tions are not present during the isolation process. It is possible however that both alkaloids exist as components, joined through the –COOR linkage, of larger molecules and that the isolated alkaloids are the result of the breakage of such links by methaonlysis.

The NMR-spectrum of **2** gave the following results: $N–CH_3$ proton signal at 2.7 ppm, $–CH_2O–$ proton signal at 4.45 ppm, $–COOCH_3$ proton signal at 3.5 ppm, olefinic proton signals at 5.4 ppm and 5.9 ppm, and 3 α to N protons signal at 2.2–2.6 ppm. The base peak in the mass spectrum of **2** was similar to that of the previous alkaloid, that is the $M^+–1$ fragment. Important (> 60%) peaks occurred for the $M^+–15$, $M^+–44$, and *m/e* 31 fragments. This last fragment is the $^+CH_2 = OH$ ion, thus confirming the presence of a primary alcohol function (IR-absorption at 3,510 cm^{-1}). Here also we observed carbonyl absorption, for the ester group (1,745 cm^{-1}). and for the α,β-unsaturated acid group (1,710 cm^{-1}).

The structure and configuration of this alkaloid was further studied by NaH (in THF) condensation[7]. 2 products formed from the condensation are traces of decarboxylation product **3** (terminal $C=CH_2$, NMR-signal 4.20 ppm) and the compound **4**. This latter product was identified as being a Dieckmann type condensation product, formed between the $^+C–COOCH_3$ and COOH groups of a conjugated system. The formation of the keto-ester **4** is characterized by the disappearance of the N–*CH*–COOR and COO*H* proton signals in the NMR-spectrum of **2**.

The spectrum of **4** is similar to that of **2** with the exception of the D-ring allylic proton signals, which are shifted 0.5 ppm downfield.

The formation of **4** shows that the C- and D-ring junction must be *cis* (H, β-axial). The position of the $COOCH_3$ group, originally unknown, is shown to be β-equatorial, the α – axial position causing a 1,3-diaxial interaction. This is necessary to make the cyclisation easier. The *cis* configuration of the double bond was determined by the olefinic proton coupling constant (6.5 Hz). The CH_2OH and $C=C–COOH$ groups are *trans*-diequatorial and CH_2OH group is β-equatorial to avoid a 1,3-diaxial interaction, present if the CH_2OH group is α-axial[8].

Résumé. Une étude à l'aide de spectroscopie de masse et de spectroscopie r.m.n., ainsi que de dégradations, a permi l'identification de la structure de quelques bases isolées de feuilles de canneberges et leur classification parmi les alkaloids de la famille indolique.

K. JANKOWSKI

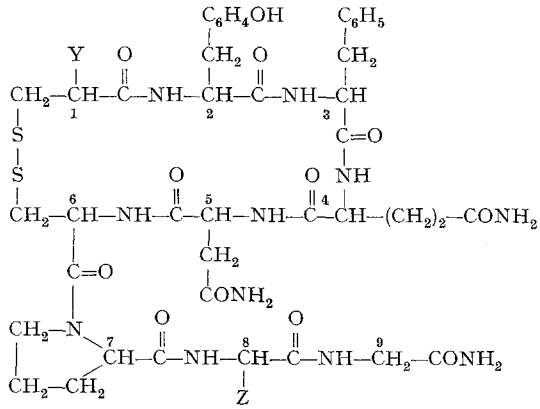
Département de Chimie, Université de Moncton, Moncton (Nouveau-Brunswick, Canada),
6 November 1972.

[7] L. J. DOLBY and Z. ESFANDIANI, J. org. Chem. *37*, 43 (1972). M. USKOKOVIC, H. BRUDERER, C. VON PLANTA, T. WILLIAMS and A. BROSSI, J. Am. chem. Soc. *86*, 3364 (1964). – J. D. HOBSON, J. RAINES and R. J. WHITEOAK, J. chem. Soc. *1963*, 3495.

[8] Author thanks Mr. G. G. ARSENAULT for the collaboration and NRC of Canada for the research grant.

Solid-Phase Synthesis of Lysine Vasopressin Analogs: [1-β-Mercaptopropionic Acid, 8- Lysine]-Vasopression and [1-β-Mercaptopropionic Acid, 8-(ε-N-*p*-Toluenesulfonyl)-Lysine]-Vasopressin[1,2]

The syntheses according to the general solid-phase procedure of MERRIFIELD[3] of [1-β-mercaptopropionic acid, 8-lysine]-vasopressin ([βSP_p^1, Lys^8]-Vpn) and [1-β-mercaptopropionic acid, 8-(ε-N-*p*-toluenesulfonyl)-lysine]-vasopressin ([βSP^1, $TosLys^8$]-Vpn) are described (Figure). These peptides are analogs of the antidiuretic hormone lysine vasopressin([Lys^8]-Vpn) in which the potential centers for cationic charges have been progressively reduced. The conformational changes evoked by these stepwise modifications have been studied by proton magnetic resonance spectroscopy[4] and have been related to the proposed conformation of [Lys^8]-Vpn[5].



Aminoacid sequence of lysine vasopressin [$Y = NH_2$; $Z = -(CH_2)_4–NH_2$], deamino-lysine vasopressin [$Y = H$; $Z = -(CH_2)_4–NH_2$], and deamino-8-tosyllysine vasopressin [$Y = H$; $Z = -(CH_2)_4–NH–SO_2–C_6H_4–CH_3$ (*p*)]; numbers indicate sequence positions of individual residues.

In detail the syntheses of the protected octapeptide derivatives of [βSP_p^1, Lys^8]-Vpn and [βSP_p^1, $TosLys^8$]-Vpn followed our earlier synthesis of arginine vasopressin [Arg^8]-Vpn[6] and of radioactively-labelled oxytocin, [Lys^8]-Vpn and [Arg^8]-Vpn[7]. The tert. butyloxycarbonyl-glycine-resin (0.49 mmole of Gly per g of copolystyrene-2% divinylbenzene resin as determined by Volhard titration[3]) was deprotected and elongated stepwise with the appropriate protected amino acid active esters to yield the protected nonapeptide attached to the resin. Ammonolysis of this material, carried out as detailed by BAXTER et al.[8], gave 0.5 g of crude S-benzyl-β-mercaptopropionyl-

[1] Supported by USPHS Grants Nos. AM-13567 and AM-10080.

[2] Abbreviations follow the rules of the IUPAC-IUB Commission on Biochemical Nomenclature in Biochemistry *6*, 362 (1967).

[3] R. B. MERRIFIELD, J. Am. chem. Soc. *85*, 2149 (1963).

[4] J. D. GLICKSON, D. W. URRY, R. T. HAVRAN and R. WALTER, Proc. natn. Acad. Sci. USA *69*, 2136 (1972).

[5] R. WALTER, J. D. GLICKSON, I. L. SCHWARTZ, R. T. HAVRAN, J. MEIENHOFER and D. W. URRY, Proc. Acad. Sci. USA *69*, 1920 (1972).

[6] J. MEIENHOFER, A. TRZECIAK, R. T. HAVRAN and R. WALTER, J. Am. chem. Soc. *92*, 7199 (1970).

[7] R. WALTER and R. T. HAVRAN, Experientia *27*, 645 (1971).

[8] J. W. M. BAXTER, M. MANNING and W. H. SAWYER, Biochemistry *8*, 3592 (1969).

O-benzyl-L-tyrosyl-L-phenylalanyl-L-glutaminlyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nε-tosyl-L-lysylglycineamide (I).

Following the procedure used for the crystallization of [Lys^8]-Vpn[9] and [Arg^8]-Vpn[6] we succeeded in securing 0.38 g of I in crystalline form from dimethylformamide (DMF)-1% formic acid (2 ml). The crystalline amide was homogeneous upon thin layer chromatography on Silica gel G with chloroform:methanol (8:2)[10]. Reduction of 250 mg of nonapeptide with sodium in liquid ammonia as applied to the synthesis of [βSP$_p{}^1$, Lys^8]-Vpn[11], followed by oxidative cyclization with ferricyanide and desalting with AG 3 × 4 yielded crude [βSP$_p{}^1$, Lys^8]-Vpn, which was purified by partition chromatography on Sephadex G-25 with n-butanol:ethanol:pyridine:water containing 1% acetic acid (6:1:1:8). Material was isolated from the symmetrical peak having an Rf of 0.33 and lyophilized to yield 118 mg of hormone. Amino acid analysis (6*N* HCl, 105°, 24 h) gave the following molar ratios, phe being taken as 1.0: Lys, 0.98; Asp, 0.99; Glu, 1.04; Pro, 1.00; Gly, 0.95; $^1/_2$-Cys, 0.57; mixed disulfide of β-mercaptopropionic acid and cysteine, 0.45; Tyr, 0.85; Phe, 1.00; NH_3, 2.77. This [βSP$_p{}^1$, Lys^8]-Vpn exhibited a rat pressor activity[12] of 132 ± 7 U/mg, which is essentially identical to the activity of 126 ± 2 U/mg, previously reported for this analog when prepared by conventional methods of peptide synthesis[11].

For the preparation of [βSP$_p{}^1$, TosLys^8]-Vpn, I (135 mg) was debenzylated by treatment with anhydrous hydrogen fluoride (6–10 ml) in the presence of 0.35 ml anisole for 1 h at room temperature[13]. Nitrogen was passed through the reaction vessel for 30 min and the syrupy material was dried in vacuo overnight over KOH. The residue was triturated with anhydrous ether and then quickly dissolved in DMF (10 ml) under a nitrogen atmosphere. In order to oxidize the dimercaptol to the disulfide[14], a solution of 34 mg of freshly recrystallized 1,2-diiodoethane in 7 ml absolute methanol was prepared under nitrogen. Both of the above solutions were added simultaneously, dropwise and with stirring, into a mixture of 25 ml absolute methanol and 5 ml DMF, under nitrogen, and within a period of 4 h. Upon removal of the methanol in vacuo at room temperature and addition of ethyl acetate, the oxidation product precipitated; it was collected by centrifugation and washed with ethyl acetate. Thin layer chromatography on Silica gel G in the upper phase of the solvent system n-butanol:benzene:acetic acid:water (3:1:1:5) of the material gave a major spot with an Rf of 0.55. The compound was purified by dissolving in acetic acid and precipitating with water. Amino acid analysis (6*N* HCl, 105°, 24 h) gave the following molar ratios, Pro being taken as 1.00: Lys, 0.81; Asp, 1.04; Glu, 0.98; Pro, 1.00; Gly, 1.04; $^1/_2$-Cys, 0.56; mixed disulfide of mercaptopropionic acid and cysteine, 0.45; Tyr, 0.72; Phe, 0.99. This compound exhibited negligible rat pressor activity.

Elemental analysis of [βSP$_p{}^1$, TosLys^8]-Vpn gave the following values: $C_{53}H_{71}O_{14}N_{12}S_3 \cdot C_2H_4O_2 \cdot 3H_2O$ (1310.56) calculated: C, 50.4; S, 7.34. Found: C, 50.3; S, 7.13.

Zusammenfassung. Mit Hilfe der Festkörpermethode nach MERRIFIELD wird die Synthese von [1-β-Mercaptopropionsäure, 8-lysin]-Vasopressin und [1-β-Mercaptopropionsäure, 8-(ε-N- -toluälsulfonyl)-lysin]-Vasopressin, beschrieben. In diesen Analogen sind die potentiellen Kationenzentren des antidiuretischen Hormons Lysin-Vasopressin schrittweise entfernt worden.

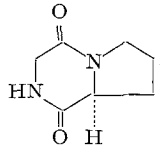
R. T. HAVRAN

Department of Physiology, Mount Sinai Graduate and Medical Schools of the City University of New York, Fifth Avenue and 100 Street, New York (N.Y. 10029, USA), 10 November 1972.

[9] J. MEIENHOFER and Y. SANO, J. Am. chem. Soc. *90*, 2996 (1968).
[10] Protected peptides and hormones were visualized on thin layer plates according to the procedure by H. ZAHN and E. REXROTH, Z. analyt. Chem. *148*, 181 (1955).
[11] R. D. KIMBROUGH, JR., W. D. CASH, L. A. BRANDA, W. Y. CHAN and V. DU VIGNEAUD, J. biol. Chem. *238*, 1411 (1963).
[12] *The Pharmacopeia of the United States;* 17th Revision (Mack Publishing Co., Easton, Pa. 1965), p. 749.
[13] S. SAKAKIBARA, in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins* (Ed. B. WEINSTEIN; Marcel Dekker, N.Y., N.Y. 1971), p. 51.
[14] I. PHOTAKI, J. Am. chem. Soc. *88*, 2292 (1966).

Isolation and Structural Elucidation of 3,6-Dioxo-Hexahydro-Pyrrolo[1,2-a]-Pyrazine from the Echinoderm *Luidia clathrata*[1]

In an intial survey of marine animals for antineoplastic components we found an ethanol extract of the Gulf of Mexico starfish *Luidia clathrata* (Echinodermata) to exhibit significant activity against experimental P-388 lymphocytic leukemia[2]. While we have not yet identified the antileukemic component, we now report the structure of a companion substance (I). A crystalline compound (m.p. 216–218°, from methanol-acetone) was isolated by careful gel permeation chromatography (Sephadex LH-20, methanol as solvent) of a water soluble fraction from the original ethanol extract.



I

The optically active amide displayed a negative plain ORD curve. The mass spectrum of I (Varian Atlas SM1B, 70eV, direct probe temp. 50°) showed major peaks at *m/e* 154.0741 ($C_7H_{10}N_2O_2$, calc. 154.0742:M, base peak); *m/e* 126.0792 ($C_6H_{10}N_2O$, calc. 126.0793:M–CO, 28% relative abundance); *m/e* 111.0685 C_6H_9NO, calc. 111.0684:M–HNCO, 71%); *m/e* 98.0481 ($C_4H_6N_2O$, calc. 98.0480:M-56, 47%); *m/e* 83.0729 (C_5H_9N, calc. 83.0735:M-71, 39%);

[1] This investigation was supported by contract No. NIH-71-2308 from Chemotherapy, National Cancer Institute, National Institutes of Health and by Public Health Service Research Grant No. CA-11451-03 from the National Cancer Institute. The authors also gratefully acknowledge support from the National Science Foundation which provided the X-ray diffractometer and mass spectrometer. For the previous contribution of this series see G. R. PETTIT, R. H. ODE and T. B. HARVEY, III, Lloydia, *36* (1973).
[2] G. R. PETTIT, J. F. DAY, J. L. HARTWELL and H. B. WOOD JR., Nature, Lond. *227*, 962 (1970).