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Note

Alternative syntheses of 2,6-dideoxy-L-*lyxo*-hexose (2-deoxy-L-fucose) and its biochemical properties

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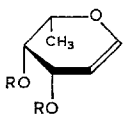
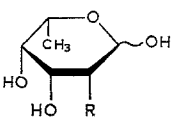
There has been considerable interest in the synthesis of 2,6-dideoxy-α-L-*lyxo*-hexose (2-deoxy-L-fucose), because it is a constituent of several natural products[1]. Also, semi-synthetic anthracycline antibiotics with antitumour properties contain this sugar in place of the naturally occurring counterpart[2]. Our interest in this sugar stems from its close structural analogy with L-fucose, which occurs as a terminal sugar in many of the glycoconjugates of the cell membrane. 6-Deoxy-6-fluoro-L-galactose ("6F-L-fucose") was incorporated into the glycoconjugate[3], and the formation of 6F-L-fucose 1-phosphate and GDP-6F-L-fucose has been indicated by h.p.l.c. of acid-soluble extracts of leukemic cells. 2-Deoxy-2-fluoro-D-fucose inhibited [^{14}C]-L-fucose incorporation into mouse fibroblasts in culture[4].

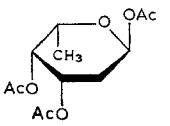
In considering methods for the synthesis of 2-deoxy-L-fucose, preference was given to procedures which would permit convenient introduction of tritium into a non-metabolisable position of the molecule for biochemical studies. 2-Deoxy-L-fucose was first synthesised[5] from the glycal **2** by acid-catalysed hydration and, more recently[1,6], from ethyl 4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranoside. However, neither method could be adapted for the introduction of tritium. Recently, improved methods for the synthesis of 2-deoxy sugars from glycals have been developed, using electrophilic additions to the double bonds; the most direct method[7] is the methanesulfonic acid-catalysed addition of acetic acid. Shortening the reaction time from that reported[7] improved the yield considerably and the conversion **1**→**4** could be effected in quantitative yield (t.l.c.). A similar result was obtained with 3,4,6-tri-*O*-acetyl-D-galactal (see Experimental).



1 R = Ac
2 R = H

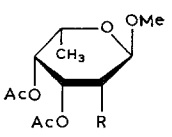


3a R = H
3c R = ^{3}H

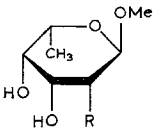


4

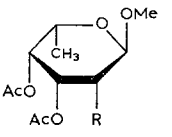
An alternative, less-direct synthesis of 2-deoxy sugars involves methoxymercuration of glycals, followed by borohydride reduction[8,9]. We have used this method for the synthesis of the title compound as well as for the introduction of deuterium and tritium at position 2. Methoxymercuration of **1** yielded solely methyl 2-acetoxymercuri-3,4-di-*O*-acetyl-2,6-dideoxy-α-L-talopyranoside (**5**). Methoxymercuration usually[10] proceeds by *trans* addition to the double bond, producing a methyl glycoside with the mercury component attached to C-2. Typically, mixtures of diaxial and diequatorial addition products are formed[10]. From the ^{1}H-n.m.r. spectrum of **5**, showing $J_{1,2}$ and $J_{2,3}$ values of 0.6 and 5.4 Hz, respectively, it can be concluded that H-2 is equatorial, and hence that MeO-1 and HgAc-2 are axial, and that only one product is formed. Also, the methoxyl protons in **5** resonate at a higher field (δ 3.34) than expected[10] for an equatorial group (δ 3.52). The ^{199}Hg–^{1}H and ^{199}Hg-^{13}C couplings have also been used for conformational assignments[11]. The $J_{Hg,3}$, $J_{Hg,2}$, and $J_{Hg,1}$ values were 454, 188, and 90.5 Hz, respectively. Although these coupling constants were significantly larger than those found for methyl 3,4,6-tri-*O*-acetyl-2-chloromercuri-2-deoxy-α-D-talopyranoside (448, 176, and 86 Hz, respectively)[11], the deviation is too small to be significant in terms of differences in the conformations of these two compounds [*i.e.*, $^1C_4$(L) and $^4C_1$(D), respectively].



5 R = HgOAc
6 R = H



7a R = H
7b R = ^{2}H
7c R = ^{3}H



8a R = H
8b R = ^{2}H
8c R = ^{3}H

The pseudo-axial AcO-4 in **1** exerts a marked influence on the stereochemistry of the electrophilic attack at C-2 and strongly favours L-*galacto* products. This applies to the acetic acid addition and the methoxymercuration reactions. When AcO-4 is pseudo-equatorial (as in 3,4,6-tri-*O*-acetyl-D-glucal), the approach of the reagents is less hindered, resulting in the loss of stereoselectivity.

Reduction of **5** with borohydride gave the acetylated methyl glycoside **8a**, which was deacetylated (**7a**) and then hydrolysed with acid, to give 2-deoxy-L-fucose (**3a**). An analogous reaction with borodeuteride gave **8b**, the configuration of which was established by n.m.r. spectroscopy. The $J_{1,2}$ value of 1.5 Hz indicates the deuterium atom to be axial and that the deuteration had proceeded with retention of configuration. Also, in the 2-methylene region of the n.m.r. spectrum of **8a**, the proton resonating at higher field had been replaced with deuterium; for 2-deoxy sugars, H-2*ax* resonates at a higher field[12] than does H-2*eq*. The high degree of retention of configuration is noteworthy, in view of the free-radical nature of the reaction[13]. Borohydride reduction of methyl 2-acetoxymercuri-3,4,6-tri-*O*-acetyl-2-

TABLE I

EFFECTS OF 2-DEOXY-L-FUCOSE AND ITS DERIVATIVES ON CELL GROWTH AND MACROMOLECULAR BIOSYNTHESIS

Compounds	*Leukemia L1210 (IC_{50}, M)*	*Mouse mammary adenocarcinoma (TA3) (IC_{50}, M)*	*P288 Leukemia*		
			Growth (% control)	*Incorporation (% control)*	
				D-*GlcN*	L-*Leu*
3a	$>10^{-3}$[a]	$>10^{-3}$	96	110	107
4	—	$>10^{-3}$	78	81	81
7	$>10^{-3}$	$>10^{-3}$	96	110	107
6	5×10^{-4}	—	43	20	9
2	—	$>10^{-3}$	92	104	99
1	—	1.2×10^{-4}	53	43	27

[a]Growth was reduced by 30% at 10^{-3}M.

deoxy-β-D-mannopyranoside was thought to proceed with retention of configuration, although the evidence was not convincing[14]. Reduction of **5** with borotritide gave **8c**, which was deprotected to give the tritiated 2-deoxy-L-fucose (**7c**).

At mM, 2-deoxy-L-fucose inhibited the growth of L1210 leukemia by 30%, had no effect on mouse-mammary adenocarcinoma (TA3) cells in culture (Table I), and inhibited the growth of SW613 mammary tumor cells to only a slight extent (11%). Since the last cell-line incorporated relatively large amounts of [^{3}H]-labelled L-fucose, it was used for competition and incorporation studies. 2-Deoxy-L-fucose at mM was ineffective as a competitor of incorporation of L-fucose. The specific activity of the tritium label of **3c** was too low to allow assessment of its incorporation into the macromolecular fraction of SW613 cells.

The acetylated derivatives and precursors of 2-deoxy-L-fucose were active as growth inhibitors and exhibited a pronounced effect on the macromolecular biosynthesis of P288 leukemia cells in culture (Table I); the acetylated derivatives had the greater inhibitory activity (*cf.* **2** and **1**, **6** and **7**, and **4** and **3**). This situation has been observed with other carbohydrate analogues and is probably related to their enhanced lipid-permeability characteristics[15]. The effects of the analogues on the viability, the growth, and the incorporation of 2-amino-2-deoxy-D-glucose and L-leucine into the macromolecular fraction of P-288 cells were also determined. The growth-inhibitory compounds **6** and **1** also have a pronounced effect on the macromolecular biosynthesis, as seen by the decrease in incorporation of 2-amino-2-deoxy-D-glucose and L-leucine, but do not show selectivity with regard to the inhibition of precursor incorporation.

EXPERIMENTAL

General. — Melting points were determined on a Mel-Temp apparatus and are uncorrected. I.r. spectra were recorded with a Perkin–Elmer 457 spectrophotometer and n.m.r. spectra for solutions in $CDCl_3$ (internal Me_4Si) with a Varian XL-100 spectrometer operating in the Fourier-transform mode. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. T.l.c. was performed on silica gel with benzene–ether (1:1) and detection by charring with sulfuric acid. Solvents were removed by using a rotary evaporator under reduced pressure.

*1,3,4-Tri-*O*-acetyl-2,6-dideoxy-*L*-lyxo-hexopyranose* (**4**). — A solution of 3,4-di-*O*-acetyl-L-fucal (**1**; 1.60 g, 7.4 mmol) in acetic acid (15 mL) containing 67mM methanesulfonic acid was stirred at room temperature for 1 h, poured into chloroform, and neutralised with aqueous $NaHCO_3$. The organic layer was washed with water, dried ($CaCl_2$), and concentrated, to give a colorless syrup (2.04 g) that gave only one spot in t.l.c. Short-path distillation (Kugelrohr, 130°/0.2 Torr) gave **4** as an oil (1.80 g, 89%) which crystallised; m.p. 198–200° (from 2-propanol), $[\alpha]_D^{22}$ −128.5° (*c* 0.26, chloroform).

Anal. Calc. for $C_{12}H_{18}O_7$: C, 52.55; H, 6.51. Found: C, 52.73; H, 6.51.

*Methyl 2-acetoxymercuri-3,4-di-*O*-acetyl-2,6-dideoxy-α-*L*-talopyranoside* (**5**). — A solution of **1** (1.07 g, 5.0 mmol) and $Hg(OAc)_2$ (1.6 g, 6.0 mmol) in methanol (20 mL) was left at room temperature for 2 h. T.l.c. then showed that no **1** remained. After removal of the solvent, the residue was crystallised from 2-propanol, to give **5** (1.52 g, 60.3%), m.p. 121–124°, $[\alpha]_D^{22}$ −22° (*c* 0.4, chloroform).

Anal. Calc. for $C_{13}H_{20}HgO_8$: C, 30.92; H, 3.99. Found: C, 31.13; H, 4.11.

*Methyl 3,4-di-*O*-acetyl-2,6-dideoxy-α-*L*-lyxo-hexopyranoside* (**6**). — A solution of **1** (1.45 g, 6.77 mmol) and $Hg(OAc)_2$ (2.16 g, 6.77 mmol) in methanol (20 mL) was left at room temperature in the dark for 3 h, cooled to 0°, and then treated with $NaBH_4$ (350 mg) in small portions with stirring. After addition of some silica gel, the solvent was evaporated, and the residue was applied to a dry column of silicic acid. Elution with benzene–ether (1:1) afforded **6** (1.56 g, 93.4%) which, after crystallisation from ethanol–light petroleum, had m.p. 66.5–67.5°, $[\alpha]_D^{22}$ −166° (*c* 0.79, chloroform).

Anal. Calc. for $C_{11}H_{18}O_6$: C, 53.65; H, 7.36. Found: C, 53.43; H, 7.29.

When NaB^2H_4 was substituted for $NaBH_4$, **8b** was obtained in excellent yield.

*Methyl 2,6-dideoxy-α-*L*-lyxo-hexopyranoside* (**7a**). — To methanolic 1% $Ba(OH)_2$ (5 mL) was added **6** (530 mg, 2.1 mmol). The solution was kept at 4° for 17 h, neutralised with CO_2, filtered, and concentrated. Acetonitrile was evaporated from the residue, to give an oily product (310 mg, 89%) which was distilled [90° (bath)/10^{-3} Torr] using a Kugelrohr. The oil crystallised on storage, and the hygroscopic crystals of **7a** had m.p. 53–55°, $[\alpha]_D^{22}$ −160° (*c* 0.19, water).

Anal. Calc. for $C_7H_{14}O_4$: C, 51.83; H, 8.70. Found: C, 51.56; H, 8.73.

*2,6-Dideoxy-*L*-lyxo-hexose* (**3a**). — A solution of **7a** (1.48 g, 9.08 mmol) in 5mM H_2SO_4 (25 mL) was heated for 6 h at 70°, cooled, neutralised with $BaCO_3$,

centrifuged, and concentrated, to give an oily residue (1.32 g, 98%). Distillation (10^{-5} Torr) yielded a clear syrup, which eventually crystallised to give **3a**, m.p. 90–92°, $[\alpha]_D^{22}$ −76° (5 min)→−57° (3 h) (*c* 0.075, water); lit.[5] m.p. 103–106°; lit.[10] m.p. 92–94°, $[\alpha]_D$ −75° (5 min)→−57° (90 min) (*c* 1.5, water).

2,6-Dideoxy-L-lyxo-[*2-*^{3}*H*]*hexose* (**3c**). — To a cooled, stirred solution of **5** (54 mg, 0.11 mmol) in ethanol (1 mL) was added sodium borotritide (0.9 mg, 0.02 mmol; 209 mCi/mg). After 30 min, the solvent was evaporated, and a solution of the residue in benzene–ether (1:1) was applied to a dry column of silica gel. Elution with benzene–ether (1:1, 35 mL) gave methyl 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-[2-^{3}H]hexopyranoside (**8c**; 24 mg, 90.5%), which was dissolved in 95% ethanol (24 mL, 150 mCi/mmol). An aliquot (4 mL) was used for the biological studies and the remainder was concentrated. To the residue was added methanolic 1% $Ba(OH)_2$ (2 mL), and the solution was kept at 4° for 3 days. After the addition of 5mM H_2SO_4 (40 mL), the mixture was heated at 70° for 6 h, cooled, neutralised with $BaCO_3$, filtered, and concentrated. A solution of the residue in ethanol was filtered and concentrated, to give **3c** (10 mg, 84%; 80 mCi/mmol) which co-chromatographed in t.l.c. with **3a**.

1,3,4,6-Tetra-O-*acetyl-2-deoxy*-α-D-lyxo-*hexose*. — 3,4,6-Tri-*O*-acetyl-D-galactal (1.5 g, 5.5 mmol) was treated with acetic acid (15 mL) containing 0.067M methanesulfonic acid, as described above for **1**. Work-up gave white crystals (1.2 g, 66%) that appeared to be homogeneous (n.m.r. spectrum). Recrystallisation from 2-propanol gave the title compound, m.p. 99–102°, $[\alpha]_D^{22}$ +118° (*c* 1.2, chloroform); lit.[7] m.p. 102–103°, $[\alpha]_D$ +123°; lit.[17] m.p. 97°, $[\alpha]_D$ +118°.

Biological evaluation. — L1210 leukemia cells were grown in stationary tube cultures in RPMI 1640 medium[18] containing 10% of heat-inactivated, foetal calf serum. Murine P288 leukemia cells were maintained as an ascites tumor in DBA/2J female mice. Periodically, cells were removed from mice, under aseptic conditions, washed twice in RPMI 1640 medium, and cultured in RPMI 1640 containing 10% of foetal calf serum. These cultures were grown in stationary tube cultures in a 90% air/10% CO_2 incubator. They were maintained *in vitro* for 1–3 months, whereupon new *in vitro* cultures were initiated from the mouse ascites tumors.

(*a*) *L1210 system.* An inoculum of 5×10^4 cells in 1 mL of RPMI 1640 medium (containing 10% of heat-inactivated, foetal calf serum and 20mM Hepes buffer) was supplemented with 1 mL of the same medium containing the compound to be tested. The tubes were incubated in an upright position for 3 days, and growth was estimated either by protein assay or cell counts (using a Coulter counter). The growth in control cultures varied from 6–10 fold after 3 days. Each concentration was tested in triplicate. For compounds found to be inhibitory, the tests were repeated at least twice. Variation between different tests was within ±10% for the 50% inhibitory concentration. The results are expressed in terms of IC_{50} (the molar concentration of the sugar analogue in the nutrient medium leading to 50% inhibition of cell growth).

(*b*) *P288 system.* For routine testing, P288 murine leukemic cells were suspended at ~10^5 cells/mL in fresh RPMI 1640 (without D-glucose and containing 10% of

heat-inactivated, foetal calf serum). Aliquots (1 mL) were transferred to disposable polyethylene tubes and placed in a CO_2/air incubator. After 1 h, sugar analogues were added to a final concentration of mM (or as otherwise stated). Cell growth and viability were monitored at later times by using a Coulter counter and Trypan blue dye exclusion, respectively. 2-Amino-2-deoxy-D-[^{14}C]glucose (2μM, 1.1 $\times$ 10^6 d.p.m.) and [^{3}H]leucine (0.3mM, 2.6 $\times$ 10^6 d.p.m.) were added to cell cultures to assess the effects of sugar analogues on protein and glycoprotein biosynthesis. Incubations were terminated 5 h later by the addition of 10% trichloroacetic acid (2 mL), and the resulting pellet was dissolved in NaOH and its radioactivity assayed by scintillation counting.

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