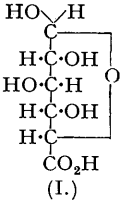
157. *Glucurone.*

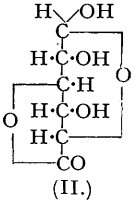
By F. Smith.

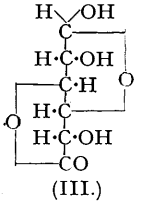
Treatment of glucurone with silver oxide and methyl iodide results in the formation of an analogue of ascorbic acid, 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (X) (Schmidt, Dippold, and Zeiser, *Ber.*, 1937, **70**, 2402; Smith, *J. Soc. Chem. Ind.*, 1938, **57**, 450; this vol., p. 510). This substance, first isolated but not characterised by Pryde and Williams (*Biochem. J.*, 1933, **27**, 1205), was accompanied by a crystalline trimethyl glucurone (IV). The latter is shown to be a 2 : 5-dimethyl methylglucofururonoside by the fact that it can be converted into the crystalline diamide (VIII) of 2 : 5-dimethyl glucosaccharic acid and also into 2 : 3 : 5-trimethyl glucosaccharo-1 : 4-lactone 6-methyl ester.

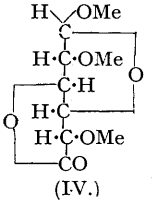
THE trimethyl glucurone (IV) can only be obtained on methylating glucurone (Pryde and Williams, *loc. cit.*) by means of silver oxide and methyl iodide if care is taken to avoid oxidation of the reducing group, probably rendered more active by the dicyclic system present in glucurone; some oxidation usually occurs and there is formed 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (X). When the glucurone was thus methylated at room temperture until the reducing group was methylated, a yield of 34% of trimethyl glucurone was obtained, and later, by the use of a still lower temperature in the initial stages of the methylation, Reeves (*J. Amer. Chem. Soc.*, 1940, **62**, 1616) obtained (IV) in a yield of 50%.

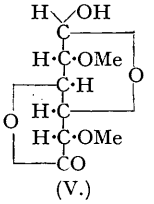
Since free sugars exist in the pyranose form, it seemed reasonable to expect that glucuronic acid would possess a six-atom ring as in (I), and that lactonisation would effect elimination of water between C_3 and C_6 (a lactone ring between C_2 and C_6 is stereochemically impossible) and afford glucurone with the structure (II). The other possible structure for glucurone (III), in which there is a 5-atom furanose ring and a 5-atom 3 : 6-lactone ring, had been considered (Smith, *loc. cit.*, 1938) but not deemed probable. It was therefore surprising to find that glucurone in the form of its trimethyl derivative contained two 5-atom rings. Subsequently, however, when investigations into similarly constituted compounds, such as the methylglycosides of 3 : 6-anhydroglucose, revealed that those derivatives containing two 5-atom rings were more stable than those with a 5- and a 6-atom ring (Haworth, Owen, and Smith, J., 1941, 88), the formulation of trimethyl glucurone as a substance with two 5-atom rings as in (III) became explicable.

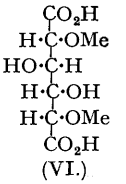

(I.)

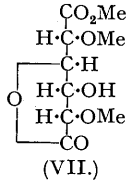

(II.)

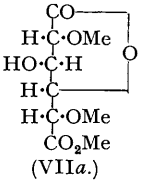

(III.)

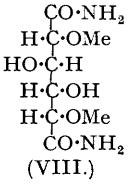

(IV.)

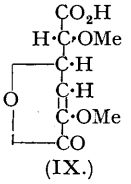

(V.)

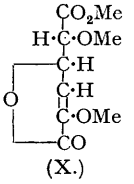

(VI.)

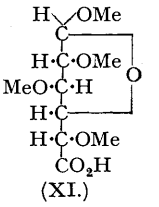

(VII.)

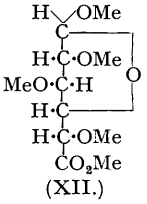

(VII*a*.)

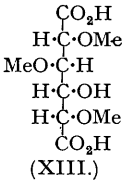

(VIII.)

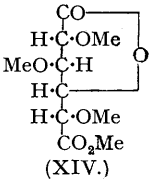

(IX.)


(X.)


(XI.)


(XII.)


(XIII.)


(XIV.)

The experimental basis upon which the structure (IV) is assigned to the crystalline trimethyl glucurone is as follows. The substance readily undergoes hydrolysis with dilute acid to give a dimethyl derivative of glucuronic acid (V). Oxidation of the latter with bromine may be considered to give the corresponding dimethyl glucosaccharic acid (VI), from which by means of methyl-alcoholic hydrogen chloride there were produced a dimethyl glucosaccharolactone methyl ester (VII, or VII*a*) and crystalline 2 : 5-dimethyl Δ^4-glucosaccharolactone (IX). The latter was identified by comparison with specimens previously made and characterised, and by its conversion into the methyl ester (X) with ethereal diazomethane (Smith, this vol., p. 515). The identification of (IX) strongly suggested that the two methyl groups in the lactone ester (VII, or VII*a*), and therefore in the trimethyl glucurone (IV), were located at C_2 and C_5. However, in view of the peculiar nature and formation of (X) from glucurone, this was not taken as good evidence for the determination of the structure of the trimethyl glucurone (cf. Reeves, *loc. cit.*), but the negative Weerman test (*Rec. Trav. chim.*, 1917, **36**, 16) displayed by the *diamide* (VIII) derived from the dimethyl glucosaccharolactone methyl ester (VII, or VII*a*) was taken as conclusive proof of the existence of the two methyl groups at C_2 and C_5 in trimethyl glucurone (IV). This clearly eliminated the structure (II) for glucurone, since this would give rise to a 2 : 4-dimethyl derivative of glucosaccharic acid. Moreover, since it is highly unlikely from stereochemical considerations that two 4-atom rings (a 1 : 3- and a 4 : 6-ring) could exist in glucurone and its trimethyl derivative, the location of the C_2 and C_5 methyl groups strongly supported the structure (III) for glucurone and (IV) for the crystalline trimethyl derivative.

Final proof of this was forthcoming from the fact that treatment of (IV) with sodium hydroxide and methyl sulphate, reagents which may open lactone rings and methylate any hydroxyl group set free, resulted in the formation of trimethyl methylglucofururonoside (XI), which was smoothly converted into the corresponding

methyl ester (XII) by means of ethereal diazomethane. Had this methyl ester (XII) contained any 2 : 3 : 4-trimethyl α-methylglucopyruronoside, it would easily have been recognised in the formation of its crystalline amide (Smith, J., 1939, 1724); when it was treated with methyl-alcoholic ammonia, however, none was detected. Oxidation of (XII) with concentrated nitric acid afforded a trimethyl glucosaccharic acid (XIII) which, by esterification and distillation, gave the characteristic crystalline 2 : 3 : 5-trimethyl glucosaccharo-1 : 4-lactone 6-methyl ester (XIV) (Smith, this vol., p. 571). The isolation of the latter having methyl groups at 2, 3, and 5 shows that, during the treatment of trimethyl glucurone (IV) with methyl sulphate and sodium hydroxide, a methyl group was introduced at C_3. Therefore, the lactone ring in trimethyl glucurone must engage C_3 and C_6, and hence the second ring of (IV) must join C_1 and C_4. Since the crystalline trimethyl glucurone (IV) shows a high positive rotation (+179°), it is believed to be an α-glycoside and hence it is designated as the 3 : 6-monolactone of 2 : 5-dimethyl α-methylglucofururonoside. The presence of two 5-atom rings in trimethyl glucurone (IV) thus being proved, it seems highly probable that this is true also for glucurone itself (III), in which case glucurone would appear to be one of the few furanose sugars existing in the free crystalline state (compare 3 : 6-anhydroglucose).

Experimental.

2 : 5-Dimethyl α-Methylglucofururonoside 3 : 6-Lactone (Trimethyl Glucurone) (IV).—Finely powdered crystalline glucurone (1·8 g.) in dry methyl alcohol (10 c.c.) and methyl iodide (2 c.c.) was treated with silver oxide (1 g.) at 15°. The mixture was shaken from time to time and the temperature was gradually raised in 1 hour to 27°, at which point methyl iodide (2 c.c.) was added. After 2½ hours the temperature had reached 35°; portions of methyl iodide (2 c.c.) and silver oxide (2 g.) were added then and again after a further hour at 35°, these additions being repeated after another 1½ hours, during which the temperature had been slowly increased to 40°. The operation was completed by heating for a further 3½ hours at 40°. The reaction mixture was filtered, and the residue washed well with hot acetone. Removal of the solvent from the combined filtrate and washings gave a pale yellow syrup which was subjected to further methylation by boiling with methyl iodide (10 c.c.) in the presence of silver oxide (5 g.) in the normal manner. The product, isolated by means of acetone, crystallised spontaneously. Purification by recrystallisation from ethyl alcohol–ether gave crystalline trimethyl glucurone, m. p. 132—133°, $[\alpha]_D^{20°}$ +179° in water (*c*, 0·4) (initial value, solution neutral to litmus); +149° (44 days; solution now acid to litmus); +134° (139 days). The substance does not reduce Fehling's solution. Retreatment of the syrup from the mother-liquors with silver oxide and methyl iodide gave a further small yield of crystalline material upon nucleation. The total yield of trimethyl glucurone was 0·75 g. (34%) (Found : C, 49·5; H, 6·5; OMe, 42·5. Calc. for $C_9H_{14}O_6$: C, 49·5; H, 6·5; OMe, 42·7%). Since this work was completed, Reeves (*loc. cit.*) has obtained (IV) in a yield of 50%.

Treatment of this trimethyl glucurone (20 mg.) with methyl-alcoholic ammonia for 24 hours at −5° gave the *amide* of 2 : 5-dimethyl methylglucofururonoside in good yield, m. p. 121° (after recrystallisation from ethyl alcohol–ether–light petroleum), $[\alpha]_D^{16°}$ +149·5° in water (*c*, 1·2) (Found : C, 46·4; H, 8·0; N, 6·1; OMe, 39·1. $C_9H_{17}O_6N$ requires C, 46·0; H, 7·3; N, 6·0; OMe, 39·6%).

Distillation of the syrup obtained from the mother-liquors after separation of trimethyl glucurone gave : Fraction I, 0·56 g., b. p. 120—125° (bath temp.)/0·02 mm., $n_D^{19°}$ 1·4470 (Found : OMe, 55·6. Calc. for the methyl ester of 2 : 3 : 5-trimethyl methylglucofururonoside : OMe, 58·7%); fraction II, 0·7 g., b. p. 140—160° (bath temp.)/0·02 mm., $n_D^{20°}$ 1·4580—1·4680, which crystallised slowly on standing. Trituration of the syrupy crystalline mass with ether–ethyl alcohol to remove the syrup, followed by recrystallisation from ethyl alcohol–ether, gave 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester (X), m. p. and mixed m. p. 89°.

Care must be exercised during the initial stages of the methylation of glucurone with Purdie's reagents, otherwise oxidation of the reducing group readily proceeds and the only crystalline product of the reaction is the unsaturated 2 : 5-dimethyl Δ^4-glucosaccharolactone 1-methyl ester.

Hydrolysis of the Monolactone (IV).—A solution of the crystalline lactone (0·92 g.) in N-sulphuric acid (20 c.c.) was heated on the boiling water-bath for 2 hours : $[\alpha]_D$ +165° (initial value); +62° (1 hr.); constant for a further hour. The solution, which at this stage reduced boiling Fehling's solution, was neutralised with barium carbonate, treated with a little charcoal, and filtered. After the residue had been washed with hot water, the filtrate was evaporated to dryness under diminished pressure. The barium 2 : 5-dimethyl glucuronate was obtained as a glassy solid which reduced Fehling's solution.

Oxidation. A solution of the glassy residue in water (15 c.c.) was treated with bromine (1 c.c.) at room temperature for 3 days and then no longer reduced Fehling's solution. Excess of bromine was removed by aeration. The solution was neutralised with silver oxide, filtered, and the filtrate was freed from silver ions by hydrogen sulphide. The solution was again filtered, and evaporated to dryness under reduced pressure, giving an acid product which also contained barium.

Esterification. The residue obtained from the previous experiment was boiled for 8 hours with 2% methyl-alcoholic hydrogen chloride (50 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to dryness. Purification of the syrupy product by extraction with ether followed by distillation gave : Fraction I (0·3 g.), b. p. 185° (bath temp.)/0·02 mm., $n_D^{12°}$ 1·4700; fraction II (0·36 g.), b. p. 220° (bath temp.)/0·02 mm., which crystallised spontaneously.

Examination of fraction I. An aqueous solution of the syrupy product gave a slightly acid reaction to litmus; it had $[\alpha]_D^{16°}$ +56° in water (*c*, 0·5) (initial value); +37° (7 hrs.); +34° (22 hrs.) [Found : OMe, 41·6. Calc. for $C_9H_{14}O_7$ (2 : 5-dimethyl glucosaccharo-3 : 6-lactone 1-methyl ester) : OMe, 39·8%]. The rest of the product was subjected to treatment with methyl-alcoholic ammonia at −5° for 2 days. The liquid was decanted from the crystals and evaporated to dryness in a vacuum. Recrystallisation of the combined crystalline products from ethyl alcohol gave the *diamide* (VIII) of 2 : 5-dimethyl glucosaccharic acid, m. p. 175°, $[\alpha]_D^{22°}$ +17° in water (*c*, 1·6) (Found : C, 41·1; H, 6·9; N, 11·5; OMe, 26·0. $C_8H_{16}O_6N_2$ requires C, 40·7; H, 6·8; N, 11·9; OMe, 26·3%). A Weerman test on this amide (20 mg.) was negative, a control test on gluconamide (20 mg.) being positive.

Examination of fraction II. Isolation of 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone. The solid distillate, after two recrystallisations from acetone–ether–light petroleum, had m. p. 171° alone or in admixture with a specimen of 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone previously prepared from silver saccharate, saccharo-3 : 6-lactone, saccharo-1 : 4-lactone, and saccharo-1 : 5-3 : 6-dilactone (Smith, this vol., p. 510). An aqueous solution of it reacted acid to Congo-red paper and showed selective absorption with the head of the band at 2290 A. (ε, 8000 approx.) (*c*, 3 mg. per 100 c.c.); it decolourised alkaline potassium permanganate in the cold but did not reduce Fehling's solution on boiling. The 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone had $[\alpha]_D^{18°}$ +76° in methyl alcohol (*c*, 0·7); $[\alpha]_D^{18°}$ +72° in water (*c*, 2·6) (initial value); equiv., 200 (by direct titration in the cold); 98 (after warming with 0·01N-sodium hydroxide) (Found : C, 47·7; H, 5·05; OMe, 31·2. Calc. for $C_8H_{10}O_6$: C, 47·5; H, 5·0; OMe, 30·7%).

Treatment of a solution of 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone (10 mg.) in methyl alcohol (1 c.c.) with a slight excess of ethereal diazomethane, followed by immediate removal of the solvent, gave 2 : 5-dimethyl Δ^4-glucosaccharo-3 : 6-lactone 1-methyl ester, m. p. and mixed m. p. 89°.

Methylation of the 3 : 6-*Monolactone of* 2 : 5-*Dimethyl Methylglucofururonoside with Methyl Sulphate.*—The crystalline lactone (0·3 g.), dissolved in acetone (10 c.c.), was treated with methyl sulphate (5 c.c.) and 30% sodium hydroxide solution (15 c.c.). The reagents were added dropwise during 1½ hours to the reaction mixture, which was well stirred and maintained at 40°. After another ½ hr.'s heating, the methylation mixture was cooled in ice, acidified with dilute sulphuric acid, and filtered to remove sodium sulphate, which was washed with chloroform, the washings being collected in the same filter flask as the aqueous filtrate. The chloroform layer was separated, and the aqueous layer extracted four times with chloroform. The chloroform extracts were combined, washed twice with a saturated solution of sodium sulphate, and then dried over anhydrous magnesium sulphate. The solution was filtered, and evaporated under slightly reduced pressure at 35°. The pale yellow, syrupy product thus obtained (0·3 g.) reacted acid to Congo-red paper. It was subjected to methylation by 6 hours' boiling with methyl iodide (5 c.c.) in the presence of silver oxide (2 g.). Isolation of the product by means of acetone, followed by removal of the solvent, gave the methyl ester of 2 : 3 : 5-trimethyl methylglucofururonoside (XII), which distilled as a colourless, mobile liquid (0·27 g.), b. p. 120° (bath temp.)/0·03 mm., $n_D^{16°}$ 1·4480, $[\alpha]_D^{18°}$ +122° in water (*c*, 0·7) (Found: OMe, 57·0; equiv., by heating with 0·01N-NaOH for 1 hour at 60°, 260. $C_{11}H_{20}O_7$ requires OMe, 58·7%; equiv., 264). This ester failed to give a crystalline amide upon treatment with methylalcoholic ammonia for 2 days at −5°.

Oxidation of the Methyl Ester of 2 : 3 : 5-*Trimethyl Methylglucofururonoside with Nitric Acid.*—The syrupy ester (0·2 g.) obtained above was heated with nitric acid (3 c.c.; *d* 1·42) for ½ hour at 60° and for 1½ hours at 90°, oxidation then appearing to be complete. The solution was diluted with water and evaporated under diminished pressure, water being added from time to time to facilitate removal of nitric acid. The last traces of the latter were removed by addition and distillation of methyl alcohol under reduced pressure. The dry, acid, syrupy residue was then esterified by dissolving it in methyl alcohol (5 c.c.) and adding a slight excess of ethereal diazomethane. Removal of solvent gave a colourless liquid, which crystallised spontaneously. It was purified by distillation in a high vacuum. Recrystallisation from ether gave 2 : 3 : 5-trimethyl glucosaccharo-1 : 4-lactone 6-methyl ester, m. p. and mixed m. p. 78—79°, $[\alpha]_D^{16°}$ −10° in water (*c*, 3·2) (Found: C, 48·6; H, 6·1; OMe, 50·15. Calc. for $C_{10}H_{16}O_7$: C, 48·4; H, 6·45; OMe, 50·0%).

A. E. Hills Laboratories,
The University, Edgbaston, Birmingham, 15.

[*Received, August 14th,* 1944.]