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Novel sesquiterpenes and norergosterol from the soft corals Nephthea erecta and Nephthea chabroli

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ABSTRACT

Chemical investigations on the acetone extract of the Formosan soft coral *Nephthea erecta* have afforded a new calamenene-type sesquiterpene with a mercaptan group at C-15, erectathiol (**1**), and a previously reported sesquiterpenoid, (+)-*trans*-calamenene (**2**). A novel *sec*-germacrane sesquiterpene (**3**), along with a novel norergosterol, chabrosterol (**4**), possessing a 19-norergostane skeleton, was isolated from the other soft coral *Nephthea chabroli*. The structures of these metabolites were elucidated through extensive spectroscopic analyses. In vitro anti-inflammatory activity of **1** and **4** (10 μ M) significantly reduced the levels of the iNOS protein (58.0 ± 6.5% and 12.4 ± 2.9%) and COX-2 protein (108.7 ± 4.5% and 45.2 ± 5.4%). In addition, metabolite **1** (166 μ g/disk) exhibited antimicrobial activities against a small panel of bacterial strains.

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The family Nephtheidae has been proved to be a rich source of bioactive sesquiterpenoids and steroids.¹⁻¹⁰ Recently, many savants have paid much attention to these secondary metabolites due to their biological activities. A number of sesquiterpenoids have shown an array of biological activities such as insecticidal² and cytotoxic activities.^{3,4} Some of steroids were reported to possess cytotoxic^{5–8} and anti-inflammatory activities.⁹ In the course of our ongoing endeavor to discover bioactive secondary metabolites from marine organisms, chemical investigations of the Formosan soft corals *Nephthea erecta* Kükenthal and *Nephthea chabroli* Audouin were undertaken.^{4,6,7}

Chromatographic separation on the acetone extract of the soft coral *N. erecta* led to the isolation of a new calamenene-type sesquiterpene, erectathiol (**1**), together with a known calamenenetype sesquiterpene, (+)-*trans*-calamenene (**2**) (Fig. 1), which was also isolated from the soft coral *Sarcophyton glaucum*^{11a} and the liverwort *Conocephalum conicum*.^{11b} We have obtained a novel *sec*-germacrane sesquiterpene, (2*E*,6*E*)-3-isopropyl-6-methyl-10oxoundeca-2,6-dienal (**3**), and a novel 19-norergosterol, chabrosterol (**4**) (Fig. 1), from the other soft coral *N. chabroli*. To the best

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of our knowledge, erectathiol (1) (possessing a rare mercaptan moiety at C-15), (2*E*,6*E*)-3-isopropyl-6-methyl-10-oxoundeca-2,6-dienal (3) (a novel *sec*-germacrane skeleton), and chabrosterol (4) (a novel 19-norergostane skeleton) are reported from marine soft corals for the first time. The details of isolation and structural elucidation of these isolated metabolites are discussed in this Letter. The in vitro anti-inflammatory activities of 1 and 4 were measured, and the antibacterial activities against *Enterobacter aerogenes* (ATCC13048), *Serratia marcescens* (ATCC25419), *Salmonella enteriti-dis* (ATCC13076), *Yersinia enterocolitica* (ATCC23715), and *Shigella sonnei* (ATCC11060) of 1 were also evaluated in vitro. Meanwhile, the plausible biosynthetic pathways for formation of these isolated metabolites were postulated, see below.

The soft corals *N. erecta* and *N. chabroli* were collected by hand using scuba at the Green Island and Siaoliouciou Island off Taiwan, respectively. Both specimens were identified by Prof. C.-F. Dai, and voucher specimens were deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

The acetone extract of *N. erecta* was concentrated to a brown gum, which was partitioned with EtOAc and H₂O. The EtOAc-soluble residue (35.0 g) was subjected to CC on silica gel using *n*-hexane–EtOAc mixtures of increasing polarity for elution, to fractionate 40 fractions. Fraction 2 (6.0 g) was subjected to CC on



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Figure 1. Structures of metabolites 1-4.

silica gel to obtain **2** (1.0 mg). Similarly, metabolite **1** (4.0 mg) was purified by NP-HPLC using *n*-hexane–CH₂Cl₂ (90:1). In the same manner, the EtOAc fraction (100.0 g) of the other soft coral *N. chabroli* was subjected to CC on silica gel to furnish 40 fractions. Duplicate samples (2.0 g) of fraction 10 (7.5 g) was fractionated over a PR-18 gel column using MeOH–H₂O mixtures of increasing polarity for elution, to separate six subfractions. Subfraction 2 (26 mg) was subjected to a PR-18 gel column to obtain a mixture (12 mg) that was further purified by RP-HPLC using 75% MeOH in H₂O to yield **3** (2 mg). Fraction 12 (4.0 g) was fractionated over Sephadex LH-20 (100% acetone) to produce 40 subfractions. Subfraction 36 (102 mg) was subjected to CC on silica gel to obtain a mixture (64 mg) that was further purified by RP-HPLC using 95% MeOH in H₂O to give **4** (2 mg).

Erectathiol $(1)^{12}$ was obtained as a light yellow oil. The molecular formula was determined to be $C_{15}H_{22}S$, as deduced from HRFABMS $(m/z 235.1521, [M+H]^+)$ and ¹³C NMR spectroscopic data (Table 1), implying the existence of five degrees of unsaturation. The NMR spectrum (Table 1) showed a trisubstituted phenyl moiety [¹H NMR $\delta_{\rm H}$ 7.16, 7.01 (*d*, *J* = 8.0 Hz, 1H each), and 7.07 (br s, 1H); ¹³C NMR $\delta_{\rm C}$ 127.0, 126.1, 135.7, 128.7, 140.2, and 142.1]. The above functionalities also account for four of the five degrees of unsaturation, suggesting a bicyclic structure in **1**. Moreover, the ¹³C NMR and DEPT spectra revealed 15 carbon signals, including three methyls, three methylenes, six methines, and three guaternary carbons. The above data of **1** were similar to those of (–)-*trans*-calamenene,^{11b} except for the replacement of the methyl by a mercaptan group [$\delta_{\rm H}$ 3.89, 3.81 (d, J = 10.8 Hz, 1H each) and $\delta_{\rm C}$ 32.9 (t)] at C-15.¹³ This was supported by HMBC spectrum that long-range ¹H-¹³C correlations were observed from H-15 to C-2, C-3, and C-4. Thus, the planar structure of 1 was established unambiguously.

Table 1 ¹H and ¹³C NMR spectroscopic data of metabolites 1 and 3^a



Figure 2. Key NOE correlations and computer-generated perspective model using MM2 force field calculations for 1 and 3.

The NOE correlations (Fig. 2) between H-7 β with the following protons of H-6 and Me-14, and Me-14 and H-8 β suggested that these protons were oriented on the same side of the cyclohexene moiety, while the isopropyl group at C-6 was oriented on the opposite side. Moreover, the absolute stereochemistry of **1** was further elucidated by comparison of its optical rotation, $[\alpha]_D^{24} + 23$ (*c* 0.4, CHCl₃), with that of (–)-*trans*-calamenene.^{11b} From the above-mentioned findings, metabolite **1** was formulated as [(6*R*,9*S*)-6-isopropyl-9-methyl-6,7,8,9-tetrahydronaphthalen-3-yl]methanethiol.

Metabolite **3**¹⁴ analyzed for a molecular formula of $C_{15}H_{24}O_2$, according to the interpretation of its HRESIMS and NMR spectroscopic data (Table 1), implies four degrees of unsaturation. The IR absorptions of **3** at 2874 and 1671 cm⁻¹ revealed the presence of a conjugated aldehyde functionality. This was further indicated from the ¹H NMR signals at δ 9.93 (1H, d, *J* = 8.0 Hz) and 5.78 (1H, d, *J* = 8.0 Hz), and ¹³C NMR signals at δ 189.5 (CH, C-1), 124.1 (CH, C-2), and 170.2 (qC, C-3). The UV absorption maxima

C/H	1				3			
	¹³ C	¹ H	COSY	HMBC	¹³ C	¹ H	COSY	HMBC
1	127.0 (d) ^b	7.16 d (8.0) ^c	2	3, 5	189.5 (d) ^b	9.93 d (8.0) ^c	2	2
2	126.1 (d)	7.01 d (8.0)	1	4, 10	124.1 (d)	5.78 d (8.0)	1	4, 13
3	135.7 (s)				170.2 (s)			
4	128.7 (d)	7.07 s		2, 10	30.5 (t)	2.63 t (8.0)	5	2, 3, 5, 6, 13
5	140.2 (s)				41.0 (t)	2.17 t (8.0)	4	6, 7
6	43.8 (d)	2.71 m	7, 11		133.6 (s)			
7	21.4 (t)	α: 1.61 m; β: 1.83 m	6, 8		123.6 (d)	5.11 t (6.4)	8	5, 8
8	30.6 (t)	α: 1.95 m; β: 1.36 m	7, 9		23.3 (t)	2.26 dd (7.6, 6.4)	7, 9	6, 7, 10
9	32.7 (d)	2.75 m	8, 14		44.0 (t)	2.46 t (7.6)	8	7, 10
10	142.1 (s)				206.1 (s)			
11	31.9 (d)	2.23 m	6, 12, 13		30.8 (q)	2.14 s		9, 10
12	17.4 (q)	0.71 d (6.8)	11	6, 11,13	17.2 (q)	1.66 br s		5, 6, 7
13	21.3 (q)	1.01 d (6.8)	11	6, 11,13	36.5 (d)	2.41 septet (6.8)		3
14	22.3 (q)	1.26 d (6.8)	9	8, 9, 10	22.4 (q)	1.11 d (6.8)		3, 13, 15
15	32.9 (t)	α: 3.89 d (10.8)						
	.,	β: 3.81 d (10.8)		2, 3, 4	22.4 (q)	1.11 d (6.8)		3, 13, 14

 $^{\rm a}$ Spectra were measured in CDCl3 (1H, 400 MHz and 13C, 100 MHz).

^b Multiplicities are deduced by HSQC and DEPT experiments.

^c J values (in Hz) are in parentheses.

Table 2	
¹ H and ¹³ C NMR spectroscopic of	data of chabrosterol $(4)^{a}$

C/H	4						
	¹³ C	¹ H	COSY	HMBC			
1	24.4 (t) ^b	α: 2.15 m; β: 2.59 m	2				
2	28.8 (t)	α: 1.69 m; β: 1.80 m	1, 3				
3	65.0 (d)	4.13 m	2, 4				
4	31.3 (t)	α: 2.52 m; β: 2.27 m	3	5			
5	158.6 (s)						
6	43.2 (t)	α : 2.01 d (16.5) ^c ; β : 2.49 dd (16.5, 3.5)		5, 7, 8, 10			
7	199.1 (s)						
8	39.4 (d)	1.82 m	9, 14				
9	46.2 (d)	2.00 m	8, 11	5, 7, 8			
10	128.8 (s)						
11	24.5 (t)	α: 1.95 m; β: 1.43 m	9, 12				
12	39.8 (t)	α: 1.29 m; β: 2.09 m	11				
13	42.5 (s)						
14	55.0 (d)	1.29 m	8, 15				
15	23.4 (t)	α: 1.58 m; β: 1.09 m	14, 16				
16	28.1 (t)	α: 1.89 m; β: 1.29 m	15, 17				
17	55.9 (d)	1.19 m	16, 20				
18	12.0 (q)	0.71 s		12, 13, 14, 17			
20	35.6 (d)	1.43 m	17, 21, 22				
21	18.6 (q)	0.96 d (6.5)	20	17, 20, 22			
22	34.6 (t)	α: 1.16 m; b: 1.53 m	20, 23				
23	30.9 (t)	α: 1.88 m; b: 2.09 m	22	24			
24	156.7 (s)						
25	33.8 (d)	2.23 septet (6.5)	26, 27				
26	21.8 (q)	1.03 d (6.5)	25	24, 25, 27			
27	22.0 (q)	1.03 d (6.5)	25	24, 25, 26			
28	106.0 (t)	α: 4.66 s; b: 4.72 s		23, 25			

^a Spectra were measured in CDCl₃ (¹H, 500 MHz and ¹³C, 125 MHz).

^b Multiplicities are deduced by HSQC and DEPT experiments.

^c J values (in Hz) are in parentheses.

241 nm suggested the aforementioned chromophore. A keto-carbonyl group was recognized as being present in **3** from its ¹³C NMR signal at δ 206.1 (qC, C-10), as well as from a broad IR absorption at 1716 cm⁻¹. Moreover, the ¹H and ¹³C NMR spectroscopic data of **3** contained resonances for a trisubstituted double bond at C-6 and C-7 [$\delta_{\rm H}$ 5.11 (t, *J* = 6.4 Hz, 1H); $\delta_{\rm C}$ 133.6 (qC) and 123.6 (CH)]. The above functionalities accounted for four of the four degrees of unsaturation, suggesting a linear structure for **3**.

By interpretation of ¹H–¹H COSY correlations, it was possible to establish four partial structures of consecutive proton systems extending from H-1 to H-2, from H₂-4 to H₂-5, from H₃-14 to H₃-15 through H-13, and from H_2 -9 to H_3 -12 through H-7 and H_2 -8, as well as long-range COSY correlation between H₃-12 and H-7. Moreover, the connectivities of these partial structures were further established by the HMBC correlations. On the basis of the above observations, the sec-germacrane structure of **3** was established unambiguously. The geometry of the trisubstituted olefins was assigned as *E* based on the γ -effect of the olefinic methyl signal for C-12 (less than 20 ppm) and the NOESY correlation between H_2 -8 and H_3 -12. The NOE correlations (Fig. 2) between H-1/ H_2 -4, H-2/H₃-14, and H-2/H₃-15 indicated the s-trans geometry of the conjugated aldehyde moiety. All the NMR spectroscopic data of metabolite 3, assigned by COSY, HMQC, HMBC, and NOESY correlations, were satisfactorily consistent with the structure shown as (2E,6E)-3-isopropyl-6-methyl-10-oxoundeca-2,6-dienal.

Chabrosterol (**4**)¹⁵ was isolated as a white amorphous powder. HRESIMS of **4** exhibited a pseudo molecular ion peak at m/z421.3085 [M+Na]⁺ (calcd for 421.3082) and established a molecular formula of C₂₇H₄₂O₂, indicating seven degrees of unsaturation. The ¹³C NMR displayed 27 carbon signals, which were identified by the assistance of the DEPT spectrum as four methyls, eleven methylenes, seven methines, and five quaternary carbons. The ¹H NMR signal [$\delta_{\rm H}$ 4.13 (m, 1H)] (Table 2) and IR absorption at 3309 cm⁻¹, together with the observation of one oxygen-bearing carbon resonance ($\delta_{\rm C}$ 65.0) in ¹³C NMR spectrum (Table 2), revealed the presence of one hydroxyl. Furthermore, one tetrasubstituted double bond ($\delta_{\rm C}$ 128.8 and 158.6), one terminal double bond ($\delta_{\rm C}$ 106.0 and 156.7), and one carbonyl carbon ($\delta_{\rm C}$ 199.1) were assigned from ¹³C NMR and DEPT spectra of **4**. The above functionalities accounted for three of the seven degrees of unsaturation, suggesting a tetracyclic skeleton for **4**.

Interpretation of the ¹H–¹H COSY spectrum led to three partial structures. The connectivities of these partial structures were further established by the HMBC correlations. Moreover, the long-range ¹H–¹³C correlations observed from H₂-6 to C-7, and H-9 to C-7 indicated the position of the carbonyl group at C-7. The COSY correlations from H₂-1 to H-3 through H₂-2 led the assignment of the one secondary hydroxyl group at C-3. The location of the tetrasubstituted double bond at C-5/C-10 was clarified by analysis of the HMBC correlations from H₂-4 to C-5, and H₂-6 to C-10, and H-9 to C-5. The NOESY correlations (Fig. 3) observed between H-3 and H-2 α and H-4 α indicated the β -oriented of the hydroxyl group at C-3. Moreover, the NOESY correlations observed between H-2 β and H-1 β , H-4 α and H-6 α , H-6 β and H-8, H-9 and H-14, H-11 β and H-12 β , H-12 β and Me-18, Me-18 and H-20, and Me-21 and



Figure 3. Key NOE correlations and computer-generated perspective model using MM2 force field calculations for 4.



Scheme 1. Plausible biosynthetic pathway for formation of 4.



Figure 4. Effect of compounds **1** and **4** at 10 μ M on the LPS-induced pro-inflammatory iNOS and on COX-2 protein expression of RAW 264.7 macrophage cells by immunoblot analysis. (A) Immunoblot of iNOS; (B) immunoblot of COX-2; (C) immunoblot of β -actin. The values are mean ± SEM. (n = 5). The relative intensity of the LPS alone stimulated group was taken as 100%. *Significantly different from LPS-stimulated (control) group (*P < 0.05).

H-12 β in **4** confirmed the relative configurations for each ring junction and chiral center. Thus, the structure of **4** was established unambiguously.

The main novelty of **1** is the presence of a calamanene skeleton with a rare mercaptan moiety at C-15. Erectathiol (**1**), possessing a rare mercaptan moiety, differs from previously calamenene-type sesquiterpenoids isolated from marine soft corals. Biosynthetically, it is suggested that a new approach for exhaustive transformation of **1**, in two steps mainly involves benzylic oxidation and acid-induced mercaptanization at C-15 of **1**. In addition, a plausible oxidation mechanism (dihydroxylation and oxidative cleavage of diols) of formation of metabolite **3** was postulated. It is worthwhile to mention that metabolite **4** represents the first 19-norergosterol. Because of biogenic considerations, metabolite **4** may be involved in the biosynthesis of **5**¹⁶ through oxidation and 19-decarboxylation of **4** as depicted in Scheme 1.

Preliminary antibacterial activity revealed that metabolite **1**, at a concentration of 166 μ g/disk, exhibited moderate activities against four bacterial strains, comprising *E. aerogenes*, *S. marcescens*, *Y. enterocolitica*, and *S. sonnei*. Under the same concentration, metabolite **1** revealed greater antibacterial potential than the positive control (ampicillin) against *S. enteritidis*. The antibacterial assays were carried out according to the procedure described previously.¹⁷

In vitro anti-inflammatory activity of metabolites **1** and **4** were tested using LPS-stimulated cells. Stimulation of RAW 264.7 cells with LPS resulted in up-regulation of the pro-inflammatory iNOS and COX-2 proteins. At a concentration of 10 μ M, metabolites **1** and **4** significantly reduced the levels of the iNOS protein (58.0 ± 6.5% and 12.4 ± 2.9%) and COX-2 protein (108.7 ± 4.5% and

45.2 ± 5.4%) compared with the control cells (LPS alone) (Fig. 4). The primary anti-inflammatory results of **1** exhibited the observed activity against iNOS protein expression, but no discernible activity against COX-2 protein expression. Besides, chabrosterol **(4)**, possessing a novel 19-norergostane skeleton, showed significantly anti-inflammatory activity against LPS-stimulated RAW 264.7 cells. The anti-inflammatory assay was carried out according to the procedure described previously.¹⁷

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- (2E,6E)-3-Isopropyl-6-methyl-10-oxoundeca-2,6-dienal (3): Primrose yellow oil; UV (MeOH) λ_{max} (log ε) 241 (3.81) nm; IR (KBr) v_{max} 2963, 2932, 2874, 1716,

1671, 1625, 1463, 1363, 1155 cm⁻¹; ¹H NMR and ¹³C NMR data, see Table 1; ESIMS m/z 259 [M+Na]⁺; HRESIMS m/z 259.1676 [M+Na]⁺ (calcd for C₁₅H₂₄O₂Na, 259.1674).

- 15. *Chabrosterol* (**4**): White amorphous powder; $[\alpha]_D^{25}$ +28.5 (*c* 0.1, CHCl₃); IR (KBr) ν_{max} 3309, 2933, 2868, 1723, 1685, 1462, 1376, 1209 cm⁻¹; ¹H NMR and ¹³C NMR data, see Table 2; ESIMS *m/z* 421 [M+Na]⁺; HRESIMS *m/z* 421.3085 [M+Na]⁺ (calcd for C₂₇H₄₂O₂Na, 421.3082).
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