Polyoxygenated Steroids from the Gorgonian *Isis hippuris*

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Eleven new hippuristanols (**1**−**4**, **7**−**9**, and **11**−**14**), along with eight known metabolites (**5**, **6**, **10**, and **15**−**19**), have been isolated from the gorgonian coral *Isis hippuris*. Single-crystal X-ray diffraction analyses supported the structure elucidation of known steroids **5** and **10**. The absolute structures of hippuristanols were established by application of modified Mosher's method on **19**. Compounds **14**−**19** have been found to exhibit significant cytotoxicity against several cancer cell lines.

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Previous studies on *Isis hippuris* have resulted in the isolation of a series of novel metabolites, including highly oxygenated spiroketal steroids that were named hippurins or hippuristanols,[1−7,9] polyoxygenated gorgosteroids,[5,6] (22*R*,-23*S*,24*S*)-polyoxygenated steroids,[7−9] and suberosane-type sesquiterpenes.[10] Some hippuristanols[9] and suberosane-type sesquiterpenes have been reported to have significant cytotoxicity against several cancer cell lines. Our continuing investigation on the chemical constituents of *I. hippuris*, collected by hand using scuba at Green Island, located off the southeast coast of Taiwan, in February 1999, has again afforded a series of hippuristanols. We describe herein the isolation, structure elucidation, and biological activity of these compounds.

Results and Discussion

The gorgonian coral *I. hippuris* was frozen immediately after collection, and the freeze-dried organism was extracted successively with *n*-hexane and CH_2Cl_2 to afford a crude extract. The crude extract was repeatedly purified by extensive column chromatography on silica gel and afforded 11 new (**1**−**4**, **7**−**9**, and **11**−**14**) and eight known steroids (**5**, **6**, **10**, and **15**−**19**, see Figure 1).

Both steroids **5** and **10** have been reported in the literatures,[3] but without supporting their structures by X-ray diffraction analyses. The results (Figure 2) of our study on single-crystal X-ray diffraction for both **5** and **10** further confirmed the overall structures of these two metabolites.

The absolute configuration of hippuristanols was determined by application of the modified Mosher's method.[13] Compound **19** was treated with (*R*)- or (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid [(*R*)- or (*S*)-MTPA] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and 4-(dimethylamino)pyridine (4-DMAP) to yield the (*R*)- and (*S*)-MTPA esters (**19b** and **19a**), respectively. The MTPA esters selectively formed at C-2 were elucidated from the ^{1}H NMR chemical shifts and coupling constants of H-2 in **19a** and **19b** (**19a**, δ 5.23, 1H, br d, J = 12.2 Hz, H-2; **19b**, δ 5.23, 1H, br d, J = 12.2 Hz, H-2), due to less hindrance of the equatorial hydroxy group attached at C-2. Comparison of the ^{1}H NMR chemical shifts for **19a** and **19b** (Δ values shown in Figure 3) led to the assignment of the *R*-configuration at C-2. Therefore, the absolute structure of **19** was determined as shown in formula **19**. Because of biogenic considerations, the absolute configurations at C-3, C-5, C-8, C-9, C-10, C-13, C-14, C-16, C-17, C-20, and C-24 of other hippuristanols, reported or discovered by the present study, were assumed to be identical with those of **19**.

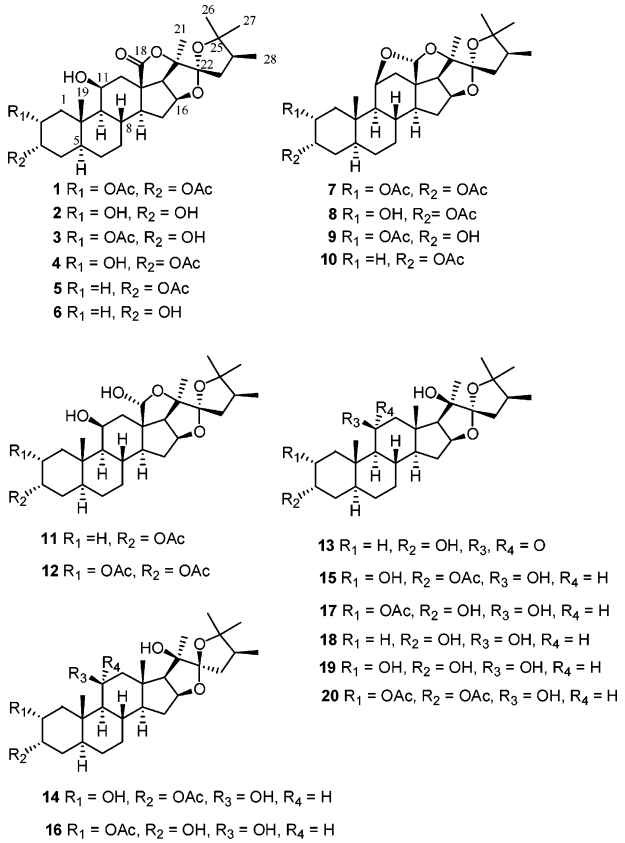

Figure 1. Structures of metabolites **1**−**20**.

Compound **1** was isolated as a white powder. The HRFABMS of **1** established a molecular formula of $C_{32}H_{46}O_9$.

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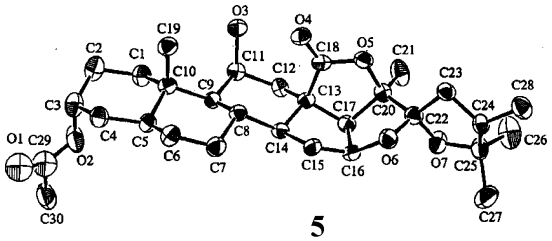
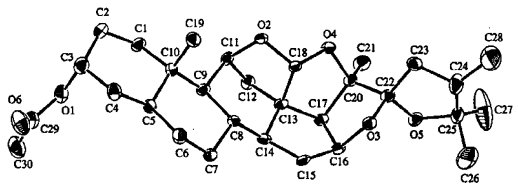
Table 1. ^{13}C NMR Spectral Data of Compounds **1**−**4**, **13**, and **14**

C #	**1**[b]	**2**[a]	**3**[a]	**4**[b]	**13**[a]	**14**[a]
1	37.5 (CH_2)[c]	40.2 (CH_2)[c]	36.6 (CH_2)[c]	41.2 (CH_2)[c]	31.0 (CH_2)[c]	41.2 (CH_2)[c]
2	69.7 (CH)	68.8 (CH)	72.4 (CH)	67.8 (CH)	29.0 (CH_2)	67.9 (CH)
3	69.4 (CH)	69.0 (CH)	67.4 (CH)	72.9 (CH)	66.4 (CH)	73.0 (CH)
4	31.8 (CH_2)	33.4 (CH_2)	33.3 (CH_2)	31.7 (CH_2)	35.4 (CH_2)	31.8 (CH_2)
5	40.1 (CH)	38.7 (CH)	38.7 (CH)	40.0 (CH)	39.0 (CH)	40.2 (CH)
6	26.6 (CH_2)	26.7 (CH_2)	26.6 (CH_2)	26.6 (CH_2)	27.9 (CH_2)	26.9 (CH_2)
7	32.2 (CH_2)	32.2 (CH_2)	32.2 (CH_2)	32.3 (CH_2)	32.7 (CH_2)	32.3 (CH_2)
8	29.1 (CH)	29.1 (CH)	29.1 (CH)	29.1 (CH)	35.9 (CH)	29.6 (CH)
9	57.7 (CH)	57.6 (CH)	57.7 (CH)	57.7 (CH)	64.7 (CH)	58.1 (CH)
10	37.6 (C)	37.3 (C)	37.5 (C)	37.7 (C)	35.9 (C)	37.1 (C)
11	67.0 (CH)	67.0 (CH)	66.9 (CH)	67.0 (CH)	210.5 (C)	68.1 (CH)
12	40.7 (CH_2)	40.6 (CH_2)	40.6 (CH_2)	40.7 (CH_2)	58.5 (CH_2)	49.9 (CH_2)
13	53.0 (C)	53.0 (C)	52.9 (C)	53.1 (C)	46.0 (C)	42.2 (C)
14	56.7 (CH)	56.7 (CH)	56.6 (CH)	56.7 (CH)	56.2 (CH)	56.9 (CH)
15	35.0 (CH_2)	34.9 (CH_2)	34.9 (CH_2)	35.0 (CH_2)	31.5 (CH_2)	34.0 (CH_2)
16	80.3 (CH)	80.2 (CH)	80.2 (CH)	80.3 (CH)	79.2 (CH)	80.1 (CH)
17	60.0 (CH)	59.9 (CH)	59.9 (CH)	60.0 (CH)	63.2 (CH)	66.1 (CH)
18	182.4 (C)	182.5 (C)	182.4 (C)	182.5 (C)	17.7 (CH_3)	18.6 (CH_3)
19	15.1 (CH_3)	14.9 (CH_3)	14.8 (CH_3)	15.2 (CH_3)	11.0 (CH_3)	15.3 (CH_3)
20	90.3 (C)	90.2 (C)	90.2 (C)	90.2 (C)	81.9 (C)	79.2 (C)
21	18.9 (CH_3)	18.8 (CH_3)	18.8 (CH_3)	18.9 (CH_3)	25.9 (CH_3)	29.1 (CH_3)
22	116.7 (C)	116.7 (C)	116.7 (C)	116.7 (C)	118.7 (C)	115.3 (C)
23	38.6 (CH_2)	38.5 (CH_2)	38.5 (CH_2)	38.6 (CH_2)	39.6 (CH_2)	40.9 (CH_2)
24	41.2 (CH)	41.1 (CH)	41.1 (CH)	41.2 (CH)	41.0 (CH)	41.9 (CH)
25	85.7 (C)	85.6 (C)	85.6 (C)	85.7 (C)	84.5 (C)	84.6 (C)
26	29.2 (CH_3)	29.0 (CH_3)	29.0 (CH_3)	29.0 (CH_3)	29.1 (CH_3)	28.4 (CH_3)
27	23.1 (CH_3)	23.0 (CH_3)	23.0 (CH_3)	23.1 (CH_3)	23.0 (CH_3)	23.0 (CH_3)
28	14.0 (CH_3)	13.9 (CH_3)	13.9 (CH_3)	14.0 (CH_3)	14.0 (CH_3)	14.7 (CH_3)
OAc	170.4 (C)		169.9 (C)	171.5 (C)		171.5 (C)
	170.3 (C)					
OAc	21.3 (CH_3)		21.3 (CH_3)	21.4 (CH_3)		21.4 (CH_3)
	21.1 (CH_3)					

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

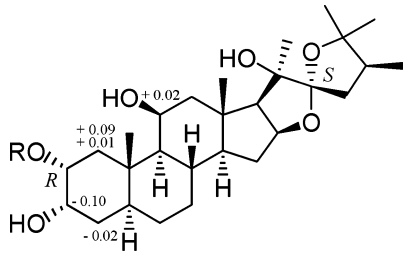


5



10

Figure 2. Computer-generated ORTEP drawings of compounds **5** and **10**. Hydrogen atoms were omitted for clarity.



19 a: R=(*S*)-MTPA
19 b: R=(*R*)-MTPA

$\Delta\delta=\delta(S)-\delta(R)$ (ppm)

Figure 3. ^1H NMR chemical shift differences [δ(*S*)-MTPA − δ(*R*)-MTPA] of the MTPA esters.

This compound was shown to be a member of hippurins by the presence of a spiroketal functionality (δ_C 116.7, s).[3] The NMR spectral data (Tables 1 and 3) also showed the presence of a lactone carbonyl (δ_C 182.4, s) and were found to be similar to those of **5**.[3] Furthermore, the presence of an additional acetoxy group at C-2 (δ_H 5.02, br d, J = 11.4 Hz; δ_C 69.7, d) was supported by COSY (H_2-1/H-2; H-2/H-3) and HMBC cross-peaks (H_3-19/C-1). The orientations of two acetoxy groups in ring A were determined to be α, as evidenced by a large coupling constant of H-2 (br d, J = 11.4 Hz) and a small coupling constant of H-3 (br d, J = 2.2 Hz). Also, by comparison of the NMR data, including chemical shifts and coupling constants, with those of the known steroid 3-acetyl-22-*epi*-hippurin-1 (**20**),[4] which possessed the same structure as that of **1** in ring A, the α-orientation for both acetoxy groups in ring A was unambiguously determined. Thus, the structure of **1** was deduced as (22*S*)-2α,3α-diacetoxy-11β-hydroxy-24-methyl-22,25-epoxy-5α-furostan-18,20β-lactone (**1**).

Compound **2** was isolated as a white powder. The HRFABMS of **2** established a molecular formula of $C_{28}H_{42}O_7$. By comparison of the NMR data of **2** with those of **1** the structure of **2** was deduced as (22*S*)-2α,3α-dihydroxy-11β-hydroxy-24-methyl-22,25-epoxy-5α-furostan-18,20β-lactone (**2**). Moreover, hydrolysis of **1** was found to get **2** as the major product and further confirmed the structure of **2**.

The molecular formula $C_{30}H_{44}O_8$ of **3** was established by the HRFABMS spectrum. By comparison of NMR data of **3** with those of **1** and **2**, together with the elucidation of the COSY (H_2-1/H-2; H-2/H-3) and HMBC cross-peaks (H_3-19/C-1), the structure of **3** was fully established. Furthermore, hydrolysis of **1** also formed **3** as a minor component.

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Table 2. ^{13}C NMR Spectral Data of Compounds **7**–**9**, **11**, and **12**

C #	**7**[b]	**8**[a]	**9**[a]	**11**[b]	**12**[b]
1	38.3 (CH_2)[c]	41.7 (CH_2)[c]	37.3 (CH_2)[c]	32.6 (CH_2)[c]	37.8 (CH_2)[c]
2	69.6 (CH)	67.6 (CH)	72.4 (CH)	25.7 (CH_2)	69.8 (CH)
3	69.2 (CH)	72.7 (CH)	67.3 (CH)	70.1 (CH)	69.4 (CH)
4	31.8 (CH_2)	31.6 (CH_2)	33.3 (CH_2)	32.2 (CH_2)	31.8 (CH_2)
5	39.5 (CH)	39.4 (CH)	38.1 (CH)	40.6 (CH)	40.0 (CH)
6	27.5 (CH_2)	27.4 (CH_2)	27.5 (CH_2)	27.5 (CH_2)	26.6 (CH_2)
7	32.2 (CH_2)	32.1 (CH_2)	32.2 (CH_2)	33.8 (CH_2)	32.0 (CH_2)
8	39.0 (CH)	38.9 (CH)	38.9 (CH)	31.2 (CH)	30.5 (CH)
9	57.9 (CH)	57.7 (CH)	57.8 (CH)	58.6 (CH)	58.6 (CH)
10	37.4 (C)	37.1 (C)	37.4 (C)	36.0 (C)	37.3 (C)
11	81.0 (CH)	81.0 (CH)	80.9 (CH)	66.1 (CH)	66.2 (CH)
12	39.6 (CH_2)	39.5 (CH_2)	39.6 (CH_2)	39.3 (CH_2)	39.2 (CH_2)
13	63.7 (C)	63.7 (C)	63.7 (C)	56.1 (C)	55.9 (C)
14	49.6 (CH)	49.4 (CH)	49.5 (CH)	56.3 (CH)	56.4 (CH)
15	36.2 (CH_2)	36.2 (CH_2)	36.2 (CH_2)	33.8 (CH_2)	33.9 (CH_2)
16	81.6 (CH)	81.9 (CH)	81.6 (CH)	80.7 (CH)	80.7 (CH)
17	57.4 (CH)	57.3 (CH)	57.3 (CH)	64.6 (CH)	64.4 (CH)
18	107.7 (CH)	107.5 (CH)	107.6 (CH)	101.4 (CH)	101.6 (CH)
19	12.8 (CH_3)	13.0 (CH_3)	12.8 (CH_3)	14.5 (CH_3)	15.6 (CH_3)
20	93.5 (C)	93.4 (C)	93.4 (C)	90.8 (C)	91.1 (C)
21	20.8 (CH_3)	20.7 (CH_3)	20.8 (CH_3)	19.7 (CH_3)	19.7 (CH_3)
22	117.1 (C)	117.0 (C)	117.0 (C)	118.0 (C)	118.0 (C)
23	39.0 (CH_2)	39.0 (CH_2)	38.9 (CH_2)	38.9 (CH_2)	38.9 (CH_2)
24	41.0 (CH)	40.9 (CH)	41.0 (CH)	41.2 (CH)	41.2 (CH)
25	85.2 (C)	85.1 (C)	85.1 (C)	84.8 (C)	84.9 (C)
26	29.3 (CH_3)	29.2 (CH_3)	29.2 (CH_3)	29.2 (CH_3)	29.2 (CH_3)
27	23.1 (CH_3)	23.0 (CH_3)	23.0 (CH_3)	23.0 (CH_3)	23.0 (CH_3)
28	14.1 (CH_3)	14.0 (CH_3)	14.0 (CH_3)	14.1 (CH_3)	14.3 (CH_3)
OAc	170.4 (C)	171.5 (C)	169.9 (C)	170.7 (C)	170.5 (C)
	170.3 (C)				170.5 (C)
OAc	21.2 (CH_3)	21.3 (CH_3)	21.3 (CH_3)	21.6 (CH_3)	21.3 (CH_3)
	21.1 (CH_3)				21.2 (CH_3)

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

Table 3. ^1H NMR Spectral Data of Compounds **1**–**4**, **13**, and **14**

C #	**1**[b]	**2**[a]	**3**[a]	**4**[b]	**13**[a]	**14**[a]
1	1.58m	1.40 t (12.0)	1.56 m	1.35 t (12.1)	1.21m	1.31 m
	1.83 m	1.94 dd (12.0, 5.0)	1.92 dd (11.5, 4.5)	2.06 m	2.27 m	1.98 m
2	5.02 br d (11.4)	3.86 br d (12.0)	5.04 br d (12.0)	3.92 br d (12.2)	1.74 m	3.90 m
					1.54 m	
3	5.28 br d (2.2)	3.97 br s	4.06 br s	5.14 br d (2.3)	4.04 br s	5.12 br s
4	1.53 m	1.54 m	2.35 m	1.59 m	1.53 m	1.55 m
	1.36 m		1.59 m		1.37 m	1.57 m
5	1.49 m	1.50 m	1.59 m	1.52 m	1.52 m	1.53 m
6	1.28 m	1.27 m	1.27 m	1.25 m	1.19 m	1.26 m
7	1.95 m	1.85 m	1.85 m	1.85 m	1.80 m	1.81 m
	0.88 m	0.89 m	0.91 m	0.88 m	1.13 m	0.90 m
8	2.50 m	2.48 m	2.50 m	2.50 m	1.90 m	1.95 m
9	0.90 m	0.93 m	0.94 m	0.94 m	1.70 m	0.81 m
10						
11	4.13 dd (12.6, 3.3)	4.20 br d (12.0)	4.15 dd (13.0, 2.5)	4.20 br d (12.6)		4.29 br s
12	2.40 m	1.70 dd (14.0, 4.0)	1.68 dd (14.0, 4.0)	1.71 dd (14.0, 3.6)	2.51 d (12.0)	2.18 br d (14.0)
	1.71 m	2.37 dd (14.0, 2.0)	2.36 dd (14.0, 2.0)	2.38 br d (14.2)	2.25 m	1.40 m
13						
14	1.51 m	1.49 m	1.50 m	1.50 m	1.67 m	0.88 m
15	2.26 m	2.32 m	2.31 m	2.31 m	2.10 m	2.02 m
	1.37 m	1.23 m	1.23 m	1.23 m	1.40 m	1.43 m
16	4.66 dt (6.6, 6.6)	4.68 dt (8.0, 7.5)	4.66 dt (8.0, 7.0)	4.67 dt (7.4, 6.9)	4.49 dt (8.0, 7.5)	4.32 m
17	2.66 d (8.6)	2.67 d (8.5)	2.66 d (8.0)	2.67 d (8.3)	2.12 d (8.0)	1.74 d (7.0)
18					1.05 s	1.39 s
19	1.17 s	1.11 s	1.16 s	1.13 s	1.01 s	1.07 s
20						
21	1.47 s	1.48 s	1.48 s	1.48 s	1.29 s	1.31 s
22						
23	2.11 m	2.12 dd (13.0, 6.0)	2.12 dd (13.0, 6.5)	2.12 m	2.02 dd (13.0, 7.0)	2.40 dd (12.0, 5.0)
	1.79 m	1.80 t (13.0)	1.80 t (13.0)	1.80 t (13.0)	1.77 t (13.0)	1.70 m
24	2.30 m	2.31 m	2.30 m	2.30 m	2.23 m	1.88 m
25						
26	1.29 s	1.30 s	1.30 s	1.30 s	1.28 s	1.22 s
27	0.99 s	1.00 s	1.00 s	1.00 s	0.98 s	1.20 s
28	0.96 d (6.8)	0.96 d (6.5)	0.96 d (6.5)	0.97 d (6.9)	0.94 d (7.0)	0.98 d (7.0)
11-OH	3.95 d (12.6)	4.00 d (12.5)	4.00 d (12.5)	3.98 d (12.7)		3.19 s (20-OH)
OAc	2.04 s		2.01 s	2.10 s		2.11 s
	1.97 s					

[a] Spectra recorded at 500 MHz in $CDCl_3$ at 25 °C. [b] 300 MHz in $CDCl_3$ at 25 °C. The values are in ppm downfield from TMS.

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Table 4. ^{1}H NMR Spectral Data of Compounds **7**−**9**, **11**, and **12**

C #	**7**[b]	**8**[a]	**9**[a]	**11**[b]	**12**[b]
1	1.58 m	1.20 t (12.0)	1.41 t (12.0)	1.34 m	1.50 m
	1.80 m	1.94 dd (12.0, 4.5)	1.80 m	1.70 m	1.87 m
2	5.02 br d (11.1)	3.92 br d (12.0)	5.01 br d (12.0)	1.70 m	5.00 br d (11.3)
3	5.29 br d (2.6)	5.13 br d (3.0)	4.05 br d (2.0)	5.01 br s	5.28 br s
4	1.55 m	1.50 m	1.54 m	1.46 m	1.54 m
5	1.53 m	1.33 m	1.54 m	1.47 m	1.47 m
6	1.26 m	1.24 m	1.36 m	1.18 m	1.26 m
7	1.78 m	1.80 m	1.78 m	1.82 m	1.87 m
	1.10 m	1.06 m	1.07 m	0.94 m	0.97 m
8	1.70 m	1.65 m	1.64 m	1.88 m	1.89 m
9	0.85 d (10.8)	0.88 br d (11.0)	0.90 br d (11.0)	0.81 br d (11.1)	0.86 br d (10.8)
10					
11	4.77 br d (5.4)	4.82 br d (5.5)	4.76 br d (5.5)	4.29 br s	4.24 br s
12	2.30 m	2.30 dd (11.5, 5.5)	2.28 dd (10.5, 5.5)	2.71 d (12.0)	2.72 d (13.9)
	1.40 m	1.38 br d (11.0)	1.37 br d (10.0)	1.68 m	1.68 m
13					
14	1.75 m	1.75 m	1.74 m	1.48 m	1.48 m
15	2.22 m	2.35 m	2.34 m	2.11 m	2.09 m
	1.37 m	1.53 m	1.56 m	1.30 m	1.30 m
16	4.58 dt (6.8, 1.7)	4.59 dt (7.0, 2.0)	4.58 dt (7.0, 2.0)	4.53 dt (7.9, 7.2)	4.54 dt (8.0, 6.6)
17	2.61 d (6.9)	2.62 d (6.5)	2.61 d (7.0)	2.62 d (8.0)	2.62 d (8.3)
18	5.31 s	5.31 s	5.31 s	5.32 s	5.33 s
19	0.91 s	0.94 s	0.97 s	1.02 s	1.09 s
20					
21	1.37 s	1.37 s	1.37 s	1.43 s	1.43 s
22					
23	2.05 m	2.04 dd (13.5, 6.5)	2.05 dd (13.0, 6.5)	2.09 m	2.10 m
	1.75 m	1.72 m	1.74 m	1.72 m	1.75 m
24	2.25 m	2.24 m	2.25 m	2.28 m	2.26 m
25					
26	1.28 s	1.28 s	1.28 s	1.30 s	1.30 s
27	0.98 s	0.98 s	0.97 s	0.97 s	0.98 s
28	0.94 d (6.8)	0.94 d (6.5)	0.94 d (7.0)	0.94 d (6.9)	0.91 d (7.2)
OAc	2.04 s	2.10 s	2.08 s	2.04 s	2.09 s
	1.98 s				1.99 s

[a] Spectra recorded at 500 MHz in $CDCl_3$ at 25°C. [b] 300 MHz in $CDCl_3$ at 25 °C. The values are in ppm downfield from TMS.

Thus, the structure of **3** was deduced as (22*S*)-2α-acetoxy-3α-hydroxy-11*β*-hydroxy-24-methyl-22,25-epoxy-5α-furostan-18,20*β*-lactone (**3**).

Compound **4** was obtained as a white powder. It gave the same formula, $C_{30}H_{44}O_8$, as that of **3** from the HR-FABMS spectrum and revealed that **4** was an isomer of **3**. Hydrolysis of **1** also obtained **4** as a minor component. Thus, from the above observations and by comparison of the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 3) with those of metabolites **1**−**3**, the structure of **4** was deduced as (22*S*)-2α-hydroxy-3α-acetoxy-11*β*-hydroxy-24-methyl-22,25-epoxy-5α-furostan-18,20*β*-lactone (**4**).

The formula of compound **7** was found to be $C_{32}H_{46}O_8$ as deduced from the HRFABMS spectrum. It was found to contain an additional acetoxy group at C-2 (δ_H 5.02, br d, J = 11.1 Hz, and δ_C 69.6, d) by COSY (H_2-1/H-2; H-2/H-3) and HMBC cross-peaks (H_3-19/C-1) as compared to **10**.[3] The two acetoxy groups attached at C-2 and C-3 were both placed on the α face by comparison of the chemical shifts and large coupling constants of H-2 and H-3 with those of **1**. Furthermore, the NMR spectra of **7** are similar to those of **10**[3] (Tables 2 and 4), except that an additional acetoxy group was found to be present at C-2 of **7**. Thus, the structure of **7** was deduced as (22S)-2α,3α-diacetoxy-24-methyl-11*β*,18;18, 20*β*;22,25-triepoxy-5α-furostane (**7**).

The molecular formula of compound **8**, $C_{30}H_{44}O_7$, was deduced from the HRFABMS spectrum. It was found that **8** contained an additional oxygen atom as compared to **10**.[3] The above evidence together with the comparison of the NMR spectral data between **8** and **10**[3] (Tables 2 and 4) revealed that **8** had one more hydroxy group than **10**. Furthermore, the analyses on the 2D NMR spectral data of **8** suggested that the hydroxy group should be positioned at C-2 (δ_C 67.6, d). The α-orientation of 2-OH was determined by NOE correlations between both H-1*β* and H_3-19 and H-2, and the large coupling constant of H-2 (J = 12.0 Hz). Thus, the structure of **8** was deduced as (22*S*)-2α-hydroxy-3α-acetoxy-24-methyl-11*β*,18;18,20*β*;22,25-triepoxy-5α-furostane (**8**).

Table 5. Cytotoxicities of **1**−**3**, **5**−**7**, **10**, **12**, and **14**−**19**

	cancer cell line[a] (IC_{50}, μg/mL)			
compound	Hep G2	Hep 3B	MCF-7	MDA-MB-231
1	>20	−[b]	19.59	>20
2	>30	23.85	>30	>30
3	27.60	>30	>30	>30
5	>20	−	>20	>20
6	>20	−	>20	>20
7	>20	−	>20	>20
10	>20	−	>20	>20
11	>20	−	12.72	>20
12	>20	−	11.39	>20
14	0.72	0.46	1.07	0.21
15	2.06	1.46	2.41	0.74
16	0.56	0.10	0.53	0.41
17	4.64	0.68	4.54	2.64
18	0.08	0.10	0.20	0.13
19	0.62	0.77	0.59	0.75

[a] Human hepatocellular carcinoma Hep G2 and Hep 3B; human breast carcinoma MCF-7 and MDA-MB-231, and human lung carcinoma A-549. [b] "−" not tested.

Compound **9**, $C_{30}H_{44}O_7$, was found to be an isomer of **8** from the HRFABMS spectrum, and the ^{1}H NMR data of **9** show clear differences at H-2 (5.01, br d, J = 12.0 Hz) and H-3 (4.05, br d, J = 2.0 Hz) as compared with those of **8** (see Tables 2 and 4). It was found that **9** has the same structure in ring A by comparison of the NMR spectral data with those of **3**. Therefore, the structure of **9** was concluded

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to be (22*S*)-2α-acetoxy,3α-hydroxy-24-methyl-11*β*,18;18,-20*β*;22,25-triepoxy-5α-furostane (**9**).

Compound **11** was isolated as a white powder and was found to be more polar than **10**, a known metabolite also isolated in the present study. Its HRFABMS established the molecular formula $C_{30}H_{46}O_7$, implying eight degrees of unsaturation. Furthermore, an acetoxy group was observed from NMR signals appearing at δ_H 2.04 (s), δ_C 170.7 (s), and 21.6 (q). The above data suggested a heptacyclic structure in the molecule of **11**. The NMR data of **11** were found to be close to those of **10**,[3] except that the carbon signal of C-18 (δ 107.7, d) in **10**[3] was converted to 101.8 (d) in **11** and that of C-11 (δ 81.0, d) in **10**[3] was converted to 66.4 in **11** (see Tables 2 and 4). H-18 was assigned as having a *β*-orientation from the key NOE correlation between H-18 and H-8. Thus, the structure of **11** was established as (22*S*)-3α-acetoxy-11*β*,18α-dihydroxy-24-methyl-18,20*β*;22,25-diepoxy-5α-furostane (**11**).

Compound **12** was obtained as a white powder. The HREIMS of **12** established a molecular formula of $C_{32}H_{48}O_9$. The ^{1}H and ^{13}C NMR spectral data of **12** were similar to those of **11** and revealed the presence of an additional acetoxy group as compared to **11**. By comparison of NMR data of **12** in ring A with those of **1** and **7** (see Tables 1−4), the structure of **12** was fully determined and assigned as (22*S*)-2α,3α-diacetoxy-11*β*,18α-dihydroxy-24-methyl-18,-20*β*;22,25-diepoxy-5α-furostane (**12**).

Compound **13** was obtained as a white powder, which gave a $[M + H]^+$ peak at *m/z* 461.3267 in the HRFABMS and thus established the molecular formula $C_{28}H_{44}O_5$. By comparison of the NMR spectral data of **13** with those of a known steroid, 22-*epi*-hippuristanol (**18**),[2] the structure of **13** was determined to be 22-*epi*-hippuristan-11-one, as the NMR spectrum of **13** (see Tables 2 and 4) did not show the signals at δ_H 4.30 (br s) and δ_C 68.0, attributable to the presence of a hydroxyl group at C-11 of **18**,[9] and instead showed the presence of a ketone functionality at δ_C 210.5 (s).

Compound **14** was obtained as a white powder that gave a $[M + H]^+$ peak at *m/z* 521.3478 in the HRFABMS spectrum. Thus, the molecular formula $C_{30}H_{48}O_7$ was established. By comparison of the NMR data of **14** with those of a known steroid, 3-acetyl-2-desacetyl-22-*epi*-hippurin-1 (**15**),[4] it was found that the structure of **14** is similar to that of **15**, except that the 22*S* configuration in **15** should be converted to 22*R* in **14**, as the carbon signal at δ_C 118.6 (attributable to the presence of the 22*S* configuration) was replaced by a carbon signal at δ_C 115.3 (s) (attributable to the presence of the 22*R* configuration)[9] (see Tables 2 and 4).

Except for compounds **4**, **8**, **9**, and **13**, the hippuristanols obtained in this study have been submitted for cytotoxicity evaluation toward cancer cell lines. The investigations showed that compounds possessing an ether or ester ring that links C-18 to C-20, such as **1**−**3**, **5**−**7**, and **10**−**12**, would possess only weak cytotoxicity. Also, it was found that the hydroxy groups attached at both C-11 and C-20, such as in **14**−**19**, could significantly enhance the cytotoxicity against the proliferation of the tested cancer cell lines.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Jasco FT-5300 infrared spectrophotometer. NMR spectra were recorded on a Bruker Avance DPX300 FT-NMR at 300 MHz for ^{1}H and 75 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as internal standard. FABMS was obtained with a VG Quattro GC/MS spectrometer. HRMS spectra were recorded on a Finnigan MAT-95XL mass spectrometer. Silica gel (Merck, 230−400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC. High-performance liquid chromatography (HPLC) was performed on a Hitachi L-7100 apparatus equipped with a Bischoff refractive index detector or a Hitachi L-7400 UV detector and with the Merck Hibar Si-60 column (250 × 21 mm, 7 μm).

Animal Material. The gorgonian coral *I. hippuris* was collected by hand using scuba at the 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 organism 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 to obtain fractions 1−31. Fraction 21 was subjected to normal-phase HPLC column chromatography (gradient EtOAc/CH_2Cl_2, 7−10%) to afford compounds **1** (15 mg) and **5** (13 mg). Compounds **13** (2 mg), **18** (5 mg), and **20** (6 mg) were obtained from fraction 22 by repeated HPLC column chromatography (acetone/hexane, 15%). Fraction 23 was subjected to repeated normal-phase HPLC column chromatography (gradient acetone/CH_2Cl_2, 13−17%) to afford compounds **7** (30 mg), **10** (8 mg), **6** (9 mg), and **11** (9 mg). Similarly, fraction 24 was chromatographed (acetone/CH_2Cl_2, 14%) to yield compound **12** (4 mg). Compounds **3**, **4**, **8**, and **9** (each 1 mg) were eluted with MeOH/CH_2Cl_2 (4−8%) from fraction 25. Repeated chromatography of fraction 28 over HPLC column (acetone/hexane, 25%) led to the isolation of compounds **14**−**17** (each 3 mg). Compounds **2** (1 mg) and **19** (6 mg) were both obtained by elution with MeOH/CH_2Cl_2 (gradient, 3−10%) from fraction 31 and fraction 30, respectively.

(22*S*)-2α,3α-Diacetoxy-11*β*-hydroxy-24-methyl-22,25-epoxy-5α-furostan-18,20*β*-lactone (1): white powder; mp 270−272 °C; $[\alpha]_D$ −8° (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3479, 1736, and 1263 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 3; FABMS *m/z* 575 ($[M + H]^+$, 2); HRFABMS *m/z* 575.3223 $[M + H]^+$ (calcd for $C_{32}H_{47}O_9$, 575.3221).

(22*S*)-2α,3α-Dihydroxy-11*β*-hydroxy-24-methyl-22,25-epoxy-5α-furostan-18,20*β*-lactone (2): white powder; mp 253−254 °C; $[\alpha]_D$ −27° (*c* 0.92, $CHCl_3$); IR (KBr) ν_{max} 3445, and 1734 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 3; FABMS *m/z* 491 ($[M + H]^+$, 1), 473 (3), 455 (1), 437 (2), 391 (3), and 149 (57); HRFABMS *m/z* 491.3012 $[M + H]^+$ (calcd for $C_{28}H_{43}O_7$, 491.3010).

(22*S*)-2α-Acetoxy-3α-hydroxy-11*β*-hydroxy-24-methyl-22,25-epoxy-5α-furostan-18,20*β*-lactone (3): white powder; mp 204−205 °C; $[\alpha]_D$ −17° (*c* 1.28, $CHCl_3$); IR (KBr) ν_{max} 3481 and 1736 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 3; FABMS *m/z* 533 ($[M + H]^+$, 48), 515 (82), 455 (25), and 437 (53); HRFABMS *m/z* 533.3108 $[M + H]^+$ (calcd for $C_{30}H_{45}O_8$, 533.3116).

(22*S*)-2α-Hydroxy,3α-acetoxy-11*β*-hydroxy-24-methyl-22,25-epoxy-5α-furostan-18,20*β*-lactone (4): white powder; mp 244−245 °C; $[\alpha]_D$ +4° (*c* 1.40, $CHCl_3$); IR (KBr) ν_{max} 3449 and 1736 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 3; FABMS *m/z* 533 ($[M + H]^+$, 3); HRFABMS *m/z* 533.3116 $[M + H]^+$ (calcd for $C_{30}H_{45}O_8$, 533.3116).

(22S)-2α,3α-Diacetoxy-24-methyl-11*β*,18;18,20*β*;22,25-triepoxy-5α-furostane (7): white powder; mp 252−253 °C; $[\alpha]_D$ −22° (*c* 0.31, $CHCl_3$); IR (KBr) ν_{max} 1726 and 1263 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 4; FABMS *m/z* 559 ($[M + H]^+$, 44), 449 (10), 439 (18); HRFABMS *m/z* 559.3271 $[M + H]^+$ (calcd for $C_{32}H_{47}O_8$, 559.3272).

(22*S*)-2α-Hydroxy-3α-acetoxy-24-methyl-11*β*,18;18,-20*β*;22,25-triepoxy-5α-furostane (8): white powder; mp 193−194 °C; $[\alpha]_D$ −30° (*c* 0.83, $CHCl_3$); IR (KBr) ν_{max} 3447, 1734, and 1246 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 4; FABMS *m/z* 517 ($[M + H]^+$, 0.5), 499 (2), and 439 (0.6); HRFABMS *m/z* 517.3155 $[M + H]^+$ (calcd for $C_{30}H_{45}O_7$, 517.3167).

(22*S*)-2α-Acetoxy,3α-hydroxy-24-methyl-11*β*,18;18,-20*β*;22,25-triepoxy-5α-furostane (9): white powder; mp 236−237 °C; $[\alpha]_D$ −27° (*c* 0.96, $CHCl_3$); IR (KBr) ν_{max} 3447 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 4; FABMS *m/z* 517 ($[M + H]^+$, 2), 499 (3), and 439 (0.6); HRFABMS *m/z* 517.3165 $[M + H]^+$ (calcd for $C_{30}H_{45}O_7$, 517.3167).

(22*S*)-3α-Acetoxy-11*β*,18α-dihydroxy-24-methyl-18,-20*β*;22,25-diepoxy-5α-furostane (11): white powder; mp 269−271 °C; $[\alpha]_D$ −43° (*c* 0.31, $CHCl_3$); IR (KBr) ν_{max} 3406, 1726, and 1265 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 4; FABMS *m/z* 519 ($[M + H]^+$); HRFABMS *m/z* 519.3328 $[M + H]^+$ (calcd for $C_{30}H_{47}O_7$, 519.3323).

(22*S*)-2α,3α-Diacetoxy-11*β*,18α-dihydroxy-24-methyl-18,20*β*;22,25-diepoxy-5α-furostane (12): white powder; mp 273−275 °C; $[\alpha]_D$ −23° (*c* 0.38, $CHCl_3$); IR (KBr) ν_{max} 3406, 1726, and 1265 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 4; FABMS *m/z* 577 ($[M + H]^+$, 10); HREIMS *m/z* 576.3300 $[M]^+$ (calcd for $C_{32}H_{48}O_9$, 576.3299).

22-*epi*-Hippuristan-11-one (13): white powder; mp 165−167 °C; $[\alpha]_D$ +38° (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} 3460 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; FABMS *m/z* 461 ($[M + H]^+$, 4); HRFABMS *m/z* 461.3267 $[M + H]^+$ (calcd for $C_{28}H_{45}O_5$, 461.3269).

3-Acetyl-2-desacetylhippurin-1 (14): white powder; mp >300 °C; $[\alpha]_D$ +27° (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 3435 and 1726 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; FABMS *m/z* 521 ($[M + H]^+$, 4); HRFABMS *m/z* 521.3478 $[M + H]^+$ (calcd for $C_{30}H_{49}O_7$, 521.3480).

Hydrolysis of 1 to Compounds 2−4. A mixture of **1** (25.0 mg), LiOH (0.5 mg), and THF (1 mL) was stirred at room temperature for 2 h, followed by removal of the solvent under reduced pressure. Then, the resultant mixture was washed with brine (5 mL) and extracted with EtOAc (3 × 5 mL). The extracts were combined and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to gave a mixture of **1**−**4**, which were chromatographed on normal-phase HPLC using MeOH/CH_2Cl_2 (gradient, 4−10%) to yield **2** (9.5 mg), **3** (4.1 mg), **4** (1.2 mg), and the reactant **1** (5 mg).

(*R*)- and (*S*)-MTPA Derivatives of 19. To a solution of compound **19** (5.0 mg, 1.0×10^{-2} mmol) in $CHCl_3$ (1.0 mL) at room temperature were added (*R*)-MTPA acid (11.7 mg, 5.0×10^{-2} mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (9.6 mg, 5.0×10^{-2} mmol), and 4-(dimethylamino)pyridine (DMAP) (0.6 mg, 5.0×10^{-3} mmol), and the resultant mixture was stirred for 24 h at room temperature. The reaction mixture was concentrated under reduced pressure to give a crude product. Further purification was performed by a short silica gel column with *n*-hexane/acetone (2:1) to give **19b** (1.5 mg) as a colorless oil. The (*S*)-MTPA ester **19a** (1.2 mg) was prepared in the same way. Selected $\Delta\delta$ values $[\delta(S) - \delta(R)]$ are as follows: H-11 = +0.02, H_2-1 = +0.09 and +0.01, H-3 = −0.10, H-4 = −0.02.

(*S*)-MTPA ester of 19: ^1H NMR ($CDCl_3$, 300 MHz), δ 5.23 (1H, br d, J = 12.2 Hz, H-2), 4.43 (1H, dt, J = 7.5, 5.4 Hz, H-16), 4.23 (1H, br s, H-11), 4.02 (1H, br s, H-3), 1.95 (1H, m, H-1a), 1.69 (1H, m, H-1b), 1.57 (2H, m, H-4), 1.34 (3H, s, H_3-18), 1.30 (3H, s, H_3-21), 1.27 (3H, s, H_3-26), 1.13 (3H, s, H_3-19), 0.98 (3H, s, H_3-27), 0.94 (3H, d, J = 6.8 Hz, H_3-28).

(*R*)-MTPA ester of 19: ^1H NMR ($CDCl_3$, 300 MHz), δ 5.22 (1H, br d, J = 12.2 Hz, H-2), 4.43 (1H, dt, J = 7.5, 5.4 Hz, H-16), 4.21 (1H, br s, H-11), 4.12 (1H, br s, H-3), 1.94 (1H, m, H-1a), 1.60 (1H, m, H-1b), 1.59 (2H, m, H-4), 1.34 (3H, s, H_3-18), 1.30 (3H, s, H_3-21), 1.27 (3H, s, H_3-26), 1.13 (3H, s, H_3-19), 0.98 (3H, s, H_3-27), 0.94 (3H, d, J = 6.8 Hz, H_3-28).

Cytotoxicity Assays. Compounds were assayed for cytotoxicity against Hep G2, Hep 3B, A549, MCF-7, and MDA-MB-231 cells using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method.[14] Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 5000−10 000 cells per well with tested compounds added from DMSO-diluted stock. After 3 days in culture, attached cells were incubated with MTT (0.5 mg/mL, 1 h) and subsequently dissolved in DMSO. The absorbency at 550 nm was then measured using a microplate reader. The IC_{50} is the concentration of agent that reduced cell growth by 50% under the experimental conditions.

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(12) Crystallography data (excluding structure factors) of **5** and **9** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC259656 and CCDC259655, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
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