On acid hydrolysis the purified peptide yielded serine, leucine, isoleucine, methionine, glycine, alanine, phenylalanine, proline, lysine, aspartic and glutamic acids in a 1:1 ratio. The chromatographic and electrophoretic spots gave the same aminoacid pattern.

The aminoacid sequence was elucidated by chemical and enzymatic degradation. No splitting of C-terminal residue was achieved by carboxypeptidase digestion and both the fluorodinitrobenzene (FDNB) and Edman techniques failed to reveal a free N terminal residue. Chymotrypsin split two peptide bonds giving a heptapeptide containing glutamic and aspartic acids, serine, proline, lysine, alanine and phenylalanine, a tripeptide composed of isoleucine, leucine and glycine and a single residue identified as methioninamide. Trypsin digestion produced two fragments one of which contained glutamic acid, serine, proline and lysine and the other the remaining seven residues. When the heptapeptide produced by chymotrypsin digestion was submitted to trypsin attack, the same tetrapeptide as above was obtained together with a tripeptide composed of alanine, phenylalanine and aspartic acid.

The structure of the two tripeptides produced by chymotrypsin and trypsin hydrolysis was made clear by the FDNB and carboxypeptidase methods and resulted respectively in Asp(OH)-Ala-Phe and Ileu-Gly-Leu.

Under the action of carboxypeptidase, lysine was split from the tetrapeptide isolated from tryptic digests; but attempts to break other peptide bonds were unsuccessful, and even at high concentrations of enzyme only lysine and an acidic peptide negative to ninhydrin could be isolated. As for eledoisin, and also for the tetrapeptide, the N-terminal degradation failed with both the Edman and FDNB procedures. The relative positions of the proline, serine and glutamic acid residues were established by partial acid hydrolysis experiments, and therefore the structure of the tetrapeptide resulted in Pyr-Pro-Ser-Lys.

Consequently, the sequence of eledoisin was clearly established as Pyr-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ileu-Gly-Leu-Met-NH_2. On the basis of digestion with leucinaminopeptidase all aminoacids appeared to have the *L*-configuration.

Pharmacological actions. Eledoisin is a powerful vasodilator and hypotensive agent in most animal species. In the dog, the threshold hypotensive dose is 0.5–2 mμg/kg, by i.v. injection. The pressure fall is proportional, both in intensity and duration, to the dose of the polypeptide, and there is no sign of tachyphylaxis. Subcutaneous doses of 10–150 μg/kg eledoisin produce a pressure fall lasting 6 to 10 h, and with doses of 300 μg/kg sometimes hypotension terminates in death due to heart failure. A persistent, controllable lowering of blood pressure may also be obtained with i.v. infusion of eledoisin, the threshold being 5–15 mμg/kg/min.

Eledoisin displays a potent vasodilator action on the musculo-cutaneous vessels of the dog's hind leg (threshold intraarterial dose 0.3–1.5 mμg), as well as on the coronary arteries and probably on the brain vessels. The vessels of the splanchnic area, lung and kidney are less affected by the polypeptide.

10–20 μg eledoisin are capable of abolishing completely the hypertensive action of 1000 μg *L*-noradrenaline; 1–2 μg counteract the hypertensive action of 3–20 μg angiotensin. Weight-for-weight eledoisin is at least 50 times more potent than acetylcholine, histamine and bradykinin in lowering the blood pressure of the dog.

Following subcutaneous doses of 100–300 μg/kg, eledoisin is detectable in the blood stream only for 10–15 min, whereas hypotension lasts for hours.

Like the kinins, eledoisin potently lowers the permeability of the skin vessels in the guinea-pig and man.

Of the many preparations of extravascular smooth muscles examined the following proved to be particularly sensitive to the polypeptide: terminal portion of the rabbit large intestine (threshold concentration 0.3–0.6 mμg/ml), guinea-pig ileum (0.3–0.6 mμg/ml), dog duodenum and large intestine (0.6–3 mμg/ml), and frog stomach (2–4 mμg/ml). On the rat duodenum eledoisin displays a purely stimulant action; the oestrus rat uterus is poorly sensitive to the polypeptide (0.3–1 μg/ml). The powerful action of eledoisin on the gastro-intestinal smooth muscle becomes apparent also *in vivo*, when high doses of the polypeptide are administered subcutaneously to intact unanaesthetized dogs.

High doses of eledoisin stimulate salivary, and probably also gastrointestinal secretion. This effect, like that on vascular and extravascular smooth muscle, is atropine-resistant.

In parallel assays eledoisin may easily be distinguished from the kinins (bradykinin, wasp kinin), from substance P, and from all other known active tissue products. The natural polypeptide which so far is most similar to eledoisin in its biological properties, especially in its action on smooth muscles, is substance P. The behaviour of the kinins is distinctly different.

Rabbit large intestine, guinea-pig ileum and dog blood pressure are particularly suitable for the quantitative estimation of crude and pure preparations of eledoisin, owing to their high sensitivity and the excellent dose/effect relationship.

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 ed il chiarimento della struttura chimica dell'*eledoisina*, l'endecapeptide attivo delle ghiandole salivari posteriori dell'*Eledone*. L'eledoisina è dotata di potente azione ipotensiva, particolarmente spiccata nel cane, e di intensa azione stimolante su alcuni preparati di muscoli lisci extravasali. L'eledoisina è facilmente distinguibile, mediante saggi paralleli, da tutti gli altri polipeptidi biogeni finora descritti.

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Synthesis of Eledoisin

The structure proposed for Eledoisin[1] (H-Pyr-Pro-Ser-Lys-Asp-(OH)-Ala-Phe-Ileu-Gly-Leu-Met-NH_2) was confirmed in our laboratories by a total synthesis, which was performed according to the scheme given in the Figure.

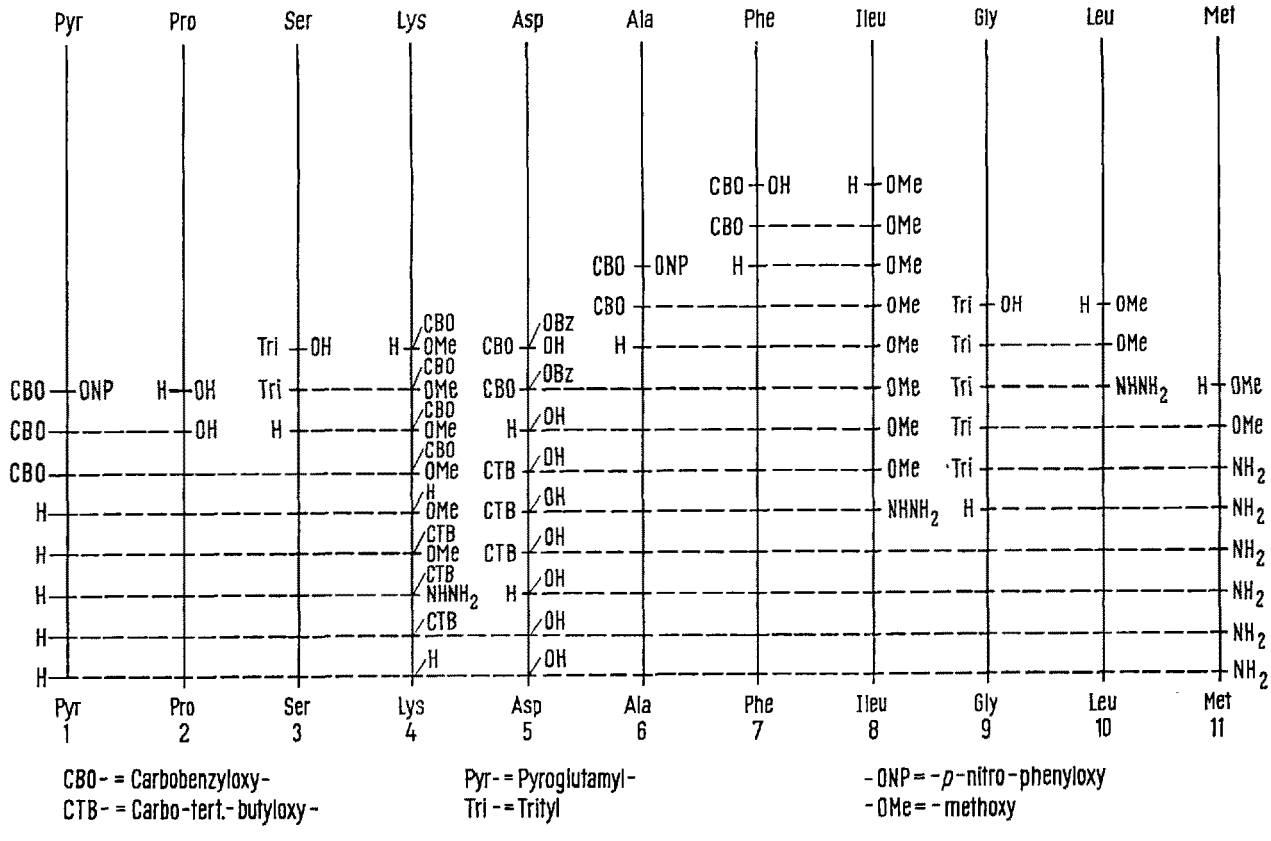
Condensation of *p*-nitrophenyl N-carbobenzoxy-*L*-pyroglutamate with *L*-proline gave N-carbobenzoxy-*L*-pyroglutamyl-*L*-proline (54% yield. M.p. 198°. $[\alpha]_D^{21} = -109°$ in 95% acetic acid. Analysis calculated for $C_{18}H_{20}O_6N_2$: C 59.8; H 5.6; O 26.7; N 7.8. Found: C 60.2; H 5.5; O 26.1; N 8.0), while condensation of N-trityl-*L*-serine with methyl ε-N-carbobenzoxy-*L*-lysinate by dicyclohexylcarbodiimide afforded methyl N-trityl-*L*-seryl-ε-N-carbobenzoxy-*L*-lysinate (66% yield. M.p. 102°. $[\alpha]_D^{22} = -45°$ in dimethylformamide. Analysis calculated for $C_{37}H_{41}O_6N_3$: C 71.3; H 6.6; O 15.4; N 6.7. Found C 71.0; H 6.9 O; 15.5;

N 6.5) which was detritylated by heating in aqueous acetic acid to methyl *L*-seryl-ε-N-carbobenzoxy-*L*-lysinate (79% yield. M.p. 80°. $[\alpha]_D^{22} = -8°$ in dimethylformamide. Analysis calculated for $C_{18}H_{27}O_6N_3$: C 56.7; H 7.1; O 25.2; N 11.0. Found: C 56.9; H 7.3; O 25.0; N 10.9). Condensation of the two dipeptides by dicyclohexylcarbodiimide resulted in methyl N-carbobenzoxy-*L*-pyroglutamyl-*L*-prolyl-*L*-seryl-ε-N-carbobenzoxy-*L*-lysinate (83% yield. $[\alpha]_D^{22} = -76°$ in 95% acetic acid. Analysis calculated for $C_{36}H_{45}O_{11}N_5$: C 59.7; H 6.3; O 24.4; N 9.7. Found: C 59.3; H 6.6; O 24.1; N 9.9), which was hydrogenated to methyl *L*-pyroglutamyl-*L*-prolyl-*L*-seryl-*L*-lysinate (75% yield). This was converted by treatment with tert.-butyl *p*-nitrophenylcarbonate to methyl *L*-pyroglutamyl-*L*-prolyl-*L*-seryl-ε-N-carbo-tert.-butoxy-*L*-lysinate (68% yield), which was transformed to the corresponding hydrazide (55% yield. M.p. 120° (with. dec.). $[\alpha]_D^{22} = -64°$ in methanol. Analysis calculated for $C_{24}H_{41}O_8N_7 + {}^1/_2\,H_2O$: C 51.0; H 7.4; O 24.1; N 17.4. Found: C 50.3; H 8.0; O 24.4; N 17.8), and from this to the azide.

The condensation of *p*-nitro-phenyl N-carbobenzoxy-*L*-alaninate with methyl *L*-phenylalanyl-*L*-isoleucinate afforded methyl N-carbobenzoxy-*L*-alanyl-*L*-phenylalanyl-*L*-isoleucinate (73% yield. M.p. 152° with transition at 130°. $[\alpha]_D^{22} = -18°$ in 95% acetic acid. Analysis calculated for $C_{27}H_{35}O_6N_3$: C 65.2; H 7.1; O 19.3; N 8.5. Found: C 64.9; H 7.2; O 19.6; N 8.6). This was hydrogenated to methyl *L*-alanyl-*L*-phenylalanyl-*L*-isoleucinate, which was condensed by dicyclohexyl-carbodiimide with β-benzyl-N-carbobenzoxy-*L*-aspartate to methyl N-carbobenzoxy-β-benzyl-*L*-aspartyl-*L*-alanyl-*L*-phenylalanyl-*L*-isoleucinate (75% yield. M.p. 136°. $[\alpha]_D^{22} = -24°$ in 95% acetic acid. Calculated for $C_{38}H_{46}O_9N_4$: C 65.0; H 6.6; O 20.5; N 8.0. Found: C 64.5; H 6.8; O 20.3; N 8.3). This was hydrogenated to methyl L-aspartyl-*L*-alanyl-*L*-phenylalanyl-*L*-isoleucinate (92% yield. M.p. 245° with dec. $[\alpha]_D^{22} = -15°$ in 95% acetic acid. Analysis calculated for $C_{23}H_{34}O_7N_4$: C 57.7; H 7.2; O 23.4; N 11.7. Found: C 57.2; H 7.5; O 23.7; N 11.7), which was reacted with tert.-butyl *p*-nitro-phenyl-carbonate to methyl N-carbo-tert.-butoxy-*L*-aspartyl-*L*-alanyl-*L*-phenylalanyl-*L*-isoleucinate (80% yield. M.p. 120° with dec. $[\alpha]_D^{22} = -55°$ in methanol. Analysis calculated for $C_{28}H_{42}O_9N_4$: C 58.1; H 7.3; O 24.9; N 9.7. Found: C 58.4; H 7.5; O 24.3; N 10.2). Reaction with hydrazine hydrate gave the hydrazide (78% yield. M.p. 220° with dec.), which was converted to the azide.

Methyl N-trityl-glycyl-*L*-leucinate was converted to the corresponding hydrazide (68% yield. M.p. 189°. $[\alpha]_D^{22} = -34°$ in 95% acetic acid. Analysis calculated for $C_{27}H_{32}O_2N_4$: C 73.0; H 7.3; O 7.2; N 12.6. Found: C 72.7; H 7.4; O 7.8; N 12.9). Transformation to the azide and condensation with methyl *L*-methionate afforded methyl N-trityl-glycyl-*L*-leucyl-*L*-methionate (66% yield. M.p. 73°. $[\alpha]_D^{22} = -8°$ in dimethylformamide. Analysis calculated for $C_{33}H_{41}O_4N_3S$: C 68.8; H 7.2; O 11.1; N 7.3; S 5.6. Found: C 69.0; H 7.3; O 11.4; N 7.3; S 5.3). Amidification gave N-trityl-glycyl-*L*-leucyl-*L*-methioninamide (54% yield. M.p. 212°. $[\alpha]_D^{22} = -4°$ in dimethylformamide. Analysis calculated for $C_{32}H_{40}O_3N_4S$: C 68.5; H 7.1; O 8.6; N 10.0; S 5.7. Found: C 68.7; H 7.2; O 8.8; N 10.5; S 6.1), which was converted by heating in aqueous acetic acid to glycyl-*L*-leucyl-*L*-methioninamide (98% yield. M.p. 130.° Analysis calculated for $C_{13}H_{26}O_3N_4S$: C 49.1; H 8.3; O 15.1; N 17.6; S 10.1. Found: C 49.1; H 8.6; O 15.7; N 17.6; S 10.1).

This tripeptide was condensed with the above-mentioned N-carbo-tert.-butoxy-*L*-aspartyl-*L*-alanyl-*L*-phenylalanyl-*L*-isoleucine azide to N-carbo-tert.-butoxy-*L*-aspartyl-*L*-alanyl-*L*-phenylalanyl-*L*-isoleucyl-glycyl-*L*-leu-



cyl-*L*-methioninamide (55% yield. M.p. 245° with dec. $[\alpha]_D^{22} = -35°$ in dimethylformamide. Analysis calculated for $C_{40}H_{64}O_{11}N_8S$: C 55.5; H 7.5; O 20.4; N 13.0; S 3.7. Found: C 54.7; H 7.7; O 20.1; N 12.8; S 3.8). Treatment with trifluoroacetic acid gave a quantitative yield of *L*-aspartyl-*L*-alanyl-*L*-phenylalanyl-*L*-isoleucyl-glycyl-*L*-leucyl-*L*-methioninamide. This was shown to possess the same electrophoretical mobilities, the same amino acid composition and the same behaviour towards chymotrypsin, as the larger of the two peptides obtained by the action of trypsin on Eledoisin[1].

Condensation of this synthetic heptapeptide with the above-mentioned *L*-pyroglutamyl-*L*-prolyl-*L*-seryl-ε-N-carbo-tert.-butoxy-*L*-lysine azide afforded L-pyroglutamyl-*L*-prolyl-*L*-seryl-ε-N-carbo-tert.-butoxy-*L*-lysyl-*L*-aspartyl-*L*-alanyl-*L*-phenylalanyl-*L*-isoleucyl-glycyl-*L*-leucyl-*L*-methioninamide (64% yield. M.p. 200° with dec. $[\alpha]_D^{22} = -57°$ in 95% acetic acid. Analysis calculated for $C_{59}H_{93}O_{17}N_{13}S + 2^1/_2\ H_2O$: C 53.0; H 7.4; O 23.4; N 13.6; S 2.4. Found: C 52.7; H 7.6; O 23.3; N 13.5; S 2.4). Treatment with trifluoroacetic acid at room temperature for 2 h and counter-current distribution in *sec*-butanol/0.1 N aqueous ammonia yielded *L*-pyroglutamyl-*L*-prolyl-*L*-seryl-*L*-lysyl-*L*-aspartyl-*L*-alanyl-*L*-phenylalanyl-*L*-isoleucyl-glycyl-L-leucyl-*L*-methioninamide (51% yield. M.p. 230° with dec. $[\alpha]_D^{22} = -44°$ in 95% acetic acid. Analysis calculated for $C_{54}H_{85}O_{15}N_{13}S + 1^1/_2\ H_2O$: C 53.4; H 7.3; O 21.7; N 14.9; S 2.6. Found: C 53.2; H 7.9; O 21.4; N 14.6; S 2.8), which was found to be homogeneous and to possess the same amino acid composition, the same electrophoretic and chromatographic mobilities, the same behaviour towards trypsin and chymotrypsin, and the same biological properties as natural Eledoisin, thus proving the correctness of the structure proposed by ERSPAMER and ANASTASI[1].

Résumé. Le *L*-pyroglutamyl-*L*-prolyl-*L*-séryl-ε-N-carbo-tert.-butoxy-*L*-lysyl azide et le *L*-aspartyl-*L*-alanyl-*L*-phénylalanyl-*L*-isoleucyl-glycyl-*L*-leucyl-*L*-méthioninamide ont été synthétisés, puis condensés en *L*-pyroglutamyl-*L*-prolyl-*L*-séryl-ε-N-carbo-tert. butoxy-*L*-lysyl-*L*-aspartyl-*L*-alanyl-*L*-phénylalanyl-*L*-isoleucyl-glycyl-*L*-leucyl-*L*-méthioninamide. Après traitement par l'acide trifluoroacétique, ce dernier a donné l'endécapeptide, *L*-pyroglutamyl-*L*-prolyl-*L*-séryl-*L*-lysyl-*L*-aspartyl-*L*-alanyl-*L*-phénylalanyl-*L*-isoleucyl-glycyl-*L*-leucyl-*L*-méthioninamide, qui s'est montré doué des mêmes propriétés chimiques, physiques et biologiques que l'élédoisine naturelle.

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Pharmazeutisch-chemische Forschungslaboratorien, SANDOZ AG, Basel (Switzerland), October 16, 1961.

[1] V. ERSPAMER and A. ANASTASI, Exper. *18*, 58 (1962).

Über die Fettsäuren aus Spinatchloroplasten

In Ergänzung früher[1] mitgeteilter Befunde über die Fettsäuren aus Chloroplasten von *Antirrhinum majus* Sippe 50, der Plastom-Mutante *prasinizans* von *Antirrhinum majus* und von *Allium porrum* konnten nun die Fettsäuren zweier verschiedener Chloroplastenpräparate[2] von *Spinacia oleracea* bestimmt werden.

Das eine der untersuchten Präparate (S) wurde mit Hilfe einer hochtourigen Zentrifuge (Sharples) dargestellt und bestand zum grössten Teil aus Chloroplastentrümmern. Die Möglichkeit der Anwesenheit auch anderer Zellelemente kann nicht ausgeschlossen werden. Im Gegensatz dazu war das andere Präparat (D) über einen Dichtegradienten aus Saccharoselösungen zentrifugiert worden und bestand vorwiegend aus gut erhaltenen Chloroplasten.

[1] H. DEBUCH, Z. Naturforschung *16b*, 246 (1961).

[2] Für die Überlassung der Chloroplastenpräparate sowie alle Förderung der Arbeit danke ich Herrn Professor Dr. MENKE, Köln, auf das herzlichste.

Tab. I. Zusammensetzung der destillierten Fettsäuregemische verschiedener Chloroplasten (in % der Gesamtfettsäuren)

Chloroplastenpräparate	C_{14} Myristinsäure	C_{15}	C_{16} Palmitinsäure	C_{16} Monoensäure[a]	C_{16} Triensäure	C_{18} Stearinsäure	C_{18} Ölsäure	C_{18} Linolsäure	C_{18} Linolensäure
Spinacia oleracea S	0,4	0,5	17,8	3,8	11,5	1,0	5,4	10,8	48,8
Spinacia oleracea D	0,5	1,3	15,5	5,8	19,5	1,5	3,1	5,0	47,8
Antirrhinum majus	—	Spur	9,5	1,3	0,2	1,0	1,6	15,1	71,3
Antirrhinum majus mut. *prasinizans*	0,7	Spur	13,8	2,4	—	1,6	1,9	15,0	64,6
Allium porrum	0,4	Spur	19,7	2,1	—	1,0	3,5	29,2	44,1

[a] Bei der C_{16}-Monoensäure dürfte es sich in allen Fällen um die kürzlich erstmals aufgefundene[3] und in den Blättern von *Antirrhinum* und *Spinacia*[4] nachgewiesene Δ^3-*trans*-Hexadecensäure handeln.

Tab. II. Zusammensetzung der destillierten Fettsäuremethylester aus Gesamtlipoiden von *Spinacia oleracea*

Spinat	C_{14}	C_{15}?	C_{16} Palmitinsäure	C_{16} Δ^3-*trans*-Monoensäure	C_{16} Triensäure	C_{18} Stearinsäure	C_{18} Ölsäure	C_{18} Linolsäure	C_{18} Linolensäure	C_{18} Tetraensäure	C_{20} Arachinsäure
Chloroplasten	0,5	1,3	15,5	5,8	19,5	1,5	3,1	5,0	47,8	—	—
Gesamtblatt	0,2	Spur	12,9	2,6	4,6	Spur	6,6	16,3	56,2	Spur	0,6