Metabolites of Demoxepam, a Chlordiazepoxide Metabolite, in Man

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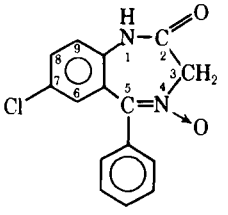
Abstract □ The biotransformation of demoxepam, 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide, was studied in two subjects. Following the administration of a 20-mg. oral dose of demoxepam-2-^{14}C, these subjects excreted 24 and 29% of the dose in the urine as unchanged drug. The urinary metabolites included "opened lactam" (formed by hydrolysis of the lactam bond), conjugated oxazepam (*N*-desoxy-3-hydroxydemoxepam), and two conjugated phenols, the 5-(4-hydroxyphenyl) derivative (I) and the 9-hydroxy derivative (II) of demoxepam. *N*-Desoxydemoxepam, an apparent intermediate in the formation of oxazepam, was the only metabolite identified in the feces. Of these metabolites, only opened lactam was previously shown to be a metabolite of chlordiazepoxide in man.

Keyphrases □ Demoxepam, radiolabeled—biotransformation, urinary and fecal metabolites, man □ Chlordiazepoxide metabolites—biotransformation of orally administered radiolabeled demoxepam, urinary and fecal metabolites, man

Demoxepam (formerly designated Ro 5-2092 or "lactam"), 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide, is a metabolite of chlordiazepoxide[1] present in the plasma (1) and excreted in the urine (2) of dog and man. It has been shown to have antianxiety activity in man (3) and, therefore, is an active chlordiazepoxide metabolite.

A recent investigation of the metabolism of demoxepam in the dog revealed that its biotransformation proceeded *via* oxidative, reductive, and hydrolytic pathways (4). Two phenolic metabolites, the 5-(4-hydroxyphenyl) derivative (I)[2] and the 9-hydroxy derivative (II)[2], resulted from the hydroxylation of demoxepam. The reduction of the *N*-oxide function yielded *N*-desoxydemoxepam[2], and the combination of oxidative and reductive pathways yielded oxazepam[3] and the *N*-desoxy phenols, Desoxy I[2] and Desoxy II[2]. In addition, an amino acid, *N*-(2-amino-5-chloro-α-phenylbenzylidene)glycine *N*-oxide ("opened lactam"), was formed by hydrolysis of the demoxepam lactam bond. The predominance of metabolites bearing the *N*-oxide function in bile, coupled with the fecal excretion of the corresponding *N*-desoxy metabolites, suggested that the intestinal tract was one site of *N*-oxide reduction.



demoxepam

The present study was designed to measure the excretion of these same metabolites in man. This information would permit the metabolism of demoxepam to be compared in the two species and would also yield additional data on chlordiazepoxide metabolism in man.

Table I—Excretion of Radioactivity by Two Subjects Given a Single 20-mg. Oral Dose of ^{14}C-Demoxepam

	—Excretion of ^{14}C in Percent of Dose Administered—					
	—Subject H-20—			—Subject H-23—		
Day	Urinary	Fecal	Total	Urinary	Fecal	Total
0–1	6.8	Nil	6.8	14.2	—[a]	14.2
0–3	23.4	1.6	25.0	32.3	4.7	37.0
0–7	44.8	11.7	56.5	49.5	11.2	60.7
Total[b]	64.0	17.7	81.7	58.9	11.6	70.5

[a] No feces excreted. [b] Duration of study: 18 days for H-20 and 16 days for H-23.

EXPERIMENTAL

Experimental Design—Two subjects were each given an oral dose of 20 mg. of labeled demoxepam in a gelatin capsule. The demoxepam-2-^{14}C, the same labeled substance as that used in the dog studies (4), was diluted with authentic demoxepam to a specific activity of 1.0 μc./mg. TLC was employed, as previously reported (4), to demonstrate a radiochemical purity of 97%. In addition, two-dimensional TLC revealed that <0.5% of the ^{14}C was associated with any of the metabolites previously identified (4).

Subject H-20 was a healthy 49-year-old, 75-kg. man who received no medication for 1 week prior to the study. He had previously been given 500 mg. q.i.d. of ampicillin for 2 weeks to treat an ear infection. The second subject, H-23, was a 35-year-old, 58-kg. woman with essential hypertension and asthmatic bronchitis. She received 300 mg. of methyprylon the night before the study started and 1 teaspoon of a glyceryl guaiacolate preparation[4] t.i.d. during the study.

Both subjects were given water during the 1st day to promote diuresis, and urine was collected over thymol in intervals of 0–2, 2–4, 4–6, 6–9, 9–12, and 12–24 hr. and then daily until the end of the study. Feces were collected daily. The studies were terminated when the excretion of radioactivity became minimal.

Determination of Radioactivity—The ^{14}C was measured in a liquid scintillation spectrometer[5], and disintegrations per minute (d.p.m.) were obtained by the external standard-channels ratio procedure for quench correction. Aquasol[6] was used as a fluor (10 ml.) for the quantitation of ^{14}C in urine and organic extracts of urine and feces. Aquasol diluted with 2 ml. of water to form a gel was used for the determination of the ^{14}C content of particles of silica gel and homogenates of feces prepared in 50% ethanol.

Estimation of Urinary and Fecal Demoxepam and Metabolites—Aliquots of pooled urine representing the total excretion of ^{14}C by

[1] Chlordiazepoxide hydrochloride, 7-chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide hydrochloride, is the active ingredient in Librium, Hoffmann–La Roche Inc., Nutley, N. J.

[2] Metabolite I is 7-chloro-1,3-dihydro-5-(4-hydroxyphenyl)-2*H*-1,4-benzodiazepin-2-one 4-oxide; Metabolite II is 7-chloro-1,3-dihydro-9-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one 4-oxide; *N*-desoxydemoxepam is 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one. Desoxy I differs from Metabolite I, as Desoxy II differs from Metabolite II, only in that the 4-oxide (*N*-oxide) is absent.

[3] Oxazepam, 7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one, is the active ingredient in Serax, Wyeth Labs., Inc., Radnor, Pa.

[4] Robitussin, Robins.

[5] Model 3380, Packard Instrument Co., Downers Grove, Ill.

[6] New England Nuclear Corp., Boston, Mass.

Table II—Total Urinary Excretion of Labeled Demoxepam and Metabolites by Two Human Subjects Given a 20-mg. Oral Dose[a]

Compound Form[b]	Urinary Excretion in Percent of Dose								
	Total[c]	Demoxepam	Metabolite I	Desoxy I	Metabolite II	Desoxy II	*N*-Desoxy-demoxepam	Oxazepam	Opened Lactam
Subject H-20									
Nonconjugated	32.4	28.7	1.2	(<0.5)[d]	(<0.5)	—	(<0.5)	(<0.5)	—
Conjugated	16.2	(<1.1)	1.5	(<1.1)	1.2	—	(<1.1)	7.6	—
Acidic	10.2	—	—	—	—	—	—	—	5.5
Unextracted	5.2	—	—	—	—	—	—	—	—
Subject H-23									
Nonconjugated	29.0	24.3	(<0.6)	(<0.6)	(<0.6)	(<0.6)	(<0.6)	(<0.6)	—
Conjugated	14.9	(<0.6)	3.0	(<0.6)	1.9	1.1	(<0.6)	2.4	—
Acidic	4.7	—	—	—	—	—	—	—	2.8
Unextracted	10.3	—	—	—	—	—	—	—	—

[a] The total urinary excretion of ^{14}C by H-20 and H-23 was 64.0 and 58.9% of the dose, respectively. [b] The nonconjugated compounds were those extracted directly at pH 7.0, the conjugated metabolites were those extractable at this pH after β-glucuronidase-sulfatase treatment, and the acidic metabolites were those extracted at pH 2.0. [c] This is the total ^{14}C excreted as each compound form. [d] The parentheses indicate that a detectable amount was not found; the limit of detection is indicated within the parentheses.

each subject were extracted serially with ethyl acetate (4); 250 ml. of H-20 urine containing 1.5×10^5 d.p.m. and 200 ml. of H-23 urine containing 2.4×10^5 d.p.m. were extracted. The extraction procedure provided for the initial removal at pH 7.0 of the drug and its lipophilic metabolites. Following the incubation of the unextracted ^{14}C with a β-glucuronidase-sulfatase preparation[7], subsequent extraction at pH 7.0 then removed the deconjugated metabolites. A final extraction at pH 2.0 removed those metabolites which, unlike the weakly acidic demoxepam (pKa 10.7), formed salts at pH 7.0. Two consecutive volumes of ethyl acetate, each equaling the aqueous phase volume, were used for each extraction.

Labeled demoxepam and those metabolites previously found in the dog were separated and quantitated by the chromatographic procedure previously described (4). Identification and quantitation were based on the demonstration that, on two-dimensional TLC with at least two different solvent system pairs, a consistent amount of extracted ^{14}C migrated as an internal reference compound. Silica gel containing a fluorescent indicator[8] was used as the adsorbent layer. The solvent systems, used in combination for two-dimensional TLC, were chloroform–acetone–ethanol (80:5:5), heptane–chloroform–ethanol–concentrated ammonia (5:5:2:0.1), heptane–ethyl acetate–ethanol (40:80:5), and heptane–ethyl acetate–ethanol (40:80:10).

A 100-ml. aliquot and a 75-ml. aliquot of pooled urine from H-20 and H-23, respectively, were analyzed for opened lactam as previously described (4). This method consisted of the extraction of opened lactam at pH 2.0 and the formation of its methyl ester prior to TLC and radioassay.

Aliquots (50 ml.) of pooled 50% ethanol homogenates of the feces excreted during the first 6 days were first diluted 1:5 with water and were then flash evaporated to remove the ethanol. These aqueous homogenates were then extracted with ethyl acetate in the same manner as the urine samples. Extracts containing sufficient ^{14}C were analyzed for labeled demoxepam and metabolites by two-dimensional TLC.

RESULTS AND DISCUSSION

The excretion of radioactivity by both subjects is summarized in Table I. The total recovery of administered ^{14}C in the excreta was 81.7% for H-20 and 70.5% for H-23. Only 6.8 and 14.2% of the dose were excreted by H-20 and H-23, respectively, during the 1st day. In both subjects, the major portion of the fecal ^{14}C was excreted after the first 3 days. This finding suggests that, as in the dog (4), biliary secretion of labeled products into the GI tract, rather than unabsorbed drug, was primarily responsible for the fecal excretion of radioactivity.

A profile of those metabolites excreted in the feces was sought by solvent extraction and two-dimensional TLC. Roughly 20% of the fecal ^{14}C of both subjects was extracted at pH 7.0 into ethyl acetate. The only metabolite identified and quantitated in these extracts was *N*-desoxydemoxepam; no intact demoxepam, Metabolite I, Desoxy I, Metabolite II, Desoxy II, or oxazepam was detected. The excretion of fecal *N*-desoxydemoxepam was estimated at 2% of the dose in H-20 and 1% in H-23. The remaining fecal ^{14}C resided mainly in polar compounds which, after β-glucuronidase-sulfatase[7] treatment, were still not extracted at either pH 7.0 or 2.0. Since the fecal excretion of *N*-desoxydemoxepam in the dog was associated with reduction of the *N*-oxide function in the GI tract (4), the human excretion of this metabolite suggests that the same process also occurs in man.

The combined characterization and quantitation by two-dimensional TLC of the labeled urinary products yielded the results shown in Table II. Demoxepam was the major labeled urinary component excreted by both subjects. It accounted for 29% of the dose administered to H-20 and 24% of that given to H-23, and it also represented 45 and 41% of the total urinary ^{14}C excreted by H-20 and H-23, respectively. Conjugated oxazepam was a prominent metabolite in H-20 urine (7.6% of the dose) and was excreted in minor, but still significant, quantities (2.4% of the dose) by H-23. Both subjects excreted small amounts of Metabolites I and II but did not excrete detectable quantities of Desoxy I or *N*-desoxydemoxepam. The Desoxy II excretion was determined only in H-23 urine and a small amount (1.1% of the dose) was found. Approximately twice the amount of the acidic metabolite, opened lactam, was excreted by H-20 (5.5% of the dose) than was excreted by H-23.

The outline of demoxepam metabolism presented for the dog (4) is also appropriate for describing its metabolism in man. While aromatic ring hydroxylation to form the phenolic Metabolite I appears to be preferred by the dog, the hydrolytic cleavage to opened lactam and the combination of *N*-oxide reduction and 3-hydroxylation to yield oxazepam appear to be more prominent pathways in man. Furthermore, since both species excreted fecal *N*-desoxydemoxepam and urinary conjugated oxazepam, the hypothesis (4) that demoxepam is reduced in the GI tract to *N*-desoxydemoxepam, which is partially absorbed and systemically converted to oxazepam, may also hold for man. Of the metabolites found in the dog, only Desoxy I was not detected in human urine or feces.

The half-life of demoxepam appears to be significantly shorter in the dog than in man: a mean of 16 ± 2.0 (*SE*) hr. in the dog (4, 6) as compared to 45 ± 14 (*SE*) hr. in man (5) with $p < 0.05$. Since demoxepam is eliminated mainly by biotransformation in the dog (4), the slower elimination in man is probably due to slower rates of formation of metabolites. The greater excretion of intact drug in man (roughly 27% of the dose) than in the dog (10% of the dose) is compatible with this hypothesis. In addition, the discontinuous falloff of plasma demoxepam previously reported (5) is compatible with a process such as the emptying of the gall bladder to yield a fresh supply of intestinal demoxepam for reabsorption. This is additional support for the postulated biliary secretion of demoxepam and metabolites in man.

This study provides further information on the metabolites of chlordiazepoxide in man. In addition to opened lactam, which was already known to be a metabolite (2), oxazepam, *N*-desoxydemoxepam, and the phenolic metabolites must be considered as potential chlordiazepoxide metabolites. The quantities formed would depend mainly on how much chlordiazepoxide was biotransformed to demoxepam. While this process was estimated to be as high as 50% in the dog (6), no such estimate is currently available for man.

[7] Glusulase, Endo Lab., Garden City, N. Y.
[8] SilicAR 7-GF-5, Mallinckrodt Chemical Works, St. Louis, Mo.

N-Desoxydemoxepam is of interest because this pharmacologically active compound (7) is a major blood metabolite of the tranquilizing agents, diazepam[9] (8, 9) and medazepam[10] (10, 11), in man. Chronic administration of either of these drugs in man yields blood levels of *N*-desoxydemoxepam (also designated *N*-desmethyldiazepam) which accumulate with time (9, 11). Since chronic chlordiazepoxide administration in man results in the accumulation of plasma demoxepam (12), it is possible that, under these conditions, *N*-desoxydemoxepam formed from demoxepam may also reach measurable plasma levels.

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[9] Diazepam, 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one, is the active ingredient in Valium, Hoffmann-La Roche Inc., Nutley, N. J.

[10] Medazepam, 7-chloro-2,3-dihydro-1-methyl-5-phenyl-1*H*-1,4-benzodiazepine, is the active ingredient in Nobrium, F. Hoffmann-La Roche and Co., A.G., Basle, Switzerland.

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Carbamates and Ureas with Potential Chemotherapeutic Properties

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Abstract □ Three series of carbanilates and ureas were prepared and tested for their antibacterial, antifungal, and antiviral activities. Twelve compounds were prepared by the action of the appropriate isocyanate on *N*-*p*-chlorobenzyl-*N*-methylaminoethanol, *N*,*N*-dibenzylaminoethanol, and benzylphenylamine. The *meta*- and *para*-tolylcarbamates and the *meta*- and *para*-chlorophenylcarbamates of *N*-*p*-chlorobenzyl-*N*-methylaminoethanol were active as antibacterial and antifungal agents. No antiviral activity was seen. The corresponding carbamates of *N*,*N*-dibenzylaminoethanol and the corresponding ureas from benzylphenylamine were found to be inactive as antibacterial, antifungal, and antiviral agents.

Keyphrases □ *N*-*p*-Chlorobenzyl-*N*-methylaminoethyl carbanilate series—prepared and tested for antibacterial, antifungal, and antiviral activities □ *N*,*N*-Dibenzylaminoethyl carbanilate series—prepared and tested for antibacterial, antifungal, and antiviral activities □ N^1-Benzylphenyl-N^3-(substituted-phenyl)-urea series—prepared and tested for antibacterial, antifungal, and antiviral activities

Earlier studies indicated the activity of *N*-aralkyl-*N*-methylaminoethyl carbanilates as antihypercholesteremic agents (1). Substitution of the benzyl group in the series of compounds was also shown to have a favorable effect on the activity (2). In this study, three series of compounds were studied for their possible antibacterial, antifungal, and antiviral activities.

The *N*-*p*-chlorobenzyl-*N*-methylaminoethyl carbanilates constituted the first series. *N*,*N*-Dibenzylaminoethyl carbanilates were in the second series. N^1-Benzylphenyl-N^3-(substituted-phenyl)-ureas comprised the third series.

These compounds were prepared by the reaction of the appropriate isocyanate on *N*-*p*-chlorobenzyl-*N*-methylaminoethanol, *N*,*N*-dibenzylaminoethanol, and benzylphenylamine. The hydrochloride salts of the first two series were formed in the usual manner.

EXPERIMENTAL[1]

***N*-*p*-Chlorobenzyl-*N*-methylaminoethanol**—To 75.1 g. (1.0 *M*) of 2-methylaminoethanol was added slowly, with cooling in an

[1] The reagents used were obtained from the Eastman Kodak Co. and Matheson, Coleman and Bell. They were of sufficient purity for immediate use. The carbon and hydrogen analyses were determined by Spang Microanalytical Laboratory, Ann Arbor, Mich. All melting points and boiling points are uncorrected. The melting points were taken on a Thomas-Hoover Unimelt melting-point apparatus.