STUDIES ON THE OXIDATIVE DEGRADATION OF FLUPHENAZINE DECANOATE IN OILY SOLUTION

by

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A thesis submitted to the Council for National Academic Awards in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

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TO

DOREEN

ROBERT

and

ANDREW

for their infinite patience and understanding

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Finally, to my family for the inevitable sacrifices made pursuant with my determination to complete this work. In this respect, I have demonstrated my deepest gratitude by dedicating this thesis to them.

ABSTRACT

Studies on the Oxidative Degradation of Fluphenazine Decanoate in Oily Solution

William F. Heyes

Oxidation of fluphenazine decanoate, a member of the neuroleptic phenothiazine drugs, is believed to occur in oily solution via the hydroperoxides formed as a result of autoxidation of the oil. Synthesis and characterisation of the expected oxidation products of the drug was undertaken so that the formation of these degradation products in oily solution, could be accurately determined. By this means the rate of oxidation of fluphenazine decanoate by various hydroperoxides was determined. Only in the case of the C-18 unsaturated fatty esters did the overall oxidation process obey simple kinetics (2nd order) enabling values for the respective rate-constants to be calculated. The presence of acid was clearly demonstrated to catalyse oxidation of the tertiary amine centres in the molecule, a finding contrary to reports in the literature. In addition, the catalytic effect was shown to be related to the pKa value of the acid.

Benzyl alcohol is commonly added as a preservative to oily formulations and thus the effect of this material on the rate of fluphenazine decanoate oxidation was investigated. Evidence for the enhanced oxidation of the drug in the presence of benzyl alcohol in a naturally ageing formulation was obtained and a plausible mechanism for this phenomenon was sought. Autoxidation of the benzyl alcohol was deemed a likely explanation and since little information on this aspect of benzyl alcohol chemistry could be found in the literature an extensive study of benzyl alcohol autoxidation was conducted. It was finally concluded that autoxidation of benzyl alcohol leads directly to hydrogen peroxide offering one viable explanation of the enhanced degradation of the drug observed in the presence of the preservative.

CONTENTS

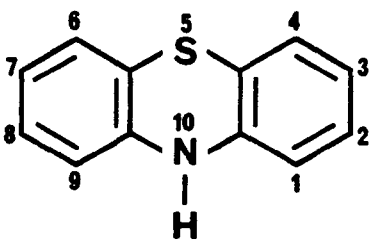
CHAPTER 1

INTRODUCTION

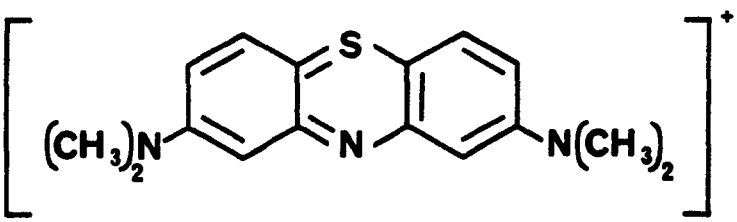
1.1. The Phenothiazines

1.1.1. Historical Development

Phenothiazine (I) was first synthesised by Bernthsen[1], nearly 100 years ago during studies aimed at elucidation of the chemical structure of methylene-blue (II). Subsequently, phenothiazine formed the basis of the chemistry of the important thiazine dyes[2].



(I)



(II)

The anthelmintic properties of phenothiazine were noted during the 1930's[3] and it has since been extensively used in veterinary medicine[4].

The origins of the modern phenothiazine drugs may be traced to the mid 1940's when a series of phenothiazine derivatives containing an amino-alkyl substituent at the 10-position were synthesised independently in France[5] and the U.S.A.[6]. By varying the nature of the amino-alkyl side chain, modification of the therapeutic activity was achieved culminating in patent rights being granted to the French group in Britain[7] (1948) and the USA[8] (1950). Included amongst the

compounds listed in the patents were diethazine (III) and promazine (IV) which were destined to become highly successful products, both commercially and therapeutically.

Additional substitution at the 2-position (aromatic ring) further modifies the pharmacological properties of the molecule, eg chlorpromazine (VII) which was first synthesised[9] in 1952 and revolutionised the therapy of the mentally ill. During the ensuing decade intensive activity in the field of phenothiazine chemistry produced literally thousands of related derivatives. Schenker and Herbst[10] reviewing the literature in 1963 listed some 3957 individual compounds.

Unfortunately, because of the labile nature of chloro group in chlorpromazine[59], the compound exhibited acute phototoxicity[11] thus limiting its therapeutic potential. Yale successfully overcame this problem by replacing the labile chloro group with the stable trifluoromethyl group synthesising[12] triflupromazine (VIII) in 1957. The discovery that the trifluoromethyl group conferred increased neuroleptic activity upon the molecule was an obvious additional advantage. Further development of the trifluoromethyl phenothiazine derivatives eventually led to the synthesis of fluphenazine[13] (XVII) which has a side chain containing the piperazine-ethanol moiety. The final stage in the development of the phenothiazine drugs was the preparation of the long-acting ester forms (1963), made possible by the presence of the alcoholic hydroxy group in fluphenazine. By esterification with decanoic acid Yale[14] produced a compound which had a duration of activity of 21 days following I.M. injection as compared with 1.5 days for unesterified fluphenazine[15]. Further discussion of this phenomenon is included in Section 1.1.2 below.

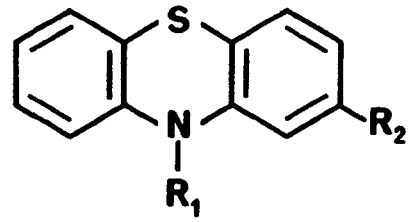
1.1.2. Structure - Activity Relationships.

1.1.2.1. Effect of Structure on Pharmacodynamic Properties

Table 1.1 lists the chemical structures of some commonly encountered phenothiazine derivatives and compares their medical uses.

Table 1.1

Some Commonly Encountered Phenothiazine Drugs



R_1	R_2	Generic Name		Medical use
$-CH_2CH_2N(C_2H_5)_2$	H	Diethazine	(III)	anticholinergic; antiparkinsonian
$-CH_2CH(CH_3)N(C_2H_5)_2$	H	Ethopropazine	(IV)	anticholinergic;antiparkinsonian
$-CH_2CH(CH_3)N(CH_3)_2$	H	Promethazine	(V)	antihistamine; antiemetic
$-CH_2CH_2CH_2N(CH_3)_2$	H	Promazine	(VI)	tranquilliser
$-CH_2CH_2CH_2N(CH_3)_2$	Cl	Chlorpromazine	(VII)	tranquilliser
$-CH_2CH_2CH_2N(CH_3)_2$	CF_3	Triflupromazine	(VIII)	tranquilliser
$-CH_2CH(CH_3)CH_2N(CH_3)_2$	H	Trimeprazine	(IX)	antipruritic

Table 1.1 (Contd..)

Some Commonly Encountered Phenothiazine Drugs

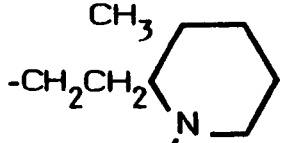
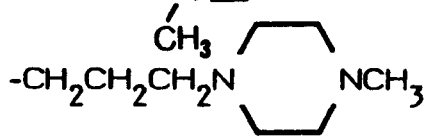
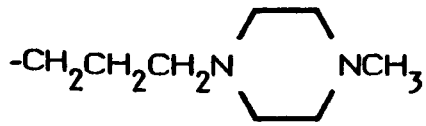
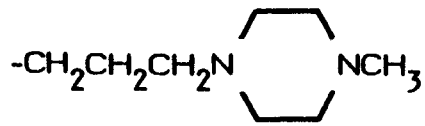
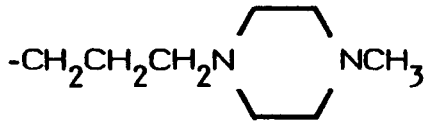
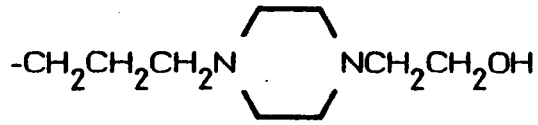
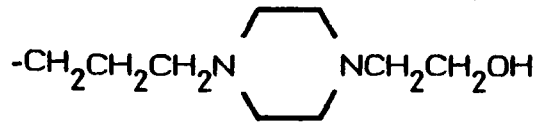
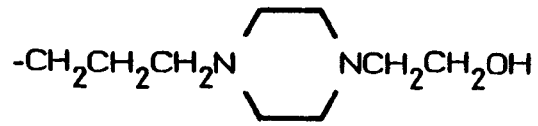
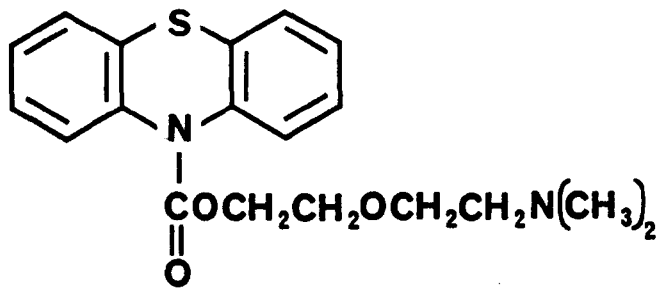
R_1	R_2	Generic Name		Medical use
$-CH_2CH(CH_3)CH_2N(CH_3)_2$	OCH_3	Methotrimeprazine	(X)	analgesic
	SCH_3	Thioridazine	(XI)	tranquilliser
	H	Perazine	(XII)	tranquilliser
	Cl	Prochlorperazine	(XIII)	tranquilliser
	CF_3	Trifluperazine	(XIV)	tranquilliser

Table 1.1 (Contd..)

Some Commonly Encountered Phenothiazine Drugs

R_1	R_2	Generic Name		Medical use
	SCH_2CH_3	Thiethylperazine	(XV)	antiemetic
	Cl	Perphenazine	(XVI)	tranquilliser
	CF_3	Fluphenazine	(XVII)	tranquilliser
	$COCH_2CH_3$	Carphenazine	(XVIII)	tranquilliser

The type of pharmacological activity shown by the compounds can be seen to vary with the nature of the substituents at both the 2 and 10 positions. A phenothiazine with an aminoalkoxy carbamate side-chain at the 10-position has ANTITUSSIVE activity, eg. dimethoxanate (XIX).[23]



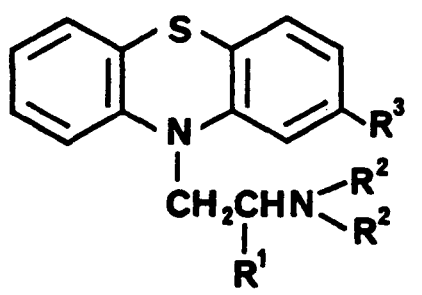
(XIX)

For a phenothiazine derivative to possess strong ANTIHISTAMINE activity two fundamental requirements must be satisfied[10].

a) the molecule should contain an aliphatic tertiary amino group in the side-chain (10-position).

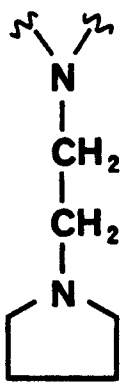
b) the side-chain should contain two carbon atoms between the phenothiazine ring N and the aliphatic amine N atom.

Even if the above requirements are met, the extent of antihistamine activity may be altered by changing the substituent groups R^1 and R^2 in the general structural formula:

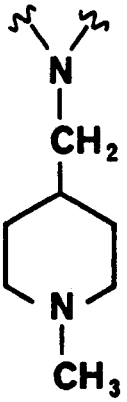


A branched-chain (ie. $R^1 = CH_3$) increases antihistamine activity whereas changing R^2 from methyl to ethyl reduces antihistamine

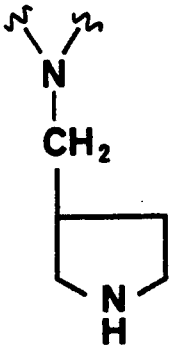
activity. Adding a 2-substituent to the aromatic ring (at R^3) also reduces antihistamine activity. Replacement of one R^2 group by a 2nd aliphatic tertiary amine group destroys the antihistamine action of the molecule[10]. Inclusion of a heterocyclic N within the side-chain renders the structure-activity relationship less predictable. Antihistamine activity ceases to be a function of the carbon-chain length between the two N atoms; thus each of compounds XX, XXI and XXII below exhibit antihistamine properties[10].



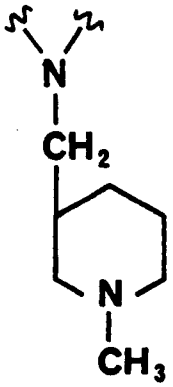
(XX)



(XXI)



(XXII)



(XXIII)

Although compounds XXII and XXIII each possess a similar C-chain length between the two N atoms, their pharmacological properties are totally different. Compound XXII is an antihistamine whereas compound XXIII is a neuroleptic.

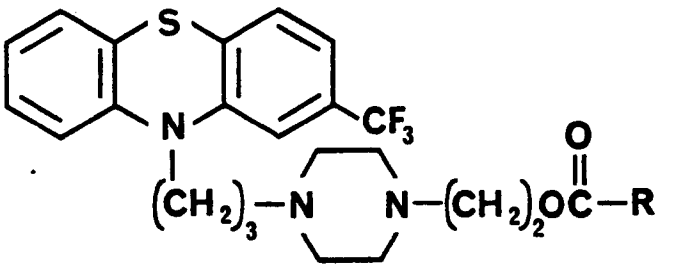
Lengthening the carbon chain between the ring N atom and the amino side-chain N to three carbon atoms results in CNS effects replacing antihistamine activity[16]. The 3-C chain is optimal for TRANQUILLISING activity[17]. Enhancement of CNS activity may be gained by inclusion of an electronegative substituent in the

aromatic ring at the 2-position[18,19]. In this respect tranquillising activity decreases in the order $CF_3 > Cl > SO_2N(CH_3)_2$[19]. Hence triflupromazine (VIII) has about five times the tranquillising activity of chlorpromazine[20](VII). The discovery concerning the effect of the trifluoromethyl group was contrary to indications given in the literature describing the structure-activity relationships of other drug molecules[21]. A further improvement in tranquillising activity may be achieved by including a piperazine-ethanol moiety in the side-chain (fluphenazine XVII). Fluphenazine has been shown to possess fifteen to thirty times the tranquillising activity of chlorpromazine(VII)[22].

1.1.2.2 Effect of Structure and Route of Administration on Duration of Activity

Of the many phenothiazine derivatives devised over the last 25 years, fluphenazine (XVII) has been shown to be the most potent neuroleptic[22]. By esterification of the alcoholic hydroxy group of fluphenazine with fatty acids[14], Yale demonstrated the prolonged activity of these novel compounds[15]. However, another equally important criteria for prolonged activity is the route of administration. Intraperitoneal injection of the hydrochloride salts of fluphenazine and fluphenazine enanthate(XXIV) results in similar potencies and duration of activity of both compounds (about 1.5 days)[15].

In order to prolong the duration of activity it is necessary to administer a sesame oil solution of the ester by intramuscular injection. By this means the duration of activity was extended to between twelve and fifteen days[15]. In control tests, similar experiments with fluphenazine showed a duration of activity of only 1.5 days ie. the same as obtained by intraperitoneal injections.

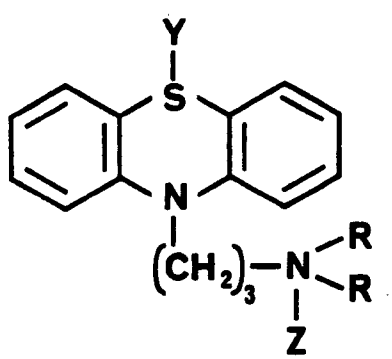


R = C_6H_{13} fluphenazine enanthate (XXlV)

R = C_9H_{19} fluphenazine decanoate (XXV)

The length of the ester C-chain is also important. The decanoate ester (C-10) migrates from the site of injection at about a half the rate of the enanthate (C-7) ester[24]. Thus fluphenazine decanoate administered I.M., as a sesame oil solution, normally has a duration of activity of about three weeks though reports of therapeutic effects lasting as long as three months have been published[25]. Hungarian workers have recently reported a new series of fluphenazine esters[26], of which the 3, 4, 5-trimethoxybenzoate ester was shown to possess a duration of activity greater than that of fluphenazine decanoate as measured by the Conditioned Avoidance Test.

1.1.2.3. Activity of Phenothiazine N- and S-Oxides



Y	Z	
O	-	sulphoxide
O_2	-	sulphone
-	O	N-oxide (t-amine oxide)

Oxidation of amino-alkyl phenothiazines can give rise to sulphoxides, sulphones or amine oxides, depending on the choice of reaction conditions (See Section 1.3 below for further details) and these compounds have been assessed for pharmacological activity.

a) Sulphoxides

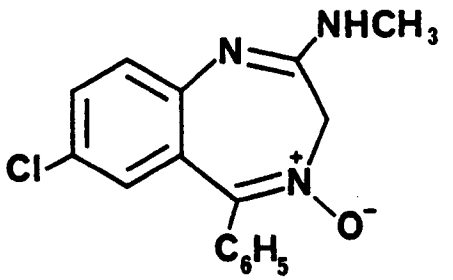
In general the sulphoxides are less active than the parent compounds. Chlorpromazine sulphoxide has been shown to be inactive (neuroleptic activity) in vitro[27, 43, 57, 84]. However, sulphoxides which have lost the tranquillising effect of the parent still retain the lactogenic activity[28].

b) Sulphones

Sulphones possess no tranquillising activity but promethazine sulphone and the sulphone of 10-(3-dimethylamino-2-methylpropyl) phenothiazine both exhibit strong antihistamine properties[29, 30]. Because of the lack of tranquillising activity and much reduced CNS activity, the sulphones have fewer associated side-effects and have been proposed as improved antihistamines.

c) N-oxides

The antidepressent properties of the N-oxide chlordiazepoxide (XXVI) are well-known and it might thus be expected that alkylamino phenothiazine N-oxides may also be pharmacologically active. Indeed, Yale has described the N-oxides of fluphenazine as having a similar tranquillising activity to the parent[31]. However, the N-oxide of chlorpromazine has been shown to be inactive in-vitro[32,33]. The neuroleptic activity found for these oxides in pharmacological test systems is therefore probably due to an enzymatic conversion to the parent compound in-vivo[32,34].

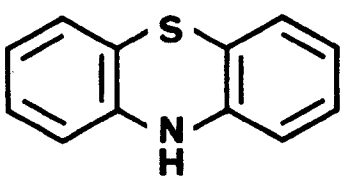


(XXVl)

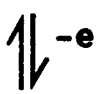
1.1.3 Oxidative Degradation

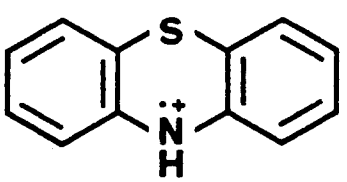
1.1.3.1 Electrochemical Oxidation - Generation of Radicals and Cations

Anodic oxidation of unsubstituted phenothiazine results in two distinct oxidation steps with half-wave potentials of + 270mv and + 750 mv respectively, measured against a Ag/AgCl reference electrode[35]. The reaction may be represented by the following equation:

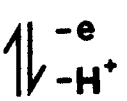


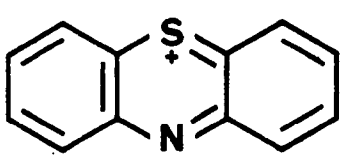
phenothiazine, R





semiquinone;
radical cation, S^+
(orange)





phenazathionium ion, T^+
(red)

The notations used to denote the various forms of the phenothiazine ring are those proposed by Cymerman-Craig[39] and Tozer[45].

Although the existence of the radical cation was postulated by Michaelis as early as 1941[36], it was not until the development of ESR spectrometry that Billon and coworkers were able to conclusively demonstrate the existence of the S^+ species[37,38]. Either of the S^+ or T^+ species may be quantitatively prepared by electrolysis of a solution of phenothiazine in acetonitrile at the necessary potential.

The redox potentials of individual phenothiazine derivatives show a pronounced dependance on the nature and position of substituent groups[39]. Substitution at the phenothiazine ring N atom renders oxidation of the molecule more difficult[40,41]. Following acylation this phenomenon would be expected, but with alkyl groups at position 10 the observation is contrary to expectation[42] as similar substitution at C atoms leads to easier oxidation. The effect can only be explained if a steric factor is taken into consideration (See Section 1.1.4). For the 10-alkylamino phenothiazine series the number of C atoms between the two N atoms is also of importance, as exemplified by the effect of pH on the redox potentials of these types of phenothiazines. Derivatives with three C atoms between the two N centres show little or no change in redox potential with pH but for similar compounds with only two C atoms the redox potentials show a strong pH dependance[40], due to the electron withdrawing effect of the protonated exocyclic N atom.

The influence of acidity on the stability of the cation radical (S^+) has been investigated polarographically[44]; an increase in pH stabilises S^+. A similar effect has been noted by other workers[45].

1.1.3.2 Chemical Oxidation - Generation of Radicals and Cations.

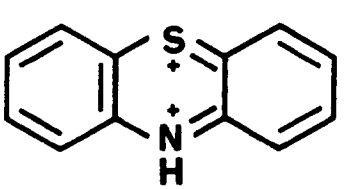
An alternative approach to the investigation of the radical species and the reaction mechanisms involved in the oxidation of phenothiazine derivatives had been adopted by Shine who generated the radical species by chemical means. Working with solutions of phenothiazine in concentrated sulphuric acid two distinct colour changes were observed[46] (Yellow to golden-orange to green) and were shown to result from the successive loss of electrons resulting in the following species:

R — phenothiazine

$\rightleftharpoons -e$

S^+ — semiquinone; radical cation

$\rightleftharpoons -e$

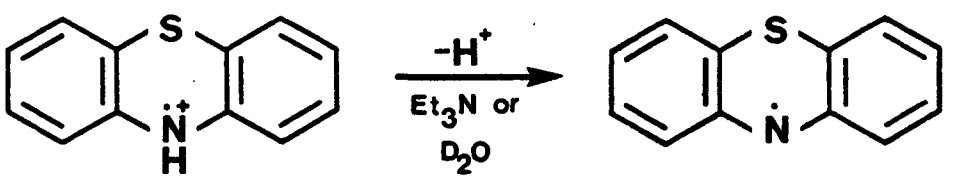


protonated phenazathionium cation, TH^{2+} (green)

The TH^{2+} species exists only in a strongly acidic anhydrous medium. In other media, the phenazathionium cation(T^+) is present, because of the prototropic equilibrium which exists between TH^{2+} and T^+ [43].

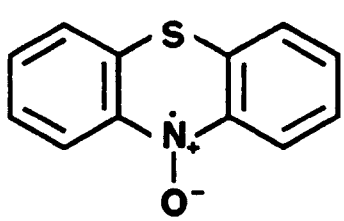
$$TH^{2+} \rightleftharpoons T^+ + H^+$$

Gilbert et al[47] have also utilised chemical oxidation to study the generation of radicals and cations from phenothiazine. These workers generated the cation radical, S^+, with perchloric acid and then demonstrated the conversion of this species to the neutral radical, S, by treatment with triethylamine or D_2O.



cation radical (S^+) neutral radical (S)

The neutral radical has been prepared in the form of an N-oxide (XXVII) by treating phenothiazine with hydroperoxides[91].



(XXVII)

Other reagents which may be employed for the chemical generation of oxidised forms of phenothiazine are:

Cerric ammonium sulphate[45]

Iron(III) salts[48]

Ti^{3+} and hydrogen peroxide[49]

Ce^{4+} and dilute sulphuric acid[50]

Fe^{3+} and dilute sulphuric acid[51]

Potassium dichromate and glacial acetic acid[52]

Using chemical methods, the effect of substituent groups of the phenothiazine molecule on radical or cation formation and stability and their relationship to the pharmacodynamic properties of the compounds have been studied. Original attempts to measure the rate of formation of the cation radical S^+ with cerric ammonium sulphate in dilute sulphuric acid were unsuccessful because of the rapid decomposition of the species[45]. However, in recent studies Gasco and Carlotti[50], again using a Ce^{4+}/dilute sulphuric acid system, determined the kinetics of S^+ formation as 2nd order when the reactants were present in equimolar concentrations, and also demonstrated a pseudo lst order formation rate when one of the reagents was present in excess. These authors were able to show a correlation between the rate constant (decay of S^+) and the reduction potential of some phenothiazine drugs. Moreover, the neuroleptic and antihistamine groups of compounds could be differentiated into two groups on the correlation plot. The same authors have also employed ferric perchlorate as oxidising agent[53] in order to study the overall rates of oxidation of promazine (VI) and promethazine (V).

The processes involved in the decay of the cation radical S^+, leading to oxidation products, has been shown to obey 2nd order kinetics[45]. The influence of substituent groups was studied and, in general, it was shown that N-substitution reduced

the rate of decay of S^+. Increasing the acidity of the solution had a similar effect. Further investigations of the kinetics of S^+ decay have been considered by Levi et al[54], particularly the influence of the disproportionation reaction with respect to 10-alkyl phenothiazines:

$$2S^+ \rightleftharpoons R + T^{2+}$$

Decay of S^+ obeyed 2nd order kinetics and the rate determining step was postulated to be the hydrolytic decomposition of T^{2+} leading to sulphoxide (See Section 1.1.3.5.below) because disproportionation and its reverse appeared to be instantaneous reactions.

Phenothiazine is a good electron donor therefore oxidation may also be achieved by interaction with suitable electron acceptors. Foster and Hanson[55] postulated that oxidation to the cation radical occurred on mixing phenothiazine with the powerful electron acceptor 2,3-dicyano-*p*-benzoquinone. Tsujino[56] subsequently demonstrated that more moderate electron acceptors, such as iodine, were sufficient to cause oxidation of phenothiazine to the S^+ species in dimethylsulphoxide solution.

1.1.3.3. Photochemical Oxidation - Generation of Radicals and Cations.

The cation radical (S^+) of phenothiazine has been generated photochemically with UV radiation in aqueous acetic acid media[46]. However, in 95% aqueous ethanol solution, the neutral radical, S, was obtained. Identification of S was made on ESR evidence.

A re-evaluation of the reaction in ethanol has been conducted by Iwaoka et al[58]. These workers confirmed that photochemical oxidation of phenothiazine in ethanolic solution

yields the neutral radical S. The process was shown to be a one photon process and the mechanism of formation was postulated to be via a molecular phenothiazine/O_2 complex of the charge-transfer type.

Phenothiazine derivatives containing a 2-chloro substituent are particularly susceptible to photo-oxidation as these molecules readily lose the chloro group via free radical formation[59]. By comparison, derivatives containing alternative 2-substituents are relatively stable.

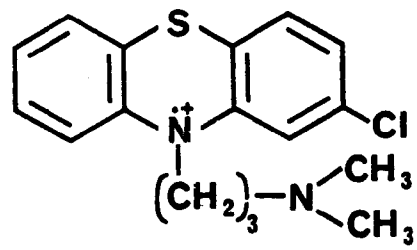
1.1.3.4 Autoxidation

The ease with which phenothiazine may be oxidised by atmospheric oxygen has been known for many years and one of the most important early industrial applications of phenothiazine was its use as an antioxidant for preventing oxidative changes in lubricating oils.

By bubbling air through an oily solution of 10-methyl phenothiazine, Brown et al[60] were able to isolate the resulting sulphoxide (10-methyl-phenothiazine-5-oxide). Colclough and Cunneen[61] subsequently demonstrated the capability of phenothiazine to react with hydroperoxides and postulated that the antioxidant action of phenothiazine involved both interference with the propagation step of the oxidative chain-reaction and decomposition of the olefinic hydroperoxide.

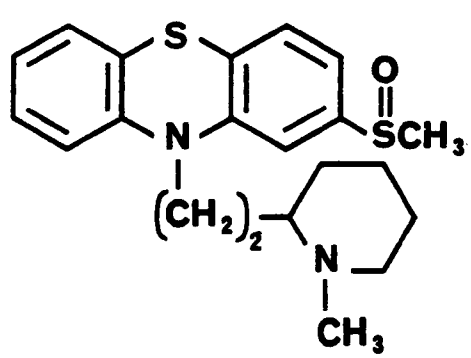
The direct photosensitised (UV) autoxidation of the pharmacologically important phenothiazine derivatives in aqueous acidic solution was originally demonstrated using chlorpromazine[62](VII). Twelve degradation products were observed of which three were identified as chlorpromazine N-oxide, chlorpromazine sulphoxide and hydroxychlorpromazine. Solution colour changes

were noted during the oxidation and are undoubtedly due to the formation of free radicals[63,64]. The radical formed has been shown to be a cation radical[65]:



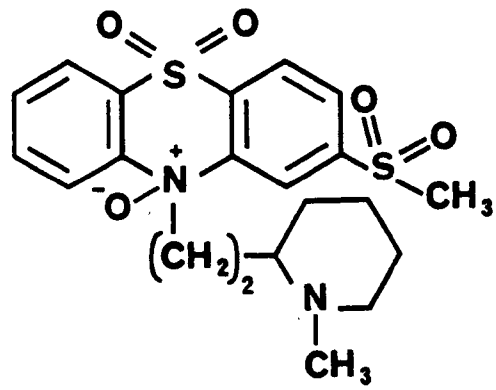
The role of free radicals in the oxidative degradation of phenothiazines is discussed below in Section 1.1.3.5.

The autoxidation of numerous phenothiazine drugs has since been investigated under a variety of conditions. In general, the compounds yielded the 5-oxide, together with other degradation products, often unidentified. Eg. promethazine[66], methopromazine[67], levopromazine[67], trifluperazine[68] and thioridazine[69]. Of particular interest are the results concerning thioridazine. Degradation in the influence of UV irradiation produced a pattern of degradation products similar to that reported for other phenothiazine drugs, but under conditions of total darkness only one major degradation product was noted. This compound was identified as a sulphoxide, but was not the expected 5-oxide. Attack of the 2-methyl thio ether occurred yielding the following compound:



(XXVIII)

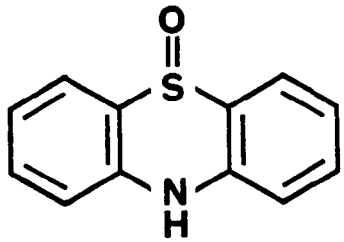
In addition, the authors also claimed to have isolated an N-oxide species in which the phenothiazine ring N was oxidised:



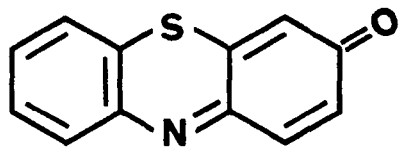
(XXIX)

Oxidation of the phenothiazine ring N in preference to the side-chain N is contrary to expectation, as substitution of an alkyl group at the 10- position causes oxidation at this point to be more difficult[42]. The absence of other literature reports concerning the preferential oxidation of the phenothiazine ring N together with the knowledge that the position of oxidation was assigned on I R evidence alone renders the structual identity of this compound suspect. The N-O band observed in the I R spectrum is more likely to arise from oxidation of the piperidine (side-chain) nitrogen atom.

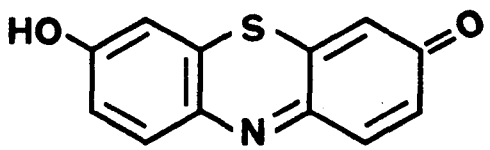
A detailed investigation of phenothiazine oxidation in an oxygen-saturated acidic media protected from light has been conducted by Roseboom and Perrin. The major degradation products were identified[70] (Fig. 1.1) and Ist order kinetics demonstrated[71] with respect to the disappearance of phenothiazine. Furthermore, by kinetic means, the authors were able to demonstrate that XXX and XXXI were formed by parallel reactions, but XXXIII was produced independantly. Hence a mechanistic pathway for the formation of the degradation products of phenothiazine was postulated[72]. By contrast, N-methyl-phenothiazine (XXXV)



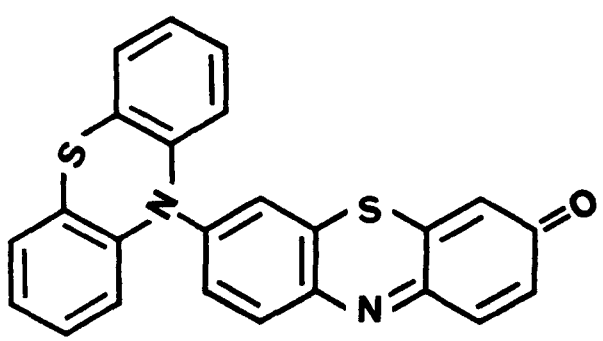
phenothiazine-5-oxide
(XXX)



3H-phenothiazine-3-one
(phenothiazinone, XXXl)



7-hydroxy-3H-phenothiazine
3-one (XXXll)



7-(10'-phenothiazinyl)-
3H-phenothiazine-
3-one (XXXlll)

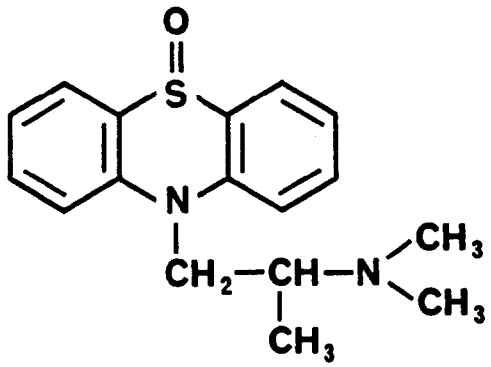
Fig.1.1 The Major Autoxidation Products of Phenothiazine[70]

formed only two degradation products[71], phenothiazine sulphoxide (XXX) and N-methyl-phenothiazine sulphoxide, which were shown to be produced by parallel reactions.

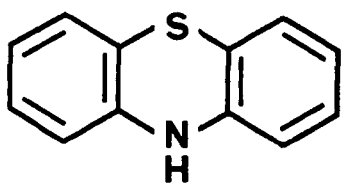
In an extension to the above study, degradation of the 10-acetyl derivative of phenothiazine under acid conditions[73] was studied (again in the dark). This compound was considered as a model for phenothiazines possessing a carbonyl group adjacent to the ring N, compounds which exhibit antiarrythmic properties[74]. None of the observed degradation products contained the acetyl group, and were identified as identical to those reported previously for phenothiazine[70] (Fig. 1.1). Kinetic investigations in the presence and absence of oxygen, together with the fact that the acetyl group was absent from all degradation products, indicated that 10-acetyl-phenothiazine degraded via a specific hydrogen-ion catalysed hydrolysis, followed by subsequent oxidation of the phenothiazine so formed.

Underberg has extensively studied the degradation of promethazine. An aqueous oxygen-saturated solution of promethazine hydrochloride was allowed to degrade in the dark, and the resulting major oxidation products were isolated and identified. Fig. 1.2 shows the identity of the compounds. Disappearance of the parent compound followed 1st order kinetics[76]. The influence of Fe and Cu ions on the degradation process was demonstrated[76] and a mechanism by which the ions could affect the degradation of the drug was postulated.

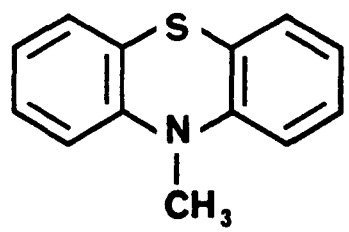
Further work aimed at correlation of the oxidative degradation pattern with the nature of the 2-substituent has been reported by Underberg et al[77]. After comparing the degradation patterns of 2-methyl promazine, triflupromazine and cyanopram-



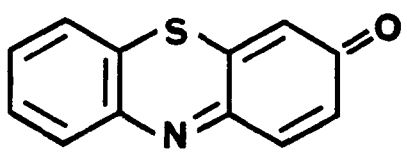
promethazine-5-oxide
(XXXlV)



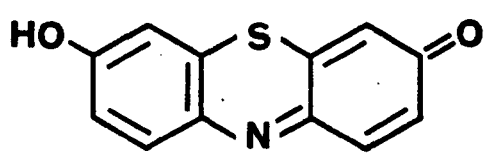
phenothiazine (I)



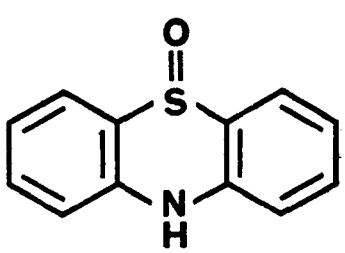
lO-methylphenothiazine
(XXXV)



3H-phenothiazine-3-one
(XXXl)



7-hydroxy-3H-phenothiazine-
3-one (XXXll)



phenothiazine-5-oxide
(XXX)

Fig. 1.2 - The Major Degradation Products of Promethazine

azine the authors postulated that with an electron-withdrawing group at the C-2 position, an interaction with the N-10 side-chain is possible, leading to demethylated degradation products; thus explaining the formation of demethylated products from triflupromazine and cyanopromazine, and not from 2-methyl promazine.

1.1.3.5. Reaction Mechanisms - The Role of Radicals and Cations

The majority of products formed during the oxidative degradation of phenothiazine derivatives may be explained in terms of free-radical and cation intermediate species. Three types of reaction may be distinguised:

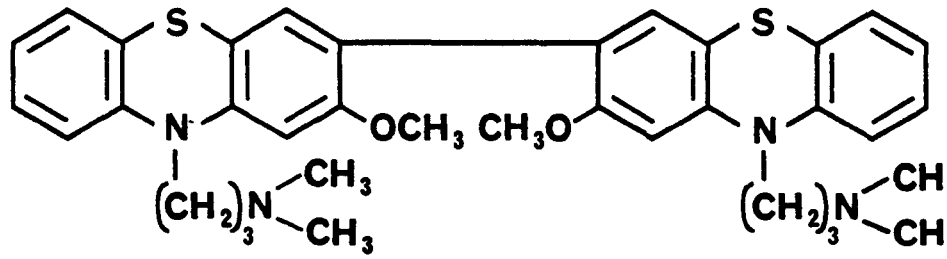
a) oxidative coupling(resulting in dimers)

b) hydrolysis of cations yielding 5-oxides

c) hydrolysis leading to 3-oxo substituents

a) Oxidative Coupling; Dimer Formation

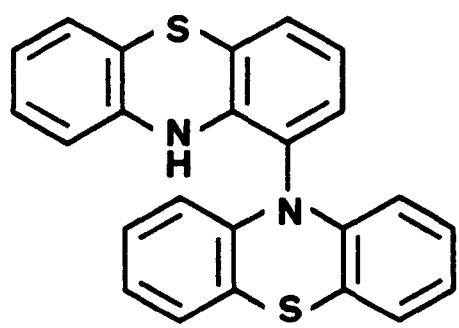
ESR studies have shown that the greatest spin-density in the unsubstituted phenothiazinyl free radical is concentrated at the N, S, C-3 and C-7 atoms[78]. The sulphur atom in the sulphide bridge shows no tendency to form a third covalency[42] and hence no S-linked dimers have been reported. Dimers linked via a N-N coupling have been prepared[79, 80, 81], but have not been reported as oxidative degradation products. Formation of a C-C linked dimer at C-3 has been claimed by Fujisawa[82], by the action of white fluorescent light on an aqueous acid solution of methoxypromazine:



(XXXVI)

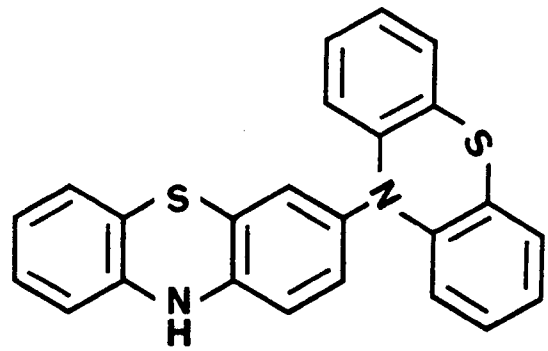
The most commonly encountered dimers occurring as degradation products are the N-C linked compounds. 7-(10'-phenothiazinyl)-3-H-phenothiazine-3-one (XXXIII, Fig. 1.1) has been identified in an ageing solution of phenothiazine[70].

When dissolved in a mixture of DMSO and acetic anhydride (deoxygenated) phenothiazine is slowly converted into two dimers (XXXVII and XXXVIII) and certain higher polymers (proposed as a trimer and tetramer)[83]. The reaction was postulated to proceed via the neutral free radical, S'. In DMSO/iodine solution the 3,10' dimer (XXXVIII) is formed in 64% yield[56].



(XXXVII)

1,10' biphenothiazine

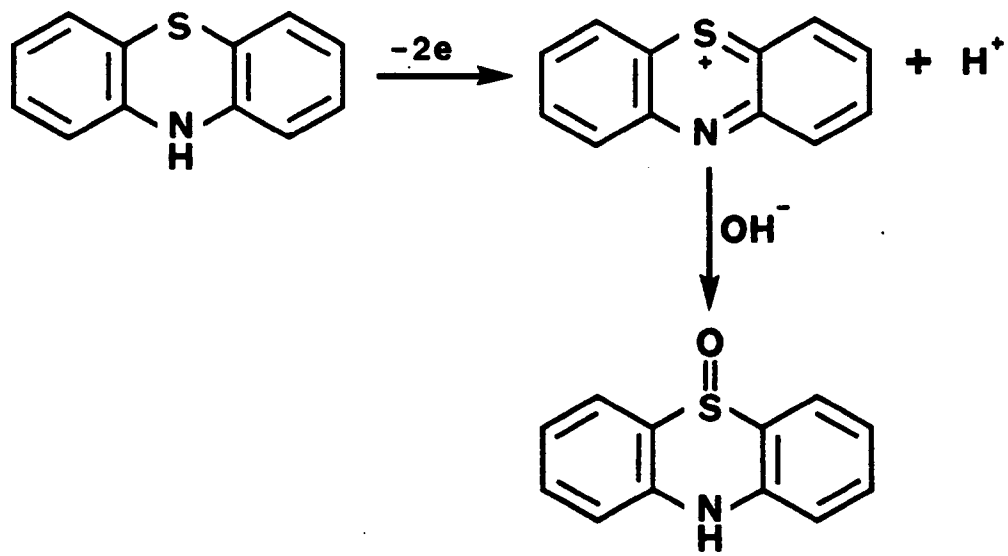


(XXXVIII)

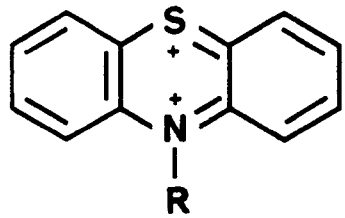
3, 10' biphenothiazine

b) Hydrolysis of Intermediate Cations; Formation of Sulphoxide

Electrochemical generation of the phenazathionium cation (T^+) from phenothiazine followed by treatment with hydroxylated bases results in the formation of phenothiazine-5-oxide[40,85].



N-substituted phenothiazine derivatives, however, form the 5-oxide with such ease that the traces of water present in the solution are sufficient to hydrolyse the intermediate cation species generated. In this latter case, the lack of a H atom in the 10 position (i.e. the ring N) prevents the elimination of H^+ and thus a highly reactive bivalent cation (XXXIX) is produced which is immediately hydrolysed to the 5-oxide[86].



(XXXIX)

Intermediate cation formation during the chemical oxidation of 10-alkylamino phenothiazines to the corresponding sulphoxide (5-oxide) has been demonstrated with such reagents as bromine[87], lead tetra acetate[88] and sulphuric acid[89].

An alternative reaction mechanism via the radical cation, S^+, leading to production of the 5-oxide has been described[85,86]. Instead of the direct oxidation of phenothiazine to the phenazathionium cation T^+ (loss of two electrons), the intermediate radical cation S^+ (loss of one electron) is formed which then disproportionates, particularly in alkaline solution, resulting in the formation of T^+. Hydrolysis of the T^+ species to the 5-oxide may then take place. The reaction pathways are summarised in Fig. 1.3.

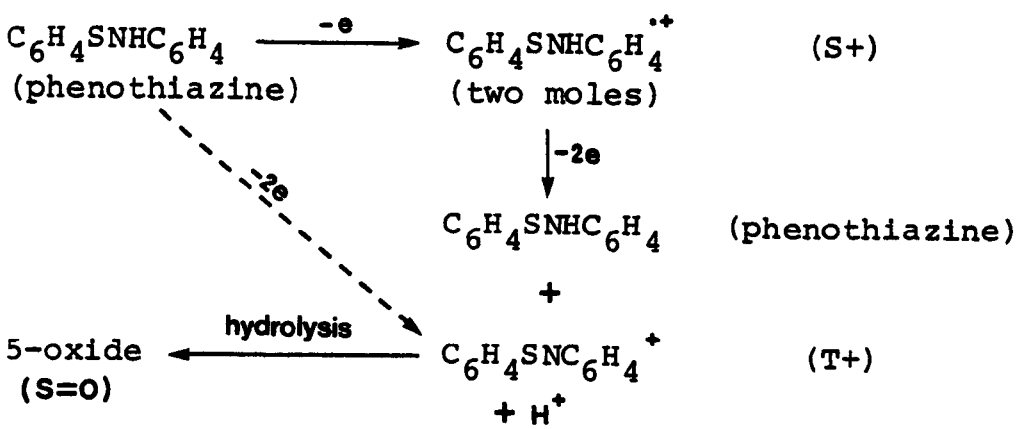
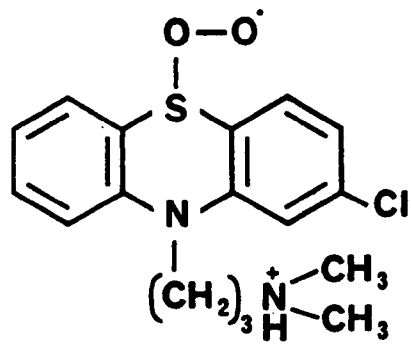


Fig. 1.3 - Reaction Pathways for S=O Formation

Hydrolysis of the phenazathionium cation (T^+) has been further investigated[90] by tracer experiments using H_2O^{18}. Photoxidation of chlorpromazine (VII) in H_2O^{18} and ethanol/H_2O^{18} solutions resulted in a 5-oxide product that did not contain O^{18}. Thus in the case of photoxidation, hydrolysis of T^+ does not appear to be the mechanistic pathway. The authors postulated a peroxyl radical (XL) as precursor to the sulphoxide on the basis of flash-photolysis experiments.



(XL)

c) Hydrolysis Leading to 3-oxo Substituents

Nucleophilic attack by water on the T^+ ion yields phenothiazine-3-one[46]. The reaction is concurrent with that of 5-oxide formation and dependant on the acidity of the solution. Strongly acid conditions favour 3-one formation because protonation of the 5-oxide leads to S^+ [46] i e. sulphoxide formation is acid reversible. Fig. 1.4 summarises the competing reactions.

1.1.3.6. Biological Oxidation

Over the past 30 years considerable effort has been devoted to the investigation of the metabolic changes undergone by phenothiazine drugs. The majority of reports concern chlorpromazine (VII) which has become the accepted model for such investigations. A recent estimation of the number of chlorpromazine metabolites observed

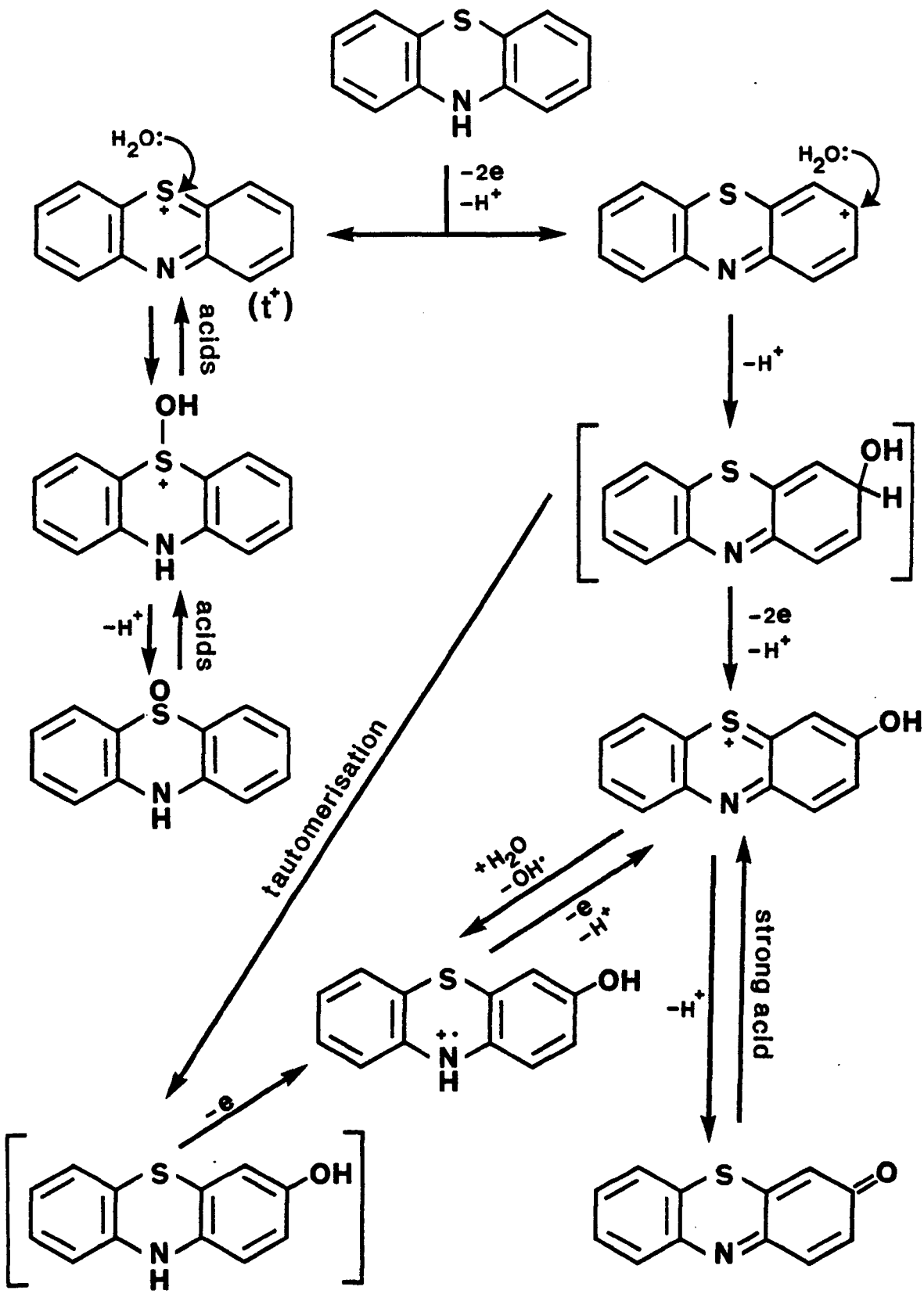
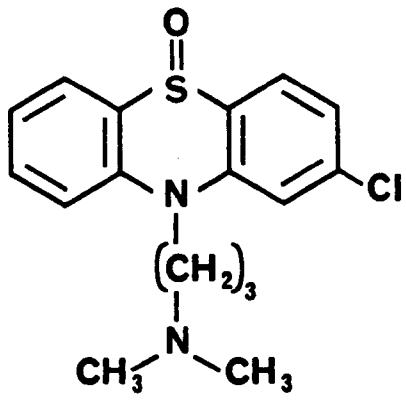


Fig. 1.4 - Competing Reactions in Hydrolysis of T^+ [42]

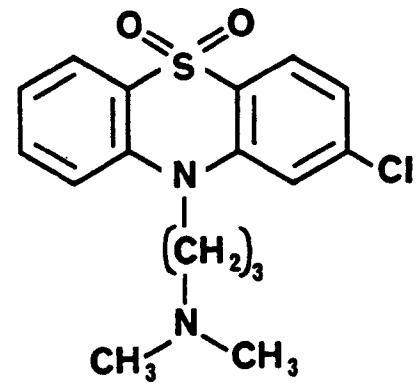
since the discovery of the drug[94] totalled some 168. However, many of the observed compounds have not yet been fully identified. In general, metabolic transformation of 10-alkylamino-phenothiazine drugs leads most usually to the 5-oxide, the 7-hydroxy derivative, the side-chain N-oxide and demethylated products[92]. Further oxidation of the sulphoxide to sulphone may also take place. Fig. 1.5 shows the structures of typical compounds. Obviously, the chemical structure of the compound will ultimately determine the metabolic profile. Eg. phenothiazines substituted at the 2-position with a sulphur-containing group (thioridazine, XI) can, and do, form the alternative sulphoxide[93]. The oxidoreductase enzymes present in the liver are believed to be responsible for the formation of the S- and N- oxides[98] and have been utilised by some workers as a means of synthesizing these compounds (See Section 1.2.1.2).

1.1.4. Molecular Configuration

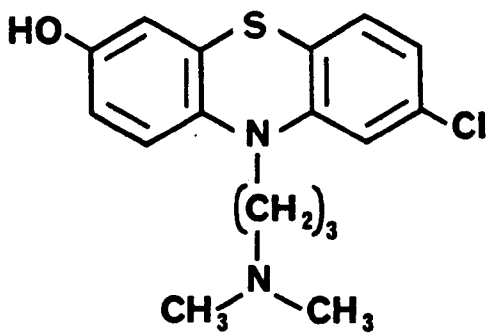
Conflicting accounts of the molecular configuration of phenothiazines are to be found in the literature. Some workers have interpreted the available evidence to indicate a planar structure whereas others believe a folded structure about the N-S axis to be the case. The folded structure is the more generally accepted. Malrieu and Pullman have considered the proposed tetragonal-folded structure of phenothiazine in detail[95]. Two extremes of conformation are possible with the 10-hydrogen atom in either the intra (endo or quasi equatorial) position, or the extra (exo or quasi axial) position - Fig. 1.6.



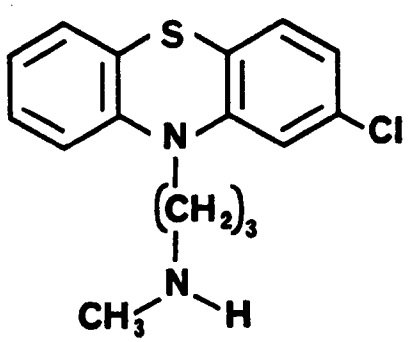
chlorpromazine sulphoxide



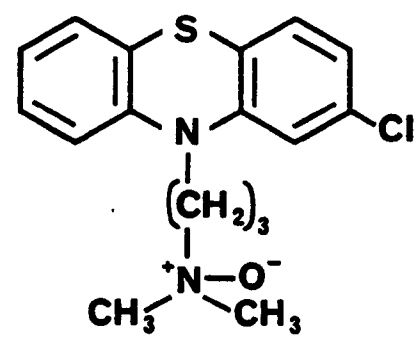
chlorpromazine sulphone.



7-hydroxychlorpromazine



desmethylchlorpromazine



chlorpromazine N-oxide

Fig. 1.5 - Some Metabolites of Chlorpromazine

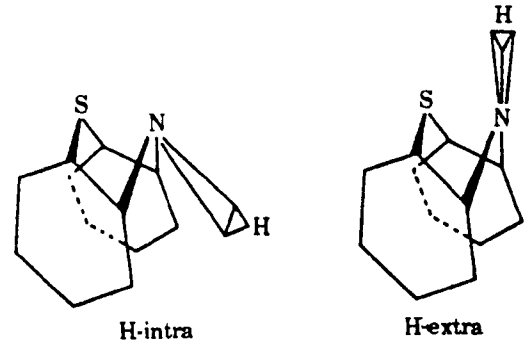


Fig. 1.6 - Configuration of Phenothiazine

Unsubstituted phenothiazine was shown to adopt the H-intra configuration by comparing the calculated and experimental values for the spin densities of the corresponding free radical. More recent considerations of dipole moments has indicated that 2-substituted phenothiazine may, in fact, exist in solution as a mixture of the conformers[96].

Substitution of an alkyl substituent at the ring N was postulated to result in formation of the 'extra' conformer because of the bulk of the substituent group[95]. The two forms 'intra' and 'extra' are not electronically equivalent because the proximity of the N lone-pair to the aromatic rings changes and thus alters the energy of the highest filled molecular-orbital[42]. It is for this reason that oxidation of N-substituted phenothiazines is rendered more difficult ie. the oxidation potential of N-substituted derivatives is increased. Following NMR experiments with S-oxidised phenothiazines, Russian workers also concluded that 10-alkyl phenothiazines adopt the 'extra' configuration[205].

Calculations by Reboul and Cristau[97], based on previously published crystallographic data, have demonstrated that the N-alkyl sidechain does indeed stretch away from the ring system and that

introduction of a 2-substituent tends to increase the folding of the 'butterfly' tricyclic system.

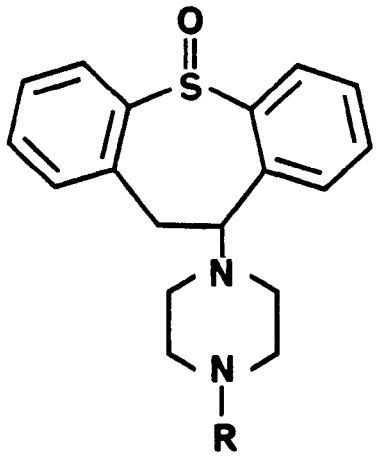
1.2. Synthesis of N- and S-Oxides

1.2.1 Practical Aspects(pertaining to tricyclic moieties)

1.2.1.1. Chemical Methods

Oxidation of substituted phenothiazine generally leads to a mixture of oxidation products[42]. Specific S-oxidation may be achieved with the hydroperoxides of tetrahydrofuran and dioxan[99], compounds which are present in these solvents following autoxidation. Alternatively, the action of HNO_3/acetic acid on the protected N-benzyl phenothiazine yields the corresponding sulphoxide, which may then be converted to phenothiazine sulphoxide by removal of the benzyl substituent via alkaline hydrolysis[100] (85% yield phenothiazine sulphoxide). Acetylation has also been utilised to protect the phenothiazine ring N during the preparation of the sulphone[101] ie. the 5, 5 dioxide.

In the specialised case of 10-aminoalkyl phenothiazines, numerous methods have been developed to allow the specific oxidation of either the ring S, or the side-chain N. (Oxidation of the phenothiazine ring N is unlikely because of the high value of the oxidation potential when substituted with an alkyl group - See Section 1.1.3) Specific S-oxidation is most commonly gained via an acetic acid/hydrogen peroxide system[102]. This procedure yields the 5-oxide in quantitative amounts on a semi-micro scale and has been applied to the detection of phenothiazines in biological fluids[102]. It is also the basis of a quantitative fluorimetric assay for phenothiazines[103]. A similar preparative procedure has been reported for the selective synthesis of the sulphoxide of piperazino dibenzothiepines[104](XLI).



(XLI)

The hydrogen peroxide/acetic acid system has the added advantage that by raising the temperature to 70°C, the corresponding sulphone may be synthesised even in the presence of a side-chain containing tertiary amino groups[105].

Other reagents which have been reported to selectively oxidise the S atom in the presence of an amino-alkyl side-chain are:

lead tetra-acetate[106,118]

periodic acid[107,109]

chromium trioxide/acetic acid[108]

potassium permanganate/acetic acid[108]

ozone[110]

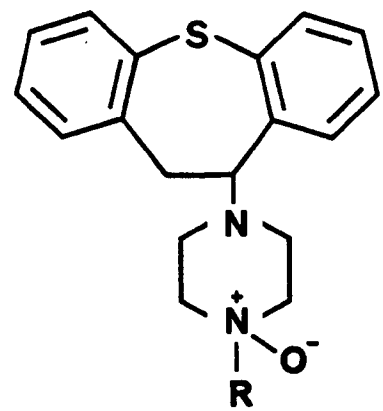
sodium nitrite/acetic acid[111]

ammonium persulphate[112]

perbenzoic acid(or its *m*-chloro derivative)[113,119]

The selective oxidation of the side-chain tertiary amino N centre(s) is less well defined. Numerous procedures exist but none has become universally accepted. A Japanese patent records the use of $Ca(OCl)_2$ for the preparation of chlorpromazine N-oxide[114]. An ethanolic solution of hydrogen peroxide,

followed by a palladium/charcoal treatment has been employed by several workers[115, 116] for the synthesis of N-oxides, although use of ethanolic hydrogen peroxide alone apparently resulted in formation of the mixed N-oxide-sulphoxide of chlorpromazine[117]. With piperazino dibenzothiepines however, ethanolic hydrogen peroxide alone yielded the N-oxide[105] (XLII). The use of *m*-chlorperbenzoic acid has been similarly described[120].



(XLII)

In contrast to the above direct oxidations, selective synthesis may also be accomplished by indirect procedures. Eg. to prepare the sulphoxide of chlorpromazine, phenothiazine sulphoxide is first synthesised then the required alkylamino side-chain is attached[121]. Many such procedures have been described in the literature, often as a means of avoiding the infringement of patent rights.

1.2.1.2. Enzymic Methods

Conversion of phenothiazine derivatives to the corresponding sulphoxide and N-oxide by the enzymes present in liver microsomes is a well-documented feature of the metabolism of these

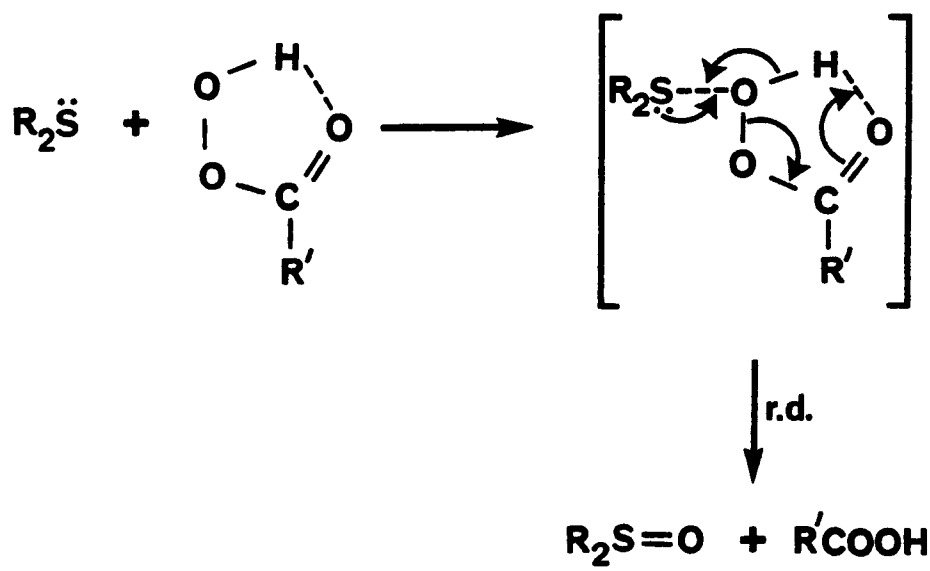
compounds[98, 123]. (See also 1.1.3.6). N-oxidation in particular is considered to be a major metabolic route of phenothiazine drugs[124]. Using pig-liver microsomes, Sofer and Zeigler[125] have developed a novel procedure for the selective preparation of the N-oxide of such compounds as fluphenazine, thiopropazine and trifluperazine. In order to achieve a useful yield the stability and activity of the oxidases was enhanced by covalent bonding to glass beads[126]. By this means 500mg quantities of the N-oxide of each compound was synthesised.

1.2.2. Kinetic and Mechanistic Aspects

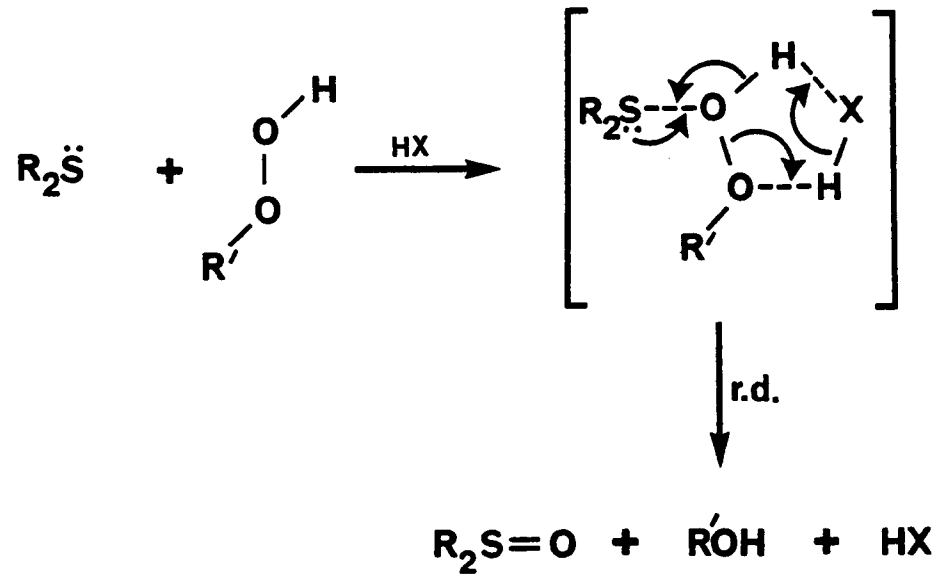
The oxidation of phenothiazines to sulphoxides by some chemical oxidising agents has been discussed previously (Section 1.1.3.5) in terms of a mechanism involving the phenazathionium cation and the phenothiazine cation radical. However, the reaction of peroxides with organic nitrogen and sulphur centres involves an entirely different mechanism and is fully discussed below.

1.2.2.1 Organic Sulphides

Oxidation of dialkyl sulphides with percarboxylic acids may be summarised by the following mechanism[127].



The reaction is usually fast and accelerated by electron-donating groups in the sulphide or electron-withdrawing groups in the peracid. The reaction shows no dependance on the presence of a strong acid[128,129]. A similar mechanism may be written for the reaction of dialkyl sulphides and hydroperoxides[130].



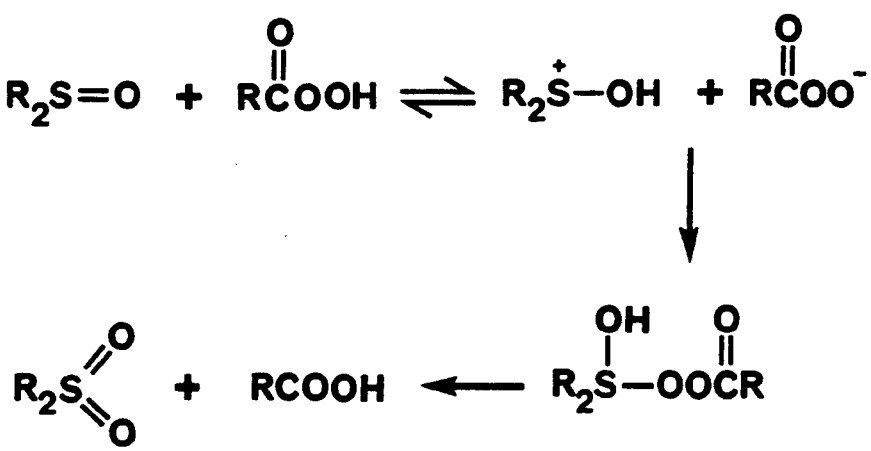
$$R_2S{=}O + R'OH + HX$$

The reaction is 1st order in both sulphide and peroxide (ie. 2nd order overall). In contrast to the reaction with peracid, hydroperoxide oxidations are strongly acid-catalysed. The role of the acid, HX, is to facilitate the loss of the leaving group ROH from the transition state. With the peracid the additional proton source is not required because the geometric characteristics of the molecule provide a low-energy cyclic transition state for the loss of RCOOH[128]. The nature of the protonic material HX can be any potential proton donor, such as the protic solvents alcohol and water. The effectiveness of HX is determined by its pKa. Work in aprotic solvents has shown that in the absence of other protonic material, the alkyl hydroperoxide can itself function as the proton donor. In this case, the oxidation rates are 2nd order in hydroperoxide The acid strengths of alkylhydroperoxides

have been found to correlate with the rate of oxidation of the sulphide[128].

On addition of strong acid to the hydroperoxide however it is the acid which is preferentially involved (as HX) in the transition state. Eg. acetic acid added to hydrogen peroxide; acetic acid acts as the HX species. The very much lower pKa of acetic acid accelerates the reaction rate. Hence a mixture of acetic acid/hydrogen peroxide has an oxidation rate comparable to that of a peracid. The effect is believed to be catalytic rather than the 'in situ' generation of peracetic acid as oxidation usually occurs faster than the rate of formation of peracetic acid[128].

Sulphoxides may be further oxidised to sulphones by percarboxylic acids or by hydroperoxides. With peracids, the conversion of sulphoxide to sulphone is slower by a factor of 100 to 1000 than the initial formation of sulphoxide from the dialkyl sulphide[86,122,129,131,138]. A reaction mechanism similar to that illustrated on p.35 was originally postulated for sulphone formation, but more recent reports indicate that the following mechanism may be more accurate[132].

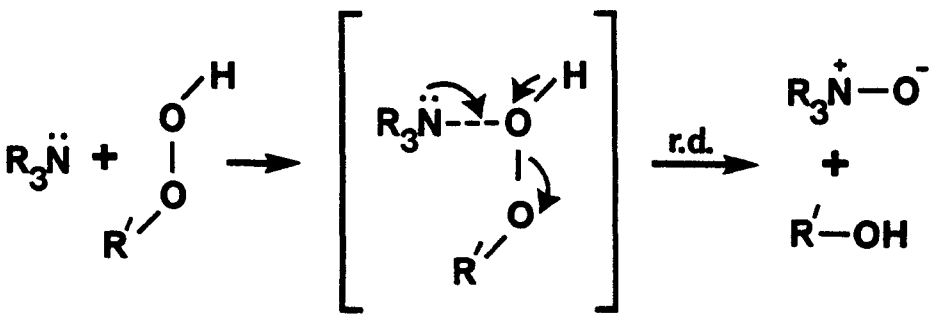


Conversion of sulphoxide to sulphone with hydroperoxides requires elevated temperatures and/or the addition of a suitable

catalyst. Temperatures above 70°C are generally used and typical catalysts include the carboxylic acids[133] and the salts of molybdenum[134], zirconium[135] and vanadium[136].

1.2.2.2. Tertiary Amines

Publications concerning the oxidation of t-amines are relatively sparse in comparison to the literature describing the many aspects of S-oxidation. However, the following mechanism has been proposed[137] for the reaction of alkylhydroperoxides with t-amines.



Aqueous hydrogen peroxide is sufficient to convert aliphatic t-amines to the corresponding oxide but less reactive compounds, such as the heterocyclic t-amines, often require peroxy acids. *m*-chloroperbenzoic acid in chloroform solution has been recommended as a means of preparing tertiary amine N-oxides[107] in almost quantitative yields.

As with the oxidation of dialkyl sulphides, the rate of the reaction increases with increasing nucleophilicity of the amine and electrophilicity of the peroxide. The reaction is not acid-catalysed, indicating the decreased dependance on hydrogen-bonding for loss of the leaving group $R\text{-}O^{-}$. A low pH is disadvantageous due to the decrease in effective concentration of the amine. Thus the attempted oxidation of t-amines with trifluoroperacetic acid results in the formation of the TFA salt

of the amine, no oxidation to the amine oxide being noted[139].

1.3. Analysis of N- and S- Oxides

The occurrence of N- and S- oxides as degradation products, and as metabolic products, of tricyclic drugs requires that suitable analytical procedures be available for the quantitation of the compounds. A variety of procedures have been reported for this purpose and are discussed individually below.

1.3.1. Spectrophotometric Procedures

Many of the colorimetric methods described for the quantitation of phenothiazines[140] may be successfully applied to the determination of the corresponding N- and S- oxides. One notable exception is the procedure involving the chelation of phenothiazine with suitable metal ions (e.g. palladium[141]) forming a coloured complex. The metal chelates at the S atom of the tricyclic ring system and thus sulphoxides and sulphones are not sensitive to this reagent.

Most of the early spectrophotometric procedures (in respect of phenothiazine oxides) were developed in order to assay the sulphoxide present in biological samples. Thus, for example, Flanagan et al[142] utilised a UV method, Hetzel[143] nitrated the compounds forming yellow-coloured products, and Gothelf and Karczmar[144] used ferric ammonium sulphate which produced a red colour. More recently Difference Spectrophotometry has been described[145] for the assay of the sulphoxide content of degraded phenothiazine formulations. The assay is specific for the sulphoxide moiety in the presence of the parent phenothiazine drug, but in order to avoid interference from other oxidation products, a selective alkaline extraction procedure was included.

The specific determination of t-amine oxides may be achieved by an indirect colorimetric method[146]. The N-O is allowed to react with SO_2 forming an adduct. Subsequent hydrolysis yields a secondary amine and an

aldehyde, the latter of which can be determined colorimetrically.

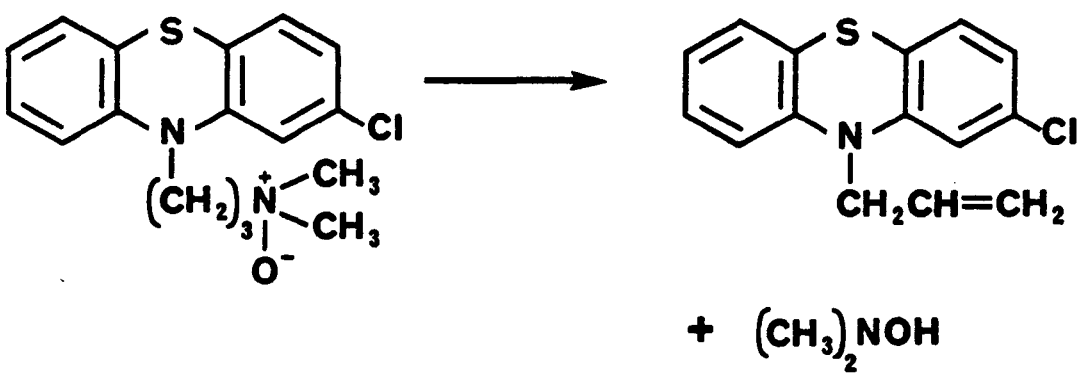
1.3.2. Fluorimetric Procedures

Phenothiazines exhibit only a weak native fluorescence. However, on conversion to the sulphoxide an intensely fluorescent molecule is obtained[103,148]. This phenomenon has been made the basis of an assay procedure for the determination of both phenothiazines[149] and thioxanthenes[150] in biological fluids.

1.3.3. Chromatographic Techniques

TLC systems have been extensively used for the separation of phenothiazine degradation products, including the sulphoxide, sulphone and side-chain N-oxide, from the parent compound[140]. Quantitation of the chromatograms may be achieved by densitometry[151]. Conversion of the parent compound to the sulphoxide on the surface of the TLC plate has been noted[168,169]. Thus particular care must be exercised when examining phenothiazines by TLC in order to avoid erroneous results.

Of the aminoalkyl phenothiazine oxides only the sulphoxide and sulphone are amenable to direct GC analysis since the N-oxide derivatives undergo thermolysis[152] yielding an allyl compound via Cope elimination[153].



However, this reaction has formed the basis of an indirect GC assay for chlorpromazine N-oxide[152]. The majority GC procedures in recent years have been applied to the determination of the metabolites of phenothiazine

drugs[140]. In order to improve the sensitivity of the GC procedure a combined GC - Mass Spectrometry method has been reported[154]. Similar procedures have been utilised for the identification of some phenothiazine metabolites[155,156].

In view of the problems encountered with the GC of the oxides, HPLC has proven to be the most useful of the chromatographic methods for the direct quantitation of tricyclic N- and S- oxides, particularly when a mixture of oxidation products is expected to be present. Eg. the separation of the oxides of thioridazine[157] (X1). Examples of the application of both normal-phase[157,158,159] and reversed-phase[160,161,162] HPLC are apparent in the literature. UV detection is normally employed, but with sulphoxide and sulphone a fluorimetric detector has also been utilised[157].

1.3.4. Electrochemical Methods

The use of electrochemical methods during the investigation of phenothiazine oxidation by Billon and co-workers has been referred to previously. However, cathodic polarography may be successively used to quantitatively determine sulphoxides in the presence of the parent compound[163]. Beckett et al[164] have determined the metabolic N-oxide, N-oxide sulphoxide and sulphoxide of chlorpromazine after prior separation by conventional partition. German workers have adopted polarography as a means of assaying chlorpromazine tablets by first oxidising the phenothiazine to the polarographically reducible sulphoxide[165].

1.3.5. Radio-immuno Assay(RIA)

Description of the application of RIA procedures to phenothiazine oxides are restricted to the determination of the level of interference during assay of the parent[166,167]. In the majority of cases the sulphoxide is almost devoid of cross-reactivity, typically about 0.6%. No doubt, if required, a specific antibody for reaction with sulphoxide could be raised, but with the many alternative methods already available, an RIA method is unlikely to be developed in the near future.

CHAPTER 2

FLUPHENAZINE DECANOATE INJECTION(MODECATE)

2.1 Introduction

Modecate injection is a depot-action phenothiazine formulation designed for the management of acute schizophrenia and marketed as a solution of the ester in sesame oil. The idea for such a novel product was conceived when Yale, working at the Squibb Institute for Medical Research in New Brunswick (USA), discovered the extended duration of activity associated with fluphenazine esters when formulated as a solution in sesame oil solution.[15]

2.2 The Formulation

The normal Modecate formulation is given below:-

Material	Quantity
Fluphenazine decanoate	25mg
Benzyl alcohol	15mg
Sesame oil	q.s. 1ml

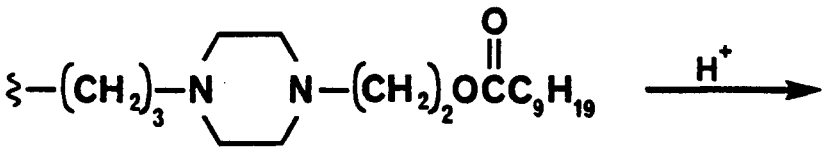
Benzyl alcohol is included in the standard formulation, its original, purpose being a preservative for the multidose pack.

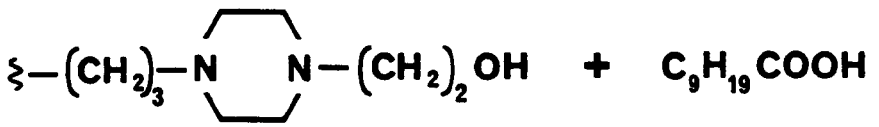
2.3 Degradation Routes

Numerous degradative pathways for fluphenazine decanoate formulated in sesame oil have been identified, all of which are predictable from a knowledge of the chemistry of the active.

2.3.1 Hydrolysis

Hydrolysis of the side-chain ester linkage in fluphenazine decanoate yields fluphenazine and decanoic acid:





This reaction is particularly detrimental to the product as relatively high concentrations of free fluphenazine are considered clinically undesirable and may lead to unacceptably high initial blood-levels[170].

2.3.2 Transesterification

Sesame oil consists of a mixture of triglycerides derived from oleic (45.4%), linoleic (40.4%), palmitic (9.1%), stearic (4.3%) and arachidic (0.8%) acids[171]. Hence interaction of fluphenazine decanoate with the oily vehicle can produce such esters as fluphenazine oleate, linoleate, palmitate and stearate by transesterification. The trans-esters may be detected by GLC[12] or HPLC[173].

2.3.3. Oxidation

The phenothiazine ring may undergo a variety of oxidation reactions (Section 1.1.3), but the only ring-oxidation product reported to date in Modecate injection is the sulphoxide. This compound may be detected in aged formulations using a TLC procedure[174].

Oxidation of the side-chain will result in the formation of a t-amine N-oxide. The occurrence of such a degradation product was confirmed in 1971[174] when small amounts were isolated from an artificially aged formulation. Preparative layer chromatography was utilised for the

isolation of the degradation products which were then examined by mass spectrometry. Two independant interpretations[174, 185] of the MS data agreed that one particular isolated zone from the TLC plate was probably the N-oxide of fluphenazine decanoate, although the thermolabile nature of the species rendered an absolute identification impossible. Attempts to confirm the identity of the N-oxide by synthesis of a compound with the proposed structure were, at that time, unsuccessful[174]. Subsequent work by Heyes[175] eventually led to the synthesis of a compound with an Rf value (TLC) identical to that of the compound believed to be the N-oxide. However, further structural investigations were not undertaken.

2.3.4. Photolysis

Storage of the product is usually in the dark, by virtue of the packaging; thus photolysis is a highly unlikely degradation route. If, however, opened packages were left exposed to light, production of the sulphoxide could be postulated by analogy with similar studies concerning chlorpromazine[90]. No investigations of this nature have been undertaken with the Modecate formulation.

2.4 Factors Affecting Formulation Stability

2.4.1 Water Content of Sesame Oil

A study of the effect of water on the levels of free fluphenazine produced by hydrolysis of the active during storage of the formulation has conclusively demonstrated a positive relationship[176]. Increasing the amount of water added to a previously dried sesame oil containing fluphenazine decanoate and benzyl alcohol, resulted in a corresponding increase in free fluphenazine during accelerated stability tests. A ten-fold increase in water content (from 0.01 to 0.1%) caused a three-fold increase in free fluphenazine levels. Above 0.1% added water, the sesame oil/water mixture

was immiscible.

2.4.2 Effect of Hydroperoxide Content of Sesame Oil

2.4.2.1 Formation of Peroxide

Reference has previously been made to the triglyceride constituents of sesame oil, the majority of which are derived from the unsaturated fatty acids (oleic 45.4%, linoleic 40.4%).

$CH_3(CH_2)_7 CH = CH (CH_2)_7COOH$

oleic acid

(cis-9-octadecenoic acid)

$CH_3(CH_2)_4 CH = CH \overset{*}{C}H_2 CH = CH(CH_2)_7COOH$

linoleic acid

(cis-9, cis-12-octadecadienoic acid)

The presence of the C=C double bond renders these molecules susceptible to autoxidation. The linoleate moiety has been reported to autoxidise about eleven times faster than oleate because of the highly activated methylene group (shown starred above) in the centre of the 1:4 pentadiene system[177].

The early stages of autoxidation are characterised by the formation of hydroperoxides via a chain reaction involving free radicals:

Initiation:	$\text{Initiator} \longrightarrow R^{\bullet}$
Propagation:	$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$
	$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$
Termination:	$R^{\bullet} + R^{\bullet} \longrightarrow R - R$
	$R^{\bullet} + ROO^{\bullet} \longrightarrow ROOR$
	$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2$

It has been suggested[177] that the reaction may be initiated either by a photochemically activated oxygen molecule, or by the direct attack of oxygen on the more reactive linoleate system. The radicals obtained from the oleate chain are shown below:

$$R - \dot{C}H - CH = CH - CH_2 - R^1$$
$$\Downarrow\Uparrow$$
$$R - CH_2 - \dot{C}H - CH = CH - R^1$$
$$\Downarrow\Uparrow$$
$$R - CH = CH - \dot{C}H - CH_2 - R^1$$
$$\Downarrow\Uparrow$$
$$R - CH_2 - CH = CH - \dot{C}H - R^1$$

These mesomeric structures account for the observed formation of the four hydroperoxides[178], the order of decreasing yield being $C_{10} > C_{11} > C_8 > C_9$.

Similar structures may be written for linoleate:

$$R - \dot{C}H - CH = CH - CH = CH - R^1$$
$$\Downarrow\Uparrow$$
$$R - CH = CH - \dot{C}H - CH = CH - R^1$$
$$\Downarrow\Uparrow$$
$$R - CH = CH - CH = CH - \dot{C}H - R^1$$

Again the structures explain the sequence of observed products. In the initial stages of oxidation, attack is almost entirely confined to C_{11}, because of the highly activated nature of this methylene group. However, the conjugated radicals are more stable than the non-conjugated form and ultimately the formation of the con-

jugated hydroperoxides predominates (90% yield).

The level of hydroperoxides in sesame oil depends on both the degree of refining and on the storage conditions of the refined oil.

2.4.2.2. Reaction of Peroxides with Fluphenazine Decanoate

The effect of increasing peroxide levels of sesame oil on the degradation of fluphenazine decanoate has been studied. A corresponding increase in the formation of the N-oxide degradation product was noted[179] even in the absence of dissolved air or oxygen (See next section).

The exact mechanism by which the N-oxides are formed from fluphenazine decanoate in the oily vehicle are unknown, although an interaction between the phenothiazine and the radical chain-propagating peroxide species has been postulated[61] in connection with the antioxidant activity of 10-methyl-phenothiazine. Alternatively, the drug may react with the true hydroperoxide(formed during the hydrogen-abstraction step) by a conventional nucleophilic/electrophilic reaction mechanism.

2.4.3 Effect of Dissolved Oxygen

Using a freshly refined sesame oil devoid of peroxide, the effect of dissolved oxygen on the formation of fluphenazine decanoate N-oxide has been investigated[179]. When compared with a deoxygenated control sample, the formulation containing dissolved oxygen was found to contain twice as much N-oxide after storage under identical conditions.

By contrast, similar solutions of the drug in coconut oil containing dissolved oxygen (as air) showed little, if any degradation to the N-oxide following accelerated stability studies[180], but the possibility of a direct reaction between dissolved oxygen and fluphenazine decanoate via a free

radical mechanism cannot be entirely eliminated on the basis of the described experimental work. However, the results are sufficient to indicate that the most likely role of oxygen is to react with the unsaturated triglycerides of the oil prior to the formation of the N-oxides of the drug via the intermediate hydroperoxides.

2.4.4 Effect of Free Fatty Acid Content of Sesame Oil

An increase in the free fatty acids present in the sesame oil vehicle could contribute to the accelerated degradation of fluphenazine decanoate as both transesterification and hydrolysis are known to be acid-catalysed reactions[181].

A factorial study[182] has confirmed the expected catalytic effect for transester formation in a sesame oil solution of fluphenazine decanoate. The same study also demonstrated that production of N-oxide and free fluphenazine was subject to acid-catalysis but to a lesser extent than transesterification.

2.4.5 Effect of Metal Ions

Metal ions have been shown to exert a profound effect upon the stability hydroperoxides, inducing decomposition via free radical mechanisms[183]. Transition metals in particular have been investigated. The presence of trace metals in an autoxidising system results in a more rapid rate of chain initiation through the following reaction:

$$2\ ROOH \xrightarrow{M^{+};\ M^{2+}} ROO^{\bullet} + H_2O + {}^{\bullet}OR$$

Cobalt, copper, iron and lead compounds have been reported in this respect[183].

The influence of copper and iron on the degradation of fluphenazine decanoate in sesame oil solution has been studied. A preliminary study[184] showed that both metals had little effect on the degradation

rate, but a subsequent factorial study[182] indicated that the presence of Fe^{3+} ions caused an increase in the production of N- and S- oxides.

Choice of metal ions used in the above investigation appears to have been arbitrary. An alternative, and perhaps more useful, approach would be to initially examine sesame oil by x-ray emission spectrometry in order to identify those metals most commonly present and to determine the approximate levels.

2.4.6 Effect of Benzyl Alcohol

Some loss of benzyl alcohol has been noted during stability studies in multidose vials (rubber stopper)[180] and, more recently, in sealed glass vials[184]. A number of possibilities exist to account for the observed loss:

a) a physical loss through the rubber vial closure, as described by Royce and Sykes[186].

b) an involvement in the degradative reactions.

c) conversion of the benzyl alcohol to the corresponding ester by reaction with the free fatty acids present.

Investigation of the above possibilities has not been undertaken.

2.5 Selection of Topics for Investigation

From the foregoing resume of work completed to date concerning the degradation of fluphenazine decanoate in sesame oil solution numerous areas for further study can be identified. The general concept of oxidation of the phenothiazine derivative was chosen for investigation, particularly the aspects of N- and S- oxidation which is thought to occur in oily solution. Little has been reported in the literature concerning such reactions except for early references to the antioxidant action of

phenothiazine and some of its derivatives. Squibb in-house R & D reports have described the tentative identification of an N-oxide in degraded oily formulations and also the observation of a sulphoxide.

Before investigations of the oxidation reaction(s) could be attempted, reference compounds of the individual oxides were required to act as markers for qualitative studies and as standards for quantitative investigations. Establishing the identity of any synthesised compound is imperative before using the material as a reference compound. Hence a full spectral characterisation of the synthesised oxides was proposed - especially since previous attempts to identify the N-oxide of fluphenazine decanoate were never satisfactorily resolved.

Studies at the Squibb Institute for Medical Research (International Development Laboratory, U.K.) have indicated that the oxidation of Modecate in sesame oil is due to the reaction of fluphenazine decanoate with the hydroperoxides formed during autoxidation of the oil. In order to gain further knowledge of the mechanism and kinetics involved it was proposed to study the reaction between fluphenazine decanoate and simplified systems of pure hydroperoxides and autoxidised olefines.

Essential to the above proposal is the availability of a suitable analytical procedure for the determination of the oxides formed. HPLC offers the most flexibility in this respect with a variety of columns and detectors being available. The technique is ideal for the chromatography of N-oxides which are often thermolabile and therefore not amenable to direct GC analysis. Thus the development of an appropriate HPLC assay procedure was required.

The role of benzyl alcohol in the formation of N- and S-oxides of the drug has been investigated by the Squibb R & D Department, but was dismissed as being of little consequence. These studies however were originally conducted using a semi-quantitative TLC procedure for

estimation of the oxides and for this reason the conclusions of the study cannot be taken as absolute. Quantitative determinations using an HPLC procedure should produce reliable and meaningful data for interpretation. In addition, a reference to the direct reaction of benzaldehyde, (traces of which are present in benzyl alcohol as an impurity, B.P. limit 0.2%) with atmospheric oxygen forming the powerful oxidant perbenzoic acid[187] is apparent in the literature.

Therefore, a study of benzyl alcohols of varying purity and their effect on the oxidative degradation of fluphenazine decanoate was proposed.

SUMMARY OF ASPECTS FOR STUDY

1. Devise synthetic routes for the preparation of the N- and S-oxides of fluphenazine and its decanoate ester.
2. Investigate the spectral properties of the synthesised oxides and conclusively identify the compounds.
3. a) Develop an HPLC procedure for the assay of small amounts of N- and S-oxides in the presence of the parent compound.
   b) Using this assay procedure, investigate the kinetics/mechanism of the reaction between fluphenazine decanoate and hydroperoxides.
4. Investigate the role of benzyl alcohol and benzaldehyde in the oxidative degradation of fluphenazine decanoate.

Each of the above aspects form the basis of a separate chapter.

CHAPTER 3

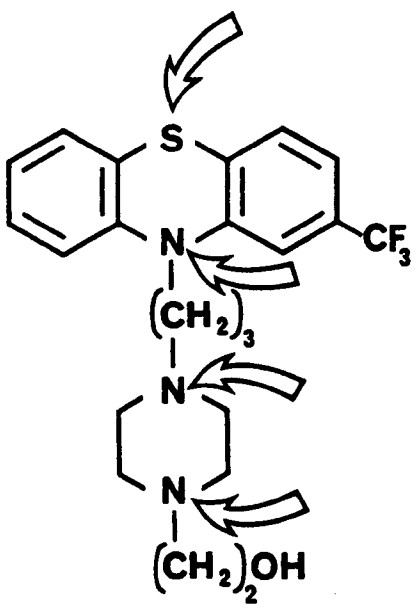
OXIDATION OF FLUPHENAZINE AND ITS DECANOATE ESTER; SYNTHESIS OF THE N- AND S-OXIDES

3.1. Synthesis of the Oxides

3.1.1 Introduction

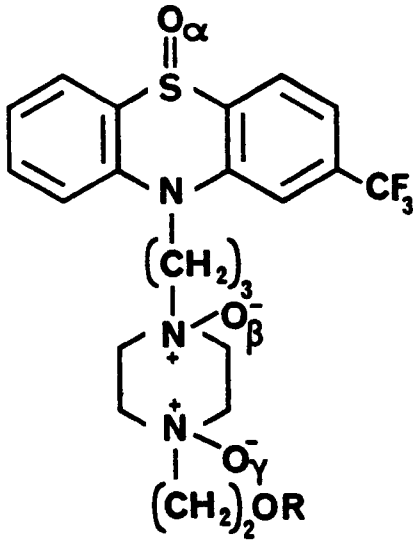
3.1.1.1 Theoretical Oxides

The chemical structure of fluphenazine (and its decanoate ester) reveals four potential sites where N- or S- oxidation may take place:



Oxidation at the ring N atom is unlikely to occur[42] thus the remaining three sites offer the possibility of a total of eleven compouds, viz. two side-chain mono N-oxides, a side-chain di N-oxide, a sulphoxide and the corresponding series of mixed sulphoxide N-oxides, a sulphone and the corresponding series of mixed sulphone N-oxides. (See Fig. 3.1).

Only two of the possible compounds have been reported previously. The preparation of a mono N-oxide of fluphenazine has been patented by Yale[31]. The synthesis of fluphenazine sulphoxide has also been described[147].



Compound	α	β	γ
mono N-oxide A	-	1	-
mono N-oxide B	-	-	1
di N-oxide	-	1	1
sulphoxide	1	-	-
sulphoxide mono N-oxide A	1	1	-
sulphoxide mono N-oxide B	1	-	1
sulphoxide di N-oxide	1	1	1
sulphone	2	-	-
sulphone mono N-oxide A	2	1	-
sulphone mono N-oxide B	2	-	1
sulphone di N-oxide	2	1	1

when R = H, fluphenazine series

R = $C(=O)C_9H_{19}$, fluphenazine decanoate series.

Fig.3.1 - Structures of Fluphenazine Oxides

3.1.1.2 Choice of Oxidants

Little information was available concerning the oxidation of fluphenazine except for two previously cited references (31 and 147). In order to gain an insight into the subject, an investigation of the oxidation of fluphenazine and its decanoate ester by selected oxidants was proposed. Three reagents seemed to offer particular advantages and were therefore chosen for the initial survey:

a) Ethanol/hydrogen peroxide - used by Yale for the synthesis of fluphenazine mono N-oxide B[31] and for the specific N-oxidation of chlorpromazine[115].

b) *m*-chloroperbenzoic acid - Reacts with piperazino-dibenzthiapines forming specifically the 'B' mono-N-oxide[120]. Also recommended for the preparation of the N-oxides of t-amines such as codeine and morphine[107].

c) Hydrogen peroxide/acetic acid - At room temperature specifically forms the sulphoxide derivative of phenothiazines[102]. At increased temperatures may produce the corresponding sulphone.

3.1.1.3 Choice of Analytical Methods

Before investigations with the selected oxidising agents could begin, a suitable analytical procedure was required with which to monitor the progress of reactions.

Chromatographic systems only were considered because of their ability to separate the potential mixture of oxidation products. Suitable TLC systems for examining fluphenazine and fluphenazine decanoate were readily available[180] and were known to separate the sulphoxide from the parent compound. TLC however is a slow analytical technique and cannot provide information on the progress of a reaction within a short time, the TLC plates requiring approximately two hours development time in the mobile phases described in the references.

In this respect HPLC is particularly advantageous as the state of a reaction mixture can be determined within a few minutes. GC is highly unsuitable because of the thermolabile nature of N-oxides. Consequently, HPLC was adopted as the method of choice, with the TLC systems held in reserve.

3.1.2. Experimental

3.1.2.1 HPLC Method Development

a) Columns

An HPLC procedure for the separation of fluphenazine from fluphenazine decanoate has been described in the literature[161]. The system comprised of a reversed-phase column, Partisil-TMS (a 10u irregular-particle material) and a mobile phase of methanol/acetonitrile/1% aqueous ammonium carbonate. Since the publication of this method, an improved column packing material with a similar short-chain alkyl phase bonded to the silica had become available. The material (SAS-Hypersil) also had the added advantages of being spherical and of being a smaller particle size (5u) resulting in improved column efficiency. Initially, 100mm x 4.6mm columns were slurry packed from a methanol/aqueous sodium acetate solultion, using a Haskel air driven fluid pump, at 4600 psi. However, in later stages of the project new batches of the packing material yielded columns with poor efficiency. Consultation with the manufacturers (Shandon Products Ltd) confirmed the problems experienced with the originally recommended slurry solvent. Substitution of isopropanol for the original slurry-solvent, followed by hexane wash was recommended and successfully resolved the problem. The new procedure also allowed the preparation of 200mm x 4.6mm columns.

Use of columns with an octadecyl stationary phase bonded to the silica was also assessed. Of the many commercial brands of ODS-silicas

available, ODS-Hypersil (Shandon Products, Runcorn) was arbitrarily chosen. 200mm x 4.6mm columns were packed by a similar procedure to that described above, but with carbon tetrachloride as slurry solvent, followed by a methanol wash.

b) Development of Mobile Phases

Throughout all the HPLC work the following equipment was uitilised:

U.V. detector	-	Cecil 212, variable wavelength
Pump	-	Altex 110A(reciprocating)
Injection mode	-	Syringe, SGE, 10ul
Recorder	-	Perkin Elmer, Model 56.

(i) Separation of fluphenazine decanoate oxidation products was attempted using a 100mm SAS-Hypersil column. Without samples of the expected oxides the development work was always subject to uncertainty. However, as a convenient means of assessing the effectiveness of particular mobile phases, a mixture of fluphenazine decanoate and *m*-chloroperbenzoic acid in methanol solution was used. A mobile phase of methanol/acetonitrile/1% ammonium carbonate[161] separated a series of peaks from the mixture, all of which were more polar than the parent ester (Fig. 3.2). Such an increase in polarity may be expected upon formation of fluphenazine decanoate oxidation products. Removal of either the methanol or the acetonitrile from the mobile phase caused considerable tailing of the peaks. Optimum chromatography was obtained with a 1:1 ratio of methanol:acetonitrile. When the same solution was chromatographed on an ODS column (200mm) tailing peaks were noted regardless of the composition of the mobile phase.

Further refinement of the HPLC procedure was required before embarking upon the quantitative aspects of the study. A full

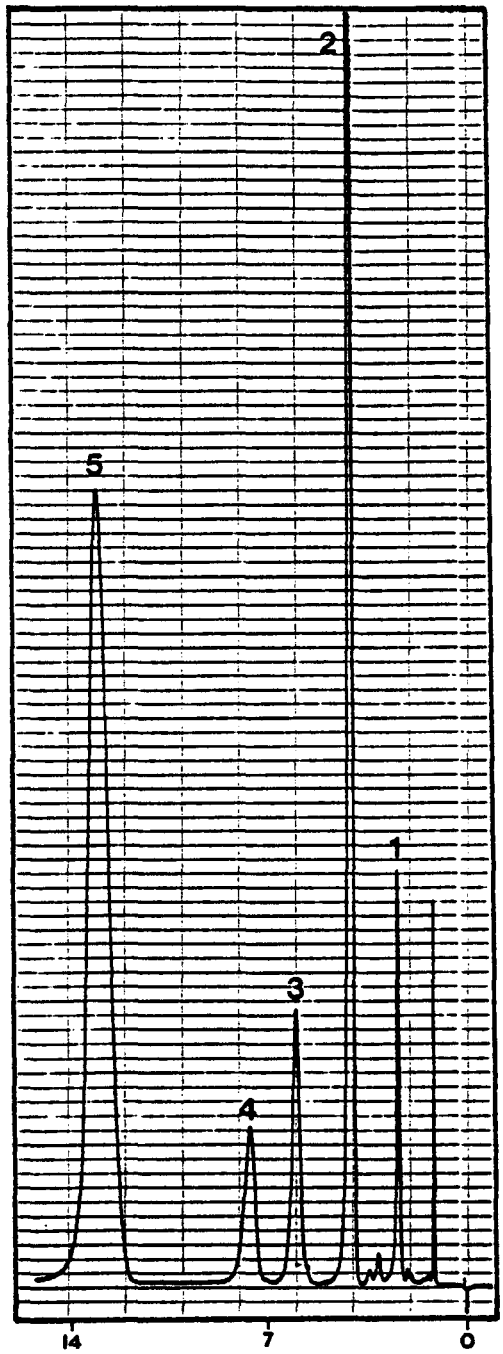


Fig. 3.2 - HPLC Chromatogram of Fluphenazine Decanoate following Oxidation with *m*-Chloroperbenzoic Acid.

1, 2, 3 and 4 = Oxidation products, 5 = fluphenazine decanoate; mobile phase, methanol-acetonitrile-1% aqueous ammonium carbonate (1-1-1); column, SAS-Hypersil.

description of this work is given in Chapter 5.

(ii) HPLC system for the separation of fluphenazine oxidation products was sought in a similar manner to that described above using a solution containing fluphenazine and *m*-chloroperbenzoic acid. Again, satisfactory chromatography was obtained with a 100mm SAS-Hypersil column together with a mobile phase of methanol/acetonitrile/1% aqueous ammonium carbonate and a series of peaks were separated following injection of the solution of fluphenazine and oxidant (Fig. 3.3) All peaks were eluted before the fluphenazine, indicating the increased polarity of the oxidation products present in the solution. One potential problem was noted from the chromatogram, namely that two of the observed peaks were only partially resolved. Increasing the column length to 200mm failed to overcome the problem. An improvement of the resolution of the two peaks was obtained by (i) removal of the acetonitrile component from the mobile phase; (ii) replacing the ammonium carbonate solution with 10% aqueous ammonium hydroxide (Fig. 3.4). Potassium chloride has been reported to improve the chromatographic resolution of phenothiazines[189]. The beneficial effect of incorporating potassium chloride in the proposed mobile phase is clearly demonstrated in Fig. 3.4. The resulting mobile phase, comprising of methanol/10% aqueous ammonium hydroxide containing 1% potassium chloride (1-1) was found to be suitable for the majority of the subsequent experimental work.

However, in the final stages of the work it became necessary to separate a complex mixture of seven fluphenazine oxides and, contrary to expectation, the reintroduction of acetonitrile was required for optimum resolution of the seven compounds. Thus in the presence of potassium chloride the originally noted detrimental

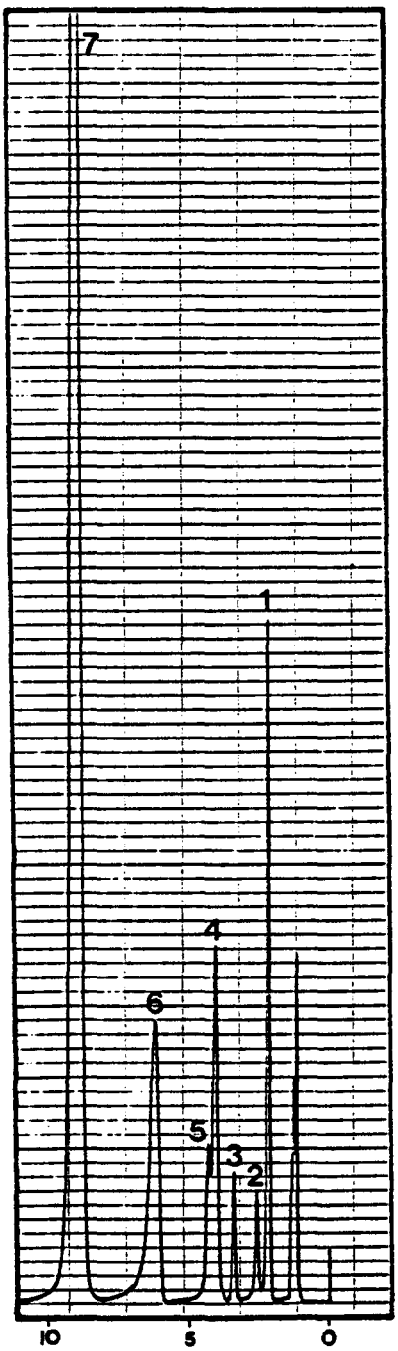


Fig. 3.3 - HPLC Chromatogram of Fluphenazine Following Oxidation with *m*-Chloroperbenzoic Acid

1, 2, 3, 4, 5 and 6 = oxidation products, 7 = fluphenazine; mobile phase, methanol-acetonitrile-1% aqueous ammonium carbonate(1-1-2); column, SAS-Hypersil.

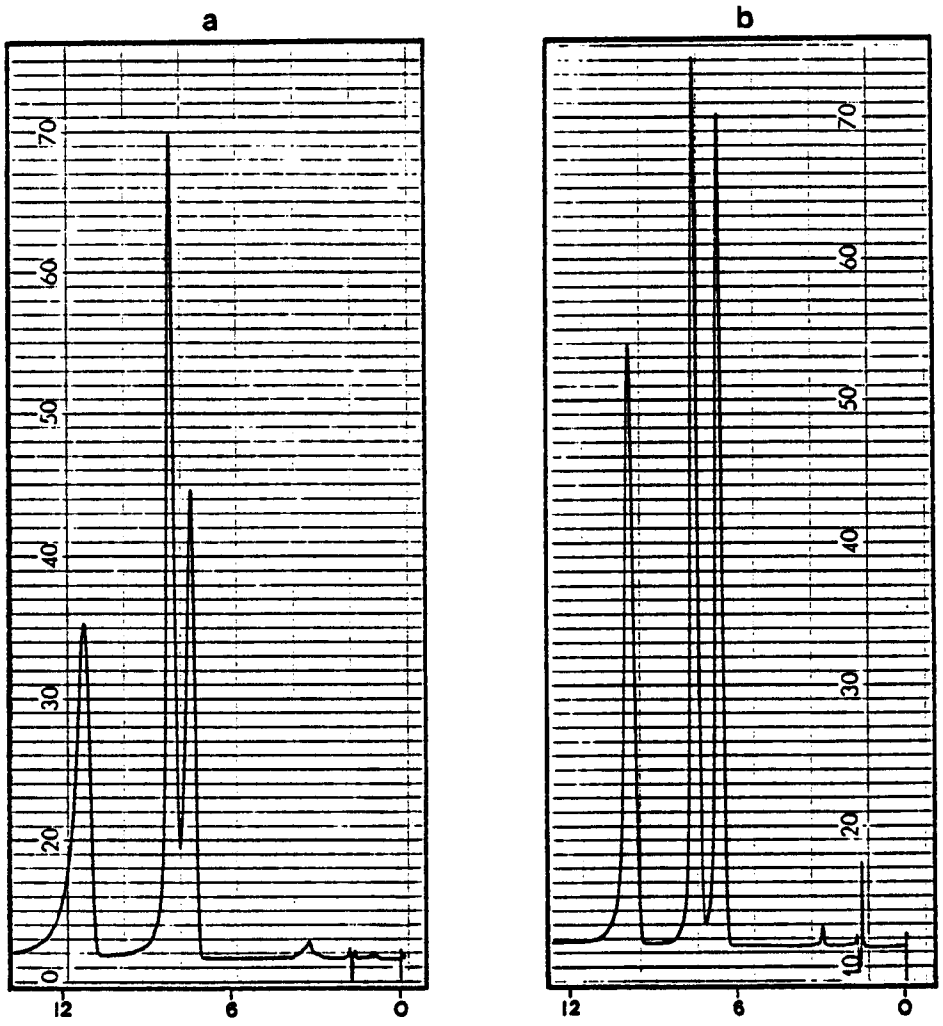


Fig. 3.4 - Chromatography of Fluphenazine Oxidation Products on SAS-Hypersil

a) methanol-10% aqueous ammonia (1-1)

b) methanol-10% aqueous ammonia containing 1% potassium chloride (1-1)

effect of acetonitrile was negated.

In view of the excellent chromatography attained using an SAS-Hypersil column, use of the alternative ODS column was not investigated.

3.1.2.2 Experiments With *m*-Chlorperbenzoic Acid

a) Oxidation of Fluphenazine Decanoate

In a preliminary experiment, *m*-chloroperbenzoic acid and fluphenzine decanoate were mixed in equimolar quantities in ethyl acetate. After heating the solution at 50°C for 2 hours, an aliquot of the reaction mixture was examined by TLC[188]. Amongst the zones observed on the TLC plate was one particular zone with an Rf value identical to that of the degradation product considered by Shand[174] to be the N-oxide. Further work indicated that the reaction of fluphenazine decanoate with *m*-chloroperbenzoic acid was an almost instantaneous reaction; thus in a detailed investigation a solution of *m*-chloroperbenzoic acid of known concentration in ethyl acetate was added dropwise from a burette to an ethyl acetate solution of fluphenazine decanoate (0.5g), at 20°C, with constant stirring. 20ul aliquots of reaction mixture were withdrawn at intervals, diluted with the HPLC mobile phase(methanol-acetonitrile-1% aqueous ammonium carbonate; 1-1-1) then 5ul injected onto the HPLC column. Monitoring the reaction in this manner revealed the formation of a series of compounds in the sequence:

$$\text{Fluphenazine decanoate} \xrightarrow{\text{fast}} A + B + C \xrightarrow{\text{fast}} C \xrightarrow{\text{fast}} D \xrightarrow{\text{slow}} E$$

The sequence is illustrated in Fig. 3.5. All the products of the reaction were observed to be more polar than fluphenazine decanoate. The chromatograms also indicated that compounds C, D and E were produced in > 95% yield.

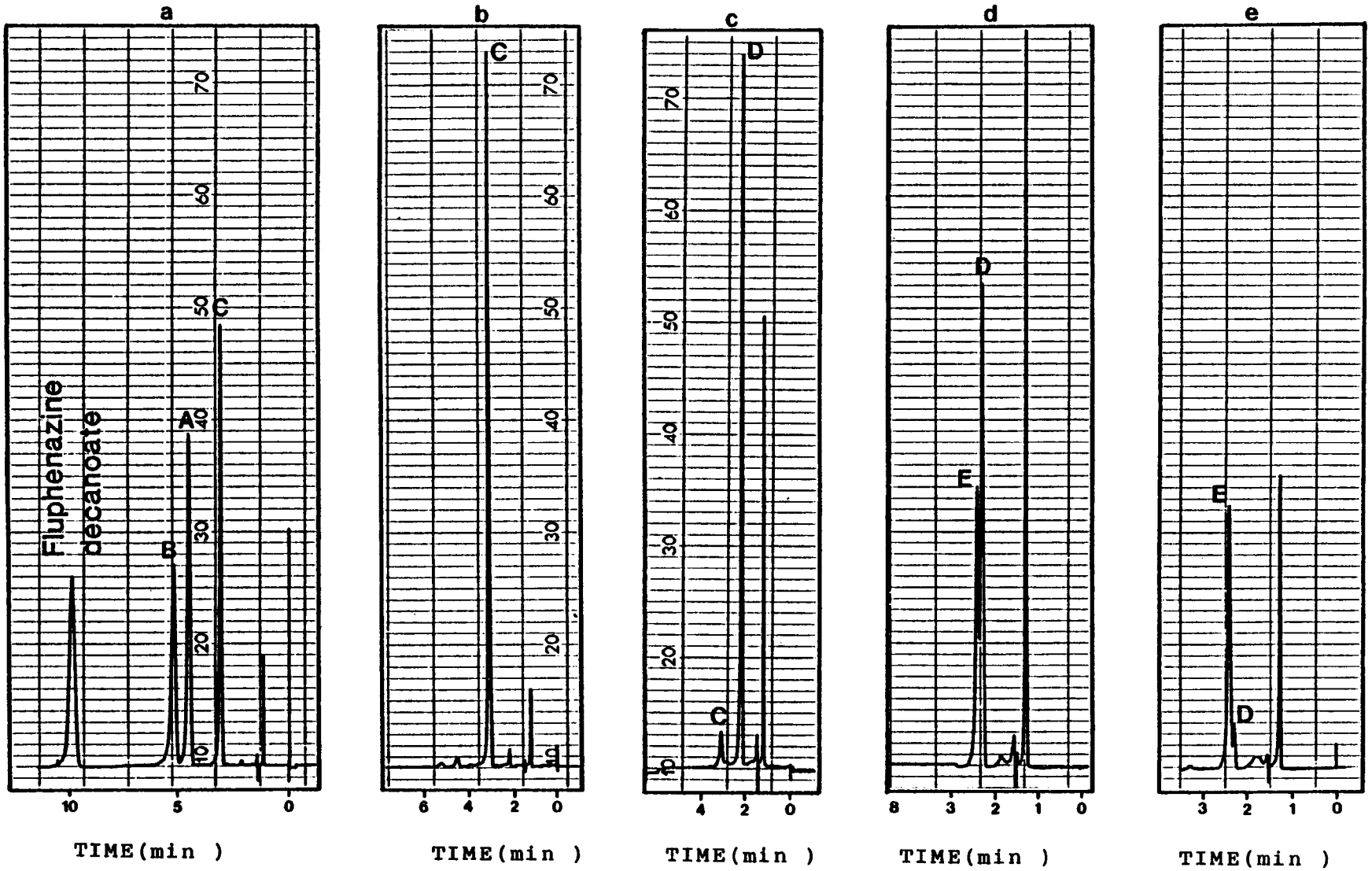


Fig. 3.5 - Oxidation of Fluphenazine Decanoate with *m*-Chloroperbenzoic Acid

a) 1 molar equivalent of oxidant; b) 2 molar equivalents of oxidant; c) 3 molar equivalents of oxidant; d) excess oxidant, 15 min; e) excess oxidant, 30 min.

As the parent ester is readily hydrolysed to fluphenazine under acid conditions the compounds A-E could be hydrolysis rather than oxidation products. The carboxylic acid most closely related to the oxidant (*m*-chlorobenzoic acid) was used to demonstrate that none of the compounds A-D further reacted in the presence of the non-oxidising acid alone, establishing that the compounds were, as expected, oxidation products of the ester.

The oxidation products of phenothiazines are many and varied in nature and in an attempt to discover which of the products observed above were likely to be oxides, solutions of the compounds were treated with zinc/methanolic hydrochloric acid. HPLC indicated that all of the compounds except E were reducible to the parent ester, suggesting that compounds A-D were N- or S-oxides[42,190]. Although compound E reacted with the zinc/HCl system this compound was converted to an unknown product F which was less polar than compound E.

From the amount of oxidant consumed during the reaction with *m*-chloroperbenzoic acid, compound C was calculated to be a dioxide and compound D a tri-oxide. Conversion of compound D to E was observed to be a slow reaction, whereas the other reactions in the sequence were virtually instantaneous. The rate of formation of sulphones via peracid oxidation is known to be 100 to 1000 times slower than sulphoxide formation[86,122,129,131,138] thus implying that compound E may be a sulphone. Additionally, oxidation of the phenothiazine ring S atom causes a bathochromic shift in the wavelength of maximum absorption (λ max.) because of the extended conjugation of the aromatic ring system. Hence the ring-oxidised phenothiazines exhibit a λ max. of 275nm in comparison to that of 260nm for the parent molecule. Comparing chromatograms of the reaction mixture determined at 260 and 275nm indicated that compounds D and E were ring-oxidised species. Compounds A, B and C

were almost undetected at 275nm, suggesting that the UV spectrum of each of these compounds was similar to that of the parent ester, as might be expected if oxidation had taken place in the N-10 side-chain.

From the fragmentary evidence available at this early stage, it was concluded that compounds A, B and C were the side-chain piperazino N-oxides, compound D was a sulphoxide and compound E was possibly a sulphone.

Because compounds A and B were produced as a mixture at room-temperature (20°C), the effect of conducting the oxidation at low-temperature was investigated. In accordance with the literature[120] the reaction with *m*-chloroperbenzoic acid was performed at -40°C using a cardice/acetone bath to cool the reaction mixture. Other experimental details were identical to those used in previous experiments. HPLC demonstrated that the ratio of A : B produced at -40°C was little different from that formed at 20°C.

Attempts to oxidise compound E by refluxing in ethyl acetate in the presence of excess *m*-chloroperbenzoic acid failed to produce any further change, as evidenced by the invariant chromatograms obtained throughout the duration of the experiment.

b) Oxidation of Fluphenazine

Before experiments with fluphenazine could be commenced, it was necessary to convert the fluphenazine dihydrochloride salt (the normal commercial form) to the base since the salt is insoluble in ethyl acetate.

Performing a similar investigation to that described above for the decanoate ester, an almost identical sequence of events was witnessed ie.

$$\text{Fluphenazine} \xrightarrow{\text{fast}} A' + B' + C' \xrightarrow{\text{fast}} C' \xrightarrow{\text{fast}} D' \xrightarrow{\text{slow}} E'$$

Once again, refluxing compound E' with excess *m*-chloroperbenzoic acid did not produce any further reaction.

The most noticeable difference between the oxidation of fluphenazine and of the decanoate ester was the ratio of the A' and B' components formed. At 20°C the ester produced an approximately 1 : 1 mixture throughout addition of the oxidant (Fig. 3.5a), but with fluphenazine compounds A' and C' appeared to be formed at the expense of B' (Fig. 3.6).

This observed difference in the behaviour of the two forms of fluphenazine with *m*-chloroperbenzoic acid was investigated at a later stage in the project. The work is fully described in section 3.2.

3.1.2.3 Oxidation with Ethanolic Hydrogen Peroxide Solution

Yale's patent for the preparation of fluphenazine N-oxide[31] claimed the selective formation of the 'B' mono N-oxide (Fig. 3.1) using an ethanol/hydrogen peroxide mixture. The reaction of this reagent with fluphenazine decanoate was therefore investigated to determine if a similar selectivity was apparent.

0.5g Fluphenazine decanoate was dissolved in 25ml ethanol, 1.5ml hydrogen peroxide (30%) added and the mixture heated under reflux for 2 hours. HPLC examination of the reaction mixture indicated that compounds A, B and C were produced simultaneously (Fig. 3.7). Subsequent treatment of the reaction mixture with Pd/charcoal had no effect on the ratio of A : B : C present in the solution.

3.1.2.4 Oxidation with Hydrogen Peroxide in Glacial Acetic Acid

a) Fluphenazine Decanoate

Fluphenazine decanoate reacts with hydrogen peroxide in glacial acetic acid solution to form the corresponding sulphoxide at room

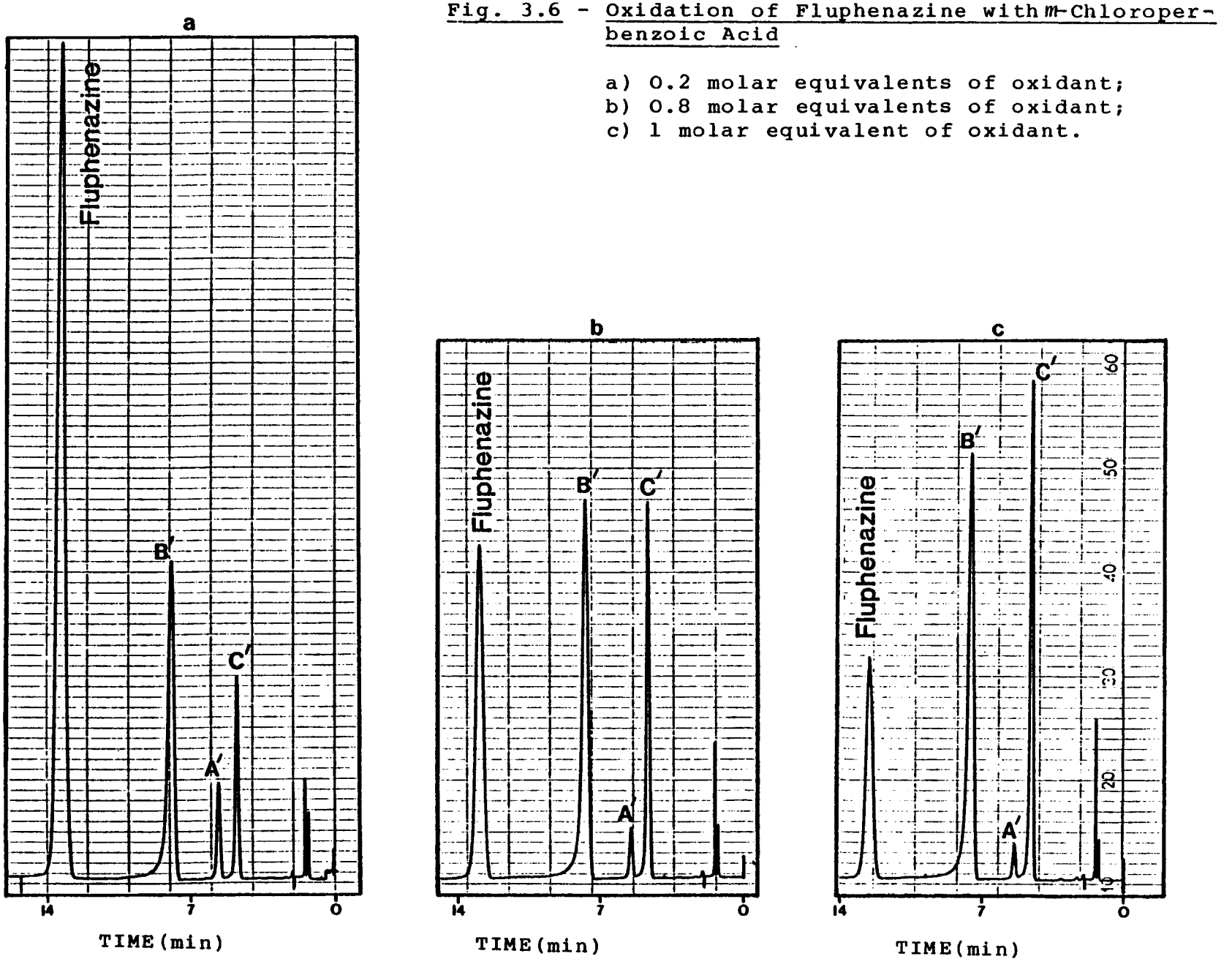


Fig. 3.6 - Oxidation of Fluphenazine with *m*-Chloroperbenzoic Acid

a) 0.2 molar equivalents of oxidant;
b) 0.8 molar equivalents of oxidant;
c) 1 molar equivalent of oxidant.

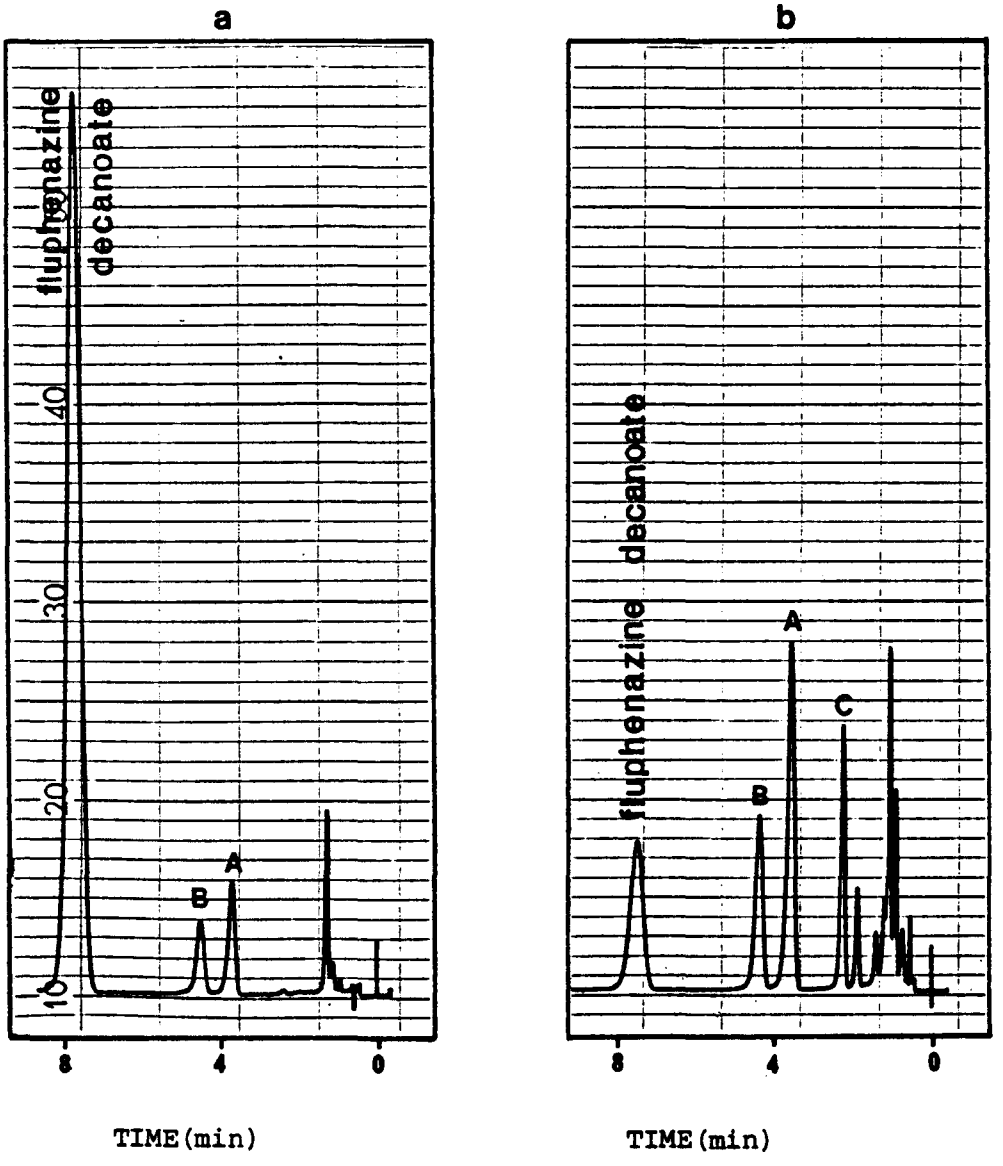


Fig. 3.7 - Oxidation of Fluphenazine Decanoate with Hydrogen Peroxide in Ethanol

a) 0.5 hr

b) 5 hr

temperature[147]. Thus 0.5g of the ester was dissolved in glacial acetic acid, 0.5ml of hydrogen peroxide (100 vol.) added and the mixture allowed to react at 20°C. Using HPLC, the progress of the reaction was monitored by withdrawing 10ul aliquots of the reaction mixture at intervals, diluting with 0.2ml of mobile phase and injecting 5ul onto the HPLC column. The resulting chromatography showed that, after an hour, one major product (compound Z) was present together with small quantities of two other compounds (X and Y). The reaction was allowed to continue at room temperature and compound Z was observed to be replaced by compounds X and Y and a fourth compound, W. After 16 hours reaction, compound W was the only component of the solution. Refluxing the solution of compound W at 60°C for 5 hours converted W into a fifth compound V. Comparison of chromatograms determined at 260 and 275nm suggested that all of the observed compounds were ring-oxidised.

Thus to summarise:

$$\text{Fluphenazine decanoate} \xrightarrow[HAc]{H_2O_2} Z + X + Y + W \xrightarrow[RT]{16\,hr} W \xrightarrow[60^{\circ}C]{5\,hr} V$$

HPLC retention times for the observed compounds indicated that compound W was equivalent to the compound D formed with -chloroperbenzoic acid, and also that compound V was the equivalent of compound E formed similarly.

Treating the solutions of compounds Z, X, Y and W with zinc/methanolic hydrochloride acid (following destruction of the excess peroxide with caustic soda) reduced the oxidation products to the parent ester, again indicating that the compounds were N- or S- oxides. Compound V however, behaved in a similar manner to the compound E formed with *m*-chloroperbenzoic acid, and was converted to an unknown

compound of lesser polarity.

b) Fluphenazine

An identical reaction sequence was observed with fluphenazine, ie.

fluphenazine $\longrightarrow$ Z' + X' + Y' + W' $\longrightarrow$ W' $\longrightarrow$ V'

Compound W' had an HPLC retention time equal to that of the compound D' formed with -chloroperbenzoic acid. Compound V' also appeared to be equivalent to compound E'.

c) Effect of Hydrochloride Salt

Conflicting recommendations are apparent in the literature concerning the form of the phenothiazine most suitable for selective oxidation to the sulphoxide; Turner[102] used the phenothiazines as bases, whereas Shand[191] and Clarke[147] preferred to use the hydrochloride salt. The difference between the oxidation of the two forms of the phenothiazine in glacial acetic acid solution was therefore investigated. The reaction sequence pertaining to oxidation of the base is described above. With the hydrochloride salt of fluphenazine, however, compound Z' was produced in almost 100% yield (Fig. 3.8). The extra components formed when using the base as starting material were noted to be absent. In addition the reaction proceeded at a faster rate in the presence of the hydrochloride salt. A similar selectivity and increased rate of formation of compound Z was observed with the dihydrochloride of fluphenazine enanthate (the only fluphenazine ester readily available in the form of the salt).

Both of the above observations are consistent with the findings of other workers. The catalytic effect of acids on the oxidation of dialkyl sulphides by hydrogen peroxide is a well documented fact. Formation of a purer product Z can be explained if the impurities X and Y represent the

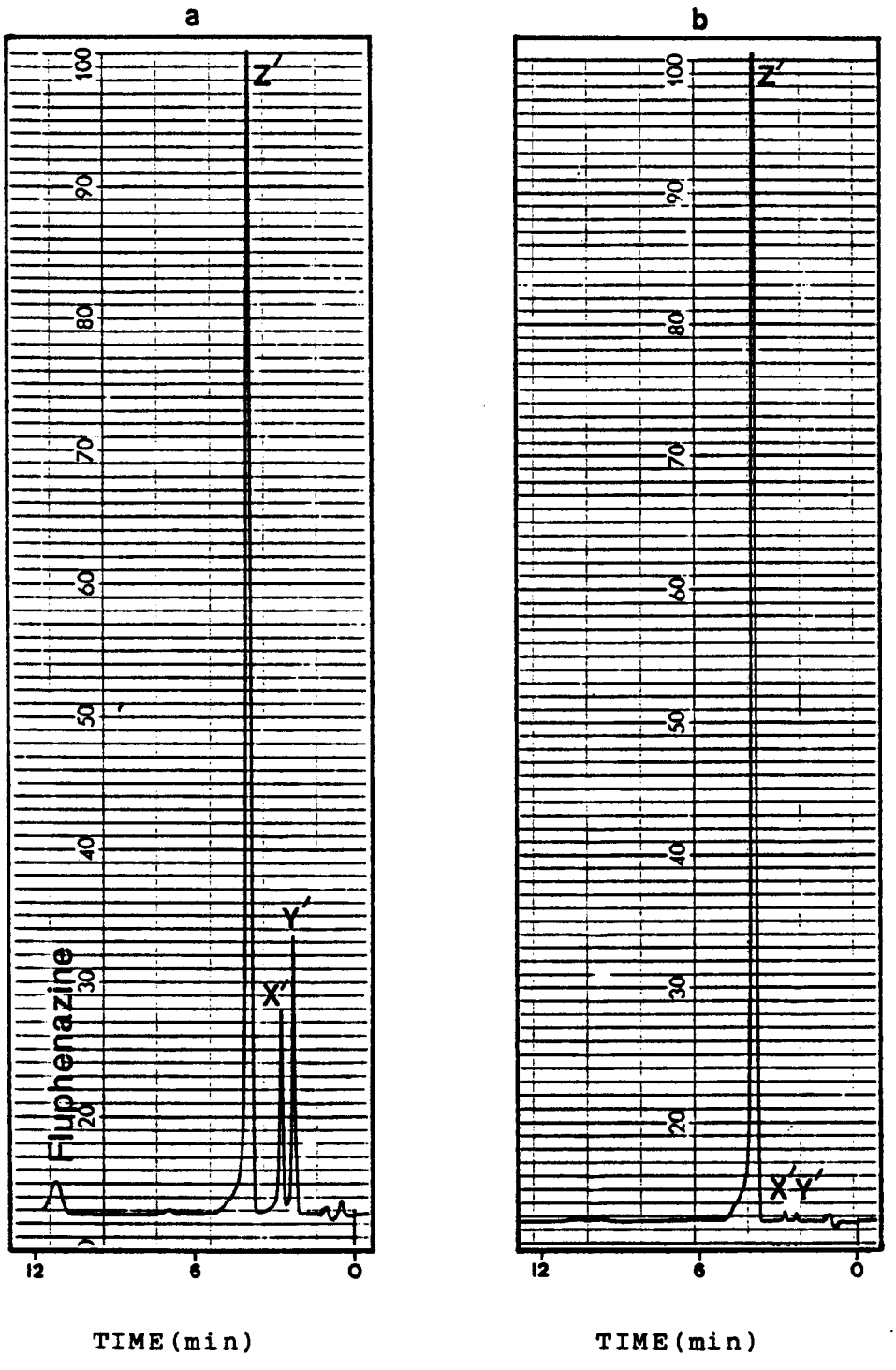


Fig. 3.8 - Oxidation of Different Forms of Fluphenazine with Hydrogen Peroxide/Glacial Acetic Acid

a) fluphenazine base

b) fluphenazine hydrochloride

further oxidation of Z (probably the sulphoxide) to the sulphoxide-N-oxide, as the formation of N-oxides by t-amines is dependant upon the basicity of the amine[190,192]. A reduction in basicity reduces the probability of N-oxide formation, and in the presence of a strong acid oxidant(eg. trifluoroperacetic acid), N-oxidation of t-amines does not occur[139].

In order to oxidise the hydrochloride salt beyond the formation of compound Z' (or Z) it was necessary to reflux the reaction mixture at 60°C. At this higher temperature the reaction proceeds via compounds X' and Y' producing compound W' after approximately 5 hours.

3.1.2.5 Combined Use Hydrogen Peroxide/Glacial Acetic Acid and *m*-Chloroperbenzoic Acid

Oxidation of the compound Z (formed initially with hydrogen peroxide in acetic acid) by either *m*-chloroperbenzoic acid or hydrogen peroxide in glacial acetic acid resulted in the formation of the same sequence of compounds, as indicated by HPLC retention times. That is,

$$Z \longrightarrow X + Y + W \longrightarrow W \longrightarrow V$$

However, as the reaction with *m*-chloroperbenzoic acid is almost instantaneous, formation of X, Y and W is more easily controlled by use of this particular reagent.

HPLC comparison of the oxidation products A, B, C and D (formed by *m*-chloroperbenzoic acid alone) with the products Z, X, Y and W established that compounds D and W have identical retention times and are therefore probably the same chemical entity. Compounds A, B, C, Z, W and Y were shown to be individual oxidation products. (Fig. 3.9).

3.1.2.6 Development of Procedures for the Preparation and Isolation of the Oxides

The above description of the oxidation of fluphenazine and its

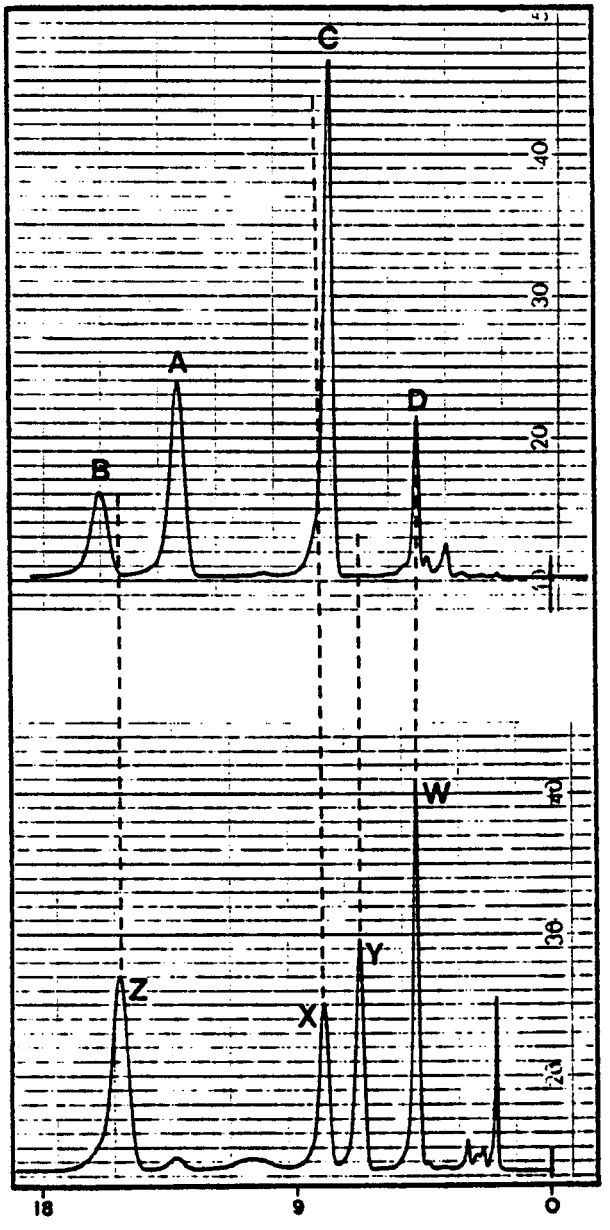


Fig. 3.9 - HPLC Comparison of Compounds ABCD and WXYZ (from Fluphenazine Decanoate)

decanoate ester has shown that the selective synthesis of oxidation products C,D,E,F,Z,W and V may be achieved, yielding compounds of >95% purity, as evidenced by the HPLC chromatograms. Only compounds A, B (A', B') and X, Y (X', Y') were formed as mixtures and therefore required a means of separation from the starting material and other oxidation products.

a) Isolation of Compounds A, B from the Reaction Mixture

A column chromatographic procedure has been described for the quantitative separation of fluphenazine decanoate from its degradation products and this procedure was adapted for the isolation of compounds A and B from the reaction mixture. The suitability of the chromatographic system(methanol/cyclohexane/methyl acetate; 40-70-100) for the separation of A and B from the starting material, fluphenazine decanoate, and from the compound C which is also present in the reaction mixture, was confirmed by thin layer chromatography on silica gel GF_{254}. TLC of the reaction mixture indicated that the compounds A and B, although separated from the parent ester and compound C, were not completely separated from each other. (Fig. 3.10). Hence, a more efficient means of separating these two materials was required. Preparative HPLC was eventually chosen for this purpose, allowing 20mg each of pure compound A and B to be isolated.

The subsequent discovery that compound B was hydrolysed at a faster rate than compound A (Chapter 4) provided a means whereby larger quantities of compound A could easily be prepared. Hydrolysis of the mixture of compounds A and B separated from the oxidation reaction mixture by column chromatography yielded a mixture of compound A, the hydrolysis products of A and B (ie. fluphenazine derivatives) and decanoic acid. Compound A was separated from this mixture by repeating the column chromatographic procedure described previously.

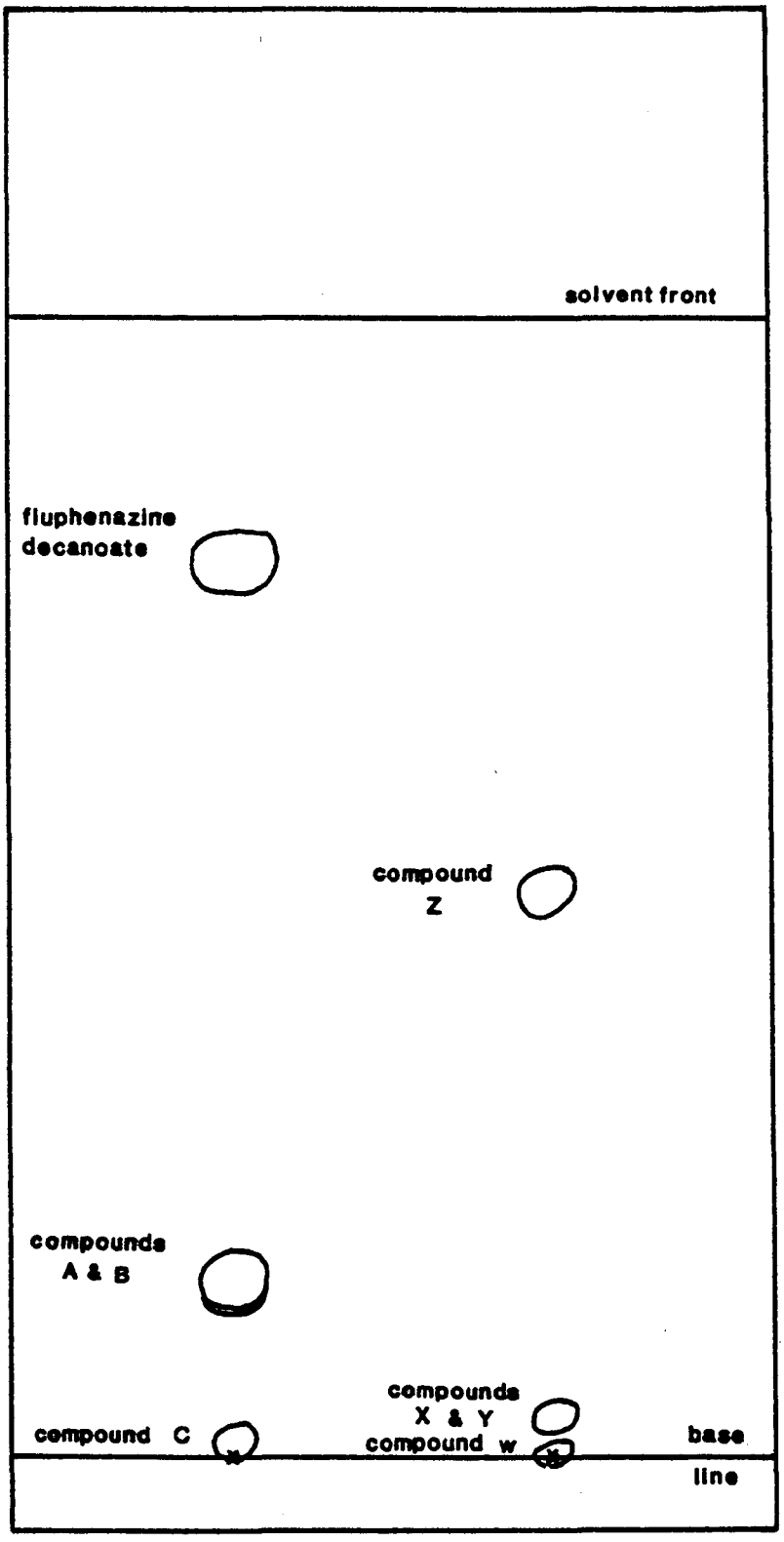


Fig. 3.10 - TLC Separation of Some Fluphenazine Decanoate Oxidation Products

mobile phase, methanol-cyclohexane-methyl acetate(40-70-100); layer, silica gel GF_{254}.

b) Isolation of Compounds X, Y from Reaction Mixture

The methanol/cyclohexane/methyl acetate mobile phase (40-70-100) proved to be a suitable system for the separation of X and Y from the reaction mixture. Again TLC indicated that these two compounds were not resolved from each other (Fig. 3.10). Therefore, after prior isolation by normal mode column chromatography, small amounts of each of compounds X and Y were separated by preparative HPLC for characterisation.

c) Isolation of Compounds A' and B' from Reaction Mixture

For isolation of the compounds A' and B' obtained from the oxidation of fluphenazine it was necessary to revert to a mobile phase of acetone/cyclohexane/ammonia (80:30:5). The A' component in the reaction mixture can be reduced to a negligible level by conducting the oxidation at -40^{o}C enabling pure compound B' to be isolated.

d) Preparation of Compound A'

As compound A' is not easily formed by the direct oxidation of fluphenazine (See Section 3.2) an alternative procedure was sought. The simplest method of obtaining A' was found to be via hydrolysis of the corresponding decanoate ester oxide (ie. compound A).

The hydrolysis reaction yields a mixture of compoundA' and decanoic acid which may be satisfactorily separated on a silica gel column with a mobile phase of the same composition as that used for the separation compound B above.

e) Preparation of Compound Y'

Isolation of compound Y' was a particularly difficult task, due mainly to the apparent instability of this compound. The method used previously for the preparation of the equivalent ester oxide (Y) proved to be impractical for preparation of Y' and it was necessary to reverse the sequence of oxidation. ie. *m*-chloroperbenzoic acid oxidation was per-

formed first (at -40°C) and following the conversion of the resulting mixture of products to the hydrochloride salts, the mixture was treated with hydrogen peroxide in glacial acetic acid. The result of this exercise was to produce a mixture comprised of compounds Z' Y' and W'. The high polarity of W' enabled this compound to be separated by partition into aqueous sodium hydroxide solution leaving compounds Z' and Y' in the organic phase for subsequent separation by preparative layer chromato graphy using the mobile phase of acetone/cyclohexane/ammonia (80-30-5). Elution of compound Y' from the silica gel was achieved with methanol, but isolation of the compound from solution caused considerable problems. Application of any degree of heating during evaporation of the solvent instantly degraded compound Y' to a red/brown syrup. Evaporation of the methanol under vacuum was found to be the most satisfactory procedure. Last traces of methanol were removed from compound Y' by addition of dichloromethane and further evaporation to dryness.

f) Isolation of Oxidation Products from Solution

The original isolation of fluphenazine N-oxide by Yale[31] was achieved by precipitating the hydrochloride salt from ethereal solution, and a similar method was proposed for the isolation of the synthesised compounds from solution.

Precipitation of the hydrochloride salts was found to be possible only if solutions of the oxidation products had not been treated with water, or the compounds exposed to the atmosphere (eg. on a chroma-tography plate), at any stage during the preparation. Treating the solutions of the oxidation products with desiccants such as anhydrous sodium sulphate or dried magnesium sulphate failed to dry the solutions sufficiently to allow the hydrochloride salt to precipitate.

The most likely explanation of this phenomenon is that the oxidation products readily form stable hydrates which are not converted

to the anhydrous form by the normal non-reactive desiccants commonly used in preparative organic chemistry. Such behaviour has been reported previously for aliphatic N-oxides[190].

An alternative salt used commercially for many phenothiazine derivatives is the maleate. This too proved to be useless in those cases where water had been used at some stage in the preparation/isolation.

When precipitation of either the hydrochloride or maleate salts could not be achieved, the oxidation product was isolated as the base (or, possibly, hydrated base) from an organic solvent by rotary evaporation. In only one case did this procedure prove to be unsuitable - that of compound V', after preparation with hydrogen peroxide. Compound V' was found to be preferentially soluble in the resulting aqueous solution and could not be extracted into an organic solvent. The product was therefore isolated from the aqueous solution by adsorption onto a resin, Amberlite XAD2, which was subsequently eluted with methanol.

g) Purification by Recrystallisation

Recrystallisation was successful only with compound C (and C') in the form of the hydrochloride salt. For this purpose a solvent mixture of ethanol/ether was used. The hydrochloride salts of other compounds, eg. D and E, could not be recovered from solution as either crystalline or amorphous solids. These compounds produced a sticky semi-solid mass, possibly due to their hygroscopic nature.

Because of these problems, the preparative reactions were carefully controlled and monitored to ensure a yield of product with a purity of not less than 95%, as shown by HPLC. By this means the need for purification via recrystallisation was avoided. A purity of 95% should be adequate for the spectral characterisation of the material, as 5% impurity would be unlikely to be detected by the majority of spectroscopic techniques. In addition, the impurity present in any of the synthesised

oxidation products represents a known entity in the form of one of the other compounds under investigation. Possible interference from the impurity could therefore be taken into account during the interpretation of the spectra.

If for any reason the purity of the isolated compound was insufficient for characterisation, preparative layer chromatography was employed to purify the material.

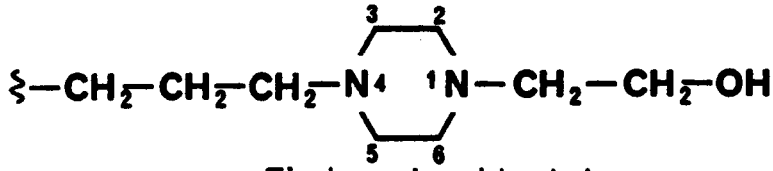
Full practical details of the procedures used for the preparation and isolation of each of the compounds A, B, C, D, E, F, Z, X, Y and W are given in Appendix I. Similar details concerning the compounds A', B', C', D', E', F', Z', Y' and W' are given in Appendix II.

3.2. Oxidation of Fluphenazine, Fluphenazine Esters and Related Compounds

3.2.1 Introduction

Fluphenazine and fluphenazine decanoate each react with *m*-chloroperbenzoic acid forming a mixture of three compounds during the initial stages of the reaction. However, a difference in the ratio of products (arbitrarily labelled A, B, C and A', B', C' with respect to the decanoate ester and fluphenazine respectively) was noted. Compounds A and B were formed in approximately equal amounts, but compound B' was produced only in minor quantities at room temperature and in negligible amounts at -40°C. (Figs. 3.5 and 3.11).

The phenothiazine N-10 side-chain of fluphenazine is composed of 4-propyl-piperazine-ethanol and the above compounds were identified (Chapter 4) as the piperazino N-oxides following isolation.



Fluphenazine side-chain

Compound A (A') was identified as the 4-oxide, compound B (B') as the 1-

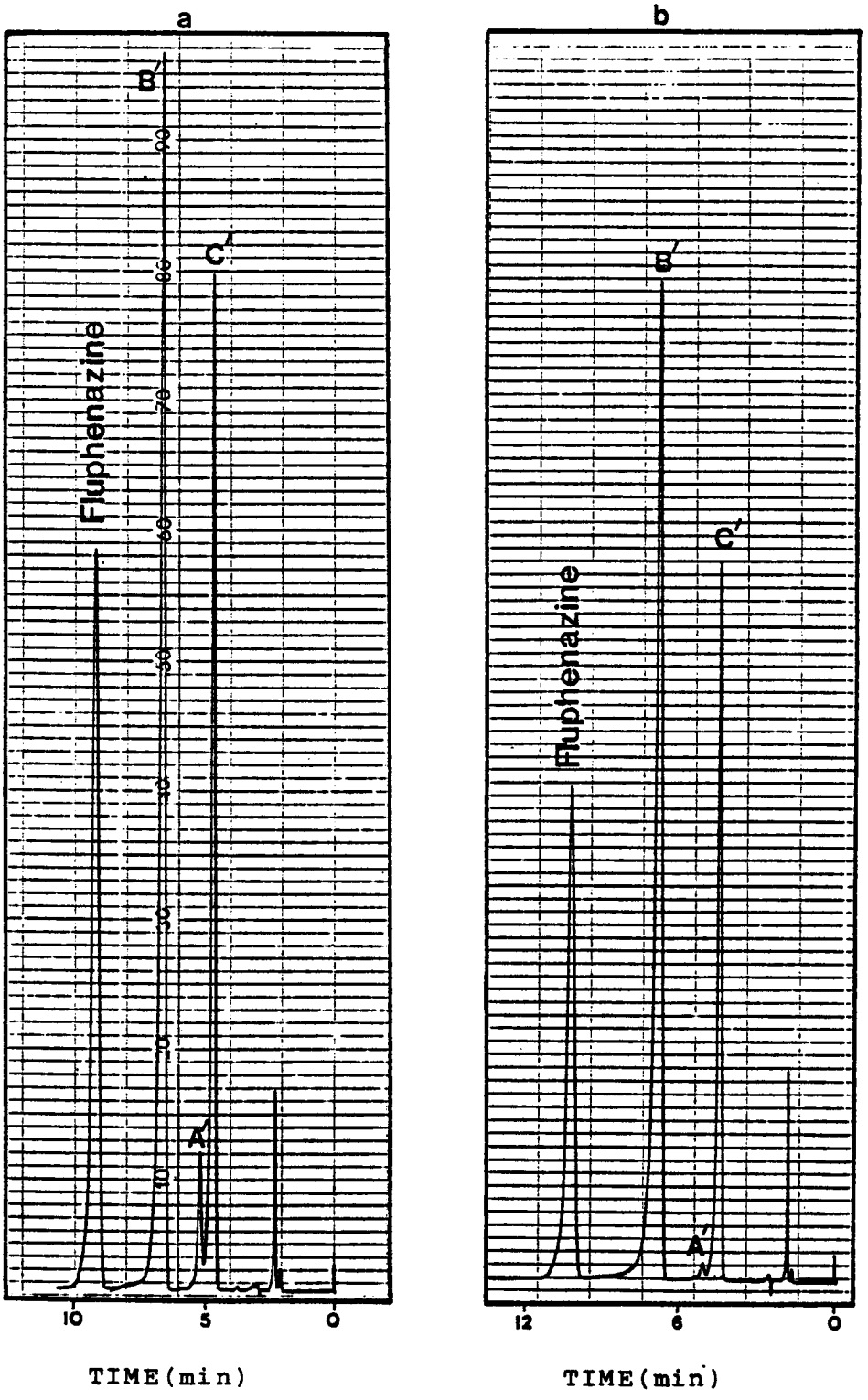


Fig. 3.11 - Oxidation of Fluphenazine with *m*-Chloroperbenzoic Acid After Addition of 1 Molar Equivalent of Oxidant

(a) 20°C; (b) -37°C.

oxide and compound C (C') as the 1,4-dioxide.

Oxidation of fluphenazine with ethanolic hydrogen peroxide has been reported to selectively form the 1-oxide[31]. Using a purified microsomal mixed-function amine oxidase, Sofer and Zeigler also reported the selective oxidation of fluphenazine to the 1-oxide[125] whereas an unpurified microsomal liver extract has been shown to oxidise trifluperazine forming a mixture of the 1-oxide and the 4-oxide[196]. In contrast to these reports, no information concerning the oxidation of the esterified piperazine-ethanol moiety was found in the literature. Thus a comparitive study of the oxidation of fluphenazine, fluphenazine esters and other closely related phenothiazines was undertaken.

3.2.2 Choice of Compounds

Compounds were chosen to represent:

a) variation of the phenothiazine ring 2-substituent

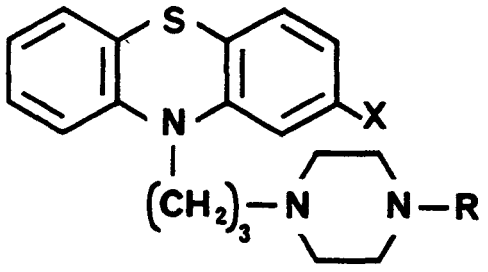
b) variation of the ester chain-length

c) variation of the piperazine 1-substituent

The piperazine 4-substituent (propyl) was kept constant throughout. Table 3.1 lists the compounds investigated.

All compounds except fluphenazine acetate were obtained from the manufacturers and were of pharmaceutical grade. Those compounds supplied as salts were converted to the equivalent bases. Fluphenazine acetate was prepared from fluphenazine and purified to >99% (HPLC assay).

Table 3.1



Compound	X	R
Fluphenazine	CF_3	$-CH_2CH_2OH$
Fluphenazine acetate	CF_3	$-CH_2CH_2OCOCH_3$
Fluphenazine enanthate	CF_3	$-CH_2CH_2OCOC_6H_{13}$
Fluphenazine decanoate	CF_3	$-CH_2CH_2OCOC_9H_{19}$
Perphenazine	Cl	$-CH_2CH_2OH$
Perphenazine acetate (Thiopropazate)	Cl	$-CH_2CH_2OCOCH_3$
Trifluperazine	CF_3	$-CH_3$
Perazine	H	$-CH_3$

3.2.3 Experimental

3.2.3.1 Conversion of Salts to Bases

The compounds received as salts were treated with 10M sodium hydroxide and the resulting base extracted with diethyl ether. The ethereal solution was dried over magnesium sulphate and then the solvent evaporated to dryness on a rotary evaporator. Finally, the isolated base was dried overnight in a vacuum oven at 60^0C.

3.2.3.2 Preparation of Fluphenazine Acetate

The preparative method was adapted from the general procedure

for esterification of fluphenazine described by Yale[194].

2.5g Fluphenazine hydrochloride was dissolved in 20ml pyridine and diluted with 100ml trichloroethane. 10ml Acetyl chloride were then added and the mixture heated under reflux for 5 hours. HPLC (SAS-Hypersil column; mobile phase, methanol-acetonitrile-1% aqueous ammonium carbonate, 1-1-1; detector 260nm) of the solution showed that the reaction was essentially complete after 2½ hours, little further esterification occurring after this time. Excess acid chloride was destroyed by treating the trichloroethane solution with 50ml of 1M sodium hydroxide and, after washing free from sodium hydroxide with successive quantities of water, the trichloroethane solution was dried over magnesium sulphate. Crude ester was isolated by evaporating the organic solvent to dryness. In order to remove residual pyridine it was necessary to perform repeated addition and evaporation of aliquots of trichloroethane. By this means pyridine may be azeotroped from the residue[195].

HPLC examination of the isolate indicated that the material contained about 8% unreacted fluphenazine. (Fig. 3.12) Purification of the ester was achieved by passing the crude product through a silica gel column (Merck, Kieselgel 60, 70-230 mesh) using a mobile phase of methanol - cyclohexane - methyl acetate (40 - 70- 100). With this system fluphenazine is retained on the silica gel whilst the ester is eluted. HPLC of the ester, isolated from solution by rotary evaporation, verified that the final material obtained was of acceptable purity (less than 1% fluphenazine, Fig. 3.12).

3.2.3.3 HPLC Systems for Monitoring Oxidative Reactions

The molecules chosen for study (Table 3.1) show widely differing polarities. It was therefore necessary to adjust the HPLC systems previously devised for fluphenazine and fluphenazine decanoate oxidation products in order to accommodate these differences. In general, the

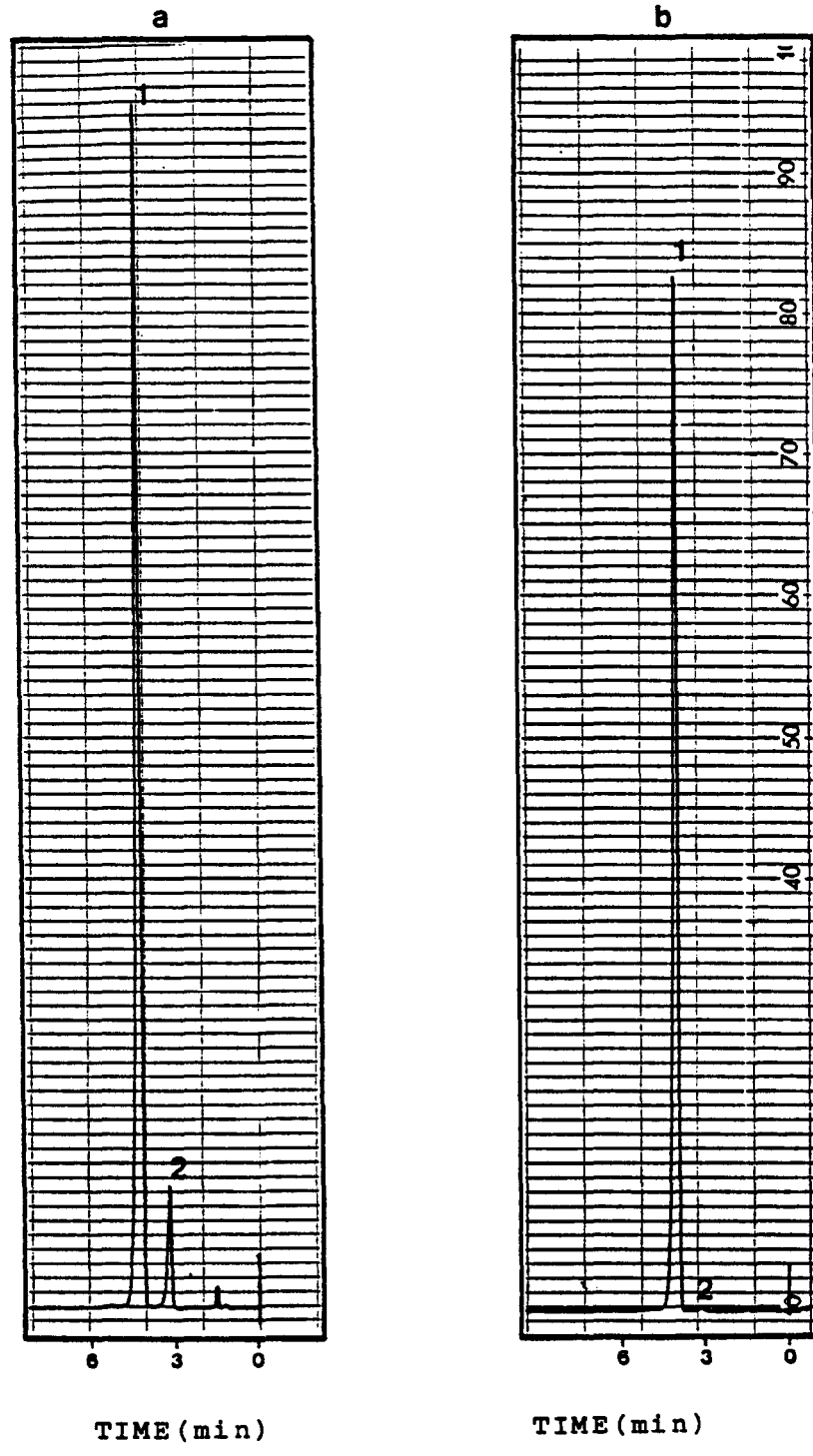


Fig. 3.12 - HPLC Examination of Prepared Fluphenazine Acetate

(a) isolated crude product; (b) purified product.

1 = fluphenazine acetate, 2 = fluphenazine

mobile phase of methanol - acetonitrile -1% aqueous ammonium carbonate was utilised for esters and that containing 10% ammonia and 1% potassium chloride for all other compounds. Altering the ratio of organic/aqueous phases produced suitable systems, details of which are summarised in Table 3.2.

Table 3.2

Mobile Phases for Chromatography of Phenothiazines and Related Oxidation Products

Column: SAS-Hypersil, 20 cm

Compound	Methanol/ acetonitrile (50:50)	1% aqueous ammonium carbonate	10% aqueous ammonia + 1% potassium chloride	Flow rate (ml/min)
Fluphenazine acetate	1	1	-	2
Fluphenazine enanthate	2	1	-	2
Fluphenazine decanoate	2	1	-	2
Perphenazine acetate	1	1.25	-	2
Fluphenazine	1	-	1	1
Perphenazine	1	-	1.5	1.5
Trifluperazine	1	-	1	1.5
Perazine	1	-	2	1.5

3.2.3.4 Oxidation Procedure

0.5g of each compound was dissolved in 50ml ethyl acetate and a solution of *m*-chloroperbenzoic acid in ethyl acetate added dropwise at a suitable temperature (usually 20°C) with constant stirring. When a low temperature (-40°C) was required, the reaction vessel was cooled in a cardice/acetone bath. The concentration of the *m*-chloroperbenzoic acid solution was adjusted in accordance with the M.W. of the compound being oxidised, such that addition of 50ml of oxidant was equivalent to a molar ratio, oxidant: phenothiazine of 1:1 (Table 3.3).

Table 3.3

Reaction Conditions for Oxidation

Compound	M.W.	mM taken (0.5g)	Oxidant g/100ml
Fluphenazine acetate	479.5	1.043	0.360
Fluphenazine enanthate	549.5	0.091	0.314
Fluphenazine decanoate	591.8	0.085	0.292
Perphenazine acetate	446.0	1.121	0.387
Fluphenazine	437.5	1.143	0.394
Perphenazine	404.0	1.238	0.427
Trifluperazine	407.5	1.227	0.423
Perazine	339.5	1.473	0.508

3.2.3.5 Quantitation of Oxidation Products

Samples of the reaction mixture were examined at intervals during the addition of *m*-chloroperbenzoic acid using the HPLC systems described in Table 3.3. Quantitation of the components present in the solution was accomplished by integration of the detected peaks, using a Spectra Physics (Minigrator) integrator. Normalisation of the resulting integrals gave the relative proportions of N-oxides produced.

3.2.3.6 Preparation of Fluphenazine Mono N-Oxide Mixture

An approximately equimolar mixture of the two fluphenazine mono N-oxides (A and B) was prepared by alkaline hydrolysis of the corresponding ester N-oxides.

0.25g Fluphenazine decanoate mono N-oxide mixture (prepared as described in Appendix 1) was dissolved in 10ml of methanol and 1ml of ammonia (SG 0.88) was added. The reaction was allowed to proceed at room temperature ($20^{o}C$) and when complete the resulting fluphenazine mono N-oxides were isolated as a mixture by the procedure described in Appendix 2.

3.2.3.7 Oxidation of Fluphenazine Mono N-oxide Mixture

0.1g of fluphenazine mono N-oxide mixture was dissolved in 25ml ethyl acetate and oxidised with *m*-chloroperbenzoic acid according to the procedure described in Section 3.2.3.4.

3.2.3.8 Oxidation of Fluphenazine in Presence of *m*-Chlorobenzoic Acid

0.5g Fluphenazine base was dissolved in 50ml of ethyl acetate, 1g of *m*-chlorobenzoic acid added and the solution oxidised with *m*-chloroperbenzoic acid according to the procedure described in Section 3.2.3.4.

3.2.4 Results

The ratio of oxides (A, B and C ie. 1-piperazino oxide, 4-piperazino oxide and 1,4 piperazino dioxide) formed by each phenothiazine examined are given in Tables 3.4 to 3.13. The same results are expressed graphically in Figs. 3.13 to 3.22.

Table 3.4

Relative Proportions of N-oxides Formed

by Fluphenazine Acetate at 20°C

m-chloroperbenzoic acid added (molar equiv.)	Mono N-O A (%)	Mono N-O B (%)	di N-O (%)
0.30	50.0	43.4	6.6
0.40	48.4	42.3	9.3
0.60	45.4	39.5	15.1
0.80	42.1	36.8	21.1
1.00	38.2	32.8	29.0
1.30	31.8	27.0	41.2
1.80	15.2	12.1	72.7

Table 3.5

Relative Proportions of N-oxides Formed

by Fluphenazine Enanthate at 20°C

m-chloroperbenzoic acid added (molar equiv.)	Mono N-O A (%)	Mono N-O B (%)	di N-O (%)
0.20	49.3	43.7	7.0
0.40	47.4	41.9	10.7
0.60	43.9	38.7	17.4
0.80	41.1	36.1	22.8
1.00	38.0	33.1	28.9
1.22	33.9	29.3	36.8
1.60	24.2	20.5	55.3

Table 3.6

Relative Proportions of N-oxides Formed

by Fluphenazine Decanoate at 20°C

m-chloroperbenzoic acid added (molar equiv.)	Mono N-O A (%)	Mono N-O B (%)	di N-O (%)
0.20	50.7	43.9	5.4
0.40	47.3	42.3	10.4
0.60	44.4	39.6	16.0
0.80	41.1	36.4	22.5
1.00	37.3	33.1	29.6
1.30	30.8	26.4	42.8
1.60	21.4	17.9	60.7

Table 3.7

Relative Proportions of N-oxides Formed

by Perphenazine Acetate at 20°C

m-chloroperbenzoic acid added (molar equiv.)	Mono N-O A (%)	Mono N-O B (%)	di N-O (%)
0.19	50.5	41.9	7.6
0.36	49.4	40.7	9.9
0.58	47.3	38.5	14.2
0.74	45.1	35.7	19.2
0.93	42.3	32.5	25.2
1.11	38.9	28.8	32.3
1.48	29.3	19.3	51.4

Table 3.8

Relative Proportions of N-oxides Formed

by Perphenazine at 20°C

m-chloroperbenzoic acid added (molar equiv.)	Mono N-O A (%)	Mono N-O B (%)	di N-O (%)
0.19	16.9	68.2	14.9
0.36	13.0	66.3	20.7
0.58	9.7	64.6	25.7
0.74	6.9	62.4	30.7
0.93	4.6	60.6	34.8
1.20	2.2	55.5	42.3
1.57	0.7	41.7	57.6

Table 3.9

Relative Proportions of N-oxides Formed

by Trifluperazine at 20°C

m-chloroperbenzoic acid added (molar equiv.)	Mono N-O A (%)	Mono N-O B (%)	di N-O (%)
0.20	14.2	79.1	6.7
0.40	13.2	76.8	10.0
0.60	12.0	74.8	13.2
0.80	10.3	72.9	16.8
1.00	8.4	69.9	21.7
1.22	5.7	64.4	29.9
1.30	4.8	61.1	34.1
1.60	1.3	42.2	56.5

Table 3.10

Relative Proportions of N-oxides Formed by Perazine at 20°C

m-chloroperbenzoic acid added (molar equiv.)	Mono N-O A (%)	Mono N-O B (%)	di N-O (%)
0.19	16.6	76.1	7.3
0.38	15.9	73.6	10.5
0.57	14.9	71.3	13.8
0.76	13.5	69.1	17.4
0.93	12.0	66.8	21.2
1.20	8.8	61.0	30.2
1.48	4.8	51.3	43.9

Table 3.11

Relative Proportions of N-oxides Formed by Fluphenazine in the Presence of *m*-chlorobenzoic Acid at 20°C

m-chloroperbenzoic acid added (molar equiv.)	Mono N-O A (%)	Mono N-O B (%)	di N-O (%)
0.21	12.3	69.1	18.6
0.43	8.6	65.7	25.7
0.74	4.8	62.1	33.1
1.10	2.6	55.8	41.6
1.52	-	43.7	56.3

Table 3.12

Relative Proportions of N-oxides Formed by Fluphenazine at Various Temperatures

Temperature (^{o}C)	*m*-chloroperbenzoic acid added (molar equiv.)	Mono N-O A %	Mono N-O B %	di N-O %
-40	0.20	4.8	81.0	14.2
	0.40	2.5	78.6	18.9
	0.60	1.8	76.6	21.6
	0.80	0.9	73.6	25.5
	1.00	0.8	69.2	30.0
	1.30	0.3	58.2	41.5
	1.60	-	41.0	59.0
+20	0.20	16.4	65.6	18.0
	0.40	11.0	67.6	21.4
	0.80	4.8	63.7	31.5
	1.00	2.9	60.3	36.8
	1.30	1.3	48.6	50.1
+37	0.20	17.6	66.7	15.7
	0.40	13.5	65.9	20.6
	0.60	10.2	64.9	24.9
	0.80	7.5	63.4	29.1
	1.00	5.0	61.0	34.0
	1.30	2.5	54.5	43.0
	1.60	-	43.7	56.3

Table 3.13

Oxidation of Fluphenazine Mono N-O

Mixture at 20°C

m-chloroperbenzoic acid added (ml)	Mono N-O A (%)	Mono N-O B (%)	di N-O (%)
0(Initial)	41.3	58.7	-
5	31.8	59.0	9.2
10	22.3	58.4	19.3
15	14.4	56.5	29.1
25	3.4	50.0	46.6
30	-	44.8	55.2
45	-	23.8	76.2
55	-	9.3	90.7
65	-	1.3	98.7

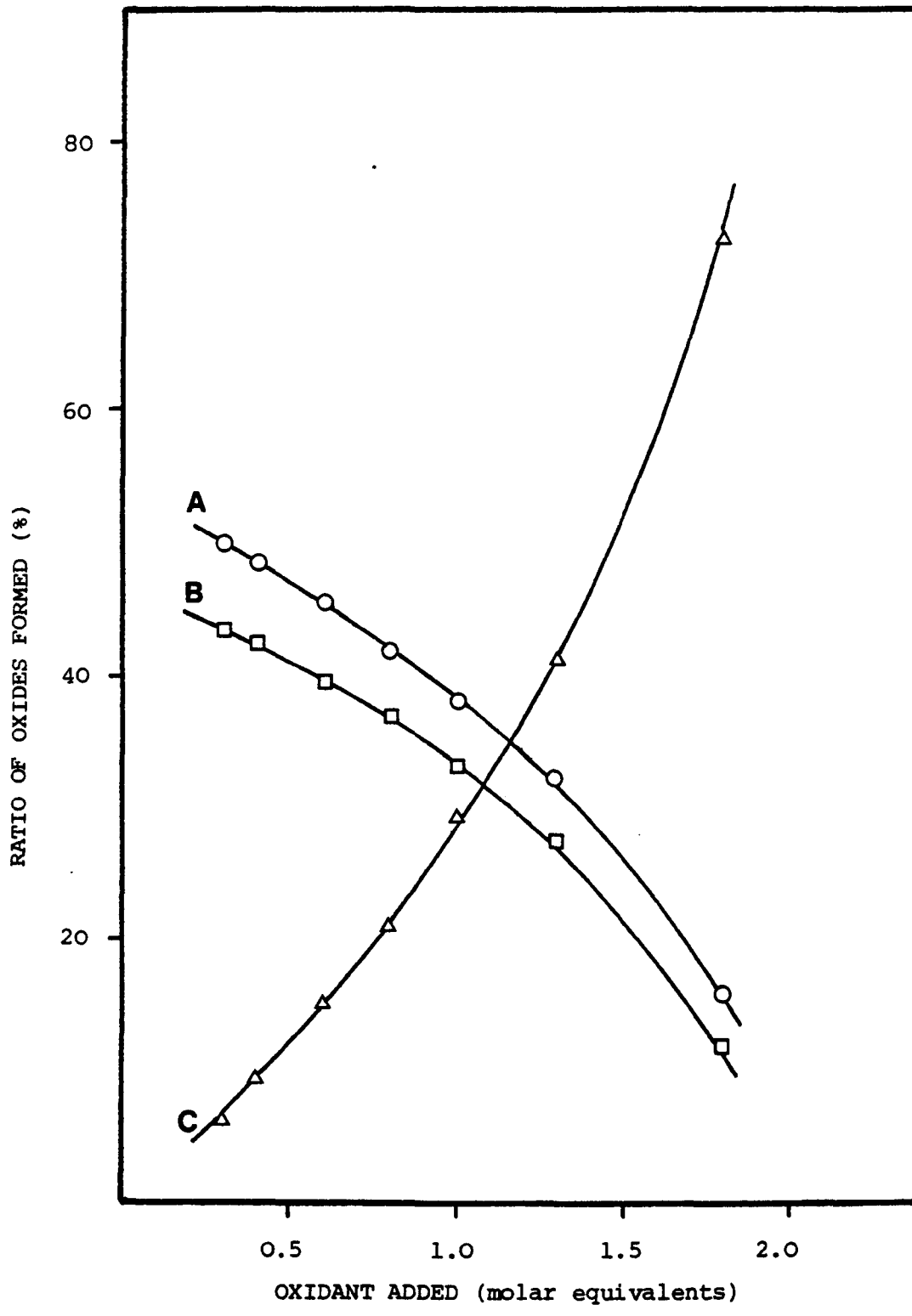


Fig. 3.13 - Relative Proportions of N-Oxides Formed During Oxidation of Fluphenazine Acetate with *m*-Chloroperbenzoic Acid

A = mono N-oxide A; B = mono N-oxide B;

C = di N-oxide.

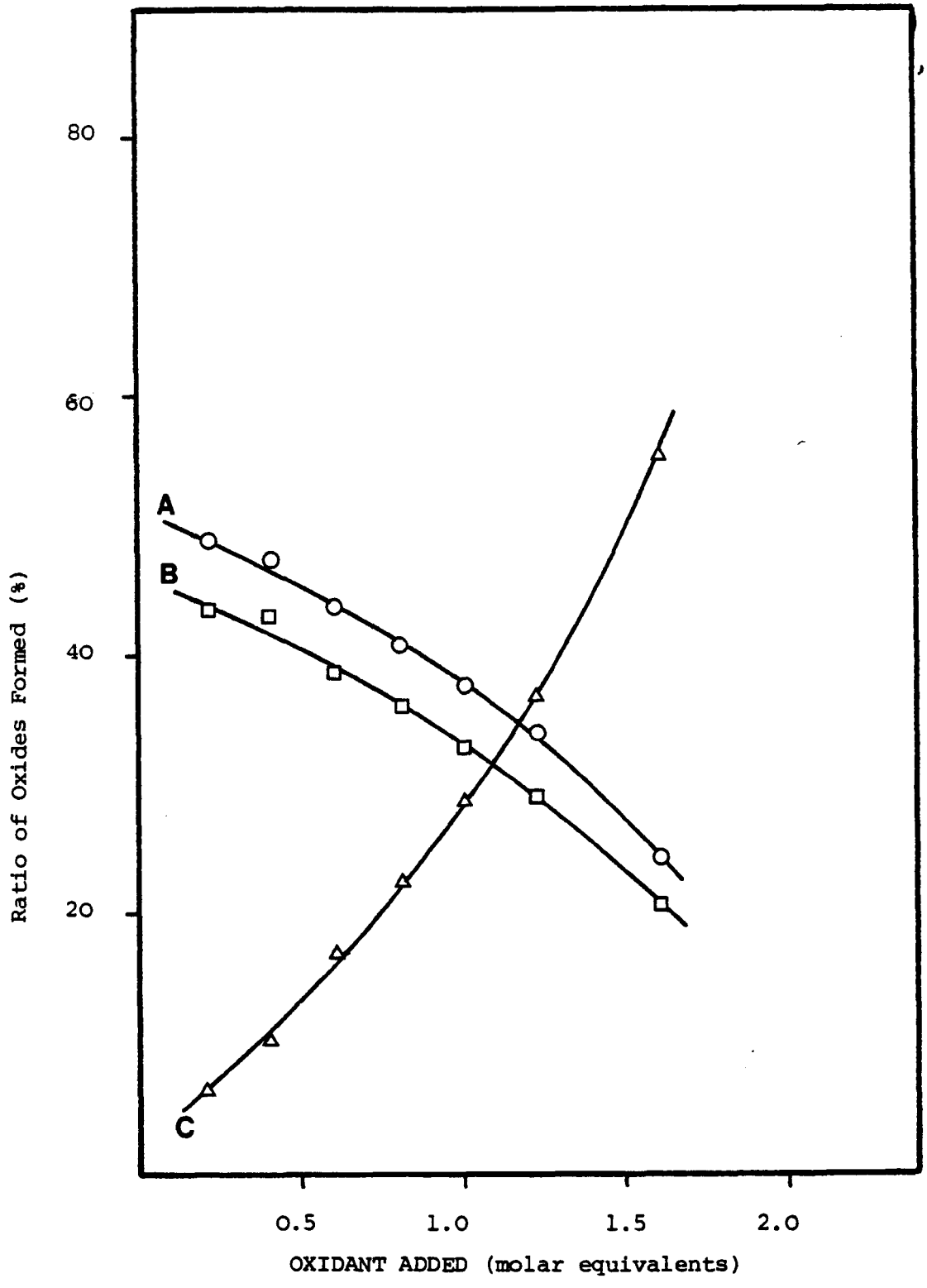


Fig. 3.14 - Relative Proportions of N-Oxides Formed During Oxidation of Fluphenazine Enanthate with *m*-Chloroperbenzoic Acid.

A = mono N-oxide A; B = mono N-oxide B;

C = di N-oxide.

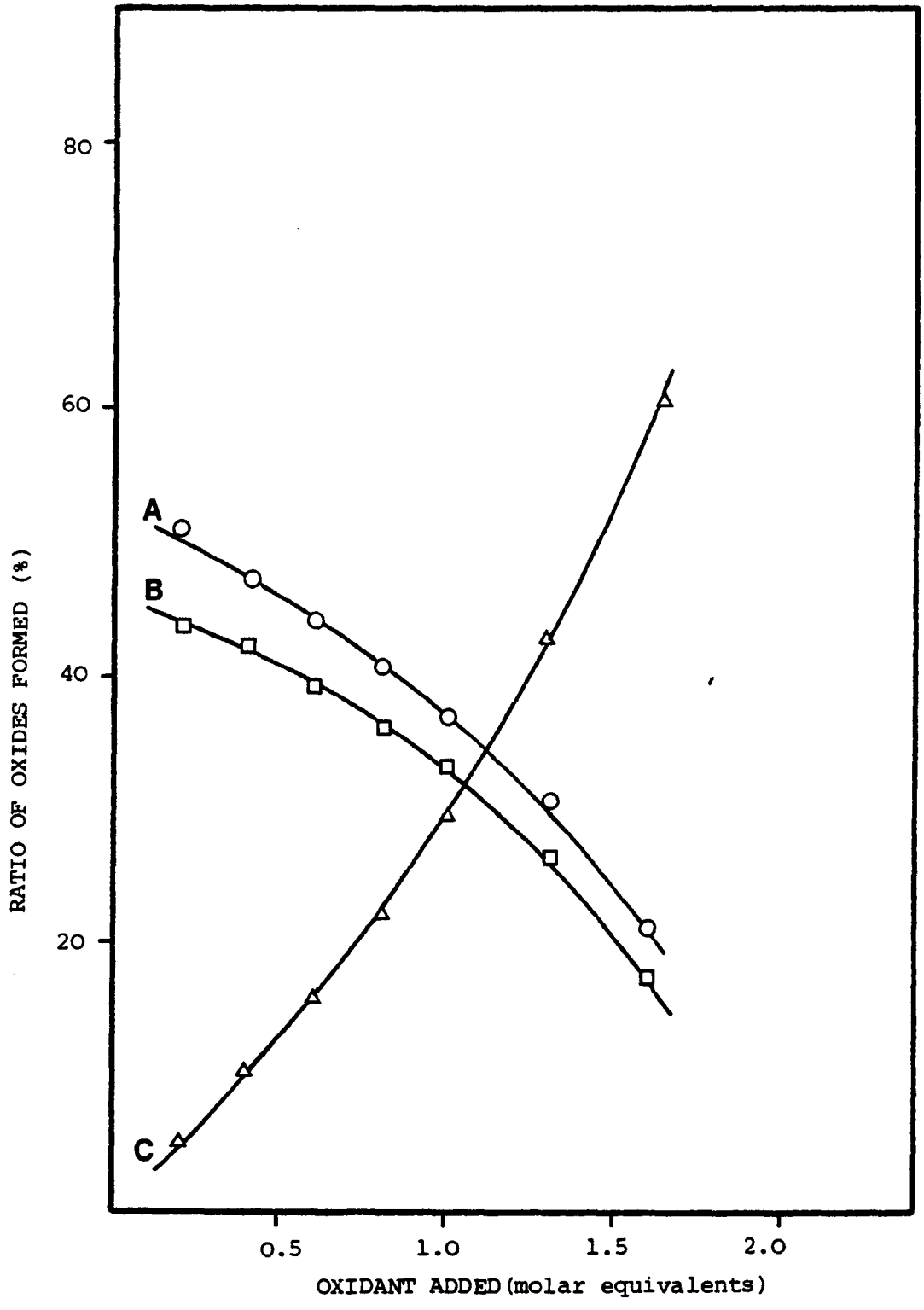


Fig. 3.15 - Relative Proportions of N-Oxides Formed During Oxidation of Fluphenazine Decanoate with *m*-Chloroperbenzoic Acid

A = mono N-oxide A; B = mono N-oxide B;

C = di N-oxide.

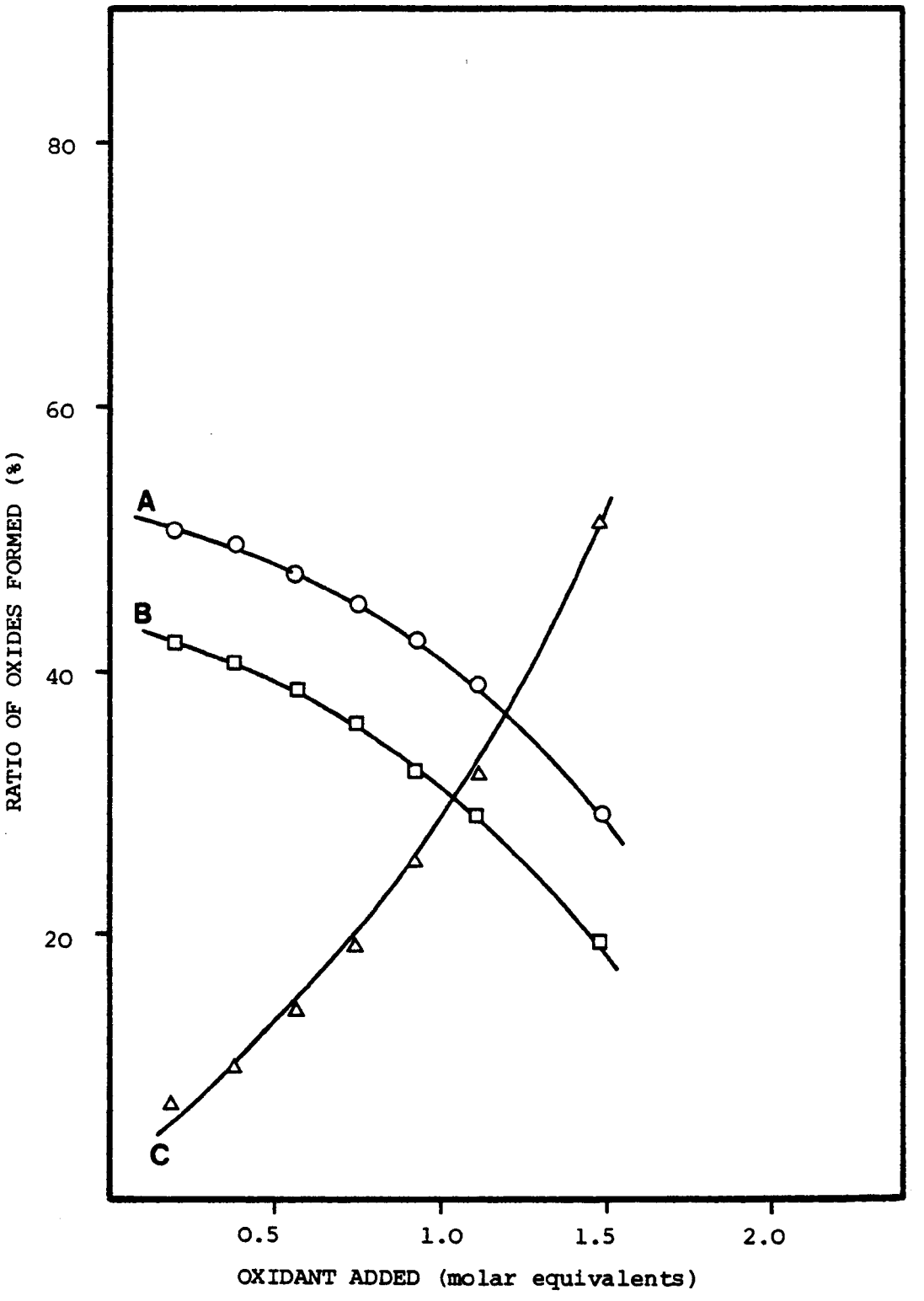


Fig.3.16 - Relative Proportions of N-Oxides Formed During Oxidation of Perphenazine Acetate with *m*-Chloroperbenzoic Acid

A = mono N-oxide A; B = mono N-oxide B;

C = di N-oxide.

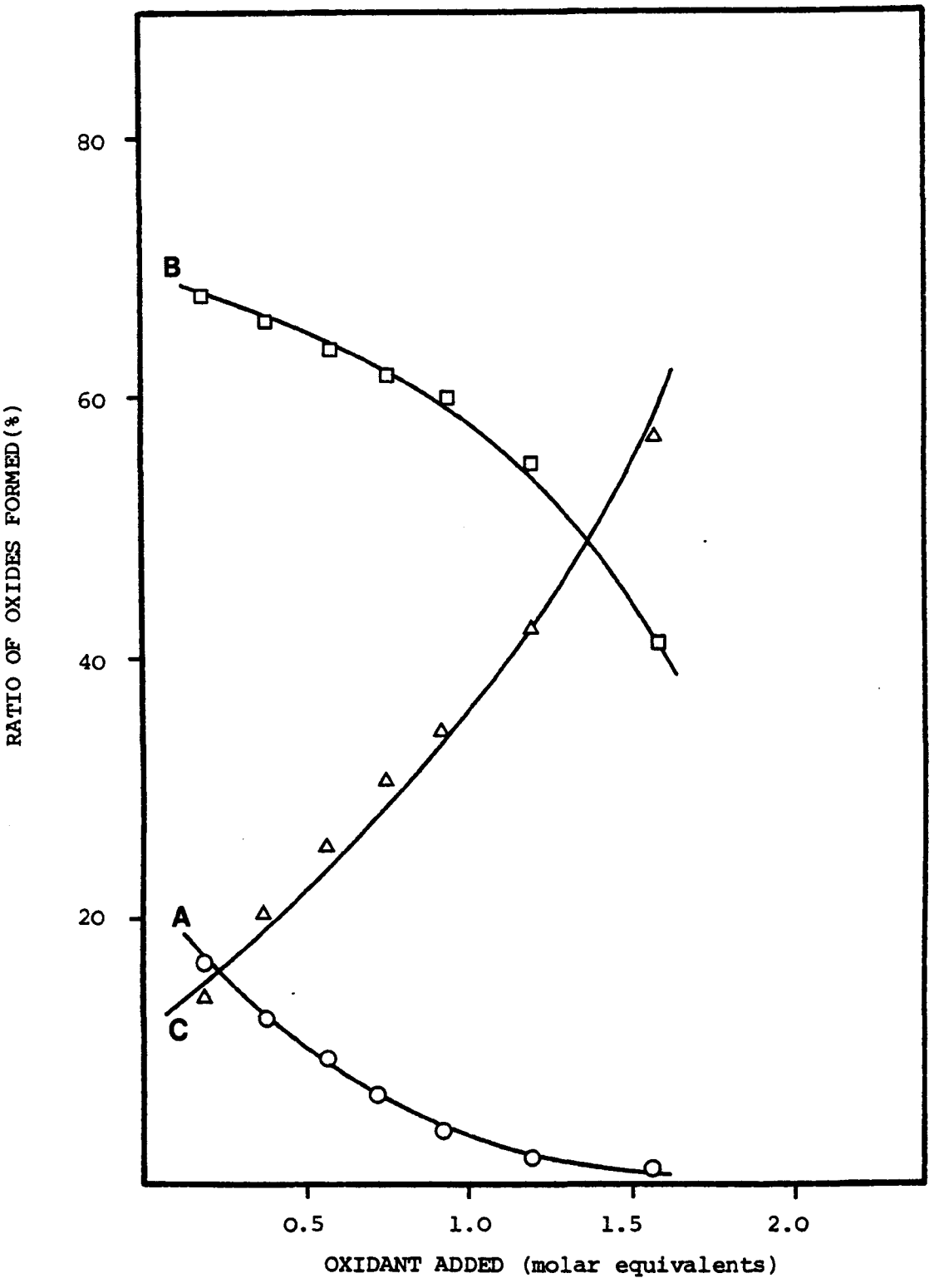


Fig.3.17 - Relative Proportions of N-Oxides Formed During Oxidation of Perphenazine with *m* -Chloroperbenzoic Acid

A = mono N-oxide A; B = mono N-oxide B;

C = di N-oxide.

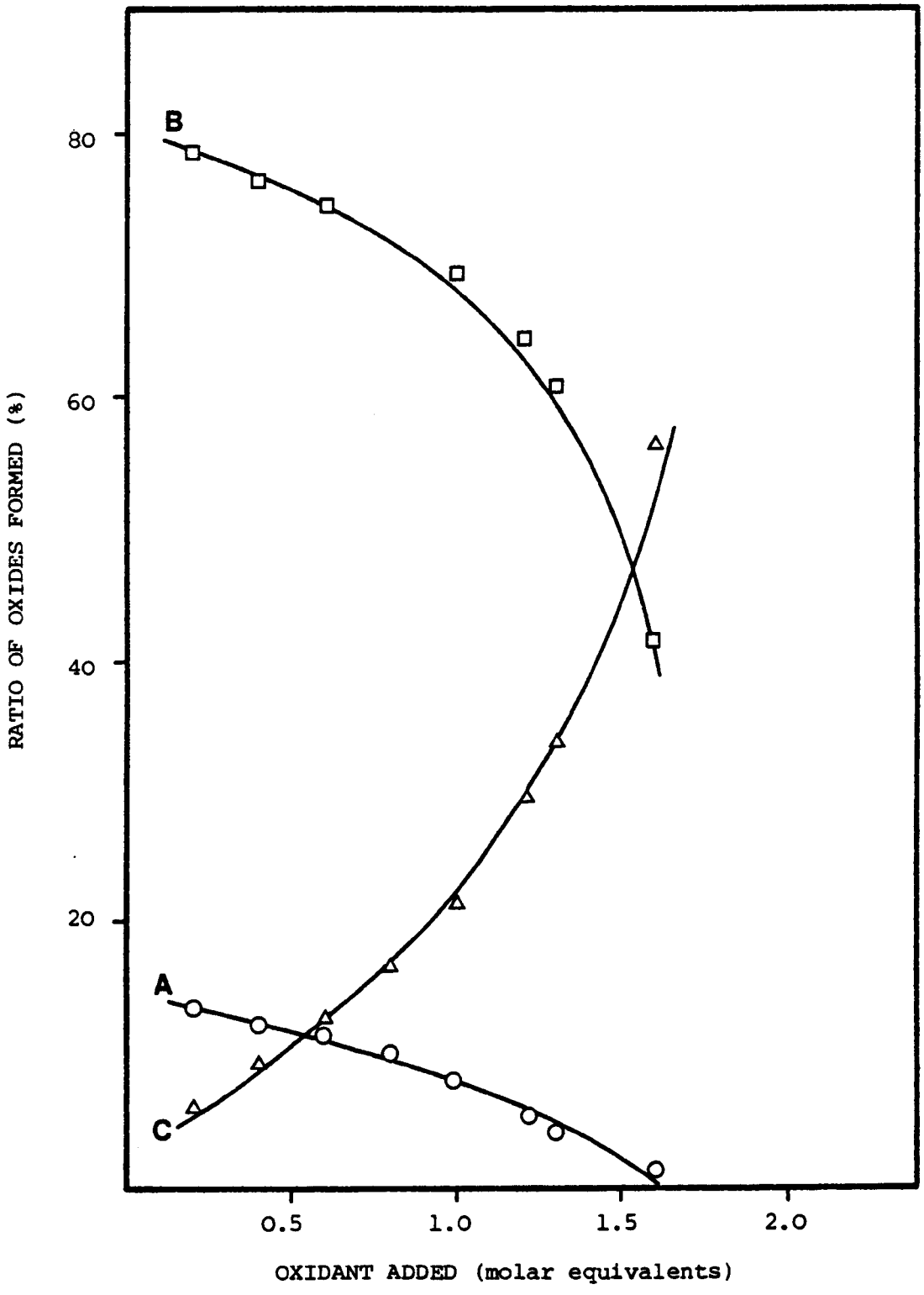


Fig.3.18 - Relative Proportions of N-Oxides Formed During Oxidation of Trifluperazine with *m*-Chloroperbenzoic Acid

A = mono N-oxide A; B = mono N-oxide B;

C = di N-oxide.

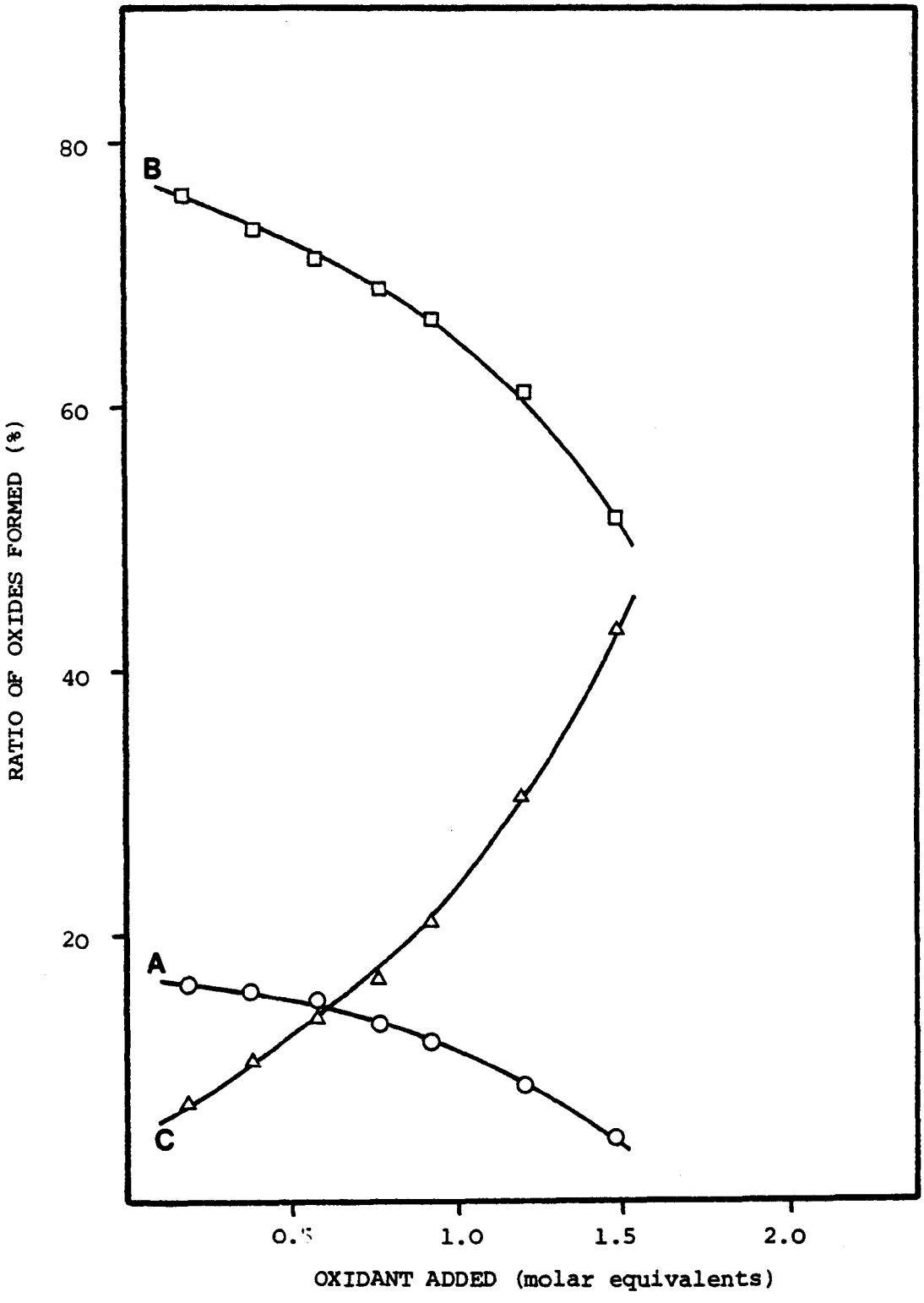


Fig.3.19 - Relative Proportions of N-Oxides Formed During Oxidation of Perazine with *m*-Chloroperbenzoic Acid

A = mono N-oxide A; B = mono N-oxide B;

C = di N-oxide.

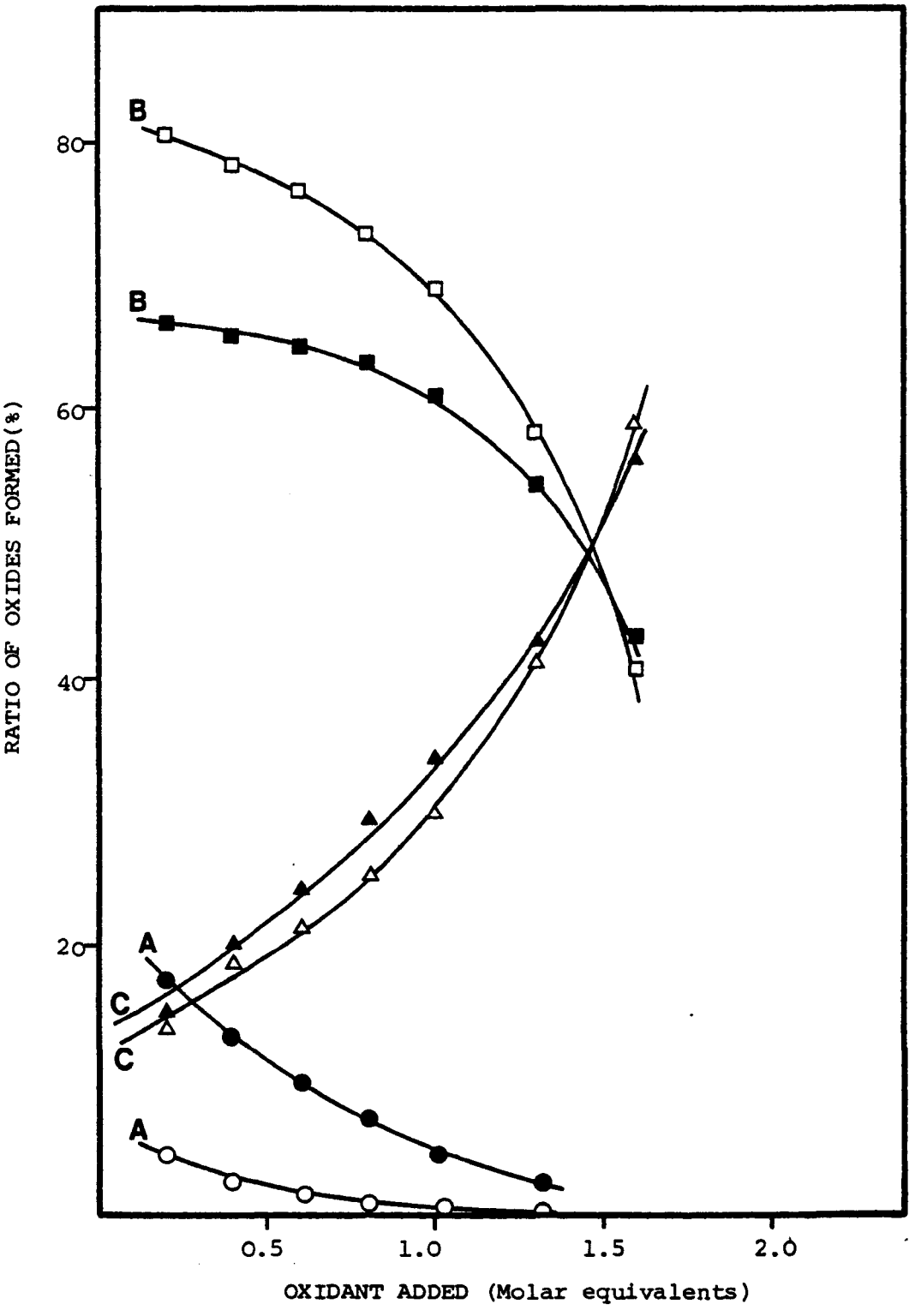


Fig.3.20 - Relative Proportions of N-Oxides Formed During Oxidation of Fluphenazine with *m*-Chloroperbenzoic acid

■●▲, 20°C ; □○△, -40°C .

A = mono N-oxide A; B = mono N-oxide B;

C = di N-oxide.

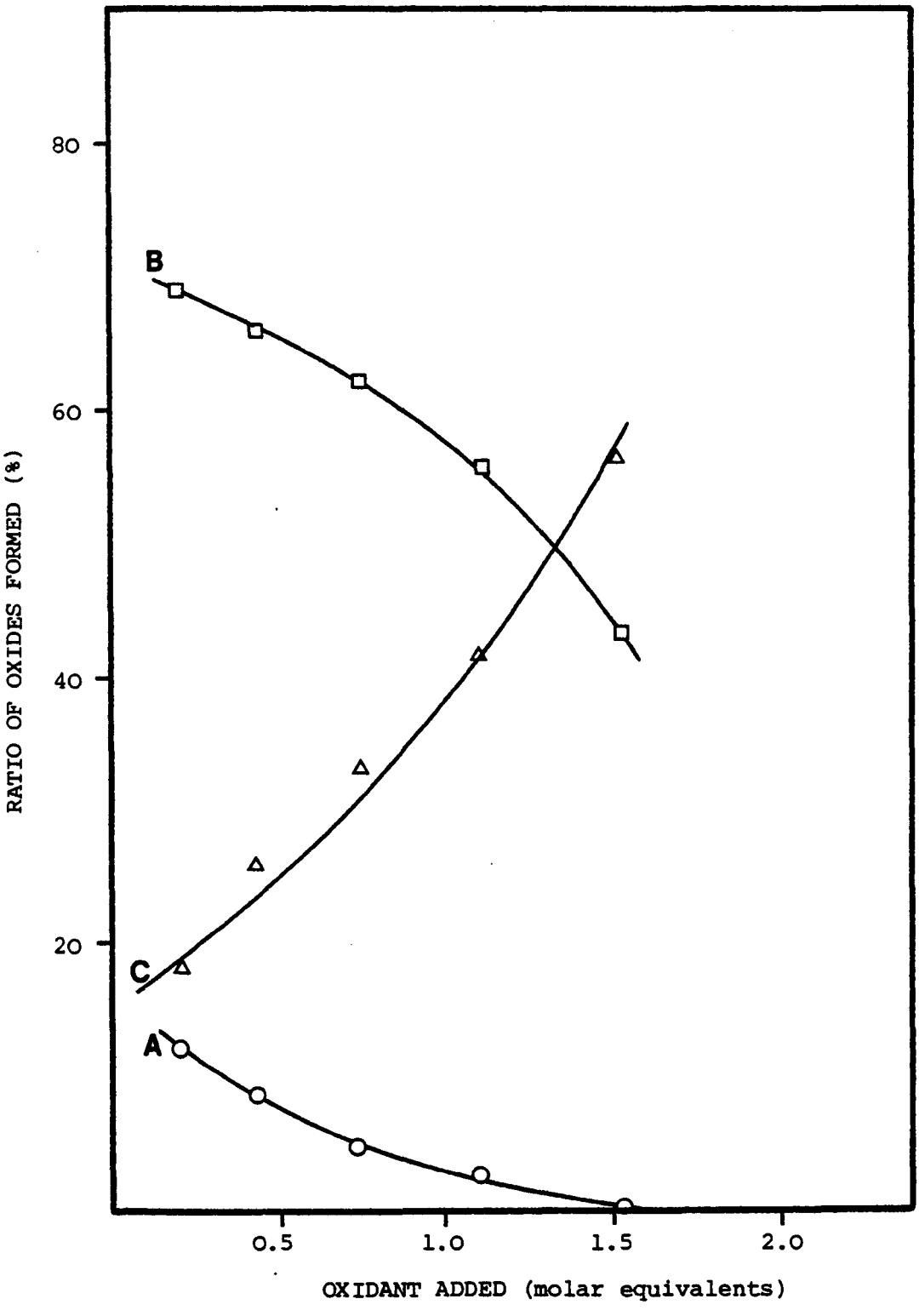


Fig. 3.21 - Relative Proportions of N-Oxides Formed During Oxidation of Fluphenazine (20°C) with *m* -Chloroperbenzoic Acid in the Presence of *m*-Chlorobenzoic Acid

A = mono N-oxide A; B = mono N-oxide B;

C = di N-oxide.

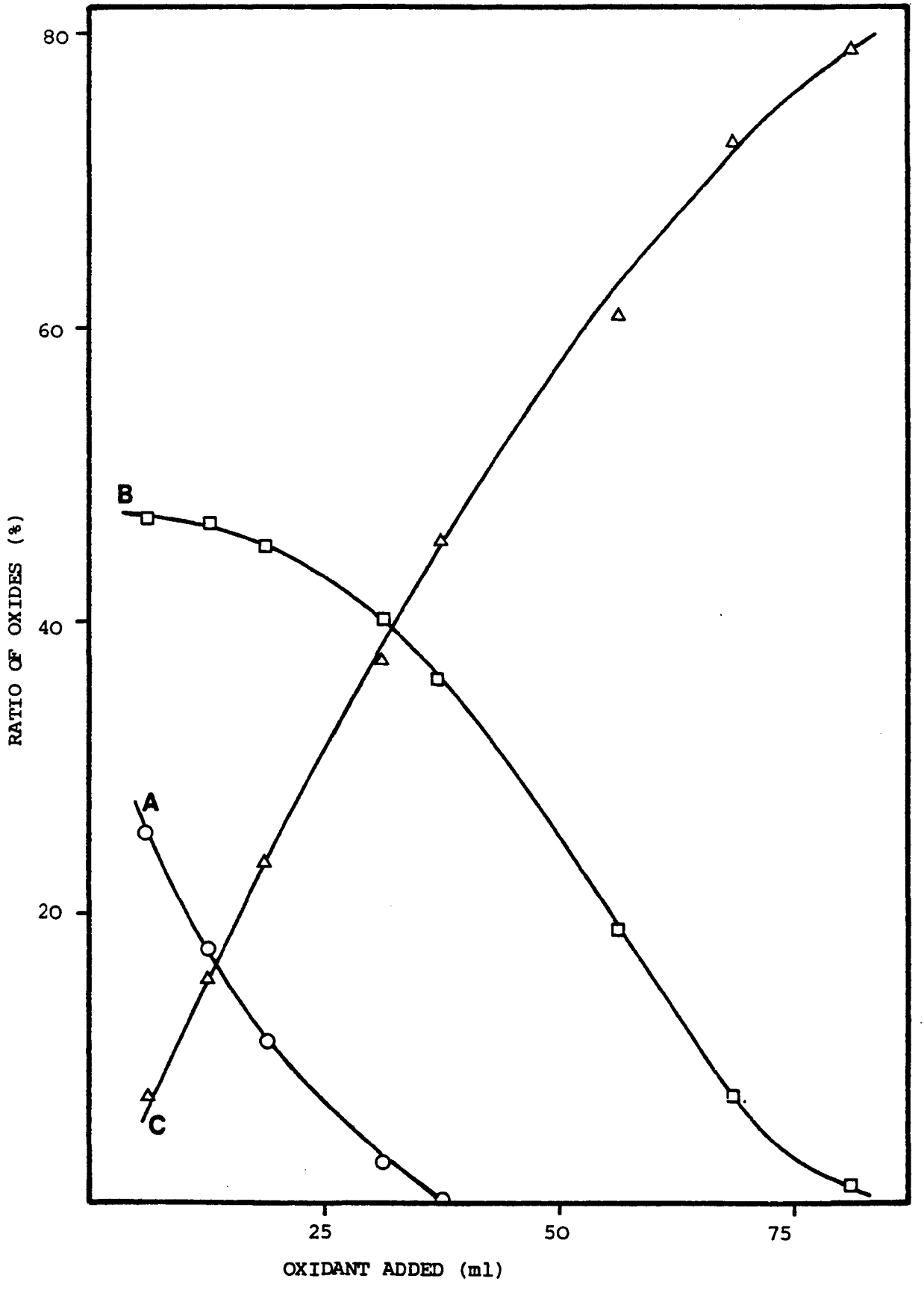


Fig.3.22 - Oxidation of a Mixture of Fluphenazine Mono N-Oxides with *m*-Chloroperbenzoic Acid

A = mono N-oxide A; B = mono N-oxide B;

C = di N-oxide.

3.2.5 Discussion of Results

The above results demonstrate that the compounds examined may be divided into two distinct groups:

(i) Esterified piperazino-phenothiazines, which form approximately equal quantities of each of the two mono N-oxides.

(ii) Non-esterified piperazino-phenothiazines, which form mainly mono N-oxide B with small amounts of mono N-oxide A (approximate ratio 90:10).

A recent paper by Rowell et al[211] has described evidence to support the theory of conformational recognition by antibodies (originally postulated by Horn and Snyder[212]) which requires that the phenothiazine side-chain is orientated toward the phenothiazine ring 2-substituent and held in this preferred conformation by Van der Waals forces.

A similar interaction between the 2-substituent and the side-chain was suggested by Underberg et al[77] to account for the change in the pattern of degradation products observed with respect to the electronegativity of the 2-substituent.

However, introduction of an electronegative substituent at the 2-position in the phenothiazine ring has little effect on the ratio of the A and B mono N-oxides formed during oxidation, suggesting that molecular conformation is unimportant in this case.

Other minor molecular alterations also have no apparent effect on the ratio of A:B formed. Thus extending the ester chain-length from acetate to decanoate does not affect the approximate 1:1 ratio of mono N-oxides produced on oxidation of the esters. Similarly, varying the terminal group attached to the piperazine ring in the non-esterified phenothiazines has little effect on the 9:1 ratio of N-oxides A:B formed.

The influence of the basicity of the piperazine t-amino groups can be excluded as the published pKa values for fluphenazine and fluphenazine esters

are similar (Table 3.14).

Table 3.14

pKa Values of Some Piperazino-Phenothiazines

Compound	Ring 2-substituent	pKa_1	pKa_2	Reference
Perazine	H	3.95	5.20	213
Prochlorperazine	Cl	4.05	6.80	213
Trifluperazine	CF_3	4.13	7.23	213
Fluphenazine	CF_3	3.90 3.60	8.10 7.80	214 215
Fluphenazine enanthate	CF_3	3.29 3.50	7.70 8.20	216 ?

Within the group of non-esterified piperazino-phenothiazines, introduction of an electronegative substituent at the 2-position in the aromatic ring system causes a distinct increase in pKa_2 value (Table 3.14), but has little effect on the ratio of mono N-oxide A:B formed during oxidation (Tables 3.4 to 3.13). The apparent relationship between pKa_2 value and electronegativity of the ring substituent maybe further evidence supporting the conformational theory of Horn and Synder[212]. The electronegative influence of the phenothiazine ring substituent would not normally be transmitted through the length of a propyl carbon-chain to influence the piperazine t-amino groups; thus a spatial effect is indicated.

With further regard to the ratio of A and B oxides produced, the alternative possibility of protonation of the basic amino groups by the acidic oxidant was also considered. Thus oxidation of fluphenazine was performed after addition of excess carboxylic acid (*m*-chlorobenzoic acid). The results (Fig. 3.20) demonstrate that this facet had little or no effect on the ratio of A:B formed and may therefore be dismissed as a non-contributory factor.

The most important factor in this respect was the temperature at which the oxidation reaction was conducted. A reduction of the reaction temperature from $20^{0}C$ to $-40^{0}C$ considerably reduced the amount of N-oxide A produced (Fig.3.21) Such an effect may be considered as indicative of steric hinderance in the region of the 4-position of the piperazine-ethanol moiety within the side-chain.

A further demonstration of the preferential oxidation of the piperazine-ethanol moiety at the 1-position was obtained by observing the oxidation of approximately equimolar quantities of mono N-oxides A and B under identical conditions to those used in previous experiments. The results (Fig.3.22) unambiguously demonstrate that N-oxide A is preferentially converted to the di-N-oxide. Indeed, the observed preference is of such magnitude that N-oxide A is selectively oxidised in the initial stages of the reaction. Hence, all available oxygen is consumed by N-oxide A until the concentration of A falls to a relatively low value (A:B;15:55) whence both compounds (A and B) are oxidised to the di-oxide at approximately equal rates.

The means by which the introduction of an ester carbonyl group into the piperazino side-chain causes a modification of the behaviour of the phenothiazines toward oxidation cannot be envisaged from the results obtained to date. Further experimental work will be required before a satisfactory explanation of this phenomenon can be proposed.

CHAPTER 4

CHARACTERISATION OF PREPARED COMPOUNDS

4.1. Purity

4.1.1 Experimental

The purity of the isolated compounds was assessed using HPLC, based upon systems described previously (Section 3.1.2.1). A 20cm column of SAS-Hypersil together with a mobile phase of methanol-acetontrile-1% aqueous ammonium carbonate in the ratio of 1:1:1 proved to be adequate for the examination of the majority of ester-related oxidation products. However, for complete resolution of compounds D and E (or V and W) it was necessary to adjust the ratio of the mobile phase components to 1:1:2.

Similarly, a mobile phase of methanol-acetonitrile-10% aqueous ammonia containing 1% potassium chloride (ratio 0.5:0.5:1) was utilised for the examination of the majority of isolated fluphenazine oxidation products, but for complete resolution of compounds D' and E' (or V' and W') the ratio was altered to 0.5:0.5:2.

4.1.2 Results

Results are summarised in Table 4.1.

Table 4.1

Estimated Purity(HPLC) of Isolated Oxdiation Products

Compound	Estimated Purity	Identity of Impurities
A (Prep.HPLC)	99%	
B (Prep.HPLC)	99%	Compound A(1%)
C (recrystallised)	99%	Compound D(1%)
D	98%	Compound E(2%)
E	99%	
F	98%	Fluphenazine decanoate(1%); Compound E(1%)
V	95%	Fluphenazine sulphoxide(4%); Unknown(1%)
W	99%	Compound V(1%)
X (Prep.HPLC)	99%	Compound Y(1%)
Y (Prep.HPLC)	99%	
Z	95%	Unknown(5%)
A'	95%	Fluphenazine(5%)
B'	98%	Fluphenazine(2%)
C'(recrystallised)	99%	Compound D'(1%)
D'	96%	Compound C'(3%); Compound E'(1%)
E'	99%	Compound D'(1%)
F'	98%	Fluphenazine(2%)
V'	95%	Compound W'(3%)
W'	Not isolated	Unknown (2%)
X'	Not prepared	-
Y'	96%	Compound B'(4%)
Z'	99%	

4.2 Infra-red Spectrophotometry

4.2.1 Experimental

The infra-red spectrum of each isolated compound was determined on a Pye-Unicam SP 1000 IR spectrophotometer as a compressed disc (1% dispersion in KBr). To ensure homogeneity of the mix, the compound being examined was added to the potassium bromide as a solution in dichloromethane (decanoate series) or methanol (fluphenazine series). The solvent was evaporated under vacuum prior to pressing the disc at a pressure of 10 tons.

4.2.2 Results

Salient features of the spectra obtained are listed in Tables 4.2 and 4.3. The full spectrum of each compound is reproduced in Appendix 3. Spectra of fluphenazine decanoate, fluphenazine hydrochloride and fluphenazine base are included for comparison.

Table 4.2

Main Features of IR Spectra

(Fluphenazine Decanoate Oxidation Products)

Compound	Diagnostic absorption band (cm^{-1})	Assignment	Fig.No. (Appendix 3)
A	960	aliphatic N-oxide	A3.2
B	960	aliphatic N-oxide	A3.3
C	975	aliphatic N-oxide	A3.4, A3.5
D	975 1030, 1060	aliphatic N-oxide sulphoxide	A3.6, A3.7
E	975 1180 & 1305	aliphatic N-oxide sulphone	A3.8, A3.9
F	1170 & 1310	sulphone	A3.10
V	975 1180 & 1310	aliphatic N-oxide sulphone	A3.11
W	975 1035, 1060	aliphatic N-oxide sulphoxide	A3.12
X	960 1030, 1055	aliphatic N-oxide sulphoxide	A3.13
Y	960 1040, 1060	aliphatic N-oxide sulphoxide	A3.14
Z	1030, 1055	sulphoxide	A3.15

Table 4.3

Main Features of IR Spectra

(Fluphenazine Oxidation Products)

Compound	Diagnostic absorption band(cm^{-1})	Assignment	Fig.No. (Appendix 3)
A'	960	aliphatic N-oxide	A3.18
B'	940	aliphatic N-oxide	A3.19
C'	970	aliphatic N-oxide	A3.20
D'	955 1025	aliphatic N-oxide sulphoxide	A3.21
E'	970 1709 & 1300	aliphatic N-oxide sulphone	A3.22, A.23
F'	1170 & 1300	sulphone	A3.24
V'	970 1170 & 1300	aliphatic N-oxide sulphone	A3.25
Y'	950 1050, 1060	aliphatic N-oxide sulphoxide	A3.26
Z'	1050, 1060	sulphoxide	A3.27

4.2.3 Discussion

In the I.R. spectra of phenothiazine N and S oxidised species, four absorption bands are important for diagnostic purposes:[102,197]

950 - 970 cm^{-1}	N - O	stretching vibration
1030 - 1070	S = O	stretching vibration
1120 - 1160	$S(=O)_2$	symmetrical stretching vibration
1300 - 1350	$S(=O)_2$	asymmetrical stretching vibration

N - O and S = O stretching frquencies are readily observed as the spectrum of the unoxidised parent shows no interfering bands in the appropriate region. The expected absorption frequency of the sulphone symmetrical stretching vibration however, overlaps other frequencies present in the spectrum of the parent molecule and the presence of this band must be inferred from the observed changes in intensity.

The pattern of absorption bands in the 1600cm^{-1} region, associated with the aromatic ring system, clearly indicate those compounds where oxidation has occurred in the phenothiazine nucleus. Thus observed absorption bands relating to oxidation of the phenothiazine S atom are accompanied by a concurrent change in the pattern of the spectrum in the 1600 cm^{-1} region (C = C in-plane stretching vibration). One unexpected feature of the IR spectra of the fluphenazine decanoate N-oxidised species (as bases) was an observed broad absorption band in the 3500 cm^{-1} region. Such an absorption band was anticipated for fluphenazine oxidation products (alcoholic OH group), but in the case of the oxides derived from the ester, the absorption band is believed to be indicative of the suspected hydrated nature of these compounds.

The main conclusions to be drawn from the IR data are included in Tables 4.2 and 4.3. It is also apparent from these tables that fluphenazine and the decanoate ester yield a similar sequence of oxidation products and that compound A' is related to compound A by addition of a decanoyl ester group.

Conversely, hydrolysis of compound A should yield A' and this relationship was confirmed by HPLC. A similar relationship was demonstrated for all the other compounds; therefore B is hydrolysed to B' etc.

Combining IR data and earlier evidence from HPLC and oxidation experiments with *m*-chloroperbenzoic acid, the following tentative conclusions may be made concerning the identity of the oxidation products:

Compounds A, A'	piperazino mono-N-oxide
Compounds B, B'	piperazino mono-N-oxide
Compounds C, C'	piperazino di N-oxide
Compounds D, D' = W, W'	sulphoxide piperazino di N-oxide
Compounds E, E' = V V'	sulphone piperazino di N-oxide
Compounds F, F'	sulphone
Compounds X, X'	sulphoxide piperazino mono N-oxide
Compounds Y, Y'	sulphoxide piperazino mono N-oxide
Compounds Z, Z'	sulphoxide

4.3 UV Spectrophotometry

4.3.1 Experimental

The UV spectra of the isolated compounds in methanolic solution were recorded on Perkin-Elmer 137 scanning spectrophotometer. Quantitative measurements were determined with a Varian model 634 spectrophotometer and the molar absorptivity calculated from the equation[198,199]

$$A = \epsilon c l$$

where A = absorbance

ϵ = molar absorptivity

c = concentration (mole/litre)

l = path-length of cell (cm)

4.3.2 Results

The results obtained are summarised in Tables 4.4 and 4.5.

Table 4.4

UV Absorption Characteristics

(Fluphenazine decanoate oxidation products)

Compound	λmax(nm)	ε
(Fluphenazine decanoate	260	34500)
A (base)	258	32000 32800
B (base)	261	
C (base)	258	31200
D (HCl Salt)	274 300 350	10900 7100 4100
E (HCl Salt)	270 296 335	13100 6900 4500
E (base)	272 298 340	
F (base)	274 302 343	
V (base)	271 295 340	
W (base)	275 301 349	
X (base)	277 305 352	
Y (base)	276 303 350	
Z (base)	277 305 352	

Table 4.5

UV Absorption Characteristics
(Fluphenazine Oxidation Products)

Compound	λmax(nm)
Fluphenazine	260
A'(base)	260
B'(base)	261
C' (HCl Salt)	258
D' (HCl Salt)	274 300 348
E' (HCl Salt)	272 298 340
F'(base)	275 302 343
V' (base)	272 298 340
Y' (base)	278 305 355
Z' (base)	278 304 352
Z' (HCl Salt)	276 303 352

4.3.3 Discussion

Numerous descriptions of the UV spectral characteristics of phenothiazine sulphoxides and sulphones are apparent in the literature[200,201,202]. All reports are in agreement that oxidation of the phenothiazine ring S atom results in a bathochromic shift of the λmax with respect to the parent phenothiazine, and that the spectra of the sulphoxides are characterised by the presence of three wavelengths of maximum absorption (at about 275, 300 and 350 nm). On further oxidation to the sulphone, the characteristic triple

absorbtion-band pattern is retained but the three λmax show a small hypochromic (blue) shift with respect to the sulphoxide. Thus, the results given in Tables 4.4(and 4.5) for compounds D-F (D' - F') and V-Z (V' - Z') are consistent with the conclusions derived from interpretation of IR spectra (Section 4.2).

No information concerning the spectral characteristics of the N-oxidised phenothiazine species could be found in the literature. However, the results given in the Tables for compounds A-C (A'-C') are consistent with the postulation that oxidation has occurred in the side-chain whence little change in λmax or ϵ would be expected.

4.4 Spectrofluorimetry

4.4.1 Experimental

Excitation and fluorescence spectra of the isolated compounds in 0.1% methanolic hydrochloric acid were recorded on a Perkin-Elmer model 204 spectrofluorimeter.

4.4.2 Results

Tables 4.6 and 4.7 summarise the results obtained.

Table 4.6

Fluorimetric Properties of Fluphenazine Decanoate and its Oxidation Products

Compound	Excitation λ max (nm)	Fluorescence λ max (nm)
Fluphenazine decanoate		V.Weak, 470
A		V.Weak, 470
B		V.Weak, 470
C		V.Weak, 470
D	275(shoulder) 295 345	Strong, 397
E	275(shoulder) 295 337	Strong, 390
F	285(shoulder) 313 353	Strong, 390
V	275(shoulder) 295 337	Strong, 390
W	275(shoulder) 295 345	Strong, 397
X	275(shoulder) 298 347	Strong, 395
Y	275 298 347	Strong, 398
Z	275(shoulder) 297 345	Strong, 393

Table 4.7

Fluorimetric Properties of Fluphenazine and its Oxidation Products

Compound	Excitation λ max (nm)	Fluorescence λ max (nm)
Fluphenazine		V.Weak, 470
B'		V.Weak, 470
C'		V.Weak, 470
D'	275(shoulder) 297 346	Strong, 396
E'	273(shoulder) 295 336	Strong, 390
F'	275(shoulder) 297 338	Strong, 390
V'	273(shoulder) 295 336	Strong, 390
Y'	275(shoulder) 297 345	Strong, 396
Z'	300 348	Strong, 398

4.4.3 Discussion

Phenothiazines, which exhibit a weak native fluorescence, are converted into strongly fluorescent sulphoxides (or sulphones) by hydrogen peroxide. In addition, the fluorescence λ max is shifted to shorter wavelengths[103]. The intense fluorescence is believed to result from the extended conjugation of the aromatic nucleus formed by substitution of oxygen at the phenothiazine-ring S atom. Tables 4.6 and 4.7 clearly indicate those compounds which possess S = O or $S\overset{=O}{\underset{=O}{}}$ groups and are in agreement with the IR and UV data quoted in the previous sections.

The further oxidation of a phenothiazine sulphoxide yielding a sulphone could alter the fluorimetric properties of the molecule, but no indications to this effect are to be found in the literature. The results given in Tables 4.6 and 4.7 imply that those compounds shown by IR to be sulphones, exhibit a λ max at slightly shorter wavelengths than the corresponding sulphoxide - compare compounds D (D') with E (E') and F (F') with Z(Z').

No information regarding the fluorimetric properties of phenothiazine N-oxides could be gleaned from the literature. However, if oxidation occurs at the side-chain piperazine N atoms, the molecule could be expected to retain the fluorimetric characteristics of the parent, ie. no extension of the conjugated system. Accordingly, compounds A, B and C (A', B' and C') are again intimated to be piperazino N-oxides.

4.5 Proton N.M.R.Spectra

4.5.1 Experimental

4.5.1.1 Conversion of Hydrochloride Salts to Bases

Compounds prepared as hydrochloride salts were converted to the corresponding bases prior to determining NMR spectra in order to avoid interference from the associated proton.

100mg Hydrochloride salt (fluphenazine decanoate oxidation products) was dissolved in 2ml methanol and excess IM aqueous ammonium hydroxide added. The precipitated base was extracted with ethyl acetate (or dichloromethane). The separated organic fraction was washed with water then dried over sodium sulphate. Evaporation of the organic solvent yielded the required base which was dried overnight at 20°C in a freeze-drier. The purity of the isolated solid material was checked by HPLC.

4.5.1.2 Determination of Spectra

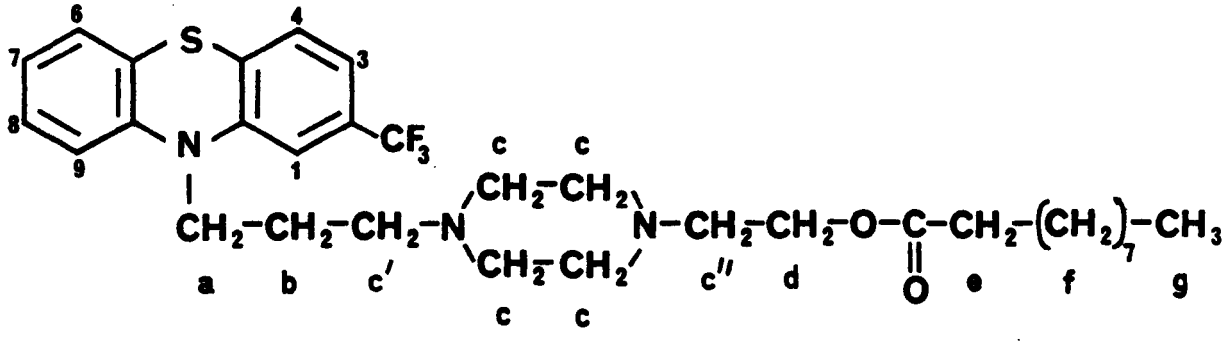
The PMR spectra of the oxidation products (decanoate ester series) were recorded by an external agency (PCMU, Harwell) on a Perkin Elmer Model R 34 220MHz NMR spectrometer using deutrochloroform to solubilise the compounds, with TMS as reference.

4.5.2 Results

Figs A3.28 - A3.35 (Appendix 3) illustrate the spectra obtained for compounds A-F and Z together with a similar spectrum of fluphenazine decanoate for comparison.

4.5.3 Discussion

For the purpose of this discussion, the proton signals of the parent ester will be labelled according to the interpretation of Florey[204]:



Protons	Integral value	ppm
aromatic	7, multiplet	6.8-7.2
a	2, triplet	3.95
b	2, triplet	1.9
c c' c"	12, broad singlet	2.5(broad band)
d	2, triplet	4.2
e	2, broad singlet	1.6
f	14, broad singlet	1.3
g	3, broad singlet	0.85

Resonance signals for methylene groups c, c' and c" were not clearly resolved and thus assignment of individual values to the three groups of protons was not attempted.

However, the 220 MHz spectrum of fluphenazine decanoate obtained in the present study distinctly resolves two triplets (integral value = 2) at 2.3 and 2.6ppm which may be attributed to the methylene protons of c' and c". The chemical shift of methylene group c" would be expected to be greatest, since this group is situated β to an oxygen atom. Hence, in the 220 MHz spectrum, the following chemical shift values are tentatively assigned to the methylene groups associated with the piperazine ring:

Protons	Integral value	ppm
c"	2, triplet	2.6
c	8, broad singlet	2.45
c'	2, triplet	2.3

i) Compounds F and Z

The PMR spectra of compounds F and Z (Figs. A3.34 and A3.35) are, in general, similar to the spectrum of the parent ester the most important difference being the perturbation of the aromatic proton signals (which are also moved downfield to 7.2-8.2 ppm). The spectrum of compound Z exhibits two doublets at 7.9 and 8.0 ppm separated from the general aromatic signal whilst in the spectrum of compound F the two doublets are shifted still further down-field (8.1 and 8.2ppm). This splitting of the aromatic proton signal is consistent with the introduction of an oxygen atom at the 5-position (S atom)[202], the two doublets representing the 4H and 6H protons[205]. Thus compound Z contains a sulphoxide group and compound F, which exhibits the greatest shift of the 4H and 6H aromatic signals is indicated to possess a second oxygen atom ie. a sulphone group. The pattern of side-chain proton signals is unchanged implying no oxidation of the piperazine N atoms in these compounds.

One interesting feature of the spectra of compounds Z and F is the down-field shift of resonance values for methylene groups a and b (4.4 and 2.1 ppm respectively). Such a shift will only be evident if the N-10 side-chain adopts the H-extra configuration[205].

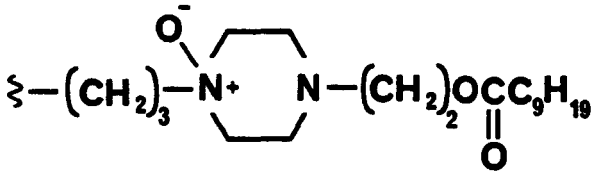
ii) Compounds A and B

From chemical evidence, compounds A and B are each indicated to contain 1 molar equivalent of oxygen. No splitting of the aromatic proton signal is observed in the spectrum of either of the compounds signifying that oxidation has occurred only in the side-chain. The main task therefore is to determine the position of N-oxidation within the piperazine-ring.

Compound A

The spectrum of compound A is difficult to interpret even though numerous chemical-shift changes are apparent relative to the parent-ester. The most easily recognised feature is the downfield shift of the signal

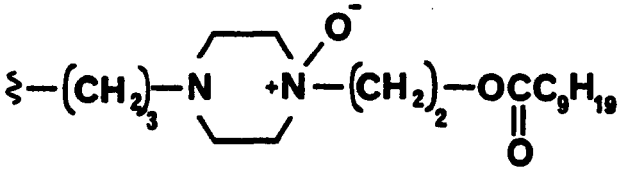
associated with methylene group a (from 3.95 to 4.2ppm), whence the a and d protons resonate at similar values and suggesting that the oxygen atom in Compound A is substituted at the 4-position within the piperazine-ethanol group:



Further interpretation of the spectrum is not possible without additional experimental work involving, perhaps, the use of spin-spin decoupling techniques. The non-availability of suitable instrumentation prevented completion of this work.

Compound B

The most noticeable difference between the spectrum of compound B and that of the parent ester is the large down-field shift (from 4.2 to 4.8 ppm) associated with the protons of methylene group d. The shift in the resonance value of methylene group a is negligible in comparison (from 3.95 to 4.05 ppm). Changes in the chemical shift values of the other methylene groups within the side chain are also evident. This pattern of chemical shifts is consistent with the postulation that oxidation has occurred at the 1-position in the piperazine-ethanol moiety:



The c' protons, however, resonate at approximately the same value as in the parent molecule which further supports the above structure.

iii)Compound C

Compound C has previously been shown to contain two molar equivalents of oxygen.

The main feature of the spectrum of compound C (Fig. A3.31) is the total change in the resonance values for the piperazine methylene groups suggesting that both of the oxygen atoms are situated in the side-chain. This postulation is further supported by the unchanged aromatic proton pattern apparent when the spectrum of compound C is compared with that of fluphenazine decanoate (Fig. A3.28).

iv) Compounds D and E

The spectra of compounds D and E (Figs. A3.32 and A3.33) show a pattern of side-chain methylene group signals similar to compound C, indicating the substitution of O at each of the piperazine N atoms.

The aromatic signals of compound D are similar to those observed with compound Z; likewise the spectrum of compound E is related to compound F.

Thus, it was inferred that compound D is the sulphoxide di N-oxide and compound E the sulphone di N-oxide of fluphenazine decanoate, a conclusion which is in complete agreement with IR data.

4.6 Carbon-13 NMR

Further evidence for the assignment of the position of oxidation in compounds A (A') and B (B') was sought using ^{13}C NMR. To simplify the spectra, use of the ester (decanoyl chain) was avoided and the equivalent fluphenazine compounds examined (ie. compounds A' and B').

4.6.1 Experimental

Spectra were determined at the University of Bath, School of Pharmacy, on a Joel NMR Spectrometer. The compounds were dissolved in deuterochloroform and TMS used as reference.

4.6.2 Results

The spectra obtained are illustrated in Figs. A3.37 and A3.38 (Appendix 3) together with a similar spectrum of fluphenazine base for comparison (Fig. A3.36).

4.6.3. Discussion

The ^{13}C NMR spectra of fluphenazine and the corresponding N-oxidised metabolite have been interpreted previously by Sofer and Zeigler[125]. The main diagnostic features of the spectra were reported to be the resonance peaks at 25 and 59ppm, which were attributed to the methylene group β to the N-propyl piperazine nitrogen atom and the methylene group β to the N-ethanol piperazine nitrogen atom respectively. Because of the gamma effect of oxygen, substitution of oxygen at a piperazine N atom will cause the resonance signal of the appropriate methylene group (β to N, therefore γ to the O atom) to move upfield.

In the ^{13}C NMR spectra of compounds A' and B', the signal representative of the methylene group β to the N-ethanol piperazine nitrogen atom (at 59ppm) is difficult to identify because of the presence of other resonance signals in this region. In contrast, the alternative diagnostic resonance signal at 25ppm is easily distinguised. Comparing the spectra of compounds A' and B' with that of the parent molecule, only compound A' shows the upfield shift of the 25ppm signal (to 20ppm) and must therefore contain an oxygen atom substituted at the N-propyl piperazine nitrogen atom, a conclusion which is in complete agreement with the structural assignment based upon proton NMR spectra.

4.7 Elemental Analysis

All determinations were undertaken by an external agency (Butterworth Laboratories Ltd.).

4.7.1 Oxygen Determination

Assay of the oxygen content of the oxides proved to be invalid because of interference from the fluorine present in the compounds.

4.7.2 CHN Determination

CHN determinations on the compounds (in the form of the base derived from the hydrochloride salt, Section 4.5.1.1) resulted in values which bore little resemblance to the theoretical values (Table 4.8)

Table 4.8

CHN Analysis of Compound C (base)

	Theoretical %	Found %
C	60.09	54.64; 54.59
H	6.88	7.48; 7.43
N	6.58	5.21; 5.52

The high values obtained for the H content combined with the low values of C and N implied contamination of the sample by water. Subsequent work (Section 4.9) confirmed that water was indeed present in the sample.

On the contrary, elemental analysis of the hydrochloride salt of compound C gave results close to theory:

Table 4.9

CHN Analysis of Compound C (HCl salt)

	Theoretical % (anhydrous)	Corrected for H_2O content	Found %
C	55.17	54.19	55.07
H	6.69	6.73	6.17
N	6.04	5.92	6.21
Cl	10.19	10.00	10.07

The above results, with the exception of the hydrogen value, are within the limits of allowable error (0.3% absolute) quoted by the manufacturer of the instrumentation. However, if the level of moisture present in compound C (1.8%, see Section 4.9) is taken into account, the values for determined carbon and hydrogen content deviate considerably from the theoretical figure (corrected values, Table 4.9).

Because of the unreliability of the CHN values, alternative techniques were sought to confirm the suspected identity of the compounds.

4.8 Mass Spectrometry

4.8.1 Experimental

Spectra were recorded by various external agencies.

Electron impact mass spectra (EI) were determined on a Kratos MS 25 mass spectrometer at the National Hospital, London.

Chemical ionisation mass spectra (CI) were determined on a Finnigan 3000 mass spectrometer using ammonia or butane as reagent gases.

Desorption Chemical Ionisation (DCI) and Fast Atom Bombardment(FAB) mass spectra were determined using a VG Analytical 70-70HS mass spectrometer coupled to a VG DS 20-50 data system (V.G. Analytical, Altrincham). The following conditions were employed:

DCI

Reagent-gas	-	ammonia
Ionisation energy	-	70 eV
Accelerating voltage	-	4.0 KV
Filament emmission	-	1mA

In negative chemical ionisation mode, Freon 113 was also introduced into the source as a supply of chlorine ions for chlorine adduction.

FAB

Samples were introduced into the ion source by means of an axially mounted direct insertion probe fitted with a stainless-steel sample support.

Xenon atom-beam source

acceleration potential	-	7KV
emmission current	-	1mA
accelerating voltage	-	4KV

4.8.2 Results

Tables 4.10 and 4.11 summarise the results obtained from the mass spectra.

Table 4.10

MS Examination of Fluphenazine Decanoate Oxidation Products

Compound	Technique	Molecular Ion (m/z)	Intensity (%)	Fig.No. (Appendix 3)
A	DCI	607	35	A3.39
B	DCI	607	10	A3.40
C	DCI	623	10	A3.41
	FAB	624(M^++1)		A3.42
D	DCI	640(M^++1)	10	A3.43
E	DCI	656(M^++1)	10	A3.44
F	DCI	624(M^++1)	100	A3.46
	EI	623	15	A3.45
X	DCI	624(M^++1)	10	A3.47
	FAB	624(M^+1)	100	A3.48
Y	DCI	624(M^++1)	15	A3.49
	FAB	624(M^++1)	70	
Z	EI	607	5	A3.50

Table 4.11

MS Examination of Fluphenazine Oxidation Products

Compound	Technique	Molecular Ion (m/z)	Intensity (%)	Fig. No. (Appendix 3)
B'	DCI	454(M^++1)	64	A3.51
C'	DCI	470(M^++1)	20	A3.52
	FAB	470(M^++1)	15	A3.53
D'	DCI	486(M^++1)	40	A3.54
	FAB	486(M^++1)	50	A3.55
E'	DCI	502(M^++1)	40	A3.56
Y'	DCI	470(M^++1)	10	A3.57
	FAB	470(M^++1)	80	A3.58
Z'	DCI	454(M^++1)	100	A3.59
	FAB	454(M^++1)	100	A3.60

4.8.3 Discussion of Results

EIMS provided molecular ion information only for those compounds not containing an N-O group (as shown by IR). This phenomenom is probably a result of the thermolabile nature of N-oxides. For the ester N-O compounds a major fragment was m/z 591, which is the molecular ion of the parent, indicating that the N-oxide readily decomposed to the parent ester at the source-temperature. The thermally labile N-oxides may also undergo re-arrangement according to defined rules. Cope elimination[153] forms an allyl derivative and it was by this means that original identification of the piperazino N-oxide as a degradation product of fluphenazine decanoate was achieved[174]. Meisenheimer rearrangement of heterocyclic N-oxides[206] involves the incorporation of the oxygen atom into the skeleton of the side-chain.

Attempts to deduce the molecular structure of the compound from the observed fragmentation pattern were unsuccessful.

Compounds Z and F however, the suspected sulphoxide and sulphone respectively, yielded small but observable peaks for the expected molecular ion. A comparison of the EI mass spectra of compounds Z, F and fluphenazine decanoate indicated that those fragments containing the intact phenothiazine ring occurred at 16 and 32 mass units higher in the spectra of compounds Z and F respectively. Fig. 4.1 summarises the observed intact-ring fragments. m/z fragments at 325, 153, 139 and 125 are indicative of the side-chain. The presence of each of these fragments in all three spectra signifies that the side-chain is unchanged in each of compounds Z and F, and thus the spectra fully support the proposed structures for these oxidation products.

Melikian et al[207] have investigated the application of CIMS to phenothiazine-oxides. The pseudo molecular ion (MH^+) was observed as base-peak for all sulphoxides examined, but in the case of the side-chain N-oxide of chlorpromazine, the molecular ion (and pseudo molecular ion) were absent. In the latter case, MH^+ of the Cope elimination product (allyl compound) was observed to be the base-peak. CI experiments with those oxidation products of fluphenazine and fluphenazine decanoate thought to be N-oxides (IR data) were similarly unsuccessful. The situation was further complicated by the probable presence of two N-O groups in compound C thus allowing the possibility of two Cope elimination products.

Because of these difficulties an alternative technique was sought in order to obtain useful molecular ion data by mass-spectrometry. The use of Field Desorption MS was briefly considered but the non-availability of suitable instrumentation hindered progress in this direction. Subsequent discussions with V.G. Analytical Ltd., revealed the existence of two new techniques which, at that time, were not generally available - DCI and FAB. DCI is

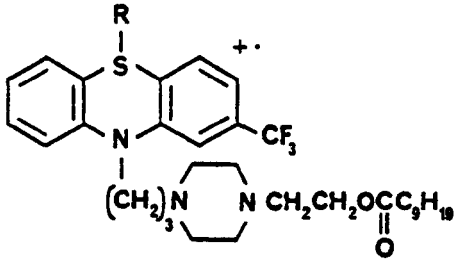
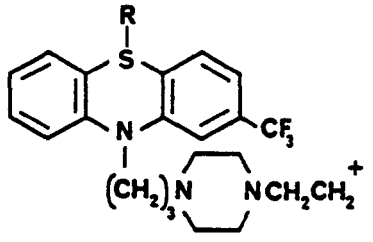
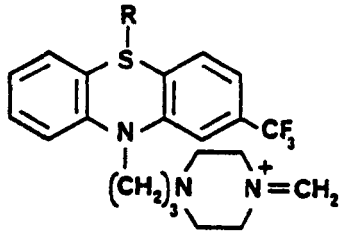
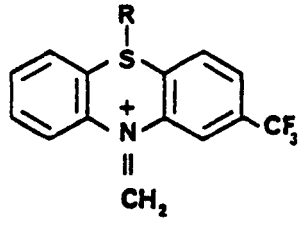
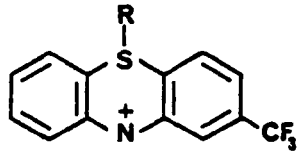
	Fluphenazine decanoate (R = -)	Fluphenazine decanoate sulphoxide (R = O)	Fluphenazine decanoate sulphone ($R = O_2$)
	m/z 591	m/z 607	m/z 623
	m/z 419	m/z 435	m/z 451
	m/z 405	m/z 421	m/z 437
	m/z 280	m/z 296	m/z 312 (V.weak)
	m/z 266	m/z 282 (V.weak)	m/z 298

Fig. 4.1 - Intact Phenothiazine-ring Fragments

similar to the technique of Field Desorption, but the MS is operated in CI mode instead of the EI mode usually associated with Field Desorption. In DCI the sample is deposited on a probe having a heatable platinum wire tip. The probe is inserted into the CI plasma beam and the wire rapidly heated. By this means, the desorption of ions occurs with much reduced fragmentation.

The technique of FAB uses the phenomenon of ion sputtering, employing a beam of fast neutral atoms of high energy, thus avoiding the use of heat to promote ionisation.

The results (Table 4.10 and 4.11) established that DCI successfully produced the required molecular ion (or pseudo molecular ion) albeit in small quantities (approx. 10% intensity). The advantages of the mild experimental conditions used with FAB, particularly when applied to the heat labile N-oxides, is also clearly demonstrated by the results.

The information obtained by mass spectrometry confirmed the expected molecular weight of the isolated oxidation products and provided further evidence for the structures postulated in Section 4.2.

4.9 Hydrated Nature of Oxides

4.9.1 Introduction

The samples of the oxide hydrochloride salts which were converted to the corresponding bases for NMR and MS examination were also subjected to elemental analysis (Section 4.6). However the CHN values obtained did not correspond to the expected values. In addition, a strong absorption band in the 3500 cm^{-1} region of the IR spectrum of the ester compounds could not be explained, except as water contamination. The hygroscopic nature of N-oxides is a commonly reported feature of these compounds[190,210], thus the determination of the water content of the samples pertinent to this study was undertaken.

4.9.2 Experimental - A Gas Chromatographic Method for Moisture Determination

The procedure is based upon a method in current use within the Squibb Institute for Medical Research[208].

4.9.2.1 GC Conditions

Column	: glass, 5ft x ¼ in.
Packing	: Poropak Q, 100-120 mesh
Oven	: 120°C
Injector heater	: 194°C
Carrier gas	: Helium, 40ml/min
Detector	: Katharometer
	Bridge current, 230mA
	Attenuation, X1

4.9.2.2 Sample Preparation

3mg of the compound under examination were accurately weighed into a 1ml reactivial (Pierce Chemicals Ltd.) and dissolved in 100ul methanol.

4.9.2.3 Standard Preparation

25ul of water were diluted to 5ml with methanol in a volumetric flask.

4.9.2.4 Analytical Procedure

5ul aliquots of each of a solvent blank, the sample and the standard were injected in duplicate onto the GC and the resultant peak-height of the peak representing water was determined. A typical chromatogram is illustrated in Fig. 4.2.

4.9.2.5 Linearity

Linearity of response was determined by preparing a series of standard solutions containing known amounts of water between 1 and 5mg/ml (the proposed range of the procedure). A linear response for

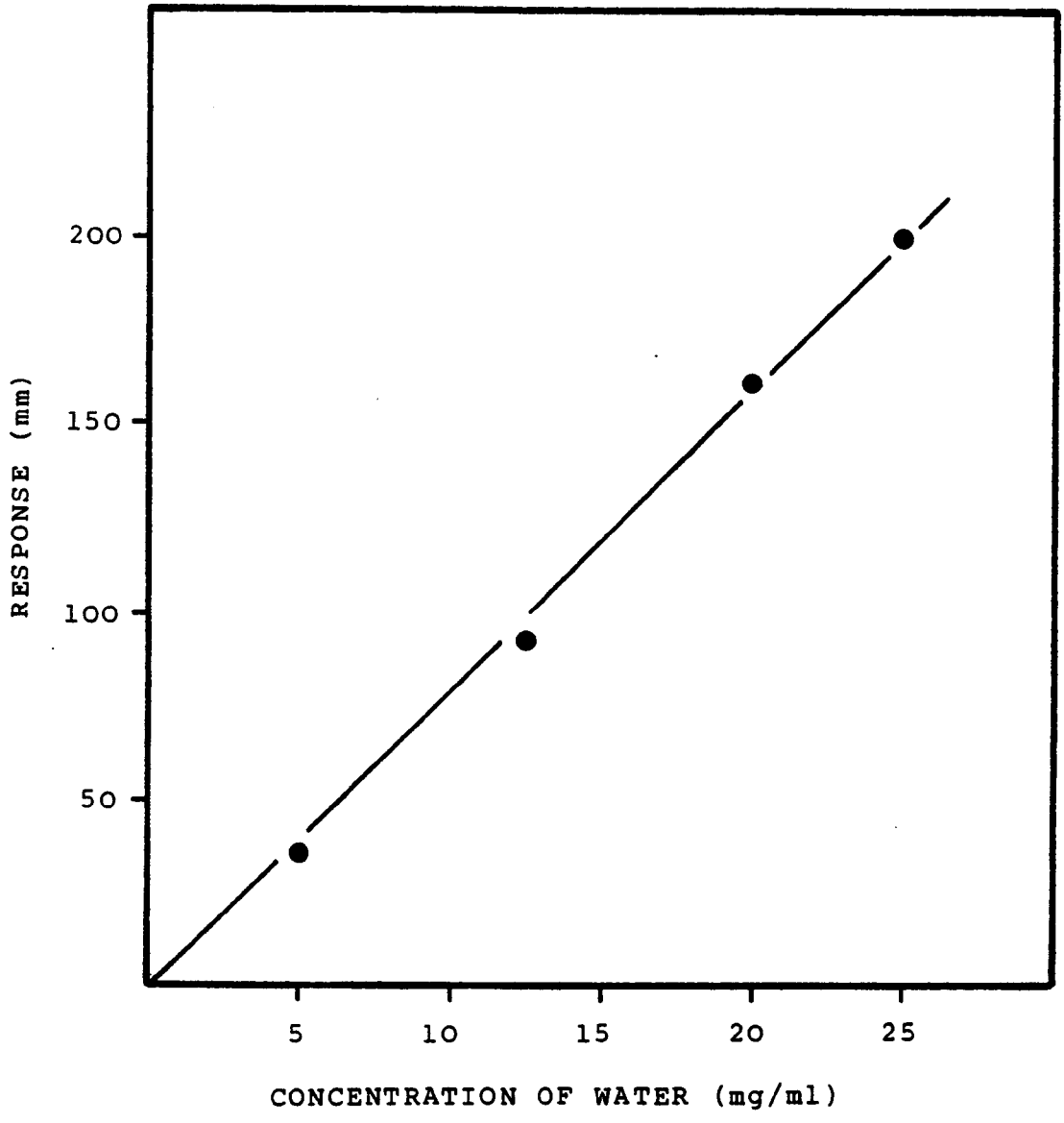


Fig. 4.2 - Linearity of Response, GC Determination of Water

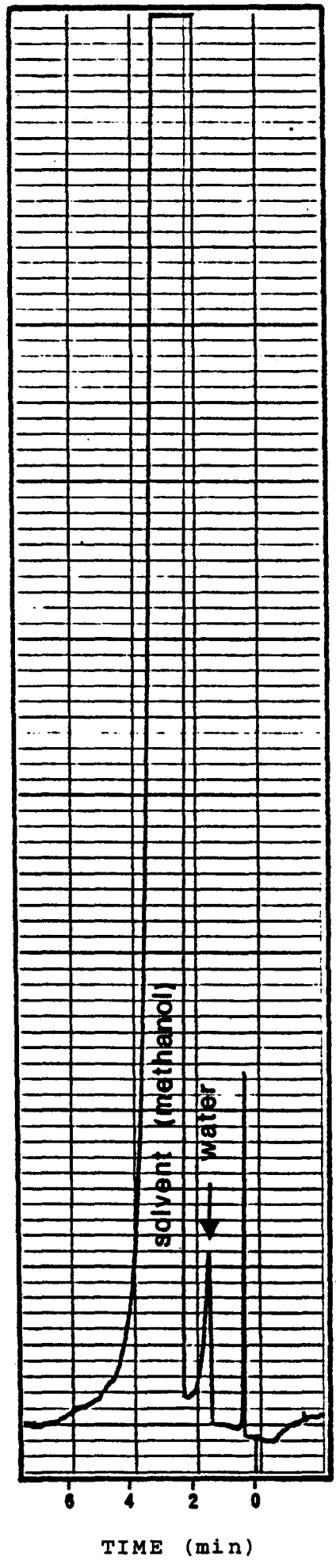


Fig. 4.3 - GC Determination of Water, Typical Chromatogram

peak-height versus concentration was obtained (Fig. 4.3). The scatter of the points about the average straight line may be attributed to the interference of atmospheric moisture with which the GC syringe is readily contaminated.

4.9.3 Results

Samples of the isolated compounds (bases and hydrochloride salts) were examined before and after treatment by various drying techniques. The results are tabulated overleaf:-

Table 4.12

Water Content of N- and S- Oxides of Fluphenazine and Fluphenazine Decanoate

a) Ester Oxide Bases (prepared from hydrochloride salts for NMR and MS examination).

Compound	Treatment	H_2O Content(%)
C	'as is' from isolation procedure (Section 4.5)	3.7
D		3.5
E		6
D	Freeze-dried (2nd treatment)	4.5
D	Solution dried over $MgSO_4$	2.8
D	Dimethoxypropane reaction	1.7

b) Original Hydrochloride Salts (Ester oxides)

Compound	Treatment	H_2O Content(%)
C	'as is'	1.8
C	Freeze dried 16 hrs @ RT	1.5
C	Dried under vacuum, 16 hrs @ 55^0C	1.0
D	'as is'	3.0
D	Freeze dried	1.9

c) Fluphenazine Oxide Hydrochloride Salts

Compound	Treatment	H_2O Content(%)
C'	as isolated	4.5
D'	as isolated	7.1
Z'	as isolated	5.5

4.9.4 Discussion of Results

The results clearly verify the expected presence of water in all the compounds examined. Furthermore, the difficulty experienced when attempting to dehydrate the samples is also adequately demonstrated.

In the majority of cases, the moisture content does not represent a stoichiometric value. However, one exception may be noted. Compound D (in methanol solution) dried over magnesium sulphate appears to form the monohydrate (2.7% theoretical water content).

Reaction with dimethoxypropane, a reagent which has been reported to quantitatively react with water[209,227], does not completely eliminate moisture from compound D in dichloromethane solution. Instead, the moisture content is reduced to a value approximating to the hemihydrate (1.4% theoretical water content).

4.10 Hydrolysis Rates of the Piperazino-Mono-N-oxides

4.10.1 Introduction

The mono N-oxides of fluphenazine decanoate (ie. compounds A and B) had been noted to hydrolyse to the corresponding oxides of fluphenazine at differing rates during earlier investigations with HPLC. Rate constants were thus determined for the hydrolysis of compounds A and B and compared with the hydrolysis rate of the parent ester (fluphenazine decanoate), determined under identical conditions. Hydrolysis was performed under alkaline conditions in order to avoid the alternative reactions that can occur with phenothiazines in acid solution; eg. formation and subsequent decomposition of the cation radical.

4.10.2 Experimental

A solution containing 0.5mg/ml of the compound to be hydrolysed was prepared in methanol. 4.0ml (pipette) were transferred to a 5ml volumetric flask and the solution diluted to volume with water. A similar dilution was

prepared using 0.5M aqueous ammonium hydroxide instead of water. Aliquots of the second (alkaline) solution were withdrawn at suitable intervals and injected directly into the HPLC (SAS-Hypersil column; mobile phase, methanol/acetonitrile/1% aqueous ammonium carbonate, 1-1-1; flow rate, 1.5ml/min; detector, 260nm). The unchanged ester component of the solution was quantitated by integration of the peak area. (Spectra Physics, Minigrator).

4.10.3 Results

Tables 4.13 to 4.15 list the results obtained.

Table 4.13

Alkaline Hydrolysis of Compound A

Time (min.)	Compound A remaining(%)
Initial	100.0
5	95.6
10	91.2
25	85.9
35	80.3
45	75.7
55	71.7
65	67.9
85	61.1
105	55.1
125	49.2

Table 4.14

Alkaline Hydrolysis of Compound B

Time (min.)	Compound B remaining(%)
Initial	100.0
5	82.9
15	60.7
25	44.4
35	32.0
45	23.3
55	16.8
65	12.8

Table 4.15

Alkaline Hydrolysis of Fluphenazine Decanoate

Time (min.)	Ester remaining(%)
Initial	100.0
10	95.4
30	87.1
50	80.4
70	74.4
90	67.9
111	62.5

In the presence of excess reagent, the hydrolysis reaction obeys 1st order kinetics (pseudo 1st order reaction)[208] and reaction rate is represented by the equation

$$\frac{-d[\text{ester}]}{dt} = k'[\text{ester}]$$

which, following integration becomes

$$\ln[\text{ester}_t] = \ln[\text{ester}_o] - k't$$

or,

$$\ln\frac{[\text{ester}_t]}{[\text{ester}_o]} = -k't$$

The above equation is of the general mathematical form $y = mx$. Thus a graph of $\ln[\text{ester}_t]/[\text{ester}_o]$ versus t will produce a straight line, the slope of which represents the rate-constant k'.

The result of plotting $\ln[\text{ester}_t]/[\text{ester}_o]$ against t for the compounds examined is shown in Fig. 4.4. The rate-constant (ie. the slope) was calculated by linear regression and the results of this exercise are given in Table 4.16.

Table 4.16

Rate-Constants for Hydroysis of Fluphenazine Decanoate and its mono N-oxides

Compound	Rate-constant (min^{-1})	Correlation coefficient
Fluphenazine	0.416×10^{-2}	0.9997
A	0.556×10^{-2}	0.9995
B	3.146×10^{-2}	0.9998

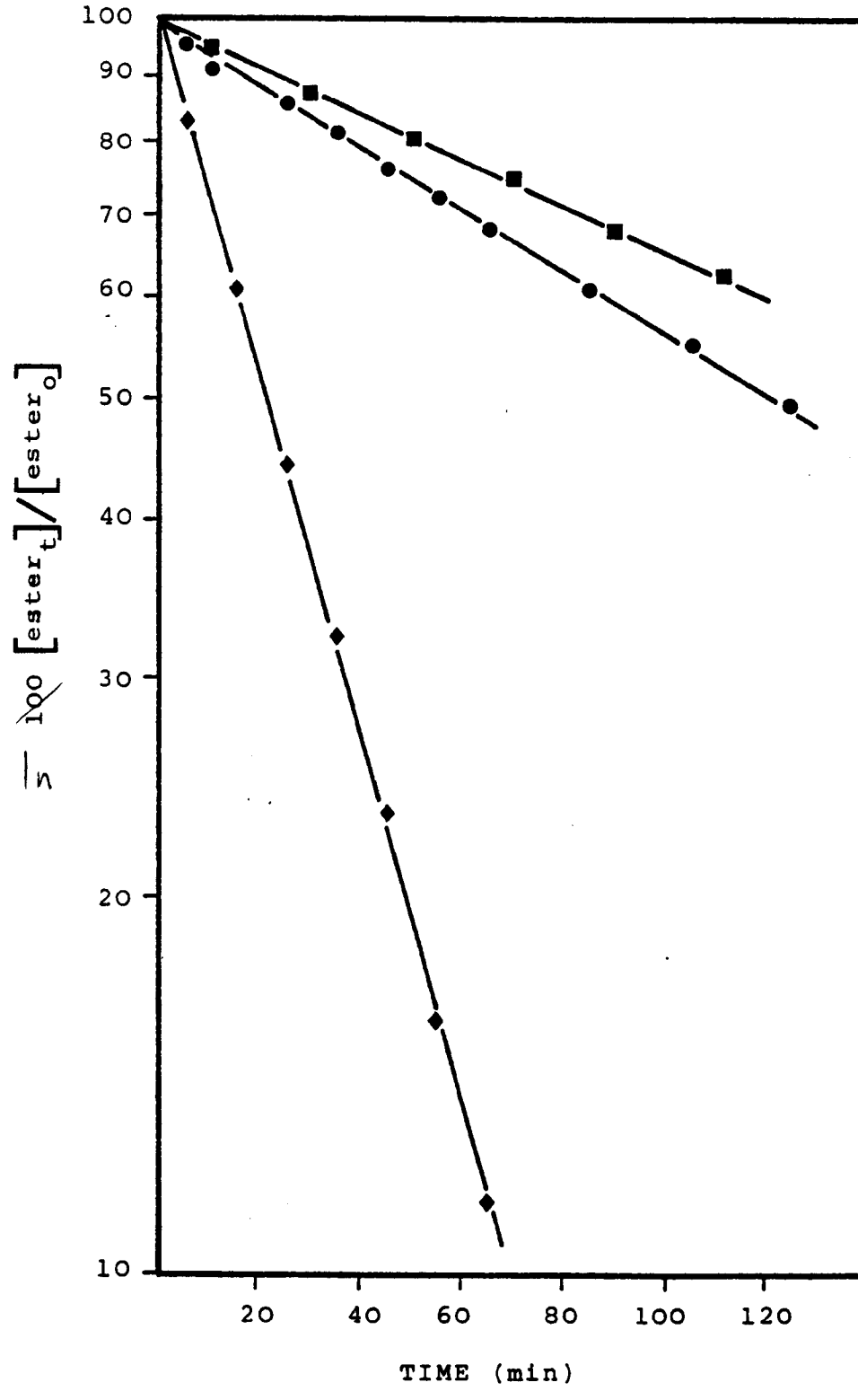


Fig. 4.4 - Rates of Hydrolysis of Fluphenazine Decanoate and Mono N-Oxides

■, fluphenazine decanoate; ●, fluphenazine decanoate mono N-oxide A; ◆, fluphenazine decanoate mono N-oxide B.

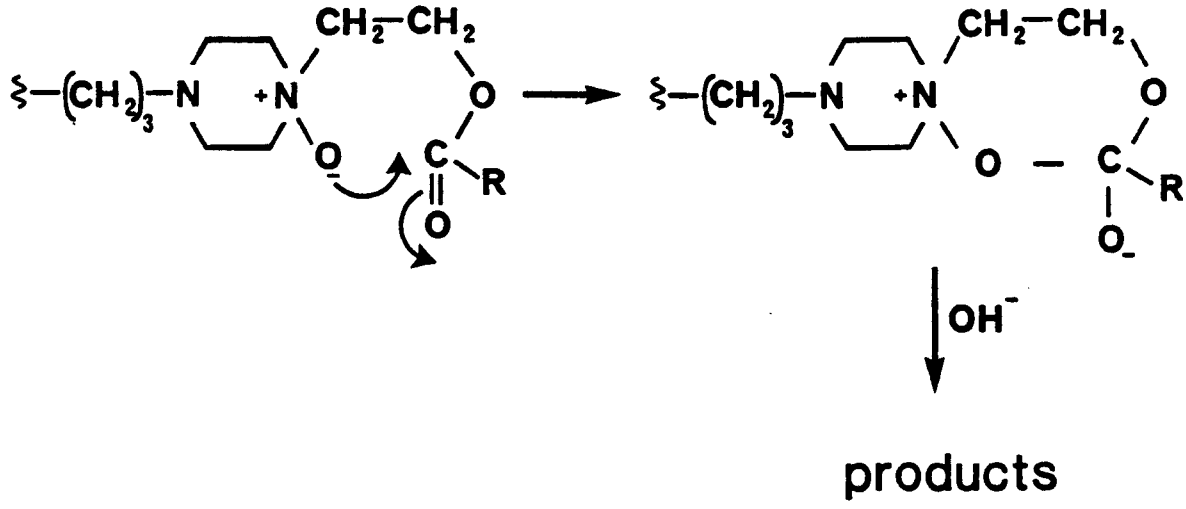
4.10.4 Discussion of Results

From the values of the rate constants given in Table 4.16 it can be seen that the hydrolysis rate of compound A is of the same order as fluphenazine decanoate whereas that of compound B is greater by a factor of five. The difference can be readily explained if compound A is assumed to be the 4-piperazine-ethanol mono N-oxide and compound B the 1-piperazine-ethanol mono N-oxide.

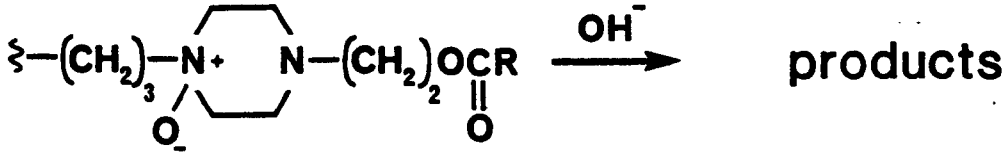
The enhanced rate of hydrolysis apparent for compound B may then be accounted for by an anchiomeric effect involving the ester carbonyl group and the formation of a stable 6-membered transition state (Fig. 4.5). The observed phenomenon provides further evidence to support the structures of compounds A and B proposed on the basis on NMR data.

With compound A, no such transition state is possible and therefore the hydrolysis rate of A would be expected to be similar to that of the parent ester.

(i) Compound B



(ii) Compound A



no cyclic intermediate
possible with this oxide

Fig. 4.5 - Postulated Cyclic Intermediate Formed During Hydrolysis of Ester Mono N-Oxides

CHAPTER 5

OXIDATION OF FLUPHENAZINE DECANOATE BY HYDROPEROXIDES

5.1 Introduction

5.1.1 Background

The antioxidant properties of phenothiazines when added to lubricating oils were reported as early as 1955 and the product of the reaction was established as the corresponding phenothiazine sulphoxide[60]. The mechanism by which the oxidation of the phenothiazine occurred in oily solution was postulated to involve the hydroperoxides formed during autoxidation of the oil[61].

Identification of a phenothiazine N-oxide formed during the accelerated degradation of a sesame oil solution of fluphenazine decanoate resulted in a similar postulation ie. that the unsaturated triglycerides present in sesame oil were autoxidised to hydroperoxides which further reacted with fluphenazine decanoate producing the N-oxidised phenothiazine species[179]. Indeed, experiments with castor-oil, which contains almost 100% fully saturated triglycerides, established that the direct reaction of dissolved oxygen with fluphenazine decanoate was unlikely[180]. The nature of the reaction mechanism is unknown. The reaction may involve radical species from the autoxidation process or, alternatively, may involve the nucleophilic attack of the peroxide species at the t-amine centre as described by Lewis[137]. An investigation of the reaction between fluphenazine decanoate and various hydroperoxides was therefore proposed. As few hydroperoxide species are available in a pure form commercially, it was decided to prepare the required hydroperoxide 'in situ' by autoxidation prior to addition of fluphenazine decanoate. The chemistry involved in the formation of hydroperoxides by

autoxidation is well-documented, thus the nature of the peroxide formed should be predictable with a moderate degree of confidence.

5.1.2 Choice of Materials

Two criteria limited the choice of materials:

1. Material must contain at least one C = C double bond to enable autoxidation to readily proceed.
2. Materials should be structurally related, however remotely, to sesame oil or its constituents.

Sesame oil, the vehicle used in Modecate Injection, consists of triglycerides composed mainly of oleic and linoleic esters (40 and 45% respectively). The chemical entities most closely related to the triglycerides and which are commercially available in a pure form are the simple esters of oleic and linoleic acids. The methyl ester was chosen arbitrarily. Fatty acids may occur as minor constituents of natural oils, thus oleic and linoleic acids were also included in the study.

To further simplify the situation, the ester group may be eliminated from the molecule resulting in simple olefins. These materials too were readily available commercially in a variety of carbon chain-lengths. Olefins less than seven carbon atoms long are extremely volatile, a property that could cause problems during autoxidation. Use of olefins was thus restricted to those molecules having a carbon-chain of between seven and eighteen atoms.

Two readily available hydroperoxides were also included in the study, t-butyl hydroperoxide and hydrogen peroxide.

5.2 Preparation of Autoxidised Media

It is imperative that the level of peroxide present in each of the degrading fluphenazine decanoate solutions be standardised to ensure a valid compari-

son of the rates of oxidation occurring in the various media. The concentration of fluphenazine decanoate in each solution must be identical to that in Modecate Injection ie. 25mg/ml. The level of peroxide required was defined as that required to achieve an equimolar ratio of peroxide: drug. On this basis, the concentration of peroxide was calculated to be 1.4mg of OOH/ml. The chosen ratio of peroxide: drug exhibits two particular advantages:

a) Complete conversion of the drug to the di N-oxide would be prevented thus allowing the ratio of mono N-oxide A: mono N-oxide B formed during the reaction to be determined in solutions of differing polarity.

b) In event of the oxidation reaction obeying 2nd order kinetics, calculation of the reaction rate would be simplified.

5.2.1 Determination of Peroxide Content of Autoxidising Material

A titrimetric procedure based on methods similar to those described in the British and European Pharmacopoeias[217,223] was adopted.

5.2.1.1 Materials and Reagents

Potassium iodide, saturated solution

Glacial acetic acid/chloroform mixture, 3:2

0.01M Sodium thiosulphate, accurately standardised.

1% starch solution.

5.2.1.2 Procedure

Pipette 1ml of the sample under examination into a 250-ml conical flask and disperse in 25ml of glacial acetic acid-chloroform mixture. Add 0.5ml potassium iodide solution and swirl to mix. After exactly 1 min. reaction time, titrate the liberated I_2 with standard sodium thiosulphate solution using starch as indicator.

5.2.1.3 Calculation

1ml 0.01M sodium thiosulphate ≡ 0.165mg hydroperoxide (OOH).

5.2.2 Autoxidation Procedure

Two procedures were designed for autoxidation of the chosen media:

a) Volatile liquids (short-chain olefins)

30ml of the sample were placed in a clear glass screw-top jar and the solution and headspace flushed with oxygen. Jars were stored in a south-facing window and autoxidation allowed to proceed. Samples were purged with oxygen at weekly intervals and the concentration of peroxide monitored by iodine titration.

b) Non-volatile liquids

30ml of sample were poured into a large test-tube in a thermostated oil bath (80°C) and air bubbled through at a rate of 50ml/min. Formation of peroxide was monitored by iodine titration.

In each case autoxidation was allowed to proceed until the peroxide content of the solution was in excess of 1.4mg OOH/ml.

5.2.3 Preparation of Standardised Media

Subsequent to autoxidation, each liquid required dilution in order to produce a solution containing the chosen standard value of peroxide (1.4mg OOH/ml). The solutions were prepared in the following manner:-

a) The peroxide content of the original bulk material was determined.

b) The peroxide content of the autoxidised portion of material was determined.

c) The relative proportions of (a) and (b) necessary to produce a medium with the correct peroxide content was calculated.

d) The calculated quantities of (a) and (b) were carefully pipetted into a suitable container and thoroughly mixed.

5.3 H P L C Method for the Determination of N- and S- Oxides in Oily Solution.

Before investigations into the reaction of fluphenazine decanoate with hydroperoxides could begin, an analytical procedure was required for quantitation of the expected mixture of oxides. HPLC seemed a logical choice of technique as suitable systems for the separation of fluphenazine decanoate oxides had been partially developed (Chapter 3) and quantitation could readily be achieved. The technique also offered the additional advantage that individual components of the expected mixture could be determined. By this means the occurrence of any change in the oxidation pattern may be observed.

5.3.1 Development of HPLC System

Both the literature and previous work with hydroperoxides during the synthesis of fluphenazine decanoate oxide reference compounds indicated that formation of sulphone was improbable at 20^{o}C, the proposed temperature at which oxidation would be performed. An HPLC system was thus sought that would provide a separation of the N-oxides, sulphoxide and mixed N-oxide sulphoxide of fluphenazine decanoate (Compounds A, B, C, D, X, Y and Z).

A mobile phase of methanol-acetonitrile-1% aqueous ammonium carbonate (1:1:1) together with a 20cm column of SAS-Hypersil resulted in the separation of six out of the seven oxides (Fig. 5.1). Compounds C and Y, the di N-oxide and sulphoxide N-oxide, chromatographed together in this system. The ratio of methanol: acetonitrile was found to be critical, the most efficient separation being obtained with the two organic components present in a ratio of 1:1.

The complete resolution of all seven compounds was achieved (Fig. 5.2) by altering the mobile phase to methanol-10% aqueous ammonia (2:1). The presence of ammonia was essential in order to prevent tailing.

Subsequent work with the same column used in the above experiments

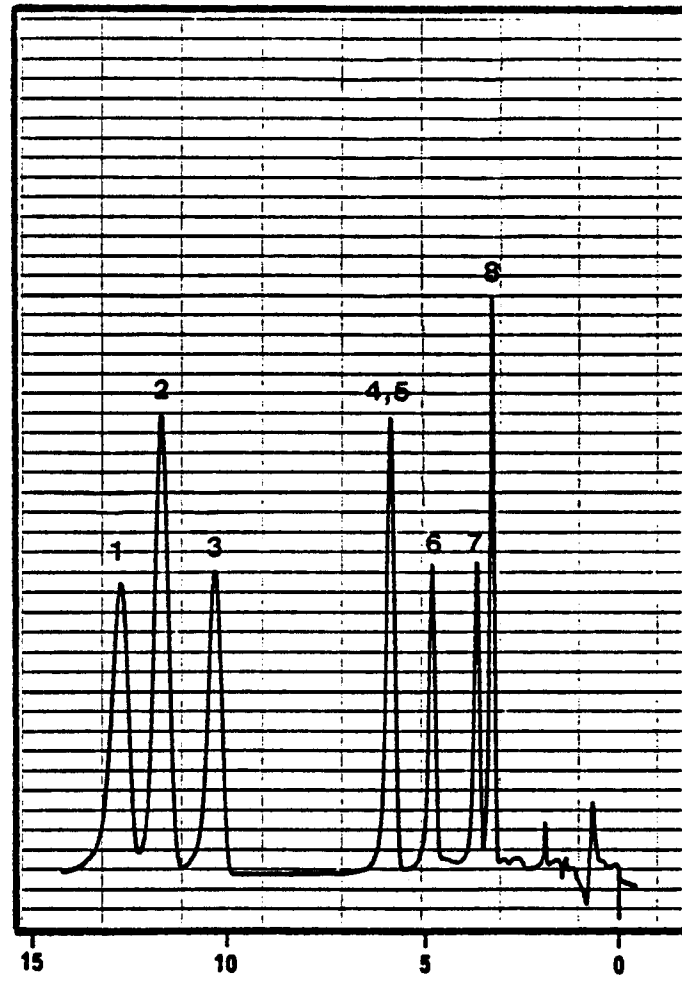


Fig. 5.1 - HPLC Separation of Fluphenazine Decanoate Oxides

Mobile phase, methanol-acetonitrile-1% aqueous ammonium carbonate (1-1-1); column, SAS-Hypersil;
1 = mono N-oxide B; 2 = sulphoxide; 3 = mono N-oxide A; 4 = di N-oxide; 5 = sulphoxide mono N-oxide B; 6 = sulphoxide mono N-oxide A; 7 = sulphone di N-oxide; 8 = sulphoxide di N-oxide.

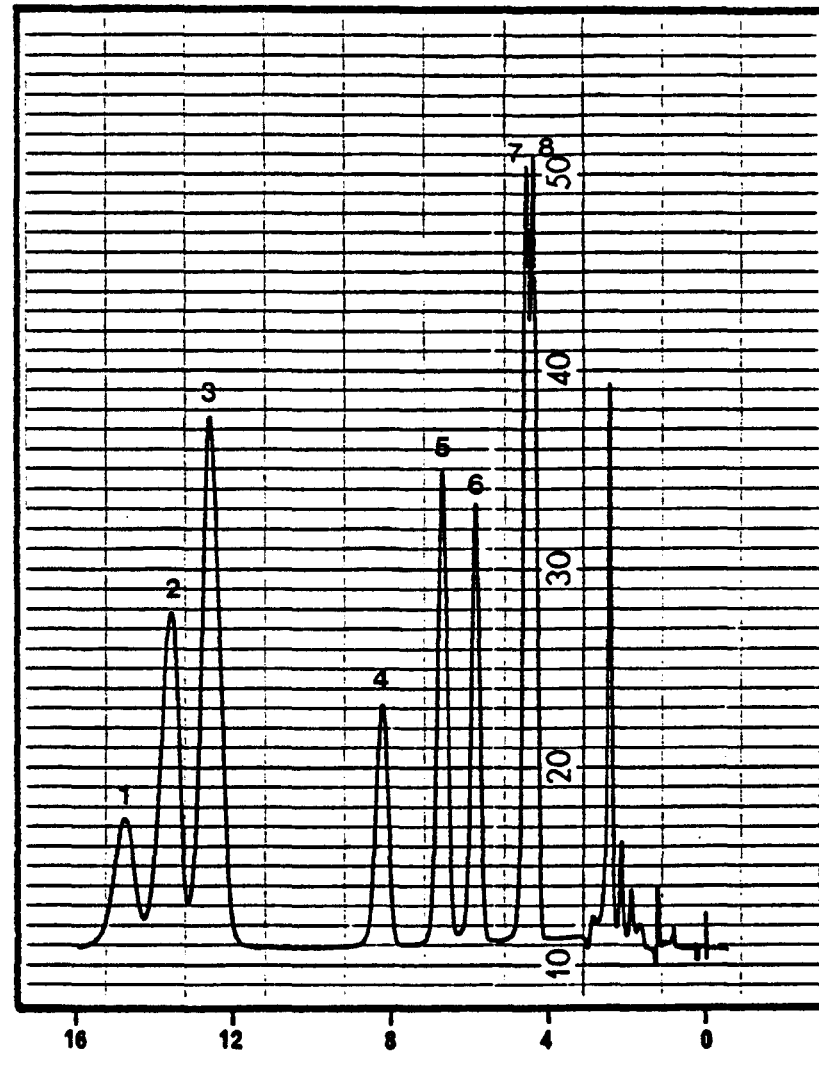


Fig. 5.2 - HPLC Separation of Fluphenazine Decanoate Oxides

Mobile phase, methanol-10% aqueous ammonia (2-1); column, SAS-Hypersil; 1 = mono N-oxide B; 2 = sulphoxide; 3 = mono N-oxide A; 4 = di N-oxide; 5 = sulphoxide mono N-oxide B; 6 = sulphoxide mono N-oxide A; 7 = sulphone di N-oxide; 8 = sulphoxide di N-oxide.

implied that ageing of the column-packing modified the chromatographic characteristics and enabled a separation of compounds C and Y to be achieved with the original mobile phase of methanol-acetonitrile-1% aqueous ammonium carbonate, 1:1:1. Compound Z (sulphoxide) eluted from the aged column between Compounds A and C.

Further investigation demonstrated that on a newly-packed SAS-Hypersil column, compound Z (sulphoxide) is co-eluted with compound B (mono N-oxide). As the column ages the relative position of compound Z changes until it is situated between compounds A and C. Probable reasons for this observed phenomenon are difficult to prove, but it is most likely that the ageing process is a result of the alkaline hydrolysis of the chemically-bonded reversed-phase leaving free silica sites on the surface of the column-packing particles. Under these modified conditions the adsorption characteristics of the sulphoxide (compound Z) and the N-oxides (compounds A and B) are altered, resulting in the changed elution pattern.

Further evidence of the chemical stripping phenomenon is provided by the observation that a newly-packed SAS-Hypersil column can be converted to the modified form by a short treatment with methanolic ammonia.

5.3.2 Effect of Ammonium Carbonate Concentration

Increasing the ammonium carbonate concentration of the mobile-phase aqueous component has been reported to cause a decrease in the retention time of nortriptyline, although the effect was not observed with fluphenazine[235].

The effect of varying the ammonium carbonate content with respect to the oxides of fluphenazine decanoate is illustrated in Fig. 5.3. Small variations in retention-time are apparent for mono N-oxides A and B and the sulphoxide. Each compound, however, was affected to a similar extent thus no overall change in the relative position of elution was observed and hence the ammonium carbonate concentration can be considered as non-critical.

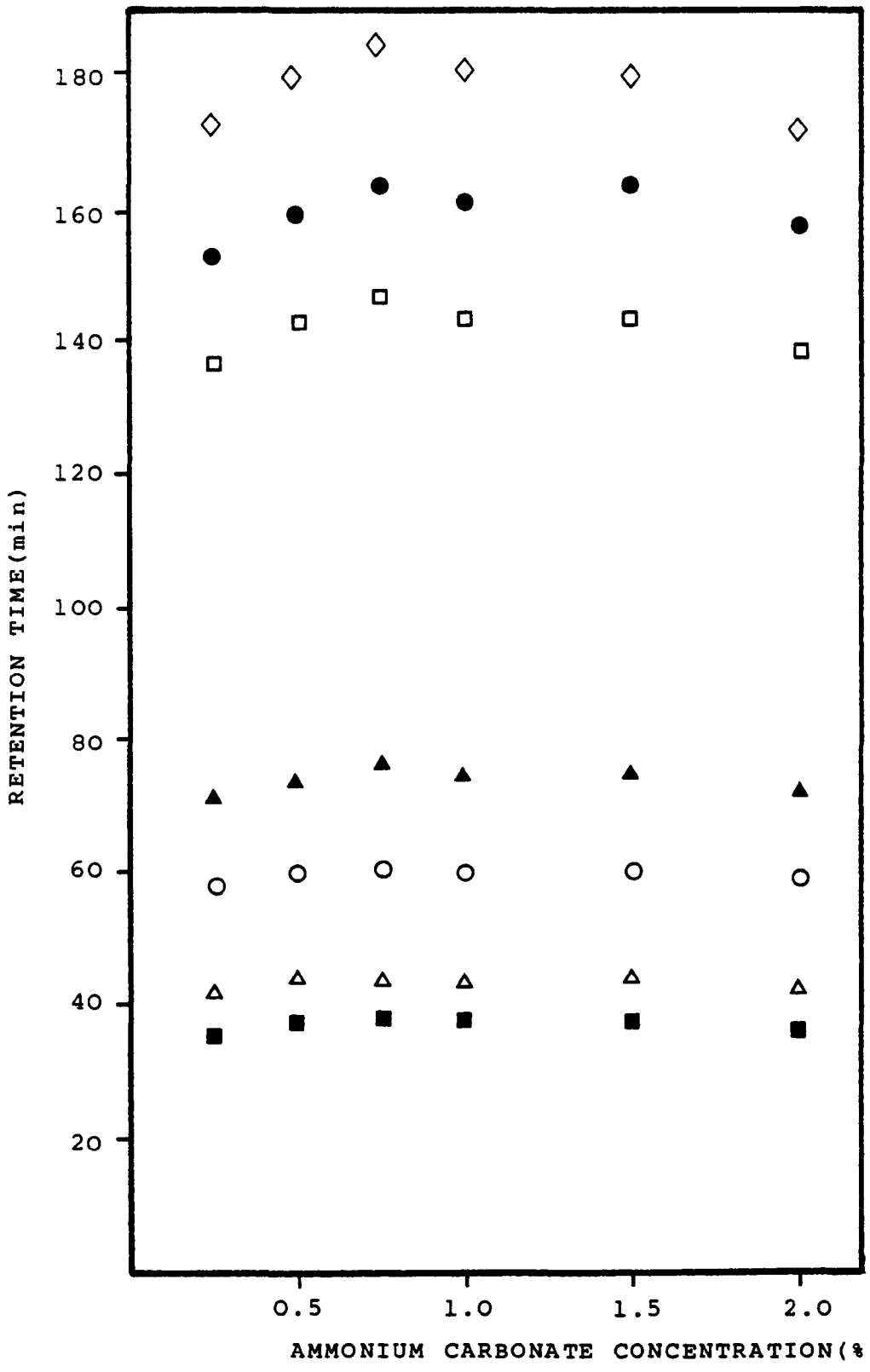


Fig. 5.3 - Effect of Ammonium Carbonate Concentration on HPLC Retention of Fluphenazine Decanoate Oxides

◇ = mono N-oxide B; ● = mono N-oxide A;
□ = sulphoxide; ▲ = di N-oxide;
o = sulphoxide mono N-oxide B;
△ = sulphoxide mono N-oxide A;
■ = sulphoxide di N-oxide.

5.3.3 Effect of Injecting Oil onto HPLC Column

In order to simplify sample preparation it was proposed to dilute the oily sample with a chloroform/methanol mixture and inject the resulting solution directly onto the HPLC column.

The effect of this treatment was to cause a small amount of peak-broadening, although resolution of the oxides was not unduly affected. However, to compensate for this small effect, an equivalent amount of oil was added to the standard solution during quantitative studies.

5.3.4 Choice of Detector Wavelength

N-oxides were detected at the λ max of the compounds (260nm) but attempts to monitor low-levels of the sulphoxide at the λ max of 275nm were thwarted because of interference from a minor impurity (λ max, 260nm) present in the fluphenazine decanoate. The interference was reduced to negligible proportions, with minimal loss of sensitivity, by offsetting the detector wavelength to 280nm.

5.3.5 Validation

5.3.5.1 Linearity of Response

Linearity of response was determined over the range 0 to 5mg oxide per ml of formulation (equivalent to 0-20% degradation of fluphenazine decanoate) at wavelengths of 260nm (N-oxide) and 280nm (sulphoxide) after dilution in the manner proposed for sample preparation (Section 5.2.6.2).

Linear responses were obtained in each case (Figs. 5.4 and 5.5).

5.3.5.2 Reproducibility

An oily solution of fluphenazine decanoate was spiked with each oxide at a level equivalent to 10% degradation and diluted according to the procedure described in Section 5.2.6.

A 5ul aliquot of each diluted solution was injected onto the HPLC column and the oxide content assayed against a standard solution contain-

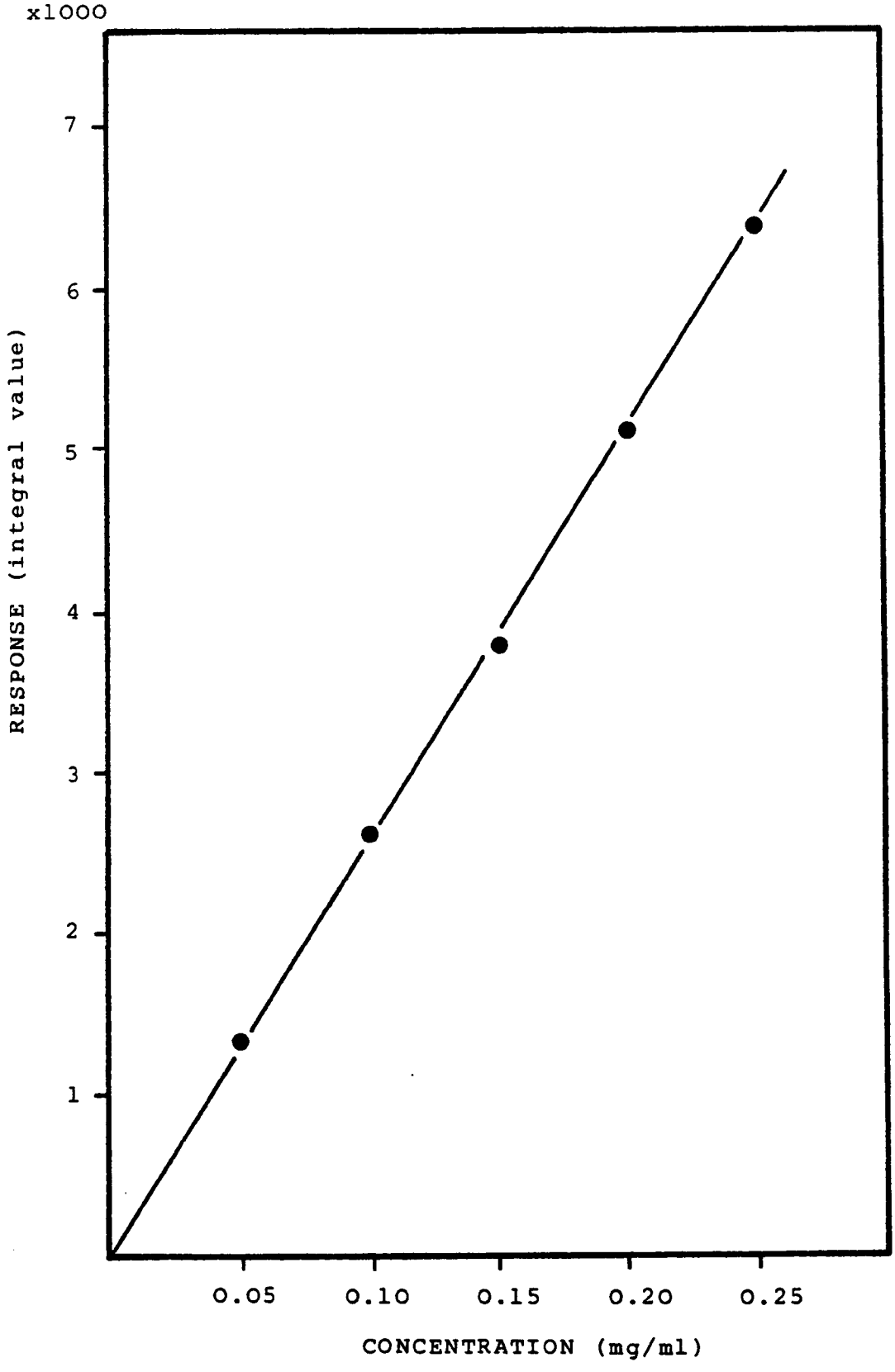


Fig. 5.4 - Linearity of Response at 260nm, N-Oxide HPLC Assay

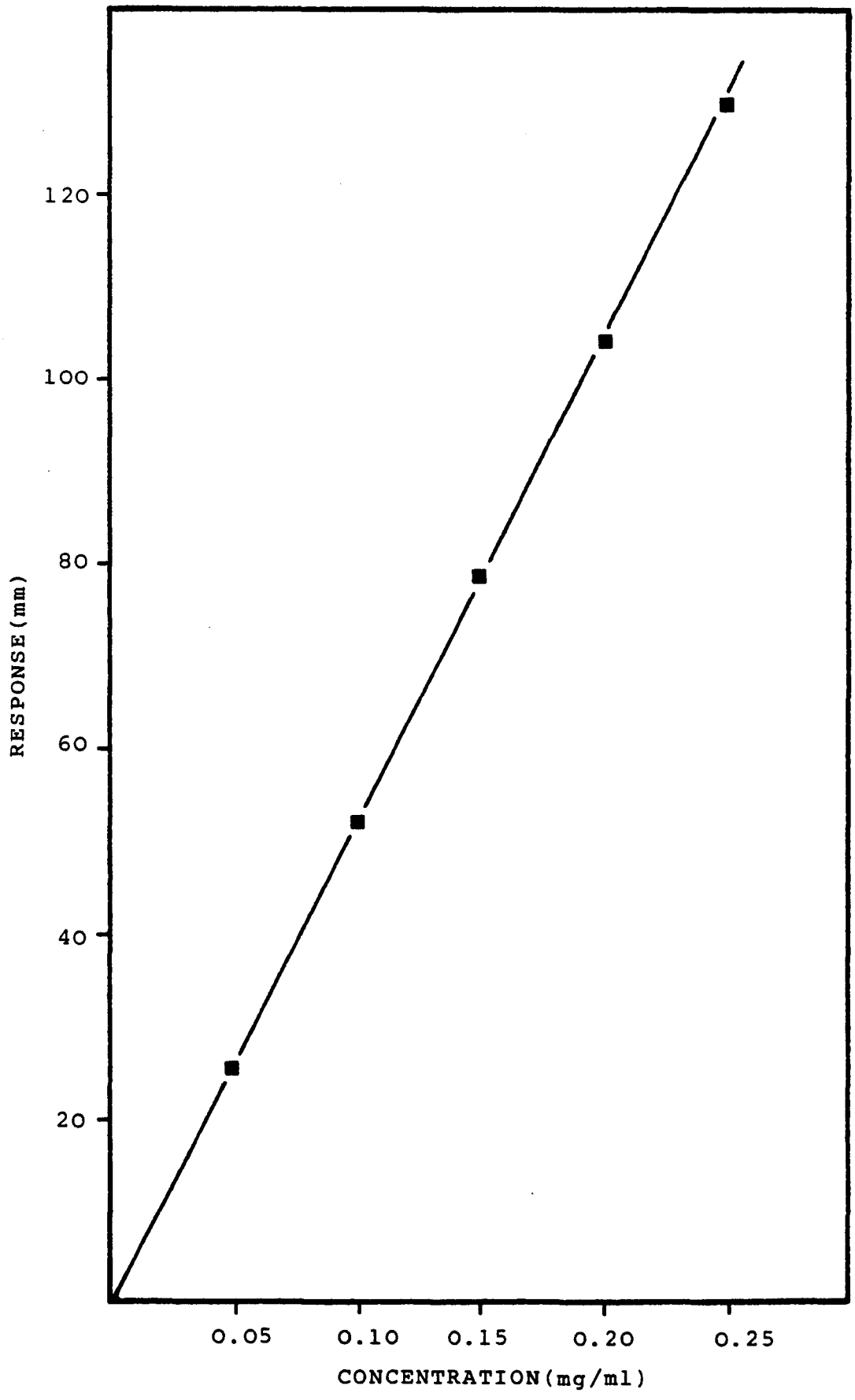


Fig. 5.5 - Linearity of Response at 280nm, Sulphoxide HPLC Assay

ing an equivalent amount of oil.

The results are given in Table 5.1.

Table 5.1

Reproducibility of HPLC Assay for Fluphenazine Decanoate Oxides

	Mono N-oxide, Total A + B (Theoretical 2.50mg/ml)	Sulphoxide (Theoretical 2.50mg/ml)
Assay (% theory)	99.9; 99.1; 100.5 101.3; 102.0; 100.5 100.4; 100.0	100.0; 102.3; 101.7 100.6; 102.3;
Average	100.5	101.4
S.D.	±0.88	±1.04

5.3.6 Analytical Procedure for Determination of Fluphenazine Decanoate Oxides in Oily Solution

5.3.6.1 Preparation of Standards

Accurately weigh 1mg of each oxide (Cahn Micro balance) into separate 10-ml volumetric flasks. Dissolve in and dilute to volume with a 50-50 mixture of methanol/chloroform. Further dilute the solution to the required level (ie. the approximate level of oxide expected in the degrading oily solution). Typical dilutions are given below:

Compound	Dilution
Mono N-O	x 2
di N-O	x 10
S = O	x 5
S = O N-O	x 5
S = O N-O	x 5

5.3.6.2 Preparation of Sample

Dilute 1ml of oily fluphenazine decanoate solution to 25ml with a 50-50 mixture of methanol/chloroform.

5.3.6.3 HPLC Procedure

Column	:	SAS-Hypersil, 20cm
Mobile phase	:	Methanol-acetonitrile-1% aqueous ammonium carbonate, 1-1-1.
Detector wavelength	:	N-oxide, 260nm; sulphoxide, 280nm
Flow	:	1-2ml/min, as required.
Injection volume	:	5ul(syringe)
Quantitation	:	Mono N-oxides) Integration of di N-O) peak area

S = O) Peak height
S = O mono N-oxides) measurement
S = O di N-Oxides)

5.3.6.4 Calculation

$$\text{Oxide, mg/ml of formulation} = \frac{\text{Sample response}}{\text{Std. response}} \times \frac{\text{Std.wt}}{\text{Std.diln.}} \times \frac{25}{1}$$

5.4 Reaction of Fluphenazine Decanoate with t-Butyl Hydroperoxide

5.4.1 Preparation of Samples

t-Butyl hydroperoxide was purchased from Aldrich Chemicals Ltd., as an aqueous solution (70% t-BuOOH). As this mixture is not miscible with organic hydrocarbons, the aqueous solution was partitioned with heptane and the organic layer separated by centrifugation. The hydroperoxide content of the heptane fraction was assayed by iodine titration then the solution diluted with heptane to produce the required standard value of hydroperoxide (1.4mg/ml). A saturated hydrocarbon was used as solvent to avoid alternative reactions that could possibly occur if an unsaturated hydrocarbon was employed. Eg. oxidation of the C = C double bond.

A solution containing 25mg/ml fluphenazine decanoate was subsequently prepared with the t-BuOOH/heptane solution as solvent. It was necessary to gently warm the mixture ($35^{o}C$) to aid dissolution of the fluphenazine decanoate (MP = 32^{o}). A similar solution omitting the peroxide was prepared as a control. The mixtures were then stored in the dark in an environment thermostated at $20^{o}C$. Storage under conditions of darkness was considered essential so that photoxidation reactions would be prevented. These conditions also simulate the normal manner of storage of Modecate Injection.

Formation of fluphenazine decanoate oxides was monitored by assaying the oxidising solultions at suitable intervals using the HPLC procedure described in Section 5.3

5.4.2 Results and Discussion

The results obtained are summarised in Table 5.2.

Table 5.2

Oxidation of Fluphenazine Decanoate with t-Butyl Hydroperoxide (Heptane Solution)

Time of storage (weeks)	Oxide Content, um/ml				
	t BuOOH Solution		Control		
	Mono N-oxide (Total)	Di N-oxide	Sulphoxide	Mono N-oxide (Total)	Sulphoxide
1	0.731	Not detected	0.058	Not detected	Not detected
2	2.303	0.353	0.120	0.027	
3	2.893	0.517	0.171	0.035	
6	4.054	1.493	0.188	0.039	Not detected

The most notable feature of the above results is the preferential oxidation of the piperazine t-amine centres. Oxidation of the phenothiazine ring S atom is almost negligible by comparison.

5.5 Reaction of Fluphenazine Decanoate with Olefinic Hydroperoxides

5.5.1 Preparation of Samples

Solutions of fluphenazine decanoate at a strength of 25mg/ml were prepared with each of the standardised olefinic hydroperoxide solutions resulting from the procedures described in Section 5.2. The solutions were gently heated (35^{o}C) to dissolve the fluphenazine decanoate, then immediately cooled to RT and stored under conditions of darkness in a thermostated environment at 20^{o}C. Samples were assayed for oxide content at regular intervals.

5.5.2 Results

The results obtained are given in Tables 5.3 to 5.8.

Table 5.3

Oxidation of Fluphenazine Decanoate with Autoxidised 1-Heptene

Time (Hours)	Oxide Content (um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
5	0.381	Not detected	Not detected
24	1.199	0.057	0.067
46	1.916	0.152	0.100
101	2.773	0.335	0.154
168	3.880	0.703	0.156
288	4.512	1.246	0.239

Table 5.4

Oxidation of Fluphenazine Decanoate with Autoxidised 2-Heptene

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
72	1.466	0.113	0.048
144	2.204	0.266	0.079
240	3.274	0.520	0.163
312	3.582	0.704	0.134

Table 5.5

Oxidation of Fluphenazine Decanoate with Autoxidised 1-Octene

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
24	1.331	0.088	0.107
48	2.339	0.189	0.150
120	3.689	0.579	0.208
192	4.526	0.852	0.232

Table 5.6

Oxidation of Fluphenazine Decanoate with Autoxidised 1-Decene

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
24	2.549	0.190	0.114
48	3.872	0.422	0.170
120	5.445	0.960	0.239
192	6.361	1.464	0.254

Table 5.7

Oxidation of Fluphenazine Decanoate with Autoxidised 1-Tetradecene

Time (Hours)	Oxide Content(um/ml) Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
24	1.646	0.092	0.107
48	2.545	0.267	0.150
120	4.013	0.717	0.186
196	4.703	1.006	0.226

Table 5.8

Oxidation of Fluphenazine Decanoate with Autoxidised 1-Octadecene

Time (Hours)	Oxide Content(um/ml) Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
24	2.305	0.218	0.134
48	3.860	0.483	0.199
120	5.025	1.320	0.262
144	5.387	1.405	0.275
192	5.710	1.785	0.290

5.5.3 Application of Kinetics

The degradation of fluphenazine decanoate calculated from the oxide assay results obeys no particular order of kinetics, the rate of degradation steadily decreasing with time. This effect is probably a result of the chemical nature of the drug. Because of the four potential oxidation sites within the molecule, hydroperoxides may further attack one of the oxidation products, effectively reducing the amount of peroxide available for reaction with the remaining drug ie. two parallel reactions occur viz. oxidation of fluphenazine decanoate and oxidation of the degradation (oxidation) product. A decrease in the degradation rate of fluphenazine decanoate is thus observed as oxidation proceeds. Although this phenomenon cannot be prevented from occurring in practice, a mathematical correction can be made when calculating values for use with kinetic-equations. From the total amount of oxygen consumed (moles) during formation of the oxides, a theoretical value may be calculated for the concentration of fluphenazine decanoate that would remain had the drug contained only one oxidation site ie. the theoretical concentration of drug which would be present in solution if all available peroxide had reacted with the drug alone. Although this treatment is not valid for stability-prediction, it will allow a comparison of varying reaction parameters on a quantitative basis.

The integrated forms of the kinetic equations are as follows:-

zero order	$kt = C_o - C$
1st order	$kt = \ln \frac{C_o}{C}$
2nd order	$kt = \frac{1}{a-b} \ln \frac{b(a-x)}{a(b-x)}$

where C_o = Initial concentration of reactant

C = Concentration of reactant at time t

a = Initial concentration of reactant a

b = Initial concentration of reactant b

x = Concentration of product at time t

For the special case of both reactants being of equal intial concentration ($a = b = C_o$) the 2nd order equation simplifies to

$$kt = \frac{1}{C} - \frac{1}{C_o}$$

A linear graph may therefore be obtained by plotting C (zero order), ln C (1st order) or 1/C (2nd order) against time.

Treatment of the results in this manner failed to reveal an obeyance of any one particular reaction order. Closest fit of the data was observed for 2nd order kinetics.

5.5.4 Discussion

In all cases the results tabulated above show a similar selectivity to that exhibited by t-butyl hydroperoxide and thus oxidation of the piperazino t-amine groups is observed in preference to oxidation of the phenothiazine ring heteroatoms. Although absolute reaction-rates could not be calculated from the results obtained, a plot of the reciprocal of the calculated theoretical drug concentration remaining versus time indicates an apparent relationship between the peroxide carbon-chain length and reaction-rate (Fig. 5.6). The autoxidised 1-decene (C-10) was a notable exception, the rate of oxidation of fluphenazine decanoate by this material being greater than that obtained with autoxidised 1-octadecene (C-18).

Formation of secondary products from hydroperoxides is an established reaction which has been documented with particular regard to the autoxidation of unsaturated fatty acids and esters[177]. Under conditions of elevated temperatures the hydroperoxides of fatty esters are known to decompose

producing saturated and unsaturated carbonyl compounds, and organic acids, which are believed to be responsible for the characteristic odour of rancid fats.

If the autoxidised olefines used in the current experiments were to decompose to different extents during autoxidation, then varying levels of secondary reaction products would be formed which could possibly influence the reaction rate of the peroxide with fluphenazine decanoate. A viable explanation of observed differences (Fig. 5.6) could then be offered.

Accordingly the olefins were examined by IR spectrophotometry and the materials before and after autoxidation compared. A scan of the full IR spectrum (625-4000 cm^{-1}) of a thin smear of each sample between NaCl plates revealed no obvious differences (Figs. 5.7 and 5.8). However, the IR spectra of a 0.1mm layer (liquid-cell) provided useful information, particularly in the 1700 cm^{-1} region (carbonyl stretching frequency). The spectra are reproduced in Figs. 5.9 and 5.10. In each case, the appearance of an absorption band at about 1700 cm^{-1} was observed in all materials following autoxidation which is attributable to the carbonyl compounds resulting from the secondary reaction of the hydroperoxides formed during autoxidation. Autoxidised 1-decene contained the greatest amount of this impurity thus proffering a plausible explanation for the curiously enhanced rate of oxidation of fluphenazine decanoate by this material.

The possibility of other trace-impurities (eg. from manufacture of the olefins) influencing the rate of oxidation could not, however, be ignored. Consequently an alternative batch of 1-decene was acquired (Sigma Chemicals) and autoxidised simultaneously with a second portion of the original batch. Solutions of fluphenazine decanoate were then prepared in accordance with the procedure described under 'Preparation of Samples', Section 5.5.1. The results of this experiment are given in Table 5.9. A graphical representation of the results (Fig. 5.11) clearly established that the

Table 5.9

Oxidation of Fluphenazine Decanoate - Comparison of Two Lots of Autoxidised 1-Decene (OOH content, 1.4 mg/ml)

Time (hours)	Original Batch			New Batch		
	Piperazino Mono N-oxide (Total) um/ml	Piperazino di N-oxide um/ml	Sulphoxide um/ml	Piperazino Mono N-oxides (Total) um/ml	Piperazino di N-oxide um/ml	Sulphoxide um/ml
24	2.769	0.259	0.179	1.451	0.130	0.127
48	4.521	0.566	0.200	2.531	0.165	0.220
120	7.290	1.549	0.343	4.498	0.625	0.320

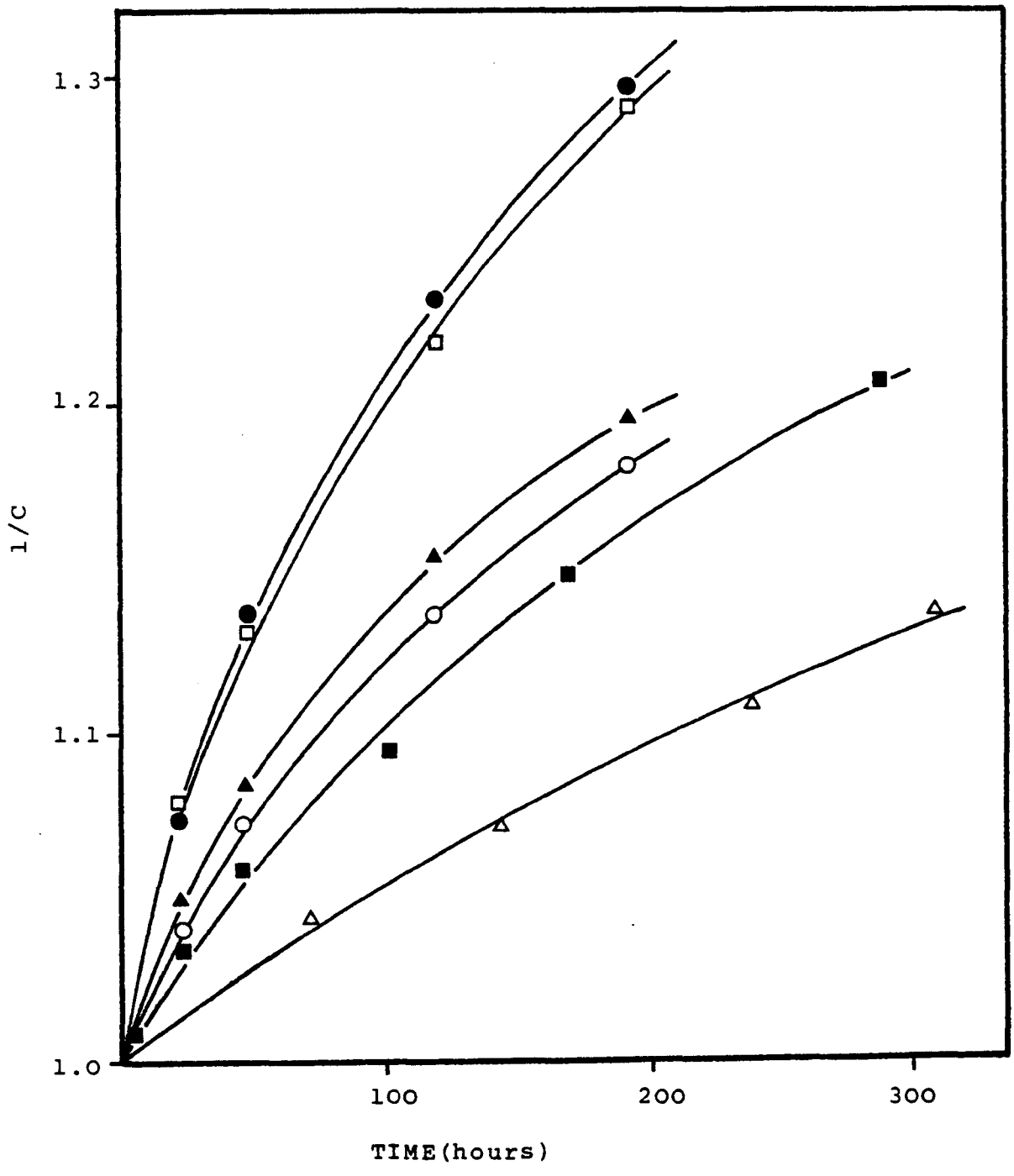


Fig. 5.6 - Oxidation of Fluphenazine Decanoate by Autoxidised Olefines (2nd order kinetics)

△ = 2-heptene; ■ = 1-heptene; o = 1-octene;

▲ = 1-tetradecene; □ = 1-octadecene;

● = 1-decene.

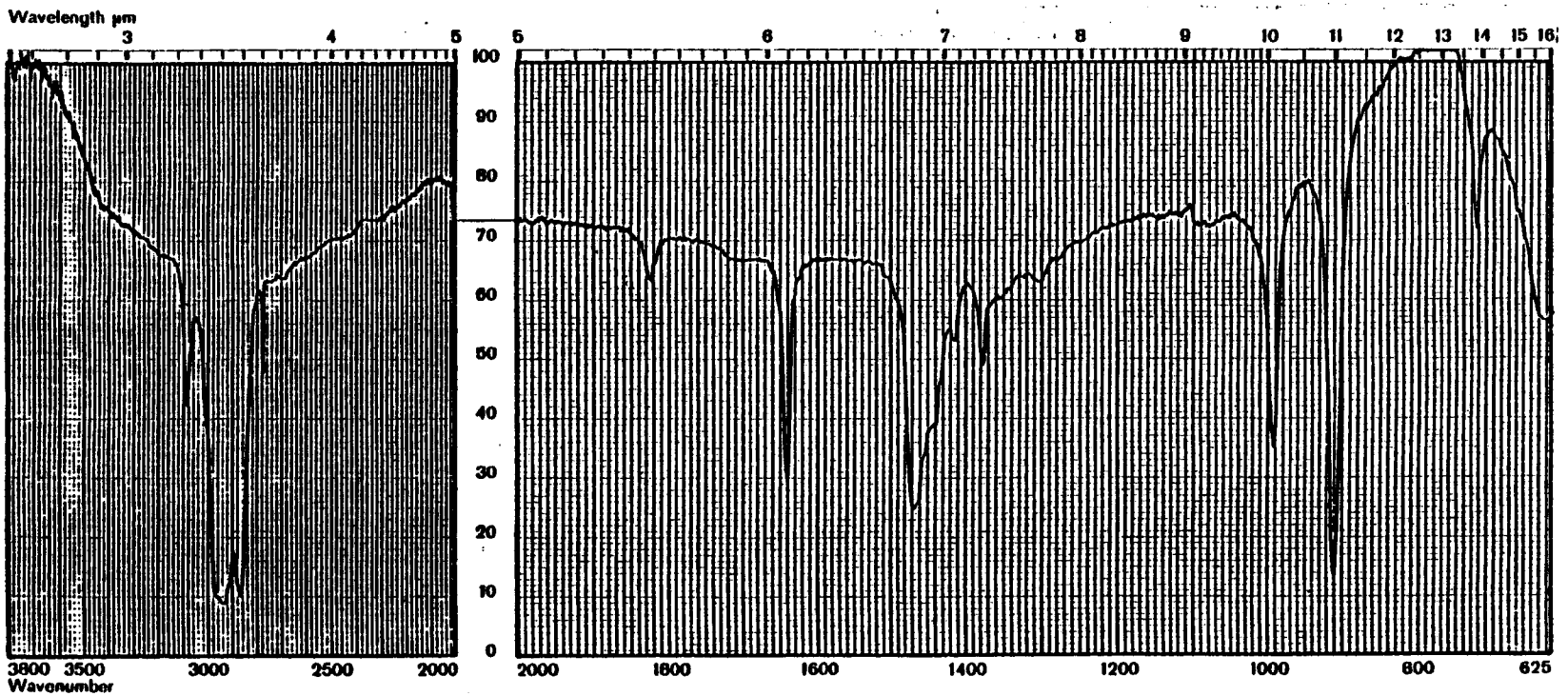


Fig. 5.7 - IR Spectrum of 1-Decene

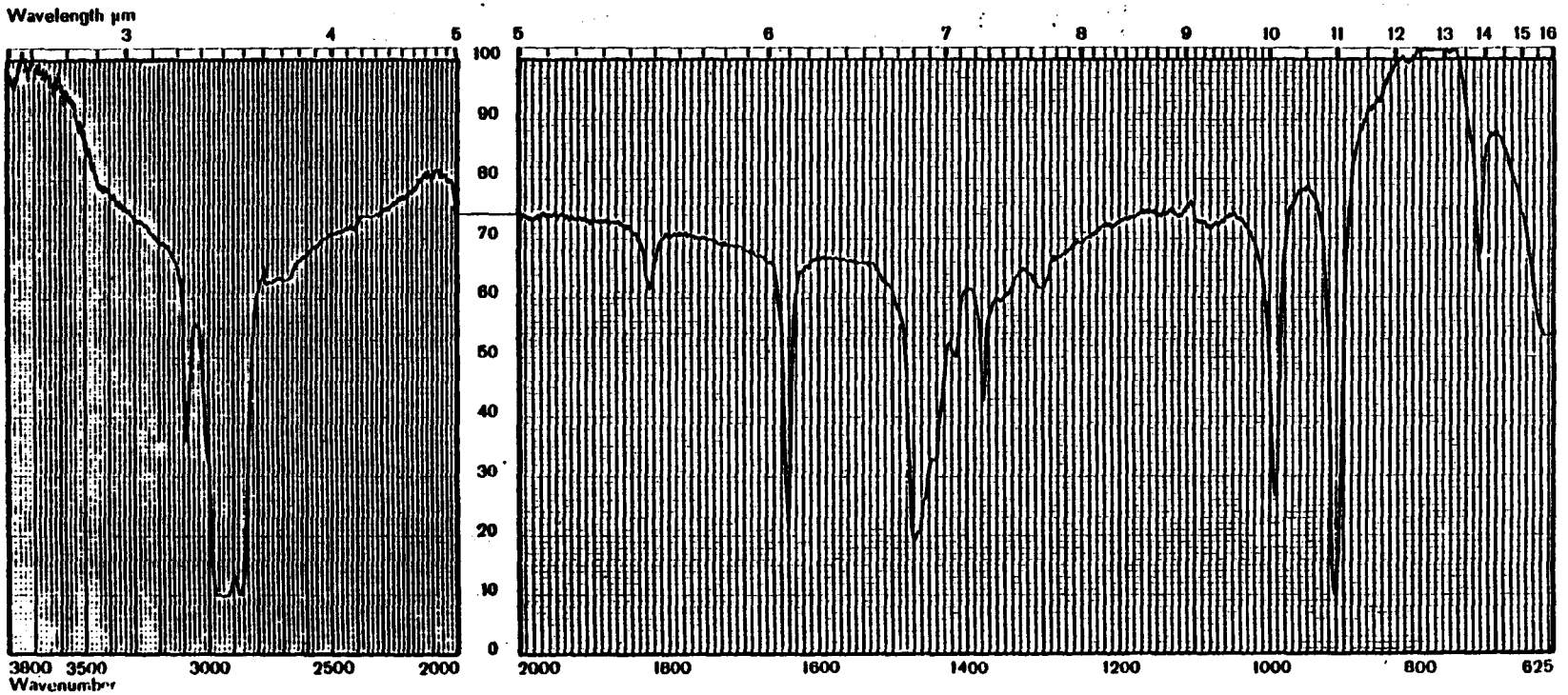
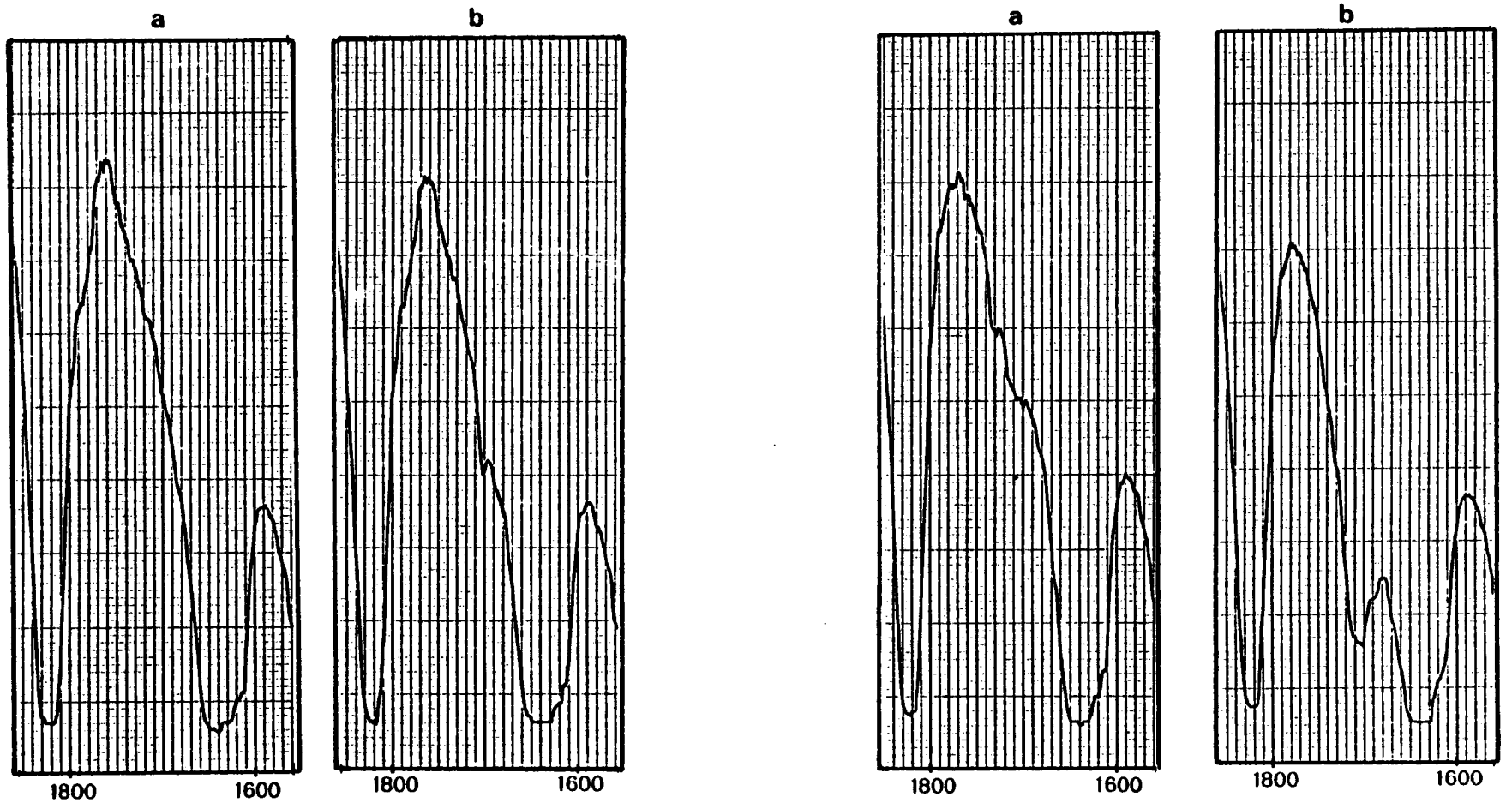


Fig. 5.8 - IR Spectrum of Autoxidised 1-Decene

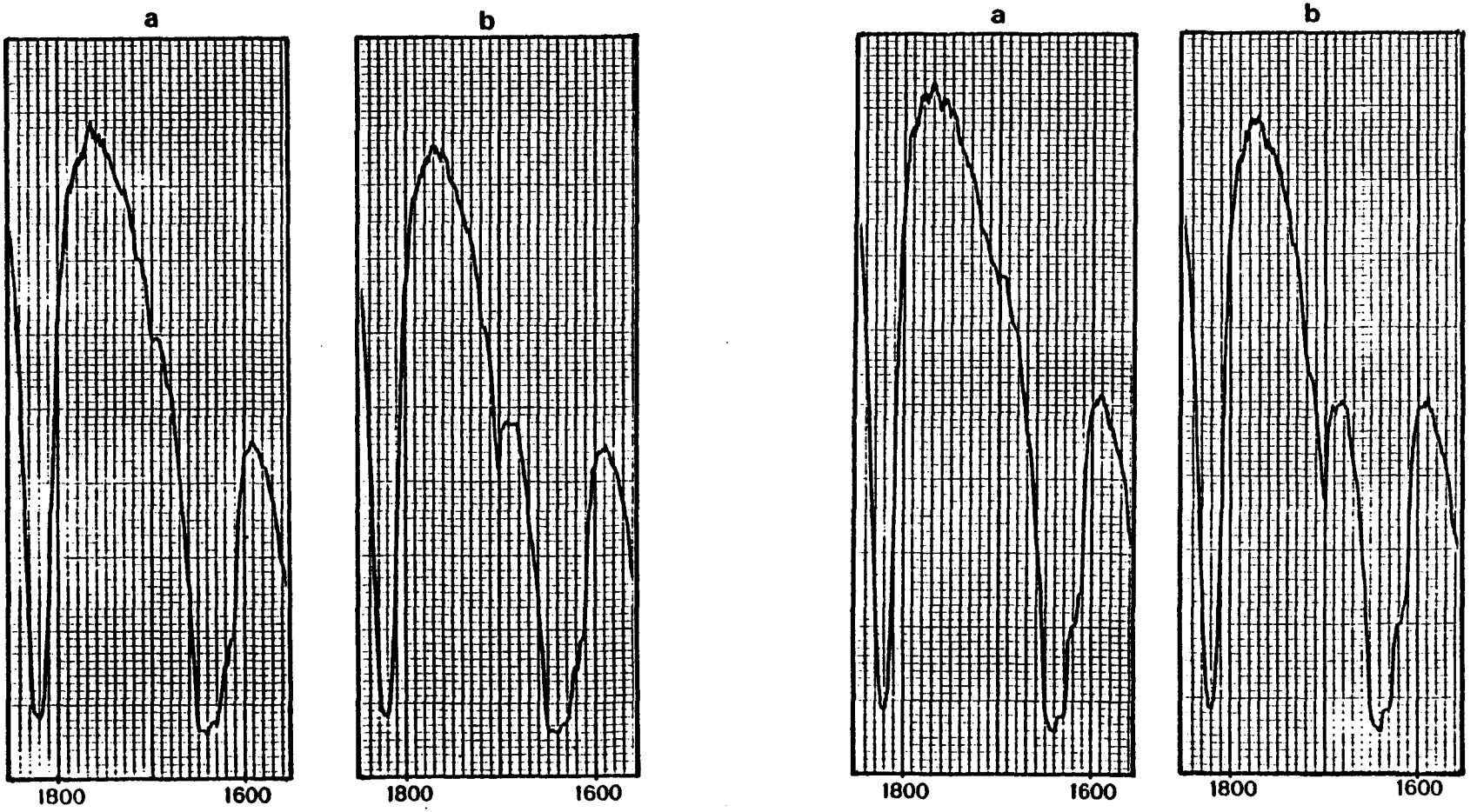


1-octene

1-decene

Fig. 5.9 - IR Comparison of Some Olefines

(a) 'as is'; (b) following autoxidation.



1-tetradecene

1-octadecene

Fig. 5.10 - IR Comparison of Some Olefines

(a) 'as is'; (b) following autoxidation

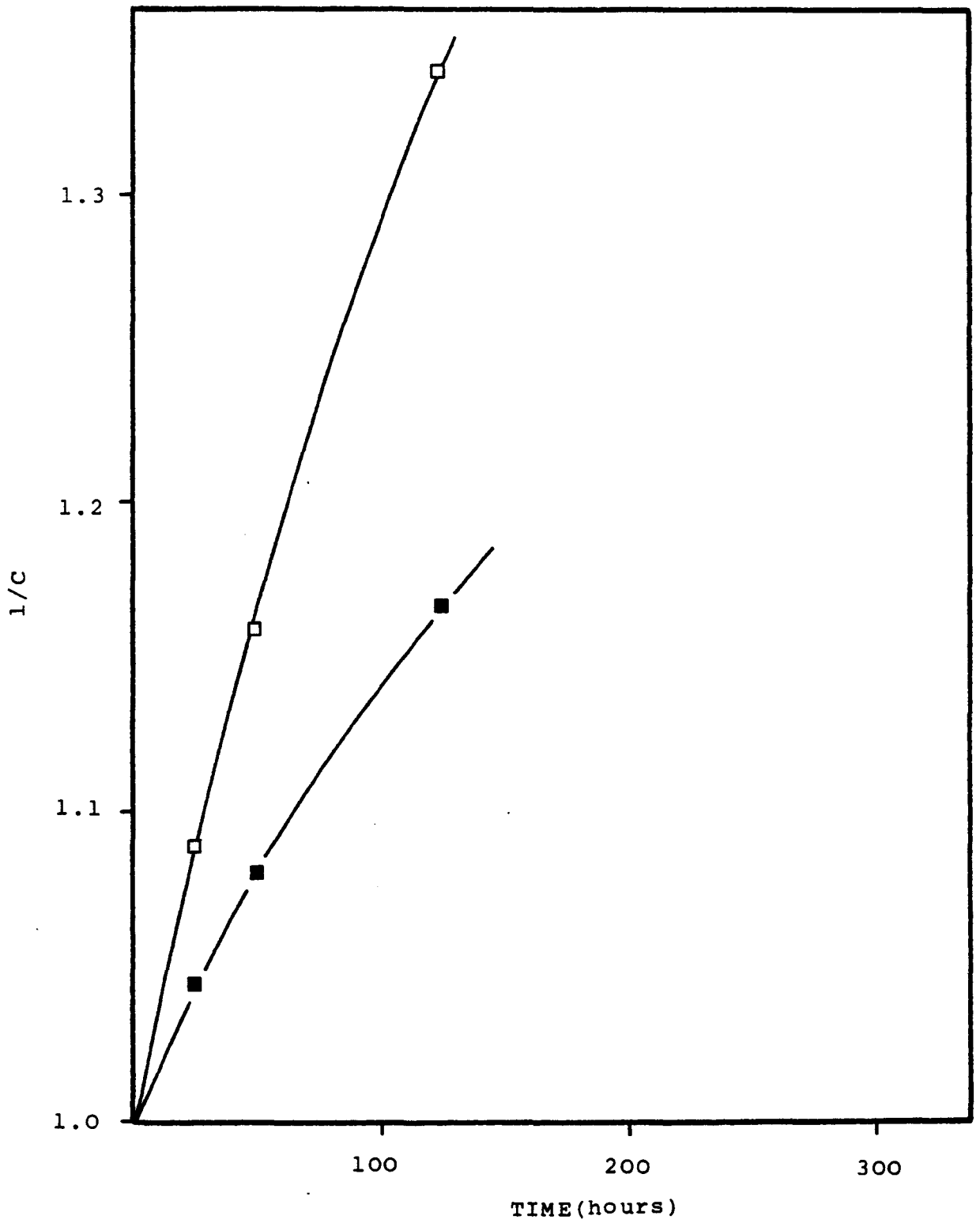


Fig.5.11 - Oxidation of Fluphenazine Decanoate by Separate Batches of 1-Decene (2nd order kinetics)

□ = Batch A(original); ■ = Batch B.

two batches of 1-decene (after autoxidation) oxidised fluphenazine decanoate at different rates. Furthermore, comparison with the original results (Fig. 5.6) demonstrated that the new batch of 1-decene exhibited a rate of oxidation in approximate agreement with the postulated relationship with regard to carbon-chain length.

The characteristics of the two lots of 1-decene were thus closely compared. A most noticeable difference upon opening the bottles was the strongly rancid odour of the original batch. The rancid odour was apparent in the material as received from the manufacturer, and as this material contained very little peroxide (OOH = 0.28mg/ml) an impurity resulting from the manufacturing process was indicated. Such odours are typical of the higher fatty acids hence the acid value of each of the olefins was determined using the method described in the British Pharmacopoeia[218]. The results are tabulated below and unequivocally show the comparatively high acidity of the original batch of 1-decene. In addition, a considerable increase in acidity was noted following autoxidation.

Table 5.10

Acid Values of the Olefins

Material	Acid Value
1-heptene	0.064
1-octene	0.031
1-decene(original)	0.258
Autoxidised 1-decene	1.272
1-decene(2nd lot)	0.038
Autoxidised	0.121
1-tetradecene	0.051
Autoxidised 1-tetradecene	0.116
1-octadecene	0.064

5.6 Reaction of Fluphenazine Decanoate with Autoxidised Fatty Acids

As a logical extension to the previous section the rate of oxidation of fluphenazine decanoate by fatty acid hydroperoxides was determined.

5.6.1 Preparation of Samples

Oleic and linoleic acids were autoxidised as described in Section 5.2 then diluted to produce standardised hydroperoxide solutions. Fluphenazine decanoate was dissolved in the prepared peroxide solutions to give a concentration of 25mg/ml and samples stored at 20°C in the dark.

5.6.2 Results and Discussion

Tables 5.11 and 5.12 list the results obtained.

Table 5.11

Oxidation of Fluphenazine Decanoate with Autoxidised Oleic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
1	0.643	Not detected	0.338
2	1.143	Not detected	0.647
4	2.465	0.032	1.191
5	3.031	0.052	1.325
6	3.633	0.078	1.702

Table 5.12

Oxidation of Fluphenazine Decanoate with Autoxidised Linoleic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
1	1.613	Not detected	0.557
2	2.832	Not detected	0.881
3.5	4.002	0.114	1.303
4.5	5.023	0.170	1.712
5.5	5.988	0.271	2.069

The results given in the above tables, when treated as described in Section 5.5.3 apparently obeyed both 1st and 2nd order kinetics equally well (Figs. 5.12 and 5.13), the degree of scatter within the available data causing the orders of reaction to be indistinguishable. Linear regression of the data also failed to identify the reaction order. Further data is obviously required to determine the correct reaction order and corresponding reaction rate.

However, the results are sufficient to demonstrate the enhanced oxidation rate of fluphenazine decanoate by the autoxidised fatty acids compared with the results of similar experiments using autoxidised olefins (Section 5.5). With the fatty acid hydroperoxides, formation of fluphenazine decanoate oxides is achieved in a matter of hours whereas the olefinic hydroperoxides required days to produce a similar level of oxidation products. Hence the suspected influence of acidity on the rate of fluphenazine decanoate oxidation by autoxidised olefins appears to be further substantiated. It could not, however, be deduced at this stage of the work whether the influence of acidity was dependant on the presence of the corresponding acid hydro-

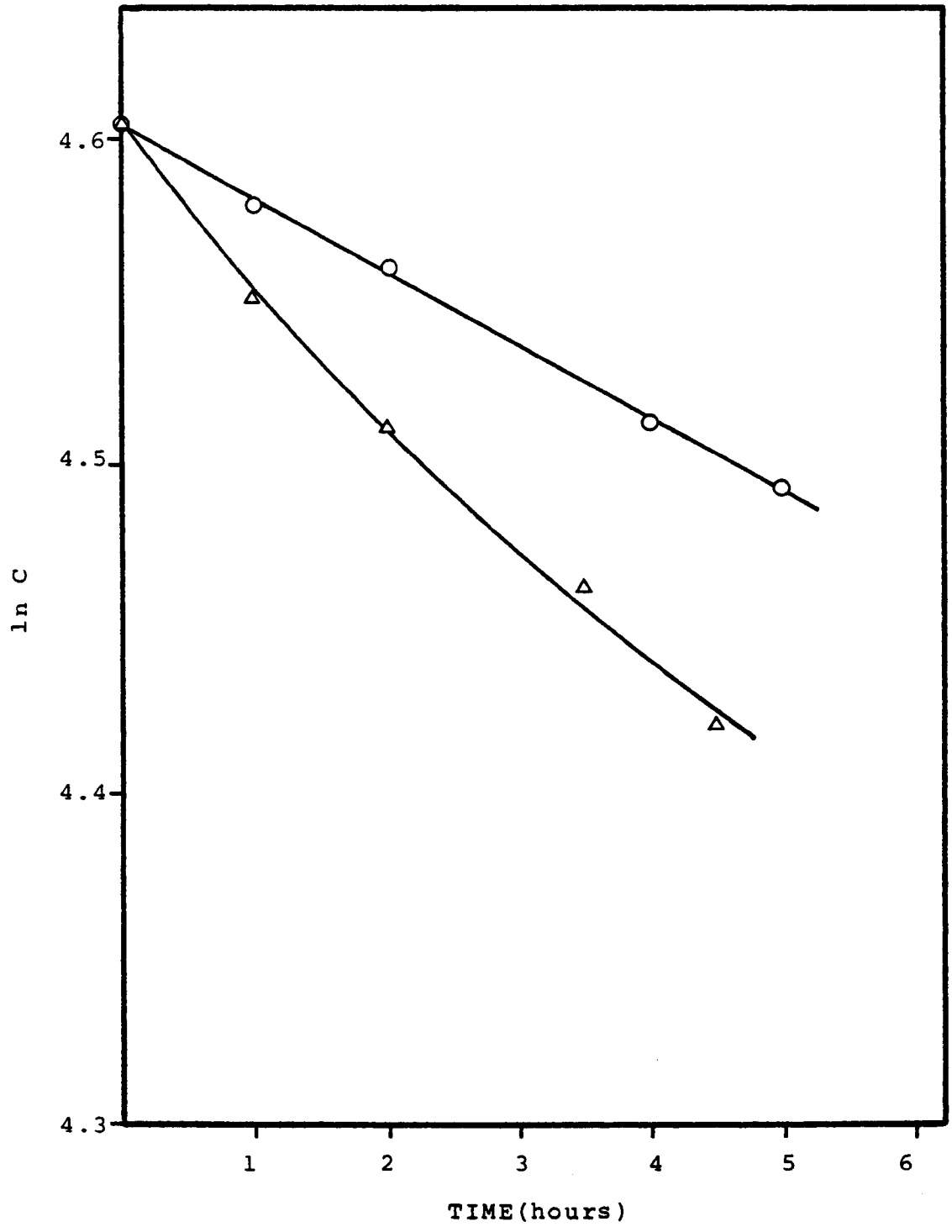


Fig. 5.12 - Oxidation of Fluphenazine Decanoate by Autoxidised Fatty Acids (1st order kinetics)

o = oleic acid, Δ = linoleic acid.

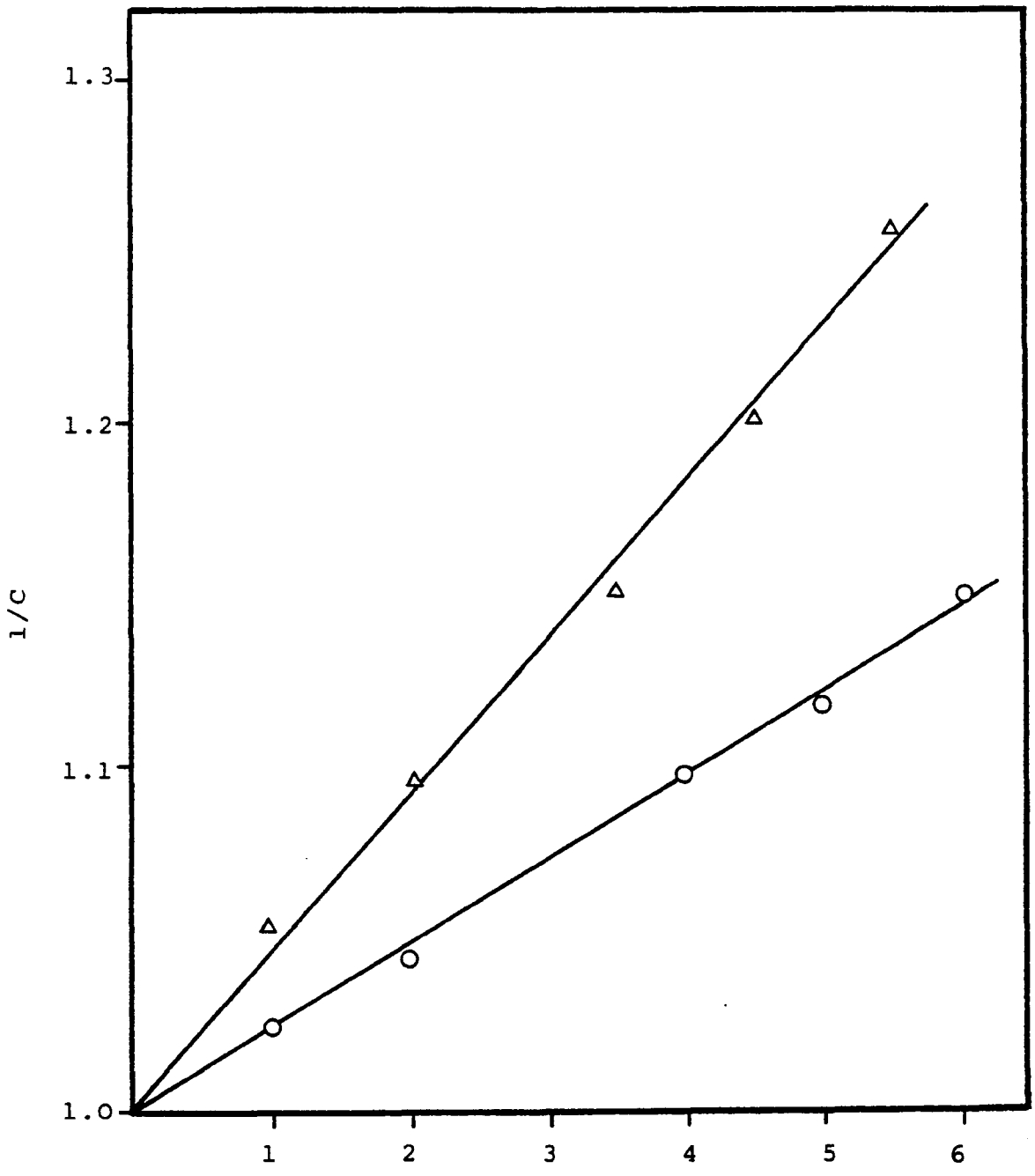


Fig. 5.13 - Oxidation of Fluphenazine Decanoate by Autoxidised Fatty Acids (2nd order kinetics)

o = oleic acid, Δ = linoleic acid.

peroxide, or whether the effect was due to the acid acting in a catalytic role.

Another feature of oxidation by the autoxidised acids is the increased amount of sulphoxide formed. Oxidation with autoxidised olefins (Section 5.5) had shown almost negligible proportions of sulphoxide to be produced. The effect of acidity in this respect may be related to either of two previously noted facts:

(i) Hydrogen peroxide oxidation of fluphenazine in a protonated form (di hydrochloride salt) exclusively yields sulphoxide (Section 3.1.2.4)

(ii) N-substituted phenothiazines in the presence of H^+ form a protonated phenazathionium ion which is readily converted to sulphoxide by traces of water.

5.7 Reaction of Fluphenazine Decanoate with Autoxidised Fatty Esters

A small amount of acid present as impurity in 1-decene, either naturally occurring or formed during autoxidation, had previously been intimated as a factor influencing the rate of oxidation of fluphenazine decanoate by the autoxidised material. In order to determine if a similar effect was evident with fatty esters, which contain small amounts of fatty acid impurities, similar experiments were conducted with methyl oleate and methyl linoleate.

A procedure for deacidification of the esters was required so that oxidation by neutral materials could be compared with materials containing natural fatty acid impurities. Because of the likelihood of acid formation during the autoxidation step the deacidification procedure would have to be performed after autoxidation of the ester. Deacidification must therefore selectively remove acid material from the ester, allowing the hydroperoxide to remain in solution. Acid values were determined by the B.P. procedure[218].

5.7.1 Method for the Deacidification of Autoxidised Fatty Esters

Using a technical grade of ethyl oleate for reasons of cost-efficiency, two procedures for the deacidification of autoxidised fatty esters were investigated.

a) Macrorecticular ion-exchange resins were successful only on a small scale with traces of acid impurity. At a realistic level of acid, results became erratic.

b) Basic alumina. A short column (30mm x 10mm) of basic alumina (Merck) adsorbed acid impurities, allowing the majority of hydroperoxide to be eluted with the ester. To test the validity of the procedure stearic acid (90mg) was added to an aliquot (30ml) of autoxidised ethyl oleate and the peroxide and acid contents determined before and after deacidification using a 30mm x 10mm alumina column.

The results are given below and indicate the validity of the deacidification procedure:

		Autoxidised Ester (as is)	Autoxidised Ester after deacidification
Peroxide (ml 0.01M $Na_2S_2O_3$)		14.7	12.1
Natural acid level	)ml 0.1M)NaOH	0.36	zero
Added Stearic acid	)	1.45	0.05

5.7.2 Preparation of Samples

5.7.2.1 Deacidified Esters

Following autoxidation, the esters were deacidified as described above (Section 5.7.1) and diluted to the standardised value of hydroperoxide with deacidified original material. Fluphenazine decanoate was then dissolved in the peroxide solution to produce solutions containing 25mg/ml of drug.

5.7.2.2 'As is' Esters

Standardised ester solutions containing 25mg/ml of fluphenazine decanoate were prepared as previously described for autoxidised olefins.

All solutions were stored at 20°C (thermostated) in the dark.

5.7.3 Modification of Assay Procedure for Oxides

No interferences had been noted when injecting the prepared olefin and fatty acid samples, in chloroform/methanol solution, directly on-column. However, methyl linoleate caused interfering peaks to be co-eluted with the two fluphenazine decanoate mono N-oxides. The nature of the interferences was unknown. To overcome this problem the oxides were separated from the ester by extraction prior to chromatography. Thus the oily sample (1ml; pipette) was dispersed in hexane (5ml) and the fluphenazine decanoate oxides extracted into an accurate volume (10 ml) of a mixture of methanol/0.1M aqueous hydrochloric acid (50-50).

Validation of the procedure was determined by dissolving a known amount of oxide in the hexane/methyl linoleate solution and after partition with methanol/0.1N HCl mixture the level of oxide extracted was determined. The following recoveries were obtained:

Mono N-oxides (A +B)	97.7% recovery
Di N-oxide	100.9% recovery
Sulphoxide	98.1% recovery

5.7.4 Results and Discussion

Tables 5.13 to 5.16 summarise the results obtained.

Table 5.13

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate - 'As is' (Acid Value = 0.44)

Time (hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
24	0.366	No detected	Not detected
120	1.736	0.038	0.074
192	2.297	0.046	0.082
288	2.890	0.088	0.130

Table 5.14

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate - Deacidified

Time (days)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
7	0.345	Not detected	Not detected
14	0.743	Not detected	Not detected
21	1.129	Not detected	0.091
26	1.251	Not detected	0.086
42	1.761	0.027	0.119
49	2.115	0.247	0.123

Table 5.15

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Linoleate - 'As is' (Acid Value = 0.19)

Time (hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
72	0.611	0.093	0.064
168	1.708	0.092	0.147
240	1.951	0.154	0.138
336	2.582	0.175	0.280

Table 5.16

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Linoleate - Deacidified

Time (days)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
6	0.599	Not detected	Not detected
13	1.246	Not detected	0.142
19	1.765	0.062	0.086
34	3.089	0.147	0.203

As found in previous experiments using olefinic hydroperoxides and t-butyl hydroperoxide, preferential oxidation of the piperazine ring was observed resulting in the almost selective formation of N-oxides. The results also confirm the enhancement of oxidation by acid which was suspected from earlier work.

5.8 Influence of Acid on Rate of Oxidation

5.8.1 Effect of Acid Concentration

5.8.1.1 Choice of Materials

Although the enhancement of fluphenazine decanoate oxidation rate in the presence of acid had been adequately demonstrated with both olefin and fatty ester hydroperoxides, the nature of the effect was unknown. Fatty acid hydroperoxides had been shown to oxidise fluphenazine decanoate at a much faster rate than the olefinic or fatty ester hydroperoxides implying one possible mechanism for the rate-enhancement effect ie. that small amounts of fatty acid hydroperoxides are formed during the autoxidation process effectively increasing the rate of oxidation. Alternatively, the acid could be acting as a catalyst in similar manner to that described in the published literature referring to the oxidation of organic sulphides (See Section 1.2.1.1).

Experiments were therefore designed in which oxidation of the acid could not readily occur. Stearic acid was chosen as it is a saturated fatty acid which would be unlikely to autoxidise at 20^{o}C throughout the duration of the experiment. The acid is also of the same carbon-chain length as oleic/linoleic esters which would be used as the media and would thus be of a similar nature to the naturally occurring fatty acids present in the oleate and linoleate fatty esters.

Oleate and linoleate esters were chosen as the solution media representative of the esters present in sesame oil, the vehicle used for Modecate Injection.

5.8.1.2 Preparation of Samples

Oleic and linoleic esters were autoxidised, deacidified then diluted to the standardised value of hydroperoxide with deacidified ester as described previously.

125mg Quantities of fluphenazine decanoate were weighed into a 5ml volumetric flask and known amounts (between 0 and 50mg) of stearic acid added, representing 0 to 1% acid impurity relative to the fatty ester. The drug and acid were then dissolved in the standardised fatty ester hydroperoxide solution and warmed gently to dissolve. The solutions were cooled to 20°C as soon as the materials had completely dissolved. Samples were stored in the dark at 20°C (thermostated environment). Aliquots of each sample were assayed at regular intervals. Because of the limited volume (5ml) of each sample available 0.4ml aliquots were diluted to 10ml in chloroform/methanol mixture (Section 5.3).

Methyl linoleate samples again showed interfering peaks on the HPLC chromatograms if injected directly on-column and thus the oxides were extracted into methanol/0.1M HCl prior to injection into the HPLC. (See Section 5.7).

5.8.1.3 Results and Discussion

The results obtained with methyl oleate hydroperoxides are given in Table 5.17.

Table 5.17

Influence of Stearic Acid on Oxidation
of Fluphenazine Decanoate by Autoxidised Methyl Oleate

a) Zero % acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
17	0.055	Not detected	Not detected
63	0.078	"	"
165	0.329	"	0.037
230	0.502	"	0.052
336	0.657	"	0.060
504	0.865	"	0.075
672	1.130	"	0.076

b) 0.25% acid (0.045 mM)

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
17	0.303	Not detected	0.012
63	0.815	Not detected	0.056
165	2.091	0.095	0.087
230	2.974	0.100	0.094

Table 5.17(Contd...)

c) 0.5% acid (0.089mM)

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
17	0.504	Not detected	0.038
63	1.563	0.029	0.080
165	3.450	0.137	0.105
230	4.908	0.233	0.155

d) 0.75% acid (0.132mM)

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
17	0.647	Not detected	0.038
63	2.081	0.051	0.072
165	4.675	0.263	0.161
230	6.175	0.431	0.198

e) 1.0% acid (0.177mM)

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
17	0.825	Not detected	0.049
63	2.651	0.073	0.104
165	5.681	0.395	0.204
230	7.278	0.631	0.241

The results were found to obey 2nd order kinetics (Figs. 5.14 and 5.15). The enhanced rate of oxidation with increasing acid content is clearly demonstrated and in this controlled experiment is unlikely to be due to the presence of fatty acid hydroperoxides. The effect must thus be attributed to acid catalysis.

To obtain confirmation of the reaction order, the experiment was repeated with a single level of acid. The results are given below:-

Table 5.18

Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Oleate in the Presence of Stearic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
17	0.529	Not detected	0.052
41	1.397	Not detected	0.059
65	2.011	Not detected	0.065
89	2.550	0.071	0.116
168	4.585	0.168	0.167
233	5.845	0.344	0.191

Graphical illustration of these results (Figs.5.16 and 5.17) confirms the 2nd order kinetics of the reaction.

A combination of stearic acid and methyl linoleate hydroperoxides had a similar effect on the oxidation rate of fluphenazine decanoate. These results are given in Table 5.19. Reproducibility of the results was demonstrated by repeating the experiment with a separately autoxidised methyl linoleate (Table 5.19). 2nd order rate-constants in good agreement, calculated as described in Section 5.5.3, were obtained (Table 5.20).

Table 5.19

Oxidation of Fluphenazine Decanoate with Methyl Linoleate Hydroperoxides in the Presence of Stearic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
Experiment 1			
72	3.518	0.161	0.176
120	5.041	0.263	0.199
168	6.473	0.510	0.283
Experiment 2			
24	1.081	Not detected	0.139
72	3.301	0.114	0.136
144	5.395	0.338	0.211
192	6.639	0.548	0.230

Table 5.20

2nd Order Rate Constants for Oxidation of Fluphenazine Decanoate by Methyl Linoleate with Added Stearic Acid

Experiment	Rate Constant (litre $mole^{-1} hr^{-1}$)	Correlation Coefficient
1	1.327×10^{-5}	0.9987
2	1.205×10^{-5}	0.9994

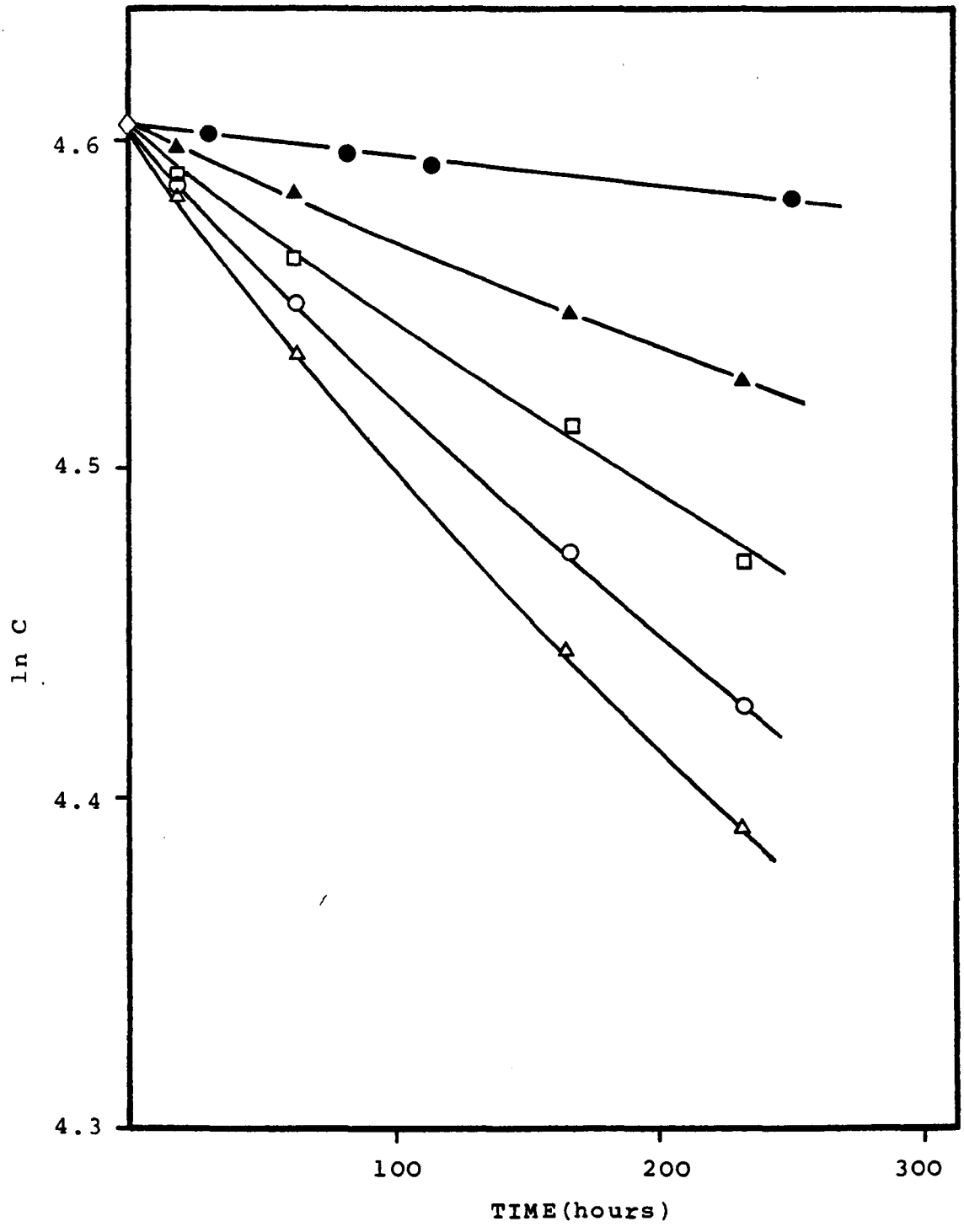


Fig. 5.14 - Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Oleate in the Presence of Stearic Acid (1st order kinetics)

● = zero, ▲ = 0.045mM, □ = 0.089mM,

○ = 0.132 mM, △ = 0.177mM of added stearic acid.

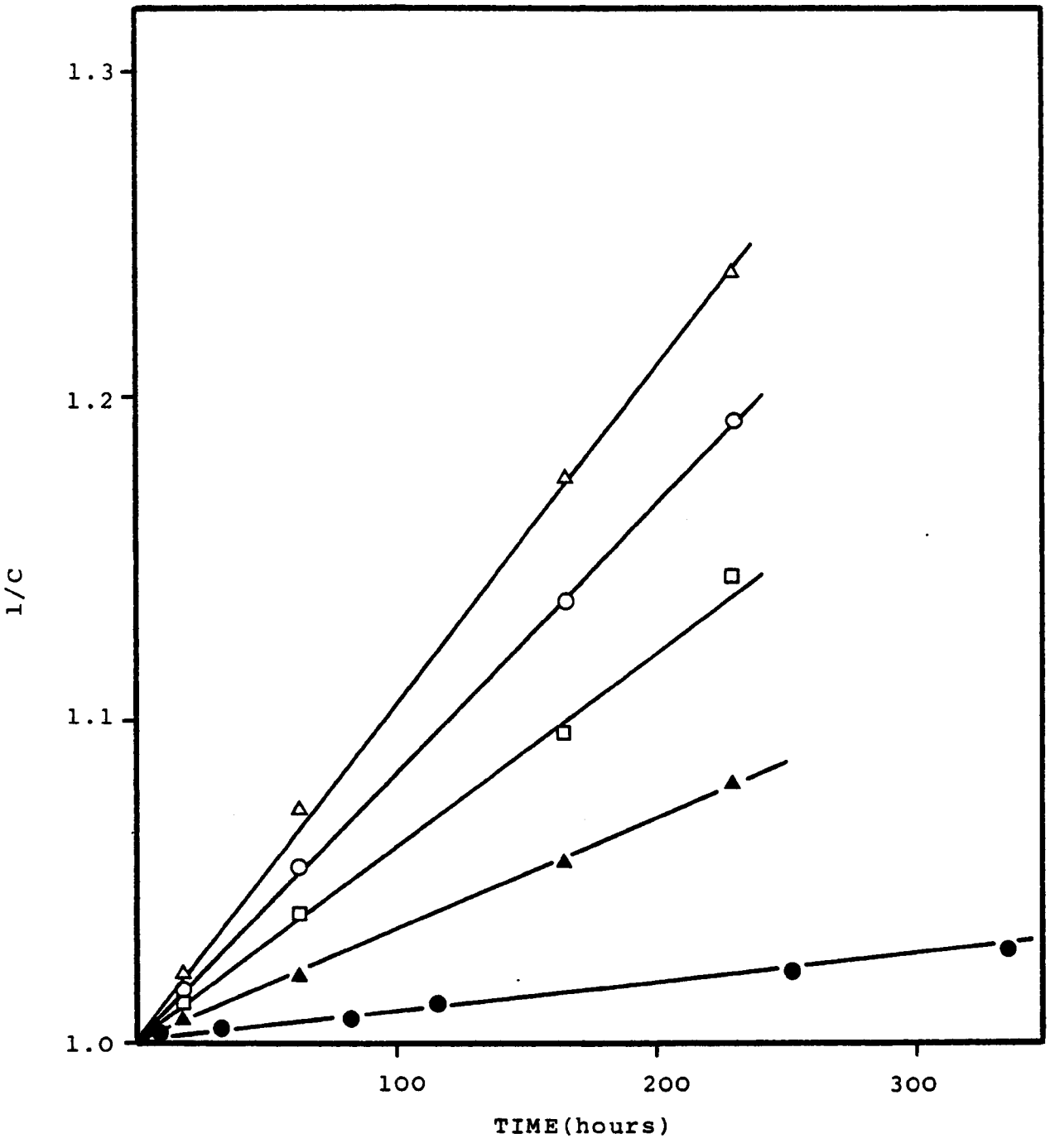


Fig. 5.15 - Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Oleate in the Presence of Stearic Acid (2nd order kinetics)

● = zero, ▲ = 0.045mM, □ = 0.089mM,

○ = 0.132mM, △ = 0.177mM of added stearic acid

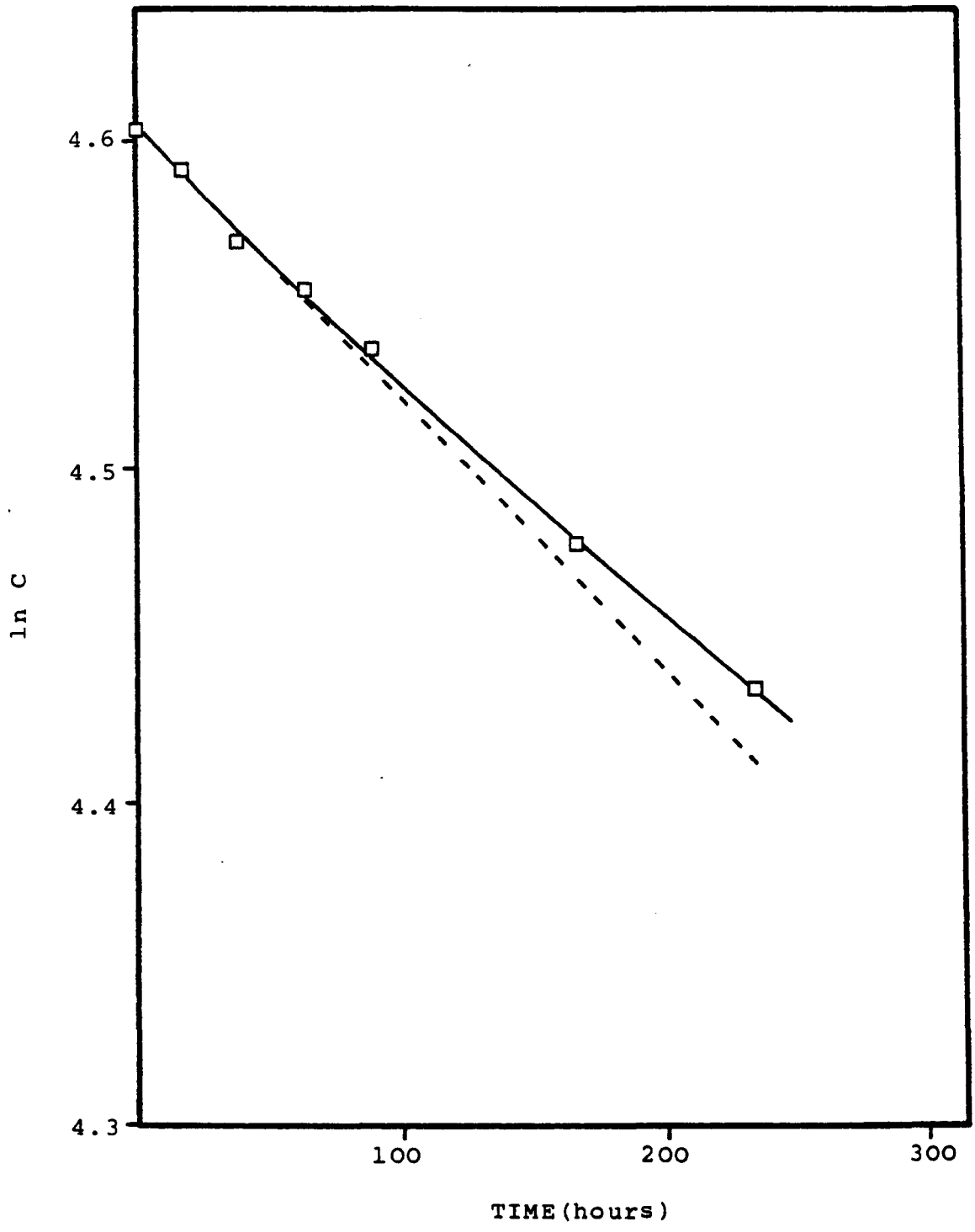


Fig. 5.16 - Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Oleate in the Presence of 0.1mM Stearic Acid (1st order kinetics)

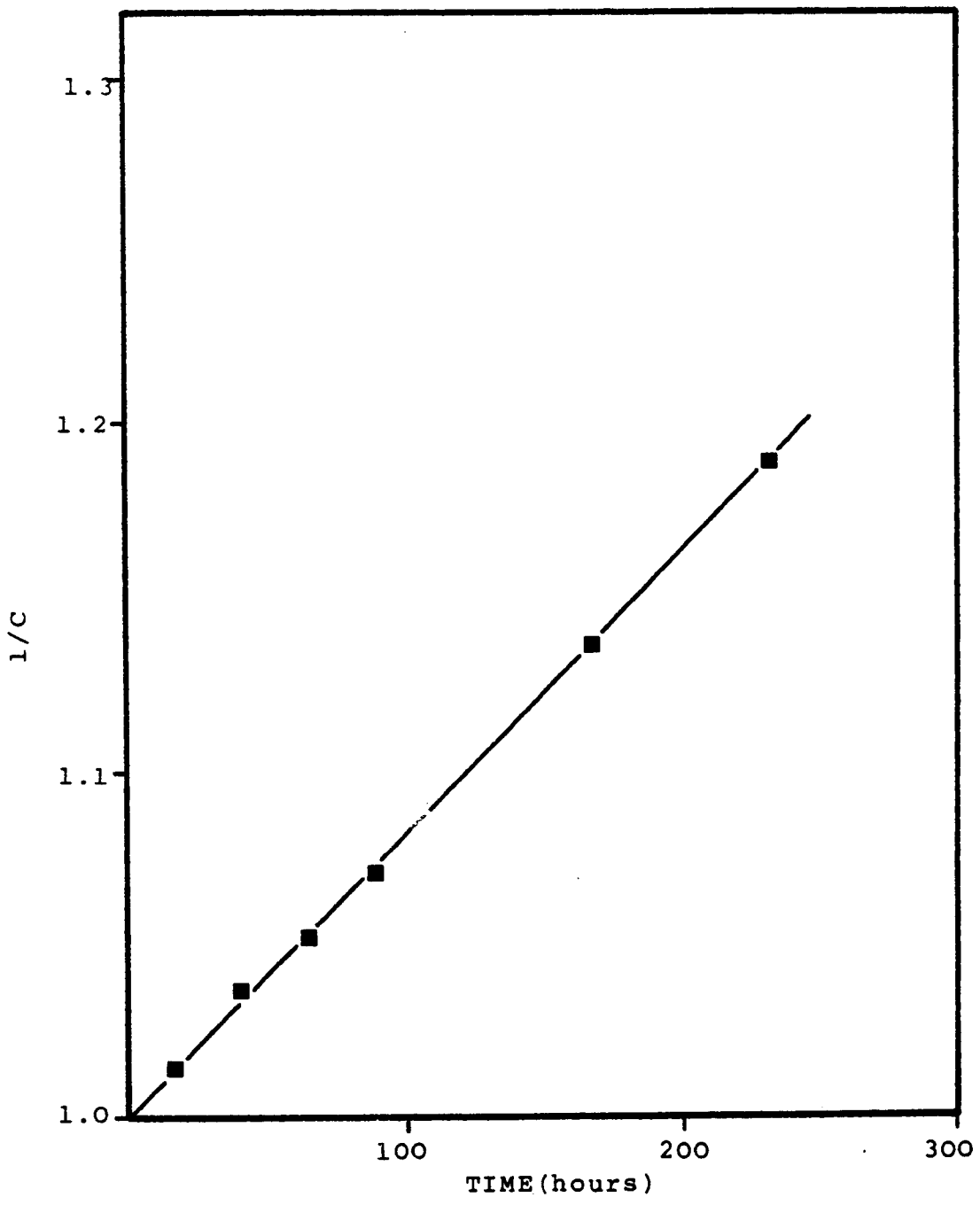


Fig. 5.17 - Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Oleate in the Presence of 0.1mM Stearic Acid (2nd order kinetics)

5.8.2 Effect of Different Carboxylic Acids

5.8.2.1 Choice of Materials

Use of stearic acid had successfully verified the catalytic effect of acidity on the oxidation rate of fluphenazine decanoate by hydroperoxides. The effect of varying the nature of the acid was next investigated. Materials were chosen to represent some of the typical carboxylic acids that may be encountered in a Modecate Injection formulation. The following acids were thus selected:

Acid	Possible origin in formulation
Oleic	)Mono and di unsaturated
Linoleic	)acids from hydrolysis of)sesame oil triglycerides.
Decanoic	Hydrolysis of fluphenazine decanoate
Benzoic	Autoxidation of benzyl alcohol(preservative)

Acetic acid was also included in order to determine the effect (if any) of the carbon-chain length. Stearic acid was used as 'control'.

5.8.2.2 Sample Preparation

Autoxidised methyl oleate was used as the oxidising medium and samples prepared in the same manner as in Section 5.8.1.2 with the modification that each acid was added at a concentration of 0.1mM per 5ml of solution. Samples were then stored under conditions identical to those used in all other experiments.

5.8.2.3 Results and Discussion

Tables 5.21 to 5.26 summarise the results obtained.

Table 5.21

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate in the Presence of Stearic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
96	3.025	0.076	Not detected
168	4.542	0.173	0.195
264	5.777	0.350	0.199

Table 5.22

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate in the Presence of Oleic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
96	2.806	0.065	Not detected
168	4.270	0.147	0.186
264	5.847	0.393	0.208

Table 5.23

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate in the Presence of Linoleic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
96	2.901	0.053	Not detected
168	4.475	0.169	0.195
264	5.704	0.362	0.208

Table 5.24

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate in the Presence of Benzoic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
96	4.074	0.124	Not detected
168	5.938	0.251	0.242
264	7.606	0.713	0.272

Table 5.25

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate in the Presence of Decanoic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
96	2.977	0.075	Not detected
168	4.327	0.163	0.186
264	6.054	0.430	0.217

Table 5.26

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate in the Presence of Acetic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
96	2.997	0.077	Not detected
168	4.433	0.170	0.186
264	6.012	0.479	0.208

From the results the 2nd order rate constants were calculated by linear regression. The calculated values are given below:

Table 5.27

2nd Order Rate Constants for the Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Oleate in the Presence of Various Acids

Acid	Rate Constant (litre $mole^{-1} hr^{-1}$)	Correlation Coefficient
Stearic	7.12×10^{-6}	0.9928
Stearic, 2nd expt.	6.92×10^{-6} 7.30×10^{-6}	0.9981 0.9992
Oleic	7.29×10^{-6}	0.9991
Linoleic	7.11×10^{-6}	0.9949
Benzoic	10.64×10^{-6}	0.9983
Benzoic, 2nd expt.	9.62×10^{-6} 10.18×10^{-6}	0.9948 0.9941
Decanoic	6.76×10^{-6}	0.9920
Acetic	7.69×10^{-6}	0.9988

The aliphatic carboxylic acids all give similar values for the rate constant of the reaction. The obvious exception in the list is benzoic acid which causes an increase in the rate of reaction by a factor of approximately 2. Consequently the reaction in the presence of benzoic acid was repeated in duplicate (Expt. 2) and yielded rate constants in good agreement with the original results, confirming the greater rate of reaction. Stearic acid was again used as 'control'. Minor differences in duplicate rate constants may be attributable to small deviations from the chosen 0.1mM acid concentration, caused by the method of sample preparation.

The most obvious difference between benzoic and the other acids utilised in this investigation was the aromaticity of the benzoic acid, the delocalising effect of which causes an increase in the dissociation constant (pKa). To

substantiate the influence of pKa on the oxidation rate, a series of acids of known pKa value were investigated.

5.8.3 Effect of Acid Dissociation Constant

5.8.3.1 Choice of Materials

Choice of acids for use in this experiment was dictated by the commercial availability of material and published values of the dissociation constant. The following range of pKa values was eventually chosen.

Acid	pKa[171]
o-Iodobenzoic	2.85
m-Chlorobenzoic	3.82
Benzoic	4.19
Caprylic	4.89

Autoxidised methyl oleate, adjusted to the standardised value of hydroperoxide was used as the oxidising media so that the results obtained would be comparable with the previous set of results (Section 5.8.2).

5.8.3.2 Preparation of Samples

Samples were prepared in a manner identical to that described in the previous experiment (Section 5.8.2).

5.8.3.3 Results and Discussion

The results obtained are summarised in Tables 5.28 to 5.31 and illustrated graphically in Fig. 5.18.

Table 5.28

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate in the Presence of *o*-Iodobenzoic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
17	1.265	Not detected	0.052
41	2.929	Not detected	0.092
65	4.041	Not detected	0.093
89	5.132	0.213	0.155

Table 5.29

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate in the Presence of*m*-Chlorobenzoic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
17	0.996	Not detected	0.039
65	3.063	Not detected	0.112
89	3.770	0.118	0.141
168	6.695	0.251	0.198

Table 5.30

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate in the Presence of Benzoic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
17	0.945	Not detected	Not detected
41	2.064	Not detected	0.086
65	2.994	Not detected	0.084
89	3.071	0.118	0.129
168	6.504	0.326	0.244

Table 5.31

Oxidation of Fluphenazine Decanoate with Autoxidised Methyl Oleate in the Presence of Caprylic Acid

Time (Hours)	Oxide Content(um/ml)		
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide
17	0.588	Not detected	Not detected
41	1.338	Not detected	0.086
65	1.982	Not detected	0.056
168	4.634	0.186	0.167

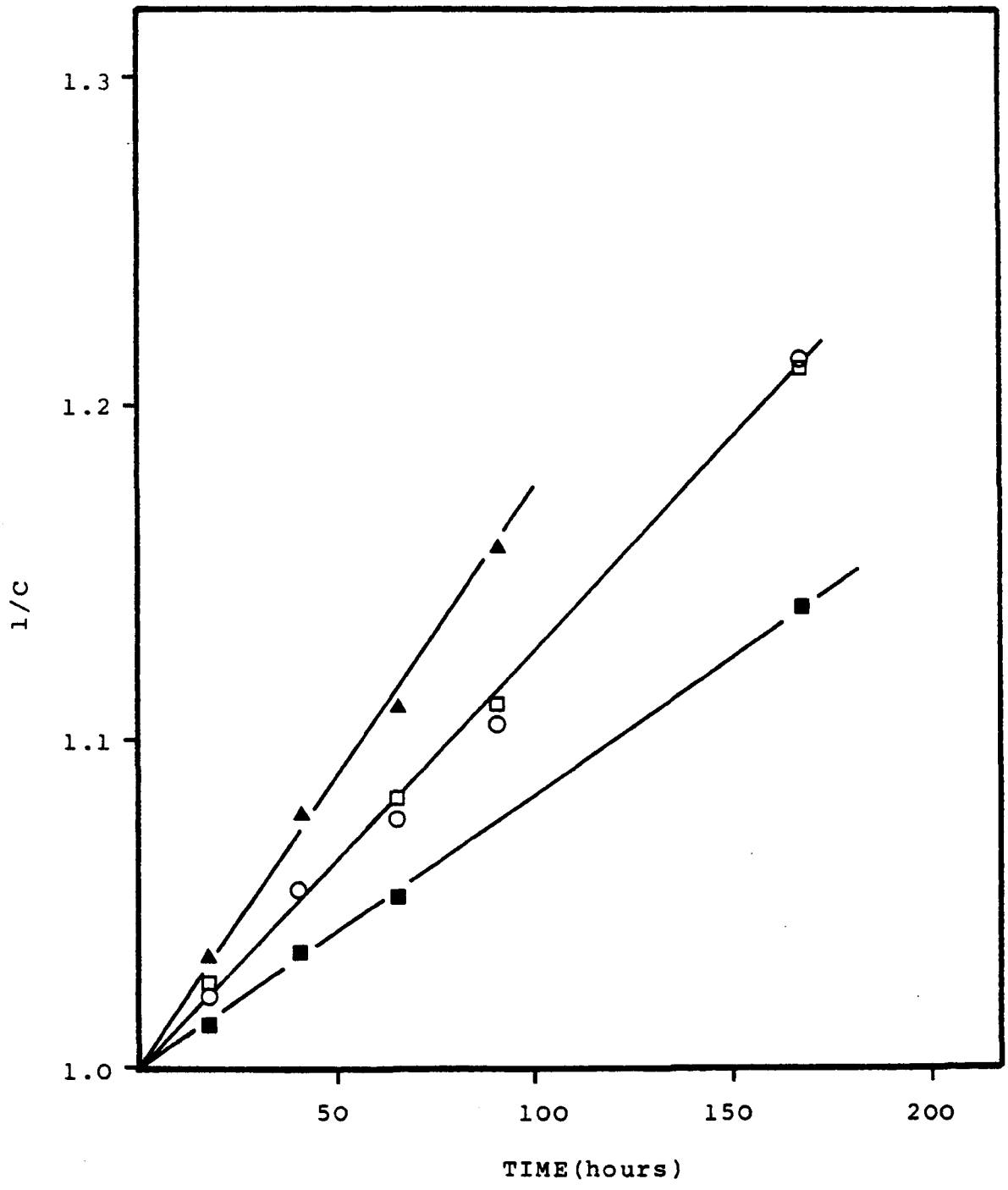


Fig. 5.18 - Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Oleate - Effect of pKa of Added Acid(2nd order kinetics)

■ = Caprylic acid, pKa = 4.89; o = benzoic acid, pKa 4.19; □ = *m*-chlorobenzoic acid, pKa 3.82; ▲ = *o*-iodobenzoic acid, pKa 2.85.

Table 5.32

2nd Order Rate Constants for Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Oleate in the Presence of Acids

Acid	pKa[171]	Rate Constant (litre $mole^{-1} hr^{-1}$)	Correlation Coefficient
o-iodobenzoic	2.85	17.20×10^{-6}	0.9982
m-chlorobenzoic	3.82	12.49×10^{-6}	0.9996
benzoic	4.19	12.50×10^{-6}	0.9992
caprylic	4.89	8.29×10^{-6}	0.9996

The results clearly demonstrate that an increase in the pKa of added acid is accompanied by a decrease in oxidation rate. The similarity of oxidation rates exhibited by fluphenazine decanoate in the presence of *m*-chlorobenzoic acid (pKa 3.8) and benzoic acid (pKa 4.2) is probably a combination of experimental design and closeness of the two pKa values. Errors during the addition of the acid to the samples by weight may be controlled but not completely eliminated, and thus acids with a greater difference in pKa value would have been more suitable. Small errors during sample preparation would then be negligible in comparison to the degree of rate-enhancement. However, when considered with the rates of oxidation attributable to the other acids used, the results are sufficient to verify the suspected effect of pKa.

5.9 Influence of Benzyl Alcohol on Rate of Fluphenazine Decanoate Oxidation by Fatty Ester Hydroperoxides

Benzyl alcohol is added to Modecate Injection as a preservative and thus the influence of this material on the rate of fluphenazine decanoate oxidation was investigated. Autoxidised fatty esters were selected as the solution

media in order to simulate typical conditions which would be apparent in the product.

5.9.1 Preparation of Samples

Freshly distilled benzyl alcohol was obtained from a suitable supplier (B.D.H.) and checked for benzaldehyde content (Squibb & B.P. limit 0.2%) by HPLC (See Chapter 6). The material was deacidified before use in order to remove possible traces of benzoic acid. 75mg of the alcohol was added to 125mg of fluphenazine decanoate (equivalent to the level of preservative in the product) in a 5ml volumetric flask. Deacidified standardised autoxidised fatty ester was added to the flask contents which were diluted to volume when the components had completely dissolved.

A second series of samples containing a known amount of stearic acid (0.1mM per 5ml) were also prepared.

All samples were stored under conditions identical to those used in previous experiments.

5.9.2 Results and Discussion

For convenience, this series of experiments was conducted concurrently with the investigations described in Sections 5.7 and 5.8. Although the results of experiments 5.7 and 5.8 have already been quoted, the relevant results are reproduced in Tables 5.33 to 5.36 so that the effect of adding benzyl alcohol to the oxidation reaction mixture may be conveniently assessed.

Table 5.33

Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Oleate (Deacidified)

Time (days)	Oxide Content - Absence of Benzyl Alcohol (um/ml)			Oxide Content - Presence of Benzyl Alcohol (um/ml)		
	Piperazino Mono N-oxide (Total)	Piperazino di N-oxide	Sulphoxide	Piperazino Mono N-oxide (Total)	Piperazino di N-oxide	Sulphoxide
7	0.345	Not detected	Not detected	0.442	Not detected	Not detected
14	0.743	Not detected	Not detected	0.901	Not detected	Not detected
21	1.129	Not detected	0.0913	1.289	Not detected	0.0913
27	1.251	Not detected	0.0859	1.752	Not detected	0.103
42	1.761	0.027	0.119	2.162	Not detected	0.132
49	2.115	0.247	0.123	2.420	0.210	0.167

Table 5.34

Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Oleate, with Added Stearic Acid

Time (days)	Oxide Content - Absence of Benzyl Alcohol (um/ml)			Oxide Content - Presence of Benzyl Alcohol (um/ml)		
	Piperazino Mono N-oxide (Total)	Piperazino di N-oxide	Sulphoxide	Piperazino Mono N-oxide (Total)	Piperazino di N-oxide	Sulphoxide
96	2.589	Not detected	0.108	2.542	Not detected	0.108
168	3.774	0.152	0.149	3.819	0.135	0.158
264	5.541	0.375	0.238	5.718	0.489	0.220

Table 5.35

Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Linoleate
(Deacidified)

Time (days)	Oxide Content - Absence of Benzyl Alcohol (um/ml)			Oxide Content - Presence of Benzyl Alcohol (um/ml)		
	Piperazino Mono N-oxide (Total)	Piperazino di N-oxide	Sulphoxide	Piperazino Mono N-oxide (Total)	Piperazino di N-oxide	Sulphoxide
6	0.599	Not detected	Not detected	0.615	Not detected	Not detected
13	1.246	Not detected	0.142	1.303	Not detected	0.213
19	1.765	0.0620	0.086	1.928	0.054	0.129
34	3.089	0.147	0.203	3.269	0.144	0.347

Table 5.36

Oxidation of Fluphenazine Decanoate by Autoxidised Methyl Linoleate with Added Stearic Acid

Time (hours)	Oxide Content - Absence of Benzyl Alcohol (um/ml)			Oxide Content - Presence of Benzyl Alchol (um/ml)		
	Piperazino Mono N-oxide (Total)	Piperazino di N-oxide	Sulphoxide	Piperazino Mono N-oxide (Total)	Piperazino di N-oxide	Sulphoxide
72	3.518	0.161	0.176	3.343	0.134	0.176
120	5.041	0.263	0.199	4.621	0.280	0.196
168	6.473	0.510	0.283	6.397	0.586	0.251

From the results, the 2nd order rate constants were calculated by linear regression and are given in Table 5.37.

Table 5.37

2nd Order Rate Constants for Fluphenazine Decanoate Oxidation in the Presence of Benzyl Alcohol

Oxidant	Conditions/ Additives	Rate Constant (litre $mole^{-1} hr^{-1}$)	Correlation Coefficient
Methyl oleate hydroperoxide	Deacidified	0.55×10^{-6}	0.9892
	Deacidified + Benzyl alcohol	0.64×10^{-6}	0.9974
	Stearic acid	6.85×10^{-6}	0.0993
	Stearic acid + Benzyl alcohol	7.33×10^{-6}	0.9970
Methyl linoleate hydroperoxide	Deacidified	1.14×10^{-6} (Expt.1) 0.96×10^{-6} (Expt.2)	0.9994 0.9990
	Deacidified + Benzyl alcohol	1.26×10^{-6} (Expt.1) 1.07×10^{-6} (Expt.2)	0.9993 0.9993
	Stearic acid	13.27×10^{-6} (Expt.1) 12.05×10^{-6} (Expt.2)	0.9987 0.9994
	Stearic acid + Benzyl alcohol	13.19×10^{-6} (Expt.1) 11.46×10^{-6} (Expt.2)	0.9964 0.9996

The results demonstrate that benzyl alcohol has little influence on the oxidation rate of fluphenazine decanoate.

5.10 Reaction of Fluphenazine Decanoate with Hydrogen Peroxide

Use of hydrogen peroxide as oxidant in this series of studies was proposed at the beginning of the programme, hydrogen peroxide being the simplest of the commercially available hydroperoxides. However, alternative investigations had meanwhile indicated that the autoxidation of benzyl alcohol (the

preservative in Modecate Injection) would ultimately result in the formation of hydrogen peroxide (See Chapter 6). To simulate the typical conditions apparent in a degraded product, the reaction was conducted in fatty ester solution. The unsaturated fatty esters could not be used in this case, because of possible side-reactions between the C=C double bond and the added peroxide. Of the saturated fatty esters methyl myristate (C_{14}) is the highest of the series which are liquid at room temperature. Thus methyl myristate was selected as solvent for the reaction.

5.10.1 Preparation of Sample

Methyl myristate (Sigma Chemicals) was deacidified through alumina then partitioned with an equal volume of 50% aqueous hydrogen peroxide (BDH Chemicals) and the organic layer separated by centrifugation. Deacidification was necessary prior to addition of hydrogen peroxide since the alumina column effectively adsorbs the total hydrogen peroxide content of the methyl myristate solution.

After drying the peroxide/methyl myristate solution over magnesium sulphate, the hydroperoxide content of the solution was determined iodometrically and subsequently diluted with deacidified methyl myristate in order to obtain a solution containing the standardised amount of OOH (1.4mg/ml).

Fluphenazine decanoate was dissolved in the prepared solution at a concentration of 25mg/ml and the final sample stored as in previous experiments.

5.10.2 Results and Discussion

The results obtained are summarised in Table 5.38 and again show preferential oxidation of the piperazine ring. In common with the olefinic hydroperoxides the results could not be fitted to any particular reaction order, although the application of 2nd order kinetics gave the shallowest curve. (Fig. 5.19).

Table 5.38

Oxidation of Fluphenazine Decanoate by Hydrogen peroxide in Methyl Myristate Solution

Time (Hours)	Oxide Content(um/ml)				
	Piperazino Mono N-oxides (Total)	Piperazino Di N-oxide	Sulphoxide	Sulphoxide mono N-oxides	Sulphoxide di N-oxide
24	0.790	Not detected	Not detected	not detected	Not detected
144	2.550	0.253	0.259	0.253	0.208
216	3.846	0.212	0.484	0.370	0.190
310	4.550	0.225	0.379	0.338	0.427

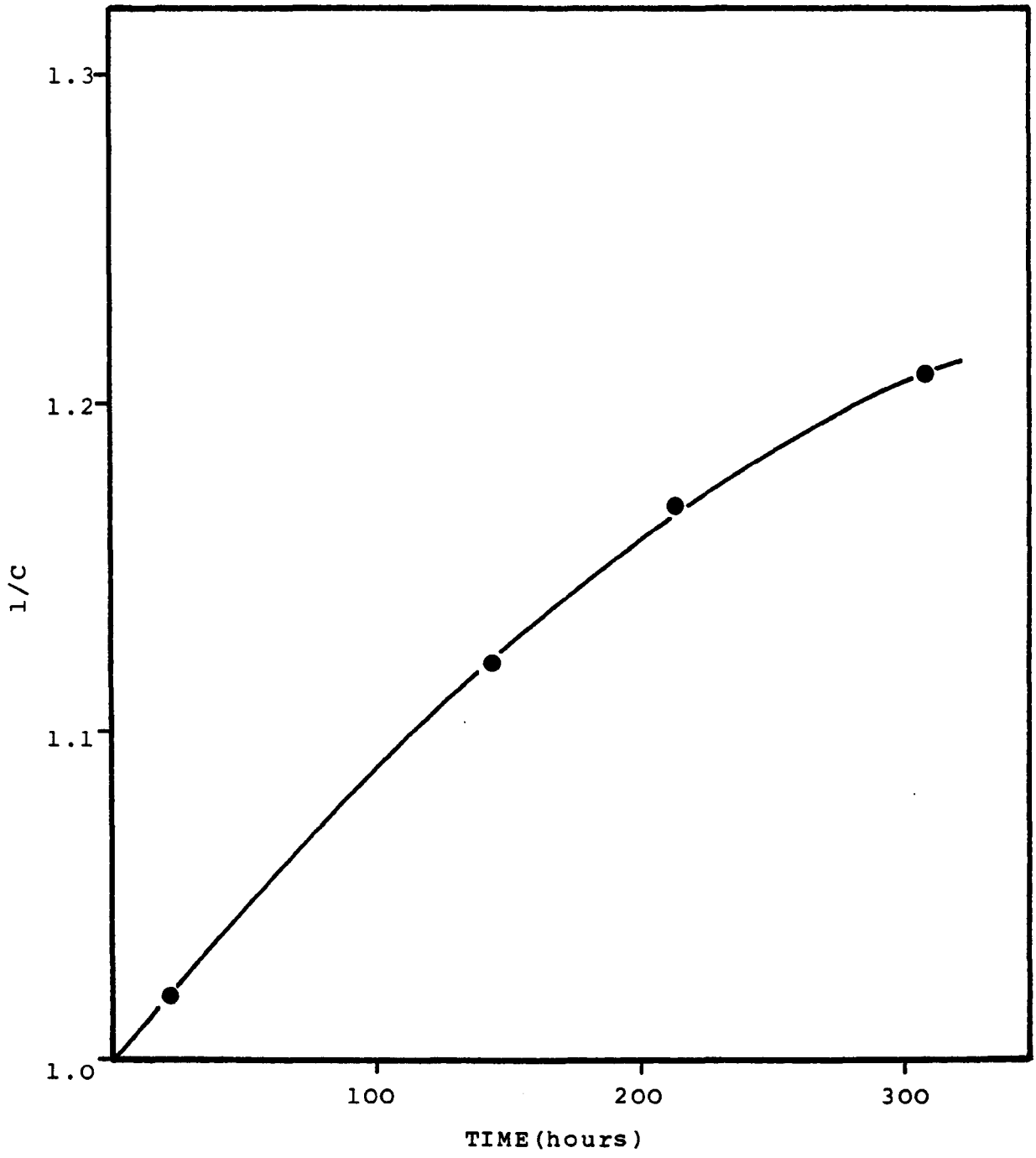


Fig. 5.19 - Oxidation of Fluphenazine Decanoate by Hydrogen Peroxide in Methyl Myristate (2nd order kinetics)

Re-examination of the standardised methyl myristate/peroxide solution remaining after sample preparation revealed that the peroxide originally present had decomposed:

original titre(ml 0.01M $Na_2S_2O_3$)	7.5 ml
Titre after 1 week @ RT (sealed amber bottle)	2.0 ml

These figures indicate the instability of the myristate/peroxide solution and may explain the deviation of the oxidation reaction from standard reaction kinetic laws. Reasons for this observed instability are unknown.

A second feature of the reaction was the formation of mixed sulphoxide-N-oxides which were not noted in previous experiments. Interestingly, only one of the two possible sulphoxide-mono-N-oxides was produced (Compound Y). No evidence for the presence of the alternative sulphoxide-mono-N-oxide (Compound X) was obtained but the sulphoxide di N-oxide (Compound D) was observed. It is thus feasible that compound Y is converted to compound D during degradation.

Formation of the mixed oxides may be associated with the presence of water resulting from either the sample preparation (incomplete drying by magnesium sulphate) or from the decomposition of hydrogen peroxide which evidently occurs during storage.

5.11 Conclusions

In all the experiments described in this Chapter, the ratio of mono N-oxides A and B formed during oxidation of fluphenazine decanoate varied little from the approximate 1:1 ratio noted in Chapter 3, regardless of the solvent (oil). Although N-oxidation was noted to be the primary degradative route, some sulphoxide was also formed. The only notable difference in the pattern of oxidation products observed in each of the oily solutions was the increased formation of sulphoxide in fatty acid solution.

The presence of small amounts of acid caused an increase in the oxidation rate of the drug, enhancing the formation of both N- and S- oxides; N-oxidation was enhanced to a far greater extent than S- oxidation. The catalytic effect of acid on the oxidation of t-amines by hydroperoxides is contrary to previously published data[192], though the acid-catalysed oxidation of dialkyl sulphides under similar conditions is well established[128].

A comparison of the 2nd order rate constants obtained during the course of the work clearly demonstrates that methyl linoleate hydroperoxides oxidised fluphenazine decanoate approximately twice as fast as methyl oleate hydroperoxides, in both acid and deacidified solution. Reasons for this observed phenomenon are not understood but three possibilities may be presented for further consideration:

(i) formation of secondary oxidation products which affect the reaction of the hydroperoxide with the drug to a greater or lesser degree.

(ii) presence of positional isomers (four from methyl oleate, viz. 8-, 9-, 10- and 11- hydroperoxide; three from methyl linoleate, viz. 9-, 11- and 13-hydroperoxide) which may react with the drug at different rates. The ratio of isomers present in the autoxidised materials used for the described experiments is unknown. An HPLC procedure for the separation and quantitation of such mixtures has been reported.[235]

(iii) Oxidation of unsaturated fatty esters is accompanied by stereomutation of the cis double-bond system[177] thus altering the spatial arrangement of the molecules.

Factors (ii) and (iii) could be of considerable importance because of the bulky nature of both the oxidant and the drug.

CHAPTER 6

AUTOXIDATION OF BENZYL ALCOHOL AND ITS INFLUENCE ON THE OXIDATIVE DEGRADATION OF FLUPHENAZINE DECANOATE

6.1 Effect of Benzyl Alcohol on the Oxidative Degradation of Fluphenazine Decanoate in Sesame Oil

6.1.1 Introduction

The preservative efficacy of benzyl alcohol has been well documented[219] and it is thus included in the Modecate formulation to ensure a sterile product, particularly where the package is designed to be a multidose container.

Preliminary experiments (Section 5.9), which compared the rate of fluphenazine decanoate oxidation by fatty ester hydroperoxides, had demonstrated that the addition of benzyl alcohol had relatively little influence on the oxidation rate. However, the data obtained may be insufficient to enable a true estimate of the effect of benzyl alcohol to be determined. The results were obtained over a period of six weeks - which is perhaps unrealistic in comparison to the product shelf-life (2 yrs).

To assess the potential of benzyl alcohol to affect the stability of the product a series of laboratory-prepared samples of Modecate Injection, with and without the preservative, were formulated using materials of normal Squibb quality.

As an alternative approach, the effect of increasing the benzaldehyde level in the preservative was also assessed since benzaldehyde has been reported to react directly with molecular oxygen forming the powerful oxidant perbenzoic acid[187]. In order that these experiments could be

directly compared with the former, solutions were prepared in sesame oil to normal product specifications. Two forms of benzyl alcohol/benzaldehyde mixture were used:

a) Naturally aged benzyl alcohols which contained benzaldehyde at varying levels.

b) Accurately prepared mixtures of pure benzyl alcohol and pure benzaldehyde.

a) provides conditions which are more closely-related to those expected in a degrading product, whereas (b) is an ideallised mixture which would enable the true effect of the benzaldehyde impurity to be assessed.

6.1.2 Materials

Sesame oil and fluphenazine decanoate	Obtained from Squibb Q.C.Dept. and tested according to the current Squibb Testing Standards. The materials complied in all respects.
Benzyl alcohol	Redistilled benzyl alcohol was was obtained from a suitable supplier(BDH) and tested in accordance with the current Squibb Testing Standard. The material complied in all respects(Note - benzaldehyde level = 0.012%; limit 0.2%)
Degraded benzyl alcohol	Naturally aged batches of benzyl alcohol were selected from various laboratories within the Squibb laboratory complex and chosen to represent increasing levels of benzaldehyde impurity. Actual levels of benzaldehyde were as follows:-

		PhCHO (%)	Original Supplier
	Batch A	0.17	Von Heyden (Germany)
	Batch B	0.56	BDH
	Batch C	1.12	BDH
	Batch D	1.27	BDH

Benzaldehyde	Analar material was obtained from BDH Chemicals and stored at $4^{o}C$ under N_2.
Benzyl alcohol/ benzaldehyde mixtures	Analar benzaldehyde was added to pure benzyl alcohol(absence of benzaldehyde impurity confirmed) at levels of 0.1, 0.5, 1.0, 5.0, 10.0 and 50% v/v.

6.1.3 Preparation of Samples

2g Fluphenazine decanoate was dissolved in 80ml sesame oil by gently heating ($35^{o}C$). The preservative, or benzyl alcohol/benzaldehyde mixture, was then added to 5ml aliquots at a concentration of 1.5% by weight, simulating the product formulation. A 5ml aliquot was reserved as control.

Solutions were stored under a headspace of air to hasten oxidative decomposition. (Modecate product is N_2 purged during manufacture and packing). Storage under conditions of darkness at temperatures of $20^{o}C$ and $40^{o}C$ were utilised.

Samples were assayed for N-oxide content at intervals using the HPLC procedure described in Section 5.3.

Bubble in air or O2 - faster

6.1.4 Results and Discussion

Results are summarised in the following tables:

Table 6.1

Effect of Benzyl Alcohol/Benzaldehyde on Fluphenazine Decanoate Oxidation in Ageing Sesame Oil Solution @ 20°C

Sample	Total N-oxide(mg/g formulation)							
	Initial	1 week	2 weeks	3 weeks	6 weeks	12 weeks	20 weeks	25 weeks
Control	Not detected	0.042	0.070	0.077	0.132	0.235	-	0.388
+ pure $PhCH_2OH$	Not detected	0.045	0.079	0.086	0.140	0.257	-	0.440
+ Aged $PhCH_2OH$ (Batch A, 0.17% pHCHO)	Not detected	0.056	-	-	-	-	0.565	-
+ Aged $PhCH_2OH$ (Batch B, 0.56% PhCHO)	Not detected	0.258	-	-	-	-	-	-
+ Aged $PhCH_2OH$ (Batch C, 1.12% PhCHO)	Not detected	0.463	0.660	0.778	0.847	0.928	-	-
+ Aged $PhCH_2OH$ (Batch D, 1.27% PhCHO)	Not detected	0.724	-	-	-	-	-	-
+ pure benzaldehyde	Not detected	0.035	0.096	0.090	0.128	0.193	-	0.321

Table 6.2

Effect of Benzyl Alcohol/Benzaldehyde on Fluphenazine Decanoate Oxidation in Ageing Sesame Oil Solution @ 40°C

Sample	Total N-oxide (mg/g formulation)						
	Initial	1 week	2 weeks	3 weeks	6 weeks	12 weeks	25 weeks
Control	Not detected	0.140	0.280	0.278	0.421	0.723	1.042
+ pure $PhCH_2OH$	Not detected	0.139	0.340	0.313	0.508	0.880	1.522
+ Aged $PhCH_2OH$ (Batch C, 1.12% PhCHO)	Not detected	0.769	0.980	0.949	1.035	1.308	-
+ pure benzaldehyde	Not detected	0.146	0.280	0.331	0.428	0.709	1.175

Table 6.3

Effect of Benzyl Alcohol/Benzaldehyde
Mixtures on Fluphenazine Decanoate Oxidation in Sesame Oil

Sample	N-oxide(mg/g formulation)	
	Initial	1 Week ($20^{\circ}C$)
Control	Not detected	0.009
+ Benzyl alcohol containing 0.1% PhCHO	Not detected	0.028
+ Benzyl alcohol containing 0.5% PhCHO	Not detected	0.036
+ Benzyl alcohol containing 1.0% PhCHO	Not detected	0.019
+ Benzyl alcohol containing 5.0% PhCHO	Not detected	0.042
+ Benzyl alcohol containing 10.0% PhCHO	Not detected	0.020
+ Benzyl alcohol containing 50.0% PhCHO	Not detected	0.025
+ PhCHO alone	Not detected	0.023

Comparison of the results obtained for the Control sample and that containing added benzyl alcohol (pure) establishes an enhanced rate of N-oxide formation in the presence of the alcohol at both $20^{\circ}C$ and $40^{\circ}C$. The effect is conveniently illustrated by plotting the difference in N-oxide level evident between the control and the sample with added benzyl alcohol against time (Fig. 6.1). The difference can be seen to increase steadily, implicating benzyl alcohol to be a contributory factor in reducing the potential stability of Modecate.

By contrast, replacing benzyl alcohol with an equivalent amount of benzaldehyde has an apparent stabilising effect at $20^{\circ}C$, whereas at $40^{\circ}C$ a

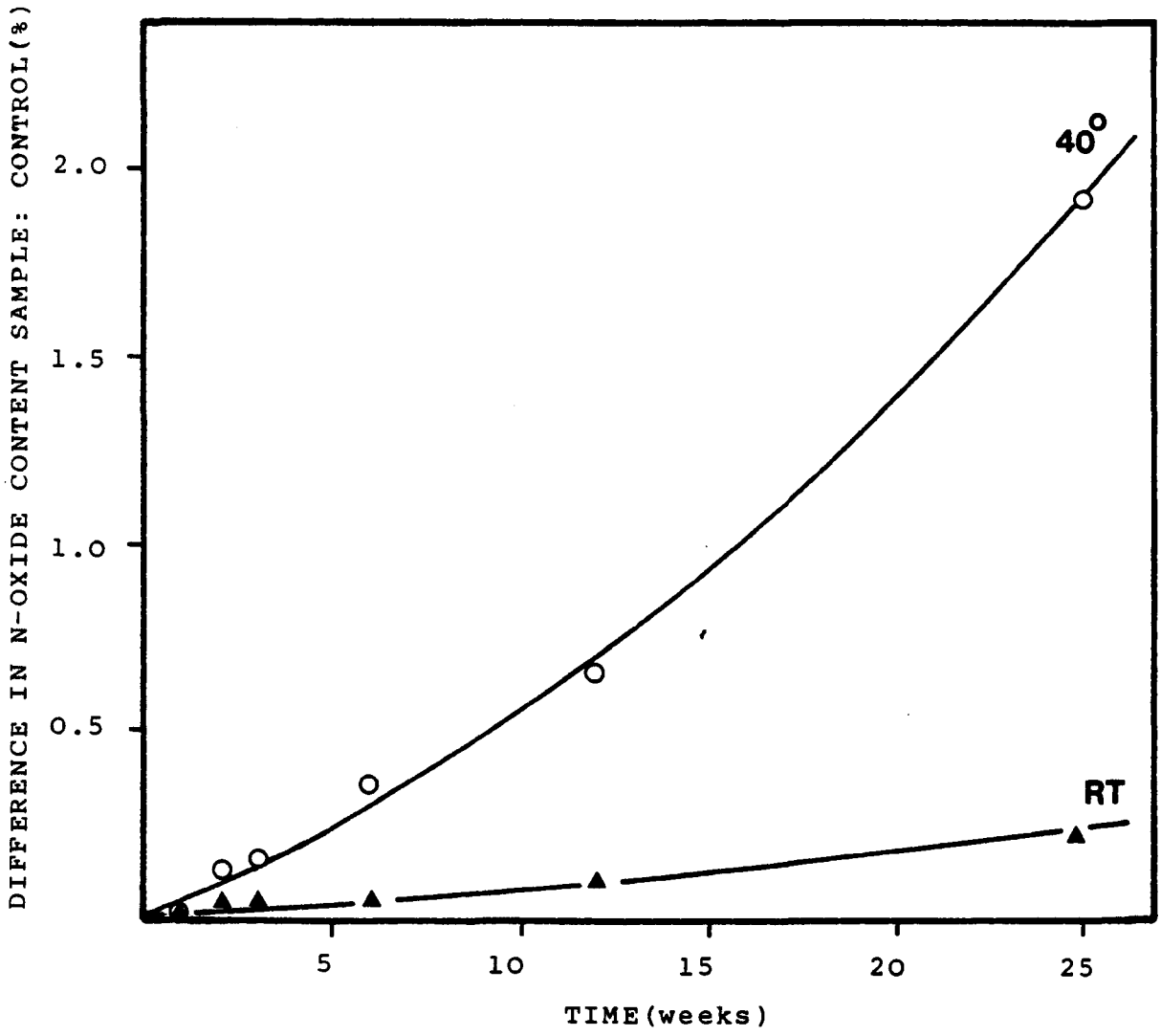


Fig. 6.1 - Effect of Adding Benzyl Alcohol to Fluphenazine Decanoate in Sesame Oil, Demonstrating the Enhanced Formation of N-Oxides in the Presence of the Preservative.

small increase in fluphenazine decanoate degradation is noted in comparison to the Control. However, assay values for N-oxide formed in the presence of benzaldehyde alone are less than those observed in the presence of pure benzyl alcohol, thus signifying that the direct reaction of oxygen with benzaldehyde, yielding perbenzoic acid[187], would be of little importance under the normal conditions of the product pack.

Addition of aged benzyl alcohols to the sesame oil solution of fluphenazine decanoate has considerable influence on the oxidation rate of the drug. The only known variable between the batches of aged benzyl alcohols was the concentration of benzaldehyde present in each. Table 6.1 indicates that the amount of fluphenazine decanoate N-oxide present after 1 week at room temperature is seemingly related to the benzaldehyde concentration, yet benzaldehyde alone had been shown to exert little effect on the oxidation of fluphenazine decanoate.

The improbable requirement of benzaldehyde and benzyl alcohol together being necessary for enhanced fluphenazine decanoate oxidation to occur was investigated using mixtures of pure benzyl alcohol and pure benzaldehyde. The results (Table 6.3) verify the remoteness of this possibility.

Consequently, the enigma of the large amounts of fluphenazine decanoate N-oxides formed in the presence of aged benzyl alcohol remained to be explained. A logical explanation of the effect is that an unidentified degradation product of benzyl alcohol was causing the observed enhancement of fluphenazine decanoate oxidation.

Work was thus orientated toward the identification of the oxidation factor present in aged benzyl alcohol and is described in Section 6.2.

6.2 Autoxidation of Benzyl alcohol

6.2.1 Introduction

Before commencing a detailed examination of the naturally aged benzyl

alcohols used in Section 6.1 above, a literature search was undertaken in order to determine the extent of knowledge concerning the natural degradation of benzyl alcohol ie. autoxidation. Surprisingly little information was gleaned from the wealth of published material connected with benzyl alcohol. Only one directly relevant report, by Howard and Korcek[220], was discovered. These authors determined the absolute rate constants for the oxidation of some -substituted toluenes, including benzyl alcohol. The autoxidation was described as proceding via an α-hydroxybenzylperoxy radical, PhCH(OH)OO·.

The formation of benzaldehyde following the autoclaving of a parental solution containing 0.9% benzyl alcohol as preservative has been noted[221] and was attributed to the oxidation of the benzyl alcohol by the oxygen present in the vial headspace. No mechanism for the reaction was considered.

An alternative possibility that benzaldehyde (formed from benzyl alcohol by an unknown mechanism) is autoxidised to benzoic acid via perbenzoic acid[222] was also identified.

Each of the above intermediate species (α-hydroxybenzyl peroxy radical and perbenzoic acid) are powerful oxidants and would readily oxidise fluphenazine decanoate. Thus the batches of naturally aged benzyl alcohols were studied in an attempt to quantitate and identify the nature of the suspected oxidant.

6.2.2 Examination of Naturally Aged Benzyl Alcohol

6.2.2.1 Peroxide Values

The peroxide value (P.V.) of each of the aged benzyl alcohols was determined by the iodometric procedure described in the British Pharmacopoeia, 1980[217]. The results (Table 6.4) clearly established the oxidising nature of the materials.

Table 6.4

Peroxide Values of Some Naturally Aged Benzyl Alcohols

Batch	Peroxide Value
A	11.4
B	113.8
C	203.4
D	345.8

By comparison, a freshly purchased benzyl alcohol (obtained from B.D.H. Chemicals) had a P.V. of zero, indicating that the oxidation factor present after ageing was attributable to a degradation product.

6.2.2.2 HPLC

An HPLC procedure reported for the determination of benzoic acid in syrups and elixirs[224] was adapted to the examination of benzyl alcohol and used in a search for the presence of perbenzoic acid in the naturally aged samples.

Experimental Conditions:

Column	:	ODS-Hypersil, 20cm
Mobile phase	:	Citrate buffer (pH4.2)-acetonitrile (9:1)

Perbenzoic acid was synthesised from benzaldehyde by photoxidation (sunlight) in an acetone solution saturated with oxygen[225]. The separation of the perbenzoic acid from benzyl alcohol and benzaldehyde under the proposed HPLC conditions was demonstrated (Fig. 6.2) prior to examination of the aged benzyl alcohols. Chromatography of each of the naturally aged materials failed to reveal the occurrence of perbenzoic acid.

To verify this observation 50ul aliquots of each aged benzyl alcohol were injected onto the HPLC column and the fractions of eluate repre-

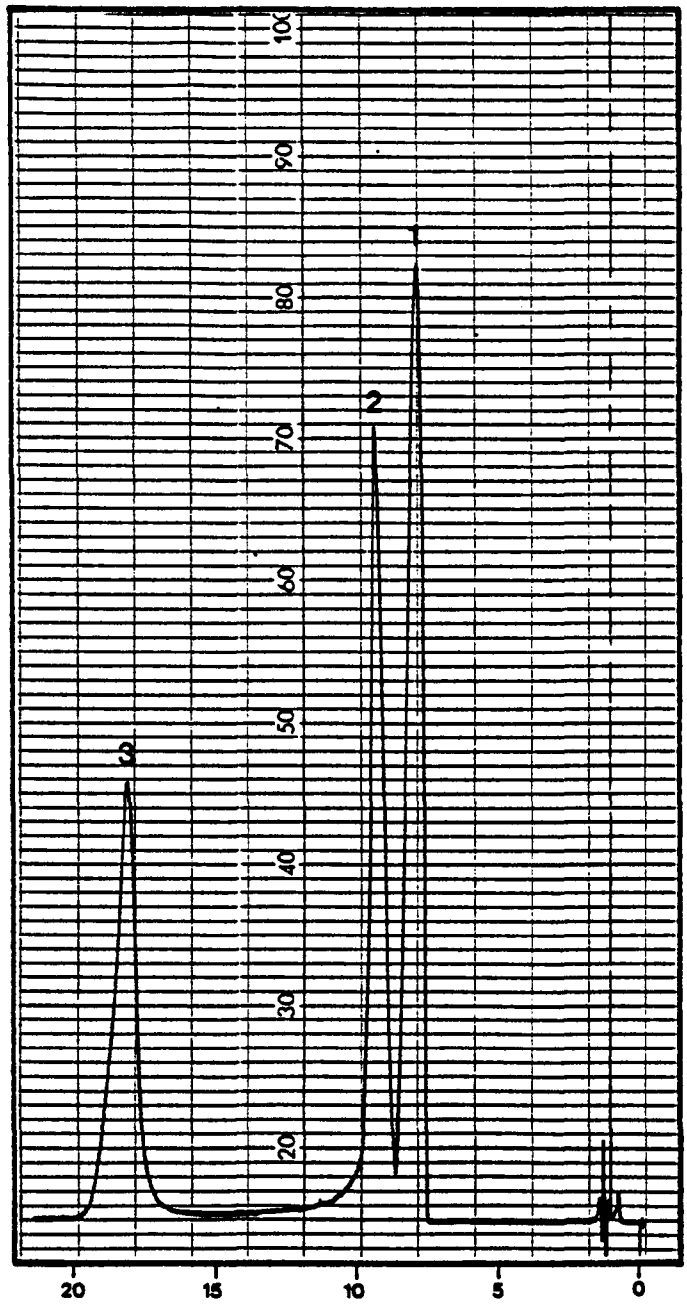


Fig. 6.2 - HPLC Separation of Perbenzoic Acid, Benzaldehyde and Benzyl Alcohol

1 = benzyl alcohol; 2 = perbenzoic acid;
3 = benzaldehyde.

senting a positive recorder response were collected and tested for the presence of peroxide with potassium iodide/acetic acid solution. The peroxide known to be present in these materials (Section 6.2.1) was shown to be eluted within the solvent-front, indicating the highly polar nature of the peroxide. No other potassium iodide-positive fractions were observed conclusively demonstrating the absence perbenzoic in the aged samples of benzyl alcohol.

6.2.2.3 Titration with Ceric Sulphate

Ce^{4+} is reduced by hydrogen peroxide according to the equation:

$$2Ce^{4+} + H_2O_2 \longrightarrow 2Ce^{3+} + 2H^+ + O_2$$

Organic hydroperoxides also react with ceric sulphate[226]; peracids, however, will not react. Hence the procedure has been utilised for the determination of hydrogen peroxide in the presence of peracetic acid. To prevent diacyl and dialkyl peroxides reacting the titration is normally conducted at 0-5°C[226].

Procedure: Approximately 2g of benzyl alcohol was accurately weighed into a 250-ml conical flask and dissolved in 10ml methanol. 50ml of 2M sulphuric acid was added, the mixture cooled to 0°C and the peroxide titrated with 0.05M ceric sulphate (BDH, Volucon) using Ferroin as indicator.

1ml 0.05M$Ce(SO_4)_2$ ≡ 0.825mg OOH

Validation: The reactivity of Ce^{4+} with chloroperbenzoic acid, benzaldehyde and freshly distilled benzyl alcohol was determined. In each case, 1g of material resulted in a titre value of zero, confirming the non-reactivity of these materials, which will thus not interfere if present during the titration of the aged benzyl alcohols.

Each of the naturally aged benzyl alcohols was subjected to the titrimetric procedure described above and the peroxide content calculated in terms of hydroperoxide (OOH). The results are given in Table 6.5 together with the results of the iodometric titre (Peroxide Value, Section 6.2.2.3) transposed from peroxide values to mg of hydroperoxide (OOH).

Table 6.5

Peroxide Content of Some Aged Benzyl Alcohols

Aged Benzyl Alcohol	OOH mg/g	
	Ce^{4+} titre($0^{o}C$)	I_2 titre
A	0.198	0.188
B	1.898	1.878
C	3.415	3.366
D	5.817	5.702

The results of the Ce^{4+} titre are in excellent agreement with the values obtained iodometrically indicating that all of the peroxide present is in the form of a hydroperoxide. These results confirm the absence of peracid (which is readily determined iodometrically[229]) thus discounting the possibility of a direct reaction between benzaldehyde and oxygen.

Each sample of aged benzyl alcohol was also titrated with Ce^{4+} at $20^{o}C$. Identical results were obtained at both $0^{o}C$ and $20^{o}C$ implying the absence of diacyl and dialkyl peroxides.

6.2.2.4 TLC

The samples of aged benzyl alcohol were examined by TLC to determine the impurity profile. Benzaldehyde and benzoyl peroxide were used as markers.

TLC Conditions:

Plate	:	Merck Silica Gel $F60_{254}$
Mobile phase	:	dichloromethane
Sample	:	50% solution of benzyl alcohol in dichloromethane. 20ul of solution was streaked onto the base-line of the TLC plate across a 2cm channel.
Visualisation:		a) UV lamp, 254nm
		b) potassium iodide/2M sulphuric acid spray.

All samples produced similar impurity profiles but with the impurities present in varying degrees. A typical chromatogram is shown in Fig. 6.3. Three important observations were noted:

1. Benzoyl peroxide was present in only trace amounts.
2. Two KI positive zones were present, one of which was less polar than benzyl alcohol and present in negligible proportions.
3. The majority of peroxide was present in the form of highly polar compound, Rf = zero.

6.2.2.5 Comparison of Benzaldehyde and Peroxide Levels

The benzaldehyde content of the aged benzyl alcohols were determined by an HPLC method[227] and compared with the level of peroxide, as determined by I_2 titration. (Section 6.2.2.3)

HPLC procedure

Column	:	ODS-Hypersil, 20cm.
Mobile phase	:	Acetonitrile - water (60 - 40)
Sample	:	1g of benzyl alcohol, accurately weighed, was diluted to 100ml with methanol.

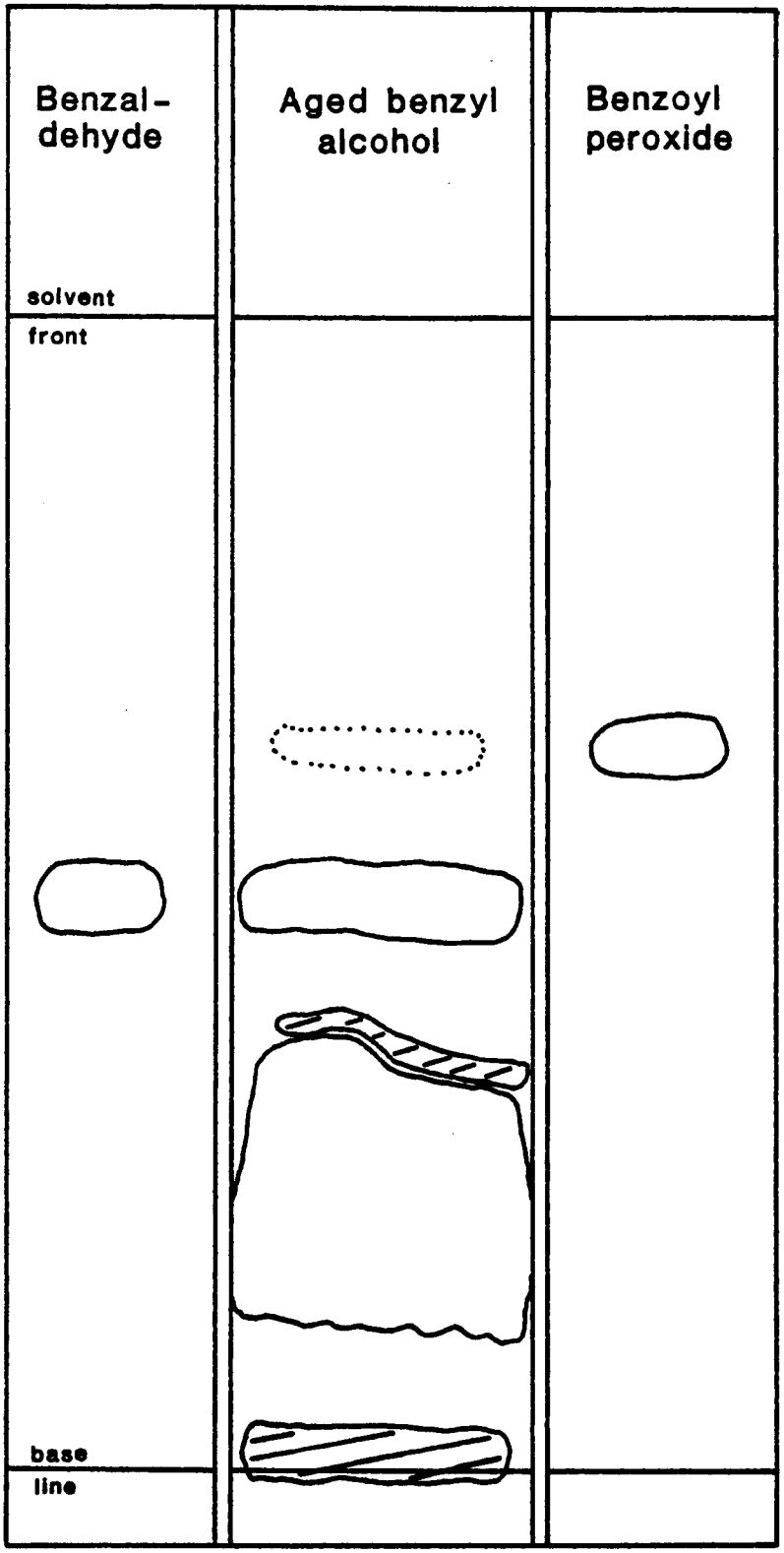


Fig. 6.3 - TLC Examination of Naturally Aged Benzyl Alcohol

Mobile phase, dichloromethane; layer, silica gel GF_{254}.

= visible under UV_{254}

= +ve to KI spray reagent.

Standard : 10ul of benzaldehyde were diluted to 100ml with methanol. 5ml of this solution were further diluted to 25ml with methanol.

The results are given in the following Table.

Table 6.6

Comparison of Benzaldehyde and Peroxide Levels of Some Aged Benzyl Alcohols

Batch	Benzaldehyde(mg/g)	Hydroperoxide(mg/g)
A	1.72	0.19
B	5.56	1.88
C	11.20	3.36
D	12.70	5.70

A graphical representation of the above results (Fig. 6.4) indicates that the levels of peroxide and benzaldehyde are related.

6.2.2.6 Discussion

The extensive examination of the aged samples of benzyl alcohol, described above, ascertained the formation of a hydroperoxide during ageing which is highly polar in nature. In addition a possible relationship between the benzaldehyde and hydroperoxide autoxidation products was identified.

Both of these observations are in agreement with the work of Howard and Korcek[220] who have suggested that benzyl alcohol does not oxidise directly to an organic hydroperoxide, as do other alcohols, but forms instead hydrogen peroxide and an aldehyde. The authors also postulated that the intermediate species is represented by α-hydroxy-

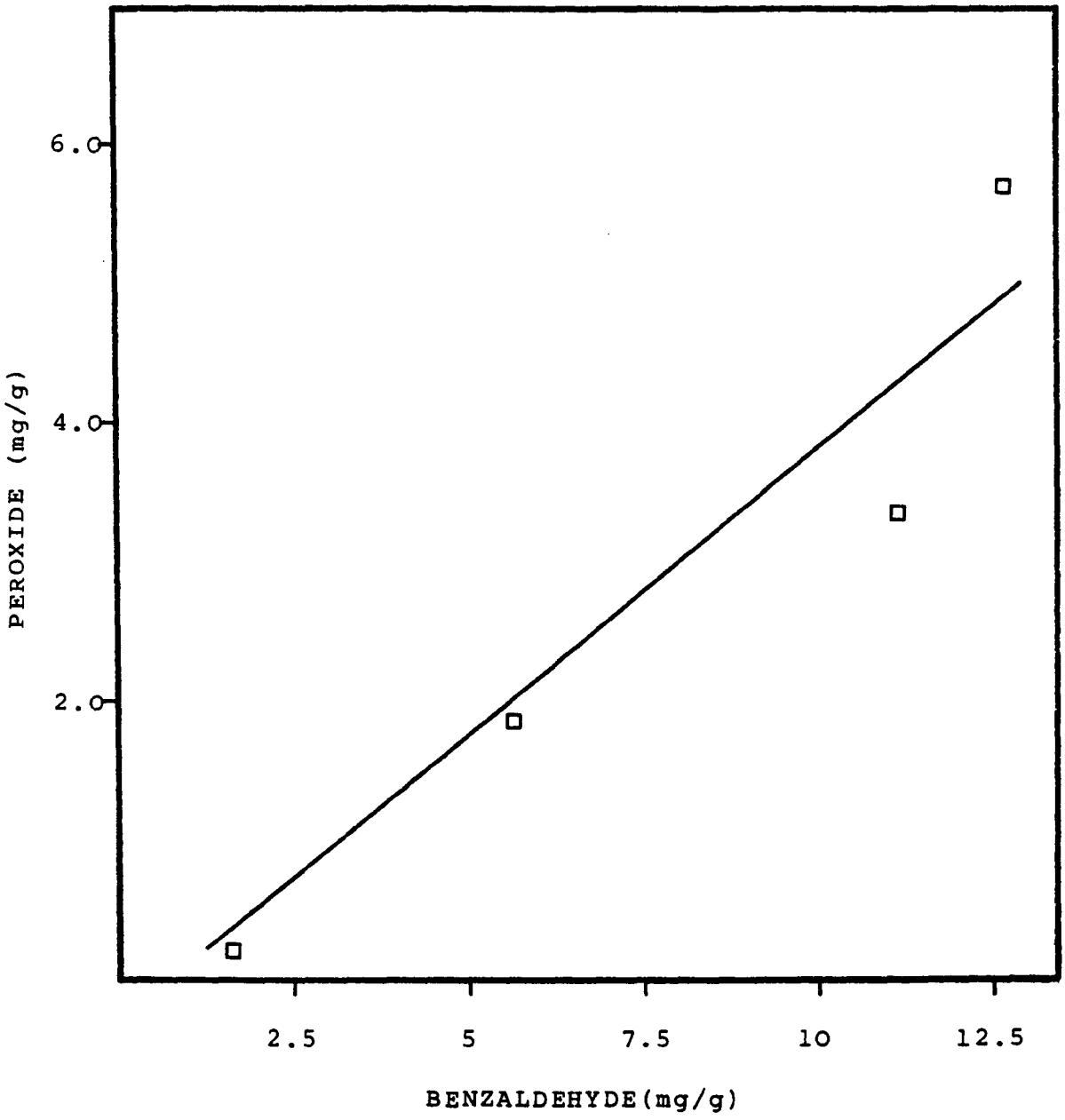
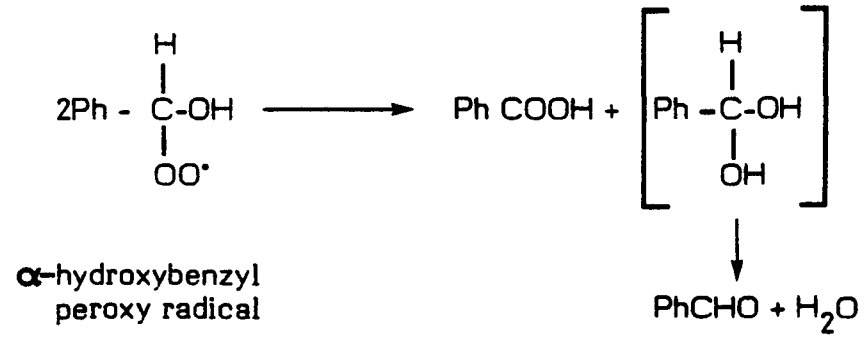


Fig. 6.4 - Relationship Between Benzaldehyde and Peroxide Content of some Naturally Aged Benzyl Alcohols.

benzylhydroperoxide and thus the following reactions were suggested to account for the formation of benzaldehyde.

a) 

b) $Ph\ CH(OH)OOH \longrightarrow PhCHO + H_2O_2$

Each of these mechanisms require the α-hydroxybenzylperoxy radical to be the chain propagating species and indeed the existence of this radical was confirmed by radical trapping experiments. Based on the observation that the total yield of carbonyl-compounds (IR measurements) was always in excess of the stoichiometric amount calculated from the concentration of oxygen absorbed during the autoxidation process, the authors concluded that carbonyl-compounds must also be formed in the radical termination step as in (a) above. If reaction (b) was the sole mode of aldehyde formation then the peroxide: aldehyde molar ratio present in the autoxidised material would be 1 : 1. From the results given in Table 6.6 the molar ratio of hydroperoxide: benzaldehyde for the samples of naturally aged benzyl alcohol was calculated to be:

Batch A	OOH: PhCHO =	1:2.82
B		1:0.96
C		1:1.04
D		1:0.69

The ratio obtained for batches B and C is evidence for only reaction (b) occurring during degradation whereas the greater than stoichiometric concentration of aldehyde present in Batch A suggests that

reaction (a) has occurred within this particular material. The anomolous results obtained for Batch D cannot be explained by reactions (a) or (b).

The nature of the peroxide formed during autoxidation of α-substituted toluenes was also considered in the paper by Howard and Korcek[220]. To differentiate between organic hydroperoxide and hydrogen peroxide an aqueous partition method was adopted, whence the organic hydroperoxide remained in the organic phase but hydrogen peroxide partitioned into the aqueous phase. By this means the authors demonstrated that the peroxide present in autoxidised benzyl alcohol was hydrogen peroxide. However, the failure to consider the aqueous-solubility of benzyl alcohol renders the conclusions suspect. Benzyl alcohol is soluble in water to the extent of 4% at 20°C. It may therefore be reasonably expected that α-hydroxybenzylhydroperoxide, which is a more polar molecule than benzyl alcohol, would have a considerable aqueous solubility. Hence the "identification" of the peroxide present in autoxidised benzyl alcohol based on the aqueous solubility of the material must be seriously questioned.

Two facets of benzyl alcohol autoxidation therefore warranted further investigation:

(i) the nature of the peroxide formed.

(ii) the reaction mechanism involved in the formation of benzaldehyde.

6.2.3 Mechanism of Benzaldehyde Formation

6.2.3.1 Benzoic Acid Content of Naturally Aged Benzyl Alcohol

The published literature had indicated two possible mechanisms by which benzaldehyde could be formed from benzyl alcohol during autoxidation (Section 6.2.2.6). If benzaldehyde is formed by the interaction of two α-hydroxybenzyl peroxyl radicals (radical termination step), then formation of benzaldehyde will be accompanied by formation of benzoic

acid (equation (a) Section 6.2.2.6) and the molar ratio of the two components will be 1:1. The benzoic acid content of the naturally aged benzyl alcohols was thus determined. A suitable analytical procedure was devised by modification of the HPLC method utilised previously for the determination of perbenzoic acid (Section 6.2.2.2). By substituting acetate buffer for the original mobile phase containing citrate buffer and increasing the pH to 4.5, an improved separation of benzoic acid from the other components present (benzaldehyde and benzyl alcohol) was achieved.

HPLC column:	ODS-Hypersil, 20cm
Acetate buffer:	3.86% sodium acetate solution adjusted to pH 4.5 with acetic acid.
Mobile phase:	pH 4.5 acetate buffer/ acetonitrile (3 - 1)
Standard:	50mg benzoic acid was dissolved in and diluted to volume with methanol. A further x 5 dilution was prepared.
Sample:	Diluted as necessary with methanol (typically x 2)

A typical sample chromatogram is shown in Fig. 6.5. The benzoic acid content of the samples examined is given in Table 6.7.

Table 6.7

Benzoic Acid Content of some Naturally Aged Benzyl Alcohols

Batch	Benzoic Acid Content	
	mg/g	uM
A	0.062	0.57
C	1.455	11.93
F	0.214	1.75

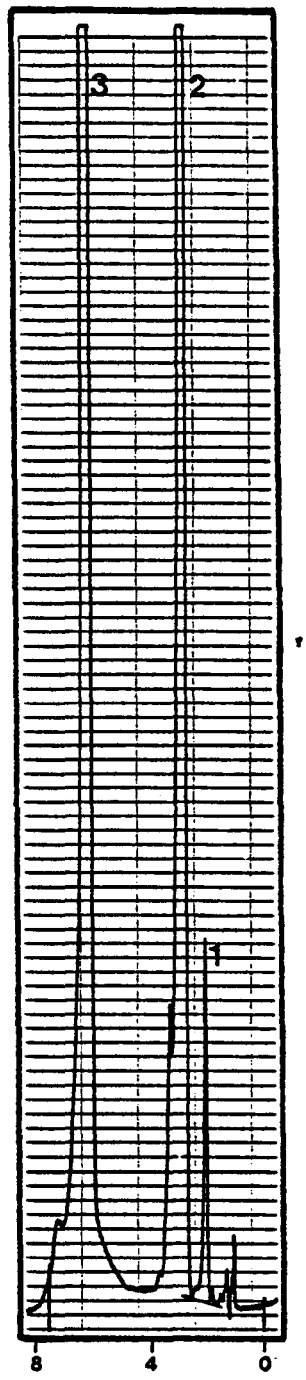


Fig. 6.5 - HPLC Determination of Benzoic Acid - Typical Chromatogram

Mobile phase, pH 4.5 acetate buffer - acetonitrile (3-1); column, ODS-Hypersil.
1 = benzoic acid; 2 = benzyl alcohol;
3 = benzaldehyde.

Assuming that the radical termination step postulated by Howard and Korcek is the only means of benzoic acid formation, equimolar quantities of benzoic acid and benzaldehyde would be produced simultaneously. The fraction of benzaldehyde formed during radical termination can therefore be predicted from the benzoic acid results. A comparison of this value with the total benzaldehyde content (determined previously) is given in Table 6.8.

Table 6.8

Comparison of Benzaldehyde Contents

Batch of benzyl alcohol	Total PhCHO Content		Predicted PhCHO formed during radical termination		
	mg/g	uM	mg/g	uM	% of Total
A	1.72	16.23	0.062	0.51	3.14
C	11.20	105.66	1.455	11.93	11.30
F	0.77	7.26	0.186	1.75	24.10

The figures above indicate that relatively little aldehyde is formed by this route in Batches A and C. Batch F however may be indicative that the mechanism is feasible in some benzyl alcohols.

6.2.3.2 Forced Autoxidation of Benzyl Alcohol - Ratio of Benzaldehyde and Peroxide

The value of the benzaldehyde: peroxide ratio is important since a reliable figure for this molar ratio could be used to confirm, or reject, the direct relationship between the two entities postulated by Howard and Korcek[220] ie. that both are formed as a result of the decomposition of the intermediate autoxidation product, α-hydroxybenzylhydroperoxide.

$$PhCH(OH)OOH \longrightarrow PhCHO + H_2O_2$$

The aldehyde/peroxide ratio of four naturally aged benzyl alcohols (examined previously) had varied considerably. As the history of these samples was unknown, it was considered likely that different modes and times of ageing could have contributed to the observed variability. Thus it was proposed to study the autoxidation of benzyl alcohol under rigidly controlled conditions. Two sets of experimental conditions were chosen to represent varying degrees of severity and can be classed as

(i) static autoxidation

(ii) dynamic autoxidation

Experimental Conditions

(i) Static autoxidation

A pure benzyl alcohol (BDH Chemicals) was tested for the absence of benzaldehyde and peroxide. 100ml of the material was transferred to a 250-ml conical flask and the liquid and headspace sparged with oxygen. The stoppered flask was then stored in the dark in a thermostated environment at 40°C. Aliquots of the alcohol were withdrawn at intervals and the benzaldehyde and peroxide contents determined simultaneously. The headspace above the alcohol was flushed with oxygen following the removal of an aliquot for assay to ensure that an atmosphere of oxygen was above the alcohol at all times. Oxygen was chosen in preference to air in order to accelerate the autoxidation reaction.

(ii) Dynamic Autoxidation

50ml of pure benzyl alcohol was transferred to a large test-tube and suspended in a thermostated oil-bath at 80°C. Compressed air was bubbled through the alcohol via a sintered-bubbler at a constant flow-rate of 50ml/min. The flow-rate was continually monitored with a flow-meter. Aliquots of benzyl alcohol were withdrawn at intervals and the benzaldehyde and peroxide levels determined simultaneously.

Benzaldehyde was determined by HPLC (Section 6.2.2.5) and peroxide by iodometric titration (Section 6.2.2.5 and 5.2.1).

Results and Discussion

The results obtained are summarised in Tables 6.9 and 6.10. At each sampling station, under either static or dynamic conditions, the ratio of peroxide to benzaldehyde is extremely close to the 1:1 value required for the direct formation of the two entities by dissociation of α-hydroxybenzylhydroperoxide.

Furthermore, benzoic acid levels in naturally aged benzyl alcohols (Section 6.2.3.1) indicated that the alternative route for benzaldehyde formation postulated by Howard and Korcek[220] is likely to be of minor importance. Hence both experiments provide evidence in support of the theory that benzaldehyde is formed mainly via an α-hydroxybenzylhydroxperoxide moiety during the autoxidation of benzyl alcohol.

6.2.4. Identity of Peroxide Formed During Benzyl Alcohol Autoxidation

Reaction of fluphenazine decanoate with a variety of autoxidised media has demonstrated the variable rate at which the drug is oxidised by differing hydroperoxides. (Chapter 5). Thus it is important to identify the peroxide present in autoxidised benzyl alcohol so that the rate of fluphenazine decanoate oxidation by this entity can be determined. The rate may then be compared with similar rates obtained for other hydroperoxides and the relative importance of the reaction, with respect to product stability, can be assessed.

The hydroperoxide formed initially during benzyl alcohol autoxidation has been reported as α-hydroxybenzylhydroperoxide but is ultimately believed to dissociate into hydrogen peroxide and benzaldehyde[220,230]. The equilibrium constant for this reaction is unknown and hence the relative proportions of the two peroxides present in ageing benzyl alcohol cannot be predicted.

Table 6.9

Autoxidation of Benzyl Alcohol - Static Conditions, 40°C

Time (days)	Benzaldehyde Content mg/ml	Benzaldehyde Content mM	Hydroperoxide Content mg/ml	Hydroperoxide Content mM	Molar Ratio OOH: PhCHO
Initial	0.039	0.368	0.0132	0.40	1: 1.09
22	0.273	2.57	0.066	2.00	1: 0.78
27	0.422	3.98	0.155	4.70	1: 1.18
33	1.088	10.26	0.317	9.60	1: 0.93
36	1.44	13.60	0.561	17.00	1: 1.25
42	3.00	28.30	1.007	30.50	1: 1.08

Table 6.10

Autoxidation of Benzyl Alcohol - Dynamic Conditions, 80°C

Time (hours)	Benzaldehyde Content mg/ml	Benzaldehyde Content mM	Hydroperoxide Content mg/ml	Hydroperoxide Content mM	Molar Ratio OOH: PhCHO
Initial	0.092	0.868	0.023	0.696	1: 1.24
1	0.113	1.066	0.037	1.000	1: 1.06
2	0.148	1.396	0.040	1.212	1: 1.15
4	0.324	3.066	0.086	2.616	1: 1.18
5	0.719	6.783	0.224	6.788	1: 1.00
6	1.649	15.56	0.475	14.393	1: 1.08

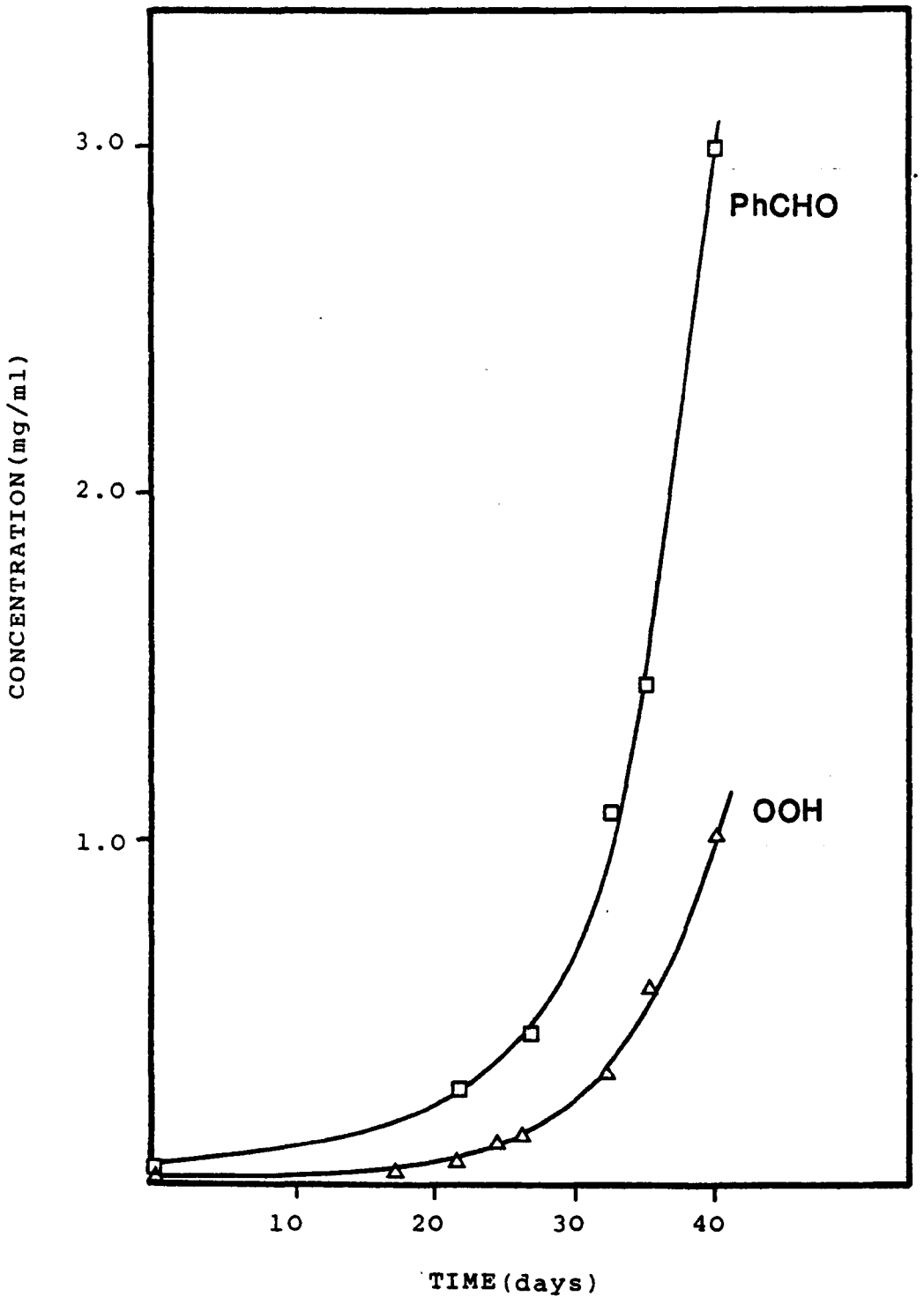


Fig. 6.6 - Comparison of Peroxide and Benzaldehyde Content of an Autoxidising Benzyl Alcohol (40°C, static conditions)

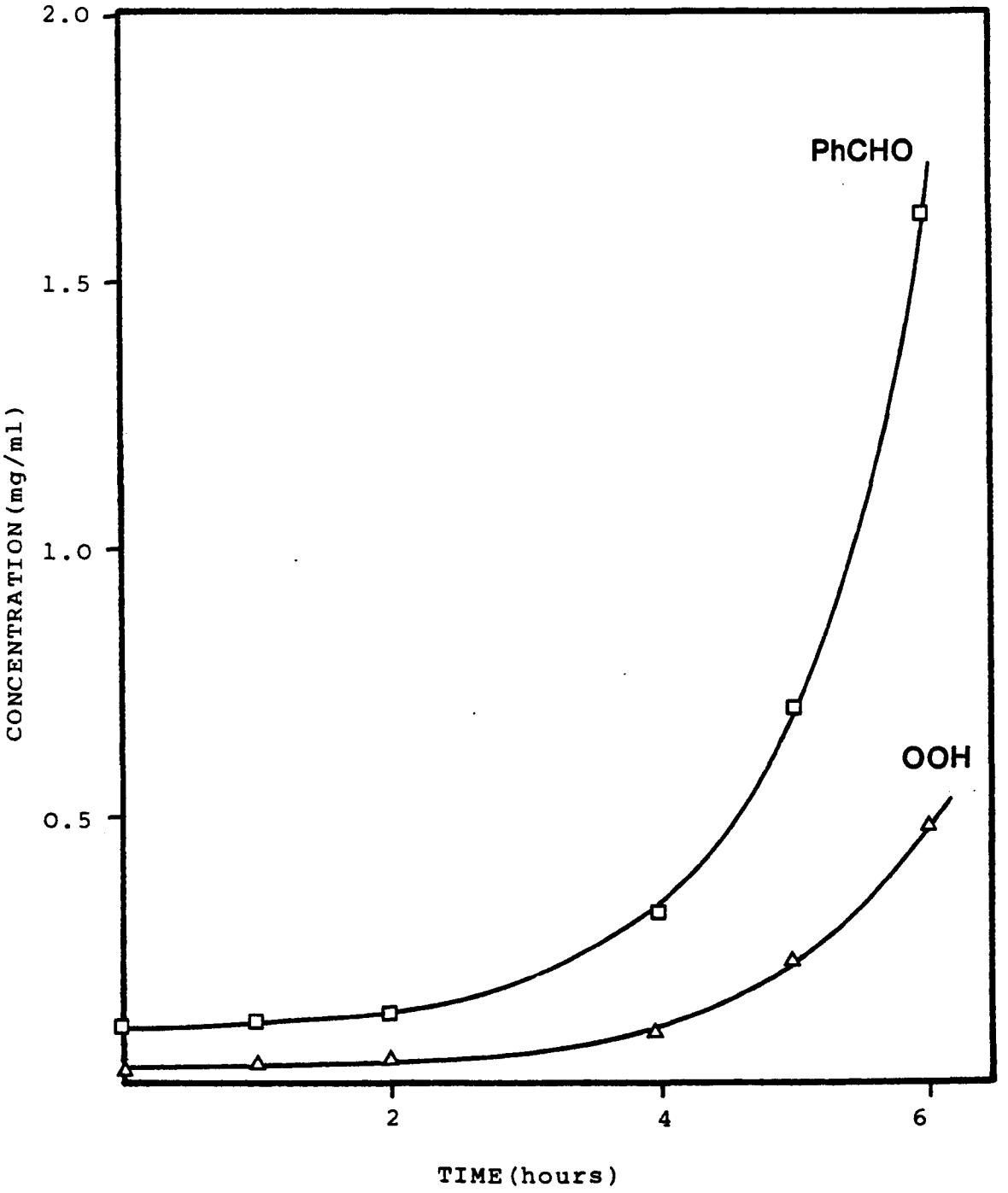


Fig. 6.7 - Comparison of Peroxide and Benzaldehyde Content of an Autoxidising Benzyl Alcohol (80°C, dynamic conditions)

Without a quantity of the pure α-hydroxy compound, it is impossible to determine a value for the equilibrium constant. Indeed, the material may be unstable, spontaneously decomposing to hydrogen peroxide and benzaldehyde and thus be impossible to isolate. As an alternative means of determining the position of equilibrium existing, the ratio of benzaldehyde: peroxide may be determined. However, the assay procedures used to quantify this value must not affect the equilibrium so that a true value of the ratio may be obtained.

Dissociation of α-hydroxyalkylhydroperoxides occurs in aqueous solution and is catalysed by acid and alkali[230]. Consequently the presence of water in the HPLC mobile phase utilised for assay of benzaldehyde, and of water and acid in the titrimetric procedure for peroxide determination could be the explanation of the consistent 1:1 ratio of peroxide: benzaldehyde noted in Section 6.2.3. The effect of sample diluent on the value obtained for benzaldehyde was thus investigated. In addition alternative assay procedures for determining benzaldehyde, which did not require use of water, acid or other highly polar solvents such as methanol, were sought.

6.2.4.1 Effect of Sample Preparation on Benzaldehyde HPLC Assay

Water is present in the HPLC mobile phase and could, if dissociation of α-hydroxyalkylhydroperoxide is a fast reaction, be converted to hydrogen peroxide and aldehyde upon injection of the organic solution onto the column. To overcome any effect which could be attributable to the mobile phase aqueous component, autoxidised benzyl alcohol (naturally aged, Batch A) was diluted with water during sample preparation. The benzaldehyde content of this solution was compared with similar solutions diluted with 1M sulphuric acid and with methanol.

The benzaldehyde content of the aqueous dilution was noted to be identical to that of a dilution prepared in methanol (Table 6.11). In contrast the benzaldehyde level of the acid dilution steadily increased

over a period of 20 min., subsequently decreasing in value until approximately equal to the initial figure (Table 6.11 and Fig. 6.8). Such an increase would be expected if the acid caused dissociation of the α-hydroxyalkylhydroperoxide to occur, but the subsequent decrease in benzaldehyde value is difficult, if not impossible to explain on these results alone.

6.2.4.2 Aqueous Extraction of Naturally Aged Benzyl Alcohol

Methanolic and aqueous dilution of naturally aged benzyl alcohols resulted in identical values for benzaldehyde upon assay. In order to determine whether the peroxide present in the aqueous solution was in the form of α-hydroxybenzylhydroperoxide or hydrogen peroxide, the peroxide was extracted from the benzyl alcohol with water and the benzaldehyde level before and after acidification of the aqueous extract determined. If α-hydroxybenzylhydroperoxide is present, the addition of acid will cause dissociation and a corresponding increase in benzaldehyde concentration.

Experimental

Naturally aged benzyl alcohol was extracted with an equal volume of water and the aqueous layer separated by centrifugation. Absence of peroxide in the benzyl alcohol layer was established using a test solution of potassium iodide and sulphuric acid. The aqueous fraction was washed (X2) with dichloromethane to remove residual benzyl alcohol, again separating the layers of centrifugation. The concentration of benzaldehyde in the aqueous extract was determined by an HPLC method before and after the addition of acid. Total peroxide content was determined iodometrically.

Results and Discussion

No benzaldehyde was detected in the aqueous extract before acidification. Following the addition of acid, benzaldehyde concentration

Table 6.11

Benzaldehyde Content of Some Aged Benzyl Alcohols(mg/ml)

Methanol solution	Aqueous solution	1M sulphuric acid solution	
2.29	2.37	Initial*	2.57
		5.0 min.	2.64
		8.4 min.	2.83
		12.2 min.	2.95
		16.0 min.	3.09
		20.2 min.	3.03
		27.4 min.	2.41
		36.0 min.	2.44

* Immediately after dilution.

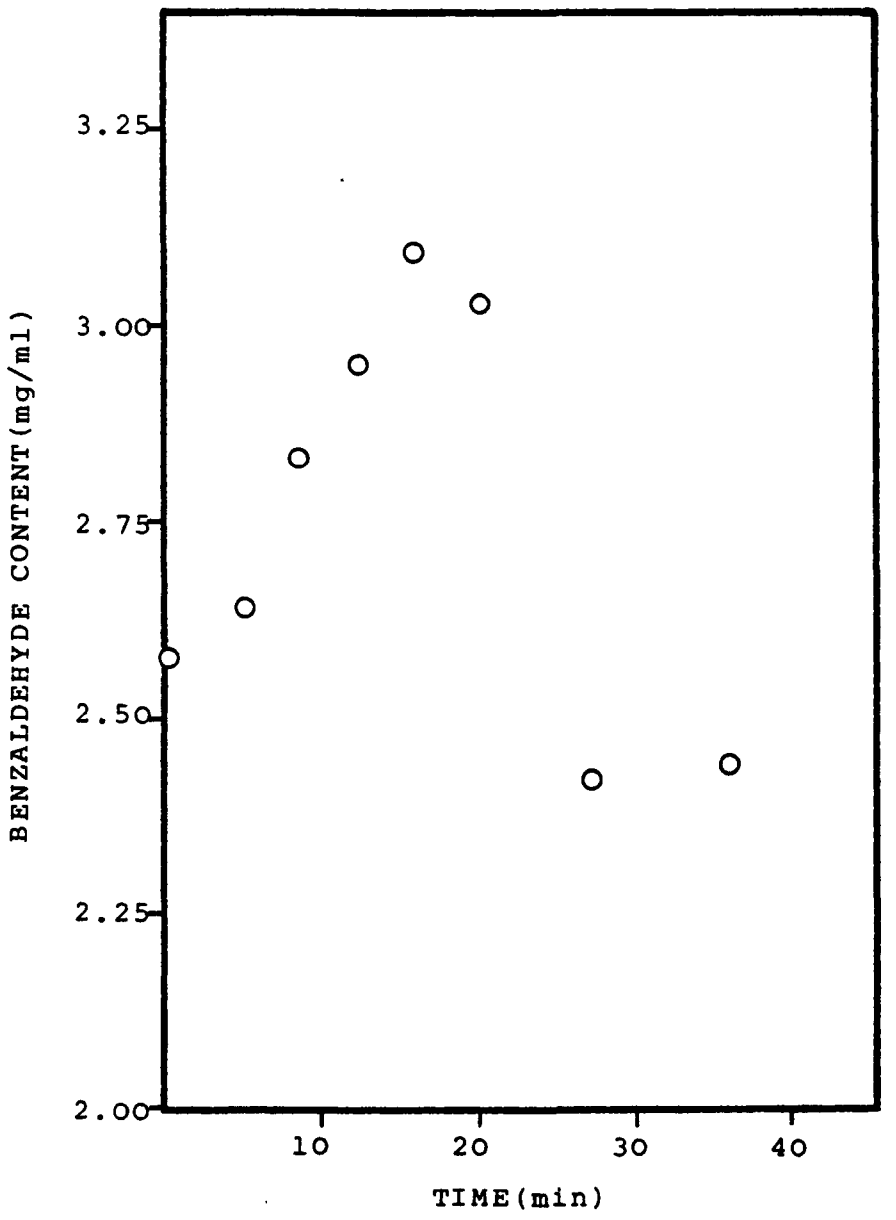


Fig. 6.8 - Effect of Addition of Acid on Benzaldehyde Content of a Naturally Aged Benzyl Alcohol

was determined to be 0.029 mg/ml. From this increase in benzaldehyde content, the equivalent concentration of α-hydroxybenzylhydroperoxide was calculated at 0.039mg/ml, which is equivalent to 0.0091mg/ml of hydroperoxide (OOH). The total hydroperoxide content of the aqueous extract, calculated as OOH, was 0.301mg/ml thus the amount of peroxide attributable to α-hydroxybenzylhydroperoxide is approximately 3% of the total. The majority of peroxide present in the aqueous extract is therefore represented by hydrogen peroxide.

6.2.4.3 Alternative Assay for Benzaldehyde - UV Spectrophotometry

As an alternative means of assessing the true level of benzaldehyde in aged benzyl alcohol, and hence the extent of dissociation of α-hydroxybenzylhydroperoxide, an assay procedure was sought which would be free from influences affecting the natural equilibrium present in the alcohol. A simple UV spectrophotometric procedure for determining the benzaldehyde content of commercial benzyl alcohol has been described in the literature[231] and was applied to the batches of naturally aged benzyl alcohol. The method is valid only in the absence of other impurities or degradation products which absorb UV radiation at 283nm.

Experimental Conditions

Naturally aged benzyl alcohol was diluted with various solvents and the UV absorption of the solution determined at 283nm. Quantitation of benzaldehyde was achieved by comparing the sample with a standard solution prepared in the same solvent.

Results and Discussion

The results of assay are given in Table 6.12.

Table 6.12

Benzaldehyde Content(mg/ml) of Some Naturally Aged Benzyl Alcohols (Spectrophotometric Assay)

Benzyl Alcohol	Diluent		
	Hexane	Methanol/ Water (50-50)	Methanol/ 2M H_2SO_4 (50-50)
Batch A	3.36	3.08	3.18
C	12.47	12.00	14.86
E	0.41	0.41	
F	2.37	2.16	

During the HPLC determination of benzaldehyde following acidification of a benzyl alcohol solution (Section 6.2.4.1), benzaldehyde concentration was observed to increase, then subsequently decrease in value over a period of 35 min. The UV absorbtion measurements above were thus determined at intervals throughout a 35 min. period. After 15 minutes only Batches B and E exhibited an increase in absorption. Batch B retained the new absorption value throughout the 35 minute interval whereas the absorbance of Batch E slowly decreased to the original value. Batches A and C, in contrast exhibited no change in absorbance with respect to time. The variable effect of acid addition observed in this and previous experiments suggests that a reaction is occurring which is related to an unknown impurity or degradation product present in only occasional batches of benzyl alcohol.

The true value of benzaldehyde concentration is most likely to be that obtained by assay of the benzyl alcohol diluted with hexane, since this non-polar inert solvent is unlikely to influence the dissociation of the α-hydroxyalkylhydroperoxide species, if present. The similar assay values apparent for hexane and methanol/water solutions of benzyl alcohol indicates that the addition of water, has had little effect on any

equilibrium that may exist within the aged benzyl alcohol. By analogy the assay values for benzaldehyde obtained via the HPLC procedure would be unaffected by the mobile-phase aqueous component and therefore also represent actual values for benzaldehyde content.

6.2.4.4 Determination of Benzaldehyde by an IR Spectrophotometric Procedure

To obtain further evidence that the HPLC assay gives a true value for the benzaldehyde content ie. the assay procedure has no influence on the equilibrium concentration, an IR assay for total carbonyl compounds was developed. Although benzoic acid will interfere, the level of this compound present in Batches A and C of the aged benzyl alcohols (determined by an HPLC method; Section 6.2.3.1) was known to be negligible in comparison to the benzaldehyde content (about 5%). A high bias may thus be expected but the procedure will be of sufficient precision for the envisaged purpose.

Experimental

Hexane was initially investigated as diluent for the benzyl alcohol samples, but was ultimately found to possess a high background absorption (1mm cell) in the 1600-1800cm^{-1} region (carbonyl stretching frequency). Dichloromethane was subsequently chosen, being almost free from background absorption in the required spectral region.

Linearity of response for benzaldehyde was determined by adding aliquots (0-10ul) of benzaldehyde to 5ml (volumetric flask) of a 50% solution of freshly distilled benzyl alcohol in dichloromethane. The IR spectrum of each solution was recorded and the response of the carbonyl stretching frequency at about 1700 cm^{-1} measured in terms of % transmission. Absorbance values were then calculated from the relationship

$$\text{absorbance} = \log \frac{1}{T} \qquad \text{where, } T = \text{transmission}$$

A plot of absorbance against concentration (Fig. 6.9) indicated that a deviation from linearity was apparent beyond a benzaldehyde concentration of 1.25mg/ml solution (equivalent to 2.5mg/ml benzaldehyde in the original benzyl alcohol).

Sample preparation was therefore adjusted by dilution so that the final concentration of benzaldehyde present in solution was within the linear range of the assay. A standard calibration graph was constructed concurrently with sample examination. A typical sample spectrum is illustrated in Fig. 6.10.

Results and Discussion

The results are tabulated below, where they are also compared with HPLC assay values (methanol solution) determined on the same day.

Table 6.13

Comparison of IR and HPLC Assay Values for the Benzaldehyde Content of some Naturally Aged Benzyl Alcohols

Benzyl Alcohol	Benzaldehyde Content (mg/ml) IR	HPLC
A	1.92	2.27
C	11.45	10.46
E	0.32	0.23
F	1.49	0.77

The IR results are in good agreement with HPLC values with the exception of Batch F. The fractionally higher results for the IR assay of batches A and C were not unexpected since these materials were known to contain small amounts of benzoic acid.

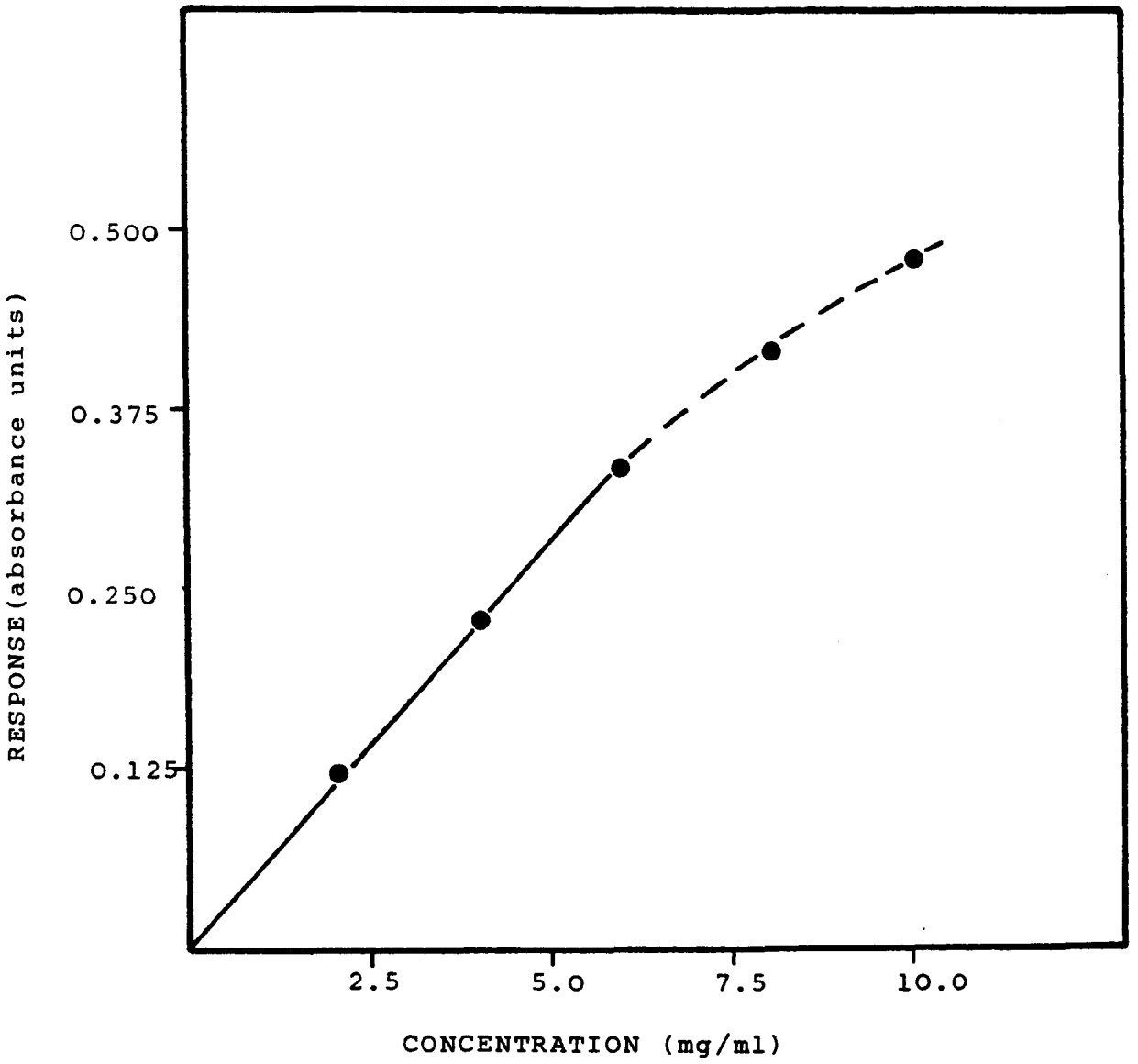


Fig. 6.9 - Linearity of Response, IR Assay for Benzaldehyde

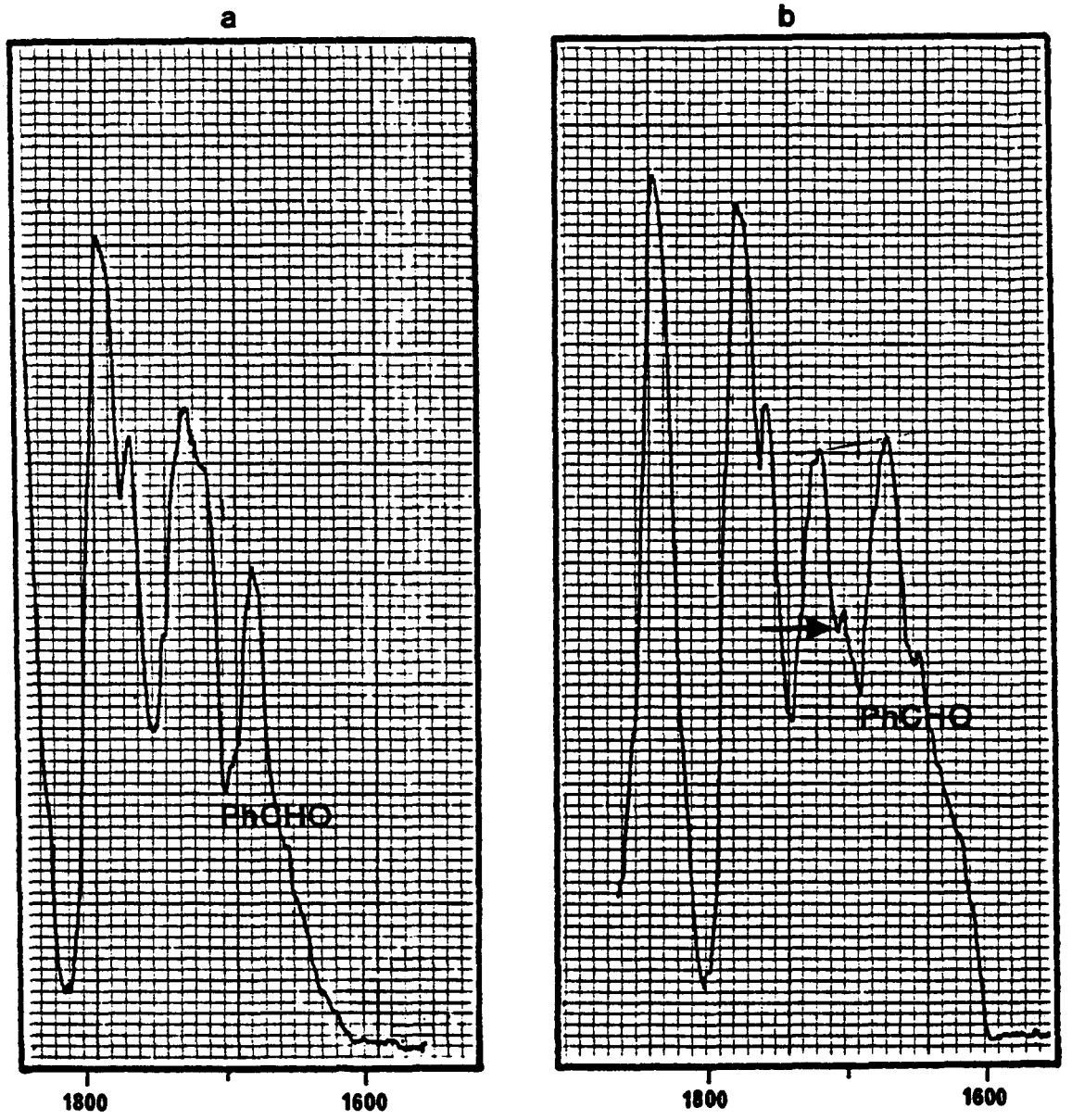


Fig. 6.10 - IR Determination of Benzaldehyde in Benzyl Alcohol

a) normal aged sample of benzyl alcohol

b) abnormal benzyl alcohol showing presence of interfering absorption.

The vastly different assay values noted for Batch F is probably due to the relatively high level of benzoic acid present in this material (approx. 25% of benzaldehyde content). Comparison of the IR spectrum obtained for Batch F with the spectra of the other aged benzyl alcohols clearly shows an interfering absorption adjacent ($1720cm^{-1}$) to the aldehyde carbonyl stretching frequency ($1705cm^{-1}$) which may be attributable to benzoic acid. (Fig. 6.10)

6.2.4.5 GC Assay for Benzaldehyde

For this determination a Squibb Q.C. procedure[232] was utilised.

Experimental Conditions

GC column	:	15% carbowax on Universal support.
Temperature	:	$150^{o}C$
Injection heater	:	$200^{o}C$
Detector	:	F.I.D.
Gases	:	N_2(carrier gas),60ml/min O_2, 600ml/min H_2, 60ml/min
Sample preparation	:	Naturally aged benzyl alcohols were diluted with cyclohexane.
Standard preparation	:	50mg benzaldehyde was diluted to 50ml with cyclohexane.

Results and Discussion

The results obtained are compared with HPLC assays of the same materials in Table 6.14. A typical chromatogram is illustrated in Fig. 6.11.

Table 6.14

Comparison of GC and HPLC Assay Values for Benzaldehyde Content of Aged Benzyl Alcohol

Benzyl Alcohol		Benzaldehyde Content mg/ml GC	HPLC
Batch	A	4.98	2.27
	C	17.16	10.46
	E	0.87	0.23
	F	2.20	0.77

The most striking feature of the results is that the GC procedure produces values which are approximately twice as high as those obtained by HPLC. Two postulates can be offered to account for this phenomenon:

(i) that α- hydroxybenzylhydroperoxide is thermally decomposed at the injection point yielding increased benzaldehyde values.

(ii) that the hydrogen peroxide present in the aged benzyl alcohol reacts in the gaseous phase at the injection point, oxidising benzyl alcohol to benzaldehyde via a mechanism other than that which requires formation of α- hydroxybenzylhydroperoxide. (Howard and Korcek[220]).

The results of Section 6.2.2.6 suggest that peroxide and benzaldehyde are present in equimolar quantities in Batch C, thus all peroxide must be present as hydrogen peroxide if the Howard & Korcek relationship is valid:

$$PhCH(OH)OOH \longrightarrow PhCHO + H_2O_2$$

Batch A contains a greater molar concentration of benzaldehyde than peroxide, thus again all peroxide present should be in the form of H_2O_2. Assuming that the results of Section 6.2.2.6 are not influenced by the assay procedure, as seems improbable from subsequent work (Section 6.2.4.3), then the additional amount of benzaldehyde found during GC assay cannot result from the

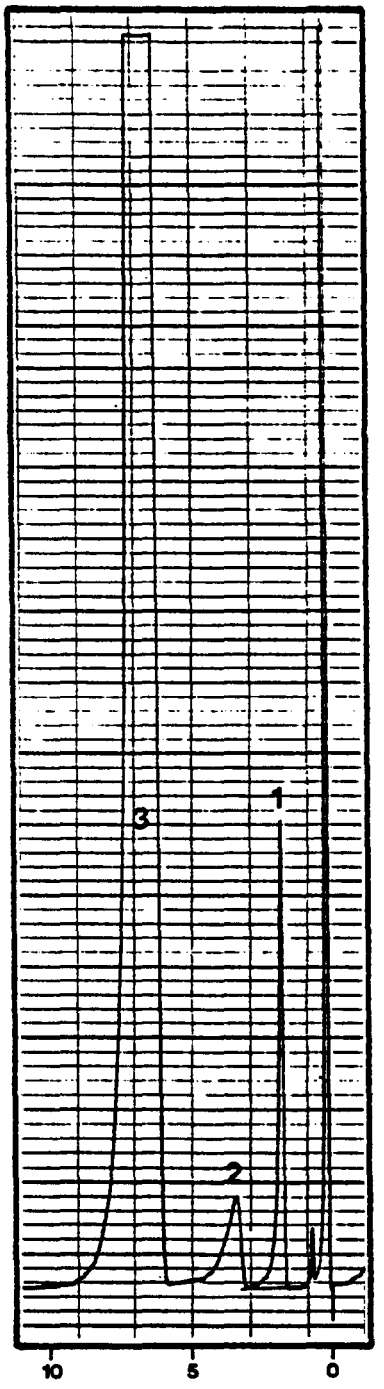


Fig. 6.11 - GC Determination of Benzaldehyde Content of Benzyl Alcohol - Typical Chromatogram

1 = benzaldehyde; 2 = unknown;
3 = benzyl alcohol.

decomposition of an α-hydroxyalkylhydroperoxide since this entity is apparently absent. The most favourable explanation of the observed phenomenon must therefore be that of (ii).

6.2.4.6 TLC of Peroxide

A TLC procedure for the chromatography of hydrogen peroxide[233] was applied to the samples of naturally aged benzyl alcohol.

Experimental Conditions

Plate	:	Merck, Cellulose F_{254}
Mobile phase	:	Water/n-butanol/ether (1-10-10)
Visulisation	:	a) UV lamp, 254nm b) KI/H_2SO_4 spray

The results are shown in Fig. 6.12. Only one peroxide zone was observed with an R_f value identical to that of hydrogen peroxide.

6.2.5 Conclusions

The formation of benzaldehyde in autoxidising benzyl alcohol probably occurs via an α-hydroxybenzylhydroperoxide intermediate, as postulated by Howard and Korcek[220]. An investigation of the peroxide present in the autoxidising material provided evidence that hydrogen peroxide is the major form of peroxide produced. The existence of small quantities of the α-hydroxybenzylhydroperoxide cannot be entirely excluded on the available evidence, but results indicate that this entity must be >95% dissociated to benzaldehyde and hydrogen peroxide.

An alternative route of benzaldehyde formation via radical termination involving two α-hydroxybenzylperoxy radicals was shown to be unlikely to occur.

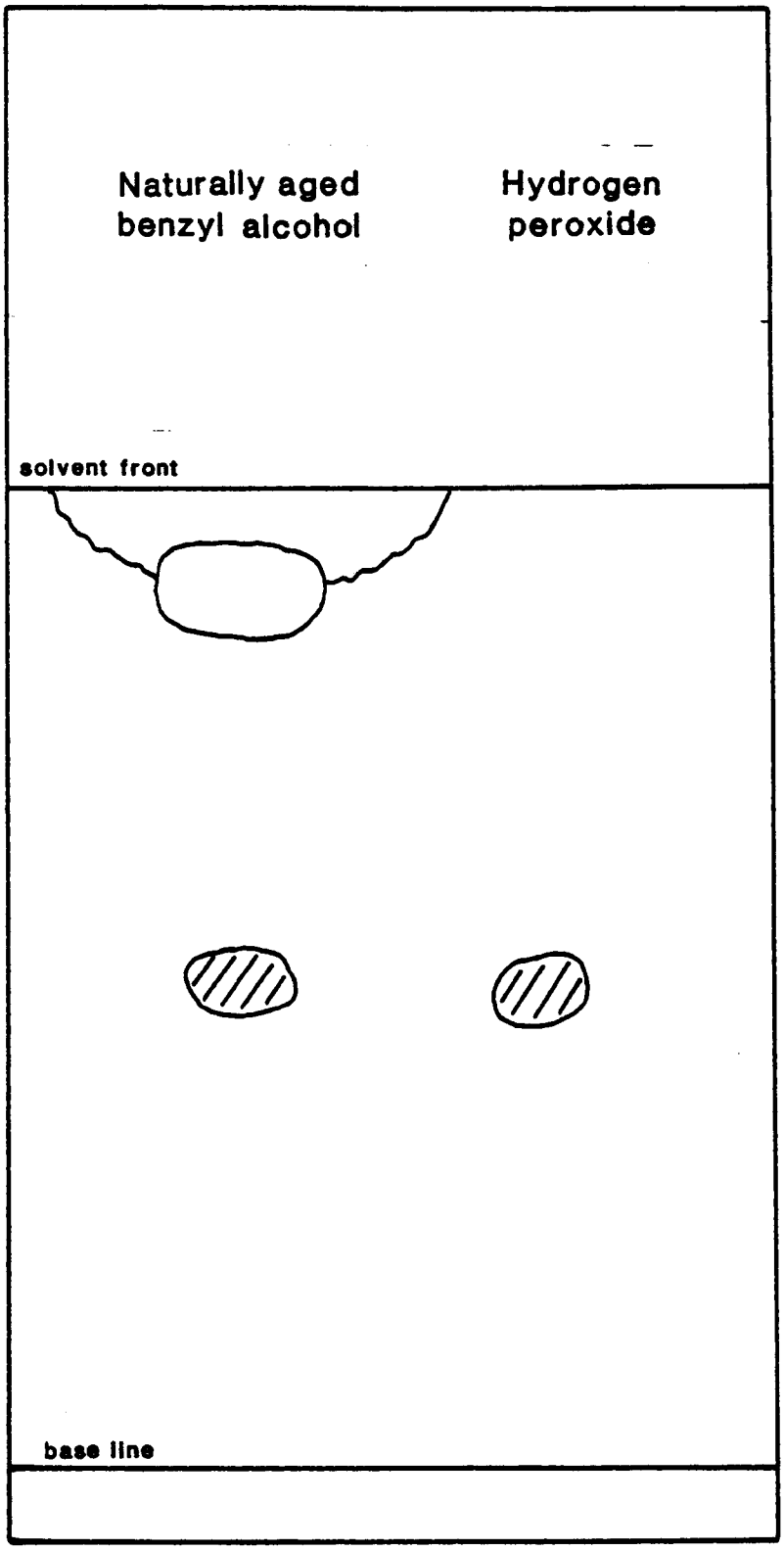


Fig. 6.12 - TLC of Naturally Aged Benzyl Alcohol

Mobile phase, water-n-butanol-diethyl ether (1-10-10); layer, cellulose F_{254}.

○ = visible under UV_{254}

▨ = +ve to KI spray reagent

Thus the identity of the peroxide which would be available to attack fluphenazine decanoate, if autoxidation of benzyl alcohol occurred within the Modecate product, is concluded to be hydrogen peroxide.

6.3. Relative Oxidation Rates of Benzyl Alcohol, Fatty Esters and Fatty Acids

Autoxidation of Modecate Injection could proceed via five possible intermediaries since there are five potential receptors of oxygen present in the formulation:

(i)	fluphenazine decanoate	(active)
(ii)	oleate triglycerides	)sesame oil
(iii)	linoleate triglycerides	)
(iv)	benzyl alcohol	(added preservative)
(v)	traces of unsaturated fatty acids.	(natural impurities of sesame oil)

Of these possible intermediaries, the direct reaction of the drug with molecular oxygen (i) has been shown to be unlikely[180], though confirmatory evidence has still to be produced. Thus in this investigation only mechanisms (ii), (iii) (iv) and (v) were considered. A knowledge of the oxidation rate of each of the materials would enable prediction of the mechanism most likely to occur in the product. Autoxidation rates however are difficult to quantify in absolute terms because of the complex reactions occurring. A typical autoxidation follows the general pattern[234]

a) initiation period

b) increasing rate of oxidation as radical propagation proceeds.

Hence the overall reaction does not obey simple kinetic laws. The commonest method of determining the relative autoxidation rates of a series of materials is to oxidise each sample under identical conditions (carefully controlled) and to compare the results graphically. Quantitation of the data is not possible, but the procedure will enable a knowledge of the relative potential for autoxidation to be gained.

6.3.1 Materials

Ideally the rates of autoxidation of the triglycerides should be determined, but these materials are not available in the pure form in the quantities required by the experimental technique. Methyl esters of oleic and linoleic acid were therefore used, these compounds being the most closely related entities available in pure form. (Sigma Chemicals).

Pure unsaturated fatty acids (oleic and linoleic) were also obtained from Sigma Chemicals. Benzyl alcohol was obtained from BDH Chemicals.

6.3.2 Autoxidation Procedure

20ml of the material to be oxidised was transferred to a large test-tube and suspended in a thermostated bath at 80^{o}C. Compressed air was allowed to bubble through the sample at a constant rate of 50ml/min, the flow rate being monitored with a suitable flow-meter (range, 0-100ml/min). Air was introduced into the liquid sample via a sintered tube.

Aliquots of the sample were withdrawn at intervals and assayed for peroxide by an iodometric procedure (Section 5.2.1).

Each material was vacuum-distilled immediately prior to autoxidation so that all samples were of an identical nature at the beginning of the experiment ie. free from peroxide.

6.3.3 Results and Discussion

Table 6.15

Autoxidation of Methyl Oleate

Time (hrs)	OOH Content (mg/ml)
0	0.05
2	0.30
4	0.61
6	0.94
8	1.39

Table 6.16

Autoxidation of Methyl Linoleate

Time (hrs)	OOH Content (mg/ml)
0	0
0.5	0.64
1.0	1.67
1.5	3.33
1.75	4.21
2.0	5.38
2.25	7.10

Table 6.17

Autoxidation of Oleic Acid

Time (hrs)	OOH Content (mg/ml)
0	0.03
1	0.30
2	0.56
4	1.24
6	1.75
7	2.10
8	2.48

Table 6.18

Autoxidation of Linoleic Acid

Time (hrs)	OOH Content (mg/ml)
0	0
0.25	0.94
0.5	1.93
0.75	2.97
1.0	4.39
1.25	5.68

Table 6.19

Autoxidation of Benzyl Alcohol

Time (hrs)	OOH Content (mg/ml)
0	0.023
1	0.037
2	0.040
4	0.086
5	0.224
6	0.475

6.3.4 Discussion and Conclusions

A graphical comparison of the autoxidation rates (Fig. 6.13) illustrates the relatively faster rate at which the diunsaturated fatty ester (linoleate) is oxidised and is in agreement with previously published data[177]. Fatty acids, which follow a similar profile to the esters, have a marginally greater rate of oxidation than the corresponding ester. In contrast, benzyl alcohol possesses the slowest rate of autoxidation.

Thus in a mixture of the components the diunsaturated compounds can be expected to preferentially autoxidise. As only trace amounts of fatty acid are present in a typical formulated product, the majority of oxidation can be predicted to occur with the diunsaturated ester. Autoxidation of the mono unsaturated ester (oleate) may occur to a limited degree. Benzyl alcohol, which exhibits the slowest rate of autoxidation and is also present in only small amounts (1.5%) would not be expected to oxidise to any great extent based on the above results. Hence the role of the preservative in enhancing degradation of the drug (Section 6.2) is still unclear.

However, two criteria must be borne in mind when attempting to extrapolate these results to Modecate formulation:

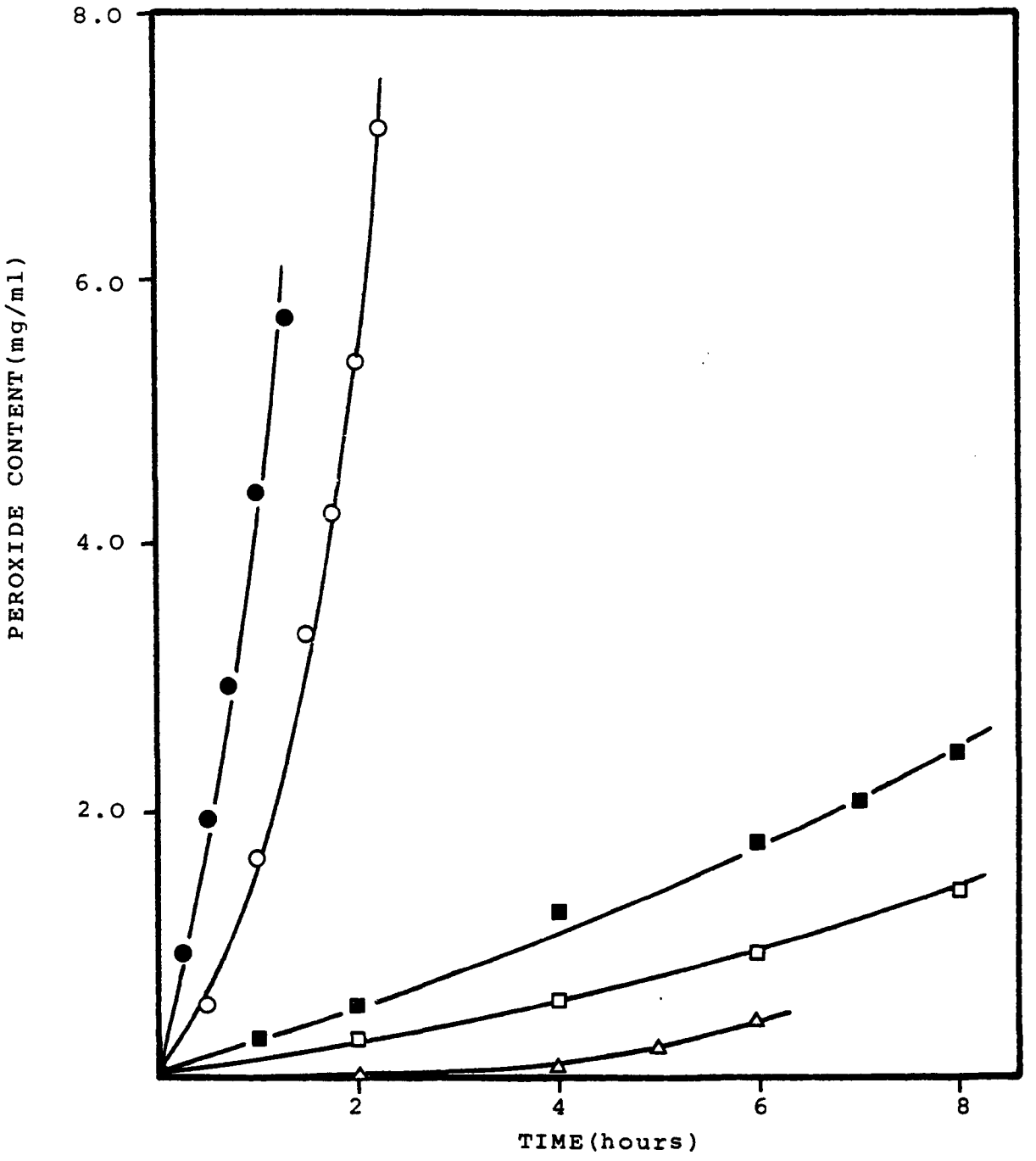


Fig. 6.13 - Comparison of Autoxidation Rates(Identical Conditions)

● = linoleic acid; ○ = methyl linoleate;

■ = oleic acid; □ = methyl oleate;

△ = benzyl alcohol.

(i) sesame oil contains natural antioxidants such as sesamolin

(ii) interaction of the peroxides (formed during autoxidation) with other components of the product.

The presence of antioxidant will undoubtedly prolong the first stage of autoxidation ie. the initiation period. The degree to which sesamolin may influence subsequent rates of oxidation is totally unknown and future work could usefully be directed towards determining the effect of antioxidants in this respect.

The second criteria can be predicted with more certainty. Peroxides formed during autoxidation will probably react directly with the active, fluphenazine decanoate, since the drug has demonstrated a ready willingness to react with hydroperoxides, even at room temperature (Chapter 5).

RECOMMENDATIONS FOR FURTHER WORK

1. That the difference in the pattern of oxidation products exhibited by piperazino-phenothiazines and by piperazino-phenothiazine esters respectively during reaction with chloroperbenzoic acid be further investigated.
2. That the ^{13}C NMR spectra of piperazino-phenothiazines be studied and, with the aid of model compounds, absolute assignments of the chemical shifts be made.
3. That the hygroscopic nature of the phenothiazine oxides be investigated.
4. That the reaction of fluphenazine decanoate with olefinic hydroperoxides be assessed following deacidification in order to determine the true effect of the carbon-chain length.
5. That the parameters affecting the rate of reaction of fluphenazine decanoate with oleate and linoleate hydroperoxides be investigated so that the difference in reaction rates, observed in the current work, be explained.
6. That the effect of antioxidants on the reaction of fluphenazine decanoate with hydroperoxides be assessed.
7. That α-hydroxybenzylhydroperoxide be synthesised and its absence in naturally autoxidised benzyl alcohol be confirmed.

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APPENDIX I

METHODS FOR THE PREPARATION AND ISOLATION OF FLUPHENAZINE DECANOATE OXIDES

A1.1. General Notes

A1.1.1 Reagents and Materials

Analar reagents and solvents were used wherever possible. In the absence of Analar material, Reagent Grade chemicals were substituted.

a) Purity of *m*-chloroperbenzoic acid, in terms of available oxygen, was found to decrease with age. In order to obtain the closest control over the oxidation with this reagent, the amount of available oxygen must be known. This value is readily determined for peracids by a simple iodometric titration[193].

Accurately weigh approximately 200mg of *m*-chloroperbenzoic acid into a 250ml iodine flask (duplicate). Dissolve in 10ml of glacial acetic acid, add 2g of potassium iodide and allow to stand for five minutes. Titrate the liberated iodine with 0.1M sodium thiosulphate solution using starch solution as indicator.

A reagent blank determination should be performed simultaneously.

1ml of 0.1M sodium thiosulphate solution ≡ 8.629mg *m*-chloroperbenzoic acid.

b) Fluphenazine Decanoate

Pharmaceutical grade fluphenazine decanoate (purity 99.5%) was used as received from the manufacturers (Linson Ltd. Eire).

A1.1.2 Equipment

HPLC equipment comprised of an Altex 110A reciprocating pump (Anachem Ltd.) and a Cecil 212 variable wavelength UV detector (Cecil Instruments Ltd.) coupled to a Perkin-Elmer Model 56 chart recorder.

Operating the HPLC under preparative conditions overloaded the UV detector because of the relatively high concentrations of product being eluted from the column. To overcome this problem, a less sensitive refractive-index detector (Waters Associates Ltd.) was substituted for the UV detector.

A1.2. Synthesis of Fluphenazine Decanoate Mono N-Oxides (Compounds A and B)

A1.2.1 Oxidation of Fluphenazine Decanoate

Dissolve 1g fluphenazine decanoate (1.69mM in 25ml of dichloromethane contained in a 250ml conical flask. Add a teflon-coated magnetic-stirrer bar and place the flask on a magnetic stirrer. Add dropwise from a burette (approx. 1 drop per sec.) with constant stirring a solution of 0.29g *m*-chloroperbenzoic in 25ml of dichloromethane. Evaporate the resulting mixture to about 10ml on a rotary film evaporator.

A1.2.2. Separation of A and B from Reaction Mixture

Prepare a 200mm x 25mm column of freshly activated (110°C) Kieselgel 60 (70-230 mesh, Merck) dispersed in chloroform. Transfer the reaction mixture, prepared as in A1.2.1 above, to the top of the column and allow the solution to percolate through the silica gel. Elute the compounds from the column with a mobile-phase of methanol-cyclohexane-methyl acetate (40 - 70 - 100). The progress of the compounds through the column may be conveniently monitored with a hand-held UV lamp (366nm), whence the fluphenazine decanoate-related bands appear bright-blue. Discard the first band eluted from the column (fluphenazine decanoate) and collect the second band (mixture of compounds A and B) in a round-bottom flask. Evaporate the solution to dryness on a rotary evaporator with gentle heat (35-40°C); an oily residue remains which slowly solidifies when stored at -20°C, resulting in a pale yellow waxy solid.

A1.2.3. Isolation of Pure A and B by Preparative HPLC

Prepare a solution of the A/B mixture at a concentration of 500mg/ml in methanol.

Using a 20cm x 3/8" HPLC column packed with SAS-Hypersil and a mobile phase of methanol-acetonitrile-1% ammonium carbonate (1-1-1)

inject 50ul aliquots of A/B solution onto the column. Collect the eluted fractions containing compound A and compound B in separate round-bottom flasks. Repeat the injection of the solution and collection of the respective fractions as necessary. Treat the flasks containing separated A and B as follows:

Evaporate under vacuum to remove the majority of the organic phase. Add about 20ml of acetonitrile and again evaporate on a rotary evaporator at about 70°C. Transfer the largely aqueous solution into a separating funnel, add an equal volume of water and extract with ethyl acetate or dichloromethane.

Separate the organic layer and dry over magnesium sulphate. Evaporate to dryness on a rotary evaporator. Final traces of solvent can be removed in a freeze-drier. The resulting oil slowly solidifies on storage at -20°C yielding a pale yellow waxy solid.

A1.3 Alternative Synthesis of Compound A

Prepare the mixture of compounds A and B as detailed in Sections A1.2.1 and A1.2.2 above. Dissolve the mixture in methanol and add 20ml of ammonium hydroxide (SG, 0.88). Allow to hydrolyse at room temperature until compound B comprises about 5% of the A/B mixture (about 48 hrs) using HPLC (SAS-Hypersil column; mobile phase, methanol-acetonitrile-1% ammonium carbonate solution, 1:1:1) to monitor the reaction.

Isolate compound A (approx. 95% pure) from the reaction mixture by the method described in Section A1.2.2.

A1.4. Synthesis of Fluphenazine Decanoate di N-Oxide (Compound D)

Dissolve 0.5g fluphenazine decanoate (0.85mM) in 25ml ethyl acetate contained in a 250-ml conical flask.

Prepare a solution of 0.44g of *m*-chloroperbenzoic acid (2.55 mM available oxygen) in 75.0ml ethyl acetate.

To the solution of fluphenazine decanoate at 20°C, add 48.0ml of oxidant dropwise from a burette, with constant stirring. Continue to add the oxidant in 0.5ml quantities monitoring the progress of the reaction by HPLC (SAS-Hypersil column; methanol-acetonitrile-1% ammonium carbonate solution, 1:1:1 as mobile phase, detector 260nm) after each addition. Cease the addition of oxidant as soon as compound D is seen to be formed. Evaporate the reaction mixture to dryness with a rotary film-evaporator and re-dissolve in diethyl ether. Add an etheral solution of hydrogen chloride dropwise from a graduated pipette until the precipitant is just present in excess (pH paper). Separate the precipitated hydrochloride salt by centrifugation and wash the solid twice with ether to remove excess hydrogen chloride. Dry the material in a vacuum oven at 60°C.

If necessary, the material may be recrystallised from a mixture of dry ether and dry absolute ethanol.

A1.5 Synthesis of Fluphenazine Decanoate Sulphoxide di N-Oxide (Compound D)

Dissolve 0.5g fluphenazine decanoate (0.85mM) in 25ml ethyl acetate in a 250-ml conical flask.

Prepare a solution of 0.59g *m*-chloroperbenzoic acid (3.42 mM available oxygen) in 100ml ethyl acetate.

To the solution of fluphenazine decanoate at 20°C, add 73.0ml of oxidant dropwise from a burette ensuring that the reaction mixture is stirred constantly. Continue to add the oxidant in 0.5ml quantities monitoring the progress of the reaction by HPLC after addition of each 0.5ml aliquot. (SAS-Hypersil column; methanol-acetonitrile-1% aqueous ammonium carbonate, 1:1:1 as mobile phase; detector 260nm). Cease addition of oxidant when the

level of compound C is reduced to approximately 2% of the reaction mixture.

Evaporate the reaction mixture to dryness on a rotary evaporator and re-dissolve the residue in diethyl ether. Add an ethereal solution of hydrogen chloride dropwise from a graduated pipette until the precipitant is just present in excess (pH paper). Separate the precipitated hydrochloride salt by centrifugation and wash the solid twice with ether. Dry the material in a vacuum oven at 60°C.

A1.6 Synthesis of Fluphenazine Decanoate Sulphone di N-Oxide (Compound E)

Dissolve 0.5g fluphenazine decanoate (0.85mM) in 25ml ethyl acetate in a 250-ml conical flask. Add 1.0g *m*-chloroperbenzoic acid (5.8mM available oxygen) and swirl to dissolve. Allow to react at room temperature (20°C). Monitor the reaction by HPLC (SAS-Hypersil column; methanol-acetonitrile-1% aqueous ammonium carbonate, 1:1:1; detector 275nm). Compound D is formed immediately and then slowly converted to Compound E. When conversion to compound E is complete, evaporate the reaction mixture to dryness on a rotary evaporator. Re-dissolve the residue in diethyl ether and precipitate the hydrochloride salt by dropwise addition of an ethereal solution of hydrogen chloride until in excess (pH paper). Separate the precipitate by centrifugation and wash the solid twice with ether. Dry the material in a vacuum oven at 60°C.

A1.7 Synthesis of Fluphenazine Decanoate Sulphone (Compound F)

Prepare Compound E as described in Section A1.6 but omitting the isolation of the hydrochloride.

To the reaction mixture (20°C) in ethyl acetate add 10ml of ethyl acetate saturated with hydrogen chloride and pieces of granulated zinc. Monitor the reaction by HPLC (SAS-Hypersil column; methanol-acetonitrile-1% aqueous

ammonium carbonate, 1:1:1; detector 260mm). When reduction of compound E is complete add excess 1M aqueous ammonium hydroxide and mix well. Separate the ethyl acetate layer, dry over magnesium sulphate, then evaporate to dryness on a rotary evaporator. Add 10-ml acetonitrile and again evaporate to dryness in order to remove residual water. Compound F remains in the flask as a light-brown oil.

The above product will be contaminated with a small amount of fluphenazine decanoate. If necessary, compound F may be purified by preparative layer chromatography:

Streak a methanolic solution of compound F onto the base-line of a 40 x 20cm silica-gel preparative layer plate (Merck; 2mm layer). Develop the plate in a mobile phase of acetone-cyclohexane-0.88 ammonia (80:30:5). Evaporate the mobile phase from the surface of the plate by placing in an oven at $100^{o}C$ for 5 mins. Scrape the main band from the plate, transfer the silica gel to a glass column and elute with methanol. Evaporate the solution to dryness with a rotary film evaporator. Remove any residual water by adding acetonitrile and again evaporating to dryness. Drying may be completed in a freeze-drier.

A1.8 Synthesis of Fluphenazine Decanoate Sulphoxide (Compound Z)

Two procedures are available for the preparation of Compound Z, either of which may be adopted:

a) Oxidation of fluphenazine decanoate yielding the impure sulphoxide, which may be purified by preparative layer chromatography.

b) Conversion of fluphenazine decanoate to the hydrochloride salt which, after isolation and dissolution in glacial acetic acid, may be selectively oxidised to the sulphoxide yielding Compound Z in almost 100% purity.

The former method only has been used:

Dissolve 0.5g fluphenazine decanoate in 5ml glacial acetic acid. Add

0.5ml of hydrogen peroxide (100 volume) and allow to react at 20°C.

Monitor the reaction by HPLC (SAS-Hypersil column; methanol-acetonitrile-1% aqueous ammonium carbonate, 1:1:1; detector 260nm). When all the fluphenazine decanoate has been oxidised isolate the crude sulphoxide by adding excess 10M sodium hydroxide whilst cooling the flask and contents in an ice-bath. Add 10ml dichloromethane to extract the sulphoxide and separate the organic layer. Dry over magnesium sulphate. Evaporate to low volume then streak the solution onto the base line of a silica gel preparative layer plate (Merck; 20 x 40cm, 0.2mm layer). Carry out the chromatographic procedure and method for the elution of the compound from the silica gel described under A1.7 above. A pale yellow viscous oil is obtained.

A1.9 Synthesis of Fluphenazine Decanoate Sulphoxide mono- N-Oxides (Compounds X and Y)

A1.9.1 Preparation and Isolation of the Mixture (Compounds X and Y)

Prepare approximately 0.5g of crude fluphenazine decanoate sulphoxide and extract from the reaction mixture into dichloromethane by following the procedure described in section A1.7 above. Dry the dichloromethane solution over magnesium sulphate then filter through a No. 3 sinter. Oxidise the resulting solution of crude sulphoxide with -chloroperbenzoic acid solution (0.2g in 60ml dichloromethane) by adding the oxidant dropwise with constant stirring at 20°C. Monitor the reaction by HPLC (SAS-Hypersil column; methanol-acetonitrile-1% aqueous ammonium carbonate, 1:1:1, detector 275nm). Stop the reaction when compound W begins to be formed in preference to compounds X and Y (25-30ml of oxidant). Evaporate to low volume on a rotary evaporator and transfer the concentrated solution to the top of a 10cm x 2.5cm column of freshly activated (110°C) silica gel (Merck, 70-230 mesh) dispersed in chloroform. Allow the solution to run onto the column then elute with a

mobile phase of methanol-cyclohexane-methyl acetate (40-70-100). Progress of the bands through the column may be conveniently followed using a hand-held UV lamp (366nm). Discard the first band eluted from the column (fluphenazine decanoate sulphoxide). Collect the second band in a round-bottom flask. Evaporate the eluate to dryness on a rotary evaporator. A pale yellow/brown viscous oil results.

A1.9.2 Separation of Pure X and Pure Y by Preparative HPLC

Prepare a 50% solution of the mixture of C and Y isolated in Section A1.8.1 above. Operate the 20cm x 3/8" SAS-Hypersil column under the conditions described in Section A1.2.3, injecting 25ul aliquots of solution. Collect the fractions containing compounds X and Y in separate flasks. Repeat the injection of the solution and collection of the respective fractions as necessary. Isolate X and Y from the mobile phase by the following procedure:

Evaporate under vacuum at about 70°C to remove the majority of the organic phase. Add 20ml acetonitrile and repeat the evaporation at 70°C. Transfer the largely aqueous solution to a separating funnel, add an equal volume of water and extract with dichloromethane. Separate the organic layer and dry over magnesium sulphate.

Evaporate the solvent on a rotary evaporator, yielding a pale yellow viscous oil. Last traces of solvent may be removed by 'drying' in a freeze-drier.

A1.10 Synthesis of Fluphenazine Decanoate Sulphoxide di N-Oxide (Compound W)

Prepare approximately 0.5g of crude fluphenazine decanoate sulphoxide and extract from the reaction mixture into ethyl acetate by following the procedure described in Section A1.7 above. Dry the ethyl acetate solution

over magnesium sulphate then filter through a No. 3 sinter. Oxidise the resulting solution of crude sulphoxide with *m*-chloroperbenzoic acid solution (0.3g in 60ml ethyl acetate) by adding the oxidant dropwise with constant stirring at 20°C. Monitor the reaction by HPLC (SAS-Hypersil column; methanol-acetonitrile-1% aqueous ammonium carbonate, 1:1:1, detector 275nm).

Add the oxidant until compounds X and Y constitute about 10% of the reaction mixture. Continue to add the oxidant dropwise, but in 0.5ml aliquots until X and Y are almost completely converted to compound W, ie. 2% reaction mixture. Wash the ethyl acetate solution with 1M sodium hydroxide and with two successive volumes of water. Dry over sodium sulphate and concentrate to low volume. Place the solution in a freezer (-20°C) and allow precipitation to occur. Separate the resulting amorphous solid by centrifugation, wash with ether and dry in a freeze-drier or efficient vacuum oven at 20°C.

APPENDIX II

METHODS FOR THE PREPARATION AND ISOLATION OF FLUPHENAZINE OXIDES

A2.1 General Notes

A2.1.1 Reagents and Materials

Analar reagents and solvents were used wherever possible. In the event of unobtainability, Reagent Grade chemicals may be substituted.

a) Purity of *m*-Chloroperbenzoic Acid

For the closest control during oxidations with this reagent, it is necessary to know the precise value of purity (in terms of available oxygen). Determine by the iodometric method described in Section A.1.1.1.

b) Purity of Fluphenazine Dihydrochloride

The pharmaceutical grade material received from the manufacturers (Linson Ltd., Eire) has a purity of not less than 99.5%.

c) Fluphenazine base is not available from the manufacturers and should be prepared from the hydrochloride by the following method:

Dissolve 10g of fluphenazine dihydrochloride in 100ml water, add excess 10M sodium hydroxide solution and extract with 50ml di-ethyl ether. Separate the ether layer, wash twice with water and dry over magnesium or sodium sulphate. Isolate fluphenazine base by evaporating the ether solution to dryness on a rotary evaporator. Finally, dry the base in a vacuum oven at $60^{o}C$ for 16 hours.

A2.2 Synthesis of Fluphenazine Mono N-Oxide (Compound B')

A2.2.1 Oxidation of Fluphenazine

Dissolve 0.5g fluphenazine base (1.14mM) in 25ml ethyl acetate contained in a 25-ml conical flask. Cool the flask and contents in a cardice-acetone bath at $-40^{o}C$. Add dropwise from a burette, 50.0ml of a solution of *m*-chloroperbenzoic acid (0.296g dissolved in 75ml ethyl acetate) ensuring constant stirring (magnetic stirrer), throughout. The temperature of the bath should be carefully monitored and kept at approximately $-40^{o}C$ by addition of small pieces of cardice during the reaction. When addition of the oxidant is

complete, allow the reaction mixture to attain room temperature then evaporate to dryness on a rotary evaporator. Dissolve the residue in 5ml of dichloromethane.

A2.2.2 Isolation of Compound B' from Reaction Mixture

Transfer the dichloromethane solution from (a) above onto the top of a 30 x 2.5cm column of freshly activated (110°C) silica gel (70-230 mesh) dispersed in chloroform. Allow the solution to run onto the column then elute with a mobile phase of acetone-cyclohexane-ammonia, SG 0.88 (80-30-5). Use a hand-held UV lamp (366nm) to follow the progress of bands through the column. Discard the first eluted band (fluphenazine). Collect the second band to elute from the column. Isolate compound B by evaporating the eluate to dryness on a rotary film evaporator (70°C). Add 50ml acetonitrile and again evaporate to dryness. This last step will ensure the removal of residual water as a water/acetonitrile azeotrope. A pale yellow viscous oil results, which slowly solidifies if stored at -20°C.

A2.3 Synthesis of Fluphenazine Mono N-Oxide (Compound A')

Compound A' cannot be prepared in any reasonable quantity by normal oxidation methods. However, this material may be obtained by hydrolysis of the equivalent fluphenazine decanoate N-oxide (Compound A):

Prepare Compound A from fluphenazine decanoate by the procedure described in Section A1.3. Dissolve 0.5g compound A in methanol (20ml) and add 2ml of ammonia (SG 0.88). Allow to react at 20°C until compound A is completely hydrolysed (about 3 days). Evaporate to dryness on a rotary evaporator and dissolve the residue in a small volume of chloroform. Isolate compound A' using the column chromatographic procedure described in Section A2.2.2.

A2.4 Synthesis of Fluphenazine di N-Oxide (Compound C')

Dissolve 0.50g fluphenazine base (1.14mM) in 25ml dichloromethane in a 250-

ml conical flask.

Dissolve 0.394g *m*-chloroperbenzoic acid (2.28mM available oxygen) in 50ml of dichloromethane. Add the oxidant dropwise to the solution of fluphenazine, with constant stirring, until the total volume of oxidant has been consumed. Wash the final reaction mixture with water in order to remove the small amount of compound D' produced during the reaction. Two or three washes should suffice - use HPLC to monitor (SAS-Hypersil column; mobile phase, methanol-10% aqueous ammonia containing 1% potassium chloride, 1:1; detector 260nm). Dry the dichloromethane solution over magnesium sulphate, then evaporate to dryness on a rotary evaporator. Dissolve the residue in diethyl ether and add ethereal hydrogen chloride until present in a small excess (pH paper). Separate the precipitated hydrochloride salt by centrifugation and wash the solid with two successive volumes of ether. Dry in a vacuum oven at 60°C.

If HPLC indicates the product to be contaminated with compound B', then the material should be recrystallised from a mixture of absolute ethanol and ether.

A2.5 Synthesis of Fluphenazine Sulphoxide di N-Oxide (Compound D')

Dissolve 0.5g fluphenazine (1.14mM) in 25ml ethyl acetate in a 250-ml conical flask.

Prepare a solution of 0.79g *m*-chloroperbenzoic acid (4.57 mM available oxygen) in 100ml ethyl acetate.

To the solution of fluphenazine decanoate at 20°C, add 73.0ml of oxidant dropwise from a burette ensuring that the reaction mixture is stirred constantly. Continue to add the oxidant in 0.5ml quantities monitoring the progress of the reaction by HPLC after addition of each 0.5ml aliquot. (SAS-Hypersil column; mobile phase, methanol-10% aqueous ammonia containing 1% potassium chloride, 1:1; detector 260nm). Cease the addition of oxidant when compound E', which appears on the tail of the peak representing compound D', begins to increase.

Evaporate the reaction mixture to dryness on a rotary evaporator and re-

dissolve the residue in diethyl ether. Add an ethereal solution of hydrogen chloride dropwise from a graduated pipette until the precipitant is just present in excess (pH paper). Separate the precipitated hydrochloride salt by centrifugation and wash the solid twice with ether. Dry the material in a vacuum oven at 60°C.

A2.6 Synthesis of Fluphenazine Sulphone di N-Oxide (Compound E')

Dissolve 0.5g fluphenazine (1.14 mM) in 25ml ethyl acetate in a 250-ml conical flask. Add 1.0g *m*-chloroperbenzoic acid (5.8 mM available oxygen) and swirl to dissolve. Allow to react at room temperature (20°C). Monitor the reaction by HPLC (SAS-Hypersil column; mobile phase, methanol-10% aqueous ammonia containing 1% potassium chloride, 1:1; detector, 275nm).

Compound D' is formed immediately and then slowly converted to Compound E'. When conversion to compound E' is complete, evaporate the reaction mixture to dryness on a rotary evaporator. Re-dissolve the residue in diethyl ether and precipitate the hydrochloride salt by dropwise addition of an ethereal solution of hydrogen chloride until in excess (pH paper). Separate the precipitate by centrifugation and wash the solid twice with ether. Dry the material in a vacuum oven at 60°C.

A2.7 Synthesis of Fluphenazine Sulphone (Compound F')

Prepare 0.5g of Compound E' as the hydrochloride salt using the method described in Section A2.6.

Dissolve the resulting product in 1M aqueous hydrochloric acid (20ml) and add pieces of granulated zinc. Allow the reaction to proceed at room temperature, monitoring the progress with HPLC (SAS-Hypersil column; mobile phase, methanol-10% aqueous ammonia containing 1% potassium chloride, 1:1; detector 260nm). When conversion of compound E' to compound F' is complete, add 5M sodium hydroxide until the solution is alkaline and extract with dichloromethane. Separate the layers by centrifugation and discard the aqueous layer. Wash the

dichloromethane free from caustic soda using water, then dry the organic solution over magnesium sulphate. Isolate compound F' by evaporating the dichloromethane solution on a rotary film evaporator to yield a pale yellow viscous oil. Final traces of solvent may be evaporated in a freeze-drier at 20°C.

A2.8 Synthesis of Fluphenazine Sulphoxide N-Oxide (Compound Y')

Carry out the procedure described in Section A2.2.1 (oxidation of fluphenazine at -40°C), but dissolve the final, residue in 10ml diethyl ether instead of dichloromethane. Add an ethereal solution of hydrogen chloride until present in excess then separate the precipitate by centrifugation. Wash the solid with two successive 10ml volumes of ether and dry in a vacuum oven at 20°C.

Dissolve the mixture of hydrochloride salts in 10ml of glacial acetic acid, add 1.0ml of hydrogen peroxide (100 volume) and allow to react at room temperature until the original components have been converted to the corresponding sulphoxides (about 2 hours) as shown by the HPLC chromatograms (SAS-Hypersil column; mobile phase, methanol-10% aqueous ammonia containing 1% potassium chloride, 1:1; detector, 260nm). Stop the reaction by adding excess 10M sodium hydroxide and extract with dichloromethane. Concentrate the dichloromethane solultion to low volume (about 1ml) then streak the solution on to the base line of a silica gel preparative layer plate (Merck; 20 x 40cm, 2mm layer). Develop the plate in a mobile phase of acetone-cyclohexane-ammonia, SG 0.88 (80-30-5). View the plate under a UV lamp and mark the position of the main band. Scrape off the marked band and transfer to a glass chromatography column. Elute the compound Y' with methanol. Carefully evaporate the solution to dryness on a rotary evaporator, without the use of heat. (Addition of aliquots of dichloromethane to the methanolic solution will help evaporate the methanol under vacuum at 20°C). A brown viscous oil results, which slowly solidifies at -20°C.

A2.9 Synthesis of Fluphenazine Sulphoxide di N-Oxide (Compound V')

A2.9.1 Oxidation Reaction

Dissolve 0.5g fluphenazine base in 5ml glacial acetic acid and add 0.5ml hydrogen peroxide (100 volume). Heat the mixture on a water bath at 80°C, monitoring the reaction by HPLC (SAS-Hypersil column; mobile phase, methanol-10% aqueous ammonia containing 1% potassium chloride, 1:1; detector 275nm). When the reaction is complete isolate the product by the following procedure.

A2.9.2 Isolation of Compound V'

Add 10M sodium hydroxide to the reaction mixture, cooled in an ice-bath, until the solution is alkaline. Allow the alkaline solution to percolate through a 20 x 25cm bed of Amberlite XAD2 resin. Wash the resin with water until the washings are neutral then draw excess water from the resin bed using a vacuum-line connected through a suitable trap. Elute the product from the resin with methanol (about 200ml) collecting the eluate in a round-bottom flask. Evaporate the majority of solvent on a rotary evaporator (70°C) then add two successive quantities of acetonitrile and evaporate in order to azeotrope the residual water. Dissolve the oily residue in dichloromethane and dry over magnesium sulphate. Finally, evaporate to dryness on a rotary evaporator to yield a white solid. Dry the resultant solid (compound V') in a freeze-drier or efficient vacuum oven at 20°.

APPENDIX III

IR, NMR AND MS SPECTRA OF THE ISOLATED OXIDES

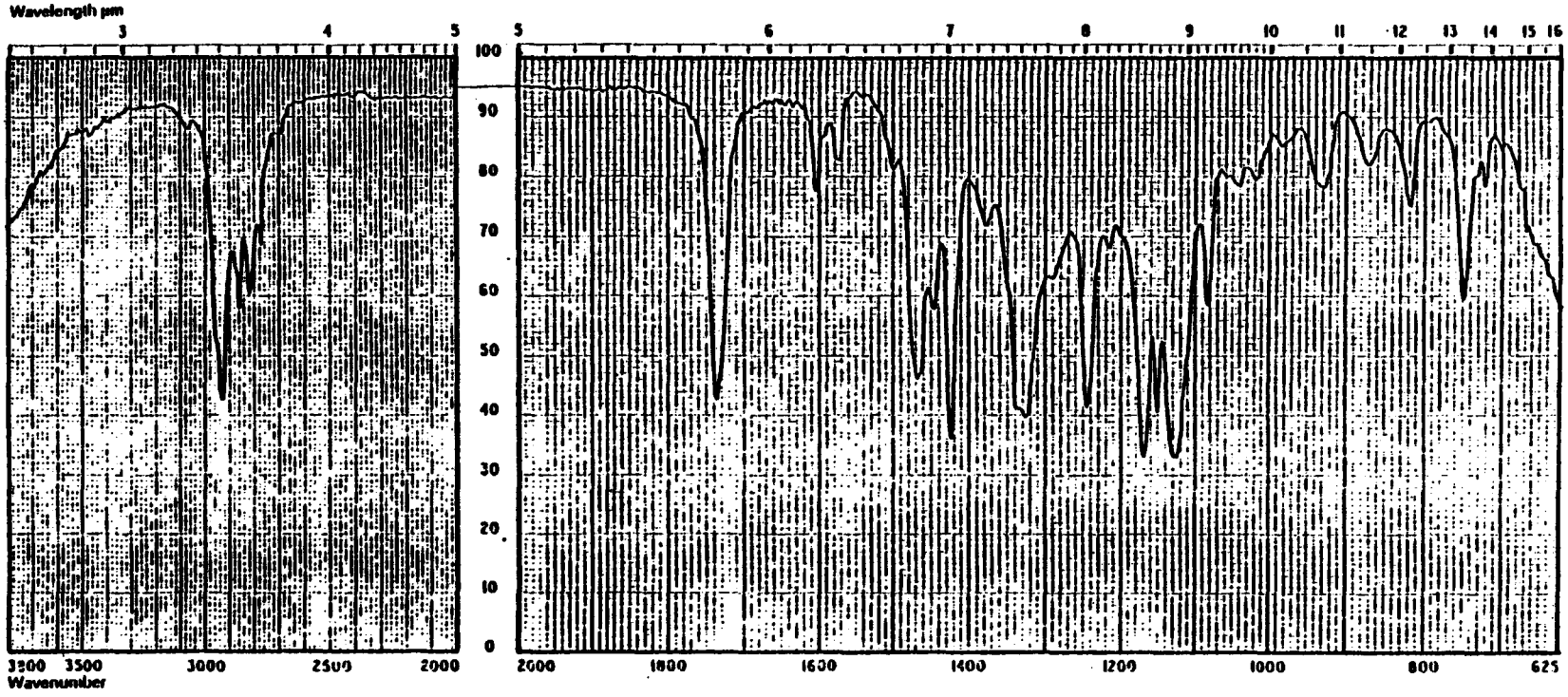

Fig. A3.1 - IR Spectrum (KBr) of Fluphenazine Decanoate

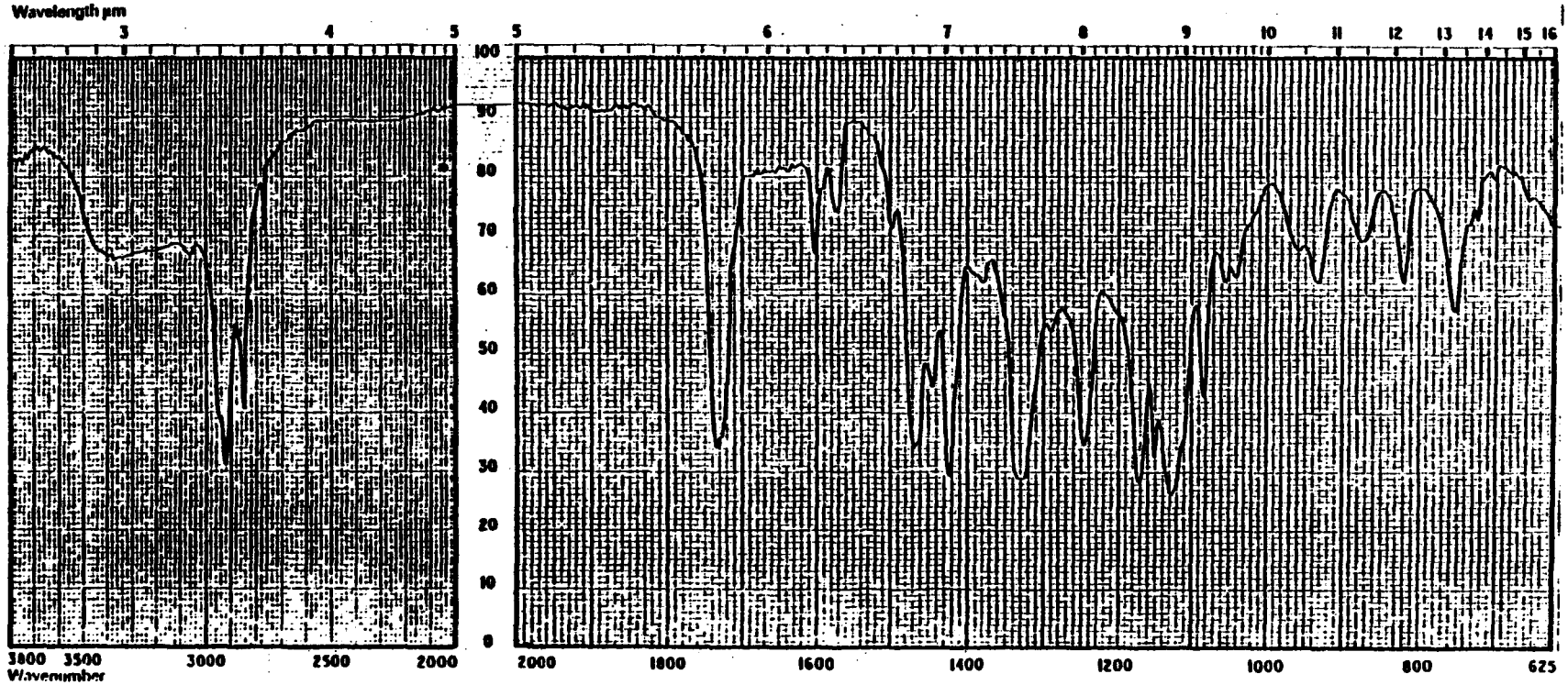

Fig. A3.2 - IR Spectrum (KBr) of Compound A (fluphenazine decanoate mono-N-oxide A)

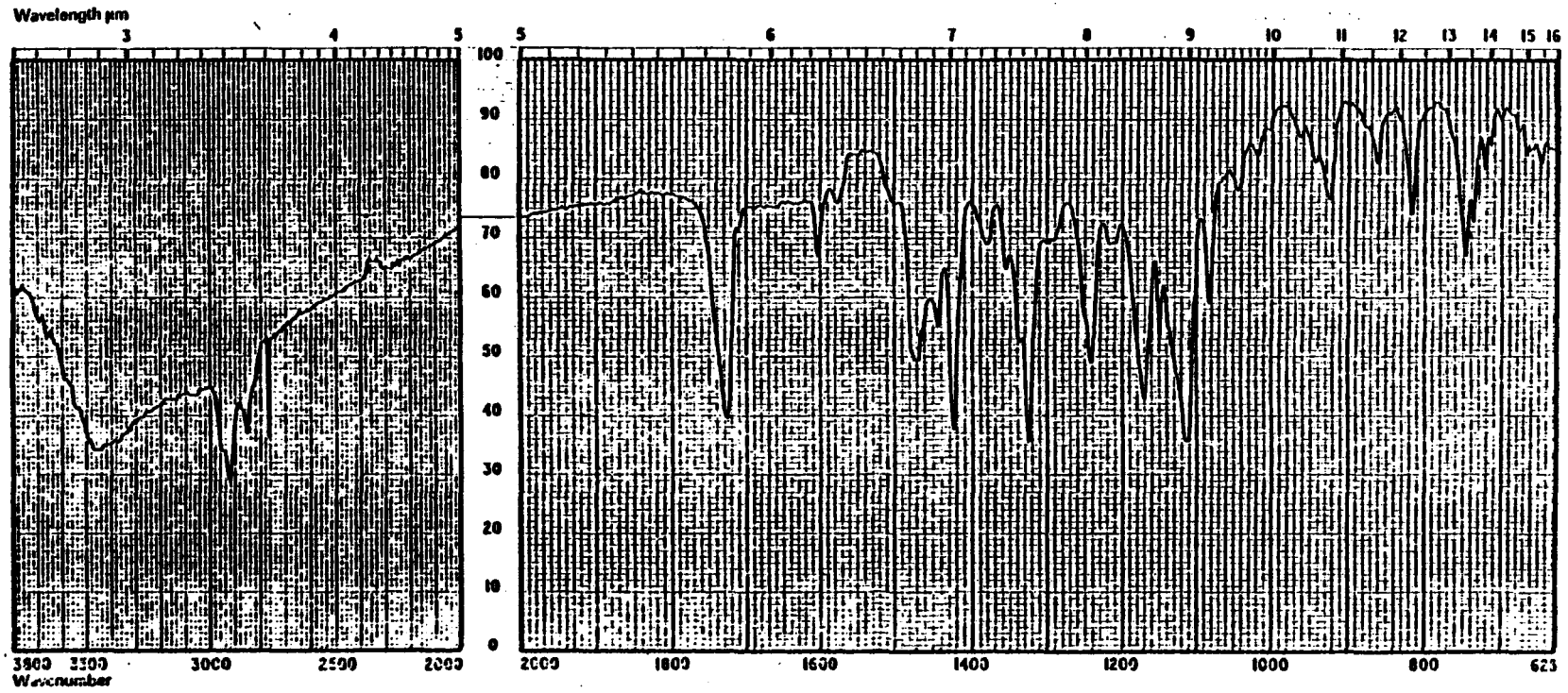

Fig. A3.3 - IR Spectrum (KBr) of Compound B (fluphenazine decanoate mono-N-oxide B)

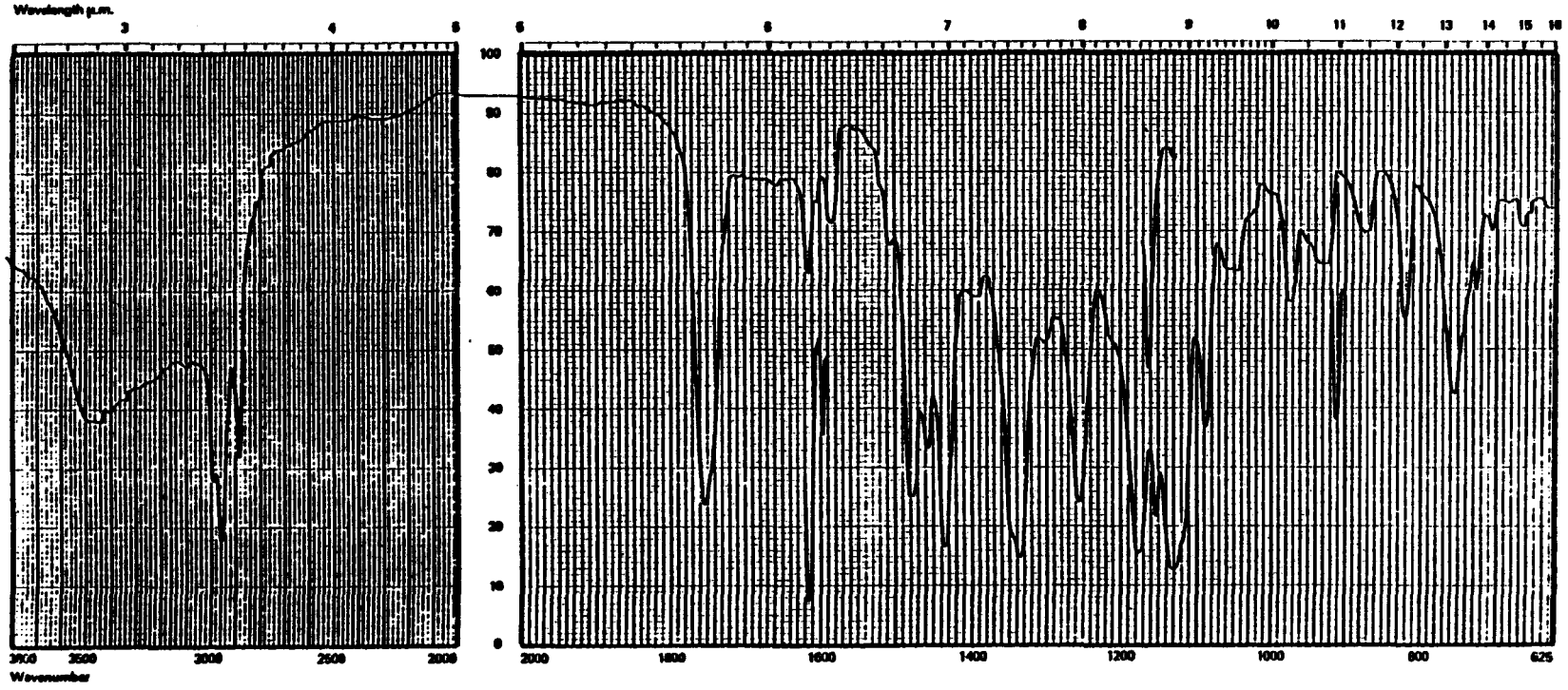

Fig. A3.4 - IR Spectrum (KBr) of Compound C (fluphenazine decanoate di-N-oxide)

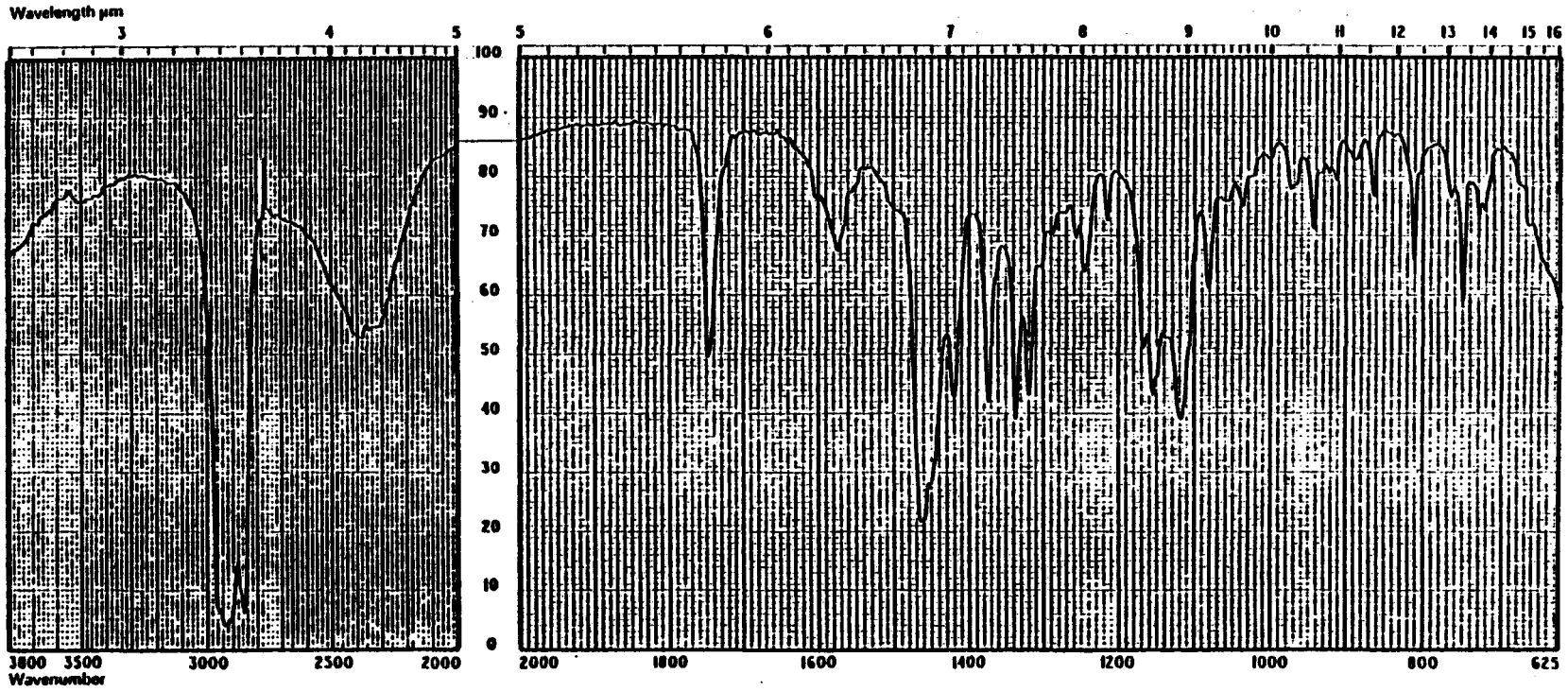

Fig. A3.5 - IR Spectrum (Nujol) of Compound C, Hydrochloride Salt. (fluphenazine decanoate di N-oxide di hydrochloride)

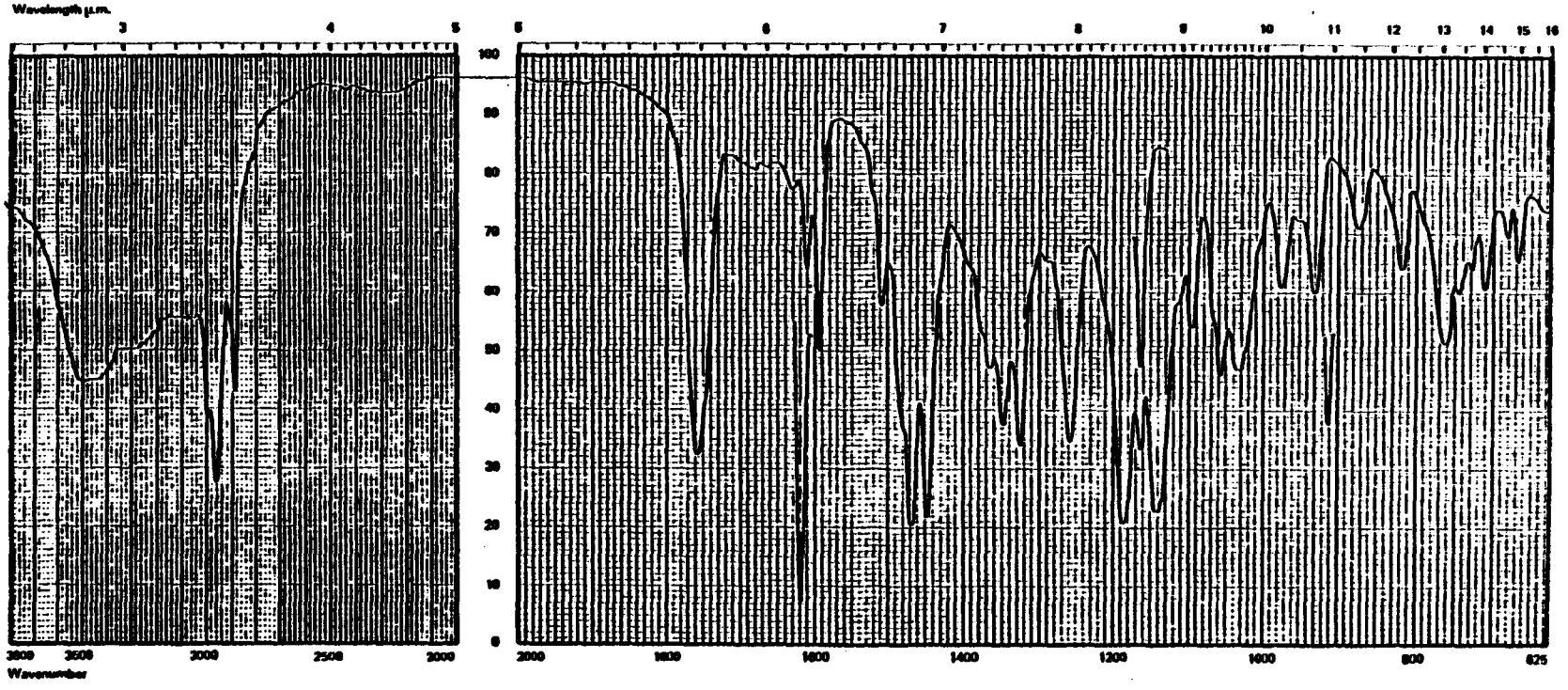

Fig. A3.6 - IR Spectrum (KBr) of Compound D (fluphenazine decanoate sulphoxide di-N-oxide.

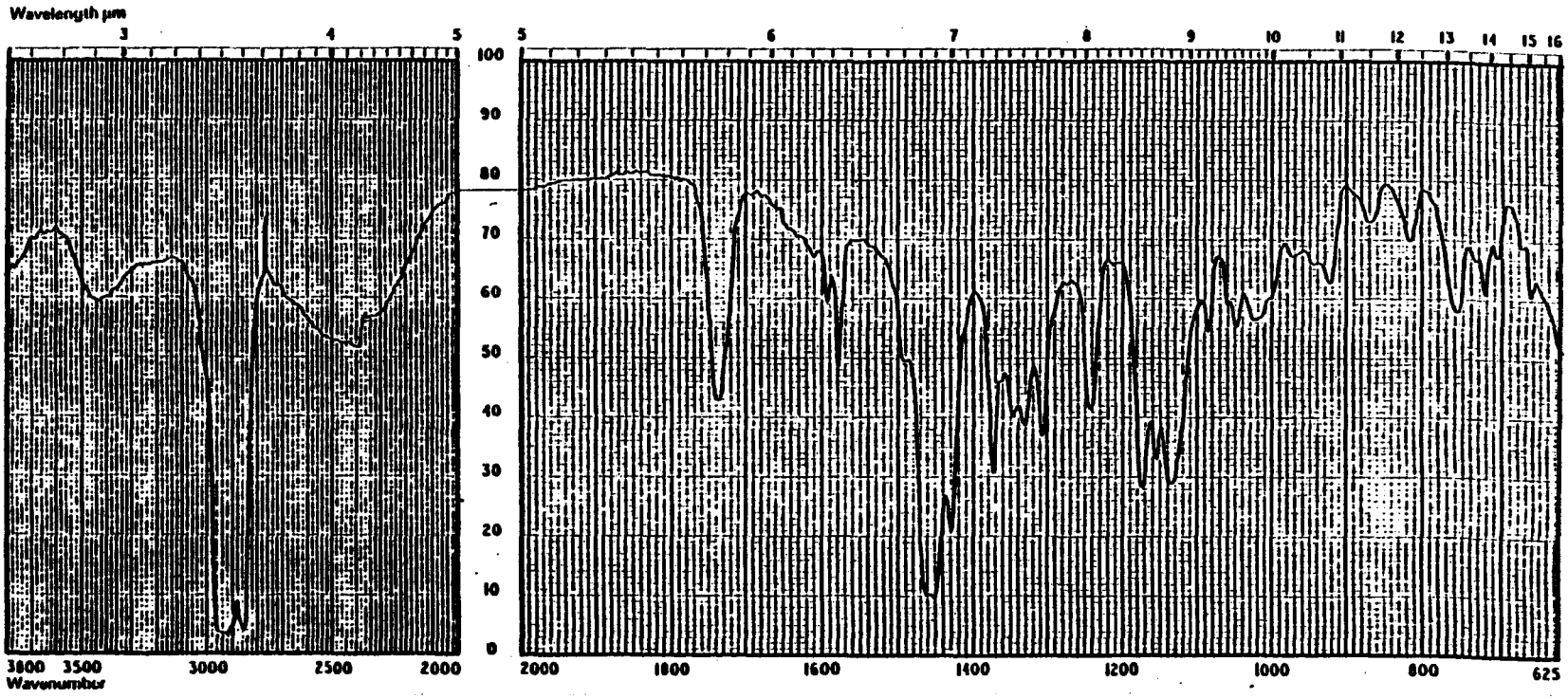

Fig. A3.7 - IR Spectrum (nujol) of Compound D Hydrochloride Salt.
(fluphenazine decanoate sulphoxide di N-oxide di hydrochloride)

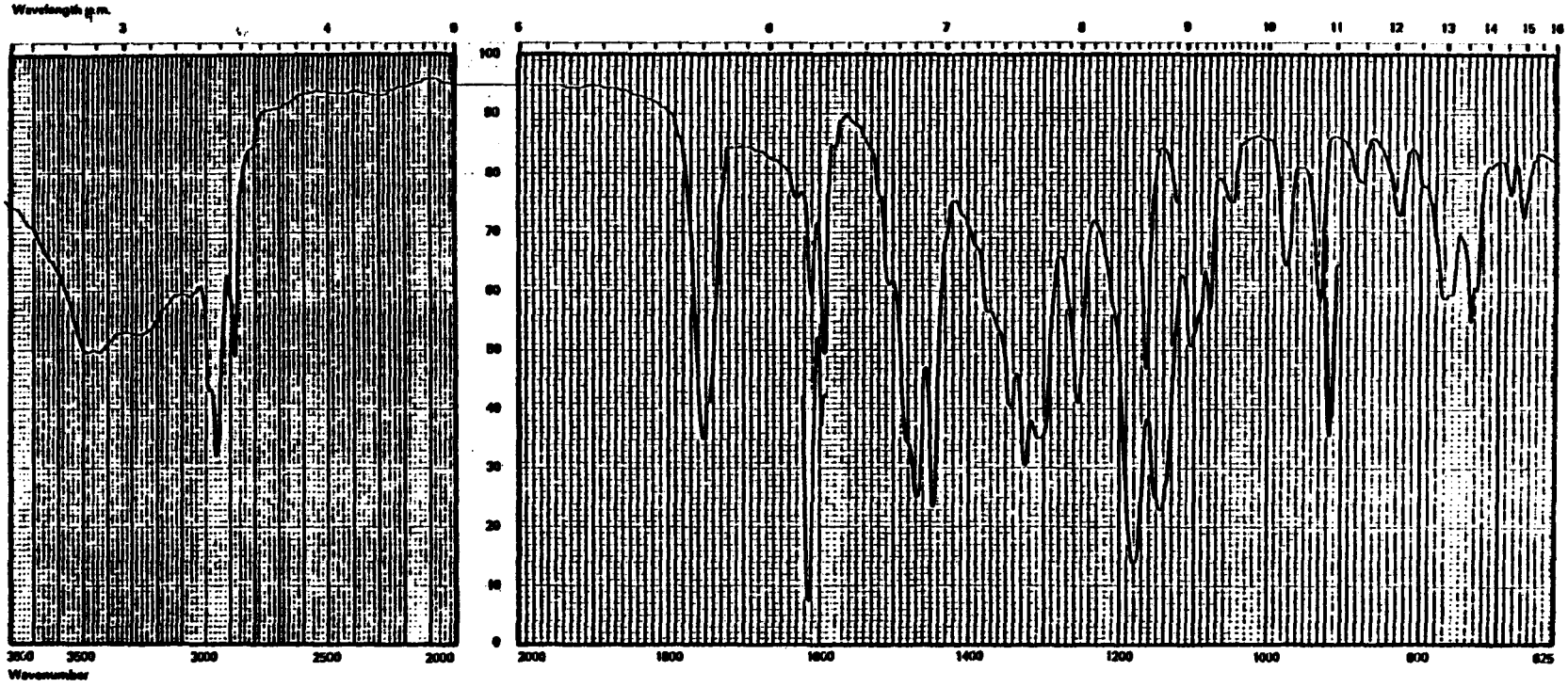

Fig. A3.8 - IR Spectrum (KBr) of Compound E (fluphenazine decanoate sulphone di-N-oxide)

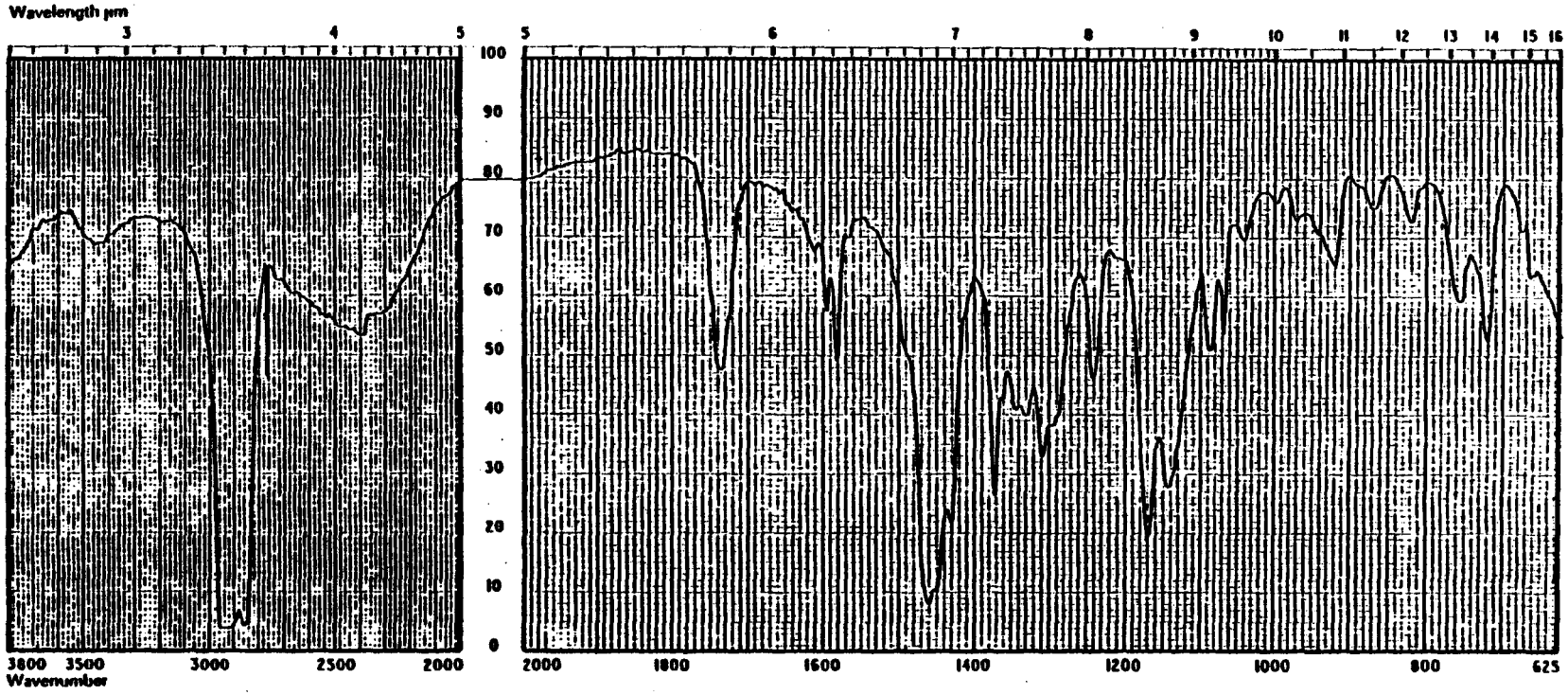

Fig. A3.9 - IR Spectrum (Nujol) of Compound E Hydrochloride Salt. (fluphenazine decanoate sulphone di-N-oxide dihydrochloride).

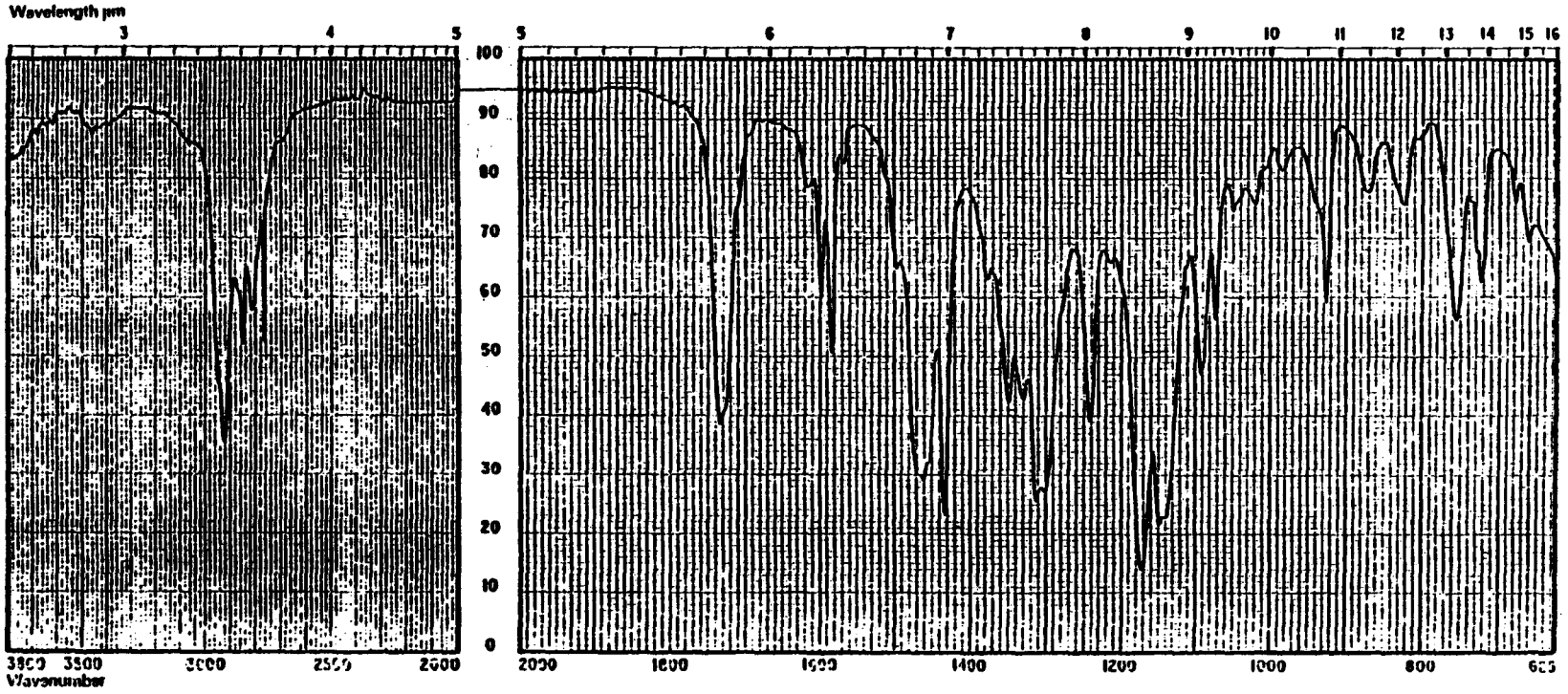

Fig. A3.10 - IR Spectrum (KBr) of Compound F (fluphenazine decanoate sulphone).

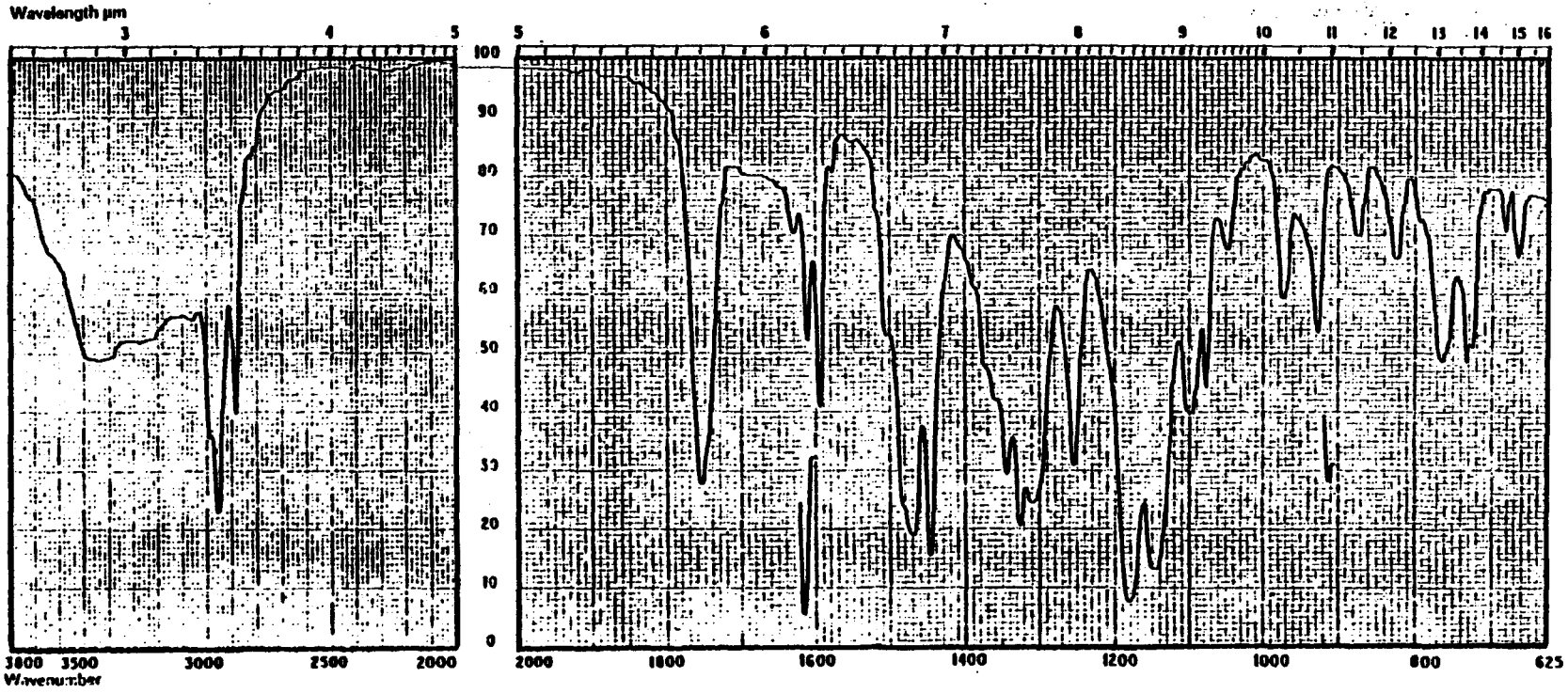

Fig. A3.11 - IR Spectrum (KBr) of Compound V (fluphenazine decanoate sulphone di-N-oxide).

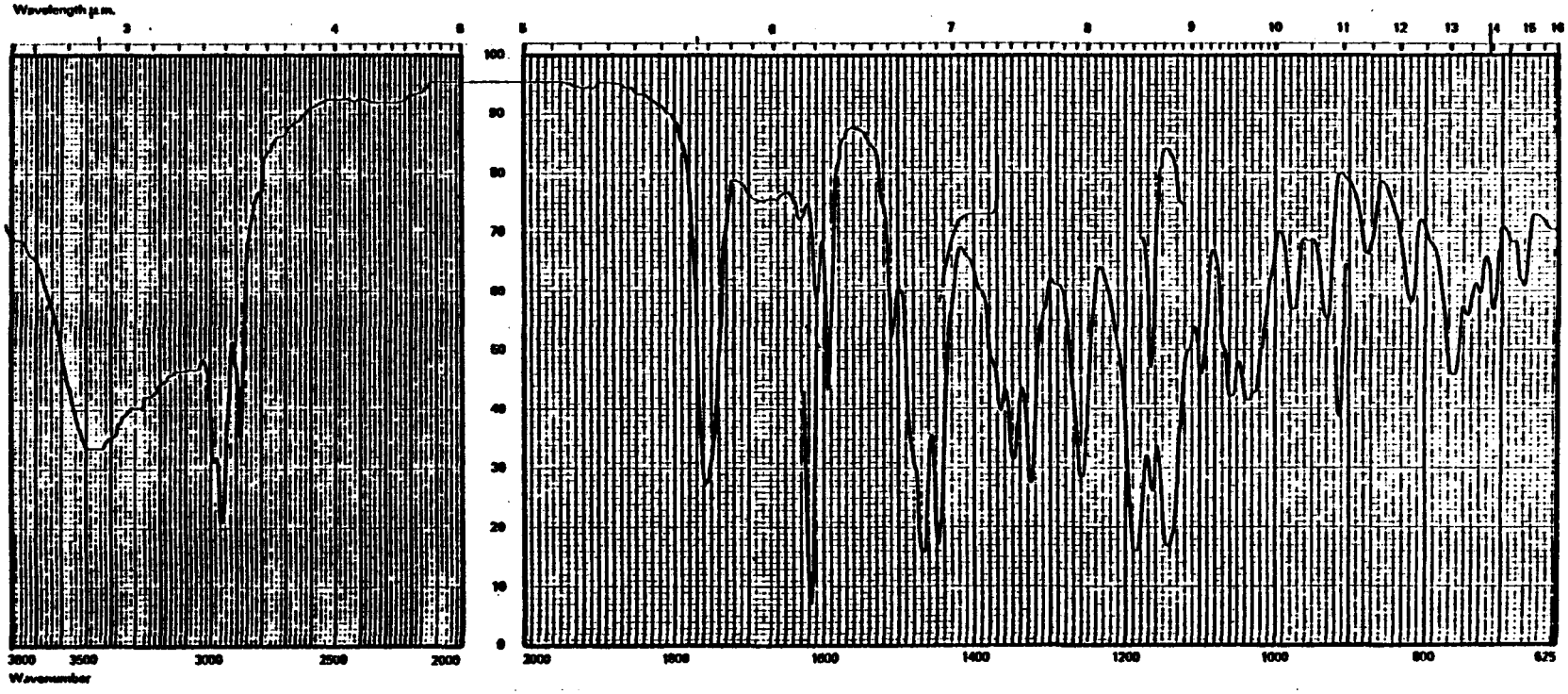

Fig. A3.12 - IR Spectrum (KBr) of Compound W (fluphenazine decanoate sulphoxide di-N-oxide).

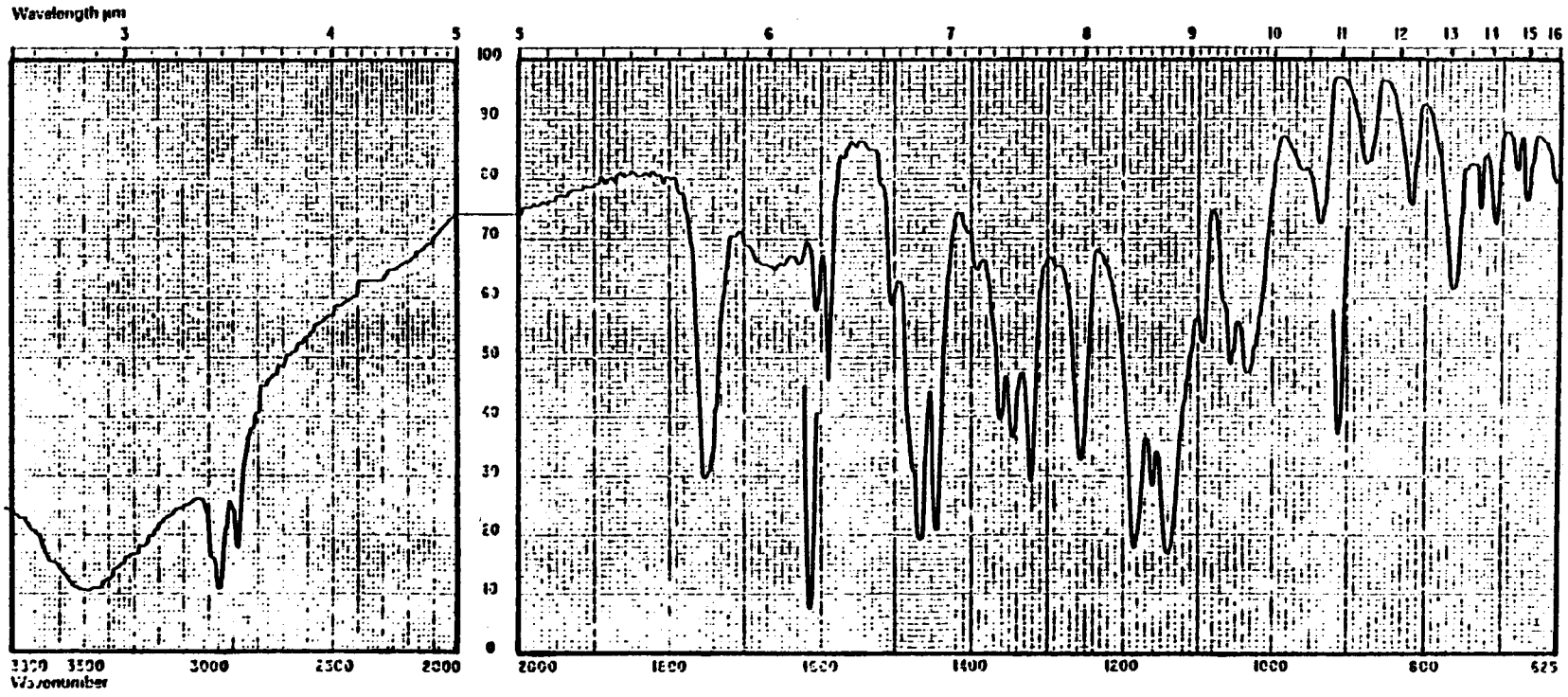

Fig. A3.13 - IR Spectrum (KBr) of Compound X (fluphenazine decanoate sulphoxide mono N-oxide B).

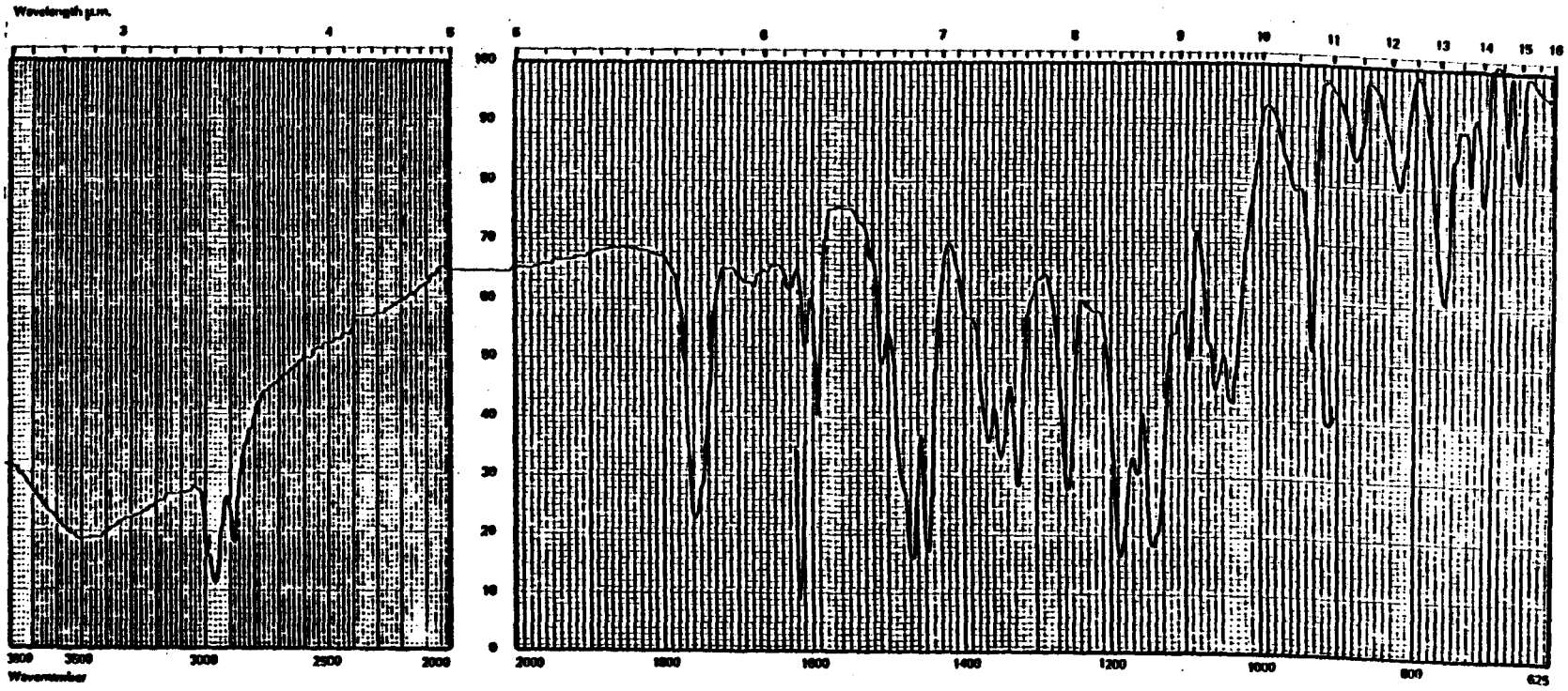

Fig. A3.14 - IR Spectrum (KBr) of Compound Y (fluphenazine decanoate sulphoxide mono-N-oxide A)

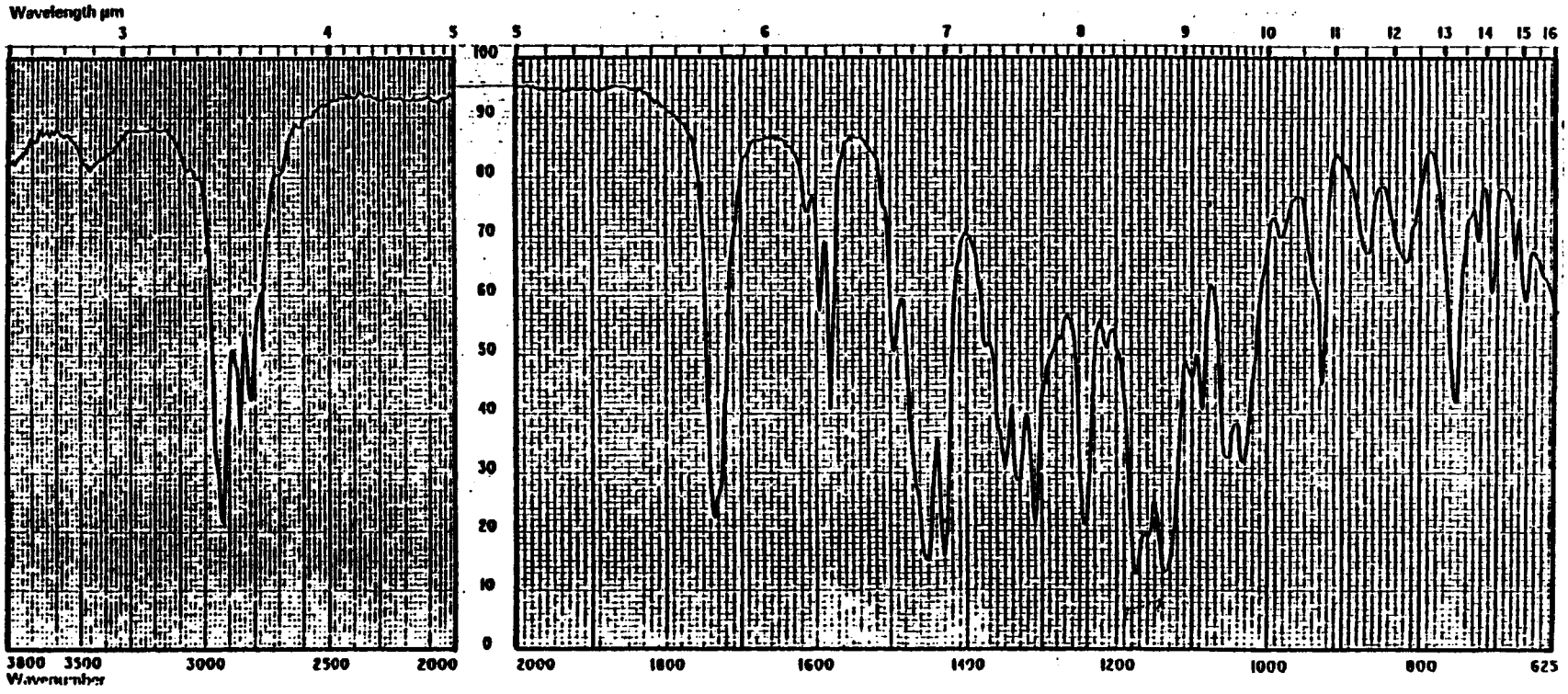

Fig. A3.15 - IR Spectrum (KBr) of Compound Z (fluphenazine decanoate sulphoxide)

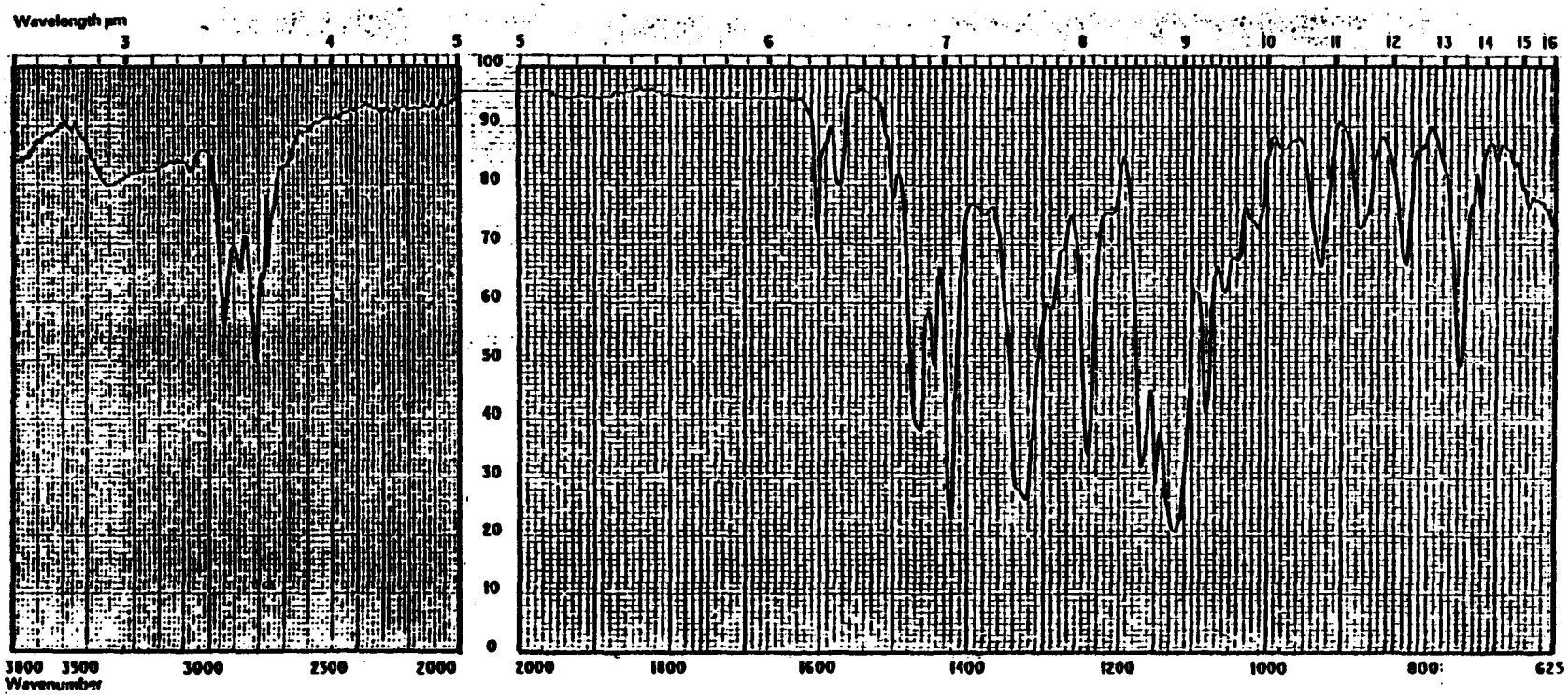

Fig. A3.16 - IR Spectrum (smear on NaCl plates) of Fluphenazine Base

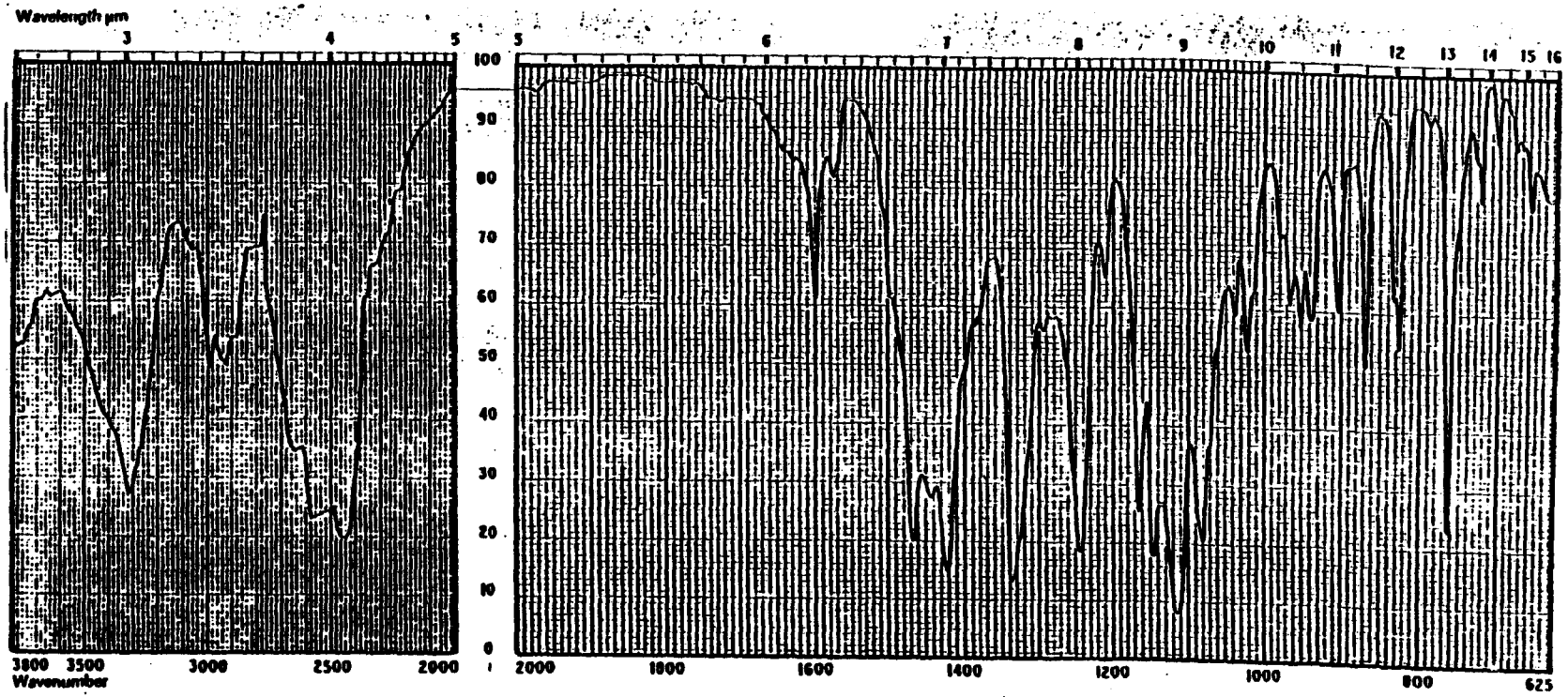

Fig. A3.17 - IR Spectrum (KBr) of Fluphenazine Dihydrochloride

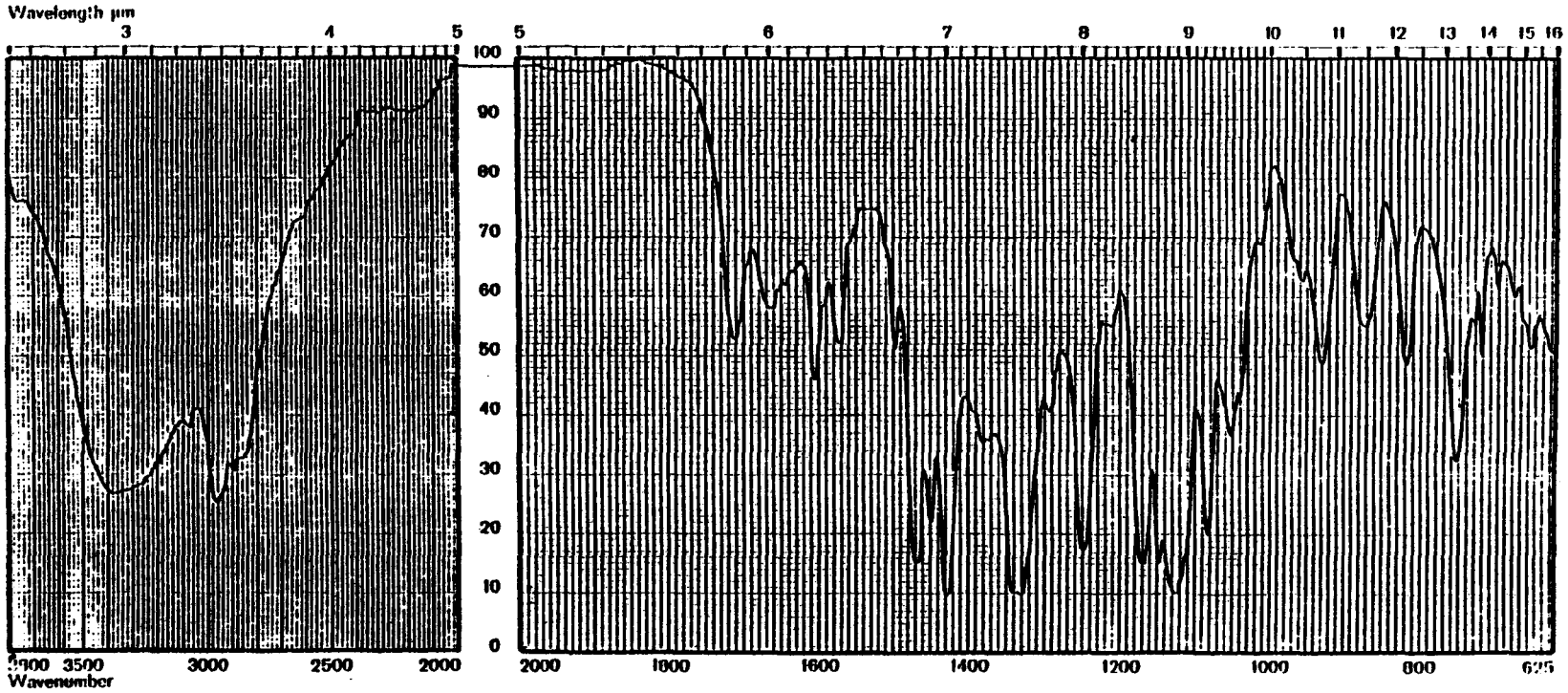

Fig. A3.18 - IR Spectrum (smear on NaCl plate) of Compound A' (fluphenazine mono-N-oxide A).

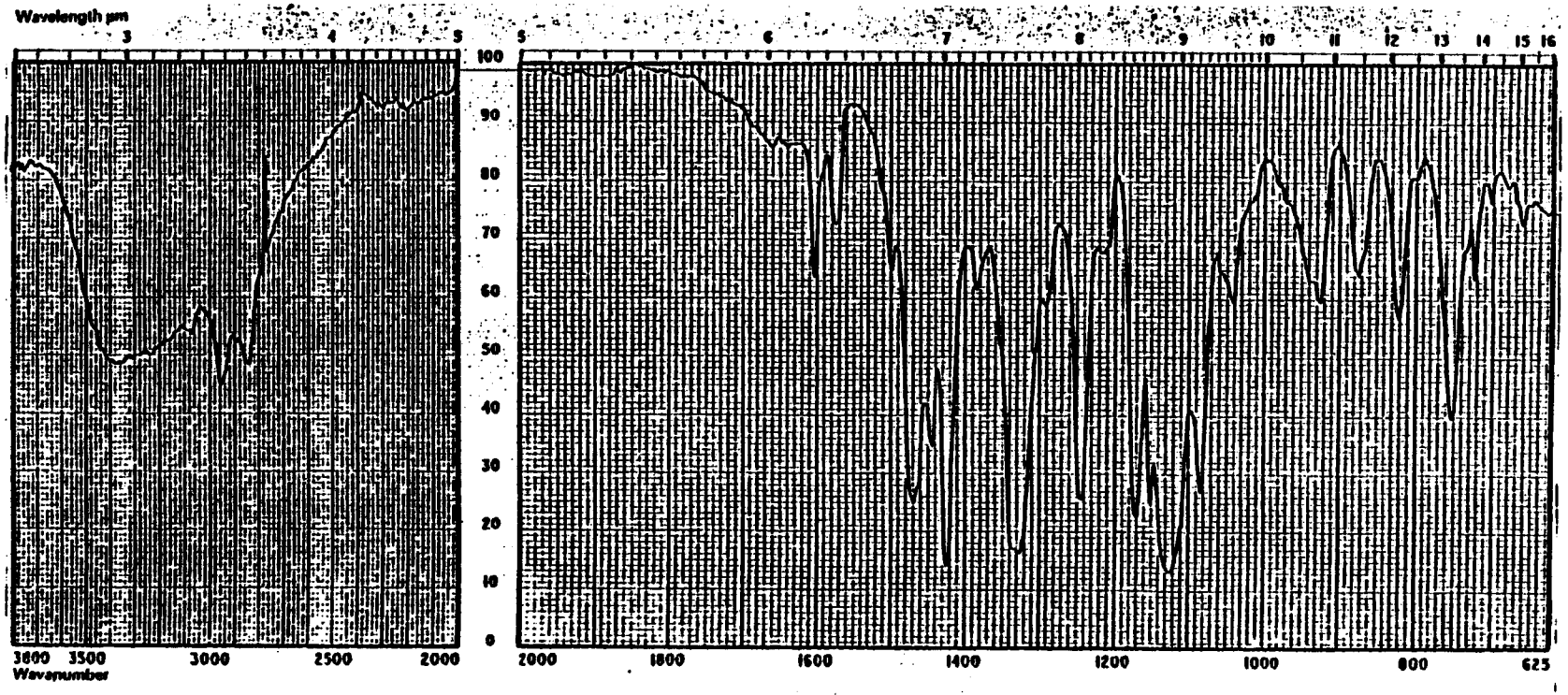

Fig. A3.19 - IR Spectrum (KBr) of Compound B' (fluphenazine mono-N-oxide B).

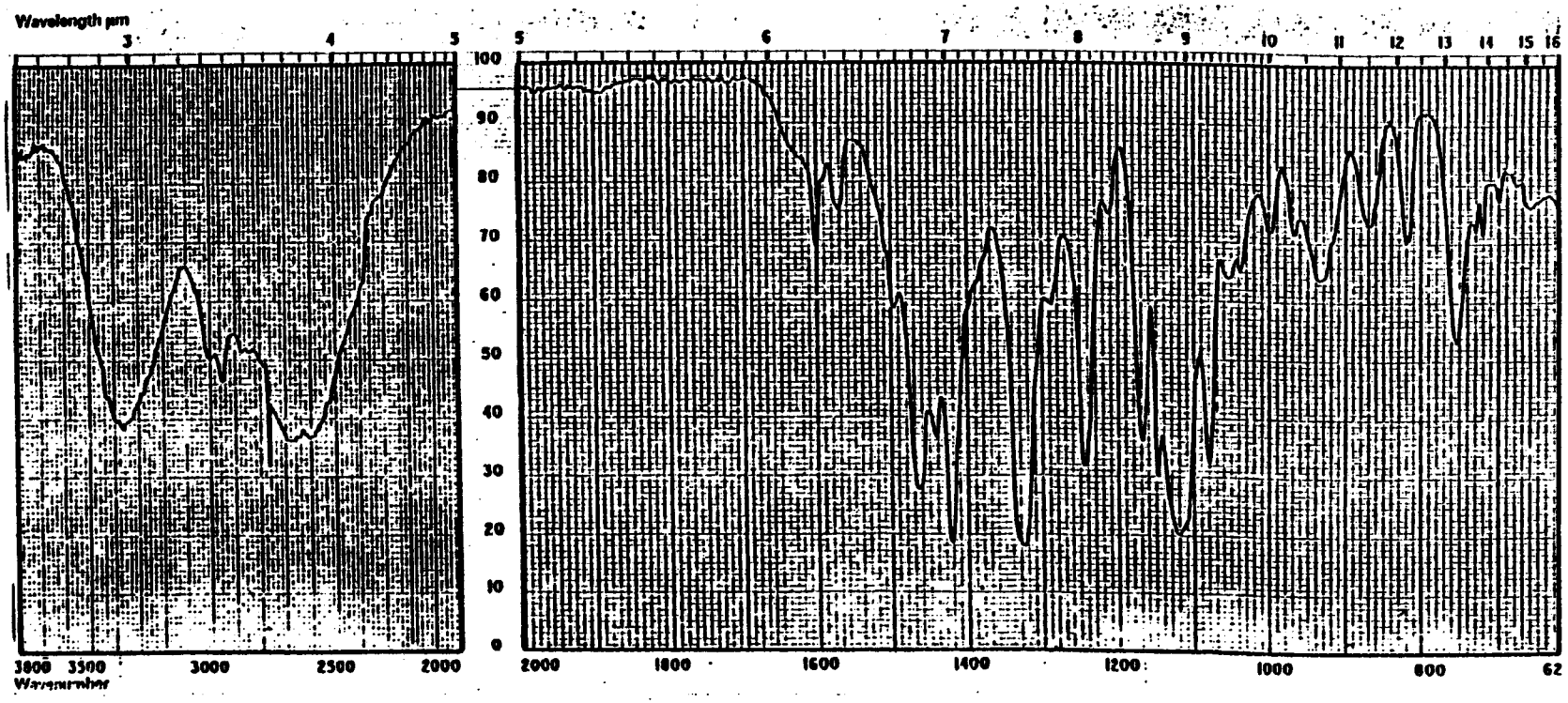

Fig. A3.20 - IR Spectrum (KBr) of Compound C' Hydrochloride Salt. (fluphenazine di-N-oxide dihydrochloride)

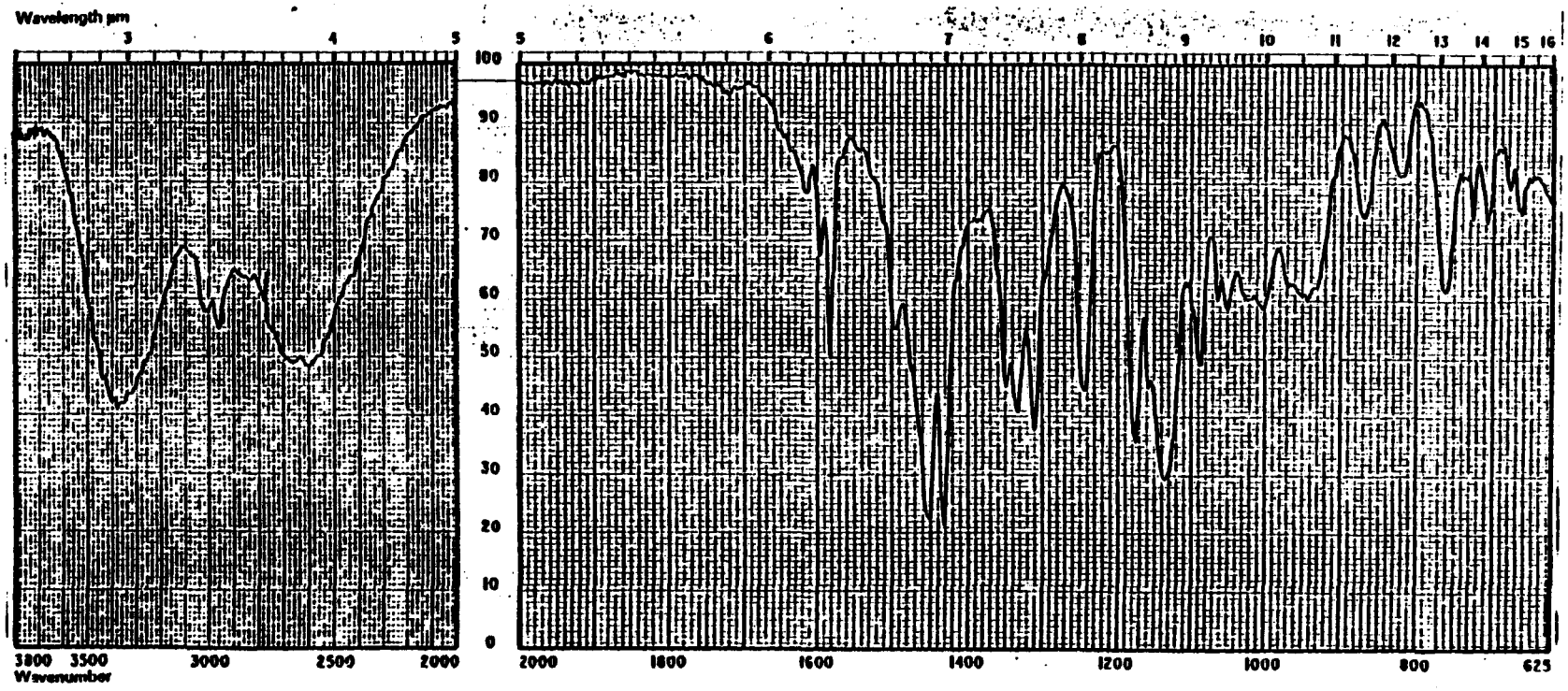

Fig. A3.21 - IR Spectrum (KBr) of Compound D' Hydrochloride Salt. (fluphenazine sulphoxide di-N-oxide dihydrochloride)

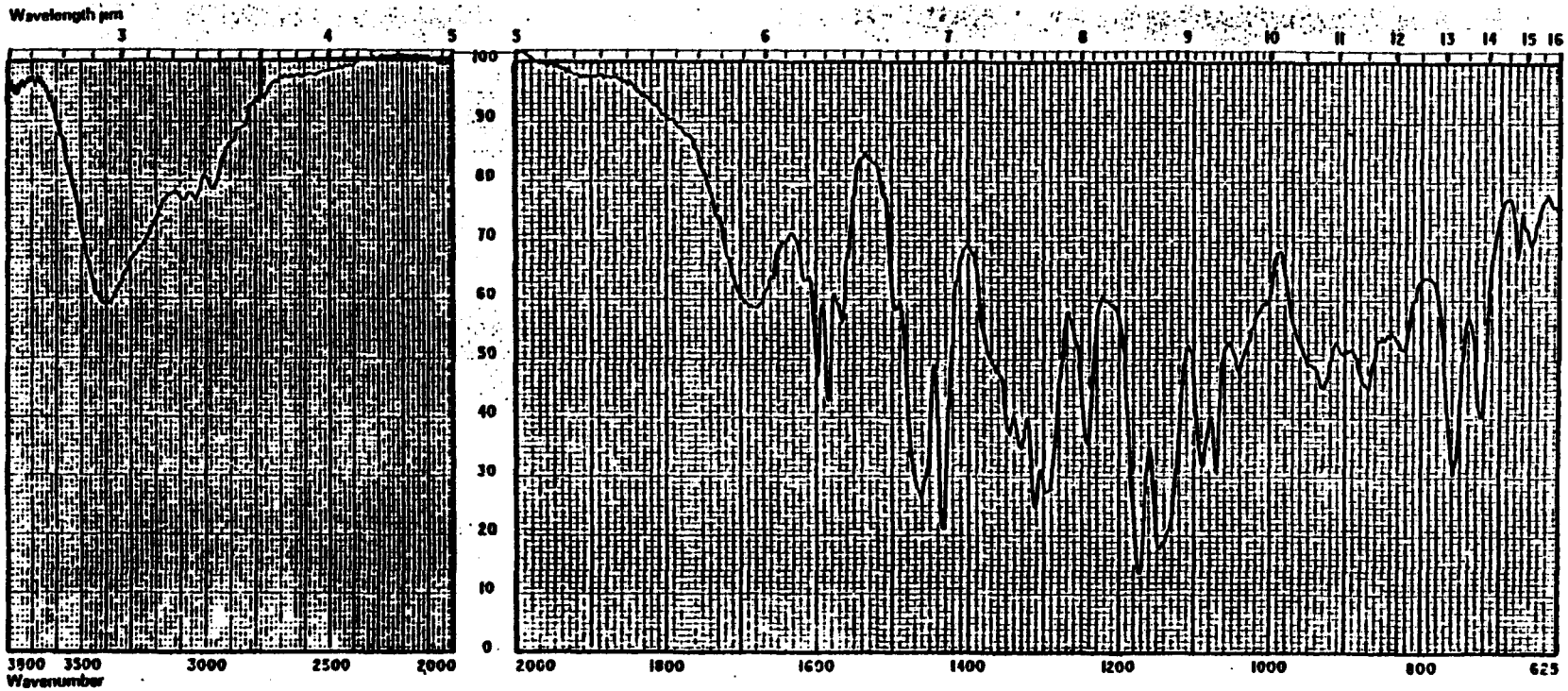

Fig. A3.22 - IR Spectrum (KBr) of Compound E' (fluphenazine sulphone di-N-oxide).

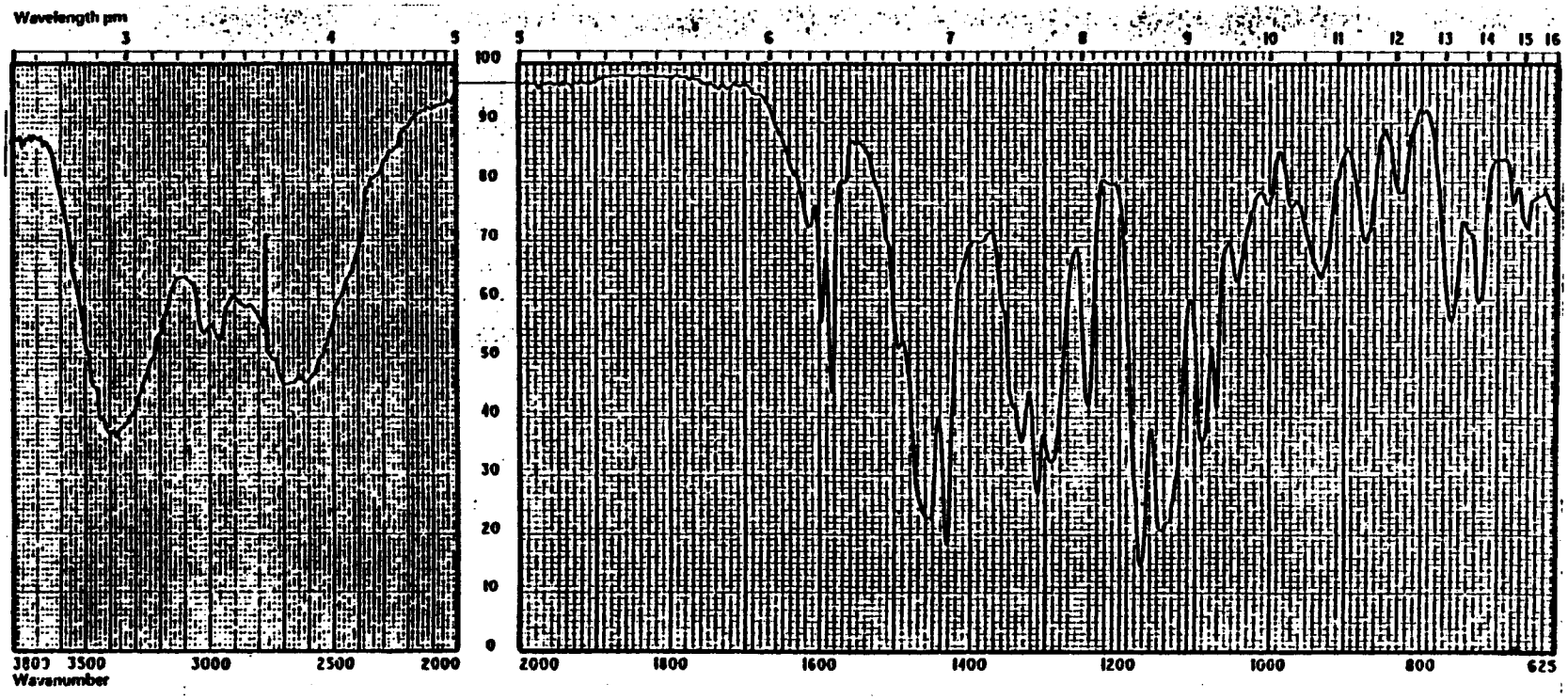

Fig. A3.23 - IR Spectrum of Recrystallised Compound E' Hydrochloride Salt (fluphenazine sulphone di-N-oxide).

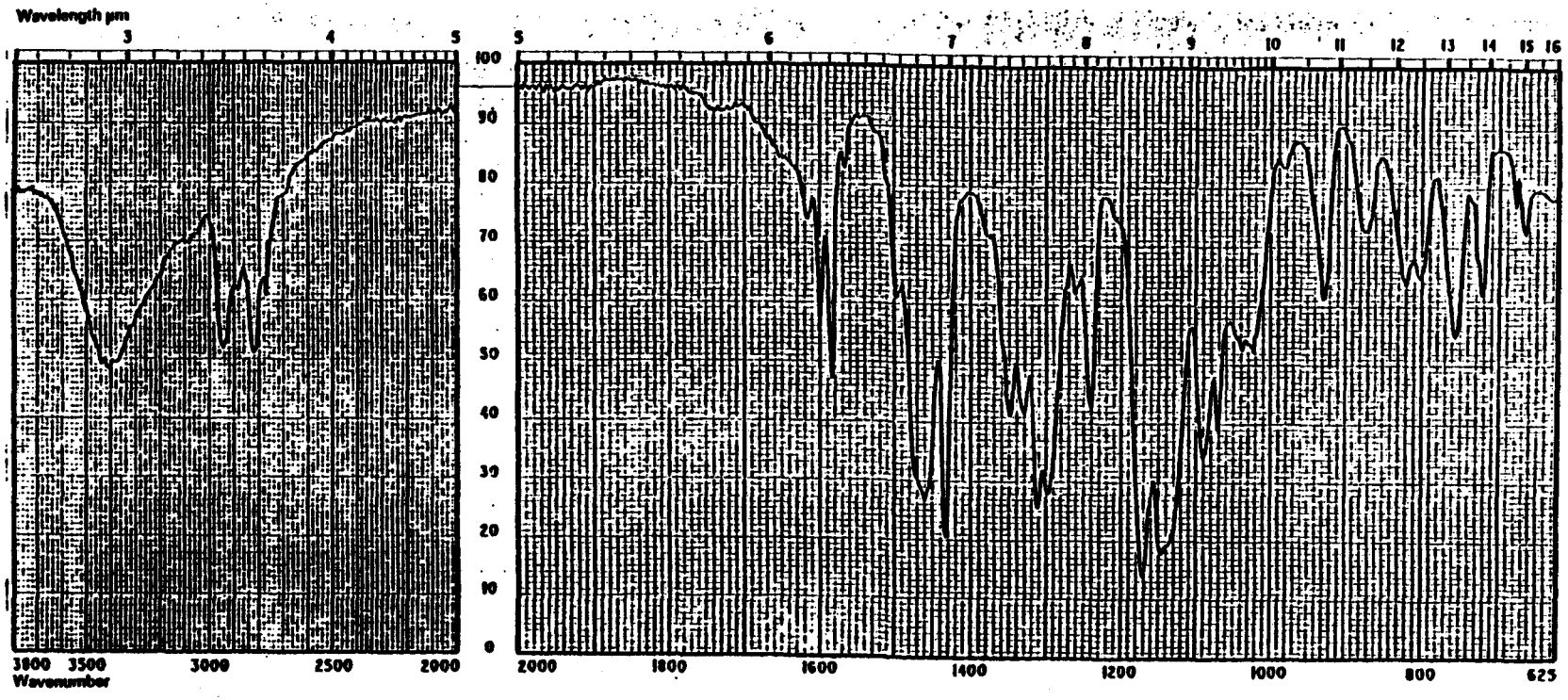

Fig. A3.24 - IR Spectrum (KBr) of Compound F' (fluphenazine sulphone)

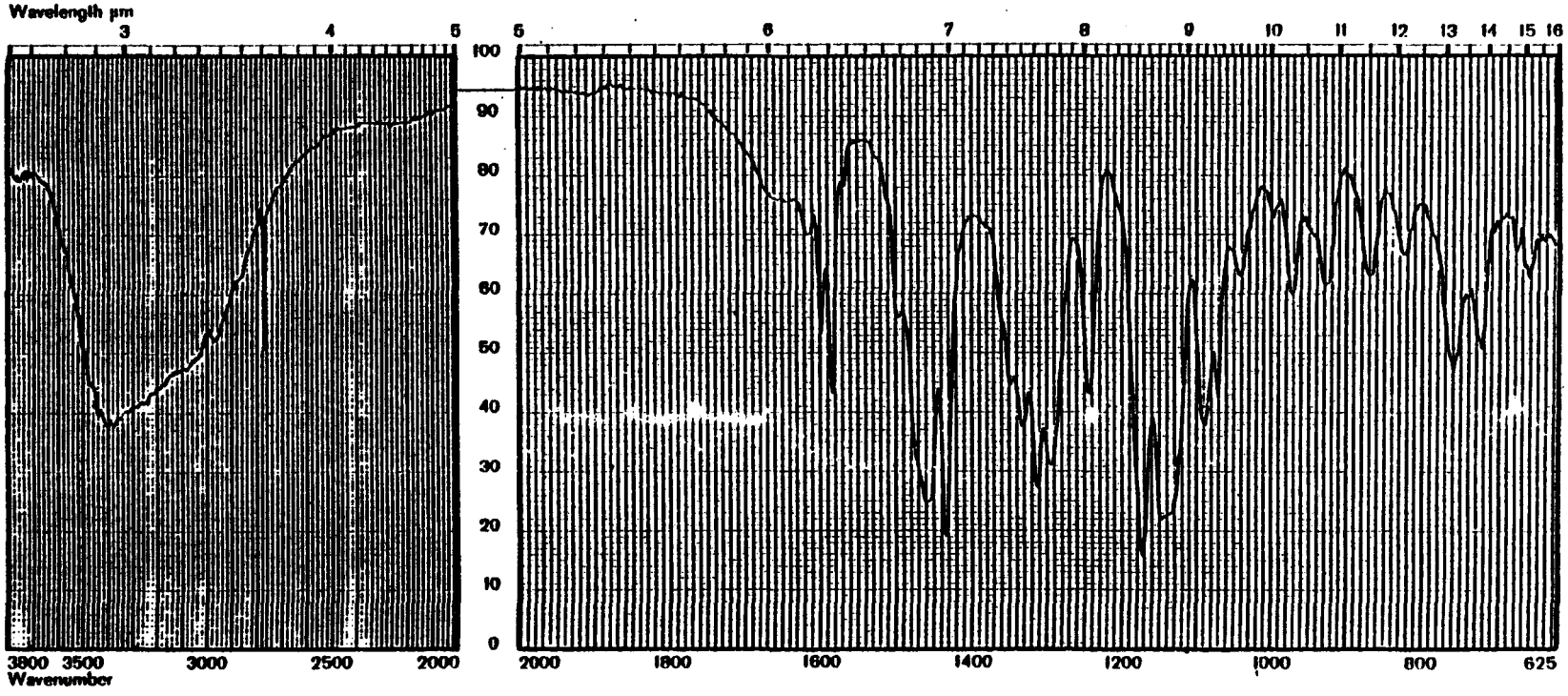

Fig. A3.25 - IR Spectrum (KBr) of Compound V' (fluphenazine sulphone di-N-oxide)

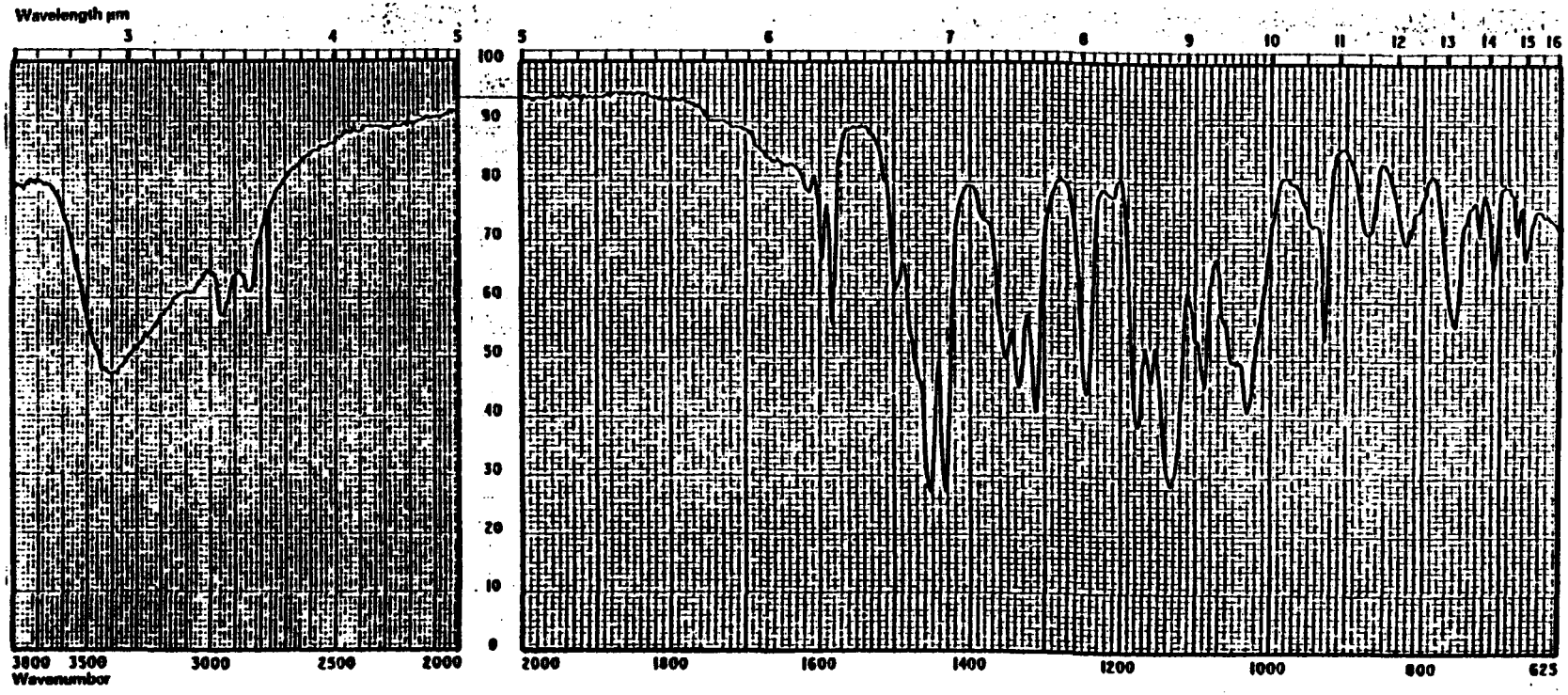

Fig. A3.26 - IR Spectrum (KBr) of Compound Y' (fluphenazine sulphoxide mono-N-oxide)

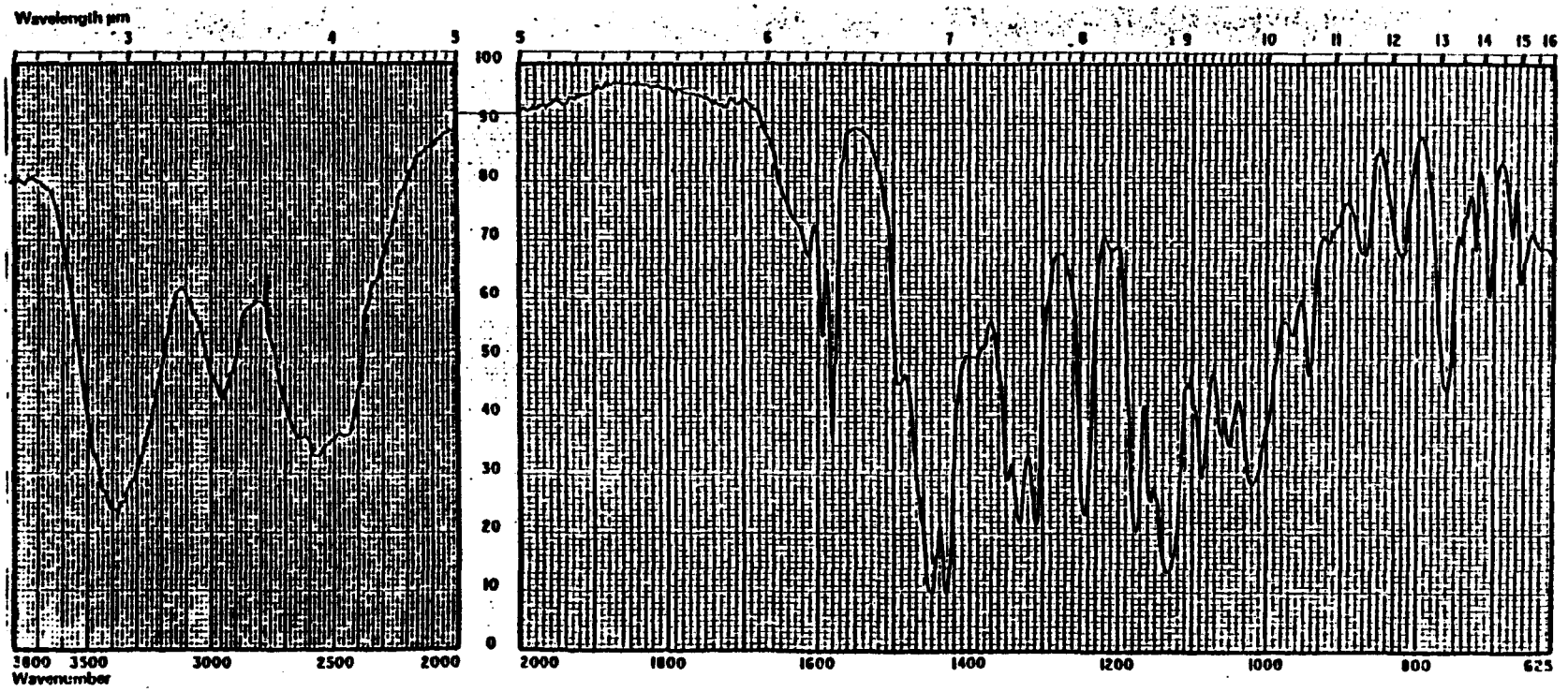

Fig. A3.27 - IR Spectrum (KBr) of Compound Z' Hydrochloride Salt. (fluphenazine sulphoxide dihydrochloride)

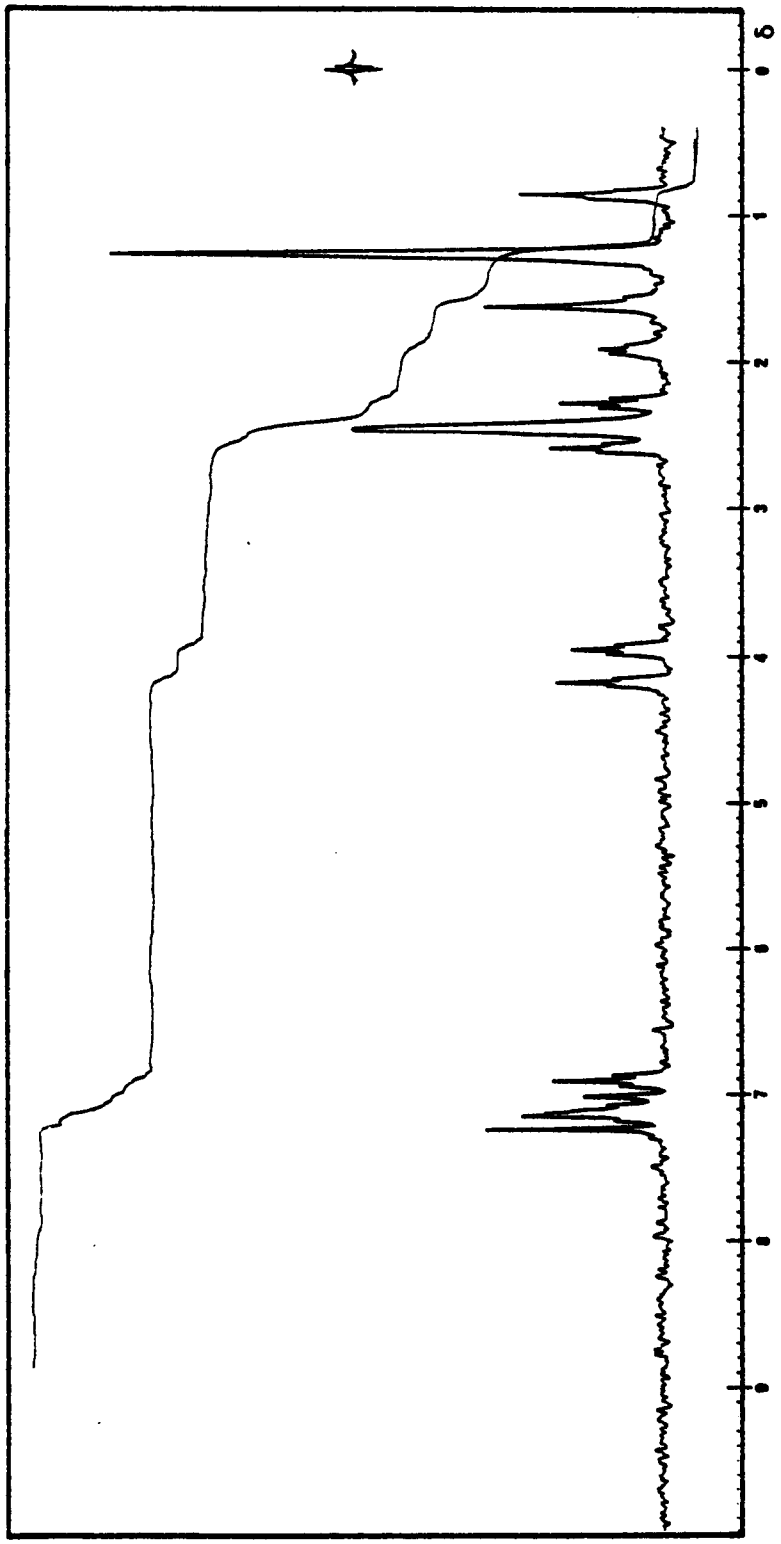

Fig. A3.28 - ^{1}H NMR Spectrum of Fluphenazine Decanoate

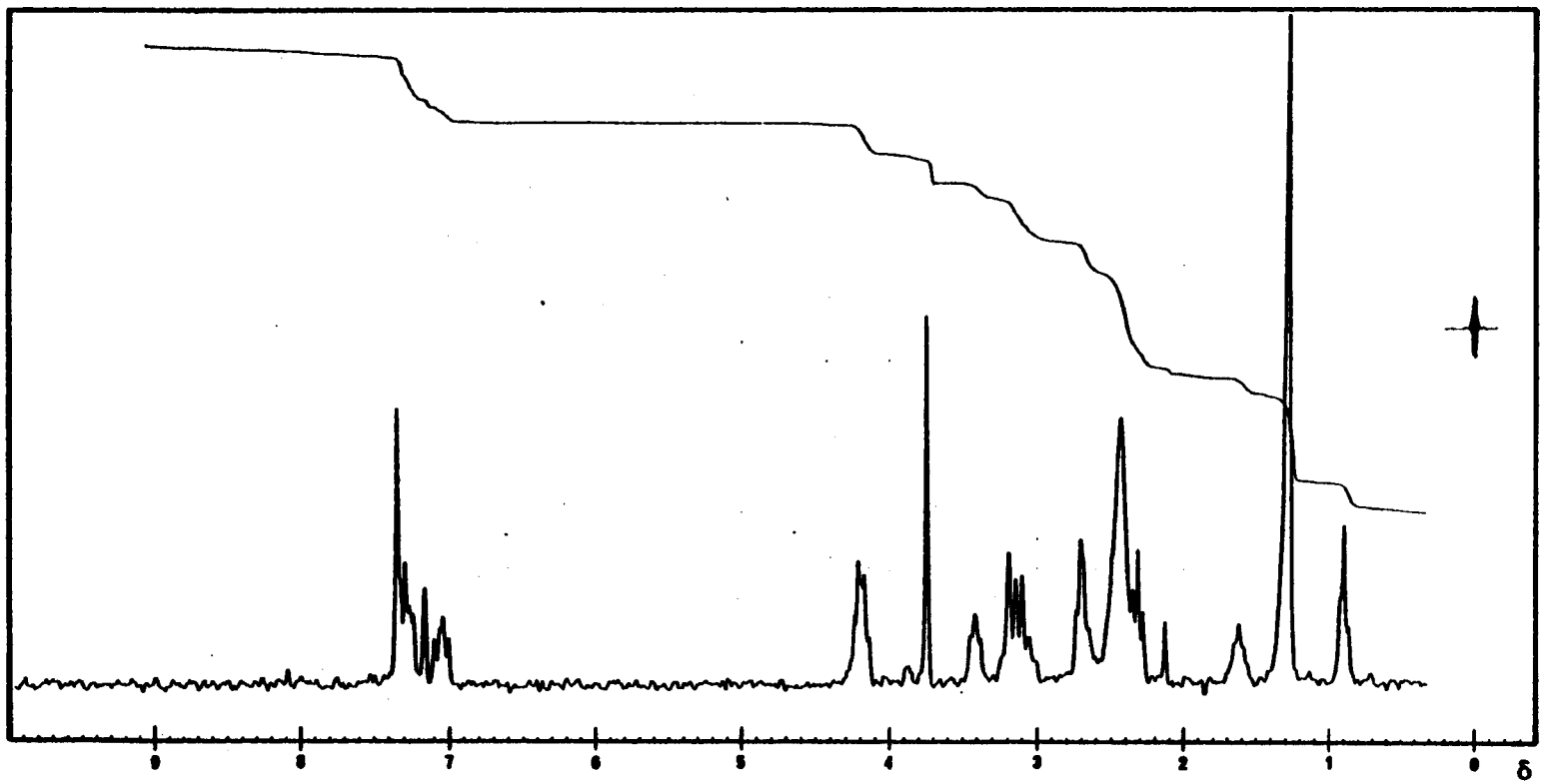

Fig. A3.29 - ^{1}H NMR Spectrum of Compound A (fluphenazine decanoate mono N-oxide).

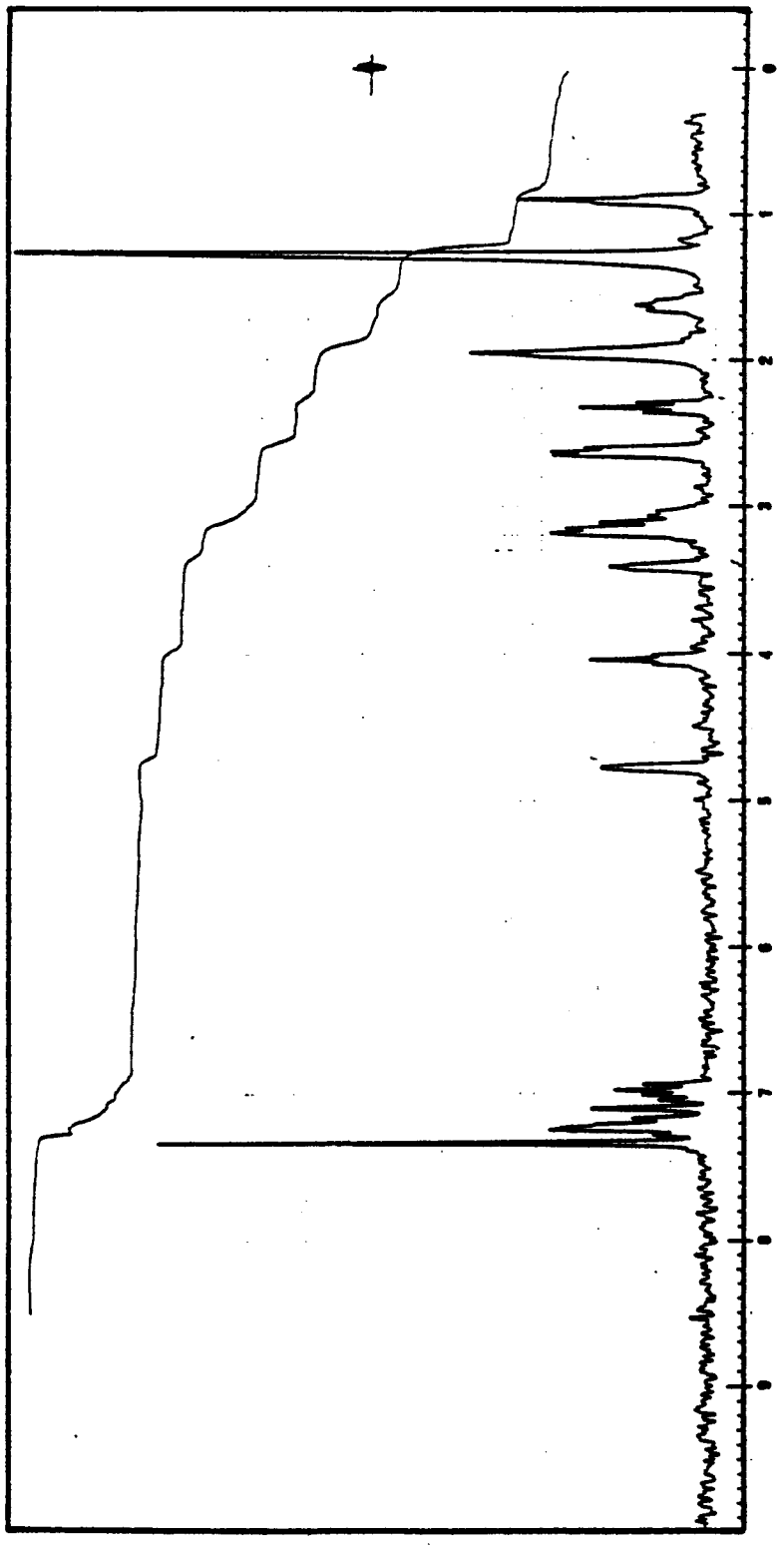

Fig. A3.30 - ^{1}H NMR Spectrum of Compound B (fluphenazine decanoate mono N-oxide B)

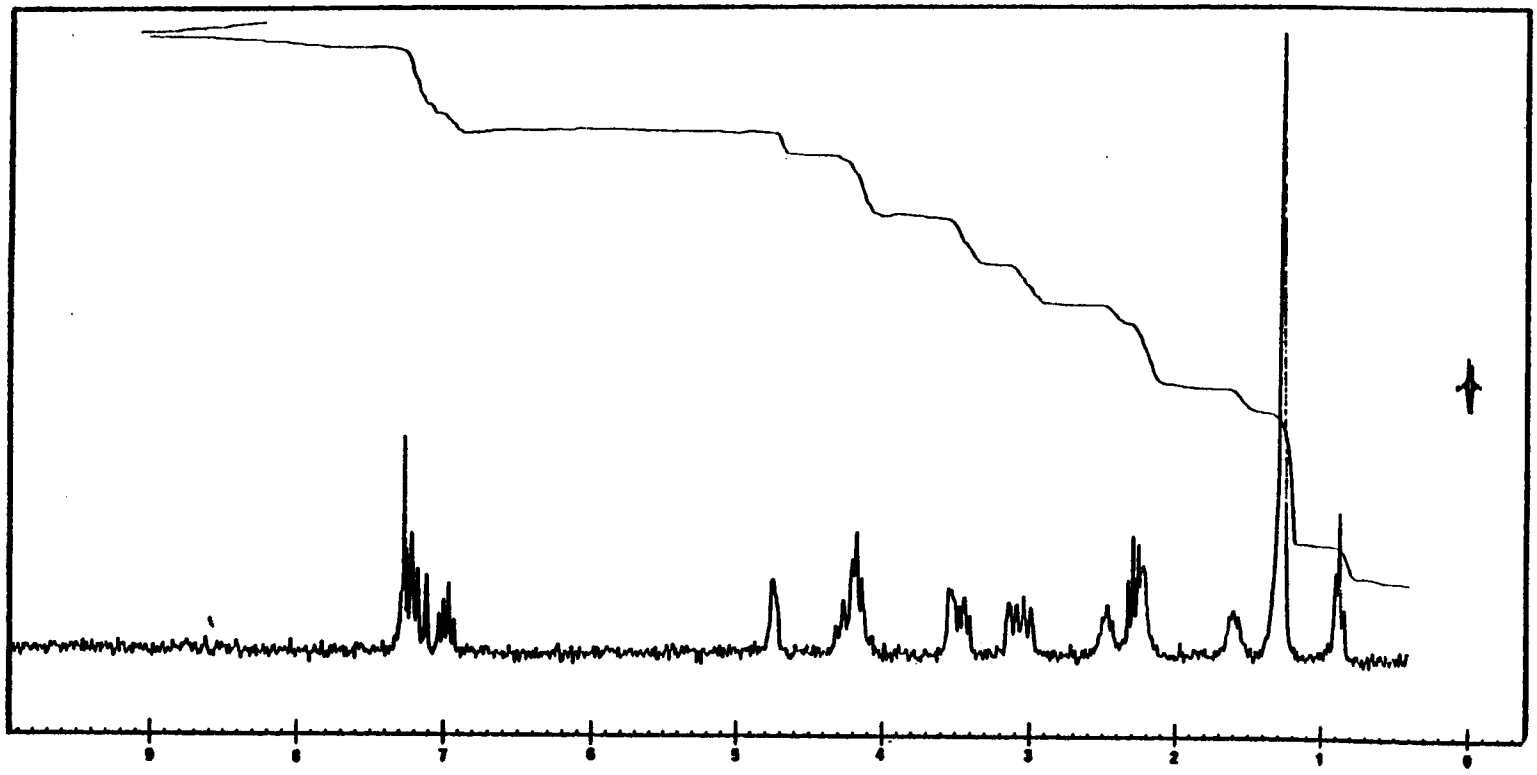

Fig. A3.31 - 'H NMR Spectrum of Compound C (fluphenazine decanoate di N-oxide)

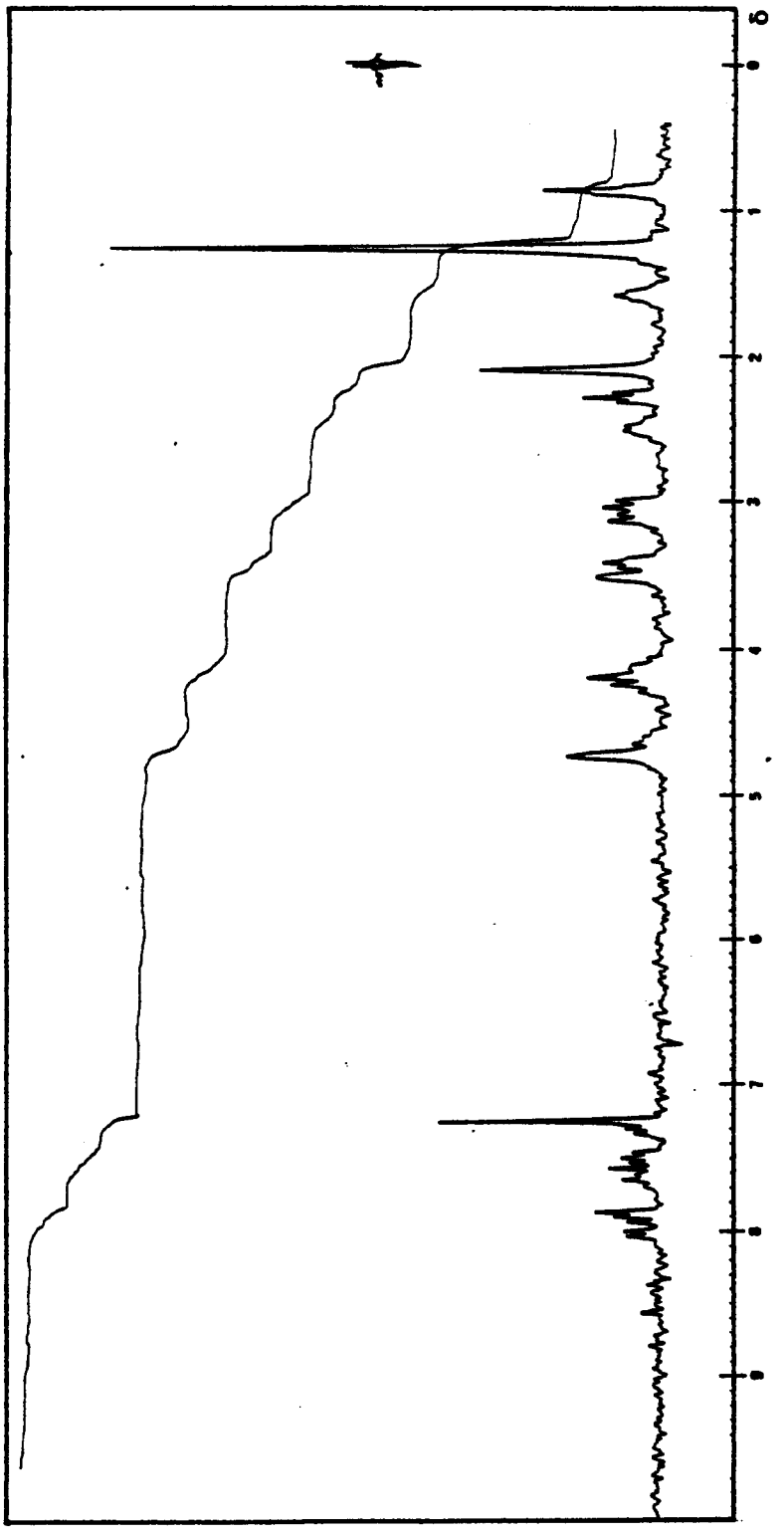

Fig. A3.32 - ^{1}H NMR Spectrum of Compound D (fluphenazine decanoate sulphoxide di-N-oxide).

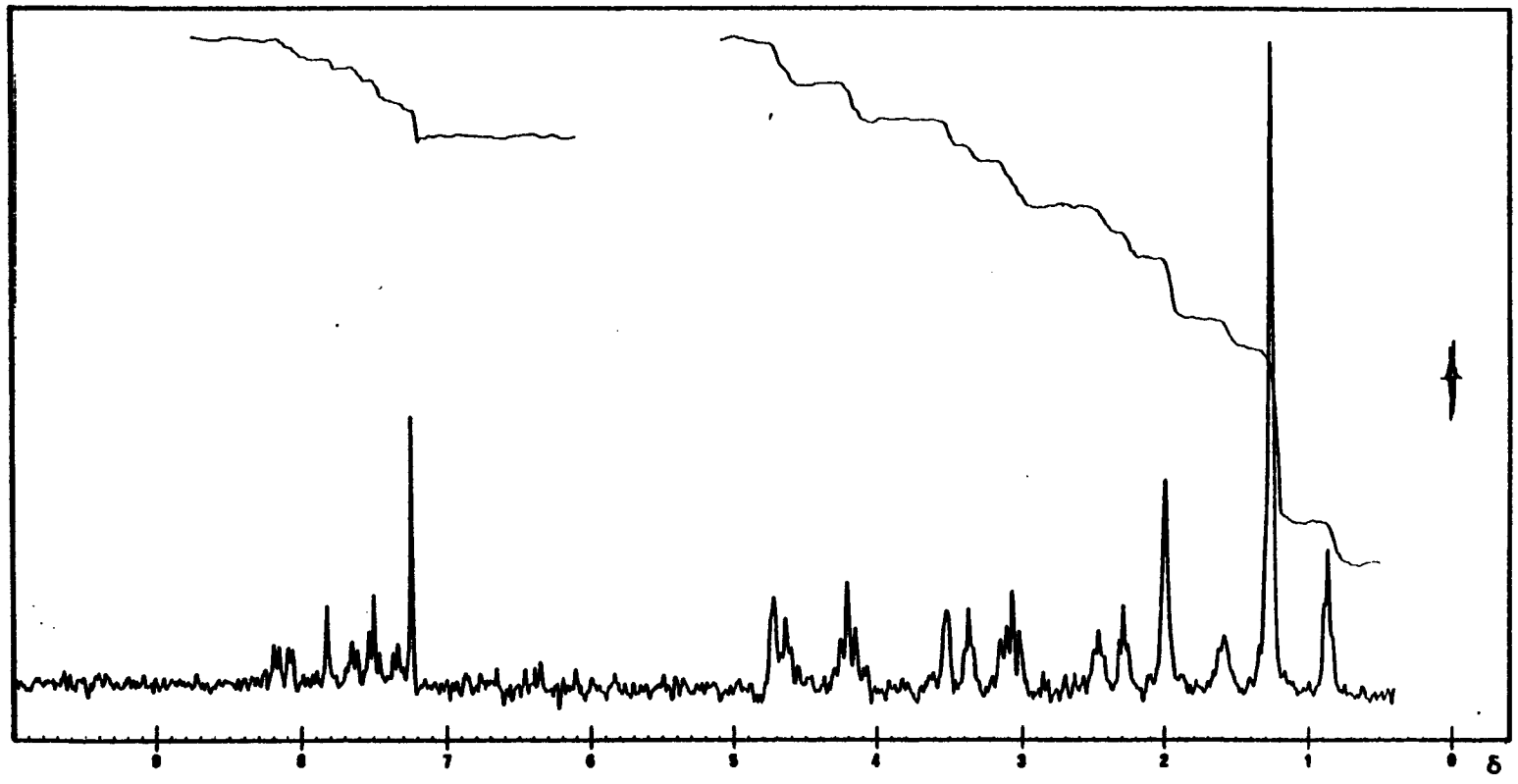

Fig. A3.33'- 'H NMR Spectrum of Compound E (fluphenazine decanoate sulphone di-N-oxide)

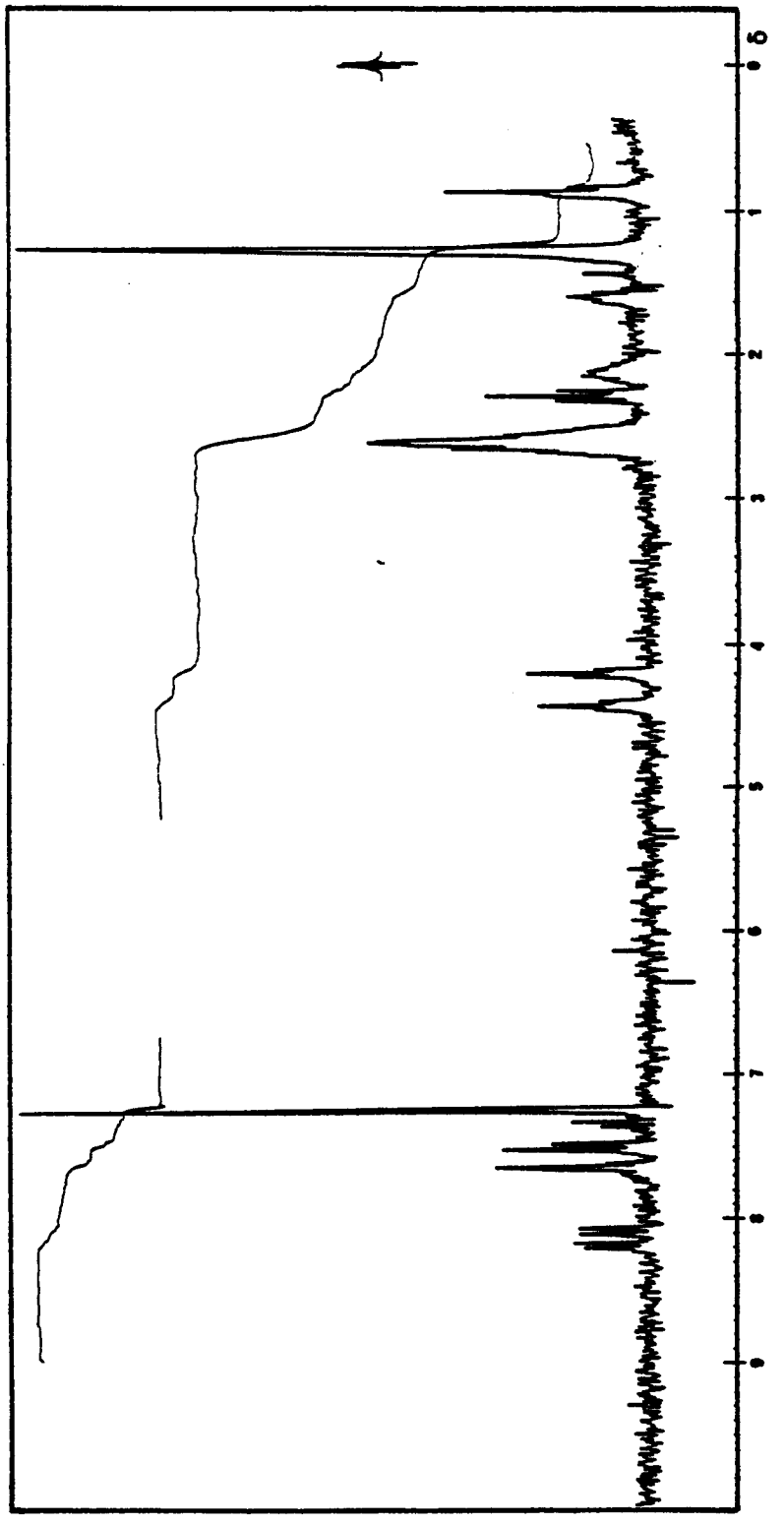

Fig. A3.34 - ^{1}H NMR Spectrum of Compound F (fluphenazine decanoate sulphone)

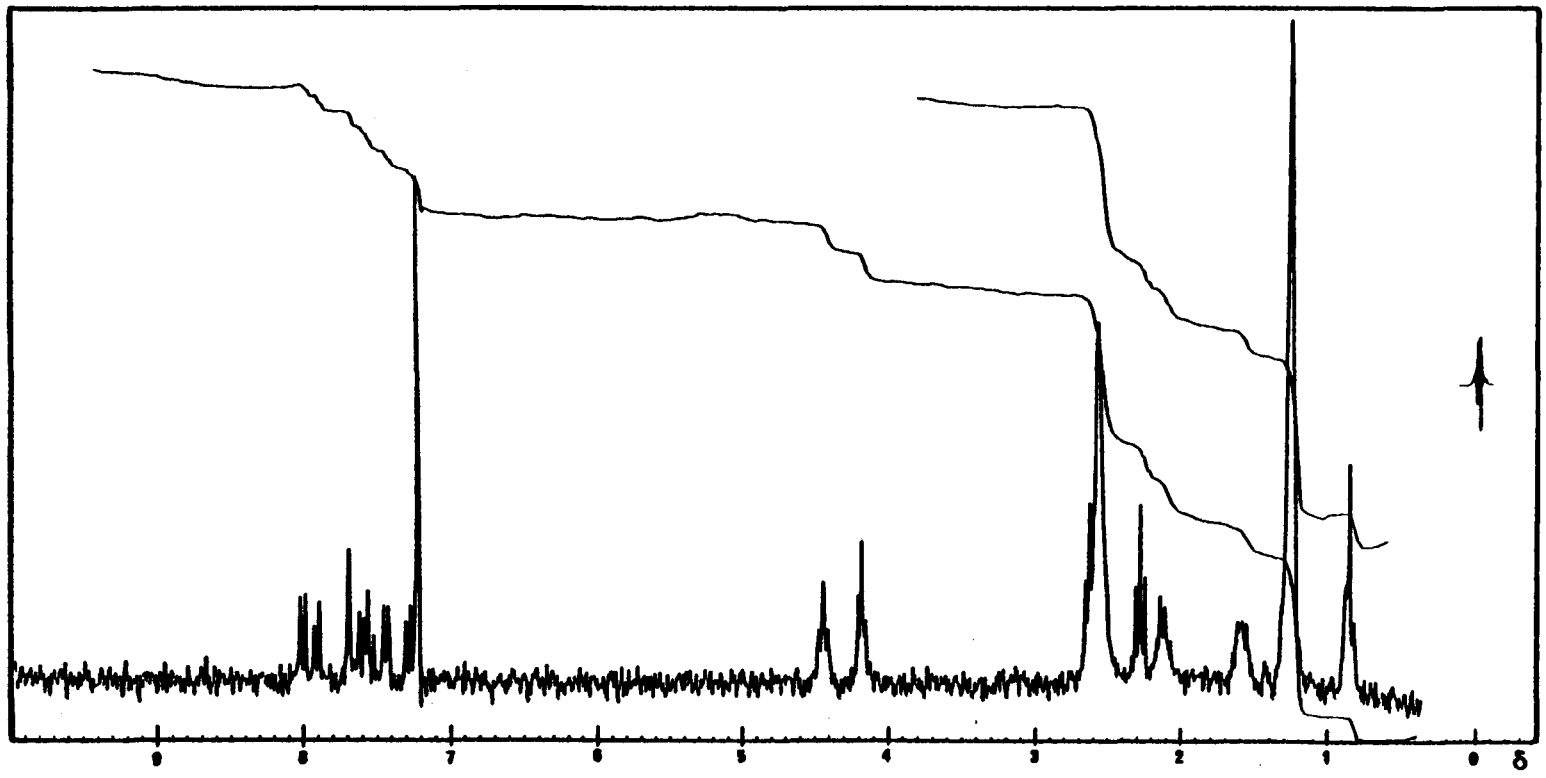

Fig. A3.35 - ^{1}H NMR Spectrum of Compound Z (fluphenazine decanoate sulphoxide)

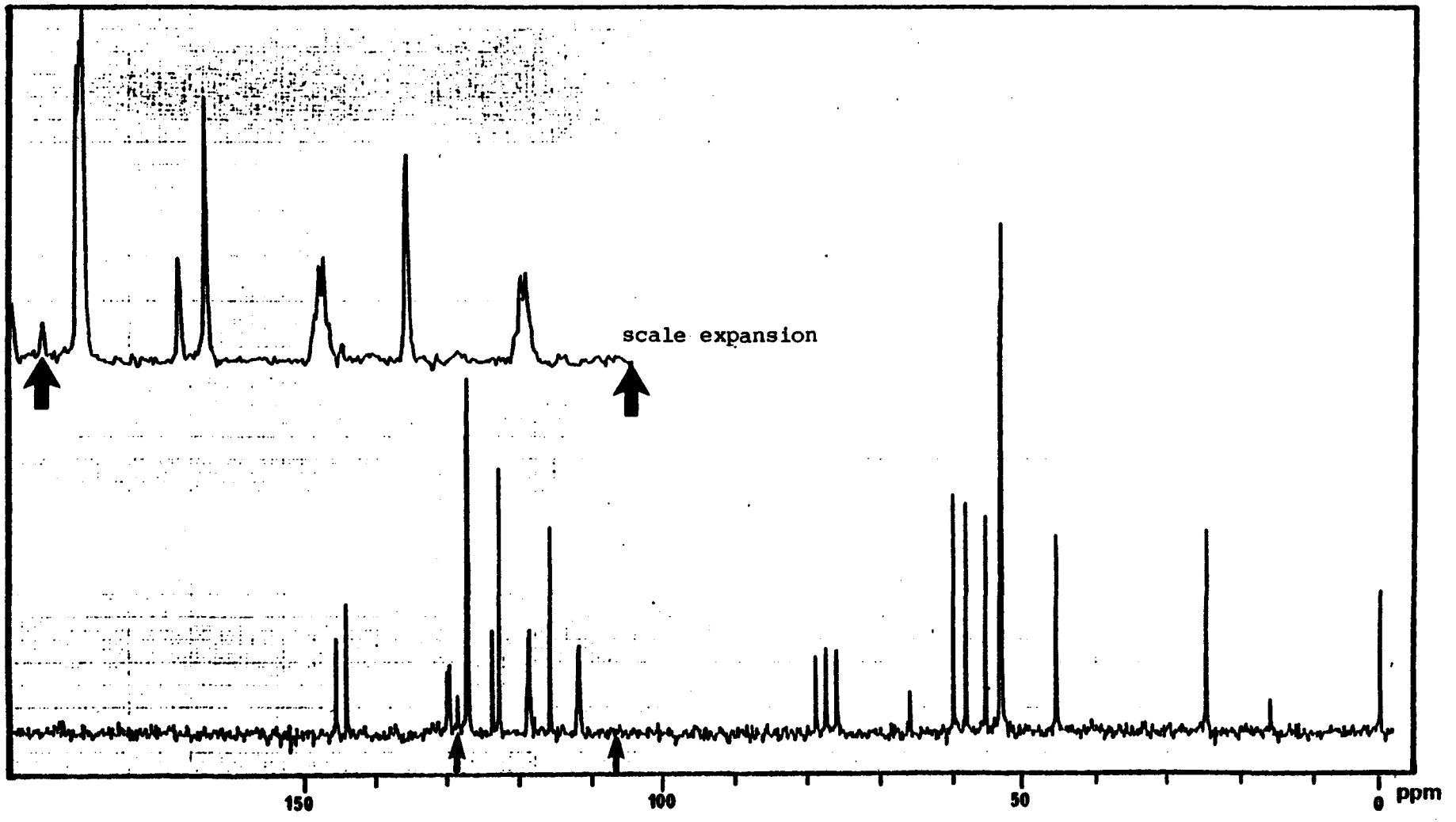

Fig. A3.36 - ^{13}C NMR Spectrum of Fluphenazine (base)

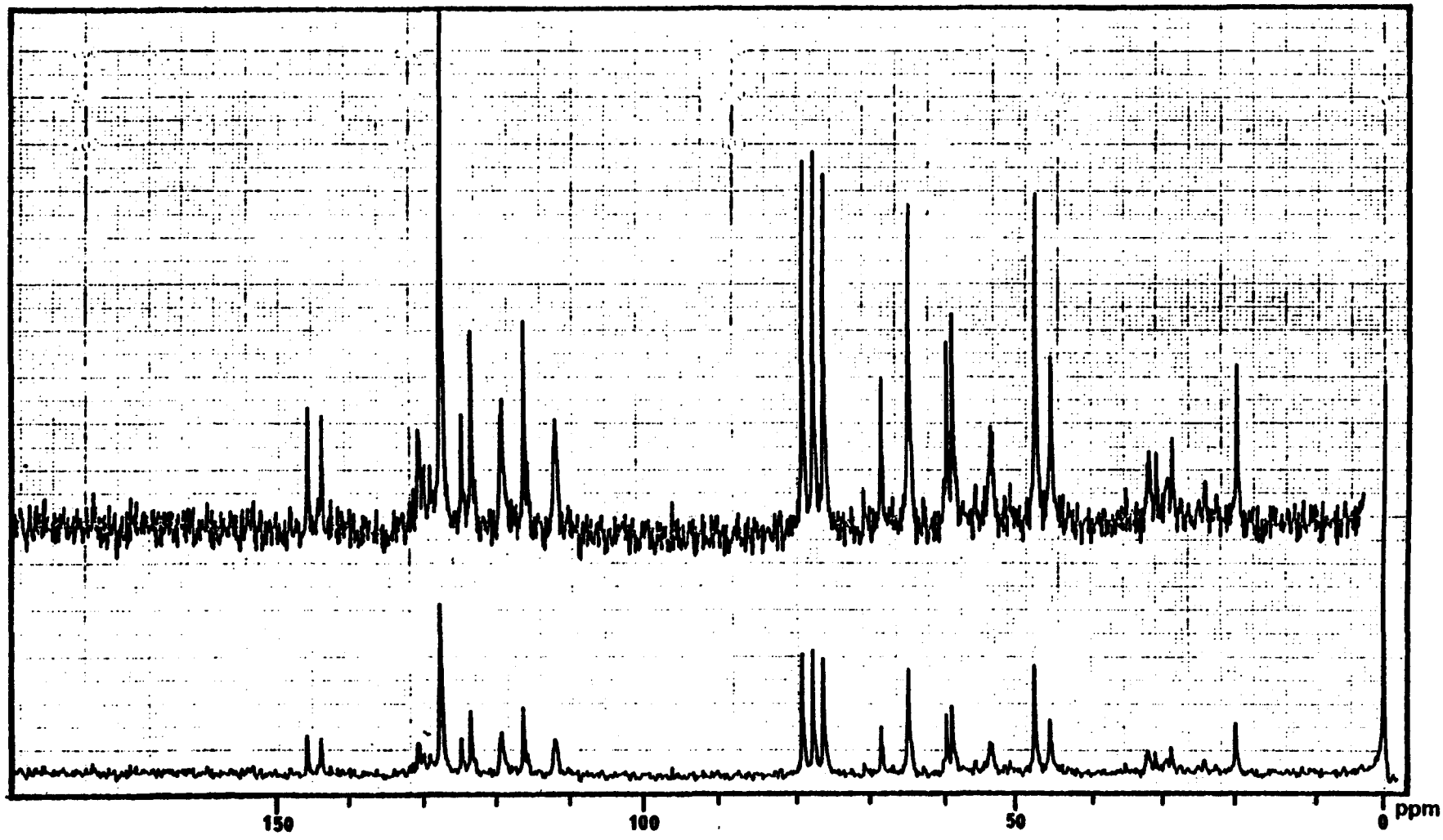

Fig. A3.37 - ^{13}C NMR Spectrum of Compound A' (fluphenazine mono N-oxide A)

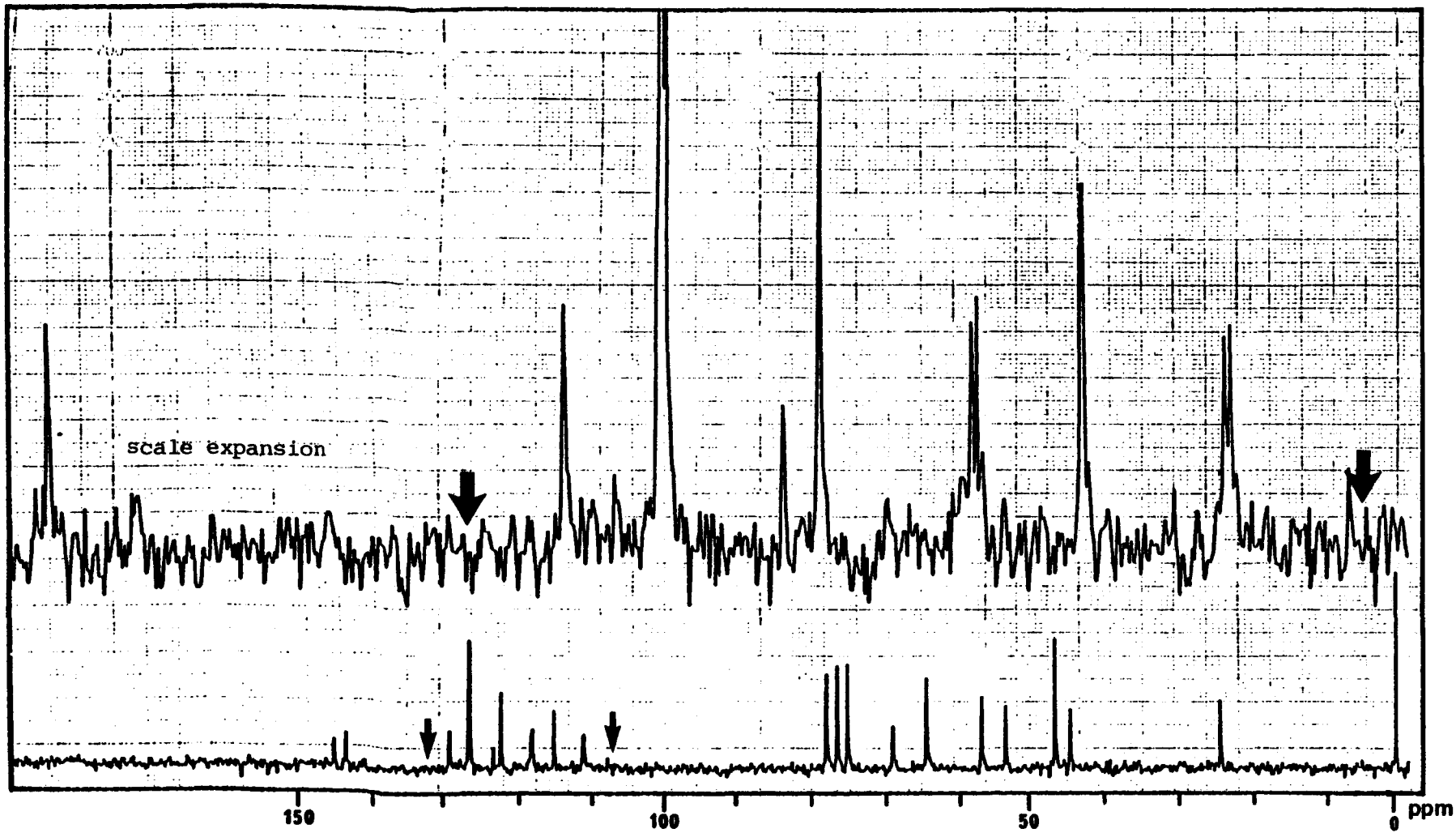

Fig. A3.38 - ^{13}C NMR Spectrum of Compound B' (fluphenazine mono N-oxide B)

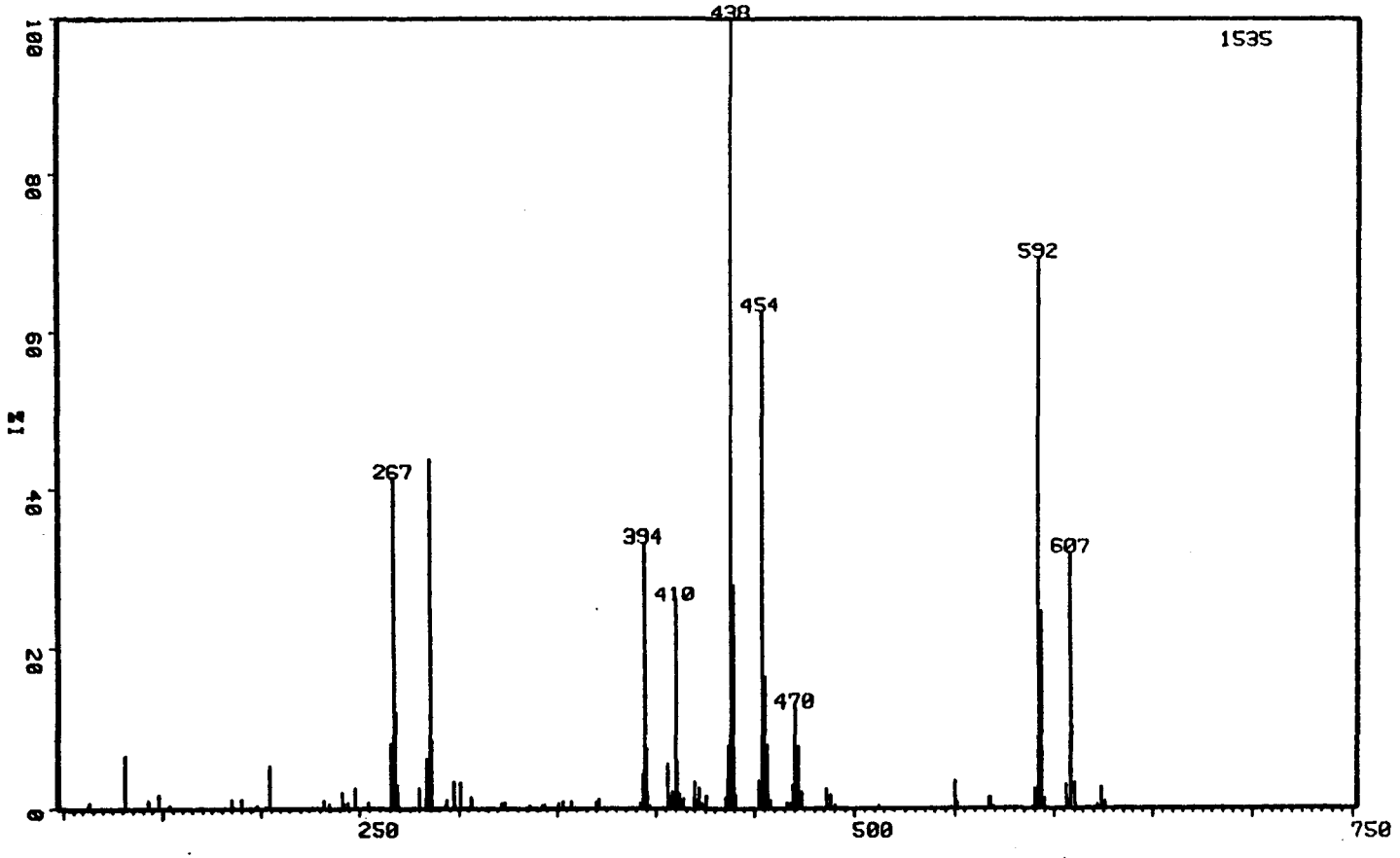

Fig. A3.39 - DCI Mass Spectrum of Compound A (fluphenazine decanoate mono N-oxide A)

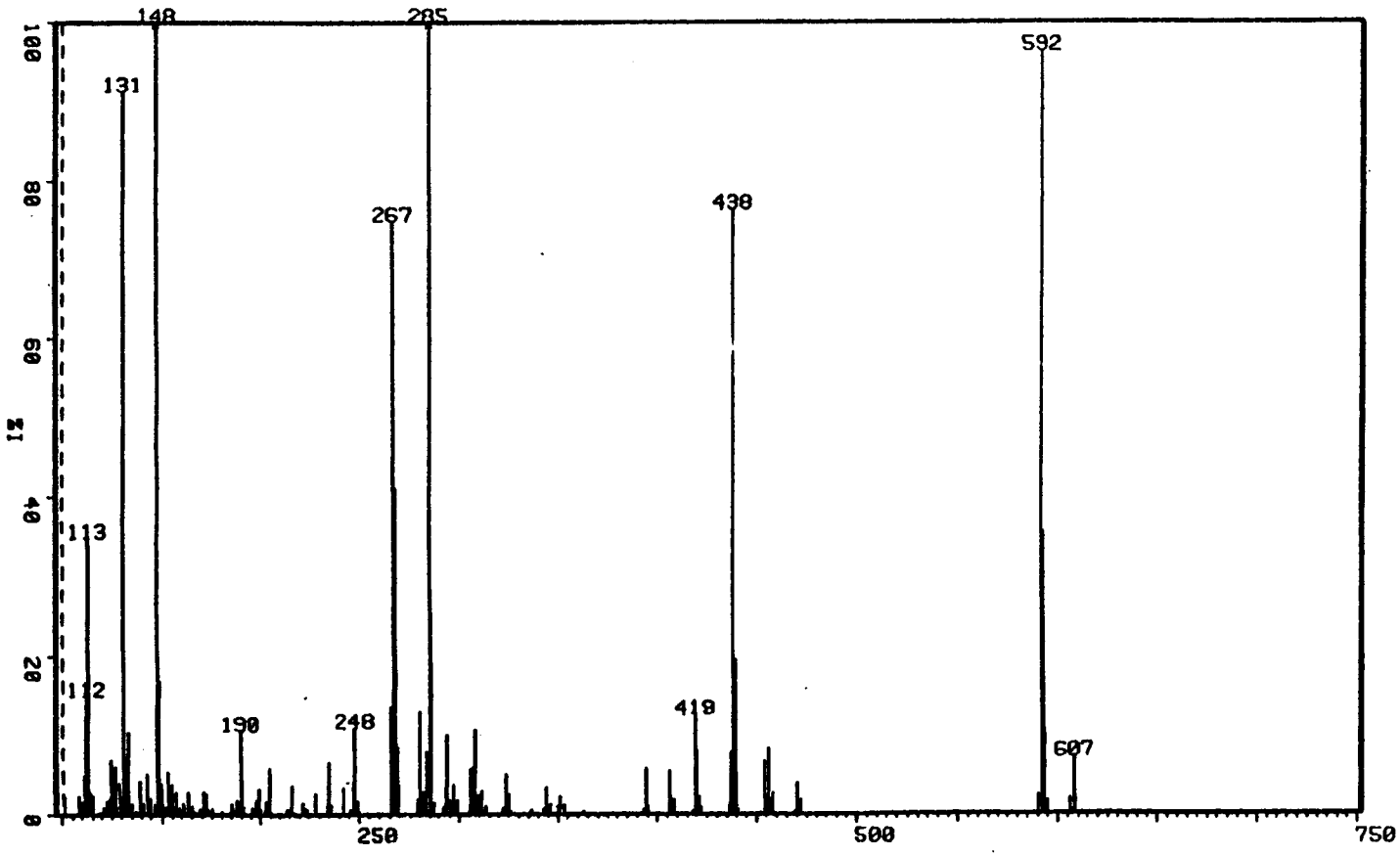

Fig. A3.40 - DCI Mass Spectrum of Compound B (fluphenazine decanoate mono N-oxide B)

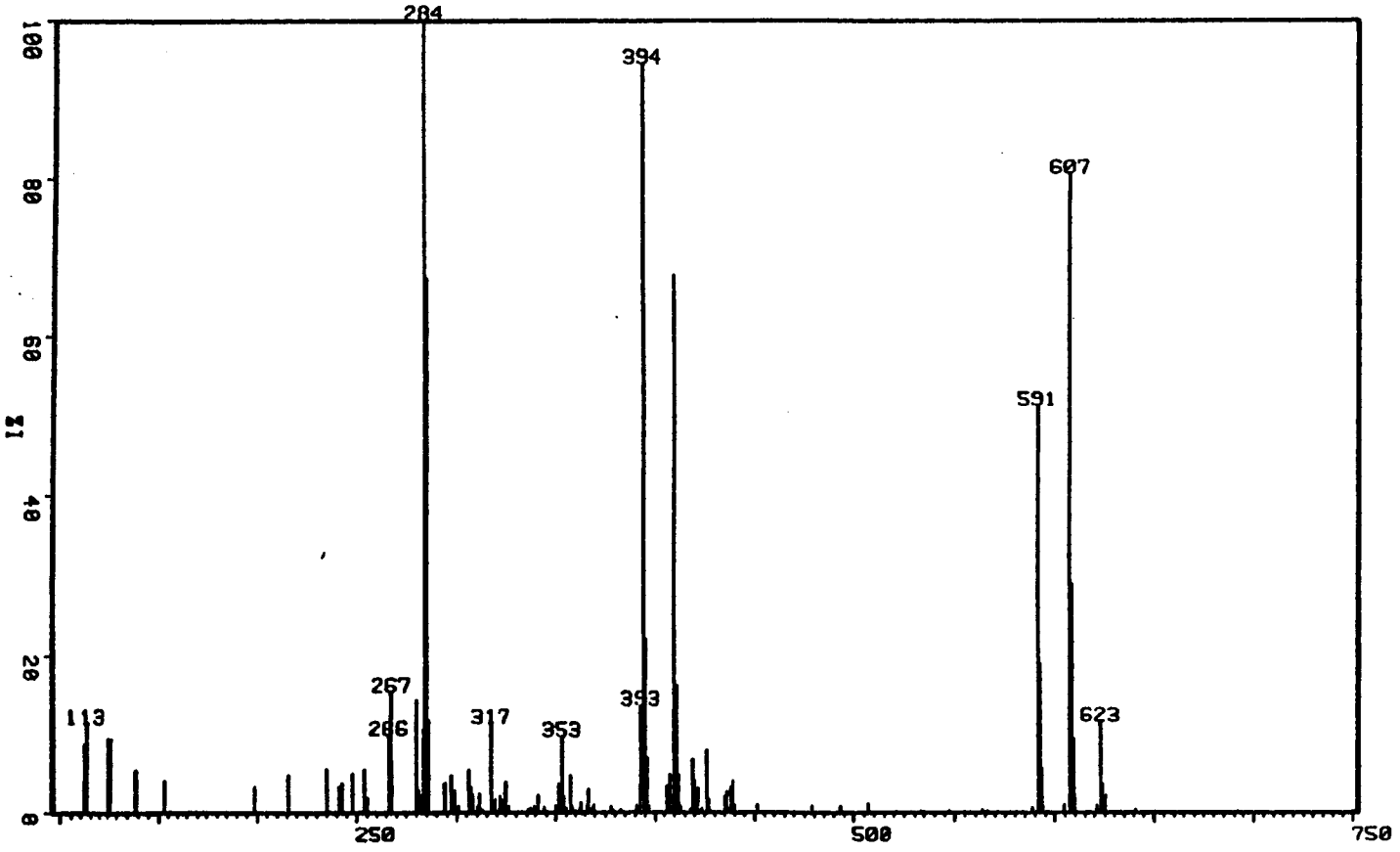

Fig. A3.41 - DCI Mass Spectrum of Compound C (fluphenazine decanoate di N-oxide)

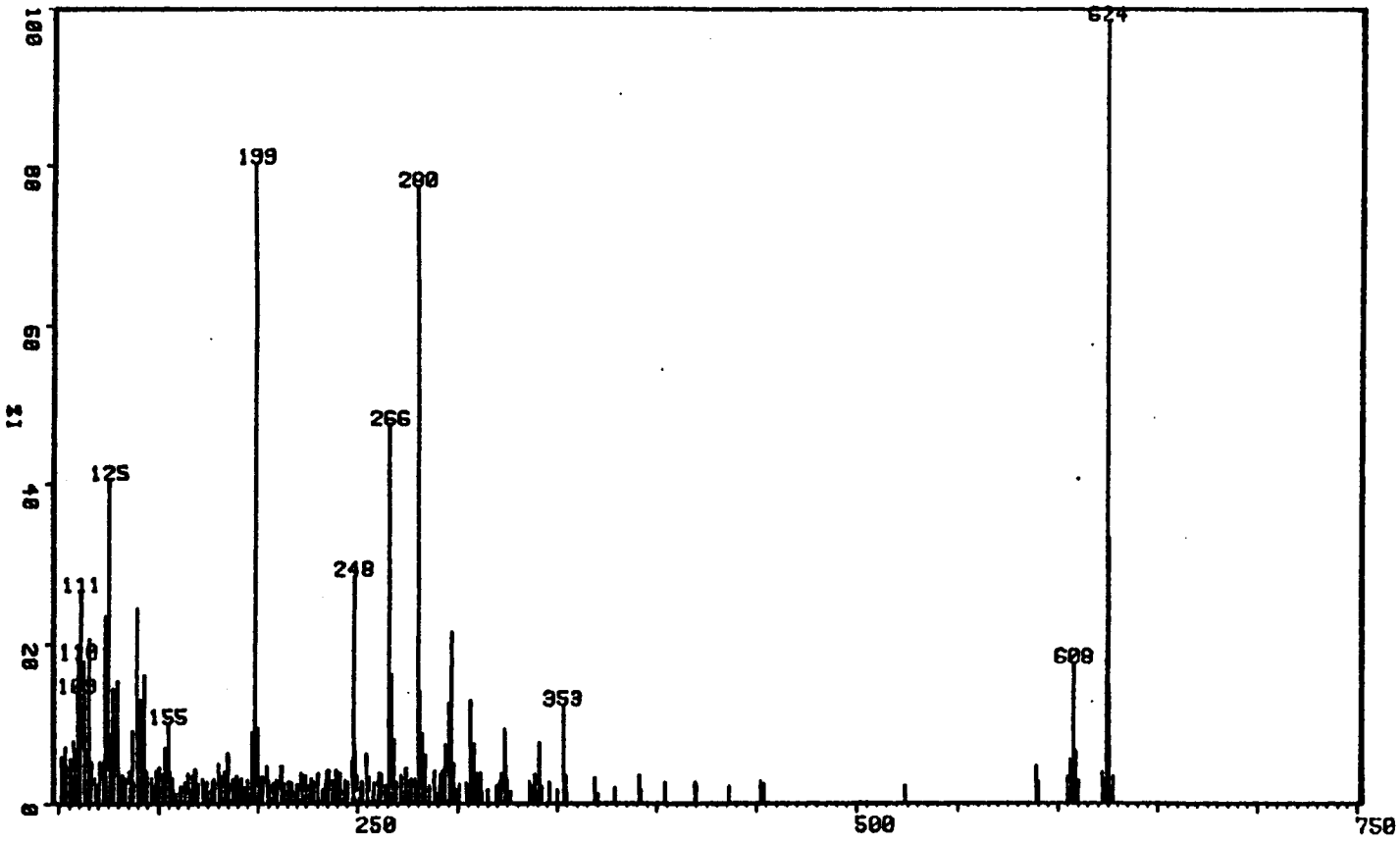

Fig. A3.42 - FAB Mass Spectrum of Compound C (fluphenazine decanoate di N-oxide)

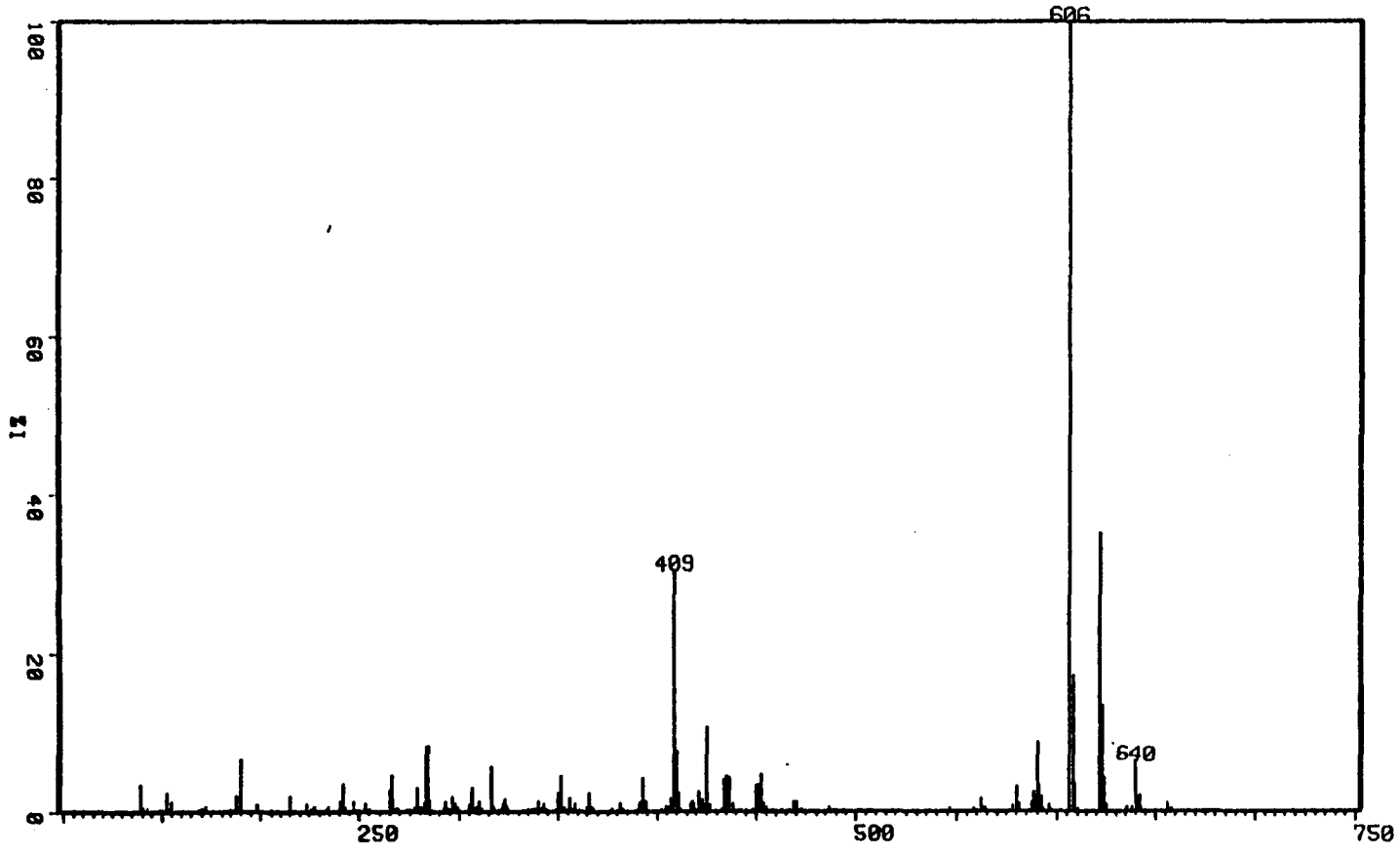

Fig. A3.43 - DCI Mass Spectrum of Compound D (fluphenazine decanoate sulphoxide di N-oxide)

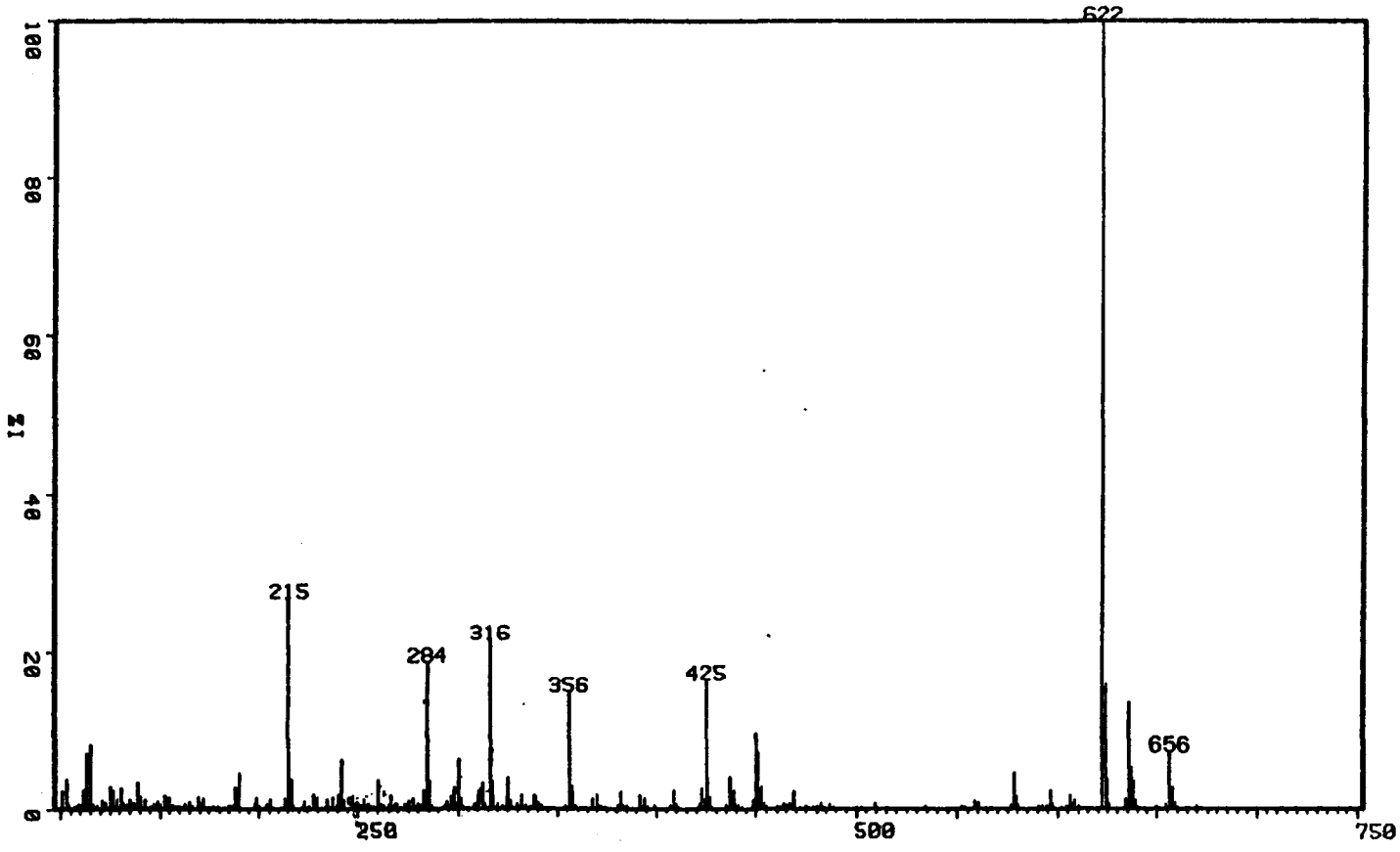

Fig. A3.44 - DCI Mass Spectrum of Compound E (fluphenazine decanoate sulphone di N-oxide)

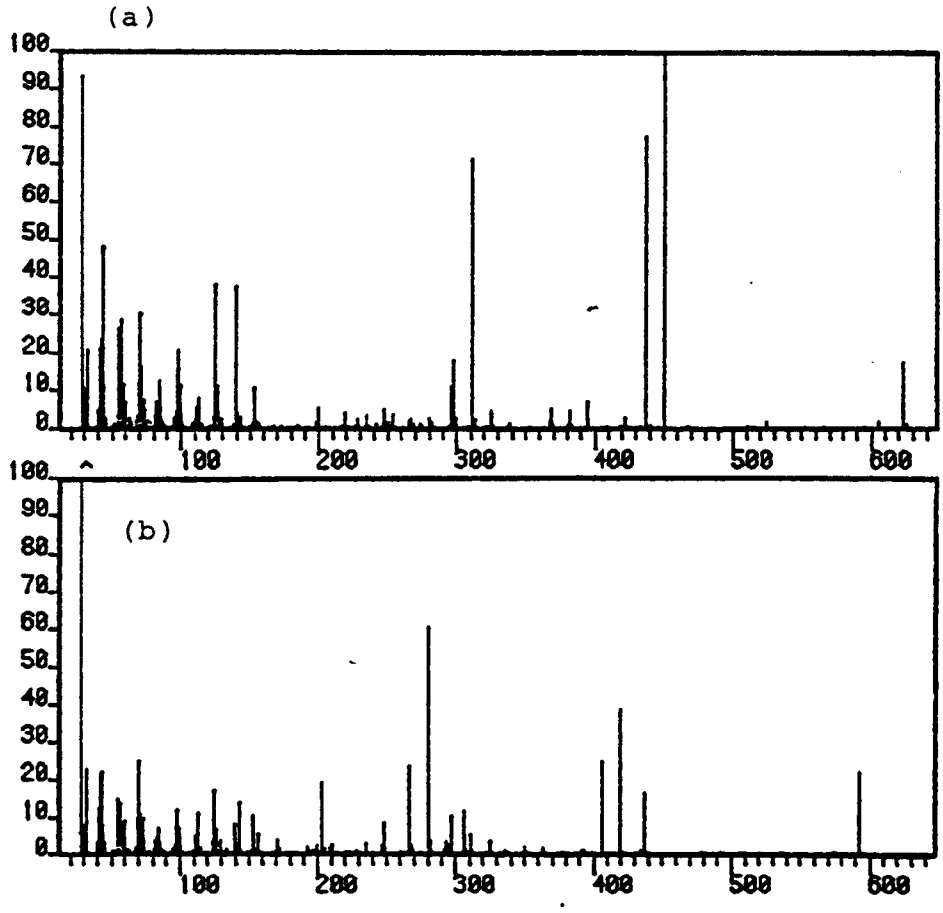

Fig. A3.45 - EI Mass Spectrum of

a) Compound F(fluphenazine decanoate sulphone

b) Fluphenazine Decanoate

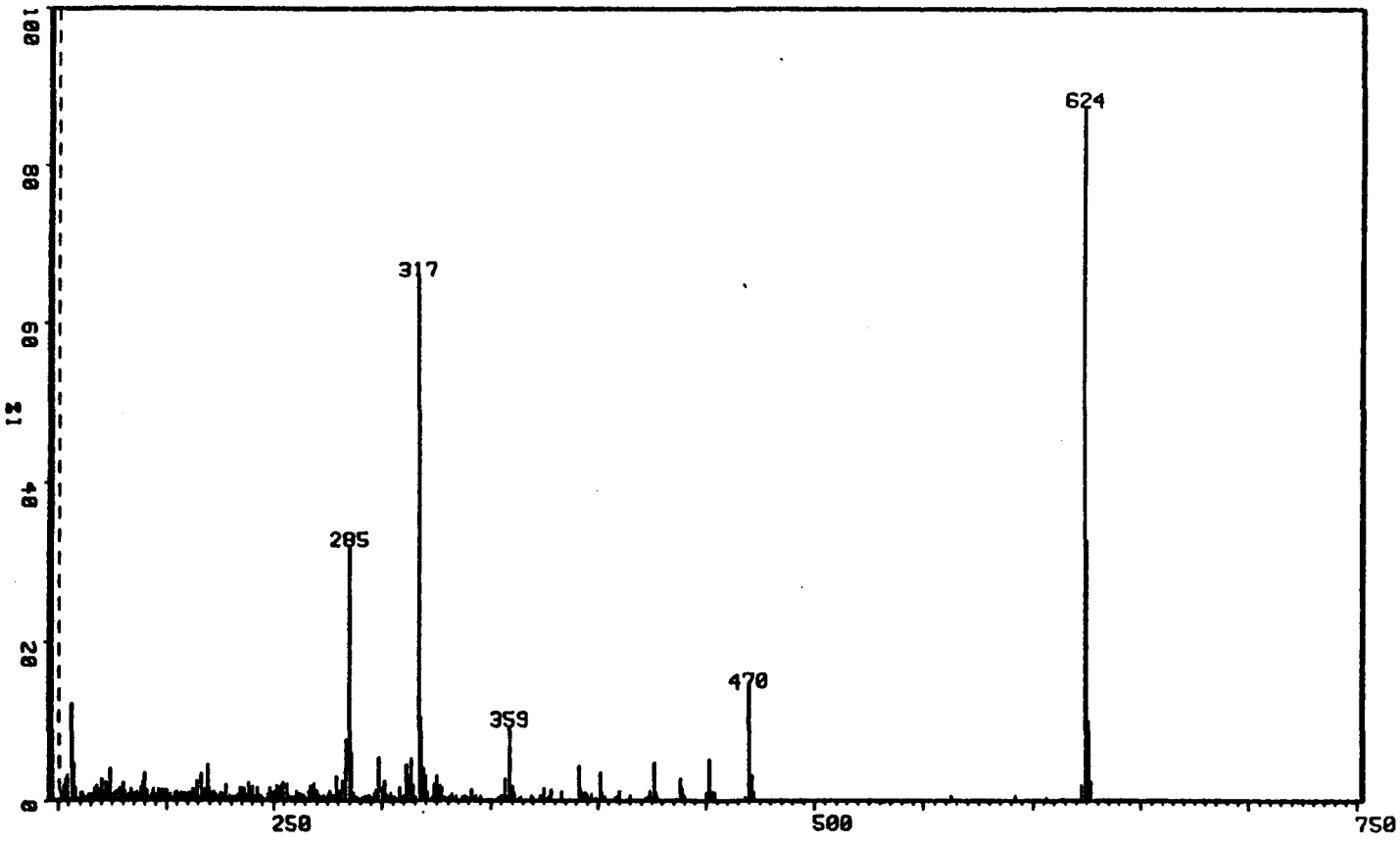

Fig. A3.46 - DCI Mass Spectrum of Compound F (fluphenazine decanoate sulphone)

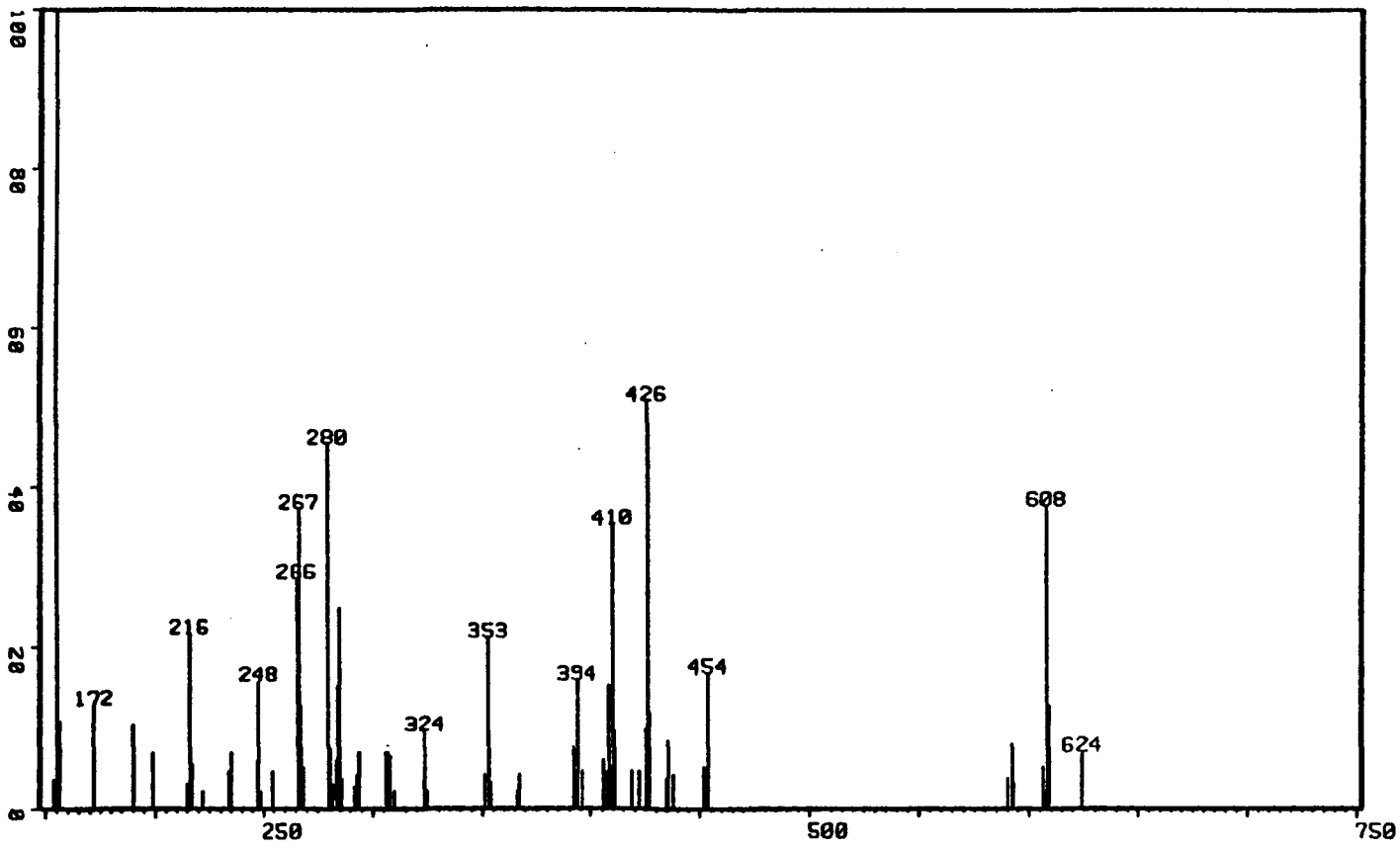

Fig. A3.47 - DCI Mass Spectrum of Compound X (fluphenazine decanoate sulphoxide mono N-oxide A)

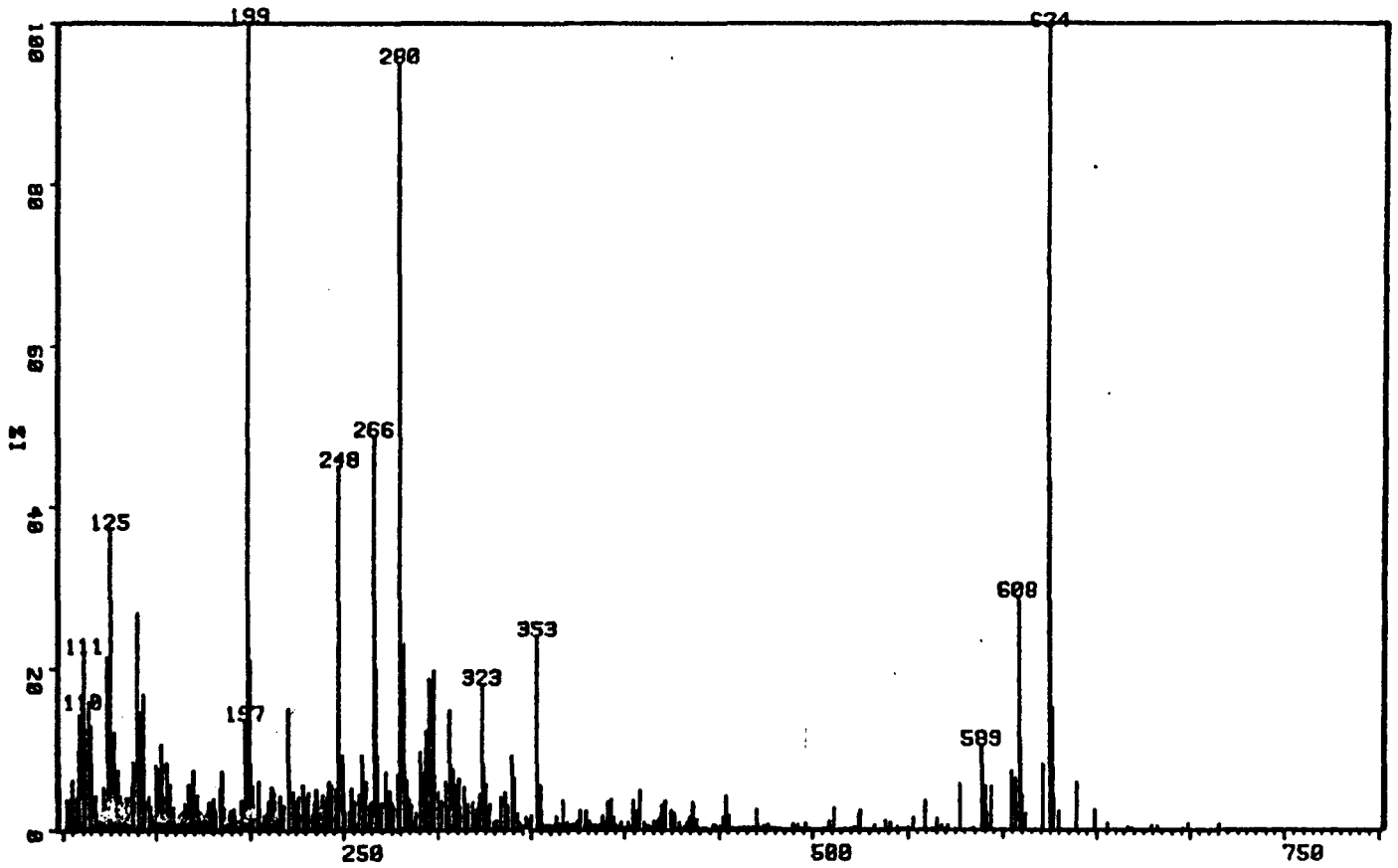

Fig. A3.48 - FAB Mass Spectrum of Compound X (fluphenazine decanoate sulphoxide mono N-oxide A)

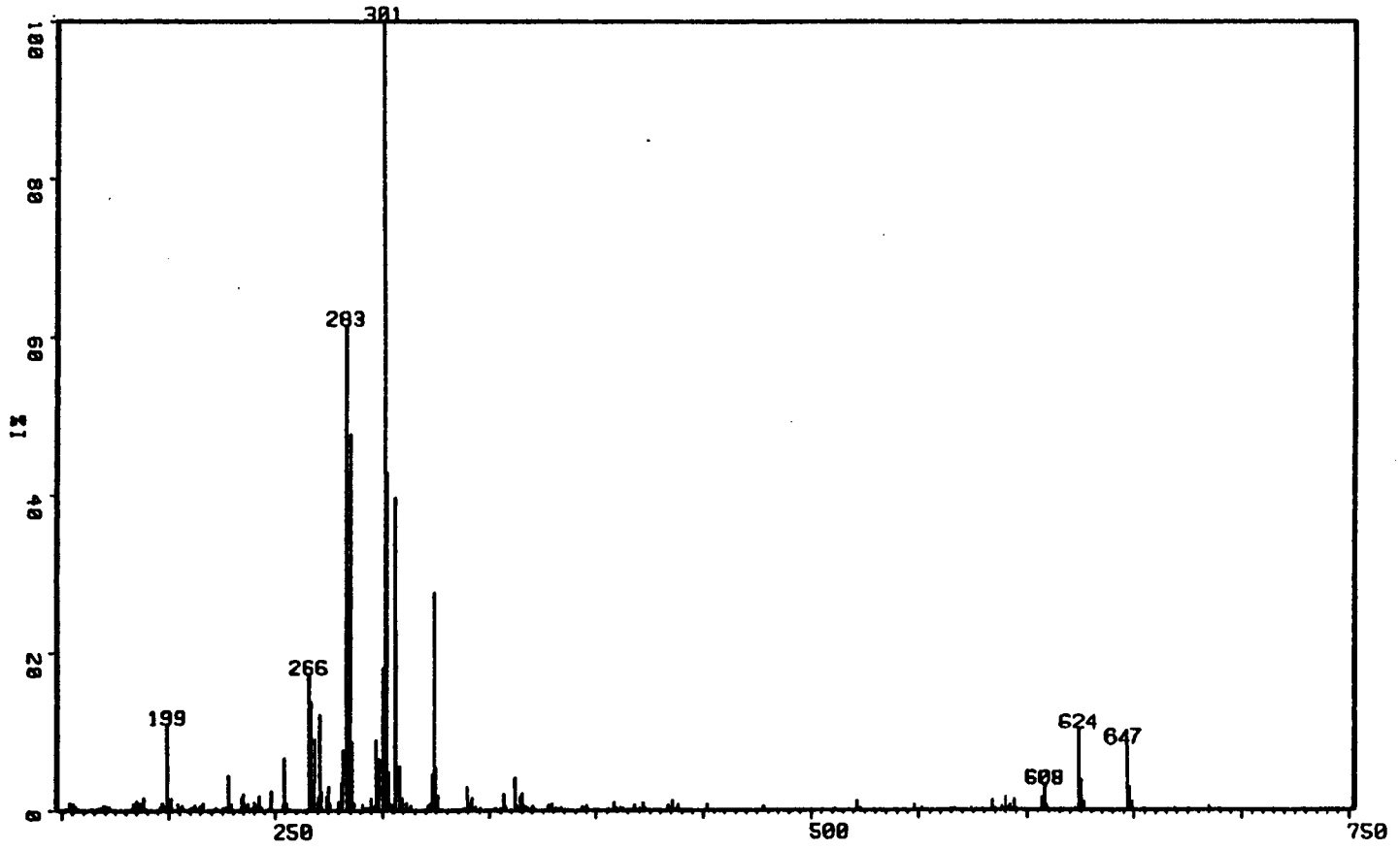

Fig. A3.49 - DCI Mass Spectrum of Compound Y (fluphenazine decanoate sulphoxide mono N-oxide B)

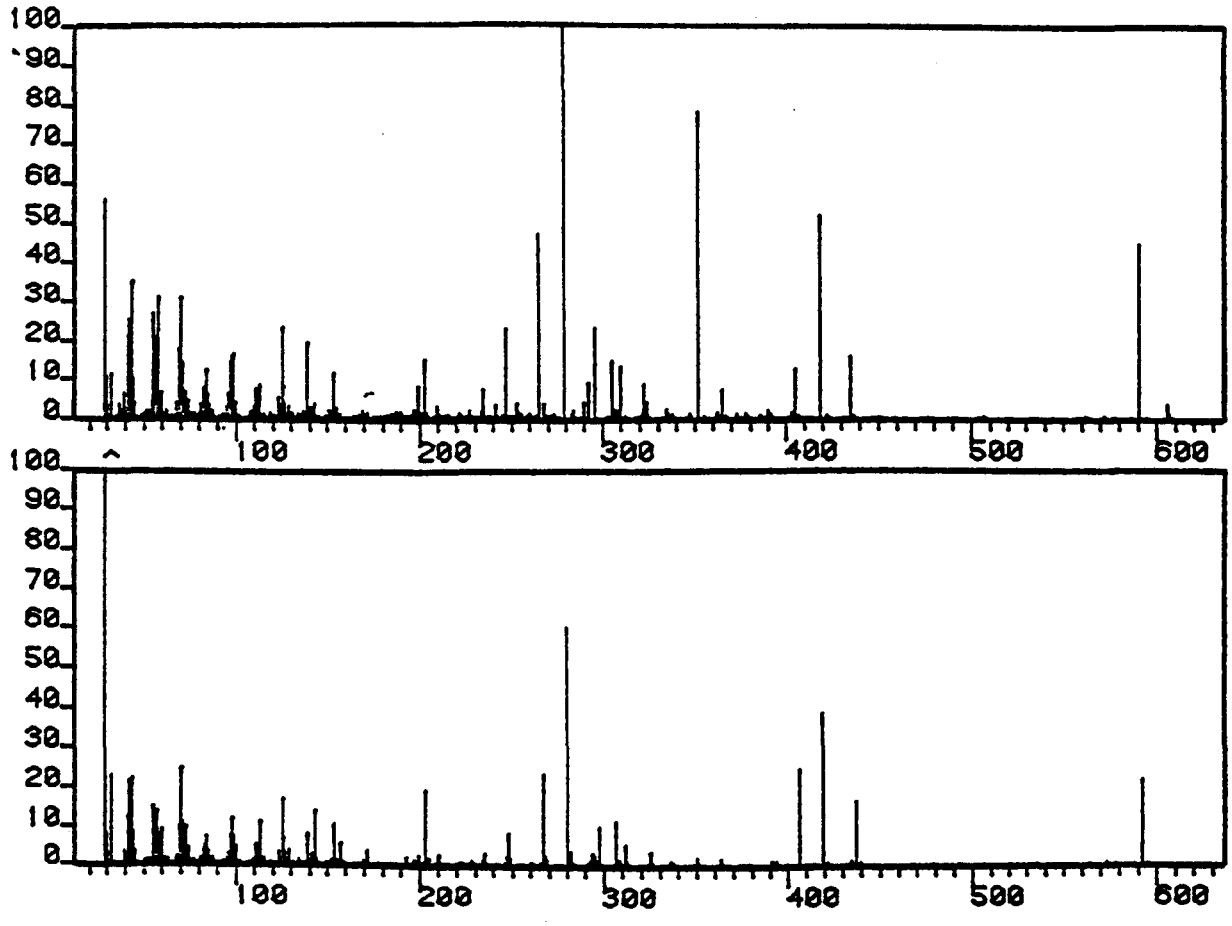

Fig. A3.50 - EI Mass Spectrum of

a) Compound Z(fluphenazine decanoate sulphoxide)

b) Fluphenazine Decanoate

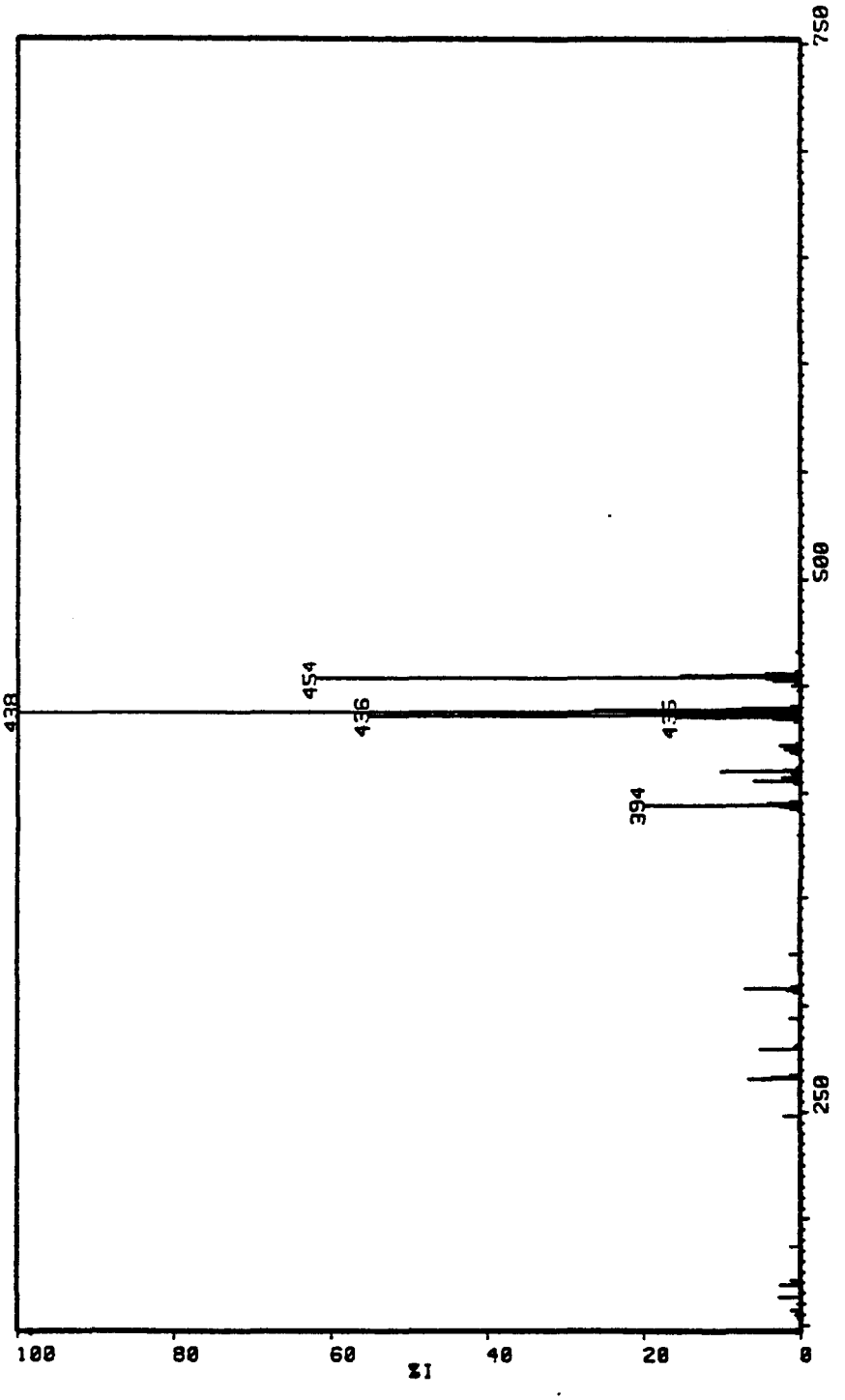

Fig. A3.51 - DCI Mass Spectrum of Compound B' (fluphenazine mono N-oxide B)

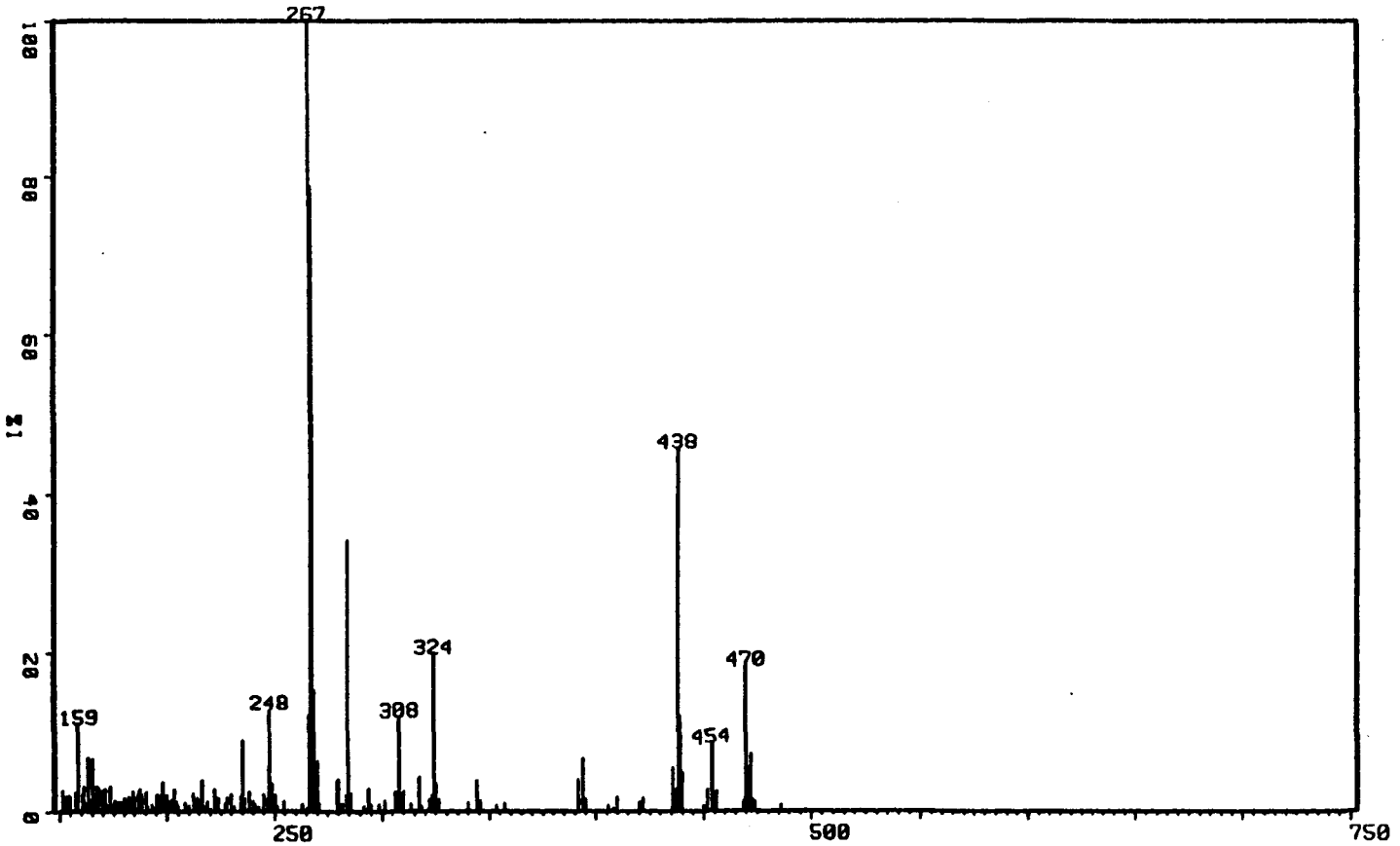

Fig. A3.52 - DCI Mass Spectrum of Compound C' (fluphenazine di N-oxide)

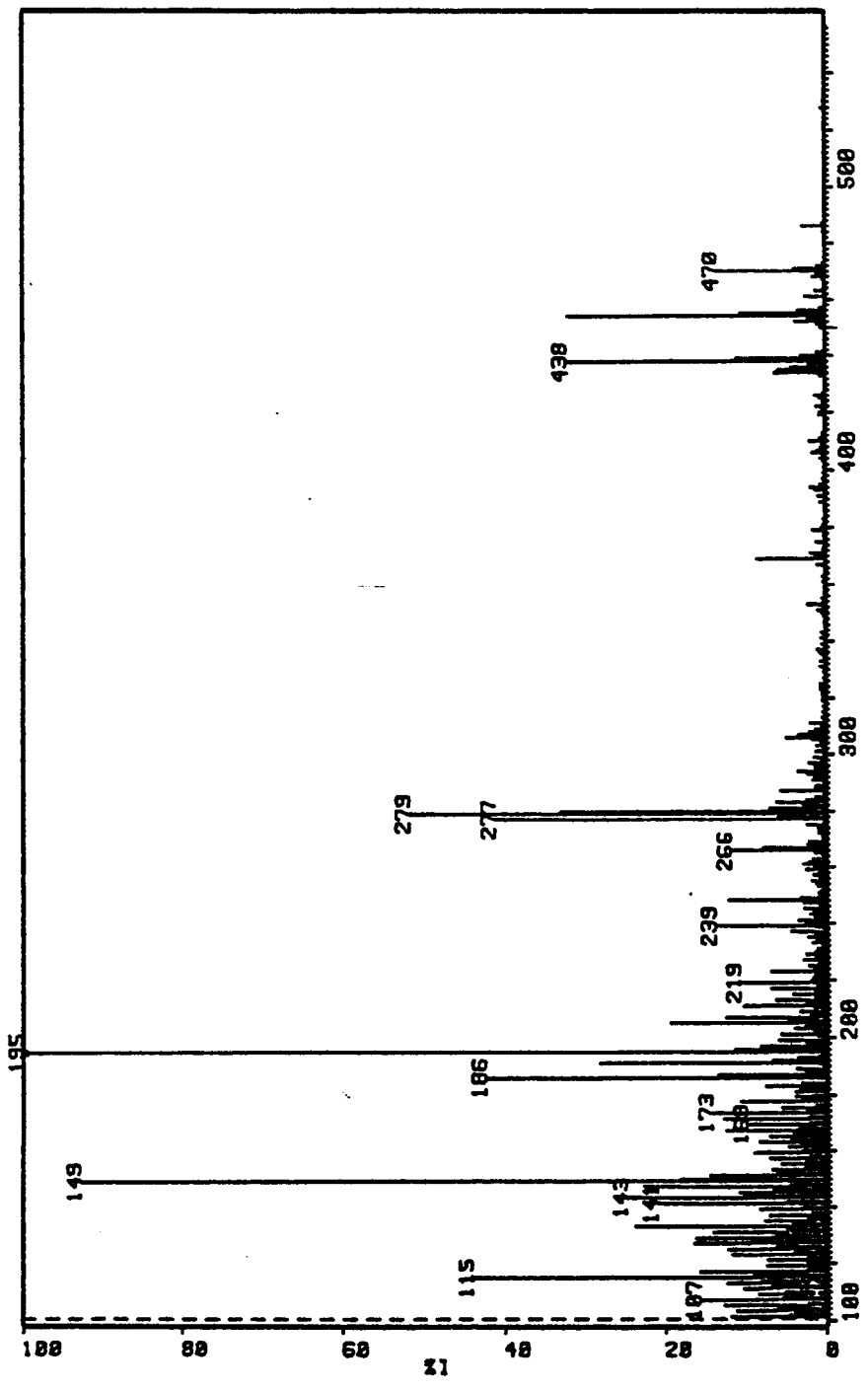

Fig. A3.53 - FAB Mass Spectrum of Compound C' (fluphenazine di N-oxide)

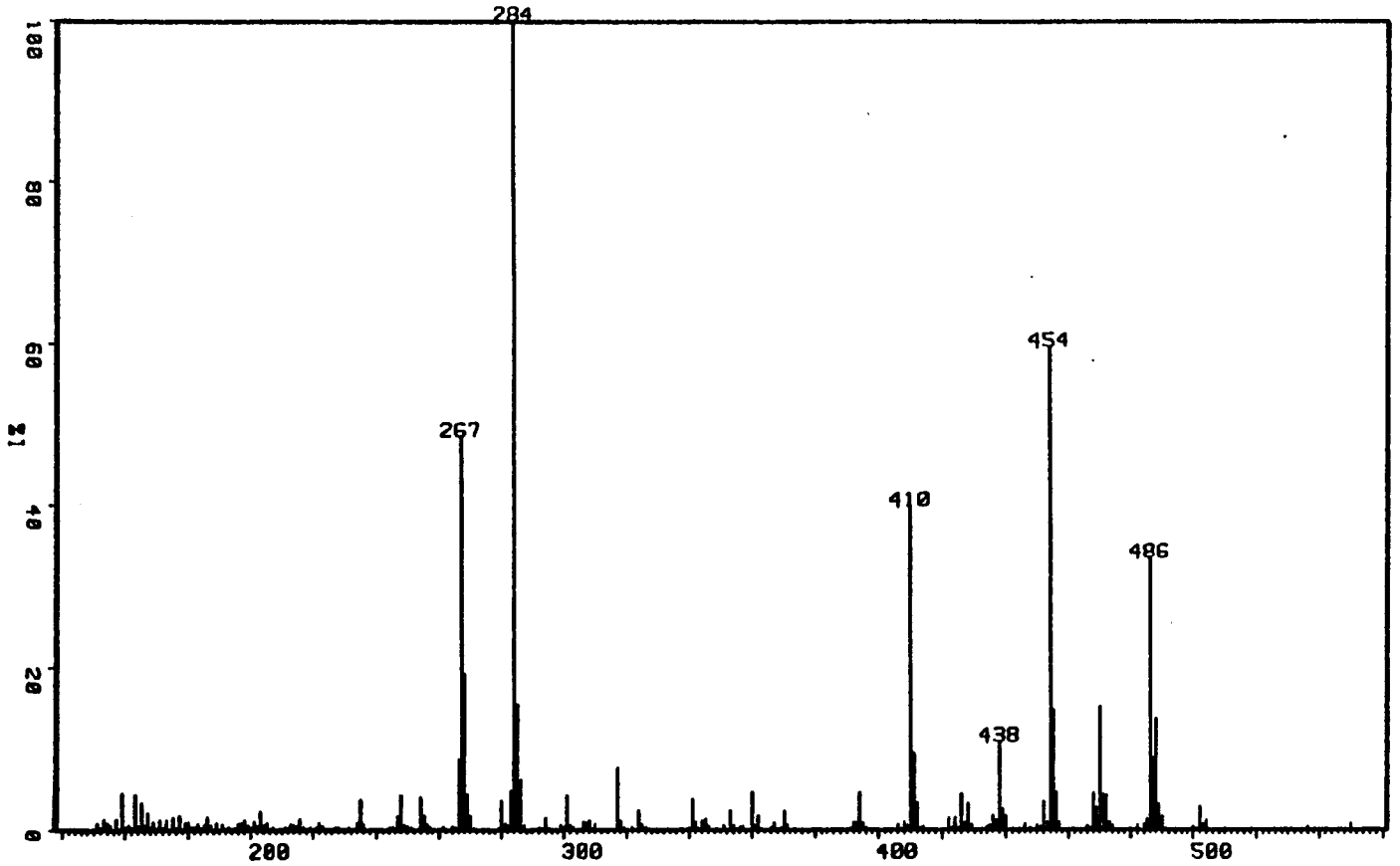

Fig.A3.54 - DCI Mass Spectrum of Compound D' (fluphenazine sulphoxide di N-oxide)

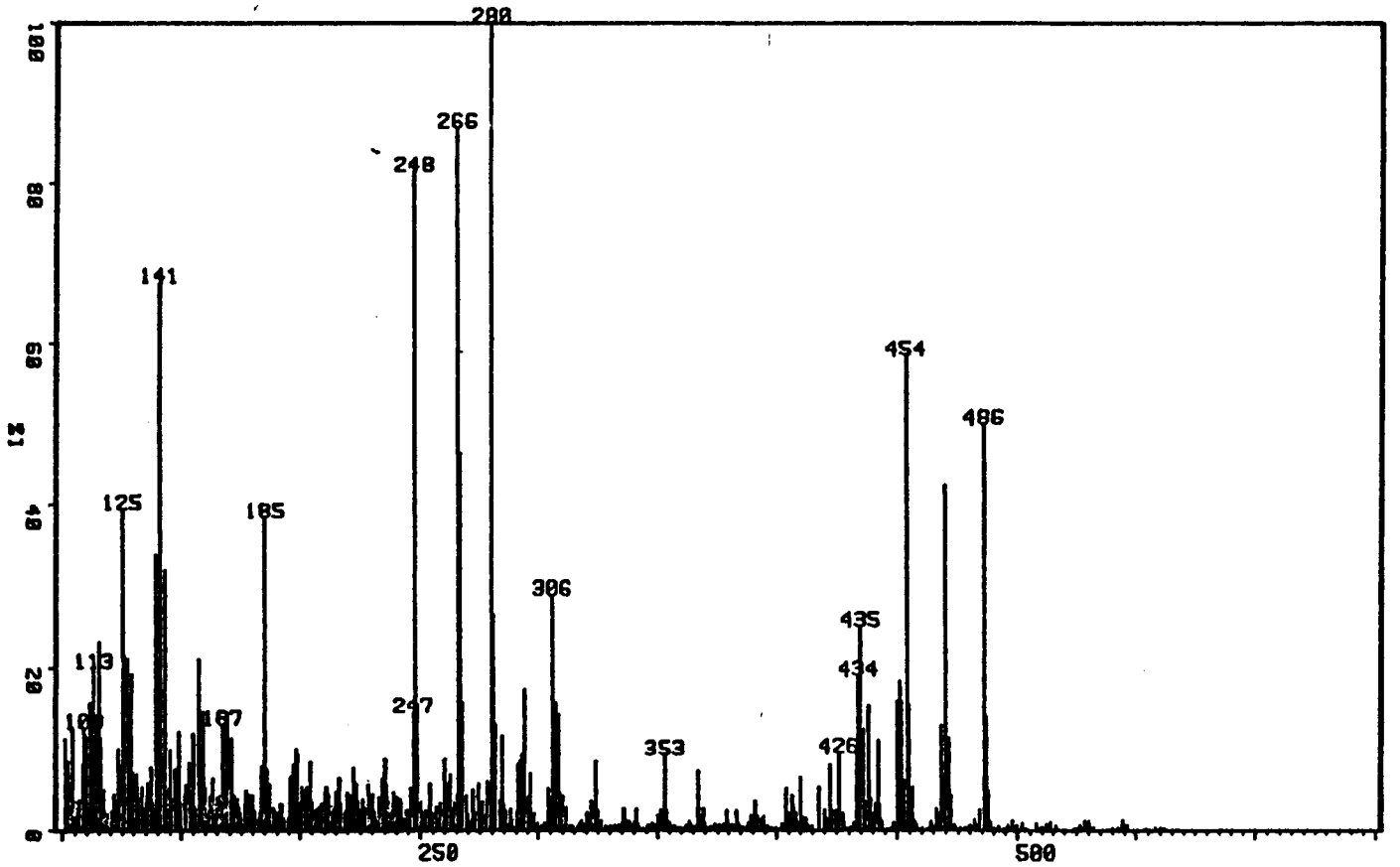

Fig. A3.55 - FAB Mass Spectrum of Compound D' (fluphenazine sulphoxide di N-oxide)

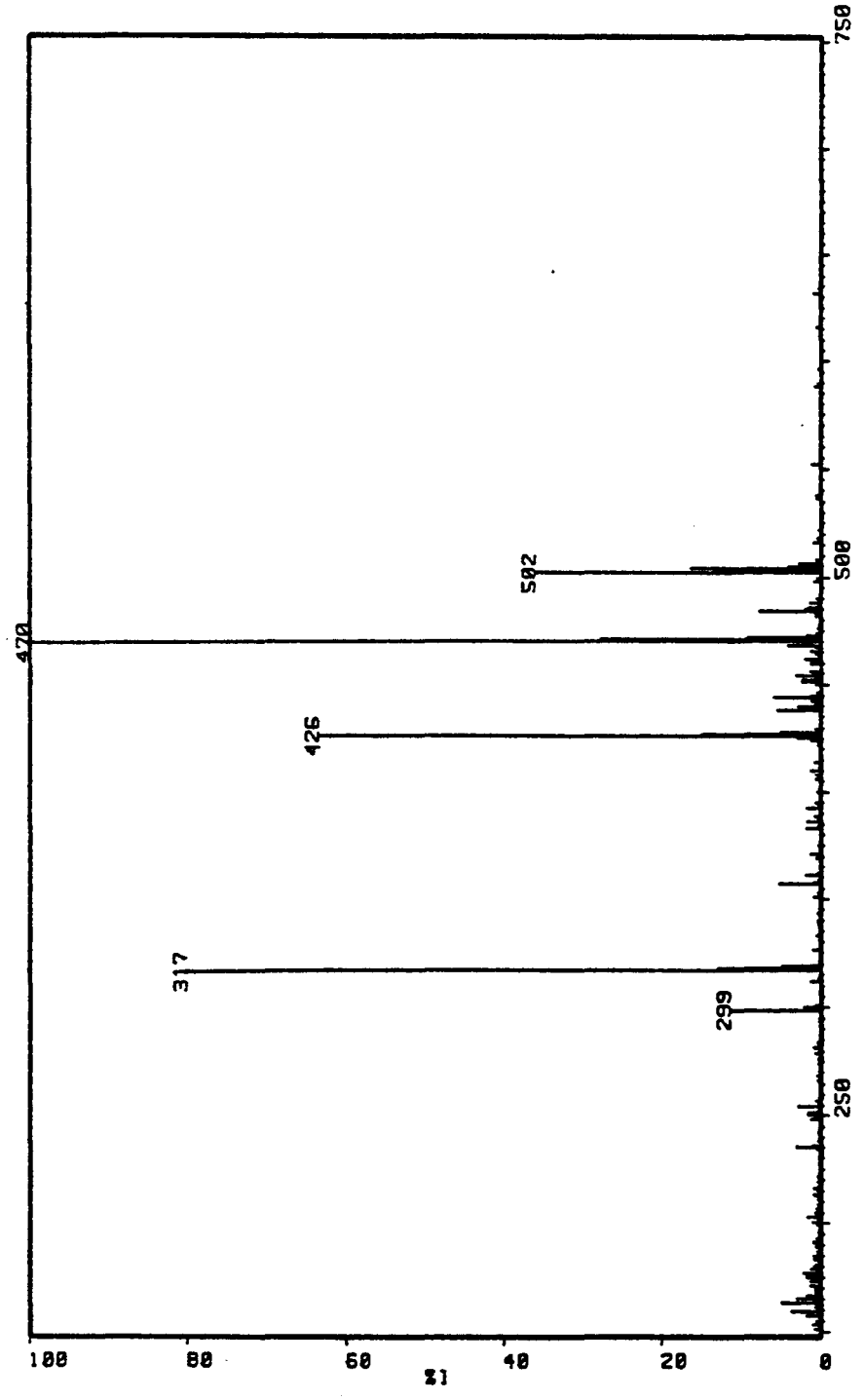

Fig. A3.56 - DCI Mass Spectrum of Compound E' (fluphenazine sulphone di N-oxide)

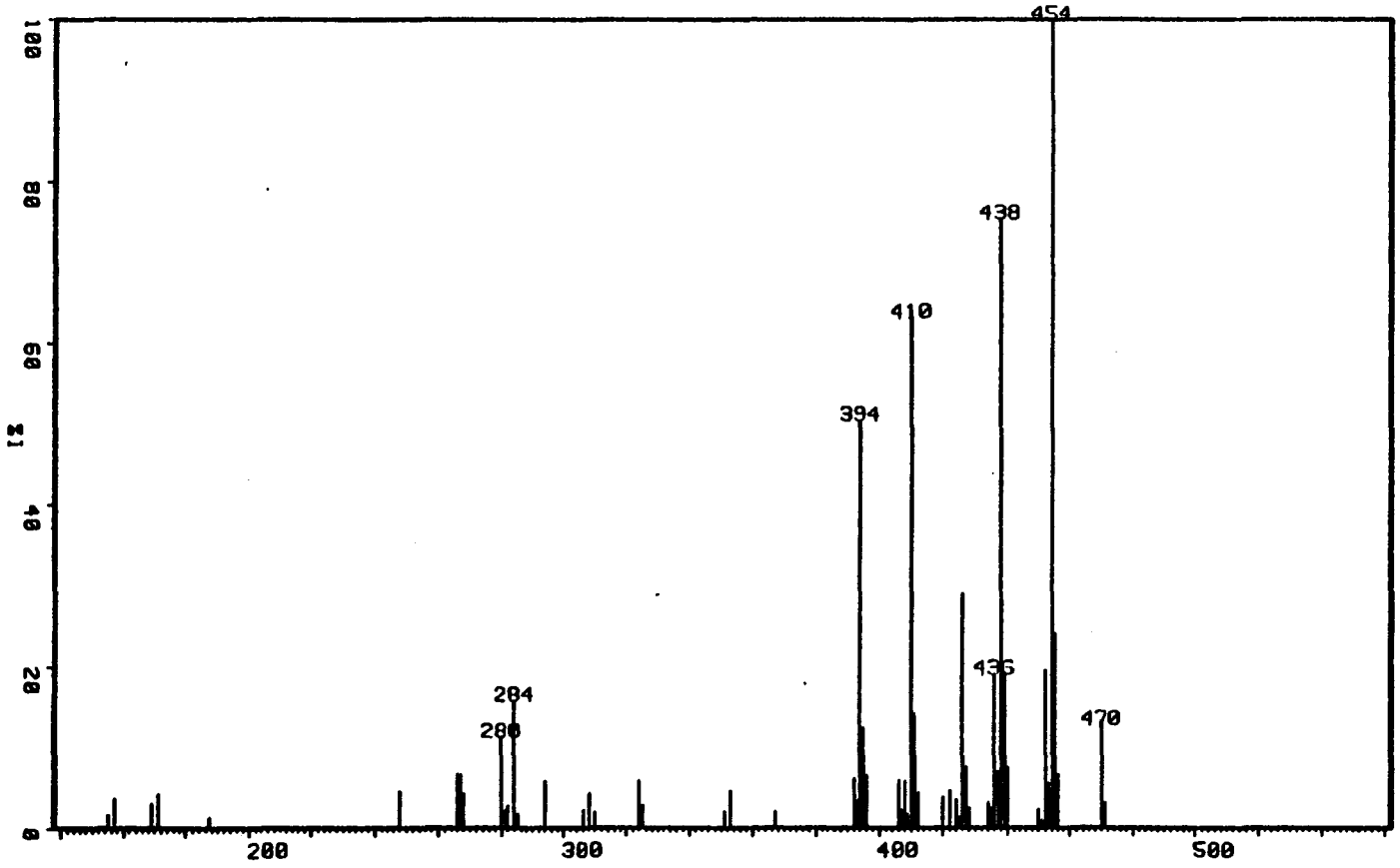

Fig. A3.57 - DCI Mass Spectrum of Compound Y' (fluphenazine sulphoxide mono N-oxide B)

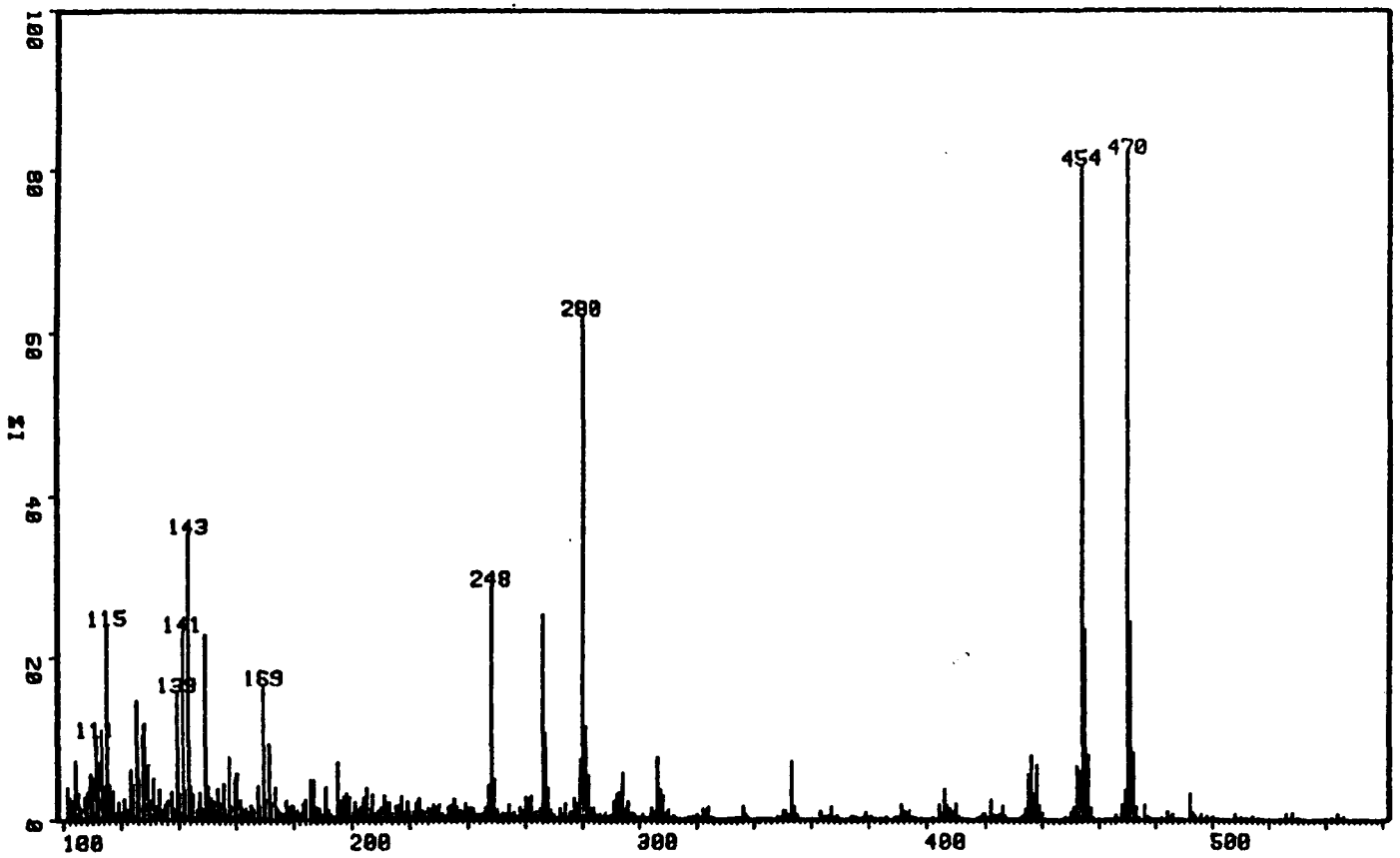

Fig. A3.58 - FAB Mass Spectrum of Compound Y' (fluphenazine sulphoxide mono N-oxide B)

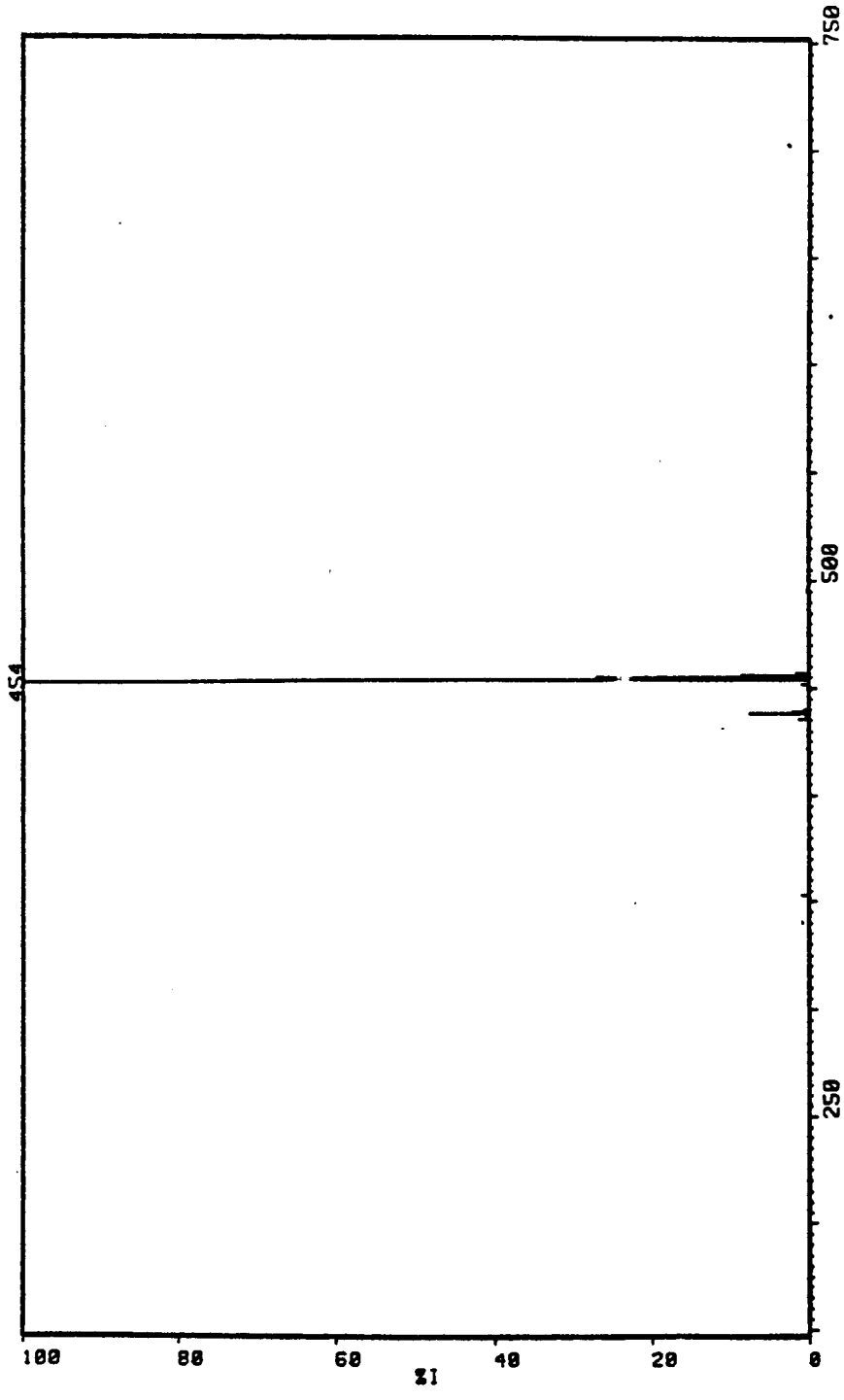

Fig. A3.59 - DCI Mass Spectrum of Compound Z' (fluphenazine sulphoxide)

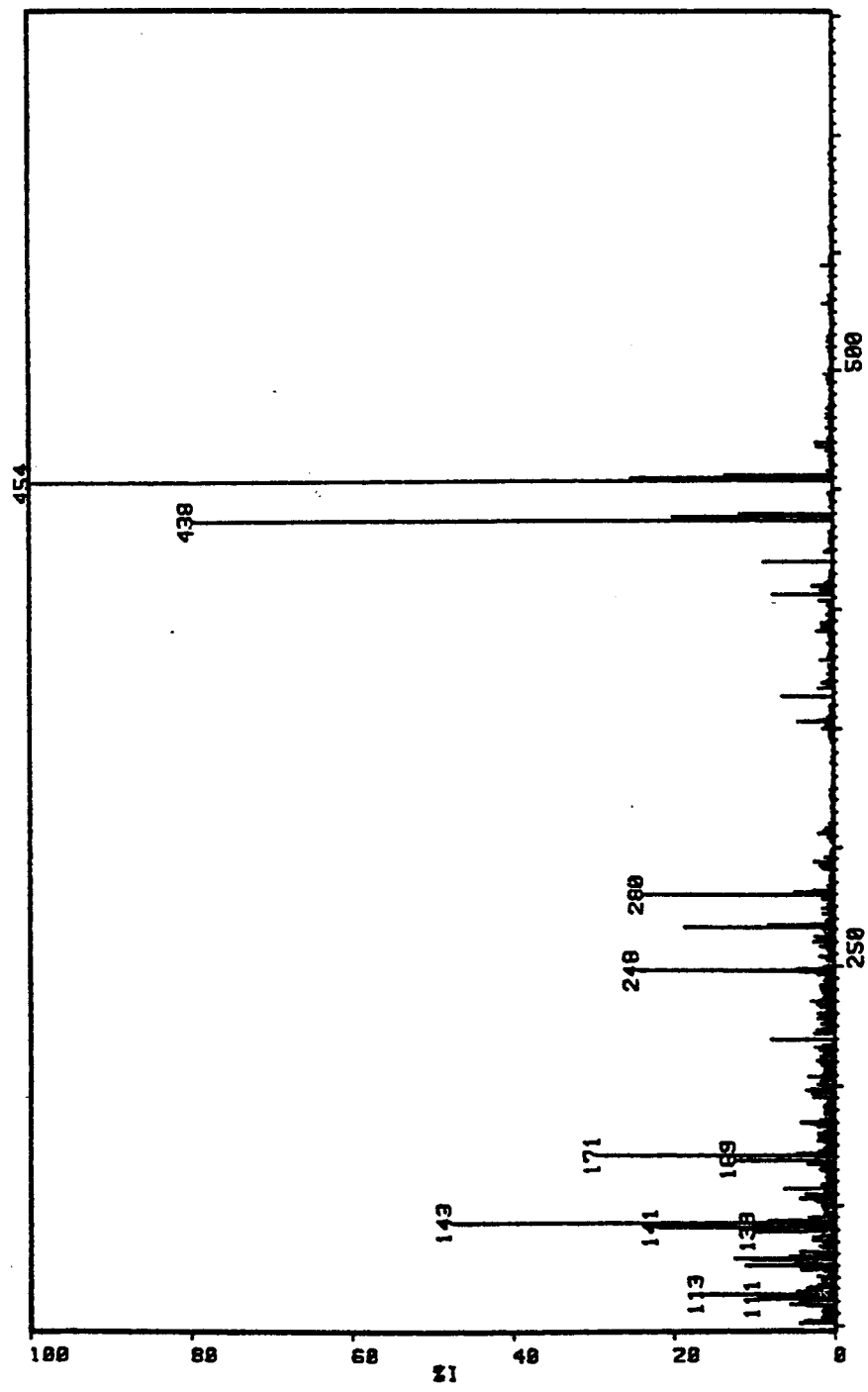

Fig. A3.60 - FAB Mass Spectrum of Compound Z' (fluphenazine sulphoxide)