Luftoxidation von $Fe(OH)_2$ im alkalischen Medium bei 20 °C, auch bei Zusatz von Al^{3+}

Nr.	Mol. Verh. $FeSO_4$: NaOH	Atom-verh. $Fe^{2+}:Al^{3+}$	Die Oxidationsprodukte								
			Farbe	% Fe_2O_3	% FeO	% Al_2O_3	% SO_3	% H_2O	Ferromagnetismus direkt	bei 300°	Röntgenbefund
1	1:3	1:0	gelbbraun	83,5	0,0	–	0,05	16,4	0	0	α-FeOOH
2	1:3	1:0,02	braun	82,4	0,2	0,8	0,03	16,6	schwach		α-FeOOH + einige schwache Magnetitlinien
3	1:3	1:0,2	dunkel-braun	75,7	1,9	7,5	0,03	14,9	deut-lich	deut-lich	α-FeOOH + Magnetit

eine Zeitlang vor der Oxidation schützt, bis dieses zur Bildung des Magnetits verbraucht wird, das übrigens kaum luftempfindlich ist. Die betreffenden Präparate waren daher FeO-haltig, ferromagnetisch und zeigten das Magnetitgitter. Bei saurer und stöchiometrischer Fällung des $Fe(OH)_2$ hatte das Al^{3+} keinen Einfluss auf die Qualität der Oxidationsprodukte.

Zwecks Ausführung der Versuche löst man 6 g $FeSO_4 \cdot 7H_2O$ in 100 cm^3 destilliertem Wasser und versetzt dieses bei 20 °C mit 1 *N* NaOH in einem Mol.-Verhältnis $FeSO_4$:NaOH = 1:3. Al^{3+}-Ionen verwendet man in Form von $Al(NO_3)_3 \cdot 9H_2O$ in einem bestimmten Atomverhältnis $Fe^{2+}:Al^{3+}$. Nach Auffüllung mit destilliertem Wasser auf 200 cm^3 behandelt man das Reaktionsgemisch 3 Std. mit einem Luftstrom (1,6 l/min) bei 20 °C. Die sorgfältig ausgewaschenen und luftgetrockneten Oxidationsprodukte wurden auf ihre Zusammensetzung geprüft, worüber die Tabelle Auskunft gibt.

Summary. It has been proved that $Fe(OH)_2$, precipitated in alkaline solution and oxidated by air-oxygen, transforms to α-FeOOH. In presence of Al^{3+}, besides α-FeOOH magnetite also appears.

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Structure and Activity of Corticotrophic Peptides: Synthesis and Biological Activity of Two Corticotrophic Peptides with Neutral Amino Acids in Positions 17 and 18

Positions 17 and 18 in the amino acid sequence[1] of ACTH are of considerable importance, since appreciable stimulation of the adrenal cortex only occurs if the chain length exceeds 16 amino acids, starting from the amino end of the molecule[2–5]. In the natural sequence these positions are occupied by two arginine residues, which are known to be strongly basic (Table I). If these are replaced, in synthetic ACTH fragments, by the less basic ornithine or lysine residues, corticotrophic activity is retained[6,7]. It is even intensified if serine in position 1 is in the D-form instead of the L-form[8–13].

In order to assess the influence of a further decrease in basicity in positions 17 and 18 with regard to biological activity, we synthesized the two nonadecapeptides Xa and Xb. They are substituted in both positions 17 and 18 by the neutral amino acids norvaline (Xa) and norleucine (Xb) and have D-serine in position 1.

Norvaline has the same alkyl side chain as arginine and ornithine, and norleucine the same alkyl side chain as lysine, both being devoid of the respective basic groups. Moreover, the length of the norleucine side chain is equal to that of the ornithine side chain (including the basic group).

Synthesis. The synthesis was carried out in the same way for both the a and b series of the compounds (numbers refer to formulae in Table II): I was reacted with hydrazine hydrate to produce the hydrazide II, which was condensed with proline amide to III by the method of HONZL and RUDINGER[14]. Catalytic hydrogenation of the benzyloxycarbonyl group led to compound IV, which was coupled with Z-Lys(Boc)-Pro-Val-Gly-Lys(Boc)-Lys(Boc)-N_3 (prepared in situ from the corresponding hydrazide V[10] by HONZL and RUDINGER's method[14]), forming the protected, crystalline nonapeptide amides VI. Removal of the benzyloxycarbonyl group, again by catalytic hydrogenation, gave VII, and condensation with Boc-D-Ser-Tyr-Ser-Met-Glu(OBut)-His-Phe-Arg-Trp-Gly-OH VIII[12] by the carbodiimide method yielded IX. These fully protected nonadecapeptide amides IXa and IXb were purified by countercurrent-distribution. Then all protecting groups were split off with trifluoroacetic acid. Finally, the resultant trifluoroacetates were

Table I. β-Corticotrophin-(1-24)-tetracosipeptide (Synacthen®)[16]

H-Ser	Tyr	Ser	Met	Glu	His	Phe	Arg	Trp	Gly	Lys	Pro	Val	Gly	Lys	Lys	Arg	Arg	Pro	Val	Lys	Val	Tyr	Pro-OH
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

Table II

No.		Chemical formula[1]	Mp.	$[\alpha]_D^{20}$ concentration solvent	Thin-layer chromatography Rf on silica gel	Elemental formula	Elemental analysis 1. found, 2. calc. C	H	N
I	a)	Z-Nva-Nva-OCH_3	124–125°	—30° 2.0% EtOH	0.65 (Cy/E 1:1), 0.76 (T/A 1:1)	$C_{19}H_{28}O_5N_2$	62.62	7.74	7.69
						(364.4)	62.46	7.60	7.83
	b)	Z-Nle-Nle-OCH_3	97–99°	—23° 1.9% EtOH	0.72 (Cy/E 1:1), 0.78 (T/A 1:1)	$C_{21}H_{32}O_5N_2$	64.26	8.22	7.14
						(392.5)	64.61	8.28	7.24
II	a)	Z-Nva-Nva-$NHNH_2$	207–208°	—9° 2.0% DMF	0.41 (C/M 9:1), 0.48 (T/A 1:1)	$C_{18}H_{28}O_4N_4$	59.32	7.74	15.37
						(364.4)	59.41	7.71	15.29
	b)	Z-Nle-Nle-$NHNH_2$	182–183°	—8° 2.0% DMF	0.46 (C/M 9:1), 0.55 (T/A 1:1)	$C_{20}H_{32}O_4N_4$	61.20	8.22	14.28
						(392.5)	61.27	8.05	14.31
III	a)	Z-Nva-Nva-Pro-NH_2	156–159°	—38° 2.1% DMF	0.38 (C/M 9:1), 0.17 (T/A 1:1)	$C_{23}H_{34}O_5N_4$	61.86	7.68	12.55
						(446.5)	61.59	7.54	12.70
	b)	Z-Nle-Nle-Pro-NH_2	157–159°	—35° 2.2% DMF	0.46 (C/M 9:1), 0.25 (T/A 1:1)	$C_{25}H_{38}O_5N_4$	63.27	8.07	11.81
						(474.6)	63.21	7.97	11.74
IV	a)	H-Nva-Nva-Pro-NH_2-tosylate	105–112° dec.	—50° 1.9% MeOH	0.38 (C/M 1:1), 0.58 (121 A)				
	b)	H-Nle-Nle-Pro-NH_2	104–105°	—47° 2.2% DMF	0.36 (102 A), 0.66 (121 A)	$C_{17}H_{32}O_3N_4$	59.97	9.48	16.46
						(340.5)	59.93	9.33	16.47
VI	a)	Z-Lys(Boc)-Pro-Val-Gly-Lys	(147°)	—75°	0.25 (C/M 9:1), 0.74 (43 A)	$C_{68}H_{113}O_{17}N_{13}$	58.98	8.23	13.15
		(Boc)-Lys(Boc)-Nva-Nva-Pro-NH_2	188–191°	1.5% MeOH	0.77 (102 A)	(1384.7)	58.69	8.33	12.91
	b)	Z-Lys(Boc)-Pro-Val-Gly-Lys	(139°)		0.29 (C/M 9:1), 0.77 (43 A)				
		(Boc)-Lys(Boc)-Nle-Nle-Pro-NH_2	164–170°		0.80 (102 A)				
VII	a)	H-Lys(Boc)-Pro-Val-Gly-Lys	(158°)	—72°	0.53 (C/M 1:1), 0.38 (102 A)	$C_{60}H_{107}O_{15}N_{13}\cdot H_2O$	56.81	8.66	14.35
		(Boc)-Lys(Boc)-Nva-Nva-Pro-NH_2	171–175° dec.	1.9% MeOH	0.44 (52 A)	(1268.61)	57.09	8.54	14.33
	b)	H-Lys(Boc)-Pro-Val-Gly-Lys	(141°)	—71°	0.71 (C/M 1:1), 0.45 (102 A)	$C_{62}H_{111}O_{15}N_{13}\cdot H_2O$	57.43	8.78	14.04
		(Boc)-Lys(Boc)-Nle-Nle-Pro-NH_2	165–169° dec.	1.9% MeOH	0.37 (52), 0.49 (52 A)	(1296.66)	57.49	8.82	13.85
		countercurrent distribution:							
IX	a)	K = 0.55 (20°)			0.77 (121), 0.29 (102 A)				
		system: MeOH-buffer*-$CHCl_3$-CCl_4 10 + 3 + 7 + 4			0.75 (37)				
	b)	K = 0.04 (20°)			0.78 (121), 0.35 (102 A)				
		system: MeOH-buffer*-$CHCl_3$ 3 + 2 + 4			0.76 (37)				
		electrophoretic mobilities on cellulose plates:			Rf on aluminium oxide:	UV in 0.1-*N* NaOH:			
X	a)**	200 V 1.5 h pH = 1.9 : 8.2 cm			0.58 (121), 0.45 (121 A)	λ_{max} = 282 nm (ε = 6050)			
		pH = 4.75 : 5.2 cm			0.39 (111 B)	290 nm (ε = 5900)			
	b)**	200 V 1.5 h pH = 1.9 : 8.2 cm			0.63 (121), 0.50 (121 A)	λ_{max} = 283 nm (ε = 7500)			
		pH = 4.75 : 5.2 cm			0.41 (111 B)	290 nm (ε = 7350)			

$$\underset{1}{R_1}\text{-}\underset{2}{D}\text{-}\underset{3}{Ser}\text{-}\underset{4}{Tyr}\text{-}\underset{5}{Ser}\text{-}\underset{6}{Met}\text{-}\underset{7}{Glu}\text{-}\underset{8}{His}\text{-}\underset{9}{Phe}\text{-}\underset{10}{Arg}\text{-}\underset{11}{Trp}\text{-}\underset{12}{Gly}\text{-}\underset{13}{Lys}\text{-}\underset{14}{Pro}\text{-}\underset{15}{Val}\text{-}\underset{16}{Gly}\text{-}\underset{17}{Lys}\text{-}\underset{18}{Lys}\text{—}X\text{—}X\text{—}\underset{19}{Pro}\text{-}NH_2$$

(OR_2 at 5; $H_2^{\oplus}$ at 8; R_1 at 11; R_1 R_1 at 15 16)

IX: R_1 = Boc, R_2 = But = protected
X: $R_1 = R_2$ = H = free

[D-Ser[1], X[17,18]]-β-corticotrophin-(1-19)-nonadecapeptide amide

a: X = Nva
b: X = Nle

Abbreviations: A, acetone; C, chloroform; Cy, cyclohexane; E, ethylacetate; M, methanol; T, toluene, solvents for thin-layer chromatography v/v:37 = *n*-butanol-pyridine-water 46 + 31 + 33, 43 A = *t*-amylalcohol-isopropanol-water 67 + 26 + 7, 52 = *n*-butanol-acetic acid-water 75 + 7.5 + 21, 52 A = do 67 + 10 + 23, 102 A = ethylacetate-methyl-ethyl-ketone-formic acid-water 50 + 30 + 10 + 10, 121 = isopropanol-ammonia conc.-water 70 + 10 + 20, 121 A = do 85 + 5 + 10, 111 B = *n*-butanol-pyridine-conc. ammonia-water 40 + 24 + 6 + 30.

* Buffer: 19.3 g ammonium acetate and 28.6 ml acetic acid in 1 l water.

** Xa and Xb after total hydrolysis (6-*N* HCl, 24 h, 110°) gave the expected amino acid ratios on a Beckman Unichrome Amino Acid Analyzer.

converted to the acetate salts and biologically assayed in the form of their lyophilizates.

Biological activity. Steroidogenesis in the adrenal cortex of male, hypophysectomized (24 h before injection) rats was taken as the criterion of biological activity. In accordance with the in vivo method described in detail by DESAULLES and RITTEL[6] the time-dependant rise in the plasma corticosterone concentration after subcutaneous administration was determined.

The new compounds Xa and Xb were compared with the following synthetic corticotrophic peptides[1]:

XI: [D-Ser[1]]-β-corticotrophin-(1-16)-hexadecapeptide amide[15]
XII: [D-Ser[1]]-β-corticotrophin-(1-19)-nonadecapeptide[15]
XIII: β-Corticotrophin-(1-24)-tetracosipeptide (Synacthen®)

Results. The reference hexadecapeptide amide XI restores the plasma corticosterone concentrations to normal (normal value: 19.4 ± 4.6 µg/100 ml at 08.00 h in intact, male rats; mean of 48 determinations) within half an hour of the injection. Subsequently the concentration declines relatively rapidly. After 1.5 h there is no longer any perceptible response. The duration of action therefore is distinctly shorter than that of peptide XIII (Synacthen)[16]. At the same time, the plasma corticosterone concentration after 1 h is significantly smaller.

The norvaline nonadecapeptide amide Xa induces a rise in the plasma corticosterone concentration to about half the normal level (after 30 min: 10.5 µg/100 ml), the duration of effect is 1.5 h (the values after 1.5 h being only slightly above the limits of accuracy). The norleucine substituted nonadecapeptide amide Xb elicits a response rather similar to that evoked by Xa, but its duration of action is much shorter. Both compounds Xa and Xb display less activity with regard to plasma corticosterone concentration than the hexadecapeptide amide XI, and only Xa has the same duration of action[17].

The reference nonadecapeptide XII acts over a period of 2 h, and raises the plasma corticosterone concentration to between 20 and 35 µg/100 ml in the first 1.5 h. Its duration of action is thus about twice that of the hexadecapeptide amide XI.

Discussion. HOFMANN et al.[18] have shown that the N-terminal hexadecapeptide amide with L-serine in position 1 is virtually devoid of adrenocortical activity[18]. Substitution with D-serine, resulting in compound XI, yields a corticotrophic peptide with a distinct, though weak, hormonal activity. Elongation of the peptide chain from 16 to 19 amino acids with the natural sequence Arg[17]-Arg[18]-Pro[19] (compound XII) increases the activity considerably (see Table III)[19]. The introduction of the neutral amino acids norvaline and norleucine in positions 17 and 18 thus leads to a considerable decrease in activity. Hence, to obtain potent corticotrophic activity not only a peptide chain of 17 or more amino acids, but also side chains exhibiting basic character seem to be necessary.

This finding lends further support to the apparant positive correlation between the net positive charge associated with peptide portion 15 to 18 on the one hand and its corticotrophic potency on the other (LI and OELOFSEN[20]). The fact that both new peptides Xa and Xb are even less potent than the hexadecapeptide amide XI (whose structure they completely incorporate) shows that the added sequences, Nva-Nva-Pro and Nle-Nle-Pro respectively, abolish some of the original activity of the 1–16 core. In terms of hormone-receptor affinity, this could be explained by a suppressed binding capacity of the hormone to a negative site of the receptor due to the added neutral moieties.

Table III. Rise in plasma corticosterone concentrations (µg/100 ml) in relation to time after a single, s.c. dose of 0.3 mg/kg

Substance	n[a]	Time (h) ½	1	1½	2	4
XI	12	22.1 ± 3.7[b]	8.5 ± 2.8	< 3	< 3	–
Xa	9	10.5 ± 1.8	7.3 ± 2.5	4.7 ± 1.7	< 3	–
Xb	9	12.4 ± 3.8	< 3	< 3	< 3	–
XII	6	27.2 ± 2.6	34.2 ± 4.1	23.4 ± 4.7	17.4 ± 7.1	< 3
XIII	18	30.3 ± 2.5	30.2 ± 3.7	14.3 ± 2.9	< 3	< 3

Base value: < 3 µg/100 ml. [a] n, number of animals. [b] Standard deviation of the mean. The peptides are listed in increasing order of chain length.

Zusammenfassung. [D-Ser[1], Nva[17,18]]-β-Corticotropin-(1–19)-nonadecapeptidamid Xa und [D-Ser[1], Nle[17,18]]-β-Corticotropin-(1–19)-nonadecapeptidamid Xb wurden synthetisiert und ihre biologischen Aktivitäten diskutiert.

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