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## REFERENCES

1. N. D. GOLDBERG and F. E. SHIDEMAN, *J. Pharmacol. exp. Ther.* **136**, 142 (1962).
2. W. C. LEE, Y.-H. SHIN and F. E. SHIDEMAN, *J. Pharmacol. exp. Ther.* **133**, 180 (1961).
3. R. W. RYALL, *Brit. J. Pharmacol.* **17**, 339 (1961).
4. J. H. BURN, in *Practical Pharmacology*, p. 35. Blackwell, Oxford (1952).
5. J. W. THOMPSON, *J. Physiol. (Lond.)* **141**, 46 (1958).
6. W. W. FLEMING and U. TRENDELENBURG, *J. Pharmacol. exp. Ther.* **133**, 41 (1961).
7. S. M. KIRPEKAR, P. CERVONI and R. FURCHGOTT, *J. Pharmacol. exp. Ther.* **135**, 180 (1962).
8. U. TRENDELENBURG, *J. Pharmacol. exp. Ther.* **134**, 8 (1961).
9. C. B. SMITH, *J. Pharmacol. exp. Ther.* **142**, 163 (1963).
10. G. PEPEU, M. ROBERTS, S. SCHANBERG and N. J. GIARMAN, *J. Pharmacol. exp. Ther.* **132**, 131 (1961).

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# Characterization of the glucuronide conjugate of chlorphenesin carbamate from the rat and from man

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THE excretion and metabolism of chlorphenesin carbamate* [1,2-propanediol,3(*p*-chlorophenoxy)-1-carbamate] in rats and humans has recently been described.[1] A major portion of the drug was excreted into the urine as a conjugate which could be hydrolyzed by incubation with β-glucuronidase. The recent characterization of the N-glucuronide derivatives of the carbamate-type drugs meprobamate[2, 3] and ethinamate,[4] however, suggested the possibility that chlorphenesin carbamate might be excreted as both a carbamate N-glucuronide as well as the conventional O-glucuronide. Therefore, it was of considerable interest to establish the nature of the chlorphenesin carbamate conjugates excreted by both the rat and by man.

## METHODS

Normal human subjects received 2 g of (±)-chlorphenesin carbamate orally in capsules, and urine was collected over a 24-hr period. In animal studies, four male Wistar rats were each given oral doses of 200 mg (±)-chlorphenesin carbamate/kg every 24 hr for 5 days. The excreted urine was collected for 6 days and pooled. The crude glucuronide fraction was isolated from the urine samples by the basic lead acetate precipitation method of Kamil *et al.*[5] The resultant glucuronide gum was methylated with ethereal diazomethane and then acetylated with acetic anhydride in the presence of pyridine. The crude glucuronide ester mixtures were purified by chromatography on either a silica gel column with ethyl ether–benzene and ethyl ether–ethyl acetate mixtures of increasing polarity or on

* Registered by The Upjohn Company under the trade name of Maolate.

a Florisil column after development with acetone–isooctane mixtures. Thin-layer chromatography of methyl acetyl glucuronides was carried out on silica gel GF in 1·5% methanol in chloroform or on alumina GF in 0·75% methanol in chloroform. Glucuronide esters were detected by their u.v. absorption and by their reaction with 1% *p*-dimethylaminobenzaldehyde in 4 N hydrochloric acid or with 50% sulfuric acid. Aliquots of urine and crude glucuronide gum were chromatographed on paper in butanol:acetic acid:water (4:1:5) and isopropanol:aqueous ammonia:water (8:1:1). Dried chromatograms were examined under u.v. light and carbamates located by spraying with 1% *p*-dimethylaminobenzaldehyde or with sodium hypochlorite and *o*-tolidine.[1]

## RESULTS AND DISCUSSION

Paper chromatography of unhydrolyzed rat or human urine showed a major zone ($R_F$ 0·49 in isopropanol–ammonia–water and 0·41 in butanol–acetic acid–water) which absorbed u.v. light, gave a positive test for carbamates[1] and, in addition, a positive naphthoresorcinol test characteristic of glucuronides.[6] In the previous studies[1] this zone was shown to correspond to a major radioactive component in the urine of rats dosed with radioactive chlorphenesin carbamate. This zone disappeared upon incubation of the urine with β-glucuronidase with a concomitant increase in the amount of unconjugated chlorphenesin carbamate.

The crude human glucuronide fraction was methylated and acetylated and then purified by chromatography on a Florisil column. Yellowish crystals were obtained from the acetone eluate; however, thin-layer chromatography on alumina demonstrated that this material was a mixture. The two components, present in approximately equal concentration, showed an $R_F$ of 0·38 and 0·47, respectively, and gave a spray reaction characteristic for a free carbamate group. Recrystallization of this mixture from aqueous methanol again produced mixtures. When the mixture was recrystallized twice from methylene chloride, however, crystals (m.p. 169°–170°) were obtained. Alumina thin-layer chromatography indicated that this isolate contained only the less polar component (designated isomer I). The nuclear magnetic resonance spectrum† was consistent with that expected for methyl (chlorphenesin carbamate tri-O-acetyl-β-D-glucoside) uronate. The spectrum showed sharply defined acetate peaks at 1·83 and 2·00 ppm (in the ratio of 1 to 2 respectively), a sharp methyl peak at 3·74 ppm, broad multiplets attributed to carbinol and amide hydrogens between 4 and 5 ppm, and a typical *p*-substituted aryl-hydrogen pattern centered at 7·02 ppm. Integration of the Nuclear Magnetic resonance spectrum accounted for all 28 protons and showed 9 acetate hydrogens, 3 methyl hydrogens, 4 aryl hydrogens, and 12 carbinol and amide hydrogens. The u.v. absorption spectrum‡ of this derivative exhibited maxima at 228 Mμ ($\epsilon$ = 13,400), 274 mμ ($\epsilon$ = 1,230), 281 mμ ($\epsilon$ = 1,630) and 280 mμ ($\epsilon$ = 1,330) which was consistent with a *p*-chlorophenoxy structure. The infrared spectrum§ with $\lambda_{max}$ at 3,460, 3,372, 1,750, 1,700, 1,610, 1,595, 1,495, 1,245, 1,225, 1,170, 1,095, and 1,040 $cm^{-1}$ was in agreement with that expected for a methyl tri-O-acetyl ester of chlorphenesin carbamate O-glucuronide. The mass spectrum¶ of this material exhibited molecular ions at 561 and 563 mass units in an approximate ratio of 3 to 1 consistent with the $Cl^{35}$ and $Cl^{37}$ derivatives of the proposed structure. The $[\alpha]_D$ was −16° (C = 0·76, $CHCl_3$).

*Anal.* Calcd. for $C_{23}H_{28}O_{13}NCl$: C, 49·16; H, 5·02; O, 37·01; N, 2·49; Cl, 6·31; $COOCH_3$, 23·02. Found: C, 49·37; H, 5·08; O, 37·40 (direct); N, 2·53; Cl, 6·26; $COOCH_3$, 23·11.

Nuclear magnetic resonance analyses of the original crystalline mixture prior to isolation of isomer I showed it to be a mixture composed of isomer I plus a second component designated isomer II. Attempts to isolate pure isomer II have resulted only in the recovery of mixtures rich in isomer II. A typical mixture obtained after several recrystallizations from benzene–isooctane had a m.p. 131°–133°; nuclear magnetic resonance analysis of this mixture indicated that it contained 70% isomer II and 30% isomer I. Isomer II was characterized by sharply defined acetate peaks at 2·00 (coinciding with

† Determined in $CDCl_3$ in a Varian model A-60 apparatus. Values reported are in parts per million (ppm) downfield from the tetramethylsilane internal standard. The author is indebted to G. Slomp and F. MacKellar, Physical and Analytical Chemistry Research, The Upjohn Co, for preparing and interpreting these analyses.

‡ Determined in ethanol in a Cary model 14 apparatus.

§ KBr pellet i.r. spectra were obtained on a Perkin-Elmer model 421 apparatus.

¶ Mass analysis carried out on an Atlas CH-4 mass spectrometer by R. J. Wnuk, Physical and Analytical Chemistry Research, The Upjohn Co.

a similar isomer I peak) and 2·05 ppm, a sharp methyl peak at 3·70 ppm, several changes in the carbinol and amide-hydrogen region (4 to 5 ppm), and a similar aryl pattern in the region of 7 ppm. The elemental analysis of this mixture was very similar to that observed for the purified isomer I, indicating that isomer II had the same composition. The i.r. spectrum of this mixture was very similar to that for isomer I except for small differences in the 1,700–1,600 $cm^{-1}$ region. The $[\alpha]_D$ of $-7°$ (C = 0·92, $CHCl_3$) for the isomer II-rich mixture suggested that isomer II was probably methyl [( + )-chlorphenesin carbamate-tri-O-acetyl-β-D-glucoside] uronate and that isomer I was the ( − ) diastereoisomer. The observed differences in the nuclear magnetic resonance spectra of these two diastereoisomers provide evidence for differences in hydrogen bonding between the two molecules.

Paper chromatographic examination of untreated urine from rats which had been dosed with chlorphenesin carbamate showed that rats produced smaller amounts of the chlorphenesin carbamate O-glucuronide ($R_F$ 0·41 in isopropanol–ammonia–water). After methylation and acetylation of the crude glucuronide fraction and purification on a silica gel column, fine colorless needles, m.p. 170°–171°, were obtained upon recrystallization twice from ethyl acetate–isooctane. The infrared and nuclear magnetic resonance spectra were identical with those obtained previously with isomer I, and the $[\alpha]_D$ was $-16°$ (C = 0·36, $CHCl_3$).

*Anal.* Calcd. for $C_{23}H_{28}O_{13}NCl$: C, 49·16; H, 5·02; N, 2·49; Cl, 6·31. Found: C, 48·99; H, 6·17; N, 3·17; Cl, 6·41.

The present experiments demonstrate that in humans who have received orally administered chlorphenesin carbamate, the bulk of the glucuronide conjugate of the drug excreted into the urine was recovered as two isomeric methyl-tri-O-acetyl esters. Analyses of these esters were in agreement with those expected for the methyl tri-O-acetyl esters of ( + ) and ( − ) chlorphenesin carbamate O-glucuronide. After administration of ( ± )-methylphenylcarbinol to rabbits, such a mixture of ( + ) and ( − ) diastereoisomers is formed upon conjugation of the drug with glucuronic acid.[7] Conjugation of chlorphenesin carbamate at the carbamate nitrogen atom, however, would be expected to form two isomeric N-glucuronides which would in turn yield tetraacetates upon acetylation. In addition, the methyl tetraacetyl N-glucuronide esters would not be able to react with *p*-dimethylaminobenzaldehyde.

The formation of an equimolar mixture of ( + ) and ( − ) diastereoisomers of chlorphenesin carbamate O-glucuronide in humans is presumed from the lack of optical activity in chlorphenesin carbamate recovered after cleavage of the glucuronides with β-glucuronidase.[1] In rats, on the other hand, thin-layer chromatography provided no evidence for the presence of the ( + ) isomer, and only the methyl [( − )-chlorphenesin carbamate-tri-O-acetyl-β-D-glucoside] uronate was isolated. The preferential formation of one isomer of chlorphenesin carbamate O-glucuronide in rats was also inferred from the isolation of optically active chlorphenesin carbamate from urine which had been treated with β-glucuronidase.[1] No evidence for the formation of chlorphenesin carbamate N-glucuronide was found in either the rat or human studies.

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## REFERENCES

1. D. R. BUHLER, *J. Pharmacol. exp. Ther.* **145**, 232 (1964).
2. B. J. LUDWIG, J. F. DOUGLAS, L. S. POWELL, M. MEYER and F. M. BERGER, *J. med. pharm. Chem.* **3**, 53 (1961).
3. H. TSUKAMOTO, H. YOSHIMURA and K. TATSUMI, *Chem. pharm. Bull.* **11**, 421 (1963).
4. *Ibid.*, p. 1134.
5. I. A. KAMIL, J. N. SMITH and R. T. WILLIAMS, *Biochem. J.* **50**, 235 (1951).
6. G. HARRIS and I. C. MACWILLIAM, *Chem. and Ind.* 249 (1954).
7. J. N. SMITH, R. H. SMITHIES and R. T. WILLIAMS, *Biochem. J.* **56**, 320 (1954).