Carbohydrate Research, 113 (1983) 166–171
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Synthesis and conformation of 5-thio-D-glucal, an inhibitor of glycosidases

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(Received August 9th, 1982; accepted for publication, August 31st, 1982)

Molecules whose geometry resembles that of the transition state in enzyme-catalyzed reactions are known to be excellent enzyme-inhibitors. Carbohydrate substrates of glycosidases are known to produce a carboxonium ion in the transition state, the geometry of which probably resembles that of a flattened, half-chair conformer. As the conformation of glycals is also a half-chair, somewhat resembling the transition state, these and similar compounds have been of considerable interest as inhibitors of glycosidases (reviewed by Flowers and Sharon[1]).

D-Glucal (1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol) was found[2] to inhibit competitively the α-D-mannosidase of sweet-almond emulsin with a K_i of 10mM. Subsequently, glycosidases were found to catalyze the hydration of the double bond in D-glycals, to afford 2-deoxy sugars, as well as rearrangement products. Thus, Lehmann and Schröter[3] reported that sweet-almond β-D-glucosidase catalyzes the hydration of D-glucal to 2-deoxy-D-*arabino*-hexose, and Hehre *et al.*[4] elucidated the stereochemistry of the hydration of D-glucal by both α- and β-D-glucosidases.

Hence, it was of interest to synthesize a sulfur analog **3** of D-glucal, which was expected to be a transition-state inhibitor of D-glucosidases and D-mannosidases, with, however, somewhat changed properties. In addition to possible effects of the sulfur atom on the conformation, the reactivity of the 1,2 double bond in **3** would be lessened, because of the lower electronegativity of sulfur compared to that of oxygen.

We have utilized the classical synthesis of glycals in which the per-*O*-acetylated bromide (**1**) of 5-thio-D-glucose was treated with Zn in acetic acid, resulting in a good yield of 3,4,6-tri-*O*-acetyl-5-thio-D-glucal (**2**). Deacetylation of **2** gave 5-thio-D-glucal (1,5-anhydro-2-deoxy-5-thio-D-*arabino*-hex-1-enitol, **3**).

The conformation of 3,4,6-tri-*O*-acetyl-D-glucal (**4**) has been established as being $^4H_5$ by X-ray crystallography[5]. ^{1}H-N.m.r. investigations of **4** in solution have been interpreted as indicating an equilibrium mixture of two half-chair forms[6], *viz.*, $^4H_5$ and $^5H_4$, although, earlier, these data had been interpreted in terms of a

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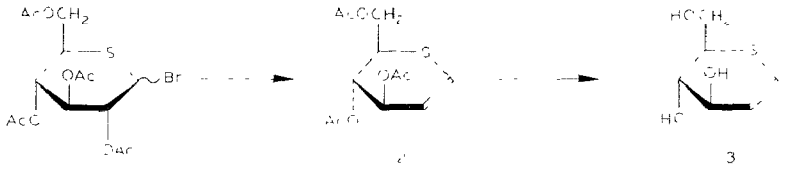
TABLE I

CHEMICAL SHIFTS AND COUPLING CONSTANTS OF 5-THIO-D-GLUCAL (3) AND DERIVATIVE

Compound	*Chemical shifts (δ)*[a] *(first-order couplings, Hz, in parentheses)*								
	H-1		*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6′*	*Acetyl CH_3*
	($J_{1,2}$)	($J_{1,3}$)	($J_{2,3}$)	($J_{3,4}$)	($J_{4,5}$)	($J_{5,6}$)	($J_{5,6'}$)	($J_{6,6'}$)	
2[b]	6.27q		5.72q	5.35 (unresolved m)	5.35 (unresolved m)	3.64o	4.33q	4.27q	2.05s, 2.08s, 2.10s
	(10.64)	(1.06)	(3.54)	(n.d.)	(7.72)	(6.85)	(6.10)	(11.23)	
3[c]	6.70q		6.18q	4.69o	4.38m	3.92m	4.38m	4.38m	
	(10.0)	(1.8)	(2.8)	(7.2)	(9.0)	(6.4)	(4.6)	(n.d.)	
D-Glucal[c]	6.86m		5.26	4.65m	4.14m	4.35m	4.31m	4.31m	
	(6.1)	(1.6)	(2.2)	(6.8)	(n.d.)	(n.d.)	(n.d.)	(n.d.)	

[a]Signal multiplicities: d, doublet; m, multiplet; o, octet; q, quartet; s, singlet. [b]Determined at 400 MHz, in $CDCl_3$, using internal tetramethylsilane. [c]Determined at 100 MHz, in D_2O, from external tetramethylsilane; n.d., not determined.



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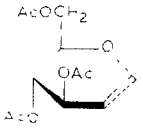

4

distorted, half-chair form[7,8]. The ^{1}H-n.m.r. spectrum of **2** (the corresponding 5-thio analog) could not be completely resolved, even at 400 MHz, but the deacetylated derivative **3** provided first-order coupling-constants for the ring protons (see Table I). A comparison of $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ of the latter (2.8, 7.2, and 9.0 Hz, respectively; see Table I) with the coupling constants published for 3,4,6-tri-*O*-acetyl-D-glucal (3.2, 5.8, and 7.8 Hz, respectively[6]) suggested that both compounds exist as a mixture of the aforementioned two half-chair forms, in which the $^4H_5$ form preponderates for the thio analog.

^{13}C-N.m.r. resonances and one-bond, C–H couplings of the 5-thio-D-glucals are summarized in Table II and are compared with those of **4** and D-glucal. Replacement of O by S dramatically affects the ring-carbon resonances, particularly those of C-1 and C-5. Likewise, the corresponding, one-bond coupling-constants are much smaller for the sulfur derivatives, as expected from the decreased electronegativity of sulfur.

The double bond in **2** and **3** was found to be much less reactive than that in ordinary glycals. Thus, **2** was resistant to fluorination[9] with XeF_2–BF_3, as well as to BF_3-catalyzed, azide formation, which is known to occur with the corresponding

TABLE II

^{13}C-N.M.R. SPECTRA OF COMPOUNDS **2**, **3**, AND **4**, AND D-GLUCAL

Compound	*C-1* (J_{CH})	*C-2* (J_{CH})	*C-3* (J_{CH})	*C-4* (J_{CH})	*C-5* (J_{CH})	*C-6* (J_{CH})
2[a]	123.4(177)	117.9(168)	68.1(154)	68.0(154)	40.4(141)	62.4(152)
3[b]	121.7(176)	123.6(172)	70.9(145)	71.7(158)	47.0(140)	61.9(145)
4[c]	145.4(192)	98.9(170)	67.4(155)	67.2(155)	73.9(152)	61.3(152)
D-Glucal[b]	144.6(188)	103.8(161)	69.3(152)	69.9(151)	79.2(150)	61.2(146)

[a]Determined at 25.2 MHz, in $CDCl_3$, from tetramethylsilane as internal standard; δ_{Me} of acetates 20.6, 20.8; δ_{CO} 169.4, 169.7. [b]Determined at 25.2 MHz, in D_2O, from tetramethylsilane as external standard. [c]Determined as in footnote a, δ_{Me} of acetates 20.8, 21.0; δ_{CO} 169.3, 170.1, 170.3.

glycals[10]. In addition, attempts to obtain the 2-deoxy derivative of 5-thio-D-glucose by the acid-catalyzed hydration of the double bond[11] in **3** were unsuccessful, due to polymerization and degradation of the starting material.

In view of this unreactivity of the double bond, and the similarity of the conformation of 5-thio-D-glucal (**3**) with that of D-glucal, it was of interest to study the interaction of **3** with D-glucosidases and D-mannosidases. On incubation for 60 h with β-D-glucosidase from almonds, 5-thio-D-glucal was recovered unchanged, whereas Lehmann and Schröter[3] found substantial hydration of the double bond in D-glucal with this enzyme. Nevertheless, D-glucal and its thio analog **3** were found to be competitive inhibitors of the enzyme, with K_i values of 9.7 (±1.7) and 6.3 (±2.8) mM, respectively. 5-Thio-D-glucal (**3**) was also found to be a competitive inhibitor of the α-D-mannosidase from jack beans, with a K_i of 3.9 ±1.0mM, which compares favorably with the reported K_i of 10mM for D-glucal, and that found by us for the latter (13.8 ±3mM, by competitive-inhibition kinetics).

Thus, 5-thio-D-glucal (**3**) was found not to be a substrate, but a competitive inhibitor of glycosidases. This may be due to the relative, chemical inertness of its double bond as compared to that in D-glucal on the one hand and its conformational similarity to it on the other. Because sulfur is a poor hydrogen-bond acceptor, a hydrogen bond to the ring-oxygen atom, also, does not appear to be important in the binding of inhibitors, or substrates, of the glycosidases investigated.

EXPERIMENTAL

General methods. — (*a*) *Chemical methods.* Melting points (uncorrected) were determined by the capillary method; i.r. spectra were recorded with a Perkin–Elmer 457 spectrophotometer, and ^{1}H-n.m.r. spectra, with a Varian XL-100 instrument operated in the F-t mode at 100 MHz. The latter instrument was also used for recording ^{13}C-n.m.r. spectra at 25.2 MHz. Positions of peaks are expressed in δ from the signal of tetramethylsilane as the internal standard. Assignment of ^{13}C-signals was achieved by off-resonance, proton decoupling. Residual coupling-constants (J_R) were measured at two decoupler offsets which flanked the proton resonances. A computer program (based on the mathematical treatment of MacDonald and Mazurek[12]) was used to calculate the decoupling intensity (K) in Hz, from a compound having a known value of $^1J_{CH}$; then the residual coupling-constants and K were used to calculate $^1J_{CH}$ and the frequency s of the associated proton for each carbon signal. Ambiguities arise where proton chemical-shifts almost coincide, or where extensive overlap in the off-resonance, ^{13}C spectrum creates errors in measurements of J_R.

Optical rotations were determined with a Perkin–Elmer 141 polarimeter. Thin-layer chromatograms were obtained with plates of Merck MF-254 silica gel, the spots being detected by u.v. absorption, or by spraying with a sulfuric acid solution.

(*b*) *Enzyme procedures.* α-D-Mannosidase (α-D-mannoside mannohydrolase, (EC 3.2.1.24) from jack beans was a commercial product (Sigma) containing 20

units of enzyme per mg of protein. The substrate used was *p*-nitrophenyl α-D-mannopyranoside, and the assay procedure, that of Li and Li[14]; K_m 5.7 ±3mM. β-D-Glucosidase (emulsin; β-D-glucoside glucohydrolase, EC 3.21.21) from almonds was a commercial product (Sigma) containing ~5 units of enzyme per mg of protein. The substrate used was *p*-nitrophenyl β-D-glucopyranoside, and it was assayed according to Schwartz *et al.*[2]; K_m 0.9 ±0.2mM.

The enzyme kinetic data were analyzed with a Hewlett-Packard HP-85 microcomputer, using a nonlinear, regression-curve-fitting package developed by Greco *et al.*[15].

3,4,6-Tri-O-acetyl-5-thio-D-glucal (**2**). — 1,2,3,4,6-Penta-*O*-acetyl-5-thio-α-D-glucopyranose (650 mg, 1.7 mmol) was dissolved in CH_2Cl_2 (10 mL), a freshly prepared, saturated solution of HBr in glacial acetic acid (2 mL) was added, and the solution kept overnight at 0°. After evaporation *in vacuo*, the residual, crude bromide[10] was dissolved in glacial acetic acid (10 mL), Zn dust (1.2 g) was added, and the mixture was stirred for 2.5 h at room temperature, filtered, and the filtrate evaporated. A solution of the residue in ethyl acetate was successively washed with $NaHCO_3$ solution and water, and dried (Na_2SO_4). Chromatography on a column of silica gel (20 g) with 2:1 ether–pet. ether gave **2** (69%), which crystallized from hexane; m.p. 43–44°, $[\alpha]_D^{22}$ 47.1° (*c* 1, chloroform).

Anal. Calc. for $C_{12}H_{16}O_6S$: C, 49.99; H, 5.59; S, 11.12. Found: C, 49.94; H, 5.42; S, 11.33.

5-Thio-D-glucal (**3**). — To a solution of **2** (186 mg, 0.65 mmol) in methanol (10 mL) was added methanolic 0.1M sodium methoxide (1 mL). After 1 h at room temperature, the solution was treated with Amberlite IR-120 (H^+) resin, and evaporated. The oily residue was dissolved in methyl formate, affording crystalline **3** (57 mg, 54%); m.p. 83–86°, $[\alpha]_D^{22}$ +17.3° (*c* 1, methanol).

Anal. Calc. for $C_6H_{10}O_3S$: C, 44.43; H, 6.21. Found: C, 44.43; H, 6.25.

ACKNOWLEDGMENT

This study was supported by USPHS Grants CA-08793 and CA-13038.

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