## 117. *Preparation of* d-*Mannuronic Acid and its Derivatives.*

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The occurrence of sugar units of the uronic acid type in many plant products and in the soluble specific polysaccharides having immunological importance has led us to seek methods for the preparation of these substances in reasonable yield in order that they may be available for synthetic work.

In the present paper the preparation of *d*-mannuronic acid from α-methylmannoside is described. We first prepared *α-methylmannoside 2 : 3-monoacetone* along with α-methylmannoside 2 : 3-4 : 6-diacetone, both of which are crystalline. The former of these serves admirably as a starting material, and alkaline permanganate oxidises the terminal group (6) to a carboxyl group, and the *potassium* salt of the resulting acid, α-methylmannuronide monoacetone, was isolated. Elimination of the acetone residue gave a crystalline potassium salt of α-methylmannuronide and also α-methylmannuronide, m. p. 108°, $[\alpha]_{5780}^{19°}$ + 65·6° in water, and finally *d*-mannuronolactone was isolated, m. p. 143—144°, $[\alpha]_{D}^{20°}$ + 95° in water. The optical rotation is slightly higher than has previously been recorded (compare Niemann and Link, *J. Biol. Chem.*, 1933, **100**, 407).

Inasmuch as subsequent studies of natural products by the methylation procedure may result in the isolation of a methylated residue of mannuronic acid we have prepared and investigated the *methyl* ester of 2 : 3 : 4-α-methylmannuronide.

### Experimental.

*α-Methylmannoside 2 : 3-Monoacetone.*—α-Methylmannoside (15 g.) was shaken for 24 hours with dry acetone (500 c.c.) containing 1% hydrochloric acid. The process was continued until all the solid had dissolved (10 days). The acetone extracts were neutralised (silver carbonate) immediately after decantation. They were finally united and evaporated in the presence of barium carbonate. The resulting syrup was extracted with boiling light petroleum (b. p. 40—60°), which removed α-methylmannoside diacetone together with condensation products of acetone. *α-Methylmannoside monoacetone* was left as a viscid syrup, b. p. 145°/0·02 mm., which soon crystallised and on recrystallisation from alcohol–light petroleum had m. p. 105°, $[\alpha]_{D}^{20°}$ + 28·3° in methyl alcohol (*c*, 4·3), $[\alpha]_{D}^{20°}$ + 24·3° in water (*c*, 4·0); yield, 6 g. The substance was readily soluble in all the usual solvents except light petroleum (Found : C, 51·1; H, 7·7; OMe, 13·3; $Me_2CO$, 24·5. $C_{10}H_{18}O_6$ requires C, 51·3; H, 7·7; OMe, 13·2; $Me_2CO$, 24·8%).

The pyranose structure of this substance was proved by its hydrolysis in the presence of *N*/100-hydrochloric acid at 15° to give quantitatively α-methylmannopyranoside, m. p. 195°, $[\alpha]_{D}$ + 79° in water (*c*, 1·0). The properties of α-methylmannoside 2 : 3-4 : 6-diacetone, m. p. 76°, will be described in a later paper.

*Oxidation of α-Methylmannoside 2 : 3-Monoacetone.*—This substance (6 g.), dissolved in water (100 c.c.), was oxidised by aqueous alkaline potassium permanganate (900 c.c. containing 8·1 g. of potassium permanganate and 3 g. of potassium hydroxide). After 24 hours at 15—20° the liquid was filtered, neutralised (carbon dioxide), and evaporated to dryness at 40°/12 mm. The resulting solid was extracted several times with alcohol, and the filtered extract concentrated to a viscid syrup. This was extracted with cold acetone and the acetone extract was evaporated to a syrup, which was dissolved in a little ethyl alcohol. On the addition of ether a viscid syrup was precipitated which, after purification by successive precipitations from alcohol, was obtained as a white amorphous hygroscopic powder. This was the *potassium* salt of α-methylmannuronide monoacetone, $[\alpha]_{5780}^{20°}$ − 4·5° in water (*c*, 3·9), (yield, 6·5 g.) (Found : OMe, 13·9; $Me_2CO$, 18·6; K, 13·7. $C_{10}H_{15}O_7K$ requires OMe, 10·8; $Me_2CO$, 20·3; K, 13·6%).

*α-Methyl* d-*Mannuronide.*—The potassium salt of α-methylmannuronide (6·5 g.) was dissolved in water and 24·8 c.c. of *N*-hydrochloric acid were added (1 mol.). Further acid was added to make the mineral acid concentration *N*/50 and the solution was kept at 50° for 1 hour. During this time the observed rotation (1 dm. tube) changed from $\alpha_{5780}^{20°}$ + 0·27° to the constant value + 1·19°. The final solution was non-reducing. After neutralisation (silver carbonate) and concentration (diminished pressure) a syrup was obtained which was slightly contaminated with mineral matter. After solution in water, filtration, and evaporation of most of the solvent, followed by addition of ethyl alcohol at 70°, the crystalline *potassium* salt of α-methyl *d*-mannur-

onide was obtained as fine needles (4·55 g.) which contained alcohol of crystallisation. The salt decomposed without melting at about 230°. $[\alpha]_{5780}^{17°}$ + 48° in water (*c*, 1·5) (Found : OR, calc. as OMe, 16·9; K, 14·6. $C_7H_{11}O_7K,\frac{1}{2}C_2H_5{\cdot}OH$ requires OR, 17·3; K, 14·5%). An amorphous variety of this salt (free from combined alcohol) was obtained by precipitation of a concentrated aqueous solution with acetone (Found : OMe, 12·5. $C_7H_{11}O_7K$ requires OMe, 12·6%). On treatment with aqueous alcohol this was transformed into the above crystalline derivative. Both salts gave a strong positive Tollens–Neuberg reaction for uronic acid.

The above potassium α-methylmannuronide (1·8 g.) in water (2 c.c.) was treated at − 10° with the exact equivalent of perchloric acid, alcohol being added to complete the precipitation of the potassium perchlorate. The filtered solution was concentrated under diminished pressure at 25° to a syrup, which was dissolved in alcohol. After filtration and removal of the alcohol a syrup remained which was crystallised from alcohol–ether at − 10°, giving the *monohydrate* of α-methylmannuronide as rosettes of long needles, m. p. 108°, $[\alpha]_{5780}^{19°}$ + 65·6° in water (*c*, 1·2). Yield, almost quantitative (Found : C, 37·4; H, 6·4; OMe, 14·5. $C_7H_{12}O_7,H_2O$ requires C, 37·2; H, 6·2; OMe, 13·7%). Aqueous solutions of the acid did not show mutarotation. The hydrated acid was readily soluble in water, moderately soluble in methyl alcohol, ethyl alcohol, and only slightly soluble in other solvents. The water of crystallisation was held firmly and was removed slowly and with difficulty by heating in a vacuum at 80—90°.

d-*Mannuronolactone*.—α-Methylmannuronide (0·8 g.) was boiled with 2·5% hydrochloric acid (25 c.c.) for 9 hours, the hydrolysis being followed polarimetrically ($[\alpha]_D^{20°}$ + 50° approx. Constant value after completion of hydrolysis). After neutralisation with silver carbonate, filtration, and removal of the dissolved silver by titration with hydrochloric acid, the solution was evaporated to a syrup (0·7 g.) under diminished pressure. Crystallisation of the *d*-mannuronolactone was brought about by very slow evaporation of an aqueous solution of the syrup containing a little ether. A similar method was used for recrystallisation. Broad plates, m. p. 143—144°. $[\alpha]_D^{20°}$ + 95° in water (*c*. 3·0). No mutarotation. The yield of recrystallised lactone was low (5—10%) (Found : C, 41·0; H, 4·7. Calc. for $C_6H_8O_6$ : C, 40·9; H, 4·6%).

*Methyl Ester of* 2 : 3 : 4-*Trimethyl* α-*Methylmannuronide*.—Potassium α-methylmannuronide (3 g.) was methylated in the usual way with methyl sulphate (18 c.c.) and potassium hydroxide (20·5 g. in 30% aqueous solution). After the completion of reaction the solution was cooled to 20° and neutralised exactly by addition of dilute sulphuric acid. After evaporation to dryness (diminished pressure) the product was extracted from the solid residue by boiling absolute alcohol. The alcoholic extract was filtered, and concentrated to a syrup, which was dissolved in the minimum amount of water. After addition of sulphuric acid the liberated 2 : 3 : 4-trimethyl α-methylmannuronide was extracted by shaking with chloroform. This gave a syrup, which was dissolved in methyl iodide and boiled with silver oxide in the usual way, giving (after one repetition of the treatment with methyl iodide and silver oxide) the *methyl* ester of 2 : 3 : 4-trimethyl α-methylmannuronide as a colourless mobile syrup, b. p. 118°/0·02 mm., $n_D^{18°}$ 1·4523; $[\alpha]_{5780}^{20°}$ + 74° in methyl alcohol (*c*, 1·0); + 61·4° in chloroform (*c*, 1·0) (Found : C, 50·0; H, 7·5; OMe, 58·3; $CO_2Me$, 22·5. $C_{11}H_{20}O_7$ requires C, 50·0; H, 7·6; OMe, 58·7; $CO_2Me$, 22·4%). The ester was readily soluble in most organic solvents and moderately soluble in water (yield, 60%).

On hydrolysis with boiling *N*-sodium hydroxide the above ester gave the corresponding sodium salt, from which 2 : 3 : 4-trimethyl α-methylmannuronide was liberated by addition of the appropriate quantity of sulphuric acid. The product was removed from the aqueous solution by shaking with chloroform, and distillation yielded the pure substance as a viscid syrup, b. p. 156—158°/0·02 mm., $[\alpha]_{5780}^{21°}$ + 73° in methyl alcohol (*c*, 1·1) (yield, almost quantitative). 2 : 3 : 4-*Trimethyl* α-*methyl*-d-*mannuronide* was soluble in water and all the usual solvents (Found : C, 48·2; H, 7·1; OMe, 50·1. $C_{10}H_{18}O_7$ requires C, 48·0; H, 7·2; OMe, 49·6%). The amide, the phenylhydrazide, and various salts were prepared and examined, but none of these has yet crystallised.

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