Synthesis of Glucosides of Isopropyl 3-Chlorocarbanilate Metabolites

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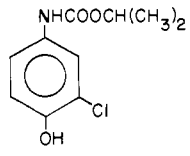
Glucosides of two previously identified soybean metabolites of isopropyl 3-chlorocarbanilate were prepared. Reaction of isopropyl 5-chloro-2-hydroxycarbanilate (1) with tetra-*O*-acetyl-α-D-glucopyranosyl bromide and sodium hydroxide in aqueous acetone for 4 hr at 23° gave 29% of the tetraacetate of the *O*-β-D-glucoside of **1**, mp 120.5–122°. Deacylation by methanol in the presence of sodium methoxide for 40 min at 23° yielded 86% of the *O*-β-D-glucoside of **1**, mp 172–173°. Similar treatment of isopropyl 3-chloro-4-hydroxycarbanilate (2) yielded 32% of the tetraacetate, mp 144–145.5°, which, upon deacylation, gave 86% of the *O*-β-D-glucoside of **2**, mp 193°.

Recently, Still and Mansager (1973) identified two metabolites of isopropyl 3-chlorocarbanilate (chlorpropham) from conjugates in soybean plants grown in a nutrient solution containing the herbicide. The aglucones were shown to be isopropyl 5-chloro-2-hydroxycarbanilate (1) and isopropyl 3-chloro-4-hydroxycarbanilate (2).

As an aid in the development of analytical methods for chlorpropham metabolites, quantities of the aglucones and their glucosides were required. Aglucone **1** was converted to its glucoside tetraacetate (3) using the Michael synthesis, and the free glucoside (5) subsequently was obtained by the Zemplen deacetylation reaction (Figure 1). By an analogous procedure, **2** was converted to its glucoside (6) *via* the tetraacetate (4).



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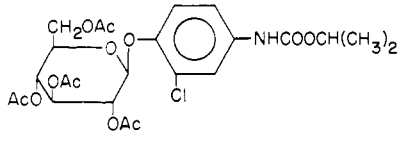

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As hydroxylation and subsequent glucoside formation is a common process in plants, and since there is no information in the literature on the synthesis of glucosides containing a carbamate function, these preparations are of general interest in carbamate metabolite studies.

EXPERIMENTAL SECTION

Elemental analyses were performed by Frank Melcher of PPG Industries and by Crobaugh Laboratories. The mass spectrum of **3** was run on a Hitachi Perkin-Elmer RMU-6D spectrometer by Morgan-Schaffer Corp. The nmr spectra were determined on a Varian DP-60 instrument using tetramethylsilane as an internal standard for **3** and **4** and as an external standard for **5** and **6**. Tetra-*O*-acetyl-α-D-glucopyranosyl bromide was prepared by a published method (Wolfrom and Tipson, 1957). Compounds **1** and **2** were prepared by the reaction of the appropriate aminochlorophenol with isopropyl chloroformate (Grunow *et al.*, 1970). The melting point of **2** was 90–91°; Grunow reported 84–86° and Bobik *et al.* (1972) reported 93–94°. The elemental analyses, ir, and nmr spectra of **1** and **2** were in accord with the proposed structures. The procedure for the preparation of **3** and **4** was based upon one found in Wolfrom and Tipson (1957) and that for deacylation to **5** and **6** was based upon one in Whistler *et al.* (1963).

2-Isopropoxycarbonylamino-4-chlorophenyl-2′,3′,4′,-6′-tetra-*O*-acetyl-*O*-β-D-glucopyranoside (3). A solution of 20.56 g (0.05 mol) of tetra-*O*-acetyl-α-D-glucopyranosyl bromide in 100 ml of acetone was added over a 10-min period to a solution of 11.48 g (0.05 mol) of **1** in 50 ml of 1.02 *N* (0.051 equiv) aqueous sodium hydroxide. The temperature was held at 20° by external cooling. The mixture was allowed to stand for 4 hr at room temperature, during which time two 20-ml portions of acetone were added in order to maintain homogeneity. The acetone was then removed *in vacuo* using a 30° water bath, and the residue treated with 50 ml of benzene and 25 ml of 1.02 *N* aqueous sodium hydroxide solution. After separation, the organic phase was washed with two 10-ml portions of water, followed by removal of the benzene at reduced pressure to give 26.5 g of brown residue. Recrystallization from methanol, followed by charcoal treatment in benzene and final recrystallization from ethanol, gave 7.98 g (29%) of white crystals: mp 120.5–122°; $[\alpha]^{20}D$ −39° (*c* 1.34, chloroform); ir (mull) 1700 shoulder (carbamate C=O), 1735 (acetate C=O), 3435 cm^{-1} (NH); nmr (acetone-d_6) δ 1.27 (d, J = 6 Hz, 6, isopropyl methyl), 1.98, 1.99, 2.00, 2.08 (4 s, 12, acetate), 4.08–4.42 (m, 3, H-5′,6′,6′), 5.00 (sept, J = 6 Hz, 1, isopropyl methine), 5.03–5.42 (m, 4, H-1′,2′,3′,4′), 6.87–8.24 (m, 3, aromatic), 7.42 (br s, 1, NH); mass spectrum (70 eV, direct introduction, 110°) *m/e* (rel intensity) 559 (0.004), 331 (8), 271 (2), 229 (2), 211 (2), 187 (3), 169 (56), 145 (5), 143 (3), 139 (4), 127 (15), 115 (4), 109 (46), 103 (4), 97 (5), 85 (3), 81 (5), 43 (100).

Anal. Calcd for $C_{24}H_{30}ClNO_{12}$: C, 51.48; H, 5.40. Found: C, 51.46; H, 5.59.

4-Isopropoxycarbonylamino-2-chlorophenyl-2′,3′,4′,-6′-tetra-*O*-acetyl-*O*-β-D-glucopyranoside (4). The reaction conditions and quantities were the same as used for **3**. As the crude product was a glass which could not be recrystallized, purification was effected by elution chromatography over 950 g of silica gel (Fisher no. S-622, as received) using a methylene chloride–ether system. The concentration of ether was slowly increased from 0 to 13%. A small amount of unreacted **2** was obtained from fractions containing 2–3% ether, and the desired product was obtained from eluent containing 10–13% ether. From 19 l. of eluent (10–13% ether) was obtained a total of 16.20 g of white solid, mp 68–134°, which was purified by fractional recrystallization from benzene–hexane solution. Slow cooling was essential to avoid the formation of fine crystals which could not be filtered efficiently. A total of 8.81 g (32%) of white crystals was obtained: mp 144–145.5°; $[\alpha]^{20}D$ −46° (*c* 1.31, chloroform); ir (mull) 1710 (carbamate C=O), 1750 (acetate C=O), 3375 cm^{-1} (NH); nmr (acetone-d_6) δ 1.25 (d, J = 6 Hz, 6, isopropyl methyl), 1.97, 2.02, 2.03, 2.03 (4 s, 12, 4 acetate), 4.03–4.42 (m, 3, H-5′,6′,6′), 4.98 (sept J = 6 Hz, 1, isopropyl methine), 5.00–5.50 (m, 4, H-1′,2′,3′,4′), 7.20–7.80 (m, 3, aromatic), 8.60 (br s, 1, NH).

Anal. Calcd for $C_{24}H_{30}ClNO_{12}$: C, 51.48; H, 5.40. Found: C, 51.77; H, 5.51.

2-Isopropoxycarbonylamino-4-chlorophenyl-*O*-β-D-glucopyranoside (5). To a mixture of 7.33 g (0.0131 mol) of **3** in 100 ml of dry methanol at room temperature was added 10 ml of 0.32 *N* methanolic sodium methylate.

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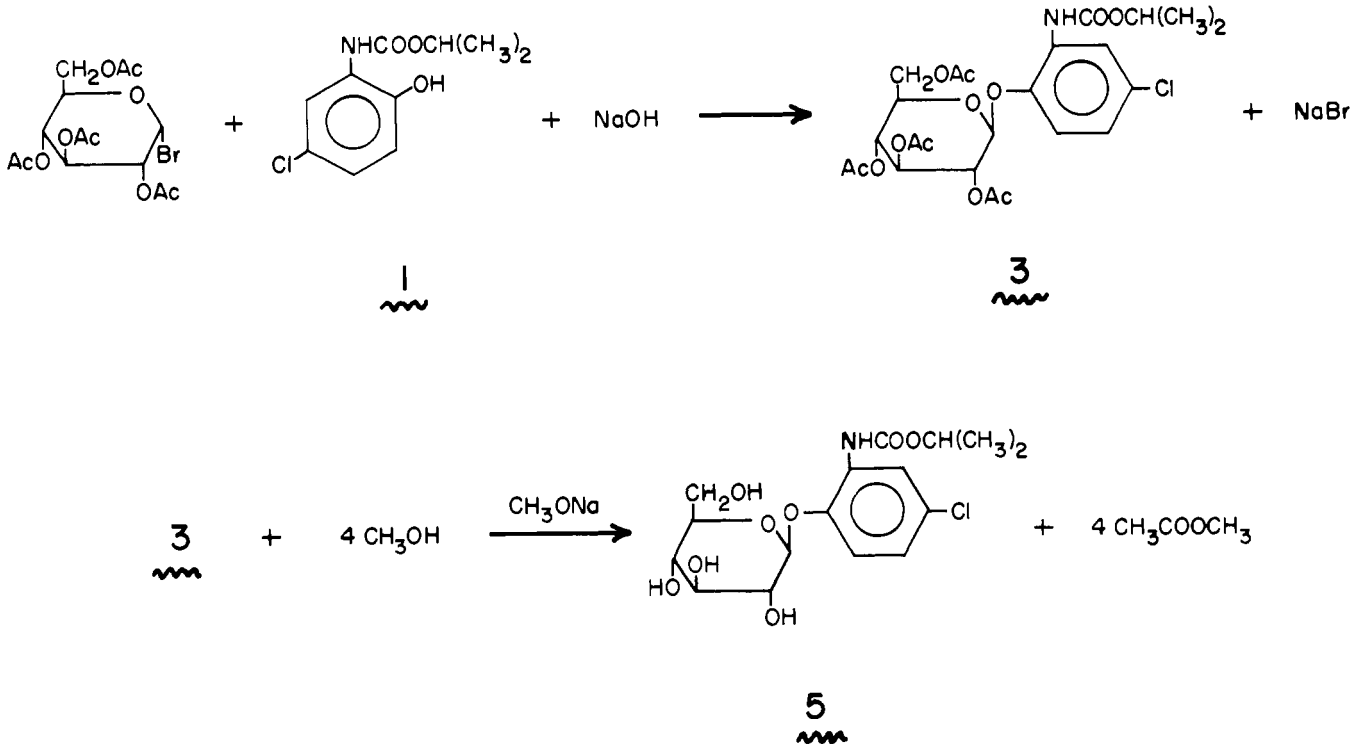

Figure 1. Glucoside synthesis route.

After standing 40 min at room temperature, the solution was added slowly to 100 ml of water kept saturated with carbon dioxide and maintained at 25°. The solvent was removed under vacuum until a residue of batter-like consistency was obtained. After filtration, the solid was again dissolved in methanol, water added, and solvent removed under vacuum to give a viscous slurry. Filtration, vacuum drying, and recrystallization from ethyl acetate gave 4.44 g (86%) of white crystals: mp 172–173°; $[\alpha]^{20}D$ −69° (*c* 1.31, 1:1 methanol-water); ir (mull) 1700 (C=O), 3380 (br, OH), 3510 cm^{-1} (NH); nmr (acetone-d_6) δ 1.11 (d, J = 6.5 Hz, 6, isopropyl methyl), 2.80–5.17 (m, H-1′,2′,3′,4′,5′,6′,6′), 4.84 (sept, J = 6.5 Hz, isopropyl methine), 6.70–8.03 (m, 3, aromatic).

Anal. Calcd for $C_{16}H_{22}ClNO_8$: C, 49.04; H, 5.66. Found: C, 48.95; H, 5.86.

4-Isopropoxycarbonylamino-2-chlorophenyl-*O*-β-D-glucopyranoside (6). The deacylation of 7.80 g (0.0139 mol) of 4 was carried out by the same procedure used for 3 except that the product was recrystallized from water instead of ethyl acetate. The 5.02 g (86%) of white needles so obtained was a monohydrate of 6 (mp 193°), which was quantitatively dehydrated by heating for 1 hr at 90° and 0.1 Torr: mp 193°; $[\alpha]^{20}D$ −64° (*c* 1.31, 1:1 methanol-water); ir (mull) 1690 (C=O), 3285 (NH), 3370 cm^{-1} (br, OH); nmr (acetone-d_6) δ 1.07 (d, J = 6.5 Hz, 6, isopropyl methyl), 2.84–5.00 (m, H-1′,2′,3′,4′,5′,6′,6′), 4.80 (sept, J = 6.5 Hz, isopropyl methine), 6.96–7.64 (m, 3, aromatic).

Anal. Calcd for $C_{16}H_{22}ClNO_8$: C, 49.04; H, 5.66. Found: C, 49.28; H, 5.76.

Ultraviolet Spectra. The uv maxima were determined in 1:3 water-methanol solutions. In neutral solution: **1**, 238 nm (ϵ 16,900) and 292 (1050); **2**, 240 (15,800) and 293 (2530); **5**, 239 (17,200) and 285 (2600); **6**, 240 (23,100) and 289 (2000); chlorpropham, 238 (16,900) and 277 (1050). In 0.1 *N* sodium hydroxide: **1**, 252 nm (10,800) and 312 (6660); **2**, 253 (15,400) and 307 (3450); **5**, 239 (17,600) and 285 (2700); **6**, 240 (23,100) and 289 (2000); chlorpropham, 238 (17,300) and 277 (1070).

Enzymatic Hydrolysis. Hydrolysis was carried out at 37–38° for 16 hr using 30 ml of a solution containing β-glucosidase (from almonds, Sigma Chemical Co.), phthalate buffer (pH 5.25), and the glucosides (18 μg of **5** and 23 μg of **6**). Analysis showed 12% hydrolysis of **5** and 99% hydrolysis of **6**. Phenyl β-D-glucopyranoside was 37% hydrolyzed under these same conditions. No hydrolysis occurred in the absence of β-glucosidase.

DISCUSSION

In the absence of any reports in the literature of syntheses of an aryl glucoside containing a carbamate function, there was uncertainty whether the carbamate group might be cleaved during the synthesis and also whether the reaction might proceed abnormally to attach the glucosyl moiety to nitrogen or to the aromatic ring.

The Michael synthesis of tetraacetates was chosen as the preferred method for the first of the two-step reaction sequence. While the yields of the tetraacetates were low (29% for **3** and 32% for **4**), it is not believed that cleavage of the carbamate group is the principal cause of the low yields, although efforts to identify the byproducts were unsuccessful. Elution chromatography of the crude products over silica gel showed the byproducts to elute after **3** and **4**, but no single component could be isolated. Modification of the Michael synthesis using the anhydrous sodium salt of **1** in ethanol or dimethylformamide gave products from which no **3** could be isolated. Also, the use of excess bromide failed to increase the yield.

Attempts to prepare the tetraacetate by other known synthetic routes were not promising. The reaction of **1** with α-glucosyl bromide in the presence of silver carbonate (Koenigs–Knorr method, deBelder *et al.*, 1962) resulted in reduction of the silver carbonate and oxidation of **1** to an insoluble material. With cadmium carbonate (Conrow and Bernstein, 1971) in place of silver carbonate the oxidation was avoided, but no **3** could be crystallized from the product. The preparation of **3** by the reaction of **1** with β-D-glucopyranose pentaacetate in the presence of boron trifluoride etherate (Helferich synthesis, Bretschneider and Beran, 1949) gave a 22% yield. Efforts to increase the yield were unsuccessful, and difficulty in separating the product from unreacted starting materials made the method less attractive than the Michael synthesis.

The possibility that compound **1** or **2** might react anom-

alously with the glucosyl bromide to give N-alkylation or phenyl alkylation was eliminated, for either possibility would result in a product containing a phenolic group. The absence of an aromatic hydroxyl was demonstrated by determining the uv spectra in neutral and in basic solution. Whereas the absorption maxima of **1** and **2** were shifted in the presence of sodium hydroxide, no shift occurred in the uv maxima of **5**, **6**, or indeed chlorpropham itself.

The Michael synthesis is known to yield β- rather than α-glucosides. Confirmation of the β configuration of compounds **3**, **4**, **5**, and **6** was obtained by determining their specific optical rotations. These rotations, ranging from $-39°$ to $-69°$, were in the range of values of the β isomers of known substituted phenyl glucosides and their tetraacetates ($-103°$ to $+45°$) rather than in the range of the corresponding α isomers ($+137°$ to $+212°$) (Bonner *et al.*, 1952). The susceptibility of **5** and **6** to hydrolysis by the enzyme, β-glucosidase, also indicates β configuration. Efforts to confirm the configuration of **3**, **4**, **5**, and **6** by their nmr spectrum were unsuccessful. The H-1′ doublet could not be observed because of signal overlap and difficulties with radiofrequency saturation.

The mass spectrum of **3** showed mass peaks corresponding to those reported by Still and Mansager (1972) for a sample of acetylated chlorpropham conjugate from soybeans.

In the final step of the reaction sequence, the carbamate group appeared to be quite stable. Deacylation occurred without any major tendency for replacement of isopropoxy by methoxy, as was evident by the 86% yield of both **5** and **6**.

The alternate route analogous to the preparation of glucuronides used by Paulson *et al.* (1972) was not considered, as the metabolites **1** and **2** were independently needed. His procedure involved the reduction of commercially available *p*-nitrophenyl-β-glucuronide, followed by reaction with isopropyl chloroformate.

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Absorption, Excretion, and Metabolism of Robenz, Robenidine Hydrochloride [1,3-Bis(*p*-Chlorobenzylideneamino)guanidine Hydrochloride], in the Rat

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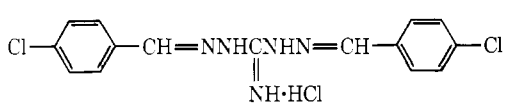
The results of the absorption, excretion, and metabolism in the rat of a new anticoccidial agent, 1,3-bis(*p*-chlorobenzylideneamino)guanidine hydrochloride [Robenz, robenidine hydrochloride], are presented. Rats given a single oral dose of robenidine hydrochloride, labeled with ^{14}C in the α-carbon atom of the *p*-chlorobenzylidene moiety, excreted approximately 58% of the radioactive dose in the feces and 20% in the urine within 24 hr. Tissue retention was low and accounted for 0.4% of the dose 96 hr after oral treatment. Two urinary metabolites were identified as *p*-chlorohippuric acid and *p*-chlorobenzoic acid and these accounted for 88 and 2%, respectively, of the total urinary radioactivity. Unmetabolized robenidine accounted for the major portion of the fecal radioactivity. Qualitatively, the metabolites isolated from the liver, kidney, and muscle were the same as those found in urine. *p*-Chlorohippuric acid appeared as the predominant metabolite in liver and kidney, while robenidine was predominant in skin and fat.

1,3-Bis(*p*-chlorobenzylideneamino)guanidine hydrochloride, the active ingredient in American Cyanamid's Robenz, robenidine hydrochloride medicated premix coccidiostat, is a new and highly effective product for preventing coccidiosis (Kantor *et al.*, 1970) and has been developed for use in broiler chickens. The absorption, excretion, and metabolism of ^{14}C-labeled robenidine hydrochloride (I) was studied in a mammal, the rat, as part of the program to aid toxicologists in evaluating robenidine data and in evaluating the safety to the consumer associated with the commercial use of this compound in poultry.



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Robenz, robenidine hydrochloride

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