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Studies in Detoxication

36. A NOTE ON THE GLUCURONIDES OF BENZENEAZOPHENOL AND BENZENEAZORESORCINOL

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Salant & Bengis (1916) found that a number of fat-soluble dyes administered to rabbits were excreted in the urine and bile, besides being deposited in adipose tissues. Since no fat was excreted in the urine with these dyes, these workers concluded that they were converted in the body into water-soluble substances, probably ethereal sulphates and glucuronides. Benzeneazophenol (oil yellow) and benzeneazoresorcinol (Sudan G 35) were fed to rabbits and the urine was examined for metabolites. Glucuronides were isolated in both cases and although melting points were recorded, the compounds were not analysed or properly described.

It appeared from the paper of Salant & Bengis that the glucuronides of benzeneazophenol and benzeneazoresorcinol would be compounds readily prepared by acidification of urine, and we thought that they might perhaps be suitable substrates for the assay of β -glucuronidase, if the glucuronides were less highly coloured than the free azophenols. We have therefore prepared and characterized these glucuronides and examined their possibilities as substrates for glucuronidase.

We have assumed that the monoglucuronide obtained from benzeneazoresorcinol is 4-benzeneazo-3-hydroxyphenylglucuronide (I) on analogy

with the biosynthetic monoglucuronides of 4-chlororesorcinol (Dodgson & Williams, 1949) and 2:4-dihydroxyacetophenone (Dodgson, 1950), which have been proved to be *para*-substituted phenyl glucuronides (see also Dodgson, Smith & Williams, 1950).

EXPERIMENTAL

4-Benzeneazophenyl- β -D-glucuronide. Benzeneazophenol (m.p. 151-152°; 2 g.) was fed to a rabbit (weight, 3 kg.). After 3 hr. the bright-yellow urine (50 ml.), which gave negative tests for aromatic amino groups and reducing substances but positive ones for glucuronic acid, was acidified with HCl. A small pale-yellow crystalline precipitate of the glucuronide separated; this, however, redissolved on further acidification.

To isolate the glucuronide, the urine was made faintly acid to litmus and extracted three times with a mixture of 1 vol. ethanol to 3 of ether. The extracts were evaporated to a syrup to which was added Na_2SO_4 to form a paste. The latter was then stirred repeatedly with small amounts of the ethanol-ether mixture until all the yellow material had been extracted. Evaporation of this solution gave

crystals of the glucuronide (200 mg.), which on recrystallization from methanol gave the 4-benzeneazophenylglucuronide

Fig. 1. Absorption spectra of benzeneazophenol (—) and its glucuronide (---), in 0.1N-NaOH. Benzeneazophenol: λ_{max} 250–270 $\text{m}\mu$, ϵ_{max} 8500 and λ_{max} 400–410 $\text{m}\mu$, ϵ_{max} 20,700. Benzeneazophenylglucuronide sesquihydrate: λ_{max} 336 $\text{m}\mu$, ϵ_{max} 22,400.

Fig. 2. Absorption spectra of benzenaezoresorcinol (—) and its monoglucuronide (---), in 0.1N-NaOH. Benzenaezoresorcinol: λ_{max} 470, ϵ_{max} 24,000. Benzenaezoresorcinol monoglucuronide monohydrate: $\lambda \sim 230 \text{ m}\mu$, ϵ 12,000; λ_{max} 333 $\text{m}\mu$, ϵ_{max} 11,100 and λ_{max} 460 $\text{m}\mu$, ϵ_{max} 11,700.

as pale-yellow needles of the *sesquihydrate*, m.p. 164°, $[\alpha]_D^{20} - 88^\circ$ (c, 0.7 in ethanol). Salant & Bengis (1916) give m.p. 164–165°. (Found: C, 54.3; H, 5.4; N, 7.3.

$\text{C}_{18}\text{H}_{18}\text{O}_7\text{N}_2 \cdot 1.5\text{H}_2\text{O}$ requires C, 54.1; H, 5.3; N, 7.0%.) It was slightly soluble in ethanol and ethyl acetate, less soluble in ether and almost insoluble in water.

4(?) - Benzenaezo-3(?) - hydroxyphenyl- β -D-glucuronide. 4-Benzenaezoresorcinol (British Drug Houses Ltd., m.p. 161°; 2 g.) was fed to a rabbit (weight 3 kg.) and the urine (100 ml.) collected for 15 hr. Tests on the urine were similar to those for benzeneazophenol urine. The urine was made acid to congo red and kept at 0° for several hours. The crystalline deposit was filtered, washed with water on the centrifuge and recrystallized from ethanol-water. The 4(?) - benzenaezo-3(?) - hydroxyphenylglucuronide monohydrate was obtained as yellow needles (200 mg.), m.p. 198°, $[\alpha]_D^{20} - 114^\circ$ (c, 1 in ethanol). Salant & Bengis (1916) give m.p. 189–190°. Its solubilities were similar to those of the benzeneazophenol derivative. (Found: C, 53.0; H, 5.3; N, 7.1. $\text{C}_{18}\text{H}_{18}\text{O}_8\text{N}_2 \cdot \text{H}_2\text{O}$ requires C, 53.2; H, 5.0; N, 6.9%.)

Spectroscopic observations

Both the azophenols and their glucuronides formed yellow solutions which were not very obviously altered in colour when made alkaline. These glucuronides could not, therefore, be used as substrates for glucuronidase in the same way as phenolphthalein glucuronide, i.e. by the use of the red colour given in alkaline solution by phenolphthalein freed from the glucuronide by the enzyme. However, the absorption spectra of the azophenols and their glucuronides were examined in alkaline solution with the Unicam spectrophotometer (see Figs. 1 and 2).

DISCUSSION

The spectra of benzeneazophenol and its glucuronide (Fig. 1) in alkaline solution show considerable differences. Benzeneazophenol has a broad maximum at 400–410 $\text{m}\mu$ with a high extinction (ϵ_{max} 20,700), but its glucuronide also has an appreciable absorption at these wavelengths which amounts to almost 10% of that of the phenol, a fact which, we feel, renders the glucuronide not very suitable for glucuronidase assay. Benzenaezoresorcinol glucuronide is unsuitable for a similar reason (see Fig. 2); benzenaezoresorcinol has a sharp maximum at 470 $\text{m}\mu$ (ϵ_{max} 24,000), but the glucuronide at this wavelength has an extinction (ϵ) of about 11,000.

SUMMARY

1. The monoglucuronides of the fat-soluble dyes, benzeneazophenol and benzenaezoresorcinol have been prepared from the urine of rabbits receiving these dyes orally, and have been characterized.
2. The spectra of the glucuronides were compared with those of the parent dyes and it was concluded that the glucuronides were not suitable as substrates for β -glucuronidase assay.

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