Potential Inhibitors of S-Adenosylmethionine-Dependent Methyltransferases. 10. Base- and Amino Acid Modified Analogues of S-Aristeromycinyl-L-homocysteine[1a]

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A series of base- and amino acid modified analogues of S-aristeromycinyl-L-homocysteine, a carbocyclic nucleoside, were synthesized and evaluated as inhibitors of S-adenosyl-L-methionine-dependent methyltransferases, including catechol O-methyltransferase, phenylethanolamine N-methyltransferase, and histamine N-methyltransferase. The base-modified analogues (8-azaadenine, 3-deazaadenine, and N^6-methyladenine) were prepared by reaction of the corresponding carbocyclic 5′-chloro-5′-deoxynucleosides with the anion of homocysteine generated in situ either from L-homocystine or S-benzyl-L-homocysteine in Na/liquid NH_3 or with DL-homocysteine thiolactone in alkaline solution. S-Aristeromycinyl-D-homocysteine was prepared with use of D-homocystine in the Na/liquid NH_3 reaction. The sulfoxide and sulfone analogues were prepared by oxidation of S-aristeromycinyl-L-homocysteine. The various base- and amino acid modified analogues of S-aristeromycinyl-L-homocysteine were inactive as inhibitors of catechol O-methyltransferase. In contrast, the 3-deaza analogue was a good inhibitor (K_i = 20.5 ± 1 μM) of phenylethanolamine N-methyltransferase whereas S-aristeromycinyl-D-homocysteine was an excellent inhibitor (K_i = 10.4 ± 2.4 μM) of histamine N-methyltransferase. On the basis of these results, it would appear that the structural requirements for the binding S-aristeromycinyl-L-homocysteine are similar to those for binding S-adenosyl-L-homocysteine. Therefore, these carbocyclic analogues have the potential of being better inhibitors in vivo, because they should be more stable to metabolism than the ribosyl analogues.

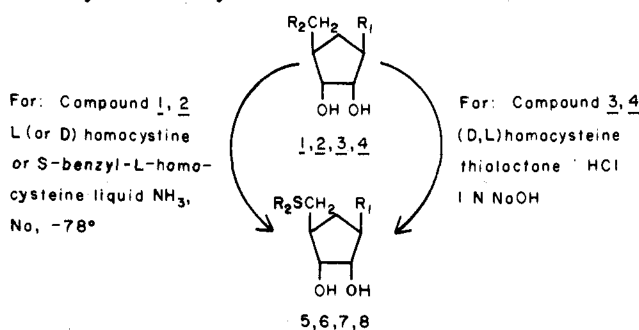
Biological transmethylations, which utilize S-adenosyl-L-methionine (AdoMet) as a methyl donor, are involved in the biosynthesis and metabolism of small molecules (e.g., dopamine, norepinephrine, epinephrine, histamine, melatonin)[2,3] and in modulating the activity of macromolecules (e.g., nucleic acids, proteins).[3] A common feature of most methyltransferases is the inhibition produced by the demethylated product S-adenosyl-L-homocysteine (L-AdoHcy).[2,3] The activities of AdoMet-dependent methyltransferases in vivo are dependent on the intracellular level of AdoHcy and the activity of the enzyme AdoHcy hydrolase (EC 3.3.1.1), which in eukaryotes catalyzes the hydrolysis of AdoHcy to homocysteine and adenosine.[4-7]

This regulatory system for AdoMet-dependent methyltransferases has attracted considerable attention as a target for drug design.[2-7] The two general approaches that have been employed include the following: (a) the synthesis of analogues of AdoHcy that function directly on a particular methyltransferase[2] and (b) the synthesis of inhibitors of AdoHcy hydrolase which cause an elevation in cellular levels of AdoHcy and subsequently produce inhibition of methyltransferases.[2,6,7]

In earlier papers in this series,[8] we have described the synthesis and biological activity of a series of AdoHcy analogues which have structural modifications in the amino acid, sugar, or base portions of the AdoHcy molecule. From these studies we concluded that there exists differences in the AdoHcy-binding sites on AdoMet-dependent methyltransferases. Subsequently, differential inhibition of these AdoMet-dependent enzyme can be achieved with use of appropriately designed AdoHcy analogues.[8]

In the current study, we have synthesized a series of S-aristeromycinyl-L-homocysteine (L-C-AdoHcy) analogues with structural modification in either the amino acid or the base portion of the molecule. L-C-AdoHcy is a carbocyclic nucleoside analogue of L-AdoHcy. The specific base- and amino acid modified analogues of L-C-AdoHcy, which were prepared in this study, were selected on the basis of the interesting biological properties of the corresponding base- and amino acid modified analogues of L-AdoHcy.[8] This carbocyclic framework was chosen because

Scheme I. Synthesis of Base-Modified Analogues of Carbocyclic AdoHcy



Compound	R_1	R_2
1	adenine	Cl
2	8-azaadenine	Cl
3	N^6-methyladenine	Cl
4	3-deazaadenine	Cl
5	adenine	(L)-$CH_2CH_2CH(NH_2)CO_2H$
6	8-azaadenine	(L)-$CH_2CH_2CH(NH_2)CO_2H$
7	N^6-methyladenine	(D,L)-$CH_2CH_2CH(NH_2)CO_2H$
8	3-deazaadenine	(D,L)-$CH_2CH_2CH(NH_2)CO_2H$
9	adenine	(D)-$CH_2CH_2CH(NH_2)CO_2H$

L-C-AdoHcy, itself, exhibits inhibitory activity toward several AdoMet-dependent methyltransferases.[8d] These

(1) (a) Taken in part from the Ph.D. dissertation submitted to the Graduate School of the University of Kansas by D. M. H., 1983. (b) Supported by NIH Predoctoral Training Grant GM-07775.
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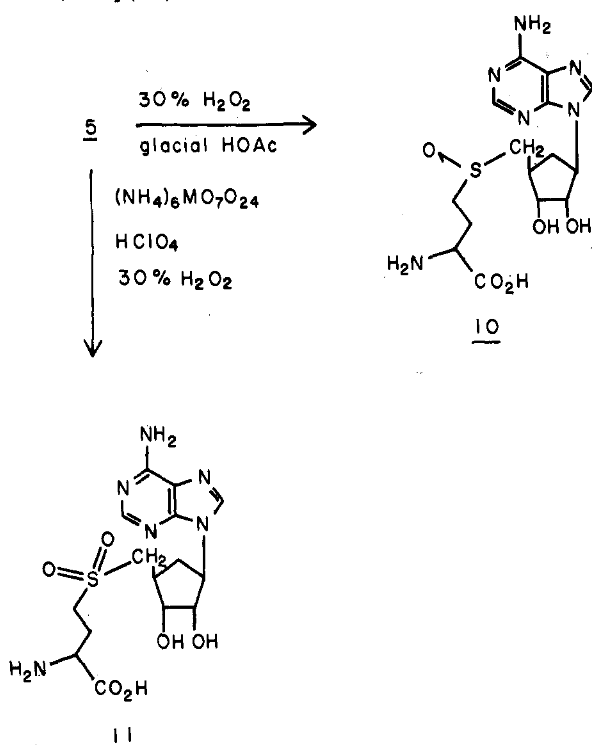
Scheme II. Synthesis of L-C-AdoHcy-50 (10) and L-C-AdoHcy-SO_2 (11)



results suggest that the 2′,3′-dihydroxycyclopentyl ring of L-C-AdoHcy sufficiently resembles the ribosyl ring of L-AdoHcy to permit binding of this analogue and related carbocyclic analogues to various methyltransferases. In addition, however, L-C-AdoHcy or its analogues may be more metabolically stable than L-AdoHcy. Crooks et al.[9] have prepared metabolically stable analogues of L-AdoHcy that have proven to be useful experimental tools.

Chemistry. The base-modified analogues of L-C-AdoHcy, which were prepared in this study, include the 8-azaadenine analogue **6**, the N^6-methyladenine analogue **7**, and the 3-deazaadenine analogue **8**. These base-modified L-C-AdoHcy analogues were prepared from the corresponding carbocyclic 5′-chloro-5′-deoxynucleosides **1–4**. Carbocyclic 5′-chloro-5′-deoxyadenosine (**1**) and carbocyclic 5′-chloro-5′-deoxy-8-azaadenosine (**2**) were condensed with the anion of L-homocysteine generated in situ from either L-homocystine or S-benzyl-L-homocysteine with use of Na in liquid NH_3 at −78 °C (Scheme I)[10] to yield the corresponding homocysteinyl analogues **5** and **6**. Preparation of the carbocyclic analogues **7** and **8** required reaction of the corresponding carbocyclic 5′-chloro-5′-deoxynucleosides **3** and **4** with D,L-homocysteine thiolactone in 1 N NaOH (Scheme I).[11] The carbocyclic 5′-chloro-5′-deoxynucleosides **1–4** were prepared from the corresponding carbocyclic nucleosides[12] with use of thionyl chloride in hexamethylphosphoramide (HMPA).[10]

The amino acid modified analogues of L-C-AdoHcy, which were prepared in this study, include D-C-AdoHcy (**9**), L-C-AdoHcy sulfoxide (L-C-AdoHcy-SO, **10**), and L-C-AdoHcy sulfone (L-C-AdoHcy-SO_2, **11**). D-C-AdoHcy (**9**) was prepared by reaction of carbocyclic 5′-chloro-5′-deoxyadenosine (**1**) with D-homocystine with use of Na in liquid NH_3 at −78 °C. L-C-AdoHcy-SO (**10**) and L-C-AdoHcy-SO_2 (**11**) were prepared by oxidation of L-C-AdoHcy (**5**) with H_2O_2 in glacial acetic acid (to sulfoxide) or with H_2O_2 in perchloric acid and ammonium molybate in glacial acetic acid (to sulfone) (Scheme II).

It should be noted that Montgomery et al.[18] have reported that the Na/NH_3 condensation procedure which was used to prepare **5**, **6**, and **9** can lead to some racemization of the α-carbon of the amino acid. Therefore, the compounds prepared in this study which are designed D or L (compounds **5**, **6**, **9**, **10**, and **11**) may, in fact, be slightly contaminated with the other enantiomer.

Biological Activity. In Table I are shown the inhibitory effects of the C-AdoHcy analogues **5–11** and the corresponding AdoHcy analogues on COMT, PNMT, and HMT. Of the methyltransferases screened, COMT appears to be most intolerant to the change of the ribosyl ring of AdoHcy to the 2′-3′-dihydroxycyclopentyl ring of C-AdoHcy. Of the ribosyl analogues tested, the most effective inhibitors of COMT were L-AdoHcy, L-N^6-methyl-AdoHcy, L-3-deaza-AdoHcy, L-AdoHcy-SO, and L-AdoHcy-SO_2. In contrast, the corresponding cyclopentyl analogues, e.g., L-C-AdoHcy (**5**), DL-C-N^6-methyl-AdoHcy (**7**), DL-C-3-deaza-AdoHcy (**8**), L-C-AdoHcy-SO (**10**), and L-C-AdoHcy-SO_2 (**11**) were all substantially less effective as inhibitors of COMT. It should be noted that our laboratory had previously reported that L-C-AdoHcy was an inhibitor of COMT.[8d] However, the data generated in this study do not confirm our earlier result but are now consistent with the data reported earlier by Coward and Slisz.[13] There are at least three possible explanations for the reduced inhibitory activity of these carbocyclic analogues: (1) the 1′,4′-oxygen bridge of the ribosyl ring of AdoHcy is directly involved in binding to the enzyme, (2) the methylene group of C-AdoHcy creates steric hindrances that prevent optimal binding at the enzymatic site, and (3) the conformation of the ribosyl ring of AdoHcy

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Table I. Inhibition of COMT, PNMT, and HMT by Base- and Amino Acid Modified Analogues of AdoHcy and C-AdoHcy[a]

	% inhibition											
	COMT				PNMT				HMT			
	C-AdoHcy analogue		AdoHcy analogue[c]		C-AdoHcy analogue		AdoHcy analogue[c]		C-AdoHcy analogue		AdoHcy analogue[c]	
compd[b]	I = 0.1 mM	I = 1.0 mM	I = 0.2 mM	I = 2.0 mM	I = 0.1 mM	I = 1.0 mM	I = 0.2 mM	I = 2.0 mM	I = 0.1 mM	I = 1.0 mM	I = 0.2 mM	I = 2.0 mM
L-AdoHcy or L-C-AdoHcy (5)	3	4	39	87	61	93	49	92	23	64	40	89
D-AdoHcy or D-C-AdoHcy (9)	9	10	5	22	3	25	14	32	64	97	73	99
L-AdoHcySO or L-C-AdoHcySO (10)	1	10	14	42	0	6	10	22	14	35	0	9
L-AdoHcySO_2 or L-C-AdoHcySO_2 (11)	3	10	8	45	0	34	13	17	28	35	0	10
L-8-aza-AdoHcy or L-C-8-aza-Ado-Hcy (6)	0	0	5	28	25	45	8	24	2	27	4	33
L-N^6-methylAdoHcy or DL-C-N^6-methylAdoHcy (7)	0	2	15	55	28	60	11	42	9	42	11	28
L-3-deazaAdoHcy or DL-C-3-deazaAdoHcy (8)	0	9	25	68	37	83	29	91	21	40	25	79

[a] COMT, PNMT, and HMT were purified and assayed as described in the Experimental Section, except in each case the AdoMet concentration = 1.0 mM. [b] The inhibitors were prepared in aqueous stock solution at a concentration of 10.0 μmol/mL. [c] Data taken from ref 8a, 8c, or 8d.

produces the proper orientation of the amino acid and base portions of the molecule for optimal fit at the enzymatic binding site, whereas the 2′,3′-dihydroxycyclopentyl ring of C-AdoHcy creates a less desirable conformation. Considering the overall high specificity of the AdoHcy binding site on COMT[8], the latter explanation seems most reasonable.

In contrast to COMT, PNMT is more tolerant of changing the ribosyl ring of AdoHcy to the 2′,3′-dihydroxycyclopentyl ring of C-AdoHcy. In fact, some of the base- and amino acid modified analogues of C-AdoHcy are more effective inhibitors of PNMT than the corresponding AdoHcy analogues (Table I). The enhanced binding of C-AdoHcy (3) (K_i = 7.59 μM) vs. AdoHcy (K_i = 29 μM) to PNMT was reported earlier by Borchardt and Wu.[8d] However, modifications in the amino acid portion of C-AdoHcy (e.g., D-C-AdoHcy (9), L-C-AdoHcy-SO (10), and L-C-AdoHcy-SO_2 (11)), result in dramatic decreases in enzyme inhibitory activity. Similar results were obtained for the corresponding amino acid modified analogues of AdoHcy.[8a] Alterations in the base portion of C-AdoHcy, in general, reduced the inhibitory activity toward PNMT (Table I) to about the same extent as that seen with similar base-modified AdoHcy analogues.[8b,e] Of those base-modified C-AdoHcy analogues evaluated, DL-C-3-deaza-AdoHcy (8) was the most potent inhibitor of PNMT. Like AdoHcy and its amino acid and base-modified analogues, C-AdoHcy and its analogues all exhibited competitive inhibition kinetics when AdoMet was the variable substrate, and the results are shown in Table II.

In earlier studies we had shown that D-AdoHcy (K_i = 10.5 μM) was a better inhibitor of HMT than L-AdoHcy (K_i = 18.1 μM).[8a] In the current study we have shown that the corresponding D-C-AdoHcy (9) (K_i = 10.4 ± 2.4 μM) is also a better inhibitor than the corresponding L-C-AdoHcy (5) (K_i = 59.8 ± 11.9 μM). It was interesting to note that L-C-AdoHcy-SO (10) and L-C-AdoHcy SO_2 (11) both showed some inhibitory activity toward HMT, in contrast to the corresponding AdoHcy analogues which were nearly inactive as inhibitors of this enzyme. These results suggest that the conformational changes brought about by incorporation of the 2′,3′-dihydroxycyclopentyl ring in place of the ribosyl ring of AdoHcy resulted in enhanced binding of these sulfur-modified analogues to HMT.

Table II. Inhibition Constants for Base- and Amino Acid Modified Analogues of AdoHcy and C-AdoHcy[a]

inhibitor	inhibn constant, μM, K_i ± SEM: PNMT	HMT
L-AdoHcy	29.0 ± 2.8[b]	18.1 ± 2.19[b]
L-C-AdoHcy (5)	7.6 ± 0.6[c]	59.8 ± 11.9[c]
D-AdoHcy	62.3 ± 4.5[b]	10.5 ± 1.3[b]
D-C-AdoHcy	ND[e]	10.4 ± 2.4
L-3-DeazaAdoHcy	81.1 ± 14.9[d]	59.2 ± 6.1[d]
DL-C-3-DeazaAdoHcy (8)	20.5 ± 1.0	ND

[a] PNMT and HMT were purified and assayed as described in the Materials and Methods section. [b] Taken from ref 8a. [c] Taken from ref 8d. [d] Taken from ref 8b. [e] ND, not determined.

Experimental Section

Biological Methods. The enzymes used in this study were purified from the following sources according to previously described procedures: COMT,[14] rat liver (male, Sprague–Dawley, 180–200 g); PNMT,[15] bovine adrenal medulla (Pel-Freez Biologicals); and HMT,[16] guinea pig brain (Pel-Freez Biologicals). In the experiments designed to screen the AdoHcy analogous as enzyme inhibitors (Table I), the assay mixtures contained AdoMet-$^{14}CH_3$ (0.05 μCi, 1.0 mM) and the appropriate acceptor molecules with their final concentrations being as follows: COMT, 3,4-dihydroxybenzoic acid (2.0 mM); PNMT, DL-β-phenylethanolamine (1.0 mM); and HMT, histamine (1.0 mM). In the kinetic experiments for determining inhibition constants (Table II), the concentrations of the acceptor substrates were held constant at the values mentioned above, but the AdoMet concentrations were varied from 24 to 210 μM. All inhibitors showed linear competitive kinetics, and the inhibition constants (K_i) were calculated according to the method of Cleland.[17]

Synthesis. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian FT-80A (80 MHz for proton nuclei) spectrophotometer. Chemical shifts are reported in δ from the internal standard tetramethylsilane (Me_4Si), δ_{Me_4Si} = 0.00. Mass spectra were recorded on a Ribermag R10-10 quadrupole mass spectrometer. Elemental analyses were performed on a F and M Model 185 C, H, N analyzer in the Department of Medicinal Chemistry, University of Kansas. Where analyses are indicated by symbols of the elements, analytical

Table III. R_f Values for Base- and Sugar-Modified Analogues of S-Aristeromycinylhomocysteine

compd	R_f values and chromatography system[a]			
	A	B	C	D
L-C-AdoHcy (**5**)	0.29	0.60	0.45	0.22
C-8-aza-AdoHcy (**6**)	0.36	0.68	0.46	0.22
C-N^6-MethylAdoHcy (**7**)	0.31	0.41	0.64	0.45
C-3-deazaAdoHcy (**8**)	0.22	0.53	0.23	0.14
D-C-AdoHcy (**9**)	0.19	0.66	0.51	0.14
C-AdoHcy-SO (**10**)	0.22	0.72	0.49	0.14
C-AdoHcy-SO_2 (**11**)	0.29	0.60	0.45	0.22

[a] The chromatography systems had the following compositions: system A, n-BuOH–H_2O–HOAc (10:6:3), 250 μm silica gel GF, Analtech; system B, 5% Na_2PO_4, 250 μm silica gel GF, Analtech; H_2O, 250 μm Avicel F, Analtech; EtOH–H_2O (2:1), 250 μm Avicel F, Analtech.

results obtained for these elements were within ±0.4% of the theoretical values. The designation of D, L, or DL in the final compounds **5–11** is based strictly on the nature of the precursor (e.g., D-homocysteine, L-homocysteine, or DL-homocysteine thiolactone) used in the preparation of the final compounds **5–11**. This is important because Montgomery et al.[18] have reported that the use of Na/NH_3 may lead to racemization of the α-carbon of the amino acid. Ion-exchange column chromatography was performed with a strong cation-exchange resin (Dowex 50-W (H^+ form), 4% cross-linked, 100–200 dry mesh]. Thin-layer chromatography was carried out on silica gel GF (Analtech, 0.25 mm) commercial glass plates and cellulose (Avicel F, Analtech, 0.25 mm) glass plates. Preparative-layer chromatography was carried out on cellulose (Avicel F, Analtech, 1.00 mm) glass plates. R_f values are reported in Table III.

General Preparation of Carbocyclic 5′-Chloro-5′-deoxynucleosides. The carbocyclic 5′-chloro-5′-deoxynucleosides **1–4** were prepared by using the general procedure described earlier by Kikugawa and Ichino.[10b] An ice-cooled solution of thionyl chloride (0.075 mL, 1.04 mmol) in HMPA (0.5 mL) was stirred under N_2 to which the carbocyclic nucleoside[12] (0.175 mmol) was added. The mixture was stirred for 5 min with cooling and then for 15–20 h at ambient temperature. The reaction solution was carefully quenched over a period of 30 min with H_2O (2 mL) and then neutralized with 2 N NH_3 to pH 7. Dowex 50-W (H^+) resin (2 mL) was added to the neutral solution and the resulting slurry was stirred for 20 min and loaded onto a Dowex 50-W (H^+) column (1.0 × 12 cm). The column was washed with 50 mL of H_2O and eluted with 100 mL of 2 N NH_3. For compounds **1**, **2**, and **4**, the alkaline eluant was reduced in vacuo to 10 mL and then cooled to give white crystals. Compounds **1**, **2**, and **4** were recrystallized from H_2O or H_2O–acetone. For the N^6-methyladenine analogue **3** the eluant was reduced to dryness and the dried oil dissolved in EtOH and cooled to give crystals.

Carbocyclic 5′-chloro-5′-deoxyadenosine (1): yield 83%; mp 194–196 °C (lit.[8d] mp 162–165 °C). Anal. ($C_{11}H_{14}O_2N_5Cl$) C, H, N.

Carbocyclic 5′-chloro-5′-deoxy-8-azaadenosine (2): yield 75%; mp 205–207 °C; ^1H NMR (Me_2SO-d_6) δ 8.25 (s, 1 H, H-2), 4.25–4.55 (m, 1 H, H-2′), 3.8–4.05 (m, 1 H, H-3′), 3.8 (d, 2 H, 2 H-5′). Anal. ($C_{10}H_{13}N_6O_2Cl$) C, H, N.

Carbocyclic 5′-chloro-5′-deoxy-N^6-methyladenosine (3): yield 82%; mp 107–110 °C; ^1H NMR (Me_2SO-d_6) δ 8.15 (2 s, 2 H, H-2 and H-8), 7.4–7.7 (m, 1 H, NHCH$_3$), 4.5–4.8 (m, 1 H, H-1′), 4.2–4.5 (m, 1 H, H-2′), 3.7–4.0 (d overlapping 1 m, 3 H, H-3′ and 2 H-5′), 3.0 (d, 3 H, NHCH_3), 1.65–2.6 (several overlapping m, 3 H, H-4′ and 2 H-6′). Anal. ($C_{12}H_{16}N_5O_2Cl\cdot H_2O$) C, H, N.

Carbocyclic 5′-chloro-5′-deoxy-3-deazaadenosine (4): yield 69%; mp 185–187 °C (lit.[10] mp 323–326 °C). Anal. ($C_{12}H_{15}N_4O_2Cl\cdot H_2O$) C, H, N.

L-C-AdoHcy (5) and D-C-AdoHcy (9). L-C-AdoHcy (**5**) was prepared by reaction of **1** with the anion of L-homocysteine generated in situ from L-homocystine with use of Na in liquid NH_3 at −78 °C as previously described by Borchardt and Wu.[8d] D-C-AdoHcy (**9**) was prepared by the same method[8d] except that D-homocystine was used in place of L-homocystine. The product was purified by chromatography on a Dowex 50-W (H^+) column followed by preparative thick-layer chromatography on Avicel F (1000 μm) eluting with EtOH–H_2O (2:1) as previously described.[8d] Crystallization from H_2O–acetone yielded **9** in 48% yield: mp 235 °C. Anal. ($C_{15}H_{22}N_6O_4S\cdot H_2O$) C, H, N. This material had identical chromatographic (R_f values, see Table III) and spectral (^1H NMR, IR, MS) properties with that of **5** prepared in the same fashion with L-homocystine. **9**: ^1H NMR (Me_2SO-d_6 plus two drops deuterium oxide) δ 8.1 (2 s, 2 H, H-2 and H-8), 4.5–4.8 (m, 1 H, H-1′), 4.2–4.5 (m, 1 H, H-2′), 3.7–3.9 (m, 1 H, H-3′), 3.1–3.5 (m, 1 H, H-α), 2.35–2.80 (2 m, 4 H, 2 H-5′ and 2 H-γ), 1.5–2.3 (several overlapping m, 5 H, H-4′, 2 H-6′ and 2 H-β); mass spectrum (DCI-CH_4), m/e 383 (MH^+), 367 ($MH^+ - NH_2$), 339 ($MH^+ - CO_2$), 280 (M − $CH_2CH_2CH(NH_2)CO_2H$), 136 (adenine H^+).

L-C-S-AdoHcy-SO (10). L-C-S-AdoHcy-SO (**10**) was prepared by using a modification of the procedure of Borchardt and Wu.[8a] To a stirred and cooled (10 °C) slurry of **5** (50 mg, 0.13 mmol) in 0.4 mL of glacial HOAC was added 0.025 mL of 30% H_2O_2. The reaction mixture was stirred for 15 min at ambient temperature and then allowed to set for an additional hour. The reaction mixture was diluted with EtOH (2 mL) and the resulting white precipitate isolated by filtration. Tituration of the white solid with EtOH (3 mL) followed by filtration and drying afforded 43 mg (83% yield) of **10**: mp 190 ° dec; for R_f values, see Table III; ^1H NMR (Me_2SO-d_6 plus two drops of deuterium oxide) δ 8.15 (2 s, 2 H, H-2 and H-8), 4.5–4.85 (m, 1 H, H-1′), 4.2–4.5 (m, 1 H, H-2′), 3.85–4.05 (m, 1 H, H-3′), 3.3–3.5 (t, 1 H, H-α), 2.7–3.2 (m, 4 H, 2 H-5′ and 2 H-γ), 1.7–2.6 (several overlapping m, 5 H, 2 H-6′, H-4′, and 2 H-β); mass spectrum (DCI-NH_3), no molecular ion detectable, m/e 355 ($MH^+ - CO_2$), 339 [$MH^+ - (CO_2+NH_2)$], (D-EI), m/e 296 (M − $CH_2CH_2CH(NH_2)CO_2H$), 136 (adenine H^+). Anal. ($C_{15}H_{22}N_6O_5\cdot HOAc\cdot {}^3/_2\,H_2O$) C, H, N.

L-C-S-AdoHcy-SO_2 (11). L-C-S-AdoHcy-SO_2 (**11**) was prepared by using a modification of the procedure of Borchardt and Wu.[8a] A solution of ammonium molybate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (20 mg, 0.016 mmol), in 0.55 mL of water and 0.14 mL of 70% perchloric acid was boiled with stirring until a precipitate formed (~2 min) and then for an additional 5 min. The resulting slurry was filtered and the filtrate cooled to 5 °C. To this cooled solution were added **5** (20 mg, 0.052 mmol) and 0.4 mL of 30% H_2O_2 (dropwise). The resulting slurry was stirred at ambient temperature until all the solid had dissolved (3–6 h). The resulting solution was neutralized to pH 4 with n-butylamine. This solution was loaded onto a Dowex 50-W (H^+) column (1.0 × 10 cm). The column was washed with 30 mL of water and then eluted with 30 mL of 2 N NH_3. The alkaline eluant was reduced in vacuo to 2 mL and diluted with acetone (4 mL) to turbidity. After cooling overnight, the crystals were collected by filtration and dried to yield 14.2 mg (65% yield): mp 235 °C dec; for R_f values, see Table III; ^1H NMR (Me_2SO-d_6 plus two drops deuterium oxide) δ 8.15 (2 s, 2 H, H-2 and H-8), 4.5–4.9 (m, 1 H, H-1′), 4.2–4.45 (m, 1 H, H-2′), 3.85–4.05 (m, 1 H, H-3′), 3.05–3.6 (several overlapping m, 5 H, H-α, 2 H-5′, and 2 H-γ), 1.75–2.75 (several overlapping m, 5 H, 2 H-6′, H-4; and 2 H-β); mass spectrum (DCI-NH_3), no molecular ion was detectable, m/e 371 ($MH^+ - CO_2$), 136 (adenine H^+). Anal. ($C_{15}H_{22}N_6O_6\cdot H_2O$) C, H, N.

C-S-8-AzaAdoHcy (6). C-S-8-AzaAdoHcy (**6**) was prepared in the same manner as **5** except that S-benzyl-L-homocysteine was used rather than L-homocystine. From the reaction of **2** (57 mg, 0.20 mmol) with S-benzyl-L-homocysteine (79 mg, 0.35 mmol, 1.75 meq) and Na in liquid NH_3 at −78 °C was isolated 50 mg (65% yield) of **6**: mp 248 °C dec; for R_f values, see Table III; ^1H NMR (Me_2SO-d_6 plus two drops deuterium oxide) δ 8.25 (s, 1 H, H-2), 4.85–5.15 (m, 1 H, H-1′), 4.3–4.5 (m, 1 H, H-2′), 3.75–4.0 (m, 1 H, H-3′), 3.15–3.45 (m, 1 H, H-α), 2.55–2.8 (d, 2 H, 2 H-5′), 2.5 (t, 2 H, 2 H-8), 1.7–2.35 (several overlapping m, 5 H, 2 H-6′, H-4′; and 2 H-β). Anal. ($C_{14}H_{21}N_7O_4S\cdot H_2O$) C, H, N.

C-S-N^6-MethylAdoHcy (7). C-S-N^6-MethylAdoHcy (**7**) was prepared by using a modification of the procedure reported earlier by Montgomery et al.[11a] for the synthesis of C-3-deazaAdoHcy (**8**). D,L-Homocysteine thiolactone hydrochloride (40 mg, 0.26 mmol) was dissolved in 1.0 mL of 1.0 N NaOH. This reaction mixture was stirred at ambient temperature for 30 min followed by addition of **3** (40 mg, 0.13 mmol). The resulting solution was heated and stirred at boiling for 3 h followed by stirring at ambient temperature for 12 h. The reaction mixture was acidified with

10% HCl to pH 4 and then loaded onto a Dowex 50-W (H^+) column (1.0 × 15 cm). The column was washed with 50 mL of H_2O and 75 mL of 2 N NH_3. Those fractions containing 7 were combined and reduced in vacuo. The product was further purified by thick-layer chromatography (1.0 mm) on Avicel F plates eluting with EtOH–H_2O (3:1). The product was isolated as a hydrochloride salt in 84% yield: for R_f values, see Table III; ^{1}H NMR (Me_2SO-d_6 plus two drops deuterium oxide) δ 8.15 (2 s, 2 H, H-2 and H-8), 4.5–4.8 (m, 1 H, H-1′), 4.25–4.5 (m, 1 H, H-2′), 3.75–3.95 (m, 1 H, H-3′), 3.2–3.45 (m, 1 H, H-α), 3.0 (s, NHC*H*$_3$), 2.55–2.8 (d, 2 H, 2 H-5′), 2.5 (t, 2 H, 2 H-γ), 1.6–2.3 (several overlapping m, 5 H, 2 H-6′, H-4′ and 2 H-β); mass spectrum (DCI-CH_4), *m/e* 397 (MH^+), 353 ($MH^+ - CO_2$), 296 ($MH^+ - CH_2CH_2CH(NH_2)CO_2H$), 262 (M − $SCH_2CH_2CH(NH_2)CO_2H$), 150 (N^6-methyladenine H^+). Anal. ($C_{16}H_{24}N_6O_4S\cdot{}^7/_2$ HCl·EtOH) C, H, N.

C-S-3-DeazaAdoHcy (8). C-S-3-DeazaAdoHcy (8) was prepared in 84% yield according to the procedure of Montgomery et al.:[11] mp 194–220 °C (220 °C < dec); for R_f values, see Table III; ^{1}H NMR (Me_2SO-d_6 plus two drops deuterium oxide) δ 8.15 (s, 1 H, H-8), 7.6 (d, 1 H, H-2), 6.8 (d, 1 H, H-3), 4.3–4.65 (m, 1 H, H-1′), 4.05–4.3 (m, 1 H, H-2′), 3.7–3.85 (m, 1 H, H-3′), 3.2–3.25 (m, 1 H, H-α), 2.55–2.8 (d, 2 H, 2 H-5′), 2.5 (t, 2 H, 2 H-γ), 1.5–2.35 (several overlapping m, 5 H, 2 H-6′, H-4′; and 2 H-β). Anal. ($C_{16}H_{23}N_5O_4\cdot 3H_2O$) C, H, N.

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A New Class of Cardiotonic Agents: Structure–Activity Correlations for Natural and Synthetic Analogues of the Alkaloid Pumiliotoxin B (8-Hydroxy-8-methyl-6-alkylidene-1-azabicyclo[4.3.0]nonanes)

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Pumiliotoxin B (PTX-B, 6-(6′,7′-dihydroxy-2′,5′-dimethyl-(*E*)-4′-octenylidene)-8-hydroxy-8-methyl-1-azabicyclo-[4.3.0]nonane) increases the force of contractures of spontaneously beating guinea pig atrial strips by 3- to 5-fold with half-maximal effects at about 3 μM and increases rates of atrial contractions by 2- to 3-fold with half-maximal effects at about 6 μM. The presence of an axial 7-hydroxy substituent (PTX 339A) decreases the efficacy but not the potency of PTX-B as a positive inotropic agent while having only slight effects on activity as a positive chronotropic agent. The presence of an equatorial 7-hydroxy substituent (PTX 339B) greatly decreases efficacy and potency of PTX-B as a positive chronotropic and inotropic agent. Pumiliotoxin A which lacks the side-chain 7′-hydroxy group of PTX-B causes only a 2-fold increase in force of contracture at 54 μM while having minimal effects on rate. The presence of an axial 7-hydroxy substituent (PTX 323B′ and 323B″, epimeric at the 6′-hydroxy) markedly enhances positive inotropic and chronotropic effects of PTX-A. Another congener, PTX 251D with a 6-(2′-methylhexylidene) side chain, and a synthetic analogue with a 6-(6′-heptenylidene) side chain are cardiac depressants. Both lack hydroxyl groups in the side chain. The presence of an ω-1 hydroxy group in the side chain of PTX 251D yields an alkaloid (267C) with weak positive inotropic effects and minimal chronotropic effects. The presence of an axial 7-hydroxy group in the indolizidine ring of PTX 251D results in a compound (PTX 267A) with very weak positive inotropic effects while retaining the negative chronotropic effects of PTX 251D. A synthetic analogue with a 6-(7′-hydroxyheptylidene) side chain is a cardiac depressant even though it contains a side-chain hydroxyl corresponding in position to the 7′-hydroxyl of the side chain of PTX-B. The positive chronotropic and inotropic effects of pumiliotoxin B are reversed only by relatively high concentrations of the calcium channel blockers nifedipine and verapamil, suggesting that pumiliotoxin B may owe its cardiotonic activities to effects on internal mobilization of calcium.

An alkaloid, pumiliotoxin B (PTX-B, Figure 1), from the neotropical frog *Dendrobates pumilio*,[1,2] has marked myotonic[3,4] and cardiotonic[4] activity. In nerve-striated muscle preparations, PTX-B (1–30 μM) markedly increased both direct and indirect elicited twitch.[3] It was markedly more potent (10-fold) in potentiating contractures with isolated muscle fibers. PTX-B had no effect on Na^+, K^+, or Cl^- conductances[3] nor on Na^+-K^+-ATPase but was reported at high concentrations to inhibit Ca^{2+}-ATPase of sarcoplasmic reticulum.[5] It was proposed that PTX-B in some manner facilitated or enhanced stimulus-evoked release of calcium in both nerve and muscle.[3] In atria, PTX-B (1.5–7.5 μM) caused marked positive inotropic and chronotropic effects which were readily reversible and not blocked by a β-antagonist.[4] The present paper concerns structure–activity correlations for this class of alkaloids with respect to positive inotropic and chronotropic effects in spontaneously beating guinea pig atria.

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