Ultraviolet and Attenuated Total Reflectance Infrared Spectra of Chlorpromazine Metabolites

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Ultraviolet absorption and attenuated total reflectance infrared spectra of chlorpromazine and 14 possible metabolites have been recorded and analyzed. Structural correlations of the absorption bands are presented and discussed. This collection provides a positive means of identifying chlorpromazine metabolites extracted from body fluids and tissues and separated by chromatographic techniques. Spectral identification can be made on microgram samples.

IDENTIFICATION of the metabolites of chlorpromazine has been the subject of considerable research effort over the past decade. As a result of this research, many, if not most, of its important metabolites have been identified. The usual procedure for identifying chlorpromazine metabolites has been comparison of either paper or thin-layer chromatograms of the metabolites, and of standards run at the same time. Very few investigators have used infrared spectroscopy for metabolite identification, principally no doubt because of the small amounts of metabolites available. With the advent of attenuated total reflectance spectroscopy (ATR), this obstacle has been effectively removed. The technique of ATR was developed by Fahrenfort (1) and has been described in detail by him and by Harrick (2). Its application to the identification of metabolites has been reported by several investigators. The ATR method provides infrared spectra from samples of the order of 10 mcg. In most cases, the unchanged sample can be recovered easily for further study. The combination of ATR and ultraviolet absorption spectroscopy provides an effective and positive means of identifying chlorpromazine metabolites and other materials from biological systems.

The purpose of this paper is to report the results of a spectral study of 14 possible metabolites of chlorpromazine. The ATR and ultraviolet spectral data presented here make possible a positive identification of these compounds.

EXPERIMENTAL

The infrared spectra were recorded on a Perkin-Elmer model 521 spectrometer equipped with a Wilks Scientific model 12 attenuated total reflectance attachment. The samples were prepared and run as natural films dispersed over KRS-5 plates by evaporation from chloroform solution. The average sample size was of the order of 75 to 100 mcg.

The ultraviolet spectra were recorded between 200 and 400 mμ with a Cary model 14 spectrophotometer using matched fused silica cells with a 1 cm. path length.

All spectral data were obtained from the same samples of analytical standards of a purity of 98% or greater.

RESULTS AND DISCUSSION

Infrared Spectra (ATR)—Although it is possible that transmission spectra and ATR spectra of the same materials may show slight differences (*e.g.*, band shape, relative intensities), no significant differences between transmission and ATR spectra have been found in this study. Figure 1 shows a comparison of the I.R. transmission spectrum of chlorpromazine (top) and the ATR spectrum of the same material (bottom). It is readily apparent that, for all practical purposes, the two spectra are identical. The remaining spectra of the reference materials are ATR spectra rather than transmission spectra because it is believed that, in most cases of metabolite study, the use of ATR rather than transmission spectra will be dictated by the size of the sample.

For infrared study the compounds can be separated into six groups: four groups are distinguished by the type of side chain on the nitrogen atom; the fifth group is made up of hydroxylated derivatives; and the sixth of *N*-oxides.

Figure 2 shows the spectrum of 2-chlorophenothiazine-5-oxide. This is the only member of the series without a side chain. The spectrum is typical of a 2-substituted phenothiazine (3). The strong S=O absorption band in the vicinity of 1000 cm.$^{-1}$ identifies the material as a sulfoxide.

Figure 3 shows the spectra of chlorpromazine derivatives with a primary amine side chain on the nitrogen atom. The side chain is the same in each case but the compounds shown from top to bottom are the sulfide, sulfoxide, and sulfone analogs. The spectra agree with the expected pattern of a 2,10-disubstituted phenothiazine (3); and a strong S=O absorption band at 1000 cm.$^{-1}$ ($\pm$20) and SO_2 bands at 1250–1300 cm.$^{-1}$ and 1140–1160 cm.$^{-1}$ identify the sulfoxide and sulfone derivatives.

Figure 4 shows the spectra of chlorpromazine analogs with a secondary amine side chain. Again, the order of spectra from top to bottom is sulfide, sulfoxide, sulfone.

Figure 5 shows chlorpromazine sulfoxide, chlorpromazine sulfone, and promazine. Chlorpromazine itself is shown in Fig. 1. The comments regarding 2,10-disubstituted phenothiazines in Figs. 3 and 4 apply to the sulfoxide and sulfone. Promazine gives the expected pattern of a 10-substituted phenothiazine, *i.e.*, the strong absorption band in the 720–770 cm.$^{-1}$ region of the 4 adjacent ring hydrogens (4).

Figure 6 shows the spectra of the 7- and 8-hydroxy derivatives of chlorpromazine, which differ in several respects from those of other metabolites. Since these two materials have ring substituents in addition to the 2-position, the aromatic substitution pattern would be expected to be different, and in fact it is. The peak in the 770–735 cm.$^{-1}$ region which is

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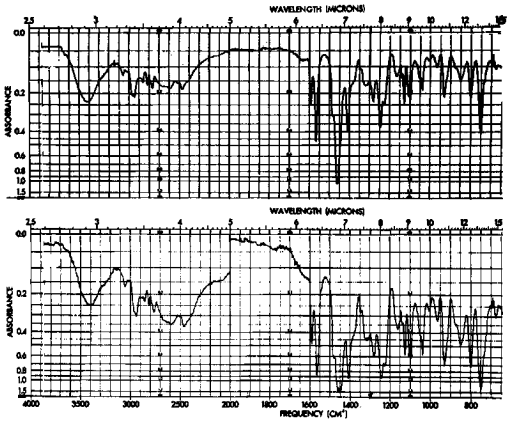


Fig. 1—I.R. transmission spectrum (top) and ATR spectrum (bottom) of chlorpromazine.

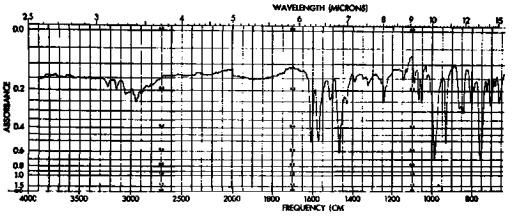


Fig. 2—ATR spectrum of 2-chlorophenothiazine-5-oxide.

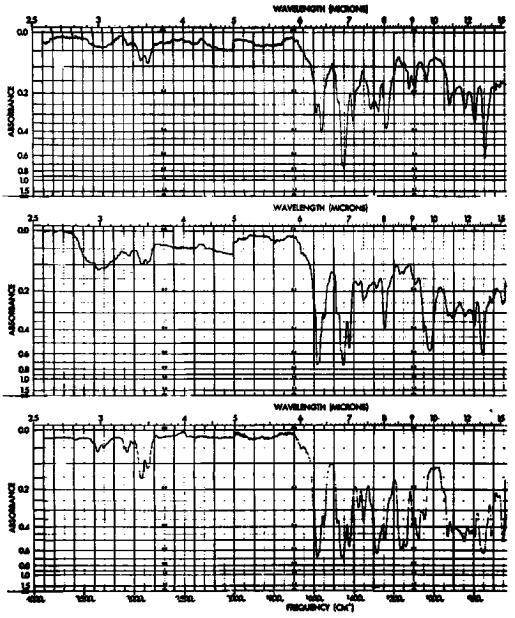


Fig. 3—ATR spectra of 2-chloro-10-(3'-aminopropyl)-phenothiazine (top), 2-chloro-10-(3'-aminopropyl)-(middle), and 2-chloro-10-(3'-aminopropyl) phenothiazine-5,5-dioxide (bottom).

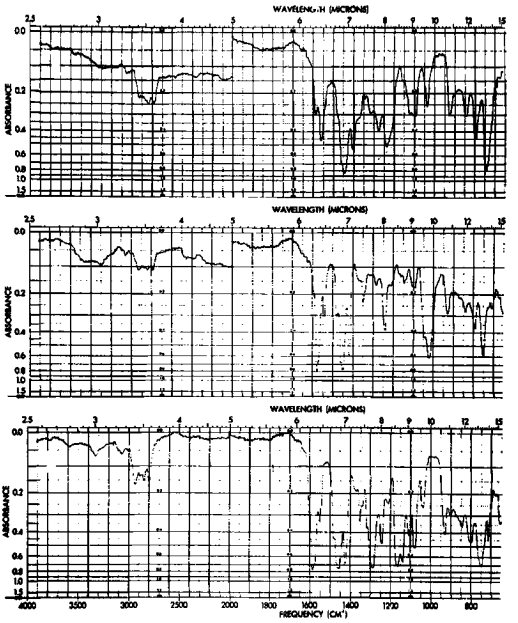


Fig. 4—ATR spectra of 2-chloro-10-(3'-methylaminopropyl)phenothiazine (top), 2-chloro-10-(3'-methylaminopropyl)phenothiazine-5-oxide (middle), and 2-chloro - 10 - (3' - methylaminopropyl)phenothiazine-5,5-dioxide (bottom).

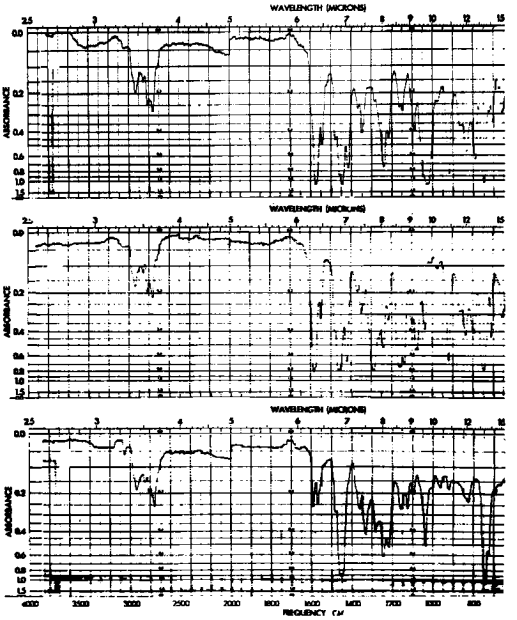


Fig. 5—ATR spectra of 2-chloro-10-(3'-dimethylaminopropyl)phenothiazine-5-oxide (top), 2-chloro-10-(3' - dimethylaminopropyl)phenothiazine - 5,5 - dioxide (middle), and 10-(3'-dimethylaminopropyl)phenothiazine (bottom).

attributed to four adjacent hydrogens (4) in 2-substituted phenothiazines disappears, and absorption bands are found at higher frequencies. This is what one should expect from the number of adjacent hydrogens in the hydroxy derivatives. The ATR spectra of the 7- and 8-hydroxy derivatives are

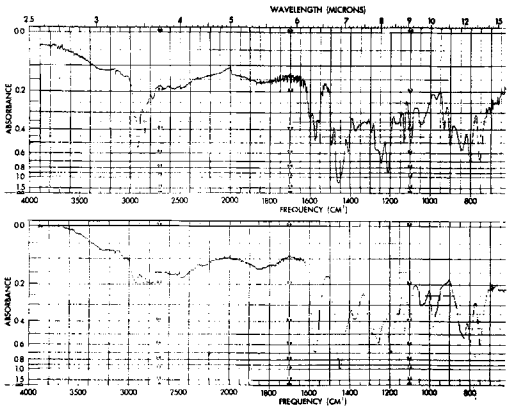


Fig. 6—ATR spectra of 2-chloro-10-(3′-dimethylaminopropyl)-7-hydroxyphenothiazine (top) and 2-chloro-10-(3′-dimethylaminopropyl)-8-hydroxyphenothiazine (bottom).

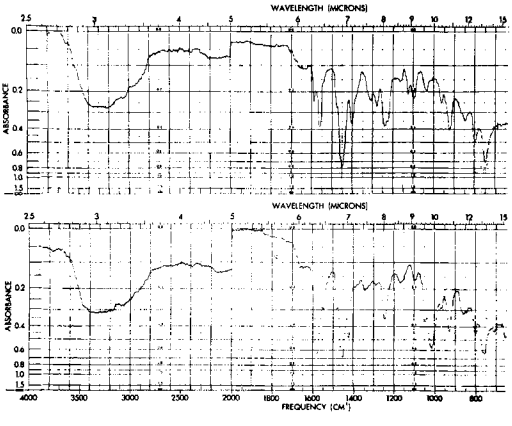


Fig. 7—ATR spectra of 2-chloro-10-(3′-dimethylaminopropyl)phenothiazine-N-oxide (top) and 2-chloro-10-(3′-dimethylaminopropyl)phenothiazine-N-oxide-5-oxide (bottom).

characteristic enough to allow differentiation from each other as well as from the other metabolites.

Figure 7 shows the ATR spectra of *N*-oxide derivatives of chlorpromazine. The *N*-oxide absorption band is located at 1200–1250 cm.$^{-1}$ and, in the case of the *N*-oxide-sulfoxide derivative, this absorption band and the strong band in the 1000 cm.$^{-1}$ region confirm the structure.

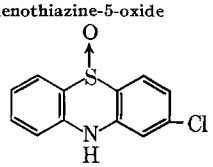
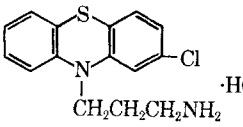
Ultraviolet Spectra—The ultraviolet spectra of chlorpromazine, chlorpromazine sulfoxide, and sulfone have been previously reported (3) by these laboratories. In addition to the ultraviolet data on these compounds, Table I gives data on the primary and secondary amine side chain analogs of these materials as well as data on hydroxy and *N*-oxide derivatives. As expected, the derivatives within a single group have the same over-all ultraviolet spectrum; thus, with the exception of hydroxy derivatives, identification of a specific sulfide, sulfoxide, or sulfone could not be made on the basis of the ultraviolet spectrum alone. Nevertheless, the ultraviolet spectra of these analogs assume greater importance when coupled with the ATR spectra, which provide positive identification of the material in question. Once identity has been established, the ultraviolet spectra provide quantitative data on the amount of material present.

The over-all differences between sulfide, sulfoxide, and sulfone are well known, as has been mentioned above. Not yet reported are the ultraviolet spectra and their differentiation of the 7- and 8-hydroxy and of the *N*-oxide derivatives. The ultraviolet data for the hydroxy compounds in acidic and basic media are given in Table I, which shows that the spectrum of either of these materials can be easily distinguished from the other metabolites listed. The shift in peak locations on change in pH is a ready identification, even in mixtures of two or more of the metabolites.

The ultraviolet spectra of the *N*-oxide derivatives are not much different from those of chlorpromazine and the sulfoxide. With the exception of a slight shift of the long wavelength peak, the spectrum of the *N*-oxide is very similar to that of chlorpromazine itself. A similar relationship is found also between the spectra of the *N*-oxide sulfoxide and chlorpromazine sulfoxide (see Table I).

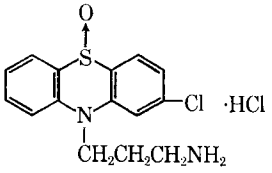
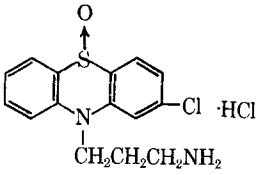
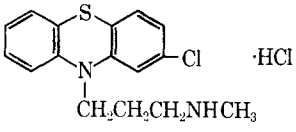
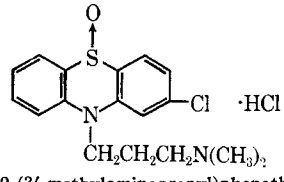
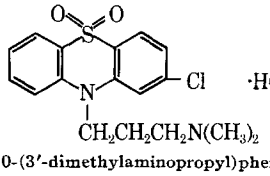
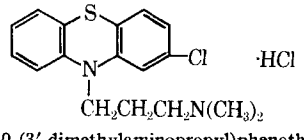
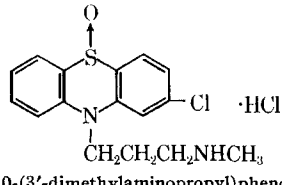
The value of these ultraviolet data is increased immeasurably after identity has been confirmed by ATR spectra, and relative amounts of the various metabolites can be calculated from standard reference data.

TABLE I—ULTRAVIOLET ABSORPTION DATA FOR CHLORPROMAZINE AND ITS METABOLITES

Compd.	$\lambda_{max.}$, mμ	Log ε	Solvent	ATR Spectrum (Free Base)
2-Chlorophenothiazine-5-oxide 	233 257 sh 273.8 304 339	4.55 4.38 4.19 3.95 3.75	95% EtOH	Fig. 2
2-Chloro-10-(3′-aminopropyl)phenothiazine hydrochloride 	256 306	4.57 3.65	95% EtOH	Fig. 3 (top)

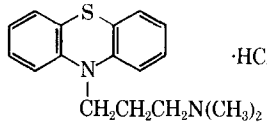
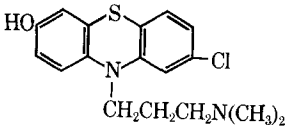
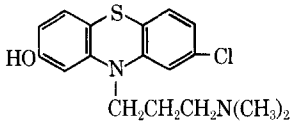
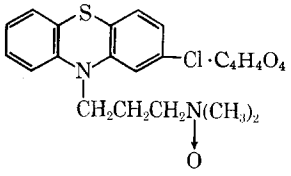
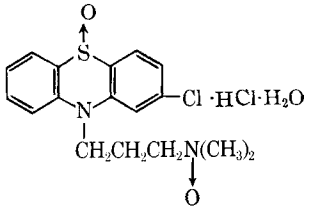
(*Continued on next page.*)

TABLE I—(*Continued.*)

Compd.	$\lambda_{max.}$, mμ	Log ε	Solvent	ATR Spectrum (Free Base)
2-Chloro-10-(3′-aminopropyl)phenothiazine-5-oxide hydrochloride 	239 275 298 340	4.56 4.10 3.94 3.78	95% EtOH	Fig. 3 (middle)
2-Chloro-10-(3′-aminopropyl)phenothiazine-5,5-dioxide hydrochloride 	233 271.5 294.5 332	4.52 4.16 3.90 3.37	95% EtOH	Fig. 3 (bottom)
2-Chloro-10-(3′-methylaminopropyl)phenothiazine hydrochloride 	255.5 306	4.57 3.65	95% EtOH	Fig. 4 (top)
2-Chloro-10-(3′-methylaminopropyl)phenothiazine-5-oxide hydrochloride 	239 275 298 341	4.56 4.08 3.92 3.76	95% EtOH	Fig. 4 (middle)
2-Chloro-10-(3′-methylaminopropyl)phenothiazine-5,5-dioxide hydrochloride 	233 271 294 332	4.55 4.18 3.91 3.75	95% EtOH	Fig. 4 (bottom)
2-Chloro-10-(3′-dimethylaminopropyl)phenothiazine hydrochloride 	256 310	4.54 3.60	95% EtOH	Fig. 1
2-Chloro-10-(3′-dimethylaminopropyl)phenothiazine-5-oxide hydrochloride 	240 275 298 342.5	4.52 4.03 3.88 3.72	95% EtOH	Fig. 5 (top)
2-Chloro-10-(3′-dimethylaminopropyl)phenothiazine-5,5-dioxide hydrochloride 	233 271 294 332	4.54 4.16 3.89 3.76	95% EtOH	Fig. 5 (middle)

(*Continued on next page.*)

TABLE I—(*Continued.*)

Compd.	$\lambda_{max.}$, mμ	Log ε	Solvent	ATR Spectrum (Free Base)
10-(3′-Dimethylaminopropyl)phenothiazine hydrochloride  ·HCl	254 306	4.53 3.64	95% EtOH	Fig. 5 (bottom)
2-Chloro-10-(3′-dimethylaminopropyl)-7-hydroxyphenothiazine 	261 322 255 310	4.41 3.86 4.47 3.79	pH 13 pH 1	Fig. 6 (top)
2-Chloro-10-(3′-dimethylaminopropyl)-8-hydroxyphenothiazine 	262 312 256 308	4.40 3.68 4.40 3.68	pH 13 pH 1	Fig. 6 (bottom)
2-Chloro-10-(3′-dimethylaminopropyl)phenothiazine-*N*-oxide maleate 	255.5 307.5	4.51 3.63	95% EtOH	Fig. 7 (top)
2-Chloro-10-(3′-dimethylaminopropyl)phenothiazine-*N*-oxide-5-oxide hydrochloride monohydrate 	239 274 296 332 sh 340	4.50 4.04 3.88 3.68 3.72	95% EtOH	Fig. 7 (bottom)

SUMMARY

Attenuated total reflectance infrared spectra and ultraviolet absorption spectra of chlorpromazine and 14 metabolites have been presented. On the basis of these data, it is possible to make an identification of any of the materials listed.

REFERENCES

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