[7] *J. Dunitz*, Acta crystallogr. *10*, 307 (1957); *V. Kupcik, S. Durovic*, Czechoslov. J. Physics *10*, 182 (1960).
[8] *L. L. Boyle*, Acta crystallogr. *A 28*, 172 (1972); Spectrochim. Acta *28A*, 1347 (1972); Spectrochim. Acta *28A*, 1355 (1972).
[9] *H. R. Stieger*, Dissertation Zürich 1973.
[10] *J. H. S. Green, D. J. Harrison*, Spectrochim. Acta *29A*, 293 (1973); *J. H. S. Green, D. J. Harrison, W. Kynaston, H. M. Paisley*, Spectrochim. Acta *26A*, 2139 (1970); *C. W. Frank, L. B. Rogers*, Inorg. Chemistry *5*, 615 (1966).
[11] *L. E. Brady*, J. chem. Educ. *46*, 301 (1969).

232. Synthesis of Some Structural Analogues of LH-RH Modified in Position 5, their *in vivo* and *in vitro* Gonadotropin-releasing Activity and Immunoreactivity[1])

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Summary. Four analogues of LH-RH modified in position 5 have been synthesized using either classical procedures or the solid phase method. The gonadotropin-releasing activities were determined by radioimmunological measurements of LH and FSH *in vivo* and *in vitro* and the immunoreactivities by a specific LH-RH radioimmunoassay (RIA). The following relative potencies and immunoreactivities were found:

	in vivo	*in vitro*	Immuno-reactivity
LH-RH:	100 %	100 %	100 %
[Tyr(Me)^5]-LH-RH:	8.6%	6.0%	40.0%
[Phe^5] -LH-RH:	42.5%	27.5%	17.0%
[Cha^5][2]) -LH-RH:	22.4%	27.8%	10.0%
[Gly^5] -LH-RH:	0.1%	0.5%	0.7%

Introduction. – The luteinizing hormone-releasing hormone (LH–RH) isolated from porcine [2] and ovine hypothalami [3] has been shown to be a decapeptide with the sequence pGlu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly–NH_2. This peptide causes the release of hypophysial luteinizing hormone (LH) and follicle-stimulating hormone (FSH) both *in vivo* and *in vitro*. Like many other polypeptide hormones, LH–RH has thus more than one distinct effect. Structural alterations may result in compounds with changed potencies and/or modified selectivity. In addition,

[1]) Part of this work was presented at the IX Acta Endocrinologica Congress, Oslo, 17th–21th June, 1973 [1].

structural changes could yield substances competing with endogenous LH–RH at the pituitary binding sites, acting thus as inhibitors of the release of either LH or FSH or both. Structure-activity studies in the field of oxytocin [4], lysine-vasopressin [5], bradykinin [6] and angiotensin [7] suggest that chemically reactive groups are involved in the interaction of a hormone with its receptor sites. Since modification of tyrosine in oxytocin had led to a partial inhibitor, we were interested in studying the effects of LH–RH analogues similarly altered. We therefore synthesized [Tyr(Me)^5]–LH–RH, [Phe^5]–LH–RH, [Cha^5]–LH–RH and [Gly^5]–LH–RH[2]).

[Gly^5]–LH–RH was prepared by solid phase synthesis according to the general procedure of *Merrifield* [8] [9] using dicyclohexylcarbodiimide (DCC) as coupling agent and the *t*-butoxycarbonyl (Boc) group as amino protecting group for all amino acids except pyroglutamic acid. The latter was introduced in the form of its pentachlorophenylester. The side chain functional group of serine was protected by the benzyl group, that of histidine by the dinitrophenyl group and that of arginine by the nitro group. The protected decapeptide was cleaved from the resin by transesterification [10]. Subsequent treatment with liquid HF [11], followed by ammonolysis and purification, yielded pure [Gly^5]–LH–RH.

The synthesis of [Phe^5]–LH–RH was carried out by condensation of the central tetrapeptide Boc–Ser(Bzl)–Phe–Gly–Leu–OH with the C-terminal tripeptide H–Arg(NO_2)–Pro–Gly–NH_2 [12–15] by means of DCC. The resulting heptapeptide was treated with trifluoroacetic acid and coupled to the N-terminal tripeptide pGlu–His–Trp–OH [16] by means of DCC. The central tetrapeptide was prepared by stepwise elongation of H–Gly–Leu–OMe [17] with Boc–Phe–OH [18] and then with Boc–Ser(Bzl)–OH [19] using the DCC method.

[Tyr(Me)^5]–LH–RH was prepared according to the same procedure using Boc–Tyr(Me)–OH instead of Boc–Phe–OH. The protecting groups of both resulting decapeptidamides were removed by liquid HF.

The synthesis of [Cha^5]–LH–RH was carried out by coupling the N-terminal tripeptide pGlu–His–Trp–OH to the central tripeptide H–Ser(But)–Cha–Gly–OEt by means of DCC. The resulting hexapeptidester was transformed into its hydrazide and coupled *via* azide to the C-terminal tetrapeptide H–Leu–Arg(Tos)–Pro–Gly–NH_2 [20] [21]. To obtain the central tripeptide, Z–Cha–OH was coupled with H–Gly–OEt to the corresponding dipeptide, which, after hydrogenation, was elongated with Z–Ser(But)–OH to the fully protected tripeptidester. Hydrogenation yielded H–Ser(But)–Cha–Gly–OEt. The protecting groups of the decapeptidamide were removed by treatment with HF.

The *in vivo* gonadotropin-releasing activities of the analogues were determined in ovariectomized, estradiol-3-benzoate, progesterone-treated rats (O.E.P.-rats) [22]. The concentration changes of serum gonadotropins were measured by radioimmunoassay (RIA). The activities of the analogues were compared with those of LH–RH.

In addition, the gonadotropin-releasing activities of the synthetic peptides were tested by an *in vitro* method [23] using pituitary halves of O.E.P.-rats. LH and FSH were determined in the incubation media by means of RIA. Since all peptides

[2]) Abbreviations were used according to [37]; Cha = cyclohexyl-L-alanine.

showed the same ratio of LH to FSH release as LH–RH, only LH-releasing activities are reported in this paper.

The ability of the peptides to inhibit the binding of iodine labelled LH–RH to a specific antiserum (immunoreactivity) was tested by means of our LH–RH RIA [24].

All these activities of the synthetic analogues are summarized in Table 1.

Table 1. In vivo *and* in vitro *LH-releasing activity and immunoreactivity of some LH-RH analogues modified in position 5*

compound	LH-releasing activity			immuno-reactivity [b] (LH-RH RIA) (%)
	our results (%)		results of others (%)	
	in vivo [a]	*in vitro* [a]		
LH-RH	100	100	100	100
[Tyr(Me)^5]-LH-RH	8.6 (4.4–15.8) 3 [c]	6.0 (0.08–11.9) 2 [c]	24 (17–32) [25] 35 [26]	40.0
[Phe^5] -LH-RH	42.5 (23.9–57) 2 [c]	27.5 (1.4–53.6) 3 [c]	44.3 (19.1–97) [27] 64 (38–108) [28]	17.0
[Cha^5] -LH-RH	22.4 (5.6–48.7) 3 [c]	28.8 (8.4–47.2) 4 [c]		10.0
[Gly^5] -LH-RH	0.1 [d]	0.5 [d]	0.1 [29]	0.7

[a] Relative potencies with 95% confidence limits.
[b] Activities expressed as percentage of response obtained by synthetic LH-RH.
[c] Number of independent experimental series.
[d] Estimation of the releasing activity; (no parallelism of the dose-response curves).

Results and Discussion. – Since the completion of this work, the syntheses of [Tyr(Me)^5]–LH–RH [25] [26], of [Phe^5]–LH–RH [27] [28] and of [Gly^5]–LH–RH [29] have been reported. The relative potencies of these analogues are compared with our results in Table 1. There is no significant difference between our *in vivo* and *in vitro* results and those of other groups with the exception of [Tyr(Me)^5]–LH–RH.

Although the results obtained by LH–RH RIA are of the same order of magnitude, the immunoreactivities differ somewhat from the releasing activities of the analogues. For example, the introduction of a methyl group in the tyrosyl residue seems to influence the ability of this analogue to compete with LH–RH for the antibody binding sites only to a small extend, while it affects its releasing activity considerably. The situation is reversed with [Cha^5]–LH–RH, which has a rather low immunoreactivity but still about 25% of the releasing activity of synthetic LH–RH.

Chang et al. [30] have proposed that the releasing activity of LH–RH and its analogues is related to the development of a charge-transfer interaction between the tyrosine side chain and the tryptophan indole-moiety. The fact that both analogues with a modified aromatic side chain in the 5-position exhibit considerable activity, whereas [Gly^5]–LH–RH shows very little, is consistent with this concept. However, the distinct releasing activity of [Cha^5]–LH–RH, the non-aromatic side chain of

which cannot participate in a charge-transfer interaction, contradicts this hypothesis. This charge-transfer interaction thus seems to be of no essential importance for the exhibition of releasing activity.

Our analogues show neither a different ratio of LH/FSH release from the parent molecule nor do they act as release inhibitors. The position 5 in LH–RH seems to be of some importance for both releasing and immunological activity. Replacement of the tyrosyl residue by another aromatic amino acid (Phe) or an amino acid with a saturated cyclic side chain (Cha) moderately reduces both activities, whereas the replacement by a glycyl residue results in an almost complete loss of releasing and immunological activity.

Experimental Part

***In vivo* assay.** – The substances, dissolved in physiological saline, were injected under light ether anaesthesia into the carotid artery of O.E.P.-rats. The animals (10 per group) were decapitated 15 min. (LH) or 40 min. (FSH) thereafter, the blood collected from the trunk, clotted at room temperature and centrifuged. The sera were kept deep-frozen until assayed. The hormone content of each individual serum was determined by appropriate RIA performed with the material and according to the instructions from the NIAMDD. Tests were run as 4- and occasionally 6-point-assays and relative potencies of the analogues were determined by the method of *Bliss* [31].

***In vitro* assay.** – The gonadotrophin-releasing activity was assayed using pituitary halves (4 per flask) of O.E.P.-rats, incubated for 4 hours in a *Krebs-Ringer*-hydrogen carbonate/glucose buffer as previously described [23]. LH and FSH were determined in the incubation media by rat LH [32] and FSH [33] RIA. The hypophysial response to the analogues was examined in 2 to 4 independent experimental series (Table 1) at least at 3 dose levels (1, 10, 100 ng/ml of incubation medium) and compared with the response to synthetic LH-RH. The relative potencies were calculated by a 4-point-assay [31].

LH-RH RIA. – The ability of the peptides to inhibit the binding of ^{131}I- or ^{125}I-labelled LH-RH to a specific antiserum was tested by RIA [24]. The antiserum was generated in guinea pigs after treatment with Al_2O_3-adsorbed LH-RH [34].

Peptide syntheses. – Melting points were determined on a *Büchi* apparatus and are uncorrected. Optical rotation was determined with a polarimeter *Perkin Elmer* 141. All free peptides, isolated by lyophilization from diluted acetic acid, contained variable amounts of water. They were used directly for optical rotation measurements. Amino acid analysis was carried out according to *Spackman et al.* [35] and *Lin et al.* [36] on a *Beckman* Unichrom apparatus. The values for Glu were taken as 1.00. All amino acids used were of L-configuration. Thin layer chromatography (TLC.) was performed on precoated silicagel and cellulose plates (*Merck*) in various systems.

Under 'usual work up' of the peptide intermediates we understand the following: filtering the reaction mixture, evaporating the filtrate, dissolving the remaining residue in ethylacetate, extracting this solution with saturated $NaHCO_3$- or 10% Na_2CO_3-solution, with 1N hydrochloric acid or 5% $KHSO_4$- or 10% citric acid-solutions depending on the protecting groups present, extracting finally with water, followed by drying over Na_2SO_4 and evaporation.

The following abbreviations are used: MeOH for methanol, EtOH for ethanol, DMF for N,N-dimethylformamide, THF for tetrahydrofuran, DCC for N,N'-dicyclohexylcarbodiimide, DCHA for dicyclohexylamine, Cha for cyclohexylalanine, pGlu for pyroglutamic acid, -OPhCl$_5$ for pentachlorophenylester. All other abbreviations are according to [37].

[Gly^5]-LH-RH. – Chloromethylated *Merrifield* resin (2% crosslinked, 0.9 mM Cl/g) was esterified [8] [9] with Boc-Gly-OH to yield a resin with 0.31 mM of Gly per gram of resin. To 4 g of this resin were subsequently coupled using a 5-fold excess: Boc-Pro-OH, Boc-Arg(NO_2)-OH, Boc-Leu-OH, 2 × Boc-Gly-OH, Boc-Ser(Bzl)-OH, Boc-Trp-OH, Boc-His(N^{im}-Dnp)-OH and pGlu-OPhCl$_5$. All coupling reactions were carried out with a 5-fold excess of DCC for 3 h according to the procedure described in [20], except the coupling of pGlu-OPhCl$_5$, which was allowed to react directly for 16 h.

The completed decapeptide resin (5.03 g) was submitted to transesterification at room temperature in 200 ml of triethylamine/MeOH 1:10 for 3 days to yield 0.92 g of protected decapeptidemethylester. This product was treated with 20 ml of liquid HF at 0° for 1 h in the presence of 2 ml of anisole. After the removal of HF and extraction of the anisole by ethylacetate, the residue was submitted directly to ammonolysis in abs. methanol (250 ml) saturated with anhydrous ammonia. After 48 h at room temperature the reaction mixture was evaporated to dryness to yield crude decapeptide, which was purified in a first step on a column of Sephadex G-25 (5 × 80 cm) using 0.2M acetic acid as eluent. The material (250 mg) obtained from this purification was further purified on a column of Amberlite CG-50 (0.8 × 9 cm) using an exponential gradient of acetic acid (H_2O – 1.0M AcOH) to provide a product (60 mg) which was finally purified by partition chromatography on Sephadex G-25 (1.5 × 30 cm) in the system butanol/acetic acid/water 4:1:5. After evaporation of the corresponding fractions and repeated lyophilization from diluted acetic acid and water, pure [Gly^5]-LH-RH was obtained (31 mg).

The product was pure and homogeneous as judged by thin layer chromatography and gave the following amino acid analysis: $Glu_{1.00}$ $His_{0.96}$ $Trp_{1.08}$ $Ser_{0.85}$ $Gly_{2.88}$ $Leu_{0.96}$ $Arg_{1.05}$ $Pro_{1.01}$.

$C_{48}H_{69}N_{17}O_{12} \cdot 2\,C_2H_4O_2 \cdot 5\,H_2O$	Calc.	C 48.55	H 6.81	N 18.51%
(1286,37)	Found	,, 48.62	,, 6.74	,, 18.72%

[Tyr(Me)^5]-LH-RH. – 1. *Boc-Tyr(Me)-OH* was prepared according to the general procedure for Boc-Tyr(Bzl)-OH of *Schnabel* [18] with H-Tyr(Me)-OH [38] [39] as starting material. The derivative was obtained in 64.8% yield after recrystallization from ether/cyclohexane. M.p. 61–63° (dec.), $[\alpha]_D^{25} = +29.7°$ ($c = 1$, EtOH).

$C_{15}H_{21}NO_5$ (295.33) Calc. C 61.00 H 7.17 N 4.74% Found C 61.08 H 7.31 N 4.52%

2. *Boc-Tyr(Me)-Gly-Leu-OMe.* Z-Gly-Leu-OMe [17] (3.5 g) was deprotected with 15% HBr in acetic acid at room temperature for 30 min. to provide after precipitation with ether 2.8 g of HBr · H-Gly-Leu-OMe [40], which was dissolved in DMF and neutralized with N-methylmorpholine (pH 8). The coupling with Boc-Tyr(Me)-OH (3.0 g) and DCC (2.1 g) was carried out at 4° for 20 h. After the usual work up, the crude product was crystallized from ether/petroleum ether to yield pure Boc-Tyr(Me)-Gly-Leu-OMe (3.4 g). M.p. 98–100°, $[\alpha]_D^{25} = -20.7°$ ($c = 1$, EtOH).

$C_{24}H_{37}N_3O_7$ (479.57) Calc. C 60.11 H 7.78 N 8.76% Found C 59.82 H 7.21 N 8.70%

3. *Boc-Ser(Bzl)-Tyr(Me)-Gly-Leu-OMe.* Boc-Tyr(Me)-Gly-Leu-OMe (3.0 g) was deprotected with trifluoroacetic acid at room temperature for 30 min. After repeated evaporation from CH_2Cl_2 the product was dissolved in CH_2Cl_2 and the pH adjusted to 8 with N-methylmorpholine. The coupling with Boc-Ser(Bzl)-OH (1.85 g) and DCC (1.4 g) was carried out at 4° for 20 h. After the usual work up, the product was crystallized from ethylacetate to provide pure Boc-Ser(Bzl)-Tyr(Me)-Gly-Leu-OMe (2.7 g). M.p. 150–151°, $[\alpha]_D^{25} = -22.3°$ ($c = 1$, EtOH).

$C_{34}H_{48}N_4O_9$ (656.78) Calc. C 62.18 H 7.37 N 8.53% Found C 62.19 H 7.42 N 8.44%

4. *Boc-Ser(Bzl)-Tyr(Me)-Gly-Leu-Arg(NO_2)-Pro-Gly-NH_2.* Boc-Ser(Bzl)-Tyr(Me)-Gly-Leu-OMe (1.65 g) was saponified at room temperature for 1 h with 3 ml of 1N KOH in a mixture of dioxane/water 5:1. Upon neutralization with HCl and evaporation to dryness, the residue was taken up in a mixture of CH_2Cl_2/MeOH 3:1. The insoluble material was separated by filtration, the filtrate evaporated and the resulting Boc-Ser(Bzl)-Tyr(Me)-Gly-Leu-OH directly used for the coupling reaction.

The deprotection of Z-Arg(NO_2)-Pro-Gly-NH_2 (1.3 g) [12–15] was performed with 15% HBr in acetic acid at room temperature for 15 min. Upon precipitation with ether the crude HBr · H-Arg(NO_2)-Pro-Gly-NH_2 was reprecipitated several times from MeOH/ether. This product was dissolved in DMF, the pH adjusted to 8 with N-methylmorpholine, and coupled with Boc-Ser(Bzl)-Tyr(Me)-Gly-Leu-OH and DCC (0.5 g) at 4° for 60 h. After the usual work up, the obtained crude product was purified on a column of silicagel using a gradient of CH_2Cl_2/EtOH as eluent (CH_2Cl_2/EtOH 95:5 – CH_2Cl_2/EtOH 80:20). Crystallization of the main fractions from a mixture of ether/EtOH 10:1 yielded pure Boc-Ser(Bzl)-Tyr(Me)-Gly-Leu-Arg(NO_2)-Pro-Gly-NH_2 (1.1 g). M.p. 155–160° (dec.), $[\alpha]_D^{25} = -27.3°$ ($c = 1$, EtOH).

$C_{46}H_{68}N_{12}O_{13}$ (997.12) Calc. C 55.41 H 6.87 N 16.85% Found C 55.86 H 6.89 N 16.52%

5. *pGlu-His-Trp-Ser-Tyr(Me)-Gly-Leu-Arg-Pro-Gly-NH$_2$.* Boc-Ser(Bzl)-Tyr(Me)-Gly-Leu-Arg(NO$_2$)-Pro-Gly-NH$_2$ (1.0 g) was deprotected with 50% trifluoroacetic acid in CH_2Cl_2 at room temperature for 30 min. After evaporation to dryness, the oily residue was redissolved in CH_2Cl_2 and evaporated again several times. The resulting product was dissolved in DMF, the pH adjusted to 8 with N-methylmorpholine, and coupled with pGlu-His-Trp-OH (0.45 g) [16] and DCC (0.2 g) at 4°. After 48 h the mixture was filtered and the filtrate evaporated to dryness to provide an oily residue (2.8 g), which was treated for 1 h at 0° with 40 ml of liquid HF in the presence of 5 ml of anisole. Upon evaporation of the HF, the residual oil was taken up in water and extracted with ethylacetate. The aqueous layer was evaporated and the resulting solid purified on a column of Sephadex G-25 (5 × 85 cm) using 0.2M acetic acid as eluent. The material (0.8 g) obtained was further purified on a column of carboxymethyl-cellulose (2.5 × 22 cm) using a linear gradient of ammonium acetate (0.02M – 0.3M) as eluent. Subsequent partition chromatography on Sephadex G-25 (3 × 35 cm) in the system butanol/ethanol/pyridine/0.2M acetic acid 4:1:1:7 provided pure pGlu-His-Trp-Ser-Tyr(Me)-Gly-Leu-Arg-Pro-Gly-NH$_2$ (42 mg).

The product was homogeneous as judged by TLC., and by dansylation [41] we could not detect any O-dansyl-tyrosine, indicating that the methylether of the tyrosine side chain was still intact.

$Glu_{1.00}$ $His_{0.85}$ $Trp_{0.95}$ $Ser_{0.95}$ $Tyr(Me)_{0.85}$ $Gly_{1.99}$ $Leu_{0.99}$ $Arg_{0.90}$ $Pro_{0.97}$. $[\alpha]_D^{25} = -44.9°$ (c = 0,5, 1% acetic acid).

$C_{56}H_{77}N_{17}O_{13} \cdot 4\ C_2H_4O_2 \cdot 6\ H_2O$	Calc.	C 49.76	H 6.85	N 15.41%
(1544.65)	Found	,, 49.25	,, 6.35	,, 15.45%

[Phe^5]-LH-RH. – 6. *Boc-Phe-Gly-Leu-OMe.* Z-Gly-Leu-OMe (5 g) was deprotected by hydrogenation with 5% Pd/C in methanol/water in the presence of a few drops of acetic acid. The obtained product (3 g) was dissolved in CH_2Cl_2, neutralized with N-methylmorpholine and coupled with Boc-Phe-OH (5.2 g) and DCC (3.1 g) at 4° for 48 h. After the usual work up Boc-Phe-Gly-Leu-OMe crystallized from benzene/petroleum ether (3.5 g). M.p. 119–121°.

$C_{23}H_{35}N_3O_6$ (449.56) Calc. C 61.45 H 7.84 N 9.34% Found C 61.77 H 8.00 N 9.40%

7. *Boc-Ser(Bzl)-Phe-Gly-Leu-OMe.* Boc-Phe-Gly-Leu-OMe (3.3 g) was deprotected with trifluoroacetic acid at room temperature for 30 min. After evaporation of the reaction mixture, the residue was dissolved in ethylacetate and extracted with 1M citric acid. The combined aqueous acidic fractions were neutralized with $NaHCO_3$ and reextracted with ethylacetate to provide H-Phe-Gly-Leu-OMe (2.4 g) which was dissolved in CH_2Cl_2. Upon adjusting the pH to 8 with N-methylmorpholine, coupling was performed with Boc-Ser(Bzl)-OH (2.1 g) and DCC (1.5 g) at 4° for 48 h. After the usual work up the crude product was crystallized from benzene (3.6 g). M.p. 124–125°.

$C_{33}H_{46}N_4O_8$ (626.75) Calc. C 63.24 H 7.40 N 8.94% Found C 63.48 H 7.50 N 8.72%

8. *Boc-Ser(Bzl)-Phe-Gly-Leu-Arg(NO$_2$)-Pro-Gly-NH$_2$.* Boc-Ser(Bzl)-Phe-Gly-Leu-OMe (1.25 g) was saponified as described in 4.) to afford the tetrapeptide acid (1.1 g), which was directly used for the coupling reaction with H-Arg(NO$_2$)-Pro-Gly-NH$_2$ (1.3 g) and DCC (0.41 g) in DMF at 4° (4.). After two days, the reaction mixture was filtered and the filtrate evaporated to dryness. The obtained residue was taken up in water and extracted with ethylacetate. The ethylacetate layer provided crude product (1.7 g) which was purified on a column (4.2 × 20 cm) of silicagel using $CHCl_3$/MeOH 10:1 as eluent. Crystallization of the main fractions from CH_2Cl_2/petroleum ether yielded pure Boc-Ser(Bzl)-Phe-Gly-Leu-Arg(NO$_2$)-Pro-Gly-NH$_2$ (0.7 g). M.p. 135–140°.

$C_{45}H_{66}N_{12}O_{12}$ (967.10) Calc. C 55.89 H 6.88 N 17.38% Found C 55.86 H 6.84 N 17.14%

9. *pGlu-His-Trp-Ser-Phe-Gly-Leu-Arg-Pro-Gly-NH$_2$.* Boc-Ser(Bzl)-Phe-Gly-Leu-Arg(NO$_2$)-Pro-Gly-NH$_2$ (0.4 g) was deprotected as described in 5.). The resulting product was dissolved in DMF, neutralized with N-methylmorpholine and coupled with pGlu-His-Trp-OH (0.19 g) and DCC (0.1 g) at 4° for 48 h. The resulting mixture was filtered and evaporated to dryness to provide an oily residue which was treated during 1 h at 0° with 40 ml of liquid HF in the presence of 4 ml of anisole. Upon removal of the HF and the anisole, the product was purified on Sephadex G-25 as outlined in 5.). Further purification was achieved by partition chromatography on Sephadex G-25 (3 × 95 cm) in the system butanol/acetic acid/water 4:1:5. The obtained pGlu-His-Trp-Ser-Phe-Gly-Leu-Arg-Pro-Gly-NH$_2$ (15 mg) was pure and homogeneous as judged by thin layer

chromatography and gave the following amino acid analysis: $Glu_{1.00}$ $His_{0.85}$ $Trp_{1.21}$ $Ser_{0.97}$ $Phe_{1.01}$ $Gly_{2.14}$ $Leu_{0.91}$ $Arg_{0.92}$ $Pro_{1.01}$. $[\alpha]_D^{25} = -40.5°$ ($c = 0.5$, 1% acetic acid).

[Cha⁵]-LH-RH. – 10. *Z-Cha-OH · DCHA.* Cyclohexylalanine hydrochloride (Cha · HCl) [42] was carbobenzoxylated by the general procedure of *Bergmann & Zervas* [43] and the resulting oily Z-Cha-OH was isolated as its dicyclohexylammonium salt in 88% yield after cristallization from ether. M.p. 157°, $[\alpha]_D^{25} = -5.4°$ ($c = 1$, MeOH).

$C_{29}H_{46}N_2O_4$ (486.70) Calc. C 71.57 H 9.53 N 5.76% Found C 71.25 H 9.54 N 5.70%

11. *Z-Cha-Gly-OEt.* Z-Cha-OH (4.6 g) (liberated from Z-Cha-OH · DCHA (7.3 g) by means of 1N sulfuric acid) was dissolved in DMF and activated at –20° for 1 min. by the addition of N-methylmorpholine (1.68 ml) and isobutylchloroformate (1.98 ml) before a precooled suspension of HCl · H-Gly-OEt (2.1 g) and N-methylmorpholine (1.68 ml) in DMF was added. The reaction mixture was stirred for 15 min. at –20° and 1 h at room temperature, and worked up in the usual way. The product was crystallized from ethylacetate/hexane to yield pure Z-Cha-Gly-OEt (5.3 g). M.p. 113–114°, $[\alpha]_D^{25} = -20.5°$ ($c = 1$, MeOH).

$C_{21}H_{30}N_2O_5$ (390.48) Calc. C 64.59 H 7.74 N 7.17% Found C 64.54 H 7.83 N 7.34%

12. *Z-Ser(But)-Cha-Gly-OEt.* Z-Ser(But)-OH (2.95 g) was dissolved in THF and activated at –20° for 1 min. by the addition of N-methylmorpholine (1.12 ml) and isobutylchloroformate (1.32 ml), before a precooled solution of AcOH · H-Cha-Gly-OEt – prepared from Z-Cha-Gly-OEt (3.9 g) by hydrogenation in methanol in the presence of Pd/C and an equivalent of acetic acid – and N-methylmorpholine (1.12 ml) in THF was added. After being stirred for 15 min. at –20° and 1 h at room temperature the reaction mixture was worked up as usual, and the protected tripeptidester (3.6 g) crystallized from ethylacetate/hexane. M.p. 96–97°, $[\alpha]_D^{25} = -24.8°$ ($c = 1$, MeOH).

$C_{28}H_{43}N_3O_7$ (533.67) Calc. C 63.02 H 8.12 N 7.87% Found C 62.68 H 8.19 N 7.97%

13. *pGlu-His-Trp-Ser(But)-Cha-Gly-OEt.* pGlu-His-Trp-OH [16] (110 mg) was coupled to H-Ser(But)-Cha-Gly-OEt – prepared by hydrogenation of Z-Ser(But)-Cha-Gly-OEt (130 mg) in methanol in the presence of Pd/C – in DMF at 0° by means of N-hydroxybenzotriazole (34 mg) and DCC (52 mg). The reaction mixture was stirred for 30 min. at 0° and for 4 h at room temperature, kept for 15 h at 4° and filtered. The filtrate was evaporated, the residue dissolved in methanol and chromatographed on a Sephadex LH-20 column (5 × 200 cm) in methanol. After evaporation of the main fractions, the compound crystallized from EtOH (114 mg). The crystalline material contained 1 mol of EtOH. M.p. 236–237°, $[\alpha]_D^{25} = -16.0°$ ($c = 0.5$, MeOH).

$C_{42}H_{59}N_9O_9 \cdot C_2H_5OH$ (880.05) Calc. C 60.05 H 7.44 N 14.32%
Found ,, 59.74 ,, 7.25 ,, 14.31%

14. *pGlu-His-Trp-Ser(But)-Cha-Gly-N_2H_3.* pGlu-His-Trp-Ser(But)-Cha-Gly-OEt (500 mg) was dissolved in ethanol and reacted with hydrazine hydrate for 3 days at room temperature. The hydrazide was precipitated with ether and cristallized from 2-propanol. Yield 225 mg. M.p. 168–173°, $[\alpha]_D^{25} = -7.2°$, ($c = 0.5$, MeOH).

$C_{40}H_{57}N_{11}O_8$ (819.96) Calc. C 58.59 H 7.01 N 18.79% Found C 58.94 H 6.97 N 18.50%

15. *pGlu-His-Trp-Ser-Cha-Gly-Leu-Arg-Pro-Gly-NH_2.* A solution of pGlu-His-Trp-Ser(But)-Cha-Gly-N_2H_3 (180 mg), 2N HCl/THF (0.7 ml) and isoamylnitrite (0.15 ml) in DMF was stirred for 30 min. at –20° before N-methylmorpholine (0.155 ml) and a precooled solution of H-Leu-Arg(Tos)-Pro-Gly-NH_2 – prepared from Z-Leu-Arg(Tos)-Pro-Gly-NH_2 [20] [21] (218 mg) by hydrogenation in methanol in the presence of Pd/C – in DMF was added. The reaction mixture was kept at –20° for 30 min. and for 15 h at 4°. It was evaporated and the residue was chromatographed on a Sephadex LH-20 column (5 × 200 cm) in methanol. The main fractions were evaporated to yield the protected decapeptide as amorphous powder (110 mg). This material (100 mg) was treated with 20 ml liquid HF at 20° for 30 min. in the presence of 1 ml of anisole. After removal of the HF and anisole, the crude material was purified by partition chromatography on a Sephadex G-25 column (1.5 × 80 cm) in the solvent system butanol/benzene/0.2M ammonium

acetate buffer (pH 5.5) 100:4:100 to yield homogeneous [Cha^5]-LH-RH (35 mg) as judged by thin layer chromatography. $Glu_{1.00}$ $His_{0.94}$ $Trp_{1.04}$ $Ser_{0.81}$ $Cha_{0.97}$ $Gly_{1.91}$ $Leu_{0.94}$ $Arg_{1.01}$ $Pro_{0.97}$. $[\alpha]_D^{25} = -56.6°$ ($c = 0.3$, 1M acetic acid).

$C_{55}H_{81}N_{17}O_{12} \cdot 4\ C_2H_4O_2 \cdot 5.5\ H_2O$	Calc.	C 50.06	H 7.20	N 15.75	H_2O 6.55%
(1511.65)	Found	,, 50.16	,, 7.20	,, 15.83	,, 6.38%

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REFERENCES

[1] *B. Kerdelhué, M. Jutisz, R. O. Studer, D. Gillessen & H. Künzi*, Acta Endocr. (Kbh) Suppl. *177*, 197 (1973).
[2] *H. Matsuo, Y. Baba, R. M. G. Nair, A. Arimura & A. V. Schally*, Biochem. biophys. Res. Commun. *43*, 1334 (1971).
[3] *R. Burgus, M. Butcher, M. Amoss, N. Ling, M. Monahan, J. Rivier, R. Fellows, R. Blackwell, W. Vale & R. Guillemin*, Proc. Nat. Acad. Sci. (US) *69*, 278 (1972).
[4] *J. Rudinger, V. Pliška & I. Krejčí*, 'Recent Progress in Hormone Research', E. B. Astwood ed., Vol. *28*, 131, Academic Press, New York, 1972.
[5] *M. Zaoral, E. Kasafírek, J. Rudinger & F. Šorm*, Coll. Czechoslov. chem. Commun. *30*, 1869 (1965).
[6] *J. M. Stewart & D. W. Wolley*, 'Hypotensive Peptides' E. G. Erdös, N. Back & F. Sicuteri eds., p. 21, Springer, Berlin, 1966.
[7] *G. R. Marshall, W. Vine & P. Needleman*, Proc. Nat. Acad. Sci. (US) *67*, 1624 (1970).
[8] *R. B. Merrifield*, J. Amer. chem. Soc. *85*, 2149 (1963), ibid. *86*, 304 (1964).
[9] *J. M. Stewart & J. D. Young*, 'Solid Phase Peptide Synthesis' W. H. Freeman, San Francisco, 1969.
[10] *H. C. Beyerman, H. Hindriks & E. W. B. de Leer*, Chem. Commun. *1968*, 1668.
[11] *S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada & H. Sugihara*, Bull. chem. Soc. Japan *40*, 2164 (1967).
[12] *N. Yanaihara, C. Yanaihara, M. Sakagami, K. Tsuji, T. Hashimoto, T. Kaneko, H. Oka, A. V. Schally, A. Arimura & T. W. Redding*, J. med. Chemistry *16*, 373 (1973).
[13] *G. R. Flouret, W. H. Arnold, J. W. Cole, R. L. Morgan, W. White, H. T. Hedlund & R. H. Rippel*, J. med. Chemistry *16*, 369 (1973).
[14] *S. Bajusz, A. Turan & I. Fauszt*, 'Chemistry and Biology of Peptides' J. Meienhofer ed. p. 325, Ann Arbor Science Publishers, Ann Arbor, 1972.
[15] *M. Fujino, T. Fukuda, S. Kobayashi & M. Obayashi*, Chem. pharm. Bull. *21*, 87 (1973).
[16] *H. Sievertsson, J. K. Chang, A. v. Klaudy, C. Bogentoft, B. Currie, K. Folkers & C. Y. Bowers*, J. med. Chemistry *15*, 222 (1972).
[17] *E. Bricas, J. van Heigenoort, M. Barber, W. A. Wolstenholme, B. C. Das & E. Lederer*, Biochemistry *4*, 2254 (1965).
[18] *E. Schnabel*, Ann. Chem. *702*, 188 (1967).
[19] *E. Wünsch & A. Zwick*, Chem. Ber. *97*, 2497 (1964).
[20] *D. Gillessen, H. Künzi, A. Trzeciak, G. Roncari & R. O. Studer*, 'Hypothalamic Hormones, Structure, Synthesis and Biological Activity', D. Gupta and W. Voelter eds., in press, Verlag Chemie, Weinheim, 1974.
[21] *Y. Okada, K. Kitamura, Y. Baba, A. Arimura & A. V. Schally*, Biochem. biophys. Res. Commun. *53*, 1180 (1973).
[22] *V. D. Ramirez & S. M. McCann*, Endocrinology *73*, 193 (1963).
[23] *M. Jutisz, B. Kerdelhué & A. Bérault*, 'The Human Testis', E. Rosemberg and C. A. Paulsen eds., p. 221, Plenum Press, New York, 1970.
[24] *B. Kerdelhué, M. Jutisz, D. Gillessen & R. O. Studer*, Biochim. biophys. Acta *297*, 540 (1973).
[25] *D. H. Coy, E. J. Coy & A. V. Schally*, J. med. Chemistry *16*, 827 (1973).
[26] *G. Grant, W. Vale & J. Rivier*, Biochem. biophys. Res. Commun. *50*, 771 (1973).

[27] *N. Yanaihara, T. Hashimoto, C. Yanaihara, K. Tsuji, Y. Kenmochi, F. Ashizawa, T. Kaneko, H. Oka, S. Saito, A. Arimura & A. V. Schally,* Biochem. biophys. Res. Commun. *52*, 64 (1973).
[28] *D. H. Coy, E. J. Coy & A. V. Schally,* J. med. Chemistry *16*, 83 (1973).
[29] *M. Monahan, J. Rivier, W. Vale, N. Ling, G. Grant, M. Amoss, R. Guillemin, R. Burgus, E. Nicolaides & M. Rebstock,* 'Chemistry and Biology of Peptides', J. Meienhofer ed., p. 601, Ann Arbor, Science Publishers, Ann Arbor, 1972.
[30] *J. Chang, R. H. Williams, A. J. Humphries, N. G. Johansson & K. Folkers,* Biochem. biophys. Res. Commun. *47*, 727 (1972).
[31] *C. I. Bliss,* 'The Statistics of Bioassay', Academic Press Inc., New York, 1952.
[32] *B. Kerdelhué, A. Bérault, C. Courte & M. Jutisz,* C. R. hebd. Seances Acad. Sci. (Paris) Ser. D. *269*, 2413 (1969).
[33] *B. Kerdelhué, G. Kann & M. Jutisz,* 'Hormones Glycoprotéiques' Colloque INSERM, M. Jutisz ed., p. 177, published by INSERM, Paris, 1972.
[34] *B. Kerdelhué & M. Jutisz,* Abstr. IVth Int. Congr. Endocrinol. Washington D.C., Excerpta Medica I.C.S. *256*, 141 (1972), (Abstr. No. 352).
[35] *D. H. Spackman, W. H. Stein & S. Moore,* Analyt. Chemistry *30*, 1190 (1958).
[36] *T. Y. Lin & Y. H. Chang,* J. biol. Chemistry *246*, 2842 (1971).
[37] IUPAC-IUB Commission, Biochemistry *5*, 2485 (1966); ibid. *6*, 362 (1967).
[38] *P. Karrer, M. Gisler, E. Horlacher, F. Locher, W. Mäder & H. Thomann,* Helv. *5*, 482 (1922).
[39] *W. Siedel, K. Sturm & R. Geiger,* Chem. Ber. *96*, 1436 (1963).
[40] *E. Kliegler & H. Gibian,* Liebigs Ann. Chem. *649*, 183 (1961).
[41] *W. R. Gray,* 'Methods in Enzymology', C.H.W. Hirs ed., Vol. XI, 139, Academic Press, New York, 1967.
[42] *E. Waser & E. Brauchli,* Helv. *7*, 740 (1924).
[43] *M. Bergmann & L. Zervas,* Chem. Ber. *65*, 1192 (1932).

233. Dicationic Diolefinic Complexes of Palladium(II) and Platinum(II)

Preliminary communication

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(12. IX. 74)

Uncharged diolefinic complexes M(diolefin)Cl_2 (**1**; M = Pt(II), Pd(II)) have been obtained by *Chatt et al.* in 1957 [1]. The coordinated diolefins are susceptible of nucleophilic attack by alcohols, amines, etc. [1–4]. In view of obtaining complexes in which the double bond is prone to be attacked by weaker nucleophiles and to give thermally 'forbidden' [2+2]-cycloadditions, we have prepared the dicationic complexes [M(diolefin)$(CH_3CN)_2$]$(PF_6)_2$ (**2**; diolefin = cycloocta-1,5-diene, 2,5-norbornadiene) and [Pd(cod)L]$(PF_6)_2$ (**3**; L = 2,2'-dipyridyl, bis(1,2-diphenylphosphine)-ethane).

The white complexes **2** are obtained in the solid state by adding a stoichiometric amount of [Ag$(CH_3CN)_2$]PF_6 to a dichloromethane solution of **1** at $-15°$, extracting **2** from AgCl with acetone and adding acetonitrile then dichloromethane. These complexes are stable when stored at $-15°$. IR.-spectra show that acetonitrile is coordinated (ν(CN) = 2328–2340 cm^{-1}, compared to 2266 for free CH_3CN and 2347