which each is bonded. This feature is also observed in Figure 2b, where the signal at 1 ppm is sharper than the one at 13 ppm. An exact assignment of C-4 and C-5 has not been made because the observed line broadening may depend on other factors.[17,18]

The ^{13}C CP-MAS spectra of pyrimidine bases, viz., cytosine, uracil, and thymine, taken with Cu^{2+} doped samples, are presented in Figure 3, while those taken in the solution state are shown in Figure 4. The measured ^{13}C chemical shifts are given in Table II. The solution spectra of the nucleic acid bases have apparently not been reported, although purine and pyrimidine nucleosides have been studied in detail before.[20] We have included the solution spectra because of the fundamental importance of these compounds. The assignment of the ^{13}C resonances in the three bases proceeds directly following those in pyrimidine nucleosides.[20] The assignments from low to high field are: cytosine·H_2O C-4, C-2, C-6, and C-5; uracil C-4, C-2, C-6, and C-5; and thymine C-4, C-2, C-6, C-5, and CH_3.

Cytosine in fact exists as a monohydrate, as it does in the present study, when recrystallized from water.[21] Thus a shorter T_1^H, compared with a $T_1^H > 100$ s expected for a nonhydrate form,[8] is expected and is observed in our study (Table I). This is consistent with an efficient proton relaxation mechanism eminating from a twofold flipping of water molecules.[22] Cu^{2+} doping is seen to have a great effect as T_1^H is reduced to millisecond order and a CP-MAS spectrum easily obtained. In cytosine·H_2O the resonances of carbon bonded to nitrogen are either split (C-4) or broadened (C-2 and C-6), while the nonbonded one (C-5) remains sharp (Figure 3a). The CP-MAS spectrum of uracil (Figure 3b) also exhibits similar features in the resonances, C-4, C-2, and C-6 being broadened and C-5 being sharp as in cytosine·H_2O. Thymine, an important constituent of DNA, differs from uracil only in the presence of a methyl group in the 5-position of the ring and its CP-MAS spectrum is essentially the same as that of uracil except for the appearance of methyl resonance at 115 ppm. As seen, the resonances of C-5 and CH_3 are quite sharp as compared to the others. The broadening of the resonances of carbons bonded to nitrogen is therefore a common feature of the pyrimidine bases as well, and an explanation to this is to be sought in the ^{14}N quadrupole effects[16-18] discussed earlier.

It is also seen that though the assignment of the ^{13}C resonances is the same as in solution, the chemical shifts are not the same (Table II). Such differences are thought to be due to conformational variations in the solid state.

Conclusions

Paramagnetic doping can be used effectively to reduce T_1^H in the solid state and so one can obtain CP-MAS spectra routinely. At moderately low Cu^{2+} content (Cu^{2+}/host molecule molar ratio $\lesssim$0.4%) the carbon resonances do not shift nor are they broadened to any significant amount. The spectra so obtained exhibit features characteristic of the solid state, such as the absence of exchange processes, the broadening of ^{13}C resonances of the carbons bonded to nitrogen etc. It is suggested that T_1^H need not be reduced very much (millisecond order) as one has to use a recycle delay of about 5-10 s in most CP-MAS experiments. A reduction in T_1^H to about 2 s should be convenient for obtaining CP-MAS spectra. For most practical cases, it is good enough to dope with only 0.04% of paramagnetic impurity, and this amount of paramagnetic impurity definitely will not cause any shift or broadening in the ^{13}C NMR spectra. Also, the dissolved oxygen in the sample (at such low concentration) will not cause any confusion in the ^{13}C spectra as the effect will be negligibly small, obviating the need to use sealed samples.

Acknowledgment. This work was generously supported by Natural Sciences and Engineering Research Council of Canada. We wish to thank Drs. E. E. Burnell and J. B. Farmer for experimental assistance.

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S-Adenosyl-L-methionine and *S*-Adenosyl-L-homocysteine, an NMR Study[1]

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Abstract: An analysis of the 360-MHz ^1H NMR spectra of the title compounds in ^2H_2O is presented. The ^3J values for the ribose vicinyl protons of (*S*)-adenosyl-L-methionine are consistent with a predominantly C_3-exo conformation and with one highly favored gauche-anti conformation about the C_4-$C_{5'}$ bond. The corresponding ^3J values for *S*-adenosyl-L-homocysteine imply a similar C_3-exo ribose ring conformation, but the orientation about the C_4-$C_{5'}$ bond is distributed between two gauche-anti rotamers. The methionine side chain of *S*-adenosyl-L-methionine has approximately equal populations of rotational isomers about the C_α-C_β and C_β-C_γ bonds, whereas the side chain of *S*-adenosyl-L-homocysteine exhibits a conformational preference for the gauche-anti conformations about the C_α-C_β bond. ^1H and ^{13}C NMR spectra of commercially available samples of (-)-*S*-adenosyl-L-methionine consistently reveal the presence of a small amount of the (+)-sulfonium diastereomer. This assignment was confirmed by synthesis of both the ^1H and ^{13}C methyl derivatives of *S*-adenosyl-L-homocysteine. Arguments are presented to explain the failure of previous workers to detect (+)-*S*-adenosyl-L-methionine in biological preparations.

S-Adenosyl-L-methionine (SAM) is ubiquitous and uniquely versatile. The extensive repertoire of this important cofactor includes methyl group transfer to oxygen, nitrogen, sulfur, or carbon in the chemical modification of members of every major class of biomolecule, including nucleic acids, proteins, lipids, and carbohydrates.[2] The coproduct of the methyl-transfer reaction is usually *S*-adenosyl-L-homocysteine (SAH).

Few extensive studies of the conformations of SAM and SAH have been reported. Klee and Mudd[3] examined the ORD curves

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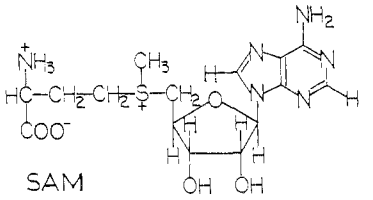
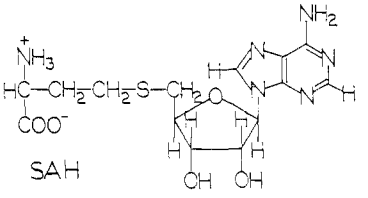


SAM



SAH

of SAM and some model compounds in the range 220–260 nm and came to the tentative conclusion that the adenine ring is in the anti conformation. Talalay et al.[4] determined the absolute configuration at the sulfonium center of a pair of diastereomeric *S*-(carboxymethyl)-L-methionine salts by X-ray crystallography. Degradation of biologically active (−)-*S*-adenosyl-L-methionine to *S*-(carboxymethyl)-L-methionine, under experimental conditions designed to retain the sulfonium center configuration, lead to the diastereomer with the *S* configuration at the sulfonium center. (The biologically most active form of SAM is designated as (−)-SAM because of its lower specific optical rotation.[5]) Attempts at X-ray crystallographic studies of intact SAM have been unsuccessful. The chemical shifts of the purine protons of SAM and SAH, obtained at 100 MHz, have been reported.[6]

This paucity of spectroscopic data is remarkable, considering the importance of SAM, and it must stem from the reported instability of SaM in solution.[7] However, we have found that decomposition is not a significant impediment to a detailed NMR investigation of this cofactor. By carefully controlling the *p*D and using low concentrations of purified SAM, we have been able to obtain FT-NMR spectra of SAM in ^2H_2O and Me_2SO-d_6. The stability of SAM at various pH values has been ascertained by Parks and Schlenk[8] and Borchardt.[9] In the pH range 4–7, SAM is inert for at least several hours at room temperature, but heating at 100 °C results in the formation of 5′-(methylthio)adenosine and α-amino-γ-butyrolactone which is subsequently converted to homoserine. In 0.1 N mineral acid, hydrolysis of the glycosidic bond occurs along with the above reactions, resulting in the formation of adenine, 5′-(methylthio)ribose, and homoserine.[10] In alkaline solution, hydrolysis is rapid but confined to the glycosidic bond; adenine and (*S*)-ribosylmethionine are the result.[9] Our NMR results confirm these observations, and the spectra of the usual hydrolysis products at pH 4–7 appear only after heating the samples. We report here a complete analysis of the 360-MHz ^1H NMR spectra of SAM and SAH obtained under mild conditions.

Experimental Section

^1H NMR spectra at 25 °C were obtained on a Nicolet 360-MHz pulsed FT-NMR spectrometer (at the University of California, Davis) and on a Brucker HXS-360 spectrometer (at Stanford University). The free induction decays, with up to 16K points, were apodized by exponential multiplication prior to Fourier transformation, and the final spectra were base-line straightened. The residual water line was suppressed from the spectra either by application of a 180°–τ–.0° pulse sequence with an interval such that the observing pulse is applied as the solvent magnetization passes through zero or by progressive saturation of the water.[11] ^{13}C NMR spectra were obtained on a Nicolet 200-MHz spectrometer (at U.C., Davis) operating at 50.3 MHz and using 16K points. ^{13}C spin–lattice relaxation times were measured by using a 180°–τ–90° inversion recovery technique where τ is the time interval between the 180 and 90° pulses. Computer simulations of the five and six spin systems by the LOACN3 algorithm[12] and least-squares iteration converged with the observed spectral lines within a RMS deviation of 0.03.

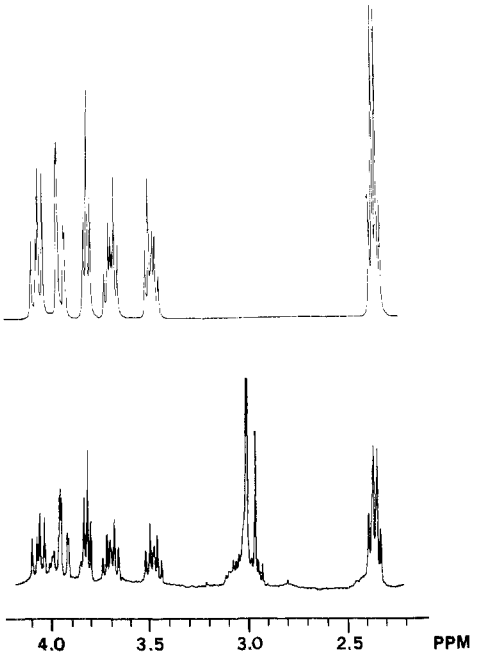


Figure 1. Lower trace: 360-MHz spectrum of the 5′, methionine, and *S*-methyl protons of 0.025 M SAM in ^2H_2O. Upper trace: Computer simulation of the spin–spin coupled protons from lower spectrum.

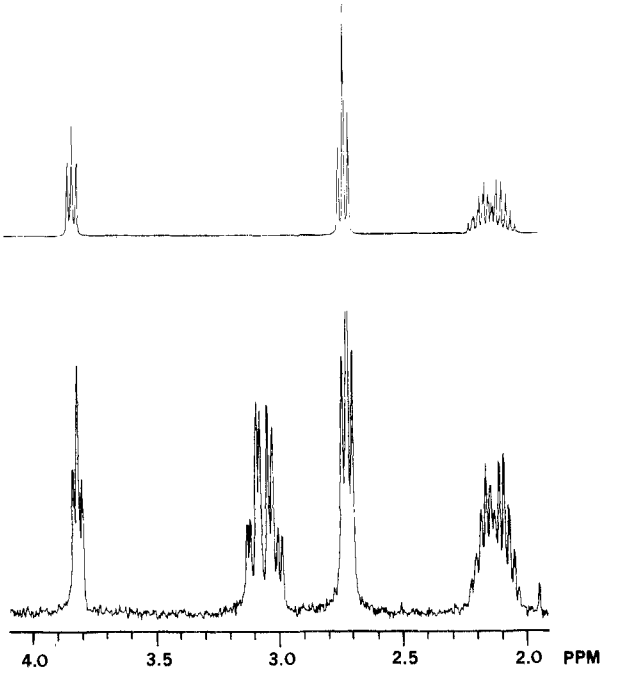


Figure 2. Lower trace: 360-MHz spectrum of the 5′- and methionine protons of 0.025 M SAH in ^2H_2O. Upper trace: Computer simultation of methionine protons of SAH.

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Table I. NMR Parameters[a,b] for SAM and Related Sulfonium Compounds

	chemical shifts,[d] δ										
compd[d]	H_α	H_β	H_γ	H_{SCH_3}	$H_{5'5''}$	$H_{4'}$	$H_{3'}$	$H_{2'}$	$H_{1'}$	H_2	H_8
SAM I^-	3.81	2.36	3.48 3.70	2.96 3.01	3.92 4.06	4.60	4.86	4.97	6.12	8.27	8.29
MMet I^-	3.89	2.39	3.45 3.54	2.97							
DMTA I^-				2.92 2.94	3.79 3.85	4.48	4.52	4.84	6.05	8.26	8.28

	coupling constants (*J*), Hz										
compd[d]	$^3J_{\alpha\beta}$	$^3J_{\alpha\beta'}$	$^2J_{\beta\beta'}$	$^3J_{\beta\gamma}$	$^2J_{\gamma\gamma'}$	$^3J_{1'2'}$	$^3J_{2'3'}$	$^3J_{3'4'}$	$^3J_{4'5'}$	$^3J_{4'5''}$	$^2J_{5'5''}$
SAM I^-	6.6	6.6	0	7.4	−11.2	4.4	5.3	<0.5	9.5	2.4	−12.8
MMet I^-	6.5	6.5	0	8.7	−14.6						
DMTA I^-						4.4	4.9	<0.5	8.8	3.1	−13.6

[a] 0.025 M solutions in ^2H_2O (pD 3.4). [b] Observed at 360 MHz. [c] Shifts in ppm from internal TSP ±0.005 ppm. [d] SAM I^-, *S*-adenosyl-L-methionine iodide (Sigma); MMet I^-, *S*-methylmethionine iodide; DMTA I^-, 5′-(dimethylthio)adenosine iodide.

Table II. NMR Parameters[a,b] for SAH and Related Thioethers

	chemical shifts,[c] δ										
compd[d]	H_α	H_β	H_γ	H_{SCH_3}	$H_{5'5''}$	$H_{4'}$	$H_{3'}$	$H_{2'}$	$H_{1'}$	H_2	H_8
SAH	3.85	2.19	2.73		2.89 2.97	4.38	4.45	4.87	6.09	8.25	8.35
Met	3.87	2.13 2.21	2.65	2.14							
Aden					3.83 3.91	4.32	4.44	4.80	6.04	8.19	8.30

	coupling constants (*J*), Hz										
compd[d]	$^3J_{\alpha\beta}$	$^3J_{\alpha\beta'}$	$^3J_{\beta\beta'}$	$^3J_{\beta\gamma}$	$^2J_{\gamma\gamma'}$	$^3J_{2'3'}$	$^3J_{3'4'}$	$^3J_{4'5'}$	$^3J_{4'5''}$	$^2J_{5'5''}$	
SAH	4.7	6.8	−14.1	7.5	0	5.2	5.0	<0.5	4.9	6.4	−14.1
Met	5.3	6.9	−14.9	7.6	0						
Aden						6.1	5.3	4.3	2.7	3.5	−12.9

[a] 0.025 M solutions in ^2H_2O (pD 3.4). [b] Observed at 360 MHz. [c] Shifts in ppm from internal TSP ±0.005 ppm. [d] SAH, *S*-adenosyl-L-homocysteine; Met, L-methionine; Aden, adenosine.

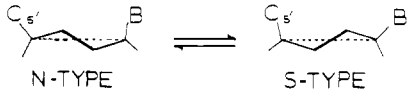
^1H chemical shifts were measured vs. internal TSP, sodium 3-(trimethylsilyl)tetradeuteriopropionate. Routine samples were twice lyophilized from ^2H_2O with the final concentration adjusted to 0.025 M. The pD (pD = pH + 0.4) was adjusted to 3.4 with the dropwise addition of ^2HCl solution.

S-Methyl-L-methioninesulfonium iodide, 5′-(methylthio)adenosine, *S*-adenosyl-L-homocysteine, (−)-*S*-adenosyl-L-methionine iodide and -chloride were purchased from Sigma Chemical Co. The latter compound was purified by ion exchange on Amberlite IRC-150 with 0.1–4 M HCl eluant. (−)-*S*-Adenosyl-L-methionine bisulfate was from Boehringer Mannheim. (±)-*S*-Adenosyl-L-methionine iodide was prepared by reaction of *S*-adenosyl-L-homocysteine with methyl iodide as described by De La Haba et al.[6] 5′-(Dimethylthio)adenosine iodide was similarly prepared.

Results and Discussion

All the proton resonances of SAM and SAH can be assigned unambiguously based on chemical shift values, spin multiplicities, decoupling experiments, and direct comparisons to the previously assigned spectra of the model compounds: adenosine, L-methionine, *S*-methyl-L-methionine iodide, and 5′-(dimethylthio)adenosine iodide. The assignments have been confirmed by computer simulation. The chemical shifts for *S*-adenosyl-L-methionine iodide, *S*-methylmethionine iodide, and 5′-(dimethylthio)adenosine iodide are given in Table I. A 360-MHz NMR spectrum of the methionine side chain of SAM iodide is given in Figure 1. The chemical shifts for SAH, L-methionine, and adenosine are in Table II, and a spectrum of the side chain of SAH is given in Figure 2. The following discussion is focused first on the conformational information, provided by vicinal proton coupling constants, which permits a comparison between SAM and SAH, and second by a discussion of the NMR evidence concerning the stereochemistry of the sulfonium methyl group of SAM.

S-Adenosyl-L-methionine (SAM). The ribose ring protons constitute a six-spin ABKMNX system where A = $H_{5''}$, B = $H_{5'}$, K = $H_{4'}$, M = $H_{3'}$, N = $H_{2'}$, and X = $H_{1'}$. Only the $H_{1'}$, $H_{2'}$ coupling interaction is first order. The observed coupling constants are as follows: $^3J_{1'2'}$ = 4.4, $^3J_{2'3'}$ = 5.3, and $^3J_{3'4'}$ = 0 Hz. Although authors have differed in their choice of Karplus parameters for estimating ribose ring dihedral angles from observed coupling constants,[13-18] all reasonable sets of parameters support the conclusion that the ribose ring of SAM is predominantly in the C_3-exo conformation, and we need not enter into the debate over the respective merits of the parameter sets. A number of approximate methods for estimating the relative contribution of the N- and S-type conformers in a rapid equilibrium situation have been proposed.[19] Davies and Danyluk[20] have shown that the



equilibrium constant for the N ⇌ S equilibrium can be calculated directly from the ratio of observed $^3J_{1'2'}$ and $^3J_{3'4'}$ values without invoking particular magnitudes for J(N) and J(S) if the effect of electronegativity on J is negligible.

$$K_{eq} = S/N = {}^3J_{1'2'}/{}^3J_{3'4'} \quad (1)$$

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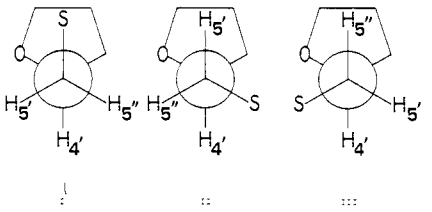
Within the confines of this reasonable assumption, our data clearly indicate the marked predominance of an S-type conformation such as $C_{3'}$-exo. Moreover, purine nucleosides and nucleotides in anti-type conformations prefer S-type conformers.[13] In SAM, the $C_{3'}$-exo conformation tips the $H_{3'}$ proton away from the bulky sulfonium center at $C_{5'}$.

The $H_{4'}$, $H_{5'}$, and $H_{5''}$ protons constitute a three-spin ABX system with coupling constants $^3J_{4'5'} = 9.5$, $^3J_{4'5'} = 2.4$, and $^2J_{5'5''} = -12.8$ Hz. An estimation of the rotameric distribution about the $C_{4'}$–$C_{5'}$ bond shows that one of the gauche–anti conformations (II or III) is very highly favored. This estimate is based on the assumptions that the observed coupling constant is equal to the weighted average coupling constant of the three staggered rotomers and that each rotomer J value can be estimated by the Karplus expression (eq 2).[13,21,22] Rotamer I is populated to about 2% and

$$J = J_0 \cos^2 \theta - 0.28 \text{ Hz}$$
$$J_0 = 9.27 \text{ Hz} \quad 0 \le \theta \le \pi/2$$
$$J_0 = 10.36 \text{ Hz} \quad \pi/2 < \theta \le \pi \qquad (2)$$

either II or III by 93%. We cannot tell from the coupling constant alone whether it is rotamer II or III that is so profoundly favored; however, CPK molecular models indicate that there is less steric interaction between the methyl group and the purine and ribose ring systems in rotamer III. Our estimate of the conformational preference for rotamer III is considerably larger than that found in most rotational isomer distributions. If rotamer III is the preferred ground-state orientation about the $C_{4'}$–$C_{5'}$ bond, then the (*S*)-methyl group can project to the less hindered side making it more accessible to nucleophiles. This may have mechanistic significance.[23]



The α protons of the SAM methionine side chain appear as a triplet ($J_{\alpha\beta} = 6.6$ Hz), the β protons as a quartet, and the γ protons as a 12-line multiplet (Figure 1). Homonuclear decoupling of the α protons leaves the β-proton signal as a triplet ($^3J_{\beta\gamma} = 7.4$ Hz) and the γ resonance unchanged. Irradiation of the β protons gives a singlet for the α resonance and an AB quartet ($^2J_{\gamma\gamma'} = -11.2$ Hz) for the γ-protons. Hence the β protons are equivalent but the γ protons, next to the assymetric sulfonium group, are nonequivalent. Computer simulation of the spectrum provided confirmation of these chemical shift and coupling constant assignments. Theoretical spectra were calculated with the LOACN3 algorithm within a RMS deviation of 0.03. Because the same coupling constant is obtained for both α–β pairs, there must be approximately equal populations of rotational isomers about the C_α–C_β bond. This is not the case for SAH and methionine. The γ-protons differ in chemical shift, but the β-γ coupling can also be described by only one coupling constant, implying free rotation about this bond as well.

***S*-Adenosyl-L-homocysteine (SAaH).** The ribose ring protons comprise a six-spin system analogous to that of SAM. The observed coupling constants are as follows: $^3J_{1'2'} = 5.21$, $^3J_{2'2'} = 5.0$, $^3J_{3'4'} = 0$, $3J_{4'5'} = 4.85$ $^3J_{4'5''} = 6.44$, and $^2J_{5'5''} = -14.13$ Hz. These values are consistent with a $C_{3'}$-exo ribose ring conformation. The rotational equilibrium about the $C_{4'}$–$C_{5'}$ bond also exhibits a preference for an anti–gauche orientation although, unlike SAM, both such orientations are significantly populated (35% and 56%). The gauche–gauche orientation is populated to about 9%.

Table III. Chemical Shifts of the *S*-Methyl Protons of Various *S*-Adenosyl-L-methionine Salts[a]

	iodide	chloride	bisulfate
(−)	3.01	3.00	3.01
(+)	2.96	2.96	2.97

[a] Chemical shifts (in ppm relative to TSP) for the (−) and (+) diastereomers 0.03 M in ^2H_2O (pH 3.4).

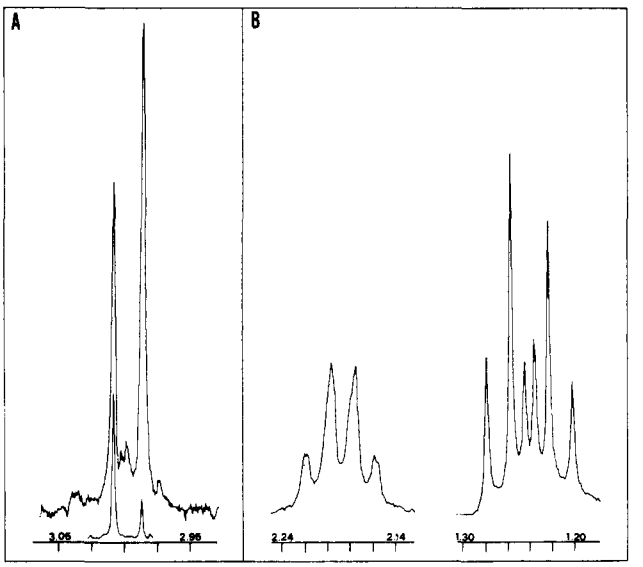
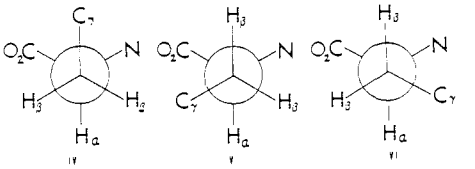


Figure 3. (A) *S*-Methyl proton resonances of synthetic (upper trace) and "biosynthetic" (lower trace) SAM. (B) *S*-Ethyl proton resonances of biosynthetic *s*-adenosyl-L-ethionine.

The α protons of the SAH homocysteine side chain appear as a 4-line multiplet ($^3J_{\alpha\beta} = 4.68$ and $^3J_{\alpha\beta'} = 6.80$ Hz), the β protons as a 24-line multiplet, and the γ protons as a triplet (Figure 2). Irradiation of the γ protons decouples the β-proton signal to eight lines and leaves the α-resonance unchanged. The β protons are nonequivalent ($^2J_{\beta\beta'} = -14.1$ Hz) whereas the γ protons have equivalent chemical shifts ($J_{\beta\gamma} = 7.45$ Hz). There is a preference for the two anti conformations about the C_α–C_β bond. The gauche–gauche conformer (IV) is populated to 8%, and the two gauche–anti conformers (V and VI) at 33% and 59% or vice versa.



The $\beta\gamma$ proton coupling is fully described by just one coupling constant; hence the C_β–C_γ bond is freely rotating in SAH. The purine ring orientation in SAM and SAH was not investigated.

Sulfonium Methyl Group. The functional portion of the SAM molecule is the sulfonium methyl group. The sharp singlet at 2.96 ppm can clearly be attributed to the methyl protons both by its chemical shift and by the absence of coupling. However, we found that the sharp singlet attributed to the *S*-methyl resonance is always accompanied by a second smaller upfield singlet regardless of the commercial source, counterion, or solvent origin (Table III). Our samples of (−)-SAM were stable at room temperature, and the relative intensities of these two peaks for a freshly prepared sample did not change over the course of several hours. Moreover, purification of SAM by column ion exchange chromatography (Amberlite-IRC-150) did not change their relative intensities (82% major component). Having accounted for all other observable spectral features, we are forced to attribute the upfield peak to the *S*-methyl protons of the "unnatural" sulfonium diastereomer (+)-*S*-adenosyl-L-methionine and the downfield one to the (−)

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diastereomer. This is confirmed by our observation that both peaks appear in the ^1H NMR spectrum of (±)-SAM prepared by the reaction of SAH with methyl iodide in formic and acetic acid.[5] The starting material did not have any extraneous resonances and was stable in cold formic acid. Moreover, the spectrum of purified (±)-SAM indicates that the (+) diastereomer is the major component (66%, see Figure 3). We also found that the ^{13}C spectrum of synthetic (±)-*S*-adenosyl-L-methionine-*methyl*-^{13}C prepared with $^{13}CH_3I$ has two intense ^{13}C resonances, at 24.93 and 24.73 ppm. The chemical shifts are consistent with those found for methylsulfonium compounds,[24] the C–H coupling constants (J_{HC} = 150 Hz) are equal, and the two ^{13}C methyl resonances have equivalent T_1 values, 300 ms. The 360-MHz ^1H NMR spectrum of *S*-adenosyl-L-ethionine of biological origin (iodide salt from Sigma) indicates two *S*-ethyl groups (Figure 3).

The more upfield peak cannot be attributed to methyl iodide or methanol because of its chemical shift and its presence even after purification. It cannot be due to any known degradation product because these would exhibit additional peaks which were not observed. A diastereomer epimeric about the methionine α carbon can be ruled out because the SAH used to synthesize (±)-SAM was cleanly the L epimer. Moreover, Schlenk has shown that SAM biosynthesized by using racemic methionine is still exclusively *S*-adenosyl-L-methionine.[25] There has been no report of the spontaneous racemization at the sulfonium center of the (–) diastereomer, and the structurally related *S*-(carboxymethyl)-L-methioninesulfonium salt racemizes at the sulfonium center only after prolonged heating ($t_{1/2}$ = 24 h, 60 °C).[4] Talalay et al. did observe that degradation of *S*-adenosyl-L-methionine-methyl-^{14}C, prepared enzymatically, yielded about 10% of some radioactive impurity, chiral at sulfur but with the opposite sulfonium configuration.[4]

We cannot say whether the (+) diastereomer in commercial samples is the result of some reaction during purification or whether both diastereomers are biosynthesized under the conditions used by the supplier.[26] However, the widely held view that the naturally occurring cofactor is only the (–) diastereomer is based upon the assumption that synthetic (±)-SAM is an equal mixture of both sulfonium diastereomers. *Figure 3 shows this is not the case.* De La Haba et al.[6] and Zappia, Zydek-Cwick, and Schlenk[27] found that synthetic (±)-SAM exhibited about half the activity of SAM of biological origin and, by applying the reasonable assumption that the (–)-SAM was active and the (+) form inactive, concluded that biological SAM was entirely the (–) form. If samples of biological origin contain 10–20% of the (+) diastereomer (see Table III) and if the synthetic samples contain an excess of the (+) diastereomer (60–70%), then the relative activity of the synthetic mixture would still be approximately half that of the biological SAM.

Comparison between SAM and SAH. The two cofactors have nearly equivalent ribose ring coupling constants and both clearly have $C_{3'}$-exo ring puckerings. The slight differences in observed coupling constants are real and probably imply differences in the extent of ring deformation and dynamics, but no more can be said with certainty. SAM exhibits one highly favored gauche–anti conformation about the $C_{4'}$–$C_{5'}$ bond whereas SAH is distributed between both gauche–anti rotamers. In almost all other nucleosides and mononucleotides, the ribose ring has been shown to prefer a $C_{2'}$-endo conformation with the $C_{4'}$–$C_{5'}$ bond in a gauche–gauche orientation.[13,28] The different steric demands of the methionine and homocysteine side chains must account for the different conformations of SAM and SAH. The methionine side chain of SAM undergoes free rotation about both the C_α–C_β and the C_β–C_γ bonds whereas SAH reveals a preference for the gauche–anti rotamers about the C_α–C_β bond. In this regard we point out that rotation is free about both side chain bonds in SAM and (*S*)-methylmethionine iodide whereas rotation about the C_α–C_β bonds of methionine and homocysteine encounters a significant torsional barrier.[29]

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(29) Rotation about the C_α–C_β bond of the methyl ester of *S*-methylmethionine iodide is hindered and the γ protons are nonequivalent: $^3J_{\alpha\beta}$ = 5.5, $^3J_{\alpha\beta'}$ = 7.9, $^2J_{\beta\beta'}$ = –14.9, $^3J_{\beta\gamma}$ = 5.9, and $^2J_{\gamma\gamma'}$ = –19.0 Hz. This suggests that electrostatic interaction between the carboxylate group and the sulfonium center is not the dominant factor in determining the side chain mobility.