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 EC50 value of 6.0 μg/mL.

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

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), hiposterol 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\textsubscript{33}H\textsubscript{52}O\textsubscript{8} was assigned to hipposterone M (1) on the basis of positive HRESIMS (found \textit{m}/\textit{z} 599.3556 [M + Na]\textsuperscript{+}), implying eight degrees of unsaturation. Its IR spectrum revealed the absorptions for hydroxyl (\textit{\nu}\textsubscript{max} 3454 cm\textsuperscript{-1}), ketone carbonyl (\textit{\nu}\textsubscript{max} 1717 cm\textsuperscript{-1}), and ester carbonyl (\textit{\nu}\textsubscript{max} 1733 cm\textsuperscript{-1}) groups. NMR data (Tables 1 and 2) of 1 indicated the presence of a ketone (\textit{\delta}_C 211.7), two ester carbonyls, two oxygenated sp\textsuperscript{3} methines, an oxygenated sp\textsuperscript{3} methylene, three oxygenated sp\textsuperscript{3} quaternary carbons, two secondary methyls, four tertiary methyls, six non-oxygenated sp\textsuperscript{3} methines, eight non-oxygenated sp\textsuperscript{3} methylenes, and two non-oxygenated sp\textsuperscript{3} quaternary carbons. NMR signals (Table 1) at \textit{\delta}_C 80.0 (qC) and 67.1 (qC) suggested the existence of a tetrasubstituted epoxy. The quaternary carbon at \textit{\delta}_C 85.5, which has HMBC correlation (Figure 3) with tertiary methyl signals at \textit{\delta}_H 1.56 (s) and 1.43 (s) (Table 2) disclosed the presence of –OC(CH\textsubscript{3})\textsubscript{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\textsubscript{2}-18 (\textit{\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 \textsuperscript{1}H and \textsuperscript{13}C NMR spectral data with those of hippuristerone A.

Hipposterone N (2) had a molecular formula of C\textsubscript{31}H\textsubscript{50}O\textsubscript{7}, as suggested by the NMR and HRESIMS data. Its IR spectrum also showed the absorptions for hydroxyl (\textit{\nu}\textsubscript{max} 3454 cm\textsuperscript{-1}), ketone carbonyl (\textit{\nu}\textsubscript{max} 1715 cm\textsuperscript{-1}), and ester carbonyl (\textit{\nu}\textsubscript{max} 1731 cm\textsuperscript{-1}) groups. NMR data (Tables 1 and 2) of 2 revealed the presence of a ketone (\textit{\delta}_C 211.7), an ester carbonyl, two oxygenated sp\textsuperscript{3} methines, an oxygenated sp\textsuperscript{3} methylene, three oxygenated sp\textsuperscript{3} quaternary carbons, two secondary methyls, four tertiary methyls, six non-oxygenated sp\textsuperscript{3} methines, eight non-oxygenated sp\textsuperscript{3} methylenes, and two non-oxygenated sp\textsuperscript{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 acetoxyl in 1 [14]. HMBC correlations (Figure 3) from H\textsubscript{3}-26 (\textit{\delta}_H 1.24) and H\textsubscript{3}-27 (\textit{\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 \textsuperscript{1}H and \textsuperscript{13}C NMR spectral data with those of hippuristerones F, H, and I isolated from \textit{I. hippuris} [16].

The positive HRESIMS of hipposterone O (3) established a molecular formula of C\textsubscript{35}H\textsubscript{54}O\textsubscript{10}. NMR data (Tables 1 and 2) of 3 showed the presence of a ketone (\textit{\delta}_C 211.5), three ester carbonyls, two oxygenated sp\textsuperscript{3} methines, two oxygenated sp\textsuperscript{3} methylene, three oxygenated sp\textsuperscript{3} quaternary carbons, two secondary methyls, three tertiary methyls, six non-oxygenated sp\textsuperscript{3} methines, eight non-oxygenated sp\textsuperscript{3} methylenes, and two non-oxygenated sp\textsuperscript{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\textsubscript{2}-18 [\textit{\delta}_H 4.23 (1H, d, \textit{J} = 11.6 Hz) and 4.30 (1H, d, \textit{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 \textsuperscript{1}H and \textsuperscript{13}C NMR spectral data with those of hippuristerones J and K previously isolated from \textit{I. hippuris} [14].
Table 1. $^{13}$C NMR data for compounds 1–5.

| C# | 1. $^{a}\delta_C$, type | 2. $^{a}\delta_C$, type | 3. $^{a}\delta_C$, type | 4. $^{b}\delta_C$, type | 5. $^{c}\delta_C$, type |
|----|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1  | 38.3, CH₂       | 38.3, CH₂       | 38.2, CH₂       | 36.7, CH₂       | 35.6, CH₂       |
| 2  | 38.1, CH₂       | 38.1, CH₂       | 38.0, CH₂       | 31.4, CH₂       | 29.2, CH₂       |
| 3  | 211.7, qC       | 211.7, qC       | 211.5, qC       | 71.2, CH       | 100.7, qC       |
| 4  | 44.5, CH₂       | 44.5, CH₂       | 44.5, CH₂       | 38.0, CH₂       | 36.2, CH₂       |
| 5  | 46.5, CH        | 46.5, CH        | 46.4, CH        | 44.7, CH       | 43.0, CH        |
| 6  | 28.6, CH₂       | 28.5, CH₂       | 28.5, CH₂       | 28.3, CH₂       | 28.8, CH₂       |
| 7  | 31.7, CH₂       | 31.7, CH₂       | 31.5, CH₂       | 31.9, CH₂       | 32.7, CH₂       |
| 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, CH₂       | 21.0, CH₂       | 21.5, CH₂       | 21.4, CH₂       | 22.0, CH₂       |
| 12 | 30.6, CH₂       | 31.0, CH₂       | 32.4, CH₂       | 32.2, CH₂       | 33.3, CH₂       |
| 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, CH₂       | 33.3, CH₂       | 33.5, CH₂       | 33.4, CH₂       | 34.4, CH₂       |
| 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, CH₂       | 61.9, CH₂       | 63.5, CH₂       | 63.5, CH₂       | 64.3, CH₂       |
| 19 | 11.3, CH₃       | 11.4, CH₃       | 11.4, CH₃       | 12.2, CH₃       | 12.0, CH₃       |
| 20 | 67.1, qC        | 67.5, qC        | 66.7, qC        | 66.4, qC       | 67.3, qC        |
| 21 | 16.1, CH₃       | 15.9, CH₃       | 16.1, CH₃       | 16.2, CH₃       | 17.1, CH₃       |
| 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, CH₃       | 30.9, CH₃       | 71.0, CH₂       | 22.8, CH₃       | 31.2, CH₃       |
| 27 | 25.1, CH₃       | 25.8, CH₃       | 20.3, CH₃       | 25.1, CH₃       | 25.9, CH₃       |
| 28 | 10.4, CH₃       | 11.4, CH₃       | 10.9, CH₃       | 10.5, CH₃       | 11.7, CH₃       |
| 29 | 11.9, CH₃       | 12.1, CH₃       | 12.3, CH₃       | 11.9, CH₃       | 12.6, CH₃       |
| OAc| 20.9, CH₃       | 20.9, CH₃       | 21.2, CH₃       | 21.2, CH₃       | 21.1, CH₃       |
|    | 171.6, qC       | 171.6, qC       | 171.1, qC       | 171.0, qC       | 170.6, qC       |
|    | 22.6, CH₃       | 21.1, CH₃       | 21.0, CH₃       | 20.9, CH₃       | 171.4, qC       |
|    | 169.8, qC       | 171.3, qC       | 171.2, qC       | 21.1, CH₃       | 22.7, CH₃       |
|    |                 |                 |                 | 170.8, qC       | 169.9, qC       |

* Spectra were measured in CDCl₃ (100 MHz); † Spectra were measured in CDCl₃ (125 MHz); ‡ Spectra were measured in C₆D₆ (125 MHz).
Table 2. $^1$H NMR data for compounds 1–5.

| H# | 1, $\delta_H$ (J in Hz) $^a$ | 2, $\delta_H$ (J in Hz) $^a$ | 3, $\delta_H$ (J in Hz) $^a$ | 4, $\delta_H$ (J in Hz) $^b$ | 5, $\delta_H$ (J in Hz) $^c$ |
|----|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1  | α: 1.39 m       | α: 1.35 m       | α: 1.32 m       | α: 1.02 m       | α: 1.33 m       |
|    | β: 2.02 m       | β: 2.00 m       | β: 1.97 m       | β: 1.69 m       | β: 1.06 m       |
| 2  | α: 2.32 m       | α: 2.31 m       | α: 2.31 m       | α: 2.18 m       | α: 1.86 m       |
|    | β: 2.38 m       | β: 2.39 m       | β: 2.37 m       | β: 1.41 m       | β: 1.41 m       |
| 3  |                |                |                | 3.60 m          |                |
| 4  | α: 2.12 dd ovvl | α: 2.09 dd ovvl | α: 2.12 dd ovvl | α: 1.58 m       | α: 1.86 dd (13.6, 3.6) |
|    | β: 2.28 t (13.6) | β: 2.27 t (13.6) | β: 2.26 t (13.6) | β: 1.29 m       | β: 1.41 dd ovvl |
| 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          | 1.78 m          | 1.82 m          | 1.78 m          | 1.54 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 | α: 1.66 m       | α: 1.65 m       | α: 1.63 m       | α: 1.60 m       | α: 1.48 m       |
|    | β: 1.48 m       | β: 1.44 m       | β: 1.33 m       | β: 1.23 m       | β: 1.23 m       |
| 12 | α: 1.28 m       | α: 1.28 m       | α: 1.34 m       | α: 1.38 m       | α: 1.44 m       |
|    | β: 2.44 m       | β: 2.44 m       | β: 2.16 m       | β: 2.17 m       | β: 2.28 m       |
| 14 | 1.36 m          | 1.18 m          | 1.23 m          | 1.31 m          | 1.28 m          |
| 15 | α: 2.23 m       | α: 2.24 m       | α: 2.21 m       | α: 2.21 m       | α: 2.22 m       |
|    | β: 1.44 m       | β: 1.46 m       | β: 1.48 m       | β: 1.46 m       | β: 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)   | 3.74 t (11.2)   | 4.23 d (11.6)   | 4.27 d (11.5)   | 4.55 d (11.5)   |
|    | 3.94 d (11.6)   | 3.94 dd (11.6, 4.60 d (10.8)  | 4.66 d (10.8)   | 4.67 d (11.0)   | 5.04 d (10.5)   |
| 22 | 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)   | 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, 3.02 s |
| OH-16 | 3.36 s, 3.43 s | 3.27 s          | 3.19 br s       | 3.83 br s       |
| OH-18 | 2.44 d ovvl    | 3.46 d ovvl    |                 |                 |                 |

$^a$ Spectra were measured in CDCl$_3$ (400 MHz); $^b$ Spectra were measured in CDCl$_3$ (500 MHz); $^c$ Spectra were measured in C$_6$D$_6$ (500 MHz).
Hiposterol G (4) was isolated as a white powder, and its molecular formula, C_{35}H_{56}O_{9}, was determined by HRESIMS. Its IR spectrum revealed the functionalities of hydroxyl (ν_{max} 3471 cm\(^{-1}\)) and ester carbonyl (ν_{max} 1734 cm\(^{-1}\)). NMR data (Tables 1 and 2) of 4 indicated the presence of three ester carbonyls, three 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) of 4 were similar to those of hippuristerone G [16] with the absence of the ketone carbon signal at δ_{C} 211.6 ppm and the presence of signal at δ_{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 5α-cholestan-3β-ol, which allowed us to propose a β orientation of OH group at C-3 (Figure 4). The stereochemistry of the side chain moiety was determined by comparison of the $^1$H and $^{13}$C NMR spectral data with those of hippuristerone A.

The molecular formula of hippuristeroketal A (5) was found to be C$_{35}$H$_{58}$O$_9$, as deduced from HRESIMS data. Its IR spectrum revealed the absorptions for hydroxyl ($\nu_{\text{max}}$ 3471 cm$^{-1}$) and ester carbonyl ($\nu_{\text{max}}$ 1731 cm$^{-1}$) groups. NMR data (Tables 1 and 2) of 5 indicated the presence of a ketal ($\delta_C$ 100.7), 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. 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 $\delta_C$ 211.6 and the presence of two methoxy signals [$\delta_H$ 3.12 (3H, s), 3.02 (3H, s) and $\delta_C$ 47.6 (CH$_3$), 47.5 (CH$_3$)] in the molecule. The HMBC correlations (Figure 3) of the methoxyl protons with C-3 [$\delta_C$ 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 $^1$H and $^{13}$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 $^1$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 IC$_{50}$ values greater than 50 μg/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 EC$_{50}$ values of 6.0 μg/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 $^1$H and 100 MHz for $^{13}$C or on a Varian Unity INOVA 500 FT-NMR spectrometer at 500 MHz for $^1$H and 125 MHz for $^{13}$C, respectively. $^1$H NMR chemical shifts are expressed in $\delta$ (ppm) referring to the solvent peaks $\delta_H$ 7.27 and 7.15 for CDCl$_3$ and C$_6$D$_6$, respectively, and coupling constants are expressed in Hz. $^{13}$C NMR chemical shifts are expressed in $\delta$ (ppm) referring to the solvent peaks $\delta_C$ 77.0 and 128.0 for CDCl$_3$ and C$_6$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 μm) were used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F$_{254}$, 0.25 mm) and precoated RP-18 F$_{254s}$ 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 μm, 250 × 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$_2$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$_2$O, 75:25 to 100:0) to give four fractions. In turn, a subfraction 28-2-2, eluted with MeOH–H$_2$O (80:20), was further purified by RP-18 HPLC (MeOH–H$_2$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$_2$O, 75:25 to 100:0) to give five subfractions. A subfraction 28-3-2 (112 mg), eluted with MeOH–H$_2$O (70:30), was further purified by RP-18 HPLC (MeOH–H$_2$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$_2$O (80:20), was purified by RP-18 HPLC (MeOH–H$_2$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 MeOH–H$_2$O (60:40 to 100:0) to give four subfractions. A subfraction 29-3-2, eluted with MeOH–H$_2$O (75:25), was further purified by RP-18 HPLC (MeOH–H$_2$O, 70:30) to afford 3 (1.0 mg), 2 (1.2 mg), and 1 (0.2 mg).

**Hipposterone M** (1): White amorphous powder; [α]$_D^{25}$ $-8$ (c 0.1, CHCl$_3$); IR (neat) $\nu$$_{max}$ 3454, 2954, 2922, 1733, 1717, 1558, 1456, 1374, 1238, 1152, 1019 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz) and $^{13}$C NMR (CDCl$_3$, 100 MHz) data in Tables 1 and 2; HRESIMS $m/z$ 599.3556 [M + Na]$^+$ (calcd for C$_{33}$H$_{52}$O$_8$Na, 599.3560).

**Hipposterone N** (2): White amorphous powder; [α]$_D^{25}$ $-11$ (c 0.1, CHCl$_3$); IR (neat) $\nu$$_{max}$ 3463, 2970, 2933, 1731, 1715, 1374, 1244, 1021, 735 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz) and $^{13}$C NMR (CDCl$_3$, 100 MHz) data in Tables 1 and 2; HRESIMS $m/z$ 557.3452 [M + Na]$^+$ (calcd for C$_{31}$H$_{50}$O$_7$Na, 557.3454).

**Hipposterone O** (3): White amorphous powder; [α]$_D^{25}$ $-5$ (c 0.1, CHCl$_3$); IR (neat) $\nu$$_{max}$ 3471, 2974, 2939, 1731, 1449, 1373, 1247, 1023, 739 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz) and $^{13}$C NMR (CDCl$_3$, 100 MHz) data in Tables 1 and 2; HRESIMS $m/z$ 657.3616 [M + Na]$^+$ (calcd for C$_{35}$H$_{54}$O$_{10}$Na, 657.3614).
**Hipposterol G** (4): White amorphous powder; \([\alpha]_D^{25} +5\) (c 0.1, CHCl\(_3\)); IR (neat) \(\nu_{\text{max}}\) 3471, 2928, 2860, 1734, 1454, 1371, 1244, 1023, 736 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 500 MHz) and \(^{13}\)C NMR (CDCl\(_3\), 125 MHz) data in Tables 1 and 2; HRESIMS \(m/z\) 643.3819 [M + Na]\(^+\) (cald for C\(_{35}\)H\(_{56}\)O\(_9\)Na, 643.3822).

**Hppuristeroketal A** (5): White amorphous powder; \([\alpha]_D^{25} +21\) (c 0.1, CHCl\(_3\)); IR (neat) \(\nu_{\text{max}}\) 3471, 2974, 1731, 1373, 1248, 1041, 739 cm\(^{-1}\); \(^1\)H NMR (C\(_6\)D\(_6\), 500 MHz) and \(^{13}\)C NMR (CDCl\(_3\), 125 MHz) data in Tables 1 and 2; HRESIMS \(m/z\) 645.3975 [M + Na]\(^+\) (cald for C\(_{35}\)H\(_{58}\)O\(_9\)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\(_{50}\) (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].

**Acknowledgments**

This research was financially supported by grants from the National Science Council (NSC99-2628-B-110-002-MY3) and Ministry of Education of Taiwan awarded to C.-Y.D.

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*Samples Availability:* Not available.

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