THE JOURNAL OF Organic Chemistry

VOLUME 48, NUMBER 24

DECEMBER 2, 1983

Cyclic Peptide Structures from the Tunicate *Lissoclinum patella* by FAB Mass Spectrometry

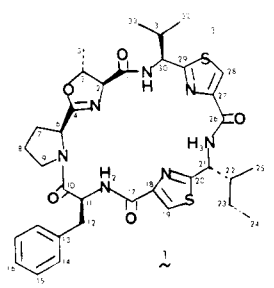
John M. Wasylyk,† Joseph E. Biskupiak,† Catherine E. Costello,‡ and Chris M. Ireland*†

Section of Medicinal Chemistry and Pharmacognosy, School of Pharmacy, University of Connecticut, Storrs, Connecticut 06268, and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

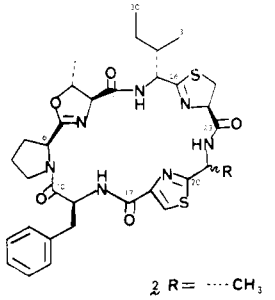
Received May 10, 1983

The structures of three cyclic peptides from the tunicate *Lissoclinum patella* are presented. The amino acid sequences of these peptides were determined by positive and negative ion fast atom bombardment (FAB) mass spectrometry. We also report a revised structure for a fourth peptide ulicyclamide on the basis of FAB data.

There has been growing interest in lipophilic cyclic peptides from marine organisms during the past 2 years, due largely to the potency of these metabolites as antineoplastic or antiviral agents. Three years ago we reported the first such peptides, ulithiacyclamide and ulicyclamide from the tunicate *Lissoclinum patella*.[1] This initial report was followed by the isolation of three additional cytotoxic peptides, the patellamides, from the same species collected at a different locale.[2] In this same time frame Rinehart and Pettit reported the isolation of very potent cytotoxic lipophilic peptides from a second tunicate (*Tridemnum sp.*)[3] and a mollusc (*Dolabella auricularia*),[4] respectively. Although *Lissoclinum* and *Tridemnum* belong to the same taxonomic family (*Didemnidae*) the peptides isolated from these tunicates bear no structural resemblance. Conversely, the dolastatins isolated from *D. auricularia* resemble the lissoclinum peptides: both series contain thiazole amino acids. We now wish to report that a further examination of column chromatography fractions from extracts of *L. patella* has yielded a new series of peptides **1**–**3**. The epimeric peptides **2** and **3** represent the first thiazoline-containing peptides in the lissoclinum series. We also report a revised structure for ulicyclamide (**4**) based on a detailed analysis of the fast atom bombardment (FAB) mass spectra of its hydrolysis product.



1



2 R = ····CH$_3$
3 R = —CH$_3$

Silica gel column chromatography (EtOAc) of combined petroleum ether, CCl_4 and $CHCl_3$ Soxhlet extracts (1.53 g) of freeze-dried *L. patella* (413 g) gave three peptide bands. Each band was subsequently resolved by high-performance liquid chromatography (RP-18; 8:2 MeOH/H_2O) into two components. The first peptide band (150 mg) was a 7:3 mixture of ulithiacyclamide and patellamide A. Band two (70 mg) contained patellamide B and a new peptide **1** in an 8:2 ratio. The third band (230 mg) contained ulicyclamide and patellamide C in a 9:1 ratio. Further elution from the original silica column with EtOAc/MeOH, 95:5, gave a fourth peptide band (624 mg) which was resolved by RP HPLC into the thiazoline peptides **2** and **3** in 2:1 ratio.

Peptides **2** and **3** had identical molecular formula $C_{33}H_{41}N_7O_5S_2$ (high-resolution EI mass measurement 679.2575 and 679.2588, respectively; calcd 679.2610). The ^1H and ^{13}C NMR spectra (Tables I and II) of **2** and **3** were very similar indicating the two peptides had identical amino acid compositions and differed in either conformation or stereochemistry. Furthermore, these data bore a striking resemblance to ulicyclamide ($C_{33}H_{39}N_7O_5S_2$), suggesting a dihydro relationship for **2** and **3** to ulicyclamide. Detailed proton decoupling studies with both **2** and **3** confirmed the presence of proline, phenylalanine, isoleucine, and alanine side chains as well as a threonine oxazoline (Table I)

† University of Connecticut.
‡ Massachusetts Institute of Technology

(1) Ireland, C.; Scheuer, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 5688.
(2) Ireland, C. M.; Durso, J. R., Jr.; Newman, R. A.; Hacker, M. P. *J. Org. Chem.* **1982**, *47*, 1807.
(3) Rinehart, K. L., Jr.; Gloer, J. B.; Cook, J. C., Jr.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857.
(4) Pettit, G. R.; Kamano, Y.; Brown, P.; Gust, D.; Inoue, M.; Herald, C. L. *J. Am. Chem. Soc.* **1982**, *104*, 905.

0022-3263/83/1948-4445$01.50/0

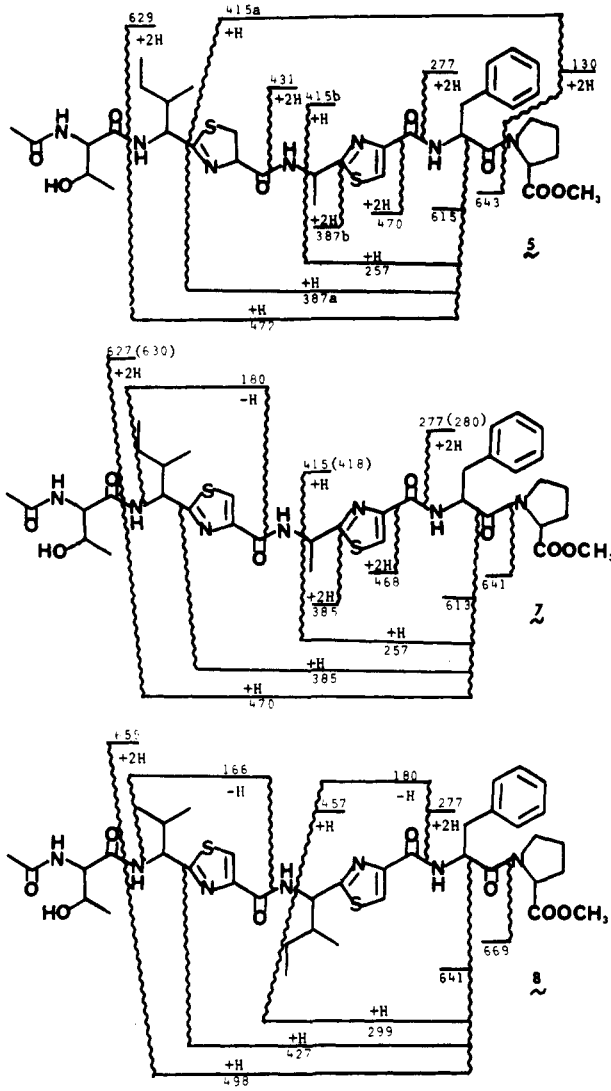
Table I. 500-MHz ^{1}H NMR Assignments for Peptides 1, 2, and 3

H at C number	1	2	3
1			
2	4.30 (d, J = 4 Hz)	4.27 (d, J = 5 Hz)	4.23 (d, J = 5 Hz)
3	4.87 (dq, J = 4, 6 Hz)	4.81 (dq, J = 5, 6 Hz)	4.77 (dq, J = 5, 6 Hz)
5	1.46 (d, J = 6 Hz)	1.51 (d, J = 6 Hz)	1.46 (d, J = 6 Hz)
6	4.58 (t, J = 8 Hz)	4.62 (t, J = 7 Hz)	4.59 (t, J = 8 Hz)
7	3.27 (m)	2.01 (m)	2.16 (m)
	2.78 (m)	2.34 (m)	1.81 (m)
8	1.87 (m, 2 H)	2.01 (m)	1.82 (m)
		2.14 (m)	1.75 (m)
9	1.64 (m, 2 H)	3.80 (m)	3.38 (m)
		3.42 (m)	2.35 (m)
10			
11	4.86 (m)	5.14 (m)	4.95 (ddd, J = 9, 8, 5 Hz)
12	3.28 (dd, J = 13, 5 Hz)	3.17 (dd, J = 14, 6 Hz)	3.21 (dd, J = 13, 5 Hz)
	2.91 (dd, J = 13, 10 Hz)	3.02 (dd, J = 14, 6 Hz)	2.95 (dd, J = 13, 9 Hz)
13			
14	7.31 (m, 5 H)	7.18 (m, 5 H)	7.30 (m, 5 H)
15			
16			
17			
18			
19	8.05 (s)	7.99 (s)	8.07 (s)
20			
21	5.35 (dd, J = 9, 6 Hz)	5.33 (dq, J = 7, 8 Hz)	5.37 (dq, J = 7, 7 Hz)
22	2.22 (m)	1.56 (d, J = 7 Hz)	1.56 (d, J = 7 Hz)
23	1.65 (m, 2 H)		
24	0.92 (t, J = 7 Hz)	5.21 (ddd, J = 11, 7, 1 Hz)	5.21 (dd, J = 11, 7 Hz)
25	1.04 (d, J = 7 Hz)	3.48 (dd, J = 11, 11 Hz)	3.73 (dd, J = 11, 11 Hz)
		3.42 (dd, J = 11, 7 Hz)	3.64 (dd, J = 11, 7 Hz)
26			
27		5.14 (m)	4.81 (m)
28	8.03 (s)	2.45 (m)	2.35 (m)
29		1.43 (m)	1.60 (m)
		1.32 (m)	1.32 (m)
30	5.15 (t, J = 10 Hz)	0.93 (t, J = 8 Hz)	0.91 (t, J = 7 Hz)
31	2.78 (dqq, J = 10, 7, 7 Hz)	1.02 (d, J = 7 Hz)	0.95 (t, J = 7 Hz)
32	1.08 (d, J = 7 Hz)		
33	0.75 (d, J = 7 Hz)		
N1	7.97 (d, J = 10 Hz)	7.56 (d, J = 10 Hz)	7.70 (d, J = 10 Hz)
N2	8.73 (d, J = 6 Hz)	7.98 (d, J = 6 Hz)	8.58 (d, J = 8 Hz)
N3	8.97 (d, J = 6 Hz)	6.70 (d, J = 8 Hz)	8.24 (d, J = 7 Hz)

analogous to ulicyclamide. However, each spectrum contained resonances for a single thiazole at δ 7.97 and 8.07, respectively. Resonances for a second thiazole were replaced by an ABX system [**2**: 5.21 (ddd, 1 H, J = 11, 7, 1 Hz), 3.64 (dd, 1 H, J = 11, 11 Hz), 3.42 (dd, 1 H, J = 11, 7 Hz); **3**: 5.25 (dd, 1 H, J = 11, 7 Hz), 3.73 (dd, 1 H, J = 11, 7 Hz), 3.64 (dd, 1 H, J = 11, 11 Hz)] characteristic of a Δ^2-thiazoline.[5] GC–MS analysis of total hydrolysis products from **2** and **3** as methyl ester trifluoroacetyl (ME–TFA) derivatives confirmed NMR assignments, yielding approximately equimolar amounts of threonine, proline, isoleucine, phenylalanine, alanine thiazole, and $^1/_2$ equiv of cystine (due to oxidation of cysteine during workup). The amino acids were identified by comparison with standards or in the case of the thiazole by mass spectral fragmentation [alanine thiazole, m/z 282 ($M^{+\cdot}$), 140 $(CH_3CHNHCOCF_3)^+$]. The stereochemistry of the α-amino acid components was assigned by GC retention time on a chiral SP-300 column (Supelco).[6] Threonine, proline, and phenylalanine were assigned L configurations and isoleucine a D configuration in both peptides **2** and **3**. Cystine, after conversion to alanine with Raney Ni, was assigned an L absolute configuration by the same method. We reported earlier that thiazole amino acids present in the lissoclinum peptides racemize during acid hydrolysis, but that the stereochemistry of the side chain can be preserved by treatment of the peptide with singlet oxygen prior to hydrolysis. Singlet oxygen adds in a [4 + 2] manner to the thiazole ring. The resulting thioozonide decomposes on acid hydrolysis to free the side chain as an α-amino acid.[7] Applying this methodology, the thiazole in **2** was degraded to D-alanine whereas the thiazole in **3** yielded L-alanine. All of these data were consistent with peptide structures **2** and **3** that were epimeric at the thiazole side chain.

Selective hydrolysis of **2** and **3** with 5% H_2SO_4/MeOH (followed by workup) gave the linear peptide **5** (racemic at the thiazole position), further confirming that the two peptides had identical amino acid sequences. The fast atom bombardment (FAB) mass spectra of **5** (positive and negative ion modes) were consistent with the amino acid sequence shown. The positive ion FAB spectrum (Figure 1) showed $(M + H)^+$ and abundant ions corresponding to sequential losses of (carbomethoxy)proline – CO and *N*-acetylthreonine at m/z 615 and 472, respectively, placing these amino acids at the C and N termini. The central tripeptide fragment (m/z 472) underwent further fragmentation from the N terminal side, losing in succession, an isoleucine side chain (m/z 387) and the thiazoline ring (m/z 257), placing isoleucine at C-2 of the thiazoline moiety. The negative ion FAB spectrum of **5** proved equally informative, showing $(M - H)^-$ and both an N-

(5) Bycroft, B. W.; Pinchin, R. *J. Chem. Soc., Chem. Commun.* **1975**, 121.
(6) Charles, R.; Bertler, B.; Gil-Av, E. *J. Chromatogr.* **1975**, *112*, 121.
(7) Biskupiak, J. E.; Ireland, C. M. *J. Org. Chem.* **1983**, *48*, 2302.

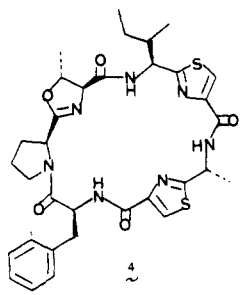


5:	MH^+	772.3202	$C_{36}H_{50}N_7O_8S_2$	Δ 4.0 mmu
		643.2384	$C_{30}H_{39}N_6O_6S_2$	1.1
		615.2439	$C_{29}H_{39}N_6O_5S_2$	1.6
		472.1843	$C_{23}H_{30}N_5O_2S_2$	0.2
		470.1848	$C_{20}H_{32}N_5O_4S_2$	-4.7
		415.0925(a)	$C_{19}H_{19}N_4O_3S_2$	2.6
		415.1584(b)	$C_{24}H_{25}N_3O_4S$	1.8
		387.0951(a)	$C_{18}H_{19}N_4O_2S_2$	2.7
		387.2011(b)	$C_{17}H_{29}N_4O_4S$	5.5
		257.0753	$C_{14}H_{13}N_2OS$	0.5
7:		470.1673	$C_{23}H_{28}N_5O_2S_2$	Δ 1.1 mmu
		257.0749	$C_{14}H_{13}N_2OS$	0.0
		180.0489	$C_9H_{10}NOS$	0.6
CD_3 ester (#):		280.1741	$C_{15}H_{18}N_2O_3D_3$	0.1
8:		498.1994	$C_{25}H_{32}N_5O_5S_2$	Δ-0.3 mmu
		299.1170	$C_{18}H_{19}N_2OS$	-1.1

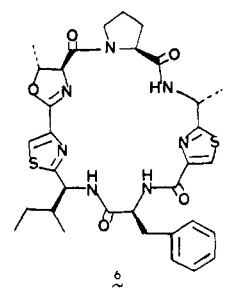
Figure 1. Positive ion FAB mass spectral fragmentation patterns for linear peptides **5**, **7**, **7** CD_3 ester, and **8**.

terminal and a C-terminal series of ions arising by cleavage at every peptide linkage with sequential loss of each amino acid (Figure 2).

The similarities in the structural and spectral properties of **2**, **3**, and ulicyclamide prompted us to reexamine our earlier structure assignment for ulicyclamide. Ulicyclamide was originally assigned structure **6**, where the isoleucine



4



6

thiazole was fused at C-2 of the oxazoline, on the basis of the multiplicity (d, J = 4 Hz) of the proton at C-4 of the oxazoline, coupled only to the adjacent proton at C-5. Oxazolines are reported to exhibit homoallylic (five bond) coupling between the C-4 proton and protons on a carbon attached at C-2.[8] This coupling was observed in ulithiacyclamide where a cystine unit is fused to C-2 of the oxazoline.[1] Since long range coupling was not observed in the ^1H NMR spectrum of ulicyclamide it was assumed that a thiazole was fused at C-2. With that limitation, structure **6** seemed most compatible with EI mass spectral data. However, reexamination of EI data for the ulicyclamide selective hydrolysis product and more importantly the availability of FAB fragmentation data has led us to reassign structure **7** to the hydrolysis product and consequently structure **4** to ulicyclamide. The amino acid sequence of **4** is identical to peptides **2** and **3** with oxidation of the thiazoline to a thiazole. Positive and negative ion FAB spectra for **7** were virtually superimposable with data from **5** with a two mass unit shift for fragments containing the isoleucine thiazole. These assignments were confirmed by examination of the FAB spectrum of the CD_3 ester derivative of **7**. The ions at m/z 627, 415, 277, and 130 in the spectrum of **7** showed a three mass unit shift in the spectrum of the CD_3 ester, confirming that this sequence was generated by fragmentation from the N terminal. The ion at m/z 280 peak matched for $C_{15}H_{18}H_2O_3D_3$ confirming the Phe-Pro-OCH_3 C-terminal (even at high resolution [1:12 500] the m/z 277 ion in the spectrum of **7** was not resolved from the isobaric glycerol peak although its presence could be demonstrated by the shift in the peak

(8) Weinberger, M. A.; Greenhalgh, R. *Can. J. Chem.* **1963**, *41*, 1038.

(9) Martin, S. A.; Costello, C. E.; Biemann, K., *Anal. Chem.* **1982**, *54*, 2362.

Table II. ^{13}C NMR Assignments for Peptides 2 and 3[a-c]

C number	2	3
1	182.56[a]	174.60[a]
2	75.62	74.91
3	80.81	81.66
4	169.13	169.04
5	21.66	21.63
6	56.65	56.30
7	28.70[b]	28.52[b]
8	26.56[b]	25.03[b]
9	47.34	46.83
10	171.84[a]	170.72[a]
11	52.03[c]	52.83[c]
12	37.77	40.27
13	135.58	135.51
14	128.20 (2C)	128.23 (2C)
15	129.91 (2C)	129.60 (2C)
16	126.71	126.94
17	171.81[a]	170.62[a]
18	148.32	148.03
19	123.68	123.32
20	159.60	159.14
21	47.02	47.29
22	21.99	24.44
23	171.72[a]	170.58[a]
24	78.71	78.75
25	36.25	35.21
26	169.14[a]	169.92[a]
27	54.16[c]	53.16[c]
28	38.46	38.85
29	25.46	24.93
30	11.70	10.18
31	14.04	14.77

[a-c] Superscript letters indicate interchangeable signals.

center position upon variation of the glycerol/sample ratio). Each of the α-amino acid components of ulicyclamide 4 was assigned the L absolute configuration by GC analysis of ME–TFA derivatives as described earlier. The alanine and isoleucine thiazoles were assigned D and L absolute configuration by their conversion to D-alanine and L-isoleucine, respectively.

The third new peptide 1 had the molecular formula $C_{35}H_{43}N_7O_5S_2$ (mass measurement 705.2835; calcd 705.2771). Detailed proton decoupling studies at 500 MHz, as well as GC and GC–MS analysis of 6 N HCl hydrolysis products, as ME–TFA derivatives, showed the presence of L-threonine, L-proline, L-phenylalanine, L-valine thiazole, and D-isoleucine thiazole in equimolar amounts. These amino acid components with threonine in the form of an oxazoline account for all of the atoms in the formula. Selective hydrolysis of the oxazoline ring by using the conditions described earlier gave linear peptide 8. The positive ion FAB mass spectrum of 8 (Figure 1) exhibited ions at m/z 641 and 498 placing (carbomethoxy)proline and *N*-acetyl threonine at the termini. The central piece (m/z 498) then underwent further fragmentation (m/z 427 and 299) analogous to the peptides 5 and 7, positioning valine thiazole at the N-terminal side of the central piece. The negative ion FAB spectrum (Figure 2) had abundant fragment ions which affirmed the sequence.

It should be pointed out that a number of the nominal m/z values assigned in the positive ion FAB spectra of 5, 7, and 8 (Figure 1) could have been assigned elsewhere in the structures presented and/or would have been compatible with alternative sequences. The assignments shown in Figure 1 are all based on exact mass measurements, and the elemental compositions are unique to the designated sites. The negative ion FAB spectral assignments (Figure 2) were made on the basis of low resolution measurements, since even the low resolution negative ion FAB spectra

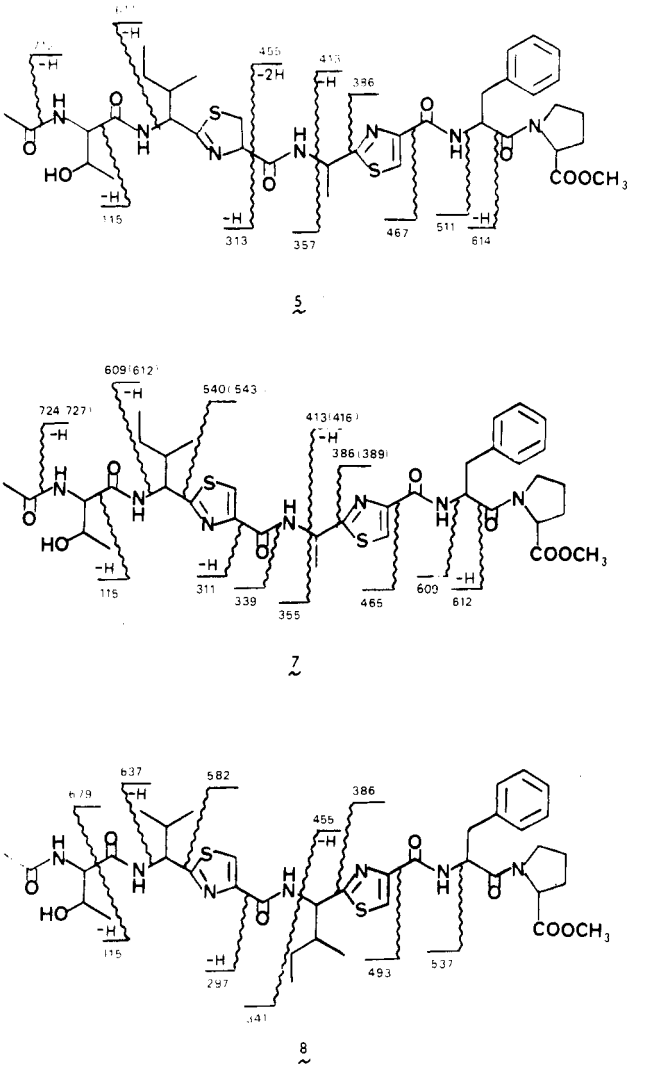


Figure 2. Negative ion FAB mass spectral fragmentation patterns for linear peptides 5, 7, 7 CD ester, and 8.

would not accommodate alternate sequences. Additionally, the hazard of assigning compositions to ions observed in the FAB spectrum of a compound on the basis of appearance of a peak at the same nominal mass in its EI spectrum is illustrated by the measurement of two ions of almost equal abundance at m/z 415 in the (+) FAB spectrum of 5 (Figure 1). In the EI spectrum of 5, however, the peak at m/z 415 was a singlet having the composition $C_{24}H_{25}N_3O_4S$. Furthermore, in the (+) FAB spectra of 7, its CD_3 analogue and 8, the ion at m/z 180, were shown to have the composition $C_9H_{10}NOS$. The ion at m/z 166 in the spectrum of 8 was the lower homologue, C_8H_9NOS. In the EI spectrum of 7, reported[1] and recently remeasured, the peak at m/z 180 was a singlet whose composition was $C_8H_8N_2OS$.

Peptides 1, 2, and 3 displayed borderline cytotoxicity in L1210 tissue culture assay, exhibiting IC_{50} values greater than 10 μg/mL.[10]

Experimental Section

Infrared spectra were recorded on a Beckman 620 MX spectrophotometer. Electron ionization mass spectra were recorded

(10) Subsequent to submission of this manuscript an article appeared describing the isolation of ulithiacyclamide and a new peptide from an unidentified didemnids species from Australia. Hamamoto, Y.; Endo, M.; Nakagawa, M.; Nakanishi, T.; Mizukawa, K. *J. Chem. Soc., Chem. Commun.* **1983**, 323.

either on an AEI MS-902 (University of Connecticut) or on photographic plates by using a CEC 110B spectrometer (Massachusetts Institute of Technology). FAB mass spectra were recorded with a MAT 731 instrument fitted with an Ion Tech neutral atom gun[9] (MIT). Samples were dissolved in (1:1) dimethyl sulfoxide/glycerol for FAB analysis. ^1H and ^{13}C NMR spectra were recorded on a Bruker WM-500 spectrometer at Yale University. Chemical shifts are reported relative to Me_4Si (δ 0). Low resolution GC–EI mass spectra were recorded on an HP-5985 spectrometer. Gas chromatograms were recorded on a Varian Model 3700 gas chromatograph. HPLC separations were performed on a Waters Model 201 system with Model 441 refractive index detector.

Collection of *Lissoclinum patella*. Colonies of *L. patella* were collected in June 1981 by snorkeling (–2 m) in Iwayama Bay (near the Continental Hotel dock), Korror Island, Western Caroline Islands. The frozen animals were lyophilized and ground to a powder (413 g) in a Wiley mill. The powdered tissue was extracted exhaustively in a Soxhlet apparatus with petroleum ether, CCl_4, and $CHCl_3$. Rotoevaporation of the solvent gave 570 mg, 460 mg, and 500 mg of oil, respectively. The combined petroleum ether, CCl_4, and $CHCl_3$ extracts (1.530 g) were chromatographed on silica gel 60 (Merck, 60 × 2.5 cm). Three peptide fractions eluted with EtOAc. A fourth band eluted in EtOAc/MeOH (95:5). Each of the four bands was subjected to HPLC (RP-18; MeOH/H_2O, 80:20) and resolved into two peptides. The first band (150 mg) gave ulithiacyclamide (95 mg) and patellamide A (40 mg). The second band (70 mg) gave patellamide B (55 mg) and 1 (14 mg). Band three (230 mg) gave ulicyclamide (4) (190 mg) and patellamide C (20 mg). The fourth and most polar band (624 mg) gave 2 (400 mg) and 3 (200 mg).

1: IR (CH_2Cl_2) 3390, 3320, 2940, 2820, 1672, 1640, 1542, 1500 cm^{-1}; high-resolution EI mass measurement, obsd 705.2835; calcd 705.2771 for $C_{35}H_{43}N_7O_5S_2$.

2: IR (CH_2Cl_2) 3380, 3315, 2985, 2935, 2860, 1674, 1638, 1530, 1440 cm^{-1}; high-resolution EI mass measurement, obsd 679.2575; calcd 679.2610 for $C_{33}H_{41}N_7O_5S_2$.

3: IR (CH_2Cl_2) 3380, 3320, 3020, 2980, 2940, 1665, 1635, 1510, 1410 cm^{-1}; high-resolution EI mass measurement, obsd 679.2588; calcd 679.2610 for $C_{33}H_{41}N_7O_5S_2$.

Total Acid Hydrolysis of *Lissoclinum* Peptides. The peptides (5 mg) and 6 N HCl (5 mL) were heated at 100 °C for 18 h in a Pyrex threaded bomb sealed with a Teflon screw cap. The cooled reaction mixture was transferred to a 25-mL round-bottom flask and rotoevaporated to dryness. The hydrolysate was dissolved in MeOH (25 mL). Anhydrous HCl (gas) was bubbled through the solution for 1 min. Afterwards the solution was refluxed for 1 h. Upon cooling, the solvent was evaporated, and the residue suspended in CH_2Cl_2 (5 mL) with trifluoroacetic anhydride (5 mL) in a Pyrex threaded bomb sealed with a Teflon screw cap. The mixture was heated at 150 °C for 30 min. The cooled reaction vessel was placed in ice and the solvent evaporated in a stream of nitrogen gas. The residues were resuspended in CH_2Cl_2 and used for GC and GC–MS analysis.

1, valine thiazole: m/z 310 ($M^{+\cdot}$), 267 ($M - C_3H_7$)$^+$, 235, 208, 171, 166, 157, 139, 69.

1, isoleucine thiazole: m/z 324 ($M^{+\cdot}$), 267, ($M - C_4H_9$)$^+$; 236, 235, 208, 171, 139, 39.

Selective Hydrolysis of the Oxazoline Ring. Conversion of 2 and 3 to 5, 4 to 7, and 1 to 8. The peptide (10 mg) was refluxed in 5% H_2SO_4/MeOH (20 mL) for 1 h. The reaction was basified to pH 12 with 10% aqueous NaOH and immediately extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to give an oil. The oil was *N*-acetylated by stirring in pyridine (100 μL) and acetic anhydride (500 μL) at room temperature for 24 h. Excess reactants were removed under vacuum. The residue was saponified by stirring with 1% KOH/MeOH at room temperature for 1 h. After acidification to pH 1 with 10% aqueous HCl the product was extracted with CH_2Cl_2 (3 × 20 mL). The CH_2Cl_2 layers were dried over Na_2SO_4 and evaporated, and the residue was methylated with CH_2N_2 or by refluxing for 1 h in CD_3OD/HCl to obtain the CD_3 ester. The crude product was purified by HPLC (silica gel, EtOAc/MeOH, 95:5).

General Procedure for Reaction of Peptides with $^1O_2$. (a) Triphenylphosphite (2.0 g, 6.4 mmol) was dissolved in CH_2Cl_2 (100 mL) in a round-bottom flask and cooled to –70 °C in a dry ice–acetone bath. A stream of ozone was bubbled through the solution until a deep blue ozone color persisted. A stream of N_2 was bubbled through the solution, still at –70 °C, to remove excess ozone. Immediately afterward, the round-bottom flask was fitted with a connecting hose adapter outleted to a second round-bottom flask through Tygon tubing and a pipet. The second flask contained the peptide (5–10 mg) dissolved in CH_2Cl_2 (30 mL) at room temperature. The flask containing triphenylphosphite ozonide was removed from the –70 °C bath and allowed to warm slowly to room temperature. Vigorous bubbling was observed almost immediately in both flasks. After gas evolution ceased the progress of the reaction was monitored by TLC. The process was repeated if starting material remained.

(b) After the reaction was completed the CH_2Cl_2 was removed under a stream of nitrogen and the crude product treated directly with 6 N HCl (6 mL) for 20 h at 100 °C in a Pyrex threaded bomb sealed with a Teflon screw cap. Afterwards the HCl was removed in vacuo to give a mixture of amino acid hydrochlorides.

(c) The mixture of amino acid hydrochlorides was dissolved in MeOH (50 mL) in a round-bottom flask and anhydrous HCl bubbled through the solution for 1 min. The flask was fitted with a reflux condenser and refluxed for 1 h. Upon cooling the solvent was removed in vacuo, and the residue suspended in CH_2Cl_2 (5 mL) and trifluoroacetic anhydride (1 mL) in a pyrex threaded bomb and heated to 150 °C for 10 min. The cooled reaction mixture was placed in an ice bath and carefully evaporated under a stream of nitrogen. The residue was taken up in CH_2Cl_2 (1 mL) and injected on the GC.

Determination of Chirality of Thiazole Amino Acids. The chirality of all thiazole amino acids was determined by GC retention times of the α-amino acid degradation products from $^1O_2$ reaction on a chiral GC column (SP-300, 12 ft × $^1/_8$ in.; program: 110–140 °C at 2 °C/min, 30 min delay at 110 °C). The general protocol called for the comparison of GC traces for the $^1O_2$ reaction product and the acid hydrolysis of the corresponding peptide without singlet oxygen treatment to define which peaks correspond to amino acids generated by thiazole degradation. The chirality of the newly formed amino acids was determined by comparison or coinjection with D,L amino acid standards.

Acknowledgment. This research was supported by a grant from the National Institutes of Health (CA 29821) to CMI. NMR spectra were run at the Northeast Regional NSF-NMR Facility, Yale University, partially supported by the NSF (Grant CHE 79-16210). The MIT mass spectrometry facility is supported by a grant from the NIH Division of Research Resources (RR00317 to K. Biemann). L 1210 assays were run by Drs. Robert A. Newman and Miles P. Hacker.

Registry No. 1, 87411-84-5; 2, 87393-58-6; 3, 87393-59-7; 4, 87393-60-0; 5, 87393-61-1; 7, 87393-62-2; 7 (CD_3 ester), 87393-63-3; 8, 87393-64-4.