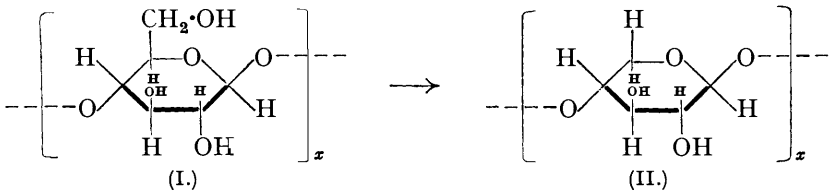
104. *Pectic Substances. Part I. The Araban and Pectic Acid of the Peanut.*

By E. L. Hirst and J. K. N. Jones.

The polysaccharides present in the seeds of *Arachis hypogæa* have been isolated and examined. They include starch, cellulose, and a complex which is shown to be a mixture of pectic acid and araban. After methylation by treatment of the thallium derivative of the complex with methyl iodide, separation can be effected; methylated araban and methylated pectic acid are described. Hydrolysis of the methylated araban gives rise to 2 : 3 : 5-trimethyl arabofuranose, 2 : 3-dimethyl arabinose, and 3-methyl arabinose, the structures of which are proved. Reasons are given for the view that araban contains solely furanose residues, and the molecular structure of the araban, which must contain branched chains, is discussed. The observations are held to throw doubt on the probability of the usually accepted mechanism for the phytochemical derivation of pentosans from hexosans, namely, the direct transformation by oxidation at the sixth carbon atom, followed by decarboxylation.

There has been much speculation concerning the origin of the pentose sugars in nature and the view most generally held is that they are derived from the corresponding hexoses by a process of oxidation at the terminal carbon atom, followed by decarboxylation. For instance, *d*-galactose, *d*-galacturonic acid, and the stereochemically related pentose *l*-arabinose are found closely associated in pectic materials and there is a corresponding structural relationship between the cellulose and the xylan constituents of woody fibres. In the latter instance, the fundamental molecular structures of the hexose and pentose units are similar, being pyranose units linked through the 1 : 4-positions, and it seemed reasonable to imagine that the long chains of xylopyranose residues (II) in xylan had originated by oxidation and decarboxylation from the long chains of the similarly united



(I.) (II.)

glucopyranose units (I) in cellulose. A difference, which may be of fundamental importance, was, however, brought to light by the discovery (Haworth, Hirst, and Oliver,

* In aqueous solution the alkali salts of *d*- and *l*-lactic acid exhibit *l*- and *d*-rotations respectively.

J., **1934**, 1917) that the long molecules of xylan were terminated by an arabofuranose residue, this being the first observed occurrence of the arabofuranose structure in products of natural origin. Furthermore, unpublished results obtained by the present writers in the course of work on damson gum show that arabofuranose and galactopyranose residues can occur together in one and the same molecule. It thus becomes all the more necessary to determine the ring structure of the pentose residues present in pectic materials, since it is not possible to derive, by simple oxidation and decarboxylation, a pentose residue containing a furanose structure from a hexopyranose residue. Such a transformation would involve change of ring structure and so far as is known at present no such transformation can occur unless the glycosidic link is broken by hydrolysis and the free sugar liberated. If, therefore, the arabans and the associated polygalacturonides and galactans differ with respect to ring structure or molecular arrangement, doubt will be cast on the validity of the above simple and direct mode of transformation.

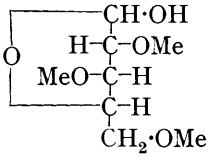
We decided to approach the problem of the phytochemical origin of the pentose sugars by instituting a comparison of the structures of the associated hexose and pentose units present in pectic substances and hemicelluloses. Little information is at present available concerning the detailed molecular constitution of any members of this group. A review of the literature revealed that a close association of *d*-galacturonic acid with *l*-arabinose was found in the seeds of many leguminous plants, the substances in question being referred to vaguely as galactoarabans. We selected for a first study the polysaccharides present in the seeds of *Arachis hypogæa* (ground nut; pea-nut). In addition to starch and cellulosic materials, we isolated a complex of pectic acid and araban which was found to be a mixture and not a single polysaccharide. In this respect, the observations are of interest in connection with those of recent workers (compare, for example, Schneider and Bock, *Ber.*, 1937, **70**, 1617), who tend to doubt the occurrence of arabinose residues as constituent parts of the pectin molecule. Our results demonstrate that the araban is composed entirely of arabofuranose residues and that the molecular structure comprises branched chains. On the other hand, the associated pectic acid is characterised by very high dextrorotation and by its extreme resistance to acid hydrolysis. It would appear, therefore, to be pyranose in structure and it seems very unlikely that any such simple generic relationship as that mentioned above can exist between the arabinose and galacturonic acid residues which are found together in *Arachis* seeds. Nevertheless, the close stereochemical relationship between *d*-galactose and *l*-arabinose remains significant and it may be that the transformation of a hexosan into a pentosan involves enzymic hydrolysis to single molecules, followed by resynthesis.

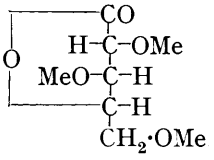
The pea-nut meal used as starting material contained some 50% of oil and 25% of protein in addition to carbohydrate material. After removal of the oil and protein the crude carbohydrate mixture was extracted with hot dilute alkali, which dissolved most of the araban–pectic acid complex, leaving the starch and cellulose. More drastic treatment with alkali removed the starch, which was purified by transformation into the acetyl derivative. The constitution of this material and of the cellulose portion will be considered in another communication. The araban–pectic acid complex appeared to be a mixture of the two components, araban and pectic acid, inasmuch as the properties of different samples varied and the components were not present in fixed stoicheiometric proportions and could be separated to some degree by extraction with aqueous alcohol or by fractional precipitation from alkaline solution by acid. Gentle hydrolysis removed the araban completely, leaving the pectic acid unattacked. On the other hand, the araban portion was best separated in a homogeneous condition after partial methylation of the mixture; the araban, which underwent etherification much more readily than the pectic acid, then became easily separable.

The preparation of derivatives of the two polysaccharides proved to be a matter of great difficulty. Methylation by the methods usually successful with polysaccharides resulted in extensive decomposition. New methods had to be sought for, and ultimately success was achieved by using a modification of Menzies's procedure (J., 1926, 937), in which the thallium derivative of a carbohydrate is heated with methyl iodide. A special advantage of this method in the case of polyuronides is that it leads directly to the forma-

tion of the methyl ester of the methylated product. Two methods of procedure were adopted, designed, as shown in the experimental section, to lead ultimately to the isolation of methylated pectic acid in the one instance and of methylated araban in the other.

The constitution of the pectic acid portion will be considered later and in the present paper we are concerned primarily with the structure of the methylated araban, which was obtained in the fully methylated condition after completion of methylation by the aid of the Purdie reagents. It was an amorphous substance, the analytical data for which corresponded to dimethyl araban. On gentle hydrolysis with methyl-alcoholic hydrogen chloride it gave rise to three products in approximately equimolecular proportions, namely, 2 : 3 : 5-*trimethyl methyl*-l-*arabinoside*, 2 : 3-*dimethyl methyl*-l-*arabinoside*, and 3-*methyl methyl*-l-*arabinoside*. The identity of the first of these was readily proved by its conversion successively into 2 : 3 : 5-trimethyl *l*-arabinose (III), 2 : 3 : 5-trimethyl γ-*l*-arabonolactone (IV), and 2 : 3 : 5-trimethyl *l*-arabonamide (V) (compare Pryde, Humphreys, and Waters, J., 1931, 1298; Haworth, Hirst, and Oliver, J., 1934, 1922).


(III.)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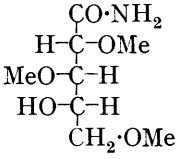
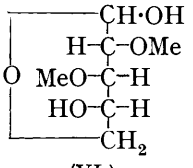

(V.)

The position of the methyl groups in the dimethyl *l*-arabinose (VI) followed from the following considerations. In the first place, the yields and properties of the *lactone* and *dimethyl* l-*arabonamide* prepared from the dimethyl sugar showed that the product was homogeneous and not a mixture of different dimethyl sugars. The high positive rotation (+ 106°) of the dimethyl sugar itself showed that the sugar could exist in the pyranose form, the rotational differences between pyranose and furanose forms in the arabinose series being by this time well established and so noteworthy that they may be utilised with certainty in assigning ring structures. The lactone (VII) derived from (VI) by bromine oxidation had a strong lævorotation, and this fact, coupled with the extremely slow rate of hydrolysis, established it as a γ-lactone. It follows, therefore, that neither position 4 nor position 5 was occupied by a methyl group and the sugar must therefore be 2 : 3-*dimethyl* l-*arabinose*. Further evidence on this point was secured by the observation that the amide (VIII) failed to give a positive Weerman test when allowed to react with sodium hypochlorite, indicating that position 2 was occupied by a methyl group.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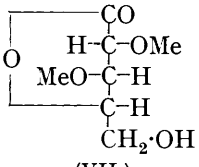
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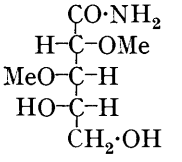
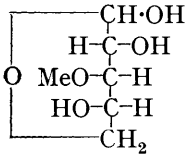

(VIII.)

The identification of the *monomethyl arabinose* depends on the following observations, and although these do not involve the use of crystalline reference compounds, the results are so clear-cut and definite that there need be no hesitation in assigning the methyl group to position 3. The free sugar possesses the high positive rotation characteristic of pyranose forms in the *l*-arabinose series and its gives a lævorotatory γ-lactone. Positions 4 and 5 therefore contain hydroxyl groups. Furthermore, comparison of the rotations of the sugar and the *lactone* with those of other corresponding derivatives of *l*-arabinose indicate that there can be no appreciable contamination with any methylated arabinose containing methyl groups in either of positions 4 and 5. The *amide* (XI) obtained from the lactone (X) gives a positive Weerman reaction, the yield of product obtained being identical with that given by *l*-arabonamide. Position 2 therefore possesses a hydroxyl group and the one methyl group must be situated at position 3. The yields of lactone and amide,

together with the rotational and other evidence, all point to the homogeneity of this monomethyl sugar, and the only structure which will account for the experimental data is (IX).



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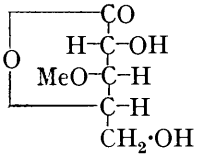

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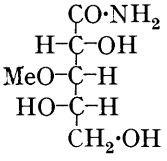
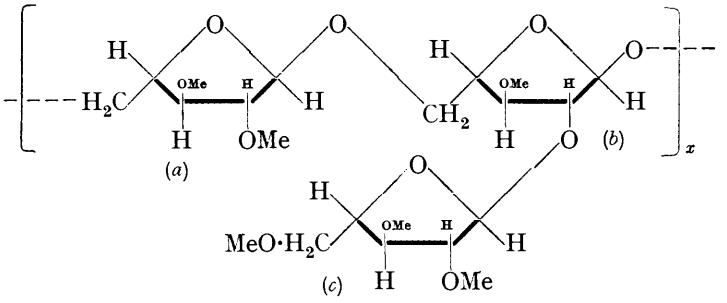


(XI.)

There remains to be discussed the problem of the molecular constitution possible for a methylated araban which gives rise to the above three sugars on hydrolysis. The data so far available are insufficient for the allocation of a unique formula, but the main features of the structure are nevertheless clear. The high viscosity of methylated araban, especially in view of the fact that the molecule is not a straight chain, points to a large molecular size and the initial problem is to combine the three residues represented by the hydrolysis products to give a repeating unit capable of forming, by primary valencies, products of high molecular weight. The residue (III) must be furanose, but (VI) and (IX) by reason of the free hydroxyl groups can be combined in either the pyranose or the furanose form. However, the extreme ease of hydrolysis and the strong lævorotation of araban point to the presence solely of furanose residues united by α-glycosidic links. The trimethyl arabinose must be attached as the terminal unit of a side chain and the simplest interpretation of the facts is obtained by postulating a main chain of arabofuranose residues linked through positions 1 and 5, with an arabofuranose residue attached by its reducing group to position 2 of every alternate unit of the main chain, giving the repeating unit shown in (XII) and diagrammatically in (XII *a*). Repeating units of the types shown diagrammatically in (XIII), (XIV), and (XV), or combinations of any of these, would also satisfy the experimental observations. Reference to models shows that (XIV) and (XV) give a highly congested array of atoms, but the structures in question do not seem to be impossible on stereochemical grounds.

With structural units of this type the method of end-group assay for determining chain length or deciding between the possible types of chain is inapplicable, since the termination of the main chain would not necessarily be signalised by the introduction of a new and easily recognisable group, as is the case with methylated cellulose and methylated starch. Conditions are indeed closely similar to those discussed in connection with the structure of yeast-mannan (Haworth, Hirst, and Isherwood, J., 1937, 784). The latter polysaccharide resembles araban in containing a repeating unit composed of three mannose residues, one pair of which must be united by a 1 : 2 link, but differs from araban in that all the mannose units of yeast-mannan are of the pyranose type.

(XII.)

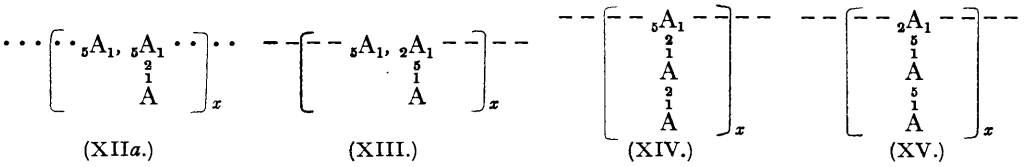


(*a*) (*b*) (*c*)

(*a*) ⟶ Dimethyl *l*-arabinose (VI) (*b*) ⟶ Monomethyl *l*-arabinose (IX)
(*c*) ⟶ Trimethyl *l*-arabofuranose (III)

In conclusion, reference must be made to a paper by Miyama (*J. Dept. Agric. Kyushu University*, 1935, **4**, 200), who has described a polysaccharide from peanuts, which appears to resemble closely the araban–pectic acid complex described above, but differs

from it in containing galactose. It is not certain, however, that the material examined by Miyama was homogeneous and he failed to recognise that galacturonic acid residues are an essential structural feature, being of the opinion that the galacturonic acid present was derived by alkaline oxidation of galactose during the process of isolation. This reaction does not take place and a simple explanation of his observations follows from the fact that recovery of the mixed polysaccharides from their solution in alkali results in loss of araban and progressive increase in the uronic acid content of the recovered material. The methods employed by Miyama in allocating structures are invalidated by a misunderstanding of the properties of partly methylated sugars. For example,



(XII*a*.) (XIII.) (XIV.) (XV.)

In (XII*a*)—(XV), A = an α-arabofuranose residue united to other residues through the numbered C-atoms.

the presence of arabopyranose residues is claimed (*loc. cit.*, p. 220) on the ground that trimethyl arabopyranose is formed on methylation of the hydrolysis products from his methylated polysaccharide. We have shown that such hydrolysis products contain 2 : 3-dimethyl and 3-monomethyl *l*-arabinose, which exist in the polysaccharide as furanose units but revert to the pyranose form on hydrolysis. Methylation of these would lead to the formation of trimethyl arabopyranose, but such a result has no bearing on the ring structure present in the polysaccharide. It is obvious that no serious consideration can be given to structural formulæ advanced on such uncertain and misleading evidence.

Experimental.

Extraction of Polysaccharides from Seeds of Arachis Hypogæa.—The seeds (peanuts) (11 kg.) were dried at 90°/10 mm. for 12 hours. The thin skins had then become brittle and were easily removed by trituration of the nuts in an air blast. The skins were collected for further examination. The nuts were then powdered, and the oil content removed partly by pressure (heated presses should not be employed) and partly by extraction with boiling alcohol (oil content, 45%). The meal was then stirred for 4 hours, with 10% aqueous sodium chloride (40 l.), and the liquid filtered through cloth. After five repetitions of this process the greater part of the protein content (*ca.* 30%) had been removed. The meal was next washed with water and then stirred with boiling 0·2% aqueous potassium hydroxide (20 l.) for 4 hours. After filtration through cloth the liquor was poured into methylated spirits (60 l.) and the precipitate was washed with alcohol and dried in a vacuum at 70°. The extraction of the meal with alkali was repeated two or three times until the polysaccharides isolated gave a faint blue colour with iodine in slightly acid solution. The products (77 g.) which gave no colour with iodine consisted mainly of a complex of pectic acid and araban (material A; see below). Traces of starch may be removed by the action of diastase on a solution of the potassium salt.

The peanut meal was next extracted with boiling 5% aqueous potassium hydroxide (20 l.). The filtered solution was poured into methylated spirits (60 l.), giving a pale yellow solid which consisted mainly of starch contaminated with material (A) and cellulosic material (protein content, nil). The extractions were repeated until the residue gave no colour with iodine. The combined starch fractions (75 g.) were then treated with pyridine and acetic anhydride; the starch went into solution, leaving the other polysaccharides undissolved. After filtration of the solution the acetylated starch was isolated in the usual way ($[\alpha]_D^{20°}$ + 170° in chloroform. Found: $CH_3 \cdot CO$, 43%). This was a typical starch acetate and further confirmation of its identity was forthcoming from its quantitative hydrolysis to crystalline glucose.

That portion of the pea-nut meal which resisted the action of boiling 5% alkali solution was mainly cellulose but was contaminated by some non-saponifiable oily material, which was removed by washing successively with alcohol (acidified with acetic acid) and then with ether. The cellulose so obtained (200 g.) gave crystalline glucose in almost quantitative yield on hydrolysis by the method of Monier-Williams. It dissolved in cuprammonium solution

and gave quantitatively on acetylation by the Barnett method a cellulose acetate having $[\alpha]_D^{20°}$ − 10° in chloroform containing 10% of alcohol.

Properties of the Araban–Pectic Acid Complex.—This material (A, p. 500) still contained traces of protein and starch. Purification was effected by drying it at 90°/10 mm. until the impurities had become insoluble in water. The solid was then stirred with water, and the flocculent insoluble residue removed in the centrifuge. This residue was washed with water, and the washings added to the aqueous solution of (A). On addition of alcohol a cream-coloured powder was precipitated. This purified potassium salt was redissolved in water, and the araban–pectic acid complex precipitated by addition with stirring of a slight excess of hydrochloric acid. The precipitate was triturated with alcohol until all the hydrochloric acid and potassium chloride had been removed, and finally it was dried at 50°/10 mm.

The araban–pectic acid complex so obtained was a white powder, insoluble in water, but soluble in dilute alkali, giving viscous solutions. It gave no colour with iodine solution or with zinc chloroiodide. Hot concentrated alkali solution caused decomposition, with formation of deep orange-coloured products. The complex had $[\alpha]_D^{20°}$ + 120° (neutral sodium salt, in water, *c*, 1·5, calc. on weight of free polysaccharide). Equiv. wt., 388 (by titration with N/10-sodium hydroxide). N, nil. OMe, nil. (On titration with alkaline iodine under the conditions given by Bergmann and Machemer for determination of iodine numbers, 1·0 g. required 2·6 c.c. of N/10-iodine.) Furfural, 31·7% (estimated both as barbiturate and as phloroglucide, after treatment of the polysaccharide with boiling 12% hydrochloric acid in the usual way). Uronic acid content, from the amount of carbon dioxide liberated on boiling with 12% hydrochloric acid, 45·8%. A substance containing 45·8% of uronic anhydride and no other acidic residues should have an equivalent weight of 383 (Found by titration of A with alkali, 388). Moreover, this proportion of uronic anhydride accounts for 10·1% of the total furfural. This leaves 21·6% of furfural contributed by the pentosan portion of the polysaccharide and since the only pentose present is arabinose (see below) the calculated araban content of (A) is 40·5% (Found: 40%, from estimation of arabinose after hydrolysis). Finally, the equiv. wt. of the pectic acid portion, which was free from araban, was found to be 214 (by carbon dioxide estimation and by direct titration), corresponding to a pectic acid content of 45·8 × 214/176, *i.e.*, 55·7%. Thus the analytical data satisfactorily account for 96·2% of polysaccharide (A).

Hydrolysis of the polysaccharide complex could be effected by boiling an aqueous solution of the substance for 20 hours. The solution darkened owing to decomposition and a little furfural was evolved. The solution was then neutralised with barium carbonate, filtered through charcoal, concentrated under diminished pressure, and poured into alcohol. The barium salt of a polygalacturonic acid was precipitated (yield, 33%) (equiv. wt., by estimation of barium content of salt, 232; furfural, 16·2%). The alcoholic filtrate contained *l*-arabinose, which was obtained, on evaporation of the solvent, in the crystalline condition, m. p. 157—158°, $[\alpha]_D^{21°}$ + 107° (equilibrium value in water, *c* 0·6), diphenylhydrazone, m. p. 202°, characteristic osazone, m. p. 166°. The yield of arabinose diphenylhydrazone actually isolated corresponded to an araban content of 27% and since under the conditions of the experiment pure arabinose gives only 66% of the theoretical yield of diphenylhydrazone the estimated araban content of the polysaccharide was 40%.

When the hydrolysis of the polysaccharide was carried out by heating it at 90° with 1% hydrochloric acid, a definite stage was reached at the end of 3 hours, and on pouring the mixture into alcohol the undissolved material was separated from the arabinose. The insoluble material (a white powder) was free from admixed araban and had all the properties of a pectic acid. Equiv. wt., by titration, 214; $[\alpha]_D^{20°}$ + 224° (as sodium salt in water); it gave typical insoluble calcium and copper salts. The high equivalent weight shows that the substance is not composed entirely of uronic acid residues. We have obtained a pectic acid with the same equivalent weight and properties from strawberry pectin.

Total hydrolysis of the polysaccharide was effected with 3% sulphuric acid at 90° in 30 hours. After neutralisation with barium carbonate the filtered solution was poured into alcohol, and filtered. The filtrate gave arabinose, estimated as the diphenylhydrazone [yield (corr.) corresponds to 40% araban]. No other sugar could be detected. The barium salt gave the characteristic derivative of galacturonic acid with *p*-bromophenylhydrazine, but no glycuronic acid could be detected. Repeated precipitation of the polysaccharide (A) from alkaline solution by dilute acid in the cold resulted in a progressive fall in the equiv. wt. and a corresponding increase in the rotation value. After five such treatments the product had $[\alpha]_D^{20°}$ + 177° (as sodium salt) and equiv. wt. 273. A similar separation was achieved by use of dilute thallium

hydroxide solution, which gave an insoluble derivative with the pectic acid portion and a more soluble one with araban. On removal of the thallium the araban had a strong negative rotation, whereas the recovered pectic acid obtained had properties similar to those recorded above.

These observations indicated that (A) was not a chemical individual but an intimate mixture of pectic acid and araban. Further confirmatory evidence in favour of this view was obtained by extracting (A) with 70% alcohol at 20°. The dissolved material was soluble in water, non-reducing, not precipitatable by acid from aqueous solution, did not contain acidic groups, gave no insoluble copper complex on addition of copper sulphate, no insoluble calcium salt, and had a negative rotation. It was hydrolysed by hot dilute hydrochloric acid with extreme ease, giving only arabinose. The portion insoluble in alcohol showed progressive rise in rotation and fall in equiv. wt. as the extraction proceeded, but the rate of extraction was slow and it was not possible by this means to obtain pectic acid free from adherent araban.

Yet further evidence of the non-homogeneity of (A) is found in the observation that samples prepared from the same supply of pea-nuts showed considerable variation in properties. Some

Preparation	1	2	3	4	5	6
$[\alpha]_D^{20°}$ (as sodium salt)	+120°	+ 79°	+140°	+171°	+177°	+224°
Equiv. wt.	388	452	359	292	273	214

typical results are recorded in the accompanying table, preparations (1), (2), and (3) being isolated directly from pea-nut meal. Products (4) and (5) were obtained when (2) was extracted by dilute alkali solution. Preparation (6), obtained by the action of dilute acid on (1), was free from araban and appeared to be composed entirely of pectic acid.

Since the equiv. wt. of the pectic acid portion is 214, it is possible to calculate from the above values the percentage of pectic acid in preparations (1), (2), (3), (4), and (5). If now these values are plotted against the observed rotation values, the latter lie approximately on a straight line. By extrapolation the rotation for araban free from pectic acid is — 50°, which may be regarded as an approximation to the rotation of the araban portion of polysaccharide (A). That this is of the right order of magnitude is shown by the observation (see below) that the fully methylated derivative of araban has $[\alpha]_D^{20°}$ — 90° in methyl alcohol.

When heated with nitric acid (*d* 1·2) under the standard conditions (compare Dorée, " Cellulose Chemistry," 1933 ed., p. 411), polysaccharide (A) gave mucic acid (yield, 21·4%). This confirmed the presence of galacturonic acid residues, but control experiments carried out under similar conditions with pectic acid (from strawberry pectin) and with lactose showed that the yields of mucic acid from combined galacturonic acid and combined galactose derivatives are variable and unreliable for quantitative analysis. Results with free galactose and free galacturonic acid are reproducible (yield in each case, *ca.* 62% of the theoretical), but high and uncertain correction factors are required in the case of polygalacturonides which are resistant to hydrolysis.

Methylation of the Araban–Pectic Acid Complex: Isolation of Methylated Araban.—Preliminary experiments showed that methylation of the polysaccharide (A) could not be effected by the usual methods. With methyl sulphate and sodium hydroxide the mixture became dark and no methylated derivative could be isolated. The material was recovered unchanged when the liquid ammonia–sodium–methyl iodide method was used and direct application of the Purdie method was equally unsuccessful.

Methylation was successfully carried out by a modification of the method employed by Menzies (*loc. cit.*) for the preparation of methyl ethers from the thallium derivatives of hydroxy-compounds. The polysaccharide (30 g.) was allowed to swell under water and to the swollen mass N-thallium hydroxide (450 c.c.) was added with stirring. After 2 hours at 20°, light being excluded, the liquid was filtered, and the solid material washed with alcohol and thoroughly dried at 60°/20 mm., again with exclusion of light. The lemon-yellow solid (98 g., containing 49 g. of thallium titratable with dilute sulphuric acid and phenolphthalein) was powdered and boiled with methyl iodide containing a little anhydrous methyl alcohol. (At this stage complete absence of moisture is essential, and the operation should be carried out in a dim light. It is frequently advantageous to carry out the reaction with methyl iodide under pressure at higher temperatures, in which case thallous oxide or silver oxide is added to the mixture to counteract the possible development of acidity.) After 12 hours the solvent was removed at 30°/10 mm. and the solid mixture of thallium iodide and partly methylated product was mixed with thallium ethoxide (60 g.) in benzene (200 c.c.). After a few minutes' shaking, the benzene was distilled at 30°/10 mm. and the remaining solid was powdered and boiled with methyl iodide with the precautions mentioned above. After removal of the methyl iodide the

product was separated from thallium iodide by solution in boiling methyl alcohol and on removal of the solvent it was obtained as a yellow solid (25·3 g.) (Found: OMe, 30·0%; $[\alpha]_D^{20°}$ + 4° in methyl alcohol, *c* 1·3). This had a higher araban content than the original material (A). Some of the pectic acid remained in the thallium iodide, from which it could be extracted in the form of a partly methylated derivative by means of hot water. The yellow solution was again treated with thallium ethoxide, followed by methyl iodide. The product was separated from thallium iodide by solution in methyl alcohol. It was then treated with ether, giving a soluble portion (B) and an insoluble portion (C). The ether-soluble portion (9·8 g.; $[\alpha]_D^{20°}$ + 6° in methyl alcohol; OMe, 40%) was separated by fractional precipitation into a stiff syrup (D) (7 g.) and a solid (2·7 g.), which was added to the material (C) insoluble in ether. (D) on distillation gave material (3 g.) with b. p. up to 200°/0·001 mm.; $[\alpha]_D$ + 4° in methyl alcohol; OMe, 54%; equiv. wt., *ca.* 140. This reduced neutral permanganate in the cold and rapidly decolorised bromine in carbon tetrachloride. It was similar to products we have obtained by treatment of pectic acid by the above procedure and is obviously composed of oxidised decomposition products.

The ether-insoluble material (C) had $[\alpha]_D^{20°}$ − 31° in methyl alcohol; OMe, 32%; equiv. wt., 800. It was remethylated with methyl iodide and silver oxide, giving a yellow solid (12·5 g.; $[\alpha]_D^{21°}$ − 45° in methyl alcohol; OMe, 37·8%), which was separated by fractional precipitation from chloroform (by addition of light petroleum) into a crisp solid (E) (7·8 g.) and a slightly viscid solid. (E) was remethylated (Purdie's reagents), giving a solid (F) (7·0 g.) having $[\alpha]_D^{20°}$ − 60° in methyl alcohol; OMe, 36·1%; equiv. wt., *ca.* 1100. The small amount of methylated pectic acid in (F) was removed by hydrolysing it with dilute alcoholic sodium hydroxide. The excess of alkali was removed by carbon dioxide, and the solution evaporated to dryness. Methylated araban (6 g.) was extracted by acetone, leaving the sodium salt of the pectic acid derivative undissolved. On removal of the acetone, methylated araban (G) was obtained as a cream-coloured powder soluble in acetone, alcohol, chloroform, benzene, and methyl alcohol, but insoluble in water, light petroleum and ether.

The above procedure leads to the isolation of pure methylated araban. In parallel series of experiments much greater amounts of thallium hydroxide were used in the initial stages. In this way some of the araban remained in solution in the thallium hydroxide liquor and was lost, but more complete formation of the thallium derivative of pectic acid took place. It was observed in consequence that increased amounts of the decomposed material (D) were obtained, but the principal feature was that the product at stage (F) of the above scheme contained a very much greater proportion of methylated pectic acid. A typical sample at this stage (yield, 8·0 g. from 25 g. of polysaccharide) had $[\alpha]_D^{20°}$ + 60° in methyl alcohol; OMe, 37%. The araban was removed by hydrolysis with 0·1% methyl-alcoholic hydrogen chloride, which has no action on methylated pectic acid. After neutralisation of the solution with silver carbonate the solvent was evaporated. The product (a sticky solid) was extracted successively with boiling light petroleum and ether, which removed the methylated arabinoses and left methylated pectic acid as a crisp solid having solubilities similar to those of methylated araban, $[\alpha]_D^{20°}$ + 120°; OMe, 33·9%; equiv. wt., 250. This substance was resistant both to further methylation and to hydrolysis by acids. It was sensitive to alkali, undergoing profound decomposition when heated with excess of N-sodium hydroxide. It is not clear from the evidence available whether this material is derived from normal or degraded pectic acid and further investigations are in progress.

Methylated Araban.—The material (G) (4·5 g.) was submitted to fractionation by precipitation from chloroform solution by addition of light petroleum. Two fractions were obtained, the first (3·2 g.) being a crisp cream-coloured powder; the second, more soluble portion (1·3 g.) was similar but very slightly sticky, on which account it was discarded. The first fraction had $[\alpha]_D^{20°}$ − 90° in methyl alcohol (*c*, 1·76); $\eta_{sp.}^{20°}/c$ 0·138 in *m*-cresol (*c* in g. per 100 c.c.; since the substance does not possess a straight chain structure, no calculation of molecular size by Staudinger's method is permissible) (Found: C, 51·8; H, 7·4; OMe, 37·4; CO_2Me, nil. Fully methylated araban, $C_7H_{12}O_4$, requires C, 52·5; H, 7·6; OMe, 38·8%. Methylated araban of methoxyl content 37·4% requires C, 52·2; H, 7·6%).

Hydrolysis of methylated araban was carried out by boiling the substance (3·0 g.) for 18 hours, with 1% methyl-alcoholic hydrogen chloride (60 c.c.). The solution was neutralised with silver carbonate and filtered, and the solvent removed by distillation. (It is advisable not to distil off the methyl alcohol under diminished pressure, since trimethyl methylarabofuranoside is volatile under these conditions.) The products of hydrolysis (2·9 g.) were then fractionally distilled, giving:

(i) 2 : 3 : 5-*Trimethyl methyl*-l-*arabofuranoside* (0·63 g.), bath temp. 87—90°/0·001 mm.; $n_D^{16\cdot5°}$ 1·4362; $[\alpha]_D^{21°}$ − 60° in water (*c*, 0·6) (Found: OMe, 60·0. $C_9H_{18}O_5$ requires OMe, 60·2%).

(ii) 2 : 3-*Dimethyl methyl*-l-*arabinoside* (0·76 g.), bath temp. 115—122°/0·001 mm.; $n_D^{16\cdot5°}$ 1·4522; $[\alpha]_D^{21°}$ + 14° in water (*c*, 0·4) (Found: OMe, 47·7. $C_8H_{16}O_5$ requires OMe, 48·4%).

(iii) 3-*Methyl methyl*-l-*arabinoside* (0·64 g.), bath temp. up to 200°/0·001 mm.; $n_D^{16°}$ 1·4710; $[\alpha]_D^{21°}$ + 46° in water (*c*, 0·47) (Found: OMe, 37·6. $C_7H_{14}O_5$ requires OMe, 34·8%. The methoxyl value indicates that this fraction contains a small amount of dimethyl methyl-*l*-arabinoside).

(iv) Residue (0·6 g.). This was a dark tar which could not be further hydrolysed by 3% methyl-alcoholic hydrogen chloride. It probably consisted of the polymerised material formed as by-products when arabinose derivatives are heated with methyl-alcoholic hydrogen chloride. The amounts of fractions (i), (ii), and (iii) indicate a ratio of 1 : 1 : 1 for the trimethyl, dimethyl, and monomethyl portions respectively, the slight deficiency of trimethyl methylarabinoside being readily accounted for by experimental loss due to the high volatility of this fraction (see Carruthers and Hirst, J., 1922, 2304), and that of the third fraction by difficulty in separating it from the non-volatile portion.

2 : 3 : 5-*Trimethyl* l-*Arabinose*.—Fraction (i) (0·57 g.) was hydrolysed by N/10-hydrochloric acid (25 c.c.) at 90° for 8 hours. $[\alpha]_D^{20°}$ − 57° (initial value); − 49° (30 mins.); − 44° (1 hr.); − 26° (2·5 hrs.); − 12° (7 hrs.; constant value). After neutralisation the solution was evaporated to dryness, and the product dissolved in chloroform. On removal of the solvent 2 : 3 : 5-trimethyl *l*-arabinose was obtained as a syrup (0·47 g.), $n_D^{18°}$ 1·4522, $[\alpha]_D^{20°}$ − 14° in water (*c*, 2·4) (Found: OMe, 48·2. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). The trimethyl *l*-arabinose (0·45 g.) in water (2 c.c.) was oxidised by bromine (1 c.c.) at 50° for 1 hour and then at 20° for 12 hours. After removal of the excess of bromine by aeration the solution was neutralised with silver carbonate and filtered immediately, hydrogen sulphide passed through it to remove dissolved silver, and the filtered solution evaporated to dryness. The product (0·41 g.) was distilled, giving 2 : 3 : 5-trimethyl γ-*l*-arabonolactone (0·33 g.), bath temp. 110—115°/0·002 mm.; $n_D^{19°}$ (of superfused solid) 1·4462; m. p. 27—28° (without recrystallisation); $[\alpha]_D^{20°}$ − 44° in water (*c*, 0·8), decreasing after 160 hours to − 30° (mutarotation still not completed) (Found: OMe, 47·8. Calc. for $C_8H_{14}O_5$: OMe, 48·9%. *M*, by titration with *N*/100-sodium hydroxide, 192. Calc., 190). On treatment with methyl-alcoholic ammonia the lactone gave quantitatively the amide of 2 : 3 : 5-trimethyl *l*-arabonic acid, m. p. and mixed m. p. with an authentic specimen, 139°; $[\alpha]_D^{20°}$ + 23° in ethyl alcohol (*c*, 0·3).

2 : 3-*Dimethyl* l-*Arabinose*.—Fraction (ii) (0·61 g.) was hydrolysed by N/10-hydrochloric acid (25 c.c.) for 5 hours at 90°. $[\alpha]_D^{20°}$ + 8° (initial value); + 50° (30 mins.); + 77° (1·5 hrs.); + 89° (2·5 hrs.); + 98° (3·5 hrs.; constant value). The product was isolated in the usual way, giving 2 : 3-*dimethyl* l-*arabinose* (0·55 g.) as a syrup, $n_D^{17°}$ 1·4650, $[\alpha]_D^{20°}$ + 106° (equilibrium value in water; *c*, 2·4) (Found: OMe, 35·7. $C_7H_{14}O_5$ requires OMe, 34·8%). When oxidised by bromine water (for conditions, see above), the sugar (0·53 g.) gave 2 : 3-*dimethyl* γ-l-*arabonolactone* (0·5 g.), obtained after distillation, bath temp. 145—155°/0·003 mm., as a colourless syrup (0·4 g.), $n_D^{17°}$ 1·4605, $[\alpha]_D^{20°}$ − 34° (initial value in water; *c*, 0·7); − 33° (3 hrs.); − 32° (16 hrs.); − 31° (1 day); − 29° (42 hrs.); − 25° (140 hrs.); − 23° (170 hrs.); − 19° (330 hrs., mutarotation still incomplete) (Found: OMe, 35·5; *M*, by titration with N/100-sodium hydroxide, 176. $C_7H_{12}O_5$ requires OMe, 35·2%; *M*, 176). On treatment with methyl-alcoholic ammonia this lactone gave in quantitative yield the *amide* of 2 : 3-dimethyl *l*-arabonic acid, m. p. 160°, $[\alpha]_D^{20°}$ + 17° in water (*c*, 2·2). It was soluble in water, methyl alcohol, ethyl alcohol, less soluble in acetone, ethyl acetate, and nearly insoluble in ether. It was readily recrystallised from ethyl alcohol (Found: C, 43·5; H, 7·7; OMe, 31·7. $C_7H_{15}O_5N$ requires C, 43·4; H, 7·8; OMe, 32·1%). The properties of this amide are in full agreement with those of a sample of 2 : 3-dimethyl *l*-arabonamide prepared from other sources in the Birmingham University laboratories by Haworth and Smith (private communication). When treated with sodium hypochlorite under the conditions given by Weerman (*Rec. Trav. chim.*, 1917, **37**, 16), the above amide gave no trace of sodium *iso*cyanate, as shown by the complete absence of hydrazodicarbonamide after subsequent addition of semicarbazide to the solution.

3-*Methyl* l-*Arabinose*.—Fraction (iii) (0·58 g.) was hydrolysed by N/10-hydrochloric acid for 6 hours at 90°. $[\alpha]_D^{20°}$ + 46° (initial value), rising to + 88° at the end of 3 hours (constant value). After neutralisation with silver carbonate the solution was evaporated to dryness, giving a syrup (0·51 g.) (mainly 3-*methyl* l-*arabinose*), $n_D^{18°}$ 1·4850, $[\alpha]_D^{20°}$ + 96° (equilibrium value in water; *c*, 2·3) (Found: OMe, 21·3 $C_6H_{12}O_5$ requires OMe, 18·9%). (The high methoxyl value indicates the presence of a little dimethyl arabinose.) The sugar (0·49 g.) was oxidised

by bromine water (for conditions, see above), giving the *lactone* as a syrup (0·4 g.), distilling at bath temp. 175°/0·003 mm.; $n_D^{17°}$ 1·4800; $[\alpha]_D^{20°}$ − 36° (initial value in water; *c*, 2·0); − 33° (95 hrs.); − 22° (133 hrs.); − 19° (160 hrs.; mutarotation still incomplete) (Found: OMe, 21·0; *M*, by titration with N/100-sodium hydroxide, 170. $C_6H_{10}O_5$ requires OMe, 19·1%; *M*, 162). When treated with methyl-alcoholic ammonia, the lactone gave the corresponding *amide* as a glassy solid, $[\alpha]_D^{20°}$ + 31° in water (*c*, 1·1) (Found: N, 7·5; OMe, 19·4. $C_6H_{13}O_5N$ requires N, 7·8; OMe, 17·3%). This amide, after treatment with sodium hypochlorite under Weerman's conditions (*loc. cit.*) and subsequent addition of semicarbazide to the solution, gave hydrazodicarbonamide, m. p. and mixed m. p. with an authentic sample, 256°, the yield being identical with that obtained in parallel experiments under the same conditions with *l*-arabonamide.

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