set of C-13-epimers can be distinctly separated from each other on the nonselective phase, although no plausible explanation is at present available for this chromatographic behavior. In addition it was found interesting that there is a relatively high degree of constancy of the observed values in both series. The contribution values for C/D-*cis* ring fusion thus established may be helpful to discriminate the structural difference in the steroid skeleton by means of gas chromatography.

*Acknowledgements*

The authors thank Miss HARUKO IKEDA for technical assistance. The present work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education (Japan), which is gratefully acknowledged.

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Received July 31st, 1967

*J. Chromatog.*, 31 (1967) 535–538

## Conversion of chlorpromazine sulfoxide to chlorpromazine by use of metals in acid solution

Previous work from this laboratory dealt with the conversion of some phenothiazine derivatives to the corresponding sulfoxides[1]. For example chlorpromazine sulfoxide[2], one of the major metabolites of chlorpromazine is the resulting product of the oxidation of the sulphur in the phenothiazine nucleus.

In routine work in the forensic laboratory the sulfoxides are normally extracted from the body tissues and from urine. Due to the similarity of the ultraviolet spectra of the various sulfoxides, difficulties are always encountered in trying to establish the identity of any particular sulfoxide. Because of this, work was commenced to study the conversion of any particular sulfoxide to the original parent phenothiazine.

*Materials*

Samples of chemically pure chlorpromazine and of chemically pure chlorpromazine sulfoxide were obtained from Smith, Kline and French laboratories, and were used throughout the experimental work.

*Apparatus*

The ultraviolet spectrophotometric data were obtained by use of a Beckman D.K. 2A Ratio Recording Spectrophotometer, while the infrared data were obtained on a Beckman IR-12 Grating Spectrophotometer. The gas chromatographic measurements were made on a Microtek GC-2500R Gas Chromatograph. A short wave ultraviolet lamp UVS-11 Ultra Violet Products Inc., San Gabriel (Calif.) was used to locate the spots on the thin-layer chromatography plates.

*Thin-layer chromatography*

Plates: 20.5 × 20.5 cm and 4.0 × 20.5 cm.

Absorbent: Silica Gel $GF_{254}$ according to STAHL, E. Merck AG., Darmstadt, Germany.

Solvent: Ammonium acetate 1.5 g ,distilled water 10 ml, methanol up to 50 ml.

Locating agent: Ultraviolet light (short wave).

Spray reagent: 8 *N* HCl.

*Gas chromatography*

Column: type: stainless steel: length 60 cm: internal diameter 7 mm: coating 3 % SE-30, support Chromosorb G, 80–100 mesh Johns Manville Products.

Carrier gas: helium; inlet pressure 30 p.s.i., outlet pressure atmospheric, flow rate 75 ml/min.

Temperature: Column 220°, inlet 280°, detector 280°.

Detector: flame ionization.

*Experimental*

The conversion of a sulphoxide to the original phenothiazine was first attempted using various concentrations of HCl at several different temperatures. As a result it was concluded that concentrated HCl at steam bath temperature would bring about conversion. The time required was in excess of 30 min. It was noted that at this point while the ultraviolet spectrum resembled that of chloropromazine (maxima at 256 and 306 m$\mu$) the $R_F$ of the converted product was slightly greater than that of the standard chlorpromazine. Table I shows the progress of the conversion as followed by the ultraviolet spectrophotometer.

The action of some reducing agents was investigated. It was found that metallic tin, zinc and aluminum in 8 *N* HCl worked quite satisfactorily. The method employed was as follows: 1 ml of 8 *N* HCl was added to 100 $\mu$g of chlorpromazine sulfoxide in aqueous solution in an evaporating dish. On standing, a deep red colour developed.

TABLE I

CONVERSION OF CHLORPROMAZINE SULFOXIDE USING CONC. HCl ON A STEAM BATH

| *Time required* | *Spectrum maxima* |
|---|---|
| Immediately | 237, 249 (s), 273, 297, 334 |
| 5 min | 256, 274 (s) |
| 15 min | 256, 276, 306 |
| 30 min | 256, 306 |

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TABLE II

PROGRESSIVE CHANGES IN THE ULTRAVIOLET SPECTRA OF CHLORPROMAZINE SULFOXIDE TO CHLORPROMAZINE WITH DIFFERENT METALS

| *Metal/conditions* | *Time and absorption maxima* | | | | |
|---|---|---|---|---|---|
| Tin, open air | 5 min<br>237, 249(s), 273(s), 298, 340 | 20 min<br>237, 249(s), 273(s), 295, 340 | 1 h<br>251, 274(s), 300 | 1 h 30 min<br>252, 274(s), 300 | 2 h<br>254, 306 |
| Tin, heated | Initial<br>238, 242(s), 272, 297, 338 | 1 min<br>254, 306 | After 2 h at room temp.<br>254, 306 | | |
| Zinc, open air | 10 min<br>237, 248(s), 268, 274, 298(s), 230 | 20 min<br>258(s), 267, 274, 320 | 30 min<br>254, 266(s), 274, 308 | 50 min<br>254, 306 | |
| Zinc, heated | 10 min<br>255, 266(s), 273, 304 | 30 min<br>254, 275(s), 304 | 50 min<br>254, 306 | After 3 h at room temp.<br>254, 306 | |
| Aluminum, open air | 10 min<br>269(s), 275, 295(s) 330 | 20 min<br>253, 274(s), 300 | 35 min<br>254, 306 | | |
| Aluminum, heated | 1 min<br>255, 274(s) | 3 min<br>254, 306 | After 1 h at room temp.<br>255, 274(s), 314 | | |

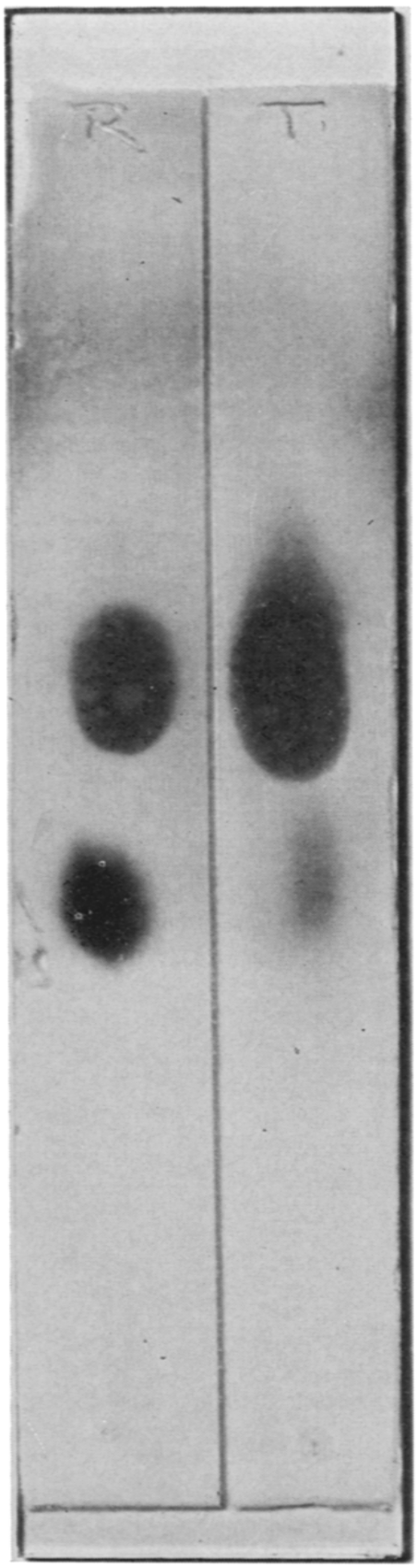


Fig. 1. Confirmation of identity of converted sulfoxide using thin layer chromatography. R, upper spot = chlorpromazine, lower spot = chlorpromazine sulfoxide. T, upper spot = converted product, lower spot = trace amounts sulfoxide.

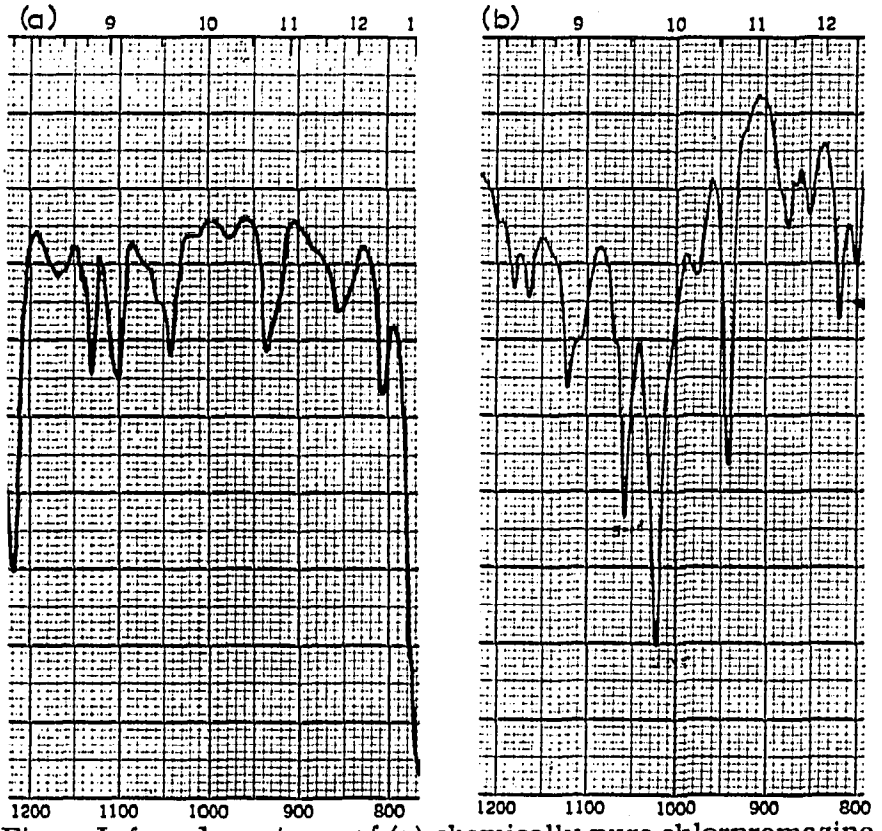


Fig. 2. Infrared spectrum of (a) chemically pure chlorpromazine and (b) chlorpromazine sulfoxide.

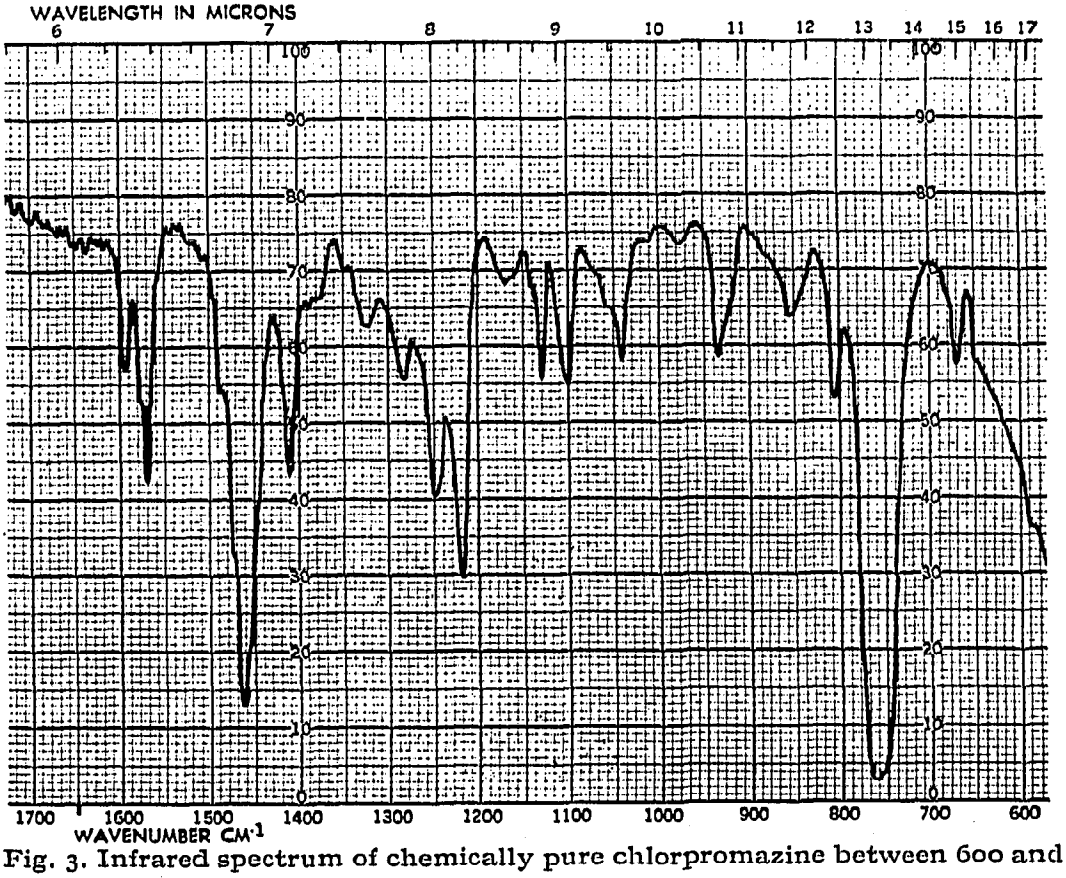


Fig. 3. Infrared spectrum of chemically pure chlorpromazine between 600 and 1700 $cm^{-1}$

At this point approximately 1 g of the finely divided metal to be used was added. The process of conversion was followed by recording the ultraviolet spectrum at various time intervals. This process was carried out at room temperature and at 100° (steam bath temperature). Table II shows the progress of the conversion from the sulfoxide to chlorpromazine as it was followed by recording changes in the ultraviolet spectrum. Each commenced with the sulfoxide curve which changed with time to the chlorpromazine spectrum with two maxima at 254 and 306 mμ. A shoulder at 274 mμ indicated that conversion was not complete. Using heat, conversion was accomplished in a considerably shorter period of time with tin and aluminum, but not with zinc. The product of conversion using tin and zinc was stable. Oxidation to the initial sulfoxide occurred with the aluminum. Because of the time element and the stability of the final product, tin was chosen for future experimentation. Lead and selenium were also used to attempt conversion. The time required was too long to be of practical use.

*Proposed method for the conversion of chlorpromazine sulfoxide to chlorpromazine.* An evaporating dish containing the chlorpromazine sulfoxide in 8 *N* HCl was heated on the steam bath together with a similar dish of 8 *N* HCl. When both were hot (approximately 100°) about 1 g of metallic tin shavings was added to the 8 *N* HCl and left for about 1 min. Then the supernatant HCl was transferred drop by drop to the sulfoxide solution until the red colour disappeared. While disappearance of the red colour indicated the conversion of the sulfoxide to the original chlorpromazine, checks for completeness were made by measurement of the ultraviolet spectrum. Further confirmation that complete conversion had been accomplished was ascertained using thin layer chromatography, gas chromatography and infrared spectrophotometry.

*Thin-layer chromatography.* The solution was first evaporated to dryness on the steam bath, dissolved in 10 % HCl in ethanol and run on thin-layer chromatographic plates using the solvent cited above, in the dark. Location of spots was first accomplished by using short wave ultraviolet light and then by spraying with 8 *N* HCl.

After being heated under a hair dryer the spots became a light pink. All plates were spotted with reference standards of a mixture of chlorpromazine and chlorpromazine sulfoxide. The $R_F$ value of the converted product and chemically pure sample chlorpromazine are identical. The trace of sulfoxide indicated on the thin-layer plate was much less than one gamma. Fig. 1. shows a typical plate after spraying.

*Gas chromatography.* Chlorpromazine hydrochloride and the converted product were extracted to give the free bases which were run on the gas chromatograph using the conditions described above. The retention time was found to be 2.3 for both the chlorpromazine and the converted material.

*Infra red spectrophotometry.* The samples of the free bases were run on the infrared spectrophotometer as a chloroform film between two salt plates.

Chlorpromazine and chlorpromazine sulfoxide differ in the spectra only in the region 1000–1100 $cm^{-1}$ (see Fig. 2). The converted product shows favourable comparison (see Figs. 3 and 4) with chlorpromazine over the entire spectrum.

## *Conclusions*

The difficulties encountered in the past in identifying the sulfoxides of various phenothiazine derivatives extracted from body tissues or urine have made positive identification of them almost impossible. In this paper a method is presented for

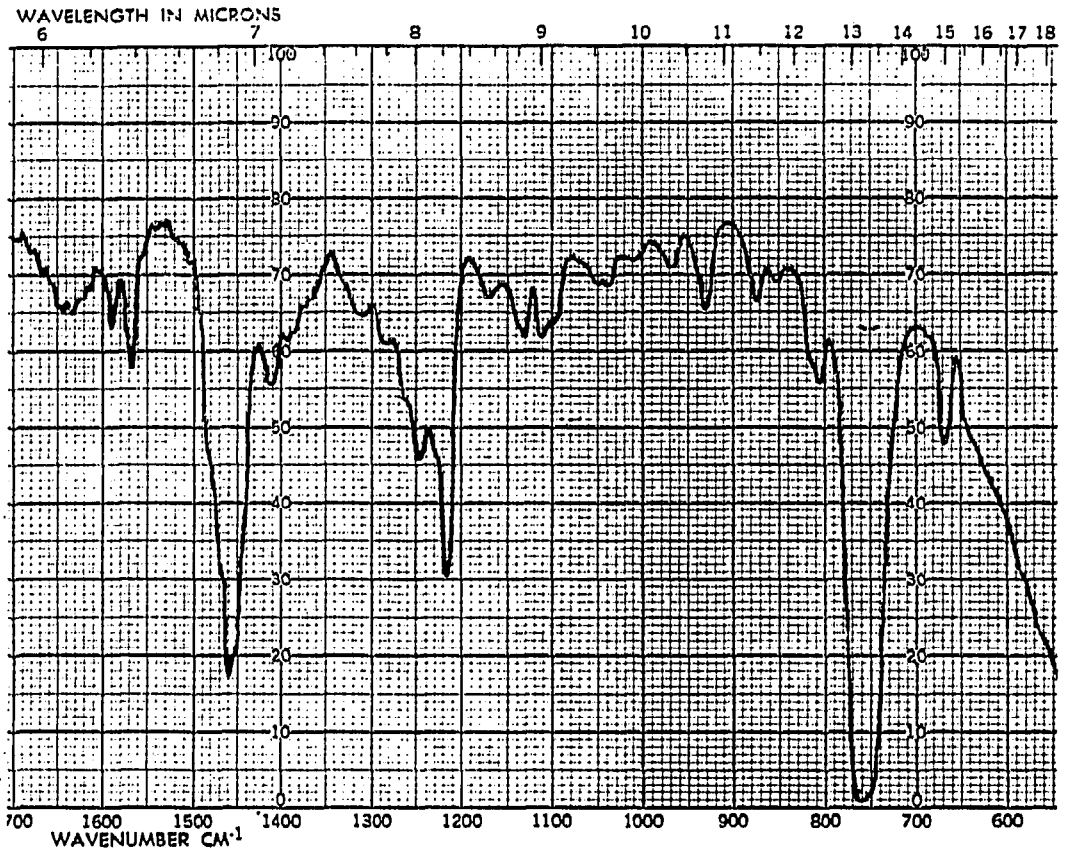


Fig. 4. Infrared spectrum of the converted product of chlorpromazine sulfoxide between 600 and 1650 $cm^{-1}$.

reducing chlorpromazine sulfoxide to chlorpromazine and hence facilitating the identification of its sulfoxide. The proposed method has been applied to the sulfoxides of promazine, promethazine, trifluoperazine, triflupromazine, fluphenazine, with promising results. Work is continuing along this line.

*Acknowledgements*

Our appreciation is expressed to Dr. H. WARD SMITH, Director of this Laboratory, for his encouragement in our work.

We are indebted to Smith Kline and French Laboratories for supplying samples of chlorpromazine and chlorpromazine sulfoxide.

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Received June 26th, 1967