Four Novel Diterpenoids, Including Nakamurol A with a Unique Thelepogane Skeleton, from the Marine Sponge *Agelas nakamurai*

Noboru Shoji, Akemi Umeyama,* Miyuki Teranaka, and Shigenobu Arihara

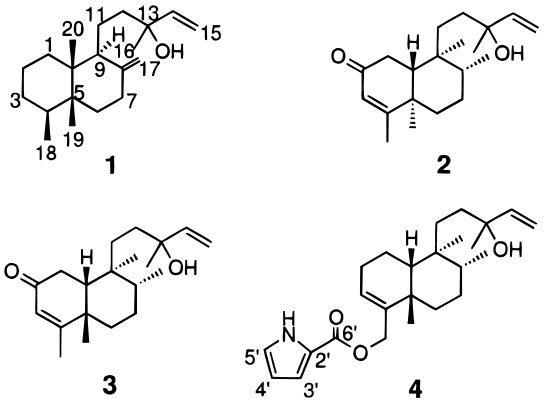
Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770, Japan

Received November 15, 1995[⊗]

Nakamurols A-D (**1**−**4**) have been isolated from the Okinawan sponge *Agelas nakamurai* Hoshino. The structures of **1**−**4** were proposed on the basis of extensive NMR experiments. Nakamurol A is the first non-alkaloid diterpene with the thelepogane skeleton.

Marine sponges are recognized as a prolific source of biologically active and structurally unique secondary metabolites.[1] In a continuation of our survey of marine organisms for pharmacologically active substances, we have isolated four novel diterpenoids from *Agelas nakamurai* Hoshino (order Agelasida, family Agelasidae), collected from Okinawa Island. We report herein on the structure elucidation of these compounds.

The lyophilized sponge was extracted with EtOAc, and the viscous residue, after evaporation of the solvent, was chromatographed on Sephadex LH-20 and Si gel columns. Final purification by reversed-phase HPLC afforded four novel diterpenoids, nakamurols A (**1**), B (**2**), C (**3**) and D (**4**), of which the first (**1**) is the major and unique metabolite.



Nakamurol A (**1**), $[\alpha]_D$ +39.1° (*c* 1.6, $CHCl_3$), was obtained as a colorless oil. The molecular formula of **1** was established as $C_{20}H_{34}O$ on the basis of HREIMS, and corresponds to four degrees of unsaturation. In the IR spectrum, a hydroxy group (3300-3500 cm^{-1}) was observed. The ^{13}C-NMR spectrum in $CDCl_3$ indicated the presence of two double bonds [δ 149.5 (s), 145.3 (d), 111.5 (t), 106.5 (t)] and one carbon bearing a hydroxy group [δ 73.6 (s)]. The ^{1}H-NMR spectrum contained three methyl singlets (δ 1.28, 0.73, 0.63), one methyl doublet [δ 0.79 (d, J = 6.6 Hz)] and five olefinic protons [δ 5.92 (dd, J = 17.6, 11.0 Hz), 5.21 (d, J = 17.6 Hz), 5.05 (d, J = 11.0 Hz), 4.84 (s), 4.51 (s)]. These data suggested **1** was a bicyclic diterpene with a labdane, clerodane, or related carbon skeleton. The ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY experiments implied the partial structures a (CH_2CHCH_3: from C-3 to C-18), b (CH_2-CH_2: from C-6 to C-7), c ($CHCH_2CH_2$: from C-9 to C-12), and d ($CH{=}CH_2$: from C-14 to C-15). The HMBC experiment revealed long-range couplings from the C-18 methyl protons to C-5; from the C-19 methyl protons to C-6 and C-10; from the C-20 methyl protons to C-1, C-5, C-9, and C-10; from the C-1 methylene protons to C-3; and from the C-2 methylene protons to C-4. This suggested a linkage between partial structures a and b. Additional HMBC correlations between H-7 and C-8, H-7 and C-17, H-17 and C-9, and H-11 and C-8 established the connectivity between partial structures b and c. Furthermore, the HMBC spectrum showed couplings between H-16 and C-12, C-13, and C-14. Thus, the planar structure of **1** was determined. The relative stereochemistry of nakamurol A was established by NOESY experiments (Figure 1). The NOEs between H-19/H-18 and H-19/H-20 established the cis A/B ring junction and all β-methyl groups. Furthermore, NOEs were detected between H-4/H-7α and H-4/H-9, confirming the α-orientation of H-9. Thus, the structure of nakamurol A was suggested to be **1** with a thelepogane skeleton. The isolation of thelepogine from the grass *Thelepogon elegansas* was reported by Crow in 1962;[2] this is the only diterpenoid alkaloid with the thelepogane skeleton that has been isolated from natural sources. To the best of our knowledge, **1** is the first non-alkaloid diterpene with the thelepogane skeleton.

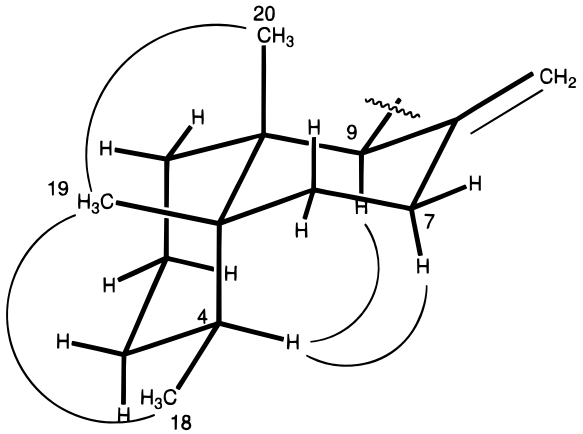


Figure 1. NOE correlations for **1**.

Nakamurol B (**2**) was isolated as a colorless oil. The molecular formula of **2** was established as $C_{20}H_{32}O_2$ on the basis of HREIMS. The IR spectrum suggested that **2** possessed a hydroxy group (3300−3500 cm^{-1}) and an α,β-unsaturated carbonyl group (1655 cm^{-1}). The ^{13}C-NMR spectrum indicated the presence of one carbonyl

* To whom correspondence, should be addressed. Phone: 0886-22-9611. FAX: 0886-55-3051.

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1996.

Table 1. ^{13}C-NMR Data of Nakamurol A-D (**1**−**4**) in $CDCl_3$

	1		**2**		**3**		**4**	
position	δ^a	m^b	δ	m	δ	m	δ	m
1	31.9	t	34.8	t	35.4	t	17.2	t
2	21.4	t	200.6	s	199.2	s	24.0	q
3	31.1	t	125.5	d	128.5	d	129.5	d
4	30.4	d	172.6	s	168.5	s	138.4	s
5	39.5	s	39.8	s	39.3	s	36.3	s
6	33.9	t	35.6	s	36.9	s	37.2	d
7	33.1	t	26.9	t	28.3	t	28.8	t
8	149.5	s	35.9	s	35.1	s	37.4	s
9	43.2	d	38.3	s	39.9	s	39.8	s
10	42.4	s	45.6	d	47.1	d	45.0	d
11	18.2	t	31.2	t	30.4	t	31.5	t
12	41.7	t	34.8	t	35.1	t	35.0	d
13	73.6	s	73.1	s	73.2	s	73.5	s
14	145.3	d	144.9	d	144.7	d	145.2	d
15	111.5	t	112.0	t	112.3	t	111.9	t
16	27.6	q	27.8	q	27.8	q	27.6	q
17	106.5	t	15.7	q	15.9	q	15.8	q
18	16.3	q	18.9	q	20.6	q	66.5	t
19	16.4	q	18.3	q	32.1	q	34.7	q
20	18.3	q	18.0	q	19.3	q	17.4	q
2′							123.0	s
3′							115.2	d
4′							110.4	d
5′							122.9	d
6′							161.2	s

^{a} δ values are recorded in ppm. ^{b} Multiplicity is given in DEPT.

group [δ 200.6 (s)], two double bonds [δ 172.6 (s), 144.9 (d), 125.5 (d), 112.0 (t)], and one carbon bearing a hydroxy group [δ 73.1 (s)]. The ^{1}H-NMR spectrum contained four methyl singlets (δ 1.88, 1.26, 1.10, 0.80), one methyl doublet [δ 0.81 (d, J = 5.9 Hz)], and four olefinic protons [δ 5.85 (dd, 1H, J = 16.6, 10.3 Hz); 5.71 (s, 1H); 5.20 (d, 1H, J = 16.6 Hz); 5.06 (d, 1H, J = 10.3 Hz)]. The ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY experiments established the following proton connectivities: H-1/H-10, H-6/H-7, H-7/H-8, H-8/H-17, H-11/H-12, and H-14/H-15. The connectivities around quaternary carbons established by a HMBC experiment (Experimental Section) led to the planar structure of **2**. The relative stereochemistry of nakamurol B was established by NOESY experiments. The NOEs between H-1α/H-19, H-1α/H-20, H-7α/H-19, and H-8/H-10 afforded a *trans*-clerodane.

Nakamurol C (**3**), a colorless oil, had the same molecular formula of $C_{20}H_{32}O_2$ as that of **2**. The ^{1}H-, ^{13}C-NMR, and IR spectra of **3** were sufficiently similar to those of **2**, suggesting that the molecules were stereoisomers. This assumption was confirmed as follows. The ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY experiments established the same proton connectivities as those of **2**. The HMBC correlations (experimental section) led to the planar structure of **3**. The stereochemistry of **3** was determined as follows. The NOEs between H-20/H-1α, H-20/H-3, H-20/H-17, H-19/H-1β, and H-19/H-10 established the cis-ring junction. The downfield shifts (about 14 ppm) of the C-19 methyl group in the ^{13}C-NMR spectrum also confirmed a *cis*-clerodane.[3]

Nakamurol D (**4**) was isolated as a pale yellow oil. The molecular formula of **4** was determined as $C_{25}H_{37}O_3N$ on the basis of HREIMS. The IR spectrum suggested that **4** possessed hydroxy (3300−3500 cm^{-1}) and ester carbonyl (1684 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR spectra contained signals at δ 6.96 (brs, 1H), 6.91 (brs, 1H), and 6.26 (brs, 1H), and δ 161.2 (s), 123.0 (s), 122.9 (d), 115.2 (d), and 110.4 (d) assigned to the pyrrole-2-carboxylic acid protons and carbons, respectively. The diterpene portion of the molecule was defined as a clerodane by the ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY (H-1/H-2, H-1/H-10, H-2/H-3, H-6/H-7, H-8/H-17, H-14/-H15, H-3′/H-4′, H-4′/H-5′) and HMBC (Experimental Section) experiments. The cross peaks of C-6′/H-18 in a COLOC experiment allowed connection between the diterpene portion and pyrrole ring. The stereochemistry of **4** was established by difference NOE experiments. The NOEs between H-20/H-2α, H-10/H-6β, H-7α/H-18, and H-19/H-10 confirmed a *cis*-clerodane skeleton. Thus, the structure of nakamurol D was deduced to be **4**. The absolute stereochemistry represented in the structure diagrams are arbitrary.

The coexistence of thelepogane and clerodane diterpenoids is interesting from a biosynthetic point of view. The pharmacological study of **1**−**4** is in progress.

Experimental Section

General Experimental Procedures. The following instruments were used: JASCO FT-IR-5300 (IR), JASCO DIP-360 polarimeter (optical rotation), JEOL JMS-HX-100 mass spectrometer (HRMS), and JEOL JNM-GX-400FT NMR or Varian UNITY 600 spectrometer (^{1}H and ^{13}C NMR).

Sponge Material. A specimen of *Agelas nakamurai* Hoshino was collected by netting at a depth of 40−70 m eff Okinawa Island. It was kept frozen (−20 °C) until used. The voucher specimen (US020) is deposited in the Herbarium of the Department of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation of Metabolites. The frozen sample of *A. nakamurai* (500 g, wet wt) was lyophilized and exhaustively extracted with MeOH/toluene (3:1) (2 L × 4) at room temperature for 1 day. The extract was concentrated, and the resulting residue was extracted with EtOAc (500 mL × 3). The EtOAc-soluble portion (4.8 g) was repeatedly subjected to Si gel flash column chromatography (using increasing concentrations of MeOH in $CHCl_3$ as eluent), followed by Sephadex LH-20 column chromatography ($CHCl_3$-MeOH 1:1) and reversed-phase HPLC (60−80% MeOH) to give **1** (33 mg, 0.0066% net wt), **2** (17 mg, 0.0034%), **3** (7 mg, 0.0014%), and **4** (24 mg, 0.0048%).

Nakamurol A (1): Colorless oil; $[\alpha]^{25}_D$ +39.1° (*c* 1.6, $CHCl_3$); FT-IR (film) 3426 (br), 3083, 2965, 2928, 2359, 1644, 1449 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.63 (s, 3H, Me-20), 0.73 (s, 3H, Me-19), 0.79 (d, 3H, J = 6.6 Hz, Me-18), 1.28 (s, 3H, Me-16), 1.29 (m, 1H, H-12), 1.34 (m, 1H, H-3), 1.35 (m, 1H, H-11), 1.36 (m, 1H, H-6), 1.42 (m, 3H, H-1, H-2), 1.48 (m, 2H, H-3, H-11), 1.53 (m, 1H, H-1), 1.59 (ddd, 1H, J = 14.5, 5.0, 2.5 Hz, H-6), 1.82 (ddd, 1H, J = 13.5, 13.5, 4.8 Hz, H-12), 2.05 (ddd, 1H, J = 14.5, 5.0, 2.5 Hz, H-7), 2.18 (ddd, 1H, J = 14.5, 14.5, 5.0 Hz, H-7), 2.33 (m, 1H, H-4), 2.56 (brd, 1H, J = 11.0 Hz, H-9), 4.51 (s, 1H, H-17), 4.84 (s, 1H, H-17), 5.05 (d, 1H, J = 11.0 Hz, H-15), 5.21 (d, 1H, J = 17.6 Hz, H-15), 5.92 (dd, 1H, J = 17.6, 11.0 Hz, H-14); HREIMS, obsd *m*/*z* 290.2604, $C_{20}H_{34}O$ calcd *m*/*z* 290.2609; HMBC (H/C) 1/3, 2/4, 6/5, 6/7, 6/8, 6/10, 6/19, 7/5, 7/6, 7/8, 7/9, 7/17, 9/8, 9/10, 9/17, 9/20, 11/8, 12/11, 12/13, 12/14, 14/13, 15/13, 15/14, 16/12, 16/13, 16/14, 17/7, 17/9, 18/3, 18/4, 18/5, 19/5, 19/6, 19/10, 20/1, 20/5, 20/9, 20/10.

Nakamurol B (2): Colorless oil; $[\alpha]^{25}_D$ −12.8° (*c* 0.8, $CHCl_3$); CD $\Delta\epsilon$ +0.38 (245 nm) (*c* 0.21, MeOH); UV λ max (MeOH) 238.5 nm (log ϵ 3.93); FT-IR (film) 3420

(br), 2965, 2874, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (s, 3H, Me-20), 0.81 (d, 3H, J = 5.9 Hz, Me-17), 1.10 (s, 3H, Me-19), 1.26 (s, 3H, Me-16), 1.28 (m, 1H, H-12), 1.32 (m, 2H, H-11), 1.34 (m, 1H, H-6), 1.36 (m, 1H, H-12), 1.46 (m, 1H, H-8), 1.48 (m, 2H, H-7), 1.81 (m, 1H, H-6), 1.84 (m, 1H, H-10), 1.88 (d, 1H, J = 1.5 Hz, Me-18), 2.34 (d, 2H, J = 9.5 Hz, H-1), 5.06 (dd, 1H, J = 10.3, 1.5 Hz, H-15), 5.20 (dd, 1H, J = 16.6, 1.5 Hz, H-15), 5.71 (s, 1H, H-3), 5.85 (dd, 1H, J = 16.6, 10.3 Hz, H-14); HREIMS, obsd m/z 304.2369, $C_{20}H_{32}O_2$ calcd m/z 304.2403; HMBC (H/C) 1/2, 1/5, 1/10, 10/1, 10/2, 10/5, 14/13, 15/13, 16/12, 16/13, 16/14, 17/7, 17/8, 17/9, 18/3, 18/4, 18/5, 19/4, 19/5, 19/6, 19/10, 20/8, 20/9, 20/10, 20/11.

Nakamurol C (3): Colorless oil; $[\alpha]^{25}_D$ −8.0° (*c* 0.4, $CHCl_3$); CD $\Delta\epsilon$ +0.064 (250 nm) (*c* 0.42, MeOH); UV λ max (MeOH) 246.5 nm (log ϵ 3.81); FT-IR (film) 3447 (br), 2963, 1651, 1435, cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.58 (s, 3H, Me-20), 0.77 (d, 3H, J = 6.6 Hz, Me-17), 1.22 (s, 3H, Me-19), 1.23 (m, 3H, H-6, H-7, H-11), 1.29 (s, 3H, Me-16), 1.31 (m, 1H, H-7), 1.38 (m, 1H, H-11), 1.40 (m, 1H, H-12), 1.46 (m, 1H, H-8), 1.49 (m, 1H, H-12), 1.84 (d, 1H, J = 6.6 Hz, H-10), 1.95 (s, 1H, Me-18), 2.09 (dd, 1H, J = 11.0, 4.0 Hz, H-6), 2.52 (d, 1H, J = 18.3 Hz, H-1), 2.70 (dd, 1H, J = 18.3, 6.6 Hz, H-1), 5.09 (d, 1H, J = 11.0 Hz, H-15), 5.21 (d, 1H, J = 17.6 Hz, H-15), 5.84 (s, 1H, H-3), 5.88 (dd, 1H, J = 17.6, 11.0 Hz, H-14); HREIMS, obsd m/z 304.2372, $C_{20}H_{32}O_2$ calcd m/z 304.2403; HMBC (H/C) 1/2, 1/5, 1/9, 1/10, 3/5, 3/18, 6/8, 8/9, 10/1, 10/2, 10/4, 10/5, 10/9, 10/19, 10/20, 12/13, 12/14, 14/13, 15/13, 15/14, 16/12, 16/13, 16/14, 17/7, 17/8, 17/9, 18/3, 18/4, 18/5, 19/4, 19/5, 19/6, 20/8, 20/9, 20/10, 20/11.

Nakamurol D (4): Pale yellow oil; $[\alpha]^{25}_D$ −8.5° (*c* 1.2, $CHCl_3$); FT-IR (film) 3500 (br), 3312, 3088, 2944, 2247, 1684, 1555 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.76 (d, 3H, J = 5.9 Hz, Me-17), 0.80 (s, 3H, Me-20), 1.15 (m, 1H, H-12), 1.16 (s, 3H, Me-19), 1.18 (m, 1H, H-6), 1.27 (m, 1H, H-11), 1.28 (m, 2H, H-7), 1.30 (s, 3H, Me-16), 1.36 (d, 1H, J = 7.0 Hz, H-1), 1.40 (m, 1H, H-12), 1.42 (m, 1H, H-8), 1.46 (m, 1H, H-11), 1.82 (m, 1H, H-1), 2.02 (m, 1H, H-1), 2.05 (m, 1H, H-6), 2.17 (m, 2H, H-2), 4.75 (d, 1H, J = 12.0 Hz, H-18), 4.81 (d, 1H, J = 12.0 Hz, H-18), 5.08 (d, 1H, J = 11.0 Hz, H-15), 5.22 (d, 1H, J = 16.9 Hz, H-15), 5.75 (brs, 1H, H-3), 5.91 (dd, 1H, J = 16.9, 11.0 Hz, H-14), 6.26 (brs, 1H, H-4′), 6.91 (brs, 1H, H-3′), 6.96 (brs, 1H, H-5′); HREIMS, obsd m/z 399.2780, $C_{25}H_{37}O_3N$ calcd m/z 399.2773; HMBC (H/C) 1/2, 1/3, 1/5, 1/9, 3/1, 3/18, 10/4, 11/8, 14/13, 15/13, 15/14, 16/12, 16/13, 16/14, 17/7, 17/8, 17/9, 18/3, 18/4, 19/4, 19/5, 19/6, 19/10, 20/8, 20/9, 20/10, 20/11.

Acknowledgment. We are grateful to Prof. P. R. Bergquist of Auckland University for her kind identification of the sponge. We thank Prof. J. Kobayashi of Hokkaido University and Mr. Z. Nagahama for help with collections. We are indebted to Ms. Y. Kan for measurements of NMR spectra and to Ms. I. Okamoto for measurements of mass spectra.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1995**, *12*, 223−269, and earlier reviews cited therein.
(2) Crow, W. D. *Aust. J. Chem.* **1962**, *15*, 159−161.
(3) Capon, R. J.; Faulkner, D. J. *J. Am. Chem. Soc.* **1984**, *106*, 1819−1822.

NP960110C