Hippuristerones E−I, New Polyoxygenated Steroids from the Gorgonian Coral *Isis hippuris*

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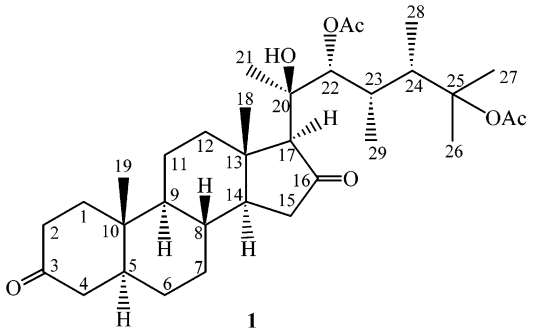
Five novel (22*R*,23*S*,24*S*)-steroids, hippuristerones E−I (**1**−**5**), have been isolated from the gorgonian coral *Isis hippuris*. The structures of steroids **1**−**5** were deduced by extensive 1D and 2D NMR studies (^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY experiments). The structure of **1** was further supported by molecular mechanics calculations.

Gorgonian corals have been known to be rich sources of polyoxygenated steroids with novel structures that exhibit a variety of biological activities.[1] As part of our ongoing study on chemical constituents of the Taiwanese gorgonian and soft corals,[2] the gorgonian coral *Isis hippuris* L. (phylum Cnidaria, order Gorgonacea, family Isididae),[3] which is a major inhabitant in Taiwanese tropical waters, has been the subject of an investigation. Previous studies on *I. hippuris* have resulted in the isolation of a series of novel metabolites including several highly oxygenated spiroketal steroids[4−8] and polyoxygenated gorgosteroids,[9,10] a (22*R*,23*S*,24*S*)-polyoxygenated steroid, hippuristerone A,[11] and six suberosane-type cytotoxic sesquiterpenes.[12] A study on an Indonesian gorgonian *I. hippuris* also has led to the discovery of a series of polyoxygenated steroids, including hippuristerones B−D.[13] Our present study on the constituents of this gorgorian coral has resulted in the isolation of five novel (22*R*,23*S*,24*S*)-steroids, hippuristerones E−I (**1**−**5**). The structures, including the relative configurations of the new metabolites **1**−**5**, were elucidated by extensive spectral analysis, and the structure of **1** was further confirmed by molecular dynamics calculations.



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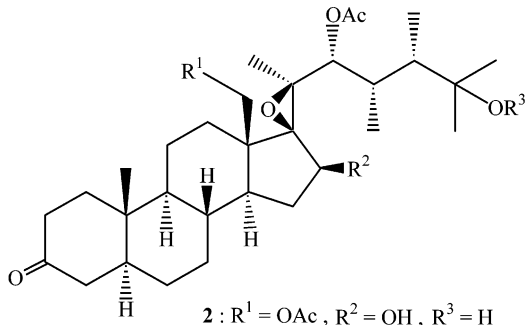

2 : R^1 = OAc , R^2 = OH , R^3 = H
3 : R^1 = OAc , R^2 = OH , R^3 = Ac
4 : R^1 = H , R^2 = OH , R^3 = H
5 : R^1, R^2, R^3 = H
6 : R^1 = H , R^2 = OH , R^3 = Ac

Results and Discussion

The gorgonian coral *I. hippuris* was frozen immediately after collection and subsequently freeze-dried. The freeze-dried organism was extracted successively with *n*-hexane and CH_2Cl_2 to afford a crude extract. The crude extract was purified by extensive column chromatography on silica gel and afforded the new steroids **1**−**5**.

Hippuristerone E (**1**) was obtained as a white powder. The HRFABMS of **1** established a molecular formula of $C_{33}H_{52}O_7$, implying eight degrees of unsaturation. The IR spectrum of **1** showed the presence of hydroxy (ν_{max} 3380 cm^{-1}) and carbonyl (ν_{max} 1720, 1717 cm^{-1}) groups in the molecule of **1**. Its ^{13}C NMR spectrum showed signals of nine methyl, eight methylene, eight methine, and eight quaternary carbons, including those of two ketones (δ 218.8, s; 211.8, s), two ester carbonyls (δ 171.4, s; 170.3, s), and three oxygenated sp^3 carbons (δ 85.7, s; 81.1, d; 77.1, s) (Table 2). By comparison of the above data with those of hippuristerone A (**6**),[11] it was found that **1** is a steroid with a structure related to that of **6**. In the ^{1}H NMR of **1** (Table 1), the doublets at δ 0.85 (3H, d, J = 7.0 Hz) and 0.88 ppm (3H, d, J = 7.0 Hz) were attributed to H_3-28 and H_3-29. Furthermore, five singlets appearing at δ 1.45 (3H), 1.43 (3H), 1.27 (3H), 1.04 (3H), and 1.00 ppm (3H) were due to the resonances of H_3-26, H_3-27, H_3-21, H_3-19, and H_3-18, respectively. Two signals that appeared at δ 2.12 (3H, s) and d 1.96 ppm (3H, s) revealed the presence of two acetoxy groups. From the ^{1}H−^{1}H COSY spectrum of **1** (Figure 1), it was possible to establish the proton sequences from H_2-1 to H_2-2; H_2-4 to H-9; H-8 to H-14; H-9 to H_2-11; H_2-11 to H_2-12; H-14 to H_2-15; H-22 to H-24; H-23 to H_3-29; and H-24 to H_3-28. The ring-junctured C-18 and C-19 methyl groups were positioned at C-10 and C-13, respectively, from the key HMBC correlations of H_3-19 with C-1, C-5, C-9, and C-10, and H_3-18 with C-12, C-13, C-14, and C-17,

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Table 1. ^{1}H NMR Chemical Shifts for Steroids **1**−**5**

proton	**1**[a]	**2**[b]	**3**[c]	**4**[c]	**5**[c]
1	2.02 m;	1.99 m;	2.00 m;	2.05 m;	2.04 m;
	1.33 m	1.34 m	1.40 m	1.33 m	1.62 m
2	2.40 m;	2.30 m;	2.38 m	2.30 m	2.32 m
	2.22 m	2.38 m			
4	2.28 m;	2.27 m;	2.30 m;	2.27 m;	2.27 m;
	2.11 m	2.11 m	2.10 m	2.10 m	2.09 m
5	1.54 m	1.56 m	1.56 m	1.56 m	1.54 m
6	1.38 m	1.40 m	1.40 m	1.40 m	1.37 m
7	1.65 m;	1.81 m;	1.80 m;	1.82 m;	1.78 m;
	1.02 m	0.91 m	0.92 m	0.92 m	0.92 m
8	1.60 m	1.71 m	1.76 m	1.56 m	1.49 m
9	0.93 m	0.78 m	0.89 m	0.70 m	0.75 m
11	1.60 m;	1.65 m	1.52 m;	1.60 m;	1.59 m;
	1.48 m		1.34 m	1.48 m	1.37 m
12	2.26 m;	2.17 m;	2.15 m;	1.82 m;	1.88 m;
	1.49 m	1.37 m	1.40 m	1.48 m	1.46 m
14	1.45 m	1.22 m	1.42 m	1.06 m	1.29 m
15	2.28 m;	2.22 m	2.28 m;	2.20 m;	1.64 m;
	1.90 m		1.45 m	1.45 m	1.40 m
16		4.09 t (7.0)	4.05 t (6.9)	4.06 t (7.2)	2.08 m;
					1.91 m
17	2.20 s				
18	1.00 s	4.31 d (11.5);	4.31 d (11.4);	0.95 s	0.92 s
		4.22 d (11.5)	4.21 d (11.4)		
19	1.04 s	1.03 s	1.02 s	1.01 s	1.00 s
21	1.27 s	1.61 s	1.59 s	1.59 s	1.55 s
22	5.36 d (2.8)[d]	4.64 d (10.5)	4.67 d (10.7)	4.62 d (10.8)	4.73 d (10.7)
23	2.22 m	1.48 m	2.30 m	2.45 m	2.33 m
24	1.92 m	1.54 m	2.05 m	1.55 m	1.59 m
26	1.45 s	1.24 s	1.56 s	1.23 s	1.28 s
27	1.43 s	1.21 s	1.42 s	1.20 s	1.20 s
28	0.85 d (7.0)	0.90 d (7.0)	0.90 d (7.4)	0.88 d (7.2)	0.91 d (7.4)
29	0.88 d (7.0)	0.86 d (7.0)	0.87 d (6.6)	0.84 d (6.9)	0.82 d (6.6)
OH-16				3.27 s	
OH-20	3.49 br s				
acetate	2.12 s;	2.13 s;	2.12 s;	2.11 s;	2.08 s;
methyls	1.96 s	2.07 s	2.07 s;		
			1.98 s		

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. [b] Spectra recorded at 500 MHz in $CDCl_3$ at 25 °C. [c] Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C. [d] J values (in Hz) in parentheses. The values are ppm downfield from TMS.

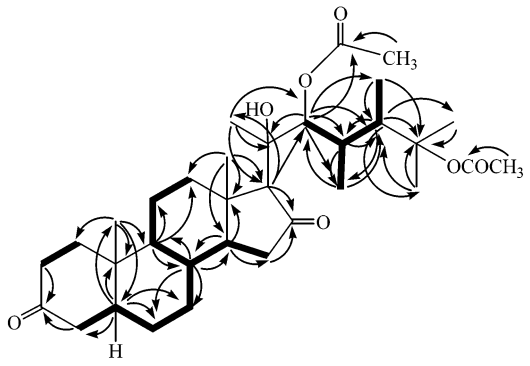

Figure 1. Selective HMBC and ^{1}H−^{1}H COSY correlations of **1**.

observed in an HMBC experiment (Figure 1). The ^{13}C NMR signals at δ 171.4 (s) and 170.3 ppm (s) correlated with the signals of the methyl protons at δ 2.12 (3H, s) and 1.96 ppm (3H, s) in the HMBC spectrum of **1** (Figure 1) and were consequently assigned as the carbon atoms of the two acetate carbonyls. The HMBC experiment of **1** further revealed the connectivity between H-22 (δ 5.36, 1H, d, J = 2.8 Hz) and the carbonyl carbon (δ 171.4, s) of an acetate unit and demonstrated the location of an acetoxy group to be at C-22. On the basis of the consideration of the molecular formula and by comparison of the NMR spectral data with **6**, the second acetoxy group should be attached at C-25 and one hydroxy group had to be placed at C-20. The two ketone groups at C-3 and C-16 were confirmed by their HMBC correlations with H_2-2 and H_2-4, and H_2-15 and H-17, respectively. The above observations and several other ^{1}H−^{1}H COSY and HMBC correlations (Table 3 and Figure 1) thus provided unambiguous evidence for the molecular framework of **1**.

The relative stereochemistry of **1** was deduced using a NOESY experiment (Table 3). In the NOESY spectrum of **1**, H_3-21 did not give correlation with H_3-18, and H-17 was found to exhibit correlations with H_3-21 and H-14. Thus, H-14, H-17, and H_3-21 should be placed on the α-face, since the C-18 methyl is the β-substituent at C-13, and the hydroxy group at C-20 should be β-oriented. Furthermore, H-8 exhibited NOE correlations with H_3-18 and H_3-19, but not with H-9 and H-14, indicating that H_3-19 and H-8 are situated on the β-face, and H-9 is situated on the α-face. Also, H-5 was found to exhibit correlations with H-9, but not with H_3-19, indicating that H-5 was α-oriented in **1**. The relative stereochemistry of the side chain of **1** is also determined by a NOESY experiment (Table 3). H-17 was found to show NOE correlations with H-22, H-23, and H-24. By detailed consideration of molecular models, it was suggested that the side chain substituents of **1** should possess orientations of C-22α, C-23α, and C-24α. Thus, hippuristerone E (**1**) is considered to be a novel 20-hydroxysteroid possessing a (22*R*,23*S*,24*S*)-23,24-dimethyl-22,25-diacetoxy side chain subunit.

Geometry optimization was performed using DISCOVER utilizing the CVFF (consistent valence force field) calculations for energy minimization. The results were visualized using INSIGHT II running on a Silicon Graphics IRIS

Table 2. ^{13}C NMR Chemical Shifts for Steroids **1**−**5**

carbon	**1**[a]	**2**[b]	**3**[c]	**4**[c]	**5**[c]
1	38.1 t	38.3 t	38.4 t	38.4 t	38.5 t
2	38.0 t	38.0 t	38.1 t	38.1 t	38.2 t
3	211.8 s	211.6 s	211.6 s	211.8 s	211.9 s
4	44.5 t	44.5 t	44.6 t	44.6 t	44.7 t
5	46.5 d	46.5 d	46.6 d	46.6 d	46.6 d
6	28.6 t	28.5 t	28.7 t	28.7 t	28.8 t
7	31.5 t	31.6 t	31.7 t	31.6 t	31.5 t
8	33.8 d	34.4 d	34.5 d	34.7 d	35.6 d
9	53.4 d	53.8 d	53.3 d	53.7 d	53.6 d
10	35.7 s	35.7 s	35.7 s	35.7 s	35.7 s
11	20.9 t	21.5 t	21.6 t	21.4 t	21.6 t
12	39.3 t	32.4 t	33.5 t	36.7 t	36.5 t
13	43.6 s	45.6 s	45.6 s	43.1 s	43.8 s
14	50.9 d	49.3 d	48.2 d	49.3 d	54.9 d
15	38.8 t	33.4 t	32.0 t	33.4 t	23.6 t
16	218.8 s	70.1 d	70.2 d	70.2 d	30.9 t
17	67.4 d	77.3 s	77.8 s	79.2 s	79.1 s
18	14.4 q	63.4 t	63.5 t	15.6 q	15.4 t
19	11.4 q	11.4 q	11.5 q	11.4 q	11.5 q
20	77.1 s	66.8 s	66.5 s	67.8 s	67.3 s
21	20.6 q	16.2 q	16.3 q	16.5 q	17.0 q
22	81.1 d	77.3 d	77.2 d	77.7 d	78.5 d
23	32.3 d	33.1 d	33.8 d	33.1 d	33.4 d
24	44.5 d	41.8 d	40.0 d	41.8 d	41.7 d
25	85.7 s	73.8 s	85.7 s	73.8 s	73.8 s
26	23.6 q	30.8 q	23.4 q	30.8 q	30.5 q
27	25.1 q	25.9 q	25.2 q	26.1 q	26.3 q
28	9.5 q	11.4 q	10.5 q	11.5 q	11.3 q
29	10.7 q	12.0 q	12.0 q	12.1 q	11.9 q
acetate	22.7 q	21.2 q	21.1 q	21.1 q	21.1 q
methyls	21.1 q	21.1 q	21.2 q		
			22.7 q		
acetate	171.4 s	171.4 s	171.3 s	171.6 s	170.7 s
carbonyls	170.3 s	171.3 s	171.1 s	171.1 s	171.1 s
			169.9 s	169.9 s	169.9 s

[a] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. [b] Spectra recorded at 125 MHz in $CDCl_3$ at 25 °C. [c] Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C. [d] Multiplicity deduced by DEPT and indicated by usual symbols. The values are ppm downfield from TMS.

(SGI) INDIGO XS24-4000. The conformational search suggested the most stable conformation as shown in Figure 2. The most stable conformation revealed the presence of a hydrogen bond between the hydroxy group at C-20 and the oxygen at C-22 (the H−O distance is 2.43 Å), so that the conformation around C-20/C-22 bond is fixed, and in turn prevents the free rotation around the C-22/C-23 bond. The distance between H-17 and H-24 was found to be 4.09 Å, which could reasonably explain the NOE correlations observed between these two protons. Furthermore, the orthogonal (torsional angle around 90°) arrangement of H-22 and H-23 protons can be used to rationalize the

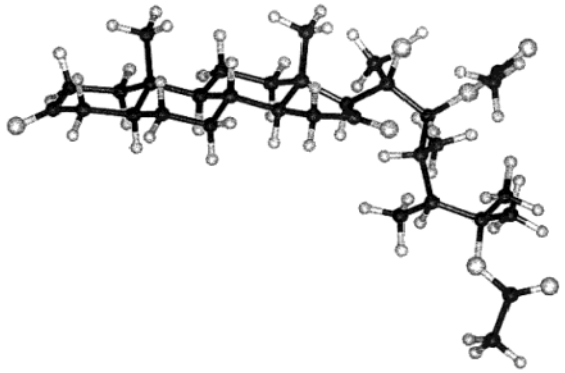

Figure 2. Stereoview of **1** generated from computer modeling.

presence of a very small coupling constant (2.8 Hz) between these two methine protons in the ^1H NMR spectrum of **1** (Table 1). On the basis of the above results, the molecular structure of **1** was established unambiguously.

Hippuristerone F (**2**) was obtained as a white amorphous solid that gave a $[M + H]^+$ peak at *m/z* 577.3742 in the HRFABMS, appropriate for a molecular formula of $C_{33}H_{52}O_8$, requiring eight degrees of unsaturation. The LRFABMS showed peaks at *m/z* 559 $[M + H - H_2O]^+$, 541 $[M + H - 2\ H_2O]^+$, 517 $[M + H - AcOH]^+$, 457 $[M + H - 2\ AcO]^+$, and 421 $[M + H - 2\ AcOH - 2\ H_2O]^+$, suggesting the presence of two hydroxy and two acetoxy groups in the molecular structure of **2**. The ^{13}C NMR spectrum of **2** showed signals of eight methyl, nine methylene, eight methine, and eight quaternary carbons, incuding one ketone (δ 211.6, s), two ester carbonyls (δ 171.4, s; 171.3, s), six carbons bonded to an oxygen (δ 77.3, s; 77.3, d; 73.8, s; 70.1, d; 66.8, s; 63.4, t), and two normal quaternary carbons (δ 45.6; 35.7) (Table 2). It was found that the above data are very similar to the carbon shifts of hippuristerone A (**6**), indicating that the structure of **2** should be close to **6**. However, it was observed that the signal of the methyl carbon C-18 in molecules of **2** disappeared and was replaced by a signal resonating at δ 63.4 (t). Also, the resonance of H_3-18 in **6** was replaced by signals that were downfield shifted to δ 4.31 (1H, d, J = 11.5 Hz) and 4.22 (1H, d, J = 11.5 Hz). Thus, the methyl group attached at C-13 in **6** should be converted to an oxygen-bearing methylene group. The ^{13}C NMR signals resonating at 171.4 and 171.3 ppm were found to be correlated with the signals of the methyl protons at δ 2.13 (3H, s) and 2.07 (3H, s) in the HMBC spectrum of **2** and consequently assigned as the carbon atoms of the two acetate carbonyls. The HMBC spectrum of **2** further revealed the connectivities between H-22 (δ 4.64, 1H, d, J = 10.5 Hz) and a carbonyl carbon (δ 171.4),

Table 3. Selective $^1H-^1H$ COSY, HMBC, and NOESY Correlations for **1**

C/H	$^1H-^1H$ COSY	HMBC	NOESY
3		H_2-2, H_2-4	
16		H_2-15, H-17	
17		H_3-18, H_3-21	H-14, H_3-21, H-22, H-23, H-24
18		H_2-12, H-14, H-17	H-8
19		H_2-2, H-5, H-9	H-8
20		H-17, H_3-21, H-22	
21		H-17, H-22	H-17, H-22
22	H-23	H-17, H_3-21, H-23, H-24, H_3-29	H-17, H_3-21, H-23, H-24
23	H-22, H-24, H_3-29	H-22, H-24, H_3-28, H_3-29	H-17, H-22
24	H-23, H_3-28	H-22, H-23, H_3-26, H_3-27, H_3-28, H_3-29	H-17, H-22
25		H-24, H_3-26, H_3-27, H_3-28	
26		H-24	
27		H-24	
28	H-24	H-23, H-24	
29	H-23	H-22, H-23, H-24	
22-OCOMe		H-22	

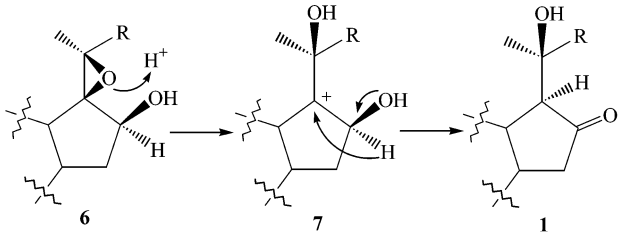

Figure 3. Possible biosynthetic pathway of hippuristerone (**1**).

and between H_2-18 and the other carbonyl carbon (δ 171.3), demonstrating the locations of two acetoxy groups to be at C-22 and C-18. On the basis of these results, the structure of hippuristerone F was established as 25-deacetoxy-25-hydroxy-18-acetoxyhippuristerone A, as described by formula **2**.

Hippuristerone G (**3**) was obtained as a white powder. The molecular formula $C_{35}H_{54}O_9$ was established by a FAB mass spectrum, which gave a $[M + H]^+$ peak at *m/z* 619, and by ^{13}C NMR spectral data, which reavealed the presence nine methyl, nine methylene, eight methine, and nine quaternary carbons, including one ketone (δ 211.6, s), three ester carbonyls (δ 171.3, s; 171.1, s; 169.9, s), six carbons attached by an oxygen (δ 85.7, s; 77.8, s; 77.2, d; 70.2, d; 66.5, s; 63.5, t), and two normal quaternary carbons (δ 45.6; 35.7). In comparison of the ^{13}C and ^1H NMR spectral data of **3** with those of **2** and **6**, the structure of hippuristerone G was established as 18-acetoxyhippuristerone A, as described by formula **3**.

Hippuristerones H (**4**) and I (**5**) had molecular formulas of $C_{31}H_{50}O_6$ and $C_{31}H_{50}O_5$, respectively, as suggested by their NMR (Tables 1 and 2) and HRFASMS data. In comparison of both ^1H and ^{13}C NMR spectral data of these two metabolites with those of hippuristerone A (**6**), it was concluded that the structures of **4** and **5** are very similar to that of **6**, except that the acetoxy group attached at C-25 of **6** was replaced by a hydroxy group in both **4** and **5**. Also, it was observed that the hydroxymethine functionality at C-16 of **4** was reduced to the corresponding methylene moiety in **5**. Thus, the structures of **4** and **5** were established unambiguously as 25-deacetoxy-25-hydroxyhippuristerone A and 16-dehydroxy-25-deacetoxy-25-hydroxylhippuristerone A, respectively.

Hippuristerone E (**1**) appeared to be biosynthesized from hippuristerone A (**6**) by a pathway as shown in Figure 3. The 17β,20β-epoxy group of **6** was ring-opened to form the intermediate **7**, which has a carbonium ion at C-17 and a β-hydroxy group at C-20. The Wagner−Meerwein shift of Hα-16 to the α-face of C-20 and the following deprotonation of the 16-hydroxy group converted the intermediate **7** into **1**.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and have not been corrected. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on Hitachi I-2001 and Jasco FT/IR-5300 infrared spectrophotometers. The NMR spectra were recorded on FT-NMR instruments at 300, 400, or 500 MHz for ^1H and 75, 100, or 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as an internal standard. Nuclear Overhauser and exchange spectroscopy (NOESY), $^1H-^1H$ correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and ^1H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) experiments were performed by using standard Bruker pulse sequences. Low-resolution mass spectra were obtained by fast atom bombardment (FAB) on a VG Quattro GC/MS spectrometer. High-resolution mass spectra (HRMS) were recorded by fast atom bombardment on a JEOL JMX-HX 110 mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography. Precoated silica gel plates (Merck Kieselgel 60 F_{254}, 2 mm) were used for analytical TLC.

Animal Material. The gorgonian coral *I. hippuris* was collected by hand using scuba at Green Island, which is located off the southeast coast of Taiwan, in February 1999, at a depth of 25 m, and was stored in a freezer until extraction. A voucher specimen was deposited in the Department of Marine Resources, National Sun Yat-Sen University (specimen no. GISC-102).

Extraction and Isolation. The gorgonian coral (4.3 kg fresh wt) was collected and freeze-dried. The freeze-dried material was minced and extracted exhaustively with *n*-hexane and CH_2Cl_2. The combined organic extract was evaporated to give a dark green residue (37.0 g), which was chromatographed on a SiO_2 column using solvents of increasing polarity from *n*-hexane to EtOAc. Steroids **1** (12.9 mg) and **2** (2.7 mg) were eluted with *n*-hexane/EtOAc (3:1). Steroid **3** (6.3 mg) was obtained by elution with *n*-hexane/EtOAc (5:1). A fraction eluted with *n*-hexane/EtOAc (3:1−3:2) was further purified by SiO_2 column using acetone/CH_2Cl_2 (1:3) to afford **4** (7.9 mg). A fraction eluted with *n*-hexane/EtOAc (4:1−3:1) was further purified by SiO_2 column using acetone/CH_2Cl_2 (1:3) to afford **5** (14.5 mg).

Hippuristerone E (1): white powder; mp 174−176 °C; $[\alpha]_D$ −92° (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3380, 1720, and 1717 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 583 $[(M + Na)^+, 2]$, 565 (0.2), 523 (1), 483 (1), 439 (1), 423 (1), 391(3), and 341 (7); HRFABMS *m/z* 583.3626 $(M + Na)^+$ (calcd for $C_{33}H_{52}O_7Na$, 583.3611).

Hippuristerone F (2): white powder; mp 125−127 °C; $[\alpha]_D$ −15° (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 3452, 1726, and 1244 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 577 $[(M + H)^+]$; HRFABMS *m/z* 577.3742 $(M + H)^+$ (calcd for $C_{33}H_{53}O_8$, 577.3726).

Hippuristerone G (3): white powder; mp 113−114 °C; $[\alpha]_D$ 23° (*c* 0.02, $CHCl_3$); IR (KBr) ν_{max} 3495, 1728, and 1248 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 619 $[(M + H)^+, 0.01]$, 551 (0.04), 499 (0.01), 481 (0.01), 439 (0.02), and 421 (0.02); HRFABMS *m/z* 641.3666 $(M + Na)^+$ (calcd for $C_{35}H_{54}O_9Na$, 641.3667).

Hippuristerone H (4): white powder; mp 122−124 °C; $[\alpha]_D$ 9° (*c* 0.31, $CHCl_3$); IR (KBr) ν_{max} 3493, 1726, and 1711 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 541 $[(M + Na)^+]$; HRFABMS *m/z* 519.3682 $(M + H)^+$ (calcd for $C_{31}H_{51}O_6$, 519.3672).

Hippuristerone I (5): white powder; mp 120−122 °C; $[\alpha]_D$ 12° (*c* 0.36, $CHCl_3$); IR (KBr) ν_{max} 1730, 1709, and 1246 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 525 $[(M + Na)^+]$; HRFABMS *m/z* 503.3734 $(M + H)^+$ (calcd for $C_{31}H_{51}O_5$, 503.3723).

Molecular Mechanics Calculations. The minimum energy conformation of hippuristerone E (**1**) was determined using the MSI Insight II/DISCOVER version 95 molecular modeling package incorporating an empirical force field, the consistent valence force field (CVFF),[14] on a Silicon Graphics IRIS Indigo XS24/R4000 workstation. All the force field calculations were carried out in vacuo (dielectric constant = 1). The conformational space of steroid **1** was scanned using the high-temperature molecular dynamics simulation technique followed by energy minimization. A 100 ps molecular dynamics simulation at 1000 K provided a set of 500 conformations of **1**. Each of them was used as a starting structure for the subsequent energy minimization (1000 steps, conjugated gradient algorithm). In the subsequent analysis, only the 15 conformations with a reasonably low energy (at most 5 kcal/mol higher with respect to the lowest energy conformer) were used. The conformer shown in Figure 2 is the lowest energy conformation of steroid **1**.

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(14) MSI Insight II/DISCOVER (version 95.0/2.97) is a molecular modeling software package of MSI Technologies Inc., Barnes Canyon Rd., San Diego, CA 92121.

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