389. *The Structure of Alginic Acid. Part I.*

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The action of methyl-alcoholic hydrogen chloride on sodium alginate has been examined. The products are shown to be a degraded alginic acid and *d*-mannuronic acid—recognised, after methylation and oxidation, as 2 : 3 : 4-trimethyl *d*-manno-saccharamide. A degraded alginic acid has been methylated by the thallium method. This methylated product was exceptionally stable towards hydrolytic agents, but

was simultaneously hydrolysed and oxidised by nitric acid, giving *i*-dimethoxysuccinic acid, recognised as its crystalline dimethyl ester and amide. Hydrolysis with methyl-alcoholic hydrogen chloride gave 2 : 3-dimethyl *d*-mannuronic acid, the constitution of which was proved by its oxidation by periodic acid, glyoxylic acid and the half aldehyde of *i*-dimethoxysuccinic acid being obtained. The latter substance was recognised after further oxidation as the crystalline methyl ester of *i*-dimethoxysuccinic acid. The results show that at least the major portion of the alginic acid molecule is composed of β-*d*-mannuronic acid residues, and reasons are given for the view that alginic acid contains solely pyranose residues which must in this case be linked through C_1 and C_4.

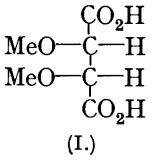
ALGINIC acid constitutes a large proportion of certain seaweeds, where it occurs as a salt, usually associated with other polysaccharides such as fucosan. Sodium alginate is of considerable importance and finds a very wide industrial application. It is noteworthy among polysaccharides in that it appears to be built entirely of *d*-mannuronic acid residues, although its resistance to hydrolysis is so great that no quantitative transformation to *d*-mannuronic acid has been achieved (Nelson and Cretcher, *J. Amer. Chem. Soc.*, 1929, **51**, 1914; 1932, **54**, 3409; Bird and Haas, *Biochem. J.*, 1931, **25**, 403; Schoeffel and Link, *J. Biol. Chem.*, 1933, **100**, 397). The results we have recently obtained from a study of the action of methyl-alcoholic hydrogen chloride on the polysaccharide show that high yields of *d*-mannuronic acid are obtainable from alginic acid and that under suitable conditions it is possible to obtain a partially degraded alginic acid of comparatively low molecular weight. This reaction resembles that in which pectic acid is simultaneously hydrolysed and degraded by methyl-alcoholic hydrogen chloride to *d*-galacturonic acid and a pectic acid of lower molecular size (Morrell, Baur, and Link, *J. Biol. Chem.*, 1934, **105**, 1).

Except for the lower viscosity of its solution, degraded alginic acid differed little from the original alginic acid (compare Morrell, Baur, and Link, *loc. cit.*), but it was more amenable to chemical treatment. From it, by use of thallium hydroxide, thallium ethoxide and methyl iodide, it was possible to obtain the fully methylated derivative. Methylation of undegraded alginic acid was rendered difficult by the very slow reaction between methyl iodide and the thallium derivative, even under pressure. The matter was further complicated by the comparative insolubility of the partly methylated alginic acid in the usual organic solvents.

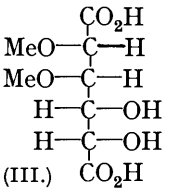
The methylated product from degraded alginic acid was exceptionally stable towards hydrolytic agents, but two methods were developed which gave insight into its chemical structure. When boiled with nitric acid, it underwent hydrolysis, followed by degradative oxidation, with the formation of *i*-dimethoxysuccinic acid (I), recognised as its crystalline methyl ester and amide. This indicates that in each of the mannuronic acid residues the methyl groups were attached either to C_2 and C_3 or C_4 and C_5.

Proof that the methyl groups were, in fact, situated at C_2 and C_3 of the mannuronic acid residues was provided in the following manner. Methylated alginic acid on drastic treatment under pressure with methyl-alcoholic hydrogen chloride gave the methyl ester of 2 : 3-*dimethyl methyl*-d-*mannuronide* (II). This underwent hydrolysis to 2 : 3-*dimethyl* d-*mannuronic acid*, with change of rotation, on heating with dilute aqueous acid, thereby proving that methoxyl groups must have been on C_2 and C_3, since, if they had been on C_4 and C_5, the mannuronic acid would have been incapable of forming a ring compound. 2 : 3-Dimethyl *d*-mannuronic acid was oxidised with bromine water to 2 : 3-*dimethyl* d-*mannosaccharic acid* (III), which in turn was oxidised with periodic acid (a reagent diagnostic for αβ-glycols) to glyoxylic acid (IV) and the half aldehyde of *i*-dimethoxysuccinic acid (V). The latter was oxidised by bromine water to *i*-dimethoxysuccinic acid (I), recognised as its crystalline dimethyl ester and amide. It would appear, therefore, that alginic acid is composed of a chain of *d*-mannuronic acid residues in each of which C_2 and C_3 are free. The glycosidic linkage must therefore be either 1 : 4 (pyranose ring structure) or 1 : 5 (furanose ring structure). It is very unlikely that the polysaccharide consists of a chain of *d*-mannuronic acid units linked as in (VI), since no polysaccharide with this type of linkage is known. Moreover, the great difficulty experienced in

hydrolysing alginic acid is not in agreement with the presence of such an acetal type of linkage, which would probably undergo hydrolysis with relative ease.



(I.)



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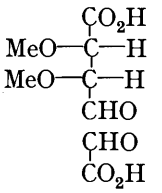
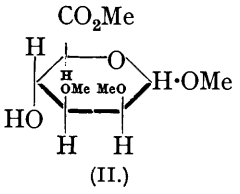
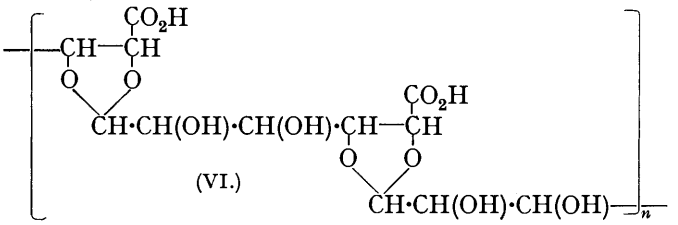


(V.) (IV.)

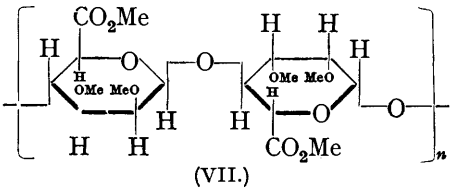


(II.)

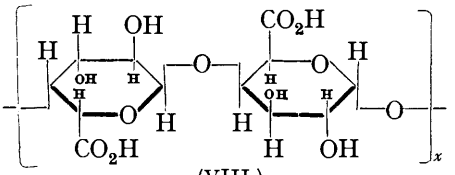


(VI.)

In view of the extreme stability of alginic acid and its large negative rotation, it is highly probable that the ring structure is pyranose and that the linkage between the residues is 1 : 4. We conclude, therefore, from the amounts of methylated mannuronic acid isolated from the hydrolysis of alginic acid and from degraded methylated alginic acid that at least 50% of the molecule is composed of β-*d*-mannuronic acid residues linked as shown in (VII).



(VII.)



(VIII.)

Owing to the experimental difficulties connected with the hydrolysis of alginic acid it is not possible to state whether the whole of the molecule is so constituted. But in view of the facts that no other sugar than *d*-mannuronic acid was isolated from the hydrolysis of the polysaccharide and that hydrolysis of the methylated degraded alginic acid gave 2 : 3-dimethyl *d*-mannuronic acid in 50% yield despite extensive decomposition during hydrolysis, it is probable that alginic acid is composed entirely or almost entirely of *d*-mannuronic acid units.

The structural resemblance between (VII) and the formula for cellulose (β-*d*-glucopyranose units linked through C_1 and C_4) is apparent. Both structural formulæ resemble closely that suggested for degraded citrus pectic acid (VIII) (Smith, *Chem. and Ind.*, 1939, **58**, 363), degraded strawberry pectic acid (Beaven and Jones, *ibid.*, p. 363), and degraded apple pectic acid (unpublished results), the residues in these instances being α-*d*-galacturonic acid (pyranose form) linked glycosidically through positions 1 and 4.

Experimental.

Alginic Acid.—Alginic acid is obtained from certain marine algæ by extraction with dilute aqueous alkali and is marketed as its sodium salt under the trade name of manucol. This is a white powder, which forms highly viscous aqueous solutions. On addition of hydrochloric acid to an aqueous solution of sodium alginate, alginic acid was precipitated as a jelly. This was filtered off and washed with water until free from mineral acid and salts. It was then passed through alcohol–water mixtures of gradually increasing alcohol concentration and dried at 60°/12 mm. This procedure served to retain the alginic acid in a friable condition and avoided the formation of horny masses which were difficult to powder.

Alginic acid was prepared from two samples of sodium alginate : (a) a sample of high

viscosity ($\eta_{sp.}/c$ 33·3 for c 1·0), (b) a sample of low viscosity ($\eta_{sp.}/c$ 12·4 for c 1·0). Both samples of the sodium salt had $[\alpha]_D^{20°}$ — 139° in water (c, 1·0). The samples of alginic acid both gave furfural (21%, estimated as phloroglucide after treatment with 12% hydrochloric acid) and had equiv. wt. 182 (by titration with N/10-sodium hydroxide). The yield of carbon dioxide from alginic acid on boiling with 12% hydrochloric acid was invariably low (*ca.* 70% of the theoretical), owing to the stability of the substance towards the acid reagent.

Attempts to hydrolyse alginic acid were complicated by the fact that the products of hydrolysis were rapidly decomposed by the hydrolytic agent under the experimental conditions necessary. It was almost unaffected by boiling dilute acids (hydrochloric, sulphuric, nitric). Heating alginic acid with water in a sealed tube at 150° led to little if any autohydrolysis (compare Hirst and Jones, J., 1938, 1174). The most satisfactory methods of hydrolysis were (a) by methyl-alcoholic hydrogen chloride (see below) and (b) by 80% sulphuric acid (method of Nelson and Cretcher, *J. Amer. Chem. Soc.*, 1929, **51**, 1914). By the latter method it was possible to isolate barium *d*-mannuronate (identified as the *p*-bromophenylosazone of the barium salt) in 50% yield.

Attempts to acetylate alginic acid, sodium alginate and methyl alginate by the use of pyridine or zinc chloride and acetic anhydride, or by Barnett's method, were unsuccessful. The method of Dillon and McGuinness (*Sci. Proc. Roy. Dublin Soc.*, 1932, **20**, 129) using hydriodic acid as a catalyst led to the production of degraded products.

Hot dilute alkali solution rapidly degraded alginic acid with formation of orange-coloured decomposition products, and in this respect alginic acid closely resembles pectic acid. In consequence, the methylation of alginic acid by methyl sulphate and 30% sodium hydroxide solution was unsatisfactory even when carried out in an oxygen-free atmosphere. In one experiment material having OMe 14·9% was isolated after three methylations (yield, 25%). The working-up of the solution was rendered difficult by the presence of large quantities of sodium sulphate and attempts to remove this by dialysis resulted in loss of product, indicating that during methylation the alginic acid had been degraded, at least in part, to molecules small enough to pass through the parchment membrane. Alginic acid formed an insoluble thallium derivative, which was most conveniently prepared by addition of excess of thallous hydroxide solution (4 equivs.) to an aqueous solution of sodium alginate. The thallium derivative was a white curdy precipitate which dried as a yellow powder. Acidification of the thallium derivative regenerated alginic acid which differed from the original material only in its lower viscosity. This thallium derivative was difficult to powder and it reacted with methyl iodide slowly and incompletely even when heated under pressure. Substitution of methyl sulphate for methyl iodide gave even less satisfactory results. In view of these difficulties a degraded alginic acid was prepared, the thallium derivative of which was much more amenable to treatment with methyl iodide.

Preparation of Degraded Alginic Acid.—Sodium alginate (50 g.) was boiled for 17 hours with 10% methyl-alcoholic hydrogen chloride (400 c.c.). The cooled solution was filtered, and the solid washed with methyl alcohol to remove sodium chloride and adsorbed hydrochloric acid, and dried (diminished pressure). It was a white powder (25 g.) soluble in water, giving viscous solutions, but with a viscosity considerably less than that of the original material. $[\alpha]_D^{20°}$ — 134° (c, 1·6 in neutral solution) [Found : furfural, 20·4; uronic acid anhydride (as carbon dioxide liberated by boiling with 12% hydrochloric acid), 86·6; OMe, 14·5; equiv. (by titration), 203. A completely esterified polyuronide requires uronic acid anhydride, 92·5; OMe, 16·3%; equiv. 190].

The filtrate from the degraded material was neutralised with silver carbonate, filtered, and evaporated to a mass of syrup and sodium chloride. The syrup (20·7 g., extracted with cold methyl alcohol) was evaporated to dryness with N-thallium hydroxide (600 c.c.) and the powdered solid (120 mesh) was boiled with methyl iodide until it no longer gave an alkaline reaction to litmus (2 days). After removal of the methyl iodide by distillation, the partially methylated product was extracted with methyl alcohol. The extracts were concentrated, dissolved in methyl iodide, and boiled (7 hours) with addition of silver oxide. The product was extracted with acetone and after two more methylations with Purdie's reagents the refractive index ($n_D^{20°}$ 1·4752) of the syrup (20·2 g.) remained constant.

This product was separated into two fractions by extraction with ether : (1) An ether-insoluble solid (1·9 g.) (Found : OMe, 28·0%); and (2) an ether-soluble syrup (18·3 g.), $n_D^{19°}$ 1·4670, which gave on distillation : Fraction I (an orange liquid, probably decomposed material) (0·1 g.), b. p. 140°(bath temp.)/0·002 mm., $n_D^{21°}$ 1·4445; fraction II, the methyl ester of trimethyl methylmannuronide (15·0 g.), b. p. 147°(bath temp.)/0·002 mm., $n_D^{20°}$ 1·4580,

$[\alpha]_D^{20°}$ + 60·0° (*c*, 10·0 in water) (Found : OMe, 58·2. Calc. for $C_{11}H_{20}O_7$: OMe, 58·7%); fraction III (2·0 g.), b. p. 160—220°(bath temp.)/0·002 mm., $n_D^{19°}$ 1·4810 (Found : OMe, 49·2%). This fraction was methylated with Purdie's reagents, and the product distilled, giving : Fraction IV (1·10 g.), b. p. 140°(bath temp.)/0·002 mm., $n_D^{20°}$ 1·4682, $[\alpha]_D^{20°}$ + 57·2° (*c*, 1·56 in water) (Found : equiv., 244; OMe, 51·7%). (This fraction may have contained incompletely methylated mannuronic acid or disaccharides.) The still residues consisted mainly of unhydrolysed polysaccharides.

The ester from fraction II (10·4 g.) was hydrolysed with N-hydrochloric acid (100 c.c.) for 12 hours, and the solution neutralised with silver carbonate, filtered before and after treatment with hydrogen sulphide, and evaporated to a syrup. The syrup was extracted with acetone, the solution filtered, and acetone removed at 40°/12 mm., leaving *trimethyl mannuronic acid* as a pale yellow, viscid syrup (8·5 g.), $[\alpha]_D^{20°}$ + 36·4° (*c*, 1·62 in water) (Found : OMe, 38·7; equiv., 242. $C_9H_{16}O_7$ requires OMe, 39·3%; equiv., 236). The acid (8·4 g.) was dissolved in water (10 c.c.) and oxidised with bromine (5 c.c.) until non-reducing, the product isolated in the usual manner and esterified with methyl-alcoholic hydrogen chloride, and the ester (8·42 g.) distilled, giving (A) (7·80 g.), b.p. 125°/0·003 mm., $n_D^{18°}$ 1·4600.

Fraction (A) was a mixture of 2 : 3 : 4-trimethyl *d*-mannosaccharolactone methyl ester and methyl 2 : 3 : 4-trimethyl *d*-mannosaccharate, $[\alpha]_D^{21°}$ + 13·7° (*c*, 4·39 in methyl alcohol), + 16·1° (*c*, 2·25 in water) (Found : OMe, 49·2; equiv., 132. Calc. for $C_{11}H_{20}O_8$: OMe, 55·3%; equiv., 140. Calc. for $C_{10}H_{16}O_7$: OMe, 50·0%; equiv., 124). Fraction (A) was dissolved in methyl alcohol, and the solution saturated with ammonia at 0°. After 7 days at — 5° the solution deposited crystals of 2 : 3 : 4-trimethyl *d*-mannosaccharodiamide; concentration of the solution gave a further crop (total yield, 62% of the theoretical); m. p. and mixed m. p. with an authentic specimen prepared by Haworth, Hirst, Isherwood, and Jones (preceding paper), 228° (decomp.), $[\alpha]_D^{20°}$ — 17° (*c*, 0·46 in methyl alcohol) (Found : C, 43·3; H, 6·9; N, 10·8; OMe, 37·2. Calc. for $C_9H_{18}O_6N_2$: C, 43·2; H, 7·2; N, 11·2; OMe, 37·2%). Treatment of an aqueous solution of the amide with sodium hypochlorite solution gave sodium cyanate, identified as hydrazodicarbonamide, proving that the substance is an α-hydroxyamide (Weerman reaction).

Methylation of Alginic Acid.—Degraded alginic acid (30 g.) was suspended in water and brought into solution by the addition of a little N-thallous hydroxide (20 c.c.). N-Thallous hydroxide (500 c.c.) was concentrated to 150 c.c. and added with stirring to the solution of alginic acid. The precipitated thallium derivative was immediately filtered off, washed once with methyl alcohol, and dried in a vacuum with exclusion of air and light. The cream-coloured solid was powdered (120 mesh) and boiled with methyl iodide until it no longer gave an alkaline reaction (60 hours). Methyl iodide was distilled off, and the solid extracted with methyl alcohol. The alcoholic extracts gave on concentration a yellow solid (11·0 g.), which was dissolved in water, the solution evaporated to dryness with N-thallous hydroxide (150 c.c.), and the residue powdered and boiled with methyl iodide. Repetition of this process, with thallium ethoxide in place of thallium hydroxide, followed by methylation with Purdie's reagents, gave a viscid solid, which was separated into two fractions : (a) a solid (5·5 g.) (Found : OMe, 43·2%), and (b) a viscid syrup (4·2 g.).

The solid (a) (degraded methylated alginic acid) had $[\alpha]_D^{22°}$ — 28° (in methyl alcohol, *c*, 0·6) [Found : equiv., 231 (by titration with N/10-alkali; methylated alginic acid gave an orange colour with alkaline reagents); uronic anhydride, 48·2, from carbon dioxide liberated on heating with 12% hydrochloric acid (the low value was due to incomplete decomposition of the methylated product). A methylated polymannuronide requires equiv., 218; uronic acid anhydride, 80·7%; OMe, 42·6%].

The methylated product was exceptionally stable to acid reagents; after treatment with 3% methyl-alcoholic hydrogen chloride at 90° for 24 hours and with 2% methyl-alcoholic hydrogen chloride at 120° in a sealed tube during 24 hours, it was recovered practically unchanged. It underwent hydrolysis and oxidation when boiled with 50% nitric acid. The methylated product (2 g.) was heated with 50% nitric acid (50 c.c.) for 18 hours; a vigorous reaction then occurred and nitrous fumes were evolved. The product (0·69 g.) was isolated in the usual way, esterified, and distilled, giving methyl *i*-dimethoxysuccinate (0·11 g.), b. p. 110°(bath temp.)/0·01 mm., $n_D^{19°}$ 1·4372, $[\alpha]_D^{20°}$ ± 0° (*c*, 2·2 in methyl alcohol) (Found : OMe, 59·0%). (The still residue appeared to consist of unaltered polysaccharide.) With ammonia in methyl alcohol, the ester gave in quantitative yield the corresponding amide, m. p. and mixed m. p. with an authentic specimen 242° (decomp.), $[\alpha]_D^{21°}$ ± 0° (*c*, 1·5 in water) (Found : OMe, 34·0. Calc. for $C_6H_{12}O_4N_2$: OMe, 35·2%). No other dimethoxysuccindiamide was detected.

The methylated derivative (1·65 g.) underwent partial hydrolysis with the formation of some decomposition products when it was heated with 4% methyl-alcoholic hydrogen chloride (40 c.c.) at 150° for 24 hours. The solution was neutralised with silver carbonate, filtered, and evaporated to a syrup (strong odour of furfural). Extraction with ether gave a syrup (0·76 g.), $n_D^{18°}$ 1·4712, which was distilled, giving the methyl ester of 2 : 3-*dimethyl methyl*-d-*mannuronide* (0·41 g.), b. p. 180°(bath temp.)/0·005 mm., $n_D^{22°}$ 1·4650, $[\alpha]_D^{20°}$ + 59° (*c*, 2·0 in water) (Found : OMe, 50·4. $C_{10}H_{18}O_7$ requires OMe, 49·5%).

The ester (0·38 g.) was hydrolysed with N-hydrochloric acid (18 c.c.) at 90—95°. The product had $[\alpha]_D^{20°}$ + 20° (*c*, 2·0 in N-hydrochloric acid, equilibrium value after hydrolysis). The solution was neutralised with silver carbonate and filtered, silver ions removed with hydrogen sulphide, and the filtered solution evaporated to a syrup (0·28 g.) consisting of 2 : 3-*dimethyl* d-*mannuronic acid*, $[\alpha]_D^{20°}$ + 30° (*c*, 1·5 in methyl alcohol), + 33° in 2% methyl-alcoholic hydrogen chloride, unaltered after 12 hours (Found : OMe, 27·9; equiv., 230. $C_8H_{14}O_7$ requires OMe, 27·9%; equiv., 222).

2 : 3-Dimethyl mannuronic acid (0·26 g.) was oxidised with bromine (1 c.c.) in water (10 c.c.) at 30° for 6 hours. The excess of bromine was removed by aeration, and the solution worked up in the usual manner. The syrupy product (0·22 g.), 2 : 3-*dimethyl* d-*mannosaccharic acid*, had equiv. wt. 118, $[\alpha]_D^{20°}$ + 16° (*c*, 4·2 in water), − 7·5° (*c*, 2·1 in alkali) (Found : OMe, 26·8. $C_8H_{14}O_8$ requires OMe, 27·3%; equiv., 119). It was dissolved (0·20 g.) in a slight excess of N/3-barium hydroxide, and aqueous periodic acid added, sufficient to liberate and oxidise the free dimethyl *d*-mannosaccharic acid. A slight rise in temperature occurred and the rotation fell to zero. After 36 hours the solution was neutralised with barium carbonate and filtered. The filtrate reduced Fehling's solution and gave a purple colour with casein and sulphuric acid, indicating the presence of glyoxylic acid. Bromine (1 c.c.) and barium carbonate (1 g.) were added to the filtrate, which was kept at 20° until it was non-reducing to Fehling's solution (3 hours). The solution was filtered, aerated, and evaporated to a small volume. Addition of a little calcium chloride to a test sample gave a precipitate of calcium oxalate (oxalic acid produced by oxidation of glyoxylic acid). The solution was filtered and evaporated to dryness, and the product esterified with methyl-alcoholic hydrogen chloride. The solution was neutralised with silver carbonate, filtered, and evaporated to a syrup (0·15 g.), which was distilled; b. p. 110°(bath temp.)/0·001 mm., $n_D^{20°}$ 1·4368. The distillate (0·11 g.) crystallised to a solid mass. On recrystallisation from ether–light petroleum, the solid (75 mg.) had m. p. 66—67°, not depressed by authentic methyl *i*-dimethoxysuccinate; $[\alpha]_D^{20°}$ ± 0° (*c*, 1·3 in methyl alcohol) (Found : OMe, 62·2. Calc. for $C_8H_{14}O_6$: OMe, 60·2%). The crystals gave with methyl-alcoholic ammonia *i*-dimethoxysuccinamide in quantitative yield, m. p. and mixed m. p. 244°.

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