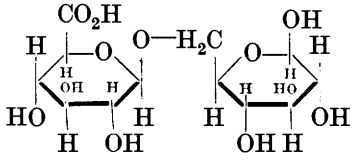
XXXIV.—*The Compound Uronic Acids. Structure of the Aldobionic Acid from Gum Arabic.*

By Sydney William Challinor, Walter Norman Haworth, and Edmund Langley Hirst.

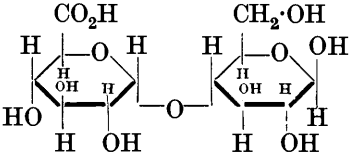
Recent work in immunology has excited renewed interest in the compound uronic acids because of the specific properties associated with some representatives of these carbohydrate derivatives. The

soluble specific substances which are produced in cultures of types II and III antipneumococcus sera (and precipitate the anti-serum of that type) are known to give rise on partial hydrolysis to " aldobionic acids." The latter have been shown to be glycuronosidoglucoses (Heidelberger and Goebel, *J. Biol. Chem.*, 1927, **74**, 613, 619). A product of similar specific activity has recently been derived by partial hydrolysis from gum arabic and, on further hydrolysis, this yields an " aldobionic acid " which contains a galactose and a glycuronic residue and is therefore an oxidation derivative of a simple biose (Butler and Cretcher, *J. Amer. Chem. Soc.*, 1929, **51**, 1519; compare C. O'Sullivan, J., 1884, **45**, 41). A crystalline " aldobionic acid " which seems to be identical with the former has been recognised, and suggestions (Heidelberger and Kendall, *J. Biol. Chem.*, 1929, **84**, 639), which leave, however, some dubiety, have been made for its structure.

It seemed to us probable that these hexose-hexo-uronic acids are structurally related to disaccharides such as maltose, cellobiose, and gentiobiose, the constitutions of which we have already determined. The oxidation products corresponding to " aldobionic acids " may therefore be recognisable as belonging to one of the two types :



Gentiobiouronic acid.



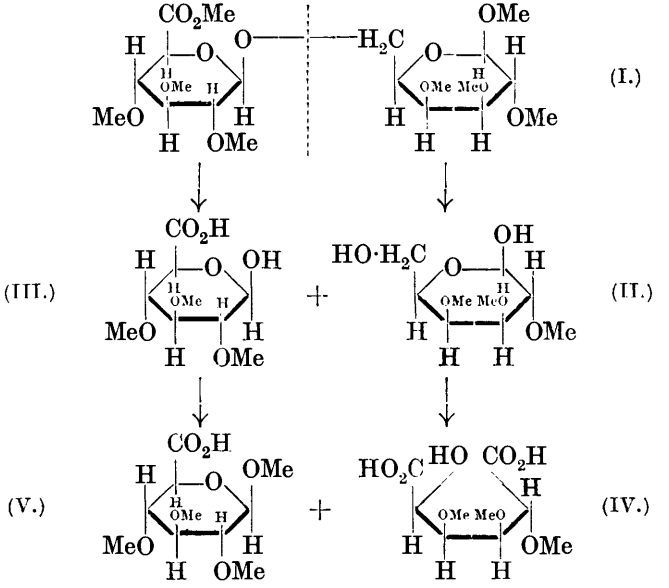
Maltobiouronic acid.

We submit the results of preliminary experiments which support the view that the aldobionic acid from gum arabic is structurally similar to the first of these possible types, having the biose linking through the side-chain. The reducing component is, however, galactopyranose.

Methylation of this aldobionic acid prepared from gum arabic (Kordofan) gave rise to a completely methylated specimen of the biose-uronic ester, of which the β-form was crystalline but the mixture of α- and β-modifications distilled as a colourless syrup. This specimen of the *methyl heptamethyl aldobionate* (*methyl* ester of *hexamethyl glycuronosidomethylgalactoside*) (I) hydrolysed with approximately the same velocity as a methylated biose, and yielded two cleavage fragments which were recognised as a trimethyl galactose (II) and a trimethyl glycuronic acid (III). The former of these products gave rise on further methylation to a high yield of 2 : 3 : 4 : 6-tetramethyl β-methylgalactoside, which was identified by its m. p. and comparison with an authentic specimen. The

product (II) yielded on oxidation a lactone which underwent hydration at the same rate as 2 : 3 : 4 : 6-tetramethyl δ-galactonolactone (Drew, Goodyear, and Haworth, J., 1927, 1237) and also gave a crystalline phenylhydrazide. On oxidation with nitric acid the trimethyl galactose yielded a trimethyl mucic acid (IV), of which the dimethyl ester was crystalline. The latter result afforded conclusive evidence of the presence of a free $CH_2 \cdot OH$ group in the trimethyl galactose. The orientation of three methyl groups in the sugar could only be represented by the 2 : 3 : 4- or 2 : 3 : 5-positions, and the latter possibility seems at present to be excluded by the evidence of the δ-lactone, although confirmation of this view will be sought by other means.

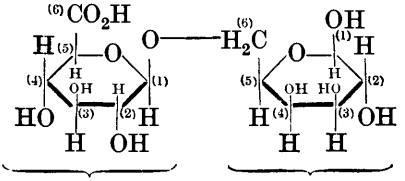
What is abundantly clear at this stage is that the 6-position of the galactose residue is joined, in the biose type of linking, with the reducing group of the glycuronic acid residue.



The trimethyl glycuronic acid (III) was converted into a characteristic crystalline derivative, recognised as 2 : 3 : 4-*trimethyl* β-*methylglycuronide* (V), which is a non-reducing methylglucosidic form of methylated glycuronic acid. For comparison, a specimen was also prepared direct from glycuronic acid and this was shown to be identical. Furthermore, the rate of hydrolysis, expressed as $k \times 10^5$ in minutes and decimal logarithms, was found to be 25, which is almost identical with that found for β-methylglucopyranoside under similar experimental conditions (Haworth and Hirst, J., 1930,

2625). The ring structure of the trimethyl glycuronic acid is therefore that of a pyranose form and the methyl groups can only be accommodated at positions 2 : 3 : 4.

The structure of the aldobionic acid isolated from gum arabic appears to be that given below. We are not, however, in a position to decide whether α- or β-glycuronic acid is involved in the biose linking, although preliminary evidence favours a β-linking.



Glycuronic acid component. Galactose component.

EXPERIMENTAL.

The aldobionic acid was prepared from gum arabic (Kordofan) by the method of Butler and Cretcher (*loc. cit.*). The gum arabic used had $[\alpha]_D^{20°}$ −30° in water. The aldobionic acid was isolated as the calcium salt, the properties of which were in agreement with those recorded by the above authors; $[\alpha]_D^{20°}$ +2·0°, constant value in water (*c*, 5·1). Before analysis the substance was dried at 100° in a vacuum (Found : Ca, 5·05. Calc. for $C_{24}H_{38}O_{24}Ca$: Ca, 5·3%).

Methylation of the Aldobionic Acid.—Acetone was added to a solution of calcium aldobionate (15 g.) in water (50 c.c.) until a slight permanent turbidity appeared. Methylation was effected by methyl sulphate (133 c.c.) and 30% aqueous sodium hydroxide (340 c.c.), one half of the reagents being added simultaneously at 35° until the reducing action disappeared (7 hours), and the remainder at 70° in the usual manner. Sufficient dilute sulphuric acid was added to the ice-cold mixture to neutralise the excess of sodium hydroxide and to liberate the organic acid from its sodium salt. After removal of the solid material by filtration, extraction of the aqueous portion with chloroform gave only 5 g. (A) of methylated aldobionic acid. Accordingly the solid residue from the filtration was washed several times with warm methylated spirit, and the combined washings were made slightly alkaline with sodium hydroxide and evaporated to small bulk (B). The aqueous portion after extraction with chloroform was made slightly alkaline and concentrated under reduced pressure to small bulk (C). (A), (B) and (C) were then united and remethylated at 60—70° in the presence of acetone (40 c.c.) by methyl sulphate (133 c.c.) and 30% aqueous sodium hydroxide (340 c.c.).

Dilute sulphuric acid was added to the cold mixture to liberate the organic acid. The greater part of the sodium sulphate was precipitated by methylated spirit. The solid was washed with methylated spirit and the combined washings were added to the aqueous portion. This was then made slightly alkaline, evaporated to small volume under diminished pressure, acidified, and extracted with chloroform (yield, 14·3 g.). It is important that no free sulphuric acid should be present during the extraction.

Methylation was completed by silver oxide and methyl iodide. After one such treatment the product was distilled, giving methyl heptamethyl aldobionate as a colourless non-reducing viscid liquid (9·4 g.), which contained an excess of the β-form; b. p. about 185°/0·02 mm., $n_D^{20°}$ 1·4715, $[\alpha]_D^{21°}$ −3·5° in water (*c*, 1·55), $[\alpha]_D^{20°}$ −24° in chloroform (*c*, 2·0). It was non-reducing (Found: C, 51·3; H, 7·8; OMe, 50·1. $C_{20}H_{36}O_{12}$ requires C, 51·3; H, 7·8; OMe, 53·0%).

When kept at 45—50° the syrupy methyl heptamethyl aldobionate gradually deposited the crystalline β-form. After 14 days the mixture of syrup and crystals was dissolved in boiling light petroleum (b. p. 40—60°). On cooling, a syrup separated, from which the supernatant liquid was decanted. The solution then deposited *methyl heptamethyl aldobionate* (or *methyl* ester of *hexamethyl* 6-*glycuronosido*-β-*methylgalactoside*) in rosettes of needles, which were recrystallised from light petroleum; m. p. 86°, $[\alpha]_D^{20°}$ −21° in water (*c*, 0·67), $[\alpha]^{20°}$ −43° in chloroform (*c*, 0·65) (Found: C, 51·0; H, 7·8%). By repeated treatments, about 20% of the syrupy ester was obtained crystalline.

Hydrolysis of Methyl Heptamethyl Aldobionate.—The ester (4·85 g.) used was a mixture containing both α- and β-forms. Hydrolysis was effected by heating for 3½ hours at 100° in 7% aqueous hydrochloric acid (100 c.c.). Polarimetric observations were made as follows: $[\alpha]_D^{20°}$ −1° (initial value); + 49° (1 hour); 58° (2 hrs.); 63° (3 hrs.); 63·5° (3½ hrs., constant value). Excess of barium carbonate was then added in order to neutralise the mineral acid and form the barium salt of trimethyl glycuronic acid. The neutral solution was evaporated to dryness under diminished pressure at 40°, the last traces of water being removed by the addition of alcohol. Removal of the alcohol under diminished pressure left a white solid, which was exhaustively extracted with boiling dry ether. The ethereal extract gave 2 : 3 : 4-*trimethyl galactose* as a pale yellow, viscid syrup which reduced boiling Fehling's solution (yield, 87% of the theoretical). The following physical constants were observed for undistilled material, purification by distillation not being attempted owing to risk of decomposition: $n_D^{16°}$ 1·4727, $[\alpha]_D^{20°}$ +

83° in water (*c*, 0·6) (Found: OMe, 38·5. $C_9H_{18}O_6$ requires OMe, 41·9%).

The solid residue was heated at 60° until free from ether and was then dissolved in water (70 c.c.). Sufficient hydrochloric acid was added to liberate 90% of the trimethyl glycuronic acid from its barium salt. The solution was concentrated to small volume at 40°/12 mm., any trace of mineral acid still present was removed by the continuous addition and evaporation of water at 40°/12 mm. for 6 hours, and the solution was evaporated to dryness at 45°/12 mm. The dry, cream-coloured powder was extracted 10 times with boiling dry ether. The combined ethereal extracts gave on evaporation 2 : 3 : 4-*trimethyl glycuronic acid* as a pale yellow, viscid syrup (1·93 g.) which could not be crystallised. Careful treatment of the solid residue with mineral acid gave a further 0·22 g. (total yield, 88% of the theoretical). Owing to the risk of decomposition of trimethyl glycuronic acid in the presence of mineral acid it is advisable to carry out the isolation in two stages as described above.

Methylation of 2 : 3 : 4-*Trimethyl Galactose.*—2 : 3 : 4-Trimethyl galactose (0·8 g.) was methylated by methyl sulphate (6·9 c.c.) and 30% aqueous sodium hydroxide (15·4 c.c.) in the presence of acetone, at 35° until the reducing action had disappeared and then at 55—60°. The product (0·77 g.) was re-methylated by methyl sulphate and alkali at 55—60°, giving a mobile syrup (0·72 g.) which immediately crystallised. The solid was spread on porous earthenware and recrystallised from light petroleum (b. p. 40—60°), giving 2 : 3 : 4 : 6-tetramethyl β-methylgalactoside, m. p. 44—45° alone and in admixture with an authentic sample. $[\alpha]_D$ + 20° in water (*c*, 0·2; *l*, 4).

2 : 3 : 4-*Trimethyl* δ-*Galactonolactone.*—A solution of 2 : 3 : 4-trimethyl galactose (1·16 g.) in water (15 c.c.) was heated with bromine (3·8 c.c.) at 35° for 24 hours and then kept at 15° for 30 hours. The solution was then non-reducing. The excess of bromine was removed by aeration, the acids were neutralised by silver oxide, and thereafter the organic acid was liberated from its silver salt by titration with *N*/2-hydrochloric acid. Evaporation of the solution under diminished pressure left a viscid syrup, from which any inorganic impurities were removed by solution in chloroform. The solvent was evaporated under diminished pressure and the syrupy residue was heated for 2 hours at 100°/12 mm., giving 2 : 3 : 4-*trimethyl* δ-*galactonolactone* (1 g.) as a viscid straw-coloured syrup (yield 86%) which could not be crystallised but was characterised as its crystalline *phenylhydrazide*. This substance was prepared by heating together at 100° equivalent quantities of the lactone and phenylhydrazine (yield, quantitative): recrystallisation from ethyl

acetate containing some ether gave colourless needles, m. p. 165—167° (Found : C, 54·7; H, 7·7; OMe, 27·9. $C_{15}H_{24}O_6N_2$ requires C, 54·9; H, 7·4; OMe, 28·4%).

The rate of hydrolysis of 2 : 3 : 4-trimethyl galactonolactone in aqueous solution was similar to that of tetramethyl δ-galactonolactone. In view of the risk of decomposition during the distillation of partly methylated sugar lactones it was considered inadvisable to attempt purification of the trimethyl lactone by distillation or any method involving distillation. The following figures, therefore, whilst indicating correctly the rate of hydrolysis of the lactone, cannot be regarded as giving accurately the initial rotation of the pure substance in water. $[\alpha]_D^{19°}$ + 80° (initial value in water; *c*, 0·7); 64° (30 mins.); 54° (1 hr.); 43° (2 hrs.); 35° (3 hrs.); 30° (4 hrs.); 23° (6 hrs.); 19° (8 hrs., constant value).

Oxidation of 2 : 3 : 4-*Trimethyl* δ-*Galactonolactone to* 2 : 3 : 4-*Trimethyl Mucic Acid.*—A solution of 2 : 3 : 4-trimethyl δ-galactonolactone (0·85 g.) in concentrated nitric acid (5 c.c., *d* 1·42) was heated at 50° until the somewhat vigorous evolution of nitrous fumes had ceased (1 hour). The temperature was then slowly raised and maintained at 90° for 4 hours. The solution, after dilution with twice its volume of water, was evaporated at 40°/12 mm. until the volume was 8 c.c. The distillation was continued with simultaneous addition of water for 12 hours, until the whole of the nitric acid had been removed. The oxidation product was converted into the methyl ester in the usual way by boiling with methyl-alcoholic hydrogen chloride. The acid was removed by silver carbonate and on evaporation the esterified oxidation product was obtained as a pale yellow, rather viscid liquid (0·62 g.) which immediately began to crystallise. After 2 days the syrupy portion was separated from the solid by repeated washing with ether, in which the crystals were only sparingly soluble. In this manner the *dimethyl* ester of 2 : 3 : 4-*trimethyl mucic acid* was obtained as well-defined colourless plates which were neutral to litmus and had m. p. 98°, $[\alpha]_D^{20°}$ + 35° in water (*c*, 0·6) [Found : C, 47·3; H, 7·6; OMe, 54·7; CO_2Me (by titration), 41·0. $C_{11}H_{20}O_8$ requires C, 47·1; H, 7·2; OMe, 55·3; CO_2Me, 42·1%].

The syrupy portion was a complex mixture of products which gave crystalline methylamides when treated with methylamine in methyl alcohol. Attempts to separate the mixed methylamides by fractional crystallisation were unsuccessful and owing to the small amount of material available these products are held over for further investigation.

2 : 3 : 4-*Trimethyl Glycuronic Acid.*—This acid, which was obtained by the method described above, reduced Fehling's solution vigorously and was acidic in reaction towards Congo-red and litmus. It had

$n_D^{16°}$ 1·4709 and $[\alpha]_D^{18°}$ +58° (equilibrium value in water; *c*, 0·63). These values are given with reserve, since the product had not been distilled, owing to the risk of decomposition (Found: OMe, 36·6. $C_9H_{16}O_7$ requires OMe, 39·4%).

Methylation of 2 : 3 : 4-*trimethyl glycuronic acid* (1·02 g.) by methyl sulphate (8·2 c.c.) and 30% aqueous sodium hydroxide (18·5 c.c.) in the presence of acetone (10 c.c.) and water (5 c.c.) at 35° until reducing action ceased (2 hours) and then at 55—60° in the usual manner gave a mixture of the α- and β-forms of 2 : 3 : 4-*trimethyl methylglycuronide* (1 g.). The product was isolated by the method previously described for methylated aldobionic acid (see above). Recrystallisation from light petroleum (b. p. 40—60°) gave 2 : 3 : 4-*trimethyl* β-*methylglycuronide* as colourless needles having a strong acid reaction; m. p. 133°, $[\alpha]_D^{23°}$ −38° in water (*c*, 0·4), $[\alpha]_D^{21°}$ −63° in chloroform (*c*, 0·6) (Found: C, 48·05; H, 7·6; OMe, 48·7. $C_{10}H_{18}O_7$ requires C, 48·0; H, 7·2; OMe, 49·6%).

Methylation of Glycuronic Acid.—Glycuronic acid was prepared from gum arabic (Kordofan) by Wienmann's method (*Ber.*, 1929, **62**, 1637). Crystallisation of the acid so obtained proceeded slowly and it was more convenient to work with the crystalline lactone (glycuron), $[\alpha]_D^{20°}$ + 18°, which separated when the syrupy glycuronic acid was kept in a vacuum desiccator. The lactone (2·5 g.) was methylated by methyl sulphate (27 c.c.) and 30% aqueous sodium hydroxide (62 c.c.) in the presence of water (30 c.c.) and acetone (40 c.c.) at 35° until the reducing action disappeared and then at 50°. The product (2·25 g.), which was isolated by the method given above, was remethylated by methyl sulphate and alkali. This gave an acidic non-reducing syrup (2 g.) which partly crystallised. Recrystallisation from ether–light petroleum (b. p. 40—60°) gave 2 : 3 : 4-trimethyl β-methylglycuronide, m. p. 133° alone or in admixture with the material described in the preceding paragraph (Found: C, 48·1; H, 7·4%).

In *N*-hydrochloric acid at 100° the velocity of hydrolysis of 2 : 3 : 4-trimethyl β-methylglycuronide was almost identical with that of β-methylglucoside under similar conditions, the value of *k*, in terms of minutes and decimal logarithms, being 25 × 10^{-5} (calc. for *N*/100-acid). $[\alpha]_D^{20°}$ − 40° (initial value), + 6° (10 mins.), + 33° (20 mins.), + 47° (30 mins.), + 54° (40 mins.), + 59° (50 mins.), + 61·5° (60 mins., constant value).

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UNIVERSITY OF BIRMINGHAM,
EDGBASTON.

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