hkl	(1) $\lvert F_{MN}\rvert$	(2) $\lvert F_{hkl}\rvert$ $\lvert F_{\bar{h}\bar{k}\bar{l}}\rvert$	(3) $\lvert F_{\bar{h}kl}\rvert$ $\lvert F_{h\bar{k}\bar{l}}\rvert$	(4) $\left(\frac{\Delta I}{\bar{I}}\right)_{obs.}$	(5) $\left(\frac{\Delta I}{\bar{I}}\right)_{calc.}$	(6) $\lvert F_{hkl}\rvert$ calc.	(7) $\lvert F_{\bar{h}\bar{k}\bar{l}}\rvert$ calc.
111	140	141 137	105 104	+ 0.56	+ 0.50	119	92
222	170	169 166	155 154	+ 0.16	+ 0.13	139	130
333	82	81 80	87 85	— 0.13	— 0.13	74	79
444	28	36 35	41 40	— 0.28	— 0.20	37	41
211	101	97 97	102 100	— 0.09	— 0.18	69	75
422	183	163 159	165 161	— 0.03	— 0.06	138	142
633	102	114 113	112 111	+ 0.07	0.00	91	91
112	28	31 32	22 22	+ 0.71	+ 0.68	23	16
224	40	45 45	39 39	+ 0.28	+ 0.45	49	39

Note that the columns of this Table contain: (1) structure amplitudes observed by MILLS and NYBURG[14]; (2), (3) structure amplitudes, based on presently measured intensity data and scaled by comparison with the data in column (1). The crystal symmetry class (222) relates the data in column (2) to the form $\{hkl\}$ and the data in column (3) to the form $\{\bar{h}\bar{k}\bar{l}\}$; (4) the intensity difference $(\Delta I/\bar{I})$, given by $2\,(I_{hkl} + I_{\bar{h}k\bar{l}} - I_{\bar{h}kl} - I_{h\bar{k}\bar{l}})/(I_{hkl} + I_{\bar{h}k\bar{l}} + I_{\bar{h}kl} + I_{h\bar{k}\bar{l}})$ where I_{hkl} etc. are observed integrated intensities of X-ray reflections. The maximum e.s.d. in $(\Delta I/\bar{I})$ from counting statistics is 0.02. The maximum error in an individual intensity measurement due to X-ray absorption is estimated to be 5%, from a consideration of the variation in intensity of $0k0$ reflections with rotation about the diffractometer Φ-axis; (5) the calculated intensity difference $(\Delta I/\bar{I})_{calc.}$, given by $2\left(\lvert F_c\rvert^2_{hkl} - \lvert F_c\rvert^2_{\bar{h}\bar{k}\bar{l}}\right)/\left(\lvert F_c\rvert^2_{hkl} + \lvert F_c\rvert^2_{\bar{h}\bar{k}\bar{l}}\right)$; (6), (7) calculated structure amplitudes, based on MILLS and NYBURG's atomic parameters. Values for atomic scattering factors, including the CuKα dispersion correction for iodine ($\Delta f' = -1.2$, $\Delta f'' = 7.0$) were taken from 'International Tables for X-ray Crystallography', Vol. 3 (Kynoch Press, 1962).

with the needle axis (b) along the Φ-axis of a G.E. single crystal orienter. Integrated intensity measurements were made using CuKα radiation and a scintillation counter as detector, with $\theta/2\theta$ scans of 2° in 2θ and background counts at each scan limit.

Zusammenfassung. Die absolute Konfiguration von (–)-Aspidospermin-N(b)-jodmethylat wurde röntgenographisch unter Anwendung der anomalen Streuung bestimmt. Dadurch ist die absolute Konfiguration von (–)-Aspidospermin festgelegt.

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Synthetic Peptides Related to Caerulein[1]. Note 1

A number of caerulein-like peptides has been synthesized in our laboratories. This first report describes the activity of the compounds listed in Table I, which shows the amino acid composition and sequence of these peptides, as well as their molecular weight and electrophoretic mobilities.

Synthesis. Compounds 3, 4, 7, 14 were synthesized by the stepwise procedure starting from the known tetrapeptide Trp-Met-Asp-Phe-NH_2[2] and adding the activated ester or the azide of the appropriate *ter*-butyloxycarbonyl amino acid, followed by treatment with AcOH/HCl.

The synthesis of compounds 5, 8, 10, 13, 15 was achieved by treating with pyridine/SO_3, the corresponding peptide having the amino group protected as trifluoroacetyl derivative. After sulphation, the protecting group (or groups) was eliminated by alkaline treatment.

Trifluoroacetyltyrosine azide was obtained according to the scheme: Tfa-Tyr → Tfa-Tyr-NHNH-Boc → Tfa-Tyr-$NHNH_2$ → Tfa-Tyr-N_3, and was condensed with

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Table I

No.		mol. wt.	$E_{1,2}$ Leu	$E_{5,8}$ Tyr(S)
1	Pyr-Gln-Asp-Tyr(S)-Thr-Gly-Trp-Met-Asp-Phe-NH_2 (caerulein)	1352		
2	Pyr-Gln-Asp-Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH_2	1272		
3	Thr-Gly-Trp-Met-Asp-Phe-NH_2	755	0.47	
4	Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH_2	930	0.42	
5	Tyr(S)-Thr-Gly-Trp-Met-Asp-Phe-NH_2	1010		0.17
6	Tyr(P)-Thr-Gly-Trp-Met-Asp-Phe-NH_2	998		0.24
7	Tyr——Gly-Trp-Met-Asp-Phe-NH_2	817	0.43	
8	Tyr(S)——Gly-Trp-Met-Asp-Phe-NH_2	897		0.21
9	Tyr(P)——Gly-Trp-Met-Asp-Phe-NH_2	897		0.31
10	Tyr(S)-Thr——Trp-Met-Asp-Phe-NH_2	941		0.19
11	Tyr(S)————Trp-Met-Asp-Phe-NH_2	840		0.23
12	Tyr(P)————Trp-Met-Asp-Phe-NH_2	840		0.30
13	Tyr(S)-*Gly*-Gly-Trp-Met-Asp-Phe-NH_2	954		0.19
14	*Met*-Gly-Trp-Met-Asp-Phe-NH_2	785	0.45	
15	Tyr(S)-*Met*-Gly-Trp-Met-Asp-Phe-NH_2	1028		0.16
16	Tyr(P)-*Met*-Gly-Trp-Met-Asp-Phe-NH_2	1028		0.24
17	*Boc*-Tyr(S)-Thr-Gly-Trp-Met-Asp-Phe-NH_2	1098		0.54
18	*Boc*-Tyr(S)-*Met*-Gly-Trp-Met-Asp-Phe-NH_2	1152		0.49
19	Gastrin I (human)	2098		

Boc = *ter*-butyloxycarbonyl. Tyr(S) = Tyrosine-*O*-sulphate. Tyr(P) = Tyrosine-*O*-phosphate.

Trp-Met-Asp-Phe-NH_2 (No. 11), Gly-Trp-Met-Asp-Phe-NH_2 (No. 8) and Met-Gly-Trp-Met-Asp-Phe-NH_2 (No. 15).

Compounds 5 and 10 were most easily obtained by condensing the appropriate tetra- and pentapeptides with Tfa-Tyr-Thr(Ac)-N_3 which was synthesized in good yields according to the scheme:

Boc-Thr → Boc-Thr-NHNH-Z $\xrightarrow{AcOH/HCl}$ $^+H_2$-Thr(Ac)-NHNH-Z ·

Cl^- $\xrightarrow{Tfa\text{-}Tyr\text{-}N_3}$ Tfa-Tyr-Thr(Ac)-NHNH-Z → Tfa-Tyr-Thr(Ac)-$NHNH_2$

→ Tfa-Tyr-Thr(Ac)-N_3

Compound 13 was obtained by condensation of Trp-Met-Asp-Phe-NH_2 with Tfa-Tyr-Gly-Gly-N_3 which was prepared according to the scheme:

Z-Gly-Gly → Z-Gly-Gly-NHNH-Boc → Gly-Gly-$NHNH_2$-Boc

$\xrightarrow{Tfa\text{-}Tyr\text{-}N_3}$ Tfa-Tyr-Gly-Gly-NHNH-Boc → Tfa-Tyr-Gly-Gly-$NHNH_2$ → Tfa-Tyr-Gly-Gly-N_3.

Boc-Tyr-ONp was treated with pyridine/SO_3 and with pyridine/$POCl_3$ to give Boc-Tyr(SO_3H)-ONp and Boc-Tyr(H_2O_3P) respectively. The latter was condensed with Trp-Met-Asp-Phe-NH_2, Gly-Trp-Met-Asp-Phe-NH_2, Thr-Gly-Trp-Met-Asp-Phe-NH_2 and Met-Gly-Trp-Met-Asp-Phe-

Table II. Relative biological activity

Polypeptide No.	Dog blood pressure	Denervated gastric pouch of the dog	Perfused rat stomach	Dog pancreas	Guinea-pig gall bladder	Dog jejunum
1 (caerulein)	100	100	100	100	100	100
2	< 1	2–5	8–10	4–4.5	< 1	< 1
3	< 1	1–2	0.2–0.4	3.5–4	< 0.05	–
4	< 1	< 1	< 1	1.2–2.5	< 0.05	< 1
5	30–40	20–40	30	60–80	30–50	–
6	< 1–2	5.5–7	0.5–1	5–10	< 1	1.5
7	< 0.5	0.5–1	0.5	1.5–3	< 0.1	–
8	0.5–1	8–20	2–5	10–30	< 0.5	< 1
9	0.5–2	2	1	5–7	< 1	< 1
10	–	2–3	2–3	3–5	10–15	7–8
11	< 1	–	2–3	1–2	< 1	< 1
12	< 1	2–3	1–1.5	2.5–3	< 1	–
13	2–3	3–4	3–4	10–15	4–10	8–9
14	1	2	1	1.5–2	< 1	–
15	70–140	10–20	60–100	65–70	80–150	–
16	2–2.5	–	1.5–2.5	3–5	< 1	–
17	40	50–60	35–45	30–40	50–60	–
18	65–70	50–75	60–80	55–75	50–75	–
Gastrin-I (human)	< 1	25–35	2–10	1.5–2.5	0.01–0.03	< 1
Cholecystokinin pancreozymin	3.5–5	–	10	6–7	7–9	6–9

– Not investigated.

NH_2 to give, after treatment with trifluoroacetic acid (to which the tyrosine *O*-phosphate bond is stable) the free peptides 12, 9, 6 and 16. Compounds 17 and 18 were obtained in good yields by condensation of Boc-Tyr(SO_3H)-ONp with the appropriate free peptide.

Pharmacological activity. The activities of the different synthetic caerulein-like peptides were assayed in parallel on the following test objects: dog blood pressure (hypotension), denervated gastric pouch of the dog (stimulation of acid secretion), perfused rat stomach preparation (stimulation of acid secretion), dog pancreas (stimulation of secretion), guinea-pig gall bladder (contraction), in situ dog jejunal loop (stimulation of movements and tone). The activity of a given weight of pure natural caerulein was always considered equal to 100, and the activity of the same weight of the other polypeptides was expressed as %. Results of parallel assays are shown in Table II.

For comparison purposes synthetic human gastrin-I and pure natural cholecystokinin-pancreozymin were also included in Table II. In the case of the last peptide a sample, possessing 3000 U of activity/mg, kindly supplied by Prof. E. JORPES of Stockholm, was used.

From the tabulated data a few preliminary conclusions may be drawn on the relationship between chemical structure and biological activity:

(1) The presence in the caerulein molecule of a sulphated tyrosyl residue is of decisive importance for the biological activity of the polypeptide. In fact, desulphation of the tyrosyl residue invariably causes a conspicuous decrease in the biological activity. This may be clearly observed in the peptide couples, No. 1–2 and No. 4–5.

(2) Substitution of the sulphuric acid with a phosphoric acid residue yields a less active compound: compare Nos. 6 and 16 with Nos. 5 and 15.

(3) Omission of the threonyl residue as in No. 8, which is incidentally the C-terminal hexapeptide of gastrin-II, gives a peptide which still retains a considerable activity on the gastric and pancreatic secretions of the dog, but which is deprived of any significant action on vascular and extravascular smooth muscle.

(4) Substitution of the threonyl residue in the C-terminal heptapeptide of caerulein (No. 5) produces different and unpredictable results, depending on the entering amino acid residue. Glycine (No. 13) is an unfavourable substituent, whereas methionine (No. 15) causes only minor but interesting changes in the activity spectrum.

(5) No significant change in activity is noted when the N-terminal residue is protected by the *ter*-butyloxycarbonyl group (*Boc*) (No. 17 and 18) or not (No. 5 and 15).

A glance at Table II seems to justify the hope that it will be possible, with opportune changes in the caerulein molecule, to dissociate the action on smooth muscle from that on secretory cells, and perhaps the action on gastric secretion from that on pancreatic secretion. This is the aim of our efforts[3].

Riassunto. Vengono descritte le proprietà di una serie di peptidi sintetici affini alla ceruleina e si discutono brevemente i rapporti fra attività e struttura.

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