Electronik recorder of 1-mv. sensitivity. Silicone Dow 710 and diethyleneglycol succinate, both adsorbed on firebrick, were the most suitable stationary phases; the other experimental conditions are listed under Fig. 1.

Inspection of Fig. 1 reveals that the oil may be separated into a total of 19 fractions, eight representing minor and 11 major components. Due to the lack of authentic blank substances, all of these ingredients have yet to be positively identified, but work is in progress. Carvone produced the most prominent peak (No. 18) which was readily identified by use of added reference standard.

Comparison of retention volumes and co-chromatography with added pure reference standard under identical experimental conditions were employed in the identification of three compounds in the oil, the presence of which had previously been disputed. Authenticated reference substances, pure and in 2% mixtures in natural oil, were gas chromatographed individually and in various combinations. The results are presented in Fig. 2.

The addition of 2% linalool to the oil produced an increase in the size of peak 12 at a retention time of 4.3 minutes, which is identical to that of pure linalool under the stated conditions. Added cineole similarly effected an increase in peak 11, which appeared only as a "shoulder" on peak 10 in the natural oil, and clearly separated it from peak 10. The retention time of 3.7 minutes for peak 11 corresponds to that of pure cineole. Pinene, with a retention time of 2.2 minutes, caused a marked increase in peak 7.

Gas chromatography was found to be a highly versatile and valuable method for the complete analysis of complex naturally occurring volatile substances.

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Separation and Investigation of a Stable Solid Free Radical of Chlorpromazine

By F. H. MERKLE, C. A. DISCHER, and A. FELMEISTER*

A solid stable free radical of chlorpromazine was prepared and characterized. The free radical nature of this compound was verified by electron spin resonance and ultraviolet spectral studies. The molecular weight and melting point of the free radical were determined.

ALTHOUGH the pharmacological importance of chlorpromazine is well established, its metabolic fate is not fully known (1). While studies of the oxidation of chlorpromazine have been reported (2, 3) and the existence of a free radical intermediate proposed (4, 5), little is known regarding the nature of this radical in the solid state (6, 7). The preparation and characterization of this free radical intermediate was undertaken as a preliminary step to the study of its role in the electrochemical and photooxidation mechanisms of chlorpromazine.

Preparation of Free Radical Intermediate

A procedure based on that outlined by Billon (8) for the preparation of a phenazothionium salt of phenothiazine was used in the preparation and separation of solid free radical. Approximately 0.5 Gm. of chlorpromazine 5-oxide hydrochloride[1] was dissolved in 10 ml. of 70% perchloric acid. The resulting dark red solution was agitated for 10 to 15 minutes and diluted first with an equal volume of acetone and then ether. Upon cooling to about −5° a strongly red-purple, fine crystalline solid separated. This solid material will be referred to in future discussions as Compound R. The solid softened over the range of 185–195°, before melting.

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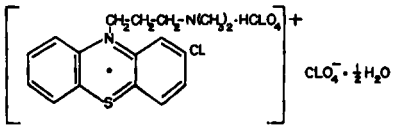
[1] Chlorpromazine hydrochloride and chlorpromazine 5-oxide were supplied by Smith Kline and French Laboratories, Philadelphia, Pa.

Characterization of Compound R

Schieser and Tuck (9) prepared a semiquinone free radical of chlorpromazine by dissolving chlorpromazine in concentrated sulfuric acid; electron spin resonance studies were used to confirm the presence of the free radical in the reaction mixture. Visible and ultraviolet spectra of the radical, prepared by the method of these workers, were obtained on a Beckman DK-2 ratio-recording spectrophotometer. These spectra were identical to those of Compound R, Fig. 1.

Electron spin resonance spectra indicate that Compound R is strongly paramagnetic.[2]

Elemental analysis of the free radical, Compound R, indicates that it probably exists as the hemihydrate of the diperchlorate salt. Structure I is



I

proposed. Calculated theoretical percentages of the elements in the proposed structure, based on a molecular weight of 527.5, are in good agreement with the analytical results, Table I.

[2] Electron spin resonance measurements were performed by Mrs. N. Steinberger, Columbia University, Department of Chemistry. The authors wish to thank Dr. G. Fraenkel and his staff for the use of their laboratory facilities in making these measurements.

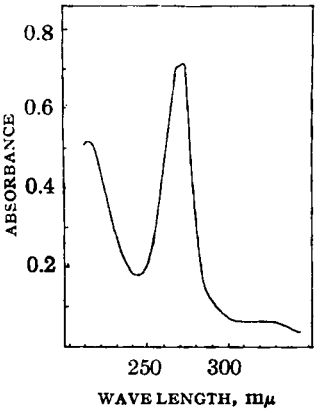


Fig. 1.—Ultraviolet spectrum of Compound R in 1 *N* sulfuric acid, immediately after solution.

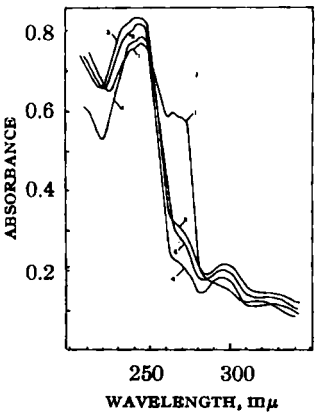


Fig. 2.—Ultraviolet spectra of an aqueous solution of Compound R. Key: Curve 1, immediately after solution; Curve 2, after 1 minute; Curve 3, after 5 minutes; Curve 4, spectrum of synthetic equimolar mixture of chlorpromazine hydrochloride and its 5-oxide.

TABLE I.—CALCULATED THEORETICAL PERCENTAGES OF THE ELEMENTS IN THE PROPOSED STRUCTURE COMPARED TO THE ANALYTICAL PERCENTAGES

Element	Per Cent Calcd.	Per Cent Found
Carbon	38.67	38.11
Hydrogen	3.98	3.77
Nitrogen	5.31	5.64
Chlorine	20.19	21.48
Sulfur	6.07	6.59
Oxygen	25.78	25.30
	100.00	100.89

The molecular weight was confirmed using a spectrophotometric technique. Solutions of chlorpromazine hydrochloride (approximately 10^{-5} *M*) were prepared in concentrated sulfuric acid. Under these conditions the chlorpromazine is converted quantitatively to the free radical, as verified by ultraviolet spectral studies. These solutions obey Beer's law at 277 mμ, with a molar absorbancy index of 4.15×10^4. Sulfuric acid solutions (9 *N*) of Compound R were then prepared. The ultraviolet spectra of these solutions of Compound R were identical to those of the free radical produced in concentrated sulfuric acid. Assuming that the molar absorbancy index of Compound R in 9 *N* acid does not differ appreciably from the molar absorbance index of the free radical produced by reaction of chlorpromazine with concentrated sulfuric acid, its molecular weight may be calculated from absorbancy measurements at 277 mμ. Three such determinations gave calculated molecular weights of 530, 519, and 547, respectively, with an average value of 532.

Reactivity of Compound R

Ultraviolet spectral studies indicate that aqueous solutions of Compound R disproportionate rapidly giving rise to equimolar mixtures of chlorpromazine and its 5-oxide. This was verified by comparison with the spectrum of a synthetic mixture of chlorpromazine hydrochloride and its 5-oxide, Fig. 2.

TABLE II.—COULOMETRICALLY DETERMINED *n* VALUES FOR THE CONTROLLED-POTENTIAL ELECTROLYSIS OF COMPOUND R

Number of Moles Taken Based on M. W. of 527.5	*n* Value Obtained
Oxidation 9.478×10^{-5}	0.98
Reduction 9.478×10^{-5}	0.94

This spontaneous disproportionation may be expressed as

2 free radicals (deep red) → 1 chlorpromazine (colorless) + 1 chlorpromazine 5-oxide (colorless) (Eq. 1)

Further evidence of the free radical nature of Compound R was obtained by controlled-potential electrolysis.[8] Samples of the free radical were oxidized and reduced electrolytically in 9 *N* sulfuric acid. The coulometrically determined *n* values (the number of equivalents per mole) are reported in Table II.

SUMMARY

On the basis of these studies it was concluded that Compound R is a free radical of chlorpromazine. The role of the free radical as an intermediate in the electrochemical and photo-oxidation of chlorpromazine will be reported shortly.

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[8] An electronic controlled-potential coulometric titrator, Model Q-2005 ORNL, was used to perform the electrolyses. Readout voltages were measured with a Non-Linear Systems 484 A digital voltmeter.