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Manzamenones G and H, New Dimeric Fatty-Acid Derivatives from the Okinawan Marine Sponge *Plakortis* Sp.

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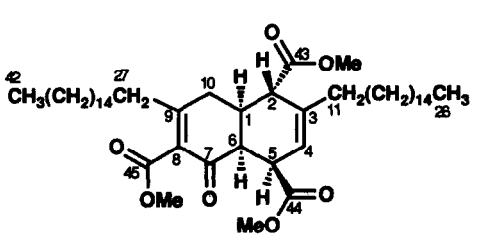
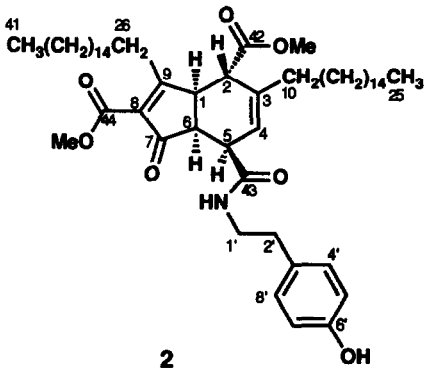
Abstract: *Manzamenone G* (**1**), *a novel dimeric fatty-acid derivative with a new carbon-skeleton, was isolated from the Okinawan marine sponge* Plakortis *sp. together with manzamenone H* (**2**), *a new tyramine-containing manzamenone congener, and their structures elucidated on the basis of spectral and chemical means.*

Marine sponges of the genus *Plakortis* have proven to afford a variety of unique peroxy aliphatic acids and esters.[1] During our studies on bioactive substances from Okinawan marine organisms,[2] we have investigated extracts of the *Plakortis* sponges and isolated plakotenin,[3] a cytotoxic metabolite, and manzamenones A ~ F,[4] unique fatty acid derivatives. Further examination of the constituents of the same *Plakortis* sponges has now resulted in the isolation of manzamenones G (**1**) and H (**2**), two new dimeric fatty acid derivatives, possessing bicyclo[4.4.0]decane and bicyclo[4.3.0]nonane skeleton, respectively. The carbon-framework of manzamenone G (**1**) is hitherto unknown. Here we describe the isolation and structure elucidation of **1** and **2**.

The sponge *Plakortis* sp.,[5] collected off Manzamo, Okinawa, was extracted with



1



2

methanol and partitioned between ethyl acetate and water. The ethyl acetate soluble fraction was subjected to silica gel columns eluted with [MeOH/$CHCl_3$ (1:9) and acetone/hexane (1:3)] followed by gel filtration on Sephadex LH-20 [MeOH/$CHCl_3$ (1:1)]. Final purification by reversed-phase HPLC [ODS; CH_3CN/$CHCl_3$ (7:3) with 0.01% TFA] afforded manzamenone G (**1**, 0.001 % yield based on wet weight). From another *Plakortis* sponge,[6] collected at Unten-harbor, Okinawa, manzamenone H (**2**, 0.001 % yield) was obtained by the similar silica gel and gel filtration chromatographies.[7]

The molecular formula of manzamenone G (**1**) was determined as $C_{48}H_{82}O_7$ by HRFABMS data [*m/z* 771.6120, $(M+H)^+$, Δ -1.9 mmu]. This composition corresponded to that having one more CH_2 unit than that of 43-*O*-methylmanzamenone A (**3**).[4] The UV and IR absortions were analogous to those of compound **3** and indicative of the presence of enone (λ_{max} 225 nm) and ester (ν_{max} 1720 cm^{-1}) groups. Exceptionally, a relatively strong IR band was observed at 1680 cm^{-1} for **1**, which was not found in the IR spectrum of **3**. The ^1H and ^{13}C NMR spectra of **1** (Table 1) suggested the presence of a ketone, three methoxycarbonyls, a trisubstituted and a tetrasubstituted double bonds, four sp^3 methines, and two long alkyl chains. The ^1H-^1H COSY spectrum of **1** revealed the presence of the identical cyclohexene moiety (cross-peaks: H-2/H-1, H-1/H-6, H-6/H-5, and H-5/H-4) with that embraced in compound **3**. In the ^1H NMR of **1** two double-doublet signals were characteristically observed at δ_H 2.79 (1H, dd, *J*=19 and 6.0 Hz; H-10a) and 2.69 (1H, dd, *J*=19 and 3.0 Hz; H-10b), which showed one-bond ^1H-^{13}C correlation to the sp^3 methylene at δ_C 32.1 (t, C-10) in the HSQC[8] spectrum of **1**. These methylene protons

Table 1. ^1H and ^{13}C NMR Data of Manzamenone G (**1**) in $CDCl_3$

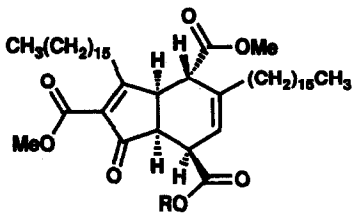
position	δ_H		*J* (Hz)	δ_C		^1H coupled with ^{13}C (HMBC correlations)
1	2.88	dddd	6.3, 6.0, 5.7, 3.0	32.8	d	
2	3.02	d	6.3	46.3	d	H-4, H-6, H-10a, H-11a
3				136.5	s	H-2, H_2-11
4	5.68	br d	3.7	119.6	d	H-2, H-5, H-6, H_2-11
5	4.09	dd	3.7, 2.8	38.8	d	H-4, H-6
6	3.22	dd	5.7, 2.8	43.6	d	H-2, H-4, H-10b
7				192.1	s	H-1, H-6
8				131.8	s	H_2-10, H_2-27
9				160.7	s	H_2-10, H_2-27
10a	2.79	dd	19, 6.0	32.1	t	H-2, H-6, H_2-27
10b	2.69	dd	19, 3.0			
11a	1.96	m		36.3	t	H-4
11b	1.89	m				
12-25	1.2~1.6	br s		22.7~31.9	each t	
27	2.18 (2H)	m		36.3	t	
28-41	1.2~1.6	br s		22.7~31.9	each t	
26 and 42	0.88 (6H)	t	6.9	14.1 (2C)	q	
43				172.4	s	H-1, H-2, 43-OMe
43-OMe	3.58 (3H)			52.2	q	
44				173.8	s	H-5, H-6, 44-OMe
44-OMe	3.80 (3H)			52.0	q	
45				166.8	s	45-OMe
45-OMe	3.71 (3H)			52.3	q	

(H_2-10) showed the COSY correlation to H-1. In the HMBC[9] spectrum of **1** long-range ^1H-^{13}C connectivities were observed for H-10a/C-2, H-10b/C-6, H_2-10/C-8, H_2-10/C-9, H-2/C-10, H-6/C-10, and H_2-27/C-10. From these results a bicyclo[4.4.0]decane skeleton was deduced for carbon-framework of manzamenone G (**1**), viz., an additional sp^3 methylene (C-10) was inserted between the β-position (C-9) of the conjugated enone and the bridge-head carbon (C-1) of 43-*O*-methylmanzamenone A (**3**). The HMBC correlations (Table 1) supported that the substituted positions of three methoxycarbonyl groups as well as two alkyl side chains were analogous to those of compound **3**. The ^{13}C NMR chemical shifts for the enone moiety [δ_C 192.1 s (C-7), 131.8 s (C-8), and 160.7 s (C-9)] of **1** coincided with those of cyclohexenone derivatives better than those of cyclopentenone derivatives.[10] The characteristic IR band at 1680 cm^{-1} for **1** was ascribable to the conjugated ketone functionality of the cyclohexenone ring. In the EIMS spectrum of **1** prominent fragment ion peaks were observed at *m/z* 619, 593, 395, and 369: the former two were assignable to the $(M - 2 \times CO_2Me - MeOH)^+$ and $(M - 3 \times CO_2Me)^+$ ions and the latter two corresponding to the ions generated by loss of a $C_{16}H_{32}$ (224 amu) unit from the ions of *m/z* 619 and 593, respectively. Since these fragmentations were also observed in the EIMS spectrum of 43-*O*-methylmanzamenone A (**3**),[4] each of the alkyl side chain was implied to be a hexadecyl [$-(CH_2)_{15}CH_3$] group. For the relative stereochemistry of **1**, the H-2 and H-10b were shown to be on the β-side of the decaline ring, while other protons on the decaline ring (H-1, H-6, H-5, and H-10a) were on the α-side, based on the following phase-sensitive NOESY correlations: H-1/H-6, H-6/H-5, H-6/H-10a, H-10a/H-1, and H-10b/H-2. These configurations were consistent with the *J*-values for these protons in the ^1H NMR of **1** (Table 1), assuming that the cyclohexene ring adopts the conformation in which the all C-1 ~ C-6 carbons are almost on the same plane. Thus the structure of manzamenone G was concluded as **1**.

Manzamenone H (**2**) was shown to have the molecular formula $C_{54}H_{87}O_7N$ by HRFABMS [*m/z* 862.6549, $(M+H)^+$, Δ -1.2 mmu]. The UV and IR spectra were suggestive

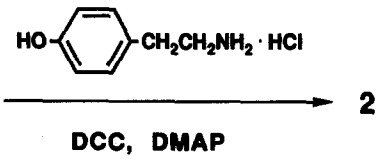
Table 2. ^1H and ^{13}C NMR Data of Manzamenone H (**2**) in $CDCl_3$

position	δ_H		*J* (Hz)	δ_C		position	δ_H		*J* (Hz)	δ_C	
1	3.06	dt	5.7, 7.9	44.1	d	25 and 41	0.88 (6H)	t	6.6	14.1 (2C)	q
2	3.42	d	5.5	46.3	d	42				170.8	s
3				136.0	s	42-OMe	3.49 (3H)	s		51.9	q
4	6.04	d	1.5	123.9	d	43				173.6	s
5	3.40	m		41.2	d	44				163.4	s
6	2.55	dt	7.7, 7.3	48.4	d	44-OMe	3.86	s		52.1	q
7				205.2	s	NH	7.35	d	5.9		
8				132.7	s	1'	3.50 (2H)	m		40.9	t
9				184.6	s	2'	2.77 (2H)	m		32.0	t
10	2.15 (2H)	m		37.0	t	3'				130.9	s
11-24	1.2~1.6	br s		22.7~31.9	each t	4',8'	7.06 (2H)	d	8.4	130.0	d
26a	3.05	m		34.9	t	5',7'	6.72 (2H)	d	8.4	115.4	d
26b	2.42	m				6'				154.3	s
27-40	1.2~1.6	br s		22.7~31.9	each t	6'-OH	5.00	br s			



3 R = Me
4 R = H

Scheme 1



of the presence of enone (λ_{max} 226 nm), ester (ν_{max} 1725 cm^{-1}), and amide (ν_{max} 1650 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **2** (Table 2) revealed the presence of a ketone, two methoxycarbonyls, an amide, a *p*-disubstituted benzene ring, a trisubstituted and a tetrasubstituted double bonds, four sp^3 methines, and two long alkyl chains. Interpretation of the ^1H-^1H COSY spectrum of **2** revealed the proton connectivities for four contiguous sp^3 methine protons and an olefinic proton (H-2/H-1/H-6/H-5/H-4). From these observations manzamenone H (**2**) was inferred to possess a unique bicyclo[4.3.0]nonane ring system, which was commonly contained in manzamenones A ~ F.[3] In the ^1H-^1H COSY spectrum of **2** the NH proton observed at δ 7.35 showed a cross-peak with sp^3 methylene protons at δ 3.50 (H_2-1'), which in turn was correlated with other methylene protons at δ 2.77 (H_2-2'). From these findings as well as the ^1H NMR signals for the aromatic portion [δ_H 7.06 and 6.72 (each 2H, d, J=8.4 Hz) and 5.00 (1H, br s, D_2O-exchangeable; OH of a phenol)] the presence of a tyramine unit was deduced. This tyramine unit was shown to be attached to the C-5 carboxyl group through an amide bond by preparation of compound **2** from manzamenone A (**4**)[4] previously obtained from the same sponge as shown in Scheme 1. Manzamenone A (**4**) was treated with tyramine hydrochloride in the presence of DCC and DMAP in CH_2Cl_2 to afford manzamenone H (**2**), which was completely identical with the natural specimen.

Manzamenones G (**1**) and H (**2**) possess biogenetically unique bicyclic ring systems (bicyclo[4.4.0]decane and bicyclo[4.3.0]nonane, respectively), presumably derived through enantioselective intermolecular cycloaddition reaction from two fatty acid-derived precursors in their biosynthetic processes. To substantiate our hypothesis[4] on the biosynthesis of manzamenones, further investigation on the minor constituents of the *Plakortis* sponges are currently in progress to obtain related compounds corresponding to a precursor or an intermediate of the biosynthetic path.

EXPERIMENTAL

General Methods. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV and IR spectra were recorded on a JASCO Ubest-35 and JASCO IR

Report-100 spectrometers, respectively. ^1H and ^{13}C NMR spectra were recorded on JEOL GX-270 and EX-400 spectrometers in chloroform-*d*. The resonances of residual $CHCl_3$ at δ_H 7.26 and δ_C 77.0 were used as internal references for ^1H and ^{13}C NMR spectra, respectively. EI and FAB mass spectra were obtained on JEOL DX-303 and JEOL HX-110 spectrometers, respectively.

Collection, Extraction, and Isolation. The sponge *Plakortis* sp. (2 kg, wet weight), collected off Manzamo, Okinawa, was extracted with methanol. Evaporation of the extract afforded a brown residue, which was partitioned between 1 M NaCl (600 mL) and EtOAc (600 mL x 3). The EtOAc-soluble fraction was evaporated under reduced pressure to give a crude residue (10.2 g), which was partially (3.8 g) subjected to a silica gel column chromatography (2.2 x 40 cm) with MeOH/$CHCl_3$ (1:9). The fraction (2.1 g) eluting from 210 mL to 260 mL was then separated by the second silica gel column (2.2 x 40 cm) with acetone/hexane (1:3). The 275-320 mL fraction (340 mg) was further purified by gel filtration on Sephadex LH-20 (1.1 x 58 cm; MeOH/$CHCl_3$, 1:1) followed by reversed-phase column (YMC ODS 60, 1.1 x 20 cm; CH_3CN/$CHCl_3$, 7:3) to give a fraction (190 mg), which was further purified by reversed-phase HPLC [Develosil ODS-5, (5 μm, 10 x 250 mm); eluent: CH_3CN/$CHCl_3$ (7:3 with 0.01 % trifluoroacetic acid); flow rate: 2.5 mL/min; detection: UV at 254 nm] to afford manzamenone G (**1**, t_R 21.2 min, 0.001 % wet weight).

Another *Plakortis* sponge (1 kg, wet weight), collected at Unten-harbor, Okinawa, was extracted with MeOH. After evaporation of the solvent the residue was partitioned between 1 M NaCl (400 mL) and EtOAc (400 mL x 3). The EtOAc-soluble portion was evaporated under reduced pressure to give a crude residue (5.3 g), which was partially (1.0 g) subjected to a silica gel column chromatography (2.4 x 36 cm) with EtOAc/hexane (2:8). The fraction eluting from 720 mL to 860 mL was further purified by a Sephadex LH-20 column (2.0 x 120 cm) with MeOH/$CHCl_3$ (1:1) to afford manzamenone H (**2**, 0.001% wet weight).

Manzamenone G (1). Colorless oil; $[\alpha]_D^{19}$ -12° (*c* 1.1, $CHCl_3$); UV (EtOH) λ_{max} 225 nm (ε 10700); IR ($CHCl_3$) ν_{max} 1720, 1680, and 1620 cm^{-1}; ^1H and ^{13}C NMR (Table 1); EIMS *m/z* 771 $(M)^+$, 739 $(M-MeOH)^+$, 707 $(M-2MeOH)^+$, 681 $(M-MeOH-CO_2Me+H)^+$, and 648 $(M-2MeOH-CO_2Me)^+$; FABMS (matrix: 3-nitrobenzylalcohol) *m/z* 771 $(M+H)^+$; HRFABMS *m/z* 771.6120, calcd for $C_{48}H_{83}O_7$ (M+H): 771.6139.

Manzamenone H (2). Colorless oil; $[\alpha]_D^{27}$ -5.7° (*c* 0.29, MeOH); UV (MeOH) λ_{max} 226 nm (ε 9700); IR (KBr) ν_{max} 3400, 1725, 1715, 1650, and 1610 cm^{-1}; ^1H and ^{13}C NMR (Table 2); EIMS *m/z* 698 $(M-CONHCH_2CH_2C_6H_4OH)^+$, 639 $(M-CONHCH_2CH_2C_6H_4OH-CO_2Me)^+$, 621 $(M-CH_2C_6H_4OH-2CO_2Me)^+$, 579 $(M-CO_2Me-C_{16}H_{32})^+$, 472 $(M-CONHCH_2CH_2C_6H_4OH-C_{16}H_{32}-H)^+$, and 414 $(M-2C_{16}H_{32}+H)^+$; FABMS (matrix: 3-nitrobenzylalcohol) *m/z* 862 $(M+H)^+$; HRFABMS *m/z* 862.6549, calcd for $C_{54}H_{88}O_7N$ (M+H): 862.6561.

Preparation of Manzameone H (2) from Manzamenone A (4). Treatment of manzamenone A (**4**, 3.0 mg) with an excess amount of tyramine hydrochloride (10 mg)

in the presence of dicyclohexylcarbodiimide (15 mg) and 4-dimethylaminopyridine (5 mg) in CH_2Cl_2 (0.5 mL) at 0 °C for 3 h afforded, after purification through a silica gel column ($CHCl_3$/acetone, 9:1), manzamenone H (**2**, 1.5 mg), which was completely identical with the natural specimen based on TLC, ^1H NMR, and EIMS data.

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5. From this *Plakortis* sponge manzamenones A ~ E[4] were previously obtained.
6. From this *Plakortis* sponge plakotenin[3] and manzamenone F[4] were previously obtained.
7. 43-*O*-Methylmanzamenone A (**3**)[4] was coisolated as a natural product for the first time in 0.0005 % yield (wet weight).
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10. The ^{13}C chemical shifts for the enone moieties: 3-methylcylcohexenone [δ_C 198.4 (C-1), 126.5 (C-2), and 162.3 (C-3)]; 3,4-dimethylcylcopentenone [δ_C 208.5 (C-1), 130.3 (C-2), and 182.2 (C-3)]. Kalinowski, H.-O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*; John Wiley & Sons: Chichester, 1984; pp 269-270.