Notes

Celenamide E, a Tripeptide Alkaloid from the Patagonian Sponge *Cliona chilensis*

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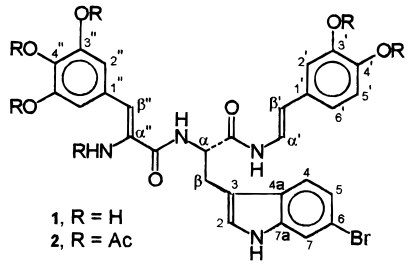
Celenamide E, a novel tripeptide alkaloid and a possible biosynthetic precursor of the previously reported celenamides A−C, was isolated from the Patagonian sponge *Cliona chilensis* collected at Rada Tilly, Chubut, Argentina. The structure of celenamide E was elucidated by spectroscopic techniques—UV, IR, 1D and 2D NMR (COSY, NOESY, HETCOR, COLOC) experiments—HRFABMS, and preparation of an hexaacetate derivative. An unusual feature of celenamide E is the presence of a N-terminal dehydroamino acid. Celenamide E shows antibiotic activity against Gram-positive bacteria at 50 μg/disk.

Sponges of the family *Clionidae* are usually burrowing organisms that excavate a variety of calcareous substrates such as rocks, coralline reefs, and oyster shells. In a previous paper[1] we reported the isolation and structure elucidation of storniamides A−D, hexacyclic aromatic alkaloids from a Patagonian sponge of the genus *Cliona*. Linear peptide alkaloids have also been reported from sponges of this family. Andersen *et al.* isolated from *Cliona celata* clionamide[2,3] and celenamides A − D,[4,5] linear peptide alkaloids of two or four amino acid units, respectively. In this paper we report the isolation and structure elucidation of celenamide E, a novel tripeptide alkaloid from the Patagonian sponge *Cliona chilensis*.

Although initially reported from Chile, *Cliona chilensis* (Thiele), a yellow massive sponge, is also widely distributed in the south Atlantic ocean, especially along Patagonian coastal waters. The crude extract of *C. chilensis* collected at Rada Tilly, Chubut, Argentina, was partitioned into polar and lipophillic extracts (see Experimental section). The polar extract showed antibiotic activity against Gram-positive bacteria. Fractionation of this extract by reversed-phase flash chromatography and final purification by Sephadex LH-20 enabled us to isolate celenamide E (**1**) as a yellow solid.

HRFABMS of **1** was consistent with a molecular formula $C_{28}H_{25}N_4O_7Br$, while initial inspection of the ^{1}H-NMR spectrum showed signals attributable to aromatic and olefinic protons with the exception of a methylene [δ 3.68 (dd, 1H, J = 15.1, 4.2 Hz); 3.22 (dd, 1H, J = 15.1, 10.1 Hz)] and a methine group [δ 4.47 (dd, 1H, J = 10.1, 4.2 Hz)] typical of a tryptophan derivative. In fact, some of the aromatic signals were characteristic of a 6-bromotryptophan derivative [δ 7.35 (s); 7.55 (d, J = 8 Hz); 7.50 (d, J = 2 Hz); 7.04 (dd, J = 8,2 Hz)]. The remaining signals indicated the presence of two additional aromatic rings, one of them tetrasubstituted [δ 6.67 (s, 2H)] and the other trisubstituted [δ 6.85 (d, J = 2 Hz); 6.70 (d, J = 8 Hz), and 6.65 (dd, J = 8; 2 Hz)]. The shielding of the aromatic protons suggested that several of these substituents were hydroxyl groups. Taking into account that the molecular formula indicated 16 double-bond equivalents, four additional unsaturations had to be present. A *trans*-disubstituted double bond was identified from the ^{1}H-NMR spectrum [δ 7.25 (d, J = 14 Hz) and 6.25 (d, J = 14 Hz)] as an enamide double bond. A singlet at 7.08 indicated the possible presence of an additional trisubstituted double bond. 2D NMR spectra proved that these three substituents were a 3,4,5-trihydroxylated aromatic ring, an amide carbonyl, and an enamino group.



The ^{13}C-NMR spectrum indicated the presence of two amide carbonyls, one of them α,β unsaturated, which completed the required 16 degrees of unsaturation. All these data suggested a tripeptide structure comprising three aromatic amino acids, of which one was 6-bro-

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Table 1. NMR Correlations for Celenamide E (1) [Me_2CO-d_6−CD_3OD (9:1)]

C	δ ^{13}C	δ ^{1}H (J = Hz)[a]	COSY	COLOC[b]
TrpC=O	168.9			
α	54.0	4.47 dd (10.1; 4.2)	H-βa; H-βb	
β	27.1	(a) 3.68 dd (15.1; 4.2)	H-α; H-βb; H-2	
		(b) 3.22 dd (15.1; 10.1)	H-α; H-βa; H-2	
2	126.7	7.35 s		
3	107.1			H-βa; H-2
4a	137.7			H-2; H-4
4	120.5	7.55 d (8)	H-5; H-7	
5	122.5	7.04 dd (8;2)	H-4; H-7	H-7
6	126.4			H-4; H-5; H-7
7	114.7	7.50 d (2)	H-4; H-5	H−5
7a	115.1			H−4
α′	121.2	7.25 d (14)	H-β′	H-β′
β′	115.3	6.25 d (14)	H-α′, H-2′	H-2′
1′	129.0			H-α′; H−6′
2′	112.7	6.85 d (2)	H-6′; H-β′	H-β′; H-6′
3′	145.2			H-2′; H-5′
4′	144.4			H-2′; H-5′; H-6′
5′	116.2	6.70 d (8)	H-6′	H-6′
6′	118.6	6.65 dd (8;2)	H-2′; H-5′	H-β′; H-2′; H-5′
PheC=O	163.9			H-β″
α″	124.7[c]			
β″	132.7	7.08 s	H-2″	H-2″
1″	125.1[c]			
2″	110.1	6.67 s	H-β″	H-β″
3″	145.6			H−2″
4″	135.2			H-2″

[a] Exchangeable protons are not shown. [b] COLOC experiment is optimized for J ^{1}H−^{13}C = 8 Hz. [c] Assignments may be interchanged.

motryptophan. There were striking similarities between compound **1** and celenamides A and B, the only difference being the absence of the aliphatic amino acid (leucine or valine, respectively). The confirmation of this tentative structure, as well as a complete assignment of the ^{1}H- and ^{13}C-NMR spectra, was accomplished by a series of 2D NMR experiments. The coupling pattern of the protons was established by ^{1}H−^{1}H COSY and long-range optimized COSY, while direct and long-range ^{1}H−^{13}C connectivities were determined by HETCOR and COLOC experiments, respectively. These correlations are indicated in Table 1. The stereochemistry of the trisubstituted double bond was established as *Z* by means of a gated decoupled ^{13}C-NMR spectrum. The carbonyl signal at δ 163.9 showed a long-range ^{1}H−^{13}C coupling to H-β″ of 4 Hz, which indicated that these two nuclei were *cis* to each other, thus establishing the *Z* geometry for the double bond. The absolute configuration of the 6-bromotryptophan residue was established as L (as in the other celenamides) by comparison of the CD curve of **1** with that of *N*-acetyl-L-tryptophanamide.

The enamine protons could not be identified in ^{1}H-NMR spectra in DMSO-d_6, because they overlapped with the five phenolic protons in a broad signal; however, conclusive evidence of the presence of the enamine functional group could be obtained after the preparation of the hexaacetate of celenamide E, obtained in low yield under standard acetylation conditions (see Experimental section). The ^{1}H-NMR spectrum of this compound showed the presence of six acetate methyls. On the basis of their chemical shifts, five of them [δ 2.36, 2.27, 2.26, and 2.20 (6H)] were undoubtedly characterized as acetoxy groups, while the upfield shift of the remaining methyl (δ 1.94) was typical of an *N*-acetyl group. Four exchangeable protons [δ 8.98 (d, J = 10 Hz), 8.73 (br s), 7.85 (br s), and 6.45 (d, J = 5 Hz)] were observable in the ^{1}H-NMR spectrum of celenamide E hexaacetate. Three of them could be easily identified by correlations observed in a COSY experiment as TrpCON*H*, Trp-H_1, and Trp-N*H*CO. No correlations could be observed in the COSY spectrum for the broad singlet at δ 7.85, the probable enamide proton bound to C-α″. A phase-sensitive NOESY experiment of the hexaacetate derivative provided conclusive evidence of the enamine functional group in celenamide E. In this spectrum, the signal of H-2″ protons showed NOE correlations to the exchangeable proton at δ 7.85 and to H-β″, thus confirming that the hexaacetate of celenamide E had an −N*H*-acetyl substituent at C-α″.

Compound **1** was named celenamide E on the basis of the structural similarities to the previously reported celenamides. An unusual feature of celenamide E is the presence of a N-terminal dehydroamino acid. Conjugation among the aromatic ring, the enamine double bond, and the peptide carbonyl, together with the mild isolation conditions employed, may account for the stability of this N-terminal dehydroamino acid unit during purification steps. Celenamide E may be the possible biogenetic precursor of celenamides A−C and represents the first tripeptide alkaloid isolated from sponges of the family *Clionidae*. Celenamide E shows antibiotic activity against Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtillis*, and *Enterococcus faecallis*) at 50 μg/disk.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Hewlett-Packard 8451A diode array spectrophotometer; IR, on a Nicolet magna-FT-IR 550 spectrometer; CD curves, on a JASCO J-20 spectropolarimeter; FABMS (matrix: glycerol, Cs 3.5 kv), on a VG-ZAB-SEQ hybrid mass spectrometer. HRFABMS

were acquired on a VG Autospec Q spectrometer; NMR experiments were recorded on Bruker AC 200 and AC 500 spectrometers using TMS as internal standard. 2D NMR experiments were performed using standard pulse sequences.

Collection and Isolation. The sponge *Cliona chilensis* was collected by scuba (−15 m) at Rada Tilly, Chubut, Argentina, and identified by one of us (E. C.). A voucher specimen is deposited at the Departamento de Biología, Universidad Nacional de la Patagonia "San Juan Bosco", Comodoro Rivadavia, Chubut, Argentina. The freshly collected sponge was frozen at −20 °C until worked up. The frozen sponge (600 g) was blended with EtOH (1 L). The extract was filtered and the residue extracted once again at room temperature with EtOH (1 L) and EtOAc (1 L). The combined extracts were evaporated to leave 18 g of crude extract, which was triturated with MeOH (400 mL) and filtered. The soluble material was partitioned between hexane and MeOH−H_2O (9:1) yielding lipophilic (hexane layer) and polar (aqueous MeOH layer) extracts. The polar extract was fractionated by reversed-phase flash chromatography using a H_2O−MeOH gradient. The fraction eluted with MeOH−H_2O (60:40) (400 mg) was chromatographed by Sephadex LH-20 (4 × 80 cm column, MeOH) to yield celenamide E (70 mg; 0.39% crude extract).

Celenamide E (**1**), obtained as an amorphous yellow solid (MeOH−H_2O); mp 212−218 °C (dec); $[\alpha]^{25}_D$ −25.2° (*c* 0.25, MeOH); UV (MeOH) λ max (log ϵ) 226 (4.50), 296 (4.17), 340 (4.30) nm; IR (KBr) ν max 3256, 2911, 2861, 2479, 1697, 1640, 1624, 1519, 1446, 1045, 953, 824 cm $^{-1}$; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; HRFABMS $(M + H)^+$ 609.1001 (calcd for $C_{28}H_{26}N_4O_7$-Br 609.0985; Δ −1.6 mDa).

Acetylation of Celenamide E (1). Celenamide E (**1**) (20 mg) was dissolved in 1 mL of dry Ac_2O−pyridine, and the mixture was allowed to stand overnight at room temperature. Preparative TLC [Si gel, cyclohexane−EtOAc (1:1)] of the crude acetylation product obtained after usual workup afforded 5 mg of celenamide E hexaacetate (**2**).

Celenamide E hexaacetate (2): obtained as an oil; $[\alpha]^{25}_D$ −20.8° (*c* 0.35, CH_2Cl_2); UV (MeOH) λ max (log ϵ) 226 (4.50), 298 (4.17), 344 (4.30) nm; IR (KBr) ν max 2921, 2859, 2473, 1733, 1720, 1687, 1633, 1624, 1510, 1453, 1041, 948, 821 cm $^{-1}$; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.97 (d, J = 10 Hz, TrpCON*H*), 8.73 (br s, H-1), 7.85 (br s, Ac−N*H*-Cα″), 7.44 (d, J = 1.6, H-7), 7.43 (dd, J = 14.5, 10, H-α′), 7.40 (d, J = 8.4, H-4), 7.33 (s, H-β″), 7.15 (dd, J = 8.4, 1.6, H-5), 7.11 (dd, J = 8.4, 2, H-6′), 7.09 (d, J = 1.6, H-2′), 7.05 (d, J = 8.4, H-5′), 7.00 (s, 2H, H-2″), 6.68 (d, J = 2.1, H-2), 6.45 (d, J = 5, Trp-N*H*), 6.26 (d, J = 14.5, H-β′), 4.54 (m, H-α), 3.17 (AB system, 2H, H-β), 2.36 (s, 3H, −O-*Ac*), 2.27 (s, 3H, −O-*Ac*), 2.26 (s, 3H, −O-*Ac*), 2.20 (s, 6H, 2 × −O-*Ac*), 1.95 (br s, 3H, −NH-*Ac*); ^{13}C NMR ($CDCl_3$; 50 MHz) δ 172.3, 171.8, 168.5, 168.4, 168.3, 167.3, 161.2, 143.2, 142.2, 140.5, 135.7, 134.3, 131.9, 130.0, 129.5, 126.3, 124.5, 124.2, 123.8, 123.5, 122.5, 121.0, 120.3, 119.3, 115.3, 114.7, 112.9, 108.7, 55.7, 29.7, 26.1, 22.9, 20.6, 20.2.

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References and Notes

(1) Palermo, J. A.; Rodríguez Brasco, M. F.; Seldes, A. M. *Tetrahedron* **1996**, *52*, 2727.
(2) Andersen, R. J. *Tetrahedron Lett.* **1978**, 2541−2544.
(3) Andersen, R. J.; Stonard, R. J. *Can J. Chem.* **1979**, *57*, 2325−2328.
(4) Stonard, R. J.; Andersen, R. J. *J. Org. Chem.* **1980**, *45*, 3687−3691.
(5) Stonard, R. J.; Andersen, R. J. *Can J. Chem.* **1980**, *58*, 2121-2126.

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