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New Cembranolide Diterpenes with a Dimethylamino Group from the Okinawan Soft Coral (*Sinularia* sp.)

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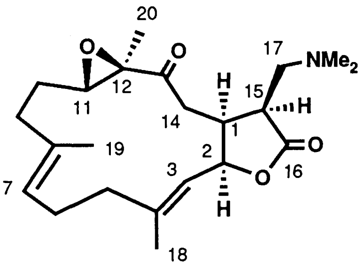
Three new cembranolide diterpenes each possessing a dimethylamino group were isolated from the Okinawan soft coral *Sinularia* sp. Their structures were determined based on data from spectroscopic analysis and chemical transformation.

Cembrane-type diterpenes from marine sources are of interest due to their structural features and biological activity.$^{1)}$ While studying bioactive substances from Okinawan marine invertebrates,$^{2)}$ three new cembranolide diterpenes, designated as sinulamine I (**1**), II (**2**), and III (**3**), were isolated from the soft coral (*Sinularia* sp.) along with known cembranolide diterpenes, **4**, **5**, and **6**. The structures of the new cembranolide diterpenes are characterized by a dimethylaminomethyl group at the α-position of the γ-butyrolactone moiety. This paper describes the structures of sinulamines as determined on the basis of spectroscopic and chemical data.

The methanol extract of specimens (wet weight 2.2 kg) of the soft coral (*Sinularia* sp.), collected on the coral reef of Ishigaki Island (Okinawa, Japan), was partitioned between ethyl acetate and water. The ethyl acetate soluble portion (26 g) was chromatographed on a silica gel column by elution with hexane-ethyl acetate, ethyl acetate and methanol in this order, to give three fractions. From fraction 2 (eluted with ethyl acetate), known cembranolide diterpenes, **4**,$^{3)}$ **5**,$^{3b,c,4)}$ and **6** (lobophytol acetate)$^{3b,5)}$ were isolated (2.8%, 2.1%, and 16% yields, respectively, based on the ethyl acetate soluble portion). From fraction 3 (eluted with methanol), sinulamine I (**1**), II (**2**), and III (**3**) were isolated in the order of increasing polarity (0.54%, 0.39% and 0.54% yields, respectively, based on the ethyl acetate soluble portion). Sinulamine I and III inhibited the proliferation of KB cells at concentration (IC_{50}) of 2.0 and 1.65 μg/ml, respectively.

Sinulamine I (**1**)$^{6)}$ was found to have a molecular formula, $C_{22}H_{33}NO_4$, based on a high resolution mass measurement. Its IR spectrum (KBr) showed absorptions at 1760 (γ-butyrolactone) and 1705 (ketone) cm^{-1}, which were confirmed to be present by ^{13}C signals at 177.3 and 208.7 ppm, respectively. A dimethylaminomethyl group was indicated by ^{1}H NMR [δ 2.19 (6H, s), 2.51 (1H, dd, *J* 8.4, 12.6 Hz), 2.67 (1H, dd, *J*



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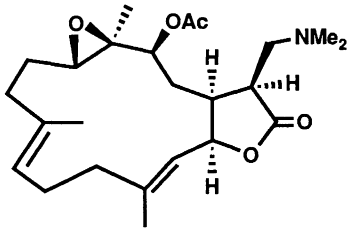

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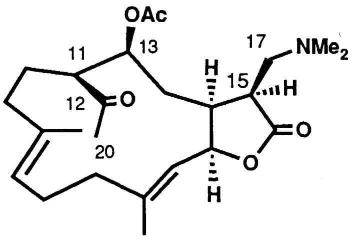

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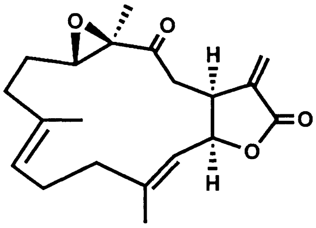

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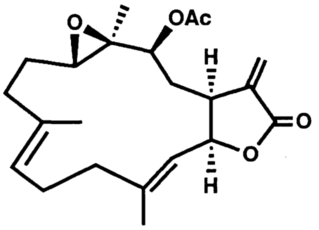

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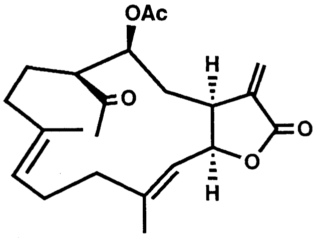

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4.4, 12.6 Hz)] (Table 1) and ^{13}C NMR [δ 45.9 (NMe_2), 59.9 ($\underline{C}H_2NMe_2$)] spectra. Signals due to two trisubstituted olefins ($CH_3C{=}CH$), a trisubstituted epoxide bearing a methyl group, five methylenes and three methines were also observed in ^{1}H and ^{13}C NMR spectra. A comparison of the ^{1}H NMR spectrum of **1** with that of **4** showed them to be quite similar, except for the signals at positions C-1, -15, and -17. The conjugated *exo*-olefin signals in **4** disappeared in **1** and those due to the dimethylaminomethyl group were newly observed in **1**, indicating it to possibly have a structure in which dimethylamine is added to the conjugated *exo*-olefin of **4**. This structure of **1** was confirmed by the following chemical reaction. Treatment of **4** with dimethylamine in methanol at 0 °C gave an adduct[7] in 92% yield. Physical properties of the adduct including the sign of optical rotation were identical with those of **4**, whose complete structure including the absolute stereochemistry has already been established.[3c] The structure of sinulamine I was thus concluded to be that of **1**, except for the stereochemistry of the chiral center at C-15.

The possibility of the β-configuration (*S* configuration) for the dimethylaminomethyl group at C-15 was strongly indicated by the absence of the diastereomeric isomer of **1** in the above Michael type addition of dimethylamine. In this reaction, protonation at C-15 in a possible intermediate (a) takes place from the less-hindered

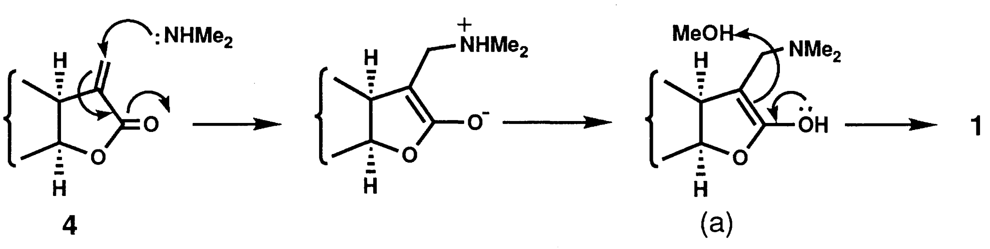

Table 1. ^1H NMR Data of Sinulamines[a)]

1	**2**	**3**
1.39 (3H, s, H-20)	1.14 (3H, s, H-20)	1.60 (3H, br s)
1.60 (3H, br s)	1.59 (3H, br s)	1.85 (3H, br s)
1.64 (3H, br s)	1.83 (3H, br s)	2.11 (3H, s)
2.19 (6H, s, NMe_2)	2.20 (3H, s, OAc)	2.17 (3H, s)
2.40 (1H, ddd, *J* 4.4, 8.4, 11.9, H-15)	2.25 (6H, s, NMe_2)	2.22 (6H, s, NMe_2)
	2.58 (2H, m, H-17)	2.53 (1H, dd, *J* 7.0, 12.9, H-17)
2.51 (1H, dd *J* 8.4, 12.6, H-17)	2.76 (1H, dd, *J* 2.6, 9.5, H-11)	2.60 (1H,dd, *J* 5.0, 12.9, H-17)
2.56 (1H, dd, *J* 11.6, 19.5, H-14)	4.93 (1H, dd, *J* 1.5, 11.5, H-13)	5.10 (2H, m, H-7, -13)
2.67 (1H, dd, *J* 4.4, 12.6, H-17)	4.98 (1H, br t, *J* 7.1, H-7)	5.20 (1H, br d, *J* 10.2, H-3)
2.90 (1H, dd, *J* 3.3, 19.5, H-14)	5.11 (1H, br d, *J* 10.7, H-3)	5.29 (1H, dd, *J* 7.8, 10.2, H-2)
2.99 (1H, dd, *J* 4.0, 7.9, H-11)	5.32 (1H, m, H-1)	
4.96 (1H, br t, *J* 7.5, H-7)		
4.99 (1H, br d, *J* 10.4, H-3)		
5.44 (1H, dd, *J* 7.9, 10.4, H-2)		

[a)] ^1H NMR spectra were measured at 400 MHz in $CDCl_3$. Chemical shifts are given in δppm based on $CHCl_3$ (7.26 ppm). *J* values in parentheses are expressed in Hz.

side of the *cis*-fused lactone, to yield the adduct with the β-oriented dimethylaminomethyl group. This was supported by NOE measurement of **1**. When irradiated at one of the methylene proton signals [δ 2.67 (dd)] at C-17, a 2.0% NOE was observed at the signal [δ 2.90 (dd)] due to one of the methylene protons at C-14, indicating a *cis* relationship between the dimethylaminomethyl and C-14 methylene groups.

Sinulamine II (**2**)[8)] ($C_{24}H_{37}NO_5$) was also shown to have a dimethylaminomethyl group by ^1H and ^{13}C NMR spectra [δ_H 2.25 (6H, s), 2.58 (2H, m); δ_C 46.2 ($2CH_3$), 57.8 (CH_2)]. The NMR data of **2** showed close agreement to those of **5** except for signals due to positions C-1, -15, and -17. The structure of sinulamine II would thus appear to be that of **2**. This was confirmed by the reaction of **5** with dimethylamine in methanol, to give **2**[9)] in quantitative yield. Since no diasteremeric isomer of **2** could be detected in this reaction and the structure of **5** including the absolute stereochemistry has already been established,[3c)] the structure of sinulamine II was concluded to be that of **2**.

In the same manner, the structure of sinulamine III (**3**)[10)] ($C_{24}H_{37}NO_5$) was suggested from the data of spectroscopic analysis, and confirmed by the reaction of lobophytol acetate (**6**) with dimethylamine to give **3**[11)] in quantitative yield.[12)] Cembranolide diterpenes possessing a 13-membered carbocyclic skeleton such as **3** are very rare.[3b,5)]

Sinulamine I, II, and III are, to the best of our knowledge, the first examples of cembrane-type diterpenes containing a nitrogen atom.

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6) **1**: Colorless needles; mp 139-140 °C; $[\alpha]_D$ +34.0°(*c* 0.40, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.3 (CH_3), 14.6 (CH_3), 15.1 (CH_3), 23.9 (CH_2), 24.4 (CH_2), 35.8 (CH_2), 36.4 (CH_2), 38.4 (CH), 40.1 (CH_2), 41.4 (CH), 45.9 ($2CH_3$), 59.9 (CH_2), 60.4 (CH), 63.5 (C), 76.4 (CH), 118.2 (CH), 125.8 (CH), 133.3 (C), 145.8 (C), 177.3 (C), 208.7 (C). Numbers of attached protons of ^{13}C signals were determined by DEPT experiments.

7) **1** (Synthetic): colorless needles; mp 142-143 °C; $[\alpha]_D$ +28.8° (*c* 0.74, $CHCl_3$).

8) **2**: Colorless needles; mp 147-148 °C; $[\alpha]_D$ +40.0° (*c* 0.1, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.5 ($2CH_3$), 15.6 (CH_3), 20.8 (CH_3), 23.8 (CH_2), 24.7 (CH_2), 28.0 (CH_2), 37.0 (CH_2), 38.7 (CH), 40.2 (CH_2), 43.3 (CH), 46.2 ($2CH_3$), 57.8 (CH_2), 60.2 (CH), 61.2 (C), 72.7 (CH), 77.3 (CH), 119.2 (CH), 125.7(CH), 134.2 (C), 144.0(C), 171.2(C), 177.6(C).

9) **2** (Synthetic): colorless needles; mp 148-149 °C; $[\alpha]_D$ +66.5° (*c* 0.4, $CHCl_3$).

10) **3**: Pale yellow viscous oil; $[\alpha]_D$ -57.7° (*c* 1.02, $CHCl_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.2 (CH_3), 15.5 (CH_3), 21.1 (CH_3), 23.5 (CH_2), 24.9 (CH_2), 29.4 (CH_3), 34.3 (CH_2), 38.6 (CH), 38.9 (CH_2), 39.9 (CH_2), 44.6 (CH), 45.8 ($2CH_3$), 57.9 (CH), 58.6 (CH_2), 70.8 (CH), 76.4 (CH), 118.5 (CH), 126.0 (CH), 133.9 (C), 145.9 (C), 170.9 (C), 177.5 (C), 209.4 (C).

11) **3** (Synthetic): pale yellow viscous oil; $[\alpha]_D$ -45.8° (*c* 1.02, $CHCl_3$).

12) In this reaction, no diastereomeric isomer of **3** was observed.

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