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The Structure of κ-Carrageenan from *Gigartina Tenella*

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An alkaline treatment of κ-carrageenan from *Gigartina tenella* resulted in the change of the proportions of galactose, 3,6-anhydro-galactose and sulfate from 100 : 91 : 124 to 95 : 96 : 119. Fragmentation of the methylated polysaccharide gave 2,4,6-tri-*O*-, 2,6-di-*O*-, and mono-*O*-methyl-D-galactose in the molar proportions of 1 : 18 : 0.8 together with 3,6-anhydro-D-galactose and its 2-*O*-methyl-derivative in the molar proportion of 1 : 4. From these and other data, it is concluded that the polysaccharide is composed of the residues of 1,3-linked β-D-galactose 4-sulfate (95 mol), 1,4-linked α-D-galactose 6-sulfate (5 mol), and 1,4-linked 3,6-anhydro-α-D-galactose (67 mol) and its 2-sulfate (24 mol), and that the 1,3- and 1,4-linked residues are repeated alternatingly to form a chain macro-molecule.

The chemical structure of κ-carrageenan obtainable from several species of red seaweeds of the *Chondrus and Gigartina* families has been extensively investigated by many workers.[1)]

Our previous studies on the κ-carrageenan from *G. tenella*[2)] showed the presence of D-galactose, 3,6-anhydro-D-galactose and ester sulfate in the molar proportions of 100 : 98 : 117, together with very small amounts of L-galactose and D-xylose. Partial methanolysis provided evidence that carrabiose (3,6-anhydro-4-*O*-β-D-galactopyranosyl-D-galactose) represented the repeating units of the backbone structure of the sulfated polysaccharide. In the present paper, further details of the structure have been investigated by means of alkali-treatment, methylation and IR spectroscopic measurement.

The κ-carrageenan used in this work has been prepared in the same manner as that described in the preceding paper.[2)] On analysis it contained galactose, 3,6-anhydro-galactose and sulfate in the molar proportions of 100 : 91 : 124, the proportions being in approximate agreement with those of the previous preparations. When this polysaccharide sample was treated with alkaline borohydride according to the method of Rees,[3)] the molar proportions of the components changed from those described above to 95 : 96 : 119. It is apparent that both galactose and sulfate decreased by 5 mol with concurrent increase of an equimolar amount of the anhydro-sugar. This could be explained by the presence of 2-linked galactose 3-or 6- sulfate or 4-linked galactose 3- or 6-sulfate residues, any of which could be convertible into 3,6-anhydro-galactose residues by the alkali treatment. However, from the general concept[4)] of chemical structures, based on extensive investigations of a variety of sulfated polysaccharides of red seaweed origin, it is most probable that 5 mol of 4-linked galactose 6-sulfate residues exist in the sulfated polysaccharide under investigation.

The sulfated polysaccharide was then methylated ten times with dimethyl sulfate and sodium hydroxide solution. The product contained 17.3% of methoxyl groups and 16.7% of sulfate groups. If the methylation was complete for the alkali-modified polysaccharide, 4-linked galactose 6-sulfate residues should have been converted into 3,6-anhydro-galactose re-

1) E. Percival and R. H. Mcdowell, "Chemistry and Enzymology of Marine Algal Polysaccharides," Academic Press, London and New York (1967).

2) S. Hirase and K. Watanabe, This Bulletin, **40**, 1442 (1967).

3) D. A. Rees, *J. Chem. Soc.*, **1961**, 5168.

4) N. S. Anderson, T. C. S. Dolan, and D. A. Rees, *Nature* **205**, 1060 (1965); D. A. Rees, *Adv. Carbohyd. Chem. Biochem.*, **24**, 267 (1969).

sidues, and consequently, the product would contain 17.2% of methoxyl groups and 20.6% of sulfate groups. That the observed sulfate content was lower than the expected one suggested that additional desulfation had occurred during the methylation.

TABLE 1. FRAGMENTATION OF METHYLATED κ-CARRAGEENAN

Methylated sugar	Molar ratio
2,4,6-Tri-*O*-methyl-D-galactose	1.0
2,6-Di-*O*-methyl-D-galactose	18.0
Mono-*O*-methyl-D-galactose	0.8
2-*O*-Methyl-3,6-anhydro-D-galactose	4.0
3,6-Anhydro-D-galactose	1.0

The complete degradation of the methylated polysaccharide gave the products shown in Table 1. 2,4,6-Tri-*O*-methyl-D-galactose and 2,6-di-*O*-methyl-D-galactose were identified by the isolation of crystalline reducing sugars. Mono-*O*-methylgalactoses were shown to be mainly 2-*O*-methyl- and 6-*O*-methyl derivatives by paper chromatography, gas liquid chromatography and paper electrophoresis. 3,6-Anhydro-D-galactose was obtained as a syrupy dimethylacetal, and was then converted into crystalline diphenylhydrazone. 2-*O*-Methyl-3,6-anhydro-D-galactose was identified by the isolation of its crystalline methyl β-glycoside. In addition, a trace amount of 2,4-di-*O*-methyl-D-galactose was also shown to be present.

Among the partially methylated D-galactoses obtained above, 2,6-di-*O*-methyl-D-galactose was the major product in accordance with the cases of κ-carrageenans of other origin.[5] This product is derived either from 3-linked D-galactose 4-sulfate or 4-linked D-galactose 3-sulfate residues. But the latter possibility is completely excluded, because the 3-sulfate residues would have been converted into 3,6-anhydro-D-galactose residues by the action of alkali during methylation. The formation of 2,4,6-tri-*O*-methylgalactose appears to indicate the presence of non-sulfated, 3-linked D-galactose residues. However, on the basis that the yield was as small as one eighteenth of that of 2,6-di-*O*-methyl-D-galactose, and also that some desulfation occurred during methylation as discussed above, it seems more probable that this tri-*O*-methylgalactose has been derived by desulfation followed by methylation from 3-linked D-galactose 4-sulfate residues. The yield of the tri-*O*-methylgalactose relative to that of the di-*O*-methylgalactose indicated that 5% of the 4-sulfate residues were desulfated during methylation. The formation of small amounts of 2-*O*-, and 6-*O*-methylgalactose could be accounted for by incomplete methylation of 3-linked D-galactose 4-sulfate residues. 2,4-Di-*O*-methyl-D-galactose was formed in so small an amount that it had no structural significance. After all, all the D-galactose residues seem to be joined 1,3 with sulfate groups on C_4 in the native polysaccharide.

On the other hand, the formation of 2-*O*-methyl-3,6-anhydro-D-galactose and non-methylated 3,6-anhydro-D-galactose as the cleavage fragments from the methylated polysaccharide indicates that all the anhydro-sugar residues are joined 1,4 as has been proved by the isolation of carrabiose derivatives in high yield[2], and also that some of the anhydro-sugar residues are 2-sulfated. The proportion of the 2-sulfated residues to the non-sulfated residues may not be directly evaluated from the relative yields of the anhydro-sugar fragments from the methylated polysaccharide, because a part of the 2-sulfate groups might have possibly been released during methylation. This possibility is supported by the fact[6] that the treatment of methyl 2-*O*-p-toluensulfonyl-3,6-anhydro-D-galactoside with strong alkali resulted in complete desulfonation without configurational inversion.

Now the problems to be solved are concerned with the proportion of 2-sulfation on anhydro-sugar residues in the native polysaccharide and also with the proportion of desulfation of these sulfate groups during methylation. These problems have been tentatively solved as shown in Table 2, which includes the summary of discussion as well. As has been already mentioned, the native polysaccharide contains galactose, anhydrogalactose and sulfate in the molar proportions of 100 : 91 : 124. Since, on the basis of the results of alkali treatment, 5 mol per 100 mol of galactose residues are joined 1,4 with 6-sulfate groups, as has been mentioned above, the residual 95 mol are joined 1,3 with 4-sulfate groups. These two residues account for 100 mol per 124 mol of total sulfate groups so that

TABLE 2. STRUCTURAL FEATURES OF THE κ-CARRAGEENAN

κ-Carrageenan	Residues in relative moles					
	3-Linked galactose		4-Linked galactose			Total
	non-S	4-S	6-S	A, non-S	A, 2-S	sulfate
Native	nil	95	5	67	24	124
		100		91		
Alkali-modified	nil	95	nil	67+5[a]	24	124−5[a]
Methylated	5[b]	95−5[b]	nil	67+5[a]+5[b]	24−5[b]	124−5[a]−10[b]

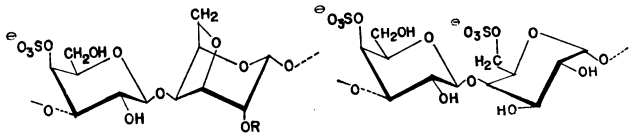
S: sulfate, A: 3,6-anhydride.
a) due to desulfation with concurrent formation of 3,6-anhydro sugar.
b) due to desulfation during methylation.

5) N. S. Anderson, T. C. S. Dolan, and D. A. Rees, *J. Chem. Soc., C*, **1968**, 596.

6) P. A. Rao and F. Smith, *ibid.*, **1944**, 229.

24 mol of sulfate groups could exist on C_2 of anhydro-sugar residues. Then the non-sulfated anhydro-sugar residues exist by 67 mol, the proportion of 2-sulfation to non-sulfation being approximately 1 : 3. When the polysaccharide was methylated, 5 mol per 95 mol of galactose 4-sulfate residues seems to have been desulfated, as was discussed above. Similarly, if it is presumed that 5 mol per 24 mol of anhydro-sugar 2-sulfate residues were desulfated during methylation, fragmentation of the methylated polysaccharide would produce 3,6-anhydro-D-galactose and its 2-methyl ether in the molar proportion of 77 : 19, which agrees with the experimental result (4 : 1), Furthermore, when such desulfation as discussed above are taken into consideration, the molar proportion of methoxyl groups to sulfate groups should be 2.48 for the completely methylated polysaccharide. This value is again reasonable for the experimental result (2.67).

In conclusion, the κ-carrageenan under investigation is a hybrid consisting of three structural units a (70%), b (25%), and c (5%) shown in Fig. 1, the α-linkages being presumed from the positive value of optical rotation of the native polysaccharide.[2] Although the indicated proportions between the units are based on the discussion about Table 2, the κ-carrageenan seems to contain a family of related polysaccharide molecules, which differ more or less in the proportions of a, b, and c, as has been pointed out by Anderson and his co-workers[7]. The structure proposed above is similar in outline to that reported for the κ-carrageenan from *Gigartina stellata* as well as from *Chondrus crisps*.[5]



(a) R=H
(b) R=$SO_3^{\ominus}$ (c)

Fig. 1. The possible structure of the κ-carrgeenan from *G. tenella*

The proposed structure is also supported by infrared spectrum, which showed absorption bands at 850 cm^{-1} and 805 cm^{-1}. The former band is attributed to the sulfate groups attached to secondary axial hydroxyl groups[8] (C_4 of D-galactose residues), while the latter band is characteristic to the 2-sulfate groups on anhydro-sugar residues.[7] The band (820 cm^{-1}) due to the sulfate groups on primary hydroxyl groups (C_6 of galactose residues) was not observed. The 6-sulfated residues exist in so small amounts that the band may be masked in the broadening region of neighboring bands.

Experimental

General Method. Solutions were concentrated under reduced pressure at 40°C in a rotary evaporator. Melting points were measured on Yanagimoto apparatus Model MP-S2. Paper electrophoresis was performed on Toyo filter paper No. 51 with 0.1 M sodium borate buffer (pH 10) and a potential gradient of 17 volts/cm. Infrared spectra were recorded with Hitachi Infrared Spectrophotometer Model 215 (sample as a potassium bromide disk).

Paper Chromatography (pc) and Thin Layer Chromatography (tlc). Pc was performed on Toyo-filter paper No. 51, and the solvents (in v/v) used were as follows: A) 1-butanol - acetic acid - water (4 : 1 : 2); B) 1-butanol - ethanol-water (5 : 1 : 4, upper layer); C) butanone - 28% ammonium hydroxide - water (20 : 0.1 : 1.7); D) cyclohexanol saturated with water; E) 1-butanol - pyridine - water (10 : 3 : 3); F) benzene - ethanol (5 : 1) saturated with water; G) ethyl acetate - pyridine - water saturated with ammonium borate (12 : 4.5 : 3); and H) 1-butanol-water saturated with ammonium borate (4 : 1 : 2). In the case of the solvents G and H, filter paper was dipped into ammonium borate solution and dried before use. Spray reagents used were a) aniline hydrogen phthalate,[9] b) *p*-anisidine hydrochloride,[10] and c) *o*-aminophenol phosphate.[11] Tlc was carried out on glass plates (20 cm in length) coated with silica gel G containing calcium sulfate as binder, using the solvents J ethyl acetate-pyridine-water (30 : 2 : 1), and K benzene-ethanol-water (40 : 15 : 1). Chromatograms were sprayed with 2N-methanolic sulfuric acid and heated at 110°C.

Gas Liquid Chromatography (glc). The apparatus used was a Hitachi Gas Chromatograph Model K53 equipped with a hydrogen flame ionization detecter, using nitrogen as a carrier gas. Analyses were carried out by the following methods depending upon the purpose: Method A: For the general purpose, methyl glycosides and dimethyl acetals of methylated sugars were analysed on a column (3 mm × 1 m) packed with 2.5% neopentylglycol succinate on chromosorb W (100—200 mesh) at 160°C. This method has the disadvantage that some of the peaks are overlapped each other especially in the analysis of a complicated mixture. Method B: For the purpose of identifying anhydro-sugar derivatives, a mixture (10 mg) of methyl glycosides and dimethyl acetals of methylated sugars was dissolved in concentrated hydrochloric acid (0.2 m*l*) at 0°C, ethanethiol (0.1 m*l*) added, and the mixture was vigorously stirred at 0°C for 15 min. By this treatment, anhydro-sugar derivatives were converted into diethyl dithioacetals, while methylated methyl galactosides were mostly unchanged. The reaction mixture was neutralized with saturated sodium bicarbonate solution, evaporated to dryness, and the trimethylsilylated with pyridine (0.5 m*l*), trimethylchlorosilane (0.1 m*l*), and hexamethyldisilazane (0.2 m*l*). The products in chloroform (3 m*l*) were washed three times with cold water (1 m*l*), dried with anhydrous magnesium sulfate, evaporated, and then analysed on a column (1 m) packed with 5% neopentyl glycol succinate on chromosorb W (100—200 mesh) at 160°C. Retention times of trimethylsilylated diethyl dithioacetals were much higher than those of trimethylsililated methyl galactosides. Method C: For the purpose of identifying galactose derivatives, the sample to be analysed was hydrolysed with 1 N-sulfuric acid at 100°C for 5 hr. During this treatment, anhydro-sugar derivatives are, if present, completely degraded. The remaining methylated galactoses were converted into alditol acetates, which were then analysed on a column (2 m)

7) N. S. Anderson, T. C. S. Dolan, A. Penman, D. A. Rees, G. P. Mueller, D. J. Stancioff, and N. F. Stanley, *ibid.*, **1968**, 602.
8) J. R. Turvey and T. P. Williams, ibid., **1962**, 2119.
9) S. M. Partridge, *Nature*, **164**, 443 (1949).
10) J. B. Pridham, *Anal. Chem.*, **28**, 1967 (1956).
11) S. Hirase, C. Araki, and S. Nakanishi, This Bulletin, **26**, 183 (1953).

packed with 3% ECNSS-M on chromosorb W.[12,13]

Column Chromatography. Effluents were collected in an automatic fraction collector, and examined for their contents by pc, tlc, and/or coloration with an anthrone-sulfuric acid reagent and a resorcinol-hydrochloric acid reagent.[14] Fractions containing the same contents were combined, evaporated and again examined by pc, tlc and/or glc.

Alkali Treatment of Polysaccharide. According to the method of Rees,[3] the polysaccharide (0.42 g) dissolved in water (250 m*l*) was treated with sodium borohydride (0.1 g) at room temperature for 24 hr. Sodium hydroxide (10 g) and sodium borohydride (1.5 g) were then added, and the mixture was heated at 80°C for 3 hr. The solution was neutralized with acetic acid, dialysed against running tap water for 2 days, concentrated, precipitated with ethanol, and finally dried *in vacuo*; yield, 0.33 g (78.5%).

The product, as well as the sample before alkali treatment, was analysed for sugars by the colorimetric method with a resorcinol reagent and an anthrone reagent after Yaphe,[14] and for sulfate by the volumetric method[15] modified as follows: the sample (2—5 mg) to be analysed was dissolved in water (3—10 m*l*), and about 0.005 N glycol chitosan solution (2 m*l*) was added in excess to precipitate the polysaccharide sulfate in a stoichiometric reaction. To this mixture, 1 N-hydrochloric acid (0.5 m*l*) and one or two drops of 0.2% toluidine blue indicator solution were added. The excess of the glycol chitosan was titrated with a standard 0.0025 N potassium polyvinyl alcohol sulfate solution (equivalent 162) until the indicator changed from blue to reddish purple. The sulfate content of the sample was calculated from the difference between the titration value and the blank value. The results were as follows: the molar proportions of galactose, 3,6-anhydro-galactose and sulfate were 1 : 0.91 : 1.24 in the native polysaccharide, and 0.95 : 0.96 : 1.19 (1 : 1.01 : 1.25) in the alkali-treated sample.

Methylation of Polysaccharide. To a solution of polysaccharide (12.4 g) in water (150 m*l*) treated with sodium borohydride (0.5 g) overnight at room temperature was added dimethyl sulfate (50 g) and 30% sodium hydroxide (80 m*l*) dropwise with vigorous stirring during 6 hr at 60°C under at atmosphere of nitrogen. The reaction mixture was left overnight. Dimethyl sulfate (50 g) and 30% alkali (80 m*l*) were again added in the same manner. The reaction mixture was left overnight, then heated at 80°C for 1 hr to decompose the excess of dimethyl sulfate, neutralized with 2 N-sulfuric acid, dialysed against running tap water for 50 hr, and concentrated. The resulting solution was treated four more times with dimethyl sulfate and alkali in the same manner as above-mentioned. Finally, the methylated polysaccharide was precipitated with 99% ethanol and dried *in vacuo*; yield, 10 g; $[\alpha]_D^{10}$ +57.3° (*c* 0.79, water), (Found: OCH_3, 17.3; SO_3, 16.7%).

Methanolysis of Methylated Polysaccharide. The methylated polysaccharide (8.1 g) was treated with 3% methanolic hydrogen chloride at 80°C for 20 hr. The reaction mixture was then neutralized with silver carbonate, filtered and evaporated. The resulting syrup was treated with 0.2 N-barium hydroxide solution (100 m*l*) at 60°C for 2 hr, neutralized with carbon dioxide, and filtered. The filtrate was passed through Amberlite IR-120 (200 m*l*) and IR-45 (300 m*l*) in succession, and the deionized solution was evaporated to a syrup; yield, 4.82 g.

Analysis of the Methanolysis Products. Examination of the methanolysis products obtained above by pc (solvent D, spray reagent c) indicated the presence of 3,6-anhydrogalactose dimethyl acetal (**1**), its 2-methyl ether (**2**), and the methyl β-glycopyranoside (**3**) corresponding to **2**. Analysis by glc (method B) showed two peaks of diethyl dithioacetals, one of which was derived from **1**, and the other from **2**. and **3**. From the peak areas measured and the molar response factor determined for authentic samples, it was shown that 3,6-anhydrogalactose and its 2-methyl ether existed in the molar proportion 1 : 4.

On the other hand, when a portion of the methanolysis products was hydrolysed and examined by pc (solvent A, B and C; spray reagent a and b), there were detected 2,6-di-*O*-methylgalactose (**4**) together with small amounts of 2,4,6-tri-*O*-methylgalactose (**5**) and mono-*O*-methylgalactose (**6**). Analysis by glc (method C) showed that **4**, **5** and **6** existed in the molar proportions of 1 : 18 : 0.8, the figure being based on the peak areas and molar response factors.

Separation of the Methanolysis Products. Methanolysis products (1.3 g) obtained above were applied to a Dowex 1×4 column (OH form, 5×36 cm) and eluted first with distilled water to give fractions I, II, and III. The column was then eluted with 0.2 M-ammonium carbonate aqueous solution, and the effluent was deionized with Amberlite IR-120 and IR-45 resins, and evaporated to give fraction IV. All the fractions were obtained in syrups.

Fraction I: Yield, 0.61 g. This fraction should be a mixture of methyl glycosides of partially methylated galactoses, as it showed color reaction with an anthrone reagent and no color reaction with a resorcinol reagent. It was combined with a similar fraction obtained by the same procedure as that mentioned above, and the resulting mixture (1.4 g) was hydrolysed with 1 N-sulfuric acid (20 m*l*) at 100°C for 30 hr. The resulting mixture of reducing sugars (1.08 g) was separated on a cellulose powder column (5×40 cm), which was eluted first with a benzene-ethanol (5 : 1) mixture saturated with water, to give fractions I-1 (0.24 g), and I-2 (0.88 g) in syrups. The proportion of the solvent mixture was then changed to 3 : 1 to give fraction I-3 (0.04 g) in a syrup.

Examination of the fraction I-1 by pc (solvent F, spray reagent b) showed two spots, one (R_f 0.73, pink brown) corresponding to 2,4,6-tri-*O*-methylgalactose, and the other (R_f 0.63, pale brown) corresponding to an unidentified compound. In tlc (solvent J and K), the former component gave a darker spot with slightly higher R_f value than the latter component. The mixture was separated to the components on an Avicel column (3×92 cm) with a benzene-ethanol (5 : 1) mixture saturated with water. The faster moving component (0.077 g) was identified as 2,4,6-tri-*O*-methyl-D-galactose, which was crystallized from ether; mp and mmp 102—103.5°C,[16] $[\alpha]_D^{10}$ +116.9°→+79.8°[16,19] (*c* 0.46, water), (Found: C, 48.66; H, 8.19%). The slower-moving component (0.07 g) was not examined further.

Fraction I-2 (0.88 g) gave a single spot corresponding to 2,6-di-*O*-methylgalactose both in pc (solvent B and C, spray reagent b) and tlc (solvent K). It crystallized readily and was recrystallized from ethanol - petroleum ether, 2,6-di-*O*-

12) J. S. Sawardeker, J. H. Sloneker, and A. Jeanes, *Anal. Chem.*, **37**, 1602 (1965).
13) H. Björndal, B. Lindberg, and S. Svensson, *Acta. Chem. Scand.*, **21**, 1801 (1967).
14) W. Yaphe, *Anal. Chem.*, **32**, 1327 (1960).
15) S. Okimasu, *Nippon Nogei Kagaku Zasshi*, **22**, 63 (1958).
16) C. Araki, *Nippon Kwagaku Kwaishi*, **58**, 1362 (1937).
17) E. T. Dewar and E. G. V. Percival, *J. Chem. Soc.* **1947**, 1622.
18) P. Andrews, L. Hough, and J. K. N. Jones, *ibid.*, **1954**, 806.
19) C. Araki and S. Hirase, This Bulletin, **33**, 291 (1960).

methyl-D-galactose was obtained as plates; mp and mmp 118—119.5°C,[17,18] $[\alpha]_D^{10}$ +47.7°→+90.7°[17,18] (*c* 0.73, water), (Found: C, 46.16; H, 7.91%).

Fraction I-3 (0.04 g) was shown by pc (solvent C and F, spray reagent b) to be a mixture of 2,6-di-*O*-methylgalactose, 2,4-di-*O*-methylgalactose and mono-*O*-methylgalactose. The mixture was separated to the components on an Avicel column (2×30 cm), which was first eluted with benzene-ethanol (5 : 1) saturated with water to give two fractions. The proportion of the solvent mixture was then changed to 3 : 1 to give an additional fraction. The first fraction (0.11 g) on crystallisation gave 2,6-di-*O*-methyl-D-galactose; mp and mmp 118—119.5°C, $[\alpha]_D^{12}$ +45.3°→+88.5° (*c* 0.46, water). The second fraction (0.005 g), $[\alpha]_D^{28}$ +76.7° (*c* 0.45, water),[18] was obtained as a syrup, which showed exactly the same behavior as that of an authentic sample of 2,4-di-*O*-methyl-D-galactose both in pc[18] (solvent C and F, spray reagent b) and glc (method C). This fraction was obtained in too small an amount to be crystallized. The last fraction (0.025 g) was obtained as a syrup, which was shown to be a mixture of 2-*O*-, 3-*O*-, 4-*O*-, and 6-*O*-methyl-galactoses both by paper electrophoresis and glc (method C), the first and last components being predominant. Isolation of the components was not attempted.

Fraction II. Yield, 0.48 g; $[\alpha]_D^{23}$ −21.6° (*c* 0.85, water). Examination of the fraction II by pc and tlc with any solvent system, detected a single spot, respectively. But examination by glc (method A) revealed the presence of three components, one of which had exactly the same retention time as that of an authentic sample of 2-*O*-methyl-3,6-anhydro-galactose dimethyl acetal. The other two seemed to be methyl 2,6-di-*O*-methyl-α- and -β-galactofuranosides. The negative value of optical rotation of this fraction II supported the above view. As the mobilities of these three were identical both on a filter paper and a thin layer chromatogram with any solvent system, the separation on a column was not attempted. Instead, the fraction was treated with methanolic hydrogen chloride to give an equilibrium mixture in the following manner. The fraction was combined with a similar fraction (1.2 g) obtained from the methanolysis products of the methylated polysaccharide, and the resulting mixture (1.68 g) was heated with 1.5% methanolic hydrogen chloride (20 m*l*) at 80°C for 23 hr, at which time optical rotation of the reaction solution reached a constant value (α_D −2.5→+0.8°). The reaction solution was treated in the usual manner, and the products were chromatographed on a Dowex 1×4 column (OH form, 3×46 cm) with distilled water as a eluting solvent, three fractions being obtained. The first fraction (0.41 g), $[\alpha]_D^{15}$ +120.2° (*c* 0.88, water) was identified as methyl 2,6-di-*O*-methyl-α- and -β-D-galactopyranosides, which on hydrolysis with N-sulfuric acid at 100°C for 5 hr gave 2,6-di-*O*-methyl-D-galactose; mp and mmp 118—119°C, $[\alpha]_D^{10}$ +47°→+86° (*c* 0.52, water). The second fraction (0.75 g), $[\alpha]_D^{15}$ −18.2° (*c* 0.61, water) was a mixture of the same components as those of the fraction II itself. The last fraction (0.29 g) was identified as methyl 2-*O*-methyl-3,6-anhydro-β-D-galactopyranoside, which was crystallized from ether; mp 65.5°C, $[\alpha]_D^{10}$ −65.1° (*c* 0.87, water).

Fraction III. Yield, 0.126 g; $[\alpha]_D^{23}$ −44.0° (*c* 1.45, water). In pc (solvent D, spray reagent c) the fraction III showed a single spot. It crystallized on standing. Recrystallization from ether gave methyl 2-*O*-methyl-3,6-anhydro-β-D-galactopyranoside. This is the first information of this compound, although its enantiomorph was reported before;[19] mp 65—65.5°C, $[\alpha]_D^{10}$ −66.3° (*c* 0.79, water), (Found: C, 50.52; H, 7.5%).

Fraction IV. Yield, 0.061 g; $[\alpha]_D^{23}$ +24.0° (*c* 1.1, water). This fraction was identified as 3,6-anhydro-D-galactose dimethyl acetal[2,20,21] by the optical rotation, pc (solvent A, B, and D, spray reagent c) and also by the hydrolysis with 0.1 N-sulfuric acid at 100°C for 2 hr to the reducing sugar, which was then converted into the diphenylhydrazone;[20] mp and mmp 156—157.5°C, $[\alpha]_D^{10}$ +40.0°→+31.9° (*c* 0.26, methanol), (Found: C, 66.07; H, 5.75; N, 7.94%).

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