

Article

## Polyhydroxylated Steroids from the Bamboo Coral *Isis hippuris*

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**Abstract:** In previous studies on the secondary metabolites of the Taiwanese octocoral *Isis hippuris*, specimens have always been collected at Green Island. In the course of our studies on bioactive compounds from marine organisms, the acetone-solubles of the Taiwanese octocoral *I. hippuris* collected at Orchid Island have led to the isolation of five new polyoxygenated steroids: hipposterone M-O (**1–3**), hipposterol G (**4**) and hippuristeroketal A (**5**). The structures of these compounds were determined on the basis of their spectroscopic and physical data. The anti-HCMV (human cytomegalovirus) activity of **1–5** and their cytotoxicity against selected cell lines were evaluated. Compound **2** exhibited inhibitory activity against HCMV, with an EC<sub>50</sub> value of 6.0 µg/mL.

**Keywords:** octocoral; *Isis hippuris*; anti-HCMV activity

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### 1. Introduction

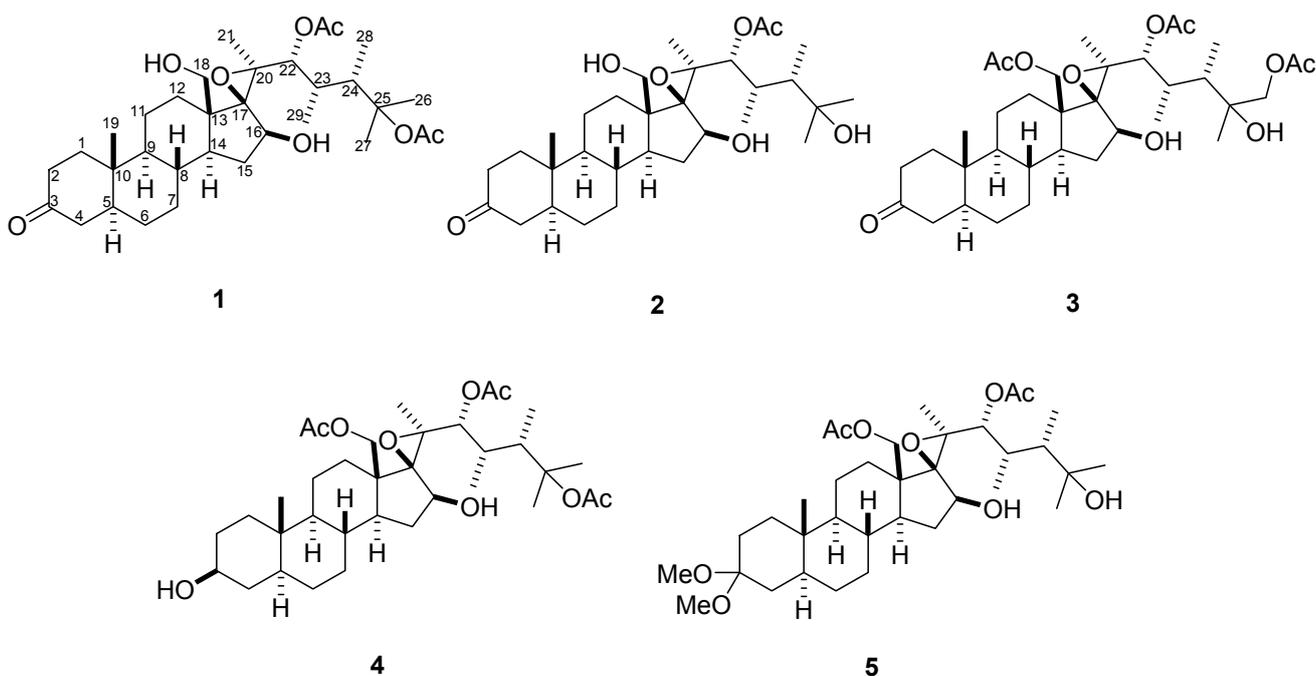
The octocoral *Isis hippuris*, distributed widely in the western Pacific, has yielded a number of polyoxygenated steroids, including hippuristanol type [1–9], gorgosterol type [10–14], hippuristerone type [3,14,15], and hippuristerol type [3,14–16]. Those of the first type were originally reported as cytotoxins and later rediscovered as selective inhibitors against the translation factor eIF4A [17,18].

Some of the second types were reported to show cytotoxicity or a reversal of multidrug resistance activity [10]. The samples for previous studies on the secondary metabolites of Taiwanese octocoral *I. hippuris* were all collected at Green Island [5–7,12,14,15]. In our continued study of the bioactive metabolites from marine organism, the Taiwanese octocoral *I. hippuris* (Figure 1) collected at Orchid Island was selected for study since its acetone extract exhibited antiviral activity against HCMV. Bioactivity-guided fractionation resulted in the isolation of five polyoxygenated steroids: hipposterone M–O (1–3), hipposterol G (4), hippuristeroketal A (5) (Figure 2). We describe herein the isolation, structure elucidation, and biological activity of these compounds.

**Figure 1.** Bamboo coral *Isis hippuris*.



**Figure 2.** Structures of compounds 1–5.



## 2. Results and Discussion

The molecular formula  $C_{33}H_{52}O_8$  was assigned to hipposterone M (**1**) on the basis of positive HRESIMS (found  $m/z$  599.3556  $[M + Na]^+$ ), implying eight degrees of unsaturation. Its IR spectrum revealed the absorptions for hydroxyl ( $\nu_{max}$  3454  $cm^{-1}$ ), ketone carbonyl ( $\nu_{max}$  1717  $cm^{-1}$ ), and ester carbonyl ( $\nu_{max}$  1733  $cm^{-1}$ ) groups. NMR data (Tables 1 and 2) of **1** indicated the presence of a ketone ( $\delta_C$  211.7), two ester carbonyls, two oxygenated  $sp^3$  methines, an oxygenated  $sp^3$  methylene, three oxygenated  $sp^3$  quaternary carbons, two secondary methyls, four tertiary methyls, six non-oxygenated  $sp^3$  methines, eight non-oxygenated  $sp^3$  methylenes, and two non-oxygenated  $sp^3$  quaternary carbons. NMR signals (Table 1) at  $\delta_C$  80.0 (qC) and 67.1 (qC) suggested the existence of a tetrasubstituted epoxy. The quaternary carbon at  $\delta_C$  85.5, which has HMBC correlation (Figure 3) with tertiary methyl signals at  $\delta_H$  1.56 (s) and 1.43 (s) (Table 2) disclosed the presence of  $-OC(CH_3)_2$ . By extensive analysis of 2D NMR spectra, including COSY, HSQC, NOESY (Figure 4) and HMBC, **1** was shown to be a derivative of hippuristerone A [15]. HMBC correlations (Figure 3) from H<sub>2</sub>-18 ( $\delta_H$  3.94 and 3.75) to C-12, C-13, C-14, and C-17 established **1** as 18-hydroxyhippuristerone A. The stereochemistry of the side chain moiety was determined by comparison of the  $^1H$  and  $^{13}C$  NMR spectral data with those of hippuristerone A.

Hipposterone N (**2**) had a molecular formula of  $C_{31}H_{50}O_7$ , as suggested by the NMR and HRESIMS data. Its IR spectrum also showed the absorptions for hydroxyl ( $\nu_{max}$  3454  $cm^{-1}$ ), ketone carbonyl ( $\nu_{max}$  1715  $cm^{-1}$ ), and ester carbonyl ( $\nu_{max}$  1731  $cm^{-1}$ ) groups. NMR data (Tables 1 and 2) of **2** revealed the presence of a ketone ( $\delta_C$  211.7), an ester carbonyl, two oxygenated  $sp^3$  methines, an oxygenated  $sp^3$  methylene, three oxygenated  $sp^3$  quaternary carbons, two secondary methyls, four tertiary methyls, six non-oxygenated  $sp^3$  methines, eight non-oxygenated  $sp^3$  methylenes, and two non-oxygenated  $sp^3$  quaternary carbons. NMR data (Tables 1 and 2) (Figure 3) of **2** resembled those of **1** except for a hydroxyl group replacing the tertiary acetoxy in **1** [14]. HMBC correlations (Figure 3) from H<sub>3</sub>-26 ( $\delta_H$  1.24) and H<sub>3</sub>-27 ( $\delta_H$  1.21) to C-25 established **2** as a 25-deacetyl-18-hydroxy derivative of hippuristerone A. The stereochemistry of the side chain moiety was determined by comparison of the  $^1H$  and  $^{13}C$  NMR data with those of hippuristerones F, H, and I isolated from *I. hippuris* [16].

The positive HRESIMS of hipposterone O (**3**) established a molecular formula of  $C_{35}H_{54}O_{10}$ . NMR data (Tables 1 and 2) of **3** showed the presence of a ketone ( $\delta_C$  211.5), three ester carbonyls, two oxygenated  $sp^3$  methines, two oxygenated  $sp^3$  methylene, three oxygenated  $sp^3$  quaternary carbons, two secondary methyls, three tertiary methyls, six non-oxygenated  $sp^3$  methines, eight non-oxygenated  $sp^3$  methylenes, and two non-oxygenated  $sp^3$  quaternary carbons. By comparison of NMR spectroscopic data (Tables 1 and 2) of **3** with those of hippuristerone J [14], the primary acetoxy group at C-21 was shift to C-18 on the basis of HMBC correlations (Figure 3) from H<sub>2</sub>-18 [ $\delta_H$  4.23 (1H, d,  $J = 11.6$  Hz) and 4.30 (1H, d,  $J = 11.6$  Hz)] to C-12, C-13, C-14, C-17, and carbonyl carbon of 18-OAc. The stereochemistry of the side chain moiety was determined by comparison of the  $^1H$  and  $^{13}C$  NMR spectral data with those of hippuristerones J and K previously isolated from *I. hippuris* [14].

**Table 1.**  $^{13}\text{C}$  NMR data for compounds 1–5.

| C#  | 1, <sup>a</sup> $\delta_{\text{C}}$ , type | 2, <sup>a</sup> $\delta_{\text{C}}$ , type | 3, <sup>a</sup> $\delta_{\text{C}}$ , type | 4, <sup>b</sup> $\delta_{\text{C}}$ , type | 5, <sup>c</sup> $\delta_{\text{C}}$ , type |
|-----|--|--|--|--|--|
| 1   | 38.3, CH <sub>2</sub>                      | 38.3, CH <sub>2</sub>                      | 38.2, CH <sub>2</sub>                      | 36.7, CH <sub>2</sub>                      | 35.6, CH <sub>2</sub>                      |
| 2   | 38.1, CH <sub>2</sub>                      | 38.1, CH <sub>2</sub>                      | 38.0, CH <sub>2</sub>                      | 31.4, CH <sub>2</sub>                      | 29.2, CH <sub>2</sub>                      |
| 3   | 211.7, qC                                  | 211.7, qC                                  | 211.5, qC                                  | 71.2, CH                                   | 100.7, qC                                  |
| 4   | 44.5, CH <sub>2</sub>                      | 44.5, CH <sub>2</sub>                      | 44.5, CH <sub>2</sub>                      | 38.0, CH <sub>2</sub>                      | 36.2, CH <sub>2</sub>                      |
| 5   | 46.5, CH                                   | 46.5, CH                                   | 46.4, CH                                   | 44.7, CH                                   | 43.0, CH                                   |
| 6   | 28.6, CH <sub>2</sub>                      | 28.5, CH <sub>2</sub>                      | 28.5, CH <sub>2</sub>                      | 28.3, CH <sub>2</sub>                      | 28.8, CH <sub>2</sub>                      |
| 7   | 31.7, CH <sub>2</sub>                      | 31.7, CH <sub>2</sub>                      | 31.5, CH <sub>2</sub>                      | 31.9, CH <sub>2</sub>                      | 32.7, CH <sub>2</sub>                      |
| 8   | 34.5, CH                                   | 34.4, CH                                   | 34.4, CH                                   | 34.5, CH                                   | 35.1, CH                                   |
| 9   | 53.1, CH                                   | 53.6, CH                                   | 53.4, CH                                   | 54.0, CH                                   | 55.1, CH                                   |
| 10  | 35.7, qC                                   | 35.7, qC                                   | 35.6, CH                                   | 35.5, qC                                   | 36.4, qC                                   |
| 11  | 21.0, CH <sub>2</sub>                      | 21.0, CH <sub>2</sub>                      | 21.5, CH <sub>2</sub>                      | 21.4, CH <sub>2</sub>                      | 22.0, CH <sub>2</sub>                      |
| 12  | 30.6, CH <sub>2</sub>                      | 31.0, CH <sub>2</sub>                      | 32.4, CH <sub>2</sub>                      | 32.2, CH <sub>2</sub>                      | 33.3, CH <sub>2</sub>                      |
| 13  | 46.8, qC                                   | 46.7, qC                                   | 45.6, qC                                   | 45.6, qC                                   | 46.5, qC                                   |
| 14  | 47.7, CH                                   | 48.7, CH                                   | 49.2, CH                                   | 48.7, CH                                   | 50.3, CH                                   |
| 15  | 33.3, CH <sub>2</sub>                      | 33.3, CH <sub>2</sub>                      | 33.5, CH <sub>2</sub>                      | 33.4, CH <sub>2</sub>                      | 34.4, CH <sub>2</sub>                      |
| 16  | 70.0, CH                                   | 70.1, CH                                   | 70.3, CH                                   | 70.1, CH                                   | 71.1, CH                                   |
| 17  | 80.0, qC                                   | 79.7, qC                                   | 77.2, qC                                   | 77.7, qC                                   | 78.6, qC                                   |
| 18  | 61.9, CH <sub>2</sub>                      | 61.9, CH <sub>2</sub>                      | 63.5, CH <sub>2</sub>                      | 63.5, CH <sub>2</sub>                      | 64.3, CH <sub>2</sub>                      |
| 19  | 11.3, CH <sub>3</sub>                      | 11.4, CH <sub>3</sub>                      | 11.4, CH <sub>3</sub>                      | 12.2, CH <sub>3</sub>                      | 12.0, CH <sub>3</sub>                      |
| 20  | 67.1, qC                                   | 67.5, qC                                   | 66.7, qC                                   | 66.4, qC                                   | 67.3, qC                                   |
| 21  | 16.1, CH <sub>3</sub>                      | 15.9, CH <sub>3</sub>                      | 16.1, CH <sub>3</sub>                      | 16.2, CH <sub>3</sub>                      | 17.1, CH <sub>3</sub>                      |
| 22  | 77.2, CH                                   | 77.2, CH                                   | 77.2, CH                                   | 77.2, CH                                   | 78.1, CH                                   |
| 23  | 33.5, CH                                   | 32.9, CH                                   | 32.5, CH                                   | 33.6, CH                                   | 33.6, CH                                   |
| 24  | 39.9, CH                                   | 41.7, CH                                   | 38.8, CH                                   | 40.1, CH                                   | 42.2, CH                                   |
| 25  | 85.5, qC                                   | 73.7, qC                                   | 74.2, qC                                   | 85.6, qC                                   | 73.5, qC                                   |
| 26  | 23.2, CH <sub>3</sub>                      | 30.9, CH <sub>3</sub>                      | 71.0, CH <sub>2</sub>                      | 22.8, CH <sub>3</sub>                      | 31.2, CH <sub>3</sub>                      |
| 27  | 25.1, CH <sub>3</sub>                      | 25.8, CH <sub>3</sub>                      | 20.3, CH <sub>3</sub>                      | 25.1, CH <sub>3</sub>                      | 25.9, CH <sub>3</sub>                      |
| 28  | 10.4, CH <sub>3</sub>                      | 11.4, CH <sub>3</sub>                      | 10.9, CH <sub>3</sub>                      | 10.5, CH <sub>3</sub>                      | 11.7, CH <sub>3</sub>                      |
| 29  | 11.9, CH <sub>3</sub>                      | 12.1, CH <sub>3</sub>                      | 12.3, CH <sub>3</sub>                      | 11.9, CH <sub>3</sub>                      | 12.6, CH <sub>3</sub>                      |
| OAc | 20.9, CH <sub>3</sub>                      | 20.9, CH <sub>3</sub>                      | 21.2, CH <sub>3</sub>                      | 21.2, CH <sub>3</sub>                      | 21.1, CH <sub>3</sub>                      |
|     | 171.6, qC                                  | 171.6, qC                                  | 171.1, qC                                  | 171.0, qC                                  | 170.6, qC                                  |
|     | 22.6, CH <sub>3</sub>                      |  | 21.1, CH <sub>3</sub>                      | 21.0, CH <sub>3</sub>                      | 20.9, CH <sub>3</sub>                      |
|     | 169.8, qC                                  |  | 171.3, qC                                  | 171.2, qC                                  | 171.4, qC                                  |
|     |  |  | 21.1, CH <sub>3</sub>                      | 22.7, CH <sub>3</sub>                      |  |
|     |  |  | 170.8, qC                                  | 169.9, qC                                  |  |
| OMe |  |  |  |  | 47.6, CH <sub>3</sub>                      |
|     |  |  |  |  | 47.5, CH <sub>3</sub>                      |

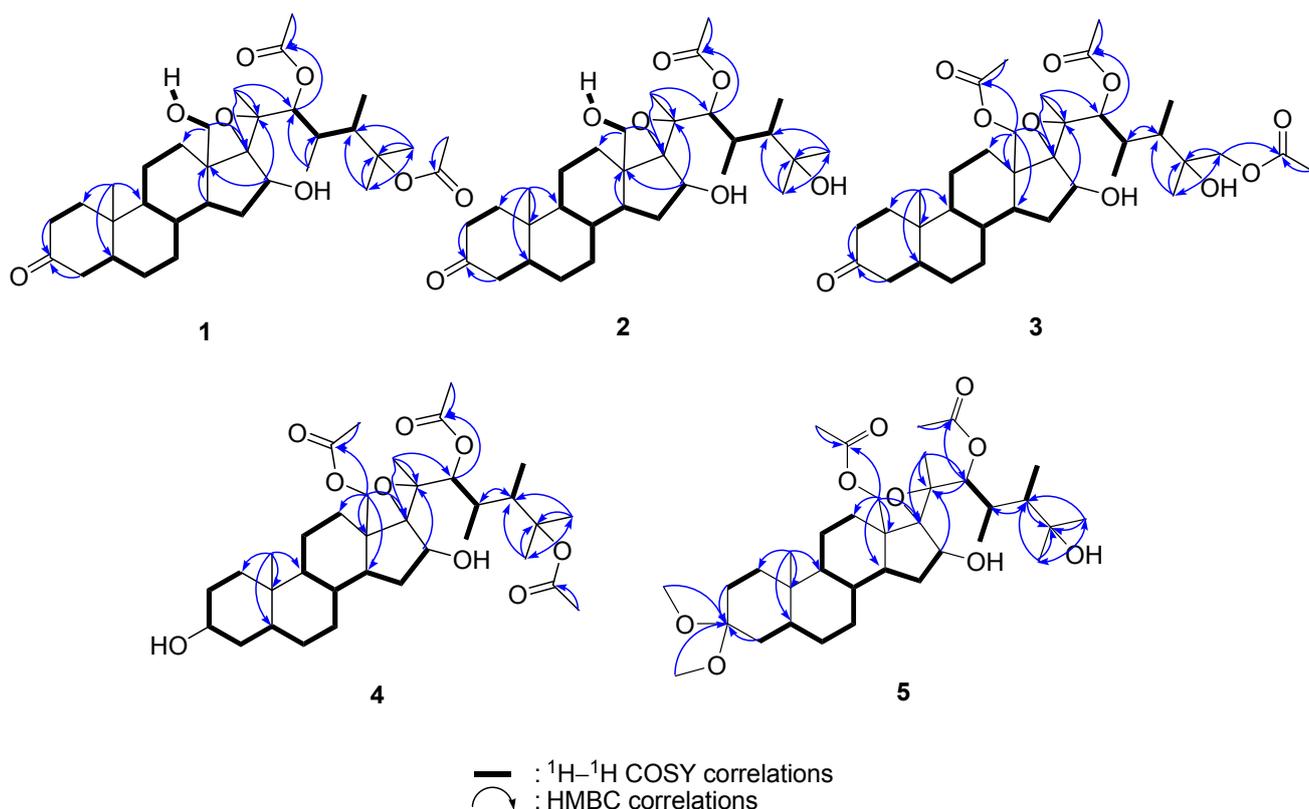
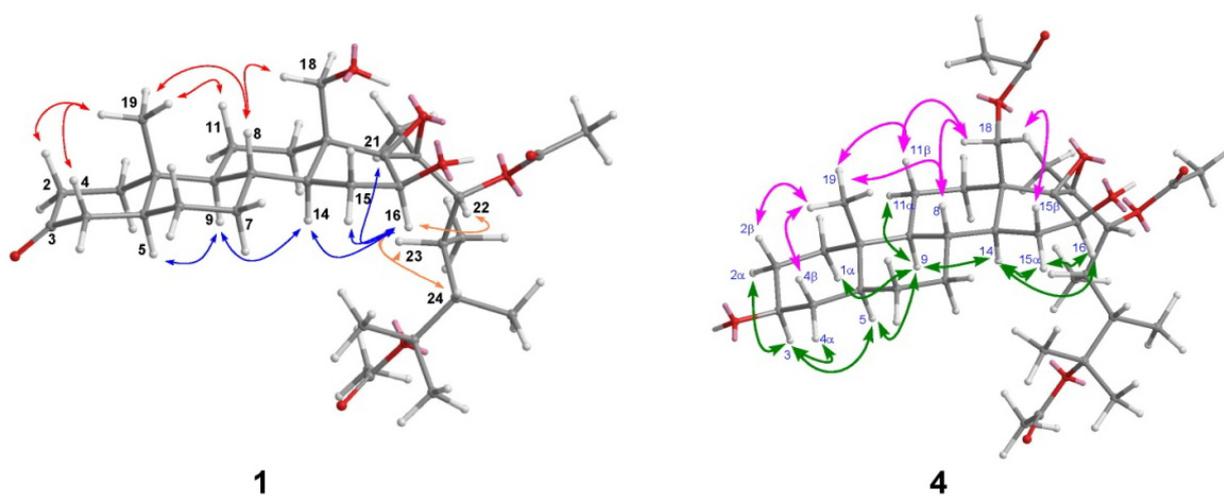
<sup>a</sup> Spectra were measured in CDCl<sub>3</sub> (100 MHz); <sup>b</sup> Spectra were measured in CDCl<sub>3</sub> (125 MHz);

<sup>c</sup> Spectra were measured in C<sub>6</sub>D<sub>6</sub> (125 MHz).

**Table 2.**  $^1\text{H}$  NMR data for compounds 1–5.

| H#    | 1, $\delta_{\text{H}}$ (J in Hz) <sup>a</sup>     | 2, $\delta_{\text{H}}$ (J in Hz) <sup>a</sup>     | 3, $\delta_{\text{H}}$ (J in Hz) <sup>a</sup>     | 4, $\delta_{\text{H}}$ (J in Hz) <sup>b</sup> | 5, $\delta_{\text{H}}$ (J in Hz) <sup>c</sup>           |
|-------|---|---|---|---|---|
| 1     | $\alpha$ : 1.39 m<br>$\beta$ : 2.02 m             | $\alpha$ : 1.35 m<br>$\beta$ : 2.00 m             | $\alpha$ : 1.32 m<br>$\beta$ : 1.97 m             | $\alpha$ : 1.02 m<br>$\beta$ : 1.69 m         | $\alpha$ : 1.33 m<br>$\beta$ : 1.06 m                   |
| 2     | $\alpha$ : 2.32 m<br>$\beta$ : 2.38 m             | $\alpha$ : 2.31 m<br>$\beta$ : 2.39 m             | $\alpha$ : 2.31 m<br>$\beta$ : 2.37 m             | $\alpha$ : 1.82 m<br>$\beta$ : 1.41 m         | $\alpha$ : 1.86 m<br>$\beta$ : 1.41 m                   |
| 3     |   |   |   | 3.60 m  |   |
| 4     | $\alpha$ : 2.12 dd ovl<br>$\beta$ : 2.28 t (13.6) | $\alpha$ : 2.09 dd ovl<br>$\beta$ : 2.27 t (13.6) | $\alpha$ : 2.12 dd ovl<br>$\beta$ : 2.26 t (13.6) | $\alpha$ : 1.58 m<br>$\beta$ : 1.29 m         | $\alpha$ : 1.86 dd (13.6, 3.6)<br>$\beta$ : 1.41 dd ovl |
| 5     | 1.56 m  | 1.54 m  | 1.55 m  | 1.54 m  | 1.34 m  |
| 6     | 1.38 m  | 1.38 m  | 1.39 m  | 1.34 m  | 1.08 m  |
| 7     | 1.79 m<br>0.93 m                                  | 1.78 m<br>0.92 m                                  | 1.82 m<br>0.95 m                                  | 1.78 m<br>0.91 m                              | 1.54 m<br>0.67 m  |
| 8     | 1.58 m  | 1.58 m  | 1.72 m  | 1.67 m  | 1.45 m  |
| 9     | 0.85 m  | 0.74 m  | 0.81 m  | 0.75 m  | 0.70 m  |
| 11    | $\alpha$ : 1.66 m<br>$\beta$ : 1.48 m             | $\alpha$ : 1.65 m<br>$\beta$ : 1.44 m             | $\alpha$ : 1.63 m<br>$\beta$ : 1.33 m             | $\alpha$ : 1.60 m<br>$\beta$ : 1.23 m         | $\alpha$ : 1.48 m<br>$\beta$ : 1.23 m                   |
| 12    | $\alpha$ : 1.28 m<br>$\beta$ : 2.44 m             | $\alpha$ : 1.28 m<br>$\beta$ : 2.44 m             | $\alpha$ : 1.34 m<br>$\beta$ : 2.16 m             | $\alpha$ : 1.38 m<br>$\beta$ : 2.17 m         | $\alpha$ : 1.44 m<br>$\beta$ : 2.28 m                   |
| 14    | 1.36 m  | 1.18 m  | 1.23 m  | 1.31 m  | 1.28 m  |
| 15    | $\alpha$ : 2.23 m<br>$\beta$ : 1.44 m             | $\alpha$ : 2.24 m<br>$\beta$ : 1.46 m             | $\alpha$ : 2.21 m<br>$\beta$ : 1.48 m             | $\alpha$ : 2.21 m<br>$\beta$ : 1.46 m         | $\alpha$ : 2.22 m<br>$\beta$ : 1.59 m                   |
| 16    | 4.10 t (7.2)                                      | 4.13 t (7.6)                                      | 4.06 t (7.6)                                      | 4.04 dd (8.0, 7.5)                            | 4.38 t (7.5)  |
| 18    | 3.75 t (10.4)<br>3.94 d (11.6)                    | 3.74 t (11.2)<br>3.94 dd (11.6, 2.4)              | 4.23 d (11.6)<br>4.30 d (11.6)                    | 4.27 d (11.5)<br>4.20 d (11.5)                | 4.55 d (11.5)<br>4.49 d (11.5)                          |
| 19    | 1.02 s  | 1.02 s  | 1.02 s  | 0.82 s  | 0.64 s  |
| 21    | 1.64 s  | 1.66 s  | 1.60 s  | 1.59 s  | 1.84 s  |
| 22    | 4.62 d (10.8)                                     | 4.60 d (10.8)                                     | 4.66 d (10.8)                                     | 4.67 d (11.0)                                 | 5.04 d (10.5)   |
| 23    | 2.28 m  | 2.47 m  | 2.50 m  | 2.29 m  | 2.43 m  |
| 24    | 1.97 q (8.0)                                      | 1.47 q (6.8)                                      | 1.64 q (6.8)                                      | 1.92 q (7.0)                                  | 1.55 q (7.5)  |
| 26    | 1.56 s  | 1.24 s  | 3.89 d (11.6)<br>4.04 d (11.6)                    | 1.56 s  | 0.88 s  |
| 27    | 1.43 s  | 1.21 s  | 1.18 s  | 1.46 s  | 0.78 s  |
| 28    | 0.90 d (8.0)                                      | 0.90 d (6.8)                                      | 0.88 d (6.8)                                      | 0.91 d (7.0)                                  | 0.65 d (7.5)  |
| 29    | 0.88 d (6.4)                                      | 0.86 d (6.4)                                      | 0.88 d (6.8)                                      | 0.87 d (7.0)                                  | 0.80 d (7.0)  |
| OAc   | 2.14 s, 1.99 s                                    | 2.14 s  | 2.07 s, 2.13 s, 2.13 s                            | 2.06 s, 2.00 s, 2.13 s                        | 1.76 s, 1.69 s  |
| OMe   |   |   |   |   | 3.12 s, 3.02s   |
| OH-16 | 3.36 s  | 3.43 s  | 3.27 br s   | 3.19 br s                                     | 3.83 br s   |
| OH-18 | 2.44 d ovl  | 3.46 d ovl  |   |   |   |

<sup>a</sup> Spectra were measured in  $\text{CDCl}_3$  (400 MHz); <sup>b</sup> Spectra were measured in  $\text{CDCl}_3$  (500 MHz); <sup>c</sup> Spectra were measured in  $\text{C}_6\text{D}_6$  (500 MHz).

**Figure 3.** COSY and HMBC correlations of compounds 1–5.**Figure 4.** NOESY correlations of compounds 1 and 4.

Hiposterol G (**4**) was isolated as a white powder, and its molecular formula,  $\text{C}_{35}\text{H}_{56}\text{O}_9$ , was determined by HRESIMS. Its IR spectrum revealed the functionalities of hydroxyl ( $\nu_{\text{max}}$   $3471\text{ cm}^{-1}$ ) and ester carbonyl ( $\nu_{\text{max}}$   $1734\text{ cm}^{-1}$ ). NMR data (Tables 1 and 2) of **4** indicated the presence of three ester carbonyls, three oxygenated  $\text{sp}^3$  methines, an oxygenated  $\text{sp}^3$  methylene, three oxygenated  $\text{sp}^3$  quaternary carbons, two secondary methyls, four tertiary methyls, six non-oxygenated  $\text{sp}^3$  methines, eight non-oxygenated  $\text{sp}^3$  methylenes, and two non-oxygenated  $\text{sp}^3$  quaternary carbons. NMR data (Tables 1 and 2) of **4** were similar to those of hippuristerone G [16] with the absence of the ketone carbon signal at  $\delta_{\text{C}}$  211.6 ppm and the presence of signal at  $\delta_{\text{H}}$  3.60 ppm NOE correlation H-3/H-5

and chemical shift values for C-1–C-7 nuclei. This is in agreement with the results reported for 5 $\alpha$ -cholestan-3 $\beta$ -ol, which allowed us to propose a  $\beta$  orientation of OH group at C-3 (Figure 4). The stereochemistry of the side chain moiety was determined by comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data with those of hippuristerone A.

The molecular formula of hippuristeroketal A (**5**) was found to be  $\text{C}_{35}\text{H}_{58}\text{O}_9$ , as deduced from HRESIMS data. Its IR spectrum revealed the absorptions for hydroxyl ( $\nu_{\text{max}}$  3471  $\text{cm}^{-1}$ ) and ester carbonyl ( $\nu_{\text{max}}$  1731  $\text{cm}^{-1}$ ) groups. NMR data (Tables 1 and 2) of **5** indicated the presence of a ketal ( $\delta_{\text{C}}$  100.7), two ester carbonyls, two oxygenated  $\text{sp}^3$  methines, an oxygenated  $\text{sp}^3$  methylene, three oxygenated  $\text{sp}^3$  quaternary carbons, two secondary methyls, four tertiary methyls, six non-oxygenated  $\text{sp}^3$  methines, eight non-oxygenated  $\text{sp}^3$  methylenes, and two non-oxygenated  $\text{sp}^3$  quaternary carbons. By comparison of the NMR spectroscopic data (Tables 1 and 2) of **5** resembled those of hippuristerone F [14] with the absence of ketone carbon at  $\delta_{\text{C}}$  211.6 and the presence of two methoxyl signals [ $\delta_{\text{H}}$  3.12 (3H, s), 3.02 (3H, s) and  $\delta_{\text{C}}$  47.6 ( $\text{CH}_3$ ), 47.5 ( $\text{CH}_3$ )] in the molecule. The HMBC correlations (Figure 3) of the methoxyl protons with C-3 [ $\delta_{\text{C}}$  100.7 (qC)], suggesting that C-3 was substituted by two methoxy groups. The stereochemistry of the side chain moiety was determined by comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data with those of hippuristerones F, H, and I previously isolated from *I. hippuris* [16]. Compound **5** was not an artifact because  $^1\text{H}$  NMR signals for the dimethylketal were observed before MeOH treatment.

Metabolites **1–5** were not cytotoxic against P-388 (mouse lymphocytic leukemia), HT-29 (human colon adenocarcinoma) tumor cells, and human embryonic lung (HEL) cells with  $\text{IC}_{50}$  values greater than 50  $\mu\text{g}/\text{mL}$ . The anti-HCMV activity and cytotoxicity against of selected cell lines of **1–5** were evaluated. Compound **2** exhibited inhibitory activity against HCMV, with an  $\text{EC}_{50}$  values of 6.0  $\mu\text{g}/\text{mL}$ .

### 3. Experimental Section

#### 3.1. General Experimental Procedures

Optical rotations were determined with a JASCO P1020 digital polarimeter. Ultraviolet (UV) and infrared (IR) spectra were obtained on JASCO V-650 and JASCO FT/IR-4100 spectrophotometers, respectively. NMR spectra were recorded on a Varian MR 400 NMR spectrometer at 400 MHz for  $^1\text{H}$  and 100 MHz for  $^{13}\text{C}$  or on a Varian Unity INOVA 500 FT-NMR spectrometer at 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$ , respectively.  $^1\text{H}$  NMR chemical shifts are expressed in  $\delta$  (ppm) referring to the solvent peaks  $\delta_{\text{H}}$  7.27 and 7.15 for  $\text{CDCl}_3$  and  $\text{C}_6\text{D}_6$ , respectively, and coupling constants are expressed in Hz.  $^{13}\text{C}$  NMR chemical shifts are expressed in  $\delta$  (ppm) referring to the solvent peaks  $\delta_{\text{C}}$  77.0 and 128.0 for  $\text{CDCl}_3$  and  $\text{C}_6\text{D}_6$ , respectively. ESI-MS were recorded by ESI FT-MS on a Bruker APEX II mass spectrometer. Silica gel 60 (Merck, Germany, 230–400 mesh) and LiChroprep RP-18 (Merck, 40–63  $\mu\text{m}$ ) were used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60  $\text{F}_{254}$ , 0.25 mm) and precoated RP-18  $\text{F}_{254\text{s}}$  plates (Merck) were used for thin-layer chromatography (TLC) analysis. High-performance liquid chromatography (HPLC) was carried out using a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector at 220 nm together with a semi-preparative reversed-phase column (Merck, Hibar LiChrospher RP-18e, 5  $\mu\text{m}$ , 250  $\times$  25 mm).

### 3.2. Biological Material

The octocoral *I. hippuris* was collected by hand using scuba at Orchid Island, 70 km off the southeastern coast of Taiwan, in August 2008 at a depth of 9 m and stored in a freezer until extraction. The voucher specimen (LY-19) was identified by Prof. Chang-Feng Dai, National Taiwan University and deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Taiwan.

### 3.3. Extraction and Isolation

A specimen of octocoral *I. hippuris* (4.0 kg, wet weight) was minced and exhaustively extracted with acetone (3 × 3 L) at room temperature. The combined acetone extracts was then partitioned between H<sub>2</sub>O and EtOAc. The resulting EtOAc extract (25.6 g) was subjected to gravity silica gel 60 column chromatography (Si 60 CC) using *n*-hexane–EtOAc and EtOAc–MeOH of increasing polarity, to give 44 fractions. Fraction 28 (0.86 g), eluted with *n*-hexane–EtOAc (1:6), was further subjected to Si 60 CC (*n*-hexane–EtOAc, 5:3) to give 4 subfractions. A subfraction 28-2 (105 mg) was separated by a RP-18 flash column (MeOH–H<sub>2</sub>O, 75:25 to 100:0) to give four fractions. In turn, a subfraction 28-2-2, eluted with MeOH–H<sub>2</sub>O (80:20), was further purified by RP-18 HPLC (MeOH–H<sub>2</sub>O–MeCN, 80:20:5) to afford **1** (3.0 mg) and **4** (0.5 mg). Similarly, the subfraction 28-3 (112 mg) was further subjected to a RP-18 flash column (MeOH–H<sub>2</sub>O, 75:25 to 100:0) to give five subfractions. A subfraction 28-3-2 (112 mg), eluted with MeOH–H<sub>2</sub>O (70:30), was further purified by RP-18 HPLC (MeOH–H<sub>2</sub>O–MeCN, 75:25:5) to obtain **1** (0.2 mg) and **4** (0.3 mg). Likewise, the subfraction 28-3-3, eluted with MeOH–H<sub>2</sub>O (80:20), was purified by RP-18 HPLC (MeOH–H<sub>2</sub>O–MeCN, 75:25:5) to give **5** (1.2 mg). Fraction 29 (0.41 g), eluted with *n*-hexane–EtOAc (1:7), was subjected to Si 60 CC (*n*-hexane–EtOAc, 8:2 to 2:8) to give four subfractions. A subfraction 29-3 (309 mg), eluted with *n*-hexane–EtOAc (2:7), was further fractionated by a RP-18 flash column (MeOH–H<sub>2</sub>O, 60:40 to 100:0) to give four subfractions. A subfraction 29-3-2, eluted with MeOH–H<sub>2</sub>O (75:25), was further purified by RP-18 HPLC (MeOH–H<sub>2</sub>O, 70:30) to afford **3** (1.0 mg), **2** (1.2 mg), and **1** (0.2 mg).

**Hippossterone M (1)**: White amorphous powder;  $[\alpha]_D^{25}$   $-8$  (*c* 0.1, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3454, 2954, 2922, 1733, 1717, 1558, 1456, 1374, 1238, 1152, 1019 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) data in Tables 1 and 2; HRESIMS *m/z* 599.3556 [M + Na]<sup>+</sup> (calcd for C<sub>33</sub>H<sub>52</sub>O<sub>8</sub>Na, 599.3560).

**Hippossterone N (2)**: White amorphous powder;  $[\alpha]_D^{25}$   $-11$  (*c* 0.1, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3463, 2970, 2933, 1731, 1715, 1374, 1244, 1021, 735 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) data in Tables 1 and 2; HRESIMS *m/z* 557.3452 [M + Na]<sup>+</sup> (calcd for C<sub>31</sub>H<sub>50</sub>O<sub>7</sub>Na, 557.3454).

**Hippossterone O (3)**: White amorphous powder;  $[\alpha]_D^{25}$   $-5$  (*c* 0.1, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3471, 2974, 2939, 1731, 1449, 1373, 1247, 1023, 739 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) data in Tables 1 and 2; HRESIMS *m/z* 657.3616 [M + Na]<sup>+</sup> (calcd for C<sub>35</sub>H<sub>54</sub>O<sub>10</sub>Na, 657.3614).

**Hipposterol G (4):** White amorphous powder;  $[\alpha]_D^{25} +5$  (*c* 0.1, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3471, 2928, 2860, 1734, 1454, 1371, 1244, 1023, 736 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) data in Tables 1 and 2; HRESIMS *m/z* 643.3819 [M + Na]<sup>+</sup> (calcd for C<sub>35</sub>H<sub>56</sub>O<sub>9</sub>Na, 643.3822).

**Hppuristeroketal A (5):** White amorphous powder;  $[\alpha]_D^{25} +21$  (*c* 0.1, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3471, 2974, 1731, 1373, 1248, 1041, 739 cm<sup>-1</sup>; <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>, 500 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) data in Tables 1 and 2; HRESIMS *m/z* 645.3975 [M + Na]<sup>+</sup> (calcd for C<sub>35</sub>H<sub>58</sub>O<sub>9</sub>Na, 645.3978).

### 3.4. Cytotoxicity Assay

Cytotoxicity was determined on P-388 (mouse lymphocytic leukemia), HT-29 (human colon adenocarcinoma), and A-549 (human lung epithelial carcinoma) tumor cells using a modification of the MTT colorimetric method according to a previously described procedure [19,20]. The provision of the P-388 cell line was supported by J.M. Pezzuto, formerly of the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago. HT-29 and A-549 cell lines were purchased from the American Type Culture Collection.

### 3.5. Anti-HCMV Assay

To determine the effects of natural products upon HCMV cytopathic effect (CPE), confluent human embryonic lung (HEL) cells grown in 24-well plates were incubated for 1 h in the presence or absence of various concentrations of tested natural products. Then, cells were infected with HCMV at an input of 1000 pfu (plaque forming units) per well of 24-well dish. Antiviral activity was expressed as IC<sub>50</sub> (50% inhibitory concentration), or compound concentration required to reduce virus induced CPE by 50% after 7 days as compared with the untreated control. To monitor the cell growth upon treating with natural products, an MTT-colorimetric assay was employed [21].

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