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Acta Cryst. (1982). B**38**, 2611–2615

The Structure of *S*-8-Azaadenosyl-L-homocysteine (8-aza-SAH): A Synthetic Methyltransferase Inhibitor

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(*Received* 11 *December* 1981; *accepted* 26 *April* 1982)

Abstract

8-aza-SAH ($C_{13}H_{19}N_7O_5S.H_2O$, M_r = 403·4), a synthetic methyltransferase inhibitor, crystallizes in the orthorhombic space group $P2_12_12_1$, with Z = 8 and a = 5·154 (1), b = 17·672 (3), c = 38·019 (7) Å, V = 3463 (1) Å^3; $F(000)$ = 1696, D_x = 1·55 g cm^{-3}, μ(Cu $K\alpha$) = 19·9 cm^{-1}. The structures of the two independent molecules in the asymmetric unit were determined by locating the two S atoms in the Patterson map, and superposition on all symmetry-related S positions. The final R after block-diagonal least-squares refinement is 0·091, for 1706 observed intensities (diffractometer data) and 488 parameters. The conformations of the two independent molecules are different.

Introduction

Methyltransferases such as hydroxyindole-*O*-methyltransferase (HIOMT) depend upon the cofactor *S*-adenosyl-L-methionine (SAM) for their activity. During the methylation reaction, a methyl substituent on the S atom of SAM is transferred to substrate, producing *S*-adenosyl-L-homocysteine (SAH). The reaction is inhibited by SAH and various analogues of SAH, such as 8-aza-SAH (Fig. 1).

Borchardt and his colleagues at the University of Kansas have synthesized a series of these SAH analogues, and have tested them for inhibitory activity on four methyltransferase enzymes, in order to determine what portions of the SAH molecule are necessary for inhibition (Borchardt, Huber & Wu, 1974, 1976; Borchardt, 1977). The amino acid moiety, the S atom, and the exocyclic amine on the base are strictly required by all the enzymes tested. However, HIOMT requires N(3) and N(7) in addition to N(6) on the adenine ring of the inhibitor (Fig. 1). It was found that 8-aza-SAH, in which N(8) replaces C(8) in the adenine ring of SAH, is a selective inhibitor for only one of the methyltransferase enzymes tested, HIOMT.

The conformations of these inhibitors are being investigated by X-ray diffraction with the aim of deducing the geometric requirements imposed by the enzyme. The results of the X-ray analysis of the structure of 8-aza-SAH show that the solid-state conformations of the two independent molecules of 8-aza-SAH are quite different from those of SAH (Shieh & Berman, 1982).

Experimental

8-aza-SAH was crystallized by dissolving a sample provided by Dr R. T. Borchardt in 5% MeOH (1·5 mg

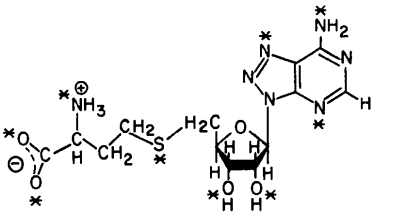

Fig. 1. Molecular formula of the 8-aza-SAH dipolar ion. Atoms which have been found to be necessary for inhibition of HIOMT are starred (Borchardt, Huber & Wu, 1974).

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0567-7408/82/102611-05$01.00

cm^{-3}), and diffusing the solution with 10% MeOH. A colorless, needle-shaped crystal of size 0·4 × 0·02 × 0·01 mm was mounted on the end of a glass fiber. Cell parameters (*Abstract*) were obtained by a least-squares analysis of 15 centered reflections obtained from diffractometer measurements.

Table 1. *Fractional positional parameters* (×10⁴) *and equivalent isotropic temperature factors of the non-hydrogen atoms of* 8-aza-SAH

	x	*y*	*z*	B_{eq} (Å²)
N(1)*A*	−2 (28)	6719 (6)	10237 (3)	3·5
C(2)*A*	−1715 (34)	6373 (7)	10448 (3)	2·7
N(3)*A*	−1997 (30)	5642 (6)	10518 (3)	3·5
C(4)*A*	26 (36)	5253 (7)	10366 (4)	3·1
C(5)*A*	1891 (33)	5532 (7)	10136 (3)	2·3
C(6)*A*	1973 (38)	6352 (7)	10071 (3)	3·6
N(6)*A*	3671 (28)	6658 (6)	9847 (3)	3·2
N(7)*A*	3522 (27)	4970 (7)	10026 (3)	3·1
N(8)*A*	2719 (27)	4334 (6)	10167 (3)	3·4
N(9)*A*	586 (27)	4477 (6)	10374 (3)	2·6
C(1′)*A*	−1012 (35)	3859 (7)	10510 (3)	3·0
O(1′)*A*	−794 (23)	3821 (5)	10880 (2)	2·7
C(2′)*A*	66 (28)	3104 (7)	10379 (3)	1·4
O(2′)*A*	−914 (21)	2925 (5)	10028 (2)	2·3
C(3′)*A*	−903 (28)	2551 (7)	10660 (3)	0·9
O(3′)*A*	−3539 (21)	2370 (5)	10607 (2)	2·0
C(4′)*A*	−636 (32)	3026 (7)	10997 (3)	2·1
C(5′)*A*	1971 (35)	2977 (8)	11168 (3)	3·1
S*A*	2820 (10)	2013 (2)	11322 (1)	2·9
C(6′)*A*	2047 (36)	2055 (8)	11795 (3)	3·4
C(7′)*A*	−840 (35)	2077 (8)	11855 (3)	3·0
C(8′)*A*	−1479 (34)	2043 (8)	12260 (3)	2·7
N(8′)*A*	−557 (28)	2774 (6)	12440 (3)	2·8
C(9′)*A*	−109 (37)	1373 (8)	12432 (4)	3·1
O(9′)*A*	1827 (25)	1498 (5)	12651 (3)	4·4
O(10′)*A*	−681 (28)	718 (5)	12324 (3)	5·8
W(1)	1534 (32)	−2 (8)	11703 (3)	9·5
W(2)	−4331 (31)	−203 (7)	12115 (3)	7·4
N(1)*B*	11157 (27)	4161 (6)	9251 (3)	2·9
C(2)*B*	12863 (40)	4421 (8)	9001 (3)	3·7
N(3)*B*	12952 (30)	5122 (6)	8868 (3)	3·2
C(4)*B*	11155 (35)	5565 (7)	9017 (3)	2·6
C(5)*B*	9315 (32)	5359 (7)	9259 (3)	2·5
C(6)*B*	9321 (33)	4601 (7)	9387 (3)	2·4
N(6)*B*	7675 (28)	4335 (6)	9625 (3)	2·5
N(7)*B*	7780 (30)	5977 (7)	9338 (3)	3·9
N(8)*B*	8571 (29)	6532 (6)	9131 (3)	3·8
N(9)*B*	10657 (29)	6311 (6)	8942 (3)	3·5
C(1′)*B*	11950 (40)	6770 (8)	8672 (4)	4·6
O(1′)*B*	12178 (24)	7519 (5)	8784 (3)	4·1
C(2′)*B*	10388 (40)	6735 (8)	8323 (4)	5·0
O(2′)*B*	12488 (31)	6713 (6)	8054 (3)	7·0
C(3′)*B*	9085 (44)	7505 (9)	8328 (4)	5·4
O(3′)*B*	8835 (29)	7804 (6)	7992 (2)	5·9
C(4′)*B*	10602 (35)	8038 (8)	8577 (4)	3·5
C(5′)*B*	8903 (38)	8471 (9)	8839 (3)	4·1
S*B*	6705 (11)	9113 (3)	8619 (1)	4·5
C(6′)*B*	8448 (40)	10040 (8)	8661 (4)	4·9
C(7′)*B*	10579 (35)	10156 (8)	8399 (4)	3·5
C(8′)*B*	9793 (32)	10164 (7)	8002 (4)	2·4
N(8′)*B*	12071 (29)	10062 (6)	7788 (3)	3·4
C(9′)*B*	8245 (33)	10937 (8)	7935 (3)	3·0
O(9′)*B*	6016 (24)	10991 (6)	8003 (3)	4·6
O(10′)*B*	9757 (25)	11421 (6)	7780 (3)	4·9

Data were collected on a Syntex $P\bar{1}$ four-circle diffractometer with Cu $K\alpha$ radiation and a highly oriented graphite monochromator (6·3° take-off angle). The θ–2θ scan technique (bisecting mode) was used to a 2θ limit of 130° (sin $\theta/\lambda = 0{\cdot}588$ Å^{-1}), at a variable scan speed (2·0 to 24° min^{-1}) which was dependent upon intensity. Three check reflections measured every 100 reflections showed no decay in intensity with time. Scan-to-background time was 3·0. Of 3425 unique reflections, 1706 with $I \geq 2\sigma(I)$ [where $\sigma(I)$ was determined from counting statistics] were considered observed. All the data were corrected for Lorentz and polarization factors (LP) and put on an absolute scale with a Wilson plot. Values of $\sigma(F)$ were calculated as $\sigma(F) = \frac{1}{2}\sigma(I)/(\text{LP} \times I)^{1/2}$. No absorption correction was applied.

The structure was solved by an automated Patterson search procedure, which led to the location of the two S positions. Superposition on all symmetry-related S positions and successive electron density maps revealed the positions of the remaining atoms.

The atomic positions and anisotropic thermal parameters of all nonhydrogen atoms were refined by a block-diagonal least-squares procedure. The quantity minimized was $\sum w(|F_o| - |F_c|)^2$ where the weights, w, were $1/\sigma^2(F)$. Atomic scattering factors for non-hydrogen atoms were those listed by Cromer & Mann (1968), and for H atoms those given by Stewart, Davidson & Simpson (1965). Correction for the anomalous scattering of all atoms was applied (*International Tables for X-ray Crystallography*, 1974). The computer programs used are part of the Crystallographic Program Library written at the Institute for Cancer Research (H. L. Carrell & H.-S. Shieh, undated).

Of the 42 H atoms in the structure, 11 were found in the difference electron density maps, and the positions of 19 more were calculated. These positions were not refined. H atoms on sugar hydroxyl groups, the amino nitrogen (molecule *B*), and the water molecules were not found in the difference map, and their positions could not be calculated. The final value of $R = \sum(||F_o| - |F_c||)/\sum|F_o|$ was 0·091; the average shift in the atomic parameters was less than one half their standard deviations. The final electron density difference map contained no interpretable features; the highest peak (0·48 e Å^{-3}) was more than 2·0 Å from any atom. The fractional coordinates of the atoms are given in Table 1.* The distances and angles are shown in Fig. 2.

* Lists of structure factors, anisotropic thermal parameters and H-atom parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36916 (11 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

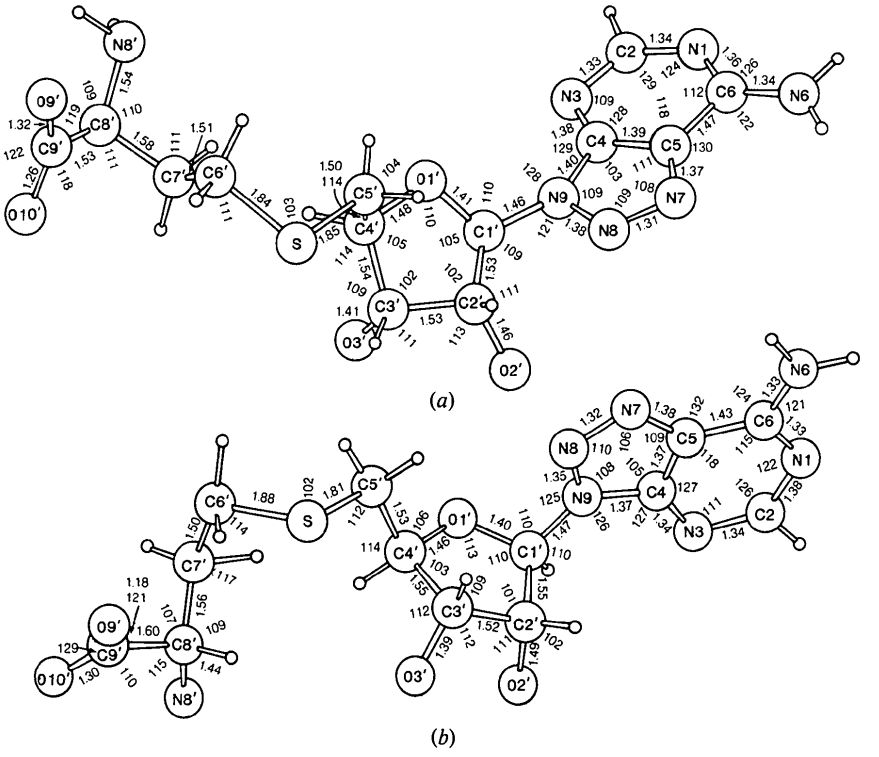

Fig. 2. Bond lengths (Å) and angles (°) in (*a*) molecule *A*, and (*b*) molecule *B* of 8-aza-SAH. Mean standard deviations are 0·02 Å and 1°.

Table 2. *Comparison of the observed torsion angles* (°) *for* 8-aza-SAH *and* SAH (*Shieh & Berman*, 1982)

The mean standard deviation is 2°.

		8-aza-SAH		SAH	
		Molecule *A*	Molecule *B*	Molecule *A*	Molecule *B*
N(8)–N(9)–C(1′)–O(1′)	(χ)	114	42	50	6
C(4′)–O(1′)–C(1′)–C(2′)	(τ_0)	−22	8	−30	4
O(1′)–C(1′)–C(2′)–C(3′)	(τ_1)	37	−18	33	−26
C(1′)–C(2′)–C(3′)–C(4′)	(τ_2)	−38	20	−24	42
C(2′)–C(3′)–C(4′)–O(1′)	(τ_3)	25	−17	8	−41
C(1′)–O(1′)–C(4′)–C(3′)	(τ_4)	−2	5	13	23
O(3′)–C(3′)–C(4′)–C(5′)		155	104	128	83
C(3′)–C(4′)–C(5′)–S		−63	−64	63	41
C(4′)–C(5′)–S–C(6′)		−99	−99	−160	−91
C(5′)–S–C(6′)–C(7′)		71	80	82	−91
S–C(6′)–C(7′)–C(8′)		175	61	179	163
C(6′)–C(7′)–C(8′)–C(9′)		−52	71	−66	70
C(6′)–C(7′)–C(8′)–N(8′)		68	−164	58	−169
C(7′)–C(8′)–C(9′)–O(9′)		110	−82	128	101
C(7′)–C(8′)–C(9′)–O(10′)		−61	102	−54	−84

Results and discussion

The two molecules in the asymmetric unit show considerable variation in torsion angles between themselves and in comparison with the two independent molecules of SAH (Table 2). Molecule *A* has C(2′)-*endo* sugar pucker, and a 'high *anti*' glycosyl (χ) torsion angle of 114°, while molecule *B* has a C(2′)-*exo*–C(3′)-*endo* sugar pucker and χ angle of 42° (Table 2). Thus, 8-aza-SAH (like SAH) follows the pattern observed in purine nucleosides of higher χ angles for C(2′)-*endo* sugars than for C(3′)-*endo* sugars (Altona & Sundaralingam, 1972). However, for both 8-aza-SAH conformations, the χ angles are considerably higher than the angles for the same ribose conformation in SAH. A high-*anti* χ angle (110°) is also observed in the crystal structure of formycin, in which there is an N atom substituted for C(8)

(Prusiner, Brennan & Sundaralingam, 1973). Another feature of the 8-aza-SAH structure is the relatively rare *gauche*$^-$ torsion angle around the C(4′)–C(5′) bond in both molecules. The higher χ values and the *gauche*$^-$ conformation both serve to increase the distance between the S atom and the electronegative N(8).

The amino acid portion of each 8-aza-SAH molecule can be described as a substituted methionine. In the crystal the conformational features of the amino acid portion of molecule *A* resemble those of the second residue of methionylmethionine (Stenkamp & Jensen, 1975); molecule *B* is similar to L-methionine (Torii & Iitaka, 1973). As has been observed in most amino acids, the C^α atom [C(8′)] and the carboxyl group are coplanar while the amino nitrogen [N(8′)] deviates by 0·1 and 0·5 Å in molecules *A* and *B*, respectively.

The crystal structure consists of two types of hydrogen-bonded columns; one contains the bases and ribose rings and the other contains the amino acids, sugars and water molecules (Table 3, Fig. 3). The adenine portions of each independent molecule are hydrogen bonded to each other through their N(6) and N(7) atoms to form slightly twisted base pairs. These base pairs form a herringbone pattern with perpendicular pairs linked by N(6)–N(1) hydrogen bonds (Fig. 3). The sugar molecules for this column are hydrogen bonded to one another *via* their hydroxyl groups. The other column contains a cyclic sugar–amino acid network [O(3′)*B* → O(9′)*A* and N(8′)*A* → O(2′)*B*] as well as other direct and indirect (*i.e.* mediated by water) amino acid–amino acid and amino acid–sugar hydrogen bonds.

Table 3. *Hydrogen-bond distances* (Å) *in* 8-aza-SAH

Average standard deviations are 0·02 Å for bond lengths and 1° for bond angles.

i	*j*	Distance *i*–*j* (Å)	Symmetry (*j*)
Base–base			
N(6)*A*	N(7)*B*	3·11	*x*,*y*,*z*
N(6)*B*	N(7)*A*	2·86	*x*,*y*,*z*
N(6)*A*	N(1)*A*	2·97	$x+\frac{1}{2}, -y+1\frac{1}{2}, -z+2$
Base–sugar			
N(6)*B*	O(2′)*A*	3·01	$x+1, y, z$
O(3′)*A*	N(1)*B*	2·76	$x-1\frac{1}{2}, -y+\frac{1}{2}, -z+2$
Sugar–sugar			
O(2′)*A*	O(2′)*A*	2·99	$x+\frac{1}{2}, -y+\frac{1}{2}, -z+2$
	O(3′)*A*	2·75	$x+\frac{1}{2}, -y+\frac{1}{2}, -z+2$
Amino acid–sugar			
N(8′)*A*	O(2′)*B*	2·96	$-x+1\frac{1}{2}, -y+1, z+\frac{1}{2}$
	O(3′)*B*	2·88	$-x+\frac{1}{2}, -y+1, z+\frac{1}{2}$
O(3′)*B*	O(9′)*A*	2·86	$-x+1\frac{1}{2}, -y+1, z-\frac{1}{2}$
Amino acid–amino acid			
N(8′)*A*	O(9′)*B*	2·87	$x-\frac{1}{2}, -y+1\frac{1}{2}, -z+2$
N(8′)*A*	O(10′)*B*	2·92	$x-1\frac{1}{2}, -y+1\frac{1}{2}, -z+2$
N(8′)*B*	O(9′)*B*	2·74	$x+1, y, z$
N(8′)*B*	O(9′)*A*	2·86	$-x+1\frac{1}{2}, -y+1, z-\frac{1}{2}$
	O(10′)*A*	2·91	$-x+1\frac{1}{2}, -y+1, z-\frac{1}{2}$
Water interactions			
W(1)	O(10′)*A*	2·91	*x*,*y*,*z*
W(1)	N(3)*B*	2·86	$x-1\frac{1}{2}, -y+\frac{1}{2}, -z+2$
W(2)	*W*(1)	2·67	$x-1, y, z$
W(2)	O(10′)*A*	2·61	*x*,*y*,*z*
N(8′)*B*	*W*(2)	2·93	$-x+\frac{1}{2}, -y+1, z$
O(2′)*B*	*W*(2)	2·90	$x+1\frac{1}{2}, -y+\frac{1}{2}, -z+2$

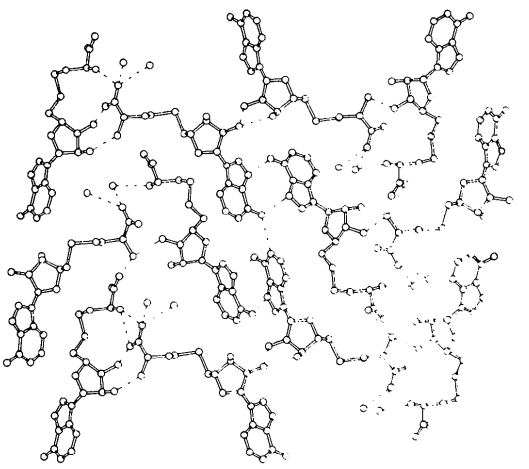

Fig. 3. The crystal structure shown projected down the *a* axis.

The charge distribution in the two molecules cannot be firmly established, since not all the H atoms were found and the standard deviations of the bond lengths and bond angles are too high. A detailed consideration of the hydrogen-bonding scheme eliminated several possibilities. Even though the angles at N(1) are larger than expected for a non-protonated adenine (Voet & Rich, 1970), N(1)*A* appears to be a hydrogen-bond acceptor from N(6)*A*, and cannot be protonated. The bond lengths in carboxyl *B* could indicate an uncharged carboxyl. However, this assumption requires a disordered water molecule in order to construct a consistent hydrogen-bonding scheme. Therefore, the best conclusion is that the two molecules are dipolar ions, with negatively charged carboxyl and positively charged amino groups, as is commonly observed in other amino acid crystal structures (Hamilton, Frey, Golič, Koetzle, Lehmann & Verbist, 1972).

This research was supported by grants from NIH GM-21589, CA-09035, CA-22780, CA-06927, RR-05539 and an appropriation from the Commonwealth of Pennsylvania. We are grateful to Ron Borchardt for supplying the materials for this study.

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Acta Cryst. (1982). B**38**, 2615–2620

Structure of the 4:1 Inclusion Compound between Deoxycholic Acid and (*E*)-*p*-Dimethylaminoazobenzene

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(*Received* 24 *March* 1982; *accepted* 27 *April* 1982)

Abstract

The title compound, $4C_{24}H_{40}O_4.C_{14}H_{15}N_3$, M_r = 1795·6, crystallizes in space group $P2_12_12_1$ with a = 25·676 (8), b = 13·731 (3), c = 7·160 (2) Å, V = 2524·3 Å^3, Z = 4, D_m = 1·17, D_x = 1·18 Mg m^{-3}, μ(Mo $K\alpha$) = 4·3 mm^{-1} (m.p. 479–480 K). The structure has been refined to $R = 0{\cdot}09_3$ and $R_w = 0{\cdot}09_9$ for 1810 observed reflections with $I > 2\sigma(I)$. The crystal packing is characterized by an assembly of pleated antiparallel bilayers, nearly equal to that of the acetophenone–choleic acid complex, which give rise to canals filled by *p*-dimethylaminoazobenzene molecules. The guest molecules, located by van der Waals energy calculations, run along **c** and have their long axes approximately parallel to **c**. The C(5), C(6), C(19), C(20), C(21) and C(22) atoms, together with their H atoms, are engaged in strong interactions with the atoms of *p*-dimethylaminoazobenzene.

Introduction

The bile acid 3α,12α-dihydroxy-5β-cholan-24-oic acid (deoxycholic acid, DCA) gives rise to host lattices of the 'canal' type in which several guest components can be accommodated, especially in the orthorhombic phases (D'Andrea, Fedeli, Giglio, Mazza & Pavel, 1981; Giglio, 1981, and references therein). In order to throw light on the possibilities of formation of the DCA

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