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ADENOSINE-*5′-d*: ROTATIONAL CONFORMATION OF THE 5′-CARBINOL GROUP*

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ABSTRACT

A synthesis of adenosine-*5′-d* (**4**), and its p.m.r. spectral characteristics, are described. The presence of deuterium in **4** gives rise to a 2:1 mixture of *R* and *S* configurations at C-5, thereby permitting specific assignments for the resonances of the residual 5′-protons. From the observed spin–spin coupling between the latter and H-4′, an estimate has been made of the rotamer population of the exocyclic 5′-carbinol group. It is shown that the *gauche–gauche* rotamer is preponderant (~70%) and the *gauche–trans* one of minor importance (~20%) in aqueous solution, which contrasts markedly with the preference for the latter rotamer exhibited by adenosine in the solid state.

INTRODUCTION

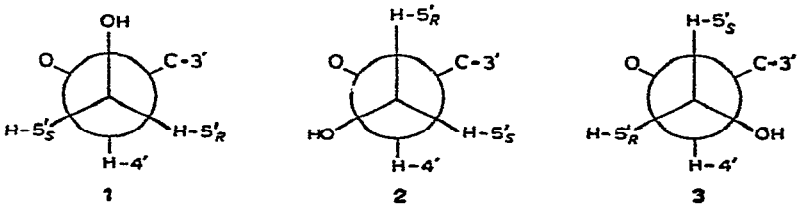
Nucleosides and nucleotides have been studied intensively as models for deducing the conformation of polynucleotides. For many years[1], n.m.r. spectroscopy has been especially valuable for examining solutions of these molecules, and has provided detailed descriptions of conformational preferences within the series[2–6]. Some ambiguity, however is attached to analyses of coupling between the 4′- and 5′-protons of the D-ribose or 2-deoxy-D-*erythro*-pentose moieties, which provides information about rotamer populations of the exocyclic 5′-carbinol group[2–6]. That is, the ^1H–^1H splittings observed are time-averaged values for all rotamers, such as the fully-staggered species **1**–**3****, and hence depend on the various torsional angles relating the protons. In **1**, each of the 5′-protons is *gauche* with respect to H-4′, whereas both *anti* and *gauche* relationships are found in **2** and **3**. For a complete spectral analysis, the two 5′-proton resonances must be differentiated. but this has not been done in most studies. Comparative measurements on some pyrimidine nucleosides and their 3′-phosphates provide a rare instance in which specific assignments have been given for the 5′-proton signals[5].

*Dedicated to the memory of Professor J. K. N. Jones, F.R.S.
**The *R* and *S* labels in these formulas are considered later.

A method is described here for unequivocally differentiating between the two resonances of the 5′-protons of adenosine. It involves synthesis of adenosine-*5′-d* (**4**) in which the absolute configuration at C′-5 is known. By analogy with an earlier study[7] on the 6-carbinol group of D-galactose, this has made it possible to specify each value of $^3J_{4',5'}$ in the spectrum of **4**, and thereby furnish a conformational description of the 5′-carbinol group of this purine nucleoside*.

RESULTS AND DISCUSSION

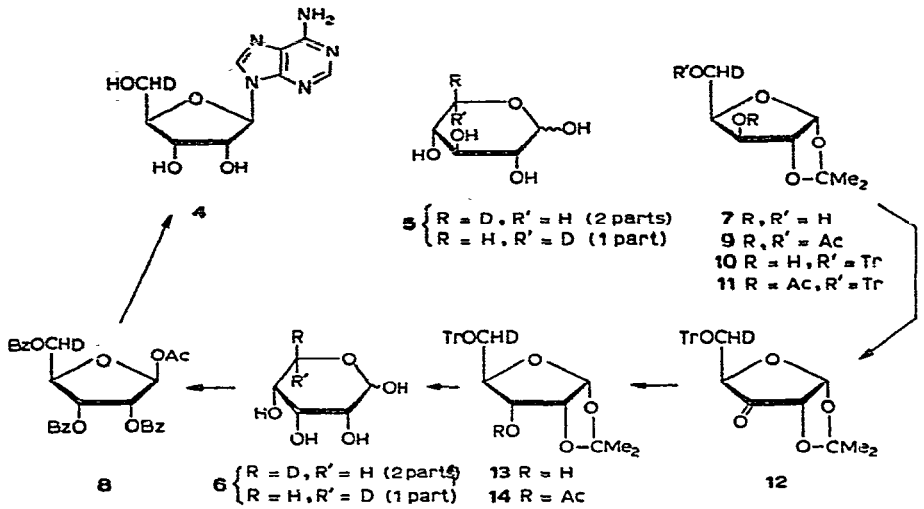
D-Xylose-*5-d*[8] (**5**) provided a starting material for the synthesis of **4**. Prepared as described previously[9], **5** incorporated a 2:1 mixture of *R* and *S* configurations at its C-5 chiral centre[9,10]. A sequence of well-known reactions[11,12] was then employed to convert **5** into D-ribose-*5-d* (**6**). Thus, 1,2-*O*-isopropylidene-α-D-xylofuranose-*5-d* (**7**) was modified[12] successively by *O*-tritylation, methyl sulfoxide oxidation, borohydride reduction, and acid hydrolysis, giving **6** as illustrated in Scheme 1. Adenosine-*5′-d* (**4**) was then synthesized from **6** by an established[13,14] route (Scheme 1) involving the intermediacy of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose-*5-d* (**8**), which was condensed with 6-benzamido(chloromercuri)purine in the presence of titanium tetrachloride, with subsequent deacylation.



As none of these transformations could have involved a direct attack on C-5, the distribution of deuterium at that position must have remained intact. Furthermore, it was evident from the integrated H-5 signals in the spectra (see later) of various intermediates in Scheme 1, that partial scrambling of the isotope did not take place during the course of the synthesis.

The 220-MHz spectrum of **4** in D_2O (Fig. 1) clearly shows two signals due to the residual 5′-protons and also, as expected, that their relative intensities are 1:2. As the $5'_R$ isomer of **1** has the higher deuterium content, it gives rise to the smaller peak, at 3.78 p.p.m. Hence the 5′-signals are definitively assigned, and their splittings due to coupling with H-4′ are seen to be 2.8 and 3.5 Hz for H-$5'_S$ and H-$5'_R$ respectively.

*A related application has involved selectively deuterated derivatives of D-glucopyranose[7a], and a general description of the conformation of the C-5–C-6 fragment in aldohexopyranoses has been presented recently[7b].



Scheme 1. Synthesis of adenosine-5′-*d* (4).

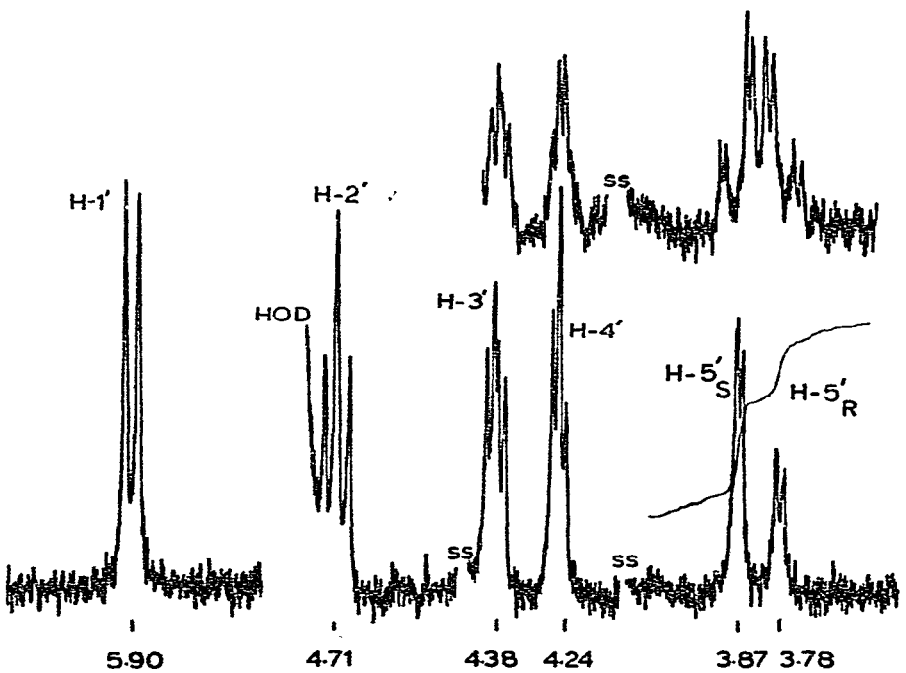


Fig. 1. Partial p.m.r. spectrum (at 220 MHz) of adenosine-5′-*d* (4) in D_2O at 18°. Signals for the 3′-, 4′-, and 5′,5″-protons of adenosine are shown in the upper trace (ss, spinning side-band).

With these spacings, the rotamer population of the 5′-carbinol group was estimated on the following basis[2]: (*a*) only fully staggered rotamers—*gauche–gauche* (**1**), *gauche–trans* (**2**) and *trans–gauche* (**3**)—were considered; (*b*) an observed coupling-constant was taken to be the sum of *gauche* and *trans* couplings, weighted by the relative contributions of **1–3**; and (*c*) $^3J_{trans} = 10$ Hz and $^3J_{gauche} = 2$ Hz. Accordingly, the rotamer population of adenosine-*5′-d* (**4**) may be expressed as a ratio of **1**:**2**:**3** of 0.7:0.2:0.1.

Although other assumptions may equally well be made in population analyses of this kind, for example that distortions of perhaps 15° from fully-staggered orientations are probable[2,5], or that the ^3J values should be slightly larger or smaller[5], they would not substantially alter the conclusion that the *gauche–gauche* rotamer (**1**) of adenosine strongly preponderates in the aqueous solution. The same preference is already known to be true for several pyrimidines[2–6], including uridine, although other rotamers may be more prominent in representing the 5′-carbinol group of some pyrimidines[4–6].

The current findings for adenosine are notably different from those obtained[15] by X-ray crystallography, because, in the solid state, the 5′-carbinol group is oriented as in **2**, a minor contributing rotamer for adenosine in solution. A theoretical treatment suggests[16] that the *gauche–gauche* rotamer (**1**) is favored energetically, although the associated calculations[16] of coupling constants for the 4′- and 5′-protons differ substantially from those actually observed. It is worth noting that, in contrast to crystalline adenosine, crystals of its hydrochloride exhibit a 5′-carbinol orientation as[17] in **1**, and that adenosine 5′-phosphate also favors **1**, in solution[18] as well as in in the crystalline modification[19]. Variations such as these draw attention to the broad question of how closely solid and liquid-state geometries resemble each other. Although extensive compilations of conformational data on nucleosides and nucleotides indicate that, *in general*, there is a close parallel between these two states[6,20], it is clear that rather large differences may exist for individual compounds, as can be seen for adenosine.

P.m.r. data are given in Table I for several compounds prepared in conjunction with the synthesis of **4**, and listed as well are rotamer populations for their individual 5′-carbinol groups calculated from the 4,5-coupling constants. Usually, the H-5_R signal is upfield of that of H-5_S, but in the spectrum of **11**, the converse holds. It can be seen also that the 4,5-couplings of **11** differ markedly from those of the non-acetylated compound (**10**). However, this is not associated with an overall difference in conformation between **10** and **11**, because δ and J values for their other protons are closely similar (except for the anticipated deshielding of H-3 in **11**). Analogous changes in chemical shift and coupling constants (and, hence, rotamer populations) are found on comparing **13** with its 3-*O*-acetyl derivative **14** (Table I). The upfield shifts of H-5_S in **11** and **14** as compared with **10** and **13**, may be related to the presence of the carbonyl group in the former pair, but a cause for the changes in orientation is more obscure: for instance although H-bonding between OH-3 and O-5 appears feasible in **10** (and would be prevented in **11**) this explanation seems much

less likely for **13**. Other substantial variations are found in comparing the *xylo* and *ribo* isomers, **10** and **13**; namely, the latter exhibits a strong preference for the *gauche–gauche* rotamer, whereas the 5-carbinol of **10** is appreciably represented by all three rotamers. Also noteworthy is the fact that a change in solvent for adenosine-*5-d* from D_2O to Me_2SO-d_6 has little effect on the rotamer population or, judging from the overall constancy of the n.m.r. parameters (see Experimental), on other conformational features of the molecule.

TABLE I

CHEMICAL SHIFTS, COUPLING CONSTANTS, AND ROTAMER POPULATIONS ASSOCIATED WITH THE EXOCYLIC CARBINOL GROUP OF ADENOSINE-5′-*d* AND RELATED COMPOUNDS

		Compound[a]							
		4	**8**	**9**	**10**	**11**	**12**	**13**	**14**
$\delta_{(p.p.m.)}$[b]	H-5′$_R$	3.78(1)	4.51(1)	4.40(1)	3.45(1)	3.44(1)	3.31(1)	3.24(1)	3.54(1)
	H-5′$_S$	3.87(2)	4.73(2)	4.51(2)	3.51(2)	3.19(2)	3.48(2)	3.39(2)	3.18(2)
^3J (Hz)	4′,5′$_R$	3.5	4.0(3.9)[c]	7.2	3.6	5.2	2.6	~2.5	6.0
	4′,5′$_S$	2.8	4.5(4.2)[c]	5.2	5.5	7.5	2.4	~1.5	6.0
Rotamers	1	0.7	0.5	0	0.4	0	0.9	0.9	0
(mole	2	0.2	0.2	0.6	0.2	0.6	<0.1	0.1	0.5
fraction)	3	0.1	0.3	0.4	0.4	0.4	<0.1	0	0.5

[a]D_2O was the solvent for **4** and $CDCl_3$ for the other compounds. [b]Numbers in parenthesis give the relative integrals. [c]In acetone-d_6.

EXPERIMENTAL

General methods. — Proton magnetic resonance spectra were recorded with a Varian HA-100 spectrometer; spectra at 220 MHz were provided by the Canadian 220 MHz Centre, Sheridan Park, Ont. ^{13}C-N.m.r. spectra were recorded at 22.63 MHz with a Bruker WH-90 FT spectrometer. Chemical shifts (δ) are reported with reference to tetramethylsilane. U.v. spectra were recorded with a Unicam SP 1800 spectrophotometer. Plates of Silica Gel G were used for thin-layer chromatography, and the developing solvents were 1:1 ethyl acetate–petroleum ether or 10:1 chloroform–methanol. Column chromatography was carried out with silica gel, using mixtures of benzene and ethyl acetate as eluants.

D-*Ribose*-5-d (**6**). — This compound was prepared essentially as described by Sowa[12] for interconversion of D-xylose into D-ribose*: 1,2-*O*-isopropylidene-α-D-xylofuranose-*5-d* (**7**) was treated in pyridine with chlorotriphenylmethane to form[21] the 5-trityl derivative (**10**); the latter was oxidized with methyl sulfoxide–*N*,*N*′-dicyclohexylcarbodiimide[22] to the corresponding 3-glycosulose (**12**), which was reduced with sodium borohydride in aqueous ethanol to give 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-ribofuranose-*5-d* (**13**). The p.m.r. data recorded for **10**, **12**, and **13** agreed well with those reported by Sowa[12], except for the new data (Table I) arising from

*An analogous procedure is reported in ref. 11.

deuteration at the 5-position. ^{13}C-N.m.r. data ($CDCl_3$) for **7**: 111.4 (CMe_2), 104.5 (C-1), 85.3 (C-2), 79.1 (C-4), 76.0 (C-3), 60.7 (t, C-5), 26.6 and 26.0 (Me_2); for **13**: 112.0 (CMe_2), 103.8 (C-1), 86.3 (CPh_3), 79.4 (C-2), 78.3 (C-4), 71.8 (C-3), 61.6 (t, C-5), 26.1 and 26.0 (Me_2). Acid hydrolysis of **13** afforded syrupy D-ribose-*5-d*; its ^{13}C n.m.r. spectrum was indistinguishable from that of D-ribose[23], aside from the fact that the C-5 signals appeared as 1:1:1 triplets (C-5–deuterium coupling).

1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose-5-d (**8**). — The procedure employed was closely similar to that of Ness, Diehl, and Fletcher[13,24]. D-Ribose-*5-d* (1.2 g) was dissolved in 1% methanolic hydrogen chloride (100 ml) and, after 1 h, an excess of silver carbonate was introduced. The solids were removed and the filtrate was concentrated; a ^{13}C n.m.r. spectrum of the residue corresponded[25] almost exclusively to a 5:1 mixture of methyl α- and β-D-ribofuranoside. This residue was treated in pyridine (30 ml) with benzoyl chloride (7.5 g), and the benzoate produced was subjected to acetolysis[26] for 1 h at ~25° in a mixture of acetic anhydride (50 ml), acetic acid (50 ml) and conc. sulfuric acid (0.5 ml). The final product crystallized from solution in methanol; yield, 1.8 g, m.p. and mixed m.p. 120–130.5° (uncorr.) (lit.[24], 129–130°, 126–129°); p.m.r. data ($CDCl_3$): 6.45 (d, 1 H, H-1β), 5.93 (q, 1 H, H-3), 5.80 (dd, 1 H, H-2), 4.8 (m, 1 H, H-4), 4.73 (d, 2/3 H, H-5_S), 4.50 (d, 1/3 H, H-5_R), 1.97 (s, 3 H, OAc), 7.1–8.1 (m, OBz), $J_{1,2}$ 1.0, $J_{2,3}$ 5.0, $J_{3,4}$ 6.2, $J_{4,5_S}$ 4.0, $J_{4,5_R}$ 4.5 Hz. ^{13}C n.m.r. data ($CDCl_3$): 86.6 (C-1), 80.0 (C-4), 73.3 (C-2), 70.0 (C-3), 62.5 (t, C-5).

Anal. Calc. for $C_{28}H_{23}DO_9$: C, 66.5; HD, 4.9. Found: C, 66.0, HD, 4.8.

Adenosine-5′-d (**4**). — A procedure closely similar to that of Reist, Fisher, and Goodman[14] was followed. A mixture of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose-*5-d* (**8**, 3.0 g), 6-benzamido(chloromercuri)purine (3.6 g), Celite (3.0 g), and 1,2-dichloroethane (230 ml) was heated under reflux until 20 ml of the solvent had been distilled off. Titanium tetrachloride (1.0 ml, 1.25 equiv.) was introduced[28] dropwise, and the mixture was heated for 12 h under reflux with efficient stirring; t.l.c. showed the presence of a major product and a minor, slower-moving product. Saturated sodium hydrogencarbonate (100 ml) was introduced into the cooled mixture, then the organic layer was passed through Celite, washed successively with water, sodium iodide solution (30%), water (twice), and dried. Evaporation of the solvent afforded an amorphous solid, which was heated under reflux in methanol (40 ml) containing sodium methoxide (5 ml, 15M) for 2.5 h. An excess of acetic acid was added, and the solution was concentrated. Isolation of the nucleoside was effected by formation of a solid picrate, and the picrate anion was then removed in water with Dowex-1 (CO_3^{2-}) ion-exchange resin[29]. An amorphous, colorless, solid was recovered on evaporation of the filtrate; yield, 0.6 g. The ^{13}C n.m.r. spectrum was indistinguishable from that of adenosine, except that the C-5′ signal was 0.2 p.p.m. upfield, and appeared as a 1:1:1 triplet ($^1J_{C-5',D}$ 22 Hz). λ_{max}^{MeOH} 259 nm (ε 12,700); p.m.r. data (D_2O): 8.09 (s, 1 H, H-8), 7.95 (s, 1 H, H-2), 5.90 (d, 1 H, H-1′), 4.71 (t, 1 H, H-2′), 4.38 (dd, 1 H, H-3′), 4.24 (m, 1 H, H-4′), 3.87 (d, 2/3 H, H-$5'_S$), 3.78 (d, 1/3 H, H-$5'_R$); $J_{1',2'}$ 6.0, $J_{2',3'}$ 5.1, $J_{3',4'}$ 3.3, $J_{4',5'_S}$ 2.8, $J_{4',5'_R}$ 3.5 Hz. In Me_2SO-d_6,

the chemical shifts were as follows: 8.32 (H-8), 8.11 (H-2), 5.88 (H-1′), 4.62 (H-2′), 4.15 (H-3′), 3.97 (H-4′), 3.66 (H-5′$_S$), 3.54 (H-5′$_R$); the observed spacings were virtually the same as in D_2O.

Calculation of rotamer populations. — The mole fractions of rotamers **1**, **2**, and **3**, expressed as *a*, *b*, and *c*, respectively, are given by

$$\begin{bmatrix} J_R^a & J_R^b & J_R^c \\ J_S^a & J_S^b & J_S^c \\ 1 & 1 & 1 \end{bmatrix} \cdot \begin{bmatrix} a \\ b \\ c \end{bmatrix} = \begin{bmatrix} J_R \\ J_S \\ 1 \end{bmatrix}$$

where $J_R^a = 2$, $J_S^a = 2$, $J_R^b = 10$, $J_S^b = 2$, $J_R^c = 2$, $J_S^c = 10$ Hz. For compound **4**, $J_R = 3.5$ and $J_S = 2.8$ Hz. Hence, the following simultaneous equations are obtained (see refs. 2, 3, 5):

$$\begin{aligned} 2a+10b+2c &= 3.5 \\ 2a+2b+10c &= 2.8 \\ a+b+c &= 1 \end{aligned}$$

Solving for *a*, *b*, and *c* gives

$$a{:}b{:}c = \mathbf{1{:}2{:}3} = 0.7{:}0.2{:}0.1$$

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