Tetrahedron, 1966, Vol. 22, pp. 705 to 710. Pergamon Press Ltd. Printed in Northern Ireland

ORIENTATION OF RIBONUCLEOSIDE DERIVATIVES BY PROTON MAGNETIC RESONANCE SPECTROSCOPY

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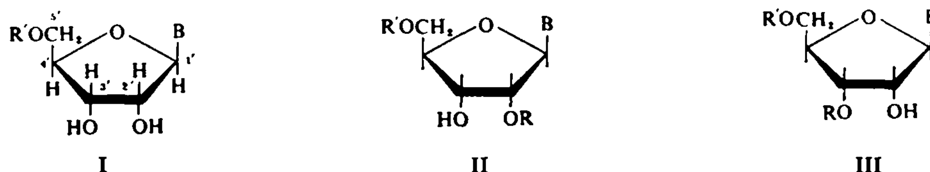
(*Received* 24 *September* 1965)

Abstract—The 60 Mc/s PMR spectra of a number of ribonucleoside derivatives have been measured. From correlations of the chemical shifts of H(1′) protons and the magnitude of their splitting ($J_{1',2'}$), two rules of general application in distinguishing between isomeric 2′- and 3′-ribonucleoside derivatives (II and III respectively) can be formulated. Other features of the spectra are discussed.



AN IMPORTANT aspect of ribonucleoside chemistry is the development of methods whereby the secondary hydroxyl groups of the sugar 2′,3′-*cis*-diol system (as in I) can be distinguished. Recently in connection with oligoribonucleotide synthesis,[1–3] a number of pure crystalline 2′- and 3′-ribonucleoside derivatives (II and III respectively) have been prepared and the orientation of most of them determined by chemical methods. However, a chemical approach to orientation may, especially in the case of purine ribosides, prove to be difficult or even unfeasible. It is the purpose of the present communication to put forward a general solution to this problem of orientation, based on PMR spectroscopy. Although several authors have used PMR spectroscopy to study the conformations[4–7] and other properties[8,9] of nucleosides, only Sonnenbichler *et al.*[10] have indicated that it may be used to distinguish between isomeric 2′- and 3′-derivatives of ribonucleosides.

The PMR spectral data, obtained with approximately 0·5 M solution of pyrimidine and purine ribosides, are listed in Tables 1 and 2. As the nucleoside derivatives had widely differing solubility properties, it was not possible to use the same solvent

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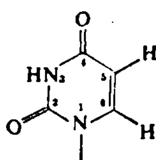

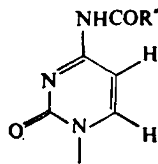

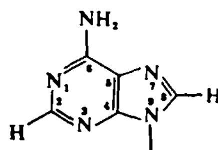

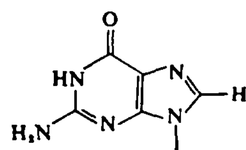

throughout. The following range of solvents was found to be suitable: deuterium oxide, dimethyl sulphoxide, dimethylcyanamide and dimethylformamide. All except the last were transparent below 6·0 τ. In order to ensure complete exchange of hydroxyl, amino and lactam protons, D_2O (10–15%) was added where possible to the

TABLE 1. CHEMICAL SHIFTS AND COUPLING CONSTANTS OF PYRIMIDINE NUCLEOSIDE DERIVATIVES

Spectrum No.	Nucleoside derivative	R	R′	B	R″	Ref.	Solv.	Chemical shifts H(6)	H(5)	H(1′)	($J_{1',2'}$)	H(2′)	H(3′)	Δ
1.	I	—	H	IV	—		1	2·16	4·14	4·12	(4·0)			
2.	I	—	H	IV	—		2	2·10	4·31	4·17	(4·8)			
3.	II	$PhCH_2$	H	IV	—	3	2	2·08	4·34	3·97	(4·9)			
4.	III	$PhCH_2$	H	IV	—	3	2	2·11	4·33	4·15	(6·2)			0·18
5.	II	SO_2Me	H	IV	—	3	1	2·12	4·12	3·93	(3·1)	4·75		
6.	III	SO_2Me	H	IV	—	11	1	2·17	4·09	4·04	(6·3)		4·82‡	0·11
7.†	II	$PO_3^{=}$	H	IV	—		1	2·12	4·08	3·99				
8.	III	$PO_3^{=}$	H	IV	—		1	2·08	4·08	4·04	(4·2)			0·05
9.†	II	Ac	H	IV	—	1	3	2·05	4·27	3·97	(5·5)			
10.	III	Ac	H	IV	—	1	3*	2·07	4·24	4·09	(6·5)		4·82‡	0·12
11.	III	Ac	H	IV	—	1	1*	2·16	4·10	4·05	(6·0)		4·82‡	
12.	I	—	t-BuCO	IV	—		3*	2·39	4·26	4·17	(3·0)			
13.	III	Ac	t-BuCO	IV	—	1	3*	2·39	4·23	4·15	(5·4)			
14.	I	—	Ac	IV	—		3	2·32	4·23	4·17	(3·5)			
15.	III	Ac	Ac	IV	—	12	3*	2·33	4·20	4·12	(5·5)		4·86	
16.	I	—	CHO	IV	—	13	3*	2·36	4·25	4·16	(3·2)			
17.†	II	Bz	CHO	IV	—	13	3*		4·21	3·84	(4·6)			
18.	III	Bz	CHO	IV	—	13	3*		4·19	3·98	(5·8)		4·57	0·14
19.	I	—	Tr	IV	—		4		4·55	3·96	(3·0)			
20.	II	Tr	Tr	IV	—	11	4		4·72	3·61	(7·5)			
21.	III	Tr	Tr	IV	—	3	4		4·45	3·86	(5·1)			0·25
22.	I	—	Ac	V	Me	13	2*	1·84	2·68	4·15	(2·1)			
23.	II	Ac	Ac	V	Me	13	2*	1·84	2·67	4·05	(3·0)	4·66‡		
24.†	III	Ac	Ac	V	Me	13	2	1·82	2·65	4·10	(4·0)		4·91	0·05
25.	I	—	Bz	V	Ph	13	5*			3·95	(1·0)			
26.†	II	Bz	Bz	V	Ph	13	5*			3·67	(2·7)			
27.	III	Bz	Bz	V	Ph	13	5*			3·79	(3·7)		4·32	0·12
28.	I	—	t-BuCO	V	t-Bu	13	5		2·51	4·00	(1·6)			
29.	I	—	t-BuCO	V	t-Bu	13	3*	1·85	2·53	4·14	(1·8)			
30.	II	Ac	t-BuCO	V	t-Bu	13	3*	1·91	2·56	4·03	(3·3)	4·66		
31.†	III	Ac	t-BuCO	V	t-Bu	13	3	1·89	2·55	4·09	(4·5)		4·89	0·06
32.	II	Bz	t-BuCO	V	t-Bu	13	5			3·68	(3·6)	4·27‡		
33.†	III	Bz	t-BuCO	V	t-Bu	13	5			3·84	(4·2)			0·16

[11] N. C. Yung and J. J. Fox, *J. Amer. Chem. Soc.* **83**, 3060 (1961).
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organic solvents. When the spectra of pure 2'- and 3'-O-acyl nucleosides were taken, the D_2O was acidified with acetic acid to minimize acyl migration. If it was required to compare the spectra of different compounds directly (see below), it was necessary to use solutions of approximately equal concentrations in the same solvent. Jardetzky and Jardetzky[4] have reported that the PMR spectra of nucleosides are concentration dependent, and we have found that the spectra of individual compounds are solvent dependent. For example, both uridine (Table 1: spectra 1, 2) and $N^4,O^{5'}$-dipivaloyl-cytidine (Table 1: spectra 28, 29) have appreciably different H(1') resonances in different solvents.

TABLE 2. CHEMICAL SHIFTS AND COUPLING CONSTANTS OF PURINE NUCLEOSIDE DERIVATIVES

Spectrum No.	Nucleoside derivative	R	R'	B	Ref.	Solv.	Chemical shifts H(2)	H(8)	H(1')	($J_{1',2'}$)	H(2')	H(3')	Δ
34.	I	—	H	VI		1*	1·64	1·80	3·95	(5·7)			
35.	I	—	H	VI		2	1·55	1·73	3·98	(5·9)			
36,†	II	PO_3^-	H	VI		1	1·71	1·99	3·88	(5·4)			
37.	III	PO_3^-	H	VI		1	1·76	2·03	3·97	(5·2)			0·09
38.†	II	Ac	H	VI	13	1*	1·70	1·90	3·80	(5·0)			
39.	III	Ac	H	VI	13	1*	1·70	1·90	3·98	(6·6)			0·18
40.	III	Ac	H	VI	13	2*	1·56	1·76	4·03	(7·2)		4·67	
41.	I	—	Ac	VI		2	1·63	1·75	4·00	(4·9)			
42.†	II	Ac	Ac	VI	13	2	1·56	1·69	3·74	(4·0)			
43.	III	Ac	Ac	VI	13	2	1·56	1·69	3·93	(6·0)			0·19
44.	I	—	t-BuCO	VII	2	2*	—	2·11	4·19	(4·8)			
45.†	II	Ac	t-BuCO	VII	13	2*	—		4·00	(~4·5)			
46.†	III	Ac	t-BuCO	VII	13	2*	—		4·21	(~6)			

Chemical shifts in ppm on τ-scale. $J_{1',2'}$ in c/s.
Solvents: 1, D_2O; 2, dimethylsulphoxide + D_2O; 3, dimethylcyanamide + D_2O; 4, dimethylformamide; 5, dimethylformamide + D_2O.
* indicates that the D_2O is molar with respect to acetic acid.
† indicates that the data were obtained from the spectrum of a mixture of isomers.
‡ indicates that the signal was a quartet.
Δ = difference in chemical shifts (ppm) between H(1') resonances of 2'- and 3'-isomers.

The principal resonances listed in Tables 1 and 2 are those of the heterocyclic base protons and the glycosidic proton, H(1'). With the exception of spectra 38, 39, 40, 42, 45 and 46, the data are based on compounds the orientations of which have been determined by chemical methods.[3,13] For uridine and cytidine* derivatives, H(5) and H(6) (see formulae IV and V) constitute an AX system with $J_{5,6}$ approximately equal to 8·1 and 7·5 c/s respectively; for adenosine derivatives,[4] H(2) and H(8) (see formula VI) appear as sharp singlets, as does H(8) for guanosine derivatives. Although the chemical shifts of the base protons often differ for 2'- and 3'-isomers, the chemical shift of the H(1') doublet and the coupling constant ($J_{1',2'}$) between the protons attached to C(1') and C(2') provide the most useful information for orientation purposes. Two generalizations emerge from the data listed in Tables 1

* In D_2O solution, the H(6) and H(5) resonances of cytidine occur at 2·20 τ and 4·02 τ (J = 7·5 c/s) respectively. Protonation of the cytosine residue leads to slight deshielding of both protons, but acylation of N(4) leads to *considerable* deshielding, especially of H(5). This effect facilitates orientation as the resonance signals of H(5) and H(1') for cytidine (and uridine) derivatives often overlap.

and 2: (1) *for a pair of* 2'- *and* 3'-*isomers, the* H(1') *resonance is at lower field for the* 2'- (II) *than for the* 3'-*isomer* (III) *and* (2), $J_{1',2'}$ *is greater for the* 3'- *than for the* 2'-*isomer*. These generalizations are referred to below as the chemical shift and coupling constant rules, respectively.

The chemical shift rule can be rationalized inasmuch as an electron-withdrawing substituent attached to the 2'-hydroxyl would be likely to have a greater deshielding effect on H(1') than it would were it attached to the 3'-hydroxyl group. No exception to this rule has been observed*, even when the substituent was not obviously electron-withdrawing. From the spectra of the uridine derivatives (II and III; B = IV) listed in Table 1, it can be seen that this effect is shown by the methanesulphonyl (spectra 5, 6), phosphoryl (spectra 7, 8), acetyl (spectra 9, 10) benzoyl (spectra 17, 18), trityl (spectra 20, 21) and benzyl (spectra 3, 4) substituents. The actual difference in chemical shift (Δ) between the H(1') resonances of 2'- and 3'-isomers, however, is rarely more than 0·2 ppm (see Tables). For cytidine derivatives (II and III; B = V), the effect is greater for a benzoyl (Table 1: spectra 26, 27 and 32, 33) than for an acetyl group (Table 1: spectra 23, 24 and 30, 31). Nevertheless, for adenosine derivatives (II and III; B = VI), the acetyl group (Table 2: spectra 38, 39 and 42, 43) shows a comparatively large difference effect of nearly 0·2 ppm.

The coupling constant rule reflects the relative effects on the conformation of the ribose ring of attaching a particular substituent either to the 2'- or the 3'-hydroxyl group. Karplus[14] has derived expressions which relate the coupling constants between two protons, attached to adjacent carbon atoms, to the dihedral angle between them. If it is assumed that the Karplus equations[14] can be applied to the ribonucleoside derivatives listed in Table 1, then the dihedral angle between H(1') and H(2') can be calculated to range from 112° (J = 1·0) for $N^4,O^{5'}$-dibenzoylcytidine (spectrum 25) to 156° (J = 7·5) for 2',5'-di-O-trityluridine (spectrum 20). It follows from the coupling constant rule that the dihedral angle between H(1') and H(2') is less for a 2'-(II) than for a 3'-isomer (III). For uridine derivatives (Table 1), the rule is exemplified by the following groups: benzyl (spectra 3, 4), methanesulphonyl (spectra 5, 6), acetyl (spectra 9, 10), and benzoyl (spectra 17, 18). However, it is not surprising that the trityl group (spectra 20, 21) should behave exceptionally, as such a bulky substituent might be expected to have an unusual effect on the conformation of the ribose ring. The cytidine derivatives examined (Table 1: spectra 23, 24; 26, 27; 30, 31; and 32, 33) show small H(1'), H(2') splittings, but they follow the coupling constant rule. From the data relating to adenosine derivatives, it can be seen (Table 2) that $J_{1',2'}$ values are comparatively large and that the rule applies to the acetyl group (spectra 38, 39 and 42, 43). However, within experimental error, $J_{1',2'}$ is the same for both adenosine 2'- and 3'-phosphates (spectra 36, 37), and is relatively small for uridine 3'-phosphate (Table 1: spectrum 8). The spectrum of the mixture of uridine 2'- and 3'-phosphates was too complex to allow an estimate of $J_{1',2'}$ for the 2'-isomer to be made. Thus although the coupling constant rule can provide valuable support in favour of a particular orientation, it should be applied with caution.

In order to apply the two orientation rules under the most favourable conditions, it is desirable that both the compound under investigation and its isomer should be

* The chemical shift rule is a generalization of an effect noted by Sonnenbichler *et al.*[10] in their NMR spectral studies on 2'(3')-O-aminoacyladenosines.

[14] M. Karplus, *J. Chem. Phys.* **30**, 11 (1959).

available. However, the orientation of a 2′- or 3′-ribonucleoside derivative can often be determined from the spectrum of a pure specimen of it (II or III) together with that of a mixture of both isomers (II and III). The signals belonging to the spectrum of the other isomer can then be found by subtraction (e.g. Table 1: spectrum 9). This approach is especially useful for ribonucleoside 2′- and 3′-carboxylic esters as a mixture of both isomers can readily be obtained from either pure isomer.[3,13] Mixtures of 2′- and 3′-isomers with non-migrating substituents are also frequently available as most known alkylation and acylation reactions of the diol system (I) are unspecific. The tritylation of uridine[3] and the tosylation of 5′-O-acetyluridine[15] may be cited as typical examples.

In connection with both orientation rules, it is relevant to compare the PMR spectra of a pair of 2′- and 3′-isomers with that of the corresponding parent diol system (I). With reference to the chemical shift rule, the H(1′) proton of the 2′-isomer (II) is invariably at lower field than that of (I). This generalization normally also holds for the 3′-isomer (III) but the effect is much smaller. With reference to the coupling constant rule, whereas $J_{1',2'}$ is usually larger for the 3′-isomer (III) than it is for (I), no general rule holds for the 2′-isomer (II). It is thus not possible to orientate a 2′- or 3′-ribonucleoside derivative with certainty by comparing its PMR spectrum with that of its parent diol system (I).

It was noted above that the H(6) and H(5) proton signals of isomeric 2′- and 3′-derivatives of pyrimidine ribosides (Table 1) often differ. This difference, which is normally very small, can sometimes be made the basis of a simple method of estimating the proportions of 2′- and 3′-isomers in a mixture. It is especially noteworthy that the H(5) signals of the 2′-derivatives of uridine and cytidine occur at higher field than those of the corresponding 3′-isomers.* No such simple correlation can be observed for the H(6) signals which, in any case, are sometimes obscured. No obvious relationship between the resonances of base protons and the orientation of purine riboside derivatives is apparent from the data listed in Table 2.

In the spectra of ribonucleosides or their 5′-derivatives (I), the resonance signals of protons attached to C(2′), C(3′), C(4′), and C(5′) all occur at higher field than 5·0 τ. However, the introduction of an electron-withdrawing substituent at the 2′- or 3′-position leads to substantial deshielding of H(2′) or H(3′) respectively†. For uridine derivatives (Table 1), this effect is illustrated by methanesulphonyl (spectra 5, 6), acetyl (spectra 10, 11, 15) and benzoyl (spectrum 18) groups, and for cytidine derivatives (Table 1), by acetyl (spectra 23, 24, 30, 31) and benzoyl (spectra 27, 32) groups. The benzoyl group shows a larger deshielding effect than the other substituents examined. In general, the H(2′) signal of a 2′-O-acyl derivative is found at lower field than the H(3′) signal of its 3′-isomer. This difference may be used to determine the proportions of 2′- and 3′-isomers in a mixture, if the respective H(2′) and H(3′) signals do not overlap. In those cases (Table 1: spectra 6, 10, 23, 32) where H(2′) or H(3′) appears as a well resolved quartet, it should be possible to

* This difference is usually about 0·02 ppm. However, for 2′, 5′- and 3′,5′-di-O-trityluridines (spectra 20, 21) it is 0·27 ppm, but for uridine 2′- and 3′-phosphates (spectra 7, 8) it is undetectable.

† A similar effect has been observed in monoacyl derivatives of both *cis*-cyclopentane-1,2-diol and *cis*-3,4-dihydroxytetrahydrofuran.[16]

[15] D. M. Brown, D. B. Parihar, Sir Alexander Todd and S. Varadarajan, *J. Chem. Soc.* 3028 (1958).
[16] H. G. Zachau, personal communication.

estimate the dihedral angles between protons other than H(1') and H(2') and thus obtain a clearer indication of the conformation of the ribose ring.

In conclusion, it is of interest to consider some of the possible applications of the orientation rules formulated in this paper. As has already been indicated, PMR spectroscopy may be used to estimate the proportions of II and III in a mixture of isomers,* obtained by the alkylation or acylation of I. The proportion of each isomer in the mixture may be estimated by integration of the H(1') doublets or, if these signals are not sufficiently well resolved, by integration of any other distinguishable signals such as those of the base protons or of H(2') and H(3'). In this way, the relative reactivities towards various reagents of the 2'- and 3'-hydroxyl groups may be determined or, if the entering substituent can undergo migration, the composition of the equilibrium mixture of 2'- and 3'-isomers may be estimated. In the latter situation, if the conditions are carefully controlled, the actual rate of migration of such a substituent may be measured. Such studies are of importance in connection with the structure of aminoacyl-s-RNA,[10,17,18] and consequently with the mechanism of *in vivo* protein synthesis. We are currently investigating the possible applications of PMR spectroscopy to the solution of such problems.

EXPERIMENTAL

PMR spectra were measured on a Perkin-Elmer Spectrophotometer, operating at 60 Mc/s with tetramethylsilane (for solutions in organic solvents) and t-butanol (for solutions in D_2O) as internal references. The preparation and the chemical methods of orientation of new compounds will be described in forthcoming publications.

Acknowledgements—This investigation was supported in part by Research Grant CA-06066 from the National Cancer Institute of the National Institutes of Health, United States Public Health Service. One of us (J. E. S.) thanks the D.S.I.R. for the award of a Research Studentship.

* The mixture of isomers should first be separated from starting material (I) and other products by silicic acid chromatography or by some other fractionation technique.

[17] R. Wolfenden, D. H. Rammler and F. Lipmann, *Biochem.* **3**, 329 (1964).
[18] C. S. McLaughlin and V. M. Ingram, *Biochem.* **4**, 1442, 1448 (1965).