CLXII. THE BIOCHEMISTRY AND PHYSIOLOGY OF GLUCURONIC ACID.

II. THE METHYLATION OF GLUCURONE OF ANIMAL ORIGIN.

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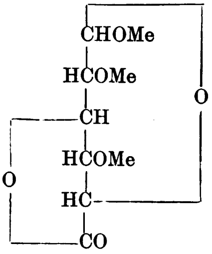
d-Glucurone is the stable anhydride of *d*-glucuronic acid and it is in the form of this lactone that glucuronic acid is generally obtained. It was first prepared by Schmiedeberg and Meyer [1879] from camphoglycuronic acid isolated from the urine of dogs fed with camphor. Free glucuronic acid was not isolated in a crystalline condition until 1925 [Ehrlich and Rehorst, 1925]. *d*-Glucurone has been isolated from both plant and animal sources, and it is clear that the products from both sources are identical. *d*-Glucurone probably possesses both a pyranoid and a furanoid ring, since most of its properties indicate it to be a γ-lactone. However, direct chemical evidence on this point is lacking.

The preparation of d-*glucurone.* Quick [1927] has hydrolysed, with dilute sulphuric acid, bornylglucuronide obtained from the urine of dogs receiving borneol. He obtained a product which by titration he found to be a mixture of 70 % of glucuronic acid and 30 % of glucurone. Adopting Quick's method in the present investigation identical results have been obtained. The lactone is separated from this mixture by crystallisation from glacial acetic acid, and the crystals so obtained are recrystallised from water.

In the work here described it was necessary to obtain the lactone in good yield and free from the acid. Quick's method was inconvenient and necessitated the use of an acid solvent—acetic acid—which tended to char the product. A method was therefore devised by means of which the glucuronic acid residue of bornylglucuronide could be obtained entirely as the lactone (see experimental section). The glucurone obtained in this way possessed the physical properties previously quoted for this compound.

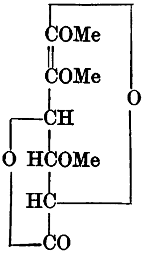
The methylation of d-*glucurone.* While this investigation was in progress, the methylation of *d*-glucurone, obtained by the hydrolysis of gum arabic, was described by Challinor *et al.* [1931]. These workers used methyl sulphate and alkali as methylating reagents and obtained trimethyl-β-methyl-*d*-glucuronide, a result which indicated that the lactone ring had been opened, as is to be expected when these methylating reagents are used. In the present investigation *d*-glucurone was methylated with silver oxide and methyl iodide. The products of methylation consisted of two crystalline solids, which at first were erroneously assumed to be stereoisomeric trimethylglucurones [Pryde and Williams, 1931; 1933, 1], and an uncrystallisable syrup. Out of a total of seven methylation experiments, crystalline trimethylglucurone was isolated in three instances, while the other four experiments yielded another crystalline solid which has been provisionally named trimethylglucuralone. In each methylation the solid product was obtained in a yield of 25 % of the glucurone used, whilst the other 75 % underwent more extensive methylation, as a result of the opening of the lactone ring. In no case were trimethylglucurone and trimethylglucuralone isolated together from the same methylation. The conditions under which either was formed seemed to be identical, but it is probable that some slight variation in the conditions of the methylation which would favour the formation of one or the other of these two solids has been overlooked by us. This problem is at present the subject of further investigation.

Trimethyl-d-*glucurone*. This compound was isolated after subjecting *d*-glucurone, dissolved in methyl alcohol, to two or three methylations, each of 8 hours' duration, with silver oxide and methyl iodide. It formed very long colourless prismatic needles of M.P. 131–132°. It was purified by recrystallisation from ether or methyl alcohol, preferably ether containing a little methyl alcohol. It was easily soluble in methyl alcohol and water but sparingly soluble in ether. It had $[\alpha]_{5461} + 197{\cdot}5°$ in water, which remained constant. Dissolved in methyl alcohol containing 1 % HCl, it showed a marked change in rotation at room temperature, the rotation falling according to a reaction of the first order. Its aqueous solution was neutral to litmus and Congo red papers, but was acid to methyl red indicator solution. It titrates as a lactone with alkali; it gives a positive reaction with naphthoresorcinol; it does not decolorise permanganate or bromine water like trimethylglucuralone (*vide infra*). Trimethylglucurone probably has the constitution:

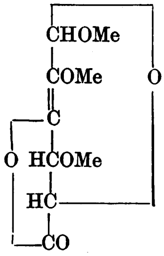


There is, however, no direct chemical evidence for the existence of the furanoid ring, although it is the most probable structure. The presence of a pyranoid ring may be inferred from the results already described in Part I of this series [Pryde and Williams, 1933, 2] and from those of Challinor, Haworth and Hirst [1931]. The writers hope to supply, in the near future, definite chemical evidence on this point.

Trimethylglucuralone. This compound was isolated as a crystalline solid from the methylation of *d*-glucurone with silver oxide and methyl iodide as has already been mentioned. It was crystallised from ether or methyl alcohol and formed elongated flat tablets, M.P. 88°, soluble in methyl alcohol and dissolving with initial difficulty in water; it is sparingly soluble in ether. Its rotation in water was $[\alpha]_{5461} + 110{\cdot}8°$, which remained constant. Unlike trimethylglucurone it does not undergo any change in rotation in methyl alcohol containing 1 % HCl, even on heating to 100° in a sealed tube. It gives a positive naphthoresorcinol reaction and titrates as a lactone with alkali. The facts that its aqueous solution decolorises permanganate immediately and bromine water a little more gradually were taken to indicate the presence of a double bond. Several analyses for C and H were carried out and although the analytical figures did not agree with those calculated for trimethylglucurone, $C_9H_{14}O_6$, they were consistent between themselves and indicated a compound with two hydrogen atoms less, $C_9H_{12}O_6$. This formula can be accommodated by a trimethylglucurone structure *minus* two hydrogen atoms. The following structure, A, is tentatively advanced, and it is suggested that this compound be called trimethylglucuralone on analogy with the glucals. A double bond is introduced to account for its action on permanganate and bromine water. This suggestion is supported by the fact that the introduction of an unsaturated linkage into a compound causes a marked change in optical rotation [*cf.* Stewart, 1919] and properties (*e.g.* rotation, trimethylglucurone + 197·5°; trimethylglucuralone + 110·8°; M.P. 132 and 88° respectively). This double bond could be in positions 1:2, 2:3, 3:4, 4:5 as shown by the structures A, B, C and D.



A



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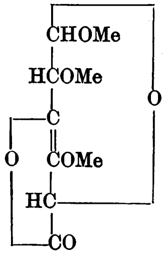
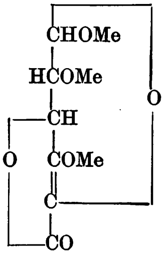
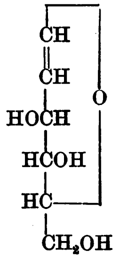


C



D



Glucal

If the compound has a double bond in the position indicated by the structure B, C or D, then, since carbon atom 1 in each of these structures possesses the mutarotating groups OMe and H, mutarotation might be expected on heating the compound with acid methyl alcohol. Reference to structure A will show the H atom of the first carbon atom to be absent, making mutarotation in this case impossible. Under the conditions already specified trimethylglucuralone shows no mutarotation, and therefore of the four structures A appears most probable and is tentatively adopted here. The introduction of a 1:2 double bond into a sugar derivative in the course of methylation is ascribed to the mild oxidising action of silver oxide.

The residual syrup. The main product of the methylation of glucurone was a yellow syrup which, after separation of the crystalline material, possessed a methoxyl value of 46–47 %. This syrup on further methylation gave a product of methoxyl value approaching the theoretical value (58·7 %) for a fully methylated glucuronic acid. Three specimens of the further methylated syrup, irrespective of whether trimethylglucurone or trimethylglucuralone had been isolated, showed rotations of $[\alpha]_{5461}$ + 30° in water and had refractive indices, $n_D^{18°}$ 1·4461, $n_D^{18°}$ 1·4465 and $n_D^{16°}$ 1·4679. The first two values for the refractive index agree with that for the α- and β-mixture of trimethylmethylglucuronide methyl ester obtained by the action of sulphuric acid in methyl alcohol on trimethylbornylglucuronide methyl ester [Pryde and Williams, 1933, 2], namely $n_D^{17°}$ 1·4469. This syrup was probably predominantly the β-isomeride of trimethylmethylglucuronide methyl ester, but it was not further investigated.

EXPERIMENTAL.

The preparation of d-*glucurone.* Bornyl glucuronide, isolated from the urine of dogs fed with borneol, was hydrolysed according to the method of Quick [1927]. The resulting mixture of glucuronic acid and its lactone was dissolved in glacial acetic acid (Quick). The crystals which separated on standing were brown in colour and were recrystallised from water, decolorising with charcoal; M.P. 177–178°; $[\alpha]_{5461}$ + 20·7° in water (c = 0·59).

Preparation of glucurone directly from bornylglucuronide and free from glucuronic acid. Quick's method does not give good yields when glucurone free from glucuronic acid is required. To attain this, 30 g. of bornylglucuronide were hydrolysed with 600 cc. of 0·2N H_2SO_4 by boiling for 4 hours. The solution was set aside to cool and the borneol recovered by filtration. The filtrate was treated with sufficient baryta solution to remove the H_2SO_4 quantitatively. The exact removal of H_2SO_4 is important since small residual quantities of this acid lower the yield of glucurone considerably. After standing for a short time the solution was filtered and concentrated *in vacuo* at 40° to a syrup. The syrup was taken up in the smallest quantity of water possible and sufficient alcohol was added to induce an incipient precipitation. The crystals formed on keeping in a refrigerator were filtered off and dried. The mother-liquors were put through the same sequence of operations and a further crop of glucurone obtained. The yield was 9–10 g.; M.P. 177–178°; $[\alpha]^{17°}_{5461}$ + 21·3° in water (c = 1·33); $[\alpha]_D$ calc. + 18°. In this way pure specimens of glucurone can be obtained without further recrystallisation.

The methylation of d-*glucurone. Isolation of trimethylglucurone and trimethylglucuralone.* A typical methylation was carried out as follows. Glucurone was methylated with excess MeI and Ag_2O, using MeOH as an extraneous solvent in the initial stages of the methylation. Each methylation was carried out for 8 hours and extraction of the products was performed with methyl alcohol and finally with dry ether. After 3 (sometimes 2) methylations, crystals were isolated in a yield of 25 % of the glucurone used. The residue after concentration of the extracts consisted of a mass of crystals embedded in a syrupy matrix. By shaking this syrupy mass with cold ether, the syrup dissolved leaving the crystalline material. The latter was recrystallised from ether containing a little methyl alcohol and dried in a desiccator.

In three cases out of seven the crystals were trimethylglucurone, M.P. 131–132° (needles); $[\alpha]^{16°}_{5461}$ + 197·5° in water (c = 0·76). Found C, 49·6; H, 6·5; OMe, 42·4 %; $C_9H_{14}O_6$ requires C, 49·5; H, 6·5; OMe, 42·7 %. 15·28 mg. of the lactone required 4·75 cc. of N/70 NaOH; calculated for $C_9H_{14}O_6$, 4·8 cc.

Trimethylglucurone (0·0467 g.) was dissolved in 10 cc. of methyl alcohol containing 1 % HCl and the change in rotation observed polarimetrically at constant temperature (20°). The initial rotation was $[\alpha]^{20°}_{5461}$ + 197° (after 5 mins.) falling to $[\alpha]^{20°}_{5461}$ + 43·5 in 190 mins. The fall in rotation followed the equation for reactions of the first order and therefore was due to mutarotation. The solution was then heated for 2 hours in a sealed tube at 100° to secure a constant equilibrium value, which was found to be $[\alpha]^{20°}_{5461}$ + 10·7°.

Trimethylglucurone did not decolorise permanganate solution or bromine water.

In the other four cases the crystalline solid was trimethylglucuralone; M.P. 88°; $[\alpha]^{18°}_{5461}$ + 110·8° in water (c = 0·63). Found C, 50·2, 50·0, 49·9, 50·2, 50·15; H, 5·6, 5·8, 5·7, 5·6, 5·65; OMe, 43·4 %. $C_9H_{12}O_6$ requires C, 50·0; H, 5·6; OMe, 43·1 %. 48·7 mg. of trimethylglucuralone required 3·3 cc. of 0·0625N NaOH; $C_9H_{12}O_6$ requires 3·5 cc.

Trimethylglucuralone (0·0346 g.) was dissolved in 10 cc. of methyl alcohol containing 1 % HCl and the rotation observed. The initial rotation was $[\alpha]^{20°}_{5461}$ + 110° and after 18·25 hours the rotation was unchanged. The solution was then heated in a sealed tube for 4 hours at 100° and the rotation still remained practically unchanged, being $[\alpha]^{20°}_{5461}$ + 107°. Trimethylglucuralone in aqueous solution decolorised permanganate solution immediately, while bromine water was decolorised gradually.

The residual syrup. On extracting with ether the mass of crystals and syrup obtained from the methylation of glucurone and evaporating the extract to dryness a yellow neutral syrup was obtained in a yield of 75 % of the glucurone methylated (found, average for six specimens, OMe, 46–47 %). These syrups were then subjected to further methylation with silver oxide and methyl iodide. After three further methylations, the syrups were recovered by ether extraction. After evaporating the solvent the resulting syrupy product was distilled in a high vacuum (found, on three specimens, OMe, 53·4, 54·7, 57·35 %; calculated for a fully methylated glucuronic acid $C_{11}H_{20}O_7$, OMe, 58·7 %); rotations $[\alpha]_{5461}^{20^\circ}$ + 29·6°, + 30·45°, + 30·5° in 20 % aqueous alcohol; refractive indices, $n_D^{18^\circ}$ 1·4465, $n_D^{18^\circ}$ 1·4461, and $n_D^{16^\circ}$ 1·4679. The data given for this syrup are quoted with reserve since it has not been prepared in a satisfactorily pure state; it is probably 2:3:4-trimethyl-β-methyl-*d*-glucuronide methyl ester, $C_{11}H_{20}O_7$.

SUMMARY.

A method is given whereby glucurone free from glucuronic acid can be prepared directly by hydrolysis of bornylglucuronide obtained from the urine of dogs fed on borneol. Methylation of glucurone with silver oxide and methyl iodide gives two crystalline solids, trimethylglucurone and an unsaturated derivative, trimethylglucuralone. Structures for both these compounds are tentatively suggested. The main bulk of the glucurone undergoes more extensive methylation owing to the opening of the lactone ring.

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