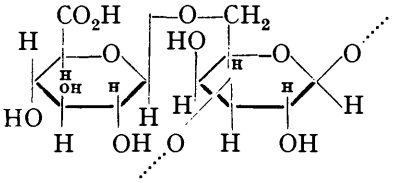
14. *The Constitution of Arabic Acid. Part III. The Isolation of Methyl Heptamethyl Aldobionate from Methylated Degraded Arabic Acid.*

By J. Jackson and F. Smith.

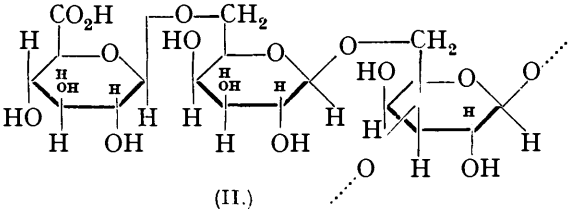
Degraded arabic acid, obtained from arabic acid by autohydrolysis, is composed of units of glucuronic acid and galactose all of which have the pyranose structure. An examination of the cleavage products of methylated degraded arabic acid has shown that the repeating unit of this degraded polysaccharide acid probably consists of a chain of galactose residues to which are attached three side chains each of which is terminated by a residue of glucuronic acid. The present work shows that graded hydrolysis of methylated degraded arabic acid gives a *hexamethyl* 6-β-*glucuronosido-galactose* (III), the constitution of which is proved by the fact that on boiling the corresponding methyl ester glycoside (IV) with methyl-alcoholic hydrogen chloride the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (V) and 2 : 3 : 4-trimethyl methylgalactoside (VII) are obtained. It is suggested, therefore, that each side chain is composed of a terminal or " end " residue of glucuronic acid which is linked through at least one galactose unit with the main galactose chain as shown in (II).

It was shown by Challinor, Haworth, and Hirst (J., 1931, 258) that the aldobionic acid obtained from arabic acid by hydrolysis is composed of a unit of glucuronic acid linked through its reducing group with position 6 of a galactose residue. This formulation was confirmed by Hotchkiss and Goebel (*J. Amer. Chem. Soc.*, 1936, **58**, 858) inasmuch as synthetic 6-β-glucuronosidogalactose proved to be identical with the aldobionic acid derived from arabic acid.

Further information concerning the constitution of arabic acid was obtained when it was demonstrated that methylated degraded arabic acid gave on hydrolysis 2 : 3 : 4 : 6-tetramethyl galactose, 2 : 3 : 4-trimethyl galactose, 2 : 4-dimethyl galactose, and 2 : 3 : 4-trimethyl glucuronic acid (Smith, J., 1939, 1724). It was pointed out that the identification of the glucuronic acid as the 2 : : 4-trimethyl derivative demonstrated that the uronic acid must be present as terminal groups in the branched-chain structure of degraded arabic acid. It was not possible at that time to define more precisely the structure of these side chains, but it is clear that the glucuronic acid residues may be attached directly by a 1 : 6-link to the main chain of galactose residues as in (I) or, alternatively, that one or more galactose units may be interposed between the terminal glucuronic acid residue and the main chain, as, for example, in formula (II), in which one galactose unit is interposed between the terminal uronic acid and the main chain of galactose units. In (II) the side chain aldobionic acid residue is joined to the main galactose chain by a 1 : 6-link, but the possibility of the side chains being joined by a 1 : 3-link must also be borne in mind.



(I.)

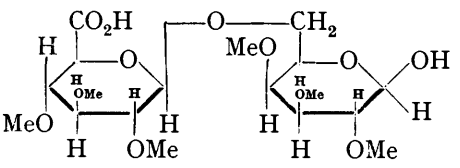


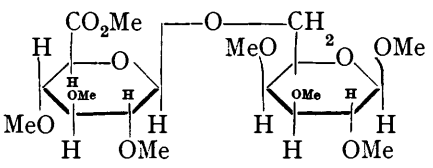
(II.)

A decision between the structures represented by (I) and (II) could be made if it were possible to isolate an aldobionic acid from methylated degraded arabic acid, and if it were possible also to ascertain the disposition of the methoxyl groups in the galactose moiety of this partially methylated aldobionic acid. If the mode of linking present in degraded arabic acid is to be represented by (I), the galactose constituent of the aldobionic acid derived from it will be the 2 : 4-dimethyl derivative, whereas if (II) is the correct representation, the galactose residue will appear in the form of 2 : 3 : 4-trimethyl galactose.

The object in view, namely, the isolation of a methylated aldobionic acid by the graded hydrolysis of methylated degraded arabic acid, has now been achieved and moreover the structure of this aldobionic acid has been established. When methylated degraded arabic acid is hydrolysed with cold 14N-sulphuric acid, a hexamethyl aldobionic acid is liberated; the experimental evidence outlined below shows that this product has the constitution represented by (III). The hexamethyl aldobionic acid was subjected to simultaneous esterification and glycoside formation by the agency of cold acid methyl

(III.)



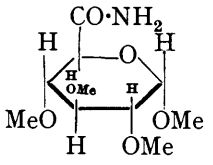


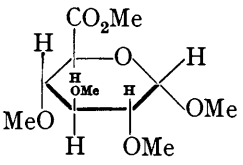
(IV.)

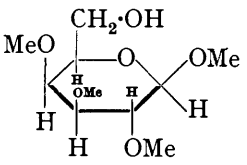
alcohol, thus forming the methyl ester glycoside (IV). The equivalent weight, methoxyl content, and boiling point of (IV) suggested that it was a methyl ester of a completely methylated aldobionic acid. When boiled with methyl-alcoholic hydrogen chloride, (IV) furnished an approximately equimolecular mixture of the methyl ester of the trimethyl methylglucuronoside (V) and the trimethyl methylgalactoside (VII).

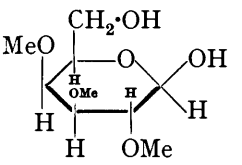
After separation of these two substances the uronic acid fragment (V) was identified by its ready conversion into the characteristic crystalline amide of 2 : 3 : 4-trimethyl α-methylglucuronoside (VI), the structure of which is known (Smith, *loc. cit.*).

The trimethyl methylgalactoside (VII) gave on hydrolysis with dilute sulphuric acid the corresponding trimethyl sugar (VIII), which was identified as 2 : 3 : 4-trimethyl


(VI.)


(V.)


(VII.)


(VIII.)

galactose since it afforded a crystalline anilide identical with an authentic specimen of 2 : 3 : 4-trimethyl galactose anilide (McCreath and Smith, J., 1939, 387).

These facts indicate that the side chains in degraded arabic acid do not consist of a single unit of glucuronic acid as shown in (I), but rather that each side chain is composed of a terminal or " end " glucuronic acid group which is linked through at least one galactose residue with the main galactose chain as shown in (II).

Experimental.

Methylation of Degraded Arabic Acid.—The barium salt of degraded arabic acid was prepared from arabic acid by autohydrolysis (Part I; J., 1939, 744). Methylation of the barium salt (120 g.) was effected according to the directions given in Part II (J., 1939, 1724) and after six methylations the product (70 g.) had $[\alpha]_D^{18°}$ — 51° in chloroform (*c*, 0·8); equiv. 825 (Found : OMe, 39·7%).

Simultaneous esterification and completion of the methylation of this methylated degraded arabic acid (63 g.) was effected by subjecting it to three treatments with silver oxide and methyl iodide, the product, after each Purdie methylation, being isolated by means of acetone (yield, 60 g.). The methyl ester of methylated degraded arabic acid was then purified by fractional precipitation as follows. The material was dissolved in chloroform (150 c.c.), and ether (150 c.c.) added. Into this solution light petroleum was slowly stirred until a convenient amount of the methylated substance had been precipitated; the liquid was then decanted and treated with more light petroleum to give a second precipitate. In this manner six fractions were obtained and each one was dissolved in acetone and precipitated as a white powder by pouring the acetone solution into excess of light petroleum; the precipitate was separated on the centrifuge, washed with dry light petroleum, and dried. The properties of the fractions indicated that the methyl ester of methylated degraded arabic acid was essentially homogeneous. These fractions showed $[\alpha]_D^{18°}$ — 56° in chloroform (*c*, 1·3), $\eta_{sp.}^{20°}$ 0·035 in *m*-cresol (*c*, 0·4) (Found : OMe, 45·6%; equiv., 810).

Hydrolysis of the Methyl Ester of Methylated Degraded Arabic Acid with 14N-*Sulphuric Acid.*—(*a*) A solution of the methyl ester of methylated degraded arabic acid (0·1 g.) in 14N-sulphuric acid underwent hydrolysis as shown by the change in rotation thus : $[\alpha]_D$ — 14° (20 mins.); + 6·5° (2½ hrs.); + 11·3° (15 hrs.); + 22·5° (19½ hrs.); + 49° (26½ hrs.); + 63° (39 hrs.); + 83·5° (50 hrs.); + 94° (63 hrs.); + 103° (112 hrs.). This solution, when neutralised with sodium hydroxide, reduced Fehling's solution actively.

(*b*) A solution of the methyl ester of methylated degraded arabic acid (10·4 g.) in 14N-sulphuric acid (100 c.c.) was kept at room temperature for 51 hours. After the hydrolysis had proceeded for 18 hours 1 c.c. of the solution reacted with 3·74 c.c. of 0·05N-iodine in alkaline solution, corresponding to an apparent molecular weight of 1100; after 42 hours the apparent molecular weight was 675 and after 51 hours it was 570. The solution was then diluted to 500 c.c. with ice-water, neutralised with barium carbonate, treated with charcoal, filtered, and evaporated to dryness under diminished pressure. The material thus obtained (7·1 g.), consisting of a mixture of methylated sugars and the barium salts of methylated uronic acids, readily reduced Fehling's solution.

Separation of the Barium Salts of the Uronic Acids and the Methylated Sugars.—To a solution of the mixture (7·1 g.) in methyl alcohol (15 c.c.), dry ether was added slowly with

stirring; the white flocculent precipitate thus produced was separated on the centrifuge, giving fraction I (3·45 g.). Addition of more ether until there was no further precipitate gave fraction II (0·445 g.). The mother-liquors were concentrated to a syrup, which was dissolved in methyl alcohol and treated with an excess of ether, whereby fraction III (0·608 g.) was precipitated. Fraction I reduced Fehling's solution actively; it had equiv. 290 (from an estimation of the barium content), and an iodine number of 32, corresponding to *M*, 630. Removal of the solvent from the mother-liquors after fraction III had been removed gave a syrup (2·44 g.) consisting of a mixture of reducing methylated sugars. The constituents of this mixture are now under investigation.

The three fractions of the barium salts were combined, dissolved in water, and treated with a slight excess of N-sulphuric acid; the barium sulphate was filtered off, and the solution neutralised with lead carbonate, filtered, and treated with hydrogen sulphide. The precipitate of lead sulphide was filtered off and the solution, containing the free organic acid, was evaporated to dryness under reduced pressure. The syrupy product thus obtained reacted acid to Congo paper. It was purified by extraction with chloroform (yield, 2·43 g.) (equiv., 455).

Fractionation of the Partially Methylated Uronic Acids.—To a solution of the syrupy methylated acids (2·43 g.) in ethyl alcohol, sufficient light petroleum was added to precipitate approximately half the material, giving fraction I (1·2 g.) (equiv., 560). Removal of the solvent from the mother-liquor gave fraction II (1·22 g.) (equiv., 415). Refractionation of fraction I by the same procedure gave a small insoluble fraction, which was rejected since it had a high equivalent weight; removal of the solvent gave fraction I*B* (1·08 g.) (equiv., 540). Fractionation of I*B* in the same way served to remove a small amount of material having a high equivalent weight, and evaporation of the solution then gave fraction I*D* (0·65 g.) (Found: OMe, 39·0%; equiv., 475).

Similarly fraction II was separated into two portions by dissolving it in ethyl alcohol and adding light petroleum. Fraction II*A* was precipitated (0·69 g.) (Found: OMe, 40·1%; equiv., 430). Concentration of the solution gave fraction II*B* (0·44 g.) (Found: OMe, 38·1%; equiv., 365). In view of its low equivalent weight this fraction probably contained some partially methylated glucuronic acid.

Simultaneous Esterification and Glycoside Formation.—A trial experiment was carried out in order to determine whether esterification and glycoside formation could be effected at room temperature without cleavage of the methylated aldobionic acid. Accordingly, a solution of fraction II*B* (0·4 g.) in 1% methyl-alcoholic hydrogen chloride (50 c.c.) was kept at room temperature until the rotation became constant. $[\alpha]_D^{18°}$ + 32·5° (initial value); + 39·5° (38 hrs.); + 46·2° (87 hrs.); + 47·5° (110 hrs.); + 47·5° (133 hrs., constant value). The solution was then neutralised with silver carbonate, filtered, and evaporated to dryness. The syrup thus obtained reacted neutral to litmus paper and did not reduce Fehling's solution. On distillation it gave: Fraction (i) the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (0·15 g.), b.p. (bath temp.) 120°/0·03 mm.; $n_D^{18°}$ 1·4490; $[\alpha]_D^{18°}$ + 78° in water (*c*, 1·0) (Found: OMe, 57·0%. Calc. for $C_{11}H_{20}O_7$: OMe, 58·7%). Fraction (ii) methyl heptamethyl aldobionate (0·16 g.), b.p. (bath temp.) *ca.* 220°/0·03 mm., $n_D^{18°}$ 1·4670 (Found: OMe, 50·0%; equiv., 450. Calc. for $C_{20}H_{36}O_{12}$: OMe, 53·0%; equiv., 468). These facts suggested that the required methylated aldobionic acid could be obtained by the application of the above procedure.

Accordingly, each of the fractions I*D* (0·57 g.) and II*A* (0·6 g.) containing the hexamethyl aldobionic acid (III) were dissolved in 1% methyl-alcoholic hydrogen chloride (50 c.c.), and the solutions kept at room temperature for 140 hours. The solution of fraction I*D* in methyl-alcoholic hydrogen chloride showed the following changes of specific rotation: $[\alpha]_D^{18°}$ + 12° (initial value); + 18·0° (19 hrs.); + 26° (44 hrs.); + 26·5° (68 hrs.); + 28° (140 hrs., constant value). The two solutions were then combined, neutralised with silver carbonate, filtered, and evaporated under diminished pressure; the syrup obtained (1·1 g.) was neutral to litmus and did not reduce Fehling's solution (Found: OMe, 50·2%). This product distilled, giving: Fraction (*a*) the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (0·34 g.), b.p. (bath temp.) 120°/0·03 mm., $n_D^{18°}$ 1·4490, $[\alpha]_D^{18°}$ + 80° in water (*c*, 1·2) (Found: OMe, 57·0%). Fraction (*b*) the methyl ester of hexamethyl 6-β-glucuronosidomethylgalactoside (0·5 g.), b.p. (bath temp.) 220°/0·03 mm., $n_D^{18°}$ 1·4685—1·4690, $[\alpha]_D^{18°}$ + 42° in water (*c*, 1·7) (Found: OMe, 52·0%; equiv., 470). This fraction was nucleated with a specimen of the β-form of the methyl ester of hexamethyl 6-β-glucuronosidomethylgalactoside {m.p. 94°, $[\alpha]_D^{25°}$ − 21° in water (*c*, 1·6)} (see Challinor, Haworth, and Hirst, *loc. cit.*), but no crystallis-

ation took place; it would appear, therefore, that fraction (*b*) probably contains preponderance of the α-form of the glycoside.

Hydrolysis of the Methyl Ester of Hexamethyl 6-β-*Glucuronosidomethylgalactoside* (IV).—A solution of fraction (*b*) (0·3 g.) in 3% methyl-alcoholic hydrogen chloride (20 c.c.) was boiled under reflux for 8 hours. The solution was then cooled, neutralised with silver carbonate, filtered, and evaporated under diminished pressure, giving a fairly mobile liquid (0·38 g.). A solution of this syrup (0·38 g.) in 0·3N-barium hydroxide (7 c.c.) was heated for 2 hours at 60° in order to convert the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside into its ether-insoluble barium salt. The excess of the barium hydroxide was then neutralised with carbon dioxide. The neutral solution was treated with a little charcoal, filtered, and evaporated to dryness under diminished pressure. The residue, consisting of a mixture of the barium salt of 2 : 3 : 4-trimethyl methylglucuronoside and 2 : 3 : 4-trimethyl methylgalactopyranoside, was exhaustively extracted with boiling dry ether; concentration of the combined ethereal extracts gave 2 : 3 : 4-trimethyl methylgalactopyranoside (VII) (0·15 g.) (Found : OMe, 50·8. Calc. for $C_{10}H_{20}O_6$: OMe, 52·6%). The ether-insoluble residue consisting of the barium salt of 2 : 3 : 4-trimethyl methylglucuronoside amounted to 0·23 g.

Identification of the 2 : 3 : 4-*Trimethyl Methylgalactoside* (VII).—A solution of the 2 : 3 : 4-trimethyl methylgalactoside (0·13 g.) in N-sulphuric acid (5 c.c.) ($[\alpha]_D$ + 135°) was heated for 4 hours on the boiling water-bath; the specific rotation then became constant (+ 108°). The solution was neutralised with barium carbonate, filtered, and evaporated to dryness under diminished pressure. The pale yellow syrup thus obtained was purified by extraction with ether; it reduced Fehling's solution on boiling (yield, 0·09 g.). To a solution of this syrup in ethyl alcohol (2 c.c.) was added freshly distilled aniline (0·04 g.; 1 mol.), and the mixture heated under reflux for 3 hours. On removal of the solvent the syrup readily crystallised, and after recrystallisation from ethyl alcohol–ether the anilide of 2 : 3 : 4-trimethyl galactose had m.p. and mixed m.p. 166° (Found : C, 60·5; H, 7·7; OMe, 31·1; N, 4·9. Calc. for $C_{15}H_{23}O_5N$: C, 60·6; H, 7·8; OMe, 31·3; N, 4·7%).

Identification of the Methyl Ester of 2 : 3 : 4-*Trimethyl Methylglucuronoside* (V).—The barium salt of 2 : 3 : 4-trimethyl methylglucuronoside (0·23 g.) was boiled for 8 hours with 3% methyl-alcoholic hydrogen chloride (10 c.c.). The solution was cooled, neutralised with silver carbonate, filtered, and evaporated to dryness under diminished pressure. To avoid loss the ester was not distilled. After purification by extraction with ether, the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside had $[\alpha]_D^{18°}$ + 80° in water (*c*, 0·9) (Found : OMe, 57·0. Calc. for $C_{11}H_{20}O_7$: OMe, 58·7%). On treating this methyl ester with methyl-alcoholic ammonia there was obtained in good yield the amide of 2 : 3 : 4-trimethyl α-methylglucuronoside (VI), m.p. and mixed m.p. 183° (Found : C, 48·4; H, 7·8; OMe, 50·1; N, 5·65. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·7; OMe, 49·8; N, 5·6%).

The Amide of Hexamethyl 6-β-*Glucuronosido*-β-*methylgalactopyranoside*.—The methyl ester of hexamethyl 6-β-glucuronosido-β-methylgalactoside prepared according to the directions of Challinor, Haworth, and Hirst (*loc. cit.*) had m.p. 94°, $[\alpha]_D^{25°}$ − 21° in water (*c*, 1·6) (after crystallisation from acetone–light petroleum) (Found : C, 51·4; H, 7·9; OMe, 53·4; equiv., 470. Calc. for $C_{20}H_{36}O_{12}$: C, 51·2; H, 7·8; OMe, 53·0%; equiv., 468). Treatment of this ester with methyl-alcoholic ammonia gave the corresponding *amide* of hexamethyl 6-β-glucuronosido-β-methylgalactopyranoside, m. p. 196°, $[\alpha]_D^{20°}$ − 18° in water (*c*, 3·7) (after crystallisation from ethyl alcohol) (Found : C, 50·4; H, 7·8; OMe, 47·7; N, 3·2. $C_{19}H_{35}O_{11}N$ requires C, 50·3; H, 7·8; OMe, 47·9; N, 3·1%).

The authors thank Professor W. N. Haworth, F.R.S., for his interest in this work, and Imperial Chemical Industries Ltd. for a grant to one of them (J. J.).

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