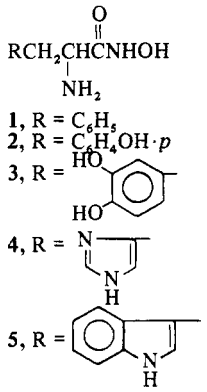

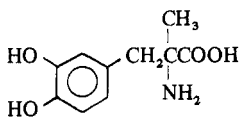

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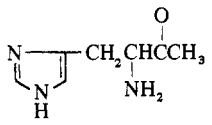

7

Biological Results. Studies on the inhibition of histidine decarboxylase and L-aromatic amino acid decarboxylase (dopa decarboxylase) utilizing the α-aminohydroxamic acids were performed.[4] Phenylalanine hydroxamic acid (**1**) was inactive as an inhibitor of either enzyme whereas the tyrosine analog **2** was able to inhibit histidine decarboxylase but not dopa decarboxylase. The dopa hydroxamic acid **3** was the most potent inhibitor of both enzyme systems. Histidine hydroxamic acid **4** showed more specificity for inhibition of the histidine decarboxylase than for the inhibition of dopa decarboxylase while the tryptophan analog **5** had little activity in the inhibition of the dopa decarboxylase but was as active as the histidine analog in the inhibition of histidine decarboxylase. The above compounds can be compared (Table I) with α-methyldihydroxyphenylalanine (**6**) as a specific inhibitor of dopa decarboxylase and with 4-(4-imidazolyl)-3-amino-2-butanone (**7**) as a specific inhibitor of histidine decarboxylase.

Experimental Section‡

L-Phenylalanine Hydroxamic Acid (1). L-Phenylalanine methyl ester·HCl (6.7 g, 0.031 mole) was dissolved in 50 ml of H_2O, 10% NaOH was added until the soln was basic, and the free base was extracted with $CHCl_3$ (3 × 50 ml). The combined $CHCl_3$ exts were dried (Na_2SO_4), and the solvent was removed yielding 1.8 g (50%) of the free base. A NH_2OH soln was prepd by adding with cooling KOH (2.2 g, 0.034 mole) in 20 ml of MeOH to a cooled soln of NH_2OH·HCl (2.5 g, 0.036 mole) in 20 ml of MeOH and the KCl formed was removed by filtration. NH_2OH soln was added to the L-phenylalanine methyl ester, the reaction mixt was allowed to stand 8 hr at 4° and filtered, and the white solid recrystd (MeOH) yielding 1.4 g (52%), mp 186–188°. *Anal.* ($C_9H_{12}N_2O_2$) C, H, N.

L-Tyrosine Hydroxamic Acid (2). A NH_2OH soln, prepd from NH_2OH·HCl (2.2 g) as above, was added to tyrosine methyl ester (3.5 g, 0.018 mole) in 25 ml of MeOH and the pH adjusted to 9 with MeOH–KOH. The reaction mixt was allowed to stand for 12 hr at 4°, the solvent was concd to about 20 ml, and starting material crystd yielding 1.1 g (31%). The filtrate was taken to dryness and the residue recrystd (MeOH) yielding 1.6 g (45%) of **2** as a white solid, mp 178–180°. *Anal.* ($C_9H_{12}N_2O_3$) C, H, N.

DL-3,4-Dihydroxyphenylalanine Hydroxamic Acid (3). A NH_2OH soln, prepd from NH_2OH·HCl (2.5 g) as above, was added under N_2 to DL-3,4-dihydroxyphenylalanine methyl ester·HCl (2.5 g, 0.010 mole) and the reaction mixt allowed to stand overnight at 4°. The solvent was removed and when Et_2O was added crystn occurred. The white solid (**3**) was washed with hot MeOH yielding 1.1 g (52%), mp 177–179°. *Anal.* ($C_9H_{12}N_2O_4$) C, H, N.

L-Histidine Hydroxamic Acid (4). L-Histidine methyl ester·2HCl (7.4 g, 0.031 mole) was placed in 100 ml of 5% NH_3–$CHCl_3$, allowed to stand for 1 hr, and filtered. The solvent was removed from the filtrate yielding 5.1 g (98%) of the free base. A NH_2OH soln from 2.5 g of NH_2OH·HCl was added to the L-histidine methyl ester and the reaction mixt allowed to stand 12 hr at 4°. It was taken to dryness yielding a white solid which was washed with $CHCl_3$ (3 × 25 ml) and recrystd (MeOH) yielding 2.6 g (47%), mp 159–161°. *Anal.* ($C_6H_{10}N_4O_2$) C, H, N.

L-Tryptophan Hydroxamic Acid (5). L-Tryptophan methyl ester·HCl (7.4 g, 0.031 mole) was placed in 100 ml of 5% NH_3–$CHCl_3$ allowed to stand for 1 hr, and filtered. The solvent was removed from the filtrate yielding 4.6 g (72%) of the free base. A NH_2OH soln from NH_2OH·HCl (2.5 g) was added to the L-tryptophan methyl ester, and after 12 hr at 4°, was worked up as above. The white solid (**5**) was recrystd (MeOH) yielding 3.2 g (67%), mp 166–168°. *Anal.* ($C_{11}H_{13}N_3O_2$) C, H, N.

Acknowledgment. The authors gratefully acknowledge the support of this project by the National Institutes of Health Grants GM-09254 and GM-01341. We wish to thank Dr. R. J. Taylor, Jr., McNeil Laboratories, Fort Washington, Pa., for the enzymatic data.

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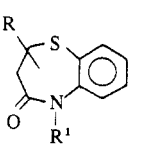
‡Melting points were det on a calibrated Thomas-Hoover capillary melting point apparatus and are corrected. Microanalyses were performed on an F and M CHN analyzer Model 185 in this department and by Midwest Microlab, Inc., Indianapolis, Ind. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4%.

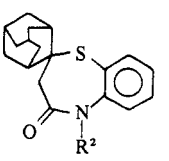
Adamantyl Analogs of the Antidepressive, 5-(2-Dimethylaminoethyl)-2,3-dihydro-2-phenyl-1,5-benzothiazepin-4(5*H*)-one†

V. L. Narayanan

The Squibb Institute for Medical Research, New Brunswick, New Jersey 08903. *Received December 23, 1971*

Several recent reports have described the synthesis and biological activity of a variety of adamantane derivatives.[1] We were interested in determining the effect on antidepressive activity achieved by replacing the planar phenyl ring of 5-(2-dimethylaminoethyl)-2,3-dihydro-2-phenyl-1,5-benzothiazepin-4(5*H*)-one [thiazesim (**1**)] with the symmetrical lipophilic adamantane moiety. This note describes the syntheses and antimuricide activity of three adamantyl analogs of **1**, which had been developed in our laboratories by Krapcho, *et al.*[2]



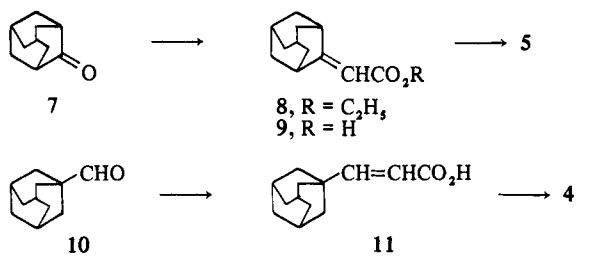


1, R = C_5H_6; R^1 = $(CH_2)_2N(CH_3)_2$
2, R = 1-adamantyl; R^1 = $(CH_2)_2N(CH_3)_2$
3, R = 1-adamantyl; R^1 = $(CH_2)_3NC_4H_8NCH_3$
4, R = 1-adamantyl; R^1 = H

5, R^2 = H
6, R^2 = $(CH_2)_2N(CH_3)_2$

Chemistry. The reaction of adamantanone (**7**) with triethyl phosphonoacetate and NaH gave Δ^2,α-adamantaneacetic acid, ethyl ester (**8**). It was determined that, by using 1.5 equiv of triethyl phosphonoacetate to an equivalent of **7** and allowing the reaction to proceed at 45°, a nearly quantitative yield of **8** could be realized. Base hydrolysis of **8** furnished Δ^2,α-adamantaneacetic acid (**9**)[3] in 98% yield. The Michael addition of **9** to 2-aminobenzenethiol, followed by cyclization, gave the spirobenzothiazepinone **5**. Alkylation of **5** with β-dimethylaminoethyl bromide gave 5′-(2-dimethylaminoethyl)spiro[adamantane-2,2′(3′*H*)-

†Thiazesim is the approved generic name for 5-(2-dimethylaminoethyl)-2,3-dihydro-2-phenyl-1,5-benzothiazepin-4(5*H*)-one.



1,5-benzothiazepin-4'(5'*H*)-one], isolated as the hydrochloride (**6**).

We have previously reported the synthesis of 1-adamantanecarboxaldehyde (**10**) by the LAH reduction of 1-(1-adamantanecarbonyl)aziridine.[4] Two other methods have been published[5] for the synthesis of **10**. However, we find it more convenient to synthesize 1-adamantanecarboxaldehyde (**10**) by the Jones oxidation of 1-adamantanemethanol,[6] obtained in quantitative yields by LAH reduction of commercially available 1-adamantanecarboxylic acid.‡ The reaction of **10** with triethyl phosphonoacetate and NaH, followed by hydrolysis, gave 1-adamantaneacrylic acid (**11**). The Michael addition cyclization of 2-aminobenzenethiol with **11** yielded 2-(1-adamantyl)-2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one (**4**). Compound **4** was alkylated with β-dimethylaminoethyl bromide and 1-(3-bromopropyl)-4-methylpiperazine to give **2** and **3**, respectively.

Pharmacology. The compounds were administered ip to rats, and the effective dose inhibiting the mouse-killing response (antimuricide activity) was determined. The depressant effect of these compounds on motor activity in the rat was measured by the rotarod test.[7] A rotarod ED_{50}/antimuricide ED_{50} ratio significantly greater than 1.0 indicates a selective antimuricide response whereas a ratio of less than 1.0 indicates a nonspecific antimuricide effect due to depressant activity. The results are shown in Table I. Compound **2** had 1.5 times the selective antimuricide activity of thiazesim.

Experimental Section

Melting points were determined on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Ir spectra were determined on a Perkin-Elmer 21 spectrometer in Nujol. Nmr spectra were obtained on a Varian A-60 spectrometer in $CDCl_3$, with TMS as the internal std. Where analyses are indicated by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

Δ²,α-Adamantaneacetic Acid (9). To a well-stirred suspension of 21.8 g (0.45 mole) of 50% NaH in 300 ml of dry 1,2-dimethoxyethane (DME), 100.9 g (0.45 mole) of triethyl phosphonoacetate was added slowly at 20°. After 2 hr of stirring at room temp, a soln of 45.0 g (0.3 mole) of **7** in 450 ml of dry DME was added rapidly. The reaction temp rose to 45° and was maintained at 45° for 2 hr and then stirred overnight at room temp. The mixt was concd, diluted with H_2O, and extd with Et_2O. The Et_2O ext was washed with H_2O, dried ($MgSO_4$), and concd to give 65.5 g (99%) of **8** as a thick yellow liquid; ir 5.83 (C=O), 6.08 μ (conjugated C=C).

The crude ester **8** was hydrolyzed by refluxing with 300 ml of 5 *N* alcoholic KOH for 4 hr. The basic soln was cooled, acidified with 5 *N* HCl, and extd with $CHCl_3$. The $CHCl_3$ soln was dried ($MgSO_4$) and evapd *in vacuo* to give 56.6 g (98%) of **9** as a brownish white powder. Crystn from aqueous Me_2CO gave a pure sample, mp 136–138°.

Spiroadamantane-2,2'(3'*H*)-1,5-benzothiazepin-4'(5'*H*)-one (5). A mixt of 5.75 g (0.03 mole) of **9**, 15.0 g of 2-aminobenzenethiol, and 5 ml of DMF was heated under N_2 at 218–222° for 3 hr. After cooling, the reaction mixt was stirred with 200 ml of Et_2O and the solid that sepd was collected and crystd twice from $CHCl_3$–Et_2O to give 2.1 g (23%) of **5** as shiny white crystals, mp 281–283°. *Anal.* ($C_{18}H_{21}NOS$) C, H, N.

‡ Aldrich Chemical Co., Milwaukee, Wis.

Table I

Compound No.	Rotarod ED_{50}, mg/kg	Antimuricide ED_{50}, mg/kg	Rotarod/antimuricide ratio
1	32	15	2.1
2	54	15	3.6
3	27.5	26.5	1.0
6	25	25	1.0

5'-(2-Dimethylaminoethyl)spiro[adamantane-2,2'(3'*H*)-1,5-benzothiazepin-4'(5'*H*)-one] Hydrochloride (6). To a suspension of 0.21 g (0.0054 mole) of $NaNH_2$ in 25 ml of dry $PhCH_3$, a suspension of 1.5 g (0.005 mole) of **5** in 50 ml of dry $PhCH_3$ was added at 10–15°, and the reaction mixt was stirred at 20° for 0.5 hr, when a practically clear soln was obtained. To the above reaction mixt, a soln of β-dimethylaminoethyl bromide in dry $PhCH_3$ (made from 1.75 g of 2-dimethylaminoethyl bromide HBr and 0.6 g of K_2CO_3) was added dropwise, and the mixt was stirred overnight. The $PhCH_3$ was washed with H_2O, dried, and concd. The thick oil obtained was taken up in dry *i*-PrOH and treated with an equiv amount of HCl to give 0.95 g (47%) of **6** as a white solid. It was crystd twice from EtOH–Et_2O to give 0.48 g (24%) of crystals, mp 250–251°. *Anal.* ($C_{22}H_{30}N_2OS \cdot HCl$) C, H, N.

1-Adamantanecarboxaldehyde (10). To a soln of 33.2 g (0.2 mole) of 1-adamantanemethanol in 700 ml of reagent grade Me_2CO, cooled to 5°, 60 ml of 8 *N* Jones reagent was added while the temp of mixt was maintained at 8–10°. After 0.25 hr of stirring, 50 ml of MeOH was added and the mixt was stirred again for an additional 0.25 hr. The sepd solids were dissolved by the addition of 700 ml of H_2O and stirring. The soln was concd *in vacuo* and the aqueous layer was extd with Et_2O. The Et_2O ext was washed with H_2O and then with satd $NaHCO_3$ soln, dried ($MgSO_4$), and concd *in vacuo* to give 24.8 g (76%) of **10** as a thick oil, which solidifies on standing; ir 5.78 μ (C=O); thiosemicarbazone, mp 193–194°. *Anal.* ($C_{12}H_{19}N_3S$) C, H, S.

Since the 1-adamantanecarboxaldehyde loses CO on standing,[8] the crude material was used immediately for the next step.

1-Adamantaneacrylic acid (11) was synthesized from **10**, using the same procedure as for **9**, mp 170–172°, yield 40%. *Anal.* ($C_{13}H_{18}O_2$) C, H.

2-(1-Adamantyl)-2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one (4) was prepd by using the same procedure as for **5**, mp 239–241°, yield 58%. *Anal.* ($C_{19}H_{23}NOS$) C, H, N, S.

2-(1-Adamantyl)-5-(2-dimethylaminoethyl)-2,3-dihydro-1,5-benzothiazepin-4(5*H*)-one hydrochloride (2) was synthesized according to the procedure for **6**, yield 50%, mp 230–232°. *Anal.* ($C_{23}H_{32}N_2OS \cdot HCl$) C, H, N, S.

2-(1-Adamantyl)-2,3-dihydro-5-[3-(4-methyl-1-piperazinyl)-propyl]-1,5-benzothiazepin-4(5*H*)-one dihydrochloride (3) was synthesized according to the procedure for **6**, yield 30%, mp 280–281°. *Anal.* ($C_{27}H_{39}N_3OS \cdot 2HCl$) N, S, Cl.

Acknowledgment. The author is indebted to Dr. J. Bernstein for his advice and encouragement during the course of this investigation. The author wishes to thank Dr. Z. P. Horovitz and his staff for the pharmacologic data, Mr. J. Alicino and his associates for the microanalyses, and Dr. A. I. Cohen and Mrs. B. Toeplitz for the spectral data.

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4,6-Didemethyl-4,6-dibromoactinomycin C_1 (D)

Frank Seela

Department of Chemistry, Yale University, New Haven, Connecticut 06520. *Received September 7, 1971*

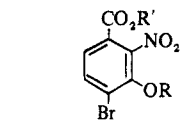
One varies the structure of the bacteriostatic and antitumor-active chromopeptide actinomycin C_1 for two reasons: improved therapeutic utility and elucidation of the inhibition mechanism. Microbiological modification of enlarged the group of actinomycins. This led to a better understanding of the inhibition mechanism, but did not result in a better therapeutic activity.

Kersten, *et al.*,[1] have shown that the antibiotic activity of actinomycin C_1 is based on its ability to inhibit mRNA synthesis by complex formation with DNA. A detailed model of complex formation was suggested by Müller and Crothers[2] and by Sobell, *et al.*[3]

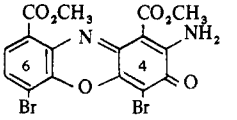
According to this model the chromophore of actinomycin and simple analogs[4] intercalates between the base pairs adjacent to guanine-cytosine. In agreement with the predictions based on this idea, the replacement of the 4,6 methyl groups by bulkier ethyl groups decreases the complex stability and the antibiotic activity; *tert*-butyl groups cause the activity to disappear.[5]

Replacement of the 4,6 methyl groups in actinomycin C_1 may also alter the electron distribution of the phenoxazone moiety and its electronic interactions with the guanine-cytosine base pair. In order to study the influence of substituent electronegativity on antibacterial activity and DNA complex stability in the absence of any major steric change and to improve our knowledge of the inhibition mechanism, the bromo analog **8** was synthesized. This compound is the first analog which has 4,6 substituents (bromine) with approximately the same van der Waals radii (r_{Br} = 1.95 Å, r_{CH_3} = 2.00 Å) as the methyl groups of actinomycin C_1, but with a higher electronegativity. Furthermore the heavy atoms should make it useful for crystallographic studies of the interaction with DNA.

The synthesis followed essentially a strategy which was successfully employed previously in synthesizing actinomycins[6] and actinomycin derivatives.[5] Nitration and esterification of 4-bromo-3-hydroxybenzoic acid[7,8] gave compound **1c**. Esterification was necessary in order to separate an isomeric side product of the nitration by crystallization. Saponification of the ester **1b** gave the acid **1a**. Treatment of the disodium salt of **1a** with excess benzyl chloride formed the benzyl ester-benzyl ether of **1a**, which was saponified to yield **1c**. *Via* the acid chloride, **1c** was coupled with L-threonine to compound **3**. Condensation of **3** and the peptide **4** with Woodward's reagent[9] gave **5a**, debenzylation of **5a** to **5b** was accomplished with hydrobromic acid in glacial acetic acid. Sodium hydroxide hydrolyzed **5b** to



1a, R = H, R′ = H
1b, R = H, R′ = CH_3
1c, R = CH_2Ph, R′ = H



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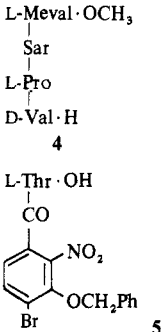

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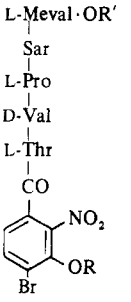

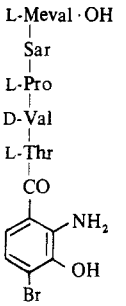

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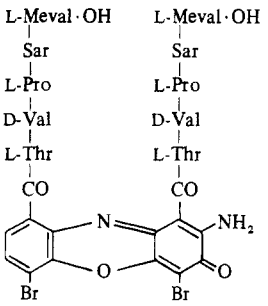
3

5a, R = CH_2Ph, R′ = CH_3
5b, R = H, R′ = CH_3
5c, R = H, R′ = H



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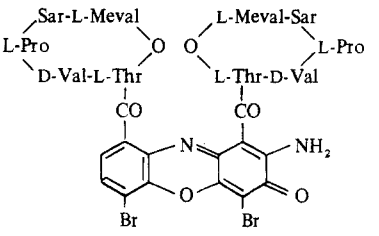

8

5c. By sodium dithionite reduction, **5c** was converted to **6** which was not isolated because of its air sensitivity, but was oxidized immediately to **7** with potassium ferricyanide. Ring closure of crude **7** with acetylchloride–acetylimidazole[10] formed **8**.

The main problem of the synthesis was the lability of the bromine substituents toward catalytic hydrogenation, a reaction which was used for removal of the benzyl protecting groups, and the conversion of the nitro to an amino group.[5] Catalytic hydrogenation of **1b** followed by oxidative condensation with potassium ferricyanide, for example, did not result in **2**. Instead, 2-amino-3*H*-phenoxazin-3-one-1,9-dicarboxylic acid methyl ester[11] was formed by loss of halogen. Hydrogenation was avoided by use of sodium dithionite for reduction of the nitro group. Using this method the compounds **2** and **7** were obtained with retention of bromine. Cleavage of the benzyl group was carried out with hydrobromic acid in glacial acetic acid.

Microbiological Testing. For antibacterial tests *Bacillus subtilis* (ATCC 6633) was used as a test organism. The method is described elsewhere.[12] The minimal concentration for the complete inhibition of the growth of *Bacillus subtilis* was 0.31 μg/ml for the actinomycin analog **8** and 0.07 μg/ml for actinomycin C_1 (standard).

This indicates that not only a larger size[5] but also a higher electronegativity of the 4,6 substituents decreases the antibacterial activity of actinomycin C_1.

Experimental Section

Melting points were determined with a Reichert apparatus and are corrected. Microanalyses were performed by Mikroanalytisches Laboratorium, Alfred Bernhardt, Germany. Where analysis is indicated by symbols of element, analytical results obtained for elements