MOLECULAR PHARMACOLOGY, 10, 319-334

Structural and Conformational Analogues of L-Methionine as Inhibitors of the Enzymatic Synthesis of S-Adenosyl-L-methionine

III. Carbocyclic and Heterocyclic Amino Acids

A. W. COULTER, J. B. LOMBARDINI,[1] JANICE R. SUFRIN, AND PAUL TALALAY

(Received August 20, 1973)

Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

(Received August 20, 1973)

SUMMARY

COULTER, A. W., LOMBARDINI, J. B., SUFRIN, JANICE R., AND TALALAY, PAUL: Structural and conformational analogues of L-methionine as inhibitors of the enzymatic synthesis of *S*-adenosyl-L-methionine. III. Carbocyclic and heterocyclic amino acids. *Mol. Pharmacol.* **10,** 319–334 (1974).

1-Aminocyclopentane-1-carboxylic acid (cycloleucine) is a competitive inhibitor of the synthesis of *S*-adenosyl-L-methionine by purified preparations of ATP:L-methionine *S*-adenosyltransferase of yeast, *Escherichia coli*, and rat liver. This inhibition is strictly dependent upon ring size, and is abolished by the introduction of 2-alkyl or 2-nitro substituents. Of the four isomers of 3-methylcycloleucine, only 1*R*-amino-3*R*-methylcyclopentane-1-carboxylic acid has inhibitory activity comparable to cycloleucine, whereas 1*S*-amino-3*R*-methylcyclopentane-1-carboxylic acid was far less active, and the enantiomers of these compounds were inferred to be inactive. Although the negative effects of certain substituents on inhibitory potency may be the result of lack of "bulk tolerance" in some topographical regions of the enzyme, other factors influencing physicochemical properties, bond angles, and conformations are probably of greater importance. The presence of the amino and carboxyl groups on a 5-membered ring appears to be a necessary condition for inhibition. Rigidification of the cyclic amino acid skeleton may influence the inhibitory properties profoundly. Thus 2-aminoadamantane-2-carboxylic acid lacks inhibitory activity. Of the four isomeric 2-aminonorbornane-2-carboxylic acids, only the 1*R*,2*R*,4*S*-isomer has significant inhibitory activity, which was slightly superior to that of cycloleucine in all three enzyme systems. The addition of an aromatic ring to the cycloleucine to create an indane skeleton depresses the inhibitory activity, whereas the addition of a second aromatic ring, as in 9-aminofluorene-9-carboxylic acid, gives an excellent inhibitor of yeast and liver enzyme with little activity for the *E. coli* enzyme. The effects of introduction of heteroatoms into the ring system lead to compounds with either enhanced or depressed activity. Thus 4-

These investigations were supported by Pharmacology-Toxicology Program Project Grant GM 16492 and Training Grant GM 1183 from the National Institutes of Health, and by a grant-in-aid from the Gustavus and Louise Pfeiffer Foundation of New York.

[1] Present address, Department of Pharmacology and Therapeutics, Texas Tech University School of Medicine, Lubbock, Texas 79409.

amino-1,2-dithiolane-4-carboxylic acid is an excellent inhibitor, superior to cycloleucine, whereas its sulfone [(*RS*)-4-amino-1,1-dioxo-1,2-dithiolane-4-carboxylic acid] is rather less active than the disulfide. Introduction of sulfur atoms into 6-membered rings, as in 5-amino-1,3-dithiane-5-carboxylic acid and (*RS*)-4-amino-1,2-dithiane-4-carboxylic acid, affords compounds with little inhibitory activity. Cucurbitine (*RS*-3-aminopyrrolidine-3-carboxylic acid) has very little inhibitory activity, and this may be the consequence of a formal positive charge on the ring nitrogen atom at physiological pH. Two new methylmercapto derivatives of cyclic amino acids were synthesized: 1*RS*-amino-3*RS*-(methylthio)cyclopentane-1-carboxylic acid, which had some inhibitory activity associated with one of its racemic pairs, and 1*RS*-amino-3*RS*-(methylthio)cyclohexane-1-carboxylic acid, which was inactive. Some of these carbocyclic and heterocyclic amino acids possess various pharmacological activities, including the capacity to inhibit tumor growth, suppress the immune response, and block amino acid transport. The structural specificities for these activities are different. Conformational, electronic, and steric factors all require consideration in the design of inhibitors for the adenosyltransferases.

INTRODUCTION

This paper describes the synthesis and evaluation of a series of carbocyclic and heterocyclic amino acids as potential inhibitors of the enzymatic synthesis of *S*-adenosyl-L-methionine by partially purified preparations of ATP:L-methionine *S*-adenosyltransferases (EC 2.5.1.6) of bakers' yeast, *Escherichia coli*, and rat liver. The studies form part of a systematic effort to obtain inhibitors of this reaction with a high degree of potency and tissue selectivity, with a view to exploring their potential as chemotherapeutic agents. In accompanying papers we report on the structural, electronic, and conformational features of aliphatic (1) and aromatic amino acid (2) analogues that are inhibitors of adenosyltransferases[2] in competition with L-methionine.

Our interest in cyclic amino acid analogues originated with the observation that 1-aminocyclopentane-1-carboxylic acid (cycloleucine) was an inhibitor of the synthesis of *S*-adenosyl-L-methionine in competition with L-methionine (3, 4). Of a number of cyclic amino acids of varying ring size examined, this inhibitory activity was confined to 5-membered ring structures, and the ring size was critical (3, 5).

Cycloleucine has attracted considerable interest because of a number of unusual pharmacological properties. This compound is an inhibitor of the growth of *E. coli* in competition with L-methionine and L-valine (6, 7). Cycloleucine retards the growth of a number of experimental tumors (8–10) and has received extensive clinical evaluation in man with some encouraging results, especially in leiomyosarcomas (11). With the single exception of the Novikoff hepatoma, for which 1-aminocyclohexane-1-carboxylic acid is active (8), the cyclopentane ring is a strict structural requirement for growth inhibition of a wide variety of transplantable tumors (9, 10) as well as for the inhibition of the adenosyltransferases (3, 5). Ring enlargement or contraction leads to dramatic reductions in these inhibitory activities, and we have suggested the possibility of a causal relationship between these properties (5, 12). The molecular mechanisms of action of cycloleucine have also been attributed to interference with valine utilization (13–15) and to inhibition of cellular amino acid uptake (16).

Cycloleucine is not significantly metabolized in animal tissues and has been used to study and to inhibit the transport of a variety of amino acids (17–21). In the rat intestine cycloleucine appears to share common transport mediators with L-valine and L-methionine (17). Studies by Oxender

[2] The abbreviations used are: adenosyltransferase, for ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6); cycloleucine, for 1-aminocyclopentane-1-carboxylic acid; I_{50}, the concentration of inhibitor required to achieve 50% reduction in adenosyltransferase activity under specified conditions.

and Christensen (18) of amino acid transport into Ehrlich ascites tumor cells indicate that under certain conditions cycloleucine inhibits the uptake of glycine, alanine, leucine, and phenylalanine. Ahmed and Scholefield (19) have concluded on the basis of competitor studies that cycloleucine and L-methionine share a common transport system in Ehrlich ascites tumors. The transport function of cyclic amino acids is not specific for a 5-membered ring structure, and also differs in certain other respects from the specificity of the structural requirements for inhibition of the adenosyltransferases (17, 20, 21).

More recently cycloleucine has evoked interest as an immunopharmacological agent. Cycloleucine inhibits antibody synthesis at the preinduction (22). This compound is effective in alleviating the severity and lowering the incidence of adjuvant arthritis and experimental allergic encephalomyelitis (3). The mode of action resembles that of immunosuppressive agents rather than anti-inflammatory agents, although the mechanism has certain unique features. Subtoxic doses of cycloleucine permit long-term retention of homografts in mice, while other immunosuppressants are less effective even when given in near-lethal dosages (24).

Experiments with cycloleucine in rodents have uncovered the interesting finding that this compound is concentrated to a high degree in the pancreas of some species (25, 26) and in certain transplantable tumors, such as the Walker 256 and the Lewis lung tumors (12). Administration of cycloleucine results in the accumulation of L-methionine in all tissues examined and lowers the *S*-adenosyl-L-methionine levels in these tissues, with the exception of liver, where both L-methionine and *S*-adenosyl-L-methionine concentrations become elevated (12). These findings are consistent with an inhibition of ATP:L-methionine *S*-adenosyltransferase *in vivo*. Possible reasons for the apparently paradoxical situation in the liver have been developed elsewhere (17, 27).

In this paper we explore in further detail the structural, electronic, and conformational requirements among cyclic amino acids for inhibition of adenosyltransferases. We have examined the effects of such modifications of cycloleucine on the inhibitory potency for the adenosyltransferases, and for their selectivity for isofunctional enzymes. The specific structural modifications include substitution of alkyl groups on the cyclopentane ring, conformational rigidification of the ring system by transannular carbon bridges or by incorporation of the cycloleucine into an extended ring system, and introduction of heteroatoms, such as nitrogen and sulfur into the ring system. The results indicate the feasibility of obtaining more effective inhibitors than cycloleucine and in achieving considerable tissue selectivity with some analogues.

EXPERIMENTAL PROCEDURE

The materials and methods used in these experiments have been described (1). The inhibitory potencies of all analogues were examined with partially purified preparations of adenosyltransferases of bakers' yeast, *E. coli*, and rat liver (1, 3). The concentrations of inhibitors required to produce 50% inhibition of enzyme activity (I_{50} values) were determined by the graphical method of Dixon (28) at L-methionine concentrations of 37.5 μM and under carefully defined conditions (3).

Sources of Amino Acid Analogues

The structures of all amino acid analogues are represented in Table 1. Each compound is assigned a Roman numeral.

Commercial. 1-Aminocyclopentane-1-carboxylic acid (I) was purchased from the Cyclo Chemical Corporation.

Gifts. A mixture of the four isomers of 1*RS*-amino-2*RS*-nitrocyclopentane-1-carboxylic acid (III) was a gift of Dr. W. B. Turner, Imperial Chemical Industries, Alderley Park, Macclesfield, Cheshire, U. K. The synthesis of III and some of its properties have been described (29, 30).

The following dithiacyclane amino acids were synthesized by Dr. H. F. Herbrandson, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, N. Y., and were supplied to us by Dr. Herbrandson and by Dr. T. Sweeney of the Walter Reed Army Medical Research Institute, Washington, D. C.: 4-amino-1,2-dithiolane-4-carboxylic acid (VI), (RS)-4-amino-1,1-dioxo-1,2-di-

thiolane-4-carboxylic acid (VII), 5-amino-1,3-dithiane-5-carboxylic acid (VIII), and (*RS*)-4-amino-1,2-dithiane-4-carboxylic acid (IX). Compound V has also been described by Shen and Walford (31).

The four isomeric 2-aminonorbornane-2-carboxylic acids (2-aminobicyclo[2.2.1]heptane-2-carboxylic acid, XII) (32) of known absolute configuration (33) were prepared by Dr. H. S. Tager and kindly supplied to us by Dr. H. N. Christensen, Department of Biological Chemistry, University of Michigan, Ann Arbor.

(*RS*)-2-Amino-5-hydroxyindane-2-carboxylic acid [XIV (34)] was a gift of Dr. R. M. Pinder, Chemical Defence Establishment, Porton Down, Salisbury, Wiltshire, U. K.

(*RS*)-1-Aminoindane-1-carboxylic acid [XV, NSC 32833 (10)] and 9-aminofluorene-9-carboxylic acid [XVI, NSC 32848 (10)] were gifts of Dr. Harry B. Wood, Jr., of the National Cancer Institute.

Syntheses

1R- and 1S-Amino-3R-methylcyclopentane-1-carboxylic acid (II). 1-*RS*-Amino-3*R*-methylcyclopentane-1-carboxylic acid was synthesized by Dr. H. Doshan from 3*R*-methylcyclopentanone according to the procedure of Zelinsky and Stadnikoff (35), but modified by saturation of the solution with ammonia to minimize formation of the iminodinitrile. The intermediate 1*RS*-amino-3*R*-methylcyclopentylcarbonitrile hydrochloride had mp 144–146° (with decomposition in a sealed tube); mass spectrum (70 eV) *m/e* (relative intensity) 124(26, M^+-HCl), 95(100), 82(56), 81(98), 69(44), 68(67), 56(75), 43(57).

	$C_7H_{13}ClN_2$
Calculated:	C 52.33, H 8.16, N 17.44, Cl 22.07
Found:	C 52.19, H 8.14, N 17.63, Cl 22.14

Acid hydrolysis (10 ml of concentrated HCl) of the above intermediate (803 mg) at reflux temperature for 2 hr, followed by evaporation and chromatograpy on a Dowex 50–X8 (H^+) column, afforded 508 mg of 1*RS*-amino-3*R*-methylcyclopentane-1-carboxylic acid: mp 304–304.5° [with decomposition in a sealed tube; recorded (35) mp 299–300°]. The automatic amino acid analyzer (36) showed two approximately equal components. The compounds, in order of their elution from the column, are referred to as isomer A (IIa) and isomer B (IIb) and were separated on a preparative high-resolution cation-exchange column. The column measured 118 × 5 cm and utilized finely graded Dowex 50 resin (Bio-Rad, Aminex Q-15S). The column was used in the Na^+ form and was eluted with sodium citrate buffer, pH 3.57, with 0.20 M Na^+ and 0.05 M citrate.

Isomer A: mp 247–251° (decomposition in a sealed tube), $[\alpha]_D^{25}$ −0.122° (*c*, 3 in H_2O), $[\alpha]_D^{25}$ −0.062° (*c*, 1.5, in 4 N HCl). Isomer B: mp 234–240° (decomposition in a sealed tube), $[\alpha]_D^{25}$ −0.130° (*c*, 4, in H_2O), $[\alpha]_D^{25}$ −0.063° (*c*, 2, in 4 N HCl).

X-ray diffraction analysis by Carrell, Gallen, and Glusker (37) of crystals of isomer A prepared from 3*R*-methylcyclopentanone permitted assignment of the absolute configuration of 1*R*-amino-3*R*-methylcyclopentane-1-carboxylic acid to isomer A, and of 1*S*-amino-3*R*-methylcyclopentane-1-carboxylic acid to isomer B.

(RS)-Cucurbitine hydrobromide [(RS)-3-aminopyrrolidine-3-carboxylic acid hydrobromide, IV]. This compound was synthesized by Dr. T. Karpetsky in this laboratory by the procedure of Sun *et al.* (38) via 1,3-dicarbethoxy-4-pyrrolidone (39). The recrystallized IV had mp 284° (decomposition) [recorded (40), 286° (decomposition)]; mass spectrum (70 eV) *m/e* (relative intensity) 130 (44, M^+ − HBr), 88(47), 85(12), 56(34), 43(100); infrared spectrum (KBr) 3225–2775, 2400–2325, 2000–1900, 1634, 1575 cm^{-1} (40); NMR (D_2O) δ 2.60–3.20 (*m*, 2), 3.80–4.70 (*m*, 4).

(RS)-3-Aminothiolane-3-carboxylic acid (V). This compound was synthesized by Dr. H. Doshan in this laboratory from thiolan-3-one by the Strecker procedure. The ketone (2.04 g, 20 mmoles) and 2 drops of pyridine were added to 15 ml of HCN that had been cooled, and stirred at 0° for 5.5 hr. Excess hydrogen cyanide was removed, and the crude product was dissolved

in 15 ml of *tert*-butyl alcohol maintained at saturation with ammonia. Stirring was continued at 4° for 66 hr. The brown suspension was filtered at 0°, and the filtrate was evaporated at 25° under reduced pressure to give a brown gelatinous mass which was dissolved in 10 ml of chloroform, cooled to 0°, and saturated with hydrogen chloride gas to precipitate the aminonitrile hydrochloride. The solvent was removed, giving a brown oil which was dissolved in water (10 ml) and washed with five 5-ml portions of ether. The aqueous phase was treated with 4 ml of concentrated HCl and refluxed for 2.3 hr to effect hydrolysis of the aminonitrile. The cooled reaction mixture was filtered, neutralized with potassium carbonate, and desalted on a Dowex 50–X8 (H^+) column. The amino acid was eluted with 1 N NH_4OH, which was evaporated to dryness. The orange residue was crystallized from water with the addition of absolute ethanol to afford 117 mg of (*RS*)-3-aminothiolane-3-carboxylic acid (V): mp 279–280 (with decomposition in a sealed tube); infrared spectrum (KBr) 3510, 3125–2940 (broad), 2040, 1680, 1626, 1575, 1510, 1375, 1255, 1220, 1185, 1144, 778 cm^{-1}; mass spectrum (70 eV) *m/e* (relative intensity) 148 (4.5), 147(54, M^+), 130(18), 119(13), 102 (100), 101(18), 100(37), 85(25), 73(12.5), 56(10), 45(20).

1-RS-Amino-3RS-(methylthio)cyclohexane-1-carboxylic acid (XI). Methyl mercaptan (20 g, 416 mmoles) was bubbled into a stirred mixture of 23.8 g (248 mmoles) of 2-cyclohexen-1-one, 200 mg of cupric acetate monohydrate, and 200 mg of hydroquinone. The temperature was maintained at 25–30° by cooling. Stirring was continued for 24 hr at room temperature. The mixture was filtered, and the filtrate was fractionated by distillation to yield 15.9 g of 2-cyclohexen-1-one and 7.8 g of 3*RS*-(methylthio)cyclohexanone: bp 62–64°/0.2 mm (65%, based on the amount of starting material consumed) [recorded (41) bp 55°/0.1 mm]; infrared spectrum ($CHCl_3$) 1715, 1445, 1418, 1310, 1225, 1200 cm^{-1}. The semicarbazone had mp 162.5° [recorded (41) mp 165°].

To a solution of 1.60 g (30 mmoles) of ammonium chloride and 1.95 g (30 mmoles) of KCN in 8 ml of water were added 2.97 g (20.6 mmoles) of 3*RS*-(methylthio)cyclohexanone dissolved in 5 ml of methanol. The mixture was cooled to 0°, saturated with ammonia gas, and stirred for 24 hr at room temperature. Water was added to dissolve a small amount of precipitate, and the solution was extracted with five 10-ml portions of ether. The ether was washed twice with 10 ml each of a saturated sodium chloride solution, dried over Na_2SO_4, and evaporated under reduced pressure to yield 2.5 g of yellow oil. The oil was dissolved in ether and cooled to 5°, and dry hydrogen chloride was bubbled into the solution to precipitate 2.39 g (56%) of 1*RS*-amino-3*RS*-(methylthio)cyclohexanecarbonitrile hydrochloride, which was crystallized from ethanol–acetonitrile–ether: infrared spectrum (KBr) 3125–2380 (broad), 2075, 1580, 1480, 1445 cm^{-1}; mass spectrum (70 eV) *m/e* (relative intensity) 170(27, M^+ − HCl), 153 (15), 123(37), 105(17), 96(25), 81(100).

	$C_8H_{15}ClN_2S$
Calculated:	C 46.48, H 7.31, N 13.55, Cl 17.15
Found:	C 46.62, H 7.19, N 13.39, Cl 17.37

A solution of 500 mg (2.4 mmoles) of 1*RS*-amino-3*RS*-(methylthio)cyclohexanecarbonitrile hydrochloride in 5 ml of 6 N HCl was refluxed for 3 hr, cooled, and adjusted to pH 5 with 1 N NaOH. The solution was desalted by adsorption onto a column of Dowex 50–X8 (H^+). After thorough washing of the column with water, the 1*RS*-amino-3*RS*-(methylthio)cyclohexane-1-carboxylic acid (XI) was eluted with 1 N NH_4OH. The eluate was evaporated to dryness under reduced pressure, and the product was dissolved in hot water and decolorized by filtration through a layer of charcoal. Upon the addition of acetone, crystallization began. A further crystallization from hot water afforded 270 mg of amino acid, mp 271–273° (with decomposition); infrared spectrum (KBr) 3200–2400, 2050, 1640, 1600, 1525, 1450, 1430, 1390, 1335, 1295 cm^{-1}; mass spectrum (70 eV) *m/e* (relative intensity) 190(4), 189(39), 172(48), 144(93), 127(35), 96(100), 87(35).

$C_8H_{15}NO_2S$
Calculated: C 50.76, H 7.99, N 7.40, S 16.94
Found: C 50.85, H 7.95, N 7.37, S 16.81

1RS - Amino - 3RS - (methylthio)cyclopentane-1-carboxylic acid (*X*). Methyl mercaptan (29 g, 605 mmoles) was bubbled into a stirred mixture of 25 g (305 mmoles) of 2-cyclopenten-1-one, 0.5 g of cupric acetate monohydrate, and 0.5 g of hydroquinone. The temperature was maintained at 35–40° during the addition, and at room temperature overnight. The mixture was filtered, and the filtrate was fractioned by distillation to yield 8.8 g of 3*RS*-(methylthio)cyclopentanone, bp 61°/1.6 mm (38%, based on the amount of starting material consumed); infrared spectrum (neat) 1745, 1433, 1400, 1280, 1250, 1163, 1130, 964, 900, 798, 755 cm^{-1}; mass spectrum (70 eV) *m/e* (relative intensity) 132(5, $C_6H_{10}O^{34}S^+$), 130(82, $C_6H_{10}O^{32}S^+$), 83(48), 82(20), 74(32), 61(18), 55(100), 54(24), 47(19), 45 (25).

$C_6H_{10}OS$
Calculated: C 55.34, H 7.74, S 24.63
Found: C 55.41, H 7.56, S 24.81

The semicarbazone had mp 137–140° (ethanol).

$C_7H_{13}N_3OS$
Calculated: C 44.90, H 7.00, N 22.44
Found: C 45.38, H 7.05, N 22.36

1*RS* - Amino - 3*RS* - (methylthio)cyclopentane-1-carboxylic acid was prepared from the ketone by the procedure described above for the homologous cyclohexyl amino acid (XI). The product was crystallized from water to afford white plates, mp 260–265° (with decomposition).

$C_7H_{13}NO_2S$
Calculated: C 47.97, H 7.48, N 7.99
Found: C 49.38, H 7.40, N 7.70

When analyzed with the amino acid analyzer, two ninhydrin-positive peaks were present in the ratio of 1:1.3 and were designated isomers A and B in order of their elution. These two diastereomeric amino acids were separated by preparative high-resolution cation-exchange chromatography and had the following properties.

Isomer A: mp 220° (with decomposition); mass spectrum (70 eV) *m/e* (relative intensity) 177(0.5, $C_7H_{13}NO_2{}^{34}S^+$), 176(1, $M^+ + 1$), 175(10, $C_7H_{13}NO_2{}^{32}S^+$), 158(4), 132 (1, $M^+ - 45$), 130(18, $M^+ - 45$), 128(18), 113(6), 88(9), 87(10), 83(11), 82(100), 55 (15).

Isomer B: mp 235–240° (with decomposition); mass spectrum (70 eV) *m/e* (relative intensity) 177(1, $C_7H_{13}NO_2{}^{34}S^+$), 176(2, $M^+ + 1$), 175(18, $C_7H_{13}NO_2{}^{32}S^+$), 158(14), 132 (2, $M^+ - 45$), 130(19, $M^+ - 45$), 128(17), 113(21), 88(12), 87(9), 83(11), 82(100), 55(15).

2-Aminoadamantane-2-carboxylic acid (*XIII*). A cooled solution of KCN (1.6 g, 25 mmoles), NH_4Cl (1.3 g, 25 mmoles), and adamantan-2-one (2.3 g, 15.5 mmoles) in 100 ml of 30% aqueous methanol was saturated with NH_3 (gas). The solution was stirred at room temperature for 10 days. After dilution with 50 ml of H_2O, the reaction mixture was extracted with ether. The combined ether extracts were washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and then brought to dryness on a rotary evaporator to give 2.4 g of a mixture containing crude 2-aminoadamantane-2-carbonitrile (0.9 g, 31%), infrared spectrum (KBr) 2240 cm^{-1} (—C≡N), and recovered adamantan-2-one (1.5 g, 65%), infrared spectrum (KBr) 1700 cm^{-1} (—C=O). The crude reaction mixture was refluxed for 18 hr in 100 ml of 6 N HCl, during which time the adamantan-2-one (1.5 g) sublimed into the reflux condenser and was recovered. The cooled acidic solution was adjusted to pH 4.5 with 2 N NaOH. It was filtered and then desalted on a Dowex 50–X8 (H^+) column, and the amino acid was eluted from the column with 1 N NH_4OH. The recovered amino acid was recrystallized from water using charcoal to give 370 mg (27% based on crude 2-aminoadamantane-2-carbonitrile); mp 271–272° (recrystallized twice from water) [recorded (42) mp 308–310°, sealed capillary]; infrared spectrum (KBr) 3100–2500 (broad, $—NH_3^+$), 2050 ($—NH_3^+$), 1660, 1640, 1580, 1520, 1465, 1360, 865, 755 cm^{-1}; mass spectrum (70 eV) *m/e* (relative intensity) 150(100, M—CO_2H); NMR (CF_3CO_2D) δ 2.55 (*s*, 2), 2.02 (*m*, 12). 2-Aminoadamantane-2-carboxylic acid, when chro-

matographed on the amino acid analyzer column under the conditions described previously (36) with appropriate standards, gave the following times and positions of elution of these amino acids: phenylalanine, 520 min; NH_3, 604 min; 2-aminoadamantane-2-carboxylic acid, 662 min; lysine, 743 min; histidine, 777 min.

$C_{11}H_{17}NO_2$	
Calculated:	C 67.66, H 8.77, N 7.17
Found:	C 67.27, H 8.70, N 6.89

RESULTS AND DISCUSSION

Effects of ring size and substitution of carbocyclic analogues. We have previously reported that 1-aminocyclopentane-1-carboxylic acid (cycloleucine, I) is a moderately potent inhibitor of several microbial and animal tissue adenosyltransferases in competition with L-methionine (3–5). An extended series of new determinations of the inhibitory potency of cycloleucine gave mean I_{50} values of 5.4, 3.8, and 2.1 mM for the yeast, *E. coli*, and rat liver enzymes, respectively, at L-methionine concentrations of 37.5 μM (Table 1), in good agreement with earlier measurements (3). There is a strong dependence of inhibitory strength on ring size, since the cyclobutane and cyclohexane amino acids are markedly less active, and the cyclopropane and cycloheptane analogues are inactive (3, 5). These findings might be considered somewhat surprising for several reasons: (a) the acyclic amino acid with the same number of carbon atoms (norleucine) is almost inactive (3); (b) the introduction of regions of appropriately placed electron density (as in 2-amino-4-hexynoic acid or 2-amino-*trans*-4-hexenoic acid) into aliphatic compounds leads to more potent inhibitors (1, 3), whereas cycloleucine lacks such an electron-dense region; (c) DL-2-methylmethionine is inactive as an inhibitor (3), suggesting that substitution of a carbon atom for the α-hydrogen at C-2 is not tolerated; and (d) cycloleucine contains no group corresponding to the *S*-methyl group of L-methionine, whereas experiments with various C_5 and C_6 amino acids have suggested that the terminal methyl group plays a critical role in augmenting the binding of aliphatic amino acid inhibitors (1, 3).

These observations suggest that the inhibitory potency of cycloleucine may reside in the ability of the ring of this molecule to assume a specific conformation that is accurately complementary to a region on the enzyme and to enter into a series of interactions by hydrophobic or dispersive forces which are effective only at very close ranges. These types of forces may be of lesser importance in the binding of more polar and flexible acyclic structures, which presumably depend for their interactions on other molecular features.

The introduction of certain substituents on the cycloleucine ring leads to impairment of the inhibitory function. 1*RS*-Amino-2*RS*-nitrocyclopentane-1-carboxylic acid (III, Table 1) is a much weaker inhibitor than the unsubstituted parent cycloleucine (I), although the I_{50} values for the nitro derivative should be interpreted in terms of four isomers which are probably present in unequal amounts. Compound III is a natural product isolated from *Aspergillus wentii* and has been shown to inhibit the growth of many plants, especially pea seedlings (29, 30). These inhibitory effects of the nitro derivative of cycloleucine can be reversed by L-leucine and to a lesser extent by L-methionine. Although plant adenosyltransferases have not been studied, our findings suggest that the mechanism of the growth-inhibitory effects of III in plants may not involve inhibition of *S*-adenosyl-L-methionine synthesis.

Introduction of 2-alkyl substituents in cycloleucine, as in 2*RS*-ethyl-1*RS*-aminocyclopentane-1-carboxylic acid (3) or 2-methylcycloleucine,[3] abolishes the inhibitory powers for the adenosyltransferases. Of the four isomeric 3-methyl derivatives of cycloleucine, only one (IIa, Table 1) had inhibitory activity comparable to that of cycloleucine itself, whereas a diastereomer [IIb (3)] was considerably less active. We have previously inferred that the enantiomers of both of these compounds, which were not available as chemical entities, are either very weakly active or inactive (3). The absolute configuration of the most active isomer is now known to be 1*R*-amino-3*R*-

[3] Unpublished observations.

TABLE 1

Inhibitory potencies of carbocyclic and heterocyclic amino acid analogues on ATP:L-methionine S-adenosyltransferases of yeast, E. coli, and rat liver

The enzyme activity was measured at 37.5 μM L-methionine according to described procedures (3).

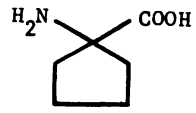
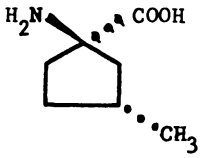
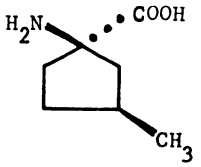
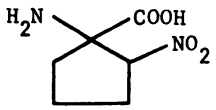
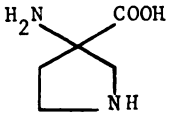
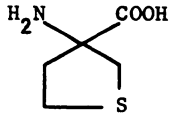
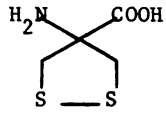
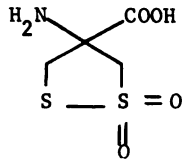
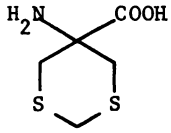
Compound No.	Compound	Structure	Maximal concentration tested	Concentration required for 50% inhibition		
				Yeast	*E. coli*	Rat liver
			mM	mM	mM	mM
I	1-Aminocyclopentane-1-carboxylic acid (cycloleucine)			5.4	3.8	2.1
IIa	1*R*-Amino-3*R*-methylcyclopentane-1-carboxylic acid (isomer A)			6.0	4.3	3.2
IIb	1*S*-Amino-3*R*-methylcyclopentane-1-carboxylic acid (isomer B)			14.6	28	4.9
III	1*RS*-Amino-2*RS*-nitrocyclopentane-1-carboxylic acid (4 isomers)			20[a]	77[a]	20[a]
IV	(*RS*)-3-Aminopyrrolidine-3-carboxylic acid (*RS*-cucurbitine)			54[a]	220[a]	14[a]
V	(*RS*)-3-Aminothiolane-3-carboxylic acid			9.4	4.6	3.2
VI	4-Amino-1,2-dithiolane-4-carboxylic acid			2.2	1.9	0.9
VII	(*RS*)-4-Amino-1,1-dioxo-1,2-dithiolane-4-carboxylic acid			13.4	12.5	2.2
VIII	5-Amino-1,3-dithiane-5-carboxylic acid		17.5	I[b]	I	I

TABLE 1—*Continued*

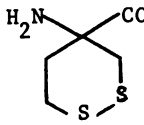
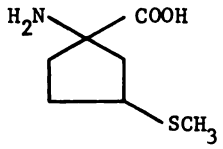
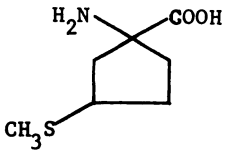
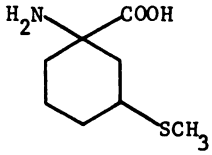
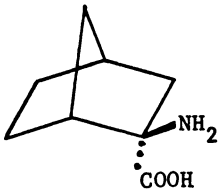
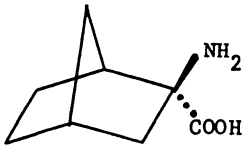
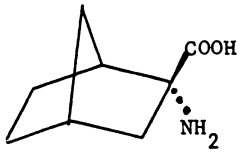
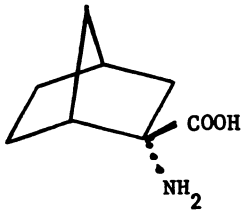
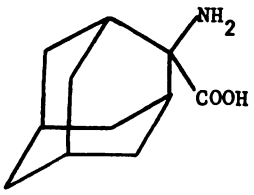
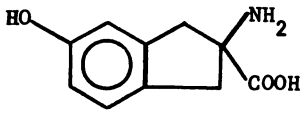
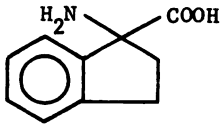
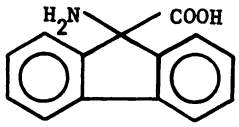
Compound No.	Compound	Structure	Maximal concentration tested	Concentration required for 50% inhibition		
				Yeast	*E. coli*	Rat liver
			mM	mM	mM	mM
IX	(*RS*)-4-Amino-1,2-dithiane-4-carboxylic acid		17.5	I	I	I
Xa	1*RS*-Amino-3*RS*-(methylthio)cyclopentane-1-carboxylic acid [(*RS*)-isomer A]			165[a]	300[a]	40.5[a]
Xb	1*RS*-Amino-3*RS*-(methylthio)cyclopentane-1-carboxylic acid [(*RS*)-isomer B]			44[a]	37.5[a]	8.7[a]
XI	1*RS*-Amino-3*RS*-(methylthio)cyclohexane-1-carboxylic acid (4 isomers)		20	I	I	I
XIIa	(1*R*, 2*R*, 4*S*)-2-Aminonorbornane-2-carboxylic acid, (−)a isomer			4.0	2.8	1.7
XIIb	(1*S*, 2*S*, 4*R*)-2-Aminonorbornane-2-carboxylic acid, (+)a isomer		35	I	I	47[a]
XIIc	(1*S*, 2*R*, 4*R*)-2-Aminonorbornane-2-carboxylic acid, (+)b isomer		60	I	I	I
XIId	(1*R*, 2*S*, 4*S*)-2-Aminonorbornane-2-carboxylic acid, (−)b isomer		35	68.5[a]	I	30.5

TABLE 1—*Continued*

Compound No.	Compound	Structure	Maximal concentration tested	Concentration required for 50% inhibition		
				Yeast	*E. coli*	Rat liver
			mM	*mM*	*mM*	*mM*
XIII	2-Aminoadamantane-2-carboxylic acid		4.4	I	I	I
XIV	(*RS*)-2-Amino-5-hydroxyindane-2-carboxylic acid			42[a]	70[a]	32[a]
XV	(*RS*)-1-Aminoindane-1-carboxylic acid			140	I	140
XVI	9-Aminofluorene-9-carboxylic acid			3.3	52[a]	1.5

[a] These values were obtained by graphical extrapolation, and are not bracketed by experimental observations.

[b] Compounds were designated as I (inactive) if less than 10% inhibition was observed at the maximum concentration that could be tested because of limitations of solubility.

methylcyclopentane-1-carboxylic acid [IIa (37)], in agreement with our inferences based on similarities to the enzyme-bound conformation of L-methionine, as deduced from earlier inhibitor studies (3). Although the results obtained with various 1-amino-3-methylcyclopentane-1-carboxylic acids might be interpreted in terms of "bulk tolerance," or lack thereof, in certain complementary regions of the enzyme, this may not be the only factor contributing to the differences in the inhibitory potency of the isomers. Based on X-ray diffraction studies of crystals of racemic IIa, Carrell, Gallen, and Glusker (37) concluded that the introduction of the 3-methyl group also reduces the thermal mobility of the 5-membered ring conformation and modifies certain of its bond angles considerably. Four of the C—C—C bond angles of the ring system (hereafter referred to as internal ring angles) of 1*R*-amino-3*R*-methylcyclopentane-1-carboxylic acid (IIa) lie between 104.6° and 107.9°, whereas the ring angle at the site of the methyl substitution is 99.9°. Crystallographic studies of the parent cycloleucine (43) reveal that the hydrate of the amino acid exists in two conformational states, both of which can be considered as envelopes. The predominant structure has internal ring angles of 104.4–109°, whereas the alternative structure has internal ring angles of 102–108.2°. Thus at least one internal ring angle of IIa is considerably smaller than those of cycloleucine.

Crystallographic studies of other cycloaliphatic amino acids (Table 2) have shown that the average ring bond lengths of the 5–8-membered cyclic structures are not materially different between those compounds that are active inhibitors (1.50–1.54 A) and those that are not (1.52–1.55 A). However,

TABLE 2

Bond lengths and bond angles of cycloaliphatic amino acids

Compound No.	Compound	Internal ring bond angle at α-carbon atom[a]	$^+H_3N—C—CO_2^-$ bond angle	Mean bond distance between ring carbon atoms	Reference
				A	
I	1-Aminocyclopentane-1-carboxylic acid·H_2O				
	Predominant[b]	104.4°	108°	1.54	43
	Alternative[b]	108.2°	108°	1.54	43
I	1-Aminocyclopentane-1-carboxylic acid·HBr	103.0°	106°	1.50	45
IIa	1*R*-Amino-3*R*-methylcyclopentane-1-carboxylic acid	104.6°	108.0°	1.52	37
	1-Aminocyclohexane-1-carboxylic acid	114.1°	104.3°	1.53	44
	1-Aminocycloheptane-1-carboxylic acid	115.9°	108.8°	1.52	44
	1-Aminocyclooctane-1-carboxylic acid	115.7°	104.9°	1.55	46
XIIa	(1*R*, 2*R*, 4*S*)-2-Aminonorbornane-2-carboxylic acid[c]	103.6°	104.9°	1.54[c]	33

[a] The α-carbon is that bearing the amino and carboxyl functions (43).

[b] The hydrate of cycloleucine exists in the crystal in two conformations, of which one predominates.

[c] This value applies to the 5-membered ring as well as to all the other bonds of the ring system.

the internal ring angles are not identical. Thus, if we consider the α-carbon atom bearing the amino and carboxyl groups, the internal ring angle for the compounds (I, IIa, XIIa) is 103–104.4° (assuming the predominant conformation of cycloleucine), whereas for the noninhibitory compounds this ring angle is about 10° larger (114.1–115.7°). In contrast, the $^+H_3N—C—CO_2^-$ angles of the same cycloaliphatic amino acids vary between 104.3° and 108°, and there appears to be no relation between this angle and inhibitory properties. Although bond angles cannot be the sole determinants of inhibitory potency (since, for example, the enantiomers of IIa and XIIa are essentially inactive), and information is available on only a limited number of compounds, closely defined limits on the internal ring of the α-carbon atom may be a necessary condition for a good inhibitor.

Methylmercapto derivatives of cyclic amino acids. Close inspection of space-filling molecular models of L-methionine and of methylmercapto derivatives of cycloleucine revealed remarkable similarities if both molecules assumed certain conformations. This observation prompted the synthesis of 1*RS*-amino-3*RS*-(methylthio)cyclopentane-1-carboxylic acid (X) and the higher homologue with a 6-membered carbocyclic ring (XI). Compound X but not compound XI displayed measurable inhibitory activity, emphasizing that ring size is a factor of critical importance in this series also and that the cyclopentyl amino acid is greatly superior to its 6-membered homologue. Neither X nor XI was a substrate for the reaction. When the two diastereomeric pairs of 1*RS*-amino-3*RS* - (methylthio)cyclopentane - 1 - carboxylic acid were separated into two racemates (Xa and Xb), the last-mentioned mixture proved to be considerably more active than Xa, although neither racemate was particularly powerful as an inhibitor. Analogies with the conformation of L-methionine deduced earlier (3) would suggest that the most powerful inhibitor is likely to have the 1*R*, 3*R* configuration and that isomer A (Xa) is in fact the racemate (1*R*, 3*R* + 1*S*, 3*S*), in which the 1-carboxyl group and the 3-substituent are on the same side of the ring (in analogy with compound IIa).

Effects of rigidification of ring system: norbornanes. Although crystallographic evidence implies that the introduction of a 3-methyl group into cycloleucine leads to a significant increase in thermal stability (37), a far more effective rigidification of the ring system is achieved either by bridging the 5-membered ring or by incorporating it into a larger ring system. Examples of both types of compounds will be presented.

The four isomeric 2-aminonorbornane-2-carbocyclic acids (2-aminobicyclo[2.2.1.]-heptane-2-carboxylic acid, XII) have be-

come available through the elegant work of Tager and Christensen (32), and their absolute configuration has been determined by Apgar and Ludwig (33) on the basis of X-ray diffraction study of the hydrobromide of the 1*R*, 2*R*, 4*S* isomer (XIIa). These compounds may for our purposes be considered modifications of cycloleucine in which the 5-membered ring system has been completely rigidified by the introduction of a 2-carbon transannular bridge extending from C-2 to C-4 (thus creating a 6-membered ring as well as an additional 5-membered ring). The inhibitory powers of these isomers are radically different (Table 1). Dixon plots of the dependence of reciprocal enzyme ac-activity on inhibitor concentrations for the four isomeric 2-aminonorbornane-2-carboxylic acids with the liver enzyme are shown in Fig. 1, and compared with cycloleucine. Among the four compounds only XIIa [the (−)a isomer] (1*R*, 2*R*, 4*S*) was a powerful inhibitor, which in all three enzyme systems was slightly but consistently superior to cycloleucine. The enantiomer XIIb [the (+)a isomer] was weakly inhibitory for the liver enzyme and not measurably active in the other two systems. The (+)b isomer was inactive, and the (−)b isomer showed some slight activity in two of the three enzyme systems.

The finding that 1*R*-amino-3*R*-methylcyclopentane-1-carboxylic acid (IIa) is the most inhibitory of the 3-methyl derivatives of cycloleucine cannot be readily reconciled with the finding that XIIa is the best inhibitor in the norbornane series, if we consider only steric homologies between bridge origins of the latter and methyl group orientations of the former. The internal ring angle (Table 2) of XIIa for the carbon atom bearing amino and carboxyl group is 103.6°, and therefore is closely similar to the corresponding angle of IIa (104.6°) and of the

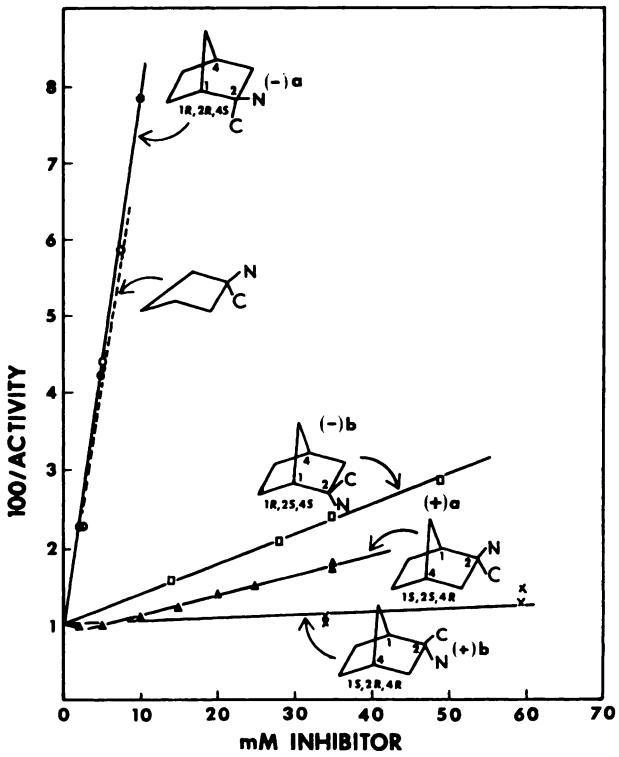


FIG. 1. *Dixon plots showing inhibition of rat liver ATP:L-methionine S-adenosyltransferase by the four isomers of 2-aminonorbornane-2-carboxylic acid and by 1-aminocyclopentane-1-carboxylic acid*

The inhibitions were measured as described under EXPERIMENTAL PROCEDURE.

predominant conformation of cycloleucine (104.4°). Consideration should also be given to the fact that other properties of these cyclic structures may influence their binding to the adenosyltransferases. Thus Tager and Christensen (32) detected differences between the (±)a and (±)b isomers of 2-aminonorbornane-2-carboxylic acids with respect to pK_a values of both carboxyl and amino groups, infrared spectra, and rate constants of hydrolysis of their *N*-formyl derivatives. The results are to a considerable extent inconsistent with the generally held views on the greater steric accessibility of the *exo* over the *endo* positions. Thus additional factors that are not completely understood influence the physicochemical properties of these compounds and may play a role in their interactions with the adenosyltransferases.

The interaction of the cyclic amino acids with the enzyme surfaces may be visualized as a two-step process in which the polar NH_3^+ and CO_2^- groups detect their complementary regions by long-range Coulombic forces and thus initiate the interaction of the enzyme with the inhibitor. When this has occurred, the shorter range van der Waals or hydrophobic forces become effective and complete the fine details of the interaction. On this basis, rigid requirements are imposed for the orientation of the NH_3^+ and CO_2^- groups in the binding to the enzyme surface. Since a 5-membered ring appears to be a cardinal feature of all cyclic inhibitors, we may examine the three-dimensional structure of compounds XIIa–d by superimposing their NH_3^+ and CO_2^- groups and observing the effects on the remainder of the molecules. This is most easily done by examination of the projection drawings of Tager and Christensen [Fig. 2: A, super-

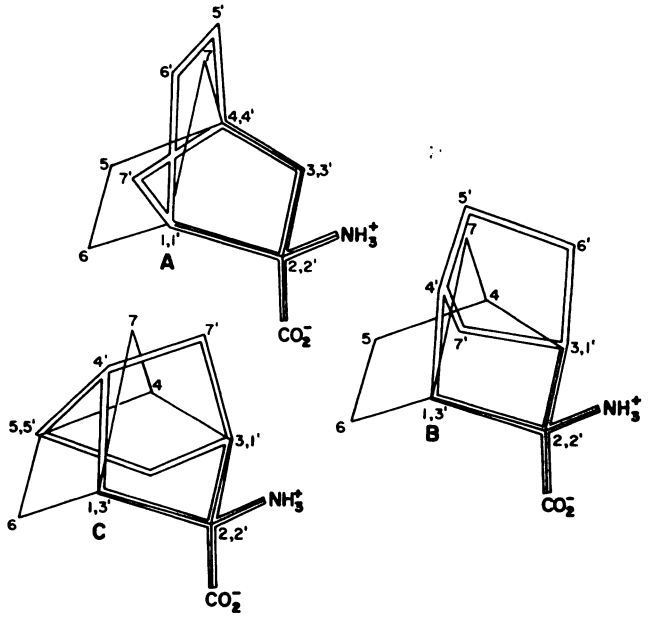


FIG. 2. *Superimposition of the four isomers of 2-aminonorbornane-2-carboxylic acid*

The amino and carboxyl groups of all isomers are drawn so as to occupy the same spatial positions. In each case the single line structure is the (−)a isomer, whereas the structure that is being superimposed is shown in double lines. A. Superimposition of (+)b on (−)a. The 5- and 6-membered rings are spatially interchanged. B. Superimposition of (−)b on (−)a. In this case the 5-membered rings have closely similar conformations, but the 2-carbon bridges point in two different directions. C. Superimposition of the (+)a on the (−)a isomer. In this case the rings point in different directions and there is very little steric similarity between these molecules. Interchange of NH_3^+ and CO_2^- groups represents: for A, (−)b on (+)a; for B, (+)b on (+)a; for C, (−)a on (+)a. [Reproduced with permission from Tager, H. S. & Christensen, H. N. (1972) *Journal of the American Chemical Society*, **94**, 968–972.]

imposition of (+)b on (−)a; B, superimposition of (−)b on (−)a; C, superimposition of (+)a on (−)a]. Only in the superimposition of (−)a and (−)b [or (+)a and (+)b] isomers do the critical 5-membered ring structures assume very similar conformation and occupy approximately the same spatial positions with respect to the NH_3^+ and CO_2^- groups. Thus the two isomers (−)a and (−)b with measurable inhibitory activities have a nearly common conformation of the 5-membered ring structure and its orientation with respect to the functional groups. However, the most potent isomer [(−)a] and the very much less potent [(−)b] possess the bulky rigidifying 2-carbon bridge on opposite sides of the cyclopentane ring bearing the amino and carboxyl groups. Thus the 2-carbon bridge appears to be much better tolerated if it is on the same side rather than on the opposite side of the plane of the 5-membered ring as the carboxyl group. However, it may also be inferred from Fig. 2 that the 5-membered rings of the (−)a and (−)b isomers have very similar conformations, and it is entirely plausible that the conformations of the bonds around the amino acid function may be more important for the binding process than any steric hindrance induced by bulky bridging groups. Tager and Christensen (32) pointed out the close spatial similarities of the pairs of isomers (−)a and (+)b or (+)a and (−)b. Precise conformational factors may be expected to be of special importance where interactions must rely on dispersive forces, which are much more susceptible to weakening by minor changes in "fit" than are electrostatic forces. It is of interest that the geometrical requirements of the amino acid receptor for insulin release by pancreatic islet cells are different from those involved in the synthesis of *S*-adenosyl-L-methionine, since only the (−)b isomer is active in the pancreatic system (47). In amino acid transport systems the (−)b isomer is also the most active compound, both as a substrate and as an inhibitor (32).

Effects of rigidification of ring systems; adamantane. We have prepared the rigid 2-aminoadamantane-2-carboxylic acid (XIII). According to expectations, this compound was totally inactive, presumably because the amino acid function is not located on a 5-membered ring. All the rings of the adamantane skeleton are 6-membered. Because of limitations on solubility, it is difficult to ascertain whether XIII is less active than 1-aminocyclohexane-1-carboxylic acid, and the effect of the additional ring system remains undefined. In this connection, it is interesting to note that Nagasawa, Elberling, and Shirota (42) recently also synthesized XIII in the hope that it might be a more powerful tumor inhibitor than cycloleucine. Although the tumor-inhibitory properties of XIII were disappointing, the compound did inhibit the transport of L-leucine and L-methionine into Ehrlich ascites cells, and in this respect was more effective than cycloleucine. It would thus appear that in this case also the geometrical requirements for tumor inhibition are quite different from those for amino acid transport. The lack of any effects on tumor growth and on the adenosyltransferases would tend to support our view (6, 12) that at least a part of the antitumor action of cycloleucine is mediated through inhibition of the synthesis of S-adenosyl-L-methionine.

Effects of rigidification of ring systems: indanes. Fusion of an aromatic ring onto cycloleucine leads to the indane ring system. Although compounds XIV and XV are not strictly comparable, because of the additional 5-hydroxyl function in XIV, this structural modification, which adds a bulky hydrophobic region and restricts pseudorotation of the cyclopentane ring, is deleterious to inhibitory activity. However, one of the most interesting observations is that the addition of a second phenyl group to the indane system, to give 9-aminofluorene-9-carboxylic acid (XVI), produces one of the most powerful inhibitors of yeast and liver enzymes, with very little inhibitory activity for the *E. coli* enzyme. In this compound the conformation of the cyclopentane ring is rigid and nearly flat, and large regions for hydrophobic interaction with the enzymes are created. The original reports (10) indicated that XVI is devoid of tumor-inhibitory properties, but it might be appropriate to re-examine this finding.

Heterocyclic analogues. We have alluded above to the fact that cycloleucine carries no

electronegative groups corresponding to the regions of unsaturation required for inhibition among the aliphatic analogues (1, 3). Efforts to introduce methylthio groups on cycloleucine led to loss rather than enhancement of inhibitory activity. It was consequently of interest to examine the effects of the introduction of sulfur atoms directly into the ring. A series of cyclic amine and amino acid disulfides were prepared by Herbrandson and Wood (48)[4] in an effort to design agents protective against ionizing radiations, although such activity was not found. Anti-inflammatory activity has been ascribed to thiolane and dithiolane amino acids, although details are not available (31).

The racemic (*RS*)-3-aminothiolane-3-carboxylic acid (V) had inhibitory properties not greatly different from those of cycloleucine itself, on the assumption that only one of the enantiomers is inhibitory. Insertion of a second sulfur atom to give a 5-membered ring system containing a disulfide bridge (4-amino-1,2-dithiolane-4-carboxylic acid, VI) leads to one of the best inhibitors thus far encountered, despite the fact that the disulfide group markedly enlarges the 5-membered ring in relation to the carbocyclic compound. The reasons for the greater efficiency of the C_5 disulfide over the C_5 monosulfide are not entirely clear. Although the enlargement of the ring system might tend to reduce inhibitory activity, electronic factors may outweigh spatial considerations. It is not unexpected that if the ring system is further expanded to 6 members with 2 sulfur atoms, the resultant isomeric compounds (5-amino-1,3-dithiane-5-carboxylic acid, VIII, and (*RS*)-4-amino-1,2-dithiane-4-carboxylic acid, IX) will be devoid of inhibitory activity. Oxidation of the sulfur in one of the two positions of the 5-membered cyclic disulfide to the sulfone [(*RS*) - 4 - amino - 1,1 - dioxo - 1,2 - dithiolane-4-carboxylic acid, VII] detracts from the inhibitory activity for the yeast and *E. coli* enzymes while retaining excellent inhibition for the liver enzyme.

In the naturally occurring amino acid cucurbitine [(*RS*)-3-aminopyrrolidine-3-carboxylic acid, IV], C-3 of cycloleucine is replaced by nitrogen. This compound is a poor inhibitor, possible because of the formal positive charge carried by the secondary amino nitrogen at physiological pH, in contrast to the relative electronegativity of the ring sulfur. Cucurbitine has been isolated from pumpkin seeds and identified as the active principle responsible for the chemotherapeutic effects of these seeds in the treatment of schistosomiasis. The chemotherapeutic activity of cucurbitine is not high, and the compound is effective only against immature schistosomes and has very limited utility in medicine (38, 40).

Specificity of analogues for isofunctional enzymes. It may be noted that the liver adenosyltransferase displays some degree of selectivity among many members of this group of compounds. The inhibitory potency for the liver enzyme is clearly superior for compounds I, IIa, IIb, IV, V, VI, VII, X, XIIa, XIId, and XVI. In some instances the differences are very great, as for XVI, which has an I_{50} value of about 52 mM for the *E. coli* enzyme and 1.5 mM for the liver enzyme. It would therefore be of considerable interest to determine whether there exist differences in susceptibility to inhibition by these componds among adenosyltransferases obtained from a variety of normal and malignant tissues.

[4] H. F. Herbrandson and R. H. Wood, personal communication.

ACKNOWLEDGMENTS

The authors are grateful to Mrs. Mary Karen Burch for skillful technical assistance. Drs. Harold Doshan and Timothy Karpetsky kindly carried out some of the syntheses indicated in the text. Generous gifts of a number of compounds are acknowledged in the body of this paper. We are grateful to our colleague, Dr. Cecil H. Robinson, for much valuable advice, and to Drs. J. P. Glusker and H. L. Carrell for discussion of their crystallographic analyses.

REFERENCES

1. Coulter, A. W., Lombardini, J. B. & Talalay, P. (1974) *Mol. Pharmacol.*, **10**, 293–304.
2. Coulter, A. W., Lombardini, J. B. & Talalay, P. (1974) *Mol. Pharmacol.*, **10**, 305–314.
3. Lombardini, J. B., Coulter, A. W. & Talalay, P. (1970) *Mcl. Pharmacol.*, **6**, 481–499.

[4. Chou, T.-C. & Talalay, P. (1972) *Biochemistry*, 11, 1065–1073.
[5. Lombardini, J. B. & Talalay, P. (1971) *Adv. Enzym. Regul.*, 9, 349–384.
6. Abshire, C. J., Larouquere, J. & Berlinguet, L. (1967) *Can. J. Biochem.*, 45, 557–561.
7. Abshire, C. J. & Pineau, R. (1967) *Can. J. Biochem.*, 45, 1637–1640.
8. Martel, F. & Berlinguet, L. (1959) *Can. J. Biochem. Physiol.*, 37, 433–439.
9. Connors, T. A., Elson, L. A., Haddow, A. & Ross, W. C. J. (1960) *Biochem. Pharmacol.*, 5, 108–129.
10. Ross, R. B., Noll, C.I., Ross, W. C. J., Nadkarni, M. V., Morrison, E. H., Jr. & Bond, H. W. (1961) *J. Med. Pharm. Chem.*, 3, 1–23.
11. Aust, J. B., Andrews, N. C., Schroeder, J. M. & Lawton, R. L. (1970) *Cancer Chemother. Rep.*, 54, 237–241.
12. Lombardini, J. B. & Talalay, P. (1973) *Mol. Pharmacol.*, 9, 542–560.
13. Berlinguet, L., Bégin, N. & Sarkar, N. K. (1962) *Nature*, 194, 1082–1083.
14. Machlin, L. J., Gordon, R. S. & Puchal, F. (1963) *Nature*, 198, 87–88.
15. Gregory, F. J., Flint, S. F., Ruelius, H. W. & Warren, G. H. (1969) *Cancer Res.*, 29, 728–729.
16. Sterling, W. R. & Henderson, J. F. (1963) *Biochem. Pharmacol.*, 12, 303–316.
17. Akedo, H. & Christensen, H. N. (1962) *J. Biol. Chem.*, 237, 113–117.
18. Oxender, D. L. & Christensen, H. N. (1963) *J. Biol. Chem.*, 238, 3686–3699.
19. Ahmed, K. & Scholefield, P. G. (1962) *Can. J. Biochem. Physiol.*, 40, 1101–1110.
20. Bégin, N. & Scholefield, P. G. (1965) *J. Biol. Chem.*, 240, 332–337.
21. Christensen, H. N. & Jones, J. C. (1962) *J. Biol. Chem.*, 237, 1203–1206.
22. Frisch, A. W. (1969) *Biochem. Pharmacol.*, 18, 256–260.
23. Rosenthale, M. E., Datko, L. J., Kassarich, J. & Rosanoff, E. I. (1972) *J. Pharmacol. Exp. Ther.*, 180, 501–513.
24. Rubin, B. A. & Tint, H. (1971) *Transplant. Proc.*, 3, 819–822.
25. Sherman, F. C., Paredes, A. M., Chambers, D. A. & Nardi, G. L. (1965) *Can. J. Biochem.*, 43, 1243–1247.
26. Berlinguet, L., Bégin, N., Nicole, L., Ho-Van-Hap, A. & Singh, P. (1966) *Can. J. Physiol. Pharmacol.*, 44, 345–351.
27. Lombardini, J. B., Chou, T.-C. & Talalay, P. (1973) *Biochem. J.*, 135, 43–57.
28. Dixon, M. (1953) *Biochem. J.*, 55, 170–171.
29. Burrows, B. F. & Turner, W. B. (1966) *J. Chem. Soc. Sect. C*, 255–260.
30. Brian, P. W., Elson, G. W., Hemming, H. G. & Radley, M. (1965) *Nature*, 207, 998–999.
31. Shen, T.-Y. & Walford, G. L. (1971) U. S. Patent 3,547,948; *Chem. Abstr.*, 75, 529, 6336j.
32. Tager, H. S. & Christensen, H. N. (1972) *J. Am. Chem. Soc.*, 94, 968–972.
33. Apgar, P. A. & Ludwig, M. L. (1972) *J. Am. Chem. Soc.*, 94, 964–967.
34. Pinder, R. M., Butcher, B. H., Buxton, D. A. & Howells, D. J. (1971) *J. Med. Chem.*, 14, 892–893.
35. Zelinsky, N. & Stadnikoff, G. (1906) *Chem. Ber.*, 39, 1722–1732.
36. Coulter, A. W. & Talalay, P. (1968) *J. Biol. Chem.*, 243, 3238–3247.
37. Carrell, H. L., Gallen, B. & Glusker, J. P. (1973) *Acta Crystallogr.*, B29, 2580–2585.
38. Sun, T.-T., Loh, S.-H., Chow, S.-W. & Kyi, Z.-Y. (1961) *Sci. Sin.*, 10, 853–859.
39. Rapoport, H. & Willson, C. D. (1962) *J. Am. Chem. Soc.*, 84, 630–635.
40. Fang, S. D., Li, L. C., Hiu, C. & Tseng, K. F. (1961) *Sci. Sin.*, 10, 845–851.
41. Bateman, L. & Shipley, F. W. (1955) *J. Chem. Soc.*, 1996–2005.
42. Nagasawa, N. T., Elberling, J. A. & Shirota, F. N. (1973) *J. Med. Chem.*, 16, 823–826.
43. Mallikarjunan, M. & Chacko, K. K. (1972) *J. Cryst. Mol. Struct.*, 2, 53–66.
44. Chacko, K. K., Srinivasan, R. & Zand, R. (1971) *J. Cryst. Mol. Struct.*, 1, 213–224, 261–269.
45. Chandrasekharan, R., Mallikarjunan, M. & Chandrasekharan, G. (1968) *Curr. Sci. (Bangalore)*, 31, 91–93.
46. Srikrishnan, T., Srinivasan, R. & Zand, R. (1971) *J. Cryst. Mol. Struct.*, 1, 199–212.
47. Tager, H. S. & Christensen, H. N. (1971) *Biochem. Biophys. Res. Commun.*, 44, 185–191.
48. Herbrandson, H. F. & Wood, R. H. (1969) *J. Med. Chem.*, 12, 617–620, 620–624.