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Three New Oxylipins Related to 3,6-Dioxo-4-docosenoic Acid from Okinawan Marine Sponges, *Plakortis* spp.

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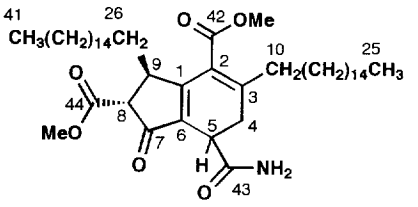
Abstract: Manzamenones J (**1**) and K (**2**) and plakoridine B (**3**), three new oxylipins with unique carbon-skeletons related to 3,6-dioxo-4-docosenoic acid, were isolated from Okinawan marine sponges, *Plakortis* spp., and their structures elucidated on the basis of spectral and chemical means. Absolute stereochemisty of manzamenone A (**4**) was investigated by appling the modified Mosher's method developed recently for secondary carboxylic acids by Kusumi.

Marine sponges of the genus *Plakortis* have been recognized as a source of novel oxidized fatty acid-derived substances known as oxylipins.[1] During our studies on bioactive substances from Okinawan marine organisms,[2] we have investigated extracts of the *Plakortis* sponges, and isolated several types of aliphatic secondary metabolites with chemically unique structures including plakorin,[3] plakotenin,[4] manzamenones,[5,6] untenone A,[7] and plakoridine A.[8] In connection with our interest in the biosynthesis of these unique lipids, we further examined the constituents of the same *Plakortis* sponges, and have now isolated three new oxylipins, manzamenones J (**1**) and K (**2**) and plakoridine B (**3**). Compound **1** is a new manzamenone congener containing a different conjugation system, while compounds **2** and **3** possess new backbone frameworks, particularly **2** consisting of cyclopentenone and dioxabicylclo[3.3.0]octane moieties. Obviously all of these metabolites are biogenetically related and 3,6-dioxo-4-docosenoic acid may be a common key intermediate. Here we also describe the study on the absolute stereochemistry of manzamenone A (**4**)[5] by application of the modified Mosher's method developed recently by Kusumi and coworkers for secondary carboxyl groups.[9]

The sponge *Plakortis* sp., collected off Manzamo, Okinawa, was extracted with MeOH and partitioned between EtOAc and water. The EtOAc-soluble fraction was subjected to silica gel column chromatography, followed by gel filtration on Sephadex LH-20. The fraction containing mainly a mixture of manzamenones[5,6] was finally purified by reversed-phase HPLC to afford manzamenones J (**1**, 0.0003 % yield based on wet weight) and K (**2**, 0.0002 %). From another *Plakortis* sponge, collected off Unten-harbor, Okinawa, the new pyrrolidine alkaloid, plakoridine B (**3**) was isolated in 0.0002 % yield (wet weight) from the EtOAc-soluble fraction of the MeOH extract after similar treatment.[10]

Manzamenone J (**1**) was shown to have the molecular formula of $C_{46}H_{79}O_6N$ by HRFABMS data [*m/z* 742.5986, $(M+H)^+$, Δ 0.0 mmu], which was the same composition as that of manzamenone D (**5**).[5] The UV and IR spectra of **1** were suggestive of the presence of conjugated dienone (λ_{max} 317 nm), ester (ν_{max} 1720 cm^{-1}), and amide (ν_{max} 1680 cm^{-1}) functionalities. Analysis of the ^1H and ^{13}C NMR spectra of **1** aided



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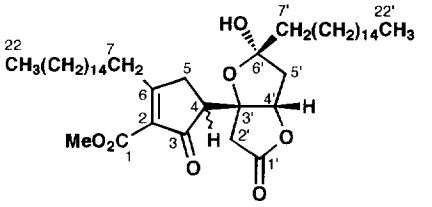

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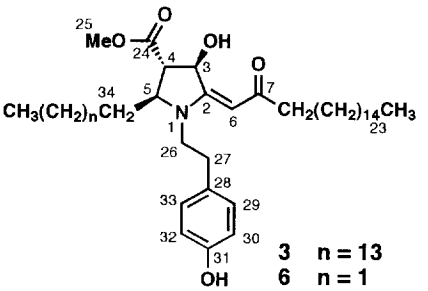
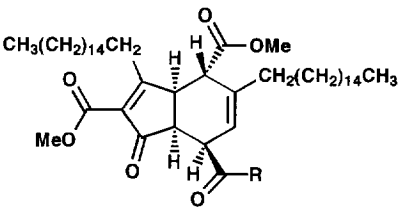
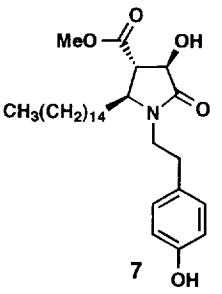


3 n = 13
6 n = 1



4 R = OH
5 R = NH_2
10 R = $NH\overset{*}{C}H(Ph)CO_2Me$



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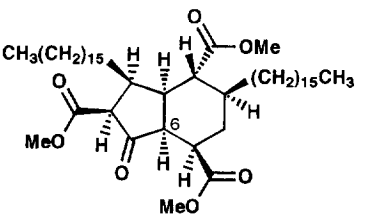
$HO_2C(CH_2)_{15}CH_3$

8



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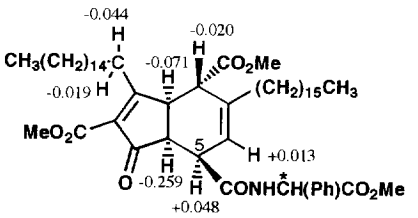
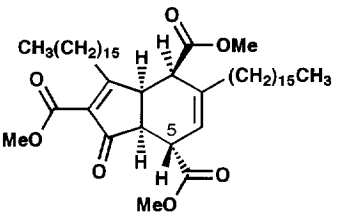


10a



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by comparison with the data of compound **5**[5] revealed that **1** contained two methoxycarbonyls, one primary amide (δ_H 6.08 and 4.40), two tetrasubstituted olefins, three methines, and two long unbranched alkyl chains. The ^1H-^1H COSY spectrum of **1** showed two proton connectivity networks; one consisted of one methylene with one methine (H_2-4 and H-5) and the other of two methine protons (H-8 and H-9) with methylene protons (H_2-26) which were further correlated to the huge methylene envelope (δ_H 1.3 ~ 1.6). In the HMBC spectrum of **1**, cross-peaks due to long-range ^1H-^{13}C couplings were observed for H_2-4/C-2, H-5/C-1, H-5/C-3, H-5/C-6, H-5/C-7, H-8/C-7, and H-9/C-1, which were diagnostic for elucidating the carbon framework of **1** to lead to a bicyclo[4.3.0]nonane ring system involving a 1(6),2-dien-7-one chromophore. The HMBC correlations for H-5/C-43, 43-NH_2/C-5, H-8/C-44, and 44-OMe/C-44 indicated that the primary amide and one of the two methoxycarbonyl groups are attached at C-5 and C-8 positions, respectively. One of the two alkyl groups was shown to be substituted on C-9 by the COSY spectrum as described above, while the other alkyl group was suggested to be on C-3 on the basis of NOESY cross-peaks observed clearly for H-4a/H_2-10 and H-4a/H_2-11. The remaining one methoxycarbonyl group therefore had to be placed on C-2. The FABMS of **1** showed intense peaks at *m/z* 516 and 441, which were ascribable to the fragment ions due to $(M - C_{16}H_{33})^+$ and $(M - CONH_2 - OMe - C_{16}H_{33})^+$, respectively, implying that each of two alkyl chains is a hexadecyl group [-$(CH_2)_{15}CH_3$]; this unit is generally contained in the oxylipins obtained from *Plakortis* sponges.[1,3-8] The vicinal methine protons on C-8 and C-9 were suggested to be anti since the NOESY spectrum of **1** showed substantial correlations from H-8 to H_2-26 and H_2-27, while the cross-peak from H-8 to H-9 was only weakly observed. The stereochemistry of C-5 position relative to C-8 and C-9 portion remained unassigned since no appreciable NOESY data were obtained between the two isolated moieties. From these results, the structure of manzamenone J was concluded as **1**.

Manzamenone K (**2**) exhibited a pseudomolecular ion $(M-H)^-$ at *m/z* 729 and an $(M-H_2O+H)^+$ ion at *m/z* 713 in the negative and positive FABMS, respectively. The high-resolution analysis of the latter ion showed the molecular formula of **2** as $C_{45}H_{78}O_7$ [*m/z* 713.5737, $(M-H_2O+H)^+$, Δ +1.7 mmu]. The UV absorption maximum of **2** (λ_{max} 225 nm) implied the presence of an enone group. Comparison of the ^{13}C NMR chemical shifts for the cross-conjugated enone moiety (C-1 ~ C-3 and C-6) with those of manzamenone A (**4**) suggested the presence of a cyclopentenone ring system substituted by a methoxycarbonyl and an aliphatic chain at the C-2 and C-6 positions, respectively, which was corroborated by the HMBC cross-peaks of **2** for CH_3O/C-1, H-4/C-3, H_2-5/C-2, H_2-7/C-2, H_2-7/C-5, and H_2-7/C-6. Except for the alkyl chains the remaining part of the molecule was inferred to consist of an oxymethine (δ_H 5.56; δ_C 86.3), an oxygenated quaternary carbon (δ_C 89.0), a hemiketal (δ_C 108.6), an ester carbonyl (δ_C 173.8), and two methylenes whose protons resonated unequivalently (δ_H 2.76/2.18; 2.35/2.20). The deuterium induced shift in the ^{13}C NMR signals between $CDCl_3$ and $CDCl_3$-D_2O solutions identified that the hydroxyl group was attached only on the hemiketal carbon (C-6'; Δ 0.09 ppm). The HMBC spectrum of **2** revealed the connectivities for H_2-2'/C-1', H_2-2'/C-4', H-4'/C-1', H-4'/C-6', H_2-7'/C-5', and H_2-7'/C-6', giving rise to the dioxabicylo-[3.3.0]octane ring system with an alkyl chain attached to the C-6' position. The γ-lactone moiety (HMBC: H-4'/C-1') had also been indicated by the IR band (ν_{max} 1750 cm^{-1}), and was also embraced in the *Plakortis* metabolites isolated recetnly.[11] Thus, compound **2** was shown to be composed of two moieties (a cyclopentenone and a dioxabicylo[3.3.0]octane rings), which were linked between C-4 and C-3' by the HMBC correlations [H-4/C-3', H-5 (δ_H 2.29)/C-3', and H-2'(δ_H 2.18)/C-4]. The EIMS of **2** showed intense peaks at *m/z* 638 $(M - H_2O - MeOH - CH_2CO)^+$ and 414, the difference of which (224 amu) corresponded to a hexadecyl group ($C_{16}H_{33}$

– H) assignable to the two aliphatic chains attached at C-6 and C-6' of **2**. The planar structure of manzamenone K was thus elucidated as **2**. The NOESY spectrum of **2** revealed a cross-peak between H-4 and H-4', indicating the C-3'/C-4' juncture to be *cis*, which was also suggested from the model consideration. The NOESY correlations were also observed from H-4 to Ha-7' (δ_H 2.32) as well as from H-4' to Hb-7' (δ_H 2.08), thus implying that H-4, H-4', and H_2-7' are located on the same side of the tetrahydrofuran ring. The relative stereochemistry of the C-4 position remains unassigned.

Plakoridine B (**3**) had a molecular formula of $C_{47}H_{81}O_5N$, revealed by the HRFABMS data [*m/z* 740.6215, $(M+H)^+$, Δ +2.2 mmu]. The ^1H and ^{13}C NMR spectra of **3** were almost identical with those of plakoridine A (**6**),[8] having a pyrrolidine skeleton with two aliphatic side chains. In the ^1H NMR spectrum of **3**, the two terminal methyl protons were observed as an overlapped triplet (6H), while the two terminal methyls of **6** were observed as two separate signals, indicating that the second alkyl chain of **3** is sufficiently long. The molecular weight of **3** was different from **6** by 168 amu, corresponding to twelve CH_2 units. Compound **3** was therefore reasonably assigned to a homologue of plakoridine A (**6**) with different length of the alkyl side chain. To elucidate the length of alkyl chains, plakoridine B (**3**) was treated with ozone and subsequently reduced with dimethyl sulfide to give a lactam (**7**)[12] and heptadecanoic acid (**8**), which were detected by EIMS analysis [*m/z* 489 (M^+ for **7**) and *m/z* 270 (M^+ for **8**)]. These findings firmly established the structure of plakoridine B as **3**. The optical rotation of plakoridine B (**3**) as well as that of plakoridine A (**6**)[8,13] was revealed as small as zero, and the CD spectra of **3** and **6** showed no characteristic curves.

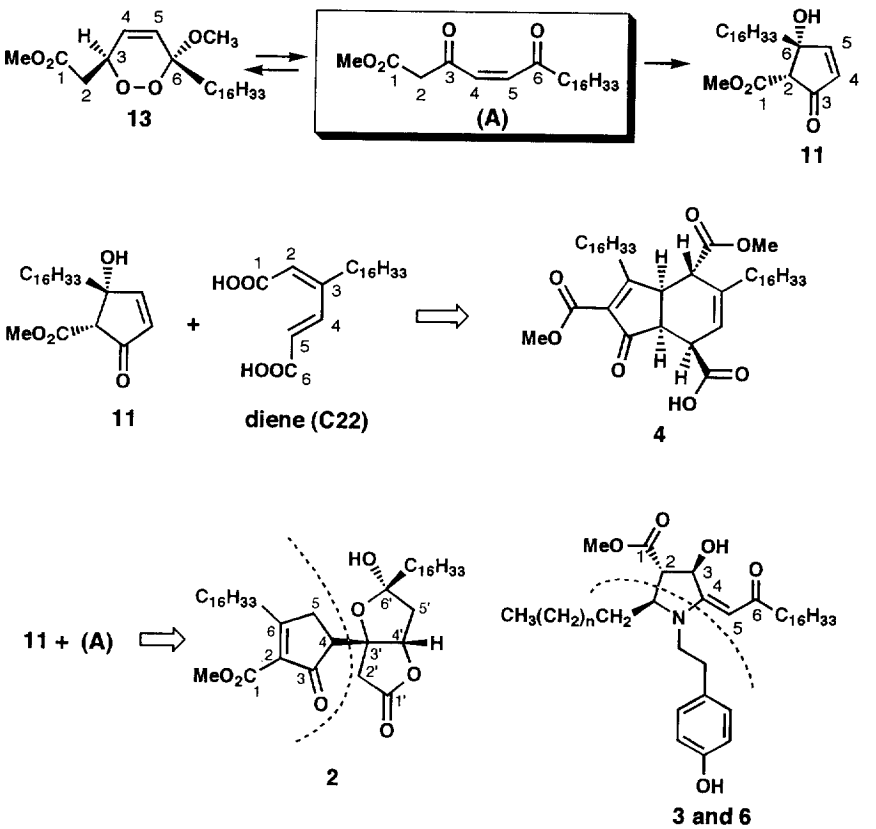
Manzamenone A (**4**) is the most abundant constituent of those having a bicyclo[4.3.0]nonane skeleton isolated from the two species of Okinawan *Plakortis*. The absolute stereochemistry of this compound has been left unassigned.[5] The CD spectrum of the tetrahydro derivative (**9**)[5] of the methyl ester of **4** was previously recorded to show a negative Cotton effect [λ_{ext} 325 nm ($\Delta\varepsilon$ -0.27) and 286 nm ($\Delta\varepsilon$ +1.0)]. Based on the chiroptic data of *cis*-hexahydroinda-1-ones,[14] 6*S*-configuration was inferred for **9**. Further investigations, however, were required to confirm the assignment since the structure of **9** is much different from those in the literature.[14] Recently Kusumi and coworkers[9] demonstrated a new methodology to determine the absolute configurations of secondary carboxyl groups based on the ^1H NMR data of their amides of (*R*)- and (*S*)-phenylglycine methyl esters (PGME),[15] which was evolved from modified Mosher's method.[16] Accordingly, manzamenone A (**4**) was treated with (*R*)- and (*S*)-PGME in the presence of BOP reagent[17] and Et_3N in CH_3CN. Although partial racemization at the PGME moiety occurred during the reaction, (*R*)- and (*S*)-PGME amides of **4**, (*R*)- and (*S*)-**10**, respectively, were obtained after HPLC separation. The $\Delta\delta$ (δ_S - δ_R) values obtained from the ^1H NMR data of (*R*)- and (*S*)-**10** in $CDCl_3$ are shown in **10a**, which implied 5*R*-configuration for **4**, being consistent with the inference from the CD data of **9**.

Here we described the gross structures of three new oxylipins isolated from Okinawan *Plakortis* sponges as well as the study on the absolute stereochemistry of manzamenone A (**4**). Manzamenone J (**1**) possesses a common bicyclo[4.3.0]nonane ring system found in manzamenones A ~ F and H, but the conjugation system [1(6),2-dien-7-one] of **1** is new. Manzamenone K (**2**) comprises two structural components, and the cyclopentenone moiety is reminiscent of the structure of untenone A (**11**), isolated from the *Plakortis* sponge by us.[7] Plakoridine B (**3**) is a homologue of plakoridine A (**6**) whose propyl group is replaced by a pentadecyl alkyl chain. The backbone carbon-frameworks of manzamenone K (**2**) and plakoridine B (**3**) are hitherto unknown. All three compounds (**1**-**3**) were revealed to have no cytotoxicity (IC_{50} > 20 μg/mL). From the *Plakortis* sp. collected off Unten-harbor, 5-epi-43-*O*-methylmanzamenone B

(**12**) was isolated in 0.0004% yield (wet weight) and its structure was assigned by spectral data (see, Experimental section).

We previously proposed[7] that untenone A (**11**) having a cyclopentenone structure might be biogenetically derived from a precursor containing 1,6-dicarboxyl groups through Dieckmann reaction. It is another possibility that the cyclopentenone may be derived from a 1,4-dicarbonyl compound, which may be closely related to chondrillin (**13**).[3,18] 1,4-Dicarbonyl compounds are known to be susceptible to intramolecular condensation to give cyclopentenones.[19] These metabolites isolated from *Plakortis* sponges are all likely to be biogenetically related to one another, and 3,6-dioxo-4-docosenoic acid (**A**, methyl ester; Chart 1) may be a common key intermediate.

Chart 1



EXPERIMENTAL

General methods. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. UV and IR spectra were taken on JASCO Ubest-35 and JASCO Report-100 infrared spectrometers, respectively. ^1H and ^{13}C NMR spectra were recorded on Bruker ARX-500 or AMX-600 spectrometers. EI and FAB mass spectra were obtained on a JEOL DX-303 and HX-110 spectrometers, respectively.

Collection, Extraction, and Isolation. The sponge *Plakortis* sp. (2 kg, wet weight), collected off Manzamo, Okinawa, was extracted with MeOH (7.5 L). Evaporation of the solvent afforded a residue (110 g), which was partitioned between 1 M NaCl (600 mL) and EtOAc (600 mL x 3). A part (4.0 g) of the EtOAc-soluble fraction (11 g) was subjected to two silica gel column chromatographies [4.0 x 40 cm, MeOH/$CHCl_3$ (1:9); 2.2 x 40 cm, acetone/hexane (1:3)]. The fraction (350 mg eluted from 590 to 760 mL) containing a mixture of manzamenones was further purified by gel filtration on Sephadex LH-20 [2.0 x 100 cm, MeOH/$CHCl_3$ (1:1)] to give two fractions [i (100-150 mL) and ii (160-460 mL)]. The former fraction (i) was successively separated with reversed-phase HPLC [Develosil ODS-5, (5 μm, 10 x 250 mm); eluent: CH_3CN/$CHCl_3$, 8:2 with 0.01 % trifluoroacetic acid; flow rate: 2.5 mL/min; UV detection at 254 nm] to afford a mixture of manzamenones D and J, which was finally purified by the same HPLC column (eluent: MeOH/$CHCl_3$/H_2O, 6:3.5:1) to give manzamenone J (**1**, t_R 46.0 min, 0.0003 % wet weight). The latter fraction (ii) of gel filtration was also purified by the same HPLC column (eluent: CH_3CN/$CHCl_3$, 8:2) to give manzamenone K (**2**, t_R 27.2 min, 0.0002 %). The EtOAc-soluble portion (5.3 g) of the MeOH extract of another *Plakortis* sponge (1 kg, wet weight), collected at Unten-harbor, Okinawa, was partially (1.0 g) subjected to a silica gel column chromatography [2.4 x 36 cm, EtOAc/hexane (2:8)]. The fraction containing a mixture of manzamenones was further purified by a Sephadex LH-20 column [2.0 x 120 cm, MeOH/$CHCl_3$ (1:1)], followed by separation with reversed-phase HPLC [Develosil ODS-5, (5 μm, 10 x 250 mm); eluent: CH_3CN/$CHCl_3$, 7:3; flow rate: 2.0 mL/min; detection: UV at 254 nm] to afford plakoridine B (**3**, t_R 18.0 min, 0.0001%) together with 5-epi-43-*O*-methylmanzamenone B (**12**, t_R 24.8 min, 0.0004 %).

Manzamenone J (1). Colorless oil; $[\alpha]_D^{17}$ -6.1° (*c* 0.23, MeOH); UV (MeOH) λ_{max} 317 (ε 23300) and 220 nm (39000); IR ($CHCl_3$) ν_{max} 3480, 3370, 1720, 1680, 1625, 1595, and 1565 cm^{-1}; ^1H NMR (C_6D_6) δ_H 6.08 and 4.40 (each 1H br s, 43-NH_2), 3.90 (1H, br d, H-9), 3.43 (3H, s, MeO-44), 3.39 (3H, s, MeO-42), 3.33 (1H, d, H-8), 3.24 (1H, d, H-5), 3.16 (1H, d, H-4a), 2.86 (1H, ddd, H-10a), 2.43 (1H, ddd, H-10b), 2.11 (1H, dd, H-4b), 1.78 (1H, m, H-26a), 1.10 (1H, m, H-26b), 1.6 ~ 1.3 (56H, br s, H_2-11 ~ 24 and H_2-27 ~ 40), and 0.96 (6H, t, H_3-25 and 41), [$J_{4a,4b}$=17.9, $J_{4a,5}$=0, $J_{4b,5}$=9.8, $J_{8,9}$=2.3, $J_{9,26a}$=10.2, $J_{10a,10b}$=15.4, and $J_{24,25}$=$J_{40,41}$=6.6 Hz]; ^{13}C NMR (C_6D_6) δ_C 202.7 (C-7), 171.5 (C-1), 170.8 (C-43), 170.5 (C-44), 166.2 (C-42), 164.5 (C-6), 132.0 (C-3), 123.3 (C-2), 59.2 (C-8), 52.5 (MeO-44), 51.3 (MeO-42), 45.3 (C-9), 36.0 (C-10), 35.0 (C-5), 33.3 (C-26), 32.3 (C-4), 32-23 (C-11 ~ C-24 and C-27 ~ C-40), 14.1 (C-25 and C-41); FABMS (matrix: 3-nitrobenzylalcohol) *m/z* 742 $(M+H)^+$, 710 $(M-OMe)^+$, 697 $(M-CONH_2)^+$, 665 $(M-CONH_2-MeOH)^+$, 516 $(M-C_{16}H_{33})^+$, and 441 $(M-CONH_2-OMe-C_{16}H_{33})^+$; HRFABMS *m/z* 742.5986, calcd for $C_{46}H_{80}O_6N$ (M+H): 742.5986.

Manzamenone K (2). Colorless oil; $[\alpha]_D^{17}$ -5.2° (*c* 0.35, MeOH); UV (MeOH) λ_{max} 225 nm (ε 1200); IR (neat) ν_{max} 3500, 2890, 1750, 1715, and 1480 cm^{-1}; ^1H NMR (C_6D_6) δ_H 5.56 (1H, dd, H-4'), 3.53 (3H, s, MeO), 2.76 (1H, d, H-2'a), 2.62 (1H, m, H-7a), 2.51 (1H, m, H-7b), 2.35 (1H, dd, H-5'a), 2.32 (1H, m, H-7'a), 2.29 (1H, dd, H-5a), 2.20 (1H, dd, H-5'b), 2.18 (1H, d, H-2'b), 2.08 (1H, d, H-7'b), 2.03 (1H, dd, H-5b), 1.81 (1H, dd, H-4), 1.5 ~ 1.1 (60H, br s, H_2-8 ~ 21 and H_2-8' ~ 21'), and 0.96

(6H, t, H_3-22 and 22'), [$J_{4,5a}$=3.1, $J_{4,5b}$=7.5, $J_{5a,5b}$=18.9, $J_{21,22}$=7.1, $J_{2'a,2'b}$=17.9, $J_{4',5'a}$=6.2, $J_{4',5'b}$= 13.3, $J_{5'a,5'b}$=16.0, and $J_{21',22'}$=7.1 Hz]; ^{13}C NMR (C_6D_6) δ_C 201.1 (C-3), 186.5 (C-6), 173.8 (C-1'), 163.7 (C-1), 133.5 (C-2), 108.6 (C-6'), 89.0 (C-3'), 86.3 (C-4'), 51.9 (C-4), 51.3 (MeO), 42.9 (C-2'), 42.3 (C-5'), 34.0 (C-5), 33.1 (C-7'), 32.3 (C-7), 31-29 (C-8 ~ 21 and C-8' ~ 21'), and 14.3 (C-22 and C-22'); FABMS (negative, glycerol matrix) *m/z* 729 $(M-H)^-$; FABMS (positive, glycerol matrix) *m/z* 713 $(M-H_2O+H)^+$ and 349; EIMS *m/z* (%) 638 [6, $(M-H_2O-CH_2CO-MeOH)^+$; exact mass, *m/z* 638.5289, $C_{42}H_{70}O_4$, Δ +1.5 mmu], 414 [13, $(M-H_2O-CH_2CO-MeOH-C_{16}H_{32})^+$; exact mass, *m/z* 414.2801, $C_{26}H_{38}O_4$, Δ +3.0 mmu], 357 (80), and 43 (100); HRFABMS *m/z* 713.5737, calcd for $C_{45}H_{77}O_6$ $(M-H_2O+H)^+$: 713.5720.

Plakoridine B (3). Colorless oil; $[\alpha]_D^{17}$ *ca.* 0° (*c* 1.6, $CHCl_3$); UV (MeOH) λ_{max} 318 (ε 16000) and 224 nm (12200); IR (KBr) ν_{max} 3320, 1745, 1620, 1525, and 1470 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ_H 7.05 (2H, d, H-29 and 33), 6.95 (1H, br s, 3-OH), 6.79 (2H, d, H-30 and 32), 5.19 (1H, d, H-3), 5.08 (1H, s, H-6), 4.75 (1H, br s, 31-OH), 3.72 (3H, s, H_3-25), 3.71 (1H, m, H-5), 3.39 (1H, m, H-26a), 3.29 (1H, m, H-26b), 2.90 (1H, dd, H-4), 2.83 (1H, m, H-27a), 2.76 (1H, m, H-27b), 2.36 (2H, m, H_2-8), 1.69 (2H, m, H_2-9), 1.3 ~ 1.2 (54H, br s, H_2-10 ~ 22 and H_2-34 ~ 47), and 0.88 (6H, t, H_3-23 and 48), [$J_{3,4}$=5.5, $J_{4,5}$=5.5, and $J_{29,30}$=$J_{32,33}$=8.5 Hz]; ^{13}C NMR ($CDCl_3$) δ_C 199.8 (C-7), 165.7 (C-2), 154.7 (C-31), 130.0 (C-28), 129.8 (C-29 and 33), 115.7 (C-30 and 32), 90.2 (C-6), 75.9 (C-3), 65.4 (C-5), 52.5 (C-25), 52.2 (C-4), 46.2 (C-26), 43.5 (C-8), 31.2 (C-27), ~30 (C-10 ~ 22 and C-34 ~ 47), 26.3 (C-9), 22.7 (C-24), 14.1 (C-23 and 48); FABMS (matrix: 3-nitrobenzylalcohol) *m/z* 740 $(M+H)^+$, 722 $(M+H-H_2O)^+$, 620 $(M+H-CH_2CH_2C_6H_4OH+H)^+$, 514 $(M-C_{16}H_{33})^+$, and 121 $(C_2H_4C_6H_4OH)^+$; HRFABMS *m/z* 740.6215, calcd for $C_{47}H_{82}O_5N$ (M+H): 740.6193.

Ozonolysis of Plakoridine B (3). A solution of the compound **3** (2.0 mg) in 1 mL of CH_2Cl_2 was bubbled with O_3 at -78 C° for 1 min. After the removal of excess ozone by bubbling N_2, a solution of Me_2S (20 μL) was added, and the whole mixture was stirred at 0 C° for 30 min. Evaporation of the solution followed by HPLC [Develosil ODS-5, (5 μm, 10 x 250 mm); eluent: $CH_3CN/CHCl_3$, 7:3; flow rate: 2.0 mL/min; detection: UV at 266 nm] to afford the lactam [**7**, 0.6 mg, EIMS *m/z* 489 (M^+), 370 $(M-CH_2CH_2C_6H_4OH+2H)^+$, and 120] and the acid [**8**, EIMS *m/z* 270 (M^+), 227, 213, 199, and 185].

Preparation of PGME Amides (10) of Manzamenone A (4). To a solution of manzamenone A (**4**, 1.7 mg) in CH_3CN (1 mL), (*R*)-PGME hydrochloride[15] (1.0 mg), triethylamine (0.7 mL), and BOP (1.0 mg) was added, and the mixture was stirred at room temperature for 3 min. After addition of brine, the mixture was extracted three times with ethyl acetate. The organic phase was washed successively with 2N HCl, water, 5% $NaHCO_3$ aqueous solution, and water, and dried over $MgSO_4$. After the solvent was removed under reduced pressure, the crude product was subjected to reversed-phase HPLC [Develosil ODS-5, (5 μm, 10 x 250 mm); eluent: $CH_3CN/CHCl_3$, 85:15 with 0.01 % trifluoroacetic acid; flow rate: 2.5 mL/min; detection: UV at 254 nm] to afford (*R*)-**10** (0.8 mg, t_R 78.0 min) and (*S*)-**10** (0.6 mg, t_R 74.4 min) in the ratio of 57:43. Using (*S*)-PGME hydrochloride, the same procedures afforded (*R*)-**10** and (*S*)-**10** in the ratio of 33:67. (*R*)-**10**: ^{1}H NMR ($CDCl_3$) δ_H 3.161 (H-1), 3.451 (H-2), 5.557 (H-4), 3.556 (H-5), 2.973 (H-6), 2.429 (H-26a), and 3.043 (H-26b); FABMS *m/z* 890 $(M+H)^+$. (*S*)-**10**: ^{1}H NMR ($CDCl_3$) δ_H 3.090 (H-1), 3.431 (H-2), 5.570 (H-4), 3.604 (H-5), 2.714 (H-6), 2.410 (H-26a), and 2.999 (H-26b); FABMS *m/z* 890 $(M+H)^+$.

5-Epi-43-*O*-Methylmanzamenone B (12). Colorless oil; $[\alpha]_D^{17}$ -12.7° (*c* 0.12, $CHCl_3$); UV (EtOH) λ_{max} 230 nm (ε 22000); IR (KBr) ν_{max} 1730, 1720, and 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ_H 5.71 (1H,

d, H-4), 4.00 (1H, d, H-1), 3.82 (3H, s, MeO), 3.76 (1H, dd, H-5), 3.69 (3H, s, MeO), 3.67 (3H, s, MeO), 3.25 (1H, dd, H-6), 3.16 (1H, s, H-2), 3.11 (1H, m, H-26a), 2.42 (1H, m, H-26b), 2.05 (2H, m, H_2-10), 1.5 ~ 1.2 (56H, br s, H_2-11 ~ 24 and H_2-27 ~ 40), and 0.88 (6H, t, H_3-25 and 41), [$J_{1,2}$=0, $J_{4,5}$=8.1, $J_{5,6}$=1.3, $J_{6,1}$=7.5, and $J_{24,25}$=$J_{40,41}$=6.6 Hz]; FABMS (matrix: 3-nitrobenzylalcohol) *m/z* 757 $(M+H)^+$, 725 $(M-OMe)^+$, 697 $(M-CO_2Me)^+$, 666 $(M-OMe-CO_2Me)^+$, and 441 $(M-OMe-CO_2Me-C_{16}H_{33})^+$; HRFABMS *m/z* 757.6029, calcd for $C_{47}H_{81}O_7$ (M+H): 757.6024.

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