

Preparation and Properties of Crystalline Acetochlorofucose and Its Conversion into the β -Glycosides and α -1-Phosphate of L-Fucose*

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ABSTRACT: The preparation and properties of aceto-chloro- α -L-fucose and its use in the synthesis of L-fucopyranosides of potential biological interest have been described. Properties of α - and β -L-fucopyranosides have been examined and the relationship of the chemically synthesized α -L-fucose 1-phosphate to the corresponding biosynthetic material (the β anomer) is

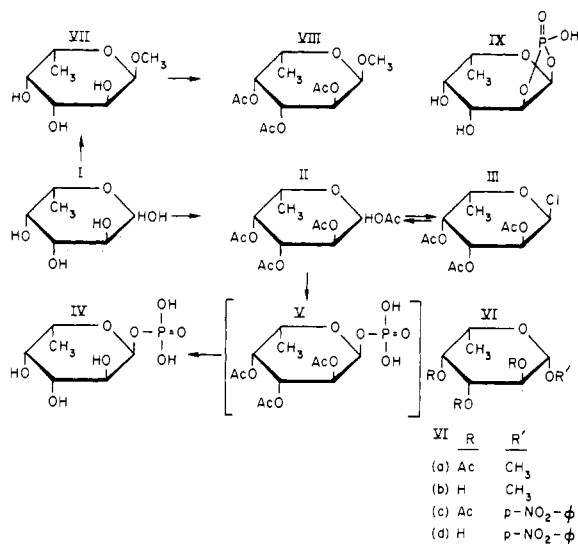
discussed. Some techniques for the examination, estimation, and isolation of relatively small quantities of sugar phosphates are described, together with some observations on the use of Ag_2HPO_4 in the synthesis of sugar phosphates. Nuclear magnetic resonance studies indicate that the expected *1C* conformation is assumed by derivatives of L-fucose in solution.

FL-fucose (6-deoxy-L-galactose) is an important constituent of many glycoproteins and oligosaccharides (Gottschalk and Graham, 1966). Studies on the biosynthesis of fucose-containing macromolecules have been hampered by the unavailability of substrate quantities of suitably labeled nucleotide derivatives such as GDP-fucose- ^{14}C . The latter compound can be made enzymatically (Ginsburg, 1961), but only in limited quantities. A study was therefore undertaken on some basic chemistry of L-fucose with the aim of preparing its glycosides and 1-phosphate esters.

There have been few reports on the synthesis of the acetohalogeno derivatives of L-fucose. Thus, an unstable, syrupy acetobromo-L-fucose was employed in the preparation of 3,4-di-*O*-acetyl-L-fucal (Iselin and Reichstein, 1944), of *p*-nitrophenyl 2,3,4-tri-*O*-acetyl- β -L-fucopyranoside (Levy and McAllan, 1961), of 2-*O*- α -L-fucopyranosyl-L-fucopyranose (Flowers *et al.*, 1967), and of 2-*O*- α -L-fucopyranosyl-D-galactose (Levy *et al.*, 1967). A partially characterized acetochloro-L-fucose was briefly reported (Heath and Elbein, 1962). In the present studies, crystalline acetochlorofucose was synthesized and used for the preparation of compounds of potential biochemical interest.

Acetylation of L-fucose by conventional methods gave the known derivative, 1,2,3,4-tetra-*O*-acetyl- α -L-fuco-

SCHEME I



pyranose (IIa) (Scheme I), and/or uncrystallizable mixtures of fucose acetates. While the crystalline α -tetraacetate (IIa) was converted into the desired crystalline acetochlorofucose (III) by either TiCl_4 in chloroform or by an acetic acid-HCl reagent, crystalline III was not obtained with an acetyl chloride-acetic acid reagent unless tetraacetate mixtures containing a high proportion of the β anomer were employed. These observations are in accord with the expectation that the 1,2-*trans*-acetate is the more labile isomer (Lemieux, 1954).¹ The crystalline chloro compound could also be obtained by treatment of the mixed tetraacetates with

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¹ Similar findings with the anomeric pentaacetates of 2-amino-2-deoxy-D-glucose suggest that the acetic acid-acetyl chloride reagent may serve as a diagnostic test for 1,2-*trans*-acetates (D. H. Leaback, unpublished results).

TABLE I: Optical Rotation of Derivatives of L-Fucopyranose.

Derivative of L-Fucopyranose	$[\alpha]_D$ (deg)	$[M]_D$ (deg)	Solvent	Ref
α -1,2,3,4-Tetra- <i>O</i> -acetate (IIa)	-130	-43,300	Acetone	<i>a</i>
	-138	-45,800	Acetone	<i>b</i>
	-113	-37,500	Chloroform	<i>b</i>
	-116	-38,500	Chloroform	<i>c</i>
β -1,2,3,4-Tetra- <i>O</i> -acetate (IIb)	-39	-12,900	Chloroform	<i>d</i>
Acetochlorofucose (III)	-215	-66,300	Chloroform	<i>c</i>
α -Methyl 2,3,4-tri- <i>O</i> -acetate glycoside (VIII)	-149	-43,110	Chloroform	<i>e</i>
	-152	-46,200	Chloroform	<i>c</i>
β -Methyl 2,3,4-tri- <i>O</i> -acetate glycoside (VIa)	+7.1	+2,160	Chloroform	<i>e</i>
	+8	+2,430	Chloroform	<i>c</i>
α -Methyl glycoside (VII)	-197	-35,070	Water	<i>e</i>
	-200	-35,600		<i>c</i>
β -Methyl glycoside (VIb)	+14.2	+2,530	Water	<i>e</i>
Dicyclohexylammonium α -1-phosphate (IV)	-77.8	-34,400	Water	<i>c</i>
Cyclohexylammonium bis(α -L-fucose 1-)-phosphate (Xb)		-72,000	Water	<i>c</i>
α - <i>p</i> -Nitrophenyl 2,3,4-tri- <i>O</i> -acetyl	-222	-91,200	Chloroform	<i>d</i>
	-228	-93,700	Chloroform	<i>b</i>
β - <i>p</i> -Nitrophenyl 2,3,4-tri- <i>O</i> -acetyl (VIc)	+1.5	+6,600	Acetone	<i>b</i>
	+2	+8,800	Acetone	<i>c</i>
α - <i>p</i> -Nitrophenyl glycoside	-317	-90,350	Acetone	<i>b</i>
β - <i>p</i> -Nitrophenyl glycoside (VID)	+100	+28,500	Ethanol	<i>b</i>
	+95	+27,100	Ethanol	<i>c</i>

^a Iselin and Reichstein (1944). ^b Levvy and McAllan (1961). ^c Present work. ^d Westphal and Feier (1956). ^e Hockett *et al.* (1939).

TABLE II: 60-MHz Proton Magnetic Resonance Spectral Data.

L-Fucopyranose Derivatives	Methoxyl Protons	C-1 Proton (Doublet)		C-2, C-3, C-4 Protons		C-5 Proton (Quartet)		C-6 Protons (3 Doublets)	
	(3 Singlets)	τ	J (cps)	τ		τ	J (cps)	τ	J (cps)
Acetochlorofucose (III)		3.66	3.5	4.55-4.75		5.6	6.5	8.83	6.5
α -Tetraacetate (IIa)		3.70	3	4.7 -4.75		5.7	6.5	8.86	6.5
β -Tetraacetate (IIb) ^a		4.27	7	4.72-4.82		5.95	6.5	8.77	6.5
α -Methyl triacetate (VIII)	6.6	5.08	3.5	4.72-4.85		5.85	6.5	8.87	6.5
β -Methyl triacetate (VIa)	6.5	5.62	7	4.80-4.92		6.1	6.5	8.77	6.5
α -Methyl glycoside (VII) ^b	6.65	5.06	3.5			5.82	6.5	8.84	6.5
β -Methyl glycoside (VIb) ^b	6.5	5.74	7			6.05	6.5	8.75	6.5

^a Conducted on syrup containing some IIa. ^b In D₂O solution; other derivatives in CDCl₃.

anhydrous HCl, followed by chromatography on silicic acid. For analytical purposes, the silicic acid column method (*cf.* Finan and Warren, 1962) was modified to a thin-layer method which proved useful for examining the purity of acetochlorofucose and other acetylated fucose derivatives (Figure 1).

Application of the Hassel-Ottar principle (Lemieux, 1964) predicts an α configuration for the stable acetochlorofucose. This prediction is consistent with the optical rotation of the derivative (Table I), and also with the reaction of the latter with methanol-silver carbonate and alkaline solutions of *p*-nitrophenol to give the corresponding β -glycosides.

In contrast to the results obtained with methanol and *p*-nitrophenol, treatment of acetochlorofucose with a variety of phosphorylating agents generally gave α -L-fucose 1-phosphate derivatives. The same results were obtained by treatment of the α -tetraacetate with anhydrous phosphoric acid (MacDonald, 1962). Possible reasons for these results are discussed below.

Materials and Methods

All materials were reagent grade commercial samples, unless otherwise specified. Solutions were con-

centrated under vacuum at bath temperatures approximating 37°. Capillary tubes were used to obtain the melting point, uncorrected. Activated silicic acid, 200–325 mesh called Unisil (Clarkson Chemical Co.), was employed without pretreatment for column chromatography. Infrared spectra were determined on KBr pellets with a Perkin-Elmer 137 Infracord spectrometer. Nuclear magnetic resonance spectra (60 and 100 MHz) were determined at ambient temperature in Varian A-60 and HA-100 spectrometers, respectively, using D₂O and CDCl₃ as solvents, and the chemical shifts were compared with signals from tetramethylsilane. Elemental analyses were performed by the Spang Microanalytical Laboratories, Ann Arbor, Mich.

Fucose (anthrone), P_i, and total phosphate were estimated by the methods of Carroll (1960), Lowry and Lopez (1946), and Chen *et al.* (1956), respectively.

Chromatographic and Electrophoretic Procedures. Paper chromatography was performed with Whatman No. 1 paper in a solvent system containing isopropyl alcohol–80% aqueous ammonia–water (7:1:2, v/v).

Thin-layer chromatography was conducted on either 20 × 20 cm² glass plates or microscope slides, using silica gel G as the adsorbent, and developed with diisopropyl ether.

Paper electrophoresis was conducted with Whatman No. 3MM paper for 30 min at 65 V/cm in a pH 6.4 buffer system containing the following components: pyridine–acetic acid–water (180:7.2:1800, v/v). Under these conditions, P_i migrated approximately 37 cm from the origin. After drying (105° for 10 min with efficient air circulation), the pyridine salts of the phosphate esters were located by the fact that they “quenched” the background fluorescence in ultraviolet light.

The following spray reagents were employed to locate the indicated substances: alkaline silver nitrate (Trevelyan *et al.*, 1950) for reducing substances; acid molybdate (Bandurski and Axelrod, 1951) followed by development under ultraviolet light for inorganic and ester phosphates; a hydroxylamine–ferric chloride reagent (Tate and Bishop, 1962) for sugar acetates.

1,2,3,4-Tetra-O-acetyl-L-fucopyranose (II). (a) Dry L-fucose (5 g) was added over 15 min to a stirred mixture of acetic anhydride (42 ml) and dry pyridine (54 ml) at 0°. After stirring for 48 hr at 0–5°, the mixture was poured onto crushed ice (about 250 g), stirred at 0° for an additional 4 hr, and extracted with chloroform (four times with 100-ml portions). The combined chloroform extracts were washed with water (six 300-ml portions), dried over CaCl₂, and concentrated to a colorless syrup which was crystallized from ethanol. Recrystallization from ethanol gave 4.6 g (45%) of the tetraacetyl derivative (IIa), mp 93°. The product gave a single discrete spot on thin-layer chromatography (Figure 1A).

Anal. Calcd for C₁₄H₂₀O₉: C, 50.60; H, 6.07. Found: C, 51.05; H, 6.15. λ_{\max} (KBr) 690, 735, 820, 900, 930, 975, 1015, 1040, 1080, 1150, 1240, 1390, 1440, 1650, 1760, and 3050 cm⁻¹; $[\alpha]_D^{20}$ –116° (c 1, chloroform).

(b) Dry L-fucose (20 g) was added over 60 min to a stirred solution of freshly fused ZnCl₂ (8 g) in cold (0°) acetic anhydride (80 ml). After stirring at 0–5° for 24 hr, water was added (160 ml), and the mixture was stirred for an additional 8 hr at 0–5° and then filtered. Following adjustment of the pH of the filtrate to 5.5 with 5% NaOH, it was extracted with chloroform (five portions of 30 ml each); the chloroform extract was washed with water (five 50-ml portions) and dried over CaCl₂. The dried solution was concentrated to a syrup which was readily crystallized from ethanol. Recrystallization from ethanol gave 25.6 g (63%) of material identical in melting point, mixture melting point, chromatographic mobility, and optical rotation with that described above. (c) Acetochlorofucose (1 g; see below), silver carbonate (1 g), and dry acetic acid (20 ml) were shaken in a stoppered flask for 16 hr in the dark. After centrifugation, the residue was washed with chloroform (three 20-ml portions); the combined chloroform supernatant fluids were washed successively with water, saturated NaHCO₃, and water, and dried over CaCl₂. The solution gave a colorless syrup upon concentration (1.2 g), $[\alpha]_D^{20}$ –56° (c 1, chloroform), which failed to crystallize from a wide variety of organic solvents. The product reduced Fehlings and alkaline silver nitrate solutions rather slowly, and gave two spots on thin-layer chromatography (Figure 1C). The nuclear magnetic resonance spectrum of the chief component of this mixture was readily distinguishable from that of the α -tetraacetate (IIa), and was consistent with that expected for the β -tetraacetate (IIb). (d) Acetochlorofucose (1 g) and mercuric acetate (0.58 g) were dissolved in dry acetic acid (15 ml), allowed to stand overnight at room temperature, and dissolved in chloroform (150 ml), and the solution was extracted, dried, and concentrated as described in preparation c above. The product, a syrup (1.5 g), exhibited $[\alpha]_D^{20}$ –54° (c 1, chloroform), but otherwise was similar to the product obtained by method c.

2,3,4-Tri-O-acetyl- α -L-fucopyranosyl Chloride (III).

(a) A solution of crystalline tetraacetate (IIa; 5 g) in dry chloroform (35 ml) was treated with TiCl₄ (3.3 g) in dry chloroform (35 ml), and the mixture was heated under reflux with the exclusion of moisture. After 3.5 hr, the brown solution was added to ice–water (about 100 g), and the chloroform layer was extracted with water (five 50-ml portions), dried over CaCl₂, decolorized with charcoal, and concentrated to a syrup. The latter was dissolved in diethyl ether, and petroleum ether (bp 40–60°) was added to turbidity. After 16 hr at 5°, the crystals were filtered, washed with a small volume of cold water, and dried under vacuum, giving 2.55 g. A second crop (0.5 g) was obtained from the mother liquor by adding more petroleum ether. Recrystallization of the combined fractions from the same solvent system gave 2.75 g (58%) of acetochlorofucose (III) which gave a single discrete spot on thin-layer chromatography (Figure 1E), and showed mp 65°.

Anal. Calcd for C₁₂H₁₇ClO₇: C, 46.68; H, 5.55; Cl, 11.49. Found: C, 46.75, 46.69; H, 5.45, 5.48; Cl, 11.48, 11.69. λ_{\max} (KBr) 660, 695, 755, 825, 880, 915,

970, 1020, 1080, 1110, 1140, 1170, 1230, 1330, 1380, 1460, 1750, and 3050 cm^{-1} ; $[\alpha]_D^{20} -215^\circ$ (c 1, chloroform).

(b) Crystalline tetraacetate IIa (1 g) was dissolved in a dry mixture of acetic acid (15 ml) and acetic anhydride (1 ml), and the solution was saturated with dry HCl at 0° . After storage at room temperature in a stoppered flask for 48 hr, cold chloroform (250 ml) was added; the solution was extracted twice with ice-water, three times with cold saturated NaHCO_3 solution, twice with water, dried over CaCl_2 , decolorized with charcoal, concentrated, and recrystallized as described above to give 0.62 g (68%) of crystalline acetochlorofucose (III); melting point and melting point of an admixture with the preparation described above, 65° .

(c) The mixture of fucose acetates described above (1.1 g of syrup, $[\alpha]_D^{20} -56^\circ$) was dissolved in 5 ml of a mixture of acetyl chloride and dry acetic acid (1:1, v/v). After 16 hr at room temperature, chloroform (100 ml) was added, and the product was isolated as described in method b above. The final product, obtained after two recrystallizations, consisted of acetochlorofucose (III, 0.54 g, 54% yield); mp 65° . When the crystalline tetraacetate was used as starting material (1.1 g), it yielded 0.81 g of unchanged starting material (II); chromatographic examination of the crude product showed little acetochlorofucose.

(d) For the possible preparation of isotopic derivatives, it was desirable to convert fucose into the chloro compound without isolation of the acetate. Thus, anhydrous L-fucose (10 g), anhydrous sodium acetate (5 g), and acetic anhydride (90 ml) were stirred at 100° for 2 hr with the exclusion of moisture.² The solution was cooled to 0° , saturated with dry HCl, and allowed to react for 48 hr at room temperature in a stoppered flask. Chloroform (300 ml) was then added, and the product purified as described under b. Concentration of the chloroform solution gave a syrup which failed to crystallize; chromatographic examination of the syrup indicated the presence of an impurity (Figure 1D). The syrup was dissolved in a minimum quantity of dry diethyl ether, applied to a silicic acid column (14×2.5 cm), and eluted with diisopropyl ether. The first fraction (approximately 40 ml) of effluent was discarded; the movement of the desired fraction through the column could be followed by refractive index effects; using this criterion, the following fraction (about 90 ml) was collected and concentrated to a syrup which crystallized readily. The crude product (mp $62-65^\circ$) was recrystallized once from ether-petroleum ether to give 10.95 g (58%) of acetochlorofucose (III), mp and mmp 65° .

(e) Dry L-fucose (10 g) was added over 60 min to a

² In three separate experiments, such mixtures were purified according to the method of Westphal and Feier (1956), but the products failed to crystallize from isopropyl alcohol, from a variety of other solvents, or after silicic acid chromatography. Thin-layer chromatography (Figure 1B) showed the presence of the α -tetraacetate (IIa), and a material that migrated more slowly, presumably the β anomer (IIb). Levy and McAllan (1961) also reported difficulty in preparing IIb by this method.

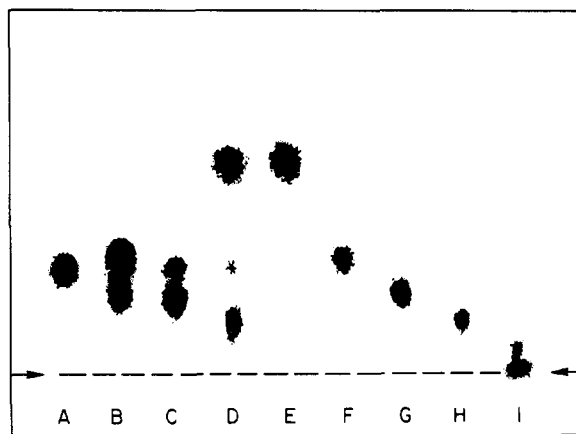


FIGURE 1: Silica gel thin-layer chromatogram of L-fucose derivatives. Samples were placed at the origin (indicated by arrows) as follows: A, α -tetraacetate (IIa); B and C, fucose-acetate mixtures; D, crude acetochlorofucose; E, pure acetochlorofucose; F, α -methyl triacetate (VIII); G, β -methyl triacetate (VIa); H, β -*p*-nitrophenyl triacetate (VIc); I, product of condensation of III with silver dibenzyl phosphate.

stirred solution of anhydrous zinc chloride (4 g) in acetic anhydride (40 ml), and the mixture stirred for an additional 23 hr at $0-5^\circ$. The mixture was then saturated with dry HCl at 0° , and left to react at room temperature in a stoppered flask for 48 hr. Chloroform (300 ml) was added, and the extraction, concentration, chromatography, and crystallization were conducted as described in d to give 9.9 g (52%) of acetochlorofucose (III); mp and mmp 65° .

Methyl 2,3,4-Tri-O-acetyl- β -L-fucopyranoside (VIa). Freshly prepared silver carbonate (4 g) was stirred for 2 hr with anhydrous calcium sulfate (2 g) and dry methanol (40 ml). Acetochlorofucose (2 g) was added, and the mixture was stirred at room temperature with the exclusion of light and moisture. After 24 hr, the mixture was filtered through filter aid and the filtrate (which showed a single spot on thin-layer chromatography, Figure 1G) was concentrated to dryness. The residue was dissolved in benzene (25 ml), filtered to remove small quantities of insoluble material, and concentrated to a residue, and the latter was crystallized from ethanol to give 0.92 g (47%) of methyl 2,3,4-tri-O-acetyl- β -L-fucopyranoside, mp $99-100^\circ$.

Anal. Calcd for $\text{C}_{13}\text{H}_{20}\text{O}_8$: C, 51.30; H, 6.63. Found: C, 51.48; H, 6.65. λ_{max} (KBr) 720, 905, 930, 980, 1060, 1150, 1180, 1240, 1380, 1460, 1640, 1760, 2850, and 3050 cm^{-1} ; $[\alpha]_D^{20} +8^\circ$ (c 1, chloroform).

Methyl β -L-Fucopyranoside (VIb). Methyl tri-O-acetyl- β -L-fucopyranoside (VIa) (0.75 g) was dissolved in dry methanol (9 ml), treated with 1 ml of 1 N sodium methoxide, allowed to stand at room temperature for 16 hr, and evaporated to dryness, and the residue was recrystallized twice from ethanol. The product, 0.39 g (89%), consisted of methyl β -L-fucopyranoside (VIb), mp 123° .

Anal. Calcd for $\text{C}_7\text{H}_{14}\text{O}_5$: C, 47.18; H, 7.92. Found: C, 47.21; H, 8.00. $[\alpha]_D^{20} +15^\circ$ (c 1, water).

p-Nitrophenyl 2,3,4-Tri-*O*-acetyl- β -L-fucopyranoside (VIc). Acetochlorofucose (1 g) was dissolved in 10 ml of cold acetone containing a suspension of 1 g of *p*-nitrophenol. The mixture was vigorously stirred, chilled to 0°, treated with 6.5 ml of 1 N NaOH (added over 30 min), maintained at 5° for 16 hr, and concentrated. The residue was triturated with chloroform (three 30-ml portions) and the combined chloroform extracts were washed with 5% NaOH solution (three 50-ml portions) and water (two 50-ml portions) and dried over CaCl₂. The chloroform solution was concentrated to a residue which crystallized from ethanol; after recrystallization, 0.34 g was obtained (26%). The product, VIc, exhibited mp 165–167°, and gave one spot on thin-layer chromatography (Figure 1H).

Anal. Calcd for C₁₈H₂₁NO₁₀: C, 52.55; H, 5.15; N, 3.41. Found: C, 52.61; H, 5.30; N, 3.41. $[\alpha]_D^{20} + 2^\circ$ (c 1, acetone).

p-Nitrophenyl β -L-Fucopyranoside (VIId). Compound VIc (0.3 g) was dissolved in dry methanol (10 ml), and treated with 2 ml of 1 N sodium methoxide. After 16 hr at room temperature, the solution was concentrated, crystallized, and recrystallized from ethanol; the product (0.17 g, 88%), *p*-nitrophenyl β -L-fucopyranoside (VIId), showed mp 186–188°.

Anal. Calcd for C₁₂H₁₅NO₇: C, 50.52; H, 5.30; N, 4.91. Found: C, 50.21; H, 5.19; N, 4.92. $[\alpha]_D^{20} + 95^\circ$ (c 1, ethanol).

Methyl α -L-Fucopyranoside (VII). Dry L-fucose (5 g), dry Dowex 50 (10 g, H⁺ form resin, 200–400 mesh), and dry methanol (125 ml) were refluxed with constant stirring and the exclusion of moisture for 16 hr. After filtration, the solution was concentrated, and the residue was recrystallized twice from isopropyl alcohol, giving 2.3 g (42%) of methyl α -L-fucopyranoside, mp 155°.

Anal. Calcd for C₇H₁₄O₅: C, 47.18; H, 7.92. Found: C, 47.30; H, 7.96. $[\alpha]_D^{20} - 200^\circ$ (c 1, water).

Methyl 2,3,4-Tri-*O*-acetyl- α -L-fucopyranoside (VIII). Methyl α -L-fucopyranoside (2 g) was dissolved in a mixture of dry pyridine (9 ml) and acetic anhydride (6.6 ml), and maintained at room temperature in a stoppered flask for 16 hr. Water (100 ml) was then added, the mixture stirred for 4 hr and extracted with chloroform (five 30-ml portions), and the combined chloroform extracts were washed successively with water, 1 N HCl, water, saturated NaHCO₃, and water. After drying over CaCl₂, the chloroform solution was evaporated to give a solid that crystallized and was recrystallized from ethanol; the product (2.4 g, 70%), methyl 2,3,4-tri-*O*-acetyl- α -L-fucopyranoside, showed mp 70°, and gave one spot on thin-layer chromatography (Figure 1F).

Anal. Calcd for C₁₃H₂₀O₈: C, 51.30; H, 6.63. Found: C, 51.23; H, 6.54. λ_{\max} (KBr) 670, 750, 805, 875, 900, 940, 970, 1040, 1140, 1170, 1230, 1375, 1460, 1640, 1750, and 2950 cm⁻¹; $[\alpha]_D^{20} - 152^\circ$ (c 1, chloroform).

The results of nuclear magnetic resonance spectral analysis of several of the derivatives described above are presented in Table II.

Preparation of L-Fucose 1-Phosphate. (a) A mixture

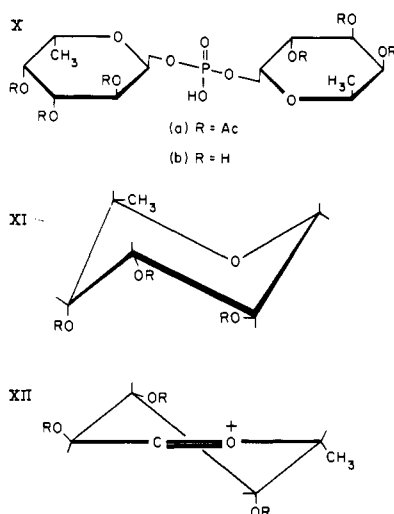
of dry benzene (5 ml), anhydrous calcium sulfate (1 g), silver dibenzyl phosphate³ (766 mg), and glass beads was shaken in a stoppered flask for 30 min while protected from light. Acetochlorofucose (309 mg) was added to the mixture, and shaking was continued for 16 hr. After the addition of benzene (10 ml), the mixture was centrifuged, the residue was washed twice with 10-ml portions of benzene, and the benzene solutions were combined (thin-layer chromatography of an aliquot gave the results shown in Figure 1I) and concentrated to a syrup. The latter was dissolved in 25 ml of ethanol and added to a flask containing 25 ml of ethanol and 200 mg of 5% Pd on charcoal in an atmosphere of N₂. After evacuation, H₂ was admitted to the flask, and H₂ uptake was followed; the reaction stopped after 8 min of vigorous stirring (H₂ uptake, 1.7 mmoles), and the mixture was filtered rapidly into a receiver containing 5 ml of 2 N LiOH at 0°. The filtrate was maintained at 5° for 16 hr, the solids were removed by filtration and washed with cold water (two 5-ml portions), and the filtrate was adjusted to pH 7.5 with Dowex-50, H⁺ form resin. After removal of the resin by filtration, the washings (5 ml) and filtrate were combined, concentrated to approximately 10 ml, and transferred to a Dowex 1, HCO₃⁻ (200–400 mesh) column (1 × 14 cm), and the latter was washed extensively with water. Elution was effected with a linear gradient according to the method of Peterson and Sober (1962), using 300 ml of water and 300 ml of 0.5 N triethylammonium bicarbonate (pH 7.5). The fractions (4 ml each) were assayed with the anthrone and acid molybdate reagents, and were examined by paper chromatography and/or electrophoresis. Fractions 3–11 (36 ml), 26–41 (78 ml), and 44–65 (83 ml) contained fucose (520 μ moles), fucose 1-phosphate (350 μ moles; $[M]_D^{20} - 28,600^\circ$), and P_i, respectively. Fractions 26–41 were combined, concentrated to dryness, and, to remove residual triethylammonium bicarbonate, concentrated repeatedly after the addition of small portions of water. Freshly redistilled cyclohexylamine (1 ml) was added, and the concentration, after the addition of water, repeated several times. The crystalline residue was dissolved in 1 ml of water, acetone was added to turbidity, and after 16 hr at 5°, the solid was filtered and dried over P₂O₅. The product (0.13 g, 29%), dicyclohexylammonium α -L-fucose 1-phosphate, which reacted slowly with Fehlings and alkaline silver nitrate solutions, exhibited R_F 0.16 on paper chromatography, and an electrophoretic mobility of 1.54×10^{-4} cm² sec⁻¹ V⁻¹ in the system described under Methods. Titration of the product (IV) gave $pK'_1 = 2$, $pK'_2 = 6.5$, and a molecular weight of 440. The apparent first-order hydrolysis rate constant for the phosphate ester was determined at 37° in 0.25 N H₂SO₄ as shown in Figure 3. The $t_{0.5} = 9$ min, and $k_{\text{hydrolysis}}$ therefore was 1.28×10^{-3} sec⁻¹. Under the same conditions, α -D-galactose 1-phosphate was 50% hydrolyzed in 68 min, and $k_{\text{hydrolysis}}$

³ The silver dibenzyl phosphate was prepared by the method of Zervas and Dilaris (1955); difficulty had been encountered in the preparation of dibenzylphosphoric acid by the method of Putnam (1963).

was $1.70 \times 10^{-4} \text{ sec}^{-1}$. The fucose phosphate was therefore hydrolyzed about 10 times more rapidly than the galacto analog.

Anal. Calcd for $\text{C}_{18}\text{H}_{39}\text{N}_2\text{O}_8\text{P}$ (442.49): C, 48.86; H, 8.88; N, 6.33; P, 7.00. Found: C, 49.05; H, 8.92; N, 6.31; P, 7.01. $[\alpha]_D^{20} -77.8^\circ$ (c 1, water).

CHART I



(b) Dry, crystalline Ag_2HPO_4 (620 mg) (Flatt and Brunisholz, 1951) was shaken for 30 min with anhydrous calcium sulfate (1 g), dry benzene (5 ml), and glass beads in a stoppered flask protected from light. Acetochlorofucose (309 mg) was then added, and the mixture was shaken for 16 hr. After adding 10 ml of benzene, the mixture was centrifuged, the residue was washed twice with 10-ml portions of benzene, and the combined solutions were concentrated to a syrup. Electrophoresis (Figure 2) showed that the syrup contained P_i and two fucose phosphate esters of lower mobility (1.37×10^{-4} and $8.55 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1} \text{ V}^{-1}$, respectively).⁴ Methanol (5 ml) was added, the solution was cooled to 0° and treated with cold 2 N LiOH (5 ml), and the mixture was left at 5° for 20 hr. After filtration, the solid was washed with 5 ml of cold water, and the combined filtrates were adjusted to pH 7.5 with Dowex 50, H^+ form resin. Electrophoresis of an aliquot (Figure 2) showed P_i and two phosphate esters that migrated somewhat more rapidly than the products prior to alkaline hydrolysis.⁴ After removal of the Dowex 50, H^+ form resin, the solution and washings were chromatographed on a Dowex 1 column as described in preparation a above. Fractions 2–10 (35 ml) contained fucose (405 μmoles), 27–41 (86 ml) contained fucose 1-phosphate (200 μmoles , $[\text{M}]_D^{20} -28,600^\circ$), and 44–70 (108 ml) contained P_i . The combined fractions 27–41 were treated as described in method a above, giving 60 mg of IV which

showed similar optical rotation, electrophoretic and chromatographic mobilities, and fucose to phosphate ratios to the product isolated by method a.

On ion-exchange chromatography, a product different from those observed in method a was detected. This substance was detected in fractions 11–24 (62 ml), and contained nonreducing fucose and organic phosphate (fucose, 275 μmoles ; phosphate, 140 μmoles). When isolated by the usual procedure, it failed to crystallize. Upon acid hydrolysis under the conditions described in Figure 3, the $t_{0.5} = 14 \text{ min}$, and the apparent $k_{\text{hydrolysis}} = 8.25 \times 10^{-4} \text{ sec}^{-1}$. This substance was therefore somewhat more resistant to hydrolysis than α -fucose 1-phosphate. Titration of the compound showed a single ionizable hydrogen, pK'_1 approximately 2. It exhibited $[\text{M}]_D^{20} -72,000^\circ$, an R_F in the paper chromatographic system of 0.43, and an electrophoretic mobility of $1.14 \times 10^{-4} \text{ cm}^2 \text{ sec}^{-1} \text{ V}^{-1}$. We therefore concluded that the unknown product was bis(α -L-fucopyranosyl 1-)phosphate (Xb) (Chart I). Repetition of this experiment using acetochlorofucose (1 mmole), Ag_2HPO_4 (2 mmoles), and cold chloroform (8 ml) and ether (3 ml) as the solvent or with 10 mmoles of Ag_2HPO_4 in benzene (25 ml) gave the results shown in Table III (expt 6 and 7). (c) Phosphoric acid (90%, 140 μl), freshly prepared Ag_3PO_4 (280 mg), and dry ether (3 ml) were vigorously stirred at 0° in a centrifuge tube protected from light. After 10 min, acetochlorofucose (309 mg) in dry chloroform (8 ml) was added and the mixture was stirred for an additional 60 min at 0° . After centrifugation, the residue was washed twice with a mixture of chloroform (8 ml) and ether (3 ml). The combined supernatant fluids contained a small quantity of unreacted acetochlorofucose as indicated by thin-layer chromatography, but were concentrated to a syrup, treated with LiOH, neutralized, and fractionated on Dowex 1, HCO_3^- , as described in method a above. Fractions 2–14 (59 ml), 25–35 (45 ml), and 45–60 (65 ml) contained fucose (710 μmoles), fucose phosphate (150 μmoles), and P_i , respectively. Fractions 25–35 ($[\text{M}]_D^{20} -28,000^\circ$) were combined and the product was isolated as described above, giving 43 mg (10%) of IV, with properties essentially the same as the properties of the material described in method a. (d) Anhydrous phosphoric acid (800 mg) was melted at 50° with the exclusion of moisture. Dry, crystalline fucose tetraacetate (IIa, 332 mg) was added to the melt, and the mixture was stirred under vacuum at 50° for 90 min. (An effective method for both stirring the melt and maintaining both the proper temperature and pressure consists of placing the flask on the moveable joint of a rotary evaporator.) Cold, dry tetrahydrofuran (10 ml) was added, and the solution was then quickly transferred to a stirred solution of cold 2 N LiOH (15 ml). After storage at 5° for 16 hr, the solid was removed by filtration and washed with cold water (10 ml), and the combined filtrates were neutralized, concentrated, and chromatographed as described in method a above. Fractions 3–12 (42 ml), 24–45 (90 ml), and 46–68 (95 ml) contained fucose (550 μmoles), fucose phosphate (350 μmoles , $[\text{M}]_D^{20} -34,000^\circ$), and P_i , respec-

⁴ Elution of the bands from the paper, followed by treatment with LiOH, neutralization (HCl), and electrophoresis, showed that the band migrating at 1.37×10^{-4} gave a product migrating at 1.54×10^{-4} , while the band migrating at 8.55×10^{-5} yielded a product migrating at $1.14 \times 10^{-4} \text{ cm}^2 \text{ sec}^{-1} \text{ V}^{-1}$.

TABLE III: Data on Fucose Phosphate Preparations.

Expt	Reactants	Solvent and Temperature	Yield of X (%)	Fucose 1-Phosphate		
				[M] _D on Crude (deg)	Crude Yield (%)	Yield of Crystalline IV (%)
1	Silver dibenzyl phosphate	In benzene at 20–25°	None	–27,900	27	15
2	(2 mmoles)–III (1 mmole)	In benzene at 20–25°	None	–28,600	35	29
3		In benzene at 20–25°	None	–31,000	33	29
4	Ag ₂ HPO ₄ (2 mmoles)–III	In benzene at 20–25°	28	–28,600	20	15
5	(1 mmole)	In benzene at 20–25°	20	–28,400	17	12
6	Ag ₂ HPO ₄ (10 mmoles)–III (1 mmoles)	In benzene at 20–25°	16	–34,000	12	5
7	Ag ₂ HPO ₄ (2 mmole)–III (1 mmole)	In ether at 0°	15	–29,200	10	7
8	“Monosilver phosphate”	In CHCl ₃ –Et ₂ O at 0°	None	–28,000	15	10
9	(2 mmoles)–III (1 mmole)	In CHCl ₃ –Et ₂ O at 0°	None	–10,000	5	2
10	H ₃ PO ₄ (82 mmoles)–	At 50°	None	–32,000	20	15
11	IIa (1 mmole)	At 50°	None	–34,000	35	29

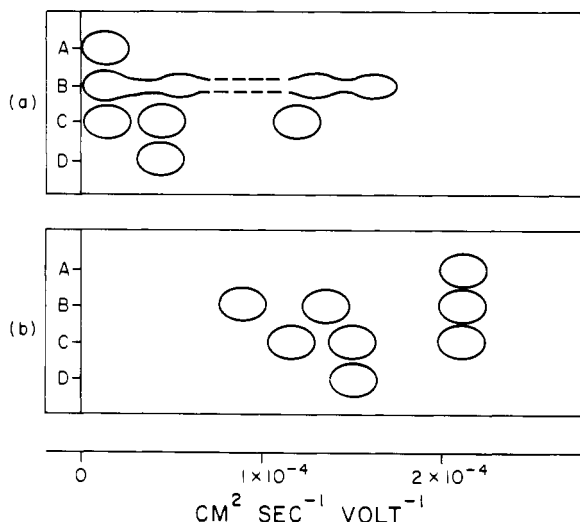


FIGURE 2: Qualitative examination of L-fucose phosphates. (a) Chromatography on Whatman No. 4 paper developed with isopropyl alcohol–ammonia–water (7:1:2, v/v). (b) Electrophoresis on Whatman No. 3MM paper using 4000 V for 30 min, and pyridinium acetate buffer (pH 6.4). Samples were A, P_i; B, crude reaction products from Ag₂HPO₄ and acetochlorofucose; C, sample B after LiOH treatment; D, α-L-fucose 1-phosphate.

tively. Purification of combined fractions 24–45 according to the procedures described in method a above gave 129 mg (29%) of IV.

Anal. Calcd for C₁₈H₃₉N₂O₈P: C, 48.86; H, 8.88; N, 6.33; P, 7.00. Found: C, 48.75; H, 8.90; N, 6.37; P, 7.00. $[\alpha]_D^{20}$ –77.5° (c 1, water).

Recently, the barium salt of α-L-fucose 1-phosphate was dissolved in D₂O and residual H₂O removed by repeated evaporation and addition of D₂O. A nuclear magnetic resonance spectrum of this sample was then obtained in concentrated D₂O solution and compared with that from a sample of L-fucose 1-phosphate ob-

tained by enzymatic synthesis (Ishihara *et al.*, 1968). The 100-MHz spectrum of α-L-fucose 1-phosphate showed H₁ resonance at τ 4.1, J = 3 cps (a quartet due to splitting by ³¹P of the phosphate); H₂, H₃, and H₄ resonances with τ 5.5–6.0; H₅ resonance as a quartet with τ 5.28, J = 7.0 cps, and a doublet with τ 8.34, J = 7.0 cps, for the CCH₃ resonances. Under similar conditions, the biosynthetic β-L-fucose 1-phosphate showed H₁ resonance at about τ 4.77 (probably a quartet but partly obscured by the HDO signal); CCH₃ resonances at τ 8.22, J = 7.0 cps; and the corresponding H₅ signals lying within the region occupied by those of H₂, H₃, and H₄ (τ values 5.5–6.0).

Results and Discussion

The optical rotations of the compounds described above are presented in Table I along with available literature values. The data clearly show that the chloro compound (III) is the α anomer; it can be derived from the corresponding tetraacetate using vigorous conditions for replacement of the 1-acetoxy group, or from the β-tetraacetate using milder conditions. As expected, the acetochlorofucose reacted with methanol and *p*-nitrophenol to give inversion at C-1, *i.e.*, the β-glycosides (Table I). Methyl β-L-fucopyranoside (VIb) was obtained from the tri-*O*-acetate and the corresponding α derivatives were prepared by improvements of the original method of Hockett *et al.* (1939).

Examination of the anomeric tetra-*O*-acetates, the anomeric methyl glycosides and their tri-*O*-acetyl derivatives by nuclear magnetic resonance spectroscopy (Table II) showed the following.

(a) Splitting of the C-1 hydrogen signal indicated (Lemieux *et al.*, 1958) a 1,2-*trans*-diaxial arrangement of the hydrogen atoms in the β series, and together with the known configuration, an equatorial-axial arrangement in the α series.

(b) The chemical shifts of the α- and β-glycosidic

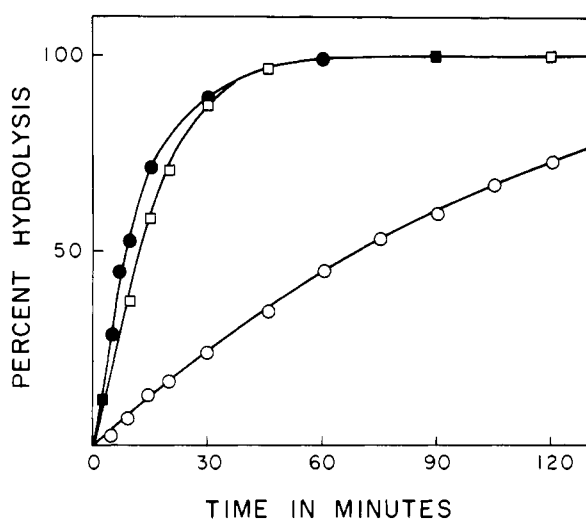


FIGURE 3: Hydrolysis of sugar phosphates at 37° in 0.25 N H_2SO_4 . α -L-Fucose 1-phosphate (●), bis(α -L-fucose 1-phosphate (□), and α -D-galactose 1-phosphate (○).

methoxyl groups indicate axial and equatorial arrangements, respectively (cf. Lemieux and Reid, 1964).

(c) The CH_3CH group present in fucopyranose structures gives fully resolved doublet (τ 8.13–8.86) and quartet (τ 5.60–6.1) signals with splittings of 6.5 cps. Since CH_3CH systems often give complex signals, the simple pattern found in these fucose derivatives is attributed to the relatively large chemical shift of the C-5 hydrogen (Anet, 1961). The chemical shift of the C-6 hydrogens is only slightly affected by axial substituents at C-1, whereas the C-5 hydrogen has a higher chemical shift induced by nonbonded interaction with axial glycosidic methoxyl groups; these results indicate the close proximity of these groups in the α series and thus exclude the possibility of a boat conformation.

The nuclear magnetic resonance spectra therefore show that the anomeric tetra-*O*-acetates of methyl α - and β -L-fucopyranosides (VII and VIb), and the corresponding tri-*O*-acetates assume the expected *1C* conformation (XI) in the solvents specified. A comparison of the chemical shifts and coupling constants (Table II) of the C-1 and C-5 hydrogens of the tetraacetylfucose (IIa) and acetochlorofucose suggests that the latter has a similar conformation; a spectrum of acetochloro- β -L-fucose would be required to validate this conclusion.

The acid lability of fucopyranosides is well known but not readily explained. The observed order of stability, *viz.*, glucopyranosides > galactopyranosides > fucopyranosides (Figure 3, Leloir and Cardini, 1959), can be deduced on purely steric grounds from a knowledge of the stable conformations of the glycosides in solution in terms of the ease of formation of ions such as XII (cf. Ferrier and Overend, 1959; Feather and Harris, 1965); it is difficult, however, to assess how much polar and electronic factors should also be taken into account (Be Miller, 1967).

Attempts to obtain confirmatory evidence concerning the configurations of the various derivatives by their infra red spectra were not satisfactory. Analysis of the

730–960- cm^{-1} region did not clearly show type 2a or 2b absorptions (cf. Neely, 1957).

An important objective of these studies was the chemical synthesis of the anomeric fucose 1-phosphate esters. Only the α anomer was isolated, despite the fact that the starting materials were α -acetochlorofucose (III) or the α -tetraacetate (IIa) and that the phosphate reagents employed frequently give inversion of configuration with other sugar acetates of acetohalogeno derivatives. However generalizations concerning the steric course of reactions of acetohalogeno sugars with phosphate reagents are unreliable and are usually based on the optical rotations of products obtained after fractionation (MacDonald, 1962; Foster and Overend, 1957). In the present studies, the crystalline dicyclohexylammonium α -L-fucose 1-phosphate was isolated in 29, 10, and 15% yields by condensing acetochlorofucose with silver dibenzyl phosphate, "monosilver phosphate," and disilver hydrogen phosphate, respectively (Table III). The MacDonald (1962) procedure, involving the condensation of the crystalline α -tetraacetate with anhydrous phosphoric acid, also gave the α -phosphate derivative in 30% yield. Optical rotations of crude fucose phosphate preparations showed, with one possible exception (expt 9, Table III), the absence of significant quantities of dextrorotatory components; by analogy with the optical rotation of methyl β -L-fucopyranoside (Table I), a $[\text{M}]_D$ of approximately $+2500^\circ$ would be expected for β -L-fucose 1-phosphate (cf. Carlson *et al.*, 1964).

The results shown in Table III are surprising in that the phosphate reagents employed in these reactions with acetochlorofucose (α anomer) generally yield inversion with 1,2-*cis*-acetohalogeno sugar. A possible explanation is suggested by the extreme acid lability of fucopyranosides; perhaps anomerization of the phosphate derivatives to the more stable α anomer takes place under milder conditions than occurs with hexoses. Some support for this suggestion is the observation that mixtures of L-fucose acetates were produced under conditions where other hexoses gave the 1,2-*trans*-acetates (Leaback and Walker, 1957; Wolfrom and Groebke, 1963).

L-Fucose is a common constituent of many glycoproteins, and is generally believed to occur in the α -anomeric form (Gottschalk and Graham, 1966). The sugar donors in the biosynthesis of glycoproteins are the corresponding sugar nucleotides, and in the case of L-fucose is apparently guanosine diphosphate fucose. While the anomeric configuration of the fucose in this sugar nucleotide has not been established with certainty, it appears to be the β anomer. This conclusion is based on the fact that the precursor of GDP-L-fucose is the α -D-mannopyranoside, GDP-mannose, and it is thought that the many steps involved in the conversion of the mannose into fucose derivative (Ginsburg, 1961) does not involve inversion at C-1. In a similar sequence of reactions, the enzymatic conversion of thymidine diphosphate D-mannose into TDP-L-rhamnose, inversion at C-1 of the aldohexose does not occur (Blumsen and Baddiley, 1961).

The enzymatic synthesis of L-fucose 1-phosphate was

recently achieved with an animal kinase and ATP (Ishihara *et al.*, 1968). The nuclear magnetic resonance spectra of the enzymatically and chemically synthesized fucose 1-phosphates were obtained as described above. A comparison of the 100-MHz spectra of the fucose phosphate preparations was complicated by the fact that the H_1 and H_5 resonances in the biosynthetic material were overlaid by other signals. It was clear, however, that the chemically synthesized and biosynthetic phosphates were different and that the H_1 and H_5 signals occur at higher field strengths in the latter compound. Thus, bearing in mind the discussion of the results in Table II and, in spite of the unobservability of the $J_{1,2}$ coupling constant in the biosynthetic material, it was possible to conclude that the spectra were consistent with the two fucose phosphates being anomers, and that they adopt the *1C* conformation in D_2O solution.

MacDonald and Fletcher (1960) introduced the use of crystalline Ag_2HPO_4 for the preparation of sugar phosphates in place of the usual, uncharacterized "monosilver phosphate." In the present studies, Ag_2HPO_4 and acetochlorofucose gave at least two fucose phosphate derivatives (Figure 2); on treatment with alkali, one of the derivatives was converted into α -L-fucose-1-P, while the other yielded bis(α -L-fucose 1-)-phosphate (X). While the latter was first thought to be the cyclic phosphate (IX), further analysis proved it to be X. The isolation of X after alkali treatment was unexpected in view of the known susceptibility of similar diesters with 1,2-equatorial-axial arrangements to give hexose 1,2-cyclic phosphates under basic conditions (Khorana, 1961). Since the yields of fucose phosphates (IV and Xb) were not significantly altered by using larger excesses of Ag_2HPO_4 and benzene, or by using ether-chloroform as solvent (Table III, expt 6 and 7), and since no diester phosphate (Xa) was detected in the products of the reaction between acetochlorofucose and "monosilver phosphate," it appears unlikely that Ag_2HPO_4 is the active agent in "monosilver phosphate" preparations as has been suggested by MacDonald and Fletcher (1960). Presumably the data of Platt and Brunisholz (1951) on Ag_2HPO_4 are not applicable to systems containing organic solvents under the conditions described above.

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