CIII. METABOLISM OF POLYCYCLIC COMPOUNDS.

II. PRODUCTION OF DIHYDROXYDIHYDROANTHRACENEGLYCURONIC ACID FROM ANTHRACENE.

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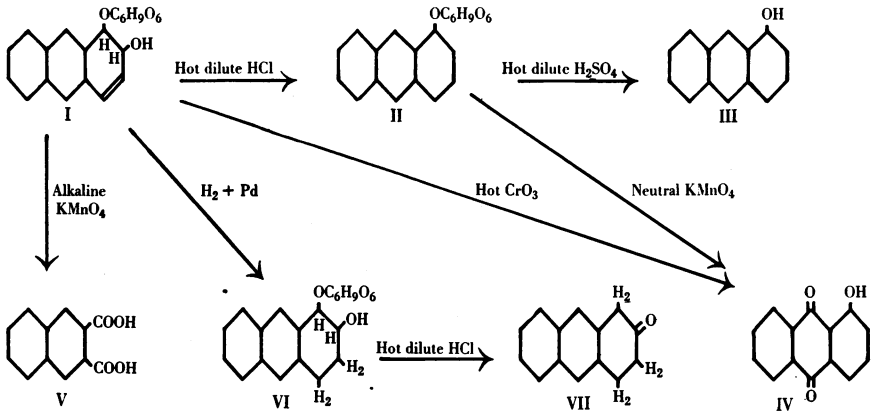
IN a previous communication it was shown that rats and rabbits fed on a diet containing anthracene excreted two different isomerides of dihydroxydihydroanthracene [Boyland and Levi, 1935]. These compounds were excreted as such and as conjugation compounds with glycuronic acid. The structure of the glycuronic acid compounds has now been determined.

The glycuronic acid compound from rabbit urine was oxidised by alkaline $KMnO_4$ to naphthalene-2:3-dicarboxylic acid (V), but treatment with acid caused loss of water to give an anthrylglycuronic acid (II), which was oxidised



by $KMnO_4$ to give α-hydroxyanthraquinone (IV). The acid must therefore be 1:2-dihydroxy-1:2-dihydroanthracene-1-glycuronic acid (I). That the oxygen atoms are attached in the 1 and 2 positions is shown by the formation of α-anthrol (III) by acid hydrolysis, and 2-keto-1:2:3:4-tetrahydroanthracene (VII) by catalytic reduction and acid hydrolysis.

The glycuronic acid from the rat urine occurred in much smaller amounts and was isolated with considerable difficulty from the acid solution. The purified product showed the chemical properties of α-anthrylglycuronic acid, but its optical activity was somewhat greater than that of the α-anthrylglycuronic acid (II) produced by acid hydrolysis of 1:2-dihydroxy-1:2-dihydroanthraceneglycuronic acid obtained from rabbit urine. It is probable that the rat urine contains a laevorotatory 1:2-dihydroxy-1:2-dihydroanthraceneglycuronic acid.

Of the two 1:2-dihydroxy-1:2-dihydroanthracenes the laevorotatory compound is excreted by rats, and it is probable that a glycuronic acid complex of this is formed. Rats excrete chiefly (0·2 g./l.) free 1:2-dihydroxy-1:2-dihydroanthracene and very little glycuronic acid. Rabbits produce little free 1:2-dihydroxy-1:2-dihydroanthracene and considerable amounts (0·5 g./l.) of the glycuronic acid complex.

Experimental.

The animals were treated in the manner described in the previous communication. After the neutral urine had been extracted with ether to remove dihydroxydihydroanthracene it was acidified with HCl until acid to Congo red and extracted with $CHCl_3$ in large separating funnels to remove mercapturic and other acids. The urine was then shaken twice with 5 g. lots of charcoal (norite), and filtered.

Glycuronic acid from rabbit urine.

The rabbit urine contained relatively large amounts of glycuronic acid (about 0·5 g./l.), which was readily extracted by shaking with 2 portions of amyl alcohol in a separating funnel. The extraction was facilitated by the addition of about 300 g. $(NH_4)_2SO_4$/l. The amyl alcohol was then extracted with 2*N* NaOH. The extract on acidifying and stirring gave a precipitate of crude glycuronic acid which was purified by crystallisation from hot water or hot alcohol.

The purified extract from acidified rabbit urine crystallised from hot water in laminae, M.P. 197° with decomposition, and from alcohol in rosettes or needles, M.P. 193–195°; $[\alpha]_D^{20°}$ in dioxan (*c*, 0·6%) +197°; $[\alpha]_D^{20°}$ Na salt in H_2O (*c*, 0·3%) +95°. Soluble in acetone and alcohol, sparingly soluble in ether and water; insoluble in benzene and chloroform. (Found (Schoeller): C, 59·4; H, 5·1%; equiv. wt. 408, 412, 407. $C_{20}H_{20}O_8$, H_2O requires C, 59·2; H, 5·4%; mol. wt. 406.)

The pure substance gave a strong naphthoresorcinol test for glycuronic acid. It did not reduce Fehling's solution except after acid hydrolysis. Boiling dil. HCl for 5 min. changed the optical activity and gave a less soluble product which appeared to be anthrylglycuronic acid (II).

Anthrylglycuronic acid. For the preparation of this substance the glycuronic acid of the rabbit is dissolved in hot water and, after the addition of a little HCl, kept at about 70° for an hour. The anthrylglycuronic acid slowly separates in shining laminae and is removed from the cooled solution, washed with benzene to remove a small amount of α-anthrol and purified from a large volume of water, or from aqueous methyl alcohol; $[\alpha]_D^{20°}$ in dioxan (*c*, 1·0%) −52°; $[\alpha]_D^{20°}$ Na salt in H_2O (*c*, 0·3%) −79°. Readily soluble in alcohol and acetone, sparingly in water and ether. The solubility is greater in alcohol and less in water than that of the original acid. (Found (Weiler): C, 62·6; H, 5·1%; equiv. wt. 392. $C_{20}H_{18}O_7$, H_2O requires C, 61·9; H, 5·1%; mol. wt. 388.)

The anthrylglycuronic acid (0·5 g.) was hydrolysed by boiling with 0·2*N* H_2SO_4 for 4 hours. On cooling, the precipitate was filtered and extracted with benzene. The extract gave crude α-anthrol (III) (0·1 g.), which was acetylated with cold acetic anhydride and pyridine. The α-acetoxyanthracene was purified by sublimation *in vacuo* giving needles, M.P. 127° (α-acetoxyanthracene has M.P. 128–130°).

The residue after benzene extraction of the hydrolysis product was readily soluble in alcohol and was recrystallised from water. It appeared to be unchanged anthrylglycuronic acid; $[\alpha]_D^{20°}$ in dioxan (*c*, 0·8%) −50°. This appears to be identical with the anthrylglycuronic acid obtained by short boiling of the

original acid with dilute mineral acid and indicates that this acid is much more resistant to acid hydrolysis than other glycuronic acids, such as bornylglycuronic acid [Quick, 1927].

Oxidation of the original glycuronic acid with CrO_3 in acetic acid at 100° gave a yellow crystalline product which was recrystallised from aqueous alcohol, and resublimed *in vacuo*, M.P. 185–186° (α-dihydroxyanthraquinone has M.P. 193°). It was soluble in alkali with an orange colour and appeared to be α-hydroxyanthraquinone (IV). Oxidation of α-anthrylglycuronic acid (obtained by acid hydrolysis of the original acid) with $KMnO_4$ in alkaline solution gave the same α-hydroxyanthraquinone (IV). This was purified by sublimation *in vacuo* and formed orange needles, M.P. 193°.

Oxidation of the original glycuronic acid with alkaline $KMnO_4$ gave naphthalene-2:3-dicarboxylic acid (V), M.P. 236–238° (M.P. in literature 240°); equiv. wt. 114 (theory = 108). On vacuum sublimation this gave the anhydride, M.P. 242–244° (M.P. in literature 245°).

The glycuronic acid was not hydrogenated under the conditions which were effective with 1:2-dihydroxy-1:2-dihydroanthracene (*i.e.* using a Pd-$BaSO_4$ catalyst), but using dioxan as solvent and a Pd catalyst prepared by reduction of $PdCl_2$ with sodium formate the glycuronic acid took up H_2. The hydrogenation product was much less soluble in dioxan and acetone than the original acid and crystallised from hot water, M.P. 188°; $[\alpha]_D$ Na salt in water (*c*, 0·14 %) − 41°. (Found (Weiler): C, 56·6; H, 6·2 %; equiv. wt. 420. $C_{20}H_{22}O_8$, $2H_2O$ requires C, 56·3; H, 6·1 %; mol. wt. 426.)

It appeared to have one molecule of hydrogen more than the original acid and was probably 1:2-dihydroxy-1:2:3:4-tetrahydroanthraceneglycuronic acid (VI).

The reduced glycuronic acid was rapidly changed with boiling 2*N* HCl and gave 2-keto-1:2:3:4-tetrahydroanthracene (VII), M.P. 149° [Brown and Bayer, 1926], identical as to M.P. with that obtained from 1:2-dihydroxy-1:2:3:4-tetrahydroanthracene [Boyland and Levi, 1935]. The keto-compound could not be recrystallised satisfactorily. (Found (Weiler): C, 85·0; H, 6·4 %; $C_{14}H_{12}O$ requires C, 86·2; H, 6·1 %.)

Glycuronic acid from rat urine.

The rat urine gave a much less intense glycuronic acid reaction with naphthoresorcinol than the rabbit urine. After removal of the 1:2-dihydroxy-1:2-dihydroanthracene by ether extraction, the urine was acidified with HCl and extracted with ether in continuous extractors for 2 or 3 days. Small amounts of crystalline glycuronic acid separated from the ether. The yields were much smaller than those obtained from rabbit urine. The glycuronic acid was repeatedly crystallised from hot water and was finally obtained in laminae, M.P. 199–200° with decomposition; $[\alpha]_D$ in dioxan (*c*, 0·5 %) − 114°; $[\alpha]_D$ Na salt in H_2O (*c*, 0·3 %) − 126°.

Soluble with violet fluorescence in alcohol and acetone but not in benzene. (Found (Schoeller): C, 61·3; H, 5·05 %; equiv. wt. 392. $C_{20}H_{20}O_8$ requires C, 61·8, H, 5·15 %; mol. wt. 388.)

The pure substance gave a strong naphthoresorcinol reaction for glycuronic acid. The glycuronic acid was heated in a sealed tube at 100° with 0·2*N* HCl for 24 hours. A dark green precipitate was filtered off, dried, dissolved in benzene and acetylated with cold acetic anhydride and pyridine. The acetoxyanthracene was sublimed *in vacuo* to give white crystals, M.P. 126° (α-acetoxyanthracene, M.P. 128°–130°; β-acetoxyanthracene, M.P. 198°).

The glycuronic acid was oxidised with $KMnO_4$ in aqueous dioxan solution. The solution was decolorised with SO_2 and concentrated by evaporation. An orange precipitate was filtered off, dried and sublimed *in vacuo*. Orange needles, M.P. 193°, agreeing with that of α-hydroxyanthraquinone, M.P. 193°.

SUMMARY.

Rabbits fed on a diet containing anthracene excrete a dextrorotatory 1:2-dihydroxy-1:2-dihydroanthracene-1-glycuronic acid. Rats fed on the same diet excrete much less glycuronic acid, but it is probable that they excrete an analogous laevorotatory compound which is readily hydrolysed to α-anthryl-glycuronic acid.

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