Three New Nardosinane Type Sesquiterpenes from an Indonesian Soft Coral *Nephthea* sp.[1)]

Magie Melanie KAPOJOS,[a,b] Remy Emile Petrus MANGINDAAN,[c] Takahiro NAKAZAWA,[b] Taiko ODA,[d] Kazuyo UKAI,[b] and Michio NAMIKOSHI*,[b]

[a] *Department of Ocean Sciences, Tokyo University of Marine Science and Technology; Minato-ku, Tokyo 108–8477, Japan:* [b] *Tohoku Pharmaceutical University; Aoba-ku, Sendai 981–8558, Japan:* [c] *Faculty of Fisheries and Marine Science, Sam Ratulangi University; Kampus Bahu, Manado 95115, Indonesia: and* [d] *Kyoritsu University of Pharmacy; Minato-ku, Tokyo 105–8512, Japan.* Received November 13, 2007; accepted December 11, 2007; published online December 12, 2007

Three new sesquiterpenes, 2-deoxy-7-*O*-methyllemnacarnol (1), 2-deoxy-12α-ethoxy-7-*O*-methyllemnacarnol (2), and 2-deoxy-12α-methoxy-7-*O*-methyllemnacarnol (3), were isolated from a soft coral *Nephthea* sp. collected in Indonesia, together with five known sesquiterpenes. The structures of the new compounds were assigned on the basis of their spectroscopic data.

Key words *Nephthea* sp.; lemnacarnol derivative; sesquiterpene; soft coral

In the course of our ongoing studies on bioactive metabolites from marine organisms of North Sulawesi, we found that the MeOH extract of a soft coral *Nephthea* sp. inhibited the colony formation of Chinese hamster V79 cells (100% inhibition at 50 μg/ml). This bioassay reflects the direct action of compounds on the cells.[2)] Bioassay-guided isolation gave three new compounds (**1**—**3**), together with the known compounds **4**—**8** (Fig. 1). We describe herein the isolation and structure elucidation of three new compounds (**1**—**3**).

The MeOH extract of *Nephthea* sp. was extracted successively with *n*-hexane, EtOAc, and *n*-BuOH. Compounds **1**—**4**, **6**, and **7** were isolated from the hexane extract and **5** and **8** from the EtOAc extract. The structures of the five known compounds (**4**—**8**) were assigned by comparing their spectroscopic data with those of the reported values for 7-epilemnalactone (**4**),[3,4)] 2-deoxy-12-oxolemnacarnol (**5**),[5,6)] **6**,[3,5)] 4-acetoxy-2,8-neolemnadiene-5-one (**7**),[7)] and 11,12-dihydroxy-6,10-eremophiladiene (**8**).[8)]

Compound **1** was obtained as a colorless oil and gave the $[M]^+$ ion at *m*/*z* 250 in the EI-MS. The molecular formula ($C_{16}H_{26}O_2$) was deduced from HR-EI-MS and NMR data (Table 1) for **1**. ^{1}H- and ^{13}C-NMR spectra of **1** were very similar to those of 2-deoxylemnacarnol,[3,5)] except that methoxy signals were observed at δ_H 3.28 and δ_C 48.6 in the spectra of **1**. HMBC correlation was detected from δ_H 3.28 (H_3-16) to δ_C 110.7 (C-7) in the HMBC spectrum of **1**. Therefore, **1** was assigned as a 7-*O*-methyl derivative of 2-deoxylemnacarnol.

NOE correlations were observed between H-6/H_3-16, H-6/H_3-13, H-6/H_3-14, H_3-13/H_3-15, and H_3-14/H_3-15 in the NOESY spectrum of **1** (Fig. 2). The relative stereochemistry of **1** was the same as that of 2-deoxylemnacarnol.[3,5)] Although reliable specific rotation of **1** was not obtained because the compound was decomposed after the measurement of NMR spectra, **1** may have an identical absolute configuration as 2-deoxylemnacarnol since the specific rotations of **4**—**8** were identical to those of the reported values. Thus, the structure of **1** was determined as 2-deoxy-7-*O*-methyllemnacarnol (Fig. 1).

Compound **2** was isolated as a colorless oil. The molecular weight (294 Da) and formula ($C_{18}H_{30}O_3$) of **2** were deter-

Table 1. ^{13}C- (100 MHz) and ^{1}H-NMR (400 MHz) Data for **1** in $CDCl_3$

C#	**1**		
	^{13}C	^{1}H (*J* in Hz)	HMBC
1	122.5	5.45, br s	2, 3, 5
2	25.4	2.95, m	1, 3, 4, 10
3	26.7	1.41, m	1, 2, 4, 5, 15
4	34.8	1.70, m	2, 3, 5, 6, 10, 14, 15
5	40.2	—	
6	57.8	1.82, m	4, 5, 7, 8, 10, 11, 12, 13, 14
7	110.7	—	
8	29.7	1.80, m	6, 7, 9, 10
		1.85, m	6, 7, 9, 10
9	26.7	2.18, m	1, 7, 8, 10
		2.33, m	1, 7, 8, 10
10	139.3	—	
11	37.2	1.84, m	5, 6, 7, 12, 13
12	72.6	3.37, dd (8.7, 5.8)	7, 11, 13
		3.84, dd (8.7, 7.2)	6, 7, 11
13	18.0	1.04, d (7.7)	6, 11, 12
14	21.7	1.05, s	4, 5, 6, 10
15	16.4	0.84, d (6.8)	3, 4, 5
16	48.6	3.28, s	7

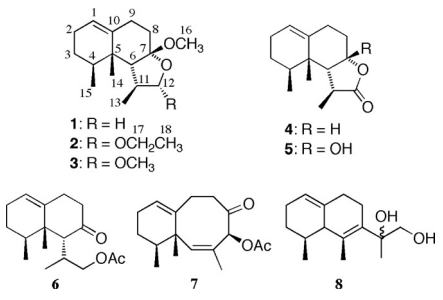

Fig. 1. Structures of Compounds **1**—**8** Isolated from *Nephthea* sp.

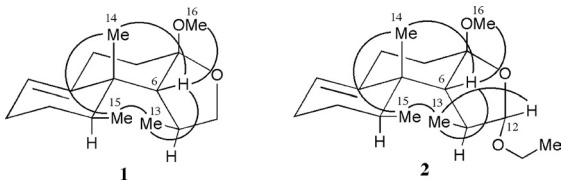

Fig. 2. Key NOESY Data for Compounds **1** and **2**

* To whom correspondence should be addressed. e-mail: mnami@tohoku-pharm.ac.jp.

Table 2. ^{13}C- (100 MHz) and ^{1}H-NMR (400 MHz) Data for **2** in $CDCl_3$

C#	**2**		
	^{13}C	^{1}H (J in Hz)	HMBC
1	123.0	5.46, br d (4.9)	2, 3, 5, 9
2	25.6	1.86, m	1, 3, 4, 10
		1.91, m	1, 3, 4, 10
3	26.9	1.40, m	1, 2, 4, 5, 15
4	35.2	1.66, m	2, 3, 5, 6, 14, 15
5	40.3	—	
6	54.1	1.95, d (10.2)	4, 5, 7, 8, 10, 11, 12, 13, 14
7	109.0	—	
8	33.2	1.89, m	6, 7, 9, 10
		1.91, m	6, 7, 9, 10
9	28.1	2.05, m	1, 7, 8, 10
		2.35, m	1, 7, 8, 10
10	139.4	—	
11	42.2	1.85, m	5, 6, 7, 12, 13
12	108.7	4.59, d (4.9)	6, 7, 11, 13, 17
13	19.5	1.16, d (6.8)	6, 11, 12
14	20.3	1.03, s	4, 5, 6, 10
15	16.5	0.81, d (6.3)	3, 4, 5
16	48.9	3.31, s	7
17	64.3	3.43, dq (14.1, 6.8)	12, 18
		3.76, dq (14.1, 6.8)	12, 18
18	15.3	1.18, t (6.8)	17

Table 3. ^{13}C- (100 MHz) and ^{1}H-NMR (400 MHz) Data for **3** in $CDCl_3$

C#	**3**		
	^{13}C	^{1}H (J in Hz)	HMBC
1	123.1	5.46, br d (4.9)	2, 3, 5, 9
2	25.6	1.98, m	1, 3, 4, 10
		2.34, m	1, 3, 4, 10
3	26.8	1.40, m	1, 2, 4, 5, 15
4	35.2	1.65, m	2, 3, 5, 6, 14, 15
5	40.4	—	
6	54.0	1.96, d (10.2)	4, 5, 7, 8, 10, 11, 12, 13, 14
7	109.2	—	
8	33.4	2.90, m	6, 7, 9, 10
		2.92, m	6, 7, 9, 10
9	28.2	2.04, m	1, 7, 8, 10
		2.34, m	1, 7, 8, 10
10	139.4	—	
11	42.2	1.84, m	5, 6, 7, 12
12	110.1	4.51, d (4.9)	7, 11, 13, 17
13	19.7	1.18, d (6.8)	6, 11, 12
14	20.2	1.05, s	4, 5, 6, 10
15	16.5	0.83, d (6.3)	3, 4, 5
16	48.9	3.33, s	7
17	56.2	3.36, s	12

mined from HR-EI-MS and NMR data (Table 2). ^{1}H- and ^{13}C-NMR data for **2** resembled those for **1**, but the signals ascribed to H-12 (d 4.59) and C-12 (d 108.7) were observed at a lower field than H_2-12 (δ 3.37, 3.84) and C-12 (δ 72.6) of **1**. These data suggested that **2** was an acetal derivative of **1** at the 12 position. The ^{1}H-NMR spectrum of **2** revealed the presence of an O–CH_2–CH_3 group by signals at δ 3.43 (H-17a, dq, J=6.8, 14.1 Hz), 3.76 (H-17b, dq, J=6.8, 14.1 Hz), and 1.18 (H_3-18, t, J=6.8 Hz), which corresponded to the difference in the molecular weight and formula between **2** and **1** (44 Da, C_2H_5O). Since HMBC correlations were observed from H_2-17 (δ 3.43, 3.76) to C-12 (δ 108.7) and from H-12 (δ 4.59) to C-17 (δ 64.3), the OEt group was attached to the 12 position. The key NOEs were detected as shown in Fig. 2 in the NOESY spectrum of **2**. Although reasonable specific rotation could not be obtained because the compound was decomposed after NMR measurement, the absolute stereochemistry of **2** may be identical to those of **1** and 2-deoxylemnacarnol. Consequently, the structure of **2** was identified as 2-deoxy-12α-ethoxy-7-*O*-methyllemnacarnol (Fig. 1).

Compound **3** was purified as a colorless oil. HR-EI-MS and NMR data (Table 3) for **3** revealed the molecular mass (280 Da) and formula ($C_{17}H_{28}O_3$) of **3**. ^{1}H- and ^{13}C-NMR spectra of **3** were very similar to those of **2**, except for OMe signals (δ_H 3.36, δ_C 56.2) in the spectra of **3** instead of OEt signals in those of **2**. HMBC correlations were detected from H_3-17 (δ 3.36) to C-12 (δ 110.1) and *vice versa*. Key NOEs were observed between the identical ^{1}H signals to those of **2**. Therefore, the structure of **3** was elucidated as 2-deoxy-12α-methoxy-7-*O*-methyllemnacarnol (Fig. 1).

Bioactivity of compounds **1**—**3** could not be examined because the compounds decomposed during recovery from NMR tubes. The colony formation of V79 cells was inhibited by **8** with the EC_{50} value of 7.0 μM, but **4**—**7** were not active against V79 cells (EC_{50} >20 μM). Compound **8** was isolated as a cytotoxic component of the Micronesian soft coral *Lemnalia africana* and showed growth inhibitory activity against P388 at 1 μg/ml (4.2 μM) and CV-1 at 10 μg/ml (42.4 μM).[7)]

Experimental

General Procedures Optical rotations were measured on a JASCO DIP-370 Digital Polarimeter. UV and IR spectra were recorded on a Hitachi U-3310 spectrophotometer and a Perkin-Elmer Spectrum One FT-IR spectrometer, respectively. NMR spectra were measured on a JEOL AL 400 NMR spectrometer. Mass spectra were obtained by a JEOL JMS-MS 700 mass spectrometer (EI or FAB mode with *m*-nitrobenzyl alcohol or glycerol as the matrix).

Soft Coral *Nephthea* sp. was collected by scuba diving at a coral reef in Manado, Indonesia. The voucher specimen is deposited at the Faculty of Fisheries and Marine Science, Sam Ratulangi University as SC1.

Extraction and Isolation The soft coral was cut into small pieces and extracted three times with MeOH. The MeOH extract was evaporated, and 10 g of the residue was dissolved in MeOH–H_2O (9 : 1, 200 ml) and extracted with *n*-hexane. The lower layer was evaporated to remove MeOH, diluted with water, and extracted successively with EtOAc and *n*-BuOH. Hexane and EtOAc extracts showed 100% inhibition against the colony formation of V79 cells at 50 μg/ml. The hexane extract (1.5 g) was separated by a SiO_2 column with *n*-hexane–EtOAc (gradient elution) into four fractions. Fractions 1 and 2 showed bioactivity against V79 cells (100% at 50 μg/ml) and were subjected to HPLC (ODS, MeOH–H_2O=4 : 1) to give compounds **1** (3.2 mg), **2** (20 mg), and **3** (4.4 mg) from fraction 1 and **4** (38 mg), **6** (10 mg), and **7** (15 mg) from fraction 2. Compounds **5** (3.9 g) and **8** (4.2 mg) were isolated from the EtOAc extract (0.4 g) by SiO_2 column chromatography (*n*-hexane–EtOAc, gradient elution) followed by HPLC (ODS, MeOH–H_2O=3 : 1).

2-Deoxy-7-*O*-methyllemnacarnol (**1**): ^{1}H- and ^{13}C-NMR data are listed in Table 1. IR (KBr) cm^{-1}: 2926, 1540, 1459, 1381, 1259, 1099. UV λ_{max} ($CHCl_3$) nm (log ε): 241 (2.56). EI-MS *m*/*z*: 250 (M^+), 218, 203, 176, 161. HR-EI-MS *m*/*z*: 250.1941 (Calcd for $C_{16}H_{26}O_2$: 250.1933).

2-Deoxy-12α-ethoxy-7-*O*-methyllemnacarnol (**2**): ^{1}H- and ^{13}C-NMR data are listed in Table 2. IR (KBr) cm^{-1}: 2965, 2929, 1456, 1381, 1310, 1256, 1198, 1104, 1010, 978. UV λ_{max} ($CHCl_3$) nm (log ε): 248 (2.64). EI-MS *m*/*z*: 294 (M^+), 280, 262, 248, 220, 190, 175, 148. HR-EI-MS *m*/*z*: 294.2200 (Calcd for $C_{18}H_{30}O_3$: 294.2195).

2-Deoxy-12α-methoxy-7-*O*-methyllemnacarnol (**3**): ^{1}H- and ^{13}C-NMR data are listed in Table 3. IR (KBr) cm^{-1}: 2956, 2929, 1684, 1663, 1455, 1381, 1358, 1305, 1254, 1198, 1104, 1023, 1006, 976. UV λ_{max} ($CHCl_3$) nm (log ε): 241 (2.45). EI-MS *m*/*z*: 280 (M^+), 248, 233, 206, 190, 175, 148. HR-EI-MS *m*/*z*: 280.2033 (Calcd for $C_{17}H_{28}O_3$: 280.2039).

Bioassay Chinese hamster V79 cells were grown as a monolayer in

Eagle's Minimum Essential Medium (MEM) (Nissui Seiyaku Co., Ltd., Tokyo, Japan) with 10% heat-inactivated FBS. Two hundred cells were seeded onto a 60/15 mm Petri dish in 4 ml MEM with 10% FBS and incubated overnight at 37 °C. Samples were dissolved in DMSO, and 4 μl of each sample were added to the dish, and incubated for another 4 d. The numbers of colonies in the sample dishes were counted and compared with those in the control dishes. The relative plating efficiency of the sample against V79 cells at 50 μg/ml was described as the ratio of the number of colonies in the sample dish to that in the control culture.[9,10]

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas 17035029 from The Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan to M. N. We thank Ms. A. Fujita for technical assistance.

References and Notes

1) Dedicated to the late Dr. Shigeo Iwasaki.
2) Oda T., Kamoshita K., Maruyama S., Masuda K., Nishimoto M., Xu J., Ukai K., Mangindaan R. E. P., Namikoshi M., *Biol. Pharm. Bull.*, **30**, 385—387 (2007).
3) Ahond A., Chiaroni A., Coll J. C., Fourneron J. D., Riche C., Braekman J. C., Daloze D., Tursch B., Dunstan P. J., *Bull. Soc. Chim. Belg.*, **88**, 313—324 (1979).
4) Ahond A., Coll J. C., Fourneron J. D., *Tetrahedron Lett.*, **20**, 1879—1880 (1979).
5) Daloze D., Braekman J. C., Georget P., Tursch B., *Bull. Soc. Chim. Belg.*, **86**, 47—54 (1977).
6) Bowden B. F., Coll J. C., Mitchell S. J., *Aust. J. Chem.*, **33**, 885—890 (1980).
7) Jurek J., Scheuer P. J., *J. Nat. Prod.*, **56**, 508—513 (1993).
8) Izac R. R., Fenical W., Tagle B., Clardy J., *Tetrahedron*, **37**, 2569—2573 (1981).
9) Sakakibara Y., Saito I., Ichinoseki K., Oda T., Kaneko M., Saito H., Kodama M., Sato Y., *Mutat. Res.*, **263**, 269—276 (1991).
10) Sato Y., Sakakibara Y., Oda T., Aizu-Yokota E., Ichinoseki I., *Chem. Pharm. Bull.*, **40**, 182—184 (1992).