Characterization of Certain Sulphur-containing Nucleosides by ^1H Nuclear Magnetic Resonance Spectroscopy using the Magnetic Anistropy Effect of the Thione Group

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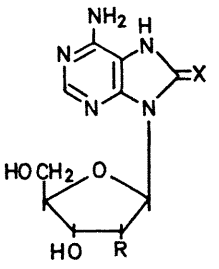
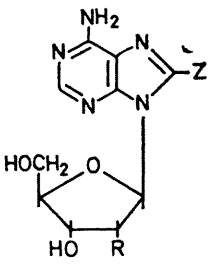
Summary A deshielding effect which has been observed for the anomeric proton of a number of thionucleosides was ascribed to the anistropy effect of a thione group and has provided a new method for the structural assignment of certain isomeric thionucleosides.

SEVERAL minor constituents of t-RNA have been identified[1] as sulphur analogues of the naturally-occurring nucleosides (uridine, adenosine, and related derivatives). This has prompted interest, not only in the chemical synthesis of closely related sulphur-containing nucleosides, but also in

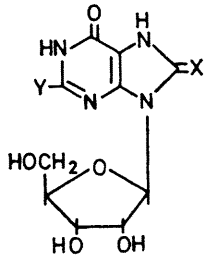
methods or procedures for determining the juxtaposition between the sulphur atom and the ribosyl moiety. Several



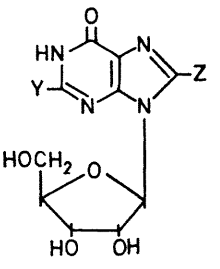
(I) X = S, R = OH
(II) X = O, R = OH
(III) X = S, R = H



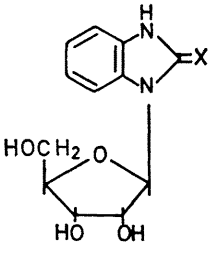
(IV) Z = SMe, R = OH
(V) Z = Br, R = OH
(VI) Z = SMe R = H
(VII) Z = Br, R = H



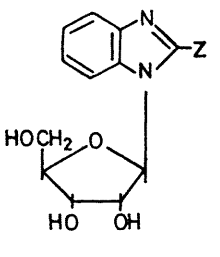
(VIII) X = S, Y = NH_2
(IX) X = O, Y = NH_2
(X) X = S, Y = H



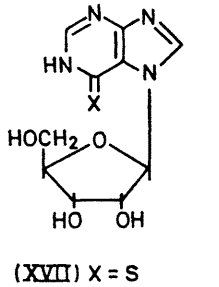
(XI) Z = OMe, Y = NH_2
(XIa) Z = NH_2, Y = H



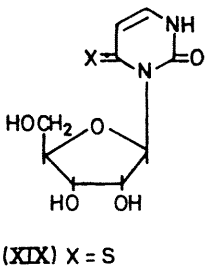
(XII) X = S
(XIII) X = O



(XIV) Z = SMe
(XV) Z = Cl
(XVI) Z = OMe



(XVII) X = S
(XVIII) X = O



(XIX) X = S
(XX) X = O

methods have been reported[2] for differentiation between 4-thiouridine, 2-thiouridine, and their respective aglycones, *e.g.*, chemical, mass spectroscopy, *etc.* We have now found that ^{1}H n.m.r. spectroscopy can also be used to differentiate between 4-thiouridine, 2-thiouridine, and several other sulphur-containing nucleosides.

The peak for the anomeric proton of 8-thioadenosine (I) was observed (see Table) downfield (doublet centred at 384 Hz) relative to the peak assigned to the anomeric proton of several other 8-substituted purine nucleosides.[3] This was obviously directly related to the introduction of a sulphur atom at C-8. The ^{1}H n.m.r. spectrum of 8-bromoadenosine, where the bromine atom has empty *d*-orbitals similar to those of sulphur, had a doublet centred at 355 Hz (Δ Hz = 29) for the anomeric proton which is very similar to the chemical shift values observed for several other 8-substituted adenosine derivatives. This suggested that magnetic anistropy effects associated with the thione group (C=S) might be responsible for the deshielding observed for the anomeric proton and appeared to eliminate any direct effect of magnetically allowed transitions due to empty *d*-orbitals. The same effect was expected for 6-amino-9-(β-D-ribofuranosyl)purin-8-one [(II), 8-hydroxy adenosine] since the carbonyl group (C=O) has previously demonstrated[4] magnetic anistropy effects. However, a ^{1}H n.m.r. spectrum of (II) revealed a doublet centred at 346 Hz for the anomeric proton; this represents a Δ Hz = 38 relative to (I). This indicated either that the observed downfield chemical shift for the anomeric proton of 8-thioadenosine was not due to magnetic anistropy effects or that there was a considerable difference in the screening environment[5] (size of the screening cones) around the C=S and C=O bonds. This prompted us to remove the magnetic anisotropy effects of the C=S group which should result in an upfield shift for the peak assigned to the anomeric proton if the observed shift was due to magnetic anistropy. 8-Methylthioadenosine (IV) had a ^{1}H n.m.r. spectrum with a doublet centred at 346 Hz which was assigned to the anomeric proton. This upfield chemical shift caused by methylation of the sulphur atom lends support to the assumption that the magnetic anistropy effect of the C=S group is directly responsible for the deshielding effect observed for the anomeric proton. This would also indicate that 8-thioadenosine exists at least partially in the thione form in solution, since if it existed entirely in the thiol form the anistropy effects on the anomeric proton would not have been observed.

A number of other nucleosides were examined and a similar downfield shift (deshielding) of the peak for the anomeric proton was observed in all cases where a molecular model showed the possibility of a proximal effect between the exocyclic sulphur atom and the anomeric proton. This was observed in every case where the exocyclic sulphur atom is attached to a carbon atom directly adjacent to the atom bearing the glycosyl moiety. However, when a sulphur atom was introduced at a position where there was no possibility of a proximal effect from the glycosyl moiety, there was observed essentially no deshielding effect on the anomeric proton (*e.g.*, 4-thiouridine). In this same ring system there was observed[6] a definite deshielding effect on the anomeric proton for the other isomer with the sulphur atom at an adjacent carbon (2-thiouridine). It appears that there is a certain degree of freedom since the above effect was observed for the 6-substituted 7-ribosylpurine (XVII) where the sulphur atom is in one ring and the ribosyl moiety is attached to a nitrogen atom of the other ring.

It is emphasized that the above effect is empirical in nature and being based on a limited number of examples may prove to have exceptions.

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TABLE[a]

	Solvent	Hz	ΔHz[b]		Solvent	Hz	ΔHz[b]
(I)	$(CD_3)_2SO$	384	—	(XII)	$(CD_3)_2SO$	392	—
(II)	"	346	38	(XIII)	"	337	55
(IV)	"	346	38	(XIV)	"	353	39
(V)	"	355	29	(XV)	"	363	29
(III)	"	411	—	(XVI)	"	351	41
(VI)	"	378	33	(XVII)	"	442	—
(VII)	"	385	26	(XVIII)	"	380	62
(VIII)	"	377	—	(XIX)	D_2O	436	—
(IX)	"	338	39	(XX)	"	382	54
(XI)	"	340	37				
(X)	"	385	—				
(XIa)	"	359	26				

[a] For the synthesis of these compounds see: (I), (IV), (VIII), (V), (VII), and (III), ref. 7e; (II) and (IX) ref. 7c; (XI) and (XIa), ref. 3; (XII), (XIII), (XIV), (XV), and (XVI), ref. 7a; (XVII) and (XVIII), ref. 7f; (XIX) and (XX), ref. 7d; (VI) and (X), ref. 7b.
[b] ΔHz = Chemical shift difference in Hz observed for the anomeric proton. Spectra were obtained on a Varian A-60 spectrophotometer at 60 MHz.

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