neuer Weg zu dieser synthetisch bisher nicht gut zugänglichen Alkaloidklasse.

1,2,3,4-Tetrahydro-6,7-dimethoxy-isochinolin-1-essigsäure-äthylester (I), der aus Homoveratrylamin leicht erhältlich ist[10,11], wird durch Methylieren mit Formaldehyd-Ameisensäure und anschliessende Verseifung in 1,2,3,4-Tetrahydro-2-methyl-6,7-dimethoxy-isochinolin-1-essigsäure(II) (Smp. des Hydrochlorids 217–218°, Zers.) umgewandelt. Cyclisierung von II mit Polyphosphorsäure liefert 1-Methyl-1, 2, 3, 7, 8, 8a-hexahydro-5, 6-dimethoxy-7-oxo-cyclopent[ij]isochinolin (III) (Smp. 102–104°; λ_{max}^{EtOH} 259 mμ, log ε 3,90; 334 mμ, log ε 3,56; IR-Carbonylbande (KBr) bei 5,88 μ). Aus III entsteht mit Chloressigsäure-methylester und Natriumamid in Äther der Glycidester IV (Smp. 158–159°), der durch siedende äthanolisch-wässrige Kalilauge in den Aldehyd V (im Hochvakuum destillierbares Öl; IR-Aldehydbanden (Film) bei 3,62 und 5,83 μ) übergeführt wird. V reagiert in Gegenwart von Natriumhydrid oder Kalium-t-butylat mit Methyläthinyl-keton unter Bildung von D,L-Pronuciferin (VI) (Smp. 148–151°). Die synthetische Verbindung stimmt hinsichtlich UV-Spektrum (Äthanol), IR-Spektrum (Chloroform) und NMR-Spektrum (Deuterochloroform) mit natürlichem (+)-Pronuciferin überein. Durch Reduktion mit Lithiumalanat und nachfolgende Säurebehandlung entsteht aus VI D,L-Nuciferin (VII) (Smp. 136–138°), identisch mit einem durch Pschorr-Synthese erhaltenen Präparat[12].

Summary. A synthesis of the alkaloid Pronuciferine (VI) is described.

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[10] A. R. BATTERSBY, H. T. OPENSHAW und H. C. S. WOOD, J. chem. Soc. *1953*, 2463.
[11] J. M. OSBOND, J. chem. Soc. *1951*, 3464.
[12] J. M. GULLAND und R. D. HAWORTH, J. chem. Soc. *1928*, 581.

The Stability of Angiotensinamide in the Presence of Urea

It has been reported that incubation of 5-L-isoleucyl angiotensin II with high concentrations of either urea or arginine results in a considerable loss of the oxytocic activity of the polypeptide (BUMPUS et al.[1]). The results of optical rotary dispersion studies on 5-L-isoleucyl angiotensin II, in the presence and absence of urea, were interpreted as indicative of secondary order in the octapeptide; additional physical data led to the hypothesis of a helical conformation for the molecule stabilized by intra-chain hydrogen bonds (SMEBY et al.[2]). Urea decreased the oxytocic activity of 5-L-isoleucyl angiotensin II presumably by rupturing the hydrogen bonds, thus destroying the helical conformation which was considered essential for biological activity (BUMPUS et al.[1]).

PAIVA et al.[3] reported that the optical rotary dispersion of 1-L-asparaginyl-5-L-valyl angiotensin II (angiotensinamide) is not affected by the presence of urea and, in addition, presented other physical data which indicate that the octapeptide is not involved with intra-chain hydrogen bonds. This latter work was published while I was studying an unrelated polypeptide suspected of being involved in hydrogen bonding; in connection with this study attempts were made to inactivate angiotensinamide by incubation with urea – but without success. The failure to inactivate angiotensinamide in this manner constitutes evidence which supports the conclusions of PAIVA et. al. and is the subject of this report.

Methods. (1) Isolated rat uterus. Virgin Wistar strain rats (180–200 g) were injected intramuscularly with diethylstilbesterol, 0.1 mg/kg, 20 h before being sacrificed. Uterine strips were suspended in 40 ml of modified Tyrode solution ('Tyrode II', SCHWARZ et al.[4]) maintained at 30°C, and aerated with a mixture of 95% O_2 and 5% CO_2.

(2) Isolated guinea-pig ileum: Segments of terminal ileum, 4 cm long, were excised from male Hartley strain guinea-pigs (290–310 g) and suspended in 40 ml of Tyrode solution maintained at 38°C and aerated with 95% O_2 and 5% CO_2.

Solutions of angiotensinamide (Hypertensin-Ciba) were prepared in the appropriate tissue bathing medium, and introduced into the baths at 5 min intervals in a volume of 0.4 ml by means of a 1 ml syringe. Bath concentrations of angiotensinamide were 0.2 ng/ml for uterus, and 2.0 ng/ml for ileum. For the incubation mixtures a 30% solution of urea was mixed with equal volumes of 40 ng/ml, and with 400 ng/ml solutions of angiotensinamide for uterus and ileum, respectively; the mixtures remained at room temperature (25°C) for 10 min before introduction into the baths in volumes of 0.4 ml each. Angiotensinamide solutions were left in contact with the tissues until the start of relaxation after which the baths were flushed three times with fresh bathing medium. All contractions were recorded on a smoked drum by means of isotonic levers. A total of 10 uterine strips (5 rats) and 9 ileal strips (9 guinea-pigs) were used.

Results. Figures 1 and 2 show contractions of rat uterus and guinea-pig ileum, respectively, to angiotensinamide and to mixtures of the polypeptide with urea. The contractions of both tissues were submaximal. For each tissue a control injection of 15% urea (bath concentration 0.15%), left in contact with the tissue for 2 min, did not influence a subsequent angiotensinamide-induced contraction. A second control injection of a mixture of the

[1] F. M. BUMPUS, P. A. KHAIRALLAH, K. ARAKAWA, I. H. PAGE, and R. R. SMEBY, Biochim. biophys. Acta *46*, 38 (1961).
[2] R. R. SMEBY, K. ARAKAWA, F. M. BUMPUS, and M. M. MARSH, Biochim. biophys. Acta *58*, 550 (1962).
[3] T. B. PAIVA, A. C. M. PAIVA, and H. A. SCHERAGA, Biochemistry *2*, 1327 (1963).
[4] H. SCHWARZ, G. M. C. MASSON, and I. H. PAGE, J. Pharmacol. exp. Therap. *114*, 418 (1955).

polypeptide and urea introduced into the bath within 5 sec of mixing resulted in a normal response of both tissues. The mixture of angiotensinamide and urea, incubated for 10 min, resulted in contractions of ileum and uterus, the amplitude of which was no different from the contractions of these tissues induced by the same concentrations of angiotensinamide alone. Furthermore, it is evident from the figures that the incubated mixtures did not increase either the latent period or the contraction time of the tissues.

Discussion. It has been demonstrated that any significant degree of pressor or oxytocic activity of 5-L-isoleucyl angiotensin II requires the presence of the carboxyl and phenyl groups of L-phenylalanine and the phenolic group of L-tyrosine. The reported decrease in the oxytocic activity of 5-L-isoleucyl angiotensin II produced by urea, even though the necessary groups were intact, led to the conclusion that the polypeptide constituents are spatially oriented in a particular manner. It was postulated that an imperfect α-helical conformation, stabilized by hydrogen bonds, would result in the close proximity of the three essential groups and so facilitate their combination with reactive tissue sites (BUMPUS et al.[1]).

This argument implies that the octapeptide exists in a particular conformation which is necessary for its combination with the reactive tissue site prior to such combination. It is possible, however, that a particular spatial configuration which may be associated with activity is assumed by the polypeptide as a result of a combination with the tissue site at one or more loci of the molecule. In any case, the experiments reported here indicate that neither the oxytocic nor the intestinal smooth muscle stimulating activity of angiotensinamide is decreased in the presence of urea. It is established that angiotensin II has both a direct and indirect excitatory effect on guinea-pig ileum, and that the latter activity is predominant and is mediated by parasympathetic nerve tissue (KHAIRALLAH and PAGE[5], Ross et al.[6], ROBERTSON and RUBIN[7]). The demonstration that urea does not diminish the activity of angiotensinamide on either rat uterus or guinea-pig ileum suggests that a conformation stabilized by hydrogen bonds is not a prerequisite for the interaction of angiotensinamide with either the muscular or nervous tissue reactive sites of these preparations. On the basis of physical measurements, PAIVA et al.[3] have concluded that neither angiotensinamide nor 5-L-valyl angiotensin II exist in an α-helical conformation stabilized by hydrogen bonds. With regard to angiotei inamide, the experiments reported here support this conclusion and serve to emphasize that the tacit assumption of an α-helical conformation for the angiotensin II octapeptides is not justified.

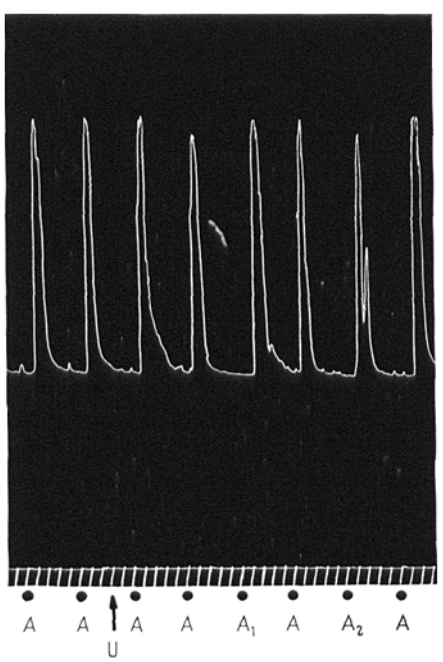

Fig. 1. Responses of isolated rat uterus to angiotensinamide. A – angiotensinamide, 0.2 ng/ml; U – urea, 0.15%; A_1 – angiotensinamide incubated 5 sec with urea; A_2 – angiotensinamide incubated 10 min with urea; time marks 1/min, drum speed 2 mm/min.

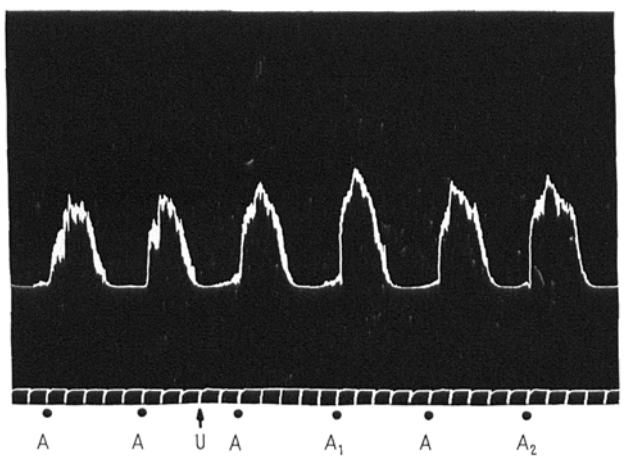

Fig. 2. Responses of isolated guinea-pig ileum to angiotensinamide. A – angiotensinamide, 2.0 ng/ml; U – urea, 0.15%; A_1 – angiotensinamide incubated 5 sec with urea; A_2 – angiotensinamide incubated 10 min with urea; time marks 1/min, drum speed 4 mm/min.

Zusammenfassung. Inkubation von 1-L-Asparaginyl-5-L-valyl-angiotensin II (Angiotensinamid) mit Harnstoff vermindert die spasmolytische Aktivität des Polypeptides weder am Meerschweinchenileum noch am Uterus der Ratte. Eine durch Wasserstoffbrückenbindungen stabilisierte räumliche Konfiguration des Polypeptids ist daher anscheinend nicht Voraussetzung für biologische Aktivität.

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[5] P. A. KHAIRALLAH and I. H. PAGE, Am. J. Physiol. *200*, 51 (1961).
[6] C. A. ROSS, C. T. LUDDEN, and C. A. STONE, Proc. Soc. exp. Biol. Med. *105*, 558 (1961).
[7] P. A. ROBERTSON and D. RUBIN, Brit. J. Pharmacol. *19*, 5 (1962).