Hepatic Infections in Female Golden Hamsters. A piece of ameba-laden absorbable sponge (about 5 × 5 × 1 mm) was inserted between the middle lobes of the livers of anesthetized hamsters during laparotomy. Untreated hamsters usually die from the resulting infection about 7 days after inoculation. Treatment was started on the day of inoculation as soon as the hamsters recovered from the surgical anesthetic. The test compounds were dissolved or suspended in 0.2% aqueous agar and administered once daily, by gavage, for 5 consecutive days. Effective regimens prevented mortality. Survival rates were corrected for nonspecific survival observed in untreated groups. An active dose was the lowest dose, expressed in mg/kg/day, which protected 50% or more of the hamsters so treated as evidenced by survival 14 days after inoculation. The results of the compounds appear in Table II, together with the effective dose of known effective drugs for comparison.

Acknowledgment. We wish to thank Mr. L. Brancone and staff for microanalyses and Mr. W. Fulmor and staff for spectral analyses.

References and Notes

(1) (a) R. Elson-Dew, *Adv. Parasitol.*, 1–62 (1968); (b) *WHO Tech. Rep. Ser.*, 421, 5–52 (1969); (c) S. J. Powell, *Bull. WHO*, 40, 953–956 (1969); (d) E. Barrett-Conner, *Calif. Med.*, 114, 1–6 (1971); (e) L. Goodman and A. Gilman, Ed., "Pharmacological Basis of Therapeutics", 5th ed, Macmillan, New York, N.Y., 1975, Chapter 54, p 1088; (f) I. M. Rollo in ref 1e, Chapter 53, pp 1069–1080.
(2) M. Rutia and P. Shubik, *J. Natl. Cancer Inst.*, 48, 721–729 (1972).
(3) P. Shubik, *Proc. Natl. Acad. Sci. U.S.A.*, 69, 1052–1055 (1972).
(4) U.S. Patents 3 133 078, 3 073 851, 3 184 482, 3 188 316, 3 182 053, and 3 135 755 (Hoffmann-La Roche).
(5) H. Bredereck, R. Gompper, K. Klemm, and H. Rempfer, *Chem. Ber.*, 92, 837 (1959).
(6) U.S. Patent 3 646 035-S (Searle).
(7) (a) F. Zumstein, Sr., E. Assmann, R. Koeingsberger, R. Holzbauer, and F. Zumstein, German Patent 2 202 942 (1971); (b) S. M. Gadekar, S. Nibi, B. D. Johnson, E. Cohen, and J. R. Cummings, *J. Med. Chem.*, 11, 453 (1968).
(8) Ciba Geigy, DT-2 202 942-Q.
(9) H. Meerwein, W. Florian, N. Schon, and G. Stopp, *Justus Liebigs Ann. Chem.*, 641, 1 (1961).
(10) J. D. Albright, E. Benz, A. E. Lanzilotti, and L. Goldman, *Chem. Commun.*, 413 (1965).

Branched-Chain Analogues of Luteinizing Hormone-Releasing Hormone

Janos Seprodi,[1] David H. Coy,* Jesus A. Vilchez-Martinez, Escipion Pedroza, and Andrew V. Schally

Department of Medicine, Tulane University School of Medicine, and Veterans Administration Hospital, New Orleans, Louisiana 70112. *Received September 23, 1977*

Benzoyl-, acetylsalicylyl-, indomethacinyl-, pyroglutamylhistidyl-, and pyroglutamyl-D-phenylalanyl-D-tryptophanylseryltyrosyl groups were attached to a moderately active inhibitory analogue of LH-RH, [D-Phe^2,D-Trp^3,D-Lys^6]-LH-RH, via the ϵ-amino group of the lysine residue. The resulting compounds were assayed for anti-LH-RH activity and for their ability to block ovulation in the rat. The decrease in polarity and increase in size of the lysine side chain resulting from addition of the aromatic acyl groups gave almost no increase in inhibitory activity. Addition of the dipeptide, <Glu-His, also had little effect on potency. However, incorporation of the pentapeptide sequence to give a branched pentadecapeptide with essentially two N termini resulted in antiovulatory activity greater than the parent peptide or any other analogue thus far tested by us. The corresponding agonist peptide, [N^{ϵ}-(<Glu-His-Trp-Ser-Tyr)-D-Lys^6]-LH-RH, was also synthesized and tested for LH- and FSH-releasing activity. Surprisingly, it was no more active than [D-Lys^6]-LH-RH itself, suggesting that an intact C terminus as well as an N terminus is necessary for the full expression of gonadotropin release.

The molecular factors responsible for the agonist or antagonist activities of LH-RH analogues are presently under intensive investigation, and the field has been the subject of a number of review articles.[2] Antagonist peptides have been made by the deletion of histidine[3] in position 2 of the decapeptide (Figure 1) or, better still, the substitution of D-phenylalanine in the same position.[4] The tryptophan residue in position 3 is essential for agonist, but not antagonist, activity. For instance, analogues containing L-phenylalanine or D-tryptophan in position 3 have decreased intrinsic LH-RH activities but still retain virtually full ability to compete for LH-RH receptors.[5,6]

Among the most interesting agonist peptides to have been discovered are those containing D-amino acids in place of glycine in position 6 of LH-RH.[7] The most potent peptides in this series are those containing aromatic D-amino acids[8] with in vivo gonadotropin-releasing activities 10–15 times higher than the natural hormone. Use has been made of this observation in the design of some very potent inhibitory analogues, such as [D-Phe^2,Phe^3,D-Phe^6]-LH-RH[5] and [D-Phe^2,D-Trp^3,D-Phe^6]-LH-RH,[6] which contain the position 2, 3, and 6 modifications within the same molecule. These compounds represent some of the most effective LH-RH antagonists presently known and are capable of blocking ovulation in the rat at doses of 4–6 mg/kg of body weight.

The reason for the increased biological activities of the D-amino acid[6] analogues is not wholly clear at present. It was originally proposed[7] that an improved receptor-binding conformation was responsible, a view which is supported by minimum free-energy calculations on LH-RH analogues.[9,10] Using this argument, one would assume that the D-aromatic amino acid[6] peptides are the most active due to improved conformations resulting from steric effects on the large side chains. It has also been found[11,12] that this type of analogue is more resistant than LH-RH to cleavage by tissue peptidases; however, this does not appear to offer an explanation for increased biological activity since several superactive LH-RH agonists with position 6 modifications have been found[13] not to exhibit prolonged plasma half-lives.

One approach for investigating structural requirements of the side chain of the position 6 D-amino acid, other than by the substitution of a wide array of naturally occurring and exotic amino acids, is to introduce various moieties by reaction with a suitable functional side group in this

0022-2623/78/1821-0276$01.00/0

Table I. Physicochemical Characteristics of LH-RH Analogues

Peptide	TLC systems A	B	C	D	$[\alpha]^{26}_D$, deg	Mol wt
I	0.2	0.45	0.4	0.7	−4.5 (*c* 0.66, 0.2 N AcOH)	1263.5
II	0.35		0.65	0.78	−11.1 (*c* 0.54, DMF)	1367.6
III	0.45	0.7	0.7	0.83	−23.3 (*c* 0.73, 0.2 N AcOH)	1425.6
IV	0.43	0.7	0.73	0.88	−11.3 (*c* 0.53, DMF)	1603.3
V	0.13	0.43	0.40	0.58		1512.1
VI	0.35	0.67	0.55	0.83	+7.1 (*c* 0.56, DMF)	1958.3
VII	0.1	0.35	0.3	0.55	−35.0 (*c* 0.75, 0.2 N AcOH)	1938.8

Table II. Amino Acid Analyses of LH-RH Analogues

Peptide	Ser	Glu	Pro	Gly	Leu	Tyr	Phe	His	Lys	NH_3	Trp	Arg
I	0.92	1.08	1.06	1.00	0.95	0.99	0.98		0.97	1.06	0.88	0.97
II	0.92	1.11	1.05	1.00	1.00	0.99	0.99		1.00			1.01
III	0.92	1.07	1.00	0.98	1.00	0.99	0.97		1.01		1.02	1.05
IV	0.95	1.08	1.09	1.00	0.97	1.00	0.99		0.98	1.01	0.91	0.99
V	0.99	2.21	1.06	1.00	1.03	1.01	1.02	0.98	1.06		0.99	1.04
VI	1.87	2.15	1.06	1.00	1.18	2.01	1.95		0.97	1.21	1.78	0.99
VII	1.84	2.14	1.08	1.00	1.08	1.97		1.85	0.95	1.25	1.73	1.00

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

1 2 3 4 5 6 7 8 9 10

Figure 1. Amino acid sequence of luteinizing hormone-releasing hormone.

position. Thus, LH-RH analogues containing D-lysine or D-ornithine[14] could be derivatized at their free amino side groups quite readily. Acylation with acetyl or lauryl groups generally resulted in slight losses in in vitro agonist activity of some analogues. However, the lauryl derivatives of certain antagonist peptides resulted in slight increases in inhibitory activities. The agonist activity of [D-Lys^6]-LH-RH attached to Dextran-2000 polymer decreased,[2b] but coupling this analogue to a water-soluble polyglutamic acid increased potency and duration of action.[15]

In the present study, we have extended this work by attaching a number of aromatic groups and some peptide chains to the inhibitory peptide [D-Phe^2,D-Trp^3,D-Lys^6]-LH-RH and the agonist peptide [D-Lys^6]-LH-RH and have examined the interesting effects on either antagonist or agonist activities.

Bioassays. All inhibitory analogues were tested for antiovulatory activity using adult female rats (SD strain, Charles River Co.) weighing about 200 g. They were kept in controlled light conditions (14 h of light and 10 h of dark), and their vaginal smears were checked daily starting 14 days after arrival. Only rats exhibiting at least two successive regular cycles (4 days) were used. On the afternoon of the proestrous day, the animals were injected sc with peptide dissolved in propylene glycol–saline solution and, on the following day, the animals were sacrificed and the oviducts inspected for ova.

The anti-LH-RH effects of the N^{ϵ}-benzoyl-, acetylsalicylyl-, indomethacinyl-, and <Glu-D-Phe-D-Trp-Ser-Tyr inhibitory peptides were screened over a 4-h period using an immature male rat system described previously.[5,16,17] The animals were injected sc with 300 μg of peptides in 20% propylene glycol–saline or with vehicle alone. Synthetic LH–RH (200 ng) or saline solution was injected at the same time or at different times thereafter. Blood was collected 30 min after LH-RH or saline injection and analyzed for LH and FSH content.

The single agonist peptide, [N^{ϵ}-(<Glu-His-Trp-Ser-Tyr)-D-Lys^6]-LH-RH, was assayed in a similar immature male rat system over a 4-h period against LH-RH and the superactive analogue, [D-Leu^6,des-Gly-NH_2^{10}]-LH-RH ethylamide.[18] Doses of 25 ng were injected sc and blood samples taken at intervals after administration.

In these experiments, LH was determined by the double antibody radioimmunoassay method of Niswender et al.[19] and FSH by the method of Daane and Parlow[20] using NIH-LH-S17 and NIAMD-rat-FSH-RP1 as standards.

Experimental Section

Unless otherwise stated, amino acids were of the L configuration. Amino acid derivatives were purchased from Bachem. Inc., Torrance, Calif.

Solid-Phase Synthesis. All amino acids were protected at their α-amino groups with the *tert*-butyloxycarbonyl group (Boc). Reactive side chains were protected as follows: Arg, tosyl; Lys, 2-chlorocarbobenzoxy; Ser, benzyl; Tyr, 2-bromocarbobenzoxy; His, Tos. The protected amino acids (3 mmol) were coupled in a Beckman Model 990 peptide synthesizer to a benzhydrylamine resin (1 mmol, 0.8 mmol/g capacity) in the presence of dicyclohexylcarbodiimide (3 mmol). The Boc protection was removed at each step by treatment with 33% TFA in CH_2Cl_2.

The completed peptides were cleaved from the resin and deprotected (45 min, 0 °C) with 10% anisole–HF and purified as described below. The homogeneity of final peptides was demonstrated by TLC on silica gel plates in the following solvent systems: A, 1-BuOH–AcOH–H_2O (4:1:5, upper phase); B, 2-PrOH–1 M AcOH (2:1); C, 1-BuOH–AcOH–H_2O–EtOAc (1:1:1:1); D, EtOAc–Pyr–AcOH–H_2O (5:5:1:3). Solvent fronts were allowed to travel 10–12 cm and spots were visualized with ninhydrin (all peptides negative apart from [D-Phe^2,D-Trp^3,D-Lys^6]-LH-RH) followed by Cl–starch–KI reagent. R_f values are given in Table I. Amino acid analyses were performed on a Beckman 119 amino acid analyzer on samples which were hydrolyzed (110 °C, 18 h) in 4 M methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole.[21] Results appear in Table II.

[D-Phe^2,D-Trp^3,D-Lys^6]-LH-RH (I). After its solid-phase synthesis and HF cleavage, peptide I was purified by gel filtration on a column (2.5 × 95 cm) of Sephadex G-25 (fine) in 0.2 M AcOH, followed by partition chromatography on Sephadex G-25 (fine) in the biphasic solvent system A (column, 2.5 × 95 cm). A major peak was obtained from elution volumes of 300–430 mL which yielded pure peptide I (35%).

Pentafluorophenyl Esters. The pentafluorophenyl esters used in the synthesis of the *N*-acyl derivatives of peptide I were freshly prepared from the corresponding aromatic carboxylic acids (1 equiv) and pentafluorophenol (1 equiv) in the presence of dicyclohexylcarbodiimide (1.1 equiv) in DMF (0 °C, 1 h). After removal of the precipitated urea and the DMF, the reaction mixture was dissolved in EtOAc, followed by removal of more urea. After removal of the solvent, the residual oil was crystallized from ethanol in 70–80% yields. The melting points of the esters of benzoic acid, acetylsalicylic acid, and indomethacin were 73–74, 94–95, and 133–134 °C, respectively. Satisfactory elemental analyses were obtained in each case. In general, acylation of I

Table III. Anti-LH-RH Activities of Analogues in Immature Male Rats[a]

Peptide	Time (h) after analogue injection	% anti-LH-releasing act.[b]	% anti-FSH-releasing act.[b]
I	1	36	37
	4	38	18
[D-Phe^2,D-Trp^3,-D-Phe^6]-LH-RH	1	84	65
	4	52	77
II	0.5	76	
	2	40	
	4	0	
III	1	57	23
	4	18	13
IV	1	7	0
	4	0	0
VI	1	62	43
	4	47	41

[a] Doses of peptides: 300 μg per rat. [b] Percent inhibition of serum gonadotropin at 30 min after administration of 200 ng of LH-RH.

by the active ester method gave far higher yields of cleaner products than by the alternative route employing dicyclohexylcarbodiimide coupling of carboxylic acid to the unprotected peptide.

[D-Phe^2,D-Trp^3,N^ϵ-benzoyl-D-Lys^6]-LH-RH (II). Freshly prepared benzoic acid pentafluorophenyl ester (0.05 mmol) and peptide I (0.05 mmol) were stirred in DMF (1 mL) for 4 h at room temperature. After evaporation of the solvent, the crude product was eluted on a column (2.5 × 95 cm) of Sephadex G-50 in 50% AcOH. The main peak at 420–490 mL gave homogeneous peptide II in 77% yield.

[D-Phe^2,D-Trp^3,N^ϵ-acetylsalicylyl-D-Lys^6]-LH-RH (III). Freshly prepared acetylsalicylic acid pentafluorophenyl ester (0.05 mmol) and peptide I (0.05 mmol) were stirred in DMF (1 mL) for 4 h at room temperature. The residual oil after evaporation was eluted on a column (2.5 × 95 cm) of Sephadex G-50 in 50% AcOH. Material from the major pek (410–460 mL) was chromatographed on a column of silica gel 60 (1.5 × 145 cm) using 1-BuOH–AcOH–H_2O (4:1:1) for elution. Fractions from 480 to 530 mL gave homogeneous peptide III in 55% yield.

[D-Phe^2,D-Trp^3,N^ϵ-indomethacinyl-D-Lys^6]-LH-RH (IV). Freshly prepared indomethacin pentafluorophenyl ester (0.05 mmol) and peptide I (0.05 mmol) were stirred in DMF (1 mL) for 4 h at room temperature. After evaporation of the solvent, the residual oil was eluted on a column (2.5 × 95 cm) of Sephadex G-50 in 50% AcOH. Material from the major peak (390–460 mL) was chromatographed on a column (1.5 × 145 cm) of silica gel 60 using 1-BuOH–AcOH–H_2O (4:1:1) for elution. Fractions from 400 to 580 mL contained homogeneous pale yellow peptide IV in 70% yield. The incorporation of indomethacin into the peptide was calculated photometrically by its characteristic absorbance at 323 nm. An 84% indomethacin content was found, which agrees well with a peptide content of 77% calculated from the amino acid analysis.

[D-Phe^2,D-Trp^3,N^ϵ-(<Glu-His)-D-Lys^6]-LH-RH (V). <Glu-His-$NHNH_2$[22] (0.15 mmol) was dissolved in 0.5 mL of DMF and HCl (0.45 mmol) and $NaNO_2$ (0.16 mmol) in concentrated aqueous solution were added at −10 °C. Five minutes later, a mixture of peptide I (0.15 mmol) and triethylamine (0.3 mmol) in DMF (0.5 mL) was added to neutral pH. The reaction mixture was stirred at −10 °C for 24 h, and, after removal of the solvent, the residual oil was eluted on a column (2.5 × 95 cm) of Sephadex G-25 in 0.2 M AcOH. Material from the major peak (260–280 mL) was chromatographed on a column (2.5 × 95 cm) of Sephadex G-25 using biphasic solvent system A (major peak at 230–350 mL), followed by elution on a column (1.5 × 145 cm) of silica gel 60 using 1-BuOH–AcOH–H_2O (4:1:1). Fractions from 980 to 1000 mL contained pure peptide V in 60% yield.

[D-Phe^2,D-Trp^3,N^ϵ-(<Glu-D-Ph-D-Trp-Ser-Tyr)-D-Lys^6]-LH-RH (VI). The branched-chain peptide VI was prepared by the usual solid-phase technique, except that bis-Boc-D-Lys[23] was incorporated in the 6 position. Simultaneous removal of the ε- and α-Boc groups allowed the remaining part of the peptide to be extended from two points. The HF-cleaved pentadecapeptide was eluted on Sephadex G-50 in 50% AcOH (column 2.5 × 95 cm), followed by chromatography on silica gel 60 (column 1.5 × 145 cm) in 1-BuOH–AcOH–H_2O (4:1:1). The major peak (220–320 mL) still contained a minor, faster moving component which was removed by chromatography on silica gel 60 (column 2.5 × 95 cm) using stepwise elution with 400 mL each of EtOAc–1-PrOH–AcOH–H_2O mixtures (16:4:1:2, 12:4:1:2, 45:20:6:11, and 30:20:6:11, respectively. Fractions from 1650 to 1900 mL gave homogeneous peptide VI (10%).

[N^ϵ-(<Glu-His-Trp-Ser-Tyr)-D-Lys^6]-LH-RH (VII). The pentadecapeptide VII was synthesized in a similar fashion to peptide VI and the HF-cleaved material was eluted on a column (2.5 × 95 cm) of Sephadex G-25 (fine) in 0.2 M AcOH. The main peak (570–640 mL) was purified by partition chromatography on Sephadex G-25 (fine) (column 2.5 × 95 cm) using the biphasic solvent system 1-BuOH–1-PrOH–AcOH–H_2O (7:1:2:10), and fractions from 440 to 600 mL gave homogeneous peptide VII (7%).

Results and Discussion

As expected from the relative agonist activities of the parent peptides, [D-Lys^6]-LH-RH (300%)[2b] and [D-Phe^6]-LH-RH (1000%),[8] [D-Phe^2,D-Trp^3,D-Lys^6]-LH-RH is a much inferior inhibitor to [D-Phe^2,D-Trp^3,D-Phe^6]-LH-RH both in the immature male rat (Table III) and blockade of ovulation (Table IV) assays. In the latter test, the D-Lys analogue exhibited no activity at a dose of 1 mg per rat. The introduction of large aromatic groups onto the ε-amino group of the D-Lys residue appeared to result in hardly any increase in anti-LH-RH activity in either assay system, clearly indicating that improved inhibitory or agonist properties are only achieved when bulky aromatic side chains are introduced adjacent to the asymmetric center of the D-amino acid. Apart from observing the effects of acylation on the antagonist activity of the D-Lys^6 peptide, we were interested in the indomethacin and aspirin conjugates for another reason. Both aspirin and indomethacin are inhibitors of prostaglandin biosynthesis,[24] and indomethacin has been shown[25] to inhibit steroid-induced LH release from the rat pituitary. The possibility existed that the attachment to the D-Lys^6 peptide might promote their transport to and concen-

Table IV. Antiovulatory Activities of LH-RH Analogues in Rats

Peptide	Dose, mg	Time	(No. of rats ovulating)/(total no. of rats)	% inhibn of ovulation	No. of ova in ovulating rats ± SE
Control	Vehicle	12 noon	10/10	0	11.31 ± 0.61
[D-Phe^2,D-Trp^3,-D-Phe^6]-LH-RH	1.0	12 noon	1/6	83	10.31 ± 0.41
	1.5	9 a.m.	2/6	67	11.25 ± 0.86
I	1.0	12 noon	7/7	0	11.28 ± 0.52
II	1.0	12 noon	8/9	11	8.0 ± 1.02
III	1.5	12 noon	8/9	11	11.37 ± 0.78
IV	1.5	12 noon	8/8	0	12.12 ± 0.85
V	1.0	12 noon	4/5	20	10.25 ± 1.31
VI	1.0	12 noon	3/11	73	5.66 ± 1.2
	1.5	9 a.m.	5/10	50	12.4 ± 0.68

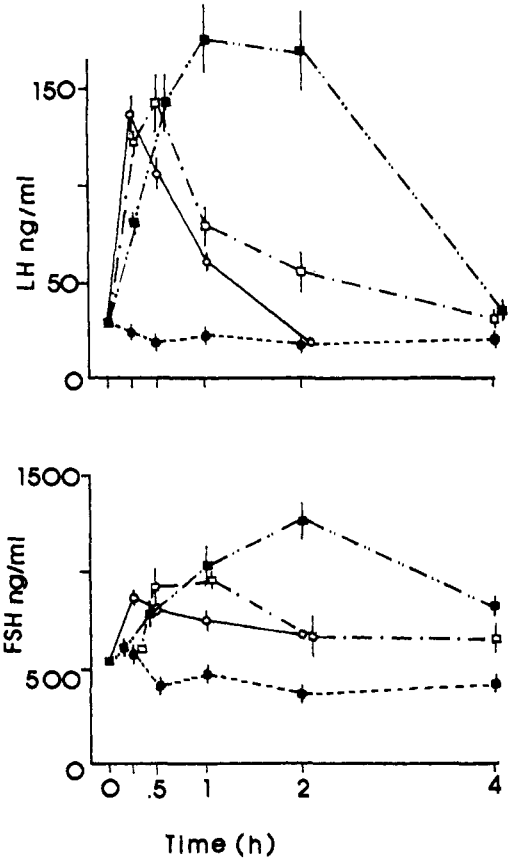


Figure 2. Serum LH and FSH concentrations at 0.25–4 h after sc injection to immature male rats with 50 ng of LH-RH (○—○), 25 ng of [D-Leu^6,des-Gly-$NH_2{}^{10}$]-LH-RH ethylamide (■–·–·–■) and [N^ϵ-(<Glu-His-Trp-Ser-Tyr)-D-Lys^6]-LH-RH (□–·–·–□), and saline (●– – – – – – –●).

tration directly at the site of action of LH-RH, thus perhaps blocking gonadotropin release which is known to be a cAMP-mediated process.[26] Since neither of the conjugated peptides had significantly improved inhibitory activities in either the immature male rat or the blockade of ovulation assays, it appears that this concept is not workable, one possible reason being a lack of cleavage of free aspirin or indomethacin from the receptor-bound peptides. In control experiments, small doses of aspirin or indomethacin alone also had no effect on LH-RH-induced gonadotropin release (Table IV).

In contrast to the simpler acylated analogues, the branched-chain peptide, [D-Phe^2,D-Trp^3,N^ϵ-(<Glu-D-Phe-Trp-Ser-Tyr)-D-Lys^6]-LH-RH, had far greater inhibitory activity than [D-Phe^2,D-Trp^3,D-Lys^6]-LH-RH in both assay systems. In immature male rats, its ability to antagonize LH-RH was essentially similar (Table IV) to [D-Phe^2,D-Trp^3,D-Phe^6]-LH-RH, the most effective inhibitory peptide to be tested by us prior to this work. Similarly, in the blockade of the ovulation test, the pentadecapeptide was equipotent with the D-Phe^6 antagonist. Since the branched peptide has a 50% higher molecular weight than the D-Phe^6 analogue, on a molar basis it is considerably more active. It is possible to attribute this increased activity to the presence of two N termini in one molecule which can interact with and block two receptor sites simultaneously. Prolonged activity due to greater biological stability might also cause greater inhibitory potency. However, this does not seem likely since the pentadecapeptide is no more active than [D-Phe^2,D-Trp^3,D-Phe^6]-LH-RH when injected at 9 a.m. rather than at 12 noon in the blockade of the ovulation test.

The corresponding agonist branched-chain peptide, [N^ϵ-(<Glu-His-Trp-Ser-Tyr)-D-Lys^6]-LH-RH, was also synthesized in the expectation that the presence of two "agonist-type" N termini would result in an increase in gonadotropin releasing activity over [D-Lys^6]-LH-RH. It was assayed (Figure 2) against LH-RH and, for comparison, [D-Leu^6,des-Gly-$NH_2{}^{10}$]-LH-RH ethylamide[18] over a 4-h period in immature male rats. LH- and FSH-releasing activity was considered proportional to the integrals of the corresponding time-release curves. In this experiment, the pentadecapeptide was only four times more active than LH-RH, possessing a similar potency to [D-Lys^6]-LH-RH[2b] itself. It showed very little tendency for the delayed peak response which is a characteristic of the superactive analogues of LH-RH, such as the ethylamide retested here. The low activity of the branched-chain agonist is hard to explain in view of the greatly increased effectiveness of the corresponding antagonist. However, these results suggest that both an intact N terminus and C terminus are necessary for triggering gonadotropin release whereas, for receptor site blocking, the N terminus alone is partially sufficient.

Peptide V, [D-Phe^2,D-Trp^3,N^ϵ-(<Glu-His)-D-Lys^6]-LH-RH, contains both an "inhibitory N terminus" and the N-terminal dipeptide sequence of LH-RH, <Glu-His. The dipeptide side chain appeared to exert little effect on either inhibitory or agonist activity, and it is assumed that the shortened chain is unable to interact with the receptors.

Acknowledgment. This research was supported by NICHD Contract 6-2841 and the Veterans Administration.

References and Notes

(1) Present address: First Institute of Biochemistry, Semmelweis University Medical School, Budapest, Hungary.
(2) (a) D. H. Coy, E. J. Coy, and A. V. Schally, *Res. Methods Neurochem.*, **3**, 393 (1975); (b) J. Rivier, M. Brown, C. Rivier, N. Ling, and W. Vale in "Peptides 1976", A. Loffet, Ed., Editions de l'Universite de Bruxelles, Brussels, 1977, p 427.
(3) W. Vale, G. Grant, J. Rivier, M. Monahan, M. Amoss, R. Blackwell, R. Burgus, and R. Guillemin, *Science*, **176**, 933 (1972).
(4) R. W. A. Rees, T. J. Foell, S. Chai, and N. Grant, *J. Med. Chem.*, **17**, 1015 (1974).
(5) A. de la Cruz, D. H. Coy, J. A. Vilchez-Martinez, A. Arimura, and A. V. Schally, *Science*, **191**, 195 (1976).
(6) D. H. Coy, J. A. Vilchez-Martinez, and A. V. Schally in "Peptides 1976", A. Loffet, Ed., Editions de l'Universite de Bruxelles, Brussels, 1977, p 660.
(7) M. Monahan, M. S. Amoss, H. A. Anderson, and W. Vale, *Biochemistry*, **12**, 4616 (1973).
(8) D. H. Coy, J. A. Vilchez-Martinez, E. J. Coy, and A. V. Schally, *J. Med. Chem.*, **19**, 423 (1976).
(9) F. A. Momany, *J. Am. Chem. Soc.*, **98**, 2990 (1976).
(10) F. A. Momany, *J. Am. Chem. Soc.*, **98**, 2996 (1976).
(11) Y. Koch, T. Baram, P. Chobsieng, and M. Fridkin, *Biochem. Biophys. Res. Commun.*, **61**, 95 (1974).
(12) Y. Koch, T. Baram, E. Hazum, and M. Fridkin, *Biochem. Biophys. Res. Commun.*, **74**, 488 (1977).
(13) J. J. Reeves, G. K. Tarnavsky, S. R. Becker, D. H. Coy, and A. V. Schally, *Endocrinology*, **101**, 540 (1977).
(14) N. Ling, J. Rivier, M. Monahan, and W. Vale, *J. Med. Chem.*, **19**, 937 (1976).
(15) M. S. Amoss, M. W. Monahan, and M. S. Verlander, *J. Clin. Endocrinol. Metab.*, **39**, 203 (1974).
(16) A. Corbin and C. W. Beattie, *Endocr. Res. Commun.*, **2**, 1 (1975).
(17) J. A. Vilchez-Martinez, A. V. Schally, D. H. Coy, E. J. Coy, C. M. Miller, and A. Arimura, *Endocrinology*, **96**, 1130 (1975).
(18) 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).

(19) G. D. Niswender, A. R. Midgeley, S. E. Monroe, and L. E. Reichert, *Proc. Soc. Exp. Biol. Med.*, **128**, 807 (1968).
(20) T. A. Daane and A. R. Parlow, *Endocrinology*, **88**, 653 (1971).
(21) S. Moore in "Chemistry and Biology of Peptides", J. Meienhoffer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 629.
(22) H. Immer, V. R. Nelson, C. Revesz, K. Sestanj, and M. Gotz, *J. Med. Chem.*, **17**, 1060 (1974).
(23) K. Hofmann, R. Schmiechen, R. D. Wells, Y. Wolman, and N. Yanaihara, *J. Am. Chem. Soc.*, **87**, 611 (1965).
(24) J. Vane, *Nature (London), New Biol.*, **231**, 232 (1971).
(25) J. C. Carlson, B. Barcikowski, V. Cargill, and J. A. McCracken, *J. Clin. Endocrinol. Metab.*, **39**, 399 (1974).
(26) P. Borgeat, F. Labrie, J. Cote, F. Ruel, A. V. Schally, D. H. Coy, E. J. Coy, and N. Yanaihara, *Mol. Cell. Endocrinol.*, 1, 7 (1974).

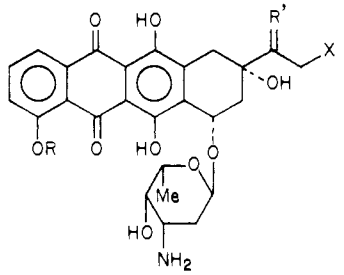
Adriamycin Analogues. 2. Synthesis of 13-Deoxyanthracyclines[1]

Thomas H. Smith,* Allan N. Fujiwara, and David W. Henry

Bio-Organic Chemistry Department, SRI International, Menlo Park, California 94025. *Received September 26, 1977*

The syntheses of several 13-deoxyanthracyclines are described. Koenigs–Knorr condensation of ε-rhodomycinone (**12**) with the protected daunosaminyl chloride **15** afforded **14** after deprotection. Efforts to decarbomethoxylate **12**, as well as attempts to selectively deoxygenate the 13 position of daunomycinone and adriamycinone, were unsuccessful as approaches to 13-deoxyanthracyclines. However, reaction of the readily available tosylhydrazones **4** and **5**, of daunorubicin and adriamycin with $NaCNBH_3$ in acidic MeOH, afforded the 13-deoxy analogues **6** and **7** in satisfactory yield. These compounds retained antitumor activity, being comparable to the parent compounds in both efficacy and potency in the P-388 mouse leukemia screen. The ε-rhodomycinone glycoside **14** was less active than **6** and **7**.

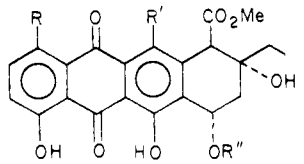
The anthracycline antibiotics, daunorubicin[2] (**1**),

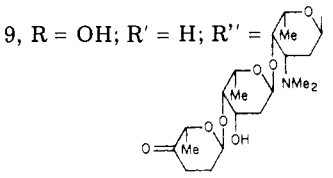


1, R = Me; R′ = O; X = H
2, R = Me; R′ = O; X = OH
3, R = X = H; R′ = O
4, R = Me; R′ = NNHTS; X = H
5, R = Me; R′ = NNHTS; X = OH
6, R = Me; R′ = H_2; X = H
7, R = Me; R′ = H_2; X = OH
8, R = Me; R′ = NNHCOPh; X = H

adriamycin[3] (**2**), and carminomycin[4] (**3**), show significant antineoplastic activity against a variety of experimental tumors with daunorubicin and especially adriamycin having clinical utility against human cancer. However, chemotherapy employing these agents is hampered by a number of undesirable side effects, the most serious being dose-related cardiotoxicity.[5] As part of our efforts to prepare related compounds having improved therapeutic properties, we now report the results of our studies of a number of synthetic approaches to 13-deoxyanthracyclines.

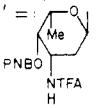
The antitumor agents **1**–**3** differ from the majority of the naturally occurring anthracyclines by the presence of the carbonyl function of C-13,[6] which is one of the principal sites of in vivo metabolism of **1** and **2**.[7] Lacking the carbonyl function at C-13, the 13-deoxy analogues might possibly display biological properties markedly different from the parent compounds and, in fact, the 13-deoxyanthracyclines, cinerubin A (**9**)[8] and aclacinomycins A and B (**10** and **11**),[9] have already demonstrated several interesting biological properties. Besides possessing potent antitumor activity, aclacinomycin A is reported to be substantially less cardiotoxic than adriamycin as determined by ECG monitoring of hamsters,[9] while cinerubin was found to possess low but reproducible activity against an adriamycin-resistant subline of P-388 leukemia.[10]





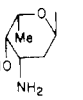
9, R = OH; R′ = H; R″ =

12, R = R″ = H; R′ = OH
13, R = H; R′ = OH; R″ =

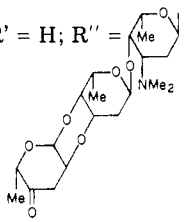


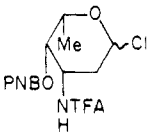
10, R = R′ = H; R″ = R″ in structure **9**

14, R = H; R′ = OH; R″ =



11, R = R′ = H; R″ =





15

Chemistry. ε-Rhodomycinone (**12**),[11] a fermentation by-product, was received as a gift from Bristol Laboratories. We hoped that this aglycon would afford a facile and economic route to 13-deoxyanthracyclines. The Koenigs–Knorr condensation of **12** with four 1 molar equiv portions of 1-chloro-3-*N*-trifluoroacetyl-4-*O*-*p*-nitrobenzoyldaunosamine (**15**)[12] afforded stereospecifically the α-glycoside **13**. The crude product was treated with aqueous NaOH in THF at 0 °C to effect removal of the sugar-protecting groups. The glycoside **14** could be separated from the now water-soluble sugar by-products of the coupling reaction by solvent extraction and was isolated as the HCl salt in 70% yield from **12**.

Attempts to use the aglycon **12** as a starting material for the synthesis of novel aglycons have been unsuccessful. As

0022-2623/78/1821-0280$01.00/0