A STUDY OF THE CONVERSION OF PHENOTHIAZINE DERIVATIVES TO THE CORRESPONDING SULFOXIDES ON THIN-LAYER PLATES

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Previous experimental work[1] reported from this laboratory on the phenothiazine derivatives has dealt mainly with color, crystal and spectrophotometric tests as a means of identification. In a recent preliminary report[2] at a meeting of the American Academy of Forensic Science attention was drawn to the value of the sulfoxides as a further means of identification of these drugs and a tentative method for preparing them quickly and accurately was described. The present paper extends the data of the preliminary report and deals with a complete study of 40 phenothiazine derivatives and their respective sulfoxides employing ultraviolet spectrophotometry, thin-layer and gas chromatography.

MATERIALS

Reference compounds

The phenothiazine derivatives and a few sulfoxides were obtained from the various manufacturers. The remaining sulfoxides were prepared according to the method of SCHMALZ AND BURGER[3]. The identity of the synthesized products was established by means of ultraviolet and infrared spectrophotometry, color tests, thin-layer and where applicable gas chromatography.

Apparatus

The ultraviolet spectrophotometric data were obtained on a Beckman DK-2A Ratio Recording Spectrophotometer, the infrared data on a Beckman IR-4 Infrared Spectrophotometer and the gas chromatographic work was done on a Microtek GC-2500 R gas chromatograph. A short-wave ultraviolet lamp, model SL 2537 manufactured by Ultraviolet Products Inc., South Pasadena, Calif., was used to locate the spots on the thin-layer chromatography plates.

Thin-layer chromatography

Plates: 20.5 × 20.5 cm.

Absorbants: Silica Gel GF-254 and Silica Gel G according to STAHL, E. Merck, Darmstadt, Germany; Adsorbasil P-2, Applied Science Laboratories Inc., State College, Pa., U.S.A.; Aluminum Oxide G, according to STAHL, E. Merck, Darmstadt, Germany.

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Solvent: ammonium acetate 1.5 g, dist. water 10.0 ml, methanol up to 50.0 ml.
Locating agents: U.V. light (short wave).
Iodoplatinic acid.

Gas chromatography

Column: type: stainless steel and glass; length 60 cm; internal diameter 7 mm; coating 3 % SE-30; support Gas Chrom Q, 80–100 mesh, Applied Science Laboratories, Inc., State College, Pa., U.S.A.

Carrier gas: helium; inlet pressure 30 p.s.i.; outlet pressure atmospheric.

Temperature: column 210° and 250°; inlet 300°; outlet 290°; detector 290°.

Detector: flame ionization.

Recorder: Minneapolis-Honeywell.

EXPERIMENTAL

Each of the following drugs in form of their salts were used in the experiments described below: chlorpromazine, thioridazine, trifluoperazine, fluphenazine, triflupromazine, promazine, promethazine.

During routine work on the identification of phenothiazines it was observed that when a developed thin-layer plate was left in the open air for some days, the ultraviolet spectrum of an eluted spot no longer resembled that of the original product, but was closer to its sulfoxide. Gas–liquid and thin-layer chromatography confirmed, that indeed the sulfoxide had been formed, and because of this, certain conditions influencing the oxidation of phenothiazines on thin-layer plates were further investigated.

Plates were prepared for ten different compounds. Each plate contained eight spots of the drug assigned to it. The plates were developed in the solvent cited above and were allowed to stand in the open air in day light at approximately 20°. Each day one spot was eluted with distilled water and the ultraviolet spectrum recorded. After an interval of 48 to 72 h, depending on the derivative under study, marked changes in the adsorption spectrum had taken place; the spectrum began to resemble that of the corresponding sulfoxide. At the end of 8 days the spectrum was that of the pure sulfoxide (see Table I).

While a change in color of the spot was noted a few minutes after the dry plate was exposed to daylight, it was not accompanied by a change in the ultraviolet spectra. Gas–liquid and thin-layer chromatography confirmed that only the sulfoxide was found.

It was found that the oxidation was dependent mainly on the availability of oxygen. If the experiments were performed without circulating air, no oxidation occurred. The same was observed when the plates, spotted in the usual manner, were kept in a tightly closed vessel in an atmosphere of nitrogen for up to two weeks.

Apart from this it was evident that neither daylight nor ultraviolet light was essential in the oxidation process; no change in the ultraviolet spectra was observed after four days of irradiation of the plates with ultraviolet light in a dark room without circulating air.

It was observed, however, that deep brownish colors had developed in the spots. A control plate placed in the same room, but excluded from ultraviolet irradia-

TABLE I

SPECTROPHOTOMETRIC DATA AT THE END OF EIGHT DAYS IN DAYLIGHT AND OPEN AIR (*ca.* 20°) OBTAINED FROM THE ELUTED SPOTS

Generic name	*Maxima of the standard drugs*	*Maxima of the sulfoxides eluted from TLC plates*
Chlorpromazine	254–305	238–274–298–340
Thioridazine	262–313	235–260–276–304
Trifluoperazine	257–308	244–275–304–336
Thioproperazine	234–264–315	249–275–304–342
Levopromazine	251–303	240–275–296–329
Perphenazine	255–307	233–275–300–340
Fluphenazine	258–308	233–255–274–302–340
Triflupromazine	257–308	233–255–274–300–340
Promazine	252–303	233–272–295–340
Promethazine	249–299	234–260–295–330

tion had acquired faint colors only and again no change in the ultraviolet spectra was noted.

Reports in the literature have indicated that the adsorbant gels may cause a chemical change in the compounds adsorbed on it. Our experience with several absorbants is, that per se they do not have any effect on the formation of the sulfoxides and it was further established that the thickness of the gels was not a decisive factor. It is therefore our opinion that the oxidation reaction appears to be associated with a relatively large increase in the surface area of the phenothiazine drugs adsorbed on the plates. Such an increase is brought about by the development of the plate after spotting with drug.

In order to check the effect of temperature on the oxidation, a series of plates were spotted and placed in an oven with circulating air at 60°. Conversion to the sulfoxide became measurable after about 2 h and was complete after 3–4 days. Contrary to our expectation, only traces of oxidized material were detectable by ultraviolet measurements at the end of three days at 120° but further experimentation in this area is in progress. No oxidation took place on a plate left at 0° for five weeks.

In all these experiments it was observed, that when spotted plates were maintained in daylight at approximately 20°, with or without oxygen, a color characristic of the compound employed developed in the spots within 15 min. In darkness the development of color was delayed. When ultraviolet irradiation was applied to spots at 20° in a dark room a color developed about as rapidly as it did in daylight. The shades were pastel at first, but with longer time of irradiation they assumed a different hue in contrast to those developed in daylight when the shade of the color increased. The temperature at which the plates were stored, played an important role in the color development. After six weeks at 0° only very faint colors were evident, but with temperatures well above 20° the colors appeared very rapidly and were more intense.

Experiments employing hydrogen peroxide as oxidizing agent

In our preliminary report[3] it was shown that spots of phenothiazine developed on thin-layer plates could be oxidized to the sulfoxide rapidly by use of a 3 % solution

of hydrogen peroxide. At that time the optimum concentration of peroxide had not been determined. Further investigations have shown that the concentration of hydrogen peroxide is not critical but that best results are obtained when the concentration is between 10 % and 20 %. The oxidation product obtained in this manner on the plate was confined to one spot only as indicated in Fig. 1 above the spot treated with 10 % to 20 % hydrogen peroxide and the R_F value corresponds to that of the pure sulfoxide. The optimum hydrogen peroxide concentration was determined as follows.

In Fig. 1 the original compound chlorpromazine HCl was spotted in such a manner as to obtain a large spot without tailing and the chromatogram allowed to develop in direction 1. The spot was outlined under ultraviolet light and one strength of peroxide was applied to the upper part and another concentration to the lower part of the spot. On line with, and next to the spot two reference spots were applied, one

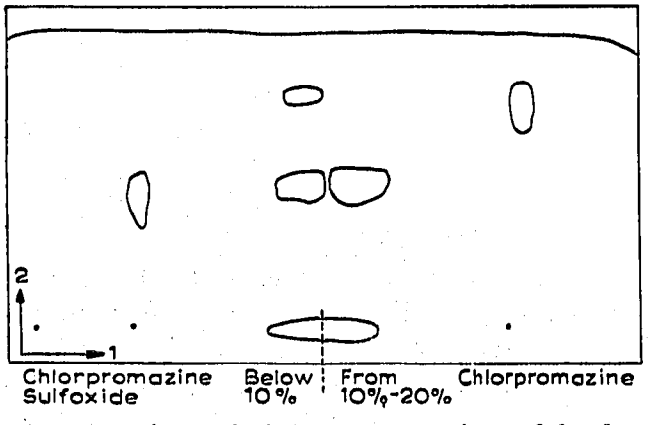

Fig. 1. Effect of the concentration of hydrogen peroxide on the complete oxidation of chlorpromazine.

of chlorpromazine and one of chlorpromazine sulfoxide. The plate was now allowed to develop in direction 2 perpendicular to the first one. This experiment indicated that in order to obtain a complete conversion to the sulfoxides for the quantity employed a concentration of at least 10 % to 20 % hydrogen peroxide was essential. Below that concentration only portions of the drug were oxidized and two spots were obtained upon rechromatographing. There was some indication that when 30 % hydrogen peroxide was employed together with hot air to dry the spot a second compound was formed, travelling behind the sulfoxide on the plate. This compound might be the corresponding sulfone. The sulfoxides were identified by their ultraviolet spectra, color tests, thin-layer chromatography and where applicable by gas–liquid chromatography.

Gas chromatography

Gas chromatography represents the most satisfactory means to date of separating and identifying the phenothiazines and their respective sulfoxides. Excellent separations of these were achieved utilizing the SE-30 column at different temperatures (Tables II and III). It should be noted, however, that only very broad peaks could be obtained for the sulfoxides of the four phenothiazines with the highest molecular weight. The retention times for these four varied from 40 to 60 min. All

TABLE II

RETENTION TIMES OF PHENOTHIAZINE DERIVATIVES

Compound	*Retention time*		
	Flow rate 80 ml/min (200°)	*Flow rate 90 ml/min (210°)*	*Flow rate 120 ml/min (250°)*
Trifluprομazine	6.8	2.4	—
Promethazine	7.8	2.8	—
Promazine	9.1	3.1	—
Chlorpromazine	—	4.5	—
Levopromazine	—	5.7	—
Trifluoperazine	—	8.6	—
Fluphenazine	—	28.7	4.3
Thioridazine	—	29.8	5.8
Perphenazine	—	—	9.6
Thioproperazine	—	—	17.6

compounds were injected in the free state as well as in salt form without any differences appearing in the chromatogram.

Proposed method for the identification of phenothiazine derivatives

The phenothiazine derivative is spotted in the lower two corners of a thin-layer plate (20 × 20 cm) approximately 3 cm from the edges. The plate is placed in the developing tank, with the spots at the bottom, until the solvent has travelled to about 3–4 cm from the top, after which time it is taken out and allowed to dry. The spots are now outlined under the short wave ultraviolet light (cited above) and the right hand one is just wetted with 10 % to 20 % hydrogen peroxide solution by adding it dropwise in small drops. The spot is dried in a hot air stream (60°) after which the plate is placed in the tank again with the peroxide treated spot at the bottom and allowed to develop in a direction perpendicular to the initial one. In this way

TABLE III

RETENTION TIMES OF PHENOTHIAZINE SULFOXIDES

Sulfoxide of	*Retention time*	
	Flow rate 90 ml/min (210°)	*Flow rate 120 ml/min (250°)*
Trifluprομazine	4.2	—
Promethazine	6.6	—
Promazine	7.8	—
Chlorpromazine	10.2	2.5
Levopromazine	11.8	3.1
Trifluoperazine	16.8	3.6
Fluphenazine	—	—
Thioridazine	—	—
Perphenazine	—	—
Thioproperazine	—	—

TABLE IV

R_F VALUES AND SPECTROPHOTOMETRIC DATA OF PHENOTHIAZINE SULFOXIDES

Generic name	*R_F value*		*Maxima of the sulfoxides obtained in the manner described*
	Original drug	*Sulfoxide*	
1 Acetophenazine	0.69	0.16	251–274S–310
2 Acetopromazine or acetylpromazine	0.58	0.39	251–272S–310–343
3 Aminopromazine or proquamazine	0.60	0.28	232–266–295–333
4 Carphenazine	0.71	0.16	246–277S–310
5 Chlorpromazine	0.62	0.48	238–273–298–340
6 Chlorproethazine	0.69	0.49	238–250S–273–298–340
7 Chlorprothixene	0.66	0.45	255–302
8 Cyamepromazine	0.61	0.39	243–274S–304–340
9 Diethazine	0.69	0.44	233–268–293–338
10 Dimethoxanate	0.56	0.41	240–274–295
11 Ethopropazine or prophenamine	0.70	0.47	233–267–292–336
12 Fluphenazine	0.75	0.59	232–273–304–343
13 Isopromethazine	0.63	0.39	233–267–291–336
14 Isothipendyl	0.64	0.41	238–273–336
15 Levopromazine	0.87	0.59	250–276S–296–333
16 Mepazine	0.58	0.39	231–272–299–342
17 Methdilazine	0.64	0.43	232–272–298–342
18 Methopromazine or methoxypromazine	0.67	0.35	244–274S–294–330
19 Methylpromazine	0.62	0.40	238–272–299–340
20 Perphenazine or chlorpiprozine	0.65	0.45	240–250S–274–342
21 Phenothiazine or fenethazine	0.69	0.37	232–266–294–334
22 Pipamazine	0.83	0.53	239–274–300–342
23 Prochlorperazine	0.55	0.15	238–274–300–340
24 Promazine	0.51	0.37	231–271–299–342
25 Promethazine	0.66	0.38	232–270–297–340
26 Propiomazine	0.77	0.54	246–265S–304–360
27 Prothipendyl	0.69	0.48	238–276–340
28 Pyrathiazine or pyrrolazate	0.64	0.43	232–269–295–336
29 Thiazinamium	0.53	0.33	232–269–294–336
30 Thiethylperazine	0.47	0.28	238–272–301–350
31 Thiopropazate	0.77	0.25	238–274–300–340
32 Thioproperazine	0.43	0.26	245–262S–275–304–342
33 Thioridazine	0.71	0.46	237–273–302–340
34 Transergan	0.53	0.33	225–266–291–330
35 Trifluoperazine	0.63	0.41	233–273–302–343
36 Triflupromazine	0.69	0.50	233–274–301–343
37 Trimeprazine or alimemazine	0.71	0.50	232–297–340
38 No. 6710 Rhône-Poulenc	0.63	0.46	251–273–298–332
39 No. 9260 Rhône-Poulenc	0.85	0.59	240–274–305
40 No. 7261 Smith Kline & French	0.78	0.27	233–272–302–340

two R_F values can be obtained, one for the phenothiazine derivative and one for the sulfoxide. The spots can now be removed from the plate and eluted with distilled water. After the ultraviolet spectra are obtained from the supernatant the original phenothiazine and its sulfoxide can be compared with reference compounds by use of thin-layer and gas–liquid chromatography. It should be noted that even after the most careful elution the gel remaining in the centrifuge tube still contains some sulfoxide which may be demonstrated by color tests[1].

In Table IV is shown the ultraviolet spectrophotometric data on the 40 phenothiazine sulfoxides investigated together with the R_F values and those of the parent compound.

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SUMMARY

The difficulty of obtaining the sulfoxides of the numerous phenothiazine derivatives now in use in medicine has limited their value in forensic chemistry as a satisfactory means of identifying these important drugs. In this paper is described a relatively simple and efficient method for oxidizing the phenothiazines to their respective sulfoxides in pure state. It also describes how the sulfoxides may be obtained quickly and satisfactory by elution from thin-layer chromatography plates so that the necessary chemical, spectrophotometric and chromatographic tests may be carried out. Extensive data on 40 phenothiazine derivatives and their sulfoxides studied to date are presented.

REFERENCES

1 G. H. W. LUCAS AND C. FABIERKIEWICZ, *J. Forensic Sci.*, 8, No. 3 (1963) 462.
2 C. KORCZAK-FABIERKIEWICZ, J. KOFOED AND G. H. W. LUCAS, *J. Forensic Sci.*, 10, No. 3 (1965) 308.
3 A. C. SCHMALZ AND A. BURGER, *J. Am. Chem. Soc.*, 76 (1954) 5456.