Carbohydrate Research, 56 (1977) 315–324

SYNTHESIS OF β-L-FUCOPYRANOSYL PHOSPHATE AND L-FUCOFURANOSYL PHOSPHATES BY THE MACDONALD PROCEDURE

HARRY S. PRIHAR, JI-HSIUNG TSAI, STEVEN R. WANAMAKER, STEPHANIE J. DUBER, AND EDWARD J. BEHRMAN*

Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210 (U. S. A.)

(Received July 7th, 1976; accepted for publication in revised form, September 18th, 1976)

ABSTRACT

Fusion of β-L-fucopyranose tetraacetate with phosphoric acid for 1 min at 50° gives a 9:1 anomeric mixture of the α- and β-pyranosyl phosphates. Longer fusion times give the α-anomer exclusively. The L-fucofuranose tetraacetates were synthesized for the first time by acetolysis of methyl-2,3,5-tri-*O*-acetyl-β-L-fucofuranoside. Fusion of the furanose tetraacetates with phosphoric acid gave a mixture of the fucofuranosyl phosphates in which the β-anomer predominated ($\beta/\alpha = 2.4$). Anomeric pairs in the fucofuranose series appear to be distinguishable by the chemical shift of the C-6 methyl protons, as already shown by Sinclair and Sleeter in the pyranose series.

INTRODUCTION

β-L-Fucopyranosyl phosphate has been made enzymically by using a fucose kinase[1] and chemically via phosphorylation of 2,3,4-tri-*O*-acetyl-β-L-fucopyranose[2]. It appeared to us that application of the MacDonald procedure[3] to tetra-*O*-acetyl-β-L-fucopyranose ought to yield the desired β-phosphate, despite the reported failures[4,5] in which only the α-anomer was obtained.

MacDonald[6] has already shown that the initial (kinetic) product in the reaction of β-D-glucopyranose pentaacetate with phosphoric acid is the expected β-phosphate, but that the β-phosphate also undergoes a slower anomerization to the α-phosphate. As pointed out by MacDonald[7] in his first paper on this subject (see also ref. 3), the reaction is probably analogous to the well-studied synthesis of acetylated glycosyl halides[8] from the peracetylated sugar and a hydrogen halide. By this analogy, the rate data collected by Capon[9], and MacDonald's results for the *gluco* series[6], one may calculate that the optimum time for reaction of β-L-fucopyranose tetraacetate with phosphoric acid at 50° so as to produce the maximal yield of the β-phosphate is of the order of 10 sec. This number was calculated by dividing the optimum time in the *gluco* series (5 min) by a kinetic factor of 3 (for conversion into the *galacto* series) and again by a kinetic factor of 10 (for conversion into the 6-deoxy series)[9].

*To whom correspondence should be addressed

Chittenden[10] reported the synthesis of β-D-galactofuranosyl phosphate by several routes. This is the only aldohexofuranosyl phosphate whose synthesis had been achieved prior to this report.

RESULTS AND DISCUSSION

Acetylation of L-*fucose.* — The MacDonald fusion with phosphoric acid is effected on the peracetylated sugar. As only the pyranose tetraacetates had been previously described in the fucose series, we undertook preliminary identification and partial characterization of the fucofuranose tetraacetates.

Acetylation of L-fucose with acetic anhydride–pyridine at 0° gave an 86:14 mixture of the α-pyranose and β-pyranose tetraacetates, from which 1,2,3,4-tetra-*O*-acetyl-α-L-fucopyranose readily crystallized.

Acetylation with acetic anhydride–sodium acetate at 70–80, 90, 100, and 140° (reflux) gave a mixture of tetraacetates of almost invariant composition: 60–65% β-pyranose; 20–25% α-pyranose and α-furanose; and 12–18% β-furanose. Westphal and Feier[11] reported crystallization of the β-pyranose tetraacetate from this mixture but we, like others[5,12], were unsuccessful in this effort. We have, however, obtained this compound crystalline by another route[2], and find physical constants in agreement with those reported by Westphal and Feier[11].

N.m.r. assignments for the fucose tetraacetates. — Leaback *et al.*[5] reported n.m.r. assignments for the fucopyranose tetraacetates. Examination of the H-1 region of the mixed tetraacetates by n.m.r. spectroscopy showed the presence of the β-pyranose (δ 5.70 d, *J* 8 Hz), a new component resonating at δ 6.2 (broad singlet), and a resonance for the α-pyranose (δ 6.30, broad singlet, $J < 2$ Hz) at δ 6.30–6.37, but which appeared as a distorted doublet, $J = 4$ Hz. The furanose assignments were made from spectra of a sample of fucofuranose tetraacetates synthesized by Chittenden's route[10] in the *galacto* series via acetolysis of the known methyl 2,3,5-tri-*O*-acetyl-β-L-fucofuranoside[13]. The mixed tetraacetates obtained contained about 80–85% of the δ 6.2 component (broad singlet) and about 15–20% of material having δ 6.35 d, $J = 4$ Hz. The major component is probably the β-furanose tetraacetate and the minor one the α-furanose tetraacetate, on the basis of the following evidence: the 1,2-*trans*-furanose (β) should be the more-stable anomer, and hence present in the larger proportion[10]; the coupling constant for the 1,2-*trans*-furanose should be in the range[14] 1–2 Hz, consistent with the broad singlet at δ 6.2; and the *cis*-anomer is expected[14] to have $J = 3$–4 Hz, consistent with the doublet at δ 6.35. The chemical-shift data for H-1 are also consistent with these assignments. Angyal and Pickles found[14] that the 1,2-*cis* anomer generally resonates downfield of the *trans* anomer.

These n.m.r. assignments for the fucofuranose tetraacetates are supported by the chemical shifts of the 3-proton doublet for H-6. The *C*-methyl-group region of the mixture shows a major doublet at δ 1.28, $J = 6.5$ Hz, and a minor doublet at δ 1.20, $J = 6.0$ Hz. Sinclair and Sleeter[15] have shown that the α and β anomers of

20 pairs of 6-deoxypyranose derivatives may be distinguished in this way, as the *C*-methyl group of the α anomer invariably resonates upfield of that of the β anomer. This correlation also appears to hold in the furanose series. Table I compares the methyl resonances for 5 pairs of fucopyranose and 4 pairs of fucofuranose derivatives.

TABLE I

C-METHYL RESONANCES IN FUCOSE DERIVATIVES

Compound	*δ*	*J*	*Solvent*	*Reference*
Methyl α-L-fucopyranoside	1.16	6.5	D_2O	5
Methyl β-L-fucopyranoside	1.25	6.5	D_2O	5
Methyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranoside	1.13	6.5	$CDCl_3$	5
Methyl 2,3,4-tri-*O*-acetyl-β-L-fucopyranoside	1.23	6.5	$CDCl_3$	5
2,3,4-Tri-*O*-acetyl-α-L-fucopyranose	1.15	6.5	$CDCl_3$	2
2,3,4-Tri-*O*-acetyl-β-L-fucopyranose	1.22	6.5	$CDCl_3$	2
1,2,3,4-Tetra-*O*-acetyl-α-L-fucopyranose	1.14	6.5	$CDCl_3$	5
1,2,3,4-Tetra-*O*-acetyl-β-L-fucopyranose	1.23	6.5	$CDCl_3$	5
α-L-Fucopyranosyl phosphate	1.17[a]	7.0	D_2O	2, 5
β-L-Fucopyranosyl phosphate	1.29[a]	7.0	D_2O	2, 5
Methyl α-L-fucofuranoside	1.17	6.0	D_2O	this paper[b]
Methyl β-L-fucofuranoside	1.22	6.0	D_2O	this paper[b]
Methyl 2,3,5-tri-*O*-acetyl α-L-fucofuranoside	1.23	6.0	$CDCl_3$	this paper[c]
Methyl 2,3,5-tri-*O*-acetyl β-L-fucofuranoside	1.30	7.0	$CDCl_3$	this paper[c]
1,2,3,5-Tetra-*O*-acetyl-α-L-fucofuranose	1.20	6.0	$CDCl_3$	this paper[d]
1,2,3,5-Tetra-*O*-acetyl-β-L-fucofuranose	1.28	6.5	$CDCl_3$	this paper[d]
α-L-Fucofuranosyl phosphate	1.10	7.0	D_2O	this paper
β-L-Fucofuranosyl phosphate	1.23	7.0	D_2O	this paper

[a]Refs. 2 and 5 report chemical shifts 0.5 p.p.m. downfield of these values because of referencing to external tetramethylsilane[15a]. [b]Other resonances: 3.38 (s, 3, OMeα); 3.38 (s, 3, OMeβ), 4.85(d, 1, J = 4 Hz, H-1α);) 4.90 (br. s, 1, H-1β). [c]Other resonances: 3.40 (s, 3, OMeα); 3.40 (s, 3, OMeβ); 5.6 (m, H-1α); 5.7, H-1β. [d]Other resonances 6.35 (d, 1, J = 4 Hz, H-1α); 6.2 (br s, 1, H-1β).

The observed specific rotation of the mixed L-fucofuranose tetraacetates was +30°, $[M]_D$ = +10,000°. This is consistent with the expected rotation for an 80–90% β-furanose/10–20% α-furanose mixture on the assumption that the same change ($\Delta[M]_D$) in $[M]_D$ takes place in passing from the pyranose to the furanose form in the fucose as in the galactose series[16] ($\Delta[M]_D$ α-D-*galacto* = −18,000; $\Delta[M]_D$ β-D-*galacto* = −26,000°). As $[M]_D$ for α-L-fucopyranose tetraacetate is −37,500° and that for β-L-fucopyranose tetraacetate is −13,900° (refs. 5 and 11), the calculated values for the α- and β-L-fucofuranose tetraacetates are −19,500 and +13,000°, respectively. An alternative calculation, using +1600° for the change from hydroxymethyl to methyl, gives substantially the same figures (−22,000 and +18,000°, respectively)*.

*Lemieux and Martin[17] have shown that this change is about 3000 in the pyranose series. We calculated 1600° for the furanose series as follows. Ethyl β-D-galactofuranoside has[18a] $[M]_D$ = −21,200. The value calculated for methyl-β-D-galactofuranoside is −19,200 from the difference between the methyl and ethyl glucofuranosides[18b] Methyl-β-L-fucofuranoside has $[M]_D$ = +20,800° (ref. 19).

MacDonald fusion of the fucose tetraacetates. — *A. With β-L-fucopyranose tetraacetate.* When β-L-fucopyranose tetraacetate[2] was fused with phosphoric acid for 90 min (a 5-min fusion gives substantially the same results), the α-L-fucopyranosyl phosphate was the only product detectable after conventional isolation[6]. On the other hand, fusion for 1 min produced a mixture of the α- and β-pyranosyl phosphates in the approximate ratio 9:1*. Quantitative estimation of the α:β ratio was made from the rotation of a sample of known content of sugar phosphate. The two components were distinguished as separate spots by electrophoresis at pH 6.5 (ref. 2). Paper chromatography in solvent systems A and B gave a single spot. This result excludes the presence of furanose phosphate, as solvent B separates the furanose and pyranose phosphates (see the next section). When the appearance of orthophosphate was monitored during acid-catalyzed hydrolysis, a linear, semi-log plot was found, as expected for the mixture of the two pyranosyl phosphates, as their rate constants differ[2] by only about 10%.

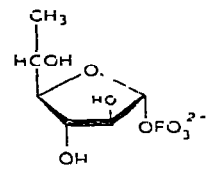
B. With the mixed L-fucose tetraacetates. When the mixed tetraacetates, containing about 65% β-pyranose, 20% α-pyranose and α-furanose, and 15% β-furanose, were fused with anhydrous phosphoric acid for 5 min or longer at 50°, only the α-pyranosyl and mixed furanosyl phosphates were formed. Fusion for 1 min produced a small quantity of the β-pyranosyl phosphate as well. The rate of appearance of orthophosphate during acid-catalyzed hydrolysis was nonlinear, consistent with a furanose–pyranose mixture. Both electrophoresis at pH 6.5 and paper chromatography in solvent B of the 1-min fusion mixture gave two spots having mobilities corresponding to those given under A and C. The mixed barium salts were examined by ^{31}P-n.m.r. spectroscopy. Both the 1- and 5-min fusions showed a single, broad resonance at δ −2.14 p.p.m. The widths at half-height were 15–20 Hz consistent with a mixture. An authentic sample of β-L-fucopyranosyl phosphate (dicyclohexylammonium salt) gave a single resonance at δ −2.18 p.p.m. width at half-height = 10 ±2 Hz.

C. With L-fucofuranose tetraacetates. — L-Fucofuranosyl phosphate free of pyranosyl phosphate was made by a MacDonald fusion of a mixture of fucofuranose tetraacetates containing about 80% of the β and 20% of the α anomer. Processing yielded the barium salt as the trihydrate in about 13% yield. The precipitations needed to be carried out with special care, as the solubility of the barium salt is close to that of barium acetate. The product showed a single spot on electrophoresis at pH 6.5, and a single spot upon chromatography in solvent B, R_{Pi} 1.57, well separated from a standard of the pyranosyl phosphate, R_{Pi} 1.17. Aspinall *et al.*[20] had previously shown the utility of this solvent for separating the arabinofuranosyl and pyranosyl phosphates. The furanosyl phosphates reacted slowly with periodate, consuming 1 mole of periodate per mole of sugar phosphate during 88 h at 25° and pH 4.3. Hydrolysis in 0.3M hydrochloric acid at 100° for 20 min gave reducing sugar and inorganic phosphate in yields of 100 ±5%. The rotation, $[M]_D$ +5680°, is consistent

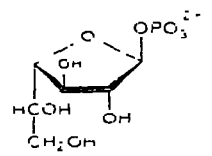
*Fusion with adequate mixing for less than one min is difficult because of the viscosity of the melt.

with a β/α ratio of about 2.4 on the basis of the calculations described in the next section. This ratio was confirmed by the ^{31}P n.m.r. spectrum, which showed two partially resolved resonances at δ −2.22 and −1.735 p.p.m., in the ratio 1:2. The widths at half-height were <10 Hz. The proton n.m.r. spectrum showed a broad doublet in the H-1 region (δ 5.46, $J = 6$ Hz) consistent with P–H coupling for the β anomer. A small multiplet at δ 5.2 was assigned to the α anomer*. The analytical sample did not contain enough of the α anomer to resolve satisfactorily the expected methyl doublet. Other samples, however, showed an unequal, four-line pattern in the methyl region with the assigned resonances δ 1.10 (α) and 1.23 (β). (See Table I.)

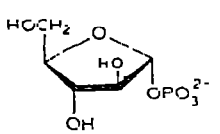
Rotations of the furanose phosphates. — The rotations expected for the fucofuranosyl phosphates have been calculated in two independent ways. Putman and Hassid[21] have shown that the 2A value for several pyranosyl phosphates is about 25,500°. The data of Khorana and coworkers in the *ribo* series suggest that this value is not very different for the furanoses[22,23]. Although the 2B value for the pyranosyl phosphates is not the same as the 2B value for the methyl pyranosides[21], data for the ribofuranoses[18,22,23] (the only sugars for which comparative data exist) suggest that the 2B value in the furanose series is about the same for the phosphates as for the glycosides. Thus, using the 2B value taken from the methyl fucofuranosides[19] (−1000°), we arrive at calculated values of $[M]_D$ for the L-fucofuranosyl phosphates of about −12,000 (α, −A−B) and +13,000° (β A−B). These values are confirmed by an independent calculation from the arabinofuranosyl phosphates (Chart I). Wright and Khorana[24] gave $[M]_D$ +20,500° for methyl α-D-arabinofuranoside. Methyl β-L-fucofuranoside has $[M]_D$ +20,800° (ref. 19). Thus, the addition of the methyl group has little effect on $[M]_D$. No arabinofuranosyl phosphate has been obtained with certain anomeric purity, but Wright and Khorana[24] reported



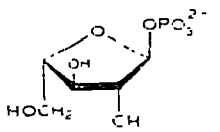
β-L-fucofuranosyl phosphate



β-D-galactofuranosyl phosphate



α-D-arabinofuranosyl phosphate



α-L-arabinofuranosyl phosphate

*The ratio of the proton resonances could not be determined very precisely because of baseline noise. It was between 2.5 and 4 to 1.

that a mixture of the anomeric D-arabinofuranosyl phosphates in which the α anomer predominated had $[M]_D = 2800°$, whereas Aspinall *et al.*[20] reported that a sample of L-arabinofuranosyl phosphate that was predominantly the α anomer had $[M]_D = -12{,}000°$. Thus β-L-fucofuranosyl phosphate must have $[M]_D$ in the vicinity of $+12{,}000°$.

These calculations also imply that Chittenden's value[10] for the rotation of β-D-galactofuranosyl phosphate is in error, as the expected value on the basis of the foregoing arguments is about $-14{,}000°$.

EXPERIMENTAL

General methods. — Paper chromatography was performed by the descending technique with the following solvents (v/v): (A) 7:1:2 2-propanol–conc. ammonium hydroxide–water[22], (B) 3:3:1:3 1-butanol–pyridine–conc. ammonium hydroxide water (containing 0.01% EDTA)[20], (C) 7:3 ethanol–M aqueous ammonium acetate (pH 7.5)[25]. Sugars were revealed by the silver nitrate dip-procedure[26] and sugar phosphates by the Hanes–Isherwood reagent[27]. T.l.c. was carried out on glass plates coated with silica gel G by using either solvent D (3:7 benzene–ethyl acetate[28]) or E (butanone saturated with water)[29]. Paper electrophoresis was conducted on Schleicher and Schuell No. 589 Orange Ribbon paper, 58-cm strips, in 0.05M sodium maleate buffer, pH 6.2 or 6.5, at about 5000 volts with a Savant Model HV 8000 apparatus. Periodate consumption was measured spectrophotometrically[30]. Reducing sugar, total phosphate, and inorganic phosphate were measured by the methods of Park and Johnson[31a], Fiske and Subbarow[31b], and Lowry and Lopez[31c], respectively. Reactions with *N,N'*-dicyclohexylcarbodiimide were carried out according to the procedure of Tener *et al.*[23]. Rotations of the sugar phosphates were measured with a precision of 0.002° by using a Perkin-Elmer 241 instrument. Other rotations were measured using a Bausch and Lomb saccharimeter with a precision of ±0.2°. ^{31}P-N.m.r. spectra (^1H broad-band decoupled) were obtained on a Bruker-HFX-5 (36.43 MHz) instrument. The reference signal was orthophosphoric acid (0 p.p.m.). Signals downfield of this reference are given negative chemical shifts. Ratios of the areas under the peaks were taken as measures of the proportions of the various types of phosphates[32] (Costello *et al.*[32] also gave data on ^{31}P spectra of three other glycosyl phosphates.) P.m.r. spectra were recorded with a Varian T-60 instrument at 35° with tetramethylsilane as internal standard in organic solvents and the HDO resonance at δ 4.67, the acetate ion resonance at δ 1.92, or acetone at δ 2.0 as the reference in D_2O.

Tetra-O-acetyl-L-fucose. — A mixture of acetic anhydride (40 ml) and anhydrous sodium acetate (4.5 g) was heated to boiling, and L-fucose (10 g, Pfanstiehl) was added in small portions. The exothermic reaction required no heating to maintain reflux during the addition. After the final addition of fucose, the mixture was heated briefly to a full boil. The solution was then cooled and poured in a thin stream into 500 ml of ice–water with stirring. The solution was extracted with chloroform

(3 × 50 ml), and the extract was dried (sodium sulfate) and then evaporated *in vacuo* to a syrup; yield 12 g (59%), $[\alpha]_D^{25}$ −27° (chloroform) that failed to crystallize from water, ethanol, ether, ethyl acetate, isopropyl alcohol, or petroleum ether. This material showed two spots (R_F 0.53 and 0.60) upon t.l.c. with solvent D, corresponding in mobility to the furanose and the pyranose tetraacetates, respectively[28].

1,2,3,4-Tetra-O-acetyl-β-L-fucopyranose was prepared by the method of Prihar and Behrman[2].

1,2,3,4-Tetra-O-acetyl-α-L-fucopyranose was prepared by low-temperature acetylation of L-fucose with acetic anhydride–pyridine[5,12].

L-*Fucose diethyl dithioacetal* was synthesized in 59% yield following the procedure of Wolfrom[33] for the galactose derivative; m.p. 166–167° (lit.[34] 167–168.5°).

L-*Fucofuranose tetraacetates*. — Methyl β-L-fucofuranoside was synthesized and isolated by the method of Gardiner and Percival[19], $[\alpha]_D^{25}$ +117° (lit.[19,29] $[\alpha]_D^{18}$ +112°) or, in better yield, from L-fucose diethyl dithioacetal. The procedure of Green and Pacsu[35] for methyl β-D-galactofuranoside yielded 2.6 g of the mixed methyl fucosides as a syrup from 4.0 g of L-fucose diethyl dithioacetal. Separation on a cellulose column[19] gave 1.97 g of methyl β-L-fucofuranoside. The proportions of the four glycosides prepared from the thioacetal and by both high- and low-temperature direct glycosidation are compared in Table II.

TABLE II

PROPORTIONS OF THE METHYL FUCOSIDES (PERCENT)

Product	*From the thioacetal*[a]	*Low-temperature glycosidation*[b]	*High-temperature glycosidation*[c]
β-Furanoside	82	44	13
α-Furanoside	9	21	6
α-Pyranoside	8	15	54
β-Pyranoside	1	20	27

[a]This paper. [b]Ref. 19. [c]Ref. 36.

Methyl β-L-fucofuranoside was acetylated to yield the crystalline methyl 2,3,5-tri-*O*-acetyl-β-L-fucofuranoside, $[\alpha]_D^{25}$ +73° in methanol (lit.[13] $[\alpha]_D^{20}$ +75.5°). The methyl triacetate was treated[10] with sulfuric acid and acetic anhydride to yield an anomeric mixture (80% β, 20% α) of the previously unreported L-fucofuranose tetraacetates, $[\alpha]_D^{25}$ +30° (*c* 0.6, chloroform). This mixture migrated as a single component in a t.l.c. system[28] (solvent D) that separates the fucofuranose (R_F 0.53) and fucopyranose (R_F 0.6) tetraacetates.

Methyl α-L-fucofuranoside was also isolated by the method of Gardiner and Percival[19]. It was shown to be free of the β-furanoside and the two pyranosides by t.l.c. on silica gel in solvent E (R_F values: β-furanoside 0.58; α-furanoside 0.41; α-pyranoside 0.26; β-pyranoside 0.11)[29]. Acetylation of this material with acetic anhydride and pyridine at room temperature gave the sample of methyl 2,3,5-tri-*O*-acetyl-α-L-fucofuranoside, whose n.m.r. characteristics are given in Table I.

MacDonald fusion reactions. — A. With the mixed fucose tetraacetates (pyranose and furanose). Crystalline phosphoric acid (2 g), which had been dried *in vacuo* over phosphorus pentaoxide, was melted in a 50-ml flask at 50°. The mixture of fucose tetraacetates [$(\alpha)_D$ −27°, 2 g) was added. The mixture was kept for 1 min at 50° and then cooled in ice. Ice-cold 2M lithium hydroxide was added (40 ml) with good mixing. After 1 h at room temperature, the precipitated lithium phosphate was filtered off. The pH of the clear, supernatant solution was lowered to 8.5 by using Dowex-50W (H^+) resin. The resin was removed by filtration. Barium acetate (1.4 g) was added and the solution concentrated to about 15 ml. Barium salts were precipitated by the addition of ethanol (60 ml) followed by acetone (20 ml). The salts were collected by centrifugation after 16 h at 4°, washed with alcohol and acetone, and air dried. These salts were dissolved in 10 ml of water and reprecipitated as before. This material (270 mg) had $[\alpha]_D$ −46° (*c* 1, water). The salts were converted into the cyclohexylammonium form by passage through a column of Dowex-50W (H^+) resin at 4° and neutralization of the effluent with cyclohexylamine. Concentration of this effluent and the addition of 12 parts of acetone gave crystals of bis(cyclohexylyammonium) α-L-fucopyranosyl phosphate, $[\alpha]_D$ −67° (*c* 1, water). The mother liquors were evaporated to a syrup. Electrophoresis of an aliquot at pH 6.5 showed two spots having $R_{Picrate}$ 1.45 (minor) and 1.54 (major). Paper chromatography in solvent B also revealed two spots having R_{P_i} 1.17 (minor) and 1.57 (major). The material that migrated more rapidly in paper chromatography was eluted and concentrated. In acetate buffer, pH 4.3, it consumed one mole of periodate per mole of sugar phosphate during 2 days. Under identical conditions, α-L-fucopyranosyl phosphate consumed 2 moles of periodate per mole of sugar phosphate in 3 h.

B. With β-L-fucopyranose tetraacetate. A sample of β-L-fucopyranose tetraacetates (1 g) was mixed with 1 g of anhydrous phosphoric acid on a rotary evaporator *in vacuo* for one min at 50°. Following the addition of cold, 2M lithium hydroxide (20 ml), the mixture was processed as in (A) to give the barium salt (100 mg, $[\alpha]_D$ −74°). Electrophoresis at pH 6.5 showed two spots having $R_{Picrate}$ 1.45 (major) and 1.54 (minor, ~15%). In contrast to (A), however, paper chromatography gave a single spot in solvents A, B, and C. Another sample was fused with phosphoric acid for 90 min and processed similarly. It showed only one spot on electrophoresis as already described, $R_{Picrate}$ 1.45. The α/β ratio (9) was determined on a sample of the barium salt contaminated by barium acetate but free from inorganic phosphate. The quantity of fucosyl phosphate was determined by measurement of the phosphate released following hydrolysis in 10mM hydrochloric acid for 20 min at 100° (sealed capillary). The $[\alpha]_D$ values were calculated on the basis of these determinations. The 1-min fusion sample had $[\alpha]_D$ −74° and the 90-min sample $[\alpha]_D$ −82°.

C. With L-fucofuranose tetraacetates. A sample of the mixed fucofuranose tetraacetates (725 mg) and 1 g of anhydrous phosphoric acid were mixed, and kept for 30 min at 55°. The mixture was cooled. Cold, 2M lithium hydroxide was added to give a pH of 11. This pH was maintained for 12 h by the periodic addition of lithium hydroxide solution. Trilithium phosphate 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* (25–30°) to about 5 ml. Barium acetate (0.58 g) was added, followed by 30 ml of ethanol. After 2 h at 0°, the precipitate was collected by centrifugation and washed with ethanol and ether. The product was dissolved in 4 ml of water, traces of insoluble material were removed by centrifugation, and the clear solution was added to 30 ml of ethanol. The precipitate was again collected by centrifugation and the precipitation cycle repeated once more. The final product was washed with 60% ethanol, acetone, and ether. It was then dried *in vacuo* over phosphorus pentaoxide overnight at room temperature to give the trihydrate of the barium salt; yield 120 mg (13%); $[\alpha]_D^{25}$ +13° (*c* 0.2, water).

Anal. Calc. for $C_6H_{11}BaO_8P \cdot 3H_2O$: C, 16.63; H, 3.92; P, 7.16. Found: C, 16.62; H, 3.81; P, 6.91.

The hydration was confirmed by showing a loss of weight equivalent to $3H_2O$ per molecule upon drying *in vacuo* over phosphorus pentaoxide for 3 days at 56°.

A preliminary account of some of this work has been published[37].

ACKNOWLEDGMENTS

We thank Prof. N. J. Lewis for the use of the Perkin–Elmer polarimeter, and the Department of Chemistry, The Ohio State University for the ^{31}P-n.m.r. spectra. This work was supported by NIH Grant GM-20002.

REFERENCES

1 H. ISHIHARA, D. J. MASSARO, AND E. C. HEATH, *J. Biol. Chem.*, 243 (1968) 1103–1109.
2 H. S. PRIHAR AND E. J. BEHRMAN, *Biochemistry*, 12 (1973) 997–1002.
3 D. L. MACDONALD, *Methods Carbohyar. Chem.*, 6 (1972) 389–392.
4 F. SCHANBACHER AND D. R. WILKEN, *Biochim. Biophys. Acta*, 141 (1967) 646–649.
5 D. H. LEABACK, E. C. HEATH, AND S. ROSEMAN, *Biochemistry*, 8 (1969) 1351–1359.
6 D. L. MACDONALD, *Carbohydr. Res.*, 3 (1966) 117–120.
7 D. L. MACDONALD, *J. Org. Chem.*, 27 (1962) 1107–1109
8 L. J. HAYNES AND F. H. NEWTH, *Adv. Carbohydr. Chem.*, 10 (1955) 207–256.
9 B. CAPON, *Chem. Rev.*, 69 (1969) 407–498, tables LII, LIV, and LXII.
10 G. J. F. CHITTENDEN, *Carbohydr. Res.*, 25 (1972) 35–41.
11 O. WESTPHAL AND H. FEIER, *Chem. Ber.*, 89 (1956) 582–588.
12 G. A. LEVVY AND A. MCALLAN, *Biochem. J.*, 80 (1961) 433–435.
13 O. KJØLBERG AND O. J. TJELTVEIT, *Acta Chem. Scand.*, 17 (1963) 1641–1645.
14 S. J. ANGYAL AND V. A. PICKLES, *Aust. J. Chem.*, 25 (1972) 1695–1710, 1711–1718.
15 H. B. SINCLAIR AND R. T. SLEETER, *Tetrahedron Lett.*, (1970) 833–836.
15(a) K. G. R. PACHLER, E. B. RATHBONE, AND A. M. STEPHEN, *Carbohydr. Res.*, 47 (1976) 155–157.
16 C. S. HUDSON AND J. M. JOHNSON, *J. Am. Chem. Soc.*, 38 (1916) 1223–1228.
17 R. U. LEMIEUX AND J. C. MARTIN, *Carbohyar. Res.*, 13 (1970) 139–161.
18 (a) J. W. GREEN AND E. PACSU, *J. Am. Chem. Soc.*, 59 (1937) 1205–1210; (b) W. N. HAWORTH, C. R. PORTER, AND A. C WAINE, *J. Chem. Soc.*, (1932) 2254–2258.
19 J. G. GARDINER AND E. PERCIVAL, *J Chem. Soc.*, (1958) 1414–1418.
20 G. O. ASPINALL, I. W. COTRELL, AND N. K. MATHESON, *Can. J. Biochem.*, 50 (1972) 574–580.
21 E. W. PUTMAN AND W. Z. HASSID, *J. Am. Chem. Soc.*, 79 (1957) 5057–5060.
22 R. S. WRIGHT AND H. G. KHORANA, *J. Am. Chem. Soc.*, 78 (1956) 811–816.
23 G. M. TENER, R. S. WRIGHT, AND H. G. KHORANA, *J. Am. Chem. Soc.*, 79 (1957) 441–443.
24 R. S. WRIGHT AND H. G. KHORANA, *J. Am. Chem. Soc.*, 80 (1958) 1994–1998.

25 A. C. PALADINI AND L. F. LELOIR, *Biochem. J.*, 51 (1952) 426–430.
26 W. E. TREVELYAN, D. P. PROCTER, AND J. S. HARRISON, *Nature*, 166 (1950) 444–445.
27 R. S. BANDURSKI AND B. AXELROD, *J. Biol. Chem.*, 193 (1951) 405–410.
28 J. O. DEFERRARI, R. M. DE LEDERKREMER, B. MATSUHIRO, AND J. F. SPROVIERO, *J. Chromatogr.*, 9 (1962) 283–290.
29 W. M. WATKINS, *J. Chem. Soc.*, (1955) 2054–2055.
30 D. H. RAMMLER AND J. C. RABINOWITZ, *Anal. Biochem.*, 4 (1962) 116–123.
31 (a) G. ASHWELL, *Methods Enzymol.*, 3 (1957) 86; (b) L. F. LELOIR AND C. E. CARDINI, *ibid.*, 843; (c) L. F. LELOIR AND C. E. CARDINI, *ibid.*, 845.
32 A. J. R. COSTELLO, T. GLONEK, M. E. SLODKI, AND F. R. SEYMOUR, *Carbohydr. Res.*, 42 (1975) 23–37.
33 M. L. WOLFROM, *J. Am. Chem. Soc.*, 52 (1930) 2464–2473.
34 E. VOTOČEK AND V. VESELÝ, *Chem. Ber.*, 47 (1914) 1515–1519.
35 J. W. GREEN AND E. PACSU, *J. Am. Chem. Soc.*, 60 (1938) 2056–2057.
36 D. F. MOWERY, JR., *Carbohydr. Res.*, 43 (1975) 233–238.
37 H. S. PRIHAR, S. R. WANAMAKER, AND E. J. BEHRMAN, *Abstr. Papers Amer. Chem. Soc. Meeting*, 170 (1975) CARB-51.