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Analogues of Methionine as Substrates and Inhibitors of the Methionine Adenosyltransferase Reaction

Deductions Concerning the Conformation of Methionine

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SUMMARY

Steric, electronic, and conformational requirements are described for analogues of L-methionine essential to their function as substrates or inhibitors of the methionine adenosyltransferase reaction (ATP:L-methionine *S*-adenosyltransferase, EC 2.4.2.13). With the aid of partially purified transferase preparations from *Escherichia coli*, bakers' yeast, and rat liver, a systematic study of substrate analogues has been undertaken. Inhibitors of the enzyme fall into three categories: (a) straight carbon chain amino acids, such as L-2-amino-4-hexenoic acid (*trans* but not the *cis* isomer) and L-2-amino-4-hexynoic acid, which are the most potent inhibitors; (b) cyclic amino acids, among which 1-aminocyclopentanecarboxylic acid and one of the four isomers of 1-amino-3-methylcyclopentanecarboxylic acid (either the 1R,3R or the 1S,3R isomer) are the most powerful; and (c) *O*-acetyl-L-serine, *O*-carbamyl-L-serine, and *S*-carbamyl-L-cysteine. Since inhibitors belonging to groups (a) and (b) possess considerable conformational rigidity by virtue of the presence of unsaturations or cyclic structures, it has been possible to draw conclusions with respect to the conformation of L-methionine at the active site of the adenosyltransferase reaction. A number of the inhibitors of the methionine adenosyltransferase reaction, such as 1-aminocyclopentanecarboxylic acid and *S*-carbamyl-L-cysteine, are known to be inhibitors of the growth of certain microorganisms and tumors. The possibility is suggested that these inhibitory activities may be mediated at least in part through the inhibition of the synthesis of *S*-adenosyl-L-methionine.

INTRODUCTION

This paper describes the steric, electronic, and conformational features of analogues of methionine essential for their function as substrates or inhibitors of the methionine adenosyltransferase reaction (ATP:L-methionine *S*-adenosyltransferase, EC 2.4.2.13). These molecular characteristics have been explored with partially purified transferase preparations obtained from *Escherichia coli*, bakers' yeast, and rat liver. The ultimate goal of these studies is the development of selective and specific inhibitors of these enzymes and their exploitation as potential chemotherapeutic agents.

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The design of antagonistic structural analogues of essential metabolites has been widely practiced since the recognition that sulfanilamide is a competitive antagonist of *p*-aminobenzoic acid and blocks the incorporation of the latter into 5,6-dihydropteroic acid in susceptible microbial systems (1, 2). Systematic guidelines for the design of such inhibitors were formalized in the monograph by Woolley (3). More recently, efforts at the rational design of chemotherapeutic agents have pointed to the critical importance of common conformational features among effective structural analogues (4, 5).

It is now abundantly clear that enzymes from diverse sources catalyzing the same reaction (*isofunctional enzymes*) may display large differences in their susceptibility to inhibitors, and that such differences may be responsible for selective toxicity (6). The classic work of Burchall and Hitchings (7) on several classes of inhibitors of folic reductases has emphasized that such inhibitors may reveal large differences in the active sites of isofunctional enzymes, and that these differences may be exploited in the design of selective chemotherapeutic agents.

Methionine, an essential amino acid for mammalian species, participates in a variety of enzymatic reactions, in which different regions of the molecule undergo activation or transformation. These reactions have received little systematic study with respect to their inhibition by structural analogues of methionine. It is known from the work of Skinner, Edelson, and Shive (8) that the *cis* (but not the *trans*) isomer of DL-2-amino-4-hexenoic acid is a weak antagonist of methionine for the growth of *E. coli*. In contrast, Mudd (9) has shown that the *trans* (but not the *cis*) isomer of DL-2-amino-4-hexenoic acid is an inhibitor of yeast methionine adenosyltransferase. In a series of studies Shive and Skinner (10) prepared a number of structural analogues of methionine, lysine, and other amino acids, in which unsaturation or ring structures imposed conformational restrictions. These workers put forward suggestions on the possible conformation of such amino acids at their "sites of utilization". Their conclusions were based on microbial nutrition experiments and could not define the precise metabolic site for these antagonisms, because of absence of information at that time on the essential enzymatic reactions in which the natural amino acids participate.

In the present work, a number of structural analogues of methionine, characterized by conformational restrictions resulting from unsaturation or ring formation, and with varying degrees of electronegativity in the region of the molecule corresponding to that occupied by the sulfur atom in methionine, have been used to probe the preferred conformation of methionine in the methionine adenosyltransferase reaction. The mechanism of this enzymatic reaction has already been studied extensively by Cantoni and Durell (11), Mudd and Cantoni (12), Mudd (13), and Mudd and Mann (14). More recently, Greene (15) has subjected the yeast transferase to kinetic analysis and established the ordered addition of reactants. Mechanistic studies of the reaction have shown that the total dephosphorylation of ATP results in the liberation of the two innermost phosphate groups of ATP as inorganic pyrophosphate, and of the terminal phosphate as inorganic orthophosphate. The enzyme also possesses tripolyphosphatase activity that is markedly accelerated by low concentrations of *S*-adenosylmethionine (16).

EXPERIMENTAL PROCEDURE

Materials

All solutions were prepared in deionized, glass-distilled water from chemicals of the best commercial grades available. All compounds that possessed significant inhibitory potency or were of particular interest were recrystallized in this laboratory. The sources of the chemicals were as follows: L-methionine, L-norleucine, DL-homoserine, L-serine, L-leucine, L-cysteine, DL-methionine sulfoxide, DL-methionine sulfone, and ammonium sulfate (special enzyme grade) were supplied by Mann Research Laboratories. D-Ethionine, L-norvaline, D-norvaline, 2-methyl-DL-methionine, D-methionine, *S*-ethyl-L-cysteine, *S*-methyl-L-cysteine, and

yeast inorganic pyrophosphatase were obtained from Nutritional Biochemicals. The commercially available calcium salt of 2-hydroxy-DL-methionine [DL-2-hydroxy-4-(methylthio)butyric acid] was converted to the free acid, purified by distillation (b.p. 62–64° at 20 μ of mercury), and converted to its methyl ester. D-Norleucine, *O*-acetyl-L-serine, L-homocysteine thiolactone hydrochloride, trifluoro-L-methionine (*S*-trifluoromethyl-L-homocysteine), *O*-carbamyl-L-serine, and 1-aminocyclopentanecarboxylic acid were purchased from Cyclo Chemical Corporation. *N*-Acetyl-DL-methionine, L-ethionine, L-methionine ethyl ester hydrochloride, *N*-formyl-DL-methionine, glutathione, and tris(hydroxymethyl)aminomethane base were obtained from Sigma Chemical Company. DL-Methioninol [DL-2-amino-4-(methylthio)butan-1-ol], DL-homocysteine (free base), and *O*-methyl-DL-serine were supplied by Pfaltz and Bauer. *S*-Carbamyl-L-cysteine, L-methionine methyl ester hydrochloride, and L-methionine-*S*(RS)-sulfoximine were obtained from Aldrich Chemical Company, Pierce Chemical Company, and Calbiochem, respectively. Adenosine 5′-triphosphate (disodium) was supplied by P-L Biochemicals. Radioactivity was determined with a liquid scintillation spectrometer with an efficiency for ^{14}C of about 60%. The primary and secondary scintillators PPO (2,5-diphenyloxazole) and POPOP {*p*-bis[2-(5-phenyloxazolyl)]benzene} were purchased from New England Nuclear. Spectroscopic quality *p*-dioxane and glycerol and reagent grade naphthalene were purchased from Matheson, Coleman, and Bell. The cation and anion exchange Dowex resins AG 50W-X2 (100–200 mesh) and AG 1-X10 (200–400 mesh) and DEAE-cellulose (Cellex D) were supplied by Bio-Rad Laboratories. Disodium ethylenediaminetetraacetate was purchased from J. T. Baker. 2-Mercaptoethanol was purchased from Eastman Organic Chemicals and distilled under reduced pressure (b.p. 58–60° at 23 mm of Hg). [^{14}C-Methyl]-L-methionine (53.6 mCi/mmole) and adenosine 5′-triphosphate-8-^{14}C tetralithium (47 mCi/mmole) were the products of Amersham/Searle Corporation and Schwarz Bio-Research. Sprague-Dawley female rats (180–200 g) were purchased from Huntington Farms. Fleischmann bakers' yeast was purchased from local food markets. *E. coli* strain B (midlogarithmic) cells were obtained frozen from Grain Processing, Muscatine, Iowa. Crystallized bovine plasma albumin was a product of Armour Pharmaceutical Company. Methyl 1-aminocyclopentanecarboxylate, 1-aminocyclohexanecarboxylic acid, 1-aminocycloheptanecarboxylic acid, 1-amino-2-ethylcyclopentanecarboxylic acid, 1-amino-*trans*-1,3-cyclopentanedicarboxylic acid, and DL-2-amino-6-(methylthio)hexanoic acid were the kind gifts of Drs. H. B. Wood, Jr., and S. Schepartz of the Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda, Md. The 1-aminocyclopropane- and 1-aminocyclobutanecarboxylic acids were gifts of Dr. W. C. J. Ross of the Chester Beatty Research Institute, London (17). *trans*-3-Dehydro-L-methionine [L-2-amino-4-(methylthio)-*trans*-3-butenoic acid] was the kind gift of Professor K. Balenović of the University Chemical Laboratory, Zagreb, Yugoslavia (18). The syntheses of DL-2-amino-4-hexynoic acid, L-2-amino-4-hexynoic acid, DL-2-amino-*trans*-4-hexenoic acid, DL-2-amino-*cis*-4-hexenoic acid, and DL-2-amino-5-chloro-*trans*-4-hexenoic acid have been described (8, 19, 20). L-2-Amino-4-pentynoic acid, DL-2-amino-4-pentenoic acid, and DL-2-amino-5-(methylthio)pentanoic acid were prepared according to published procedures (21–23).

There are four isomeric 1-amino-3-methylcyclopentanecarboxylic acids (Fig. 4). The 1R,3R and 1S,3R diastereomers were prepared in this laboratory by Strecker synthesis from (+)-3-methylcyclopentanone (3R), and separated by high resolution cation exchange chromatography.[1] The compounds, in order of their elution from the column, will be referred to as isomers A and B, respectively, although assignment of absolute configuration cannot be made at present. The two racemic mixtures (1R,3R + 1S,3S) and (1S,3R + 1R,3S) of 1-amino-3-methylcyclopentanecarboxylic acid were also synthesized in this laboratory and

[1] H. Doshan, manuscript in preparation.

separated from each other.[1] We are indebted to Dr. H. Doshan of this department for gifts of the isomeric 1-amino-3-methylcyclopentanecarboxylic acids. All new compounds were characterized by mass, infrared, and nuclear resonance spectroscopy. The purity of these compounds was established on an amino acid analyzer.

Methods

Preparation of Methionine Adenosyltransferase

E. coli strain B. The enzyme was prepared according to the procedure of the Tabors (24). All operations were conducted at 0–4°, except when otherwise indicated. A 150-g portion of frozen midlogarithmic *E. coli* strain B cells was thawed in 350 ml of 10 mM potassium phosphate buffer (pH 7.0) containing 5 mM 2-mercaptoethanol. Small batches of this suspension were then sonically disrupted for 15 min at 9 kc with an MSE ultrasonic disintegrator, model 60W, while the temperature was maintained below 10°. Saturated ammonium sulfate solution (neutralized with NH_4OH) containing 5 mM 2-mercaptoethanol was added to give 33% final saturation, and the mixture was allowed to stir slowly overnight. The precipitate was removed by centrifugation for 20 min at 12,000 × g_{max} and discarded. Neutral saturated ammonium sulfate solution was added to the supernatant fluid to give a final saturation of 50%, and the mixture was allowed to stir for 2 hr and centrifuged. The precipitate (33–50% ammonium sulfate) was dissolved in 10 mM potassium phosphate buffer (pH 7.0) containing 5 mM 2-mercaptoethanol and allowed to stir slowly overnight. The supernatant fluid was fractionated with the above ammonium sulfate solution, and the precipitate obtained between 33 and 38% saturation was dissolved in 10 mM potassium phosphate–5 mM 2-mercaptoethanol (pH 7.0). An approximately 40-fold purification with a recovery of about 15% of the total enzyme activity was achieved. The specific activity was 2.5 units (see below)/mg of protein. At this stage of purification the enzyme is stable for at least 6 months at −15°.

Bakers' yeast. This methionine adenosyltransferase was prepared by modification of the procedure of Stekol (25). Dried Fleischmann's yeast (600 g) was homogenized with 1800 ml of 67 mM K_2HPO_4 containing 3.6 g of DL-methionine in a cooled (0–4°) Gifford-Wood mill for 15 min at 30 V with a 0.005-inch aperture. The mixture was then incubated for 4 hr at 32°, cooled, and centrifuged at 4900 × g_{max} for 20 min in a Servall GSA centrifuge head. An acetone fractionation was performed, and the precipitate obtained between 24 and 45% acetone (by volume) was suspended in 20 mM potassium phosphate buffer (pH 6.6) and then dialyzed overnight against 25 volumes of 1 mM potassium phosphate (pH 7.0) containing 5 mM 2-mercaptoethanol. The dialyzed material was chromatographed on a column (2.5 × 47 cm) packed with DEAE-cellulose previously washed by the procedure of Peterson and Sober (26), and then equilibrated with 1 mM potassium phosphate buffer (pH 7.0) containing 5 mM 2-mercaptoethanol. An elution gradient was generated with a constant volume mixing chamber containing 800 ml of the equilibrating buffer, connected to a reservoir containing 0.4 M potassium phosphate (pH 7.0) and 5 mM 2-mercaptoethanol. Fractions (8 ml) containing significant quantities of enzyme with high specific activity were combined and fractionated with a saturated neutralized ammonium sulfate solution. The precipitate (50–75% saturation with ammonium sulfate) was dissolved in 1 mM potassium phosphate (pH 7.0)–5 mM 2-mercaptoethanol. At this stage it is critical to measure and adjust the protein concentration of the enzyme solution to approximately 10 mg/ml before a series of negative bentonite adsorptions are undertaken. Bentonite was added in four different stages with constant monitoring of the enzyme activity. For each milliliter of enzyme solution, 30 mg of bentonite were added, the mixture was stirred for 20 min and centrifuged for 10 min at 12,000 × g_{max}, and the precipitate was discarded. This step was repeated three more times with 15 mg of bentonite per milliliter of enzyme. The bentonite reduced the protein concentrations to about 3 mg/ml without significantly reducing the enzyme activity. The enzyme

was then fractionated with saturated neutral ammonium sulfate. The fraction precipitating between 53 and 84% saturation was dissolved in 1 mM potassium phosphate (pH 7.0)–5 mM 2-mercaptoethanol and could be stored for at least 6 months at −15°. An approximately 180-fold purification over the initial homogenate was obtained with a recovery of about 15% of the total enzyme activity. The specific activity was 5.2 units/mg of protein.

Rat liver. The preparation of the methionine adenosyltransferase from rat liver followed essentially the procedures of Cantoni and Durell (11) and Pan and Tarver (27). From female Sprague-Dawley rats, weighing approximately 180–200 g, 78 g of liver were obtained and homogenized in 0.01 N acetic acid and then centrifuged at 27,000 × g_{max} for 30 min. The supernatant fluid was fractionated with saturated ammonium sulfate (pH 7.0) containing 5 mM 2-mercaptoethanol and 0.2 mM EDTA. The fraction precipitating between 33 and 45% saturation was then dissolved in, and dialyzed for 8 hr against, 50 mM potassium phosphate buffer (pH 7.0) containing 5 mM 2-mercaptoethanol and 0.2 mM EDTA. The dialyzed enzyme solution was mixed with glycerol to a final concentration of 20% of glycerol by volume and then fractionated on a DEAE-cellulose column (2.7 × 40 cm) previously equilibrated with 50 mM potassium phosphate (pH 7.0) containing 5 mM 2-mercaptoethanol, 0.2 mM EDTA, and 20% glycerol. The gradient was generated by means of a constant volume mixing chamber of 800 ml containing the equilibrating buffer, and was connected to a reservoir containing buffer of the same composition but 0.2 M with respect to potassium phosphate (pH 7.0). The eluted fractions (7 ml each) containing significant quantities of enzyme activity with high specific activity were stored at −15° and were stable for at least 6 months under these conditions. The specific activity of the enzyme is approximately 6.0 units/mg of protein and represents about a 40-fold purification with a recovery of 40% of the initial enzyme activity.

Enzymatic Assays

Methionine adenosyltransferase assays as described by Cantoni and Durell (11) were carried out in a final volume of 0.25 ml, containing (in micromoles) KCl, 50; ATP, 3–5; L-methionine, 0.00938, 0.1, or 5.0, depending upon the purpose of the experiment; Tris-HCl of pH 7.6, 40; $MgCl_2$, 75; glutathione, 2; and enzyme, which was used to initiate the reaction. The incubations were carried out in glass-stoppered tubes at 37° with agitation. One unit of enzyme activity produces 1 μmole of *S*-adenosylmethionine in 30 min under these conditions (11).

The original assay of Cantoni and Durell (11), utilizing an anion exchange resin which quantitatively removed unreacted ATP, thus leaving *S*-adenosylmethionine in solution to be analyzed spectrophotometrically at 256 mμ, was not employed because of high optical density blanks caused by self-perpetuating impurities in the resin. This method is also not adequate for inhibition studies, as it cannot detect the formation of low levels of *S*-adenosylmethionine when very low methionine concentrations are used. Therefore, *S*-adenosylmethionine formation was determined by one of the two following methods, both taking advantage of the positive charge on the sulfonium atom. First, a modification of the procedure of Mudd *et al.* (28) utilized radioactive L-methionine as substrate. In this highly sensitive assay the incubation is terminated by diluting the incubation mixture with a large volume (10 ml) of cold water and passing the solution through Dowex AG 50W-X2 (100–200 mesh) columns (6 × 30 mm) in the ammonium form. The ^{14}C-L-methionine does not exchange with the resin at neutral pH and passes through the column in the water wash (100 ml). *S*-Adenosylmethionine is absorbed onto the resin and may be quantitatively eluted with two aliquots (5 ml each) of concentrated ammonium hydroxide, rather than 3 N ammonium hydroxide as described by Mudd *et al.* (28). To each 5-ml aliquot of concentrated ammonium hydroxide, 15 ml of Bray's scintillation fluid (29) are added, and the total radioactivity is determined in a liquid scintillation counter. The amount of *S*-adenosylmethionine

formed in the reaction can be calculated from the original specific activity of the [^{14}C-methyl]-L-methionine.

Inhibition studies were generally carried out with the radioactive methionine assay because of its sensitivity (it is capable of measuring less than 1 mμmole of *S*-adenosylmethionine), which permits lowering of the methionine concentration far below the recorded (12, 15, 27) K_m values of approximately 0.4–2.5 mM (depending upon the source of enzyme). Unless otherwise noted, in the inhibition studies the methionine concentration was 37.5 μM and each reaction vessel contained approximately 380,000 cpm.

A second method designed in this laboratory is also a modification of the original spectrophotometric method of Cantoni and Durell (11) but employs radioactive ATP. In this procedure ATP-8-^{14}C (250,000 cpm) is used in the standard incubation mixture. The reaction is terminated by addition of 2 ml of a 67% slurry (v/v) of Dowex AG 1-X10 (200–400 mesh) in the chloride cycle (neutral pH) and sufficient water to give a final volume of 10 ml. The contents are shaken and centrifuged, and an aliquot of the supernatant fluid is counted. The radioactive ATP is taken up by the anion exchange resin while the radioactive product, *S*-adenosylmethionine, remains in solution. The quantity of *S*-adenosylmethionine formed may then be calculated on the basis of the specific activity of the ATP-^{14}C.

The purification of the transferases from yeast, *E. coli*, and rat liver was monitored with the radioactive ATP assay procedure, since it was considerably less time-consuming than the radioactive methionine assay. The assay systems used to follow the purification were incubated for 30 min. In the assay of the rat liver enzyme, 3 units of yeast inorganic pyrophosphatase were routinely added to the incubation mixture (11). Inorganic pyrophosphatase was not added for the inhibitor studies (37.5 μM ^{14}C-L-methionine), as the amount of pyrophosphate formed was very low and inorganic pyrophosphatase had no stimulating effect.

In the inhibition studies all three enzymes were diluted with bovine serum albumin (10 mg/ml) containing 5 mM 2-mercaptoethanol.

Protein Determinations

Protein was determined either by the biuret method (30) with bovine serum albumin as standard or by the ultraviolet absorption method of Warburg and Christian (31).

RESULTS

Substrate Specificity of Methionine Adenosyltransferase

Sulfur-containing analogues of methionine as substrates. Several studies of the amino acid specificities of yeast and liver methionine adenosyltransferase have been reported (11, 12, 27, 32). In order to define the structural features required for substrate activity, a systematic examination of a number of sulfur analogues of methionine was undertaken (Table 1). Since radioactive compounds were not readily available, the tests were carried out with ATP-8-^{14}C, at a final concentration of 12 mM, and the potential substrates were tested at 20 and 40 mM for L- and DL-compounds, respectively. The reaction velocities were compared with the rates obtained with 20 mM L-methionine for yeast, *E. coli*, and rat liver enzymes.

The methyl and ethyl esters and the *N*-formyl derivatives of methionine are substrates for all three transferases. *N*-acetyl-DL-methionine is a substrate for the *E. coli* and rat liver enzymes, but is not a substrate for the yeast system, as has already been reported by Mudd and Cantoni (12). The possible, although unlikely, hydrolysis of these derivatives to methionine in the course of the incubation has not been ruled out, since the products were not identified. We have confirmed that L-ethionine is a substrate for the yeast (12) and liver enzymes (27), but is barely active with the *E. coli* enzyme (33).

In addition, seleno-L-methionine and seleno-L-ethionine, while not tested in this laboratory, have been shown to be activated by the yeast and liver enzymes (27, 34, 35), but no information on these compounds is available for the *E. coli* enzyme.

Sulfur-containing analogues of methionine

TABLE 1

Analogues of methionine as substrates of methionine adenosyltransferase

The assays were performed with ATP-8-^{14}C in the system described under *Methods*, except that the final ATP concentration was 12 mM. Reaction mixtures were incubated for 30 min with 45.7, 144, and 15 μg of the yeast, *E. coli*, and rat liver enzymes, respectively. The amounts of *S*-adenosyl-L-methionine formed by the above enzymes in the control incubations were 0.252, 0.370, and 0.102 μmole, respectively.

Compound	Concentration	Relative reaction velocity		
		Yeast	*E. coli*	Rat liver
	mM			
L-Methionine	20	100	100	100
L-Methionine methyl ester	20	38	56	80
L-Methionine ethyl ester	20	14	30	31
N-Formyl-DL-methionine	40	39	53	65
N-Acetyl-DL-methionine	40	0	23	8
L-Ethionine	20	16	4	30

that are neither substrates nor inhibitors. A number of sulfur-containing analogues of methionine were examined in the above assay systems and were found to be incapable of replacing L-methionine, at least at the level of detection (about 5% of the activity of L-methionine). The following compounds were all inactive in the three enzyme systems: 2-methyl-DL-methionine, 2-hydroxy-DL-methionine, 2-hydroxy-DL-methionine methyl ester, *trans*-3-dehydro-L-methionine, DL-2-amino-4-(methylthio)-butan-1-ol (methioninol), D-methionine,[2] D-ethionine,[2] DL-methionine sulfoxide, DL-methionine sulfone, L-methionine-*S*(RS)-sulfoximine, L-homocysteine (thiolactone and free base), L-cysteine, and *S*-ethyl-L-cysteine. Also, DL-2-amino-5-(methylthio)-pentanoic acid (homomethionine) and DL-2-amino-6-(thiomethyl)hexanoic acid (bishomomethionine) cannot replace methionine. The former finding confirms the report of Stekol (38). *S*-Isoamylhomocysteine and *S*-*n*-propylhomocysteine have been reported by Stekol (32) to be inactive with the liver enzyme.

These compounds, many of which are close structural analogues of methionine, also appear to be unable to bind to the active site of the enzyme since they did not inhibit the activation of L-methionine. The majority of determinations of inhibitory potency were made using 12 mM ATP-8-^{14}C and 0.4 mM L-methionine, with the potential inhibitors at 20 mM (D- or L-compounds) and 40 mM (DL-compounds).

Inhibitors of Methionine Adenosyltransferase

Conditions of assay. In order to detect even weak inhibitors of the enzyme, nonsaturating levels of ^{14}C-L-methionine (37.5

[2] It was previously reported that D-methionine and D-ethionine are converted to the corresponding *S*-adenosyl derivatives (27, 36, 37). Pan and Tarver (27) observed with partially purified rat liver transferase that the K_m for D-methionine was 47 times higher than that for L-methionine, and that the rate of activation of the D-isomer was only 13% of that for the L-isomer when both were tested at 10 mM. The K_m for D-ethionine was not determined, because of low activity; however, D-ethionine at a 20 mM concentration reacted at 8% of the rate of L-methionine (10 mM). Schlenk and DePalma (36) have extracted *S*-adenosyl-D-methionine from *Torulopsis utilis* that had been cultured in the presence of D-methionine. Upon degradation of the isolated *S*-adenosylmethionine by base hydrolysis and D-amino acid oxidase, it was established that the D-isomer of methionine was present in the *S*-adenosylmethionine. Stekol *et al.* (37) reported that "Fleischmann's baker's yeast synthesizes only about one-tenth as much *S*-adenosylmethionine or *S*-adenosylethionine from D-methionine or D-ethionine, respectively, as it does from the corresponding L-enantiomorphs." The discrepancies between our findings and those of other authors might be ascribed to a variety of factors, such as differences in the purities or properties of enzymes and enantiomorphic purities of substrates.

μM) were used in the standard assay system. The formation of product was linear with respect to time for more than 45 min in the case of the *E. coli* enzyme, and for less than 10 min for the liver and yeast enzymes (Fig. 1). Under conditions of incubation which gave linear product formation with time, the formation of *S*-adenosylmethionine as a function of protein concentration is shown in Fig. 2. On the basis of these findings, the incubation times were chosen to be 5 min for both yeast and liver enzymes, and 30 min for *E. coli*. To each reaction vessel were added 5 μg of each of the former enzymes and 2.5 μg of purified *E. coli* protein.

Determination of inhibitory potency. Since kinetic analysis of the relation between reaction velocity and methionine concentration gave significant deviations from linearity in double-reciprocal plots for each of the three enzymes, it was not possible to determine the K_m for L-methionine in a simple manner, and consequently K_i values were not accurately obtainable. For the purpose of the present studies, inhibitors were tested at a series of concentrations in the presence of 37.5 μM

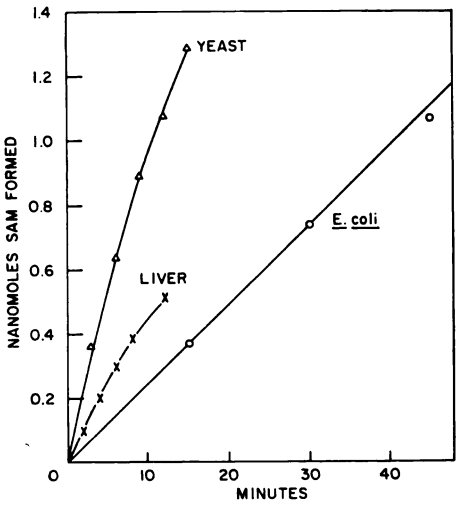


FIG. 1. *Time course of formation of S-adenosylmethionine (SAM) at low concentrations of L-methionine*

Incubations were carried out with 37.5 μM ^{14}C-L-methionine (380,000 cpm). For conditions, see *Methods*. The amounts of enzyme protein in the incubation systems were 4.6 μg (yeast), 4.6 μg (liver), and 2.4 μg (*E. coli*).

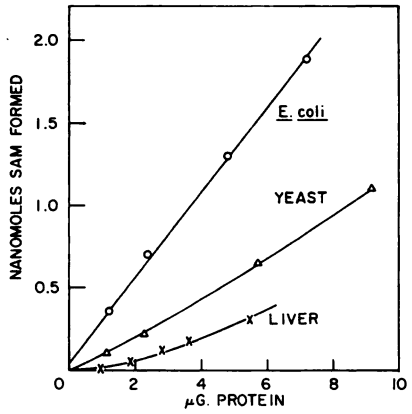


FIG. 2. *Relation between formation of S-adenosylmethionine (SAM) and protein concentration at low concentrations of L-methionine*

Incubations were carried out with 37.5 μM ^{14}C-methionine (380,000 cpm). For conditions, see *Methods*. Incubations were conducted for 5 min (yeast), 5 min (rat liver), and 30 min (*E. coli*).

^{14}C-L-methionine, and the reaction rates were related (as a percentage) to those obtained in the absence of inhibitor. By plotting the activity or its reciprocal (39) against inhibitor concentration, the I_{50} (the concentration of inhibitor giving 50% of control activity) could be determined graphically (Fig. 3A and B). It was noted that at low concentrations of inhibitors the activity of the liver enzyme was stimulated by many inhibitors.

Inhibition by S-trifluoromethyl-L-homocysteine. This compound (also known as trifluoro-L-methionine) was first prepared by Dannley and Taborsky (40). It has been claimed by Stekol (32) that it can serve as a substrate for rat liver methionine adenosyltransferase. However, we were unable to obtain any detectable activation of this compound with any of the three enzyme systems. *S*-Trifluoromethyl-L-homocysteine was a reasonably good inhibitor of the methionine adenosyltransferase of yeast (I_{50} = 11 mM) and of rat liver (I_{50} = 11.4 mM) but less effective with *E. coli* (I_{50} = 34 mM). This compound has been described as an inhibitor of methionine adenosyltransferase of *Salmonella typhimurium* by Cox and Smith (41).

Alkane, alkene, and alkyne analogues of methionine as inhibitors. Skinner *et al.* (8)

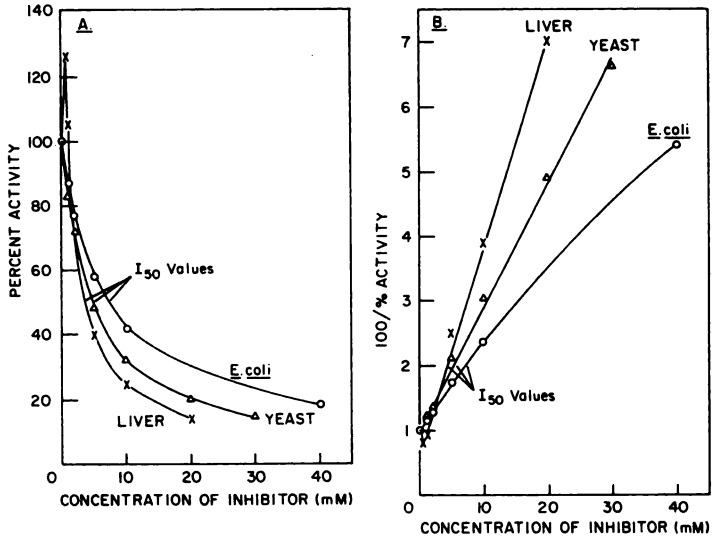


FIG. 3. *Inhibition of methionine adenosyltransferase by various concentrations of* DL-*2-amino-4-hexynoic acid*

The assays were carried out with 37.5 μM ^{14}C-L-methionine and 0.5–40 mM inhibitor in the reaction system described under *Methods*. Yeast, 4.6 μg of protein, 5-min incubation; liver, 4.6 μg of protein, 5-min incubation; *E. coli*, 2.4 μg of protein, 30-min incubation.

A. Plot of activity (as a percentage of control) with respect to inhibitor concentration. B. Same results as A, plotted as a reciprocal of activity with respect to inhibitor concentration (Dixon plot).

reported in 1961 that DL-2-amino-*cis*-4-hexenoic acid was a weak inhibitor of the growth of *E. coli*, competitive with methionine, and that at a ratio of inhibitor to methionine of about 50:1, 50% inhibition of growth was observed. Since the *trans* isomer was almost inactive as an inhibitor, these authors concluded that the conformation of methionine at the unknown site(s) of utilization spatially resembled the *cis* isomer. However, when Mudd (9) examined both geometrical isomers of DL-2-amino-4-hexenoic acid as inhibitors of the methionine adenosyltransferase of yeast, he found the DL-*trans* isomer to be an inhibitor competitive with methionine whereas the *cis* isomer had minimal inhibitory activity. These studies were complicated by the fact that the *cis* isomer prepared by the procedure of Skinner *et al.* (8) was contaminated by dehydroisoleucine and dehydroalloisoleucine. We have confirmed that the *trans* isomer of DL-2-amino-4-hexenoic acid is a far more potent inhibitor of methionine adenosyltransferase of yeast than the *cis* form. Table 2 also shows that the homologous enzymes from *E. coli* and rat liver are likewise inhibited by this compound. The corresponding saturated straight chain amino acid, norleucine (2-aminohexanoic acid), was a very much weaker inhibitor, but interestingly the L-isomer was considerably more potent than its antipode.

The acetylenic DL-2-amino-4-hexynoic acid proved to be almost twice as powerful an inhibitor as the DL-2-amino-*trans*-4-hexenoic acid. Upon resolution of the acetylenic amino acid, it was evident that the inhibitory property resided almost entirely in the L-isomer. The 5-chloro derivative of DL-2-amino-*trans*-4-hexenoic acid is intermediate in inhibitory potency between the *trans* double-bonded and the acetylenic amino acids.

In addition to the unsaturation which introduces electronegativity at the C-4 to C-5 region, the terminal methyl group also appears to be important for inhibitory properties. Thus, DL-2-amino-4-pentenoic acid and L-2-amino-4-pentynoic acid are much less effective inhibitors than their 6-carbon analogues (Table 2). Minor differ-

TABLE 2

Inhibition of methionine adenosyltransferase of yeast, E. coli, and rat liver by 2-aminoalkane, -alkene, and -alkyne carboxylic acids with 5 and 6 carbon atoms

The methionine adenosyltransferase was measured with 37.5 μM ^{14}C-methionine under conditions described under *Methods*.

Inhibitor	Concentration required for 50% inhibition		
	Yeast	*E. coli*	Rat liver
	mM	mM	mM
6-Carbon compounds			
L-Leucine	40[a]	45[a]	70[a]
D-Norleucine	170[a]	118[a]	310[a]
L-Norleucine	56[a]	38[a]	23[a]
DL-2-Amino-*cis*-4-hexenoic acid	170[a]	55[a]	80[a]
DL-2-Amino-*trans*-4-hexenoic acid	11	12.5	6.5
DL-2-Amino-5-chloro-*trans*-4-hexenoic acid	8.5	8.5	5.3
DL-2-Amino-4-hexynoic acid	5.2	7.3	4.2
L-2-Amino-4-hexynoic acid	2.7	3.5	1.8
5-Carbon compounds			
D-Norvaline	122[a]	45[a]	76[a]
L-Norvaline	144[a]	45[a]	124[a]
DL-2-Amino-4-pentenoic acid	110[a]	27[a]	65[a]
L-2-Amino-4-pentynoic acid	26	9	20.5

[a] These values were determined by extrapolation and were not bracketed by experimental observations.

ences in inhibitory power have been observed with the enzymes derived from different sources.

Inhibition by serine and cysteine derivatives. A series of substituted cysteine and serine analogues were found to be relatively potent inhibitors of the methionine adenosyltransferase reaction. Whereas L-serine and L-cysteine are quite weak inhibitors of the enzyme, *O*-methyl-DL-serine and *S*-methyl-L-cysteine are somewhat more powerful, and DL-homoserine and L-homocysteine are considerably more active in this respect. The substituted derivatives, *O*-carbamyl-L-serine, *O*-acetyl-L-serine, and *S*-carbamyl-L-cysteine, are inhibitors of activity comparable to DL-2-amino-*trans*-4-hexenoic acid (Table 3).

Inhibitory cyclic analogues of methionine. An unexpected finding was the inhibitory power of 1-aminocyclopentanecarboxylic acid (cycloleucine). This compound has been used as a nonmetabolizable amino acid transport monitor (42–44), and was discovered to be a tumor inhibitor during routine screening programs (45–47). It has been reported that inhibition of bacterial growth by 1-aminocyclopentanecarboxylic acid is reversed by L-methionine (48). Table 4 shows that this compound is almost as efficient an inhibitor of methionine adenosyltransferase as L-2-amino-4-hexynoic acid in all three enzyme systems. It is interesting that this inhibition is critically dependent on ring size and on the presence of ring substituents. Thus, 1-aminocyclopropanecarboxylic acid, 1-aminocyclobutanecarboxylic acid, and 1-aminocyclohexanecarboxylic acid are very much weaker inhibitors, and 1-aminocycloheptanecarboxylic acid is virtually inactive (Table 4). Moreover, 1-amino-2-ethylcyclopentanecarboxylic acid is not inhibitory.

Since elimination of the terminal methyl group of the 6-carbon alkane, alkene, and alkyne analogues of L-methionine markedly reduces inhibitory activity (Table 2), various isomers of 1-amino-3-methylcyclopentanecarboxylic acid were prepared on the assumption that the methyl ring substituent might occupy a spatial position corresponding to that of the terminal methyl group of

methionine. Two of the four isomers are available as steric entities, and the other two only as racemates (see *Methods*). Figure 4 shows the spatial configurations of the four isomers of 1-amino-3-methylcyclopentanecarboxylic acid and their relation to the conformation of L-methionine which most closely corresponds to the ethylenic,

TABLE 3

Inhibition of methionine adenosyltransferase of yeast, E. coli, and rat liver by serine and cysteine analogues of methionine

The enzyme activity was measured with 37.5 μM ^{14}C-L-methionine under conditions described under *Methods*.

Inhibitor	Concentration required for 50% inhibiton		
	Yeast	*E. coli*	Rat liver
	mM	mM	mM
L-Serine	510[a]	200[a]	440[a]
O-Methyl-DL-serine	410[a] (205)	78[a] (39)	260[a] (130)
DL-Homoserine	78[a] (39)	65[a] (33)	70[a] (35)
O-Acetyl-L-serine	10	15	8.3
O-Carbamyl-L-serine	13.5	4	15.2
L-Cysteine	310[a]	60[a]	186[a]
S-Methyl-L-cysteine	144[a]	65[a]	97[a]
DL-Homocysteine (free base)	44[a] (22)	34[a] (17)	
L-Homocysteine thiolactone	33[a]	46[a]	29[a]
S-Carbamyl-L-cysteine	27	12.5	22

[a] These values were determined by extrapolation and were not bracketed by experimental observations. The figures in parentheses refer to the probable values for the L-isomer, on the assumption that the D-isomer is inactive.

TABLE 4

Inhibition of methionine adenosyltransferase of yeast, E. coli, and rat liver by cyclic analogues of methionine

The methionine adenosyltransferase was measured with 37.5 μM ^{14}C-L-methionine under conditions described under *Methods*.

Inhibitor	Concentration required for 50% inhibition		
	Yeast	*E. coli*	Rat liver
	mM	mM	mM
1-Aminocyclopropanecarboxylic acid	132[a]	35[a]	97[a]
1-Aminocyclobutanecarboxylic acid	32	9.2	20
1-Aminocyclopentanecarboxylic acid	5.7	4.1	2.4
1-Aminocyclohexanecarboxylic acid	110[a]	83[a]	56[a]
1-Aminocycloheptanecarboxylic acid	Inactive	Inactive	Inactive
1-Amino-3-methylcyclopentanecarboxylic acid, isomer A (1S,3R or 1R,3R)	6.0	4.3	3.2
1-Amino-3-methylcyclopentanecarboxylic acid, (±)-isomer A	12.3	5.7	5.2
1-Amino-3-methylcyclopentanecarboxylic acid, isomer B (1S,3R or 1R,3R)	14.6	28	4.9
1-Amino-3-methylcyclopentanecarboxylic acid, (±)-isomer B	31.5	27	9.2

[a] These values were determined by extrapolation and were not bracketed by experimental observations.

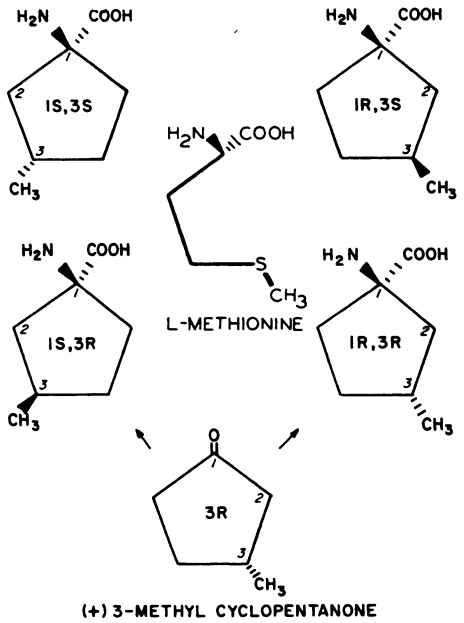


FIG. 4. *Structures of the four isomeric 1-amino-3-methylcyclopentanecarboxylic acids*

The absolute configurations of each compound (1S,3S; 1R,3S; 1S,3R; 1R,3R) are designated within the cyclopentane rings. The relationship of these structures to the conformation of L-methionine deduced from the inhibitor studies is shown. Each compound is displayed so that the amino and carboxyl groups are all oriented in the same way.

acetylenic, and cyclic inhibitors. Although the conformation of the terminal methyl group cannot be ascertained from these inhibitor studies, the rather strong inhibitory properties of L-2-amino-4-hexynoic acid suggest that the terminal methyl group may be in the same plane as C-2, C-3, C-4, and the sulfur atom of L-methionine.

Isomer A is much more highly inhibitory for the enzyme than isomer B (Table 4), and since correspondence of the methyl group to the position of the *S*-methyl group of methionine in the conformation required by the other inhibitors (see DISCUSSION and Fig. 4) occurs only with the 1R,3R diastereomer, we are inclined tentatively to assign the configuration 1R,3R to isomer A, and the configuration 1S,3R to isomer B, both of which were prepared from the (+)-3-methylcyclopentanone (3R). With yeast and liver enzymes the racemic mixtures of isomers A and B were almost exactly one-half as active as the optically active forms derived from the 3R ketone. Consequently, it seems likely that both the 3S isomers are virtually inactive as inhibitors. The *E. coli* enzyme appears to be less discriminating in this regard. These findings indicate that there exist serious steric limitations on substituent tolerance at position 3 of 1-aminocyclopentanecarboxylic acid, if inhibitory properties are to be retained.

DISCUSSION

Substrates

The structural requirements for activation and for binding to the methionine adenosyltransferase are a thioether sulfur atom located at a critical distance of two methylene groups from a carbon atom bearing a free hydrogen, an amino group in the L-configuration, and a carboxyl group or its ester. The optical specificity is rigid. Substitution of the amino group by an *N*-formyl or to a lesser extent an *N*-acetyl group appears to be tolerated. However, reduction of the carboxyl group to an alcohol, substitution of the α-hydrogen by a methyl group, or replacement of the amino group by a hydroxyl moiety all lead to compounds which are neither substrates nor inhibitors for the reaction. These structural and steric requirements are summarized schematically in Fig. 5. A methyl group substituent on the sulfur atom must be present, since L-homocysteine is not appreciably activated and only very poorly bound. If the alkyl substituent is enlarged to an ethyl group (ethionine), the rate of activation of the compound falls off (27, 32), especially with the *E. coli* enzyme (33). Furthermore, Stekol (32) has reported that *S*-*n*-propylhomocysteine and *S*-isoamylhomocysteine are not activated by the liver enzyme. It is also known that selenomethionine and selenoethionine are activated by methionine adenosyltransferase (27, 34, 35, 38). As might be anticipated, the oxidation state of the sulfur atom is also critical, since methionine sulfone, sulfoxide, and sulfoximine are not activated.

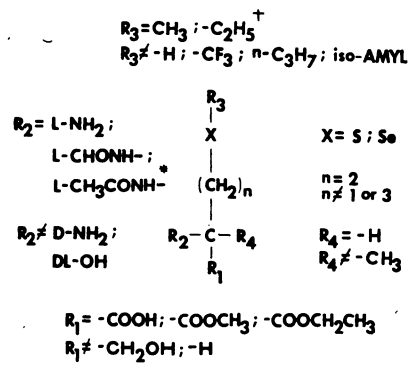


FIG. 5. *Schematic representation of the structural requirements for substrates of the methionine adenosyltransferase reaction*

For L-methionine R_1 = COOH, R_2 = L-NH_2, R_3 = CH_3, R_4 = H, and n = 2. Those structural changes of the substituents R_1, R_2, R_3, and R_4 which lead to active substrates are designated as =, whereas those that produce inactive compounds are shown as ≠.

† R_3 may be —C_2H_5 for the yeast and rat liver enzymes, but not for *E. coli*.

* R_2 may be an acetyl group for *E. coli* and liver enzymes, but the reactivity with the yeast enzyme is very poor.

Inhibitors

The compounds which have been found to inhibit methionine adenosyltransferase fall into three structural categories: the straight carbon chain amino acids, the serine and cysteine derivatives, and the cyclic amino acids. The only close structural analogue of methionine that has so far been found to be an inhibitor is *S*-trifluoromethyl-L-homocysteine.

Straight carbon chain compounds. Whereas the C_6 saturated amino acid norleucine is a poor inhibitor, the introduction of a double bond at C-4 converts this compound into a relatively potent inhibitor provided that the geometrical configuration is *trans* and not *cis*. Although methionine can assume the conformation of either *cis* or *trans* isomers of L-2-amino-4-hexenoic acid (Fig. 6, panel *I*), these findings strongly suggest that the conformation of L-methionine at the active enzyme site resembles that of L-2-amino-*trans*-4-hexenoic acid (Fig. 6, panel *I*). This is consistent with the proposition that the electronegative region around the double bond lies in a position structurally analogous to the sulfur atom of methionine, and this view is reinforced by the finding that L-2-amino-4-hexynoic acid, which can only assume an extended conformation, is an even more powerful inhibitor than the *trans* olefinic amino acid. If we assume that the electronegative region of unsaturation corresponds to the sulfur atom of L-methionine, the increase in electronegativity produces more powerful inhibitors, and even the bulky chlorine group of DL-2-amino-5-chloro-*trans*-4-hexenoic acid appears to provide some enhancement of inhibitory potency.

Furthermore, studies with these compounds not only considerably restrict the probable conformation of L-methionine at the activating site of the enzyme, but also point to the importance of the *S*-methyl group for inhibition. Thus all the C_5 compounds (L-norvaline, D-norvaline, DL-2-amino-4-pentenoic acid, and L-2-amino-4-pentynoic acid) are much less powerful inhibitors than the analogues bearing a terminal methyl substituent; however, in the C_5 series, as in the C_6 series, a progressive increase in inhibitory power is also observed as the degree of unsaturation increases (Table 2). The Courtauld models (Fig. 6, panels *II* and *III*) illustrate the correspondence of the region of unsaturation in L-2-amino-*trans*-4-hexenoic acid and in L-2-amino-4-hexynoic acid to the sulfur region of L-methionine and the general similarities in the sizes and shapes of these molecules.

Cyclic amino acids. The surprising finding that 1-aminocyclopentanecarboxylic acid is an inhibitor rivaling L-2-amino-4-hexynoic acid in potency is of considerable interest. Since this cyclic amino acid lacks a region of electronegativity and has no alkyl group which corresponds in space to the *S*-methyl group of methionine (Fig. 6, panels *II* and *III*), it must be assumed that the rigid cyclic structure provides the possibility for highly efficient van der Waals binding that can compensate for the lack of the two above-mentioned features. This view is reinforced by the fact that the ring size of cyclic compounds is absolutely critical (the 1-aminocyclopropane-, 1-aminocyclobutane-,

1-aminocyclohexane-, and 1-aminocycloheptanecarboxylic acids are much weaker inhibitors or essentially noninhibitory), and this suggests a close correspondence of each carbon of the cyclic ring to a complementary binding site. In this connection, it might be mentioned that 2-methyl-DL-methionine is not bound to the enzyme surface, yet the methyl substituent on C-2 could assume a position corresponding to one of the methylene groups of the cyclopentane ring. Thus the mere presence of such a carbon atom is not sufficient for binding, and the cyclic structure presumably exerts its effect by creating a rigid ring system, several components of which are closely complementary to the binding surface. A number of substituted derivatives of 1-aminocyclopentanecarboxylic acid, such as the methyl ester and the *N*-methyl, the 3-carboxyl, and the 2-ethyl derivatives, are all completely inactive as inhibitors (no results presented).

As may be seen from Table 4, isomer A of 1-amino-3-methylcyclopentanecarboxylic acid (probably the 1R,3R enantiomer) is no more powerful as an inhibitor than the unsubstituted cyclic compound, although its methyl group is in the proper position to correspond to the *S*-methyl group of methionine. It is possible that the relatively rigidly oriented methyl substituent, which is at an angle to the plane of the cyclopentane ring, cannot assume the required conformation corresponding to the *S*-methyl group of methionine on the enzyme surface. Alternatively, the rigid ring system is able to enhance the binding affinity to such a degree that the terminal methyl group does not contribute a significant increment in binding strength. Courtauld models of 1-aminocyclopentanecarboxylic acid are shown in Fig. 6 (*II* and *III*) to display the general similarity to the inhibitory acyclic compounds already discussed. L-Methionine in the conformation required to correspond to these inhibitors may be regarded as forming a partial 5-membered ring composed of C-2, C-3, C-4, and the sulfur atom.

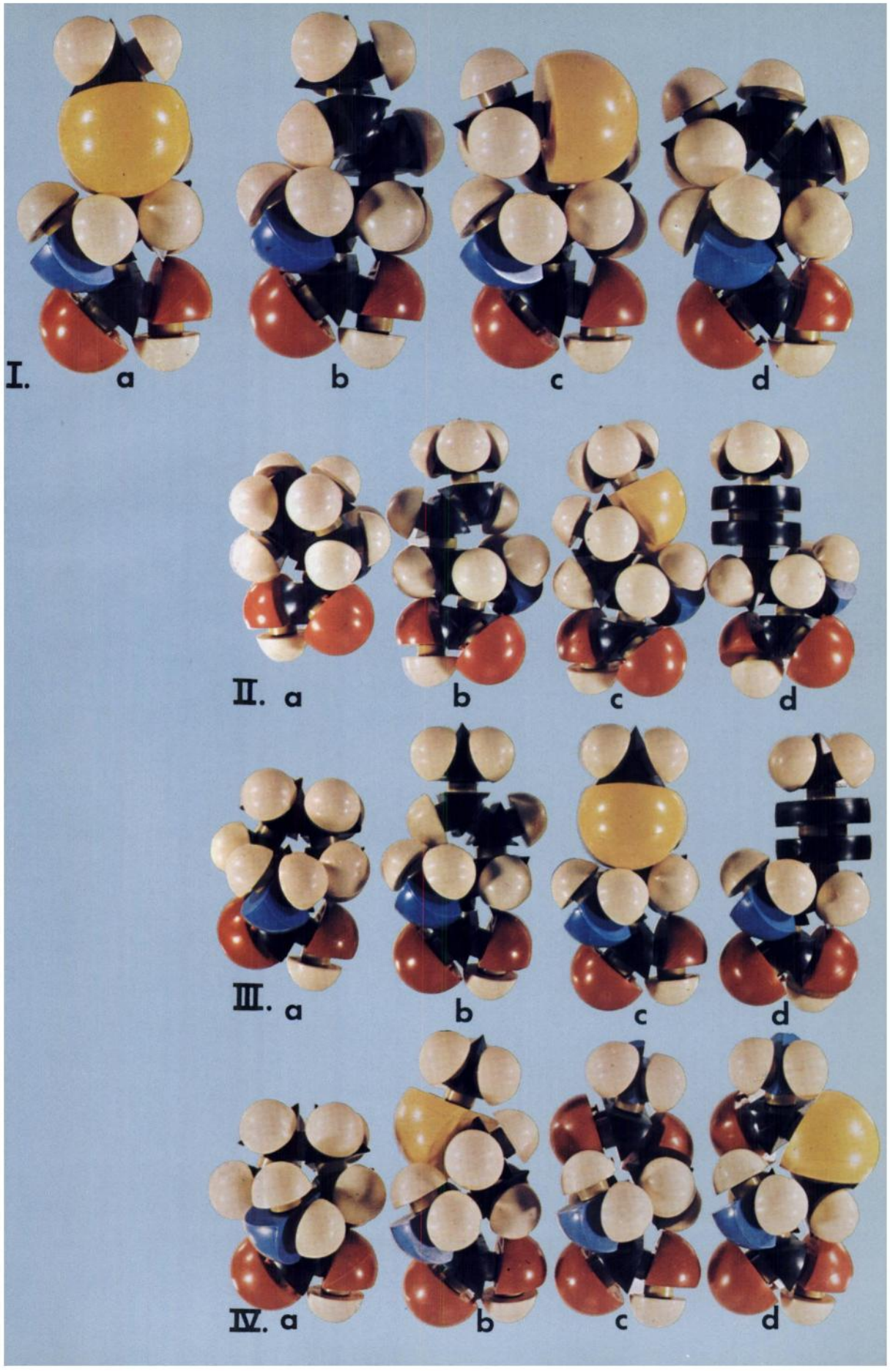
Serine and cysteine derivatives. It can be seen from Table 3 that both L-serine and L-cysteine are very weak inhibitors of methionine adenosyltransferase and that the higher homologues, homoserine and homocysteine, are 2–10 times more potent inhibitors, again suggesting the need for an electronegative center in a position closely corresponding to that occupied by the sulfur atom of methionine. *O*- or *S*-Methyl substitution of serine and cysteine, respectively, results in a 2–3-fold further improvement in inhibitory potency over the unsubstituted parent compounds. However, significantly better inhibitors are obtained when a carboxyl-carrying substituent, such as either a carbamyl or acetyl group, is present on the oxygen or sulfur atoms of serine or cysteine. This finding might be rationalized on the basis that the carbon atom of the carbonyl group which now lies in a position corresponding to the sulfur of methionine is flanked by electronegative atoms (sulfur or oxygen). Moreover, the general size and shape of these compounds resemble L-methionine conformationally (Fig. 6, panel *IV*).

O-Carbamylserine is a somewhat better inhibitor than *S*-carbamylcysteine, and this observation might be related to the fact that the carbon-oxygen bond distance is 1.43 A whereas the carbon-sulfur bond length is 1.81 A. Consequently, *O*-carbamyl-L-serine can assume a better fit than the *S*-carbamyl-L-cysteine to the ring of 1-aminocyclopentanecarboxylic acid (carbon-carbon bond length = 1.54 A).

Pharmacological activities of inhibitors of methionine adenosyltransferase. It is of some

FIG. 6. *Courtauld models of L-methionine and analogues that inhibit the methionine adenosyltransferase reaction, shown to emphasize conformational similarities*

The carbon atoms are black, nitrogen blue, oxygen red, hydrogen white, and sulfur yellow. Panel I: (*a*) L-methionine and (*b*) L-2-amino-*trans*-4-hexenoic acid, both shown in extended conformation; (*c*) L-methionine and (*d*) L-2-amino-*cis*-4-hexenoic acid, both shown in folded conformation. Panels II and III: two views of (*a*) I-aminocyclopentanecarboxylic acid, (*b*) L-2-amino-*trans*-4-hexenoic acid, (*c*) L-methionine, and (*d*) L-2-amino-4-hexynoic acid. Panel IV: (*a*) 1-Aminocyclopentanecarboxylic acid, (*b*) L-methionine, (*c*) *O*-carbamyl-L-serine, and (*d*) *S*-carbamyl-L-cysteine.


I. a b c d
II. a b c d
III. a b c d
IV. a b c d

interest that a number of the compounds which have been found to be inhibitors of methionine adenosyltransferase activity are known to possess important pharmacological properties. 1-Aminocyclopentanecarboxylic acid has been shown by Abshire *et al.* (48) to be a competitive antagonist of L-methionine for the growth of *E. coli*. These authors showed that the toxicity of the cyclic amino acid could be attributed neither to inhibition of the entry of essential amino acids into the cells nor to facilitation of the exit of methionine, phenylalanine, and valine. Thus, it was assumed that the mechanism of toxicity of this compound must involve the inhibition of an essential intracellular biosynthetic pathway. Upon further investigation of the toxicity of 1-aminocyclopentanecarboxylic acid for *E. coli* 9723, Abshire and Pineau (49) found that L-valine in smaller quantities than L-methionine can reverse the deleterious effects of this cyclic amino acid. It has also been reported (50) that 1-aminocyclopentanecarboxylic acid toxicity in the chicken can be reversed by L-valine.

1-Aminocyclopentanecarboxylic acid has been found to be an inhibitor of the growth of Novikoff hepatoma (45) and Walker carcinoma 256 (46) in the rat, and Sarcoma 180, Carcinoma 755, and Leukemia L1210 tumors (47) in the mouse. Although this compound is a powerful tumor inhibitor, the high doses which are required cause appreciable toxicity and loss of weight to the animal. Gregory *et al.* (51) have observed that L-valine can reverse the antileukemic action and toxicity of 1-aminocyclopentanecarboxylic acid in mice with Leukemia L1210, thus again suggesting an antagonistic action to an amino acid other than methionine. A mechanism of antitumor action has been proposed by Berlinguet *et al.* (52), who observed that 1-aminocyclopentanecarboxylic acid prevents the charging of L-valine to tRNA and may thus interfere with protein synthesis. Clinical tests with 1-aminocyclopentanecarboxylic acid have given mixed results (53–55). Some multiple myeloma patients reported subjective improvement after treatment. In a large number of cyclic amino acids screened as tumor inhibitors, this activity was strictly dependent upon ring size, since compounds containing rings smaller or larger than cyclopentane were totally inactive. Moreover, substituents on the ring nearly universally destroyed antitumor activity (although the 1-amino-3-methylcyclopentanecarboxylic acids have not been tested). Since the inhibition of methionine adenosyltransferase is also critically dependent upon a 5-membered ring (Table 4) and inhibitory properties are destroyed by a number of ring substitutions, we suggest the possibility that one mechanism of the antitumor action of the cyclic amino acid may be through the inhibition of *S*-adenosylmethionine synthesis. This proposal is supported by our finding that the levels of methionine adenosyltransferase activity are quite low (5–10%) in the Walker 256 and Lewis lung tumors and in the B-16 melanoma, when compared to liver homogenates from the same animals. Our findings contrast with those of Hancock (56) and Sheid and Bilik (57), who were unable to detect methionine adenosyltransferase activity in mouse hepatoma BW 7756 and Novikoff rat hepatoma in solid and ascites forms. The low enzyme content in tumors and the inhibition of the enzyme by 1-aminocyclopentanecarboxylic acid may account for the chemotherapeutic action of this compound. It may be noted that *S*-carbamyl-L-cysteine, which is another inhibitor of the transferase, is also a tumor inhibitor (58). It would be clearly worth examining the possible chemotherapeutic properties of a number of the methionine adenosyltransferase inhibitors described in this paper.

S-Trifluoromethyl-DL-homocysteine inhibited the growth of various microorganisms, and in some instances the inhibition could be reversed by methionine (59). *S*-Carbamyl-L-cysteine is a growth inhibitor of *Streptococcus lactis* and *Lactobacillus arabinosus* (60), but the inhibitions are only partial and are not competitively prevented by glutamine. This observation is in contrast to *O*-carbamyl-L-serine, which is also a growth antagonist of the two above microorganisms as well as *E. coli*, but whose antagonistic properties are competitively reversed by L-glutamine in *S. lactis* (61).

1-Aminocyclopentanecarboxylic acid (also known as cycloleucine) has received exten-

sive attention as a nonmetabolizable amino acid transport model. Akedo and Christensen (42) found that this compound probably shares a common transport mediator with valine and methionine in the rat intestine. Sterling and Henderson (62) observed that 1-aminocyclopentanecarboxylic acid inhibited the transport of natural amino acids into ascites tumor cells. Christensen and Jones (43) have also shown that this compound is not metabolized by the rat and that uphill tubular reabsorption almost certainly occurs for this amino acid, since it is cleared from the body very slowly.

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