444. *Disaccharides from Fucoidin.*

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A sample of fucoidin, a seaweed material made up of sulphated fucose, has been partially hydrolysed by acetolysis. Amongst the products liberated, three disaccharides have been isolated and characterised as 2-, 3-, and 4-*O*-α-L-fucopyranosyl-L-fucose. Comment is made concerning the structure of fucoidin in the light of these and earlier results.

FUCOIDIN, found in several types of seaweed, is commonly designated as a polyfucose ethereal sulphate. From methylation studies, Conchie and Percival suggested that 1 : 2-linked fucose units are the main linkages present but others also contribute to the fucoidin structure.[1] Indeed, after acetolysis of fucoidin and reduction, O'Neill was able to isolate and characterise by periodate oxidation a fucobi-itol containing a 1 : 2-linkage.[2] It was thought that such a polysaccharide would be a suitable source of different fucose-containing disaccharides, which were needed as reference compounds to assist in the identification of partial acid hydrolysis products from blood-group substances.[3]

After acetolysis of fucoidin, we have isolated three disaccharides A, B, and C. None of the corresponding fucobi-itols could be detected on chromatograms by the acidic benzidine spray [4] which quickly reveals non-reducing furanoside structures such as sucrose and methyl fucofuranosides or galactofuranosides. This behaviour suggests the presence of a pyranose ring in the non-reducing end. By treatment with an enzyme preparation, which hydrolysed methyl α-L- and to a smaller extent β-L-fucopyranoside,[5] fucose was easily liberated from each disaccharide. The strongly negative optical rotation of all the disaccharides indicates that the fucose molecules are joined in the α-configuration, and the similarity of the rotations points to the probable identity of their ring structures. Moreover, from the known optical rotations of the methyl fucopyranosides [6] and fucofuranosides,[7] it appears that the $2B$ values for furanose and pyranose rings are respectively +850° and −32,500°, while the mean $2A$ value for fucosides lies around −38,380°. The molecular-rotation values of the three fucobi-itols fall between −35,000° and −38,600°. On the reasonable assumption that the aglycone group should have a minor influence on the partial rotation of the anomeric carbon atom of the cyclic structure, only an α-pyranose ring can closely fit Hudson's isorotation rules. The results of periodate oxidation, in good

[1] Conchie and Percival, *J.*, 1950, 827.
[2] O'Neill, *J. Amer. Chem. Soc.*, 1954, **76**, 5074.
[3] Côté and Morgan, *Nature*, 1956, **178**, 1171.
[4] Bacon and Edelman, *Biochem. J.*, 1951, **48**, 174.
[5] Clancy, personal communication.
[6] Hockett, Phelps, and Hudson, *J. Amer. Chem. Soc.*, 1939, **61**, 1658.
[7] Watkins, *J.*, 1955, 2054.

agreement with the general conclusions reached by Hough and Perry,[8] support these deductions and confirm the positions of the linkages. The periodate–benzidine spray [9] can be useful in indicating a particular structural feature. The white spots left on the blue background by the benzidine spray appear as bright-yellow fluorescent areas under ultraviolet light in the instances of 1 : 2-fucobiose, 2-acetamido-2-deoxyglucose, 1 : 3-fucobi-itol, and maltitol, but not of methyl galactofuranoside under the usual conditions: such compounds are susceptible to a quick degradation to a substituted malondialdehyde (or a hemiacetal form of it).

Disaccharide (A) is 2-*O*-α-L-fucopyranosyl-L-fucose. Besides a very low copper reducing value, it has a low M_G value,[10] as has 2-*O*-methyl-L-fucose, and gives a negative test with triphenyltetrazolium chloride,[11,12] properties which are consistent with substitution on $C_{(2)}$. This structure is also indicated by the overoxidation which occurs with periodate (>7 mols. consumed with liberation of 5 mols. of formic acid after 48 hr.), whereas, after reduction, only one mol. of acetaldehyde was released and no formaldehyde (44 hr.).

Disaccharide (B), 3-*O*-α-L-fucopyranosyl-L-fucose, gives a positive triphenyltetrazolium chloride test and has a much higher M_G value than (A) or (C) (3-*O*-methyl-L-fucose has also a high M_G value), which suggests a 1 : 3-linkage. After treatment with periodate for a limited time, 5-deoxy-L-lyxose was liberated by hydrolysis of the oxidised disaccharide, which indicates a relatively resistant 1 : 3-linkage.[13] As was expected for such a structure, the corresponding alcohol was overoxidised by periodate to yield 1·5 mols. of acetaldehyde and one of formaldehyde (44 hr.).

Disaccharide (C) is 4-*O*-α-L-fucopyranosyl-L-fucose. The compound has a low M_G value, but it produces a positive triphenyltetrazolium chloride test which may be due to a 1 : 4-linkage. It reduced up to 3·8 mols. of periodate, and released 2·4 mols. of formic acid (48 hr.); after reduction, the product consumed just under 4 mols. of periodate, yielding no acetaldehyde but one mol. of formaldehyde and nearly 2 mols. of formic acid (44 hr.). This behaviour can only be explained by a non-reducing pyranose ring 1 : 4-linked to the reducing unit.

The fucobi-itols, which are rapidly separated from the bioses by ionophoresis, are not readily distinguished from each other under these conditions as they have similarly high M_G values, although the net charges in each case should result from different complexes. However, a clear distinction between these alcohols can be obtained by paper chromatography with Rees and Reynolds's mixture which was elaborated to separate glucose and sorbitol,[14] and its use here allows a further check on the homogeneity of the preparations. While sugar alcohols show unpredictable R_F values with the usual solvent systems, they often have much higher R_F values than those of the parent sugars when Rees and Reynolds's solvent system is used. Such behaviour is probably due mainly to one component of the mixture, boric acid, which can form complexes in a different way with a sugar and its reduced form. Maltose (or lactose) has no appreciable effect on the conductivity of boric acid solutions because no furanose forms are possible,[15] and this would explain the remarkable enhancement of R_F between maltitol and maltose, maltotri-itol, and maltotriose.[16] Similarly, a large difference in R_F has been observed between 1 : 4-fucobi-itol and 1 : 4-fucobiose, which have R_F values in the ratio of 2·9 while the ratio is 1·5 and 1·3 for the corresponding 1 : 2- and 1 : 3-pairs. Moreover, 5-deoxy-L-lyxitol has approximately the same R_F as 5-deoxy-L-lyxose, but ribitol, which has a very low effect on the conductivity

[8] Hough and Perry, *Chem. and Ind.*, 1956, 768.
[9] Cifonelli and Smith, *Analyt. Chem.*, 1954, **26**, 1132.
[10] Foster, *J.*, 1953, 952.
[11] Wallenfels, *Naturwiss.*, 1950, **37**, 491.
[12] Bell and Dedonder, *J.*, 1954, 2866.
[13] Barker and Smith, *Chem. and Ind.*, 1952, 1035.
[14] Rees and Reynolds, *Nature*, 1958, **181**, 767.
[15] Böeseken, *Adv. Carbohydrate Chem.*, 1949, **4**, 189.
[16] Walker and Whelan, *Biochem. J.*, 1957, **67**, 548.

of boric acid solutions,[15] does not show a large increase in R_F and runs even more slowly than ribose (which has a strong tendency to exist in furanose forms) under those conditions.

The present findings can, with reservations, contribute to a structural study of fucoidin. The " linkage analysis method " [17] does not always give a fair estimate of the ratios of the different units present. The fragments released cannot always be recovered quantitatively, as complete purification of the products can result in losses which vary with the fractionation procedures used. Moreover, the hydrolytic conditions for maximum yields vary according to the sugars and their linkages. For instance, fucose is rather quickly split off from some materials by N-acetic acid at 100°;[3,18,19] the fucobioses (and fucobi-itols) are also easily hydrolysed and, apparently, at different speeds. However, the relative rates of acetolysis may not be the same, and, moreover, a sulphate group is present in fucoidin. The presence of oligosaccharides in minor quantities after acid-treatment can also be due to reversion, which is usually characterised[20,21] by the predominance of products arising by condensation at the most reactive position (although the proportions between synthesis and hydrolysis through reversion might sometimes differ), with a minority of other complex structures. It is possible to produce fucose disaccharides by acid reversion and the three types mentioned above have been detected after such a process, the 1 : 2-α-biose being predominant owing to the great reactivity of $C_{(2)}$. However, the conditions used in acetolysis differ greatly from those suitable for extensive reversion, which results in a very complex mixture. Finally, the homogeneity of polysaccharide preparations is not easily proved in a satisfactory way and the detection of a variety of linkages even in a sample made up of a single type of sugar requires confirmation by isolation of larger fragments which embody more than one type of linkage or by a careful methylation.

The first knowledge about the arrangement of fucose units in fucoidin was obtained by methylation. Conchie and Percival [1] isolated 3-*O*-methyl-L-fucose as a major component (*ca.* 60%) after hydrolysis of methylated fucoidin and suggested that the main residue is made up of 1 : 2-pyranose units carrying a 4-sulphate group, as alkali would lead to the elimination of sulphate from position $C_{(2)}$ or $C_{(3)}$ (vicinal *trans*-hydroxyl). The α-configuration was suggested because of the strong lævorotation of this polysaccharide. These conclusions were confirmed by O'Neill who, on the basis of periodate oxidation, characterised the fucobi-itol isolated as 2-*O*-α-L-fucopyranosyl-L-fucoitol, the α-configuration again being inferred from the highly negative rotation. The present work has led to the isolation and identification of the corresponding biose. To explain the presence of free fucose after methylation, Conchie and Percival suggested, without excluding the possibility of an incomplete methylation, the presence of branching points at $C_{(3)}$ on the main (2-linked) residue, or the presence of fucose molecules with two sulphate groups. The branching points, if any, can represent only a minor fraction of the linkages and the presence of 1 : 3-disaccharide units in the acetolysate is in line with this as the yield was small (though no attempt was made to improve it). Alternatively, it could mean branching points on 1 : 4-units, carrying a 2-sulphate group, as the present work affords strong evidence for the occurrence of many 1 : 4-linkages in fucoidin. The possibility cannot be rejected that these two main kinds of linkage, 1 : 2 and 1 : 4, might arise here from different polysaccharides present together in various proportions. This aspect was not investigated by us, but it is noteworthy that O'Neill noticed two components on electrophoresis of a purified sample of fucoidin.[2] Conchie and Percival pointed to the presence of 1 : 4-linkages as a chain of the general structure of fucoidin, having cautiously described their last fraction of methylated fucoidin (*ca.* 20%; $[\alpha]_D$ +4·6°) as 2 : 3-di-*O*-methyl-L-fucose after the preparation of suitable derivatives. Schmidt and Wernicke [22] had, however,

[17] Peat, Whelan, and Edwards, *J.*, 1955, 355.
[18] Kuhn, Baer, and Gauhe, *Chem. Ber.*, 1955, **88**, 1135.
[19] Aminoff, Morgan, and Watkins, *Biochem. J.*, 1950, **46**, 426.
[20] Jones and Nicholson, *J.*, 1958, 27; Ball and Jones, *ibid.*, p. 33.
[21] Thompson, Anno, Wolfrom, and Inatome, *J. Amer. Chem. Soc.*, 1954, **76**, 1309.
[22] Schmidt and Wernicke, *Annalen*, 1944, **556**, 179.

synthesised 2 : 3-di-*O*-methyl-D-fucose with $[\alpha]_D$ +73°, and recently Gardiner and Percival have reported $[\alpha]_D$ −97° for the L-isomer obtained by partial methylation.[23] Thus this fraction from methylated fucoidin remains largely unidentified and, according to other data, the contaminant(s) in it would not be any of the fucofuranose dimethyl ethers with positive optical rotation.[23] Since even N-sodium hydroxide at 100° could remove appreciable amounts of sulphate from fucoidin,[1] the positive optical rotation could be explained by the presence of a methylated fucose epimer produced by Walden inversion [24] on removal of the sulphate group on 1 : 4-linked units during methylation. The small quantity of dimethylfucose obtained might also have been produced by terminal non-reducing fucose molecules with a (stable) 4-sulphate group. As regard the minor features of fucoidin, glucose, galactose, etc., are probably constituents of contaminating polysaccharides. The probable isolation of 3-*O*-methyl-L-fucose from crude fucoidin [25] has not been confirmed (see p. 2252). That methylated L-fucose occurs naturally has been recently suggested by the isolation of 2-*O*-methyl-L-fucose from plum leaf [26] and possibly another plant.[25]

Experimental

General Methods.—Adsorption chromatography was carried out with different preparations of charcoal: (1) B.D.H., "acid-washed," with moderate adsorptive power; (2) Hopkin and Williams, that was treated successively with boiling 20% acetic acid for 1 hr., washed with water and hot aqueous ethanol (25%), and dried at 150° in air. The latter material was a strong adsorbent even for fucose. For use, either type of charcoal was mixed with washed Celite (1 : 1, w/w) and elution was with aqueous ethanol.[27]

Preparative paper chromatography by the descending technique was usually made with Whatman sheets no. 17 (18¼ × 22½ in.), which can easily accommodate 0·5 g. of material each. Glass rods were fixed to provide a smooth bend to the sheets, which were clamped one on each side of a stainless steel frame dipping into the solvent contained in a 1-l. trough. This very thick paper offers a great rate of flow and is at the same time reasonably even. After detection, elution of the sugar bands was made either by sewing on strips of Whatman no. 3 mm. as wicks for descending technique [28] or by pulping the strips in a Waring blender. Whatman paper no. 1 was used for qualitative chromatography by the descending technique. The following solvent systems were used: (*a*) butan-1-ol–pyridine–water (3 : 1 : 1, v/v),[29] (*b*) butan-1-ol–acetic acid–water (4 : 1 : 5, v/v),[30] and (*c*) ethyl methyl ketone–acetic acid–water saturated with boric acid (9 : 1 : 1, v/v).[14] The spraying reagents were benzidine–trichloroacetic acid,[4] triphenyltetrazolium chloride,[11] and benzidine–periodate.[9]

Ionophoresis was carried out with an apparatus of the type described by Foster,[31] used as a semi-open one by insertion of a suitable rubber gasket between the glass plates. The paper (no. 3MM, 57 cm. in length) was dipped completely in the buffer before being put between the plates for draining and spotting. To allow long runs (5 v/cm., 15 hr.), the starting line was placed 25 cm. from the cathode side. M_G values were measured, as by Foster, in presence of Sørensen's borate buffer (pH 10) (*viz.*, 7·44 g. of boric acid in 1 l. of 0·1N-sodium hydroxide). Non-reducing materials were detected with the benzidine–periodate spray [8] after the dried paper had been first treated by acetone containing 5% of acetic acid.

Oxidations were made in the dark at room temperature in 0·2M-sodium metaperiodate. Periodate consumption was measured by iodometry at pH 7 [32] or in acid medium,[33] formic acid by iodometry, formaldehyde by chromotropic acid,[34] and acetaldehyde by piperazine–sodium

[23] Gardiner and Percival, *J.*, 1958, 1414.
[24] Peat, *Adv. Carbohydrate Chem.*, 1946, **2**, 38.
[25] Springer, "Chemistry and Biology of Mucopolysaccharides," Ciba Foundation Symposium, Churchill, London, 1958, p. 216.
[26] Anderson, Andrews, and Hough, *Chem. and Ind.*, 1957, 1453.
[27] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677.
[28] Brownell, Hamilton, and Casselman, *Analyt. Chem.*, 1957, **29**, 550.
[29] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[30] Partridge, *Biochem. J.*, 1948, **42**, 238.
[31] Foster, *Chem. and Ind.*, 1952, 1050.
[32] Neumüller and Vasseur, *Arkiv Kemi*, 1953, **5**, 235.
[33] Hughes and Nevell, *Trans. Faraday Soc.*, 1948, **44**, 941.
[34] Frisell, Meech, and Mackenzie, *J. Biol. Chem.*, 1954, **207**, 709.

nitroprusside.[35] Reductions were by sodium borohydride,[36] and boric acid was removed by methanol in the usual way after de-ionisation by resins.

The degree of polymerisation was measured in a manner similar to that described by Peat, Whelan, and Roberts.[37] As fucitol produces a negligible colour, the ratios of fucose in the compounds before and after reduction can be measured by Dische and Shettles's method for methylpentoses, which was used, as modified by Gibbons,[38] with samples of 1 : 2- and 1 : 4-fucobiose previously treated according to Peat *et al.*[37] The average values were 2·05 and 2·00 for the 1 : 2- and 1 : 4-bioses respectively. Determination of fucose is possible in the usual range (10—50 μg.), as the inhibiting effect of inactivated sodium borohydride on the development of colour is small. With the third substance (1 : 3-linkage), the ratio of fucose to fucitol was directly measured. A pure sample (*ca.* 3 mg.) of the fucobi-itol was hydrolysed by N-acetic acid and spotted on paper (solvent *c*), and the bands corresponding to fucose and fucitol were eluted. Aliquot parts equivalent to 70—130 μg. of acetaldehyde were treated by 0·05M-periodate in phosphate buffer (pH 7, 90 min., 25°), and acetaldehyde was measured as before. The net values obtained from the fucose and fucitol levels had a ratio of 1·10, while uniform and low blanks were shown with a control sheet of paper identically treated.

Hydrolysis of the disaccharides as 1—2% solutions in N-acetic acid at 100° was followed by paper chromatography which indicated 75% hydrolysis in ~2 hr. for the 1 : 2-biose, in ~9 hr. for the 1 : 3-biose, and ~7 hr. for the 1 : 4-biose.

All solutions were concentrated under reduced pressure at <20° in a rotary evaporator with a seal provided by two glass tubes ground into each other, the mobile one (diam. 15 mm.) being set in a self-aligning ball-bearing. Optical rotations were measured in water at 22°.

Acetolysis and Fractionation of Fucoidin.—A commercial sample of fucoidin, prepared from *F. vesiculosus* as described by Black, Dewar, and Woodward,[39] dried at 50° *in vacuo* for 36 hr., contained 35% of fucose [38] and had $[\alpha]_D$ −93° (*c* 1). Complete hydrolysis showed the presence of small amounts of glucose, mannitol (chromatographic evidence after development with solvent *c*), xylose (identified by its R_F and M_G), galactose, mannose (?), and uronic acid(s). A fast component had R_F similar to that of 3-*O*-methylfucose in solvent *b*, but of quite different M_G. A first batch (25 g.) was acetolysed and deacetylated by O'Neill's method.[2] After neutralisation, the solution was freed from methanol by evaporation, diluted to 600 ml. with water, and fractionated by the frontal analysis method [40] as follows: Four Celite–charcoal (no. 2) filters of 30 ml., separated by 2 cm., were set up on top of each other in a glass tube (diam. 2·5 cm.). The sugar solution was percolated through this column and followed by 200 ml. of water. Each section was extruded and eluted with 25% ethanol. The bottom filter (IV) was saturated with fucose, the one above (III) contained mainly disaccharide (C), the second filter retained mostly disaccharide (A) (on first evidence), and the top section contained various components. Combined eluates from (I) and (II) were spotted on Whatman paper no. 17 which was irrigated for 30 hr. with solvent *a*. The syrup recovered after elution and usual purification was dissolved in moist methanol, from which crystals of disaccharide (A) separated quickly (0·15 g.). On addition of ethyl acetate, the mother-liquors yielded some more material, shown to be a mixture of disaccharides (A) and (B). Disaccharide (A) has $[\alpha]_D$ −160° (0·5 hr.) ⟶ −169° (*c* 1) and m. p. 185—190° (decomp.), M_G 0·34, R_{fuc} 0·78, 0·74, 0·45 in solvents *a*, *b*, and *c* respectively (galactose has 0·56, 0·51, and 0·38). With the benzidine spray, it produces a yellow-orange colour. Acid-hydrolysis of the corresponding fucobi-itol, which crystallised easily from moist ethanol, liberated fucose and fucitol, which are readily distinguishable with all solvents used: fucitol has R_{fuc} 1·2, 1·3, and 2·4 in solvents *a*, *b*, and *c* respectively. The fucobi-itol has $[\alpha]_D$ −124° (*c* 1), m. p. 191—192° (lit.,[2] $[\alpha]_D$ −118°, m. p. 190—192°), M_G 0·59, R_{fuc} 0·79, 0·78, and 0·68 in solvents *a*, *b*, and *c* respectively, and is identical (mixed m. p. and R_F in solvent *c*) with the fucobi-itol isolated by O'Neill. The contents of filter (III) were similarly purified by partition with solvent *a* (40 hr.), disaccharide (C) being obtained as a semi-amorphous product by precipitation with ethyl acetate from methanol (0·10 g.) (see below).

A second acetolysis was carried out as before, with another sample of fucoidin (50 g.). After

[35] Fromageot and Heitz, *Mikrochim. Acta*, 1938, **3**, 52.
[36] Abdel-Akher, Hamilton, and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 4691.
[37] Peat, Whelan, and Roberts, *J.*, 1956, 2258.
[38] Gibbons, *Analyst*, 1955, **80**, 268.
[39] Black, Dewar, and Woodward, *J. Sci. Food Agric.*, 1952, **3**, 122.
[40] Tiselius, *Kolloid Z.*, 1943, **105**, 101.

deacetylation with barium methoxide at 0° for 48 hr. and neutralisation, methanol was removed and the filtered solution diluted to 1500 ml. with water. The solution was passed through two filters of charcoal (no. 2)–Celite (180 and 70 ml.) which were washed with water (900 ml.). The smaller filter contained only fucose (*ca.* 0·45 g.). From the first filter, about 1·5 g. of material (containing a trace of fucose) with similar elution properties were collected in a volume of 400 ml. by displacement analysis [40] with a 0·5% solution of raffinose in 15% ethanol. The mixture was refractionated on a charcoal (no. 2)–Celite column (150 ml.) with a gradient elution [41] $x = 25\%$, $v = 1$ l. The first peak, at 10% ethanol, was due to disaccharide (C), the purification of which was completed by partition on a Celite column [42] (300 g.; diam. 5 cm.) with 5 l. of solvent. The sugar was located by spraying a print taken on the extruded column with a no. 3MM paper strip and recovered after elution as described previously (0·47 g.). Heating *in vacuo* at 76° caused the material to lose weight equivalent to one mol. of water, and the final product was a semi-amorphous solid (according to a *X*-ray diffraction photograph), $[\alpha]_D$ −170° (*c* 1). It has M_G 0·28, R_{fuc} 0·64, 0·62, and 0·28 in solvents *a*, *b*, and *c* respectively, and gives a bright yellow spot with benzidine. The corresponding alcohol (amorphous) has $[\alpha]_D$ −115° (*c* 1), M_G 0·62, R_{fuc} 0·79, 0·77, and 0·82 in solvents *a*, *b*, and *c*. Hydrolysis by acetic acid afforded fucose and fucitol.

The second peak eluted from the charcoal column was mainly a mixture of disaccharides (A) and (B). Small amounts of contaminants with clearly different R_F were first eliminated by partition chromatography on Whatman paper no. 17 (solvent *a* for 28 hr.). The final syrup (*ca.* 0·30 g.) was fractionated on a charcoal (no. 1)–Celite filter (75 ml.) in the presence of 0·025M-sodium tetraborate [43] (pH 9·2) with a gradient of ethanol, $x = 10\%$, $v = 1$ l. Disaccharide (B) started to come from the column at about 2½% ethanol and was clearly separated from disaccharide (A). Each pool was run through a column of Zeo-Karb 225 (H^+), the effluent being freed from boric acid as usual. Disaccharide (A) (0·12 g.) crystallised from methanol. Disaccharide (B) had to be further purified by paper chromatography on Whatman paper no. 3MM with solvent *b* and passed through a small charcoal column. The final syrup crystallised slowly in presence of ethanol (0·06 g.), then having m. p. 198—200°, $[\alpha]_D$ −200° ⟶ −191° (*c* 1). Disaccharide (B) produces a yellow-brown colour with the benzidine spray and has M_G 0·52, R_{fuc} 0·80, 0·75, and 0·43 in solvents *a*, *b*, and *c*. The corresponding fucobi-itol (a syrup) had $[\alpha]_D$ −112° (*c* 1), M_G 0·60, R_{fuc} 0·75, 0·74, and 0·56 in solvents *a*, *b*, and *c*, and also gave fucose and fucitol on acid-hydrolysis. The fucobiose was oxidised by periodate (5-fold excess) at 0° for 90 min.; after de-ionisation and hydrolysis by 0·1N-hydrochloric acid for 3 hr. 5-deoxylyxose was identified on paper chromatograms.

5-Deoxy-L-lyxose.—L-Fucose was treated with bromine in presence of calcium carbonate, and the non-reducing solution run successively through columns of Zeo-Karb 225 (H^+ form) and DeAcidite E (AcO^- form); L-fuconolactone, obtained on evaporation of the final effluent, was transformed into L-fuconamide as described by Clark.[44] Weerman degradation of this product led to 5-deoxy-L-lyxose,[45] but several non-reducing materials were also detected by chromatography in the final syrup. The mixture, in phosphate buffer (pH 7·5), was fractionated on charcoal (no. 2)–Celite and then by partition on Celite (water-saturated butanol), to yield 5-deoxy-L-lyxose as a chromatographically pure syrup (yield, 8%). As a result of observations on the oxidation of monosaccharides by periodate,[46,47] this methyltetrose was also obtained by oxidation of L-fucose with one equivalent of periodate at room temperature. After de-ionisation and hydrolysis, unchanged fucose (*ca.* 50%) was removed by partition chromatography on Whatman paper no. 17 (water-saturated butanol for 20 hr.) and the material was finally purified by chromatography on charcoal (yield, 5%). 5-Deoxy-L-lyxose has $[\alpha]_D$ −35° (*c* 1) (lit.,[48] $[\alpha]_D$ +32° for 5-deoxy-D-lyxose), M_G 0·78, R_{fuc} 1·7 in solvents *a* and *b*. With benzidine spray, it gives a brownish-red spot, strongly fluorescent in ultraviolet light; treatment on paper with the Ehrlich reagent at 80° produces an orange-purple spot which distinguishes it from other oxidation products of fucose.

[41] Alms, Williams, and Tiselius, *Acta Chem. Scand.*, 1952, **6**, 826.
[42] Lemieux, Bishop, and Pelletier, *Canad. J. Chem.*, 1956, **34**, 1365.
[43] Barker, Bourne, and Theander, *J.*, 1955, 4276.
[44] Clark, *J. Biol. Chem.*, 1922, **54**, 65.
[45] Schmidt, Mayer, and Distelmaier, *Annalen*, 1943, **555**, 26.
[46] Courtois and Guernet, *Bull. Soc. chim. France*, 1957, 1388.
[47] Warsi and Whelan, *Chem. and Ind.*, 1958, 71.
[48] Votoček and Valentin, *Coll. Czech. Chem. Comm.*, 1930, **2**, 36; *Chem. Zentr.*, 1930, I, 2543.

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