246. *Some Derivatives of* D-*Galacturonic Acid.*

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The synthesis is described of some derivatives of D-galacturonic acid, in particular 2-*methyl α-methyl-*D*-galacturonoside methyl ester*. This compound was oxidised to 2-*methyl galactosaccharic acid* (2-methyl mucic acid) a useful reference compound, while under stronger oxidising conditions some demethylation occurred with formation of galactosaccharic acid (mucic acid).

In addition to its widespread occurrence in plant gums and pectins, D-galacturonic acid forms a constituent of certain bacterial polysaccharides, *e.g.*, the Type I *pneumococcus* specific polysaccharide (Heidelberger, Goebel, and Avery, *J. Exp. Med.*, 1925, **42**, 701), and the polysaccharide of *Coccidioides immitis* (Hassid, Baker, and McCready, *J. Biol. Chem.*, 1943, **149**, 303).

It was necessary to synthesise partly methylated derivatives of D-galacturonic acid to serve as reference compounds in determination of bacterial polysaccharide structure. D-Galacturonic acid, obtained both by synthesis from D-galactose (Stacey, *J.*, 1939, 1529) and from citrus pectin by enzymic degradation, was converted into α-methyl-D-galacturonoside methyl ester [which has the pyranose structure (Levene and Kreider, *J. Biol. Chem.*, 1937, **120**, 597)] by boiling it with 1% methanolic hydrogen chloride for 8 hours. Treatment of this ester for 15 hours with acetone containing either 0·4% hydrochloric acid or anhydrous copper sulphate with a trace of concentrated sulphuric acid converted it into 3 : 4-*monoacetone α-methyl-*D*-galacturonoside methyl ester* (m. p. 107°; *monohydrate*, m. p. 70°).

This product was transformed on methylation with silver oxide and methyl iodide into the syrupy 2-methyl derivative. On being kept in a moist atmosphere the syrup crystallised in the form of its *monohydrate*. Removal of the acetone groups gave syrupy 2-*methyl α-methyl-*D*-galacturonoside methyl ester* (*amide*, m. p. 174°) which on being oxidised with nitric acid under moderate conditions gave 2-*methyl galactosaccharic acid*, a liquid (*amide*, m. p. 200°). Under more drastic oxidation conditions with nitric acid there was observed an unusual case of demethylation with production of free galactosaccharic acid in fairly good yield; cf. Anderson and Otis (*J. Amer. Chem. Soc.*, 1930, **52**, 4462), who effected the removal of non-glycosidic methyl residues from a mesquite gum by means of 72% sulphuric acid.

EXPERIMENTAL.

D-*Galacturonic Acid.*—This was prepared both from citrus pectin by the action of the enzyme, " Pectinol IOM," and from D-galactose by the synthetic method of Stacey (*loc. cit.*).

*α-Methyl-*D*-galacturonoside Methyl Ester.*—D-Galacturonic acid (5 g.) was boiled for 8 hours with 1% methanolic hydrogen chloride. The solution, neutralised with lead carbonate, was filtered and evaporated under diminished pressure to a syrup which rapidly crystallised. When recrystallised from methyl alcohol–acetone the substance had m. p. 147°, yield 50%, $[\alpha]_D^{20^\circ}$ +128° (*c*, 1·0 in water); cf. Morell and Link (*J. Biol. Chem.*, 1933, **100**, 385).

3 : 4-*Monoacetone α-Methyl-*D*-galacturonoside Methyl Ester.*—(*a*) α-Methyl-D-galacturonoside methyl ester (1·2 g.) was shaken with acetone (50 c.c.) containing hydrogen chloride (0·2 g.) for 15 hours. The clear solution was poured into aqueous sodium hydrogen carbonate and the 3 : 4-monoacetone derivative extracted with chloroform. The extracts were dried ($MgSO_4$), and the filtered solution was evaporated in a vacuum. The *compound* (1·1 g.) had m. p. 107° (Found : C, 50·1; H, 6·8; OMe, 23·4. $C_{11}H_{18}O_7$ requires C, 50·4; H, 6·9; OMe, 23·6%).

(*b*) α-Methyl-D-galacturonoside methyl ester (5 g.) was shaken for 12 hours in acetone (200 c.c.) containing anhydrous copper sulphate (5 g.) and concentrated sulphuric acid (0·5 g.). The acid was neutralised with copper carbonate, and the filtered solution on evaporation yielded a crystalline product. When recrystallised from acetone–ligroin the product (4·5 g.) formed large prisms, m. p. 74°. On being heated or on being kept in a vacuum desiccator the crystals lost one molecule of water giving a product in the form of a fine white powder, m. p. 107°, $[\alpha]_D^{20^\circ}$ +118° (*c*, 1·0 in water) (Found : C, 50·0; H, 6·9; OMe, 23·6%).

3 : 4-*Monoacetone 2-Methyl α-Methyl-*D*-galacturonoside Methyl Ester.*—The above monoacetone compound (3·0 g.) was dried and methylated thrice with silver oxide and methyl iodide, giving the *ester* as a syrup (3·0 g.), b. p. 124° (bath temp.)/0·3 mm., $n_D^{26^\circ}$ 1·4210 (Found : OMe, 33·5. $C_{12}H_{20}O_7$ requires OMe, 33·7%). On exposure to a moist atmosphere the syrup slowly crystallised. When recrystallised from ethyl alcohol–ligroin the *monohydrate* had m. p. 38°, $[\alpha]_D^{20^\circ}$ +116° (*c*, 1·1 in water) (Found : C, 49·2; H, 7·5; OMe, 32·4. $C_{12}H_{20}O_7,H_2O$ requires C, 49·1; H, 7·6; OMe, 31·6%).

2-*Methyl α-Methyl-*D*-galacturonoside Methyl Ester.*—A sample of the above compound (0·33 g.) was hydrolysed with boiling 33% acetic acid which converted it into a syrupy mixture of 2-methyl α-methyl-D-glacturonic acid and its methyl ester (Found : OMe, 35·7. Calc. for $C_8H_{14}O_7$: OMe, 27·7. Calc. for $C_9H_{16}O_7$: OMe, 39·5%). The syrup was dissolved in methyl alcohol and esterified by the addition of excess of diazomethane. After 2 hours the solvent was removed under reduced pressure and the residual syrup distilled; b. p. 190° (bath temp.)/0·3 mm., $n_D^{21^\circ}$ 1·4732, $[\alpha]_D^{20^\circ}$ +80° (*c*, 1·1 in water) (Found : OMe, 37·5. $C_9H_{16}O_7$ requires OMe, 39·5%). The *ester* was converted in the usual way into the *amide* which slowly crystallised; m. p. 174°, $[\alpha]_D^{18^\circ}$ +55° (*c*, 0·05 in alcohol) (after recrystallisation from absolute ethanol–ether) (Found : N, 6·3; OMe, 27·5. $C_8H_{15}O_6N$ requires N, 6·3; OMe, 28·1%).

Oxidation of 2-*Methyl* 3 : 4-*Monoacetone* α-*Methyl*-D-*galacturonoside Methyl Ester.*—(*a*) A sample of the syrupy monoacetone compound (1·3 g.) was oxidised by treating it at 100° for 35 minutes with nitric acid (*d* 1·3). There was a copious deposit of crystalline material (0·5 g.) which was difficult to recrystallise. It had m. p. 219° (decomp.) alone or in admixture with galactosaccharic acid.

From the mother liquor there was obtained a small amount of oxalic acid and a syrup (0·5 g.) which was a mixture of ill-defined products.

(*b*) A further carefully redistilled fraction of the monoacetone compound (0·6 g.) was hydrolysed and the products were oxidised by heating them at 80° for 15 minutes with nitric acid (*d* 1·3). On being kept overnight the solution deposited only a trace of galactosaccharic acid. The nitric acid was distilled off with water and the syrupy product converted to the methyl ester (0·8 g.) which was distilled in a high vacuum; b. p. 160—162° (bath temp.)/0·07 mm., $[\alpha]_D^{20^\circ}$ $-19°$ (*c*, 0·27 in water), yield 0·5 g. The ester was converted into an *amide* which crystallised from water in the form of long needles, m. p. 200°, and gave a positive Weerman reaction (Found : C, 38·0; H, 6·5; N, 12·3; OMe, 14·4. $C_7H_{14}O_6N_2$ requires C, 37·8; H, 6·3; N, 12·6; OMe, 14·2%).

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