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STUDIES ON THE $\Delta\delta$ CRITERION FOR DETERMINING THE ANOMERIC CONFIGURATION OF RIBOFURANOSYL NUCLEOSIDES

BERNARD RAYNER, CLAUDE TAPIERO, AND JEAN-LOUIS IMBACH
Université des Sciences et Techniques du Languedoc, Laboratoire de Chimie Bio-Organique, Place E. Bataillon, 34060 Montpellier Cédex (France)
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ABSTRACT

Studies on the origin of the criterion for determination of the anomeric configuration of D-ribofuranosyl nucleosides, based on the differences in the chemical shift ($\Delta\delta$) between the Me signals of the corresponding 2′,3′-*O*-isopropylidene derivatives, is discussed. Proof of the anisotropic influence of the aglycon group on the chemical shifts of the Me groups is given by a comparison of the chemical shifts of anomeric pairs of nucleosides having a reduced aglycon group with those of the non-reduced heterocycle. Therefore, the criterion appears to be limited to ribofuranosyl compounds having an unsaturated heterocyclic aglycon.

INTRODUCTION

Of the aldofuranosyl nucleosides, the most important is the ribofuranosyl series. General synthetic approaches to ribofuranosyl nucleosides involve the condensation of a suitably protected sugar with a heterocycle. Such reactions often lack stereospecificity[1], and anomeric mixtures are sometimes obtained. However, the β derivatives usually preponderate due to neighbouring-group participation of an acetyl or benzoyl group in the ribofuranosyl moiety. A major problem is the establishment of the anomeric configuration of synthetic ribofuranosyl nucleosides. Various methods have been suggested, but so far none of them seems to be of general use except our recently published criterion[2–6].

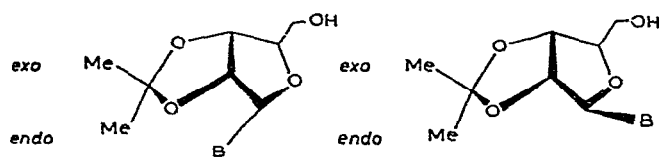
Nishimura and Shimizu[7] noticed that the anomeric proton of α-D-ribofuranosyl nucleosides resonates at a lower field than the anomeric proton of the β compounds. However, this approach requires the availability of *both* anomers. Although no exceptions to this rule have been found for ribonucleosides, we have recently shown it is not always true for ribofuranosyl derivatives[6]. The $J_{1',2'}$ coupling constants have been widely used[8], and it has been suggested[8–11] that the β configuration should be assigned only if $J_{1',2'} < 1$ Hz, which is often not the case. Leonard and Laursen's approach[12], which used the 2′,3′-*O*-isopropylidene derivatives and the same coupling constant ($J_{1',2'}$) since a decrease in the J value is generally observed, has the same

limitations, although the problem can be solved in some cases. The anisotropic effect of the heterocycle on the resonance of AcO-2′ was used by Cushley and Fox[13–15]. This approach needs a comparison of the p.m.r. spectrum of the pyrmidine nucleoside with that of the corresponding 5,6-dihydro derivative. Whereas this approach is generally applicable to such aglycons as pyrimidines, it is not readily applicable to purine nucleosides where a selective reduction of the purine ring is difficult. Montgomery[16] proposed that the chemical shift of AcO-2′ could be used to determine the configuration of furanosyl nucleosides. This approach, which is also based on the anisotropic effect of the aglycon, has exceptions involving certain particular ribofuranosyl nucleosides[6].

We have shown[5,6] that the differences in the chemical shift ($\Delta\delta$) between the methyl signals of 2′,3′-*O*-isopropylidene derivatives of certain nucleoside pairs are >0.15 p.p.m. for the β derivatives and <0.15 p.p.m. for the α anomers. However, the basis for this rule has not yet been firmly established, although we postulate that it should be related to the anisotropic effect of the aglycon group in α anomers. We now report studies on the basis for this general method for determining the anomeric configuration of ribofuranosyl nucleosides.

RESULTS AND DISCUSSION

The 2′,3′-*O*-isopropylidene derivatives of anomeric D-ribofuranosyl nucleosides are shown in **1** and **2**.



1 (α anomer) 2 (β anomer)

For each form (α and β), the main factors which affect the chemical shifts of the Me signals are the conformation of the carbohydrate moiety and the anisotropy of the aglycon group. The conformation of such isopropylidene nucleosides is well documented; the 2,2-dimethyl-1,3-dioxolane ring produces a general flattening of the furanose ring which provides conformational rigidity to the sugar ring[17,18]. Therefore, we can expect that the effect of the deformation of the sugar moiety on the Me chemical-shift is negligible.

This prompted us to study the anisotropic effect of the aglycon group on the Me groups of the isopropylidene moiety. However, it was first necessary to obtain a definitive assignment of the signals for the *exo* and *endo* Me groups. For such 1,3-dioxolane rings, the different chemical-shifts of the methyl groups have been studied by Brimacombe *et al.*[19], who assigned the low-field signal to the *endo*-Me and the high-field signal to the *exo*-Me. These assignments have been confirmed by

TABLE I

METHYL RESONANCES OF 2',3'-*O*-ISOPROPYLIDENE DERIVATIVES OF 1-(α- AND β-D-RIBOFURANOSYL)NUCLEOSIDES IN $(CD_3)_2SO$

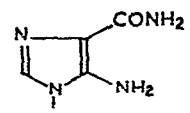
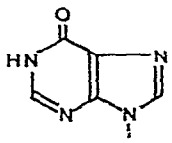
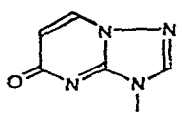
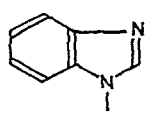
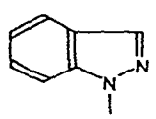
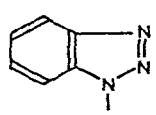
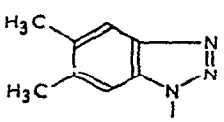
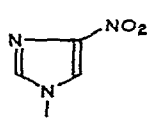
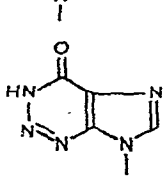
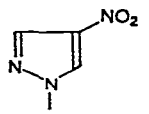
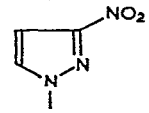
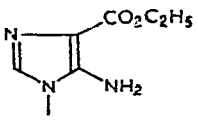
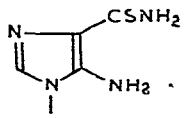
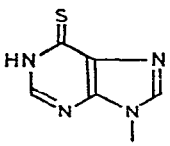
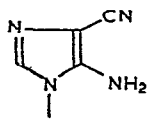
Aglycon group	*α Anomers*			*β Anomers*			*Δ'δ* endo	*Δ'δ* exo	*Ref.*
	δMe		*Δδ*	*δMe*		*Δδ*			
	endo	exo		endo	exo				
Group I									
	1.36	1.27	0.09	1.55	1.33	0.22	0.19	0.06	22
	1.37	1.27	0.10	1.56	1.35	0.21	0.19	0.08	22
	1.37	1.27	0.10	1.53	1.33	0.20	0.16	0.06	2
Group II									
	1.33	1.33	0.00	1.60	1.37	0.23	0.27	0.04	2
	1.21	1.21	0.00	1.59	1.41	0.18	0.38	0.20	2
	1.20	1.20	0.00	1.60	1.40	0.20	0.40	0.20	2
	1.20	1.20	0.00	1.60	1.42	0.18	0.40	0.22	2
	1.27	1.27	0.00	1.52	1.32	0.20	0.25	0.05	2
	1.24	1.24	0.00	1.57	1.36	0.21	0.33	0.12	22

TABLE II

METHYL RESONANCES OF 2′,3′-*O*-ISOPROPYLIDENE DERIVATIVES OF 1-(α- AND β-D-RIBOFURANOSYL)NUCLEOSIDES IN $(CD_3)_2SO$

Aglycon group	*α Anomers*		*β Anomers*			*Ref.*
	δMe	*Δδ*	*δMe*		*Δδ*	
			endo	exo		
Group III						
	1.28 1.31	0.05	1.55	1.32	0.23	2
	1.25 1.30	0.05	1.53	1.35	0.18	2
	1.27 1.34	0.07	1.55	1.34	0.21	2
	1.25 1.33	0.08	1.55	1.33	0.22	23
	1.24 1.34	0.10	1.55	1.34	0.21	23
	1.27 1.29	0.02	1.53	1.32	0.21	23

Sable and co-workers[20,21]* for the *gem*-dimethyldioxolane/cyclopentane series. Furthermore, these authors[20,21] have shown that the chemical-shift difference ($\Delta\delta$) of the Me groups is influenced by the anisotropy of the substituents on the cyclopentane ring.

For the β compounds listed in Tables I and II, the influence of the aglycon group on the *exo* or *endo* Me groups should be relatively small as indicated from an examination of Dreiding models (see also **1** and **2**). The situation is not so straightforward for the α compounds. In fact, three groupings could be seen according to the

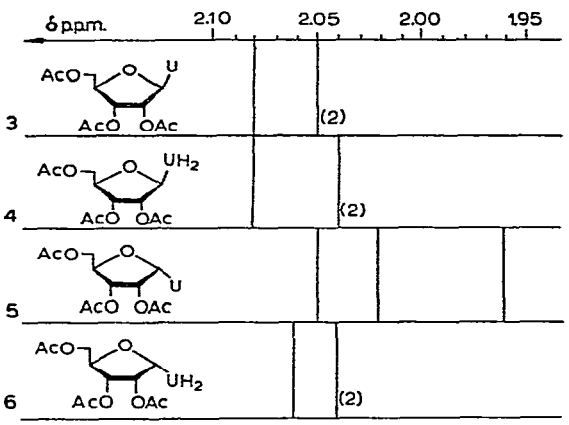
*In Sable's model, the *endo*-Me group gives the most variable n.m.r. signal at lower field, that Me group being the closest to the anisotropic center.

position of the Me signals of α anomers compared to those of the β anomers. For the first group of compounds (Table I), the lower-field signal of an α anomer was at a lower field than the *exo*-Me signal of the corresponding β anomer. In the second group, both Me signals of an α anomer coincide and are at a higher field than either Me signal of the corresponding β anomer.

Thus, for these two groupings, it was possible to obtain information on the specific shielding of each Me group caused by the juxtaposition of the base in going from the β to the α anomer.

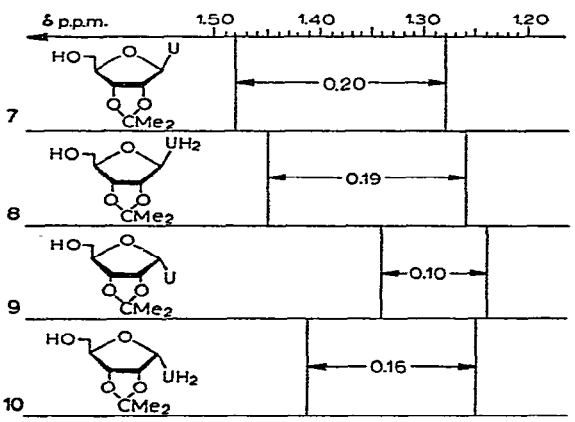
TABLE III

ACETYL RESONANCES OF ACETYLATED DERIVATIVES OF 1-(α- AND β-D-RIBOFURANOSYL)NUCLEOSIDES IN $(CD_3)_2SO$[a]



[a]Numbers in parentheses refer to the number of acetyl signals at a particular δ value. U, uracil; UH_2, 5,6-dihydrouracil.

METHYL RESONANCES OF 2′,3′-*O*-ISOPROPYLIDENE DERIVATIVES OF 1-(α- AND β-D-RIBOFURANOSYL)NUCLEOSIDES IN $(CD_3)_2SO$



The corresponding $\Delta\delta'$ values are given in Table I and show that the *endo*-Me groups are always much more shielded than the *exo* groups by an inversion of anomeric configuration from β to α. This indicates that the anisotropy of the aglycon group is responsible for this criterion of anomeric assignment.

For the third group of compounds (Table II), both Me groups of the *O*-isopropylidene group of the α anomers are more shielded than the corresponding Me groups in the β anomer. As a cross-over of the signals is possible, we prefer not to assign the corresponding methyl groups as *endo* and *exo* in the α anomers.

To determine the origin of our criterion with greater certainty, we used an approach similar to that employed by Cushley and Fox[14,16], *i.e.*, to compare the chemical shifts of the isopropylidene Me groups in anomeric nucleoside pairs with and without the 5,6-double bond in the aglycon group. Table III presents the acetyl and isopropylidene resonances of the anomeric uracil nucleosides and also the corresponding 5,6-dihydrouracil derivatives. For the α-acetylated compounds **5** and **6**, a large paramagnetic shift is observed for the AcO-2′ resonance upon removal of the anisotropy of the 5,6-double bond, which is in agreement with the Cushley and Fox data[14–16]. With the β compounds **7** and **8**, reduction of the aglycon group gives no displacement, except for a slight upfield shift as was observed previously for the AcO-2′ signal. However, for the α pair **9** and **10**, the *endo*-Me group is deshielded by 0.07 p.p.m. on reducing the 5,6-double bond, giving rise to a $\Delta\delta$ value of 0.16 p.p.m. for **10**.

It appears that the major basis for our criterion is due to the influence of the anisotropy of the aglycon group on the *endo*-Me group in the α anomer, and is therefore limited to ribofuranosyl compounds having an unsaturated base as the aglycon*.

EXPERIMENTAL SECTION

General. — P.m.r. spectra were obtained with a Varian HA-100 spectrometer, using methyl sulphoxide-d_6 as solvent and tetramethylsilane as internal reference. Values are given in δ and are accurate to ± 0.01 p.p.m. M.p's. were obtained with a Gallenkamp apparatus and are not corrected. U.v. spectra were obtained with an Optica 10 spectrometer. Elemental analyses were made by the Service Central de Microanalyse du C.N.R.S., Montpellier.

*1-(2,3,5-Tri-*O*-acetyl-α-*D*-ribofuranosyl)uracil* (**5**). — A suspension of 0.71 g (2.91 mmol) of 1-α-D-ribofuranosyluracil[24] in 5 ml of acetic anhydride and 4 drops of pyridine was gently warmed with stirring until dissolution occurred. Stirring was continued for 1.5 h, methanol (12 ml) was then added, and stirring was continued for 3 h. The solvents were evaporated *in vacuo* and the resulting syrup was dissolved in 12 ml of water. This solution was neutralised with sodium hydrogen carbonate and then extracted with chloroform (2×25 ml).

*We would like to point out again that our criterion is limited to ribofuranosyl compounds without a 5′-substituent (for a discussion, see ref. 6). Compounds having a 5′-substituent may have anisotropy which could influence $\Delta\delta$.

The combined extracts were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Elution of the resulting syrup from a column (1 × 25 cm) of silica gel with methanol–chloroform (1:49) yielded **5** as a white foam (0.92 g, 85%), $[\alpha]_D^{20}$ −25° (*c* 1, methanol), λ_{max} 262 nm (ε 8500). P.m.r. data: δ 7.64 (d, $J_{5,6}$ 8 Hz, H-6), 6.33 (d, $J_{1',2'}$ 4.5 Hz, H-1'), 5.64 (d, H-5), 5.43 (m, H-2',3'), 4.53 (m, H-4'), 4.22 (m, H-5',5').

Anal. Calc. for $C_{15}H_{18}N_2O_9$: C, 48.65; H, 4.90; N, 7.57. Found: C, 48.52; H, 4.86; N, 7.45.

Preparation of 5,6-dihydro derivatives. — A solution of **5** (0.25 g, 0.676 mmol) in 50 ml of ethanol was hydrogenated over 0.125 g of 5% rhodium-on alumina at room temperature and atmospheric pressure[25]. The reaction was monitored by u.v. spectroscopy. The absence of selective absorption at 260 nm showed that hydrogenation of the 5,6-double bond was essentially complete.

Stirring was continued for 15 h. The mixture was then filtered through Celite and concentrated *in vacuo*. The resulting syrup was eluted from a column (1 × 25 cm) of silica gel with methanol–chloroform (1:49) to give 5,6-dihydro-1-(2,3,5-tri-*O*-acetyl-α-D-ribofuranosyl)uracil (**6**; 0.242 g, 97%) as a white foam, $[\alpha]_D^{20}$ +57° (*c* 1.08, methanol). P.m.r. data: δ 6.19 (d, $J_{1',2'}$ 5.5 Hz, H-1'), 5.38 (m, H-2',3'), 4.38 (m, H-4'), 4.16 (m, H-5',5'), 3.46 (t, H-6,6), 2.50 (m, H-5,5 and methyl sulphoxide-d_5).

Anal. Calc. for $C_{15}H_{20}N_2O_9$: C, 48.38; H, 5.41; N, 7.52. Found: C, 48.21; H, 5.54; N, 7.48.

By essentially the foregoing procedure, 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)uracil (**7**; 0.2 g, 0.70 mmol) was hydrogenated for 3 h. The mixture was filtered through Celite and concentrated *in vacuo* to give 5,6-dihydro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)uracil (**8**; 0.180 g, 90%), $[\alpha]_D^{20}$ −34° (*c* 1, methanol). P.m.r. data: δ 5.74 ($J_{1',2'}$ 3.1 Hz, H-1'), 4.91 (t, HO-5'), 4.70 (m, H-2',3') 3.84 (dd, H-4'), 3.52 (m, H-5',5',6,6), 2.50 (m, H-5,5 and methyl sulphoxide-d_5).

Anal. Calc. for $C_{12}H_{18}N_2O_6$: C, 50.34; H, 6.34; N, 9.79. Found: C, 50.40; H, 6.22; N, 9.71.

Likewise, 1-(2,3-*O*-isopropylidene-α-D-ribofuranosyl)uracil[26] (**9**; 0.25 g, 0.88 mmol) was hydrogenated at room temperature and at 70 atmos. with stirring for 18 h. The mixture was filtered through Celite and concentrated *in vacuo*. The residue was crystallized from chloroform–ether to afford 5,6-dihydro-1-(2,3-*O*-isopropylidene-α-D-ribofuranosyl)uracil (0.226 g, 90%), m.p. 162–164°, $[\alpha]_D^{20}$ −31° (*c* 1, methanol). P.m.r. data: δ 5.95 (d, $J_{1',2'}$ 3.7 Hz, H-1'), 5.12 (t, HO), 4.72 (m, H-2',3'), 3.74 (m, H-5,5',6,6), 2.47 (m, H-5,5 and methyl sulphoxide-d_5).

Anal. Calc. for $C_{12}H_{18}N_2O_6$: C, 50.34; H, 6.34; N, 9.79. Found: C, 50.10; H, 6.30; N, 9.65.

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