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Potential Inhibitors of *S*-Adenosylmethionine-Dependent Methyltransferases. 3. Modifications of the Sugar Portion of *S*-Adenosylhomocysteine

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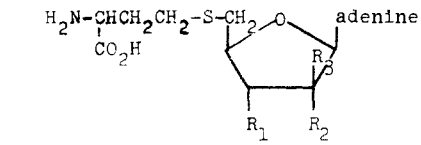
Structural analogs of *S*-adenosyl-L-homocysteine (L-SAH), with modification in the ribose portion of the molecule, have been synthesized and their abilities to inhibit catechol *O*-methyltransferase (COMT), phenylethanolamine *N*-methyltransferase (PNMT), histamine *N*-methyltransferase (HMT), and hydroxyindole *O*-methyltransferase (HIOMT) have been investigated. From these studies it was concluded that, in general, the 2′-hydroxyl and 3′-hydroxyl groups of the ribose moiety of SAH play crucial roles in the binding of this molecule to most methyltransferases. However, several interesting exceptions to this strict structural specificity have been observed. While *S*-3′-deoxyadenosyl-L-homocysteine produced no inhibition of HMT and HIOMT, it produced strong inhibition of the transmethylation catalyzed by PNMT and COMT. Likewise, *S*-2′-deoxyadenosyl-L-homocysteine and *S*-5′-[9-(arabinofuranosyl)adenyl]-L-homocysteine had little or no effect of COMT, HMT, and HIOMT but were potent inhibitors of PNMT. The significance of these data relative to the nature of the SAH binding sites and the potential for *in vivo* differential inhibition of methyltransferases will be discussed.

S-Adenosyl-L-homocysteine (SAH)‡ produces strong product inhibition of most *S*-adenosylmethionine (SAM)-dependent methyltransferases.[1] In the preceding papers of this series,[2,3] we described the synthesis and enzymatic evaluation of SAH analogs with modifications in the homocysteine or base portions of the SAH molecule. From the results of these studies[2,3] it was concluded that there exist at least four functional groups on SAH, which play a primary role in its binding to methyltransferases. These points of attachment appear to be the terminal carboxyl, the terminal amino and the sulfur atom of the homocysteine portion, and the 6-amino group of the adenine moiety. In an effort to further elucidate the nature of the intermolecular forces involved in enzymatic binding of SAH, we have synthesized a series of SAH derivatives with minor modifications in the sugar portion of this molecule (Chart I). Coward and coworkers[4,5] previously prepared analogs in which the ribose moiety of SAH was replaced by a cyclopentyl group, a 2,3-dihydroxycyclopentyl group, or a five-carbon acyclic bridge. However, these analogs were nearly inactive as inhibitors of SAM-dependent methyltransferases. In the present study we have made very minor changes in the ribose moiety of SAH in an effort to elucidate the important structural features of this portion of SAH in its binding to methyltransferases. Using these sugar-modified SAH analogs (Chart I) as probes of the active sites of COMT, PNMT, HMT, and HIOMT, we have delineated the contribution of the ribose moiety in the enzymatic binding of SAH, which is the subject of this paper.

Chart I. Sugar-Modified Analogs of SAH Synthesized to Probe the Binding Sites on COMT, PMNT, HMT, and HIOMT



Compd	R_1	R_2	R_3
SAH	OH	OH	H
2′-deoxy-SAH	OH	H	H
3′-deoxy-SAH	H	OH	H
SAraAH	OH	H	OH

Experimental Section

Melting points were obtained on a calibrated Thomas-Hoover Uni-Melt and were corrected. Microanalyses were conducted on an F & M Model 185 C, H, N analyzer, The University of Kansas, Lawrence, Kan. Unless otherwise stated, the ir, nmr, and uv data were consistent with the assigned structures. Ir data were recorded on a Beckman IR-33 spectrophotometer, nmr data on a Varian Associates Model T-60 spectrophotometer (TMS), and uv data on a Cary Model 14 spectrophotometer. Scintillation counting was done on a Beckman LS-150 scintillation counter. Tlc were run on Analtech silica gel GF (250 μ) or Avicel F (250 μ). Spots were detected by visual examination under uv light and/or ninhydrin for compounds containing amino moieties.

†This work was done during the tenure of an Established Investigatorship of the American Heart Association.

‡Abbreviations used are SAM, *S*-adenosyl-L-methionine; SAH, *S*-adenosyl-L-homocysteine; 2′-deoxy-SAH, *S*-2′-deoxyadenosyl-L-homocysteine; 3′-deoxy-SAH, *S*-3′-deoxyadenosyl-L-homocysteine; SAraAH, *S*-5′-[9-(arabinofuranosyl)adenyl]-L-homocysteine; COMT, catechol *O*-methyltransferase (E.C. 2.1.1.6); PNMT, phenylethanolamine *N*-methyltransferase (E.C. 2.1.1); HMT, histamine *N*-methyltransferase (E.C. 2.1.1.8); HIOMT, hydroxyindole *O*-methyltransferase (E.C. 2.1.1.4); K_{is}, inhibition constant for the slope.

Materials. SAM-$^{14}CH_3$ (New England Nuclear, 55.0 mCi/mmol) was diluted to a concentration of 10 μCi/ml and stored at −20°F. SAM iodide (Sigma) was stored as a 0.01 *M* aqueous stock solution. Phosphate buffers were prepared as 0.5 *M* stock solutions.

The following compounds were commercially available from the indicated sources: 2′-deoxyadenosine, 3′-deoxyadenosine, and *S*-benzyl-L-homocysteine (Sigma). 1′-β-D-Arabinofuranosyladenine was a gift from Dr. H. Wood, Drug Research and Development, Division of Cancer Treatment, NCI.

***S*-2′-Deoxyadenosyl-L-homocysteine (2′-Deoxy-SAH).** 5′-*O*-*p*-Toluenesulfonyl-2′-deoxyadenine was prepared from 2′-deoxyadenosine and *p*-toluenesulfonyl chloride according to the procedure previously described by Robins, *et al.*[6,7] The 5′-tosylate (435 mg, 1.07 mmol) was condensed with *S*-benzyl-L-homocysteine (216 mg, 96 mmol) in Na and liquid NH_3 according to the previously described procedure.[2,3,8] The crude 2′-deoxy-SAH was purified by thick-layer chromatography on cellulose (Analtech, 1000 μ) eluting with EtOH–H_2O (3:1). The product was removed from the cellulose by extraction with H_2O followed by lyophilization. The lyophilized product was homogeneous on tlc [cellulose, EtOH–H_2O (3:2)] and was crystallized (H_2O–acetone) to yield 240 mg (68%): mp 191°; nmr (D_2O) δ 7.93, 7.77 (2 s, 2 H, C_2-H, C_8-H), 6.00 (t, 1 H, $C_{1'}$-H), 3.80 (m, 1 H, $C_{3'}$-H), 4.20 (m, 1 H, $C_{4'}$-H), 3.48 (t, 1 H, C_α-H), 2.10–2.77 (m, 6 H, $C_{2'}$-H_2, $C_{5'}$-H_2, and C_γ-H_2), and 1.33–2.10 (m, 2 H, C_β-H_2). *Anal.* ($C_{14}H_{20}O_4N_6S \cdot H_2O$) C, H, N.

***S*-3′-Deoxyadenosyl-L-homocysteine (3′-Deoxy-SAH).** 3′-Deoxyadenosine (50 mg, 0.18 mmol) was dissolved in 4 ml of dry pyridine, freshly distilled from barium oxide, and the resulting solution was cooled to *ca.* 0–5° in an ice–salt bath. Freshly recrystallized *p*-toluenesulfonyl chloride (55 mg, 0.28 mmol) was added in portions over a 1-hr period, after which the solution was kept for 8 hr at 5–10° and another 4 hr at ambient temperature. The reaction mixture was poured into an ice–water–$CHCl_3$ mixture and the $CHCl_3$ layer was separated and washed with cold 1.0 *N* H_2SO_4, cold saturated $NaHCO_3$, and ice–H_2O. The $CHCl_3$ solution was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The concentrated $CHCl_3$ solution was added dropwise to cold hexane and the white solid which formed was filtered and recrystallized ($CHCl_3$) to yield 30 mg (41%) of the desired 5′-*O*-*p*-toluenesulfonyl-3′-deoxyadenine: mp 158–160°; nmr (DMSO-d_6) δ 8.10 (s, 2 H, C_2-H, C_8-H), 7.69 (d, 2 H, phenyl), 7.27 (d, 2 H, phenyl), 5.87 (s, 1 H, $C_{1'}$-H), 4.60 (br, 1 H, $C_{2'}$-OH), 4.53 (m, 2 H, $C_{2'}$-H, $C_{4'}$-H), 4.03–4.37 (m, 2 H, $C_{5'}$-H), 2.31 (s, 3 H, $-CH_3$), and 1.92–2.30 (m, 2 H, $C_{3'}$-H_2). Spectral data were consistent with the proposed structure, but an accurate analysis could not be obtained because of the instability of the compound when recrystallization was attempted.

5′-*O*-*p*-Toluenesulfonyl-3′-deoxyadenine (30 mg, 0.074 mmol) was condensed with *S*-benzyl-L-homocysteine (25 mg, 0.148 mmol) in Na and liquid NH_3 as described above for 2′-deoxy-SAH. The crude 3′-deoxy-SAH was purified by thick-layer chromatography on cellulose (Analtech, 1000 μ), double developing with EtOH–H_2O (3:1). The product was removed from the cellulose by extraction with H_2O followed by lyophilization to yield 10 mg (37%). The product was homogeneous on tlc [cellulose, EtOH–H_2O (3:2)] and was crystallized (water–acetone) to yield 3′-deoxy-SAH: mp 211° dec; nmr (D_2O) δ 7.93, 7.97 (2 s, 2 H, C_2-H, C_8-H), 5.78 (d, 1 H, $C_{1'}$-H), 4.23 (m, 2 H, $C_{2'}$-H, $C_{4'}$-H), 3.43 (t, 1 H, C_α-H), 2.47–2.78 (m, 2 H, $C_{5'}$-H_2), 2.13–2.47 (m, 2 H, C_γ-H_2), and 1.50–2.10 (m, 4 H, $C_{3'}$-H_2, C_β-H_2). *Anal.* ($C_{14}H_{20}O_4N_6S \cdot H_2O$) C, H, N.

9-(5′-*O*-*p*-Toluenesulfonyl-β-D-arabinofuranosyl)adenine (1). Compound 1 was prepared by a modification of the procedure of Robins, *et al.*[6] Anhydrous 1-β-D-arabinofuranosyladenine (1.27 g, 5 mmol) was dissolved in 94 ml of boiling pyridine, freshly distilled from barium oxide. To the pyridine solution, cooled to *ca.* 0–5° in an ice–salt bath, was added dropwise over 1 hr a solution of *p*-toluenesulfonyl chloride (1.44 g, 7.5 mmol) in 36 ml of $CHCl_3$–pyridine (1:1). After addition was completed the solution was stirred at 0° for 8 hr. The reaction mixture was poured into a solution of saturated aqueous $NaHCO_3$ at 0–4° and the $CHCl_3$ layer was separated and the aqueous phase extracted with two 20-ml portions of cold $CHCl_3$. The combined $CHCl_3$ fractions were washed with water and dried (Na_2SO_4). After filtration the $CHCl_3$ was removed under pressure at 0° and the residue was dissolved in 1 ml of $CHCl_3$ and added dropwise to cold hexane. The precipitate was collected by filtration to yield 1.04 g (51%): mp 156–157°; nmr (DMSO-d_6) δ 8.17, 7.98, (2 s, 2 H, C_2-H, C_8-H), 7.80 (d, 2 H, phenyl), 7.40 (d, 2 H, phenyl), 6.28 (d, 1 H, $C_{1'}$-H), 5.57–6.03 (m, 2 H, $C_{2'}$-OH, $C_{3'}$-OH), 4.36 (d, 2 H, $C_{5'}$-H), 3.80–4.50 (m, 3 H, $C_{2'}$-H $C_{3'}$-H, $C_{4'}$-H), and 2.38 (s, 3 H, $-CH_3$). Various attempts to crystallize this material for C, H, and N analysis resulted in decomposition. Therefore, it was converted to the diacetyl derivative **2**.

9-(2′,3′-*O*-Diacetyl-5′-*O*-*p*-toluenesulfonyl-β-D-arabinofuranosyl)adenine (2). To a solution of 5′-tosylate 1 (500 mg, 1.2 mmol) in 4 ml of pyridine was added 4 ml of Ac_2O and the solution was stirred at 0° for 6 hr. The reaction mixture was poured into an ice–H_2O–$CHCl_3$ mixture and the $CHCl_3$ layer was separated and the aqueous phase extracted several times with cold $CHCl_3$. The $CHCl_3$ solutions were combined, washed with H_2O, and dried (Na_2SO_4). The $CHCl_3$ was removed under reduced pressure and the desired tosylate **2** crystallized (hexane–$CHCl_3$) to yield 430 mg (71%): mp 226–228°. *Anal.* ($C_{21}H_{23}N_5O_8S$) C, H, N.

Attempted Synthesis of *S*-5′-[9-(Arabinofuranosyl)adenyl]-L-homocysteine (SAraAH). (a) From 9-(5′-*O*-*p*-Toluenesulfonyl-β-D-arabinofuranosyl)adenine (1). The tosylate 1 (95 mg, 0.23 mmol) was condensed with *S*-benzyl-L-homocysteine (53 mg, 0.23 mmol) in Na and liquid NH_3 according to the previously described procedure.[2,3,8] After the NH_3 had evaporated, the residue was dissolved in 1 ml of H_2O, neutralized (1.0 *N* HCl), and cooled (*ca.* 0–4°). The solid which formed was filtered and recrystallized (H_2O) to yield 46 mg (80%), mp 298° dec. The ir, uv, and nmr spectra indicated that the product obtained was 9-(2′,5′-anhydro-β-D-arabinofuranosyl)adenine (**3**) (lit.[6,9] mp was reported as darkened near 220° and liquified with decomposition at 300°). *Anal.* ($C_{10}H_{11}N_5O_3$) C, H, N.

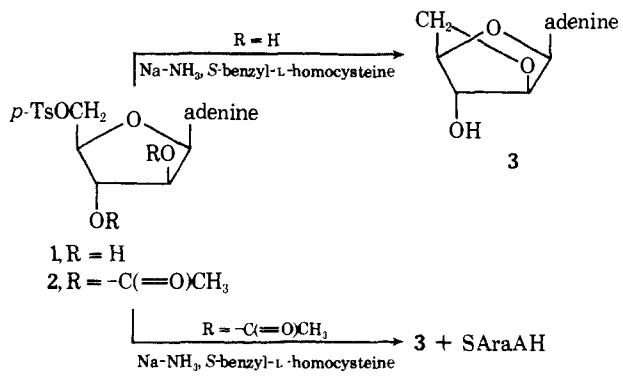
(B) From 9-(2′,3′-*O*-Diacetyl-5′-*O*-*p*-toluenesulfonyl-β-D-arabinofuranosyl)adenine (2). Using the standard condensation procedure,[2,3,8] tosylate **2** (218 mg, 0.41 mmol) was allowed to react with *S*-benzyl-L-homocysteine (90 mg, 0.4 mmol) in Na and liquid NH_3. After the NH_3 had evaporated, the residue was dissolved in 5 ml of cold H_2O and the aqueous layer extracted several times with $CHCl_3$. The aqueous layer was lyophilized and the product purified by thick-layer chromatography on cellulose (Analtech, 1000 μ) eluting with EtOH–H_2O (3:1). Fraction A (R_f 0.64) was extracted with H_2O and crystallized (H_2O) to yield 65 mg (66%) of 9-(2′,5′-anhydro-β-D-arabinofuranosyl)adenine (**3**), mp 298°. Fraction B (R_f 0.23) was extracted from the cellulose with H_2O and the H_2O removed by lyophilization to yield 45 mg of the crude product, which contained the desired SAraAH and homocysteine. This product was further purified by thick-layer chromatography on cellulose (Analtech, 1000 μ) eluting with a mixed solvent system containing nine parts of EtOH–AcOH–H_2O (50:3:5) and one part of phosphate buffer, pH 7.0 (0.02 *M*), and then rechromatography on cellulose eluting with EtOH–H_2O (3:1). The desired SAraAH was extracted with H_2O and the H_2O removed by lyophilization to yield 9 mg (6%) of the product: mp 235° dec; nmr (CF_3CO_2D) δ 9.68, 9.35 (2 s, 2 H, C_2-H, C_8-H), 7.10 (s, 1 H, $C_{1'}$-H), 5.50–6.07 (m, 3 H, $C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H), 5.17–5.50 (m, 1 H, C_α-H), 4.05–5.02 (m, 4 H, $C_{5'}$-H_2, C_γ-H_2), and 3.13–3.87 (m, 2 H, C_β-H_2). (The unexpected peak positions observed for SAraAH appear to be the result of the nmr solvent (CF_3CO_2D), since shifts to similar positions were observed for SAH and homocysteine when the spectrums were recorded in CF_3CO_2D); uv λ max (H_2O) 260 nm (ε 10,200). *Anal.* ($C_{14}H_{20}O_5N_6S$) C, H, N.

Enzyme Purification Assay. The enzymes used in this study were purified from the following sources according previously described procedures: COMT,[10,11] rat liver (male, Sprague–Dawley, 180–200 g); PNMT,[12] bovine adrenal medulla (Pel-Freez Biologicals); HMT,[13] guinea pig brain (Pel-Freez Biologicals); and HIOMT,[14] bovine pineal glands (Pel-Freez Biologicals). COMT, PNMT, HMT, and HIOMT were assayed and the analogs of SAH evaluated as inhibitors using the radiochemical techniques described in the preceding papers of this series.[2,3] The assay mixtures for each of the methyltransferases contained SAM-$^{14}CH_3$ (0.05 μCi) and SAM (1.0 m*M*). The acceptor molecules and their final concentrations in the assay mixtures were as follows: COMT, dihydroxybenzoate (2.0 m*M*); PNMT, DL-β-phenylethanolamine (1.0 m*M*); HMT, histamine (1.0 m*M*); and HIOMT, *N*-acetylserotonin (1.0 m*M*). Processing of the kinetic data was accomplished as previously described.[2,3,11,15,16]

Results and Discussion

Chemistry. The various sugar-modified analogs of SAH prepared in this study are listed in Chart I. 2′-Deoxy-SAH and 3′-deoxy-SAH were prepared by the general synthetic procedures outlined in a preceding paper of this series.[3] The appropriate nucleosides were converted to their 5′-to-

Scheme I



sylates and condensed directly with *S*-benzyl-L-homocysteine to yield the desired SAH derivatives (2′-deoxy-SAH and 3′-deoxy-SAH). During these condensation reactions it was not necessary to protect the 2′- or 3′-hydroxyl groups of the nucleoside 5′-tosylates. However, difficulty was encountered in the synthesis of SAraAH, because the condensation of 9-(5′-*O*-*p*-toluenesulfonyl-β-D-arabinofuranosyl)adenine (1) with *S*-benzyl-L-homocysteine in sodium and liquid NH_3 afforded 9-(2′,5′-anhydro-β-D-arabinofuranosyl)adenine (3) rather than the desired SAraAH (Scheme I). In fact, this intramolecular cyclization of tosylate 1 to 2′,5′-anhydronucleoside 3 took place in sodium and liquid NH_3 alone. This intramolecular cyclization had previously been observed to occur in ethanol with sodium methoxide at room temperature.[9,17] In an attempt to prevent this cyclization, 5′-tosylate 1 was converted to 9-(2′,3′-*O*-diacetyl-5′-*O*-*p*-toluenesulfonyl-β-D-arabinofuranosyl)adenine (2) using acetic anhydride in pyridine. Condensation of 2 with *S*-benzyl-L-homocysteine afforded a mixture of the 2′,5′-anhydronucleoside 3 and the desired SAraAH. That the major product was again the 2′,5′-anhydronucleoside 3 suggests that the acetyl-protecting groups of 2 were rapidly removed under the condensation conditions permitting rapid cyclization to 3. However, displacement of the 5′-tosylate of 2 by L-homocysteine anion must have been competitive with the intramolecular cyclization, since a significant amount of SAraAH was also isolated. The 2′,5′-anhydronucleoside 3 appears to arise from an intramolecular displacement of the 5′-tosylate rather than from SAraAH, since SAraAH was stable under the conditions used for consideration reaction. All of the SAH derivatives and their synthetic intermediates were characterized by their ir, nmr, and uv spectra, their chromatographic properties, and elemental analyses.

***In Vitro* Inhibition Studies.** The various sugar-modified analogs of SAH, which were synthesized as part of this study, were tested as inhibitors of COMT, PNMT, HMT, and HIOMT and the results are shown in Table I. Included for comparison are data for the inhibition of these enzymes by SAH. The enzyme showing the highest specificity for the structural features of the ribose portion of SAH was HIOMT, since none of the analogs listed in Table I inhibited this enzyme appreciably. HIOMT appears to have an extremely high specificity for all of the structural features of SAH, since analogs with modifications in the amino acid or base portions of this molecule also did not inhibit.[2,3] With HMT the various sugar-modified SAH analogs showed very slight inhibitory activities, indicating that both the 2′-hydroxyl and 3′-hydroxyl groups must be important in binding to this enzyme. In contrast, PNMT, an enzyme which exhibits high specificity for the structural features of the base and amino acid portions of SAH,[2,3] was very susceptible to inhibition by 2′-deoxy-SAH, 3′-deoxy-SAH, and SAraAH, thus showing little specificity for the 2′- and 3′-hydroxyl groups of the sugar moiety. This appears to be particularly true for the 3′-hydroxyl group, since 3′-deoxy-SAH is as potent as SAH itself in inhibiting this enzyme. However, the 2′-hydroxyl group may be somewhat important in binding, since 2′-deoxy-SAH and SAraAH were less potent inhibitors than SAH. With COMT the 2′-hydroxyl group appears necessary for the optimal binding of SAH, since 2′-deoxy-SAH and SAraAH were completely devoid of inhibitory activity. However, the 3′-hydroxyl group must not be directly involved in the binding, since 3′-deoxy-SAH exhibited substantial inhibitory activity toward this enzyme.

Table I. Inhibition of COMT, PNMT, HMT, and HIOMT by Sugar-Modified Analogs of SAH[a]

Compd	Inhibitor[b] concn, m*M*	% inhibition COMT	PNMT	HMT	HIOMT
SAH	0.2	39	49	40	71
	2.0	87	92	89	94
2′-Deoxy-SAH	0.2	0	9	7	0
	2.0	2	49	25	5
3′-Deoxy-SAH	0.2	22	52	10	2
	2.0	59	90	29	9
SAraAH	0.2	0	11	5	6
	2.0	0	33	13	14

[a] COMT, PNMT, HMT, and HIOMT were purified and assayed as described in the Experimental Section except in each case the SAM concentration = 1.0 m*M*. [b] The inhibitors were prepared in aqueous stock solutions (10.0 μmol/ml).

The kinetic patterns of inhibition of COMT, PNMT, and HMT by SAH, 2′-deoxy-SAH, 3′-deoxy-SAH, and SAraAH were determined and the resulting inhibition constants are listed in Table II. In all cases linear competitive patterns of inhibition were observed when SAM was the variable substrate. Of particular interest is the potent inhibitory activity of 3′-deoxy-SAH toward PNMT, where the inhibition constant of 42.7 ± 2.45 μ*M* for this sugar analog is comparable to that for SAH (K_{is} = 29.0 ± 2.84 μ*M*). With 2′-deoxy-SAH and SAraAH the inhibition constants toward PNMT are about ten times greater than that for SAH, indicating that some of the interactions critical to formation of the PNMT-inhibitor complex have been lost by removal of the 2′-hydroxyl or inversion at C-2. 3′-Deoxy-SAH is also a potent inhibitor of COMT, but nearly inactive with HMT. These apparent differences in the specificity for the ribose portion of SAH at the enzymatic binding sites could be potentially useful in obtaining differential inhibition *in vivo*.

The competitive kinetic data discussed above for SAH and 3′-deoxy-SAH inhibition of PNMT suggest that both of these inhibitors are binding to the same site on this enzyme. To provide evidence in support of this assumption a study of the kinetics of multiple inhibition of PNMT by SAH and 3′-deoxy-SAH was conducted using the procedures of Yonetani and Theorall.[18] As shown in Figure 1, a series of parallel straight lines was obtained when reciprocal velocities were plotted *vs.* SAH concentrations at varying concentrations of 3′-deoxy-SAH, indicating that these two inhibitors are competing for the same site on PNMT.

Conclusions

The present study has attempted to elucidate the importance of the 2′- and 3′-hydroxyl groups of the ribose moiety

Table II. Inhibition Constants for SAH, 2′-Deoxy-SAH, 3′-Deoxy-SAH, and SAraAH toward COMT, PNMT, and HMT[a]

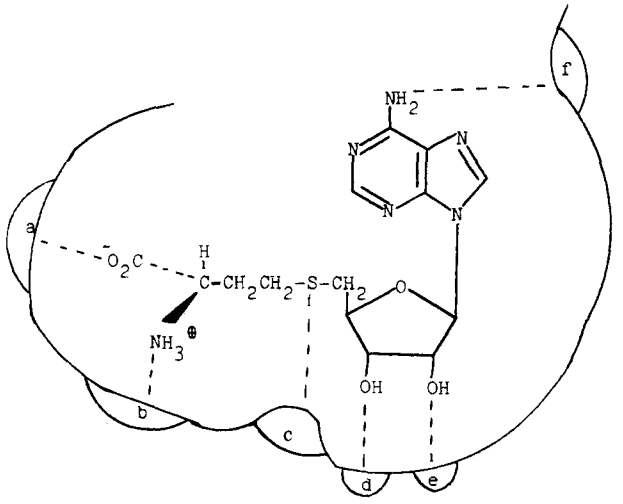
Inhibitor	Inhibition constants,[b,c] μM, K_{is} ± S.E.M.		
	COMT	PNMT	HMT
SAH	36.3 ± 2.20	29.0 ± 2.84	18.1 ± 2.19
2′-Deoxy-SAH		278 ± 12.2	
3′-Deoxy-SAH	138 ± 31.2	42.7 ± 2.45	2070 ± 864
SAraAH		206 ± 27	

[a] COMT, PNMT, and HMT were purified and assayed as described in the Experimental Section. SAM concentration, 24-210 μM. [b] Each inhibitor showed linear competitive kinetics and the inhibition constants were calculated as previously described.[2,3,11,15,16] [c] If low inhibitory activity was observed from the preliminary studies, no attempt was made to determine the kinetic inhibition constants.

of SAH in the binding of this molecule to COMT, PNMT, HMT, and HIOMT. The SAH analogs of interest in this study were 2′-deoxy-SAH, 3′-deoxy-SAH, and SAraAH. From the inhibitory activities of these sugar-modified analogs, it can be concluded that HMT and HIOMT show strict specificity for the structural features of the ribose portion of SAH. However, the binding sites of PNMT and COMT appear to be less sensitive to changes in the ribose moiety, since strong inhibition of PNMT by 2′-deoxy-SAH and 3′-deoxy-SAH and moderate inhibition of COMT by 3′-deoxy-SAH were observed. These interesting exceptions to the normal strict specificity for SAH binding may permit differential inhibition *in vivo* of these catecholamine metabolizing enzymes from other methyltransferases.

From the inhibitory activities observed for the various amino acid, base, and sugar-modified analogs of SAH prepared in our laboratory,[1-3] it can be generally concluded that methyltransferases show strict specificity for the structural features of SAH. However, several interesting exceptions to this strict specificity have been observed which may permit differential inhibition of methyltransferases *in vivo*. Chart II shows a schematic representation of a possible enzymatic binding site for SAH. This proposed

Chart II. Proposed Binding Sites for SAH



binding site for SAH is similar to that proposed by Zappia, *et al.*,[19] for the enzymatic binding of SAM. The structural features of primary importance in the binding of SAH are

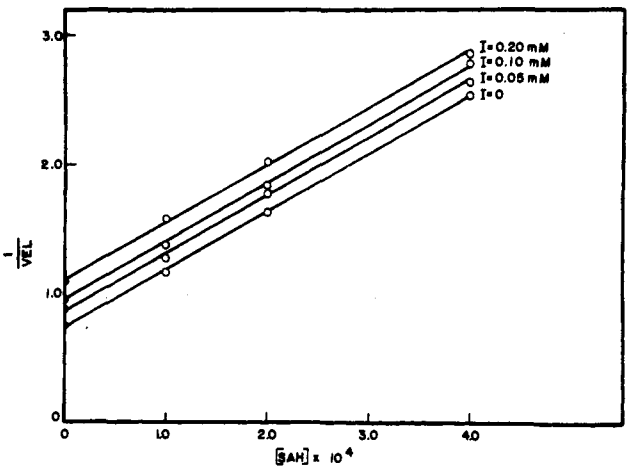


Figure 1. Reciprocal PNMT velocity *vs.* SAH concentration with varying 3′-deoxy-SAH concentration. DL-β-Phenylethanolamine concentration, 1.0 m*M*. SAM concentration, 1.0 m*M*. Velocity = nmol of product/mg of protein/min.

the terminal carboxyl (site a), terminal amino (site b), and sulfur atom (site c) of the homocysteine portion; the 6-amino group (site f) of the base portion; and the 3′-hydroxyl (site d) and 2′-hydroxyl (site e) groups of the ribose portion of SAH. The binding of SAH through the terminal amino group (site b) and the 6-amino group of adenine (site f) appears to be particularly important for all methyltransferases studied. For the optimal binding of SAH to HMT and tRNA methyltransferase all of the functional groups shown in Chart II except the terminal carboxyl group (site a) and the configuration of the amino acid asymmetric carbon are required.[2,20-22] For the binding of SAH to COMT, the sulfur atom (site c) and the 3′-hydroxyl group (site d) do not appear crucial for optimal binding; however, the other functional groups shown in Chart II are absolute requirements.[2] For PNMT the 2′-hydroxyl group (site e) and 3′-hydroxyl group (site d) of SAH do not appear to be required for maximum binding, since removal of either of these groups does not result in loss activity. However, all of the other attachment sites on SAH are crucial in optimal binding to PNMT. The enzyme HIOMT shows a very high specificity for all the structural features of SAH and only certain base-modified derivatives retain significant inhibitory activity.[3] The differences in the specificity of the SAH binding sites, which have been detected in our studies, are being further explored in an attempt to design specific inhibitors of these enzymes.

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Norepinephrine *N*-Methyltransferase Inhibition by Benzamidines, Phenylacetamidines, Benzylguanidines, and Phenylethylguanidines

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Norepinephrine *N*-methyltransferase (NMT) from rabbit adrenal glands was inhibited by benzylamine and phenethylamine analogs in which the nitrogen was replaced by an amidino or guanidino group. Mono and dichloro derivatives of benzamidines, phenylacetamidines, benzylguanidines, and phenethylguanidines were studied. The two most potent NMT inhibitors among the compounds examined were 2,3-dichlorobenzamidine and 3,4-dichlorophenylacetamidine, with pI_{50} values of 5.55 and 5.36, respectively. These inhibitors were reversible and were competitive with norepinephrine as the variable substrate. They inhibited NMT from human, rat, and bovine adrenal glands but were slightly less effective against those enzymes than against the rabbit adrenal enzyme. In exercised rats, 2,3-dichlorobenzamidine had no significant effect on adrenal catecholamine levels. 3,4-Dichlorophenylacetamidine slightly reduced epinephrine levels in the adrenal glands of exercised rats, but the effect may have been due to release rather than inhibition of synthesis, since heart norepinephrine levels were also reduced significantly by that agent (which is from a chemical series known to release catecholamines). Thus, whereas these compounds are reasonably potent inhibitors of NMT *in vitro*, they apparently are not effective in blocking enzyme activity *in vivo*.

Norepinephrine *N*-methyltransferase† (NMT) catalyzes the terminal step of epinephrine biosynthesis in the adrenal medulla. This enzyme is an ideal target for inhibiting epinephrine formation; an inhibitor of NMT would not affect the synthesis of dopamine or norepinephrine, which have physiologic functions of their own in addition to being precursors of epinephrine. The pharmacologic consequences of blocking NMT are unknown, since agents that specifically inhibit this enzyme *in vivo* have not been reported. For several years we have been searching for inhibitors of NMT and have found that phenethylamines and benzylamines are among the most effective inhibitors *in vitro*.[6,7] This paper describes some compounds of those types in which the amine function has been replaced by an amidino or guanidino group. The compounds studied have the structures

$$\mathrm{RC(=NH)NH_2} \quad \text{or} \quad \mathrm{RNHC(=NH)NH_2}$$

where R = phenyl, benzyl, or phenethyl.

Inhibition by Analogs of Benzylamines. Table I shows the inhibition of NMT by chlorinated benzamidines. Included for comparison are data for the inhibition by benzylamines and α-methylbenzylamines (taken from ref 7). The benzamidines were slightly weaker inhibitors of NMT than were the benzylamines, but the order of potency of the various chlorine-substituted isomers was generally similar. The 2,3-dichloro compound was the most active inhibitor in all three series. The 3-chloro compound was next most potent with the 2-chloro just behind it in the benzamidine series and the benzylamine series. Among the α-methylbenzylamines, the 2-chloro compound was more potent than expected probably due to steric interaction between the α-methyl and the bulky 2-chloro substituent. The 4-chloro compound was least potent of the chloro compounds in all three groups.

Table I. *In Vitro* Inhibition (pI_{50} Values) of NMT by Benzylamines, α-Methylbenzylamines, and Benzamidines

	Aromatic substituent					
Series	None	2-Cl	3-Cl	4-Cl	3,4-Cl	2,3-Cl
$C_6H_5CH_2NH_2$	3.12	4.66	5.07	4.07	4.97	6.23
$C_6H_5CHNH_2$	3.04[c]	5.29	4.72	3.70		6.42
CH_3						
$C_6H_5CNH_2$	2.62[b]	4.09[c]	4.25	3.02[b]	3.83	5.55
NH	(1)[d]	(2)	(3)	(4)	(5)	(6)

[a] *d* isomer 3.17, *l* isomer 2.2. [b] Marshallton Research Laboratories. [c] Reference 8. [d] Compound numbers are shown in parentheses in this table and Table II.

†This enzyme has previously been referred to as phenylethanolamine *N*-methyltransferase.[1] However, norepinephrine is a much better substrate (lower K_m) than phenylethanolamine[2-4] and is perhaps the only physiological substrate for the enzyme. Thus the 1972 recommendations of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry list noradrenalin *N*-methyltransferase as their recommended name for this enzyme (E.C. 2.1.1.28).[5] Following their recommendations and replacing norepinephrine (U. S.) for noradrenalin (British), we intend to refer to this enzyme as norepinephrine *N*-methyltransferase (NMT for norepinephrine methyltransferase) in the future, despite the extensive use of the name phenylethanolamine *N*-methyltransferase in the prior literature.