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L-2-AMINO-4-METHOXY-cis-BUT-3-ENOIC ACID, A POTENT INHIBITOR OF THE ENZYMATIC SYNTHESIS OF S-ADENOSYLMETHIONINE

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L-2-Amino-4-methoxy-cis-but-3-enoic acid was synthesized and found to be an extremely potent inhibitor of ATP:L-methionine adenosyltransferase isozymes of rat liver and Novikoff solid hepatoma. The inhibitory potency of L-2-amino-4-methoxy-cis-but-3-enoic acid was found to be significantly higher than that of its structural analogues, L-methoxinine and L-2-amino-4-methoxy-trans-but-3-enoic acid. The K_i values of L-2-amino-4-methoxy-cis-but-3-enoic acid and the K_m values of L-methionine are comparable for the rat liver and tumor isozymes, suggesting a close correlation between the enzyme-bound conformations of these two molecules.

S-Adenosylmethionine occupies a strategic position in biochemical pathways essential to cell growth and function. This can be attributed to its participation as methyl donor in numerous transmethylation processes, and after decarboxylation, as propylamine donor in polyamine biosynthesis. The chemotherapeutic potential of designing selective inhibitors of the enzymatic synthesis of Ado-Met[4] has been studied extensively by Talalay and coworkers (1-5) whose studies on L-methionine analogue inhibitors of isofunctional ATP:L-methionine S-adenosyltransferases have defined steric, electronic and conformational properties of L-methionine that can be correlated with

4. S-Adenosyl-L-methionine (Ado-Met); ATP:L-methionine S-adenosyltransferase (EC 2.5.1.6) (methionine adenosyltransferase, MAT); cis and trans isomers of L-2-amino-4-methoxy-but-3-enoic acid (L-cis-AMB and L-trans-AMB); methionine adenosyltransferase isozymes of rat liver (rat liver iso 1, rat liver iso 2 and rat liver iso 3) and of Novikoff solid hepatoma (tumor iso 1 and tumor iso 2); 1-aminocyclopentane-1-carboxylic acid (cycloleucine).

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substrate and inhibitory activities. Our interest has continued in identifying new methionine analogue inhibitors of methionine adenosyltransferases as an approach to the development of selective chemotherapeutic agents.

In this communication we report the synthesis of L-2-amino-4-methoxy-cis-but-3-enoic acid (L-cis-AMB) and the observation that this molecule is a potent in vitro inhibitor of methionine adenosyltransferase isozymes from rat liver and Novikoff hepatoma. This finding complements earlier studies (6,7) of the antimicrobial and growth inhibitory properties of the corresponding trans-isomer, L-2-amino-4-methoxy-trans-but-3-enoic acid (L-trans-AMB), a natural product isolated from the fermentation broth of Pseudomonas aeruginosa (6). In particular, Tisdale (7) has found that L-trans-AMB is an in vitro inhibitor of protein methylation in Walker carcinoma, but that the mechanism of this inhibition does not correlate with any effects on the intracellular levels of Ado-Met. Our findings of the unexceptional in vitro inhibitory activities of L-trans-AMB towards methionine adenosyltransferases of rat liver and Novikoff hepatoma support Tisdale's claim that the trans-isomer does not inhibit methylation via direct effects on the enzymatic biosynthesis of Ado-Met.

MATERIALS AND METHODS

The inhibitory potencies of all amino acid analogues were examined by assay of the separated and partially purified isozymes of rat liver (rat liver iso 1, rat liver iso 2 and rat liver iso 3) and Novikoff solid hepatoma (tumor iso 1 and tumor iso 2). These isozymes were obtained using methods previously reported by Hoffman and Kunz (8,9). The concentrations of inhibitors required to produce 50% inhibition of activity for each isozyme were determined using Dixon plots at subsaturating levels of L-methionine, specified for each isozyme in Table 1 and under well-defined assay conditions described by us previously (10). The structures of the amino acid analogues are shown in Figure 1. The sources of amino acid analogues are indicated: cycloleucine[4] (Sigma Chemical Company, St. Louis, Mo.); L-methoxinine (Hoffmann-LaRoche, Inc., Nutley, N.J.); L-2-amino-4-methoxy-trans-but-3-enoic acid was prepared according to published procedures (11); the synthesis of L-2-amino-4-methoxy-cis-but-3-enoic acid is described in detail that follows.

L-2-amino-4-methoxy-cis-but-3-enoic acid (L-cis-AMB). A solution consisting of 0.6 g (3.2. mmol) of D,L-methyl-2-acetamido-4-methoxy-cis-but-3-enoate (11), 3.91 ml of 0.82 N lithium hydroxide and 6 ml of methanol was allowed to stand at ambient temperature for 3 1/2 h. It was concentrated in vacuo and the residue dissolved in 10.7 ml of deionized water. The pH was adjusted to 7.4 with 6N hydrochloric acid, 2.0 mg of hog kidney acylase I (Sigma Chemical Co.) was added and the resultant solution stirred at 40° for

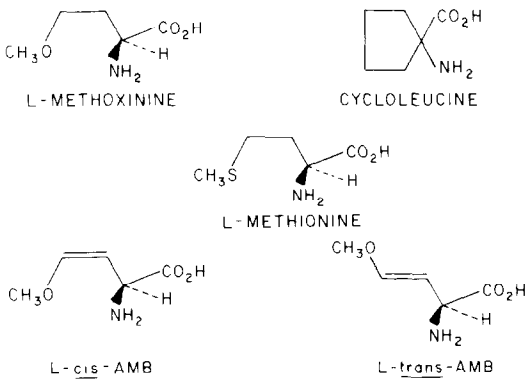

Fig. 1. Structures of amino acid analogues.

16 h. The pH of the solution was adjusted to 10.4 with 25% aqueous trimethylamine and applied to an ion exchange column (AG 1-X8; 100-200 mesh; acetate form; 7.5 ml of resin bed). The column was washed with 30 ml of 25% aqueous trimethylamine and 50 ml of water. The L-amino acid was eluted with 0.1N acetic acid. Concentration of the acetic acid fraction gave 124 mg of a pale yellow solid which was crystallized from methanol/ethanol/ether to give 50 mg of L-2-amino-4-methoxy-cis-but-3-enoic acid: mp 182-185° dec; $[\alpha]_D^{25}$ + 105.8° (c 0.992, H_2O); NMR (D_2O) δ 6.91 (d, 1H, J = 5 Hz, -OCH=CH-), 5.16 and 5.04 (m, 2H, -OCH=CH- and =CH-CH<), 4.19 (3H, s, CH_3O-); mass spectrum m/e 86 (M^+-COOH).

Anal. Calcd. for $C_5H_9NO_3$: C, 45.80; H, 6.92; N, 10.68. Found: C, 45.69; H, 7.16; N, 10.61.

RESULTS AND DISCUSSION

The inhibitory properties of L-cis-AMB have been compared to those of cycloleucine, L-methoxinine and L-trans-AMB. Cycloleucine, a well-studied antitumor agent, was first recognized as a methionine adenosyltransferase inhibitor by Lombardini et al. (1) and has been used in these studies as a standard measure for MAT inhibition. Both L-cis-AMB and L-trans-AMB are constrained analogues of L-methoxinine (Fig. 1) whose comparative inhibitory properties are therefore of interest. As seen in Table 1 and Table 2, both the K_i and I_{50} values of cycloleucine and L-methoxinine are comparable for the three rat liver MAT isozymes. In contrast, a comparison of the K_i values of L-cis-AMB and L-methoxinine indicates that the values for L-cis-AMB are 14X, 3.5X and 4.5X lower for rat liver iso 1, rat liver iso 2 and rat liver iso 3, respectively. A similar comparison of their I_{50} values towards rat liver and tumor MAT isozymes shows L-cis-AMB to be 2 - 8.5 times more potent than L-methoxinine. On the other hand, the I_{50} values towards rat liver and tumor

Table 1

Kinetic inhibition constants for methionine analogue inhibitors of methionine adenosyltransferase isozymes of rat liver and Novikoff solid hepatoma

Measured at ATP concentration of 5 mM.

Compound	K_i (μM)				
	rat liver			tumor	
	iso 1	iso 2	iso 3	iso 1	iso 2
L-Methionine (K_m)	34.5±4.4	11.1±3.3	93±5	11.0±1.6	5.78±0.24
Cycloleucine	516±36	263±22	1633±73	382±131	209±10
L-Methoxinine	788	170	1223	---	---
L-cis-AMB	56±14	49±8	275±71	23±6	28±2

Means ± SEM (N=3)

MAT isozymes indicate L-trans-AMB is 12 - 28 times less potent than L-methoxinine. These observations of the increased inhibitory potency of L-cis-AMB and the reduced inhibitory potency of L-trans-AMB relative to that of L-methoxinine are significant and suggest that the presence of a conformational restraint, as well as the critical orientation of the cis-methoxy substituent

Table 2

Inhibitory potencies of methionine analogues on methionine adenosyltransferase isozymes of rat liver and Novikoff solid hepatoma. The enzyme activity was measured at a subsaturating level of L-methionine at least 50% below the respective K_m (L-methionine) value of each isozyme: [1 μM] : rat liver iso 2, tumor iso 1 and tumor iso 2; 5 μM: rat liver iso 1; 37.5 μM: rat liver iso 3.

Compound	Concentration required for 50% inhibition (mM)				
	rat liver			tumor	
	iso 1	iso 2	iso 3	iso 1	iso 2
Cycloleucine	0.29±0.02	0.18±0.01	2.5±0.14	0.19±0.03	0.14±0.01
L-Methoxinine	0.24±0.03	0.27±0.04	3.6±0.55	0.11±0.02	0.09±0.01
L-cis-AMB	0.046±0.004	0.036±0.001	0.43±0.07	0.012±0.003	0.041±0.002
L-trans-AMB	7.2±1.4	4.6±1.6	43.8±16.4	2.8±0.3	2.2±0.1

Means ± SEM (N > 3)

in L-cis-AMB facilitate a good correlation between its enzyme-bound conformation with that of the natural substrate, L-methionine. Furthermore, the close correspondence between the K_i values of L-cis-AMB and the K_m values of L-methionine towards rat liver and tumor methionine adenosyltransferase isozymes strongly suggests that the use of L-cis-AMB to study inhibition of transmethylation processes might be preferred to that of cycloleucine, which has been used previously for this purpose in a number of well-defined biologic systems (12,13). It is also encouraging that the K_i values of L-cis-AMB are 2-10 fold lower for the tumor MAT isozymes, as compared to the rat liver isozymes, suggesting some degree of selectivity for the tumor-derived forms. It remains to be seen whether the very potent inhibitory effects of L-cis-AMB are a general phenomenon for other isofunctional methionine adenosyltransferases of microbial and plant origin, or whether these effects are limited to the mammalian enzymes.

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