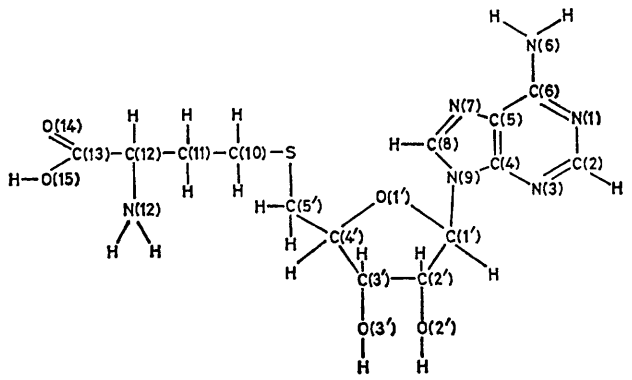
Three-dimensional *X*-Ray Crystal Structure of *S*-Adenosyl-L-homocysteine, a Potent Inhibitor of *S*-Adenosylmethionine-dependent Methyltransferases

By TOSHIMASA ISHIDA,* HIROKO MORIMOTO, and MASATOSHI INOUE
(*Osaka College of Pharmacy*, 2-10-65 *Kawai, Matsubara-City, Osaka* 580, *Japan*)
and TAKAJI FUJIWARA and KEN-ICHI TOMITA
(*Faculty of Pharmaceutical Sciences, Osaka University*, 133-1 *Yamadakami, Suita-City, Osaka* 565, *Japan*)

Summary In order to obtain information on the probable spatial arrangement of the biologically important *S*-adenosylmethionine, the *X*-ray crystal structure of *S*-adenosyl-L-homocysteine has been determined; the conformations of the two independent molecules are *anti* for the glycosidic bond, C(3′)-*endo* or C(1′)-*exo*-C(2′)-*endo* sugar puckering, *gauche-gauche* for the orientation about the C(4′)–C(5′) bond, and extended in the L-homocysteine moiety.

A GENERAL feature of most *S*-adenosylmethionine (SAM)-dependent methyltransferases is the inhibition produced by the demethylated product *S*-adenosyl-L-homocysteine (SAH) (**1**).[1] In view of this inhibitory characteristic and



(1)

the equivalent levels of SAH and SAM in tissue,[2] it has been suggested that SAH is a regulator of biological methylation.[3] The conformational characteristics of SAH may provide information as to the stereochemistry of SAM and the substrate specificity of methyltransferases.

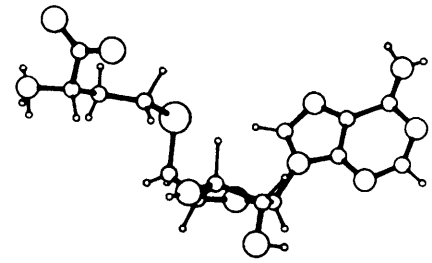
SAH crystallized as thin needles from aqueous solution. A single crystal (0·04 × 0·5 × 0·2 mm) was sealed in a thin-walled glass capillary tube containing mother liquor to prevent deterioration by loss of water of crystallization. *X*-Ray data were recorded with a Rigaku four-circle diffractometer with graphite-monochromated Cu-K_α radiation using the ω–2θ scan procedure.

Crystal data: $C_{14}H_{20}N_6O_5.3H_2O$, M = 438·46, monoclinic, space group $C2$, a = 45·910(1), b = 5·688(1), c = 15·620(1) Å, β = 100·22(1)°, U = 4014·3(1) Å^3, D_m = 1·446(1), D_c = 1·451 g cm^{-3}, Z = 8. A total of 3665 independent reflections with $I \geqslant 3\sigma(I)$ were measured. The structure was solved by direct methods using the symbolic addition procedure[4] and refined by block-diagonal least-squares to R = 0·12.† Hydrogen atoms were not included in the refinement but they were fixed at their expected positions.

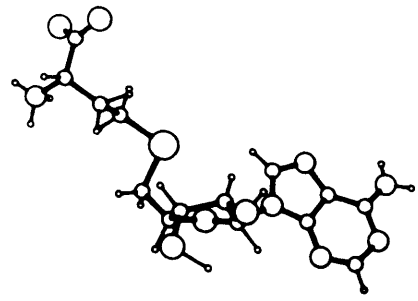
TABLE. Torsion angles (°) of the L-homocysteine moiety of SAH.

	(A)	(B)
C(4')–C(5')–S–C(10)	−91.5	−156.6
C(5')–S–C(10)–C(11)	−89.0	82.4
S–C(10)–C(11)–C(12)	167.9	178.3
C(10)–C(11)–C(12)–C(13)	66.2	−66.7
C(10)–C(11)–C(12)–N(12)	−173.7	60.5
C(11)–C(12)–C(13)–O(14)	105.2	127.6
C(11)–C(12)–C(13)–O(15)	−74.8	−52.9
N(12)–C(12)–C(13)–O(14)	−12.9	0.1
N(12)–C(12)–C(13)–O(15)	167.1	179.5

There are two crystallographically independent molecules (A) and (B) in the structure, and these exhibit significant differences in bonding parameters; the maximum differences between the two molecules are 0·14 Å for the bond length C(4')–C(5') and 11° for the angle C(4')–C(5')–S. The molecular conformations are illustrated in the Figure. The most significant difference between molecules (A) and (B) is in the puckering of the ribose ring: molecule (A) is in the commonly occuring C(3')-*endo* form, while molecule (B) has a C(1')-*exo*-C(2')-*endo* form. The latter form is quite unusual in comparison with other crystal structures of adenosine derivatives. The pseudorotational phase angle



(A)



(B)

FIGURE. The conformation of SAH molecules: both molecules (A) and (B) are viewed parallel to the plane of C(1')–O(1')–C(4').

P is 24·0° and the maximum amplitude of puckering ϕ_m is 32·3° in molecule (A), with corresponding values of 137·5° and 33·2° in molecule (B). The torsion angle, χ, O(1')–C(1')–N(9)–C(8) is 7·7° for (A) and 50·8° for (B). The orientation about the glycosidic bond is therefore *anti* for both molecules, which is commonly observed in crystal structures of nucleotides.[5] The orientations about the exocyclic C(4')–C(5') bond for (A) and (B) are: S–C(5')–C(4')–O(1') = −67·1° and −60·6°, S–C(5')–C(4')–C(3') = 45·9° and 61·5°, respectively, *i.e.*, the two independent SAH molecules have *gauche–gauche* conformations about the C(4')–C(5') bond, unlike that of the related compound 5'-methylthioadenosine in which the conformation is *trans–gauche*.[6] The molecular conformations found in the solid state here are in good agreement with the preferred conformations observed in aqueous solution by ^{1}H n.m.r. spectroscopy.[7]

The L-homocysteine moieties of molecules (A) and (B) are in extended conformations, although the torsion angles of both molecules are significantly different, as seen in the Table. This conformational difference in the L-homo-

† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

cysteine moieties seems to be mainly due to their different hydrogen-bonding modes between the α-amino- or α-carboxy-groups and the neighbouring adenine base: the N(12) and O(14) atoms of (A) are hydrogen-bonded to N(7) and (N6), respectively [N(12)···N(7) = 2·88, O(14)···N(6) = 2·93 Å], while these atoms in (B) are hydrogen-bonded to N(1) and N(6), respectively [N(12)···N(1) = 2·87, O(14)···N(6) = 3·04 Å].

(*Received*, 30*th March* 1981; *Com.* 361.)

[1] R. T. Borchardt, 'The Biochemistry of S-Adenosylmethionine,' Columbia University Press, New York, 1977.
[2] F. Salvatore, R. Utili, and V. Zappia, *Anal. Biochem.*, 1971, **41**, 16; J. Hoffman, *ibid.*, 1975, **68**, 552.
[3] T. Deguchi and J. Brachas, *J. Biol. Chem.*, 1971, **246**, 3175; S. J. Kerr, *ibid.*, 1972, **247**, 4248; J. K. Coward, D. L. Bussolotti, and C. D. Chang, *J. Med. Chem.*, 1974, **17**, 1286; C. S. G. Pugh, R. T. Borchardt, and H. O. Stone, *Biochemistry*, 1977, **16**, 3928.
[4] J. Karle and I. L. Karle, *Acta Crystallogr.*, 1966, **21**, 849.
[5] C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, 1972, **94**, 8205.
[6] N. Borkakoti and R. A. Palmer, *Acta Crystallogr., Sect. B.*, 1978, **34**, 867.
[7] H. Follmann and G. Gremels, *Eur. J. Biochem.*, 1974, **47**, 187.