228. *Pectic Substances. Part V. The Molecular Structure of Strawberry and Apple Pectic Acids.*

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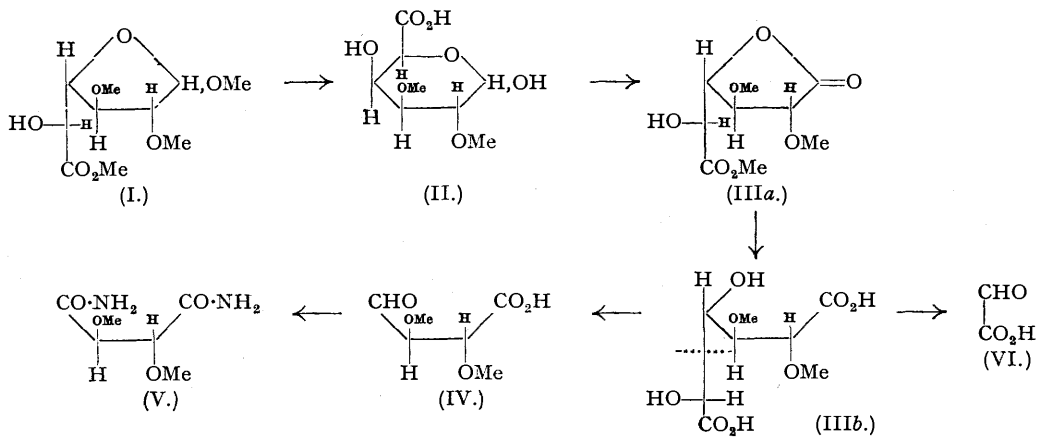
Degradation products prepared from strawberry pectin and apple pectin by boiling with methanolic hydrogen chloride have been converted into their methylated derivatives. Hydrolysis of the latter with methanolic hydrogen chloride yielded the methyl ester of 2 : 3-dimethyl methyl-*d*-galacturonoside as the main product. The bearing of these observations on the chemical structure of pectic acid is discussed.

Pectins commonly occur in fruit and have been the subject of chemical investigation by many workers, and it is now generally accepted that they are mixtures of polysaccharides, amongst which galactan, araban, and polygalacturonosides predominate (for a review of the evidence see the article by Hirst and Jones, " Advances in Carbohydrate Chemistry ", Vol. II, 1946). These polysaccharides are in close physical union, and great difficulty is experienced in separating the components in a pure condition, particularly those of the polygalacturonoside fraction. Arabans from peanut, apple, and citrus pectins have been shown to consist of *l*-arabofuranose residues linked to form a branched chain polymer (Hirst and Jones, *J.*, 1938, 496; 1939, 453, 454; Beavan, Hirst, and Jones, *J.*, 1939, 1865), whilst the galactan from *Lupinus albus* consists of β-*d*-galactopyranose residues in the form of a linear polymer (Hirst, Jones, and Walder, *J.*, forthcoming publications). These two polyssaccharides could not therefore be derived one from the other by oxidation and decarboxylation at C_6 of the pyranose residues. In view of these results it became of special interest to determine whether any correlation exists between the structures of the pectic acid and of either of the polysaccharides associated with it, and whether pectic acids from different sources possess similar structures

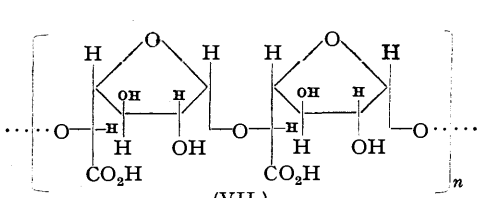
The investigation of the pectic acid component was rendered all the more difficult by reason of the special physical and chemical properties of the polysaccharide. In a previous publication (Beavan and Jones, *Chem. and Ind.*, 1939, **58**, 363) attention was drawn to the difficulties encountered in methylating pectic acid both by the methyl sulphate and by the thallium methylation technique. This difficulty was in part overcome by methylating a degraded pectic acid derivative prepared by boiling crude pectin with methyl alcoholic hydrogen chloride (Morrel, Bauer, and Link, *J. Biol. Chem.*, 1935, **105**, 1; Hirst, Jones, and Jones, *J.*, 1939, 1880). This procedure destroyed the more labile araban and galactan and converted the pectic acid into the methyl ester of a degraded polygalacturonide of approximately eighteen residues. The physical properties of this material, designated " polyester " by Morrel, Bauer, and Link, made it more amenable to methylation by the thallium technique (Menzies, *J.*, 1926, 937; Hirst and Jones, *J.*, 1938, 497) and by this method the methylated derivatives of strawberry and apple polyesters were prepared and examined. The present results indicate that these polyesters are similar in constitution to the methylated polygalacturonic acids which have been previously examined (Smith, *Chem. and Ind.*, 1939, **58**, 363; Beavan and Jones, *loc. cit.*; Luckett and Smith, *J.*, 1940, 1106).



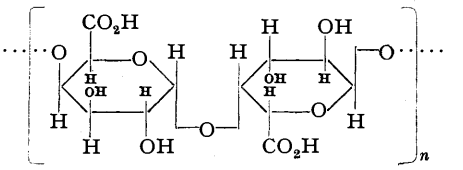
Proof of the constitution of the methylated polyester was furnished by the following observations : the methylated polyuronide gave, on hydrolysis with methyl alcoholic hydrogen

chloride under pressure, 2 : 3-dimethyl methyl-*d*-galacturonoside methyl ester (I) as a mixture of its pyranose and furanose forms (cf. Luckett and Smith, *loc. cit.*). On hydrolysis with aqueous acid (I) was converted into 2 : 3-dimethyl *d*-galacturonic acid (II) identified after oxidation and esterification as the crystalline γ-lactone methyl ester (III*a*) of 2 : 3-dimethyl *d*-mucic acid (III*b*). The constitution of this crystalline product followed from its reaction with periodic acid, which gave the half-aldehyde of *l*(+)-dimethoxysuccinic acid (IV), identified as *l*(+)-dimethoxysuccinamide (V), and glyoxylic acid (VI), identified by its colour reaction with casein and sulphuric acid.

The polyester from both strawberry and apple pectin must therefore be a polymer built up of *d*-galacturonic acid residues in which the hydroxyl groups on C_2 and C_3 are free. The two structures which are in agreement with this evidence are (VII) and (VIII), of which the pyranose form (VIII) is by far the more probable since the pyranose structure alone would display the resistance to acidic hydrolysis so characteristic of the pectic acid molecule. That the linkage



(VII.)



(VIII.)

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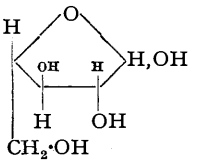

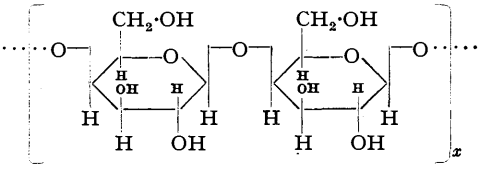

(X.)

between the galacturonic acid residues is of the α-type is shown by the high positive rotation of pectic acid. It is clear, therefore, that a polygalacturonide with this structure cannot be transformed directly by decarboxylation at C_6 into an araban of the type found associated with pectic acid, since this araban is known to possess a branched chain structure of *l*-α-arabofuranose residues (IX) linked to each other in three different ways—namely, through C_1 (A1 . . . .), through C_1 and C_5 (. . 5A1 . .), and through C_1C_3 and C_5 ($\vdots\overset{5}{3}$A1 . . .). Nor can the pectic acid arise from the galactan (X) by oxidation of the primary alcohol groups, since the sugar residues in (X) are linked by β-links. It follows, therefore, that if galactan (X) is the source of araban, then hydrolysis followed by re-synthesis of the oxidised and decarboxylated sugar must intervene.

Experimental.

Strawberry Pectic Acid.—The pectin was obtained from strawberry juice, for a supply of which we wish to express our thanks to Dr. V. L. S. Charley and Carter and Co. of Bristol. The crude pectin was contaminated with kieselguhr; it was triturated with 90% alcohol and filtered to remove colouring matter. The solid was dried at 40°/12 mm. and extracted with the calculated quantity of dilute sodium hydroxide solution. The sludge was spun on the centrifuge and the top clear, light blue solution poured with stirring into 5 vols. of 90% alcohol acidified with hydrochloric acid. Pectic acid was precipitated; it was filtered off, washed with alcohol until free from hydrochloric acid, and dried under reduced pressure; $[\alpha]_D^{20°}$ + 251° (in neutral aqueous solution) [Found: Uronic anhydride, 84·0 (calc. from the yield of carbon dioxide evolved on boiling with 12% hydrochloric acid); pentosan, 4·1 (calc. from the yields of furfuraldehyde and carbon dioxide evolved on boiling with 12% hydrochloric acid); galactan, 9·9 (by difference); OMe, 0·2%; equiv., 225].

Strawberry Pectin Polyester (see Morrel, Bauer, and Link, *loc. cit.*).—Strawberry pectic acid (100 g.) was refluxed with stirring with 3% methyl alcoholic hydrogen chloride (1 l.). After 24 hours sufficient concentrated methyl alcoholic hydrogen chloride was added to bring the concentration up to 5% and the refluxing continued for a further 66 hours. The cooled reaction mixture was spun on the centrifuge and the dark slimy solid thus obtained washed with methyl alcohol until free from hydrogen chloride. The solid was dried at 60°/12 mm. and then extracted thrice with boiling water (500 c.c.). The extracts were spun on the centrifuge and the clear solution was poured into alcohol (2½ l.). The precipitated polyester (65 g.) was separated on the centrifuge, washed with alcohol and acetone, and dried at 50°/12 mm.; $[\alpha]_D^{20°}$ + 234° (*c*, 1·2) (Found: OMe, 17·4%; equiv., 200).

A sample of the polyester was titrated with the calculated quantity of barium hydroxide and the resulting barium *salt* precipitated with alcohol. The white solid was filtered off, washed with alcohol,

and dried in a vacuum (Found: Ba, 25·1; OMe, 1·8. A methylpolygalacturonoside methyl ester containing eight units requires Ba, 27·7; OMe, 1·6%).

Methylation of Strawberry Polyester.—The polyester (5·7 g.) was dissolved in water (20 c.c.) and to it was added a hot concentrated solution of thallium hydroxide (30 c.c. of 6N). The cream-coloured precipitate was filtered off, washed with methyl alcohol and ether, and dried at 40°/12 mm. The orange solid was ground to a powder (120 mesh) and boiled with methyl iodide for 72 hours under reflux with the exclusion of light and moisture. Excess of methyl iodide was distilled off and the residue extracted exhaustively with methyl alcohol. The extracts on concentration gave partially methylated strawberry polyester as a pale brown solid (4·6 g.). The partially methylated material was dissolved in alcohol and evaporated to dryness with the addition of a benzene solution of thallous ethoxide (5 equivs.). The resulting brown solid was powdered (120 mesh) and boiled with methyl iodide until the thallium iodide was neutral to litmus. Methylated strawberry polyester was isolated, as described above, as a stiff brown syrup (4·35 g.) (Found: OMe, 42·4%). The syrup was separated into two fractions by precipitation from a chloroform solution with ether. (A) Ether-insoluble (2·15 g.); $[\alpha]_D^{20°}$ + 144° (*c*, 0·66 in methyl alcohol) (Found: OMe, 39·9%. A dimethyl uronic methyl ester polymer requires OMe, 42·7%). (B) A stiff brown syrup obtained on evaporation of the ethereal extract (2·20 g.), which was not further examined.

Hydrolysis. The methylated polyester was extremely resistant to hydrolysis, but this could be effected by the use of methyl alcoholic hydrogen chloride under pressure. The material (2·10 g.) was dissolved in 3% methyl alcoholic hydrogen chloride (35 c.c.) and heated at 140° in a sealed tube for 24 hours. The hydrolysis mixture was nearly neutral and a considerable pressure developed in the tube owing to the formation of methyl chloride and carbon dioxide. The solution was neutralised with silver carbonate, filtered, and evaporated under diminished pressure to a dark brown syrup (1·85 g.). This was separated into two fractions by extraction with ether—(1) an ether insoluble-solid which consisted of incompletely hydrolysed material, and (2) an ether-soluble syrup consisting of the methyl ester of 2:3-dimethyl methyl-*d*-galacturonoside [1·5 g., $n_D^{18°}$ 1·473, $[\alpha]_D^{20°}$ − 27° (in acetone)]. This was fractionally distilled in a vacuum giving: (*a*) the methyl ester of 2:3-dimethyl methyl-*d*-galacturonoside (0·90 g.), b. p. 150°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4618, $[\alpha]_D^{21°}$ − 38° (*c*, 3·0 in water) (Found: OMe, 48·2; equiv., 254. Calc. for $C_{10}H_{18}O_7$: OMe, 49·6%; equiv., 250); (*b*) a fraction, b. p. 180°/0·01 (0·3 g.), $n_D^{19°}$ 1·4738, which was partly crystalline, the crystals which separated after tiling having m. p. 211° not raised on recrystallisation from ether; (*c*) still residue (0·3 g.).

The distilled ester (0·87 g.) was hydrolysed with N-hydrochloric acid (30 c.c.) at 90—95°: $[\alpha]_D^{21°}$ − 38° (initial value); + 31° (25 minutes); + 52° (1 hour); + 59° (1½ hours, constant value). The solution was neutralised with silver carbonate and filtered before and after the passage of hydrogen sulphide. Removal of the solvent at 40°/12 mm. left 2:3-dimethyl *d*-galacturonic acid (0·8 g.), $[\alpha]_D^{20°}$ + 63° (*c* 1·11 in water) (Found: OMe, 25·9: equiv., 212. Calc. for $C_8H_{14}O_7$: OMe, 27·9%; equiv., 222), which was oxidised with bromine water; the resultant 2:3-dimethyl *d*-mucic acid (0·8 g.) was isolated as a syrup, $[\alpha]_D^{21°}$ − 20° (*c*, 0·99 in methyl alcohol) (Found: OMe, 25·0; equiv., 122. Calc. for $C_8H_{14}O_8$: OMe, 26·9%; equiv., 119). The acid (0·70 g.) was esterified by boiling with 2% methyl alcoholic hydrogen chloride for 15 hours. The cooled solution was neutralised with silver carbonate, filtered, and evaporated to a syrup, which was extracted with ether. The solvent was distilled off, leaving the syrupy ester (0·72 g.), $n_D^{18°}$ 1·4688, $[\alpha]_D^{20°}$ − 5° (*c*, 0·78 in methyl alcohol) (Found: OMe, 38·8%), a portion of which (0·40 g.) was distilled under reduced pressure giving the methyl ester of the 1:4-lactone of 2:3-dimethyl *d*-mucic acid (0·33 g.), b. p. 160°/0·001 mm. (bath temp.), $n_D^{18°}$ 1·4658, $[\alpha]_D^{20°}$ − 40° (in water) (Found: equiv., 118). This crystallised on standing and was recrystallised from ether. It had m. p. 77—78°, raised to 96° on drying in a vacuum (Found, on a dried sample: C, 46·2; H, 5·8; OMe, 38·4. Calc. for $C_9H_{14}O_7$: C, 46·2; H, 6·0; OMe, 39·7%).

The ester lactone (0·26 g.), m. p. 96°, was oxidised with the calculated quantity of periodic acid in water (10 c.c.): $[\alpha]_D^{22}$ − 40° (initial value); − 10° (5 minutes); + 12° (12 hours, constant value). The solution was neutralised with barium carbonate, filtered, and concentrated to about 5 c.c. A small portion of the solution at this stage gave with casein and sulphuric acid a strong positive test for glyoxylic acid. Barium carbonate (1 g.) and bromine (1 c.c.) were added to the solution which became non-reducing towards Fehling's solution after 12 hours. The solution was aerated to remove excess of bromine and filtered. The filtrate was evaporated to dryness and the residue esterified by boiling with 2% methyl alcoholic hydrogen chloride (90 c.c.). The cooled solution was neutralised with silver carbonate and filtered, and the solvent evaporated at 60°/12 mm. The solid residue was exhaustively extracted with ether and the extracts were concentrated in a vacuum to a syrup (0·20 g., $n_D^{20°}$ 1·4348) which was distilled giving the dimethyl ester of *l*(+)-dimethoxysuccinic acid (0·15 g.), b. p. 90—110°/0·001 mm. (bath temp.), $n_D^{21°}$ 1·4322, $[\alpha]_D^{20°}$ + 77° (*c*, 2·46 in methyl alcohol) (Found: OMe, 58·2. Calc. for $C_8H_{14}O_6$: OMe, 60·2%). The ester (120 mg.) with methyl alcoholic ammonia gave needle-shaped crystals of the diamide of *l*(+)-dimethoxysuccinic acid (75 mg.), m. p. and mixed m. p. 280° (decomp.), $[\alpha]_D^{20°}$ + 96° (*c*, 0·73 in water).

Apple Pectin Polyester.—Apple pectic acid (Hirst and Jones, *J.*, 1939, 456) (uronic anhydride, 73·0; OMe, 10·2%; equiv., 250; $[\alpha]_D^{23°}$ + 230°) was converted by boiling with methyl alcoholic hydrogen chloride in the usual manner into the polyester which was isolated, as described above, as a snow-white powder. Yield, 65%; $[\alpha]_D^{20°}$ + 226° (in neutral aqueous solution) (Found: OMe, 17·4%; equiv., 195). A sample of the polyester was treated with the calculated quantity of barium hydroxide and the resulting barium salts were precipitated by alcohol. The white solid was filtered off, washed with alcohol, and dried in a vacuum (Found: Ba, 24·6; OMe, 1·5. A methylpolygalacturonoside methyl ester containing eight units requires Ba, 27·7; OMe, 1·6%).

Methylation. The polyester (6·0 g.) was dissolved in water (30 c.c.) and a hot solution of 2N-thallous hydroxide (100 c.c.) was added. The precipitated white thallium derivative was filtered off, washed with alcohol and ether, and dried at 60°/12 mm. The powdered solid (120 mesh) was boiled with methyl iodide under reflux with the exclusion of light and moisture until the solid no longer had an alkaline

reaction. The solution was worked up as described for the strawberry polyester. Two further methylations with thallous ethoxide solution gave methylated apple polyester as a syrup (5·4 g.). This was dissolved in chloroform and separated by the addition of ether into (*a*) a crisp brown solid {1·8 g., $[\alpha]_D^{20°}$ + 146° (in methyl alcohol); OMe, 39·9%}, and (*b*) a syrup which was not further examined.

Hydrolysis. Methylated apple polyester (1·10 g.) was dissolved in 4% methyl alcoholic hydrogen chloride (25 c.c.) and heated under pressure at 140—150° for 24 hours. The cooled solution was neutralised with silver carbonate, filtered, and evaporated to a syrup (0·95 g.) which was exhaustively extracted with ether. Evaporation of the ether left a syrup (0·80 g.), $n_D^{21°}$ 1·4842, $[\alpha]_D^{20°}$ − 15° (*c*, 1·0 in acetone), which was distilled in a vacuum giving: (1) 0·10 g., b. p. up to 130°/0·001 mm. (bath temp.), $n_D^{21°}$ 1·4805; (2) the methyl ester of 2 : 3-dimethyl methyl-*d*-galacturonoside (0·39 g.), b. p. 160°/0·001 mm. (bath temp.), $n_D^{19°}$ 1·4645, $[\alpha]_D^{20°}$ − 30° (*c*, 1·5 in water) (Found: OMe, 48·1%); (3) 0·1 g. of syrup which partly crystallised, b. p. 175—200°/0·001 mm.

Fraction (2) (0·37 g.) was hydrolysed with N-hydrochloric acid (25 c.c.): $[\alpha]_D^{20°}$ − 30° (initial value); + 47° (1½ hours); + 51° (3·75 hours, constant value). The cooled solution was neutralised with silver carbonate, and filtered before and after the passage of hydrogen sulphide. Evaporation of the solvent gave 2 : 3-dimethyl *d*-galacturonic acid (0·32 g.), $[\alpha]_D^{20°}$ + 35° rising to + 58° in 1 hour (*c*, 6·0 in water) (Found: OMe, 26·9; equiv., 226. Calc. for $C_8H_{14}O_7$: OMe, 27·9%; equiv., 222). The acid (0·30 g.) was dissolved in water (7 c.c.) and oxidised with bromine (1 c.c.) for 12 hours. The solution was then non-reducing. Bromine was removed by aeration and the solution neutralised with silver carbonate and filtered before and after the passage of hydrogen sulphide. Evaporation of the solution gave 2 : 3-dimethyl *d*-mucic acid (0·30 g.) which was converted into the methyl ester of the 1 : 4-lactone of 2 : 3-dimethyl *d*-mucic acid by boiling 3% methyl alcoholic hydrogen chloride (50 c.c., 7 hours). The solution was worked up in the usual manner and the crude ester lactone (0·31 g.) distilled giving the methyl ester of the 1 : 4-lactone of 2 : 3-dimethyl *d*-mucic acid (0·26 g.), b. p. 160°/0·001 mm. (bath temp.), $n_D^{20°}$ 1·4650. The distillate crystallised on nucleation with an authentic specimen of the ester lactone. Trituration with ether and filtration gave crystals (0·20 g.), m. p. and mixed m. p. with an authentic specimen, 96° (Found: OMe, 38·6%).

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