New Oxygenated Sesquiterpenes from the Indonesian Soft Coral *Nephthea chabrolii*

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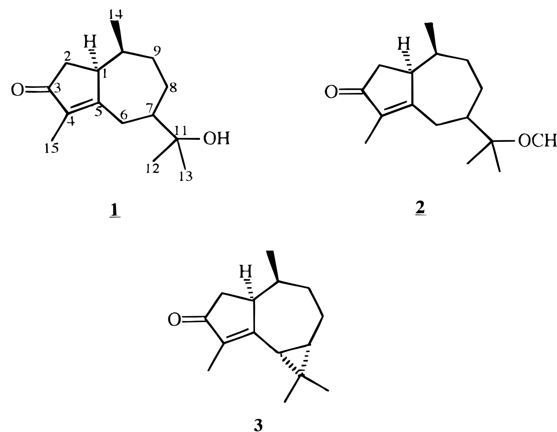
The Indonesian soft coral *Nephthea chabrolii* afforded two new oxygenated sesquiterpenes: hydroxycolorenone (**1**) and methoxycolorenone (**2**), as well as the known liverwort metabolite (+)-cyclocolorenone (**3**). This is the first report of the occurrence of such terrestrial liverwort metabolites in marine soft corals. The structures of the new compounds were unambiguously established on the basis of NMR spectroscopic data (^{1}H, ^{13}C, COSY, ^{1}H-detected direct and long-range ^{13}C−^{1}H correlations). Compound **1** exhibited insecticidal activity towards neonate larvae of the polyphagous pest insect *Spodoptera littoralis*, with an EC_{50} of 8.8 ppm and a LC_{50} of 453 ppm, when incorporated in artificial diet and offered to larvae in a chronic feeding bioassay.

Soft corals of the genus *Nephthea* (Alcyonacea, Nephtheidae) have yielded a variety of bioactive sesquiterpenes and diterpenes.[1−7] In the present paper, we describe the isolation and structure elucidation of two new oxygenated sesquiterpenes,[12] hydroxycolorenone (**1**), which has a strong antifeedant activity, and methoxycolorenone (**2**), and the known sesquiterpene, (+)-cyclocolorenone (**3**). These were obtained from the Indonesian soft coral *Nephthea chabrolii* Andouin, 1828. The same species has been previously reported to yield cembranoid diterpenes[7] and caryophyllene-based diterpenes.[6] This is the first report of the occurrence of colorenone sesquiterpenes in this species of soft corals. The oxygenated sesquiterpene (+)-cyclocolorenone is a known metabolite of terrestrial liverworts of the genera *Plagiochila*[8,9] and *Bazzania*,[10] which include widespread liverwort species in Southeast Asia. The liverworts (Hepaticae) are classified phylogenetically between vascular plants and algae,[10] and it may thus be speculated that epiphytic or symbiotic marine algae are the true sources of these compounds isolated from the soft coral.

Three major fractions were obtained by column chromatography on Si gel of the EtOAc-soluble material of the crude extract from the soft coral. The first fraction, a non-polar one, yielded the known compound **3**, the second fraction, a semi-polar one, yielded compound **2**, and the third fraction, a polar one, yielded compound **1**.

The known compound **3** was readily identified from its spectroscopic data and by comparison with published



data.[8,10] Through-bond homonuclear (^{1}H COSY) and heteronuclear (^{1}H-detected one-bond and multiple-bond ^{13}C multiple coherence) correlations were used to establish unambiguously the assignments and atom connectivities in compound **3**, and these assignments were then applied to compounds **1** and **2**. In particular, the presence of a long-range correlation of H-6 to C-13 and C-12 established the nature of the three-membered ring in **3**, and the absence of this correlation confirmed the lack of a three-membered ring in **1** and **2**. Similar correlations between the methyl group H-14 and carbons C-1, C-9, and C-10 in all compounds confirmed the seven-membered ring, the position of the methyl substituent, and the correct assignment of the methylene groups (Table 2, footnote *e*). These data indicated the close relationship of the two new metabolites with the known compound **3**, cyclocolorenone.

Compound **1** showed a protonated molecular ion [M + H]$^+$ at m/z 237, which is compatible with the molecular composition of $C_{15}H_{24}O_2$. The ^{1}H-NMR spectrum of **1** showed two complex signals for the C-6 protons at δ 2.24 and δ 3.14, with a large geminal coupling constant of 18.3 Hz and vicinal coupling constants of 12.3 Hz and <2 Hz, respectively, in contrast to a broad doublet at δ 1.54 (^3J = 8.2 Hz) found in **3**,

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Table 1. ^{13}C-NMR Data of Compounds **1**−**3** in Me_2CO-d_6

C[a]	1		2		3	
1	46.2	d	46.1	d	42.8	d
2	41.8	t	41.8	t	40.5	t
3	207.0	s	206.9	s	206.6	s
4	137.8	s	137.9	s	140.6	s
5	175.7	s	175.2	s	175.2	s
6	34.3	t	34.0	t	28.9	d
7	48.9	d	44.9	d	32.8	d
8	27.7	t	27.3	t	21.7	t[b]
9	37.6	t	37.5	t	33.3	t[b]
10	36.4	d	36.5	d	32.4	d
11	72.5	s	77.4	s	26.1	s
12	26.3	q	22.0	q	16.7	q
13	27.4	q	22.5	q	29.2	q
14	12.4	q	12.4	q	17.7	q
15	8.0	q	8.0	q	8.2	q
16			48.7	q		

[a] Signal multiplicities were established from DEPT-135 spectra. [b] The assignments of these signals are interchanged as compared with that of Wu and Chen.[10]

which signify the opening of the cyclopropane system. This was confirmed from the ^{13}C data, as C-6 appeared as a methylene carbon at δ 34.3. The close similarity of the ^{1}H shifts, coupling constants, and signal forms (Table 2) of H-1, H-2A, and H-2B argues for the same relative conformation and configuration at C-1 and C-10 in **1** and **3**. The magnitude of the the vicinal couplings between H-6A/H-6B and H-7 indicates an equatorial disposition of the substituent at C-7, and the equatorial disposition of H-6A and H-8A follows from the observation of a long-range W-coupling.

Compound **2** is the methoxyl analogue of **1** and shows a protonated molecular ion $[M + H]^+$ at m/z 251, which is compatible with the molecular composition of $C_{16}H_{26}O_2$. The ^{1}H-NMR spectrum showed an additional singlet signal at δ 3.13 corresponding to $-OCH_3$. The ^{13}C-NMR spectrum was comparable to that of **1**, except for an additional methoxyl signal at δ 48.8, which is positioned at C-11 from the 4- to 5-ppm highfield shifts of C-7, C-12, and C-13.

The ^{13}C chemical shift changes in Table 1 are in keeping with the increased conformational freedom of the seven-membered ring on going from **3** to **1** and **2**, and compatible with the same relative stereochemistries for all three compounds. Although we could not unambiguously establish through NOE experiments that the stereochemistry of all three compounds are the same at carbons C-1, C-7, and C-10, it is reasonable to assume that the similar ^{1}H coupling constants for H-2A, H-2B, H-9B and the low intensity of the cross peak in the COSY spectra of H-9B and H-10 in all three compounds indicate that the relative stereochemistries, at least at C-1 and C-10, are maintained.

Because the soft coral was kept and stored in MeOH after collection, it is possible that **2** is an artifact arising through methylation of **1**. A further possibility is that **1** and **2** are artifacts derived from the ring-opening of **3** in the presence of H_2O and MeOH. This was definitely not the case, as a 48-h treatment of compound **3** with aqueous MeOH in the presence of acid (TFA, pH 2) did not generate **1** or **2**, as shown from TLC and HPLC of the reaction mixture.

All compounds isolated were analyzed for insecticidal activity by incorporating each compound into an artificial diet at an arbitrarily chosen concentration (530 ppm) and offering the spiked diet to neonate larvae of the vigorous pest insect *Spodoptera littoralis* in a chronic feeding experiment. After 6 days of exposure, larval survival and larval weight were monitored and compared to controls. Only compound **1** was active and inhibited the growth of the larvae of *S. littoralis*. Compound **1** caused 94% inhibition of larval growth at a concentration at 530 ppm. From the dose−response curve obtained, the EC_{50} for growth inhibition and LC_{50} were calculated by probit analysis as 8.8 [±0.26 SE] ppm and 453 [±0.43 SE] ppm, respectively. Thus, opening of the three-membered ring and the attachment of the hydroxyl group at C-11 transformed **3** to the

Table 2. ^{1}H-NMR Data of Compounds **1**−**3** in Me_2CO-d_6

H[a]	1[b]		2[c]		3[d]	
1	3.16	brm	3.19	brm	3.06	brm
2A	2.45	ddd, J = 18.5, 6.7, 1.2 Hz	2.45	ddd, J = 18.4, 6.7, 1.1 Hz	2.39	dd, J = 18.3, 6.8 Hz
2B	1.92	ddd, J = 18.5, 1.9, 1.0 Hz	1.92	ddd, J = 18.4, 1.5, 1.0 Hz	1.95	dd, J = 18.5, 2.3 Hz
6A	3.14	dm, J = 18.3 Hz	2.97	dm	1.54	dm, J = 8.2 Hz
6B	2.24	ddm, J = 18.3, 12.3 Hz	2.24	ddm, J = 19.4, 12.1 Hz		
7	1.72	m	1.88	m	1.29[e]	ddd, J = 10.0, 8.0, 7.0 Hz
8A	1.99	dm, J = 14.1 Hz	2.06	m	1.93[e]	m
8B	1.25	m	1.26	m	1.68[e]	dddd, J = 15.0, 13.3, 10.2, 3.2 Hz
9A	1.83	dddd, J = 14.0, 4.6, 3.4, 3.3 Hz	1.84	dddd, J = 13.7, 4.3, 3.4, 3.4 Hz	2.09[e]	m
9B	1.72	dddd, J = 13.9, 13.9, 3,8, 3.8 Hz	1.73	dddd, J = 13.7, 13.3, 3.5, 3.5 Hz	1.43[e]	dddd, J = 13.5, 13.5, 3.3, 3.3 Hz
10	2.11	brm	2.12	m	2.02[e]	m
12	1.22	s	1.16	s	1.05	s
13	1.18	s	1.13	s	1.25	s
14	0.62	d, J = 7.1 Hz	0.63	d, J = 7.1 Hz	0.80	d, J = 6.9 Hz
15	1.60	ddd, J = ca. 1.6 Hz × 3	1.60	ddd, J = 1.6 Hz × 3	1.66	d, J = 2.0 Hz
16			3.13	s		

[a] Signal multiplicities are abbreviated br = broad, d = doublet, m = multiplet, s = singlet. [b] In the COSY spectrum (signal width, $\Delta_{0.5}$, ca. 14 Hz) H-1 shows couplings to H-2A and H-2B (and probably H-6A, H-6B, and H-15; see footnote c); H-6A several small couplings to H-7 (<2 Hz), H-8A and H-15; H-6B small coupling to H-15; H-7 couplings to H-6A, H-6B, H-8A, and H-8B; H-8A several small couplings to H-6A, H-9A and H-9B; H-8B is a broad signal overlapped with H-12 and couples to H-7, H-8A, H-9A, H-9B; H-9B overlaps H-7 and couples with H-8A, H-8B, H-9A and H-10; H-10 is a very broad multiplet with couplings to H-9A, H-9B, and H-14 (The coupling of H-10 to H-1 was not visible in the COSY spectrum, but, it is vicinal to this proton as there is an unambiguous 3-bond long range coupling between H-14 and C-1 in the HMBC correlation); H-15 several couplings to H-1, H-6A, and H-6B. [c] The COSY responses are very similar to those in **1** and only the differences are noted. H-1 ($\Delta_{0.5}$ 13 Hz) shows couplings to H-2A, H-2B, H-6A, H-6B, H-10, and H-15; H-7 is overlapped by H-2B and H-9A; H-8A is overlapped by the residual solvent signal. [d] In the COSY spectrum ($\Delta_{0.5}$ 13 Hz) H-1 shows couplings to H-2A, H-2B, H-6, and H-15; H-6 shows several small couplings to H-1, H-2B, and H-15; H-8A is overlapped by H-2B; H-15 has an unresolved coupling to H-6. [e] The shifts of H-7 and H-10, and H-8 and H-9 are interchanged compared with the assignments of Wu and Chen.[10] In the present case, the spin system H-6 to H-10 was unambiguously established from the COSY spectrum where H-6 showed a homoallylic coupling with H-15 and a large vicinal coupling (8.2 Hz) to H-7.

active derivative **1**. In contrast, methylation of the hydroxyl group as in **2** results in a loss of insecticidal activity.

Experimental Section

General Experimental Procedures. ^{1}H-NMR and ^{13}C-NMR spectra (chemical shifts in ppm) were recorded on Bruker WM 400 NMR and AVANCE DMX 600 NMR spectrometers, respectively. Mass spectra (EIMS) were measured on a Finnigan MAT 8430 mass spectrometer. FABMS (glycerine as matrix) were measured on an AMD Intectra 402 mass spectrometer. Optical rotations were determined on a Perkin-Elmer 241 MC Polarimeter. UV spectra were recorded in MeOH. Percent purity of isolated compounds was analyzed by HPLC. For HPLC analysis, samples were injected into a HPLC system (Pharmacia, LKB, Sweden) coupled to a photodiode-array detector (Waters Millipore GmbH, Eschborn, Germany). Routine detection was at 254 nm. The separation column (125 × 4 mm, i.d.) was prefilled with Nova-Pak C-18 (Waters Millipore GmbH, Eschborn, Germany).

Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC was performed on precoated TLC plates with Si gel 60 F_{254} (Merck, Darmstadt, Germany). The compounds were detected from their UV absorbance at 254 and 366 nm and by spraying the TLC plates with anisaldehyde reagent.

Animal Material. Specimens of *N. chabrolii* were collected by snorkelling off the shores of Sinyaru Island of West Sumatra, Indonesia, in April 1994. The samples were immersed in MeOH immediately after collection and were transported to the University of Würzburg, Germany. A voucher fragment is kept in 70% MeOH under the voucher no. DH16 in the Nationaal Natuurhistorisch Museum, Leiden.

Extraction and Isolation. The samples of *N. chabrolii* (ca. 1 kg, wet wt) were extracted successively with Me_2CO and MeOH (300 mL × 2 for each). The total extract was evaporated under reduced pressure and was partitioned between EtOAc (50 mL × 5) and H_2O (50 mL). The organic fraction was taken to dryness (5 g) and chromatographed over a Si gel column (mobile phase hexane−EtOAc, 70:30), and three major fractions were obtained. The non-polar fraction (fraction 1) and the semi-polar fraction (fraction 2) were separated from chlorophyll on a Sephadex column (LH-20) using Me_2CO. The fractions were taken to dryness and further purified by column chromatography on Sephadex (LH-20) and on RP-18 Lobar (Merck, Darmstadt, Germany). Fraction 1 afforded **3** (198 mg, 0.04%) and was obtained after a series of chromatographic separations on a Sephadex column (MeOH−CH_2Cl_2, 1:1) and on RP-18 Lobar (MeOH−H_2O, 90:10). Fraction 2 yielded **2** (5 mg, 0.001%) and was purified by column chromatography on RP-18 Lobar (MeOH−H_2O, 80:10). The third fraction, a polar fraction, yielded **1** (18 mg, 0.004%). The identity of the fractions was confirmed by HPLC and UV spectra recorded online.

Hydroxycolorenone (1): colorless oil; purity 97%; UV λ_{max} (MeOH) 248 (ϵ 17 000); $[\alpha]_D$ +58.6° (*c* 0.46, $CHCl_3$); ($C_{15}H_{24}O_2$) FABMS *m/z* $[M + H]^+$ 237; EIMS (70 eV) *m/z* $[M - 18]^+$ 218 (100), 203 (11), 178 (35), 163 (37), 149 (50), 110 (40), 91 (21), 73 (33), 59 (66), 43 (28).

Methoxycolorenone (2): colorless oil; purity 95%; UV λ_{max} (MeOH) 250 (ϵ 15 000); $[\alpha]_D$ +57.4° (*c* 0.31, $CHCl_3$); ($C_{16}H_{26}O_2$) FABMS *m/z* $[M + H]^+$ 251; EIMS (70 eV) *m/z* $[M - 32]^+$ 218 (14), 178 (15), 73 (100).

Experiments with Insects. Larvae of *S. littoralis* were from a laboratory colony reared on artificial diet under controlled conditions as described previously.[11] Feeding studies were conducted with neonate larvae ($n = 20$) kept on an artificial diet that had been treated with various concentrations of the compounds under study. After 6 days, survival of the larvae and weight of the surviving larvae were protocolled and compared to controls. ED_{50}s were calculated from the dose−response curves by probit analysis.

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References and Notes

(1) Vanderah, D. J.; Rutledge, N.; Schmitz, F. J.; Ciereszko, L. S. *J. Org. Chem.* **1978**, *43*, 1641−1616.
(2) Coll, J. C.; Bowden, B. F.; Tapiolas, D. M.; Willis, R. H.; Djura, P.; Streamer, M.; Trott, L. *Tetrahedron* **1985**, *41*, 1085−1092.
(3) Poet, S. V.; Ravi, B. N. *Aust. J. Chem.* **1982**, *35*, 77−83.
(4) Kitagawa, I.; Cui, Z.; Son, B. W.; Kobayashi, M.; Kyoboku, Y. *Chem. Pharm. Bull.* **1987**, *35*, 124−135.
(5) Blackman, A. J.; Bowden, B. F.; Coll, J. C.; Frick, B.; Mahendran, M.; Mitchell, S. J. *Aust. J. Chem.* **1982**, *35*, 1873−1880.
(6) Ahond, A.; Bowden, B. F.; Coll, J. C.; Fourneron, J.; Mitchell, S. J. *Aust. J. Chem.* **1981**, *34*, 2657−2664.
(7) Bowden, B. F.; Coll, J. C.; Mitchell, S. J. *Aust. J. Chem.* **1980**, *33*, 1833−1839.
(8) Matsuo, A.; Nakayama, M.; Sato, S.; Nakamoto, T.; Uto, S.; Hayashi, S. *Experientia* **1974**, *30*, 321−322.
(9) Asakawa, Y.; Toyota, M.; Takemoto, T.; Kubo, I.; Nakanishi, K. *Phytochemistry* **1992**, *19*, 2147−2154.
(10) Wu, C.; Chen, C. *Phytochemistry* **1992**, *31*, 4213−4217.
(11) Srivastava, R. P.; Proksch, P. *Entomol. Gener.* **1991**, *15*, 265−274.

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