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SYNTHESIS OF L-FUCOSE 2-, 3-, AND 4-SULPHATES[1]*

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ABSTRACT

Treatment of L-fucose with an excess of pyridine–sulphur trioxide gave an equilibrium mixture of mono-, di-, and tri-sulphates. L-Fucose was sulphated under optimal conditions for monosulphate formation, and the monoester fraction was isolated by chromatography on DEAE-cellulose. The isomeric L-fucose 2-, 3-, and 4-sulphates (**1**–**3**) were separated on a DEAE-cellulose column by elution with borate buffer. The structures of **1**–**3** were established by electrophoresis, colour tests, periodate oxidation, and, for the 2-isomer, by comparison with a specimen of **1** that had been definitively synthesised *via* methyl 3,4-*O*-isopropylidene-α-L-fucopyranoside (**6**) and methyl α-L-fucopyranoside 2-(barium sulphate) (**5**). The latter was rapidly hydrolysed in hot, dilute acetic acid to **1** and methyl α-L-fucopyranoside (**4**).

INTRODUCTION

L-Fucose sulphate residues are widely encountered in polysaccharides, especially those present in algae, but although the isolation of L-fucose 4-sulphate from an acid hydrolysate of the brown seaweed *Pelvetia wrightii* has been reported[2], no synthetic routes to this and the other two isomeric monosulphates of L-fucopyranose have been described.

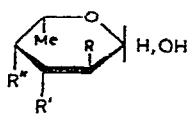
Recently, we isolated from the viscera of *Patella vulgata*[3] a sulphohydrolase which, when tested against a large number of carbohydrate sulphates, exhibited specific hydrolase activity towards the sulphate esters of L-fucose; the substrate was a mixture of isomers obtained by direct sulphation of L-fucose[4]. For more-detailed specificity studies, pure specimens of L-fucose monosulphates were required, and we now describe their synthesis.

DISCUSSION

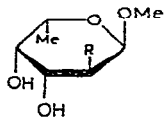
Sulphation of hexoses with pyridine–sulphur trioxide leads to preferential esterification of primary hydroxyl groups, although smaller amounts of secondary

*Dedicated to the memory of Professor Edward J. Bourne.

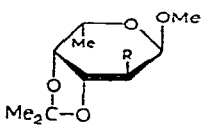
sulphates may be formed. When excess reagent is employed, di-, tri-, and tetra-sulphate esters[5–7] are obtained. Earlier studies on L-fucose[4] and L-rhamnose, which contain no primary hydroxyl group, suggested that mono-, di-, and tri-sulphates are formed at closely similar rates. This work also indicated a possible method for the separation of the mono-sulphated isomers based upon electrophoresis of their borate or molybdate complexes or an allied technique. Thus, direct sulphation of L-fucose and separation of the products was investigated as a rapid route to L-fucose 2- (**1**), 3- (**2**), and 4-sulphates (**3**). The assumption was made that the glycose would react in the pyranose form, and there was no indication, at any stage, that any L-fuco-furanose 5-sulphate was formed.



1 R = SO_4^- $\frac{1}{2}Ba^{2+}$, R' = R'' = OH
2 R = R'' = OH, R' = SO_4^- $\frac{1}{2}Ba^{2+}$
3 R = R' = OH, R'' = SO_4^- $\frac{1}{2}Ba^{2+}$



4 R = OH
5 R = SO_4^- $\frac{1}{2}Ba^{2+}$



6 R = OH
7 R = SO_4^- $\frac{1}{2}Ba^{2+}$

In preliminary studies, L-fucose was sulphated with various amounts of reagent (1.5–10 mol.) at various temperatures (20, 35, 65°), for varying times, and the products were estimated colorimetrically after electrophoretic separation. Mono-sulphation appeared to be optimal when sulphation was performed using 5 mol. of reagent, at low temperatures for short periods of time. For a subsequent, quantitative study, these conditions were employed and the relative amounts of mono-, di-, and tri-esters formed after various times are shown in Fig. 1. Two procedures were adopted. In the first, the products were stirred with water, to destroy excess reagent, and applied directly on the electrophoresis paper. Then followed electrophoretic separation[4] and assay[8,9] of the separated zones. The second procedure parallelled the normal preparative method, and involved mixing with water, neutralisation, $Ba(OH)_2$ neutralisation, removal of pyridine, Ba^{2+} removal, and evaporation. The two procedures gave identical results. It is apparent that, since most of the pyridine–sulphur trioxide is destroyed by water within a few minutes, a considerable amount of L-fucose monosulphate is formed almost immediately on mixing. There is evidence that, under the above conditions, sulphation may be reversible[10,11] and that, on storage, an equilibrium mixture of mono- and poly-sulphates results. This would seem to be true for L-fucose, after 1–2 h (Fig. 1), the concentrations of both mono- and di-sulphates fall as that of trisulphate increases, but some sugar remains unesterified.

On the preparative scale, L-fucose was sulphated with pyridine–sulphur trioxide for 1 h at room temperature and the reaction products were isolated as a mixture of their barium salts. These salts were fractionated on a DEAE-cellulose column. L-Fucose passed directly through the column, and the monosulphates were eluted, free from fucose and polysulphates, with M ammonium hydroxide; this method of

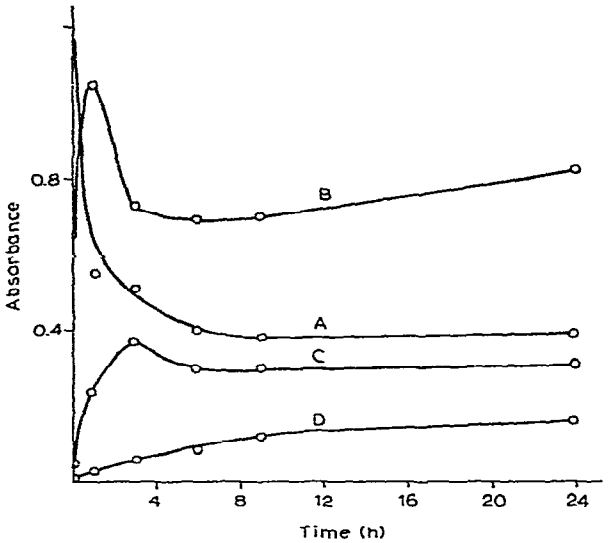


Fig. 1. Sulphation of L-fucose with the pyridine–sulphur trioxide reagent: A, L-fucose; B, L-fucose monosulphates; C, L-fucose disulphates; D, L-fucose trisulphate(s).

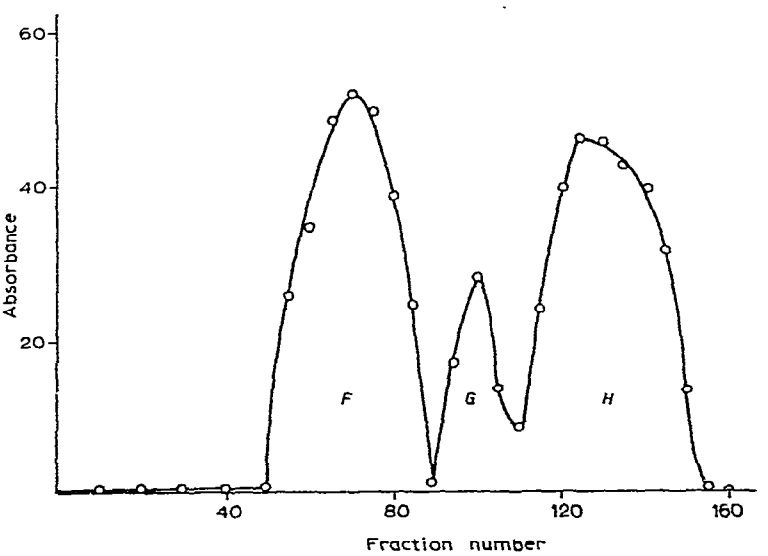


Fig. 2. Separation of L-fucose 2-, 3-, and 4-sulphates on DEAE-cellulose in 0.1M borate buffer (pH 10).

isolation of glycose monosulphates is of general application (*e.g.*, glucose, galactose, and rhamnose). Disulphates were eluted with 3M ammonium hydroxide.

The monosulphate fraction, isolated as the ammonium salts, was fractionated on a DEAE-cellulose column, using borate buffer as eluent (Fig. 2). The three monosulphate fractions were freed from borate by passage through Sephadex G10, and isolated as their barium salts which were analytically pure white powders of indefinite m.p. They could be distinguished by colour tests, chromatography on DEAE-cellulose paper (irrigation with 0.1M borate, pH 10), their optical rotations and infrared spectra, and prolonged electrophoresis in borate buffer. There was not a close correspondence of M_G values (in borate) with those of D-galactose 2-, 3-, and 4-sulphates (1.02, 1.33, and 1.04, respectively[7,13,14]), but is it significant that the introduction of a methoxyl group at position 6 in D-galactose 4-sulphate[15] decreases its ability to complex with borate (M_G 0.86).

Structures were assigned as follows. Product *H* (from Peak *H*, Fig. 2) was identical with L-fucose 2-sulphate whose synthesis is described below. On chromatograms, *F* and *G*, but not *H*, gave a pink colour with the triphenyltetrazolium hydroxide reagent[16], indicating a free hydroxyl group on C-2; *F* gave a blue colour with the diphenylamine–aniline–phosphoric acid reagent[17], thus confirming that HO-4 was substituted, whereas *G* and *H* gave grey colours, with that for *G* having a brown tint indicative of a substituent at C-3. These tests are applicable to sugar sulphates[15].

Although many assignments of structure for sugar sulphates have been made using infrared spectroscopy[7], this method may not always be reliable[18]. Products *F*, *G*, and *H* each gave the characteristic broad S=O stretching vibration at $\sim 1250\ cm^{-1}$, and weaker absorptions for the C–O–S vibrational mode at 843 (*F*), 856 (*G*), and $852\ cm^{-1}$ (*H*). There was a good correlation between the values obtained for *F* and those reported[2] for the L-fucose 4-sulphate isolated from *Pelvetia*. It was concluded that *F* was the 4-sulphate (**3**), *G* the 3-sulphate (**2**), and *H* the 2-sulphate (**1**), and these conclusions were supported by periodate-oxidation studies.

The results of periodate oxidation of *F*–*H* are shown in Fig. 3. To avoid precipitation of barium periodate, the esters were converted into their sodium salts prior to oxidation. Studies by other workers[13,14,19,20] suggested that it is not possible to predict whether sugar sulphates will be oxidised *via* the cyclic or open-chain forms, or mixtures of both. Our results suggest that oxidation of **1** and **2**, and possibly **3**, proceeds *via* the pyranoid ring form. *F* rapidly consumed 2 mol. of oxidant. This result would be expected if oxidation proceeded *via* the cyclic form (**3**) to give 4-deoxy-3-*O*-formyl-L-erythrose 2-sulphate, which would slowly hydrolyse to products that would not be oxidised further. The open-chain form would follow a similar oxidation pattern, except that 4-deoxy-L-erythrose 2-sulphate would be formed directly. *G* rapidly consumed 1 mol. of periodate and a second mol. during the next 24 h. This result is consistent with oxidation *via* the pyranose form with initial formation of 5-deoxy-4-*O*-formyl-L-lyxose 2-sulphate, which, as the formic ester hydrolyses, consumes a further mol. of oxidant to give acetaldehyde and tartronaldehyde 2-sulphate. Over a longer period, the last product would be susceptible to over-oxidation.

This behaviour could not be accounted for by oxidation *via* the acyclic form which would rapidly consume 2 mol. of oxidant. *H* rapidly consumed 1 mol. of oxidant followed by a second mol. at a somewhat lower rate. For the open-chain form, a rapid uptake of 2 mol. is anticipated, with formation of formic acid, acetaldehyde, and tartronaldehyde 2-sulphate. The cyclic from **1** would be expected to reduce one 1 mol. of oxidant immediately, but the reuslting mixed acetal would be hydrolysed rapidly to tartronaldehyde 2-sulphate and 2-hydroxypropionaldehyde with further oxidation (1 mol.) of the latter. None of the products exhibited the behaviour expected of L-fucose 5-sulphate (*i.e.*, rapid uptake of 2 mol. of oxidant with fairly rapid uptake of a further mol.).

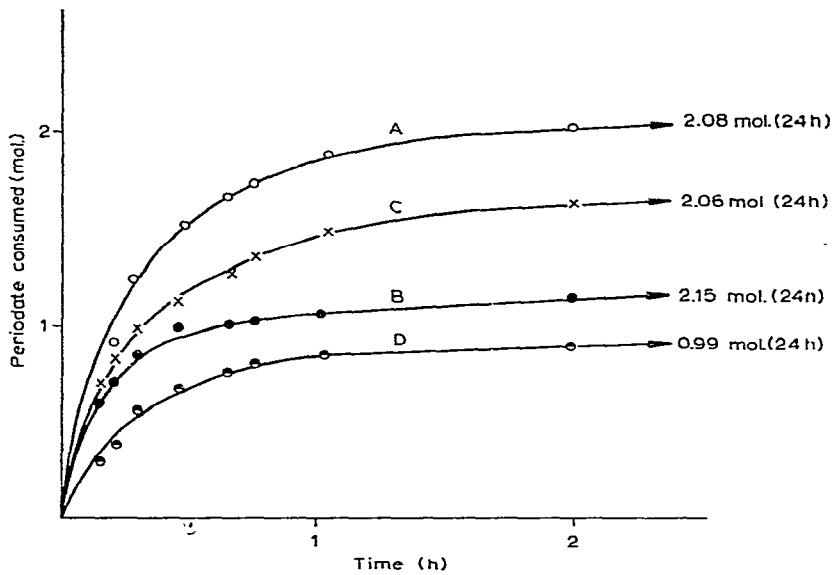


Fig. 3. Oxidation of L-fucose monosulphates with sodium metaperiodate: A, L-fucose 4-sulphate (*F*); B, L-fucose 3-sulphate (*G*); C, L-fucose 2-sulphate (*H*); D, methyl α-L-fucopyranoside 2-sulphate.

L-Fucose 2-sulphate was synthesised definitively as follows. Methyl-α-L-fucopyranoside[21] (**4**) was converted into methyl 3,4-*O*-isopropylidene-α-L-fucopyranoside[22] (**6**), which was then treated with pyridine–sulphur trioxide. The resulting 2-sulphate was isolated as the barium salt **7**, but **7** was rather unstable and was immediately de-acetalated using very mild acid conditions to give methyl α-L-fucopyranoside 2-(barium sulphate) (**5**). Compound **5** reduced 1 mol. of sodium metaperiodate (Fig. 3), as expected, but was much more readily hydrolysed by dilute mineral acids than had been expected; treatment with 0.25M sulphuric acid at 100° rapidly gave a mixture of L-fucose, the required product **1**, and the desulphated glycoside **4**. When **5** was heated with 1% acetic acid at 100° for 2 h, it underwent hydrolysis to give only **4** (60%) and **1** (40%); neither the starting material nor the

product of total hydrolysis, L-fucose, could be detected by chromatography. Compound **1** was isolated by preparative layer chromatography and was identical (M_G, i.r., $[\alpha]_D$, periodate oxidation) with *H* described above.

The facile hydrolysis of **5** requires comment. Extension of our studies[12] to other glycoside 2-sulphates suggests that this class of compound is generally much more susceptible to acid hydrolysis than glycoside 3-, 4-, or 6-sulphates[23–28]. A strongly acidic sulphate ester group at C-2 may, through an electrostatic field effect, facilitate protonation of the adjacent glycosidic oxygen and hence hydrolysis proceeding by an A1 (or A2) mechanism. Alternatively, a process of general acid catalysis may apply in the hydrolysis of glycoside 2-sulphates, which would explain the effectiveness of acetic acid.

The acid-lability of the 2-sulphate group is less easily understood; HO-2 is normally more acidic than other hydroxyl groups in glycosides, because of its proximity to the acetal carbon. The same electron-withdrawing effect may render the 2-sulphate more susceptible to acid hydrolysis by the additional polarisation of the sulphur–oxygen bond in the conjugate acid $C_2–\overset{+}{\underset{|}{O}}\underset{H}{}–SO_2–$ that is expected to be formed prior to sulphur–oxygen bond scission. Other interpretations are possible but, like this one, none of them explain clearly why the parent glycose 2-sulphates are considerably less acid-labile.

EXPERIMENTAL

General methods. — For paper electrophoresis, 0.1M borate buffer (pH 10) or pyridine–acetic acid buffer (made by adding pyridine to 0.1M acetic acid until the pH was 6.5) was used, in a water-cooled apparatus[29] as already described[4], using Whatman No. 3 paper and a *p*-anisidine hydrochloride[30] spray. For thin-layer electrophoresis[31], plates coated with a layer (0.5 mm) of Silica Gel G were used, with detection by *p*-anisidine–conc. sulphuric acid[32]. This reagent and silica-gel plates were also used for t.l.c. DEAE-cellulose (D.E.50, Whatman) and Sephadex (G10, Pharmacia) columns were monitored quantitatively, using the phenol–sulphuric acid method[33], on 1-ml aliquots. For the detection of borate, six spots were superimposed on a filter paper which was then sprayed, first with methanolic HCl [made by adding conc. HCl (4 ml) to methanol (100 ml)] and then, after drying, with tumeric solution [made by shaking tumeric (5 g) with ethanol (100 ml), filtering, and adding water (100 ml) to the filtrate]; the paper was gently heated and the appearance of a pink colour denoted the presence of borate.

Unless otherwise stated, all optical rotations (sodium D line) were measured at 20° in 1-dm tubes in water (c, 0.2) on a Perkin–Elmer 141 polarimeter.

Preliminary sulphation studies. — (*a*) Dry L-fucose (3 g) was dissolved in anhydrous pyridine (30 ml), and 2-ml aliquots of this solution were transferred to each of 12 tubes. Pyridine–sulphur trioxide[34] (1 g) was added to each tube and the

sides were washed down with pyridine (1 ml). After each tube had been kept at room temperature for an appropriate time, water (3 ml) was added, the mixture was allowed to stand for 1 h to destroy excess reagent, and the volume was then made up to 10 ml with water. Using an Agla syringe, six (5 μl) spots were placed along the transverse centre-line of a Whatman No. 3 paper strip (68 × 16.5 cm), and the specimens were subjected to electrophoresis in pyridine–acetic acid buffer for 1 h at 2000 V. The paper was removed, dried, dipped into aniline hydrogen phthalate reagent[8], evenly pressed between filter-paper strips to remove excess reagent, and heated at 115° for 15 min. The bands corresponding to L-fucose and its mono-, di-, and tri-sulphates were cut out (together with a blank strip from the same paper) and each strip (16.5 × 2 cm) was extracted with 0.7M hydrogen chloride in 80% ethanol[9] (4 ml). The absorbance at 380 nm was recorded on a Unicam SP500 spectrophotometer, using the blank-strip extract as control solution. The results are shown in Fig. 1.

(*b*) After the treatment in (*a*) with water for 1 h, the pH was adjusted to 9 with barium hydroxide, and the filtered solution was evaporated to a small volume several times (with addition of further water) to remove pyridine. Barium was removed by addition of solid CO_2 and filtration, the solution was evaporated to dryness, and the product was dissolved in water (10 ml). From this point, the procedure described in (*a*) was followed. The results of the two procedures (*a*) and (*b*) were practically identical.

Sulphation of L-*fucose.* — To dry L-fucose (10.0 g) in dry pyridine (180 ml) was added pyridine–sulphur trioxide (50 g), and the mixture was stirred at room temperature for 1 h under anhydrous conditions. An equal volume of water was added and the solution stirred for 1 h to destroy excess reagent. Aqueous barium hydroxide was added to raise the pH to 9.0, the precipitated barium sulphate was removed by centrifugation, and the solution was concentrated *in vacuo*, more water being added from time to time to ensure the complete removal of pyridine; the pH was checked periodically, and, if necessary, maintained at 7.0 by addition of barium hydroxide. Excess barium was removed by the addition of solid CO_2 and filtration, the filtrate was concentrated to a small volume, and the sulphated product (13.2 g) was isolated as a white solid by addition of ethanol (20 vol.).

A solution of this product (4.00 g) in the minimal volume of water was introduced on to a column (100 × 7.5 cm) of DEAE-cellulose (HO^- form, washed with M ammonium hydroxide and water), and the column was eluted with M ammonium hydroxide. Fractions (25 ml) were collected and assayed for carbohydrate. Fractions 10–21 (*A*), 30–45 (*B*), 60–120 (*C*), 131–198 (*D*), and 199–280 (*E*) were combined, and evaporated *in vacuo*. The products were identified by electrophoresis as fucose (*A*), fucose monosulphates (*B*), and fucose disulphates (*C*, *D*, and *E*). The yield of L-fucose monosulphates (ammonium salts) was 1.10 g.

A solution of the L-fucose monosulphate fraction (300 mg) in the minimal volume of water was introduced onto a column (90 × 2.5 cm) of DEAE-cellulose (borate form) and eluted with 0.1M borate buffer (pH 10). Fractions (5 ml) were collected and assayed for carbohydrate (Fig. 2). Fractions 38–80 (*F*), 85–122 (*G*), and 130–170 (*H*)

were combined, and desalted by passage through columns (40 × 2 cm) of Sephadex G10. The eluate was tested (10-ml fractions) for the presence of carbohydrate and borate, and the fractions containing carbohydrate were combined, percolated through Zeocarb 225 (H^+) resin, neutralised with barium hydroxide, and reduced in volume *in vacuo*. The sugar sulphates were then precipitated as the barium salts by addition of ethanol.

The following products were obtained as white powders. From fraction *F*, L-fucose 4-(barium sulphate) (**3**, 83 mg), indefinite m.p., $[\alpha]_D^{20}$ −51.2°, M_G 1.2 (borate); ν_{max} (Nujol) 1240 (s), 843 cm^{-1} (w); lit.[2] $[\alpha]_D^{16}$ −66° (*c* 0.6, water), ν_{max} (KBr disc) 1240, 845 cm^{-1} [Found: C, 22.7; H, 3.8; S, 9.8. $(C_6H_{11}O_8S)_2Ba$ calc.: C, 23.1; H, 3.6; S, 10.3%].

From fraction *G*, L-fucose 3-(barium sulphate) (**2**, 36 mg), indefinite m.p., $[\alpha]_D^{20}$ −41.0°, M_G 1.25 (borate); ν_{max} (Nujol) 1255 (s), 856 cm^{-1} (vw) (Found: C, 23.0; H, 3.7; S, 10.6%).

From fraction *H*, L-fucose 2-(barium sulphate) (**1**, 68 mg), indefinite m.p., $[\alpha]_D^{20}$ −49.6°, M_G 1.56 (borate); ν_{max} (Nujol) 1240 (s), 852 cm^{-1} (w) (Found: C, 22.9; H, 3.8; S, 10.0%).

Periodate oxidation. — A solution of the barium salt of the sugar sulphate was prepared containing 2–4 mg (6–12 μmol) of sugar/ml. Sodium sulphate in 10% excess was weighed into the solution, and the mixture was stirred and warmed, and the precipitate removed by centrifugation.

An aliquot (1 ml) of the supernatant solution was incubated in the dark at room temperature with unbuffered sodium metaperiodate solution (2 ml, 0.03M containing 60 μmol of periodate). Samples (10 μl) were periodically withdrawn, and dispersed in water (3 ml) accurately measured into a spectrophotometer cell. The absorbances of the resulting solutions were measured at 223 nm against distilled water in a Unicam SP500 spectrophotometer, and compared with a standard calibration curve to determine the extent of conversion of periodate into iodate[35]. The results of the oxidation of **1**, **2**, **3**, and **5** are given in Fig. 3.

Methyl α-L-fucopyranoside 2-(barium sulphate) (**5**). — A solution of methyl 3,4-*O*-isopropylidene-α-L-fucopyranoside[21] (**6**, 3.0 g), $[\alpha]_D^{20}$ −113.2°, in dry pyridine (30 ml) was treated with pyridine–sulphur trioxide reagent (3.8 g), and kept at 40° for 24 h. Water was then added, and the sulphated product was isolated as the amorphous barium salt (**7**, 4.0 g) by the method described above (sulphation of L-fucose). This was rather unstable, and consistent analyses could not be obtained. A solution of **7** in water (100 ml) was adjusted to pH 3 by addition of sulphuric acid, and then heated at 60° for 5 h. Barium hydroxide was added, until the pH was 8, and the precipitated barium sulphate was removed by centrifugation. After removal of excess barium as barium carbonate by addition of solid CO_2, the solution was concentrated to a small volume *in vacuo*, and the product was precipitated by addition of ethanol and recrystallised from aqueous ethanol to give **5** (3.1 g), m.p. 103°, $[\alpha]_D^{20}$ 71.5°, ν_{max} (Nujol) 844 cm^{-1} [Found: C, 25.9; H, 3.9; S, 9.5. $(C_7H_{13}O_8S)_2Ba$ calc.: C, 25.8; H, 4.0; S, 9.5%].

Hydrolysis of methyl α-L-fucopyranoside 2-(barium sulphate) (**5**). — A solution of **5** (500 mg) in 1% (w/v) acetic acid (100 ml) was heated at 100° for 2 h, and then evaporated to dryness *in vacuo*. Thin-layer electrophoresis (borate) and t.l.c. (ethyl acetate–acetic acid–water, 6:1:1) then established that **1** (R_F 0.02) and **4** (R_F 0.29) were present, and **5** (R_F 0.10) and L-fucose (R_F 0.19) were absent. Compounds **1** and **4** were separated by preparative layer chromatography (ethyl acetate–2-propanol–water 5:3:1); (R_F values: L-Fucose, 0.31; **5**, 0.08; **4**, 0.17; **1**, 0.02). Guide strips were sprayed with *p*-anisidine–conc. sulphuric acid, and the silica gel from zones corresponding to each coloured band was extracted with 85% ethanol. Evaporation of the solution from the slower band gave L-fucose 2-(barium sulphate) (158 mg), $[\alpha]_D^{20}$ −49° (Found: C, 22.6; H, 3.6; S, 9.9%). This was identical (M_G, i.r., periodate oxidation) with **1** whose preparation is described above.

Evaporation of the second solution gave methyl α-L-fucopyranoside (**4**, 150 mg), m.p. and mixture m.p. 154°, $[\alpha]_D^{22}$ −190°; lit.[21] m.p. 154°, $[\alpha]_D^{21}$ −191°.

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