

## Communications to the Editor

### S-Aristeromycinyl-L-homocysteine, a Potent Inhibitor of S-Adenosylmethionine-Dependent Transmethylations

Sir:

The observation that S-adenosylhomocysteine (SAH) inhibits S-adenosylmethionine (SAM)-dependent methyltransferases has stimulated considerable interest in chemically modifying the structure of SAH. Such analogs of SAH have been shown to be effective inhibitors in vitro of the enzymes which catalyze this type of transmethylation.<sup>1</sup> In earlier papers from our laboratory we have described the synthesis and enzymatic activity of SAH analogs with modifications in the amino acid, base, or sugar portions of this molecule.<sup>2-4</sup> Some of the more active analogs of SAH were those with modifications in the sugar moiety [e.g., 2'-deoxy-SAH, 3'-deoxy-SAH, and S-5'-[9-(arabinofuranosyl)adenyl]-L-homocysteine (S-AraAH)].<sup>4</sup> Of the methyltransferases investigated, phenylethanolamine N-methyltransferase (PNMT) appeared best able to accommodate such changes in the sugar moiety of SAH, since 2'-deoxy-SAH, 3'-deoxy-SAH, and S-AraAH were potent inhibitors of this enzyme. Coward and coworkers<sup>5,6</sup> previously prepared analogs of SAH in which the ribose moiety was replaced by a cyclopentyl group, a 2,3-dihydroxycyclopentyl group, or a five-carbon acyclic bridge. These analogs were found, however, to be nearly inactive as inhibitors of catechol O-methyltransferase (COMT). These results suggested that the 1',4'-oxygen bridge was involved in the enzymatic binding or more likely involved in maintaining the proper orientation of the base and amino acid portions of SAH during its interaction with COMT. After observing that the various sugar modified analogs of SAH (2'-deoxy-SAH, 3'-deoxy-SAH, and S-AraAH) reported in our studies<sup>4</sup> were potent inhibitors of methyltransferases, we decided to synthesize and to reexamine the activity of S-aristeromycinyl-L-homocysteine (SAMH) as a potential inhibitor of SAM-dependent methyltransferases.

Thanks to the generosity of Dr. T. Kishi of Takeda Chemical Industries, we were able to obtain a sample of aristeromycin, which we used to prepare SAMH. Coward and coworkers<sup>6</sup> had previously reported the synthesis of SAMH from aristeromycin through the intermediate formation of the 2',3'-isopropylidene 5'-O-tosylate derivative. In contrast, we have used a general two-step synthesis to prepare SAMH, which affords a better yield of this SAH analog.<sup>7</sup> The synthesis involves the formation of the intermediate 5'-chloro-5'-deoxyaristeromycin using a general procedure previously described by Kikugawa and Ichino.<sup>8</sup> The 5'-chloro-5'-deoxyaristeromycin was subsequently allowed to react with L-homocysteine in sodium and liquid ammonia to afford SAMH in 56% yield. The 5'-chloro-5'-deoxyaristeromycin was prepared by reaction of aristeromycin (100 mg, 0.377 mmol) with thionyl chloride (0.2 ml) in hexamethylphosphoramide (2 ml) for 15 hr to yield 106 mg (~100%) of the desired intermediate: mp 162-165°; NMR (Me<sub>2</sub>SO-d<sub>6</sub>)  $\delta$  1.57-2.47 (m, 2 H, -C<sub>1</sub>-CH<sub>2</sub>-C<sub>4</sub>-), 3.67-4.13 (m, 3 H, C<sub>4</sub>-H and C<sub>5</sub>-H<sub>2</sub>), 4.13-5.00 (m, 5 H, C<sub>1</sub>-H, C<sub>2</sub>-H, C<sub>3</sub>-H and C<sub>2</sub>-OH, C<sub>3</sub>-OH), 6.87 (broad, 2 H, -NH<sub>2</sub>), 8.17, 8.23 (2 s, 2 H, C<sub>2</sub>H and C<sub>8</sub>-H). Anal. (C<sub>11</sub>H<sub>14</sub>O<sub>2</sub>N<sub>5</sub>Cl) C, H, N.

The 5'-chloro-5'-deoxyaristeromycin (60 mg, 0.21 mmol) was condensed with L-homocysteine anion which was

generated from L-homocysteine (55 mg, 0.30 mmol) in sodium and liquid ammonia according to a general procedure previously described by our laboratory.<sup>7</sup> The desired SAMH was purified by thick-layer chromatography<sup>4,7</sup> to yield 45 mg (56%): mp 240 dec°; one spot material on TLC with *R<sub>f</sub>* 0.54 [silica gel GF, 250  $\mu$ , solvent 1 (EtOH-HOAc-H<sub>2</sub>O, 50:3:5) and 9 parts (pH 7.0, 0.02 M phosphate buffer)]; NMR (D<sub>2</sub>O)  $\delta$  1.87-2.50 (m, 4 H, C<sub>3</sub>-H<sub>2</sub> and C<sub>4</sub>-CH<sub>2</sub>-C<sub>1</sub>-), 2.37-3.00 (m, 4 H, C<sub>5</sub>-H<sub>2</sub> and C<sub>7</sub>-H<sub>2</sub>), 3.47-3.99 (m, 1 H, C<sub>4</sub>-H), 3.88 (m, 1 H, C<sub>4</sub>-H), 8.07, 8.13 (2 s, 2 H, C<sub>2</sub>H and C<sub>8</sub>-H). Anal. (C<sub>15</sub>H<sub>22</sub>N<sub>6</sub>O<sub>4</sub>S·2.5H<sub>2</sub>O) C, H, N. Since the earlier reported synthesis of SAMH gives uv spectral data (*V*<sub>max</sub> = 261 nm)<sup>6</sup> and no analytical data for the final product, a comparison with our material was difficult. However, our uv data were consistent with that reported earlier.<sup>6</sup> In addition, our NMR and analytical data support the assigned structure for SAMH.

SAMH was then tested as an inhibitor of COMT, PNMT, histamine N-methyltransferase (HMT), and hydroxyindole O-methyltransferase (HIOMT). In contrast to the earlier reports on the inactivity of SAMH,<sup>6</sup> we have found that SAMH is a potent inhibitor of various methyltransferases including COMT. The kinetic patterns of inhibition of COMT, PNMT, HMT, and HIOMT by SAMH were determined and the resulting inhibition constants are listed in Table I. Included for comparison are the inhibition constants for SAH. In all cases linear competitive patterns of inhibition were observed when SAM was the variable substrate and SAMH the inhibitor. Of particular interest is the observation that SAMH (*K<sub>i</sub>* = 7.50  $\pm$  0.62  $\mu$ M) was a significantly better inhibitor of PNMT than SAH (*K<sub>i</sub>* = 29.0  $\pm$  2.84  $\mu$ M). In addition, however, SAMH was also found to have inhibitory activity somewhat comparable to SAH itself toward COMT, HMT, and HIOMT. SAMH is an SAH analog in which the 1',4'-oxygen bridge is replaced by a methylene group and has a carbon-nitrogen linkage between the adenine base and cyclopentyl moiety instead of the usual N-glycosidic linkage which is found in the natural nucleosides. The strong inhibitory activity of SAMH toward these methyltransferases would suggest that the 1',4'-oxygen bridge is not involved in binding to COMT, PNMT, HMT, and HIOMT and that the usual N-glycosidic linkage is not an absolute requirement for maintaining the active conformation of SAH when binding to these enzymes. The extremely potent inhibitory activity of SAMH toward PNMT is consistent with our earlier observations<sup>4</sup> that PNMT can tolerate changes in the sugar portion of SAH without these modifications drastically affecting enzymatic binding. This very potent inhibitory activity of SAMH toward PNMT provides another example of the fact that the SAH binding sites on methyltransferases show differences in specificity toward the structural features of SAH and such differences can be used for the design of specific inhibitors of these enzymes.

The inhibitory activity of SAMH toward COMT reported above contradicts the results reported earlier by Coward et al.<sup>6</sup> Testing the SAMH prepared in our laboratory under conditions identical with those previously reported by Coward et al.<sup>6</sup> (SAM concentration = 1.00 mM), we find that a concentration of 1.5 mM SAMH produced 71% inhibition of the COMT-catalyzed reaction in contrast to the lack of inhibitory activity previously

Table I. Kinetic Inhibition Constants for SAH and SAmH toward COMT, PNMT, HMT, and HIOMT<sup>a</sup>

$\begin{array}{c} \text{HO}_2\text{CCHCH}_2\text{CH}_2\text{SCH}_2 \\   \\ \text{NH}_2 \end{array} \quad \begin{array}{c} \text{adenine} \\ \diagup \quad \diagdown \\ \text{X} \\ \diagdown \quad \diagup \\ \text{OH} \quad \text{OH} \end{array}$					
Inhibition constants, <sup>b</sup> $\mu\text{M}$ , $K_{is} \pm \text{SEM}$					
Compd	X	COMT	PNMT	HMT	HIOMT
SAH	O	36.3 $\pm$ 2.2	29.0 $\pm$ 2.84	18.1 $\pm$ 2.19	18.5 $\pm$ 1.9
SAmH	CH <sub>2</sub>	168 $\pm$ 39	7.59 $\pm$ 0.62	59.8 $\pm$ 11.9	51.8 $\pm$ 6.3

<sup>a</sup> COMT, PNMT, HMT, and HIOMT were purified and assayed as described earlier.<sup>2-4</sup> SAM concentration, 24–210  $\mu\text{M}$ .

<sup>b</sup> Each inhibitor showed linear competitive kinetic patterns and the inhibition constants were calculated as previously described.<sup>2-4</sup>

reported.<sup>6</sup> The differences between our results and those of Coward et al.<sup>6</sup> are difficult to explain.

SAmH was also converted to the corresponding S-aristeromycinyl-L-methionine-methyl-<sup>14</sup>C (SAMM-<sup>14</sup>CH<sub>3</sub>) using methyl-<sup>14</sup>C iodide in a mixture of formic acid and acetic acid.<sup>9</sup> SAMM was found to be a good substrate for COMT, PNMT, HMT, and HIOMT with kinetic constants ( $K_m$  and  $V_{max}$ ) comparable to SAM itself. It would appear, therefore, that for the binding of SAM and for the enzyme-catalyzed methyl transfer reaction the 1',4'-oxygen bridge of SAM is not absolutely crucial. Further work is continuing in our laboratory in an effort to further define the structural requirements for the binding of SAH and SAM to these methyltransferases.

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