In the present paper we are reporting on some of the analogues or partial sequences which have been found to be highly active, in some cases even more so than eledoisin itself. The Table indicates the level of activity on guinea-pig ileum, and, where comparisons have been made, on rabbits' and cats' blood pressure; all data referred to eledoisin taken as 100. (Where no standard deviation is given, data may be regarded as good approximations only.) In addition, the physical properties and elemental analysis of these peptides are given. The methods used for the synthesis of these peptides are described elsewhere in detail[5].

The data given in [4] have furnished evidence that any alteration to the six amino acids of the C-terminal moiety of the eledoisin molecule (i.e. -Ala-Phe-Ile-Gly-Leu-Met-NH_2) is likely to yield biologically inactive peptides. This was recently confirmed by another group of workers[6]. From the present data it will be evident that alterations to the five amino acids of the N-terminal moiety of eledoisin (i.e. H-Pyr-Pro-Ser-Lys-Asp(OH)-), even changes affecting the acid or basic groups, do not interfere to any great extent with biological activity. It thus appears that almost one-half of the peptide chain can be modified or even left out without substantial loss of biological activity. Nevertheless, such modifications do affect the relation between the biological activities, as measured in the different tests, in an unpredictable manner[7].

Zusammenfassung. Die Eigenschaften einer Serie von synthetischen Peptiden, die mit Eledoisin strukturell und wirkungsmässig verwandt sind, werden beschrieben.

E. STÜRMER, ED. SANDRIN, and R. A. BOISSONNAS

Medizinisch-biologische und Pharmazeutisch-chemische Forschungslaboratorien der Sandoz AG., Basel (Switzerland), March 13, 1964.

[5] ED. SANDRIN and R. A. BOISSONNAS, Helv. chim. acta *46*, 1637 (1963); *47*, 417 and in press (1964).
[6] E. SCHRÖDER and K. LÜBKE, Exper. *20*, 19 (1964).
[7] Further analogues of eledoisin are described and discussed in the following paper (Part III), by L. BERNARDI, G. BOSISIO, F. CHILLEMI, G. DE CARO, R. DE CASTIGLIONE, V. ERSPARMER, A. GLAESSER, and O. GOFFREDO (Exper. *20*, 306 (1964)). The results reported by these authors are in line with our own findings.

Synthetic Peptides Related to Eledoisin[1]

In a previous paper[2] a first group of synthetic peptides related to eledoisin was presented. We wish now to report briefly some chemical data and biological actions of a new group of peptides similarly related to eledoisin or to its fragments[3]. While the problem of the relationship between the chemical structure and the biological activity of eledoisin-like polypeptides will be discussed in detail elsewhere, we wish, on the grounds of the former[2] and present data, to call attention here to a few essential points:

(1) It is possible to reduce consistently the size of the eledoisin molecule, without any drastic reduction in its biological activity, by means of a progressive elimination of the N-terminal amino acid residue. A minimum of five amino acid residues is needed in order to have an appreciable activity (No. 8). This increases sharply in the C-terminal hexapeptide (No. 7) and reaches a high level in the octapeptide (No. 5). Maximum activity is attained in the nonapeptide (No. 4), which is approximately twice as active as eledoisin, even if assayed in the dog blood pressure.

(2) The structure of the highly active hexapeptide Ala-Phe-Ile-Gly-Leu-Met-NH_2 (No. 7) has been altered stepwise by changing amino acid residues; from the data reported in the Table it may be seen that substitution of the phenylalanine residue produces a tremendous decay in the specific biological activity. The same is true for any substitution of the leucine and methioninamide residues. The only striking exceptions are so far represented by compounds Nos. 39 and 40, where the substitution of the methioninamide with ethioninamide has caused a 5- to 6-fold increase in activity[4].

Changes in biological activity produced by substitution of one of the three remaining amino acids are more irregular and apparently unpredictable. A high degree of activity is present in compounds Nos. 26, 27 and 29, where isoleucine has been replaced by valine and phenylalanine. However, compounds Nos. 25 and 28, where isoleucine has been replaced by leucine and alanine, are practically devoid of activity. Likewise substitution of glycine furnishes in some cases active compounds (Nos. 30 and 32), in other cases inactive compounds (No. 31). Finally, alanine can be replaced, often with advantage, by a number of amino acids, the most interesting being lysine (No. 14), which considerably enhances the biological activity. It may be further seen from the Table that through suitable substitution of two amino acid residues (Nos. 29 and 40) it is possible to obtain hexapeptides which are more active, even on molar basis, than eledoisin itself.

(3) The introduction of a D. amino acid residue into the molecule does not necessarily destroy the biological activity. In the case of No. 20 as compared with No. 16 this activity is rather enhanced.

(4) Compounds Nos. 41 to 47 show that a free terminal amino group is not necessary for the biological activity.

(5) The activity ratio between eledoisin and a given eledoisin-like polypeptide may vary conspicuously according to the different preparation of test-objects used in the bioassay.

Riassunto. Vengono descritte le proprietà di una serie di peptidi sintetici affini all'Eledoisina sia per la struttura che per l'attività.

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[1] Part III. For part II see [3]; for part I see [2].
[2] B. CAMERINO, G. DE CARO, R. A. BOISSONNAS, ED. SANDRIN, and E. STÜRMER, Exper. *19*, 339 (1963).
[3] In the paper by E. STÜRMER, ED. SANDRIN, and R. A. BOISSONNAS (Part II) in this same issue (Exper. *20*, 303 (1964)) another large group of peptides related to eledoisin is presented and discussed.
[4] The biological activities of various polypeptides having as C-terminal amino acid S-alkyl-homocysteinamides and their homologues will be the subject of a later report.

No.	Chemical formula	Relative biological activities: Hypotensive effect in dog	Contraction of rabbit large intestine	Contraction of guinea-pig ileum	Mp. (in dec.)	$[\alpha]_D^{22}$ in 95% AcOH (c = 0.8)	Electrophoretic mobility[d]	Elemental formula		Elemental analysis: C	H	N
1	H-Pyr-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu-Met-NH_2 (Eledoisin)	100[e]	100[e]	100[e]								
2	H—Ala-Phe-Ile-Gly-Ala-Phe-Ile-Gly-Leu-Met-NH_2	30	–	60	~260°	−40°[c]	0.60	$C_{51}H_{79}O_{10}N_{11}S$ · HCl	C F	57.03 56.81	7.50 7.89	14.34 13.98
3	H—Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu-Met-NH_2	150	180–240	170	~250°	−18°[a]	0.65	$C_{49}H_{80}O_{13}N_{12}S$ · $2CF_3COOH$	C F	48.76 48.85	6.33 6.80	12.88 13.08
4	H—Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu-Met-NH_2	175	350–700	210	~245°	−15°[a]	0.70	$C_{44}H_{73}O_{12}N_{11}S$ · $2CF_3COOH \cdot 2H_2O$	C F	46.33 46.38	6.40 6.58	12.38 12.27
5	H—Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu-Met-NH_2	80–100	120	110	214–216°	−18°	0.77	$C_{41}H_{68}O_{10}N_{10}S$ · $2CF_3COOH \cdot H_2O$	C F	47.45 47.45	6.50 6.71	12.27 12.07
6	H—Asp(OH)-Ala-Phe-Ile-Gly-Leu-Met-NH_2	30	60	35	~230°	−17°[a]	0.66	$C_{35}H_{56}O_9N_8S$ · HBr	C F	49.70 49.15	6.79 7.21	13.25 12.89
7	H—Ala-Phe-Ile-Gly-Leu-Met-NH_2	15	30	15	~225°	−20°	0.50	$C_{31}H_{51}O_6N_7S$	C F	57.30 57.00	7.90 8.10	15.10 14.80
8	H—Phe-Ile-Gly-Leu-Met-NH_2	1–1.5	3	1.5	~230°	−20°	0.55	$C_{28}H_{46}O_5N_6S$ · HCl	C F	54.66 54.55	7.69 7.79	13.66 13.57
9	H—*Lys*—Ala-Phe-Ile-Gly-Leu-Met-NH_2	45	240	55	244–248°	−27°	0.78	$C_{37}H_{63}O_7N_9S$ · 2HCl	C F	52.22 52.61	7.70 7.69	
10	H—(*D*)*Asp*(NH$_2$)-Ala-Phe-Ile-Gly-Leu-Met-NH_2	90	270	125	252–254°	−48°	0.50	$C_{35}H_{57}O_8N_9S$ · HCl	C F	52.51 52.77	7.32 7.34	
11	H—*Gly*-Ala-Phe-Ile-Gly-Leu-Met-NH_2	50	160	65	284–285°	−44°	0.55	$C_{33}H_{54}O_7N_8S$ · HCl	C F	53.32 52.88	7.46 7.38	
12	H-*Pyr*—Ala-Phe-Ile-Gly-Leu-Met-NH_2	145	310	240	263–265°	−48°	0.15	$C_{36}H_{56}O_8N_8S$	C F	56.82 57.18	7.42 7.36	
13	H—*Asp*(NH$_2$)-Lys-Phe-Ile-Gly-Leu-Met-NH_2	200	460	220	255–256°	−31°	0.83	$C_{38}H_{64}O_8N_{10}S$ · $2HCl \cdot H_2O$	C F	50.03 49.77	7.53 7.51	
14	H—*Lys*—Phe-Ile-Gly-Leu-Met-NH_2	60–70	175	110	240–243°	−17°	0.81	$C_{34}H_{56}O_6N_8S$ · $2HCl \cdot 2H_2O$	C F	50.05 50.17	7.91 8.05	13.73 13.20
15	H—*Ser*-Phe-Ile-Gly-Leu-Met-NH_2	15	55	25	218–220°	−19°	0.51	$C_{31}H_{51}O_7N_7S$ · $CH_3COOH \cdot {}^1/_2H_2O$	C F	53.93 54.01	7.68 7.88	13.34 13.09
16	H—*Asp*(NH$_2$)-Phe-Ile-Gly-Leu-Met-NH_2	15	25	20	240–245°	−17°	0.53	$C_{32}H_{52}O_7N_8S$ · HCl	C F	52.69 52.68	7.33 7.68	

No.	Chemical formula	Relative biological activities: Hypotensive effect in dog	Contraction of rabbit large intestine	Contraction of guinea-pig ileum	Mp. (in dec.)	$[\alpha]_{22}^{D}$ in 95% AcOH (c = 0.8)	Electrophoretic mobility[a]	Elemental formula		Elemental analysis: C	H	N
17	H—*Phe*-Phe-Ile-Gly-Leu-Met-NH_2	20	55	60	287–290°	−18°	0.47	$C_{37}H_{55}O_6N_7S$ · HCl	C	58.28	7.40	
									F	58.09	7.36	
18	H—*Pro*-Phe-Ile-Gly-Leu-Met-NH_2	40	60	20	260–262°	−48°	0.53	$C_{33}H_{53}O_6N_7S$ · HCl	C	55.64	7.64	
									F	55.84	7.71	
19	H—*Met*-Phe-Ile-Gly-Leu-Met-NH_2	20	90	55	315–316°	−20°	0.44	$C_{33}H_{55}O_6N_7S_2$ · HCl	C	53.09	7.58	
									F	53.38	7.52	
20	H—(*D*)*Asp*(NH$_2$)-Phe-Ile-Gly-Leu-Met-NH_2	20	70	50	~240°	−32°	0.50	$C_{32}H_{52}O_7N_8S$ · HCl · H_2O	C	51.42	7.42	15.00
									F	52.00	7.30	14.86
21	H—*Ala*-(*D*)*Phe*-Ile-Gly-Leu-Met-NH_2	<0.2	<0.2	<0.1	~190°	−27°	0.50	$C_{31}H_{51}O_6N_7S$ · HCl	C	54.24	7.64	14.28
									F	53.95	7.91	13.95
22	H—Ala-*Try*-Ile-Gly-Leu-Met-NH_2	2.5	4	3.0	~180°	−13°	0.46	$C_{33}H_{52}O_6N_8S$ · CF_3COOH	C	52.35	6.65	13.96
									F	52.13	7.06	13.61
23	H—Ala-*Ala*-Ile-Gly-Leu-Met-NH_2	<0.1	0.3	<0.01	230–235°	−44°	0.50	$C_{25}H_{47}O_6N_7S$ · HCl	C	49.21	7.91	16.07
									F	48.87	8.21	15.78
24	H—Lys—*Ile*-Gly-Leu-Met-NH_2	<0.2	<0.1	<0.1	223–227°	−26°	0.89	$C_{25}H_{49}O_5N_7S$ · 2HCl	C	47.45	8.14	
									F	47.44	8.30	
25	H—Ala-Phe-*Leu*-Gly-Leu-Met-NH_2	1	1.5	1	195–198°	−20°	0.49	$C_{31}H_{51}O_6N_7S$ · HCl · $2\frac{1}{2}H_2O$	C	50.90	7.85	
									F	50.84	7.98	
26	H—Ala-Phe-*Val*-Gly-Leu-Met-NH_2	15	30	15	285–288°	−23°	0.50	$C_{30}H_{49}O_6N_7S$ · HCl · $1\frac{1}{2}H_2O$	C	51.52	7.64	
									F	51.62	7.55	
27	H—Ala-Phe-*Phe*-Gly-Leu-Met-NH_2	10	50	25	284–286°	−20°[b]	0.49	$C_{34}H_{49}O_6N_7S$ · HCl · H_2O	C	55.29	7.11	
									F	55.19	7.14	
28	H—Ala-Phe-*Ala*-Gly-Leu-Met-NH_2	<0.2	–	<0.3	160–163°	−12°[b]	0.48	$C_{28}H_{45}O_6N_7S$ · HCl	C	52.19	7.21	
									F	51.94	7.64	
29	H—Lys—Phe-*Phe*-Gly-Leu-Met-NH_2	200–250	–	100	165–170°	−16°[b]	0.82	$C_{37}H_{56}O_6N_8S$ · 2HCl · $1\frac{1}{2}H_2O$	C	52.84	7.33	
									F	52.92	7.58	
30	H—Ala-Phe-Ile-*Ala*-Leu-Met-NH_2	20	65	45	309–310°	−33°	0.44	$C_{32}H_{53}O_6N_7S$ · HCl · H_2O	C	53.50	7.71	
									F	53.38	8.06	
31	H—Ala-Phe-Ile-*Phe*-Leu-Met-NH_2	0.4	0.5	0.5	282–285°	−35°	0.38	$C_{38}H_{57}O_6N_7S$ · HCl · H_2O	C	57.44	7.63	
									F	57.22	7.58	
32	H—Ala-Phe-Ile-*Sar*-Leu-Met-NH_2	20	60	55	117–119°	−30°	0.45	$C_{32}H_{52}O_6N_7S$ · HCl · $2H_2O$	C	52.27	7.81	
									F	52.18	7.92	

33	H—Ala-Phe-*Ile*-Gly-*Ile*-Met-NH_2	<0.1	<0.2	<0.1	294–296°	−28°	0.48	$C_{31}H_{51}O_6N_7S$ · HCl	C F	54.25 54.52	7.64 7.91	
34	H—Ala-Phe-Ile-Gly-*Ser*-Met-NH_2	<0.2	<0.3	<0.1	211–212°	−13°[a]	0.53	$C_{28}H_{45}O_7N_7S$ · CF_3COOH · H_2O	C F	47.66 47.34	6.41 6.23	
35	H—Ala-Phe-Ile-Gly-*Ala*-Met-NH_2	0.25	3	0.1	290–292°	−24°	0.53	$C_{28}H_{45}O_6N_7S$ · HCl · H_2O	C F	50.78 50.76	7.31 7.39	
36	H—Ala-Phe-Ile-Gly-*Asp*(NH$_2$)-Met-NH_2	<0.1	0.25	<0.1	249–251°	−18°	0.51	$C_{29}H_{46}O_6N_8S$ · HCl · $3H_2O$	C F	48.01 48.28	7.38 7.08	
37	H—Ala-Phe-Ile-Gly-Leu-Met-$N(CH_3)_2$	<0.2	<0.2	<0.1	284–286°	−20°	0.42	$C_{33}H_{55}O_6N_7S$ · HCl · $2H_2O$	C F	52.82 52.84	8.08 8.18	13.07 12.76
38	H—Ala-Phe-Ile-Gly-Leu-(*D*)*Met*-NH_2	<0.1	<0.2	0.1	288–289°	− 8°	0.48	$C_{31}H_{51}O_6N_7S$ · HCl · H_2O	C F	52.86 52.72	7.74 7.85	
39	H—Ala-Phe-Ile-Gly-Leu-*Eti*-NH_2	65–100	220	85	293–295°	−25°	0.49	$C_{32}H_{53}O_6N_7S$ · HCl · $2H_2O$	C F	52.20 52.62	7.93 7.86	13.31 13.07
40	H—Lys—Phe-Ile-Gly-Leu-*Eti*-NH_2	180–220	600	155	246–247°	−14°	0.82	$C_{35}H_{60}O_6N_8S$ · 2HCl · $4H_2O$	C F	48.54 48.88	7.92 8.10	
41	CTB—(*D*)*Asp*(NH$_2$)-Ala-Phe-Ile-Gly-Leu-Met-NH_2	25	230	85	262–263°	−17°[a]	0	$C_{40}H_{65}O_{10}N_9S$ · H_2O	C F	54.96 54.81	7.74 7.82	
42	CTB—*Gly*-Ala-Phe-Ile-Gly-Leu-Met-NH_2	30	190	160	266–268°	−30°[a]	0	$C_{38}H_{62}O_9N_8S$	C F	56.55 56.64	7.74 7.81	
43	CTB—Ala-Phe-Ile-Gly-Leu-Met-NH_2	25	180	145–170	254–256°	−28°[a]	0	$C_{36}H_{59}O_8N_7S$	C F	57.65 57.40	7.93 8.07	
44	CBO—Ala-Phe-Ile-Gly-Leu-Met-NH_2	15	230	95	242–244°	−26°[a]	0	$C_{39}H_{57}O_8N_7S$	C F	59.74 59.74	7.32 7.38	12.51 12.32
45	CTB—Ala-Phe-Ile-*Sar*-Leu-Met-NH_2	25	–	–	193–195°	−42°[a]	0	$C_{37}H_{60}O_8N_7S$	C F	58.25 58.48	7.93 8.04	
46	CTB—*Pro*-Phe-Ile-Gly-Leu-Met-NH_2	35	600	260	242–243°	−43°[a]	0	$C_{38}H_{61}O_8N_7S$	C F	58.81 58.64	7.92 7.99	12.64 12.65
47	CTB—*Lys*(CTB)-Phe-Ile-Gly-Leu-Met-NH_2	5	200	105	244–245°	−28°[a]	0	$C_{44}H_{74}O_{10}N_8S$	C F	58.25 58.10	8.22 8.41	
48	CTB—*Asp*(NH$_2$)-Phe-Ile-Gly-Leu-Met-NH_2	15	115	50	227–230°	−29°	0	$C_{37}H_{60}O_9N_8S$	C F	56.03 55.82	7.64 7.98	

[a] In DMF c = 0.5%. [b] In MeOH 50% c = 0.5%. [c] In HCOOH c = 0.5%. [d] In HCOOH/CH_3COOH/H_2O (15:10:75) with leucine as standard.

[e] The activity of Eledoisin is arbitrarily taken as 100; the activity of the other peptides is compared on a weight base and expressed in percentage

Abbreviations: Eti = ethionyl, CBO = carbobenzyloxy, CTB = carbo-tert.-butyloxy.