Alternative syntheses of isotocin (Ic) and mesotocin (Id) were carried out by the same route[12].

The free peptides showed satisfactory elemental and amino-acid analyses. The optical rotations, distribution coefficients, and Rf values are given in Table II. The gel filtration experiment showed that all the products were monomeric.

Oxytocic activity was measured with isolated uteri from stilboestrol-treated rats[19] by the method of HOLTON[20] using suspension fluids[21] containing no magnesium or 0.5 m*M* $MgCl_2$. Milk ejection activity was determined in the anaesthetized rat[22]. Chicken vasodepressor potencies were determined by a modification[23] of the method of COON[24]. Antidiuretic activity was measured in ethanol-anaesthetized rats by a modification[25] of the method of JEFFERS, LIVEZEY and AUSTIN[26]. The effect on water permeability was measured with isolated toad (*Bufo marinus*) bladders according to BENTLEY[27]. The results are given in Table II. Where comparable assay conditions were used the biological activities of our isotocin and mesotocin are comparable with those reported earlier[9-11, 28-30], particularly if it is kept in mind that the peptide content of the earlier samples is uncertain.

Of the newly prepared peptides, [4-proline, 8-isoleucine]-oxytocin (Ia) showed very low activities in all the standard assay systems, as had been anticipated. A similar low degree of activity is also shown by [4-proline]-oxytocin[7]. Although the (as yet unknown) physiological target organs of the oxytocin-like principles in lower vertebrates may well have specificity requirements different from any of those represented in the standard range of assays, all of the peptides isolated to date have at least moderate potencies in these tests. It therefore seems unlikely that a peptide 10^4 times less active than the natural hormones in all the assays would be a member of the same biological series. On the other hand, the [4-leucine, 8-isoleucine]-oxytocin (Ib), though less active than mesotocin or isotocin in all the standard assays, has appreciable potency in several tests so that its occurrence as a natural hormone cannot be discounted on the same grounds.

Characteristic properties of [4-leucine, 8-isoleucine]-oxytocin which should help in any search for its natural occurrence are the rather high partition coefficient and Rf value; the high ratio of avian depressor activity to activity on the rat uterus in vitro in the absence of magnesium; the high degree of magnesium potentiation (though in the case of glumitocin[31] this parameter has recently proved less constant than had been assumed); and the diuretic rather than antidiuretic effect together with natriuretic action[32] in the hydrated rat, properties which it shares with [4-leucine]-oxytocin[6].

If an evolutionary intermediate of the type discussed still survives, it is likely that it will be found among the elasmobranch fishes, since the primitive holocephalians seem to elaborate oxytocin itself[33] (with glutamine in position 4) while the more recent selachians secrete glumitocin (with serine in position 4)[34].

Zusammenfassung. [4-Prolin, 8-Isoleucin]-Oxytocin und [4-Leucin, 8-Isoleucin]-Oxytocin wurden als mögliche Glieder in der entwicklungsgeschichtlichen Reihe der Neurohypophysenhormone synthetisiert und pharmakologisch geprüft.

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[19] B. K. FOLLETT and P. J. BENTLEY, J. Endocr. *29*, 277 (1964).
[20] P. HOLTON, Br. J. Pharmac. *3*, 328 (1948).
[21] R. A. MUNSICK, Endocrinology *66*, 451 (1960).
[22] G. W. BISSET, B. J. CLARK, J. HALDAR, M. C. HARRIS, G. P. LEWIS and M. ROCHA E SILVA, Br. J. Pharmac. *31*, 537 (1967).
[23] B. K. FOLLETT and H. HELLER, J. Physiol. (Lond.) *172*, 74 (1964).
[24] J. M. COON, Arch. int. Pharmacodyn. Thér. *62*, 79 (1939).
[25] R. E. J. DYBALL, G. J. LANE and R. G. MORRIS, J. Physiol. (Lond.) *186*, 43P (1966).
[26] W. A. JEFFERS, M. M. LIVEZEY and J. H. AUSTIN, Proc. Soc. exp. Biol. Med. *50*, 184 (1942).
[27] P. J. BENTLEY, J. Endocr. *17*, 201 (1958).
[28] B. BERDE and H. KONZETT, Medna exp. *2*, 317 (1960).
[29] R. ACHER, J. CHAUVET, M. T. CHAUVET and D. CREPY, Biochim. biophys. Acta *58*, 624 (1962).
[30] H. RASMUSSEN, I. L. SCHWARTZ, R. YOUNG and J. MARC-AURELE, J. gen. Physiol. *46*, 1171 (1963).
[31] W. H. SAWYER, M. MANNING, E. HEINICKE and A. M. PERKS, Gen. comp. Endocr., in press.
[32] Unpublished results by Dr. J. H. CORT, Prague.
[33] W. H. SAWYER, R. J. FREER and T.-C. TSENG, Gen. comp. Endocr. *9*, 31 (1967).
[34] The authors are obliged to Academician F. ŠORM and Professor H. HELLER for their interest and encouragement, and to Professor W. H. SAWYER for information about his results in advance of publication.
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Hexalure, an Insect Sex Attractant Discovered by Empirical Screening

Chemical attractants have become an indispensable tool in the detection[1] and control[2] of certain destructive insect pests. The sex attractants emitted by the virgin females of several species of Lepidoptera have been shown to be C_{12-16} alkenol acetates[3,4]. Isolation and identification of minute amounts of natural lure is an arduous, often frustrating task, and we therefore undertook to supplement our isolation program with a search for new insect attractants by a strictly empirical approach.

A large number of C_{12}, C_{14} and C_{16} alken-1-ol acetates were synthetized and evaluated as attractants for several insect species. One of these, *cis*-7-hexadecen-1-ol acetate, has proved to be an outstanding attractant for male

[1] M. JACOBSON, *Insect Sex Attractants* (Interscience Publishers, Inc., New York 1965), p. 104.
[2] M. JACOBSON, *Insect Sex Attractants* (Interscience Publishers, Inc., New York 1965), p. 112. – L. K. GASTON, H. H. SHOREY and C. A. SAARIO, Nature *213*, 155 (1967).
[3] R. S. BERGER, Ann. ent. Soc. Am. *59*, 767 (1966). – A. A. SEKUL and A. N. SPARKS, J. econ. Ent. *60*, 1270 (1967). – W. L. ROELOFS and H. ARN, Nature *219*, 513 (1968).

pink bollworm moths, *Pectinophora gossypiella* (Saunders), eliciting a copulatory response in laboratory tests and luring large numbers to field traps. As such, it becomes the first sex attractant to be discovered by empirical means. The attractiveness of this compound, which we have named hexalure, is highly unusual, since propylure[4], the natural pink bollworm sex pheromone, is a C_{16} alkadienol acetate which has a branched chain and *trans* configuration.

Hexalure is far more attractive than propylure, which requires admixture with an activator[5] before it can lure males in the field. In tests with several species of insects, hexalure attracted only the pink bollworm, and this insect was not attracted to the *trans* isomer of hexalure.

Hexalure was synthetized by first condensing 2-(7-octynyloxy)-tetrahydropyran[6] with 1-bromooctane, according to previously published procedures[7], to give a 40% yield of 2-(7-hexadecynyloxy)-tetrahydropyran (bp, 140–145 °C at 0.001 mm Hg; n_D^{25}, 1.4636). This product was refluxed with acetic acid-acetyl chloride[6,7] to give a quantitative yield of 7-hexadecyn-1-ol acetate (bp, 117–121 °C at 0.001 mm; n_D^{25}, 1.4532), which was semihydrogenated to the desired product. Hexalure (bp, 100–104 °C at 0.001 mm, 121.5–124.5 °C at 0.08–0.14 mm; n_D^{25}, 1.4484) is a clear, colorless liquid with a mild odor reminiscent of freshly cut grass. Analysis of one lot by a new gas chromatographic method[8] showed the isomeric composition to be roughly 80% *cis* and 20% *trans*.

The efficacy of *cis*-7-hexadecen-1-ol acetate as an attractant for pink bollworm moths was evaluated in laboratory and field tests at Brownsville, Texas, and Phoenix, Arizona. In a preliminary test, a trap baited with 6 mg of hexalure caught, during 7 days, 51% of the male moths caught by the natural sex attractant extracted from 25 virgin females. Over a 14-day period the synthetic/natural catch ratio was 42%. In extensive field tests 60 mg of hexalure was initially about equal to 50 female equivalents of natural lure but after 5–7 days the synthetic lure at all test dosages was superior to either natural lure or to live caged virgin female moths.

Hexalure is now being used by the U.S. Department of Agriculture's Plant Pest Control Division to combat the pink bollworm in Florida and several southwestern states. It has been found to be more stable, more convenient to use, and much more economical than the previously used lure, which is a crude methylene chloride extract of the terminal abdominal segments of virgin female moths.

Zusammenfassung. Empirisch wurde ein Sexuallockstoff – *cis*-7-Hexadecen-1-ol-acetat – gefunden, der auf Männchen der roten Baumwollkapselmotte, *Pectinophora gossypiella* (Saunders), auffallend stark wirkt.

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4 W. A. JONES, M. JACOBSON and D. F. MARTIN, Science *152*, 1516 (1966).
5 W. A. JONES and M. JACOBSON, Science *159*, 99 (1968).
6 N. GREEN, M. JACOBSON, T. J. HENNEBERRY and A. N. KISHABA, J. med. Chem. *10*, 533 (1967).
7 D. WARTHEN and M. JACOBSON, J. med. Chem. *10*, 1190 (1967); D. WARTHEN and M. JACOBSON, J. med. Chem. *11*, 373 (1968); D. WARTHEN, J. med. Chem. *11*, 371 (1968).
8 D. WARTHEN and N. GREEN, J. Am. Oil Chem. Soc. *46*, 191 (1969).

Nephrocalcinosis in Rats After Forced Weaning

After forced weaning of the young, certain biochemical changes appear in the mammary glands, bones[1], blood[2] and urine[3] of the suckling rat mothers. Rats fed on the so-called Larsen diet were also found to have morphological changes in the aorta[2]. A further manifestation of this period were changes found in the kidneys having the character of nephrocalcinosis.

Thirteen rat mothers of the Wistar strain, aged 3–4 months, were allowed to suckle 12 young each after their first gestation. This group, together with a control group of virgin females, were fed on a Larsen diet[2] containing 1.4% calcium and 0.5% phosphorus. A second group of 21 rat mothers reared under the same conditions and a control group, were fed on a Larsen diet enriched with calcium (1.8% calcium and 0.5% phosphorus). On the day of weaning, which was always on the twenty-first day of lactation, 6 mothers from the first group were killed and 10 from the second. The remaining rats of both groups were killed 24 h after weaning. Blood for determining calcium and phosphorus was obtained by opening the heart. An excision was taken from one or both kidneys for histological examination. The renal calcium, phosphorus and citrate content was determined quantitatively in most animals.

At post mortem examination urolithiasis was observed in the form of gravel (composed of calcium carbonate), most often in the bladder. Nearly all these cases showed varying degrees of dilatation of the ureters and pelves. Acute suppurative pyelonephritis accompanying the above 2 changes was a frequent finding in the second group. In most animals killed 24 h after weaning the surface of the kidneys showed a typical form of yellowish red marbling. It was only in these animals that nephrocalcinosis was found histologically (Table I) as follows: calcification was found in the epithelium of numerous proximal tubules and in the basement membrane and sometimes in Bowman's capsule. The tubules were always affected in the area of the cortex corticis, but changes reached into the deeper layers of the cortex between the medullary processes. No differences were found between the experimental animals and the controls in the PAS-reaction and on staining with Alcian blue.

The biochemical findings show good correlation (Table II) with the occurrence of nephrocalcinosis. Changes in the calcium and phosphorus levels in the serum

1 A. KOMÁRKOVÁ, Z. ZÁHOŘ and V. CZABANOVÁ, J. Lab. clin. Med. *69*, 102 (1967).
2 Z. ZÁHOŘ, R. VANĚČEK, V. CZABANOVÁ, J. MÜLLER and A. KOMÁRKOVÁ, J. Atheroscler. Res. *7*, 25 (1967).
3 L. I. PUGSLEY, Biochem. J. *30*, 1271 (1936).