

## Isolation of a Novel Diterpene Triacetate from Two Soft Corals of the Order Alcyonacea

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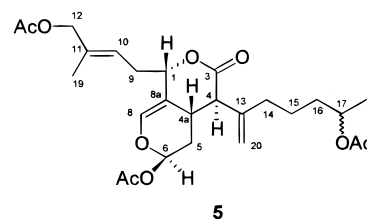
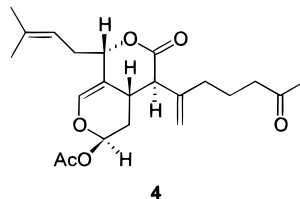
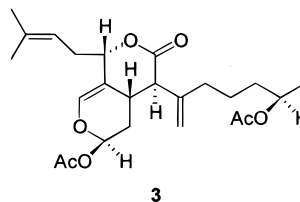
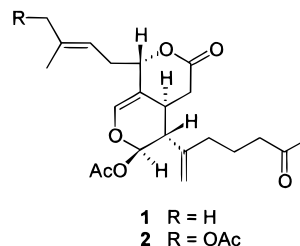
Alcyonolide **5** (**5**), a novel diterpene triacetate, has been isolated from two soft corals of the order Alcyonacea.

Soft corals of the subclass Alcyonaria are abundant in tropical seas, and, together with the gorgonians, constitute a major component of reef fauna.<sup>1</sup> They are also regarded as true soft corals.<sup>2</sup> Previously, Kobayashi et al.<sup>3</sup> isolated diterpene **1** from an *Alcyonium* sp. and ascribed to it the trivial name alcyonolide. Later, Bowden et al.<sup>4</sup> isolated diterpenes **2**, **3**, and **4** from several species of *Efflatounaria* (family Xenidiidae). These compounds are believed to be derived from a xenicin-type precursor. We now report the isolation of the triacetate **5** from two soft corals of the order Alcyonacea<sup>5</sup> that were collected from Lamont Reef in the Capricorn Bunker Group, during our investigation of the chemistry of marine invertebrates of the Great Barrier Reef. We propose that compounds **2**, **3**, **4**, and **5** be assigned the trivial names alcyonolides 2–5, respectively.

The crude extracts of both corals were separately chromatographed on Si gel using hexane–EtOAc mixtures and yielded the same single major metabolite. The <sup>13</sup>C NMR spectrum of this metabolite contained resonances for 26 carbons, including four ester-like carbonyl carbons (approximately 170 ppm) and six olefinic carbons. The <sup>1</sup>H NMR contained signals for two olefinic methine protons ( $\delta$  5.5 and  $\delta$  6.34), a methine proton at lowfield ( $\delta$  6.26), oxygenated methylene hydrogens at  $\delta$  4.45, and olefinic methylene hydrogens at  $\delta$  4.99 and 5.05. In addition there were three acetate methyl singlet signals at  $\delta$  2.01, 2.05, and 2.10; a vinyl methyl singlet at  $\delta$  1.66; and a methyl doublet at  $\delta$  1.18.

An analysis of the DEPT-135 and <sup>13</sup>C NMR spectra gave the partial formula of C<sub>26</sub>H<sub>36</sub>, indicating there could be a maximum of nine degrees of unsaturation in the molecule. Seven were satisfied by four ester functional groups and three carbon–carbon double bonds; therefore, the structure of **5** may contain two carbocyclic rings. The molecular weight, 493.2444 [M + H]<sup>+</sup>, was obtained from the HRESIMS. This information, together with the <sup>13</sup>C and DEPT-NMR data, revealed the molecular formula of **5** to be C<sub>26</sub>H<sub>36</sub>O<sub>9</sub>.

The NMR spectra of **5** were similar to the corresponding data reported for both **2** and **3** (see Table 1) and suggested **5** was a derivative of one of these compounds. From the chemical shifts of the signals in the <sup>1</sup>H and <sup>13</sup>C NMR spectra and the COSY, HMQC, and HMBC



data, a bicyclic pyranopyranone function, common to both **2** and **3**, was present in **5**.

The deshielding of H-6 ( $\delta$  6.26) in **5** suggested attachment to a carbon that was bonded to two oxygen atoms. The HMBC correlations from this proton resonance to both C-8 (137.1 ppm) and an acetate carbonyl carbon (approximately 170 ppm) confirmed this and were consistent with the presence of a 6-acetoxy substituent (as observed in both **2** and **3**). An additional HMBC correlation between H-6 and a methylene carbon (31.2 ppm), suggested **5**, like **3**, was substituted at C-4 rather than C-5.

The methylene protons at  $\delta$  4.45 (H-12) showed a HMBC correlation with an acetate carbonyl carbon and

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**Table 1.** Selected  $^{13}\text{C}$  and  $^1\text{H}$  NMR Resonances of **2**, **3** and **5**

carbon	<b>2</b>	<b>3</b>	<b>5</b>
1	78.5 (4.85)	77.7 (5.0)	77.2 (5.02)
6	92.5 (5.97)	88.6 (6.3)	88.7 (6.26)
8	137.5 (6.42)	136.9 (6.38)	137.1 (6.34)
8a	109.7	110.6	110.5
10	121.8 (5.46)	117.2 (5.17)	122.4 (5.50)
11	133.9	135.2	133.8
12	69.1 (4.49, 4.46)	25	69.4 (4.45)
13	144.6	143.6	143.7
17	207.0	70.4 (4.9)	70.6 (4.88)
20	113.4 (5.04, 4.94)	114.4 (5.07, 5.0)	114.6 (5.05, 4.99)

exhibited allylic coupling to an olefinic methine proton. This methine had additional allylic and vicinal coupling to the sole vinyl methyl protons ( $\delta$  1.66) and the methylene protons at  $\delta$  2.55, respectively, indicating the presence of a 4-acetoxy-3-methyl-2-butenyl substituent in **5**. The COSY and HMBC spectra correlated this side chain with C-1 (77.2 ppm). The  $^{13}\text{C}$  NMR chemical shift of the vinyl methyl was 14.9 ppm. This suggested an *E* configuration of the double bond.<sup>6</sup>

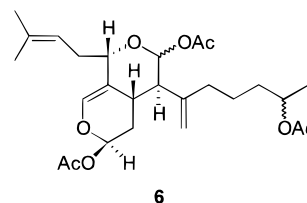
The methyl ketone side chain present in **2** and **4** was absent in **5**. Instead, a methyl doublet resonance at  $\delta$  1.18 was coupled to a methine proton multiplet at  $\delta$  4.88. The HMBC spectrum contained long-range correlations from this signal to an acetate carbonyl carbon and a methylene carbon at 23.2 ppm. This suggested a secondary acetoxy side chain, as seen in **3**, was present in **5**. The vinyl methylene protons at  $\delta$  5.05, 4.99 had HMBC correlations with a quaternary olefinic carbon (143.7 ppm), C-4 (54.4 ppm), and a methylene carbon (35.4 ppm) with attached protons that resonated at  $\delta$  1.80, 1.55. The remaining, so far unaccounted, methylene must be part of a three-carbon chain that links the secondary acetoxy functionality with the vinyl substituent at C-4.

The relative stereochemistry of the four chiral centers of the pyranopyranone ring has been defined by the NOESY data and by comparison of the magnitude of the coupling constants of **5** with the data reported for **3**. NOESY correlations were observed between the H-10 vinyl methine proton ( $\delta$  5.50) and H-1 and between H-9 and H-8. This suggests H-1 is most likely in the axial position. The coupling constant of the H-4 doublet resonance ( $J = 9$  Hz) is consistent with *trans*-diaxial coupling with H-4a. Therefore, C-4 and C-4a, given the structure of **5**, have opposite relative stereochemistry. Additional evidence supporting this was obtained from the NOESY spectrum. The signals at  $\delta$  5 in the  $^1\text{H}$  NMR spectrum of **5** had NOESY correlations with both the H-4 and H-4a resonances. Because H-4 and H-4a were *trans*-diaxial, the observed correlations were most likely from the vinyl methylene protons (H-20) to H-4 and from H-1 to H-4a.

In **5**, H-4a resonated as a broad doublet of doublets in the  $^1\text{H}$  NMR spectrum, due to the two diaxial coupling constants,  $J_{\text{H-4a}, 5\alpha}$  and  $J_{\text{H-4a}, 4}$  ( $J = 13$  Hz and  $J = 9$  Hz, respectively). Bowden et al. had suggested<sup>4</sup> only a small coupling constant existed between both H-5 protons and H-4a in **3**; however, the results of decoupling experiments reported<sup>4</sup> for **3** contradict<sup>7</sup> this assessment. As H-6 resonated as a triplet ( $J = 2$  Hz) in the  $^1\text{H}$  NMR spectrum, the two dihedral angles formed between H-6 and both H-5 methylene protons must be small (approx  $60^\circ$ ) and nearly equal. Therefore, H-6 is

equatorial, and in this instance C-6 has the same relative stereochemistry as C-4. The chiral carbons of the bicyclic function, therefore, have the same relative stereochemistry as those of **3**.

The stereochemistry of C-17 could not be determined from the NOESY spectrum. Bowden et al. found that when **4** was reduced and then acetylated, the product—triacetate **6**—was indistinguishable (by NMR and optical rotation) from the product obtained after reduction and acetylation of **3**. This was despite **6** being racemic at C-17. They concluded<sup>4</sup> that a racemic chiral center removed from the pyranopyranone function did not affect the spectral and optical properties. The stereochemistry about C-17 of **5**, therefore, cannot be determined from spectroscopic or optical data alone. Further evidence, for example, X-ray crystallographic data, will be required.

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## Experimental Section

**General Experimental Procedures.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker AMX-300 NMR at 300 and 75 MHz, respectively, in  $\text{CDCl}_3$  calibrated to  $\delta$  7.27 and 77.0 ppm, respectively. LREIMS were recorded on a Shimadzu QP2000A spectrometer and HRESIMS were recorded on a Micromass Autospec spectrometer. FT-IR spectra were recorded on a Perkin-Elmer 1600 spectrometer, optical rotations on a Perkin-Elmer 341 polarimeter, and UV spectra on a Cary 4E spectrophotometer. Column chromatography was performed on Merck Kieselgel 60 (230–400 mesh).

**Animal Material.** The specimens were collected by scuba diving in about 8 m of water from Lamont Reef in the Capricorn Bunker Group, Great Barrier Reef, in 1994. The corals were kept frozen prior to extraction. The first colony had the general colony form of *Efflatounaria*. The sample had no obvious sclerites in the tissues, and polyps were tightly contracted, preventing the observation of pinnules on the tentacles. Strange crystalline particles were found within the tissues. The second colony had the general colony form of *Cespitularia*. It also had no sclerites in the tissues and contained the same type of crystalline material within the tissues as observed in the first colony. There were 11 pinnules on the tentacles. There are no morphological differences between soft corals of the genus *Cespitularia* and a noncontracted *Efflatounaria*. Both colonies appeared black when alive, and most likely the two specimens are of the same species. Voucher specimens NTMC12534 and NTMC12535, respectively, have been deposited at the Museum and Art Gallery of the Northern Territory, Darwin, Australia.

**Extraction and Isolation.** A sample (73 g, wet and frozen) of the first colony (*Efflatounaria* form) was exhaustively extracted with  $\text{Me}_2\text{CO}$ . The crude extract was evaporated, then partitioned between  $\text{H}_2\text{O}$  and  $\text{EtOAc}$ . The second colony was freeze-dried (4.4 g dry) and exhaustively extracted with  $\text{CH}_2\text{Cl}_2$ . The  $\text{EtOAc}$

and  $\text{CH}_2\text{Cl}_2$  extracts were then evaporated to residues (0.23 g in each case). The two extracts were individually chromatographed on Si gel using a solution of 15% EtOAc in hexane to yield **5** (35 mg).

**Alcyonolide 5 (5):** isolated as a pale yellow gum;  $[\alpha]_{\text{D}}^{25} +92.6^\circ$  (*c* 0.0074,  $\text{CHCl}_3$ ); UV (EtOH)  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 236 (3.5) 200 (4.3) nm; IR (KBr disk)  $\nu_{\text{max}}$  2928, 1736, 1375, 1248, 1023  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.34 (1H, t,  $J = 2$  Hz, H-8), 6.26 (1H, t,  $J = 2$  Hz, H-6), 5.50 (1H, t,  $J = 7$  Hz, H-10), 5.05, 4.99 (each 1H, brs, H-20), 5.02 (1H, t,  $J = 7$  Hz, H-1), 4.88 (1H, m, H-17), 4.45 (2H, s, H-12), 2.95 (1H, d,  $J = 9$  Hz, H-4), 2.85 (1H, br dd,  $J = 13, 9$  Hz, H-4a), 2.55 (2H, m, H-9), 2.05/1.65 (2H, m, H-5), 2.10, 2.05, 2.01 (each 3H, s, OAc), 1.80, 1.55 (each 1H, m, H-14), 1.62 (2H, m, H-15), 1.85, 1.6 (each 1H, m, H-16), 1.66 (3H, s, H-19), 1.18 (3H, d,  $J = 6$  Hz, H-18);  $^{13}\text{C}$  NMR (75 MHz)  $\delta$  170.7, 170.3, 170.1 (each  $\text{CH}_3\text{CO}$ ), 169.3 (C-3), 143.7 (C-13), 137.1 (C-8), 133.8 (C-11), 122.4 (C-10), 114.6 (C-20), 110.5 (C-8a), 88.7 (C-6), 77.2 (C-1), 70.6 (C-17), 69.4 (C-12), 54.4 (C-4), 35.4 (C-14), 32.8 (C-9), 31.9 (C-15), 31.2 (C-5), 26.9 (C-4a), 23.2 (C-16), 21.3, 21.0, 20.9 (each  $\text{CH}_3\text{CO}$ ), 19.9 (C-18), 14.3 (C-19); EIMS  $m/z$  434  $[\text{M}-58]^+$  (0.2), 365 (0.5), 323 (0.2), 305 (0.3), 287 (0.5), 263 (3.3), 259 (3.7),

245 (9.5), 219 (5.2), 201 (2.9), 175 (3.5); HRESIMS  $m/z$  515.2246 (calcd for  $\text{C}_{26}\text{H}_{36}\text{O}_9\text{Na}$ , 515.2257), 510.2679 (calcd for  $\text{C}_{26}\text{H}_{40}\text{O}_9\text{N}$ , 510.2703), 493.2444 (calcd for  $\text{C}_{26}\text{H}_{37}\text{O}_9$ , 493.2438).

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#### References and Notes

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