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The Crystal and Molecular Structure of 2-Ethylthio-8-methylinosine Monohydrate

By Nobuya Nagashima and Kyoko Wakabayashi

Central Research Laboratories, Ajinomoto Co. Inc., Kawasaki, Japan

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2-Ethylthio-8-methylinosine monohydrate crystallizes in the space group $P2_1$ with unit-cell dimensions: $a=10{\cdot}81$, $b=6{\cdot}93$, $c=10{\cdot}58$ Å, $\beta=96{\cdot}1°$. The structure was determined by Patterson and Fourier syntheses, and refined by block-diagonal least-squares calculations. The molecule was found to exist in the *syn* conformation. The sugar ring exhibits a C(2′)-*endo* puckering, and the conformation about the C(4′)–C(5′) bond is *gauche–trans*. The purine rings are nearly normal to the *b* axis and are stacked along the twofold screw axis by overlapping of the planes, each of which involves the ring and the S atom. There are two kinds of intermolecular hydrogen bond between the symmetry-related molecules; one connects N(1)–H of the base to O(2′) of the ribose, and the other connects O(6) of the base to O(5′)–H of the ribose. N(7) of the base is hydrogen bonded to the water molecule which is fixed in its position by three hydrogen bonds.

Introduction

As part of the work of our group on the substituent effect of 5′-nucleotides on flavouring activity, Yamazaki *et al.* have synthesized various substituted analogues of 5′-purine nucleotides, and evaluated their activity. They have found that 2-methylthio- or 2-ethylthio-5′-inosinic acid has a more enhanced synergistic effect than 5′-guanilic acid when used with monosodium glutamate (Yamazaki, Kumashiro & Takenishi, 1968), while its 8-methylated analogue, 2-methylthio- or 2-ethylthio-8-methyl-5′-inosinic acid, has no such activity. These results suggest that the conformation of the 5′-nucleotides is considerably different from that of the 8-methyl-5′-nucleotides because of the substituent effect of the methyl group at the C(8) position, and that the disappearance of flavouring activity is probably due to the conformational change.

Various attempts were made to obtain good crystals of several analogues of the 8-methylpurine nucleotides, but no crystal suitable for an X-ray diffraction study was obtained. However, good crystals of the 8-methyl nucleoside, 2-ethylthio-8-methylinosine, were obtained and we have therefore carried out the crystal structure analysis on this nucleoside.

Experimental

The fine crystals of 2-ethylthio-8-methylinosine* were crystallized by slow cooling of a saturated aqueous solution at about 30°C. After filtering out these fine crystals, long thin needles of the monohydrate were recrystallized from the mother liquid by slow evaporation.

The unit-cell dimensions determined by precession photographs are $a=10{\cdot}81$, $b=6{\cdot}93$, $c=10{\cdot}58$ Å, $\beta=96{\cdot}1°$. Systematic extinction suggested the space group $P2_1$. The calculated density assuming two structure units of chemical composition $C_{13}H_{18}N_4O_5S.H_2O$ per unit cell, is $1{\cdot}536$ g cm^{-3}, and the observed density by flotation is $1{\cdot}520$ g cm^{-3}. Intensity data ($h0l\sim h5l$) were collected on equi-inclination Weissenberg photographs with nickel-filtered Cu $K\alpha$ radiation. The intensities were measured on multiple-film photographs with a Narumi microphotometer, and 1387 independent structure factors were obtained which correspond to 67·3% of the total possible in the Cu $K\alpha$ sphere. The crystal could not be rotated around another axis because of its slenderness and the interlayer scale factors were adjusted later during the isotropic refinement process. No absorption corrections were applied. The size of the crystal used for the diffraction study was approximately 0·1 × 0·05 × 1 mm.

Determination of the structure

Several Harker peaks expected for the vectors between the sulphur atoms were examined. Correct assignment of these peaks could be made by comparing each Fourier synthesis phased by several trial sets of coordinates of sulphur atoms.

Two subsequent cycles of Fourier syntheses revealed all atomic positions of the molecule except those of the hydrogen atoms. The structure was then refined by block-matrix least-squares calculations initially with isotropic temperature factors and later with anisotropic values. The weighting scheme was essentially that proposed by Hughes, in which $4F_{\min}=20$. A difference Fourier synthesis was calculated at this stage and the positional parameters for 18 out of 20 hydrogen atoms were determined.

Finally, two cycles of block-matrix least-squares refinement were carried out in which the coordinates

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* The compound was kindly supplied by K. Suzaki and A. Yamazaki.

Table 1. *The final positional parameters and temperature factors and their estimated standard deviations for non-hydrogen atoms*

The temperature factors are in the form $T=\exp\{-10^{-4}(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)\}$.

	X	Y	Z	$S(X)$	$S(Y)$	$S(Z)$	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
S	0·0939	0·0	0·2176	0·002 Å	0·0 Å	0·001 Å	53 (1)	141 (5)	35 (1)	−9 (3)	4 (1)	8 (3)
O(6)	−0·2956	−0·0166	−0·0572	0·004	0·008	0·005	39 (4)	224 (17)	70 (4)	−19 (9)	14 (3)	−25 (9)
O(1′)	0·2377	−0·1549	−0·2755	0·005	0·006	0·005	41 (4)	91 (17)	89 (5)	1 (6)	−3 (4)	−9 (7)
O(2′)	0·2320	0·3602	−0·2695	0·005	0·006	0·005	56 (4)	79 (15)	60 (4)	−5 (6)	19 (3)	−11 (6)
O(3′)	0·4072	0·1641	−0·3880	0·004	0·006	0·005	41 (4)	116 (16)	72 (5)	6 (6)	21 (3)	13 (7)
O(5′)	0·4135	−0·4191	−0·1455	0·005	0·006	0·005	68 (5)	52 (15)	76 (5)	−22 (6)	4 (4)	6 (6)
N(1)	−0·1041	−0·0121	0·0541	0·005	0·009	0·005	51 (4)	135 (17)	45 (4)	5 (9)	17 (3)	12 (9)
N(3)	0·0867	0·0042	−0·0367	0·005	0·009	0·005	38 (4)	134 (17)	51 (4)	−11 (9)	12 (3)	5 (10)
N(7)	−0·1544	0·0086	−0·2926	0·005	0·009	0·005	34 (4)	117 (17)	55 (4)	−5 (9)	−7 (3)	−5 (10)
N(9)	0·0524	0·0194	−0·2671	0·004	0·007	0·005	33 (4)	59 (17)	48 (4)	−15 (7)	2 (3)	1 (8)
C(2)	0·0210	−0·0056	0·0599	0·006	0·011	0·006	49 (5)	135 (21)	45 (5)	−20 (12)	5 (4)	9 (12)
C(4)	0·0125	0·0078	−0·1478	0·005	0·010	0·006	44 (4)	43 (18)	53 (5)	−19 (10)	13 (4)	−13 (10)
C(5)	−0·1146	−0·0005	−0·1640	0·006	0·010	0·006	50 (5)	70 (19)	54 (5)	−3 (11)	11 (4)	−20 (11)
C(6)	−0·1832	−0·0095	−0·0582	0·006	0·011	0·006	46 (5)	95 (20)	57 (5)	−6 (11)	17 (4)	−6 (12)
C(8)	−0·0546	0·0215	−0·3494	0·006	0·009	0·006	51 (5)	42 (21)	53 (5)	−21 (9)	−3 (4)	−13 (9)
C(10)	0·2592	−0·0121	0·1938	0·007	0·013	0·007	47 (5)	257 (27)	68 (6)	14 (14)	−2 (4)	6 (15)
C(11)	0·3319	−0·0124	0·3210	0·008	0·015	0·008	86 (7)	271 (32)	86 (8)	−41 (17)	−18 (6)	12 (17)
C(12)	−0·0552	0·0370	−0·4912	0·007	0·011	0·007	72 (6)	187 (31)	47 (6)	1 (11)	−7 (5)	6 (10)
C(1′)	0·1800	0·0296	−0·3006	0·006	0·009	0·006	45 (5)	57 (23)	45 (5)	16 (8)	4 (4)	0 (8)
C(2′)	0·2649	0·1728	−0·2303	0·006	0·008	0·006	46 (5)	55 (20)	32 (5)	10 (8)	−3 (4)	−5 (7)
C(3′)	0·3897	0·0951	−0·2666	0·006	0·009	0·006	26 (4)	124 (21)	49 (5)	6 (8)	1 (4)	−11 (9)
C(4′)	0·3693	−0·1213	−0·2631	0·007	0·009	0·007	52 (6)	90 (20)	54 (6)	7 (9)	20 (5)	−13 (9)
C(5′)	0·4294	−0·2172	−0·1439	0·008	0·010	0·008	66 (7)	128 (25)	85 (8)	−10 (10)	3 (6)	6 (11)
O(*W*)	0·6149	−0·9938	−0·4604	0·005	0·009	0·006	52 (4)	250 (20)	108 (6)	32 (10)	−9 (4)	−53 (12)

and isotropic temperature factors for the hydrogen atoms were kept fixed. The *R* value decreased to 6·5%. The temperature factors for the hydrogen atoms were chosen to be approximately equal to the isotropic temperature factors of the atoms to which they are covalently bonded.

The final positional and temperature factors for non-hydrogen atoms are given in Table 1, and those of the hydrogen atoms are in Table 2. The observed and calculated structure factors are listed in Table 3.

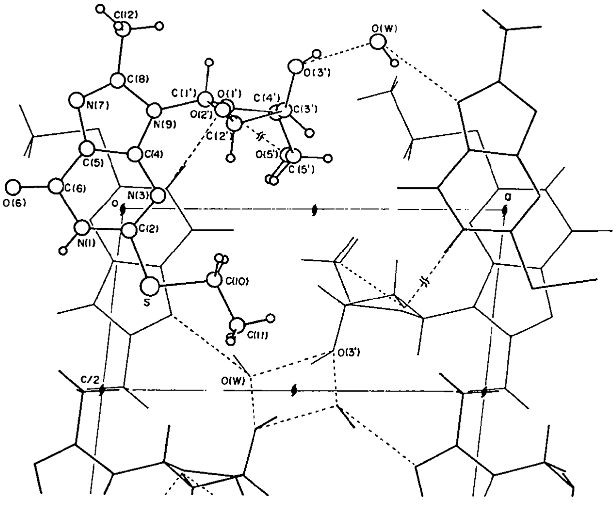

Fig. 1. The structure of 2-ethylthio-8-methylinosine viewed down the *b* axis. Dashed lines indicate probable hydrogen bonds.

Table 2. *The hydrogen-atom parameters*

The assumed temperature factors *B* are approximately equal to the isotropic temperature factors of the heavy atoms to which the hydrogen atoms are bonded.

	X	Y	Z	B
H(N1)	−0·155	−0·025	0·120	2·33
H1(C10)	0·280	−0·130	0·140	3·15
H2(C10)	0·260	0·120	0·140	3·15
H1(C11)	0·420	−0·025	0·290	3·98
H2(C11)	0·320	−0·150	0·365	3·98
H3(C11)	0·315	0·110	0·387	3·98
H1(C12)	−0·120	−0·010	−0·540	3·12
H2(C12)	0·035	0·020	0·490	3·12
H3(C12)	−0·070	0·180	−0·510	3·12
H(C1′)	0·190	0·040	−0·400	1·62
H(C2′)	0·270	0·170	−0·130	1·60
H(C3′)	0·460	0·100	−0·210	1·63
H(C4′)	0·400	−0·175	−0·337	1·73
H1(C5′)	0·380	−0·180	−0·080	2·55
H2(C5′)	0·510	−0·200	−0·150	2·55
H(O3′)	0·480	0·110	−0·400	2·39
H(O5′)	0·370	−0·450	−0·070	2·53
H(O*W*)	0·680	−0·980	−0·420	3·52

Results and discussion

Hydrogen bonding and packing

The packing of this structure and the probable hydrogen bonding are shown in Fig. 1. The base plane is stacked approximately perpendicular to the *b* axis at a distance of about 3·46 Å. The closest contacts between the rings are N(1)···N(3) = 3·36 and 3·57 Å, and S···C(5) = 3·52 and 3·53 Å. The hydrogen-bond lengths in the crystal are given in Table 4.

Table 3. *The observed and calculated structure factors*

The values have been multiplied by 10. The reflexions marked with an asterisk were given a weight other than unity.

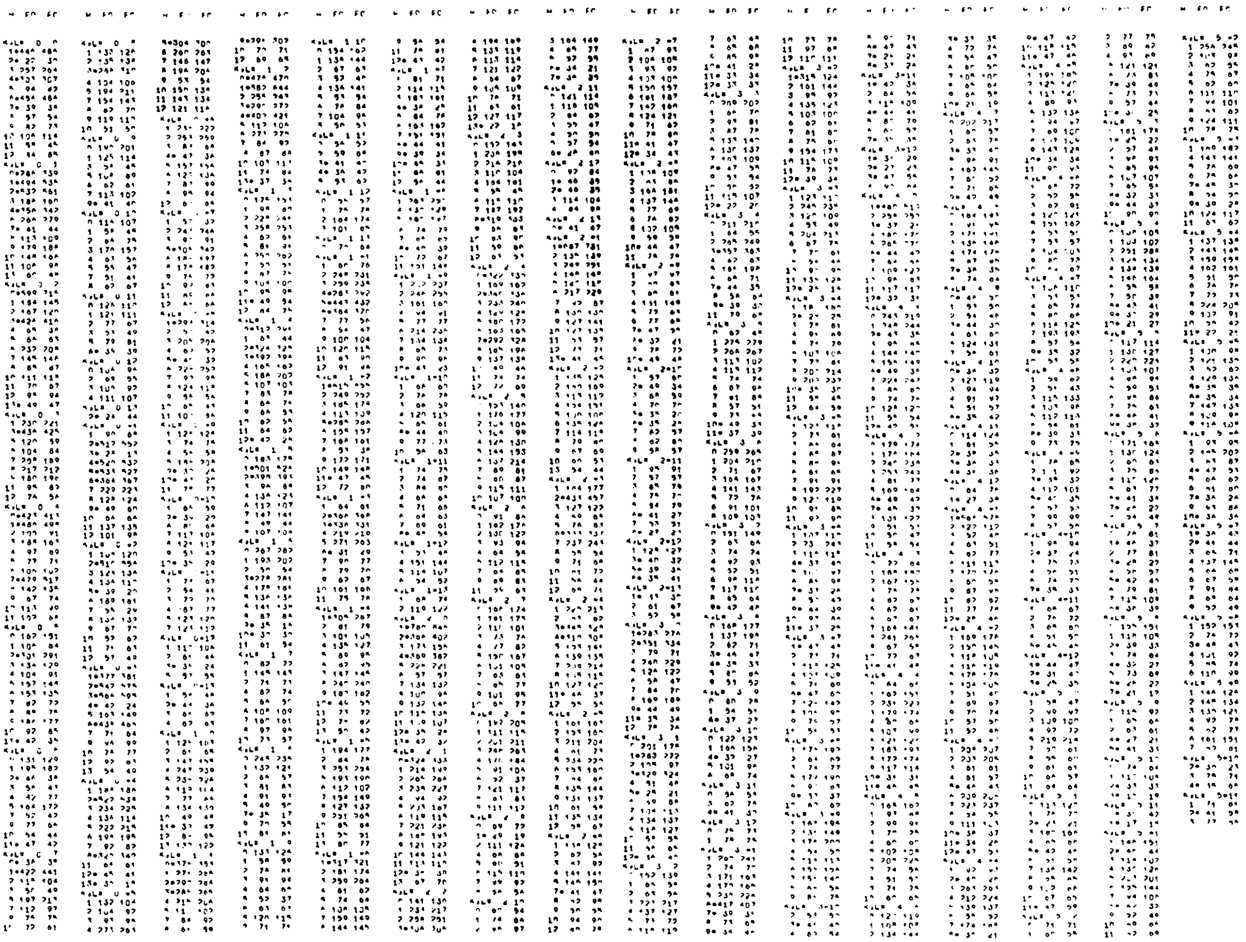

Table 4. *Hydrogen-bond lengths*

Atoms		Length
Donor	Acceptor	(Å)
N(1) (1)* · · · ·	O(2′)(3)	2·92
O(5′) (1)· · · · ·	O(6) (3)	2·69
O(2′) (1)· · · · ·	O(5′) (2)	2·71
O(*W*) (4)· · · ·	N(7) (1)	2·90
O(3′) (1)· · · · ·	O(*W*) (1)	2·68
O(*W*) (5)· · · ·	O(3′) (1)	2·86

* The value in parentheses following the atom name denotes its equivalent position in fractional coordinates, as follows: (1) x,y,z; (2) $-x,y+\frac{1}{2},-z$; (3) $-x,y-\frac{1}{2},-z$; (4) $x-1,y,z$; (5) $-x+1,y+\frac{1}{2},-z-1$.

There are two kinds of hydrogen bond, N(1)· · ·O(2′) and O(6)· · ·O(5′), between the base and the ribose units of the nucleosides related by the twofold screw axes. The hydrogen bond between O(2′) and O(5′) of the adjacent molecule links the molecules along the *b* axis. The water molecule is involved in three hydrogen bonds to N(7), O(3′) and O(3′) of different molecules The hydrogen-bond system between O(3′) and the water generates a hydrogen-bonded spiral around a twofold screw axis.

The molecular structure

The bond lengths and angles of 2-ethylthio-8-methylinosine are shown in Fig. 2. The estimated standard deviations are about 0·01 Å for bond lengths and 0·6° for bond angles. These lengths and angles are reasonable. Some torsion angles of the molecule are listed in Table 5.

The sugar-base relationship is *syn*, and the torsion angle (φ_{CN}) is 110°. Least-squares planes have been calculated for the purine and ribose rings (Table 6). The purine ring is planar within experimental error, and the ethylthio group lies approximately on the base plane. The ribose puckering is C(2′)-*endo*, C(2′) being 0·66 Å out of the least-squares plane formed by the other four atoms of the ribose.

With the notation defined by Shefter & Trueblood (1965), the torsion angles about the C(4′)–C(5′) bond are $\varphi_{OO}=62°$ and $\varphi_{OC}=182°$, and the conformation is described as *gauche–trans*. There is no intramolecular hydrogen bond between the O(5′) hydroxyl group and the N(3) purine ring nitrogen, although it is usually found in other nucleosides having the *syn* conformation.

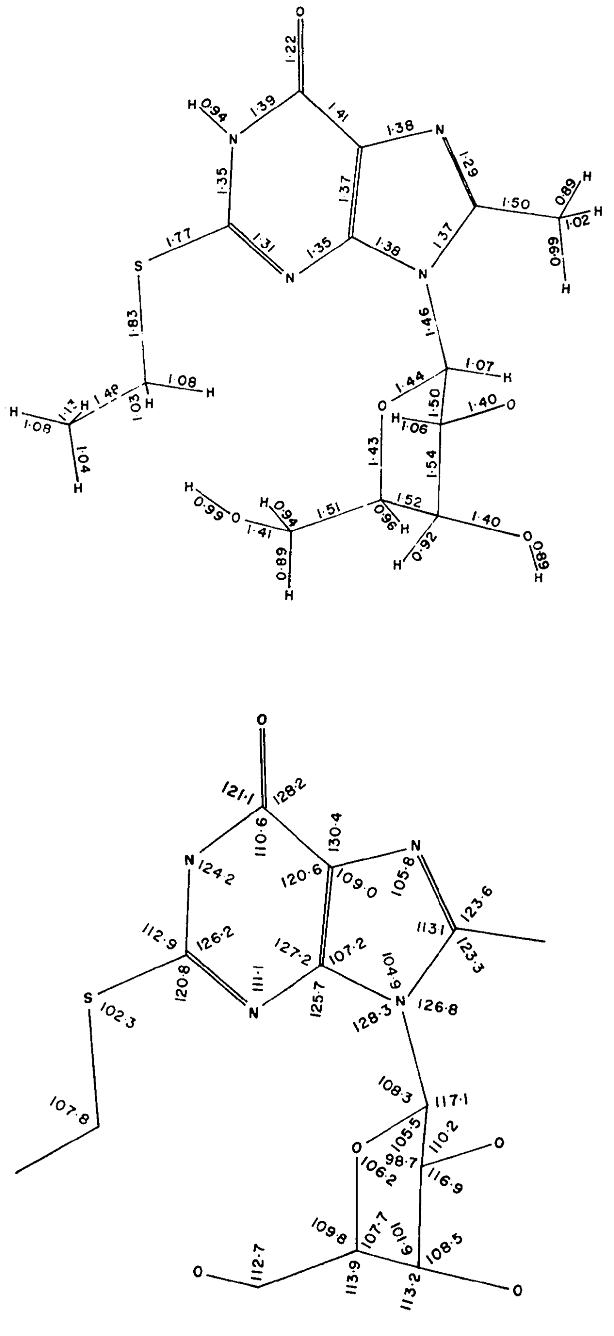

Fig. 2. The bond lengths and angles in 2-ethylthio-8-methylinosine.

Table 5. *Some torsion angles*

C(8)–N(9)–C(1′)–O(1′) ($-\varphi_{CN}$)*	−110°
N(9)–C(1)′–C(2′)–C(3)′	165
O(1′)–C(1′)–C(2′)–C(3′)	44
C(1′)–C(2′)–C(3′)–C(4′)	−38
O(2′)–C(2′)–C(3′)–O(3′)	−37
C(2′)–C(3′)–C(4′)–O(1′)	21
C(2′)–C(3′)–C(4′)–C(5′)	−101
C(3′)–C(4′)–C(5′)–O(5′) (φ_{OC})	−178
O(1′)–C(4′)–C(5′)–O(5′) (φ_{OO})	62
C(3′)–C(4′)–O(1′)–C(1′)	6
C(4′)–O(1′)–C(1′)–C(2′)	−33

* This torsion angle has the opposite sign of φ_{CN} by definition.

Table 6. *Deviations of atoms from the least-squares planes*

Deviation (Å)			
Purine ring		Ribose ring	
N(1)	0·008	O(1′)	0·036
C(2)	0·004	C(1′)	−0·023
N(3)	−0·004	C(2′)*	0·660
C(4)	−0·005	C(3′)	0·022
C(5)	−0·017	C(4′)	−0·035
C(6)	0·002		
N(7)	−0·003		
C(8)	0·016		
N(9)	−0·001		
S*	0·092		
C(10)*	−0·072		
C(11)*	−0·040		
O(6)*	−0·000		
C(12)*	0·052		
C(1′)	−0·000		

* Atoms excluded from the calculation of the least-squares plane.

Some of the significant structural parameters of the purine nucleotides and nucleosides having the *syn* conformation are listed in Table 7. All the compounds, except for the last five, exist in the *gauche–gauche* conformation about the C(4′)–C(5′) bond, and have an intramolecular hydrogen bond between N(3) and O(5′) [N(5′) in the case of 5′-methylammonium-5′-deoxyadenosine], and this hydrogen bond is considered to stabilize the *syn* conformation. In the present molecule the *syn* conformation is not stabilized by such an intramolecular hydrogen bond.

The barrier hindering the rotation about the C(4′)–C(5′) bond may be the repulsion between the O(5′) atom and the N(3) atom on the ring or the C(10) atom of the ethylthio substituent. In the *syn* conformation, close contacts of O(5′) with the methylene group [C(10) and its hydrogen atoms] during rotation about the C(4′)–C(5′) bond, occur in the *gauche* range of φ_{OC}, and these close contacts may exclude the *gauche–gauche* conformation, which is considered to be the most stable in the *syn* conformation. Similar considerations for the rotation about the glycosidic bond indicate that numerous close contacts, for example, C(12) (methyl carbon)···C(2′), C(12)···O(2′), H(C12) (hydrogen atom in methyl group)···C(2′), H(C12)···O(1′) and H(C12)···O(2′) *etc.*, occur in the *anti* range because of the presence of the 8-methyl group.

Cyclic 3′,5′-adenosine phosphate (Watenpaugh, Dow, Jensen & Furberg, 1968), inosine and guanosine (Thewalt, Bugg & Marsh, 1970), in which both *syn* and *anti* conformations exist in the same crystal, show, as Haschemeyer & Rich (1967) have concluded, that the barriers to the rotation about the glycosidic bond are not high and the base–sugar conformation (φ_{CN}) depends on its environment and the crystal packing. In the present compound, the close steric contacts introduced by the methyl group at the 8-position may be responsible for the *syn* conformation.

To date, only two structure determinations of 8-substituted purine nucleosides, 8-bromoguanosine and 8-bromoadenosine (Tavale & Sobell, 1970) have been reported, both of which have the *syn* conformation. For purine nucleotides or nucleosides without an 8-substituent, the *anti* conformation appears most frequently, but, because of its environmental condition, the *syn* conformation also appears, as shown in Fig. 3. It may be concluded, therefore, that an 8-substituted purine nucleoside or nucleotide is unable to take an *anti* conformation, and may only take the *syn* conformation. Ikehara (1970) investigated the conformation of the present compound by means of circular dichroism in an aqueous solution, and suggested that this compound is also in the *syn* conformation.

It is interesting that 2-ethylthio-8-methylinosine-5′-phosphate has no flavouring activity. It seems that this is due to the *syn* conformation of this compound. Tavale & Sobell (1970) suggested a close relationship between the rotational isomer and the activity in biochemical reactions, *i.e.*, the polymerization reaction for which the Qβ replicase and *E. coli* transcriptase enzymes are unable to utilize 8-bromoguanosine triphosphate as a substrate.

Kuninaka (1960) reported that a structural requirement of nucleotides essential to flavouring activity is the presence of a purine 5′-mononucleotide with a hydroxyl group in the 6-position, *e.g.* 5′-guanilic acid and 5′-inosinic acid. In the light of the present study, the '*anti*' conformation is another feature that should be added to the above requirements for flavouring activity.

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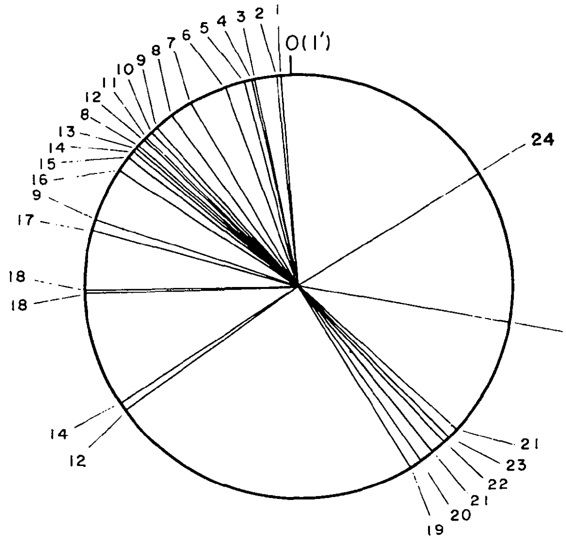

Fig. 3. The distribution of the glycosyl torsion angle φ_{CN} for the purine nucleotides and nucleosides without an 8-substituent. The torsion angles are viewed down the direction N(9)–C(1′), and are considered positive for a clockwise rotation. The compounds and torsion angles are as follows: (1) Deoxyadenosine (Watson, Sutor & Tollin, 1965), −3°. (2) Adenosine-3′-phosphate (Sundaralingam, 1966), −3·9°. (3) Adenosine (Lai & Marsh, 1972), −9·9°, and adenosine complexed with 5-bromouridine (Haschemeyer & Sobell, 1965*a*), −10°. (4) Inosine (Munns & Tollin, 1970), −10·6°. (5) Guanosine-5′-phosphate (Murayama, Nagashima & Shimizu, 1969), −12·4°. (6) Adenosine-5′-phosphate (Kraut & Jensen, 1963), −18°. (7) 3′-Deoxy-3′-(dihydroxyphosphinylmethyl)adenosine (Hecht & Sundaralingam, 1972), −28·1°. (8) Barium inosine-5′-phosphate (Nagashima & Iitaka, 1968), −34° and −46°. (9) Disodium adenosine triphosphate (Kennard, Isaacs, Motherwell, Coppola, Wampler, Larson & Watson, 1971), −39° and −69°. (10) Sodium inosine-5′-phosphate (Rao & Sundaralingam, 1969), −40·9°. (11) Disodium inosine-5′-phosphate (Nagashima & Iitaka, 1968), −43°. (12) Guanosine (Thewalt, Bugg & Marsh, 1970), −43·9° and −121·2°. (13) 5′-Bromo-5′-deoxyadenosine complexed with riboflavin (Voet & Rich, 1971), −47°. (14) Inosine (Thewalt, Bugg & Marsh, 1970), −47·8° and −121·2°. (15) Cyclic 3′,5′-adenosine monophosphate (Watenpaugh, Dow, Jensen & Furburg, 1968), −50° and 102°. (16) Adenosine moiety in adenosine-2′-uridine-5′-phosphate (Shefter, Barlow, Sparks & Trueblood, 1964), −55°. (17) Lithium 2-methylthio-inosine-5′-phosphate (Nagashima & Wakabayashi, 1974), −72°. (18) Deoxyguanosine complexed with actinomycin (Sobell & Jain, 1972), −88·6° and −89·0°. (19) 5′-Methylammonium-5′-deoxyadenosine (Saenger, 1971), 151·2°. (20) Formycin (Koyama, Maeda, Umezawa & Iitaka, 1966), 148°. (21) 6-Thioinosine (Shefter, 1968), 135° and 144°. (22) 3′-*O*-Acetyladenosine (Rao & Sundaralingam, 1970), 139·9°. (23) Deoxyguanosine complexed with 5-bromodeoxycytidine (Haschemeyer & Sobell, 1965*b*), 138°. (24) α-D-2′-Amino-2′-deoxyadenosine (Rohrer & Sundaralingam, 1970), 59·8°.

Table 7. *Some structural parameters in the purine nucleotides or nucleosides having the syn conformation*

	φ_{CN}	φ_{OO}	φ_{OC}	Ribose conformation	Reference
2-EtS-8-Me-Inosine. H_2O	110°	62°	182°	C(2')-*endo*	This work
8-Bromoguanosine. $2H_2O$	125	−65	54	C(2')-*endo*	*a*
8-Bromoadenosine	119	−74	46	C(2')-*endo*	*b*
2'-Deoxyguanosine*	138	−71	43	C(2')-*endo*	*c*
3'-*O*-Acetyladenosine	139·9	−61	58	C(2')-*endo*	*d*
6-Thioinosine, mol. 1	135	−63	55	C(2')-*endo*	*e*
mol. 2	144	−63	57	C(2')-*endo*	
5'-Methylammonium-5'-deoxyadenosine	151·2	43·0	158·3	C(2')-*endo* C(3')-*exo*	*f*
Formycine HBr	148	*gauche*	*trans*	C(2')-*endo*	*g*
Cyclic 3',5'-adenosine monophosphate, mol. II	102	*trans*	*gauche*	C(4')-*endo*	*h*
Guanosine, mol. *A*	−123·3	−51	68	C(2')-*endo*	*i*
Inosine, mol. *A*	−121·2	−55	64	C(2')-*endo*	*i*

* In the complex with 5-bromodeoxycytidine.

(*a*) Bugg & Thewalt (1969); Tavale & Sobell (1970). (*b*) Tavale & Sobell (1970). (*c*) Haschemeyer & Sobell (1965*b*). (*d*) Rao & Sundaralingam (1970). (*e*) Shefter (1968). (*f*) Saenger (1971). (*g*) Koyama, Maeda, Umezawa & Iitaka (1966). (*h*) Watenpaugh, Dow, Jensen & Furberg (1968). (*i*) Thewalt, Bugg & Marsh (1970).

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