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CONFORMATION OF SOME RIBOFURANOSYL IMIDAZOLE AND URACIL NUCLEOSIDES: A ^{1}H AND ^{13}C NMR STUDY

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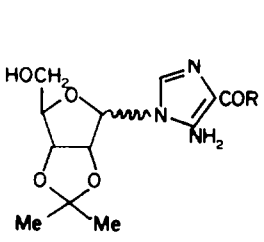
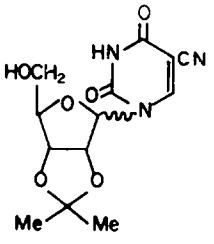
Abstract—100 and 220 MHz ^{1}H NMR spectra, recorded in DMSO-d_6 solution and including some measurements at 343 K, of three anomeric pairs of novel 2′, 3′-*O*-isopropylidene nucleosides (two ribofuranosyl imidazoles, **1** and **2**, and one uridine **3**, all related to intermediates in *de novo* nucleotide biosynthesis) have been analysed, mostly as overlapping ABX spin systems. Chemical shifts and couplings have enabled the α- and β-anomers to be identified; the sugar-ring vicinal H–H coupling constants indicate that the 2′,3′-ketal blocking group locks the α and β anomers in predominantly S and approximately 1:1 N/S ribofuranoside conformations, respectively. Among the ^{13}C shifts reported for **3** in DMSO-d_6, those of the 2′, 3′-*O*-isopropylidene CH_3 in the α anomer (shift difference 1.4 ppm) are around 1.6 ppm upfield of those for the β anomer (which have a shift difference of 1.8 ppm).



1: R = OEt
2: R = NH_2



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The α and β anomers of the novel N-glycosyl nucleosides ethyl-5-amino-1-(2,3-*O*-isopropylidene-α and β-D-ribofuranosyl) imidazole-4-carboxylate (**1**α and **1**β) and 5-amino-1-(2,3-*O*-isopropylidene-α and β-D-ribofuranosyl)imidazole-4 carboxamide (**2**α and **2**β) are related to active intermediates in the *de novo* biosynthesis of purine nucleotides; 5-cyano-1-(2, 3-*O*-isopropylidene-α and β-D-ribofuranosyl)uracil (**3**α and **3**β) are related to reactive intermediates in the *de novo* biosynthesis of pyrimidine nucleotides and to several naturally occurring nucleosides with anti-viral and anti-tumor activity.[1,2] Such activities of nucleoside derivatives appear to be largely a consequence of the inhibition of biosynthetic pathways leading to pyrimidine or purine nucleotides or to incorporation of nucleotide analogues into nucleic acids. In turn, inhibitory action depends on the anomeric configuration of the analogues; most materials of these types known to be active have a configuration related to the usual β form present in natural nucleotides.

Nuclear magnetic resonance (NMR) chemical shifts and coupling constants provide a means for investigation of sugar-ring puckering, primary-alcohol group conformation, anomeric configuration, and relative orientation of sugar and base rings. In this paper, we report high-field ^{1}H NMR measurements on the α and β anomers of **1–3** together with preliminary ^{13}C measurements on **3**α and **3**β and discuss these in terms of anomeric glycosidic configuration and conformation of the ribose ring.

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EXPERIMENTAL

The nucleosides were prepared as described previously.[1] 100 and 220 MHz ^{1}H NMR spectra **1–3** were recorded in external-lock mode at ambient probe temperature (302 K) for the JEOL MH-100 spectrometer and 295 K for the Varian HR-220 spectrometer; some 100 MHz spectra were also recorded at 343 K. 22.63 MHz ^{1}H broad-band-decoupled ^{13}C spectra of **3**α and **3**β were recorded at the PCMU, Harwell, and at Queen Mary College, London. All samples were examined as *ca.* 5–10% w/v solutions in DMSO-d_6 dried over molecular sieve and stored in a dry box. NMR sample tubes were outgassed with dry nitrogen gas and sealed with Gallenkamp "Parafilm" tissue. As a result of residual moisture in the DMSO-d_6, the HOD solvent peak at δ 3.0–4.5 obscured some of the H(2′)-H(5′) peaks and also exchange occurred with NH_2 and CH_2OH hydrogens of the solutes. 100 MHz decoupling experiments confirmed preliminary chemical-shift assignments and enabled vicinal coupling constants to be deduced. Shifts and couplings were then computer-analyzed with the LAOCOON 4-SPIN and LAOCOON 1968 programs[3] on the Bradford University ICL 1904 A computer.

RESULTS AND DISCUSSION

^{1}*H spectra of the ribofuranosyl imidazoles* **1** *and* **2**

In the ribose moeties in each of the α anomers of **1–3**, the chemical shift differences (Table 1) between H(2′) and H(3′) and between H(5′) and H(5″) are small or zero, as is the coupling between H(3′) and H(4′) (Table 2). Consequently, H(1′), H(2′) and H(3′) form a deceptively simple ABX spin system, with AB protons appearing in the spectrum (Fig. 1) as a doublet and X proton as a triplet, from which only the combined coupling $|J_{AX}+J_{BX}|$ may be extracted.[4] Similarly, the other ribose protons H(4′) H(5′), H(5″), and OH(5′) constitute an ABMX system, from which decoupling of either H(4′) or OH(5′) can allow observation of one or other of two deceptively simple ABX systems, H(4′), H(5′), or H(5′), H(5″), and OH(5′). Since irradiation at H(5′, 5″) reduced the H(4′) "triplet" to a broad (1 Hz) singlet, then $J_{3'4'}=0$ (compare isopropylidenes).[4,5]

For the β anomers, on the other hand, $\Delta\delta(2'3')\neq 0$, so that H(1′) appears in the ^{1}H spectrum (Fig. 2) as a doublet (actually a doublet of closely spaced doublets, typical of X of ABX), while H(2′) and H(3′) are the other components of what is virtually an AMX system, i.e. they appear as doublets of doublets. Similarity of the H(5′, 5″) shifts and non-zero coupling between H(3′)

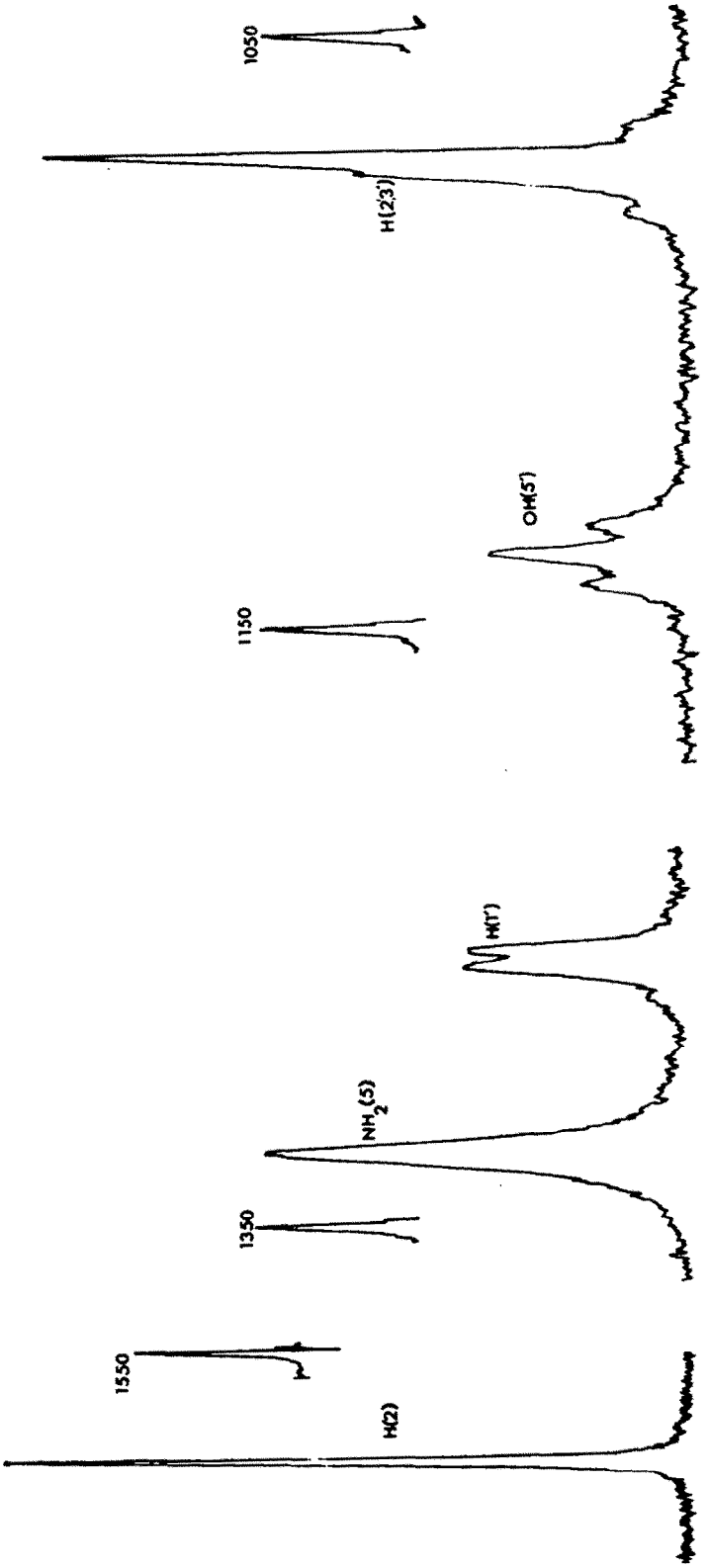


Fig. 1.

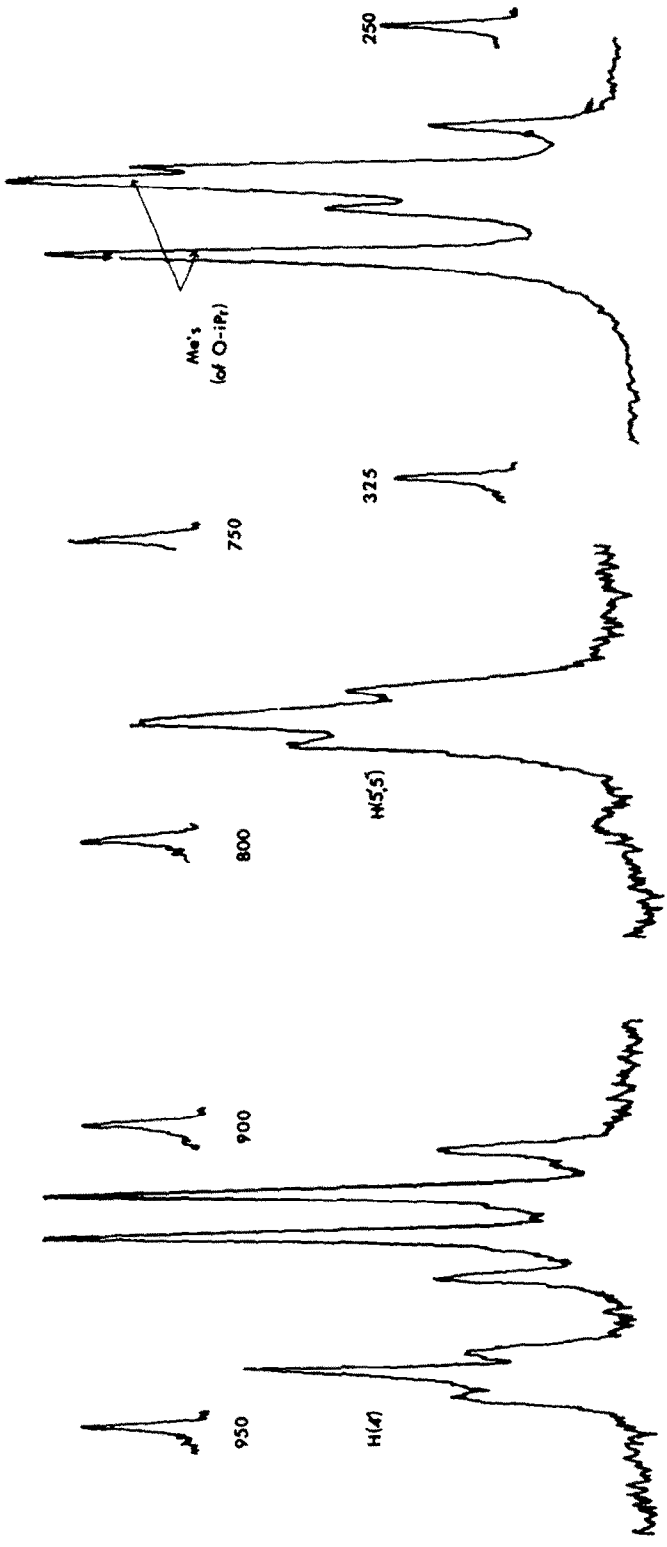


Fig. 1. 220 MHz ^1H spectrum of 1*a* in DMSO-d_6 solution; frequency markers indicate downfield shifts from TMS in Hz.

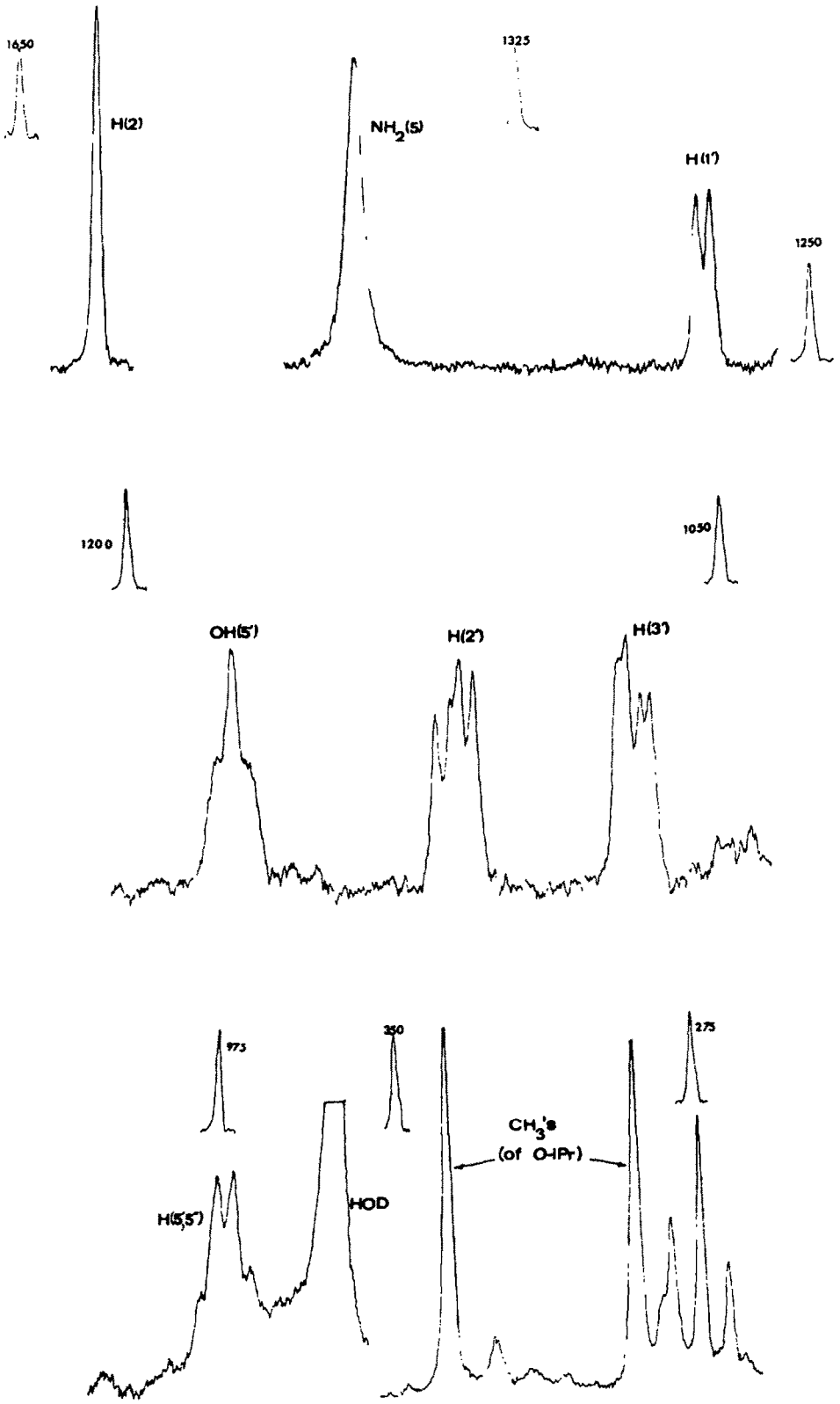


Fig. 2. 220 MHz ^{1}H spectrum of 1β in DMSO-d_6 solution; frequency markers indicate downfield shifts from TMS in Hz; H(4') and $CO_2CH_2CH_3$ resonances omitted.

Table 1. ^{1}H chemical shifts (ppm downfield from internal TMS) of α and β anomers 1, 2 and 3 in DMSO-d_6 (at 293 K, unless otherwise specified)

Proton(s)	1α		1β					
	293 K	343 K	293 K	343 K	2α	2β	3α	3β
CH_3 (of CO_2Et)	1.24	1.24	1.24	1.24	—	—	—	—
CH_3 (of O–iPr)	1.26	1.26	1.32	1.33	1.27	1.33	1.27	1.32
	1.32	1.32	1.54	1.54	1.36	1.55	1.34	1.50
H(5′, 5″)	3.55	3.56	3.52	3.54	3.54	3.55	3.60	3.59
CH_2 (of CO_2Et)	4.16	4.17	4.17	4.16	—	—	—	—
H(4′)	4.28	4.25	4.14	4.14	4.22	4.12	4.45	4.17
H(3′)	4.87	4.88	4.88	4.90	4.87	4.88	4.81	4.71
H(2′)	4.88	4.88	5.11	5.10	4.89	5.10	4.81	4.90
OH(5′)	5.18	4.93	5.34	5.21	5.09	5.27	N.O.[a]	N.O.[a]
H(1′)	5.94	5.93	5.80	5.81	5.89	5.79	6.07	5.76
NH_2(5)	6.08	5.91	6.22	6.09	5.80	5.94	—	—
H(2)	7.13	7.14	7.42	7.41	7.12	7.39	—	—
H(6)	—	—	—	—	—	—	8.35	8.67
$CONH_2$(4)	—	—	—	—	6.68	6.74	—	—

[a]NH(3) and OH(5′) not observed in 3α and β because of exchange with moisture in DMSO-d_6 solvent.

cause H(4′) to appear as a complex multiplet (a doublet of pseudotriplets). As with the α anomers, the H(5′)/H(5″) resonances from a complex multiplet (unless decoupled), owing to simultaneous coupling to H(4′) and OH(5′) (H(4′) is not included in Fig. 2).

The rather small temperature dependence of the ^{1}H chemical shifts and coupling constants of 1α and 1β (Table 1) suggests that the overall molecular conformation is stable over the temperature range 293–343 K, perhaps because of the 'locking' effect of the ketal blocking group.

For each compound 1 and 2, the pairs of α and β anomers have virtually the same ^{1}H shifts for the ribose H(5′, 5″) and H(4′) and for the imidazole 4-substituents. The principal differences from α to β are (i) shielding of ribose H(4′); (ii) deshielding of H(2), NH_2(5), H(2′) and OH(5′) (though part of the differences in α- and β-anomeric shifts for NH_2(5) and OH (5′) could be ascribed to the exchange effects of DMSO-d_6 of slightly different moisture content); and (iii) differential deshielding of the methyls of the 2′, 3′-*O*-isopropylidene group. Among the coupling constants (Table 2), $J_{1'2'}$ is almost the same (around 3.5 Hz) within each pair of anomers, so that the major J_{HH} difference between α and β anomers is in $J_{3'4'}$ (zero for the α forms); values of this, and of $J_{2'3'}$, were estimated from linewidths, by use of molecular models, and by comparison with analogous structures. For $J_{4'5'}$ and $J_{4'5''}$, only the combined coupling is accessible.

Differentiation between α and β N-glycosides is thus achieved by (a) the larger H(1′) chemical shift of the α (0.14 ppm in 1, 0.10 in 2), and (b) the smaller shielding difference between the *exo* and *endo* methyl groups of the *O*-isopropylidene group by the imidazole base for the α anomer than the β[7]: $\Delta\delta^\alpha = 0.06$ (0.07) ppm, $\Delta\delta^\beta = 0.22$ (0.21) for 1; and $\Delta\delta^\alpha = 0.09$ (0.10), $\Delta\delta^\beta = 0.22$ (0.21) for 2 (the figures in parentheses are those reported by Imbach).[7] In addition, the appearance of the H(4′) signal, a triplet in the α and a complex multiplet in the β, provides a useful practical means of distinguishing between anomers of 2′,3′-*O*-isopropylidene nucleosides, even if the explanation is uncertain. The apparent triplet is a consequence of $J_{3'4'} = 0$, implying that the dihedral angle $\phi_{3'4'} = 90°$; while such a conformation is said to lead to a decreased steric interaction between the *endo* methyl of the ketal group and the unsaturated base at C(1′),[8] our molecular models show an increased interaction, unless the sugar ring is predominately in a severely buckled S-type conformation (C(2′) *endo*, O(1′) *exo*).

Table 2. Vicinal H–H coupling constants/Hz for α and β anomers of 1, 2 and 3

Compound	$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$(J_{4'5'} + J_{4'5''})$
1α	3.2	6.0	0	7.2
2α	3.9	6.5	0	8.4
3α	3.9	6.3	0	6.0
1β	3.5	6.6	2.6	7.9
2β	3.5	6.2	2.8	7.6
3β	3.2	6.1	3.0	8.0

Indications of the relative orientation (*syn* or *anti*) of imidazole base to sugar ring, i.e. the glycosidic torsion angle χ_{CN}, can be gleaned from the magnitude of long-range coupling between base H(2) and ribose H(1′). When H(1′) was decoupled from H(2′) (in the β anomers) or from H(2′, 3′) (α anomers), the broadened H(1′) signal implied a small (~0.3 Hz) residual coupling, presumably to H(2′). Since the linewidth of the decoupled H(1′) signal remained constant at *ca.* 0.8 Hz, implying no change in $^3J_{H(1')\text{-}H(2)}$, we deduced that the glycosidic conformation angle, χ_{CN}, was the same (or differed by π) in α and β anomers; study of molecular models indicates that the imidazole base is in the preferred *anti* range.

Ribose-ring conformation of 1 *and* 2

The magnitudes of the $J_{2'3'} = 6.4$ Hz coupling, comparable with the 6 ± 0.5 Hz found by Gaudemer *et al.*[9] in a series of 2′, 3′-*O*-isopropylidenoadenosine derivatives, and $(J_{1'2'} + J_{3'4'}) = 6.2$ Hz coupling for the β anomers of 1 and 2 imply that the ribose ring is flatter[10] in the 2′, 3′-*O*-isopropylidene nucleosides than in their parent nucleosides[6]. Molecular models for plausible N and S geometries of 1 and 2 yielded static dihedral angles, ϕ_{HH},

Table 3. Model geometries and calculated coupling constants, J_{HH}/Hz, for N and S conformers of 2',3'-*O*-isopropylidene nucleosides

	Type N (C(3') *endo*) Torsion angle, ϕ_{HH}	J_{HH}	Type S (C(2') *endo*) Torsion angle, ϕ_{HH}	J_{HH}
$1'2'_{trans}$	95° (85°)	0.2 (0.1)	135° (138°)	5.5 (6.2)
$1'2'_{cis}$	25°	7.2	15°	8.2
2'3'	30° (33°)	6.6 (6.2)	−25° (−23°)	7.2 (7.5)
3'4'	−140° (−138°)	6.4 (6.2)	−100° (−101°)	0.5 (0.5)

Values in parentheses are from de Kok *et al.*[12]

between hydrogen substituents of the ribose ring (Table 3), from which coupling constants, J_{HH}, were calculated by the modified[10] Karplus equation (1),

$$J_\phi = A\cos^2\phi - B\cos\phi + C \text{ (in Hz)}. \quad (1)$$

in which $C = 0$, $B = 0.9$ and $A = 9.8$.[11] In the crystal structure of 2',3'-*O*-methyloxymethyleneuridine,[12] the ribose ring, which is somewhat analogous to those of **1** and **2**, assumes an S-type conformation with pseudo-rotational angle $P = 162.8°$ and maximal degree of puckering[10] $\tau_m = 23.1°$, i.e. within the normal pseudo-rotational range for S nucleosides but with rather little ring puckering. Preliminary X-ray crystallographic data[13] for **1β** indicate that the ring pucker in the solid may be described as C(2') *endo*, O(1') *endo*, with C(2') well out of the ribose-ring plane.

Combination of the calculated $J_{1'2'}$ for N and S conformers (Table 3) with the observed $J_{1'2'}$ (Table 2) enables the relative populations X_N, X_S of the two conformers to be estimated from equation (2):

$$J^{obs}_{1'2'} = X_S(J^S_{1'2'}) + (1 - X_S)J^N_{1'2'}. \quad (2)$$

The ratio $X_S/X_N = 0.63/0.37$ then gives the equilibrium constant K_{eq} for the process N⟷S in **1β**. Similarly, from $J_{3'4'}$ we obtain another estimate for $X_S = 0.64$ and $K_{eq} = 1.8$ in **1β**. The conclusion by Gaudemer *et al*[9]. that solutions of the (presumably β) 2',3'-*O*-isopropylideneadenosines with sp^3 bonding at C(5') [the first sub-group of these authors' first group] contain rather more N than S conformers derives from their surprising finding than $J_{1'2'} < J_{3'4'}$; possibly these two couplings could be interchanged. The $J_{2'3'} = 6.8$ Hz calculated on the basis of these equilibrium composition agrees well with the 6.6 Hz observed. Results for **1β**, **2β** and **3β** are summarized in Table 4.

For the α-anomers, the vicinal coupling constants (Table 2) extracted from the second-order spectra due to $\Delta\delta(2',3') \sim 0$ are less reliable than for the β-anomers. The overwhelming preponderance of an S-type conformer (as in Gaudemer's 1st subgroup of the first group (presumably β) isopropylidene adenosines in DMSO)[9] in the equilibrium mixture implied by $J_{3'4'} = 0$ appears to conflict with $J_{1'2'} = 3.2$ and 3.9 Hz, respectively, observed for **1α** and **2α**, since the geometries of Table 3 would require $J^{cis}_{1'2'} > 7$ Hz for all K_{eq}. Evidently the solution geometries of the α anomers may differ from those of the β anomers in involving two or more conformers in which the O(1') atom leaves the C(4')-*O*(1')-C(1') plane, e.g. (C(2')*endo*, *O*(1')*exo* (^2_OT) and C(3')*endo*, *O*(1')*endo* (^3_OT).

The rather bigger $J_{1'2'}$ for **2α** than **1α** (Table 2) and the slightly greater chemical non-equivalence of H(2',3') in **2α** than in **1α** (Table 1) point to some difference in ribose equilibrium conformation which, apparently, is also reflected in the relative proportions of the *gg* conformer in the exocyclic CH_2OH groups of **1α** and **2α**. When, as in both anomers of **1–3**, deceptively simple ABX/ABC spectra allow only the sum ($J_{AX} + J_{BX}$) or ($J_{4'5'} + J_{4'5''}$) to be extracted, the relative population of the *gauche–gauche* classical rotamer may still be estimated from the approximate equation.[14, 15]

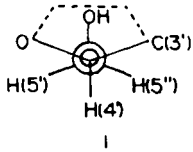
$$P_{gg} = [13 - (J_{4'5'} + J_{4'5''})]/10. \quad (3)$$

This yields $P_{gg} = 0.5$ for **1β** and **2β**, and 0.6, 0.45 for **1α**, **2α**, i.e. this conformer is preferred, as is usual in both the solid state and in solution,[16, 17] but rotation of the CH_2OH group is fairly free. The lower P_{gg} for **2α** than **1α** may be a consequence of increased steric interaction between the CH_2OH group and the ribose protons, caused by the buckled ribose-ring conformation in the α-anomers (as apparently required by $J_{3'4'} = 0$). Interactions of CH_2OH with the base and with the ribose protons H(2') and H(3') also tend to make P_{gg} lower for β than α, even with a preferred *anti* conformation about the glycosidic bond.

Table 4. Fractional conformer populations, X_S and X_N, and equilibrium compositions K_{eq} for **1β**, **2β** and **3β**

	1β	**2β**	**3β**
X_S	0.64	0.64	0.58
X_N	0.36	0.38	0.42
$K_{eq} = X_S/X_N$	1.8	1.6	1.4

X_S is the fractional population of S-type conformer, i.e. $(X_S + X_N) = 1$.



gauche–gauche

Spectra of the ribofuranosyl uridines **3**

The ^{1}H shifts (Table 1) and coupling constants (Table 2) of the α and β anomers of **3** closely parallel those discussed for compounds **1** and **2**. In particular, from **3α**

to 3β, the H(1') shift decreases by 0.31 ppm (compared with 0.14 and 0.10 for 1 and 2), the H(4') shift decreases by 0.31 ppm (0.14 and 0.12 for 1 and 2), while the H(6) uridine shift increases by 0.32 between 3α and 3β (analogous to the increases of 0.29 and 0.27 ppm for the H(2) of the imidazole in 1 and 2). Further support for anomeric identification of 3β was provided by the somewhat large downfield shift of C(1') (Table 5) than in 3α[18] and by the larger difference (1.8 rather than 1.4 ppm in the α) between the two 2',3'-*O*-isopropylidene CH_3 resonances (which are about 1.6 ppm downfield of those of the α anomer[19]) (Figs 3 and 4).

Indications of the glycosidic torsion angle can come from long-range sugar-base coupling and from ribose chemical shifts. Explicit long-range coupling between H(1') and H(6) was not discerned for either anomer, but, as in 1 and 2, the linewidths of the H(1') signal, when decoupled from H(2'), indicated a small (~0.3 Hz) four-bond coupling in both anomers. By comparison with the ribose ^1H shifts of the parent, 2',3'-*O*-isopropylidene uridine,[2] those of 3β are *all* relatively shielded, an indication that the *anti* conformation is preferred, as models suggest is also the case for 3α and β.

If the shifts of H(1') (all close to 5.78 ppm) and H(4') (all close to 4.15 ppm) of the β-anomers (taken to be in *anti* conformation, with appreciable populations of N and S conformers), are accepted as a reference, then changes in these chemical shifts in the α anomer can be ascribed to a combination of altered ribose conformation and changes in the *syn*/*anti* glycosidic conformation. The excess of 0.17–0.20 ppm in the β→α deshielding of 3 (0.31 ppm for both H(1') and H(4')) over the corresponding deshielding for 1 (0.14 and 0.14 ppm) and 2 (0.10 and 0.12 ppm) (predominantly attributable to much the same β→α changes in ribose conformation in 1–3, may tentatively be ascribed to the anisotropy of the 2-keto group of the pyrimidine base, which would imply some *syn* conformational contribution to 3α.

The equilibrium ribose conformation of 3β, as with those of 1 and 2β, shows a slight preference (here 57%) for the S-type conformer (Table 4). For 3α, as with 1 and 2α, the inference, in the absence of much crystallographic data on α-nucleosides, is of a predominantly S-type (C(2')*endo*) conformation, with little evidence of equilibrium with an N-type conformation.

Application of equation (3) to the coupling constants of 3α and β (Table 2) yields approximate fractional populations of 0.7 and 0.5, respectively, of the preferred *gg* rotamer, consistent with fewer sugar-nucleobase interactions in the α anomer. While P_{gg} also depends on the preferred ribose puckering mode (which also affects the glycosidic configuration), we consider that the relative *gg* rotamer populations are influenced primarily by the anomeric configuration.

Table 5. ^{13}C chemical shifts (ppm downfield of internal TMS) for 3α and β in DMSO-d_6

Carbon	3α	3β
C(4)	160.0	160.0
C(2)	150.1	150.5
C(6)	148.0	148.9
CN(5)	114.0	114.2
C(O–iPr)	112.3	112.7
C(5), C(1')[a]	87.4, 87.1	92.5, 88.1
C(2'), C(3')[a]	83.6, 81.4	87.7, 84.5
C(4')	79.0	80.2
C(5')	62.1	60.9
CH_3(O–iPr)	25.1, 23.7	26.9, 25.1

[a]Assignments C(5), C(1') may be interchanged; similarly C(2'), C(3').

CONCLUSIONS

In solution, the β anomers of the 2',3'-*O*-isopropylidene nucleosides 1, 2 and 3 assume a dynamic conformational equilibrium between N and S-type furanose-ring puckering modes with slight predominance of the S conformer. The preferred glycosidic rotational conformer is *anti* and the preferred exocyclic CH_2OH group conformation is the *gg* (*gauche–gauche*). The furanose-ring conformations of the α anomers appear to be predominantly (>95%) S-type, again with a preferred *gg* exocyclic CH_2OH conformation and, with the possible exception of the pyrimidine 3β, an *anti* conformation about the glycosidic base.

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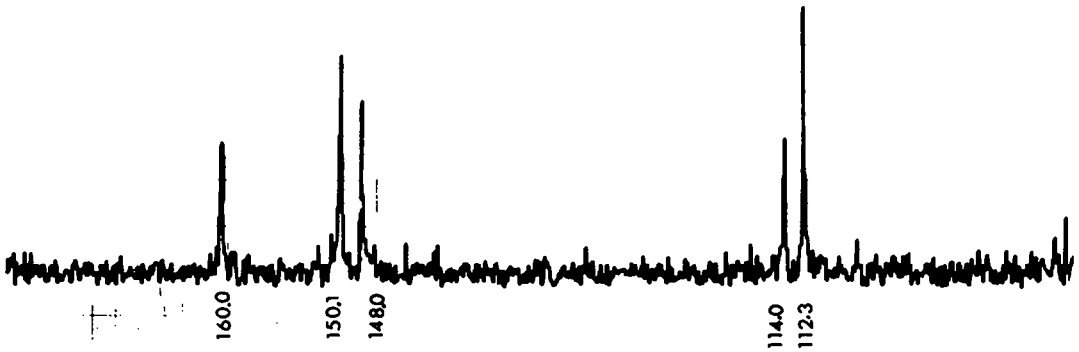

Fig. 3. 22.63 MHz ^{1}H-decoupled ^{13}C spectrum of 3α in DMSO-d_6 solution; shifts in ppm downfield from TMS.

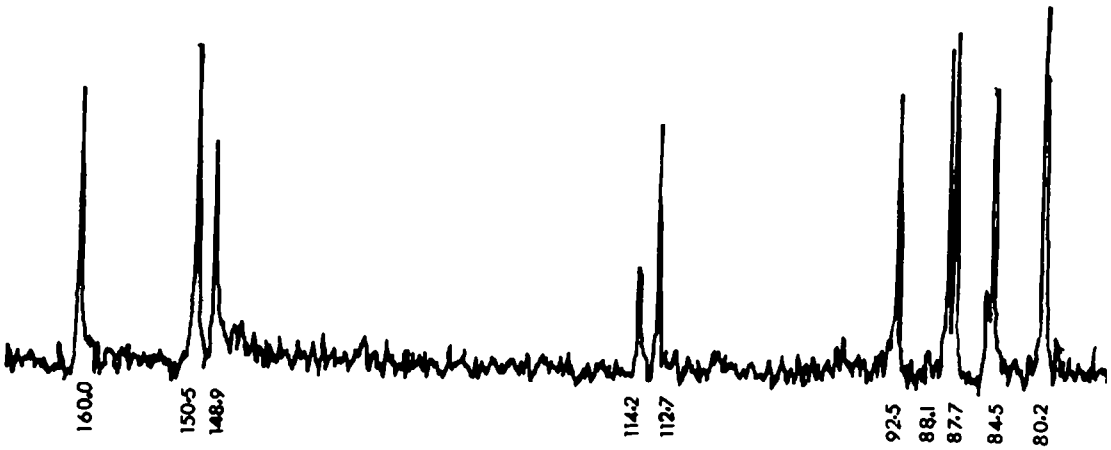

Fig. 4. 22.63 MHz ^{1}H-decoupled ^{13}C spectrum of 3β in DMSO-d_6 solution; shifts in ppm downfield from TMS.

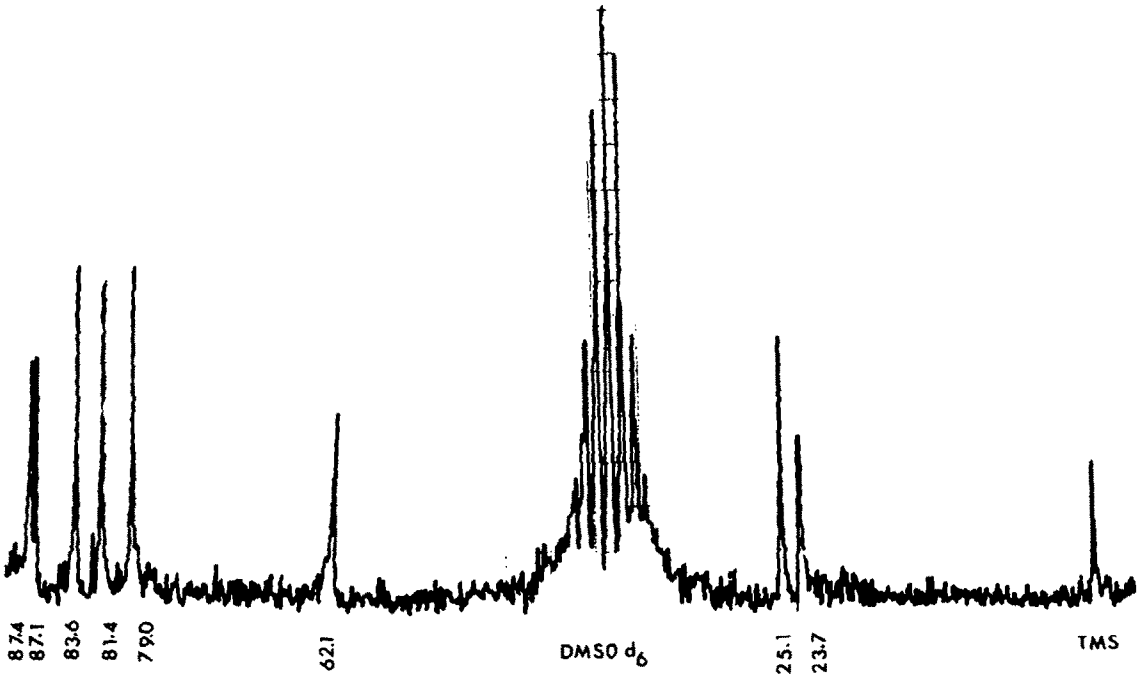

Fig. 3(*Contd*).

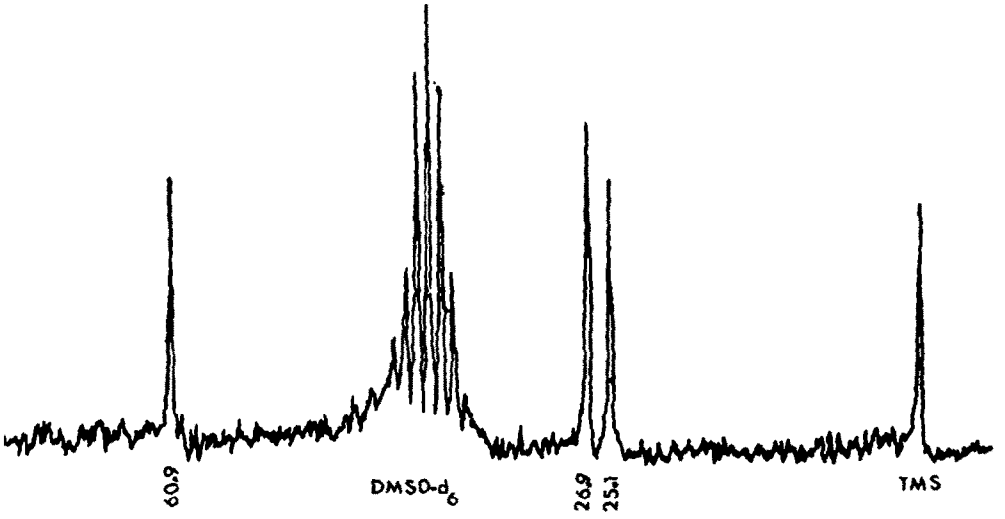

Fig. 4 (*Contd*).