flash chromatography E. Merck silica gel 60 (230–400 mesh) was used.

Arylation. The general procedure is exemplified with arylation of 4,6-di-*O*-acetyl-1,5-anhydro-2-deoxy-D-*erythro*-hex-1-en-3-ulose (1). In a round-bottomed flask 684 mg (3 mmol) of enone 1 was dissolved in a solution of 20 mL of acetic acid and 36 mL of benzene, and 672 mg (3 mmol) of palladium acetate was added. The mixture was kept at 110 °C in a thermoregulated oil bath, and the reaction was followed by TLC.

After completion the solution was filtered on paper, in order to recover the palladium which can be reoxidized and reused, washed with water, and extracted with ether. The organic phase was then neutralized with a 5% solution of sodium bicarbonate.

The solution was cooled to 0 °C, and 100 mg of sodium borohydride was added to reduce the remaining traces of palladium salts (brown). The solution turned colorless, and a fine precipitate of Pd(0) was filtered off.

The organic phase was washed again, dried over anhydrous $MgSO_4$ and filtered. A crude mixture of arylated products was obtained by evaporating the solvent and purified by flash chromatography (ether–pentane).

The following new compounds were isolated and characterized as described below.

(4,6-Di-*O*-acetyl-2-deoxy-D-*erythro*-hex-1-enopyranos-3-ulos-1-yl)benzene (1a) could not be separated from **1b**, which was isolated only after hydrogenation of **1a** into **1e**.

(4,6-Di-*O*-acetyl-2-deoxy-α-D-*erythro*-hexopyranos-3-ulos-1-yl)benzene (1b): 191 mg (30% yield); oil; R_f 0.30; $[\alpha]^{20}_D$ 111.2° (*c* 1; EtOH); IR (cm^{-1}) 3040–3020, 1760 (C=O), 1740 (ester), 1250 (CO), 1100–1050, 740–710 (Ar). Anal. Calcd for $C_{16}H_{18}O_6$: C, 62.74; H, 5.88. Found: C, 62.51; H, 6.08.

Arylation of **2** afforded **(4,6-di-*O*-acetyl-2-deoxy-D-*threo*-hex-1-enopyranos-3-ulos-1-yl)benzene (2a)** [351 mg (38.5% yield); oil; R_f 0.49; $[\alpha]^{20}_D$ 46.3° (*c* 1.1; $CHCl_3$); IR (cm^{-1}) 3100–2900, 1740 (ester), 1670 (enone), 1600 (C=C), 1240 (CO), 1100–1050, 780–700 (Ar). Anal. Calcd for $C_{16}H_{18}O_6$: C, 62.74; H, 5.88. Found: C, 62.48; H, 5.78] and **(4,6-di-*O*-acetyl-2-deoxy-α-D-*threo*-hexopyranos-3-ulos-1-yl)benzene (2b)** [288 mg (31.5% yield); oil; R_f 0.51; $[\alpha]^{20}_D$ −53° (*c* 1.1; $CHCl_3$); IR (cm^{-1}) 3100–2900, 1760 (C=O), 1740 (ester), 1230 (CO), 1100–1050, 770–700 (Ar). Anal. Calcd for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30. Found: C, 63.08; H, 5.40].

Arylation of **3** afforded **(4-*O*-acetyl-2,6-dideoxy-L-*erythro*-hex-1-enopyranos-3-ulos-1-yl)benzene (3a)** [463 mg (63% yield); mp 84–86 °C; R_f 0.38; $[\alpha]^{20}_D$ −321° (*c* 0.7; $CHCl_3$); IR (cm^{-1}) 3100–2900, 1720 (C=O), 1700 (enone), 1600 (C=C), 1240 (CO), 1140–1050, 790–700 (Ar). Anal. Calcd for $C_{14}H_{14}O_4$: C, 68.29; H, 5.69. Found: C, 68.07; H, 5.68] and **(4-*O*-acetyl-2,6-dideoxy-α-L-*erythro*-hexopyranos-3-ulos-1-yl)benzene (3b)** [198 mg (27% yield); oil; R_f 0.4; $[\alpha]^{20}_D$ −110° (*c* 1; $CHCl_3$); IR (cm^{-1}) 3100–2900, 1750 (C=O), 1720 (ester), 1230 (CO), 1150–1050, 800–700 (Ar). Anal. Calcd for $C_{14}H_{16}O_4$: C, 67.74; H, 6.45. Found: C, 67.59; H, 6.49].

Reduction of Arylated Enones. Three mmol of enone **1b**, **2b**, or **3b** was dissolved in 90 mL of ethyl acetate, and 200 mg of 10% palladium on charcoal was added. The mixture was vigorously stirred in a hydrogenating apparatus, and the course of the reaction was followed by TLC and GLC. After completion the catalyst was filtered off and the solvent was evaporated.

Direct acetylation of the reduction product was carried out using 2 mL (20 mmol) of acetic anhydride in 7 mL (86 mmol) of pyridine. After 24 h at room temperature, the solution was evaporated and treated with toluene in order to remove traces of pyridine or Ac_2O. The residue was dissolved in 30 mL of CH_2Cl_2, washed with water, and neutralized with a 5% solution of $NaHCO_3$. The organic phase was washed again and dried over $MgSO_4$. Filtration and evaporation of the solvent yielded the corresponding arylated triesters **1e** and **2e** and diester **3e**.

(3,4,6-Tri-*O*-acetyl-2-deoxy-β-D-*arabino*-hexopyranosyl)benzene (1e). A total of 280 mg (80% yield) was obtained from **1a**: oil; R_f 0.28; $[\alpha]^{20}_D$ 3.07° (*c* 1.5; EtOH); IR (cm^{-1}) 3040–3000, 1740 (ester), 1250 (CO), 1100–1050, 740–710 (Ar). Anal. Calcd for $C_{18}H_{22}O_7$: C, 61.71; H, 6.28. Found: C, 61.54; H, 6.08.

(3,4,6-Tri-*O*-acetyl-2-deoxy-β-D-*lyxo*-hexopyranosyl)benzene (2e). A total of 332 mg (95% yield) was obtained from **2a**: oil; R_f 0.8; $[\alpha]^{20}_D$ 16.7° (*c* 1; $CHCl_3$); IR (cm^{-1}) 3100–2900, 1740 (ester), 1230 (CO), 1100–1050, 770–700 (Ar). Anal. Calcd for $C_{18}H_{22}O_7$: C, 61.71; H, 6.28. Found: C, 61.85; H, 6.17.

(3,4-Di-*O*-acetyl-2-deoxy-β-L-*ribo*-hexopyranosyl)benzene (3e). A total of 277 mg (95% yield) was obtained from **3a**: oil; R_f 0.43; $[\alpha]^{20}_D$ −28° (*c* 1; $CHCl_3$); IR (cm^{-1}) 3100–2900, 1740 (ester), 1240 (CO), 1100–1050, 780–700 (Ar). Anal. Calcd for $C_{16}H_{20}O_4$: C, 65.75; H, 6.85. Found: C, 65.95; H, 6.98.

Tawicyclamides A and B, New Cyclic Peptides from the Ascidian *Lissoclinum patella*: Studies on the Solution- and Solid-State Conformations

Leonard A. McDonald, Mark P. Foster, Dennis R. Phillips, and Chris M. Ireland*[,1]

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

Angela Y. Lee and Jon Clardy*

Department of Chemistry–Baker Laboratory, Cornell University, Ithaca, New York 14853-1301

Received February 4, 1992

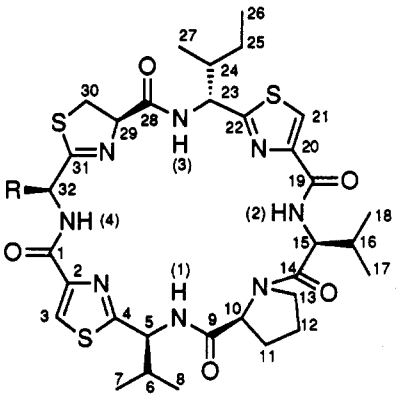
Two new cytotoxic cyclic peptides, tawicyclamides A and B (1–2), were isolated from the ascidian *Lissoclinum patella* collected in the Philippine Islands and their structures determined by NMR spectroscopy, oxidation studies, and tandem mass spectrometry. Absolute configurations were determined by HPLC analysis of derivatized constituent amino acids obtained from acid hydrolysis. X-ray crystallography confirmed the structure of tawicyclamide B and showed that the compound assumes an unusual conformation facilitated by a *cis*-valine–proline amide bond and stabilized by an intramolecular hydrogen bond. Tawicyclamides A and B represent a new family of cyclic octapeptides, possessing thiazole and thiazoline amino acids but lacking the oxazoline ring characteristic of previously reported cyclic peptides from *L. patella*. Isomerization of the valine–proline amide bond from cis to trans is among the conformational changes occurring upon oxidation of the thiazoline ring to a thiazole. A variety of NMR data supports these changes. Molecular modeling studies allowed us to establish the solution conformations of these compounds and to evaluate these conformational interpretations. Tawicyclamides A and B were weakly but equally cytotoxic against human colon tumor cells in vitro.

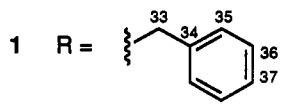
Ascidians have proven to be a rich source of bioactive amino acid-derived secondary metabolites.[2] The prolific Didemnidae family has produced several classes of peptide metabolites such as the didemnins[3] and the lissoclinum

0022-3263/92/1957-4616$03.00/0

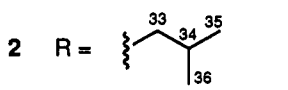
peptides.[4] Didemnin B, isolated from *Trididemnum solidum*, exhibited strong in vitro and in vivo antileukemic activities and was the first marine natural product evaluated in clinical trials as an anticancer agent.[3,5] The peptides from *Lissoclinum patella*, characterized by the presence of thiazole and oxazoline amino acids, fall into two general groups—the heptapeptide lissoclinamides and the octapeptide patellamides/ulithiacyclamides.[4] These peptides were shown to exhibit in vitro cytotoxicity, with the presence of the oxazoline ring proving important to their potency.[6]

Continuing investigations of the chemistry of *L. patella* have led to the isolation of tawicyclamides A and B (1–2), two new cyclic peptides from a Philippine collection of the ascidian. We report here the structure determination of these peptides by a combination of NMR spectroscopy, oxidation studies, and tandem mass spectrometry (MS/MS). X-ray crystallography confirmed the structure of



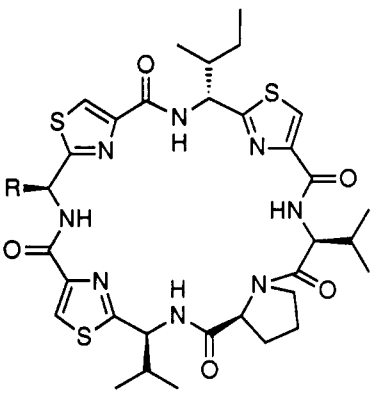


1 R =

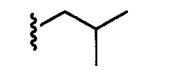


2 R =

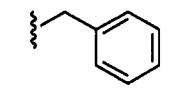
tawicyclamide B (2), thereby firmly establishing this new family of cyclic peptides. This family lacks the characteristic oxazoline ring, but possesses a thiazoline ring and a *cis*-valine–proline amide bond that facilitates an unusual three-dimensional conformation. A conformational reorganization occurring upon oxidation of the thiazoline ring to a thiazole prompted an investigation of the conformations of tawicyclamide B (2) and its oxidized analog, dehydrotawicyclamide B (3), by molecular modeling. These studies established the solution conformations of both tawicyclamide B and its dehydro analog 3, and by analogy also established the conformations of tawicyclamide A (1) and dehydrotawicyclamide A (4). Tawicyclamide



3 R =



4 R =



B assumes a conformation in which the valine–proline peptide bond is cis while dehydrotawicyclamide B assumes an all-trans amide bond conformation. This is reminiscent of patellin 2, a thiazoline containing cyclic peptide from *L. patella* that undergoes cis–trans isomerization of a valine–proline amide bond leading to two solution conformations.[7]

The variability of *L. patella* peptide metabolites with location has been noted previously,[4] and is again demonstrated by our findings. This is of interest since algal symbionts associated with *L. patella* have been implicated in the transfer of amino acids to their ascidian host.[8] It is tempting to speculate that the lissoclinum peptides may be produced by algal symbionts. To this effect, westiellamide, a compound recently isolated from the terrestrial blue-green alga *Westiellopsis prolifica*, is apparently identical to trisoxazoline, a bistratamide-type cyclic peptide previously reported from the ascidian *Lissoclinum bistratum*.[9]

Results and Discussion

The methanol extract of *L. patella* was concentrated and partitioned between a series of solvents of increasing polarity. Repeated silica gel flash chromatography of the chloroform-soluble fraction, followed by reversed-phase HPLC, yielded the new peptides, tawicyclamides A (1) and B (2), and the known peptides, patellamides A (5) and B (6) and ulithiacyclamide (7).[4a]

HR FAB mass spectral analysis showed a protonated molecular ion at m/z 807.3135 for tawicyclamide A (1). In agreement with the molecular formula $C_{39}H_{51}N_8O_5S_3$ (Δ 1.0 mmu), the ^{13}C NMR spectrum of 1 contained 37 res-

(1) NIH Career Development Awardee, 1987–1992.

(2) (a) Ireland, C. M.; Molinski, T. F.; Roll, D. M.; Zabriskie, T. M.; McKee, T. C.; Swersey, J. C.; Foster, M. P. In *Bioorganic Marine Chemistry*; Scheuer, P. J., Ed.; Springer-Verlag: Berlin, 1989; Vol. 3, pp 1–46. (b) Ireland, C. M.; Roll, D. M.; Molinski, T. F.; McKee, T. C.; Zabriskie, T. M.; Swersey, J. C. In *Biomedical Importance of Marine Organisms*; Fautin, D. G., Ed.; California Academy of Sciences: San Francisco, 1988; No. 13, pp 41–57.

(3) Rinehart, K. L.; Gloer, J. B.; Cook, J. C.; Mizsac, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857.

(4) (a) Sesin, D. F.; Gaskell, S. J.; Ireland, C. M. *Bull. Soc. Chim. Belg.* **1986**, *95*, 853. (b) Hawkins, C. J.; Lavin, M. F.; Marshall, K. A.; van den Brenk, A. L.; Watters, D. J. *J. Med. Chem.* **1990**, *33*, 1634. (c) Schmitz, F. J.; Ksebati, M. B.; Chang, J. S.; Wang, J. L.; Hossain, M. B.; van der Helm, D.; Engel, M. H.; Sarban, A.; Silfer, J. A. *J. Org. Chem.* **1989**, *54*, 3463. (d) Williams, D. E.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **1989**, *52*, 732. (e) Ireland, C. M.; Scheuer, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 5688. (f) Wasylyk, J. M.; Biskupiak, J. E.; Costello, C. E.; Ireland, C. M. *J. Org. Chem.* **1983**, *48*, 4445. (g) Hamamoto, Y.; Endo, M.; Nakagawa, M.; Nakanishi, T.; Mizukawa, K. *J. Chem. Soc., Chem. Commun.* **1983**, 323. (h) Ireland, C. M.; Durso, A. R.; Newman, R. A.; Hacker, M. P. *J. Org. Chem.* **1982**, *47*, 1807.

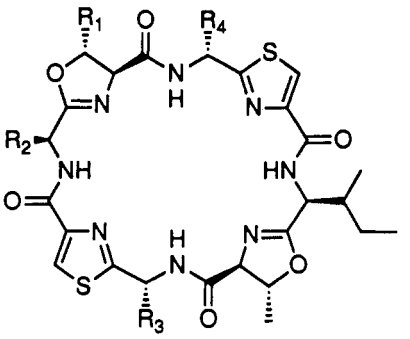
(5) Dorr, F. A.; Kuhn, J. G.; Phillips, J.; von Hoff, D. D. *Eur. J. Cancer Clin. Oncol.* **1988**, *24*, 1699.

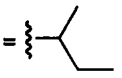
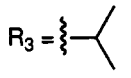
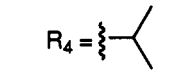
(6) Shioiri, T.; Hamada, Y.; Kato, S.; Shibata, M.; Kondo, Y.; Nakagawa, H.; Kohda, K. *Biochem. Pharmacol.* **1987**, *36*, 4181.

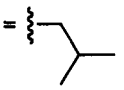
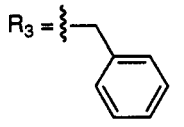
(7) Zabriskie, T. M.; Foster, M. P.; Stout, T. J.; Clardy, J.; Ireland, C. M. *J. Am. Chem. Soc.* **1990**, *112*, 8080.

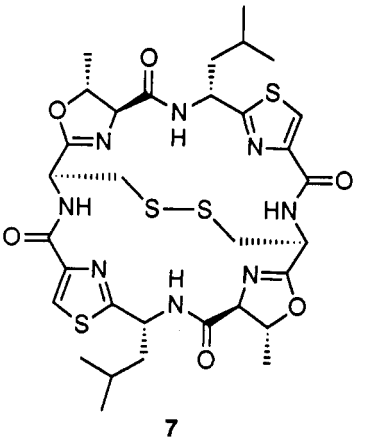
(8) (a) Pardy, R. L.; Lewin, R. A. *Bull. Marine Sci.* **1981**, *31*, 817. (b) Kremer, B. P.; Pardy, R.; Lewin, R. A. *Phycologia* **1982**, *21*, 258.

(9) Prinsep, M. R.; Moore, R. E.; Levine, I. A.; Patterson, G. M. L. *J. Nat. Prod.* **1992**, *55*, 140.



5 R_1 = H R_2 =  R_3 =  R_4 = 

6 R_1 = CH_3 R_2 =  R_3 =  R_4 = CH_3



7

onances, including signals for two degenerate phenyl carbons at 130.18 and 129.08 ppm. A DEPT[10] experiment established the multiplicities of the carbon resonances while an HMQC[11] experiment permitted assignment of the attached protons. Characteristic peptide resonances in the ^{1}H NMR spectrum of 1 included doublets at 7.41, 7.59, 8.00, and 8.34 ppm, attributable to amide NH protons, and doublets of doublets between 5.89 and 4.62 ppm corresponding to peptide α protons. Further evidence establishing 1 as a peptide was provided by strong bands in the IR spectrum at 3362 cm^{-1} (indicative of secondary amide NH stretching vibrations), amide I bands at 1666 and 1641 cm^{-1}, and amide II bands at 1536 and 1514 cm^{-1}. The absence of IR bands corresponding to a carboxylate or ammonium ion suggested that 1 was cyclic or had end terminal modifications that rendered it nonpolar. The former proved correct when the partial structures of 1, as established by NMR (Figure 1), accounted for all but one degree of unsaturation required by the molecular formula.

A singlet resonance at 7.02 ppm (H3) and a fine doublet at 7.42 ppm (H21; J = 0.8 Hz) in the proton spectrum of 1 implied the presence of two thiazole rings. A PS-DQF-COSY[12] experiment established the presence of phenylalanine, isoleucine, proline, and two valine residues. An

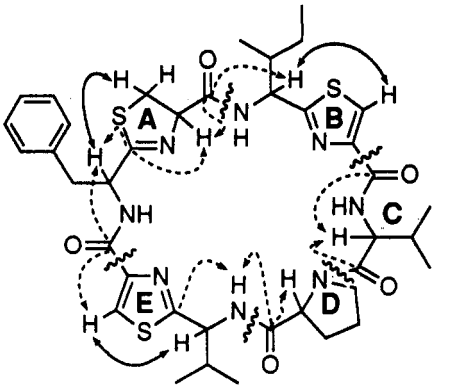


Figure 1. Partial structures for tawicyclamide A (1), established by PS DQF COSY, COLOC, and HMBC experiments. Solid arrows represent COSY correlations. Dashed arrows represent key HMBC correlations.

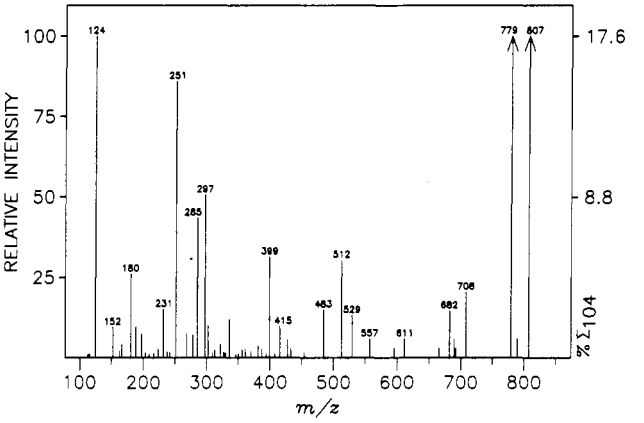
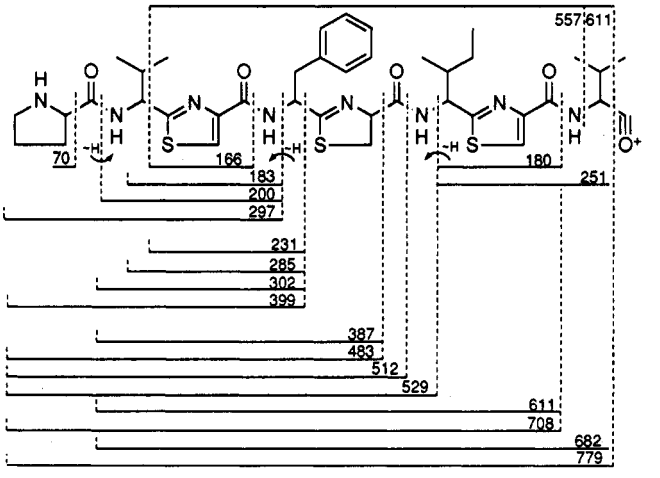


Figure 2. CID spectrum of the (M + H)$^+$ ion, m/z 807, from tawicyclamide A (1).

Scheme I



additional spin network consisting of an α proton (5.14 ppm; dd, J = 9.2, 1.3 Hz; δ ^{13}C = 77.94) coupled to geminal diastereotopic β protons (4.03 ppm, dd, J = 11.3, 1.3 Hz and 3.05 ppm, dd, J = 11.3, 9.2 Hz; δ ^{13}C = 38.47) was attributed to a thiazoline ring. This ring was confirmed by nickel peroxide oxidation of tawicyclamide A (1) to form dehydrotawicyclamide A (4). Complete NMR assignments based on COSY, HMQC, COLOC[13] and HMBC[14] data are

(10) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.
(11) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285.
(12) Rance, M.; Sørensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 458.
(13) (a) Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331. (b) Kessler, H.; Bermel, W.; Griesinger, C. *J. Am. Chem. Soc.* **1985**, *107*, 1083.
(14) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2094.

Table I. Proton NMR Assignments for 1–4 in C_6D_6

atom	δ ^{1}H (mult, J (Hz)) 1	2	3	4
3	7.02 (s)	7.12 (s)	7.49 (s)	7.43 (s)
5	5.37 (dd, 6.7, 3.3)	5.38 (dd, 6.5, 3.0)	5.15 (dd, 7.9, 6.6)	5.14 (dd, 7.9, 6.5)
6	1.89 (m)	1.94 (m)	2.16 (m)	2.19 (m)
7	0.51 (d, 6.8)	0.54 (d, 6.5)	0.71 (d, 6.8)	0.73 (d, 6.8)
8	0.91 (d, 6.8)	0.91 (d, 6.5)	0.79 (d, 6.8)	0.81 (d, 6.8)
10	4.62 (dd, 8.5, 2.1)	4.57 (dd, 8.5, 1.5)	4.44 (d, 7.9)	4.42 (d, 7.9)
11a	1.77 (m)	1.72 (m)	0.72 (m)	0.74 (m)
11b	2.10 (m)	2.06 (m)	2.51 (dd, 12.2, 6.4)	2.51 (dd, 12.3, 6.1)
12a	1.34 (m)	1.30 (m)	1.18 (m)	1.14 (m)
12b	1.60 (m)	1.58 (m)	1.53 (m)	1.57 (m)
13a	3.27 (m)	3.22 (m)	2.91 (m)	2.93 (m)
13b	3.84 (m)	3.79 (m)	2.91 (m)	2.93 (m)
15	4.97 (dd, 10.0, 8.5)	4.95 (dd, 10.0, 8.0)	5.03 (dd, 8.8, 6.2)	5.00 (dd, 9.0, 6.7)
16	2.20 (m)	2.12 (m)	2.25 (m)	2.25 (m)
17	0.83 (d, 6.8)	0.80 (d, 6.5)	1.31 (d, 6.8)	1.20 (d, 6.8)
18	0.76 (d, 6.8)	0.72 (d, 6.5)	1.22 (d, 6.8)	1.15 (d, 6.8)
21	7.42 (d, 0.8)	7.44 (s)	7.71 (s)	7.73 (s)
23	5.79 (dd, 10.2, 2.8)	5.83 (dd, 10.5, 2.5)	5.39 (dd, 7.7, 4.6)	5.47 (dd, 7.9, 4.7)
24	1.87 (m)	1.90 (m)	1.96 (m)	1.97 (m)
25a	1.35 (m)	1.39 (m)	1.52 (m)	1.51 (m)
25b	1.59 (m)	1.65 (m)	1.52 (m)	1.51 (m)
26	0.96 (t, 7.3)	1.00 (t, 7.3)	0.60 (t, 6.8)	0.62 (t, 7.3)
27	1.29 (d, 6.8)	1.32 (d, 7.0)	0.70 (d, 6.5)	0.72 (d, 6.8)
29	5.14 (dd, 9.2, 1.3)	5.28 (dd, 9.3, 1.3)	–	–
30a	3.05 (dd, 11.3, 9.2)	3.12 (dd, 11.3, 9.3)	7.85 (s)	7.77 (s)
30b	4.03 (dd, 11.3, 1.3)	4.04 (dd, 11.3, 1.3)	–	–
32	5.89 (dd, 8.0, 8.0)	5.73 (dd, 8.0, 8.0)	5.54 (ddd, 8.4, 7.4, 7.4)	5.64 (ddd, 8.4, 7.4, 7.4)
33a	3.66 (m)	2.25 (m)	1.95 (m)	3.35 (dd, 13.5, 7.4)
33b	3.66 (m)	2.25 (m)	2.04 (m)	3.44 (dd, 13.5, 8.4)
34	–	1.83 (m)	1.51 (m)	–
35	7.36 (dd, 8.2, 1.3)	0.98 (d, 6.5)	0.74 (d, 6.6)	6.99 (m)
36	7.06 (ddd, 8.2, 7.5, 1.3)	0.88 (d, 6.5)	0.69 (d, 6.6)	6.93 (m)
37	6.97 (dt, 7.5, 1.3)	–	–	6.90 (m)
N1	7.41 (d, 6.7)	7.43 (d, 6.5)	8.46 (d, 7.9)	8.55 (d, 7.9)
N2	8.00 (d, 10.0)	7.96 (d, 10.0)	8.67 (d, 8.8)	8.70 (d, 9.0)
N3	7.59 (d, 10.2)	7.64 (d, 10.5)	8.85 (d, 7.7)	8.86 (d, 7.9)
N4	8.34 (d, 8.0)	8.26 (d, 8.0)	8.05 (d, 7.4)	8.26 (d, 7.4)

provided in Tables I and II. Additional details are available as supplementary material.

Further evidence supporting structure 1 was provided by tandem mass spectrometry through examination of the collision-induced dissociation (CID) mass spectrum of the $(M + H)^+$ and fragment ions.[15] The CID spectrum of m/z 807, the protonated molecular ion of 1, was dominated by fragment ions originating from the linear acylium ion depicted in Scheme I. The major fragmentation sequence involved the loss of C-terminus fragments from the acylium ion to produce m/z 779, 708, 529, 512, 483, 399, and 297 ions (Scheme I and Figure 2). Subsequent fragmentation of these ions resulted in the additional peaks in the mass spectrum. Decarbonylation of the m/z 807 acylium ion resulted in the m/z 779 immonium ion from which arose another predominant ion series. This series is due primarily to loss of the N-terminus proline residue to form the m/z 682 product ion and subsequent fragmentation of this ion.

The absolute stereochemistry of 1 was determined by comparing the (1-fluoro-2,4-dinitrophen-5-yl)-L-alanineamide (FDAA) derivatized amino acids from the acid hydrolysate of the peptide with similarly derivatized standard amino acids by HPLC according to Marfey's procedure.[16] This procedure established L-proline, L-valine, and L-phenylalanine as constituents of 1. The absolute configurations of the thiazole amino acids were determined to be D-isoleucinylthiazole and L-valinylthiazole using a previously described method.[17,18]

HR FABMS, showed $(M + H)^+$ at m/z 773.3345 for tawicyclamide B (2), furnishing the molecular formula $C_{36}H_{53}N_8O_5S_3$ (Δ 4.4 mmu). The ^{13}C spectrum of 2 (Table II) showed 36 unique resonances in agreement with this formula. Tawicyclamide B showed remarkable spectral similarities to tawicyclamide A, the most significant differences being the absence of phenylalanine resonances and the presence of two new methyl resonances (0.98 and 22.99; and 0.88 and 23.06 ppm in the ^1H and ^{13}C spectra, respectively). These data, coupled with a 34 mass unit decrease in the molecular weight, allow the conclusion that 2 differs from 1 by replacement of phenylalanine in 1 with leucine to form 2. COSY data supported a leucine spin network with long-range coupling to the thiazoline ring in 2. This thiazoline ring was also confirmed by oxidation to a thiazole to give dehydrotawicyclamide B (3). Tables I and II contain the proton and carbon assignments for 2 and 3. HPLC analysis of the FDAA derivatized hydrolysate of 2 revealed the presence of L-proline, L-valine, and L-leucine with a significant amount of D-leucine resulting from partial racemization of the L-leucine from L-leucinylthiazoline.[19]

Single-crystal X-ray analysis was carried out on tawi-

(15) (a) Biemann, K. *Methods Enzymol.* **1990**, *193*, 455. (b) Eckart, K.; Schwartz, H.; Tomer, K. B.; Gross, M. L. *J. Am. Chem. Soc.* **1985**, *107*, 6765.

(16) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.

(17) McDonald, L. A.; Ireland, C. M. *J. Nat. Prod.* **1992**, *55*, 376.

(18) Biskupiak, J. E.; Ireland, C. M. *J. Org. Chem.* **1983**, *48*, 2302.

(19) The L/D-leucine ratio was 1.38 due to the partial racemization. The 16% ee of the L isomer did, however, support its presence in the natural product. Additionally, whereas partial racemization of L-leucine from L-leucinylthiazoline occurred upon acid hydrolysis of 2, analysis of the hydrolysate of ozonized 2 showed only L-leucine.

Table II. Carbon NMR Assignments for 1–4 in C_6D_6

	δ ^{13}C			
atom	1	2	3	4
1	161.57	161.66	160.45	160.46
2	148.69	149.01	150.20	149.95
3	124.02	124.07	123.26	123.30
4	170.44	170.68	167.96	167.90
5	57.08	57.34	56.45	56.47
6	36.59	36.53	34.94	34.83
7	16.88	17.05	18.52	18.59
8	19.84	19.84	18.73	18.74
9	171.19	171.29	170.05	169.94
10	63.32	63.23	60.74	60.78
11	32.24	32.21	25.49	25.47
12	22.89	22.80	24.77	24.81
13	47.07	46.83	47.58	47.69
14	174.24	174.06	173.36	173.59
15	56.22	56.14	55.55	55.69
16	33.60	33.60	33.15	33.25
17	19.10	19.02	18.35	18.13
18	19.60	19.57	20.01	19.99
19	160.32	160.31	160.83	160.79
20	149.62	149.69	149.52	149.64
21	124.17	124.13	123.39	123.46
22	172.66	172.73	169.16	169.35
23	54.55	54.59	56.20	56.12
24	40.71	40.82	41.40	41.54
25	28.14	28.16	25.35	25.48
26	12.66	12.67	11.75	11.75
27	15.46	15.48	15.13	15.10
28	172.50	172.61	160.51	160.48
29	77.94	78.02	150.97	150.72
30	38.47	38.39	123.68	124.11
31	177.42	178.31	171.16	170.59
32	56.13	53.20	49.60	53.15
33	38.61	41.22	43.93	41.37
34	138.18	25.87	25.28	137.16
35	130.18	22.99	22.08	129.34
36	129.08	23.06	22.35	128.69
37	127.46	-	-	127.03

cyclamide B (2), and a computer-generated perspective drawing of the final X-ray model is given in Figure 3. Only the relative stereochemistry could be determined in the X-ray experiment, and the absolute configuration shown was set by the known configurations of the L-proline, L-valine, and L-leucine residues. The X-ray analysis confirmed the spectroscopically determined structure but also revealed the three-dimensional structure—a shape that could not have been easily predicted. The easiest way to describe the shape of tawicyclamide B is to note that the "peptide" chain, a circle in representation 2, has the three-dimensional shape of a tennis ball seam. The distortion is most easily imagined by taking the two thiazole rings on the opposite sides of 2 and placing them on top of each other. The rings are essentially parallel with an interplanar angle of only 13°. The centers of the rings are separated by 3.7 Å, a typical aromatic stacking distance. The resulting conformation has the hydrophobic side chains pointing away from the internal cavity; the isopropyl group of one valine points up, while the other valyl and leucyl side chains point down. This conformation also has a *cis*-valine–proline peptide bond. Hydrogen bonding may also play a role in stabilizing the solid state conformation seen for 2 as there is a weak intramolecular hydrogen bond from N4H to O3 of 2.15 Å (148°). There were no intermolecular hydrogen bonds.

Oxidation of the tawicyclamides appears to result in conformational changes in parts of the molecules that are remote from the site at which oxidation takes place. Several pieces of NMR data for 3 suggested that it adopted a drastically different conformation than its parent peptide 2, in C_6D_6 solution. The dissimilarity between the ROESY[20] and ^{13}C NMR data of the two peptides, especially the proline residue, indicated a significant change in chemical environment. These changes suggested possible isomerization of the valine–proline amide bond from cis to trans upon oxidation of the thiazoline ring to a thiazole. These observations prompted the investigation of 2 and 3 conformations by molecular modeling.

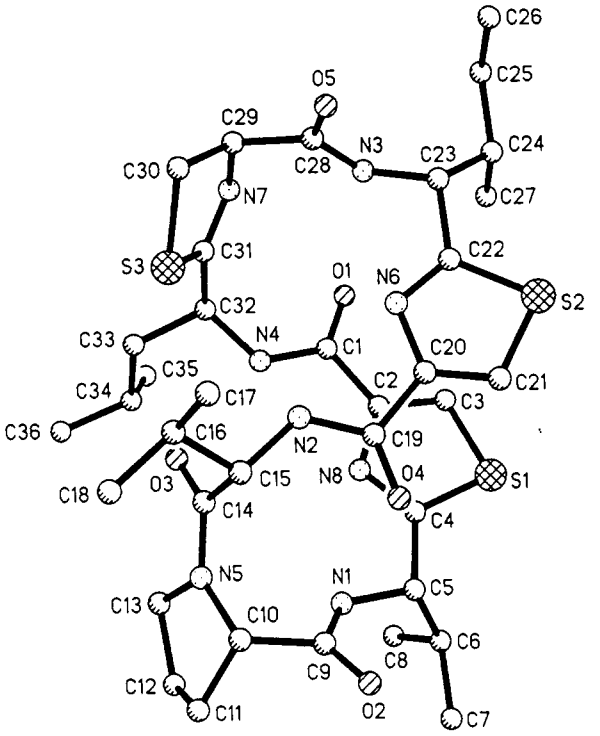


Figure 3. Computer-generated perspective drawing of the final X-ray model of tawicyclamide B (2). Hydrogens were omitted for clarity, and the absolute configuration was set by the known configurations of the L-valine, L-leucine, and L-proline.

Tawicyclamide B was modeled starting from the X-ray coordinates using a minimization–molecular dynamics–minimization (min–md–min) procedure.[21] Initial energy minimization using the ABNR (adopted basis-set Newton–Raphson) technique was run to locate the lowest energy conformer near the starting geometry. A molecular dynamics (MD) simulation at 300 K was run using this low-energy conformer. In order to evaluate the conformational space available to the molecule under experimental conditions, the effect of solvent was approximated by using a constant dielectric (ε 2.284 for benzene at 20 °C). Minimization of several dynamics data sets resulted in convergence to a structure practically identical to the starting low energy conformer and quite similar to the crystal structure.[22] The optimal conformation for 2 (Figure 4) has a *cis*-valine–proline amide bond (C15–C14–N5–C10 dihedral = −10.3°), an intramolecular hydrogen bond from N4H to O3 of 2.05 Å (145°), and was in excellent agreement with NMR data. The stability of this conformer is illustrated by the fact that both stacking

(20) (a) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207. (b) Kessler, H.; Griesinger, C.; Kressebaum, R.; Wagner, K.; Ernst, R. R. *J. Am. Chem. Soc.* **1987**, *109*, 607. (c) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811.

(21) (a) Brown, F. K.; Hempel, J. C.; Dixon, J. S.; Amato, S.; Mueller, L.; Jeffs, P. W. *J. Am. Chem. Soc.* **1989**, *111*, 7328. (b) Howard, A. E.; Kollman, P. A. *J. Med. Chem.* **1989**, *31*, 1669.

(22) During a 10-ps MD simulation, 100 data sets were collected at 0.1-ps intervals throughout the trajectory. A subset of 10 data sets (every 10th set) were minimized. The overall rms deviation between these structures was 0.0033 Å. The structure shown in Figure 4 is representative.

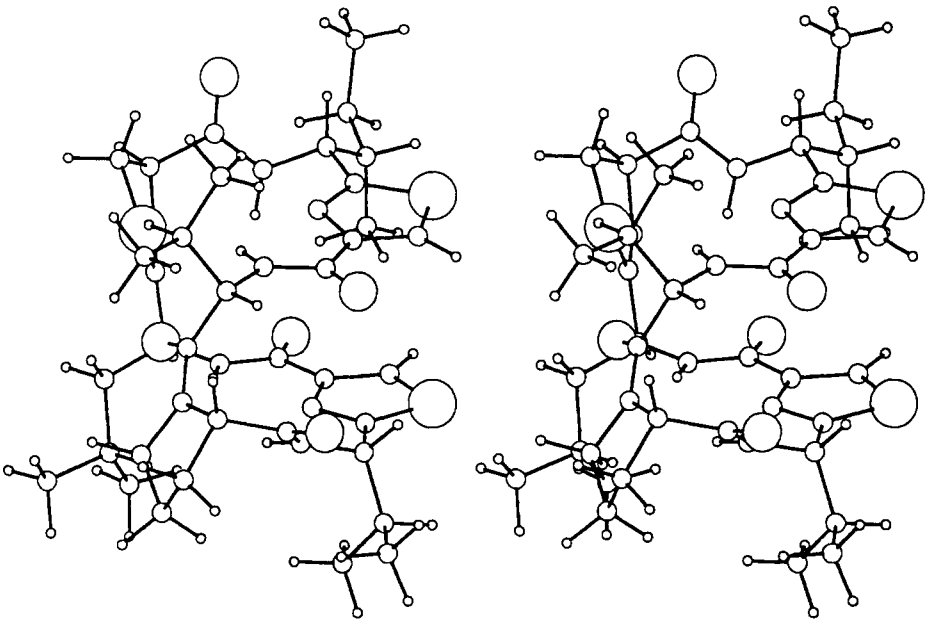


Figure 4. Computer-generated stereodrawing of the lowest energy conformer of tawicyclamide B (**2**).

interaction and hydrogen bonding persisted throughout the min–md–min procedure. Furthermore, proline allows for the possibility of cis–trans isomerism, yet only one conformer appears to be present in C_6D_6 solution—as evidenced by a single set of resonances in the NMR spectrum of **2**.[23] A distance of 2.2 Å between H10 and H15 in this model is consistent with the very strong ROESY cross peak between these two protons. This key NOE implies a cis orientation about the valine–proline amide bond. Additionally, the ^{13}C chemical shift difference between the proline β and γ carbons ($\Delta\delta_{\beta\gamma}$ = 9.5) supported the *cis*-valine–proline amide bond.[24] A more striking confirmation of the proposed solution conformer is the NOE between one thiazole aromatic proton (H3) and the γ-methyl group of isoleucine (H27) which, in this model, are separated by 2.5 Å at closest approach. The relative downfield position of H27 is due primarily to anisotropic deshielding by the thiazole ring and is fully consistent with this model. Further evidence supporting the proposed solution conformer of **2** is provided by an NOE between one leucine δ methyl group (H35) and the proline δ protons (H13) which approach each other to within 2.4 Å.

A 10-ps MD simulation at 1000 K carried out to escape local minimum energy wells, allowed for sampling of a larger conformational space, and generated a reasonable number of starting structures for subsequent minimization. Convergence to an average structure similar to the starting conformer and to the crystal structure was again observed, although the side chains were somewhat flexible, with larger deviations than the main chain atoms.[25] The average energy of these minimized conformers was, however, 3.5 kcal/mol higher than the starting low-energy conformer shown in Figure 4, as a result of large deviations of the amino acid side chains.

The starting model of *cis*-dehydrotawicyclamide B was taken from the X-ray structure of tawicyclamide B by removing two hydrogens from the thiazoline ring. Modeling was again carried out using a constant dielectric corresponding to benzene. Energy minimizations were run to locate the lowest conformer near the starting geometry. This structure, not surprisingly, retained a *cis*-valine–proline amide bond after minimization with the ABNR technique. Since this was inconsistent with the experimental NMR data, the following protocol was used to generate the trans conformer. The molecule was first heated to 1000 K for 1.0 ps, equilibrated for 1.0 ps, and finally simulated at 1000 K for 10 ps. Following minimizations, two populations of conformers were obtained: one similar to the starting cis structure, the other having a *trans*-valine–proline amide bond. Interestingly, convergence to the trans structures occurred toward the end of the 10-ps simulation. It was disappointing, however, that even though this trans isomer appears to fit the NMR data, its energy was higher, by 0.1 kcal/mol, than the starting cis isomer. Since the calculations failed to give results energetically consistent with experimental data, another approach to finding a starting conformation was explored. The valine–proline amide bond was constrained to 180° and subjected to an ABNR–MD–ABNR protocol. The dihedral constraint was removed after the first series of minimizations, and the molecular dynamics simulations were run to let the whole molecule relax. The molecule was heated to 300 K for 0.3 ps, equilibrated for 0.3 ps, and finally simulated at 300 K for 3 ps. During the MD simulation, 30 data sets from throughout the trajectory were collected. Ten data sets were minimized with an overall root mean square (rms) deviation of 1.456 Å. A convergence to a lower energy structure was observed toward the end of the trajectory.[26] After minimization, the resulting

(23) The activation energy barrier to rotation about the valine–proline amide bond was determined to be 14.9 kcal/mol, a value comparable to those reported for X-valine (X = any amino acid) rotation barrier; see, for example: MacArthur, M. W.; Thornton, J. M. *J. Molec. Biol.* **1991**, 218, 397.

(24) (a) Siemion, I. Z.; Wieland, T.; Pook, K.-H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 702. (b) Pook et al. have shown that for X–Pro (X = any amino acid), there is a linear dependence of the difference in the chemical shifts of the β and γ carbons of proline ($\Delta\delta_{\beta\gamma}$) with dihedral angle $\theta = (\psi - 60°)$ according to the equation; $\Delta\delta_{\beta\gamma} = 0.081|\theta| + 2.47$ for a cis orientation about the X–pro amide bond or $\Delta\delta_{\beta\gamma} = 0.036|\theta| + 0.73$ for a trans orientation. (c) The observed $\Delta\delta_{\beta\gamma}$ value of 9.5 for tawicyclamide B places it in the *cis*-X–Pro series. A θ (O2–C9–C10–C11) of −79.7° in the modeled cis conformer predicts a $\Delta\delta_{\beta\gamma}$ value of 8.9. (d) $\Delta\delta_{\beta\gamma}$ of 0.7 places dehydrotawicyclamide B in the *trans*-X–Pro series. A θ of −8.2° in the trans conformer predicts a $\Delta\delta_{\beta\gamma}$ value of 1.0.

(25) Similar to the 300 K MD simulation, a subset of 10 structures was minimized. The overall rms deviation between these structures was 0.0130 Å. The rms difference between the average structure and that shown in Figure 4 is 0.8927 Å.

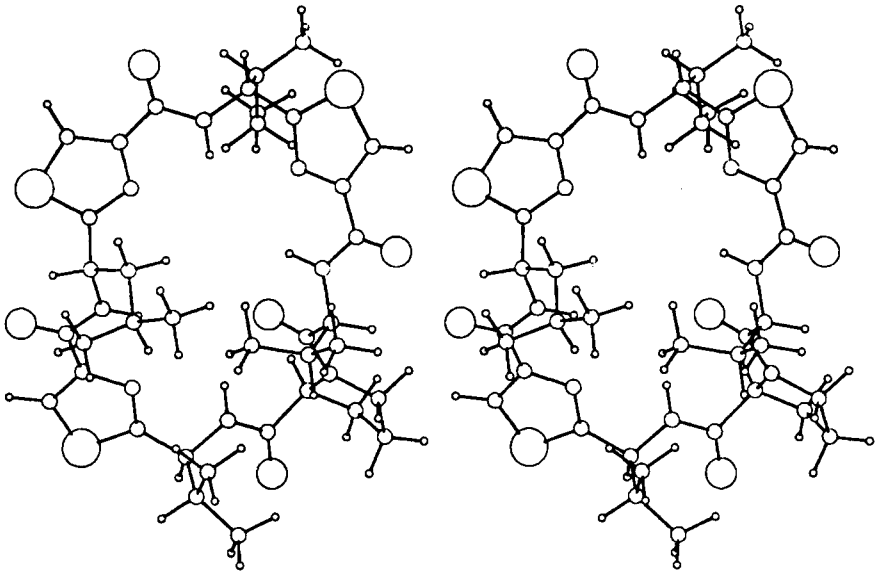


Figure 5. Computer-generated stereodrawing of the lowest energy conformer of dehydrotawicyclamide B (3).

structure contained the trans amide bond and was very similar to that obtained from dynamics simulation starting from the *cis*-dehydrotawicyclamide conformer. Compared to the cis, this trans conformer was more stable by 0.9 kcal/mol. These computational results can be most easily explained if the potential energy hyperspace of dehydrotawicyclamide B (3) has many local minima and the local minima reached are sensitive to starting conditions. Completely exploring the various local minima and defining the global minimum would be a difficult problem.

The lowest energy trans conformer of 3 is shown in Figure 5. The model shows that in 3 the all trans peptide backbone forms a rectangle, with the three thiazole rings and the proline defining its corners. This shape is similar to that reported for ascidiacyclamide,[27] although somewhat distorted from the "saddle" shape described. The isoleucine side chain protrudes below the plane of the macrocyclic ring while the leucine and both valine side chains extend above the ring. An interesting feature of this conformer is that all the NH bonds point toward the center of the ring, away from the hydrophobic environment of the solvent. The stacking of the thiazole rings and the stabilizing hydrogen bonding present in the parent are absent in the dehydro isomer. This structure agrees quite well with NMR data. For example, a strong ROESY cross peak between both δ proline protons (H13) and the α valine proton (H15) supports the trans conformer. In the modeled structure these protons are separated by 2.2 and 2.5 Å, respectively. The model also shows that H10 and N1H approach each other close enough (2.8 Å) to lead to the strong cross peak observed in the ROESY spectrum. The absence of a crosspeak between H10 and H15 in the ROESY spectrum of 3 further supports the proposed trans conformer in which these hydrogens are now 4.4 Å apart. Interestingly, upon changing the thiazoline ring to a thiazole, the resulting aromatization causes the adjacent carbonyl to come into resonance with the aromatic ring and become coplanar with the ring. This undoubtedly contributes to the drastic conformational changes observed upon oxidation.

In our modeling studies, the effects of solvent were approximated by using a uniform dielectric constant, which reflects only the polarizability of the solvents and cannot account for detailed solvent–solute interaction. This is illustrated by the chemical shift changes of the β proline protons (1.01 ppm upfield and 0.45 ppm downfield, respectively) upon oxidation of the thiazoline ring that can in part be attributed to anisotropic shielding and deshielding by the NMR solvent. This dramatic effect, which could not have been predicted by our modeling studies, may have resulted from increased access of the solvent to hydrophobic portions of the molecule such as the proline side chain. Inspection of the structures in Figures 4 and 5 reveals that the trans dehydro conformer has a larger solvent accessible surface area than the cis conformer adopted by the parent peptide.

The tawicyclamides and their dehydro analogs (3 and 4) are weakly cytotoxic against human colon tumor cells, all with IC_{50}'s of 31 μg/mL. These results are consistent with structure–activity studies that show that the oxazoline ring is essential for the cytotoxicity of this class of compounds.[6]

Conclusions. A new class of cyclic lissoclinum peptides that lack the characteristic oxazoline ring has been discovered. These peptides have a thiazoline ring and a *cis*-valine–proline amide bond that facilitates a rather unusual three-dimensional conformation. The conformation of tawicyclamide B was established both in the solid and solution state by X-ray and molecular modeling, respectively. The results of studies on tawicyclamide B and dehydrotawicyclamide B have demonstrated that oxidation of the thiazoline to a thiazole has profound conformational effects on the molecule, including isomerization of the valine–proline amide bond from cis to trans. With firm ^{13}C and NOE spectral evidence supporting these conformational interpretations, a three-dimensional solution structure for dehydrotawicyclamide B was also established.

Experimental Section

General Procedures. The ^1H and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively, on a Varian Unity 500 spectrometer or an IBM AF 200 spectrometer at 200 and 50 MHz, respectively. ^1H chemical shifts are reported in ppm relative

(26) Convergence to a final low-energy structure at the end of the MD simulation is reflected in the energies of the 10 representative minimized data sets. The first eight data sets minimized to a similar average conformer (0.138 Å overall rms deviation) while the final three data sets minimized to lower energy average conformer with an overall rms deviation of 0.0233 Å. The model shown in Figure 5 is representative of the latter set of low energy conformers.

(27) Ishida, T.; Tanaka, M.; Nabae, M.; Inoue, M. *J. Org. Chem.* 1988, *53*, 107.

to residual undeuterated benzene resonance at 7.15 ppm. ^{13}C chemical shifts are reported in ppm relative to solvent resonance at 128.0 ppm. IR spectra were recorded on a Perkin-Elmer 1600 FT spectrophotometer. UV spectra were obtained in methanol on a Beckman DU-8 spectrophotometer. Optical rotations were measured with a JASCO DIP-370 polarimeter in a 100-mm cell. High- and low-resolution FAB MS were run on a Varian MAT-731 spectrometer in a glycerol matrix. Tandem mass spectra were obtaned with a VG 70-SEQ (EBqQ geometry) spectrometer. Samples were dissolved in glycerol/3-nitrobenzyl alcohol (1:1), and 1 μL was placed on the tip of a direct insertion FAB probe. The samples were ionized by FAB using a 7–8 keV xenon beam. The CID mass spectra were obtained by selecting the precursor ion with MS-I followed by collisional activation with krypton gas (reducing the primary beam by 50%) and scanning MS-II. The precursor ion translational energy was 42 eV.

Extraction and Isolation Procedures. Specimens of *L. patella* were collected by SCUBA off Tawitawi Island in the Southern Philippines. The methanol extract of 118.4 g of freeze-dried ascidian was concentrated in vacuo to a volume of 200 mL and partitioned according to a modified Kupchan scheme.[28] Briefly, a 10% aqueous methanol solution was partitioned with hexane (5 × 110 mL). Increasing the aqueous content of the lower phase to 26% and partitioning with chloroform (3 × 110 mL) afforded a combined chloroform layer which was concentrated in vacuo to yield 4.9 g (4.1%) of a crude solid. Vacuum flash chromatography (60 mL sintered glass funnel; 20 g silica gel G; step gradient elution from 0 to 10% methanol/chloroform), carried out on a 2.77 g portion of this residue, yielded a fraction (505 mg; eluting with 2.5% methanol/chloroform) which showed ^1H NMR resonances characteristic of peptides. TLC of this fraction (silica gel; 100% ethyl acetate) revealed a series of spots (R_f = 0.20–0.40) visible with 254-nm UV light and giving positive responses to chlorine-tolidine for nitrogen-containing compounds.[29] Further silica gel chromatography using hexane/acetone mixtures followed by reversed-phase HPLC using acetonitrile/water mixtures yielded 8 mg of ulithiacyclamide (**7**), 24 mg of patellamide A (**5**), 100 mg of patellamide B (**6**), tawicyclamide A (**1**), and tawicyclamide B (**2**).

1: clear colorless solid; 63 mg (0.10% dry weight); IR (film) 3362, 3332, 2963, 2930, 2874, 1666, 1641, 1609, 1536, 1514, 1484, 1428 cm^{-1}; $[\alpha]^{25}_D$ −15.0° (c = 0.427, $CHCl_3$); UV(MeOH) λ_{max} 249.7 nm (ε 16 530); MS (FAB) m/z 807 $(M + H)^+$, 779, 682, 529, 484, 399, 297, 251, 197, 180; HR FABMS m/z 807.3135 $(M + H)^+$, $C_{39}H_{51}N_8O_5S_3$ requires 807.3145; ^1H and ^{13}C NMR see Tables I and II.

2: clear colorless solid; 69 mg (0.10% dry weight); IR (film) 3360, 3332, 2960, 2927, 2872, 1667, 1642, 1537, 1514 cm^{-1}; $[\alpha]^{25}_D$ +2.1° (c = 0.347, $CHCl_3$); UV(MeOH) λ_{max} 249.7 nm (ε 15 029); MS (FAB) m/z 773 $(M + H)^+$, 745, 297, 251, 197, 180; HR FABMS m/z 773.3345 $(M + H)^+$, $C_{36}H_{53}N_8O_5S_3$ requires 773.3301; ^1H and ^{13}C NMR see Table I and II.

Single-Crystal X-ray Diffraction Analysis of Tawicyclamide B (2). A colorless platelike crystal with dimensions 0.4 × 0.1 × 0.1 mm, grown from aqueous acetonitrile, was chosen for analysis. All measurements were done on a Nicolet R3M diffractometer using graphite-monochromated CuKα radiation (1.541 80 Å) at approximately −15 °C. Preliminary diffraction photographs illustrated orthorhombic symmetry. Accurate cell constants of a = 9.612 (3) Å, b = 12.293 (3) Å, and c = 34.186 (8) Å were obtained by a least-squares fit of 25 centered reflections in the range 30° ≤ 2θ ≤ 45°. The space group was uniquely determined as $P2_12_12_1$ from systematic absences and optical activity, and an approximate density indicated one molecule of $C_{36}H_{52}N_8O_5S_3$, in an asymmetric unit (Z = 4). All unique diffraction maxima with 2θ ≤ 116° were measured using variable speed (1.5–29.5°/min) 1° θ:2θ scans; three check reflections were monitored periodically, and displayed no significant variation in intensity. No absorption or decomposition corrections were made. After correction for Lorentz, polarization, and background effects, 2632 (83%) out of the total measurement of 3168 unique reflections were judged observed ($|F_o| \geq 4\sigma(F_o)$). The structure was solved by direct methods and refined by full-matrix least-squares using the SHELXTL program set. Anisotropic thermal parameters were employed for non-hydrogen atoms, and hydrogen atoms were fixed geometrically with riding constraints. The final agreement factors were R = 0.049, wR = 0.046 with weighting scheme $w = [s^2(F) + 0.0000F^2]^{-1}$ for observed data. The goodness of fit was 1.84 with a data/parameter ratio of 5.6:1. The difference electron density was essentially featureless with maximum and minimum values less than ±0.43 $eÅ^{-3}$. Additional crystallographic details are available as described in the supplementary material.

General Oxidation Procedure. Nickel peroxide was prepared,[30] and available oxygen was determined by titration of iodine liberated from potassium iodide solution. The peptides were dissolved in benzene and treated with nickel peroxide for 23 h at room temperature. TLC (silica gel; 100% ethyl acetate) indicated quantitative conversion of the starting materials to single products. Filtration of the reaction mixture through Celite and concentration of the filtrate afforded the pure oxidized peptides.

Preparation of Dehydrotawicyclamide B (3). NiO_2 (722 mg; 65.2 μmol available O_2) was stirred with **2** (12.3 mg; 15.9 μmol) in 6 mL of C_6H_6 for 23 h at 25 °C to yield oxidation product **3** (3.7 mg, 30% recovery) as a clear colorless solid: FABMS m/z 771 $(M + H)^+$, 743, 672, 493, 483, 297; HR FABMS m/z 771.3153 $(M + H)^+$, $C_{36}H_{51}N_8O_5S_3$ requires 771.3145; ^1H and ^{13}C NMR see Tables I and II.

Preparation of Dehydrotawicyclamide A (4). **1** (9.8 mg; 12.2 μmol) in 6 mL of C_6H_6 was stirred with 751 mg (67.8 μmol available O_2) of NiO_2 for 23 h at 25 °C and yielded oxidation product **4** (2.5 mg; 26% recovered) as a clear colorless solid: FABMS m/z 805 $(M + H)^+$, 777, 680, 527, 484, 399, 297; HR FABMS m/z 805.3017 $(M + H)^+$, $C_{39}H_{49}N_8O_5S_3$ requires 805.2988; ^1H and ^{13}C NMR see Tables I and II.

Ozonolysis of 1. A slow stream of O_3 was bubbled into a 10-mL CH_2Cl_2 solution of **1** (1.5 μmol) placed in a threaded bomb at 25 °C for approximately 15 min. Upon removal of the solvent under a stream of N_2, the residue was subjected to hydrolysis and derivatization as described below.

Hydrolysis and Derivatization Procedures. Hydrolysis of the peptide was carried out in 4 mL of 6 N HCl under a nitrogen atmosphere in a sealed bomb at 104 °C for 23 h. After traces of HCl were removed by repeated evaporation in vacuo, the residual hydrolysates were suspended in 300 μL of H_2O and derivatized with (1-fluoro-2,4-dinitrophen-5-yl) L-alanineamide (FDAA).[16] HPLC analysis (Waters NOVAPAK C_{18}; 4.6- × 100-mm column; linear gradient elution, triethylammonium phosphate (50 mM, pH 3.0)/acetonitrile, 90:10–60:40 in 45 min; 1.5 mL/min; UV detection at 340 nm) of the FDAA derivatized hydrolysates in conjunction with similarly derivatized amino acid standards established the stereochemistry of the constituent amino acids.

Stereochemistry of Tawicyclamide A (1). Coinjection with standard amino acid derivatives established the presence of L-proline, L-valine, and L-phenylalanine in the hydrolysate of **1**. One additional equivalence of L-valine and D-isoleucine was further discovered in the hydrolysate of ozonized **1**.

Stereochemistry of Tawicyclamide B (2). HPLC analysis of the FDAA derivatized hydrolysate of **2** revealed the presence of L-proline, L-valine, and L-leucine.

Molecular Modeling. Molecular modeling studies were carried out using Quanta/CHARMm[31,32] implementation of a molecular mechanics force field on a SGI Personal Iris 4D-25G computer. The effect of solvent was approximated by using a constant dielectric of 2.284 (benzene), and all energy terms were calculated. Modeling involved a minimization–molecular dynamics–minimization protocol. The structures were subjected to adopted-basis-set Newton–Raphson energy minimization which was terminated when the energy value gradient between cycles was less than 0.001 kcal/mol Å. The nonbonded interactions were switched off between 6.5 and 7.5 Å with atom pairs up to 8 Å included in the nonbonded list. The time step of integration was

(28) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Siegel, C. W. *J. Org. Chem.* **1973**, *38*, 178.
(29) Krebs, K. G.; Heusser, D.; Wimmer, H. In *Thin Layer Chromatography*; Stahl, E., Ed.; Springer-Verlag: Berlin, 1969; p 862.
(30) Nakagawa, K.; Konaka, R.; Nakata, T. *J. Org. Chem.* **1962**, *27*, 1597.
(31) Polygen Corp. 200 Fifth Ave., Waltham, MA 02254.
(32) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.

1 fs, and the nonbonded integration list was updated every 25 fs. Bond lengths involving hydrogen atoms were kept fixed using the SHAKE[33] algorithm. The initial coordinates for the atoms of 2 and 3 were taken from the X-ray structure. Prior to modeling, two hydrogens (H29 and H30) were removed from the thiazoline ring which was then explicitly aromatized. Typical molecular dynamics simulations involved a 1-ps heating period during which time the system was heated to 300 or 1000 K followed by a 1-ps equilibration period and then 10 ps of dynamics simulation at the appropriate elevated temperature. Energy barrier to rotation was calculated by constraining the valine–proline dihedral to 90° and minimizing. The energy of this structure without constraints less the energy of the unconstrained energy-minimized cis structure is taken as the barrier to rotation.

Acknowledgment. This work was supported by grants CA36622 (C.M.I.), CA24487 (J.C.), and CA50750 (J. C. and C.M.I.) awarded by the National Institutes of Health. L.A.M. acknowledges support from an NIH predoctoral fellowship supported by grant CA36622 and thanks Mr. Jay Olsen for enlightening discussions on NMR techniques. COLOC NMR studies were performed on an IBM AF 200 spectrometer purchased with funds from the NSF (PCM 8400801) and partially supported by funds from The University of Utah Cancer Center Core Grant (5P30 CA 042014). Partial funding for the Varian Unity 500 NMR spectrometer was provided by NIH grant S10 RR06262 (C.M.I.). MS/MS experiments were performed on a VG 70-SEQ spectrometer (in the laboratory of Dr. J. A. McCloskey) supported by grant GM 21584 from the National Institute for General Medical Sciences. We thank Dr. N. L. Lindquist, University of California, San Diego for collecting and identifying *L. patella* and Bristol-Myers Squibb for cytotoxicity testing.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, bond distances, and bond angles for tawicyclamide B and tables of HMBC correlation data for compounds 1–4 (14 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(33) Ryckaert, J. P.; Cicotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327.

The Natural Polypropionate-Derived Esters of the Mollusc *Onchidium* sp.

Jaime Rodríguez and Ricardo Riguera*

Departamento de Química Orgánica, Facultad de Química, Universidad de Santiago, Santiago de Compostela, 15706, Spain

Cécile Debitus

Centre ORSTOM, B. P. A5, Noumeá Cedex, New Caledonia

Received January 27, 1992

Eight new polypropionate-derived esters (**5**–**8** and **10**–**13**) have been isolated from pulmonate molluscs of the genus *Onchidium* collected in the South Pacific. The structures of these compounds were determined spectroscopically in particular by one- and two-dimensional NMR and low and high EIMS. The absolute stereochemistry of the seven asymmetric centers was determined by using the Trost–Mosher methodology. Saponification afforded two triols named onchitriol I and II (**4** and **9**, respectively). Compounds **4**–**13** displayed in vitro antitumor activity against several cell lines. Onchitriol I and II had antiviral activity also.

Introduction

The marine mollusc phylum has been the object of intense chemical scrutiny by several research groups. The initial interest was prompted by reports that colorful, shellless molluscs which appear to be highly vulnerable to predation might utilize defensive secretions.[1] The pulmonates of the family Onchidiacea inhabit the rocky intertidal zones of many tropical shorelines and are known to contain epidermal glands described as "repugnatorial". These molluscs have proved to be a rich source of in vitro cytotoxic[2] and in vivo antineoplastic[3] substances of novel molecular types. One of the first examples was onchidal a defensive allomone of *Onchidella binneyi*.[4]

The most interesting compounds isolated from these species have a propionate-based biogenetic origin and posses a linear or cyclic polypropionate carbon skeleton. According to Faulkner, three general classes can be distinguished:[5] the simple polypropionates, such as denticulatin A;[6] the α-pyrones, exemplified by diemenesin;[7] and the γ-pyrones like siphonarins A and B.[8] The γ-pyrones include the polyhydroxylated peroniatriols I and II (**1**, **2**) and ilikonapyrone (**3**), which were isolated from the saponified extracts of *Peronia peronii*[9] and *Onchidium verruculatum*,[10] respectively; these compounds have a linear structure containing two γ-pyrone rings, three hydroxyl groups, and other asymmetric centers.

The general structure and relative stereochemistry of the acetonide of **3** were established by X-ray analysis, and

(1) Thompson, T. E. *J. Mar. Biol. Ass. UK* **1960**, *39*, 123.
(2) Kigoshi, H.; Imamura, Y.; Yoshikawa, K.; Yamada, K. *Tetrahedron Lett.* **1990**, *31*, 4911.
(3) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Dufresne, C.; Cerny, R. L.; Herald, D. L.; Schmidt, J. M.; Kizu, H. *J. Am. Chem. Soc.* **1989**, *111*, 5015.
(4) Ireland, C. M.; Faulkner, D. J. *J. Bioorg. Chem.* **1978**, *7*, 125.
(5) Manker, D. C.; Faulkner, D. J.; Stout, T. J.; Clardy, J. *J. Org. Chem.* **1989**, *54*, 5371.
(6) Hochlowski, J. E.; Faulkner, D. J.; Matsumoto, G. K.; Clardy, J. *J. Am. Chem. Soc.* **1983**, *105*, 7413.
(7) Howcholowski, J. E.; Faulkner, D. J. *Tetrahedron Lett.* **1983**, *24*, 1917.
(8) Howcholowski, J. E.; Coll, J. C.; Faulkner, D. J.; Biskupiak, J. E.; Ireland, C. M.; Zheng, Q.; He, C.; Clardy, J. *J. Am. Chem. Soc.* **1984**, *106*, 6748.
(9) Biskupiak, J. E.; Ireland, C. M. *Tetrahedron Lett.* **1985**, *26*, 4307.
(10) Ireland, C. M.; Biskupiak, J. E.; Hite, G. J.; Rapposch, M.; Scheuer, P. J.; Ruble, J. R. *J. Org. Chem.* **1984**, *49*, 559.

0022-3263/92/1957-4624$03.00/0