214–215°, $[\alpha]_D$ −71.5°, c 2.5, DMF). The N-decarbobenzoxylation of the latter by means of HBr in acetic acid followed by step-by-step peptide synthesis, using each time the appropriate carbobenzoxy amino acid *p*-nitrophenyl ester[6], afforded the fully protected nonapeptide I (m.p. 227–229°, $[\alpha]_D$ −64.5°, c 1, DMF). By a similar procedure, other fully protected nonapeptides[7], having the same sequence of amino acids[8], have been prepared, e.g. II (m.p. 224–226°, $[\alpha]_D$ −60.6°, c 1, DMF), III (m.p. 217–220°, $[\alpha]_D$ −55.2°, c 1, DMF), and IV (m.p. 226–229°, $[\alpha]_D$ −56.6°, c 1, DMF). Removal of the S-protecting acyl groups from the protected nonapeptides I and II by methanolysis in the presence of sodium methoxide gave the N-carbobenzoxy-nonapeptide V with free –SH groups (m.p. 234–235°, $[\alpha]_D$ −46.2°, c 1, DMF). The compound V may be called N-carbobenzoxy-oxytoceine according to the nomenclature proposed by du Vigneaud. Using as starting materials III and IV, the N-t-butyloxycarbonyl- and the N-*o*-nitrophenylsulphenyl-oxytoceines have been prepared in the same manner.

The details of the synthesis of all the above compounds and their transformation to oxytocin will be described in a forthcoming publication[9].

Zusammenfassung. Es wird die Synthese von N-substituierten Oxytoceinen beschrieben. In einem Fall wurde die Synthese durch Benutzung des S-Benzoyl- und S-Carbobenzoxy-L-cysteins ermöglicht.

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Laboratory of Organic Chemistry, University of Athens (Greece), May 11, 1964.

[6] M. BODANSZKY and V. DU VIGNEAUD, J. Am. chem. Soc. *81*, 5688 (1959).

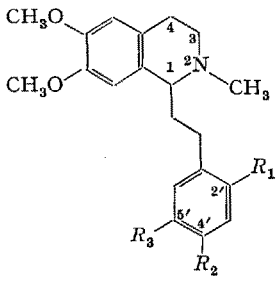
[7] Dicyclohexylammonium salt of N-t-butyloxycarbonyl-S-benzoyl-L-cysteine (m.p. 151–153°, $[\alpha]_D$ +18°, c 1, DMF), and *p*-nitrophenyl-S-benzoyl-N-*o*-nitrophenylsulfenyl-L-cysteinate (m.p. 101–102°. $[\alpha]_D$ −51.5°, c 2, DMF) were used (unpublished work of this laboratory) for the last step of the synthesis of nonapeptides III and IV, respectively.

[8] For the abbreviations used to denote amino acid residues see: Report of the Committee on Nomenclature, *Peptides*, Proceedings of the Fifth European Peptide Symposium, Oxford (September 1962) (Ed. G. T. YOUNG, Pergamon Press, Oxford 1963), p. 261.

[9] This investigation was supported by the Royal Hellenic Research Foundation, to which I am greatly indebted. I also thank Dr. H. MANTZOS for the microanalyses and Mr. V. BARDAKOS for helpful technical assistance.

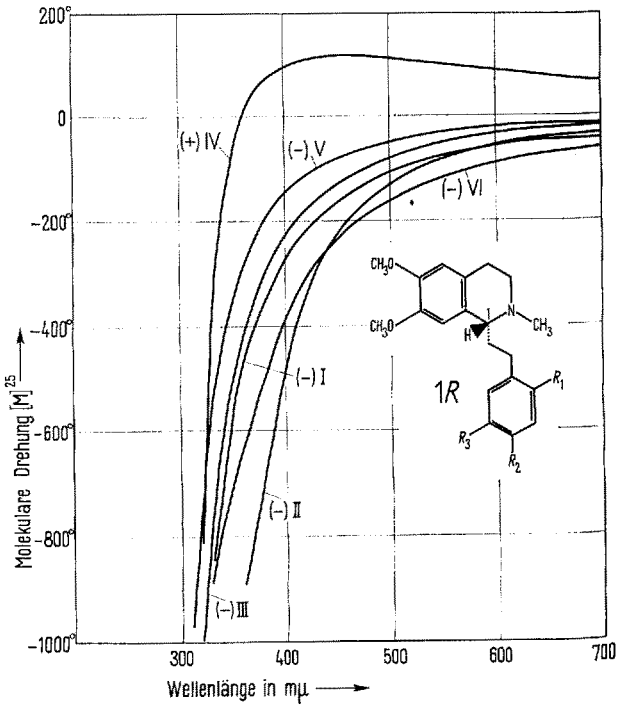
Konfiguration und pharmakologische Aktivität bei 1-phenäthylsubstituierten 1,2,3,4-Tetrahydroisochinolinen

Verschiedene im Phenylrest der Phenäthylseitenkette substituierte 1-Phenäthyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisochinolinderivate zeichnen sich durch pharmakologische Aktivität aus. Als Analgetika beanspruchen die 4'-Chlor-(I)[1–3] und die 4'-Nitroverbindung (II)[4], als Antitussiva die 3',4'-Dichlor-(III)[1] und die 2',4',5'-Trichlorverbindung (IV) besonderes Interesse. Zur Abklärung der Zusammenhänge zwischen Konfiguration und Wirkung wurden diese vier Racemate in ihre optischen Antipoden gespalten. Die pharmakologische Auswertung ergab, dass praktisch die gesamte analgetische Aktivität[5] jeweils nur einem Antipoden zukommt[6]. Der gleiche Antipode zeigt auch hustenlindernde Eigenschaften[7], während die analgetisch inaktiven Enantiomeren höchstens schwach antitussiv wirken[8]. Die analgetische Aktivität nimmt in der Reihe IV < III < I < II zu, die antitussive dagegen in der Reihe I < III < IV < II.



I $R_1 = R_3 = H$, $R_2 = Cl$
II $R_1 = R_3 = H$, $R_2 = NO_2$
III $R_1 = H$, $R_2 = R_3 = Cl$
IV $R_1 = R_2 = R_3 = Cl$
V $R_1 = R_2 = R_3 = H$
VI $R_1 = R_3 = H$, $R_2 = NH_2$

Bei den Verbindungen I, II und III war jeweils der im Na-Licht negativ drehende[9] Antipode der analgetisch und antitussiv wirksame Bestandteil; im Falle von IV erwies sich hingegen der positiv drehende Antipode als die aktive Komponente. Es war deshalb von Interesse, alle diese optisch aktiven Substanzen durch chemische Reaktionen



Molekulare Rotationsdispersionskurven

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

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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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