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Ultraviolet Spectrophotometric Determination of Chlorprothixene in Biologic Specimens

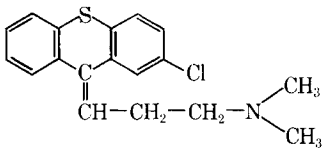
By JACK E. WALLACE

A rapid spectrophotometric method for determining chlorprothixene permits analysis of the drug in biologic specimens in the presence of its metabolites and other alkaline drugs without preliminary separation. The procedure is based upon the oxidation of chlorprothixene and its immediate metabolites to a mixture of reaction products by means of alkaline permanganate. In hexane the products have a characteristic ultraviolet absorption curve with a well-defined maximum at 233 mμ. The strong absorption at this wavelength is utilized as a basis for the analytical procedure. The method is sufficiently sensitive for the determination of chlorprothixene in urine 3 days after the ingestion of a single 50-mg. dose. Observations concerning the distribution of chlorprothixene in the rat and the excretion of the drug in man are presented.

CHLORPROTHIXENE,[1] *trans* isomer of 2-chloro-9-(13-dimethylaminopropylidene) thioxanthene, is a potent tranquilizing agent not only in acute and chronic schizophrenia but in severe psychoneuotic conditions. The chemical structure of chlorprothixene resembles that of the phenothiazines. Few methods for the determination of the drug are available in the scientific literature.

Ferrari and Toth (1) described a thin-layer chromatographic technique which identifies urinary metabolites of chlorpromazine, chlorprothixene, imipramine, and amitriptyline. Although the method is specific, it is time consuming and is not quantitative. Fluorometric procedures for determining chlorprothixene in blood and urine (2) are very sensitive, detecting 0.25-mcg. amounts of the drug, but they require the utilization of specialized analytical instruments.

Chlorprothixene strongly absorbs ultraviolet radiations, therefore, it can be determined spectrophotometrically in biologic extracts, but its strongest absorption is at 227 mμ. At that wavelength the spectral curve of the drug is often affected by background absorption and sulfoxide metabolites which induce a wide degree of variance in the ultraviolet absorbance curve, unless extensive purification of the drug extract is achieved. The method reported here is a specific spectrometric assay which requires no extensive purification procedure or no separation of unchanged drug and sulfoxides (3). The method permits a reliable evaluation of drug intake by patients treated with chlorprothixene.



Chlorprothixene

EXPERIMENTAL

Instrumentation—A Beckman DK-2A ratio recording spectrophotometer with linear presentation of the wavelength was used for the ultraviolet absorption measurements. The sample path was 10 mm. throughout. A Beckman IR-4 doublebeam infrared spectrophotometer was used for infrared spectral characterization of functional groups in the reaction products. Gas chromatographic analyses were performed by means of a Barber-Colman model 5000 gas chromatograph utilizing a 6-ft. U-shaped glass column containing 2% Carbowax 20 M on Gas-Chrom Q, 100–120 mesh. An F & M model

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[1] Marketed as Taractan by Hoffmann-LaRoche, Inc., Nutley, N. J.

185 carbon-hydrogen-nitrogen analyzer was used for elemental analysis of the chlorprothixene reaction products.

Method—Ten milliliters of whole oxalated blood, serum, or gastric contents are adjusted to pH 7.0–7.5 by the addition of a dilute aqueous solution of sodium hyroxide. Ten to 50 ml. of urine or 10 Gm. of homogenized tissue specimens are adjusted to a pH of 11.0 to 12.0 with 5 *N* sodium hydroxide. The resulting solution or homogenate is shaken vigorously for 3 min. with 200 ml. of *n*-hexane (spectroanalyzed) in a 500-ml. separator. The hexane layer is placed in a graduated cylinder by filtration through Whatman No. 541 filter paper. The volume of hexane recovered is recorded, and the lost hexane is considered in the final calculations. Five milliliters of 0.5 *N* HCl is added to the filtered hexane, and this mixture is shaken for 3 min. Four milliliters of the aqueous layer is placed in a 500-ml. round-bottom flask to which are added 20 ml. of 1% $KMnO_4$ buffered at pH 12.4 with 0.4 *M* sodium triphosphate–0.4 *M* sodium hydroxide buffer, 50 ml. of *n*-hexane (spectrophotometric grade), and a Teflon-coated magnetic stirring bar. The contents of the flask are refluxed for 30 min. with constant magnetic stirring. After cooling, the *n*-hexane is separated from the aqueous permanganate layer, washed with 5 ml. of 0.5 *N* HCl, and read in the spectrophotometer at 5 mμ intervals from 220 to 340 mμ against a blank of *n*-hexane. At least three maxima should be observed. If absorption at a single wavelength is desired, it may be determined at 233 mμ. Should the *n*-hexane contain less than 2.5 mcg./ml. of chlorprothixene as the reaction product, the following concentration technique should be performed. The recovered *n*-hexane, whose total volume is recorded, is placed in a 125-ml. flask which is then attached to a rotary vacuum evaporator. The solvent is evaporated by vacuum while the flask, partially submerged, rotates in a 50° water bath. Depending on the absorbance previously observed, the residue is dissolved in 2, 4, or 8 ml. of *n*-hexane. A standard curve is prepared from aqueous solutions of the drug which have been carried through the procedure described, including the concentration technique for solutions containing small amounts of chlorprothixene.

RESULTS AND DISCUSSION

At 233 mμ a linear relationship exists between the absorbance of the reaction products and the concentration of the drug in the original sample. This relationship is demonstrated in Table I. The ultraviolet absorption spectra of chlorprothixene

TABLE I—STANDARD CURVE DATA FOR CHLORPROTHIXENE REACTION PRODUCT

Chloroprothixene in Sample, mcg./ml.	Absorbance of Reaction Product in Hexane[a]	Absorbance/ Concn.
10.0	0.84	0.084
8.0	0.67	0.084
6.0	0.50	0.083
4.0	0.33	0.083
2.0	0.16	0.080

[a] Read at 233 mμ.

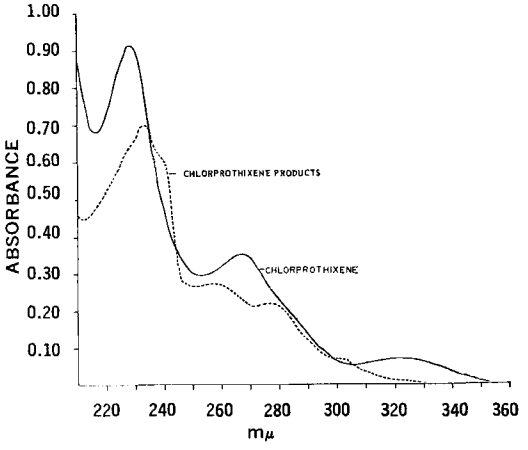

Fig. 1—Ultraviolet absorption spectra of chlorprothixene, 10 mcg./ml. in water, and of the chlorprothixene reaction products corresponding to a sample of equivalent concentration in hexane.

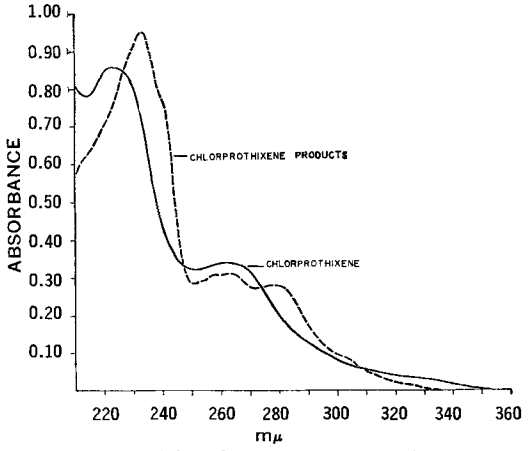

Fig. 2—Ultraviolet absorption spectra of chloroprothixene in water and of the chlorprothixene reaction products in hexane both obtained with chlorprothixene and its metabolites extracted from liver of rats.

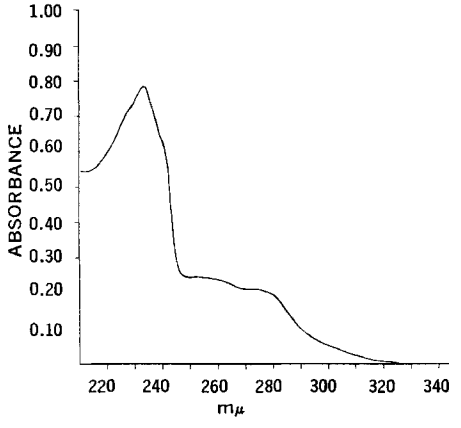

Fig. 3—Ultraviolet absorption spectrum of chlorprothixene reaction products obtained with chlorprothixene and its metabolites extracted from urine of humans.

and of its oxidation products are shown in Fig. 1. The absorption curve of the products is well defined, with absorbance maxima in hexane or ethanol at 278, 258, and 233 mμ and a shoulder at 302 mμ. The reaction products of chlorprothixene extracted from human urine and from the tissues of animals which have received the drug yield almost identical absorption curves to that observed for the reaction products of pure drug (Figs. 2 and 3). Direct analysis of chlorprothixene and its metabolic products from urine and animal tissues does not afford an ultraviolet absorption pattern from which accurate qualitative and quantitative data can be obtained (Fig. 2). The infrared spectrum of the reaction products of pure chlorprothixene is similar to the spectrum obtained from the reaction products of chlorprothixene extracted from human urine. Infrared spectra of chlorprothixene (Fig. 4) and of its reaction products (Fig. 5) show several distinct differences, of which the most significant is the appearance of a band at 1682 cm.$^{-1}$ in the spectrum of the reaction products. This band is thought to be produced by a carbonyl group (4) formed by mild permanganate oxidation of the olefinic carbon-to-carbon bond in the parent compound. Very strong absorption at 1150 and 1305 cm.$^{-1}$ in the spectrum of the chlorprothixene products suggests that a majority of the sulfur is oxidized to a sulfone group. Schrieber (5) examined the spectra of 13 sulfones and found two bands in the sulfones which were absent in the corresponding sulfides. These were at 1120–1160 cm.$^{-1}$ and at 1300–1350 cm.$^{-1}$. It seems likely that some sulfoxide is also present in the reaction products, since a strong band is obtained at 1020–1060 cm.$^{-1}$ (Fig. 5). Most normal sulfoxides will be found to give a strong band in the range 1020–1060 cm.$^{-1}$ in the solid state. The weak absorption

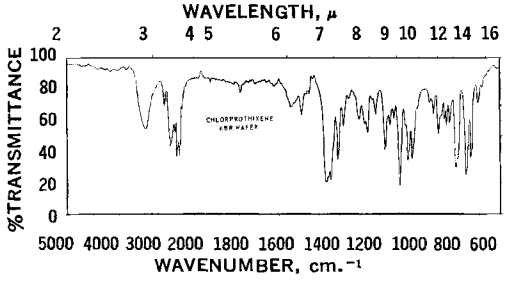

Fig. 4—Infrared absorption spectrum of chlorprothixene, 2 mg./400 mg. of potassium bromide.

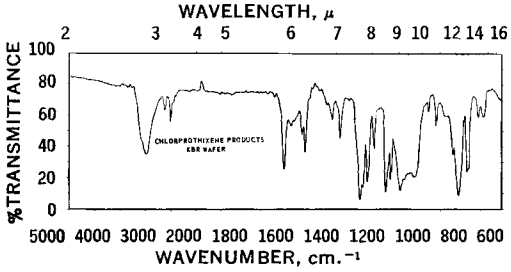

Fig. 5—Infrared absorption spectrum of chlorprothixene reaction products, 2 mg./400 mg. of potassium bromide.

TABLE II—COMPOUNDS INVESTIGATED FOR INTERFERENCE WITH THE DETERMINATION OF CHLORPROTHIXENE[a]

Compd.	Absorbance of Hexane Extract[b]
Chlorprothixene	1.61
Blank	0.02
Amitriptyline (250 mμ)	1.35
Amphetamine	0.02
Antazoline	0.06
Azacyclonol (247 mμ)	0.52
Caffeine	0.02
Captodiamine (247 mμ)	1.41
Carbinoxamine (270 mμ)	0.87
Chlordiazepoxide	0.05
Chlorothen	0.02
Chlorpromazine	0.02
Cyclizine (247 mμ)	1.24
Diazepam	0.18
Diphenhydramine (247 mμ)	1.34
Doxylamine	0.02
Ephedrine	0.02
Hydroxyzine (254 mμ)	0.85
Imipramine	0.02
Lidocaine	0.02
Methoxamine	0.02
Methylphenidate	0.02
Morphine	0.02
Nortriptyline (250 mμ)	1.08
Phenaglycodol	0.04
Phenindamine	0.02
Pheniramine	0.10
Phenmetrazine	0.02
Pilocarpine	0.02
Pipradrol (247 mμ)	0.85
Propoxyphene	0.09
Pyrilamine	0.05
Pyrrobutamine	0.20
Reserpine	0.02
Thenyldiamine	0.02
Tricyclamol	0.02
Triprolidine	0.15

[a] The absorbance in each case corresponds to a concentration of 20 mcg. of the compound per ml. of sample. [b] Read at 233 mμ unless otherwise indicated.

by the products in the 2800–3000 cm.$^{-1}$ band area to which carbon-hydrogen stretching vibrations have characteristic assignment (6) implies that the dimethylaminopropylidene group is removed during the oxidation process. Hydrogen, carbon, and nitrogen analysis of two fractions obtained by fractional crystallization of a saturated solution of the products in hexane supports the infrared data. One fraction appears to contain a carbonyl–sulfoxide product, the other a carbonyl–sulfone product.

Many organic bases were investigated for possible interference with quantitative determination of chlorprothixene. Several produced a reaction product(s) which gave considerable absorption at 233 mμ (Table II). However, none showed maximum absorption at 233 mμ, nor did any exhibit the absorption pattern characteristic of the chlorprothixene reaction products (Fig. 1). The reaction products of compounds listed in Table II which absorb maximally at 247 mμ, except that from captodiamine, are all benzophenone, and they can be identified as such by gas chromatography (7). The chlorprothixene reaction products chromatographed under the same conditions exhibit no chromatographic peak (Table III).

TABLE III—RELATIVE RETENTION TIMES OF REACTION PRODUCTS BY GAS CHROMATOGRAPHY[a]

Compd.	Max. Ultraviolet Absorption,[b] mμ	Reaction Ratio Reaction Product/Benzophenone Peak 1	Peak 2
Chlorprothixene	233	...[c]	...
Amitriptyline	250	4.5	5.5
Azacyclonol	247	1.0	...
Captodiamine	247	...	...
Carbinoxamine	270	3.0	...
Cyclizine	247	1.0	...
Diphenhydramine	247	1.0	...
Hydroxyzine	254	2.0	...
Nortriptyline	250	4.5	5.5
Pipradrol	247	1.0	...

[a] Chromatographic conditions: 6-ft. glass column packed with 100–120 mesh Gas-Chrom Q (Applied Science Laboratories) coated with 2% by weight Carbowax 20M (AnaLabs, Inc.). Column temperature 200°; injector temperature, 240°. Flow rate 50 ml. nitrogen/min. [b] In hexane. [c] No peak observed.

Recoveries of known amounts of chlorprothixene added to whole blood and urine are summarized in Table IV. Standard aqueous solutions of chlorprothixene hydrochloride were added to the biologic material to provide concentrations ranging from 0.50 to 10.0 mg. %. Ten milliliters of blood or 20 ml. of urine with added drug was analyzed. Recoveries of greater than 90% were obtained in each instance. It was observed very early in this work that the pH range 7.0–7.5 used in preliminary recovery studies was inadequate for maximum extraction of chlorprothixene and its metabolites from the urine of individuals who had taken the drug. Table V presents data from a healthy male who had taken a single 50-mg. oral dose, and whose urine was collected during the period 24–48 hr. after the dose. Samples of equivalent amounts were assayed for chlorprothixene, varying the pH for the initial extraction of the drug from 7.0 to greater than 12.5. Optimum recovery was obtained at pH 10.0 or greater.

TABLE IV—RECOVERY OF CHLORPROTHIXENE AFTER *In Vitro* ADDITION TO BLOOD AND URINE

Amt. Added, mcg./ml.	No. of Detn.	Recovery, Mean ± Std. (mcg./ml.) Whole Blood[a]	Urine[b]
50.0	10	47.1 ± 1.5	46.9 ± 1.7
25.0	12	23.7 ± 0.3	24.0 ± 0.5
10.0	11	9.2 ± 0.2	9.0 ± 0.3

[a] Extracted at pH 7.0–7.5. [b] Extracted at pH 11.0–12.0.

TABLE V—EFFECT OF pH ON EFFICIENCY OF EXTRACTION OF CHLORPROTHIXENE FROM HUMAN URINE[a]

pH of Extraction	Concn. Determined, mcg./ml.
7.0	0.29
8.0	0.53
9.0	0.75
10.0	0.92
11.0	0.96
12.0	0.93
12.5–13.0	0.94

[a] Urine obtained during the period 24–48 hr. after an oral dose of 50 mg.

To determine the optimum pH of extraction for biologic specimens other than urine, white albino rats were fed *via* stomach tube 70 mg. of chlorprothixene per kilogram of body weight. They were killed after 3, 4.5, 6, 12, and 24 hr. Tissues were assayed for chlorprothixene in duplicate, using a pH of either 7.0–7.5 or of 11.0–12.0 for the initial extraction (Table VI). In all tissues much more drug was recovered when the specimens were initially extracted at pH 11.0–12.0. Since chlorprothixene itself is efficiently recovered from biologic materials at pH 7.0–7.5 (Table IV) it seems reasonable to assume that certain metabolites in the tissues and urine necessitate the strongly alkaline extraction.

Table VII shows the distribution of the drug among various tissues of the rat 6 to 24 hr. after the animals had received the 70 mg./Kg. of body weight dose. The levels of drug and metabolites in various biologic fluids and organs in decreasing order are urine, liver, brain, fat, skeletal muscle, and blood. With the exception of the data for concentrations of the drug in muscle and fat tissues, the distribution ratios are in good agreement with those previously reported (8).

In three adult human males, each of whom received 50 mg. of chlorprothixene by mouth, the

TABLE VII—CHLORPROTHIXENE[a] DISTRIBUTION IN RAT

Tissue or Biologic Fluid[b]	Distribution Ratio[c] Concn. in Tissue or Fluid Concn. in Liver
Blood	0.02
Brain	0.37
Fat	0.23
Kidney	0.83
Liver	1.00
Muscle	0.16
Urine	2.50

[a] Chlorprothixene and metabolities. [b] Specimens obtained 6 to 24 hr. after rats had ingested 70 mg./Kg. of body weight. Blood initially extracted at pH 7.0–7.5. Remaining specimens initially extracted at pH 11.0–12.0. [c] Average of results from 16 rats.

TABLE VI—pH DEPENDENCE OF CHLORPROTHIXENE EXTRACTION[a]

Time, hr.	Extracted at pH	Chlorprothixene Recovered,[b] mcg./ml. or Gm. Blood	Brain	Fat	Kidney	Liver	Muscle
3	7.0–7.5	0.9	10.9	10.7	15.5	27.4	5.1
	11.0–12.0	...[c]	16.5	10.5	39.5	47.2	8.6
4.5	7.0–7.5	0.7	4.4	4.1	16.9	26.6	3.3
	11.0–12.0	...	12.5	10.0	30.7	37.1	4.9
6	7.0–7.5	0.8	6.4	6.3	16.3	16.0	3.3
	11.0–12.0	...	13.5	10.5	27.3	32.8	5.1

[a] Tissues from rats given chlorprothixene 70 mg./Kg. by stomach tube. [b] Pooled tissues from three rats. [c] Not determined.

TABLE VIII—CHLORPROTHIXENE RECOVERED FROM HUMAN URINE[a]

Time, hr.	Adult Male Subject I	II	III
	mcg./ml.		
4	0.14	0.55	3.33
8	1.46	3.76	7.59
12	1.48	4.45	3.99
24	4.19	2.32	2.26
48	2.21	0.88	1.14
72	1.19	0.20	0.35
Total recovered, mg.	3.6	3.4	3.6

[a] Each subject received 50 mg. chlorprothixene by mouth.

concentration of chlorprothixene measured in the urine over a 72-hr. period varied considerably from one subject to another (Table VIII). The total amount excreted in the urine by the three subjects during the 72 hr. was less variable: 3.4 to 3.6 mg.

Although the method for analysis which has been described does not distinguish between chlorprothixene and its sulfoxide metabolite, this lack of specificity is not likely to carry medical or legal importance. Sensitivity of the method is sufficient to permit the analyst to detect the drug and its metabolites in the urine 3 days after an individual has taken a single 50-mg. dose.

SUMMARY

A quantitative ultraviolet spectrophotometric procedure has been developed for the determination of chlorprothixene and its principal sulfoxide metabolite in biologic specimens. In the procedure, the drug is oxidized with buffered permanganate to a carbonyl derivative which has a well-defined ultraviolet absorption spectra. The method is sensitive and the combined ultraviolet spectrum of the oxidation products is sufficiently specific to establish qualitative identification of the drug. Results of a study to establish the excretion pattern of chlorprothixene and its metabolites in man, as well as their distribution in the tissues, and biologic fluids of the rat are presented.

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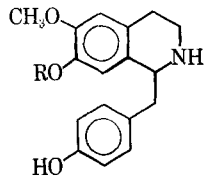
1-Benzyl-1,2,3,4-tetrahydroisoquinolines

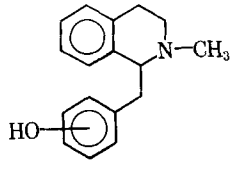
By JOSEPH SAM and A. J. BEJ*

The syntheses of 1-benzyl-1,2,3,4-tetrahydroisoquinolines, particularly phenolic derivatives, are described. Preliminary pharmacological data also are reported.

NUMEROUS CHEMICAL and biological investigations have been conducted with synthetic as well as naturally occurring 1-benzylisoquinolines (1). Limited biological information is available, however, on phenolic 1-benzyl-1,2,3,4-tetrahydroisoquinolines. Kupchan and co-workers (2) noted weak analgesic activity with (−)-*N*-normarmepavine (I*a*). Colacurine (I*b*) was tested for curare-like activity in dogs but manifested no activity (3). Both of these alkaloids are secondary amines. Often the biological activity of secondary amines are lower than the corresponding *N*-methyl or tertiary amines (4). Accordingly, a program was initiated to synthesize and evaluate phenolic 1-benzyl-2-methyl-1,2,3,4-tetrahydroisoquinolines (II) for biological activity.



I*a*, R = CH_3
b, R = H



II

DISCUSSION

The relationship of phenolic 1-benzyl-2-methyl-1,2,3,4-tetrahydroisoquinolines, both structurally (not sterically) and biogenetically (5), to morphine (III) gave additional impetus to the investigation. In this connection, Besendorf and co-workers (6) noted pronounced analgesic activity in 6,7-dimethoxy-1-(4-chlorophenethyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline (IV).

The preparation of 1-(4-hydroxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline (V) was investigated

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