(4) K. Engelmann, W. Raake, and A. Petter, *Arzneim.-Forsch.*, 24, 759 (1974).
(5) P. H. Morgan and I. W. Mathison, *J. Pharm. Sci.*, 65, 467, 635 (1976), and references cited therein.
(6) (a) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, 86, 5175 (1964); (b) J. Iwasa, T. Fujita, and C. Hansch, *J. Med. Chem.*, 8, 150 (1965).
(7) C. Hansch, *Acc. Chem. Res.*, 2, 232 (1969).
(8) D. E. Williamson and G. W. Everett, Jr., *J. Am. Chem. Soc.*, 97, 2397 (1975), and references cited therein.
(9) G. Fraenkel, B. Appleman, and J. G. Ray, *J. Am. Chem. Soc.*, 96, 5113 (1974).
(10) H. Nozaki, T. Aratani, T. Toraya, and R. Noyori, *Tetrahedron*, 27, 905 (1971).
(11) (a) N. J. Leonard, P. D. Thomas, and V. W. Gash, *J. Am. Chem. Soc.*, 77, 1552 (1955); (b) P. C. Ruenitz, Ph.D. Thesis, University of Kansas, Lawrence, Kan., 1974; *Diss. Abstr. Int. B*, 36, 735 (1975).
(12) (a) E. E. Smissman and P. C. Ruenitz, *J. Org. Chem.*, 41, 1593 (1976); (b) P. C. Ruenitz and E. E. Smissman, *J. Heterocycl. Chem.*, 13, 1111 (1976); (c) *J. Org. Chem.*, 42, 937 (1977).
(13) (a) W. W. Ewing, E. Klinger, and J. D. Brandner, *J. Am. Chem. Soc.*, 56, 1053 (1934); (b) H. Bassett and H. S. Taylor, *J. Chem. Soc.*, 576 (1912).
(14) E. C. Horning, W. J. A. VandenHeuvel, and B. G. Creech, *Methods Biochem. Anal.*, 11, 69 (1963).
(15) J. W. Lawson, *J. Pharmacol. Exp. Ther.*, 160, 22 (1968).
(16) J. E. Berger, personal communication.
(17) W. J. Dixon, *J. Am. Stat. Assoc.*, 60, 967 (1965).

Potential Histidine Decarboxylase Inhibitors. 1. α- and β-Substituted Histidine Analogues

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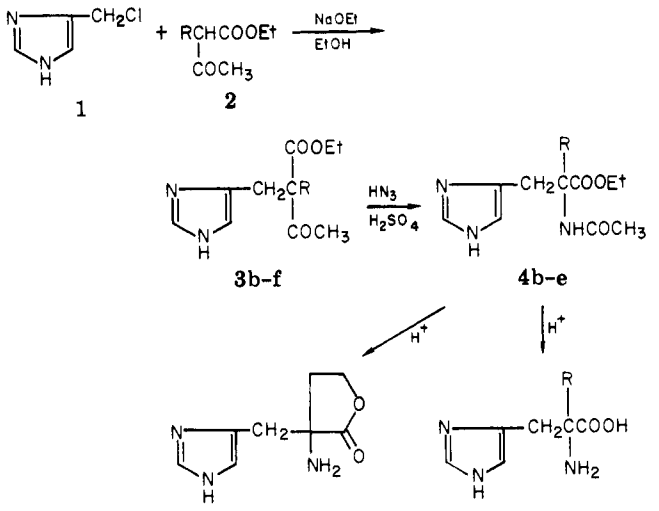
Histidine analogues with alkyl substitution at C_α and C_β were prepared as potential inhibitors of specific histidine decarboxylase. Activity was assessed in vitro using extracts of rat pyloric stomach and a radioisotopic assay of $^{14}CO_2$ evolved from carboxyl-^{14}C-labeled histidine. α-Substituted analogues (C_2–C_4) including 2-hydroxyethyl were less potent than α-methylhistidine; the α-*n*-butyl analogue was completely inactive at 10^{-3} M. Similarly, β,β-dimethylhistidine and homohistidine failed to exhibit activity at 10^{-3} M.

A major source of the histamine contained in most mammalial tissues is presumably the decarboxylation of histidine by a specific decarboxylase.[1] Inhibitors of this enzyme are of interest both as research tools and as potentially useful therapeutic agents for minimizing effects mediated at both H_1- and H_2-histamine receptors.[2] Although potent inhibitors of the specific decarboxylase are available, none are unequivocally adequate in terms of both specificity and in vivo effectiveness; in particular, those whose activity is based on reaction with pyridoxal phosphate cofactor lack specificity[1] even in terms of other enzyme reactions involved in histamine metabolism.[3]

A logical approach to the design of potent, specific inhibitors would be based on close structural similarity to the natural substrate, histidine. Such reasoning is supported by indications of specificity in the case of α-methylhistidine.[1] The present investigation was designed to assess the degree of steric tolerance toward substitution at the α and β positions of histidine with respect to retention of affinity for the enzyme active site and enhancement of inhibitor properties. Accordingly, a series of α-alkylhistidines **5a–e**, β,β-dimethylhistidine (**13**), and homohistidine was prepared and evaluated as inhibitors of specific histidine decarboxylase obtained from rat pyloric stomach (Table I). α-Methylhistidine (**5a**), a relatively weak inhibitor, was the best in this series, showing 40% inhibition at 10^{-3} M and 30% at 10^{-4} M. An increase in the size of the substituent caused a substantial decline in inhibitory potency, such that the *n*-butyl analogue **5d** was totally inactive at 10^{-3} M. β,β-Dimethylhistidine (**13**) and homohistidine (**14**) were also inactive at 10^{-3} M. Synthetic difficulties prevented evaluation of the α-allyl or β-methyl analogues. The results do not allow a definite conclusion regarding β-substitution but indicate rather poor steric tolerance at the α position. The stringent requirements for acceptance at the enzyme binding site are further borne out by the complete lack of activity by the homohistidine.

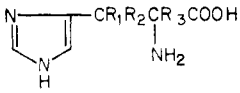
Scheme I



a, R = CH_3
b, R = C_2H_5
c, R = $CH(CH_3)_2$
d, R = C_4H_9
e, R = CH_2CH_2OH (as lactone)
f, R = $CH_2CH{=}CH_2$

The route utilized for synthesis of the α-substituted histidines is outlined in Scheme I. 4-Chloromethylimidazole hydrochloride[4] (**1**) was added to a solution of an appropriate 2-alkyl acetoacetate (**2b–d,f**) or 2-acetylbutyrolactone (**2e**) in ethanol solution containing 2 equiv of sodium ethoxide to afford the 2-alkyl 2-(4-imidazolylmethyl)acetoacetate (**3**). When the keto ester **3** was allowed to react with a slight excess of hydrazoic acid in sulfuric acid solution, the *N*-acetylhistidine esters **4b–e** were obtained in 50–70% yields. Under the acidic conditions employed, the α-allyl analogue **3f** was apparently subjected to additional attack on the vinyl moiety, leading

Table I. Inhibition of Specific Histidine Decarboxylase by Histidine Analogues



No.	R_1	R_2	R_3	Mp, °C	Formula[a]	% inhibn at[e] indicated concn[c] 10^{-3} M	% inhibn at[e] indicated concn[c] 10^{-4} M
5a	H	H	CH_3[b]			40	30
5b	H	H	C_2H_5	220-223 eff	$C_8H_{13}N_3O_2 \cdot 2HCl \cdot 0.5H_2O$	14	6
5c	H	H	$CH(CH_3)_2$	174-177	$C_9H_{15}N_3O_2 \cdot 2HCl \cdot 0.5H_2O$	1	0
5d	H	H	n-C_4H_9	250-255 dec	$C_{10}H_{17}N_3O_2 \cdot 1.5HCl$	0	
5e	H	H	CH_2CH_2OH[f]	211-218 eff	$C_8H_{11}N_3O_2 \cdot 2HCl \cdot 0.5H_2O$	19	12
13	CH_3	CH_3	H	253-256	$C_8H_{13}N_3O_2 \cdot 0.25H_2O$	0	
14 (homohistidine[d])						0	

[a] Compounds 5b-e and 13 were analyzed for C, H, and N; values ±0.4% of theory except the following: 5b, C, 35.6 (36.2), N, 16.3 (15.8), confirmed by MS (*m/e* 138, M - COOH; 184, M + H); 5d, GC-MS on bis(trimethylsilyl) derivative (*m/e* 340, M - CH_3); 5e, MS (*m/e* 137, M - CO_2; 181, M; 182, M + H); 13, GC-MS on Me_3Si (*m/e* 384, M - CH_3). [b] Regis Chemical Co. [c] Compounds were evaluated in vitro using extracts of rat pyloric stomach, carboxyl-^{14}C-labeled histidine, and the radioisotopic method of R. J. Levine and M. Watts, *Biochem. Pharmacol.*, 15, 841 (1966). Results are means of two to three determinations. Replicate determinations generally varied by less than 15%. [d] Prepared by the method of F. Schneider, *Z. Physiol. Chem.*, 334, 26 (1963). [e] Since submission of this manuscript two additional papers on this subject have appeared by Kelley et al.[11,12] These authors found that some other α-substituted histidines were equally poor inhibitors, in agreement with our study. They were able to obtain β-methylhistidine by a different route and found it to be inactive at 10^{-4} M. [f] Lactone.

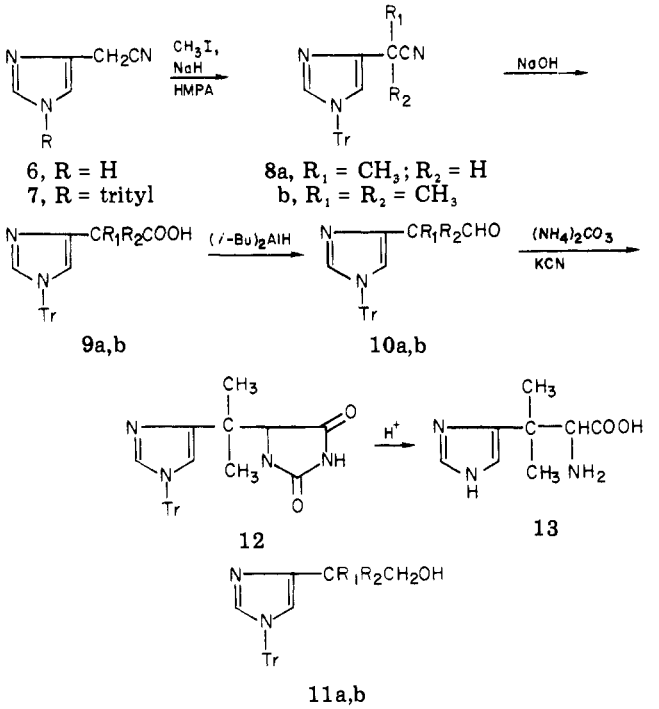
to an unidentified product rather than the anticipated product **4f**. Schmidt[5] had qualitatively shown that in β-keto esters the HN_3 reagent afforded selective attack on the ketone carbonyl with subsequent rearrangement occurring at the α carbon. Our NMR measurements on the crude products **4** did not detect evidence of alternative rearrangement from the methyl side of the ketone. Synthesis of the histidines **5b–e** was completed by acid hydrolysis of the amide and ester functions. This route seems to have been little used in the synthesis of α-substituted amino acids, but we found it to be especially valuable in the histidine series.

The β,β-dimethylhistidine (**13**) was prepared by the procedure shown in Scheme II. 4-Cyanomethylimidazole (**6**) was conveniently obtained in 70% yield from histidine by decarboxylation in sodium hypochlorite solution.[6] Treatment of **6** with bis(trimethylsilyl)acetamide reagent afforded an N-Me_3Si intermediate which when allowed to react with trityl chloride gave an N-trityl-4-cyanomethylimidazole isomer **7** in 77% yield from **6**. Although shown as the 1-N-trityl derivative in formula **7**, the true position (N^1 or N^3) of tritylation was not established.

The blocked nitrile **7** was converted to the anion by treatment with 1 equiv of sodium hydride in hexamethylphosphoramide (HMPA) solution. Subsequent reaction with methyl iodide at room temperature afforded a mixture of methylation products containing approximately 77% of the monomethylnitrile **8a**. Pure monomethyl product **8a** was obtained by fractional crystallization. The dimethylnitrile **8b** was similarly obtained, but exhaustive retreatments were usually required to ensure complete methylation. Hydrolysis of the nitriles was effected by potassium hydroxide in hot 90% 2-methoxyethanol to afford the carboxylic acids **9a** and **9b** in 84 and 86% yields, respectively.

Reduction of the dimethyl acid **9b** with diisobutylaluminum hydride in toluene gave a mixture shown to contain about 75% of the aldehyde **10b** by NMR analysis. The crude product was chromatographed to remove unreacted acid **9b** and effect a separation of aldehyde from another substance regarded as the alcohol **11b**. The crystalline aldehyde was allowed to react with KCN-$(NH_4)_2CO_3$ at 110 °C in a bomb tube[7] to yield the hydantoin **12** as a crystalline product. Acid hydrolysis of

Scheme II



12 yielded β,β-dimethylhistidine (**13**).

The monomethyl acid **9a** could not be carried through the above processes to give β-methylhistidine. Reduction of the acid **9a** with $(i\text{-Bu})_2AlH$ was unsuccessful, giving an unidentified product devoid of aldehyde as shown by NMR analysis. It should be noted that attempts to reduce the nitriles **8** to aldehydes **9** by various hydride reagents [including $(i\text{-Bu})_2AlH$] were unsuccessful causing substantial attack on the imidazole ring. Reduction of carboxylic acid **9a** to alcohol **11a** followed by oxidation with a CrO_3–pyridine reagent[8] was also unrewarding.

Experimental Section

2-Alkyl-2-acetylimidazolepropionates 3. To a solution of NaOEt in EtOH (from 643 mg of Na, 28 mg-atoms) was added 4.42 g (28 mmol) of ethyl α-ethylacetoacetate. After 10 min, 2.14 g (14 mmol) of 4-chloromethylimidazole hydrochloride[4] (**1**) was added over 5 min with stirring. The mixture was heated at 60

°C for 1 h and evaporated in vacuo. The residue was partitioned between 15 mL of 5% HCl and 10 mL of CH_2Cl_2. After an additional wash by 10 mL of $MeCl_2$, the aqueous portion was adjusted to pH 7–8 with solid $NaHCO_3$ and twice extracted with 10-mL portions of CH_2Cl_2. The extract was dried ($MgSO_4$) and evaporated in vacuo to leave 2.44 g (73%) of the syrupy keto ester **3b**: NMR (δ, ppm) 7.55 (1 H, s, Im-2-H), 6.75 (1 H, s, Im-5-H), 4.20 (2 H, q, OCH_2CH_3), 3.35 (2 H, m, $ImCH_2$), 2.00 (5 H, m, $COCH_3$, CH_2CH_3), 1.25 (3 H, t, OCH_2CH_3), 1.82 (3 H, t, CH_2CH_3). The other keto esters were similarly obtained in 40–90% yields by alkylation of appropriate α-alkyl acetoacetates or 2-acetylbutyrolactone for **3e**;[9] alkylation also proceeded smoothly when conducted at room temperature for 15 h. Ethyl α-allylacetoacetate (**2f**) was prepared by the literature procedure.[10]

α-Alkyl-*N*-acetylhistidine Esters 4. To an ice-cold, stirred solution of 1.10 g (4.37 mmol) of ethyl 2-isopropyl-2-acetyl(4-imidazole)propionate (**3c**) in 1.8 mL of concentrated H_2SO_4 was added 3 mL of CH_3CN followed by the addition of 296 mg (4.59 mmol) of ground-up NaN_3 in six portions over 1 h. The mixture was stirred at ambient temperature for 6 h and cooled to 0–5 °C. The mixture was treated with a little ice and the aqueous solution was alkalized to pH 7–8 and extracted with three 20-mL portions of CH_2Cl_2. The extract was dried over $MgSO_4$ and evaporated to leave 742 mg of a foamy residue, which was chromatographed on 75 g of silica gel. Elution by $CHCl_3$–MeOH (9:1) afforded 585 mg (50%) of a colorless gum: NMR ($CDCl_3$, D_2O exchanged) 7.50 (1 H, s, Im-2-H), 6.70 (1 H, s, Im-5-H), 4.29 (2 H, q, CH_2CH_3, J = 5.5 Hz), 3.36 and 3.85[13] (2 H, 2 doublets, $ImCH_2$, J_{gem} = 15 Hz), 2.60 (1 H, m, Ip-H), 1.95 (3 H, s, $COCH_3$), 1.35 (3 H, t, CH_2CH_3), 1.05, 0.94[13] (6 H, 2 doublets, isopropyl CH_3); IR 3.05, 3.15 (NH), 5.75 (ester C=O), 6.00 μ (amide C=O). The other *N*-acetyl esters **4b–e** were obtained as syrups in a similar manner in yields of 50–70%. The allyl keto ester **3f** was unstable to the strongly acidic conditions and failed to yield any of the amido ester **4f**. No signal for $-CONHCH_3$ was seen in the NMR of the crude reaction products.

α-Alkylhistidines 5. The *N*-acetylhistidine esters **4b–e** were refluxed in 3 N HCl for 15 h. The solutions were evaporated in vacuo to leave the amino acids as hydrochloride salts. The salts showed single spots in paper chromatograms (BuOH–HOAc–H_2O, 5:2:3) sprayed with ninhydrin reagent. Compounds **5b,c,e** and **13** were crystallized from EtOH to afford analytical samples. Compound **5d** had partial loss of HCl during crystallization from 2-PrOH but was confirmed by GC–MS on the trimethylsilyl derivative. Data are listed in Table I.

***N*-Trityl-4-cyanomethylimidazole (7).** 4-Cyanomethylimidazole (**6**) was prepared in 70% yield by decarboxylation of histidine in 5% NaOCl solution by the procedure of Hirsch and Richardson.[6] A mixture of 11.0 g (0.10 mol) of **6** and 12.0 g (59 mmol) of bis(trimethylsilyl)acetamide (Aldrich) was warmed at 50–60 °C until disappearance of solid material. After 1 h 10 mL of THF was added, followed by a solution of 27.9 g (0.10 mol) of trityl chloride in 40 mL of THF. The solution was heated at reflux for 15 h and evaporated in vacuo. The dark residue was partitioned between 250 mL of CH_2Cl_2 and 100 mL of saturated $NaHCO_3$. The $MeCl_2$ extract was twice washed with 100-mL portions of water, dried over $MgSO_4$, and evaporated to leave 35.3 g (98%) of tan crystals. Recrystallization from acetone (115 mL) afforded 27.0 g (77%), mp 157–160 °C. An analytical sample, mp 158–160 °C, was similarly obtained: NMR ($CDCl_3$) 7.30 (16 H, m, Tr-H, Im-2-H), 6.90 (1 H, s, Im-4 or 5 H), 3.70 (2 H, s, CH_2). Anal. ($C_{24}H_{19}N_3$) C, H, N.

***N*-Trityl-α-methyl- and -α,α-dimethyl-4-imidazolacetonitriles (8a,b).** A mixture of 9.0 g (26 mmol) of **7**, 1.26 g (29 mmol) of NaH (55% in oil), and 63 mL of HMPA was stirred for 30 min, followed by the addition of 2.3 mL (37 mmol) of methyl iodide. After 20 h, the mixture was partitioned between 500 mL of saturated saline and 100 mL of Et_2O. From the Et_2O layer was obtained 1.9 g (20%) of white crystals, mp 149–151 °C, identified as **8a**: NMR ($CdCl_3$) 7.35 (16 H, m, Tr-H, Im-2H), 6.90 (1 H, s, Im-4 or 5 H), 3.95 (1 H, q, CH), 1.56 (3 H, d, CH_3). Anal. ($C_{25}H_{21}N_3$) C, H, N. The Et_2O extract was washed with water, dried ($MgSO_4$), and evaporated to leave 6.0 g of crystals shown by NMR to be a mixture containing **8a** and **8b** in a 5:2 ratio.

Similar treatment of **7** (40 g, 0.115 mol) with 11.2 g (0.256 mol) of NaH (55%) and 18.2 mL (0.29 mol) of CH_3I in 570 mL of HMPA gave a crystalline crude product, containing 40% of **8b**. Retreatment under the same conditions gave 26.0 g (66%) of crystals which appeared to be pure **8b** by NMR and TLC (silica; EtOAc–$CHCl_3$, 1:9) analysis. A portion was recrystallized from cyclohexane: mp 135–137 °C; NMR ($CDCl_3$) 1.73 (6 H, s, CH_3). Anal. ($C_{26}H_{23}N_3$) C, H, N.

***N*-Trityl-α-methyl- and -α,α-dimethylimidazolacetic Acids (9a,b).** A mixture of 1.70 g of nitrile **8a**, 5 g of KOH, 40 mL of 2-methoxyethanol, and 5 mL of H_2O was heated for 18 h on the steam bath. The solution was diluted with 100 mL of H_2O, washed with two 75-mL portions of Et_2O, and carefully adjusted to pH 5 with concentrated HCl. After extraction with two 75-mL portions of Et_2O, the extract was washed with three 50-mL portions of water. The extract was dried ($MgSO_4$) and evaporated to leave 1.50 g (84%) of **9a** as off-white crystals. Recrystallization from benzene gave an analytical sample, mp 167–170 °C. Anal. ($C_{25}H_{22}N_2O_2$) C, H, N.

The dimethylnitrile **8b** (16.2 g) was hydrolyzed in a similar manner, except that CH_2Cl_2 was used as the extracting solvent in the work-up, to afford 14.1 g (86%) of **9b** as white crystals, mp 212–214 °C. An analytical sample, mp 213–214 °C, was obtained from the toluene–acetone mixture. Anal. ($C_{26}H_{24}N_2O_2$) C, H, N.

***N*-Trityl-α,α-dimethyl-4-imidazolylacetaldehyde (10b).** A mixture of 7.8 g (19.6 mmol) of the *N*-trityl acid **9b** and 500 mL of toluene was cooled to 0 °C, followed by the addition of 36 mL (47 mmol) of 20% $(i\text{-}Bu)_2AlH$ in hexane over a 30-min period. The mixture was stirred at ambient temperature for 3 h, cooled in ice, and treated with 225 mL of 2 N acetic acid. The toluene layer was separated, washed with 150 mL of H_2O and 150 mL of saturated $NaHCO_3$, dried over $MgSO_4$, and evaporated to leave 7.7 g of a foam; TLC and NMR showed a mixture of alcohol **11b** and aldehyde **10b** in an approximate 1:3 ratio. The material was chromatographed on 400 g of silica gel with elution by Et_2O to remove the aldehyde (2.3 g) as a syrup which crystallized on standing. A portion was recrystallized from Et_2O–hexane to give white crystals, mp 118–119 °C. Anal. ($C_{26}H_{24}N_2O$) C, H, N.

5-(*N*-Trityl-α,α-dimethyl-4-imidazolyl)methylhydantoin (12). The aldehyde **10b** (2.0 g, 5.25 mmol), 300 mg (6.0 mmol) of NaCN, 1.5 g (15.5 mmol) of $(NH_4)_2CO_3$, and 40 mL of 50% EtOH were heated at 110 °C in a steel tube for 15 h. Water (40 mL) was added, followed by adjustment of the pH to 7 by addition of HOAc. The crystalline precipitate was collected, washed with water, and dried to leave 2.30 g (96%). Recrystallization from 85% EtOH gave 1.90 g (82%) of white crystals: mp 227–231 °C; NMR (Me_2SO-d_6) 10.66 (1 H, s, CONHCO), 7.74 (1 H, s, NH), 7.25 (1 H, s, Im-2-H), 7.30 (15 H, m, Tr-H), 6.62 (1 H, s, Im-4 or 5 H), 4.10 (1 H, s, CH), 1.30, 1.24[13] (each 3 H, s, CH_3); IR 3.15 (NH), 5.60, 5.73 μ (CONH). Anal. ($C_{28}H_{26}N_4O_2 \cdot 0.5H_2O$) C, H, N.

β,β-Dimethylhistidine (13). A mixture of 405 mg of the hydantoin **12** and 8 mL of 12 N HCl was stirred at reflux for 66 h. The solution was evaporated to dryness in vacuo and the residue dissolved in 10 mL of absolute EtOH saturated with dry HCl. The solution was refluxed 15 h and evaporated in vacuo. The ester salt was dissolved in 5 mL of H_2O and the solution adjusted to pH 10 by addition of saturated K_2CO_3. The mixture was extracted with 5 × 5 mL portions of $CHCl_3$–2-PrOH (4:1) and solvent removed to leave 88 mg of the ester free base, which was dissolved in 2 mL of H_2O and heated at 100 °C for 40 h. Evaporation afforded the acid as a gum (84 mg) which crystallized from absolute EtOH: paper chromatogram (BuOH–HOAc–H_2O, 5:2:3) single spot, R_f 0.40; data are listed in Table I.

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References and Notes

(1) C. Maslinski, *Agents Actions*, **5**, 89 (1975).
(2) J. W. Black, W. A. M. Duncan, C. J. Durant, C. R. Ganellin, and E. M. Parsons, *Nature (London)*, **236**, 385 (1972).
(3) D. V. Maudsley and K. Kobayashi, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **28**, 353 (1969); H. L. Johnson, *J. Pharmacol. Exp. Ther.*, **177**, 579 (1971).

(4) R. A. Turner, C. F. Huebner, and C. R. Scholz, *J. Am. Chem. Soc.*, **71**, 2801 (1949).
(5) K. F. Schmidt, *Chem. Ber.*, **57**, 704 (1924).
(6) A. Hirsch and K. Richardson, *J. Appl. Chem.*, **19**, 83 (1969).
(7) L. H. Goodson, I. L. Honigberg, J. J. Lehman, and W. H. Burton, *J. Org. Chem.*, **25**, 1920 (1960).
(8) R. Ratcliffe and R. Rodehorst, *J. Org. Chem.*, **35**, 4000 (1970).
(9) G. V. Chelintsev, *Chem. Abstr.*, **35**, 6591 (1961).
(10) M. S. Schechter, N. Green, and F. B. Laforge, *J. Am. Chem. Soc.*, **71**, 3165 (1949).
(11) J. L. Kelley, C. A. Miller, and H. L. White, *J. Med. Chem.*, **20**, 506 (1977).
(12) J. L. Kelley, C. A. Miller, and E. W. McLean, *J. Med. Chem.*, **20**, 721 (1977).
(13) Due to the asymmetric center at the α carbon, adjacent groups are magnetically nonequivalent.

Nonapeptide Ethylamide Inhibitors of the Luteinizing Hormone-Releasing Hormone (LH-RH) Having a D-Alanyl Residue in Position 6 and Variations at Positions 2 and 3[1]

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A series of ten analogues, of structure des-Gly[10]-[amino acid[2],amino acid[3],D-Ala[6]]-LH-RH ethylamide, was synthesized by solid-phase methods. L-Aromatic and alkylamino acids were substituted into position 2 and alkylamino acids into position 3. Highest in vitro inhibition of LH-RH action was obtained with analogues having aromatic residues in position 2. Des-Gly[10]-[Trp[2],Leu[3],D-Ala[6]]-LH-RH ethylamide inhibited the action of 0.6 ng/mL of LH-RH, in an isolated pituitary assay, at a dosage as low as 1 μg/mL, and the corresponding Phe[2] analogue inhibited the effect of 0.3 ng of LH-RH at 0.1 μg/mL. The Trp[2] analogue inhibited ovulation in rats at the dosage of 1.5 mg per rat.

As may be predicted, a priori, an LH-RH inhibitor, which is a good candidate for antiovulation studies in which spontaneous ovulation is suppressed, should have high in vitro potency of inhibition, minimal in vitro agonist activity, no ovulation-inducing activity, and relatively prolonged bioactivity. The first reported inhibitory analogues of LH-RH, des-His[2]-LH-RH and [Gly[2]]-LH-RH, reported by Vale et al.,[2a] several 2- or 3-monosubstituted LH-RH analogues, reported by Monahan et al.,[2b] and [Leu[2],Leu[3]]-LH-RH, reported by Humphries et al.,[3] did not satisfy all of these requirements. Of first concern was the very low observed in vitro inhibitory potencies of all of these analogues. The high agonist activity associated with [Gly[2]]-LH-RH[2a] also eliminated this inhibitor from further consideration.

Analogue programs were initiated in several laboratories to achieve ways of altering the LH-RH sequence in order to increase inhibition potency. Monahan et al.[2b] reported that the incorporation of a D-Ala residue into position 6 of LH-RH led to an increase in agonist potency and that des-His[2]-[D-Ala[6]]-LH-RH was threefold more potent as an inhibitor than des-His[2]-LH-RH in a monolayer, in vitro assay.

Fujino et al.[4] reported that the replacement of the C-terminal Pro-Gly-NH_2 moiety by a Pro-NHEt group, as in des-Gly[10]-LH-RH ethylamide, increased the agonist potency by three- to fivefold, and later studies[5] showed that the activity of this analogue was prolonged. Coy et al.[6,7] found that the incorporation of this modification into the des-His[2]-LH-RH sequence, which gave des-His[2],des-Gly[10]-LH-RH ethylamide, produced an analogue which could inhibit the release of LH and FSH by LH-RH, in vivo, in rats; des-His[2]-LH-RH appeared to be inactive in this assay.

Incorporation of both the 6 position[2b] and C-terminal position[4] modifications into the des-His[2]-LH-RH sequence has given active inhibitors.[8-10]

We decided to synthesize some LH-RH analogues based upon our [Leu[2],Leu[3]]-LH-RH sequence,[3] having a D-Ala residue in position 6[2b] and also the C-terminal modification,[4] and to assay these analogues in the isolated pituitary assay. Wan et al.[11] have shown that the inhibitory potency of [Leu[2],Leu[3],D-Ala[6]]-LH-RH was up to tenfold higher than that of [Leu[2],Leu[3]]-LH-RH, in vitro. In particular, we were interested in varying the amino acid residues in positions 2 and 3 to further enhance inhibitory potency.

After this work was completed, Rees et al.[12] reported upon the advantage for inhibition of incorporating a D-Phe residue into position 2 of the LH-RH sequence, and Corbin and Beattie[13] showed that [D-Phe[2],D-Ala[6]]-LH-RH was an effective inhibitor of ovulation in rats and rabbits. However, Yardley et al.[14] later showed that although [D-Phe[2],D-Ala[6]]-LH-RH and des-Gly[10]-[D-Phe[2],D-Ala[6]]-LH-RH ethylamide both strongly inhibited the action of LH-RH, in vitro, in monolayer cultures, with the ethylamide analogue being the more potent inhibitor, only [D-Phe[2],D-Ala[6]]-LH-RH was effective in preventing ovulation in 4-day cycling rats. Studies have shown that this apparent lack of interassay correspondence could be rationalized when the ability of the inhibitors to suppress the proestrous preovulatory surge of LH was considered, for then only [D-Phe[2],D-Ala[6]]-LH-RH was effective.[14]

Experimental Section

Synthetic procedures with the Beckman Model 990 peptide synthesizer have been described.[15] Product yields (percent) were estimated from the starting amino acid-resin. On chromatography, the product of the major peaks was examined by TLC. Usually only those fractions corresponding to the upper parts of the peaks were taken, and consequently the percentage yields will be low. TLC on silica gel was used to evaluate product purity, with baths from the systems R_f[1], 1-BuOH–AcOH–EtOAc–H_2O (1:1:1:1 v/v);