Eunicellin-Based Diterpenoids, Australins A−D, Isolated from the Soft Coral *Cladiella australis*

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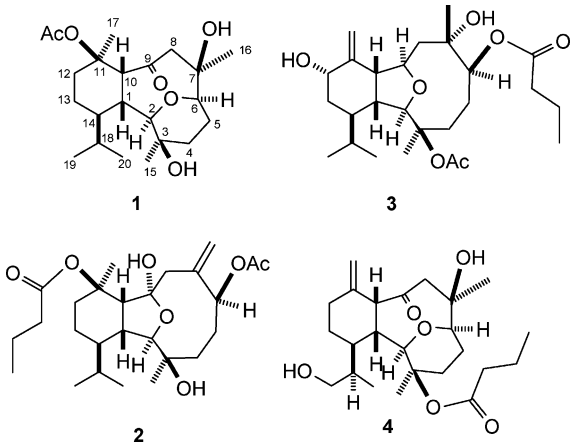
The EtOAc extract of a Taiwanese soft coral, *Caldiella australis*, yielded four new eunicellin-based diterpenoids, australins A−D (**1**−**4**). The chemical structures of these metabolites were determined on the basis of extensive spectroscopic (including 1D and 2D NMR) analyses and by comparison of their NMR spectral data with those of related metabolites. Metabolite **2** was found to possess a moderate cytotoxic activity against selected breast and liver cancer cell lines.

Worldwide chemical investigations on marine invertebrates have demonstrated structural diversity in the terpenoid constituents of octocorals.[1] In the course of our chemical study on the octocorals, metabolites with promising cytotoxic activity, including cembrane-,[2] C-4 norcembrane-,[3] xeniaphyllene-,[4] briarane-,[5] and eunicellin[6,7]-based diterpenoids, have been isolated and identified and some of these metabolites are cytotoxic against selected cancer cell lines.[2−7] Previous reports on the chemical constituents of soft corals belonging to the genus *Cladiella*,[8−16] including *C. australis*,[15,16] have illustrated the predominance of eunicellin-based diterpenoids as secondary metabolites. Our investigation on the EtOAc extract of *Cladiella australis* (Macfadyen, 1936), a soft coral collected from the southern Taiwanese waters, has led to the isolation of four new eunicellin-based diterpenoids, australins A−D (**1**−**4**). The molecular structures of these metabolites, including their relative stereochemistries, were established on the basis of extensive spectroscopic methods (including 1D and 2D NMR) and by comparison of their NMR spectral data with those of related metabolites. Metabolite **2** has been shown to exhibit moderate cytotoxic activity against MCF-7, MDA-MB-231 (human breast cancer), and HepaG2/DMEM-12 (human liver cancer) cell lines.



Results and Discussion

Frozen specimen of *C. australis* was homogenized with EtOAc, and the organic layer was separated and concentrated. The residue was subjected to fractionation and purification, utilizing a series of normal-phased chromatographic systems to afford compounds **1**−**4**.

Australin A (**1**) was isolated as a colorless oil with a molecular formula of $C_{22}H_{36}O_6$, implying five degrees of unsaturation, as established by the HRFABMS *m/z* 397.2589 $(M + H)^+$. The IR absorption bands at ν_{max} 3447 (broad), 1732, and 1716 cm^{-1} revealed the presence of hydroxy and carbonyl functionalities. The ion peaks appeared in the FABMS at *m/z* 337 $[M - AcOH + H]^+$ and 301 $[M - AcOH - 2H_2O + H]^+$, suggesting the presence of an acetate and two hydroxy groups in the molecule of **1**. The ^{13}C NMR spectrum measured in $CDCl_3$ showed signals of 22 carbons (Table 1), which were assigned by the assistance of the DEPT spectrum to six methyls, five methylenes, six methines (including two oxymethines), two carbonyls, and three oxygenated quaternary carbons. The 3H singlet appearing at δ 2.01 in the ^1H NMR spectrum and the carbonyl carbon signal at δ 170.7 in the ^{13}C NMR spectrum were ascribable to an acetate. Therefore, the remaining four degrees of unsaturation identified compound **1** as a tricyclic ketonic diterpenoid. The ^1H NMR data of **1** (Table 2) showed three signals of tertiary methyls bonded to oxygenated carbons (δ 1.40, 1.23, and 1.12, each 3H, s) and two secondary methyls (δ 0.96 and 0.71, 3H each, d, J = 6.9 Hz) of an isopropyl moiety. Inspection of the HMQC spectrum showed that proton signals appearing at δ 2.30 (1H, dd, J = 12.0, 4.0 Hz), 4.54 (1H, dd, J = 4.0, 2.0 Hz), δ 3.91 (1H, s), and 3.73 (1H, dd, J = 11.6, 6.0 Hz) were correlated to two ring-juncture methine carbons at δ 49.4 and 53.6 (CH, each) and two oxymethine carbons at δ 75.8 and 79.9 (CH, each), respectively. The planar structure of this metabolite was further deduced from the $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectral correlations (Figures1 and 2). The $^1H-^1H$ COSY experiment assigned two isolated consecutive proton spin systems. One was found to extend from H-10 to H-12 through H-14 and further extend to the isopropyl moiety. The other was shown to extend from H_2-4 to H-6 (Figure 1). Moreover, the HMBC long-range correlations observed from H-2 to C-10 and C-14 and H-6 to C-2 established the 2,6-ether

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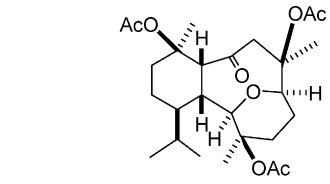
Table 1. ^{13}C NMR Chemical Shifts of Compounds **1**−**4**

C#	**1**[a]	**2**[b]	**3**[c]	**4**[c]
1	49.4 (CH)[d]	39.8 (CH)	44.8 (CH)	54.5 (CH)
2	75.8 (CH)	90.0 (CH)	91.4 (CH)	77.7 (CH)
3	69.5 (C)	73.9 (C)	86.5 (C)	81.3 (C)
4	33.5 (CH2)	34.1 (CH2)	35.5 (CH2)	27.6 (CH2)
5	20.2 (CH2)	32.2 (CH2)	29.3 (CH2)	20.5 (CH2)
6	79.9 (CH)	75.4 (CH)	84.2 (CH)	80.4 (CH)
7	85.6 (C)	146.9 (C)	75.5 (C)	84.8 (C)
8	49.8 (CH2)	49.7 (CH2)	46.2 (CH2)	48.1 (CH2)
9	212.3 (C)	106.5 (C)	80.0 (CH)	210.7 (C)
10	53.6 (CH)	47.0 (CH)	51.6 (CH)	56.7 (CH)
11	83.8 (C)	83.7 (C)	147.8 (C)	147.3 (C)
12	32.0 (CH2)	33.7 (CH2)	71.3 (CH)	31.1 (CH2)
13	19.4 (CH2)	18.0 (CH2)	30.5 (CH2)	26.2 (CH2)
14	35.7 (CH)	43.0 (CH)	35.5 (CH)	32.9 (CH)
15	30.2 (CH3)	26.8 (CH3)	23.1 (CH3)	23.6 (CH3)
16	22.9 (CH3)	119.1 (CH2)	23.6 (CH3)	23.0 (CH3)
17	22.4 (CH3)	24.1 (CH3)	113.4 (CH2)	109.3 (CH2)
18	26.9 (CH)	28.2 (CH)	28.6 (CH)	35.9 (CH)
19	21.6 (CH3)	21.9 (CH3)	21.7 (CH3)	10.0 (CH3)
20	14.4 (CH3)	14.8 (CH3)	15.5 (CH3)	67.4 (CH2)
Ac	22.6 (CH3)	22.7 (CH3)	21.4 (CH3)	
	170.7 (C)	170.6 (C)	171.8 (C)	
n-Bu		13.7 (CH3)	13.7 (CH3)	13.7 (CH3)
		18.5 (CH2)	18.4 (CH2)	18.7 (CH2)
		36.5 (CH2)	37.4 (CH2)	37.7 (CH2)
		173.5 (C)	172.2 (C)	172.3 (C)

[a] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. [b] Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C. [c] Spectra recorded at 125 MHz, in $CDCl_3$ at 25 °C. [d] Attached protons were determined by DEPT experiments. The values are in ppm downfield from TMS.

linkage of the tetrahydropyran moiety. Also, the HMBC correlation from H_2-8 to the carbon atoms resonating at δ 212.3 (C), 85.6 (C), and 79.9 (CH) indicated the C-9 location of the ketone. The downfield chemical shifts for H_3-17 (δ 1.40) and C-11 (δ 83.8) determined the C-11 location of the acetate. Furthermore, comparison of NMR data of **1** with those of **5**[17] revealed that **1** could be the 3,7-diol derivative of **5**. This was evidenced from the upfield chemical shifts induced at C-3 ($\Delta\delta$ −14.2) and C-7 ($\Delta\delta$ −7.1) in **1** relative to those of **5**. Therefore, the gross structure of **1** was suggested.



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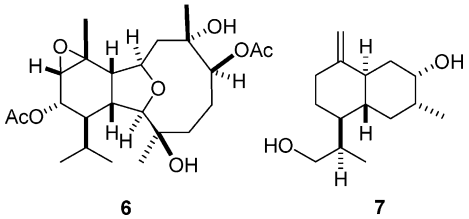

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The relative stereochemistry of **1** was established by the analyses of NOE correlations (Figure 2) observed in the NOESY spectrum of **1**. Assuming the β-orientation of the ring-juncture H-10, it was found that one of the methylene protons at C-8 (1H, δ 2.99, J = 12.0 Hz) exhibited a NOE interaction with H-10, and therefore it was assigned as H-8β, and the other C-8 proton (1H, δ 1.94, J = 12.0 Hz) as H-8α. The NOE correlations observed between H-8α and H_3-16, H_3-16 and H-6, H-6 and H-2, and H-2 and H_3-15 suggested the α-orientations of H_3-16, H-6, H-2, and H_3-15, respectively. The equatorial and α-oriented configuration of the methyl group at C-11 was evidenced from the NOE responses found between H-8α and H_3-17 (δ 1.40, 3H, s), and H_3-17 and H-10. In addition, NOE interactions displayed between H-10 and H-1 and between H-1 and the methyl (3H, δ 2.01, s) of the acetate at C-11 indicated the *cis* orientation of H-1, H-10, and 11-OAc. The lack of NOE interactions between H-1 and H-14 suggested the β-orientation of the C-14-linked isopropyl group. The stereochemistry of the cyclohexane moiety was further supported from the NOE responses observed between the protons of one isopropyl methyl and 11-OAc. The significant NOE interactions between H-1 and H_3-15, H_3-15 and H-2, and H-2 and H-1 disclosed the β-orientation of the tetrahydropyran moiety in **1**. From the above findings and other NOE correlations (Figure 2), the structure of **1** was found to possess the (1*R**,2*R**,3*R**,6*R**,7*R**,10*R**,11*R**,14*R**)-configuration.

Austarlin B (**2**) was obtained as a white solid. On the basis of its HRFABMS (*m/z* 467.3009, $[M + H]^+$) and NMR spectral data (Tables 1 and 2), the molecular formula of **2** was established as $C_{26}H_{42}O_7$, consistent with six degrees of unsaturation. The IR spectrum of **2** showed the presence of hydroxy (ν_{max} 3398 cm^{-1}, broad) and carbonyl functionalities (ν_{max} 1735 and 1703 cm^{-1}). The ion peaks appearing in the FABMS at *m/z* 407 ($[M - AcOH + H]^+$) and 379 ($[M - C_3H_7COOH + H]^+$) are due to the elimination of an acetic and a butyric acid, respectively. The NMR spectral data of **2** showed the appearance of an 1,1-disubstituted carbon−carbon double bond (δ_C 119.1, CH_2 and 146.9, C; δ_H 5.33, s and 5.43, s). Two ester carbonyls (δ170.6, C and 173.5, C) were also assigned from the ^{13}C NMR spectrum and were HMBC correlated with the acetate methyl (δ 1.99, 3H s) and methylenes (δ 1.63, 2H, m and 2.19, 2H, dd, J = 15.0, 7.5 Hz) of a *n*-butyrate, respectively. Therefore, the remaining three degrees of unsaturation revealed that **2** is a tricyclic compound. Furthermore, the ^{13}C NMR data of **2** showed good similarity with those of **1**, supporting its eunicellin-based skeleton. The main differences arised from the replacement of a tertiary methyl group and a ketone group in **1** by an exomethylene and a hemiketal (δ 106.5, C) in **2**, respectively. Their locations were proved to be at C-7 and C-9, on the basis of HMBC correlations observed from H_2-8 (δ 2.87, 2H, s) to C-7 (δ 146.9, C) and C-9 (δ 106.5) and from H-10 (δ 3.14, 1H, d, J = 8.1 Hz) to C-9. Moreover, a proton (δ 3.72, 1H, br s) linked to an oxymethine carbon (δ 90.0, CH) showed correlations with C-10 and C-14 and was assigned as H-2. The correlation observed from H-2 to C-9 directly indicated the formation of the 2,9-ether linkage of a tetrahydrofuran moiety. Furthermore, the HMBC correlations detected from the methyl protons at δ 1.26 (3H, br s) and the quaternary oxycarbon at δ 73.9 and C-2 suggested a second tertiary hydroxy group at C-3. Therefore, the acetyl and *n*-butyryloxy moiety should be linked to the remaining oxygenated carbons (δ 75.4, CH and 83.7, C), which were identified, by HMBC experiment, as C-6 and C-11, respectively. Their location was further evidenced by NOE spectral analyses (discussed later). The above findings combined with more detailed analyses on the ^1H and ^{13}C NMR spectral data and the detected 2D correlations in $^1H-^1H$ COSY and HMBC spectra led to the establishment of the gross structure of **2** (see Tables 1 and 2 and Figure 1).

The NOE correlations displayed in the NOESY spectrum of **2** confirmed the β-orientation of H-1 and H-10 and the α-orientation of H-2, H-6, H-14, H_3-15, and CH_3-17, as

Table 2. ^{1}H NMR Chemical Shifts of Compounds **1**−**4**

H#	**1**[a]	**2**[b]	**3**[c]	**4**[c]
1	2.30 dd (12.0, 4.0)[d]	2.23 dd (8.1, 7.5)	2.22 d (9.5, 7.5)	2.27 dd (12.5, 4.5)
2	3.91 s	3.72 br s	3.74 s	3.90 s
4α	1.53 ddd (13.6, 13.6, 3.2)	1.56 m	2.06 m	1.42 t (12.5)
4*β*	1.70 ddd (13.6, 3.2, 3.	1.79 m	2.59 dd (14.5, 9.5	2.99 dd (12.0, 4.5)
5α	1.60 m	2.02 m	1.54 t (10.0)	1.69 br dd (12.5, 7.0)
5*β*	1.90 m	1.94 m	1.48 dd (10.0, 6.5)	1.35 t (12.5)
6	3.73 dd (11.6, 6.0)	5.25 dd (11.2, 4.8)	5.64 d (6.5)	3.83 dd (12.5. 7.0)
8α	1.94 d (12.0)	2.87 s	1.88 m	2.03 d (12.0)
8*β*	2.99 d (12.0)	2.87 s	1.88 m	2.76 d (12.0)
9			4.50 dd (15.0, 7.5)	
10	4.54 dd (4.0, 2.0)	3.14 d (8.1)	2.99 t (7.5)	4.25 d (4.5)
12α	2.23 ddd (13.6, 13.6, 4.4)	1.98−2.02 m		2.85 ddd (13.5, 13.5, 4.5)
12*β*	1.89 m	1.98−2.02 m	4.42 br t (3.5)	2.22 br d (13.5)
13α	1.60 m	1.46 m	1.89 m	1.75 m
13*β*	1.30 m	1.38 m	1.32 m	1.14 ddd (13.5, 4.5, 4.5)
14	2.03 m	1.52 m	1.84 m	2.44 m
15	1.23 3H, s	1.26 3H, s	1.42 3H, s	1.53 3H, s
16	1.12 3H, s	5.43 s	1.20 3H, s	1.13 3H, s
		5.33 s		
17	1.40 3H, s	1.69 3H, s	5.02 s	4.73 s
			4.84 s	4.61 s
18	1.89 m	1.81 m	1.81 m	1.92 br dd (12.5, 7)
19	0.71 3H, d (6.9)	0.76 3H, d (6.9)	0.80 3H, d (7.0)	0.77 3H, d (7.0)
20	0.96 3H, d (6.9)	0.95 3H, d (6.9)	0.99 3H, d (7.0)	3.66 dd (10.0, 7.0)
				3.51 dd (10.0, 7.0)
Ac	2.01 3H, s	1.99 3H, s	2.08 3H, s	
n-Bu		0.99 3H, t (7.5)	0.99 3H, t (7.5)	1.02 3H, t (7.5)
		1.63 2H, m	1.67 2H, m	1.74 2H, m
		2.19 2H, dd (15.0, 7.5)	2.35 dd (15.0, 7.5)	2.39 2H, dd (15.0, 7.5)
			2.28 dd (15.0, 7.5)	

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. [b] Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C. [c] Spectra recorded at 500 MHz in $CDCl_3$ at 25 °C. [d] The *J* values are in Hz in parentheses.

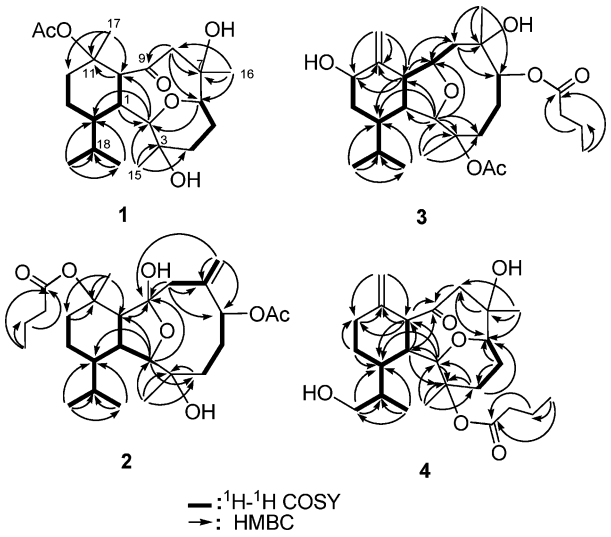


Figure 1. ^{1}H−^{1}H COSY and HMBC correlations for **1**−**4**.

illustrated in Figure 2. When the ^{1}H NMR spectrum of **2** was measured in C_5D_5N, it was found that a significant pyridine-induced downfield shift ($\Delta\delta = \delta CDCl_3 - \delta C_5D_5N$) was experienced by H_3-17 ($\Delta\delta = -0.26$ ppm), which could be achieved only when H_3-17 and 9-OH are axially oriented on the same face of the molecule.[18,19] The NOE interaction found between the α-methylene protons (δ 2.19, 2H, dd, J = 15.0, 7.5 Hz) of the *n*-butyrate and protons of one isopropyl methyl (δ 0.95, 3H, d, J = 6.9 Hz) further indicated the C-11 location and *β*-orientation of the butyrate moiety. The NOE response detected between the acetate methyl protons at δ 1.99 (3H, s) and the olefinic proton at δ 5.43 (1H, s), which in turn NOE interacts with H-5*β* also suggested the C-6 location and *β*-orientation of the acetate group. Therefore, the structure of **2** with a

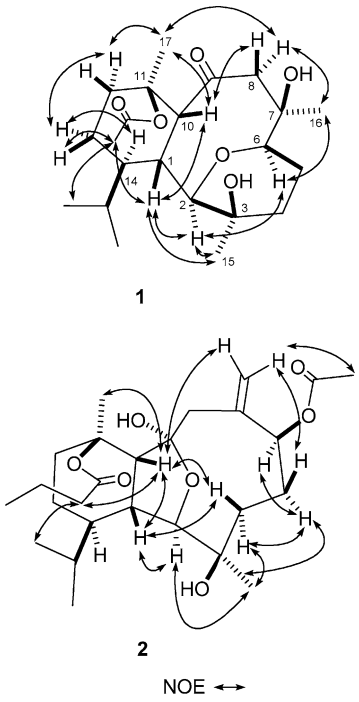
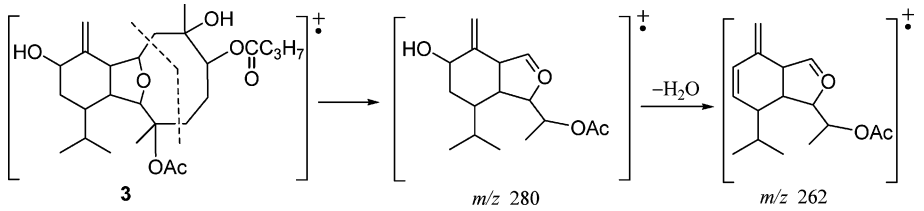


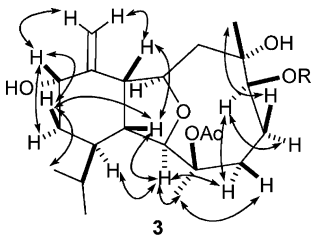
Figure 2. Key NOESY correlations of **1** and **2**.

(1*R**,2*R**,3*R**,6*S**,9*R**,10*R**,11*R**,14*R**)-configuration was fully determined.

Australin C (**3**) was obtained as a colorless viscous oil. Its molecular formula $C_{26}H_{42}O_7$ was determined by the HRFABMS (*m/z* 467.3009, $[M + H]^+$). Thus, **2** and **3** have the same molecular formula. IR absorption bands, FABMS fragmentation pattern, and the NMR spectral data (Tables 1 and 2) suggested that both **2** and **3** possess the same substituents and are geometrical isomers. The NMR spectra of **3** showed the appearance of two new oxygenated

Scheme 1. Proposed Fragmentations of **3** in FABMS





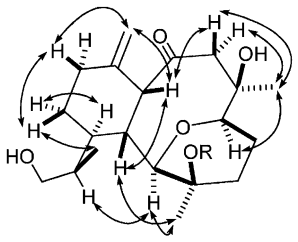


Figure 3. Key NOESY correlations of **3** and **4**.

methines (^{13}C NMR: δ 80.0, CH and 71.3, CH; ^{1}H NMR: δ 4.50, 1H, dd, J = 15.0, 7.5 Hz and 4.42, 1H, t, J = 3.5 Hz), instead of the hemiketal (δ 106.5, C) and the sp^3 methylene group at C-9 and C-12 in **2**, respectively. The C-9 and C12 locations of these two new oxygenated methine protons were determined on the basis of the ^{1}H−^{1}H COSY correlations establishing a consecutive proton spin system extending from H_2-8 to H-12 through H-10, H-1, and H-14. The HMBC correlations observed from the olefinic protons (δ 4.84 and 5.02, 1H, s, each) to C-10 (δ 51.6, CH) and C-12 (δ 71.3, CH) suggested that the exomethylene group should be positioned at C-11. Analyses of the ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY correlations led to the establishment of the carbon skeleton as seen in **3**. However, it was found to be difficult to determine the exact locations of the acetoxyl and the *n*-butyryloxyl, at the downfield shifted C-3 and C-6, from the 2D NMR spectral analyses. Inspection of the MS spectral data revealed that two peaks appearing at *m/z* 280 and 262 should arise from the fission of the 10-membered ring between C-3 and C-4, and C-8 and C-9, and the following dehydration (Scheme 1). Thus, the acetoxyl and the *n*-butyryloxyl should be positioned at C-3 and C-6, respectively. Furthermore, the relative stereochemistry of **3** was established by careful examination of the NOE correlations (Figure 3). The NOE correlations detected between the α-oriented H-2 (δ_H 3.74, 1H, s) and H-4α (δ_H 2.06, 1H, m), and between H-4α and H-6 (δ 5.64, 1H, d, J = 6.5 Hz), implied the β-configuration of the 6-*n*-butyryloxy group. Also, H_3-16 (δ 1.20, 3H, s) exhibited an NOE response with H-5β (δ 1.48, 1H, dd, J = 10.0, 6.5 Hz) but not with H-6α and revealed the α-orientation of hydroxy group at C-7. Moreover, it was found that the ^{1}H and ^{13}C NMR spectral data of **3** showed high similarity in the 10-membered ring part, especially for the data of chemical shifts of H_2-5, H-6, H_3-16, C-5, C-6, C-7, and C-16 and coupling constant of H-6, in comparison with those of a known metabolite, epoxycladine B (**6**),[14] and further confirmed the relative stereochemistry at C-6 and C-7 of **3**. Therefore, the structure of **3** was determined and was found to possess a (1*R**,2*R**,3*R**,6*S**,7*S**,9*R**,10*R**,12*S**)-configuration.

The HRFABMS (*m/z* 423.2749, $[M + H]^+$) of the most polar metabolite, australin D (**4**), established the molecular formula $C_{24}H_{38}O_6$, consistent with six degrees of unsaturation. Again, the IR indicated the existence of hydroxy (ν_{max} 3445 cm^{-1}, broad) and carbonyl (ν_{max} 1734 cm^{-1}) functionalities. The FABMS of **4** showed a peak at *m/z* 335 ($[M - C_3H_7COOH + H]^+$), due to the presence of a butyrate ester. This was supported by the appearance of *n*-butyryloxy signals (δ_C 13.7, CH_3; 18.7, CH_2; 37.7, CH_2; and 172.3, C; δ_H 1.02, 3H, t, J = 7.5 Hz, 1.74, 2H, m, and 2.39, 2H, J = 15.0, 7.5 Hz). Moreover, the ^{13}C NMR data (Table 1) designated a ketone carbonyl (δ 210.7, C) and an exomethylene (δ 109.3, CH_2 and 147.3, C) in **4**. Therefore, **4** is a tricyclic diterpenoid. Comparison of the NMR spectral data of **4** with those of **1**−**3** clearly indicated the replacement of one secondary methyl at C-18 by an oxymethylene (δ_C 67.4, CH_2; δ_H 3.51 and 3.66 [each 1H, dd, J = 10.0, 7.0 Hz]). The HMBC correlations displayed from H_2-8 (δ 2.03 and 2.76, each 1H, d, J = 12.0 Hz) and the ring-juncture H-10 (δ 4.25, 1H, d, J = 4.5 Hz) to the carbonyl carbon (δ 210.7, C) and from the olefinic H_2-17 (δ 4.61 and 4.73, each 1H, s) to C-10 (δ 56.7, CH) positioned the ketone and the exomethylene at C-9 and C-11, respectively. Moreover, it was found that the NMR spectral data of **4** closely resembled those of **1** from C-1 to C-10 except for the marked downfield shifts induced at C-3 ($\Delta\delta_C$ +11.8) and H_3-15 ($\Delta\delta_H$ +0.30) relative to **1**, indicating the attachment of C-3 with the butyrate in **4**. This can be further seen from the strong anisotropic effect induced on the neighboring H_2-4 by the ester carbonyl at C-3. As a result, **4** was suggested as an euncillin-based metabolite with a hydroxymethylene substituent at C-18. Also, the chemical shifts of C-18 (δ 35.9, CH), C-19 (δ 10.0, CH_3), and C-20 (δ 67.4, CH_2) in **4** were found to be very close to the hydroxyisopropyl group of **7** (δ 34.6, 10.2, and 67.4, respectively).[20] From the above findings together with the partial structures deduced from the ^{1}H−^{1}H COSY correlations and connectivities interpreted from the HMBC spectrum (Figure 1) the planar structure of australin D (**4**)was thus determined.

The relative stereochemistry of **4** was elucidated by careful interpretation of the NOESY correlations (Figure 3) and other ^{1}H NMR parameters (δ and J values) and was found to be the same as that of **1** at C-1, C-2, C-3, C-6, C-7, C-10, and C-14. The chiral center at C-18 was proved to be *R**-configured on the basis of NOE interactions displayed between the β-oriented H-1 and H_3-19 and between the α-oriented H-2 and H-18. On the basis of the above-mentioned observations together with other NOE responses (Figure 3), the (1*R**,2*R**,3*R**,6*R**,7*R**, 10*R**,-

14*R**, 18*R**)-configuration of **4** was assigned and the structure of **4** was established.

Several structure-related compounds have been shown to exhibit cytotoxicity against the growth of some cancer cell lines.[7,17] Our evaluation of in vitro cytotoxic activity of metabolites **2** and **4** also reveled that **2** showed moderate cytotoxicity against MDA-MB-231, MCF-7, and HepaG2/DMEM-12 cell lines (ED_{50}'s 6.4, 8.6, and 2.4 μg/mL, respectively), while **4** exhibited only weak activity against MDA-MB-231 (ED_{50} 24.4 μg/mL).

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and were uncorrected. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. Ultraviolet spectra were recorded on a Hitachi U-3210 UV spectrophotometer, and IR spectra were recorded on a Jasco FT/IR-5300 infrared spectrophotometer. EIMS was obtained with a VG Quattro GC/MS spectrometer. HRMS spectra were recorded on a Finnigan MAT-95XL mass spectrometer. The NMR spectra were recorded on a Bruker AVANCE DPX300 FT-NMR, a Bruker AMX-400 FT-NMR, or a Varian Unity INOVA 500 FT-NMR, in $CDCl_3$ using TMS as internal standard, unless otherwise indicated. Si gel (Merck, 230−400 mesh) was used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC.

Animal Material. The soft coral *C. australis* was collected at a depth of 15−20 m off the coast of the southernmost tip of Taiwan in February 2001 and frozen until use. A voucher specimen was deposited at the Department of Marine Resources, National Sun Yat-sen University.

Extraction and Separation. Frozen soft coral (1.6 kg, wet wt) was homogenized with EtOAc (3 L × 3) and then filtered. The organic layer was separated, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to yield a brown viscous residue (23.5 g). The residue was subjected to column chromatography on silica gel, and elution was performed with EtOAc−*n*-hexane (stepwise, 0−100% EtOAc) followed by MeOH−EtOAc (stepwise, 0−50% MeOH) to yield 76 fractions. Fractions 44 eluted with EtOAc−*n*-hexane (3:7) was purified by normal-phase HPLC, using acetone−dichloromethane−*n*-hexane (1:1:10) to obtain **2** (3.5 mg). Fraction 55 eluted with EtOAc−*n*-hexane (2:3) was rechromatographed by normal-phase HPLC, using EtOAc−*n*-hexane (gradient, 1:4 to 1:1) to yield **1** (8.5 mg). Fraction 67 eluted with EtOAc−*n*-hexane (1:1) was purified by silica gel column chromatography to yield a mixture, which was purified by normal-phase HPLC using acetone−*n*-hexane (1:4) to afford **3** (1.5 mg). Fraction 72 eluted with EtOAc−*n*-hexane (7:3) was subjected to silica gel column chromatography using acetone−*n*-hexane (gradient, 1:4 to 1:3) to yield a mixture, which was purified by normal-phase HPLC using acetone−*n*-hexane (gradient, 1:2 to 1:1) to afford **4** (2 mg).

Australin A (1): colorless oil; $[\alpha]^{25}_D$ −60° (*c* 0.58, $CHCl_3$); IR (neat) ν_{max} 3447, 2961, 2934, 2874, 1732, 1716, 1665, 1454, 1368, 1253, 1093 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2; FABMS *m/z* 419 (3.8, $[M + Na]^+$), 397 (100, $[M + H]^+$), 337 (57.7, $[M - AcOH + H]^+$), 319 (15.4, $[M - AcOH - H_2O + H]^+$), 301 (3.6, $[M - AcOH - 2H_2O + H]^+$); HRFABMS *m/z* 397.2589 (calcd for $C_{22}H_{37}O_6$, 397.2591).

Australin B (2): white solid; mp 137−139; $[\alpha]^{25}_D$ −91° (*c* 1.32, $CHCl_3$); IR (neat) ν_{max} 3399, 2959, 2932, 2874, 1735, 1703, 1650, 1443, 1370, 1277, 1032 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Tables 1 and 2; FABMS *m/z* 489 (0.2, $[M + Na]^+$), 467 (0.1, $[M + H]^+$), 407 (0.7, $[M - AcOH + H]^+$), 389 (9.1, $[M - AcOH - H_2O + H]^+$), 379 (1.1, $[M - CH_3CH_2CH_2COOH + H]^+$), 319 (3.5, $[M - AcOH - CH_3CH_2CH_2COOH + H]^+$), 301 (3.5, $[M - AcOH - CH_3CH_2CH_2COOH - H_2O + H]^+$); HRFABMS *m/z* 467.3009 (calcd for $C_{26}H_{43}O_7$ 467.3010).

Australin C (3): colorless oil; $[\alpha]^{25}_D$ +35° (*c* 0.46, $CHCl_3$); IR (neat) ν_{max} 3443, 2961, 2950, 2851, 1732, 1702, 1456, 1371, 1257, 1062 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; FABMS *m/z* 489 (0.1, $[M + Na]^+$), 467 (0.1, $[M + H]^+$), 407 (6.5, $[M - AcOH + H]^+$), 389 (0.6, $[M - AcOH - H_2O + H]^+$), 319 (0.9, $[M - AcOH - CH_3CH_2CH_2COOH + H]^+$), 280 (5.3, $[M - C_{10}H_{18}O_3]^+$), 262 (2.2, $[M - C_{10}H_{18}O_3 - H_2O]^+$); HRFABMS *m/z* 467.3009 (calcd for $C_{26}H_{43}O_7$ 467.3010).

Australin D (4): colorless oil; $[\alpha]^{25}_D$ −54° (*c* 0.50, $CHCl_3$); IR (neat) ν_{max} 3445, 2960, 2930, 2890, 1734, 1669, 1456, 1539, 1457, 1377, 1262, 1099, 1059 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; FABMS *m/z* 445 (0.6, $[M + Na]^+$), 423 (2.5, $[M + H]^+$), 363 335 (1.0, $[M - CH_3CH_2CH_2COOH + H]^+$), 317 (1.1, $[M - CH_3CH_2CH_2COOH - H_2O + H]^+$), 299 (0.6, $[M - CH_3CH_2CH_2COOH - 2H_2O + H]^+$); HRFABMS *m/z* 423.2749 (calcd for $C_{24}H_{39}O_6$ 423.2748).

Cytotoxicity Testing. Hepa59T/VGH and KB cells were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of the test compounds **2** and **4** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[21,22]

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