103. *Pectic Substances. Part III. Composition of Apple Pectin and the Molecular Structure of the Araban Component of Apple Pectin.*

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The properties of pectin obtained from apple pomace have been studied and evidence has been obtained in favour of the view that this pectin is a mixture containing araban, galactan, and the methyl ester of pectic acid. The pectic acid is composed mainly, if not entirely, of anhydrogalacturonic acid residues. The araban portion has been isolated in the form of its methylated derivative, which on hydrolysis gives rise, in equimolecular proportions, to 2 : 3 : 5-trimethyl *l*-arabinose, 2 : 3-dimethyl *l*-arabinose, and 3-methyl *l*-arabinose. Proofs of the structure of these sugars are given and it appears that all three are combined in the polysaccharide in the furanose form. The constitution of the araban present in apple pectin is discussed in the light of the above observations and it is shown that in the main features of its structure this araban is identical with the araban which can be isolated from the pectic materials of the pea-nut.

It is well established that pectins of various origin contain pentosan constituents and amongst these, derivatives of *l*-arabinose are of common occurrence. We have shown in previous papers (J., 1938, 496; preceding paper) that the polysaccharides present in the pea-nut include an araban which is found in close association with pectic acid and we have undertaken structural investigations on a series of arabans obtained from various natural sources with the object of ascertaining whether or not the same type of chemical constitution is common to them all. The present paper is concerned with the composition of apple pectin and the structure of its araban constituent. The evidence now presented indicates that this araban and the one present in the pectic material of the pea-nut possess the same fundamental chemical structure.

The apple pectin required for the investigation was extracted from cider apple pomace and was obtained in the form of a cream-coloured powder, analysis of which indicated the presence of anhydrogalacturonic acid residues (49·2%), araban (20%), and galactan (*ca.* 30%). The method of extraction used was such that the pectic acid remained in the form of its methyl ester and the pectin was freely soluble in water, giving viscous solutions. Evidence has been obtained in favour of the view that the above-mentioned constituents of apple pectin are not in chemical combination with each other and we are therefore in general agreement with the views of Schneider and Bock (*Ber.*, 1937, **70**, 1617) and of Norris and Resch (*Biochem. J.*, 1937, **31**, 1950) concerning the mode of occurrence of the pentosans and other carbohydrates which are found in pectic substances. In this connexion a comparison may be made between the apple pectin described in this paper and the corresponding material prepared by the latter authors (*loc. cit.*). It is apparent that the use of different methods of extraction and purification has resulted in the isolation of substances having widely different properties.

	OMe, %.	Uronic anhydride, %.	Anhydroarabinose, %.
Apple pectin (present work)	9·5	49·2	18
Apple pectin (Norris and Resch) ...	10·0	76·2	10·4

When the apple pectin was extracted by hot 70% alcohol, the pectic acid remained almost entirely undissolved and the solution contained a mixture of araban and galactan. Attempts to separate the araban and the galactan, either in the free state or as their acetyl derivatives, were unsuccessful, but this separation was effected by methylation in alkaline solution (see experimental section), methylated araban then being isolated. It follows that neither the araban nor the galactan is chemically combined with the pectic acid and furthermore the araban is not chemically bound to the galactan. The action of dilute acid on the araban–galactan mixture resulted in hydrolysis of the pentosan, leaving the galactan. Indeed the araban is by far the most readily attacked by hydrolytic agents of any of the components of apple pectin and by treatment with N/20-mineral acid at 95—100° it is possible to remove the whole of the pentosan material, which is transformed

into *l*-arabinose, the methyl pectate and the portion containing the galactose residues remaining almost unaltered. This observation, coupled with the behaviour of methylated araban on hydrolysis, provides strong evidence that all the arabinose residues are present in the furanose form. On the other hand, since the galacturonic acid and galactan residues are highly resistant to hydrolysis and possess large positive rotations, they are probably pyranose in structure.

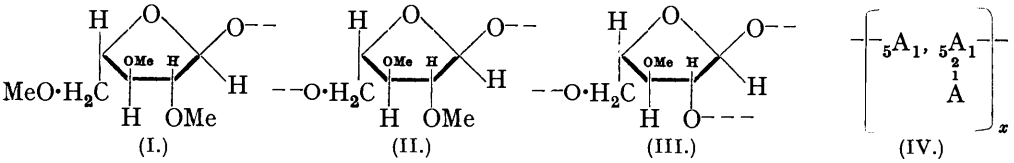
After removal of the araban portion of the pectin by preferential hydrolysis there remained a methyl pectate–galactan complex the properties of which are given under (B) in Table I of the experimental section. From this the galactan was removed by forming the sodium salt of the pectic acid and precipitating the free acid. After several repetitions of this process a pectic acid was obtained which had an equivalent weight of 185, $[\alpha]_D^{20^\circ}$ +276°, and a uronic anhydride content of 96·7%. This represents the closest approach we have yet made to a pure polygalacturonic anhydride of equivalent weight 176. Whether the remaining 3—4% consists of other organic material, for example, galactan, or whether it consists of closely bound water, difficult to remove by ordinary drying, is not yet certain. It has, however, been observed that some poly-uronic anhydrides do retain water very tenaciously, as is shown by the work of Pauli and Sternbach (*Kolloid-Z.*, 1938, **84**, 291) on the viscous form of alginic acid, which could not be dehydrated beyond the stage represented by $(C_6H_8O_6,H_2O)_n$.

The araban is stable towards alkali under conditions which produce extensive decomposition of the pectic acid. We accordingly made use of this fact to obtain in a pure condition the methyl derivative of the araban. When the apple pectin was heated with methyl sulphate and sodium hydroxide, the pectic acid was largely destroyed, the galactan was comparatively resistant to methylation, and the product consisted mainly of methylated araban. Methylation was completed by the use of thallium ethoxide and methyl iodide (see Part I, *loc. cit.*). After purification of the product by fractional precipitation from solvents, a fully methylated substance was obtained with properties closely similar to those of the methylated araban from the pea-nut. The structural identity of the two methylated derivatives was shown by the behaviour of the new methylated araban on hydrolysis. An equimolecular mixture of three different methylated arabinoses was obtained, in the form of their methyl glycosides, when the methylated araban was heated with methyl-alcoholic hydrogen chloride and the products were separated by fractional distillation. The identity of the first of these, 2 : 3 : 5-trimethyl *l*-arabo-furanose, was fully substantiated by its oxidation to the crystalline γ-lactone of 2 : 3 : 5-trimethyl *l*-arabonic acid, from which the corresponding characteristic amide was prepared. The second sugar was 2 : 3-dimethyl *l*-arabinose and the identity of this followed from the following considerations. The free sugar had a high positive rotation and was present therefore in the pyranose form. On oxidation by bromine water, the sugar gave in good yield the γ-lactone of 2 : 3-dimethyl *l*-arabonic acid, the identity of which was confirmed by its transformation into the corresponding crystalline amide (Hirst and Jones, *loc. cit.*). From these observations it is clear that in the dimethyl sugar there can be no attachment of methoxyl groups at C_4 or C_5, and it must therefore be 2 : 3-dimethyl *l*-arabinose.

As was the case also with the corresponding sugar from methylated pea-nut araban, difficulties were encountered in providing a rigid proof of the constitution of the third hydrolysis product owing to the fact that crystalline reference compounds could not be obtained. There is, however, strong evidence pointing to the identification of the sugar as 3-methyl *l*-arabinose, this structure being ascribed on the basis of the following observations. The sugar is a monomethyl arabinose with a strong positive rotation; the methyl group is not, therefore, attached to the hydroxyl at C_5. It gives an *iso*propylidene derivative by condensation with acetone and it yields a γ-lactone when oxidised by bromine water. Furthermore, this lactone gives rise to a monomethyl *l*-arabonamide, which gives a strong positive Weerman test, the yield of sodium cyanate produced being similar to that normally given by α-hydroxy-amides of the sugar group. It follows that there are free hydroxyl groups attached at C_2 and at C_4, and the sugar must be 3-methyl *l*-arabinose. No evidence could be obtained of the presence of isomeric monomethyl derivatives.

The specific rotation, $[\alpha]_D$ — 86° in methyl alcohol, of the methylated araban, together

with the ease of hydrolysis and the nature of the hydrolysis products, indicates that all the arabinose residues in the polysaccharide are furanose in structure and probably of the α-configuration. It appears, therefore, that the hydrolysis products are identical with those obtained from methylated pea-nut araban, and consequently the structural problem is the same as that considered in our former paper. The two arabans appear to be identical in their fundamental chemical structure, the principal features of which have now been ascertained. The methylated polysaccharide consists of a main chain with branches, the trimethyl *l*-arabofuranose residues (I) being the terminal units of the branch chains. In addition, the repeating unit contains a dimethyl *l*-arabofuranose residue (II) joined to



(I.) (II.) (III.) (IV.)

other arabofuranose residues through the hydroxyl groups at C_1 and C_5, and a third *l*-arabofuranose residue (III) attached to other residues through hydroxyl groups at C_1, C_2, and C_5. It is of special interest that in this araban we have a further example of a natural substance in which sugar residues are linked together glycosidically through positions 1 and 2. At present no evidence is available concerning the molecular or particle weight of these arabans, nor is it possible to differentiate between the various possible ways of building up a polysaccharide from the residues mentioned above. One such structure is indicated above (IV), the symbols used being those adopted in our earlier paper, in which will be found a fuller discussion of the various possibilities. Work on the comparative molecular sizes of arabans from different sources will be reported in a later communication.

Experimental.

Preparation and Examination of Apple Pectin.—The pectin was obtained from cider apple pomace, for a supply of which we wish to express our thanks to Professor B. T. P. Barker and Mr. V. L. S. Charley of the Long Ashton Research Station. The pomace was stirred for 5 hours with boiling water and, after cooling, the mixture was pressed in a linen bag and the liquid poured into three times its volume of methylated spirit containing 1% of hydrochloric acid. The precipitated pectin was filtered off and triturated with methylated spirit until free from hydrochloric acid. It was then dried in a vacuum oven at 40°. Further purification was effected by solution of the pectin in water, filtration through a bed of kieselguhr, and precipitation with alcohol. Apple pectin so prepared was a nearly colourless powder, soluble in water. The method of preparation was such that the methyl ester groupings remained intact and in consequence the pectin showed a high methoxyl content. It was analysed by the standard procedures and the results are collected in the accompanying table. (Substance A).

Table I.

Substance	Apple pectin (A)	Methyl pectate–galactan complex (B)	Pectic acid (C)	Araban–galactan mixture (D)
Equiv. wt.	364	250	185	Not acidic
$[\alpha]_D$ in water	+178°	+230°	+276°	−30°
OMe, %	9·5	10·2	nil	nil
Yield of furfural, % (when boiled with 12% HCl)	19·9	16·4	—	28
Uronic anhydride, % (from yield of CO_2 on boiling with hydrochloric acid)	49·2	73	96·7	nil
Pentosan, % (calc. as araban)	20	nil	nil	54
Galactan, % (approx. only: estimated by difference)	25	21	(<4)	46

These figures indicate that the galacturonic acid residues in this sample of apple pectin are present almost exclusively as the methyl ester, an observation which serves to explain the ready solubility of the pectin. From the figures for furfural and uronic anhydride it appears

that the amount of pentosan present is *ca.* 20%. The pentosan portion was removed by gentle hydrolysis of the pectin by N/20-sulphuric acid at 90° for 4 hours. Reducing sugar was liberated and the viscosity of the solution greatly diminished. The filtered solution was poured into alcohol and the insoluble pectic ester–galactan complex (B) was washed until free from sulphuric acid. The alcoholic solution contained free arabinose, identified as the diphenylhydrazone, m. p. 196° (yield of reducing sugar, *ca.* 20% of weight of pectin). No sugar other than *l*-arabinose was identified, but it is not impossible that others may have been present in small quantity. The pectic ester–galactan complex had $[\alpha]_D$ + 230° (*c*, 1·48 in water). It is somewhat remarkable that the methyl ester group had not hydrolysed during the treatment with acid (Found: OMe, 10·2%). The equiv. wt. of (B) (by titration) was 250—255, in good agreement with the uronic anhydride content (73%) estimated in the usual manner. Furfural: 16·4, equivalent to the presence of 74% of uronic anhydride. These figures show that the whole of the pentosan had been removed. The complex (B) gave 44% of mucic acid on oxidation with nitric acid, but the quantitative significance of this figure is doubtful, in view of the uncertainty as to the yield of mucic acid from polysaccharides which hydrolyse with difficulty.

Preparation of Pectic Acid.—The pectic ester–galactan complex (B) (3·6 g.) was dissolved in N-sodium hydroxide, and the pectic acid precipitated from dilute solution (600 c.c.) by hydrochloric acid, washed with water, reprecipitated four times from the calculated quantity of dilute sodium hydroxide solution, and then converted into the sodium salt, which was dissolved in water (600 c.c.). Addition of calcium chloride solution gave the insoluble calcium salt, which was washed free from calcium chloride and decomposed by trituration with alcoholic hydrogen chloride. The resulting acid was once more dissolved in sodium hydroxide solution and precipitated as pectic acid (C) (Table I), which was washed with water and then with alcohol and dried at 100°/12 mm.; $[\alpha]_D^{20°}$ + 276° (*c*, 1·8 in dilute sodium hydroxide solution), equiv. wt. 185 (calc., 176), uronic anhydride (from carbon dioxide liberated on boiling with 12% hydrochloric acid) 96·7%.

Extraction of Apple Pectin with 70% *Alcohol.*—The araban portion could be partly removed along with some galactan by repeated extraction of the pectin (A) with hot 70% alcohol. Six extractions of 90 g. of pectin with 800 c.c. portions of 70% alcohol gave a solution of the polysaccharides, which were precipitated as a light yellow solid (D) (3·2 g.) by addition of a large volume of acetone. The solid was very soluble in water, giving a neutral opalescent solution; it had $[\alpha]_D^{20°}$ − 30° (*c*, 0·9 in water) and contained no acid or ester group. On boiling with 12% hydrochloric acid under the standard conditions it gave furfural corresponding to the presence of 54% of araban. On oxidation with nitric acid (*d* 1·2), it gave mucic acid in a yield of 14·5%, indicating the presence of at least 25% of galactan.

Hydrolysis of Araban–Galactan Mixture (*D*).—The mixed polysaccharides (D) (0·40 g.) underwent partial hydrolysis on heating at 90—95° with N/20-sulphuric acid (40 c.c.). $[\alpha]_D^{18°}$ − 30° (initial value); + 8·0° (1 hr.); + 46·0° (2 hrs.); + 78° ($3\frac{3}{4}$ hrs.); + 94° (6 hrs., constant value). The increase in the amount of reducing sugar was followed by the method of Baker and Hulton: Iodine titre (in c.c. of N/10-iodine per 0·40 g. of polysaccharide), 2·6 (initial value); 12·0 (1 hr.); 18·0 (2 hrs.); 28·0 ($3\frac{3}{4}$ hrs.); 38·0 (6 hrs., constant value). The last figure is equivalent to hydrolysis of 62·0% of the polysaccharide (calculated on the assumption that only pentose sugars are liberated). The cooled solution was neutralised with barium carbonate, filtered, and concentrated to a syrup, which was extracted with methyl alcohol to remove reducing sugars. The alcohol-insoluble polysaccharide had a high positive rotation and contained galactose, identified after oxidation to mucic acid. Concentration of the alcoholic extract gave a syrup (0·20 g.), which had $[\alpha]_D^{20°}$ + 99·0° (*c*, 0·7 in water) and contained 85% of *l*-arabinose, estimated and identified as *l*-arabinose diphenylhydrazone, m. p. and mixed m. p. 195°. These figures indicate the possibility of effecting complete removal of the araban from the galactan by preferential hydrolysis.

Acetylation of Araban–Galactan Mixture (*D*).—The mixture of araban and galactan (0·29 g.), on acetylation with pyridine (2 c.c.) and acetic anhydride (1 c.c.), gave a mixture of acetates (0·40 g.) which could not be separated by fractionation. This had $[\alpha]_D^{20°}$ − 43·6° (*c*, 1·0 in acetone) and was not fully acetylated, since it contained only 29·2% of $CO{\cdot}CH_3$ (calc. for araban diacetate, 39·8%). On distillation with 12% hydrochloric acid under the standard conditions, the acetate gave an amount of furfural equivalent to the presence of 47·0% of an araban acetate (of the above acetyl content). It follows that no effective separation of the galactan and araban constituents had taken place during acetylation. It is of interest to note for comparison that pure araban acetate obtained from the pea-nut has $[\alpha]_D^{20°}$ − 90° (*c*, 0·74 in acetone).

Methylation of Apple Pectin. Preparation of Methylated Araban.—Apple pectin (in portions

of 20 g.) was dissolved in the minimum quantity of aqueous sodium hydroxide and to the solution, vigorously stirred in an atmosphere of nitrogen, were added at room temperature at intervals of an hour five separate portions of methyl sulphate (25 c.c.) and 30% sodium hydroxide solution (45 c.c.); stirring was then continued for several hours, the temperature raised to 60°, and another five additions of reagents made at hourly intervals. After a final hour at 100°, the mixture was cooled and after neutralisation of the excess of alkali the sodium sulphate was separated from the organic matter by dialysis against running water in large parchment bags. The dialysed solution was concentrated at 60° under diminished pressure and the product was remethylated in the above way except that the temperature was kept throughout at 50°. This time dialysis was not necessary, since on neutralisation the methylated product separated from the hot solution. It was rapidly filtered off, washed with boiling water, and dissolved in acetone, the filtered solution evaporated to dryness, and the product dissolved in methyl alcohol. Some inorganic material was then removed in the centrifuge and the solution was evaporated to dryness at 50° under diminished pressure. The product (6·8 g. from 60 g. of pectin) was dissolved in the minimum quantity of absolute ethyl alcohol, and a slight excess of a benzene solution of thallium ethoxide added (100 c.c. of 1·12N-thallium ethoxide). The solution was evaporated at 50° under diminished pressure, and the dry solid powdered (caution: during this operation rubber gloves should be worn, and the utmost care taken against inhaling any of the powder), and boiled for 60 hours with methyl iodide. The excess of methyl iodide was recovered by distillation, and the product exhaustively extracted with boiling methyl alcohol. Removal of the solvent left 4·6 g. of methylated polysaccharide slightly contaminated with colloidal thallous iodide. The treatment with thallous ethoxide in benzene was repeated and finally the product was methylated with silver oxide and methyl iodide by the standard procedure. This effected little if any additional methylation, but served to free the product from traces of colloidal thallous iodide, which are extremely difficult to remove. The crude methylated product (4·3 g.) at this stage had $[\alpha]_D$ — 63° in methyl alcohol and OMe, 35·6%.

The solid methylated product was dissolved in acetone, the filtered solution concentrated, and by successive additions of light petroleum (b. p. 40—60°) three fractions of methylated polysaccharide obtained: (1) a brown solid (0·45 g.), $[\alpha]_D$ + 5° in methyl alcohol (*c*, 0·6) (OMe, 33·4%); (2) a light yellow solid (3·40 g.), $[\alpha]_D$ — 84° in methyl alcohol (*c*, 0·5) (OMe, 39·6%); (3) a viscid yellowish mass (0·30 g.), not further investigated. Fractions (1) and (2) were combined and boiled with N-sodium hydroxide (10 c.c.). After carbon dioxide had been passed through it, the solution was evaporated in a vacuum, and the methylated araban extracted from the solid residue with acetone, leaving the methylated polyuronide present to some degree in fraction (1). On evaporation of the acetone, *methylated araban* (2·85 g.) was obtained as an almost colourless powder having $[\alpha]_D^{20°}$ — 86° in methyl alcohol (*c*, 1·7) (Found: C, 52·8; H, 7·5; OMe, 38·4. $C_7H_{12}O_4$ requires C, 52·5; H, 7·6; OMe, 38·8%). It was soluble in acetone.

Methylated araban (2·70 g.) was boiled with 2% methyl-alcoholic hydrogen chloride for 20 hours. The solution was then neutralised with silver carbonate, filtered, and concentrated at ordinary pressure to a syrup (3·0 g.), which was fractionally distilled, giving (A) 1·22 g., bath temp. 100°/0·002 mm., $n_D^{20°}$ 1·4382 (Found: OMe, 56·4%); (B) 0·65 g., bath temp. 140°/0·002 mm., $n_D^{20°}$ 1·4580 (Found: OMe, 46·6%); (C) 0·74 g., bath temp. 150—200°/0·002 mm., $n_D^{20°}$ 1·4735 (Found: OMe, 38·5%); (D) still residue, 0·4 g. Estimations based on n_D and OMe values of the above fractions indicated that their compositions were approximately as follows:

	Trimethyl methyl-arabofuranoside, g.	Dimethyl methyl-arabofuranoside, g.	Monomethyl methyl-arabofuranoside, g.
Fraction A	0·98	0·24	—
Fraction B	—	0·52	0·13
Fraction C	—	0·18	0·56

These yields correspond respectively to 92% of the calculated amount of trimethyl sugar, 94% of the calculated amount of dimethyl sugar, and 75% of the calculated amount of monomethyl sugar, estimated on the assumption that the methylated araban gives on hydrolysis equimolecular proportions of tri-, di-, and mono-methyl arabinose. Since some monomethyl derivative unavoidably remained in the still residue, owing to the high temperature required for distillation, these figures are held to be in agreement with the view that this sample of methylated araban, like the methylated araban from pea-nuts (Hirst and Jones, *loc. cit.*), does give these three methylated arabinoses in equimolecular proportions. Proof of the identity of the three hydrolysis products was obtained in the following ways.

2 : 3 : 5-*Trimethyl Arabinose*.—Fraction (A) (1·02 g.) was hydrolysed by 0·5N-hydrochloric acid (50 c.c.) at 90—95°; $[\alpha]_D^{21°}$ — 60° (initial value), changing after 1 hour to the constant value — 11°. The mixed free sugars on isolation in the usual way (0·85 g.) had $n_D^{19°}$ 1·4578, $[\alpha]_D^{21°}$ — 12° in water (OMe, 44·3%). The methoxyl value and the rotation value (since 2 : 3 : 5-trimethyl arabinose has $[\alpha]_D$ — 42° and 2 : 3-dimethyl arabinose has $[\alpha]_D$ + 106°) confirm the estimate given above of the composition of fraction (A). The mixed sugars (0·83 g.) were fractionally distilled, giving a distillate (0·61 g.), bath temp. 140°/0·002 mm., $n_D^{20°}$ 1·4510, $[\alpha]_D^{21°}$ — 32° in water (*c*, 0·8) (Found: OMe, 49·0. Calc. for $C_8H_{16}O_5$: OMe, 48·5%). This was 2 : 3 : 5-trimethyl arabinose in almost pure condition (purity 95%, calculated from the rotation value). On oxidation with bromine water for 6 hours at 60°, it was transformed in excellent yield into crystalline 2 : 3 : 5-trimethyl γ-arabonolactone, b. p. (bath temp.) 115—120°/0·002 mm., $n_D^{20°}$ 1·4460 (superfused solid), m.p. 30° (before recrystallisation, not raised by recrystallisation and not depressed on admixture with an authentic sample), $[\alpha]_D^{20°}$ — 44° in water (*c*, 0·7), falling to — 34° after 70 hours (mutarotation still unfinished) (Found: equiv. by titration, 193; OMe, 48·0. Calc.: equiv., 190; OMe, 48·9%). On treatment with liquid ammonia, the lactone gave quantitatively 2 : 3 : 5-trimethyl arabonamide, m. p. 139° alone or when mixed with an authentic specimen.

The syrup (0·22 g.) remaining after removal of the trimethyl arabinose, $n_D^{20°}$ 1·4720, was almost entirely dimethyl arabinose and was mixed with the syrup (0·55 g.), $n_D^{23°}$ 1·4728, $[\alpha]_D^{22°}$ + 100° in water (*c*, 1·5) (Found: OMe, 34·0. Calc. for dimethyl arabinose: OMe, 34·8%), obtained by the hydrolysis of fraction B (0·62 g.) (see above) by N/2-hydrochloric acid (40 c.c.) at 95° for 3 hours (initial rotation of fraction B before hydrolysis, $[\alpha]_D^{20°}$ + 14°). The combined syrups (0·75 g.) were oxidised by bromine water at 60° for 6 hours. The product, isolated in the usual way, gave on distillation 2 : 3-dimethyl γ-arabonolactone (0·53 g.), b. p. (bath temp.) 150—160°/0·002 mm., $n_D^{21°}$ 1·4590, $[\alpha]_D^{22°}$ — 32° in water (*c*, 1·0), initial value; — 26° after 58 hours (mutarotation still not completed) (Found: OMe, 35·7. Calc. for $C_7H_{12}O_5$: OMe, 35·2%). On treatment with liquid ammonia this lactone gave quantitatively 2 : 3-dimethyl *l*-arabonamide, m. p. 159—160° alone or when mixed with an authentic sample (compare Hirst and Jones, *loc. cit.*), $[\alpha]_D^{20°}$ + 17° in water (*c*, 3·0), + 23° in ethyl alcohol (*c*, 0·9).

The identity of the monomethyl sugar was established by examination of fraction C. This (0·71 g.) was hydrolysed by heating at 90° for 4 hours with N/2-hydrochloric acid, the rotation changing from $[\alpha]_D$ + 74° to + 94°. The product (0·67 g., mixture of dimethyl and monomethyl arabinose) had $n_D^{21°}$ 1·4852, $[\alpha]_D$ + 100° in water (*c*, 1·2). It was shaken for 80 hours with acetone (50 c.c.) containing concentrated sulphuric acid (1 c.c.) and anhydrous copper sulphate (10 g.). Excess of anhydrous potassium carbonate was then added and the filtered solution was evaporated to a fairly mobile syrup (0·84 g.), which was fractionally distilled, giving (E) (0·41 g.), bath temp. 120—150°/0·002 mm., $n_D^{20°}$ 1·4670; (F) (0·19 g.), bath temp. up to 190°/0·002 mm., $n_D^{16°}$ 1·4700 (OMe, 36%). (F) was mainly the dimethyl arabinose present in fraction (C), and (E) consisted mainly of the monoacetone derivative of the monomethyl arabinose. On hydrolysis with N/10-hydrochloric acid at 90° for 6 hours, the rotation altered from $[\alpha]_D$ + 25° to + 74°. Worked up in the usual way, 3-monomethyl arabinose was obtained as a viscid syrup (0·32 g.), $[\alpha]_D^{20°}$ + 98° in water (*c*, 1·3) (Found: OMe, 20·0. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). The sugar (0·29 g.) was oxidised by bromine water at 60° for 6 hours, giving a syrupy lactone (0·25 g.) which still contained some unoxidised sugar as shown by its slight reducing power, b. p. (bath temp.) 190°/0·002 mm., $n_D^{22°}$ 1·4790, $[\alpha]_D^{21°}$ ± 0°, rising to + 5° after 137 hours (mutarotation incomplete). It behaved on titration as a stable lactone, equiv. wt. 180 (calc., 162) (Found: OMe, 20·0. Calc. for $C_6H_{10}O_5$: OMe, 19·3%). The behaviour was therefore that of a γ-lactone and the different rotations shown by this substance and by the 3-methyl γ-arabonolactone described by Hirst and Jones (*loc. cit.*) are ascribable to the presence in this sample of some unoxidised monomethyl arabinose with high positive rotation (+ 98°). The high positive rotation of the free sugar and the low rotation and slow, positively directed mutarotation of the lactone indicate that the methyl group is situated neither at C_4 nor at C_5. The lactone gave with liquid ammonia an amorphous amide (Found: OMe, 19·1. Calc. for $C_6H_{13}O_5N$: OMe, 17·3%), which gave a strong positive Weerman reaction on treatment with alkaline sodium hypochlorite under the standard conditions. On addition of semicarbazide in acetic acid, the yield of hydrazodicarbonamide, m. p. and mixed m. p. 256°, was 5·2 mg. from 21 mg. of amide. Under identical conditions, *d*-mannonamide (20·0 g.) gave 5·6 mg. of hydrazodicarbonamide and similar yields are given by other α-hydroxy-amides of the sugar series. It appears, therefore, that the amide now under discussion must have consisted of an α-hydroxy-amide to the extent of at least 75%. It follows that the monomethyl arabinose in

fraction (C) was essentially homogeneous and that its methyl group was not attached at C_2. From the above evidence the sugar must be 3-methyl arabinose. Control experiments showed that no hydrazodicarbonamide was formed when (*a*) 2 : 3 : 5-trimethyl arabonamide or (*b*) the product obtained by the action of liquid ammonia on arabinose was subjected to the Weerman reaction. The latter control showed that no interference with the validity of the Weerman test could arise from the small amount of reducing sugar known to be present in the lactone prepared from fraction (C).

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