322. *The Synthesis of Uronic Acids.*

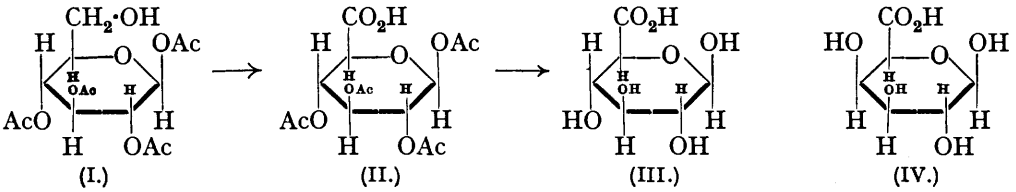
By M. Stacey.

A convenient method has been devised for the synthesis of *d*-glucuronic acid. The steps involved are: glucose ⟶ 1 : 2 : 3 : 4-tetra-acetyl 6-trityl β-*d*-glucose ⟶ 1 : 2 : 3 : 4-tetra-acetyl β-*d*-glucose ⟶ 1 : 2 : 3 : 4-tetra-acetyl *d*-glucuronic acid ⟶ *d*-glucuronic acid (isolated as *d*-glucurone). The method is applicable to the synthesis of *d*-galacturonic acid from *d*-galactose.

The increasing importance of the rôle played by glucuronic acid in many aspects of immunological chemistry, particularly those involving the preparation of synthetic antigens of the type described by Goebel (*Nature*, 1939, **143**, 77), emphasises the need for a convenient method of synthesis of this uronic acid. Previously known methods of preparation, for example, from the hydrolysis of gum arabic and the synthetic method of Zervas and Sessler (*Ber.*, 1933, **66**, 1326), have been fully explored in these laboratories and found to be unsatisfactory; the latter method, despite the improvements in technique that have been made, remains cumbersome and costly. The biological methods of Quick (*J. Biol. Chem.*, 1927, **74**, 331) and of Williams (*Nature*, 1939, **143**, 641) yield excellent results and, in the author's experience, glucurone is readily isolated by their means, but they are of necessity limited in their application and apply only to glucuronic acid.

A simple synthetic method which appears to be of general application has now been devised and full details are given for the case of glucose as the initial material. Oxidation in glacial acetic acid solution with potassium permanganate converted 1 : 2 : 3 : 4-tetra-acetyl β-*d*-glucose (I) into 1 : 2 : 3 : 4-tetra-acetyl *d*-glucuronic acid (II). The isolation of the latter was not essential, since by the action of barium hydroxide in aqueous solution deacetylation with simultaneous formation of barium glucuronate took place readily. Purification of the barium salt and removal of the barium gave in good yield

d-glucuronic acid (III), which readily crystallised in the form of its stable lactone, glucurone. From glucose, overall yields of 20% of glucurone were obtained.



By the same method, starting with *d*-galactose, *d*-galacturonic acid (IV) was prepared in approximately 20% yield, and this method of synthesis compares favourably in its ease and simplicity with that of the oxidation of galactose diacetone (Ohle and Berend, *Ber.*, 1925, **58**, 2585). Applications of this method to the synthesis of other uronic acids are in progress.

Experimental.

1 : 2 : 3 : 4-*Tetra-acetyl* 6-*Trityl* β-d-*Glucose*.—Freshly dried, anhydrous glucose (50 g.), suspended in pyridine (250 c.c., five times distilled over phosphoric oxide), was dissolved by shaking and cautious heating to the b. p. of the solvent. The solution was cooled to 40°, dry triphenylmethyl chloride (80 g.) or triphenylmethyl bromide (93 g.) added, and the solution kept at 20° for 12 hours, acetic anhydride (250 c.c.) then being admitted; after 24 hours at 20°, the yellow solution was poured into 10 vols. of water and the precipitated white granular powder was washed with warm water until essentially free from the acetylating agents, dried in a vacuum desiccator, and dissolved in ether (1200 c.c.), The solution was washed (twice) with dilute acetic acid and with water, dried over anhydrous magnesium sulphate, and evaporated until crystals appeared. The semi-solid mass was stirred in contact with dry methyl alcohol (200 c.c.), and the mixture set aside to crystallise further for 24 hours. The crystals were purified twice from methyl alcohol and showed $[\alpha]_D^{20°}$ + 45° in chloroform (*c*, 1·1) and m. p. 166°. Yield, 95 g. The mother-liquors contained mainly 1 : 2 : 3 : 4-tetra-acetyl 6-trityl α-*d*-glucose.

1 : 2 : 3 : 4-*Tetra-acetyl* β-d-*Glucose*.—The method used for eliminating the 6-trityl group was essentially that of Helferich and Klein (*Annalen*, 1926, **450**, 219). The product, recrystallised from ether–light petroleum, had m. p. 128°, and $[\alpha]_D$ + 11° in chloroform (*c*, 1·6). Yield, 30 g. from 100 g. of tetra-acetyl 6-trityl glucose. The triphenylmethyl bromide was recovered in almost quantitative amount, m. p. 152° after recrystallisation from glacial acetic acid containing a little hydrobromic acid.

Oxidation.—To a solution of the tetra-acetyl glucose (2·0 g.) in glacial acetic acid (20 c.c.), A.R. potassium permanganate (0·5 g.), dissolved in acetone (20 c.c.), was added. The liquid was slowly stirred at 15—18° for 6 hours, and a further amount of potassium permanganate (1·6 g.) added in small portions during 2 days. Acetone (40 c.c.) was added to the solution, which was then centrifuged until it was clear. The deposit of manganese oxides was washed with acetone–glacial acetic acid until it no longer gave the naphtharesorcinol test for uronic acids. The combined liquid and washings were evaporated under diminished pressure at 25°, giving a syrup. This was dissolved in chloroform, shaken (twice) with 0·1N-sulphuric acid and with water, and dried over anhydrous magnesium sulphate. After filtration and removal of the chloroform under diminished pressure at 25° a colourless syrup (2·0 g.) remained, consisting mainly of tetra-acetyl *d*-glucuronic acid, $[\alpha]_D$ +6° in chloroform (*c*, 1·1). This was dissolved in the minimum quantity of acetone, a saturated aqueous solution of barium hydroxide added in slight excess, and after 6 hours the solution was acidified with a few drops of acetic acid and evaporated to small volume under diminished pressure, and the barium salts precipitated by addition of 6 vols. of absolute alcohol. They were separated (centrifuge), redissolved in water, and reprecipitated by alcohol. The precipitate was dissolved in water (20 c.c.), a slight excess of N/10-sulphuric acid added, and the barium sulphate collected (centrifuge) and washed with water. The excess of sulphuric acid was neutralised with a few drops of a saturated aqueous solution of lead acetate (A.R.), the lead sulphate removed (centrifuge), and the lead in solution precipitated by hydrogen sulphide; the precipitate was washed with water. Concentration under diminished pressure of the combined filtrate and washings at 40° left a clear colourless syrup (0·85 g.), which crystallised rapidly on nucleation with an authentic specimen of glucurone. This product (0·75 g.), after being washed with methyl alcohol, dried in a desiccator, and recrystallised from ethyl alcohol, had m. p. 176—178° alone or in admixture with a specimen of

glucurone prepared from ammonium menthylglucuronate (cf. Williams, *loc. cit.*), $[\alpha]_D^{20°}$ +20° in water (*c*, 0·6) (Found: C, 41·2; H, 4·6. Calc. for $C_6H_8O_6$: C, 40·9; H, 4·6%). In a second experiment 2·0 g. of glucurone were obtained from 5 g. of 1 : 2 : 3 : 4-tetra-acetyl β-*d*-glucose.

Preparation of d-*Galacturonic Acid.*—1 : 2 : 3 : 4-Tetra-acetyl 6-trityl β-*d*-galactose (85 g.) was prepared from galactose (50 g.) in the manner described for the corresponding glucose compound. It was recrystallised from aqueous alcohol and had m. p. 76—77°, $[\alpha]_D^{20°}$ − 20° in chloroform (*c*, 1·0). After elimination of the 6-trityl group, mainly 1 : 2 : 3 : 4-tetra-acetyl β-*d*-galactose was obtained as a colourless glass insoluble in water and light petroleum but soluble in most organic solvents. It showed $[\alpha]_D^{20°}$ + 40° in chloroform (*c*, 3·0). Yield, 5·2 g. from 20 g. of the trityl compound. Oxidation of 1 : 2 : 3 : 4-tetra-acetyl β-*d*-galactose (3 g.) by the method described above yielded mainly 1 : 2 : 3 : 4-tetra-acetyl β-*d*-galacturonic acid as an intermediate stage, but its isolation and purification were unnecessary. *d*-Galacturonic acid (1·3 g.) was obtained as a colourless syrup, which rapidly crystallised on nucleation with *d*-galacturonic acid monohydrate and rubbing with a few drops of glacial acetic acid. It was recrystallised from a small volume of aqueous alcohol and obtained as the monohydrate, m. p. 157—158° after softening at 115°. After dehydration in a vacuum drier the crystals had m. p. 158° alone or in admixture with an authentic specimen prepared from citrus pectin, $[\alpha]_D^{21°}$ in water + 100° ⟶ +68° (equilibrium value after 4 hours) (Found: C, 37·4; H, 5·0. $C_6H_{10}O_7$ requires C, 37·1; H, 5·2%). On addition of a drop of basic lead acetate solution to an aqueous solution of the crystals, a voluminous white precipitate, which rapidly turned brick-red on warming, was obtained (the test appears to be specific for galacturonic acid).

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