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Palmerolide A, a Cytotoxic Macrolide from the Antarctic Tunicate *Synoicum adareanum*

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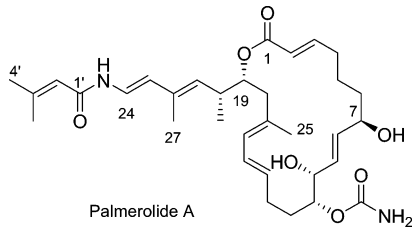
Antarctica is among the least accessible of marine habitats but supports a thriving community of invertebrates and algae, organisms widely recognized as potential sources of new chemical diversity. The Antarctic Polar Front has effectively isolated the Antarctic marine ecosystem for more than 20 million years. This isolation has led to a fauna which is largely indigenous and an ecology that reflects a rich, cold-adapted, biodiversity characterized by an unusual invertebrate-predator-dominated trophic hierarchy.[1] Antarctic invertebrates are of particular interest in the search for chemical diversity since defensive strategies evolved by invertebrate fauna in response to invertebrate predators may differ significantly from those evolved for vertebrate predators.[2] We have studied *Synoicum adareanum*, a circumpolar tunicate found commonly in the shallow waters around Anvers Island (64° 46′ S, 64° 03′ W) on the Antarctic Peninsula,[3] and report herein a new enamide-bearing polyketide, palmerolide A.[4] Palmerolide A targets melanoma (e.g., UACC-62, LC_{50} = 18 nM) in the National Cancer Institute's (NCI) 60 cell line panel with 3 orders of magnitude greater sensitivity relative to other cell lines tested.



Palmerolide A

Palmerolide A was isolated as a white amorphous solid from the 1:1 methanol/ethyl acetate fraction eluting from silica gel chromatography of the crude 1:1 methanol/dichloromethane extract. Mass spectrometric analysis provided a molecular formula of $C_{33}H_{48}N_2O_7$ (HRFABMS *m*/*z* 585.3539, Δ 0.1 mmu for $[M + H]^+$). The C-1 ester carbonyl of palmerolide A was found to be conjugated to an *E*-olefin (C-2/C-3, J_{HH} = 15.2 Hz) based on correlations in the gHMBC spectrum (Table 1) from both H-2 and H-3 to C-1. Three methylene carbons (C-4 to C-6, δ_C 32.6, 25.7, and 38.5) could be shown to intervene between the C-2/C-3 olefin and a hydroxymethine at δ 74.5 (C-7) based on gCOSY and gHMBC data. The next structural feature, an *E*-disubstituted olefin (J = 15.5 Hz), could be placed between the C-7 hydroxymethine and another at δ 69.9 (C-10). While H-10 (δ 4.15) showed no gHMBC correlations, H-8, H-9, and H-11 each displayed connectivity to C-10. H-11 could be further extended to C-12/C-13, which are coincident at δ_C 30.1, as well as to an ester-type carbonyl (OCOX) which displayed no further connectivity. In the gHMBC spectrum, H-13 coupled to C-14 and C-15. The C-14/C-15 *E*-olefin (J = 14.6 Hz) was shown to be conjugated to a trisubstituted olefin by gHMBC correlations of H-14, H-15, H-18, and H-19, as well as H_3-25 to C-16 and/or C-17. The C-16/C-17 olefin must be *E* based on NOE data which demonstrated the proximity (Figure 1) of H_3-25 to H-15. Further, the protons of a methylene group (C-18) correlated to C-16 and C-17 and to an oxygen-bearing methine (C-19, δ 75.8). The 20-membered macrocycle was completed based on gHMBC coupling between H-19 and the C-1 (δ 166.1) ester carbonyl.

Pendant on the macrocycle is the C-19 side chain. The H-20 multiplet, correlating to C-19 (gHMBC), was shown by gCOSY to couple to a methyl group (C-26, δ_H 0.90) and to a conjugated diene based on H-19 and H-20 gHMBC correlations to olefinic C-21 (δ_C 130.5). Both the C-21/C-22 and the C-23/C-24 olefins

Table 1. NMR Data for Palmerolide A (DMSO-d_6, 500 MHz)

position		δ ^{1}H (ppm, mult, J (Hz))	δ ^{13}C	gHMBC
1			166.1	
2		5.78 (1H, d, 15.2)	121.3	1, 4
3		6.72 (1H, ddd, 5.0, 9.9, 15.2)	150.0	1, 2, 4, 5
4		2.11 (2H, m)	32.6	2, 3, 5, 6
5	a	1.30 (1H, m)	25.7	7
	b	1.05 (1H, m)		6
6	a	1.50 (1H, ddd, 4.5, 8.2, 11.2)	38.5	5, 7, 8
	b	1.30 (1H, m)		5, 7, 8
7		3.83 (1H, ddd, 4.4, 7.4, 7.6)	74.5	5, 9
8		5.55 (1H, dd, 7.7, 15.5)	134.3	6, 7, 9, 10
9		5.50 (1H, dd, 2.9, 15.5)	129.6	7, 8, 10
10		4.15 (1H, br s)	69.9	
11		4.49 (1H, ddd, 2.2, 5.0, 10.5)	73.2	9, 10, 12/13, *C*ONH$_2$
12	a	1.59 (1H, m)	30.1	12/13
	b	0.98 (1H, m)		11, 12/13
13		1.96 (2H, m)	30.1	12/13, 14, 15
14		5.42 (1H, ddd, 4.7, 10.1, 14.6)	132.7	12/13, 16
15		6.05 (1H, dd, 11.1, 14.6)	128.4	12/13, 16, 17
16		5.60 (1H, d, 11.4)	127.1	14, 15, 18, 25
17			132.3	
18	a	2.17 (1H, dd, 1.3, 13.2)	43.9	16, 17, 19, 25
	b	2.00 (1H, dd, 11.2, 13.2)		16, 17, 19, 20, 25
19		4.85 (1H, ddd, 1.3, 7.4, 11.2)	75.8	1, 17, 18, 20, 21, 26
20		2.69 (1H, qdd, 6.5, 7.4, 9.6)	37.3	18, 19, 21, 22, 26
21		5.14 (1H, d, 9.6)	130.5	19, 20, 23, 26, 25
22			133.3	
23		5.85 (1H, d, 14.2)	117.2	21, 22, 24, 27
24		6.86 (1H, dd, 10.1, 14.2)	122.9	22, 23, 1′
25		1.60 (3H, s)	16.9	16, 17, 18
26		0.90 (3H, d, 6.5)	17.7	19, 20, 21
27		1.71 (3H, s)	13.3	21, 22, 23
1′			163.9	
2′		5.70 (1H, br s, 1.0)	118.8	1′, 3′, 4′, 5′
3′			152.5	
4′		1.83 (3H, s)	27.7	1′, 2′, 3′, 5′
5′		2.13 (3H, s)	20.4	1′, 2′, 3′, 4′
*C*ONH$_2$		6.49 (2H, br)	157.3	
24-N*H*		9.84 (1H, d, 10.1)		23, 24, 1′
10-O*H*		5.18 (1H, d, 4.9)		9, 10, 11
7-O*H*		4.69 (1H, d, 3.9)		6, 7, 8

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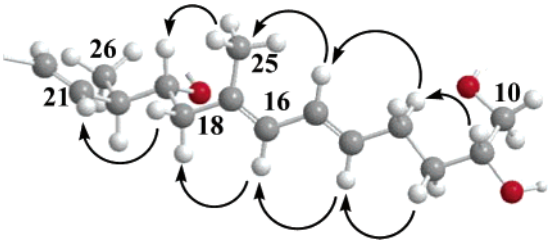
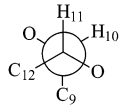
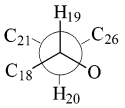


Figure 1. Selected NOE correlations relating the relative stereochemistry between C-11 and C-19. The conformation of C-11 is constrained (see Table 2), enabling the configuration of C-19 to be defined from NOE enhancements. Note that the requirement that C-10 and C-19 complete the macrocycle precludes the C-19/C-20 diastereomer.

Table 2. Parameters from Conformational Analysis[a]

fragment		$^3J_{HH}$	selected $^2J_{CH}$	NOE
C-10/C-11		2.2	C-11/H-10 = -5.5 C-10/H-11 = -4.2	H-10 to H-11 H-9 to H-12a
C-19/C-20		7.4		H-18a to H-21 H-18b to H-21

[a] $^2J_{CH}$ determined by HETLOC.[8]

were assigned as *E*, based, in the former case, on a ROESY correlation between H_3-27 and H-20 and, in the latter case, based on vicinal couplings ($J = 14.2$ Hz). Connectivity of the C-23/C-24 olefin was established based on gHMBC correlations of H-23 to C-21, C-22, C-24, and C-27. C-24 marked the terminus of the contiguous carbon chain and could be shown to bear an −NH group due to gHMBC correlations of an amide proton at δ 9.84 to carbons C-23, C-24, and the amide carbonyl, C-1′ (δ 163.9).

The isopentenoyl substructure C-1′ to C-5′ was assigned based on gHMBC correlations of the methyl protons (H_3-4′, δ 1.83, H_3-5′, δ 2.13) to the two vinyl carbons at δ 118.8 (C-2′) and 152.5 (C-3′) as well as correlation of the vinyl proton at δ 5.70 to the amide carbonyl (δ 163.9).

The connectivity described above established the full planar structure of palmerolide A with the exception of a single open valence on the ester carbonyl attached to the macrolide at C-11. Remaining to be accounted from the molecular formula is $-NH_2$, establishing a carbamate functionality at C-11.

(*R*)- and (*S*)-MTPA esters[6] of palmerolide A demonstrated both C-7 and C-10 to bear the *R* configuration (see Supporting Information). Conformational analysis[7] of the C-10/C-11 fragment (Table 2) identified a gauche relationship between H-10 and H-11, based on the small $^3J_{HH}$ and large $^2J_{CH}$ for both the H-10/C-11 and the H-11/C-10 relationships, defining the absolute stereochemistry of C-11 as *R*.

Conformational analysis of the C-19/C-20 system suggested an anti relationship of the respective protons, based on the large $^3J_{HH}$, which precludes further *J*-based analysis since two diastereomers exist with anti-disposed protons and the same expected $^2J_{CH}$ and $^3J_{CH}$ coupling relationships.[7] The relative configuration of this fragment was therefore secured by NOE difference spectroscopy. Irradiation of either H-18a or H-18b resulted in enhancement of H-21, while neither enhanced H_3-26, supporting the relative configuration 19*R**, 20*R** (Table 2).

The four olefins in the macrocycle constrain the flexibility often found in macrolides, facilitating stereochemical analysis by NOE studies. Further analysis of the NOE data revealed the macrolide to adopt two largely planar sides of a tear-drop-shaped cycle, one side consisting of C-1 through C-6, the other C-11 through C-19, with C-7 through C-10 providing a curvilinear connection. In particular, H-19, H_3-25, H-15, and H_2-13 (Figure 1) are sequentially correlated in the ROESY spectrum, as are H-21, H_2-18, H-16, H-14, and H-12, defining the periphery of the top and bottom face of the western hemisphere. H-11 correlates only to the top series of protons, a result consistent only with C-19 adopting the *R* configuration. The absolute stereochemistry of the C-19/C-20 fragment is therefore 19*R*, 20*R*.

Palmerolide A is stable for extended time periods and does not readily yield a single hydrolysis product. Repeated attempts at crystallization were unsuccessful.

Besides melanoma, one colon cancer cell line (HCC-2998, $LC_{50} = 6.5$ μM) and one renal cancer cell line (RXF 393, $LC_{50} = 6.5$ μM), palmerolide A was largely devoid of cytotoxicity ($LC_{50} > 10$ μM), representing a selectivity index among tested cell lines of 10^3 for the most sensitive cells. Palmerolide A displayed a 60 cell line activity profile which correlated, using the NCI COMPARE algorithm, to several vacuolar ATPase inhibitors.[9] In ongoing studies, we have found palmerolide A to inhibit V-ATPase with an IC_{50} of 2 nM and to be active in NCI's hollow-fiber assay (http://dtp.nci.nih.gov/branches/btb/hfa.html), details of which will be published elsewhere.

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Supporting Information Available: ^{1}H, ^{13}C, DEPT135, gCOSY, gHMQC, gHMBC, ROESY, selected NOE and HETLOC expansions, and NMR spectra for palmerolide A; experimental data on MTPA esters. This material is available free of charge via the Internet at http://pubs.acs.org.

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(3) The tunicate was identified as *Synoicum adareanum* (Herdman 1902; family Polyclinidae) by Dr. Linda Cole, Smithsonian Institution, Washington, DC. A voucher specimen is held at USF.
(4) The National Science Foundation's facility on Anvers Island, Palmer Station, is named for the American sealer Nathaniel B. Palmer, considered to be among the first to observe the continent of Antarctica.
(5) White solid, 0.01% of dry mass; $[\alpha]_{24}^{D}$ −1.6 (*c* 0.5, MeOH); IR (thin film) cm^{-1}: 3360 (br), 2925, 2856, 1696, 1633, 1517, 1392, 1275, 1190 and 1080. UV (MeOH) λ_{max} (ϵ): 224 (2670), 242 (2800), 296 (1775); ^{1}H and ^{13}C NMR see Table 1; HRFABMS *m/z* 585.3539 ($C_{33}H_{49}N_2O_7$ requires 585.3540).
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