167. *Fucoidin. Part II. The Hydrolysis of a Methylated Fucoidin prepared from* Fucus vesiculosus.

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L-Fucose (*ca.* 1 part), 3-methyl L-fucose (*ca.* 3 parts) and 2 : 3-*dimethyl* L-*fucose* (*ca.* 1 part) have been identified in the carbohydrate portion of the hydrolysate of a methylated fucoidin. A branched structure in which 1 : 2-α-links predominate is suggested for the polysaccharide.

The fucoidin used in this series of experiments was prepared from dried, ground *Fucus vesiculosus* collected in the summer months of 1945. The product was comparable with the specimens of fucoidin described in Part I (*J.*, 1950, 717) but the intensive purification was not applied. It had $[\alpha]_D^{15}$ −118°, gave 38% of L-fucose on hydrolysis, and contained Ca 5·9, Mg 0·2, Na 0·9, K 0·1—0·2, and SO_4 32·8%, the sulphate of the ash accounting for half the total sulphate. It required 36 hours at 100° to remove 36% of the total sulphate with N-sodium hydroxide.

Methylation with sodium hydroxide and methyl sulphate gave a product having $[\alpha]_D^{15}$ −107° and containing Ca 5·1, Mg 0·2, Na 0·8, K 0·05, SO_4 25·9, and OMe 15·8%, in which the sulphate in the ash comprised 57% of the total. Evidently, therefore, as for the carragheen polysaccharides (Buchanan, Percival, and Percival, *J.*, 1943, 51; Dewar and Percival, *J.*, 1947, 1622) the metals combined with the sulphate group were not displaced by sodium during the methylation process. That all the available hydroxyl groups were substituted in the above product was shown by the fact that on treatment with acetic anhydride and pyridine an insignificant amount of acetylation occurred. The acetyl content of fucoidin acetate was found to be 18·0%. If the basic formula is assumed to be $(C_6H_9O_3SO_4Ca_{0·5})_n$ the methylated derivative would contain OMe 12·0%, and the acetate OAc 15·0%. The slightly higher values obtained in practice are attributed to the presence of small amounts of more highly substituted " foreign " polysaccharides rather than to the loss of sulphate groups from the substituted fucose units during methylation.

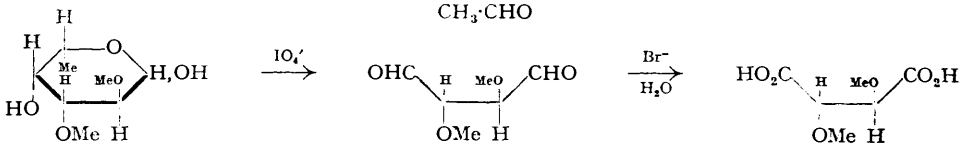
The methylated fucoidin was hydrolysed, and the products were converted into methyl-glycosides and separated by solvent extraction (Brown and Jones, *J.*, 1947, 1344) and on an alumina column. The only compounds to be isolated in quantity were methyl L-fucosides, and the following were found present : methyl-L-fucosides (*ca.* 20%), 3-methyl L-fucosides (*ca.* 57%), and 2 : 3-dimethyl L-fucosides (*ca.* 20%).

The monomethyl sugar was shown to be a derivative of L-fucose by conversion into 2 : 3 : 4-trimethyl α-methyl-L-fucoside. The methyl group was assigned to $C_{(3)}$ for the following reasons. Oxidation gave a lactone, m. p. 136°, $[\alpha]_D$ + 25° ⟶ +75° (in water; 62 hours), with constants comparable with those of 3-methyl D-fuconolactone (digitalonolactone), m. p. 136—140°, $[\alpha]$ −22° ⟶ −70° (60 hours), a specimen of which was prepared from digitalose isolated from *iso*emicymarin. The derived *amide* gave a positive Weerman test.

Further support for the view that we were dealing with 3-methyl L-fucose was derived from a direct comparison on the paper chromatogram with 2-methyl L-fucose, synthesised by the method of McPhillamy and Elderfield (*J. Org. Chem.*, 1939, **4**, 150), and with digitalose (3-methyl D-fucose). There was a distinct separation between 2-methyl L-fucose (R_G 0·56) (Hirst, Hough, and Jones, *J.*, 1949, 928) and digitalose (R_G 0·45), and the material under test was indistinguishable from the latter on the chromatogram. A small quantity of a monomethyl α-methyl-L-fucoside, m. p. 130—132°, $[\alpha]_D$ −173°, which was isolated on one occasion gave on hydrolysis a sugar chromatographically identical with digitalose and must therefore be designated *3-methyl α-methyl-L-fucoside*.

The dimethyl methylglycoside was shown to be a L-fucopyranose derivative by complete methylation to give trimethyl α-methyl-L-fucopyranoside. The methyl groups were assigned to $C_{(2)}$ and $C_{(3)}$ for the following reasons. Oxidation of the dimethyl sugar gave a syrupy lactone, $[\alpha]_D$ +9° ⟶ +47° (22 hours), which was undoubtedly a furanolactone on account of its positive rotation [cf. 2 : 3 : 4-trimethyl L-fuconolactone, $[\alpha]_D$ −138° ⟶ −36° (James and Smith, *J.*, 1945, 746)], thus excluding substitution on $C_{(4)}$. Synthetic 3 : 4-dimethyl L-fucose (Percival and Percival, *J.*, 1950, 690) failed to cause crystallisation on nucleation and 3 : 4-dimethyl α-methyl-L-fucoside had constants markedly different from the crystalline dimethyl methyl-L-fucoside, isolated from the dimethyl fraction in these experiments, which had m. p. 49—51° and $[\alpha]_D$ −190° and is designated 2 : 3-*dimethyl α-methyl-L-fucoside*.

Direct chromatographic comparison with 3 : 4-dimethyl L-fucose (R_G 0·67—0·68) showed that although the rate of travel of the dimethyl fucose in question was closely similar (R_G 0·65), by running the paper strips for 48 hours with butanol–ethanol as solvent, the spots developed on spraying with aniline oxalate were distinctly differentiated. Evidence that a methoxyl group was present on $C_{(2)}$ was also given by the fact that the crystalline *dimethyl fuconamide* gave a negative Weerman test, and oxidation of the free sugar with periodate, followed by bromine water, esterification, and amide formation gave D(−)-dimethoxysuccinamide. This product could only arise if methoxyl groups were present on $C_{(2)}$ and $C_{(3)}$: as in

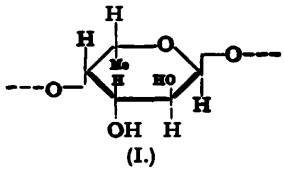


It was only the low yield of amide, attributed to the difficulties which occasionally arise on the oxidation of certain methylated sugars with periodate, which prevented this experiment from being quite conclusive; if the other evidence outlined above is also considered, there seems no reasonable doubt that the dimethyl sugar was, in fact, 2 : 3-dimethyl fucose.

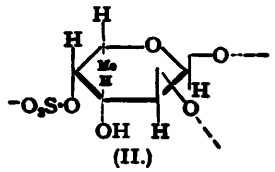
In connection with the above work on partly methylated fucoses, attention should be drawn to the virtual failure of the periodate oxidation method as a test for the presence of a free hydroxyl group on $C_{(4)}$ in partly methylated 6-deoxyhexoses. The method for estimating fucose by periodate oxidation and conversion into acetaldehyde (Nicolet and Shinn, *J. Amer. Chem. Soc.*, 1941, **63**, 1456; Cameron, Ross, and Percival, *J. Soc. Chem. Ind.*, 1948, **67**, 161) was applied to the monomethyl fucose (3-methyl fucose) and the dimethyl fucose (2 : 3-dimethyl fucose). Only one-third of the theoretical yield of acetaldehyde was obtained in each case, although this is much greater than the yield (5%) from 2 : 3-dimethyl rhamnose (Percival and Percival, *loc. cit.*). These results recall similar difficulties with partly methylated hexoses, for Jeanloz (*Helv. Chim. Acta*, 1944, **27**, 1509) and Bell, Palmer, and Johns (*J.*, 1948, 992) showed that in the oxidation of methylated sugars such as 2 : 3 : 4-trimethyl glucose, 2 : 4-dimethyl galactose, and 3-methyl glucose by periodate in buffered solutions, the yield of formaldehyde was much lower than the theoretical.

Before an attempt is made to draw a preliminary picture of the possible structure of fucoidin, one important fact should be noted. Since the sulphate groups are stable to alkali (apart from the fairly rapid initial removal of *ca.* 10% of the total sulphate which will require further

study), it is clear that the main linkage of L-fucopyranose units cannot be through the hydroxyl group on $C_{(4)}$ as the hydroxyl groups on $C_{(2)}$ and $C_{(3)}$ are *trans* to one another (I). A sulphate



(I.)

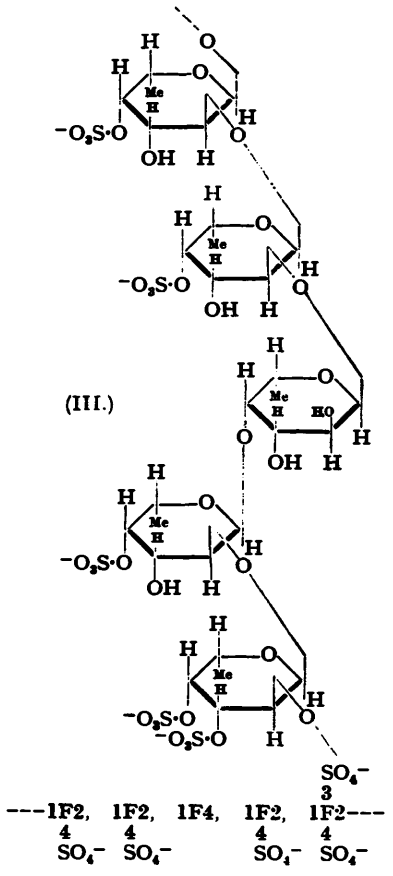


(II.)

group in either of these positions would be readily eliminated by alkali (Percival, *Quart. Rev.*, 1949, **3**, 369). The isolation of 3-methyl L-fucose leaves only one possible arrangement (II) for those residues carrying one free hydroxyl group, namely a 1 : 2-linkage with the sulphate on $C_{(4)}$. Because of the strongly negative rotations of fucoidin and its derivatives, these junctions are considered to be predominantly of the α-type.

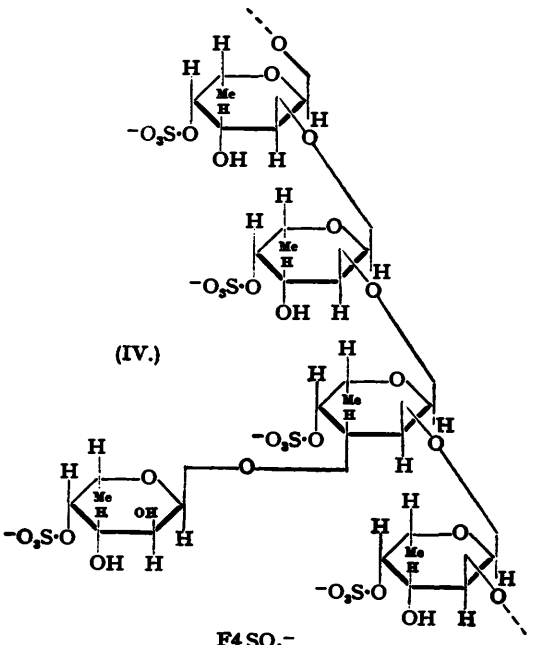
There are two possible ways of accounting for the free fucose residues in methylated fucoidin, excluding the possibility of incomplete methylation owing to steric effects. First, some fucose residues might carry two sulphate groups, a possibility by no means out of the question since the proportion of sulphate to fucose in the original fucoidin is somewhat higher than is demanded by a 1 : 1 ratio, although contamination with a galactan sulphate would also explain this. The 2 : 3-dimethyl fucose could be explained by the appearance of occasional 1 : 4-linkages, and these residues would require to be unsubstituted by sulphate groups (cf. III).

If the conclusions as to the main structural feature are correct, the 2 : 3-dimethyl fucose could not arise as a result of the loss of sulphate groups during methylation since this would produce the 3 : 4-isomer.



(III.)

---1F2, 1F2, 1F4, 1F2, 1F2---
4 4 4 4
SO_4^- SO_4^- SO_4^- SO_4^-
3
SO_4^-
3 H



(IV.)

F4 SO_4^-
1
3
---1F2, 1F2, 1F2, 1F2---
4 4 4 4
SO_4^- SO_4^- SO_4^- SO_4^-

F = L-Fucopyranose.

Another possible explanation is that the free fucose originates from branching points carrying terminal groups having free hydroxyls on $C_{(2)}$ and $C_{(3)}$. From the proportions of the various sugars isolated there would be an average of one such branch in every five fucose units (cf. IV).

For either of the two possibilities it will be observed that one equivalent of periodate would be consumed for each unit of five fucose residues. Because of the presence of impurities, this figure cannot be determined with any accuracy at present, but, if the galactose, xylose, and uronic acid known to be present are assumed to be linked by 1 : 4-linkages and a correction is made for the consumption of periodate by these materials, fucoidin reacts with M/10-sodium periodate in 24 hours so that one equivalent of periodate is accounted for by 880 g. of the " ideal " polysaccharide, which corresponds to between three and four monosaccharide units.

Before anything approaching the last word on the constitution of fucoidin can be written, it will be necessary to study more highly purified specimens, and preparations from different algae. Progress will undoubtedly be slow for technical reasons, but this preliminary study may stimulate other workers to attack this problem, which has added interest owing to the discovery by Vasseur (*Acta Chim. Scand.*, 1948, **2**, 900) of fucosan sulphates in the jelly coat of sea-urchin eggs of the species *Strongylocentrus droebachiensis*, *Echinocardium cordatum*, and *Paracentrotus lividus*.

Experimental.

Preparation of the Polysaccharide.—The polysaccharide was extracted as described in Part I (*loc. cit.*). For this investigation specimens of *Fucus vesiculosus* (May, June, July, 1945 samples) kindly supplied by The Scottish Seaweed Research Association, Musselburgh, were used. Purification was effected by allowing a solution of the polysaccharide in water to stand over " Filter-Cel " for several days, followed by filtration, concentration, and pouring into alcohol. The process was repeated till a constant fucose content was obtained. The resulting white fibrous product had $[\alpha]_D^{15}$ −118°. For all quantitative work the polysaccharide was dried over phosphoric oxide at 50°/15 mm. to constant weight [Found : ash (as sulphate), 24·1; Ca, 5·9; Na, 0·9; Mg, 0·2; K, 0·1 — 0·2; SO_4 (total), 32·8%; SO_4 (in ash), 17·6%]. Estimation of fucose by periodic acid oxidation (Nicolet and Shinn, *J. Amer. Chem. Soc.*, 1941, **63**, 1456; Cameron, Ross, and Percival, *J. Soc. Chem. Ind.*, 1948, **67**, 161) gave 37·8% of fucose.

Hydrolysis of Fucoidin with N-Sodium Hydroxide at 100°.—The polysaccharide (1·1082 g.) was heated with N-sodium hydroxide (200 c.c.) at 100° in the presence of barium chloride (1·283 g.). At definite intervals, samples (15 c.c.) were withdrawn, water (15 c.c.) and dilute acetic acid (5 c.c.) were added, and the solution was centrifuged. The residual combined sulphate in 50 c.c. of the solution was then determined by hydrolysis with hydrochloric acid and weighing of the barium sulphate produced :

Time (hrs.)	0	½	1	4	10	20	30	36	48	56	72
Residual sulphate ($BaSO_4$, mg.)	48·5	45·6	43·0	41·2	38·9	38·2	32·0	30·9	25·1	24·1	20·0
Hydrolysis, %	0	6·0	11·4	15·0	19·8	26·1	34·1	36·4	48·2	50·3	58·7

Acetylation of Fucoidin.—The polysaccharide (2 g.) was dissolved in distilled water (30 c.c.), pyridine (120 c.c.) was added, and the pyridine–water azeotrope distilled off at 50°/15 mm (Pacsu and Mullen, *J. Amer. Chem. Soc.*, 1941, **63**, 1487). The distillation was continued till the volume of residual solution was *ca.* 30 c.c., a brown gelatinous mass being obtained. Pyridine was added to bring the volume to 50 c.c., followed by acetic anhydride (25 c.c.) added slowly, with shaking and cooling. After being left in the dark (2 days) the acetate was separated at the centrifuge, washed with pyridine, alcohol, and ether, and dried, to give a white powder (2 g.) [Found : $CH_3{\cdot}CO$, 18·0. Calc. for $C_6H_8O_7(CO{\cdot}CH_3)SCa_{0\cdot5}$: $CH_3{\cdot}CO$, 15·0%).

Typical Methylation.—Deacetylation and methylation proving no more satisfactory than direct methylation, the direct method was used throughout the investigation.

The polysaccharide (10 g.), dissolved in the minimum of water, was treated with methyl sulphate (240 c.c.) and sodium hydroxide (672 c.c.; 30%) in one-eighth portions every 2 minutes, followed by methyl sulphate (120 c.c.) and sodium hydroxide (208 c.c. : 30%) in one-eighth portions every 10 minutes, the solution being stirred and the temperature kept at 50° during the methylation. The solution was then heated to 85° for 30 minutes, cooled, neutralised with glacial acetic acid, and dialysed against running water till sulphate-free (10 days). On evaporation at 45°/15 mm. a brown glass was obtained (Found : OMe, 7·2%). This glass was taken up in water and the methylation procedure repeated 3 times, after which the methoxyl content remained unchanged on further methylation (Found : OMe, 15·8%); yield, 6·8 g.

To ascertain whether or not the polysaccharide was fully methylated, it was acetylated as described above. Examination of the product obtained (Found : CH_3CO, <1%) appeared to indicate, within experimental error, that the polysaccharide was fully methylated. The product, after drying over phosphoric oxide at 50°/15 mm., had $(\alpha]_D^{15}$ −107° (*c*, 1·0 in water) [Found : ash (as sulphate) 21·2; Ca, 5·1; Na, 0·8; Mg, 0·2; K, 0·05; SO_4 (total), 25·9; SO_4 (in ash), 14·8%].

Hydrolysis of the Methylated Polysaccharide.—The above product (5·01 g.) was hydrolysed at 100° with oxalic acid (200 c.c.; 3%) to constant rotation. The solution was neutralised with barium carbonate and worked up in the usual way to give a yellow syrup (3·3 g.), $[\alpha]_D^{15}$ −54·0° (*c* 1·0 in water) (Found : OMe, 20·4%). Estimation of fucose by periodic acid oxidation (Cameron, Ross, and Percival, *loc. cit.*) gave 30·4% as the apparent fucose content or 33·0% calculated as monomethyl fucose.

Glycopyranoside Formation.—The hydrolysed methylated polysaccharide (8·9 g.) was boiled with methanolic hydrogen chloride (400 c.c. : 2%) until non-reducing (5 hours), neutralised with silver carbonate, evaporated, and extracted with alcohol, to give on evaporation a yellow syrup (5·8 g.). Fractionation by distillation in a high vacuum was unsuccessful, but one fraction (b. p. 125—150°/0·1

mm.; n_D^{11} 1·4717; OMe, 29·8%) gave, on hydrolysis and osazone formation, a small yield of an osazone, m. p. 178—179° (decomp.) (Found: OMe, 9·2. Calc. for $C_{19}H_{24}O_3N_4$: OMe, 8·7%), which was presumed to be 3-methyl phenylfucosazone.

Fractionation by Chromatographic Adsorption on Alumina.—The glycosides were dissolved in chloroform–light petroleum (1 : 1) and introduced into a column (30 × 2 cm.) of activated alumina (Spence and Sons: Grade H) suspended in the same solvent. The column was developed first with the same mixture, then with increasing proportions of chloroform, followed by mixtures of chloroform and methanol, and finally pure methanol. Six fractions were obtained: (1) 1·036 g., n_D^{13} 1·4590, (2) 0·124 g. (partly crystalline), n_D^{12} 1·4580, (3) 1·680 g. (partly crystalline), n_D^{12} 1·4670, (4) 1·192 g., n_D^{12} 1·4688, (5) 0·878 g., and (6) 0·182 g. Recovery was 88%.

Examination of fraction (1). This fraction (0·97 g.) on distillation gave fractions (1*a*) 0·478 g., b. p 75—80°/0·03 mm., n_D^{13} 1·4549 (Found: OMe, 46·6%), and (1*b*) 0·385 g., b. p. 90—110°/0·03 mm.; n_D^{13} 1·4601 [Found: OMe, 47·7. Calc. for $C_6H_9O_2(OCH_3)_3$: OMe, 45·1%]. Later experiments using the paper chromatogram showed the two fractions to be identical.

Formation of 2 : 3 : 4-*trimethyl* α-*methyl*-L-*fucoside from fraction* 1a. This fraction (0·2 g.), after two treatments with Purdie's reagents, gave white needles (0·18 g.), m. p. 84—89°, subliming *in vacuo*, $[\alpha]_D^{14}$ −192° (*c*, 1·08) [Found: OMe, 56·1. Calc. for $C_{10}H_{20}O_5$: OMe, 56·3%]. A mixed m. p. determination with authentic 2 : 3 : 4-trimethyl α-methyl-L-fucoside showed no depression.

Periodic acid oxidation of fraction 1*b*. *Isolation of* D(−)-*dimethoxysuccinamide.* The syrup (0·2 g.) was hydrolysed with sulphuric acid (50 c.c.; 0·1N.) for 8 hours at 100° ($[\alpha]_D$ −61° ⟶ −1°) and the solution worked up in the usual manner. To the syrup so obtained was added periodic acid (20 c.c.: 0·5M.), and the solution was set aside for 20 hours at room temperature. After thorough aeration to remove the acetaldehyde, barium chloride (1·22 g.) was added to the solution, followed by barium carbonate in excess. The residue, after filtration, was extracted with a small quantity of water, and the extract added to the filtrate. Oxidation with bromine (3 c.c.) gave a small quantity of syrup which was boiled with methanolic hydrogen chloride (50 c.c.; 2·5%) for 5 hours and worked up in the usual way. Treatment of the resulting syrup with methanolic ammonia (2 c.c.) for 3 days at 0° gave a crystalline solid mixed with syrup. The long needle-shaped crystals (12 mg.) were washed with alcohol and dried, and then had m. p. 262—263° and $[\alpha]_D^{15}$ −92° (*c*, 2·01 in water) (Found: OMe, 34·8. Calc. for $C_6H_{12}O_4N_2$: OMe, 35·3%). A mixed m. p. determination with authentic D(−)-dimethoxysuccinamide, m. p. 265—268°, $[\alpha]_D$ −94°, showed no depression, but one with (±)-dimethoxysuccinamide showed a large depression.

Examination of crystalline portion of fraction 2. 2 : 3-*Dimethyl* α-*methyl*-L-*fucoside.* After fraction 2 had been dried on a tile and recrystallised from light petroleum (b. p. 40—60°) a small quantity of crystals (12 mg.) was obtained, having m. p. 49—51°, $[\alpha]_D^{15}$ −190° (*c*, 1·4 in water) (Found: C, 52·3; H, 8·6; OMe, 44·3. $C_9H_{18}O_5$ requires C, 52·4; H, 8·7; OMe, 45·1%).

Examination of fraction 3. *Isolation of* 3-*methyl* α-*methyl*-L-*fucoside.* This fraction was partly crystalline, and an attempt was made to obtain a greater yield of crystals by fractionation using a column of alumina and developing with chloroform–methanol (3 : 1). The crystalline fraction so obtained was dried on a porous tile and recrystallised from ethyl acetate, and then had m. p. 130—132°, $[\alpha]_D^{14}$ −173° (*c*, 0·4 in water) (Found: C, 50·3; H, 8·4; OMe, 32·1. $C_8H_{16}O_5$ requires C, 50·0; H, 8·3; OMe, 32·3%).

Hydrolysis with sulphuric acid (0·1N.) for 8 hours gave the free sugar as a syrup, $[\alpha]_D^{15}$ −94° (c, 0·51) Found: OMe, 17·8. $C_7H_{14}O_5$ requires OMe, 17·4%). This sugar was compared on the same paper) chromatogram with synthetic 2-methyl L-fucose (R_G 0·56) and digitalose (3-methyl D-fucose) (R_G 0·45), obtained from *iso*emicymarin (see below). The R_G value (0·45) obtained for the sugar was identical with that of digitalose.

Examination of fractions 4, 5, *and* 6. Fraction 4, when examined by paper chromatography, proved to be a mixture of di- and mono-methyl methylfucosides with a little free methylfucoside. An attempted separation by means of cold extraction with chloroform did not give very satisfactory results, though from one monomethyl fraction an osazone, m. p. 172—176° (decomp.), which contained a methoxyl group, was obtained, indicating the presence of a free hydroxyl group on $C_{(2)}$.

Fractions 5 and 6 gave, on storage, a quantity of crystals of α-methyl-L-fucoside which were separated, drained on a tile, and recrystallised from ethyl acetate, to give m. p. 154—156°, $[\alpha]_D^{15}$ −197° (*c*, 1·1 in water) (Found: OMe, 17·2. Calc. for $C_7H_{14}O_5$: OMe, 17·4%) The remaining syrup was found, on hydrolysis with sulphuric acid (0·1N.) and examination by paper chromatography, to contain 3-methyl fucose, fucose, and a trace of hexose. Fucose estimations on these hydrolysed syrups gave: fraction (5) 93·1%; fraction (6) 87·8% calculated as fucose.

Fractionation by Solvent Extraction.—Methylated glycosides from the methylated polysaccharide (5 g.) were dissolved in water (25 c.c.) and extracted for 15·5 hours with light petroleum (b. p. 40—60°) to give fraction *A* (0·38 g.), n_D^{13} 1·4499. This fraction was distilled in a high vacuum, giving a clear syrup (0·28 g.), b. p. 83—87°/0·04 mm., n_D^{15} 1·4442 (Found: OMe, 49·8%). Extraction with chloroform for (*a*) 4½ hours gave fraction *B* (1·80 g.), n_D^{15} 1·4610 (Found: OMe, 40·0%), (*b*) 23½ hours gave fraction *C* (1·66 g.), n_D^{15} 1·4735 (Found: OMe, 28·6%), and (*c*) 34 hours gave fraction *D* (0·24 g.), partly crystalline (Found: OMe, 30·9%). Evaporation of the aqueous solution gave fraction *E* (0·97 g.) (Found: OMe, 16·4%). Recovery was 98%.

Examination of fraction A. This fraction on hydrolysis and examination by paper chromatography was found to consist of the dimethyl fucose already examined and traces of 2 : 3 : 4-trimethyl fucose and one or more highly methylated hexoses or pentoses. There was also a possible indication of the presence of a small amount of a methylated uronic acid.

Fractionation and examination of fraction B. This portion was subjected to further fractionation by passage through an alumina column (20 × 2 cm.), using for development first chloroform and then methanol. Two fractions were obtained: (*a*) with chloroform, fraction *B*1 (0·79 g.), b. p. 100—110°/0·04 mm., n_D^{16} 1·4590 [Found: OMe, 46·3%; apparent percentage of fucose (see Introduction), 32·4%], and (*b*) with methanol, fraction *B*2 (0·87 g.), n_D^{16} 1·4669 (Found: OMe, 31·1%; apparent percentage of fucose, 34·2).

Fraction *B*1 was hydrolysed with sulphuric acid (0·1N.), giving a syrup, $[\alpha]_D^{15}$ +4·6° (*c*, 3·0 in water) (Found: OMe, 32·1%), which did not crystallise even on storage for several months. Nucleation with authentic 3 : 4-dimethyl L-fucose did not induce crystallisation. Comparison of the two sugars by paper chromatography showed they had very similar R_G values (*J.*, 1950, 690), but on prolonging the experiment [48 hours in butanol–ethanol–water (40 : 10 : 50)] it was possible to differentiate between the spots after spraying with aniline oxalate.

Formation of lactone from hydrolysed fraction B1. 2 : 3-*Dimethyl* L-*fucofuranolactone*. The dimethyl fucose (0·18 g.) in water (5 c.c.) was treated with bromine (1·5 c.c.) at room temperature till non-reducing (30 hours). After aeration, neutralisation with silver carbonate, treatment with hydrogen sulphide, etc., and lactonisation, a clear syrup (0·1 g.) was obtained, having b. p. 130°/0·04 mm. (Found: OMe, 34·8%; 16 mg. required 1·67 c.c. of 0·027N-sodium hydroxide for neutralisation. $C_8H_{14}O_5$ requires OMe, 32·6%; 1·79 c.c.). The lactone showed $[\alpha]_D^{13}$ +9° (5 minutes), +10° (30 minutes), +11° (1 hour), +23° (3 hours), +28° (4 hours), +34° (8 hours), +37° (12 hours), +40° (15 hours), +47° (22 hours, constant) (*c*, 1·6 in water), the positive rotation indicating the presence of a dimethyl γ-L-fuconolactone; cf. 2 : 3 : 4-trimethyl L-fuconolactone $[\alpha]_D$ −138° ⟶ −36° (James and Smith, *loc. cit.*).

2 : 3-*Dimethyl* L-*fuconamide*. The above lactone (40·4 mg.) was treated with methanolic ammonia (2 c.c.) at 0° for 48 hours. On evaporation of the solvent at 14°/15 mm. the entire solution crystallised, giving a white solid which, recrystallised from light petroleum (b. p. 60—80°), had m. p. 78—79°, $[\alpha]_D^{15}$ +30·2° (*c*, 0·86 in water) (Found: OMe, 30·0. $C_8H_{15}O_5N$ requires OMe, 30·3%). The *amide* gave a negative Weerman test, indicating that the hydroxyl group on $C_{(2)}$ was substituted by methoxyl.

3-*Methyl* L-*fucofuranolactone and its amide*. The hydrolysed fraction *B*2 was oxidised with bromine till non-reducing. After the usual treatment followed by lactonisation, a white crystalline product, m. p. 132—136°, was obtained. This gave $[\alpha]_D^{14}$ +25° (10 minutes), +37° (30 minutes), +46° (1 hour), +46° (2 hours), +47° (3 hours), +50° (7 hours), +53° (12 hours), +61° (19 hours), +74° (32 hours), +75° (48 hours), +75° (62 hours, constant), (*c*, 0·95 in water). This indicated a γ-lactone, and thus $C_{(4)}$ was occupied by a hydroxyl group in the methylated fucose.

The lactone gave a syrupy *amide*, $[\alpha]_D^{15}$ +17·2° (*c*, 0·6 in water) (Found: OMe, 16·7. $C_7H_{15}O_5N$ requires OMe, 16·1%). The amide (40 mg.) yielded hydrazodicarbonamide (15 mg.), m. p. 253—255° unchanged on admixture with an authentic specimen.

Paper chromatography. Examination of hydrolysed fraction *B*2 and comparison with 2-methyl L-fucose and digitalose showed that, as with fraction 3, the R_G value (0·45) was the same as that of digitalose.

Fractions C *and* D. Both fractions were observed, on hydrolysis and examination by paper chromatography, to be identical with fraction *B*2. Periodic acid oxidation of hydrolysed fraction *E* gave acetaldehyde corresponding to 34·5% of monomethyl fucose.

Derivatives from fraction C. 3-*Methyl* L-*fuconamide*. Oxidation with bromine, followed by the usual treatment, gave the lactone as a syrup, b. p. 180°/0·04 mm., n_D^{15} 1·4750 [Found: OMe, 18·1%; 14 mg. required 2·67 c.c. of 0·025N-sodium hydroxide for neutralisation. Calc. for $C_7H_{12}O_5$: OMe, 17·6%; 2·96 c.c.]. This lactone in water showed $[\alpha]_D$ +20° (20 minutes), +20° (30 minutes), +22° (1 hour), +26° (2 hours), +28° (3 hours), +29° (4 hours), +34° (6 hours), +45° (12 hours), +50° (18 hours), +52° (21 hours), +59° (28 hours), +67° (36 hours), +74° (42 hours, constant) (*c*, 0·7 in water). 2 C.c. of the above solution required 2·66 c.c. of 0·02505N-sodium hydroxide (Calc.: 2·96 c.c.).

Treatment of the above lactone with methanolic ammonia gave a crystalline *amide*, m. p. 176—180°, $[\alpha]_D^{14}$ +16·4° (*c*, 0·58 in water) (Found: OMe, 16·4. $C_7H_{15}O_5N$ requires OMe, 16·1%). This amide (43 mg.) gave hydrazodicarbonamide (20 mg.), m. p. 256° (decomp.) unchanged on admixture with an authentic speciman.

Fraction E. *Isolation of* α-*methyl* L-*fucoside*. This fraction partly crystallised, and after drying on a porous tile and recrystallisation from ethyl acetate had m. p. 154—155° not depressed on admixture with authentic α-methyl-L-fucoside, $[\alpha]_D^{15}$ −194° (*c*, 1·1) (Found: OMe, 17·0. Calc. for $C_7H_{14}O_5$: OMe, 17·4%). Examination by paper chromatography after hydrolysis showed the fraction to consist of fucose with a trace of a hexose. A fucose estimation with periodic acid gave 90·3% fucose.

Extraction of 3-*Methyl* D-*Fucose from* iso*Emicymarin*.—The cardiac glycoside emicymarin is a much better source of digitalose (3-methyl D-fucose), but unfortunately no samples of this compound were available.

*iso*Emicymarin (0·3 g.), obtained through the kindness of Dr. S. Smith, Wellcome Chemical Research Laboratories, Beckenham, was dissolved in 50% alcohol (150 c.c.) containing 5% of hydrochloric acid and hydrolysed for 4 hours on a boiling water-bath. After extraction several times with chloroform, the aqueous fraction was neutralised with silver carbonate. Removal of the silver salts and evaporation gave a small yield of syrup (60 mg.).

Preparation of 3-*Methyl* D-*Fuconolactone*.—Part of the syrup (54 mg.) was oxidised with bromine for 3 days. After the usual treatment and lactonisation a small amount of needle-shaped crystals, m. p. 136—140°, was obtained. This gave $[\alpha]_D^{13}$ −22° (5 minutes), −24° (1 hour), −27° (3 hours), −31° (5 hours), −35° (9 hours), −38° (12 hours), −45° (17 hours), −53° (24 hours), −63° (36 hours), −68° (42 hours), −70° (60 hours, constant) (*c*, 0·9 in water).

Oxidation of Fucoidin with Sodium Periodate [*with* A. G. Ross].—Fucoidin (0·520 g.) from *Himanthalea lorea* (see Part I, *loc. cit.*) was dissolved in sodium metaperiodate solution (40 c.c.; 0·1M.) and continuously shaken. Samples (5 c.c.) were removed at intervals and the remaining periodate estimated, after 15 minutes, with iodine (0·1N.) after the addition of sodium hydrogen carbonate (1 g.), sodium arsenite (20 c.c.; 0·1N.), and potassium iodide (1 g.) (Found: uptake of sodium periodate in g.-equivs./100 g. of fucoidin, 4 hours 0·088, 24 hours 0·131).

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