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Structural and Conformational Analogues of L-Methionine as Inhibitors of the Enzymatic Synthesis of S-Adenosyl-L-Methionine. IV. Further Mono-, Bi- and Tricyclic Amino Acids

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SUMMARY

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A series of mono-, bi- and tricyclic amino acids was synthesized and examined for their ability to inhibit the enzymatic conversion of L-methionine to *S*-adenosyl-L-methionine by partially purified preparations of ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6) of bakers' yeast, *Escherichia coli* and rat liver. These studies are part of a program to define the topography of the amino acid binding site of these enzymes and to design selective inhibitors of potential chemotherapeutic value. The synthetic amino acids are structurally and conformationally related to 1-aminocyclopentane-1-carboxylic acid (cycloleucine), a highly effective inhibitor of *S*-adenosyl-L-methionine formation. They were designed to provide more precise information about the space-filling and conformational requirements for complementarity at the active sites of the enzymes. Cyclopentaneglycine, although less inhibitory than cycloleucine, has modest activity that is attributed to its conformational relationship to the highly active (*1R,2R,4S*)-isomer of 2-aminonorbornane-2-carboxylic acid. The (*1R,2R,4S*)-isomer of 2-amino-5,6-*exo*-trimethylenenorbornane-2-carboxylic acid appears to be a more potent inhibitor than its 2-aminonorbornane-2-carboxylic acid analogue. It is presumed that the 5,6-*exo*-trimethylene extension of the norbornane framework increases binding capacity because of positive hydrophobic interactions with the enzyme surface in this region. The most active enzyme inhibitor found in this study was (+)-2-aminobicyclo[2.1.1]hexane-2-carboxylic acid, which was even more potent than its close structural analogue, the active 2-aminonorbornane-2-carboxylic acid isomer. This enhancement of inhibitory activity may be a reflection of the size of the bridgehead angle of the bicyclo[2.1.1]hexane derivative which is almost 7° smaller than the corresponding bridgehead angle of the norbornane derivative, and apparently contributes to a more precise complementarity of the bicyclo[2.1.1]hexane amino acid at the surface of the enzyme. The idea that this internal bridgehead angle

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must not exceed a critical value if binding is to occur is further supported by the fact that the analogous isomer of 2-aminobicyclo[3.2.1]octane-2-carboxylic acid, which has an internal bridgehead angle 6° larger than the norbornane derivative, is inactive. Other amino acids which have contributed to the elucidation of the conformational requirements of the enzyme active site include 7-aminonorbornane-7-carboxylic acid, 3-aminonortricyclene-3-carboxylic acid, 1-amino-2,5-dimethylcyclopentane-1-carboxylic acid, 1-amino-3,4-dimethylcyclopentane-1-carboxylic acid and 3-aminobicyclo[3.2.0]heptane-3-carboxylic acid; the latter analogue possessed significant inhibitory activity.

INTRODUCTION

The enzymatic conversion of L-methionine to the biologically reactive and metabolically versatile derivative, Ado-Met[3], has been the subject of extensive biochemical investigation (1–7). In a series of earlier studies from our laboratory (8–13), we have examined the effects of a large number of structural and conformational analogues of L-methionine as potential inhibitors of ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6) in an effort to obtain information about the topography of the amino acid binding region of the active site and to design selective inhibitors of isofunctional enzymes from various sources. It was reasoned that if such selective inhibitors could be obtained, they might serve as chemotherapeutic agents. Three basic types of amino acids differing in their conformational, electronic and steric relationships to L-methionine have been found to inhibit the adenosyltransferase reaction in competition with L-methionine. These inhibitory amino acids include: (a) saturated and unsaturated aliphatic amino acids endowed with critical regions of unsaturation and/or substitutions by electronegative groups (8, 9); (b) aromatic amino acids, including a series of substituted *O*-phenyl derivatives of L-homoserine, in which the inhibitory potency was closely correlated with the lipophilicity and the Hammet sigma values of the substituents (10, 11); and (c) carbocyclic and heterocyclic compounds in which the amino acid function was located on 5-membered rings (12, 13).

The analysis of the structural features of these enzyme inhibitors has allowed certain deductions concerning the topography of the active site of the adenosyltransferases of bakers' yeast, *Escherichia coli* and rat liver. Of particular interest was the observation that 1-aminocyclopentane-1-carboxylic acid (cycloleucine) (I) was an effective adenosyltransferase inhibitor despite its apparent lack of any obvious structural and electronic features analogous to the terminal *S*-methyl group of L-methionine (8). It was concluded that the inhibitory activity of cycloleucine resulted from highly efficient van der Waals' binding forces provided by the somewhat conformationally restricted 5-membered ring structure (12, 13). The low inhibitory potency of the other members of the series of carbocyclic amino acids containing 3, 4, 6 or 7 carbon atoms in their ring confirms the critical requirement for the 5-membered ring to enable complementary binding at the active site of the enzyme (14).

Administration of some of the most potent adenosyltransferase inhibitors to rodents *in vivo* resulted in the expected accumulations of L-methionine and depressions in the levels of Ado-Met in several of the tissues examined (15). Ado-Met plays a central role as the biological methyl group donor (16, 17), and as the source of aminopropyl groups in polyamine biosynthesis (18) and of aminocarboxypropyl groups in certain modified RNA bases (19). Ado-Met participates in the chemotaxis of bacteria (20, 21), in the control of the activity of DNA restriction enzymes (22), and regulates the synthesis of methionine and of

[3] The abbreviations used are: Ado-Met, (*S*)-*S*-adenosyl-L-methionine; (the absolute configuration at the sulfonium pole has been determined by Cornforth, J. W., S. A. Reichard, P. Talalay, H. Carrell and J. P. Glusker, *J. Am. Chem. Soc. 99:* 7292–7300, 1977); adenosyltransferase, ATP: L-methionine *S*-adenosyltransferase (EC 2.5.1.6); cycloleucine, 1-aminocyclopentane-1-carboxylic acid; I_{50}, the concentration of inhibitor required to achieve 50% reduction in adenosyltransferase activity under specified conditions.

Ado-Met itself (3, 14). Analogues of L-methionine, inhibitory for the synthesis of Ado-Met, have already become valuable tools in the analysis of a number of phenomena in which this sulfonium compound participates. Aberrant methylation of tRNA has been found to be associated with neoplasia (23) and with the transformation of cells by oncogenic viruses (24), and it is therefore of interest that the methylation of certain RNA species can be inhibited by cycloleucine (25). It has also been shown that cycloleucine modifies the chemotactic behavior of certain microorganisms, presumably by blocking the synthesis of Ado-Met and thereby affecting the methylation of protein carboxyl groups (21, 26).

The present study extends earlier investigations of inhibitors of adenosyltransferases and includes information on the syntheses and enzyme inhibitory activities of an additional series of cyclic and polycyclic amino acids. These amino acid analogues were chosen because of their ability to provide more precise information about the conformational requirements of the 5-membered ring structure required for optimal interaction at the binding site of the target enzymes.

EXPERIMENTAL PROCEDURE

Many of the materials and methods used in these experiments have been described previously (8, 9). The inhibitory potencies of all analogues were examined with partially purified preparations of adenosyltransferases of bakers' yeast, *E. coli* and rat liver (8, 9). The purification procedures for these enzymes were identical to those described in previous studies from this laboratory (8, 9) in order to maintain strictly comparable conditions for evaluating inhibitory potencies. However, recent work indicates that liver (27) and various yeasts (7, 28) contain at least two species of adenosyltransferase and it is quite possible that similar situations exist in *E. coli* (29). The isoenzymatic composition of our enzyme preparation is unknown and the possibility that various types of adenosyltransferase may display differential susceptibility to inhibitors needs to be kept in mind. The concentrations of inhibitors required to produce 50% inhibition of enzyme activity (I_{50} values) were determined by the graphical method of Dixon (30) at L-methionine concentrations of 37.5 μM and under carefully specified conditions (8, 9).

Amino Acid Analogues

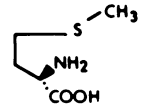
The structures of all amino acid analogues prepared for these studies are represented in Table 1, and are identified by Roman numerals. Isomeric amino acids are further designated with the letters a, b and c to indicate their increasing order of elution from the amino acid analyzer column. In addition, a number of amino acid analogues (I, IV and IX) described in previous studies (12) are included in Table 1 to document structural relationships which are relevant to the discussion relating structure and activity.

Syntheses

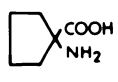
General Procedure for Preparation of Amino Acids. The carbocyclic amino acids represented in Table 1 were synthesized from the corresponding ketones by the use of the following general Strecker procedure. The ketone (1 equiv), ammonium chloride (1.5 equiv) and potassium cyanide (1.5 equiv) were added to sufficient 50% aqueous methanol to dissolve all reactants. After cooling in ice, the reaction mixture was saturated with ammonia gas. The reaction flask was stoppered and stirred at room temperature for 3 to 14 days. The more hindered ketones required the longer reaction time. The solution was extracted 4 times with ether. The extract was washed with saturated aqueous sodium chloride, dried over magnesium sulfate, filtered and evaporated under vacuum to yield the intermediate aminonitrile.

The aminonitrile was converted to the amino acid by refluxing in 6 N HCl for 15 hr. The resulting solution was adjusted to pH 4.5, desalted by adsorption on a column of Dowex 50 (H^+) ion exchange resin, and after thorough washing of the column bed with H_2O, the amino acid was eluted with 1 N NH_4OH. The recovered amino acid was recrystallized from an appropriate solvent after charcoal treatment.

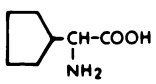
TABLE 1



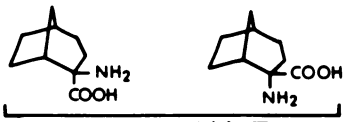
L-Methionine



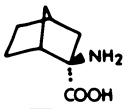
I



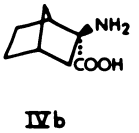
(±)-II



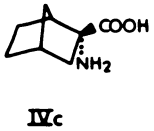
(±)-IIIa and (±)-IIIb



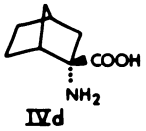
IVa



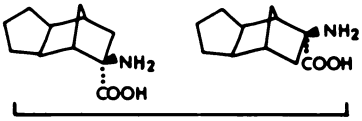
IVb



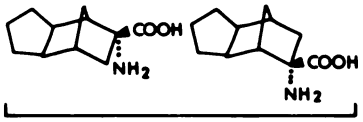
IVc



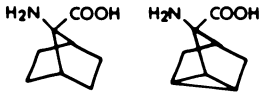
IVd



(±)-Va

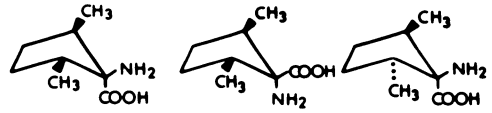


(±)-Vb



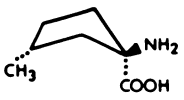
VI

(±)-VII

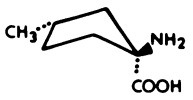


VIIIa and VIIIc

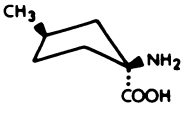
(±)-VIIIb



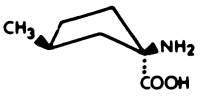
IXa



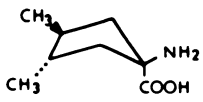
IXb



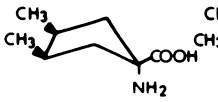
IXc



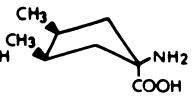
IXd



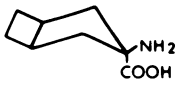
(±)-X



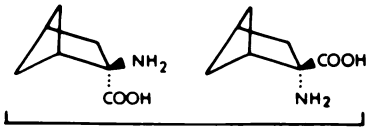
XIa



XIb



XII



(±)-XIII

All amino acids were characterized by infrared, mass and nuclear magnetic resonance spectroscopy. Melting points were determined and analytical chromatography was carried out on the amino acid analyzer (31). All amino acids not previously reported in the literature were further characterized by C, H, N elemental analysis. Mass spectroscopic data are not reported for X, XIa and XIb because these analogues did not undergo volatilization.

(RS)-Cyclopentaneglycine (32, 33) (*II*). Cyclopentylmethanal was converted to II in 67% yield by Strecker synthesis. II had mp 280–284° (lit. (32) mp 284–286°); mass spectrum (70 eV) m/e (rel. intensity) 39(20), 41(27), 56(26), 75(30), 98 (100, M^+ – COOH).

2-Aminobicyclo[3.2.1]octane-2-carboxylic acid (34, 35) (*III*). A Strecker synthesis on (*RS*)-bicyclo[3.2.1]octan-2-one (Aldrich) gave a mixture of diastereomers IIIa and IIIb in relative amounts of 73% IIIa and 27% IIIb as determined on the amino acid analyzer. Mass spectrum (70 eV) m/e (rel. intensity) 41 (10), 54 (10), 79 (10), 124 (100, M^+ – COOH), 125 (12). Anal. Calculated for $C_9H_{15}NO_2$: C 63.88, H 8.93, N 8.28. Found: C 63.76, H 9.12, N 8.70. Recrystallization of III from water gave the pure IIIb racemate which had mp 235–237°. A previous Bucherer synthesis (34) of III via the spirohydantoin intermediate gave only one of the two possible racemates of III with a reported mp 258–260° (34) and with a configuration in which the amino group is *exo* and the carboxyl group is *endo*, as determined by X-ray diffraction (35).

2-Amino-5,6-exo-trimethylenenorbornane-2-carboxylic acid. (V). A Strecker synthesis on (*RS*)-5,6-*exo*-trimethylene-2-norbornanone (Aldrich) yielded diastereomers Va and Vb in relative amounts of 80% Va and 20% Vb, as determined on the amino acid analyzer. A Bucherer synthesis on the identical ketone according to the procedure of Tager and Christensen (36) yielded a mixture containing 15% Va and 85% Vb. These results may be correlated with those of Tager and Christensen (36) who performed both Strecker and Bucherer syntheses on (*RS*)-2-norbornanone to give the two diastereomeric pairs of 2-amino-norbornane-2-carboxylic acid. They designated their isomers a and b in increasing order of elution from the amino acid analyzer column. Strecker reaction gave relative amounts of 81% IVa and 19% IVb, whereas Bucherer synthesis gave 7% IVa and 93% IVb. Thus Strecker synthesis on (*RS*)-2-norbornanone or (*RS*)-5,6-*exo*-trimethylene-2-norbornanone gives predominantly the racemate which is eluted first, whereas Bucherer synthesis of the same pair of ketones gives largely the racemate which is eluted last. Since both ketones are structurally identical at the molecular site of reaction and should yield similar product distribution, it is a reasonable assumption that the absolute configurations of the products can be correlated. On the basis of the X-ray crystallographic studies of Apgar and Ludwig (37), Tager and Christensen (36) assigned to the (±) a isomers the configuration in which the amino group is *exo* and the carboxyl group is *endo*. Accordingly, their (±) b isomers have the *endo* amino group and the *exo* carboxyl group. In other words, it is highly likely that Va is a racemic mixture of *1R, 2R, 4S* plus *1S, 2S, 4R*, in which the amino group occupies the *exo*-position, whereas Vb is a racemic mixture of *1S, 2R, 4R* plus *1R, 2S, 4S*, in which the amino group is in the *endo*-configuration. Fractional crystallization from water of the Strecker mixture of Va and Vb provided pure Va isomer, as determined by chromatography on the amino acid analyzer. The Vb isomer was not separated from Va, but was tested in the enzyme inhibition assay as an equimolar mixture of Va and Vb. Recrystallized (H_2O) Va had mp 274–277°; mass spectrum (70 eV) m/e (rel. intensity) 79 (20), 87 (45), 88 (33), 150 (100, M^+ – COOH), 195 (10). Anal. Calculated for $C_{11}H_{17}NO_2$: C 67.66, H 8.78, N 7.17. Found: C 67.59, H 9.10, N 7.66.

7-Aminonorbornane-7-carboxylic acid (VI). Sodium dichromate oxidation of 7-hydroxynorbornane (38) gave 7-norbornanone (39, 40) which was subjected to Strecker synthesis to give VI (10% yield) which had m.p. 218–220° after recrystallization from water; mass spectrum (70 eV) m/e (rel. intensity) 40 (31), 87 (52), 100 (70), 109 (45), 110 (100, M^+ – COOH), 155 (35). Anal.

Calculated for $C_8H_{13}NO_2$: C 61.91, H 8.44, N 9.02. Found: C 62.13, H 8.45, N 8.88.

(RS)-3-Aminonortricyclene-3-carboxylic acid (VII). Nortricyclanone (41, 42) was prepared by conversion of 3-bromonortricyclene to 3-hydroxynortricyclene as described by Roberts *et al.* (41), followed by Jones oxidation of the alcohol (42) to give the tricyclic ketone. Strecker synthesis on nortricyclanone gave VII in 31% yield. Recrystallization from 1-propanol gave product which had mp 254–256° (dec); mass spectrum (70 eV) m/e (rel. intensity) 60 (100), 81 (43), 82 (40), 107 (77), 108 (79, M^+ – COOH), 126 (32), 153 (39); nmr (D_2O, ext. TMS): δ 4.25 (q, 1H), 3.28 (t, 1H), 2.36 (m, 2H), 1.9 (m, 2H), 1.4 (q, 1H), 1.1 (t, 1H). Anal. Calculated for $C_8H_{11}NO_2 \cdot H_2O$: C 56.13, H 7.65, N 8.18. Found: C 56.43, H 7.50, N 8.60.

1-Amino-2,5-dimethylcyclopentane-1-carboxylic acid (VIII). An isomeric mixture of *cis-* and *trans*-2,5-dimethylcyclopentanone (Aldrich) was converted to the Strecker intermediates, *cis-* and *trans*-1-amino-2,5-dimethylcyclopentane-1-carboxynitrile in 80% yield. The three isomeric aminonitriles contained in this mixture were separated on a dry column of silica gel (100 g silica gel/g product) by elution with petroleum ether/ethyl acetate (9:5 by vol). The aminonitriles were designated a, b or c according to their order of elution from the column. Isomer c was a minor component. Each aminonitrile was then hydrolyzed to give the corresponding amino acid. When a mixture of the amino acids was chromatographed on the amino acid analyzer column, the order of elution was the same as that observed for the silica gel chromatography of the corresponding aminonitriles.

Isomer VIIIa: Recrystallization from water gave product which had mp 200–201° (dec); ir (KBr) 3400–2400 (broad, s), 1625 (m), 1565 (m), 1540 (s), 1400 (s) cm^{-1}; mass spectrum (70 eV) m/e (rel. intensity) 38 (87), 54 (43), 96 (42), 100 (43), 112 (100, M^+ – COOH); nmr (D_2O, ext. TMS): δ 3.08 (m, 2H), 2.58 (m, 2H), 1.82 (m, 2H), 1.44 (t, 6H).

Isomer VIIIb: Recrystallization from an acetone/water mixture gave product with mp 253–255°; ir (KBr) 3500–2350 (broad, s), 1640 (m), 1595 (s), 1525 (s), 1375 (s), 1340 (s) cm^{-1}; mass spectrum (70 eV) m/e (rel. intensity) 36 (100), 38 (36), 55 (22), 112 (67, M^+ – COOH); nmr (D_2O, ext. TMS): δ 2.62 (m, 1H), 2.04 (m, 3H), 1.5 (m, 2H), 1.22 (d, 6H).

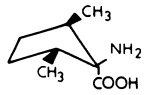
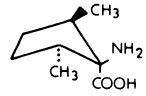
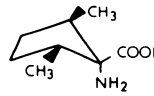
Isomer VIIIc: Recrystallization from acetone/water gave product with mp 260–262°; ir (KBr) 3400–2300 (broad s), 1640 (shoulder), 1680 (s), 1445 (m), 1400 (s), 1320 (s) cm^{-1}; mass spectrum (70 eV) m/e (rel. intensity) 36 (100), 54 (16), 87 (11), 96 (16), 100 (16), 112 (5, M^+ – COOH); nmr (D_2O, ext. TMS): δ 2.13 (m, 4H), 1.77 (m, 2H), 1.23 (t, 6H). Anal. Calculated for $C_8H_{15}NO_2 \cdot H_2O$: C 54.84, H 9.77. Found: C 55.19, H 9.49.

Assignment of configuration to VIIIb: A previously reported synthesis of 1-amino-2,5-dimethylcyclopentane-1-carboxylic acid (43) did not give any indication of the isomeric composition of the starting material or the product obtained. The reported (43) mp 320–322° does not correspond to that of any of the isomers separated in these studies. Although the configurational assignments of VIIIa and VIIIc have been hampered by the lack of distinguishing physical and spectral characteristics, this is not crucial to the present discussion, since both lack inhibitory activity for the adenosyltransferases. However, it has been possible to assign the structure of VIIIb by ^{13}C-nmr spectroscopy (see Table 2) as (*RS*)-1-amino-*trans*-2,5-dimethylcyclopentane-1-carboxylic acid. The evidence for this is as follows: There are two isomers of VIII with the 2,5-dimethyl substituents *cis* to one another. These isomers are symmetrical molecules and accordingly, their ^{13}C-nmr spectra should reflect this by showing the equivalence of the carbon atom pairs: C(2),C(5); C(3),C(4); and C(6),C(7). This is seen in the observed chemical shifts for isomers VIIIa and VIIIc. On the other hand, VIIIb, in which the 2,5-dimethyl substituents are *trans* to one another, is asymmetrical, with nonequivalent carbon atoms at all positions, and this is reflected in the observed chemical shifts.

(RS)-1-Amino-trans-3,4-dimethylcyclopentane-1-carboxylic acid (X). Strecker synthesis on *trans*-3,4-dimethylcyclopen-

TABLE 2

^{13}C chemical shifts (ppm) for isomers of 1-amino-2,5-dimethylcyclopentane-1-carboxylic acid (VIII)*

Product	Structure	C(2), C(5)	C(3), C(4)	C(6), C(7)
VIIIa		43.0	30.0	14.5
VIIIb		41.09 45.03	32.68 32.87	14.41 15.82
VIIIc		43.0	29.9	13.8

* Spectra taken in D_2O/HCl with dioxane as internal reference in the decoupled mode.

tanone yielded X in 75% yield. Recrystallization from water gave a product with mp 269–272°; ir (KBr) 3350 (s), 2900 (s), 1600 (s), 1550 (s), 1440 (m), 1400 (m) cm^{-1}; nmr (D_2O, ext. TMS): δ 2.85 (m, 2H) 2.1 (m, 4H), 1.85 (d, 6H).

1-Amino-cis-3,4-dimethylcyclopentane-1-(cis and trans)-carboxylic acid (XIa and XIb). Strecker synthesis on *cis*-3,4-dimethylcyclopentanone produced only one of the two possible isomers of XI in 31% yield. A modification of conditions, involving 4 hr reflux of the ammoniacal reaction solution before proceeding with the isolation yielded both isomers XIa and XIb in relative amounts of 1:7.5. Dry silica gel column chromatography (petroleum ether/ethyl acetate, 9:5 by vol) of the mixture of intermediate aminonitriles, followed by hydrolysis, yielded the separate isomers, XIa and XIb.

Isomer XIa: Recrystallization from water gave a product with mp 248–251°; ir (KBr) 3400–2400 (broad, s), 1615 (shoulder), 1600 (s), 1515 (s), 1390 (s) cm^{-1}.

Isomer XIb: Recrystallization from water/acetone gave a product with mp 270–273°; ir (KBr) 3300 (s), 2900 (s), 1670 (shoulder), 1600 (s), 1545 (s), 1360 (s), 1245 (s) cm^{-1}; nmr (D_2O, ext. TMS): δ 2.75 (m, 2H), 2.25 (2d, 4H), 1.34 (d, 6H). Anal. Calculated for $C_8H_{15}NO_2 \cdot \frac{1}{2}H_2O$: C 57.80, H 9.70. Found: C 57.87, H 9.84.

3-Aminobicyclo[3.2.0]heptane-3-carboxylic acid (XII). Bicyclo[3.2.0]hept-6-en-3-one (44) was prepared photolytically as described, and hydrogenated with platinum oxide as catalyst to give bicyclo[3.2.0]heptan-3-one (45). Strecker synthesis on this ketone gave XII in 27% yield. After recrystallization from water, XII had mp 240–241° (dec); ir (KBr) 3500–2300 (broad, s), 1610 (s), 1560 (s), 1525 (s), 1380 (s), 1300 (s) cm^{-1}; mass spectrum (70 eV) m/e (rel. intensity) 36 (100), 38 (34), 110 (22, M^+ – COOH). Anal. Calculated for $C_8H_{13}NO_2$: C 61.91, H 8.44, N 9.02. Found: C 62.06, H 8.29, N 9.02. Although XII has two possible structural isomers, only one isomer was apparently formed during Strecker synthesis.

(RS)-2-Aminobicyclo[2.1.1]hexane-2-carboxylic acid (XIII). Bicyclo[2.2.1]-hexan-2-one (46, 47) prepared by the procedure of Meinwald and Chapman (46), was converted to XIII by Strecker synthesis in 76% yield. The racemate had mp 228–232° when recrystallized from water; mass spectrum (70 eV) m/e (rel. intensity) 39 (35), 54 (100), 77 (33), 87 (55), 96 (76, M^+ – COOH), 100 (79), 141 (4). Anal. Calculated for $C_7H_{11}NO_2 \cdot \frac{1}{4}H_2O$: C 57.72, H 7.97, N 9.62. Found: C 57.90, H 8.10, N 9.76.

(+)-2-Aminobicyclo[2.1.1]hexane-2-carboxylic acid [(+)-XIII]. XIII was converted to its *N*-acetyl derivative, 2-acetamidobicyclo[2.1.1]hexane-2-carboxylic acid which had mp 195–196°; mass spectrum (70 eV) m/e (rel. intensity) 39 (36), 41 (26), 43 (100), 54 (35), 96 (57), 100 (20), 183 (1). Anal. Calculated for $C_9H_{13}NO_3$: C 59.00, H 7.15. Found: C 59.07, H 7.13. Resolution (48) of the *N*-acetyl derivative was accomplished as follows: A solution of 1.31 g (7.16 mmole) of 2-acetamidobicyclo[2.1.1]hexane-2-carboxylic acid and 967 mg (7.16 mmole) of (−)-amphetamine in 215 ml of refluxing 95% ethanol was allowed to cool and remain at room temperature for 48 hr. The resulting solid was collected, giving 810 mg of white crystals. Recrystallization from 25 ml 95% ethanol gave 510 mg of a solid phase. Two further recrystallizations of the solid phase from 25 ml 95% ethanol were performed. The final product was dissolved in water, passed through a Dowex 50 (H^+) column to remove (−)-amphetamine, and the eluate was evaporated to give 133 mg of (+)-2-acetamidobicyclo[2.1.1]hexane-2-

carboxylic acid. Acid hydrolysis yielded (+)-XIII ($[\alpha]_{216}^{20}$ = + 335° at c, 0.374 mg/ml in 0.01 N HCl) which proved to be the active isomer of XIII in the enzyme-inhibition assays (See Table 3).

RESULTS AND DISCUSSION

Quantitation of inhibitory activities. The inhibitory potencies of the amino acid analogues in these studies (structures presented in Table 1) for the partially purified isofunctional adenosyltransferases of yeast, *E. coli* and rat liver are given in Table 3. Several analogues from previous studies (8, 12) have been included in this table for purposes of comparison and discussion.

Effect of exocyclic displacement of the amino acid function. The discovery (8) that 1-aminocyclopentane-1-carboxylic acid (I), despite its lack of obvious structural similarity to L-methionine, was an effective competitive inhibitor of the adenosyltransferases of yeast, *E. coli*, and rat liver, prompted the examination of the inhibitory potencies of an extensive series of amino acid analogues containing the cyclopentane structure but modified by substitution and ring rigidification. The inhibitory potency was highly sensitive to ring size, being optimal for the cyclopentane derivative. The conclusion was drawn that the most favorable interactions of the somewhat flexible cyclopentane ring with the enzyme occurred in a highly specific conformation which enabled the molecule to enter into accurate complementarity with the enzyme active site by short range van der Waals' and/or hydrophobic interactions. Since the amino acid function is essential for inhibitory activity, it was assumed that the interaction of the cyclic amino acids is a two-step process in which, first, long range Coulombic forces initiate the recognition of the polar NH_3^+ and CO_2^- by complementary ionic binding groups on the enzyme surface, and that as the ligand approaches its target, powerful short range forces at multiple points then complete the interaction. This proposed mechanism imposes strict requirements on the spatial orientation of the NH_3^+ and CO_2^- groups with respect to the remainder of the molecule. It is not surprising, therefore, that cyclopentaneglycine (II) in which the amino acid function is displaced by the insertion of a C—C bond (1.54 A°) from the five-membered ring system, is a much less powerful inhibitor than cycloleucine (I), even if allowance is made for the fact that probably only one of the enantiomers of II has any inhibitory activity (9).

Nevertheless, cyclopentaneglycine has some capacity for interaction with the adenosyltransferases, and insight into its possible conformation during this process may be obtained by structural comparison with IVa, which is the most inhibitory isomer (*1R, 2R, 4S*) of 2-aminonorbornane-2-carboxylic acid (12). Projection drawings in Fig. 1 show that superimposition of one enantiomer of cyclopentaneglycine upon IVa enables complete correspondence of the semirigid structure of II within that of the larger, rigid norbornane analogue IVa. This suggests that the modest inhibitory power of cyclopentaneglycine may lie in its ability to assume the conformation of the norbornane framework.

Effects of rigidification of the ring system: norbornane analogues. The effects of rigidification of the cycloleucine ring on inhibitory potency were clearly demonstrated with the four isomers of 2-aminonorbornane-2-carboxylic acid, IVa, IVb, IVc and IVd (12). Only IVa was a powerful inhibitor of all three isofunctional adenosyltransferases, while IVd had moderate activity in the yeast and rat liver enzyme systems. However, their respective enantiomers, IVb and IVc, were virtually inactive in all three enzyme systems (Table 3). Comparisons were made of the projection drawings of the three isomers IVb-IVd with IVa, the most active isomer, by superimposing their NH_3^+ and CO_2^- groups and observing the orientations of the rest of their molecular structures. Upon superimposition (Fig. 2A), the two most active inhibitors, IVa and IVd, appeared to have a similar conformation and orientation of their 5-membered ring structure. The much reduced inhibitory potency of IVd when compared to IVa was attributed to the ability of the enzyme to tolerate steric bulk

TABLE 3

Inhibitory potencies of cyclic, bicyclic and tricyclic 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 (8).

Compound no.	Compound	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[a]	3.8[a]	2.1[a]
II	(*RS*)-Cyclopentaneglycine	30	I[c]	60[b]	30
IIIa + IIIb	2-Aminobicyclo[3.2.1]octane-2-carboxylic acid (73% Isomer a, 27% Isomer b)	6	I	I	I
IIIb	2-Aminobicyclo[3.2.1]octane-2-carboxylic acid (Isomer b)	7.5	I	I	I
IVa	(*1R,2R,4S*)-2-Aminonorbornane-2-carboxylic acid		4.0[a]	2.8[a]	1.7[a]
IVb	(*1S,2S,4R*)-2-Aminonorbornane-2-carboxylic acid	35[a]	I[a]	I[a]	47[a]
IVc	(*1S,2R,4R*)-2-Aminonorbornane-2-carboxylic acid	60[a]	I[a]	I[a]	I[a]
IVd	(*1R,2R,4S*)-2-Aminonorbornane 2-carboxylic acid	35[a]	68.5[a]	I[a]	30.5[a]
Va + Vb	2-Amino-5,6-*exo*-trimethylenenorbornane-2-carboxylic acid (54% Isomer a, 47% Isomer b)	4.2	5.2[b]	10.5[b]	4.1
Va	2-Amino-5,6-*exo*-trimethylenenorbornane-2-carboxylic acid (Isomer a)		2.65	1.95	1.55
VI	7-Aminonorbornane-7-carboxylic acid	18.6	61[b]	88[b]	23.5[b]
VII	(*RS*)-3-Aminonortricyclene-3-carboxylic acid	60	I	I	80[b]
VIIIa	1-Amino-*cis*-2,5-dimethylcyclopentane-1-carboxylic acid (Isomer a)	30	I	I	I
VIIIb	(*RS*)-1-Amino-*trans*-2,5-dimethylcyclopentane-1-carboxylic acid (Isomer b)	60	132[b]	I	31
VIIIc	1-Amino-*cis*-2,5-dimethylcyclopentane-1-carboxylic acid (Isomer c)	60	I	126[b]	I
IXc	(*1S*)-Amino-(*3R*)-methylcyclopentane-1-carboxylic acid		14.6[a]	28[a]	4.9[a]
X	(*RS*)-1-Amino-*trans*-3,4-dimethylcyclopentane-1-carboxylic acid		53	41	20
XIb	1-Amino-*cis*-3,4-dimethylcyclopentane-1-carboxylic acid (Isomer b)	60	83[b]	74[b]	51[b]
XII	3-Aminobicyclo[3.2.0]heptane-3-carboxylic acid		16.5	22.2	6.7
XIII	(*RS*)-2-Aminobicyclo[2.1.1]hexane-2-carboxylic acid		2.55	1.1	0.65
(+)-XIII	(+)-2-Aminobicyclo[2.1.1]hexane-2-carboxylic acid		1.4	0.85	0.3

[a] See Reference 12.

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

[c] 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.

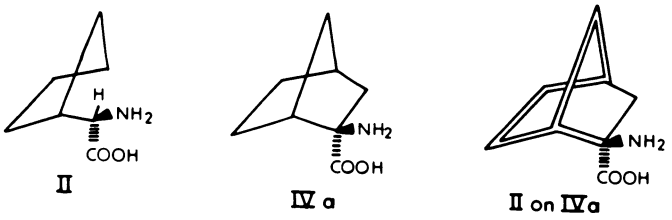


FIG. 1. *Structural comparison of cyclopentaneglycine and 2-aminonorbornane-2-carboxylic acid*

The structures of one enantiomer of cyclopentaneglycine (II) and of the most active isomer of 2-aminonorbornane-2-carboxylic acid (IVa) are shown. In their superimposition (II on IVa) the double lined portion of the structure corresponds to the cyclopentaneglycine framework.

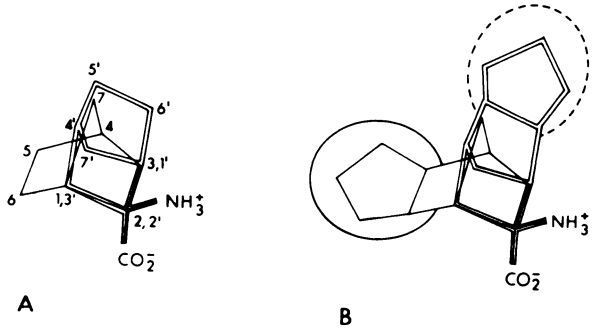


FIG. 2. *Superimposition of the two active isomers of 2-aminonorbornane-2-carboxylic acid and of the corresponding isomers of 2-amino-exo-5,6-trimethylenenorbornane-2-carboxylic acid*

The amino and carboxylic groups of all isomers are drawn so as to occupy the same spatial positions. A. Superimposition of IVd (double line) on IVa (single line). B. Superimposition of analogous isomers Vb and Va. The encircled region indicates the location of regions for positive hydrophobic interaction with the enzyme. The dotted, encircled region indicates an area in which the enzyme does not tolerate steric bulk.

more easily in the region of the 2-carbon bridge of IVa than in the region of the 2-carbon bridge of IVd (Fig. 2A). This supposition is supported by consideration of the closely related 2-amino-5,6-*exo*-trimethylenenorbornane-2-carboxylic acids, Va and Vb, in which the norbornane skeleton is extended with a cyclopentane ring. Thus, Va is the racemic 5,6-*exo*-trimethylene analogue of IVa and IVb; racemic Vb, the corresponding analogue of the IVc and IVd racemate. The inhibitory potency of racemate Va was excellent in all three enzyme systems. These results appear even more impressive, if it is assumed that the enantiomer of Va which corresponds structurally to IVb, is inactive (as was found for IVb), and that inhibitory potency resides exclusively in the other enantiomer of Va. Resolution should then provide an isomer of Va with I_{50} values of approximately 1.3, 1.0 and 0.78 mM, respectively, in yeast, *E. coli* and rat liver enzyme preparations. Whereas the inhibitory potencies of the norbornane analogue IVa in these systems was only slightly better than those observed for cycloleucine (I), the active Va isomer appears to have significantly increased potency when compared to both I and IVa. It is concluded that IVa has slightly higher inhibitory potency than I because its 2-carbon bridge (Fig. 2A) provides appropriately oriented steric bulk for a positive hydrophobic interaction with the enzyme. The addition of a 5,6-*exo*-trimethylene bridge to IVa to give the Va (active isomer) structure augments this positive hydrophobic action, as reflected in the increment of inhibitory activity (Fig. 2B). Thus hydrophobic substituents in this region appear to favor binding of inhibitors.

On the other hand, it follows from the original conclusion (12) that the norbornane amino acid isomer IVd had only moderate inhibitory potency because its 2-carbon bridge was in a topographic region

where substituents are not well tolerated by the enzyme, that its analogous trimethylene derivative, Vb, with increased steric bulk in this region, would be inactive (Fig. 2B). Racemic Vb was tested for inhibitory potency only as an equimolar mixture of Va and Vb. In fact, a comparison of the inhibitory potency of this equimolar mixture (Va + Vb) with that of Va in yeast and rat liver preparations shows an approximate doubling of I_{50} values. This is consistent with the suggestion that both enantiomers of Vb are inactive.

Displacement of amino acid function to norbornane bridgehead. In 7-aminonorbornane-7-carboxylic acid (VI) and (*RS*)-3-aminonortricyclene-3-carboxylic acid (VII), the NH_3^+ and CO_2^- functionalities are located on the 1-carbon bridgeheads of the molecule as opposed to the 2-carbon bridges in the isomeric 2-aminonorbornane-2-carboxylic acids (IVa–IVd). This displacement results in a sharp reduction of inhibitory potency of VI and VII, when compared to that of IVa.

One obvious and critical change resulting from the displacement of the functional groups is a decrease in the internal ring angles at the points of attachment of the NH_3^+ and CO_2^- groups. The internal bond angles have been determined to be 94.2° and 103.6° at C(2) and C(7), respectively, in 2-aminonorbornane-2-carboxylic acid (37). It may be assumed that these angles are not appreciably changed by the presence or absence of the CO_2^- and NH_3^+ groups. Thus, the internal bond angle at the carbon to which CO_2^- and NH_3^+ groups are attached may be close to 94.2° in VI and 103.6° in VIa. Consideration has been given previously (12) to the importance of the internal bond angle of the cyclopentane ring at the α-carbon atom bearing the amino and carboxyl substituents in promoting binding to the enzyme. Comparison of this bond angle in several cyclic amino acid derivatives showed that binding to the enzyme was most effective when this internal bond angle was maintained within a range of 103–104.4°.

3-Aminonortricyclene-3-carboxylic acid (VII) bears a structural relationship to both IVa and VI. Although crystallographic information on the internal bond angle of the carbon atom bearing the amino acid functions is not available for VII, it is highly probable that this angle is much closer to the corresponding angle of VI than of IVa, and that this difference could account for the fact that VII is virtually inactive as an inhibitor. Nevertheless, it should be recognized that the introduction of the additional restraining bond between C(2) and C(6) in VI is likely to affect the geometry of the norbornane ring system in multiple ways which might also influence the affinity of the enzymes for VII.

Effects of alkyl ring substitution in cyclopentane analogues. The effects of introducing substituents on the cycloleucine ring have been examined in detail in previous studies (12). In general, it was observed that introduction of substituents at the 2-position of the ring either impaired or completely abolished inhibitory activity towards the adenosyltransferases, whereas in specific instances, introduction of substituents at the 3-position could be well tolerated by the enzyme. In the present study, selected dimethylcycloleucine derivatives have been prepared because they provide structural comparisons to the rigidified analogues VI and XII.

1-Amino-2,5-dimethylcyclopentane-1-carboxylic acid (VIII) has four possible isomeric configurations (see EXPERIMENTAL PROCEDURES for discussion of structural assignments). In isomers VIIIa and VIIIc the 2,5-dimethyl substituents are in a *cis* relationship to one another and differ only in the orientations of the amino and carboxyl groups to the two methyl groups. In isomer VIIIb, the 2,5-dimethyl substituents are *trans* oriented, and because of the presence of an asymmetric center at C(1), this derivative is a racemate. Although assignment of the absolute configuration of isomers VIIIa and VIIIc has not been possible, this is not crucial for our purposes since both VIIIa and VIIIc are almost devoid of inhibitory activity. The relevance of VIIIa and VIIIc to our discussions lies in their structural relationship to VI. Thus construction of a transannular bridge by covalent linkage of the *cis*-dimethyl substituents in either VIIIa or VIIIc produces the structural ar-

rangement found in 7-aminonorbornane-7-carboxylic acid (VI). *A priori*, one would not expect either VIIIa or VIIIc to inhibit the adenosyltransferases since it was observed previously that a 2-methyl or 2-ethyl substituent on the cycloleucine ring significantly impairs inhibitory potency (12). The effect of two *cis*-dimethyl substituents directly adjacent to either the carboxyl groups or the amino groups as in VIIIa and VIIIc, appears to introduce even more severe steric restrictions on the accessibility of these polar groups to their complementary binding site on the enzyme surface. Indeed, both VIIIa and VIIIc were inactive as enzyme inhibitors. The bridging of these *cis*-dimethyl groups of VIIIa and VIIIc relieves this steric hindrance sufficiently to enable binding of the amino and carboxyl groups to the enzyme, as seen by the inhibitory activities recorded for VI.

Interestingly VIIIb (in which the two methyl groups are *trans*) was the only 2,5-dimethylcycloleucine isomer for which any inhibitory activity was observed. The *trans* orientation of the 2,5-dimethyl substituents (VIIIb), apparently alleviates, to a small degree, the steric restrictions on the polar amino and carboxyl groups, and permits binding, although not with great efficiency.

An analogous series of 1-amino-3,4-dimethylcyclopentane-1-carboxylic acids, X, XIa and XIb has been prepared. These structural isomers exhibit the same *cis-trans* relationships of their dimethyl, amino and carboxyl substituents as observed for the 2,5-dimethylcyclopentane analogues of VIII. The availability of *trans*-3,4-dimethylcyclopentanone, separated from its *cis*-isomer, enabled an unambiguous synthesis of (*RS*)-1-amino-*trans*-3,4-dimethylcyclopentane-1-carboxylic acid (X). The corresponding *cis* isomers, (XIa and XIb) were prepared from *cis*-3,4-dimethylcyclopentanone, and were separated, but as in the case of VIIIa and VIIIc, spectroscopic data did not permit absolute assignment of structure. XIb was, under the general reaction conditions described in this paper, the only product of Strecker synthesis. Although recent mechanistic studies (49, 50) have confirmed that the stereochemical course of addition in the Strecker reaction is greatly influenced by steric hindrance in another part of the molecule, the final product distribution is nevertheless thermodynamically controlled. For XIb, because one face of the reacting ketone is sterically hindered by the presence of the dimethyl substituents, it might be possible to predict the stereochemistry of the initially formed aminonitrile intermediate but equilibration in alkaline medium with its isomeric aminonitrile is so rapid that it precludes isolation and identification of the kinetically controlled isomer. However, a tentative structural assignment will be proposed for XIb which is consistent with comparable properties of related amino acid analogues. Isomers XIa and XIb provide a structural relationship to the bicyclic amino acid analogue XII. A covalent linkage between the *cis*-3,4-dimethyl substituents of XIa or XIb gives the rigidified bicyclo[3.2.0]heptyl framework of XII, 3-aminobicyclo[3.2.0]-heptane-3-carboxylic acid. Although XII has two possible isomeric configurations, Strecker synthesis on bicyclo[3.2.0]heptan-3-one provided only one of the two possible isomers. It is highly likely that the isomer of XII obtained is the exact structural analogue of XIb, since under the same reaction conditions, they are the *only* products of thermodynamic control in the Strecker reaction. Thus the 5,6-dimethylene bridge of XII and the 3,4-*cis*-dimethyl substituents of XIb most probably share the same orientation with respect to the amino (and carboxyl) groups.[4] We conclude that rigidi-

[4] If this assumption is made, the orientation of the amino and carboxyl substituents might then be determined by relating the structure of XII to an amino acid analogue of known configuration. In this regard, there is a striking correlation between the I_{50} values observed for XII in yeast, *E. coli* and rat liver preparations and the corresponding values observed for IXc, a 3-methylcyclopentane derivative in which the carboxyl group is *trans* to the 3-methyl group. Previous analysis (12) of the structure-activity relationships of the four isomeric 3-methylcyclopentane amino acids strongly suggests that a close structural correspondence must also prevail between IXc and XII. Accordingly XII is tentatively assigned the configuration depicted in Table 1, in which the carboxyl group is *trans* to the *exo*-3,4-dimethylene bridge. The configuration proposed for XIb is analogous (Table 1) with the carboxyl group *trans* to the 3,4-dimethyl substit-

fication through bridging of the 3,4-dimethyl substituents causes a substantial increase in inhibitory activity for all three adenosyltransferase preparations. This may be attributed to two factors: (1) the bicyclic structure of XII reduces the conformational freedom of the cyclopentane ring, and it appears that the resulting allowed conformations favor the binding capabilities of the amino acid terminus; (2) the transannular 3,4-dimethylene bridge of XII occupies a smaller proscribed volume than the corresponding *cis*-3,4-dimethyl substituents of XIb. The latter may be a particularly important factor in alleviating negative steric interactions with the enzyme surface, since it was observed in previous studies with the four isomers of 1-amino-3-methylcyclopentane-1-carboxylic acid, IXa-IXd, that differences in the orientations of the 3-methyl substituents were associated with marked changes in inhibitory potencies (12).

Further effects of rigidification of cyclopentane ring system: bicyclo[2.1.1]hexane and bicyclo[3.2.1]octane. Synthetic 2-aminobicyclo[2.1.1]hexane-2-carboxylic acid (XIII) is an excellent inhibitor of all three adenosyltransferases (13). In fact, among all the methionine analogues tested during these extensive studies (8–13), (+)-XIII was found to be the most effective inhibitor of these enzymes. The cumulative evidence derived from the striking differences in structure-activity relationships observed for closely related, semirigid cyclic amino acid analogues discussed in this study strongly suggests that the enhanced inhibitory potency of (+)-XIII can be attributed to highly precise complementarity between the occupied volume of XIII and the surface contour of the enzyme at the binding site. This is more clearly revealed by a detailed comparison of XIII, with its close structural analogue, IVa. For purposes of this comparison, only IVa, the most inhibitory isomer of 2-aminonorbornane-2-carboxylic acid, and the enantiomer of XIII which appears analogous by virtue of the superimposibility of its dimethylene bridge and its NH_3^+ and CO_2^- groups on those of IVa, will be considered. Although the absolute configuration of (+)-XIII is not known, the structure-activity relationships strongly suggest that the active enantiomer of XIII has the *2R* configuration and shares this configurational correspondence with IVa. It is recalled, once again, that IVa was an active inhibitor of yeast, *E. coli* and rat liver adenosyltransferases; yet (+)-XIII, with a bicyclo[2.1.1]hexane structure representing only a small modification of IVa, is significantly more active than this norbornane analogue. A comparison, based on results obtained by gas phase electron diffraction (51, 52), of bond angles and bond lengths found in bicyclo[2.1.1]hexane and bicyclo[2.2.1]heptane (norbornane) is presented in Table 4, and a graphic comparison of these same molecular parameters is shown in Fig. 3.[5] The first point to be made

TABLE 4

Comparison of bond angles and bond lengths for bicyclo[2.1.1]hexane and bicyclo[2.2.1]heptane (norbornane)

	Bond angles (degrees)	
	bicyclo-[2.1.1]hexane (52)	bicyclo-[2.2.1]heptane (51)
C(1)-C(2)-C(3)	102.1	104.3
C(5)-C(4)-C(6)	78.2	
C(5)-C(4)-C(7)		101.4
C(1)-C(6)-C(4)	89.4	
C(1)-C(7)-C(4)		96
	Bond lengths (Angstroms)	
	bicyclo-[2.1.1]hexane (52)	bicyclo-[2.2.1]heptane (51)
C(1)-C(2)	1.565	1.556
C(2)-C(3)	1.513	1.551
C(1)-C(7)		1.559
C(1)-C(6)	1.544	1.556

uents. It is interesting that the elution patterns, on the amino acid analyzer, of XIa-XIb and IXa-IXc are consistent with this assignment. Thus, whether the cyclopentane ring is monomethyl or *cis*-dimethyl substituted, the amino acid with its carboxyl group *trans* to these methyl substituents is eluted second.

[5] Although bond lengths and bond angles of 2-aminonorbornane-2-carboxylic acid, Isomer IVa, are available through X-ray crystallographic studies (37), the effect of placing amino and carboxyl substituents at the 2-position of the norbornane ring causes very slight distortions in the symmetry of the molecule which is

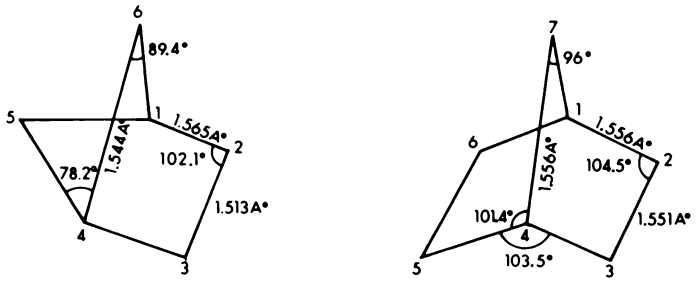


FIG. 3. *The structures of bicyclo[2.1.1]hexane and bicyclo[2.2.1]heptane (norbornane)*
The bond angles and bond lengths for these two closely related bicyclic molecules have been obtained from gas-phase electron diffraction studies (51, 52).

about the structures of IVa and XIII is that there is a very close correspondence in bond lengths and bond angles at the region of C(2), which is the point of attachment for the NH_3^+ and CO_2^- substituents which are assumed to initiate the Coulombic interactions with the enzyme. This close correlation between the bond lengths of C(1)-C(2), C(2)-C(3) and C(3)-C(4) and the internal C(1)-C(2)-C(3) bond angle in both molecules seems to satisfy the optimum conformational requirements that assure initiation of the primary event involved in enzyme-inhibitor recognition. It appears that the factors underlying the increased inhibitory potency of XIII reside in another region of the bicyclic structural framework. In this regard, a most significant difference between the two structures is reflected in the size of the corresponding internal bridgehead bond angles, C(1)-C(6)-C(4) of bicyclo[2.1.1]hexane and C(1)-C(7)-C(4) of norbornane. The former is almost 7° smaller than the latter, and herein lies the most probable cause for the enhancement of activity seen for XIII. The size and orientation of steric bulk in this region of the bridgehead carbon was previously shown to have critical effects on resulting inhibitory potency, as evidenced in the structure-activity relationships of the four isomers of IV (see Fig. 2 of ref. 12). The inactivity of IVc was attributed to the bulk and orientation of the C(5)-C(6) dimethylene bridge that apparently could not be tolerated by the enzyme. With XIII it is assumed that the smaller C(1)-C(6)-C(4) bond angle orients the bridgehead methylene group and the C(6)-C(4) bond so that they fit more closely the enzyme contour at this location, resulting in a positive interaction that significantly increases the total binding efficiency of the molecule. Additional evidence to support the idea that small changes in the internal angle at the norbornane bridgehead produce large changes in inhibitory activity is provided by the four isomers of 2-aminobicyclo[3.2.1]octane-2-carboxylic acid (IIIa and IIIb). Racemates IIIa and IIIb showed no inhibitory activity for the adenosyltransferases. This indicates that the enantiomer of III which, by virtue of its *2R* configurational correspondence to IVa, might be expected to have some binding capacity, appears inactive as an inhibitor. However, the one-carbon expansion of the transannular bridge to which the amino and carboxyl groups are attached (formally producing III from IV) is accommodated by an increase of the internal bridgehead angle at C(8) in III. Recent X-ray crystallographic studies have determined that this bridgehead angle of III is 102.2° (35), apparently exceeding the critical requirements for complementary fit of the molecule at the enzyme surface.

The relative orientations of the C(5) methylene group of XIII and the homologous C(5)-C(6) dimethylene bridge of IVa, are quite different. This region has been delineated in this study as one in which a positive hydrophobic interaction with the enzyme may occur, but apparently there is

reflected in slight changes in the bond lengths and bond angles of IVa when compared to norbornane itself. Similar changes would be expected for XIII, when compared to bicyclo[2.1.1.]hexane, but X-ray diffraction data for XIII are unavailable to substantiate this supposition.

no difficulty in accommodating this steric bulk in the conformation presented by either XIII or IVa.

Conclusions. The analysis of the inhibitory potencies of an additional series of mono-, bi- and tricyclic amino acid analogues on yeast, *E. coli* and rat liver adenosyltransferase preparations has refined our understanding of the topographic features of the active sites of these enzymes. A region has been delineated in which positive hydrophobic interactions occur between the enzyme and the appropriately designed inhibitor. This is illustrated by the inhibitory activity observed for 2-aminonorbornane-2-carboxylic acid (isomer IVa) and the apparently augmented potency of its 5,6-*exo*-trimethylene analogue, Va. Another region has been defined in which precise complementarity between the enzyme and the bridgehead carbon of the highly inhibitory bicyclohexane amino acid analogue XIII has been observed. This supplies significant information about the shape of the enzyme contour in this region.

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