465. *Pectic Substances. Part VIII. The Araban Component of Sugar-beet Pectin.*

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Methylation of crude pectic material isolated from sugar beet gave a product from which could be separated material which closely resembled the methylated araban obtained from other plant pectins. On methanolysis, this methylated araban yielded in approximately equimolecular proportions 2 : 3 : 5-trimethyl methyl-L-arabinoside, 2 : 3-dimethyl methyl-L-arabinoside, and 2-methyl methyl-L-arabinoside. The ease of hydrolysis indicates that the polysaccharide is built up of L-arabofuranose residues and it appears to be identical in structure with the araban present in the peanut (Hirst and Jones, *J.*, 1947, 1221).

The presence in sugar beet of a polysaccharide containing L-arabinose residues was suggested some 75 years ago by Scheibler (*Ber.*, 1873, **6**, 612), who found that extraction of the beet with lime water yielded a solution of a gum which, on acidic hydrolysis, gave a good yield of L-arabinose. Later work by Ehrlich and Schubert (*Ber.*, 1929, **62**, 1974) confirmed this observation. Ehrlich considered that the araban was of comparatively low molecular weight, and Gaponenkov (*J. Gen. Chem. Russia*, 1937, **7**, 1729) reported that the polysaccharide had a molecular weight of the order of 6000. It was not, however, until 1945 that Ingelman (*Communications Swedish Sugar Corp.*, 1945, **1**, 179) detailed the actual isolation of the araban from sugar beet and described its physical properties. The optical rotation ($[\alpha]_D$ *ca.* $-130°$) suggested that this araban was closely similar to, if not identical with, the araban present in pectic materials isolated from other plant sources.

In the present experiments, attempts were made first of all to extract the araban by the methods detailed by earlier workers (Scheibler, *loc. cit.*), but difficulty was experienced in obtaining it in a sufficiently high degree of purity when fresh beet, or sugar beet chips from which the sucrose had been removed by extraction, were used as source. Earlier work on the pectic components of the peanut (Hirst and Jones, *J.*, 1938, 496; 1939, 452) had shown that, under the usual conditions of methylation with sodium hydroxide and methyl sulphate, the pectic acid component is largely destroyed, and that the araban component is converted into an insoluble methyl ether derivative much more rapidly than is the galactan component. Application of this methylation procedure to a sample of crude araban which had been isolated by extraction of sugar beet chips with lime water according to the method of Scheibler (*loc. cit.*) led to the isolation of a methylated araban. This material had $[\alpha]_D$ *ca.* $-150°$ in acetone, compared with *ca.* $-170°$ for the purest sample of methyl araban isolated from peanut araban, but somewhat lower figures have been obtained for other samples of methylated peanut araban and for methylated apple araban (Hirst and Jones, *J.*, 1939, 454). The presence of a small amount of contaminant was shown in that after methanolysis of the methylated beet araban there remained a small quantity of a difficultly hydrolysable residue, and it is probable that the difference in optical rotation of the samples of methylated araban is due to the presence of this impurity. It is clear that traces of methylated galactan or pectic acid, with their high positive rotations, would account for the variations.

Methanolysis of the methylated araban, followed by fractional distillation of the products, gave three fractions in approximately equimolecular proportions. These were (*a*) 2 : 3 : 5-trimethyl methyl-L-arabofuranoside, which was further characterised as the crystalline lactone and the crystalline amide of 2 : 3 : 5-trimethyl L-arabonic acid (Baker and Haworth, *J.*, 1925, 365); (*b*) 2 : 3-dimethyl methyl-L-arabinoside, identified as the crystalline anilide of 2 : 3-dimethyl L-arabinose and as the crystalline amide of 2 : 3-dimethyl L-arabonic acid (Smith, *J.*, 1939, 753); and (*c*) 2-methyl methyl-L-arabinoside characterised as the crystalline phenylhydrazone of 2-methyl L-arabinose and as the crystalline amide of 2-methyl L-arabonic acid (Hirst and Jones, *J.*, 1947, 1221). Confirmation of the presence of three methylated derivatives of arabinose was obtained from a study of the reducing sugars produced on hydrolysis of methylated araban. A solution of the sugar derivatives on examination on the paper chromatogram showed three widely separated spots of approximately equal intensity. The isolation of these three derivatives of L-arabinose in approximately equimolecular proportions shows that the araban possesses a branched chain structure. The isolation of the 2 : 3 : 5-trimethyl methyl-L-arabinoside proves that at least one-third of the pentose residues is in the furanose form. The high negative rotation of the methylated araban, coupled with its ease of methanolysis, indicates that in all probability all the L-arabinose residues are present as furanosides in the polysaccharide. The position reached in the delineation of the possible

structures which satisfy the experimental observations is exactly the same as in the case of peanut araban discussed earlier (Hirst and Jones, *loc. cit.*). It is clear that one-third of the arabinose residues are attached in the form A,1 . . . and therefore are end groups. Another set of arabinose residues is present in the form . . . 5A,1 . . ., linked through positions 1 and 5, and the remaining arabinose residues occur as $\cdots\overset{3}{5}$,A,1 . . ., linked through positions 1 and 3. It is clear also that sugar-beet pectin resembles the other pectins we have examined, derived from citrus fruit, peanut, and apple, in that it contains a polymer built up of L-arabofuranose residues which cannot arise by direct decarboxylation of the uronic acid residues in the pectic acid, which is intimately associated with the araban.

Experimental.

(All b. p.s recorded in distillations are bath temperatures.)

Sugar-beet chips, which had previously been extracted with water to remove sucrose, were used as the source of araban. The extracted material (500 g.) was mixed with water (4 l.) containing calcium hydroxide (100 g.) and the whole heated on the steam-bath for 12 hours. The slurry was filtered through cloth and the filtrate was centrifuged. The clear solution was then acidified with acetic acid, and the solution filtered to remove degraded pectic acid. On addition of alcohol, crude araban, contaminated with calcium acetate, was precipitated. The product was purified by dissolving it in water (250 c.c.); the insoluble material was filtered off, and the araban reprecipitated by the addition of alcohol. This crude product (*ca.* 5 g.) was used in subsequent experiments.

Acetylation.—The crude araban (1 g.) was suspended in pyridine (50 c.c.), and acetic anhydride (15 c.c.) added. The mixture was heated on the steam-bath for 6 hours, cooled, and the insoluble material removed on the centrifuge. Crude araban acetate was precipitated on pouring the pyridine solution into water. It was filtered off, washed with water, and purified by reprecipitation from acetone solution by the addition of water. The product (0·4 g.) had $[\alpha]_D^{20°}$ − 84° (*c*, 0·36 in acetone) (Found: OAc, 39. Calc. for a diacetyl araban: OAc, 37·2%). On deacetylation by the method of Zemplén and Kunz (*Ber.*, 1923, **56**, 1705), an araban (0·1 g.), $[\alpha]_D^{20°}$ − 114° (*c*, 0·4 in water), was isolated. Ingelman reports $[\alpha]_D$ − 129° for an araban isolated from sugar beet.

Methylation.—The crude araban (10 g.) was methylated with sodium hydroxide and methyl sulphate, and the crude product (6 g.) purified, after 6 methylations, by solution in acetone followed by filtration. Methylation of the acetone-soluble fraction was completed by boiling it with silver oxide and methyl iodide. The product (5·2 g.; OMe, 36·7%) was fractionated by addition of light petroleum (b. p. 40—60°) to its acetone solution. Fraction (I) was a yellow solid (2·4 g.), $[\alpha]_D^{20°}$ −148° (*c*, 1·3 in acetone) (Found: OMe, 36·2. Calc. for dimethyl araban: OMe, 38·8%). Fraction (II) was a yellow solid (1·5 g.), $[\alpha]_D^{20°}$ −146° (*c*, 0·55 in acetone) (Found: OMe, 36·8%). Fraction (III) (1·3 g.) was a sticky yellow solid which was not further examined.

Hydrolysis.—(a) *Qualitative.* Methylated araban [Fraction (I)] (12 mg.) was dissolved in methyl alcohol (1 c.c.) and 3N-hydrochloric acid (0·5 c.c.) added. The solution was heated at 70° for 12 hours. The temperature was then raised to 90° and the solution concentrated to *ca.* 0·2 c.c. A spot of the solution was withdrawn, mixed with one spot of ammonia (*d* 0·88), and the mixture of sugars separated on a paper chromatogram by the method of Partridge (*Nature*, 1946, **158**, 270), as used by Flood, Hirst, and Jones for methylated sugars (*ibid.*, 1947, **160**, 86) with a mixture of butanol (50%), ethanol (10%), and water (40%) for development. After 20 hours the paper was removed from the apparatus, dried in a steam-oven, and sprayed with ammoniacal silver nitrate. After removal of the solvent in the steam-oven, the position of the reducing sugars was indicated by the formation of a brown stain of silver. Three well separated spots of approximately equal intensity were produced, the presence of three different reducing sugars being thus indicated.

(b) *Quantitative.* Fraction (I) (2·18 g.) was boiled with methanolic hydrogen chloride (100 c.c.; 1% w/v) for 12 hours. It was not possible to follow the change in optical rotation, owing to darkening of the solution. The cooled solution was neutralised with silver carbonate and filtered, and the filtrate concentrated to a syrup (2·39 g.). This syrup was submitted to fractional extraction from aqueous solution in an all-glass continuous extraction apparatus (Brown and Jones, *J.*, 1947, 1344) with light petroleum (b. p. 39—40°) and then with ether, yielding (*a*) a portion soluble in light petroleum (1·38 g.), and (*b*) a portion soluble in ether (0·40 g.). The residue (0·4 g.) in the water was obtained on concentration of the aqueous solution. Portion (*a*) was distilled in a vacuum, giving Fraction (*A*) (0·52 g.), b. p. 90°/0·1 mm., $n_D^{20°}$ 1·4365 (Found: OMe, 59·4%). Portion (*b*) was added to the still residue and the distillation continued, giving Fraction (*B*) (0·67 g.), b. p. 120—130°/0·1 mm., $n_D^{20°}$ 1·4478 (Found: OMe, 49·7%). The water-soluble residue was added to the still residue and the distillation continued, giving Fraction (*C*) (0·46 g.), b. p. 130—140°/0·1 mm., $n_D^{20°}$ 1·4629 (Found: OMe, 38·4%), and Fraction (*D*) (0·31 g.), b. p. 140—160°/0·1 mm., $n_D^{20°}$ 1·4743 (Found: OMe, 34·0%). The still residue (0·28 g.) was not further examined.

Examination of the fractions. Fraction (*A*) (0·52 g.) was hydrolysed with boiling N-sulphuric acid; $[\alpha]_D^{20°}$ changed from −57° to −17° (6 hours; constant value). The syrupy sugar (0·45 g.), $n_D^{20°}$ 1·4568, was isolated by continuous extraction of the neutralised solution with chloroform (Found: OMe, 45·8%). On oxidation with bromine water this fraction gave in good yield crystalline 2 : 3 : 5-trimethyl L-arabonolactone, m. p. and mixed m. p. with an authentic sample, 29°. On reaction with liquid ammonia, the lactone was converted into 2 : 3 : 5-trimethyl L-arabonamide, m. p. and mixed m. p. with an authentic sample, 139°. The figures for the methoxyl content and the optical rotation show that this fraction could contain some 15% of 2 : 3-dimethyl L-arabinose.

Fraction (*B*) (0·67 g.) was boiled (6 hours) with N-sulphuric acid until the optical rotation had become constant; $[\alpha]_D^{20°}$ −48° ⟶ +76°. The reducing sugar (0·59 g.), $[\alpha]_D$ +82° in water, was isolated in the

usual manner (Found: OMe, 36·7%). On boiling a portion of the syrup with alcoholic aniline, 2 : 3-dimethyl L-arabinose anilide was produced, m. p. and mixed m. p., with an authentic sample, 138°. On oxidation with bromine water the sugar gave 2 : 3-dimethyl L-arabonolactone characterised as its crystalline amide, m. p. and mixed m. p. with an authentic sample, 162°. From the optical rotation and methoxyl content, it may be calculated that this fraction contains some 16% of 2 : 3 : 5-dimethyl L-arabinose.

Fraction (*C*) (0·45 g.) was hydrolysed with boiling N-sulphuric acid; $[\alpha]_D$ +5° ⟶ +8·4, yielding a syrup (0·4 g.), $[\alpha]_D^{20°}$ +90°, $n_D^{22°}$ 1·4702 (Found: OMe, 23·6%), which was a mixture of 2 : 3-dimethyl L-arabinose (25%) and 2-methyl L-arabinose (75%). On oxidation with bromine water, it yielded a lactone which partly crystallised after distillation and on nucleation with 2 : 3-dimethyl L-arabonolactone. The crystals were separated on a porous plate and were purified by recrystallisation from ether–light petroleum. They then had m. p. 34°, not depressed on admixture with an authentic sample.

Fraction (*D*) (0·31 g.), on hydrolysis with N-sulphuric acid, yielded a syrup (0·22 g.), $[\alpha]_D$ +100° (Found: OMe, 19·6%); this, on being heated with alcoholic phenylhydrazine, yielded 2-methyl L-arabinose phenylhydrazone, m. p. 116°, not depressed on admixture with an authentic specimen. The methoxyl content indicated that this fraction is a pure monomethyl pentose.

	Wt. of L-arabinose derivatives (g.) calcd. as		
Fraction.	2 : 3 : 5-Trimethyl methyl-L-arabinoside.	2 : 3-Dimethyl methyl-L-arabinoside.	2-Methyl methyl-L-arabinoside.
(*A*)	0·465	0·055	—
(*B*)	0·107	0·563	—
(*C*)	—	0·065	0·385
(*D*)	—	—	0·310
Total	0·572	0·683	0·695
Ratio in g.-mols.	0·8	1·0	1·1

The quantitative data relating to the hydrolysis products obtained from Fraction (I) of the methylated araban are collected together in the Table, which shows the amounts of 2 : 3 : 5-trimethyl methyl-arabinoside, 2 : 3-dimethyl methylarabinoside and 2-methyl methylarabinoside contained in the Fractions (*A*), (*B*), (*C*), and (*D*). The three sugars are present in the mixture of hydrolysis products in approximately equimolecular proportions.

Fraction (II) (1·44 g.) was hydrolysed by boiling it with methanolic hydrogen chloride (50 c.c., 4%) for 20 hours; ($[\alpha]_D^{20°}$ −20°, constant value). The cooled solution was treated with diazomethane and concentrated to a syrup under reduced pressure. The residual yellow syrup (1·6 g.) was then dissolved in water, and some insoluble brown material was filtered off. The filtrate was made up to 50 c.c. with water and extracted with light petroleum (b. p. 40—60°) in a double extractor of the type described by Brown and Jones (*loc. cit.*). Very little methylated sugar was being removed from the aqueous solution after extraction for 24 hours, and at this stage the light petroleum solution was evaporated to a syrup (*X*, 0·63 g., $n_D^{20°}$ 1·4360) and extraction of the aqueous solution was continued with ether. 24 Hours' extraction sufficed to remove all the ether-soluble material (*Y*, 0·41 g.). Concentration of the residual aqueous solution left a syrup (*Z*, 0·51 g.).

These fractions were further purified by distillation in a vacuum. Fraction (*X*) was distilled, yielding fraction (*E*) (0·48 g.), $n_D^{20°}$ 1·4358, b. p. 100°/0·1 mm. (Found: OMe, 60%). Fraction (*Y*) was then added, and the distillation continued, yielding fraction (*F*) (0·51 g.), b. p. 130°/0·1 mm., $n_D^{20°}$ 1·4568 (Found: OMe, 49·2%). The still residue (80 mg.) was added to the syrup obtained on concentration of the residual aqueous solution and purified by distillation, giving fraction (*G*) (0·47 g.), b. p. 170°/0·1 mm., $n_D^{20°}$ 1·4738 (Found: OMe, 35·2%). The still residue (0·11 g.) was not further examined.

When Fraction (*E*) (0·47 g.) was hydrolysed with N-sulphuric acid, $[\alpha]_D$ changed from −83° to −40° (constant value). The syrupy sugar (0·4 g.), isolated in the usual manner, was oxidised with bromine water, and the resultant 2 : 3 : 5-trimethyl L-arabonolactone, m. p. 30° $[\alpha]_D$ −46° (*c*, 1·31 in water; initial value), was isolated in the manner already described (yield, 0·41 g.) (Found: OMe, 48·7; equiv., 188. Calc. for $C_8H_{14}O_5$: OMe, 48·9%; equiv., 190).

Fraction (*F*) (0·5 g.) was hydrolysed by boiling with N-sulphuric acid; $[\alpha]_D$ −17° (initial value) rising to +90° (constant value). A sample (0·1 g.) of the sugar (0·44 g., $[\alpha]_D$ +96°) gave, on boiling with alcoholic aniline, 2 : 3-dimethyl L-arabinose anilide. m. p. 138°, not depressed on admixture with an authentic specimen. The sugar (0·2 g.), on oxidation with bromine water, was converted into 2 : 3-dimethyl L-arabonolactone, ($[\alpha]_D$ −38°, falling to −27°; *c*, 1·1 in water), characterised as the crystalline 2 : 3-dimethyl L-arabonamide, m. p. and mixed m. p. with an authentic sample, 162°.

Fraction (*G*) (0·4 g., $[\alpha]_D$ −10° in water) was hydrolysed with boiling N-sulphuric acid until the rotation remained constant ($[\alpha]_D$ +94° after 6 hours). The sugar thereby obtained (0·2 g.) (Found: OMe, 19·1%) gave, with alcoholic phenylhydrazine, a poor yield of 2-methyl L-arabinose phenylhydrazone, m. p. and mixed m. p. with an authentic sample, 114°. No crystalline anilide could be obtained. Oxidation of a sample of the sugar (0·1 g.) with bromine water gave a lactone, $[\alpha]_D$ −44° ⟶ −40° (48 hours) (Found: OMe, 19; equiv., 164. Calc. for $C_6H_{10}O_5$: OMe, 19·1%; equiv., 162). The lactone with alcoholic ammonia gave in small yield 2-methyl L-arabonamide, m. p. 131°, $[\alpha]_D$ +52° (*c*, 0·7 in water) (Found: OMe, 17·1%).

Examination of the constants of fractions (*E*), (*F*), and (*G*) indicate that methylated polysaccharide [Fraction (II)] yields 2 : 3 : 5-trimethyl methyl-L-arabinoside (0·51 g.), 2 : 3-dimethyl methyl-L-arabinoside (0·48 g.), and 2-methyl methyl-L-arabinoside (0·47 g.) in approximately equimolecular proportions.

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