(±)-2-(*N*,*N*-Dimethylamino)-1-propyl Ethyl Ether Methiodide (5). Compound **2** (0.25 g, 0.0092 mol) in 50 ml of anhydrous EtOH was hydrogenated at room temperature in the presence of 0.25 g of 10% Pd/C at an initial pressure of 50 psig. The reduction was complete in 1 h. Removal of the catalyst and evaporation of the solvent under reduced pressure afforded a white solid which was recrystallized from *n*-BuOH–hexane to give 0.212 g (85%) of white crystals: mp 148.3°; NMR (Me_2SO-d_6) δ 1.26 (center) (m, 6 H, $CHCH_3$, CH_2CH_3), 3.13 [s, 9 H, $N(CH_3)_3$]. Anal. ($C_8H_{20}INO$) C, H, N.

Literature[5] Reaction of Dimethylaminoisopropyl Chloride (11) with Sodium Ethoxide. To a solution of 79 g (0.5 mol) of dimethylaminoisopropyl chloride hydrochloride in 500 ml of anhydrous EtOH was added a solution of 25.3 g (1.1 g-atom) of Na in 500 ml of anhydrous EtOH. The reaction mixture was heated on a H_2O bath for 24 h; then it was cooled and filtered. The filtrate was distilled through a 32-cm Vigreux column to give 28 g (43%) of a clear liquid: bp 133–135° (750 mm) (lit.[5] bp 133–135°).. This product (2.5 g, 0.019 mol) in 20 ml of anhydrous Et_2O was treated with 3.5 g (0.025 mol) of MeI and the white solid which formed was recrystallized from amyl alcohol–amyl acetate to give 4.6 g (86%) of a white powder: mp 145–146° (lit.[5] mp 144.5°). An ir spectrum (Nujol) of this material was superimposable upon a similar spectrum of **5**. An NMR spectrum (Me_2SO-d_6) was superimposable upon a similar spectrum of **5**, showing the characteristic broadening of the CCH_3 multiplet at δ 1.26.

(±)-1-(*N*,*N*-Dimethylamino)-2-propyl Ethyl Ether (21). Method A. Compound **10** (2.0 g, 0.015 mol) in 50 ml of anhydrous EtOH was hydrogenated at room temperature in the presence of 0.25 g of 10% Pd/C at an initial pressure of 50 psig. The calculated amount of H_2 was taken up in 1.5 min, and the reaction was allowed to continue for an additional 2 min. Filtration and concentration of the filtrate under reduced pressure gave 15 ml of crude product which was used in the next step without purification.

Method B. A mixture of 54 ml of Me_2SO (distilled from CaH) and 4.5 g of a 57% mineral oil dispersion of NaH (which had been washed three times with hexane) was heated at 70–75° until evolution of H_2 ceased (ca. 45 min). This deep gray mixture and 0.05 g of triphenylmethane were added to 9 g (0.087 mol) of 1-(*N*,*N*-dimethylamino)-2-propanol in 10 ml of Me_2SO. The resulting deep red-brown solution was treated with 13.6 g (0.088 mol) of diethyl sulfate, and the resulting mixture was stirred with cooling for 10 min. Then, 15 ml of CH_2Cl_2 and 15 ml of H_2O were added; the aqueous layer was separated and extracted repeatedly with CH_2Cl_2. The combined organic phases were washed several times with H_2O and dried ($MgSO_4$). Filtration and evaporation of the filtrate afforded a gummy residue and a supernatant liquid which were extracted with anhydrous Et_2O to give, after removal of the Et_2O, a yellow liquid for which TLC analysis (alumina, Me_2CO–$CHCl_3$, 1:1) showed two components. This material was chromatographed on 230 g of alumina and eluted with Me_2CO–$CHCl_3$ (1:1). Forty-two 15-ml fractions were collected and monitored by TLC. Fractions 12–33 were pooled and the solvent was evaporated to give a light yellow liquid which was used in the next step without purification.

(±)-1-(*N*,*N*-Dimethylamino)-2-propyl Ethyl Ether Methiodide (6). The products **21** of methods A and B were quaternized separately as described for **1**. The material from method B afforded a dark brown liquid which solidified upon standing in the cold. This was washed several times with anhydrous Et_2O and was recrystallized from *n*-BuOH–hexane to afford 0.24 g (1%) of white crystals: mp 97–98°; NMR (Me_2SO-d_6) δ 1.13 (m, 6 H, $CHCH_3$, CH_2CH_3), 3.20 [s, 9 H, $N(CH_3)_3$]. Anal. ($C_8H_{20}INO$) C, H, N. The material from method A gave a brown solid which upon recrystallization from *n*-BuOH–hexane gave 3.4 g (81%) of white crystals: mp 99.8°. NMR (Me_2SO-d_6) of this material was identical with that of the quaternization of the method B product.

References and Notes

(1) (a) A preliminary account of this work was presented at the 170th National Meeting of the American Chemical Society, Chicago, Ill., August 25, 1975. (b) Abstracted in part from a thesis submitted by A.G. in partial fulfillment of the requirements for the Ph.D. degree, University of Iowa, 1975.
(2) C. Y. Chiou, *Arch. Int. Pharmacodyn. Ther.*, **185**, 25 (1970).
(3) W. H. Watanabe and L. E. Conlon, *J. Am. Chem. Soc.*, **79**, 2828 (1957).
(4) R. T. Major and J. K. Kline, *J. Am. Chem. Soc.*, **55**, 2504 (1933).
(5) A. R. Goldfarb, *J. Am. Chem. Soc.*, **63**, 2880 (1941).
(6) R. Hunt and R. R. Renshaw, *J. Pharmacol. Exp. Ther.*, **58**, 140 (1936).
(7) A. Simonart, *J. Pharmacol. Exp. Ther.*, **46**, 157 (1932); **50**, 140 (1934).
(8) D. J. Finney, "Experimental Design and Its Statistical Basis", University of Chicago Press, Chicago, Ill., 1955.
(9) L. Knorr and H. Matthes, *Chem. Ber.*, **32**, 736 (1899).
(10) A. C. Cope and E. R. Trumbull, *Org. React.*, **11**, 380 (1960).
(11) Knorr and Matthes[9] prepared this compound, but did not report a melting point, and reported analysis for iodine only.
(12) H. Z. Sommer, H. I. Lipp, and L. L. Jackson, *J. Org. Chem.*, **36**, 824 (1971).
(13) D. L. Cottle, A. E. Jeltsch, T. H. Stoudt, and D. R. Walters, *J. Org. Chem.*, **11**, 286 (1946).
(14) R. F. Church, R. E. Ireland, and J. A. Marshall, *J. Org. Chem.*, **31**, 2526 (1966).
(15) H. R. Ing, P. Kordik, and D. P. H. Tudor Williams, *Br. J. Pharmacol. Chemother.*, **7**, 103 (1952).
(16) L. Knorr, *Chem. Ber.*, **37**, 3498 (1904).

Analogues of Luteinizing Hormone Releasing Factor Modified at Positions 2, 6, and 10

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Eighteen analogues of luteinizing hormone releasing factor (LRF, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) were synthesized. The ten agonistic analogues were [D-Lys^6], [D-Orn^6], [D-Lys^6,des-Gly^{10},Pro^9-NHEt], [D-Orn^6,-des-Gly^{10},Pro^9-NHEt]-LRF plus their respective lauric acid conjugates as well as [(N^ϵ-Ac)-D-Lys^6] and [(N^δ-Ac)-D-Orn^6]-LRF. The eight antagonistic analogues were [des-His^2,D-Lys^6], [des-His^2,D-Orn^6], [des-His^2,D-Lys^6,des-Gly^{10},Pro^9-NHEt], [des-His^2,D-Orn^6,des-Gly^{10},Pro^9-NHEt]-LRF as well as their respective lauric acid conjugates. Biological activities of these analogues were determined in vitro, using LRF as the standard for the agonists and [des-His^2,D-Ala^6]-LRF for the antagonists. The potency of the agonists ranged from 1 to 17 times the activity of LRF while the antagonists had between 1 and 3 times the potency of [des-His^2,D-Ala^6]-LRF.

Following the discovery in our laboratory that substitution of luteinizing hormone releasing factor (LRF, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2[1]) with D-alanine at the 6 position rendered the resulting compound more potent,[2a] many analogues incorporating this modification as well as other D-amino acids have been synthesized. Monahan et al.[2a] have replaced the glycine at the 6 position with D-valine and D-proline while Vil-

chez-Martinez et al.[2b] and Fujino et al.[3] have substituted the same residue with D-leucine. Rivier et al.[4] and Vale et al.[5] have systematically substituted the 6 residue with most of the commercially available D-amino acids and found that [D-Trp^6]-LRF was by far the most potent LRF analogue singly modified. On the other hand, when the corresponding L-amino acids were incorporated at the same position, much less potent compounds were obtained.[2a,4] The increased activity of [D-Ala^6]-LRF was originally attributed to a preferred conformation involving a β-II type bend in the sequence -Ser-Tyr-D-Ala-Leu- which was thought to be favored by the hormone receptor.[2a] However, recently studies[6] on [D-Ala^6,(N^{α}-Me)Leu^7]-LRF and [(N^{α}-Me)Leu^7]-LRF tended to rule out this hypothesis. A more plausible explanation seemed to be that the D-alanine residue increased the peptide's resistance to enzymic degradation[5,7] and thus prolonged the biological half-life of the compound. This fact is reflected in the much higher activity of [D-Ala^6]-LRF observed in the in vivo ovulation–induction assay vs. the in vitro system[8] and the fact that [D-Ala^6,(N^{α}-Me)Leu^7]-LRF has a protracted action in vivo as compared with LRF and [D-Ala^6]-LRF.[5]

Analogues containing the [des-Gly^{10},Pro^9-NHEt] modification[9] in combination with D-amino acid substitution at the 6 position have also been prepared.[2b,3-5,8,10,11] The most interesting compounds are [D-Ala^6,des-Gly^{10},-Pro^9-NHEt]-LRF,[2b,4,5,8,10] [D-Leu^6,des-Gly^{10},Pro^9-NHEt]-LRF,[2b,3-5,11,12] [D-Abu^6,des-Gly^{10},Pro^9-NHEt]-LRF,[3] [D-Nva^6,des-Gly^{10},Pro^9-NHEt]-LRF,[3] [D-Ser^6,-des-Gly^{10},Pro^9-NHEt]-LRF,[3,11] and [D-Ser(Bu^t)^6,des-Gly^{10},Pro^9-NHEt]-LRF[11] which are at least 5000% more active than LRF in the in vivo ovulation–induction assay. Coy et al.[13] have incorporated the [D-Ala^6] substitution in [des-Gly^{10},Pro^9-NHCH$_2$CF$_3$]-LRF and [des-Gly^{10},Pro^9-NHCH$_2$CF$_2$CF$_3$]-LRF but found the former to be equipotent and the latter less potent than [D-Ala^6,des-Gly^{10},Pro^9-NHEt]-LRF. The most potent analogue with substitutions at position 6 and 10 determined in our in vitro assay system is [D-Trp^6,des-Gly^{10},Pro^9-NHEt]-LRF.[5]

In addition to having a D-amino acid at the 6 position, compounds containing the [des-Gly^{10},Pro^9-NHEt] modification[9] plus one or more substitutions on other residues have likewise been synthesized.[3,8,11,14] All these reported compounds are either equally or less active than [D-Ala^6,des-Gly^{10},Pro^9-NHEt]-LRF.

Competitive antagonists to LRF incorporating the [D-Ala^6] substitution as well as other D-amino acids at position 6 have also appeared in the literature.[2a,4,5,15-17] So far the most powerful antagonist that has been published[5] is [D-Phe^2,D-Trp^6]-LRF which is 30 times more potent than [des-His^2,D-Ala^6]-LRF. Moreover, an affinity-labeling antagonist of LRF has been synthesized which possesses the [D-Ala^6] modification.[18]

With our overall effort of finding long-acting, highly potent LRF analogues as well as competitive antagonists, we have further explored the effect of substituting D-lysine and D-ornithine at the 6 position of LRF. In addition, the [des-Gly^{10},Pro^9-NHEt] modification as well as the deletion of histidine[19] has also been incorporated into our studies. The use of dibasic amino acids yielded LRF analogues that could be coupled to water-soluble polymers so as to prolong the biological half-life of the molecule through resistance to kidney elimination. When coupled to large antigenic proteins, these analogues could be used to raise antibodies that would bind LRF. Furthermore, conjugation of these analogues to insoluble polymers could lead to the new technique of affinity chromatography[20] for purifying LRF binding antibodies and receptors. Highly hydrophobic groups could be introduced to these compounds by coupling with long-chain fatty acids to yield fat-soluble substances. When dispersed in a suitable vehicle and injected subcutaneously, these substances would migrate slowly to the blood stream and thus maintain a continuous supply of the hormone in circulation for a prolonged period. A note reporting the synthesis and biological activity of [D-Lys^6]-LRF conjugated to poly-L-glutamic acid has been published.[21] The present paper describes the synthesis and in vitro biological activity of [D-Lys^6]-LRF, [D-Orn^6]-LRF, [D-Lys^6,des-Gly^{10},Pro^9-NHEt]-LRF, [D-Orn^6,des-Gly^{10},Pro^9-NHEt]-LRF, [des-His^2,D-Lys^6]-LRF, [des-His^2,D-Orn^6]-LRF, [des-His^2,D-Lys^6,des-Gly^{10},Pro^9-NHEt]-LRF, and [des-His^2,D-Orn^6,des-Gly^{10},Pro^9-NHEt]-LRF as well as their lauric acid and two acetylated derivatives.[22]

Synthesis. Solid-phase methodology[23a] was used to synthesize these analogues. Compounds containing the glycinamide C terminus were prepared from benzhydrylamine resin[23b] as described previously.[24] For the [des-Gly^{10},Pro^9-NHEt] modification, chloromethyl resin[23a] was used and the ethylamide introduced by treatment of the protected peptide-resin with ethylamine.[25] The protecting group was removed with 10% anisole in liquid HF.[26] The deprotected peptides were first purified by ion-exchange chromatography followed by one or more steps of partition chromatography.[27] After purification, the peptides obtained in about 30% yield were characterized by thin-layer chromatography, amino acid analysis, and optical rotation. The lauryl derivatives were prepared by treating the respective LRF analogues with pentachlorophenyl laurate and a trace of triethylamine in methanol at room temperature. Analogues containing histidine, however, gave an extra compound which migrated faster than the major product on TLC when detected with Pauly reagent. Since no such compound was detected in the [des-His^2] analogues, the by-product was presumed to have an additional lauryl group on the imidazole ring. This assumption was substantiated by conversion of the by-product to the desired compound in 0.33 N sodium hydroxide in methanol at room temperature for 1 h. The lauryl derivatives were purified by gel-filtration chromatography[28] on Sephadex LH-20 followed by partition chromatography.[27] The purified compounds obtained in about 50% yield were characterized by thin-layer chromatography, amino acid analysis, and optical rotation.

Biological Assays. Biological assays utilized primary cell cultures of enzymatically dissociated rat anterior pituitary cells.[29,30] Multiple dose levels of peptides and LRF standard were tested for their abilities to increase the luteinizing hormone secretion rates by these cells. Potencies relative to LRF and 95% confidence limits were calculated using the Harvard University biological assay program. The ability of analogues to inhibit the release of luteinizing hormone stimulated by a constant concentration of LRF (usually 3 nM) was the basis of the antagonist assay,[19,30] where [des-His^2,D-Ala^6]-LRF was used as a standard and arbitrarily assigned a potency of 100%.

Results and Discussion

The potencies relative to LRF of the peptides with agonistic activity are presented in Table I. As was the case with other D-amino acid substitutions,[2-5] the replacement of glycine at the 6 position with D-lysine or D-ornithine yields peptides with higher potencies than LRF. Even though the two amino acids differ only by a methylene group, [D-Lys^6]-LRF has a greater potency than [D-Orn^6]-LRF. Coupling of an acetyl group to the free

Table I. In Vitro Relative Potencies of LRF Agonists

	Analogue	Rel potency with confidence limits (LRF = 100)
1	pGlu-His-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH_2	383 (278-525)
2	pGlu-His-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-Gly-NH_2	182 (141-234)
3	pGlu-His-Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-NHEt	1726 (1261-2360)
4	pGlu-His-Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-NHEt	682 (538-865)
1a	pGlu-His-Trp-Ser-Tyr-(N^{ϵ}-lauryl)-D-Lys-Leu-Arg-Pro-Gly-NH_2	164 (127-213)
1b	pGlu-His-Trp-Ser-Tyr-(N^{ϵ}-Ac)-D-Lys-Leu-Arg-Pro-Gly-NH_2	274 (149-499)
2a	pGlu-His-Trp-Ser-Tyr-(N^{δ}-lauryl)-D-Orn-Leu-Arg-Pro-Gly-NH_2	290 (224-376)
2b	pGlu-His-Trp-Ser-Tyr-(N^{δ}-Ac)-D-Orn-Leu-Arg-Pro-Gly-NH_2	101 (78-130)
3a	pGlu-His-Trp-Ser-Tyr-(N^{ϵ}-lauryl)-D-Lys-Leu-Arg-Pro-NHEt	1038 (802-1344)
4a	pGlu-His-Trp-Ser-Tyr-(N^{δ}-lauryl)-D-Orn-Leu-Arg-Pro-NHEt	490 (379-636)

Table II. In Vitro Relative Potencies of LRF Antagonists

	Analogue	Rel potency with confidence limits ([des-His^2,D-Ala^6]-LRF = 100)
5	pGlu- - -Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH_2	123 (80-192)
6	pGlu- - -Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-Gly-NH_2	134 (90-196)
7	pGlu- - -Trp-Ser-Tyr-D-Lys-Leu-Arg-Pro-NHEt	97 (55-172)
8	pGlu- - -Trp-Ser-Tyr-D-Orn-Leu-Arg-Pro-NHEt	105 (50-180)
5a	pGlu- - -Trp-Ser-Tyr-(N^{ϵ}-lauryl)-D-Lys-Leu-Arg-Pro-Gly-NH_2	284 (169-487)
6a	pGlu- - -Trp-Ser-Tyr-(N^{δ}-lauryl)-D-Orn-Leu-Arg-Pro-Gly-NH_2	278 (170-454)
7a	pGlu- - -Trp-Ser-Tyr-(N^{ϵ}-lauryl)-D-Lys-Leu-Arg-Pro-NHEt	276 (163-473)
8a	pGlu- - -Trp-Ser-Tyr-(N^{δ}-lauryl)-D-Orn-Leu-Arg-Pro-NHEt	153 (92-250)

Table III. Amino Acid Ratios of LRF Analogues

Analogue	Glu	His	Trp	Ser	Tyr	X	Leu	Arg	Pro	Gly	NH_3	$EtNH_2$
1	1.03	0.98	0.99	0.89	1.02	1.00[a]	1.00	0.93	1.07	1.04	1.24	
1a	1.04	1.01	0.78	0.89	0.97	0.99[a]	1.00	0.90	1.00	1.06	1.29	
1b	1.01	0.96	0.98	0.82	1.02	1.00[a]	1.00	0.94	1.06	1.04	1.28	
2	1.04	0.98	1.07	0.89	1.03	1.00[b]	1.00	0.95	1.10	1.04	1.16	
2a	1.04	1.00	0.77	0.90	1.01	1.01[b]	1.00	0.94	1.11	1.07	1.27	
2b	1.05	0.98	0.96	0.88	1.03	1.00[b]	1.00	0.93	1.06	1.04	1.24	
3	1.04	0.99	0.79	0.92	1.02	1.02[a]	1.00	0.94	1.07			1.00
3a	0.99	0.95	0.65	0.87	1.04	1.04[a]	1.00	0.89	1.03			0.85
4	1.10	1.03	0.91	0.95	1.07	1.04[b]	1.00	0.95	1.08			0.99
4a	1.03	1.01	0.81	0.88	1.04	1.08[b]	1.00	0.99	1.07			0.82
5	1.03		1.02	0.90	1.03	0.98[a]	1.00	0.94	1.09	1.05	1.17	
5a	1.02		0.79	0.88	0.99	1.02[a]	1.00	0.93	1.15	1.07	1.40	
6	1.04		1.05	0.89	1.03	0.97[b]	1.00	0.94	1.07	1.04	1.11	
6a	1.04		0.78	0.91	1.03	1.00[b]	1.00	0.90	1.02	1.05	1.19	
7	1.04		0.81	0.91	0.95	0.99[a]	1.00	0.95	1.04			1.02
7a	0.99		0.66	0.85	1.01	1.03[a]	1.00	0.89	1.02			0.86
8	1.03		0.77	0.92	1.00	0.99[b]	1.00	0.92	1.06			0.96
8a	0.94		0.68	0.91	0.98	1.03[b]	1.00	0.90	1.07			0.78

[a] Lys. [b] Orn.

amino group of [D-Lys^6]-LRF or [D-Orn^6]-LRF results in some loss of biological activity. And, when a lauryl group is incorporated, more pronounced lowering of activity is observed. This effect is probably not solely related to the lipophilicity of the 6 position substitution since the lipophilic [D-Trp^6]-LRF is the most potent (36 × LRF) singly modified analogue reported[5] to date. Incorporation of the [des-Gly^{10},Pro^9-NHEt] modification first described by Fujino et al.[9] in the agonistic LRF analogues containing D-lysine or D-ornithine at the 6 position enhances their activity even more, bringing the potency of [D-Lys^6,des-Gly^{10},Pro^9-NHEt]-LRF to 17 times that of LRF.

The potencies of the analogues with antagonistic activity relative to [des-His^2,D-Ala^6]-LRF are shown in Table II. Substitution of the glycine residue at the 6 position in [des-His^2]-LRF (relative potency = 15%) with D-lysine, D-ornithine, or D-alanine increases the potency of the resultant antagonists to about the same extent. In contrast to the agonists, incorporation of the [des-Gly^{10},Pro^9-NHEt] modification[9] does not increase the potencies of these antagonists. Also, in contradistinction to the agonistic analogues, coupling of a lauryl group to the [D-Lys^6] or [D-Orn^6] residue in appropriate LRF antagonists raises rather than lowers their antagonistic potencies as assayed in vitro. The high lipid solubility of the peptides with the [(N^{ϵ}-lauryl)-D-Lys^6] modification may make them suitable for administration in oil or other slowly absorbed vehicles.

Experimental Section

Melting points (mp) were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared (ir) data were recorded in a Perkin-Elmer 237B grating infrared spectrophotometer. Mass spectra (MS) were obtained from a Varian Mat CH-5 single-focusing mass spectrometer with the direct inlet system. The electron current was set at 1 mA with an ionizing energy of 70 eV. Amino acid analyses were determined on peptide hydrolysates using a Beckman/Spinco Model 119 amino acid analyzer (Table III). Hydrolyses were performed in 6 N HCl containing 2.5% thioglycolic acid at 110° in evacuated sealed tubes for 20 h. Optical rotations were measured in a Perkin-Elmer Model 141 polarimeter. Ascending TLC on silica gel was performed with Eastman Chromagram Sheet No. 13181 containing fluorescent indicator (Table IV). About 20–30-μg samples were spotted and the solvent front was allowed to travel 10–15 cm. The spots were detected by ultraviolet light and ninhydrin and Pauly reagents. Amino acid derivatives used for the synthesis were of the L configuration unless stated otherwise and were purchased from Bachem, Inc. The α-amino function

Table IV. Physical Constants of LRF Analogues

Analogue	R_f value of TLC systems[c]							
	BAW	2-BA	BPyA	EtPyAW	2-BW	2-BN	BINEt	BEtAW	$[\alpha]^{25}$D, deg
1	0.31	0.24	0.42	0.58			0.24	0.57	−35.1[a]
2	0.35	0.24	0.45	0.60			0.25	0.62	−36.7[a]
3	0.40	0.27	0.53	0.63			0.31	0.66	−34.2[a]
4	0.26	0.29	0.47	0.69			0.34	0.65	−38.6[a]
5	0.35	0.33	0.47	0.71	0.25		0.26	0.73	−30.9[a]
6	0.46	0.31	0.54	0.67	0.24		0.28	0.74	−34.0[a]
7	0.49	0.35	0.59	0.71	0.31		0.37	0.76	−29.5[a]
8	0.54	0.36	0.63	0.71	0.28		0.38	0.78	−32.9[a]
1a	0.40	0.38	0.56	0.35	0.37	0.37	0.44	0.74	−23.1[b]
1b	0.39	0.34	0.56	0.69	0.34	0.33	0.43	0.73	−34.9[a]
2a	0.40	0.39	0.59	0.34	0.38	0.36	0.43	0.72	−23.9[b]
2b	0.38	0.34	0.57	0.66	0.33	0.34	0.43	0.70	−34.7[a]
3a	0.42	0.43	0.61	0.40	0.39	0.38	0.47	0.72	−28.4[b]
4a	0.45	0.43	0.62	0.38	0.40	0.37	0.46	0.72	−27.4[b]
5a	0.52	0.46	0.66	0.48	0.44	0.43	0.44	0.78	−19.9[b]
6a	0.53	0.47	0.67	0.48	0.45	0.43	0.44	0.78	−20.0[b]
7a	0.63	0.52	0.73	0.53	0.46	0.45	0.47	0.83	−24.7[b]
8a	0.64	0.51	0.71	0.55	0.47	0.46	0.49	0.84	−24.8[b]

[a] Optical rotation measured at 1% concentration in 1% acetic acid. [b] Optical rotation measured at 1% concentration in glacial acetic acid. [c] BAW = 1-butanol-acetic acid-water (4:1:5, upper phase); 2-BA = 2-butanol-0.1 N acetic acid (1:1, upper phase); BPyA = 1-butanol-pyridine-0.1% acetic acid (5:3:11, upper phase); EtPyAW = ethyl acetate-pyridine-acetic acid-water (5:5:1:3); 2-BW = 2-butanol-water (1:1, upper phase); 2-BN = 2-butanol-0.1 N ammonium hydroxide (1:1, upper phase); BINEt = 1-butanol-2-propanol-1 N ammonium hydroxide-ethyl acetate (1:1.2.5:1, upper phase); BEtAW = 1-butanol-ethyl acetate-acetic acid-water (1:1:1:1).

was protected exclusively with the Boc group. Other protecting groups consisted of tosyl for Arg and His, Cbz or 2-chloro-Cbz for D-Lys and D-Orn, and Bzl for Tyr and Ser.

Synthesis of Peptides. Synthesis of LRF analogues on the benzhydrylamine resin has been described in detail previously.[24,31,32] Coupling of Boc-Pro to the chloromethyl resin[23a] (0.75 mequiv of Cl/g, Lab Systems, Inc.) was performed by a modification[33] of the Monahan and Gilon procedure.[34] Building of the protected peptide chain on the chloromethyl resin was done similarly to the synthesis on the benzhydrylamine resin. The C-terminal ethylamide was introduced by stirring the protected peptide-resin in 50 ml of ethylamine overnight in the cold room. The resin was filtered and washed with methanol. Evaporation of the methanol solution yielded the slightly yellow protected peptide-ethylamide. The protecting groups were removed with HF containing 10% anisole[26] and the peptide was purified by ion-exchange chromatography on Whatman microgranular CM-32 carboxymethylcellulose followed by one or more steps of partition chromatography on Sephadex G-25F (Pharmacia). Details of the purification steps have been described previously.[24]

Pentachlorophenyl Laurate. Lauric acid (10.0 g, 50 mmol) and pentachlorophenol (13.3 g, 50 mmol) were stirred with dicyclohexylcarbodiimide (12.4 g, 60 mmol) in DMF (100 ml) and CH_2Cl_2 (40 ml) at room temperature overnight. The resulting white precipitate was filtered and washed with CH_2Cl_2. Evaporation of the filtrate left an oil which was partitioned between CH_2Cl_2 and 10% Na_2CO_3. The CH_2Cl_2 phase was further washed with 10% Na_2CO_3 and water. Drying with Na_2SO_4 and evaporation of the CH_2Cl_2 gave the desired compound which was recrystallized from EtOH to yield 12.8 g (57.1%) of product: mp 65–66°; ir (KBr) ν 1776 cm^{-1} (ester C=O); MS *m/e* 446 (M^+, 3), 264 (26), 235 (11), 228 (9), 200 (9), 184 (58), 183 (100), 165 (25), 155 (39), 127 (51), 123 (48), 109 (74). Anal. Calcd mass for $C_{18}H_{23}O_2{}^{35}Cl_5$, 446; found, 446.

Conjugation of Peptides 1–4 to Lauric Acid. Peptide 1 (50 mg, 0.0349 mmol) was dissolved in warm MeOH (20 ml). Triethylamine (14.5 μl, 0.1047 mmol) and pentachlorophenyl laurate (78.3 mg, 0.1745 mmol) dissolved in THF (2 ml) were added and the mixture was kept at room temperature for 2 days. The solvent was evaporated and the residue redissolved in MeOH (20 ml). NaOH (1 N) in MeOH (10 ml) was added and the mixture stirred at room temperature for 1 h. The pH of the solution was adjusted to 4 with 6 N HCl at 0° and the precipitated NaCl filtered. Evaporation of the filtrate left a white residue which was loaded on a Sephadex LH-20 (Pharmacia) column (2 × 102 cm) and eluted with MeOH. Fractions of 3 ml each were collected and evaluated by TLC in the system 2-butanol–H_2O (1:1, upper phase). The fractions (tubes 30–35) containing the major compound were pooled and evaporated. The residue was further purified by partition chromatography on a Sephadex G-25F (Pharmacia) column (2 × 102 cm, V_{bed} = 252 ml, V_{void} = 88 ml) and eluted with the solvent system 1-butanol–H_2O (1:1). Fractions of 3 ml each were collected and analyzed by TLC similarly. Tubes 40–52 were found to contain the desired compound and they were pooled, evaporated, and lyophilized from HOAc to give 29.3 mg of **1a** (54%). An identical procedure was used to prepare peptides **2a**, **3a**, and 4a from analogues **2**, **3**, and **4**.

Conjugation of Peptides 5–8 to Lauric Acid. A modification of the above method was used to prepare the lauryl derivatives of peptides 5–8. After coupling of the lauryl group on the free amino function of lysine or ornithine, the unreacted pentachlorophenyl laurate was extracted repetitively from the dried reaction mixture with warm Et_2O and the residue purified by Sephadex LH-20 and G-25F as before.

Acetylation of Peptides 1 and 2. Peptide 1 or 2 (100 mg) was suspended in 2 N NaOAc (1 ml) at 0°. Ac_2O (3 × 100 μl) was added over 1 h under stirring. After 1 h more at 0° the slurry was dried under high vacuum and extracted with the upper phase of the 1-butanol–HOAc–H_2O (4:1:5) solvent system (4 ml). This extract was applied directly onto a Sephadex G-25F partition column and eluted with the same solvent system. Fractions were collected and the elution pattern was observed at 280 nm. One main peak was obtained which was lyophilized to yield 60 mg of product (60%).

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References and Notes

(1) Amino acid abbreviations are according to IUPAC-IUB Commission on Biochemical Nomenclature published in *Biochemistry*, 14, 449 (1975).
(2) (a) M. W. Monahan, M. S. Amoss, H. A. Anderson, and W. Vale, *Biochemistry*, **12**, 4616 (1973); (b) J. A. Vilchez-Martinez, D. H. Coy, A. Arimura, E. J. Coy, Y. Hirotsu, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **59**, 1226 (1974).
(3) M. Fujino, T. Fukuda, S. Shinagawa, S. Kobayashi, I. Yamazaki, R. Nakayama, J. H. Seely, W. F. White, and R. H. Rippel, *Biochem. Biophys. Res. Commun.*, **60**, 406 (1974).
(4) J. Rivier, N. Ling, M. Monahan, C. Rivier, M. Brown, and

W. Vale in "Peptides: Chemistry, Structure and Biology", R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1976, p 863.
(5) W. Vale, C. Rivier, M. Brown, J. Leppaluoto, N. Ling, M. Monahan, and J. Rivier, *Clin. Endocrinol. (Oxford), (Suppl.)*, in press.
(6) N. Ling and W. Vale, *Biochem. Biophys. Res. Commun.*, **63**, 801 (1975).
(7) N. Marks and F. Stern, *Biochem. Biophys. Res. Commun.*, **61**, 1458 (1974).
(8) M. Fujino, I. Yamazaki, S. Kobayashi, T. Fukuda, S. Shinagawa, R. Nakayama, W. F. White, and R. H. Rippel, *Biochem. Biophys. Res. Commun.*, **57**, 1248 (1974).
(9) M. Fujino, S. Kobayashi, M. Obayashi, S. Shinagawa, T. Fukuda, C. Kitada, R. Nakayama, I. Yamazaki, W. F. White, and R. H. Rippel, *Biochem. Biophys. Res. Commun.*, **49**, 863 (1972).
(10) D. H. Coy, E. J. Coy, A. V. Schally, J. A. Vilchez-Martinez, Y. Hirotsu, and A. Arimura, *Biochem. Biophys. Res. Commun.*, **57**, 335 (1974).
(11) W. König, J. Sandow, and R. Geiger, in "Peptides: Chemistry, Structure and Biology", R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1976, p 883.
(12) R. H. Rippel, E. S. Johnson, W. F. White, M. Fujino, T. Fukuda, and S. Kobayashi, *Proc. Soc. Exp. Biol. Med.*, **148**, 1193 (1975).
(13) D. H. Coy, J. A. Vilchez-Martinez, E. J. Coy, N. Nishi, A. Arimura, and A. V. Schally, *Biochemistry*, **14**, 1848 (1975).
(14) C. R. Beddell, P. J. Fraser, D. Gilbert, P. J. Goodford, L. A. Lowe, and S. Wilkinson, *J. Med. Chem.*, **18**, 417 (1975).
(15) J. A. Vilchez-Martinez, A. V. Schally, D. H. Coy, E. J. Coy, C. M. Miller, and A. Arimura, *Endocrinology*, **96**, 1130 (1975).
(16) A. de la Cruz, D. H. Coy, A. V. Schally, E. J. Coy, K. G. de la Cruz, and A. Arimura, *Proc. Soc. Exp. Biol. Med.*, **149**, 576 (1975).
(17) K. U. Prasad, R. W. Roeske, and C. Y. Bowers, *Biochem. Biophys. Res. Commun.*, **66**, 336 (1975).
(18) C. Y. Bowers, Y.-P. Wan, J. Humphries, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **61**, 698 (1974).
(19) W. Vale, G. Grant, J. Rivier, M. Monahan, M. Amoss, R. Blackwell, R. Burgus, and R. Guillemin, *Science*, **176**, 933 (1972).
(20) P. Cuatrecasas, M. Wilchek, and C. B. Anfinsen, *Proc. Natl. Acad. Sci. U.S.A.*, **61**, 636 (1968).
(21) M. Amoss, M. Monahan, and M. Verlander, *J. Clin. Endocrinol. Metab.*, **39**, 187 (1974).
(22) Some of the results have been presented at the 4th American Peptide Symposium in New York[4] and at the Endocrinology 75 Conference in London.[5]
(23) (a) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963); (b) P. G. Pietta and G. R. Marshall, *Chem. Commun.*, 650 (1970).
(24) J. Rivier, W. Vale, R. Burgus, N. Ling, M. Amoss, R. Blackwell, and R. Guillemin, *J. Med. Chem.*, **16**, 545 (1973).
(25) D. H. Coy, E. J. Coy, A. V. Schally, J. A. Vilchez-Martinez, L. Debeljuk, W. M. Carter, and A. Arimura, *Biochemistry*, **13**, 323 (1974).
(26) S. Sakakibara, Y. Shimonishi, M. Okada, and Y. Kishida in "Peptides", H. C. Beyerman, A. van der Linde, and W. M. van der Brink, Ed., North-Holland Publishing Co., Amsterdam, 1967, p 44.
(27) D. Yamashiro, *Nature (London)*, **201**, 76 (1964).
(28) J. Porath and P. Flodin, *Nature (London)*, **183**, 1657 (1959).
(29) W. Vale, G. Grant, M. Amoss, R. Blackwell, and R. Guillemin, *Endocrinology*, **91**, 562 (1972).
(30) W. Vale and G. Grant, *Methods Enzymol.*, **37**, 82 (1974).
(31) J. Rivier, M. Monahan, W. Vale, G. Grant, M. Amoss, R. Blackwell, R. Guillemin, and R. Burgus, *Chimia*, **26**, 300 (1972).
(32) J. Rivier, M. Amoss, C. Rivier, and W. Vale, *J. Med. Chem.*, **17**, 230 (1974).
(33) J. Rivier, M. Brown, and W. Vale, *Biochem. Biophys. Res. Commun.*, **65**, 746 (1975).
(34) M. Monahan and C. Gilon, *Biopolymers*, **12**, 2513 (1973).

6,11-Dihydro-11-oxodibenz[*b,e*]oxepinacetic Acids with Potent Antiinflammatory Activity

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A series of 6,11-dihydro-11-oxodibenz[*b,e*]oxepinacetic acids was synthesized and the antiinflammatory activity determined. Studies on 29 compounds revealed certain structure–activity relationships. In the carrageenan edema test, eight compounds exhibited higher antiinflammatory activities than did indomethacin. Several compounds (**2**, **9**, **14**, **22**, **25**) also proved to have activities superior or comparable to indomethacin in suppressing chronic as well as acute inflammation and carrageenan-induced hyperesthesia. Gastric irritation and lethality rates were less frequently observed with these compounds.

Among the various nonsteroidal antiinflammatory drugs, acetic acid derivatives of aromatic and heteroaromatic compounds[1] are reported to be particularly effective in suppressing inflammation. Shen[2] has proposed a most interesting hypothesis concerning the receptor site for 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid (indomethacin). Our own research in this field led us to synthesize tricyclic dibenzoxepinacetic acids since these acids in which two benzene rings lack coplanarity were considered to fit the receptor site as is the case with indomethacin. The structure–activity relationships of these derivatives are discussed herein and pharmacological properties of five selected compounds having considerable antiinflammatory activity are described. Recently, analogous studies on dibenzoxepin derivatives have been reported by Hoechst's researchers[3] independently of our work.[4]

Chemistry. Various dibenz[*b,e*]oxepin derivatives (III) were synthesized by the general route outlined in Scheme I and are listed in Table I. Intermediates, 2-carboxybenzyloxyphenylacetic acids (II), were obtained mainly by condensation of phthalides (I) with phenols (method D). Several compounds of type II were also prepared by the reaction of benzyl halides (IV) with phenols, followed by hydrolyses of the resulting benzyloxyphenylacetic acid derivatives (V) (methods F and E). Cyclization of II according to methods A–C described in the Experimental Section gave III. The physical properties of II and V are shown in Table II. Some new compounds were also prepared by the following methods. By esterification, **2**