Sesquiterpenoids and Artificial 19-Oxygenated Steroids from the Formosan Soft Coral
Nephthea erecta

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Chemical investigations on the acetone and MeOH solubles of the soft coral Nephthea erecta have afforded five new sesquiterpenoids (1–5), one known sesquiterpene, kelsoene (6), and two known 19-oxygenated steroids (10 and 11). In addition, three unexpected artificial 19-oxygenated steroids (7–9) were obtained by letting 10 and 11 stand in CDCl3 for prolonged periods of time. The structures of 1–9 were elucidated by extensive spectroscopic analyses, and their cytotoxicity against selected cancer cells was measured in vitro.

The family Nephtheidae has been proved to be a rich source of bioactive compounds.1–9 The ongoing search for bioactive constituents prompted us to reinvestigate the secondary metabolites of the soft coral Nephthea erecta Kükenthal (Nephtheidae).9 Compounds 1–6,10,11,14–17 10,12 and 1113 were isolated from the soft coral N. erecta while 7–9 are artifacts obtained by allowing 10 and 11 to stand in CDCl3 overnight. Compound 7 was subsequently converted to 8 after 7 days. In the same conditions, 11 was transformed into 9 in CDCl3 after a week through epoxidation (Scheme 1). However, no reactions occurred when 10 and 11 were treated in pyridine-d5 for 2 months, implying 10 and 11 were stable under slightly basic solvent. In this article, we report the structure elucidation and the cytotoxicity of these metabolites.

Results and Discussion

Compound 1 was isolated as a colorless, viscous oil. HRESIMS of 1 exhibited a [M + Na]+ peak at m/z 275.1625 and established a molecular formula of C13H24O3, implying four degrees of unsaturation. The 1H NMR spectrum of 1 (Table 1) showed signals corresponding to an oxygenated methine proton [δH 4.77 (1H, ddd, J = 3.5, 2.0, 1.5 Hz)], an olefinic proton [δH 6.21 (1H, d, J = 1.5 Hz)], a secondary methyl [δH 1.00 (3H, d, J = 7.0 Hz)], and three tertiary methyls [δH 1.44 (3H, s); δH 1.47 (3H, s); δH 0.89 (3H, s)], respectively. The 13C NMR displayed 15 carbon resonances, and the DEPT spectrum (Table 1) was consistent with the presence of a methine [δC 71.4 (CH)], a quaternary carbon [δC 81.5 (qC)] bearing a peroxide ring, a quaternary carbon [δC 70.7 (qC)] bearing a hydroxyl, and trisubstituted olefinic carbons [δC 124.2 (CH) and 149.5 (qC)], as well as four methyls, four methylenes, three methines, and four quaternary carbons. The above data of 1 were similar to those of 5α,8α-epidioxy-6-eudesmen-14, except for the presence of a tertiary hydroxyl at C-11. This was supported by the HMBC spectrum, which shows correlations between H-7, H-11, H-12, and H-13 to C-11 (Figure 1). On the basis of the above evidence, the planar structure of 1 was unambiguously established. The computer-modeled structure of 1 was generated by CS Chem 3D version 9.0 using MM2 force field calculations for energy minimization (Figure 2). The results were consistent with the stereochemistry of 1 as established by the NOESY experiments. The NOESY correlations between H-14 and all protons of H-1α, H-4, H-8, and H-9α positioned all these protons on the same side of the molecule and revealed the β-orientation of H-15. Therefore, the structure of 5β,8β-epidioxy-11-hydroxy-6-eudesmen-14 was characterized as 1.

5β,8β-Epidioxy-11-hydroperoxy-6-eudesmen-14 (2) was isolated as a colorless, viscous oil, and its molecular formula was determined to be C13H24O5, as deduced from HRESIMS spectroscopic data. The 1H NMR spectrum of 2 showed a signal at δH 7.72 (1H, br s) that suggested the presence of a hydroperoxy group, while the hydroperoxy could be located at C-11, as a result of the HMBC correlations (Figure 1). The carbon signals of Me-12 and Me-13 of 1 were at a lower field when compared to 2, and the signal of C-11 was shifted downfield (ΔδC 11.1 ppm). The 13C NMR

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spectroscopic data of 1 and 2 were in good accordance with those of compounds with a similar side chain with a hydroxyl or a hydroperoxyl. Consequently, the structure of 2 was deduced unambiguously.

Compound 3 was obtained as a colorless, viscous oil, which analyzed for the molecular formula C_{15}H_{24}O_{2} by HRESIMS coupled with the DEPT and \textsuperscript{13}C NMR spectroscopic data (Table 1). A broad IR spectrum absorption at 3321 cm\textsuperscript{-1} indicated the presence of a hydroxy group. The \textsuperscript{1}H and \textsuperscript{13}C NMR spectra of 3 contained resonances for a trisubstituted double bond at C-1 and C-2 [\textit{\delta}_{\text{H}} 5.27 (ddd, J = 3.5, 2.0, 1.5 Hz, 1H); \textit{\delta}_{\text{C}} 145.3 (qC) and 116.3 (CH)].
and a disubstituted epoxide ($\delta_H$ 3.46 (dd, $J = 3.7, 2.5$ Hz, 1H) and 2.77 (d, $J = 3.7$ Hz, 1H); $\delta_C$ 50.4 (CH) and 62.1 (CH)) at C-3 and C-4. From the above evidences, 3 was suggested to be a tricyclic sesquiterpenoid. From the COSY spectrum of 3 (Figure 1), it was possible to establish that the proton sequence connects from H-2 to H-4 and from H-6 to H-14. The $^1$H–$^1$H COSY correlations further observed between H-2 and H-10 showed further the allylic coupling of the above protons. The connectivities between C-1/C-5, C-4/C-5, and C-5/C-6 were confirmed by the HMBC correlations of H-15 with C-1, C-4, C-5, and C-6. In addition, the HMBC correlations (Figure 1) from H-12 and H-13 to C-7 and C-11 proved the attachment of the isopropanol group attached at C-11. Furthermore, NOE correlations could be observed between H-9a and H-14, H-15, and H-3, indicating the $\beta$-orientations of H-3 and H-4 and the $\beta$-orientation of the isopropanol group attached at C-11. Therefore, H-14 and H-15 should be placed on the $\alpha$-face. From the aforementioned observations, 3 was formulated as (3R*,4S*,5R*,7R*,10R*)-3,4-epoxy-11-hydroxy-1-pseudoguaiane.

The molecular formula of 4 was assigned as C$_{13}$H$_{20}$O, as derived from its HRESIMS and in agreement with the NMR data. By comparison of the $^{13}$C NMR spectroscopic data of 4 with those of the known sesquiterpene prespataene,$^{10,11}$ it was found that C-8 ($\delta_C$ 50.3 d) in prespataene was converted to a tertiary hydroxyl ($\delta_C$ 87.8 s) in 4, as also confirmed by the HMBC correlations (H-13/C-8, C-11, and C-12). Thus, the structure of 4 was established and named $\beta$-hydroxyxyprespatane. From the NOESY spectrum of 4, cross-peaks for the signals with H-2, H-6, and H-10 (at $\delta_H$ 1.60) fixed the three rings in a chair or chair conformation, while the NOE interactions between H-15 and all protons of H-7, H-13, and H-14, which in turn showed correlation with H-10a, positioned the above protons on the same side of the molecule and revealed the $\beta$-orientation of the 8-OH.

HRESIMS of $\beta$-hydroxyxyprespatane (5), a colorless, viscous oil, established a molecular formula of C$_{15}$H$_{24}$O$_2$. By comparison of the NMR spectroscopic data of 5 with those of 4, it was found that the $^1$H and $^{13}$C spectroscopic data of both compounds were nearly the same, except that the carbon shift of the tertiary hydroperoxy at C-8 ($\delta_C$ 100.2, s) of 5 was shifted downfield relative to the signal of C-8 ($\delta_C$ 87.8, s) of 4.$^{16,17}$ Thus, the structure of 5 was established unambiguously.

Compound 7 was obtained from 10 by letting the latter stand in CDCl$_3$ overnight. The $^1$H and $^{13}$C NMR spectroscopic data of 7 revealed the presence of a conjugated triene ($\delta_C$ 126.4 CH and 124.2 CH; $\delta_H$ 5.77 m and 5.92 m; $\delta_C$ 121.7 CH and 137.8 qC; $\delta_H$ 5.77 d, $J = 5.5$ Hz; $\delta_C$ 128.3 CH and 132.5 CH; $\delta_H$ 6.05 dd, $J = 9.6, 2.4$ Hz and 5.68 d, $J = 9.6$ Hz) in rings A and B. This was confirmed by 2D NMR spectroscopic analyses. Interpretation of the $^1$H–$^1$H COSY spectrum led to partial structures I, II, and III (Figure 3). Partial structures I–III were connected by HMBC correlations.

$^{24}$-Methylenecholesta-4,6-dien-3β,19-epoxy-2β-ol (8) was obtained from 7 by letting the latter stand in CDCl$_3$ for 1 week. The $^{13}$C NMR and DEPT spectroscopic data of 8 showed signals for six olefin carbons, four methyl carbons, eight methylene carbons, eight methine carbons, and two quaternary carbons. The above functionalities account for three of the eight degrees of unsaturation, suggesting that 8 is a tetracyclic compound with a 3β,19-epoxy moiety. The $^1$H–$^1$H COSY spectrum correlations of 8 were similar as that of 4.

Table 2. $^1$H and $^{13}$C NMR Spectroscopic Data of Compounds 4 and 5

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<td>7.71 br s</td>
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$^a$ Spectra were measured in CDCl$_3$ ($^1$H, 300 MHz and $^{13}$C, 75 MHz). $^b$ Multiplicities are deduced by HSQC and DEPT experiments. $^c$ $J$ values (in Hz) are in parentheses.
HRESIMS of 24-methylenecholesta-6/8,19-epoxy-3/6,5α-diol (9) was obtained from 11 by letting the latter stand in CDCl₃ for 1 week. By comparison of the NMR spectroscopic data (Table 3) of 9 with those of 11, it was found that hydroxy groups attached to C-6 and C-19 in 11 were converted to a 6β,19-oxide ring in 9. The position of the oxide group at C-6/C-19 was confirmed by the HMBC correlation (Figure 3) from H-6 to C-19.

Preliminary cytotoxicity screening revealed that 2, 5, and 6 exhibited significant cytotoxicity against P-388 (mouse lymphocytic leukemia) and HT-29 (human colon adenocarcinoma) cells (Table 4). The other tested compounds were not cytotoxic to P-388 and HT-29 cells. The results of further biological activity screening will be reported elsewhere in the future.

### Experimental Section

**General Experimental Procedures.** Optical rotations were determined on a JASCO P1020 polarimeter. UV spectra were obtained on a Hitachi U-3210 spectrophotometer, and IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. The NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer at 300 MHz for 1H and 75 MHz for 13C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for 1H and 125 MHz for 13C, respectively, using TMS as internal standard. Chemical shifts are given in δ (ppm) and coupling constants in Hz. ESIMS were recorded by ESI FT-MS on a Bruker.

**to those of 7. These data, together with the HMBC correlations (Figure 3), established the structure of 8.**

**Animal Material.** The soft coral *N. erecta* was collected by hand using scuba at Green Island located on 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. A voucher specimen (GN-80) was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

**Extraction and Isolation.** A specimen of *N. erecta* was extracted sequentially with acetone and MeOH. After removal of solvent in vacuo, the acetone-soluble residue was partitioned between H₂O and EtOAc.
The dried EtOAc extract (35.0 g) was chromatographed over a Si column using n-hexane, n-hexane/EtOAc, and EtOAc/MeOH mixtures of increasing polarity. Elution with n-hexane gave fractions containing compound 6, that with n-hexane/EtOAc (90:10) gave fractions containing compounds 4 and 5, that with n-hexane/EtOAc (85:15) gave fractions containing compound 3, that with EtOAc/MeOH (20:1) gave fractions containing compound 10, and that with EtOAc/MeOH (10:1) gave fractions containing compound 11. Compound 6 (3 mg) was further purified by HPLC (Si) by eluting with n-hexane. Compounds 4 (2 mg) and 5 (5 mg) were further purified by HPLC (Si) by eluting with n-hexane/EtOAc (90:10) and n-hexane/CH2Cl2 (50:50), respectively. Compound 3 (2 mg) was purified by repeated HPLC (Si) by eluting with n-hexane/EtOAc (85:15). Compound 10 (7.0 mg) was further purified by RP-HPLC by eluting with MeOH/H2O (85:15). Compound 11 (5.0 mg) was further purified by RP-HPLC by eluting with MeOH/H2O (85:15).

Compound 10 was fully transformed into compound 7 during NMR experiments in CDCl3. Compound 7 was then converted to a mixture containing compounds 7 and 8. Compound 8 (2 mg) was further purified by RP-18 HPLC by eluting with MeOH/H2O (90:10). Under the same conditions, compound 11 was transformed into a mixture containing compounds 11 and 9 in CDCl3 in 7 days. Compound 9 (2 mg) was further purified by RP-18 HPLC by eluting with MeOH/H2O (85:15).

The MeOH-soluble residue (320 mg) was partitioned between H2O and EtOAc. The dried EtOAc layer was then subjected to column chromatography on silica gel using CH2Cl2 and CH2Cl2/MeOH mixtures of increasing polarity. Elution with CH2Cl2/MeOH (80:1) gave fractions containing compounds 1 and 2. Compounds 1 (7 mg) and 2 (5 mg) were further purified by RP-HPLC by eluting with MeOH/H2O (60:40).

6/8/9-Epideoxy-11-hydroxy-6-eudesmenne (1): colorless, viscous oil; [α]20D +10 (c 0.7, CH2Cl2); IR (KBr) νmax 3322, 2887, 1457, 1384, 1239, 1040, 927, 739 cm⁻¹; 1H NMR and 13C NMR data, see Table 1; ESIMS m/z 275 [M + Na]+; HRESIMS m/z 275.1625 [M + Na]+ (calcd for C15H24O2Na, 259.1676 [M + Na]+). Compound 10 (5 mg) was transformed into compound 11 (2 mg) by treatment with 28% H2O2, CH2Cl2 in CDCl3 in 7 days.

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