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Note

Microbial oxidation as a route for cyclization of acyclic-sugar nucleosides*†

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The synthesis of nucleoside analogs of potential biological significance has been a sustained theme of interest in this laboratory[2]. These endeavors have focused extensively on acyclic-sugar nucleosides whose sugar chains may conformationally mimic their cyclic-sugar counterparts[2,3], and have also included "homonucleosides" in which a carbon bridge is interposed between the nucleoside base and the sugar ring. It has been shown that[4], given suitable stereochemistry of the sugar chain, an acyclic-sugar nucleoside may be cyclized to a homonucleoside by kinetically controlled closure of the chain to form an oxolane ring. Similar strategy is likewise effective for generation of appropriate, cyclic-sugar structures prior to attachment of the nitrogen heterocycle for access to homonucleosides[5] and *C*-nucleosides[6].

The purpose of the present investigation was to explore the feasibility of microbiological oxidation of the sugar chain of an acyclic-sugar nucleoside, using an organism of established regio- and stereo-specific proclivity, to effect a conversion that would generate a cyclic-sugar nucleoside structure sterically analogous to that present in such clinically important, antiviral and antitumor agents as 9-β-D-arabinofuranosyladenine ("ara-A") and 1-β-D-arabinofuranosylcytosine ("ara-C"). Toward this objective, oxidation of the well characterized, acyclic-sugar nucleoside 1-(6-chloropurin-9-yl)-1-*S*-ethyl-1-thio-D-*glycero*-D-*ido*-hexitol[3,7] (**1**) by the aerobic bacterium *Acetobacter suboxydans* was evaluated.

The microbiological oxidation of carbohydrates and their derivatives by *A. suboxydans* has been extensively studied[8], and the general course observed has involved specific oxidation at the penultimate carbon atom of a nonreducing, terminal D-*erythro* sequence (Bertrand–Hudson rule[9]), although some exceptions have been recorded[10]. Oxidation of **1** in accordance with the Bertrand–Hudson rule would convert the acyclic-sugar nucleoside into its 5′-keto analog, which would predictably

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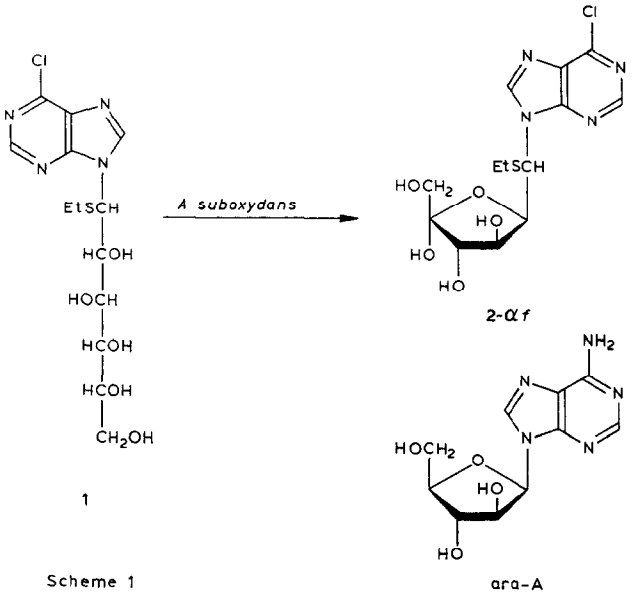

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Scheme 1

tautomerize to the anomeric 2′-ketofuranos-6′-yl derivatives. The structural analogy between these products, especially the anomer (**2**-αf) having C-1′ and C-6′ *cis*-disposed, and ara-A is clearly evident in the accompanying scheme (Scheme 1). Furthermore, the anticipated product, in addition to being a homonucleoside and having a 6-substituent amenable to useful conversions (NH_2, SH, NMe_2, and the like), also bears a formal resemblance to the 4′-fluoronucleoside nucleocidin[11] in having an anomeric carbon position at the position conventionally numbered 4′ in nucleosides.

The results communicated here show that microbiological oxidation of **1** indeed follows the course indicated, and that the resulting "5′-ketonucleoside" (formally a 6-substituted 2-ketose) does in fact tautomerize to favor a cyclic structure (**2**-αf) whose sugar ring is closely isosteric with those of ara-A and nucleocidin.

RESULTS AND DISCUSSION

Oxidation of carbohydrate derivatives by *A. suboxydans* is generally conducted by the live-culture method, whereby a broth containing substrate is inoculated with a culture of the organism grown on D-glucitol. Several trial oxidations of compound **1** were performed by this method. Whereas the procedure proceeded satisfactorily with the model compound D-glucose diethyl dithioacetal, and the yield of 5-keto product was comparable with that reported[12], there was no significant oxidation of **1** under the same conditions. Evidently, compound **1** is perceived by the organism as too foreign a structure for effective metabolic transformation. However, the isolated-cell method, wherein an active culture is suddenly deprived of its substrate (D-glucitol)

and offered the compound whose oxidation is desired (in this instance, compound **1**), proved to be effective for oxidation of **1**. By monitoring the reaction by t.l.c. (3:2 acetone–benzene), the progressive transformation of **1** into a faster-migrating product was observed over the course of 2–3 days, and the conversion approached 35%. The yield of oxidized product was significantly raised (to 70%) when the culture suspension was shaken under oxygen, instead of air. The oxidation product (**2**) was isolated in analytically pure condition, as a levorotatory syrup (by preparative t.l.c.), and its elemental analysis corresponded to the loss of two hydrogen atoms from **1**.

Detailed analysis of the ^{1}H- and ^{13}C-n.m.r. spectra of **2** confirmed that oxidation at C-5′ had, indeed, been effected by the organism, in accordance with the Bertrand–Hudson rule[9], and that the product existed as an equilibrium mixture of three tautomers, the acyclic form being predictably minor[13]; the ratio between the two furanose forms was strongly biased in favor of the one having C-1′ and C-6′ *cis*-disposed (α anomer*).

The tautomeric composition of **2** was established by ^{1}H- and, especially, ^{13}C-n.m.r. spectroscopy. Most of the proton signals for the three tautomers (see Experimental section) were superposed at the field-strength (90 MHz) used, but separate singlet resonances (δ 8.82, 8.77, and 8.71) were observed for H-2 of the purine in the three tautomers. Observation of the H-6′ signal (δ 5.87) as a doublet ($J_{5',6'}$ 5.3 Hz) clearly precluded any possibility that the microbial oxidation had taken place at C-5′, as such oxidation would have shifted the H-6′ signal from its characteristic[3,7] field position and have collapsed it to a singlet.

The ^{13}C-n.m.r. spectrum recorded for **2** in dimethyl sulfoxide-d_6 proved to be of the greatest value in establishing the site of oxidation and for identifying the three tautomers and their relative proportions (see Table I and Experimental section). The possibility of oxidation at C-1′ was clearly ruled out by the appearance of off-resonance triplets at characteristic field for -CH_2OH groups. Oxidation at C-3′ or C-4′ could be excluded, from among other considerations, by observation of doublets

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*Following established nomenclature, product **2** is regarded as a 2-ketose derivative, so that the sugar-chain numbering is reversed from that in the precursor **1**. The four asymmetric centers (C-3, 4,5,6) have the D-*ido* relative stereochemistry *if* N-9 of the purine is regarded as part of the backbone chain. Furanose ring-closure involving O-5 and C-2 leads to tautomers having O-5 and O-2 formally *cis* (α) or *trans* (β) in the Fischer projection. The major tautomer (α by this convention) could be designated 6-(6-chloropurin-9-yl)-6-*S*-ethyl-6-thio-D-*glycero*-α-L-*xylo*-hexulofuranose by following the British–American Rules for Carbohydrate Nomenclature[14], and this convention is used here for assigning α,β symbolism. An equally unambiguous name, introducing no assumptions beyond published rules, would be (6*S*)-6-(6-chloropurin-9-yl)-6-*S*-ethyl-6-thio-α-L-*xylo*-hexulofuranose, and this leads to the same anomeric symbolism.

In contrast, the IUPAC–IUB Tentative Rules for Carbohydrate Nomenclature[15] develop the anomeric symbol by reference to the highest-numbered, asymmetric center (in this instance, C-6), which would be quite ambiguous for the compounds described here, even though the chirality (6*S*) at C-6 is firmly established[3]. As there are numerous examples of acyclic-sugar nucleosides whose chirality at the point of attachment of the base remains to be established[2,16], it is clear that this asymmetric center is totally unsuitable as the point of reference for specifying the anomeric designation.

TABLE I

^{13}C-N.M.R. CHEMICAL-SHIFT DATA[a] FOR SOLUTIONS OF D-FRUCTOSE AND NUCLEOSIDE ANALOGS **1** AND **2**

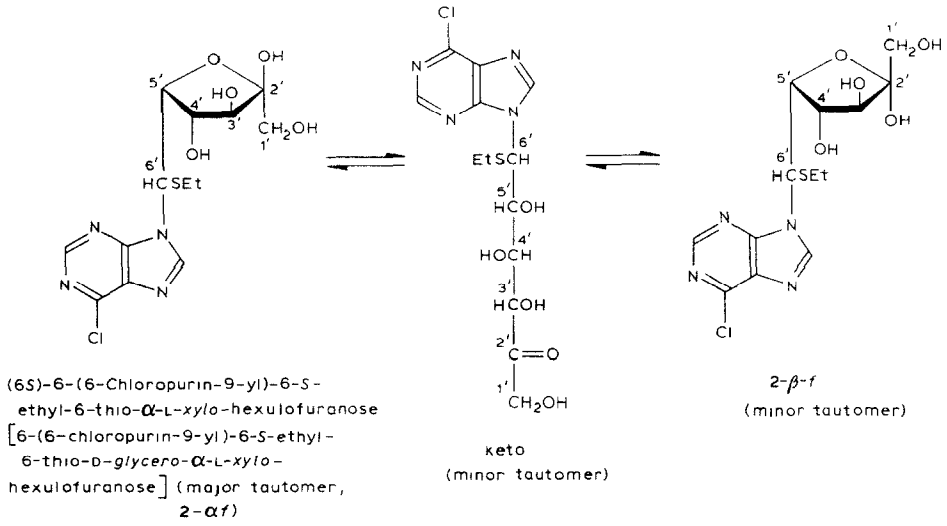
Tautomer	*Atom*[b]	D-*Fructose*[b]	**2**	**1**
Furanose		α-D	β-L	
	C-1′	61.4	62.6	
	C-2′	104.4	106.7	
	C-3′	81.2[c]	79.5 } [c]	
	C-4′	83.1	80.1 }	
	C-5′	76.0	n.o.[d]	
	C-6′	61.4	60.4	
	S*CH*$_2$CH$_3$		24.9	
	SCH$_2$*CH*$_3$		14.3	
Furanose		β-D	α-L	
	C-1′	63.3[e]	63.9	
	C-2′	102.2	103.6	
	C-3′	76.0	78.1 } [c]	
	C-4′	82.1	76.2 }	
	C-5′	75.6	75.5 }	
	C-6′	63.4[e]	59.7	
	S*CH*$_2$CH$_3$		24.9	
	SCH$_2$*CH*$_3$		14.3	
Acyclic				
	C-1′	[f]	66.2	63.2[g]
	C-2′		211.6	71.3[e]
	C-3′		n.o.[d]	71.4[e]
	C-4′		n.o.[d]	70.0
	C-5′		72.5	73.5
	C-6′		61.2	61.5
	S*CH*$_2$CH$_3$		24.5	24.5
	SCH$_2$*CH*$_3$		14.3	14.3

[a]In dimethyl sulfoxide-d_6, in p.p.m. downfield from Me$_4$Si. [b]From ref. 19; the numbering for **2** is shown in Scheme 2. [c]Not assigned. [d]Not observed (not separated from major signals). [e]Assignments could be reversed. [f]Not available. [g]The numbering for **1** is reversed to correspond to that for **2**.

for these signals in the off-resonance spectra. Reference to the detailed, ^{13}C correlations of Szarek and co-workers[17] for oxidation products of asymmetric alditols further consolidated the assignments.

Diagnostic ^{13}C signals for the three tautomers and their approximate proportions were the free carbonyl resonance (δ 211.6) for the acyclic form, the anomeric carbon signals (δ 106.7 and 103.6) of the furanose forms, and three different doublets near δ 60 (C-6′) and triplets near δ 63–66 (C-1′). By averaging the respective signal-intensities[18], the equilibrium composition was estimated to be ~7% of the acyclic form, ~11% of the β-furanose, and ~82% of the α-furanose (see Scheme 2).

Differentiation of the major cyclic tautomer ($\delta_{C-2'}$ 103.6, α-L-*xylo*) from the minor one ($\delta_{C-2'}$ 106.7, β-L-*xylo*) was made by comparison (see Table I) of the C-2′ chemical shift of the major cyclic tautomer with the C-2 shifts of β-D-fructofuranose



Scheme 2. Three tautomeric forms of 2 at equilibrium in neutral solution

(δ_{C-2} 102.2) and its α anomer (δ_{C-2} 104.4); the α-L-*xylo* tautomer of **2** is isosteric with β-D-fructofuranose at all points except the C-5′ substituent. In contrast, the C-2 resonance of α-D-fructofuranose appears at lower field (δ 104.4). It may reasonably be assumed that the relative order of the anomeric carbon signals is not altered by stereochemical change at the remote position (C-5′). It has been reported[20,21] that the chemical shifts of anomeric carbon atoms are closely dependent on the dispositions of neighboring hydroxyl groups, but are insensitive to configurational changes elsewhere in the ring. In further support of these conclusions, it may be noted that the C-2 resonance[22] of α-L-sorbofuranose, which is fully isosteric with the α-L-furanose form of **2**, also lies at comparable field (δ 101.7) to that observed for the major tautomer of **2**.

Conventional acetylation of **2** with acetic anhydride–pyridine gave, after chromatographic purification, in rather poor yield, a syrupy, homogeneous tetraacetate **3**, whose n.m.r. spectra indicated it to be one of the acetylated furanoses, or, possibly, a mixture of the two. The low yield may be attributed to removal of by-

products of elimination and of the acetate of the acyclic form during the chromatographic purification.

The electron-impact, mass spectrum of **3** was used to confirm the molecular formula, and qualitative inspection of this spectrum, in comparison with that[3] of the peracetate of the acyclic precursor (**1**), showed major qualitative differences that could be correlated with the presence of a cyclized sugar component in **3**. The 90-MHz, ^{1}H-n.m.r. spectrum of **3** in chloroform-*d* showed four distinct, 3-proton singlets for acetate groups (δ 2.21, 2.12, 2.10, and 2.07), but the low-field region was not readily analyzed. In acetone-d_6, the H-6′ signal resonated as a doublet at δ 6.05, thus resembling the H-1′ signal in typical acyclic-sugar nucleosides. A doublet of doublets was observed for H-5′ (showing $J_{4',5'}$ 9.5 Hz), indicating the near-eclipsed[23] disposition of H-4′ and H-5′. Signals for H-3′ and H-4′ overlapped, but those for H-1′ gave rise to a typical AB system (J_{AB} 11.5 Hz). In the absence of an anomeric proton, it was not possible to specify the structure further from the ^{1}H-n.m.r. data, but the sharpness of the representative signals suggested that the tetraacetate **3** may be the α anomer only, rather than an anomeric mixture.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer Model 141 recording polarimeter. I.r. and u.v. spectra were recorded with Perkin–Elmer Infracord and Cary 15 spectrophotometers, respectively. T.l.c. was performed on silica gel 60 F-254 (No. 5765, E. Merck). Preparative t.l.c. was performed on chromatoplates (200 × 200 × 2.5 mm) of silica gel 60 PF-254 (No. 7747, E. Merck) containing 1% of Lumilux Green 25. ^{1}H-N.m.r. spectra were recorded with a Bruker HX-90 spectrometer at ~25°. The following conventions are used to refer to n.m.r. spectra: d, doublet; dd, doublet of doublets; m, multiplet; q, quartet; and t, triplet. ^{13}C-N.m.r. spectra were recorded by C. Cottrell with a Bruker WP-80 spectrometer operating at 20 MHz in the Fourier-transform mode at ~25°. Chemical shifts are reported in p.p.m. relative to Me_4Si. Electron-impact mass spectra were recorded by C. R. Weisenberger with an AEI MS-9 double-focusing, mass spectrometer at an ionization potential of 70 eV and an accelerating potential of 8 kV.

Organism. — *Acetobacter suboxydans* (ATCC No. 621H), purchased from the American Type Culture Collection, Rockville, MD, was maintained at 4° on an agar slant containing D-glucitol (5%, w/v) and agar (1.5%, w/v).

All cells used were grown by heavily inoculating a standard broth of D-glucitol (50 mL of broth in 250-mL, Erlenmeyer flasks) containing D-glucitol (7.2%, v/v, of 70% D-glucitol solution, ICI America, Inc.), yeast-extract powder (0.5%, w/v), potassium dihydrogenphosphate (0.05%, w/v), and D-glucose (0.05%, w/v). The flasks were covered with lint squares, and sterilized. Inoculations were performed in sterilized air in a hood to avoid contamination. The shaker used was a portable gyratory shaker (Model G-2, New Brunswick Scientific Co., Inc.). The broths were

shaken (300 r.p.m.) for 2–3 days at 34°, and then harvested and used immediately.

Oxidation of 1-(6-chloropurin-9-yl)-1-S-ethyl-1-thio-D-glycero-D-ido-*hexitol* [*(1S)-1-(6-chloropurin-9-yl)-1-S-ethyl-1-thio*-D-*glucitol*] (**1**) *to 6-(6-chloropurin-9-yl)-6-S-ethyl-6-thio*-D-ido-*hexulose* [*(6S)-6-(6-chloropurin-9-yl)-6-S-ethyl-6-thio*-L-xylo-*hexulose*] (**2**). — *Method A.* Standard broths of D-glucitol (600 mL, with 50 mL in each 250-mL Erlenmeyer flask) were inoculated, and grown for 3 days, and the cells were collected by centrifugation at 2,000*g* for 30 min at 25°. The cells were then washed with 0.01M phosphate buffer, and re-collected by centrifugation. The washing procedure was repeated 2–3 times to remove L-sorbose and nutrients. The collected bacteria (~6 mL of wet-packed cells) were suspended in substrate-containing solution, prepared by dissolving compound **1** (300 mg) in distilled water (50 mL) in a 250-mL, Erlenmeyer flask without sterilization. The solution was then incubated for 10 days at 34° with continuous, rotary shaking (300 r.p.m.). The oxidation was terminated by adding ethanol (150 mL), and the mixture was kept for 1 h at ~25°. A small amount of activated charcoal was added, the suspension was boiled for a few min, and filtered. The filtrate was evaporated somewhat (underd iminished pressure) to remove ethanol, and the water remaining was removed by freeze-drying. The resulting, crude syrup was applied to a preparative, t.l.c. plate (200 × 200 × 2.5 mm), which was developed twice with 3:2 acetone–benzene. The faster-migrating band was excised, and extracted, first with warm ethanol, and then with cold methanol. (The other band was the starting compound **1**.) The extract was evaporated to give **2** as a homogeneous syrup, yield 0.10 g (33%); R_F 0.47 (3:2 acetone–benzene); $[\alpha]_D^{27}$ −53° (*c* 0.6, methanol); λ_{max}^{MeOH} 267 nm (ε_{mM} 3.50); ν_{max}^{film} 3500–3200 (OH), 2980, 2940 (C–H), 1730 (C=O, weak), 1595, 1570 (purine ring), 1490, 1400, 1345, and 1150–1050 cm^{-1} (C–O–C); ^{1}H-n.m.r. (D_2O): δ 8.81, 8.77, 8.72 (H-2), 8.59 (H-8), 5.87 (d, $J_{5',6'}$ 5.3 Hz, H-6′), 4.13–4.00 (m, H-3′,4′), 3.51 (m, H-1′,1″), 2.36 (q, SCH_2CH_3), and 0.94 (t, SCH_2CH_3); ^{13}C-n.m.r. (Me_2SO-d_6): δ 14.3 (SCH_2CH_3), 24.5, 24.9 (SCH_2CH_3), 59.7, 60.4, 61.9 (C-6′), 62.6, 63.9, 66.2 (C-1′), 72.5, 79.5, 80.1 (C-3′–5′), 75.5, 76.2, 78.1 (C-3′–5′, α anomer), 103.6 (C-2′, α anomer), 106.7 (C-2′, β anomer), 211.6 (C-2′, acyclic form), 130.7 (C-5), 146.8 (C-8), 149.2 (C-4), and 151.5 (C-2,6).

Anal. Calc. for $C_{13}H_{17}ClN_4O_5S$: C, 41.48; H, 4.56; Cl, 9.30; N, 14.89. Found: C, 41.56; H, 4.72; Cl, 9.47; N, 15.03.

Method B. The substrate **1** (300 mg) was dissolved in distilled water (50 mL) in a 250-mL, Erlenmeyer flask that was tightly stoppered, and connected to an oxygen reservoir. The same amount of cells as described in Method A was suspended, and the incubation was performed under an atmosphere of oxygen, instead of air. The oxygen was replenished at 1–2-day intervals, and the oxidation was terminated after 8 days. By following the same procedure of purification as in A, a homogeneous syrup was obtained; yield, 0.21 g (70%), identical with that produced by Method A.

(6S)-1,2,3,4-Tetra-O-*acetyl-6-(6-chloropurin-9-yl)-6-S-ethyl-6-thio-α,β*-L-xylo-*hexulofuranose* [*1,2,3,4-tetra*-O-*acetyl-6-(6-chloropurin-9-yl)-6-S-ethyl-6-thio*-D-glycero-α,β-L-xylo-*hexulofuranose*] (**3**). — A solution of compound **2** (50 mg, 0.13 mmol) in pyridine (2 mL) was cooled to 0°, and acetic anhydride (0.25 mL) was added. The

solution became dark on being kept overnight at room temperature. Cold water was then added dropwise to the solution, cooled in an ice bath, until heavy precipitation occurred. The orange precipitate was filtered off, the filtrate extracted with portions of chloroform, and the precipitate dissolved in the combined extracts. The solution was concentrated, and the concentrate was applied to a preparative chromatoplate of silica gel, which was developed with 17:3 chloroform–acetone. The u.v.-active, orange band, which migrated slightly faster than a u.v.-inactive yellow band, was excised, and extracted with several portions of acetone. Evaporation of the combined extracts gave chromatographically homogeneous 3 as a light-yellow glass; yield, 20 mg (28%); R_F 0.48 (6:1 chloroform–acetone); $[\alpha]_D^{27}$ −69° (*c* 0.05, acetone); ν_{max}^{KBr} 2880, 2800 (C–H), 1740 (C=O of acetate), 1575, 1540 (purine ring), 1360, 1200, and 1090–1000 cm^{-1} (C-O-C); ^{1}H-n.m.r. (90 MHz, acetone-d_6): δ 8.74 (H-2,8), 6.05 (d, H-6′, $J_{5',6'}$ 5.40 Hz), 5.27 (dd, H-5′, $J_{4',5'}$ 9.5 Hz), 5.66–5.47 (m, H-3′,4′), 4.74 (d, H-1′), 4.45 (d, H-1″, $J_{1',1''}$ 11.5 Hz), 2.55 (q, SCH_2CH_3), and 1.18 (t, SCH_2CH_3).

Anal. Calc. for $C_{21}H_{25}ClN_4O_9S$ (544.1031). Found: 544.1041 (exact mass).

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