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Metabolism of 3-(p-Chlorophenoxy)-2-Methoxypropyl Carbamate in the Rat. (32122)

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Alkyl ethers are reported to be quite stable in animals and are usually excreted unchanged (1). Such ethers, in addition, do not serve as substrates for the liver microsomal enzyme responsible for cleavage of aromatic ethers (2) although glycerol ethers of long chain fatty alcohols can be split by a similar microsomal system(3).

Recently it has been shown that the muscle relaxant drug chlorphenesin carbamate† [3-(p-chlorophenoxy)-2-hydroxypropyl carbamate], a compound potentially capable of forming both O- and N-glucuronides(4) *in vivo,* was excreted only as the O-glucuronide from the rat and human(5). The methyl ether of chlorphenesin carbamate has now been investigated to determine if blockage of the hydroxyl group would result in N-glucuronide formation. The present report describes the fate of 3-(p-chlorophenoxy)-2-methoxypropyl carbamate‡ (MCC, Fig. 1) in the rat.

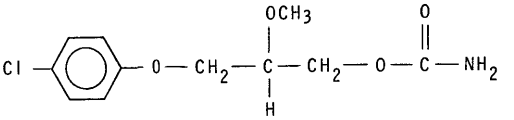

FIG. 1. 3-(p-Chlorophenoxy) - 2 - methoxypropyl carbamate.

Experimental. Four male Wistar rats weighing 260-290 g were each given an oral dose of 54 mg MCC in 2 ml homogeneous saline suspension (dose approximately 190 mg/kg). Administration was repeated every 24 hours for 5 days until the animals had received a total dose of 1.08 g of drug. Following initial dosing, the animals were placed in individual metabolism cages and urine was collected under toluene for a period of 7 days. Urine samples were removed every 24 hours, pooled and frozen.

The pooled 7-day urine was thawed, adjusted to pH 4 with glacial acetic acid and extracted 4 times with ½ volume of chloroform. The chloroform extract containing the nonconjugated metabolites then was fractionated into nonconjugated neutral and non-

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† Maolate is The Upjohn Co. trademark for chlorphenesin carbamate.

‡ Synthesized by G. Youngdale, Chemistry Research, The Upjohn Co.

conjugated acidic fractions(6). The urine residue from the chloroform extractions was subjected to the basic lead acetate precipitation of Kamil *et al*(7) to give the crude glucuronide fraction. The resultant glucuronide gum was treated with ethereal diazomethane and then acetylated with acetic anhydride in the presence of pyridine. The mixture of glucuronide esters was separated on a silica gel column by elution with ethyl ether-benzene and ethyl ether-ethyl acetate mixtures of increasing polarity.

In vitro studies on the hydrolysis of MCC and related compounds were carried out with a rat liver microsomal system(2). Male Sprague-Dawley rats were sacrificed, the livers removed and homogenized in 2 volumes of cold 0.154 M KCl. The homogenate was centrifuged at 10,000 $\times$ *g* for 20 minutes and the supernatant fraction containing the microsomes was employed for enzyme studies. One ml of the supernatant fraction was incubated for 1 hour at 37°C in air with 10 μmoles of substrate (MCC, chlorphenesin or chlorphenesin carbamate), 100 μmoles nicotinamide, 75 μmoles $MgCl_2$, 25 μmoles glucose-6-phosphate, 0.4 μmoles TPN and 360 μmoles phosphate buffer pH 7.4 in a total volume of 5 ml. The reaction was terminated by addition of dilute HCl, the incubation mixture saturated with NaCl, extracted twice with chloroform and separated into neutral and acidic subfractions(6). Neutral extracts were chromatographed on silica gel thin-layer plates in methanol-chloroform (1:19). Developed plates were examined first under ultraviolet light and then sprayed to detect carbamate-type compounds (6). Acidic fractions were chromatographed on silica gel thin-layer plates in chloroform-methanol-formic acid-water (1000:100:4:96). Acids were detected following spraying with bromophenol blue indicator and phenolic compounds were visualized by spraying with diazotized sulfanilic acid. Appropriate reference standards were run on each plate.

Results. Paper chromatography of untreated urine from rats following administration of MCC showed a pattern very similar to that observed from rats which had received chlorphenesin carbamate(5). In both cases, a major UV absorbing, carbamate spray positive(6) zone was detected in butanol-acetic acid-water (4:1:5) at R_f 0.42 and in isopropanol-aqueous ammonia-water (8:1:1) (IAW) at R_f 0.41. This spot had been found previously to correspond to a glucuronide conjugate of chlorphenesin carbamate(5).

Chromatography of the nonconjugated neutral extract also showed a pattern identical to that observed when chlorphenesin carbamate was administered to rats(6) with a single carbamate spray-positive zone having a mobility of 30.0 cm in the Bush B-5 system(8) and 28.8 cm in the FBW system(9). These properties corresponded exactly to those of authentic chlorphenesin carbamate. No evidence was obtained for the presence of any unchanged MCC in the nonconjugated neutral extract. Recrystallization of the nonconjugated neutral fraction twice from methylene chloride gave crystals m.p. 87-89° which exhibited an infrared spectrum identical to that of authentic chlorphenesin carbamate.

Chromatography of the nonconjugated acidic extract fraction in isopropanol-aqueous ammonia-water (8:1:1) and benzene-acetic acid-water (1:1:2) systems gave a pattern identical in all respects to that observed when chlorphenesin carbamate was administered to rats(6). The presence of p-chlorophenol, p-chlorophenoxyacetic acid and p-chlorophenoxylactic acid was readily detectable.

Fractionation of the methylated and acetylated glucuronide fraction on silica gel afforded 457 mg of glassy product, which upon recrystallization twice from ethyl acetate-isooctane gave needles m.p. 171-2°. The infrared spectrum of this material was identical to that observed for methyl [(-)-chlorphenesin carbamate-tri-O-acetyl-β-D-glucoside]uronate isolated from rats and from humans(5). The $(\alpha)_D$ was $-12°$ (C = 0.48, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{28}O_{13}NCl$: C, 49.16; H, 5.02; Cl, 6.31. Found: C, 49.31; H, 4.95; Cl, 6.51.

When MCC and related compounds were incubated with a rat liver microsomal preparation under the conditions employed by Axelrod(2), about 5 to 10% of the MCC was converted to chlorphenesin carbamate. How-

ever, the microsomal system failed to cleave the aryl ether linkage of MCC, chlorphenesin or chlorphenesin carbamate.

Discussion. Results of the present study suggest that the O-demethylation of MCC and its metabolites which occurs *in vivo* may be catalyzed by an enzyme system found in liver microsomes. However, the possible contribution of enzymes from other tissues or the gut microflora has not been excluded.

Cleavage of such alkyl-alkyl ethers *in vivo* is relatively uncommon in nature. Diethyl ether and divinyl ether are excreted unchanged by dogs and rabbits(10) and ethyl, methyl and vinyl cyclopropyl ethers are eliminated intact in the breath of humans(11). However, the drug diphenhydramine is dealkylated *in vivo* to yield benzhydrol and dimethylaminoethanol(12,13) and evidence for the O-demethylation of 2,2-dichloro-1,1-difluoroethyl methyl ether by the intact rat and rat liver preparations has been recently presented(14,15). Although the aliphatic ethers 3-methoxypropylamine, methoxyacetic acid and 1,2-dimethoxyethane were not hydrolyzed by liver microsomes(2), hydrolysis of long chain alkyl ethers of glycerol has been observed with a similar microsomal system supplemented with tetrahydropteridine(3). These results suggest that hydrolysis of alkyl-alkyl ethers by liver microsomes may require reduced pteridines for maximal activity.

Although phenolic cleavage products of MCC were formed by rats *in vivo,* hydrolysis of the aryl-alkyl linkage of MCC, chlorphenesin or chlorphenesin carbamate could not be demonstrated in a liver microsomal system. It is, of course, possible that optimum *in vitro* assay conditions were not employed and that studies with liver slices or a perfused liver preparation would show activity. However, recent studies by McMahon *et al* (16) suggest that the rate of cleavage of aryl-alkyl ethers by rat liver microsomes decreased with increasing length and electron withdrawing ability of the alkyl side chain. Consequently, the rate of aryl-alkyl ether cleavage of MCC by liver microsomes would probably be too low to detect *in vitro* or to account for the extent of *in vivo* hydrolysis. Therefore, the site for cleavage of the aryl-alkyl ether linkage of MCC and its metabolites *in vivo* observed in the present study remains yet to be established.

Summary. The results from the present study with orally administered 3-(p-chlorophenoxy)-2-methoxypropyl carbamate (MCC) in rats indicate that the absorbed drug was completely demethylated *in vivo* since no metabolites with intact methoxyl groups were detected. The principal urinary metabolite in the rat was chlorphenesin carbamate O-glucuronide; however, a significant portion of the MCC or its metabolites also were dealkylated *in vivo* to yield p-chlorophenol. In addition, no evidence for formation of an N-glucuronide conjugate was obtained. *In vitro* studies have shown that while cleavage of the methoxyl group of MCC or its metabolites can take place in liver microsomes, the enzymatic hydrolysis of the aryl-alkyl ether linkage of MCC probably occurs at some other site.

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