[Chem. Pharm. Bull. 36(10)3780—3786(1988)]

Marine Terpenes and Terpenoids. VI.[1] Isolation of Several Plausible Precursors of Marine Cembranolides, from the Soft Coral, *Sinularia mayi*

MASARU KOBAYASHI* and TAKUYA HAMAGUCHI

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-ku, Sapporo 060, Japan

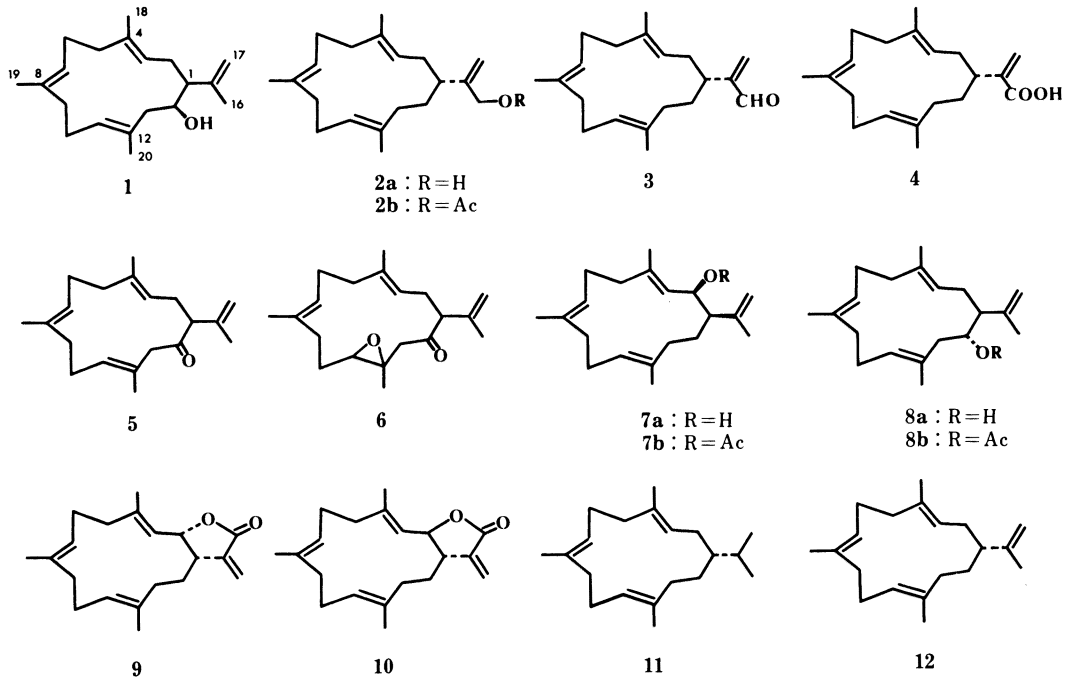
(Received February 19, 1988)

Six new cembranoid diterpenes, sinulariols C and D (**1** and **2a**), sinularial A (**3**), sinularic acid A (**4**), and sinularones A and B (**5** and **6**) were isolated from the lipid extract of the soft coral *Sinularia mayi*, together with four known compounds. The structures of the new compounds were elucidated by means of spectroscopic analyses, Horeau determination, and chemical conversion. Sinulariol C (**1**) was found to be an C-14 isomer of "13-hydroxyneocembrene" (**8a**) and gave sinularone A (**5**) on oxidation. Sinularial A (**3**) is the first example of a cembranoid aldehyde isolated from marine sources. Compounds **1** to **4** are plausible precursors to the cembranoid lactones found in various soft corals.

Keywords——soft coral; *Sinularia mayi*; cembranoid; sinulariol C; sinulariol D; sinularial A; sinularic acid A; sinularone A; sinularone B

Soft corals are a rich source of terpenoids, notably cembrane-type diterpenes. Their abundant production and accumulation of diterpenoids are intriguing, and it seems unlikely that these compounds act solely as repellents against predators. Rather, the diterpenoids may play an as yet unknown physiological role in these benthic animals, in view of their quite low contents of such common lipids as glycerides or fatty acid esters,[2] which are of vital importance in evolutionarily higher animals functioning in connective tissues and as energy sources. Our previous reports showed that the southern Japan soft coral *Sinularia mayi*, a typical example of *Sinularia* spp., which are abundant in coral reefs of Indo-Pacific coastal waters, contains a variety of cembranoid α-methylene-γ-lactones including a novel seco-cembranoid,[3] and also small amounts of simple cembranoid diols, sinulariol A (**18**) and sinulariol B (**17**), which are plausible precursors.[4] Further investigation of the less polar cembranoids of *S. mayi* resulted in the isolation of six new compounds (**1**—**6**), including plausible precursors of various soft coral cembranolides, along with four known components [**7a** (mayol),[5] **8a** (14-hydroxycembrene A or "13-hydroxyneocembrene" according to the original nomenclature),[6,7] **9**,[8] **10**[8]]. They were obtained by repeated flash chromatography and the new compounds were designated sinulariol C (**1**), sinulariol D (**2a**), sinularial A (**3**), sinularic acid A (**4**), sinularone A (**5**), and sinularone B (**6**).

Compound **1**, $C_{20}H_{32}O$, is a monohydroxycembratetraene and its infrared (IR) spectrum showed hydroxyl (3350 cm^{-1}) and terminal methylene (900 cm^{-1}) absorptions. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed signals due to three trisubstituted olefin bonds, each having a methyl group (δ 1.58, 6H; 1.65, 3H; 4.93, 5.15 and 5.03, each 1H), one hydroxymethine (δ 3.84), and one isopropenyl group (δ 1.80, 3H; 4.78 and 4.98, each 1H). These are typical values found in a derivative of the biogenetically common precursor cembrene A (3*E*,7*E*,11*E*,15-cembratetraene, **12**), which is generally believed to be derived by cyclization of all-*E*-geranylgeranyl pyrophosphate.[9] High-field chemical shifts of the olefinic methyl groups at C-4,8,12 (δ 15.3, 15.6, 18.4) in the carbon-13 nuclear magnetic



1 2a : R=H 2b : R=Ac 3 4

5 6 7a : R=H 7b : R=Ac 8a : R=H 8b : R=Ac

9 10 11 12

Chart 1

resonance (^{13}C-NMR) spectrum support the *E*-geometry of these olefin bonds.[13] The secondary hydroxyl group did not exert deshielding β-effects on any of the olefin carbons so that its only possible position is C-14; this leads to the structure which is diastereomeric with that of the known component 14-hydroxycembrene A (**8a**),[6,7] whose stereochemistry remained unsettled. This was confirmed by oxidizing **1** with pyridinium chlorochromate (PCC) to the ketone **5**, which was identical with sinularone A (**5**) simultaneously isolated from *S. mayi* (Experimental). This ketone was spectroscopically identical with the one ($[\alpha]_D$ +482.4°) which Aoki *et al.* obtained by oxidation of **8a**,[6] but showed the opposite specific rotation (−460°). This meant that **1** and **8a** bear the same configuration at C-14 and are isomeric at C-1. However, Horeau determination gave contradictory results and their absolute configurations were 14*S* (**1**) and 14*R* (**8a**). PCC oxidation of **8a**, which showed optical and spectral data in agreement with those recorded in the literature,[6] in fact gave **5** with a negative specific rotation (−480°) in conflict with the literature, and was identical with that obtained from **1**. Compound **1** was thus shown to be isomeric at C-14 with **8a**. Compound **5** was previously converted to *dl*-cembrene A by Wolf–Kishner reduction.[6] Attempted conversion of **1**, **8a**, and **5** to optically active cembrene A was, however, unsuccessful. The methanesulfonate and *p*-toluenesulfonate of **1** and **8a** were unreactive to lithium aluminum hydride reduction and gave only the hydrolysis product on prolonged reaction. Preparation of the tosylhydrazone and ethylene dithioketal of **5**, for attempts at deoxygenation, also failed.

Sinulariol D (**2a**), $C_{20}H_{32}O$, is also a monohydroxycembratetraene and afforded a monoacetate (**2b**) on acetylation. The ^{1}H-NMR signals of **2a** arising from the nuclear double bonds (3,7,11-H: δ 4.97, 5.06 and 5.17; 18,19,20-H: 1.56 (6H) and 1.59) were almost the same with those of **1** but that of the olefinic methyl of the isopropenyl group was absent. The ^{13}C-NMR spectrum showed close similarity to that of **1** concerning C-2 to C-11, and C-18 to C-19 (Experimental). The signals of the side chain at C-1 are those of terminal methylene and

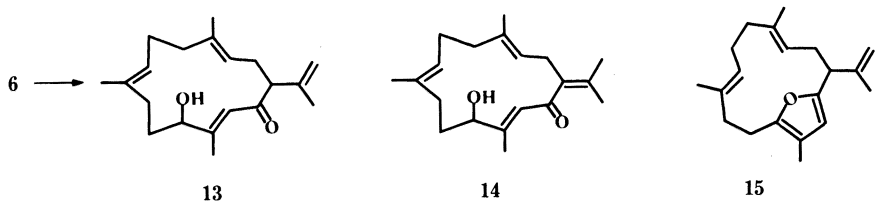
hydroxymethyl groups [^{1}H-NMR: δ 4.09, 2H, s; 4.90 and 5.09, each 1H, br s; ^{13}C-NMR: 153.1 (C-15), 65.0 (C-16), 108.6 (C-17)]. Similar chemical shifts were previously found in the case of sinulariol A (**18**).[4] Attempted correlation to **12**, by tosylation and reduction, was unsuccessful due to the low yield of tosylate, which was resistant to lithium aluminum hydride reduction. Absolute configuration at C-1 was determined indirectly by correlation to **3**.

Sinularial A (**3**), $C_{20}H_{30}O$, was found to be an aldehyde derivative of **2a**. It showed virtually the same ^{1}H- and ^{13}C-NMR signals as **2a** except for those of the side chain at C-1, and the presence of a conjugated aldehyde group was indicated [^{1}H-NMR: δ 6.02, 6.24 and 9.55 each 1H, s; ^{13}C-NMR: δ 154.5 (C-15), 194.8 (C-16), 133.5 (C-17). IR: 2700, 1695, 1620 cm^{-1}]. Lithium aluminum hydride reduction of **3** afforded **2a** and, conversely, PCC oxidation of **2a** gave **3** indicating the common configuration at C-1. Wolf–Kishner reduction of **3** was unsuccessful since the decomposition of the hydrazone did not take place. However, sodium borohydride reduction of its *p*-tosylhydrazone afforded 15,17-dihydro-(−)-cembrene A (**11**), $[\alpha]_D$ −17° (lit.[14] −17.8°) which established that the configuration at C-1 of **2a** and **3** is *R*. This is the first isolation of a cembranoid aldehyde from marine sources, though there are precedents in terrestrial *Eremophila* plants.[15a] Unlike other terpenoids having the same α-methylene aldehyde moiety,[15b–e] the ultraviolet (UV) absorption of **3** is quite weak and is masked in the strong end-absorption bands. This indicates that the coplanarity of the carbonyl and its conjugated methylene group is significantly obstructed by a steric barrier. Three *E*-double bonds in the 14-membered ring restrict the flexibility of the ring, and would force it to take a conformation quite different from that of cyclotetradecane. Perhaps similar steric hindrance would explain the unexpected difficulties encountered above in the attempted conversion of compounds **1**, **2a**, **3**, **5**, and **8a**.

Sinularic acid A (**4**), $C_{20}H_{30}O_2$ is a carboxylic acid derivative of **2a**. Its ^{1}H- and ^{13}C-NMR signals are also virtually the same as those of **2a** and **3** (Experimental) except for those of the side chain at C-1 [^{1}H-NMR: δ 5.63 and 6.35. ^{13}C-NMR: 144.3 (C-15), 172.9 (C-16), 126.0 (C-17). IR: 2700—3600 cm^{-1}]. The structure and the configuration at C-1 (*R*) were confirmed by converting **4** to **2a** by lithium aluminum hydride reduction. Discrepancies of ^{13}C-NMR chemical shifts concerning C-2 to C-13 and C-18 to C-20 in compounds **2a**, **3** and **4** were mostly less than 0.2 ppm and indicated closely related conformations.

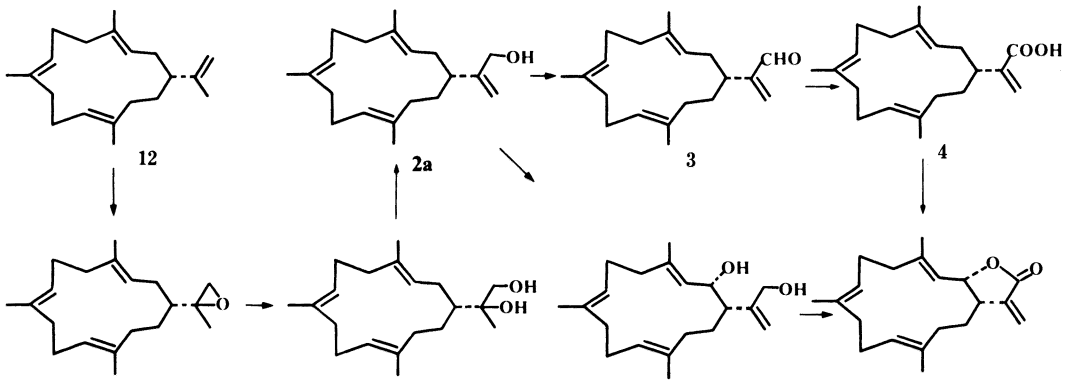
Sinularone B (**6**), $C_{20}H_{30}O_2$, is a ketone and its spectroscopic data suggested structural similarity with **5** in which one of the three double bonds in the ring was replaced by a trisubstituted epoxide (δ 1.33, 3H, s; 2.63, 1H, dd, J=6.3, 2.9 Hz). The carbonyl group is also unconjugated and the adjacent methylene protons (δ 2.66 and 2.82, each d, J=15.0 Hz) showed only the geminal coupling. This and the strongly deshielded C-1 signal (^{13}C-NMR: δ 60.7 or 61.6) as found in **5** (δ 60.4) limit the location of the carbonyl group to C-14.[16] Brief treatment of **6** with dilute KOH in aqueous *tert*-BuOH at room temperature readily caused cleavage of the epoxide ring and afforded the β-methyl-γ-hydroxy-α,β-conjugated ketone **13** [UV: 236 nm (ε, 5000); ^{1}H-NMR: δ 2.00 (3H, d, J=1.5 Hz, 20-H), 4.02 (1H, m, 11-H), 6.20 (1H, s, 13-H)] and its dienone (**14**) and furan [**15**, UV 226 nm (ε, 8800); ^{1}H-NMR: δ 1.87 (3H, s, H-20), 5.73 (1H, s, H-13)] derivatives. These results established the structure of **6** to be the one having an 11,12-epoxy-14-keto group. Its absolute stereochemistry is unknown at present, due to the minute amount isolated (Chart 2).

The major cembranolides previously isolated from *S. mayi* are formally derivatives of 1,2-*Z*-α-methylene-γ-lactone **9** but the absolute stereochemistry of most of them is unknown.[3,4] Uchio *et al.* determined that the absolute configuration at C-1 of **9** is the same as that of (1*R*)-cembrene A (**12**) by correlation to the known compound mukulol.[17] This also corresponds to those of compounds **2a**, **3** and **4** in the present study, and **17** and **18** isolated previously from *S. mayi*. However, the absolute configuration at C-1 of **7a** (mayol) was shown, synthetically, to be opposite to that of **12**.[5b] Thus the initial cyclization of



13 14 15

Chart 2



16 17 18 9

Chart 3

geranylgeranyl pyrophosphate[9] is not entirely stereospecific in this organism. This is unusual but is not unprecedented, having been found in some labdane-type diterpenes occurring alongside each other as both the enantiomeric ring fusion derivatives in the same plant species.[18] Characterization of compounds **2a**, **3** and **4** in *S. mayi* also suggests that, starting from the primary precursor cembrene A (**12**), several intermediates are possible for the completion of the α-methylene-γ-lactone ring as found in **9**. In view of the occurrence of sinulariol A (**18**) and sinulariol B (**17**) in *S. mayi*, we postulated that the initial step is the formation of 15,16-epoxycembrene A (**16**), which is converted to the glycol **17** by hydrolysis, and then to **2** by dehydration.[4] In other words, the hydroxymethyl carbons in **2a**, **17** and **18**, and hence the carbonyl carbons of many soft coral cembranolides, derive from the secondary carbon of the isopropenyl group of **12** rather than its primary carbon; the latter would be expected in the case of simple allylic oxygenation of **12** (Chart 3). Also, compound **18** seemed to indicate the next step to be the oxygenation at C-3. The present discovery of a set of three compounds, namely, the allylic alcohol (**2a**), allylic aldehyde (**3**) and allylic acid (**4**) suggests, however, that the biogenesis of **9** may be a duplicated one, since oxygenation of **4** and oxidation of **18** equally lead to the basic α-methylene-γ-lactone moiety in **9** and various other soft coral cembranolides (Chart 3).

Experimental

Optical rotations were determined on a JASCO DIP-4 digital polarimeter in $CHCl_3$ solution. ^1H- and ^{13}C-NMR spectra were determined in $CDCl_3$ solution with tetramethylsilane as an internal standard, on a JNM GX-270 spectrometer at 270 MHz (^1H) and on a JNM FX-90Q spectrometer at 22.5 MHz (^{13}C). Mass spectra (MS) were determined on a JEOL JMS D300 spectrometer. IR spectra were taken on a JASCO A 102 spectrometer. UV spectra were determined on a Hitachi EPS-3T spectrometer. Chromatography was carried out on a column of silica gel by the flash chromatography method.

Fractionation of *S. mayi* Extract——The MeOH extract (175 g) which was described in the previous report[4] was

dissolved in $CHCl_3$ (1 l) and insoluble material was removed. Silica gel (500 g) was added to the solution and the mixture was evaporated to dryness. The residue was charged on a column of silica gel (1 kg) and eluted with a mixture of ethyl acetate and hexane (1.5 l/fraction (fr.)) as follows: Fractions 1—5 (1 : 19, 1.9 g), 6—22 (1 : 9, 7.4 g), 23—26 (13 : 87, 3.6 g), 27—35 (15 : 85, 13.5 g), 36—45 (20 : 80, 17.4 g), 46—51 (25 : 75, 4.7 g), 52—58 (30 : 70, 3.9 g), 59—64 (50 : 50, 6.3 g), 64 (MeOH, 6 l, 74.35 g). Compounds **3** and **5** were eluted mainly in frs. 2 (920 mg) and 3 (560 mg). They were purified by column chromatography with ethyl acetate–hexane (1 : 40), and then 7% silver nitrate-impregnated silica gel with ethyl acetate–hexane (1 : 20). Repeated chromatography of frs. 4 (200 mg) and 5 (130 mg) over a column of 7% silver nitrate-impregnated silica gel with ethyl acetate–hexane (2 : 3) gave first 34.5 mg of **6** and then 47.5 mg of **1**. Fraction 9 (2.2 g) contained compounds **2**, **4**, **7**—**10**. Repeated column chromatography of a portion (0.74 g) of this with ethyl acetate–hexane (17 : 83) gave first a mixture of **7** and **8**, then a mixture of **9** and **10**, and **2** (139 mg), **4** (140 mg), and a mixture (16 mg) of stearic, oleic and linoleic acids in that order. Known compounds **7a**—**10** were isolated from a similar mixture obtained previously,[4] by 7% silver nitrate-impregnated silica gel with ethyl acetate–hexane (15 : 85).

Sinulariol C (1)——Oil, $[\alpha]_D$ +12.2° (c=1.5). ^{1}H-NMR δ: 1.58 (6H, s), 1.65 (3H, s), 1.80 (3H, s, 16-H), 3.84 (1H, dt, J=6.5, 5.0 Hz, 14-H), 4.78 and 4.98 (each 1H, br s, 17-H), 4.93 (1H, m), 5.03 (1H, m, overlapped with a signal at δ 4.98), 5.15 (1H, br t, J=7.0 Hz). ^{13}C-NMR δ: C-1 (50.2), C-2 (29.2), C-3,7,11 (123.4, 125.1 126.0), C-4,8,12 (132.0, 133.4, 135.3), C-5,9 (38.9, 39.4), C-6,10 (24.0, 24.9), C-13 (43.4), C-14 (70.8), C-15 (146.7), C-16 (23.3), C-17 (113.3), C-18,19,20 (15.3, 15.6, 18.4). IR ν_{max}^{neat} cm^{-1}: 3450, 1638, 900. MS m/z: 288 (M^+), 273, 270, 255, 245. High-resolution MS [Found (Calcd)] m/z: $C_{20}H_{32}O$ (M^+), 288.2469 (288.2453). Compound **1** (21.2 mg) was esterified at 25 °C with 43.4 mg of α-phenylbutyric anhydride in pyridine (0.3 ml) for 6 h. Thin-layer chromatography (TLC) of the mixture showed about a half of **1** was esterified. The mixture was worked up according the standard method[19] and the resultant benzene solution (1.5 ml) of the excess acid showed rotation of −0.03° and accordingly, 14*S* configuration. The blank test showed the rotation of less than ±0.005°.

Sinulariol D (2a)——Oil, $[\alpha]_D$ +14° (c=0.84). ^{1}H-NMR δ: 1.56 (6H, s), 1.59 (3H, s), 4.09 (2H, s, 16-H), 4.90 and 5.09 (each 1H, br s, 17-H), 4.97 (1H, br t, J=5.5 Hz), 5.06 (1H, overlapped br t, J=6.2 Hz), 5.17 (1H, br t, J=6.6 Hz). ^{13}C-NMR δ: C-1 (42.2), C-2 (29.2), C-3,7,11 (122.3, 123.9, 126.0), C-4,8,12 (133.5, 133.8, 135.2), C-5,9 (39.0, 39.5), C-6,10 (23.8, 24.9), C-13,14 (33.1, 34.2), C-15 (153.1), C-16 (65.0), C-17 (108.6), C-18,19,20 (15.3, 15.6, 17.9). IR ν_{max}^{neat} cm^{-1}: 3350, 1645, 895. MS m/z: 288 (M^+), 273, 270, 257, 255. High-resolution MS [Found (Calcd)] m/z $C_{20}H_{32}O$ (M^+), 288.24578 (288.24528). Usual acetylation of **2a** (Ac_2O–pyridine) gave **2b**. Oil, $[\alpha]_D$ +4° (c=0.50). ^{1}H-NMR δ: 1.56 (6H, s), 1.59 (3H, s), 2.09 (3H, s), 4.56 and 4.51 (each 1H, d, J=13.5 Hz, 16-H), 4.95 (1H, br s, 17-H), 5.07 (1H, q, J=1.0 Hz, 17-H), 4.96 (1H, overlapped, m), 5.05 (1H, overlapped, m), 5.17 (1H, br dd, J=7.3, 7.0 Hz). IR ν_{max}^{neat} cm^{-1}: 1730, 1630, 880. MS m/z: 330 (M^+), 315, 270, 257, 255.

Sinularial A (3)——Oil, $[\alpha]_D$ +12.5° (c=0.64). ^{1}H-NMR δ: 1.53, 1.58, 1.60 (each 3H, s), 2.62 (1H, m, 1-H), 4.97 (1H, br dd, J=6.5, 5.5 Hz), 5.07 (1H, m), 5.15 (1H, br dd, J=7.7, 7.0 Hz), 6.02 and 6.24 (each 1H, s, 17-H), 9.55 (1H, s, 16-H). ^{13}C-NMR δ: C-1 (36.6), C-2 (29.1), C-3,7,11 (122.6, 123.3, 125.9), C-4,8,12 (133.5, 133.8, 135.7), C-5,9 (39.0, 39.5), C-6,10 (23.8, 24.9), C-13,14 (32.4, 34.3), C-15 (154.5), C-16 (194.8), C-17 (133.5), C-18,19,20 (15.3, 15.6, 17.7). UV λ_{max}^{EtOH}: end-absorption only. IR ν_{max}^{neat} cm^{-1}: 2700, 1695, 1620, 940. MS m/z: 286 (M^+), 271. High-resolution MS [Found (Calcd)] m/z: $C_{20}H_{30}O$ (M^+), 286.23020 (286.22970).

Sinularic Acid A (4)——Oil, $[\alpha]_D$ +19.7° (c=0.71). ^{1}H-NMR δ: 1.55, 1.58, 1.59 (each 3H, s), 2.58 (1H, m, 1-H), 4.97 (1H, br dd, J=6.2, 5.5 Hz), 5.07 (1H, br dd, J=6.6, 5.5 Hz), 5.17 (1H, br dd, J=7.7, 7.3 Hz), 5.63 and 6.35 (each 1H, s, 17-H). ^{13}C-NMR δ: C-1 (39.7), C-2 (29.3), C-3,7,11 (122.5, 123.5, 125.9), C-4,8,12 (133.5, 134.0, 135.6), C-5,9 (39.1, 39.6), C-6,10 (23.9, 24.9), C-13,14 (32.9, 34.3), C-15 (144.3), C-16 (172.9), C-17 (126.0), C-18,19,20 (15.3, 15.6, 17.7). IR ν_{max}^{neat} cm^{-1}: 2700—3600 (br), 1698, 1625, 950. MS m/z: 302 (M^+), 287, 257, 219. High-resolution MS [Found (Calcd)] m/z: $C_{20}H_{30}O_2$ (M^+), 302.22656 (302.22456).

Sinularone A (5)——Oil, $[\alpha]_D$ −410° (c=1.59. The sample contained small amounts of persistent impurities composed of fatty acid derivatives). ^{1}H-NMR δ: 1.57, 1.58, 1.66 and 1.69 (each 3H, s), 2.63 (1H, m), 2.72 and 3.24 (each 1H, d, J=12.3 Hz, 13-H), 3.30 (1H, dd, J=11.4, 1.8 Hz, 1-H), 4.85 and 4.89 (each 1H, s, 17-H), 4.87 (1H, masked by 17-H), 4.96 (1H, br t, J=7.0 Hz), 5.06 (1H, m). ^{13}C-NMR δ: C-1 (60.4), C-2 (28.6), C-3,7,11 (123.0, 124.4, 128.3), C-4,8 (133.3, 134.9), C-5,9 (38.1, 38.7), C-6,10 (24.6, 24.9), C-12 (128.6), C-13 (51.0), C-14 (207.4), C-15 (143.8), C-16 (20.4), C-17 (113.9), C-18,19,20 (15.6, 16.4, 17.6). MS m/z: 286 (M^+), 271, 258, 243. High-resolution MS [Found (Calcd)] m/z: $C_{20}H_{30}O$ (M^+), 286.2296 (286.2297).

Sinularone B (6)——Oil, $[\alpha]_D$ −108° (c=1.46). ^{1}H-NMR δ: 1.33 (3H, s, 20-H), 1.61, 1.62 and 1.70 (each 3H, s), 2.63 (1H, dd, J=6.3, 2.9 Hz, 11-H), 2.66 and 2.82 (each 1H, d, J=15.0 Hz, 13-H), 3.33 (1H, dd, J=11.3, 2.6 Hz, 1-H), 4.83 and 4.89 (each 1H, s, 17-H), 4.96 and 5.05 (each 1H, br t, J=7.0 Hz). ^{13}C-NMR δ: C-1,11 (60.7, 61.6), C-2 (28.9), C-3,7 (123.0, 126.2), C-4,8 (132.5, 135.5), C-5 (39.1), C-6,10 (24.6), C-12 (58.8), C-13 (49.4), C-14 (207.8), C-15 (143.5), C-16,20 (19.1, 20.7), C-17 (114.2), C-18,19 (15.5). IR ν_{max}^{neat} cm^{-1}: 1710, 1640, 893. MS m/z: 302 (M^+), 284, 267, 250. High-resolution MS [Found (Calcd)] m/z: $C_{20}H_{30}O_2$ (M^+), 302.2252 (302.2246).

Mayol (7a)——Oil, $[\alpha]_D$ +150° (c=1.01) (lit.,[5b] +156°). ^{1}H-NMR δ: 1.56, 1.58, 1.60 (each 3H, s), 1.81 (3H, s, 16-H), 4.43 (1H, dd, J=8.1, 1.1 Hz, 2-H), 4.80 and 4.96 (each 1H, br s, 17-H), 5.00 (1H, m), 5.07 (1H, br t, J=6.5 Hz), 5.39 (1H, d, J=8.4 Hz, 3-H). IR ν_{max}^{neat} cm^{-1}: 3450, 1643, 885. MS m/z: 288 (M^+), 273, 270, 255, 204. High-resolution

MS [Found (Calcd)] *m/z*: $C_{20}H_{32}O$ (M^+), 288.24749 (288.24529). Acetylation of **7a** by a usual method (Ac_2O–pyridine) gave **7b**, oil, $[\alpha]_D$ +74° (*c*=0.65) (lit.,[5a] +76.3°). ^{1}H-NMR δ: 1.57 (6H, s), 1.68 and 1.71 (each 3H, s), 1.99 (3H, s), 4.70 and 4.82 (each 1H, br s, 17-H), 4.97 (1H, m), 5.07 (1H, br t, *J*=7.0 Hz), 5.29 (1H, br d, *J*=8.4 Hz, 3-H), 5.58 (1H, dd, *J*=8.4, 1.1 Hz, 2-H). IR ν_{max}^{neat} cm^{-1}: 1735, 1640, 885. MS *m/z*: 330 (M^+), 270, 255.

14-Hydroxycembrene A (8a)——Oil, $[\alpha]_D$ −30° (*c*=0.57) (lit.,[6] −30.3°). ^{1}H-NMR δ: 1.54, 1.58, 1.64 (each 3H, s), 1.79 (3H, s, 16-H), 3.84 (1H, m, 14-H), 4.85 and 4.94 (each 1H, br s, 17-H), 4.88 (1H, m), 5.06 (2H, m). IR ν_{max}^{neat} cm^{-1}: 3420, 1640, 885. MS *m/z*: 288 (M^+), 273, 270, 255. High-resolution MS [Found (Calcd)] *m/z*: $C_{20}H_{32}O$ (M^+), 288.24711 (288.24529). Usual acetylation of **8a** (Ac_2O–pyridine) gave **8b**, $[\alpha]_D$ −30.° (*c*=0.63). ^{1}H-NMR δ: 1.54, 1.58, 1.68, 1.74, and 2.03 (each 3H, s), 4.73 and 4.85 (each 1H, s, 17-H), 4.91, 5.04 and 5.13 (each 1H, m), 5.08 (1H, ddd, *J*=8.0, 6.0, 2.0 Hz, 14-H). IR ν_{max}^{neat} cm^{-1}: 1740, 1642, 1240, 890. MS *m/z*: 330 (M^+), 270, 255. A solution of **8a** (24.8 mg) in 0.4 ml of pyridine was treated with 107.9 mg of α-phenylbutyric anhydride at room temperature for 4 h and worked up in the same way as described for **1**. The resultant excess acid showed a rotation of +0.05° and thus, *R* configuration.

Compound 9——mp 100.5—101 °C, $[\alpha]_D$ +85.3° (*c*=0.75) (lit.,[8a] mp 101—102 °C, $[\alpha]_D$ +77.9°). ^{1}H-NMR δ: 1.57, 1.58, 1.67 (each 3H, s), 3.05 (1H, m, 1-H), 4.78 (1H, br d, *J*=9.0 Hz), 4.93 (1H, br t, *J*=7.5 Hz), 4.99 (1H, br d, *J*=10.0 Hz, 3-H), 5.40 (1H, dd, *J*=10.0, 7.7 Hz, 2-H), 5.51 (1H, d, *J*=3.0 Hz, 17-H), 6.24 (1H, d, *J*=3.3 Hz, 17-H). MS *m/z*: 300 (M^+), 285, 271, 217, 193, 81.

Compound 10——Oil, $[\alpha]_D$ −16° (*c*=0.50) (lit.,[8b] −29°). ^{1}H-NMR δ: 1.58 and 1.60 (each 3H, s), 1.72 (3H, d, *J*=1.5 Hz), 2.66 (1H, m, 1-H), 4.88 (1H, dd, *J*=9.5, 3.7 Hz, 2-H), 4.89 (1H, m, overlapped), 4.98 (1H, m), 5.06 (1H, br d, *J*=9.5 Hz, 3-H), 5.57 and 6.23 (each 1H, d, *J*=2.5 Hz, 17-H). MS *m/z*: 300 (M^+), 285.

PCC Oxidation of 1 and 8a——(a) A solution of **1** (16 mg) in 1 ml of CH_2Cl_2 was treated with 12.6 mg, of PCC at room temperature for 4 h. The mixture was diluted with Et_2O and washed with H_2O. The mixture was frozen and the Et_2O layer was removed by decantation. It was worked up as usual and the crude product was purified on a small column of silica gel with hexane to give 11.4 mg of **5**, $[\alpha]_D$ −460° (*c*=0.92). It was identical with natural **5** (^{1}H-NMR and MS, and behavior in several TLC systems).

(b) A solution of 41.8 mg of **8a** in 2 ml of CH_2Cl_2 was treated with 131 mg of PCC at room temperture for 2 h and worked up as described in (a). The mixture was purified as in (a) and gave 29.4 mg of **5**, $[\alpha]_D$ −480° (*c*=0.48). This was identical with natural **5** (^{1}H-NMR spectrum and behavior in several TLC systems).

PCC Oxidation of 2a——A solution of **2a** (226.5 mg) in 5 ml of CH_2Cl_2 was stirred with 262 mg of PCC at room temperature for 6 h. The mixture was diluted with Et_2O and washed with H_2O, and the Et_2O layer was separated. After usual work-up, the crude product was purified by chromatography with ethyl acetate–hexane (1 : 19) to give 55.5 mg of **3**.

Conversion of 3 and 4 to 2a——(a) $LiAlH_4$ (1.5 mg) was added in portions to a solution of **3** (10.5 mg) in tetrahydrofuran (THF) (1 ml). After 2 h, excess reagent was decomposed with moist Et_2O and the Et_2O layer was washed with H_2O, 5% HCl, H_2O, and saturated NaCl solution, and the solvent was evaporated off. Column chromatography of the residue with ethyl acetate–hexane (1 : 10) gave **2a** (2.8 mg), $[\alpha]_D$ +14° (*c*=0.28). It was identical with natural **2a** (^{1}H-NMR spectrum and behavior in several TLC systems).

(b) Compound **4** (10.4 mg) was reduced with 1.7 mg of $LiAlH_4$ and the product was purified in the same way as in (a), giving 6.7 mg, of **2a**, $[\alpha]_D$ +15° (*c*=0.21). Identification was done in the same way as in (a).

Conversion of 3 to (−)-Dihydrocembrene A (11)——A mixture of **3** (56 mg), hydrazine hydrate (100%, 0.56 ml) and KOH (500 mg) in diethylene glycol was heated at 210 °C for 6 h. Although TLC indicated the formation of the hydrazone of **3**, its decomposition was not observed. The mixture was worked up in a usual way, and the crude hydrazone (47.2 mg) was isolated by short column chromatography with hexane. It was treated in pyridine (0.2 ml) with 60 mg of *p*-toluenesulfonyl chloride. After usual work-up, the crude tosylhydrazone in dioxane (3 ml) was refluxed with 52 mg of $NaBH_4$ for 4 h. After usual work-up, the crude product was submitted to 10% $AgNO_3$-impregnated silica gel column chromatography with ethyl acetate–hexane (1 : 9) to give 4.6 mg of **11** as an oil, $[\alpha]_D$ −17° (*c*=0.46). ^{1}H-NMR δ: 0.82 and 0.91 (each 3H, d, *J*=7.0 Hz, 16,17-H), 1.54, 1.56 and 1.57 (each 3H, s), 4.94 (1H, br t, *J*=6.6 Hz), 5.02 (1H, br t, *J*=7.0 Hz), 5.11 (1H, br t, *J*=7.0 Hz). MS *m/z*: 274 (M^+), 259, 231.

Alkaline Treatment of 6——A solution of **6** (10 mg) in *tert*-BuOH (1 ml) was mixed with 50% KOH solution (20 μl) and the mixture was stirred at 30—35 °C for 5 min. The mixture was diluted with Et_2O, then washed with H_2O and saturated NaCl solution, and the Et_2O solution was evaporated to dryness. Column chromatography of the mixture with hexane gave (3.0 mg) of **15**. Further elution with ethyl acetate–hexane (1 : 9) gave **13** (1.6 mg) and **14** (0.4 mg). **13**: Oil, $[\alpha]_D$ −18° (*c*=0.16). ^{1}H-NMR δ: 1.60 and 1.61 (each 3H, d, *J*=1.5 Hz), 1.76 (3H, s, 16-H), 2.00 (3H, d, *J*=1.5 Hz, 20-H), 2.72 (1H, ddd, *J*=13.9, 12.1, 9.5 Hz, 2-H), 3.30 (1H, dd, *J*=12.1, 3.7 Hz, 1-H), 4.02 (1H, m, 11-H), 4.87 (2H, s, 17-H), 4.89 (1H, m), 5.15 (1H, br dd, *J*=8.4, 4.8 Hz), 6.20 (1H, s, 13-H). UV λ_{max}^{EtOH} nm (ε): 236 (5000). MS *m/z*: 302 (M^+), 287, 284, 269, 259. **14**: Oil. ^{1}H-NMR δ: 1.59 and 1.62 (each 3H, s), 1.82 and 1.90 (each 3H, s, 16,17-H), 2.07 (3H, d, *J*=1.5 Hz, 20-H), 3.02 (2H, m, 2-H), 3.97 (1H, m, 11-H), 4.96 (1H, m), 5.13 (1H, br t, *J*=7.5 Hz), 6.22 (1H, s, 13-H). UV λ_{max}^{EtOH} nm (ε): 264 (20000). MS *m/z*: 302 (M^+), 287, 284, 269, 259. **15**: Oil, $[\alpha]_D$ −53° (*c*=0.30). ^{1}H-NMR δ: 1.37 (3H, s), 1.54 (3H, s), 1.75 (3H, s, 16-H), 1.87 (3H, s, 20-H), 3.24 (1H, dd, *J*=11.7, 4.0 Hz, 1-H), 4.79 and 4.89 (each 1H, s, 17-H), 4.82 and 4.98 (each 1H, m), 5.73 (1H, s, 13-H). UV λ_{max}^{EtOH} nm (ε): 226 (shoulder,

8800). MS *m/z*: 284 (M^+), 269, 201, 173, 148 (base peak), 133.

References and Notes

1) Part V: M. Kobayashi, K. Kondo, K. Osabe, and H. Mitsuhashi, *Chem. Pharm. Bull.*, **36**, 2331 (1988).
2) Cembranoids represent the majority of the lipids of two soft corals, *S. mayi* and *Sarcophyton glaucum*,[1] we collected in Ishigaki Island. Although small amounts of free C_{18} fatty acids and batyl alcohol (monooctadecyl glyceryl ether) were isolated, TLC examination of these two lipid samples indicated that the spots corresponding to triglycerides and fatty acid esters were essentially negligible.
3) M. Kobayashi, *Chem. Pharm. Bull.*, **36**, 488 (1988).
4) M. Kobayashi, T. Isizaka, N. Miura, and H. Mitsuhashi, *Chem. Pharm. Bull.*, **35**, 2314 (1987).
5) *a*) Y. Uchio, H. Nabeya, M. Nakayama, S. Hayashi, and T. Hase, *Tetrahedron Lett.*, **22**, 1689 (1981); *b*) M. Aoki, T. Uyehara, T. Kato, K. Kakubo, and S. Yamaguchi, *Chemistry Lett.*, **1983**, 1121.
6) M. Aoki, T. kato, Y. Uchio, M. Nakayama, and M. Kodama, *Bull. Chem. Soc. Jpn.*, **58**, 779 (1985).
7) The compound was originally designated "13-hydroxyneocembrene" on the basis of their numbering system. In view of the consistency with our and other reports, we adopt here the numbering system and the trivial name of cembrene A, which are currently more widely accepted.
8) *a*) J. C. Coll, S. J. Mitchell, and G. J. Stokie, *Aust. J. Chem.*, **30**, 1859 (1977); *b*) Y. Uchio, S. Equchi, M. Nakayama, and T. Hase, *Chemistry Lett.*, **1982**, 277.
9) Recent studies on the biosynthesis of cyclic monoterpenes using acyclic precursors showed that linalyl pyrophosphate is a more efficient precursor than geranyl or neryl derivatives,[10] though in this case only the *Z*-double bond is possible. Occurrence of (3*Z*)-cembrene A in a termite secretion,[11] and its sesterterpene derivatives in scale insects,[12] suggests that geranyllinalyl pyrophosphate is also a plausible precursor to both (3*E*)- and (3*Z*)-cembrene A.
10) *a*) R. Croteau, *Chem. Rev.*, **87**, 929 (1987); *b*) T. Suga, T. Hirata, T. Aoki, and T. Shishibori, *Phytochemistry*, **25**, 2769 (1986).
11) D. F. Wiemer and J. Meinwald, *J. Org. Chem.*, **44**, 3950 (1979).
12) J. K. Pawlak, M. S. Tempesta, T. Iwashita, K. Nakanishi, and Y. Naya, *Chemistry Lett.*, **1983**, 1069.
13) J. B. Stothers, "Carbon-13 NMR spectroscopy," ed. by A. T. Blomquist and H. Wasserman, Academic Press, New York, 1972, p. 434.
14) R. S. Prasad and S. Dev, *Tetrahedron*, **32**, 1437 (1976).
15) *a*) F. L. Ghisalberti, P. R. Jefferies, and T. A. Mori, *Aust. J. Chem.*, **39**, 1703 (1986); *b*) T. Toda, Y. S. Cheng, and T. Nozoe, *Chem. Pharm. Bull.*, **15**, 903 (1967); *c*) W.-H. Hui and M.-M. Li, *Phytochemistry*, **15**, 1741 (1976); *d*) B. Maurer and A. Grieder, *Helv. Chim. Acta*, **60**, 2177 (1977); *e*) S. Ito, K. Endo, H. Homma, and K. Oka, *Tetrahedron Lett.* **1965**, 3777.
16) An 3,4-epoxy derivative of **5** has been reported. The recorded ^{13}C-NMR signals corresponding to C-1 and C-3 were δ 58.50 and 38.99, but the latter value is probably an error since it is up-field shifted as compared with that of **12** (δ 46.0). B. N. Ravi and D. J. Faulkner, *J. Org. Chem.*, **43**, 2127 (1978).
17) Y. Uchio, S. Eguchi, M. Nakayama, and T. Hase, Abstracts of Papers, 49th Annual Meeting of the Chemical Society of Japan, April 1984, II, p. 1349.
18) J. R. Hanson, "The Chemistry of Terpenes and Terpenoids," ed. by A. A. Newman, Academic Press, London and New York, 1972, p. 158.
19) A. Horeau, "Stereochemistry," Vol. 3, ed. by H. B. Kagan, George Thieme Publishers, Stuttgart, 1977, p. 51.