Plakoridine A, a New Tyramine-Containing Pyrrolidine Alkaloid from the Okinawan Marine Sponge *Plakortis* sp.

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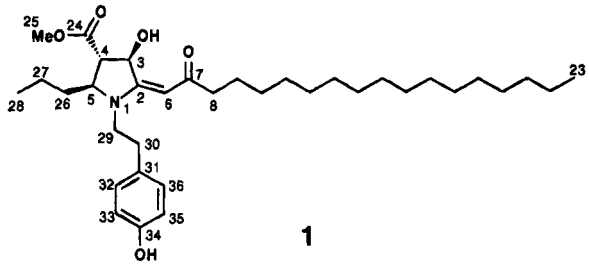
Marine sponges of the genus *Plakortis* have afforded a variety of unique peroxy aliphatic acids and esters.[1] During our studies on bioactive substances from Okinawan marine organisms,[2] we have investigated extracts of *Plakortis* sponges and have isolated several unique secondary metabolites (*e.g.*, plakotenin,[3] manzamenones,[4] and untenone A[5]). We proposed biogenetic pathways for these compounds involving intra-[3] and intermolecular[4,5] cycloaddition processes. Further examination of the constituents of the same *Plakortis* sponge has now led to the isolation of plakoridine A (**1**), a novel tyramine-containing pyrrolidine alkaloid, whose nitrogenated carbon framework is hitherto unknown. This paper describes the isolation and structure elucidation of **1**.



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The sponge *Plakortis* sp., collected at Unten Harbor, Okinawa, was extracted with methanol and partitioned between ethyl acetate and water. The ethyl acetate-soluble fraction was fractionated by chromatography on silica gel (hexane/EtOAc), followed by Sephadex LH-20 (MeOH). Further purification by preparative TLC (hexane/EtOAc, 1:1) followed by reversed-phase HPLC (ODS; $CH_3CN/CHCl_3$, 6:4) yielded plakoridine A (**1**, 0.0005% yield based on wet weight).

Plakoridine A (**1**) had a molecular formula of $C_{35}H_{57}O_5N$ as revealed by HRFABMS [m/z 572.4337, $(M + H)^+$, Δ +2.2 mmu]. The ^1H and ^{13}C NMR spectra of **1** (Table 1) suggested the presence of a ketone, a methyl ester, a trisubstituted double bond, a para-substituted phenol, three sp^3 methines, two terminal methyls, and many sp^3 methylene groups. Since seven out of the eight unsaturations were thus characterized, compound **1** was inferred to be monocyclic except for the phenol moiety. Extensive 2D NMR experiments ($^1H-^1H$ COSY, HSQC,[6] HMBC,[7] and NOESY) were carried out in three solvents ($CDCl_3$, C_6D_6, and CD_3OD), and it was revealed that the phenol moiety was attached to two sp^3 methylenes (HMBC correlations: H_2-30/C-32(36) and H_2-29/C-31), and the ^1H and ^{13}C chemical shifts of the C-29 methylene (δ_H 3.41 and 3.30; δ_C 46.1) implied that this carbon was connected to a nitrogen atom, thus giving rise to a tyramine unit. The $^1H-^1H$ COSY spectrum of **1** showed the proton connectivities from H-3 to H-5, and the H-5 was further coupled to an *n*-propyl group (H_2-26–H_3-28). A hydroxyl group was present on C-3 [COSY correlation: H-3/OH-3 (D_2O-exchangeable)], and H-3 also showed allylic coupling with H-6. The HMBC spectrum revealed $^1H-^{13}C$ long-range correlations suggesting that a carbomethoxy group was attached to C-4 (C-24/H-3, H-4, H-5, and H_3-25). The ^{13}C NMR signals resonating at δ_C 166.6 (C-2), 90.2 (C-6), and 199.9 (C-7) were assignable to a β-amino-substituted enone functionality,[8,9] and the HMBC correlations from H-6 to C-2, C-3, and C-7 suggested that the enone moiety was connected to C-3. The HMBC spectrum showed crosspeaks for H_2-29/C-5 and H_2-29/C-2, thus indicating linkage of C-2 and C-5 to the tyramine nitrogen atom to form a pyrrolidine ring. In the HMBC spectrum, C-7 showed crosspeaks with H_2-8 and H_2-9, the latter of which in turn showed COSY correlations to the sp^3 methylene envelope (δ_H 1.2–1.3). Thus, an unbranched aliphatic chain ($C_{16}H_{33}$) was connected to ketone carbon (C-7). The

Table 1. ^1H and ^{13}C NMR Data of Plakoridine A(1) in $CDCl_3$

position	δ_H	J(Hz)	δ_C	HMBC (^1H)[a]
2			165.7 s	H-6, H_2-29
3	5.20 d	5.5	75.9 d	H-4, H-6
3-OH	6.90 br s			
4	2.89 dd	5.9, 5.5	52.2 d	H-3, H_2-26
5	3.70 m		65.3 d	H-4, H_2-26, H_2-27, H_2-29
6	5.08 s		90.2 d	
7			199.9 s	H-6, H_2-8, H_2-9
8	2.35 m (2H)		43.5 t	H_2-9
9	1.69 m (2H)		26.3 t	H_2-8
10–21	1.2–1.3 br s		~29 t	
22	1.27 m (2H)		22.7 t	H_3-23
23	0.88 t (3H)	6.7	14.1 q	
24			172.8 s	H-3, H-4, H-5, H_3-25
25	3.74 s (3H)		52.6 q	
26	1.70 m 1.50 m		35.3 t	H-4, H_2-27, H_3-28
27	1.30 m (2H)		17.7 t	H_2-26
28	0.92 t (3H)	7.0	14.0 q	H_2-26, H_2-27
29	3.41 m 3.30 m		46.1 t	H_2-30
30	2.83 m 2.76 m		31.2 t	H_2-29
31			129.9 s	H_2-29
32,36	7.03 d (2H)	8.5	129.8 d	H_2-30, H-33(35)
33,35	6.79 d (2H)	8.5	115.7 d	H-32(36)
34			154.7 s	H-32(36), H-33(35)

[a] The HMBC experiment was optimized for $^nJ_{CH} = 8$ Hz in C_6D_6 with the F1 width (^{13}C NMR axis) of 110.433 ppm, which was about half of the conventional width to enhance the digital resolution, giving a spectrum with folded signals.

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(9) The UV absorption maximum of **1** (λ_{max} 317 nm) is characteristic of the presence of a β-amino substituted enone: Scott, A. I. *Interpretation of the Ultraviolet Spectra of Natural Products*; Pergamon Press: Oxford, 1964; p 58.

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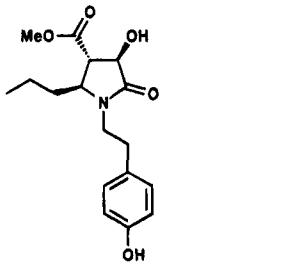
FABMS and EIMS data for **1** and its diacetate afforded several fragment ion peaks indicating the presence of a heptadecanoyl ($COC_{16}H_{33}$) group.[10] In order to further verify the length of the aliphatic chain, compound **1** was treated with ozone and subsequently reduced with dimethyl sulfide to give a lactam (**2**) and heptadecanoic acid



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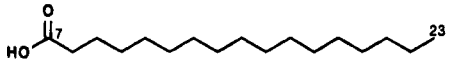

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(**3**), which were detected by EIMS analysis [**2**, *m/z* 321 (M^+); **3**, *m/z* 270 (M^+)]. The relative stereochemistry of the substitutents on the pyrrolidine nucleus was deduced as 3,4-trans and 4,5-trans on the basis of the NOESY correlations for H-3/H-5, H-4/H_2-26, and H-4/H_2-27. The $\Delta^{2(6)}$-double bond was assigned the *E*-configuration based on the NOESY data (H-6/H_2-29 and H-6/H_2-30). From all of these results, the structure of plakoridine A was established as **1**.[11]

Plakoridine A (**1**) possesses a structurally unique fully-substituted pyrrolidine ring system. The hexadecanyl aliphatic chain was commonly embraced in manzamenones[4] and untenone A,[5] while a tyramine unit was also contained in manzamenone H.[4b] Compound **1** was found to be weakly cytotoxic against murine lymphoma L1210 cells in vitro (IC_{50} 10 μg/mL).

(10) For MS data of **1**, see Experimental Section. EIMS of diacetate of **1**: *m/z* 655 (M, 0.1)$^+$, 595 (M − AcOH, 10)$^+$, 342 (M − AcOH − $COC_{16}H_{33}$, 100)$^+$, and 121 (55).

(11) The absolute configuration of **1** remained undetermined. Benzoate or MTPA ester at the C-3 hydroxyl group of **1** for CD or NMR studies was not prepared because the sample quantity of **1** was not enough for such chemical derivatizations and acetylation of **1** proceeded in low yield, presumably due to steric hindrance from the substitutents at C-2 and C-4.

Experimental Section

General Methods. A total of 256 increments of 1 K data points was collected for each 2D NMR experiment. The COSY and HMBC spectra were recorded in the absolute mode, while the HSQC and NOESY spectra were acquired in the phase-sensitive mode.

Collection, Extraction, and Isolation. The sponge *Plakortis* sp. (1 kg wet weight), collected at Unten Harbor, Okinawa, was extracted with MeOH. After evaporation of the solvent, the residue was partitioned between 1 M NaCl (400 mL) and EtOAc (400 mL × 3). The EtOAc-soluble fraction was evaporated under reduced pressure to give a crude residue (9.0 g), which was partially (1.0 g) subjected to silica gel column chromatography (2.4 × 36 cm) with EtOAc/hexane (2:8−3:7). The fraction eluting from 740 mL to 860 mL was then separated on a Sephadex LH-20 column (2.0 × 120 cm) with MeOH. The fraction containing **1** (125−150 mL) was further purified by preparative TLC (hexane/EtOAc, 1:1; R_f 0.7) followed by reversed-phase HPLC (Develosil ODS-5, 5 mm, 10 × 250 mm; eluent: $CH_3CN/CHCl_3$, 6:4; flow rate: 2.0 mL/min; detection: UV at 254 nm) to give plakoridine A (**1**, t_R 7.8 min, 0.0005% wet weight).

Plakoridine A (1): colorless oil; $[\alpha]^{19}_D$ −0.4° (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3400, 1740, 1610, and 1510 cm^{-1}; UV (MeOH) λ_{max} 317 nm (ε 22 000); ^1H and ^{13}C NMR (Table 1); FABMS (matrix: 3-nitrobenzyl alcohol) *m/z* 572 (M + H, 100)$^+$, 452 (M − $CH_2CH_2C_6H_4O$ + H, 44)$^+$, 346 (M − $C_{16}H_{33}$, 12)$^+$, and 121 ($CH_2CH_2C_6H_4OH$, 30)$^+$; EIMS *m/z* 553 (M − H_2O, 3)$^+$, 452 (M − $CH_2CH_2C_6H_4O$ + H, 72)$^+$, 300 (M − H_2O − $COC_{16}H_{33}$, 47)$^+$, and 121 (56); HRFABMS *m/z* 572.4337 (M + H; calcd for $C_{35}H_{58}O_5N$, 572.4315).

Ozonolysis of 1. A solution of plakoridine A (**1**, 0.5 mg) in MeOH (2 mL) was bubbled with O_3 at −78 °C for 1 min. After the removal of excess ozone by bubbling N_2, Me_2S (0.02 mL) was added and the mixture was stirred for 30 min at 0 °C and then at room temperature for 30 min. After evaporation of the solvent, the residue was subjected to EIMS analysis, in which the lactam **2** and the acid **3** were detected separately at different probe temperatures. **2**: *m/z* 321 (M^+, 9), 290 (M − OMe, 12)$^+$, 230 (M − C_6H_4OH + 2H, 7)$^+$, 202 (M − $CH_2CH_2C_6H_4OH$, 100)$^+$, and 121 ($CH_2CH_2C_6H_4OH$, 99)$^+$. **3**: *m/z* 270 (M^+, 40), 253 (M − OH, 12)$^+$, and 227 ($C_{16}H_{33}$ + 2H, 32)$^+$.

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Supplementary Material Available: UV spectrum and copies of 2-D NMR spectra of **1** (6 pages). This material is contained in 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.