

Metabolism of 3-(α,α,α -Trifluoro-*m*-tolylloxy)-1,2-propanediol 1-Carbamate in Man

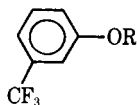
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Received November 30, 1970

3-(α,α,α -Trifluoro-*m*-tolylloxy)-1,2-propanediol 1-carbamate was rapidly and efficiently absorbed in man. Peak serum levels were observed 1–4 hr after oral administration and detectable levels persisted for at least 8 hr. Over 65% of the dose was excreted in the urine within 24 hr as the *O*-glucuronide. Small amounts were excreted as: (a) intact drug, (b) a sulfate conjugate, (c) 3-(α,α,α -trifluoro-*m*-tolylloxy)lactic acid, (d) α,α,α -trifluoro-*m*-tolylloxyacetic acid, and (e) α,α,α -trifluoro-*m*-cresol. A stereospecific metabolism was suggested.

3-(α,α,α -Trifluoro-*m*-tolylloxy)-1,2-propanediol 1-carbamate (I) is a centrally acting muscle relaxant^{1,2} similar to chlorphenesin carbamate, [3-(*p*-chlorophenoxy)-1,2-propanediol 1-carbamate].³ The metabolism of chlorphenesin carbamate in man and in animals has been reported.^{4–6} The present study was undertaken to determine the magnitude and duration of circulating drug levels and to characterize the urinary metabolite pattern in man following oral administration of large single doses of I.



I, R = CH₂CHOHCH₂OCONH₂
 II, R = CH₂CHOHCH₂OCONH₂;
 G = glucuronide
 III, R = CH₂CHOHCH₂OH
 IV, R = CH₂CHOHCO₂H
 V, R = CH₂CO₂H
 VI, R = H

Results and Discussion

Serum Drug Levels.—Concns of I in the serum of the 8 subjects over the 10-hr sampling interval are shown in Table I. Peak levels were observed 1–4 hr after

TABLE I
SERUM LEVELS OF I^a FOLLOWING ORAL ADMINISTRATION

Time, hr	Subject									Mean
	Fe	Sz	Ma	Mi	Mo	Pr	Br	Jo		
1	9.7	3.1	12.7	26.6	5.4	10.4	11.4	9.4		11.1
2	11.4	7.1	20.8	19.8	9.8	12.9	8.5	14.1		13.1
3	14.5	6.0	21.0	14.8	10.5	12.6	8.5	12.6		12.6
4	13.5	7.5	23.6	11.0	7.6	11.3	7.8	8.5		11.4
5	11.9	5.1	19.4	10.0	6.0	9.6	6.0	3.5		8.9
6	7.1	4.8	15.9	6.8	5.6	7.1	5.0	6.5		7.4
8	5.8	5.2	12.5	3.8	5.3	5.7	4.7	4.3		5.9
10	5.1	5.0	9.4	4.0	0	5.7	5.0	0		4.3

^a μ g/ml after 2-g oral dose.

oral administration indicating rapid absorption. Measurable levels were present in all subjects for at least 8 hr. However, for most of the subjects, the elimination of I could not be considered kinetically first

order. Deviations, suggestive of enterohepatic recirculation, occurred beyond 6 hr. Consequently, no attempt has been made to fit the data to a pharmacokinetic model.

Mean serum levels of I are shown in Figure 1; mean serum chlorphenesin carbamate levels found in another study with the same subjects after a 2-g dose of that drug are presented for comparison. Similarities of the two curves indicate that the rate of absorption, the distribution, and the rate of elimination of I closely approximate those for chlorphenesin carbamate. Durations of action for I and chlorphenesin carbamate should be similar.

Urinary Metabolite Pattern.—A pooled urine sample representing one-half of the 0- to 24-hr collection from each of two subjects was fractionated according to the procedure of Buhler.⁴ Distribution of materials among the 6 fractions is shown in Table II with similar data

TABLE II
DISTRIBUTION OF MATERIAL EXTRACTED FROM URINE

Fraction	Weight, mg	
	I-treated ^a	Control ^b
Nonconjugated neutral	61	97
Glucuronide neutral	1489	263
Sulfate neutral	194	263
Nonconjugated acidic	119	72
Glucuronide acidic	129	151
Sulfate acidic	59	151

^a 2-g oral dose. ^b Ref 4.

for control urine. Most of the drug-related material was excreted in the glucuronide neutral fraction.

Paper chromatog examination of the 3 neutral fractions indicated that each contained a single major metabolite which gave positive reactions for the intact carbamate group with *p*-dimethylaminobenzaldehyde as well as with the NaOCl-*o*-tolidine reagent and which was chromatographically indistinguishable from I. Crystalline material, isolated from the glucuronide neutral fraction, was identified as I by elemental and spectral analyses. Over 1300 mg of I was isolated (2 crystalline crops). Thus, over 65% of orally administered I was excreted as a glucuronide conjugate with additional small amts excreted intact and as a sulfate conjugate during 24 hr. Efficient oral absorption was indicated. No evidence for the excretion of 3-(α,α,α -trifluoro-*m*-tolylloxy)-1,2-propanediol (III), the hydrolysis product of I, was found.

Paper chromatog examination of the acidic fractions revealed small amounts of 5 metabolites, all of which were present in each of the fractions. Chromatog

(1) G. A. Youngdale, U. S. Patent 3,103,533 (1963); *Chem. Abstr.*, **59**, 11361 (1963).

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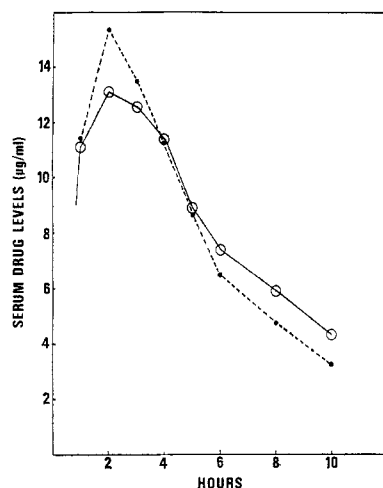


Figure 1.—Mean serum drug levels for 8 subjects following oral administration of 2 g of I (O—O) and chlorphenesin carbamate (---).

mobilities and responses to detection reactions are summarized in Table III. Comparisons with standards

TABLE III
PAPER CHROMATOGRAPHIC MOBILITIES AND DETECTION REACTIONS OF ACIDIC METABOLITES

Metabolite	Identity	<i>R_f</i>		Detection		
		IAW	BZAW	Uv ^a	BPB	DZSA
A		0.19	0.14	Bf	+	Yellow
B		0.47		Ab	+	
C	IV	0.60	0.60	Ab	+	
D	V	0.60	0.82	Ab	+	
E	VI	0.87	0.91	Ab		Yellow-brown

^a Bf = blue fluorescence; Ab = absorbing at 254 mμ.

have resulted in the tentative identification of metabolites C, D, and E as 3-(α,α,α -trifluoro-*m*-tolylloxy)lactic acid (IV), α,α,α -trifluoro-*m*-tolylloxyacetic acid (V), and α,α,α -trifluoro-*m*-cresol (VI). Metabolites A and B have not been identified.

To establish the nature of the glucuronide conjugate, an intact glucuronide fraction was isolated from a 0- to 48-hr urine sample by the basic lead acetate precipitation method of Kamil, *et al.*⁷ The crude glucuronide fraction was then methylated and acetylated.⁵ Tlc of the resulting product showed one *p*-dimethylamino-benzaldehyde positive zone with a color reaction and a mobility very similar to authentic methyl [3-(*p*-chlorophenoxy)-1,2-propanediol 1-carbamate 2-*O*-(tri-*O*-acetyl- β -D-glucosid)]uronate.⁵ Purification of this derivative by column chromatography gave a crystalline product characterized by elemental, functional group, and spectral analyses as methyl [3-(α,α,α -trifluoro-*m*-tolylloxy)-1,2-propanediol 1-carbamate 2-*O*-(tri-*O*-acetyl- β -D-glucosid)]uronate. Excretion of I as the *O*-glucuronide (II) was confirmed.

Metabolism of I in man closely resembles that of chlorphenesin carbamate. In each case the major urinary excretion product is the *O*-glucuronide. With each drug small amounts are excreted: (a) intact, (b) as a sulfate conjugate, (c) as the corresponding lactic and acetic acids produced by carbamate hydrolysis and

subsequent oxidation, and (d) as the corresponding phenols produced by *O*-dealkylation.

One difference between the disposition of I and of chlorphenesin carbamate in man was observed. In the latter case, following administration of racemic drug, optically inactive drug was isolated from the glucuronide neutral fraction. However, in the present study, I isolated from the glucuronide neutral fraction was optically active. This suggests either a preferential conjugation of one isomer of I or an enrichment in one isomer through selective absorption, distribution, hydrolysis and/or metabolism of the other. The present study does not permit a choice between alternatives. Such a stereochemical specificity in metabolism and excretion of chlorphenesin carbamate was found in the rat.^{4,5}

Experimental Section⁸

Study Design.—Eight normal adult male volunteers were fasted from 6:00 PM of the previous day until 11:00 PM of the test day; regular diet was permitted thereafter. Racemic I was administered orally as four 500-mg compressed tablets to each subject (except Pr who received four 500-mg hard-filled capsules) at 8:00 AM of the test day. H₂O was consumed at the rate of 230 ml every 2 hr for the first 12 hr and *ad lib* thereafter. Serum samples were collected at 0, 1, 2, 3, 4, 5, 6, 8, and 10 hr; samples were frozen until analyzed. Urine was collected under PhMe during the 0- to 24- and 24- to 48-hr periods and frozen for subsequent examination.

Serum Analyses.—Serum concns of I were detd by a modification of the procedure of Morgan, *et al.*⁹ Two ml each of serum and 0.2 N NaOH were mixed in a 32-ml glass-stoppered centrifuge tube, held 30 min in a boiling H₂O bath, rapidly cooled to room temp, and vigorously shaken with 25 ml of CHCl₃. Phases were sep'd by centrifugation for 5 min at 2000 rpm, the aq phase was discarded, and 20 ml of the CHCl₃ ext was transferred to a 50-ml round-bottom flask and carefully evap'd at atm pressure in a stream of N₂. H₃IO₆ (1 ml, 0.005 M) in 1 M H₂SO₄ was added to the residue, oxidn was conducted for 1 hr at room temp, and 0.5 ml of 0.1 M NaAsO₂ was added. After at least 15 min, 4 ml of chromotropic acid reagent (1.25 g in 20 ml of H₂O carefully dild to 200 ml with 12.5 M H₂SO₄) was added and thoroughly mixed; the soln was held in a boiling H₂O bath for 30 min, then rapidly cooled. Absorbance was detd at 570 nm *vs.* a reagent blank. Calens were based on a std curve obt'd by the anal. of serum samples contg known amts of added I. Such a curve was linear over the range 3–30 µg of I/ml of serum with a mean absorbance/µg per ml of 0.0104 at 570 nm and a coeff of variation of 5.1%. Results for each subject were corrected for the response obt'd for the pretreatment sample (serum blank) for the same subject. The mean serum blank for the 8 subjects was equiv to 3.8 µg of I/ml. The method is specific for I in the presence of its detected metabolites.

Fractionation of Metabolites.—One-half of the 0- to 24-hr urine sample from each of 2 subjects was combined and metabolites were fractionated by the method of Buhler.⁴ The non-conjugated fraction was extd from acidified urine with CHCl₃. The residual urine was then hydrolyzed with β -glucuronidase (Ketodase, Warner-Chilcott Co., Morris Plains, N. J.) and the glucuronide fraction was extd with CHCl₃. The resulting urine residue was in turn hydrolyzed with sulfatase (Glusulase, Endo Laboratories, Inc., Richmond Hill, N. Y.) and the sulfate fraction was extd with CHCl₃. Each CHCl₃ ext was further sep'd into neutral and acidic fractions.

Characterization of I from the Glucuronide Neutral Fraction.—The glucuronide neutral fraction was recrystd from EtOH–H₂O and then from CH₂Cl₂ to give 1011 mg of white crystals: mp

(8) Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements or functions, anal. results obtained for those elements or functions were within $\pm 0.4\%$ of the theor values. Spectra were obtained on Cary Model 14 (uv), Perkin-Elmer Model 421 (ir), and Varian Associates Model A60 (nmr) instruments.

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109–111.5° (lit.¹ mp 108–109°); $[\alpha]_D -6^\circ$ (c 0.64, CHCl_3); ir (mull) and nmr ($\text{DMSO}-d_6$) spectra identical with those for authentic I. *Anal.* ($\text{C}_{11}\text{H}_{12}\text{F}_3\text{NO}_4$) C, H, F, N.

Characterization of the Glucuronide of I.—A crude glucuronide fraction was isolated from the combined 0- to 48-hr urine of one subject using the basic lead acetate procedure.⁷ The glucuronide gum was methylated with CH_2N_2 in Et_2O , and the resulting ester was acetylated with pyridine- Ac_2O .⁵ The crude product was purified by chromatog on a column (2.3×29 cm) contg 50 g of 100–200 mesh Florisil (Fisher Scientific Co., Fair Lawn, N. J.). Sample was introduced to the column in CH_2Cl_2 and elution accomplished with Me_2CO -isooctane mixtures of increasing polarity. A yield of 458 mg of crude crystals was obtained. This material was recrystd twice from C_6H_6 -isooctane to give 285 mg of crystals: mp 168.5–169°; $[\alpha]_D -17^\circ$ (c 0.78, CHCl_3); uv indistinguishable from that of I (λ_{max} and ϵ); ir very similar to the corresponding methyl tri-*O*-acetyl derivative of chlorphenesin carbamate *O*-glucuronide⁵ and of the glucuronides of *p*-aminosalicylic acid,¹⁰ *p*-hydroxybenzoic acid,¹¹ and 3-hydroxycarbazole;¹² nmr (CDCl_3) consistent with the structure methyl [3-(α, α -trifluoro-*m*-tolxyloxy)-1,2-propanediol 1-carbamate 2-*O*-(tri-*O*-acetyl- β -*D*-glucosid)]uronate based on sharply defined acetate peaks at δ 1.83 and 2.03 (in the ratio of 1:2), a sharp peak at 3.75, broad multiplets attributed to carbinol and amide H between 3.5 and 5.5 and a typical meta-substituted aryl H pattern centered at 7.21 (all values in ppm, δ , downfield from Me_4Si); integration of the nmr spectrum accounted for 28 protons as 9 acetate, 3 Me, 4 Ar, and 12 carbinol and amide H. *Anal.* ($\text{C}_{12}\text{H}_{25}\text{F}_3\text{NO}_{13}$) C, H, N, Ac; F: calcd, 9.57; found, 10.06.

Chromatography.—Paper chromatog of the neutral fractions was carried out by the descending method on Whatman No. 2

paper (86 cm) in the Bush B-5 system (sheet equilibrated overnight at 34° in the vapor from a mixed solvent composed of C_6H_6 - MeOH - H_2O , 2:1:1, and developed with the C_6H_6 phase)¹³ and in the Mattox I system (sheet satd with MeOH -formamide, 1:1, dried 15 min at 37°, and developed with *n*- BuOAc -formamide- H_2O , 20:1:1).¹⁴ Acidic metabolites were chromatographed on Whatman No. 1 paper with *i*- PrOH - NH_4OH - H_2O , 8:1:1 (IAW)¹⁵ and with C_6H_6 - AcOH - H_2O , 1:1:2 (BZAW). All chromatograms were viewed with a short-wavelength scanner¹⁶ to detect zones absorbing uv light. Carbamate-contg metabolites were detected by spraying with *p*-dimethylaminobenzaldehyde or with NaOCl and *o*-tolidine.⁴ Acidic metabolites were sprayed with 0.1% bromphenol blue (BPB) to detect acids and with diazotized sulfanilic acid (DZSA) for visualization of phenols.

Tlc of methyl tri-*O*-acetyl glucuronides was carried out on silica gel GF (Brinkmann Instrument Co., Great Neck, N. Y.) using 1.5% (v/v) MeOH in CHCl_3 or on alumina GF with 0.75% (v/v) MeOH in CHCl_3 . Glucuronide esters were located by spraying the dried plates with 1% *p*-dimethylaminobenzaldehyde in 4 *N* HCl followed by 50% H_2SO_4 and subsequent heating to yield reddish zones.

Acknowledgments.—The authors thank Drs. S. S. Stubbs, E. W. Young, J. P. Webb, and H. L. Oster and Mr. M. J. White for assistance with the clinical portion of the study, Dr. G. A. Youngdale for synthesis of authentic compound IV, and Dr. G. Slomp for the nmr analyses.

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Synthesis and Analgetic Properties of Compounds Containing an Exocyclic Basic Center

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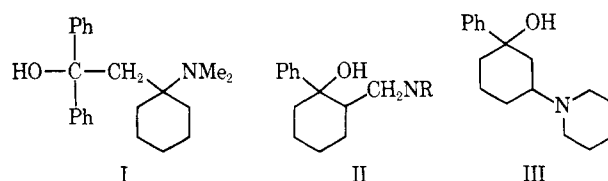
Received December 23, 1970

1-Dimethylamino-1-(2,2-diphenyl-2-hydroxyethyl)cyclohexane and 1-phenyl-3-piperidinocyclohexan-1-ol have been prepared and tested for analgetic activity. In the latter case the two geometrical isomers were separated and esterified. Analgetic activity was present only in the trans (*O/N*) isomers. The conclusion is drawn that steric restrictions in the environment of the receptor site may be responsible for lack of activity in the cis isomers.

A very large number of pethidine reversed ester compounds have been prepared in the search for synthetic analgetics of high potency and low toxicity.¹ Potency equal to or greater than morphine has often been achieved, but toxic side effects have always accompanied an increase and no compd of real clinical importance has emerged since the original reversed esters of α - and β -prodine were prepared by Ziering and Lee² in 1947.

It is my belief that an improvement in therapeutic ratio is more likely to be achieved with compds which show a more dramatic change in parent structure. The bulk of the evidence so far accumulated seems to indicate that an analgetic must possess a basic center and an aromatic ring which are separated by a suitable distance.

With this in mind, 3 structures were first considered in which the piperidine nitrogen of the reversed ester compds was moved outside the ring. Of these, various



structures of type II had already been prepared and found to possess little or no activity.³ Therefore, the syntheses of I and III were undertaken. During the course of this work, IV has been reported⁴ with an

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