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# Shermilamine A: A Pentacyclic Alkaloid from a Tunicate[1]

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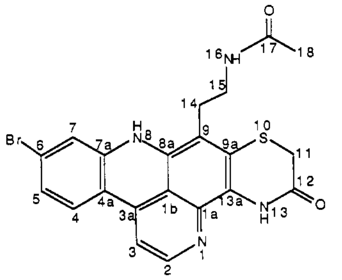
Tunicates are sessile marine invertebrates best known for their ability to concentrate vanadium and other metals from sea water.[3] More recently, tunicate-derived peptides, notably the didemnins, have attracted attention because of their desirable antiviral and anticancer bioactivities.[4] We report here the structure of a new tunicate-derived thiazinone-containing pentacyclic alkaloid, which—while reminiscent of 2-bromoleptoclinidinone[5a] and ascididemnin[5b]—has no close analogues among known marine metabolites.

The leathery purple colonial tunicate, *Trididemnum* sp., was collected in Pago Bay, Guam,[6] between −2 and −10 m and frozen. Extraction with methanol followed by solvent partition[7] furnished an orange pigment in the chloroform extract. Gel filtration, flash chromatography,[8] and HPLC yielded shermilamine A (1),[9] *N*-[2-(6-bromo-8,11,12,13-tetrahydro-12-oxopyrido[4,3,2-*mn*][1,4]thiazino[3,2-*b*]acridin-9-yl)ethyl]acetamide,[10] rectangular orange plates, mp 135 °C, after recrystallization from chloroform/methanol (96:4).



1

The IR spectrum was uninformative except for a series of amide signals. The UV spectrum undergoes a reversible bathochromic shift upon addition of acid. Mass spectrometry secured a molecular formula, $C_{21}H_{17}{}^{79}BrN_4O_2S$, for a compound with 15 elements of unsaturation. Since the $^{81}Br$ peak obscures $^{34}S$, the presence of sulfur was discovered only during X-ray diffraction.

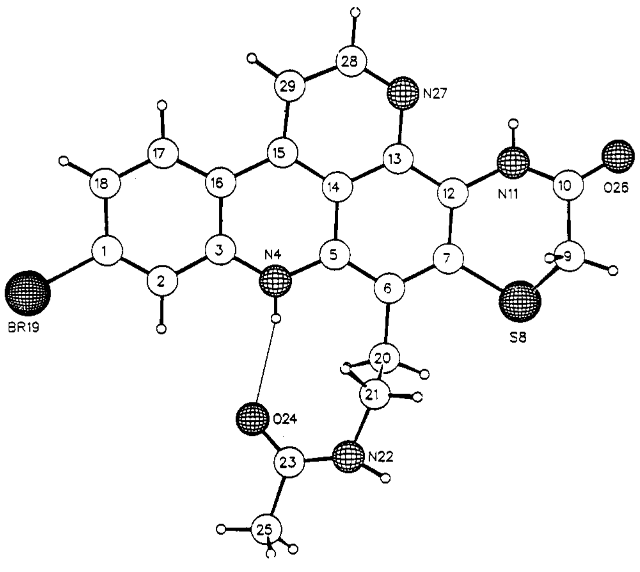
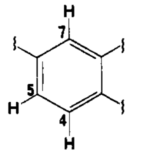
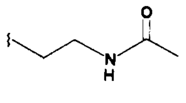


**Figure 1.** Computer-generated perspective drawing of shermilamine A.

$^1H$ NMR data revealed three exchangeable protons ($\delta$ 10.3, 9.0, 6.3), two ortho-coupled doublets in a pyridine ring,[11] three aromatic protons compatible with part structure a, an isolated and two vicinal methylenes, and an acetyl methyl, thereby accounting for all 17 hydrogen atoms (Table I). $^{13}C$ NMR[12] (Table II) combined with COSY, DEPT, and NOE (Table III) data permitted delineation of the side chain b. Establishment of unambiguous carbon connectivities was hampered by the presence of 12 quarternary carbons including two in the carbonyl region. The structural problem therefore was solved by X-ray diffraction techniques.



a



b

A single crystal of 1 was selected for X-ray diffraction analysis. Preliminary diffraction photographs displayed monoclinic symmetry, and diffractometer-measured $2\theta$ values were used to obtain accurate lattice constants of $a = 19.961$ (4) Å, $b = 9.598$ (1) Å, $c = 23.963$ (5) Å, and $\beta = 94.21$ (2)°. The possible space groups were *Cc* or *C*2/*c*, and, while we were confused about the exact molecular

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[1] A preliminary account of this work was presented at the Gordon Research Conference on Marine Natural Products, Oxnard, CA, Feb 29–March 4, 1988.

[2] On leave from the Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary.

[3] Barnes, R. D. *Invertebrate Zoology*, 3rd ed.; W. B. Saunders: Philadelphia, 1974; p 813.

[4] Rinehart, K. L., Jr.; Gloer, J. B.; Hughes, R. G., Jr.; Renis, H. E.; McGovern, J. P.; Swynenberg, E. B.; Stringfellow, D. A.; Kuentzel, S. L.; Li, L. H. *Science* (*Washington, D.C.*) 1981, *212*, 933–935.

[5] (a) Bloor, S.; Schmitz, F. J. *J. Am. Chem. Soc.* 1987, *109*, 6134–6136. (b) Kobayashi, J.; Cheng, J.-F.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* 1988, *29*, 1177–1180.

[6] We thank Drs. P. Karuso and A. Poiner for the collections in September 1986 and October 1987.

[7] Kupchan, S. M.; Britton, R. W.; Ziegler, H. F.; Sigel, C. W. *J. Org. Chem.* 1973, *38*, 178–179.

[8] Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* 1978, *43*, 2923–2925.

[9] Shermila is a character in the Indian epic *Mahabaratha*. It is also the name of N.M.C.'s daughter with whose birth the solution of this structure competed. Shermila won the race. She is rewarded by having the new compound named after her.

[10] The systematic name was kindly provided by Dr. Kurt L. Loening of Chemical Abstracts Service.

[11] Pretsch, E.; Seibl, J.; Simon, W.; Clerc, T. *Tabellen zur Strukturaufk*lärung *Organischer Verbindungen mit Spektroskopischen Methoden*; Springer-Verlag: Berlin, 1981; p H275.

[12] All crystallographic calculations were done on a PRIME 9955 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were the following: FOBS86, by G. Van Duyne, Cornell University, 1986; MULTAN 80 and RANTAN 80, written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, University of York, England, 1980; BLS78A, an anisotropic block diagonal least square refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; PLIPLOT, by G. Van Duyne, Cornell University, 1984; TABLES, by G. Van Duyne, Cornell University, 1986.

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Table I. $^1H$ NMR Data for Shermilamine A (1)[a]

| CD$_2$Cl$_2$ | | | | DMSO-$d_6$ | | |
|---|---|---|---|---|---|---|
| δ | multiplicity | $J$, Hz | proton no. | δ | multiplicity | $J$, Hz |
| 10.30* | s | | H-8 | 10.24* | s | |
| 9.00* | s | | H-13 | 9.26* | s | |
| 8.50 | d | 5.0 | H-2 | 8.40 | d | 4.5 |
| 7.71 | d | 8.6 | H-4 | 6.95 | dd | 8.0, 2.0 |
| 7.58 | d | 2.0 | H-7 | 7.40 | m | |
| 7.25 | d | 5.0 | H-3 | | | |
| 7.11 | dd | 8.6, 2.0 | H-5 | 8.00 | d | 8.0 |
| 6.30 | br s | | H-16 | 8.53* | br t | |
| 3.50 | s | | H$_2$-11 | 3.33 | s | |
| 3.20 | m | | H$_2$-15 | 3.00 | m | |
| 3.00 | m | | H$_2$-14 | 2.82 | m | |
| 2.10 | s | | H$_3$-18 | 1.90 | s | |

[a] (*) exchangeable.

Table II. $^{13}C$ NMR Data for Shermilamine A (1) (DMSO-$d_6$)

| carbon no. | chem shift, δ | mult | carbon no. | chem shift, δ | mult |
|---|---|---|---|---|---|
| 1a | *139.30 | s | 8a | 136.60 | s |
| 1b | 108.56 | s | 9 | §116.27 | s |
| 2 | 150.56 | d | 9a | +121.21 | s |
| 3 | 107.07 | d | 11 | 29.25 | t |
| 3a | +121.40 | s | 12 | 163.41 | s |
| 4a | 115.34 | s | 13a | 131.07 | s |
| 4 | 131.80 | d | 14 | 27.66 | t |
| 5 | 123.87 | d | 15 | 37.07 | t |
| 6 | §116.43 | s | 17 | 171.52 | s |
| 7 | 120.76 | d | 18 | 22.38 | q |
| 7a | *139.90 | s | | | |

[a] (*,+,§) values are interchangeable.

Table III. NOE Data of Shermilamine A (1)

| proton irradiated | proton enhanced |
|---|---|
| H-8 | H-7 (15), H$_2$-14 (4%) |
| H-13 | none |
| H-2 | H-3 (9%) |
| H-4 | H-3 (21%), H-5 (15%) |
| H-5 | H-4 (9%), H-7 (11%) |
| H$_2$-14 | H-8 (16%), H-16 (9%) |

formula, there appeared to be eight molecules in the unit cell. Eventually, the crystal composition was established as $C_{21}H_{17}BrN_4O_2S{\cdot}CHCl_3$.

All unique diffraction maxima with $2\theta < 114°$ were collected on a computer-controlled four-circle diffractometer using graphite monochromated Cu K$\bar{\alpha}$ radiation (1.541 78 Å) and variable-speed, 1° $\omega$-scans. After correction for Lorentz, polarization, and background effects, 2625 (76%) of the reflections were considered observed ($|F_o| > 3\sigma(F_o)$).[12]

A phasing model was quickly found in $C2/c$, and the eventual successful refinements indicate that this is the correct space group. The structure was refined in block diagonal least squares with anisotropic heavy atoms and isotropic hydrogens. The current crystallographic residual is 0.078 for the observed data. Additional crystallographic details can be found in the supplementary material.

A perspective drawing of the final X-ray model of compound 1 is given in Figure 1. Not included in that drawing is a chloroform of crystallization. Most of the molecule is planar within experimental error, but there is substantial puckering of the six-membered ring containing sulfur and nitrogen. The ring is roughly in a 2-fold conformation with 2-fold axis intersecting the N11–C12 and S8–C9 bonds. The torsional angle at the N11–C12 bond is −31°. The side-chain carbonyl is hydrogen bonded (H···O, 2.21 Å) back to the main planar array. The amide carbonyl (O26) has intermolecular hydrogen bonds to N11–H of 2.14 Å and N22–H of 1.98 Å.

## Experimental Section

**General Procedures.** Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 1420 spectrometer and ultraviolet spectra on a Hewlett-Packard Model 8452A diode array spectrophotometer. Mass spectra were measured on a VG-70SE instrument and NMR spectra on a Nicolet NT-300 instrument at 300 MHz ($^1H$) and 75 MHz ($^{13}C$), respectively. Solvents were freshly distilled before use.

**Isolation.** Animals were collected in November 1986, and September 1987, in Pago Bay, Guam. Frozen tunicates (1.15 kg) were thawed and extracted with MeOH. The resulting aqueous mixture was filtered and concentrated. The aqueous residue was consecutively partitioned against hexane, chloroform, and $n$-BuOH. Organic constituents were retained in the chloroform portion, which was purified on Sephadex LH-20 (chloroform/MeOH 1:1), yielding a bright red solid (900 mg, 0.08%), which was subjected to flash chromatography[8] on Florisil (chloroform/EtOH, 96:4), and then by HPLC (Partisil Magnum 10/25, chloroform/EtOH, 96:4), resulting in shermilamine A, orange plates from chloroform/MeOH, 96:4, mp 135 °C (60 mg, 0.005%).

IR (film from chloroform): $\nu_{max}$ 3290, 3265, 2980, 1640, 1630 (br, s), 1600 (br, s), 1580 (sh, m), 1550, 1450, 1420, 1350, 1310, 1190 (sh, s), 1120, 1070, 900, 840, 800, 740, 650 cm$^{-1}$.

UV (MeOH): $\lambda_{max}$ 240 (log $\epsilon$ 4.45), 284 (4.38), 350 (393), 390 (3.79), 468 nm (3.70). UV (MO$^+$H$_2$): $\lambda_{max}$ 244 (log $\epsilon$ 4.44), 286 (4.38), 3.82 (3.90), 536 nm (3.88).

HREIMS: $m/z$ 468.0260 ($C_{21}H_{17}{}^{79}BrN_4O_2S$ requires 468.0255), 390.1204 ($C_{21}H_{18}N_4O_2S$ requires 390.1259), 318.0645 ($C_{18}H_{12}N_3OS$ requires 318.0590). EIMS: $m/z$ 470 (13), 469 (3), 468 (11), 390 (4), 318 (3), 267, 212, 162.

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**Supplementary Material Available:** Tables of fractional coordinates, thermal parameters, interatomic distances, interatomic angles, and torsional angles for 1 (7 pages). Ordering information is given on any current masthead page.