Potential Latentiation Forms of Biologically Active Compounds Based on Action of Leucine Aminopeptidase. Dipeptide Derivatives of the Tricycloaliphatic α-Amino Acid, Adamantanine

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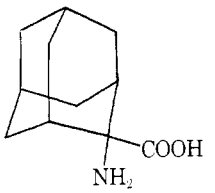
Some glycine, leucine, and phenylalanine dipeptide derivatives of the transport inhibitory, tricycloaliphatic α-amino acid, adamantanine (1), have been synthesized using classical methods of peptide synthesis with the aim of improving the latter's bioavailability. Although test doses of glycyladamantanine and L-leucyladamantanine appeared to be absorbed in vivo as evidenced by its appearance in the urine following intraperitoneal administration, they were not hydrolyzed by a purified preparation of leucine aminopeptidase in vitro. Indeed, they were inhibitors of this enzyme. Adamantanylglycine, adamantanyl-L-leucine, and adamantanyl-L-phenylalanine were also not hydrolyzed by leucine aminopeptidase.

We have previously described[1] the synthesis of 2-aminoadamantane-2-carboxylic acid (adamantanine, 1), a tricycloaliphatic amino acid analog of the antitumor, immunopharmacologic, and transport-inhibitory amino acid, 1-aminocyclopentanecarboxylic acid (cycloleucine, 2).[2,3] The crystal structure of the hydrobromide of 1 has also been reported.[4] Although adamantanine (1) is inactive in inhibiting the ATP:L-methionine S-adenosyltransferase (S-adenosylmethionine synthetase) of rat liver, *Escherichia coli*, or yeast,[5] it is nearly six times as active on a molar basis as 2 in inhibiting the transport of methionine and leucine into Ehrlich ascites carcinoma cells in vitro. Whether 1 is itself actively transported into tumor cells is not yet known. The extreme insolubility of 1 in physiological systems at pH 7 (<1 m*M*) impeded further biochemical and pharmacological studies; e.g., LD_{50} determinations were hindered by precipitation of the amino acid in the peritoneal cavity of mice. This lack of bioavailability may be the basis for the absence of in vivo antitumor activity of 1 in mice bearing L1210 lymphoid leukemia.[1]



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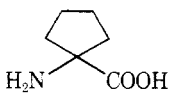

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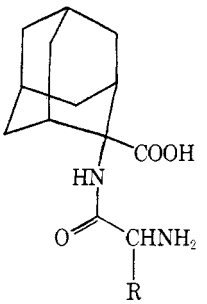
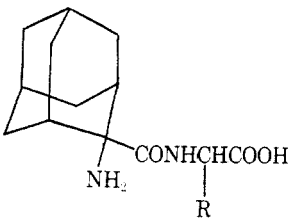
Conners et al.[6] have reported that glycine peptides of cycloleucine exhibited in vivo antitumor activity comparable to that shown by 2 itself. Although there is evidence that some dipeptides such as glycylglycine are transported intact into Ehrlich ascites cells,[7] whether the glycine peptides of 2 are similarly transported or whether they are themselves intrinsically active has not been studied. It is possible that these glycine peptides of cycloleucine are hydrolyzed by tissue aminopeptidases liberating 2 in vivo, since other structural modifications of 2 led to compounds which uniformly lacked antitumor activity,[6] and other peptide derivatives of 2 show varying antitumor activities.[8]

Greenstein et al.[9] earlier speculated that a correlation might exist between the sites of carcinogenicity of amino acid amide derivatives of certain carcinogenic aromatic amines and their susceptibility to hydrolysis at these sites. The premise upon which this was based, viz., that these aromatic amines were themselves "ultimate" carcinogens, has subsequently been shown to be in error;[10] but Hill and Smith[11] did demonstrate that those conjugates derived from *aliphatic* amino acids were in fact hydrolyzed by a purified preparation of leucine aminopeptidase. Similarly, glycyl and substituted glycyl derivatives of the nitrogen mustard, *N,N*-bis(2-chloroethyl)-*p*-phenylenediamine, were found to be rapidly hydrolyzed by an aminopeptidase found in homogenates of kidney, ileum, spleen, and liver of rats, although selective toxicity against Walker 256 tumor in vivo was not attained because of the low level of this enzyme in the tumor.[12] More recently, certain amino acid amide derivatives of dopamine—especially those derived from L-isoleucine—were found to have renal vasodilator activity in both dogs and monkeys upon oral administration.[13] This activity was correlated with free plasma dopamine levels resulting from hydrolysis of these derivatives by aminoacylarylamidases found in the small intestine and kidneys.[14]

Based on these considerations, the following dipeptide derivatives of adamantanine (1) were synthesized: viz., glycyl- (3) and L-leucyladamantanine (4), as well as adamantanylglycine (5), adamantanyl-L-leucine (6), and adamantanyl-L-phenylalanine (7). These were specifically designed



3, R = H
4, R = *i*-Bu

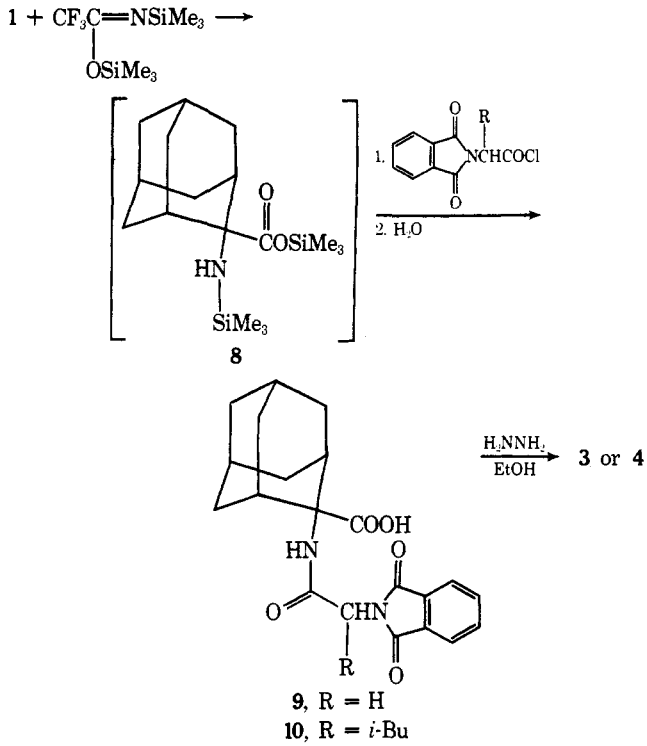


5, R = H
6, R = *i*-Bu
7, R = $CH_2C_6H_5$

to increase the bioavailability of 1 by (a) conversion to peptide derivatives with increased water solubility, (b) exploitation of the natural transport system for dipeptides to carry the dipeptide derivatives of 1 across tissue membranes, and (c) reliance on tissue amino peptidases to release the amino acid at tumor sites.

Chemistry. Glycyladamantanine (3) and L-leucyladamantanine (4) were prepared by acylating the *N,O*-bis(trimethylsilyl) derivative of 1, viz., 8, prepared in situ in acetonitrile, with phthaloylglycyl chloride and phthaloyl-L-leucyl chloride, respectively, followed by dephthaloylation of the resulting phthaloylglycyladamantanine (9) and phthaloyl-L-leucyladamantanine (10) with hydrazine (Scheme I). The use of this procedure developed by Birkhoffer and Ritter[15] for the acylation of sterically hindered α-amino acids in aprotic medium was necessary for 1; e.g., when the acylation of 1 was attempted in aqueous alkali,

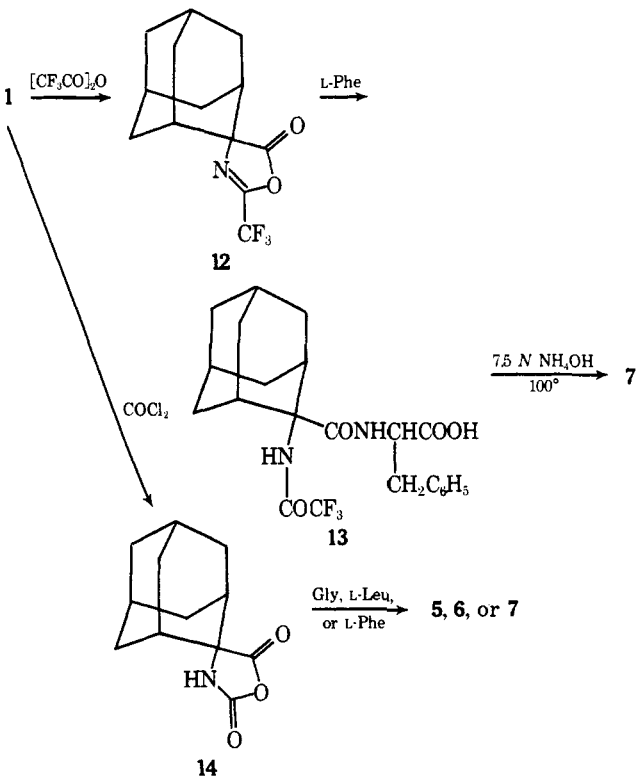
Scheme I



hydrolysis of the N-protected aminoacyl halide to the carboxylic acid predominated over peptide coupling. Alternatively, **3** was prepared in 87% overall yield (vs. 32% via phthaloylglycyl chloride) by acylating **8** with chloroacetyl chloride, followed by displacement of the α-chloro group of the product (**11**) with ammonia.

For the preparation of the *adamantanyl* dipeptides, **5, 6,** and **7,** N-protected derivatives of **1** suitable for use in peptide coupling reactions were required. The trifluoromethyl azlactone **12,** prepared by reaction of **1** with excess trifluoroacetic anhydride (Scheme II), appeared to be ideally suit-

Scheme II



ed in this regard, since the azlactone molecule is inherently N-protected and carbonyl activated at the same time. The ir spectrum of **12** was consistent with the assigned structure, as was the electron ionization (EI) mass spectrum. Condensation of **12** with L-phenylalanine provided the expected *N*-trifluoroacetyladamantanyl-L-phenylalanine (**13**). However, removal of the trifluoroacetyl protective group—usually effected under mild alkaline conditions[16]—required drastic measures for **13** due to the steric bulk surrounding this trifluoroacetamide bond.

A more useful carbonyl-activated, N-protected **1**, which did not require a separate deblocking step, was the *N*-carboxyanhydride **14,** prepared readily by treatment of **1** with phosgene. The EI mass spectrum of **14** displayed a molecular ion $[M\cdot^+]$ at the expected *m/e* of 221, and characteristic fragment ions at *m/e* 193 $[M - CO]\cdot^+$, 178 $[M - HNCO]\cdot^+$, and 149, the first two paths being verified by the presence of appropriate metastable peaks. The fragmentation of **14** on chemical ionization (CI, methane) was similar except that the ions were heavier by one atomic mass unit as expected, viz., *m/e* 222 $[MH]^+$, 194 $[MH - CO]^+$, and 179 $[MH - HNCO]^+$.

Reaction of **14** with glycine, L-leucine, or L-phenylalanine in a heterogeneous system of acetonitrile–aqueous Na_2CO_3–NaOH[17] gave the desired adamantanylglycine (**5**), adamantanyl-L-leucine (**6**), and adamantanyl-L-phenylalanine (**7**), respectively (Scheme II). The CI mass spectra of these dipeptides displayed prominent quasimolecular ions $[MH]^+$ (Table I); moreover, all three N-terminal adamantanyl dipeptides gave rise to a characteristic *m/e* 150 fragment ion which was weak or absent when the adamantanine moiety was C-terminal.

Biological Results. In qualitative in vivo experiments, glycyladamantanine (**3**) and L-leucyladamantanine (**4**) were found to be excreted in the urine of rats when test doses of 400 mg/kg were administered orally. This constitutes evidence that **3** and **4** can be absorbed intact from the gastrointestinal tract. The dipeptides were separated from normal urinary constituents by two-dimensional TLC and detected with ninhydrin reagent (Figure 1, see paragraph at end of paper regarding supplementary material). The urinary excretion of test doses of the *adamantanyl* dipeptides, viz., adamantanylglycine (**6**) and adamantanyl-L-leucine (**7**), could not be determined because these peptides did not produce the usual strong violet color with ninhydrin and did not yield fluorescent products with fluorescamine.[18]

The susceptibility of the peptides **3–7** to enzymatic cleavage by leucine aminopeptidase, an enzyme that hydrolyzes N-terminal leucyl dipeptides as well as certain amino acid amides and found in kidney, intestinal mucosa, lung, spleen, plasma, and ascites tumor cells,[19] was tested in vitro using a partially purified preparation of this enzyme from hog kidney. No hydrolysis was detectable with any of the peptides above under conditions where L-leucylamide and L-leucyl-*p*-nitroanilide were hydrolyzed. Apparently, the peptide bonds of the adamantanine dipeptides **3–7** are sterically hindered from enzymatic attack by leucine aminopeptidase. Indeed, these dipeptides of **1** were inhibitors of this enzyme. The $[I/S]_{0.5}$ values with L-leucyl-*p*-nitroanilide as substrate are listed in Table I. A more detailed kinetic analysis[20] of L-leucyladamantanine (**4**), one of the better inhibitors, indicated that **4** was a competitive inhibitor with an apparent K_i of 1.19 ± 0.10 m*M* (Figures 2 and 3). Adamantanyl-L-phenylalanine (**7**), the most active inhibitor in this series, exhibited complex kinetics, which were not further analyzed.

In a further in vitro study, the adamantanine peptides **3–7** were tested for growth inhibitory activity against P-388

Table I. Dipeptide Derivatives of Adamantanine

Name	Formula	Analyses	Mp (cor),[a] °C	Recrystn solvent	$[\alpha]^{26}D$[b]	[MH⁺][c]	Inhibition of: Leucine aminopeptidase,[d] $[I/S]_{0.5}$	Inhibition of: P-388 lymphoid leukemia in vitro,[e] I_{50} (m*M*)
Glycyladamantanine (**3**)	$C_{13}H_{20}N_2O_3 \cdot H_2O$	C, H, N	312–316	H_2O		252	6.6	>1.6
L-Leucyladamantanine (**4**)	$C_{17}H_{28}N_2O_3$	C, H, N	303–305	EtOH–H_2O	+41.6	309	3.0[g]	>0.5
Adamantanylglycine (**5**)	$C_{13}H_{20}N_2O_3$	C, H, N	324–329	H_2O		253	7.4	>4
Adamantanyl-L-leucine (**6**)	$C_{17}H_{28}N_2O_3$	C, H, N[f]	300–305	MeOH–H_2O	−34.9	309	4.7	>1
Adamantanyl-L-phenylalanine (**7**)	$C_{20}H_{26}N_2O_3$	C, H, N	286–288	MeOH–H_2O	−47.4	343	1.0	>1
Adamantanine (**1**)							10.5	1.0
Cycloleucine (**2**)								8.3

[a] Sealed capillary. [b] *c* 1, 1.0 *N* HCl. [c] Quasimolecular ion by CI mass spectrometry. For **5**, **6**, and **7**, a characteristic *m/e* 150 peak was also present. [d] Where [S] = 2.0 m*M*. None of these compounds were hydrolyzed by this enzyme as determined spectrophotometrically (see Experimental Section). [e] Highest concentrations tested where indicated by >. Because of the low activity, no further tests were conducted. Under these conditions, *N*-hydroxyurea had I_{50} of 1.6×10^{-5} *M* (1.2 μg/ml) while 5-fluorodeoxyuridine exhibited I_{50} of $<4.9 \times 10^{-6}$ *M* (<0.12 μg/ml). [f] Isolated as the monohydrate, which also analyzed correctly for C, H, and N. Vigorous drying gave the anhydrous sample. [g] Competitive inhibitor with $K_i = 1.19 \pm 0.10$ m*M*. For detailed kinetic analysis, see Figures 2 and 3.

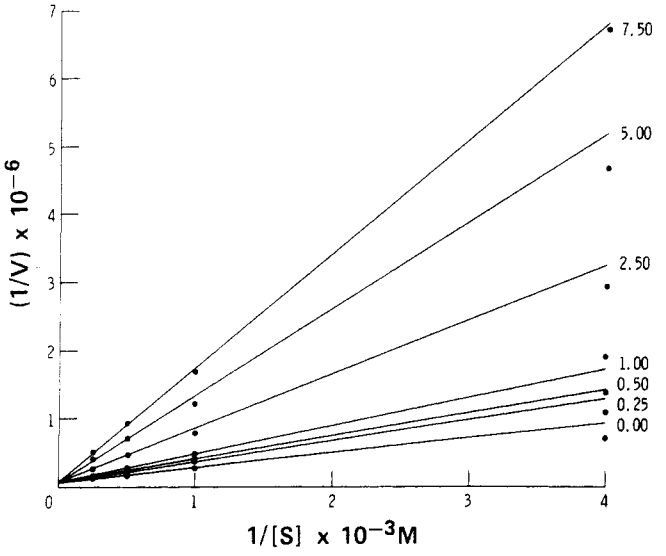


Figure 2. Lineweaver–Burk plot showing competitive inhibition by L-leucyladamantanine (**4**) in the leucine aminopeptidase catalyzed hydrolysis of L-leucyl-*p*-nitroanilide. Velocities are expressed as the increase in molar concentration per minute of *p*-nitroaniline after addition of enzyme. Inhibitor concentrations are millimolar.

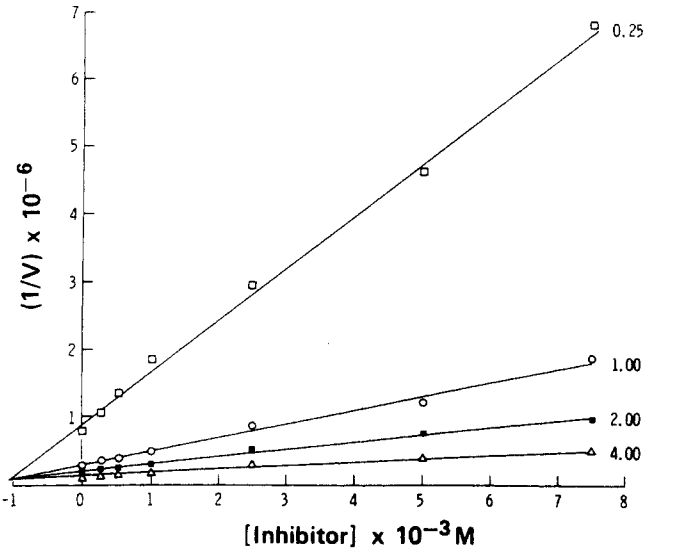


Figure 3. Dixon plot showing competitive inhibition of the hydrolysis of L-leucyl-*p*-nitroanilide by L-leucyladamantanine. Substrate concentrations are millimolar. Other parameters as in Figure 2. Apparent $K_i = 1.19 \pm 0.10$ m*M*.

lymphocytic leukemia cells in tissue culture.[21] All of the compounds tested can be considered inactive in this system (Table I). The surprising lack of activity of cycloleucine (**2**) itself in this in vitro tissue culture system (Table I), which is sensitive to other known antitumor agents (see footnote *e*, Table I), suggests that for amino acids and peptide, this test system is not a good prognosticator for in vivo activity, the in vivo antitumor activity of **2** being well documented.[2] Peptides **3**–**6** in hydroxypropylcellulose were also administered intraperitoneally to host BDF_1 mice bearing 10^5 L1210 lymphoid leukemia cells, but mean survival times were not significantly increased, even at doses of 400 mg/kg once daily for 9 days.

Discussion

The biochemical and pharmacological principles of drug latentiation based on conjugate attachment of groups that can be selectively removed by enzyme action have been advanced independently by Danielli,[22] Harper,[23] and Albert.[24] This subject was recently reviewed by Sinkula and Yalkowsky.[25] Despite our lack of success in latentiating adamantanine as its dipeptide derivatives, latentiation of biologically active compounds possessing an amino functional group in the molecule by conjugation with α-amino acids to the corresponding α-aminoacylamides and allowing tissue peptidases to release the active compound in vivo shows potential for systematic development.

Thus, the intestinal mucosa is especially rich in (a) aminopeptidases[26,27] that hydrolyze α-aminoacylamides and certain dipeptide amides, (b) dipeptidases[27,28] of wide specificity that hydrolyze the amide bonds of dipeptides, and (c) arylamidases[29,30] that hydrolyze α-aminoacyl aromatic amides such as L-alanyl-α-naphthylamide or β-naphthylamide and L-leucyl-*p*-nitroanilide. The presence of isozymic forms of aminopeptidase and arylamidase in other metabolic organs such as the liver and kidney is well known. The observed stability of adamantanine peptides to enzymatic hydrolysis by a purified preparation of leucine

aminopeptidase very likely reflects the steric hindrance of the peptide bonds involved and may be unique to adamantanine peptides. However, the rationale advanced may be generally applicable to a vast number of pharmacologically active agents. It may be especially useful for (a) substances with high in vitro activity but which encounter bioavailability or transport problems in vivo, and/or (b) certain drugs that display marked and nonselective systemic toxicity, such as antitumor agents.

Experimental Section

Melting points (corrected) were determined on a Mettler FP-2 hot-stage apparatus or in a Mel-Temp apparatus in sealed capillary tubes with the head space above the sample filled with a glass rod to minimize sublimation. All solvent evaporations were carried out in vacuo on a mechanical rotating evaporator using a water aspirator. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn. Spectrophotometers used were ir, Beckman IR-10; EI MS, Hitachi Perkin-Elmer RMU-6 (ionization energy, 70 eV; ion source temperatures as indicated). CI mass spectra were provided by Dr. Roger Foltz, Battelle Columbus Laboratories, Columbus, Ohio, using an AEI-MS-902 mass spectrometer equipped with an SRIC Model CIS-2 combined CI–EI ion source.

Phthaloyl-L-leucyladamantanine (10). To a suspension of adamantanine (1, 6.72 g, 34 mmol) in 150 ml of CH_3CN was added *N,O*-bis(trimethylsilyl)trifluoroacetamide (10.4 g, 40 mmol). After heating under reflux for 2 hr, the now clear solution was cooled, and to it was added a solution of phthaloyl-L-leucyl chloride (prepared from reaction of phthaloyl-L-leucine, 8.90 g, 0.034 mol, with $SOCl_2$) in 60 ml of CH_3CN. The mixture was heated additionally for 2 hr, then cooled, filtered, and concentrated nearly to dryness. The oily residue was hydrolyzed by stirring for 30 min with 200 ml of H_2O; the mixture was then made basic to pH 10 with 5% Na_2CO_3 and extracted with ether (3 × 50 ml). Acidification of the aqueous phase precipitated solids which were collected and recrystallized from THF–hexane to give 11.4 g (76.5% yield) of **10** as fine colorless crystals: mp 181–183°. Anal. ($C_{25}H_{30}H_2O_5 \cdot H_2O$) C, H, N.

L-Leucyladamantanine (4). To a solution of **10** (4.38 g, 0.010 mol) in 100 ml of EtOH was added 95% hydrazine (0.40 g, 0.012 mol) and the mixture was heated under reflux for 2 hr. After evaporation of the solvent to dryness, the residue was extracted twice with 0.01 *N* HCl (100 ml), and the combined extracts were filtered through Celite and neutralized to pH 7. The solids that precipitated on evaporation of the solvent were collected in three crops. Recrystallization of the combined products from EtOH–H_2O (decolorized with charcoal) gave colorless crystals which were collected and dried under vacuum at 60° for 18 hr. Elemental analysis indicated that this product still contained adsorbed H_2O. Further drying at 100° for 30 hr gave 1.04 g (34% yield) of anhydrous **4.**

Phthaloylglycyladamantanine (9). 1 (3.10 g, 0.020 mol) in 125 ml of CH_3CN was trimethylsilylated and the product, which was not isolated, was acylated with 4.47 g of phthaloylglycyl chloride as in the preparation of **10** above. The yield of **9** was 6.82 g (89%): mp 240.5–241°. Anal. ($C_{21}H_{11}N_2O_5$) C, H, N.

***N*-(Chloroacetyl)adamantanine (11).** 1 (5.86 g, 0.030 mol) in 150 ml CH_3CN was trimethylsilylated as described above and the product chloroacetylated by addition of chloroacetyl chloride (3.50 g, 0.031 mol) and heating the reaction mixture under reflux for 2 hr. After evaporation of the solvent to dryness, the residue was hydrolyzed by stirring in 200 ml of H_2O for 1 hr, and the resulting solids were collected. Recrystallization from THF yielded 7.30 g (90%) of **11.** The analytical sample was recrystallized from THF: mp 206.5–207.5°. Anal. ($C_{13}H_{18}NO_3Cl$) C, H, N.

Glycyladamantanine (3). Procedure A. A suspension of **9** (3.83 g, 0.010 mol) in 50 ml of EtOH was dephthaloylated as for **4** above. The crude product was dissolved in 900 ml of boiling H_2O and the mixture filtered to remove a small amount of dark residue. The filtrate was concentrated and the solids which precipitated were collected at intervals during the evaporation. The combined solids were dried at 80° under vacuum for 18 hr to give 0.81 g of **3** (32% yield).

Procedure B. A solution of **11** (7.30 g, 0.027 mol) in 250 ml of H_2O and 300 ml of concentrated NH_4OH was heated on a steam bath for 2 hr. The reaction mixture was decolorized with charcoal and the solvent evaporated to give **3** as colorless crystals which were collected in three crops and dried under vacuum at 70° for 24 hr: yield 6.35 g (87%).

***N*-Carboxyadamantanine Anhydride (14).** A steady stream of gaseous phosgene was led into a stirred, refluxing suspension of 1 (9.76 g, 0.050 mol) in a mixture of 300 ml of dioxane and 100 ml of THF for 2.5 hr. After distilling off 150 ml of solvent, the concentrate was filtered, and the filtrate was evaporated to dryness. Recrystallization of the solid residue from THF–hexane gave 9.90 g (90% yield) of **14** as colorless plates in two crops: mp 263–265° (sealed capillary); ir (KBr) 3410 (NH), 1850, 1790 cm^{-1} (C=O); mass spectra (EI, 210°) *m/e* (rel intensity >20%) 221 (62, $M\cdot^+$), 193 [43 $(M - CO)\cdot^+$], 178 [100 $(M - HNCO)\cdot^+$], 176 (33), 149 (28), 143 (37), metastable ions at *m/e* 168, 143.5 (calcd 168.5, 143.5); mass spectra (CI, methane) *m/e* (rel intensity >10%) 222 (14, MH^+), 194 [100 $(MH - CO)^+$], 179 [35 $(MH - HNCO)^+$]; mass spectra (CI, isobutane) 194 [100 $(MH - CO)^+$]. Anal. ($C_{12}H_{15}NO_3$) C, H, N.

Adamantanylglycine (5). A solution of glycine (2.25 g, 0.030 mol) and Na_2CO_3 (3.00 g, 0.030 mol) in 150 ml of 0.2 *N* NaOH was cooled to −5° in an ice–salt bath. Acetonitrile (120 ml) and **14** (6.60 g, 0.030 mol) were added and the mixture was stirred at <0° for 5 hr. The solution was concentrated to remove CH_3CN and then neutralized to pH 7 whereupon solids precipitated. The solid product was collected, recrystallized from water, and dried under vacuum at 70° for 18 hr to yield 2.06 g (44%) of **5.**

Adamantanyl-L-leucine (6). L-Leucine (1.97 g, 0.015 mol) was allowed to react with **14** in the same manner as for **5** above except that the reaction was allowed to proceed for 64 hr at 3°. The crude product was recrystallized from MeOH–H_2O and dried under vacuum at 26° for 18 hr to yield 3.70 g (76%) of **6.**

***N*-Trifluoroacetyladamantanine Azlactone (12).** A mixture of 1 (3.00 g, 15 mmol) and trifluoroacetic anhydride (6.71 g, 15 mmol) in 51 ml of CH_2Cl_2 was heated under reflux for 10 min. The resulting clear solution was evaporated to dryness and the solid residue was recrystallized from isopentane to yield 3.85 g (91%) of **12:** mp 97–98°; ir (KBr) 1840 (C=O), 1700 cm^{-1} (C=N); mass spectrum (EI, 170°) *m/e* (>140 amu, rel intensity) 273 (8, $M\cdot^+$), 245 [27 $(M - CO)\cdot^+$], 176 [100 $(M - CO - CF_3)^+$], metastable ions at *m/e* 220, 126 (calcd 219.9, 126.4). Anal. ($C_{13}H_{14}NO_2F_3$) C, H, N.

***N*-Trifluoroacetyladamantanyl-L-phenylalanine (13).** A mixture of L-phenylalanine (0.40 g, 2.4 mmol) and *N,O*-bis(trimethylsilyl)acetamide (2.0 ml) in a heavy walled screw-capped acylation tube was heated at 110° for 1 hr. After cooling, **12** (0.30 g, 1.1 mmol) was added, and heating was continued at 110° for 30 min. The reaction mixture was concentrated until trimethylsilylacetamide condensed in the vacuum line and then diluted with 15 ml of H_2O, and the pH was adjusted to 10. The solution was extracted with ether (3 × 30 ml) and the aqueous phase decolorized with charcoal and acidified to pH 2. The product (**13**) which precipitated was collected and recrystallized from $CHCl_3$–hexane: 0.36 g (73% yield); mp 205.5–207°. Anal. ($C_{22}H_{25}N_2O_4F_3$) C, H, N.

Adamantanyl-L-phenylalanine (7). 13 (200 mg, 4.6 mmol) was heated in an acylation tube with 4 ml of 7.5 *N* NH_4OH at 100° for 3 hr. The reaction mixture was diluted with 10 ml of H_2O and concentrated to incipient dryness. The solids which precipitated were collected and recrystallized from MeOH–ether to yield 0.099 g (63%) of **7** after drying for 30 hr under vacuum at 65°.

In Vivo Metabolism Studies. Solutions of peptides 3–7 dissolved in dilute aqueous HCl (2.5 ml, pH 1) were administered orally at a dose of 400 mg/kg to male Sprague–Dawley rats weighing approximately 250 g, and 24-hr urines were collected in stainless steel metabolism cages. The 24-hr urines from each rat prior to administration of the peptides served as control urines. The urines were spotted on 8 × 8 silica gel F_{254} plates (Merck-Darmstadt) and developed two dimensionally, first in $CHCl_3$–CH_3OH–17% NH_3 (2:2:1) and then in *n*-BuOH–HOAc–H_2O (4:1:1), and the chromatograms were visualized with 0.3% ethanolic ninhydrin spray reagent. These were compared to the chromatograms of control urines and control urines to which the individual peptides were added. The results appear as Figure 1 (see paragraph at end of paper regarding supplementary material).

In Vitro Enzyme Inhibition and Growth Inhibition Studies. Hog kidney leucine aminopeptidase (Sigma Chemical Co.) was assayed spectrophotometrically with leucineamide as substrate[31] (specific activity found, 106 μmol/min/mg). Under identical conditions, the dipeptides 3–7 were not hydrolyzed over 20–30 min. The hydrolysis of L-leucine-*p*-nitroanilide[32] (observed K_m at pH 8.0, 3.12 m*M;* reported, 3.15 m*M*[32]) and its inhibition by peptides 3–7 at varying concentrations were determined at a fixed substrate concentration of 2.0 m*M*. The $[I/S]_{0.5}$ values appear in Table I. A more detailed kinetic treatment[20] was applied to inhibition by L-leucyladamantanine (**4**) (Figures 2 and 3). Growth inhibition of

P-388 lymphoid leukemia cells in tissue culture[21] was determined using varying concentrations of peptides 3–7.

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Supplementary Material Available. Figure 1 will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JMED-75-826.

References and Notes

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Notes

Potential Histamine H_2-Receptor Antagonists.[1] 2. N^{α}-Guanylhistamine

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The agonist molecule, histamine, has been used as a starting point for the design of potential H_2-receptor antagonists. Converting the side-chain amino group into a guanidine yielded the first histamine H_2-receptor antagonist, N^{α}-guanylhistamine. Antagonism of H_2 receptors was demonstrated by the inhibition of histamine-stimulated gastric acid secretion in the rat at high dose levels (approximate ID_{50} 800 μmol/kg, iv) and by the inhibition of histamine-stimulated tachycardia of guinea-pig right atrium (pA_2 = 3.9). Guanylhistamine behaves as a partial agonist at histamine H_2 receptors.

In previous publications from these laboratories[1,2] we have described some preliminary investigations in a program aimed at the discovery of compounds that could specifically antagonize certain pharmacological actions of histamine that are not blocked by conventional antihistamine drugs such as pyrilamine. This work led to the eventual synthesis and characterization of the histamine H_2-receptor antagonists, burimamide,[3] metiamide,[4] and cimetidine.[5] The purpose of this series of publications is to describe various approaches that have been used in our quest for the