A ^{1}H NMR Study of the *Syn-Anti* Dynamic Equilibrium in Adenine Nucleosides and Nucleotides with the Aid of Some Synthetic Model Analogues with Fixed Conformations

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The *syn-anti* equilibrium about the glycosidic bond in adenosine and some related analogues was studied by means of ^{1}H NMR spectroscopy, with the aid of several model analogues fixed in given conformations either by intramolecular bonding, or by introduction of a bulky substituent. A model unambiguously and exclusively in the *syn* conformation is 8-(α-hydroxyisopropyl) adenosine; while one fixed in the *anti* conformation is 8,5′-anhydro-8-oxoadenosine. A new analogue, fixed in the *high anti* conformation, is 8,2′-O-isopropylidenearabinofuranosyladenine. Several additional new model compounds were synthesized and their properties are described.

With the aid of these models, the *syn-anti* dynamic equilibrium was examined for adenosine and some related compounds in different solvent systems, and the conformer populations evaluated quantitatively. The validity of the procedure applied, and the accuracy of the results, are critically examined, and compared with findings obtained by other procedures. Available literature data on the *syn-anti* equilibrium in other 8-substituted adenosines are re-analyzed in the light of the present results. An analysis is also presented of the interdependence of the various conformational parameters, *i. e.* conformation about the glycosidic bond and those of the sugar ring and exocyclic carbinol group, in adenosine and 2′,3′-O-isopropylideneadenosine.

The conformation of nucleosides and nucleotides about the glycosidic bond is of considerable biological significance [1–7]. In solution there is a rapid state of equilibrium between the *anti* and *syn* conformations and various physico-chemical methods have been employed to determine, usually, the predominant or preferred conformation, *e. g.* circular dichroism [8–11], ultrasonic relaxation techniques [12–14]. The most widely applied procedures are based largely on NMR spectroscopy, including: comparison of the chemical shifts of the protons [3, 15–17] or carbons [18] of the carbohydrate moiety and/or the aglycone [19, 20], the effect of phosphate ionization in nucleotides on the chemical shifts of the aglycone protons [3, 16, 21–23] the Nuclear Overhauser Enhancement effect [3, 24–26], proton relaxation techniques [26–29], broadening of the aglycone proton signals by paramagnetic ions linked to the 5′-position of the sugar [3, 16, 30] vicinal carbon-hydrogen coupling constants [31, 32], etc. With the possible exception of proton relaxation and NOE methods, the results are largely qualitative and are expressed in terms of the "preferred" conformation.

In the case of purine nucleosides and nucleotides, introduction of a bulky substituent at C(8) has long been considered to constrain the molecule to the *syn* conformation, first established for the 8-bromo derivatives of adenosine and guanosine in the solid state [33, 34]. The predominance of the *syn* form in solution is belived due to unfavourable steric and electrostatic interactions between the 8-bromo substituent and the sugar ring [16, 35, 36]. These 8-bromo derivatives are still widely employed as models of nucleosides and nucleotides in the *syn* form, and it has generally been ignored that 8-bromoadenosine diphosphoribose, when bound to the active site of alcohol dehydrogenase, is in the conformation *anti* [37]. Furthermore, although 8-dimethylaminoadenosine [17], and its 5′-phosphate appear to be *syn* in aqueous medium, there is in-

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Abbreviations: araA, 9-β-D-arabinofuranosyladenine; DSS, sodium 2,2-dimethyl-2-silapentane sulfonate; DMSO, dimethyl sulfoxide; TMS, tetramethyl silane.

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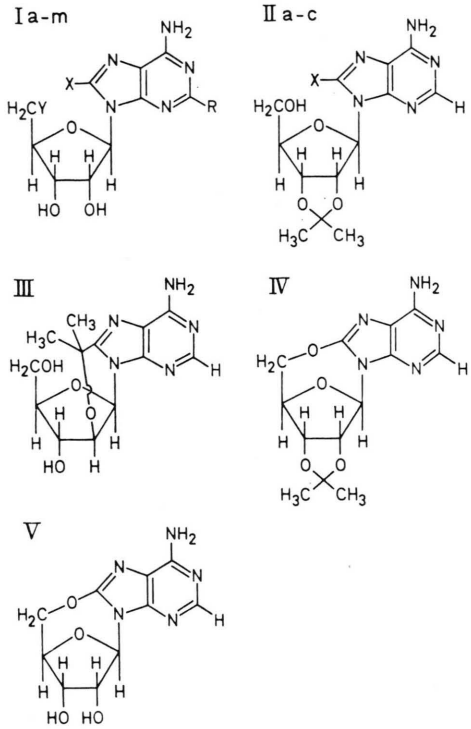
direct evidence that, at some stage during its action as a coenzyme inhibitor, the nucleotide is "stabilized" in the *anti* conformation [3].

It consequently appeared of interest to examine the influence on the *syn-anti* equilibrium of a bulkier and more neutral substituent, and we have elsewhere described the solid state structure and conformation of 8-(α-hydroxyisopropyl)adenosine (**Ie**) [38, 39]. We have now extended this to a study of the solution conformation of this nucleoside, its nucleotide, and of some derivatives locked in given conformations about the glycosidic bond *via* anhydro linkages between the sugar and the base (Scheme 1). The use of such models makes possible quantitative evaluations of the *anti* (or *syn* by difference) conformer population in adenosine and 2′,3′-O-isopropylideneadenosine. The possible influence on these results of effects deriving from the conformation of the sugar ring, the exocyclic 5′-CH_2OH group, or differences in glycosidic torsion angles, are discussed in detail.



Scheme 1.

Ia	R=H	X=H	Y=OH
b	R=H	X=CH_3	Y=OH
c	R=H	X=Br	Y=OH
d	R=H	X=$N(CH_3)_2$	Y=OH
e	R=H	X=$C(CH_3)_2OH$	Y=OH
f	R=$C(CH_3)_2OH$	X=$C(CH_3)_2OH$	Y=OH
g	R=H	X=H	Y=Cl
h	R=H	X=Br	Y=Cl
i	R=H	X=$C(CH_3)_2OH$	Y=Cl
j	R=H	X=H	Y=PO_4^{-2}
k	R=H	X=Br	Y=PO_4^{-2}
l	R=H	X=$N(CH_3)_2$	Y=PO_4^{-2}
m	R=H	X=$C(CH_3)_2OH$	Y=PO_4^{-2}

IIa X=H
b X=$C(CH_3)_2OH$
c X=Br

Results and Discussion

Synthetic procedures

8-Bromoadenosine (**Ic**) and 8-bromoadenosine-5′-phosphate (**Ik**) were prepared as described by Ikehara *et al.* [40].

5′-Chloro-5′-deoxyadenosine (**Ig**) and 8-bromo-5′-chloro-5′-deoxyadenosine (**Ih**) were obtained by treatment of adenosine and 8-bromoadenosine, respectively, with thionyl chloride and hexamethylphosphoramide. The published procedure [41] was somewhat modified to give the final products in essentially quantitative yield [42].

8-(α-hydroxyisopropyl)adenosine derivatives were prepared by photochemical addition of isopropanol, with di-*tert*-butyl peroxide as the initiator [43] as follows:

Irradiation of adenosine under the foregoing conditions gave, in addition to **Ie**, a low yield of 2,8-di-(α-hydroxyisopropyl)adenosine (**If**). The two products were fractionated from each other, and from unreacted adenosine, by chromatography on a strongly basic Dowex (OH^-) column [44], the sequence of elution being **If**, **Ie** and adenosine. The products were identified by elementary analysis, and UV and NMR spectroscopy.

8-(α-hydroxyisopropyl)-5′-AMP (**Im**) was obtained by an analogous procedure, the starting compound being the tri-*n*-butylamine salt of 5′-AMP, which is very soluble in isopropanol [45]. The reaction mixture was fractionated on a column of Dowex 1×4 ($HCOO^-$) with a 0 – 0.2 M gradient of HCOOH. Of some interest is the sequence of elution of the 5′-AMP derivatives on such a column, *viz* an unidentified product at 0.07 M formic acid, **Ie** at 0.14 M acid, whereas **Ij** required up to 0.50 M acid. Presumably this is related to the pK for protonation of the aglycone in these derivatives.

The same procedure was employed to obtain 5′-chloro-5′-deoxy-8-(α-hydroxyisopropyl)adenosine (**Ii**) from **Ig** [46]. The product was purified by chromatography on silica gel and identified by elementary analysis and NMR spectroscopy. Here as well there was formation of minor products including, presumably, the 2,8-di-(α-hydroxyisopropyl) derivative, but no attempt was made to isolate the latter.

8,2′-isopropylidene-ara A (**III**) was obtained from **Ie** by means of a cyclization reaction, *via* the 2′,3′-cyclic carbonate of the latter [47, 48]. Subsequent addition of ether precipitated both product and unreacted **Ie**, but the former preferentially crystallized from water and two such crystallizations gave an analytically pure product, further identified from its NMR spectrum.

The 2′,3′-O-isopropylidene derivatives of 8-bromoadenosine and 8-(α-hydroxyisopropyl)adenosine (**IIc** and **IIb**, respectively) were prepared with the aid of a very effective reaction system [49], using a mixture of acetone and 2,2-dimethoxypropane with *p*-toluenosulfonic acid as catalyst. The reaction was terminated by addition of a small amount of concentrated NH_4OH, and the product obtained in purified form by crystallization from aqueous methanol. Passage of the mother liquors over Dowex (OH^-) led to an increase in overall yield, the catalyst and unreacted substrate being retained on such a column.

Cyclization of 8-bromo-2′,3′-O-isopropylideneadenosine in anhydrous dioxane with sodium hydride led to the desired 8,5′-anhydro-8-oxo-2′3′-O-isopropylideneadenosine (**IV** [50]). In contrast to the published procedure [51], this reaction was accompanied by formation of another product in comparable yield, with a structure different from that of **IV**, but as yet unidentified. Deblocking of **IV** in 1 N H_2SO_4 [50] gave 8,5′-anhydro-8-oxoadenosine (**V**), with properties identical to those previously reported [50].

^1H NMR analysis

The ^1H NMR spectra were recorded, as described below in the Experimental section, for most of the adenine nucleoside and nucleotide analogues exhibited in Scheme 1. The chemical shifts of the individual protons are listed in Table I. The vicinal

Table I. Proton chemical shifts (in ppm vs internal TMS in DMSO or C^2HCl_3, or vs internal DSS in ^2H_2O) for various adenine nucleoside and nucleotide analogues in appropriate solvents at 22 °C [a].

Compound	Solvent	H(2)	H(8)	H(1′)	H(2′)	H(3′)	H(4′)	H(5′)	H(5″)	NH_2	OH [b]	CH_3 [b]
I a	$(C^2H_3)_2SO$	8.16	8.37	5.90	4.62	4.17	3.98	3.70	3.58	7.34	5.3–5.7 [c]	—
I a	^2H_2O	8.16	8.28	6.05	4.78	4.42	4.29	3.92	3.84	—	—	—
I e	$(C^2H_3)_2SO$	8.06	—	6.77	4.98	4.22	3.98	3.70	3.57	7.19	5.06 5.20 5.71 5.94	1.63 1.67
I e	^2H_2O	8.17	—	6.85	5.14	4.53	4.31	3.91 [d]			—	1.78(2)
I f	^2H_2O	—	—	6.84	5.35	4.70	4.20	3.96	3.88	—	—	1.58(2) 1.79(2)
I g	$(C^2H_3)_2SO$	8.17	8.35	5.95	4.76	4.24	4.10	3.95	3.85	7.30	5.45 5.60	—
I i	$(C^2H_3)_2SO$	8.15	—	6.82	5.20	4.45	4.07	4.01	3.92	7.10	5.33(2) 5.78	1.63 1.67
I j [e]	^2H_2O	8.26	8.62	6.12	4.80	4.50	4.36	4.01	3.99	—	—	—
I m	^2H_2O	8.22	—	6.76	5.35	4.61	4.25	4.10 [d]			—	1.77(2)
II a	C^2HCl_3	8.31	7.84	5.87	5.22	5.12	4.54	3.97	3.79	5.95	[f]	1.38 1.65
II a	$(C^2H_3)_2SO$	8.17	8.34	6.14	5.35	4.98	4.24	3.56 [d]			5.23	1.34 1.56
II b	C^2HCl_3	8.24	—	6.88	5.35	5.10	4.48	3.96	3.78	5.75	[f]	1.37 1.65 1.76(2)
II b	$(C^2H_3)_2SO$	8.09	—	7.03	5.41	5.03	4.18	3.67	3.56	7.56	5.50 5.87	1.37 1.60 1.68(2)
III	$(C^2H_3)_2SO$	8.15	—	5.92	4.42	4.07	3.91	3.53 [d]			4.82 5.72	1.55 1.64
V	^2H_2O	8.16	—	6.17	4.75	4.55	4.78	4.71	4.31	—	—	—
V [g]	$(C^2H_3)_2SO$	8.10	—	5.97	4.54	4.50	4.25	4.58	4.08	6.99	5.29 5.59	—
IV	$(C^2H_3)_2SO$	8.13	—	6.06	5.10	4.88	4.74	4.65	4.13	7.10	—	1.31 1.48
IV	C^2HCl_3	8.31	—	6.41	5.10	4.77	4.71	4.51	4.22	5.50	—	1.36 1.57

[a] Concentrations 0.2 M, or 0.05 M for poorly soluble compounds.
[b] For compounds with more than one OH (or CH_3) group with identical chemical shifts, the number of such is given in brackets.
[c] Broad unresolved band due to incomplete exchange with ^2H.
[d] Centre of unresolved band.
[e] Data from R. H. Sarma *et al.*, J. Am. Chem. Soc. **96,** 7337 (1974).
[f] Signals not visible because of partial exchange of ^1H by ^2H.
[g] Data from M. Ikehara and Y. Ogiso, Chem. Pharm. Bull. (Japan) **23,** 1114 (1975). There is probably a printer's error in these results. Our analysis suggests that the values of the chemical shifts for H(3′) and H(4′) should be reversed.

proton-proton coupling constants, and the results of conformational analyses of the carbohydrate moieties, are compiled in Table II. The conformational analysis in each instance was based on the two-state model of Altona and Sundaralingam [52] for the sugar ring conformation. For those analogues with an *intra*molecular bond in the sugar ring, or between the sugar and the aglycone, the conformational assignment was that for which the coupling constants calculated from the Karplus relation [53] most adequately corresponded to the experimental values. The *gauche-gauche* populations of the exocyclic 5′-carbinol groups were calculated from the known relationship [16]:

$$\%\ gauche\text{-}gauche = 10 \times [13 - (J(4',5') + J(4',5''))].$$

Suitable literature data were profited from for the chemical shifts, and conformational analyses, of 5′-AMP [16], 8-methyladenosine [54, 55], the 8-bromo and 8-dimethylamino-derivatives of adenosine and 5′-AMP [17, 3], araA [56], and 8,5′-anhydro-8-oxoadenosine in DMSO-d_6 [57].

8-Bromoadenosine (**Ic**) *and 8-bromoadenosine-5′-phosphate* (**Ik**)

If we compare the chemical shifts of H(1′), H(2′) and H(3′) of these compounds with the corresponding values for the parent adenosine and 5′-AMP (Table IV), several characteristic features are revealed. Bromination of adenosine leads to pronounced deshielding of H(2′) by 0.27 ppm, and, to a lesser extent, of H(3′) by 0.07 ppm and H(1′) by 0.09 ppm. Similar changes in chemical shifts accompany bromination of 5′-AMP, the deshielding of H(2′) being even more marked (0.50 ppm). These deshielding effects are ascribed to the change in conformation of the base about the glycosidic bond from *anti*, which is the preferred form in adenosine and 5′-AMP, to the preferred *syn* form in the 8-bromo derivatives [16].

Table II. Proton-proton vicinal coupling constants (in Hz) and associated conformational analysis for analogues of adenine nucleosides and nucleotides in various solvents at 22 °C [a].

Compound	Solvent	J(1′,2′)	J(2′,3′)	J(3′,4′)	J(4′,5′)	J(4′,5″)	J(5′,5″)	Sugar conform.	Sugar %	Exocyclic CH_2OH *gauche-gauche* population %
I a	$(C^2H_3)_2SO$	6.5	5.1	3.1	3.0	3.6	−12.0	C(2′)*endo*	67	65
I a	^2H_2O	6.0	5.3	3.5	2.9	3.8	−12.5	C(2′)*endo*	63	63
I e	$(C^2H_3)_2SO$	7.1	5.6	2.0	2.5	4.0	−11.8	C(2′)*endo*	75	65
I e	^2H_2O	7.5	5.6	1.8	4.5 [b,c]		[c]	C(2′)*endo*	78	85
I f	^2H_2O	5.7	6.0	4.0	2.7	4.6	−12.0	C(2′)*endo*	60	57
I g	$(C^2H_3)_2SO$	5.4	5.0	4.0	3.8	5.8	−12.0	C(2′)*endo*	57	30 [d]
I i	$(C^2H_3)_2SO$	5.2	5.0	4.3	[e]	[e]	[e]	C(2′)*endo*	55	—
I j [f]	^2H_2O	6.0	5.1	3.5	3.9	2.6	−11.5	C(2′)*endo*	67	65
I m	^2H_2O	5.5	6.1	4.0	[g]	[g]	[g]	C(2′)*endo*	58	—
II a	C^2HCl_3	4.5	6.0	0.9	1.4	1.8	−12.5	C(3′)*exo* [h]	80 [i]	100
II a	$(C^2H_3)_2SO$	3.3	6.1	2.4	9.0 [b,c]		[c]	C(3′)*exo* [h]	50 [i]	40
II b	C^2HCl_3	5.7	6.0	0.6	1.4	1.8	−12.5	C(3′)*exo* [h]	80 [i]	100
II b	$(C^2H_3)_2SO$	3.6	6.0	2.6	4.2	4.6	−11.6	C(3′)*exo* [h]	50 [i]	40
III	^2H_2O	2.5	0.0	2.0	12.0 [b,c]		[c]	C(1′)*exo*		10
V	^2H_2O	0.0	6.2	0.0	2.0	1.5	−13.5	O(1′)*exo*-C(4′)*endo* [j]		100
V [k]	$(C^2H_3)_2SO$	0.0	6.0	0.0	2.0	1.5	−13.0	O(1′)*exo*-C(4′)*endo* [j]		100
IV	$(C^2H_3)_2SO$	0.0	5.7	0.0	2.2	0.6	−13.2	O(1′)*exo*-C(4′)*endo* [j]		100
IV	C^2HCl_3	0.0	5.6	0.0	2.3	0.7	−12.9	O(1′)*exo*-C(4′)*endo* [j]		100

[a] Concentration 0.2 M, or 0.05 for less soluble analogues.
[b] Sum of J(4′,5′) and J(4′,5″).
[c] Accurate determination of coupling constants not feasible because of close proximity of signals due to H(5′) and H(5″).
[d] Calculated without taking account of replacement of −OH by Cl.
[e] Coupling not observable because of overlapping of H(4′) and H(5′), H(5″) system.
[f] Data from R. H. Sarma *et al.*, J. Am. Chem. Soc. **96,** 7337 (1974).
[g] Not determined because of coupling with ^{31}P.
[h] Non-typical C(3′)*exo* because of "flattening" of the ring by isopropylidene group (see text for details).
[i] This value is a first approximation, for reasons discussed in text.
[j] The coupling constants in this instance are also consistent with the conformations O(1′)*exo* and O(1′)*exo*-C(1′)*endo*.
[k] Data from M. Ikehara and Y. Ogiso, Chem. Pharm. Bull. (Japan) **23,** 1114 (1975).

8-Methyladenosine (**Ib**)

This analogue is of particular interest because the van der Waal's radius of the 8-methyl (2.0 Å) is close to that of Br (1.95 Å). The change in chemical shift of H(2′) relative to adenosine, in aqueous medium, is 0.20 ppm, hence qualitatively similar to 8-bromoadenosine. Quantitatively, however, it is lower than for 8-bromoadenosine, and the other 8-substituted derivatives discussed below. There is only a minimal change in the shift of H(3′), −0.03 ppm, while that for H(1′) is −0.15 ppm [54]. Analogous, but somewhat smaller, changes in chemical shifts occur in DMSO [55], *viz* deshielding of H(2′) by 0.16 ppm and H(3′) by 0.02 ppm, and shielding of H(1′) by 0.05 ppm. These effects have been ascribed to constrainment of the nucleoside to the form *syn* by the 8-methyl substituent [54, 55], the validity of which will be discussed further, below.

8-Dimethylaminoadenosine (**Id**) *and 8-dimethylaminoadenosine-5′-phosphate* (**II**)

The dimethylamino substituent, with a van der Waals' radius appreciably greater than Br, would be expected to offer considerably more steric hindrance in its interaction with the ribose ring. The observed changes in chemical shifts of H(2′) in the nucleoside (0.36 ppm) and nucleotide (0.56 ppm) are in fact, higher than for the corresponding 8-bromo derivatives (see above). The smaller changes in shifts of H(3′), relative to the parent nucleoside and nucleotide, are similar to those for the 8-bromo-derivatives. Somewhat striking, however, are the changes in shifts of H(1′); these are not only greater than for the 8-bromo derivatives, but opposite in sign (−0.12 ppm for the nucleoside and −0.33 ppm for the nucleotide). The observed changes in chemical shifts, especially of H(2′), have been ascribed to a change in conformation, with a strong preference for the form *syn* [3, 17].

The foregoing results do not necessarily exclude the possibility of the existence of some proportion of these 8-substituted nucleosides and nucleotides in the form *anti*. The changes in shifts of H(2′), regarded as a measure of the change in conformation of the base about the glycosidic bond, differ for the 8-bromo and 8-dimethylamino derivatives. They do not, however, provide a value for the change in chemical shift corresponding to a 100% change from *anti* to *syn*. It should be recalled that 8-Br-ADP-ribose, in a crystalline complex with alcohol dehydrogenase, is in the form *anti* [37], while kinetic studies indicate that, at some stage during its interaction with lactate dehydrogenase, 8-dimethylamino-5′-AMP is probably also *anti* [3].

8-(α-hydroxyisopropyl)adenosine (**Ie**) *and 8-(α-hydroxyisopropyl)-5′-AMP* (**Im**)

With a C(8)-substituent as bulky as α-hydroxyisopropyl (van der Waals radius 3.5 − 4.0 Å) it is readily seen from an examination of CPK models that its interaction with the ribose ring is such that **Ie** and **Im** must exist *exclusively* in the forms *syn*. Direct experimental evidence for this is forthcoming from an examination of the newly synthesized 8,2′-O-isopropylidene-araA (**III**), the conformation of which should be "*high anti*".

An examination was made of the chemical shifts of H(1′), H(2′) and H(3′) in **III**, 8-(α-hydroxyisopropyl)adenosine (**Ie**), adenosine and araA. The differences in chemical shifts of these protons, between araA and adenosine (Table III), on the assumption of similar conformations about the glycosidic bonds for these two nucleosides, define the effects of a change in configuration at C(2′) on

Table III. Changes in chemical shifts (in ppm vs internal DSS, in ^2H_2O at pD 8 at 22 °C) of H(1′), H(2′), H(3′) resulting from a change in configuration at C(2′) of the sugar ring, *i. e.* conversion of the ribose ring to arabinose.

Conversion		Change in chemical shift		
from	to	H(1′)	H(2′)	H(3′)
araA [a]	adenosine	+0.38	−0.22	−0.06
8,2′-isopropylidene-araA	8-(α-hydroxyisopropyl) adenosine	−0.71	−0.47	−0.23
8,2′-isopropylidene-araA	adenosine	+0.10	−0.13	−0.12

[a] M. Remin *et al.*, Biochim. Biophys. Acta **435**, 405 (1976).

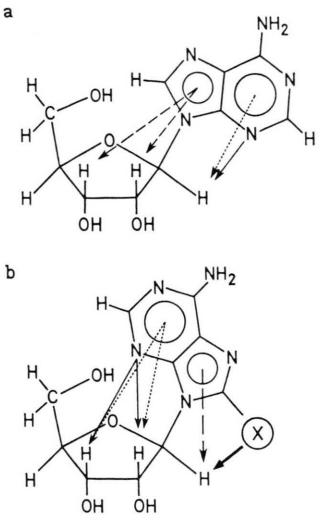
the values of the chemical shifts. Analogous differences in chemical shifts are noted between **III** and adenosine (Table III) (this also supports the predominant *anti* conformation of adenosine).

By contrast, the corresponding differences in chemical shifts between **III** and 8-(α-hydroxyisopropyl) adenosine (**Ie**) (in particular of H(1′)) relative to araA and **Ia**, and **III**, and **Ia**, indicate that, apart from the effect of a change in configuration of the sugar ring at C(2′), there is also a change in orientation of the base about the glycosidic bond. Since **III** possesses the conformation "*high anti*", 8-(α-hydroxyisopropyl) adenosine must be *syn*.

For the nucleoside **Ie**, H(2′) is deshielded by 0.36 ppm in aqueous medium and in DMSO, while for the nucleotide **Im** in aqueous medium the deshielding is 0.55 ppm. Only minor deshielding of H(3′) occurs, ~0.1 ppm. On the other hand H(1′) undergoes pronounced deshielding, 0.80 ppm for the nucleoside (**Ie**) and 0.64 ppm for the nucleotide (**Im**). In addition, the changes in chemical shifts of H(2′) and H(3′) are almost the same as those between 8-bromoadenosine and adenosine, and 8-dimethylaminoadenosine and adenosine (and for the corresponding nucleotide pairs).

The foregoing observations may be interpreted as follows: (a) The change in chemical shift of H(2′) is due to the influence on this proton of the ring current of the base, and the anisotropy of the ring N(3), when the orientation of the base changes from *anti* to *syn:* (b) the change in chemical shift of H(1′) is the resultant of the foregoing effects, plus the influence of the anisotropic and polarization interactions with the C(8)-substituent which, in the form *syn*, is in the vicinity of H(1′); (c) the change in chemical shift of H(3′) derives from the same factors as for H(2′), but is quantitatively smaller because of its larger displacement from the heterocyclic ring than H(2′) (Scheme 2).

It follows that the changes in chemical shifts of H(2′) and H(3′) are not dependent on the nature of the C(8)-substituent [58]. The chemical shift of H(1′), on the other hand, is very sensitive to the nature of the substituent since, with the conformation *syn*, they are in the immediate vicinity of each other. The observed changes in chemical shifts, with various C(8)-substituents, are in full accord with these postulates. A satisfactory, quantitative, measure of the change in conformation from *anti* to *syn* is provided by the change in chemical shift of H(2′),



Scheme 2. Illustrating the factors which influence the chemical shifts of the pentose protons in adenosine analogues: (a) in the conformation *anti;* (b) in the conformation *syn*.

since that of H(3′) is less sensitive to the change in conformation, while that of H(1′) is dependent on the nature of the substituent. From these criteria it may be concluded that 8-dimethylaminoadenosine (**Id**) and its 5′-phosphate (**Il**) are exclusively in the form *syn*. The chemical shift of H(2′) in 8-bromoadenosine differs by 0.09 ppm, and in 8-bromo-5′-AMP by 0.05 ppm, from those for the other two 8-substituted nucleosides (**Ie**, **Id**) and nucleotides (**Il**, **In**). A decrease in pH from 8 to 2 leads to an increase in the chemical shift of H(2′) in 8-bromoadenosine from 0.27 ppm to 0.33 ppm, *i. e.* to a value identical with that for H(2′) in **Ie** at pH 2 (see Table IV). This protonation effect is less marked and opposite in sign in **Ie** [58], and points to the absence of a stabilized configuration of the aglycone about the glycosidic bond in the 8-bromo derivative with a change in solvent. The lower value of the chemical shift of H(2′) in **Ic** and **Ik**, by comparison with **Ie** and **Id**, and **Il** and **Im**, as well as its dependence on solvent, points to existence of the conformation *anti*, or a different value of the glycosidic bond angle, or a combination of both of these, in the 8-bromo derivatives. Since the *syn* conformation is markedly predominant, it is difficult to distinguish between the two on the basis of the observed differences in chemical shifts. The results do point to existence of a small proportion of the *anti* conformers in 8-bromoadenosine and 8-bromoadenosine-5′-phosphate.

The appreciably smaller change in chemical shift of H(2′) in 8-methyladenosine (**Ib**); together with the negligible change in shift of H(3′), indicate that this analogue is not fully *syn*, but rather that there is an increase in the population of the *syn* conformer relative to adenosine. The change in chemical shift of H(1′) is the resultant of the effect of the 8-methyl in the conformation *syn* and that of rotation of the base about the glycosidic bond. Since the *steric* interaction with the ribose ring is virtually identical for an 8-methyl and 8-bromo substituent (difference in van der Waals' radii 0.05 Å), the more pronounced preference for the form *syn* in 8-bromoadenosine must be due to its more effective electrostatic interaction with the sugar ring and the exocyclic 5′-CH_2OH.

If we neglect the effect of the methyl substituent on the chemical shift of H(2′), it is possible, with the aid of the formula given below, to estimate the population of the *syn* conformer in 8-methyladenosine, which comes to about 50% both in DMSO and in aqueous medium.

Analogous differences between the two analogues, relative to adenosine, are forthcoming from the data of Uesugi and Ikehara [18] on ^{13}C chemical shifts. The changes in ^{13}C chemical shifts of the sugar carbons as between adenosine and its 8-substituted derivatives were ascribed to an increase in population of the *syn* conformer. The largest change in chemical shift was exhibited by C(2′) which, in the case of 8-methyladenosine (**Ib**), was -1.40 relative to adenosine, whereas in 8-bromoadenosine it was -2.34 ppm. This is in accord with the conclusion regarding the greater preference of the form *syn* for 8-bromoadenosine relative to 8-methyladenosine based on the changes in chemical shifts of H(2′).

Effect of phosphate hydroxyl ionization

One of the procedures for studying the conformation of the base in 5′-nucleotides of adenine involves titration of the phosphate group in the region of secondary ionization and noting the accompanying changes in chemical shifts of H(2) and H(8). With a view to evaluating the validity of this method, 8-(α-hydroxyisopropyl)adenosine-5′-phosphate (**Im**) was titrated over the pH range 4.5 – 9.0. The accompanying change in chemical shift of H(2) did not exceed 0.03 ppm, while no observable effect was noted on the protons of the hydroxyisopropyl substituent. The latter observation is consistent with the form *syn* for **Im**. But the small effect on H(2), in the case of a nucleotide unambiguously in the form *syn*, undermines the validity of this procedure, since it shows that the absence of any change in chemical shift of H(2) in 5′-AMP does not exclude the presence of the *syn* conformation [59].

The absence of a change in chemical shift of H(2) is clearly due to its large displacement from the phosphate group, even with the *syn* conformation. In nucleotides with a large C(8)-substituent there is an additional increase in the distance between H(2) and the exocyclic oxygen as a result of the marked decrease in the population *gauche-gauche* [16, 59], due to repulsive interaction between the six-membered pyrimidine ring and the exocyclic group. Similar interaction is present in 5′-AMP with the *syn* form. The resultant displacement of H(2) from the phosphate group renders unlikely any effect of ionization of the latter on the chemical shift of the former, whether the nucleotide is in the form *syn* or not.

8-(α-hydroxyisopropyl)-5′-chloro-5′-deoxyadenosine (**Ii**)

The proton chemical shifts for this nucleoside, relative to 5′-chloro-5′-deoxyadenosine (**Ig**), are analogous to those for the corresponding nucleosides with a free 5′-OH (Table IV). We may therefore conclude that (**Ii**) is also exclusively *syn*. The larger change in chemical shift of H(2′), 0.44 ppm as compared to 0.36, may be due to a lower population of the form *syn* in **Ig** as compared to adenosine (see below).

2,8-di-(α-hydroxyisopropyl)adenosine (**If**)

The values of the chemical shifts for the ribose protons in this analogue clearly show that it is in the form *syn*. There is the characteristic increase in deshielding of H(2′) by 0.20 ppm, as well as a change in sign in the difference in shifts of H(3′) by comparison with the monohydroxyisopropyl derivative (**Ie**) (Table IV). The shifts of H(1′) are identical for both nucleosides. These effects are readily interpretable in terms of the anisotropic and polarizing effects of the hydroxyisopropyl substituent at C(2) in the conformation *syn*. It is clear that, with the simultaneous presence of two bulky

Table IV. Changes in chemical shifts (in ppm vs internal DSS in ^2H_2O, p^{2}H = 8, and vs internal TMS in DMSO and chloroform, at 22 °C) of H(1′), H(2′), H(3′) in various adenine nucleosides and nucleotides following introduction of the bulky substituents CH_3, Br, $N(CH_3)_2$ or $C(CH_3)_2OH$ at C(8).

Nucleoside or nucleotide	Solvent	Change in chemical shift		
		H(1′)	H(2′)	H(3′)
8-methyladenosine (**I b**)[a]	^2H_2O	−0.15	0.20	−0.03
	$(C^2H_3)_2SO$	−0.05	0.16	0.02
8-bromoadenosine (**I c**)[b]	^2H_2O	0.09	0.27	0.07
	^2H_2O (p^{2}H 2)	0.01	0.33	0.10
8-dimethylaminoadenosine (**I d**)[c]	^2H_2O	−0.12	0.36	0.02
8-(α-hydroxyisopropyl) adenosine (**I e**)	^2H_2O	0.80	0.36	0.11
	^2H_2O (p^{2}H 2)	0.70	0.33	0.16
	$(C^2H_3)_2SO$	0.87	0.36	0.05
8,2-di-(α-hydroxyisopropyl) adenosine (**I f**)	^2H_2O	0.79	0.57	0.28
5′-deoxy-5′-chloro-8-(α-hydroxyisopropyl) adenosine (**I i**)	$(C^2H_3)_2SO$	0.87	0.44	0.21
2′,3′-O-isopropylidene-8-(α-hydroxyisopropyl) adenosine (**II b**)	C^2HCl_3	1.01	0.13	−0.02
	$(C^2H_3)_2SO$	0.89	0.06	0.05
8-bromoadenosine-5′-phosphate (**I k**)[b]	^2H_2O	−0.03	0.50	0.08
8-dimethylaminoadenosine-5′-phosphate (**I l**)[d]	^2H_2O	−0.33	0.56	0.0
8-(α-hydroxyisopropyl) adenosine-5′-phosphate (**I m**)	^2H_2O	0.64	0.55	0.11

[a] Data from C. Giessner-Prettre and B. Pullmann, J. Theor. Biol. **65**, 171 (1977); M. Ikehara, W. Limn, and T. Fukui, Chem. Pharm. Bull. (Japan) **25**, 270 (1977).
[b] Data from R. H. Sarma *et al.*, J. Am. Chem. Soc. **96**, 7337 (1974).
[c] Data from F. Jordan and H. Niv, Biochim. Biophys. Acta **476**, 265 (1977).
[d] Data from F. Evans and N. O. Kaplan, J. Biol. Chem. **251**, 6791 (1976).

substituents at C(2) and C(8), it is the substituent at C(8) which determines the conformation about the glycosidic bond.

2′,3′-O-isopropylidene-8-(α-hydroxyisopropyl)-adenosine (**IIb**)

The replacement of H(8) in 2′,3′-O-isopropylideneadenosine by α-hydroxyisopropyl leads to qualitatively analogous changes in chemical shifts of the ribose protons, in chloroform solution and in DMSO, as with adenosine. There are, however, marked quantitative differences: in C^2HCl_3 deshielding of H(1′) is greater by 0.20 ppm, while that of H(2′) is only 0.13 ppm. The analogous changes in DMSO amount to 0.89 ppm for H(1′) and 0.06 ppm for H(2′). A change in solvent medium consequently leads to quantitative changes in differences in chemical shifts between the 8-(α-hydroxyisopropyl) derivative and the parent nucleoside, the general character of these changes being characteristic for changes in the orientation of the base towards a preference for the form *syn* for derivatives with a substituent at C(8). It should be emphasized that the change in chemical shift of H(2′) is even lower in DMSO than in C^2HCl_3. The decisive factor here is the chemical shift of H(2′), which is quantitatively related to the orientation of the aglycone. The observed differences between the 2′,3′-O-isopropylidene derivative and that with free *cis*-hydroxyls cannot be ascribed to differences in solvent, since the difference in chemical shifts of H(2′) between 8-(α-hydroxyisopropyl)-adenosine and adenosine is identical in aqueous medium and in DMSO. Furthermore the conformations of the sugar rings in the 2′,3′-O-isopropylidene derivatives, and those with free *cis*-hydroxyls, are similar, with a preference for the form S (see Table II, and next paragraph). As will be shown below, the observed differences derive from the higher proportion of the population *syn* in 2′,3′-O-isopropyleneadenosine in C^2HCl_3, and an even higher proportion in DMSO.

Particularly pertinent is assignment of the sugar ring conformation. Introduction of the 2′,3′-O-isopropylidene substituent renders such an assignment difficult because of modifications of the conformers in the pseudorotational model normally applied to nucleosides not constrained by intramolecular bonding. The parameters in the Karplus relation likewise undergo modification. This problem is extensively discussed by Plochocka *et al.* [60], from which we assume an equilibrium for the isopropylidene derivative of C(3′)exo $\rightleftharpoons$ C(2′)exo, but with the *proviso* that these differ from those for the free ribose ring because of "flattening" of the ring by the isopro-

pylidene substituent. In chloroform solution the coupling constants point to a strong preference for the conformation C(3′)exo(~80%), whereas in DMSO the two populations are comparable (~50%).

Conformation of the 8-(α-hydroxyisopropyl) substituent

A phenomenon of particular interest was the observed magnetic non-equivalence of the CH_3 groups of the 8-(α-hydroxyisopropyl) substituent of **Ie** and **Ii** in DMSO-d_6. The difference in chemical shifts between the protons of the two groups amounted to 0.04 ppm (Table I). A similar situation prevails for 8-(α-hydroxyisopropyl)guanosine, where the difference in chemical shifts is 0.02 ppm (unpublished results). For **Ie** in ^2H_2O the chemical shifts of the protons of the two methyl groups are identical, as is also the case for **Im** in ^2H_2O and for **IIb** in DMSO and C^2HCl_3.

In view of the non-symmetry of the hydroxyisopropyl substituent, identical values of the chemical shifts for the methyl protons represent a special case involving a distribution of rotational conformers about the C(8)-C bond [61].

Interaction of the hydroxyisopropyl substituent with the solvent and/or the sugar ring may modify the distribution of rotational conformers and, by favouring one of these, lead to the observed magnetic non-equivalence of the methyl group. Since the non-equivalence, for a given nucleoside, is observed in a solvent of low polarity, and not in water; and since the introduction of a 2′,3′-O-isopropylidene group liquidates the non-equivalence of the methyl groups, one reasonable interpretation is the stabilized interaction of the pentose 2′-OH with the hydroxyisopropyl OH. In support of this is the observation of Dr. M. Remin that the non-equivalence of the two methyl groups in **Ie** is also manifested in aqueous medium which has been brought to sufficiently alkaline p^{2}H to dissociate the 2′ hydroxyl; under such conditions a strong hydrogen bond may form intramolecularly between the hydroxyisopropyl OH as donor and the $O(2')^{(-)}$ as acceptor, leading to stabilization of one conformer and resulting in the non-equivalence of the two methyl groups.

Calculation of conformer populations

We now proceed to an application of the foregoing data to calculate the relative populations of the *syn* and *anti* conformations of adenosine and 2′,3′-O-isopropylideneadenosine. While adenosine is known to be predominantly *anti*, the presence of some proportion of the form *syn* is testified to not only by the differences in chemical shifts of the sugar protons relative to the 8-substituted analogues exclusively in the *syn* form, but also by ultrasonic relaxation methods [12], which point to the existence of a dynamic equilibrium *syn* ⇌ *anti*.

The relative populations of the forms *syn* and *anti*, for adenosine in DMSO and ^2H_2O and for 2′,3′-O-isopropyladenosine in chloroform and in DMSO were calculated from the chemical shifts of H(2′). Under conditions where the dynamic equilibrium between the two forms is rapid on the NMR time scale, the experimentally observed chemical shift δ_{obs} is as follow:

$$\delta_{obs} = P_{syn}\,\delta_{syn} + P_{anti}\,\delta_{anti}$$

where the P's are the relative populations of the two forms, and the δ values are the chemical shifts for the fixed forms *syn* and *anti*, respectively. Such a relationship holds for the case where there are two well-defined conformations *syn* and *anti* with clearly defined narrow energy minima. The values adopted for δ_{syn} were those for the 8-α-hydroxyisopropyl derivatives. The corresponding values of δ_{anti} were the chemical shifts of H(2′) for 8,5′-anhydro-8-oxoadenosine (**IV**) and 8,5′-anhydro-8-oxo-2′,3′-O-isopropylideneadenosine (**V**). The selection of these two nucleoside analogues as reference compounds for the fixed *anti* forms is based on the fact that, with the cyclic bond involving the oxygen, the exocyclic sugar side chain is in a conformation close to *gauche-gauche*. It should be recalled (Table II) that adenosine (**Ia**), 2′,3′-O-isopropylideneadenosine (**IIa**) in C^2HCl_3, and their 8-α-hydroxyisopropyl derivatives, also prefer the *gauche-gauche* conformations.

From the above equation, and the chemical shift values for H(2′) from Table I, we find that the population of the *anti* conformer of adenosine is 80% in DMSO and 90% in aqueous medium, while that of 2′,3′-O-isopropylideneadenosine is 50% in chloroform and 20% in DMSO. For adenosine the difference in chemical shifts for H(2′) between the fixed forms *syn* and *anti* is 0.44 ppm in DMSO-d_6 and 0.39 ppm in ^2H_2O. For 2′,3′-O-isopropylideneadenosine it is only 0.25 ppm in C^2HCl_3 and 0.31 ppm in DMSO-d_6, most likely as a result of "flattening" of the sugar ring by the isopropylidene

substituent, so that the sugar ring protons are displaced away from the base. The influence of ring currents and the anisotropy of the ring N(3), which determine the changes in chemical shifts as between the *syn* and *anti* forms, are both strongly dependent on distance. The calculated conformer populations for 2′,3′-O-isopropylideneadenosine are consequently less accurate than in the case of adenosine.

The foregoing procedure clearly requires further refinement by taking account of the influence on the chemical shifts of a change in conformation of the sugar ring (for nucleosides in the *anti* conformation), of the exocyclic 5′-CH_2OH, and of the glycosidic angle.

The model compounds with the *anti* conformation of the bases, **IV** and **V**, possess a somewhat different conformation of the sugar rings than the other nucleosides (Table II). Accurate assignments of the populations would require a determination of the influence of changes in the sugar ring conformation on the chemical shifts of H(2′). It may be assumed that the major effect on the shift of H(2′) is defined by its orientation relative to the 3′-OH and the plane of the heterocyclic base, which is determined by the torsion angles O(3′)-C(3′)-C(2′)-H(2′) and H(2′)-C(2′)-C(1′)-N(9), respectively. A change in conformation from 65% C(2′)*endo* to C(4′)*endo*-O(1′)*exo* would not affect the former; while the latter would change by about 30° (from 20° to −10°), so that the distance of H(2′) from the plane of the base is, to a first approximation, unaltered. From Table III it will be seen that the chemical shifts of H(2′) in adenosine and araA differ by 0.21 ppm. Changes in the configuration at C(2′) correspond to changes in torsion angles of O(3′)-C(3′)-C(2′)-H(2′) by 130°, and H(2′)-C(2′)-C(18)-N(9) by 120°. It may be concluded that, with the minor changes in torsion angles accompanying a change in sugar ring conformation from 65% C(2′)*endo* to C(4′)*endo*-O(1′)*exo*, the accompanying change in chemical shift of H(2′) is negligible.

For the 2′,3′-O-isopropylidene derivatives in chloroform, all three nucleosides, *i.e.* 2′,3′-O-isopropylideneadenosine and the model analogues with the fixed conformations *anti* (**V**) and *syn* (**IIb**) exhibit 100% *gauche-gauche* populations of the exocyclic 5′-CH_2OH group, so that no correction for this is necessary. For the other systems, however, adequate account of the exocyclic group conformer populations may be necessary to obtain reasonable results. For adenosine in aqueous medium and in DMSO, where the exocyclic group conformation is about 65% *gauche-gauche* (Table II), the changes in population should be only minimal. More pronounced changes would be anticipated for 2′,3′-O-isopropylideneadenosine in DMSO, where it and its 8-(α-hydroxyisopropyl) derivative show a very marked decrease in the *gauche-gauche* population to about 40% (Table II). Since the correction should be similar for both of these, the difference in chemical shifts of H(2′) for both will remain unaltered. Since there is marked preference for the conformation *syn*, one would not expect any major quantitative changes in the calculated populations.

The lack of a model analogue in the conformation *anti* for 5′-AMP excludes any quantitative evaluation of its glycosidic bond conformation. The values of the chemical shifts of the sugar protons point only to a preference for the form *anti*. Application of the above procedure for nucleosides to nucleotides is under study.

While virtually all methods hithero employed for establishment of the *syn-anti* equilibrium in nucleosides and nucleotides have been qualitative, they are generally consistent with the results reported in this study. One exception is that involving the use of proton relaxation techniques, leading to the conclusion that 5′-nucleotides are in the form *syn*, with a glycosidic angle of 70° [27]. Application of the Nuclear Overhauser Effect to 2′,3′-O-isopropylideneadenosine in DMSO-d_6 indicated a population for the form *syn* of 70 – 80% [24]; a similar study with adenosine suggested that 60 – 70% is in the *syn* form [60]. There is consequently quantitative agreement between the NOE results, and those based on proton chemical shifts, for 2′,3′-O-isopropylideneadenosine, but not for adenosine. The NOE method is known to be subject to considerable error in experimental measurement of the signal intensities. However, as pointed out above, the procedure based on chemical shifts is also subject to some systematic errors due to neglect of the influence of the value of the glycosidic torsion angle. Both methods consequently require further refinement.

The calculated populations permit of some inferences regarding mutual interrelationships between the conformation about the glycosidic bond and the sugar and exocyclic group conformations. The decisive factor in establishment of the *syn* ⇌ *anti*

equilibrium is the conformation of the sugar ring. Marked "flattening" of the sugar ring, *e. g.* by introduction of the 2′,3′-O-isopropylidene group, appreciably increases the proportion of the *syn* conformer. In 2′,3′-O-isopropylideneadenosine itself, a change in sugar conformation from C(3′)*exo* in chloroform to an equilibrium C(3′)*exo* ⇌ C(2′)*exo* in DMSO leads to an increase in the *syn* conformer population of about 30%. Furthermore, the absence of any change in conformation of the ribose ring in adenosine with change in solvent is accompanied by only a minimal change in the equilibrium *syn-anti* (~10%).

The enhanced differences in chemical shifts of H(2′) between 8-(α-hydroxyisopropyl)-5′-deoxy-5′-chloroadenosine (**Ii**) and 5′-deoxy-5′-chloroadenosine (**Ig**) in DMSO relative to adenosine (0.44 ppm, see Table IV), points to an increase in the population *anti* for 5′-deoxy-5′-chloroadenosine relative to adenosine in DMSO. This suggests stabilization of the form *syn* by means of intramolecular hydrogen bonding between the exocyclic 5′-OH and the adenine ring N(3), since there is a simultaneous drop in the *gauche-gauche* population of 5′-deoxy-5′-chloroadenosine. Such stabilization is certainly not decisive in establishment of the conformation about the glycosidic bond, since adenosine itself strongly prefers the conformation *anti*. A change in solvent only, from DMSO to aqueous medium, is accompanied by a change in the population of the *syn* conformation, but without a change in the conformation of the exocyclic group. Furthermore, in 2′,3′-O-isopropylideneadenosine, an increase in the *syn* population is accompanied by a decrease in the *gauche-gauche* population, by as much as 50%. It is, consequently, obvious that the conformation of the sugar ring is decisive, and that of the exocyclic group much less so, in establishment of the conformation of the purine ring about the glycosidic bond. We are probably dealing here with a weak stabilizing interaction strongly dependent on the nature of the solvent. More concrete deduction(s) about this could probably be derived from more accurate determinations of the populations in the *syn* ⇌ *anti* equilibria. Considerable significance undoubtedly attaches to reasonably accurate determinations of the glycosidic bond angles, *e. g.* by means of ^{13}C-^{1}H coupling constants, and evaluation of the effects to changes in these angles on the chemical shifts of the sugar protons.

Experimental

Melting points (uncorr.) were measured on a Boetius microscope hot stage. UV spectra were recorded on a Unicam SP-8000 or a Zeiss (Jena, GDR) Specord UV-VIS.

Elementary analyses for C, H, N were carried out on a Perkin-Elmer 240 Model.

Preparative photochemical reactions were conducted in a reactor with an Astra-Lux (Vienna) 700 W mercury source, using a quartz vessel surrounded by a concentric 2-mm layer of adenine in dimethylformamide (1.0 g/l) to cut off at about 280 nm, or in a pyrex vessel where the glass itself acts as a filter to cut off short wavelengths.

Thin-layer chromatography made use of plastic sheets cellulose F_{254} and plastic sheets Silica gel $60F_{254}$ (Merck, Darmstadt, GFR). Chromatography was carried out with solvent A: chloroform-methanol (4 : 1); solvent B: chloroform-methanol (9 : 1); solvent C (cellulose: isopropanol-water-NH_3 aq. (7 : 2 : 1); and solvent D: acetone-benzene (2 : 1).

^{1}H NMR spectra were recorded on Brucker-90 and JEOL JNM-4H-100 instruments at a temperature of 22 °C, unless otherwise indicated, with solutions at concentrations of 0.2 M or, for less soluble compounds, 0.05 M. Solutions were made up in $^{2}H_2O$ (Merck, GFR, 99.7 mol-% ^{2}H) with internal DSS, in DMSO-d_6 (Merck, 99.5 mol-% ^{2}H) with internal TMS, and in $C^{2}HCl_3$ (Merck, 99.7 mol-% ^{2}H) with internal TMS. Measured values of chemical shifts are accurate to ± 0.01 ppm, and of coupling constants to ± 0.2 Hz.

5′-chloro-5′-deoxyadenosine (**Ig**)

To a mixture of 20 ml hexamethylphosphoramide and 3 ml (41.8 mmol) thionyl chloride was added, over a period of 20 min, 2 g (7.5 mmol) adenosine. The course of the reaction was followed by TLC on silica gel with solvent A, R_F of adenosine 0.30, and of sulfinated product 0.80 [42]. The reaction went to completion in 19 h at room temperature. The reaction mixture was added to 200 ml water, and the resulting solution deposited on a 3 × 40 cm column of 200/400 mesh Dowex 50 W × 2 (H^+). The column was washed with 1 litre water, and then eluted with a solution of 10% NH_3 in 50% aqueous ethanol. The UV-absorbing fractions were pooled

and brought to dryness on a rotary evaporator. The residue was taken up in a small volume of hot water, from which it crystallized as long needles, yield 2.3 g (95%), m.p. 106 – 108 °C. Anal. Found: C, 40.98; H, 4.20; N, 23.81. Calcd. for the semihydrate $C_{10}H_{12}N_5O_3Cl \times 0.5\ H_2O$: C, 40.75; H, 4.45; N, 23.77. UV (water); λ_{max} 259 nm (ε_{max} 14.2×10^3). R_F in solvent A, 0.47. NMR, see Table I.

8-Bromo-5′-chloro-5′-deoxyadenosine (**Ih**)

To a mixture of 6 ml hexamethylphosphoramide and 0.75 ml (~10 mmol) thionyl chloride was added 0.5 g (1.44 mmol) 8-bromoadenosine as above. The reaction was followed by TLC as above and was complete after 18 h. The product was isolated as above and crystallized from water to yield 360 mg (70%) as large granules, leaving in the supernatant an unidentified product with a UV spectrum characteristic of 5′,N_3-cycloadenosine. The granules were recrystallized from aqueous acetone in the form of platelets, chromatographically homogeneous, m.p. 103 – 108 °C. Anal. Found: C, 33.30; H, 3.15; N, 19.53. Calcd. for $C_{10}H_{11}N_5O_3$ BrCl: C, 32.98; H, 3.02; N, 19.24. R_F in solvent B, 0.40. UV (water): λ_{max} 262 nm (ε_{max} 16.3×10^3). NMR, see Table I.

8-(α-hydroxyisopropyl)adenosine (**Ie**) *and 2,8-di-(α-hydroxyisopropyl)adenosine* (**If**)

To a solution of 3.1 g (11.6 mmol) adenosine in 300 ml 80% isopropanol was added 4 ml di-*tert*-butyl peroxide. This solution was irradiated for 60 h with addition of 4 ml DTBP at 10 h intervals to a total of 24 ml. The course of the reaction was followed by disappearance of adenosine, using TLC on silica gel with solvent A, and was 90% complete after 60 h. The solution was brought to dryness to yield a pale yellow syrup, which was crystallized from hot water to yield 2.0 g (53%) of **Ie** in the form of rhomboids, m.p. 224 – 226 °C (liter. 227 – 230 °C) [43]. The mother liquors were fractionated on 40×2.5 cm column of Dowex AG1 × 4 (OH^-) with 50% aqueous methanol at a flow-rate of 7 ml/min and collection of 21 ml fractions. Fractions 40 – 53 contained 60 mg (1.7%) of a product identified as **If**; fractions 70 – 165 yielded 640 mg of **Ie** (overall yield 70%); fractions 348 – 400 contained unreacted adenosine. Product **If** was obtained as a colorless glass, which could not be crystallized, but was chromatographically homogeneous (R_F 0.22 with solvent B). Anal. Found: C, 45.76; H, 6.98; N, 16.50. Calcd. for $C_{16}H_{25}N_5O_6 \cdot {}^2H_2O$: C, 45.82; H. 6.92; N, 16.71. UV spectrum virtually identical with that for **Ie** and adenosine. NMR spectra of **Ie** and **If**, see Table I.

8,2′-O-isopropylidene-araA (**III**)

A solution of 100 mg (0.3 mmol) 8-(α-hydroxyisopropyl)adenosine (**Ie**), 100 mg (0.47 mmol) diphenyl carbonate and 5 mg $NaHCO_3$ in 0.25 ml dimethylformamide was heated for 30 min on an oil bath at 140 – 145 °C. Addition of 7 ml diethylether at room temperature led to precipitation of product and unreacted **Ie**. Two crystallizations from water gave the desired product, **III**, as long parallelopipeds, 50 mg (55%), m.p. 308 – 310 °C. Anal. Found: C, 50.81; H 5.47; N, 23.03. Calcd. for $C_{13}H_{17}N_5O_4 \times H_2O$: C, 50.81; H, 5.54; N, 22.80. R_F with solvent B, 0.22. UV (water): λ_{max} 261.5 nm (ε_{max} 17.7×10^3); pH 2, λ_{max} 260 nm (ε_{max} 17.5×10^3), 266.5 nm (sh). NMR spectrum, Table I.

8-(α-hydroxyisopropyl)-5′-AMP (**Im**)

The a solution of 700 mg (2 mmol) 5′-AMP and 0.5 ml (5 mmol) tri-*n*-butylamine in 10 ml water was added 50 ml isopropanol and 2 ml di-*tert*-butyl peroxide. The solution was irradiated for 30 h, the disappearance of starting substance being followed by chromatography on cellulose with solvent C. The irradiated solution was deposited on a 35×2.0 cm column of 200/400 mesh Dowex AG1 × 4 ($HCOO^-$). The column was washed with water and elution then carried out with 0.05 M formic acid at a rate of 3 ml/min, and collection of 50 ml fractions. The desired product was found in fractions 22 – 27 (12×10^3 OD_{260} units, about 40%), and unreacted 5′-AMP in fractions 47 – 52 (about 10%). Two additional minor peaks in fractions 12 – 19 and 34 – 41 were not identified. Identification of **Im** was based on its ^1H NMR spectrum (Table I). It was readily dephosphorylated to the nucleoside (**Ie**) by alkaline phosphatase; but, like the analogous guanine nucleotides [39], was fully resistant to snake venom 5′-nucleotidase.

8-(α-hydroxyisopropyl)-2′,3′-O-isopropylidene-adenosine (**IIb**)

To a suspension of 650 mg (2 mmol) 8-(α-hydroxyisopropyl) adenosine (**Ie**) in 20 ml anhydrous acetone was added 480 mg (2.4 mmol) *p*-toluenosulfonic acid hydrate, followed (with vigorous stirring) by 2 ml (16 mmol) 2,2-dimethoxypropane. Chromatography on silica gel with solvent B demonstrated 95% conversion of starting substance after 1 h. To the reaction mixture was added 1 ml conc. NH_4OH and 5 ml water. This was brought to dryness and the residue dissolved in hot water, from which crystallization proceeded to give hexagonal plates, m.p. 140 – 142 °C. The mother liquors were percolated through a column containing 2.5 ml Dowex $1\times4(OH^-)$, following which elution was carried out with MeOH. The fractions containing UV-absorbing material were pooled, brought to dryness, and the residue crystallized from hot water to give an additional 144 mg (overall yield 96%). R_F with solvent B 0.64, as compared to 0.27 for **Ie**. UV spectrum identical with starting substance. NMR data, Table I.

5′-chloro-5′-deoxy-8-(α-hydroxyisopropyl)-adenosine (**Ii**)

A solution of 865 mg (3 mmol) of 5′-chloro-5′-deoxyadenosine (**Ig**) and 4 ml di-*tert*-butyl peroxide in 50 ml 80% aqueous isopropanol was irradiated for 17 h, at which time **Ig** had virtually disappeared. Evaporation of solvent yielded about 1 g of a foamy substane, which was subjected to chromatography on 5 20 × 20 cm plates of PF_{254} gel with solvent A. Under a dark UV lamp there was an intense band (R_F 0.4), together with two fainter fast-migrating bands. The main band was eluted with methanol and crystallized from hot water to yield 500 mg (49%) of irregular shaped crystals, m.p. 187 °C (decomp.). Anal. Found: C, 20.03; H, 5.40; N, 45.68. Calcd. for $C_{13}H_{18}N_5O_4Cl$: C, 20.38; H, 5.24; N, 45.41. R_F with solvent B, 0.26. UV (water): λ_{max} 261 nm (ε_{max} 15.2×10^3; pH 2, λ_{max} 260 nm (ε_{max} 15.8×10^3), 265 nm (sh); NMR, Table I.

8-Bromo-2′,3′-O-isopropylideneadenosine (**IIc**)

To a suspension of 3.12 g (9 mmol) 8-bromoadenosine (**Ic**) in 60 ml anhydrous acetone was added 2.16 g (10.8 mmol) *p*-toluenosulfonic acid hydrate and 16 ml 2,2-dimethoxypropane. Chromatography on silica gel with solvent D demonstrated total conversion of starting substance after 1 h. In addition to the desired product, a faster migrating spot was visible. To the reaction mixture was added 2 ml conc. NH_4OH, and the whole brought to dryness. The residue was suspended in several ml water, deposited on a Buchner funnel, and washed with water to remove *p*-toluenosulfonate (NH_4^+ salt). Crystallization from methanol, followed by washing with chloroform and drying, gave 2.96 g (85%), m.p. 234 – 235 °C. The mother liquors and chloroform washings were chromatographed on five 20 × 20 cm plates of PF_{254} silica gel with acetone-benzene (1 : 1) to give a weak slowly migrating band and an intense band with higher R_F. The latter was eluted with $CHCl_3$-MeOH (1 : 1), the eluate brought to dryness, and the residue crystallized from hot methanol to yield 514 mg (12.5%) of a by-product, in the form of long needles m.p. 158 – 159 °C, identified from its NMR spectrum as 8-bromo-2′,3′-O-isopropylidene-5′-methoxyisopropyladenosine. Anal. Found: C, 44.42; H, 5.23; N, 15.25. Calcd. for $C_{17}H_{24}N_5O_5Br$: C, 44.50; H, 5.25; N, 15.50. R_F, with solvent D, 0.69. UV spectrum identical with **IIc**.

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