fered with the hexachlorophene absorbance. The wavelength of hexachlorophene absorbance is pH dependent. In the system reported here (pH 0.8/11.5), the absorbance was at 320 mμ. Using the U.S.P. XVII buffer–acid system, the absorbance is at 312 mμ, causing slight interference with the *p*-hydroxybenzoate absorbance.

### RESULTS

The recovery of standard hexachlorophene carried through this method was 99 ± 2%. The accuracy and reproducibility of the spectrophotometric "difference" method have already been well documented (5).

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# Identification of 1-, 2-, 3-, and 4-Chlorophenothiazine Isomers

By I. B. EISDORFER, R. J. WARREN, W. E. THOMPSON, and J. E. ZAREMBO

**The infrared and ultraviolet spectral data for four monochloroisomers of phenothiazine are presented and discussed. From these data it is possible to make a positive and rapid identification of any of the isomers with a minimum amount of sample. The method can be used to identify the isomers alone or in combination.**

THE PRESENCE of isomers in the preparation of chlorinated phenothiazines is always a possibility. The problem of determining the presence of an isomer and identifying the specific isomer present is a frequent analytical problem. A simple, rapid method for identifying the 1-, 2-, 3-, and 4-chlorophenothiazine isomers is presented.

### EXPERIMENTAL

**Reagents.** — 1 - Chlorophenothiazine, 2-chlorophenothiazine, 3-chlorophenothiazine, and 4-chlorophenothiazine. All chemicals are of analytical grade as prepared at Smith Kline & French Laboratories.

**Spectrophotometers.**—The infrared spectra were recorded with a Perkin-Elmer model 21 double beam spectrophotometer with a sodium chloride prism. The phenothiazines studied were prepared as mineral oil mulls.

The ultraviolet spectra were recorded with a Cary model 14 recording spectrophotometer using matched fused silica cells with a 1-cm. light path.

### RESULTS AND DISCUSSION

**Infrared Spectra.**—Figure 1 shows the infrared spectra obtained for the four chlorophenothiazine isomers. The area of greatest interest is the region between 1000 and 650 cm.$^{-1}$. This is the region which contains absorption bands due to C–H out-of-plane deformations in aromatic ring systems. Each of the isomers has its own unique pattern in this area owing to the particular position of the chlorine atom on the ring. The infrared pattern here is specific enough to distinguish one isomer from another, and the absorption bands are so located as to permit detection of one or more isomers in the presence of another.

Spectrum A, which is that of the 1-chloro isomer, shows strong absorption bands between 770 and 700 cm.$^{-1}$ which are assignable to 3 and 4 adjacent free hydrogen atoms in an aromatic ring (1). Spectrum B is that of the 2-chloro isomer with absorption

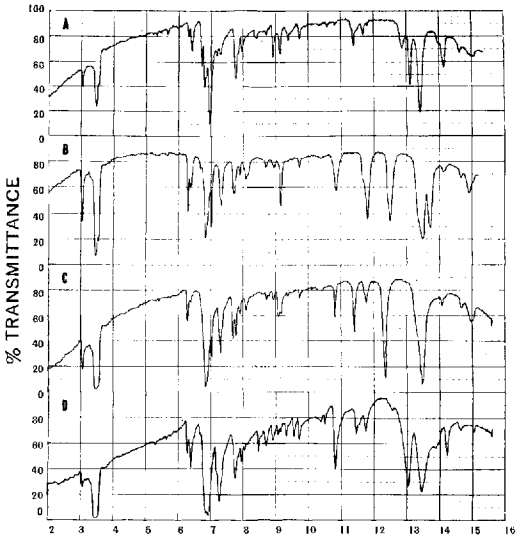


Fig. 1.—Key: A, 1-chlorophenothiazine; B, 2-chlorophenothiazine; C, 3-chlorophenothiazine; D, 4-chlorophenothiazine.

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bands at 750, 800, 825, and 920 cm.$^{-1}$ which are assignable to a 2-substituted phenothiazine (2). Spectrum C is the 3-chloro isomer with absorption bands at 750, 810, 850, 875, and 925 cm.$^{-1}$ which are all assignable to 4 and 2 adjacent free hydrogen atoms and one free hydrogen atom on an aromatic ring (1). Spectrum D is the 4-chloro isomer with strong absorption bands at 740 and 765 cm.$^{-1}$ which are in agreement with out-of-plane bending vibrations of 3 and 4 adjacent free hydrogen atoms on an aromatic ring system (1). From these infrared data it is possible not only to identify each isomer alone but also in combination with the other three isomers. An illustration of how these data may be used to identify the isomers in combination is shown in Fig. 2. Spectrum A is that of 2-chlorophenothiazine. Spectrum B is a mixture containing 6% of 3-chlorophenothiazine. Spectrum C is the spectrum of a 10% mixture of the 3-isomer in 2-chlorophenothiazine. There is no absorption whatever at 11.45 $\mu$ in the 2-chloro isomer but in the spectra of both mixtures an absorption band at 11.45 $\mu$ is noted. This band is due to the presence of the 3-chloro isomer (see spectrum C in Fig. 1). Preparation of a series of standards or mixtures of known concentrations makes it possible to prepare a calibration curve from which quantitative data and minor components could be derived.

The infrared spectra of the four isomers complements the ultraviolet data which follow and is very valuable indeed in identifying chromatographic fractions from gas chromatography or spots from thin-layer or paper chromatography. It also provides a rapid means of identification of isomers in a given preparation of chlorinated phenothiazines.

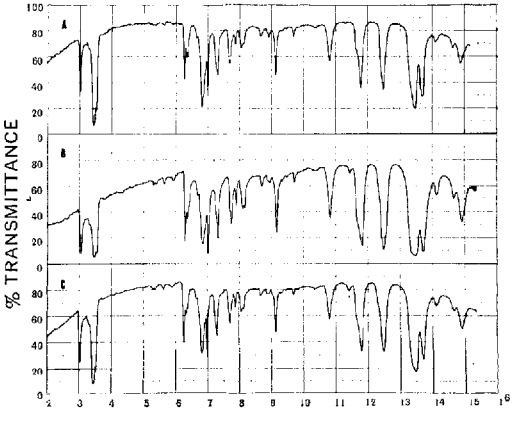
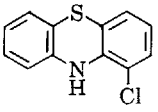
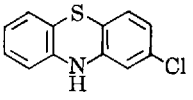
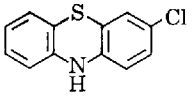
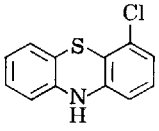



Fig. 2.—Key: A, 2-chlorophenothiazine; B, 6% 3-chlorophenothiazine in 2-chlorophenothiazine; C, 10% 3-chlorophenothiazine in 2-chlorophenothiazine.

**Ultraviolet Spectra.**—Table I lists the ultraviolet maxima and corresponding log $\epsilon$ values for the four

TABLE I.—U.V. SPECTRAL DATA FOR CHLOROPHENOTHIAZINES[a]

| Compd. | λ, mμ | Log ε |
|---|---|---|
|  1-Chlorophenothiazine | 318 | 3.62 |
| | 257 | 4.63 |
|  2-Chlorophenothiazine | 320 | 3.69 |
| | 256 | 4.71 |
|  3-Chlorophenothiazine | 323 | 3.67 |
| | 258 | 4.68 |
|  4-Chlorophenothiazine | 325 | 3.65 |
| | 259.5 | 4.66 |

[a] U. V. spectra run in 95% ethanol.

chloro isomers. The effect of the position of the chlorine on the ring can readily be seen in the various wavelength shifts. The infrared data complemented by these ultraviolet data for the isomers make possible a quantitative estimation of the amount of various isomers present in a given preparation.

## SUMMARY

Data are given to show that 1-, 2-, 3-, and 4-chlorophenothiazines each possess a unique infrared and ultraviolet spectrum. This information provides a basis for determining the presence or absence of isomers in a given preparation of chlorinated phenothiazine.

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