Tetrahedron Vol. 40, No. 4, pp. 709 to 713, 1984
Printed in Great Britain.

0040-4020/84 $3.00 + .00
Pergamon Press Ltd.

CONFORMATIONAL STUDIES OF SOME CARBOCYCLIC NUCLEOSIDE ANALOGUES

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(*Received in France* 1 *March* 1983)

Abstract--Proton NMR spectra of some carbocyclic nucleoside analogs of tubercidin have been analyzed at 100 MHz in DMSO-d_6. The spectral characteristics and nuclear Overhauser effects indicate a preferential *syn* conformation about the glycosidic bond when a hydroxyl group, oriented toward the base, is available for intramolecular hydrogen bond formation.

Carbocyclic nucleosides are nucleoside analogs in which the tetrahydrofuran ring has been replaced by a cyclopentane ring, as in aristeromycin, a natural carbocyclic analog of adenosine.[1-7] We recently prepared some new carbocyclic analogs of tubercidin in order to evaluate their antiviral and antitumor properties[8] (Fig. 1). These molecules exhibit interesting conformational properties which have been investigated by proton NMR and compared with those of the corresponding tetrahydrofuran nucleoside derivatives.

RESULTS AND DISCUSSION

Chemical shifts. The proton NMR spectra have been recorded in DMSO-d_6 at 100 MHz with a Varian XL 100 spectrometer operating in the Fourier transform mode and have been analyzed by standard homonuclear decoupling techniques. Chemical shifts and coupling constants are presented in Table 1. They fully support the proposed chemical structures.

Interestingly, the chemical shift of $OH_{2'}$ in **3b** and $OH_{3'}$ in **2b** are exceptionally high. The shift of $OH_{3'}$ in **2a** is also increased but to a lower extent as compared with the values observed in the case of **1a** or **1b**. This effect can be ascribed to an intramolecular hydrogen bond involving the nitrogen N_1 of the base. Such an hydrogen bond is possible only when the hydroxyl is oriented toward the base and is preferentially located at position 3', as in **2a** or **2b**. This phenomenon is not concentration dependent and is less intense with the chloro derivatives **2a** and **3a** than with the amino compounds **2b** and **3b** where the electron donating effect of the amino group at position 4 increases the basicity at N_1, and, hence, its hydrogen bonding ability. The formation of this intramolecular bond requires a *syn* conformation about the glycosidic bond. In **1a** and **1b**, the average chemical shifts of the $H_{5'}$ and $H_{A,B}$ protons of the carbocyclic sugar are lower and have nearer values within each methylene pair, suggesting a preferred *anti*-conformation about the glycosidic bond which lowers the ring current effects of the base upon these protons. In the 4-piperidino derivatives **3c**, the chemical shift of $OH_{2'}$ is also exceptionally downfield (5.80 ppm) as in the amino derivative **3b**.

Nuclear Overhauser effect measurements. The presence of a methyl group at position 6 of the base

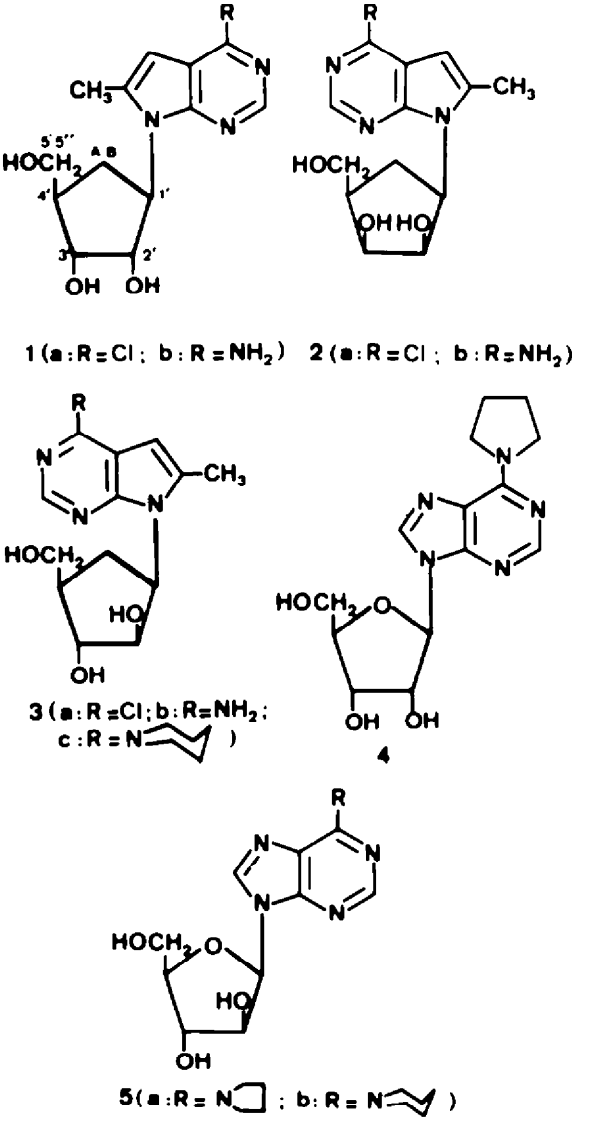

Fig. 1. Nucleosides examined in the present study. The synthesis of the carbocyclic analogs of tubercidin **1**, **2** and **3a, b** has already been published.[8] The synthesis of **3c**, **4** and **5** is described in the present paper. The various nucleosides are drawn in the preferred syn or anti conformation as deduced in this work. Numbering of carbocyclic derivatives **1**, **2** and **3** is specified for **1** and has been used for NMR assignments.

†Chercheur INSERM.
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Table 1. ^{1}H NMR data for compounds 1*a*–3*b* in Me_2SO-d_6 at 100 MHz

	H_2	NH_2	H_5	$OH_{2'}$	$OH_{3'}$	$OH_{5'}$	$H_{2'}$	$H_{1'}$	$H_{3'}$	$H_{5'}$	$H_{5''}$	CH_3	H_A	H_B	$H_{4'}$
1a	8.40 (s)	—	6.34 (q)	4.55 - 4.50 (BR)			4.64 $J_{2',3'}$ = 4.5	4.56 $J_{1',2'}$ = 9.0	3.82 $J_{3',4'}$ = 3.5	3.55 $J_{5',5''}$ = 10.6 $J_{4',5'}$ = $J_{4',5''}$ ~5.7	3.45	2.42 (D) $J_{CH_3,5}$ = 1	2.05 - 2.10 (BR)		
2a	8.51 (s)	—	6.42 (q)	4.67 (D) $J_{2'OH_{2'}}$ = 5.7	5.28 (D) $J_{3'OH_{3'}}$ = 8.1	4.40 (T) $J_{5'OH_{5'}}$ = 5.1	4.22 $J_{2',3'}$ = 5.0	4.98 $J_{1',2'}$ = 9.7	4.02 $J_{3',4'}$ = 3.5	3.75 $J_{4',5'}$ = 6.5 $J_{4',5''}$ = 6 $J_{5',5''}$ = 10.5	3.52	2.52 (D) $J_{CH_3,5}$ = 1	2.52 $J_{1',A}$ = 9.7	2.07 $J_{1',B}$ = 8.3	1.92
3a	8.49 (s)	—	6.38 (q)	4.87 (D) $J_{2',OH_{2'}}$ = 4.7	4.88 (D) $J_{3',OH_{3'}}$ = 5	4.53 (T) $J_{5',OH_{5'}}$ = 5.1	4.0-3.84 —	5.06 $J_{1',2'}$ = 7.3	4.0-3.84 —	3.69 $J_{5',5''}$ = 10.4 $J_{4',5'}$ = 5.0 $J_{4',5''}$ = 7.2	3.54	2.54 (D)	2.78 $J_{A,B}$ = 12.7 $J_{1',B}$ = 7.5 $J_{1',A}$ = 10.7	2.18	1.88 $J_{4',A}$ = 10.8 $J_{4',B}$ = 8.2
1b	7.95 (s)	6.57	6.23 (q) J ~1	4.6-4.65 (BR)	4.48 (D) $J_{3',OH_{3'}}$ = 4.4	4.6-4.65 (BR)	4.66 $J_{2',3'}$ = 4.5	4.58 $J_{1',2'}$ = 8.5	3.87 —	3.56 $J_{5',5''}$ = 10.5 $J_{4',5'}$ = $J_{4',5''}$ ~5.5	3.48	2.39 (D)	2.10 - 2.05 (BR)		
2b	7.94 (s)	6.85 (s)	6.25 (q)	4.48 (D) $J_{2',OH_{2'}}$ = 6.0	6.51 (D) $J_{3',OH_{3'}}$ = 10.0	4.36 (T) $J_{5',OH_{5'}}$ = 5.6	4.20 $J_{2',3'}$ = 4.6	4.76 $J_{1',2'}$ = 9.4	3.91 $J_{3',4'}$ = 4.0	3.72 $J_{5',5''}$ = 10.2 $J_{4',5'}$ = 6.8 $J_{4',5''}$ = 5.8	3.46	2.35 (D) $J_{CH3,5}$ = 1	2.25	2.0 $J_{1',B}$ = $J_{1',A}$ ~9	1.90
3b	7.96 (s)	6.80 (s)	6.28 (q) J ~1	6.02 (BR)	4.88 (D) $J_{3',OH_{3'}}$ ~5.0	4.55 (BR)	3.90 $J_{2',3'}$ = 3.6	4.85 $J_{1',2'}$ = 5.6	3.80 $J_{3',4'}$ = 4.2	3.62 - 3.49 $J_{5',5''}$ = 10.5 $J_{4',5'}$ ~ 6 $J_{4',5''}$ ~ 7.5		2.39 (D)	2.30 $J_{1',A}$ = 10.8	2.10 $J_{1',B}$ = 8	1.90

[A] ABBREVIATIONS USED : S, SINGLET ; D, DOUBLET ; T, TRIPLET ; Q, QUADRUPLET ; BR, BROAD.
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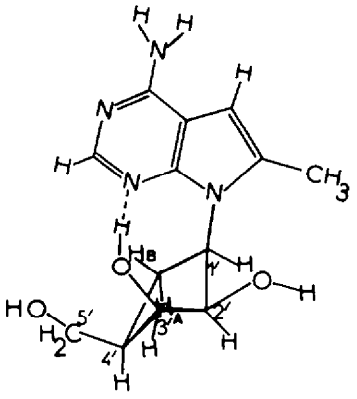
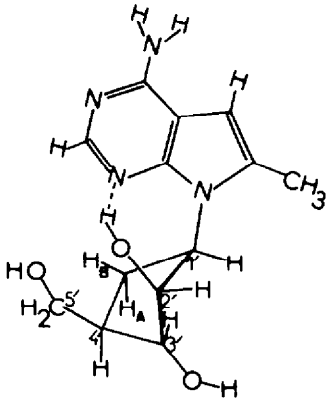


2b



3b

allowed us to investigate the *syn-anti* equilibrium about the glycosidic bond through intramolecular nuclear Overhauser effects. A positive enhancement of the $H_{1'}$ resonance measured by integration of the line upon irradiation of this methyl group can be interpreted on the basis of a *syn* conformation.[9] Such a large effect was observed in compounds **2b**, **3b** and **3c** but was not measurable in **3a**. All other NOE's were lower than 5% (Table 2). In the three former compounds, the relaxation of $H_{1'}$ is thus dominated by a dipole-dipole interaction with the methyl protons. This indicates a rigid structure, i.e. a nearly pure *syn* conformation, with a maximum internuclear distance of 2.80–2.95 Å.[9] On the contrary, the *syn-anti* equilibrium in compound **3a** should be largely displaced toward the *anti* conformation, as already suggeted by the absence of a large perturbation of the $OH_{2'}$ resonance (< 0.2 ppm).

There is therefore a good correlation between the glycosidic conformation and the ability to form an intramolecular hydrogen bond.

The fact that strong hydrogen bonding able to stabilize the *syn* conformation occurs in compound **2b** preferentially at $OH_{3'}$ and in compounds **3b** and **3c** at $OH_{2'}$ may appear contradictory with the large NOE enhancement of the $H_{1'}$ resonance observed in both cases upon saturation of the base methyl. This should correspond to similar intranuclear distances involving necessarily some conformational changes in the carbocyclic ring.

Coupling constants. The sugar ring puckering can be estimated by analysis of the NMR coupling constants. Vicinal couplings of the protons are related to the dihedral angle in the corresponding H–C–C′–H′ bonding system according to the Karplus equation.[10] They should also depend upon the nature of the substituents. In the present study however, only the stereochemistry of the substituents of the cyclopentane ring varies and the observed differences should depend mainly on the conformation.

In **1a** and **1b**, $J_{1'2'}$ is large (9 Hz and 8.5 Hz re-

Table 2. NOE experiments carried out at 100 MHz on degassed solution of **2b**, **3a**, **3b** and **3c** in DMSO-d_6

Compound	Irradiated	Observed	Percentage of enhancement[a]
2b	CH_3	$H_{1'}$	17.5 ± 2
3a	CH_3	$H_{1'}$	< 5
	H_A	$H_{1'}$	< 5
	H_B	$H_{1'}$	< 5
3b	CH_3	$H_{1'}$	17 ± 2
	H_A	$H_{1'}$	< 5
	H_B	$H_{1'}$	< 5
3c	CH_3	$H_{1'}$	17 ± 2

[a] The integration of the $H_{1'}$ resonance was repeated four independent times and compared with similar experiments using 1000 Hz off-resonance decoupling.

spectively) and should correspond to a *trans* diaxial configuration of the corresponding protons, i.e. to a $C_{2'}$ *endo* conformation of the ring in which the 2'-carbon is out of the plane formed by the four other carbons. This "envelope-like" conformation is consistent with the observed $J_{2'3'}$ and $J_{3'4'}$ couplings (Table 1, $J_{3'4'}$ was not observable for **1b**). The couplings of the nearly degenerated resonances of H_A, H_B and $H_{4'}$ are unfortunately not resolved for these compounds. The large couplings (> 10 Hz) observed for $H_{1'}$, H_A and $H_{4'}$ in compounds **3a** and **3b** (where $J_{4'A}$ is not resolved) should correspond to a conformation in which one of the methylene bridge protons is either strictly *cis* (eclipsed) or strictly *trans* with respect to $H_{1'}$ and $H_{4'}$. The *trans* situation, which should correspond to an out-of-plane situation of the methylene bridge, is not compatible with the observed $J_{1'2'}$ couplings. Compounds **3a** and **3b** thus exhibit an "envelope-like" conformation similar to that of **1a** and **1b**, i.e. with an *endo* 2'-carbon and H_A *cis* with respect to both $H_{1'}$ and $H_{4'}$. This conformation is further confirmed by equatorial-axial couplings of the *trans* $H_{3'}$ and $H_{4'}$ protons ($J_{3'4'} = 4.2$ Hz in **3b**, not resolved in **3a**) and of the *cis* $H_{2'}$ and $H_{1'}$ protons ($J_{1'2'} = 7.3$ and 5.6 Hz).

Intramolecular hydrogen bonding occurs preferentially at $OH_{3'}$ both in **2a** and **2b**. This requires a change in the conformation of the cyclopentane ring. The resolved couplings indicate indeed a $C_{3'}$ *endo* conformation which pushes the hydroxyl group toward the base. The $H_{1'}$ proton is then nearly eclipsed both with one of the protons of the methylene bridge ($J_{1'A} = 9.7$ and 9 Hz respectively) and with $H_{2'}$ ($J_{1'2'} = 9.7$ and 9.4 Hz). The puckering at $C_{3'}$ is further confirmed by the *cis* equatorial-axial conformation of both $H_{3'}$ and $H_{4'}$ ($J_{3'4'} = 3.5$ and 4.0 Hz) and $H_{3'}$ and $H_{2'}$ ($J_{2'3'} = 5.0$ and 4.6 Hz). There is therefore a balance between the preferential hydrogen bonding of $OH_{3'}$ and the less stable conformation of the ring with the main puckering at $C_{3'}$ instead of $C_{2'}$.

CONCLUSION

Examination of Dreiding models confirms these conformations and further indicates that hydrogen bonding of $OH_{3'}$ must be sterically easier than that of $OH_{2'}$ and that the change of conformation involves very little rotation of the carbocycle around the glycosidic bond as indicated by the NOE experiments.

The difference in energy between the two ring conformations should be low and there may exist a thermal equilibrium, especially in the absence of hydrogen bonding of the cyclopentane moiety with the base. Temperature dependent experiments, using high field NMR in order to resolve all the coupling constants are necessary to investigate more accurately these conformational equilibria.

It is interesting to notice that no such intramolecular hydrogen bond has been observed in the arabinofuranosyl derivatives **5a** and **5b** (to be compared to **3c**) which are known also to present conformers in equilibrium in solution of the types S($C_{2'}$ *endo*, $C_{3'}$ *exo*) ⇄ N($C_{2'}$ *exo*, $C_{3'}$ *endo*).[11] The hydroxylic protons in these compounds do not exhibit any downfield shift as compared to those of **4**, a furanose analog of **1b**. Compounds **4**, **5a** and **5b** have a nitrogen at position 5 of the base, but this supplementary nitrogen has a much lower effect on the pK value of N_1 than that of the substitution of an amino group at C_4. The possibility of intramolecular hydrogen bonding and consequently of a *syn* conformation stable in solution is thus specific of the carbocyclic nucleosides.

EXPERIMENTAL

6-Pyrrolidino-9-(β-D-ribofuranosyl) purine **4**

A mixture of 6-chloro-9-(β-D-ribofuranosyl) purine (Aldrich) (2 g, 7 mmol) and pyrrolidine (1 g, 14 mmol) in methylcellosolve (30 ml) was refluxed for 1 h. After evaporation of the solvents under reduced pressure, crystallization from methanol afforded the pure compound. m.p. 144–146° (litt.: m.p. = 145°).[12] yield: 80%; 1H NMR spectrum (DMSO-d_6): H_8 (8.34; s); H_2 (8.20; s); $H_{1'}$ (5.90; d; J = 6.2 Hz); $OH_{2'}$ (5.41; d; J = 5.8 Hz); $OH_{3'}$ (5.15; d; J = 4.8 Hz); $H_{2'}$ (4.60; m; $J_{2'3'} = 4.9$ Hz); $H_{3'}$ (4.18; m; $J_{3'4'} = 3.2$ Hz); $H_{4'}$ (4.01; m); $H_{5'}H_{5''}$ (3.71–3.57; m; $J_{4'5'} \sim 3.4$ Hz); CH_2 α pyrrol. (3.7–3.9); CH_2 β pyrrol. (2.01).

6-Pyrrolidino-9-(β-D-arabinofuranosyl) purine **5a** *and 6-piperidino-9-(β-D-arabinofuranosyl) purine* **5b**

Both compounds were obtained from 6-chloro-9-(2', 3', 5'-tri-O-acetyl - β - D-arabinofuranosyl) purine. The 6-chloro derivative was prepared from 9-β-D-arabinofuranosylhypoxanthine (Pfanstiehl Lab Inc) by acetylation in pyridine/acetic anhydride and subsequent chlorination by DMF–$SOCl_2$ in $CHCl_3$. Substitutions by pyrrolidine or piperidine in excess were performed in dry DMF at room temperature overnight. Evaporation of the solvents and subsequent crystallization from water gave **5a** in 62% yield, m.p. = 238° and **5b** in 48% yield, m.p. = 192–195°. 1H NMR (DMSO-d_6) **5a**: H_8 (8.19; s); H_2 (8.16; s); $H_{1'}$ (6.28; d; J = 4.2 Hz); OH (5.56; J = 5.1 Hz); OH (5.47; J = 4.3 Hz); $OH_{5'}$ (5.05; t; J = 5.3 Hz); $H_{2'}$, $H_{3'}$, $H_{4'}$ (4.14; m); CH_2 α pyrrol. (3.81; m); $H_{5'}$, $H_{5''}$ (3.68; m); CH_2 β pyrrol. (1.95; m). For $C_{14}H_{19}N_5O_4$ calc C, 52.33; H 5.96; N, 21.80; found C, 52.57; H, 6.01; N, 21.69%. 1H NMR (DMSO-d_6) **5b**: H_8 (8.20; s); H_2 (8.19; s); $H_{1'}$ (6.30; d; J = 4.4 Hz); $OH_{3'}$ (5.57; d; J = 5.2 Hz); $OH_{2'}$ (5.48; d; J = 4 Hz); $OH_{5'}$ (5.05; t; J = 5.4 Hz); $H_{2'}$, $H_{3'}$, $H_{4'}$, CH_2 α piperid. (4.12; m); $H_{5'5''}$ (3.86–3.60; m); CH_2 β and CH_2 γ (1.63; m). For $C_{15}H_{21}N_5O_4$, Calc C, 53.72; H, 6.31; N, 20.89; found C, 53.80; H, 6.33; N, 20.79%.

(±)-4-Piperidino-6-methyl 7[(1α, 2α, 3β, 4α)-2, 3-dihydroxy-4-(hydroxymethyl) cyclopent-1α-yl)] (7H) pyrrolo [2, 3-d] pyrimidine **3c**.

A solution of (±)-4-chloro-6-methyl 7-[1α, 2α, 3β, 4α)-2, 3-dihydroxy - 4 - (hydroxymethyl) cyclopent-1α–yl)] (7H) pyrrolo [2, 3-d] pyrimidine **3a** (1 g, 3.36 mmol),[8] 10 ml of piperidine, in 25 ml of methylcellosolve was refluxed overnight. The resulting mixture was evaporated to an oil which was subjected to alumina column chromatography ($CHCl_3$–EtOH 9:1, v/v). Trituration with diethylether afforded **3c**, yield 38%, m.p. 151–154°. 1H NMR (DMSO-d_6): H_2 (8.07; s); H_5 (6.35; q); $OH_{2'}$ (5.80; d; J = 4.5 Hz); $H_{1'}$ (5.05; m); $OH_{3'}$ (4.87; d; J = 4.5 Hz); $OH_{5'}$ (4.53; t; J = 5.2 Hz); $H_{2'}$ (3.89; m); $H_{3'}$ (3.83; m) CH_2 α pyrrol. (3.85: m); $H_{5'}$ (3.63; m); $H_{5''}$ (3.49; m); CH_3 (2.42; d); H_A (2.35; m); H_B (2; m); $H_{4'}$ (1.88; m); CH_2 β pyrrol. (1.63; m). For $C_{18}H_{26}N_4O_3$, Calc C, 62.40; H, 7.56; N, 16.17; found C, 62.57; H, 7.48; N, 16.28%.

Acknowledgements—We thank the Institut National de la Santé et de la Recherche Médicale (INSERM) for finanical support: CRL 813 013.

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