METABOLISM OF CHLORPROMAZINE.

V. CONFIRMATION OF POSITION 7 AS THE MAJOR SITE OF HYDROXYLATION*

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Chlorpromazine and related phenothiazine tranquilizers are metabolized to form numerous products whose structures, excretion patterns and possible pharmacological activity have attracted widespread interest (for reviews, see Carr, 1962; Domino, 1962; Emmerson and Miya, 1963). It has been shown that the phenothiazine nucleus of chlorpromazine is subject to sulfoxidation and hydroxylation, while the N-dimethylaminopropyl side chain can be either partially or completely demethylated, or converted to the N-oxide. The hydroxylated compounds, referred to as "phenols", are excreted in three identifiable forms, i.e., conjugated to glucuronic or sulfuric acid, or unconjugated. The situation is further complicated by possible hydroxylation of the nucleus at a number of isomeric positions, and by dihydroxylation. Since these alterations can occur singly or in combination, innumerable metabolites are theoretically possible. Actual practice reveals that animals (dogs, rabbits and probably rats) have fairly simple excretion patterns, in contrast to humans who excrete a more complex array of metabolites (Goldenberg and Fishman, 1961; Goldenberg **et al.**, 1963).

In the preceding paper of this series (Fishman and Goldenberg, 1963) chlorpromazine was shown to undergo 7-hydroxylation (Fig. 1). Analogous promazine metabolites were also isolated and later identified unequivocally as 3-hydroxypromazine† and its desmethyl and sulfoxide derivatives (Goldenberg **et al.**, 1963). The 3-HO-P assignment‡ is compatible with independent observations made by Posner **et al.** (1963). However, these authors came to a different conclusion concerning chlorpromazine phenol formation, placing the hydroxyl group at position 8. Subsequently, Posner **et al.** (1963a) modified their stand, noting that their findings could be interpreted to support hydroxylation at either position 8 or 7, or possibly 3. We have examined these data and wish to reaffirm our original placement of the hydroxyl group at the 7-position.

The conflicting evidence reported by Posner and associates was obtained when the metabolites in question (designated "CPOH" and other "chloroform-soluble chlorpromazine metabolites" released by β-glucuronidase) were tested on paper chromatograms with color reagents in an effort to locate the hydroxyl group. 1-, 2-, 3- and 4-HO-P were used as reference standards, due to the unavailability

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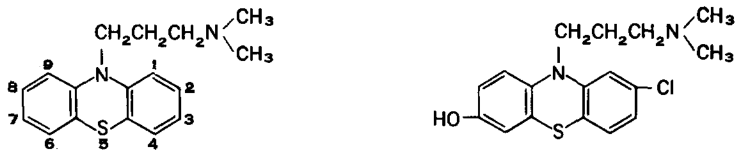
†Due to the symmetry of promazine (Fig. 1), the following isomers are equivalent: 3-HO-P and 7-HO-P, 2-HO-P and 8-HO-P, 1-HO-P and 9-HO-P, and 4-HO-P and 6-HO-P.

‡The following abbreviations are used: P = promazine, CP = chlorpromazine, CPSO = chlorpromazine sulfoxide, nor_1 = desmonomethyl, nor_2 = desdimethyl, TLC = thin-layer chromatography, GLC = gas-liquid chromatography.



Promazine **7-Hydroxychlorpromazine**

Fig. 1. Structures of promazine and 7-hydroxychlorpromazine (7-HO-CP).

of isomers in the chlorpromazine series. When tested with sodium metaperiodate, only 3-HO-P matched the metabolites in color (purple). This indicated substitution at the 3- or 7-position. Using Gibbs' reagent (2,6-dichloroquinone chloroimide), blue colors were reported for 1-HO-P and 4-HO-P, green for 2-HO-P, and purple for 3-HO-P. The metabolites yielded a green color, which suggested hydroxylation at the 8-position (chlorine atom is present at position 2). On repeating Gibbs' test in our laboratory we observed a subtle difference from the results obtained by Posner. Although CPOH—referred to in Table I and in our preceding paper as L3— gave a green color, 2-HO-P was colored green **with a decided grey cast.** The question therefore arose whether the rough agreement in color was fortuitous, and further, whether the absence of the chlorine atom in the promazine standards could be ignored when using tests based on intermolecular coupling reactions. For purposes of comparison, another coupling reaction was run using diazotized p-nitroaniline. As seen in Table I (diazo reagent), **the metabolite produced a color unlike any of the promazine derivatives.** It is clear from these results that the use of promazine isomers as color standards for verifying chlorpromazine substitution products is suspect, unless proven otherwise.

Table I

COMPARATIVE COLOR REACTIONS OF PROMAZINE AND CHLORPROMAZINE PHENOLS

Reagent	1-HO-P	2-HO-P	3-HO-P	4-HO-P	7-HO-CP	L3
Gibbs'	grey-blue	grey-green	grey-purple	deep blue	green	green
Diazo	grey	faint green	purple	orange	orchid	orchid
Persulfate	tan	orchid	lavender	tan	lavender	lavender
25% H_2SO_4	tan^{435}	orchid^{548}	lavender^{564}	tan^{489}	lavender^{562}	lavender^{562}

See Bray and Thorpe (1954) for preparation of Gibbs' reagent, Fishman and Goldenberg (1962) for diazo stain, and Smith (1958) for persulfate reagent. Superscripts on bottom line of table represent absorption maxima (mμ) of colored sulfuric acid solutions.

This conclusion was confirmed when authentic 7-HO-CP recently became available. The 7-HO-CP did not react like 3-HO-P (equivalent to 7-HO-P) to give a purple or grey-purple color with Gibbs' reagent, but instead gave a **green** color identical to L3 (Table I). Furthermore, the 7-HO-CP was indistinguishable from L3 by the diazo, persulfate and sulfuric acid tests. This provides substantial evidence for fixing the hydroxyl group in L3 at the 7-position. Its alternative placement at position 3 was discounted earlier when the O-methyl ether of L3 was shown to agree in persulfate color reaction and chromatographic properties with 7-MeO-CP, and not with 3-MeO-CP (Fishman and Goldenberg, 1963).[§]

The availability of authentic 7-HO-CP made possible a direct chromatographic comparison of this compound with L3. Analytical methods were sought which would discriminate between the isomeric hydroxypromazines. Posner **et al.** (1963a) reported being unable to resolve 2-, 3- and 4-HO-P, but we found this was readily accomplished by paper, thin-layer and gas chromatography. All systems listed in Table II effectively separated 2-HO-P and 3-HO-P as well as 1-HO-P and 3-HO-P. The 3- and 4-isomers, which were refractive to one-dimensional TLC, were easily separated on paper (system EdBzF) and by GLC. Despite their demonstrated resolving power, **none of these chromatographic systems could detect any difference in mobility between L3 and 7-HO-CP.** These data provide strong support for our earlier contention that L3 is 7-HO-CP. Further corroboration was presented in our previous paper in the form of spot tests, chemical reactions and chromatographic behavior of derivatives of L3.

Table II

CHROMATOGRAPHIC PROPERTIES OF PROMAZINE AND CHLORPROMAZINE PHENOLS

Chromatographic system[*]	R_f or retention time					
	1-HO-P	2-HO-P	3-HO-P	4-HO-P	7-HO-CP	L3
Thin-layer						
A	0.67	0.27	0.11	0.15	0.12	0.12
B	0.69	0.62	0.25	0.30	0.24	0.24
C	0.76	0.63	0.52	0.56	0.57	0.57
D	0.26	0.25	0.38	0.41	0.52	0.52
E	0.76	0.72	0.61	0.57	0.62	0.62
Paper						
BzA	0.63	0.41	0.12	0.13	0.22	0.22
EdBzF[†]	—	—	(6.0)	(11.0)	(9.0)	(9.0)
Gas[‡]						
2% XE-60	6.8	17.8	13.3	6.0	22.7	22.7
4% SE-30	10.3	16.8	11.0	7.8	17.6	17.6

[*] **A** = chloroform-acetone-diethylamine (8.8:0.2:1). **B** = acetone-diethylamine (9:1). **C** = ethanol-dioxane-benzene-58% ammonia (5:40:50:5). **D** = acetone-isopropanol-1% ammonia (9:7:4). **E** = ethyl acetate-methanol-diethylamine (14:4:3). **BzA** = benzene-acetic acid-water (2:2:1), descending. **EdBzF** = ethylene dichloride-benzene-formic acid-water (3:1:4:2), descending.

[†] Solvent permitted to pass off paper, carrying 1-HO-P and 2-HO-P with it. Running time, 48 hrs. Figures in parentheses represent cm. of travel.

[‡] Retention time in minutes. Phenols injected as 0.5% solutions in tetrahydrofuran (2 microliters). Support, Gas-Chrom P, acid-washed and silanized, 100/120 mesh. Column, U-shaped glass tube, 6 ft. x 4 mm., 224°; inlet pressure, 18 p.s.i.; flash heater, 275°; detector, 250°. Model 15 Barber-Colman gas chromatograph used, equipped with argon ionization detector.

[§] The 3-MeO-CP standard ran ahead of O-methyl L3 (and 7-MeO-CP) in two critical solvent systems which were included in Table II of the indicated reference, viz., 20% KCl and AmMe 1:1. 3-MeO-CP and O-methyl L3 (7-MeO-CP) were also separable by TLC using cyclohexane-diethylamine (9:1).

The phenolic derivatives of chlorpromazine are excreted largely as glucuronides, from which they may be recovered by enzymic hydrolysis and then resolved. A typical silica gel chromatoplate is given in Fig. 2. There are indications that eight or more metabolites possess the 7-hydroxyl configuration. These compounds yield a lavender or periwinkle color with persulfate reagent, depending on whether they are sulfides or sulfoxides. The principal members of the lavender series are 7-HO-CP, 7-HO-Nor$_1$CP, 7-HO-Nor$_2$CP, L4 and L5. L4 and L5 have been tentatively identified and will be discussed in a later paper. Pwk 3, Pwk 2 and Pwk 1 are sulfoxides and minor components. They correspond to 7-HO-CPSO, 7-HO-Nor$_1$CPSO and 7-HO-Nor$_2$CPSO, respectively. The blue (stippled) spots may represent dihydroxylated structures, according to Posner. It is not unlikely that some of the remaining trace metabolites contain the hydroxyl group at a position other than 7. This is under current investigation.

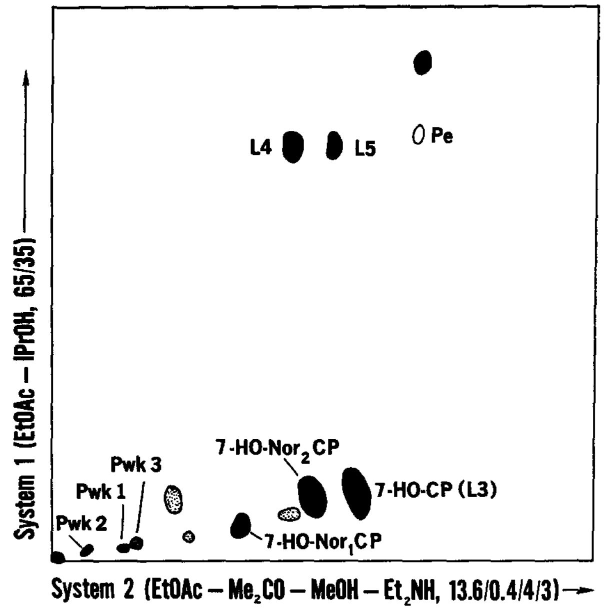


Fig. 2. Thin-layer chromatogram of chlorpromazine aglycones, obtained from human urine. Pe = unidentified peach-colored metabolite. See Fishman and Goldenberg (1963) for isolation procedure.

REFERENCES

Bray, H. G., and Thorpe, W. V., in Methods of Biochemical Analysis (ed. by D. Glick), **1**, 873 (1954). Interscience Publishers, Inc., New York.

Carr, C. J., Ann. N. Y. Acad. Sci., **96**, 170 (1962).

Domino, E. F., Clin. Pharmacol. Therap., **3**, 599 (1962).

Emmerson, J. L., and Miya, T. S., J. Pharm. Sci., **52**, 411 (1963).

Fishman, V., and Goldenberg, H., Proc. Soc. Exptl. Biol. Med., **110**, 187 (1962).

Fishman, V., and Goldenberg H., Proc. Soc. Exptl. Biol. Med., **112**, 501 (1963).

Goldenberg, H., and Fishman, V., Proc. Soc. Exptl. Biol. Med., **108**, 178 (1961).

Goldenberg, H., Fishman, V., Heaton, H., and Burnett, R., submitted for publication.

Posner, H. S., Culpan, R., and Levine, J., Federation Proc., **22**, 540 (1963).

Posner, H. S., Culpan, R., and Levine, J., J. Pharmacol. Exptl. Therap., **141**, 377 (1963a).

Smith, I., Chromatographic Techniques, 1958, p. 125, Interscience Publishers, Inc., New York.