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Note

^{13}C-N.m.r.-spectral study of some biologically relevant, synthetic, thio sugars

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Thio sugars are carbohydrates that contain a sulfur atom instead of an oxygen atom attached to the carbon skeleton of the molecule[1]. For aldoses, this replacement may occur at C-1 (to form 1-thioaldoses), C-4 or C-5 within the ring system (to afford 4-thioaldofuranoses or 5-thioaldopyranoses), or at the terminal carbon atom (to give ω-thio sugar derivatives)[1]. These thio sugars are isoelectronic with their oxy homologs. The replacement of an oxygen atom by a sulfur atom in the carbon skeleton apparently has only a slight effect on the conformation of the ring system; however, it is known to affect the rate of mutarotation of the system, and usually, as in the case of the 1-thio- and 5-thio-D-glucoses, only one preponderant, anomeric state is found in solution[1].

Thio sugars are found throughout Nature. For instance, glycosides of 1-thio-D-glucose are found in mustard-seed oils and various vegetables, and have been located in animal tissue after administration of various thio compounds[1]. (Thioglyco)peptides have also been isolated from human urine[2,3]. ω-Thio sugars (terminal derivatives) have been observed in Nature in the form of natural products, and as "active" intermediates in chemical reactions, and are also involved in biochemical methylations[1].

Recently, thio sugars (and their derivatives) have been examined as possible therapeutic agents. Gold(I)-1-thio-D-glucose compounds have been tested as antiarthritic agents[1,4]. Testing of this thio sugar as a possible antileukemia agent has been conducted[5], and it has been shown to stimulate the release of insulin in rats[6]. 5-Thio-D-glucopyranose is known to inhibit the transport of D-glucose[7], and has been found to kill tumor cells selectively under hypoxic conditions[8].

In view of the importance of thio sugars, we decided to obtain ^{13}C-n.m.r.-

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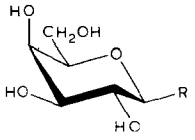
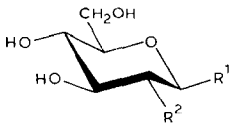
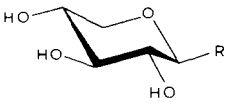


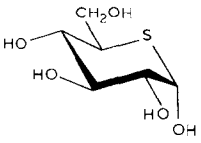
1 R = SNa^+
2 R = $SCHMe_2$
3 R = $SC_6H_4NH_2$-*p*
4 R = $SCH_2C_6H_4NH_2$-*p*
5 R = S-β-D-galactopyranoside
6 R = $SC_6H_4NO_2$-*p*
7 R = OH
8 R = OMe



9 R^1 = SNa^+, R^2 = OH
10 R^1 = $SCHMe_2$, R^2 = OH
11 R^1 = $SC_6H_4NH_2$-*p*, R^2 = OH
12 R^1 = $SC_6H_4NO_2$-*p*, R^2 = OH
13 R^1 = $SC_6H_4NH_2$-*p*, R^2 = NHAc
14 R^1 = OH, R^2 = OH
15 R^1 = OMe, R^2 = OH



16 R = $SC_6H_4NH_2$-*p*
17 R = OMe



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spectral data for some of these compounds, Presented herein are ^{13}C-n.m.r.-spectral data for 1-thio-β-D-galactose (**1**), 1-thio-β-D-glucose (**9**), a 1-thio-β-D-xyloside (**16**), and 5-thio-α-D-glucose (**18**), as well as for some of their derivatives. The chemical-shift data may facilitate the use of ^{13}C-n.m.r. spectroscopy as an analytical tool for the analysis of thio sugar derivatives in urine, as well as a tool for *in vivo* studies of ^{13}C-enriched, thio sugar drugs.

EXPERIMENTAL

Materials and methods. — The 1-thio-β-D-glucose, 1-thio-β-D-galactose, 1-thio-β-D-xyloside, and 5-thio-α-D-glucose, as well as their various derivatives, were purchased from Sigma Chemical Co., St. Louis, MO. Deuterium oxide and methanol-d_4 were obtained from Cambridge Isotope Laboratories, Cambridge, MA.

The ^{13}C-n.m.r. spectra of the thio sugars were recorded with a Varian XL-100 spectrometer operated at 25.2 MHz, using 12-mm sample-tubes. The data were collected in 16,384 addresses, using a recycle time of 2 s. Sample concentrations were typically 0.13 to 0.25M (in H_2O containing ~5% of D_2O) in the pH range of 6.5 to 7.1, unless otherwise stated in the respective Table of data, and temperatures

of samples were typically 27-31°. The ^{13}C-n.m.r.-spectrum of 5-thio-α-D-glucose was recorded with a JEOL-FX90Q instrument operated at 22.5 MHz, as previously described[9].

Coupling constants ($^1J_{C-H}$) were obtained from the ^{13}C-coupled spectra of these sugars, as described[10]. Chemical shifts are given relative to a trace of internal 1,4-dioxane, whose chemical shift[11] was taken to be 67.86 p.p.m

TABLE I

CARBON-13-N.M.R. CHEMICAL-SHIFT DATA[a] FOR SOME 1-THIO-β-D-ALDOPYRANOSIDES AND 5-THIO-α-D-GLUCOPYRANOSE

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
1[b]	85.8	77.4	75.1	71.0	80.1	62.7
	(150)	(143)	(140)	(144)	(140)	(144)
	δ 0.47	δ 0.41	δ 0.13	δ 0.20	δ 0.0	δ 0.12
2	86.8	71.2	75.4	70.2	80.1	62.4
	δ 0.0	δ 0.11	δ 0.14	δ 0.11	δ 0.03	δ 0.13
3	90.4	70.5[c]	75.5	70.1[c]	80.3	62.4
4	85.7	70.9	75.4	70.2	80.2	62.4
5	84.9	71.1	75.4	70.3	80.4	62.7
6	88.5	71.1	76.6	70.8	81.2	63.2
7[d]	97.7	73.3	74.2	70.1	76.3	62.3
8[d]	104.9	71.8	73.9	69.8	76.2	62.2
9	85.1	79.7	78.3	71.7	81.1	62.6
	(152)	(145)	(144)	(144)	(143)	(144)
	δ 0.34	δ 0.33	δ 0.16	δ 0.18	δ 0.0	δ 0.14
10	85.8	73.8	78.7	71.0	81.1	62.3
	δ 0.0	δ 0.09	δ 0.15	δ 0.09	δ 0.01	δ 0.10
11	89.3	72.9	78.7	70.8	81.2	62.3
12	87.9	74.2	80.0	71.6	82.5	63.1
13	84.5	56.2	77.8	72.5	82.4	63.4
14[d]	96.5	74.8	76.4	70.5	76.6	61.5
15[d]	104.3	74.2	76.9	70.8	76.9	61.9
16	90.0	72.9	78.8	70.4	70.1	
	(156)	(145)	(146)	(147)	(146)	
	δ 0.03	δ 0.23	δ 0.20	δ 0.15	δ 0.03	
17[d]	105.1	74.2	76.9	70.4	66.3	
18	74.5	75.0[c]	76.7[c]	75.0[c]	44.4	61.6
	(159)	(143)	(141)	(141)	(141)	(145)
	δ 0.13	δ 0.25	δ 0.15	δ 0.25	δ 0.08	δ 0.10

[a]Chemical shifts for these compounds are given at neutral pH (6.5–7.1), except for **1** (pH 9.0), **9** (pH 8.5), and **18** (pH 5.0). Compounds **6**, **12**, and **13** were dissolved in CD_3OD, because of their low solubility in H_2O. For some of the compounds, the datum in parentheses under the chemical shift is the coupling constant (J_{CH}) for that carbon atom; the datum under the coupling constant (signified by δ) is the differential isotope-shift (chemical shift in H_2O–D_2O) observed for that carbon atom. [b]The chemical shift of C-1 is 81.4 p.p.m. at pH 6.3. [c]Assignments may have to be reversed. [d]Data obtained from ref. 12

RESULTS AND DISCUSSION

Table I gives the ^{13}C-chemical-shift data, some ^{13}C–^{1}H coupling-constants, and some deuterium-induced, differential isotope-shift data for 1-thio-β-D-galactose (**1**), 1-thio-β-D-glucose (**9**), 1-thio-β-D-xyloside (**16**), and 5-thio-α-D-glucose (**18**), as well as for some of their derivatives (*p*-aminophenyl, *p*-nitrophenyl, *p*-aminobenzyl, and isopropyl). The ^{13}C-chemical-shift data given in Table I for D-glucose, D-galactose, and D-xylose were taken from work by Gorin and Mazurek[12].

The solvent employed for compounds **6, 12,** and **13** was CD_3OD instead of H_2O–D_2O, because of the limited solubility of these compounds in H_2O. However, it is considered that the chemical-shift difference for the carbohydrate carbon atoms in the two media is small. This conclusion is based on the fact that a difference of <1.0 p.p.m. was observed for the chemical shifts of the carbohydrate carbon atoms of compound **4** in H_2O and CD_3OD, and the resonance pattern remained essentially the same. The assignments of the resonances to specific, carbohydrate carbon atoms was based on chemical-shift comparisons of the various thio compounds (and, to some extent, of their oxy analogs), ^{13}C–^{1}H coupling-constants and coupling patterns, and deuterium-induced, differential, isotope-shift effects (see later). Our results agree well with data published for related compounds[13–16].

The anomeric states of the thio sugars studied were known. However, the assignment of a specific resonance to the anomeric carbon atom was difficult, because of its position near the resonances of the other carbohydrate carbon atoms (see Table I). In the coupled spectra of these compounds, one resonance typically showed a larger $^{1}J_{CH}$ value (~10 Hz) than the others; this had also been observed for the anomeric carbon atoms of the oxy analogs[15]. The assignment, then, of C-1 in the present spectra was based on the larger coupling-constant. The splitting patterns in the ^{13}C coupled spectra were also used, in order to assign some of the resonances unambiguously (*e.g.*, C-6 will give a triplet).

The deuterium-induced, differential, isotope-shift effect has previously been used to aid in the assignments of ^{13}C resonances to specific, carbohydrate carbon atoms[17–20]. It was useful in our case in order to assign C-5 specifically; it was expected that C-5 would have a negligible, deuterium-induced, differential, isotope-shift effect[17–20] (see Table I).

Another method that helps in assigning ^{13}C resonances to specific carbohydrate carbon atoms consists of chemical-shift comparisons of various thio sugars and also those of the related oxygen analogs; such data are presented in Table II. The patterns of chemical-shift difference for various thio sugars (**1, 9; 2, 10;** and **11, 16;**) are similar to those observed for their oxygen analogs (**7, 14; 8, 15;** and **15, 17;**).

These similarities in chemical-shift patterns, along with deuterium-induced, differential, isotope-shift effects, and coupling patterns, aided in making the assignments given in Table I. The similarity of the patterns of chemical-shift differ-

TABLE II

COMPARISONS OF ^{13}C-CHEMICAL SHIFTS FOR 1-THIOALDO-PYRANOSES AND -PYRANOSIDES AND THEIR OXYGEN ANALOGS

$\Delta\delta$ for	Carbon atom					
indicated compounds	C-1	C-2	C-3	C-4	C-5	C-6
δ(**1**) − δ(**9**)	0.7	−2.3	−3.2	−0.7	−1.0	0.1
δ(**2**) − δ(**10**)	0.6	−2.6	−3.3	−0.8	−1.0	0.1
δ(**3**) − δ(**11**)	1.0	−2.4	−3.2	−0.6	−0.9	−0.1
δ(**3**) − δ(**16**)	0.2	−2.4	−3.3	−0.2	10.9	—
δ(**11**) − δ(**16**)	−0.7	0.0	0.0	0.4	11.0	0.8
δ(**7**) − δ(**14**)	1.2	−1.5	−2.2	−0.2	−0.3	−0.2
δ(**8**) − δ(**15**)	0.6	−2.4	−3.0	−1.0	−0.7	—
δ(**8**) − δ(**17**)	0.2	−2.2	−3.0	−0.6	9.9	—
δ(**15**) − δ(**17**)	0.8	0.2	0.0	0.4	10.6	—

ences also indicates that the conformations of the thio sugars approximate those of the oxy sugars.

In conclusion, we have presented ^{13}C-n.m.r.-spectral data for a variety of 1-thio-β-D-aldopyranose derivatives and a 5-thio-α-D-aldopyranose. These data will facilitate the use of ^{13}C-n.m.r. spectroscopy as an analytical tool for analysis of thio sugars in body fluids, and also have potential use in experiments[21] employing ^{13}C-enriched, thio sugar drugs *in vivo*.

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