283. *Methyl Ethers of* L-*Fucose.*

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The four methyl L-fucosides have been synthesised and separated on cellulose. Partial methylation of methyl β-L-fucofuranoside and of methyl α-L-fucopyranoside followed by hydrolysis and separation on cellulose have led to the isolation of eight of the eleven possible methyl ethers of fucose.

The occurrence of L-fucose in a variety of natural polysaccharides led to this study of the properties of its methyl ethers. Treatment with 0·8% methanolic hydrogen chloride at room temperature until the rotation reached a maximum positive value gave the best yield (64·6%) of methyl α- and β-L-furanosides. The product after separation on a cellulose column gave methyl β-L-fucofuranoside (44%), α-L-fucofuranoside (20·6%), α-L-fucopyranoside (15·4%), and β-L-fucopyranoside (20%).

While this work was in progress Miss Watkins [1] recorded the separation of the four glycosides after treatment of fucose with hot 0·025% methanolic hydrogen chloride but she gave no indication of the relative proportions of the four products. We found that glycosidation in the hot gave a higher proportion of pyranosides.

Incomplete methylation of methyl β-L-fucofuranoside and of α-L-fucopyranoside with Purdie reagents followed by hydrolysis and partition on cellulose led to the isolation of the methylated fucoses shown in the Table.

Bell and Dedonder [2] have shown that sugars substituted at the position adjacent to the reducing group do not form a water-insoluble formazan with triphenyltetrazolium hydroxide and that a paper chromatogram sprayed with this reagent reveals only those reducing aldoses in which position 2 is unsubstituted. Only those derivatives marked with an asterisk in the Table gave red spots in this way.

[1] Watkins, *J.*, 1955, 2054.

[2] Dedonder, personal communication quoted by Bell in "Modern Methods of Plant Analysis," ed. Paech and Tracey, Springer-Verlag, Berlin, Vol. II, p. 9; Bell and Dedonder, *J.*, 1954, 2866.

The only methyl ether of L-fucose which was not obtained was the 4-*O*-methyl-L-fucose. In keeping with the recognised reactivity at position 2 in sugars it is noteworthy that the 2-*O*-mono- and 2 : 3-di-*O*-methyl ethers were obtained in highest yield from both methylations. Whereas the methyl ethers with a pyranose structure all had negative rotations, those containing a furanose ring were positive.

	Fraction numbers †	From β-L-fucofuranoside (%)	Fraction numbers †	From α-L-fucopyranoside (%)
2 : 3 : 4-Tri-*O*-methyl-L-fucose	—	—	P1	5·5
2 : 3 : 5-Tri-*O*-methyl-L-fucose	F1	5·5	—	—
2 : 3-Di-*O*-methyl-L-fucose	F3	17·5	P2(a)	27·5
2 : 4-Di-*O*-methyl-L-fucose	—	—	P3	11·5
2 : 5-Di-*O*-methyl-L-fucose 3 : 5-Di-*O*-methyl-L-fucose *	F2	19·0	—	—
3 : 4-Di-*O*-methyl-L-fucose *	—	—	P4	2·5
5-*O*-Methyl-L-fucose *	F4	7·0	—	—
2-*O*-Methyl-L-fucose	F5	36·0	P5	43·5
3-*O*-Methyl-L-fucose *	F6	15·0	P7	9·5

† See Experimental.

2 : 3 : 5-Tri-*O*-methyl-L-fucose was prepared by methylation of methyl β-L-fucofuranoside in dimethylformamide. Complete methylation proved difficult and after removal of the glycosidic methoxyl group the tri-*O*-methylfucose was separated from less methylated derivatives by partition on a cellulose column. It gave correct analyses for a tri-*O*-methyl-L-fucose and did not reduce periodate. It was an exceedingly hygroscopic and volatile syrup.

The first fraction (F1) separated as a syrup from the partition of the hydrolysate of the partly methylated furanoside, gave a single spot on ionophoresis, was chromatographically identical with the above 2 : 3 : 5-tri-*O*-methyl-L-fucose, and did not reduce periodate. Owing to its high volatility it was exceedingly difficult to dry and a large proportion was lost in an attempt to dry it over phosphoric oxide in a vacuum-desiccator. Consequently the recorded rotation and methoxyl contents are on moisture-containing material.

Fraction F3 and fraction P2(a) (Table) had $[\alpha]_D$ −97° and −100° respectively (cf. 2 : 3 : 4-tri-*O*-methylfucose, $[\alpha]_D$ −128°). They both gave correct analyses for di-*O*-methyl derivatives and had identical properties. The only dimethylfucose which can be synthesised from both a fucofuranoside and a fucopyranoside is the 2 : 3-di-*O*-methyl derivative. Further proof of the structure was obtained by oxidative preparation of D(−)-dimethoxysuccinic acid and its crystalline amide. The syrupy lactone, $[\alpha]_D$ +10° ⟶ +50° (96 hr.), isolated after oxidation of the dimethyl-sugar with bromine water was undoubtedly a furanolactone.[3] It is clear that the supposed 2 : 3-di-*O*-methyl-L-fucose ($[\alpha]$ +4·6°) isolated from methylated fucoidin by Conchie and Percival [3] by hydrolysis of fraction B contained a considerable quantity of impurity. Indeed these authors comment on the very low yield of D(−)-dimethoxysuccindiamide they isolated after oxidation of their material.

From the fucopyranoside hydrolysate crystalline 3 : 4- and 2 : 4-di-*O*-methyl-L-fucose were also separated. The former was identical with authentic material.[4] This 2 : 4-di-*O*-methyl-L-fucose was characterised by its methoxyl content and its resistance to periodate. The rotation ($[\alpha]_D$ −27° ⟶ −15°) of the derived lactone indicated a 1 : 5-ring structure. The di-*O*-methylfucose isolated from the methylated extracellular polysaccharide of *Aerobacter aerogenes* by Aspinall, Jamieson, and Wilkinson [5] and thought from the available evidence to be the 3 : 5-di-*O*-methyl-L-fucose has now been shown to be 2 : 4-di-*O*-methyl-L-fucose.

[3] Conchie and Percival, *J.*, 1950, 827.
[4] Percival and Percival, *J.*, 1950, 690.
[5] Aspinall, Jamieson, and Wilkinson, *J.*, 1956, 3483.

Fraction F2 (Table) gave a single spot on a paper chromatogram irrigated with solvents (1), (2), or (3) (see below), gave a red spot on a paper chromatogram sprayed with triphenyltetrazolium hydroxide, and gave analyses correct for a di-*O*-methylfucose. However, on paper ionophoresis [6] (borate buffer, pH 10) two spots were obtained with M_G 0·65 and 0·02. Foster [7] attributes high mobility under these conditions of ionophoresis to the presence of free hydroxyl groups on $C_{(1)}$ and $C_{(2)}$ and the ability of the substance to form complexes across these two atoms. He has shown that neither 2 : 3- nor 2 : 4-di-*O*-methylrhamnose ($M_G < 0·05$) has an appreciable mobility and that of the 2 : 3-, 2 : 4-, and 3 : 4-di-*O*-methylglucoses only the latter has an M_G (0·31) at all comparable with that recorded by us for this fraction. It appears therefore that the spot of M_G 0·65 corresponds to the 3 : 5-di-*O*-methyl-L-fucose and that the spot of M_G 0·02 is due to the presence of some 2 : 5-di-*O*-methyl-L-fucose.

Crystalline 2-*O*-methyl- [8] and 3-*O*-methyl-L-fucose [3] (the latter crystalline for the first time) were isolated from the hydrolysates of both methylated fucosides. In addition 5-*O*-methyl-L-fucose has been separated and characterised.

The reduction of periodate by each of the methylated derivatives was measured.[9] 2 : 3 : 5-Tri-*O*-methyl- and 2 : 4-di-*O*-methyl-fucose, in keeping with their structures, were not oxidised; of the remaining derivatives only the mixture of 2 : 5- and 3 : 5-dimethyl ethers and the 3 : 4-di-*O*-methylfucose were reduced in an approximately theoretical manner. Anomalous results have previously been obtained for partly methylated 6-deoxyhexoses [3,4] and hexoses.[10]

Experimental

Evaporations were done at 40° under reduced pressure. Paper-partition chromatography was done on Whatman No. 1 filter paper with the upper layers of the following v/v solvent systems: (1) butan-1-ol–ethanol–water (4 : 1 : 5), (2) benzene–butan-1-ol–pyridine–water (1 : 5 : 3 : 3), (3) ethyl methyl ketone half saturated with water plus ammonia (99 : 1); and the reducing sugars were located by aniline oxalate (AO), and the non-reducing sugars with aniline oxalate containing 3% (v/v) syrupy phosphoric acid (AP). Methylated sugars in which position 2 was unsubstituted were revealed by triphenyltetrazolium hydroxide.[11] R_G, R_F, and R_{fu} are the rates of travel relative to tetramethylglucose, the solvent front, and fucose respectively. Ionophoresis was carried out according to the conditions used by Percival and Fisher.[6] Rotations were measured in water at 18°.

Preparation of Methyl L-*Fucosides.*—(A) L-Fucose (m. p. 145°) (10 g.) was dissolved in 0·8% methanolic hydrogen chloride (400 c.c.) at 15° and the change in rotation followed polarimetrically to a maximum $[\alpha]_D$ of +10·4° (66 hr.). Neutralisation with silver carbonate and evaporation of the filtrate gave a syrup (10·80 g.).

(B) L-Fucose (2 g.) in dry methanol (80 c.c.) was agitated with Amberlite resin (IR-100H) (3 g.) at 15°. After 13 days the solution had a maximum rotation of $[\alpha]_D$ +8·0°. Removal of solvent gave a syrup (2·19 g.). Paper chromatography of both syrups with solvent (1) for 40 hr. and spray (AP) showed 4 components with R_F 0·57, 0·51, 0·44, and 0·39 respectively. In addition, the syrup B, $[\alpha]_D$ +8·0°, contained a little free fucose.

The syrup A (2 g.) was separated on a cellulose column (85 × 2·7 cm.) by using ethyl methyl ketone saturated with water. Four fractions were collected and their R_G values measured with solvent (1). Fractions II, III, and IV were recrystallised from methanol: fraction I (0·7—1·1 l.), syrupy methyl β-L-fucofuranoside (0·86 g.), R_G 0·70, $[\alpha]_D$ +112° (*c* 7·0); fraction II (1·6—1·92 l.), methyl α-L-fucofuranoside (0·40 g.), R_G 0·62, m. p. 127—128°, $[\alpha]_D$ −108° (*c* 2·0), −115° (*c* 2·0 in MeOH); fraction III (2·14—2·44 l.), methyl α-L-fucopyranoside (0·30 g.), R_G 0·53, m. p. 158—159°, $[\alpha]_D$ −191° (*c* 2·0); fraction IV (2·551—4·0 l.), methyl β-L-fucopyranoside (0·38 g.), R_G 0·48, m. p. 126—127°, $[\alpha]_D$ +10·5 (*c* 1·0). After addition of ethyl methyl ketone (50 c.c.)

[6] Fisher and Percival, *J.*, 1957, 2666.
[7] Foster, *J.*, 1953, 982; Foster and Stacey, *J.*, 1955, 1778.
[8] Nelson and Percival, *J.*, 1957, 2191.
[9] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.
[10] Jeanloz, *Helv. Chim. Acta*, 1944, **27**, 1509; Bell, *J.*, 1948, 992; Greville and Northcote, *J.*, 1952, 1945.
[11] Wallenfels, *Naturwiss.*, 1950, **37**, 491.

the initial syrup A, $[\alpha]_D$ +10·5° (5 g.), deposited crystals of methyl α-L-fucopyranoside (0·47 g.), m. p. and mixed m. p. 158—159°.

Partial Methylation of Methyl β-L-*Fucofuranoside.*—The furanoside (3 g.) was methylated twice with methyl iodide (60 c.c.) and silver oxide (11·5 g.) at 45°. The product was heated at 100° with 0·3N-sulphuric acid (150 c.c.) until the rotation was constant (45 min.). Neutralisation, with barium carbonate, of the cooled solution, filtration, and evaporation gave a syrup (2·6 g.). Chromatographic analysis [solvent (1)] showed 7 spots. This syrup (2·5 g.) was separated on a cellulose column (85 × 3 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3, v/v) saturated with water. After 3 l. had been collected the ratio of solvents was changed to 6·5 : 3·5, after another 2·5 l. to 6 : 4, and after another 1·5 l. finally to 1 : 1. The following fractions were collected. Each fraction appeared to be homogeneous and gave a single spot on paper chromatograms run in solvents (1), (2), and (3). The R_G values given are for solvent (1).

Fraction F1 (0·60—1·28 l.), a hygroscopic syrup (0·109 g.), R_G 1·05, $[\alpha]_D$ +47° (*c* 1·0), M_G 0·0 {2 : 3 : 5-tri-*O*-methyl-L-fucose had R_G 1·05, $[\alpha]_D$ +70° (*c* 1·3)} (Found: OMe, 36·3. Calc. for $C_9H_{18}O_5$: OMe, 45·1%); this syrup showed no appreciable reduction of periodate on prolonged standing with the oxidant.

Fraction F2 (1·30—2·00 l.), a syrup (0·384 g.), had R_G 0·92, M_G (two components) 0·65, 0·02, $[\alpha]_D$ +38° (*c* 2·5) (Found: OMe, 32·3. Calc. for $C_8H_{16}O_5$: OMe, 32·0%). One mole of this dimethyl sugar reduced the following number of moles of periodate: 0·36 (18 hr.); 0·46 (42 hr.); 0·71 (90 hr.); 1·04 (180 hr.). A portion of the syrup (90 mg.) was oxidised with bromine water at 37°. After 5 days the solution no longer reduced Fehling's solution. Treatment in the usual manner gave a mixture of syrupy lactones, $[\alpha]_D$ +20° ⟶ +36°.

Fraction F3 (2·50—3·50 l.), syrupy 2 : 3-*di*-O-*methyl*-L-*fucose* (0·3516 g.), had R_G 0·75, $[\alpha]_D$ −97° (*c* 3·0) (Found: OMe, 31·5. $C_8H_{16}O_5$ requires OMe, 32·05%). One mole reduced 0·23 (18 hr.), 0·39 (42 hr.), 0·64 (90 hr.), 0·82 (180 hr.) mole of periodate. Oxidation of a portion (0·05 g.) with bromine water at 37° for 6 days gave a syrupy lactone which had $[\alpha]_D$ +10° ⟶ +50° (96 hr.) (*c* 2·0). A portion (0·409 g.) was converted into D(−)-αβ-dimethoxysuccindiamide by oxidation first with 0·6M-sodium metaperiodate (25 c.c.) and then with bromine (7 days) according to the conditions used by Arni and Percival [12] for the oxidation of 3 : 4-di-*O*-methylfructose. Distillation of the ester (0·23 g.) (bath temp. 150°/0·05 mm.) gave a mobile syrup (0·158 g.), n_D^{20} 1·4318, $[\alpha]_D$ −73° (*c* 1·0 in MeOH). The derived amide (67 mg. from 97 mg.) had $[\alpha]_D$ −90° (*c* 0·6), m. p. and mixed m. p. with D(−)-dimethoxysuccindiamide 278°. Mixed m. p. with (±)-dimethoxysuccindiamide 240—246°.

Fraction F4 (3·92—4·56 l.), syrupy 5-O-*methyl*-L-*fucose* (0·1404 g.), had R_G 0·70, $[\alpha]_D$ +28·3° (*c* 0·8) (Found: OMe, 17·2. $C_7H_{14}O_5$ requires OMe, 17·4%). One mole reduced 1·83 (0·5 hr.), 1·99 (1·75 hr.), 2·22 (5·75 hr.), 2·70 (25 hr.), 2·70 (48 hr.) moles of periodate. The derived osazone had m. p. 190° and gave a positive test for OMe.

Fraction F5 (6·00—8·56 l.), 2-*O*-methylfucose (0·7262 g.), had R_G 0·60, m. p. and mixed m. p. 151°, $[\alpha]_D$ −87·2° (*c* 1·4). One mole reduced 1·04 (0·5 hr.), 1·17 (1·3 hr.), 1·30 (3·08 hr.), 1·98 (7 hr.), 2·93 (18 hr.), 3·8 (470 hr.) moles of periodate.

Fraction F6 (10·92—11·72 l.), syrupy 3-*O*-methyl-L-fucose (0·3056 g.), had R_G 0·48. This fraction crystallised when seeded with a crystal of Fraction P7 and had m. p. and mixed m. p. 110°, $[\alpha]_D$ −97° (*c* 1·0). One mole used 0·91 (18 hr.), 0·96 (42 hr.), 1·21 (90 hr.), 1·27 (160 hr.) moles of periodate. For further characterisation see Fraction P7.

Fucose (0·17 g.) was recovered from the water-washings of the column.

Partial Methylation of Methyl α-L-*Fucopyranoside.*—The pyranoside (5 g.) was methylated thrice with methyl iodide (40 c.c.) and silver oxide (10 g.) at 45°. Hydrolysis of the product as for the methylated furanoside gave a syrup (4·6 g.) which showed 7 spots on chromatography. The syrup was separated on a cellulose column with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3, v/v) saturated with water. After 2·25 l. had been collected the ratio of solvents was changed to 6·5 : 3·5 and after another 1 l. had passed to 6 : 4 (5·75 l.). The R_G and R_F values of each fraction were measured in solvents (1) and (2) respectively.

Fraction P1 (1·00—1·40 l.), syrupy 2 : 3 : 4-tri-*O*-methyl-L-fucose (0·123 g.), had R_G 0·92, R_F 0·79, $[\alpha]_D$ −128° (*c* 1·1). Chromatography showed a single spot in solvents (1), (2), and (3), identical with those of authentic 2 : 3 : 4-tri-*O*-methyl-L-fucose. The derived glycoside after purification by sublimation had m. p. and mixed m. p. 95—96°, $[\alpha]_D$ −200° (*c* 1·0).

[12] Arni and Percival, *J.*, 1951, 1822.

Fraction P2 (1·70—2·22 l.) was a syrup (0·928 g.), R_G 0·75, R_F 0·70, $[\alpha]_D$ −106° (*c* 7·7). Paper chromatography [solvent (3)] showed two spots, of R_F 0·47 and 0·32. This fraction (0·70 g.) was partitioned on a cellulose column (40 × 1·5 cm.) with solvent (3) and collected in 20 c.c. fractions. The solvent was removed from the respective fractions at 40° in a stream of nitrogen. Fraction P2(a) (180—240 c.c.) was syrupy 2 : 3-di-*O*-methyl-L-fucose (0·348 g.), $[\alpha]_D$ −95° ⟶ −100° (19 hr. const.) (*c*, 2·1). Chromatographic analysis showed a single spot in solvents (1), (2), and (3) identical with the spot given by fraction F3. The R_F in solvent (3) was 0·47 (Found: OMe, 30·5. Calc. for $C_8H_{16}O_5$: OMe, 32·2%). The syrup consumed 0·44 (43 hr.), 0·66 (55 hr.), 0·8 (160 hr.) mole of periodate per $C_8H_{16}O_5$ unit. Fraction P2(b) (260—280 c.c.) was a syrup (0·144 g.), shown by paper chromatography [solvent (3)] to be a mixture of fractions P2(a) and P2(c). Fraction P2(c) (300—400 c.c.) was a syrup (0·73 g.) which crystallised and had m. p. and mixed m. p. 131—132° with fraction P3 [recrystallised from chloroform–light petroleum (b. p. 40—60°)].

Fraction P3 (2·25—2·75 l.) was 2 : 4-*di*-O-*methyl*-L-*fucose* (0·127 g.), R_G 0·75, R_F 0·73, m. p. 131—132° [recrystallised from chloroform–light petroleum (b. p. 40—60°)], $[\alpha]_D$ −85° (*c* 0 85) (Found: OMe, 32·0%). This dimethyl-sugar was not oxidised by periodate during 300 hr.

Fraction P4 (3·15—4·00 l.) was 3 : 4-di-*O*-methyl-L-fucose (0·051 g.), R_G 0·7, R_F 0·67, m. p. and mixed m. p. 82° (cf. ref. 4). It moved at the same speed on a paper chromatogram as authentic 3 : 4-di-*O*-methyl-L-fucose in solvents (1), (2), and (3) and had $[\alpha]_D$ −118° (*c* 2·2). One mole took up 0·85 mole of periodate (55 hr.), 0·95 mole (160 hr.).

Fraction P5 (4·50—6·70 l.) was 2-*O*-methyl-L-fucose (0·932 g.), R_G 0·56, R_F 0·63, m. p. and mixed m. p. 150—152° (recrystallised from ethanol), $[\alpha]_D$ −68° (20 min.) ⟶ −85° (2·5 hr. const.) (*c* 1·0), −42° (*c* 1·0 in EtOH). The derived fucoside (2% MeOH–HCl under reflux) had $[\alpha]_D^{18}$ −57·7° (*c* 0·52) and reduced 0·45 (9 hr.), 0·67 (48 hr.) mole of periodate for every $C_8H_{16}O_5$ unit.

Fraction P6 (6·70—82·0 l.) was a colourless syrup (0·244 g.) shown by paper chromatography to be a mixture of fractions P5 and P7.

Fraction P7 (8·20—9·05 l.) was syrupy 3-*O*-methyl-L-fucose (0·59 g.), R_G 0·45, R_F 0·56, which crystallised slowly, then having m. p. 109—110°, $[\alpha]_D$ −97° (*c* 4·2), −60·8° (*c* 3·8 in EtOH) (Found: OMe, 17·8. Calc. for $C_7H_{14}O_5$: OMe, 17·4%). The derived osazone had m. p. 175°. The derived syrupy glycoside (2% MeOH–HCl under reflux) had $[\alpha]_D$ −66° (*c* 1·1) and did not reduce periodate.

Fraction P8 (9·05—10·50 l.) was fucose (0·6588 g.), m. p. and mixed m. p. 143°.

2 : 3 : 5-*Tri*-O-*methyl*-L-*fucose*.—Methyl β-L-fucofuranoside (0·60 g.), stirred vigorously with redistilled dimethylformamide (20 c.c.) and methyl iodide (5 c.c.), was treated with silver oxide (5 g.) added in small portions during 30 min.[13] The stirring was continued at room temperature for 17 hr. Inorganic salts were removed and washed with dimethylformamide (2 × 7 c.c.) and chloroform (4 × 15 c.c.), and residual silver salts were removed from the solution and washings by agitation with 1% aqueous sodium cyanide (50 c.c.). The organic phase was separated and the aqueous phase extracted with chloroform (6 × 20 c.c.). The combined solution and extracts were concentrated to a syrup and the methylation was repeated (yield, 0·66 g. Found: OMe, 48·2%). Hydrolysis with 0·3N-sulphuric acid (50 c.c.) at 100° for 30 min. gave a reducing syrup (0·50 g.). Paper chromatography with solvent (1) showed three components with R_{fu} 2·31, 2·70, and 3·03 respectively. Separation of a portion (0·40 g.) on a cellulose column (38 × 2·2 cm.) with butan-1-ol–light-petroleum (b. p. 100—120°) (70 : 30 v/v) gave a fraction, syrupy 2 : 3 : 5-*tri*-O-*methyl*-L-*fucose* (0·27 g.) which gave a single pinkish-red spot on chromatography, R_{fu} 3·03 [solvent (1); spray AO], had n_D^{25} 1·4452, $[\alpha]_D^{18}$ +70° (*c* 1·5), +55° (*c* 1·4 in EtOH) (Found: OMe, 44·8. $C_9H_{18}O_5$ requires OMe, 45·1%), and did not reduce periodate.

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[13] Kuhn, *Chem. Ber.*, 1955, **88**, 1540.