Corticatic Acids A-C, Antifungal Acetylenic Acids from the Marine Sponge, Petrosia corticata

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CORTICATIC ACIDS A–C, ANTIFUNGAL ACETYLENIC ACIDS FROM THE MARINE SPONGE, *PETROSIA CORTICATA*[1]

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ABSTRACT.—Three new acetylenic acids, corticatic acids A–C [**1–3**] have been isolated from the marine sponge, *Petrosia corticata*. Their structures were determined by spectroscopic methods, and **1–3** exhibited antifungal activity against *Mortieralla ramanniana*.

Sponges of the genera *Petrosia* and *Xestospongia* (family Petrosiidae, order Petrosida/Haplosclerida) often contain polyacetylenic alcohols (2–5) and acids (4, 6–10). These acetylenic acids are frequently brominated (6–9). Recently, oligobrominated acetylenic acids have been reported from an Indonesian sponge, *Oceanapia* sp. (10). Marine acetylenic compounds have shown a variety of biological activities, such as antimicrobial, cytotoxic, H^+, K^+-ATPase inhibitory, and HIV inhibitory effects (2–10). In our continuing search for biologically active compounds from Japanese marine invertebrates, we found that a lipophilic extract of the marine sponge, *Petrosia corticata* Wilson, collected off Hachijo-jima Island, exhibited antifungal effects against *Mortieralla ramanniana*. Bioassay-guided isolation afforded three new acetylenic acids, corticatic acids A–C [**1–3**].

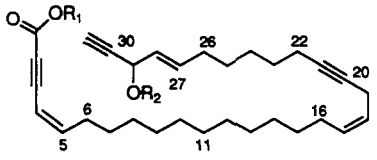
The EtOH extract (823 mg) of the frozen sponge (100 g) was fractionated on a Si gel column with a $CHCl_3$/MeOH system, followed by ODS hplc with 84% MeOH containing 0.05% TFA to afford **1** (5.6×10^{-3}%; yield based on wet wt of sponge) and **3** (1.1×10^{-3}%), and with 78% MeOH to furnish **2** (9.0×10^{-4}%).

Corticatic acid A [**1**] had a molecular formula of $C_{31}H_{44}O_3$, which was established by hrfabms and ^{13}C-nmr data. The ir spectrum showed an acetylenic absorption at 2240 cm^{-1} and a carboxylic band at 1690 cm^{-1}. The uv maxima (λ max 253, 246, and 227 nm) suggested the presence of an enyne carboxylic acid system (2). Although the carboxylic acid carbon was not observed in the ^{13}C-nmr spectrum, the presence of a carboxylic acid was confirmed by transformation to a methyl ester [**4**] with CH_2N_2. The ^{13}C-nmr spectrum indicated the presence of one mono-substituted and two disubstituted acetylenes.

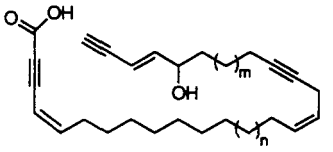
Interpretation of the ^{1}H-^{1}H COSY and ^{13}C-nmr spectra (Table 1) led to three partial structures, units **a** (C-26–C-31), **b** (C-16–C-22), and **c** (C-1–C-6). A large coupling constant (J=15.2 Hz) between H-27 and H-28 indicated 27*E*-geometry. The remaining disubstituted olefin [δ 6.23 (dt, J=10.5 and 6.7 Hz) and 5.53 (d, J=10.5 Hz)] must therefore be attached to an acetylene, thereby completing the uv chromophore of **1** (unit **c**). The HMQC-HOHAHA nmr spectrum gave two conspicuous cross-peaks (C-26/H_2-22 and C-22/H_2-26), which implied connectivity between units **a** and **b**; units **b** and **c** must be connected as shown to complete the structure. Furthermore, a cross-peak (H_2-26/H_2-23) in the two-step relayed-COSY nmr spectrum revealed that there were three methylenes between C-22 and C-26. Therefore, units **b** and **c** had to be connected through a *n*-nonyl unit to satisfy the molecular formula.

In order to determine the absolute configuration at C-29, **1** was converted to the *p*-bromobenzoyl ethyl ester **6** by treatment with EtI/DMF followed by *p*-

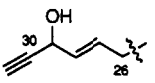
[1]Part 60 of the series 'Bioactive Marine Metabolites.' For Part 59, see Fusetani *et al.* (1).



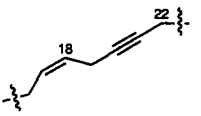
1 R_1=H, R_2=H
2 R_1=H, R_2=H, (4*E*)
4 R_1=R_2=Me
5 R_1=Et, R_2=H
6 R_1=Et, R_2=*p*-BrBz



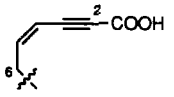
3 m+n=4



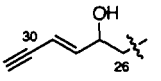
a



b



c



d

TABLE 1. Nmr Data of Corticatic Acid A [**1**] [δ in ppm and *J* in Hz ($CDCl_3$)].[a]

Position	^{1}H	^{13}C
C (1)		[b]
CH (4)	5.53 (d, *J*=10.5)	107.8
CH (5)	6.32 (td, *J*=10.5 and 6.7)	151.2
CH_2 (6)	2.36 (dt, *J*=6.6 and 7.2)	31.6
CH_2 (7)	1.40 (m)	31.6
CH_2 (15)	1.32 (m)	[c]
CH_2 (16)	2.02 (dt, *J*=7.1 and 6.2)	28.0
CH (17)	5.41 (m)	132.1
CH (18)	5.38 (m)	126.3
CH_2 (19)	2.88 (m)	17.7
CH_2 (22)	2.13 (tt, *J*=7.1 and 2.5)	19.4
CH_2 (23)	1.45 (m)	[c]
CH_2 (24)	1.32 (m)	[c]
CH_2 (25)	1.37 (m)	[c]
CH_2 (26)	2.06 (dt, *J*=7.2 and 6.9)	32.9
CH (27)	5.89 (dt, *J*=15.2 and 6.7)	134.1
CH (28)	5.58 (dd, *J*=15.8 and 6.9)	130.7
CH (29)	4.83 (br d, *J*=5.4)	63.2
C (30)		84.8
CH (31)	2.54 (d, *J*=2.0)	74.4

[a]Chemical shifts for the acetylenic carbons δ 82.5, 80.6, 79.4, 74.5; for methylenic carbons δ 39.6–30.6.
[b]Not observed.
[c]Not assigned.

bromobenzoyl chloride/DMAP in pyridine. Although all spectral data supported the structure, **6**, when dissolved in dioxane (insoluble in other solvents, e.g., hexane, MeOH, MeCN, and EtOH) did not exhibit a split cd spectrum. Quite recently, it was reported that the exciton chirality method cannot be applied to secondary alcohols flanked by two chromophores (13).

Corticatic acid B [**2**] had the same molecular formula as **1**, which was established by hrfabms and nmr spectra. The nmr and uv spectra were almost identical with those of **1**, except for a coupling constant of 16.0 Hz between H-4 and H-5. Therefore, **2** was the 4*E* isomer of **1**.

Corticatic acid C [**3**] also had a molecular formula of $C_{31}H_{44}O_3$. The uv spectrum (λ max 255, 245, 238, and 226 nm) suggested the presence of both enyne and enyne carboxylic acid systems (2,8). The ^{1}H-^{1}H COSY spectrum indicated that **3** had structural unit **d** (C-26–C-31) in addition to units **b** and **c**. 4*Z*,17*Z*-Geometry was assigned on the basis of a ^{1}H,^{1}H coupling constant of 10.8 Hz between H-4 and H-5 and a ^{13}C-nmr chemical shift of δ 28.1 ppm for C-16. *E*-Geometry of the C-27, -28 double bond was secured by a coupling constant of *J*=15.8 Hz. Although HMQC-HOHAHA cross-peaks of C-26/H_2-22 and C-22/H_2-26 suggested that units **d**

and **b** were connected through several methylene carbons, the number of methylene carbons between these units remains to be determined.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were measured on a Jasco-IR-G infrared spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were recorded on either a Bruker AM-600, a JEOL GX-500, or a Bruker AC-300 nmr spectrometer. ^{1}H- and ^{13}C-nmr chemical shifts are referenced to solvent ($CDCl_3$) peaks (δ_H 7.24 and δ_C 77.0). Optical rotations were determined on a Jasco DIP-371 digital polarimeter. Fabms were measured on a JEOL JMX-SX102 mass spectrometer with glycerol as the matrix. The cd spectrum was measured on a Jasco J-20C automatic recording spectropolarimeter. Si gel chromatography was performed using precoated Merck F_{254} plates and C-300 powder (Wako Pure Chemical). Hplc used Tosoh HLC-803D [Cosmosil 5-C_{18}AR column (1.0×25 cm); flow rate 2 ml/min; uv (240 nm) detection]. Tlc was visualized by spraying with 5% H_2SO_4 in EtOH solution followed by heating at 100° for 5–10 min.

ANIMAL MATERIAL.—Sponge samples were collected by scuba at a depth of 15 m off Hachijo-jima Island, 300 km south of Tokyo, immediately frozen, and kept frozen until processed. The sponge, *Petrosia corticata*, was yellow to white, with yellowish-dark surfaces. A voucher specimen (ZMA POR 10598) was deposited at the Institute of Zoological Taxonomy, University of Amsterdam.

ANTIFUNGAL ASSAY.—Previously described by Li *et al.* (14).

EXTRACTION AND ISOLATION.—The frozen sponge (100 g) was homogenized and extracted with EtOH (4×100 ml). The extract was concentrated to give a brownish residue (823 mg), which was subjected to open cc on Si gel (stepwise elution from $CHCl_3$ to MeOH). The antifungal fraction eluted with 10% MeOH/$CHCl_3$ was separated by reversed-phase hplc with 84% MeOH containing 0.05% TFA to afford **1** (5.6 mg) and **3** (1.1 mg), and another antifungal fraction eluted with 20% MeOH/$CHCl_3$ from the Si gel column was purified by ODS hplc with 78% MeOH to afford **2** (0.9 mg).

Corticatic acid A [**1**].—Colorless oil; $[\alpha]^{23}$D +28° (*c*=0.13, $CHCl_3$); fabms (negative, *m*-nitrobenzyl alcohol) *m/z* 463 $(M-H)^-$, 393 $[M-H-(C{\equiv}C\text{-}OOH)]^-$; hrfabms (negative) *m/z* 463.3239 (Δ −2.7 mmu) for $C_{31}H_{43}O_3$, 393.3168 (Δ −1.1 mmu) for $C_{28}H_{41}O$; uv (MeOH) λ max 253 sh (ε 8500), 246 sh (11200), 227 nm (11500); ir (film) ν max 3600–2400, 3300, 2850, 2750, 2240, 1690, 1460, 1250 cm^{-1}; ^{1}H and ^{13}C nmr, see Table 1.

Corticatic acid B [**2**].—Colorless oil; $[\alpha]^{23}$D +9° (*c*=0.04, $CHCl_3$; fabms (negative) *m*-nitrobenzyl alcohol *m/z* 463 $(M-H)^-$, 393 $[M-H-(C{\equiv}C\text{-}OOH)]^-$; hrfabms (negative *m/z* 463.3214 (Δ 0.2 mmu) for $C_{31}H_{43}O_3$; uv (MeOH) λ max 255 sh (ε 8300), 245 sh (11000), 226 nm (12100); ir (film) ν max 3600–2400, 3300, 2850, 2750, 2240, 1690 cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 6.35 (1H, dt, *J*=15.9 and 7.3 Hz, H-5), 5.92 (1H, dt, *J*=15.2 and 6.6 Hz, H-27), 5.63 (1H, dd, *J*=15.4 and 6.0 Hz, H-28), 5.60 (1H, d, *J*=16.2 Hz, H-4), 5.41 (2H, m, H-17, 18), 4.87 (1H, d, *J*=5.1 Hz, H-29), 2.89 (2H, m, H_2-19), 2.54 (1H, d, *J*=2.1 Hz, H-31), 2.3–2.10 (4H, m), 2.06 (1H, m, H-26), 1.3–1.5 (8H), 1.28 (14H).

Corticatic acid C [**3**].—Colorless oil; $[\alpha]^{23}$D +7° (*c*=0.05, $CHCl_3$); fabms (negative) *m*-nitrobenzyl alcohol *m/z* 463 $(M-H)^-$; hrfabms (negative) *m/z* 463.3186 (Δ −2.6 mmu) for $C_{31}H_{43}O_3$; uv (MeOH) λ max 255 sh (ε 8700), 245 (11500), 238 (8700), 226 nm (18900); ir (film) ν max 3600–2400, 2950, 2850, 2200, 1700, 1193 cm^{-1}; ^{1}H nmr (600 MHz, $CDCl_3$) δ 6.33 (1H, dt, *J*=11.0 and 7.4 Hz, H-5), 6.24 (1H, dd, *J*=15.8 and 6.0 Hz, H-28), 5.72 (1H, d, *J*=15.8 Hz, H-29), 5.63 (1H, d, *J*=10.8 Hz, H-4), 5.48 (1H, m), 5.41 (1H, m), 4.21 (1H, dt, *J*=6.0 and 6.7 Hz, H-27), 2.93 (3H, m, H_2-19, 31), 2.42 (1H, dt, *J*=6.7 and 7.2 Hz, H-6), 2.30–2.10 (4H, m), 1.3–1.5 (8H), 1.28 (14H).

Methylation of corticatic acid A [**1**].—To **1** (0.9 mg), 0.2 ml CH_2N_2 in Et_2O was added. After 1 min at room temperature, the solution was evaporated to dryness *in vacuo*. Compound **4** was obtained as an oil. Fabms (positive, glycerol) *m/z* 493 $(M+H)^+$; ir (film) ν max 2850, 2750, 2240, 1715, 1460, 1250 cm^{-1}; ^{1}H nmr (500 MHz, $CDCl_3$) δ 6.25 (1H, dt, *J*=11.0 and 7.6 Hz, H-5), 5.88 (1H, dt, *J*=15.3 and 7.2 Hz, H-27), 5.63 (1H, dd, *J*=15.3 and 7.1 Hz, H-28), 5.60 (1H, d, *J*=11.0 Hz, H-4), 5.41 (1H, m, H-17), 5.38 (1H, m, H-18), 4.83 (1H, d, *J*=5.4 Hz, H-29), 3.78 (3H, s, COOMe), 3.35 (3H, s, OMe), 2.87 (2H, m, H_2-19), 2.53 (1H, d, *J*=2.2 Hz, H-31), 2.35 (1H, dt, *J*=7.1 and 7.2 Hz, H-6), 2.12 (1H, m, H-22), 2.06 (1H, m, H-26), 2.02 (1H, dt, *J*=7.1 and 7.2 Hz, H-16), 1.45–1.2 (24H, m).

Ethylation of Corticatic Acid A [**1**].—To a solution of **1** (2.1 mg) in DMF (0.3 ml) was added dropwise EtI (0.05 ml), and then $NaHCO_3$ (20 mg). The mixture was stirred for 12 h at room temperature. The solvent was lyophilized. The residue was taken up in Et_2O, and the extract was washed with H_2O, dried ($MgSO_4$), and evaporated to give the ethyl ester **5** (2.0 mg, 90%): oil; ^{1}H nmr (500 MHz, $CDCl_3$) δ 6.19 (1H, dt, *J*=10.9 and 7.3 Hz, H-5), 5.88 (1H, dt, *J*=15.1 and 6.9 Hz,

H-27), 5.60 (1H, d, J=10.8 Hz, H-4), 5.53 (1H, dd, J=15.0 and 5.8 Hz, H-28), 5.38 (2H, m, H-17, 18), 4.77 (1H, d, J=5.3 Hz, H-29), 4.18 (2H, quint., J=7.2 Hz, COOCH_2Me), 2.89 (2H, m, H_2-19), 2.54 (1H, d, J=2.1 Hz, H-31), 2.38 (1H, dt, J=6.9 and 7.3 Hz, H-6), 2.12 (2H, m), 2.01 (2H, m), 1.30–1.50 (8H), 1.28 (14H), 0.81 (3H, t, J=7.2 Hz, COOCH$_2$*Me*).

p-*Bromobenzoylation of* **5**.—To a solution of **5** (2.0 mg) in pyridine (0.3 ml) was added *p*-bromobenzoyl chloride (15 mg) and DMAP (10 mg). The mixture was stirred overnight, then the solvent was removed by freeze-drying. The residue was subjected to cc over a Si gel open column. Compound **6** was obtained from the $CHCl_3$ fraction as an oil (2.2 mg, 81%). ^{1}H nmr (500 MHz, $CDCl_3$) δ 7.89 (2H, d, J=8.5 Hz), 7.57 (2H, d, J=8.5 Hz), 6.22 (1H, dt, J=10.7 and 7.3 Hz, H-5), 6.08 (1H, dt, J=15.2 and 6.7 Hz, H-27), 6.02 (1H, d, J=5.2 Hz, H-29), 5.64 (1H, dd, J=15.2 and 6.2 Hz, H-28), 5.52 (1H, d, J=10.9 Hz, H-4), 5.40 (2H, m, H-17, 18), 4.22 (2H, quint., J=7.2 Hz, COOCH_2Me), 2.88 (2H, m, H_2-19), 2.58 (1H, d, J=2.5 Hz, H-31), 2.38 (1H, dt, J=6.9 and 7.3 Hz, H-6), 2.10 (2H, m), 2.01 (2H, m), 1.30–1.50 (8H), 1.28 (14H), 0.82 (3H, t, J=7.2 Hz, COOCH$_2$*Me*).

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