vitamin A. Conditions for the applicability of this assay of the vitamin A bodypool to animal populations without further modifications are constant absorptions by the animal livers of the injected labelled vitamin A from the blood and constant long storage half life times for the vitamin A in the total body.

The observations of low vitamin A reserves in vitamin E-deficient rats, which is in agreement with the findings of several other laboratories[6], illustrates the usefulness of this assay. Further investigations are in progress.

Zusammenfassung. Es wird eine Isotopen-Verdünnungstechnik zur Berechnung des Vitamin-A-Körpervorrates in Ratten beschrieben. Aus der Verdünnung einer i.v. Dosis radioaktiven Vitamins A durch das unmarkierte Vitamin A des gesamten Körpers kann der Vitamin-A-Körpervorrat berechnet werden. Die so erhaltenen Werte sind mit denen der analytischen Vitamin-A-Bestimmungen in den entsprechenden Lebern vergleichbar.

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[6] S. R. AMES, Am. J. clin. Nutr. *22*, 934 (1969).
[7] J. KAHAN, Scand. J. clin. Lab. Invest. *18*, 679 (1966).
[8] F. KALBERER and J. RUTSCHMANN, Helv. chim. Acta *44*, 1956 (1961).

Synthesis of [4-Leucine]-Arginine-vasotocin, a Natriuretic Analogue of Arginine-Vasotocin

Oxytocin (I) and the vasopressins have been reported to be natriuretic in mammals under certain conditions[1]. The natriuretic properties are accentuated in the synthetic hormone analogues[2] [Leu^4]-oxytocin[3-5] (IIa), [Leu^4]-mesotocin[6,7] (= [Leu^4, Ile^8]-oxytocin, IIb), [Leu^3, Leu^4]-oxytocin[8] (IIc), [Leu^2, Leu^4]-[9] and [Ile^2, Ile^4]-oxytocin[5,10] (IIIb, c) and, to a lesser extent, [Ile^4]-oxytocin[5] (IIIa).

Since of all the natural neurohypophysial hormones it is arginine-vasotocin (= [Arg^8]-oxytocin, IVa), the endogenous hormone of the lower vertebrates, which has the greatest effect on the transport of sodium by amphibian membranes (skin, bladder) as well as the amphibian kidney[11] it appeared of interest to carry out a similar structural change – replacement of glutamine by leucine in sequence position 4 – also in the molecule of arginine-vasotocin and to examine the properties of the resulting analogue, [Leu^4]-arginine-vasotocin (= [Leu^4, Arg^8]-oxytocin or [Ile^3, Leu^4]-arginine-vasopressin, IVb).

Physical constants of new synthetic intermediates[a]

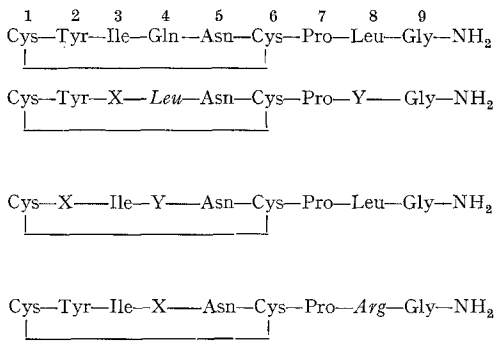
No.	Compound	M.p. (°C)[b] $[\alpha]_D^{25}$[c]
V	Z-Leu-Asn-Cys-Pro-Arg-Gly-NH_2 (Bzl, Tos)	183–185 / −41.6°
VI	Z-Ile-Leu-Asn-Cys-Pro-Arg-Gly-NH_2 (Bzl, Tos)	211–212 / −41.9°
VII	Z-Tyr-Ile-Leu-Asn-Cys-Pro-Arg-Gly-NH_2 (Bzl, Bzl, Tos)	237–238 / −36.2°
VIII	Z-Cys-Tyr-Ile-Leu-Asn-Cys-Pro-Arg-Gly-NH_2[d] (Bzl, Bzl, Tos)	220–223 / −42.6°
IXa	Z-Leu-Asn-Cys-OMe (Bzl)	195–197 / −28.0°
IXb	Z-Leu-Asn-Cys-N_2H_3 (Bzl)	230–234 / −38.2°

[a] Satisfactory elemental analyses were obtained for all the compounds listed. [b] Melting-points were determined on a microscope hot-stage. [c] In dimethylformamide, c = 0.5 (V–VIII) or 1.0 (IXa, b). [d] Monohydrate.

The protected nonapeptide VIII (Table) was prepared from the known[12] carboxyl-terminal pentapeptide derivative, Z-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2, by a stepwise procedure using benzyloxycarbonyl-protected *p*-nitrophenyl esters for addition of the individual amino-acid residues and hydrogen bromide in acetic acid for cleavage of the benzyloxycarbonyl protecting groups[13]; the intermediates are listed in the Table. In an alternative synthesis, VIII was obtained from 3 tripeptide units: The hexapeptide V was prepared from Pro-Arg(Tos)-Gly-NH_2[14] and the azide obtained from the tripeptide hydrazide IXb (made in turn from the ester IXa) and, after removal of the benzyloxycarbonyl group, condensed with the azide prepared from Z-Cys(Bzl)-Tyr-Ile-N_2H_3[15].

Removal of the protecting groups from VIII and formation of the disulphide bridge by standard procedures[16]

[1] W. Y. CHAN, Endocrinology *77*, 1097 (1965).
[2] Standard abbreviations are used for amino-acid residues and protecting groups; analogues of the natural hormones are designated in accordance with the IUPAC-IUB Tentative Rules [see e.g. Eur. J. Biochem. *1*, 375 and 379 (1967)].
[3] W. Y. CHAN, V. J. HRUBY, G. FLOURET and V. DU VIGNEAUD, Science *161*, 280 (1968).
[4] V. J. HRUBY, G. FLOURET and V. DU VIGNEAUD, J. biol. Chem. *244*, 3890 (1969).
[5] W. Y. CHAN and V. DU VIGNEAUD, J. Pharmac. exp. Ther. *174*, 541 (1970).
[6] J. RUDINGER, O. V. KESAREV, K. PODUŠKA, B. T. PICKERING, R. E. J. DYBALL, D. R. FERGUSON and W. R. WARD, Experientia *25*, 680 (1969).
[7] E. SEDLÁKOVÁ, B. LICHARDUS and J. H. CORT, Science *164*, 580 (1969).
[8] M. A. WILLE, V. DU VIGNEAUD and W. Y. CHAN, J. med. Chem. *15*, 11 (1972).
[9] V. J. HRUBY and W. Y. CHAN, J. med. Chem. *14*, 1050 (1971).
[10] V. J. HRUBY, V. DU VIGNEAUD and W. Y. CHAN, J. med. Chem. *13*, 185 (1970).
[11] F. MOREL and S. JARD, in *Handbook of Experimental Pharmacology* (Ed. B. BERDE, Springer-Verlag, Berlin-Heidelberg-New York 1968), vol. 23, p. 655.
[12] R. L. HUGUENIN and R. A. BOISSONNAS, Helv. chim. Acta *49*, 695 (1966).
[13] M. BODANSZKY and V. DU VIGNEAUD, J. Am. chem. Soc. *81*, 5688 (1959).
[14] R. L. HUGUENIN and R. A. BOISSONNAS, Helv. chim. Acta *45*, 1629 (1962).
[15] R. A. BOISSONNAS, S. GUTTMANN, P.-A. JAQUENOUD and J.-P. WALLER, Helv. chim. Acta *38*, 1491 (1955).
[16] D. B. HOPE, V. V. S. MURTI and V. DU VIGNEAUD, J. biol. Chem. *237*, 1563 (1962).



Amino-acids differing from those in oxytocin are in italic type.

afforded the crude peptide IVb which was purified either by partition chromatography[17,18] on Sephadex G-25 using the solvent systems 1-butanol-pyridine-0.2*M* acetic acid (10:7:24) and 1-butanol-ethanol-pyridine-0.2*M* acetic acid (16:1:2:28), or by ion exchange chromatography on Amberlite CG-50 using 0.5 ammonium acetate, pH 6.4, for elution[19].

The [Leu^4]-arginine-vasotocin (IVb) was isolated as the diacetate and characterised by elemental analysis, optical rotation ($[\alpha]_D^{25}$ + 4.0 ± 2°, recalculated for free peptide; c = 1 in 1*M* AcOH), paper electrophoresis at pH 6.0 (m_{Arg} = 0.51) and pH 1.7 (m_{Arg} = 0.52) and by TLC on silica gel and cellulose powder in several solvent systems; in every case the peptide appeared to contain less than 2% of impurities reacting with ninhydrin, the Pauly reagent, or the Sakaguchi reagent. An acid hydrolysate (6*M* HCl, 110°, 72 h) showed the molar ratios: Asp 1.00, Pro 1.06, Gly 0.91, Cys 1.89, Ile 0.99, Leu 1.01, Tyr 0.90, Arg 1.06, NH_3 1.80.

The parmacological properties of this peptide, in particular its natriuretic and diuretic action, are described in the two following communications[20,21].

[17] D. YAMASHIRO, Nature *201*, 76 (1964).
[18] D. YAMASHIRO, D. GILLESSEN and V. DU VIGNEAUD, J. Am. chem. Soc. *88*, 1310 (1966).
[19] J. MEIENHOFER and V. DU VIGNEAUD, J. Am. chem. Soc. *82*, 2279 (1960).
[20] V. PLIŠKA, J. VAŠÁK, M. RUFER and J. RUDINGER, Experientia *29*, 171 (1973).
[21] J. H. CORT, K. M. STRUB, G. HÄUSLER and J. RUDINGER, Experientia *29*, 173 (1973).
[22] F. Hoffmann-La Roche & Co. Ltd, CH-4002 Basel (Switzerland).
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Zusammenfassung. Es wird die Synthese des 4-Leucin-Analogen des Arginin-vasotocins (= [Leu^4, Arg^8]-oxytocin) beschrieben.

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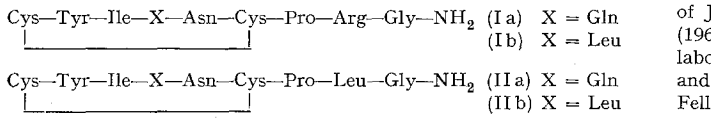
Some Pharmacological Properties of [4-Leucine]-Arginine-Vasotocin

The preceding paper[1] describes the synthesis of [Leu^4]-arginine-vasotocin (Ib), an analogue of the natural hormone arginine-vasotocin (Ia). The pharmacological properties of Ib in some standard assay systems are summarized in this paper and compared with the properties of the parent hormone Ia, of oxytocin (IIa), and of [Leu^4]-oxytocin (IIb). The diuretic and natriuretic properties of Ib are described in the following paper[2].

The action on the uterus was studied with organs from virgin rats in natural oestrus[3] in media[4] without magnesium or with 0.5 m*M* Mg^{2+}; contractions were measured isotonically. Pressor activity was assayed[5] in phenoxybenzamine-treated rats using a pressure transducer, antidiuretic activity in ethanol-anaesthetized, water-loaded rats by a modification[6] of the method of SAWYER[7]. Sodium transport by frog skin was measured as the short-circuit current[8] across isolated ventral skin of *Rana ridibunda* using voltage-clamp equipment[9]. The hydroosmotic effect was determined from the rate of osmotic water flow across frog bladder (*R. esculenta*)[10].



[1] D. GILLESSEN, R. O. STUDER and J. RUDINGER, Experientia *29*, 170 (1973).
[2] J. H. CORT, K. M. STRUB, G. HÄUSLER and J. RUDINGER, Experientia *29*, 173 (1973).
[3] P. HOLTON, Br. J. Pharmac. *3*, 328 (1948).
[4] R. A. MUNSICK, Endocrinology *66*, 451 (1960).
[5] J. DEKANSKI, Br. J. Pharmac. *7*, 567 (1952).
[6] V. PLIŠKA and I. RYCHLÍK, Acta endocrin. *54*, 129 (1967).
[7] W. H. SAWYER, Endocrinology *63*, 694 (1958).
[8] H. H. USSING and K. ZERAHN, Acta physiol. scand. *23*, 110 (1951).
[9] Measurements carried out at the Departement de physiologie, Université de Genève. We wish to thank Dr. R. DE SOUSA for the hospitality of his laboratory and helpful advice.
[10] These and other measurements were performed at the Laboratoire de physiologie cellulaire, Collège de France, Paris, by the technique of J. BOURGUET and S. JARD [Biochim. biophys. Acta *88*, 442 (1964)]. We are grateful to Prof. F. MOREL for the hospitality of his laboratory, to him and Dr. S. JARD for their interest and advice, and to the European Molecular Biology Organisation (EMBO) for a Fellowship to one of us (M.R.).