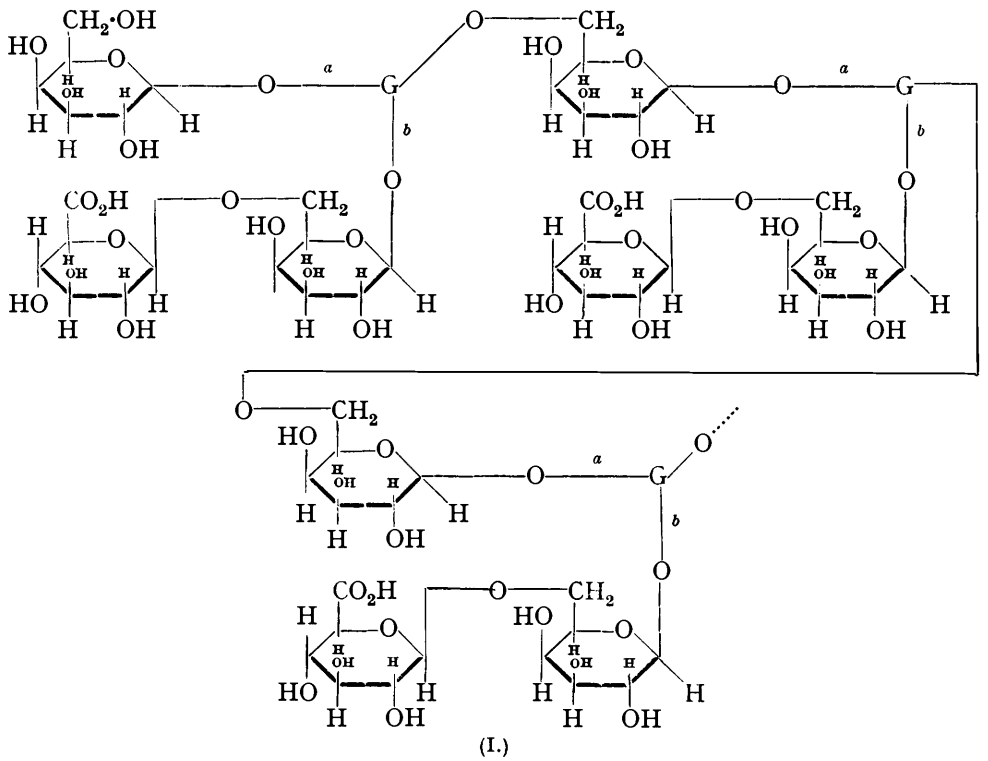
15. *The Constitution of Arabic Acid. Part IV. The Formation of 3-Galactosidogalactose by Hydrolysis of Degraded Arabic Acid.*

By J. JACKSON and F. SMITH.

The cleavage fragments derived from methylated degraded arabic acid consist of 2 : 3 : 4-trimethyl glucuronic acid (3 molecular proportions), 2 : 3 : 4 : 6-tetramethyl galactose (1 molecular proportion), 2 : 3 : 4-trimethyl galactose (5 molecular proportions) and 2 : 4-dimethyl galactose (3 molecular proportions). The identification of 2 : 4-dimethyl and 2 : 3 : 4-trimethyl galactose shows that both the 1 : 6- and the 1 : 3-type of linkage are present in degraded arabic acid. Some of the galactose units are mutually joined by 1 : 6-links, and the formation of 3-galactosidogalactose (II) by prolonged autohydrolysis of degraded arabic acid has now proved that some of the galactose units are also joined by 1 : 3-links. The structure (II) assigned to the galactose disaccharide follows from the fact that when treated with methyl sulphate and sodium hydroxide it gives *octamethyl* 3-*galactosidogalactose* (III). The latter, when boiled with methyl-alcoholic hydrogen chloride, furnishes 2 : 3 : 4 : 6-tetramethyl methylgalactoside (IV) and 2 : 4 : 6-trimethyl methylgalactoside (V), the structures of which are proved. A tentative structure (I) is suggested for degraded arabic acid.

It has been shown that the repeating unit of degraded arabic acid consists of twelve monosaccharide residues, nine of them being galactose units and three being glucuronic acid residues; all the residues have pyranose structures. The mode of linking of these twelve residues involves two types of glycosidic union, namely, 1 : 3- and 1 : 6-linkages. The repeating unit contains four terminal groups, one of galactose and three of glucuronic acid. The " end " galactose residue terminates a chain of galactose units to which are



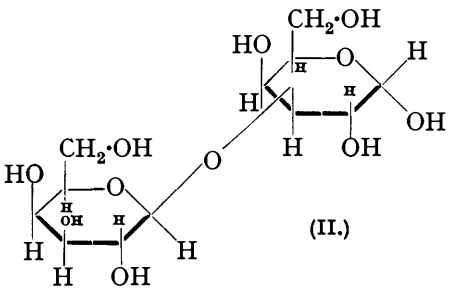
(I.)

(G = galactose unit)

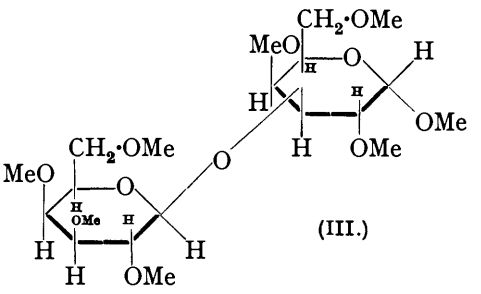
attached three side chains each of which is terminated by a unit of glucuronic acid (Part II; J., 1939, 1724).

In Part III (preceding paper) evidence was advanced that some at least of the side chains which are terminated by glucuronic acid residues consist of aldobionic acid groups so constituted that the glucuronic acid unit is joined through its reducing group to position 6 of a galactose unit. If it be assumed, at this stage, that the whole of the acid constituent of degraded arabic acid is in this aldobionic acid form, it follows that the acid side chains account for six of the twelve monosaccharide residues in the repeating unit and the latter may be tentatively represented by (I). The symmetry of the distribution of the aldobionic acid side chains in (I) is arbitrary and represents the simplest interpretation of the experimental facts. There remain to be determined the positions of the glycosidic links represented in (I) by (*a*) and by (*b*). The three molecular proportions of 2 : 4-dimethyl galactose isolated from methylated degraded arabic acid (see Part II) can only arise from the galactose units represented by G in formula (I). Hence it follows that the linkages (*a*) and (*b*) must be either of the 1 : 3- or of the 1 : 6-type, although it cannot yet be said which of the two types of linkage is represented by (*a*) and which by (*b*).

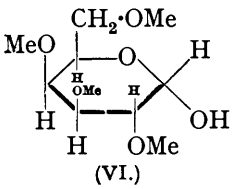
The three side chains of aldobionic acid account for 3 of the 5 molecular proportions of 2 : 3 : 4-trimethyl galactose furnished by the methylated degraded arabic acid; it is clear, therefore, that the other two residues of 2 : 3 : 4-trimethyl galactose can only arise as a result of the mutual union of galactose units by 1 : 6-linkages. The existence of the 1 : 3-type of linkage between galactose residues has now been established by the isolation of 3-galactosidogalactose in the form of its octamethyl derivative (II). This disaccharide was prepared by subjecting degraded arabic acid to prolonged autohydrolysis. The acidic and the non-acidic fragments were separated by utilising the fact that the barium salts of the acidic fragments are insoluble in aqueous alcohol and those of the non-acidic fragments are soluble. The non-acidic portion, composed of a mixture of reducing sugars, was methylated first with methyl sulphate and sodium hydroxide and then with Purdie's reagents to complete the process. Fractional distillation of the product gave an octamethyl derivative of the galactose disaccharide (III), the structure of which was deduced from the following observations. On boiling the methylated disaccharide (III) with methyl-alcoholic hydrogen chloride an equimolecular mixture of the tetramethyl methylgalactoside (IV) and the trimethyl methylgalactoside (V) was obtained.



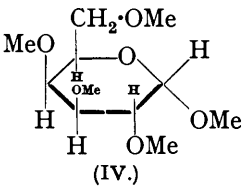
(II.)



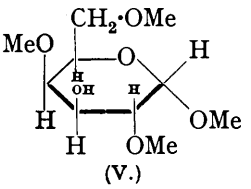
(III.)



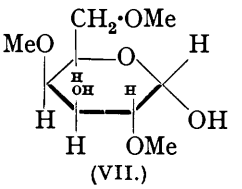
(VI.)



(IV.)



(V.)



(VII.)

Hydrolysis of the tetramethyl methylgalactoside (IV) with dilute sulphuric acid gave the corresponding tetramethyl sugar (VI), which proved to be 2 : 3 : 4 : 6-tetramethyl galactose since on treatment with ethyl-alcoholic aniline it yielded the characteristic

crystalline anilide of 2 : 3 : 4 : 6-tetramethyl galactose. Similarly, hydrolysis of the trimethyl methylgalactoside (V) with dilute sulphuric acid removed the glycosidic methyl residue with the formation of the trimethyl sugar (VII). The latter was readily identified as 2 : 4 : 6-trimethyl galactose, since it afforded a crystalline anilide identical with that obtained from the 2 : 4 : 6-trimethyl galactose of Percival and Somerville (J., 1937, 1615).

Since the *octamethyl* 3-*galactosidogalactose* shows only a slight positive rotation, it may well be that the biose link is of the β-type.

Experimental.

Autohydrolysis of Degraded Arabic Acid.—(*a*) A solution of degraded arabic acid (Smith, J., 1939, 744) (20 g.) in water (200 c.c.) was heated on the boiling water-bath; at suitable intervals a portion was withdrawn and cooled, and the iodine number and rotation determined. [For the determination of the iodine number, 1 c.c. of the solution was treated with 0·1N-iodine (15 c.c.) and N-sodium hydroxide (4·5 c.c.) for 30 minutes at room temperature; the solution was then acidified with N-sulphuric acid (5·5 c.c.), and the excess of the iodine back-titrated with 0·1N-sodium thiosulphate. A blank experiment was carried out simultaneously and the number of c.c. of 0·1N-iodine equivalent to 1 g. of material was calculated.] The aqueous solution of the degraded arabic acid showed $[\alpha]_D$ − 8·0° (initial value); $[\alpha]_D$ ± 0°, iodine number 13·4 (after 16 hrs.); + 3·0°, 18·5 (24 hrs.); + 6·3°, 27·5 (40 hrs.); + 7·6° (47 hrs.); + 9·6°, 35 (62 hrs.); + 11°, 40 (71 hrs.); + 15°, 49 (93 hrs.). At this stage the solution had become brown and accurate polarimetric readings could not be taken and hence further autohydrolysis was followed only by the iodine values. Thus, after 118 hours the iodine number was 58; 59 (127 hrs.); 64 (141 hrs.); 71 (165 hrs.); 76·5 (189 hrs.); 82·5 (213 hrs.); 93·5 (238 hrs.). These figures indicate that there is no arrest in the breakdown of the degraded arabic acid during autohydrolysis.

(*b*) In a subsequent experiment a solution of degraded arabic acid (100 g.) in water (750 c.c.) was heated on the boiling water-bath for 95 hours; the iodine number was then 54·4, corresponding to *M*, 365. The solution was neutralised with barium carbonate, filtered, concentrated to a small bulk, and poured with stirring into methyl alcohol. A few drops of an aqueous solution of calcium chloride were added in order to coagulate the barium salts of the uronic acids. The precipitate of barium salts was separated on the centrifuge (yield, 75 g.) and the aqueous methyl-alcoholic solution was evaporated to dryness under diminished pressure. In this way there was obtained a non-crystalline residue (28 g.) consisting of a mixture of reducing sugars (Found : iodine number, *ca.* 65, corresponding to *M*, 307).

Methylation of the Mixture of Sugars.—The mixture of sugars (14 g.) containing the 3-galactosidogalactose (III) was dissolved in water (15 c.c.) and treated with methyl sulphate (120 c.c.) and a 30% aqueous solution of sodium hydroxide (360 c.c.) with vigorous stirring. The reagents were slowly added in one-tenth portions during 3 hours, three-tenths being added at room temperature; when the reducing power of the solution had disappeared, the remaining seven-tenths were added at 45°. Small amounts of acetone were added from time to time to facilitate the process of methylation. The methylation was completed by heating for 15 minutes at 80°; the solution was then cooled and extracted six times with chloroform. The combined chloroform extracts were washed once with water, dried over anhydrous magnesium sulphate, filtered, and evaporated to dryness (yield of methylated sugars, 4·8 g.). The aqueous solution which had been extracted with chloroform and the water used for washing the chloroform extract were combined, cooled in ice, and almost neutralised with 10N-sulphuric acid. The sodium sulphate was filtered off and the filtrate was concentrated under reduced pressure to a suitable bulk; the residue thus obtained, consisting of sodium sulphate and some incompletely methylated sugars, was remethylated at 45° during 90 minutes, the same quantities of methyl sulphate and sodium hydroxide being used as in the first methylation. This methylation furnished 2·2 g. of methylated product.

To effect complete methylation of the sugars, the combined methylated products (7·0 g.) were given three treatments with Purdie's reagents. The product, isolated after each methylation by means of acetone, gave on distillation : Fraction I (2 : 3 : 4 : 6-tetramethyl methylgalactoside), 5·5 g., b. p. (bath temp.) 120°/0·04 mm., $n_D^{19°}$ 1·4440 (Found : OMe, 61·0. Calc. for $C_{11}H_{22}O_6$: OMe, 61·9%). Fraction II, 0·28 g., b. p. (bath temp.) 150—205°/0·03 mm., $n_D^{19°}$ 1·4490—1·4620. Fraction III (*octamethyl* 3-*galactosidogalactose*) (IV), 0·67 g., b. p.

(bath temp.) 215—230°/0·03 mm., $n_D^{20°}$ 1·4660, $[\alpha]_D^{19°}$ *ca.* + 0·5° in water (*c*, 2·2) (Found: OMe, 53·3. $C_{20}H_{38}O_{11}$ requires OMe, 54·6%).

Hydrolysis of Octamethyl 3-Galactosidogalactose (IV).—The fully methylated 3-galactosidogalactose (0·65 g.) was boiled for 8 hours with 4% methyl-alcoholic hydrogen chloride (32 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to dryness under reduced pressure (yield, 0·67 g.). The syrupy mixture of methylated galactosides was distilled, giving: Fraction I (2 : 3 : 4 : 6-tetramethyl methylgalactoside) (V), 0·3 g., b. p. (bath temp.) 110—120°/0·02 mm., $n_D^{21°}$ 1·4484—1·4510 (Found: OMe, 58·1. Calc. for $C_{11}H_{22}O_6$: OMe, 61·9%). Fraction II (2 : 4 : 6-trimethyl methylgalactoside) (VI), 0·27 g., b. p. (bath temp.) 145—150°/0·02 mm., $n_D^{19°}$ 1·4610—1·4630 (Found: OMe, 52·1. Calc. for $C_{10}H_{20}O_6$: OMe, 52·6%).

Identification of 2 : 3 : 4 : 6-*Tetramethyl Galactose* (VII).—A solution of fraction I (0·28 g.) in N-sulphuric acid (10 c.c.) was heated for 4 hours on the boiling water-bath. The solution was then neutralised with barium carbonate, filtered, and evaporated to dryness. The 2 : 3 : 4 : 6-tetramethyl galactose thus obtained was purified by extraction with ether (yield, 0·21 g.). It showed $[\alpha]_D^{18°}$ + 100° in water (*c*, 1·0) (Found: OMe, 50·6. Calc. for $C_{10}H_{20}O_6$: OMe, 52·6%).

When the 2 : 3 : 4 : 6-tetramethyl galactose (0·1 g.) was boiled with ethyl alcohol containing aniline (0·06 g.) for 3 hours, the corresponding anilide was obtained; the excess of the solvent was removed and the 2 : 3 : 4 : 6-tetramethyl galactose anilide (crystallised from ethyl alcohol–ether) had m. p. and mixed m. p. 192° (Found: OMe, 40·0; N, 4·6. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·8; N, 4·5%).

Identification of 2 : 4 : 6-*Trimethyl Galactose* (VIII).—A solution of fraction II (0·25 g.) in N-sulphuric acid (5 c.c.) was heated on the boiling water-bath for 5 hours. The solution was cooled, neutralised with barium carbonate, filtered, and evaporated to dryness under diminished pressure, giving 2 : 4 : 6-trimethyl galactose as a syrup, which was purified by extraction with ether (yield, 0·19 g.) (Found: OMe, 41·0. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

On boiling for 3 hours in ethyl alcohol (2 c.c.) containing aniline (0·08 g.), this syrupy 2 : 4 : 6-trimethyl galactose (0·15 g.) gave the corresponding anilide, which crystallised on removal of the excess of the solvent. After crystallisation from ethyl alcohol–ether the 2 : 4 : 6-trimethyl galactose anilide had m. p. 178° alone or in admixture with an authentic specimen supplied by Dr. E. G. V. Percival of Edinburgh University (Found: C, 60·7; H, 7·7; OMe, 31·1; N, 4·8. Calc. for $C_{15}H_{23}O_5N$: C, 60·6; H, 7·8; OMe, 31·3; N, 4·7%).

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THE A. E. HILLS LABORATORIES,
UNIVERSITY OF BIRMINGHAM, EDGBASTON.

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