Biochemical Pharmacology, Vol. 17, pp. 2125–2136. Pergamon Press. 1968. Printed in Great Britain

IDENTIFICATION OF DIPHENHYDRAMINE URINARY METABOLITES IN THE RHESUS MONKEY*

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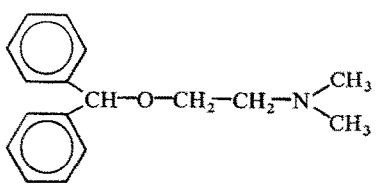
(*Received* 4 *December* 1967; *accepted* 16 *February* 1968)

Abstract—The products of diphenhydramine metabolism which were found in the urine of rhesus monkeys included unchanged diphenhydramine, the primary and secondary amine analogs of diphenhydramine, diphenhydramine-*N*-oxide, a glucuronide of unknown structure, (diphenylmethoxy)acetic acid and the glutamine conjugate of this acid. As a part of indentification criteria, the glutamine conjugate of (diphenylmethoxy)acetic acid was synthesized and characterized. The conjugate was the major excretion product and together with (diphenylmethoxy)acetic acid accounted for nearly two-thirds of the urinary metabolites. Identification of this conjugate provides the first direct evidence that the rhesus monkey forms glutamine conjugates with carboxylic acids other than arylacetic acids.

DIPHENYDRAMINE,† 2-(diphenylmethoxy)-*N*,*N*-dimethylethylamine (DPHM),‡ is a well known antihistamine drug which has been used clinically for many years. However, relatively little information is available on its metabolic fate.



Diphenhydramine

After oral administration of very high doses of this drug to human subjects (400 mg), McGavack *et al.*[1] found that an average of 46 per cent of the dose was excreted unchanged in the urine. Similar studies by Hald,[2] by Way *et al.*[3] and by Glazko and Dill[4] at lower dose levels (50–100 mg) indicated that a much smaller fraction of the dose was excreted as the unchanged drug. In addition, Glazko *et al.*[5] reported that the rat excreted not only unchanged DPHM but also several other metabolites which did not behave as organic bases.

* Portions of this work were previously reported at a meeting of the *Am. Soc. Pharmac. exp. Therap.* Chicago, Ill. April (1967).

† Registered under the trade name Benadryl (Parke, Davis & Co.).

‡ Abbreviations used in this work are: DPHM, diphenhydramine; DPMA, (diphenylmethoxy)-acetic acid; DPMA–GLN, N^2–[(diphenylmethoxy)acetyl]-L-glutamine; EDC, 1,2-dichloroethane; and THF, tetrahydrofuran.

Experiments *in vitro* by Glazko and Dill,[6] in which DPHM was incubated with rat, rabbit or guinea pig tissue homogenates, also showed that DPHM lost its basic properties as measured by the methyl orange technique. Later, by use of similar techniques, Kikkawa *et al.*[7] indicated that the compound was hydrolyzed to yield benzhydrol and 2-dimethylaminoethanol; however, these compounds were not found in the urine of animals injected with DPHM. More recently, Roozemond *et al.*[8] reported that incubation of DPHM with rat liver microsomes resulted in the formation of formaldehyde, indicating that *N*-demethylation probably had taken place.

In a study on the related compound orphenadrine (the *o*-tolyl analog of diphenhydramine), Hespe *et al.*[9] observed five metabolites in the urine of rats. The primary and secondary amine analogs of orphenadrine as well as 2-methylbenzhydrol were identified. Recently, in accord with the report of Titus *et al.* on 4-(3-diethylaminopropylamino)-7-chloroquinoline,[10] the work of Rodriguez and Johnson on chlorpromazine,[11] and our previous report on DPHM,[12] Hespe *et al.*[13] indicated that the demethylated side chain of orphenadrine undergoes further degradation *in vivo*.

The results of the present investigation demonstrate that an oxidative degradation of the side chain is the primary route of DPHM metabolism in the rhesus monkey leading to the formation of a carboxylic acid which is conjugated with glutamine.

EXPERIMENTAL

Reference compounds

The following compounds of established structure, which had been synthesized previously in the Parke, Davis Laboratories, were used as reference standards: 2-(diphenylmethoxy)-*N*,*N*-dimethylethylamine (DPHM, Lot No. 563,981); 2-(diphenylmethoxy)-*N*-methylethylamine (*N*-demethyl-DPHM, prepared by Dr. G. Rieveschl); 2-(diphenylmethoxy)ethylamine (*N*,*N*-didemethyl-DPHM, prepared by Dr. G. Rieveschl); 2-(diphenylmethoxy)-*N*,*N*-dimethylethylamine-*N*-oxide (DPHM-*N*-oxide, prepared by Dr. E. F. Elslager); and (diphenylmethoxy)acetic acid (prepared by Mrs. H. Beatty).

Labeled compound

Tritiated diphenhydramine hydrochloride with a sp. act. of 6·0 mc/m-mole was prepared in this laboratory as described by Blackburn and Ober.[14] The radioactivity was in the diphenylmethoxy moiety, as the compound was synthesized from acid-catalyzed, exchange-labeled benzhydrol. The compound appeared to be radiochemically stable in biological systems and was not affected by standing overnight at room temperature in predose monkey urine. For metabolic studies it was usually diluted with unlabeled compound to a sp. act. of 0·4 to 1·1 mc/m-mole. The six monkeys from which metabolites were isolated received DPHM having a sp. act. of 2×10^{-3} mc/m-mole.

Collection of urine samples

Rhesus monkeys (*Macaca mulatta*) were fasted for 16 hr before dosing. Urine collections were made in metabolism chairs designed so that urine and feces could be collected separately. Samples were collected at intervals never exceeding 12 hr and frozen.

Collections from multiple-dosed animals were made throughout the period of dosing and for 1 day thereafter. The urine from single-dosed animals was collected for 2 days after dosage.

Enzymic hydrolysis

Metabolite fractions were incubated for 1 hr at 37° in 0·20 M phosphate buffer (pH 6·9) with 400 units/ml of β-glucuronidase (β-D-glucuronide glucuronohydrolase, E.C. 3.2.1.31), bacterial type II (Sigma Chemical Co., St. Louis, Mo.). After incubation, reference compounds were added to the solution containing the radioactive hydrolysis products and the mixture was examined by thin-layer chromatography using solvent systems A and B (see legend to Table 1).

Chromatography

(1) *Thin-layer chromatography (TLC).* Thin-layer plates were coated with 0·25 mm of Silica gel GF_{254} (E. Merck AG., Darmstadt, Germany) and dried at room temperature. These were developed by the ascending technique with the solvent systems described in the legend to Table 1. Examination under u.v. light revealed the reference compounds which appeared as dark spots. The radioactive metabolites were detected by scraping 5-mm widths of the gel into counting vials and extracting with 1 or 2 ml methanol containing 10% 0·5 N HCl. Twelve ml of a solution of 110 g naphthalene, 8 g 2,5-diphenyloxazole and 0·1 g 1,4-*bis*-2-(5-phenyloxazolyl) benzene in 1 l. of *p*-dioxane was added and the vials were counted in a Packard liquid scintillation spectrometer. This procedure resulted in quantitative detection of DPHM and all metabolites originally spotted on the plates.

(2) *Gas-liquid chromatography (GLC).* Diphenhydramine and its metabolites were separated on a Barber–Coleman model 10 instrument with a 6 ft U-shaped column with a 3 mm i.d. For DPHM and the primary and secondary amine analogs, the column was packed with 80/100 mesh Gas-Chrom P containing 1% ECNSS-M (Applied Science Laboratories, Inc., State College, Pa.) and maintained at 143° with a nitrogen flow rate of 24 ml/min. The flash heater and detector cell were kept at 200°, while effluent materials were monitored by hydrogen-flame ionization. Although these conditions yielded good separation of the above compounds, DPHM-*N*-oxide decomposed extensively to give at least three additional compounds which were of some value in identification of the *N*-oxide.* Lowering the temperature of the flash heater to 140°, using 80/100 mesh Gas Chrom Q containing 5% XE-60 (Applied Science Labs.) as the column packing and eluting with 80 ml/min of air at 150° greatly reduced thermal decomposition. Under these conditions DPHM-*N*-oxide accounted for 85–90 per cent of the total mass detected. A decomposition product accounted for the remainder, chromatographing as DPHM.

3) *Ion-exchange chromatography (IEC).* Amino acid identification was made with a Beckman model 120C amino acid analyzer. One-tenth μmole of amino acid standards or unknown amino acid in a volume of 0·1 ml or less was introduced onto the top of a 0·9 × 54 cm bed of PA-28 resin (Beckman Instruments, Inc.). The column was eluted with 0·20 N citrate buffer (pH 3·18) at a flow rate of 50 ml/hr at 32·5°. At 100 min the

* The reference compound DPHM-*N*-oxide decomposed to yield compounds having retention times of 3·0, 4·2, 7·6 and 11·0 min. The metabolite decomposition products chromatographed with retention times of 2·8, 4·2, 7·5 and 11·0 min. The values are averages of 5 determinations.

temperature was increased to 55° and at 175 min the buffer was changed to 0·20 N citrate (pH 4·25). The amino acids were detected by reaction with ninhydrin.

RESULTS

Chromatographic identification of metabolites. Urine was obtained from both male and female monkeys which had received single, 10 mg/kg, i.v. doses of ^{3}H-DPHM·HCl. The metabolites were characterized initially by TCL of an aliquot of a 0–24 hr urine sample to which known reference compounds had been added. Results shown in Fig. 1 illustrate the presence of unchanged DPHM (VI), the primary (V) and secondary

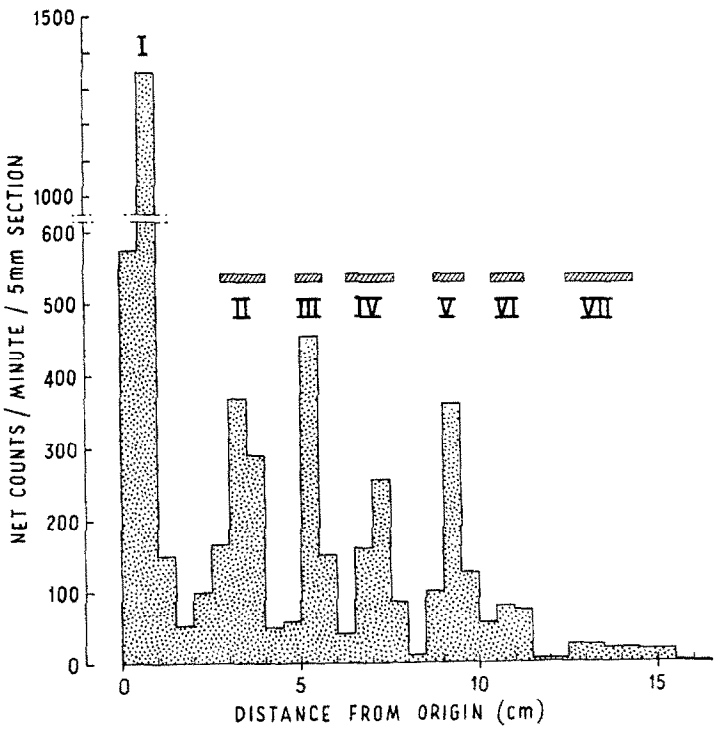


FIG. 1. TLC analysis of 0–24 hr monkey urine after i.v. dosage with ^{3}H-DPHM·HCl. The urine plus reference compounds was chromatographed in solvent system B. The radioactive metabolites, denoted by numbers, are indicated by the dotted histogram. Ultraviolet absorption areas of the reference compounds are represented by the crosshatched rectangles. In order of increasing R_f, the reference compounds are: DPHM-*N*-oxide (II), (diphenylmethoxy)acetic acid (III), *N*-demethyl-DPHM (IV), *N*,*N*-didemethyl-DPHM (V), DPHM (VI), and benzhydrol (VII). Metabolite I was later identified as the glutamine conjugate of (III).

(IV) amine analogs of DPHM, DPHM-*N*-oxide (II), DPMA (III), a metabolite of unknown structure (I), and a trace of benzhydrol (VII). In addition, it was found that in this solvent system a glucuronide of unknown structure chromatographed with the same R_f value as metabolite I. More thorough characterization was accomplished by TLC of urine plus reference compounds in four different solvent systems (Table 1). In all cases the radioactive areas were found in the same locations as the reference compounds.

TABLE 1. TLC OF MONKEY URINARY METABOLITES AND REFERENCE COMPOUNDS*

Compound	R_f			
	Solvent A	Solvent B	Solvent C	Solvent D
Metabolite I	0·58	0·11		
DPHM-*N*-oxide	0·36	0·16	0·25	0·60
Metabolite II	0·36	0·16	0·25	0·60
DPMA	0·71	0·24	0·79	
Metabolite III	0·71	0·24	0·79	
N-demethyl-DPHM	0·32	0·35	0·38	0·21
Metabolite IV	0·32	0·35	0·38	
N,*N*-didemethyl-DPHM	0·51	0·52	0·46	0·21
Metabolite V	0·52	0·52	0·46	
DPHM	0·57	0·56	0·30	0·21
Metabolite VI	0·57	0·57	0·30	

* Known reference compounds were added to urine samples from monkeys dosed intravenously with ^{3}H-DPHM-HCl. Chromatography was performed on Silicia gel GF with solvent systems: A, ethanol:1 N ammonia (20:1); B, 1-butanol:15 N ammonia (50:1); C, ethanol:1 N acetic acid (20:1); D, methanol:0·15 N ammonia (1:1). Reference compounds were detected by examination of plates under u.v. light; metabolites by liquid scintillation counting. Values are averages of at least 2 determinations.

The relative amounts of metabolites in the 0–48 hr urine collections were determined for two male monkeys, one of which received a 10 mg/kg i.v. dose, the other a 10 mg/kg oral dose. Metabolite I (later identified as the glutamine conjugate of DPMA) was the major urinary metabolite (45–50 per cent), with lesser amounts of DPMA (10–20 per cent), DPHM-*N*-oxide (7–13 per cent), a glucuronide which was not characterized (3–14 per cent), unchanged DPHM (2–8 per cent), *N*-demethyl-DPHM (5–7 per cent), *N*,*N*-didemethyl-DPHM (3–6 per cent) and benzhydrol (1–2 per cent).

Isolation and identification of basic metabolites (metabolites II, IV, V, VI). Pooled urine from 6 male rhesus monkeys, which had been dosed orally twice daily for 3 days with 25 mg/kg of ^{3}H-DPHM·HCl, was adjusted to pH 8·4 with 1 N NaOH and extracted twice with 1 vol. of EDC. The solvent was evaporated to a small volume, streaked on preparative-layer plates coated with 0·5 mm of Silica gel PF_{254} (E. Merck AG., Darmstadt, Germany) and developed in solvent system B. Four u.v. absorbing radioactive bands were detected whose R_f values corresponded to DPHM, DPHM-*N*-oxide and the primary and secondary amine analogs of DPHM. These were eluted from the gel by using chloroform:methanol (1:1). The solvent was evaporated, the residues were dissolved in $CHCl_3$ and the retention times of the metabolites and reference compounds were determined. Table 2 shows agreement of GLC times for the metabolites and reference compounds. These mass peaks were not detected in predose monkey urine.

Isolation and identification of metabolite III. The pooled urine which had been extracted with EDC at pH 8·4 was found to contain a substantial amount of unextracted radioactivity. Therefore it was acidified to pH 2 with 6 N HCl and extracted three times with an equal volume of $CHCl_3$. The extracts were combined, concentrated for countercurrent distribution and partitioned between $CHCl_3$ and 0·2 M phosphate buffer (pH 6·5). TLC and radioactivity measurement after 30 transfers revealed a

TABLE 2. GLC ANALYSIS OB THE BASIC METABOLITES FROM MONKEY URINE*

Compound	Retention time (min)	
	Column 1	Column 2
DPHM-*N*-oxide	Extensive decomposition	8·0
Metabolite II	Extensive decomposition	8·0
N-demethyl-DPHM	13·2	
Metabolite IV	13·1	
N,*N*-didemethyl-DPHM	16·8	
Metabolite V	16·7	
DPHM	9·1	21·8
Metabolite VI	9·2	

* The basic metabolites were extracted from monkey urine and the extract was chromatographed to obtain the 4 metabolites. Column 1 was the 1% ECNSS-M, nitrogen-eluted column and Column 2 was the 5% XE-60, air-eluted column described in Experimental. Values are averages of at least 2 determinations.

good separation between metabolite III and a small amount of metabolite I. The fractions containing metabolite III were pooled, acidified and extracted with $CHCl_3$. The $CHCl_3$ extracts were concentrated and further purified by preparative TLC. After development in solvent system B, the radioactive band was eluted with 0·1 N NaOH, washed with petroleum ether, acidified to pH 2 and extracted into $CHCl_3$. The $CHCl_3$ was evaporated under a stream of dry nitrogen yielding relatively pure crystals of metabolite III. The i.r. absorption spectra of these crystals of metabolite III and synthetic DPMA show excellent agreement (Fig. 2), thus establishing the structure of metabolite III as (diphenylmethoxy)acetic acid.

Isolation of metabolite I. The urine remaining after the extraction of metabolite III contained approximately one-half of the radioactivity originally present. This was removed by extracting 3 times with an equal volume of isopentyl alcohol. After evaporation of the solvent, the residue was dissolved in methanol, chromatographed on Silica gel GF in solvent system B and the main radioactive band was eluted with methanol. The sequence of chromatography and elution of the labeled metabolite was repeated twice, developing the chromatograms first in solvent system A and then with 1-butanol:ethanol:1 N ammonia (10:10:1). After chromatography in the latter system, the metabolite was eluted with 0·05 N NaOH. This basic solution was washed with petroleum ether, acidified to pH 2 and the metabolite extracted with isopentyl alcohol. The solvent was evaporated under a stream of dry nitrogen, yielding metabolite I as an oil which would not crystallize because of the presence of impurities.

Preliminary identification of metabolite I. The extraction properties and chromatographic characteristics of metabolite I indicated that it was more polar than the other metabolites. The i.r. absorption spectrum (Fig. 3) of this compound was similar to that of DPMA but with additional absorbance at 1540, 1660 and 3400 cm^{-1}. Because these peaks were indicative of amide absorption, we investigated the possibility that metabolite I was an amino acid conjugate of DPMA.

Tritiated metabolite I was hydrolyzed in 6 N HCl for 1 hr at 100°. Radioactive hydrolysis products were removed from the solution by extraction with ethyl acetate. TLC of the extract in benzene:methanol (20:1) revealed the presence of tritiated

benzhydrol and benzophenone, resulting from the hydrolysis of ether as well as amide bonds. An aliquot of the aqueous phase remaining after ethyl acetate extraction was chromatographed on Silica gel GF in water-saturated phenol, along with several reference amino acids. Spraying with 0·2% ninhydrin in acetone containing 5% collidine revealed a violet spot corresponding in location with glutamic acid (glycine chromatographed with a greater R_f and gave a yellow spot). In addition, an aliquot

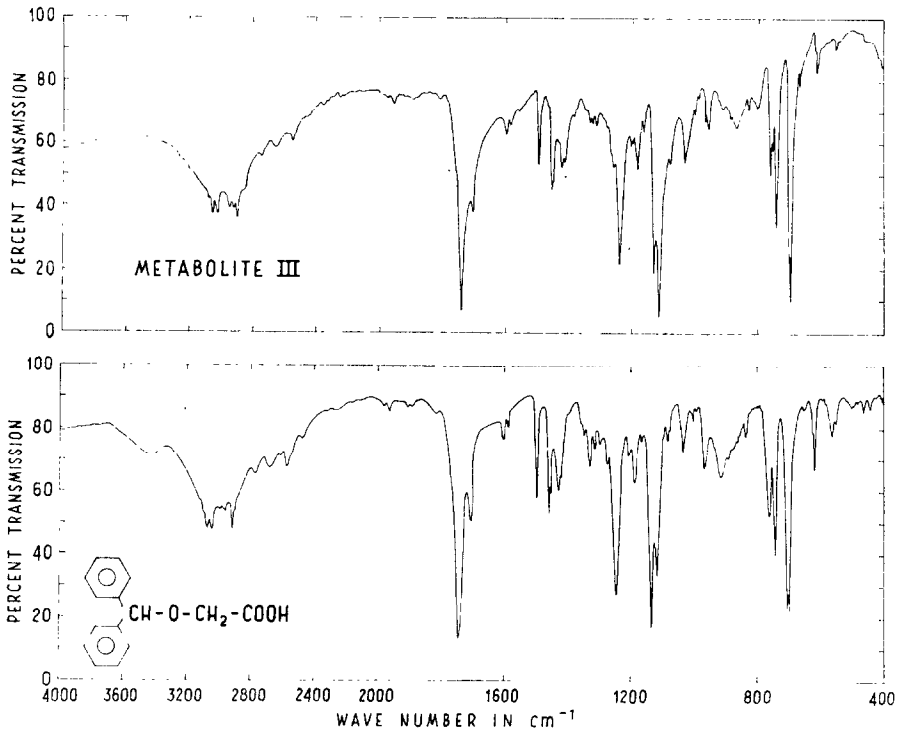


FIG. 2. Infrared absorption spectra of synthetic DPMA and of metabolite III which was isolated from monkey urine. The compounds were incorporated into KBr discs and spectra were determined in a Beckman IR9 infrared spectrophotometer.

of the aqueous solution was analyzed by IEC on an amino acid analyzer. This detected a single ninhydrin-positive peak whose retention time corresponded to that of glutamic acid. Since glutamic acid would be produced from glutamine under the hydrolysis conditions employed and because glutamine conjugates are known to be formed in higher primates from arylacetic acids such as indoleacetic acid,[15] metabolite I was examined for the possible presence of glutamine.

Sufficient metabolite I was isolated to permit nitrogen and titration analyses. These showed nitrogen amounts consistent with the glutamine conjugate structure and established that metabolite I was a monocarboxylic acid. Therefore we synthesized the glutamine conjugate of DPMA for comparison of its physical and chemical properties with those of metabolite I.

Synthesis of the glutamine conjugate. N^2-[(diphenylmethoxy)acetyl]-L-glutamine was synthesized for the first time by using the mixed anhydride procedure of Wieland and

Hörlein.[16] Thus, 2·4 g DPMA (prepared by a method similar to that of Djerassi and Scholz[17]) and 1·4 ml triethylamine were dissolved in 30 ml of dry THF in an open round-bottom flask. The solution was cooled in an ice bath, stirred magnetically and 0·95 ml ethyl chloroformate was added dropwise but in a rapid manner. After 5 min, 8 ml of a solution containing 1·46 g L-glutamine and 0·4 g NaOH was added. Stirring in the cold was continued for 30 min after which the mixture was allowed to

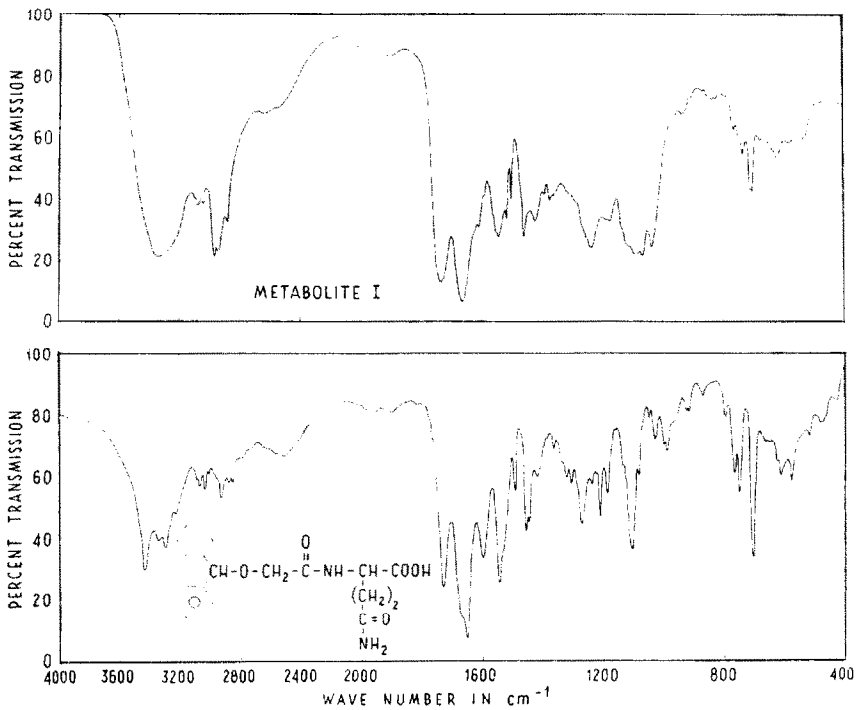


FIG. 3. Infrared absorption spectra of synthetic DPMA-GLN and of metabolite I isolated from monkey urine. The spectrum of metabolite I was determined from liquid film on a KBr salt plate. The synthetic DPMA-GLN was incorporated into a KBr disc.

warm to room temperature. The THF was removed *in vacuo* and sufficient water added to dissolve the triethylamine hydrochloride. The solution was cooled and 1 N HCl was added until no further precipitation occurred. The precipitated oil was extracted into a large volume of $CHCl_3$ and washed with very dilute HCl. The solvent was evaporated *in vacuo* and the residual oil crystallized upon standing. A part of this material was dissolved in methanol and chromatographed on Silica gel GF_{254} in solvent system B to remove the unreacted DPMA. The gel was eluted with methanol, the methanol was evaporated *in vacuo* and the resulting material was dissolved in water. The solution was cooled and 1 N HCl was added until no more precipitation occurred. The precipitate was collected on a sintered glass filter, washed with water and dried over KOH *in vacuo*. The white solid melted at 153–154°. (uncorr.).

Anal. Calcd: for $C_{20}H_{22}N_2O_5$: C, 64·8; H, 5·99; N, 7·56. Found: C, 64·5; H, 5·90; N, 7·38.

Structure of metabolite I. The i.r. absorption spectrum of synthetic DPMA-GLN is shown in Fig. 3, together with the spectrum of a preparation of metabolite I isolated from monkey urine. The spectrum of the synthetic compound showed good resolution of the absorption peaks due to amide, carboxylic acid, ether and monosubstituted phenyl group functions. The resolution of the peaks of metabolite I was somewhat obscured owing to the presence of impurities which were extremely difficult to eliminate. Also, because the i.r. absorption peaks of primary and secondary amides are poorly resolved, the γ-amide of the glutamine moiety could not be positively identified. Nonetheless, peaks corresponding to the amide, carboxylic acid, ether and monosubstituted phenyl absorptions of DPMA-GLN were found in the spectrum of metabolite I. This, together with the evidence showing that the metabolite was hydrolyzed to yield benzhydrol and glutamic acid, established metabolite I as a glutamine or glutamic acid conjugate of DPMA.

Supporting evidence for the glutamine structure was obtained from the titration analyses, which demonstrated that metabolite I was a monocarboxylic acid with a pK^a of 4·1 in 50% methanol. The pK^a of synthetic DPMA-GLN was also found to be 4·1. In addition, the ratio of per cent carbon to per cent nitrogen was 8·8 for metabolite I compared to 8·7 for the synthetic compound. The chromatographic properties of the isolated and synthetic compounds also showed excellent agreement. When samples of each were spotted side by side on thin-layer plates and chromatographed in solvent system A, both compounds migrated with R_f values of 0·58. Likewise in solvent system B, both isolated and synthetic compounds chromatographed with R_f values of 0·11.

Glucuronide metabolite. The presence of an additional acidic polar compound was observed when urine was chromatographed in solvent system A and also during the isolation of metabolite I. The R_f corresponded to that of DPMA-GLN in solvent system B, but in solvent system A was 0·12 compared to 0·58 for DPMA-GLN. This fraction was obtained free of other metabolites by TLC in solvent system A during the isolation of metabolite I. After elution with methanol from the Silica gel and incubation with β-glucuronidase, the hydrolysis products were analyzed by TLC. In solvent system A the labeled moiety chromatographed with an R_f value of 0·50 and in solvent system B with a value of 0·76. In the absence of β-glucuronidase no chromatographic changes were noted. Since the labeled moiety of the metabolite did not resemble any available reference compound in its chromatographic characteristics, its identification was not completed.

DISCUSSION

The present work establishes the general pathway of diphenhydramine metabolism in the rhesus monkey with a fair degree of certainty. The biotransformations which appear to be involved are outlined in Fig. 4.

Approximately one-half the dose of DPHM was excreted in the urine of rhesus monkeys,[12] with only a small amount of the unchanged drug present. The major portion of the drug was converted to (diphenylmethoxy)acetic acid and then conjugated with glutamine. It is not known whether the carboxylic acid was formed directly from DPHM, or from the secondary or primary amine after progressive dealkylation. While the primary amine appears to be a good possibility, some tertiary amines—like the butyrophenone amines—appear to be degraded directly to the corresponding carboxylic acids.[18]

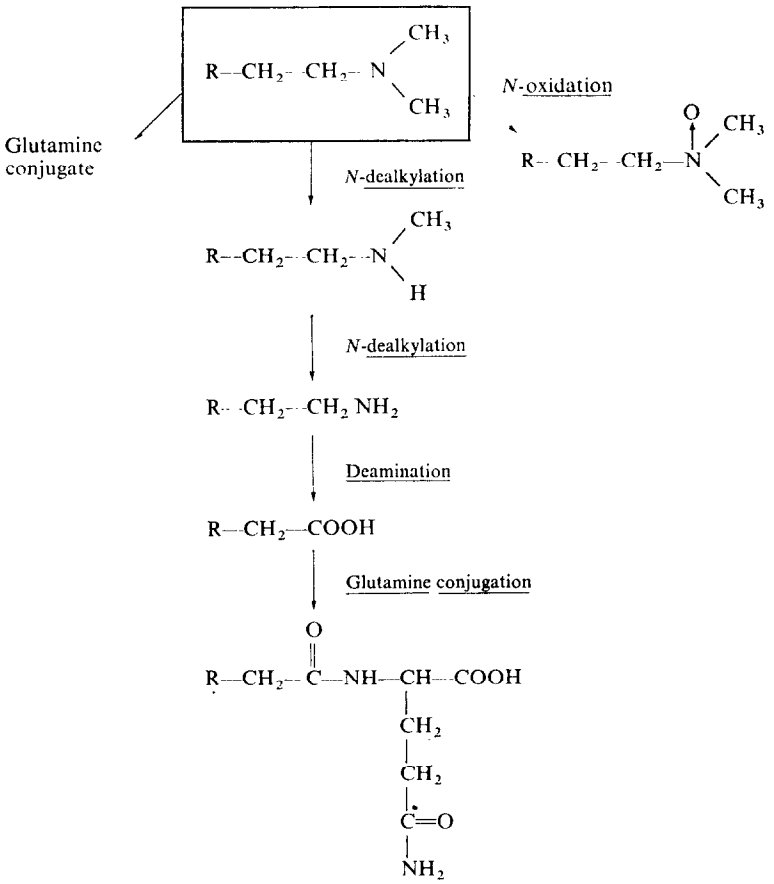


FIG. 4. Postulated pathway of diphenhydramine metabolism in the rhesus monkey. The (diphenylmethoxy) moiety is denoted by R.

The oxidative deamination of phenyl-substituted aliphatic amines and subsequent conjugation with amino acids is an established pathway.[19] Also, until recently it was generally thought that glutamine conjugation was a pathway limited to man and the chimpanzee, and did not occur in rhesus monkeys or phylogenetically lower species.[20] Recently, however, Patel and Crawford[21] have shown that both phenylacetic acid and indole-3-acetic acid can be conjugated with glutamine in several species of old world monkeys. Williams[15] has extended this to include the rhesus monkey. Our observations provide additional evidence that the rhesus monkey conjugates carboxylic acids with glutamine. Furthermore, because phenylacetic, indole-3-acetic and (3,4-dihydroxy-5-methoxyphenyl)acetic acid are the only carboxylic acids which have been reported as forming glutamine conjugates,[22] the isolation of DPMA-GLN demonstrates that glutamine conjugation is not restricted to arylacetic acids. This suggests that the glutamine conjugation pathway may occur more extensively than previously expected.

Other experiments concerning DPHM metabolism had indicated that *N*-demethylation was a metabolic pathway, by demonstrating that the basic property of DPHM decreased[6] and that formaldehyde was produced[8] during incubations *in vitro*. The

identification of the primary and secondary amine analogs of DPHM in monkey urine has now established the occurrence *in vivo* of the *N*-demethylation pathway.

This work also demonstrated that an additional amine analog, DPHM-*N*-oxide, occurred as a urinary metabolite. Other drugs for which *N*-oxides have been demonstrated as urinary metabolites include imipramine, chlorpromazine and chlorcyclizine.[23, 24] The *N*-oxidation of tertiary amines *in vitro* also has been established for trimethylamine, dimethylaniline, tremorine, imipramine and chlorpromazine.[23, 25–27] The question of the involvement of *N*-oxides as intermediates in *N*-demethylation has been explored by several investigators (see Gillette[23]). Recently, Kuntzman *et al.*[24] deduced that although a small amount of chlorcyclizine-*N*-oxide was demethylated *in vivo*, the formation of norchlorcyclizine could be accounted for by reduction of the *N*-oxide and subsequent *N*-demethylation of the resulting chlorcyclizine. Likewise Bickel and Baggiolini[25] and Coccia and Westerfeld,[27] working with imipramine and chlorpromazine respectively, reported that the *N*-oxides did not appear to be intermediates in monodemethylation pathways. On the other hand, Ziegler and Pettit[28] concluded that the *N*-oxide of dimethylaniline was an intermediate in an *N*-demethylation pathway of pork liver microsomes. Whether DPHM-*N*-oxide undergoes similar metabolic changes cannot be answered by the present study.

Although benzhydrol has been implicated as a metabolite of DPHM by experiments *in vitro*,[7] only traces were found in the present study when urine samples were analyzed directly by TLC. However, when metabolites or reference compounds were fractionated, especially if acidic extractions were used, benzhydrol was always detected during subsequent TLC. Thus benzhydrol was probably produced during handling procedures by direct hydrolysis of DPHM and its metabolites. Nevertheless, a small amount of benzhydrol may have arisen metabolically, but escaped detection because of the formation of its glucuronide which is acid labile.[29]

Acknowledgements—We wish to acknowledge the cooperation of Mr. D. E. Roll and R. C. Bonczyk for animal procedures, Dr. J. M. Vandenbelt for titration analyses, Mr. E. J. Schoeb for infrared analyses, Mr. R. Brooks for automatic amino acid analyses, and Mr. C. E. Childs for elemental analyses.

We also wish to thank especially Dr. A. J. Glazko for his continuing interest and advice in the course of this work. The project was supported by NIH Contract PH 43-65-1069.

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