Peloruside A: A Potent Cytotoxic Macrolide Isolated from the New Zealand Marine Sponge *Mycale* sp.

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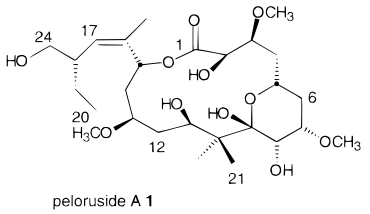
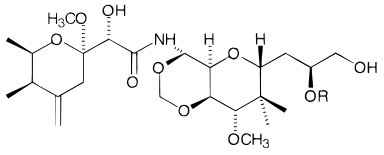
A novel, polyoxygenated, pyranose ring containing 16-membered macrolide peloruside A (**1**) exhibiting cytotoxic activity in the nanomolar range was isolated from the New Zealand marine sponge *Mycale* sp. The structure of **1** and relative stereochemistry of the 10 stereogenic centers were determined on a 3 mg sample using a variety of spectroscopic methods. Compound **1** was isolated along with the previously reported cytotoxins mycalamide A (**2**) and pateamine (**3**) from a single specimen of this sponge.

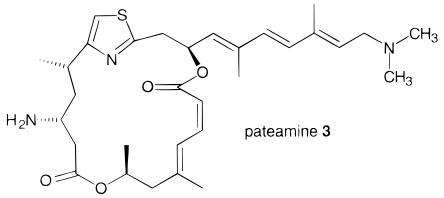
Marine sponges of the genus *Mycale* (*Carmia*) are a rich source of bioactive secondary metabolites of diverse structures. The mycalisines[1] and mycalolides[2−4] are examples isolated from members of this genus. In particular a *Mycale* species occurring in Otago Harbor on the southeast coast of the South Island of New Zealand was found to produce the antiviral and antitumor agents mycalamides A (**2**) and B (**4**).[5,6] In a subsequent study of a population of the same species collected in Thompson Sound, on the southwest coast of the South Island, the cytotoxic macrolide pateamine (**3**) was isolated.[7] The total synthesis of pateamine has been recently reported along with its interesting immunosuppressive properties.[8] No mycalamides were detected in the specimens collected from Thompson Sound. Recently we have examined specimens of the same species of *Mycale* collected from Pelorus Sound on the north coast of the South Island. NMR-guided isolation yielded the novel cytotoxic macrolide peloruside A (**1**) together with mycalamide A (**2**) and pateamine (**3**). Methanolic extracts of the sponge were fractionated on polymeric reversed-phase chromatographic material to yield 3.0 mg of peloruside A (**1**), 10.6 mg of mycalamide A (**2**), and 11.7 mg of pateamine (**3**). The previously reported compounds were identified by comparison of ^{1}H and ^{13}C NMR data to those published.



peloruside A **1**



R = H mycalamide A **2**
R = CH_3 mycalamide B **4**



pateamine **3**

The molecular formula of peloruside A was established as $C_{27}H_{48}O_{11}$ from the observation of a parent ion in the HRFABMS (571.308 26, $[M + Na]^+$ Δ −2.11 ppm) consistent with both the ^{13}C and ^{1}H NMR spectra. The presence of three oxymethyl groups (δ_H 3.48, δ_C 59.1; δ_H 3.38, δ_C 55.7; δ_H 3.31, δ_C 56.1), a saturated ester or lactone (δ_C 174.0; IR 1738 cm^{-1}), and a trisubstituted C−C double bond (δ_H 5.05, δ_C 131.1, δ_C 136.1) were evident from the IR and NMR spectra, suggesting the possibility of a highly oxygenated macrolide.

Cross-peaks in a COSY spectrum established the connectivity between two oxygenated methines, C-2 (δ_H 4.53, δ_C 70.3), C-3 (δ_H 4.22, δ_C 78.3), and a methylene, C-4 (δ_H 2.13, 1.78; δ_C 32.6). Similarly, COSY cross-peaks revealed a linear system comprised of an oxymethine, C-5 (δ_H 4.25, δ_C 63.5), a methylene, C-6 (δ_H 1.78, 1.53; δ_C 31.7), and two oxymethines, C-7 (δ_H 3.82, δ_C 75.9) and C-8 (δ_H 4.02, δ_C 66.8). Three oxymethines, C-11 (δ_H 4.89, δ_C 73.9), C-13 (δ_H 3.99, δ_C 77.9), and C-15 (δ_H 5.68, δ_C 70.9), together with two methylenes, C-12 (δ_H 2.07, 1.40; δ_C 33.9) and C-14 (δ_H 2.15, 2.02, δ_C 35.7), were assigned to a linear subunit from interpretation of COSY cross-

(1) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1985**, *26*, 3483−3486.
(2) Fusetani, N.; Yasumuro, K.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1989**, *30*, 2809−2812.
(3) Matsunaga, S.; Nogata, Y.; Fusetani, N. *J. Nat. Prod.* **1998**, *61*, 663−666.
(4) Matsunaga, S.; Sugawara, T.; Fusetani, N. *J. Nat. Prod.* **1998**, *61*, 1164−1167.
(5) Perry, N. B.; Blunt, J. W.; Munro, H. G. *J. Am. Chem. Soc.* **1988**, *110*, 4850−4851.
(6) Perry, N. B.; Blunt, J. W.; Munro, H. G.; Thompson, A. M. *J. Org. Chem.* **1990**, *55*, 223−227.
(7) Northcote, P. T.; Blunt, J. W.; Munro, H. G. *Tetrahedron Lett.* **1991**, *32*, 6411−6414.
(8) Romo, D.; Rzasa, R. M.; Shea, H. A.; Park, K.; Langenhan, S. L.; Akhiezer, A.; Liu, J. O. *J. Am. Chem. Soc.* **1998**, *120*, 12237−12254.

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peaks and a series of 1D TOCSY experiments. Connectivity between C-11 and C-12, and between C-14 and C-15, was evident from COSY cross-peaks, but spectral overlap of the C-12 and C-14 proton resonances made it difficult to establish connectivity at C-13. Selective irradiation of H-11 in a series of 1D TOCSY experiments sequentially revealed proton resonances assigned to the C-12 methylene, C-13 methine, and C-15 methine as the mixing time was increased from 20 to 80 ms. Similarly, selective irradiation of H-15 sequentially revealed the H-14 methylene pair, H-13, the upfield C-12 methylene resonance (δ_H 1.40), and H-11, clearly establishing the linear sequence of this subunit.

The remaining proton couplings evident in the COSY spectrum of **1** were assigned to a branched seven-carbon subunit. A linear arrangement of an oxymethylene, C-24 (δ_H 3.64, 3.36; δ_C 66.9), methine, C-18 (δ_H 2.61, δ_C 43.3), methylene C-19 (δ_H 1.44, 1.17; δ_C 24.6) and a primary methyl group, C-20 (δ_H 0.85, δ_C 12.2), was evident from vicinal couplings. The H-18 methine resonance also showed coupling to an olefinic proton resonance at C-17 (δ_H 5.05, δ_C 131.1) of the trisubstituted C−C double bond. Allylic coupling between this olefinic proton and the C-23 methyl proton resonance evident in the COSY spectrum and a ROESY correlation established a *cis* relationship across the double bond of these two substituents.

Long-range proton−carbon correlations from the oxymethyl protons to oxygenated methines at C-3, C-7, and C-13 observed in an HMBC experiment revealed the attachment points of the three oxymethyl groups (OCH_3-3, δ_H 3.31; OCH_3-7, δ_H 3.38; OCH_3-13, δ_H 3.48). Further HMBC correlations provided supportive evidence for the four subunits, and revealed their connectivity, along with the remaining carbonyl, ketal, or hemiketal carbon, two tertiary methyl groups, and a quaternary carbon, in a contiguous carbon chain (C-1 to C-20). Correlations were observed from the oxymethine protons at C-2 and C-3 to the carbonyl carbon C-1 (δ_C 174.0), establishing the unusual α,*β*-dioxyester moiety. Connection between C-4 and C-5 was revealed by correlations from the upfield H-6 methylene resonance (δ_H 1.53) to C-5 and C-4, and the downfield H-4 resonance (δ_H 2.13) to C-5 and C-6. Selective excitation of H-7 (δ_H 3.81) in a series of 1D TOCSY experiments with increasing mixing times sequentially revealed both C-6 methylene protons, H-5, and the downfield C-4 methylene proton (δ_H 2.13).

An HMBC correlation from the olefinic methyl protons of C-23 to the oxygenated methine C-15 established a link between C-15 and the substituted olefinic carbon (C-16). Weak COSY and 1D TOCSY correlations between H-17 and H-15 confirmed the allylic relationship of these two protons. Both aliphatic tertiary methyl groups (C-21, δ_H 1.08, δ_C 15.8; C-22 δ_H 1.12, δ_C 20.8) showed proton correlations to the remaining quaternary carbon (C-10, δ_C 43.6) and to each other's carbon resonance, which revealed the presence a *gem*-dimethyl moiety. Both *gem*-dimethyl proton resonances also correlated in the HMBC experiment to the C-9 ketal or hemiketal and the oxymethine carbon at C-11 (δ_C 73.9), establishing the C-9 to C-10 and C-10 to C-11 linkages. The final carbon−carbon connectivity between C-8 and C-9 was supported by an HMBC correlation observed between the H-8 and C-9 resonances.

With all carbon connectivities accounted for, the remaining two degrees of unsaturation of the molecular formula required a bioxycyclic structure. An HMBC correlation from H-15 to the carbonyl carbon C-1 established **1** as a 16-membered macrolide, leaving a single ether linkage to be accounted for. Addition of H_2O/D_2O, 1:1 (50 μL), to a sample of **1** dissolved in $CDCl_3$ (600 μL) caused observable splitting or broadening of the ^{13}C resonances of C-8, C-9, and C-11, indicating hydroxyl attachment at these centers.[9] A COSY correlation was observed at −10 °C from the oxygenated methine proton at C-2 to a proton resonance at δ 6.75 that lacked a correlation to carbon in the HSQC experiment, establishing the α-hydroxyester functionality at C-1 and C-2. Oxygens at C-5, C-9, and C-24 remained as possible sites for an ether linkage. Chemical reasoning suggested a link between C-5 and C-9 to form a stable six-membered cyclic hemiketal ring. An analysis of $^1H-^1H$ coupling constants and NOE enhancements observed for protons attached to carbons C-5 through C-8 provided strong evidence for a six-membered ring in a chair conformation. An HMBC correlation from H-5 to C-9 through the ether linkage was not observed in three separate experiments optimized for $^nJ_{CH}$ coupling constants of 4, 6, and 9 Hz. The lack of HMBC correlations across a pyran(ose) ring has been reported and rationalized as caused by an unfavorable dihedral angle.[9]

Chemical shift differences of methylene protons and the presence of both large and small $^1H-^1H$ coupling constants at centers throughout the molecule which did not change with temperature provided evidence of a single predominant conformer.[10] This suggested that a spectroscopic assignment of relative stereochemistry would be possible from a combination of NOE and vicinal coupling.[11] NOE correlations were detected with ROESY and selective 1D GOESY[12] experiments. Approximate $^1H-^1H$ coupling constants were determined for all but five of the nonexchangeable proton resonances from an analysis of ^1H and HOMO2DJ experiments at 500 and 300 MHz, and 1D TOCSY, 1D GOESY, and homonuclear decoupling at 300 MHz.

The resonance of one of the methylene protons attached to C-6 appeared as a quartet of 12 Hz attributed to one geminal and two large vicinal couplings. H-5, H-6a, and H-7 could be assigned as axial on the basis of these H-6a vicinal couplings of greater than 10 Hz, placing H-5 and H-7 on the β side of the six-membered ring. NOEs observed between H-5 and H-7 confirmed their 1−3 diaxial arrangement. The oxygenated methine proton (H-8) was assigned as equatorial on the basis of its small coupling to H-7 (3 Hz) and the observation of a W coupling to the equatorial methylene proton at C-6 (δ_H 1.78) in a COSY experiment optimized for long-range coupling.

An analysis of coupling constants and NOE enhancements revealed that C-3 and C-4 form an extended zigzag chain with carbons C-5 to C-7 of the six-membered ring (Figure 1). A large vicinal coupling (11.5 Hz) between H-5 and H-4b established an antiperiplanar arrangement of these two protons consistent with the observation of an NOE enhancement observed between H-6a and H-4b. A smaller coupling (4.5 Hz) and the presence of an NOE

(9) Bauer, I.; Maranda, L.; Young, K. A.; Shimizu, Y. *J. Org. Chem.* **1995**, *60*, 1084−1086.
(10) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512−523.
(11) Eberstadt, M.; Gemmecker, G.; Mierke, D. F.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1671−1695.
(12) Stonehouse, J.; Adell, P.; Keeler, J.; Shaka, A. J. *J. Am. Chem. Soc.* **1994**, *116*, 6037−6038.

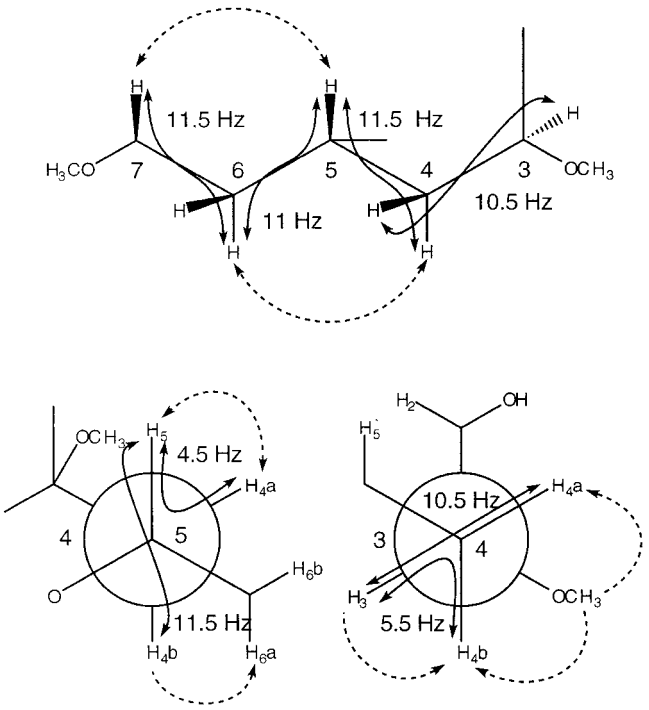

Figure 1. Relative stereochemistry of C-3 to C-7 of peloruside A (**1**). Important NOEs are illustrated with dashed arrows and vicinal couplings with solid arrows. Coupling constants are given in hertz.

enhancement between H-5 and H-4a established a *syn*[13] relationship between them. Similarly, a large vicinal coupling (10.5 Hz) between H-3 and H-4a, a smaller coupling (5.5 Hz) between H-3 and H-4b, and NOE enhancements (H-3 to H-4b, H-4a to C-3OCH_3, H-4b to C-3OCH_3) established antiperiplanar relationships of H-3 and H-4a, and H-4b and C-2. These correlations assign the relative stereochemistry of C-5 to C-3 as drawn in Figure 1 (1−3 *syn* oxygen substitution at C-5 and C-3 with the C-2 to C-6 chain in an extended zigzag conformation).

The hydroxyl group attached to C-9 (Figure 2) can be assigned as axial above the six-membered ring on the basis of an NOE correlation from H-3 to CH_3-22 on the underside of both rings as drawn in Figure 3. The placement of C-10 is consistent with the anomeric effect, the placement of the bulky *gem*-dimethyl group equatorial upon ring closure and the observation of NOE enhancements from the equatorial H-8 to both CH_3-21 and CH_3-22. These NOE correlations also assign CH_3-22 above and CH_3-21 below the macrocyclic ring. An NOE correlation between CH_3-22 and H-11 places H-11 on the underside of the macrocycle with the C-11 hydroxyl group *syn* to the hydroxyl on C-9. This stereochemical assignment is confirmed by an NOE correlation observed between H-3 and H-11 across the underside of the macrocyclic ring. Large (11 Hz) and small (<1 Hz) vicinal couplings were observed between H-11 and the downfield (H-12b, δ 2.07) and upfield (H-12a, δ 1.40) resonances, respectively, assigned to the C-12 methylene. These couplings, along with NOE correlations observed from the geminal methyls to these protons (CH_3-21 to H-12b, CH_3-22 to H-12a), revealed an extended zigzag conformation of the macrolide ring from C-9 to C-13 with H-12b above and H-12a below the plane and extending away from the center. Small vicinal couplings (4.5 Hz, <1 Hz) and strong NOE enhancements observed from H-13 to H-12a and H-12b indicate a turn in the macrolide skeleton at C-13, with H-13 bisecting H-12a and H-12b. The oxymethyl group at C-13 could be assigned *syn* to the C-11 hydroxyl on the β side of the macrolide ring on the basis of an NOE observed from this oxymethyl group to H-12b. Once again an analysis of vicinal couplings and NOE correlations revealed that H-13 bisects H-14a and H-14b. A large coupling to H-14b (9.5 Hz) and a small coupling to H-14a (<1 Hz) from H-13 and an NOE observed to H-14b indicate a near 90° angle between H-13 and H-14a and a near 0° angle between H-13 and H-14b, assigning the slightly downfield resonance (H-14b, δ 2.15) to the β side of the macrocycle. This assignment was confirmed by the observation of an NOE between H-14a and H-12a on the α side of the macrocycle. A large vicinal coupling between H-14a and H-15 and an NOE observed between OCH_3-13 and H-15 place the C-16 to C-20 side chain α with H-15 *trans* diaxial to H-14a.

The relative stereochemistry of C-2 could not be determined directly from correlations to C-3 or C-4. A small coupling constant of less than 1 Hz between H-2 and H-3 together with NOE correlations (H-2 to H-3, H-2 to H-5, and H2 to H11) indicates that H-2 is on the inside of the macrocycle. These observations are consistent with both possible epimers at C-2. An NOE observed between CH_3-23 and H-3, however, is only consistent with the relative stereochemistry at C-2 as drawn. The other possible isomer would place the C-2 hydroxyl directly between CH_3-23 and H-3.

An examination of proton couplings and NOE enhancements observed on the C-16 to C-20 side revealed that rotation is restricted in solution. In particular, the bonds C-17−C-18 and C-18−C-24 appear to be fixed. A large coupling (10 Hz) between H-18 and H-17 and an NOE enhancement (H-24a to H-18) clearly place H-17 and H-18 antiperiplanar. Similarly the pattern of large (H-24b−H-18, 10.5 Hz) and small (H-24a−H-18, 4 Hz) couplings indicates restricted rotation. Observation of NOE enhacements between H-18 and H-19a, H-19b, and CH_3-20, however, indicates some flexibility of the ethyl moiety.

Observations of NOE enhancements (H-18 to H-15, CH_3-23 to H-3) establish the coplanarity of H-15, H-18, and CH_3-23. An NOE correlation observed between CH_3-20 and H-14a indicates that the ethyl side chain sits below the plane of the macrolide ring. These observations lead to the assignment of the stereochemistry at C-18 as *S**. On the basis of the above arguements, we propose the relative stereochemistry of peloruside (**1**) as 2*R**,-3*S**,5*S**,7*S**,8*S**,9*S**,11*R**,13*R**,15*R**,16*Z*, 18*S**. All NOE correlations observed in the ROESY spectrum and a series of GOESY spectra are consistent with this configurational assignment in a single conformation.

Peloruside A (**1**) was found to be cytotoxic to P388 murine leukemia cells at approximately 10 ng/mL (18 nM). Although it bears some structural features of both mycalamides (*gem*-dimethyls and polyhydroxylation) and pateamine (macrolide ring), it does not appear to be closely related biochemically. Interestingly, while all sponge individuals tested revealed consistent levels of mycalamide A, and varying amounts of pateamine, only

(13) Masamune, S.; Ali, Sk. A.; Snitman, D. L.; Garvey, D. S. *Angew. Chem., Int. Ed., Engl.* **1980**, *19*, 557.

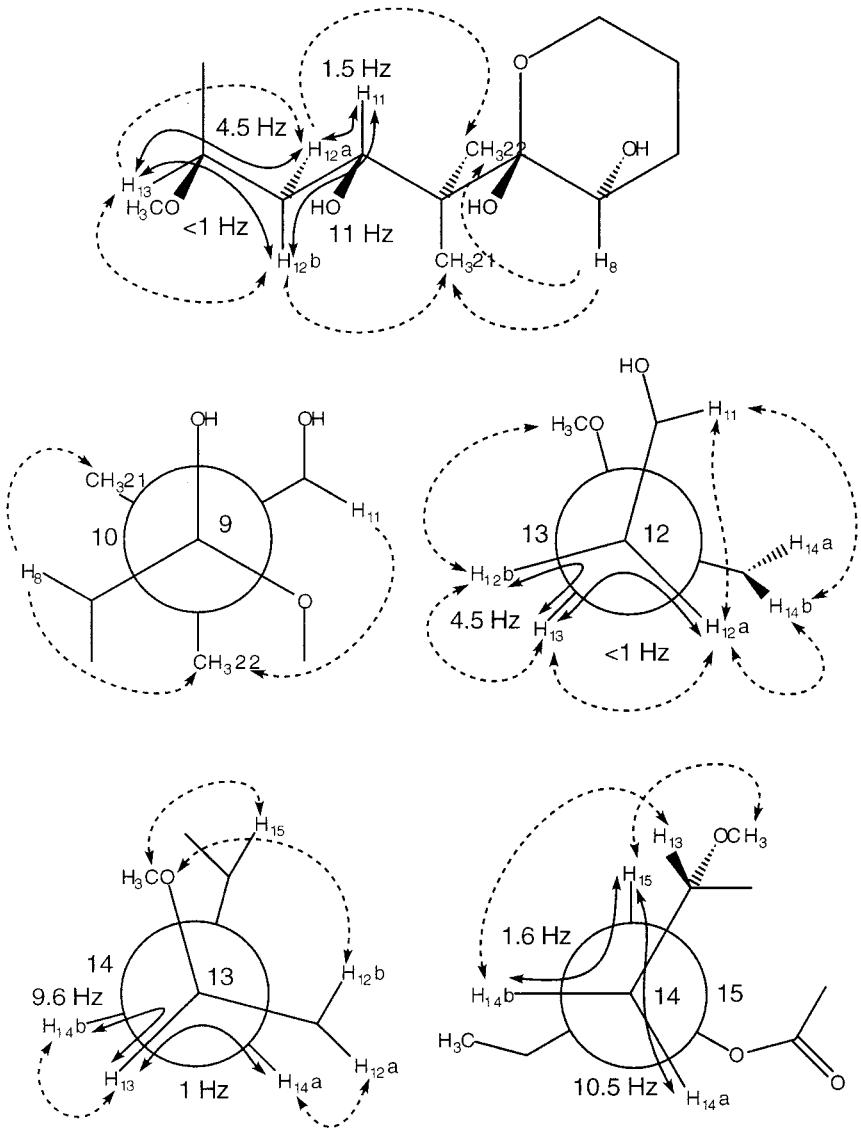

Figure 2. Relative stereochemistry of C-8 to C-15 of peloruside A (**1**). Important NOEs are illustrated with dashed arrows and vicinal couplings with solid arrows. Coupling constants are given in hertz.

specimens collected at the deeper range of the population contained detectable amounts of peloruside A.

Experimental Section

NMR Experiments. ^{1}H and ^{13}C NMR spectra were measured with 300 and 500 MHz spectrometers. All chemical shifts (δ) are referenced to the solvent peak ($CDCl_3$: ^{1}H, δ 7.25 ppm; ^{13}C, δ 77.0 ppm).[14] Short- and long-range ^{1}H−^{13}C correlations were determined with gradient-enhanced inverse-detected HSQC and HMBC experiments. 1D TOCSY experiments were performed with shaped pulse-selective excitation and spin lock mixing times of 15−80 ms. NOE enhancements were detected with a GOESY experiment with Gaussian-selective excitation and a 0.5 s mixing time.

Collection, Extraction, and Isolation. Sponge specimens were collected by SCUBA in Pelorus Sound, South Island, New Zealand, at depths of 7−15 m. A single frozen specimen (170 g wet weight, NIWA no. 95DBMYC 2-6) was cut into small segments and extracted with methanol (2 × 600 mL) for 24 h. The second and first methanolic extracts were passed through a glass column packed with 75 mL of Diaion HP20 poly-

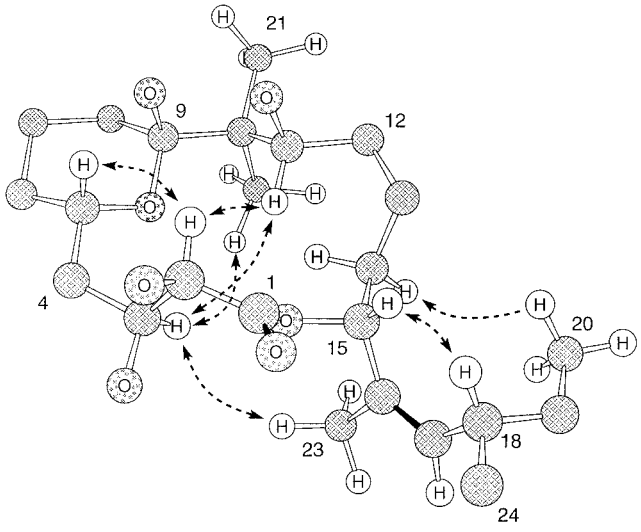

Figure 3. Probable conformation of the carbon skeleton of peloruside A (**1**). Selected NOEs are illustrated with dashed arrows.

(14) Gottieb, H. E.; Kotlar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512.

Table 1. ^{1}H and ^{13}C NMR Assignments of 1 in $CDCl_3$

	^{13}C		^{1}H	
position	δ (ppm)	mult	δ (ppm)	mult, J (Hz)
1	174.0	s		
2	70.3	d	4.53	s
3	78.3	d	4.22	dd (10.5, 5.5)
4a	32.6	t	1.78	m
4b			2.13	m
5	63.5	d	4.25	tdd (11, 4.5, 2.5)
6a	31.7	t	1.53	q (12)
6b			1.78	ddd (12.5, 5.5, 2.5)
7	75.9	d	3.82	ddd (11.5, 5, 3)
8	66.8	d	4.02	d (3)
9	101.9	s		
10	43.6	s		
11	73.9	d	4.89	br d (10)
12a	33.9	t	1.40	d (14.5)
12b			2.07	ddd (15, 11.5, 4.5)
13	77.9	d	3.99	br d (9.5)
14a	35.7	t	2.02	ddd (15.5, 11.5, 1)
14b			2.15	ddd (15.5, 10, 1.5)
15	70.9	d	5.68	d (10.5)
16	136.1	s		
17	131.1	d	5.05	d (10)
18	43.3	d	2.61	m
19a	24.6	t	1.17	m
19b			1.44	m
20	12.2	q	0.85[a]	t (7.5)
21	15.8	q	1.08[a]	s
22	20.8	q	1.12[a]	s
23	17.5	q	1.67[a]	d (1)
24a	66.9	t	3.36	t (10.5)
24b			3.64	dd (10.5, 4)
3OCH_3	56.1	q	3.31[a]	s
7OCH_3	55.7	q	3.38[a]	s
13OCH_3	59.1	q	3.48[a]	s
2OH			6.75	s

[a] 3H.

(styrene−divinylbenzene) beads preequilibrated with methanol. The eluents were combined and passed through the same column. The resulting eluent was diluted with water (100 mL) and passed through the column. Finally the resulting eluent was diluted with water (2.8 L) and passed back through the same column. The column was then washed with water (100 mL) and eluted with 150 mL fractions of (1) 20% acetone/water, (2) 55% acetone/water, (3) 55% acetone/0.2 M NH_4OH, and (4) 55% acetone/0.2 M NH_4OH adjusted to pH 4.0 with AcOH. Fraction 2 was diluted with water (250 mL) and passed through a glass column packed with 35 mL of HP20 preequilibrated with water. The column was washed with water (50 mL) and eluted with 100 mL of acetone. The acetone eluent was concentrated to dryness to yield 78.8 mg of a viscous brown oil. The resulting oil was dissolved in methanol (25 mL) and passed through a small glass column containing 250 mg of TosoHass Amberchrom. The column eluent was diluted with water (100 mL) and passed back through the column. The column was washed with water (20 mL), and the Amberchrom was transferred on top of a 20 × 1.5 cm Amberchrom column preequilibrated with water. The column was eluted with increasing concentrations of acetone in water in a stepped gradient fashion. The 32−34% acetone/water fractions were concentrated to dryness to yield **1** as a colorless oil (3.0 mg). The 38−40% acetone/water fractions were concentrated to dryness to yield 10.6 mg of mycalamide A (**2**). The fourth fraction eluted from the original HP20 column at pH 4.0 was diluted with 150 mL of water, adjusted to pH 7.0 with aqueous NH_3, and passed through a glass column packed with 30 mL of HP20 preequilibrated with water. The column was washed with water (50 mL) and eluted with 100 mL of acetone. The acetone eluent was concentrated to dryness to yield 38 mg of a yellow oil. The oil was dissolved in methanol (10 mL) and passed through 2.5 mL of amino-bonded phase packing material. The eluent was concentrated to dryness to yield 11.7 mg of pateamine (**3**).

Peloruside A (1): a colorless oil; $[\alpha]^{20}_D$ +16°(*c* 0.30 CH_2Cl_2); IR (KBr) ν_{max} 3336, 2919, 2849, 1738, 1667, 1462, 1385, 1225, 1155, 1082 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); HRFABMS *m*/*z* 571.308 26 (calcd for $C_{27}H_{48}O_{11}$ (M + Na)$^+$, 571.309 46).

Supporting Information Available: NMR spectra of **1** including ^{1}H, ^{13}C, DEPT, COSY, ROESY, HSQC, HMBC, and selected 1D TOCSY and GOESY. This material is available free of charge via the Internet at http://pubs.acs.org.

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