

Steroids from the South China Sea Gorgonian Coral *Muricella flexuosa*

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Four new steroidal glycosides and a new steroid linked with a 4-hydroxyphenylmethylene group, namely muricellasteroids A–E (**1–5**), together with one known steroidal glycoside analog, 22 α ,2',3',4'-*O*-tetraacetyl-27-*O*-[β -D-arabino-pyranosyl-oxy]-20 β -hydroxy-cholest-4-ene-3-one (**6**), were isolated from the EtOH/CH₂Cl₂ extracts of the South China Sea gorgonian coral *Muricella flexuosa*. The structures of **1–5** were established by means of 1D- and 2D-NMR and other spectroscopic analyses. Compounds **1–3** and **5–6** showed moderate cytotoxicity against A375, K562, and A549 cancer cell lines.

Key words: *Muricella flexuosa*, Steroid, Steroidal Glycoside, Cytotoxicity

Introduction

Gorgonians are recognized to produce some functionalized novel steroids, such as hippuristanol-type representatives having a spiroketal [1], gorgosterol-type compounds possessing a cyclopropane [2], 9,11-secosteroids [3], and polyhydroxylated steroid 24-ketals [4]. Some β -D-arabinopyranosides were also found in gorgonians [5–8]. Gorgonians belonging to the *Muricella* genus were reported to produce steroids and cladiellane-class diterpenoids [9–14]. Now during the course of our investigations on the chemical constituents of the South China Sea gorgonian *Muricella flexuosa*, four new steroidal glycosides and a new steroid linked with a 4-hydroxyphenylmethylene group, namely muricellasteroids A–E (**1–5**), along with a known steroidal glycoside analog, 22 α ,2',3',4'-*O*-tetraacetyl-27-*O*-[β -D-arabino-pyranosyl-oxy]-20 β -hydroxy-cholest-4-ene-3-one, namely bebrycoside (**6**) [8] were obtained. Cytotoxicity of **1–6** against A375, K562, and A549 cancer cell lines was evaluated. This paper describes the isolation, structure elucidation and cytotoxicity of these compounds.

Results and Discussion

Compound **1** showed the molecular formula of C₃₈H₅₈O₁₁ as determined by ESI-MS and NMR spec-

tra. The ¹H NMR spectrum (Table 1) exhibited signals for three tertiary methyls at δ_H = 0.91, 1.18 and 1.25 (each 3H, s), a secondary methyl group at δ_H = 0.95 (3H, d, *J* = 6.5 Hz), three acetate methyls at δ_H = 2.07, 2.10 and 2.13 (each 3H, s), and of a trisubstituted double bond at δ_H = 5.73 (1H, s). The ¹³C DEPT-NMR spectra (Table 2) displayed 38 carbon signals, including 27 basic skeleton carbons, a pentose unit [δ_C = 99.1 (d), 69.3 (d), 70.4 (d), 67.5 (d), 60.8 (t)] and three acetyl groups [δ_C = 170.3 (s), 171.4 (s), 172.4 (s), 20.8 (q), 20.9 (q), 21.1 (q)]. In the 27 basic skeleton carbons, the signals at δ_C = 123.8(d), 171.4(s) and 199.6(s) indicated the presence of an $\alpha\beta$ -unsaturated ketone group, and signals at δ_C = 73.5 (t), 77.3 (s) and 79.2 (d) suggested that three carbons were oxygenated. These ¹H and ¹³C NMR spectral data of **1** (Tables 1 and 2) showed great similarity to those of bebrycoside (**6**) [8], except for one acetyl group, which suggested that **1** should have the same cholest-type monoglycoside basic skeleton as **6**.

This suggestion was proved by the HMBC spectrum of **1**, which showed correlations of H-4 (δ_H = 5.72, 1H, s) with C-2 (δ_C = 32.9, t), C-3 (δ_C = 199.6, s), and C-10 (δ_C = 38.6, s), of Me-19 (δ_H = 1.18) with C-1 (δ_C = 35.7), C-5 (δ_C = 171.4 s) and C-10, and of H-7

Table 1. ^1H NMR spectral data of compounds **1**–**5**^a.

H	1	2	3	4	5
1	2.02, 1.68 (m)	2.01, 1.67 (m)	2.02, 1.68 (m)	2.02, 1.68 (m)	2.02, 1.68 (m)
2	2.29, 2.39 (m)	2.39, 2.29 (m)	2.39, 2.29 (m)	2.32, 2.22 (m)	2.38, 2.29 (m)
3	—	—	—	—	—
4	5.72 (s)	5.73 (s)	5.73 (s)	5.73 (s)	5.73 (s)
5	—	—	—	—	—
6	2.42, 2.36 (m)	2.42, 2.36 (m)	2.09, 2.01 (m)	2.46, 2.33 (m)	2.42, 2.36 (m)
7	1.86, 1.05 (m)	1.86, 1.05 (m)	1.39, 1.11 (m)	1.88, 1.06 (m)	1.86, 1.05 (m)
8	1.57 (m)	1.57 (m)	1.41 (m)	1.57 (m)	1.57 (m)
9	0.91 (m)	0.92 (m)	0.93 (m)	0.92 (m)	0.92 (m)
10	—	—	—	—	—
11	1.53, 1.46 (m)	1.53, .46 (m)	1.54, 1.47 (m)	1.53, 1.44 (m)	1.53, 1.46 (m)
12	2.14, 1.25 (m)	2.14, 1.23 (m)	1.49, 1.25, m	2.12, 1.26 (m)	2.15, 1.24 (m)
13	—	—	—	—	—
14	1.04 (m)	1.02 (m)	1.05 (m)	1.05 (m)	1.02 (m)
15	1.56, 1.36 (m)	1.56, 1.36 (m)	1.56, 1.32 (m)	1.53, 1.35 (m)	1.56, 1.36 (m)
16	1.86, 1.64 (m)	1.86, 1.64 (m)	1.80, 1.64 (m)	1.92, 1.65 (m)	1.84, 1.65 (m)
17	1.46 (m)	1.45 (m)	1.52 (m)	1.52 (m)	1.46 (m)
18	0.91 (s)	0.91 (s)	0.92 (s)	0.92 (s)	0.89 (s)
19	1.18 (s)	1.18 (s)	1.24 (s)	1.20 (s)	1.18 (s)
20	—	—	—	—	—
21	1.25 (s)	1.25 (s)	1.25 (s)	1.26 (s)	1.25 (s)
22	4.80 (d, 10.5)	4.81 (d, 10.5)	4.85 (d, 10.5)	3.21 (t, 6.5)	4.80 (d, 10.2)
23	1.24, 1.68 (m)	1.68, 1.24 (m)	1.68, 1.29 (m)	1.29, 1.68 (m)	1.86, 1.64 (m)
24	1.40, 1.06 (m)	1.40, 1.06 (m)	1.32, 0.98 (m)	1.37, 0.99 (m)	1.14 (m)
25	1.71 (m)	1.71 (m)	1.69 (m)	1.69 (m)	1.52 (m)
26	0.95 (d, 6.5)	0.92 (d, 7.0)	0.97 (d, 6.5)	0.98 (d, 6.5)	0.91 (d, 6.5)
27	3.60, 3.26 (dd, 9.5, 6.5)	3.13, 3.55 (dd, 9.0, 5.5)	3.23, 3.52 (dd, 9.0, 6.0)	3.57, 3.25 (dd, 9.5, 6.0)	0.87 (d, 7.0)
1'	4.91 (d, 3.5)	4.97 (d, 3.5)	4.77 (d, 2.5)	4.77 (d, 2.5)	
2'	3.96 (br d, 10.0)	5.00 (dd, 3.5, 11.0)	3.78 (overlap)	3.79 (overlap)	7.72 (d, 8.5)
3'	5.09 (dd, 3.0, 10.0)	4.15 (br d, 10.5)	3.79 (overlap)	3.86 (overlap)	7.54 (d, 8.5)
4'	5.25 (br s)	5.15 (br s)	3.88 (br s)	3.88 (overlap)	
5'	3.66 (dd, 2.0, 13.0)	3.73 (dd, 2.0, 12.5)	3.59 (dd, 2.0, 12.5)	3.59 (dd, 2.0, 12.0)	7.54 (d, 8.5)
	3.85 (d, 13.0)	3.85 (d, 12.5)	3.81 (d, 13.0)	3.87 (overlap)	
6'					7.72 (d, 8.5)
7'					4.62 (br s)
22-OAc	2.10 (s)	2.14 (s)	2.10 (s)		2.10 (s)
	2.07, 2.14 (s)	2.19, 2.17 (s)			
	(3', 4'-OAc)	(2', 4'-OAc)			

^a All compounds were measured at 500 MHz with TMS as internal standard; compounds **1**, **2** and **5** were measured in CDCl_3 , and **3**, **4** were measured in CD_3OD ; chemical shift values δ are in ppm, and coupling constant values J in Hz.

($\delta_{\text{H}} = 1.86, 1.05$) with C-5, thus supported the presence of a double bond between C-4/C-5 and a C-3 ketone group. HMBC correlations of Me-21 ($\delta_{\text{H}} = 1.25, 3\text{H}, \text{s}$) with $\delta_{\text{C}} = 77.3$ (s), 79.2 (d), 55.3 (C-17), of H-17 ($\delta_{\text{H}} = 1.46$) with $\delta_{\text{C}} = 77.3, 79.2, 13.6$ (C-18), and of H-22 ($\delta_{\text{H}} = 4.80$) with $\delta_{\text{C}} = 77.3, 55.3$ indicated the oxygenation of C-20 ($\delta_{\text{C}} = 77.3$) and C-22 ($\delta_{\text{C}} = 79.2$), respectively. In addition, HMBC correlations of $\delta_{\text{H}} = 4.80$ (1H, d, $J = 10.5$ Hz, H-22) and 2.10 (3H, s) with $\delta_{\text{C}} = 170.3$ (s) suggested that C-22 was acetylated. The HMBC spectrum also showed the correlations of $\delta_{\text{H}} = 0.95$ (3H, d, $J = 6.5$ Hz, H-26) with $\delta_{\text{C}} = 33.3$ (d, C-25), 73.5 (t), and of $\delta_{\text{H}} = 1.71$ (1H, m, H-25) with $\delta_{\text{C}} = 17.5$

(q, C-26), 73.5 (t), which indicated the oxygenation of C-27 ($\delta_{\text{C}} = 73.5$).

Furthermore, upon a comparison of the NMR data of the sugar moiety in **6** [8] and in juncellosesides A–D [7] the NMR data of a pentose unit [$\delta_{\text{C}} = 99.1$ (d, C-1'), 67.5 (d, C-2'), 70.4 (d, C-3'), 69.3 (d, C-4'), 60.8 (t, C-5'); $\delta_{\text{H}} = 4.91$ (1H, d, $J = 3.5$ Hz, H-1'), 3.96 (1H, br d, $J = 10.0$ Hz, H-2'), 5.09 (1H, dd, $J = 3.0, 10.0$ Hz, H-3'), 5.25 (1H, br s, H-4'), 3.66 (1H, dd, $J = 2.0, 13.0$ Hz, H-5'a), 3.85 (1H, d, $J = 13.0$ Hz, H-5'b)] in **1** indicated the existence of a β -D-arabinopyranosyl group. The J value of the anomeric proton ($J = 3.5$ Hz) indicated the β -configuration of the arabinose. The

C	1	2	3	4	5	6
1	35.7 t	35.7 t	36.0 t	36.8 t	35.7 t	35.8 t
2	32.9 t	32.9 t	34.0 t	34.1 t	34.0 t	32.9 t
3	199.6 s	199.7 s	202.4 s	202.6 s	199.6 s	200.0 s
4	123.8 d	123.8 d	124.2 d	124.1 d	123.8 d	123.9 d
5	171.4 s	171.4 s	173.0 s	175.6 s	171.5 s	171.4 s
6	34.0 t	34.0 t	34.8 t	34.8 t	34.0 t	34.0 t
7	31.9 t	31.9 t	33.3 t	33.3 t	31.9 t	31.9 t
8	34.9 d	34.9 d	36.2 d	36.7 d	34.9 d	34.9 d
9	53.8 d	53.8 d	55.3 d	55.4 d	53.8 d	53.8 d
10	38.6 s	38.6 s	40.0 s	40.0 s	38.6 s	38.6 s
11	20.9 t	20.9 t	22.1 t	22.1 t	20.9 t	20.9 t
12	40.2 t	40.2 t	41.0 t	41.6 t	40.2 t	40.2 t
13	43.4 s	43.4 s	44.6 s	44.4 s	43.4 s	43.5 s
14	56.0 d	55.9 d	57.3 d	57.4 d	55.9 d	56.0 d
15	27.7 t	27.5 t	28.8 t	29.6 t	27.8 t	27.6 t
16	22.1 t	22.1 t	23.0 t	23.1 t	22.0 t	22.1 t
17	55.3 d	55.4 d	56.6 d	56.3 d	55.3 d	55.4 d
18	13.6 q	13.6 q	14.2 q	14.1 q	13.6 q	13.6 q
19	17.4 q	17.2 q	17.6 q	17.7 q	17.4 q	17.4 q
20	77.3 s	77.2 s	77.4 s	77.9 s	77.2 s	77.0 s
21	20.9 q	20.9 q	21.3 q	20.8 q	21.1 q	20.8 q
22	79.2 d	79.1 d	80.4 d	78.1 d	79.2 d	79.8 d
23	23.9 t	23.9 t	25.0 t	24.9 t	23.9 t	23.9 t
24	30.6 t	30.4 t	31.9 t	32.3 t	32.9 t	30.5 t
25	33.3 d	33.3 d	34.3 d	34.6 d	29.6 d	33.4 d
26	17.5 q	17.4 q	17.7 q	17.9 q	22.3 q	17.2 q
27	73.5 t	73.6 t	74.4 t	74.4 t	22.9 t	73.8 t
1'	99.1 d	96.9 d	101.0 d	101.3 d	131.4 s	96.9 d
2'	67.5 d	72.0 d	70.9 d	70.8 d	131.3 d	69.1 d
3'	70.4 d	66.4 d	70.7 d	70.9 d	129.2 d	68.9 d
4'	69.3 d	71.9 d	70.4 d	70.5 d	167.3 s	67.3 d
5'	60.8 t	60.4 t	62.3 t	64.3 t	129.2 d	60.4 t
6'					131.3 d	
7'					63.2 t	
22-OAc	172.4 s	172.3 s	175.4 s		172.5 s	172.3 s
	20.8 q	21.0 q	21.4 q		20.7 q	21.1 q
-OAc	170.8 s, 170.4 s, 20.9 q, 21.1 q (3', 4'-OAc)	171.2 s, 170.9 s, 21.2 q, 20.8 q (2', 4'-OAc)				170.4 s, 170.3 s, 170.1 s, 20.7 q, 20.8 q, 20.9 q (2', 3', 4'-OAc)

Table 2. ^{13}C NMR spectral data for compounds **1**–**6**^a.

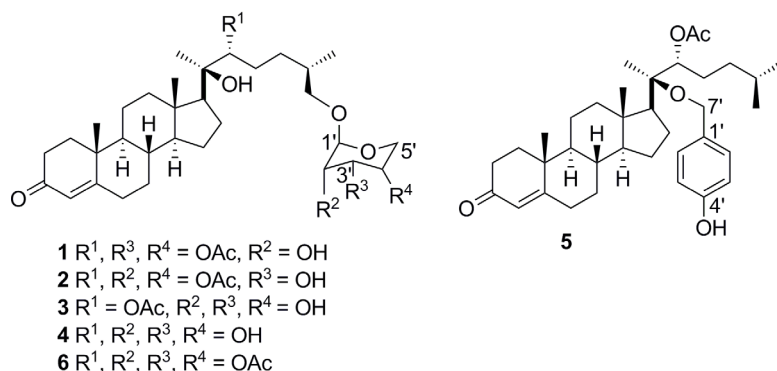
^a All compounds were measured at 125 MHz with TMS as internal standard; compounds **1**, **2**, **5**, and **6** were measured in CDCl_3 , and **3**, **4** were measured in MeOD; chemical shifts δ are in ppm.

down-field chemical shifts of H-3' and H-4', and the HMBC correlations of H-3' with $\delta_{\text{C}} = 170.4$ and of H-4' with $\delta_{\text{C}} = 170.8$ in the HMBC spectrum, indicated that C-3' and C-4' were acetylated. In addition, HMBC correlations of H-27 ($\delta_{\text{H}} = 3.60, 3.26$, each 1H, dd, $J = 9.5, 6.5$ Hz) with C-1', of H-1' with C-27 and C-5', and of H-5' with C-1' suggested that the β -arabinopyranosyl unit was linked to the aglycone C-27.

The relative stereochemistry of C-20 and C-22 was determined to be the same as that of bebrycoside(**6**) [8] and muricasteroid [11] by comparison of their NMR data. This was proved by the NOESY spectrum of **1** NOE correlation of Me-18 with H-22 suggested the β -configuration of H-22; no NOE correlation between Me-18 and Me-21 was observed, but Me-21 showed an

NOE correlation with H-17, which suggested the α -orientation of Me-21 and the β -orientation of OH-20. Based on extensive 1D and 2D NMR data analysis, including HSQC, HMBC, ^1H - ^1H COSY and NOESY spectra, the ^1H and ^{13}C NMR data of **1** were assigned (Tables 1 and 2), and its structure was elucidated to be 22 α 3',4'-*O*-triacyl-27-*O*-[β -D-arabino-pyranosyl-oxy]-20 β -hydroxy-cholest-4-ene-3-one (Fig. 1), simply named muricellasteroid A.

Compound **2** also exhibited the molecular formula of $\text{C}_{38}\text{H}_{58}\text{O}_{11}$ as deduced from NMR and ESI-MS spectra. The ^1H and ^{13}C NMR spectral data of **2** showed great similarity to those of **1** (Tables 1 and 2), except for the substituent position of two acetyl groups in the pentose unit. On comparison with **1**,

Fig. 1. Structures of compounds **1**–**6**.

the ^{13}C NMR spectra of **2** showed a down-field shift of the signals of C-2' and C-4' at $\delta_{\text{C}} = 72.0$ and 71.9 , instead of at $\delta_{\text{C}} = 67.5$ and 69.3 , respectively. HMBC correlations of $\delta_{\text{H}} = 5.0$ (d, $J = 3.5, 11.0$ Hz, H-2') and 2.19 (3H, s) with $\delta_{\text{C}} = 171.2$, and of $\delta_{\text{H}} = 5.15$ (br s H-4') and 2.17 (3H, s) with $\delta_{\text{C}} = 170.9$ were also observed in the HMBC spectrum of **2**. This result implied that two acetyl groups were attached to C-2' and C-4'. On the basis of 1D and 2D NMR experiments, the structure of **2** was confirmed to be 22 α 2',4'-*O*-triacetyl-27-*O*-[β -D-arabino-pyranosyl-oxy]-20 β -hydroxy-cholest-4-ene-3-one (Fig. 1) named muricellasteroid B.

Compound **3** exhibited the molecular formula of $\text{C}_{34}\text{H}_{54}\text{O}_9$ as deduced from NMR and ESI-MS spectra. The ^1H and ^{13}C NMR spectral data of **3** were very similar to those of **1**, except for the lack of two acetyl groups in the pentose unit of **3**. By extensive 1D and 2D NMR data analysis, the ^1H and ^{13}C NMR data of **3** were assigned (Tables 1 and 2), and its structure was deduced to be 22 α -*O*-acetyl-27-*O*-[β -D-arabino-pyranosyl-oxy]-20 β -hydroxy-cholest-4-ene-3-one (Fig. 1), named muricellasteroid C.

Compound **4** was assigned the molecular formula of $\text{C}_{32}\text{H}_{52}\text{O}_8$ on the basis of NMR and ESI-MS spectra. The ^1H and ^{13}C NMR spectral data of **4** showed great similarity to those of **3**, with the only difference of having only one acetyl group (at in C-22). No HMBC correlation of H-22 ($\delta_{\text{H}} = 3.21$, t, $J = 6.5$ Hz) with an acetyl was observed in the HMBC spectrum, and an upfield signal of C-22 at $\delta_{\text{C}} = 78.1$ was observed in the ^{13}C NMR spectrum which suggested that C-22 was hydroxylated rather than acetylated. So the structure of **4** was deduced to be 27-*O*-[β -D-arabino-pyranosyl-oxy]-20 β 22 α -dihydroxy-cholest-4-ene-3-one (Fig. 1), named muricellasteroid D.

Compound **5** was assigned the molecular formula of $\text{C}_{36}\text{H}_{52}\text{O}_5$ according to NMR and ESI-MS spectra. The ^1H and ^{13}C NMR spectral data of **5** showed similarity to those of **1** (Tables 1 and 2), however, there were no NMR signals of a pentose unit in **5**, and the presence of NMR signals at $\delta_{\text{C}} = 131.4$ (s, C-1'), 167.3 (s, C-4'), 129.2 (d, C-3', 5'), 131.3 (d, C-2', 6'), and $\delta_{\text{H}} = 7.72$ (2H, d, $J = 85$ Hz H-2', 6') and 7.54 (2H, d, $J = 85$ Hz H-3', 5') indicated the existence of a 1,4-di-substituted phenyl ring. In addition in the HMBC spectrum, correlation of $\delta_{\text{H}} = 4.62$ (2H, br s) with C-1'/C-2' / C-20 ($\delta_{\text{C}} = 77.2$, s) suggested that the phenyl group was attached to C-20 by an OCH_2 -unit. Based on the ^1H NMR ^{13}C NMR, HSQC, HMBC, ^1H - ^1H COSY and NOESY spectral data, the structure of **5** was confirmed to be 22 α -*O*-acetyl-2 β -*O*-methylene-[4'-hydroxy-phenyl]-cholest-4-ene-3-one (Fig. 1), named muricellasteroid E.

The cytotoxicity of **1**–**6** against human melanoma cell line A375, human erythroleukaemic cell line K562, and human lung carcinoma A549 was evaluated. Compounds **1**–**3** and **5**–**6** showed weak cytotoxicity against A375, K562 and A549 cancer cell lines with IC_{50} values of $32.3 \sim 100.8 \mu\text{g mL}^{-1}$. It appears that the presence of an acetyl substituent in the pentose unit improves the cytotoxicity of **1**–**3** and **6**, however, the number and position of acetyl substituents had no obvious effect on the cytotoxicity.

Compounds **1**–**6** had the same basic skeleton of 20 β ,22 α -dihydroxy-cholest-4-ene-3-one, and the only difference between **1**–**4** and **6** was the number and position of acetyl substituents in the pentose unit. Although 4-hydroxyphenyl and its derivatives are commonly present as substituents in marine natural compounds, such as zyzzyanones B–D [15] and perspic-amides A, B [16], we report for the first time the appearance of a 4-hydroxyphenylene substituent at the

oxygenated C-20 of the steroid. We had obtained **6** from the South China Sea *Bebryce indica* [8]. Zhang *et al.* had obtained 22 α -O-acetyl-20 β -hydroxy-cholest-4-ene-3-one (namely, muricestroid) from the South China Sea gorgonian *M. flexuosa* [4]. The isolation of **1–5** has now further provided a series of natural analogs of the above basic skeleton, and the first cytotoxicity evaluation is presented for this type of steroids.

Experimental Section

General experimental procedures

Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were measured with a Shimadzu double-beam 210A spectrophotometer in MeOH solution. ^1H , ^{13}C NMR and 2D NMR spectra were recorded on a Bruker AV-500 MHz NMR spectrometer with TMS as internal standard. MS spectral data were obtained on an LCQDECA XP HPLC/MSn spectrometer by ESI techniques. Silica gel (200–300 mesh) for column chromatography and GF254 for TLC were obtained from the Qindao Marine Chemical Factory, Qindao, People's Republic of China.

Animal material

The South China Sea gorgonian coral *Muricella flexuosa* was collected in Sanya, Hainan province, China, in July 2008 and identified by Prof. Hui Hunag, the South China Sea Institute of Oceanology, Academia Sinica. A voucher specimen (No. 0801) was deposited in the South China Sea Institute of Oceanology, Academia Sinica, Guangzhou, China.

Extraction and isolation

The frozen specimen of the South China Sea gorgonian coral *Muricella flexuosa* (20 kg, wet weight) was extracted with EtOH/CH₂Cl₂ (2:1) three times at r.t., and the solvent was evaporated *in vacuo*. The residue was partitioned in H₂O and extracted with EtOAc and *n*-BuOH three times each. The EtOAc and *n*-BuOH extracts were concentrated *in vacuo* to afford 45.2 and 40.6 g of residue, respectively. The EtOAc portion was subjected to column chromatography (CC) on silica gel, using petroleum ether/EtOAc (from 10:1 to 0:10) as eluent. After combining the fractions, 12 fractions were obtained with TLC (GF254). Fraction 3 was subjected to CC on silica gel and eluted with CHCl₃/Me₂CO (15:1) to afford **5** (7.5 mg). Fraction 5 was subjected to CC on silica gel, eluted with CHCl₃/Me₂CO (from 10:1 to 9:1), to give **6** (136 mg) and **1** (7.9 mg). Fraction 7 was chromatographed over Sephadex LH-20 eluting

with CHCl₃/MeOH (1:1), then subjected to CC on silica gel, eluted with CHCl₃/Me₂CO (4:1), to give **2** (3.8 mg). Fraction 10 was subjected to CC on silica gel, eluted with CHCl₃/MeOH (from 10:1 to 8:2), to give sub-fractions (a–c). Fractions a and c were chromatographed over Sephadex LH-20 eluting with CHCl₃/MeOH (1:1). Then fraction a was subjected to CC on silica gel, eluted with CHCl₃/MeOH (8:1), to yield **3** (27.2 mg). Fraction c was subjected to CC on silica gel, eluted with CHCl₃/MeOH (7:1), to yield **4** (8.6 mg).

Muricellasteroid A (1): Colorless oil. – $[\alpha]_{\text{D}}^{20} = -12.7^\circ$ ($c = 0.8$, CHCl₃). – UV (MeCN): $\lambda = 207, 235, 322$ nm. – IR (KBr): $\nu_{\text{max}} = 3356, 1735, 1681, 1466, 1232$ cm⁻¹. – ^1H and ^{13}C NMR data, see Tables 1 and 2. – HRMS ((+)-ESI): $m/z = 691.3974$ (calcd 691.3979 for C₃₈H₅₉O₁₁, [M+H]⁺).

Muricellasteroid B (2): Colorless oil. – $[\alpha]_{\text{D}}^{20} = -25.8^\circ$ ($c = 0.38$, CHCl₃). – UV (MeCN): $\lambda = 207, 235, 322$ nm. – ^1H and ^{13}C NMR data, see Tables 1 and 2. – HRMS ((+)-ESI): $m/z = 691.3970$ (calcd 691.3979 for C₃₈H₅₉O₁₁, [M+H]⁺).

Muricellasteroid C (3): Colorless oil. – $[\alpha]_{\text{D}}^{20} = -21.3^\circ$ ($c = 2.7$, MeOH). – UV (MeOH): $\lambda = 205, 240, 320$ nm. – ^1H and ^{13}C NMR data, see Tables 1 and 2. – HRMS ((+)-ESI): $m/z = 607.3761$ (calcd 607.3767 for C₃₄H₅₅O₉, [M+H]⁺).

Muricellasteroid D (4): Colorless oil. – $[\alpha]_{\text{D}}^{20} = -4.7^\circ$ ($c = 0.86$, MeOH). – UV (MeOH): $\lambda = 205, 245, 315$ nm. – ^1H and ^{13}C NMR data, see Tables 1 and 2. – HRMS ((+)-ESI): $m/z = 565.3658$ (calcd 565.3662 for C₃₂H₅₃O₈, [M+H]⁺).

Muricellasteroid E (5): Colorless oil. – $[\alpha]_{\text{D}}^{20} = 18.4^\circ$ ($c = 1.0$, MeOH). – UV (MeCN): $\lambda = 205, 240, 280, 312$ nm. – ^1H and ^{13}C NMR data, see Tables 1 and 2. – HRMS ((+)-ESI): $m/z = 565.3822$ (calcd 565.3814 for C₃₆H₅₃O₅, [M+H]⁺).

Cytotoxicity bioassays

Human lung carcinoma A549, human erythroleukaemic cell line K562, and human melanoma cell line A375 were purchased from the AMERICAN Type Culture Collection (ATCC, Rockville, MD). Cytotoxicity assays were measured by MTT methods as described previously [17].

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