Revision of the Absolute Configuration at C(23) of Lanostanoids and Isolation of Secondary Metabolites from Formosan Soft Coral *Nephthea erecta*

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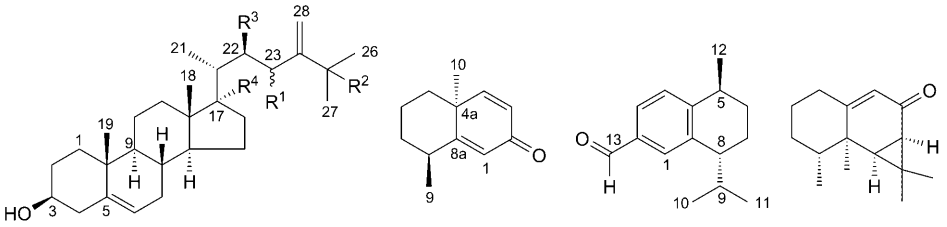
Three new oxygenated ergostanoids, **1**–**3**, one known ergostanoid, **4**, one new trinoreudesmadienone, **5**, one new calamenene type sesquiterpene, **6**, and one known aristolane-type sesquiterpene, (−)-aristolone (**7**), have been isolated from the AcOEt extract of the soft coral *Nephthea erecta*. The structures of these compounds were determined by extensive spectroscopic and X-ray crystallographic analysis, as well as *Mosher*'s method. We revised the absolute configuration at C(23) in the side chain of some lanostanoids as a result of the *Mosher*'s products of **1** (*i.e.*, **1a** and **1b**). The cytotoxicities against selected cancer cells and the anti-inflammatory effects of these tested metabolites **1**–**7** were determined *in vitro*.

Introduction. – Soft corals belonging to the genus *Nephthea* have been found to be a rich source of terpenoids and steroids [1–13]. A number of terpenoids have shown an array of biological activities such as insecticidal [12] and cytotoxic activities [5][7][13]. Earlier studies of marine soft corals have led to the isolation of steroids, some of which have shown cytotoxic [6] and anti-inflammatory activities [14a]. In the course of our ongoing search to explore bioactive substances from marine resources, chromatographic separation of the AcOEt extracts from the soft coral *N. erecta* led to the isolation of three new oxygenated ergostanoids, **1**–**3**, one known ergostanoid, **4**, [14b], one new trinoreudesmadienone, **5**, one new calamenene type sesquiterpene, **6**, and one known aristolane type sesquiterpene, (−)-aristolone (**7**) [15]. We describe here the isolation, structural elucidation, cytotoxicity, and anti-inflammatory activity of these metabolites. In addition, we revised the absolute configuration at C(23) in the side chain of some lanostanoids isolated from the bark and leaves of *Amentotaxus formosana* [16][17] by using a modified *Mosher*'s method [18][19] for **1**.

Results and Discussion. – (3*β*,23*S*)-Ergosta-5,24(28)-diene-3,23-diol (**1**) was isolated as a white amorphous powder. HR-ESI-MS of **1** exhibited a *pseudo*-molecular ion peak at *m*/*z* 437.3392 ([*M*+Na]$^+$; calc. for 437.3395) and established a molecular



1 R^1 = (*S*)-OH, R^2 = H, R^3 = H, R^4 = H
2 R^1 = (*R*)-OH, R^2 = H, R^3 = H, R^4 = H
3 R^1 = H, R^2 = H, R^3 = OH, R^4 = OH
4 R^1 = H, R^2 = OH, R^3 = H, R^4 = H

5 **6** **7**

formula of $C_{28}H_{46}O_2$, indicating six degrees of unsaturation. The ^{13}C-NMR and DEPT spectra displayed 28 signals, including those of five Me, ten CH_2, nine CH groups, and four quaternary C-atoms. ^{1}H-NMR Signals (δ(H) 4.20 (*t*, *J* = 6.7, 1 H) and 3.49–3.56 (*m*, 1 H)) (*Table 1*) and an IR absorption at 3323 cm^{-1}, together with the observation of two O-bearing C-atom resonances (δ(C) 74.4 and 71.8) in ^{13}C-NMR spectrum (*Table 1*), revealed the presence of two OH groups. Furthermore, a trisubstituted C=C bond (δ(C) 121.6 and 140.7) and a terminal C=C bond (δ(C) 108.1 and 159.2) were assigned on the basis of ^{13}C-NMR and DEPT spectra of **1**. The above functionalities accounted for two of the six degrees of unsaturation of **1**, which possesses a tetracyclic skeleton. The structure of **1** was established by extensive 2D-NMR analysis (^{1}H,^{1}H-COSY, HMQC, and HMBC). Interpretation of the ^{1}H,^{1}H-COSY spectrum led to partial structures **I**, **II**, and **III** (*Fig. 1*). Partial structures **I** and **II** were connected, on the

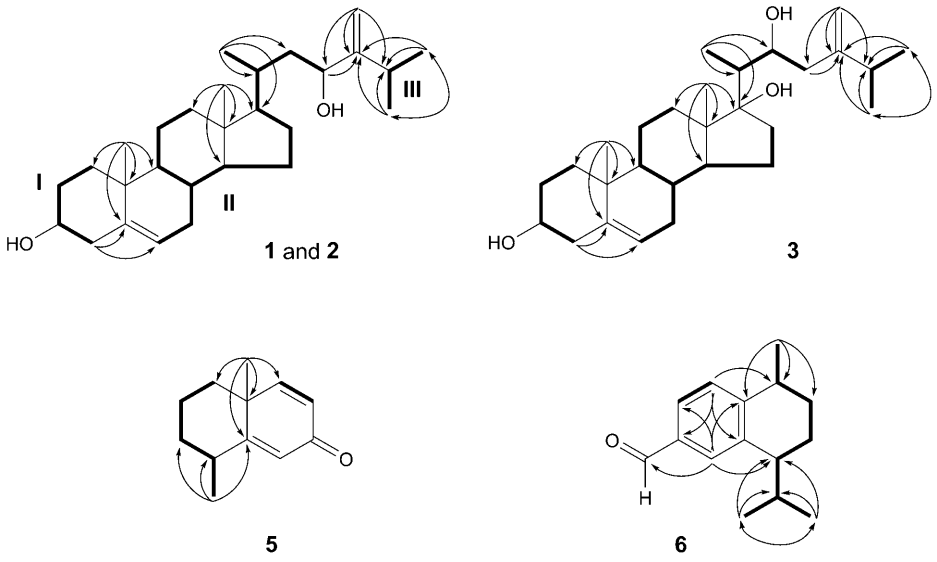


Fig. 1. *Key ^{1}H,^{1}H-COSY* (—) *and HMBC correlations* (H→C) *of* **1**–**3**, **5**, *and* **6**

Table 1. *^{1}H- and ^{13}C-NMR Data of Compounds* **1**–**3**. δ in ppm, J in Hz.

Position	**1**[a])		**2**[a])		**3**[b])	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
1	37.2 (*t*)	1.04–1.14 (*m*, H_α), 1.82–1.92 (*m*, H_β)	37.2 (*t*)	1.04–1.14 (*m*, H_α), 1.82–1.92 (*m*, H_β)	37.2 (*t*)	1.04–1.14 (*m*, H_α), 1.81–1.88 (*m*, H_β)
2	31.6 (*t*)	1.79–1.89 (*m*, H_α), 1.38–1.48 (*m*, H_β)	31.6 (*t*)	1.81–1.91 (*m*, H_α), 1.37–1.47 (*m*, H_β)	31.6 (*t*)	1.80–1.87 (*m*, H_α), 1.46–1.54 (*m*, H_β)
3	71.8 (*d*)	3.49–3.56 (*m*)	71.8 (*d*)	3.49–3.56 (*m*)	71.7 (*d*)	3.51–3.57 (*m*)
4	42.2 (*t*)	2.26–2.34 (*m*, H_α), 2.19–2.26 (*m*, H_β)	42.3 (*t*)	2.26–2.34 (*m*, H_α), 2.19–2.26 (*m*, H_β)	42.2 (*t*)	2.26–2.33 (*m*, H_α), 2.19–2.26 (*m*, H_β)
5	140.7 (*s*)		140.7 (*s*)		140.6 (*s*)	
6	121.6 (*d*)	5.35 (br. *d*, J=4.9)	121.7 (*d*)	5.35 (br. *d*, J = 4.5)	121.6 (*d*)	5.36 (br. *d*, J=5.0)
7	31.8 (*t*)	1.55–1.64 (*m*, H_α), 1.93–2.05 (*m*, H_β)	31.9 (*t*)	1.59–1.68 (*m*, H_α), 1.95–2.07 (*m*, H_β)	31.8 (*t*)	1.56–1.65 (*m*, H_α), 1.94–2.03 (*m*, H_β)
8	31.8 (*d*)	1.47–1.54 (*m*)	31.9 (*d*)	1.48–1.55 (*m*)	32.3 (*d*)	1.45–1.52 (*m*)
9	50.0 (*d*)	0.86–1.00 (*m*)	50.1 (*d*)	0.90–1.04 (*m*)	49.6 (*d*)	0.94–1.02 (*m*)
10	36.5 (*s*)		36.5 (*s*)		36.4 (*s*)	
11	21.0 (*t*)	1.49–1.57 (*m*, H_α), 1.38–1.48 (*m*, H_β)	21.1 (*t*)	1.51–1.59 (*m*, H_α), 1.41–1.51 (*m*, H_β)	20.9 (*t*)	1.54–1.62 (*m*, H_α), 1.42–1.50 (*m*, H_β)
12	39.7 (*t*)	1.12–1.24 (*m*, H_α), 1.98–2.09 (*m*, H_β)	39.8 (*t*)	1.12–1.24 (*m*, H_α), 2.00–2.11 (*m*, H_β)	32.3 (*t*)	1.42–1.50 (*m*, H_α), 1.67–1.75 (*m*, H_β)
13	42.4 (*s*)		42.5 (*s*)		47.3 (*s*)	
14	56.7 (*d*)	0.95–1.10 (*m*)	56.7 (*d*)	0.97–1.12 (*m*)	50.9 (*d*)	1.68–1.76 (*m*)
15	24.2 (*t*)	1.55–1.67 (*m*, H_α), 1.13–1.21 (*m*, H_β)	24.2 (*t*)	1.57–1.69 (*m*, H_α), 1.13–1.21 (*m*, H_β)	23.6 (*t*)	1.71–1.79 (*m*, H_α), 1.12–1.20 (*m*, H_β)
16	28.4 (*t*)	1.84–1.98 (*m*, H_α), 1.20–1.30 (*m*, H_β)	28.4 (*t*)	1.82–1.96 (*m*, H_α), 1.22–1.32 (*m*, H_β)	38.0 (*t*)	1.86–1.92 (*m*, H_α), 1.83–1.86 (*m*, H_β)
17	56.8 (*d*)	1.10–1.26 (*m*)	56.8 (*d*)	1.13–1.29 (*m*)	87.5 (*s*)	
18	11.8 (*q*)	0.67 (*s*)	11.9 (*q*)	0.73 (*s*)	14.5 (*q*)	0.76 (*s*)
19	19.4 (*q*)	1.00 (*s*)	19.4 (*q*)	1.01 (*s*)	19.4 (*q*)	1.02 (*s*)
20	34.1 (*d*)	1.36–1.49 (*m*)	33.0 (*d*)	1.71–1.84 (*m*)	41.6 (*d*)	1.69–1.77 (*m*)
21	19.5 (*q*)	1.00 (*d*, J = 6.5)	18.5 (*q*)	1.02 (*d*, J=6.5)	7.5 (*q*)	1.01 (*d*, J = 7.5)
22	42.6 (*t*)	1.72–1.83 (*m*), 1.21–1.35 (*m*)	43.6 (*t*)	1.52–1.63 (*m*), 1.11–1.25 (*m*)	71.5 (*d*)	4.19 (*dd*, J = 8.0, 5.0)
23	74.4 (*d*)	4.20 (*t*, J=6.7)	71.1 (*d*)	4.16 (*d*, J=10.2)	40.6 (*t*)	2.28 (*dd*, J = 14.0, 5.0), 2.10 (*dd*, J=14.0, 5.0)
24	159.2 (*s*)		160.6 (*s*)		152.7 (*s*)	
25	29.8 (*d*)	2.18–2.40 (*m*)	30.6 (*d*)	2.09–2.31 (*m*)	33.6 (*d*)	2.18–2.28 (*m*)
26	23.1 (*q*)	1.08 (*d*, J = 6.8)	22.5 (*q*)	1.05 (*d*, J=7.0)	21.8 (*q*)	1.04 (*d*, J=6.5)
27	23.6 (*q*)	1.09 (*d*, J=6.8)	23.3 (*q*)	1.08 (*d*, J=7.0)	21.9 (*q*)	1.06 (*d*, J = 6.5)
28	108.1 (*t*)	5.02 (*s*), 4.92 (*s*)	105.7 (*t*)	5.06 (*s*), 4.88 (*s*)	109.5 (*t*)	4.90 (*s*), 4.79 (*s*)

[a]) Spectra were recorded in $CDCl_3$ (^{1}H: 300 MHz and ^{13}C: 75 MHz). [b]) Spectra were recorded in $CDCl_3$ (^{1}H: 500 MHz and ^{13}C: 125 MHz).

basis of HMBC correlations, through CH_2(4)/C(5) and CH_2(4)/C(6). In addition, **II** and **III** were connected through the terminal methylidene based on HMBC correlations between Me(26), Me(27)/C(24), C(25), and between CH_2(28)/C(23), C(24), C(25). Rings *A* and *B* were elucidated on the basis of HMBC cross-peaks between Me(19)/C(1), C(5), C(9), C(10) and CH_2(4)/C(3), whereas rings *C* and *D* were determined

based on HMBC correlations between Me(18)/C(12), C(13), C(14), C(17) (*Fig. 1*). NOESY Correlations observed between H_β−C(11) and H−C(8), H_β−C(11) and Me(18), H_β−C(11) and Me(19), H−C(9) and H−C(14), Me(18) and H−C(8), Me(19) and H−C(8), Me(18) and H−C(20), H−C(3) and H_α−C(1), Me(21) and H_β−C(12), and Me(19) and H_β−C(4) in **1** confirmed the relative configurations for each ring junction and stereogenic center. By using a modified *Mosher*'s method, the absolute configurations at C(3) and C(23) were established. Analysis of the $\Delta\delta$ ($=\delta_{(S)}-\delta_{(R)}$) values (*Table 2*) according to the *Mosher* model [18][19] pointed to an (*S*)-configuration for C(23) of **1**, because H−C(25), Me(26), Me(27), and CH_2(28) of (*R*)-MTPA ester **1b** were more shielded by the phenyl ring of MTPA ($=\alpha$-methoxy-α-(trifluoromethyl)phenylacetic acid) products. We thereby revised the absolute configuration at C(23) in the side chain of some lanostanoids which were isolated from the bark and leaves of *Amentotaxus formosana* [16][17]. Thus, the structure of **1** was established unambiguously.

Compound **2**, a white amorphous powder, was found to possess the same molecular formula as that of **1** ($C_{28}H_{46}O_2$), as revealed by HR-ESI-MS. The spectral data of **2** (*Table 1*) differed from **1** only for C(23) (δ(H) 4.20 (*t*, *J* = 6.7, 1 H), δ(C) 74.4 of **1** and δ(H) 4.16 (*d*, *J* = 10.2, 1 H), δ(C) 71.1 of **2**). The above result, together with the comparison of 2D-NMR spectral data of **2** with those of **1**, disclosed that the two compounds were epimers. The significant ^{1}H and ^{13}C chemical-shift differences between **1** and **2** demonstrated that C(23) of **2** possessed the (*R*)-configuration. From

Table 2. *Selected ^{1}H-NMR Data of* Mosher*'s Esters of* **1** *and* **3**. δ in ppm.

Position	**1**[a])			Position	**3**[a])		
	(*S*)-MTPA **1a**	(*R*)-MTPA **1b**	$\Delta\delta$		(*S*)-MTPA **3a**	(*R*)-MTPA **3b**	$\Delta\delta$
2	1.9814, 1.5857	2.0195, 1.6786	−0.0381, −0.0929	2	1.8579, 1.5713	1.9147, 1.6853	−0.0568, −0.1140
4	2.5685, 2.5385	2.5227, 2.4623	+0.0458, +0.0762	4	2.4941, 2.4435	2.4679, 2.4059	+0.0251, +0.0376
6	5.4314	5.3997	+0.0317	6	5.3490	5.3100	+0.0390
7	1.9118, 1.5470	1.8645, 1.5113	+0.0473, +0.0357	7	1.8579, 1.5385	1.8216, 1.5054	+0.0363, +0.0331
18	0.6442	0.6682	−0.0240	18	0.7751	0.7530	+0.0221
19	0.9123	0.9161	−0.0038	19	0.8976	0.8878	+0.0098
20	1.4855	1.5391	−0.0536	20	2.2400	2.1343	+0.1057
21	1.0191	1.0375	−0.0184	21	1.2850	1.2406	+0.0444
22	1.9363, 1.6348	2.0013, 1.6612	−0.0650, −0.0264	23	2.4941, 2.2320	2.6356, 2.4679	−0.1415, −0.1490
25	2.4619	2.3914	+0.0705	25	2.3801	2.4059	−0.0258
26	1.1232	1.1113	+0.0119	26	1.0140	1.0396	−0.0256
27	1.1557	1.1332	+0.0225	27	1.0366	1.0587	−0.0221
28	5.3134, 5.1600	5.2047, 5.0908	+0.1087, +0.0692	28	4.8689	4.992	−0.1231

[a]) Spectra were recorded in C_5D_5N (300 MHz).

the aforementioned data, the structure of **2** was assigned as (3*β*,23*R*)-ergosta-5,24(28)-diene-3,23-diol.

Compound **3** had a molecular formula of $C_{28}H_{46}O_3$ as determined by HR-ESI-MS. IR Spectrum showed the presence of OH group(s) (3332 cm^{-1}). The presence of two CH−O groups and one O-bearing quaternary C-atom were inferred from ^{1}H-NMR (*δ*(H) 3.51–3.57 (*m*, 1 H), 4.19 (*dd*, *J*=8.0, 5.0, 1 H)) and ^{13}C-NMR (*δ*(C) 71.7, 71.5, and 87.5) spectra. ^{1}H- and ^{13}C-NMR spectra also exhibited signals due to five Me groups (*δ*(H) 0.76 (*s*, 3 H), 1.02 (*s*, 3 H), 1.04 (*d*, *J*=6.5, 3 H), 1.06 (*d*, *J*=6.5, 3 H), 1.01 (*d*, *J*=7.5, 3 H)), and a trisubstituted C=C bond (*δ*(H) 5.36 (br. *d*, *J*=5.0, 1 H), *δ*(C) 121.6 and 140.6), and a terminal C=C bond (*δ*(H) 4.90, 4.79 (*s*, each 1 H), *δ*(C) 109.5 and 152.7). ^{13}C-NMR and DEPT spectra of **3** also exhibited 28 signals, including those of five Me, ten CH_2, eight CH groups, and five quaternary C-atoms. This evidence suggested that **3** possessed the similar steroid skeleton (*A*, *B*, *C*, and *D* rings) as of ergosta-5,24(28)-dien-3*β*-ol except for the presence of additional OH groups at C(17) and C(22), which were confirmed by COSY and HMBC spectra of **3** (*Fig. 1*). By using *Mosher*'s method [18][19], the absolute configuration at C(22) was readily defined by the analysis of NMR-shift data of the corresponding C(22) (*S*)- and (*R*)-MTPA esters. The significant ^{1}H-NMR chemical-shift differences between the (*S*)- and (*R*)-MTPA esters **3a** and **3b** demonstrated that C(22) possessed the (*S*)-configuration (*Table 2*), because CH(25), Me(26), Me(27), and CH_2(28) of (*R*)-MTPA ester **3b** were more shielded by the phenyl ring of MTPA products. In addition, the Δ value ($\delta_{CDCl_3} - \delta_{C_5D_5N}$) of the Me(18) H-atoms (300 MHz) of **3** in two different solvents ($CDCl_3$ and (D_5)pyridine (C_5D_5N)) is small (−0.02 ppm), whereas the dihedral angle between the OH group and the Me group is large, indicating the *α*-orientation of OH at C(17) [20]. Therefore, the structure of **3** was assigned as (3*β*,22*S*)-ergosta-5,24(28)-diene-3,17,22-triol.

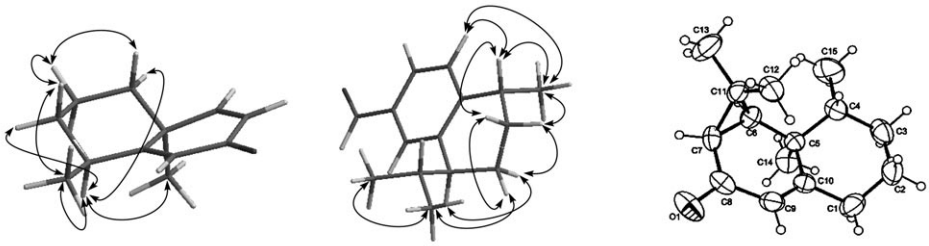
Compound **5** was obtained as a colorless oil. According to HR-EI-MS (*m*/*z* 176.1202 (M^+)) and ^{13}C-NMR data, its molecular formula was established as $C_{12}H_{16}O$, indicating five degrees of unsaturation. UV Spectrum displayed a λ_{max} (MeOH) value of 241 nm, indicating the presence of a cyclohexa-2,5-dienone moiety, supported by an IR absorption at 1657 cm^{-1} (conjugated C=O group). In addition, the ^{1}H- and ^{13}C-NMR spectra (*Table 3*) of **5** contained resonances for a cyclohexa-2,5-dienone moiety (*δ*(H) 6.12 (*d*, *J* = 1.5, 1 H), 6.22 (*dd*, *J* = 10.0, 1.5, 1 H), and 6.78 (*d*, *J* = 10.0, 1 H); *δ*(C) 171.0, 121.5, 187.5, 126.4, and 158.1). Thus, **5** was suggested to be a bicyclic structure. From the COSY spectrum of **5** (*Fig. 1*), it was possible to establish the H-atom sequence from CH_2(5) to Me(9). ^{1}H,^{1}H-COSY correlations further observed between CH(3) and CH(4) indicated the (*Z*)-form olefinic coupling (*J* = 10.0 Hz). The connectivities between C(4a) and C(4), C(5), and C(8a) were confirmed by HMBC correlations of Me(10) with C(4), C(4a), C(5), and C(8a). Furthermore, HMBC correlations from Me(9) to C(8) and C(8a), suggested that C(8) and C(8a) were connected. The relative configuration of **5** was determined by NOESY data (*Fig. 2*). NOE Correlations from H−C(8) to H_α−C(7), H_α−C(5), and Me(10) suggested that these H-atoms were oriented on the same side. NOE Correlations also indicated that H_β−C(5) and H_β−C(6), H_β−C(6) and H_β−C(7), and H_β−C(7) and Me(9) were on the opposite side. From the aforementioned data, **5** was determined as (4a*S**,8*S**)-5,6,7,8-tetrahydro-4a,8-dimethylnaphthalen-2(4a*H*)-one. An isomer (4a*R**,8*S**)-5,6,7,8-tetrahydro-4a,8-dimethylnaphthalen-2(4a*H*)-one of **5** had been

Table 3. *^{1}H- and ^{13}C-NMR Data of* **5** *and* **6**. At 500 and 300 MHz, resp., δ in ppm, J in Hz.

Position	**5**[a])		**6**[a])	
	δ(C)	δ(H)	δ(C)	δ(H)
1	121.5 (*d*)	6.12 (*d*, J=1.5)	130.0 (*d*)	7.73 (br. *s*)
2	187.5 (*s*)		141.1 (*s*)	
3	126.4 (*d*)	6.22 (*dd*, J=10.0, 1.5)	126.7 (*d*)	7.64 (br. *d*, J=7.9)
4	158.1 (*d*)	6.78 (*d*, J=10.0)	127.9 (*d*)	7.39 (*d*, J=7.9)
4a	41.2 (*s*)		150.7 (*s*)	
5	38.5 (*t*)	1.80–1.86 (*m*, H_α), 1.33–1.35 (*m*, H_β)	33.4 (*d*)	2.83–2.93 (*m*)
6	21.0 (*t*)	1.78–1.85 (*m*, H_α), 1.66–1.73 (*m*, H_β)	30.1 (*t*)	1.32–1.42 (*m*, H_α), 1.92–2.08 (*m*, H_β)
7	37.0 (*t*)	1.95–2.03 (*m*, H_α), 1.04–1.10 (*m*, H_β)	21.1 (*t*)	1.59–1.69 (*m*, H_β)
8	34.1 (*d*)	2.46–2.54 (*m*)	43.8 (*d*)	2.79 (br. *dd*, J = 12.5, 6.3)
8a	171.0 (*s*)		134.0 (*s*)	
9	17.7 (*q*)	1.14 (*d*, J=7.0)	32.0 (*d*)	2.22–2.32 (*m*)
10	23.4 (*q*)	1.27 (*s*)	17.4 (*q*)	0.73 (*d*, J=6.8)
11			21.2 (*q*)	1.03 (*d*, J=6.8)
12			22.1 (*q*)	1.31 (*d*, J=7.0)
13			192.5 (*d*)	9.96 (*s*)

[a]) Spectra were recorded in $CDCl_3$.

prepared as an intermediate in a spirovetivane synthesis [21]. The assignments of **5** were disclosed (*Table 3*) by extensive 2D-NMR data analyses for the first time.



5 **6** **7**

Fig. 2. *Selected NOESY correlations of* **5** *and* **6**, *and X-ray ORTEP representation of* **7**

HR-ESI-MS of (+)-*trans*-calamenen-13-al (**6**), a canary yellow syrup, showed a molecular formula of $C_{15}H_{20}O$, implying six degrees of unsaturation. IR Spectrum of **6** indicated the presence of a conjugated benzaldehyde function (2867 and 1695 cm^{-1}). The UV absorption maximum at 262 nm confirmed the aforementioned function. The NMR spectra (*Table 3*) indicated a trisubstituted phenyl moiety (^{1}H-NMR: δ(H) 7.39, 7.64 (*d*, J=7.9, 1 H each), and 7.73 (br. *s*, 1 H); ^{13}C-NMR δ(C) 127.9, 126.7, 130.0, 141.1, 134.0, and 150.7). The ^{13}C-NMR and DEPT spectra revealed 15 signals, including those for three Me, two CH_2, seven CH groups, and three quaternary C-atoms. The above

data of **6** were similar to those of (−)-*trans*-calamenene [22], except for the replacement of the Me by an carbaldehyde group (δ(H) 9.96 (*s*, 1 H) and δ(C) 192.5 (*d*)) at C(2). This was supported by the HMBC spectrum (*Fig. 1*) and long-range $^{1}H,^{13}C$-correlations from CH(1) to C(13)). NOESY Correlations (*Fig. 2*) between $H_{\alpha}-C(6)$ and the H-atoms of $H_{\alpha}-C(7)$ and Me(12), and between $H_{\alpha}-C(7)$ and H−C(8) suggested that these H-atoms were oriented on the same side of the cyclohexene moiety, in which the i-Pr group at C(8) was oriented on the opposite side. The absolute configuration of **6** was further established by comparison of its optical rotation with that of (−)-*trans*-calamenene [22]. On the basis of these evidences, the structure of **6** was established unambiguously.

Compound **7**, identified by comparison of spectral data with those of (−)-aristolone [15], was obtained as a colorless hexagonal crystal from hexane. The absolute configuration of aristol-9-en-8-one (**7**) was further confirmed by X-ray diffraction analyses (*Fig. 2*) [23].

Compounds **1**–**4** and **7** were tested for cytotoxicity against P-388 (mouse lymphocytic leukemia) and HT-29 (human colon adenocarcinoma) cancer cell lines. The results showed that compounds **1**, **2**, **4**, and **7** were not cytotoxic against these two cancer cell lines. However, compound **3** exhibited cytotoxicity against P-388 cell line with an ED_{50} value of 3.7 μg/ml. The anticancer agent mithramycin was used as the positive control and exhibited an ED_{50} value of 0.06 and 0.08 μg/ml against P-388 and HT-29 cells, respectively. In addition, as shown in *Fig. 3*, the *in vitro* anti-inflammatory activity of compounds **1**–**7** was tested using LPS-stimulated cell. Stimulation of RAW 264.7 cell with LPS resulted in up-regulation of the pro-inflammatory iNOS (inducible nitric oxide synthetase) and COX-2 (cyclooxygenase-2) protein. Both compounds **1** and **3**, at a concentration of 10 μM, significantly reduced the levels of the iNOS (45.8 ± 9.9 and 33.6 ± 20.6%, resp.) and COX-2 protein (68.1 ± 2.3 and 10.3 ± 6.2%, resp.) compared with the control cells stimulated with LPS. At the same concentration, compounds **2** and **4** did not inhibit the COX-2 protein expression, but significantly

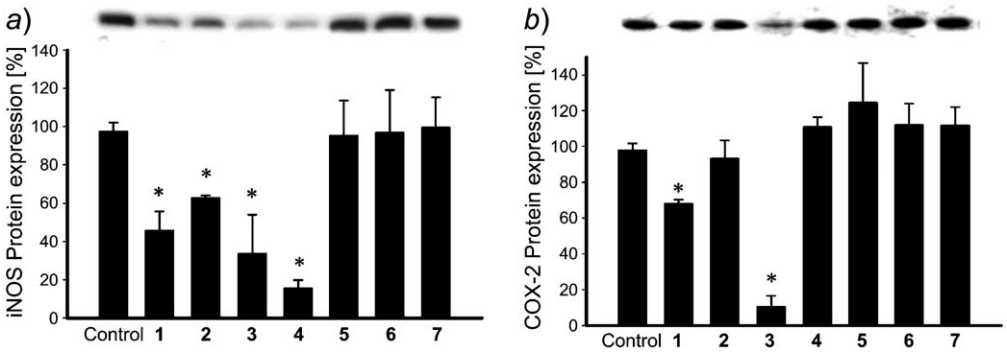


Fig. 3. *Effect of compounds* **1**–**7** *at 10* μM *concentration on the LPS-induced pro-inflammatory iNOS and COX-2 protein expression of RAW264.7 macrophage cells by immunoblot analysis. a*) Immunoblot of iNOS; *b*) immunoblot of COX-2. The values are mean ± SEM ($n=5$). Relative intensity of the LPS-alone-stimulated group was taken as 100%. *: Significantly different from LPS-stimulated (control) group ($P<0.05$).

inhibited iNOS protein expression (62.8 ± 1.3 and 15.6 ± 4.1%, resp., compared with control cells) by LPS stimulation. Moreover, cell survival was not changed by the presence of compounds **1**–**7** at the same concentration. Under the same experimental conditions, 10 μM CAPE (caffeic acid phenylthyl ester; *Sigma Chemical. Company*, St. Louis, MO) reduced the levels of the iNOS and COX-2 protein to 1.5 ± 2.1 and 70.2 ± 11.5%, respectively, relative to the control cells stimulated with LPS.

Financial support was provided by *Ministry of Education* (95C030313) and *National Science Council of Taiwan* (96–2320-B-110-003-MY3) awarded to *C.-Y. D.*

Experimental Part

General. Silica gel *60* (*Merck*, 230–400 mesh) was used for column chromatography (CC); precoated Si gel plates (*Merck*, Kieselgel *60* F_{254}, 0.25 mm) were used for TLC. High-performance liquid chromatography (HPLC): a *Hitachi L-7420* UV detector *L-7100* pump apparatus equipped with *Merck Hibar RP-18e* column (250 × 10 mm, 5 μm). Optical rotations: *JASCO P1020* polarimeter. UV Spectra: *Hitachi U-3210* spectrophotometer. IR Spectra: *JASCO FT/IR-4100* spectrophotometer. NMR Spectra: *Bruker Avance 300 NMR* spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C, or *Varian Unity INOVA 500 FT-NMR* spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C, with TMS as internal standard; chemical shifts in δ (ppm) and coupling constants *J* in Hz. ESI-MS: ESI-FT-MS on a *Bruker APEX II* mass spectrometer. EI-MS: *JEOL JMSSX/SX 102A* mass spectrometer at 70 eV.

Soft Coral Material. The soft coral *N. erecta* was collected by hand using scuba at Green Island located in the southeast coast of Taiwan, in July 2005, at a depth of 10 m, and was stored in a freezer for 5 weeks until extraction. This specimen was identified by Prof. *Chang-Feng Dai*, and a voucher specimen (GN-80) was deposited with the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Isolation. A specimen of *N. erecta* was extracted sequentially with fresh acetone and MeOH at r.t. The combined acetone extracts were concentrated to a brown gum, which was partitioned between H_2O and AcOEt. The AcOEt extract (35.0 g) was chromatographed over a silica-gel column using hexane, hexane/AcOEt, and AcOEt/MeOH mixtures of increasing polarity. Elution with hexane gave fractions containing compounds **6** and **7**, and elution with hexane/AcOEt 90:10 gave fractions containing compounds **1**, **2**, **3**, and **4**. Compounds **6** (5 mg) and **7** (5 mg) were further purified by HPLC (Si) by eluting with hexane. Compounds **1** (7 mg), **2** (2 mg), **3** (14 mg), and **4** (10 mg) were further purified by *RP-18* HPLC (95% MeOH in H_2O). In addition, the combined MeOH extract was concentrated to a brown gum, which was partitioned between H_2O and AcOEt. The AcOEt extract was chromatographed over a silica-gel column using CH_2Cl_2 and MeOH mixtures of increasing polarity. A fraction (eluted from 90% CH_2Cl_2 in MeOH) was subjected to repeated *RP-18* HPLC (60% MeOH in H_2O) to afford **5** (1 mg).

*(3β,23*S*)-Ergosta-5,24(28)-diene-3,23-diol* (**1**). White amorphous powder. $[\alpha]_D^{23} = +16$ ($c = 0.7$, $CHCl_3$). IR (KBr): 3323, 2925, 1465, 1374, 1055, 901, 799. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 437 ($[M+Na]^+$). HR-ESI-MS: 437.3392 ($[M+Na]^+$, $C_{28}H_{46}NaO_2^+$; calc. 437.3395).

*(3β,23*R*)-Ergosta-5,24(28)-diene-3,23-diol* (**2**). White amorphous powder. $[\alpha]_D^{23} = +96$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3332, 2929, 1465, 1374, 1059, 902, 803. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 437 ($[M+Na]^+$). HR-ESI-MS: 437.3394 ($[M+Na]^+$, $C_{28}H_{46}NaO_2^+$; calc. 437.3395).

*(3β,22*S*)-Ergosta-5,24(28)-diene-3,17,22-triol* (**3**). White amorphous powder. $[\alpha]_D^{23} = +14$ ($c = 0.7$, $CHCl_3$). IR (KBr): 3332, 2929, 1438, 1369, 1095, 1054, 892, 767. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 453 ($[M+Na]^+$). HR-ESI-MS: 453.3345 ($[M+Na]^+$, $C_{28}H_{46}NaO_3^+$; calc. 453.3344).

*(4a*S**,8*S**)-5,6,7,8-Tetrahydro-4a,8-dimethylnaphthalen-2(4a*H*)-one* (**5**). Colorless oil. $[\alpha]_D^{23} = +128$ ($c = 0.1$, $CHCl_3$). UV (MeOH): 241 (3.98). IR (KBr): 2926, 1657, 1461, 1378, 749. ^{1}H- and ^{13}C-NMR: see *Table 3*. EI-MS: 176 (M^+). HR-EI-MS: 176.1202 (M^+, $C_{12}H_{16}O^+$; calc. 176.1201).

*(5*S*,8*R*)-5,6,7,8-Tetrahydro-5-methyl-8-(1-methylethyl)naphthalene-2-carbaldehyde* (**6**). Canary yellow syrup. $[\alpha]_D^{23} = +42$ ($c = 0.5$, $CHCl_3$). UV (MeOH): 262 (3.18). IR (KBr): 2952, 2867, 1695, 1603,

1564, 1456, 1379, 902, 824. ^{1}H- and ^{13}C-NMR: see *Table 3*. ESI-MS (pos.): 217 ($[M+H]^+$). HR-ESI-MS: 217.1594 ($[M+H]^+$, $C_{15}H_{21}O^+$; calc. 217.1592).

*Preparation of (*R*)- and (*S*)-MTPA (=α-Methoxy-α-(trifluoromethyl)phenylacetic Acid) Esters of* **1** *and* **3**. Two aliquots of **1** (1 mg) were dissolved in (D_5)pyridine (C_5D_5N; 0.6 ml) and allowed to react overnight with (*R*)- and (*S*)-MTPA chloride (one drop), affording the (*S*)- and (*R*)-MTPA esters **1a** and **1b**, resp. In the same manner, **3** (1 mg) was dissolved in C_5D_5N (0.6 ml) and reacted with (*R*)- and (*S*)-MTPA chloride to afford (*S*)-MTPA ester **3a** and (*R*)-MTPA ester **3b**, resp. Selected $\Delta\delta$ values were shown in *Table 2*.

Cytotoxicity Testing. P-388 Cells were kindly supplied by Prof. *J. M. Pezzuto*, formerly of the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago; HT-29 cells were purchased from the *American Type Culture Collection* (*ATCC*). Cytotoxicity assays of the tested compounds were performed on the basis of a MTT assay method. The experimental details about the biological assay were carried out according to the procedure described in [24].

In vitro *Anti-Inflammatory Assay*. The anti-inflammatory assay was modified from *Ho et al.* [25] and *Park et al.* [26]. Murine RAW 264.7 macrophages were obtained from *ATCC* (No. TIB-71). The cells were activated by incubation in a medium containing *Escherichia coli* LPS (0.01 μg/ml; *Sigma*) for 16 h in the presence or absence of various compounds. Then, cells were washed with ice-cold phosphate-buffered saline (PBS), lysed in ice cold lysis buffer, and then centrifuged at 20000*g* for 30 min at 4°. The supernatant was decanted from the pellet and retained for *Western* blot analysis. Protein concentrations were determined by the DC protein assay kit (*Bio-Rad*) modified by the method of *Lowry et al.* [27]. Samples containing equal quantities of protein were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and the separated proteins were electrophoretically transferred to polyvinylidene difluoride membranes (PVDF; *Immobilon-P*, *Millipore*, 0.45-μm pore size). The resultant PVDF membranes were incubated with blocking soln. and incubated for 180 min with antibody against inducible nitric oxide synthase (iNOS; 1:1000 dilution; *Transduction Laboratories*) and cyclooxygenase-2 (COX-2; 1:1000 dilution; *Cayman Chemical*) protein. The blots were detected using enhanced chemiluminescence (ECL) detection reagents (*Perkin-Elmer*, *Western Blot Chemiluminescence Reagent Plus*) according to the manufacturer instructions.

REFERENCES

[1] J. W. Blunt, B. R. Copp, W.-P. Hu, M. H. G. Munro, P. T. Northcote, M. R. Prinsep, *Nat. Prod. Rep.* **2007**, *24*, 31, and refs. cit. in previous reviews.
[2] J. C. Coll, B. F. Bowden, D. M. Tapiolas, R. H. Willis, *Tetrahedron* **1985**, *41*, 1085.
[3] B. F. Bowden, J. C. Coll, S. J. Mitchell, *Aust. J. Chem.* **1980**, *33*, 1833.
[4] A. J. Blackman, B. F. Bowden, J. C. Coll, B. Frick, M. Mahendran, S. J. Mitchell, *Aust. J. Chem.* **1982**, *35*, 1873.
[5] C.-Y. Duh, S.-K. Wang, Y.-L. Weng, *Tetrahedron Lett.* **2000**, *41*, 1401.
[6] C.-Y. Duh, S.-K. Wang, M.-J. Chu, J.-H. Sheu, *J. Nat. Prod.* **1998**, *61*, 1022.
[7] A. A. H. El-Gamal, S.-K. Wang, C.-F. Dai, C.-Y. Duh, *J. Nat. Prod.* **2004**, *67*, 1455.
[8] W.-H. Zhang, I. D. Williams, C.-T. Che, *Tetrahedron Lett.* **2001**, *42*, 4681.
[9] I. Kitagawa, Z. Cui, B. W. Son, M. Kobayashi, Y. Kyogoku, *Chem. Pharm. Bull.* **1987**, *35*, 124.
[10] M. R. Rao, U. Venkatesham, Y. Venkateswarlu, *J. Nat. Prod.* **1999**, *62*, 1584.
[11] S. E. Poet, B. N. Ravi, *Aust. J. Chem.* **1982**, *35*, 77.
[12] D. Handayani, R. A. Edrada, P. Proksch, V. Wray, L. Witte, *J. Nat. Prod.* **1997**, *60*, 716.
[13] C.-Y. Duh, S.-K. Wang, Y.-L. Weng, M. Y. Chiang, C.-F. Dai, *J. Nat. Prod.* **1999**, *62*, 1518.
[14] a) A. F. Ahmed, Y.-T. Hsieh, Z.-H. Wen, Y.-C. Wu, J.-H. Sheu, *J. Nat. Prod.* **2006**, *69*, 1275; b) S. C. Sinha, A. Ali, A. Bagchi, M. Sahai, A. B. Ray, *Planta Med.* **1987**, *53*, 55.
[15] E. Piers, R. W. Britton, W. de Waal, *Can. J. Chem.* **1969**, *47*, 831; G. G. Harrigan, A. Ahmad, N. Baj, T. E. Glass, A. A. L. Gunatilaka, D. G. I. Kingston, *J. Nat. Prod.* **1993**, *56*, 921.
[16] H.-J. Su, S.-H. Day, S.-Z. Yang, M. Y. Chiang, C.-N. Lin, *J. Nat. Prod.* **2002**, *65*, 79.

[17] H.-J. Su, L.-W. Wang, C.-N. Lin, S.-H. Day, B.-L. Wei, S.-Z. Yang, S.-J. Won, *Helv. Chim. Acta* **2003**, *86*, 2645.
[18] I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc.* **1991**, *113*, 4092.
[19] A. H. Banskota, Y. Tezuka, K. Q. Tran, K. Tanaka, I. Saiki, S. Kadota, *J. Nat. Prod.* **2000**, *63*, 57.
[20] P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari, E. Wenkert, *J. Am. Chem. Soc.* **1968**, *90*, 5480.
[21] D. Gaine, A. A. Boucugnani, W. R. Pennington, *J. Org. Chem.* **1976**, *41*, 3632.
[22] St. Melching, N. Bülow, K. Wihstutz, St. Jung, W. A. König, *Phytochemistry* **1997**, *44*, 1291.
[23] F. H. Allen, O. Kennard, J. Trotter, *Acta Crystallogr., Sect. B* **1973**, *29*, 1451.
[24] R.-S. Hou, C.-Y. Duh, M. Y. Chiang, C.-N. Lin, *J. Nat. Prod.* **1995**, *58*, 1126.
[25] F. M. Ho, C. C. Lai, L. J. Huang, T. C. Kuo, C. M. Chao, W. W. Lin, *Br. J. Pharmacol.* **2004**, *141*, 1037.
[26] E. K. Park, Y. W. Shin, H. U. Lee, S. S. Kim, Y. C. Lee, B. Y. Lee, D. H. Kim, *Biol. Pharm. Bull.* **2005**, *28*, 652.
[27] D. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **1951**, *193*, 265.

Received October 22, 2007