A New Acyclic Diketotriterpenoid Isolated from the Indonesian Marine Sponge *Hyrtios erectus*

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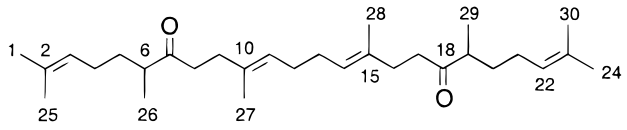
The structure of diketotriterpenoid **1**, isolated from the marine sponge *Hyrtios erectus* collected in Indonesia, has been elucidated by spectroscopic analysis.

Marine sponges continue to attract attention as a rich source of structurally novel secondary metabolites that are potential lead compounds for the development of new anticancer drugs.[1] As part of our ongoing search for new bioactive metabolites from tropical sponges,[2] it was found that crude extracts of the Indonesian sponge *Hyrtios erectus* (Keller, 1881) (order Dictyoceratida, family Thorectidae) exhibited significant antimitotic activity.[3] Bioassay-guided fractionation of the *H. erectus* extract led to the isolation of the antimitotic agent in only trace quantities that proved to be inadequate for either total structure elucidation or dereplication purposes. However, one of the biologically inactive chromatography fractions obtained from the *H. erectus* extract contained the new triterpenoid **1**, whose structure is described below.



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Hyrtios erectus (Keller, 1881) was harvested by hand using scuba on the inner reef at Ujung Pandang, Sulawesi, Indonesia. Freshly collected sponge was repeatedly extracted with EtOH. The combined extracts were concentrated in vacuo, and the resulting aqueous suspension was partitioned between H_2O and EtOAc. Repeated fractionation of the EtOAc-soluble materials via Sephadex LH-20 and flash silica gel column chromatography followed by reversed-phase HPLC gave a pure sample of the diketotriterpenoid **1** (1.3 mg).

Diketone **1**, obtained as a clear, optically active amorphous solid, gave an $[M + H]^+$ ion in the HRFABMS at *m*/*z* 443.3872 that was appropriate for a molecular formula of $C_{30}H_{50}O_2$. Only 15 carbon resonances with 25 attached hydrogen atoms were observed in the ^{13}C/DEPT/HMQC NMR data obtained for **1** (4 × CH_3, 5 × CH_2, 3 × CH, 3 × C). Comparison of carbon and hydrogen count obtained from the ^{13}C NMR data ($C_{15}H_{25}$) with the molecular formula ($C_{30}H_{50}O_2$) indicated that **1** contained a 2-fold element of symmetry. A series of downfield ^{13}C NMR resonances were routinely assigned to two olefins (δ 125.1 (CH), 125.8 (CH), 133.0 (C), 135.3 (C) ppm) and a saturated ketone (δ 217.0 ppm). Additional upfield ^1H and ^{13}C NMR resonances could be assigned to three allylic methyls (δ ^1H (^{13}C) 1.58, bs (17.8); 1.60, bs (16.2); 1.67, d, J = 1.0 Hz (25.8)), three allylic methylenes (δ 1.94, dd, J = 14.6, 7.5 Hz (26.8); 2.00, m (29.0); 2.20, t, J = 7.4 Hz (34.5)), and one aliphatic methyl (δ 1.04, d, J = 6.9 Hz (16.8)). The remaining NMR resonances were assigned to an aliphatic methylene (δ 1.32, m and 1.67, m (34.2)) residue and methylene (δ 2.55, m (41.0)) and methine (δ 2.58, m (46.9)) residues that were both adjacent to a ketone. All six sites of unsaturation demanded by the molecular formula of **1** were accounted for by the single ketone and two alkene functional groups described above in combination with the 2-fold symmetry required by the data. Furthermore, the presence of eight methyl appendages and a total of 30 carbon atoms in **1** suggested that the molecule was an acyclic triterpenoid.

COSY data routinely established the spin system extending from Me-1 and Me-25 through to H-5. Although the COSY correlations between H-5 and H-6 were weak, HMBC correlations observed between the Me-26 resonance at δ 1.04 and the C-5 (δ 34.2) resonance, between the allylic H-4 methylene proton resonance at δ 1.94 and both the C-5 (δ 34.2) and C-6 (δ 46.9) resonances, and between the H-6 methine resonance at δ 2.58 and both the C-4 (δ 26.8) and C-5 (δ 34.2) resonances, unambiguously established the connectivity between C-5 and C-6. Similarly, COSY correlations identified the spin system extending from H-8 through H-12, including Me-27, and the constitution of this fragment was confirmed by the HMBC data. The Me-26 doublet at δ 1.04, the methylene resonances of H-5 (δ 1.32 and 1.67 ppm), H-8 (δ 2.55), and H-9 (δ 2.20), as well as the H-6 methine resonance at δ 2.58 all showed HMBC correlations to the ketone resonance at δ 217.0 (C-7), demonstrating that the two structural fragments encompassing C-1 to C-6 and C-8 to C-12 represented the two alkyl substituents on the saturated ketone. Finally, the H-12 methylene resonance at δ 2.00 showed both HMQC and HMBC correlations to the same methylene carbon resonance at δ 29.0. This observation confirmed that the axis of symmetry in the molecule was situated in the C-12 to C-13 bond, leading to the assigned structure **1** for the new triterpenoid. Difference NOE experiments established the *E* configuration for the $\Delta^{10,11}$ and $\Delta^{14,15}$ olefins in **1**. The limited quantity of **1** (1.3 mg) that was available precluded determination of the configuration at C-6/C-19.

Previous chemical studies of sponges in the genus *Hyrtios* have resulted in the isolation of numerous sesterterpenoids[4] as well as the sesquiterpenoid/shikimate-derived metabolite puupehenone and several analogues of

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Table 1. 500 MHz NMR Data for Diketone **1** Recorded in MeOH-d_4

atom	δ ^{1}H	δ ^{13}C
1, 24	1.67 d (1.0), 6H	25.8
2, 23		133.0
3, 22	5.08 m, 2H	125.1
4, 21	1.94 q (7.5), 4H	26.8
5, 20	1.32 m, 2H	34.2
5′, 20′	1.67, 2H	
6, 19	2.58 m, 2H	46.9
7, 18		217.0
8, 17	2.55 m, 4H	41.0
9, 16	2.20 t (7.4), 4H	34.5
10, 15		135.3
11, 14	5.13 m, 2H	125.8
12, 13	2.00 m, 4H	29.0
25, 30	1.58 bs, 6H	17.8
26, 29	1.04 d (6.9), 6H	16.8
27, 28	1.60 bs, 6H	16.2

puupehenone, including dimers.[5] Triterpenoids are relatively common sponge metabolites;[6] however, the diketone **1** is apparently the first triterpenoid known from the genus *Hyrtios*. Okinawan specimens of *H. altum* have yielded potent antimitotic agents in the spongistatin (altohytrin) family.[7] Related macrolides may have been responsible for the antimitotic activity exhibited by the crude extract of the *H. erectus* specimens examined in the current study.

Experimental Section

General Experimental Procedures. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-500 spectrometer. ^{1}H chemical shifts are referenced to the residual MeOH-d_4 signal (δ 3.30 ppm), and ^{13}C chemical shifts are referenced to the MeOH-d_4 solvent peak (δ 49.0 ppm). Low- and high-resolution FABMS were recorded on a Kratos Concept II HQ mass spectrometer with xenon as the bombarding gas and a 3-NBA sample matrix. Optical rotations were measured using a JASCO J-710 spectrophotometer.

Merck type 5554 silica gel plates and Whatman MKC18F plates were used for analytical thin-layer chromatography. Merck silica gel G60 (230−400 mesh) was used for normal-phase flash chromatography. Reversed-phase HPLC purifications were performed on a Waters 600E system controller liquid chromatograph attached to a Waters 410 differential refractometer. All solvents used for HPLC were Fisher HPLC grade.

Animal Material. Specimens of *H. erectus* (Keller, 1881) were collected by hand using scuba at a depth of 10−15 m on the inner reef of Ujung Padang, Sulawesi, Indonesia, in October 1996. The sponge was identified by Professor Rob van Soest, University of Amsterdam, and a voucher sample has been deposited at the Zoological Museum of Amsterdam (ZMA POR 13006).

Isolation of Diketone 1. Freshly collected sponge (40 g) was cut into small pieces and extracted repeatedly with EtOH (3 × 150 mL) in Indonesia. The combined ethanolic extracts were concentrated in vacuo, and the resultant brown gum (455 mg) was shipped to Vancouver. The extract was partitioned between EtOAc (3 × 15 mL) and H_2O (45 mL), and the combined EtOAc fractions were evaporated to dryness to give 229.4 mg of dark brown oil. The oil was chromatographed on Sephadex LH-20 in MeOH/CH_2Cl_2 (4:1) to give 62.1 mg of an early eluting antimitotic fraction. This material was further fractionated using silica gel flash chromatography, employing a step gradient from CH_2Cl_2 to MeOH/CH_2Cl_2 (1:19) and on to MeOH. An 11.7 mg fraction, eluting with MeOH/CH_2Cl_2 (1:19), elicited antimitotic activity (at 1 μg/mL) and contained a bright purple staining spot when TLC plates were visualized with a standard vanillin/H_2SO_4 spray reagent. Pure triterpene **1** (1.3 mg), the vanillin staining material, was obtained from this mixture via C_{18} reversed-phase HPLC using an analytical Alltech Econosil 5 μm column, with MeOH/H_2O (9:1) as eluent.

Triterpene 1: Isolated as a clear amorphous solid; $[\alpha]^{25}_D$ −371° (*c* 0.055, CH_2Cl_2); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; positive ion HRFABMS $[M + H]^+$*m*/*z* 443.3872 ($C_{30}H_{51}O_2$ ΔM −3.76 ppm).

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