Biotransformation of the Sedative 1-(*p*-Chlorophenyl)-2,2-dimethyl-1,3-propanediol 3-Carbamate in Humans and Five Animal Species

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The sedative 1-(*p*-chlorophenyl)-2,2-dimethyl-1,3-propanediol 3-carbamate and its biotransformation products are excreted almost exclusively as glucuronide conjugates in the urine of man, monkey, pig, rabbit, dog, and rat. Urinary metabolites were cleaved by enzymatic hydrolysis and separated and isolated in quantities sufficient for characterization and structure elucidation by mass spectrometry. The drug is degraded, *in vivo*, by hydrolytic as well as oxidative pathways. In the different species these metabolic reactions take place to different extents, and no two species have exactly identical metabolite patterns. The transformations are partially stereoselective, leading to optically active metabolites. The following compounds were isolated and identified by mass spectrometry: 1-(*p*-chlorophenyl)-2,2-dimethyl-1,3-propanediol, 1-(*p*-chlorophenyl)-2-hydroxymethyl-2-methyl-1,3-propanediol 3-carbamate (occurring only in animal species), 2-carboxy-1-(*p*-chlorophenyl-2-methyl-1,3-propanediol 3-carbamate (occurring only in the dog), and 2-(*p*-chlorobenzoyl)-2-methyl-1,3-propanediol 3-carbamate.

In connection with the evaluation of 1-(*p*-chlorophenyl)-2,2-dimethyl-1,3-propanediol 3-carbamate[1] as a sedative, the biotransformation of this compound in humans, monkeys, pigs, rabbits, dogs, and rats was investigated. Considerable species differences were found, and in no 2 species were the patterns of urinary excretion products qualitatively and quantitatively the same. The detection, isolation, and structure elucidation of the various metabolites form the subject of this publication. A brief account of the mass spectrometric identification of these metabolites has been presented previously.[2]

Experimental Section

Samples of the drug, 1-(*p*-chlorophenyl)-2,2-dimethyl-1,3-propanediol 3-carbamate (reference compd A), and of 1-(*p*-chlorophenyl)-2,2-dimethyl-1,3-propanediol (reference compd B) were made available by Fritzsche Brothers, Inc., New York, N. Y.

Drug Administration and Sample Collections.—The drug was administered orally in 5 animal species as noted in Table I. In rats and rabbits this was done by intubation of a suspension in 0.4% CM-cellulose soln. In the other animal species the dosing was *via* gelatin capsule.

Total urine collections were made every 24 hr during the treatment period, and the samples were kept frozen until examd. Pretreatment urine specimens were collected for the 48-hr period preceding the initiation of drug treatment.

In humans, the drug was given orally in the form of a tablet to groups of 6–8 men, 2 hr after breakfast. The dose levels used were 200, 400, 800, and 1600 mg. The 800-mg dose was given, after a 1-week interval, to the same subjects who had received the 400-mg dose.

Blood samples were taken before and at 0.5, 1, 3, 6, and 12 hr after drug administration. Urine and feces were collected for the 48-hr period following drug administration. All of these specimens were maintained in the frozen state until examd.

Extraction of Urines.—Metabolites were isolated from the urine specimens as indicated in Scheme I. Two β-glucuronidase prepns were used; Mann Research Laboratories [catalog 05300-1045 (marine mollusc)], and Ketodase from the General Diagnostics Division of Warner-Chilcott. Mylase P, Mann Research Laboratories (catalog 2761), was used as a glucuronidase-free source of sulfatase, and except that the initial hydrolysis was at pH 6, Scheme I was followed in examg urine specimens for the presence of sulfate conjugates.

Extraction of Feces.—The entire 48-hr collection was homogenized in H_2O, and 0.1 of the homogenate was extd with 2 vol of CH_2Cl_2. The ext was taken to dryness and reconstituted with a small amount of Me_2Co, and aliquots were applied to a tlc plate.

Tlc.—Analytical sepns were carried out on glass plates precoated with a 250-μ layer of silica gel F-254 (E. Merck, Darmstadt; Brinkmann 681000). Preparative chromatog was performed on plates precoated with a 2-mm layer of silica gel F-254 (Brinkmann 681100). A mixt of $CHCl_3$ and Me_2CO (4:1 by volume) was used as the developing solvent, and it was necessary to develop the plates twice in order to obtain satisfactory resolution.

Metabolites were located on the fluorescent plates by their quenching effect when viewed under shortwave uv light. In addition, most of the metabolites were visualized with a Clorox–tolidine reagent[3] or with the H_2SO_4–*p*-dimethylaminobenzaldehyde spray described by Olesen.[4]

For prep tlc, the ext from 50–200 ml of hydrolyzed urine was streaked across the plate, which was then developed, and the metabolites were marked under shortwave uv light. The silica gel layer was removed from the plate in the marked area, the metabolite was eluted with 10 ml of Me_2CO, and the eluate was taken to dryness in a vacuum desiccator over silica gel.

Analytical Procedures.—The levels of the drug in blood serum were assayed by the colorimetric method for meprobamate described by Hoffman and Ludwig,[5] except that the final C_6H_6 diln step was eliminated and a Gilford 300 microsample spectrophotometer was used. These modifications permitted the detn of the drug in concns from 0.5 to 10 μg/ml.

Melting points were obtained with a Thomas-Hoover capillary apparatus and are uncor.

All detns of optical activity were performed on 1% solns in EtOH.

Mass spectra were detd with an MS 902Cl high-resoln mass spectrometer. High-resoln data were obtained at a resolving power of 10,000 by scanning directly into an attached PDP-8 computer. All mass measurements were within 5 ppm of the exact masses corresponding to the assigned elemental compns. Samples were introduced by the direct technique, with a source temp of 130°, and ionization was effected by a 70-eV beam.

Results

Detection and Separation of Metabolites.—Several compounds were detected in solvent extracts from β-glucuronidase-treated urines of animals and humans receiving the drug which were not found in similarly

(1) K. Kulka, U.S. Patent 3,415,844, Dec 1968; *Chem. Abstr.*, **71**, 91102 (1969).

(2) S. R. Shrader, J. T. Rees, F. V. Hadley, and H. W. Ruelius, International Conference on Mass Spectrometry and Allied Topics, Kyoto, Japan, 1969.

(3) H. W. Ruelius, J. M. Lee, and H. E. Alburn, *Arch. Biochem. Biophys.*, **111**, 376 (1965).

(4) O. V. Olesen, *Acta Pharmacol. Toxicol.*, **24**, 183 (1966).

(5) A. J. Hoffman and B. J. Ludwig, *J. Amer. Pharm. Ass., Sci. Ed.*, **48**, 740 (1959).

TABLE I
DRUG TREATMENT AND SPECIES STUDIED

Species	No. of animals—Male	No. of animals—Female	Wt range, kg	Dose, mg/kg per day	Duration of treatment, days
Rats, Charles River COBS strain	6	6	0.244–0.425	200	28
Rabbits, Dutch Belted	2	2	1.22–1.65	200	30
Dogs, Beagle	2	2	9.4–16.3	200	26
Miniature swine, Pitman–Moore strain	1	1	7.7–16.5	200	42
Monkeys, African Green	1	1	1.9–3.1	100	11

SCHEME I
EXTRACTION OF URINARY METABOLITES

A. urine (adjusted to pH 7): extd 3 times with 0.5 vol of CH_2Cl_2.

→ org phase: discard.

B. aq phase: adjust to pH 4.7, add β-glucuronidase (1000 Fishman units/ml), and incubate at 37° for 2–4 days.

adjust to pH 7 and ext 4 times with 0.25 vol of toluene.

C. aq phase: ext 4 times with 0.25 vol of CH_2Cl_2.

toluene ext: ext twice with 0.25 vol of 0.1 *N* NaOH.

D. CH_2Cl_2 phase: evap to dryness under reduced pressure; cryst residue from CH_2Cl_2 or Me_2CO (III and trace of V).

aq phase: discard.

toluene phase: evap to dryness under reduced pressure, dissolve residue in Me_2CO, and apply to tlc plate (I, II, V).

aq phase: neutralize to pH 7 and ext 3 times with 0.5 vol of CH_2Cl_2. Evap org phase under reduced pressure, dissolve residue in Me_2CO, and apply to tlc plate (IV).

extracted control urines. Only traces of these same compounds were found in unhydrolyzed urine samples.

Cleavage experiments with sulfatase demonstrated that some urines contained no conjugated sulfates, while others from the same species contained small amounts of sulfates. Acid hydrolysis of solvent-extracted urines did not result in the release of additional drug-related compounds, nor did it increase the amount of such compounds when compared with enzymatic hydrolysis, indicating that no other conjugates were present.

All the drug-related compounds were found to be present in CH_2Cl_2 extracts of the glucuronidase-hydrolyzed urine. The extraction procedure shown in Scheme I was useful in isolating III in sufficient quantity for physical measurements and in separating I and IV, which had similar R_f values. R_f values and staining reactions of all the compounds are presented in Table II. Scheme I indicates in which fraction each compound was found.

The isolation techniques used did not lend themselves to quantitative recovery. Thus, losses had to be taken, especially with tlc, in order to achieve the desired separation and purification. For this reason no exact recovery data can be given; however, about 0.5 of the administered dose was recovered in some instances.

Examination of the feces from human subjects revealed only traces of the free drug (I).

The R_f values and staining characteristics of I and II were identical with those of the drug (ref compd A) and II (ref compd B), resp (Table II). These identities were confirmed by mass spectrometry. The structures of III, IV, and V were determined by the latter technique. The structures of all compounds identified in β-glucuronidase-treated urines are presented in Figure 1.

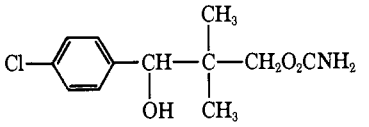
TABLE II
R_f VALUES AND STAINING REACTIONS OF THE REFERENCE COMPOUNDS AND METABOLITES RECOVERED AFTER β-GLUCURONIDASE HYDROLYSIS OF URINES

Compd	R_f value[a]	Clorox–tolidine	Acid DMB[b]	Acid DMB + heat
Ref compd A[c]	0.33	Dark blue	None	Red-brown
Ref compd B[d]	0.46	None	Yellow	Red-brown
Metabolites				
I	0.33	Dark blue	None	Red-brown
II	0.46	None	Yellow	Red-brown
III	0.09	Dark blue	None	Bright pink
IV	0.30	Dark blue	None	None
V	0.20	None[e]	None	None

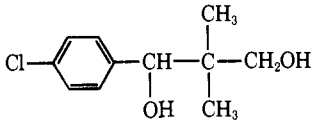
[a] Measured on Brinkmann 250-μ silica gel anal. tlc plate after 2 developments in 4:1 (by vol) $CHCl_3$–Me_2CO solvent system. [b] DMB = *p*-dimethylaminobenzaldehyde reagent (O. V. Olesen, *Acta Pharmacol. Toxicol.*, **24**, 183 (1966). [c] Compd I. [d] Compd II. [e] Failure to react may be due to small amounts present.

Mass Spectrometry.—The mass spectrum of the parent compd I exhibits a number of structurally significant peaks (Figure 2). A very weak molecular ion occurs at *m/e* 257; the base peak at *m/e* 141 corresponds to the benzylic ion A; elimination of carbamic acid yields the ion of mass 196; and an unusual rearrangement, resulting in elimination of C_4H_7, leads to the peak at *m/e* 202.

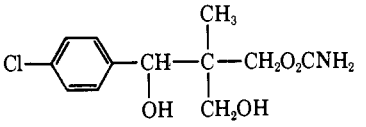
The mass spectrum of the hydrolysis product II (Figure 3) exhibits no molecular ion, but the intense peak at *m/e* 141, also shown by I. M − H_2O and M − CH_2OH ions appear at *m/e* 196 and 183, resp.



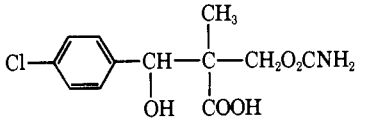
I



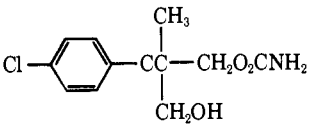
II



III

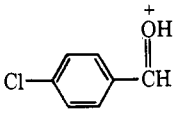


IV



V

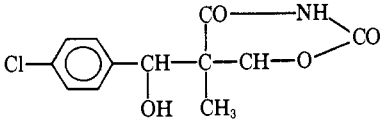
Figure 1.—Compounds identified in glucuronidase-treated urine.



A, *m/e* 141

The mass spectrum of III (Figure 4) is very similar to that of I (Figure 2), except that the molecular and M − CH_3O_2N ions are shifted up in mass by 16 amu. The monohydroxylated status of III is indicated by the occurrence of the base peak at *m/e* 141 (a phenolic derivative would have this peak shifted to *m/e* 157) and of the M − C_4H_7O ion at *m/e* 202.

The mass spectrum of IV (Figure 5) exhibits the familiar peaks at *m/e* 141, characteristic of the *p*-chlorobenzyl grouping, and *m/e* 202, the rearrangement ion. The carboxylic acid structure IV best explains the mass spectral data, with the ion at *m/e* 269 corresponding to M − H_2O. However, IV may exist as the corresponding imide under certain conditions.



m/e 269

An equilibrium between the carboxylic acid and the imide would explain why the compd can be extd into organic solvents at pH 7 and back-extd into an aq phase at pH 11.

The mass spectrum of V (Figure 6) is remarkable in that the base peak has been shifted to *m/e* 139, indicating that oxidn of the benzylic position has occurred. Except for the shift of 2 amu, this spectrum is very similar to that in Figure 4. The rearrangement elimination of C_4H_7O results in the peak at *m/e* 200, and the ion at *m/e* 210 corresponds to M − CH_3O_2N. No molecular ion is observed.

Optical Activity of Metabolites.—1-(*p*-Chlorophenyl)-2,2-dimethyl-1,3-propanediol 3-carbamate (I) is a racemate contg an asymmetric C. I, as isolated from human, pig, and dog urine, was optically active. The sp rotations of these 3 isolates were −13°, −14°, and −15°, resp. Compd I from rabbit urine was inactive and could not be recovered in sufficient quantity for detn of optical activity from rat and monkey urine. The optically active prepns gave mass spectra identical with those of the racemate.

Compd II could be isolated in a quant sufficient for the detn of optical activity only from human urine. The sp rotation of this prepn was −5.3°.

Compd III contains an additional asymmetric C. Isolates from rat, rabbit, monkey, and pig urine were optically inactive and melted between 190 and 194° dec. Those from dog urine, however, showed various degrees of optical activity, the sp rotation ranging from +20° to +31°. Furthermore, they melted over an extremely wide range (140–190°). Attempts to sep these mixts into optically pure components were only partially successful; on recrysn, the optically active isomer tended to accumulate in the mother liquors. Optically active and inactive preparations had the same elementary composition and identical ir and mass spectra. *Anal.* ($C_{12}H_{16}ClNO_4$) C, H, N.

Species Differences in the Biotransformation of the Drug.—Table III shows the occurrence of these compounds in the urines of the 6 species and gives a semiquantitative estimate of the relative quantity of each, based on the intensity of the staining reactions and the quantity recovered after prep tlc. In the nonhuman species, there were no differences in the excretion patterns found on the first day of drug administration and those found after 2–3 weeks of continuous administration. In the human, excretion patterns were detd only after administration of a single dose, except for the 800-mg dose, which was given, after a 1-week interval, to the same subjects who had received 400 mg. These subjects showed no differences in excretion pattern after the second dose.

Concentration of I in Human Serum.—The average serum levels of the free drug (I) after single dose administration are depicted in Table IV. No glucuronides or other metabolites were detected in these samples. In the 400- and 800-mg treatment groups, where the same subjects were dosed twice, there was no evidence of an increased rate of drug elimination after the second administration.

Discussion

A comparison of the biotransformation products of I eliminated by the 6 species reveals some common features as well as marked differences (*cf.* Table III). The "primary" transformations are the result of 3 metabolic reactions: hydrolytic cleavage of the carbamate group, oxidation of one of the Me groups, and oxidation of the 1-OH to C=O. The combination of the last 2 transformations constitutes a very minor pathway and leads to V in all species investigated.

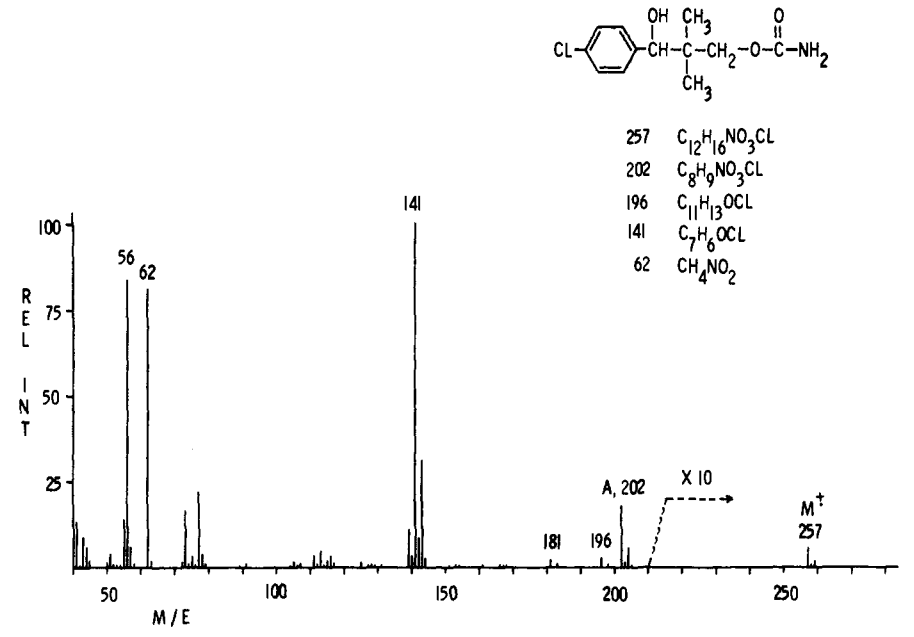


Figure 2.—Mass spectrum of 1-(*p*-chlorophenyl)-2,2-dimethyl-1,3-propanediol 3-carbamate.

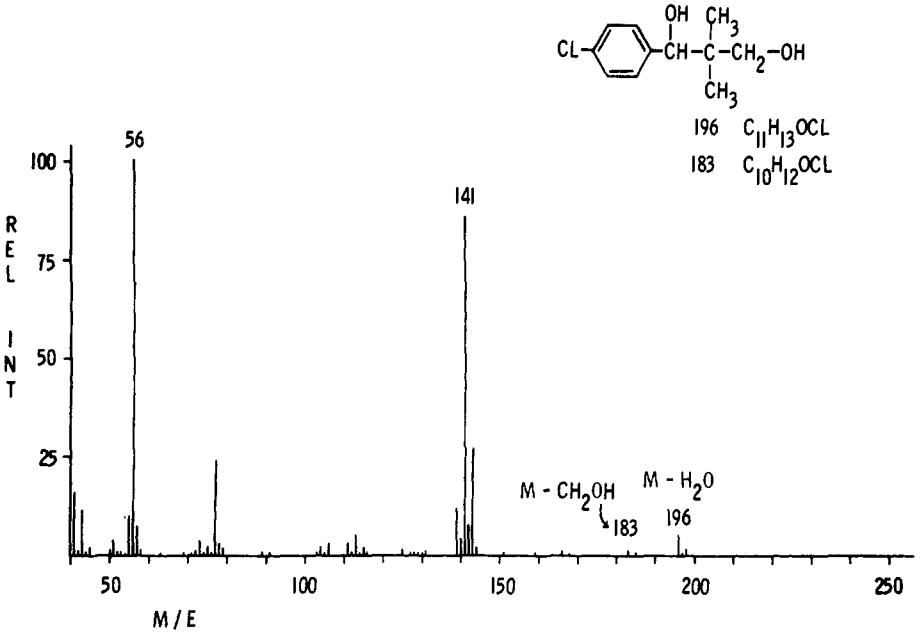


Figure 3.—Mass spectrum of 1-(*p*-chlorophenyl)-2,2-dimethyl-1,3-propanediol.

TABLE III

EXCRETION PATTERNS OF 1-(*p*-CHLOROPHENYL)-2,2-DIMETHYL-1,3-PROPANEDIOL 3-CARBAMATE AND ITS METABOLITES IN VARIOUS SPECIES

Metabolite	Man	Monkey	Pig	Rabbit	Dog	Rat
I glucuronide	+++[a,b]	++++	++++[b]	+++	++[b]	++
II glucuronide	++++[b]	+++	++	++	+	+
III glucuronide	0	++	+++	++++	++++[b]	+++++
IV glucuronide	0	0	0	0	++	0
V glucuronide	Trace	Trace	Trace	Trace	Trace	Trace

[a] Number of plus marks indicates relative quantity of each metabolite. "Trace" indicates that the quantity was less than 1%. [b] Optically active.

Hydrolytic cleavage, resulting in the formation of the diol II, is also common to all species, but its extent varies considerably from one species to another. Thus, it is the major biotransformation taking place in man, but plays only a minor role in the dog and the rat. In the other species this metabolic pathway is of intermediate importance. Oxidation of a Me group (without concurrent oxidation of the 1-OH) does not occur at all in humans but is significant in the monkey and pig, resulting in the formation of the hydroxymethyl derivative III by these species. This oxidation becomes the major pathway of metabolic transforma-

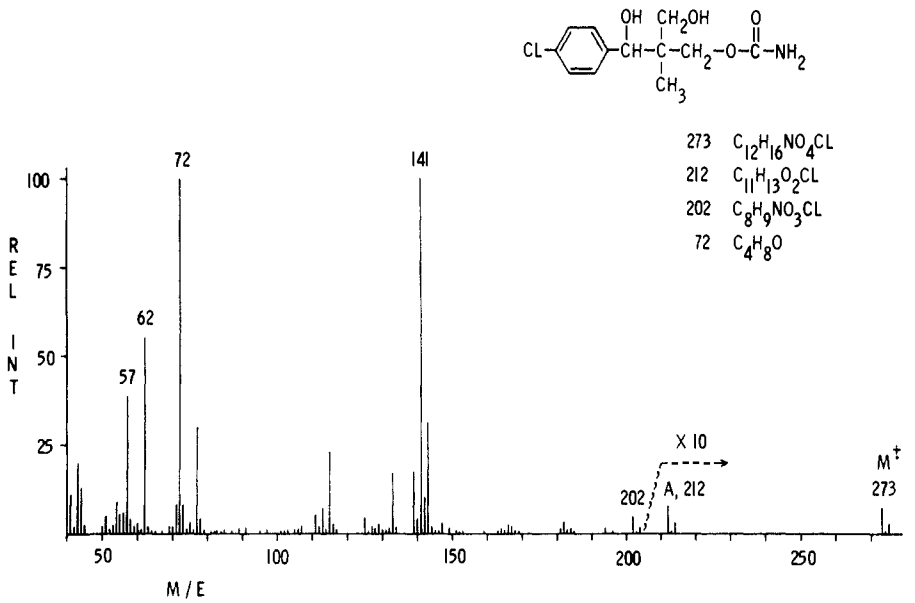


Figure 4.—Mass spectrum of 1-(*p*-chlorophenyl)-2-hydroxymethyl-2-methyl-1,3-propanediol 3-carbamate.

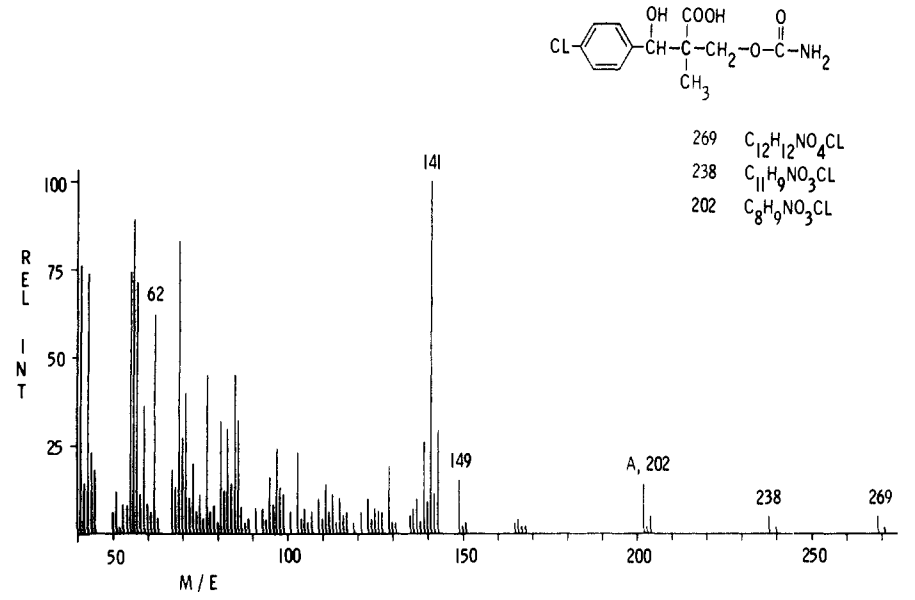


Figure 5.—Mass spectrum of 2-carboxy-1-(*p*-chlorophenyl)-2-methyl-1,3-propanediol 3-carbamate.

tion in the rabbit, rat, and dog. In the latter species, oxidative degradation goes one step further, leading to the carboxylic acid IV (which was not detected in any other species).

When the different ways by which the drug is transformed in the 6 species are compared, it appears that oxidative degradation becomes more important as hydrolytic cleavage recedes, and *vice versa*. No metabolites resulting from a combination of oxidative and hydrolytic transformations have been detected. In all species, a substantial fraction of the drug is neither oxidized nor degraded by hydrolytic cleavage, but is, instead, directly conjugated with glucuronic acid. Thus, the glucuronide of I is the major metabolite in the monkey and the pig. It is of intermediate importance in man and the rabbit, while the dog and rat excrete smaller (but still significant) amounts of this substance.

These findings indicate that monkeys and pigs metabolize this drug in a similar manner, while the biotransformations in the rabbit resemble those in the rat. However, no two species metabolize the drug in an exactly identical manner. The similarities and differences discussed above are of potential importance in the evaluation of pharmacological and toxicological observations in these species.

The elimination of I as the glucuronide is a feature that this compound has in common with other carbamate drugs possessing an OH group, *e.g.*, mephenesin carbamate,[6] chlorphenesin carbamate,[7-9] and methocarbamol.[10] However, the presence of significant amounts of the diol II in the urine of all species inves-

(6) A. P. Richardson, P. S. Jones, and H. A. Walker, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **12**, 361 (1953).
(7) D. R. Buhler, *J. Pharmacol. Exp. Ther.*, **145**, 232 (1964).
(8) D. R. Buhler, *Biochem. Pharmacol.*, **14**, 371 (1965).
(9) D. R. Buhler, H. Harpootlian, and R. L. Johnston, *ibid.*, **15**, 1507 (1966).
(10) A. D. Campbell, F. K. Coles, L. L. Eubank, and E. G. Huf, *J. Pharmacol. Exp. Ther.*, **131**, 18 (1961).

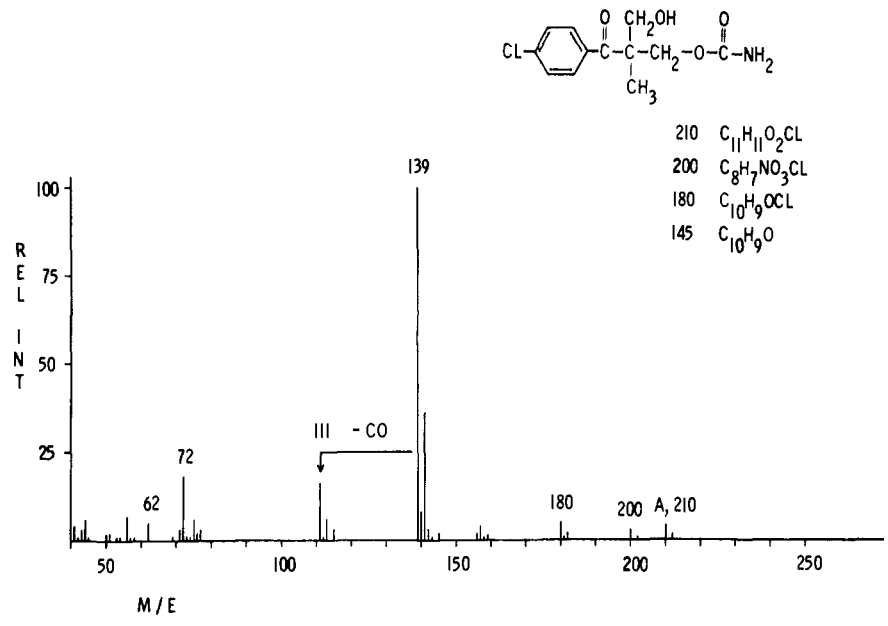


Figure 6.—Mass spectrum of 2-(*p*-chlorobenzoyl)-2-methyl-1,3-propanediol 3-carbamate.

TABLE IV

AVERAGE SERUM CONCENTRATIONS (μg/ml) OF THE FREE DRUG (I) IN HUMANS AFTER SINGLE DOSE ADMINISTRATION

No. of subjects	Dose, mg	0.5 hr	1 hr	3 hr	6 hr	12 hr
8	200	0.52	1.08	1.39	1.39	0.70
8	400	0.44	1.02	2.79	2.62	1.85
7	800	0.91	2.96	7.06	6.53	3.96
6	1600	2.58	6.08	10.07	10.97	8.37

tigated indicates that the carbamate group in I is not as stable *in vivo* as in some other carbamate tranquilizers, *e.g.*, meprobamate[11] and mephenesin carbamate.[6] No metabolites resulting from the hydrolytic cleavage of a carbamate group have been reported for these compounds.

Stepwise oxidation of the aliphatic side chain leading to carboxylic acids has been reported for several carbamates with a structure similar to that of I, *e.g.*, chlorphenesin carbamate[7,9] and phenprobamate.[12] In the present investigation, only one carboxylic acid (IV) was detected, and its formation occurred only in the dog.

The recovery of optically active metabolites from several species after administration of a racemic mixture indicates that at least part of the biotransformation process is stereoselective. Buhler[7-9] has reported similar findings for chlorphenesin carbamate.

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(11) S. S. Walkenstein, C. M. Knebel, J. A. MacMullen, and J. Seifter, *J. Pharmacol. Exp. Ther.*, **128**, 254 (1958).

(12) F. Schatz and U. Jahn, *Arzneim.-Forsch.*, **16**, 866 (1966).