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Syntheses of the β-D-Glucosides of 4- and 5-Hydroxy-1-naphthyl *N*-Methylcarbamate

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4- and 5-(*N*-Methylcarbamoyloxy)-1-naphthyl tetra-*O*-acetyl-β-D-glucopyranoside were synthesized by the condensation of the appropriate hydroxy-1-naphthyl *N*-methylcarbamate with β-D(+)-glucose pentaacetate in the presence of catalytic amounts of boron trifluoride–ether complex. Reaction of the acetylated β-D-glucosides with barium methoxide in methanol gave the corresponding β-D-glucosides. The ir, nmr, and mass spectra of the β-D-glucosides and their acetylated analogs are reported and compared. When administered ip to mice, 4-hydroxyl-1-naphthyl *N*-methylcarbamate was 28 times more toxic than its β-D-glucoside and 5-hydroxy-1-naphthyl *N*-methylcarbamate was 19 times more toxic than its β-D-glucoside. The methyl ester of the β-D-glucuronide of 5-hydroxy-1-naphthyl *N*-methylcarbamate was prepared in a manner similar to the glucosides. However, attempts to demethylate the product while leaving the carbamate ester intact were unsuccessful. Attempts to synthesize the glucoside of the 3-hydroxy derivative of carbofuran also were unsuccessful.

The metabolism of carbaryl (Sevin, 1-naphthyl *N*-methylcarbamate) in plants has been intensively investigated (Abdel-Wahab *et al.*, 1966; Dorough and Casida, 1964; Dorough and Wiggins, 1969; Kuhr and Casida, 1967; Mumma *et al.*, 1971). A review of the pathways of carbaryl and other methylcarbamate insecticide metabolism in plants has been recently reported (Kuhr, 1970). The predominant pathway involves oxidative metabolism, while hydrolysis usually occurs to a relatively small extent.

Oxidative metabolites of carbaryl which result from hydroxylation of the ring and the *N*-methyl group are not recovered as such, but are rapidly converted into stable water-soluble products by conjugation, mainly as glycosides (Kuhr and Casida, 1967; Mumma *et al.*, 1971). The major aglycones are 4-hydroxy-1-naphthyl *N*-methylcarbamate (4-hydroxycarbaryl), 5-hydroxy-1-naphthyl *N*-methylcarbamate (5-hydroxycarbaryl), 1-naphthyl *N*-hydroxymethylcarbamate (*N*-hydroxymethylcarbaryl), and *trans*-5,6-dihydro-5,6-dihydroxy-1-naphthyl *N*-methylcarbamate. While these metabolites also are present in mammals and insects they are readily eliminated, whereas plants store them as conjugates for a considerable length of time (Kuhr, 1970).

Although it has been reported that the water-soluble metabolites of carbaryl in bean plants were readily eliminated from the rat (Dorough and Wiggins, 1969), the metabolism and toxicological properties of carbaryl conjugate metabolites have not been critically evaluated. Since the aglycones possess anticholinesterase activity (Dorough, 1970), and their release from the conjugated form could occur in mammalian systems, the need for further study of these compounds is apparent. Such a study would be greatly facilitated by the chemical syntheses of the intact conjugate metabolites. This has been accomplished with certain carbaryl metabolites in the current investigation.

Chemical syntheses and acute toxicity of the β-D-glucosides of carbaryl metabolites, 4-(*N*-methylcarbamoyloxy)-1-naphthyl β-D-glucopyranoside and 5-(*N*-methylcarbamoyloxy)-1-naphthyl β-D-glucopyranoside, are reported. The preparation of their respective decarbamylated products, 4-hydroxy-1-naphthyl β-D-glucopyranoside and 5-hydroxy-1-naphthyl β-D-glucopyranoside, is also reported. These syntheses can provide material for toxicological evaluation and will enable identification of the intact plant conjugates without resorting to hydrolysis of the glycones. The synthetic conjugates may serve as standards to aid in the determination of the sugar moiety of carbaryl plant conjugates, an important consideration which up to this time has been lacking.

MATERIALS AND METHODS

Chemicals. 1,4-Naphthalenediol and 1,5-naphthalenediol were purchased from Eastman Kodak Co. 4- and 5-Hydroxycarbaryl were synthesized by the reaction of the corresponding naphthalenediol with methyl isocyanate (Knaak *et al.*, 1965). β-D(+)-Glucose pentaacetate, α-glucosidase, and β-glucosidase were purchased from Sigma Chemical Co., and boron trifluoride–ether complex (98%) was purchased from Matheson Coleman and Bell. Methyl tetra-*O*-acetyl-β-D-glucopyranuronate was synthesized by

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the reaction of a mixture of glucuronolactone, sodium methoxide, and methanol with acetic anhydride and perchloric acid (Bollenback *et al.*, 1955). Anhydrous methanol was prepared by the distillation of pesticide quality methanol over magnesium turnings (Fieser, 1957). Benzene used in the syntheses was dried over sodium.

Chromatography. Thin-layer chromatography was used to follow the progress of all reactions and to determine the purity of reaction products.

Silica gel F-254 precoated plates (0.25-mm thickness, Brinkmann Instruments, Inc., Westbury, N. Y.) developed in either benzene–ether (7:3) or petroleum ether–chloroform–ethanol (7:2:1) were used to separate 4- and 5-hydroxycarbaryl glucoside tetraacetates from their respective starting materials and other reaction products. The R_f values for the 4- and 5-hydroxycarbaryl glucoside tetraacetates were identical in the benzene–ether system (R_f 0.16) and in the petroleum ether–chloroform–ethanol mixture (R_f 0.46). Spots were detected by visualization under ultraviolet and by spraying with 10% methanolic sulfuric acid with subsequent baking at 140° for 3 min.

The glucosides of 4- and 5-hydroxycarbaryl were chromatographed on aluminum oxide F-254, Type T, precoated plates (0.25-mm thickness, Brinkmann Instruments, Inc., Westbury, N. Y.) developed in chloroform–methanol–acetic acid (75:15:10). The R_f values for the 4- and 5-hydroxycarbaryl glucosides in this system were also identical, R_f 0.49. However, upon spraying the chromatograms with 10% methanolic sulfuric acid and baking them at 140°, the 4-hydroxycarbaryl glucoside stained red, whereas the 5-hydroxycarbaryl glucoside stained a dark gray. The glycosides of 4- and 5-hydroxycarbaryl isolated from bean plants had identical R_f values on silica gel plates developed in chloroform–methanol–water (65:25:4 v/v) (Mumma *et al.*, 1971).

Enzyme Studies. The synthetic 4- and 5-hydroxycarbaryl glucosides were subjected to enzymatic treatment. Each glucoside, 250 μg, was placed in a 25-ml flask along with 6 ml of citrate buffer (pH 5.0) and 2 mg of β-glucosidase. The same was done using 2 mg of α-glucosidase in phosphate buffer (pH 6.8). The flasks were incubated, with shaking, for 1 hr at 37° and the mixtures extracted twice with ether. The ether extracts were applied to tlc plates and developed in a 7:3 mixture of benzene and ether. Products on the tlc were located by viewing under ultraviolet light and each was extracted from the gel with methanol and analyzed by mass spectrometry.

Instrumentation. The infrared data were obtained using KBr pellets with a Beckman IR5A infrared spectrophotometer. The nmr spectra were determined with a Varian Model T-60 spectrometer using tetramethylsilane as the internal standard. The mass spectral data were recorded with a Finnigan Series 1015C mass spectrometer at 70 eV. The high-resolution mass spectral data were obtained on a Hitachi RMU-7 mass spectrometer. Specific rotations were determined with a Bendix ETL-NPL Automatic Polarimeter, Type 143A. Melting points were determined with an Electrothermal capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

SYNTHESES

Preparation of 4-Hydroxycarbaryl Glucoside Tetraacetate (I) and 5-Hydroxycarbaryl Glucoside Tetraacetate (II). (*1*). *4-(N-Methylcarbamoyloxy)-1-naphthyl Tetra-O-acetyl-β-D-glucopyranoside* (*I*). A mixture of 7.2 g (0.033 mol) of 4-hydroxycarbaryl, 11.7 g (0.03 mol) of β-D(+)-glucose pentaacetate, 0.38 ml (0.003 mol) of boron trifluoride–ether complex (98%), and 350 ml of anhydrous benzene was refluxed with stirring for 2 hr. The dark red solution was cooled to 4° and the excess 4-hydroxycarbaryl was removed by filtration.

The cooled filtrate was extracted with a cold solution of 0.5 *N* sodium hydroxide (2 × 75 ml). The organic layer was separated, washed with ice water (4 × 100 ml), dried over magnesium sulfate, and concentrated. The dark red syrupy residue was dissolved in 40 ml of hot ethanol. The solid which formed on standing was filtered to give 8.70 g of a mixture of I and glucose pentaacetate, mp 95–125°. The mixture was stirred for 2 hr with 400 ml of diethyl ether in order to extract the unreacted glucose pentaacetate. Filtration gave 3.14 g (19%) of I, mp 165–169°. A single recrystallization from ethanol gave 2.94 g (18%) of I as tiny, off-white needles: mp 169–171°; ir 3311 (m, NH), 1745 (s, C=O), 1235 (s), 1045 (CO), 839 (m), 778 (m), 766 cm^{-1} (m, CH of naphthyl ring).

Anal. Calcd for $C_{26}H_{29}O_{12}N$: C, 57.04; H, 5.34; N, 2.56. Found: C, 57.11; H, 5.26; N, 2.51.

(*2*). *5-(N-Methylcarbamoyloxy)-1-naphthyl Tetra-O-acetyl-β-D-glucopyranoside* (*II*). A mixture of 7.2 g (0.033 mol) of 5-hydroxycarbaryl, 11.7 g (0.03 mol) of β-D(+)-glucose pentaacetate, 0.38 ml (0.003 mol) of boron trifluoride–ether complex (98%), and 350 ml of anhydrous benzene was refluxed with stirring for 1 hr. The dark red mixture was allowed to cool to room temperature and filtered to give 2.0 g (28%) of crude unreacted 5-hydroxycarbaryl, mp 155–165°. Recrystallization from ethyl acetate–hexane gave 1.51 g (21%) of 5-hydroxycarbaryl, mp 166–168° (lit. mp 166–167°; Knaak *et al.* (1965)).

The filtrate from the reaction mixture was cooled, washed successively with a cold solution of 0.5 *N* sodium hydroxide (2 × 75 ml) and ice water (4 × 100 ml), then dried over magnesium sulfate, and concentrated. The yellow syrupy residue crystallized upon addition of 1 l. of diethyl ether. Filtration afforded 4.20 g of a colorless solid which was recrystallized from ethanol to give 3.84 g (23%) of II as tiny colorless needles, mp 165–168°. Recrystallization from ethyl acetate did not change the melting point: ir 3247 (m, NH), 1745 (s, C=O), 1230 (s), 1056 (s, CO), 791 cm^{-1} (s, CH of naphthyl ring).

Anal. Calcd for $C_{26}H_{29}O_{12}N$: C, 57.04; H, 5.34; N, 2.56. Found: C, 56.94; H, 5.27; N, 2.48.

Evaporation of the diethyl ether filtrate gave 7.55 g (65%) of crude unreacted glucose pentaacetate, mp 115–122°. Recrystallization from ethanol gave 5.60 g (48%) of glucose pentaacetate, mp 129–130° (lit. mp 134°; Weast (1968)).

Preparation of 4-Hydroxycarbaryl Glucoside (III) and 5-Hydroxycarbaryl Glucoside (IV). (*1*). *4-(N-Methylcarbamoyloxy)-1-naphthyl β-D-Glucopyranoside* (*III*). A mixture of 2.62 g (0.0048 mol) of 4-hydroxycarbaryl glucoside tetraacetate (I) and 300 ml of anhydrous methanol was cooled to 4°, and 1.04 ml (0.00048 mol) of a 0.463 *N* barium methylate solution (Mitchell, 1941) was added. The reaction temperature was maintained at 4°, and after 7 hr of intermittent shaking a yellow solution was obtained. The solution was neutralized by the addition of an exact equivalent of standard 1 *N* sulfuric acid (0.48 ml). Removal of the almost colloidal barium sulfate which formed was facilitated by the addition of charcoal, followed by filtration through Celite.

Evaporation of the filtrate left a syrupy residue which solidified upon being stirred with diethyl ether (350 ml). Filtration gave 1.45 g (80%) of III, mp 204–208°. Two recrystallizations from methanol–diethyl ether (1:2) afforded 0.63 g (35%) of III as off-white crystals: mp 216–218°; $[\alpha]^{24°}_{5461}$ −90.0° (c 0.4, water); ir 3311 (s, OH and NH), 1724 (s, C=O), 1238 (s), 1087 (s, CO), 831 (m), 773 (s), 763 cm^{-1} (sh, CH of naphthyl ring); nmr (DMSO-d_6, D_2O added) δ 2.75 (s, 3 H, $NDCH_3$), 3.15–3.98 (m, 6 H, aliphatic CH and CH_2), 5.07 (d, 1 H, aliphatic CH of glucosidic bond), 7.18 (s, 2 H, aromatic CH, carbon atoms 2 and 3), 7.38–8.02 (m, 3 H, aromatic CH), and 8.18–8.55 (m, 1 H, aromatic CH).

Anal. Calcd for $C_{18}H_{21}O_8N$: C, 56.99; H, 5.58; N, 3.69. Found: C, 56.95; H, 5.59; N, 3.60.

(2). *5-(N-Methylcarbamoyloxy)-1-naphthyl β-D-Glucopyranoside (IV).* A mixture of 1.12 g (0.00205 mol) of 5-hydroxycarbaryl glucoside tetraacetate (II) and 70 ml of anhydrous methanol was cooled to 4°, and 0.44 ml (0.000205 mol) of a 0.463 *N* barium methylate solution was added. The reaction temperature was maintained at 4°, and after 3 hr of intermittent shaking a light pink solution was obtained.

The reaction mixture was processed in a manner similar to that described above for 4-hydroxycarbaryl glucoside (III); 0.57 g (73%) of IV as a colorless solid, mp 178–182°, was obtained. A single recrystallization from methanol–diethyl ether (1:3) gave 0.31 g (40%) of IV as off-white crystals, mp 197–200°. A second recrystallization from methanol–diethyl ether did not change the melting point; $[\alpha]^{24}{}^{\circ}{}_{5461}$ −89.0° (c 0.5, water); ir 3289 (s, OH and NH), 1721 (s, C=O), 1259 (s) 1091 (s, CO), 778 cm^{-1} (s, CH of naphthyl ring); nmr (DMSO-d_6, D_2O added) δ 2.73 (s, 3 H, $NDCH_3$), 3.05–3.82 (m, 6 H, aliphatic CH and CH_2), 5.04 (d, 1 H, aliphatic CH of glucosidic bond), and 6.99–7.85 (m, 5 H, aromatic CH), 8.00–8.32 (m, 1 H, aromatic CH).

Anal. Calcd for $C_{18}H_{21}O_8N$: C, 56.99; H, 5.58; N, 3.69. Found: C, 57.10; H, 5.65; N, 3.66.

Preparation of 4-Hydroxynaphthyl Glucoside (V) and 5-Hydroxynaphthyl Glucoside (VI). (*1*). *4-Hydroxy-1-naphthyl β-D-Glucopyranoside (V).* A solution of 0.50 g (0.0013 mol) of 4-hydroxycarbaryl glucoside (III), 20 ml of methanol, and 4.8 ml (0.0013 mol) of a 0.277 *N* barium hydroxide solution was kept at room temperature for ½ hr. The solution was neutralized with 0.71 ml (0.0014 mol) of a 2.02 *N* oxalic acid solution. The mixture was cooled to 4° to allow for complete precipitation of the barium oxalate. Charcoal was added and the cooled mixture was filtered through Celite. The filtrate was evaporated to give 0.42 g of a brown solid, mp 235–240°. A single recrystallization from methanol gave 0.13 g (30%) of V: mp 262–264°; ir 3257 (s, OH), 1046 (s, CO), 823 (m), 785 (m), 772 (s), 760 cm^{-1} (s, CH of naphthyl ring).

Anal. Calcd for $C_{16}H_{18}O_7$: C, 59.62; H, 5.63. Found: C, 59.57; H, 5.71.

(2). *5-Hydroxy-1-naphthyl β-D-Glucopyranoside (VI).* 5-Hydroxycarbaryl glucoside (IV), reacted in the same manner as described above, gave 0.23 g (53%) of VI as tan crystals, mp 232–234°. Recrystallization from isopropyl alcohol gave the analytical sample: mp 236–239°; ir cm^{-1} 3279 (s, OH), 1052 (s, CO), and 777 cm^{-1} (s, CH of naphthyl ring).

Anal. Calcd for $C_{16}H_{18}O_7$: C, 59.62; H, 5.63. Found: C, 59.61; H, 5.65.

Preparation of Methyl [5-(*N*-Methylcarbamoyloxy)-1-naphthyl Tri-*O*-acetyl-β-D-glucopyranosid] Uronate (VII). A mixture of 5.20 g (0.024 mol) of 5-hydroxycarbaryl, 7.52 g (0.020 mol) of methyl tetra-*O*-acetyl-β-D-glucopyranuronate, 0.28 ml (0.0022 mol) of boron trifluoride–ether complex (98%), and 250 ml of anhydrous benzene was refluxed with stirring for 16 hr. The dark red mixture was cooled to 4° and the unreacted 5-hydroxycarbaryl was filtered.

The filtrate was washed successively with a cold solution of 0.5 *N* sodium hydroxide (2 × 60 ml) and ice water (4 × 100 ml), then dried over magnesium sulfate, and concentrated. The residue crystallized upon addition of 50 ml of diethyl ether and was filtered to give 4.7 g of an off-white solid. Tlc (silica gel; 7:3 benzene–ether) showed this solid to be a mixture of methyl tetra-*O*-acetyl-β-D-glucopyranuronate and VII. The solid was dissolved in 50 ml of acetone, and 6 g of Florisil (60–100 mesh) was added. The mixture was concentrated to dryness by rotary evaporation and placed in a glass column containing 155 g of Florisil. The column was eluted with 2 l. of diethyl ether. The ether eluate was evaporated to give 2.5 g (33%) of unreacted methyl tetra-*O*-acetyl-β-D-glucopyranuronate, mp 171–175° (lit. mp 176.5–178°; Bollenback *et al.* (1955)). Further elution of the column with 1.2 l. of a solution of 10% acetone in benzene, followed by evaporation of the eluate, gave 1.77 g (16%) of VII, mp 167–172°. Recrystallization of a small amount of this solid from isopropyl alcohol gave the analytical sample, as off-white crystals, mp 174–177°: ir 3311 (m, NH), 1742 (s, C=O), 1232 (s), 1047 (s, CO), 781 cm^{-1} (s, CH of naphthyl ring); nmr ($CDCl_3$) δ 2.03 (s, 9 H, $COCH_3$), 2.90 (d, 3 H, $NHCH_3$), 3.72 (s, 3 H, $COOCH_3$), 4.29 (m, 1 H, aliphatic CH, carbon atom 5), 5.16–5.68 (m, 5 H, NH and aliphatic CH), 6.98–8.13 (m, 6 H, aromatic CH).

Anal. Calcd for $C_{25}H_{27}NO_{12}$: C, 56.29; H, 5.10; N, 2.63. Found: C, 56.05; H, 5.14; N, 2.59.

Toxicity Studies. The acute toxicity of 4- and 5-hydroxycarbaryl and their β-D-glucosides to male white mice (Swiss-Webster, 20 g) was evaluated by intraperitoneal injections of 0.1 ml of dimethylsulfoxide. The LD_{50}'s of the compounds were determined by plotting the mean percent mortalities converted to probits *vs.* log dosage. Four mice were used at each dosage level and observed for 3 weeks after treatment.

RESULTS AND DISCUSSION

The β-D-glucosides of 4- and 5-hydroxycarbaryl were synthesized by the scheme outlined in Figure 1. 4- and 5-Hydroxycarbaryl were prepared by the reaction of the corresponding naphthalenediol with methyl isocyanate. Condensation of 1,5-naphthalenediol with β-D(+)-glucose pentaacetate and catalytic amounts of boron trifluoride–ether complex in anhydrous benzene at room temperature for 4 days gave the diglucoside of 1,5-naphthalenediol in low yield. The diglucoside was identified by its ir and nmr spectra. Because of the diglucoside formation when the naphthalenediols were used, the *N*-methylcarbamoyl group in 4- and 5-hydroxycarbaryl was conveniently used as a protecting group.

The boron trifluoride method was evaluated since it had been used as a catalyst for the condensation of β-D(+)-glucose pentaacetate with various phenols (Bretschneider and Beran, 1949). For example, 1-naphthyl-β-D-glucoside tetraacetate was obtained in 58% yield when the reaction was carried out in benzene at room temperature for 2 days. In the current study the reaction of 5-hydroxycarbaryl with β-D(+)-glucose pentaacetate and catalytic amounts of boron trifluoride–ether complex in anhydrous benzene at room temperature for 5 days gave an 8% yield of 5-hydroxycarbaryl glucoside tetraacetate (II). Since the yield was low, the separation of II from the starting material, glucose pentaacetate, was difficult. It was subsequently found that by refluxing the reaction mixture for 1 hr, a 23% yield of II could be obtained; refluxing for longer periods of time, up to 24 hr, did not improve the yield. This modification, besides almost tripling the yield, significantly reduced the reaction time and also permitted the facile removal of glucose pentaacetate from II by its extraction with diethyl ether. 4-Hydroxycarbaryl glucoside tetraacetate I was obtained in a similar manner from 4-hydroxycarbaryl in 19% yield. I and II were identified by their elemental analyses and by their ir, nmr, and mass spectra.

Although the ir spectra of I and II were similar, they were distinguished by the out-of-plane CH bending vibrations of the naphthyl ring. The frequency of the CH out-of-plane vibration is determined by the number of adjacent hydrogens on the ring (Williams and Fleming, 1966). Therefore, the ir spectra of II contained a single strong absorption at 791 cm^{-1}, while the ir spectra of I gave several

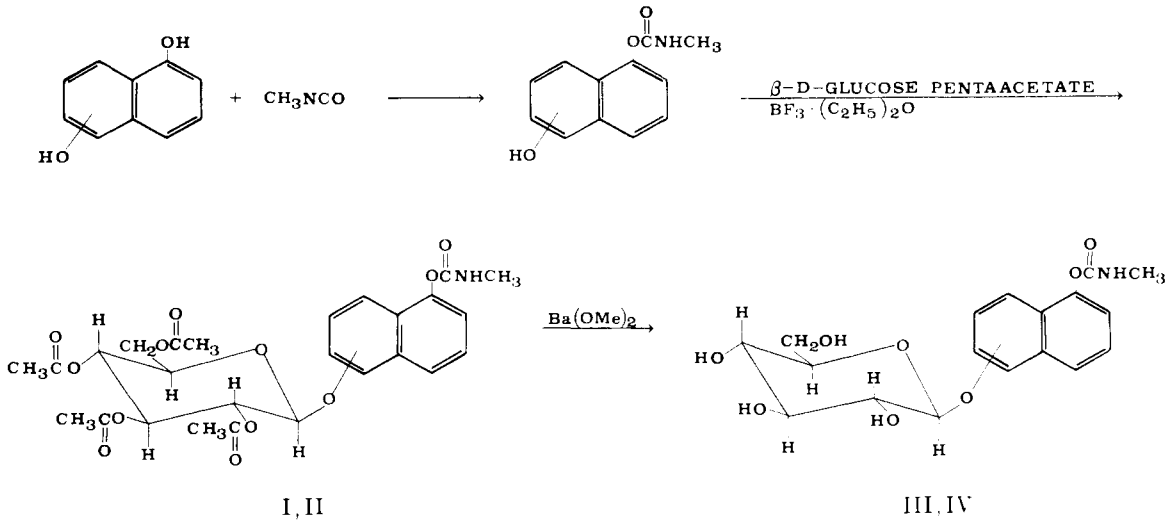


Figure 1. General reaction scheme for the syntheses of the β-D-glucosides of 4- and 5-hydroxycarbaryl. I = 4-hydroxycarbaryl glucoside tetraacetate, II = 5-hydroxycarbaryl glucoside tetraacetate, III = 4-hydroxycarbaryl glucoside, and IV = 5-hydroxycarbaryl glucoside.

Table I. The Toxicity of 4- and 5-Hydroxycarbaryl and Their Respective β-D-Glucosides (III) and (IV) to Mice[a]

Compound	LD_{50}, mg/kg[b]
4-Hydroxycarbaryl	55
4-Hydroxycarbaryl glucoside (III)	1550
5-Hydroxycarbaryl	50
5-Hydroxycarbaryl glucoside (IV)	950

[a] Compounds were administered by intraperitoneal injection using dimethylsulfoxide as carrier. [b] Mortality was recorded after 24 hr.

absorptions at 839, 778, and 766 cm^{-1} due to this vibration.

The nmr spectra of I and II (Figure 2) were taken in acetone-d_6 and, upon shaking with deuterium oxide, the NH resonance disappeared and the *N*-methyl group resonated as a singlet. Although almost identical, they were differentiated by their resonance in the aromatic region. The protons on the carbon atoms 2 and 3 of the naphthyl ring in the spectrum of I resonated as a 2-proton singlet at δ 7.19; the remainder of the aromatic protons in the spectrum of I resonated as a 4-proton multiplet at δ 7.37–8.23. However, the aromatic protons in the spectrum of II gave a 6-proton multiplet at δ 7.13–8.07.

The mass spectra of I and II both contained a weak molecular ion at *m/e* 547 and a low intensity ion at *m/e* 490 due to the "parent" ion minus CH_3NCO. An intense peak corresponding to the CH_3NCO fragment at *m/e* 57 was also present in both spectra. The base peak in each spectrum, which occurred at *m/e* 43, was due to the acylium ion (CH_3CO+). These spectra also contained a series of ions, derived from the acetylated sugar moiety, at *m/e* 331, 271, 229, 211, 187, 169, 127, 109, and 81. They form the series of pyronium ions in the spectra of pentose acetates (Smale and Waight, 1966). The remainder of the most abundant ions in the mass spectrum of I that arose from the aglycone moiety were as follows: *m/e* 160 (1,4-naphthalenediol, A), 132 (A-CO), 131 (A-COH), 105 (A-C_3H_3O), 104, 103, 102, and 77 (C_6H_5+). The peaks in the mass spectrum of II that arose from the aglycone moiety were similar to those obtained in the mass spectrum of I with the following exceptions. The ion at *m/e* 105 was 24% of the base peak in the spectrum of I and only 1% of the base peak in the spectrum of II; and the ion at *m/e* 131 was twice as intense in the spectrum of II as it was in the spectrum of I. These differences were also noted by other workers who have compared the mass spectra of 4-hydroxycarbaryl with that of 5-hydroxycarbaryl (Durden and Bartley, 1971; Mumma *et al.*, 1971).

The acetylated glucosides I and II were deacetylated with catalytic amounts of barium methoxide in anhydrous methanol at 4°. The reaction conditions permitted the removal of the acetate groups without causing significant hydrolysis of the base labile carbamate group. Therefore, 4-hydroxycarbaryl glucoside III was obtained in 80% yield by the reaction of I with barium methoxide in anhydrous methanol for 7 hr at 4°. 5-Hydroxycarbaryl glucoside (IV) was obtained in 73% yield by the similar hydrolysis of II. Both glucosides contained only trace quantities of impurities at this point. III and IV were identified by their elemental analyses and by their ir, nmr, and mass spectra.

Enzymatic cleavage of III and IV yielded only 4-hydroxycarbaryl and 5-hydroxycarbaryl, respectively. These compounds were identified by tlc and mass spectrometry. α-Glucosidase, or preparations with no enzyme, did not cleave the glucosides. These assays showed that the carbamates were in their original form and that the glucosides were β isomers.

The ir spectra of III and IV were similar but, as with the acetylated glucosides I and II, they could be distinguished by their respective aromatic protons out-of-plane deformation. The nmr spectra of III and IV were also similar, but again they were differentiated by the 2-proton singlet in the aromatic region of the nmr spectrum of III. Evidence for the β-isomeric structure of III and IV was provided by the nmr spectra. The coupling constants of the anomeric hydrogen doublets in III and IV, J = 5.7 Hz and J = 6.0 Hz, respectively, were consistent with J values obtained for the C-1,2 sugar protons of β-glucopyranosides (Coffey, 1967).

The mass spectra of III and IV (Figure 3) proved to be very interesting. Even though glucosides are very polar organic compounds, a weak molecular ion was obtained for III and IV at *m/e* 379. A weak ion at *m/e* 322, due to the "parent" ion minus CH_3NCO, was also present in III and IV. The CH_3NCO fragment was detected as a relatively intense fragment at *m/e* 57 in both spectra. The base peak in the mass spectra of III and IV occurred at *m/e* 160 and was due to 1,4-naphthalenediol and 1,5-naphthalenediol, respectively. The corresponding sugar moiety was obtained at *m/e* 163 ($C_6H_{11}O_5+$). The latter peak was of low intensity, but evidence was obtained for an intermolecular rearrangement.

It was shown that transcarbamylation had occurred be-

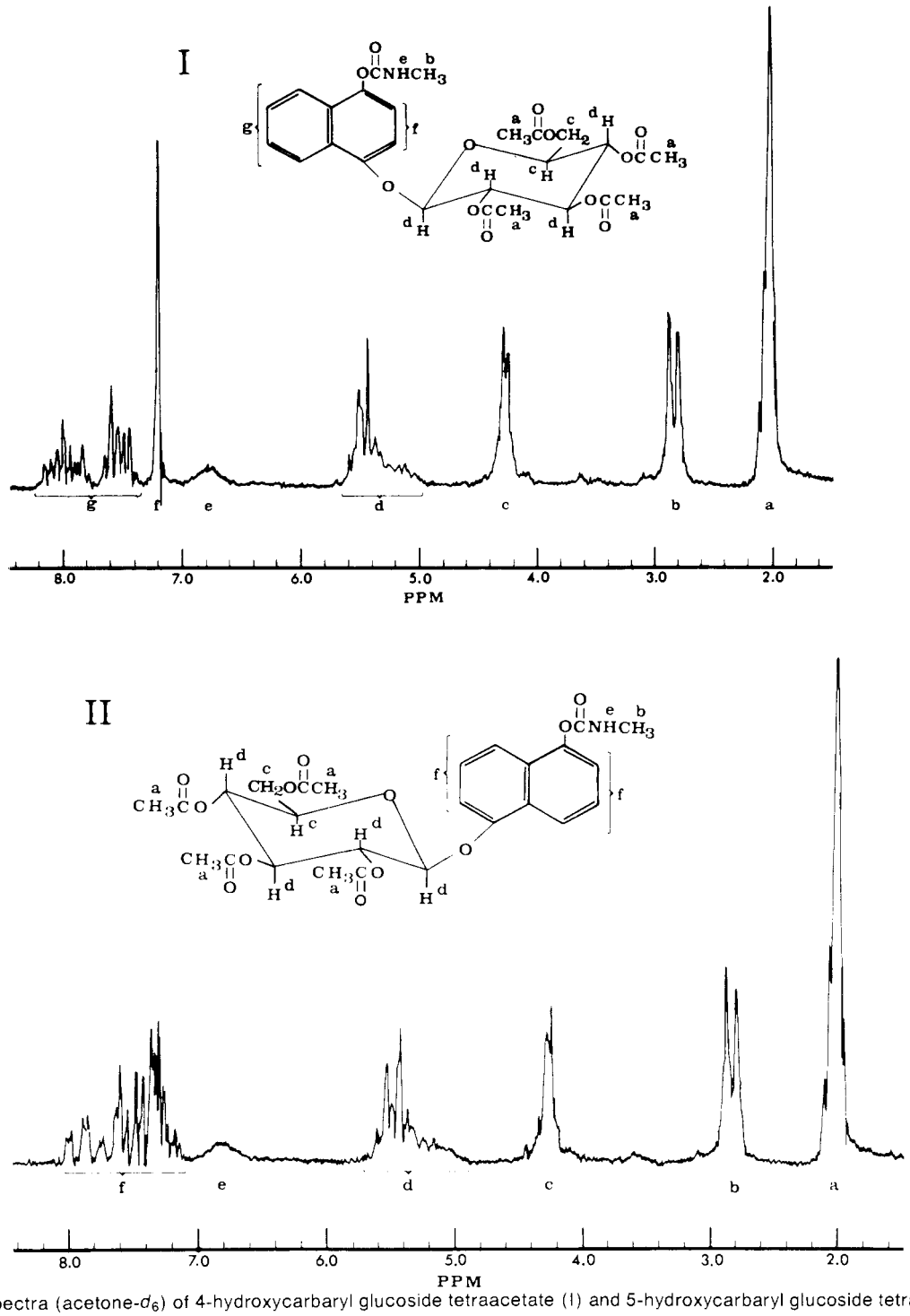
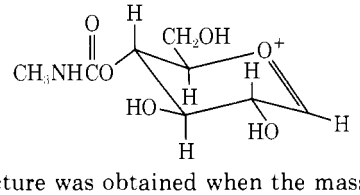


Figure 2. Nmr spectra (acetone-d_6) of 4-hydroxycarbaryl glucoside tetraacetate (I) and 5-hydroxycarbaryl glucoside tetraacetate (II).

tween the phenolic oxygen of the naphthalene ring and one of the hydroxyl oxygens on the sugar moiety. A peak, which was approximately five times as intense as the 163 peak, was obtained at m/e 220 in the spectra of III and IV. High-resolution mass spectral analysis determined the elemental composition of this peak to be either $C_8H_{14}O_6N_1$ or $C_{16}H_{12}O_1$. The latter composition was discarded on the basis of the chemical structure of the glucosides, and therefore the peak at 220 was of elemental composition $C_8H_{14}O_6N_1$ (theoretical mass 220.0821; actual mass for III, 220.089 ± 0.010, for IV, 220.080 ± 0.010). This elemental composition was consistent with the structure shown below.



It should be noted that the *N*-methylcarbamoyl group is not necessarily where shown, but could be on any one of the four hydroxyl oxygens of the sugar. Further evidence for this structure was obtained when the mass spectrum of a mixture of 1-naphthyl β-D-glucoside and 5-hydroxycarbaryl was shown to contain a peak at m/e 220. When the mass spectra of 1-naphthyl β-D-glucoside and 5-hydroxycarbaryl were taken separately, neither spectrum contained an ion at this mass. The intermolecular rearrangement appears to have occurred thermally in the solid probe of the mass spectrometer. At low voltages, for example 10 eV, the peak at m/e 220 was the second most intense ion in the mass spectra of III and IV. Since this

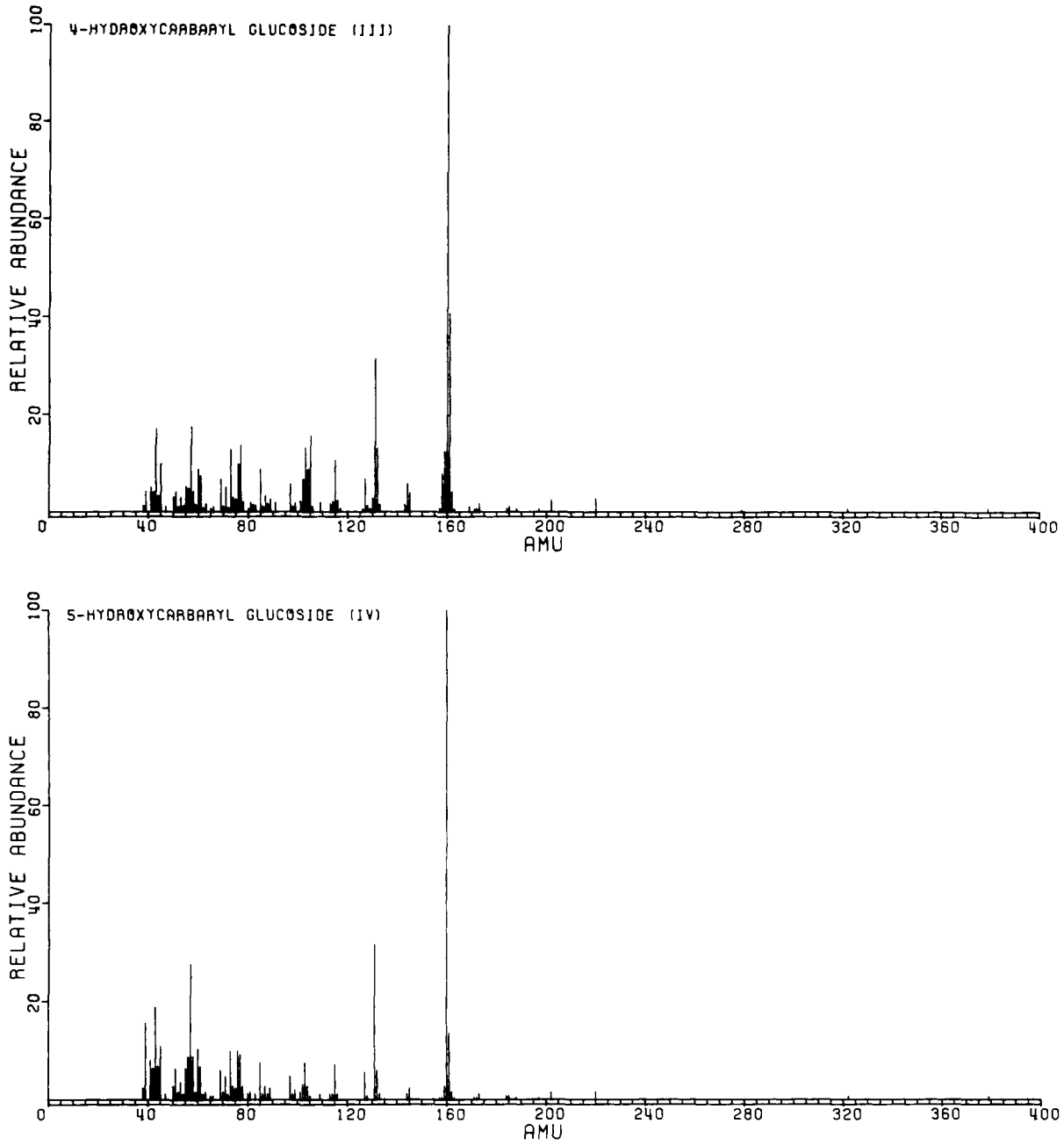


Figure 3. Mass spectra of 4-hydroxycarbaryl glucoside (III) and 5-hydroxycarbaryl glucoside (IV). Peaks in the mass spectra of III, excluding the base peak, are increased to three times their actual length.

peak was much greater in relative intensity at low voltages than at high voltages, thermal rearrangement rather than electron impact likely accounted for its formation. The base peak in the mass spectra of III and IV at low voltages was the same as that obtained at high voltages (*m/e* 160), and suggested that this ion may also be due to thermal decomposition. The ion at *m/e* 202 in the spectra of III and IV was due to the ion at *m/e* 220 minus water.

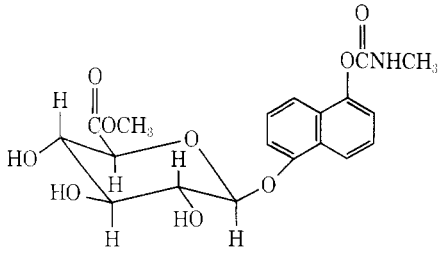
Like the mass spectra of their acetylated glucosides I and II, the mass spectra of 4-hydroxycarbaryl glucoside (III) and 5-hydroxycarbaryl glucoside (IV) were differentiated by the ions at *m/e* 105 and *m/e* 131. The peak at *m/e* 105 in the mass spectrum of IV was less than 1% of the base peak, whereas it was 5% of the base peak in the mass spectrum of III. The peak at *m/e* 131 was three times as intense in the mass spectrum of IV as it was in the spectrum of III. Also, the peak at *m/e* 132 in the mass spectrum of III was one-half as intense as the peak at *m/e* 131, while it was only one-fifth as intense as the peak at *m/e* 131 in the spectrum of IV. These spectral differences make possible the identification of 4-hydroxycarbaryl glucoside (III) and 5-hydroxycarbaryl glucoside (IV) at quantities which may be conveniently isolated from plants.

Samples of III, mp 204–208°, and IV, mp 178–182°, prepared by the barium methoxide procedure, were shown by aluminum oxide tlc to contain an impurity which traveled just below III and IV. These impurities were identified as their respective decarbamylated products, 4-hydroxy-1-naphthyl β-D-glucopyranoside (V) and 5-hydroxy-1-naphthyl β-D-glucopyranoside (VI). The latter compounds were independently synthesized by the decarbamylation of III and IV with a barium hydroxide solution in methanol at room temperature. V and VI were identified by their elemental analyses, tlc, and their ir spectra, which were similar to the ir spectra of III and IV, but did not contain a carbonyl-stretching frequency at approximately 1720 cm^{-1}. The instability of the carbamate ester in basic solutions was demonstrated when approximately 50% hydrolysis was obtained with catalytic amounts of sodium methoxide in methanol at room temperature.

The reaction of 5-hydroxycarbaryl with methyl tetra-*O*-acetyl-β-D-glucopyranouronate and catalytic amounts of boron trifluoride–ether complex in refluxing anhydrous benzene for 16 hr gave a 15% yield of methyl [5-(*N*-methylcarbamoyloxy)-1-naphthyl tri-*O*-acetyl-β-D-glucopyranosid]uronate (VII). This reaction was significant since aryl-β-D-glucuronides are more commonly prepared by the condensation of methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate with a phenol in the presence of an acidic metal catalyst (Bollenback *et al.*, 1955; Coffey, 1967; Conrow and Bernstein, 1971), or by the fusion of the acetylated methyl ester sugar with a phenol in the presence of an acidic catalyst at reduced pressures (Bollenback *et al.*, 1955).

The structural assignment for VII is supported by its elemental analysis and by its ir, nmr, and mass spectra. The mass spectrum contained a weak molecular ion at *m/e* 533. A weak ion at *m/e* 476 due to the molecular ion minus methylisocyanate was obtained. A relatively intense peak due to the latter fragment at *m/e* 57 was also present. The base peak in the spectrum occurred at *m/e* 43 (CH_3CO+). The major peaks in the spectrum corresponding to the series of pyronium ions which resulted from the fragmentation of the acetylated methyl ester sugar moiety were obtained at *m/e* 317, 257, 215, 197, 173, 155, and 127. The remainder of the most abundant ions were derived from the fragmentation of 1,5-naphthalenediol.

VII was deacetylated as described for the glucoside with catalytic amounts of barium methoxide in methanol at 4° to yield the methyl ester VIII, mp 143–145°. However, attempts to remove the methyl ester without hydrolyzing the carbamate ester were not successful. The use of barium hydroxide at 4° hydrolyzed the carbamate group along with the methyl ester. The mass spectrum of VIII had a weak molecular ion at *m/e* 407. The base peak was at *m/e* 160 (1,5-naphthalenediol). The mass spectrum also showed a peak at *m/e* 248, which corresponded to the thermal rearrangement peak at *m/e* 220 in the mass spectra of III and IV. Thermal intermolecular rearrangements of this type may prove quite common in the mass spectra of carbamate glucosides, and therefore may be useful in their identification.



VIII

The attempted preparation of the β-D-glucoside of *N*-hydroxymethylcarbaryl by a procedure similar to that used for the preparation of 4- and 5-hydroxycarbaryl glucoside was unsuccessful. *N*-Hydroxymethylcarbaryl was not stable in benzene solutions containing catalytic amounts of boron trifluoride. Silica gel tlc showed that it decomposed almost immediately in the acidic mixture to at least four products.

Preliminary experiments showed that the β-D-glucoside of 3-hydroxycarbofuran (2,3-dihydro-2,2-dimethyl-3-hydroxylbenzofuranyl-7 *N*-methylcarbamate) could not be synthesized using the procedure described herein. 3-Hydroxycarbofuran, β-D(+)-glucose pentaacetate, and catalytic amounts of boron trifluoride–ether complex were refluxed in anhydrous benzene for 16 hr. The reaction mixture was processed in a manner similar to that described for 4- and 5-hydroxycarbaryl glucoside tetraacetates. The major product isolated, mp 235–240° dec, did not contain the acetylated sugar moiety. Its elemental analysis, and its ir and nmr spectra indicated that it was a self-condensation product of 3-hydroxycarbofuran.

The toxicity of 4- and 5-hydroxycarbaryl and their respective β-D-glucosides III and IV to mice is reported in Table I. In all cases mortalities occurred within 2 hr after administration of the test compounds, with no further mortalities occurring during an observation period of 3 weeks. No mortalities occurred in the control mice. As expected, glucoside formation greatly decreased the toxicity of 4- and 5-hydroxycarbaryl. Although the differences were not statistically evaluated, the data indicate that the aglycones differed only slightly in toxicity, while their glucosides showed a relatively greater difference. This could possibly indicate that some of the toxicity of IV was due to the intact glucoside, and was not entirely the result of cleavage of the conjugated form to yield the toxic aglycone. However, it is possible that 5-hydroxycarbaryl glucoside is cleaved faster than its 4-hydroxy analog in mice, resulting in an effectively higher concentration of 5-hydroxycarbaryl. Further studies using the synthetic conjugates will enhance the elucidation of this point and allow the general toxicological nature of the carbaryl glucosides to be more completely defined.

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