323. *The Polysaccharides of Carragheen. Part II. The* Gigartina stellata *Polysaccharide.*

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The water-soluble polysaccharide of *Gigartina stellata* is shown to resemble the *Chondrus crispus* polysaccharides. Crystalline 2 : 6-dimethyl β-*d*-galactose has been isolated by the hydrolysis of the methylated polysaccharide.

Although some authorities ("British Pharmaceutical Codex", London, 1934, p. 320; "Thorpe's Dictionary of Applied Chemistry", Longmans, 4th. Edn. 1937, p. 199) appear to reserve the term carragheen for the red alga *Chondrus crispus*, and it was used in this sense in Part I (*J.*, 1943, 51), others, for example Tseng (*Science*, 1945, **101**, 2633) and Newton (*Endeavour*, 1945, *IV*, **14**, 69) also include *Gigartina stellata*, Batt. in this description on account of the close morphological resemblances between the two algæ. It is not surprising therefore that the polysaccharides extracted by hot water from these two red seaweeds prove, as the present investigation shows, to be of the same type.

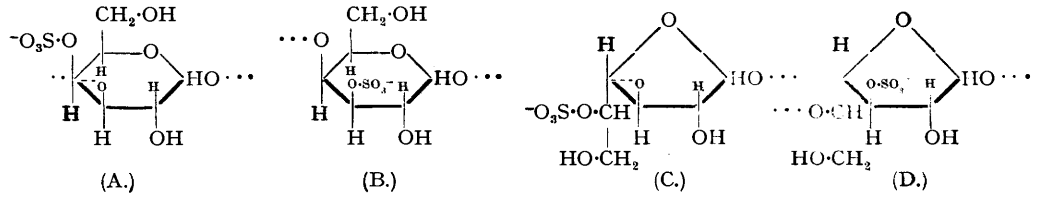
The polysaccharide obtained by the extraction of *Gigartina stellata* with hot water mainly as the calcium salt, but containing in addition magnesium, sodium, and potassium, gave *d*-galactose (40%) on hydrolysis, and had $[\alpha]_D^{15°}$ + 51° in water (SO_4, 23·9%); the hot extract from *Chondrus crispus* had $[\alpha]_D^{18°}$ + 63° (SO_4, 23·8; galactose 36·9%) (*J.*, 1943, 51). In its stability to sodium hydroxide (N) at 100° the polysaccharide also resembled the *Chondrus* extracts, three days being necessary to remove 62% of the sulphate residues. The free acid polysaccharide was isolated ($[\alpha]_D^{19°}$ + 43°; SO_4, 25·9%; neutralisation equivalent, 371) and potentiometric titration showed it to be a typically strong acid. Acetylation was accomplished by a modification of the method of Pacsu and Mullen (*J. Amer. Chem. Soc.*, 1941, **63**, 1487), deacetylation and methylation of the acetate (CH_3·CO, 19·1%) giving a partially methylated polysaccharide (OMe, 16·2%) from which after repeated methylation with sodium hydroxide and methyl sulphate a product, $[\alpha]_D^{15°}$ + 43° (OMe, 18·6; Ca, 3·8; Mg, 0·9; SO_4, 24·7%), was obtained, the retention of the ethercal sulphate emphasising the stability of that grouping to alkali.

Hydrolysis of the methylated polysaccharide gave a crystalline dimethyl galactose which was identified as 2 : 6-dimethyl galactose as follows. Oxidation gave a crystalline *dimethyl galactonic acid* which on distillation yielded a lactone, proved to be a γ-lactone because of its slow hydrolysis in water. A free hydroxyl group was therefore present on C_4, and this fact was confirmed by the inversion of the sign of rotation of the free sugar on methylgalactofuranoside formation. The *amide* prepared from the lactone gave a negative Weerman test, proving substitution by methoxyl on C_2, a fact confirmed on osazone formation since 6-methyl galactosazone (Oldham and Bell, *J. Amer. Chem. Soc.*, 1938, **60**, 324) was obtained. Proof of the substitution of the primary alcohol residue by methoxyl was afforded by the fact that no iodine entered the molecule when the ditosyl dimethyl methylgalactoside was treated with sodium iodide in acetone (Oldham and Rutherford, *ibid.*, 1932, **54**, 366). The isolation of a crystalline dimethyl monoacetone β-methylgalactopyranoside also proved the presence of free hydroxyl groups on C_3 and C_4.

The only fact at variance with the proposed assignment of structure was that, although the specific rotations were almost identical, the melting point of 131° for 2 : 6-dimethyl galactose recorded by Oldham and Bell (*loc. cit.*) was much higher than the value (119—120°) we obtained for our crystalline dimethyl galactose. The facts were re-examined by Dr. D. J. Bell (*J.*, 1945, 692) who repeated and extended the earlier work, and mixed melting point determinations of our dimethyl galactonic acid phenylhydrazide with the corresponding derivative prepared from 2 : 6-dimethyl galactose, the structure of which had been proved unequivocally both by the synthetic route employed and by periodate oxidation, showed no depression. Furthermore satisfactory agreement was obtained in respect of the melting points of the dimethyl β-methylgalactoside and the corresponding 3 : 4-monoacetone derivative. It may be recalled that evidence was presented (Part I, *loc. cit.*) that the syrupy dimethyl galactose produced on hydrolysing the methylated *Chondrus crispus* polysaccharides was also 2 : 6-dimethyl galactose. In these polysaccharide sulphates, therefore, the exposed hydroxyl groups on the galactose residues are on C_2 and C_6. If we assume that the sulphate groups are directly attached to the galactose residues four possible formulæ must be considered (see opposite page).

It has been shown recently (Percival and Duff, *Nature*, 1946, **158**, 29) that barium 3-methyl 1 : 2-monoacetone glucofuranose 6-sulphate is readily transformed into the corresponding 5 : 6-anhydride by sodium methoxide. It is highly probable therefore that the sulphate groups

in (B), (C), and (D) would be readily eliminated by alkali, with formation of ethylene oxide rings in the first instance. In addition (B) would also form a 3 : 6-anhydride by analogy with the



(A.) (B.) (C.) (D.)

methylglucofuranoside 3-sulphates (*J.*, 1945, 119). The most likely arrangement is therefore (A) in which the galactopyranose residues are linked through the 1 and 3 positions as in agar, and it may be that this type of linkage is typical of the galactans of the red seaweeds. The above arguments, based on the resistance to hydrolysis with alkali of the sulphate groups, apply only if these residues are directly attached to the galactose units. It is conceivable, however, that the sulphate groups are attached to those building units (X) of the molecule which have not, as yet, been identified, with these units (X) interposing between the sulphate groups and the triply linked galactose residues. This point could be decided by removing the sulphate groups and following this by methylation, but attempts to remove the sulphate residues have been unsuccessful so far and it seems that a final decision must await the identification of (X).

The substance, or mixture of substances, (X) is clearly not a simple hexose since the methoxyl content of the methylated polysaccharide is only slightly higher than would be required for the dimethyl galactose residues found, and it will be surprising if it contains more than one free hydroxyl group. That (X) might be an anhydro- or a deoxy-sugar has not been lost sight of, but the decomposition it undergoes under acid hydrolytic conditions has prevented progress so far. The hydrolysis of the methylated polysaccharide with methanolic hydrogen chloride was unexpectedly difficult, and only derivatives of methylated galactose could be identified among the products of hydrolysis. In the case of the *Chondrus crispus* polysaccharide, Young and Rice (*J. Biol. Chem.*, 1946, **164**, 35) have isolated diacetone 2-ketogluconic acid in small yield on hydrolysis and treatment with acetone and copper sulphate, but we have not yet succeeded in isolating this material from either the *Chondrus* or the *Gigartina* polysaccharides.

Experimental.

Preparation of the Polysaccharide.—The air-dried seaweed, kindly supplied by Dr. A. P. Orr of the Marine Station, Millport, was washed in muslin bags in 500 g. lots with running water for 10 days. Repeated extraction with water on the steam-bath followed until the extracts were no longer appreciably viscous. The combined extracts were filtered hot and concentrated at 50°/15 mm. to small volume, and the brown viscous solution was added slowly, with mechanical stirring, to ethanol. The white fibrous material was dehydrated with fresh ethanol, washed with ether, and dried in a vacuum desiccator. For all quantitative work the substance was purified by dialysis against running water for six days, filtered, concentrated, reprecipitated, and dried over phosphoric oxide at 50°/15 mm. to constant weight. The product was neutral, non-reducing and had $[\alpha]_D^{15°} + 51°$ (*c*, 0·5 in water) [Found : Ash (as sulphate), 17·6; Ca, 3·6; Mg, 1·0; Na, 0·2; K, 0·1; SO_4, 23·9%].

Isolation of the Acid Polysaccharide.—The polysaccharide (hereafter designated H.E.) (0·75 g.) in water (50 c.c.) was treated with hydrochloric acid to bring the concentration to N, and the solution in a cellophane bag was dialysed against hydrochloric acid (N) until free from calcium (9 days). Dialysis was continued against distilled water until free from chloride (5 days), and the solution was then diluted to 250 c.c. in a standard flask. By titration against sodium hydroxide (0·05N) to phenolphthalein, the solution was found to be $7·49 \times 10^{-3}$ N; by weighing the sodium salt obtained on evaporation the equivalent of the sodium salt was calculated as 393 and that of the free acid as 371. The solution of the acid was non-reducing, but became reducing on heating at 100° for 10 minutes; $[\alpha]_D^{19°} + 43°$ (*c*, 0·14 in water) (Found : SO_4, 25·9%).

Potentiometric Titration.—The table shows a comparison between the titration of the above acid solution (*a*) against sodium hydroxide ($8·22 \times 10^{-3}$N) and hydrochloric acid ($5·67 \times 10^{-3}$N), (*b*) against sodium hydroxide ($5·13 \times 10^{-3}$N) using a quinhydrone electrode.

	NaOH added (c.c.).	e.m.f. (mv.).	pH.
	—	308	2·58
	10	304	2·65
	20	296	2·79
a)	30	284	3·00
	35	270	3·24
	40	242	3·73
	43	182	4·77
	46	12	7·71

	NaOH added (c.c.).	e.m.f. (mv.).	pH.
	—	304	2·65
	10	302	2·69
	20	298	2·76
(*b*)	30	292	2·86
	40	280	3·07
	44	268	3·28
	46	258	3·45
	50	232	3·90
	52	160	5·15
	54	70	6·71
	56	8	7·78

Hydrolyses of H.E.—(1) H.E. (4·98 g.) was hydrolysed at 100° with oxalic acid (210 c.c.; 0·5N) to constant rotation (26 hours, $[\alpha]_D^{15°}$ + 25°). The solution was decolorised with charcoal, neutralised with calcium carbonate, filtered, and evaporated at 35°/15 mm. to a syrup admixed with calcium sulphate. Extraction with small quantities of water, filtration, and evaporation gave a syrup (4·52 g.). Galactose was determined quantitatively by dissolving the syrup in water (45 c.c.), adding ethanol (45 c.c.), acetic acid (0·5 c.c.), and phenylmethylhydrazine (3·75 c.c.), and keeping at 0° for 4 days. The crystalline galactose phenylmethylhydrazone was filtered off, washed with water, ethanol, and ether, and dried for 3 days in a vacuum over phosphoric oxide, m. p. 186—187° (3·055 g.). Under identical conditions *d*-galactose (3·219 g.) gave galactose phenylmethylhydrazone, m. p. 186° (4·978 g.), *i.e.*, galactose from H.E., 39·6%.

(2) H.E. (11·1 g.) gave galactose phenylmethylhydrazone (6·83 g.), *i.e.*, galactose from H.E., 39·9%.

(3) H.E. (10·23 g.) was heated at 100° with a mixture of oxalic acid (0·1N) and potassium oxalate (400 c.c.; 0·1N) for 26 hours in a nitrogen atmosphere after Young and Rice (*loc. cit.*) and the procedure described by those authors for the isolation of diacetone 2-ketogluconic acid from Irish moss carried out. No identifiable products apart from galactose were obtained.

The filtrates from (1) and (2) were treated with benzaldehyde according to Lüdtke (*Biochem. Z.*, 1929, 419) to yield a glass, $[\alpha]_D$ ± 0° in water, which reduced Fehling's and Barfoed's solution, gave Schiff's test, the Seliwanoff, Bredereck, and selenium dioxide ketose reactions, and the iodoform reaction in the cold; tests for pentoses, methylpentoses, and uronic acids were negative. Salts of organic acids were present, but attempts to isolate esters by treating the free acids with diazomethane followed by methylation were not successful.

Hydrolysis of H.E. with N-*Sodium Hydroxide at* 100°.—H.E. (1·521 g.) was heated with N-sodium hydroxide (200 c.c.) at 100° in the presence of barium chloride (1·533 g.). At definite intervals samples (25 c.c.) were withdrawn, water (25 c.c.) and dilute acetic acid (10 c.c.) added, and the solution centrifuged. The residual combined sulphate in 50 c.c. of the solution was then determined by hydrolysis with hydrochloric acid and weighing the barium sulphate produced.

Time, hrs.	4	10	32	56	72
Residual sulphate ($BaSO_4$, mg.)	70·2	65·6	47·4	38·0	34·4
Hydrolysis, %	23	28	48	58	62

Acetylation of H.E.—To H.E. (15·9 g.) dissolved in water (200 c.c.), pyridine (700 c.c.) was added, and the pyridine–water azeotrope distilled off at 50°/15 mm. (Pacsu and Mullen, *loc. cit.*) to a volume of about 250 c.c. To the brown jelly so obtained pyridine (50 c.c.) and acetic anhydride (200 c.c.) were added, slowly, with shaking and cooling. After 12 days at room temperature the acetate was separated at the centrifuge and washed with pyridine, ethanol, and ether. The white powder (18 g.) so produced had $[\alpha]_D^{18°}$ + 46° (*c*, 0·8 in water) (Found: CH_3·CO, 19·1%).

Deacetylation and Methylation.—The above acetate in water (300 c.c.) was treated with methyl sulphate (120 c.c.) and sodium hydroxide (300 c.c.; 30%) in one-twelfth portions every ½ hour, the temperature being kept below 20°. The solution was then heated to 80° for 30 minutes, cooled, neutralised with acetic acid, and dialysed against running water until sulphate-free (10 days). The dialysed solution was evaporated at 45°/15 mm. to a glass (OMe, 16·2%). This glass was taken up in water (100 c.c.), worked up with pyridine as described above, and reacetylated (CH_3·CO, 5·9%). Simultaneous deacetylation and methylation as before increased the methoxyl content to 17·2%. This was followed by a third acetylation (CH_3·CO, 4·4%) and methylation (OMe, 17·6%), and a fourth acetylation (CH_3·CO, 3·5%) and methylation (OMe, 18·2%) (14 g.).

Attempts to increase the methoxyl content of this specimen by three direct methylations with methyl sulphate and sodium hydroxide in the usual way were unsuccessful, as was the application of the thallium method (Hirst *et al.*, *J.*, 1939, 1884).

The methylated polysaccharide was obtained by precipitation from aqueous solution (after dialysis) by ethanol, as a white fibrous solid, $[\alpha]_D^{15°}$ + 43° (*c*, 1·6 in water) [Found: Ash (as sulphate), 18·2; Ca, 3·75; Mg, 0·94; Na, 0·31; SO_4, 24·7; OMe, 18·6%].

Hydrolysis of the Methylated Polysaccharide.—The above product (5·13 g.; OMe, 18·6%) was hydrolysed at 100° with oxalic acid (200 c.c., N/2) until the rotation was constant ($[\alpha]_D^{15°}$ + 37°). The solution was neutralised with calcium carbonate and worked up in the usual way to a brown syrup (4·0 g., OMe, 26·3%).

Glycopyranoside Formation.—This syrup was boiled with methanolic hydrogen chloride (160 c.c.; 6%) until non-reducing (8 hours), neutralised with silver carbonate, evaporated, and extracted with ether to give a syrup (2·34 g., OMe, 36·0%, $n_D^{11°}$ 1·4665) which was fractionated to give (1) 0·09 g., b. p. 100°/0·1 mm., $n_D^{10°}$ 1·4295; (2) 1·9 g., b. p. 145—155°/0·07 mm., $n_D^{10°}$ 1·4737, $[\alpha]_D^{12°}$ + 70° (*c*, 1·4 in water) (Found: OMe, 40·4. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). Fraction (1) was identified as methyl lævulate isolated as the dinitrophenylhydrazone, m. p. 136° not depressed on admixture with an authentic specimen.

Preparation of Tetramethyl d-*Galactopyranose Anilide from* (2).—Fraction (2) (0·57 g.) was methylated with methyl sulphate and sodium hydroxide followed by two treatments with Purdie's reagents to give a mobile syrup (0·47 g.), distilled, 90—110°/0·02 mm., to yield a colourless liquid (0·4 g.; $n_D^{11°}$ 1·4495) which was hydrolysed with sulphuric acid (15 c.c., N) at 100° for 6 hours. From the reducing syrup (0·35 g.) isolated in the usual way, treatment with aniline in alcohol gave an anilide (0·23 g.), m. p. 193—194°, not depressed by authentic tetramethyl galactopyranose anilide; $[\alpha]_D^{15°}$ — 80° (*c*, 0·6 in acetone).

Isolation of Crystalline Dimethyl Galactose.—Fraction (2) (0·81 g.) was hydrolysed with sulphuric acid (30 c.c.; N) for 5 hours, $[\alpha]_D^{14°}$ + 82°. Neutralisation with barium carbonate, filtration, and evaporation gave a crystalline product (0·65 g.), m. p. 119—120° after three recrystallisations from dry ethyl acetate, $[\alpha]_D^{15°}$ + 48° (10 mins.), + 87° (240 mins., constant; *c*, 0·67 in water) (Found: C, 46·5; H, 7·5; OMe, 29·1. Calc. for $C_8H_{16}O_6$: C, 46·2; H, 7·7; OMe, 29·8%). Normally crystallising as needles, this

dimethyl galactose was obtained in the form of large plates from ethyl acetate–light petroleum (b. p. 40—60°).

Isolation of 6-Methyl Galactosazone.—The free sugar (0·34 g.) in water (5 c.c.) gave several crops (0·26 g.) of an osazone which on recrystallisation had m. p. 201—203° not depressed by authentic 6-methyl galactosazone (Found : OMe, 7·1. Calc. for $C_{19}H_{24}O_4N_4$: OMe, 8·3%).

Galactofuranoside Formation.—The sugar in methanolic hydrogen chloride (1%; *c*, 1·675) showed the following rotational changes (time in hours) : $[\alpha]_D^{13°}$ + 42° (0·75); 23° (18); 12·5° (25); − 9·5° (42); − 15·5° (48); − 29° (66); − 39° (92); − 43° (114; constant).

Evidence for Substitution on C_6.—Fraction (2) was treated with an excess of toluene-*p*-sulphonyl chloride in pyridine to yield a syrupy *product*, $[\alpha]_D^{15°}$ + 32° (*c*, 2·3, in chloroform) (Found : OMe, 15·5. $C_{23}H_{30}O_{10}S_2$ requires OMe, 17·6%).

Treatment with sodium iodide in acetone according to Oldham and Rutherford (*loc. cit.*) gave a product devoid of iodine. Oxidation of the crystalline dimethyl galactose with sodium periodate gave no formaldehyde.

Derivatives of 2 : 6-*Dimethyl Galactose.*—2 : 6-*Dimethyl galactonic acid.* The dimethyl galactose (1·59 g.) in water (25 c.c.) was treated with bromine (1·75 c.c.) at room temperature until non-reducing (6 days). After aeration, neutralisation with silver carbonate, and treatment with hydrogen sulphide, etc., a viscous syrup (1·66 g.), $[\alpha]_D^{17°}$ + 26° (*c*, 1·6 in water), was obtained (Found : C, 42·5; H, 7·1; OMe, 24·8. $C_8H_{16}O_7$ requires C, 42·9; H, 7·2; OMe, 27·7%).

2 : 6-*Dimethyl γ-galactonolactone.* The above crystalline *acid* (0·96 g.) distilled at 180°/0·02 mm. to give a syrup (0·9 g.), $n_D^{18°}$ 1·4760, $[\alpha]_D^{17°}$ − 49° (initial); − 24° (28 days; *c*, 1·09 in water) (Found : OMe, 28·6; equiv., 205. Calc. for $C_8H_{14}O_6$: OMe, 30·1%; equiv., 206). Titration with sodium hydroxide was characteristic of a γ-lactone.

2 : 6-*Dimethyl galactonamide.* The above lactone (0·34 g.) was treated with methanolic ammonia (5 c.c.) at 0° for 2 days; removal of solvent gave a crystalline amide (0·365 g.) which after three recrystallisations from ethanol gave small needles, m. p. 154—155°, $[\alpha]_D^{16°}$ + 46° (*c*, 0·85, in water) (Found : C, 43·5; H, 7·3; N, 6·0; OMe, 26·7. $C_{18}H_{17}O_6N$ requires C, 43·1; H, 7·7; N, 6·3; OMe, 27·8%). This *amide* (0·1 g.) gave a negative Weerman reaction.

2 : 6-*Dimethyl galactonic acid phenylhydrazide.* The lactone (0·2 g.) was allowed to react with phenylhydrazine (1 mol.) in boiling ether for 15 minutes. On removing the solvent and heating at 85—90° for 2 hours, a crystalline product was obtained which was recrystallised (0·25 g.) from ethanol–ether, m. p. 140° alone and admixed with an authentic specimen of 2 : 6-dimethyl galactonic acid phenylhydrazide supplied by Dr. D. J. Bell (Found : C, 53·1; H, 7·2; N, 8·9; OMe, 19·0. Calc. for $C_{14}H_{22}O_6N_2$: C, 53·5; H, 7·1; N, 8·9; OMe, 19·8%).

2 : 6-*Dimethyl β-methyl*-d-*galactoside.* The dimethyl galactose (0·5 g.) was acetylated at room temperature with pyridine and acetic anhydride in the usual way to give a crystalline acetate which was treated with hydrogen bromide in acetic acid followed by silver carbonate and methanol to give a non-reducing syrup (0·66 g.), $[\alpha]_D^{18°}$ + 19° (*c*, 6·6 in chloroform). This was deacetylated with sodium hydroxide (0·1N) and the product distilled at 145—150°/0·05 mm. to give a syrup (0·38 g.), $n_D^{19°}$ 1·4763, which crystallised on standing. After two recrystallisations from ether–light petroleum (b. p. 40—60°), hygroscopic needles, m. p. 72°, were obtained, $[\alpha]_D^{19°}$ − 22° (*c*, 0·9 in chloroform) (cf. Bell, *loc. cit.*) (Found : OMe, 41·0. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

2 : 6-*Dimethyl* 3 : 4-*monoacetone β-methylgalactoside.* The mother liquors from the above recrystallisations were evaporated to a syrup which crystallised slowly (0·2 g.) and was dissolved in dry acetone (50 c.c.) and shaken for 4 days with anhydrous copper sulphate (2 g.). After the usual treatment, distillation at 100°/0·1 mm. gave a crystalline product, m. p. 55° (cf. Bell, *loc. cit.*) (Found : OMe, 34·5. Calc. for $C_{12}H_{22}O_6$: OMe, 35·5%).

2 : 6-*Dimethyl galactose anilide.* The dimethyl galactose (0·31 g.) in ethanol (7 c.c.) was heated with aniline (1 mol.) at 80° for 3·5 hours to give a crystalline *anilide* (0·42 g.). Three recrystallisations from ethanol gave needles, m. p. 121—122°, $[\alpha]_D^{17°}$ + 15° (*c*, 0·7 in ethanol) (Found : N, 5·2; OMe, 22·2. $C_{14}H_{21}O_5N$ requires N, 4·9; OMe, 21·9%). A satisfactory elementary analysis for carbon could not be obtained from this material (see also Bell, *loc. cit.*).

Hydrolysis of Methylated H.E. followed by Furanoside Formation.—Methylated H.E. (5·23 g.; OMe, 18·6%) was hydrolysed with oxalic acid (0·5N) as before to give a syrup (3·54 g.) which was treated with methanolic hydrogen chloride (100 c.c.; 2%) at room temperature. After 8 days the solution was non-reducing {$[\alpha]_D^{17°}$ − 30° (*c*, 0·7)}. Neutralisation with silver carbonate, etc., gave a syrup (2·75 g.) which was distilled in the presence of barium carbonate, giving : (1) 0·05 g., b. p. 100°/15 mm., $n_D^{16°}$ 1·4302; (2) 1·98 g., b. p. 120—145°/0·04 mm., $[\alpha]_D^{16°}$ − 35° (*c*, 3·8; in water), $n_D^{16°}$ 1·4672 (OMe, 39·0%); (3) 0·3 g., b. p. 145—165°/0·02 mm., $n_D^{16°}$ 1·4710; (4) 0·13 g., b. p. 165—205°/0·02 mm., $n_D^{16°}$ 1·4813, $[\alpha]_D^{16°}$ − 8° (*c*, 1·1 in water) (OMe, 33·9%). Fraction (2) was a mixture of 2 : 6-dimethyl methylgalactofuranosides and gave on hydrolysis (1·66 g.) crystalline 2 : 6-dimethyl galactose (1·2 g.), m. p. 110° without recrystallisation. Fraction (4) was thought to be an impure monomethyl methylgalactoside, but attempts to secure evidence of this by osazone formation were not successful.

Hydrolysis of Methylated H.E. with Methanolic Hydrogen Chloride.—Methylated H.E. (4·65 g.; OMe, 20·2%) was heated with methanolic hydrogen chloride (200 c.c.; 1·2%) for 24 hours in the presence of barium chloride (5·1 g.). After neutralisation with barium carbonate a non-reducing glass (4·1 g.) was isolated which still contained sulphur (OMe, 33·5%); this was boiled for 16 hours with methanolic hydrogen chloride (100 c.c.; 2%) in the presence of barium chloride. After neutralisation and suitable treatment an ether-soluble fraction (A) (1·15 g.) and a glass (B) (2·6 g.) were isolated. (B) was further hydrolysed for 24 hours with methanolic hydrogen chloride (100 c.c.; 2·7%) and barium chloride from which an ether-soluble fraction (C) (0·23 g.) and an ethanol-soluble fraction (D) (2·1 g.; OMe, 32·0%) were obtained. (A) and (C) were combined and distilled at 135—155°/0·05 mm. to give mainly 2 : 6-dimethyl methylgalactosides (1·11 g.), $n_D^{13°}$ 1·4748 (OMe, 38·1%), which gave crystalline 2 : 6-dimethyl galactose in good yield on hydrolysis.

Fraction (D) (1·75 g.) was dissolved in methano and methylated five times with silver oxide and

methyl iodide. Distillation gave: (1) 0·79 g., b. p. 90—95°/0·02 mm., $n_D^{17°}$ 1·4481 (OMe, 57·6%); (2) 0·54 g., b. p. 95—100°/0·02 mm., $n_D^{17°}$ 1·4478 (OMe, 56·9%); (3) 0·12 g., b. p. 100—125°/0·02 mm., $n_D^{17°}$ 1·4483 (OMe, 55·3%).

Fraction (1) (0·43 g.) was hydrolysed with sulphuric acid (N) and the reducing syrup (0·3 g.) treated with aniline in ethanol to give tetramethyl *d*-galactopyranose anilide (0·14 g.), m. p. 195°, $[\alpha]_D^{16°}$ − 78° (*c*, 0·7 in acetone). The other component of the mixture could not be identified.

Fractions (1), (2), and (3) (0·9 g.) were combined, and, after hydrolysis and treatment with aniline, tetramethyl *d*-galactopyranose anilide (0·3 g.), m. p. 196—197°, $[\alpha]_D^{18°}$ − 81° (*c*, 0·7 in acetone), was obtained. No other products could be identified.

A further hydrolysis with methanolic hydrogen chloride (4%, in nitrogen) resulted in the isolation after a chromatographic separation on aluminium oxide of 2 : 6-dimethyl galactose, m. p. 112° (30%). No other identifiable products could be isolated, and a search for methylated derivatives of 2-ketogluconic acid after complete methylation was abortive.

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