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Metabolism of phenothiazines: identification of *N*-oxygenated products by gas chromatography and mass spectrometry

E. E. ESSIEN*, D. A. COWAN AND A. H. BECKETT

Department of Pharmacy, Chelsea College, University of London, Manresa Road, London, SW3 6LX, U.K.

The solid inlet mass spectra of the *N*-oxide and *N*-oxide sulphoxide metabolites of promazine, chlorpromazine and methotrimeprazine have been determined at different temperatures and compared with the mass spectra of the main thermolytic product of the *N*-oxides and *N*-oxide sulphoxides obtained during combined gas chromatography-mass spectrometry. Diagnostic ions were produced by direct insertion of the compounds at ambient temperature into the mass spectrometer. These ions distinguish the *N*-oxides of arylalkyl tertiary amines from the *N*-oxygenated derivatives of primary and secondary arylalkylamines.

N-Oxidation of tertiary arylalkylamines to form *N*-oxides occurs widely in nature (Bickel, 1969). These amines may be *N*-dealkylated and the resulting products *N*-oxidized to form hydroxylamines (Beckett & Essien, 1973; Beckett & Al-Sarraj, unpublished results). All of these *N*-oxygenated compounds are thermolabile and may break down during gas chromatographic analysis (Craig, Mary & Roy, 1964; Cope & Trumbull, 1960). The hydroxylamines are converted to oximes or nitrones depending on whether the hydroxylamine is primary [RNHOH] or secondary [RN(OH)R'] (Beckett, Coutts & Ogunbona, 1973). The alkyl dimethyl *N*-oxides with a β-hydrogen undergo Cope elimination to form the corresponding allyl derivatives and dimethylhydroxylamine. The *N*-oxide may, instead, lose an oxygen atom to form the corresponding tertiary amine or it may undergo a Meisenheimer rearrangement to the corresponding *N*-alkoxylamine. This rearrangement is preferred to Cope elimination in the absence of a β-hydrogen, e.g. trimethylamine-*N*-oxide, or with the *N*-oxides of the l-benzylpiperidine-1-oxide type (Castagnoli, Craig & others, 1970).

The dimethylaminoalkyl phenothiazines are *N*-oxidized and *S*-oxidized chemically and metabolically to form the corresponding *N*-oxides and sulphoxides (Weir & Sanford, 1969; Usdin, 1971). In addition, they are demethylated and then *N*-oxidized to form the secondary and primary hydroxylamines (Beckett & Essien, 1973). The thermolabile *N*-oxides, including their *N*-oxide sulphoxides, were identified and characterized using gas-chromatography and mass spectrometry.

MATERIALS AND METHODS

Compounds and reagents

Chlorpromazine hydrochloride B.P. (Largactil) and methotrimeprazine maleate

*Present address: The Faculty of Pharmacy, University of Ife, Ile-Ife, Nigeria, West Africa.

were obtained from May and Baker Ltd. and promazine hydrochloride B.P. was from Wyeth Ltd.

Hydrogen peroxide (50% w/v), manganese dioxide powder, *m*-chloroperbenzoic acid, titanous chloride reagent ($TiCl_3$ 30% w/v in HCl 24% w/v), ammonia (0·88) and liquefied sulphur dioxide were from BDH.

Synthesis of compounds

All reactions were carried out under subdued light to prevent photodecomposition. The tertiary amine bases promazine (I), chlorpromazine (II) and methotrimeprazine (III) were extracted at alkaline pH from aqueous solutions of their salts.

N-*Oxides.* Promazine and methotrimeprazine bases were dissolved in ethanol, and an ethanol–methanol mixture (1 : 1), respectively; each solution was made alkaline with 5% 0·88 ammonia and oxidized with 10 equivalents of a 50% w/v hydrogen peroxide solution. Chlorpromazine base was oxidized similarly in ethanol solution except that the ammonia solution was omitted. The oxidation was stopped after 2 to 3 h for promazine, 4 h for methotrimeprazine and 60 h for chlorpromazine by the decomposition of excess hydrogen peroxide with powdered manganese dioxide. The solutions were filtered, solvents evaporated off under reduced pressure at 25° and the residue dissolved in a small volume of dry methanol and purified by chromatography (t.l.c.) (R_F 0·3 approx. for all three *N*-oxides) using the system described below.

N-*Oxide sulphoxides.* Using the procedure described above for the preparation of each *N*-oxide, the corresponding *N*-oxide sulphoxide is also produced in small quantities (about 10%). To increase the yield of the *N*-oxide sulphoxide, 20 equivalents of hydrogen peroxide was used, no ammonia added, and the oxidation time increased to 4 h for promazine, 5 h for methotrimeprazine and 84 h for chlorpromazine. The *N*-oxide sulphoxide was separated from the mixed oxidation products by t.l.c. (R_F 0·15 approximately, for all three *N*-oxide sulphoxides).

All the synthesized compounds gave single spots when examined by t.l.c. The *N*-oxides and *N*-oxide sulphoxides were reduced with titanous chloride reagent to the corresponding tertiary amines only (Beckett, Essien & Franklin-Smyth, 1974), as shown by t.l.c. and g.l.c. examination. Treatment of an aqueous solution of each *N*-oxide with sulphur dioxide produced the parent tertiary amine as the reduction product and the secondary amine as the *N*-demethylated product (Beckett, Essien & Antiri-Penrose, 1973; Fok & Ziegler, 1970). Similar treatment of the *N*-oxide sulphoxides produced the corresponding tertiary and secondary amine sulphoxides.

Thin-layer chromatography (t.l.c.). 20 × 20 cm glass plates were coated with 0·5 mm of a mixture of silica gel G (Merck) and water (1 : 2) and heated for 1 h at 110°. All chromatograms were run in the dark at ambient temperature, in benzene–methanol–diethylamine (75 : 15 : 10 by volume). Spots were visualized by spraying with 50% v/v sulphuric acid.

Gas-liquid chromatography (g.l.c.). The compounds were chromatographed on a Perkin-Elmer model F11 instrument with flame ionization detection. A 1 metre 0·25 inch o.d. glass column containing 3% OV17 on acid washed, DMCS treated Gas-Chrom Q (80–100 mesh) was used with nitrogen as carrier gas (108 ml min^{-1}); oven temperature 215° and injection port temperature 275°.

Combined gas chromatography/mass spectrometry. Mass spectra were recorded on a Perkin-Elmer model 270 instrument using a 1 metre 0·25 inch o.d. glass column filled with 2% OV17 on Gas-Chrom Q (100–120 mesh, A/W, DMCS treated) and

helium as carrier gas (10 p.s.i.). The following temperatures were used: oven 210–230°, injection port 220–240°, manifold 215°, gas inlet 145°, ion source 185°. An ionization potential of 70 eV was employed.

Direct inlet mass spectrometry. Direct inlet mass spectra were recorded on a Perkin-Elmer model 270 instrument at an ionization potential of 70 eV. The inlet temperature was varied between 50° and 200° depending on the sample studied. Accurate mass measurements were determined using an A.E.I. MS–902 mass spectrometer by the peak matching method.

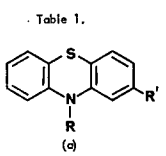
RESULTS AND DISCUSSION

Spectra are given for chlorpromazine *N*-oxide and *N*-oxide sulphoxide. The spectra of the corresponding derivatives of methotrimeprazine and promazine are available on request.

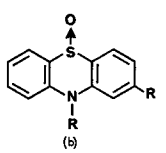
Direct inlet mass spectra

The mass spectra of the tertiary amines, promazine I, chlorpromazine II and methotrimeprazine III, their *N*-oxides IVa, Va and VIa respectively and N-oxide sulphoxides IVb, Vb and VIb respectively (Table 1) were obtained by direct inlet into the mass spectrometer at probe temperatures of 50° to 60° and above 100°. Small molecular ions were present in the spectra of compounds I, II, III, IVa, Va, Vb and VIa, irrespective of the operating conditions, but were not observed for the *N*-oxide sulphoxides IVb and VIb.

Table 1.



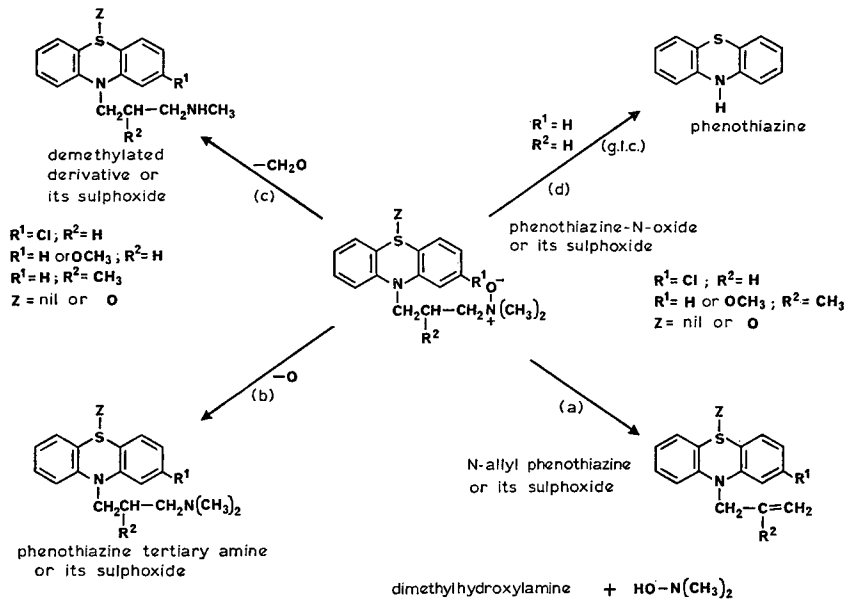
(a)



(b)

	R'	R	R'	R
I	-H	$-CH_2CH_2CH_2N(CH_3)_2$		
II	-Cl	$-CH_2CH_2CH_2N(CH_3)_2$		
III	$-OCH_3$	$-CH_2CH(CH_3)CH_2N(CH_3)_2$		
IV	-H	$-CH_2CH_2CH_2\overset{\overset{O}{\uparrow}}{N}(CH_3)_2$	-H	$-CH_2CH_2CH_2\overset{\overset{O}{\uparrow}}{N}(CH_3)_2$
V	-Cl	$-CH_2CH_2CH_2\overset{\overset{O}{\uparrow}}{N}(CH_3)_2$	-Cl	$-CH_2CH_2CH_2\overset{\overset{O}{\uparrow}}{N}(CH_3)_2$
VI	$-OCH_3$	$-CH_2CH(CH_3)CH_2\overset{\overset{O}{\uparrow}}{N}(CH_3)_2$	$-OCH_3$	$-CH_2CH(CH_3)CH_2\overset{\overset{O}{\uparrow}}{N}(CH_3)_2$

At a probe temperature of 50° all the *N*-oxides underwent Cope elimination (Scheme 1, route (a)) to liberate dimethylhydroxylamine which produced ions at *m/e* 61 [C_2H_7NO] and *m/e* 60 [C_2H_6NO] (see Figs 1a, and 2a, and Scheme 2). The ion of 60 a.m.u. fragmented further by loss of water to produce the ion at *m/e* 42 [C_2H_4N]. However, on raising the probe temperature above 100° the ion peaks at *m/e* 61 and 60, from dimethylhydroxylamine, disappeared and were replaced by an ion peak at *m/e* 58 [C_3H_8N] (see Figs 1b and 2b) resulting from the loss of an oxygen atom before ionization in the mass spectrometer (Scheme 1, route (b) and Scheme 2). In addition, a prominent ion was observed at *m/e* 44 at high temperature which probably arose from the secondary amine formed by the loss of formaldehyde before ionization (Scheme 1, route (c) and Scheme 2). The presence of the ion peaks at *m/e* 29 (M–1), *m/e* 30 (M^+) and 31 (M + 1) in the spectra of the *N*-oxides and *N*-oxide sulphoxides was in agreement with this release of formaldehyde.



SCHEME 1. Differences in the thermolysis of some 10-alkyl-dimethylaminophenothiazine-*N*-oxides and their sulphoxides.

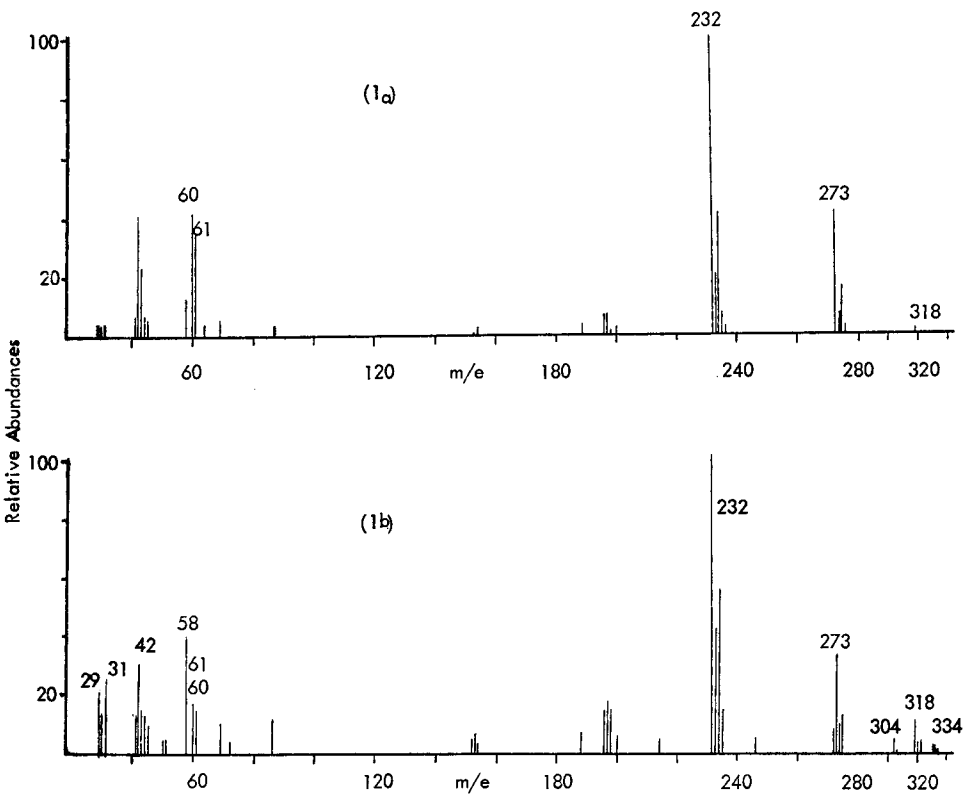
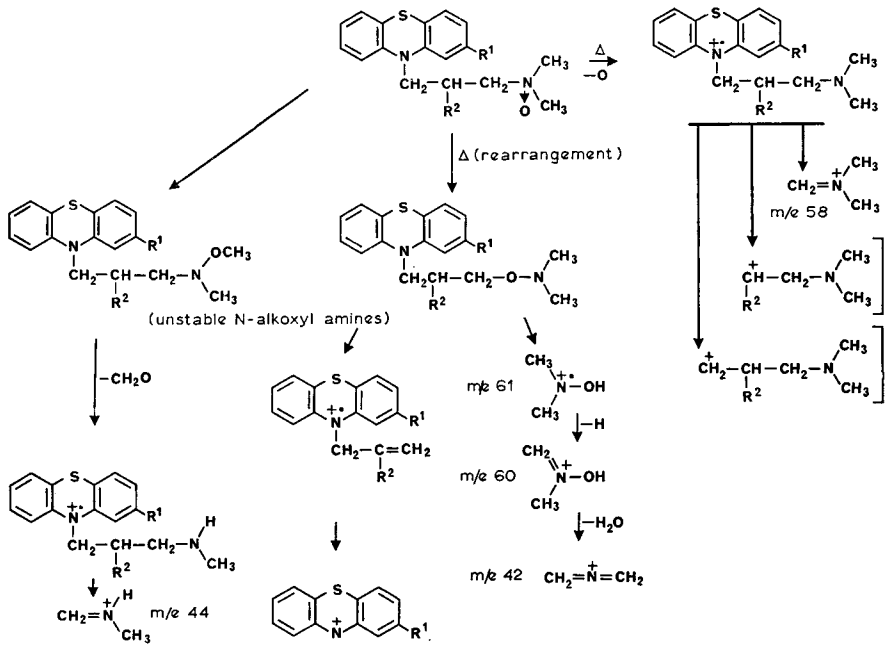


FIG. 1. The effects of temperature on the mass fragmentation of chlorpromazine-*N*-oxide (solid inlet). Temperatures °C: 1a, 60; 1b, 100.



SCHEME 2. Routes of thermolysis and fragmentation of some 10-alkyl-dimethylaminophenothiazine-*N*-oxides in the mass spectrometer ion source.

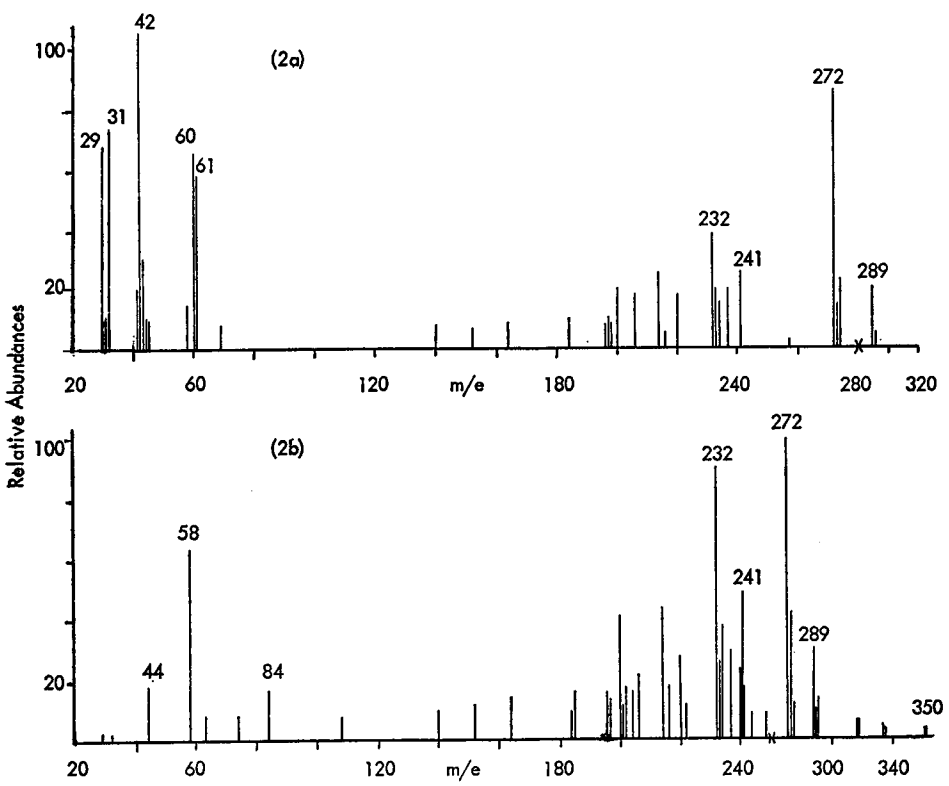
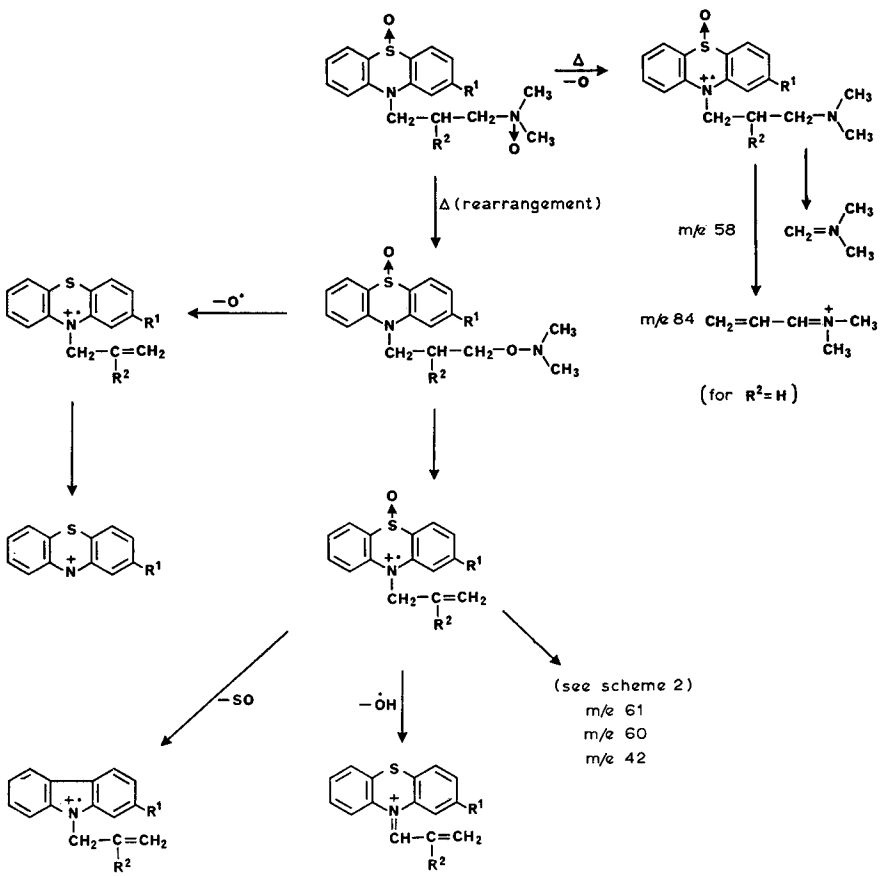


FIG. 2. The effects of temperature on the mass fragmentation of chlorpromazine-*N*-oxide sulphoxide (solid inlet). Temperatures °C: 2a, 50; 2b, 170–200.

The ion at *m/e* 60 in the low temperature spectra of these alkyldimethyl-*N*-oxides is comparable to the characteristic ion of 60 a.m.u. produced by the hydroxylamines of the type $RCH(CH_3)N(R')OH$ (R′ = H, ethyl or longer alkyl group) (Beckett & others, 1973). However, the presence of the additional ion at *m/e* 61 distinguishes the spectra of these *N*-oxides from those of the hydroxylamines which produce the ion at *m/e* 60 only.

The base peak in the 50° spectrum of each of the *N*-oxides (Fig 1a) corresponded to the complete loss of the side chain. A prominent peak, corresponding to the molecular ion of the allyl derivative (Scheme 1, route (a), Z = nil) resulting from thermolysis in the ion source, was observed for each *N*-oxide. The base peak probably arose from this molecular ion, since the spectrum of authentic 10-allyl-2-chlorophenothiazine with the expected molecular ion gave an ion peak at *m/e* 232 as the base peak corresponding to the complete loss of the allylic side chain. Increasing the probe temperature produced an ion corresponding to the phenothiazine nucleus plus a hydrogen atom (Fig. 1b).

The spectrum of each of the *N*-oxide sulphoxides (Fig. 2a & b) was markedly different from that of the corresponding *N*-oxide. An odd electron ion corresponding to the molecular ion of the sulphoxide of the allyl derivative (Scheme 1, route (a)



SCHEME 3. Routes of thermolysis and fragmentation of some 10-alkyl-dimethylaminophenothiazine-*N*-oxide sulphoxides in the mass spectrometer ion source.

Z = O) was observed for each of the *N*-oxide sulphoxides. These ions readily lost an hydroxyl radical to produce a prominent even electron ion corresponding to the allylic sulphoxide less a hydrogen atom (Scheme 3). However, in the mass fragmentation of methotrimeprazine-*N*-oxide sulphoxide an odd electron ion at *m/e* 251, resulting from the loss of SO from the allyl derivative gave the base peak at temperatures above 100°. The high intensity of the latter must be due to the influence of the methoxy substituent in position 2 of the phenothiazine ring system. The corresponding fragment ions in the spectra of the *N*-oxide sulphoxides of chlorpromazine and promazine were present at *m/e* 241 (50%) and *m/e* 207 (20–25%), respectively.

Ion peaks corresponding to the sulphoxide of the phenothiazine nucleus with and without the hydrogen atom attached to the ring nitrogen atom were present to an appreciable extent in the spectrum of promazine-*N*-oxide sulphoxide. However, the spectrum of chlorpromazine-*N*-oxide sulphoxide showed a greater abundance of the nucleus sulphoxide ion, without a hydrogen atom at the ring nitrogen (*m/e* 248, 20%), than the ion corresponding to *m/e* 249 (2–3%); whereas, similar ion peaks (*m/e* 244 and 245) were of negligible intensity in the spectrum of methotrimeprazine-*N*-oxide sulphoxide. Instead, the latter spectrum contained an ion peak corresponding to the methotrimeprazine nucleus with a $= CH_2$ attached to the nitrogen atom (*m/e* 242; cf. methotrimeprazine-*N*-oxide spectrum).

At temperatures above 100° the loss of the oxygen atom of the alkyl *N*-oxide group became an important route of decomposition of the above *N*-oxide sulphoxides to give the alkyl-*N*-dimethylamine side chain. The mass fragmentation of the latter gave a prominent peak at *m/e* 58.

Gas chromatography linked mass spectrometry

The g.c.-mass spectra of the single eluted peaks of the main Cope elimination products of the *N*-oxides (Va & VIa) and *N*-oxide sulphoxides (Vb & VIb) of chlorpromazine (Fig. 3b & c) and methotrimeprazine were in general the same as those obtained by direct inlet of these compounds into the mass spectrometer. However, the following ion peaks were absent from the g.c.-mass spectra: (i) ion fragments derived from dimethylhydroxylamine (*m/e* 61, 60 and 42); (ii) the ion at *m/e* 58 corresponding to C–C β-cleavage of the alkyl tertiary amine side chain produced by loss of the oxygen atom of the *N*-oxide group; (iii) the ion at *m/e* 44 corresponding to C–C β-cleavage of the secondary amine side chain produced by *N*-demethylation of the *N*-oxide; (iv) ion fragments corresponding to fragmentation of formaldehyde (*m/e* 29, 30 & 31) produced by demethylation of the *N*-oxide (Scheme 1, route (c)).

The direct inlet mass spectrum of authentic 10-allyl-2-chloro-phenothiazine was identical to the g.c.-mass spectrum of the elimination product of chlorpromazine–*N*-oxide (Va). The base peak in these spectra resulted from the complete loss of the side chain to produce the ion at *m/e* 232. The base peak in the g.c.-mass spectrum of the elimination product of chlorpromazine-*N*-oxide sulphoxide (Vb) corresponded to this ion (*m/e* 232) and resulted from complete loss of the side chain and oxygen atom of the sulphoxide group. Similar fragmentation of the g.c. elimination products of the *N*-oxide (VIa) and *N*-oxide sulphoxide (VIb) of methotrimeprazine occurred to give the base peak at *m/e* 228 for VIa and VIb. The molecular ions obtained from the elimination products of Va, Vb, VIa and VIb corresponded to the *N*-allyl derivatives of the respective phenothiazine nucleus and its sulphoxide. The g.c.-mass spectra and g.c. retention times of the eluted peaks of the elimination

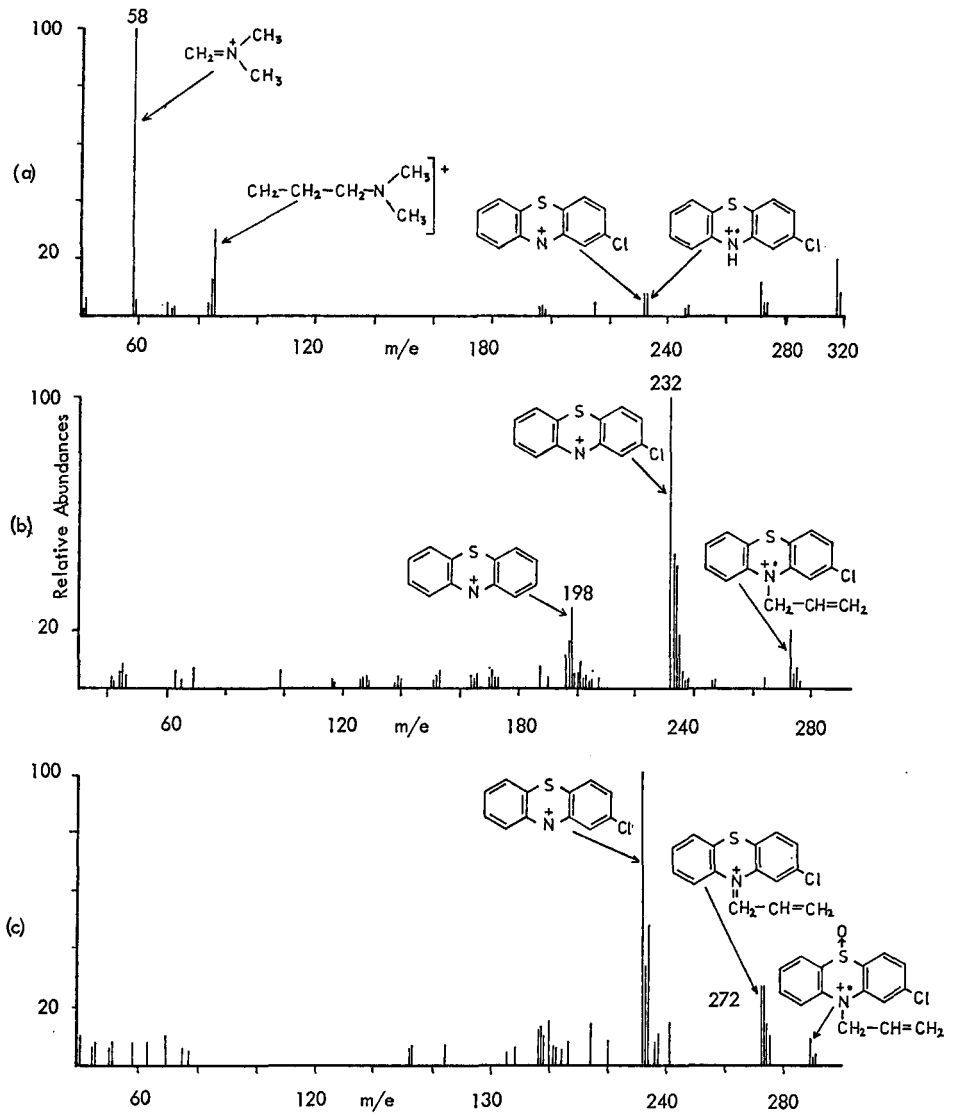


FIG. 3. Comparison of the g.c.—mass spectra of chlorpromazine and the elimination products of its *N*-oxide and *N*-oxide sulphoxide. (a) Chlorpromazine; (b) *N*-oxide elimination product; (c) *N*-oxide sulphoxide elimination product.

products from promazine-*N*-oxide (IVa) and promazine-*N*-oxide sulphoxide (IVb) were identical to those of phenothiazine itself obtained under similar conditions. The base peak at *m/e* 199 for IVa and IVb was the ion of the highest *m/e* value in the g.c.-mass spectra, and corresponded to the complete loss of the alkyl side chain of IVa and loss of the side chain and an oxygen atom from IVb to produce the nucleus plus the reattachment of a hydrogen atom to the latter. The ion at *m/e* 199 was the molecular ion of phenothiazine itself. Thus, promazine-*N*-oxide and promazine-*N*-oxide sulphoxide break down under the gas chromatographic conditions to phenothiazine itself.

The mass spectra of the amine sulphoxide and phenolic sulphoxide metabolites of promazine (I) and chlorpromazine (II) have been shown to contain an ion of 84 a.m.u., corresponding to the dimethylaminopropyl side chain less two hydrogen atoms, which is absent from the mass spectra of analogous compounds lacking the sulphoxide group (Brookes, Holmes & others, 1971; Duffield, 1974). We have shown the characteristic mass spectral features distinguishing the dimethylaminoalkyl phenothiazine-*N*-oxide and corresponding *N*-oxide sulphoxide metabolites from the parent phenothiazine tertiary amines and their sulphoxides.

Acknowledgement

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