289. *The Synthesis of Conjugated Hexuronic Acids.*

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Hexosides in neutral aqueous solution were converted by gaseous oxygen in the presence of a platinum catalyst into the corresponding conjugated hexuronic acids. By this method, (—)-menthyl-α- and -β-D-glucuronosides, and methyl-α- and -β-D-galacturonosides and -D-mannuronosides, were prepared, but attempts to oxidise phenyl-α- and -β-D-glucosides failed. The same procedure was used to oxidise α- and β-glucose-1 phosphate to the corresponding glucuronic esters, but the β-compound was not obtained in a pure state. The method appears to be specific for the oxidation of primary alcohol groups to carboxyl in sugars in which the reducing group has been protected by conjugation with an alcohol or by esterification.

IT has been reported that glycosides can be oxidised to conjugated hexuronic acids by dinitrogen tetroxide (Maurer and Drehfahl, *Ber.*, 1942, **75**, 1489; Hardegger and Spitz, *Helv. Chim. Acta*, 1949, **32**, 2165; 1950, **33**, 337; Peterman, U.S.P. 2 520 255, 2 520 256),

alkaline hypobromite (Bergmann and Wolff, *Ber.*, 1923, **56**, 1060; Smolenski, *Roczn. Chem.*, 1924, **3**, 153; Jackson and Hudson, *J. Amer. Chem. Soc.*, 1937, **59**, 994), hydrogen peroxide in the presence of traces of ferric salts (Smolenski, *loc. cit.*), and electrolytic oxidation (Niemann and Link, *J. Biol. Chem.*, 1934, **104**, 195). By all these methods the yields obtained were poor, owing to the general difficulty, particularly in aqueous media, of selective oxidation of the primary alcohol group to carboxyl. In the absence of a suitable, specific oxidising agent, methods of synthesis involving initial protection of all the secondary alcohol groups by conversion into acetoxy- or *iso*propylidenedioxy-groups have generally been used (see Smith, Stacey, and Wilson, *J.*, 1944, 131), although Ault, Haworth, and Hirst (*J.*, 1935, 517) oxidised 2 : 3-*iso*propylidene methyl-α-D-mannopyranoside to the corresponding mannuronic acid derivative with alkaline potassium permanganate.

The use of gaseous oxygen in neutral or mildly alkaline solution, in the presence of a platinum catalyst, for the preparation of the corresponding saccharic acids from D-glucose (Mehltretter, Rist, and Alexander, U.S.P. 2 472 168) and 2 : 3-*iso*propylidene L-sorbose (Merck and Co., U.S.P. 2 483 251) has very recently been extended to the synthesis of 1 : 2-*iso*propylidene D-glucuronic acid from the mono*iso*propylidene derivative of D-glucose (Fernandez-Garcia *et al.*, *El Crisol, Puerto Rico*, 1950, **4**, 40; *Chem. Abs.*, 1951, **45**, 555; Mehltretter, Alexander, Mellies, and Rist, *J. Amer. Chem. Soc.*, 1951, **73**, 2424). After mild acid hydrolysis, good yields of glucuronic acid, in the form of the γ-lactone, were obtained.

This method seemed applicable to the oxidation of hexosides to the corresponding conjugated hexuronic acids, since the former are more stable to acids than *iso*propylidene derivatives of the sugars (Freudenberg, Durr, and Hochstetter, *Ber.*, 1928, **61**, 1735). The compounds initially chosen for oxidation were (−)-menthyl-α- and -β-D-glucopyranosides, as the corresponding hexuronosides are only slightly soluble in water and are therefore easily isolated. Good yields of these hexuronosides were obtained. Attempts to oxidise phenyl-α- and -β-D-glucopyranosides in this way were unsuccessful; the trace of free phenol liberated by slight hydrolysis was apparently sufficient to render the catalyst completely inactive after a very short time. The preparation of the methyl-α- and -β-glycosides of D-galacturonic and D-mannuronic acids, however, presented no difficulty. In the preparation of methyl-α-D-galacturonoside it was found that gaseous oxygen could be replaced by dilute hydrogen peroxide, but the yield was lower. The methyl-α- and -β-D-mannuronosides were hydrolysed to D-mannuronic lactone, m. p. 188° (decomp.), $[\alpha]_D^{22}$ +92°, and m. p. 189—191° (decomp.), $[\alpha]_D^{20}$ +89° respectively, whereas Ault, Haworth, and Hirst (*loc. cit.*) give m. p. 143—144°, $[\alpha]_D^{20}$ +95°. According to Professor F. Smith (personal communication), this lactone exists in two crystalline forms having identical optical rotations, the higher-melting isomer being more stable; an authentic specimen of the latter, m. p. 189° (decomp.), gave no depression when mixed with our products.

By catalytic oxidation of 3-methyl 1 : 2-*iso*propylidene D-glucose, 3-methyl 1 : 2-*iso*propylidene D-glucuronic acid was obtained as the sodium salt, which on hydrolysis yielded a product believed to be 3-methyl D-glucuronic acid. The method may thus be of importance in providing reference compounds for the identification of methylated glucuronic acids obtained in studies of polysaccharide structures (cf. Lythgoe and Trippett, *J.*, 1950, 1983; Overend, Shafizadeh, and Stacey, *J.*, 1951, 1487; Smith, *ibid.*, p. 2646).

The very mild nature of this reaction suggested that it might be applicable to the oxidation of the more labile glucose-1 phosphates. α-Glucuronic acid 1-phosphate was obtained, in the form of its tripotassium salt, without difficulty, but the product isolated from the oxidation of synthetic β-glucose-1 phosphate appeared to be unstable, and attempts at purification were unsuccessful. The properties of the crude product nevertheless indicate that it is largely composed of β-glucuronic acid 1-phosphate.

Synthetic (−)-menthyl-β-D-glucopyruronoside was identical in melting point and optical rotation with the urinary glucuronoside excreted after oral administration of (−)-menthol (Fromm and Clemens, *Z. physiol. Chem.*, 1901—2, **34**, 385), and this establishes the pyranose structure of the biosynthetic compound. Pryde and Williams (*Nature*, 1931, **128**, 187) have suggested from methylation studies that biosynthetic (+)-bornyl-β-D-glucuronoside is in the pyranose form, whereas Neuberg and Neimann (*Z. physiol.*

Chem., 1905, **44**, 114) claimed to have synthesised euxanthic acid and phenyl-β-D-glucuronoside, identical with the biosynthetic products, by condensation of acetobromoglucuronic lactone with the appropriate aglycones in the presence of potassium methoxide. This lactone has a furanose structure (Smith, *J.*, 1944, 584). The last-mentioned synthesis has not, however, been confirmed (Goebel and Babers, *J. Biol. Chem.*, 1933, **100**, 743).

Preliminary accounts of this work have already been published (Marsh, *Nature*, 1951, **168**, 602; *Biochem. J.*, 1951, **50**, xi). Barker, Bourne, and Stacey (*Chem. and Ind.*, 1951, **45**, 970) have independently described the oxidation of methyl-α- and -β-D-glucosides and α-glucose-1 phosphate by this method.

Experimental

(Determinations of carbon and hydrogen are by Drs. Weiler and Strauss. M. p.s are corrected.)

General Procedure.—The powdered platinum catalyst was prepared in aqueous suspension from Adams's platinum oxide catalyst (Johnson, Matthey, and Co., Ltd.) by hydrogenation at atmospheric pressure; it could be stored under water without undue loss of activity for about a week, after which its efficiency decreased. The aqueous solution or suspension of the hexoside or hexose ester was placed in a narrow cylindrical vessel tapering at the base to a coarse sintered-glass disc through which oxygen was forced from a cylinder into the reaction mixture; the vessel was heated by a water-bath. Vigorous mechanical stirring was necessary to disperse the heavy particles of catalyst. Small samples of liquid were withdrawn at frequent intervals for pH measurement, and neutrality was maintained by addition of alkali carbonate or bicarbonate, until no further pH change occurred. After removal of the catalyst, the product was treated as described for individual compounds. Evaporations of all aqueous solutions were conducted below 50° *in vacuo*.

(−)-*Menthyl-α-D-glucuronoside.*—A suspension of (−)-menthyl-α-D-glucoside (0·5 g.) and catalyst (0·1 g.) in water (80 c.c.) was oxidised at 65°, with addition of 0·5N-sodium hydrogen carbonate (3·0 c.c.) during 10 hours, after which no further pH change occurred, and all the glucoside had passed into solution. After removal of the catalyst, acidification with 2N-hydrochloric acid precipitated (−)-menthyl-α-D-glucuronoside (0·163 g., 31%), m. p. 135—136°. On recrystallisation from ethyl acetate–light petroleum (b. p. 100—120°), the dihydrate (0·144 g., 27%) melted at 141—142°, $[\alpha]_D^{21}$ +46° (*c*, 20 in alcohol). The mixed m. p. with an authentic specimen, m. p. 137—138° (Bergmann and Wolff, *loc. cit.*), was 137—140° (Found, for anhydrous compound: C, 58·3; H, 9·0. Calc. for $C_{16}H_{28}O_7$: C, 57·8; H, 8·5%). Bergmann and Wolff (*loc. cit.*) give m. p. 140° (decomp.), $[\alpha]_D^{18}$ +51·9° (in alcohol).

(−)-*Menthyl-β-D-glucuronoside.*—The oxidation of a solution of (−)-menthyl-β-D-glucoside (0·5 g.) (Treibs and Franke, *Annalen*, 1950, **570**, 76) in water (50 c.c.) with catalyst (0·1 g.) and neutralisation with sodium hydrogen carbonate (0·5N; 3·1 c.c.) was complete in 4 hours at 60° (−)-Menthyl β-D-glucuronoside was precipitated as the ammonium salt (0·3 g.) and converted into the free acid by Quick's method (*J. Biol. Chem.*, 1924, **61**, 667). Two recrystallisations from water yielded a pure product (*ca.* 30%), m. p. 75—77° (decomp. 114°), $[\alpha]_D^{21}$ −99° (*c*, 10 in alcohol). The mixed m. p. with a biosynthetic specimen, m. p. 79—80°, $[\alpha]_D^{21}$ −102° (*c*, 10 in alcohol), was 78—80°. Both compounds contained *ca.* $1\frac{1}{2}H_2O$ (Found, for anhydrous compound: C, 58·0; H, 8·5. Calc. for $C_{16}H_{28}O_7$: C, 57·8; H, 8·5%). According to Fromm and Clemens (*loc. cit.*), the biosynthetic material has m. p. 87—88°, but this appears to depend on the degree of hydration.

Methyl-α-D-galacturonoside.—(*a*) Oxidation of a solution of methyl-α-D-galactoside monohydrate (1·06 g.) in water (20 c.c.) with catalyst (0·1 g.), and neutralisation with N-sodium hydrogen carbonate (4·9 c.c.), was completed in 5 hours at 60°. The filtrate was made definitely alkaline with aqueous ammonia, and excess of basic lead acetate solution added, precipitating the lead salt of methyl-α-D-galacturonoside. The product was washed thrice with water (total 60 c.c.) on the centrifuge. The moist product, suspended in water (25 c.c.), was saturated with hydrogen sulphide at 0°, and after removal of lead sulphide evaporation of the colourless solution yielded a gum which crystallised when dried *in vacuo*. Recrystallisation from 95% alcohol gave methyl-α-D-galacturonoside dihydrate, m. p. 107—108° (0·441 g.), together with a second crop, m. p. 101—103° (0·075 g., total 42%). After a second recrystallisation the m. p. was 110°, with sintering at 105° and decomp. at 126°, $[\alpha]_D^{25}$ +128° (*c*, 2 in water) (Found, for anhydrous product: C, 41·0; H, 6·2. Calc. for $C_7H_{12}O_7$: C, 40·4; H, 5·8%). According to Morell and

Link (*J. Biol. Chem.*, 1933, **100**, 385), m. p. of the dihydrate is 112—114° with sintering at 109° and decomp. at 120° and $[\alpha]_D^{28}$ is +127·6° (*c*, 2—3 in water).

(*b*) To a solution of methyl-α-D-galactoside (1·06 g.) in water (40 c.c.) with catalyst (0·1 g.) was added 20-vols. hydrogen peroxide (50 c.c.) during 4 hours with stirring at 20°, the mixture being kept neutral with N-sodium hydrogen carbonate (3·8 c.c.). After filtration, the product was treated as in the preceding preparation, giving a crude gummy product (0·48 g.), which on recrystallisation from 95% alcohol had m. p. 105—107° (alone or mixed with the above product) (0·20 g., 16%), $[\alpha]_D^{20}$ +126° (*c*, 2 in water).

Methyl-β-D-galacturonoside.—Methyl-β-D-galactoside (0·97 g.) in water (20 c.c.) with platinum catalyst (0·1 g.) was completely oxidised in 6 hours at 65°, neutralisation being with N-sodium hydrogen carbonate solution (4·8 c.c.). The subsequent procedure was as for methyl-α-D-galacturonoside. The product, recrystallised from 95% alcohol, had m. p. 160—162° (0·247 g., 22%). A second recrystallisation gave the monohydrate, m. p. 163°, with sintering at 100—110°, decomp. at 182°, $[\alpha]_D^{25}$ −38° (*c*, 2·5 in water) (Found, for the anhydrous product: C, 40·3; H, 5·8. Calc. for $C_7H_{12}O_7$: C, 40·4; H, 5·8%). Morell, Baur, and Link (*ibid.*, 1935, **110**, 719) give m. p. 163—165°, decomp. at 180°, $[\alpha]_D^{25}$ −39·6° (*c*, 1·5—2·0 in water).

Methyl-α-D-mannuronoside.—Methyl-α-D-mannoside (3·88 g.) in water (50 c.c.) with catalyst (0·2 g.) was completely oxidised in 18 hours at 65°; 10% aqueous potassium carbonate (11·8 c.c.) was used for neutralization. Evaporation after removal of catalyst yielded a yellow gum, which solidified on addition of methyl alcohol (10 c.c.). The white crystalline potassium salt of methyl-α-D-mannuronoside (2·84 g.) decomposed slowly above 200°, with sintering at 100°, $[\alpha]_D^{20}$ +45° (*c*, 2 in water) (Found, for anhydrous compound: K, 15·3. Calc. for $C_7H_{11}O_7K$: K, 15·8%). Ault, Haworth, and Hirst (*loc. cit.*) give $[\alpha]_D^{17}$ +48° (*c*, 1·5 in water).

The potassium salt (1·8 g.), dissolved in water (2 c.c.), was treated at −5° with one equivalent of 25% perchloric acid, and alcohol (5 c.c.) was added. The filtered solution was evaporated to a colourless syrup, dissolved in alcohol (15 c.c.), and filtered from residual potassium perchlorate. Evaporation yielded a colourless gum which rapidly crystallised on addition of ether (1 c.c.) and then had m. p. 102—104° (1·25 g.; calc. on methyl-α-D-mannoside, 44%). Recrystallisation from alcohol–ether (2 : 1) gave the monohydrate, m. p. 106—107°, $[\alpha]_D^{20}$ +62° (*c*, 2 in water) (Found, for anhydrous compound: C, 41·0; H, 6·1. Calc. for $C_7H_{12}O_7$: C, 40·4; H, 5·8%). Ault, Haworth, and Hirst (*loc. cit.*) give m. p. 108°, $[\alpha]_D^{19}$ +65·6° (*c*, 1·2 in water).

Methyl-α-D-mannuronoside monohydrate (1·1 g.) on hydrolysis by Ault, Haworth and Hirst's method (*loc. cit.*) yielded a yellow gum (0·328 g.) which partly solidified. Recrystallisation from water gave a colourless product, m. p. 188° (decomp.) (0·26 g.), $[\alpha]_D^{22}$ +92° (*c*, 3 in water). This gave a strong Tollens reaction for uronic acid, and a positive test for reducing sugar (Found: C, 41·4; H, 4·6. Calc. for $C_6H_8O_6$: C, 40·9; H, 4·6%). The mixed m. p. with a specimen of mannuronic lactone, m. p. 189° (decomp.), supplied by Prof. F. Smith, was 187—190° (decomp.). Ault, Haworth, and Hirst (*loc. cit.*) give $[\alpha]_D^{20}$ +95° (*c*, 3 in water).

Methyl-β-D-mannuronoside.—Methyl-β-D-mannoside *iso*propanol solvate (1·68 g.) (Isbell and Frush, *J. Res. Nat. Bur. Stand.*, 1940, **24**, 125), heated at 100° (6 hours), gave the solvent-free product (1·06 g.) as a colourless gum; the solution of this in water (30 c.c.) with catalyst (0·2 g.) was oxidised at 70°, and potassium carbonate solution (0·75N; 6·9 c.c.) added as necessary; no further pH change occurred after 6 hours. The filtered solution was evaporated to a syrup, from which a gum was precipitated on addition of methyl alcohol (15 c.c.). After 5 hours at 0° the *potassium* salt of methyl β-D-mannuronoside had solidified and was filtered off and dried *in vacuo* (1·12 g.). Precipitation from water with methyl alcohol gave the product as a yellowish, very deliquescent solid (0·78 g.), decomp. above 160°, $[\alpha]_D^{20}$ +15° (*c*, 6 in water) (Found, for anhydrous product: K, 16·0. $C_7H_{11}O_7K$ requires K, 15·8%). The neutral solution in water gave a strong Tollens test for uronic acid; the reaction for reducing sugar was only positive after hydrolysis with 3N-hydrochloric acid (5 minutes at 100°).

The potassium salt (0·160 g.), dissolved in water (2 c.c.), was converted into the free acid as for the corresponding α-isomer (see above). The reaction product, after removal of potassium perchlorate, was evaporated to a syrup which yielded a white gum on addition of alcohol (3 c.c.); this hardened to a solid (88 mg.) after 12 hours at 0° (Found, for anhydrous product: C, 39·6; H, 5·7. $C_7H_{12}O_7$ requires C, 40·4; H, 5·8%), $[\alpha]_D^{19}$ +25° (*c*, 2 in water). The strongly acid aqueous solution of *methyl-β-D-mannuronoside* gave a Tollens reaction for uronic acid, and the test for reducing sugar was only positive after hydrolysis with N-hydrochloric acid (5 minutes at 100°). Attempted recrystallisation from water–alcohol yielded a gum which on being dried *in vacuo* gave slightly positive tests for reducing sugar. The methyl-β-D-mannuronoside thus appears to be readily hydrolysed in aqueous solution.

Crude methyl-β-D-mannuronoside (0·11 g.) was hydrolysed with 2·5% hydrochloric acid to D-mannuronic lactone, m. p. and mixed m. p. 179—181° (decomp.) (28 mg.), $[\alpha]_D^{20}$ + 89° (*c*, 3 in water).

3-*Methyl* 1 : 2-iso*Propylidene Glucuronic Acid.*—A solution of 3-methyl 1 : 2-*iso*propylidene glucose (0·59 g.) (Vischer and Reichstein, *Helv. Chim. Acta*, 1944, **27**, 1332) in water (15 c.c.) with catalyst (0·1 g.) was oxidised at 60°, N-sodium hydrogen carbonate (2·6 c.c.) being used for neutralization; the reaction was complete in 1 hour. Evaporation of the filtered solution gave a yellowish gum; on dissolution of this in alcohol and precipitation with ether *sodium* 3-*methyl* 1 : 2-iso*propylidene glucuronate* was precipitated as an amorphous power, $[\alpha]_D^{21}$ −35° (*c*, 4 in water) (0·45 g., 69%) (Found : Na, 8·1. $C_{10}H_{15}O_7Na$ requires Na, 8·5%). This gave a strong Tollens reaction for uronic acid; the reaction for reducing sugar was only positive after hydrolysis with 0·5N-hydrochloric acid (1 minute at 100°).

A solution of the sodium salt (0·45 g.) in water (10 c.c.) was passed through a column of cation exchange resin (Amberlite 100), and glacial acetic acid (1 c.c.) added to the pale yellow solution. When kept at 70° the solution became less lævorotatory until after 24 hours the rotation was very slightly positive, and no further change occurred. Evaporation (charcoal) gave a yellow gum, which, dried *in vacuo* over potassium hydroxide, had $[\alpha]_D^{21}$ +6° (*c*, 5 in water) (0·30 g.). The strongly acid aqueous solution gave a strong Tollens test for uronic acid and a positive test for reducing sugar.. Neither the free acid nor its alkali salts could be crystallised (cf. Levene and Meyer, *J. Biol. Chem.*, 1924, **60**, 173).

α-*Glucuronic Acid* 1-*Phosphate.*—A solution of α-glucose-1 (dipotassium phosphate) (0·336 g.) in water (20 c.c.) and catalyst (0·1 g.) was oxidised at 40—45°, with addition of 1% aqueous potassium carbonate (4·3 c.c.) to keep the pH at 7·5—9·0; no further reaction occurred after 5 hours. Evaporation of the filtered solution yielded a gum, which was dissolved in 50% aqueous methyl alcohol (3 c.c.); absolute methyl alcohol (1·5 c.c.) was added, precipitating an oil which rapidly crystallised at 0° (0·273 g.). Recrystallisation from aqueous methyl alcohol by the same procedure gave pure *tripotassium* α-*glucuronate* 1-*phosphate* as the dihydrate, $[\alpha]_D^{20}$ +51° (*c*, 2·4 in water), decomp. above 150°, with sintering at 100°. It gave a strong Tollens reaction for uronic acid; the reaction for reducing sugar was only positive after hydrolysis with 3N-hydrochloric acid (15 minutes at 100°) [Found : loss at 78°, 9·3. $C_6H_8O_{10}PK_3,2H_2O$ requires H_2O, 8·5. Found, for anhydrous compound : Total P, 7·75; inorganic P, <0·1; K (Abul-Fadl, *Biochem. J.*, 1949, **44**, 282), 29·6; glucuronic acid (Hanson, Mills, and Williams, *ibid.*, 1944, **38**, 274), 49. $C_6H_8O_{10}PK_3$ requires P, 7·98; K, 30·1; glucuronic acid, 50%].

Oxidation of β-*Glucose*-1 (*Dipotassium Phosphate*).—The dibrucine salt of β-glucose-1 phosphate was prepared by the method of Wolfrom, Smith, Pletcher, and Brown (*J. Amer. Chem. Soc.*, 1942, **64**, 23), and converted into the dipotasium salt by addition of two equivalents of 10% aqueous potassium hydroxide; the final pH was 8·8. After 2 hours at 0° and removal of brucine, the solution was evaporated to dryness at 30°, and alcohol (30 c.c.) was added to redissolve residual brucine. The microcrystalline, deliquescent dipotassium salt (0·31 g.) was filtered off, dried *in vacuo*, and used without further purification. A solution in water (0·140 g., 15 c.c.) together with catalyst (0·1 g.) was oxidised at 50°, with addition of 1% aqueous potassium carbonate (2·1 c.c.) to keep the pH at 8—9; reaction ceased after 3 hours. The catalyst was removed, and the solution evaporated at 35° to a syrup, from which a gum was precipitated on addition of methyl alcohol (5 c.c.). After two further precipitations from aqueous solution with methyl alcohol the colourless gum gave a very deliquescent yellowish powder (0·086 g.) when dried *in vacuo* (Found, after drying at 78° : Total P, 8·36; inorganic P, 1·06; K, 27·7; glucuronic acid, 33%). The Tollens test for uronic acid was strong, and a positive test for reducing sugar was obtained only after hydrolysis with 3N-hydrochloric acid (5 minutes at 100°). Attempts to purify the product from methyl alcohol–water reduced the glucuronic acid content.

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