201. Synthesis and Biological Properties of Enkephalin-like Peptides Containing Adamantylalanine in Position 4 and 5[1])

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Summary

The syntheses of seven [D-ala^2]-enkephalin analogues, H-Tyr-ala-Gly-X-Y, with X-Y = Phe-Ada-OH, Phe-ada-OH, Phe-Ada-NH_2, Phe-ada-NH_2, Ada-Leu-OH, Ada-Leu-NH_2, and Ada-Ada-NH_2 are described. The compounds with Y = Ada (= L-Ada) or ada (= D-Ada) show pronounced morphine-like activities in certain bioassays, whereas those with X = Ada were almost inactive. The analogues were used to explore the influence of steric, electronic, and hydrophobic properties of the side chains of X and Y on opioid activity (see [14]).

1. Introduction. – The observation that the replacement of phenylalanine by the *pseudoaromatic* 'fat' amino acid [3] *o*-carboranylalanine (Car) in a chymotrypsin inhibitor [4] and in [Leu^5]-enkephalin [5] enhances the respective affinities to the enzyme and to opiate receptors prompted us to investigate the effect of the *aliphatic* 'fat' amino acid adamantylalanine (Ada, which is practically isosteric with Car [6]) on the biologic activity of enkephalins.

For this purpose, the seven analogues of [ala^2, Leu^5]-enkephalin [7], H-Tyr-ala-Gly-X-Y, shown in *Table 1* (**1b–7b**)[1]) were synthesized. In the peptides **1b–4b**, and **7b**, adamantylalanine or adamantylalanine amide in either the D or the L configuration was used to replace leucine (Y). In peptides **5b** and **6b**, however, only Ada[1]) was chosen as substitute for Phe (X), because it is known that, in this position, D-residues of Phe and Trp abolish activity [8].

2. Syntheses. – The synthetic scheme was similar to that employed for the preparation of [ala^2, Car^4]-enkephalin analogues [9]: The tripeptide Boc-Tyr-ala-Gly-OH [9] [10] was condensed with the appropriate dipeptide derivatives, H-X-Y-OMe and H-X-Y-NH_2, having free α-amino groups and possessing structures corresponding to those of **1–7** *(Table 1)*. The fully protected dipeptides, Boc-X-Y-OMe and Boc-X-Y-NH_2 (**1**, **3–7**, and Boc-Phe-ada-OMe, corresponding to **2**) were prepared from

[1]) Abbreviations are according to the IUPAC-IUB Commission on Biochemical Nomenclature [1] and *Houben-Weyl* [2] or are specified in the text; Ada = L-adamantylalanyl, ada = D-adamantylalanyl; ala = D-alanyl.

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Boc-Phe-OH and Boc-Ada-OH [6] as *N*-terminal components, and H-Ada-OME, H-ada-OMe, H-Ada-NH_2 [6], H-ada-NH_2 [6], H-Leu-OMe, or H-Leu-NH_2 [2] as *C*-terminal components. All condensations were effected with *N,N'*-dicyclohexylcarbodiimide [11] and 1-hydroxy-benzotriazole [12]. The *t*-butoxycarbonyl groups were removed with 0.1 N HCl in formic acid [13]. All products were chromatographically pure (TLC., *Table 1* and exper. part). The high Rf-values and the fact that the pentapeptides can be analyzed and purified by high pressure liquid chromatography on silica gel with chloroform methanol are indicative of the relatively strong hydrophobicity caused by the adamantylalanine residues (for a more detailed analysis, see [14]). All products were either macrocrystalline (**1–7**) or solid (microcrystalline? **1a–7a**, **1b–7b**, *Table 2*). The elemental analyses *(Table 3)* tended towards reduced (C^-), sometimes enhanced (C^+) values of C-atom, perhaps due to insufficient drying (danger of decomposition under more stringent conditions).

3. Biologic activity. – All seven compounds **1b–7b** were investigated for their enkephalin – and morphine-like action with two *in vitro* pharmacological systems: (i) the inhibition of electrically evoked contractions of the guinea pig ileum longitudinal muscle – myenteric plexus preparation (GPI), and (ii) displacement of [^{3}H] naloxone from its binding sites in rat brain homogenates ('stereospecific receptor affinity', NAL).[2])

A comparison of the peptides H-Tyr-ala-Gly-Phe-Y, with Y = Leu-OH, Met-OH, ada-OH (**2b**), and Ada-OH (**1b**) in the GPI assay indicated increasing potencies in this sequence, **1b** being about 3-times as potent as the peptide with Y = Leu-OH [14]. The same is approximately true for the NAL assay. A similar increase is observed in the GPI test for the series Y = Met-NH_2, Bug-NH_2 (*t*-butylglycine amide), ada-NH_2, and Ada-NH_2, the last compound being almost 3.5-times as potent as the first. Despite the different bulk of the side-chains, the increase of the potencies within the two series correlates excellently ($r = 0.998$ and 0.981) with the increase of the over-all pentapeptide hydrophobicity. (The ada peptides **2b** and **4b** are less hydrophobic – and less potent – than those with Ada **1b** and **3b**, probably because of differences in the conformer population.)

All peptides with adamantylalanine in position 5 are strongly resistant towards the hydrolytic action of enzymes such as chymotrypsin, neutral protease, pronase, and thermolysine, which readily degrade H-Tyr-ala-Gly-Phe-Met-NH_2. (On the other hand, H-Tyr-ala-Gly-Ada-Leu-NH_2 (**6b**) is strongly resistant only towards chymotrypsin and neutral protease, much less so against pronase and thermolysine [14].) Because of the conditions of the bio-assays, the different stabilities towards enzymes are not of great importance in determining the potency, an assumption that is confirmed by the excellent correlation found between the hydrophobicity and the potency in the two assays, especially for **1b** and **2b**, and **3b** and **4b**.

The three H-Tyr-ala-Gly-Ada-Y peptides (**5b–7b**) are practically inactive in these tests, despite their enhanced hydrophobicity. It was concluded that the

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[2]) Both assays were performed in the Laboratoire de Recherche sur les Polypeptides, Institut de Recherches Cliniques de Montréal, Montréal, Canada. We thank Prof. Dr. *P. W. Schiller* for his collaboration (see [14]).

peptide hydrophobicity caused by amino acid no. 5 is of great importance in determining the potency of enkephalin-like peptides in these assays, but that over-all hydrophobicity is not decisive when caused by amino acid no. 4 (here, aromaticity is the determining factor [14]).

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Experimental Part

General. – Product characteristics are displayed in *Tables 1–3*. All solvent ratios are in volume parts. References to well-known procedures of peptide chemistry and intermediates are usually not explicitly given, but can be found in *Houben-Weyl* [2]. Removal of solvents was carried out by evaporation at 0.1–10 Torr and low bath temperatures (30°) in rotatory evaporators. Acidifications were performed with an aqueous solution containing equal amounts (5% by weight) of $KHSO_4$ and K_2SO_4 (pH 2). Extraction into organic solvents was followed by appropriate steps, such as washing with hydrogencarbonate, acid, and water, drying, and evaporation, as usual. RT. is room temperature, about 20°. See also [9]

Table 1. *Amino acid sequence, thin layer chromatography* (Rf-values[a]) in solvents A-D[b]), *and high performance liquid chromatography* (retention times, T_r[c]) *of adamantylalanine peptides*

		A	B	C	D	T_r
1	Boc-Phe-Ada-OMe			0.79	0.82	
2	HCl, H-Phe-ada-OMe			0.65		
3	Boc-Phe-Ada-NH_2			0.76	0.70	
4	Boc-Phe-ada-NH_2	0.68		0.76	0.69	
5	Boc-Ada-Leu-OMe	0.42			0.75	
6	Boc-Ada-Leu-NH_2	0.59			0.76	
7	Boc-Ada-Ada-NH_2			0.69		
1a	Boc-Tyr-ala-Gly-Phe-Ada-OMe	0.50		0.75		
2a	Boc-Tyr-ala-Gly-Phe-ada-OMe	0.42		0.75	0.77	
3a	Boc-Tyr-ala-Gly-Phe-Ada-NH_2			0.65	0.72	
4a	Boc-Tyr-ala-Gly-Phe-ada-NH_2	0.64		0.73		
5a	Boc-Tyr-ala-Gly-Ada-Leu-OMe	0.43			0.78	
6a	Boc-Tyr-ala-Gly-Ada-Leu-NH_2	0.33			0.69	
7a	Boc-Tyr-ala-Gly-Ada-Ada-NH_2			0.78	0.69	
1b	H-Tyr-ala-Gly-Phe-Ada-OH · HCl · H_2O		0.23	0.58	0.79	1.85
2b	H-Tyr-ala-Gly-Phe-ada-OH · HCl · H_2O		0.28	0.46	0.64	1.92
3b	H-Tyr-ala-Gly-Phe-Ada-NH_2 · HCl · H_2O		0.37	0.54	0.65	2.05
4b	H-Tyr-ala-Gly-Phe-ada-NH_2 · HCl · H_2O		0.29	0.51	0.56	2.85
5b	H-Tyr-ala-Gly-ada-Leu-OH · HCl · H_2O		0.14		0.72	1.50
6b	H-Tyr-ala-Gly-Ada-Leu-NH_2 · HCl · H_2O		0.24	0.36	0.67	1.80
7b	H-Tyr-ala-Gly-Ada-Ada-NH_2 · HCl · H_2O			0.53	0.75	

a) Only one spot was observed on *Merck* F524 silica gel plates. Detection with I_2, *Reindel-Hoppe* reagent, ninhydrin, fluorescence quenching in UV. *etc.*

b) $CHCl_3$/MeOH 9:1 (A), 7:3 (B), 1:1 (C), 2-butanol/AcOH/H_2O 10:1:3 (D).

c) Stainless steel column (3.2 × 25 mm) with silica gel (*Lichrosorb Si60, 5μ*). Eluant: $CHCl_3$/MeOH 3:2, 2 ml/min T_r in min between the appearance of the solvent and compound peak (UV. detection).

Table 2. *Physical data.* Aspect (A): s = solid, c = crystalline; solvent for crystallization (S); m.p.[a]); specific rotation, $[\alpha]_D^{23}$ (c = 1, MeOH)

	A	S	M.p. [°C]	$[\alpha]_D^{23}$ [deg.]
1	c	EtOAc/Et_2O	157	−24.4
2	c	EtOAc/Et_2O	218	+46.3
3	c	EtOAc/Et_2O	137	−27.3
4	c	EtOAc/Et_2O	169	+25.9
5	c	Et_2O/pentane	135	−35.4
6	c	EtOAc/Et_2O	175	−29.9
7	c	EtOH/pentane	151	−31.9
1a	s	EtOH/Et_2O	150[b])	− 2.9
2a	s	EtOAc/Et_2O	130	+ 1.7
3a	s	EtOAc/Et_2O	150[b])	− 3.1
4a	s	EtOH/Et_2O	140[b])	+ 2.1
5a	s	EtOAc/Et_2O	142	− 4.9
6a	s	EtOH/Et_2O	205[b])	− 5.1
7a	s	EtOAc/Et_2O	192	− 5.1
1b	s	EtOH/Et_2O	195[b])	− 4.1
2b	s	EtOH/Et_2O	195[b])	+ 5.0
3b	s	EtOH/Et_2O	190[b])	− 2.9
4b	s	EtOH/Et_2O	180[b])	+ 5.6
5b	s	EtOH/Et_2O	184[b])	− 4.4
6b	s	EtOH/Et_2O	205[b])	− 3.5
7b	s	EtOH/Et_2O	210[b])	− 4.6

[a]) Uncorrected; open capillary tube.
[b]) With decomposition.

Preparation of methyl D- *and* L-*adamantylalaninate hydrochlorides.* According to the esterification method of *Brenner* [15], thionyl chloride (1 ml) was added dropwise to methanol (10 ml) at −10°. Then, D- or L-adamantylalanine (1.1 g, 4 mmol) were slowly dissolved in this mixture, taking care to keep the temperature below −5°. After complete dissolution of solids, the solvent was evaporated and the residue recrystallized from 2-propanol/diisopropyl ether. Yield 0.77 g (70%) of colourless crystals. M.p. 155°. TLC.: Rf 0.65 ($HCCl_3$/MeOH 1:1), 0.60 (2-butanol/AcOH/H_2O (10:1:3). $[\alpha]_D^{24}$ = +24.8° (L), −24.7° (D) (c = 1, MeOH).

$C_{14}H_{24}ClO_2N$ (L) (273.8) Calc. C 61.41 H 8.84 N 5.12% Found C 61.21 H 8.65 N 5.04%

Preparation of the dipeptide derivatives **1–7**. – The Boc-derivative of the amino acid destined for position 4 (Boc-Phe-OH, Boc-Ada-OH [6], or Boc-ada-OH [6]) and the hydrochloride of the amino acid methyl ester or amino acid amide destined for position 5 (H-Ada-OMe, H-ada-OMe, H-Ada-NH_2 [6], H-ada-NH_2 [6], H-Leu-OMe, or H-Leu-NH_2) were condensed according to the procedure of *König & Geiger* [2] [12]: The amino acid derivatives (2 mmol each) were dissolved in dry DMF (20 ml) and treated with *N*-ethylmorpholine and 1-hydroxybenzotriazole (2 mmol each, RT.), followed at 0° by DCC (2.2 mmol). After 1 h at 0° and 15 h at RT., a few drops of acetic acid were added and the dicyclohexylurea removed by filtration. The filtrate was evaporated and the product isolated by extraction into ethyl acetate, washing with 5% aq. $NaHCO_3$-solution and acid at pH2, drying and evaporating, as usual. After crystallization from the solvents of *Table 2*, the products were pure; the yields ranged from 75–95%.

The Boc-group was removed by dissolving the dipeptide derivative so obtained in 0.4N HCl in HCOOH (1.2 mol-equiv.) and keeping the solution for 20 min at RT. Evaporation and repeated trituration with ether gave pure hydrochlorides in 90–100% yields. They were used directly for the next condensation step after confirming their necessary purity with TLC. (only compound **2** was further analyzed and identified, see *Tables 2* and *3*).

Preparation of the pentapeptide derivatives **1a–7a**. Boc-Tyr-ala-Gly-OH [9] (409.4 mg, 1 mmol) and the *C*-terminal dipeptide methyl ester or amide hydrochloride (above, 1 mmol) were dissolved in dry DMF (10 ml) and condensed exactly as described above for the preparation of **1–7**. Residual amounts of

Table 3. *Analytical data* (microanalyses[a])): C, H, N, Cl (% Calc./% Found), deviation greater than 0.30% (dev.), drying: 40°/0.01 Torr, 15 h, amino acid analysis[b])

		C	H	N	Cl	Dev.
1	$C_{28}H_{40}O_5N_2$ (484.6)	69.39/69.08	8.32/8.47	5.78/5.42		C–, N–
2	$C_{23}H_{33}ClO_3N_2$ (421.0)	65.62/65.23	7.90/8.14	6.65/6.82	8.42/8.18	C–
3	$C_{27}H_{39}O_4N_3$ (469.6)	69.06/68.66	8.37/8.49	8.95/8.76		C–
4	$C_{27}H_{39}O_4N_3$ (469.6)	69.06/68.89	8.37/8.53	8.95/9.03		
5	$C_{25}H_{42}O_5N_2$ (450.6)	66.63/66.49	9.40/9.42	6.22/6.20		
6	$C_{24}H_{41}O_4N_3$ (435.6)	66.17/65.98	9.49/9.51	9.65/9.59		
7	$C_{31}H_{49}O_4N_3$ (527.7)	70.55/70.03	9.36/9.39	7.96/7.82		C–
1a	$C_{42}H_{57}O_9N_5$ (775.9)	65.01/64.68	7.40/7.47	9.03/8.82		C–
2a	$C_{42}H_{57}O_9N_5$ (775.9)	65.01/64.89	7.40/7.41	9.03/9.00		
3a	$C_{41}H_{56}O_8N_6$ (760.9)	64.72/63.97	7.42/7.52	11.04/10.52		C–, N–
4a	$C_{41}H_{56}O_8N_6$ (760.9)	64.72/63.90	7.42/7.62	11.04/10.87		C–
5a	$C_{39}H_{59}O_9N_5$ (741.9)	63.14/62.90	8.01/7.98	9.44/9.14		
6a	$C_{38}H_{58}O_8N_6$ (726.9)	62.78/62.86	8.04/8.25	11.56/11.16		N–
7a	$C_{45}H_{66}O_8N_6$ (819.1)	65.99/65.82	8.12/8.25	10.26/10.44		
1b	$C_{36}H_{50}ClO_8N_5$ (716.3)	60.37/60.67	7.04/7.05	9.78/9.56	4.95/4.39	Cl–
2b	$C_{36}H_{50}ClO_8N_5$ (716.3)	60.37/60.80	7.04/6.83	9.78/9.77	4.95/5.17	C+
3b	$C_{36}H_{51}ClO_7N_6$ (715.3)	60.45/60.98	7.19/7.06	11.75/11.66	4.96/4.92	C+
4b	$C_{36}H_{51}ClO_7N_6$ (715.3)	60.45/60.42	7.19/7.15	11.75/11.79	4.96/4.72	
5b	$C_{33}H_{52}ClO_8N_5$ (682.3)	58.10/58.26	7.68/7.53	10.26/10.45		
6b	$C_{33}H_{53}ClO_7N_6$ (681.3)	58.18/58.84	7.84/7.65	12.33/12.19		C+
7b	$C_{40}H_{61}ClO_7N_6$ (773.4)	62.12/61.94	7.82/7.96	10.86/10.69	4.58/4.87	

[a]) Performed in the Laboratorium für organische Chemie ETHZ (*D. Manser*).

[b]) Amino acid analyses carried out in our institute in the laboratory of Prof. Dr. *H. Zuber* (found values, the calculated value is always 1.00); **1b**: Gly 0.95, ala 1.00, Tyr 1.02, Phe 1.01; **2b**: Gly 1.00, ala 1.02, Phe 0.96; **5b**: Gly 0.95, ala 0.99, Leu 1.40, Tyr 0.96.

DCU, remaining after extraction into ethyl acetate and washing, were separated hy column chromatography over silica gel (2 × 45 cm). Chloroform removed the contaminant; the pentapeptide derivatives were eluted with $CHCl_3$/MeOH 9:1. The pure peptides were precipitated from the solvent mixtures of *Table 2*. The yields of (microcrystalline?) solids **1a–7a** varied between 60 and 80%.

Preparation of the pentapeptides **1b, 2b,** *and* **5b**. The methyl ester group was hydrolyzed in 0.2N KOH in dioxane/water 2:1 [16] (20 ml for 1 mmol of peptide derivative) and agitation at RT. for 15 min. After neutralization to pH 7 with 0.1N HCl, the solvents were evaporated, the residue mixed with water and acid at pH 2, and extracted into ethyl ether. The dried solution was evaporated, the residue dissolved in methanol and the product precipitated with ether. The Boc-group was removed exactly as described for the dipeptide derivatives **1–7**, except that the hydrochloride of the pentapeptides were precipitated from the HCl/HCOOH by addition of 5 vol. of ether. Centrifugation, resuspension in ether, and centrifugation yielded the pure pentapeptide hydrochlorides **1b, 2b,** and **5b** as (microcrystalline?) solids presumably containing 1 mol water *(Table 3)*. Total yields 70–90%.

Preparation of the pentapeptides **3b, 4b, 6b,** *and* **7b**. The Boc-groups were removed from compounds **3a, 4a, 6a,** and **7a** with 0.4N HCl in HCOOH (1.2 mol-equiv.) and the products purified exactly as described for the dipeptide derivatives **1–7** and the pentapeptides **1b, 2b,** and **5b**. The pentapeptide hydrochlorides **3b, 4b, 6b**, and **7b** were obtained as (microcrystalline?) solids presumably containing 1 mol water *(Table 3)*. Yields 85–98%.

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