Table I. Reduction of Aromatic Nitro Compounds to Aromatic Amines by Me_3SiSNa

nitro compound	amine	yield (%)
nitrobenzene (1)	aniline (2)	88
2-methylnitrobenzene (3)	2-methylaniline (4)	85
4-ethylnitrobenzene (5)	4-ethylaniline (6)	83
2-nitrobiphenyl (7)	2-aminobiphenyl (8)	93
2-nitroanisole (9)	2-aminophenol (10)	96
4-nitrophenyl phenyl ether (11)	4-phenoxyaniline (12)	88
1-fluoro-3-nitrobenzene (13)	3-fluoroaniline (14)	94
2-nitro-α,α,α-trifluorotoluene (15)	2-aminobenzotrifluoride (16)	87
3-nitro-α,α,α-trifluorotoluene (17)	3-aminobenzotrifluoride (18)	84
N,*N*-dimethyl-1,3-nitroaniline (19)	*N*,*N*-dimethyl-1,3-phenylenediamine (20)	93
4-nitro-*o*-xylene (21)	3,4-dimethylaniline (22)	86
2,5-diethoxynitrobenzene (23)	2,5-diethoxyaniline (24)	91
4-fluoro-2-nitrotoluene (25)	5-fluoro-2-methylaniline (26)	98
3-nitropyridine (27)	3-aminopyridine (28)	78
2-methoxy-3-nitropyridine (29)	3-amino-2-methoxypyridine (30)	68
2-methoxy-5-nitropyridine (31)	5-amino-2-methoxypyridine (32)	70

Table II. Yields of 2-Aminoanisole and 2-Aminophenol (10) Obtained from the Reaction of 2-Nitroanisole (9, 1.0 equiv) with Different Amounts of Me_3SiSNa

equiv of Me_3SiSNa	2-aminoanisole (%)	2-aminophenol (10, %)
1.0	57	9
1.5	51	37
2.1	34	50
3.1	3	84
4.0	not detectable	96

amines exclusively. For a special substrate 2-nitrobiphenyl, Zon et al.[4] reduced it with $Me_3SiSiMe_3$ at 240 °C to give some 9*H*-carbazole. This byproduct was not generated under our conditions, as determined by GC and TLC. The diversities between Zon's and our results may come from the difference of reaction temperature and solvent.

In control experiments, we reduced nitroarenes in 1,3-dimethyl-2-imidazolidinone at 185 °C by using $Me_3SiSSiMe_3$ alone (i.e., without adding NaOMe). The desired arylamines were obtained in 20–30% yields only. Thus we conclude that reduction of nitroarenes proceeds more efficient by using a sulfide[15] (e.g., Me_3SiSNa) than a thioether (RSR′).

Chemoselectivity and Rate. Reagent Me_3SiSNa can efficiently remove the methyl group from aryl methyl ethers.[3] For nitrobenzene containing a methoxy group (e.g., **9**), we found that reduction of the nitro group proceeded faster than O-demethylation. Table II lists the results from our systematic study.

Reduction of nitropyridines (2–3 h) proceeded faster than that of nitroarenes (~24 h). We believe that the nitrogen atom of the pyridine ring exerted some electronic and inductive effects to enhance the electrophilicity of the nitrogen atom of the nitro group. This rate enhancement may also be responsible for the successful reduction of the nitro group in methoxypyridines **29** and **31**, without competitive removal of the methyl unit. Thus the corresponding (amino)pyridines **30** and **32** were obtained in 68% and 70% yields, respectively.

Experimental Section

Standard Procedure for the Reduction of Aromatic Nitro Compounds to Aromatic Amines. A solution containing dry sodium methoxide (2.1 equiv), hexamethyldisilathiane (2.1 equiv), and anhydrous 1,3-dimethyl-2-imidazolidinone (2.0 mL) was stirred at room temperature under nitrogen for 1.5 h. The mixture was then transferred to a Pyrex combustion tube under argon. An aromatic nitro compound (1.0 equiv, ~100 mg) in 1,3-dimethyl-2-imidazolidinone (1.0 mL) was injected into the tube, which was then sealed. The sealed tube was heated in an oven at 185 °C (24 h for nitroarenes and 2–3 h for nitropyridines), during which the tube was shaken thoroughly once at 60 °C. The reaction mixture was diluted with water at room temperature, neutralized with 10% HCl, and extracted with Et_2O (15 mL × 5). The combined ethereal solutions were washed with water and saturated aqueous NaCl, dried over $MgSO_4$(s), filtered, and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel to give a pure aromatic amine. The physical properties and spectroscopic characteristics of the isolated aromatic amines, including **2**, **4**, **6**, **8**,[4] **10**, **12**, **14**, **16**, **18**, **20**, **22**, **24**,[16] **26**, **28**, **30**,[17] and **32** were consistent with those of an authentic sample[18] or published data.[4,16,17]

Acknowledgment. We are grateful to the National Science Council of Republic of China (grant No. NSC 81-0208-M007-59) and Academia Sinica for financial support.

Registry No. 1, 98-95-3; 2, 62-53-3; 3, 88-72-2; 4, 95-53-4; 5, 100-12-9; 6, 589-16-2; 7, 86-00-0; 8, 90-41-5; 9, 91-23-6; 10, 95-55-6; 11, 620-88-2; 12, 139-59-3; 13, 402-67-5; 14, 372-19-0; 15, 384-22-5; 16, 88-17-5; 17, 98-46-4; 18, 98-16-8; 19, 619-31-8; 20, 2836-04-6; 21, 99-51-4; 22, 95-64-7; 23, 119-23-3; 24, 94-85-9; 25, 446-10-6; 26, 367-29-3; 27, 2530-26-9; 28, 462-08-8; 29, 20265-35-4; 30, 20265-38-7; 31, 5446-92-4; 32, 6628-77-9; Me_3SiSNa, 87495-22-5; $Me_3SiSSiMe_3$, 3385-94-2.

(15) cf. Hojo, M.; Takagi, Y.; Ogata, Y. *J. Am. Chem. Soc.* **1960**, *82*, 2459.

(16) Braude, E. A.; Linstead, R. P.; Wooldridge, K. R. H. *J. Chem. Soc.* **1954**, 3586.

(17) Barlin, G. B.; Pfleiderer, W. *J. Chem. Soc. B* **1971**, 1425.

(18) Compounds are available from Aldrich Chemical Co.

Manzamenones A–F from the Okinawan Marine Sponges *Plakortis* sp.: Novel Dimeric Fatty Acid Derivatives Possessing a Bicyclo[4.3.0]nonane Skeleton

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Marine sponges of the genus *Plakortis* have been a rich source of unique bioactive secondary metabolites such as polycyclic aromatic alkaloids[1] or peroxy aliphatic acids and esters.[2] We have also isolated plakorin,[3] a cyclic peroxide

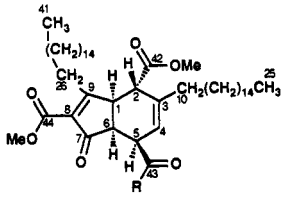
(1) (a) Inman, W. D.; O'Neill-Johnson, M.; Crews, P. *J. Am. Chem. Soc.* **1990**, *112*, 1–4. (b) West, R. R.; Mayne, C. L.; Ireland, C. M.; Brinen, L. S.; Clardy, J. *Tetrahedron Lett.* **1990**, *31*, 3271–3274.

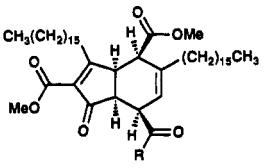
0022-3263/92/1957-5255$03.00/0

Table I. ^1H and ^{13}C NMR Data of 43-*O*-Methylmanzamenone A (1a) in $CDCl_3$

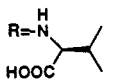
position	δ_C		J (Hz)	δ_C		^1H coupled with ^{13}C (HMBC correlations)
1	3.17	dd	7.9, 6.0	42.9	d	H-26b
2	3.43	d	6.0	46.8	d	H-4
3				136.8	s	H-2, H-4
4	5.76	br d	2.1	123.1	d	H-2
5	3.67	dd	6.4, 2.1	41.7	d	
6	3.31	dd	7.9, 6.4	45.9	d	
7				202.1	s	H-1, H-6
8				132.9	s	H-1, H-26a
9				182.8	s	H_2-26
10	2.12 (2 H)	m		36.9	t	H-2, H-4
11–24	1.2–1.6	br s		31.9–22.7	each t	
26a	2.40	ddd	13, 9.3, 5.4	30.2	t	
26b	3.06	ddd	13, 10, 7.1			
27–40	1.2–1.6	br s		31.9–22.7	each t	
25 and 41	0.86 (6 H)	t	7.1	14.1 (2 C)	q	
42				170.7	s	H-1, H-2, 42-OMe
42-OMe	3.48 (3 H)	s		51.9	q	
43				174.3	s	H-6, 43-OMe
43-OMe	3.80 (3 H)	s		52.6	s	
44				163.6	s	44-OMe
44-OMe	3.82 (3 H)	s		51.9	q	

with potent Ca^{2+}-ATPase activating activity, and plakotenin,[4] a cytotoxic carboxylic acid, from Okinawan *Plakortis* sponges. During our studies on bioactive substances from Okinawan marine organisms,[5] we further investigated extracts of the sponges of the genus *Plakortis* and have isolated six novel fatty acid derivatives, manzamenones A–F (**1**–**6**) possessing a previously unknown carbon skeleton. This paper describes the isolation and structure elucidation of **1**–**6**.

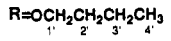




1 R=OH
1a R=OMe
3 R=OEt
4 R=NH_2



5



6

The methanol extract of the sponge *Plakortis* sp. collected off Manzamo, Okinawa, was partitioned between EtOAc and water. The EtOAc-soluble material was repeatedly subjected to silica gel, gel filtration on Sephadex LH-20, and reversed-phase column chromatographies. The final purification was carried out with reversed-phase HPLC to afford manzamenones A (**1**, 0.003%, wet weight), B (**2**, 0.0008%), C (**3**, 0.0007%), D (**4**, 0.0002%), and E (**5**, 0.0005%). From another *Plakortis* sponge collected at Unten-harbor,[6] Okinawa, manzamenone F (**6**, 0.008%) was obtained by the similar procedure. During the isolation process a mixture of manzamenones A (**1**) and B (**2**) was partially treated with diazomethane to give their methyl esters (**1a** and **2a**, respectively), which were used for structural studies.

Manzamenone A methyl ester (**1a**) was shown to have the molecular formula $C_{47}H_{80}O_7$ by HREIMS data [m/z 756.5907 (M^+), Δ +0.3 mmu]. The UV absorption at λ_{max} 228 nm of **1a** suggested the presence of an enone chromophore. The IR spectrum of **1a** was indicative of the presence of carbonyl group(s) (1730 cm^{-1}) and the absence of hydroxyl groups. The ^1H and ^{13}C NMR spectra of **1a** (Table I) showed the presence of one ketone and three methoxycarbonyl groups, two (one tri- and one tetrasubstituted) double bonds, four sp^3 methines, and two long alkyl side chains. Since six out of eight unsaturations were thus accounted for, compound **1a** was inferred to contain two rings. The ^1H–^1H COSY spectrum of **1a** revealed a proton network consisting of four sp^3 methine protons and a vinyl proton [δ_H 3.43 (H-2), 3.17 (H-1), 3.31 (H-6), 3.67 (H-5), and 5.76 (H-4), connected in this sequence]. One-bond ^1H–^{13}C correlations were determined by the HSQC[7] spectrum to make assignments of all protonated carbons of **1a**. The HMBC[8] spectrum of **1a** revealed that both H-2 and H-4 showed long-range ^1H–^{13}C connectivities with an sp^2 quaternary carbon at δ_C 136.8 (s, C-3) and H-2 also showed a cross-peak with the sp^2 methine carbon at δ_C 123.1 (C-4), indicating the presence of a cyclohexene ring for **1a**. The presence of a cyclopentenone moiety in **1a** was deduced with the aid of spectral investigations of the tetrahydro derivative (**1b**) prepared by hydrogenation of **1a**. The IR absorption band at 1755 cm^{-1} for **1b** suggested the presence of a cyclopentanone ring. In the ^1H–^1H COSY spectrum of **1b** the signal due to H-1 showed a cross-peak with H-9, which in turn was correlated with H-8.[9] Since the ^{13}C signal at δ_C 205.8 for **1b** showed the HMBC correlations with H-1, H-6, and H-8, this ^{13}C signal was as-

(2) (a) Phillipson, D. W.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1983**, *105*, 7735–7736. (b) Sakemi, S.; Higa, T.; Anthoni, U.; Christophersen, C. *Tetrahedron* **1987**, *43*, 263–268. (c) Kushlan, D. M.; Faulkner, D. J. *J. Nat. Prod.* **1991**, *54*, 1451–1454. (d) Davidson, B. S. *J. Org. Chem.* **1991**, *56*, 6722–6724.

(3) Murayama, T.; Ohizumi, Y.; Nakamura, H.; Sasaki, T.; Kobayashi, J. *Experientia* **1989**, *45*, 898–899.

(4) Kobayashi, J.; Takeuchi, S.; Ishibashi, M.; Shigemori, H.; Sasaki, T. *Tetrahedron Lett.* **1992**, *33*, 2579–2580.

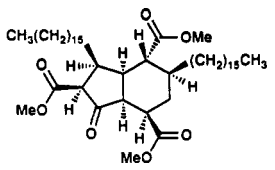
(5) (a) Kobayashi, J.; Kanda, F.; Ishibashi, M.; Shigemori, H. *J. Org. Chem.* **1991**, *56*, 4574–4576. (b) Kobayashi, J.; Shigemori, H.; Ishibashi, M.; Yamasu, T.; Hirota, H.; Sasaki, T. *J. Org. Chem.* **1991**, *56*, 5221–5224. (c) Kobayashi, J.; Itagaki, F.; Shigemori, H.; Ishibashi, M.; Takahashi, K.; Ogura, M.; Nagasawa, S.; Nakamura, T.; Hirota, H.; Ohta, T.; Nozoe, S. *J. Am. Chem. Soc.* **1991**, *113*, 7812–7813. (d) Kondo, K.; Shigemori, H.; Kikuchi, Y.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1992**, *57*, 2480–2483. (e) Tsuda, M.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1992**, *57*, 3503–3507.

(6) From this *Plakortis* sponge plakotenin was coisolated.[4]

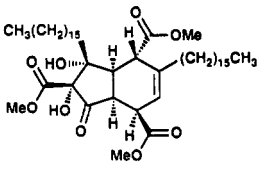
(7) Otting, G.; Wüthrich, K. *J. Magn. Reson.* **1988**, *76*, 569–574.

(8) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.

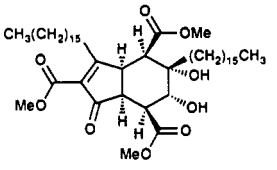
(9) It was likely that hydrogenation on $\Delta^{3,4}$- and $\Delta^{9,9}$-double bonds occurred from the same side as H-1 (convex side). On irradiation of H-9 of **1b** a 12% NOE was observed at H-1.



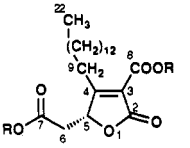
1b



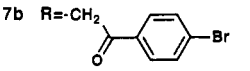
1c



2b



7 R=H
7a R=Me



7b

signed to a ketone (C-7) in a 5-membered ring. The ^{13}C NMR spectrum of the methyl ester (**1a**) revealed two sp^2 quaternary carbons at δ_C 132.9 and 182.8 due to C-8 and C-9, respectively, which were conjugated with the ketone at C-7 (δ_C 202.1),[10] and this C-7 signal of **1a** showed the HMBC correlations to two protons (H-1 and H-6) on the cyclohexene ring. From these results the presence of bicyclo[4.3.0]nona-3,8-dien-7-one moiety was revealed for **1a**. The HMBC spectrum of **1a** showed cross-peaks from the sp^3 methylene carbon (C-10, δ_C 36.9, t) to H-2 and H-4, and the HMBC correlations were also observed from C-9 to methylene protons on C-26 [δ_H 2.40 (H-26a) and 3.06 (H-26b)]. The difference NOE experiments on irradiation at H-2 revealed appreciable enhancements at signals due to H_2-10 and H-26a. These observations implied that two alkyl side chains were attached to C-3 and C-9 positions, and three methoxycarbonyl groups, therefore, had to be placed on C-2, C-5, and C-8 positions. Naturally occurring manzamenone A (**1**, $C_{46}H_{78}O_7$ based on HRFABMS) possesses one carboxyl group, and one of three methoxy groups in **1a** was derived from diazomethane. The carboxyl group of **1** was deduced to be located at C-5 by analysis of the HMBC spectrum of **1** [cross peaks: C-42 (δ_C 170.3)/H-1, C-42/H-2, and C-42/H_3CO (δ_H 3.54); C-43 (δ_C 174.4)/H-5 and C-43/H-6; C-44 (δ_C 162.9)/H_3CO (δ_H 3.87)], only C-43 being not correlated with methoxy protons. Each of alkyl side chains at C-3 and C-9 was suggested to be a hexadecyl [$-(CH_2)_{15}CH_3$] group on the basis of the EIMS data of **1a**, in which fragment ions were observed at m/z 665 ($C_{44}H_{73}O_4$), 639 ($C_{43}H_{75}O_3$), 441 ($C_{28}H_{41}O_4$), and 415 ($C_{27}H_{43}O_3$). Each of the differences of the compositions for m/z 665/441 and 639/415 was shown to correspond to $C_{16}H_{32}$ (224 amu) by HREIMS data.

Spectral data of manzamenone B methyl ester (**2a**) corresponded well to those of manzamenone A methyl ester (**1a**) and suggested that **2a** is a stereoisomer of **1a** with different configuration of a substituent on the cyclohexene ring moiety. Difference NOE experiments of **1a** showed NOE correlations for H-1/H-6, H-6/H-5, and H-5/H-4, implying that the H-1, H-6, and H-5 were on the same side of the cyclohexene ring and bridgehead protons (H-1 and H-6) were therefore oriented cis. The proton–proton coupling constants of **1a** and **2a** for H-1/H-6, H-6/H-5, and H-5/H-4 were almost the same (**1a**: $J_{1,6}$ = 7.9 Hz, $J_{6,5}$ = 6.4 Hz, and $J_{5,4}$ = 2.1 Hz; **2a**: $J_{1,6}$ = 7.1 Hz, $J_{6,5}$ = 5.9 Hz, and $J_{5,4}$ = 2.1 Hz), while those between H-1 and H-2 were quite different (**1a**: $J_{1,2}$ = 6.0 Hz; **2a**: $J_{1,2}$ = 0.5 Hz). In the difference NOE experiment of **2a** irradiation of H-2 caused a significant NOE for H-1. These observations revealed that the configuration at C-1 position was different, viz., H-1 and H-2 were trans for **1a** and cis for **2a**. These findings were coincident with the fact that treatment of **1a** and **2a** with 1 equiv of osmium tetraoxide afforded a 8,9-diol (**1c**) and a 3,4-diol (**2b**), respectively. The convex side of the $\Delta^{3,4}$-double bond of **1a** was hindered by the methoxycarbonyl group on C-2, whereas this steric effect was small for **2a**. Thus, the structure of manzamenone A was concluded to be **1**.[11]

The HRFABMS of manzamenone C (**3**) showed that its molecular formula was $C_{48}H_{82}O_7$, having one CH_2 unit more than that of 43-*O*-methylmanzamenone A (**1a**). The ^1H NMR spectrum of **3** was almost superimposable with that of **1a**. The difference was the presence of an ethoxy group [δ_H 4.25 (2 H, m) and 1.32 (3 H, t, J = 7.0 Hz)] for **3** in place of one of three methoxy groups for **1a**. The HMBC spectrum of **3** showed cross-peaks from C-43 (δ_C 173.8) to H-4, H-5, H-6, and the methylene protons (δ_H 4.25) of the ethoxy group, thus establishing that manzamenone C is 43-*O*-ethylmanzamenone A (**3**). On treatment with 2 N HCl/MeOH at room temperature **3** was converted into **1a**, which was identified by the HPLC analysis.

The molecular formula of manzamenone D (**4**) was shown as $C_{46}H_{79}NO_6$ by HRFABMS. The nitrogen atom was ascribed to an amide group because of the IR absorption bands at 3470 and 1680 cm^{-1} and two D_2O-exchangeable proton signals at δ_H 5.34 and 7.44 (each 1 H, br d, J = 1 Hz). The HMBC correlations for C-5/H_2N and C-43/H-5 revealed that the amide group was attached on C-5.

Manzamenone E (**5**) also contained an amide group, which was suggested by IR (3300 and 1670 cm^{-1}) and ^1H NMR [δ_H 8.11 (1 H, d, J = 7.5 Hz, exchangeable)] spectra. Interpretation of the ^1H–^1H COSY spectrum of **5** showed the presence of valine [δ_H 8.11 (NH), 4.43 (α), 2.33 (β), and 1.06 and 1.07 (each 3 H, d, J = 6.8 Hz; CH_3 × 2)], which was verified by amino acid analysis of the hydrolysate of **5**. This valine residue was defined to be L by the chiral GC method. The HMBC spectrum of **5** showed the connectivities from α-H of the valine residue to a carbonyl carbon at δ_H 174.2 (C-43), which was further correlated with H-4, H-5, and H-6, suggesting that the valine residue was connected through an amide bond with the carboxyl group on C-5.

The HRFABMS of manzamenone F (**6**), isolated from the *Plakortis* sponge collected at Unten-harbor, indicated its molecular formula to be $C_{50}H_{86}O_7$, possessing three more CH_2 units than that of 43-*O*-methylmanzamenone A (**1a**). Comparison of the ^1H–^1H COSY spectrum of **6** with that of **1a** revealed the presence of an *n*-butyl ester [δ_H 4.19 (2 H, m; H_2-1′), 1.65 (2 H, m; H_2-2′), 1.41 (2 H, m; H_2-3′), and 0.95 (3 H, t, J = 7.0 Hz; H_2-4′)] for **6** in place of one of three methyl ester groups for **1a**. The ester carbonyl carbon on C-5 (δ_H 173.9; C-43) showed HMBC correlates with H-4, H-6, and H_2-1′ of the *n*-butyl group. Manzamenone F was therefore revealed to be 43-*O*-*n*-butylmanzamenone A (**6**). The *n*-butyl ester group of **6** was exchanged with methyl ester by treatment with 2 N HCl/MeOH under reflux to give **1a** together with a small amount of **2a**, which was derived through an inversion of the methoxycarbonyl group on C-2.[12]

(10) The ^{13}C chemical shifts of C-7–C-9 of **1a** coincided with those of 3,4-dimethylcyclopentenone [δ_C 208.5 (C-1), 130.5 (C-2), and 182.2 (C-3)]: Kalinowski, H.-O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*; John Wiley & Sons: Chichester, 1984; p 269.

(11) Manzamenone A (**1**) showed no cytotoxicity against L1210 murine leukemia cells and KB human epidermoid carcinoma cells in vitro, while compound **1** exhibited weak inhibitory activity on protein kinase C.

In connection with the biogenetic path of manzamenones, we isolated 3-carboxy-5-(carboxymethyl)-4-tetradecyl-1-oxacyclopent-3-en-2-one (**7**) from the same *Plakortis* sponge collected off Manzamo. The structure of compound **7** was defined on the basis of extensive spectroscopic analyses. The molecular formula of **7** was determined by HREIMS as $C_{21}H_{34}O_6$. The ^1H and ^{13}C NMR spectrum of **7** revealed the presence of three ester or acid carbonyls, one tetrasubstituted double bond, one sp^3 oxymethine, one sp^3 methylene, and an alkyl chain. Four of five unsaturations were thus characterized, and compound **7** was therefore monocyclic. On treatment with diazomethane compound **7** was converted into a dimethyl ester **7a**, indicating the presence of two carboxylic acids. The ^1H–^1H COSY spectrum of **7** showed that one sp^3 methylene (δ_H 2.53 and 2.89; H_2-6) was adjacent to the sp^3 oxymethine (δ_H 5.24; H-5). The ^1H chemical shifts suggested that H_2-6 were α to a carboxyl group, which was confirmed by the ^1H–^{13}C long-range connectivities from H_2-6 to a carboxyl group (C-7) observed in the HMBC spectrum of **7**. The ^1H chemical shift of H-5 implied that C-5 was oxygenated as well as being allylic and H-5 showed HMBC correlations to two sp^2 carbons (C-3 and C-4).[13] From these observations, compound **7** was suggested to have a γ-butenolide structure with a carboxymethyl group attached to C-5. The long alkyl chain (a tetradecyl group) was shown to be connected to C-4 by the HMBC cross-peaks from H_2-9 to C-4 and C-5. Another carboxyl group (δ_C 170.0, C-8), therefore, had to be placed on C-3. The absolute configuration of the chiral center at C-5 was deduced as *R*, since the di-*p*-bromophenacyl ester **7b**, prepared from **7**, exhibited a positive Cotton effect in the CD spectrum [λ_{ext} 247 nm ($\Delta\epsilon$ +25) and 227 nm ($\Delta\epsilon$ −17)], resulting from the positive chirality due to the two *p*-bromophenacyl groups.[14]

Manzamenones could be assumed as being biosynthetically generated from two fatty acid-derived precursors (**A** and **B**, Scheme I). Both **A** and **B** might be derived through condensation of malonate with 4-oxo-2,3-dehydro carboxylic acid **C**. A butenolide **8**, which is an equivalent compound to **C**, was previously isolated from a Micronesian *Plakortis* sponge.[15] It may be proposed that manzamenones were yielded through an enantioselective intermolecular *endo*-type [4 + 2] cycloaddition between **A** and **B**. Compound **7** could be also assumed to be biosynthesized through a condensation of malonate and the hypothetical precursor **C** (a homologue with two less CH_2 units).

Experimental Section

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 × 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 × 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 × 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 × 58 cm; MeOH/$CHCl_3$ (1:1)) followed by reversed-phase column (YMC ODS 60, 1.1 × 20 cm; CH_3CN/$CHCl_3$ (7:3)) to give a fraction (190 mg) mainly containing manzamenones, which was partially purified by reversed-phase HPLC [Develosil ODS-5 (5 μm, 10 × 250 mm); eluent: CH_3CN/$CHCl_3$ (8:2 or 7:3 with 0.01% trifluoroacetic acid); flow rate: 2.5 mL/min; detection: UV at 254 nm] to afford manzamenone A (**1**, 0.003% wet weight), B (**2**, 0.0008%), C (**3**, 0.0007%), E (**5**, 0.0005%), and a crude mixture containing mainly manzamenone D, which was further purified by the same HPLC column using MeOH/$CHCl_3$/H_2O (6:3.5:1) as eluent to give manzamenone D (**4**, 0.0002%). The 460–570-mL fraction (165 mg) of the second silica gel column was further purified successively by Sephadex LH-20 (1.1 × 60 cm; MeOH/$CHCl_3$, (1:1), reversed-phase column (YMC ODS 60, 1.1 × 20 cm; 95% MeOH), and reversed-phase HPLC [Develosil ODS-5 (5 μm, 10 × 250 mm); eluent: MeOH/H_2O (92:8 with 0.01% trifluoroacetic acid); flow rate: 2.5 mL/min; detection: UV at 254 nm] to give compound **7** (0.003%).

During the separation procedure described above a part of a crude mixture of manzamenones A and B (47 mg) was dissolved in methanol (1 mL) and treated with diazomethane[16] in ether (2 mL) at room temperature for 15 min. After the solvent was evaporated off, the residue was purified by a silica gel column chromatography (1.1 × 20 cm) with MeOH/$CHCl_3$ (3:97) to afford a mixture of methyl esters and manzamenone A and B (40 mg), which was further separated by HPLC [Develosil ODS-5 (5 μm, 10 × 250 mm); eluent; MeOH/H_2O (80:20); flow rate: 2.0 mL/min; detection: UV at 254 nm] to afford 43-*O*-methylmanzamenone A (**1a**, 29 mg) and 43-*O*-methylmanzamenone B (**2a**, 3.7 mg).

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 × 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 × 36 cm) with EtOAc/hexane (2:8). The fraction eluting from 340 to 420 mL was further purified by a Sephadex LH-20 column (2.0 × 108 cm) with MeOH/$CHCl_3$ (1:1) to afford manzamenone F (**6**, 0.008% wet weight).

Manzamenone A (1): a colorless oil; $[\alpha]^{17}_D$ −3.0° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1730 and 1690 cm^{-1}; UV (EtOH) λ_{max} 228 nm (ϵ 10 000); ^1H NMR ($CDCl_3$) δ_H 3.20 (1 H, dd, *J* = 7.9 and 6.0 Hz; H-1), 3.50 (1 H, d, *J* = 6.0 Hz; H-2), 6.16 (1 H, br d, *J* = 2.1 Hz; H-4), 3.62 (1 H, dd, *J* = 8.6 and 2.1 Hz; H-5), 2.95 (1 H, dd, *J* = 8.6 and 7.9 Hz; H-6), 2.20 (2 H, m; H_2-10), 2.45 (1 H, ddd, *J* = 13, 9.7, and 5.4 Hz; H-26a), 3.12 (1 H, ddd, *J* = 13, 10, and 6.4 Hz; H-26b), 1.22–1.64 (56 H, br s; H_2-11–H_2-24 and H_2-27–H_2-40), 0.88 (6 H, t, *J* = 7.1 Hz; H_3-25 and H_3-41), 3.54 (3 H, s; 42-OMe), and 3.87 (3 H, s; 44-OMe); ^{13}C NMR ($CDCl_3$) δ_C 44.0 (d, C-1), 46.4 (d, C-2), 137.1 (s, C-3), 123.2 (d, C-4), 41.3 (d, C-5), 46.1 (d, C-6), 206.1 (s, C-7), 132.5 (s, C-8), 186.6 (s, C-9), 36.8 (t, C-10), 30.5 (t, C-26), 31.9, 29.7 (many carbons overlapped), 27.8, 27.1, and 22.6 (each t, C-11–C-24 and C-27–C-40), 14.1 (2C, q, C-25 and C-41), 170.3 (s, C-42), 52.0 (q, 42-OMe), 174.4 (s, C-43), 162.9 (s, C-44), and 52.0 (q, 44-OMe); FABMS *m/z* 765 $(M + Na)^+$ and 743 $(M + H)^+$; HRFABMS *m/z* 743.5798, calcd for $C_{46}H_{79}O_7$ (M + H) 743.5826.

Manzamenone B (2): a colorless oil; $[\alpha]^{17}_D$ +7.7° (*c* 0.26, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1720 cm^{-1}; UV (EtOH) λ_{max} 231 nm (ϵ 10 000); ^1H NMR ($CDCl_3$) δ_H 3.88 (1 H, dd, *J* = 7.1 and 0.5 Hz; H-1), 3.24 (1 H, d, *J* = 0.5 Hz, H-2), 6.11 (1 H, br d, *J* = 2.1 Hz; H-4), 3.41 (1 H, m; H-5), 3.46 (1 H, dd, *J* = 7.1 and 5.0 Hz; H-6), 2.06 (2 H, m; H_2-10), 2.36 (1 H, ddd, *J* = 13, 9.3, and 5.7 Hz; H-26a), 3.15 (1 H, ddd, *J* = 13, 10, and 6.4 Hz; H-26b), 1.2–1.6 (56 H, br s; H_2-11–H_2-24 and H_2-27–H_2-40), 0.88 (6 H, t, *J* = 6.8 Hz; H_3-25 and H_3-41), and 3.74 and 3.78 (each 3 H, s; 42-OMe and 44-OMe); FABMS *m/z* 765 $(M + Na)^+$ and 743 $(M + H)^+$; HRFABMS *m/z* 743.5797, calcd for $C_{46}H_{79}O_7$ (M + H) 743.5826.

Manzamenone C (3): a colorless oil; $[\alpha]^{17}_D$ −1.0° (*c* 0.54, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1725 cm^{-1}; UV (EtOH) λ_{max} 224 nm (ϵ 10 400); CD (EtOH) λ_{ext} 246 ($\Delta\epsilon$ +2.3) and 220 (−12); ^1H NMR ($CDCl_3$) δ_H 3.17 (1 H, dd, *J* = 7.8 and 6.2 Hz; H-1), 3.43 (1 H, d, *J* = 6.2 Hz; H-2), 5.76 (1 H, br d, *J* = 2.2 Hz; H-4), 3.64 (1 H, dd, *J* = 6.0 and 2.2 Hz; H-5), 3.32 (1 H, dd, *J* = 7.8 and 6.0 Hz; H-6),

(12) The ratio of **1a** and **2a** after 10 min of reflux was approximately 9:1 and changed into almost 1:1 after 2 h of reflux.

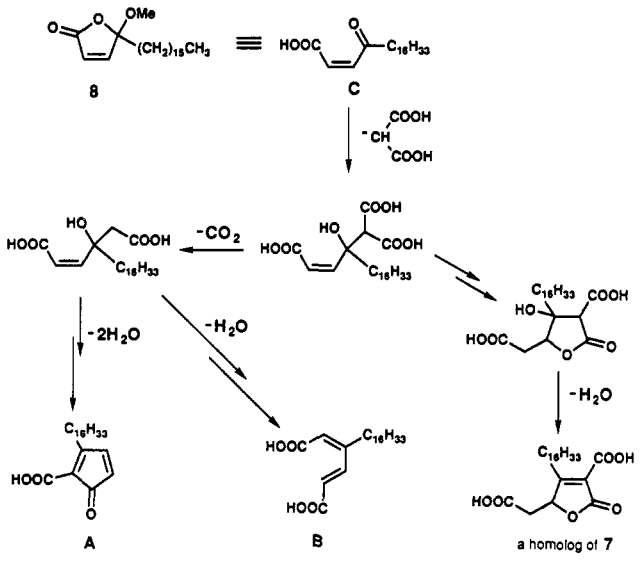
(13) Assignment of C-3 and C-4 signals was based on the observation of the HMBC correlation (three-bond coupling) between C-4 (δ_C 133.8) and one of the methylene protons on C-6 (δ_H 2.89).

(14) Tanaka, T.; Morimoto, S.; Nonaka, G.; Nishioka, I.; Yokozawa, T.; Chung, H. Y.; Oura, H. *Chem. Pharm. Bull.* **1989**, *37*, 340–344.

(15) De Guzman, F. S.; Schmitz, F. J. *J. Nat. Prod.* **1990**, *53*, 926–931.

(16) For a cautionary note on the use of diazomethane, see: Moore, J. A.; Reed, D. E. *Organic Synthesis*; John Wiley and Sons: New York, 1973; Collect. Vol. V, pp 351–355.

Scheme I



2.13 (2 H, m; H_2-10), 2.40 (1 H, ddd, J = 13, 10, and 5.5 Hz; H-26a), 3.06 (1 H, ddd, J = 13, 10, and 7.0 Hz; H-26b), 1.2–1.6 (56 H, br s; H_2-11–H_2-24 and H_2-27–H_2-40), 0.88 (6 H, t, J = 7.0 Hz; H_3-25 and H_3-41), 3.72 (3 H, s; 42-OMe), 4.25 (2 H, m; H_2-1′ of 43-OEt), 1.32 (3 H, t, J = 7.0 Hz; H_3-2′ of 43-OEt), and 3.82 (3 H, s; 44-OMe); ^{13}C NMR ($CDCl_3$) δ_C 42.9 (d, C-1), 46.8 (d, C-2), 136.8 (s, C-3), 123.3 (d, C-4), 41.9 (d, C-5), 45.9 (d, C-6), 202.2 (s, C-7), 132.9 (s, C-8), 182.9 (s, C-9), 36.9 (t, C-10), 30.2 (t, C-26), 31.9, 29.7 (many carbons overlapped), 27.8, 27.3, and 22.7 (each t, C-11–C-24 and C-27–C-40), 14.1 (2 C, q, C-25 and C-41), 170.7 (s, C-42), 51.9 (q, 42-OMe), 173.8 (s, C-43), 61.5 (t, C-1′ of 43-OEt), 14.1 (q, C-2′ of 43-OEt), 163.7 (s, C-44), and 51.9 (q, 44-OMe); EIMS m/z 771 (M^+), 739, 713, 666, 640, and 415; HREIMS m/z 770.6021, calcd for $C_{48}H_{82}O_7$ (M) 770.6060.

Manzamenone D (4): a colorless oil; $[\alpha]^{17}_D$ −9.5° (c 1.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3470, 3325, 1720, 1710, 1680, 1610, 1590, and 1425 cm^{-1}; UV (EtOH) λ_{max} 228 nm (ϵ 11600); CD (EtOH) λ_{ext} 320 ($\Delta\epsilon$ −3.1), 250 (+1.5), and 223 (−31); ^{1}H NMR ($CDCl_3$) δ_H 3.14 (1 H, dd, J = 7.7 and 5.9 Hz; H-1), 3.46 (1 H, d, J = 5.9 Hz; H-2), 6.10 (1 H, br d, J = 1.9 Hz; H-4), 3.53 (1 H, dd, J = 5.9 and 1.9 Hz; H-5), 2.82 (1 H, dd, J = 7.7 and 5.9 Hz; H-6), 2.18 (2 H, m; H_2-10), 2.44 (1 H, ddd, J = 13, 9.4, and 5.4 Hz; H-26a), 3.08 (1 H, ddd, J = 13, 9.7, and 6.6 Hz; H-26b), 1.2–1.6 (56 H, br s; H_2-11–H_2-24 and H_2-27–H_2-40), 0.88 (6 H, t, J = 6.9 Hz; H_3-25 and H_3-41), 3.52 (3 H, s; 42-OMe), 5.34 and 7.44 (each 1 H, br d, J = 1 Hz; 43-NH_2), and 3.85 (3 H, s; 44-OMe); ^{13}C NMR ($CDCl_3$) δ_C 44.3 (d, C-1), 46.3 (d, C-2), 136.3 (s, C-3), 123.8 (d, C-4), 40.7 (d, C-5), 48.3 (d, C-6), 205.3 (s, C-7), 132.7 (s, C-8), 185.0 (s, C-9), 37.0 (t, C-10), 30.4 (t, C-26), 31.9, 29.7 (many carbons overlapped), 27.8, 27.2, and 22.7 (each t, C-11–C-24 and C-27–C-40), 14.1 (2 C, q, C-25 and C-41), 170.7 (s, C-42), 51.9 (q, 42-OMe), 175.4 (s, C-43), 163.2 (s, C-44), and 52.0 (q, 44-OMe); EIMS m/z 682 (M − CO_2Me)$^+$, 640, 623, 582, 457, and 415; FABMS m/z 764 (M + Na)$^+$ and 742 (M + H)$^+$; HRFABMS m/z 742.6005, calcd for $C_{46}H_{80}NO_6$ (M + H) 742.5985.

Manzamenone E (5): a colorless oil; $[\alpha]^{19}_D$ +75° (c 0.31, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3300, 1720, 1710, 1670, and 1530 cm^{-1}; UV (EtOH) λ_{max} 227 nm (ϵ 9500); CD (EtOH) λ_{ext} 315 ($\Delta\epsilon$ +13), 283 (+4.3), and 221 (+180); ^{1}H NMR ($CDCl_3$) δ_H 3.15 (1 H, dd, J = 7.0 and 5.0 Hz; H-1), 3.46 (1 H, d, J = 5.0 Hz; H-2), 6.13 (1 H, br s; H-4), 3.58 (1 H, br d, J = 7.0 Hz; H-5), 2.87 (1 H, dd, J = 7.0 Hz; H-6), 2.18 (2 H, m; H_2-10), 2.43 (1 H, m; H-26a), 3.02 (1 H, m; H-26b), 1.2–1.6 (56 H, br s; H_2-11–H_2-24 and H_2-27–H_2-40), 0.88 (6 H, t, J = 6.7 Hz; H_3-25 and H_3-41), 3.52 (3 H, s; 42-OMe), 8.11 (1 H, d, J = 7.5 Hz; 43-NH), 4.43 (1 H, dd, J = 6.8 and 5.0 Hz; H-α of Val), 2.33 (1 H, dt, J = 5.0 and 6.8 Hz; H-β of Val), 1.06 and 1.07 (each 3 H, d, J = 6.8 Hz; Me × 2 of Val), and 3.85 (3 H, s; 44-OMe); ^{13}C NMR ($CDCl_3$) δ_C 44.2 (d, C-1), 46.5 (d, C-2), 136.2 (s, C-3), 123.5 (d, C-4), 40.9 (d, C-5), 47.9 (d, C-6), 205.7 (s, C-7), 132.8 (s, C-8), 184.9 (s, C-9), 37.0 (t, C-10), 30.5 (t, C-26), 32.0, 29.7 (many carbons overlapped), 27.8, 27.2, and 22.7 (each t, C-11–C-24 and C-27–C-40), 14.1 (2 C, q, C-25 and C-41), 170.8 (s, C-42), 51.9 (q, 42-OMe), 174.2 (s, C-43), 185.1 (s, COOH of Val), 58.0 (d, C-α of Val), 29.8 (d, C-β of Val), 17.9 and 19.5 (each q, Me × 2 of Val), 163.3 (s, C-44), and 52.1 (q, 44-OMe); EIMS m/z 823 (M − H_2O)$^+$, 795, 765, 721, 638, 496, and 414; FABMS m/z 864 (M + Na)$^+$ and 842 (M + H)$^+$; HRFABMS m/z 842.6456, calcd for $C_{51}H_{88}O_8N$ (M + H) 842.6510.

Manzamenone F (6): a colorless oil; $[\alpha]^{19}_D$ −3.3° (c 1.0, MeOH); IR (KBr) ν_{max} 1730 and 1720 cm^{-1}; UV (MeOH) λ_{max} 225 nm (ϵ 8000); CD (EtOH) λ_{ext} 236 ($\Delta\epsilon$ +0.56); ^{1}H NMR ($CDCl_3$) δ_H 3.17 (1 H, dd, J = 7.7 and 6.4 Hz; H-1), 3.44 (1 H, d, J = 6.4 Hz; H-2), 5.75 (1 H, br s; H-4), 3.64 (1 H, dd, J = 6.4 and 2.7 Hz; H-5), 3.32 (1 H, dd, J = 7.7 and 6.4 Hz; H-6), 2.12 (2 H, m; H_2-10), 2.38 (1 H, ddd, J = 15, 9.5, and 5.4 Hz; H-26a), 3.06 (1 H, ddd, J = 15, 8.8, and 6.4 Hz; H-26b), 1.2–1.6 (56 H, br s; H_2-11–H_2-24 and H_2-27–H_2-40), 0.88 (6 H, t, J = 6.6 Hz; H_3-25 and H_3-41), 3.48 (3 H, s; 42-OMe), 4.19 (2 H, m; H_2-1′ of 43-OBu), 1.65 (2 H, m; H_2-2′ of 43-OBu), 1.41 (2 H, m; H_2-3′ of 43-OBu), 0.95 (3 H, t, J = 7.0 Hz; H_3-4′ of 43-OBu), and 3.82 (3 H, s; 44-OMe); ^{13}C NMR ($CDCl_3$) δ_C 42.9 (d, C-1), 46.8 (d, C-2), 136.7 (s, C-3), 123.2 (d, C-4), 41.9 (d, C-5), 45.8 (d, C-6), 202.0 (s, C-7), 132.9 (s, C-8), 182.7 (s, C-9), 36.9 (t, C-10), 30.2 (t, C-26), 31.9, 29.7 (many carbons overlapped), 27.8, 27.3, and 22.7 (each t, C-11–C-24 and C-27–C-40), 14.1 (2 C, q, C-25 and C-41), 170.7 (s, C-42), 51.9 (q, 42-OMe), 173.9 (s, C-43), 65.1 (t, C-1′ of 43-OBu), 30.0 (t, C-2′ of 43-OBu), 19.1 (t, C-3′ of 43-OBu), 13.7 (q, C-2′ of 43-OBu), 163.6 (s, C-44), and 51.9 (q, 44-OMe); EIMS m/z 740 (M − CO_2Me)$^+$, 639, 515, and 415; FABMS m/z 799 (M + H)$^+$; HRFABMS m/z 799.6521, calcd for $C_{50}H_{87}O_7$ (M + H) 799.6452.

43-*O*-Methylmanzamenone A (1a): a colorless solid; mp 63–64 °C; IR ($CHCl_3$) 1730 cm^{-1}; UV (EtOH) λ_{max} 228 nm (ϵ 13000); CD (EtOH) λ_{ext} 235 ($\Delta\epsilon$ −2.9), 231 (−1.1), 222 (−8.6), 220 (−3.4), and 212 (−9.7); ^{1}H and ^{13}C NMR (Table I); EIMS m/z 757 (M^+), 725, 699, 666, 640, 441, and 415; HREIMS m/z 756.5907, calcd for $C_{47}H_{80}O_7$ (M) 756.5904, m/z 665.5500, calcd for $C_{44}H_{73}O_4$ (M − MeOH − CO_2Me) 665.5509, m/z 639.5693, calcd for $C_{43}H_{75}O_3$ (M − 2CO_2Me + H) 639.5716, m/z 441.3024, calcd for $C_{28}H_{41}O_4$ (M − MeOH − CO_2Me − $C_{16}H_{32}$) 441.3005, and m/z 415.3236, calcd for $C_{27}H_{43}O_3$ (M − 2CO_2Me + H − $C_{16}H_{32}$) 415.3212.

43-*O*-Methylmanzamenone B (2a): a colorless oil; UV (EtOH) λ_{max} 230 nm (ϵ 19000); ^{1}H NMR ($CDCl_3$) δ_H 3.85 (1 H, dd, J = 7.1 and 0.5 Hz; H-1), 3.24 (1 H, d, J = 0.5 Hz; H-2), 6.15 (1 H, br d, J = 2.1 Hz; H-4), 3.34 (1 H, m; H-5), 3.42 (1 H, dd, J = 7.1 and 5.9 Hz; H-6), 2.06 (2 H, m; H_2-10), 2.35 (1 H, ddd, J = 13, 10, and 5.4 Hz; H-26a), 3.13 (1 H, ddd, J = 13, 10, and 6.4 Hz; H-26b), 1.2–1.6 (56 H, br s; H_2-11–H_2-24 and H_2-27–H_2-40), 0.88 (6 H, t, J = 6.9 Hz; H_3-25 and H_3-41), 3.72 (3 H, s; 42-OMe), 3.80 (3 H, s; 43-OMe), and 3.78 (3 H, s; 44-OMe); ^{13}C NMR ($CDCl_3$) δ_C 43.7 (d, C-1), 45.4 (d, C-2), 136.3 (s, C-3), 123.3 (d, C-4), 39.3 (d, C-5), 47.5 (d, C-6), 202.3 (s, C-7), 133.6 (s, C-8), 187.4 (s, C-9), 36.7 (t, C-10), 30.1 (t, C-26), 31.9, 29.7 (many carbons overlapped), 28.1, 27.0, and 22.7 (each t, C-11–C-24 and C-27–C-40), 14.1 (2 C, q, C-25 and C-41), 171.8 (s, C-42), 52.7 (q, 42-OMe), 172.1 (s, C-43), 52.1 (q, 43-OMe), 166.1 (s, C-44), and 51.9 (q, 44-OMe); EIMS m/z 757 (M^+), 725, 699, 666, 640, 441, and 415; HREIMS m/z 756.5954, calcd for $C_{47}H_{80}O_7$ (M) 756.5904.

43-*O*-Methyl-3,4,8,9-tetrahydromanzamenone A (1b). A solution of 43-*O*-methylmanzamenone A (**1a**, 6.5 mg) in MeOH (2 mL) in the presence of 10% Pd/C catalyst (1 mg) was stirred under an atmosphere of hydrogen for 2 days. After removal of the catalysts by filtration and evaporation of the solvent, the residue was purified by HPLC [Develosil ODS-5 (5 μm, 10 × 250 mm); eluent: $CH_3CN/CHCl_3$ (7:3); flow rate: 2.5 mL/min; detection: refractive index] to give the tetrahydro derivative **1b** (3.4 mg): IR ($CHCl_3$) ν_{max} 1755 and 1720 cm^{-1}; CD (EtOH) λ_{ext} 325 nm ($\Delta\epsilon$ −0.27), 311 (0), 286 (+1.0), 249 (0), 243 (−0.10), and 235 (0); ^{1}H NMR (C_6D_6) δ_H 2.73 (1 H, td, J = 7.9 and 5.7 Hz; H-1), 2.79 (1 H, dd, J = 5.7 and 4.3 Hz; H-2), 1.69 (1 H, m; H-3), 1.93 (2 H, m; H_2-4), 3.73 (1 H, br t, J = 4.3 Hz; H-5), 3.24 (1 H, d, J = 7.9 Hz; H-6), 3.41 (1 H, d, J = 11 Hz; H-8), 2.98 (1 H, dq, J = 11 and 7.9 Hz; H-9), 1.2–1.6 (60 H, br s; H_2-10–H_2-24 and H_2-26–H_2-40), 0.96 (6 H, t, J = 6.4 Hz; H_3-25 and H_3-41), 3.22 (3 H, s; 42-OMe), 3.36 (3 H, s; 43-OMe), and 3.42 (3 H, s; 44-OMe); ^{13}C NMR (C_6D_6) δ_C 39.2 (d, C-1), 43.3 (d, C-2), 36.0 (d, C-3), 25.6 (t, C-4), 37.6 (d, C-5), 51.2 (d, C-6), 205.6 (s, C-7), 58.6 (d, C-8), 42.8 (d, C-9), 34.3, 32.3, 30.1 (many carbons overlapped), 28.5, 27.2, and 23.1 (each t, C-10–C-24 and C-26–C-40), 14.3 (2 C, q,

C-25 and C-41), 174.8 (s, C-42), 50.6 (q, 42-OMe), 175.3 (s, C-43), 51.6 (q, 43-OMe), 171.3 (s, C-44), and 52.0 (q, 44-OMe); EIMS m/z 761 (M^+), 729, 701, and 671.

8,9-Dihydroxy-43-*O*-methylmanzamenone A (1c). To the solution of 43-*O*-methylmanzamenone A (**1a**, 26.6 mg, 0.035 mmol) in THF (1.2 mL) and pyridine (0.3 mL) was added 10.5 mg (0.041 mmol) osmium tetraoxide in THF (105 μL), and the mixture was stirred for 2 h at room temperature. After addition of saturated aqueous sodium bisulfite solution (1.6 mL), stirring was continued for 1 h. Then the mixture was partitioned between ethyl acetate and water. The ethyl acetate layer was dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by a silica gel column with acetone/hexane (1:3) to give the 8,9-diol **1c** (27.1 mg): ^{1}H NMR ($CDCl_3$) δ_H 4.08 (1 H, dd, J = 7.3 and 5.8 Hz; H-1), 3.54 (1 H, d, J = 5.8 Hz; H-2), 4.98 (1 H, d, J = 4.0 Hz; H-4), 3.88 (1 H, dd, J = 11 and 4.0 Hz; H-5), 3.61 (1 H, dd, J = 11 and 7.3 Hz; H-6), 1.87 (1 H, m; H-10a), 2.09 (1 H, m; H-10b), 2.41 (1 H, ddd, J = 13, 9.5, and 5.2 Hz; H-26a), 3.02 (1 H, ddd, J = 13, 9.5, and 6.3 Hz; H-26b), 1.2–1.6 (56 H, br s; H_2-11–H_2-24 and H_2-27–H_2-40), 0.88 (6 H, t, J = 6.7 Hz; H_3-25 and H_3-41), 3.79, 3.74, and 3.53 (each 3 H, s; 42-OMe, 43-OMe, and 44-OMe); ^{13}C NMR ($CDCl_3$) δ_C 42.8 (d, C-1), 45.9 (d, C-2), 132.3 (s, C-3), 125.3 (d, C-4), 41.6 (d, C-5), 44.1 (d, C-6), 93.2 (s, C-8), 91.9 (s, C-9), 39.4 (t, C-10), 31.9, 30.4, 30.3, 29.7 (many carbons overlapped), 27.7, 23.0, and 22.7 (each t, C-11–C-24 and C-26–C-40), 14.1 (2 C, q, C-25 and C-41), 173.2, 172.3, and 164.1 (each s, C-42, C-43, and C-44), 51.9, 51.6, and 51.5 (each q, 42-OMe, 43-OMe, and 44-OMe); FABMS m/z 896 (M + diethanolamine + H)$^+$, 864, and 846.

3,4-Dihydroxy-43-*O*-methylmanzamenone B (2b). 43-*O*-Methylmanzamenone B (**2a**, 6.2 mg, 0.008 mmol) was treated with OsO_4 (2.5 mg, 0.010 mmol) by the same procedure as above to afford the 3,4-diol **2b** (1.9 mg): ^{1}H NMR ($CDCl_3$) δ_H 3.66 (1 H, dd, J = 11 and 6.7 Hz; H-1), 2.11 (1 H, d, J = 11 Hz; H-2), 2.48 (1 H, s; 3-OH), 3.70 (1 H, dd, J = 6.7 and 3.0 Hz; H-4), 3.82 (1 H, d, J = 3.0 Hz; 4-OH), 3.25 (1 H, dd, J = 11 and 6.7 Hz; H-5), 3.36 (1 H, t, J = 6.7 Hz; H-6), 1.12 (1 H, m; H-10a), 2.02 (1 H, m; H-10b), 1.93 (1 H, ddd, J = 13, 9.0, and 5.0 Hz; H-26a), 3.22 (1 H, ddd, J = 13, 10, and 5.5 Hz; H-26b), 1.2–1.6 (56 H, br s; H_2-11–H_2-24 and H_2-27–H_2-40), 0.88 (6 H, t, J = 6.7 Hz; H_3-25 and H_3-41), 3.77, 3.79, and 3.82 (each 3 H, s; 42-OMe, 43-OMe, and 44-OMe); FABMS m/z 896 (M + diethanolamine + H)$^+$ and 864.

Ester Exchange of Manzamenone C (3). Manzamenone C (**3**, 0.1 mg) was treated with 2 N HCl/MeOH (0.1 mL) at room temperature for 10 min. After evaporation of the solvent, the residue was analyzed by HPLC [Develosil ODS-5 (5 μm, 10 × 250 mm); eluent: MeOH/$CHCl_3$ (7:3); flow rate: 2.0 mL/min; detection: UV (254 nm)] to show a peak at t_R 20 min together with a small peak at t_R 22 min in the ratio of 8:1, which were ascribed to 43-*O*-methylmanzamenone A (**1a**) and the starting material **3**, respectively.

Determination of Amino Acid Residue of Manzamenone E (5). Manzamenone E (**5**, 50 μg) was heated in 6 N HCl at 110 °C for 24 h. The hydrolyzate was analyzed by a Hitachi amino acid autoanalyzer (Model 835) to show the presence of valine (t_R 55.1 min). For the chiral GC analysis, the acid hydrolysate of manzamenone E (**5**, 50 μm) was treated with 10% HCl/MeOH (0.5 mL) at 100 °C for 30 min. After the reaction mixture was evaporated under vacuum, the residue was heated in a mixture of trifluoroacetic anhydride (0.3 mL) and CH_2Cl_2 (0.3 mL) at 100 °C for 5 min and then evaporated. The residue was dissolved in CH_2Cl_2 and subjected to capillary GC analysis [Chirasil-Val column (Alltech, 0.32 mm × 25 m); carrier gas: nitrogen; program rate: 50–200 °C at 4 °C/min] to show a peak at t_R 6.6 min, which was ascribed to L-valine by comparison with the peaks of TFA/Me derivatives of authentic D- and L-valines (t_R 6.2 and 6.6 min, respectively).

Ester Exchange of Manzamenone F (6). Manzamenone F (**6**, 1 mg) was treated with 2 N HCl/MeOH (5 mL) under reflux for 10 min. After evaporation of the solvent, the residue was analyzed by HPLC [Develosil ODS-5 (5 μm, 10 × 250 mm); eluent: CH_3CN/$CHCl_3$ (7:3); flow rate: 2.0 mL/min; detection: UV (254 nm)] to show a peak at t_R 32.6 min together with a small peak at t_R 29.0 min in the ratio of ca. 9:1, which were ascribed to 43-*O*-methylmanzamenone A (**1a**) and 43-*O*-methylmanzamenone B (**2a**), respectively. The major product **1a** was isolated by HPLC (the same conditions as above) and firmly identified by comparison of TLC [R_f 0.45, hexane/EtOAc (3:1)], ^{1}H NMR, and EIMS data with those of authentic sample. After reflux for 2 h under the same conditions, the ratio of the products (**1a** and **2a**) was shown to be ca. 1:1 by HPLC analysis.

3-Carboxy-5-(carboxymethyl)-4-tetradecyl-1-oxacyclopent-3-en-2-one (7): a colorless oil; $[\alpha]^{25}_D$ +12° (c 0.78, $CHCl_3$); IR ($CHCl_3$) 3200, 1740, and 1720 cm^{-1}; UV (MeOH) λ_{max} 235 nm (ϵ 5500); ^{1}H NMR ($CDCl_3$) δ_H 5.24 (1 H, dd, J = 8.9 and 3.6 Hz; H-5), 2.53 (1 H, dd, J = 16 and 8.9 Hz; H-6a), 2.89 (1 H, dd, J = 16 and 3.6 Hz; H-6b), 2.13 (1 H, ddd, J = 15, 9.0, and 6.0 Hz; H-9a), 2.49 (1 H, ddd, J = 15, 9.0, and 7.1 Hz; H-9b), 1.2–1.6 (24 H, br s; H_2-10–H_2-21), and 0.88 (3 H, t, J = 6.9 Hz; H_3-22); ^{13}C NMR ($CDCl_3$) δ_C 173.4 (s, C-2), 137.7 (s, C-3), 133.8 (s, C-4), 76.8 (d, C-5), 24.5 (t, C-6), 173.4 (s, C-7), 170.0 (s, C-8), 29.7 (many carbons overlapped), 27.1, and 22.7 (each t, C-9–C-21), and 14.1 (q, C-22); EIMS m/z 382 (M^+), 338, and 293; HREIMS m/z 382.2372, calcd for $C_{21}H_{34}O_6$ (M) 382.2355.

Dimethyl Ester 7a. Compound **7** (0.5 mg) in methanol (0.5 mL) was treated with diazomethane in ether (1 mL) at room temperature for 20 min. After evaporation of the solvent, the residue was purified by a silica gel column chromatography (0.5 × 4 cm) with $CHCl_3$ to afford the dimethyl ester **7a** (0.5 mg): ^{1}H NMR ($CDCl_3$) δ_H 5.24 (1 H, dd, J = 8.9 and 3.6 Hz; H-5), 2.53 (1 H, dd, J = 16 and 8.9 Hz; H-6a), 2.89 (1 H, dd, J = 16 and 3.6 Hz; H-6b), 2.13 (1 H, ddd, J = 15, 9.0, and 6.0 Hz; H-9a), 2.49 (1 H, ddd, J = 15, 9.0, and 7.1 Hz; H-9b), 3.74 and 3.95 (each 3 H, s; 7-OMe and 8-OMe), 1.2–1.6 (24 H, br s; H_2-10–H_2-21), and 0.88 (3 H, t, J = 6.6 Hz; H_3-22); EIMS m/z 410 (M^+).

Di-*p*-bromophenacyl Ester (7b). Compound **7** (2.0 mg) was treated with *p*-bromophenacyl bromide (8.0 mg) in dimethylformamide (0.2 mL) containing potassium fluoride (3.0 mg) at room temperature for 2 h. After addition of H_2O (0.5 mL), the reaction mixture was extracted with ether (1 mL × 3). The ether layer was washed with H_2O (0.5 mL × 5), dried over sodium sulfate, and evaporated under reduced pressure. The residue was purified by a silica gel column chromatography (1.1 × 4 cm) with hexane/ether (1:1) to give the *p*-bromophenacyl ester **7b** (0.6 mg): UV (EtOH) λ_{max} 255 nm (ϵ 37 000); CD (EtOH) λ_{ext} 247 nm ($\Delta\epsilon$ +25) and 227 (−17); ^{1}H NMR ($CDCl_3$) δ_H 5.24 (1 H, dd, J = 8.9 and 3.6 Hz; H-5), 2.72 (1 H, dd, J = 16 and 8.2 Hz; H-6a), 3.06 (1 H, dd, J = 16 and 4.0 Hz; H-6b), 2.23 (1 H, m; H-9a), 2.69 (1 H, m; H-9b), 1.2–1.6 (24 H, br s; H_2-10–H_2-21), and 0.88 (3 H, t, J = 6.6 Hz; H_3-22), 5.27, 5.41, 5.54, and 5.64 (each 1 H, d, J = 17 Hz), 7.61 and 7.64 (each 2 H, d, J = 8.3 Hz), and 7.75 (4 H, d, J = 8.3 Hz); EIMS m/z 776 (M^+).

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Supplementary Material Available: All spectra of **1a** and **7** and ^{1}H NMR spectra of manzamenones A–F (**1–6**) (24 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Bryostatins Revisited: A New Bryostatin 3 and the Use of NMR To Determine Stereochemistry in the C-20–C-23 Area

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During our continued isolation studies of the bryostatins present in lyophilized *B. neritina*, a new compound, **1**, was isolated. It has the same exact mass (by HR FABMS) as

