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The first *A*-nor-hippuristanol and two novel 4,5-secosuberosanoids from the Gorgonian *Isis hippuris*

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Abstract—The first *A*-nor-hippuristanol, *A*-nor-22-*epi*-hippurin-2α-carboxylic acid (**1**), and two 4,5-secosuberosane sesquiterpenoids, isishippuric acids A and B (**2** and **3**), have been isolated from the gorgonian coral *Isis hippuris*. Those structures were deduced by extensive 1D and 2D NMR studies. The structure of **1** was further supported by a single crystal X-ray diffraction analysis. Isishippuric acid B has been shown to exhibit potent cytotoxicity toward a limited panel of cancer cells.

Previous studies on *Isis hippuris* have resulted in the isolation of a series of novel metabolites including several highly oxygenated spiroketal steroids, which were named as hippurins or hippuristanols,[1–5,9] polyoxygenated gorgosteroids,[5,6] (22*R*,23*S*,24*S*)-polyoxygenated steroids,[7–9] and six suberosane-type sesquiterpenes.[10] Some hippuristanols[9] and suberosane-type sesquiterpenes[10] have been reported to have significant antitumor activity. Recently, our continuing investigation on the chemical constituents of *I. hippuris*, collected by hand using scuba at Green Island, which is located off the southeast coast of Taiwan, in February 1999, has again afforded three novel compounds, including one hippurin-derived steroid and two 4,5-secosuberosanes (Fig. 1). We describe herein the isolation, structure elucidation, and biological activity of these compounds.

The organism of *I. hippuris* was frozen immediately after collection and the freeze dried organism was extracted sequentially with *n*-hexane and CH_2Cl_2. The CH_2Cl_2 extract was separated by silica gel chromatography. A fraction eluted with MeOH/EtOAc (1:5) was further purified by reversed-phase HPLC using MeOH/H_2O (4:1) to afford compound **1**. The *n*-hexane extract was also fractionated on silica gel. Compound **2** was eluted with EtOAc/*n*-hexane (1:3), and **3** was eluted with MeOH/EtOAc (1:2).

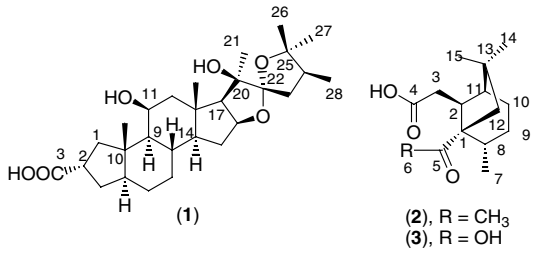

Figure 1. Structures of metabolites **1**–**3**.

Compound **1**[11] was obtained as a colorless crystal. The HRFABMS of **1** established a molecular formula $C_{28}H_{44}O_6$, implying seven degrees of unsaturation. The LRFABMS showed peaks at *m*/*z* 477 $[M+H]^+$, 459 $[M+H-H_2O]^+$, 441 $[M+H-2H_2O]^+$, suggesting the presence of two hydroxyl groups in **1**. The ^{13}C NMR and DEPT spectra of **1** showed signals of six methyl, seven methylene, nine methine, and six quaternary carbons including one carbon of a carboxylic acid (δ 182.4, s). Among these 28 carbons, 5 oxygenated

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Table 1. ^{1}H and ^{13}C NMR spectral data of **1**

C/H		^{1}H[a]	^{13}C[b]
1	α	1.45 m	44.8 (t)
	β	2.06 m	
2		2.88 m	41.5 (d)
3			182.4 (s)
4	α	1.88 m	33.1 (t)
	β	1.64 dd (12.0, 12.0)[c]	
5		1.36 m	52.3 (d)
6		1.30 m	26.0 (t)
		1.56 br d (12.0)	
7		0.90 m	33.7 (t)
		1.81 m	
8		1.92 m	31.9 (d)
9		0.83 dd (11.0, 3.0)	60.1 (d)
10			45.7 (s)
11		4.11 br d (2.0)	70.9 (d)
12	α	2.23 dd (12.0, 2.0)	49.1 (t)
	β	1.34 m	
13			43.8 (s)
14		1.03 m	59.5 (d)
15	α	2.03 m	33.2 (t)
	β	1.42 m	
16		4.39 ddd (7.5, 4.5, 4.5)	80.6 (d)
17		1.72 d (7.5)	68.9 (d)
18		1.31 s	19.1 (q)
19		0.96 s	16.2 (q)
20			82.8 (s)
21		1.27 s	28.7 (q)
22			120.0 (s)
23	β	1.85 m	
	α	2.00 m	41.5 (t)
24		2.19 m	42.4 (d)
25			84.9 (s)
26		0.98 s	23.3 (q)
27		1.24 s	29.3 (q)
28		0.95 d (7.0)	14.3 (q)

[a] Spectra recorded at 500 MHz in CD_3OD at 25 °C.
[b] Spectra recorded at 125 MHz in CD_3OD at 25 °C.
[c] J value (in Hz) in parentheses.

sp^3 carbons (δ 70.9, d; 80.6, d; 82.8, s; 84.9, s; 120.0, s) were identified. Furthermore, **1** was shown to be a member of hippuristanols by the presence of the signal resonating at δ 120.0 (s). By comparison of the ^{1}H and ^{13}C NMR spectral data of **1** (Table 1) with those of the known hippuristanols, three carbon signals appearing at δ 82.8 (s), 84.9 (s), and 120.0 (s) were assigned to C-20, C-25, and C-22, respectively. Six methyl signals observed at δ 1.31 (3H, s), 1.27 (3H, s), 1.24 (3H, s), 0.98 (3H, s), 0.96 (3H, s), and 0.95 (3H, d, J = 7.0 Hz) were assigned as H_3-18, H_3-21, H_3-27, H_3-26, H_3-19, and H_3-28, respectively. From the COSY spectrum of **1** (Fig. 2), it

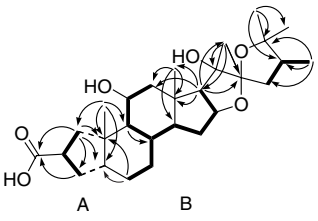


Figure 2. Selective ^{1}H–^{1}H COSY and HMBC correlations of **1**.

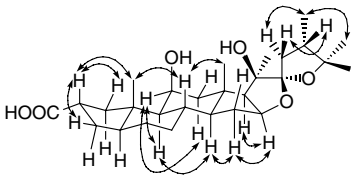


Figure 3. Selective NOESY correlations of **1**.

was possible to establish the proton sequences from H_2-6 to H-9; H-9 to H-11; H-11 to H_2-12; H-8 to H-14; H-14 to H-17. All of the above observations and the key HMBC correlations of H_3-18 with C-12, C-13, and C-14; H-17 with C-12, C-13, C-18, C-21, and C-22; and H_3-21 with C-17, C-20, and C-22 establish the planar structure of moiety B as showed in Figure 2. The moiety A was established from COSY correlations between H-2 and both H_2-1 and H_2-4, and the carboxylic acid functionality attaching at C-2 was confirmed by the HMBC correlations of both H_2-1 and H_2-4 with C-3 (δ 182.4). Furthermore, moieties A and B were connected by both the HMBC correlations of H_3-19 with C-1 and H_2-1 with C-5. Therefore, the planar structure of **1** was established unambiguously.

The stereochemistry of **1** was elucidated on the basis of the observed key NOE correlations (Fig. 3). In the NOESY spectrum of **1**, H-8 was found to show NOE interactions with both H_3-18 and H_3-19, and H_3-19 exhibited an NOE correlation with H-2, suggesting the β orientations of H-2 and H-8, and the α orientation of the carboxylic acid attached at C-2. Furthermore, NOE interactions could be observed between H-9 and H-11, H-9 and H-14, H-14 and H-15α, H-15α and H-16, H-16 and H-17, and H-9 did not show NOE interaction with either H-8 or H_3-19. Therefore, H-9, H-11, H-14, H-16, and H-17 should be placed on the α face. The structure of **1** was further confirmed by a single crystal X-ray diffraction analysis (Fig. 4).[12] Thus, the structure of **1** was fully determined, and assigned as *A*-nor-22-*epi*-hippurin-2α-carboxylic acid (**1**).

Isishippuric acid A (**2**)[13] was assigned the molecular formula of $C_{15}H_{24}O_3$, as deduced from HREIMS. Thus, four degrees of unsaturation were determined for the molecule of **2**. The IR spectrum of **2** revealed two strong absorptions at ν_{max} 1701 and 1698 cm^{-1}, suggesting the presence of two carbonyl groups, and a broad absorption at ν_{max} 2800–3600 cm^{-1}, implying the presence of a carboxylic acid. A close inspection on the ^{1}H and ^{13}C NMR spectral data (Table 2) by the assistance of

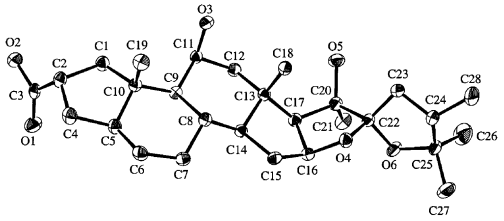


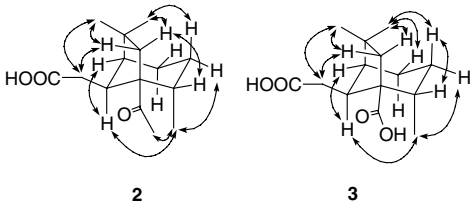
Figure 4. X-ray crystal structure of **1**.

Table 2. ^{1}H and ^{13}C NMR spectral data of **2** and **3**

C/H	2		3	
	^{1}H[a]	^{13}C[b]	^{1}H[c]	^{13}C[d]
1		66.2 (s)		60.3 (s)
2	2.54 dd (8.0, 4.0)[e]	40.2 (d)	3.16 dd (7.0, 7.0)	44.0 (d)
3	2.27 dd (16.0, 4.0)	39.9 (t)	3.65 dd (16.0, 7.0)	40.2 (t)
	2.76 dd (16.0, 8.0)		2.73 dd (16.0, 7.0)	
4		177.1 (s)		176.4 (s)
5		213.0 (s)		178.8 (s)
6	2.04 s	26.7 (q)		
7	0.92 d (7.0)	17.4 (q)	1.36 d (7.0)	19.2 (q)
8	2.06 m	38.5 (d)	2.40 m	40.5 (d)
9α	1.36 dd (14.0, 5.5)	26.4 (t)	1.25 m	27.3 (t)
9β	2.09 dd (14.0, 7.0)		2.09 dd (7.0, 7.0)	
10α	1.68 m	27.5 (t)	1.78 m	28.5 (t)
10β			1.61 m	
11	1.65 br d	52.8 (d)	1.86 br s	52.3 (d)
12α	1.79 d (13.5)	48.0 (t)	1.96 d (14.0)	50.1 (t)
12β	1.96 d (13.5)		2.45 d (14.0)	
13		38.2 (s)		38.4 (s)
14	1.18 s	26.2 (q)	1.17 s	27.1 (q)
15	1.14 s	34.1 (q)	1.27 s	35.2 (q)

[a] Spectra recorded at 500 MHz in $CDCl_3$.
[b] Spectra recorded at 125.7 MHz in $CDCl_3$.
[c] Spectra recorded at 500 MHz in pyridine-d_5.
[d] Spectra recorded at 125.7 MHz in pyridine-d_5.
[e] J values (in Hz) in parentheses.

DEPT and HMQC experiments revealed the presence of four methyl groups, including one methyl attached to a carbonyl group, one secondary, and two tertiary methyls, four sp^3-hybridized methylenes, three sp^3 methines, two sp^3 quaternary carbons, and two carbonyl carbons. The above observations suggested that **2** should be a bicyclic sesquiterpene. From the ^{1}H–^{1}H COSY spectrum of **2**, it was possible to establish the proton sequence from H-2 to H-3, H-7 to H-8, H-8 to H9, H-9 to H-10, and H-10 to H-11. The molecular framewok of **2** was further established by an HMBC experiment, which showed the following typical correlations: H-2 to C-1, C-3, C-4, C-11, C-12 and C-13, H-3 to C-2 and C-4, H-6 to C-5, H-7 to C-1, C-8 and C-9, H-8 to C-2, C-7, C-9, C-10 and C-12, H-9 to C-1, C-8 and C-11, H-10 to C-8, C-9, C-11 and C-13, H-11 to C-1, C-2, C-3, C-12, C-13 and C-15, H-12 to C-1, C-8, C-11, C-13, C-14 and C-15, and H-14 (and H-15) to C-11, C-12 and C-13. On the basis of the above results, the planar structure of **2** was elucidated. The relative stereochemistry for **2** was established by the NOESY experiment, which showed key NOE correlations as shown in Figure 5. It was found that NOEs from H-2 to H-7 and H-11, H-3 to H-12β and H-15, H-6 to H-7, H-7 to H-9α, H-8 to H-12α, H-9β to H-14, H-11 to H-15, H-12β to H-3 and H-15, H-12α to H-8, H-9β, and H-14 could be observed. Thus, the molecular structure of **2**, including the relative configuration, was fully determined. This compound was found to be a novel 4,5-secosuberosane sesquiterpene.



2 **3**

Figure 5. Selective NOE correlations of **2** and **3**.

Isishippuric acids B (**3**)[14] was found to be more polar than **2** and was assigned the molecular formula of $C_{14}H_{22}O_4$ based on the spectral evidences. ^{1}H and ^{13}C NMR spectral data (Table 2) deduced by the assistance of DEPT and 2D NMR (^{1}H–^{1}H COSY and HMQC) experiments showed that **3** contained one secondary and two tertiary methyls, four sp^3-hybridized methylenes, three sp^3 methines, two sp^3 quaternary carbons, and two carbonyl carbons. Also, the chemical shifts and splitting patterns in NMR spectra of **3** were closed to those of **2**. Thus, the structure of **3** should be very similar to that of **2** except that the proton and carbon signals at C-6 were disappeared. On the basis of the above observations, it was assumed that the acetyl group attaching at C-1 of **2** was replaced by a carboxylic acid. Furthermore, both ^{1}H–^{1}H COSY and HMBC experiments supported that **3** has very similar planar structure as that of **2**. The NOE correlations (Fig. 5) obtained from a NOESY experiment of **3** also confirmed the relative stereochemistry of isishippuric acid B. It was found that metabolite **3** is an unprecedented 4,5-seco-16-norsuberosanoid.

The cytotoxicity of **1** and **3** toward a limited panel of cancer cell lines was evaluated and the results showed that steroid **1** possessed significant cytotoxicity against the growth of HepG2 and Hep3B (both human hepatocellular carcinoma) cell lines with ED_{50} values at 3.6, and 6.9 μg/mL, respectively. Metabolites **3** exhibited

potent cytotoxicity toward P-388 (mouse lymphocytic leukemia), A549 (human lung adenocarcinoma), and HT-29 (human colon adenocarcinoma) cancer cell lines with ED_{50} values less than 0.1 μg/mL toward the above cell lines. These results suggest that suberosane-related compound **3** may warrant further antitumor studies.

Acknowledgements

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11. *A*-Nor-22-*epi*-hippurin-2α-carboxylic acid (**1**): colorless crystal; mp 263–264 °C; $[\alpha]_D^{25}$ −20 (*c* 1.04, CH_3OH); IR (KBr) ν_{max} 3487, 1712 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; FABMS *m/z* 477 $[M+H]^+$; HRFABMS *m/z* 477.3220 $[M+H]^+$ (calcd for $C_{28}H_{45}O_6$, 477.3218).
12. Crystallography data (excluding structure factors) of **1** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC239466. 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].
13. Isishippuric acid A (**2**): colorless oil; $[\alpha]_D^{25}$ −107 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2800–3600 (br), 1701, and 1698 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; EIMS *m/z* 252 $[M]^+$; HREIMS *m/z* 252.1724 $[M]^+$ (calcd for $C_{15}H_{24}O_3$, 252.1726).
14. Isishippuric acid B (**3**): white powder; mp >300 °C; $[\alpha]_D^{25}$ −115 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3000–3600 (br), 1704, and 1695 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; EIMS *m/z* 236 $[M-H_2O]^+$; HREIMS *m/z* 236.1412 $[M-H_2O]^+$ (calcd for $C_{14}H_{20}O_3$, 236.1413).