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.

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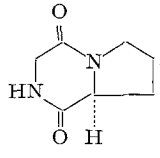
[9] J. MEIENHOFER and Y. SANO, J. Am. chem. Soc. *90*, 2996 (1968).
[10] Protected peptides and hormones were visualized on thin layer plates according to the procedure by H. ZAHN and E. REXROTH, Z. analyt. Chem. *148*, 181 (1955).
[11] R. D. KIMBROUGH, JR., W. D. CASH, L. A. BRANDA, W. Y. CHAN and V. 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%);

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[2] G. R. PETTIT, J. F. DAY, J. L. HARTWELL and H. B. WOOD JR., Nature, Lond. *227*, 962 (1970).

and at *m/e* 70 (39%), 69 (45%), 56 (25%), 55 (35%), 43 (27%), 42 (32%) and 41 (30%). Metastable ion peaks were detected for the following transitions: *m/e* 154 → 126 + CO (m* = 103.1); *m/e* 154 → 111 + HNCO (m* = 80.0); *m/e* 111 → 83 + CO (m* = 62.1); and *m/e* 83 → 55 (*m** = 36.4). Expulsion of the elements of HNCO from the molecular ion strongly suggested a cyclic lactam structure containing other unsaturated groups in the ring.

Excellent crystals of the amide were obtained by slow cooling of a methanol-acetone-hexane (trace) solution. The observed Laué symmetry and extinctions correspond to the space group $P2_12_12_1$ with a = 9.666 ± 0.006, b = 5.870 ± 0.004, c = 13.067 ± 0.010, Å; Z = 4; ϱ calc. = 1.381 g/cm³; and ϱ obsd. = 1.37 g/cm³. Diffraction intensities were measured in the variable speed θ–2θ scan mode with monochomated MoK_α radiation on a Syntex Pi diffractometer; of the 1280 independent reflections investigated ($2\theta \leq 60.0°$), a total of 1234 were retained as objectively observed. No corrections were applied for either absorption or extinction.

The equal atom structure was solved by direct methods[3,4] using a computerized[5] multiple-formula single-solution procedure based on the generalized tangent formula[6]. Full-matrix least-squares refinement of the structure (130 independent variables) with anisotropic thermal parameters and hydrogen positional parameters yielded a standard residual $R = 0.046$ (for all observed data and a weighted residual $R_w = 0.054$). Refinement of the structure without the hydrogen atoms yielded the residuals $R = 0.109$ and $R_w = 0.143$.

The perspective view in the Figure represents fully the molecular configuration and conformation of Gly-L-Pro lactam[7,8]. This is, to the best of our knowledge, the first time this dipeptide lactam has been isolated from a marine organism or indeed from any natural source[8,9]. Since the natural specimen gives the same ORD spectrum as the synthetic material prepared from L-proline, the absolute configuration is assigned as (S)–C_2.

As can be seen from the Figure neither of the rings in the molecule is planar; both in fact show pronounced folding. In the pyrrolidine ring the atoms C_2, N_1, C_8, and C_7 are quite coplanar (average deviation 0.015 Å) with C_9 considerably out of the plane (0.55 Å). This particular ring conformation has been observed only in 2,3-*cis*-3,4-*trans*-3,4-dihydroxyproline[10]; typically C_8 is out of the plane[11–13]. The dioxopiperazine ring has a pronounced fold about the line C_2–C_5; the dihedral angle between the planes $C_2, C_3, O_3, N_4, H_4, C_5$ and $C_2, N_1, C_7, C_6, O_6, C_5$ is 38.3 deg. The peptide bond in the first of these planes is flat with a dihedral angle about N_4–C_3 of only 0.8° ($\omega = 179.2°$); the atoms in this plane show average deviations of only 0.005 Å. The second plane is considerably less planar (average deviation 0.030 Å) due in part to a slight twist of 7.2° about the peptide bond N_1–C_6 ($\omega = 172.8°$). The only other example of a nonplanar dioxopiperazine ring is that from L-Ala-L-Ala[14], where the dihedral angle between the nearly planar peptide groups is 25.7°.

Zusammenfassung. Es wird über die Isolierung und Charakterisierung der Titelverbindung (Gly-L-Pro Lactam, I) aus dem Seestern *Luidia clathrata* berichtet. Die Kristallstruktur dieses Dioxopiperazinderivats wurde ermittelt. Der Piperazinring liegt in der Wannen-Konformation vor.

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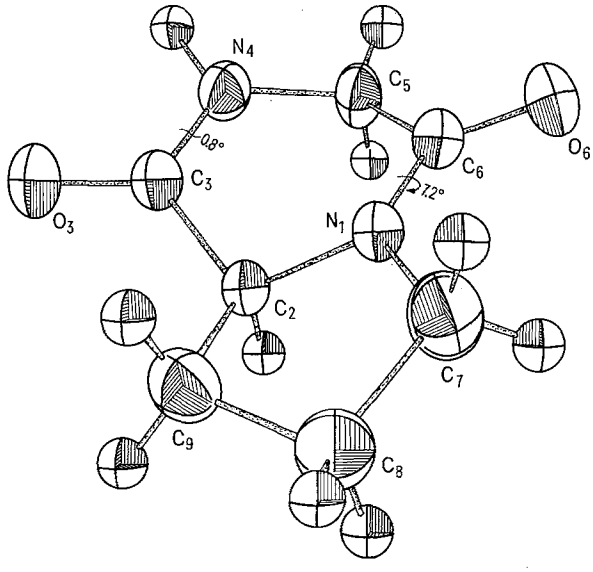
9 October 1972.



A perspective representation of the structure of Gly-L-Pro lactam.

[3] J. KARLE and I. L. KARLE, Acta, crystallogr. *21*, 849 (1966).
[4] J. KARLE, Acta crystallogr., Sect. B, *24*, 182 (1968).
[5] C. C. TSAI, private communication.
[6] C. C. TSAI and D. M. COLLINS, Acta crystallogr., in press.
[7] V. A. SHIBNEV, T. D. KOZARENKO and K. T. POROSHIN, Izvest. Akad Nauk. SSSR, Otdel. Khim. Nauk, *1960*, 1500; Chem. Abstr. *53*, 21693f (1960).
[8] H. N. RYDON and P. W. G. SMITH, J. chem. Soc. *1956*, 3642. In the citations of ref. [8] will be found syntheses of dioxopiperazine I under the term Gly-Pro anhydride, m.p. 203–204° and 213°, $[\alpha]_D$ -217°. The same substance (I) has been prepared by *Streptomyces* protease hydrolysis of gelatin, milk casein or polypeptone as described in ref. [9]. Whether a similar protease and protein (or dipeptide ester) presursor is the source of I in *Luidia clathrata* is unknown. Alternatively, lactam I may represent a biologically significant component of this starfish.
[9] Y. KOAZE, Bull. agric. chem. Soc. Japan, *24*, 449 (1960).
[10] I. L. KARLE, Acta. crystallogr. *26*, 765 (1970).
[11] T. MATSUZAKI and Y. IITAKA, Acta. crystallogr. *27*, 507 (1971) and references therein.
[12] J. DONAHUE and K. N. TRUEBLOOD, Acta. Crystallogr. *5*, 419 (1952).
[13] Y. C. LEUNG and R. E. MARSH, Acta. crystallogr. *11*, 17 (1958).
[14] E. SLETTEN, J. Am. chem. Soc. *92*, 172 (1970).

Anthraquinones in the Fungus *Talaromyces stipitatus*

In the course of morphological studies on the genus *Talaromyces*[1], it was observed that the strain CBS 349.72 of *Talaromyces stipitatus* C. R. Benjamin ex STOLK and SAMSON (st. con. *Penicillium stipitatum* Thom) showed different pigmentation from the other strains examined. This strain developed a red-brown reverse while the others became yellowish. It was decided to investigate the nature of this red colour.