miteinander zu verknüpfen, um ihre absoluten Konfigurationen bestimmen zu können.

Die negativ drehenden Antipoden von I (Smp. 77–78°, $[\alpha]_D^{25} = -17{,}4° \pm 0{,}5°$)[1,10] und III (Smp. 55–56°, $[\alpha]_D^{25} = -10{,}6° \pm 1{,}0°$)[10] sowie der positiv drehende Antipode von IV (Smp. 105–106°, $[\alpha]_D^{25} = +21{,}6° \pm 1{,}0°$) wurden mit Natrium in siedendem Äthanol reduktiv dehalogeniert und lieferten das gemeinsame Produkt V (Sdp. 155–165° bei 0,05 Torr, $[\alpha]_D^{25} = -7{,}6° \pm 0{,}7°$; Hydrobromid: Smp. 169–171°, $[\alpha]_D^{25} = -15{,}7° \pm 0{,}5°$).

Die Reduktion des negativ drehenden Antipoden von II (Smp. 114–115°, $[\alpha]_D^{25} = -17{,}4°$)[4] mit PtO_2 in Methanol zu VI (Öl, $[\alpha]_D^{25} = -29{,}5°$) und die nachfolgende Deaminierung von VI durch Diazotieren und Reduktion mit H_3PO_2 führte wiederum zu V. Somit weisen (–)I, (–)II, (–)III, (+)IV und (–)V am Asymmetriezentrum C-1 die gleiche Konfiguration auf. Dies zeigt sich auch im ähnlichen Verlauf der Rotationsdispersionskurven (Figur). Nur bei grössern Wellenlängen als 360 mμ erreicht die Kurve des wirksamen Antipoden von IV positive Drehwerte.

Da die absolute Konfiguration für (–)I bereits bewiesen wurde[11], besitzen also auch (–)II, (–)III, (+)IV und (–)V die in der Figur angegebene 1*R*-Konfiguration.

Summary. The absolute configuration of four pharmacologically active tetrahydroisoquinolines has been determined. It was shown that both the levorotatory antipodes of I, II and III and the dextrorotatory enantiomer of IV possess *R*-configuration at the asymmetric center.

A. Rheiner jr. und A. Brossi

Chemische Forschungsabteilung der F. Hoffmann-La Roche & Co. AG, Basel (Schweiz), 3. Juni 1964.

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[7] Elektrische Reizung des N. laryngeus sup. der Katze nach R. Domenjoz, Arch. exp. Path. Pharmak. *215*, 19 (1952).
[8] In der Morphinanreihe bewirken dagegen auch die analgetisch unwirksamen Enantiomeren eine deutliche Hustenhemmung.
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Structure and Pharmacological Actions of Physalaemin, the Main Active Polypeptide of the Skin of *Physalaemus fuscumaculatus*[1]

It was shown[2] that methanol extracts of the skin of the South American amphibian *Physalaemus fuscumaculatus* contain an eledoisin-like polypeptide which, like eledoisin, exerts a powerful hypotensive action and stimulates extravascular smooth muscle. This polypeptide, called *physalaemin*, has now been isolated in pure form and identified as the endecapeptide Pyr-Ala-Asp(OH)-Pro-Asp(NH_2)-Lys-Phe-Tyr-Gly-Leu-Met-NH_2.

Synthesis confirmed the above constitution[3], closely resembling that of eledoisin[4,5].

Isolation procedure and determination of structure. 471 fresh skins of *Physalaemus fuscumaculatus* weighing 206 g and 323 dried skins weighing 32.8 g (physalaemin withstands drying perfectly) were extracted twice with 80% methanol. The combined filtered extracts were kept in a refrigerator and served as standard crude extract for the isolation of physalaemin.

The first step in the purification of physalaemin was the absorption of the crude polypeptide dissolved in 95% ethanol on alkaline alumina and subsequent elution with decreasing concentrations of ethanol. Physalaemin was eluted by 60% ethanol, whilst one or two minor active polypeptides were eluted with 80 and 70% ethanol.

Final purification was achieved by submitting the partially purified material to a double 40 transfers counter-current distribution using two *n*-butanol water systems with pH 2 and 9 respectively in the water phase. The yield was 70 to 80%.

Pure physalaemin was homogeneous in paper chromatography and electrophoresis, showing a single spot with ninhydrin, chlorine, the iodoplatinate reagent of sulphur amino acids[6] and the α-nitroso-β-naphthol reagent of tyrosine[7]. In ascending paper chromatograms with the system *n*-butanol:acetic acid:water (4:1:1), physalaemin had Rf 0.41. On electropherograms it migrated towards the cathode at acid pH and showed no mobility at neutral pH. Acid hydrolysis yielded glutamic and aspartic acids, alanine, proline, lysine, phenylalanine, tyrosine, glycine, leucine and methionine. Ten out of eleven residues were present in a 1:1 mole ratio, and only the aspartyl residue was found to be present in a 2:1 ratio with respect to the other amino acids.

The sequence was elucidated through chemical and enzymatic degradation. Physalaemin, like eledoisin, was resistant to enzymatic attack at both the C and N terminus. The Sanger and Edman techniques failed to reveal a free N-terminal residue. Upon digestion with chymotrypsin, the consecutive appearance of the following fragments was observed in the reaction mixtures:

C_1 [Glu, Ala, 2 Asp, Pro, Lys, Phe, Tyr],	C_2 [Gly, Leu, Met],
C_3 [Glu, Ala, 2 Asp, Pro],	C_4 [Lys, Phe, Tyr],
C_5 [Lys, Phe],	C_6 H-Tyr-OH,
C_7 [Gly, Leu]	and C_8 H-Met-NH_2

It followed that chymotrypsin caused a rapid cleavage at the carboxyl bond of tyrosine with release of the C-

[1] Supported in part by a grant from the Consiglio Nazionale delle Richerche, Roma.
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terminal tripeptide C_2, then a bond was split between the C end of the N-terminal pentapeptide and the amino group of lysine. Upon further action of the enzyme, tyrosine was slowly released from C_4 and finally a very slow and incomplete splitting of methionamide from C_2 was observed.

Tryptic digestion was anomalous, since two bonds were split, one of which was a lysil and the other a tyrosil bond, and the rapid and simultaneous formation of the following three peptides was observed:

T_1 [Glu, Ala, 2 Asp, Pro, Lys], T_2 [Phe, Tyr] and T_3 [Gly, Leu, Met]

From the above results the sequence of the C-terminal hexapeptide was deduced, the position of the single residues being established by the specificity of the enzymes and by the composition of the fragments. The sequence Gly-Leu was determined by the fluorodinitrobenzene procedure on the tripeptide Gly-Leu-Met-NH_2.

Under the action of carboxypeptidase lysine was slowly split from the tryptic hexapeptide T_1, thus confirming the position of lysine already deduced by the specificity of trypsine cleavage. The sequence of the five N-terminal amino acids was found by submitting T_1 to partial acid hydrolysis with 0.5 *N* acetic acid. The presence of the following fragments was consecutively observed in the acid hydrolysates:

Ac_1 [Glu, Ala], Ac_2 [H-Asp-$(OH)_2$], Ac_3 [Pro, Asp(NH_2)]-Lys-OH, Ac_4 Pyr-OH, Ac_5 H-Ala-OH, Ac_6 [Pro, Asp$(NH)_2$] and H-Lys-OH

The sequence -Pro-Asp-NH_2 was deduced by the positive reaction to isatine of the spots Ac_3 and Ac_6. The status of the glutamyl and aspartyl residues was established by the relative electrophoretic mobilities of the fragments in which they occurred.

Pharmacological actions. Physalaemin is a powerful vasodilator and hypotensive agent in dog and rabbit. It is 3 to 4 times as active as eledoisin. In the dog the i.v. threshold hypotensive dose is approximately 1 ng/kg. The pressure fall is proportional, both in intensity and even more in duration, to the dose of the polypeptide, and tachyphylaxis is completely lacking. A persistent, controllable lowering of blood pressure may be obtained with i.v. infusion of physalaemin, the threshold being 2–5 ng/kg/min. An appreciable lowering of blood pressure is also produced by subcutaneous doses of 1 µg/kg physalaemin. In the majority of experiments, hypotension produced by medium i.v. doses of physalaemin is of shorter duration than that produced by equiactive doses of eledoisin.

1 µg physalaemin is capable of counteracting completely the hypertensive action of 50–100 µg L-adrenaline or L-noradrenaline, 10–15 µg angiotensin, 30–50 µg Lys-8-vasopressin, or 3 mg nicotine. On a weight basis physalaemin is 100–700 times more potent than bradykinin, and 200–400 times more than histamine. The effect on the blood pressure of the dog and rabbit of 1 µg physalaemin is equivalent to 100–200 units of substance P.

Both in the intact and in the decapitated chicken, physalaemin is predominantly hypertensive. Pressure rise seems to be mainly due to liberation of catecholamines.

In increasing the permeability of the skin vessels of the human forearm, physalaemin is slightly more active than eledoisin[8].

Generally, physalaemin stimulates the same extravascular smooth muscles which are stimulated by eledoisin but, with the exception of the rabbit large intestine and the guinea-pig ileum, the effect is weaker than with eledoisin and tachyphylaxis is not infrequent. The rat uterus is poorly sensitive to physalaemin, and even the rabbit uterus is several times less responsive to physalaemin than to eledoisin.

Large doses of physalaemin stimulate salivary secretion in the dog, but this effect seems less intense compared with eledoisin.

Whole blood slowly inactivates physalaemin, homogenates of liver and kidney far more quickly. Generally, physalaemin is inactivated more rapidly than eledoisin[9].

In parallel assays physalaemin may clearly be distinguished from known natural polypeptides active on plain muscle, including substance P and eledoisin, which in most of their biological properties are very similar to physalaemin.

Physalaemin or strictly related polypeptides are also contained in the skin extracts of *Physalaemus centralis*, *Physalaemus bresslaui* and *Physalaemus cuvieri*.

Full reports of the experiments and results described in this paper will be published elsewhere.

Riassunto. Viene descritto il procedimento che ha permesso l'isolamento e il chiarimento della struttura chimica della *fisalemina*, il principale dei polipeptidi attivi della pelle di *Physalaemus fuscumaculatus*, anfibio dell'Argentina. La fisalemina, strettamente vicina all'eledoisina anche da un punto di vista chimico è, come questa, dotata di potente azione sulla muscolatura liscia vasale ed extravasale. Essa è nettamente distinguibile, mediante saggi paralleli, da tutti gli altri polipeptidi biogeni finora noti.

V. ERSPAMER, A. ANASTASI, G. BERTACCINI, and J. M. CEI

Istituto di Farmacologia, Università di Parma, Laboratori Ricerche Farmitalia, Milano (Italia), and Instituto de Biologia, Universidad Nacional de Cuyo, Mendoza (Argentina), May 28, 1964.

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[9] M. B. NOBILI, to be published.

Synthesis of Physalaemin

We report the synthesis of a peptide of a formula H-Pyr-Ala-Asp-(OH)-Pro-Asp-NH_2-Lys-Phe-Tyr-Gly-Leu-Met-NH_2 according to scheme I[1]. The product was identical with natural physalaemin[2].

Condensation of glycine ethylester hydrochloride with N,O-bis-CBO-tyrosine in methylene chloride, with one equiv. of TEA, by DCCI gave ethyl N,O-bis-CBO-tyrosylglycinate (I) (82% yield; m.p. 165–166°; $[\alpha]_D^{20}$ −25°, c 0.5,

[1] All the amino acids have the L-configuration. The following abbreviations are used throughout this paper: CBO = carbobenzyloxy; CTB = carbo-ter-butyloxy; OtBu = ter-butylester; Pyr = pyroglutamyl; TEA = triethylamine; DCCI = dicyclohexylcarbodiimide; DCEA = dicyclohexylamine; E° = electrophoretic mobility of a sample pre-treated with HBr/AcOH or TFA according to the protecting group (CBO or CTB); DMF = dimethylformamide; TFA = trifluoroacetic acid.

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