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Polyhydroxylated steroids from the octocoral *Isis hippuris*

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ABSTRACT

The specimens for previous studies on the secondary metabolites of the Formosan octocoral *Isis hippuris* were all collected at Green Island. In the course of our studies on bioactive compounds from marine organisms, the acetone-solubles of the Formosan octocoral *I. hippuris* collected at Orchid Island has led to the isolation of six new polyoxygenated steroids (**1**–**6**), along with a known compound **7**. Compound **6** possesses a new type of steroid side chain moiety. The structures of these compounds were determined on the basis of their spectroscopic and physical data. The anti-HCMV (human cytomegalovirus) activity and cytotoxicity against selected cell lines of **1**–**7** were evaluated. Compound **3** exhibited inhibitory activity against HCMV, with an EC_{50} values of 2.0 μg/mL, respectively. Compound **7** displayed cytotoxicity against P-388 and A-549 cell lines with ED_{50} values of 3.2 and 3.86 μg/mL, respectively.

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 Formosan octocoral *I. hippuris* were all collected at Green Island. In our continued study of the bioactive metabolites from marine organism, the Formosan octocoral *I. hippuris* collected at Orchid Island was selected for study since its acetone extract exhibited antiviral activity against HCMV. Our chemical examinations on the Orchid Island collection of this organism led to the isolation of six polyoxygenated steroids (**1**–**6**), along with a known compound **7**.[13] Compound **6** possesses a new type of steroid side chain moiety. We describe herein the isolation, structure elucidation, and biological activity of these compounds.

2. Results and discussion

The positive HRESIMS of **1** exhibited a pseudomolecular ion peak at *m*/*z* 543.3296 $[M+Na]^+$, consistent with the molecular formula $C_{30}H_{48}O_7$, implying 7° of unsaturation.

The IR spectrum showed the presence of hydroxyl (3450 cm^{-1}) and ester carbonyl (1734 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra (Table 1) displayed 30 signals: six methyls, nine methylenes, nine methines, and six quaternary carbons including one acetoxy carbonyl (δ_C 170.9, qC). Extensive 1D and 2D NMR data analysis indicated that the structure of **1** should be close to 22-epihippuristanol.[2]

However, its ^1H NMR data (Table 2) showed that one of the methyl groups at C-25 disappeared and was replaced by a signal resonating at δ_C 67.7 (CH_2) and δ_H 3.95 (1H, d, J=11.2 Hz) and δ 3.88 (1H, d, J=11.2 Hz). NOESY correlations from H-24 to H_3-26 and from H_3-28 to H_2-27 established **1** to be 27-acetoxyl-22-epihippuristanol. Compound **2** was found to possess the same molecular formula, $C_{30}H_{48}O_7$, as that of **1** on the basis of the positive HRESIMS (*m*/*z* 543.3299 $[M+Na]^+$) and NMR data (Tables 1 and 2). Comparison of the 1D and 2D NMR data (including COSY, HMQC, NOESY, and HMBC) of **2** with those of **1** showed that **1** is 26-acetoxyl-22-epihippuristanol.

Compound **3** showed the $[M+Na]^+$ ion peak at *m*/*z* 557.3820 in HRESIMS, and the molecular formula $C_{32}H_{54}O_6$ was determined. The ^{13}C NMR and DEPT spectra (Table 1) showed signals of eight methyl, seven methylene, twelve methine, and five quaternary carbons including an acetoxy carbonyl. The upfield region of the ^1H NMR spectrum (Table 2) afforded two signals at δ_H −0.11 (1H, dd, J=5.2, 4.4 Hz) and 0.47 (1H, dd, J=9.2, 4.4 Hz) corresponding to the C-30 cyclopropane ring protons, and two signals at δ_H 0.38 (1H, td, J=9.2, 5.6 Hz) and 0.28 (1H, m) corresponding to the C-22 and C-24 methine protons, indicated the presence of the gorgostane skeleton side chain. Comparison of its 1D and 2D NMR data with the gorgostan-3β,5α,6β,11α-tetrol 12-acetate[12] revealed that **3** was also

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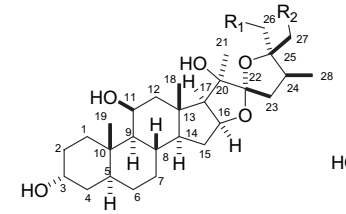
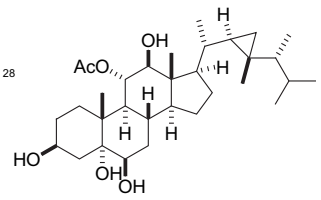


1 R_1=H, R_2=OAc
2 R_1=OAc, R_2=H



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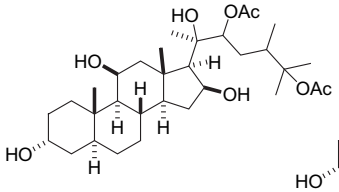

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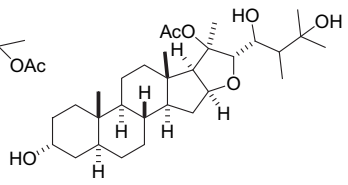

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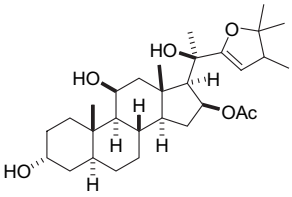

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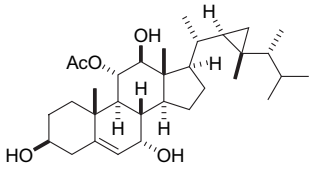

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Table 1
^{13}C NMR spectral data of **1**–**6**

C#	**1**,[a] δ_C, type	**2**,[a] δ_C, type	**3**,[a] δ_C, type	**4**,[b] δ_C, type	**5**,[a] δ_C, type	**6**,[b] δ_C, type
1	31.8, CH_2	31.8, CH_2	33.0, CH_2	31.8, CH_2	32.1, CH_2	31.8, CH_2
2	28.7, CH_2	28.6, CH_2	31.3, CH_2	28.6, CH_2	29.0, CH_2	28.7, CH_2
3	66.4, CH	66.4, CH	67.0, CH	66.4, CH	66.5, CH	66.4, CH
4	35.3, CH_2	35.3, CH_2	41.3, CH_2	35.3, CH_2	35.8, CH_2	35.3, CH_2
5	39.9, CH	39.9 (CH	76.5, qC	39.9, CH	39.1, CH	39.9, CH
6	27.9, CH_2	27.8, CH_2	76.1, CH	27.9, CH_2	28.4, CH_2	27.8, CH_2
7	32.3, CH_2	32.3, CH_2	34.6, CH_2	32.3, CH_2	32.0, CH_2	32.4, CH_2
8	30.2, CH	30.2, CH	29.0, CH	30.2, CH	34.3, CH	30.3, CH
9	58.1, CH	58.0, CH	48.1, CH	58.3, CH	54.1, CH	58.1, CH
10	36.5, qC	36.3, qC	40.0, qC	36.2, qC	36.1, qC	36.2, qC
11	68.0, CH	68.0, CH	76.5, CH	68.1, CH	20.4,. CH_2	67.9, CH
12	48.7, CH_2	48.7, CH_2	83.5, CH	49.6, CH_2	39.8, CH_2	48.4, CH_2
13	42.1, qC	42.1, qC	48.0, qC	43.0, qC	43.0, qC	41.6, qC
14	58.4, CH	58.4, CH	52.0, CH	55.9, CH	56.1, CH	57.5, CH
15	31.6, CH_2	31.4, CH_2	23.8, CH_2	36.6, CH_2	34.3, CH_2	34.2, CH_2
16	79.2, CH	79.2, CH	27.6, CH_2	73.9, CH	81.9, CH	83.3, CH
17	66.7, CH	66.4, CH	58.1, CH	58.2, CH	67.3, CH	64.9, CH
18	19.3, CH_3	19.5, CH_3	9.4, CH_3	17.8, CH_3	14.6, CH_3	17.2, CH_3
19	14.2, CH_3	14.2, CH_3	16.6, CH_3	14.3, CH_3	11.2, CH_3	14.2, CH_3
20	82.7, qC	82.6, qC	33.3, CH	78.1, qC	88.6, qC	78.1, qC
21	27.1, CH_3	27.0, CH_3	22.5, CH_3	21.0, CH_3	22.2, CH_3	31.4, CH_3
22	119.0, Qc	118.6, qC	30.2, CH	78.7, CH	83.4, CH	162.6, qC
23	40.5, CH_2	38.4, CH_2	25.2, qC	32.1, CH_2	70.6, CH	93.9, CH
24	41.1, CH	36.4, CH	50.5, CH	41.4, CH	42.4, CH	38.9, CH
25	83.5, qC	84.2, qC	32.1, CH	85.8, qC	73.5, qC	84.8, qC
26	24.8, CH_3	69.7, CH_2	21.4, CH_3	21.8, CH_3	28.5, CH_3	24.0, CH_3
27	67.7, CH_2	18.7, CH_3	22.2, CH_3	23.9, CH_3	29.2, CH_3	22.7, CH_3
28	13.2, CH_3	14.3, CH_3	15.2, CH_3	15.7, CH_3	7.0, CH_3	15.2, CH_3
29			13.7, CH_3			
30			21.6, CH_2			
OAc	21.1, CH_3	21.0, CH_3	22.0, CH_3	21.4, CH_3	22.2, CH_3	22.5, CH_3
	170.9, qC	171.0, qC	173.1, qC	172.2, qC	171.0, qC	170.6, qC
				22.6, CH_3		
				170.2, qC		

[a] Spectra were measured in $CDCl_3$ (100 MHz).
[b] Spectra were measured in $CDCl_3$ (125 MHz).

oxygenated at 3β,5α,6β,,11α,12β. HMBC correlations from H-11 to C-10 and carbonyl carbon of 11-OAc and NOESY correlations of H-11/H_3-18, H_3-19 and H-12/H-9, H-14, H-17 established **3** as gorgostan-3β,5α,6β,11α-tetrol 11-acetate.

The positive HRESIMS of **4** established a molecular formula of $C_{32}H_{54}O_8$. Comparison of the NMR spectral data (Table 1) with hippuristerol D[3], **4** possesses two additional hydroxyls at C-11 and C-16 on the basis of COSY correlations of H-11/H-9 and H-16/H-17 as well as HMBC correlations from H_3-19 to C-9 and H_3-18 to C-17. Compound **4** was finally established as 11β,16β-dihydroxyhippuristerol D by NOESY correlations of H-9/H-11, H-9/H-5, H-9/H-14, H-14/H-17, and H-17/H-16.

Compound **5** was isolated as a white powder. Its HREIMS exhibited a molecular ion peak at *m*/*z* 529.3507, corresponding to the molecular formula $C_{30}H_{50}O_6$. The IR spectrum showed absorption bands of hydroxyl (3417 cm^{-1}, broad) and ester carbonyl (1735 cm^{-1}) groups. The ^{13}C NMR spectrum (Table 2) displayed 30 signals, which were identified by the assistance of a DEPT spectrum as seven methyl, eight methylene, 10 methine, and five quaternary carbons including an acetoxy carbonyl. Thus, compound **5** must be pentacyclic. Comparison of NMR data of **5** with those of hippuristanols revealed that **5** was an analogue of 11-dehydroxyhippuristanol[3] except for the side chain moiety. The gross structure of the side chain was elucidated mainly by the connectivity of H-22/H-23 in the ^{1}H–^{1}H COSY spectrum and the key HMBC correlations from H_3-28 to C-25, C-24, and C-23, from H-16 to C-22, from H-22 to C-16, and from H_3-21 to C-17, C-20, and C-22. On the basis of the above results, together with the NOE correlations of H_3-21/H-17, H_3-21/H-16, H_3-21/H-23, H-17/H-14 of **5**, the relative configuration of C-16, C-17, C-20, and C-22 were thus established.

The HRESIMS (*m*/*z* 527.3350 $[M+Na]^+$) of compound **6** established the molecular formula of $C_{30}H_{48}O_6Na$, appropriate for 7° of unsaturation. The IR spectrum of **6** exhibited the presence of hydroxyl and carbonyl functionalities from the absorptions at ν_{max} of 3447 and 1732 cm^{-1}. The ^{13}C NMR spectrum (Table 1) showed signals of 30 carbons, which were characterized by the DEPT spectrum as seven methyl, seven methylene, 10 methine, and six quaternary carbons including one acetoxy carbonyl. Compound **6** was thus pentacyclic. Comparison of NMR data of **6** with those of hippuristanol revealed that **6** was an analogue of hippuristanol[3] except for the replacement of the spiroketal by a dihydrofuran and a secondly acetoxyl. HMBC correlations from δ_H 1.79 (H-17), 1.41 (H3-21), and 4.16 (H-23) with C-22 (δ_c 162.6, qC) confirmed the connectivity of trimethyldihydrofuran moiety with the steroid side chain via C-22. Compound **6** was the first steroid possessing a trimethyldihydrofuran moiety on the side chain. The possibility for **6** or **5** to be formed as an artifact during extraction or isolation process is negative due to all previously reported hippuristanols did not contain an acetoxyl group at C-16 or C-20.[3,14–16]

The anti-HCMV activity and cytotoxicity against of selected cell lines of **1**–**7** were evaluated. Compound **3** exhibit inhibitory activity against HCMV, with an EC_{50} values of 2.0 μg/mL, respectively. Compound **7** displayed cytotoxicity against P-388 and A-549 cell lines with ED_{50} values of 3.2 and 3.86 μg/mL, respectively.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined with a JASCO P1020 digital polarimeter. Ultraviolet (UV) and infrared (IR) spectra were obtained on a JASCO V-650 and JASCO FT/IR-4100 spectrophotometer, respectively. The NMR spectra were recorded on Bruker Avance 300 NMR/Varian 400 MR NMR/Varian Unity INOVA 500 FT-NMR spectrometers (300/400/500 MHz for ^{1}H and 75/100/

Table 2
^{1}H NMR spectral data of **1**–**6**

H#	**1**, δ_H[a] (*J* in Hz)	**2**, δ_H[a] (*J* in Hz)	**3**, δ_H[a] (*J* in Hz)	**4**, δ_H[a] (*J* in Hz)	**5**, δ_H[a] (*J* in Hz)	**6**, δ_H[b] (*J* in Hz)
1	1.59m	1.59m	α: 1.79m	α: 1.45m	α: 1.31m	1.61m
	1.45m	1.45m	β: 1.15m	β: 1.61m	β: 1.45m	1.48m
2	α; 1.65m	α; 1.64m	α: 1.82m	α: 1.66m	α: 1.64m	α: 1.66m
	β; 1.72m	β; 1.71m	β: 1.50m	β: 1.76m	β: 1.70 m	β: 1.74m
3	4.06 br s	4.05 br s	4.05m	4.06 br s	4.05 br s	4.06 br s
4	α; 1.36m	α; 1.35m	α: 1.62m	α: 1.38m	α: 1.39m	α: 1.38m
	β; 1.53m	β; 1.53m	β: 2.09m	β: 1.55m	β: 1.53m	β: 1.55m
5	1.52m	1.52m		1.54m	1.52m	1.53m
6	1.20m	1.20m	3.52 br s	1.23m	1.22m	1.22m
7	1.79m	1.80m	α: 1.76m	1.79m	1.65m	1.81m
	0.95m	0.94m	β: 1.63m	0.93m	0.92m	0.97m
8	1.94m	1.93m	1.88m	1.90m	1.53m	1.94m
9	0.79m	0.78dd (11.6, 2.8)	1.88m	0.82m	0.75m	0.82m
11	4.31 br d (2.8)	4.31 br s	5.05dd (10.0,8.8)	4.32 br s	α: 1.56m	4.33 br d (3.0)
					β: 1.32m	
12	α; 1.40m	α; 1.38m	3.36dd (8.0, 7.2)	α: 1.38m	α: 1.17m	α: 1.41m
	β; 2.15m	β; 2.12m		β: 2.28m	β: 1.80m	β: 2.17dd (14.0, 2.5)
14	1.04m	1.01m	1.22m	0.77m	1.05m	1.03m
15	α; 2.06m	α; 2.03m	α: 1.67m	α: 2.31m	α: 2.08m	α: 2.23m
	β; 1.42m	β; 1.41m	β: 1.20m	β: 1.45m	β: 1.39m	β: 1.57m
16	4.46 td (7.6, 5.2)	4.38 td (7.6, 5.2)	1.58m	4.63m	4.43 td (7.0, 4.0)	4.65 td (7.0, 4.0)
			1.90m			
17	1.80m	1.78m	1.52m	1.16m	2.21d (7.0)	1.79d (7.0)
18	1.34s	1.33s	0.76s	1.35s	1.10s	1.22s
19	1.04s	1.03s	1.29s	1.04s	0.80s	1.03s
20			1.20m			
21	1.31s	1.29s	1.10d (6.4)	1.23s	1.74s	1.41s
22			0.38 td (9.2, 5.6)	5.25d (9.0)	4.05d (9.5)	
23	1.88m	1.73m		1.25m	4.20d (9.5)	4.16d (9.5)
	2.02m	2.02m		1.76m		
24	2.39m	2.42m	0.28m	2.18m	1.68m	2.91 dq (9.5, 7.0)
25			1.58m			
26	1.28s	3.85d (11.6)	0.86d (6.8)	1.34s	1.22s	1.43s
		4.19d (11.6)				
27	3.88d (11.2)	1.01s	0.95d (6.4)	1.46s	1.38s	1.39s
	3.95d (11.2)					
28	1.03d (6.0)	0.98d (6.8)	0.93d (6.4)	0.92d (7.0)	1.03d (7.0)	0.93d (7.0)
29			0.90s			
30			−0.11dd (5.2, 4.4)			
			0.47dd (9.2, 4.4)			
OAc	2.08s	2.08s	2.07s	2.11s	2.09s	1.96s
				1.97s		
OH			2.02d (7.2)	4.53 br s	3.01 br s, 3.67 br s	

[a] Spectra were measured in $CDCl_3$ (400 MHz).
[b] Spectra were measured in $CDCl_3$ (500 MHz).

125 MHz for ^{13}C, respectively). Chemical shifts are expressed in δ parts per million (ppm) referring to the solvent peaks δ_H 7.15 and δ_C 128.5 for C_6D_6, and δ_H 7.27 and δ_C 77.0 for $CDCl_3$, respectively, and coupling constants are expressed in hertz. ESIMS were recorded by ESI FT-MS on a Bruker APEX II mass spectrometer. Silica gel 60 (Merck, 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, 1.05560) were used for TLC analyses. High-performance liquid chromatography (HPLC) was performed on a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector at 220 nm and a preparative reversed-phase column (Merck, Licrosorb RP-18e, 5 μm, 250×25 mm).

3.2. Organism

The octocoral *I. hippuris* was collected by hand using scuba at Orchid Island off Taiwan in August 2008 at a depth of 8–10 m and stored in a freezer until extraction. The voucher specimen (LY-19) was identified by Prof. Chang-Feng Dai, Institute of Oceanography, 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_2O and EtOAc. The resulting EtOAc extract (25.6 g) was subjected to gravity 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 four subfractions. A subfraction 28-2 (105 mg) was separated by an RP-18 flash column (MeOH–H_2O, 75/25 to 100/0) to give four fractions. In turn, a subfraction 28-2-2, eluted with MeOH–H_2O (80/20), was further purified by RP-18 HPLC (MeOH–H_2O–MeCN, 80/20/5) to afford **2** (0.8 mg). Similarly, the subfraction 28-3 (112 mg) was further subjected to an RP-18 flash column (MeOH–H_2O, 75/25 to 100/0) to give five subfractions. A subfraction 28-3-2 (112 mg), eluted with MeOH–H_2O (70/30), was further purified by RP-18 HPLC (MeOH–H_2O–MeCN, 75/25/5) to obtain **1** (1.1 mg). Similarly, the subfraction 28-3-3, eluted with MeOH–H_2O (80/20), was purified by RP-18 HPLC (MeOH–H_2O–MeCN, 75/25/5) to give **6** (0.6 mg) and **5** (0.5 mg). Fraction 29 (0.41 g), eluted with *n*-hexane–EtOAc (1/7), was subjected to Si 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 subjected to an RP-18 flash column (MeOH–H_2O, 60/40 to 100/0) to give four subfractions. A subfraction 29-3-2, eluted with MeOH–H_2O (75/25), was further purified by RP-18 HPLC (MeOH–H_2O, 70/30) to afford **4** (0.7 mg). Fraction 38 (0.36 g), eluted with EtOAc–MeOH (10/1), was subjected to RP-18 flash column (MeOH–H_2O, 57/43 to 100/0) to afford four subfractions. In turn, a subfractions 39-4, eluted with EtOAc–MeOH (90/10), was separated by RP-18 HPLC (MeOH–H_2O, 85/15) to afford **3** (2.7 mg) and **7** (1.4 mg).

*3.3.1. 27-Acetoxyl-22-epihippuristanol (**1**).* White amorphous powder; $[\alpha]_D^{25}$ −10 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3450, 2955, 2921, 2852, 1734, 1717, 1562, 1456, 1373, 1243, 1037, 969 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 543.3296 $[M+Na]^+$ (calcd for $C_{30}H_{48}O_7Na$, 543.3298).

*3.3.2. 26-Acetoxyl-22-epihippuristanol (**2**).* White amorphous powder; $[\alpha]_D^{25}$ −32 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3447, 2953, 2923, 2853, 1733, 1717, 1559, 1456, 1376, 1247, 1036, 971 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 543.3299 $[M+Na]^+$ (calcd for $C_{30}H_{48}O_7Na$, 543.3298).

*3.3.3. Gorgostan-3β,5α,6β,11α-tetrol 11-acetate (**3**).* White amorphous powder; $[\alpha]_D^{25}$ −21 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3410, 2954, 2873, 1715, 1457, 1373, 1263, 1024 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 557.3820 $[M+Na]^+$ (calcd for $C_{32}H_{54}O_6Na$, 557.3818).

*3.3.4. 11β,16β-Dihydroxyhippuristerol D (**4**).* White amorphous powder; $[\alpha]_D^{25}$ −12 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3447, 2953, 2853, 1733, 1717, 1559, 1456, 1376, 1247, 1036 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 589.3713 $[M+Na]^+$ (calcd for $C_{32}H_{54}O_8Na$, 589.3716).

*3.3.5. 20β-Acetoxy-24-methyl-3α,22,25-trihydroxy-5α-furostane (**5**).* White amorphous powder; $[\alpha]_D^{25}$ −72 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3417, 2955, 2924, 2852, 1735, 1716, 1566, 1376, 1247, 1163, 1050, 735 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 529.3507 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_6Na$, 529.3505).

*3.3.6. 16β-Acetoxy-22,25-epoxy-24-methyl-3α,11β,20-trihydroxy-5α-furost-22-ene (**6**).* White amorphous powder; $[\alpha]_D^{25}$ −27 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3447, 2932, 2858, 1732, 1447, 1373, 1242, 1034 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 527.3350 $[M+Na]^+$ (calcd for $C_{30}H_{48}O_6Na$, 5227.3348).

*3.3.7. Gorgost-5-en-3β,7α,11α,12β-tetrol 11-acetate (**7**).* White amorphous powder; $[\alpha]_D^{25}$ −42 (*c* 0.1, $CHCl_3$).

3.4. Cytotoxicity testing

Cytotoxicity was determined against P-388 (mouse lymphocytic leukemia), HT-29 (human colon adenocarcinoma) tumor cells, and HEL (human embryonic lung) cells using the MTT assay method. The experimental details of this assay were carried out according to a previously described procedure.[19]

3.5. Anti-cytomegalovirus assay

To determine the effects of **1**–**7** upon human cytomegalovirus (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 compounds. Metabolites **1**–**7** were not cytotoxic against HEL cells with ED_{50} values greater than 50 μg/mL. Then, HEL cells were infected with HCMV at an input of 1000 pfu (plaque forming units) per well of the 24-well dish. Antiviral activity is expressed as the IC_{50} value (50% inhibitory concentration), or the 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.[20]

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