ture since unsaturation has been excluded. b) IR-spectroscopy (as film on a Perkin Elmer model 125) revealed a characteristic band at 9.8 μm.

The localization of the propane ring was determined by GLC (Aerograph model 204 A equiped with a 240 cm column of 10% SE-30 on Chromosorb W, programming of temperature 100–260°, rise of 2°/min, N_2 flow rate 200 ml/min) after oxidative degradation by refluxing with potassium permanganate in acetone[13]. A series of homologous fatty acids up to $C_{7:0}$ and from $C_{10:cycl}$ to $C_{17:cycl}$ was obtained (see Figure 2). From the absence of C_8 and C_9 fatty acids, the C_{17} cyclopropane fatty acid was decided to be 9,10-methylene hexadecanoic acid. The change of character of the acids with increase of carbon number from straight chain to cyclopropane was clearly demonstrated by co-chromatography of a series of even numbered straight chain alkanoic acid methyl esters up to $C_{16:0}$.

The neutral lipid fraction of non-PHB consisted of a variety of components. Only small amounts of long-chain fatty acids were obtained by saponification. GLC of these fatty acids (as methyl esters) revealed C_{17} cyclopropane acid as the major component (98.4%, w/w) besides palmitic acid (1.6%, w/w).

C_{17} cyclopropane acid has been detected in a number of bacteria, predominantly gram-negative (listed in[12]), the localization of the propane ring, however, has not been determined in all these cases. In cases known so far, the propane ring is situated between C-9 and C-10.

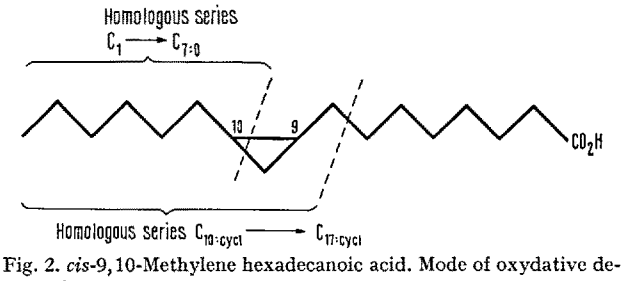

Fig. 2. *cis*-9,10-Methylene hexadecanoic acid. Mode of oxydative degradation.

It is well established[14] that *cis*-cyclopropane acids in bacteria are synthesized by transfer to the corresponding *cis*-monoenoic acid of a methylene group from S-adenosyl methionine. It is worthy of note that both *cis*-9,10-methylene hexadecanoic acid and lactobacillic acid (i.e. *cis*-11,12 methylene octadecanoic acid, present in a variety of bacterial species) belong to the same ω-family (with propane ring at the same position from the terminal methyl group). This fact is consistent with the finding that bacteria can synthesize long-chain monoenoic fatty acids by elongation of already unsaturated precursors[15].

Zusammenfassung. Knallgasbakterien (*Hydrogenomonas* H 16) wurden unter Speicherbedingungen in Submerskultur herangezogen. Die dabei gespeicherte Poly-β-hydroxybuttersäure wurde zusammen mit anderen «lokker gebundenen» Lipiden mit Chloroform extrahiert. Die daraus abgetrennten polaren Lipide bestanden ausschliesslich aus Phosphatidyläthanolamin. Die Analyse der Fettsäurezusammensetzung dieses Phosphatids ergab einen Gehalt von 42% (bezogen auf das Gewicht der Gesamtfettsäuren) einer C_{17}-Cyclopropansäure. Durch spektroskopische Untersuchungen und oxydativen Abbau wurde letztere als *cis*-9,10-Methylenhexadecansäure identifiziert.

O. W. Thiele[16]

Physiologisch-Chemisches Institut der Universität, Humboldtallee 7, D-34 Göttingen (Germany), 3 June 1971.

[13] K. E. Murray, Aust. J. Chem. *12*, 657 (1959).

[14] J. H. Law, H. Zalkin and T. Kaneshiro, Biochim. biophys. Acta *70*, 143 (1963). S. Pohl, J. H. Law and R. Ryhage, Biochim. biophys. Acta *70*, 583 (1963).

[15] K. Bloch, P. Baranowsky, H. Goldfine, W. J. Lennarz, R. Light, A. T. Norris and G. Scheuerbrandt, Fedn. Proc. *20*, 921 (1961). – G. Scheuerbrandt and K. Bloch, J. biol. Chem. *237*, 2064 (1962). – J. Erwin and K. Bloch, Science *143*, 1006 (1964).

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Optical Rotatory Dispersion of Proline-Rich Peptides from the Venom of *Bothrops jararaca*

The observation that the venom of the Brazilian snake Bothrops jararaca potentiates bradykinin[1] and also inhibits the enzyme[2] that converts angiotensin I into angiotensin II led to the isolation[3,4] of a series of peptides responsible for these activities. Subsequently, the sequences of peptides I–V (Table I) were elucidated and proved by synthesis[4]. Compound VI is a synthetic peptide prepared to determine what is the active 'core' of IV, while VII is an equiactive analog of the pentapeptide isolated by Greene and Ferreira[3] to which they assigned the structure Pyr-Lys-Trp-Ala-Pro.

A conspicuous feature of the venom peptides I-V is the frequency with which proline occurs in them. Compound VI, a pentapeptide, contains two proline residues, while there is only one in peptide VII. Yet, a second structural feature is equally remarkable: in each peptide isolated from the venom so far, pyroglutamic acid is the N-terminal residue. Pyroglutamic acid resembles proline; its pyrrolidone ring is probably even more rigid than the pyrrolidine in proline. The combination of several constrained areas in a sequence could result in a more or less well-defined geometry of the chain and therefore it was intriguing to explore, with the aid of ord and cd spectra, the existence of a preferred conformation in these peptides.

Not quite unexpectedly, the ord spectra of peptides I-VII resemble those of proline oligomers[6] and of polyproline[7], though no mutarotation could be observed in acetic acid. All spectra (Figure) exhibit, as a principal feature, a trough around 215 nm, although the mean residue rotation at this wavelength is different from pep-

[1] S. H. Ferreira, Br. J. Pharmac. Chemother. *24*, 163 (1965).

[2] Y. S. Bakhle, Nature, Lond. *220*, 919 (1968).

[3] S. H. Ferreira, D. C. Bartelt and L. J. Greene, Biochemistry *9*, 2583 (1970).

[4] M. A. Ondetti, N. J. Williams, E. F. Sabo, J. Pluscec, E. R. Weaver and O. Kocy, 2nd American Peptide Symposium, Cleveland, Ohio, August, 1970 (in press).

[5] D. W. Cushman and H. S. Cheung, Fedn. Proc. *28*, Abstr. 3019 (1969).

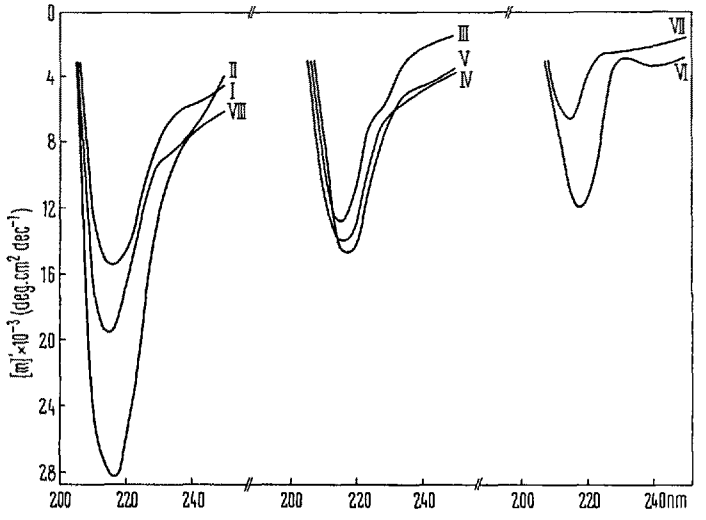
[6] H. Okabayashi, T. Isemura and S. Sakakibara, Biopolymers *6*, 307 (1968).

[7] J. P. Carver and E. R. Blout in *Treatise on Collagen* (G. Ramachandran; Academic press, New York, 1967), vol. 1, p. 441.

Sequences, mean residue rotations and inhibitory potencies of venom peptides and their analogs

Venom peptide	Amino acid sequence	Mean residue rotation at 215 nm	Inhibition I_{50}[a]
I	Pyr-Asn-Trp-Pro-His-Pro-Gln-Iln-Pro-Pro	–15,300	8
II	Pyr-Ser-Trp-Pro-Gly-Pro-Asn-Ile-Pro-Pro	–19,500	35
III	Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	–12,900	1
IV	Pyr-Trp-Pro-Arg-Pro-Thr-Pro-Gln-Ile-Pro-Pro	–14,000	5
V	Pyr-Asn-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	–14,500	3
VI	Pyr-Trp-Pro-Arg-Pro	–12,000	14
VII	Pyr-Lys-Phe-Ala-Pro	– 6,000	0.05

[a] Amount (in μg per ml) needed to inhibit 50% of the angiotensin-converting enzyme activity under standard conditions[5].



Optical rotatory dispersion curves of snake venom peptides (I-V), synthetic analogs (VI and VII) and polyproline (VIII) (Mann Research Laboratories) in aqueous solution, at 25 °C, in the concentration range of 0.03–0.04%, measured with a Carey 60 recording spectropolarimeter in quartz cells of 1 mm pathlength.

tide to peptide. The cd spectra of the venom peptides were also recorded and showed the negative Cotton effect (205 nm) characteristic of aqueous solutions of polyproline[7], but reduced in size. The weak positive band at 221 nm could be clearly identified only in peptide VI. An attempt to establish, in the IR-spectra of the venom peptides, the presence or absence of the features characteristic for the poly- and oligoprolines[8] failed because of interference from non-proline side chains.

Hydrogen bonds seem not to play a major role in the forces determining the preferred conformation in I–VII as shown by a comparison of the spectra taken in methanol and in water: no significant differences could be observed. The fact that compound II, with no basic amino acid in its sequence, produced ord spectra quite similar to those of the other venom peptides, each containing a basic residue, indicates that ionic forces do not contribute to the geometry. Thus the architecture that seems to be present in peptides I–VII should be the consequence of the restrictions caused by the proline and pyroglutamic acid residues, and perhaps by the bulky side chains between the prolines as well. A high content of proline alone does not necessarily result in a similar conformation, e.g., the 6-glycine analog of bradykinin[9], Arg-Pro-Pro-Gly-Phe-Gly-Pro-Phe-Arg, shows an ord spectrum[10] that sets it apart in this respect from peptides I–VII.

Despite the lack of quantitative correlations, it is not impossible that the preferred conformation revealed in the ord spectra of the venom peptides is in some way related to their inhibitory activity: the most active compound, VII, shows the least rigidity, while peptide II, with the most pronounced polyproline character (as expressed by the depth of the trough at 215 nm), is the weakest inhibitor of the converting enzyme[11].

Zusammenfassung. Die optische Rotationsdispersion mehrer prolinreicher Oligopeptide aus dem Gift der Schlange *Bothrops jararaca* wurde gemessen und mit jener von Oligoprolinen verglichen. Es konnte nur eine qualitative Beziehung zwischen der angedeuteten Strukturrigidität und der biologischen Wirksamkeit der Peptide festgestellt werden.

A. BODANSZKY, M. A. ONDETTI, C. A. RALOFSKY and M. BODANSZKY

Department of Chemistry, Case Western Reserve University, Cleveland (Ohio 44106, USA);
The Squibb Institute for Medical Research, New Brunswick (New Jersey 08903, USA), 24 May 1971.

[8] T. ISEMURA, H. OKABAYASHI and S. SAKAKIBARA, Biopolymers *6*, 317 (1968).

[9] M. BODANSZKY, M. A. ONDETTI, J. T. SHEEHAN and S. LANDE, J. Am. chem. Soc. *85*, 991 (1963).

[10] A. BODANSZKY, M. BODANSZKY, E. J. JORPES, V. MUTT and M. A. ONDETTI, Experientia *26*, 948 (1970).

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