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Note

A new aspect of the use of 2′,3′-*O*-isopropylidene ribonucleosides for investigation of anomeric configuration*

MALCOLM MACCOSS†, MORRIS J. ROBINS‡, BERNARD RAYNER**, AND JEAN-LOUIS IMBACH**
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Although a number of chiroptical and nuclear magnetic resonance (n.m.r.) methods have been described for determining the anomeric configuration of ribonucleosides[1], the use of their 2′,3′-isopropylidene acetals has only recently received wide attention. Initially, Leonard and Laursen used these derivatives to restrict the conformational mobility of the unprotected furanose ring[2] and invoked the decreased anomeric coupling-constant of the α anomers to assign their anomeric configuration. However, values of $J_{1',2'}$ in the range 2–3.5 Hz for the β anomers are not uncommon, and this causes uncertainty in assignments. Recently, a more-general utilization of these 2′,3′-isopropylidene acetals for determination of anomeric configuration has involved the measurement of the chemical-shift difference between the two singlet methyl resonances ($\Delta\delta$)[3–7]. This $\Delta\delta$ criterion states that the α anomers have a small chemical-shift difference between the isopropylidene methyl groups ($\Delta\delta < 0.15$), whereas the β anomers exhibit a larger shift-difference ($\Delta\delta > 0.15$)[6]. In the α anomers, the base is in close proximity to the *endo* methyl group[6] and the anisotropy effect of the base on the chemical shift of this methyl resonance has been shown to be the basis for the $\Delta\delta$ criterion[6,7]. The currently defined limitations to this method of determining anomeric configuration are that it is restricted to compounds lacking a 5′-substituent[6] and to those[1,7,8] having an unsaturated base at C-1′.

The isopropylidene acetals of the common β-ribonucleosides have been investigated by n.m.r. methods[9]. The sugar rings of these compounds have been shown to be constrained and flattened relative to the parent nucleosides (which exhibit a ${}^2E \rightleftarrows {}^3E$ equilibrium[10]). This flattened conformation in the β series gives rise to a multiplet (higher than triplet) for the H-4′ resonance, because of significant coupling with H-3′ as well as with H-5′ and H-5″.

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†Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, U. S. A.
‡Department of Chemistry, The University of Alberta, Edmonton, Alberta T6G 2G2, Canada.
**Université des Sciences et Techniques du Languedoc, Laboratoire de Chimie Bio-Organique, Place E. Bataillon, 34060 Montpellier cédex, France.

The 100-MHz spectra of all of the α-ribonucleoside derivatives we have studied (see Table I) display an "apparent triplet" resonance peak for H-4′. Homonuclear decoupling experiments confirmed that the coupling between H-4′ and H-3′ ($J_{3',4'}$) for these α anomers is very small (irradiation of H-3′ had virtually no effect on the H-4′ peaks). The triplet appearance of the H-4′ resonance is the result of "apparent equivalence" of $J_{4',5'}$ and $J_{4',5''}$ (the chemical shifts of H-5′ and H-5″ are virtually identical for the α anomers that could be readily evaluated, namely, those having these peaks separated from other resonance lines). A decrease in the dihedral angle between H-3′ and H-4′ ($\phi_{3',4'}$) approaching[11,12] 90° is not unreasonable in the α anomers.

TABLE I

H-4′ RESONANCE PEAKS OF (2,3-*O*-ISOPROPYLIDENE-α- AND β-D-RIBOFURANOSYL)NUCLEOSIDE DERIVATIVES IN Me_2SO-d_6[a]

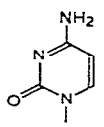
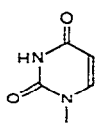
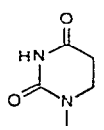
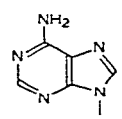
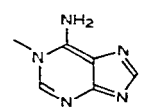
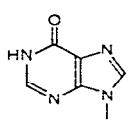
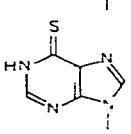
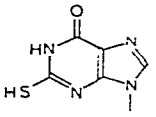
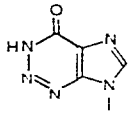
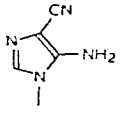
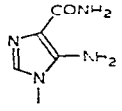
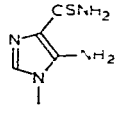
C-1′ substituent	*α Anomer*		*β Anomer*
	δ	J_{app} *(Hz)*	*δ*
	4.19 (t)	3.5	4.07 (m)
	4.31 (t)	3.0	4.07 (m)
	4.08 (t)	4.0	3.84 (m)
	4.30 (t)	3.4	4.25 (m)
	4.48 (t)	3.5	4.10 (m)
	4.36 (t)	3.2	4.23 (m)
	4.39 (t)	3.0	4.27 (m)

TABLE I (*continued*)

C–1′ substituent	*α Anomer*		*β Anomer*
	δ	J_{app} (*Hz*)	*δ*
	4.22 (t)	3.5	4.20 (m)
	4.49 (t)	3.1	4.33 (m)
	4.29 (t)	3.7	4.13 (m)
	4.23 (t)	3.7	4.11 (m)
	4.29 (t)	3.5	4.15 (m)

[a]Signal multiplicities: t, triplet; m, multiplet (higher than triplet).

Inspection of molecular models reveals that such a conformational adjustment decreases the steric interaction between the base and the *endo* methyl group. A steric restriction of rotation of the base is presumably reflected in the enhanced circular-dichroism spectra observed with these compounds[13]. Such a change in conformation is also compatible with observed coupling-values for the anomeric proton. However, conformational analysis by first-order inspection of the spectra is precluded, as the H-2′ and H-3′ resonances are considerably overlapped (in contrast to the β anomers) and those of H-5′ and H-5″ are completely superposed. These chemical-shift equivalence effects may result in pronounced "virtual coupling". In addition, uncertainties in the Karplus relationship[11] for these 2,2-dimethyl-1,3-dioxolane derivatives are well documented[12].

The H-4′ resonance peak in the n.m.r. spectra of 12 anomeric pairs of 2′,3′-*O*-isopropylidene ribonucleoside derivatives examined (Table I) appears as a triplet for the α anomer and as a higher multiplet for the β anomer in all examples. Even the 5,6-dihydrouridine compounds that do not obey the $\Delta\delta$ criterion[1,7] (see later) exhibit compatible H-4′ multiplicities. Thus, the increased flexibility of the aglycon caused by saturation of the 5,6-double bond is tolerated in this determination. Furthermore, it is noteworthy that the anomers of 2,3-*O*-isopropylidene-D-

ribofuranosylamine *p*-toluenesulfonate and 3-carbamoyl-1-(2,3-*O*-isopropylidene-ribofuranosyl)pyridinium chloride also exhibit compatible H-4′ multiplicities, but do not obey the $\Delta\delta$ criterion[14]. Moffat and co-workers have made similar observations in the carbohydrate field[15], again in situations where the $\Delta\delta$ criterion is inapplicable.

One technical exception to these observations involves the anomeric 6-*N*-(2,3-*O*-isopropylidene-D-ribofuranosyl)adenines, in which adenine is linked to the sugar moiety via the exocyclic amino group[16]. The spectra of these compounds have peaks for H-4′ of the α anomer at δ 3.98 (t, $J = 4.0$ Hz) and of the β anomer at δ 4.08 (t, $J = 4.0$ Hz). However, this is not a "normal" nucleoside, and the discrepancy for the β anomer may be rationalized by the much greater conformational flexibility about the C-1′ region conferred by introduction of an extra atom in the linkage from the sugar to the rigid heterocyclic base.

Thus, the multiplicity of the H-4′ n.m.r. resonance-peak of 2′,3′-isopropylidene acetals provides an additional item of spectroscopic evidence for investigation of the anomeric configuration of ribonucleosides, in conjunction with the $\Delta\delta$ criterion. In instances where the $\Delta\delta$ criterion is not applicable because of saturation of the aglycon, this multiplicity effect apparently remains valid. However, it must be emphasized that this is an empirical observation, and the C-1′ substituent should be of sufficient bulk and rigidity to ensure that enough steric interaction exists between the 5′-CH_2OH group and the base of the β anomers to prevent a decrease in $\phi_{3',4'}$ towards 90°, with consequent diminution of $J_{3',4'}$.

EXPERIMENTAL

The isopropylidene acetals of the ribonucleosides were prepared by conventional methods[7,17]. N.m.r. spectra were recorded at 100 MHz with a Varian HA-100 spectrometer in Me_2SO-d_6 and chemical shifts were measured in δ units downfield from internal tetramethylsilane.

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