New Terpenoid Components from the Volatile Oils of the Soft Corals *Clavularia viridis* and *Sarcophyton acutangulum*

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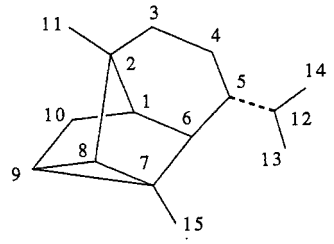
A new tetracyclic terpenoid hydrocarbon, cyclosinularane (**1**), and (+)-alloaromadendrene (**2**), the enantiomer of terrestrial (−)-alloaromadendrene, have been isolated from the volatile oil of the soft corals *Clavularia viridis* and *Sarcophyton acutangulum*, respectively. Their structures have been determined on the basis of NMR spectral analysis and their chiroptical properties.

An increasing number of marine natural products, the structures of which are quite different from the terrestrial organisms, have been reported.[1] Some marine organisms use simple hydrocarbons or fatty acids that are practically insoluble in seawater as chemical signals, for example, sex pheromones[2] and allelopathic substances.[3] In the investigation of the chemical components of marine organisms the organisms are usually freeze-dried or extracted with organic solvents. By these operations, the volatile components having physiologically important activity may be lost because of the high vacuum needed for freeze-drying or distillation during concentration of the extract on a rotary evaporator. Previously, we reported isolation of a terpenoid obtained from the oil floating on the surface of the aqueous condensate in the reservoir of an evaporator, after the methanol extract of a soft coral was concentrated.[4]

In the research reported herein, we noticed oily materials on the surface of the ice formed on the coldfinger of the lyophilizer during freeze-drying of the soft corals *Clavularia viridis* (Clavulariidae) and *Sarcophyton acutangulum* (Alcyoniidae). This paper deals with the structures and biological activity of the constituents of these oils.

Frozen soft corals *C. viridis* and *S. acutangulum*, collected off Ishigaki Island, were freeze-dried at 10^{-2} Torr, and the solid condensate (mainly ice) from each organism was, after it was thawed, extracted with CH_2Cl_2. By careful evaporation of the solvent at <20 °C on a rotary evaporator, an oily residue was obtained from each extract. The respective oils were separated by HPLC, and compounds **1**, designated cyclosinularane, and **2**, (+)-alloaromadendrene, were obtained from *C. viridis* and *S. acutangulum*, respectively.



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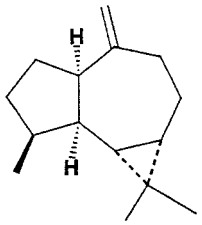

2

The HREIMS of cyclosinularane (**1**), $[\alpha]_D$ +22.2°, exhibited a molecular ion at *m/z* 204.1852, establishing its molecular formula $C_{15}H_{24}$. Because there are only sp^3-hybridized carbons in this molecule (^{13}C NMR spectrum), the compound must be tetracyclic. The ^1H NMR spectrum of **1** ($CDCl_3$) shows the signals of two methyls [δ 0.80, 1.11 (both s)] and an isopropyl group [δ 0.85, 0.91 (both 3H, d, J = 7 Hz), 1.53 (1H, dsept, J = 11, 7 Hz)]. Four rings must be formed by the residual 10 carbons. One of the rings should be a cyclopropane, because two highly shielded signals appear at δ 0.44 (1H, d, J = 5 Hz) and 0.86 (1H, d, J = 5 Hz). The ^{13}C NMR signal at δ 21.21 (s), which is unusually upfield for a quaternary carbon, also supports the presence of a cyclopropane ring. The $^1H-^1H$ COSY spectrum did not give much information about the proton networks due to overlapping of the key signals and the absence of couplings between some vicinal protons, which is characteristic of rigid polycyclic rings (vide infra).[5] Eventually, the tetracyclic structure was elucidated by detailed analysis of the HMBC spectrum (Figure 1: **1a**) after full assignment of the carbon and proton signals (Table 1) by the HSQC spectrum.

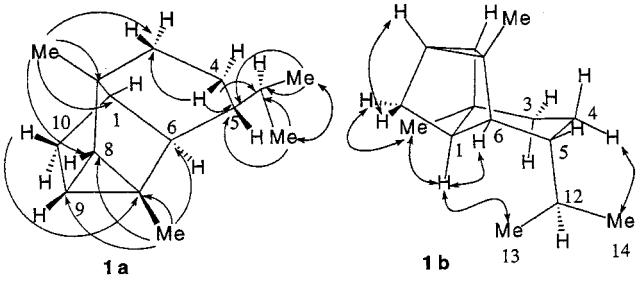

Figure 1. Correlation found in the HMBC (**1a**) and NOESY (**1b**) spectra of cylclosinularane. The spectra were recorded in $CDCl_3$ at 400 MHz. The structure **1b** is viewed from this direction to show the axial orientation of the isopropyl group clearly.

Because of the rigid tetracyclic nature of **1**, the relative stereochemistry of the asymmetric centers is automatically assigned except for that at C-5. To determine the orientation of the isopropyl group, coupling patterns of the protons at C-3, -4, -5, and 6 were needed. The patterns of the overlapped signals were determined by the proton-coupled HSQC.[6] Two examples of the cross sections are shown in Figure 2.

From the coupling constants observed for the protons on C-3, -4, and -6, the chair form of the cyclohexane ring was assumed. Although the coupling patterns of H-5 and H-4α were not fully resolved, the coupling constants between H-5/H-6 and H-5/H-4β ($J_{4\beta-5}$ = 6 Hz, J_{6-5} = 4 Hz) were deducible from the patterns of H-4β and H-6. These coupling constants suggested the axial orientation of the isopropyl group. The NOESY spectrum eventually con-

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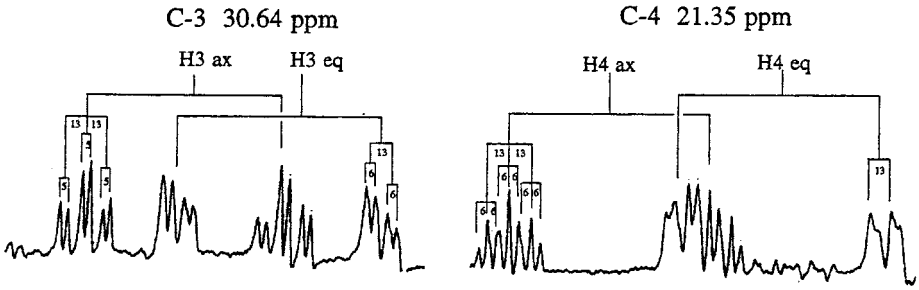


Figure 2. Cross sections of the HSQC spectrum of **1** sliced at 30.64 (C-3) and 21.35 (C-4) ppm (400 MHz, $CDCl_3$) (digital resolution: 1 Hz/point).

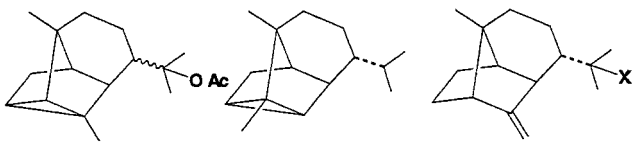
Table 1. ^{13}C and ^{1}H NMR Data (100 and 400 MHz, $CDCl_3$) of Cyclosinularane (**1**)

position	δ_C	δ_H	J (Hz)
1	40.36 d	1.24 s	
2	44.22 s		
3	30.64 t	1.25 dd	13, 6
		1.38 td	13, 5
4	21.35 t	1.43 bd	13
		1.68 tt	13, 6
5	39.64 d	1.13 m	
6	47.77 d	1.52 d	4
7	21.21 s		
8	26.09 d	0.44 d	5
9	19.84 d	0.86 d	5
10	31.17 t	1.09 d	11
		1.57 d	11
11	21.77 q	0.80 s	
12	28.23 d	1.53 dsept	11, 7
13	20.78 q	0.85 d	7
14	22.14 q	0.91 d	7
15	13.37 q	1.11 s	

firmed the stereochemical feature of **1** (Figure 1: **1b**). The NOEs between H-1 and Me-13 as well as H-4α and Me-14 reinforced the axial configuration assignment of the isopropyl group.

It should be noted that (i) H-1 appears as a singlet (no couplings with H-6, H-10α, and H-10β) and (ii) H-9 appears as a doublet ($J = 5$ Hz) (coupled with H-8 but no couplings with H-10α and H-10β). The $^{1}H-^{1}H$ COSY spectrum exhibits an intense cross-peak between H-8 and H-10β owing to a W-type coupling.

Biogenetically, cyclosinularane (**1**) may be a rearranged sesquiterpene, because its structure does not agree with the head-to-tail isoprene rule. There is only one precedent sesquiterpene, 12-acetoxycyclosinularane (**3**),[7] having the same carbon framework as **1**, which was isolated from *Clavularia koellikeri*. Cyclocopacamphane (**4**),[8] a constituent of vetiver oil, and sinularene (**5**)[9] and 12-acetoxysinularene (**6**),[7] both from soft corals, are also closely related to **1**. The absolute configuration of sinularene (**5**) has been established by Djerassi et al.[9] If sinularene (**5**) is a precursor of **1**, the absolute configuration of **1** would be the one shown as structure **1**. Experiments to confirm the absolute configuration of **1** are in progress.



3 **4** **5: X = H** **6: X = OAc**

(+)-Alloaromadendrene (**2**) showed ^{1}H and ^{13}C NMR spectra exactly the same as those of (−)-alloaromadendrene,[10] a component of terrestrial plants such as *Ledum paluster*. However, the present material shows an optical rotation $[\alpha]_D$ +25.8°, which is opposite in sign of that of (−)-alloaromadendrene. It is interesting that the soft coral produces the enantiomer of the terrestrial plants' metabolite.

While a small amount of **2** was found in the residual extract of *S. acutangulum* after rotary evaporation, no **1** was found in the equivalent extract of *C. viridis*. The present findings indicate that the volatile compounds such as **1** and **2** may be lost during freeze-drying procedure unless care is taken.

Cyclosinularane (**1**) and (+)-alloaromadendrene (**2**) showed toxicity in brine shrimp assay at an LD_{50} of 4 and 3 μg/mL, respectively. These volatile compounds may act as the repellents against the predators of the soft corals.

Experimental Section

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker ARX-400 NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to $CHCl_3$ (δ 7.25), and coupling constants are given in hertz. The specific rotations were measured on a JASCO DIP-370 instrument. Mass spectra were recorded on JEOL JMS-SX102A and JEOL JMS-AM150 mass spectrometers.

Animal Material. The soft corals *C. viridis* (11 kg, wet weight) and *S. acutangulum*[11] (10 kg, wet weight) (voucher specimens preserved in this laboratory; voucher nos. CV-10 and SA-10) were collected off Ishigaki Island, Okinawa, in October 1997. After collection they were immediately frozen with dry ice. Both soft corals were freeze-dried at 10^{-2} Torr for 18 h.

Extraction and Isolation. The ice cake formed on the coldfinger during freeze-drying of *C. viridis* was thawed at room temperature, and the resulting aqueous mixture (7 L) was extracted twice with CH_2Cl_2 (14 L). The solvent was removed on a rotary evaporator without immersing the flask in a water bath. A thin layer of frost was formed on the wall of the flask during the condensation procedure. When the solvent was gone and the frost melted, the flask was disconnected. The dichloromethane extract residue (7 g) was partitioned between hexane−MeOH (85:10). The hexane-soluble part (4 g) was separated by Si gel (120 g) column chromatography. Elution with hexane (200 mL) gave four fractions. The first fraction afforded a colorless oil [A] (1 g). By the same procedure, a slightly yellow oil [B] (900 mg) was obtained from *S. acutangulum*.

Oil [A] (100 mg) was subjected to HPLC (Hibar RT250−25, LiChrosorb Si60; hexane) to give cyclosinularane (**1**: 5 mg) together with inflatene (clavukerin B)[12] (2 mg) and β-elemene[13] (1 mg). The structures of the latter two compounds were deduced by comparing their ^{1}H NMR spectral data with those in the literature.

By separation of oil [B] using Si gel column chromatography, (+)-alloaromadendrene (300 mg) was obtained.

Cyclosinularane (1): colorless oil; $[\alpha]_D$ +22.2° (*c* 0.1, $CHCl_3$); NMR data are summarized in Table 1; GCMS *m*/*z* 204 (60) (M^+), 189 (20) ($M^+ - Me$), 161 (70) ($M^+ -$ isopropyl), 119

(55), 105 (100), 93 (90), 91 (98), 69 (35), 33 (35), 41 (40). HREIMS *m*/*z* 204.1871 (calcd for $C_{15}H_{24}$, 204.1878).

(+)-Alloaromadendrene (2): colorless oil; $[\alpha]_D$ +25.8° (*c* 1.6, $CHCl_3$). Compound **2** was identified with (−)-alloaromadendrene by comparison of its NMR properties with the literature values.[10] The ^{1}H NMR and ^{13}C NMR data are in complete agreement with those of the reported values. HREIMS *m*/*z* 204.1852 (calcd for $C_{15}H_{24}$, 204.1878).

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