## 697. *The Constitution of Mesquite Gum. Part IV.* Determination of the Structure of the Amide of 6-β-(4-Methyl D-Glucopyruronosyl) α-Methyl-D-galactopyranoside.*

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Controlled hydrolysis of mesquite gum was shown previously to give a monomethyl aldobiuronic acid (I) and 4-methyl D-glucuronic acid (Smith, *J.*, 1951, 2646). The crystalline amide (II) obtained from (I) is shown to be derived from 6-β-(4-methyl D-glucopyruronosyl) α-methyl-D-galactopyranoside.

Methanolysis of degraded mesquitic acid or the aldobiuronic acids obtained from it yielded 4-methyl methyl-D-glucopyruronoside methyl ester (White, *J. Amer. Chem. Soc.*, 1948, **70**, 367) and the methyl ester (II) of a monomethyl aldobiuronoside which was isolated as a crystalline amide (III). The determination of the structure of (III) forms the subject of this communication.

When (III) was hydrolysed with sulphuric acid it gave rise to 4-methyl D-glucuronic acid and D-galactose, as shown by paper chromatography, and prolonged methanolysis followed by treatment with ammonia yielded the known crystalline amide of 4-methyl α-methyl-D-glucopyruronoside (Smith, *loc. cit.*). There was thus reason to believe that (III) was composed of a unit of D-galactose and one of 4-methyl D-glucuronic acid, each unit being of the pyranose form.

Three molecular proportions of sodium periodate were consumed by (III) at 5°, with the liberation of one molecular proportion of formic acid. Since the 4-methyl D-glucuronic acid moiety is joined through its reducing group it is clear that it will consume one molecular proportion of periodate without the formation of formic acid, cleavage occurring between positions 2 and 3 (see VI). The methylgalactoside residue, being responsible for the consumption of two molecular proportions of periodate and the production of one molecular proportion of formic acid, was therefore most likely joined through position 6 to the reducing group of the 4-methyl D-glucuronic acid residue.

Confirmation of the presence of the 1 : 6-linkage in (III) was forthcoming from the fact that the fully methylated aldobiuronoside (IV), obtained either by methylation of (II) first with methyl sulphate and alkali and then with diazomethane or by treatment of the acid from (III) successively with diazomethane and Purdie's reagents, underwent smooth reduction with lithium aluminium hydride according to conditions previously used (Abdel Akher and Smith, *Nature*, 1950, **166**, 1037; Smith, *loc. cit.*; cf. Lythgoe and Trippett, *J.*, 1950, 1983) to give the crystalline methylglycoside of a hexamethyl disaccharide (VIII). The latter was recognized as 6-(2 : 3 : 4-trimethyl D-glucopyranosyl) 2 : 3 : 4-trimethyl methyl-D-galactopyranoside since on hydrolysis it yielded 2 : 3 : 4-trimethyl D-glucose and 2 : 3 : 4-trimethyl D-galactose. The crystalline disaccharide (VIII) differed from the crystalline disaccharide, 6-β-(2 : 3 : 4-trimethyl D-glucopyranosyl) 2 : 3 : 4-trimethyl β-methyl-D-galactopyranoside (VII), obtained by lithium aluminium hydride reduction of 6-β-(2 : 3 : 4-trimethyl D-glucopyruronosyl) 2 : 3 : 4-trimethyl β-methyl-D-galactopyranoside methyl ester (V) (Jackson and Smith, *loc. cit.*), although it gave the same hydrolysis products, namely, 2 : 3 : 4-trimethyl D-glucose and 2 : 3 : 4-trimethyl D-galactose.

It was also established that the fully methylated aldobiuronoside (IV) afforded a crystalline amide (IX) which was distinct in melting point and specific rotation from the amide derived from (V) whose structure was known (Jackson and Smith, *J.*, 1940, 74; Cunneen and Smith, *J.*, 1948, 1141). Since (VIII) has been shown above to be derived from 6-(2 : 3 : 4-trimethyl D-glucopyruronosyl) 2 : 3 : 4-trimethyl methyl-D-galactopyranoside it follows that the difference between the new aldobiuronamide derivative (IX) and the known amide from (V), and likewise the difference between the methylated disaccharides (VIII) and (VII), is to be traced either to the stereochemistry at the biose linkage or to that at the anomeric $C_{(1)}$ position. Inasmuch as the amide from (V) is the only 1 : 6-linked aldobiuronoside derivative obtained when the monomethyl aldobiuronic

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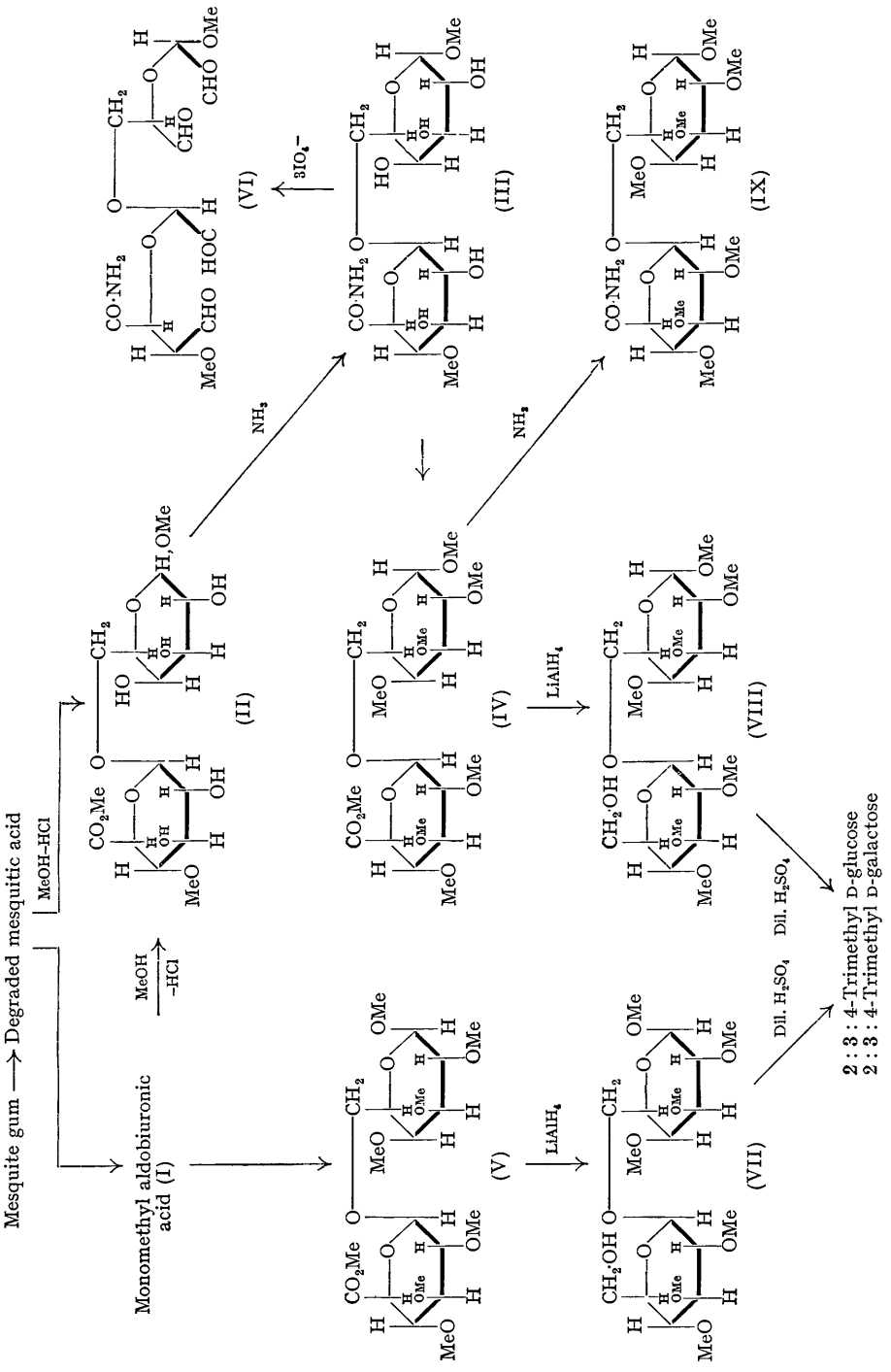
Mesquite gum
Degraded mesquitic acid
MeOH–HCl
Monomethyl aldobiuronic acid (I)
MeOH
–HCl
(II)
NH₃
(III)
3IO₄⁻
(VI)
(IV)
LiAlH₄
(V)
LiAlH₄
(VIII)
NH₃
(IX)
(VII)
Dil. H₂SO₄
Dil. H₂SO₄
2 : 3 : 4-Trimethyl D-glucose
2 : 3 : 4-Trimethyl D-galactose

acid (I) derived from mesquite gum is methylated directly with methyl sulphate and alkali and converted into an amide (Cunneen and Smith, *loc. cit.*), it is apparent that the two amides and the corresponding neutral disaccharides are derived from one and the same 1 : 6-β-linked aldobiuronic acid. The only difference between the amides and between the disaccharides must therefore lie in the position of the hydrogen and methoxyl groups at $C_{(1)}$. Since the amide from (V) and the stereochemically related disaccharide (VII) are β-glycosides it follows that (IX) and (VIII) must be α-methylglycosides. Consequently (III) is the amide of 6-β-(4-methyl D-glucopyruronosyl) α-methyl-D-galactopyranoside.

## Experimental

*Oxidation of the Amide* (III) *of* 6-β-(4-*Methyl* D-*Glucopyruronosyl*) α-*Methyl*-D-*galactopyranoside with Sodium Periodate.*—The amide (III) (50·7 mg.; prepared according to Smith, *loc. cit.*) was dissolved in 0·083N-sodium periodate (50 ml.) and kept at 5° (see Abdel Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 994). The rate of oxidation was followed by withdrawing portions (5 ml.) of the solution at suitable intervals and adding 0·01N-sodium arsenite (50 ml.), sodium hydrogen carbonate (1—2 g.), and a crystal of potassium iodide, and after 10 minutes back-titrating it with 0·01N-iodine, with the following results: 1·17 mols. (after 9 minutes); 1·58 (32 minutes); 1·89 (60 minutes); 2·11 (117 minutes); 2·41 (309 minutes); 2·98 (30 hours). After 68 hours and destruction of the excess of periodate with ethylene glycol (0·5 ml.), titration of an aliquot (5 ml.) of the solution with 0·01N-sodium hydroxide (indicator, methyl-red) showed that 1·1 mols. of formic acid were produced.

*Hydrolysis of the Amide* (III).—A solution of the amide (10 mg.) in N-sulphuric acid (0·5 ml.) in a small sealed tube was heated on the boiling-water bath for 18 hours, then neutralized with barium carbonate, filtered, passed through a column of " Dowex 50 " cation-exchange resin, and evaporated *in vacuo* to a syrup. This syrup, along with standard samples of D-galactose and 4-methyl D-glucuronic acid (prepared from the amide of 4-methyl methyl-D-glucopyruronoside by acid hydrolysis in the above manner), were subjected to partition chromatography on Whatman No. 1 filter paper with the upper layer of *n*-butanol–5N-formic acid (1 : 1) as the irrigating solvent. The syrup contained two components whose $R_F$ values corresponded to D-galactose and 4-methyl D-glucuronic acid.

*Treatment of the Amide* (III) *with Methyl-alcoholic Hydrogen Chloride.*—The amide (15 mg.) was heated for 20 hours at 100° with 8·0% methanolic hydrogen chloride (2 ml.). The solution was neutralized with silver carbonate, filtered, and evaporated to dryness *in vacuo*. The product was transformed into the amide in the usual way with methanolic ammonia. On removal of the solvent, the amide of 4-methyl α-methyl-D-glucopyruronoside crystallized, m. p. 229—230° alone or on admixture with an authentic specimen (after crystallization from methanol).

*Methylation of the Amide* (III).—A solution of the amide (225 mg.) was methylated three times in the usual way at 40—50° with methyl sulphate (9 ml.) and 30% sodium hydroxide (24 ml.), the product being extracted with chloroform after each methylation. The syrupy product (195 mg.) was further methylated by the Purdie procedure and distilled, yielding 6-β-(2 : 3 : 4-trimethyl D-glucopyruronosyl) 2 : 3 : 4-trimethyl α-methyl-D-galactopyranoside methyl ester (IV) as a fairly mobile colourless liquid (0·158 g.), b. p. (bath-temp.) 196°/0·02 mm., $n_D^{29}$ 1·4670, $[\alpha]_D^{23}$ +15·5° (*c*, 1·5 in methanol). The product did not crystallize when nucleated with 6-β-(2 : 3 : 4-trimethyl D-glucopyruronosyl) 2 : 3 : 4-trimethyl β-methyl-D-galactopyranoside methyl ester (V) which had m. p. 93°, $[\alpha]_D^{23}$ −40° (*c*, 1·2 in methanol) (Jackson and Smith, *loc. cit.*).

*The Amide* (IX) *of* 6-β-(2 : 3 : 4-*Trimethyl* D-*Glucopyruronosyl*) 2 : 3 : 4-*Trimethyl* α-*Methyl*-D-*galactopyranoside.*—A solution of the methylated aldobiuronoside (IV) (120 mg.) in methanol (4 ml.) was cooled to 0°, saturated with ammonia, and kept for 12 hours at 5° and for 5 hours at 25°. Removal of the solvent *in vacuo* yielded the crystalline *amide* (IX) of 6-β-(2 : 3 : 4-trimethyl D-glucopyruronosyl) 2 : 3 : 4-trimethyl α-methyl-D-galactopyranoside, which crystallized from ethanol–ether as needles, m. p. 160°, $[\alpha]_D^{23}$ +40·5° (*c*, 0·65 in water) (Found: OMe, 48·1. $C_{19}H_{35}O_{11}N$ requires OMe, 47·9%).

6-β-(2 : 3 : 4-*Trimethyl* D-*Glucopyranosyl*) 2 : 3 : 4-*Trimethyl* α-*Methyl*-D-*galactopyranoside* (VIII).—(i) *The methyl ester* (IV) *of* 6-β-(2 : 3 : 4-*trimethyl* D-*glucopyruronosyl*) 2 : 3 : 4-*trimethyl* α-*methyl*-D-*galactopyranoside.* (*a*) A solution of the amide (III) of 6-β-(4-methyl D-glucopyruronosyl) α-methyl-D-galactopyranoside (198 mg.) in 0·1N-sodium hydroxide (10 ml.) was heated at 50—60° until all ammonia was evolved (litmus). The solution was treated with

0·1N-sulphuric acid (9·9 ml.) and evaporated to dryness *in vacuo*. The product was extracted with methanol, treated with a slight excess of ethereal diazomethane and, after removal of solvent, subjected to three treatments with Purdie's reagents in the usual way. A small amount of methanol was used in the first treatment to increase the solubility of the methyl ester. Distillation of the product yielded the *methyl* ester (IV) of 6-β-(2 : 3 : 4-trimethyl D-glucopyruronosyl) 2 : 3 : 4-trimethyl α-methyl-D-galactopyranoside, b. p. (bath-temp.) 210°/0·01 mm., $n_D^{29}$ 1·4670, $[\alpha]_D^{20}$ +17·5° (*c*, 2·4 in methanol) (Found : OMe, 50·8. $C_{20}H_{36}O_{12}$ requires OMe, 52·9%).

(*b*) A solution of the fully methylated aldobionamide (IX) (20 mg.) in N-sodium hydroxide (5 ml.) was warmed at 50—60° and nitrogen was passed through the solution until no more ammonia was evolved (litmus). The solution was acidified with N-sulphuric acid (5·5 ml.), and the product extracted repeatedly with chloroform. The chloroform extract was dried ($MgSO_4$), filtered, and concentrated to a syrup. The latter was dissolved in ether (10 ml.) and esterified by treatment with a slight excess of ethereal diazomethane (persistent yellow colour). Removal of the solvent gave the methyl ester of 6-β-(2 : 3 : 4-trimethyl D-glucopyruronosyl) 2 : 3 : 4-trimethyl α-methyl-D-galactopyranoside (IV) as above.

(ii) *Reduction of* 6-β-(2 : 3 : 4-*trimethyl* D-*glucopyruronosyl*) 2 : 3 : 4-*trimethyl* α-*methyl*-D-*galactopyranoside methyl ester* (IV) *with lithium aluminium hydride*. A solution of the methyl ester (IV), obtained as in (*b*) above, in ether (5 ml.), was added dropwise with stirring to a solution of lithium aluminium hydride (0·3 g.) in ether (10 ml.). The reaction mixture was kept at 25° for 10 hours, refluxed for 4 hours, cooled, poured into water (50 ml.), acidified with dilute hydrochloric acid, and extracted three times with chloroform. The combined chloroform extract was dried ($Na_2SO_4$) and concentrated *in vacuo*. The syrup crystallized spontaneously, yielding 6-β-(2 : 3 : 4-*trimethyl* D-*glucopyranosyl*) 2 : 3 : 4-*trimethyl* α-*methyl*-D-*galactopyranoside* (VIII), needles (from ether–light petroleum), m. p. 114—115°, $[\alpha]_D^{24}$ +50° (*c*, 0·5 in water) (Found: C, 51·7; H, 8·2. $C_{19}H_{36}O_{11}$ requires C, 51·8; H, 8·2%).

*Hydrolysis of* 6-β-(2 : 3 : 4-*Trimethyl* D-*Glucopyranosyl*) 2 : 3 : 4-*Trimethyl* α-*Methyl*-D-*galactopyranoside* (VIII).—A solution of the methylated disaccharide (VIII) (16·5 mg.) in N-sulphuric acid (1 ml.) was heated in a sealed tube for 20 hours on a boiling-water bath. The hydrolysate was diluted with water and neutralized by passage through a column of "Duolite A-4" anion-exchange resin. Evaporation of the solution to dryness *in vacuo* gave a neutral syrup. A small portion of the syrup was chromatographed on Whatman No. 1 filter paper with ethyl methyl ketone–water azeotrope as the irrigating solvent. After about 1 hour the paper was dried in the air for about 30 minutes, sprayed with the *NN*[1]-dimethyl-*p*-phenylenediamine hydrochloride reagent (see Boggs *et al.*, *Nature*, 1951, **166**, 520) and heated for about 5 minutes at 130°. Two well-defined spots appeared, with $R_F$ 0·65 and 0·42 respectively, and corresponded exactly with spots produced on the same chromatogram by authentic specimens of 2 : 3 : 4-trimethyl D-glucose and 2 : 3 : 4-trimethyl D-galactose respectively. The rest of the mixture was treated with aniline (20 mg.) in boiling ethanol (1 ml.) for 3 hours. Removal of the excess of the solvent *in vacuo* yielded crystalline 2 : 3 : 4-trimethyl D-galactose anilide as rhombic plates, m. p. 164° not depressed on admixture with an authentic specimen.

*Reduction of the Methyl Ester* (V) *with Lithium Aluminium Hydride*.—A solution of the ester (V) (Jackson and Smith, *loc. cit.*) (173 mg.) in dry ether (10 ml.) was added slowly to a solution of lithium aluminium hydride (500 mg.) in ether (20 ml.) (see Smith, *loc. cit.*). After 30 minutes at room temperature the solution gave a negative hydroxamic acid test for esters (see Abdel Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859). The reaction mixture was poured into water, and the aqueous phase extracted repeatedly with ether. The combined extracts were dried ($Na_2SO_4$), filtered, and concentrated *in vacuo*. Crystallization occurred spontaneously, yielding 6-β-(2 : 3 : 4-*trimethyl* D-*glucopyranosyl*) 2 : 3 : 4-*trimethyl* β-*methyl*-D-*galactopyranoside* (VII), m. p. 145·5°, $[\alpha]_D^{25}$ −39° (*c*, 3·9 in chloroform) (after recrystallization from ether–light petroleum) (Found: C, 51·84; H, 8·34. $C_{19}H_{36}O_{11}$ requires C, 51·8; H, 8·3%). Saponification for 2 hours with 0·1N-sodium hydroxide was without effect on (VII).

A solution of the above methylated disaccharide (VII) (10 mg.) in N-sulphuric acid (1 ml.) was heated for 6 hours in a boiling-water bath. The solution was cooled, neutralized with "Duolite A-4" anion-exchange resin, and concentrated *in vacuo* to a syrup. Paper-chromatographic analysis, with ethyl methyl ketone–water azeotrope as the irrigating solvent, revealed the presence of two components which corresponded to 2 : 3 : 4-trimethyl D-glucose ($R_F$ 0·61) and 2 : 3 : 4-trimethyl D-galactose ($R_F$ 0·40).

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