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Manzamenone O, New Trimeric Fatty Acid Derivative from a Marine Sponge *Plakortis* sp.

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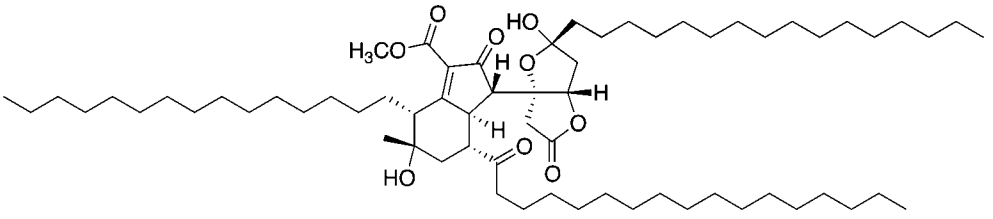
ABSTRACT



manzamenone O (**1**)

A new structurally unique trimeric fatty acid derivative, manzamenone O (1), was isolated from a marine sponge *Plakortis* sp. Manzamenone O (1) has a novel skeleton consisting of C−C bonded octahydroindenone and dioxabicyclo[3.3.0]octane moieties and three long aliphatic chains. The structure of 1 was elucidated on the basis of spectroscopic data and conformational analysis. Manzamenone O (1) exhibited antimicrobial activity against *Micrococcus luteus*, *Aspergillis niger*, and *Trichophyton mentagrophytes*.

Marine sponges have been recognized as a rich source of interesting bioactive metabolites with various chemical structures.[1] Among them, members of the genus *Plakortis* (family Plakinidae) are particularly fascinating with respect to the variety of unusual metabolites which they generate.[2] During our search for new metabolites from Okinawan marine sponges, we have reported some polyketides with unique chemical structures from *Plakortis* spp.[3] Recently, we have also reported a piperidine alkaloid (plakoridine C), *N*-methylpyridinium alkaloids (platisidines A−C), and dimeric fatty acid derivatives (manzamenones L−N) from the extracts of *Plakortis* sp. (SS-11).[4] Further investigation of the extracts from another lot of SS-11 resulted in the isolation of a new fatty acid derivative, manzamenone O (**1**). In this Letter, we describe the isolation and structure elucidation of **1**.

The sponge *Plakortis* sp. (SS-11, 1.0 kg wet weight) collected off Manzamo, Okinawa, was extracted with MeOH and then with $CHCl_3$/MeOH (1:1). The $CHCl_3$/MeOH extracts were partitioned between EtOAc and water. Manzamenone O (**1**, 0.00015%, wet weight) was isolated from the organic layer using silica gel and C_{18} column chromatography and silica gel HPLC. Three known compounds, manzamenone K,[3e] untenone A,[3c] and untenolide A,[3f] were isolated in the purification process of **1**.

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Manzamenone O (**1**)[5] was obtained as a colorless amorphous solid. The molecular formula of **1**, $C_{66}H_{116}O_9$, was established by the HRESIMS (*m*/*z* 1087.83410 $[M + Cl]^-$, Δ+2.76 mmu). The IR spectrum implied the presence of hydroxy (3517 cm^{-1}) and carbonyl functionalities (1761, 1740, and 1702 cm^{-1}). The ^1H and ^{13}C NMR spectra displayed the resonances due to aliphatic chains as well as four carbonyl groups, one double bond, one methoxy group, three sp^3 quaternary carbons, five sp^3 methines, three sp^3 methylenes, one singlet methyl, and three triplet methyls (Table 1). Among them, three sp^3 quaternary carbons (C-3′, C-6′, and C-2″) and one sp^3 methine (C-4′) were ascribed to those bearing an oxygen atom. From these spectral features, manzamenone O (**1**) was presumed to be a fatty acid derivative.

The gross structure of manzamenone O (**1**) possessing two partial structures (units A and B) was elucidated as follows. Comparison of ^1H and ^{13}C NMR data for **1** with those for manzamenone K,[3e] a dimeric fatty acid derivative isolated from a sponge *Plakortis* sp., suggested that **1** has a dioxabicyclo[3.3.0]octane moiety (unit A, C-1′−C-6′). This was confirmed by analysis of the $^1H-^1H$ COSY and HMBC spectra (Figure 1). The analysis also indicated the presence of an alkyl chain at C-6′.

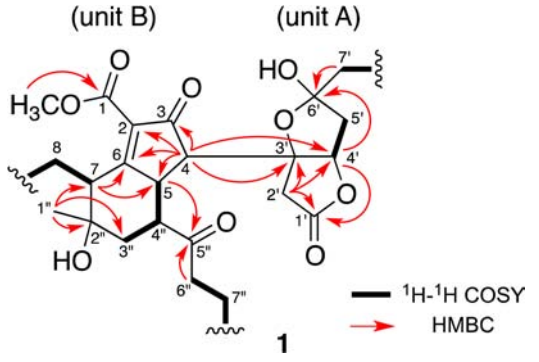


Figure 1. Selected 2D NMR correlations for manzamenone O (**1**).

The existence of an octahydroindenone ring (unit B, C-2−C-7 and C-2″−C-4″) was disclosed by interpretation of the 2D NMR spectra (Figure 1). In addition, HMBC cross-peaks of H_3-1″ to C-7, C-2″, and C-3″ revealed that a methyl and a hydroxy group were attached to C-2″. The connectivities of an alkyl chain (C-8) to C-7 and of an acyl chain (C-5″) to C-4″ were revealed by a $^1H-^1H$ COSY cross-peak of H-7/H_2-8 and HMBC correlations for H-5 to C-5″ and H_2-6″ to C-5″, respectively. The chemical shifts for C-1, C-2, and C-3 were similar to those for manzamenone K,[3e] implying that a methoxy carbonyl group was attached to C-2. The direct linkage of C-3′ (unit A) to C-4 (unit B) was revealed by HMBC cross-peaks of H-4/C-3′ and H-4/C-4′.

To elucidate three aliphatic chains in manzamenone O (**1**), FABMS/MS analysis of **1** was carried out (Figure 2). The fragmentation patterns from *m*/*z* 1075 to 850 implied three unbranched aliphatic chains, while an ion peak at *m*/*z* 822 indicated the acyl chain at C-4″ to be a heptadecanoyl group. The alkyl chains at C-7 and C-6′ were elucidated to be a pentadecanyl and a hexadecanyl group, respectively, based on the fragmentation patterns at *m*/*z* 807 (+H) and 794 (+H) in unit B and at *m*/*z* 791 and 708 in unit A. Thus, the gross structure of manzamenone O was assigned as **1**.

The relative stereochemistry manzamenone O (**1**) was assigned as follows. Resemblance of the ^{13}C chemical shifts for unit A (C-1′−C-7′) with the corresponding position of manzamenone K[3e] implied that the relative stereochemistry was identical. On the other hand, in unit B, ROESY correlations for H_3-1″/H-4″, H-3″a/H-5, H-3″a/H-8a, and H-5/H-8a suggested the axial orientations of these protons (Figure 3). This was supported by values of $^3J_{H\text{-}3''/H\text{-}4''}$ and $^3J_{H\text{-}4''/H\text{-}5}$ (12.0 Hz, each). Thus, the cyclohexane ring (C-5−C-7 and C-2″−C-4″) adopts the pseudochair conformation. The ^1H NMR resonance of H-4 was seen as a singlet, indicating that the dihedral angle of H-4/H-5 was close to 90°, while ROESY cross-peaks of H-4/H-4″ and

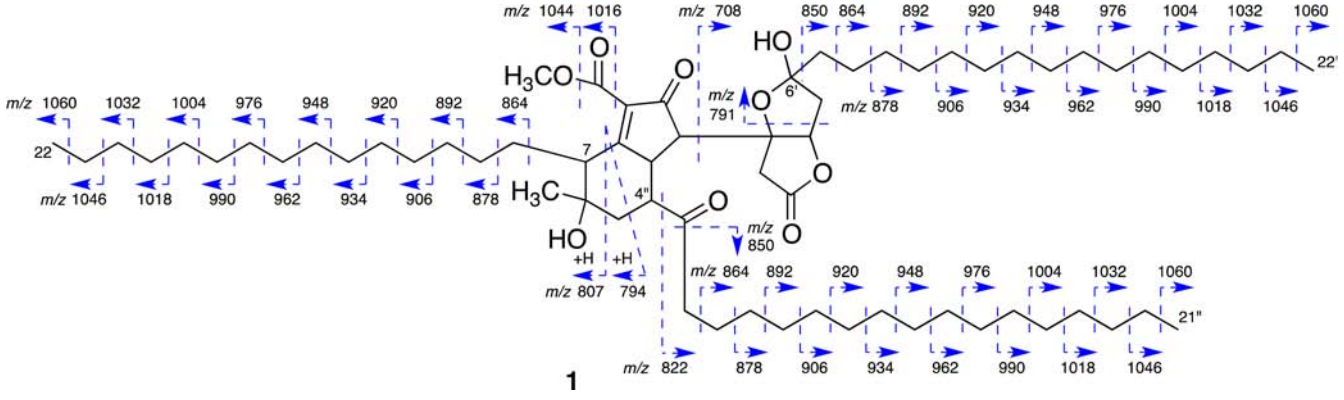


Figure 2. Fragmentation patterns observed in positive ion FABMS/MS spectrum of manzamenone O (**1**) (precursor ion, *m*/*z* 1075 $[M + Na]^+$).

Table 1. ^{1}H and ^{13}C NMR Data for Manzamenone O (**1**) in C_5D_5N and $CDCl_3$

position	δ_C (in C_5D_5N)	δ_H (in C_5D_5N)	δ_C (in $CDCl_3$)	δ_H (in $CDCl_3$)
1	164.0	–	162.8	–
2	134.6	–	133.4	–
3	202.8	–	201.7	–
4	57.4	3.08 (1H, s)	57.0	2.15 (1H, s)
5	42.5	3.82 (1H, d, J = 12.0 Hz)	41.5	3.21 (1H, d, J = 13.2 Hz)
6	190.3	–	190.0	–
7	51.4	3.94 (1H, dd, J = 11.7, 3.4 Hz)	50.1	3.58 (1H, dd, J = 11.5, 3.7 Hz)
8	27.0	2.04 (2H, m)	28.1	1.61 (2H, m)
9–21	22.9–34.0 (13C)	1.14–1.52 (26H, m)	22.7–31.9 (13C)	1.08–1.38 (26H, m)
22	14.3	0.86 (3H, t, J = 6.0 Hz)	14.1	0.87 (3H, t, J = 7.0 Hz)
1′	175.5	–	174.4	–
2′	43.9	3.45 (1H, d, J = 18.8 Hz)	42.6	2.88 (1H, d, J = 18.8 Hz)
		3.41 (1H, d, J = 18.8 Hz)		2.81 (1H, d, J = 18.8 Hz)
3′	90.2	–	89.8	–
4′	86.6	6.04 (1H, d, J = 5.9 Hz)	85.8	5.58 (1H, d, J = 6.0 Hz)
5′	42.5	2.49 (1H, d, J = 13.7 Hz)	41.5	2.21 (1H, d, J = 14.3 Hz)
		2.31 (1H, dd, J = 13.7, 5.9 Hz)		1.98 (1H, dd, J = 14.3, 6.0 Hz)
6′	109.1	–	108.8	–
7′	40.8	2.03 (1H, m)	40.2	1.56 (1H, m)
		1.94 (1H, m)		1.51 (1H, m)
8′–21′	22.9–34.0 (14C)	1.14–1.52 (28H, m)	22.7–31.9 (14C)	1.08–1.38 (28H, m)
22′	14.3	0.86 (3H, t, J = 6.0 Hz)	14.1	0.87 (3H, t, J = 7.0 Hz)
1″	28.8	1.61 (3H, s)	28.2	1.30 (3H, s)
2″	73.5	–	74.1	–
3″	38.9	2.37 (1H, t, J = 12.0 Hz)	37.9	1.86 (1H, t, J = 13.2 Hz)
		2.19 (1H, dd, J = 12.0, 3.2 Hz)		1.74 (1H, dd, J = 13.2, 3.2 Hz)
4″	54.4	2.99 (1H, td, J = 12.0, 3.2 Hz)	54.3	2.35 (1H, m)
5″	211.7	–	210.1	–
6″	43.3	2.82 (1H, m)	43.0	2.56 (1H, m)
		2.62 (1H, m)		2.31 (1H, m)
7″–20″	22.9–34.0 (14C)	1.14–1.52 (28H, m)	22.7–31.9 (14C)	1.08–1.38 (28H, m)
21″	14.3	0.86 (3H, t, J = 6.0 Hz)	14.1	0.87 (3H, t, J = 7.0 Hz)
1-OMe	51.8	3.88 (3H, s)	52.0	3.82 (3H, s)

H-4/H-5 were observed. These observations indicated the relative configuration of C-4 to be S^*.

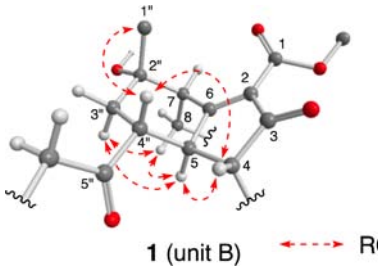


Figure 3. Relative stereochemistry and selected ROESY correlations for unit B (C-2–C-7 and C-2″–C-4″) of manzamenone O (**1**).

To assign the relative relationship for units A and B, bonded through a free rotatable C–C bond, a conformational search on the MacroModel program (MMFFs force field) was performed on two possible diastereomers. The most stable conformers **1a** (4S^*,3′R^*,4′S^*,6′S^*) and **1b** (4S^*,3′S^*,4′R^*,6′R^*) are shown in Figure 4. Stable

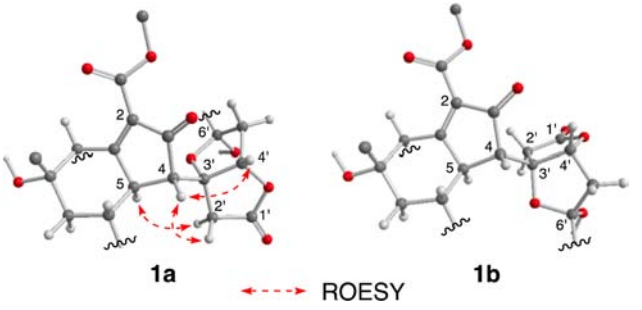
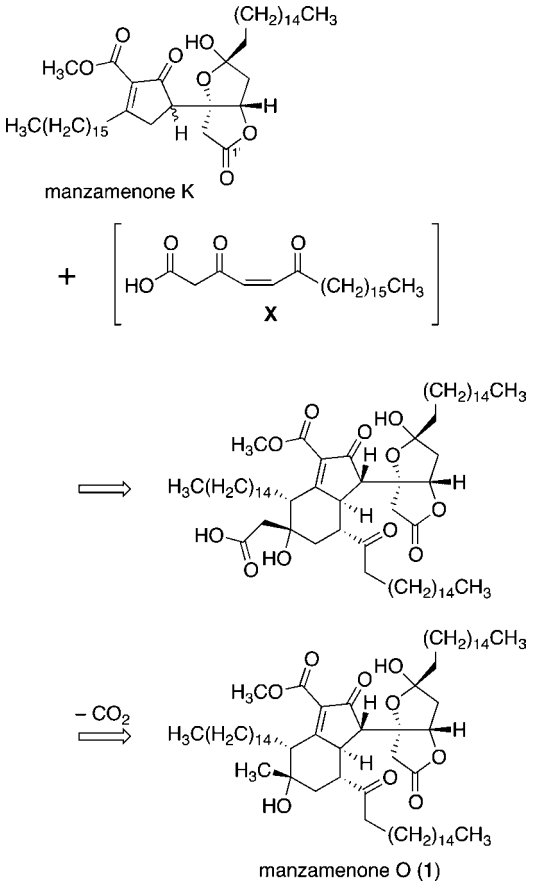


Figure 4. Most stable conformers for two possible diastereomers **1a** (4S^*,3′R^*,4′S^*,6′S^*) and **1b** (4S^*,3′S^*,4′R^*,6′R^*) of manzamenone O (**1**).

conformers found in each conformational search, which appeared within 3 kcal/mol from the most stable conformers, showed superimposable conformations for the core

(5) Manzamenone O (**1**): Colorless amorphous solid; $[\alpha]^{24}_{D} \approx 0$ (c 0.73, $CHCl_3$); IR (film) ν_{max} 3517, 1761, 1740, and 1702 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); HRESIMS: m/z 1087.83410 $[M + Cl]^-$ (calcd for $C_{66}H_{116}O_9Cl$, 1087.83134).

Scheme 1. Possible Biogenetic Pathway of Manzamenone O (**1**)



structures (C-2−C-7, C-1′−C-6′, and C-2″−C-4″) on **1a** and **1b**, respectively. Therefore, stereochemical analysis was carried out on **1a** and **1b**. Given ROESY correlations for H-4/H-4′, H-5/H-2′a, and H-4/H-2′b, the relative stereochemistry of **1** was concluded to be **1a**, whereas the distance of H-4/H_2-2′ in **1b** was not sufficiently close to show a ROESY correlation.

We previously proposed that manzamenone K[3e] might be biogenetically derived from two molecules of 3,6-dioxo-4-docosenoic acid (**X**), which may be a common key intermediate for some oxylipins from *Plakortis* spp.[3f,4a] Manzamenone O (**1**) seems to be derived from manzamenone K and one molecule of **X** as shown in Scheme 1.

Thus, manzamenone O (**1**) is a structurally unique fatty acid derivative with a novel skeleton consisting of C−C bonded octahydroindenone and dioxabicyclo[3.3.0]octane moieties and three long aliphatic side chains. To the best of our knowledge, manzamenone O (**1**) is the first example of a trimer of the hypothetical biosynthetic precursor, 3,6-dioxo-4-docosenoic acid (**X**).

Manzamenone O (**1**) exhibited antimicrobial activity against *Micrococcus luteus* (MIC 4 μg/mL), *Aspergillis niger* (IC_{50} 8 μg/mL), and *Trichophyton mentagrophytes* (IC_{50} 8 μg/mL).

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Supporting Information Available. Experimental section and 1D and 2D NMR spectra for manzamenone O. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.