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Note

Synthesis of β-L-fucopyranosyl phosphate from L-fucose orthoacetates

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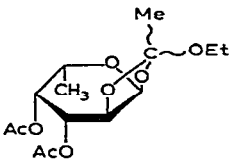
We report in this Note the synthesis of 3,4-di-*O*-acetyl-1,2-*O*-(ethyl orthoacetyl)-α-L-fucopyranose (**1a,b**), of crystalline peracetyl-α-L-fucopyranosyl bromide (**2**), and the conversion of **1a,b** into β-L-fucopyranosyl phosphate (**3**) by treatment with phosphoric acid. We also discuss some ^{1}H- and ^{13}C-n.m.r. data for these compounds and the four methyl fucosides.

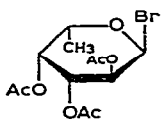
2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl bromide (**2**) had been previously obtained only as an impure syrup[1]. L-Fucopyranose tetraacetates, consisting mainly of the α-anomer[2], were treated with hydrogen bromide in acetic acid to yield the crystalline, acetylated glycosyl bromide in ~70% yield. The molecular rotation was −93,500°. This corresponds closely with the value of −90,200° calculated from the rotation of the chloro analog[3] (−66,300°), and the difference between the rotations (23,900°) of the corresponding chloro[4] and bromo[5] derivatives in the *galacto* series. The n.m.r. spectrum showed the H-1 signal as a doublet at δ 6.72, $J_{1,2}$ 4 Hz.

Ortho esters of sugars have considerable synthetic utility[6], but fucose ortho esters do not appear to have been described previously. Treatment of the bromide **2** with 2:5 (v/v) ethanol–*sym*-collidine gave a mixture of the *exo*- (**1a**) and *endo*-(**1b**)-isomers of 3,4-di-*O*-acetyl-1,2-*O*-(ethyl orthoacetyl)-α-L-fucopyranose, together with ethyl 2,3,4-tri-*O*-acetyl-β-L-fucopyranoside in the ratio of 4.3:1:3, as shown by the ^{1}H-n.m.r. spectrum. The proportion of glycoside in the product mixture could be increased by increasing the ethanol:collidine ratio. For example, 4:1 ethanol–collidine gave a glycoside–ortho ester ratio of 2:1. The ethyl tri-*O*-acetylfucopyranoside was separated from the mixture of ortho esters by column chromatography on neutral alumina with 1:1 ether–benzene as eluant. Attempts to separate the *exo*- and *endo*-ortho esters failed. The ethyl 2,3,4-tri-*O*-acetyl-β-L-fucopyranoside was identified by the similarity of its ^{1}H-n.m.r. spectrum with that of the corresponding methyl pyranoside described by Leaback *et al.*[3]. It showed the characteristic doublet for H-1β at δ 4.47, J = 7.5 Hz. N.m.r. assignments for the orthoacetates could be made by comparison with the data of Perlin[7]. Separate resonances for the *exo* and *endo* isomers were particularly clear for H-1 and the CH_3-C group. Perlin[7] has shown that these resonances for the *exo* isomer are downfield of the corresponding *endo*

resonances. The *exo* isomer is also the one that is generally formed in greater proportion. Assigning according to these generalizations, the H-1 region showed doublets at δ 5.78 (*exo*, **1a**) and 5.67 (*endo*, **1b**). The CH_3-C resonances occurred as singlets at δ 1.65 (*exo*, **1a**) and 1.57 (*endo*, **1b**). The ^{13}C-n.m.r. spectrum of the mixed ortho esters showed a pair of resonances at 98.7 (*endo*) and 98.0 p.p.m. (*exo*) for C-1. A single C-6 resonance was observed at 16.1 p.p.m.



1a (exo), 1b (endo)



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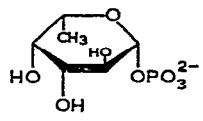

3

The mixed orthoesters (**1a,b**) were used for a new synthesis of β-L-fucopyranosyl phosphate. It had previously been shown that *trans*-1,2-glycosyl phosphates could be synthesized by treatment of a sugar ortho-ester with either dibenzyl or diphenyl hydrogenphosphate followed by removal of protecting groups[8]. We find that this synthesis may be simplified by use of unsubstituted, anhydrous phosphoric acid. For this purpose, the crude orthoester **1a,b** obtained by treating **2** with alcohol and collidine may be used; it is not necessary to remove the glycoside, as this material is removed during the purification of the sugar phosphate. Thus, treatment of the ortho ester **1a,b** in tetrahydrofuran with a five-fold excess of phosphoric acid gave, after conventional isolation, a crude barium salt. This material showed a single spot upon electrophoresis at pH 6.5. No phosphoric diester, such as that isolated by Leaback *et al.*[3], was observed. Conversion into the cyclohexylammonium salt and crystallization from ethanol–acetone gave a sample of β-L-fucopyranosyl phosphate **3** as its salt, in about 20% overall yield.

TABLE I

^{13}C-N.M.R. DATA[a]

Fucose derivative	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*-OCH$_3$*
α-Furanosyl phosphate	95.7	74.9	71.5	85.4	69.0	17.3	—
β-Furanosyl phosphate	101.7	81.4	76.8	87.5	67.9	17.5	—
α-Pyranosyl phosphate	94.1	69.1	70.0	72.3	67.2	15.8	—
β-Pyranosyl phosphate	97.7	71.9	72.8	71.7	71.0	15.9	—
Methyl α-furanoside	103.3	79.2	76.5	87.2	69.6	19.0	55.4
Methyl β-furanoside	109.5	82.4	78.8	88.2	67.6	19.6	54.7
Methyl α-pyranoside	100.3	68.9	70.4	72.3	66.5	16.1	55.3
Methyl β-pyranoside	105.3	72.3	74.9	72.0	71.2	16.8	56.4

[a]The data are expressed as p.p.m. relative to $(CD_3)_2CO$ at 29.8 p.p.m. Solvents were D_2O (pD 8.5) for the phosphates (barium salts) and 19:1 $(CD_3)_2CO$–D_2O for the methyl fucosides.

^{13}C-N.m.r. data for the α- and β-fuco-pyranosyl and -furanosyl phosphates are compared with corresponding data for the four methyl fucosides in Table I. The data for the methyl fucopyranosides agree closely with those of Gorin and Mazurek[9]. The assignments for the methyl fucofuranosides were made by comparison with the data of Ritchie *et al.*[10] and of Gorin and Mazurek[9] for the methyl galactofuranosides. The assignments for the phosphates were made by comparison with the data for the methyl fucosides. In view of the difficulties[9,11] in assigning the closely spaced resonances for C-2,3,4,5, particularly for the pyranoses, these assignments should be considered tentative. It may be seen that examination of the C-1 and C-6 resonances serves to identify both the anomeric configuration and the ring size. The C-6 resonances for the furanoses lie significantly downfield of those for the pyranoses; ring size decided, the C-1 resonance specifies the orientation at C-1, as the resonance for the 1,2-*trans* arrangement is downfield of that for the *cis*. These generalizations should be helpful in identifying fucosyl residues in polysaccharides. Our data show that the resonances for the fucosyl phosphates follow the same pattern as those for the methyl fucosides, except that the C-1 and C-2 resonances are doublets, because of splitting by phosphorus, $J = 4$–7 Hz. Some preliminary ^{13}C-n.m.r. data on glycosyl phosphates have been presented by Nunez and co-workers[12].

EXPERIMENTAL

General methods. — Proton n.m.r. spectra were recorded on Varian T-60 or HA-100 instruments; and ^{1}H-decoupled ^{13}C-n.m.r. spectra were taken on a Bruker 90 instrument (22.63 MHz). Optical rotations were measured with a Perkin–Elmer 141 instrument. T.l.c. was carried out on glass plates coated with silica gel G using either solvent *A* (1:1 ether–petroleum ether) or solvent *B* (1:1 ether–benzene). The fucosyl phosphates and methyl fucosides were prepared as previously described[2,13]. Methyl α-L-fucopyranoside was also synthesized by Mowery's method[14] and methyl β-L-fucopyranoside by treatment of the ortho esters (**1a,b**) with 4:1 methanol–collidine.

2,3,4-Tri-O-acetyl-α-L-fucopyranosyl bromide (**2**). — A solution of hydrogen bromide in acetic acid (30–32% w/v, 5 ml) was added to 2 g of the anomeric mixture of L-fucose tetraacetates obtained by low-temperature acetylation with acetic anhydride–pyridine[2]. The clear solution was stirred for 90 min at 25° with protection from atmospheric moisture. The resultant yellow solution was poured into 20 ml of ice–water, stirred, and extracted with 20 ml of carbon tetrachloride. This extract was washed successively with 15 ml of ice–water, 15 ml of a cold, saturated solution of sodium hydrogencarbonate, and finally with two 15-ml portions of ice–water. The solution in carbon tetrachloride was dried for 30 min with calcium chloride, filtered, and evaporated to dryness using an oil pump. The bromide **2** crystallized in the flask and was recrystallized from ether–petroleum ether to give a yield of 1.45 g (68%), m.p. 64–66°, $[\alpha]_D^{25}$ −265° (*c* 2, chloroform); R_F 0.6 (solvent *A*); n.m.r. ($CDCl_3$, Me_4Si); δ 6.72 (d, *J* 4 Hz, H-1), 5.55–4.99 (m, H-2,3,4), 4.44 (dd, H-5), 2.22, 2.12, and 2.02 (3s, CH_3CO), 1.17 (d, *J* 6 Hz, H-6).

Anal. Calc. for $C_{12}H_{17}BrO_7$: C, 40.80; H, 4.82; Br, 22.64. Found: C, 40.95; H, 4.81; Br, 22.11.

3,4-Di-O-acetyl-1,2-O-(ethyl orthoacetyl)-α-L-fucopyranose (**1a,b**) *and ethyl 2,3,4-tri-O-acetyl-β-L-fucopyranoside.* — The bromide **2** (2 g, 5.67 mmol) was dissolved in a mixture of *sym*-collidine (10 ml) and dry ethanol (4 ml). The homogeneous solution was stirred at 25°. A precipitate of *sym*-collidinium hydrobromide began to form after 3 h. The mixture was evaporated *in vacuo* after 24 h. Diethyl ether (15 ml) was added and the collidinium hydrobromide filtered off. The excess of collidine was removed from the filtrate by vacuum evaporation at 45°. The yellow syrup that resulted was chromatographed on a column (1 × 20 cm) of neutral alumina with 1:1 benzene–ether as eluant. The ortho ester **1a,b** was the first component to be eluted; yield 750 mg (41%) of a syrup: $[\alpha]_D^{25}$ −100° (*c* 2.5, chloroform), R_F 0.7 (solvent B); n.m.r. ($CDCl_3$, Me_4Si): δ 1.1–1.24 (m CH_3-CH, CH_3-CH_2), 1.57 (s, CH_3-C_{endo}), 1.65 (s, CH_3C_{exo}), 2.03, 2.1 (2s, CH_3CO), 3.55 (q, CH_3CH_{2endo}), 3.58 (q, CH_3CH_{2exo}), 4.18–4.30 (m, H-2, H-5), 5.02 (dd, H-3), 5.24 (dd, H-4), 5.65 (d, *J* 4.5 Hz, H-1_{endo}), and 5.78 (d, *J* 4.5 Hz, H-1_{exo}).

Anal. Calc. for $C_{14}H_{22}O_8$: C, 52.83; H, 6.92. Found: C, 52.57; H, 6.80.

The second component to be eluted was ethyl tri-*O*-acetyl-β-L-fucopyranoside, also obtained as a syrup; yield 420 mg (23%); $[\alpha]_D^{25}$ −4.4° (*c* 1.8, chloroform); R_F 0.6 (solvent B); n.m.r.: δ 1.2 (m, CH_3-CH, CH_3-CH_2), 1.96, 2.00, 2.15 (3s, CH_3CO), 3.6–4.1 (m, H-5, CH_3CH_2), 4.47 (d, *J* 7.5 Hz, H-1), and 5.09–5.25 (m, H-2,3,4).

Anal.: Calc. for $C_{14}H_{22}O_8$: C, 52.83; H, 6.92. Found: C, 52.72; H, 6.92.

Bis(cyclohexylammonium)-β-L-fucopyranosyl phosphate. — To a solution of anhydrous, crystalline orthophosphoric acid (600 mg, 6.1 mmol) in dry tetrahydrofuran (15 ml) was added a solution of the crude ethyl orthoacetates **1** (394 mg, 1.2 mmol, but containing also the ethyl glycoside) in 10 ml of dry tetrahydrofuran. This solution was stirred for 40 min at 25°. Cold, M aqueous lithium hydroxide was then added with stirring to give a pH of 11. This pH was maintained for 12 h by the periodic addition of lithium hydroxide. The trilithium phosphate precipitated was filtered off and the pH of the filtrate adjusted to 8.5 with Dowex-50 (H^+) resin. The resin was removed by filtration and the solution concentrated *in vacuo* below 30° to about 10 ml. Barium acetate (600 mg) was added to the solution, followed by 10 ml of ethanol. After 2 h at 0°, the precipitate was collected by centrifugation. The crude barium salt was dissolved in 20 ml of water and converted into the cyclohexylammonium form by the addition of an equivalent of dicyclohexylammonium sulfate. The barium sulfate was removed by centrifugation. The supernatant solution was dried *in vacuo* for 12 h at 50°. This treatment removed the excess of cyclohexylammonium acetate. The resulting solid was dissolved in 10 ml of water and precipitated by the addition of ethanol and acetone, yield: 95 mg (19% from the ortho ester); $[\alpha]_D^{25}$ −26° (*c* 1.1, water) (lit.[13] −20.5°). Hydrolysis in 0.3 M hydrochloric acid for 15 min at 100° gave a reducing sugar: inorganic phosphate ratio of 0.97:1.

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