153. *The Constitution of Arabic Acid. Part I. The Isolation of* 3-d-*Galactosido*-l-*arabinose.*

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Autohydrolysis of arabic acid yields a mixture of sugars and degraded arabic acid in approximately equal quantities. The mixture of sugars consists of *l*-arabinose, *l*-rhamnose, and a disaccharide, 3-*d*-galactopyranosido-*l*-arabinose, the structure of which is proved. This is apparently the first case in which a disaccharide with a 1 : 3-biose link has been obtained from natural sources. According to the conditions of methylation employed, this disaccharide can be obtained as *heptamethyl* 3-d-*galacto-pyranosido*-l-*arabopyranose* (I) or *heptamethyl* 3-d-*galactopyranosido*-l-*arabofuranose* (VI). These two heptamethyl derivatives yield on hydrolysis 2 : 4-dimethyl methyl-*l*-arabinoside (II) and 2 : 5-dimethyl methyl-*l*-arabinoside (VII) respectively, derivatives of which have been prepared and their structures determined. 3-β-*d*-Galacto-pyranosido-*l*-arabinose has been prepared from lactose and subjected to methylation, giving the corresponding *heptamethyl* derivative, which furnishes on hydrolysis 2 : 3 : 4 : 6-tetramethyl galactose and 2 : 4-*dimethyl* d-*arabinose* (XIII). A comparison of the derivatives of the latter with those of 2 : 4-dimethyl *l*-arabinose proves that the structure assigned to (II) is correct. It is suggested that in arabic acid the disaccharide is attached to the rest of the molecule as 3-*d*-galactopyranosido-*l*-arabofuranose (IX) through a link involving position 1 of the *l*-arabofuranose residue.

POLYSACCHARIDES which exist in pneumococcus capsular material yield on hydrolysis aldobionic acids (Heidelberger and Goebel, *J. Biol. Chem.*, 1927, **74**, 613, 619) and furthermore a polysaccharide acid obtained from gum arabic by acid degradation was shown to give a precipitin reaction with Types II and III antipneumococcus serum comparable with the bacteriological soluble specific substances themselves (Heidelberger, Avery, and Goebel, *J. Exp. Med.*, 1929, **49**, 847). It will be seen therefore that any evidence which can be obtained concerning the structure of arabic acid will be of assistance in the study of the much rarer specific polysaccharides which play such an important rôle in immunological reactions.

Arabic acid yields on acid hydrolysis an aldobionic acid the constitution of which has been shown to be 6-β-*d*-glucuronosido-*d*-galactose (Haworth, Hirst, and Challinor, J., **1931**, 258), a conclusion which has been verified by Hotchkiss and Goebel, who synthesised this compound (*J. Amer. Chem. Soc.*, 1936, **58**, 858; *J. Biol. Chem.*, 1936, **115**, 285). That *l*-arabinose is one of the products of hydrolysis of arabic acid has long been known (Scheibler, *Ber.*, 1873, **6**, 614; O'Sullivan, J., 1884, **45**, 41), and there is evidence to indicate that rhamnose also is produced (Butler and Cretcher, *J. Amer. Chem. Soc.*, 1929, **51**, 519). Kiliani (*Ber.*, 1880, **13**, 2304; 1882, **15**, 34) and Claesson (*Ber.*, 1881, **14**, 1270), working independently, isolated as a hydrolysis product of gum arabic another sugar which had the properties of galactose. The presence of the latter was confirmed by O'Sullivan (*loc. cit.*) and later by Butler and Cretcher (*loc. cit.*). Gum arabic is the salt of an organic acid, arabic acid, with metals such as calcium, magnesium, and potassium (Neubauer, *J. pr. Chem.*, 1854, **62**, 193). The free acid is obtained by the addition of a slight excess of

mineral acid to a solution of the gum in cold water, and when the aqueous acid solution is poured into alcohol the arabic acid is obtained as an amorphous powder.

When a solution of arabic acid in 0·1N-sulphuric acid is heated, hydrolysis takes place with the formation of a mixture of sugars (from which *l*-arabinose is readily isolated; Carrington, Haworth, and Hirst, J., 1934, **1653**) and a degraded arabic acid in approximately equal proportion. Further degradation takes place if the heating is prolonged, or if a more concentrated acid is employed, and eventually aldobionic acid is obtained (Butler and Cretcher, *loc. cit.*). The aldobionic acid is somewhat resistant to hydrolytic agents, but more drastic treatment yields finally glucuronic acid (Neumann, *Biochem. Z.*, **1931**, **236**, 90). Degraded arabic acids have been described by O'Sullivan (*loc. cit.*), but the method of preparation used was such that the presence of acids of quite small molecular size could not be avoided. An aqueous solution of arabic acid (*c*, 10·0) has, however, a p_H of 2·2 and it was found that hydrolysis of the arabic acid could be smoothly effected by simply heating an aqueous solution, and as far as can be ascertained there is produced the same mixture of sugars and the same degraded arabic acid as obtained by hydrolysing arabic acid with 0·1N-sulphuric acid. This autohydrolysis proceeds more slowly than the hydrolysis of arabic acid with 0·01N-sulphuric acid, but its advantage lies in the fact that the process is easily reproducible. It will be seen therefore that it is very important to avoid the application of heat in the preparation of the free polysaccharide acid, arabic acid, otherwise partial hydrolysis will ensue. Similar precautions should be exercised when other gum acids are being prepared by a similar method, especially those which contain labile sugar residues (compare O'Sullivan, *loc. cit.*; Norman, *Biochem. J.*, 1929, **23**, 529).

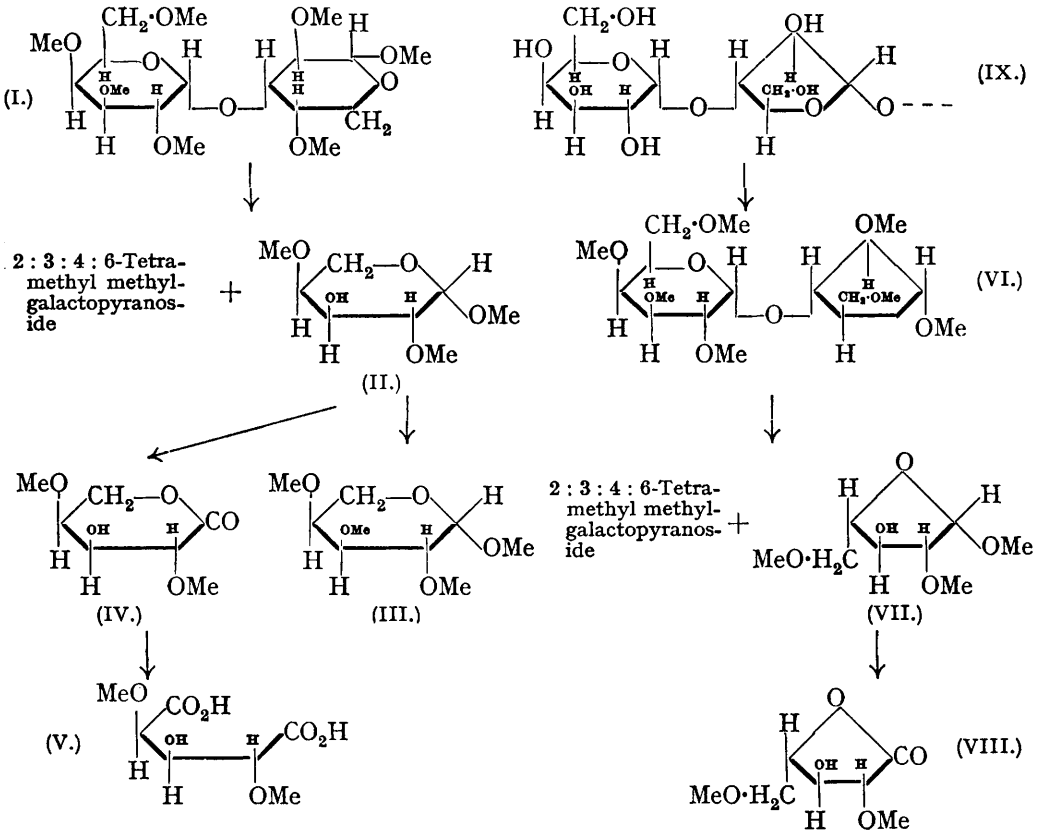
The present paper is concerned with the nature of the sugars produced in the autohydrolysis of arabic acid. The degraded arabic acid will form the subject of a subsequent communication.

In view of the difficulty experienced in attempting to characterise sugars by means of osazones, which tend to form anhydro-compounds, it was decided to identify the sugars by the preparation of well-defined crystalline methyl derivatives. Accordingly, the mixture of sugars was methylated first with methyl sulphate and sodium hydroxide solution and subsequently with Purdie's reagents. The methylated syrup thus produced yielded, on distillation, 2 : 3 : 4-trimethyl methyl-*l*-arabopyranoside, 2 : 3 : 4-trimethyl methyl-*l*-rhamnopyranoside, and a methylated disaccharide (I). The presence of the arabinose and rhamnose residues in arabic acid was proved by hydrolysis of the methyl derivatives to give 2 : 3 : 4-trimethyl *l*-arabinose and 2 : 3 : 4-trimethyl *l*-rhamnose. These in turn were oxidised to the corresponding lactones and the latter were converted into the known phenylhydrazides of 2 : 3 : 4-trimethyl *l*-arabonic acid and 2 : 3 : 4-trimethyl *l*-rhamnonic acid respectively. The arabinose residue was also identified by the preparation of 2 : 3 : 4-trimethyl *l*-arabonamide.

The methylated disaccharide proved to be *heptamethyl* 3-d-*galactopyranosido*-l-*arabopyranose* (I). It was obtained as a crystalline substance (m. p. 82°) and in view of its rather high positive rotation (+ 162°) the biose link may be of the α-type. When boiled with methyl-alcoholic hydrogen chloride, (I) yielded an equimolecular mixture of 2 : 3 : 4 : 6-tetramethyl methylgalactopyranoside and a dimethyl methyl-*l*-arabopyranoside (II). Acid hydrolysis of this mixture of glycosides gave the corresponding mixture of free sugars, which was subjected to methylation with Purdie's reagents and there was obtained crystalline 2 : 3 : 4 : 6-tetramethyl β-methylgalactopyranoside (identified by comparison with an authentic specimen) and 2 : 3 : 4-trimethyl methyl-*l*-arabopyranoside (III). The latter was characterised by the isolation of 2 : 3 : 4-trimethyl *l*-arabonic acid phenylhydrazide, and the presence of a 2 : 3 : 4 : 6-tetramethyl galactose residue was confirmed by conversion into the corresponding anilide. Since the latter could be obtained from the mixture of reducing methylated sugars before methylation with Purdie's reagents, it was evident that the methylated disaccharide consisted of a molecule of 2 : 3 : 4 : 6-tetramethyl galactose joined through its reducing group to a molecule of dimethyl *l*-arabinose.

The mixture of methylglycosides derived from (I) could not be resolved into its constituents by fractional distillation, and the corresponding reducing methylated sugars also formed a constant-boiling mixture. The free sugars could be separated to a great extent

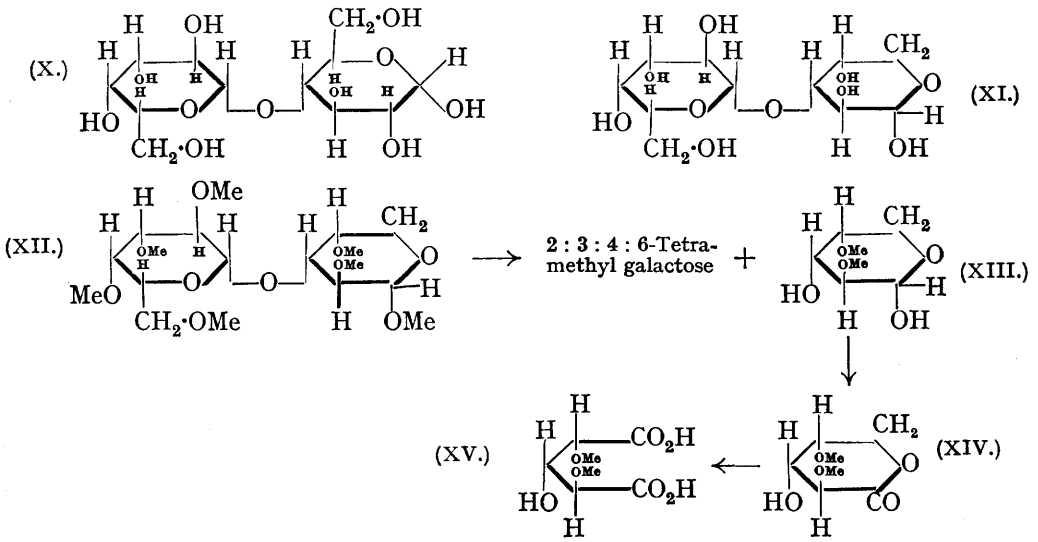
by taking advantage of the ready formation of 2 : 3 : 4 : 6-tetramethyl galactose anilide. Bromine oxidation of the mixture of sugars at room temperature proved, however, to be a better method, for the reason that the dimethyl *l*-arabinose readily formed a dimethyl *l*-arabonolactone (IV) while, under the conditions employed, the tetramethyl galactose remained unchanged. The rapid mutarotation of the lactone (IV) in water indicated that it belonged to the δ-series and hence suggested the presence of a free hydroxyl group in position 5 and a methoxyl in position 4. On treatment with ammonia, the lactone yielded a *dimethyl* l-*arabonamide* which gave a negative Weerman reaction (*Rec. Trav. chim.*, **1917**, **36**, 16), thus establishing the presence of a methoxyl group in position 2. The dimethyl *l*-arabinose is therefore to be designated 2 : 4-dimethyl *l*-arabinose and it follows that the biose link of the disaccharide (I) engages C_1 of the galactose residue and C_3 of the *l*-arabinose residue. The disposition of the two methyl residues in the arabinose moiety was confirmed by subjecting the 2 : 4-dimethyl *l*-arabinose to oxidation with nitric acid; there was then obtained β-hydroxy-αγ-dimethoxy-*l*-araboglutaric acid (V). The latter was transformed into the corresponding *diamide*, which gave a negative Weerman reaction, showing that both carbon atoms 2 and 4 adjacent to the amide groups carry methoxyl residues.



When the mixture of sugars obtained by autohydrolysis of arabic acid was treated first with cold methyl-alcoholic hydrogen chloride and then with methyl sulphate and sodium hydroxide solution, the methylated disaccharide was obtained as a methylfuranoside (VI), because on hydrolysis it yielded 2 : 3 : 4 : 6-tetramethyl methylgalactoside and 2 : 5-dimethyl methyl-*l*-arabinoside (VII). When oxidised with bromine, the dimethyl-*l*-arabinose derived from the latter gave rise to a dimethyl *l*-arabonolactone (VIII), which underwent slow mutarotation in aqueous solution, indicating the presence of a γ-lactone ring and therefore the presence in the original sugar of a free hydroxyl group in position 4.

Moreover, the lactone (VIII) readily yielded the characteristic 2 : 3 : 5-trimethyl *γ-l*-arabonolactone on treatment with silver oxide and methyl iodide. When allowed to react with ammonia, (VIII) furnished a dimethyl *l*-arabonamide which gave a negative Weerman reaction, thus proving that a methoxyl residue was attached at position 2. It is clear therefore that the methyl groups occupy the positions 2 and 5 or 2 and 3. It has been shown that direct methylation of the disaccharide with methyl sulphate and sodium hydroxide yields a crystalline heptamethyl derivative (I) in which the biose link engages position 3 of the *l*-arabinose residue and hence the methyl groups in the *dimethyl* l-*arabinose* now under discussion can only be in the positions 2 and 5. Moreover 2 : 3-dimethyl *l*-arabinose has been prepared (following paper) and an examination of its derivatives shows that it is quite different from the 2 : 4-dimethyl and the 2 : 5-dimethyl *l*-arabinose mentioned above.

In order to confirm the above results, lactose (X) was degraded by Wohl's method (Zemplén, *Ber.*, 1926, **59**, 2405) to 3-β-*d*-galactopyranosido-*d*-arabopyranose (XI) and the



latter was methylated with methyl sulphate and sodium hydroxide. There was thus produced the *heptamethyl* derivative (XII), which yielded on hydrolysis an equimolecular constant-boiling mixture of 2 : 3 : 4 : 6-tetramethyl galactose and 2 : 4-dimethyl *d*-arabinose (XIII). The mixture was resolved into its constituents by fractional crystallisation of the anilides. Oxidation of (XIII) with bromine furnished 2 : 4-*dimethyl* δ-d-*arabonolactone* (XIV), which on further oxidation gave β-hydroxy-αγ-dimethoxy-*d*-araboglutaric acid (XV). The crystalline *amides* of (XIV) and (XV) proved to be the enantiomorphs of the amides derived from (IV) and (V) respectively.

These facts show clearly that the disaccharide produced by mild acid or autohydrolysis of arabic acid is 3-*d*-galactopyranosido-*l*-arabinose, and since the latter is removed so easily from the basic nucleus of the polysaccharide acid, it is probably attached as 3-*d*-galactopyranosido-*l*-arabofuranose as shown in (IX).

Experimental.

Preparation of Arabic Acid from Gum Arabic.—The gum (500 g.) was dissolved in cold water (3 l.) and treated with a slight excess of dilute hydrochloric acid (tested with Congo paper). The mixture was poured slowly into alcohol (9 l.) and the curdy white precipitate was filtered off and pressed to remove most of the solvent. After three such treatments the arabic acid was free from inorganic salts. Mineral acid was then removed by reprecipitation of the material from an aqueous solution by addition of alcohol. The white amorphous product was washed with alcohol and ether and dried in a vacuum (yield, 400 g.). It was non-reducing and showed p_H 2·2 (10% solution); $[\alpha]_D^{20°}$ — 28° in water (*c*, 2·0), equiv. 1400 (determined by direct

titration with alkali). After drying in a vacuum at 110° for 20 hours, the material had $[\alpha]_D^{20°}$ — 30° in water (*c*, 1·0), equiv. *ca.* 1300, iodine number *ca.* 2·0.

Hydrolysis of Arabic Acid.—(a) *With 0·01N-sulphuric acid.* Arabic acid (100 g.) was dissolved in 0·01N-sulphuric acid (1000 c.c.) and heated on a boiling water-bath until the specific rotation became constant: $[\alpha]_D$ — 30° (initial value); — 24° (½ hour); — 6° (1½ hours); + 4° (2½ hours); + 13° (3½ hours); + 21·5° (5 hours); + 26° (6 hours); + 32° (8 hours); + 36° (10 hours); + 39° (12 hours); + 40·5° (14 hours); + 41·5° (15 hours); + 42° (18 hours) (constant for several hours). Prolonged heating caused a slow increase in the rotation of the solution. The solution ($[\alpha]_D$ + 42°) was neutralised with barium carbonate, filtered, and poured into excess of alcohol. The barium salt of degraded arabic acid was filtered off, purified by reprecipitation, and dried in a vacuum. The white amorphous barium salt (58 g.) reduced Fehling's solution and had $[\alpha]_D$ — 9·0° in water (*c*, 1·0). The alcoholic mother-liquors were combined and evaporated to dryness and the sugars were extracted from the residue with methyl alcohol. A small quantity of the barium salt of degraded arabic acid remained insoluble. Removal of the solvent left a strongly reducing syrup A (48 g.).

(b) *Autohydrolysis.* Arabic acid (100 g.), dissolved in water (1000 c.c.) and heated on a boiling water-bath, underwent autohydrolysis, the progress of which was observed polarimetrically: $[\alpha]_D^{20°}$ — 28° (initial value); + 22·5° (10 hours); + 40·0° (22 hours); + 41·5° (29 hours); + 42·5° (34 hours; constant for 12 hours). The solution was neutralised with barium carbonate, filtered, and poured into alcohol. The barium salt of degraded arabic acid was filtered off and the reducing syrup (A) (45 g.) was obtained when the filtrate was evaporated under reduced pressure.

Examination of the Reducing Syrup (A).—The syrup ($[\alpha]_D$ — 10° in water) was triturated with a small quantity of methyl alcohol and nucleated with *l*-arabinose. After several days crystallisation was complete and *l*-arabinose, having been triturated with methyl alcohol to remove adhering syrup, was filtered off, washed with ethyl alcohol and ether, and dried (yield, 17·5 g.); after recrystallisation from methyl alcohol it had m. p. 154°, $[\alpha]_D^{18°}$ + 104° (equilibrium value in water, *c*, 1·0).

The syrup (A) (49 g.), obtained from arabic acid (110 g.) by autohydrolysis, was methylated with methyl sulphate (250 c.c.) and sodium hydroxide solution (550 c.c., 30%) in the presence of some acetone, the time taken being about 4 hours. The methylation was conducted at 20° until the solution had no action on boiling Fehling's solution, and thereafter at 35°. The solution was then maintained at 80° for 15 minutes to remove acetone and to destroy any sodium methyl sulphate, cooled, filtered from sodium sulphate, and exhaustively extracted with chloroform. The extract, dried over anhydrous magnesium sulphate, filtered, and evaporated under diminished pressure, gave a syrup (27 g.). The aqueous alkaline layer after chloroform extraction was almost neutralised with 5N-sulphuric acid, cooled in ice, filtered from sodium sulphate, and evaporated to a suitable bulk. This residue, which still contained incompletely methylated material, was again treated with methyl sulphate and sodium hydroxide solution as above, and the product extracted with chloroform (yield, 13 g.). Completion of the methylation was effected by two treatments of the syrup (40 g.) with methyl iodide and silver oxide. The mixture of methylated sugars was distilled, giving: Fraction I (a mixture of 2 : 3 : 4-trimethyl methyl-*l*-arabinoside and 2 : 3 : 4-trimethyl methyl-*l*-rhamnoside) (30·3 g.), b. p. (bath temp.) 120°/0·05 mm., $n_D^{18°}$ 1·4440 to 1·4490 (Found: OMe, 57%). Fraction II (heptamethyl 3-*d*-galactopyranosido-*l*-arabopyranose; 9 g.), b. p. (bath temp.) 180°/0·03 mm., $n_D^{20°}$ 1·4670, $[\alpha]_D^{20°}$ + 139·5° in water (*c*, 1·0) (Found: OMe, 51·3. Calc. for $C_{18}H_{34}O_{10}$: OMe, 52·9%).

Isolation of Crystalline Derivatives of 2 : 3 : 4-Trimethyl l-Rhamnose and 2 : 3 : 4-Trimethyl l-Arabinose from Fraction I.—A portion of fraction I was dissolved in dilute sulphuric acid (3·5%) and heated on a boiling water-bath until the rotation had become constant. The solution was neutralised with barium carbonate, filtered, and evaporated to dryness, giving a reducing syrup, which was distilled in a vacuum. The product had $n_D^{19°}$ 1·4633, $[\alpha]_D^{23°}$ + 61° in water (*c*, 0·8). When the syrup was digested with aniline in alcoholic solution, no crystalline anilide was produced. If, however, the original hydrolysis of the arabic acid had been effected with 0·01N-sulphuric acid, the syrup at this stage yielded sometimes a very small quantity of 2 : 3 : 4 : 6-tetramethyl galactose anilide, m. p. 190°.

The mixture of reducing sugars was allowed to react with bromine (3 mols.) in aqueous solution at 30° for 48 hours and excess of bromine was removed by aeration. The solution was neutralised with silver oxide and filtered, the organic acids liberated with hydrogen sulphide, and the filtered solution evaporated to a syrup, which was purified by extraction with ether. Removal of the ether yielded a pale yellow syrup, which distilled at (bath temp.) 120°/0·13 mm.; $n_D^{20°}$ 1·4550—1·4600, $[\alpha]_D$ + 62° (initial value); + 56° (after ½ hour); + 6·2 (3½ hours);

— 14·9° (4½ hours); — 19·9° (18 hours); — 9·9° (69 hours); — 6·2° (75 hours). The distillate was acidic, behaved as a lactone on titration with sodium hydroxide, and did not reduce Fehling's solution. The phenylhydrazides of 2 : 3 : 4-trimethyl *l*-arabonic acid and 2 : 3 : 4-trimethyl *l*-rhamnonic acid were obtained by treating the syrupy mixture of lactones (150 mg.) with phenylhydrazine (1·1 mols.) first in boiling ether for ½ hour and then, after removal of ether, for 3 hours at 90°. The crystalline product (50 mg.) was separated from adhering syrup by trituration with ether. After recrystallisation from alcohol–ether–light petroleum the product had m. p. 156°, alone or in admixture with authentic phenylhydrazide of 2 : 3 : 4-trimethyl *l*-arabonic acid (Found: C, 56·6; H, 7·0; OMe, 31·0; N, 9·8. Calc. for $C_{14}H_{22}O_5N_2$: C, 56·4; H, 7·4; OMe, 31·2; N, 9·4%). Evaporation of the mother-liquors yielded another crop of crystals (25 mg.), which after recrystallisation from alcohol–ether had m. p. 177°, alone or in admixture with authentic phenylhydrazide of 2 : 3 : 4-trimethyl *l*-rhamnonic acid (Found: C, 57·8; H, 7·8; OMe, 29·4; N, 9·1. Calc. for $C_{15}H_{24}O_5N_2$: C, 57·7; H, 7·75; OMe, 29·8; N, 9·0%).

On treatment with methyl-alcoholic ammonia the syrupy mixture of lactones yielded a syrup, which crystallised on keeping. The crystals were freed from adhering syrup by trituration with alcohol–ether and after recrystallisation from acetone–ether–light petroleum, the amide of 2 : 3 : 4-trimethyl *l*-arabonic acid had m. p. 107°, $[\alpha]_D^{22°}$ + 24·4° in water (*c*, 1·1), $[\alpha]_D^{20°}$ + 45° in methyl alcohol (*c*, 1·2) (Found: C, 46·8; H, 8·4; OMe, 44·1; N, 6·4. Calc. for $C_8H_{17}O_5N$: C, 46·4; H, 8·3; OMe, 45·0; N, 6·8%).

Heptamethyl 3-d-*Galactopyranosido*-l-*arabopyranose from Fraction II.*—Redistillation of fraction II gave a colourless viscous oil, b. p. (bath temp.) 180°/0·07 mm., $n_D^{16°}$ 1·4678, which crystallised spontaneously. After recrystallisation from light petroleum *heptamethyl* 3-d-*galactopyranosido*-l-*arabopyranose* (I) had m. p. 82°, $[\alpha]_D^{18°}$ + 162° in water (*c*, 2·0) (Found: C, 52·8; H, 8·2; OMe, 51·3. $C_{18}H_{34}O_{10}$ requires C, 52·7; H, 8·4; OMe, 52·9%).

The crystalline disaccharide (8 g.) was boiled for 10 hours with methyl alcohol (280 c.c.) containing hydrogen chloride (10 g.). The solution was neutralised with silver carbonate, filtered, and evaporated to a syrup, which was distilled, giving 7·7 g., b. p. (bath temp.) 100—105°/0·3 mm., $n_D^{18°}$ 1·4530. The product did not reduce Fehling's solution and had $[\alpha]_D^{18°}$ + 108° in water (*c*, 1·0). The glycosides of 2 : 3 : 4 : 6-tetramethyl galactose and 2 : 4-dimethyl *l*-arabinose formed a constant-boiling mixture and separation by distillation could not be effected (Found: OMe, 55·4. Calc. for an equimolecular mixture of tetramethyl methylgalactoside and dimethyl methylarabinoside: OMe, 56·1%).

The mixture of glycosides (7·3 g.) obtained from the previous experiment was dissolved in dilute sulphuric acid (200 c.c., 7%) and heated on a boiling water-bath until the rotation became constant ($[\alpha]_D$ + 95°). The solution was neutralised with barium carbonate, filtered, and evaporated to dryness under diminished pressure. Distillation of the syrupy residue gave a colourless oil (6·7 g.), b. p. (bath temp.) 125°/0·02 mm., $n_D^{21°}$ 1·4700, $[\alpha]_D^{18°}$ + 102° in water (*c*, 0·5). Separation of this mixture of reducing methylated sugars (B) by distillation was also impossible (Found: OMe, 43·7. Calc. for an equimolecular mixture of tetramethyl galactose and dimethyl arabinose: OMe, 44·9%).

When subjected to complete methylation with silver oxide and methyl iodide, the mixture of sugars (dimethyl arabinose and tetramethyl galactose) yielded a mobile non-reducing syrup, b. p. (bath temp.) 105°/0·25 mm., $n_D^{18°}$ 1·4453, from which there separated 2 : 3 : 4 : 6-tetramethyl β-methylgalactopyranoside, m. p. 47—48° alone or in admixture with an authentic specimen. The mother-liquors, containing all the 2 : 3 : 4-trimethyl methyl-*l*-arabinoside and some 2 : 3 : 4 : 6-tetramethyl methylgalactoside, were freed from solvent, and hydrolysed with dilute sulphuric acid (6%), giving the corresponding reducing sugars. The solution was neutralised with barium carbonate and the sugars were isolated by evaporation under diminished pressure. The mixture was oxidised with bromine (3 mols.) in water at 30—35° until the solution was non-reducing. The acidic product was isolated in the usual way, and the mixture of lactones distilled in a vacuum. On treatment with phenylhydrazine in the manner previously described, the lactone mixture gave small quantities of the phenylhydrazide of 2 : 3 : 4 : 6-tetramethyl galactonic acid, m. p. 138°, and the phenylhydrazide of 2 : 3 : 4-trimethyl *l*-arabonic acid, m. p. 156°, thus showing that the disaccharide consisted of one molecule of galactose united by a glycosidic link with one molecule of arabinose.

The mixture of methylated sugars (B) (0·5 g.) was dissolved in water (10 c.c.) and treated with bromine (3 mols.) at 35° for 2 days. The lactone so produced was isolated as above and distilled, b. p. (bath temp.) 170°/0·03 mm., $n_D^{15°}$ 1·4612 (0·3 g.). The distillate was acidic and did not reduce boiling Fehling's solution. The syrupy lactone mixture was treated with methyl-alcoholic ammonia for 12 hours at 0°. Removal of the excess of solvent left a residue, which

crystallised on trituration with ether–alcohol. The 2 : 4-*dimethyl* l-*arabonamide* was filtered off, dried, and recrystallised from alcohol–ether. It had m. p. 158°, $[\alpha]_D^{17°}$ + 58° in water (*c*, 1·4) (Found: C, 43·55; H, 7·6; OMe, 31·4; N, 7·2. $C_{17}H_{15}O_5N$ requires C, 43·5; H, 7·85; OMe, 32·1; N, 7·25%). Removal of the solvent from the mother-liquors gave another crystalline substance, which was recrystallised from alcohol–ether–light petroleum. It had m. p. 121—122°, alone or in admixture with authentic 2 : 3 : 4 : 6-tetramethyl galactonamide.

Separation of 2 : 3 : 4 : 6-*Tetramethyl Galactose and* 2 : 4-*Dimethyl* l-*Arabinose.*— (a) *By means of aniline.* The mixture of sugars (3·3 g.) was refluxed in alcoholic solution with aniline (1·7 g.) for 5 hours. The tetramethyl galactose anilide (m. p. 193°) was filtered off and washed with ether. The mother-liquors were evaporated and more crystalline material was obtained. The process was repeated until no more 2 : 3 : 4 : 6-tetramethyl galactose anilide separated (total yield, 1·9 g.). The syrupy residual anilide of dimethyl arabinose was dissolved in dilute sulphuric acid (3%) and heated on a boiling water-bath for 2 hours. The solution was neutralised with barium carbonate, filtered, extracted several times with ether to remove aniline, and evaporated to dryness under diminished pressure. The pale yellow syrup (1·3 g.) thus obtained still contained some tetramethyl galactose. It had $[\alpha]_D^{21°}$ + 94° in water (*c*, 1·8) (Found: OMe, 37·4. Calc. for $C_7H_{14}O_5$: OMe, 34·8%).

(b) *Preferential bromine oxidation.* Trial experiments indicated that bromine oxidation of 2 : 4-dimethyl *l*-arabinose proceeded with great facility at 20°. The mixture of sugars (1 g.) was therefore oxidised with bromine (1·1 mols.) in aqueous solution (15 c.c.) for 1 hour at 20° and 1 hour at 35°. The excess of bromine was removed and the solution, which still had reducing properties, was neutralised with silver oxide and filtered. The filtrate was treated with hydrogen sulphide, filtered, and concentrated under diminished pressure at 35° to 50 c.c. The acid solution was neutralised with barium carbonate, filtered, and evaporated to dryness. The residue was exhaustively extracted with ether to remove tetramethyl galactose, and the ether-insoluble barium 2 : 4-dimethyl *l*-arabonate was dissolved in water and treated with a slight deficiency of dilute sulphuric acid. After addition of a little charcoal the solution was filtered and evaporated to a syrup, which was distilled in a vacuum; $n_D^{21°}$ 1·4700, $[\alpha]_D^{18°}$ + 85° (initial value in water, *c*, 1·4); $[\alpha]_D$ + 27° (14½ hours, constant value). The distillate was acid to Congo paper (Found: OMe, 35·5. $C_7H_{12}O_5$ requires OMe, 35·2%). On treatment with methyl-alcoholic ammonia the *lactone* gave 2 : 4-dimethyl *l*-arabonamide in good yield, m. p. 158°. This amide gave no sodium *iso*cyanate when treated with a standard hypochlorite solution, since, on addition of semicarbazide, the highly characteristic hydrazodicarbonamide was not obtained.

Oxidation of 2 : 4-*Dimethyl* l-*Arabinose with Nitric Acid.*—The 2 : 4-dimethyl *l*-arabinose (1·4 g.) was dissolved in dilute nitric acid (25 c.c., *d* 1·2), and the solution warmed for 20 hours at 50°. It was then diluted with water and evaporated under diminished pressure to remove nitric acid, water, and finally methyl alcohol, being added to facilitate the process. The dry acid residue was boiled for 6 hours with 1% methyl-alcoholic hydrogen chloride (100 c.c.) to effect esterification. The solution was then cooled, neutralised with silver carbonate, filtered, and evaporated to dryness. The *methyl* β-*hydroxy*-αγ-*dimethoxy*-l-*araboglutarate* thus obtained was distilled, giving a colourless mobile oil (0·9 g.), b. p. (bath temp.) 115°/0·02 mm., $n_D^{22°}$ 1·4450, $[\alpha]_D^{23°}$ + 41·3° in methyl alcohol (*c*, 0·8) (Found: OMe, 50·8; CO_2Me, 49·7. $C_9H_{16}O_7$ requires OMe, 52·6; CO_2Me, 50·0%). When the methyl ester was treated with methyl-alcoholic ammonia for 24 hours at − 5°, the *diamide* of β-*hydroxy*-αγ-*dimethoxy*-l-*araboglutaric acid* was readily formed in good yield. Removal of the excess of solvent left a crystalline residue of needles, which was recrystallised from aqueous alcohol. The product had m. p. 285° (decomp.), $[\alpha]_D^{17°}$ + 62·1° in water (*c*, 1·6). It was sparingly soluble in alcohol and insoluble in acetone, ether, and light petroleum (Found: C, 40·6; H, 6·7; OMe, 30·2; N, 13·9. $C_7H_{14}O_5N_2$ requires C, 40·7; H, 6·8; OMe, 30·1; N, 13·6%).

Heptamethyl 3-d-*Galactopyranosido*-l-*arabofuranose* (VI).—The reducing syrup (A) (50 g.) produced by autohydrolysis of arabic acid was dissolved in methyl alcohol (2000 c.c.) containing hydrogen chloride (10 g.), and the mixture kept at 18°. After 7 days, when the solution was non-reducing and the specific rotation had become constant (slightly negative), it was neutralised with lead carbonate, filtered, and evaporated to dryness under diminished pressure. The syrupy residue was dissolved in alcohol, filtered to remove a small quantity of inorganic impurity, and again freed from solvent. The dry syrup was dissolved in methyl iodide containing a small quantity of methyl alcohol and refluxed for 10 hours in the presence of excess of silver oxide. The solution was then filtered, and the residue washed well with hot methyl alcohol. Removal of solvent from the filtrate gave a pale yellow syrup. After three such methylations the product was completely soluble in methyl iodide and it was then subjected

to three more methylations with silver oxide and methyl iodide without addition of methyl alcohol. The material isolated by means of acetone (53·6 g.) was distilled, giving: Fraction I (40 g.), b. p. (bath temp.) 100—120°/0·01 mm., $n_D^{20°}$ 1·4240—1·4540, consisting mainly of 2 : 3 : 5-trimethyl *l*-arabofuranoside and 2 : 3 : 5-trimethyl methyl-*l*-rhamnofuranoside. Fraction II, *heptamethyl* 3-d-*galactopyranosido*-l-*arabofuranose* (10·3 g.), b. p. (bath temp.) 170—180°/0·01 mm., $n_D^{20°}$ 1·4670, $[\alpha]_D$ + 102° in water (*c*, 2·9) (Found: OMe, 51·0. $C_{18}H_{34}O_{10}$ requires OMe, 52·9%).

Hydrolysis of Heptamethyl 3-d-*Galactopyranosido*-l-*arabofuranose.*—A solution of methylated disaccharide (fraction II) (2·9 g.) in water (100 c.c.) containing concentrated sulphuric acid (3 g.) was heated on a boiling water-bath until the rotation became constant ($[\alpha]_D$ + 64·5° after 6 hours). The solution was neutralised with barium carbonate, filtered, and freed from solvent by distillation under diminished pressure. The product was dissolved in alcohol, and a small amount of barium carbonate filtered off. Removal of solvent from the filtrate furnished a reducing syrup, containing 2 : 3 : 4 : 6-tetramethyl galactose and 2 : 5-*dimethyl* l-*arabinose*. The syrupy mixture of sugars was extracted six times with boiling light petroleum (30 c.c.); the combined extracts gave on removal of solvent a syrup (0·6 g.) consisting chiefly of 2 : 3 : 4 : 6-tetramethyl galactose. It had $[\alpha]_D^{15°}$ + 102° in water (*c*, 0·6) (Found: OMe, 48·0. Calc. for $C_{10}H_{20}O_6$: OMe, 52·7%). The syrup readily yielded on boiling with alcoholic aniline the characteristic anilide of 2 : 3 : 4 : 6-tetramethyl galactose, m. p. 192° alone or in admixture with an authentic specimen. The portion of the syrup insoluble in light petroleum (1·7 g.) containing the 2 : 5-dimethyl *l*-arabinose had $[\alpha]_D^{16°}$ + 46·6° in water (*c*, 0·7) (Found: OMe, 38·0. $C_7H_{14}O_5$ requires OMe, 34·8%).

Oxidation of 2 : 5-*Dimethyl* l-*Arabinose with Bromine.*—The syrup (1·7 g.) in water (20 c.c.) was allowed to react with bromine (3 c.c.) at room temperature for 2 days; the solution then had no action on boiling Fehling's solution. The oxidation product, isolated as in previous cases, was distilled, giving a mixture consisting chiefly of 2 : 5-dimethyl γ-*l*-arabonolactone and some 2 : 3 : 4 : 6-tetramethyl δ-galactonolactone (1·4 g.), b. p. (bath temp.) 160°/0·05 mm., $n_D^{18°}$ 1·4630; $[\alpha]_D^{16°}$ + 8·7° (initial value); — 47·3° (3 hours); — 24° (19 hours); — 22° (44 hours); — 19° (98 hours); —16° (168 hours) (Found: OMe, 42·7. Calc. for $C_7H_{12}O_5$: OMe, 35·2%. Calc. for $C_{10}H_{18}O_6$: OMe, 53·0%). 2 : 5-*Dimethyl* l-*arabonophenylhydrazide* was obtained when the lactone mixture (1·3 g.) was heated with phenylhydrazine (1 g.) for 3 hours at 90°. After recrystallisation from alcohol–ether, the crystalline product (0·9 g.) had m. p. 163° (Found: C, 54·9; H, 7·3; OMe, 21·4; N, 10·0. $C_7H_{15}O_5N$ requires C, 54·9; H, 6·9; OMe, 21·8; N, 9·9%). Evaporation of the mother-liquors gave a small amount (0·1 g.) of the phenylhydrazide of 2 : 3 : 4 : 6-tetramethyl galactonic acid, which after recrystallisation from alcohol–ether had m. p. 138°, alone or in admixture with an authentic specimen (Found: C, 56·3; H, 7·7; OMe, 37·0; N, 8·5. Calc. for $C_{16}H_{26}O_6N_2$: C, 56·1; H, 7·7; OMe, 36·2; N, 8·2%).

Regeneration of 2 : 5-*dimethyl* γ-l-*arabonolactone* was effected by heating the phenylhydrazide (0·8 g.) in 0·1N-sulphuric acid (50 c.c.) for 12 hours at 75°. The solution was cooled, neutralised with barium carbonate, filtered, and exhaustively extracted with ether to remove phenylhydrazine. The aqueous solution was treated with a slight excess of dilute sulphuric acid, neutralised with lead carbonate, and filtered, and the dissolved lead precipitated by hydrogen sulphide. After filtration the solution was evaporated to a syrup, which was distilled (0·3 g.), b. p. (bath temp.) 145°/0·07 mm. The colourless distillate readily crystallised on keeping and after recrystallisation from ether 2 : 5-dimethyl γ-*l*-arabonolactone had m. p. 60°; $[\alpha]_D^{15°}$ — 59·7° (initial value in water, *c*, 1·0); —54·2° (6 hours); —52·2° (100 hours); —49·0° (180 hours); —44·8° (320 hours, mutarotation still incomplete). The free acid, liberated from its sodium salt, had $[\alpha]_D^{15°}$ + 25·8° [initial value in acidified (sulphuric acid) aqueous solution, *c*, 1·0]; —16·0° (120 hours, constant value). A freshly prepared aqueous solution of this lactone was not acid to Congo-red or litmus, but gradually developed acidic properties on keeping (Found: C, 47·3; H, 6·8; OMe, 35·0. $C_7H_{12}O_5$ requires C, 47·7; H, 6·9; OMe, 35·2%). 2 : 5-*Dimethyl* l-*arabonamide* was obtained from the crystalline lactone or the crude syrupy lactone on treatment with methyl-alcoholic ammonia at — 5° for 24 hours. The solid amide obtained on removal of the solvent separated in needles from ethyl alcohol–ether and had m. p. 131°, $[\alpha]_D^{18°}$ + 38·0° in water (*c*, 1·4). A Weerman test for α-hydroxy-amides on this amide was negative. When treated with sodium hypochlorite, it gave no sodium *iso*cyanate (tested with semicarbazide) (Found: C, 43·8; H, 8·3; OMe, 31·9; N, 7·2. $C_7H_{15}O_5N$ requires C, 43·5; H, 7·85; OMe, 32·1; N, 7·25%).

When the 2 : 5-dimethyl γ-*l*-arabonolactone (0·1 g.) was methylated by one treatment with methyl iodide and silver oxide, 2 : 3 : 5-trimethyl γ-*l*-arabonolactone was obtained. The lactone

was distilled, b. p. (bath temp.) 120°/0·03 mm. (0·1 g.), and it crystallised immediately on nucleation with 2 : 3 : 5-trimethyl γ-*l*-arabonolactone. After recrystallisation from light petroleum it had m. p. 30°, $[\alpha]_D^{16°}$ — 44° (initial value in water; *c*, 1·0). When treated with methyl-alcoholic ammonia, the lactone gave the characteristic amide of 2 : 3 : 5-trimethyl *l*-arabonic acid, m. p. 137°, $[\alpha]_D$ + 18° in water (*c*, 2·0) (yield, quantitative) (Found: C, 46·5; H, 7·9; OMe, 44·5; N, 6·8. Calc. for $C_8H_{17}O_5N$: C, 46·4; H, 8·3; OMe, 44·9; N, 6·8%).

Preparation of Heptamethyl 3-β-d-*Galactopyranosido*-d-*arabopyranose*.—Lactose was converted by the method of Wohl into 3-β-*d*-galactosido-*d*-arabinose according to the directions given by Zemplén (*Ber.*, 1926, **59**, 2405) and isolated as the crystalline benzylphenylhydrazone. From the latter (26 g.) the free sugar was regenerated by heating with benzaldehyde (35 c.c.) in aqueous alcohol (3·5 l. of water and 100 c.c. of ethyl alcohol) for 1½ hours on a boiling water-bath The solution was cooled, and the benzaldehydebenzylphenylhydrazone filtered off. The aqueous solution was exhaustively extracted with ether to remove the excess of benzaldehyde and any benzaldehydebenzylphenylhydrazone. It was then concentrated to 50 c.c. under diminished pressure at 35°, giving a solution which reduced Fehling's solution actively on boiling.

The aqueous solution containing the 3-β-*d*-galactosido-*d*-arabinose was methylated with methyl sulphate (100 c.c.) and sodium hydroxide solution (250 c.c. of 30%) in the presence of a small quantity of acetone, four-tenths of the reagents being added at 20° and the remaining six-tenths at 40° during 4 hours. The methyl derivative was extracted with chloroform and, after the combined chloroform extracts had been dried over anhydrous magnesium sulphate, the solution was evaporated to give a syrup (14 g.). Methylation of this was completed by means of silver oxide and methyl iodide (two treatments) in the usual manner. The *heptamethyl* 3-β-d-*galactopyranosido*-d-*arabopyranose* was then distilled (11·3 g.), b. p. (bath temp.) 175—180°/0·05 mm.; $n_D^{18°}$ 1·4685; $[\alpha]_D^{16°}$ — 28·4° in methyl alcohol (*c*, 1·5) (Found: OMe, 50·6. $C_{18}H_{34}O_{10}$ requires OMe, 53·0%). Partial crystallisation of the distillate took place on keeping and after recrystallisation from alcohol–ether–light petroleum it had m. p. 136°; $[\alpha]_D^{18°}$ — 12·1° in methyl alcohol (*c*, 1·1). The material appeared to be a *hexamethyl* derivative (Found: OMe, 46·7. $C_{17}H_{32}O_{10}$ requires OMe, 47·0%. $C_{18}H_{34}O_{10}$ requires OMe, 52·9%).

A solution of the methylated disaccharide (10·8 g.) in dilute sulphuric acid (250 c.c., 4%) was heated for 5 hours on a boiling water-bath, the rotation, $[\alpha]_D$ — 15° (initial value), changing to + 18° (constant value). The solution was neutralised with barium carbonate, filtered, and evaporated to dryness. The syrup obtained, consisting of 2 : 3 : 4 : 6-tetramethyl galactose and 2 : 4-dimethyl *d*-arabinose, formed a constant-boiling mixture (9·43 g.), b. p. (bath temp.) 130°/0·10 mm., $n_D^{19°}$ 1·4705; $[\alpha]_D^{18°}$ + 38·7° in water (*c*, 1·4). The distillate reduced Fehling's solution on boiling (Found: OMe, 44·8. Calc.: OMe, 44·9%).

2 : 4-*Dimethyl* d-*Arabinose*.—The dry mixture of methyl sugars (9·38 g.) was boiled for 5 hours in absolute alcohol (50 c.c.) containing aniline (4·8 g.). The solution was cooled, and the 2 : 3 : 4 : 6-tetramethyl galactose anilide filtered off, washed with ethyl alcohol–ether, and dried (5·9 g., m. p. 192°). The filtrate and washings were evaporated to a syrup, which crystallised on trituration with ether–light petroleum (yield, 2·6 g.). The crystals had m. p. 135° and no increase in m. p. was observed after repeated crystallisation from alcohol–ether. Slow crystallisation from absolute alcohol gave two sets of crystals: (a) needles of 2 : 3 : 4 : 6-tetramethyl galactose anilide, m. p. 192°; (b) plates, m. p. 141°. These two crops were mechanically separated and after recrystallisation from ethyl alcohol the plates (b) had m. p. 142—143°. The product was 2 : 4-*dimethyl* d-*arabinose anilide* (Found: C, 61·8; H, 7·6; OMe, 24·9; N, 5·6. $C_{13}H_{19}O_4N$ requires C, 61·65; H, 7·6; OMe, 24·5; N, 5·5%). Scission of the aniline residue from the crude 2 : 4-dimethyl *d*-arabinose anilide, which still contained some 2 : 3 : 4 : 6-tetramethyl galactose anilide, was effected by heating with dilute sulphuric acid (3%) at 80°. The solution was neutralised with barium carbonate, filtered, and evaporated to dryness under diminished pressure at 40°. The syrupy product, consisting chiefly of 2 : 4-dimethyl *d*-arabinose (see also Zemplén and Braun, *Ber.*, 1926, **59**, 2240), was purified by distillation, b. p. (bath temp.) 140°/0·09 mm., $n_D^{17°}$ 1·4800; $[\alpha]_D^{17°}$ — 30·8° in water (*c*, 2·4); $[\alpha]_D^{17°}$ — 37·8° in methyl alcohol (*c*, 1·9). The syrup reduced Fehling's solution on boiling.

Oxidation of 2 : 4-*Dimethyl* d-*Arabinose with Bromine*.—2 : 4-Dimethyl *d*-arabinose (0·9 g.), containing a little 2 : 3 : 4 : 6-tetramethyl galactose, was dissolved in water (20 c.c.) and treated with bromine (0·4 c.c.) for 1 hour at 20° and 2 hours at 40°. The lactone, isolated by the method previously employed, was distilled (0·5 g.), b. p. (bath temp.) 140°/0·15 mm., $n_D^{17°}$ 1·4750; $[\alpha]_D^{18°}$ — 65° (initial value); — 22° (8 hours, constant value). The distillate had a slight reducing action on boiling Fehling's solution owing to a small amount of 2 : 3 : 4 : 6-tetramethyl galactose being present. An aqueous solution of the distillate was acid to Congo-red. The lactone was

converted into the amide by treatment with methyl-alcoholic ammonia at 0°. After recrystallisation from alcohol 2 : 4-*dimethyl* d-*arabonamide* had m. p. 158°, $[\alpha]_D^{17°}$ — 58·8° in water (*c*, 1·1) (Found : C, 43·7; H, 8·0; OMe, 32·0; N, 7·0. $C_7H_{15}O_5N$ requires C, 43·5; H, 7·85; OMe, 32·1; N, 7·25%). Regeneration of the lactone from the crystalline amide (50 mg.) by hydrolysis with barium hydroxide gave a small quantity of crystalline lactone which had $[\alpha]_D^{22°}$ — 85° (initial value in water, *c*, 1·0); — 33·0° (18 hours, constant value).

Oxidation of 2 : 4-*Dimethyl* d-*Arabinose with Nitric Acid.*—The sugar (1·4 g.) was heated with nitric acid (25 c.c., *d* 1·2) for 24 hours at 55°. The solution was diluted with water and freed from nitric acid by distillation under diminished pressure at 50° as in previous cases. The dry acid syrup was esterified by boiling for 6 hours with 3% methyl-alcoholic hydrogen chloride (100 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to a syrup. The *methyl* β-*hydroxy*-αγ-*dimethoxy*-d-*araboglutarate* was distilled, giving a colourless oil (0·95 g.), b. p. (bath temp.) 135°/0·12 mm., $n_D^{20°}$ 1·4475; $[\alpha]_D^{18°}$ — 32° in methyl alcohol (*c*, 0·8) (Found : OMe, 50·9; CO_2Me, 46·0. $C_9H_{16}O_7$ requires OMe, 52·6; CO_2Me, 50·0%). The ester was converted by means of methyl-alcoholic ammonia into the crystalline diamide (β-*hydroxy*-αγ-*dimethoxy*-d-*araboglutaramide*), which after recrystallisation from aqueous methyl alcohol had m. p. 286° (decomp.); $[\alpha]_D^{17°}$ — 62·8° in water (*c*, 1·42) (Found : C, 40·9; H, 7·0; OMe, 30·6; N, 13·5. $C_7H_{14}O_5N_2$ requires C, 40·7; H, 6·8; OMe, 30·1; N, 13·6%).

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