Patellamide E: a New Cyclic Peptide from the Ascidian Lissoclinum patella

Leonard A. McDonald, and Chris M. Ireland

J. Nat. Prod., **1992**, 55 (3), 376-379• DOI:
10.1021/np50081a016 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 3, pp. 376-379, March 1992

PATELLAMIDE E: A NEW CYCLIC PEPTIDE FROM THE ASCIDIAN *LISSOCLINUM PATELLA*

LEONARD A. MCDONALD and CHRIS M. IRELAND*,[1]

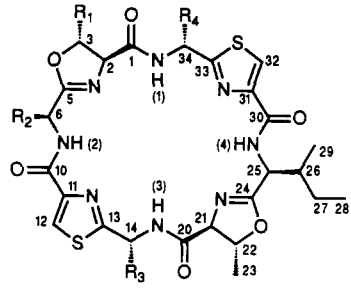
Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah 84112

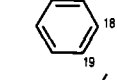
ABSTRACT.—A new cyclic peptide, patellamide E [**1**], was isolated from the ascidian *Lissoclinum patella* collected at Pulau Salu, Singapore. Its structure was determined by nmr spectroscopy, and its absolute configuration by acid hydrolysis and analysis of the derivatized constituent amino acids by hplc. Patellamide E was mildly cytotoxic against human colon tumor cells in vitro.

Ascidians produce a variety of bioactive amino-acid-derived secondary metabolites (1,2) such as the didemnins (3) and the lissoclinum peptides (4–9). Didemnin B, isolated from *Tridemnum solidum*, is the first marine natural product evaluated in clinical trials as an anticancer agent (14). The heptapeptide lissoclinamides and the octapeptide patellamides and ulithiacyclamides (5–9) from *Lissoclinum patella* Gottschaldt (Didemnidae) are characterized by unusual thiazole and oxazoline amino acids and exhibit strong in vitro cytotoxicity.

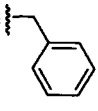
In our continuing search for potential anticancer agents from marine sources, we have isolated a new cyclic peptide, patellamide E [**1**], in addition to several known peptides, from *L. patella* collected at Pulau Salu, Singapore. We report the structure determination of **1** by extensive 1D and 2D nmr spectroscopic analyses.

The $CHCl_3$ extract of *L. patella* was purified by Si gel flash chromatography followed by Si gel hplc to yield patellamide E [**1**]. The extract also yielded patellamides A [**2**] and B [**3**] (5, 10–13), and ulithiacyclamide (4). The positive ion fabms of **1** indicated a mass of



1 R_1=Me, R_2=  , R_3=  , R_4= 

2 R_1=H, R_2=  , R_3=R_4= 

3 R_1=R_4=Me, R_2=  , R_3= 

[1]NIH Career Development Awardee, 1987–1992.

790 with hrfabms analysis supporting the molecular formula $C_{39}H_{50}N_8O_6S_2$ for patellamide E. The ir spectrum of **1** showed stretches at 3371, 3326, 1666, and 1537 cm^{-1}, indicative of a peptide. Inspection of the ^1H- and ^{13}C-nmr spectra of **1** revealed striking similarities to the patellamides, and a 14 mass unit difference suggested that **1** was a homologue of patellamide B [**3**]. In agreement with the molecular formula, the ^{13}C-nmr spectrum of **1** contained 37 resonances, including two corresponding to degenerate phenyl carbons at 129.17 and 128.55 ppm. Additional evidence from a PS-DQF-COSY (15) experiment established a phenylalanine, an isoleucine, two methyloxazolines, and two valine residues. Two broad singlet resonances in the proton spectrum of **1** at 7.46 and 7.52 ppm (H-12 and H-32, respectively) were indicative of two thiazole rings. Carbon resonances at 147.77 (C-11), 123.81 (C-12), and 170.20 (C-13) and 148.39 (C-31), 123.03 (C-32), and 170.51 (C-33) further supported two thiazole rings. With these structural units accounting for all the mass of **1**, a DEPT (16) experiment established the multiplicities of the carbon resonances while an HMQC (17) experiment permitted assignment of the attached protons. Assignment of the quaternary carbon resonances and establishment of the larger structural units A and B (Figure 1) were accomplished with an HMBC (18) experiment (Table 1). Neither HMBC experiments, optimized for $^nJ_{CH}$ of 2 to 15 Hz, nor similarly optimized selective INEPT (19) experiments were able to establish the C-10–C-11 or C-30–C-31 bonds, apparently leaving two possible ways of connecting structural units A and B. The upfield chemical shifts of the C-10 and C-30 amide carbonyl resonances however, support conjugation to the thiazole rings and allow partial structures A and B to be connected in only one way to yield structure **1** for patellamide E.

The absolute stereochemistry of **1** was

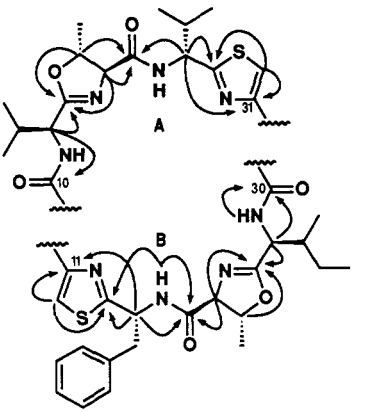


FIGURE 1. Partial structures and selected HMBC correlations for Patellamide E [**1**].

determined by comparing the amino acids obtained from acid hydrolysis with standard amino acids, both suitably derivatized for hplc analysis (20). This procedure established the presence of L-threonine, L-valine, and L-isoleucine. The absolute configurations of the thiazole amino acids were determined by a procedure greatly simplified from that previously published (21). This procedure uses ozone to destroy the aromaticity of the thiazole in order to facilitate hydrolysis and prevent racemization. This procedure involves bubbling ozone through a solution of peptide, followed by acid hydrolysis, derivatization, and hplc analysis. In addition to the previously identified amino acids, D-phenylalanine and D-valine were found in the hydrolysate of ozonized **1**, establishing the presence of (D-phenylalanine)-thiazole and (D-valine)-thiazole.

Patellamide E was weakly cytotoxic (IC_{50} 125 μg/ml) against human colon tumor cells in vitro.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were obtained on a Varian Unity 500 spectrometer. ^1H chemical shifts are reported in ppm relative to residual undeuterated solvent. Ir spectra were recorded on a Perkin-Elmer 1600 FT spectrophotometer. Uv spectra were obtained in MeOH on a Beckman DU-8 spectrophotometer.

TABLE 1. Nmr Assignments for Patellamide E [1].[a]

Position	δ ^{13}C	Multiplicity[b]	δ 1H (Multiplicity, J [Hz])	HMBC correlations[c]
1	172.97	s		
2	73.83	d	4.29 d, 4.0	C-1, C-5
3	82.21	d	4.88 m	C-1, C-5
4	21.52	q	1.40 d, 6.5	
5	168.09	s		
6	54.67	d	4.56 dd, 8.0, 8.0	C-5, C-10
7	28.74	d	2.17 m	C-5
8	18.98	q	0.87 d, 6.5	
9	18.99	q	0.92 d, 6.5	
10	160.96	s		
11	147.77			
12	123.81	d	7.46 bs	C-11, C-13
13	170.20			
14	52.11	d	5.43 ddd, 9.5, 9.5, 6.5	C-11[d], C-13, C-16, C-20
15	41.24	t	3.37 dd, 14.0, 9.5	C-13, C-16, C-17
			3.20 dd, 14.0, 6.5	C-13, C-16, C-17
16	136.18	s		
17	129.17	d	7.25 m	
18	128.55	d	7.25 m	
19	126.97	d	7.19 m	C-16, C-17, C-18
20	172.57	s		
21	73.54	d	4.16 d, 3.5	C-20, C-24
22	82.11	d	4.86 m	C-24
23	21.06	q	1.35 d, 6.5	
24	168.21	s		
25	53.00	d	4.66 dd, 9.0, 8.0	C-24, C-30
26	34.30	d	2.08 m	
27	24.90	t	1.46 m	
			1.26 m	
28	9.30	q	0.76 t, 7.5	
29	14.99	q	0.88 d, 6.9[e]	
30	161.37	s		
31	148.39	s		
32	123.03	d	7.52 bs	C-31, C-33
33	170.51	s		
34	55.68	d	5.14 dd, 10.5, 4.5	C-1, C-31, C-33
35	32.23	d	2.26 m	C-33
36	19.95	q	1.06 d, 7.0	
37	17.06	q	1.07 d, 7.0	
N-1			7.20 d, 10.5[e]	
N-2			7.69 d, 8.0[e]	
N-3			7.65 d, 9.5[e]	C-13, C-14, C-20
N-4			7.64 d, 9.0[e]	C-30

[a]Solvent was $CDCl_3$

[b]From a DEPT experiment.

[c]From an HMBC experiment optimized for an $^{n}J_{CH}$ of 8.5 Hz.

[d]Weak correlation.

[e]Obtained from a PS-DQF-COSY experiment due to severe overlap in the ^{1}H spectrum.

Optical rotations were measured with a Jasco DIP-370 polarimeter in a 100 mm cell. High and low resolution fabms were run on a Varian MAT-731 spectrometer in a glycerol matrix.

EXTRACTION AND ISOLATION PROCEDURES.—Specimens of *L. Patella* were collected by SCUBA off Pulau Salu, Singapore. The $CHCl_3$ extract of 22.6 g of freeze-dried ascidian (1.094 g) was purified by flash chromatography on Si gel (75% EtOAc/25% hexane) to yield a 191-mg fraction which was further purified by hplc

(Rainin DYNAMAX silica; 10 × 250 mm; 30% Me_2CO/70% hexane; 5.0 ml/min; ri detection) to yield 120 mg (0.53% dry wt) patellamide E [**1**].

PHYSICAL AND SPECTRAL PROPERTIES OF PATELLAMIDE E [**1**].—White amorphous solid; ir (film) ν max 3371, 3326, 2966, 2931, 2875, 1666, 1537, 1513, 1484 cm^{-1}; $[\alpha]^{25}D$ 48.6° (c = 0.58, $CHCl_3$); uv (MeOH) λ max 235 nm (ϵ 12305); fabms m/z $[M+H]^+$ 791, 763, 748, 700, 285, 214; hrfabms m/z $[M+H]^+$ 791.3359, $C_{39}H_{51}N_8O_6S_2$ (Δ = 1.7 mmu); ^1H and ^{13}C nmr see Table 1.

OZONOLYSIS OF **1**.—A slow stream of O_3 was bubbled into a 10 ml CH_2Cl_2 solution of **1** (1.7 mg; 2.15×10^{-3} mmol) in a threaded bomb at 25° for approximately 8 min. After removal of the solvent under a stream of N_2, the residue was subjected to hydrolysis and derivatization as described below.

STEREOCHEMISTRY OF **1**.—Hydrolysis of **1** (1.7 mg; 2.15×10^{-3} mmol) was carried out in 5 ml 6 N HCl under N_2 atmosphere in a sealed bomb at 110° for 18 h. After removal of traces of HCl by repeated evaporation in vacuo, the residual hydrolysate was suspended in 300 μl H_2O and derivatized with 1-fluoro-2,4-dinitrophenyl-5-L-alanineamide (FDAA) (20). Hplc analysis [Waters NOVAPAK C_{18}; 4.6 × 100 mm column; linear gradient elution, triethylammonium phosphate (50 mM, pH 3.0)/MeCN, 90:10 to 60:40 in 45 min; 1.5 ml/min; uv detection at λ 340 nm] of the FDAA-derivatized hydrolysate of **1** established the presence of L-threonine, L-valine and L-isoleucine by co-injection with similarly derivatized amino acid standards. Similar analyses confirmed D-valine and D-phenylalanine in addition to the previously identified amino acids in the acid hydrolysate of ozonized **1**.

ACKNOWLEDGMENTS

This work was supported by grants CA-36622 and CA-50750 (C.M.I.) awarded by the National Institutes of Health. L.A.M. acknowledges support from an NIH predoctoral fellowship supported by grant CA-36622. We thank Dr. Ralph A. Lewin for his generous gift of *L. Patella* and taxonomic identification. We gratefully acknowledge the Bristol-Myers Squibb Company for providing the HCT116 cell line.

LITERATURE CITED

1. C.M. Ireland, T.F. Molinski, D.M. Roll, T.M. Zabriskie, T.C. McKee, J.C. Swersey, and M.P. Foster, in: "Bioorganic Marine Chemistry." Ed. by P.J. Scheuer, Springer-Verlag, Berlin, 1989, Vol. 3, pp. 1–46.
2. C.M. Ireland, D.M. Roll, T.F. Molinski, T.C. McKee, T.M. Zabriskie, and J.C. Swersey, in: "Biomedical Importance of Marine Organisms." Ed. by D.G. Fautin, California Academy of Sciences, San Francisco, 1988, No. 13, pp. 41–57.
3. K.L. Rinehart, J.B. Gloer, J.C. Cook, S.A. Mizsac, and T.A. Scahill, *J. Am. Chem. Soc.*, **103**, 1857 (1981).
4. C. Ireland and P.J. Scheuer, *J. Am. Chem. Soc.*, **102**, 5688 (1980).
5. C.M. Ireland, A.R. Durso, R.A. Newman, and M.P. Hacker, *J. Org. Chem.*, **47**, 1807 (1982).
6. D.E. Williams, R.E. Moore, and V.J. Paul, *J. Nat. Prod.*, **52**, 732 (1989).
7. F.J. Schmitz, M.B. Ksebati, J.S. Chang, J.L. Wang, M.B. Hossain, D. van der Helm, M.H. Engel, A. Serban, and J.A. Silfer, *J. Org. Chem.*, **54**, 3463 (1989).
8. B.M. Degnan, C.J. Hawkins, M.F. Lavin, E.J. McCaffrey, D.L. Parry, A.L. van den Brenk, and D.J. Watters, *J. Med. Chem.*, **32**, 1349 (1989).
9. C.J. Hawkins, M.F. Lavin, K.A. Marshall, A.L. van den Brenk, and D.J. Watters, *J. Med. Chem.*, **33**, 1634 (1990).
10. Y. Hamada, M. Shibata, and T. Shioiri, *Tetrahedron Lett.*, **26**, 5155 (1985).
11. Y. Hamada, M. Shibata, and T. Shioiri, *Tetrahedron Lett.*, **26**, 5159 (1985).
12. U. Schmidt and H. Griesser, *Tetrahedron Lett.*, **27**, 163 (1986).
13. D.F. Sesin, S.J. Gaskell, and C.M. Ireland, *Bull. Soc. Chim. Belg.*, **95**, 853 (1986).
14. F.A. Dorr, J.G. Kuhn, J. Phillips, and D.D. von Hoff, *Eur. J. Cancer Clin. Oncol.*, **24**, 1699 (1988).
15. M. Rance, O.W. Sørenson, G. Bodenhausen, G. Wagner, R.R. Ernst, and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, **117**, 479 (1983).
16. D.M. Doddrell, D.J. Pegg, and M.R. Bendall, *J. Magn. Reson.*, **48**, 323 (1982).
17. M.F. Summers, L.G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, **108**, 4285 (1986).
18. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1988).
19. A. Bax, *J. Magn. Reson.*, **57**, 314 (1984).
20. P. Marfey, *Carlsberg Res. Commun.*, **49**, 591 (1984).
21. J.E. Biskupiak and C.M. Ireland, *J. Org. Chem.*, **48**, 2304 (1983).

Received 15 July 1991