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ELECTRON IMPACT PROMOTED FRAGMENTATION OF SOME PHENOTHIAZINE DERIVATIVES

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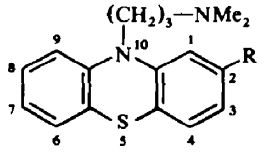
Abstract—The mass spectra of phenothiazines variously substituted with chloro, hydroxy, methoxy and acetoxy functions have been measured. Mass spectrometry is able to differentiate readily between oxygenation at C-3 *versus* C-6, C-7 and C-8 in 2-chloro-10-(3'-dimethylaminopropyl)phenothiazine (chlorpromazine) and 2-chlorophenothiazine, although (with the exception of OMe substitution in the latter series) it will not unambiguously distinguish between isomers oxygenated at C-6, C-7 or C-8. Possible mechanistic rationalizations are presented for the origin of the principal ions in the mass spectra of the compounds investigated.

MASS spectrometers, by virtue of their small sample requirement and the application of rapid scan techniques, have found extensive use in obtaining mass spectra of the effluents from gas chromatographic columns.[1] This technique is particularly useful for the identification, provided reference spectra are available, of traces of metabolites often encountered in biomedical research.[2,3] The object of the present study was to record, interpret and compare the fragmentation subsequent to electron impact of phenothiazine derivatives variously substituted with chloro, hydroxy, methoxy and acetoxy functions in the aromatic rings. No previous mass spectral data has appeared in the literature on this class of heteroaromatic compounds which are of importance because of their extensive use in medicine.[4]

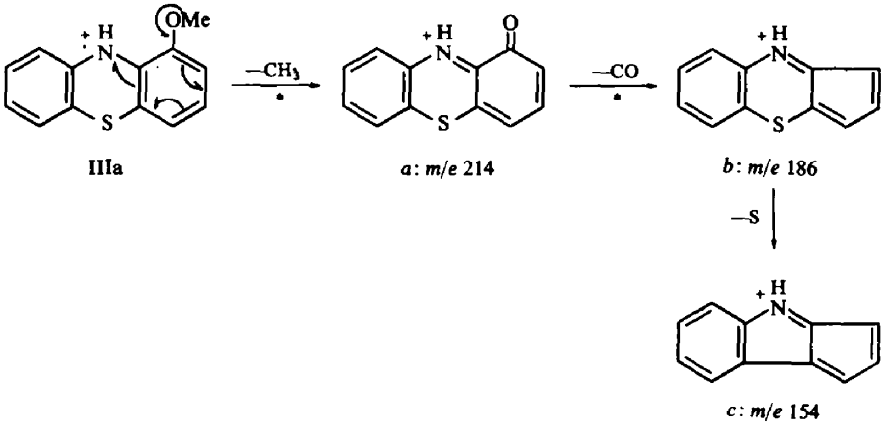
Two of the most important phenothiazine based drugs, promazine (I) and chlorpromazine (II), are known to give rise to phenolic metabolites with suggested sites of hydroxylation at C-3, C-7 or C-8.[5–7] A knowledge of the mass spectra of phenolic derivatives of phenothiazine would therefore also provide information useful in the direct identification of trace metabolites of e.g. promazine and chlorpromazine, using combined gas chromatographic-mass spectrometric techniques.



I: R = H
II: R = Cl

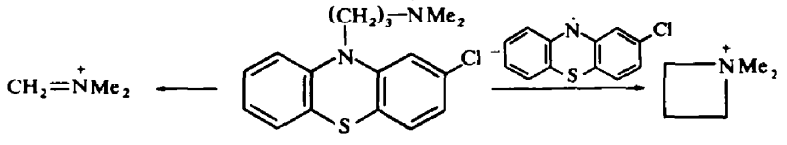
Discussion of mass spectra

The mass spectra (Figs 1 and 2) of 1- and 3-methoxyphenothiazine (III and IV) are quite similar, but show quantitative differences in the M-15 peak heights which can be used to distinguish between them. Both eliminate a Me radical* followed by successive expulsion of carbon monoxide and then a S atom (IIIa → *a* → *b* → *c*). The ion associated with the loss of a Me radical from the molecular ion is twice as intense in the case of the 1-OMe derivative IV (Fig. 2) as compared to 3-methoxyphenothiazine (III; Fig. 1). Both spectra have their most abundant peak corresponding to the molecular ion and also display a comparatively intense (12% relative abundance) doubly charged molecular ion peak which testifies to the overall stability of the phenothiazine ring system to electron impact.



IIIa *a*: *m/e* 214 *b*: *m/e* 186 *c*: *m/e* 154

Introduction of a dimethylaminopropyl group as in chlorpromazine (II) has a dramatic effect on the mass spectral fragmentation processes (Fig. 3) when compared to those compounds lacking this side chain. The mass spectrum (Fig. 3) of chlorpromazine (II) is dominated by the peaks at *m/e* 58 and 86 which must arise from fragmentation of the side chain and can be assigned structures *d* (*m/e* 58) and *e* (*m/e* 86) respectively. The ion *d* thus corresponds to the ubiquitous α-cleavage process of alkyl amines[9] while formation of *e* can best be attributed to the benzylically stabilized radical eliminated in this ion's formation.



d: *m/e* 58 II *e*: *m/e* 86

The ion of mass 85 in the spectrum (Fig. 3) of chlorpromazine (II) evidently does not arise from the loss of an H atom from *e* as the ratio of the abundances of *m/e* 85 : *m/e* 86 remains virtually constant at 15 and 70 eV which suggests that the species

* According to accepted convention "fish-hook arrows" (⌒) refer to single electron shifts.[8] The presence of an asterisk indicates that the appropriate metastable ion for a fragmentation process was observed.

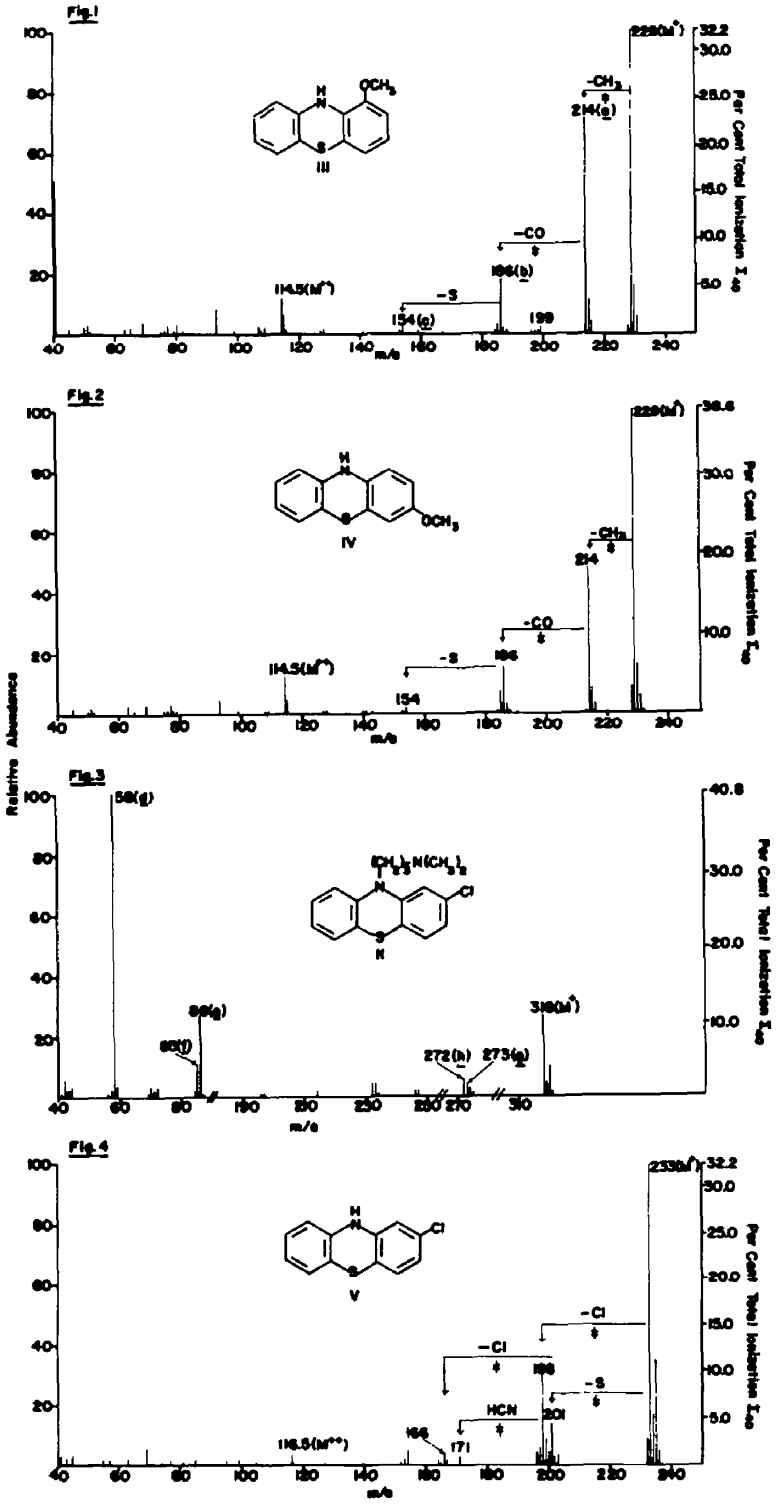
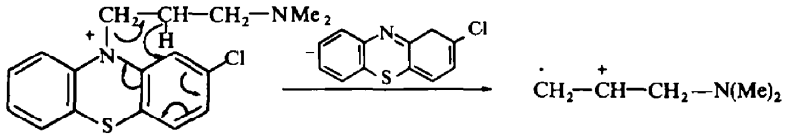


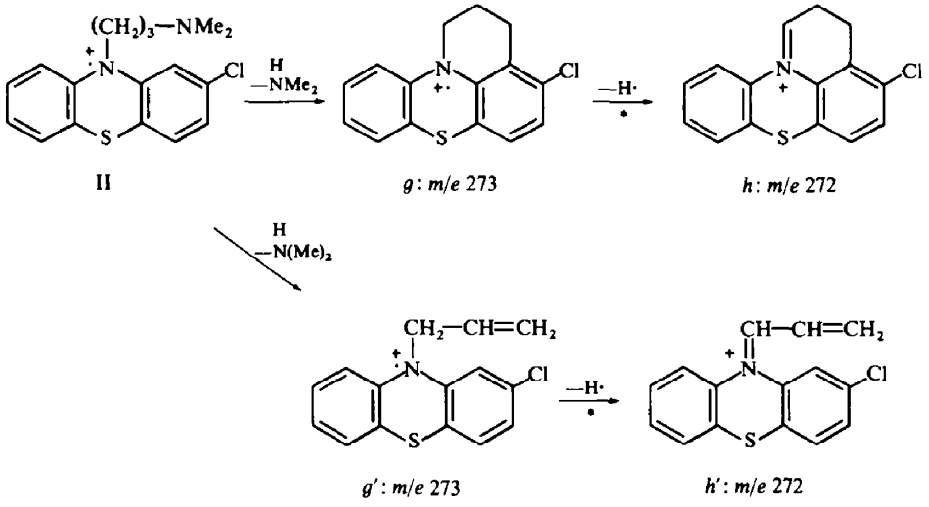
FIG. 1 Mass spectrum of 1-methoxyphenothiazine
FIG. 2 Mass spectrum of 3-methoxyphenothiazine
FIG. 3 Mass spectrum of chlorpromazine
FIG. 4 Mass spectrum of 2-chlorophenothiazine

of mass 85 arises directly from the molecular ion by a hydrogen transfer process which we postulate as II → *f*, *m/e* 85. The precise location of the itinerant hydrogen atom is unknown and in the process depicted for the origin of *f* hydrogen transfer could also occur by a 4-membered intermediate to the N atom.



II *f*: *m/e* 85

The fragment ion of highest mass in the spectrum (Fig. 3) of chlorpromazine (II) occurs at *m/e* 273 (M-45) and could correspond to the elimination of dimethylamine from the molecular ion of II such that the charged species can be assigned structure *g* or *g'*, *m/e* 273. Elimination of an H atom from *g* or *g'* is responsible for the genesis of the ion of mass 272 (*h* or *h'*) and in conformity with this postulated series of events the ion yield at mass 272 decreased relative to that at mass 273 at low (17 eV) ionizing voltage.



II *g*: *m/e* 273 *h*: *m/e* 272

g': *m/e* 273 *h'*: *m/e* 272

The absence of a dimethylaminopropyl side chain as in 2-chlorophenothiazine V is responsible for a totally different mass spectrum (Fig. 4) than that (Fig. 3) generated by chlorpromazine (II). Thus the direct loss of a S atom and chlorine radical from the molecular ion of V is responsible for peaks at *m/e* 201 and 198 in Fig. 4. Further loss of HCN from the ion of mass 198 and of a chlorine radical from the ion of mass 201 produces the fragments at *m/e* 171 and 166 respectively. A comparatively intense peak at *m/e* 116·5 corresponds to the doubly charged molecular ion of V.

The mass spectra of 2-chlorophenothiazines containing a phenolic group at either C-3, C-6, C-7 or C-8 have been determined. Sufficient differences between the mass

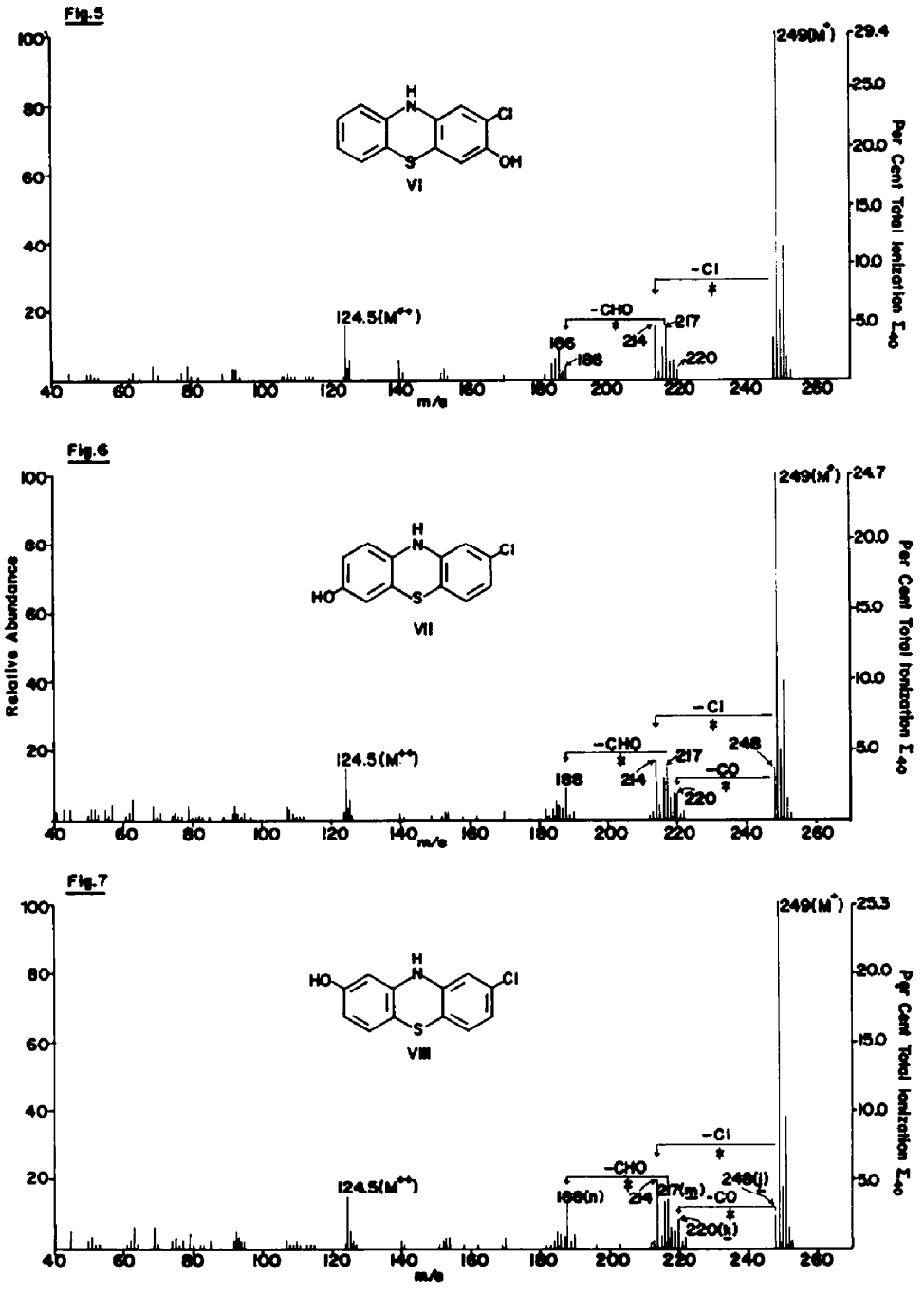
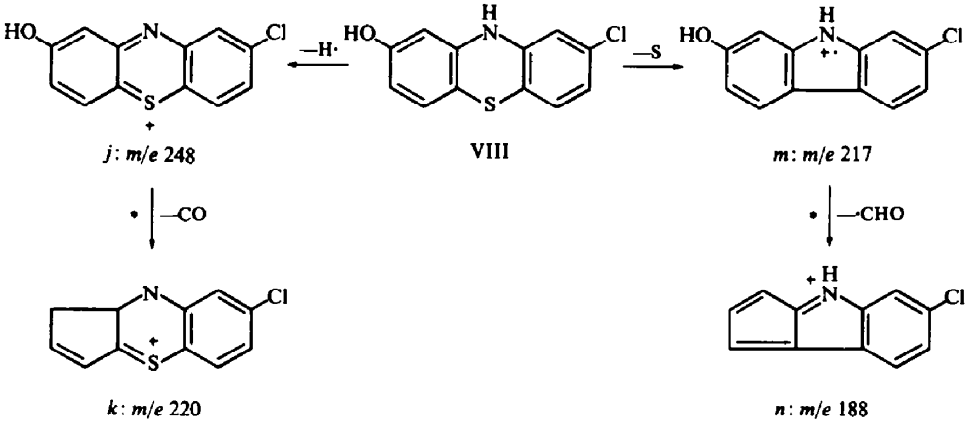


FIG. 5 Mass spectrum of 2-chloro-3-hydroxyphenothiazine
FIG. 6 Mass spectrum of 2-chloro-7-hydroxyphenothiazine
FIG. 7 Mass spectrum of 2-chloro-8-hydroxyphenothiazine

spectra of these compounds were apparent for the positive identification of the C-3 isomer VI (Fig. 5) but only minor discrepancies could be observed between the spectra (Fig. 6) of 2-chloro-7-hydroxyphenothiazine (VII) and its 6- and 8-hydroxy analogues (Fig. 7), the latter two affording identical mass spectra. The mass spectrum (Fig. 5) of 2-chloro-3-hydroxyphenothiazine (VI) is distinguished from the 6,7, and 8-derivatives by having only very low abundance peaks present at *m/e* 220 and 188. Only a minor intensity difference in the peak at *m/e* 188, less intense in the 7-hydroxy compound, serves to tentatively identify this compound from its 6- and 8-positional isomers.

The mass spectra of the four hydroxylated 2-chlorophenothiazines are easily rationalized and the 8-hydroxy compound (VIII) will serve as an example. Loss of hydrogen from the molecular ion (*m/e* 248, Fig. 7) can best be accommodated by elimination of the H atom attached to nitrogen (*j*, *m/e* 248) since the alternative possibility—loss of the H atom from the phenolic group—can be discounted as the corresponding O-methoxylated derivatives also have an M-1 ion present in their spectra. Additional support for this hypothesis is the absence of an M-1 species in the hydroxylated derivatives examined of chlorpromazine (II). Expulsion of CO from *j* yields the ion *k* of mass 220 while elimination of a chlorine radical and a S atom from the molecular ion of VIII is responsible for the ions of mass 214 and 217 (*m*) respectively. The ion *m* then eliminates CHO to yield the species *n*, *m/e* 188.



The two most intense peaks in the mass spectra (Figs 8 and 9) of 3-hydroxy and 8-hydroxychlorpromazine (IX and X) occur at *m/e* 58 and 86 and can be attributed to the species *d* and *f* respectively. Fragmentation of the 3-hydroxy derivative (IX) occurs by the elimination of the dimethylaminopropyl side chain to afford the ion of mass 248 which then consecutively eliminates CO and a chlorine radical. An identical series of fragmentations can be observed in the mass spectra of 6-, 7- and 8-hydroxy chlorpromazine. One significant difference between the mass spectra of 3- and 6-, 7- and 8-hydroxy chlorpromazine respectively is the absence of an ion of mass 289 (M-45) in the spectrum (Fig. 8) of the 3-hydroxy isomer. The ion of mass 289 in the 6,7 and 8-hydroxy analogues is thus similar to the species *g* or *g'* in

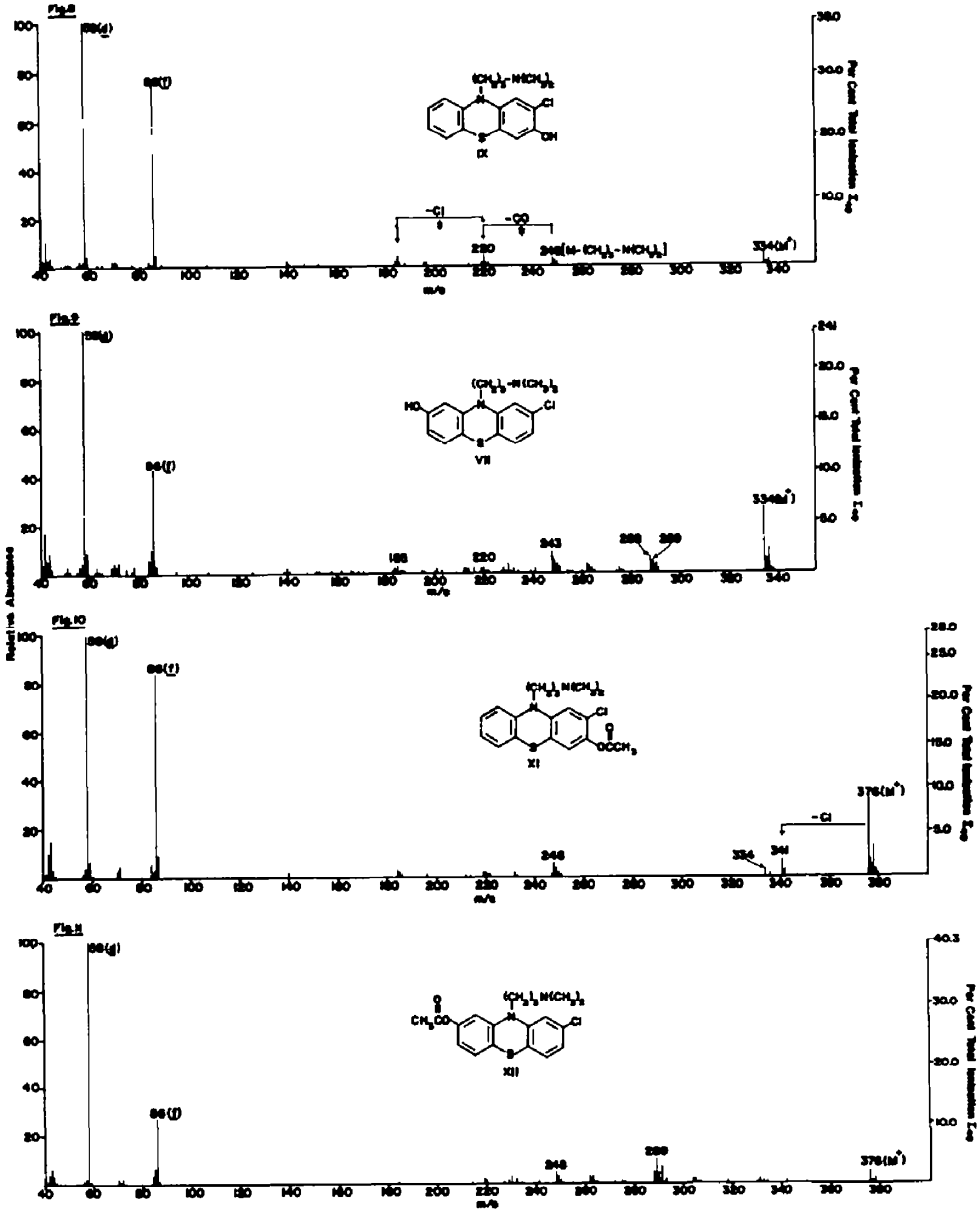
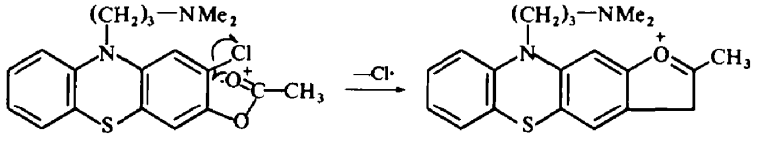


FIG. 8 Mass spectrum of 3-hydroxychlorpromazine
FIG. 9 Mass spectrum of 8-hydroxychlorpromazine
FIG. 10 Mass spectrum of 3-acetoxychlorpromazine
FIG. 11 Mass spectrum of 8-acetoxychlorpromazine

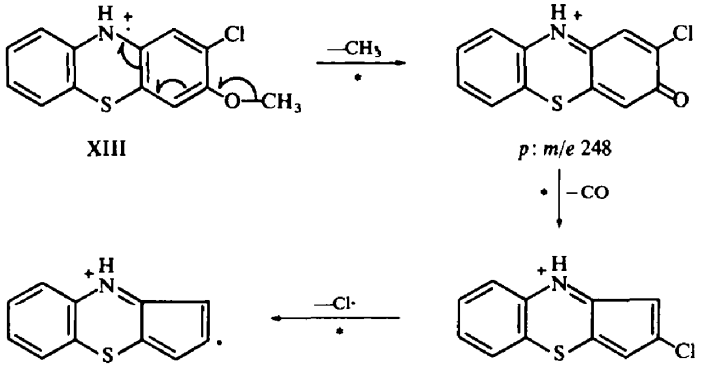
the spectrum (Fig. 3) of chlorpromazine (II) and requires no additional discussion while the further loss of an H atom produces the fragment at mass 288.

Because of the low volatility of the hydroxylated chlorpromazine derivatives studied, which may preclude the application of gas chromatographic-mass spectrometric techniques to these compounds, we have determined the mass spectra of their acetates.* As anticipated in view of our previous results mass spectrometry readily differentiates the 3-acetoxy isomer XI (Fig. 10) from the 6,7 and 8-acetoxy compounds —the latter three yielding almost identical mass spectrometric fragmentation patterns (see Fig. 11 for the mass spectrum of 8-acetoxychlorpromazine XII). Once again the spectra (Figs 10 and 11) are dominated by the peaks at *m/e* 58, 86 and 376 which correspond to the ions *d* and *f* and the parent peak respectively. The prominent ion at mass 341 in the spectrum (Fig. 10) of 3-acetoxychlorpromazine (XI) is due to the expulsion of a chlorine radical from the molecular ion. No loss of chlorine from the molecular ion of 3-hydroxychlorpromazine (IX) (Fig. 8) could be discerned which suggests that the elimination of chlorine in the acetate XI may occur by the process XI → *o*, *m/e* 341.



XI *o*: *m/e* 341

A comparison of the mass spectra (Figs 12, 13 and 14) of 3-, 7- and 8-methoxy-2-chlorophenothiazine (XIII, XIV and XV) demonstrates that mass spectrometry can be used to differentiate between these isomers. The sequential loss of a Me radical, carbon monoxide and a chlorine radical are common to all three mass spectra (Figs 12, 13 and 14) and can be rationalized in each instance by a scheme similar to that depicted for 2-chloro-3-methoxyphenothiazine in XIII → *p*, *m/e* 248 → *q*, *m/e* 220 → *r*, *m/e* 185. However, sufficient disparity in the relative abundances of these ions can be



XIII *p*: *m/e* 248

r: *m/e* 185 *q*: *m/e* 220

* Readily prepared on a micro scale by brief refluxing of the phenol with acetic anhydride.

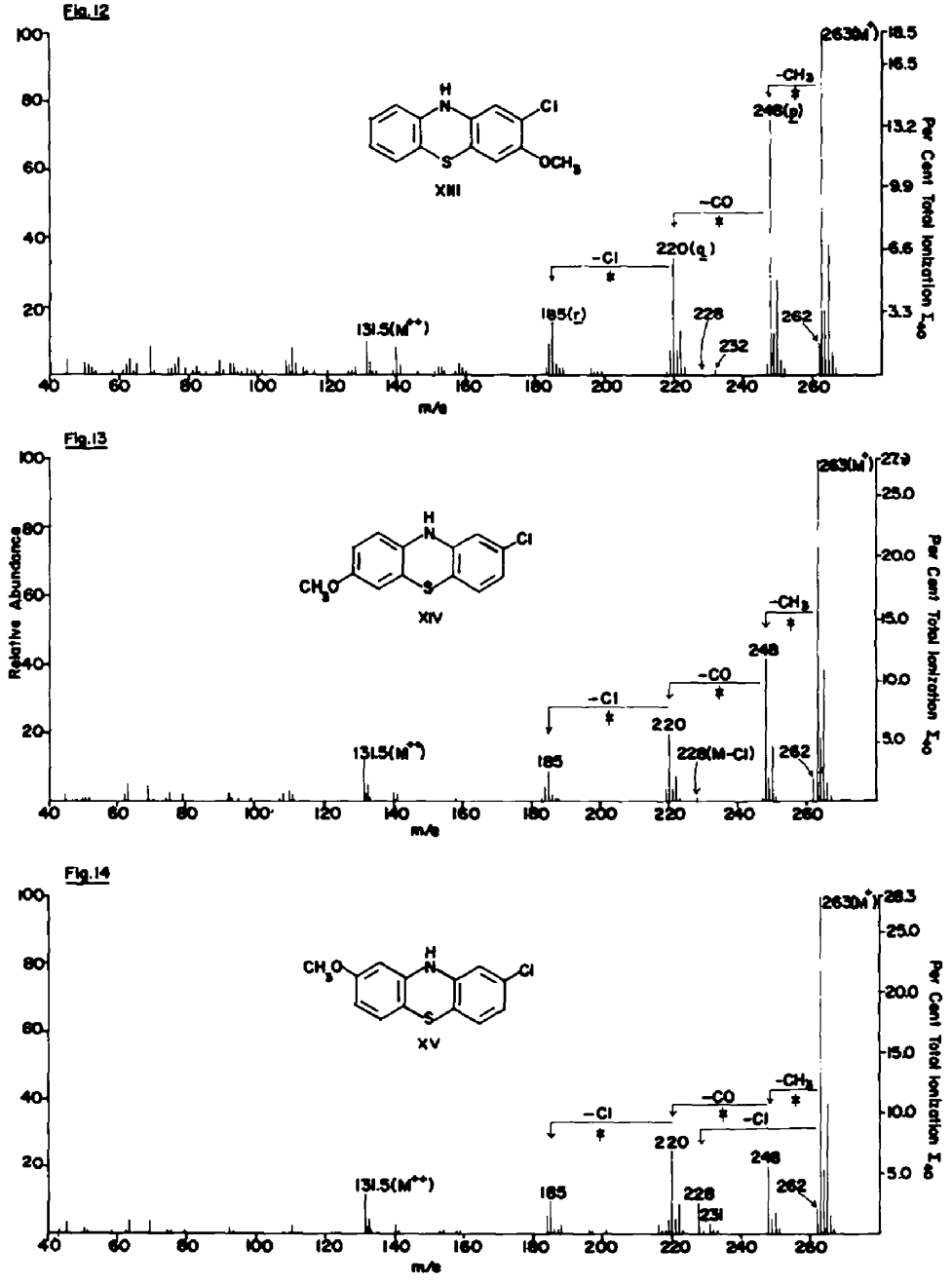


FIG. 12 Mass spectrum of 2-chloro-3-methoxyphenothiazine
FIG. 13 Mass spectrum of 2-chloro-7-methoxyphenothiazine
FIG. 14 Mass spectrum of 2-chloro-8-methoxyphenothiazine

observed for the three methyl ethers examined for the positive identification of the 3-methoxy isomer since it has a larger peak at *m/e* 248 (Fig. 12, 74% relative abundance) than either the 7- or 8-methoxy analogues (Figs 13 and 14, 42% and 21% relative abundance respectively). In addition the mass spectrum (Fig. 14) of 2-chloro-8-methoxyphenothiazine (XV) contains a peak at *m/e* 228 (10% relative abundance) due to the loss of a chlorine radical from its molecular ion and this peak only attains 1% relative abundance in the mass spectrum (Fig. 13) of the 7-methoxy isomer (XIV), and thus provides a method of distinguishing between these two compounds by mass spectrometry.

EXPERIMENTAL

Mass spectra were determined with either an A.E.I. MS-9 (ion source temp 180°) or Atlas CH-4 (compounds II, V, XI and XII ion source temp ~100°) mass spectrometer using direct sample introduction into the ion source in all cases. The compounds used in this investigation were of analytical purity and their preparation has been described.[10]

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