Homo- and *Nor*-Plakotenin, New Carboxylic Acids from the Palauan Sponge *Plakortis lita*

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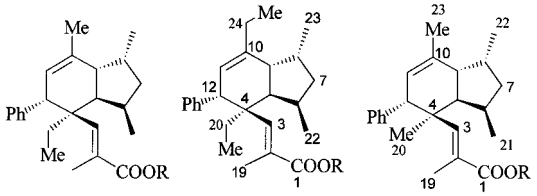
A specimen of *Plakortis lita* from Palau yielded the new carboxylic acids, *homo*-plakotenin (**2a**), the sodium salt of *homo*-plakotenin (**2b**), the sodium salt of *nor*-plakotenin (**3**), the sodium salt of plakotenin (**1b**), and the known compound plakotenin (**1a**). The structures of the new acids were elucidated by interpretation of spectral data and by comparison with the known compound. Compounds **1a**, **1b**, and **2a** were found to significantly reduce proliferation of rheumatoid synovial fibroblasts.

Marine sponges of the family Plakinidae are known to be rich sources of cyclic peroxides,[1−4] peroxylactones,[5] peroxyketals,[6] and aliphatic peroxy esters.[7] In our continuing search for new bioactive compounds, we examined 272 extracts of marine invertebrates collected in Palau in 1995, using an assay testing for the inhibition of proliferation of arthritic cells. Arthritis is a general term for inflammation of the joints, with rheumatoid arthritis being one of the most common forms of inflammatory arthritis. The crude methanolic extract of the reddish brown encrusting sponge *Plakortis lita* de Laubenfels, 1954 (Plakinidae) displayed activity in this assay. A second collection of *P. lita* was made in 1997, also from Palau. In addition to isolating the known compound plakotenin (**1a**),[8] we now report the isolation and identification of the sodium salt of plakotenin (**1b**), *homo*-plakotenin (**2a**), the sodium salt of *homo*-plakotenin (**2b**), and the sodium salt of *nor*-plakotenin (**3**).



1a R = H
1b R = Na

2a R = H
2b R = Na
2c R = CH_3

3 R = Na

Specimens of the sponge *Plakortis lita* were collected by hand in Palau and kept frozen until extraction with methanol. A concentrated methanolic extract, which was obtained after soaking the diced sponge tissue (300 g wet wt), was partitioned between ethyl acetate and water. The ethyl acetate extract was found to be active in the assay. The ethyl acetate-soluble material was chromatographed on Si gel followed by normal-phase HPLC to obtain plakotenin (**1a**, 14 mg, 4.7×10^{-3} % wet wt) and *homo*-plakotenin (**2a**, 25 mg, 8.3×10^{-3} % wet wt).

Homo-plakotenin (**2a**) was isolated as an optically active colorless oil. The molecular formula, $C_{25}H_{34}O_2$, which requires nine degrees of unsaturation, was established by HREIMS, *m*/*z* 366.2559 (M^+) (Δ 0 mmu), and by interpretation of the ^1H and ^{13}C NMR spectra. The UV and IR spectra were suggestive of a conjugated carboxyl group (λ_{max} 217 nm; ν_{max} 1680 cm^{-1}). The ^1H NMR spectrum (Table 1, $CDCl_3$) contained five methyl signals at δ 2.08 (s, 3 H, Me-19), 0.80 (t, 3 H, J = 7.1 Hz, Me-21), 1.19 (d, 3 H, J = 4.9 Hz, Me-22), 0.99 (d, 3 H, J = 6.1 Hz, Me-23), and 1.03 (t, 3 H, J = 7.1 Hz, Me-25). Also observed were methylene signals at δ 2.19 (H-24) and 1.77 and 1.08 (H-20). Comparison of the ^1H NMR spectrum with that of plakotenin **1a**[8] suggested the presence of a homologue, with an ethyl group at C-10. A COSY correlation between H-24 (δ 2.19) and Me-25 (1.03) provided further evidence of an ethyl group at C-10. Allylic coupling between H-3 and Me-19 was observed, as well as between H-11 and H-9, H-11 and H-24, and homoallylic coupling between H-12 and H-9. The ^{13}C NMR spectrum (Table 1, $CDCl_3$) displayed 23 distinct signals, two of which were assigned to the degenerate positions of a monosubstituted benzene ring (δ 127.7 and 130.9). The ^{13}C NMR data, together with the results of ^1H NMR and HMQC experiments, indicated the presence of two trisubstituted double bonds (δ 122.9 and 149.4), a carboxyl group (174.9), five methine, three methylene, and five methyl groups. The remaining quaternary carbons included an allylic carbon (δ 127.0) and an *ipso* aromatic carbon (142.8). In comparison to the ^{13}C NMR data of **1a**[8] the extra methylene group at δ 27.6 in **2a** could readily be assigned to C-24. Methylation of **2a** with diazomethane yielded **2c**, which further confirmed the presence of a carboxyl group. Comparison with the known compound **1a** confirmed the presence of a bicyclo-[4,3,0]-ring system. The relative stereochemistry of **2a** was assumed to be identical to that of **1a** based on spectral data and optical rotation measurements.

The aqueous extract was partitioned against *n*-BuOH, and the *n*-BuOH extract further chromatographed on Sephadex LH20 followed by reversed-phase HPLC to obtain plakotenin sodium salt (**1b**, 10 mg, 3.3×10^{-3} % wet wt), *homo*-plakotenin sodium salt (**2b**, 3.8 mg, 1.3×10^{-3} % wet wt), and *nor*-plakotenin sodium salt (**3**, 1.7 mg, 5.7×10^{-3} % wet wt). ESIMS of all three compounds displayed an $[M - Na]^-$ ion in the negative mode and an $[M + Na]^+$ ion in the positive mode, with **1b** also giving rise to an $[M - 1 + 2Na]^+$ in the positive mode. Also observed was the presence of the dimer, trimer, tetramer, and pentamer ions in both positive and negative modes, the oligomerization

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Table 1. ^{1}H (300 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR Data for **2a** and ^{1}H (300 MHz, CD_3OD) NMR Data for **2b**

	2a				**2b**	
C no.	δ_C	δ_H	mult, *J* (Hz)	HMBC	δ_H	mult, *J* (Hz)
1	174.9					
2	127.0					
3	149.4	7.05	s, 1H	C1, C12, C19	6.89	s, 1H
4	48.0					
5	51.6	1.78	m, 1H	C10, C12	1.79	m, 1H
6	34.7	1.86	m, 1H		1.79	m, 1H
7	45.2	1.57	dd, 2H, 7.3, 7.3	C5, C6, C8, C9, C22, C23	1.57	dd, 2H, 7.3
8	31.3	1.89	m, 1H		1.87	m, 1H
9	55.4	1.80	m, 1H		1.87	m, 1H
10	141.9					
11	122.9	5.22	m, 1H	C4	5.24	d, 1H, 4.6
12	51.4	3.76	d, 1H, 3.9	C4, C11, C13, C18	3.76	d, 1H, 4.6
13	142.8					
14	130.9	7.28	m, 1H	C13, C15	7.25	m, 1H
15	127.7	7.28	m, 1H	C16	7.25	m, 1H
16	126.4	7.22	s, 1H	C15, C17	7.25	m, 1H
17	127.7	7.28	m, 1H	C16	7.25	m, 1H
18	130.9	7.28	m, 1H	C12, C17	7.25	m, 1H
19	13.8	2.08	s, 3H	C1, C2, C3	2.02	s, 3H
20	27.0	1.77	m, 1H		1.79	m, 1H
		1.08	m, 1H	C5	1.02	m, 1H
21	9.3	0.80	t, 3H, 7.1	C4	0.80	t, 3H, 7.3
22	21.8	1.19	d, 3H, 4.9	C5, C7	1.18	d, 3H, 5.4
23	23.1	0.99	d, 3H, 6.1		0.97	d, 3H, 5.9
24	27.6	2.19	dd, 2H, 14.4, 7.6		2.19	m, 2H
25	12.8	1.03	5, 3H, 7.1	C24	1.02	t, 3H, 7.3

Table 2. ^{1}H (300 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) Assignments for *nor*-Plakotenin, Sodium Salt **3**

C no.	δ_C	δ_H	mult, *J* (Hz)	HMBC
1	174.4[a]			
2	129.6			
3	148.9	7.09	s, 1H	C-1, C-2, C-12, C-19
4	49.6[b]			
5	53.1	1.87	m, 1H	C-10, C-12
6	35.7	1.89	m, 1H	
7	45.9	1.59	m, 2H	C-6, C-9, C-22
8	33.3	1.89	m, 1H	
9	56.7	1.87	m, 1H	
10	138.3			
11	126.1	5.20	m, 1H	
12	54.6	3.61	br s, 1H	C-14, C-18
13	143.1			
14	131.7	7.24	m, 1H	C-12, C-16
15	128.7	7.23	m, 1H	C-14, C-16
16	127.6	7.23	m, 1H	C-13, C-15, C-17
17	128.7	7.23	m, 1H	C-16, C-18
18	131.7	7.24	m, 1H	C-12, C-16
19	15.1	2.08	s, 3H	C-1, C-2, C-3
20	25.3	0.88	br s, 3H	C-3, C-12
21	22.4	1.21	d, 3H, 5.6	C-5, C-6, C-7
22	23.1	0.91	d, 3H, 6.3	C-7, C-8, C-9
23	22.5	1.86	br s, 3H	C-10, C-11

[a] From HMBC data. [b] Buried under CD_3OD signals.

Table 3. The Ability of Rheumatoid Synovial Fibroblasts to Proliferate in Response to 1 nM PDGF−BB in the Presence of **1a**−**3** as Measured by the Incorporation of [^{3}H]Thymidine

compound	(μg/mL)	inhibition of DNA synthesis in rheumatoid synovial fibroblasts (%)
1a	1	76.8[a]
1b	1	35.7[a]
2a	1	73.5[a]
2b	1	27.8[b]
3	1	0

[a] Active compound (determined to be statistically significant as $p < 0.05$). [b] Borderline activity (determined to be not statistically significant as $p > 0.05$).

being induced in the mass spectrometer. Compounds **1b** and **2b** were more polar than their acids isolated from the ethyl acetate extract, but the ^{1}H NMR spectra were identical.

Nor-plakotenin (**3**) was isolated as the sodium salt. The molecular formula $C_{23}H_{29}O_2Na$ was established by ESIMS as well as HRFABMS, and by interpretation of the ^{1}H and ^{13}C NMR data. The UV and IR spectra again suggested the presence of a conjugated carboxyl group (λ_{max} 217 nm; ν_{max} 1680 cm^{-1}). The ^{1}H NMR spectrum (Table 2, CD_3OD) also contained five methyl signals, this time at δ 2.08 (s, 3 H, Me-19), 0.88 (br s, 3 H, Me-20), 1.21 (d, 3 H, J = 5.6 Hz, Me-21), 0.91 (d, 3 H, J = 6.3 Hz, Me-22), and 1.86 (br s, 3 H, Me-23). Only one methylene signal each was observed in the ^{1}H and ^{13}C spectra, that being at δ 1.59 and 45.9 (C-7), respectively. Coupling in the COSY spectrum between δ 0.88 and 1.87 (H-5) suggested the loss of the methylene unit from C-4. Coupling was also observed between H-3 and Me-19, H-11 and Me-23, and H-12 and H-9 in the COSY spectrum. Long-range HMBC correlations were observed between Me-20 and C-3, C-5, and C-12. The data are in full accord with the proposed structure.

The proliferation of rheumatoid synovial fibroblasts in response to platelet-derived growth factor-BB (PDGF-BB) was significantly reduced in the presence of 1 μg/mL of **1a**, **1b**, and **2a** as measured by the lack of [^{3}H]-thymidine incorporation as compared to controls (Table 3). At similar concentrations, **2b** and **3** had no effect on DNA synthesis, although it did appear **2b** may be active at higher concentrations. None was able to inhibit proliferation at 0.1 μg/mL, suggesting the compounds may be active over only a limited range of concentrations.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Autopol III polarimeter. IR and UV spectra were recorded on Perkin−Elmer 1600 FT−IR and Lambda 3B instruments, respectively. The ^{1}H, G(radient)COSY, GHMQC, and GHMBC spectra were re-

corded on a Varian Inova 300 MHz spectrometer, and the ^{13}C and DEPT experiments, on a Varian Gemini 400 MHz spectrometer. ESIMS were performed on a Finnigan MAT LCQ spectrometer. HRMS data were obtained from the UC Riverside Regional Mass Spectrometry Facility. All solvents were distilled prior to use.

Animal Material. Specimens of *Plakortis lita* (Homosclerophorida, Plakinidae) were collected by hand using scuba (at depths of tide to 30 ft) at Koror Island in Palau, Western Caroline Islands, in 1995 and 1997, immediately frozen after collection, and kept frozen until used. The sponge has a smooth outer surface, brownish red exterior, and consistency and texture of cooked chicken liver. The physical description and spicule measurements are in general agreement with those described in the literature.[9]

Extraction and Purification. The frozen sponge (300 g wet wt) was diced and extracted with MeOH (3 × 300 mL). The combined MeOH extracts were concentrated and partitioned between 1:1 EtOAc−H_2O (2 × 300 mL). The combined EtOAc extracts were concentrated under vacuum and chromatographed on Si gel using a gradient of 100% hexane to 50% hexane in EtOAc as eluent, followed by HPLC on a Si gel column (Microsorb, 5 μ, 10 mm × 250 mm) with 90% hexane in EtOAc to yield plakotenin (**1a**, 14 mg, 4.7×10^{-3} % wet wt) and *homo*-plakotenin (**2a**, 25 mg, 8.3×10^{-3} % wet wt). The aqueous extract was partitioned against *n*-BuOH. The *n*-BuOH extract was concentrated under vacuum and chromatographed on Sephadex LH-20 using MeOH as eluent, followed by purification by HPLC on a C_{18} column (Dynamax-60 Å, 5 μ, two 10 mm × 250 mm columns in series) using 90% MeOH in H_2O to give the sodium salts of plakotenin (**1b**, 10 mg, 3.3×10^{-3} % wet wt), *homo*-plakotenin (**2b**, 3.8 mg, 1.3×10^{-3} % wet wt), and *nor*-plakotenin (**3**, 1.7 mg, 5.7×10^{-4}% wet wt).

Plakotenin sodium salt (1b): colorless solid; $[\alpha]_D$ +224° (*c* 0.45, MeOH); UV (MeOH) λ_{max} 217 nm (ϵ 22 000); IR (film) ν_{max} 2930, 2860, 2350, 1680, 1450, 1370, 1270 cm^{-1}; ^1H NMR identical to that for **1a**;[8] FABMS *m/z* (rel int) 351 $[M - Na]^+$ (69.8); ESIMS *m/z* 351 $[M - Na]^+$, 725, 1099, 1473, 1848; 397 $[M + Na]^+$, 771, 1145, 1520, 1895; HRFABMS *m/z* 351.2332 (calcd for $C_{24}H_{31}O_2$, 351.2324).

***homo*-Plakotenin (2a):** colorless oil; $[\alpha]_D$ +183° (*c* 0.5, MeOH); UV (hexane) λ_{max} 217 nm (ϵ 22 000); IR (film) ν_{max} 2920, 2360, 1680, 1450, 1370, 1270, 1250 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 1; GC−MS *m/z* (rel int) 366 $[M]^+$ (96); HREIMS *m/z* 366.2559 (calcd for $C_{25}H_{34}O_2$, 366.2559).

***homo*-Plakotenin, sodium salt (2b):** colorless solid; $[\alpha]_D$ +211° (*c* 0.5, MeOH); UV (MeOH) λ_{max} 217 nm (ϵ 22 600); ^1H NMR, see Table 1; FABMS *m/z* (rel int) 365 $[M - Na]^+$ (6.6), ESIMS *m/z* 365 $[M - Na]^-$, 753, 1141, 1530, 1918; 411 $[M + Na]^+$, 799, 1187, 1563, 1951; HRFABMS *m/z* 365.2480 (calcd for $C_{25}H_{33}O_2$, 365.2481).

***nor*-Plakotenin, sodium salt (3):** colorless solid; $[\alpha]_D$ +101° (*c* 0.08, MeOH); UV (MeOH) λ_{max} 217 nm (ϵ 14 900); IR (film) ν_{max} 2930, 2860, 2355, 1680, 1450, 1370, 1270 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 2; FABMS *m/z* (rel int) 337 $[M - Na]^+$ (17.8), ESIMS *m/z* 337 $[M - Na]^-$, 697, 1057, 1417, 1778; 383 $[M + Na]^+$, 743, 1103, 1463, 1824; HRFABMS *m/z* 337.2186 (calcd for $C_{23}H_{29}O_2$, 337.2168).

Methylation of *homo*-Plakotenin (2a). Excess CH_2N_2 was distilled into a solution of **2a** (2 mg) in Et_2O (2 mL) at 5 °C, and the solution was allowed to warm to room temperature. After 30 min, the solution was evaporated by blowing with N_2. The residue was purified by Si gel chromatography using a disposable pipet column with 100% hexane as eluent to give **2c**: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.2−7.3 (m, 5H), 6.84 (s, 1H), 5.23 (s, 1H), 3.77 (s, 3H), 3.69 (m, 1H), 2.18 (dd, 2H, J = 15.4, 8.0 Hz), 2.07 (s, 3H), 1.85 (m. 5H), 1.59 (m, 2H), 1.19 (m, 4H), 0.99 (m, 6H), 0.79 (m, 3H); CIMS *m/z* (rel int) 381 $[MH]^+$ (77); HRCIMS *m/z* 381.2794 (calcd for $C_{26}H_{37}O_2$, 381.2800).

[^{3}H]-Thymidine incorporation assay. Rheumatoid synovial fibroblasts (a gift from Dr. Gene Mochan, Pennsylvania College of Osteopathic Medicine, Philadelphia, PA, through an agreement with Dr. Lisa A. Marshall, Department of Immunology, SmithKline Beecham Pharmaceuticals) were plated at 1.2×10^4 cells/well in 96-well cell culture plates (Nunclon, Roskilde, Denmark) in minimum essential medium with Earle's salts (EMEM) (Gibco BRL, Grand Island, NY), containing 10% heat-inactivated fetal bovine serum (FBS) (HyClone, Logan, Utah) and 10 units penstrep (Gibco BRL, Grand Island, NY), as described in Butler *et al.*[10] The cells were incubated for 6 h at 37 °C, 5% CO_2 and allowed to adhere. The media were then removed and replaced with EMEM containing 0.2% heat-inactivated FBS and 10 units penstrep. The cells were incubated for 24 h at 37 °C, 5% CO_2, where any cell growth in response to mitogens in the serum was arrested because of the low serum concentration. A total of 100 μg of **1a**, **1b**, **2a**, **2b**, and **3** (1 mg/mL stocks diluted in 100% MeOH) was transferred into Eppendorf tubes, and the MeOH was evaporated under nitrogen. The compounds were resuspended in 1 mL of DMSO, yielding a final concentration of 100 μg/mL for each compound, and subsequent dilutions in DMSO were made. Compounds were added to the appropriate wells, and the cells were incubated for 15 min at room temperature (0.5% DMSO final concentration). The cells were then stimulated with 1 nM PDGF−BB (R&D, Minneapolis, MN) diluted in EMEM containing 10% FBS and 10 units penstrep and returned to incubator for 24 h. Cells were pulsed with 0.5 μCi/well [^{3}H]-thymidine (NEN, Boston, MA) diluted in EMEM containing 10% FBS and 10 units penstrep and allowed to incubate for an additional 24 h. Assay was terminated by the addition of 200 μL of 0.25% Trypsin and 1 mM EDTA solution (Gibco BRL, Grand Island, NY), and the cells were allowed to detach by incubating for 5−10 min at room temperature. The fibroblasts were then harvested onto 96-well glass filter plates (Packard, Meriden, CT) using a Packard microplate cell harvester (Packard, Meriden, CT). The cells were lysed by subsequently rinsing and reharvesting the plates five times with distilled H_2O and four times with 70% EtOH. The filter plates were dried to completion, and 50 μL of scintillation cocktail (Packard, Meriden, CT) was added to each well in the filter plates. [^{3}H]-thymidine incorporation into the DNA was measured on a Packard microplate scintillation counter (Packard, Meriden, CT).

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