ponses ocurred with IBA given either simultaneously with sucrose or as 16 h pretreatment. Likewise, IBA alone did not elicit any differential growth. On the other hand, bending of the internodes caused by $0.3M$ sucrose was nil in the presence of $0.2M$ ammonium nitrate, $0.2M$ urea or $10^{-4}M$ gibberellic acid (GA_3).

Considering these results they seem to corroborate the hypothesis that the balance sucrose/gibberellin is responsible for the differentiation of diageotropic stems in *Cynodon sp.* However, for this to occur nitrogen there must not be above a certain level. The ratio sucrose/nitrogen as determining whether one stem bud would become a stolon or a tiller is a new factor in the geotropic process, albeit old in other aspects of plant physiology. A recent article[3] points out that the growth direction of *Agropyron repens* rhizomes depends upon nitrogen supply. This finding supports the conclusion that nitrogen also plays a rôle in the plagiotropism of grasses.

Admitting a priori the futility of interpreting the mode of action of these substances, on the ground that the geotropic mechanism in plants is far from being elucidated, a brief speculation may be of value for future work. The hypothesis previously stated [1,2] that in presence of high concentration of sucrose an antihormone would be synthetized on the low side of the stem, counteracting the effect of some growth factor, is still valid. Nitrogen could divert the metabolic sucrose pathway to another process and consequently shift the balance towards the growth promotor.

According to the hormonal theory, the difference in growth rate between the two sides of the curving stem is causally correlated with a differential distribution of auxin. However, considering the inactivity of the auxin in this and other experiments[1,2], it is easier to accept the proposed idea that an anti-hormone is present in the low side of the stems of *Cynodon sp.* The following facts further support this hypothesis: a) Sucrose induces epinasty in the presence or absence of saturating amounts of exogenous auxin. b) Sucrose keeps stolons and rhizomes growing horizontally under similar condition. c) Proofs have been reported[2] that auxins are taken up and distributed to the whole stem piece. d) Auxin destruction is excluded on the basis of its chemical nature. It is unlikely, then, that IBA were not present during the differential growth leading to the epinasty of the internodes. In this case, it is also very difficult to conceive an uneven and subtle distribution of the exogenous auxin in the stems, considering the high concentration utilized and the small differences required to elicit an unequal growth rate on both sides of the internodes.

Summing up, in the plagiotropism phenomenon of *Cynodon sp.*, only the presence of geosensors in the cells can be considered as an obvious fact. Their behaviour depends on the gravitational force and non-specific chemical substances like sucrose and nitrogen compounds, acting probably through an unknown hormone-antihormone system, in which the gibberellins are involved[4].

Zusammenfassung. Nachweis, dass Saccharose in Ausläufern von *Cynodon plectostachyum* Epinastie verursacht, was durch Gibberellinsäure und stickstoffhaltige Verbindungen antagonisiert wird. Indol-3-Buttersäure veränderte die Wirkung des Zuckers nicht.

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8 January 1973.

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STUDIORUM PROGRESSUS

Inhibition of Angiotensin-Converting Enzyme by Analogs of Peptides from *Bothrops jararaca* Venom

The product of the reaction catalyzed by the kidney enzyme renin is a biologically inactive decapeptide, angiotensin I[1]. Further cleavage of this decapeptide to form the vasopressor octapeptide angiotensin II is achieved by action of angiotensin-converting enzyme, a carboxypeptidyl dipeptide hydrolase found in blood and most other tissues[2-8]. This 'converting enzyme' is not specific in its action on angiotensin I, but cleaves dipeptide residues from the C-terminal end of many peptides, including bradykinin[9-14].

The probable physiological importance of angiotensin-converting enzyme, both for production of angiotensin II and for destruction of bradykinin, has prompted us to search for inhibitors of its action. Although many peptides and some nonpeptidic compounds are moderately potent inhibitors of angiotensin-converting enzyme[2,10-12,15-17], the most potent and specific inhibitors by far are several structurally similar peptides that have been isolated from the venoms of the South American pit viper *Bothrops jararaca*[18-21] and the Japanese pit viper *Agkistrodon halys blomhoffii*[22-25]. These venom peptides, the structures of which are shown in Table I, have been demonstrated to be potent inhibitors of angiotensin-converting enzyme in vitro[6-8,10-12,14,26-33] and in vivo[27-29,34-40]; they are competitive with substrates such as angiotensin I and bradykinin[8,26,33].

With the hope of obtaining a better understanding of the structural requirements for inhibition of angiotensin-converting enzyme by venom peptides, we have prepared and tested a large number of analogs of the peptides from *Bothrops jararaca* venom. The structures of these analogs (Table II) are all related to those of the naturally occurring pentapeptide (**1**), nonapeptide (**31**), or decapeptide (**46**); all peptides are aligned to permit direct comparison of their C-terminal amino acid residues. Most of the analogs of the pentapeptide (**2–28**) contain the more stable amino acid phenylalanine, rather than tryptophan, but this substitution does not appear to affect inhibitory activity (cf. peptides **1** and **2**). Peptides were synthesized by solid-phase methods, or in solution by fragment-condensation methods[41,42]. Their inhibitory potencies were tested against activities of crude or homogenous preparations of angiotensin-converting enzyme of rabbit lung, assayed spectrophotometrically or spectrofluorometrically[8,9,26].

Although angiotensin-converting enzyme cleaves dipeptide residues from its substrates, it is an exopeptida-

se[9–14], for which the presence on the substrate of a free C-terminal carboxyl group is an absolute requirement for activity. The same requirement is observed for inhibition of angiotensin-converting enzyme by the venom peptides or their analogs; peptides **18** and **45**, which differ from the potent inhibitors **2** and **31**, respectively, only by their lack of a C-terminal carboxyl group (pyrrolidine replaces proline), are completely inactive. Methyl ester or amide derivatives of peptide **2** retain only 5 and 2%, respectively, of its inhibitory activity.

Other structural features that affect the ability of a peptide to serve as a substrate for angiotensin-converting enzyme seem to be confined to the amino acid residues composing the C-terminal tripeptide. This would indicate that only the last three amino acid residues of substrates interact significantly with the active site of the enzyme; in agreement with this hypothesis, the protected tripeptide Z-Phe-His-Leu[9] has a K_m value (binding affinity) equal to that of the complete decapeptide angiotensin I[8,17,26]. As long as substrates possess a free carboxyl group, many different C-terminal dipeptide residues appear to be equally suitable as leaving groups[11–14], but angiotensin-converting enzyme does not readily hydrolyze peptides containing a penultimate imino acid such as proline, or a C-terminal dicarboxylic amino acid such as glutamic acid[13]. Substrates containing a phenylalanine residue in the third position from the C-terminus are bound much more tightly to the enzyme than those having other residues in this position[11–14]. Similar structure-activity correlations are obtained for the C-terminal tripeptide residues of the inhibitors, indicating that these residues are bound to the enzyme in a manner analogous to the corresponding residues of substrates. Thus, a penultimate proline residue (peptides **15**, **23**, and most analogs of **31** or **46**) or a C-terminal glutamic acid residue (peptide **17**) greatly decreases the activity of the peptidic inhibitors. Inhibitor analogs lacking a phenylalanine or tryptophan residue in the third position from the C-terminal end are much less active (peptides **9–11**, and most analogs of peptides **31** and **46**) than are those that contain such an aromatic residue. Inversion of the optical configuration at this position also results in loss of inhibitory activity (**12**).

Table I. Snake-venom Peptides that inhibit angiotensin-converting enzyme[a]

Bothrops jararaca	
< Glu-Lys-Trp-Ala-Pro	ref.[18,19]
< Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	ref.[20,21]
< Glu-Asn-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	ref.[20,21]
< Glu-Trp-Pro-Arg-Pro-Thr-Pro-Gln-Ile-Pro-Pro	ref.[20,21]
< Glu-Asn-Trp-Pro-His-Pro-Gln-Ile-Pro-Pro	ref.[20,21]
< Glu-Gly-Gly-Trp-Pro-Arg-Pro-Gly-Pro-Glu-Ile-Pro-Pro	ref.[20,21]
< Glu-Ser-Trp-Pro-Gly-Pro-Asn-Ile-Pro-Pro	ref.[20,21]
Agkistrodon halys blomhoffii	
< Glu-Gly-Leu-Pro-Pro-Arg-Pro-Lys-Ile-Pro-Pro	ref.[22–25]
< Glu-Gly-Leu-Pro-Pro-Gly-Pro-Pro-Ile-Pro-Pro	ref.[22–25]
< Glu-Lys-Trp-Asp-Pro-Pro-Pro-Val-Ser-Pro-Pro	ref.[22–25]

[a] < Glu is one of the designations for pyrrolidone-5-carboxylic acid (pyroglutamic acid) suggested by the IUPAC-IUB Commission on Biochemical Nomenclature (ref.[43]).

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Although pentapeptide **1** or its phenylalanine analog **2** (the model for most of our analogs) does not, at first sight, appear to be structurally related to the longer naturally occurring peptides such as **31**, our studies with analogs of both types of peptides indicate that the pentapeptides interact with the enzyme in a manner similar to that of the longer peptides. Nonapeptide **31** lacks an aromatic amino acid residue in the third position from its C-terminal end and has a proline residue in its penultimate position; it is, accordingly, much less potent as an inhibitor than is peptide **2**. However, as the C-terminal portion of its structure is altered by substitution of the desirable aromatic amino acid residue (**43**), by replacement of the undesirable proline with an alanine residue (**44**), or by both (**30**), the inhibitory activity of the resulting analogs approaches that of pentapeptide **2**. Similarly, as the C-terminal portion of the pentapeptide **2** is altered to look more like that of the longer peptides such as **31** (peptides **10** and **15**), the inhibitory potency of the resulting analogs is decreased accordingly.

As discussed above, the C-terminal tripeptide of the venom peptides competes with the analogous C-terminal tripeptides of substrates for binding to the active site of the angiotensin-converting enzyme; for this reason, we will refer to these three amino acid residues collectively as the 'competitive portion' of the structure of the venom peptides. The venom peptides, however, are bound at least 50-fold more tightly to the angiotensin-converting enzyme than are fragments consisting of only their C-terminal tripeptide sequences, and they are bound 100- to 1000-fold more tightly than are substrates normally employed for assay of the enzyme[8–10, 26]. This extra binding affinity, above and beyond that conferred by the competitive C-terminal tripeptide, must be due to amino acid residues in the remaining portion of the molecule. The greater inhibitory potency of pentapeptide **2** compared with its C-terminal fragments **19** and **20** indicates clearly the importance of the N-terminal residue <Glu-Lys for the inhibitory activity of this pentapeptide. Many substitutions for these two amino acid residues of peptide **2** yield peptides that are no more potent as inhibitors than are the C-terminal fragments alone (**4**, **7**, **8**). Glutamine or norleucine (**5**, **6**), however, may be substituted for lysine, and cycloalkane carboxylic acids (e.g. **3**) may be substituted for pyrrolidone carboxylic acid to give highly potent analogs. The analogous N-terminal portion of the longer venom peptide **31** is <Glu-Trp-Pro-Arg-Pro-Gln-; the importance of this residue for inhibition of the converting enzyme is evidenced by the fact that the C-terminal tripeptide fragments of peptide **31** are not bound tightly enough to the enzyme by themselves to produce significant inhibition. When one examines the inhibitory activity of peptides with the same competitive C-terminal tripeptide, but with N-terminal portions consisting of either <Glu-Lys- or <Glu-Trp-Pro-Arg-Pro-Gln- (**10** and **15** vs **44** and **43**), the latter sequence appears to confer greater affinity for the enzyme. Substitutions in the N-terminal portion of peptide **31** (peptides **32–42**) indicate that tryptophan is one of the most important residues. The pyrrolidone carboxylic acid residue of nonapeptide **31** may be replaced by a cyclopentane carboxylic acid residue with significant increase in inhibitory activity.

Several peptides that represent fragments of or additions to the structures of the naturally occurring peptides tend to confirm some of the structure-activity correlations discussed above. Most such analogs of the pentapeptide (**19–30**) were much less potent than the parent compound, but several that incorporated the unaltered competitive C-terminal tripeptide sequence Phe-Ala-Pro were reasonably active, including the unsubstituted tripeptide it-

Table II. Inhibition of angiotensin-converting enzyme by synthetic analogs of peptides from *Bothrops jararaca* venom

Number	Structure	I_{50} (μg/ml)
1	**< Glu-Lys-Trp-Ala-Pro**	0.05
2	< Glu-Lys-Phe-Ala-Pro	0.05
3	Cpc-Lys-Phe-Ala-Pro	0.06
4	Boc-Lys-Phe-Ala-Pro	5.2
5	< Glu-Nle-Phe-Ala-Pro	0.2
6	< Glu-Gln-Phe-Ala-Pro	0.4
7	< Glu-Glu-Phe-Ala-Pro	3.0
8	< Glu-Thr-Phe-Ala-Pro	5.5
9	< Glu-Lys-Pro-Ala-Pro	1.1
10	< Glu-Lys-Ile-Ala-Pro	1.6
11	< Glu-Lys-Ser-Ala-Pro	2.4
12	< Glu-Lys-*trp*-Ala-Pro	72
13	< Glu-Lys-Phe-Lac-Pro	0.06
14	< Glu-Lys-Phe-Gly-Pro	0.1
15	<Glu-Lys-Phe-Pro-Pro	3.3
16	< Glu-Lys-Phe-Ala-Ala	0.06
17	< Glu-Lys-Phe-Ala-Glu	2.0
18	< Glu-Lys-Phe-Ala-Pyn	>200
19	Lys-Trp-Ala-Pro	1.2
20	Phe-Ala-Pro	1.4
21	Ala-Pro	50
22	< Glu-Phe-Ala-Pro	2.7
23	< Glu-Lys-Phe-Ala-Pro-Pro	4.8
24	< Glu-Lys-Phe-Ala	7.5
25	Boc-Phe-Ala-Pro	17
26	< Glu-Lys-Phe	50
27	< Glu-Lys-Phe-Pro	>200
28	< Glu-Ile-Pro-Pro-Lys-Phe-Ala-Pro	0.3
29	< Glu-Lys-Phe-Ala-Pro-Gln-Ile-Pro-Pro	3.3
30	<Glu-Trp-Pro-Arg-Pro-Lys-Phe-Ala-Pro	0.05
31	**< Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro**	1.1
32	Cpc-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	0.2
33	<Glu-Tyr-Pro-Arg-Pro-Gln-Ile-Pro-Pro	2.6
34	< Glu-Phe-Pro-Arg-Pro-Gln-Ile-Pro-Pro	2.8
35	< Glu-Leu-Pro-Arg-Pro-Gln-Ile-Pro-Pro	6.5
36	< Glu-Gly-Pro-Arg-Pro-Gln-Ile-Pro-Pro	6.5
37	< Glu-*trp*-Pro-Arg-Pro-Gln-Ile-Pro-Pro	7.0
38	< Glu-Trp-Pro-Lys-Pro-Gln-Ile-Pro-Pro	1.5
39	< Glu-Trp-Pro-His-Pro-Gln-Ile-Pro-Pro	1.9
40	< Glu-Trp-Pro-Gly-Pro-Gln-Ile-Pro-Pro	1.9
41	< Glu-Trp-Pro-Orn-Pro-Gln-Ile-Pro-Pro	2.5
42	< Glu-Trp-Pro-*arg*-Pro-Gln-Ile-Pro-Pro	9.0
43	< Glu-Trp-Pro-Arg-Pro-Gln-Phe-Pro-Pro	0.3
44	< Glu-Trp-Pro-Arg-Pro-Gln-Ile-Ala-Pro	0.4
45	< Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pyn	>200
46	**< Glu-Asn-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro**	3.7
47	< Glu-Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	4.5
48	< Glu-Lys-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	5.0
49	Pro-Arg-Pro-Gln-Ile-Pro-Pro	9.6
50	Arg-Pro-Gln-Ile-Pro-Pro	32
51	Z-Pro-Gln-Ile-Pro-Pro	78
52	< Glu-Ile-Pro-Pro	>200
53	< Glu-Trp-Pro-Arg-Pro	14
54	Pro-Arg-Pro-Gln-Ile	77
55	< Glu-Arg-Pro-Gln-Ile-Pro	>200
56	< Glu-Trp-Pro	>200
57	< Glu-Trp	>200

Naturally occurring peptides are shown in boldface, and D-amino acids are written uncapitalized in italics; abbreviations are: <Glu, pyrrolidone-5-carbonyl; Cpc, cyclopentylcarbonyl; Boc, *t*-butyloxycarbonyl; Lac, Lactyl; Pyn, pyrrolidinyl; Z, benzyloxycarbonyl.

self (**20**). Peptides **23** and **29** incorporate peptide sequence **2**, but contain additional C-terminal residues. However, as viewed from the C-terminal end, the enzyme's point of view, they are very different in structure from pentapeptide **2**, and they are about 100-fold less potent as inhibitors. Peptides **28** and **30**, each of which incorporates essentially the sequence of peptide **2** at the C-terminal end of its structure, have inhibitory potencies similar to that of pentapeptide **2**. Peptides **49** to **57** are fragments of the nonapeptide **31**. In this case, it appears that at least the C-terminal pentapeptide sequence –Pro-Gln-Ile-Pro-Pro is required for significant inhibition (peptide **51**).

Kinetic data reported elsewhere[26], as well as the structure-activity correlations described in this paper, indicate that all of the peptides found in the venom of *Bothrops jararaca* are bound to angiotensin-converting enzyme in a manner that is, in part, identical to the binding of substrates for this enzyme; this competitive interaction is necessary, but not sufficient, for inhibition. The venom peptides, unlike substrates, are also bound to a second portion of the enzyme; this second interaction greatly increases their inhibitory potency. These two interactions of the venom peptides with angiotensin-converting enzyme combine to produce extremely potent, as well as highly specific, inhibitors.

Zusammenfassung. Nachweis mit Analogen der im Gifte von *Bothrops jararaca* gefundenen Peptide, dass die Inhibition des Angiotensins «converting enzyme» von zwei eindeutigen Teilsequenzen dieser Peptide abhängig ist. Die hohe spezifische kompetitive Inhibition, hervorgerufen durch die Peptide von *Bothrops jararaca*, wird der Bindung ihrer Tripeptidreste vom Carboxyterminus mit dem aktiven Zentrum des Enzyms zugeschrieben, die in gleicher Weise wie die Peptidsubstrate mit dem Enzym gebunden werden. Die Wirksamkeit der Giftpeptide hängt von der Bindung eines zweiten Teiles der Peptide mit dem Enzym ab.

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COGITATIONES

L-Methionin als Regulator der Spannung von Collagen

Das Macromolekül Collagen, das im Körper in vielen Organen als Fibrillen und in reinster Form in den Sehnenfasern vorhanden ist, hat eine charakteristische Stabilität. Diese erklärt man als die Folge von intra- und intermolekularen kovalenten «crosslinks» (Vernetzungen) zwischen den helikal angeordneten Protein-Fäden.

Bei Zerstörung von H-Brücken durch Temperatur-Wirkung, sowie von «covalenten crosslinks» erscheinen Spannungen. Isotonisch gemessen zeigt sich Schrumpfung (Verkürzung); isometrisch gemessen, Spannungen von sehr erheblicher Grösse. Diese sind nicht reversibel.

Demgegenüber kann man ähnlich grosse Spannungen erhalten, wenn man aus Collagen mit hypertonen (hochmolaren) Salzlösungen, (wie $NaClO_4$ 5 *M* oder KJ, 2 bis 7 *M* etc.), der Faser H_2O entzieht. Diese Spannung ist in Wasser, in Ringer-Lösung oder in physiologischem NaCl (0.15 *M*) reversibel. Die Collagen Fibrille kehrt in ihren vorigen Zustand zurück (Figuren 1 und 3). Die Re-

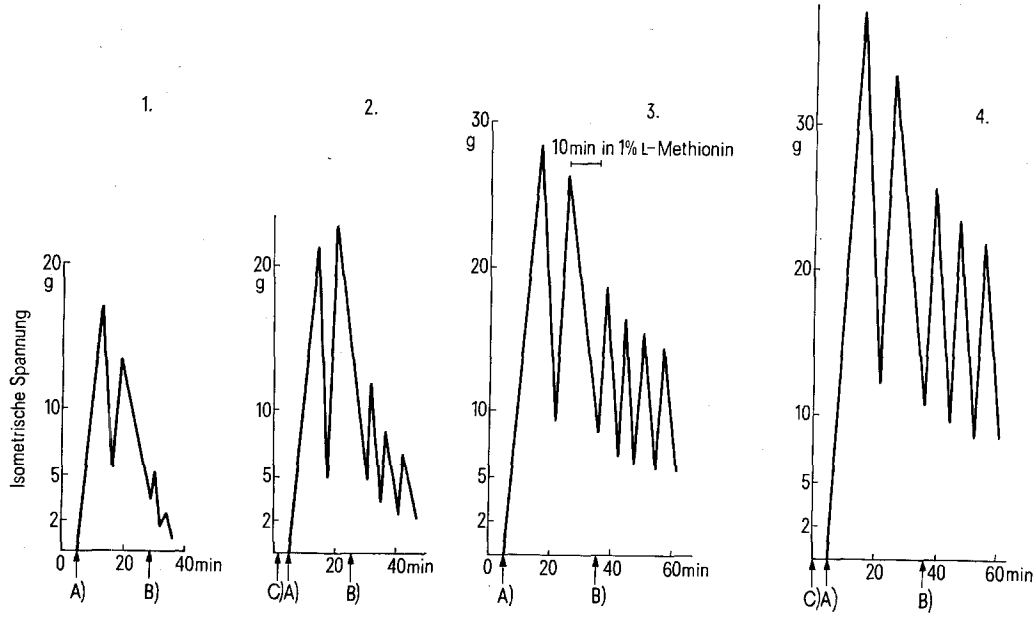

Fig. 1–4. A) Isometrische Spannungs-Entwicklung durch ↑ 5*M* $NaClO_4$ und danach Erschlaffung ↓ mit physiol. NaCl (0.15*M*). Zweimal wiederholt. B) Einlegen in 1% Methionin (10 min) vermindert die Spannungsentwicklung durch 5*M* $NaClO_4$. Mehrfach wiederholt. C) In Figuren 2 und 4 verstärkt die Vorbehandlung der Sehne mit 0.035% HCOH die Spannungsentwicklung. Figuren 1 und 2: 6 Monate (junge), Figuren 3 und 4: 28 Monate (alte) Tiere. Vertikal: Spannung in g; horizontal: Zeit in min.