588. *The Constitution of Mesquite Gum. Part III.* The Structure of the Monomethyl Glucuronic Acid Component.*

By F. Smith.

Prolonged hydrolysis of mesquite gum affords 4-methyl D-glucuronic acid, which has been characterised by means of the α- (VI) and the β-form (V) of 4-methyl methyl-D-glucuronoside amide. These have been converted into the corresponding crystalline amides (IX) and (VIII) of 2 : 3 : 4-trimethyl methyl-D-glucuronoside. Oxidation of the methyl ester (III) of 4-methyl methyl-D-glucuronoside first with sodium periodate and then with bromine, followed by hydrolysis, gives L-*erythro*-2-hydroxy-3-methoxy-succinic acid (X) and glyoxylic acid (XI). Reduction of (III) catalytically or by lithium aluminium hydride yields 4-methyl methyl-D-glucoside (I) and this on hydrolysis gives 4-methyl D-glucose. The last affords crystalline 4-methyl D-glucose phenylosazone identical with an authentic specimen prepared by an unambiguous synthetic route.

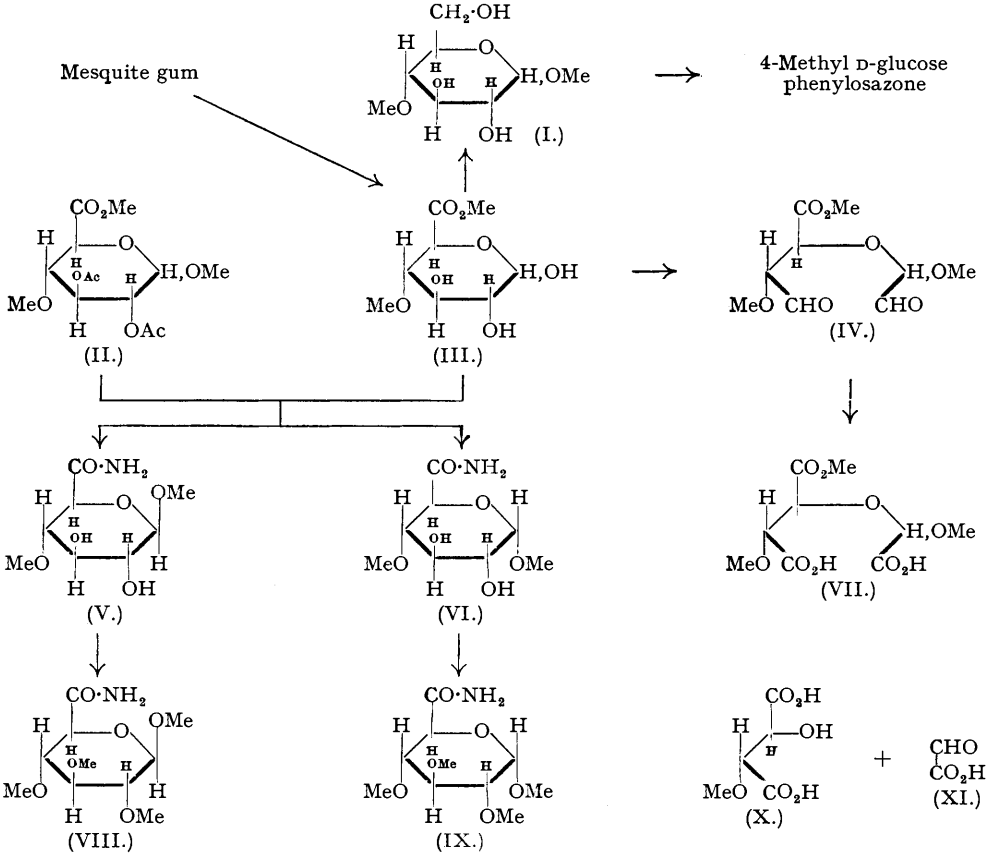
Mesquite gum, exuded by the mesquite tree (*Prosopis Juliflora* D.C. and related species) (Procter, *Amer. J. Pharm.*, 1855, **27**, 14, 223; Morfit, *Amer. J. Sci.*, 1885, **19**, 264), is the neutral salt of a complex acidic polysaccharide (Anderson, Sands, and Sturgis, *Amer. J. Pharm.*, 1925, **97**, 589), which owes the acidic character to the presence of a monomethyl D-glucuronic acid constituent (Anderson and Otis, *J. Amer. Chem. Soc.*, 1930, **52**, 4461). In addition to the latter, mesquite gum contains D-galactose and L-arabinose. Methylation studies (Cunneen and Smith, Part II; * White, *J. Amer. Chem. Soc.*, 1946, **68**, 272; 1947, **69**, 622) and other investigations (White, *ibid.*, 1947, **69**, 715) show that the L-arabinose is present as furanose units while the D-galactose and methyl D-glucuronic acid residues have pyranose structures.

* Part II, *J.*, 1948, 1146.

Mild acid hydrolysis removes the L-arabofuranose residues (Anderson and Sands, *ibid.*, 1926, **48**, 3172) and leaves intact a stable framework or nucleus of degraded mesquitic acid which is composed of the pyranose units of D-galactose and of monomethyl D-glucuronic acid (White, *ibid.*, 1947, **69**, 622; Rao and Smith, unpublished work). More drastic hydrolysis of the degraded mesquitic acid leads to D-galactose and mixed aldobionic acids (Cunneen and Smith, *J.*, 1948, 1141; White, *J. Amer. Chem. Soc.*, 1947, **69**, 2264). The latter are composed of one unit of D-galactose and one of monomethyl D-glucuronic acid for by still further hydrolysis they give rise to D-galactose and a monomethyl D-glucuronic acid (Anderson and Otis, *loc. cit.*).

The present paper deals with the structure of the methyl glucuronic acid residues of mesquite gum. The results confirm those of White (*ibid.*, 1948, **70**, 367) and provide additional proof that the methyl group of the uronic acid residues is at $C_{(4)}$.

When either degraded mesquitic acid or the derived aldobionic acid was subjected to prolonged boiling with methanolic hydrogen chloride simultaneous methanolysis and esterification gave a mixture containing methyl-D-galactoside and the methyl esters of 4-methyl α- and β-methyl-D-glucuronoside (III) and of a methylated aldobiuronoside. The glucuronic and aldobionic acid components could be separated by fractional distillation directly as their ester glycosides or after conversion into the corresponding acetates. Treatment of the methyl ester of the methyl methyl-D-glucuronosides (III) or the acetate (II) with methanolic ammonia yielded a mixture of the amide (VI) of methyl α- and that (V) of the methyl β-methyl-D-glucuronoside. The crystalline forms of these two amides proved to be quite different and their separation by fractional crystallisation was relatively easy.



The methyl ester and acetate of the methylated aldobiuronoside also afforded a crystalline amide, the structure and constitutional significance of which will be dealt with in a later communication.

The structure of the amides (V) and (VI) is based on the following facts. The β-form (V)

was transformed into the α-form (VI) by boiling it with methanolic hydrogen chloride and treating the resulting ester with ammonia. When the amides (V) and (VI) were converted into the corresponding acids and the latter methylated with silver oxide and methyl iodide, the methyl esters of 2 : 3 : 4-trimethyl β- and α-methyl-D-glucuronoside respectively were formed, and these with ammonia yielded the characteristic crystalline amides (VIII) and (IX) respectively of 2 : 3 : 4-trimethyl methyl-D-glucuronoside (Smith, *J.*, 1939, 1724). This proved that the original amides (V) and (VI) were anomers, that they possessed six-membered rings, and that the methyl group occupied position 2, or 3, or 4.

The two forms (VI) and (V) each reacted with one mole of sodium periodate to give dialdehydes (Fleury, Hérissey, and Joly, *J. Pharm. Chim.*, 1934, **20**, 149; Jackson and Hudson, *J. Amer. Chem. Soc.*, 1937, **59**, 994). Similarly the methyl ester (III) consumed one mole of periodate to give the dialdehyde (IV). No formic acid was produced. Since a methyl group at $C_{(3)}$ would render the molecule immune to periodate oxidation, the uptake of one mole indicated that the methyl group was located at either $C_{(2)}$ or $C_{(4)}$. It is of interest that while oxidation of (V) and (VI) proceeded normally with sodium periodate, over-oxidation occurred with periodic acid. Similarly, normal oxidation of the methyl ester took place with either sodium periodate at 5° and at 25° or with periodic acid at 5°, but with the latter extensive over-oxidation took place at 25° (see Huebner *et al.*, *J. Biol. Chem.*, 1945, **159**, 503; Sprinson and Chargaff, *ibid.*, 1946, **164**, 443; Grangaard *et al.*, *Paper Trade J.*, 1942, **115**, Vol. 7, 41; Halsall, Hirst, and Jones, *J.*, 1947, 1427).

Oxidation of the dialdehyde (IV) with bromine in the usual way afforded the corresponding dibasic acid (VII). Compared with the dialdehydes derived from simple sugar glycosides which are cleaved by dilute acid at room temperature the acid proved to be surprisingly stable to acid hydrolysis (cf. Jackson and Hudson, *J. Amer. Chem. Soc.*, 1940, **62**, 958), but prolonged heating with N-hydrochloric acid at 95° gave a mixture of L-*erythro*-2-hydroxy-3-methoxysuccinic acid (X) and glyoxylic acid (XI). Esterification of the former and treatment of the ester with methanolic methylamine gave the known crystalline bismethylamide of L-*erythro*-2-hydroxy-3-methoxysuccinic acid (Heslop, Salt, and Smith, *J.*, 1944, 225). The location of the methyl group in this four-carbon fragment and the formation of glyoxylic acid can only be explained by the presence of the methyl group at $C_{(4)}$ in the methyl glycuronic acid derivatives. The acidic component of mesquite gum is therefore 4-methyl D-glucuronic acid as already stated by White (*J. Amer. Chem. Soc.*, 1948, **70**, 367).

Further proof of the structure assigned to the methyl glucuronic derivatives came from the observation that reduction of the methyl ester of the methyl methyl-D-glucuronoside (III) either by hydrogenation under pressure at 180° in the presence of copper chromite (cf. Levene *et al.*, *J. Biol. Chem.*, 1937, **121**, 155; 1937, **122**, 199, 203) or by treatment with lithium aluminium hydride in tetrahydrofuran (Abdel-Akher and Smith, *Nature*, 1950, **166**, 1037; cf. Lythgoe and Trippett, *J.*, 1950, 1983) gave methyl methyl-D-glucoside (I). The lithium aluminium hydride reduction (Nystrom and Brown, *J. Amer. Chem. Soc.*, 1947, **69**, 1197, 2548; Finholt, Bond, and Schlesinger, *ibid.*, p. 1199) is much superior to hydrogenation under pressure because the yield is high, the reaction takes but a few minutes, and hydrogenolysis is avoided (Abdel-Akher and Smith, *loc. cit.*). Hydrolysis of the methyl glucoside (I) proceeded smoothly with N-sulphuric acid at a rate indicative of the presence of a pyranoside structure, to give 4-methyl D-glucose, characterised as its phenylosazone (McDonald and Jackson, *J. Res. Nat. Bur. Stand.*, 1940, **24**, 181; Schmidt and Müller, *Ber.*, 1943, **76**, 344; Shinle, *Ber.*, 1932, **65**, 315; Knauf, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1941, **63**, 1447; Munro and Percival, *J.*, 1935, 873). The structure of the osazone was confirmed by its unequivocal synthesis as follows : methyl-α-D-mannopyranoside ⟶ 2 : 3-*iso*propylidene methyl-α-D-mannoside ⟶ 2 : 3-*iso*propylidene 6-trityl methyl-α-D-mannoside ⟶ 4-methyl 2 : 3-*iso*propylidene 6-trityl methyl-α-D-mannoside ⟶ 4-methyl methyl-D-mannoside ⟶ 4-methyl D-mannose ⟶ 4-methyl D-mannose (or -glucose) phenylosazone.

Experimental.

Isolation of the Amide of 4-Methyl Methyl-α- and -β-D-glucuronoside.—(*a*) A solution of mesquite gum (250 g.) in 0·2N-sulphuric acid (1 l.) was heated for 2 days on a boiling water-bath and then dialysed for 2 days against running tap water. The solution was filtered and poured with stirring into methanol (3 volumes). The precipitate was triturated with methanol until it became granular, filtered off, washed with methanol and, while still containing some solvent, boiled for 20 hours with 7—8 parts of 7·5% methyl-alcoholic hydrogen chloride. The crude methylglucuronoside was isolated by neutralisation (silver carbonate), filtration, evaporation *in vacuo* to dryness, and extraction with acetone. Distillation of the crude, pale yellow, thick product gave a viscous liquid (23·4 g.), b. p. 165—170° (bath temp.)/0·05 mm., n_D^{28} 1·4775—1·4790, $[\alpha]_{5461}^{25}$ +48° in methanol (*c*, 2·7) (Found : OMe, 32·0%; equiv., 324.

Calc. for $C_9H_{16}O_7$: OMe, 39·4%; equiv., 236). The low methoxyl value and high equivalent weight are caused by the presence of methyl-α- and -β-D-galactosides, both of which are obtained crystalline from the mother-liquors after separation of the amides of the methylated glucuronoside (see below).

Treatment of the crude ester with methyl-alcoholic ammonia gave a crystalline amide (13·5 g.), m. p. 206—212°. Crystallisation from ethanol containing a few drops of water yielded the *amide* of 4-methyl methyl-α-D-glucuronoside as thick plates, m. p. 236°, $[\alpha]_D^{20}$ +150° in water (*c*, 1·0) [Found: C, 43·55; H, 6·9; N, 6·2; OMe, 27·8%; equiv., 224. $C_8H_{15}O_6N$ requires C, 43·5; H, 6·85; N, 6·3; OMe, 28·05%; equiv., 221 (determined by heating the amide with an excess of 0·02N-NaOH on the water-bath in a gentle current of nitrogen and back-titrating the excess of the alkali with 0·02N-H_2SO_4)]. From the mother-liquors the *amide* of 4-methyl methyl-β-D-glucuronoside was obtained as needles, m. p. 232°, $[\alpha]_D^{20}$ −50° in water (*c*, 1·1) (after crystallisation from methanol) (Found: C, 42·9; H, 7·0; N, 6·4; OMe, 28·0%; equiv., 223. $C_8H_{15}O_6N$ requires C, 43·55; H, 6·9; N, 6·3; OMe, 28·05%; equiv., 221).

(*b*) A solution of mesquite gum (200 g.) in 0·5N-sulphuric acid (1 l.) was heated for 12 hours on the boiling-water-bath. Neutralisation (barium hydroxide solution), filtration, evaporation *in vacuo* to a thin syrup, and addition of an excess of methanol gave a precipitate of barium salts. The latter were filtered off and purified by one precipitation from water with methanol. The dried salts were then boiled for 6 hours with 3% methanolic hydrogen chloride. The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to dryness *in vacuo*. The residue was dissolved in methanol (100 c.c.), and ether added cautiously to precipitate inorganic impurity. Removal of the latter on the centrifuge and concentration of the solution *in vacuo* gave a pale yellow liquid which was dissolved in pyridine (50 c.c.) and treated with acetic anhydride (50 c.c., added slowly with shaking). After 10 hours at room temperature and 2 hours at 60° the reaction mixture was poured into ice-water, and the product extracted with chloroform. The combined chloroform extracts were washed successively with N-sulphuric acid, water, and sodium hydrogen carbonate solution, and dried ($MgSO_4$). Removal of the solvent gave a viscous liquid which was distilled giving fraction I (a mixture of the methyl ester of 2 : 3-diacetyl 4-methyl methyl-D-glucuronoside and 2 : 3 : 4 : 6-tetra-acetyl methyl-D-galactoside) (19·9 g.), b. p. 155—165° (bath temp.)/0·03 mm., n_D^{24} 1·4520, $[\alpha]_D^{26}$ +79° in methanol (*c*, 4·5) (Found: OMe, 14·2%; equiv., 90), and fraction II (mainly the methyl ester of a penta-acetyl monomethyl methyl-aldobiuronoside) (2·43 g.), b. p. 260—270°/0·03 mm. (sets to a glassy solid), $[\alpha]_D^{26}$ +64° in methanol (*c*, 2·5) (Found: OMe, 13·3%; equiv., 104. Calc. for $C_{25}H_{36}O_{17}$: OMe, 15·3%; equiv., 101·3).

Treatment of fraction I. This was heated for 2 hours at 60° with an excess of barium hydroxide in 70% methanol in an atmosphere of nitrogen. The solution was neutralised with carbon dioxide, filtered, and evaporated *in vacuo* to dryness. The residue was dissolved in methanol (100 c.c.), and acetone added until precipitation was complete. The precipitate was separated (centrifuge), washed successively with acetone and ether, and dried. The barium salt was boiled for 8 hours with 2% methanolic hydrogen chloride, and the methyl ester of 4-methyl methyl-D-glucuronoside, isolated as described above, was purified by extraction with acetone and finally by distillation (3·7 g.), then having b. p. 165—170°/0·05 mm., n_D^{24} 1·4715—1·4737, $[\alpha]_{5461}^{25}$ +50° in methanol (*c*, 2·7) (Found: OMe, 38·4%; equiv., 240. Calc. for $C_9H_{16}O_7$: OMe, 39·4%; equiv., 236). Treatment of this ester with methyl-alcoholic ammonia gave a good yield of the *amide* of the α- and β-forms of 4-methyl methyl-D-glucuronoside. Trituration of the mixture of amides with ethanol extracted the β-isomer. Crystallisation of the residue from methanol gave the α-form, m. p. 236°, while the β-isomer (m. p. 232°) was readily isolated from the ethanol extract.

Treatment of fraction II. A solution of fraction II in methanolic ammonia was kept for 10 hours at room temperature and then evaporated *in vacuo* to remove solvent and acetamide. Crystallisation of the residue from aqueous methanol gave the *amide* of a methyl D-glucuronosyl(methyl-D-galactoside), m. p. 267° (decomp.), $[\alpha]_D^{18}$ +25° in water (*c*, 0·6) (Found: OMe, 16·5. $C_{14}H_{25}O_{11}N$ requires OMe, 16·2%).

The barium salt of a methyl aldobionic acid (12·5 g.) [Found: OMe, 7·6; Ba, 15·2 (sulphated ash). Calc. for $C_{12}H_{18}O_{11}(OMe)_2Ba$: OMe, 7·1; Ba, 15·6%] was prepared from mesquite gum (150 g.) by a previous method (Cunneen and Smith, *J.*, 1948, 1141). When boiled for 5 hours with 1·2% methanolic hydrogen chloride, this salt gave an ester which was isolated in the usual way (Found: OMe, 24·9. Calc. for $C_{15}H_{26}O_{12}$: OMe, 23·4%). Direct treatment of this ester with methyl-alcoholic ammonia afforded in good yield the *amide* of the methyl D-glucuronosyl(methyl-D-galactoside), m. p. 267° alone or mixed with the aldobionamide prepared through the acetate (after recrystallisation from aqueous ethanol) (Found: C, 43·1; H, 6·6; N, 3·8; OMe, 16·8. $C_{14}H_{25}O_{11}N$ requires C, 43·8; H, 6·6; N, 3·7; OMe, 16·2%). Small amounts of the α- and β-isomers of the amide of 4-methyl methyl-D-glucuronoside were isolated from the mother-liquors.

The structure and constitutional significance of this amide of methyl D-glucuronosyl(methyl-galactoside) will be dealt with in a later communication.

Determination of the Structure of the Amide of 4-Methyl Methyl-α- and -β-D-glucuronoside.—Methylation: (a) *The α-form of the amide.* A solution of the amide of 4-methyl methyl-α-D-glucuronoside (0·47 g.) in water (25 c.c.) was heated for 3—4 hours at 60—70° with 0·1025N-sodium hydroxide (30 c.c.) in a stream of nitrogen (bubbled through the solution), until all the ammonia was expelled. The solution was neutralized with 0·107N-sulphuric acid (9·2 c.c. required which corresponds to an equiv. wt. of 224 for the amide. Calc.: equiv., 221). More acid (18·8 c.c.; 0·107N.) was added to liberate the organic acid which was isolated by evaporation to dryness *in vacuo* and extraction with ethanol. Two treatments of the 4-methyl methylglucuronoside thus isolated with Purdie's reagents gave the *methyl* ester of 2 : 3 : 4-trimethyl methyl-α-D-glucuronoside (0·3 g.), b. p. (bath-temp.) 100°/0·1 mm., n_D^{27} 1·4475, $[\alpha]_D^{26}$ +156° in methanol (*c*, 2·0) (Found: OMe, 58·0. $C_{11}H_{20}O_7$ requires OMe, 58·7%). Treatment of this ester with methanolic ammonia gave the corresponding *amide* of 2 : 3 : 4-trimethyl

methyl-α-D-glucuronoside, m. p. 188—189°, $[\alpha]_D^{25}$ +149° in water (*c*, 0·25) (after recrystallisation from ethanol–ether–light petroleum) (Found : C, 48·1; H, 7·9; N, 6·0; OMe, 49·4. $C_{10}H_{19}O_6N$ requires C, 48·2; H, 7·7; N, 5·6; OMe, 49·8%). A specimen (m. p. 183°; $[\alpha]_D^{20}$ +138° in water) of this amide prepared from a mixture of the α- and the β-form of the methyl ester of 2 : 3 : 4-trimethyl methyl-D-glucuronoside was evidently not pure (Smith, *J.*, 1939, 1724).

(b) *The β-form of the amide.* The amide of 4-methyl methyl-β-D-glucuronoside (109·5 mg.) was treated with dilute aqueous sodium hydroxide as for the α-isomer (4·6 c.c. of 0·107N. required, corresponding to equiv., 223). The acid was isolated and methylated as above to give the methyl ester of 2 : 3 : 4-trimethyl methyl-β-D-glucuronoside (140 mg.), b. p. (bath-temp.) 120°/0·15 mm., n_D^{27} 1·4520, which afforded the amide of 2 : 3 : 4-trimethyl methyl-β-D-glucuronoside, m. p. and mixed m. p. 190° (after recrystallisation from ethyl acetate or dioxan–ether) (cf. Smith, *loc. cit.*). A mixture of this fully methylated β-isomer with an equal amount of the fully methylated α-form obtained as in (a) had m. p. 158° (cf. Hirst and Jones, *J.*, 1938, 1174; Lythgoe and Trippett, *loc. cit.*).

Oxidation with sodium periodate : (a) *The α-form of the amide.* A solution of the amide of 4-methyl methyl-α-D-glucuronoside (55·2 mg.) in water (5 c.c.) was treated at room temperature with sodium metaperiodate (10 c.c.; 0·27N.). After 2—3 hours (rotation constant, $[\alpha]_D^{20}$ −16°), 1·06 moles of periodate had been consumed per mole of amide. No formic acid was formed. When the reaction mixture was kept in the dark for as long as 70 hours the rotation remained constant and no further uptake of periodate occurred.

(b) *The β-form of the amide.* When the amide of 4-methyl methyl-β-D-glucuronoside (42·9 mg.) was treated with 0·083N-sodium metaperiodate (50 c.c.), the periodate consumption per mole of amide was noted as follows : 0·2 (after 15 mins.); 0·28 (51 mins.); 0·8 (147 mins.); 1·05 (470 mins.); 1·05 moles (24 hours). No acidity was produced in the solution.

Oxidation with periodic acid : (a) *The α-form of the amide.* When the amide of 4-methyl methyl-α-D-glucuronoside (52·6 mg.) in 0·08N-periodic acid (45 c.c.) was kept at room temperature in daylight, oxidation was rapid and overoxidation occurred thus : 0·50 mole of HIO_4 per mole of amide after 6 mins.; 0·85 (after 30 mins.); 1·13 (60 mins.); 1·35 (120 mins.); 1·45 (180 mins.).

When carried out in the dark the oxidation proceeded more slowly but overoxidation was again noticeable and there was no arrest at the stage corresponding to the consumption of 1 mole of acid per mole of amide : 0·2 mole (after 15 mins.); 0·38 (30 mins.); 0·67 (60 mins.); 0·82 (90 mins.); 0·92 (120 mins.); 0·97 (150 mins.); 1·0 (180 mins.); 1·05 (240 mins.); 1·1 (300 mins.); 1·15 (360 mins.); 1·18 (420 mins.).

(b) *The β-form of the amide.* The results were similar to those with the α-isomer. *Interconversion of the α- and the β-forms of the amide of 4-methyl methyl-D-glucuronoside.* The amide of 4-methyl methyl-β-D-glucuronoside (80 mg.) was boiled for 6 hours with 1·5% methyl-alcoholic hydrogen chloride (3 c.c.). The solution was cooled, neutralised with ethereal diazomethane, filtered to remove a small amount of flocculent precipitate, and evaporated *in vacuo* to dryness. Treatment of the syrupy product with methanolic ammonia in the usual way gave a crystalline amide. Slow crystallisation of this from methanol afforded two distinct types of crystal, needles and plates in about equal amounts. On washing by decantation with cold (0—5°) methanol the needles dissolved while the plates of the α-form of the amide remained [m. p. and mixed m. p. 236° (from ethanol)]. Evaporation of the methanolic washings gave the original β-form, m. p. and mixed m. p. 232° (from methanol).

Oxidation of the amide of 4-methyl methyl-D-glucuronoside with sodium periodate and identification of 2-hydroxy-3-methoxy-L-erythrosuccinic acid. A solution of the amide of 4-methyl methyl-α- and -β-D-glucuronoside (3·0 g.) in 0·27N-sodium periodate (125 c.c.) was kept at 25° until the rotation became constant : $[\alpha]_{5461}^{25}$ +115° (after 3 mins.); +92° (5 mins.); +65° (8 mins.); +35° (11 mins.); +4·6° (17 mins.); −21° (23 mins.); −42·5° (34 mins.); −60° (52 mins.); −72° (103 mins.); −72·5° (143 mins.); −60° (356 mins.); −39° (1090 mins.); −35° (1500 mins.); −31·5° (1850 mins.); −28° (3000 mins.), constant for 16 hours. Examination of the solution after 2850 mins. showed that 0·98 mole of periodate had been consumed per mole of amide. The reaction mixture was treated with dilute barium acetate solution until precipitation was complete. Removal of the precipitate (centrifuge) and the solvent (evaporation *in vacuo* at 35—40°) gave a colourless residue which restored the colour to Schiff's reagent but did not reduce boiling Fehling's solution.

A solution of the residue obtained above in water (35 c.c.) was treated for 2 days in the dark at 25° with bromine (1 c.c.) in the presence of barium carbonate (3 g.). The excess of the bromine was removed by aeration and the solution concentrated under diminished pressure. The solution (Schiff's test negative) was treated with hydrochloric acid (30 c.c.; *d* 1·2) and heated for 16 hours on the boiling-water-bath until the rotation became constant ($[\alpha]_{5461}^{20}$ +37·5°). Compared with the dialdehyde prepared from sugar glycosides this dialdehyde formed from 4-methyl methylglucuronoside thus proved to be surprisingly stable. The solution now restored the colour to Schiff's reagent and the test for glyoxylic acid (tryptophan–H_2SO_4) was positive. The solution was neutralised with N-sodium hydroxide and evaporated *in vacuo* to dryness. This residue, containing the salt of 2-hydroxy-3-methoxy-L-*erythro*-succinic acid, was boiled for 6 hours with 3·0% methanolic hydrogen chloride (200 c.c.). Ether (200 c.c.) was added to precipitate inorganic salts. The solution was evaporated *in vacuo* to half-volume, neutralised with silver carbonate, filtered, and evaporated *in vacuo* to dryness. The syrupy residue was further purified by dissolution in acetone (50 c.c.) and addition of ether (175 c.c.). Filtration and removal of solvent afforded a mobile liquid which on distillation gave methyl 2-hydroxy-3-methoxy-L-*erythro*succinate (1·1 g.), b. p. (bath-temp.) 120°/0·5 mm., n_D^{29} 1·4415, $[\alpha]_D^{25}$ +41° in methanol (*c*, 2·0) (Found : OMe, 49·0%; equiv., 107. Calc. for $C_7H_{12}O_6$: OMe, 48·5%; equiv., 96). $[\alpha]_D^{18}$ −43° in methanol, n_D^{17} 1·4440, has been recorded for this ester (Haworth, Heslop, Salt, and Smith, *J.*, 1944, 217).

Treatment of the ester with methanolic methylamine in the usual way gave a good yield of the

crystalline bismethylamide, m. p. 137°, $[\alpha]_D^{20}$ −10·5° in water (*c*, 1·5) (Found : C, 44·0; H, 7·3; OMe, 16·7. Calc. for $C_7H_{14}O_4N_2$: C, 44·2; H, 7·4; OMe, 16·3%). Mixed with the enantiomorph (m. p. 137°; $[\alpha]_D^{18}$ +10·5° in water; Heslop, Salt, and Smith, *J.*, 1944, 225) it had m. p. 122—125° (cf. White, *J. Amer. Chem. Soc.*, 1948, **70**, 367).

Methyl Ester of 4-Methyl Methyl-α-D-glucuronoside.—A solution of the amide of 4-methyl methyl-α-D-glucuronoside (1·06 g.) in 0·1N-sodium hydroxide (80 c.c.) was heated for 5 hours at 60—70° in nitrogen, bubbled through the liquid until no more ammonia was evolved. The solution was treated with 0·1N-sulphuric acid (79 c.c.) and evaporated to dryness *in vacuo*. Extraction with acetone gave 4-methyl methyl-α-D-glucuronoside as a colourless viscous liquid. Attempted lactonisation of a small portion (0·1 g.) of this product by distillation in a high vacuum resulted in decomposition : a small amount of an unidentified crystalline sublimate was produced (m. p. 118°) which was acidic in character and reduced Fehling's solution actively; this may be related to the unsaturated derivatives of glucosaccharolactone (Heslop and Smith, *J.*, 1944, 637).

Treatment of the main portion of the 4-methyl methyl-α-D-glucuronoside with ethereal diazomethane afforded the corresponding *methyl* ester (0·8 g.), b. p. (bath-temp.) 160—170°/0·05 mm., n_D^{29} 1·4720, $[\alpha]_D^{26}$ +128° in water (*c*, 1·0) (Found : OMe, 39·7. $C_9H_{16}O_7$ requires OMe, 39·4%).

Oxidation : (a) *With sodium periodate.* When the methyl ester (58·2 mg.) was allowed to react with 0·08N-sodium periodate at room temperature in the dark the oxidation proceeded according to the following results : 0·06 mole of periodate (after 10 mins.); 0·55 (65 mins.); 0·78 (110 mins.); 1·01 (270 mins.). No further consumption of periodate was observed after 34 hours. A second experiment showed that 1 mole of the ester consumed 0·99 mole of sodium periodate under the same conditions.

(b) *With periodic acid.* At room temperature and in the dark the consumption of periodate (0·08N.) in moles per mole of ester was : 0·93 (50 mins.); 1·38 (290 mins.); 1·57 (450 mins.); 1·75 (2 days). There was thus overoxidation.

At 0° in the dark and with 0·01N-periodic acid the reaction was slower and reached a constant value corresponding to the consumption of 1 mole of periodate per mole of ester : 0·08 (38 mins.); 0·25 (5·5 hrs.); 0·37 (9·7 hrs.); 0·55 (21·5 hrs.); 0·82 (45·5 hrs.); 0·94 (69·5 hrs.); 1·05 (95·3 hrs.), constant for 70 hours.

In a second experiment with 0·01N-acid, 1·05 moles of periodate were consumed per mole of ester in 90 hours (constant for a further 140 hours). When the temperature of this reaction mixture was allowed to rise to 25° (in the dark) overoxidation occurred : 1·23 moles of periodate consumed per mole of ester (after 50·5 hrs.); 1·31 moles (72 hrs.); 1·4 (120 hrs.); 1·69 (174 hrs.).

It is apparent that at room temperature it is better to use sodium periodate than periodic acid, while at 0° both periodic acid and its sodium salt may be used.

Reduction of the Methyl Ester of 4-Methyl Methyl-D-glucuronoside to 4-Methyl Methyl-D-glucoside.—(a) *By hydrogenation.* A solution of the methyl ester of 4-methyl methyl-α- and -β-D-glucuronoside (2·5 g.) in methanol (140 c.c.) was hydrogenated at 175°/4000 lbs. per sq. in. in the presence of copper chromite (2·5 g.). Filtration and removal of solvent gave a colourless viscous liquid (2·3 g.), $[\alpha]_D^{28}$ +78·5° in water (*c*, 2·5) (Found : OMe, 26·2. Calc. for $C_8H_{16}O_6$: OMe, 29·8%). No ester group could be detected by hydrolysis at 50—60°, thus showing hydrogenation was complete. The low methoxyl value is probably due to the fact that some hydrogenolysis occurred.

When a solution of the above 4-methyl methyl-α- and -β-D-glucoside (2·1 g.) in N-sulphuric acid (25 c.c.) was heated on the water-bath for 24 hours the rotation changed from $[\alpha]_D^{25}$ +78° (initial value) to +47° (final). Neutralisation [$Ba(OH)_2$ and then $BaCO_3$], followed by removal of the barium sulphate and the solvent gave 4-methyl D-glucose as a yellow viscous liquid (2·0 g.), $[\alpha]_D^{26}$ +13·5° in water (*c*, 5·0) (Found : OMe, 15·5. Calc. for $C_7H_{14}O_6$: OMe, 16·0%). The low rotation (+47°, equilibrium value of hydrolysis solution) of this 4-methyl D-glucose as compared with that (+59°) shown by the 4-methyl D-glucose obtained from 4-methyl methyl-α-D-glucoside prepared from the 4-methyl methyl-α-D-glucuronoside by reduction with lithium aluminium hydride (see below) is probably due to the presence of some 4-methyl sorbitol arising from hydrogenolysis [Munro and Percival (*loc. cit.*) give $[\alpha]_D$ +53° in water for 4-methyl D-glucose]. The discrepancy between the specific rotation (+47°) of the hydrolysis mixture and that (+13·5°) shown by the syrupy 4-methyl D-glucose after isolation would appear to indicate that the barium hydroxide effected a partial Lobry du Bruyn transformation.

The 4-methyl D-glucose (0·28 g.) in water (5 c.c.) containing acetic acid (0·5 c.c.) was heated for 2 hours at 80—85° with phenylhydrazine (0·8 c.c.). The reaction mixture was poured into water and the precipitate separated on the centrifuge. Recrystallisation from 20% aqueous acetone gave 4-methyl D-glucose phenylosazone as pale yellow needles, m. p. 158°, $[\alpha]_D^{25}$ −32·5° initial value in ethanol (*c*, 0·6), changing in 20 hours to −1·5° (approx.) (Found : C, 61·4; H, 6·6; N, 15·2; OMe, 8·8. Calc. for $C_{19}H_{24}O_4N_4$: C, 61·3; H, 6·5; N, 15·1; OMe, 8·3%).

(b) *With lithium aluminium hydride.* A solution of the methyl ester of 4-methyl methyl-α-D-glucuronoside (200 mg.) in dry tetrahydrofuran (10 c.c.) was slowly added with stirring to a solution of lithium aluminium hydride (300 mg.) in dry tetrahydrofuran (20 c.c.). After 15 minutes an excess of water was added to the reaction mixture which was filtered, and the residue was washed. Evaporation of the filtrate under reduced pressure gave a syrup (160 mg.) which was purified by dissolution in ethanol (1 c.c.) and addition of ether (8 c.c.). Filtration to remove a small amount of precipitate and concentration to dryness *in vacuo* gave *4-methyl methyl-α-D-glucoside* as a colourless, neutral, non-reducing liquid which showed n_D^{27} 1·4790, $[\alpha]_D^{27}$ +133° in water (*c*, 3·5). It did not react with warm 0·01N-sodium hydroxide. After being boiled with 1·5N-sulphuric acid for a few minutes the solution reduced boiling Fehling' solution (Found : OMe, 29·4. $C_8H_{16}O_6$ requires OMe, 29·8%).

When a solution of the 4-methyl methyl-D-glucoside (93 mg.) in N-sulphuric acid (5 c.c.) was heated on the boiling-water-bath the rotation changed in 15 hours from $[\alpha]_D^{25}$ +135° (initial value) to $[\alpha]_D^{25}$ +59°

(constant). The solution was neutralised by passing it through a column of " Duolite " A_4 anion-exchange resin and evaporated *in vacuo* to dryness. The 4-methyl glucose thus obtained (70 mg.) showed $[\alpha]_D^{25}$ +80° in methanol (*c*, 1·3) (Found : OMe, 17·0. Calc. for $C_7H_{14}O_6$: OMe, 16·0%).

The 4-methyl D-glucose (50 mg.) was warmed in water (2·5 c.c.) containing acetic acid (0·25 c.c.) for 2 hours at 80° with phenylhydrazine (0·2 c.c.). On cooling, 4-methyl D-glucose phenylosazone readily crystallised as long needles, m. p. 158—159°, $[\alpha]_D^{25}$ −31°, changing to −2° (equilibrium value) in ethanol (*c*, 0·5) (after crystallisation from 30% aqueous acetone) (Found : N, 14·7. Calc. for $C_{19}H_{24}O_4N_4$: N, 15·1%).

Synthesis of 4-Methyl D-*Glucose Phenylosazone.*—A solution of 2 : 3-*iso*propylidene methyl-α-D-mannoside (1·0 g.), prepared by the method of Ault, Haworth, and Hirst (*J.*, 1935, 317), in dry pyridine (10 c.c.) was treated with triphenylmethyl chloride (1·1 g., 1 mol.). After 1 day at room temperature the reaction mixture was warmed for 1 hour at 55—60°, cooled, and poured into water. The syrupy product was triturated, washed by decantation with water, and dissolved in benzene (50 c.c.), and the solution washed successively with N-sulphuric acid (four times), dilute sodium hydrogen carbonate solution, and water. After being dried ($CaCl_2$), evaporation to dryness *in vacuo* gave 2 : 3-iso*propylidene* 6-*trityl methyl*-α-D-*mannoside* as a colourless glassy solid (yield, almost quantitative), $[\alpha]_D^{27}$ +11° in ethanol (*c*, 1·1) (Found : OMe, 6·5. $C_{29}H_{32}O_6$ requires OMe, 6·5%).

Two treatments of the above compound with silver oxide and methyl iodide in the usual way furnished 4-*methyl* 2 : 3-iso*propylidene* 6-*trityl methyl*-α-D-*mannoside* as a viscous pale yellow liquid (1·9 g.), $[\alpha]_D^{27}$ +16·5° in methanol (*c*, 3·3) (Found : OMe, 12·95. $C_{30}H_{34}O_6$ requires OMe, 12·65%).

This product (1·8 g.) was boiled for 8 hours with 3·3% methanolic hydrogen chloride (20 c.c.). Neutralisation (Ag_2CO_3) of the solution followed by removal of solvent gave a residue which was shaken with ether (50 c.c.)–water (20 c.c.). Separation of the aqueous layer, followed by its evaporation to dryness *in vacuo*, yielded syrupy 4-methyl methyl-D-mannoside (0·63 g.), $[\alpha]_D^{27}$ +60·5° in water (*c*, 1·8) (Found : OMe, 31·0. Calc. for $C_8H_{16}O_6$: OMe, 29·8%).

When a solution of the 4-methyl methyl-D-mannoside (0·6 g.) in N-sulphuric acid (25 c.c.) was heated for 12 hours on the boiling-water-bath the rotation reached a constant value of $[\alpha]_D^{25}$ +17°. Neutralisation with N-sodium hydroxide, followed by evaporation *in vacuo* to dryness and extraction with ethanol, gave 4-methyl D-mannose as a viscous syrup (0·53 g.), $[\alpha]_D^{25}$ −2° (approx.) in water (*c*, 3·0).

A portion (0·1 g.) of the 4-methyl D-mannose was treated with phenylhydrazine in dilute acetic acid as described above. Crystallisation of the crude product from aqueous acetone yielded 4-methyl D-mannose phenylosazone in the form of long yellow needles, m. p. 157°. There was no depression of the m. p. when mixed with the two samples of 4-methyl D-glucose phenylosazone described above. The 4-methyl D-mannose phenylosazone showed $[\alpha]_D^{23}$ −34°, initial value in ethanol (*c*, 2·5), changing in 24 hours to −1·5° (approx.) (equilibrium value) (cf. Schmidt and Müller, *loc. cit.*) (Found : C, 61·4; H, 6·6; OMe, 8·3. Calc. for $C_{19}H_{24}O_4N_4$: C, 61·3; H, 6·5; OMe, 8·3%).

The author thanks Mr. J. A. Kent, of the University of Birmingham, for assistance in the preparation of 4-methyl methyl-D-glucuronoside.

THE CHEMISTRY DEPARTMENT, THE UNIVERSITY,
EDGBASTON, BIRMINGHAM, 15.
DIVISION OF AGRICULTURAL BIOCHEMISTRY, THE UNIVERSITY OF MINNESOTA,
ST. PAUL, MINNESOTA, U.S.A.
[*Received, May 15th*, 1951.]