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Marine Terpenes and Terpenoids. IV.[1] Isolation of New Cembranoid and Secocembranoid Lactones from the Soft Coral *Sinularia mayi*

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Four α-methylene-γ-lactone derivatives, designated as mayolide A to mayolide D, were isolated from the soft coral *Sinularia mayi*. From the spectroscopic data and correlations to known compounds, they were shown to be cembranoid (**2**, **3**, **4**) and unprecedented secocembranoid (**1**) lactone derivatives. Their conformations and relative configurations are discussed on the basis of the common and characteristic nuclear Overhauser effect (NOE) features.

Keywords——Coelenterata; soft coral; *Sinularia mayi*; cembranoid; secocembranoid; α-methylene-γ-lactone; mayolide

Cembranoid diterpenes are found, often in quite large amounts, in the lipids of marine soft corals (alcyonarian) and gorgonians. It is possible to roughly classify soft corals, including their symbiotic zooxanthellae, into two types, those which mainly produce α-methylene lactonic cembranoids and those which produce other types of cembranoids with an intact isopropyl side chain or its derivatives.[2] The most common soft coral, *Sarcophyton* spp., belong to the latter type. Interestingly, sarcophytol A, a simple monohydroxycembratetraene which we isolated from *S. glaucum* many years ago,[3a] was recently shown to have potent anti-tumor-promoting activity in a two-stage carcinogenesis experiment.[3b] In contrast, *Sinularia* spp., which are also common and abundant in coral reefs of Indo-Pacific coastal waters, mainly contain α-methylene lactone derivatives. The previous report showed that the soft coral *Sinularia mayi*, a common species in coral reefs of southern Japan, contains a variety of cembranoid lactones, and small amounts of two new cembranoid diols which are plausible precursors of the α-methylene-γ-lactone derivatives.[1] Further investigation of the more polar cembranoids of *S. mayi* resulted in the isolation of four new compounds, one of which has an unprecedented secocembranoid skeleton. They were obtained by repeated flash chromatography of the lipids of *S. mayi*, in smaller amounts compared with the non-polar cembrane lactones isolated before,[1] and designated as mayolide A to mayolide D (**1**—**4**).

Mayolide A (**1a**), $C_{20}H_{30}O_4$, is a α-methylene-γ-lactone derivative as indicated by its typical infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) [IR: 1760, 1660 cm^{-1}; ^{1}H-NMR: 6.27 (1H, d, J=3.0 Hz), 5.60 (1H, d, J=2.5 Hz)] spectra. The ^{1}H-NMR spectrum showed the presence of two olefinic methyl groups linked to trisubstituted double bonds [δ 1.74 (3H, d, J=1.5 Hz), 1.58 (3H, d, J=1.0 Hz), 5.12 (1H, br d, J=9.5 Hz), 5.06 (1H, br t, J=7.0 Hz)], one primary hydroxyl group (δ 3.67, 1H, dt, J=11.0, 6.5 Hz; 3.71, 1H, dt, J=11.0, 6.5 Hz) and one methylketo group (δ 2.14, s, IR, 1710 cm^{-1}). On acetylation, it gave a monoacetate **1b**. The α-methylene protons of the lactone ring were coupled with a methine (C-1) at δ 3.30 which in turn was coupled with an oxy-methine proton at C-2 [δ 5.34 (dd, J= 9.5, 7.3 Hz)]. This oxy-methine proton was further coupled with a olefinic proton at δ 5.12 (C-3). There were clear nuclear Overhauser effects (NOE) between the protons at δ 3.30 and 5.34, showing the *Z*-fusion of the lactone ring, and between the protons at δ 5.34 and 1.74. Another

NOE was observed between the C-1 proton and the hydroxymethyl group. The spectral data thus obtained satisfied the requisite functionalities for a cembranoid skeleton and also suggested it to be a derivative having terminal hydroxymethyl and methylketo groups due to cleavage at C-12 and C-13. The mass spectrum (MS) and high-resolution mass spectrum of **1a** supported this and showed the molecular ion peak (M^+) at *m/z* 334 and other ions at *m/z* 316 ($M^+ - H_2O$), 289 ($M^+ - C_2H_4OH$), 178 ($C_{11}H_{14}O_2$), 138 ($C_9H_{14}O$), 95 (138 − CH_3CO) and 81 (138 − CH_3COCH_2). The prominent ion at *m/z* 138 is probably due to the cleavage of the doubly allylic bond between C-5 and C-6 with one hydrogen transfer to the fragment which, with a loss of H_2O, appeared at *m/z* 178. This permits the allocation of the trisubstituted double bond at C-7, which is expected from the biogenesis of cembranoids derived from the cyclization of geranylgeranyl pyrophosphate. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1a** (Experimental) fully supported this assignment. The chemical shift of C-19 (16.7 or 15.9 ppm) showed the geometry of C-7 double bond to be *E*,[4] which is also biogenetically normal. This is the first isolation of a secocembranoid from marine sources. However, similar examples have been reported in the steroid field. 9,11-Seco-9-oxo-11-hydroxy marine sterol derivatives were isolated from the gorgonian *Pseudopterogorgia americana*,[5] and a soft coral belonging to *Sinularia* sp.[6] The occurrence of 9,11-dihydroxygorgosterol in gorgonians suggested it to be a precursor to one of the secosteroids found in *P. americana*.[7] It is noteworthy that *Sinularia* sp. has a common cleavage process acting on a cembranoid and on steroids, two different classes of compounds.

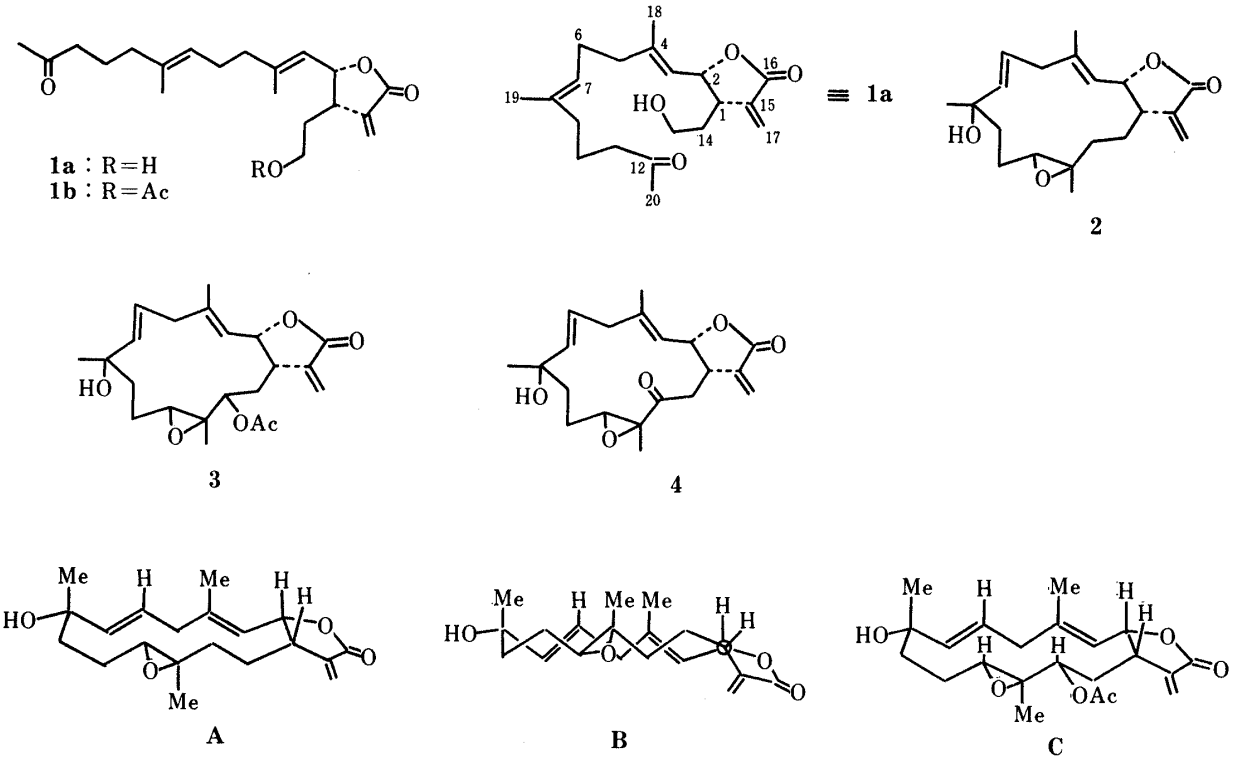

Chart 1

Mayolide B to mayolide D are derivatives of normal cembranoids which had previously been isolated from *S. mayi*.[1,8] Mayolide B (**2**), $C_{20}H_{28}O_4$, was a hydroxy lactone and its IR and ^1H-NMR spectra showed it to contain an α-methylene-γ-lactone ring [IR, 1755, 1660 cm^{-1}; ^1H-NMR, δ 6.27 (1H, d, *J* = 3.0 Hz), 5.54 (1H, d, *J* = 3.0 Hz)]. The signals of the protons at C-1 (δ 3.11, m), C-2 (5.29, dd, *J* = 10.0, 8.5 Hz), C-3 (5.14, br d, *J* = 10.0 Hz), and C-18 (1.79, d, *J* = 1.0 Hz)] were the same as those of **1a**. Other ^1H-NMR signals were those of an *E*-disubstituted double bond (5.51, dt, *J* = 16.0, 1.5 Hz; 5.78, ddd, *J* = 16.0, 6.5, 5.5 Hz),

trisubstituted epoxide (δ 2.87, dd, J=7.0, 4.5 Hz) and two singlets due to methyl groups which are adjacent to oxygen (1.25 and 1.31). The NOE enhancements were observed between the C-1 and C-2 protons, and also between the C-2 and C-18 protons as in **1a**. The C-5 methylene protons [δ 2.83, dd, J=15.0, 6.5 Hz and 2.76, dd, J=15.0, 5.5 Hz) were doubly allylic and coupled with the olefin proton at δ 5.78 (C-6). Irradiation at δ 5.78 showed NOE at the methyl groups at δ 1.79 and 1.31 (C-19). One of the C-5 protons (δ 2.83) showed NOE at the C-3 and C-7 (δ 5.51) protons. The chemical shifts of the epoxide proton and C-5 methylene protons were very similar but they were distinguishable, since no NOE at the epoxy proton was observed when the C-3 proton was irradiated. Weak NOE was observed between C-7 and the epoxide protons. These results clearly indicate the location of the disubstituted double bond and epoxide as shown in **2**. The ^{13}C-NMR signals of **2** due to C-9 to C-14 were virtually the same as those of the previously isolated compound **5** having the same partial structure.[1] The absence of NOE between the C-11 and C-20 protons shows that the C-11 epoxide ring is *E*.

Mayolide C (**3**), $C_{22}H_{30}O_6$, is an acetoxy derivative of **2**. Its IR absorptions (Experimental) showed the presence of an α-methylene-γ-lactone ring as in **2**. Also, its ^{1}H- and ^{13}C-NMR (Experimental) spectra exhibited close similarity with those of **2**, as regards the protons at C-1 to C-7, C-11, and at C-17 to C-20, except that **3** had a secondary acetoxyl group whose methine proton appeared at δ 4.73 (dd, J=10.5, 1.0 Hz). An NOE experiment was carried out in the same way as had been done for **2**, and similar results were obtained. The position of the acetoxyl group was shown to be at C-13 from the NOE found on the protons at C-1, C-2, C-11 and C-18 when the acetoxy methine was irradiated. It appears that the acetoxy methine is sterically close to these protons and is oriented in the same direction with respect to the cembrane ring. The epoxide at C-11 was shown to be *E* from the absence of NOE between the C-11 and C-20 protons.

Mayolide D (**4**), $C_{20}H_{26}O_5$, is also a monooxo derivative of **2**. The IR and ^{1}H- and ^{13}C-NMR data (Experimental) of **4** concerning the common functional groups, as described above for compound **3**, were quite similar to those of **2**. The NOE experiment was carried out in the same way as done for **2** and **3**, and virtually the same results were obtained, supporting the common structure of **2** and **4** except for the presence of one extra carbonyl group in **4**. The major difference was that the C-1 methine proton of **4** was shifted downfield at δ 3.64 and coupled with methylene protons at δ 2.59 (dd, J=20.0, 11.0 Hz) and 2.73 (dd, J=20.0, 4.0 Hz) which are adjacent to a carbonyl group. Prominent NOE was observed between the C-1 proton and the proton at δ 2.73, and between the epoxy proton and the proton at δ 2.59. These results show the location of the carbonyl group at C-13 and indicate the structure of mayolide D to be the 13-oxo derivative of mayolide B. An *E*-junction of the C-11 epoxide was also indicated from the NOE experiment.

The structures of compounds **2** to **4** were supported by their correlation to the known major components (**5**, **6**, **7**) of *S. mayi* isolated previously.[1] Alkaline hydrolysis of **6** in methanol followed by Jones' oxidation gave a ketone **8**. Compound **7** gave **8** on similar alkaline treatment and it was shown to have the same configuration at C-11 and C-12 as **6**. Catalytic hydrogenation of **6** with PtO_2 in ethyl acetate and acetic acid afforded a mixture in which the hexahydro derivative **10** was one of the major product. Dehydration of **3** with phosphoryl chloride followed by catalytic hydrogenation gave a mixture of products from which **10** was isolated and shown to be identical with **10** obtained from **6**. Hydrogenation of **5** and **7** was carried out in ethanol solution in view of the low selectivity in ethyl acetate–acetic acid medium. However, the products were again mixtures in which carboxylic acids predominated due to the hydrogenolysis at C-2. The major products from **5** and **7** obtained after methylation and purification were the octahydro derivatives **9** and **11**, respectively. The products were shown by ^{1}H-NMR to be 1 : 1 (**9**) and 2 : 1 (**11**) isomeric mixtures. Mayolide B (**2**) and mayolide D (**4**) were dehydrated ($POCl_3$–pyridine) and hydrogenated (PtO_2–ethanol),

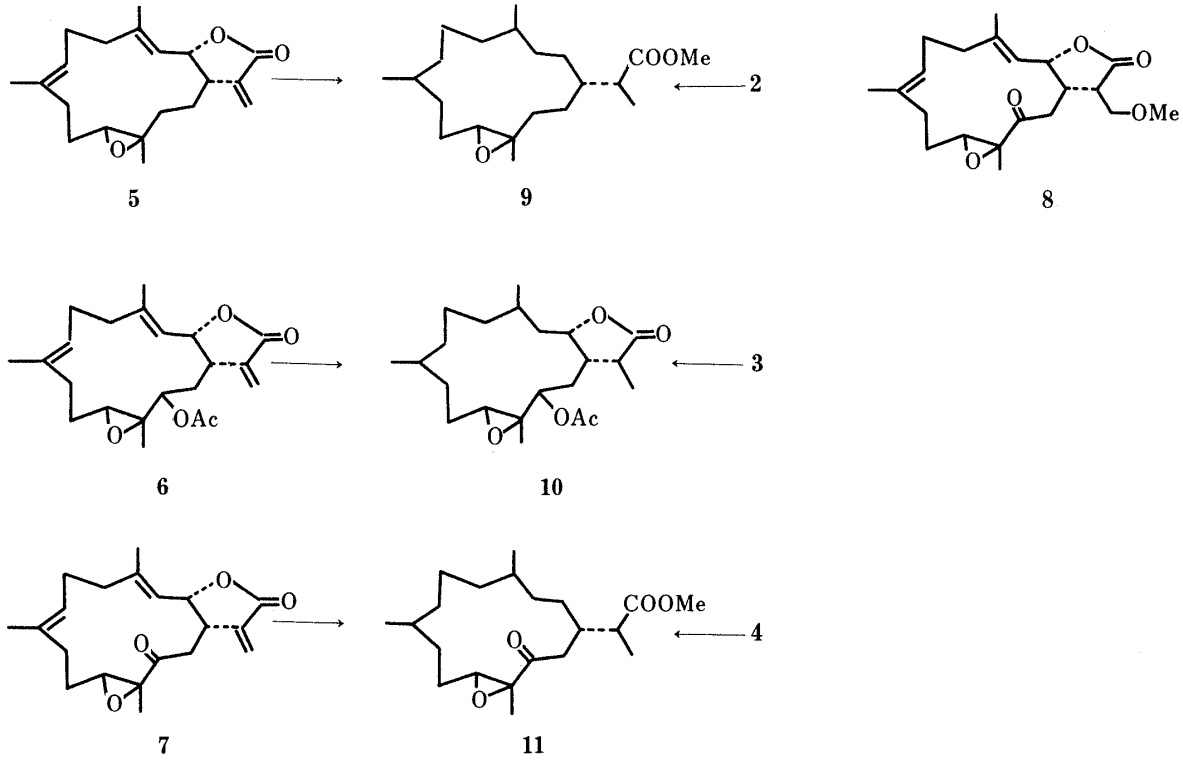

Chart 2

and the products were methylated. Chromatography of the mixtures afforded the methyl esters **9** and **11**, which were identical with those from **5** and **7**, respectively.

The results of the NOE experiments obtained for compounds **2** to **4** enable one to assign some of their conformations in solution and relative configurations. X-Ray crystallographic data of several related cembranoids having similar functionality at C-1 to C-4,[9] show that the C-2 proton and C-4 methyl group are nearly parallel and directed upward from the 14-membered ring, while the C-3 olefin proton is directed downward from the ring. The presence of NOE between one of the C-5 protons and the C-3 and C-7 protons, and between the C-6 proton and the C-18 and C-19 protons suggests that the orientations of the C-6 proton and the methyl group at C-8 are upward, and those of the C-7 proton and one or both of the C-5 methylene protons are downward, with respect to the cembrane ring, in compounds **2**, **3** and **4**. If we assume that the *E*-epoxide rings are oriented outward from the ring, then the conformation of **2** could be roughly presented as A or B (Chart 1) and at least the relative configuration at C-8 in compounds **2** to **4** could be assignable as shown in the chart. Compound **3** is a monoacetoxy derivative of **2**, and only the structure C would be consistent with the spatial arrangement needed for the simultaneous NOE enhancements found at the C-1, C-2, C-11 and C-18 protons on irradiation of the acetoxy methine proton. The configuration of the epoxide group of **4** was assigned as the same as in **3** by indirect correlation through **6** and **7**.

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were determined on a JASCO DIP-4 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were determined, unless otherwise specified, on JNM GX-270 (^{1}H) and JNM FX-90Q (^{13}C) spectrometers at 270 MHz and 22.5 MHz respectively, in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. Mass spectra were determined on a JEOL JMS D300 spectrometer. IR spectra were taken on a JASCO A-102 spectrometer. Column chromatography was carried out by the flash chromatography method.

Isolation of Compounds 1a, 2, 3, and 4——The fractionation of the *S. mayi* extract was described in the previous report and the mixture designated fraction m (4.5 g) was used as the starting material.[1] It was separated on a column of silica gel (4.4 × 13 cm) with a mixture of acetone–$CHCl_3$ (15 : 75) and fractions (16 ml each) were collected. Fractions 13—15 (290 mg) contained a mixture of unidentified carboxylic acids. Fractions 16—28 (660 mg) contained a complex mixture of compounds including the cembranoids **1a**, **2**, **3** and **4**. They were combined and separated into subfractions 1, 2, and 3 on a column of silica gel by elution with ethyl acetate–hexane (6 : 4). Subfraction 1 contained **4**. It was purified by repeated chromatography with 0.6% MeOH in $CHCl_3$ to give 55 mg of **4**. Subfraction 2 contained **2** and **3**. It was purified by chromatography with acetone–$CHCl_3$ (1 : 9) and then acetone–$CHCl_3$ (1 : 12) to give **3** (63 mg) and **2** (137 mg). Subfraction 3 contained **1a**. It was purified by chromatography with acetone–$CHCl_3$ (1 : 9) to give 123 mg of **1a**.

Mayolide A (1a)——Oil, $[\alpha]_D$ −52° (c = 1.76, $CHCl_3$). ^{1}H-NMR δ: see text. ^{13}C-NMR δ: C-1 (40.0), C-2 (77.8), C-3 (124.0), C-4 (135.0), C-5 (38.8 or 39.5), C-6 (25.8), C-7 (119.0), C-8 (139.1), C-9 (39.5 or 38.8), C-10 (21.9), C-11 (43.0), C-12 (209.4), C-13 (59.4), C-14 (31.1), C-15 (143.4), C-16 (170.4), C-17 (121.3), C-18 (15.9 or 16.7), C-19 (16.7 or 15.9), C-20 (30.0). UV λ_{max}^{MeOH} nm (ε): 206 (17000). IR ν_{max}^{neat} cm^{-1}: 3450, 1760, 1710, 1660, 955. MS m/z: 334 (M^+), 316 ($M^+ - H_2O$), 289 ($M^+ - C_2H_4OH$), 178 ($C_{11}H_{14}O_2$), 138 ($C_9H_{14}O$), 121, 95, 81, 43. High-resolution MS [Found (Calcd)] m/z: $C_{20}H_{28}O_3$ ($M^+ - H_2O$), 316.20392 (316.20382); $C_{11}H_{14}O_2$, 178.10023 (178.09943); $C_9H_{14}O$, 138.10513 (138.10453); C_9H_{13}, 121.10160 (121.10170). Compound **1a** (16.8 mg) was acetylated in a mixture of acetic anhydride–pyridine (1 : 4) at room temperature overnight. After the usual work-up, the mixture was purified by chromatography with $CHCl_3$ to give **1b** (9.9 mg). Oil, $[\alpha]_D$ −58° (c = 0.99, $CHCl_3$). ^{1}H-NMR δ: 1.58 (3H, br s, 19-H), 1.76 (3H, d, J = 1.0 Hz, 18-H), 2.05 (3H, s, OAc), 2.13 (3H, s, 20-H), 2.38 (2H, t, J = 7.3 Hz, 11-H), 3.18 (1H, qt, J = 7.3, 3.0 Hz, 1-H), 4.08 (1H, dt, J = 11.5, 6.5 Hz, 13-H), 4.11 (1H, dt, J = 11.5, 6.5 Hz, 13-H), 5.06 (1H, br t, J = 7.0 Hz, 7-H), 5.10 (1H, br d, J = 10.0 Hz, 3-H), 5.33 (1H, dd, J = 10.0, 7.3 Hz, 2-H), 5.58 (1H, d, J = 2.5 Hz, 17-H), 6.28 (1H, d, J = 3.0 Hz, 17-H). UV λ_{max}^{MeOH} nm (ε): 206 (17000). IR ν_{max}^{neat} cm^{-1}: 1760, 1735, 1710, 1660, 1240. MS m/z: 376 (M^+), 358 (probably $M^+ - H_2O$ after recombination of the lactone ring), 316 (M^+ − AcOH), 205, 178, 138, 95, 81.

Mayolide B (2)——Oil, $[\alpha]_D$ +35° (c = 1.07, $CHCl_3$). ^{1}H-NMR (500 MHz) δ: 1.25 (3H, s, 20-H), 1.31 (3H, s, 19-H), 1.79 (3H, d, J = 1.0 Hz, 18-H), 2.76 (1H, dd, J = 15.0, 5.5 Hz, 5-H), 2.83 (1H, dd, J = 15.0, 6.5 Hz, 5-H), 2.87 (1H, dd, J = 7.0, 4.5 Hz, 11-H), 3.11 (1H, m, 1-H), 5.14 (1H, br d, J = 10.0 Hz, 3-H), 5.29 (1H, dd, J = 10.0, 8.5 Hz, 2-H), 5.51 (1H, dt, J = 16.0, 1.5 Hz, 7-H), 5.54 (1H, d, J = 3.0 Hz, 17-H), 5.78 (1H, ddd, J = 16.0, 6.5, 5.5 Hz, 6-H), 6.27 (1H, d, J = 3.0 Hz, 17-H). ^{13}C-NMR δ: C-1 (41.8), C-2 (77.3), C-3 (124.3), C-4 (138.3), C-5 (42.7 or 40.5), C-6 (120.8), C-7 (137.9), C-8 (72.6), C-9 (40.5 or 42.7), C-10 (24.0), C-11 (60.4), C-12 (60.6), C-13 (32.9), C-14 (23.2), C-15 (140.7), C-16 (170.3), C-17 (121.7), C-18 (16.8), C-19 (28.5), C-20 (18.4). UV λ_{max}^{MeOH} nm (ε): 206 (13000). IR ν_{max}^{neat} cm^{-1}: 3400, 1755, 1660, 960. MS m/z: 332 (M^+), 314 ($M^+ - H_2O$), 299 ($M^+ - H_2O$, CH_3). High-resolution MS [Found (Calcd)] m/z: $C_{20}H_{28}O_4$ (M^+), 332.19772 (332.19872).

Mayolide C (3)——Oil, $[\alpha]_D$ +19° (c = 1.98, $CHCl_3$). ^{1}H-NMR (500 MHz) δ: 1.20 (3H, s, 20-H), 1.32 (3H, s, 19-H), 1.88 (3H, d, J = 1.0 Hz, 18-H), 2.15 (3H, s, OAc), 2.74 (1H, dd, J = 14.5, 8.0 Hz, 5-H), 2.80 (1H, dd, J = 14.5, 6.0 Hz, 5-H), 2.80 (1H, t, J = 6.0 Hz, 11-H), 3.20 (1H, m, 1-H), 4.73 (1H, dd, J = 10.5, 1.0 Hz, 13-H), 5.07 (1H, br d, J = 10.5 Hz, 3-H), 5.38 (1H, dd, J = 10.5, 8.0 Hz, 2-H), 5.52 (1H, d, J = 3.5 Hz, 17-H), 5.53 (1H, dt, J = 15.5, 1.0 Hz, 7-H), 5.78 (1H, ddd, J = 15.5, 7.5, 6.0 Hz, 6-H), 6.27 (1H, d, J = 3.5 Hz, 17-H). ^{13}C-NMR δ: C-1 (38.7), C-2 (76.9), C-3 (124.4), C-4 (138.4), C-5 (43.0 or 39.9), C-6 (119.9), C-7 (138.6), C-8 (72.4), C-9 (39.9 or 43.0), C-10 (23.8), C-11 (61.5), C-12 (61.8), C-13 (73.0), C-14 (28.0), C-15 (142.6), C-16 (169.9), C-17 (121.1), C-18 (16.7), C-19 (28.7), C-20 (13.1), OAc (20.8). UV λ_{max}^{MeOH} nm (ε): 206 (18000). IR ν_{max}^{neat} cm^{-1}: 3450, 1760, 1735, 1600, 1240, 970. MS m/z: 390 (M^+), 372 ($M^+ - H_2O$), 330 (M^+ − AcOH). High-resolution MS [Found (Calcd)] m/z: $C_{22}H_{30}O_6$ (M^+), 390.20263 (390.20413).

Mayolide D (4)——Oil, $[\alpha]_D$ +33° (c = 0.84, $CHCl_3$). ^{1}H-NMR δ: 1.33 (3H, s, 19-H), 1.41 (3H, s, 20-H), 1.68 (3H, d, J = 1.5 Hz, 18-H), 2.59 (1H, dd, J = 20.0, 11.0 Hz, 14-H), 2.70 (1H, dd, J = 15.0, 7.5 Hz, 5-H), 2.73 (1H, dd, J = 20.0, 4.0 Hz, 14-H), 2.78 (1H, dd, J = 15.0, 5.0 Hz, 5-H), 3.13 (1H, dd, J = 7.0, 5.5 Hz, 11-H), 3.64 (1H, m, 1-H), 4.86 (1H, br d, J = 10.5 Hz, 3-H), 5.47 (1H, br d, J = 16.0 Hz, 7-H), 5.50 (1H, d, J = 3.0 Hz, 17-H), 5.56 (1H, dd, J = 10.5, 8.0 Hz, 2-H), 5.68 (1H, ddd, J = 16.0, 7.5, 5.0 Hz, 6-H), 6.28 (1H, d, J = 3.5 Hz, 17-H). ^{13}C-NMR δ: C-1 (36.9), C-2 (76.7), C-3 (124.9), C-4 (137.6), C-5 (43.3 or 39.8), C-6 (119.5), C-7 (138.6), C-8 (72.5), C-9 (39.8 or 43.3), C-10 (24.1), C-11 (60.8), C-12 (63.9), C-13 (208.2), C-14 (35.8), C-15 (143.0), C-16 (169.8), C-17 (121.1), C-18 (16.1), C-19 (28.7), C-20 (12.3). UV λ_{max}^{MeOH} nm (ε): 207 (18000). IR ν_{max}^{neat} cm^{-1}: 3450, 1760, 1710, 1660, 960. MS m/z: 346 (M^+), 328 ($M^+ - H_2O$), 318, 317, 289, 271, 257. High-resolution MS [Found (Calcd)] m/z: $C_{20}H_{26}O_5$ (M^+), 346.17535 (346.17795).

Conversion of 6 and 3 to 10——(a) A solution of **6** (161 mg) in a mixture of ethyl acetate–acetic acid (3 : 1, 5 ml) was hydrogenated with PtO_2 catalyst (80 mg) for 2 h. The mixture was filtered, the solvent was evaporated off under reduced pressure, and the residue was submitted to silica gel column chromatography. Elution with ethyl acetate–hexane (3 : 7) gave more polar (45 mg) and less polar (114 mg) mixtures. A portion (37.4 mg) of the less polar mixture was submitted to preparative thin-layer chromatography (TLC) and developed with $CHCl_3$ twice, giving two bands. Extraction of the upper band with ethyl acetate gave the major product **10** (17 mg). $[\alpha]_D$ −11° (c = 1.69, $CHCl_3$). ^{1}H-NMR δ: 0.93 (6H, d, J = 6.6 Hz, 18, 19-H), 1.15 (3H, d, J = 7.4 Hz, 17-H), 1.23 (3H, s, 20-H), 2.16 (3H, s, OAc), 2.36

(1H, m, 1-H), 2.79 (1H, quint, $J=7.3$ Hz, 15-H), 3.01 (1H, dd, $J=8.5$, 4.5 Hz, 11-H), 4.50 (1H, ddd, $J=12.0$, 5.0, 2.5 Hz, 2-H), 5.35 (1H, br d, $J=9.7$ Hz, 13-H). MS *m/z*: 338, 320 ($M^+ - AcOH$), 310, 292, 265, 264, 252, 95. FD-MS *m/z*: 381 (MH^+), 380 (M^+).

(b) Phosphoryl chloride (10 μg) was added to a solution of **3** (5.3 mg) in 0.1 ml of pyridine. After 30 min, three drops of H_2O were added and the mixture was extracted with Et_2O. The Et_2O layer was washed with H_2O, 5% HCl solution, H_2O, and saturated NaCl solution and the solvent was evaporated off. The residue was hydrogenated in ethyl acetate–acetic acid (3 : 1, 1.8 ml) with PtO_2 (5 mg). The mixture was filtered, the filtrate was evaporated, and the residue was submitted to preparative TLC as done for **6** in (**a**) The band corresponding to **10** was extracted with ethyl acetate. Evaporation of the solvent gave 0.5 mg of pure material which was shown to be identical with **10** by silica gel TLC [$CHCl_3$; ethyl acetate–hexane (3 : 7); Et_2O–hexane (1 : 1); MeOH–benzene (5 : 95)]. MS *m/z*: 338, 320 ($M^+ - AcOH$), 310, 292, 265, 264, 252, 95. FD-MS *m/z*: 381 (MH^+), 380 (M^+).

Conversion of 5 and 2 to 9——(a) A solution of **5** (98.9 mg) in ethanol (4 ml) was hydrogenated with PtO_2 catalyst (9.6 mg) for 1 h. The mixture was filtered, the filtrate was evaporated under reduced pressure, and the residue was dissolved in ethereal diazomethane solution. After 10 min, the solvent was evaporated off and the residue was submitted to silica gel column chromatography. Elution with ethyl acetate–hexane (3 : 97) afforded 33.8 mg of **9** which was homogeneous on several TLC systems but was found to be a 1 : 1 isomeric mixture by ^{1}H-NMR. ^{1}H-NMR δ: 0.83, 0.85, 0.89, 0.92 (each d, $J=6.5$ Hz, 18, 19-H), 1.10, 1.12 (each d, $J=7.0$ Hz, 17-H), 1.26 (s, 20-H), 2.52 (m, 15-H), 2.84 (dd, $J=8.1$, 4.8 Hz, 11-H), 2.70 (dd, $J=9.5$, 2.5 Hz, 11-H), 3.67, 3.68 (OCH_3). MS *m/z*: 338 (M^+), 320, 306, 279, 250, 243.

(b) Phosphoryl chloride (25 μg) was added to a solution of **2** (8.0 mg) in pyridine (0.1 ml) and the mixture was kept at room temperature for 30 min. The mixture was worked up in the same way as done for 3 above. The product obtained was hydrogenated in ethanol (1 ml) with 2.2 mg of PtO_2 catalyst for 1 h and the mixture was filtered. The filtrate was evaporated and the residue was dissolved in ethereal diazomethane solution. After 10 min the solvent was evaporated off and the residue was submitted to silica gel column chromatography. Elution with ethyl acetate–hexane (3 : 97) gave 0.7 mg of **9** which was identical with **9** obtained from **5** as judged by TLC [ethyl acetate–hexane (12 : 88)] and MS. MS *m/z*: 338 (M^+), 320, 306, 279, 250, 243.

Conversion of 7 and 4 to 11——(a) A solution of **7** (153.4 mg) in ethanol (3.5 ml) was hydrogenated with PtO_2 catalyst (13.4 mg) for 1 h. The mixture was filtered, the filtrate was evaporated under reduced pressure and the residue was dissolved in ethereal diazomethane solution. After 10 min the solvent was evaporated off and the residue was submitted to silica gel column chromatography. Elution with ethyl acetate–hexane (3 : 97) afforded 54 mg of **11**. It was homogeneous on TLC but was found by ^{1}H-NMR to be a 2 : 1 isomeric mixture. ^{1}H-NMR δ: 0.89, 0.92 (each d, $J=6.5$ Hz, 18, 19-H), 1.13 (d, $J=7.0$ Hz, 17-H), 1.45 (s, 20-H), 1.87 (dd, $J=17.2$, 4.2 Hz, 14-H), 2.36 (m, 1-H), 2.53 (quint, $J=7$ Hz, 15-H), 2.68 (dd, $J=17.2$, 9.3 Hz, 14-H), 3.14 (dd, $J=6.5$, 6.0 Hz, 11-H). MS *m/z*: 352 (M^+), 321, 320, 309, 302, 292, 279, 264, 237, 198.

(b) Phosphoryl chloride (17 μl) was added to the solution of **4** (8.3 mg) in pyridine (0.15 ml) and kept at room temperature for 90 min. The mixture was worked up in the same way as done for **3** above. The product obtained was hydrogenated in ethanol (0.6 ml) with PtO_2 catalyst (1.6 mg) for 1 h. The mixture was filtered and the filtrate was evaporated. The residue was dissolved in ethereal diazomethane solution. After 10 min, the solvent was evaporated off and the residue was submitted to silica gel column chromatography. Elution with ethyl acetate–hexane (1 : 30) afforded 1.5 mg of **11**, which was identical with the compound obtained from **7** as judged by TLC [ethyl acetate–hexane (1 : 9); Et_2O–hexane (15 : 85)] and MS. MS *m/z*: 352 (M^+), 321, 320, 309, 302, 292, 279, 237, 198.

Conversion of 6 and 7 to 8——(a) Compound **6** (61 mg) was dissolved in 3% KOH in methanol solution (4 ml) and kept at room temperature for 1 h. The mixture was diluted with H_2O, extracted with Et_2O and worked up as usual. Jones' reagent (2 ml) was added gently to the solution and the mixture was stirred for 30 s. The mixture was washed with H_2O, 1 N NaOH solution, 5% HCl solution, H_2O, and saturated NaCl solution. The solvent was evaporated off and the residue gave, after purification by silica gel column chromatography [ethyl acetate–hexane (15 : 85)], 41 mg of **8**. $[\alpha]_D$ +10° ($c=0.88$, $CHCl_3$). ^{1}H-NMR δ: 1.41 (3H, s, 20-H), 1.62, 1.65 (each 3H, s, 18, 19-H), 2.49 (1H, ddd, $J=12.1$, 5.9, 4.0 Hz, 15-H), 2.57 (1H, dd, $J=19.5$, 11.5 Hz, 14-H), 2.75 (1H, dd, $J=19.5$, 3.3 Hz, 14-H), 3.01 (1H, dd, $J=8.1$, 4.0 Hz, 11-H), 3.12 (1H, m, 1-H), 3.33 (3H, s, OCH_3), 3.59 (1H, dd, $J=9.9$, 5.9 Hz, 17-H), 3.66 (1H, dd, $J=9.9$, 4.0 Hz, 17-H), 4.97 (1H, t, $J=7$ Hz, 7-H), 4.99 (1H, d, $J=10.6$ Hz, 3-H), 5.49 (1H, dd, $J=10.6$, 8.0 Hz, 2-H). MS *m/z*: 362 (M^+), 347, 344, 330, 312, 299, 259.

(b) Compound **7** (90 mg) was dissolved in 3% KOH in methanol solution and kept at room temperature for 30 min. The mixture was diluted with H_2O and extracted with Et_2O. After the usual work-up, the solvent was evaporated off. Silica gel column chromatography of the residue with ethyl acetate–hexane (15 : 85) afforded 90 mg of **8**, $[\alpha]_D$ +10° ($c=1.04$, $CHCl_3$). The ^{1}H-NMR spectrum and MS were identical with those of the product obtained in (a).

References and Notes

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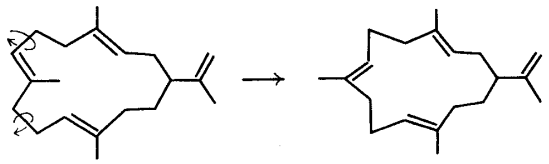
8) The representation A, currently used for the simple hydrocarbon cembrene A for instance, is inconvenient because the carbons in the 14-membered ring are not connected equally by zig-zag lines and also because it gives the unrealistic impression that one of the methyl groups (C-19) is oriented to the center of the ring. This is the reason why we adopt in the present work, and recommend to other workers, the less defective representation B, derived simply by rotating the C-6, 7 and C-9, 10 bonds of the conventional representation (A).



A B

Chart 3

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