5-Thio-D-mannose from the Marine Sponge *Clathria pyramida* (Lendenfeld). The First Example of a Naturally Occurring 5-Thiosugar

Robert J. Capon* and John K. MacLeod

Research School of Chemistry, Australian National University, GPO Box 4, Canberra, A.C.T. 2601, Australia

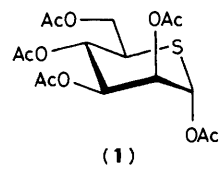
The isolation and identification of the first naturally-occurring 5-thiosugar, 5-thio-D-mannose, is reported.

Although a large number of modified sugars that exhibit significant biological activities have been isolated from terrestrial sources, reports of such compounds from the marine environment are rare.[1] In this communication we report the isolation of 5-thio-D-mannose from the marine sponge *Clathria pyramida* (Lendenfeld)† which, in addition to being the first reported modified free sugar from a sponge, represents the only naturally occurring example of this unique class of thiosugar. In the absence of a natural source, synthetic chemists provided[2] the first example of this class of compound, 5-thio-D-glucose, in 1962. Amongst its more significant biological properties 5-thio-D-glucose was found to inhibit the release of insulin[3] as well as the transport of glucose,[4] and also to cause reversible inhibition of sperm-cell development without displaying acute toxicity.[5] Thus 5-thio-D-glucose and other synthetic examples of this class of compound earned recognition as 'antimetabolites' owing to their capacity to interrupt the metabolism of natural analogues.

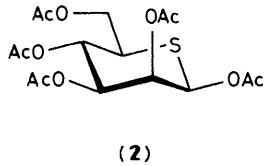
C. pyramida is an orange encrusting sponge that was observed growing over boulders at depths varying from 4 to 20 m off Durras Island on the mid-south coast of New South Wales. A crude ethanol extract of a collection of *C. pyramida* (74.8 g dry wt.) was found to inhibit the growth of both gram positive, *Bacillus subtilis*, and gram negative, *Escherichia coli*, bacteria. Partitioning of the concentrated crude extract into H_2O and lipid (CH_2Cl_2) phases revealed only the former to exhibit activity. After freeze drying, the active material was extracted into MeOH, evaporated to dryness, and fractionated by elution through Sephadex G-10 with H_2O. This resulted in the isolation of elemental sulphur (S_8) (17 mg, 0.02% dry wt.) which was determined to be responsible for the antimicrobial activity of the crude extract. Of greater significance was the presence of substantial quantities of a reducing sugar, coeluting with the leading front of sulphur on Sephadex G-10 chromatography. Although ^{1}H n.m.r. spectra of this crude material suggested the major component to be a simple pyranose sugar, ^{13}C n.m.r. analysis failed to reveal anomeric carbon resonances in the expected δ 90 to 100 range. After a number of unsuccessful attempts to resolve the mixture by gel filtration and reverse phase h.p.l.c., the crude material was treated with Ac_2O–pyridine at room temperature overnight. The resulting acetates were subsequently partitioned into EtOAc and resolved by silica m.p.l.c. (eluant, 50% EtOAc–hexane) into one major and two minor fractions.‡ Reverse phase h.p.l.c. (C18, eluant, 50% H_2O–MeOH) of the major fraction returned two sulphur-containing hexopyranose acetates.

These two compounds showed the same molecular weight by chemical ionization mass spectrometry ($[M + NH_4]^+$ at *m/z* 424), while high resolution mass measurements on the highest ion, *m/z* 347 $[M - OAc]^+$, in electron impact mass spectra (e.i.m.s.) confirmed a common molecular formulae of $C_{16}H_{22}O_{10}S$. Both isomers displayed signals in their ^{1}H and ^{13}C n.m.r. spectra consistent with five acetate moieties, accounting for all but one of the required six degrees of unsaturation. Remaining resonances in the ^{1}H n.m.r. spectra, together with values of the ^{1}H n.m.r. coupling constants,§ could be attributed to α- and β- anomers of a modified mannopyranose (Table 1). Significant upfield chemical shifts for H-5 suggested that the ring oxygen had been replaced by sulphur. Confirmation of this assignment came from their ^{13}C n.m.r. spectra in which both isomers displayed substantial upfield shifts for both C-1 and C-5 (Table 2), consistent with those previously observed[6] for the anomers of 5-thio-D-glucopyranose. As with mannopyranose penta-acetate, the α-anomer of 5-thio-mannopyranose penta-acetate (**1**) is the predominant anomer. This was established by the longer T_1 value (2.6 s) for the major anomeric proton at δ 5.85 compared with that (1.8 s) for the minor anomeric proton at δ 6.05.[7] In addition, observation of nuclear Overhauser enhancement (5%) between 5-H and 1-H in the minor anomer (**2**) required a 1,3-diaxial interaction and hence a β-pyranose configuration. The observed 86:14 ratio of α- to β-anomers of 5-thiomannopyranose penta-acetate, determined by ^{1}H n.m.r. spectroscopy is comparable to that observed[8] for 5-thio-D-glucopyranose penta-acetate (85:15, α:β).

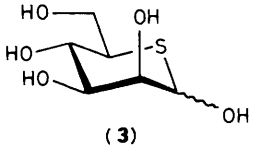
Techniques have recently been described[9] for calculating molecular rotations for 5-thio-α-D-glycopyranoses. Using these procedures the calculated value for 5-thio-α-D-mannopyranose penta-acetate ($[M]_D$ = +550 calc.) is in excellent agreement with that observed ($[M]_D$ = +590 obs.) for the major isomer described above. Deacetylation of the mixture of penta-acetate anomers with methanolic ammonia yielded a 94:6 ratio of α- and β-anomers of the free sugar (140 mg,



(1)



(2)



(3)

† A specimen of *C. (Dendrocia) pyramida* Lendenfeld lodged with the Northern Territory Museum of Arts and Sciences, Darwin, has been assigned the registry No. NTM Z2667.

‡ The minor fractions were identified as glycerol acetate (51 mg) and ethyl-2,3,4,6-tetra-*O*-acetyl-D-mannopyranoside (67 mg). Examination of other specimens of *C. pyramida* revealed varying ratios of D-mannose and the corresponding ethyl glycoside, suggesting the latter to be an artifact of the extraction procedure.

§ In the case of the α-anomer (**1**), which would appear to adopt a regular chair conformation, a 'mannopyranose' stereochemistry can be inferred from the large $J_{5,4}$ and $J_{4,3}$ values, and the small $J_{3,2}$ and $J_{2,1}$ values. Although depicted as such as in (**2**), the β-anomer appears not to adopt a regular chair conformation as evidenced from the smaller $J_{5,4}$ and $J_{4,3}$ values.

Table 1. ^{1}H N.m.r. assignments[a] for the α- (**1**) and β- (**2**) anomers of 5-thio-D-mannopyranose penta-acetate.

	(1)		(2)	
1-H	5.85	d, *J* 4.0 Hz	6.05	d, *J* 3.4 Hz
2-H	5.38	dd, *J* 4.0, 3.0 Hz	5.54	dd, *J* 3.4, 3.4 Hz
3-H	5.28	dd, *J* 3.0, 10.0 Hz	5.22	dd, *J* 3.4, 5.1 Hz
4-H	5.49	dd, *J* 10.0, 10.0 Hz	5.40	dd, *J* 5.1, 5.1 Hz
5-H	3.55	ddd, *J* 10.0, 5.8, 3.5 Hz	3.18	ddd, *J* 5.1, 8.4, 7.0 Hz
6-H	4.32	dd, *J* 5.8, 12.0 Hz	4.54	dd, *J* 8.4, 11.5 Hz
	4.09	dd, *J* 3.5, 12.0 Hz	4.47	dd, *J* 7.0, 11.5 Hz

[a] Spectra were recorded at 300 MHz in $CDCl_3$. All CH_3CO signals were present between δ 2.0 and 2.20.

Table 2. ^{13}C N.m.r. assignments[a] for the α- (**1**) and β- (**2**) anomers of 5-thio-D-mannopyranose penta-acetate, and 5-thio-α-D-mannose (**3**).

	(1)[b]	(2)[b]	(3)[c]
C-1	72.6	69.7	77.8
C-2	69.6	68.4[d]	74.8
C-3	68.5	68.2[d]	73.5
C-4	70.0	68.0[d]	72.0
C-5	40.2	40.0	45.7
C-6	61.6	64.1	62.9

[a] Spectra were recorded at 75 MHz and assignments based on 2D-HETCOR analysis. All acetate methyl resonances were present between δ 20.5 and 21.0 and acetate carbonyl signals between δ 168.0 and 170.2. [b] In $CDCl_3$. [c] In CD_3OD. [d] Assignments may be interchanged.

0.19% dry wt.).¶ Comparison of the spectroscopic data of the free sugar with that of crude material prior to acetylation confirmed 5-thio-D-mannose (**3**) to be the major natural free sugar present in the extract of *C. pyramida*.‖

¶ $[\alpha]_D$ +49.3 (*c* 0.75, H_2O); ^{1}H n.m.r. (CD_3OD, 300 MHz) for the β-anomer δ 5.02 (d, *J* 1.8 Hz, T_1 1.2 s, 1-H); for the α-anomer δ 4.77 (d, *J* 3.9 Hz, T_1 5.8 s, 1-H), 4.05 (dd, *J* 2.7, 3.9 Hz, 2-H), 3.89 (dd, *J* 4.2, 11.1 Hz, 6a-H), 3.77 (dd, *J* 6.3, 11.1 Hz, 6b-H), 3.77 (dd, *J* 9.6, 9.3 Hz, 4-H), 3.68 (dd, *J* 2.7, 9.3 Hz, 3-H), 3.17 (ddd, *J* 4.2, 6.3, 9.6 Hz, 5-H); e.i.m.s., *m/z*, 196.0406 (M^+, $C_6H_{12}O_5S$ requires 196.0405, <1%), 165 (16), 119 (34), 101 (37), 89 (35), 73 (100), 60 (65).

‖ *Note added in proof:* Since the submission of this manuscript, 5-thio-D-mannose, identical in all respects to the naturally occurring compound, has been prepared by molybdic acid isomerisation of commercially available 5-thio-D-glucose.

The possibility that 5-thio-D-mannose may not be a metabolite of *C. pyramida* but rather of a symbiont cannot be entirely excluded. The absence of chlorophylls in any extracts precludes photosynthetic symbionts such as cyanobacteria while the presence of elemental sulphur would support the presence of sulphate-reducing bacteria. In either case a biological system capable of producing 5-thio-D-mannose is at present unique and would be worthy of detailed examination. Characterisation of the microbial communities associated with *C. pyramida* is necessary to confirm their role, if any, in the biosynthesis of 5-thio-D-mannose, as well as to investigate any ecological role for this thiosugar.

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References

1 D. J. Faulkner, *Tetrahedron*, 1977, **33**, 1421; D. J. Faulkner, *Nat. Prod. Rep.*, 1984, **1**, 551; 1986, **3**, 1.
2 R. L. Whistler, M. S. Feather, and D. L. Ingles, *J. Am. Chem. Soc.*, 1962, **84**, 122.
3 B. Hellman, A. Lernmark, J. Sehlin, J. B. Taljedal, and R. L. Whistler, *Biochem. Pharmacol.*, 1973, **22**, 29.
4 R. L. Whistler and W. C. Lake, *J. Biochem.*, 1972, **130**, 919.
5 R. L. Whistler, U.S. Patent No. 3 998 949, 1973.
6 J. B. Lambert and S. M. Wharry, *J. Org. Chem.*, 1981, **46**, 3193.
7 L. D. Hall and C. Preston, *J. Chem. Soc., Chem. Commun.*, 1972, 1319.
8 M. Suzuki and R. L. Whistler, *Carbohydr. Res.*, 1972, **22**, 473.
9 Bo-Long Poh, *Carbohydr. Res.*, 1982, **108**, 197; 1982, **111**, 163; *Aust. J. Chem.*, 1984, **37**, 971.