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PATELLAMIDE F, A NEW CYTOTOXIC CYCLIC PEPTIDE FROM THE COLONIAL ASCIDIAN *LISSOCLINUM PATELLA*

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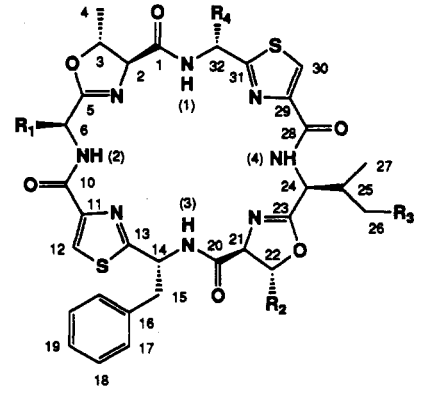
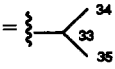
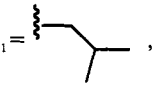
ABSTRACT.—Cytotoxicity-directed fractionation of an organic extract of the tunicate *Lissoclinum patella* afforded a new cyclic octapeptide, patellamide F [**1**]. The structure and absolute stereochemistry of **1** were established by a combination of spectroscopic and chemical methods. Three known cyclic peptides, patellamide B [**2**], ulithiacyclamide, and lissoclinamide 3, were also isolated and identified.

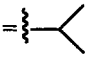
Colonial ascidians from the genus *Lissoclinum* are prolific sources of biologically active secondary metabolites (1,2). Over 40 compounds, including cyclic peptides (3–17), macrolides (18–20), thioalkaloids (21–23), and diterpenoids (24,25) have been reported from *Lissoclinum* species. *Lissoclinum*-derived cyclic peptides are characterized by unusual amino acids containing thiazole and oxazoline moieties and many of these metabolites exhibit cytotoxic activity. In our continuing search for potential anticancer drugs from natural sources (26), an extract of *Lissoclinum patella* Gottschaldt (Didemnidae) from northwestern Australia was found to be modestly cytotoxic in the NCI's 60-cell line antitumor assay. Cytotoxicity-directed fractionation of the extract by sequential application of solvent-solvent partitioning, vlc on diol bonded-phase, and Si gel hplc provided, as the principal active constituents, a new cyclic octapeptide patellamide F [**1**], and the known octapeptides patellamide B [**2**] (4,6), ulithiacyclamide (3), and the heptapeptide lissoclinamide 3 (5,6). The known compounds were identified by comparison of their spectral data with published values, while the structure of patellamide F [**1**] was assigned by spectral analysis and chemical methods.

Patellamide F [**1**] provided a fabms pseudomolecular ion (MH^+) at *m/z* 763.3049, which supported a molecular formula of $C_{37}H_{46}N_8O_6S_2$. The ^{13}C-nmr spectrum confirmed the presence of 37 carbons, while DEPT and HMQC experiments indicated that 42 of the 46 protons in **1** were attached to carbons (Table 1). The four doublet proton resonances at δ 7.27, 7.72, 7.75, and 7.83 that did not show $^1J_{CH}$ correlations could be assigned as NH protons that did not exhibit appreciable exchange with the solvent. Both the ^1H- and ^{13}C-nmr spectra of **1** showed close correspondence with the nmr data reported for patellamides B [**2**] (4,6) and E [**3**] (17). The mol wt of **1** differed by 14 and 28 daltons from compounds **2** and **3**, respectively, suggesting that **1** was a homo-



1 R_1=  , R_2=R_3=H, R_4= 

2 R_1=  , R_2=R_3=R_4=Me

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

logue of these peptides. ^{1}H-^{1}H connectivities observed in a COSY 45 nmr experiment and spectral comparisons with **2** and **3** established phenylalanine, oxazoline, methyloxazoline, and three valine residues in **1**. In addition, two disubstituted thiazole moieties could be inferred from characteristic ^{13}C-nmr resonances and singlet ^{1}H-nmr signals at δ 7.68 and 7.82 (17). Detailed spectral analysis allowed complete assignment of the ^{1}H- and ^{13}C-nmr resonances of patellamide F [**1**]. These closely matched the nmr assignments previously made for the related peptides **2** and **3** (4,6,17). Connectivities of the amino acid residues in **1** were elucidated primarily from HMBC experiments (Table 1). Correla-

TABLE 1. Nmr Data for Patellamide F [**1**].[a]

Position	δ ^{13}C	δ ^{1}H[b]	HMBC Correlations[c]
1	175.5		
2	73.0	4.47 d, 3.5	C-1, C-4, C-5
3	81.6	4.97 dq, 6.8, 3.5	C-1, C-5
4	21.1	1.48 d, 6.8	C-2, C-3
5	170.2		
6	56.3	4.49 dd, 10.7, 7.3	C-5, C-7, C-10
7	29.3	2.34 m	C-5, C-6, C-8, C-9
8	19.1	1.04 d, 6.8	
9	19.5	1.07 d, 6.8	
10	163.7		
11	148.9		
12	125.9	7.82 s	C-10, C-11, C-13
13	172.5		
14	54.0	5.51 dt, 9.8, 8.3	C-13*, C-16*, C-20*
15	41.5	3.43 (2H) d, 8.3	C-13, C-14, C-16, C-17
16	138.0		
17, 17′	130.2	7.43 d, 7.4	C-15, C-16, C-18
18, 18′	129.5	7.34 t, 7.4	C-16, C-17, C-18
19	127.9	7.25 d, 7.4	C-17, C-18
20	175.5		
21	68.4	4.68 dd, 9.5, 2.9	C-20, C-22*, C-23
22	74.7	4.60 t, 9.5	C-20, C-21
		4.69 dd, 9.5, 2.9	C-20, C-23
23	171.5		
24	56.6	4.51 dd, 7.3, 7.3	C-23, C-28*
25	29.3	2.34 m	C-23*, C-24*, C-26, C-27
26	19.4	1.05 d, 6.4	
27	18.8	1.11 d, 6.3	C-24*, C-25*
28	163.0		
29	148.2		
30	126.6	7.68 s	C-28, C-29, C-31
31	172.5		
32	57.6	5.21 dd, 10.3, 4.0	C-1, C-31, C -33, C -34, C-35
33	32.6	2.41 ddd, 7.3, 6.3, 4.0	C-31*, C-32*, C-34*, C-35*
34	17.2	1.16 d, 7.3	C-32, C-33
35	20.5	1.15 d, 6.3	C-32, C-33
N-1		7.27 d, 10.3	C-1
N-2		7.72 d, 7.3	C-6, C-7, C-10, C-11
N-3		7.83 d, 9.8	C-20
N-4		7.75 d, 7.3	C-24, C-25, C-28, C-29*

[a]Spectra were acquired in CD_3OD and referenced to the residual solvent signal.

[b]δ, multiplicity, J (Hz).

[c]From HMBC experiments optimized for $^nJ_{CH}$=8.3 or 5.0 Hz. Correlations with asterisks (*) were observed with DMSO-d_6 as solvent.

tions to carbons two- and three-bonds removed from the NH protons, the thiazole protons, and the α-methine protons established the complete cyclic amino acid sequence of patellamide F [**1**]. The unambiguous assignment of structure **1** was only possible after HMBC experiments were run in CD_3OD optimized for $^nJ_{CH}$=8.3 and 5.0 Hz and in DMSO-d_6 optimized for $^nJ_{CH}$=8.3 Hz.

The absolute stereochemistry of patellamide F [**1**] was determined by a method developed in Ireland's laboratory (17). Acid hydrolysis of **1** provided threonine, serine, and two equivalents of valine. Derivatization of the hydrolysis products with Marfey's reagent (27), and comparative hplc analyses with similarly derivatized standard D- and L-amino acids, established an L-configuration for each of these amino acids. No sign of D-valine was evident from this hydrolysis. A second sample of **1** was treated with ozone to destroy the aromatic character of the thiazoles, and then hydrolyzed. Similar derivatization and hplc analyses revealed D-phenylalanine and D-valine, in addition to the previously determined L-amino acids. This established the presence of (D-phenylalanine)-thiazole and (D-valine)-thiazole moieties in **1**.

The pure compounds patellamide B [**2**], patellamide F [**1**], and ulithiacyclamide showed only modest general cytotoxicity in the NCI's 60 human tumor cell line panel (average LC_{50} values of 48 μM, 13 μM, and 3 μM, respectively). None of the compounds produced any pattern of differential cytotoxicity of sufficient interest for further study based upon the NCI screen.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Varian VXR 500 spectrometer and chemical shifts were referenced relative to the residual undeuterated solvent signal. The number of attached protons for the ^{13}C-nmr signals was determined from a DEPT experiment. Proton-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC}$=140 Hz) and HMBC (optimized for $^nJ_{HC}$=8.3 and 5.0 Hz) pulse sequences. Whenever possible, H-H connectivities were confirmed by COSY 45 nmr experiments. Infrared spectra were obtained on a Perkin-Elmer 1600 Ft-ir spectrometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Mass spectra were recorded with a JEOL SX102 spectrometer.

ANIMAL MATERIAL.—Samples of *L. patella* were collected around Monte Bello Island, northwest Australia in August 1988, by Dr. Peter Murphy of the Australian Institute of Marine Science (AIMS). Voucher specimens from this collection are maintained at AIMS.

EXTRACTION AND ISOLATION.—Fresh samples were frozen immediately after collection and stored at −20° until processed. Frozen samples were ground to a coarse powder (221.2 g) with dry ice and extracted with H_2O. The marc was then refrozen, lyophilized, and sequentially extracted with MeOH-CH_2Cl_2 (1:1) and 100% MeOH. The combined organic extracts were evaporated at reduced pressure to give 4.13 g of a dark green gum.

A 3.0-g portion of the organic extract was subjected to a four-step solvent-solvent partitioning protocol (28), which concentrated the cytotoxic activity in a CCl_4-soluble fraction (1.0 g). This material was separated by gradient elution vlc on diol bonded-phase (YMC) using successively more polar mixtures of hexane, EtOAc, and MeOH. The cytotoxic fractions which eluted with 60–70% EtOAc in hexane were pooled (300 mg total). A portion of this material (120 mg) was separated by Si gel hplc (1×25 cm column) using hexane-*i*-PrOH (4:1; 4 ml/min) to afford patellamide F [**1**] (36 mg), patellamide B [**2**] (39 mg), and ulithiacyclamide (22 mg). The material that eluted from the diol vlc column with 80% EtOAc in hexane (66 mg) was separated by C_{18} hplc using MeOH-H_2O (4:1) to give lissoclinamide 3 (6 mg).

Patellamide F [**1**].—White amorphous solid: [α]D +40° (*c*=0.1, MeOH); hrfabms *m/z* 763.3049 (MH^+, $C_{37}H_{47}N_8O_6S_2$, calcd 763.3058); uv (MeOH) λ max 235 nm (ε 13700); ir (film) ν max 3370, 2965, 1666, 1537, 1221 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Table 1.

ACID HYDROLYSIS OF **1**.—A 2-mg sample of patellamide F [**1**] was treated with 200 μl of 6 N HCl and 0.1% phenol at 110° for 18 h in an evacuated vial. The solution was lyophilized and an aliquot was analyzed on an automated amino acid analyzer with ninhydrin post-column derivatization. The remainder of the hydrolysis product was used for stereochemical studies.

STEREOCHEMICAL DETERMINATION OF **1**.—The HCl hydrolysate of **1** was derivatized with 1-fluoro-2,4-dinitrophenyl-5-L-alanineamide

(FDAA) (27). Hplc analyses of the FDAA derivatized amino acids were performed on a 0.46×25 cm Rainin Dynamax C_{18} column eluted with a linear gradient of triethylammonium phosphate (50 mM, pH 3.0)-CH_3CN from 9:1 to 3:2 over 90 min at 1.0 ml/min, uv detection at 340 nm. FDAA derivatives of standard D- and L-amino acids were similarly prepared and analyzed by hplc. Based upon hplc retention time, integration of peak areas, and co-injection with the standard amino acid derivatives, the hydrolysate of **1** provided L-serine (1 equivalent), L-threonine (1 equivalent), and L-valine (2 equivalents). Similar analyses following ozonolysis of **1** confirmed D-valine (1 equivalent) and D-phenylalanine (1 equivalent), in addition to the previously identified amino acids.

OZONOLYSIS OF **1**.—A slow stream of O_3 was bubbled into a 10 ml CH_2Cl_2 solution of **1** (1.2 mg; 1.57 μmol) in a 50-ml tube at room temperature for 8 min. Additional CH_2Cl_2 was added at 4 min to compensate for evaporation. Solvent was removed by a stream of N_2 and the residue was subjected to acid hydrolysis and FDAA derivatization as previously described.

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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. B.S. Davidson, *Chem. Rev.*, **93**, 1771 (1993).
3. C.M. Ireland and P.J. Scheuer, *J. Am. Chem. Soc.*, **102**, 5688 (1980).
4. C.M. Ireland, A.R. Durso, Jr., R.A. Newman, and M.P. Hacker, *J. Org. Chem.*, **47**, 1807 (1982).
5. J.M. Wasylyk, J.E. Biskupiak, C.E. Costello, and C.M. Ireland, *J. Org. Chem.*, **48**, 4445 (1983).
6. D.F. Sesin, S.J. Gaskell, and C.M. Ireland, *Bull. Soc. Chim. Belg.*, **95**, 853 (1986).
7. D.E. Williams, R.E. Moore, and V.J. Paul, *J. Nat. Prod.*, **52**, 732 (1989).
8. F.J. Schmitz, M.B. Ksebati, J.S. Chang, J.L. Wang, M.B. Hossain, D. van der Helm, H.M. Engel, A. Serban, and J.A. Silfer, *J. Org. Chem.*, **54**, 3463 (1989).
9. B.M. Degnan, C.J. Hawkins, M.F. Lavin, E.J. McCaffery, D.L. Parry, A.L. van der Brenk, and D.J. Watters, *J. Med. Chem.*, **32**, 1349 (1989).
10. B.M. Degnan, C.J. Hawkins, M.F. Lavin, E.J. McCaffery, D.L. Parry, A.L. van der Brenk, and D.J. Watters, *J. Med. Chem.*, **32**, 1354 (1989).
11. C.J. Hawkins, M.F. Lavin, K.A. Marshall, A.L. van der Brenk, and D.J. Watters, *J. Med. Chem.*, **33**, 1634 (1990).
12. M.P. Foster, G.P. Concepción, G.B. Caraan, and C.M. Ireland, *J. Org. Chem.*, **57**, 6671 (1992).
13. L.A. McDonald, M.P. Foster, D.R. Phillips, C.M. Ireland, A.Y. Lee, and J. Clardy, *J. Org. Chem.*, **57**, 4616 (1992).
14. T.W. Hambley, C.J. Hawkins, M.F. Lavin, A. van der Brenk, and D.J. Watters, *Tetrahedron*, **48**, 341 (1992).
15. M.P. Foster and C.M. Ireland, *Tetrahedron Lett.*, **34**, 2871 (1993).
16. T.M. Zabriskie, M.P. Foster, T.J. Stout, J. Clardy, and C.M. Ireland, *J. Am. Chem. Soc.*, **112**, 8080 (1990).
17. L.A. McDonald and C.M. Ireland, *J. Nat. Prod.*, **55**, 376 (1992).
18. D.G. Corley, R.E. Moore, and V.J. Paul, *J. Am. Chem. Soc.*, **110**, 7920 (1988).
19. T.M. Zabriskie, C.L. Mayne, and C.M. Ireland, *J. Am. Chem. Soc.*, **110**, 7919 (1988).
20. M.P. Foster, C.L. Mayne, R. Dunkel, R.J. Pugmire, D.M. Grant, J. Kornprobst, J. Verbist, J. Biard, and C.M. Ireland, *J. Am. Chem. Soc.*, **114**, 1110 (1992).
21. T.F. Molinski and C.M. Ireland, *J. Org. Chem.*, **54**, 4256 (1989).
22. B.S. Davidson, T.F. Molinski, L.R. Barrows, and C.M. Ireland, *J. Am. Chem. Soc.*, **113**, 4709 (1991).
23. M. Litaudon and M. Guyot, *Tetrahedron Lett.*, **32**, 911 (1991).
24. C. Malochet-Grivois, P. Cotelle, J.F. Biard, J.P. Hénichart, C. Débitus, C. Roussakis, and J.F. Verbist, *Tetrahedron Lett.*, **32**, 6701 (1991).
25. J.F. Biard, C. Malochet-Grivois, C. Roussakis, P. Cotelle, J.P. Hénichart, C. Débitus, and J.F. Verbist, *Nat. Prod. Lett.*, **4**, 43 (1994).
26. M.R. Boyd, in: "Cancer, Principles and Practice of Oncology." Ed. by V.T. DeVita, Jr., S. Hellman, and S.A. Rosenberg, J.B. Lippincott Co., Philadelphia, 1989, Vol. 3, No. 10, pp. 1–12.
27. P. Marfey, *Carlsberg Res. Comm.*, **49**, 591 (1984).
28. B.C. Van Wagenen, R. Larsen, J.H. Cardellina II, D. Randazzo, Z.C. Lidert, and C. Swithenbank, *J. Org. Chem.*, **58**, 335 (1993).

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