Studies in Detoxication

28. THE BIOSYNTHESIS OF *o*-, *m*- AND *p*-CYANOPHENYLGLUCURONIDES

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Studies in progress in this laboratory on the fate of cyanobenzene in the rabbit (Smith & Williams, 1949) required as reference compounds the glucuronides of the *o*-, *m*- and *p*-cyanophenols. These compounds were made biosynthetically by feeding the necessary phenols to rabbits and isolating the glucuronides from the urine. Only *p*-cyanophenylglucuronide was isolated as such, the *o*- and *m*-glucuronides being obtained as crystalline amides and triacetyl methyl esters.

EXPERIMENTAL

o-*Cyanophenylglucuronide*

o-Cyanophenol (1·5 g.; m.p. 90°)* was fed in aqueous suspension to a 3·5 kg. rabbit. No ill effects were noted, and after 4 hr. the urine (70 ml.) was collected. The urine gave negative tests with Benedict reagent, $FeCl_3$ and dichloroquinonechloroimide, but an intense test with naphthoresorcinol. The basic Pb acetate precipitate of the urine was prepared as described in earlier papers (e.g. Smith & Williams, 1948). The Pb was removed with H_2S and the aqueous residue taken to a gum *in vacuo*. This gum (*o-cyanophenylglucuronide*) was soluble in 95% ethanol and water, but attempts to crystallize it or to form crystalline salts with benzylamine or triethanolamine were not successful. Urine collected after 4 hr. contained only traces of the glucuronide. The gum was dissolved in 5 ml. 95% ethanol and methylated by shaking for an hour with an excess of diazomethane in 150 ml. diethyl ether. The mixture was filtered and the ether removed *in vacuo* leaving a neutral gum which was presumably the *methyl ester* of *o*-cyanophenylglucuronide. This ester was now dissolved in a mixture of 10 ml. acetic anhydride and 10 ml. pyridine. After 20 hr. at room temperature, the mixture was diluted to 100 ml. with water and cooled to 0°. Crystals (1·35 g. or 25% of the dose) of the *methyl ester* of *triacetyl β-o-cyanophenyl-D-glucuronide* were thrown out. These were collected and recrystallized from absolute ethanol-ligroin as long, colourless needles, m.p. 151–152° and $[\alpha]_D^{20°} - 71°$ (*c*, 4·9 in $CHCl_3$). (Found: C, 55·45; H, 4·8; N, 3·4; OMe, 7·2. $C_{20}H_{21}O_{10}N$ requires C, 55·2; H, 4·9; N, 3·2; OMe, 7·1%.) The compound was sparingly soluble in hot water, hot ligroin and CCl_4, easily soluble in $CHCl_3$, acetone, ethanol and ethyl acetate and insoluble in light petroleum.

β-o-Cyanophenyl-D-glucuronidamide. The above acetylated ester (0·1 g.) was dissolved in 5 ml. absolute ethanol, and the solution saturated with NH_3 at 0°. On standing overnight *β-o-cyanophenyl-D-glucuronidamide hemihydrate* was deposited as fine colourless needles. It was recrystallized from ethanol/light petroleum and had m.p. 195° and $[\alpha]_D^{20°} - 78°$ (*c*, 1·3 in 50% aqueous ethanol). (Found: C, 51·3; H, 5·0; N, 9·7; $C_{13}H_{14}O_6N_2 \cdot 0{\cdot}5\ H_2O$ requires C, 51·5; H, 5·0; N, 9·5%.) The compound was soluble in water, sparingly soluble in ethanol and insoluble in ether and light petroleum.

m-*Cyanophenylglucuronide*

m-Cyanophenol (2 g.; m.p. 83°) was fed, as a suspension in water, to a 3 kg. rabbit and the clear yellow 4 hr. urine (100 ml.), which gave only the naphthoresorcinol test, was worked up as for the *o*-compound. *m-Cyanophenylglucuronide* was obtained as a gelatinous solid which dried to a non-crystalline powder soluble in 95% ethanol and water. The powder was methylated in ethanol for 3 hr. with excess ethereal diazomethane to give a gummy *methyl ester*. This ester was acetylated as above and yielded 2·9 g. (40% of dose) of the crystalline *methyl ester* of *triacetyl-β-m-cyanophenyl-D-glucuronide*. Its solubility was similar to that of the *o*-compound. On recrystallization from absolute ethanol the compound formed colourless needles, m.p. 156–157° and $[\alpha]_D^{20°} - 37°$ (*c*, 3·2 in $CHCl_3$). (Found: C, 55·3; H, 5·2; N, 3·7; OCH_3, 7·2. $C_{20}H_{21}O_{10}N$ requires C, 55·2; H, 4·9; N, 3·2; OCH_3, 7·1%.)

β-m-Cyanophenyl-D-glucuronidamide was obtained by treating the above ester (0·25 g.) in ethanol (4 ml.) with NH_3, and after keeping the solution overnight the amide (0·1 g.) was precipitated from the solution by ether. On recrystallization from hot ethanol, the *amide* formed colourless needles of the monohydrate, m.p. 207° (decomp.) and $[\alpha]_D^{28°} - 63°$ (*c*, 0·4 in water). (Found: C, 50·4; H, 5·3; N, 9·5. $C_{13}H_{14}O_6N_2 \cdot H_2O$ requires C, 50·0; H, 5·2; N, 9·0%.) It was soluble in water and ethanol but not in benzene or ether.

p-*Cyanophenylglucuronide*

p-Cyanophenol (1·5 g., m.p. 110°) was fed to a 3 kg. rabbit as before. A 4 hr. urine (100 ml.) was collected which gave no colour with $FeCl_3$, no reduction with Benedict reagent but an intense naphthoresorcinol test. On extracting the untreated urine with ether, an extract was obtained which gave feebly, with $FeCl_3$, the colours characteristic of a catechol derivative. The glucuronide fraction of the urine was separated by the Pb acetate procedure and the Pb-free filtrate was taken down *in vacuo* to a thin syrup, which solidified on standing. The solid was treated with a little water and the crystals (230 mg. or 6% of the dose) of *p-cyanophenylglucuronide monohydrate* filtered off. The compound on recrystallization from water formed colourless needles, m.p. 140° (decomp.) and $[\alpha]_D^{20°} - 92°$ (*c*, 3·5 in

* All melting points are uncorrected.

Table 1. *Melting points and optical rotations of biosynthetic cyanophenylglucuronides and derivatives*

	Glucuronide		Triacetyl methyl ester of glucuronide		Glucuronidamide	
Cyanophenol	M.p.	$[\alpha]_D^{20°}$ in water	M.p.	$[\alpha]_D^{20°}$ in $CHCl_3$	M.p.	$[\alpha]_D^{20°}$ in water
ortho-	Not crystallized		151°	−71°	195°*	−78°†
meta-	Not crystallized		156–157°	−37°	207°‡	−63°
para-	140°‡	−92°	130–135°	−42°	210–213°	−72°

* Hemihydrate. † In 50% aqueous ethanol. ‡ Monohydrates.

water). (Found: C, 50·15; H, 4·9; N, 4·45. $C_{13}H_{13}O_7N$, H_2O requires C, 49·85; H, 4·8; N, 4·5%.) The glucuronide was soluble to the extent of 5% in water; it was more soluble in ethanol but insoluble in ether.

The mother liquors after the separation of the crystalline glucuronide were evaporated to dryness, and the residue dissolved in a little 95% ethanol. The solution, on treatment with ethereal diazomethane as before, gave a gummy *methyl ester*. The latter, on acetylation with pyridine and acetic anhydride for 48 hr. at room temperature and then pouring into water, yielded the crystalline *methyl ester* of *triacetyl* β-p-*cyanophenyl*-D-*glucuronide* (0·51 g. or 9% of the dose). On recrystallization from ethanol-light petroleum the compound formed colourless needles, m.p. 130–135° and $[\alpha]_D^{20°}$ −42° (*c*, 1·4 in $CHCl_3$). (Found: C, 54·8; H, 4·75; N, 3·65. $C_{20}H_{21}O_{10}N$ requires C, 55·2; H, 4·9; N, 3·2%.)

β-p-*Cyanophenyl*-D-*glucuronidamide*. Treatment of the above acetylated ester (0·3 g.) with ethanol saturated with NH_3 at 0°, yielded after keeping overnight no crystalline material. However, on diluting the solution with light petroleum, the *amide* was thrown out as an oil which gradually solidified (yield 0·15 g.). It was recrystallized from ethanol-benzene and formed fine colourless needles, m.p. 210–213° (decomp.) and $[\alpha]_D^{20°}$ −72° (*c*, 2·6 in water). (Found: C, 53·3; H, 5·1; N, 9·5. $C_{13}H_{14}O_6N_2$ requires C, 53·05; H, 4·8; N, 9·5%.)

DISCUSSION

The properties of the *o*-, *m*- and *p*-cyanophenylglucuronides and their derivatives are summarized in Table 1.

The only figure calling for comment is the specific optical rotation of the methyl ester of triacetyl *o*-cyanophenylglucuronide which is nearly twice that of its isomers. The rotations of the three amides are, however, of the same order. It is known that the tetra-acetates of some *ortho*-substituted phenyl-β-glucosides have anomalous rotations (see Pigman & Goepp, 1948) when compared with the *m*- and *p*-isomers. In view of this, the high negative rotation of triacetyl *o*-cyanophenyl-β-glucuronide methyl ester compared with the *m*- and *p*-isomers is not unexpected. It may be noted also that the methyl ester of triacetyl β-*o*-methoxyphenylglucuronide (Garton & Williams, 1948, 1949) has $[M]_D$ − 21,560 in ethanol, while the corresponding derivatives of β-*m*-acetoxyphenyl- and β-*p*-acetoxyphenyl-glucuronides have $[M]_D$ values about half of this (− 11,466 and − 10,296, respectively) in acetone.

One further point in this work is worthy of comment and that is that the main bulk of the cyanophenylglucuronides was excreted within 4 hr. of administering the cyanophenols, none of which was toxic at 0·5 g./kg.

SUMMARY

1. The preparation of *p*-cyanophenylglucuronide, by feeding *p*-cyanophenol to rabbits, is described.

2. The characterization of the biosynthetic *o*-, *m*- and *p*-cyanophenylglucuronides as their amides and triacetyl methyl esters, is described.

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