ORGANIC
LETTERS

2004
Vol. 6, No. 15
2543−2545

Callynormine A, a New Marine Cyclic Peptide of a Novel Class

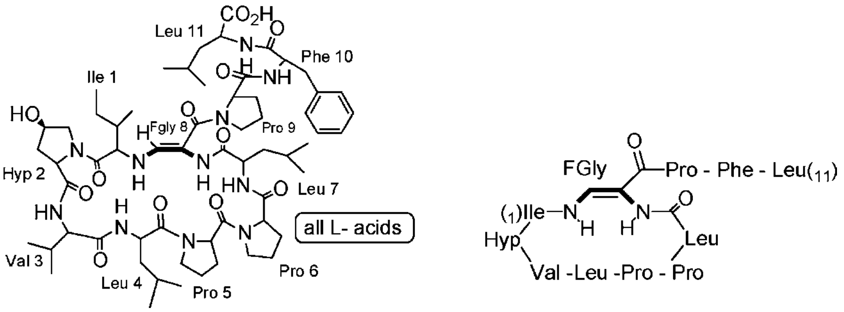
Nelly Berer,[†] Amira Rudi,[†] Israel Goldberg,[†] Yehuda Benayahu,[‡] and Yoel Kashman*,[†]

*School of Chemistry and Department of Zoology, Tel-Aviv University,
Ramat Aviv 69978, Israel*

kashman@post.tau.ac.il

Received May 5, 2004

ABSTRACT



A novel cyclic peptide, callynormine A, was isolated from the Kenyan marine sponge *Callyspongia abnormis* and its structure elucidated by interpretation of its NMR data and X-ray diffraction analysis. Callynormine A represents a new class of heterodetic cyclic peptides (designated endiamino peptides) possessing an α-amido-*β*-aminoacrylamide cyclization functionality.

Nonribosomal cyclic peptides and depsipeptides are a large group of compounds known from microorganisms (first reported from *Fusarium* and *Streptomyces*) as well as marine organisms.[1−3] In fact, it is suggested that marine peptides also originate from symbiotic microorganisms living within the marine invertebrates.[4,5] These nonribosomal peptides are a large family of natural products, with a considerable variety of unusual amino acids and other building blocks, that include many medicinally important compounds.[4,5] A recent review by Matsunaga and Fusetani[5] arranged these (over 190) marine peptides according to structural classes based on the IUPAC−IUB (biochemical nomenclature).[6] Accordingly, compound **1** belongs to the heterodectic cyclic peptides.

From the southern Kenyan (Shimoni reef) sponge *Callyspongia (Cladochaline) abnormis* (Pulitzer-Finali) (KB1010) collected by scuba (2−6 m), we isolated callynormine A (**1**, 18 mg, $[\alpha]_D$ −19 ($c = 0.01$, MeOH), mp > 300 °C, 0.05% dry weight) following solvent partition and Sephadex LH-20 and RP-18 HPLC chromatographies. The FABMS of compound **1** exhibited a pseudomolecular ion $[M + H]^+$ at m/z 1188 which is consistent with $C_{61}H_{93}N_{11}O_{13}$. A peptide structure was evident from the NMR data (in deuteriomethanol) (Table 1), i.e., the appearance of 11 carbonyls (δ_C 170.5, 172.6, 173.6, 174.6, 174.7, 174.9, 175.0, 175.1, 175.4, 176.3 and 178.2 ppm) and 10 α-amino acid carbon atom resonances (δ_C 51.2, 53.1, 53.6, 56.7, 62.1, 63.5, 64.2, 64.7, 66.3 and 64.4 ppm—all doublets carrying protons resonating in the δ_H 4.17−4.96 ppm range). The absence of the eleventh α-methine or methylene group is explained below. In addition, the NMR spectra established the presence of 10 methyl groups (four isopropyl groups, an H_3-doublet and an

---

* To whom correspondence should be addressed: Tel: +972-3-6408419. Fax: +972-3-6409293.

[†] School of Chemistry, Tel-Aviv University.

[‡] Department of Zoology, Tel Aviv University.

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1−49 and previous reviews in this series.

(2) Schemjakin, M. M. *Angew Chem.* **1960**, *72*, 342−343.

(3) Munro, M. H. G.; Blunt, J. W. Marine Literature Database, Department of Chemistry, University of Canterbury.

(4) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793−1806.

(5) Fusetani, N.; Matsunaga, S. *Curr. Org. Chem.* **2003**, *7*, 945−966.

(6) The nomenclature of amino acids and peptides is located at http://www.chem.qmul.ac.uk/iupac/AminoAcid/.

10.1021/ol0491787 CCC: $27.50

Table 1. ^{1}H and ^{13}C NMR Data of Callynormine A[a]

	δ_C	δ_H	mult (J, Hz)		δ_C	δ_H	mult
Ile[1]				4	26.5	1.69	m
CO	175.4					1.83	m
2	66.4	4.37	m	5	51.1	3.41	m
3	39.2	1.98	m			3.65	m
3-Me	17.6	1.08	d (6.8)	Leu[7]			
4	24.3	1.11	m	CO	176.3		
		1.31	m	2	53.1	4.96	dt (3.7, 9.8)
4-Me	12.3	0.92	t (7.9)	3	42.6	1.90	m
NH		5.58	m			2.00	m
Hyp[2]				4	26.8	1.83	m
CO	174.9			4-Me	21.9	0.98	d (6.7)
2	64.2	4.40	m	4-Me	24.0	1.05	d (6.6)
3	28.5	2.08	m	NH		7.84	d (9.5)
		2.31	m	FGly[8]			
4	71.3	4.57	m	CO	170.5		
5	57.1	3.72	d (11.1)	2	100.5		
		4.01	dd (3.6, 11.3)	3	143.8	7.17[b]	d (5.0)[b]
Val[3]				NH		8.64	s
CO	173.6			Pro[9]			
2	62.1	4.17	d (4.2)	CO	174.7		
3	30.9	2.28	m	2	66.3	4.57	m
3-Me	19.1	1.05	d (7.3)	3	30.8	2.08	m
3-Me	19.6	1.05	d (7.3)			2.28	m
NH		7.06	d (5.5)	4	26.5	2.18	m
Leu[4]						2.24	m
CO	172.6			5	48.2	3.61	m
2	51.2	4.76	m			3.86	t (8.9)
3	43.2	1.56	m	Phe[10]			
		1.69	m	CO	174.6		
4	26.2	1.69	m	2	56.7	4.61	dt (4.3, 12.2)
4-Me	23.5	1.05	d (6.2)	3	38.9	3.02	t (12.9)
4-Me	23.5	0.99	d (5.9)			3.33	m
NH		7.53	d (7.3)	4	138.8		
Pro[5]				5, 9	130.6	7.33	d (7.4)
CO	175.0			6, 8	130.0	7.40	t (7.6)
2	64.7	4.46	m	7	128.3	7.27	t (7.27)
3	30.4	1.87	m	NH		7.73	m
		2.50	m	Leu[11]			
4	27.3	2.08	m	CO	178.2		
		2.08	m	2	53.6	4.46	m
5	49.2	3.39	m	3	43.2	1.63	m
		3.77	m	4	26.3	1.83	m
Pro[6]				4-Me	21.4	0.97	d (6.7)
CO	175.0.1			4-Me	24.8	0.96	d (6.8)
2	63.5	4.26	dd (10.0, 7.5)	NH		7.73	m
3	38.9	2.08	m				

[a] Spectra were recorded in MeOH-d_4 at 500 MHz for ^{13}C and ^{1}H NMR. [b] Changes slowly to a singlet.

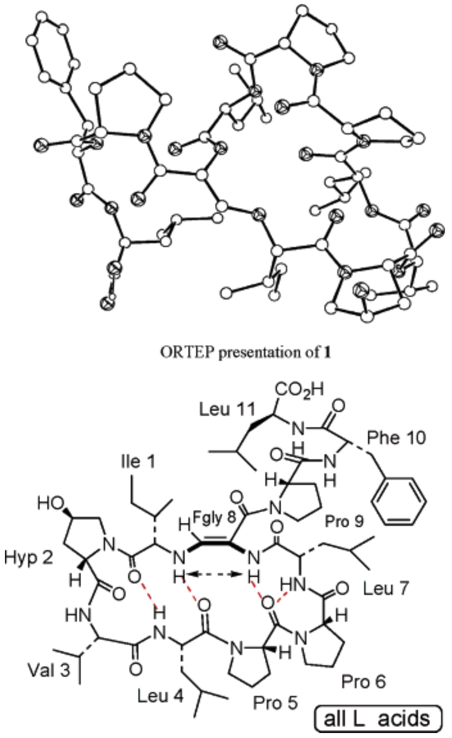


Figure 1. Callynormine A (- - -, hydrogen bonds; bold, the new class of bond; −, NOE).

H_3-triplet), four CH_2N groups, a phenyl, a methinoxy functionality, and, unusually a double bond conjugated to one of the amide carbonyl groups (δ_C 100.5 s, the α-, and 143.8 d, the β-carbon, δ_H 7.17). The NMR data enabled the determination of the major segments of **1**. Obtaining suitable crystals from **1**, from CH_3CN, made possible an X-ray diffraction analysis which established the complete structure of callynormine A, as shown[7,8] in Figure 1.

As seen here, **1** comprises a cyclic peptide (Ile (1) to FGly (8)) with a linear tripeptide side chain (Pro(9)-Phe-Leu-OH(11)) joined together by a naturally unprecedented α-amido-β-aminoacrylamide (“dehydropeptide”)[9] functional group. Evidently, the latter functionality is formed by condensation of the aldehyde of FGly, obtained from oxidation of Ser or Cys, and the N-terminal amino acid amino group [Ile(1)], to give the conjugated acrylamide functionality via

---

(7) The diffraction measurements were carried out on a Nonius KappaCCD diffractometer, using graphite-monochromated Mo Kα radiation ($\lambda = 0.7107$ Å). The analyzed crystalline sample was covered with a thin layer of light oil and freeze-cooled to ca. 110 K in order to minimize thermal motion effects, possible structural disorder, and to increase the precision of the results. The crystal structure was solved by direct methods (SHELXD-97) and refined by full-matrix least-squares on F^2 (SHELXL-97).[8] All non-hydrogen atoms were refined anisotropically. Most of the hydrogens were located in idealized positions, and some from a difference Fourier map. They were refined using a riding model with fixed thermal parameters [$U_{ij} = 1.2U_{ij}$ (equiv) for the atom to which they are bonded]. The polypeptide crystallized as biacetonitrile solvate. Crystal Data: $C_{61}H_{93}N_{11}O_{13}\cdot 2CH_3CN$, molecular weight 1270.57, orthorhombic, space group $P2_12_12_1$, $a = 12.3140(2)$ Å, $b = 15.6380(2)$ Å, $c = 36.7510(7)$ Å, $V = 7077.0(2)$ Å^3, $Z = 4$, $T = 110$ K, $D_{calc} = 1.193$ g·cm^{-3}, μ(Mo Kα) = 0.08 mm^{-1}, 20 208 measured and 10 714 unique reflections ($R_{int} = 0.018$, $2\theta_{max} = 50.0°$). Final $R1 = 0.073$ for 7073 observations with $F_0 > 4\sigma(F_0)$, $R1 = 0.118$ (w$R2 = 0.204$) for all unique data, $|\Delta p| \leq 0.61$ e/Å^3. The benzyl group of the phenylalanine fragment was found disordered between two differently oriented and equally probable positions. The acetonitrile crystallization solvent is also affected by partial disorder.

(8) Sheldrick, G. M.; SHELXD-97 and SHELXL-97. Programs for the SOlution and Refinement of Crystal Structures from Diffraction Data, University of Göttingen, Germany, 1997.

(9) Svete, J.; Aljaz-Rozic, M.; Stanovnik, B. *J. Heterocycl. Chem.* **1997**, *34*, 177−193.

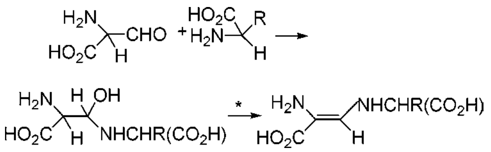


Figure 2. Suggested mechanism for the formation of the cyclization endiamino bond. *A Schiff base intermediate is also possible.

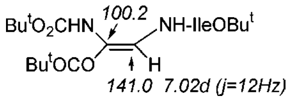
a carbinol amine directly or through isomerization of the Schiff base (Figure 2).

According to Murphy's method[10] and the X-ray data, all amino acids are of the L-configuration. According to its structure, callynormine A belongs to the heterodetic cyclic peptides[5] as, in addition to the eupeptide bonds, it includes a different bond as part of the backbone. We suggest designating this new class of cyclic peptides cyclic endiaminopeptides.

The conformation of the cyclic part of **1** in soluion, as deduced from the NH to ND exchange rate in MeOH-d_4, resembles the crystal structure. Namely, three NH groups of Leu-(4), Ile-(1), and FGly-(8) do not exchange due to hydrogen bonds to the amide CO groups of Ile-(1), Pro-(5), and Pro-(6), respectively (2.1, 2.2, and 2.0 Å in the crystal, respectively).[11]

The side chain, however, is mobile and occupies, in addition to the two crystal positions,[7] another conformation in which the side chain is close to Val(3) and Leu(4). The latter conformation is suggested on the basis of ROESY cross-peaks between Pro(9), of the side chain, and Val(3) and Leu(4).[12]

α-Formyl glycine (FGly) has already recently been reported for both eukaryotic and prokaryotic sulfatases—located within the catalytic site of the enzyme.[13,14] It was shown that the formylglycine is generated by oxidation of cysteine or serine and furthermore that the FGly hydrate is covalently sulfated[13] or covalently phosphorylated[14] during catalysis. To the best of our knowledge there are no reports of the highly likely internal (in the catalytic site or closing loops of the enzyme or peptide) or external bonds of the active FGly aldehyde group to amines (e.g., of Lys or Orn) to afford imines or the acrylamide moiety as in **1** (Figure 3). The possibility of obtaining the latter α-amido-β-aminoacrylamide functionality from protected FGly and the free amino group of Ile-OBu^t was demonstrated in the preparation of



A recent patent describes the synthesis of the closely related α-amino acid amides.[15] Like the disulfide bond, which probably is the most general of Nature's peptide groups that impose conformational restraints, the α-amido-β-aminoacrylamide functionality may also serve the same purpose. Possible ways in which the endiamino moiety can be inserted into peptide structures are shown in Figure 3; e.g., bonds may be formed between the aldehyde of FGly and an amino group of Lys or Orn. Other bonds are also possible.

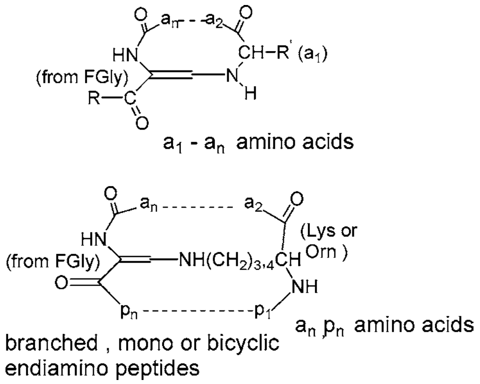


Figure 3. Possible cyclic or linear (branched) endiaminopeptides R = OR1 or NHR2, or in the case of **1**, cyclic, $n = 7$, R = Pro-Phe-LeuOH.

Acknowledgment. We thank PharmaMar Madrid for financial support and Adi Zvirdling, Coral Farm Ltd., Kenya, for logistic support and friendship. Special thanks are due to Mr. Charles O. Oduol, Assistant Director of Fisheries, Mombasa, Ministry of Agriculture and Rural Development, Fisheries Department, Kenya, for issuing the collection permit for the research expedition. Shimrit Perkol and Tali Yacobovitch are acknowledged for assistance in the fieldwork. The help of Prof. George Sheldrick in solving this difficult crystal structure is gratefully acknowledged.

Supporting Information Available: NMR data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0491787

---

(10) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596. L-Pro, L-Phe, and L-Ile were identified by this method.

(11) NH[Leu(7)] does not exchange due to a hydrogen bond to CO[Pro(6)] (2.7 Å in the crystal).

(12) ROESY correlations between Leu(4) H-2 and NH to Pro(9) H-5 and between Val (3) H-2 to Pro(9) H-2.

(13) Dierks, T.; Miech, C.; Hummerjohann, J.; Schmidt, B.; Kertesz, M. A.; von Figura, K. *J. Biol. Chem.* **1998**, *273*, 25560−25564.

(14) Chruszcz, M.; Laidler, P.; Monkiewicz, M.; Ortlund, E.; Lebioda, L.; Lewinski, K. *J. Inorg. Biochem.* **2003**, *96*, 386-392.

(15) Singh, R.; Zhou, N.; Reddy, A. V. N.; Thomas, G.; Ding, Q.; Kaleta, J.; Micetich, R. G.; Whittaker, M. US Patent 6,635,621 B1, 2003.