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Jaspiferals A ~ G, New Cytotoxic Isomalabaricane-type Nortriterpenoids from Okinawan Marine Sponge *Jaspis stellifera*

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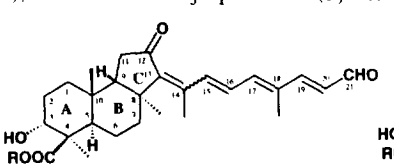
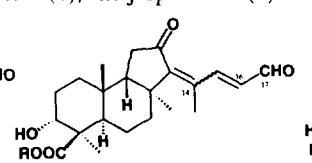
Abstract: New cytotoxic isomalabaricane-type nortriterpenoids with a 3α-hydroxy group, jaspiferals A ~ G (**1** ~ **7**), were isolated from the Okinawan marine sponge *Jaspis stellifera* and the structures were determined on the basis of spectroscopic data and chemical means.

Marine sponges belonging to the genus *Jaspis* (order Choristida, family Jaspidae) have been a rich source of structurally unique compounds.[1~6] In our studies on bioactive substances from marine organisms,[7~9] we previously isolated new isomalabaricane-type triterpenoids[10], stelliferins A ~ F, from the Okinawan marine sponge *J. stellifera*.[11] Further investigation on the constituents of this sponge resulted in isolation of new cytotoxic isomalabaricane-type nortriterpenoids with a 3α-hydroxy group, jaspiferals A ~ G (**1** ~ **7**). This paper describes the isolation and structure elucidation of **1** ~ **7**.

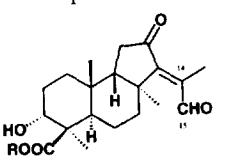
MeOH extracts of the sponge *Jaspis stellifera* collected off Ishigaki Island, Okinawa, were partitioned between EtOAc and H_2O. The EtOAc-soluble materials were subjected to silica gel columns followed by silica gel and C_{18} HPLC to afford a 1:1 mixture of jaspiferals A (**1**) and B (**2**), a 5.5:4.5 mixture of jaspiferals C (**3**) and D (**4**), a 1:1 mixture of jaspiferals E (**5**) and F (**6**), and jaspiferal G (**7**). Since separation of each mixture



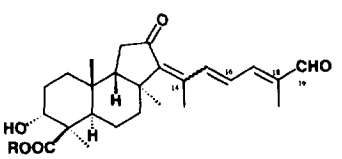
1 R = H, Δ^{13} = *Z*
2 R = H, Δ^{13} = *E*
8 R = CH_3, Δ^{13} = *Z*
9 R = CH_3, Δ^{13} = *E*



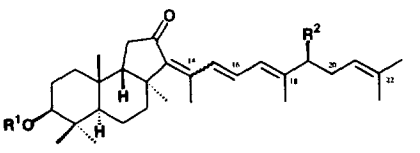
5 R = H, Δ^{13} = *Z*
6 R = H, Δ^{13} = *E*
12 R = CH_3, Δ^{13} = *Z*
13 R = CH_3, Δ^{13} = *E*



7 R = H
14 R = CH_3



3 R = H, Δ^{13} = *Z*
4 R = H, Δ^{13} = *E*
10 R = CH_3, Δ^{13} = *Z*
11 R = CH_3, Δ^{13} = *E*



15 R^1 = Ac, R^2 = OH, Δ^{13} = *Z*
16 R^1 = Ac, R^2 = OH, Δ^{13} = *E*
17 R^1 = H, R^2 = H, Δ^{13} = *Z*

Table 1. ^1H and ^{13}C NMR Data of Jaspiferals A (**1**), B (**2**), C (**3**), and D (**4**) in $CDCl_3$/MeOH (9:1).

	1		**2**		**3**		**4**	
positn	δ_H (m, *J*)	δ_C (m)	δ_H (m, *J*)	δ_C (m)	δ_H (m, *J*)	δ_C (m)	δ_H (m, *J*)	δ_C (m)
1	1.81 (m) 1.00 (brd, 14.7)	28.7 (t)	1.81 (m) 1.00 (brd, 14.7)	28.7 (t)	1.81 (m) 1.06 (brd, 13.1)	28.7 (t)	1.81 (m) 1.06 (brd, 13.1)	28.7 (t)
2	1.63 (brd, 13.7) 2.12 (m)	27.4 (t)	1.63 (brd, 13.7) 2.12 (m)	27.4 (t)	1.63 (brd, 13.7) 2.12 (m)	27.3 (t)	1.63 (brd, 13.7) 2.12 (m)	27.3 (t)
3	4.07 (dd, 2.2, 4.4)	70.3 (d)	4.07 (dd, 2.2, 4.4)	70.3 (d)	4.07 (dd, 2.2, 4.4)	70.3 (d)	4.07 (dd, 2.2, 4.4)	70.3 (d)
4		47.5 (s)		47.5 (s)		47.5 (s)		47.5 (s)
5	2.40 (brt, 10.3)	40.2 (d)	2.40 (brt, 10.3)	40.3 (d)	2.40 (brt, 10.3)	40.2 (d)	2.40 (brt, 10.3)	40.3 (d)
6	1.82 (m) 1.77 (m)	20.2 (t)	1.82 (m) 1.77 (m)	20.1 (t)	1.82[b] (m)	20.1 (t)	1.82[b] (m)	20.2 (t)
7	2.05[a] (m)	40.1 (t)	2.11[a] (m)	38.5 (t)	2.08[b] (m)	40.1 (t)	2.15[b] (m)	38.4 (t)
8		44.9 (s)		45.0 (s)		44.9 (s)		45.1 (s)
9	1.82 (m)	49.3 (d)	1.82 (m)	49.4 (d)	1.82 (m)	49.2 (d)	1.82 (m)	49.3 (d)
10		35.8 (s)		35.8 (s)		35.9 (s)		35.9 (s)
11	2.35 (dd, 4.4, 15.6) 2.11 (m)	36.9 (t)	2.35 (dd, 4.4, 15.6) 2.11 (m)	36.7 (t)	2.19 (dd, 3.9, 15.6) 2.12 (m)	36.8 (t)	2.19 (dd, 3.9, 15.6) 2.12 (m)	36.7 (t)
12		208.5 (s)		207.6 (s)		208.5 (s)		207.6 (s)
13		148.5 (s)		149.0 (s)		149.7 (s)		150.3 (s)
14		141.5 (s)		140.5 (s)		140.6 (s)		139.6 (s)
15	8.16 (d, 15.1)	138.1 (d)	6.82 (d, 14.6)	138.1 (d)	8.30 (d, 14.7)	140.7 (d)	6.99 (d, 14.7)	140.7 (d)
16	6.86 (dd, 11.2, 15.1)	129.1 (d)	6.94 (dd, 10.7, 14.6)	130.5 (d)	7.00 (dd, 11.2, 14.7)	127.9 (d)	6.90 (dd, 11.2, 14.7)	130.5 (d)
17	6.63 (brd, 11.2)	141.4 (d)	6.60 (brd, 10.7)	140.5 (d)	7.00 (brd, 11.2)	149.4 (d)	6.97 (brd, 11.2)	148.1 (d)
18		135.8 (s)		136.3 (s)		138.9 (s)		139.3 (s)
19	7.16 (d, 15.6)	156.5 (d)	7.15 (d, 15.6)	157.1 (d)	9.40 (s)	195.2 (d)	9.43 (s)	194.9 (d)
20	6.15 (dd, 7.8, 15.6)	127.7 (d)	6.19 (dd, 7.8, 15.6)	128.3 (d)				
21	9.49 (d, 7.8)	194.1 (d)	9.51 (d, 7.8)	194.4 (d)				
4-Me	1.23 (s)	23.5 (q)	1.22 (s)	23.5 (q)	1.23 (s)	23.5 (q)	1.22 (s)	23.5 (q)
4-COOH		180.45 (s)		180.5 (s)		180.5 (s)		180.5 (s)
8-Me	1.36 (s)	24.2 (q)	1.39 (s)	25.8 (q)	1.36 (s)	24.2 (q)	1.39 (s)	25.8 (q)
10-Me	0.88 (s)	19.68 (q)	0.88 (s)	19.7 (q)	0.88 (s)	19.7 (q)	0.88 (s)	19.7 (q)
14-Me	2.00 (s)	15.8 (q)	2.25 (s)	14.3 (q)	2.01 (s)	15.8 (q)	2.26 (s)	14.2(q)
18-Me	1.96 (brs)	12.6 (q)	1.92 (brs)	12.8 (q)	1.82 (brs)	9.4 (q)	1.86 (brs)	9.6 (q)

a) 2H

of the stereoisomers (**1** and **2**, **3** and **4**, and **5** and **6**) by using silica gel or reversed-phase HPLC was not successful, these mixtures were converted into the corresponding methyl esters (**8** and **9**, **10** and **11**, and **12** and **13**) with diazomethane, and separated by HPLC using a phenyl group-bound silica gel column. Structure determination was carried out mainly with each mixture of the stereoisomers (**1** and **2**, **3** and **4**, and **5** and **6**), while geometries of the double bonds of **1** ~ **6** were elucidated on the basis of the ^1H NMR data of each methyl ester **8** ~ **13**.

The 1:1 mixture of jaspiferals A (**1**) and B (**2**) showed the molecular ion peak at *m/z* 440 in the EIMS spectrum, and the common molecular formula, $C_{27}H_{36}O_5$, of **1** and **2** was established by the HREIMS (*m/z* 440.2535, M^+, Δ -2.7 mmu). The IR absorptions at 3400 and 1670 cm^{-1} were attributed to hydroxy and unsaturated carbonyl groups, respectively. The UV absorption at 374 nm (ε 41000) suggested the presence of a polyene chromophore. The ^1H and ^{13}C NMR (Table 1) spectra of the mixture of **1** and **2** in $CDCl_3/CD_3OD$ (9:1) showed a pair of signals due to a ketone, an aldehyde, a carboxyl, eight sp^2 (four quaternary and four methine), eleven sp^3 (three quaternary, three methine, and five methylene), and five methyl carbons. Seven of ten unsaturation degrees were accounted for, thus indicating that **1** and **2** possessed a common tricyclic core and a geometrically isomeric polyene moiety.

The structures of jaspiferals A (**1**) and B (**2**) were elucidated by detailed analyses of ^1H-^1H COSY, HMBC, and NOESY spectra of the 1:1 mixture. The ^1H-^1H COSY spectrum revealed that **1** and **2** possessed the following proton networks in common: H_2-1 ~ H-3, H-5 ~ H_2-7, H-9 ~ H_2-11, H-15 ~ 18-Me, and H-19 ~ H-21. The tetraenal side chain (C-14 ~ C-21) at C-13 was deduced from NOESY cross-peaks for H-17/H-19 and 18-Me/H-20 and HMBC correlations for 18-Me/C-17, 8-Me/C-13, 14-Me/C-13, 14-Me/C-14, and H-15/14-Me. Geometries of the four double bonds in the side chain of jaspiferal A (**1**) were assigned as 13*Z*, 15*E*, 17*E*, and 19*E* from the ^1H coupling constants ($J_{15,16}$ = 15.1 Hz; $J_{19,20}$ = 15.6 Hz) as well as comparison of the chemical shifts of the methyl ester **8** with those of stelliferin A (**15**), while the four double bonds of jaspiferal B (**2**) were similarly found to have all *E*-geometry like those of stelliferin B (**16**). The *trans-syn-trans* junction of A ~ C rings of **1** and **2** was assigned by NOESY data as well as comparison of the ^{13}C chemical shifts of tricyclic core of **1** and **2** with those of stelliferins A (**15**) and D (**17**). The coupling

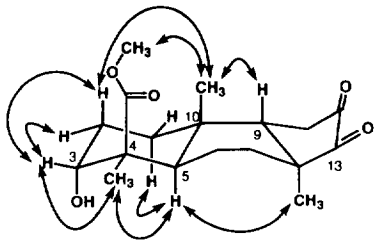


Chart I. Relative Stereochemistry of Ozonolysis Product (18) of Jaspiferals A and B Methyl Esters (8 and 9) and NOEs (arrows).

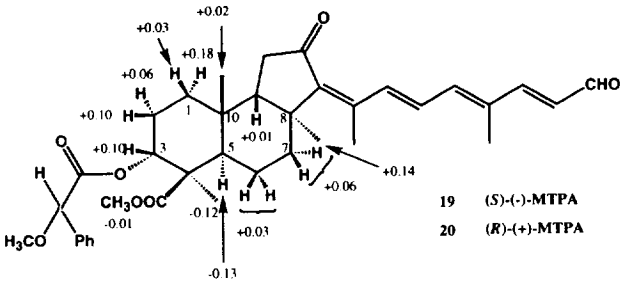


Chart II. Δδ Values [Δδ (in ppm) = δ_S -δ_R] Obtained for (*S*)- and (*R*)-MTPA Esters (19 and 20) of Jaspiferal A Methyl Ester (8).

constants ($J_{2,3}$ = 2.2 and 4.4 Hz) between of H-2 and H-3 for **1** and **2** were quite different from those ($J_{2,3}$ = 5.5 and 10.6 Hz) of **17** with a 3β-hydroxy group, impling that jaspiferals A (**1**) and B (**2**) had a 3α-hydroxy group. This was also supported by NOESY data (Chart I) and proton coupling constants of α-diketone (**18**) generated from **8** and **9** by oxidation with ozone. Thus the structures of jaspiferals A and B including the relative stereochemistry were elucidated to be **1** and **2**.

For consideration of the absolute configuration at C-3 of jaspiferal A (**1**), differences of ^{1}H chemical shifts of (*S*)- and (*R*)-MTPA esters (**19** and **20**, respectively) of **8** were obtained as shown in Chart II. The profile of chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) for the MTPA esters of **8** was similar to that reported for the MTPA esters of 3α-choresterol but different from that for those of fridelan-3β-ol.[12] However the absolute configuration at C-3 of **8** may be not directly derived from this observation, since **8** is generally a compound ruled out for MTPA method.

The HREIMS data of the 5.5: 4.5 mixture of jaspiferals C (**3**) and D (**4**), the 1:1 mixture of E (**5**) and F (**6**), and G (**7**) revealed that they had the molecular formulas, $C_{25}H_{34}O_5$, $C_{22}H_{30}O_5$, and $C_{20}H_{28}O_5$, respectively. These compounds were found to possess the same tricyclic core as that of **1** or **2**, since ozonolysis of the corresponding methyl esters (**10** ~ **14**) afforded the common α-diketone **18**.

The ^{1}H and ^{13}C NMR data (Table 1) of the mixture of **3** and **4** revealed the presence of a trienal group at C-13, which was supported by the UV absorption at 344 nm (ε 40000). A pair of signals due to an aldehyde proton (δ_H 9.43 for **3** and δ_H 9.40 for **4**) was assigned as H-19 by the following NOE difference experiments; irradiation of H-19 yielded NOE at H-17 [16 (**3**) and 14 % (**4**), respectively]. Comparison of the chemical shifts of H-15 and 14-Me of the methyl esters **10** (δ_H 8.89 and 1.70, respectively) and **11** (δ_H 6.86 and 2.49 , respectively) indicated that **11** and **12** possessed 13*Z*,15*E*,17*E*- and 13*E*,15*E*,17*E*-geometry, respectively. Thus the structures of jaspiferals D and E were assigned to be **3** and **4**, respectively.

The ^{1}H NMR (Table 2) spectrum of the mixture of jaspiferals E (**5**) and F (**6**) showed a pair of proton signals due to two olefin (δ_H 8.75 and 6.30 for **5**; δ_H 7.53 and 6.41 for **6**), an aldehyde (δ_H 9.60 for **5**; δ_H 9.62 for **6**), and a vinyl methyl group (δ_H 1.97 for **5**; δ_H 2.20 for **6**). The UV absorption at 304 nm (ε 41000) in addition to the ^{1}H NMR data implied the presence of a diene system conjugated with an aldehyde, which was supported by the cross-peak of H-15 to H-17 in the ^{1}H-^{1}H COSY spectrum. 15*E*-Geometry of **5** and **6** was assigned by the coupling constant of H-15/H-16 [$J_{15,16}$ = 16.1 (**5**) and 15.6 (**6**) Hz]. The ^{1}H chemical shifts at Me-14 and H-15 (δ_H 2.03 and 8.83, respectively) of **12** indicated 13*Z*-geometry, while 13*E*-geometry of **13** was deduced from the ^{1}H chemical shifts at Me-14 and H-15 (δ_H 2.29 and 7.57, respctively). The structures of jaspiferals E and F were therefore concluded to be **5** and **6**, respectively

Jaspiferal G (**7**) was isolated as a geometrically pure compound. The ^{1}H NMR data (Table 2) of **7** contained proton signals due to an aldehyde (δ_H 10.14) and a vinyl methyl group (δ_H 2.05). The vinyl methyl proton (14-Me) showed ^{1}H-^{13}C long-range correlations to C-13, C-14, and C-15 in the HMBC spectrum, indicating that the aldehyde group was attached to C-14. An NOE observed for 8-Me/H-15 revealed 13*E*-geometry. Thus the structure of jaspiferal G was elucidated to be **7**.

Table 2. ^1H and ^{13}C NMR Data of Jaspiferals E (**5**), F (**6**), and G (**7**) in $CDCl_3$/MeOH (9:1).

	5		**6**		**7**	
positn	δ_H (m, *J*)	δ_C (m)	δ_H (m, *J*)	δ_C (m)	δ_H (m, *J*)	δ_C (m)
1	1.81 (m) 1.06 (brd, 13.1)	28.7 (t)	1.81 (m) 1.06 (brd, 13.1)	28.7(t)	1.80 (m) 1.06 (brd, 13.1)	28.7(t)
2	2.12 (brd, 13.0) 1.65 (m)	27.3 (t)	2.12 (brd, 13.0) 1.65 (m)	27.3 (t)	2.12 (brd, 13.1) 1.65 (m)	27.3 (t)
3	4.06 (brs)	70.2 (d)	4.06 (brs)	70.2 (d)	4.06 (brs)	70.2 (d)
4		47.5 (s)		47.5 (s)		47.5 (s)
5	2.42 (dd, 8.2, 2.4)	40.2 (d)	2.39 (dd, 8.2, 2.4)	40.2 (d)	2.41 (dd, 8.2, 2.4)	40.5 (d)
6	1.80[a] (m)	20.2 (t)	1.80[a] (m)	20.0 (t)	1.75[a] (m)	21.2 (t)
7	2.05[a] (m)	38.1 (t)	2.12[a] (m)	41.8 (t)	2.22[a] (m)	43.4 (t)
8		45.1 (s)		45.4 (s)		45.4 (s)
9	1.92 (m)	49.2 (d)	1.87 (m)	49.1 (d)	1.94 (m)	49.7 (d)
10		35.9 (s)		35.9 (s)		35.9 (s)
11	2.26 (m) 2.12 (m)	36.5 (t)	2.24 (m) 2.12 (m)	36.1 (t)	2.18 (m) 2.14 (m)	35.4 (t)
12		207.4 (s)		208.2 (s)		208.9 (s)
13		152.6 (s)		153.5 (s)		158.9 (s)
14		137.4 (s)		138.3 (s)		137.3 (s)
15	8.75 (d, 16.1)	151.6 (d)	7.53 (d, 15.6)	151.5 (d)	10.14 (s)	194.8 (d)
16	6.30 (dd, 7.8, 16.1)	132.0 (d)	6.41 (dd, 7.3, 15.6)	133.3 (d)		
17	9.60 (d, 7.8)	195.4 (d)	9.62 (d, 7.3)	193.8 (d)		
4-Me	1.25 (s)	23.5 (q)	1.25 (s)	23.5 (q)	1.25 (s)	23.9 (q)
4-COOH		180.3 (s)		180.3 (s)		180.3 (s)
8-Me	1.37 (s)	23.9 (q)	1.42 (s)	26.1 (q)	1.48 (s)	27.6 (q)
10-Me	0.94 (s)	19.7 (q)	0.94 (s)	20.0 (q)	0.95 (s)	20.2 (q)
14-Me	1.97 (s)	15.8 (q)	2.20 (s)	14.3 (q)	2.05 (s)	11.0 (q)

a) 2H

Table 3. Cytotoxicity (IC_{50}, μg/mL) of Jaspiferals A ~ G (**1** ~ **7**) and the Methyl Esters (**8** ~ **11**).

	1 and **2**	**3** and **4**	**5** and **6**	**7**	**8** and **9**	**10** and **11**
L1210	3.8	4.3	3.1	0.54	0.77	2.0
KB	>10	>10	5.5	1.8	4.4	4.8

Jaspiferals A ~ G (**1** ~ **7**) are the first examples of isomalabaricane-type nortriterpenoids with a 3α-hydroxy group, while stelliferins A ~ F isolated previously from this sponge are isomalabaricane-type triterpenoids with a 3β-hydroxy or 3β-acetoxy group. To our knowledge it is very rare that cyclic terpenoids with a 3α-hydroxy group and those with a 3β-hydroxy group have been isolated from the same marine organism.[13] The 3α-hydroxy group in **1** ~ **7** may be biosynthetically generated from stelliferin D (**17**)-type precursors, e.g. through oxidation of 4β-methyl group followed by lactonization between 3β-hydroxy and 4β-carboxyl groups and hydration at C-3. On the other hand, the enal side chain in **1** ~ **7** may be also derived from stelliferin-type triterpenoids through oxidative cleavage of the side chain double bonds. Compounds **5** ~ **11** exhibited cytotoxicity against murine lymphoma L1210 cells and epidermoid carcinoma KB cells in vitro, while compounds **1** ~ **4** showed cytotoxicity against L1210 cells (Table 3). Jaspiferal G (**7**) exhibited antifungal activity against *Cryptococcus neoformans* (MIC, 50 μg/mL) and *Trichophyton memtagrophytes* (MIC, 12.5 μg/mL), and antibacterial activity against *Sarcina lutea* (MIC, 50 μg/mL), while the mixture of jaspiferals E (**5**) and F (**6**) showed antifugal activity against *T. memtagrophytes* (MIC, 50 μg/mL).

EXPERIMENTAL

Collection, Extraction, and Isolation. The brown-colored sponge, *J. stellifera*, was collected off Ishigaki Island, Okinawa, and stored at -20 °C until used. The sponge (1.0 kg wet weight) was extracted with MeOH (1 L x 2) and then evaporated under reduced pressure to give a residue (48.2 g). The EtOAc soluble material (5.66 g) of the extract was subjected to a silica gel column with $CHCl_3$/MeOH (9:1) and then hexane/EtOAc (3:7). The fraction was further separated by silica gel HPLC (Senshu Pack Silica-4251-S, Senshu Scientific, 10 x 250 mm; flow rate, 3.0 mL/min; UV detection at 230 nm) with hexane/$CHCl_3$/MeOH (60:37:3) followed by C_{18} HPLC (YMC Pack AM-323 ODS, YMC Co. Ltd., 10 x 250 mm; flow rate, 2.0 mL/min; UV detection at 230 nm) with MeOH/H_2O (75:25) to afford the mixture of jaspiferals A and B (**1** and **2**, 22.6 mg, 0.01

%, wet weight, t_R 18.0 min), the mixture of jaspiferals C and D (**3** and **4**, 14.9 mg, 0.007 %, t_R 11.4 min), the mixture of jaspiferals E and F (**5** and **6**, 21.8 mg, 0.01 %, t_R 8.4 min), and jaspiferal G (**7**, 10.9 mg, 0.007 %, t_R 7.5 min).

1:1 Mixture of Jaspiferals A (1) and B (2). A yellow powder; mp. 245 ~ 247 °C; $[\alpha]_D^{20}$ -41.2° (*c* 1.1, $CHCl_3$/MeOH, 9:1); IR (film) ν_{max} 3400 and 1670 cm^{-1}; UV (EtOH) λ_{max} 273 (ε 10000), 374 (41000), and 392 nm (sh); ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* 440 (M^+) and 442 ($M-H_2O)^+$; HREIMS *m/z* 440.2535 (M^+, calcd for $C_{27}H_{36}O_5$, 440.2562).

5.5:4.5 Mixture of Jaspiferals C (3) and D (4). A pale yellow powder; mp. 220 ~ 222 °C; $[\alpha]_D^{20}$ -47° (*c* 0.72, $CHCl_3$/MeOH, 9:1); IR (film) ν_{max} 3450 and 1670 cm^{-1}; UV (MeOH) λ_{max} 251 (ε 7800), 344 (40000), and 357 nm (sh); ^1H and ^{13}C NMR (see Table 1); EIMS *m/z* 414 (M^+); HREIMS *m/z* 414.2421 (M^+, calcd for $C_{25}H_{34}O_5$, 414.2407).

1:1 Mixture of Jaspiferals E (5) and F (6). A pale yellow powder; mp. 196 ~ 198 °C; $[\alpha]_D^{20}$ -60° (*c* 0.75, $CHCl_3$/MeOH, 9:1); IR (film) ν_{max} 3450 and 1680 cm^{-1}; UV (MeOH) λ_{max} 218 (ε 17800) and 304 nm (41300); ^1H and ^{13}C NMR (see Table 2); EIMS *m/z* 374 (M^+); HREIMS *m/z* 374.2108 (M^+, calcd for $C_{22}H_{30}O_5$, 374.2094).

Jaspiferal G (7). A colorless powder; mp. 145 ~ 147 °C; $[\alpha]_D^{20}$ -54° (*c* 0.30, $CHCl_3$/MeOH, 9:1); IR (film) ν_{max} 3450 and 1700 cm^{-1}; UV (MeOH) λ_{max} 202 (ε 7000) and 266 nm (14000); ^1H and ^{13}C NMR (see Table 2); EIMS *m/z* 348 (M^+); HREIMS *m/z* 348.2421 (M^+, calcd for $C_{20}H_{28}O_5$, 348.2432).

Methyl Esters (8 and 9) of Jaspiferals A (1) and B (2). An acetone solution (1 mL) of the mixture of jaspiferals A and B (**1** and **2**, 15.0 mg) was treated with diazomethane in ethyl ether (0.4 mL) at room tremperature for 30 min. After evaporating reagent and solvent, the residue was subjected to a silica gel column (hexane/EtOAc, 4:1) to give a mixture of the methyl esters (10.8 mg), part of which (2.4 mg) was further purified by reversed phase HPLC [Develosil Ph-5, Nomura Chemical, 4.6 x 250 mm; flow rate, 1.0 mL/min; UV detection at 254 nm; eluent, MeOH/H_2O (80:20)] to afford **8** (0.9 mg, t_R 18.4 min) and **9** (0.7 mg, t_R 15.8 min) as yellowish oil. **Compound 8.** $[\alpha]_D^{22}$ -18.9° (*c* 0.09, C_6H_6); IR (neat) ν_{max} 3500, 1710, and 1670 cm^{-1}; UV (EtOH) λ_{max} 276 (ε 10000), 374 (70000), and 388 nm (sh); ^1H NMR (C_6D_6) δ 0.71 (3H, s, 10-Me), 1.39 (3H, s, 4-Me), 1.46 (3H, s, 8-Me), 1.53 (1H, br.s, 18-Me), 1.76 (3H, s, 14-Me), 3.35 (3H, s, 4-COOMe), 4.12 (1H, dd, *J* = 2.2 and 4.4 Hz, H-3), 6.12 (1H, dd, *J* = 7.3 and 15.6 Hz, H-20), 6.34 (1H, d, *J* = 11.2Hz, H-17), 6.53 (1H, d, *J* = 15.6 Hz, H-21), 6.70 (1H, dd, *J* = 11.2 and 15.1 Hz, H-16), 8.90 (1H, d, *J* = 15.1 Hz, H-15), and 9.59 (1H, d, *J* = 7.3 Hz, , H-21); EIMS *m/z* 454 (M^+) and 395 ($M-CO_2CH_3)^+$; HREIMS *m/z* 454.2704 (M^+, calcd for $C_{28}H_{38}O_5$, 454.2719). **Compound 9.** $[\alpha]_D^{22}$ -123.7° (*c* 0.07, C_6H_6); IR (neat) ν_{max} 3500, 1710, and 1670 cm^{-1}; UV (EtOH) λ_{max} 275 (ε 10000), 374 (70000), and 388 nm (sh); ^1H NMR (C_6D_6) δ 0.68 (3H, s, 10-Me), 1.27 (3H, s, 4-Me), 1.28 (3H, s, 8-Me), 1.53 (1H, br.s, 18-Me), 2.55 (3H, s, 14-Me), 3.35 (3H, s, 4-COOMe), 4.25 (1H, dd, *J* = 2.2 and 4.4 Hz, H-3), 6.15 (1H, dd, *J* = 7.3 and 15.6 Hz, H-20), 6.22 (1H, d, *J* = 11.2Hz, H-17), 6.66 (1H, d, *J* = 15.6 Hz, H-21), 6.76 (1H, dd, *J* = 11.2 and 14.7 Hz, H-16), 6.86 (1H, d, *J* = 14.7 Hz, H-15), and 9.59 (1H, d, *J* = 7.3 Hz, H-21); EIMS *m/z* 454 (M^+) and 395 ($M-CO_2CH_3)^+$; HREIMS *m/z* 454.2726 (M^+, calcd for $C_{28}H_{38}O_5$, 454.2719).

Methyl Esters (10 and 11) of Jaspiferals C (3) and D (4). The mixture of jaspiferals C and D (**3** and **4**, 5.0 mg) was treated with CH_2N_2 to give the methyl esters, which were separated by the same procedure as described above to afford **10** (1.4 mg, t_R 15.2 min) and **11** (1.4 mg, t_R 14 min) as pale yellow oil. **Compound 10.** $[\alpha]_D^{19}$ -24° (*c* 0.14, C_6H_6); IR (neat) ν_{max} 3500, 1710, and 1670 cm^{-1}; UV (EtOH) λ_{max} 251 (ε 9000), 344 (34000), and 359 nm (sh); ^1H NMR (C_6D_6) δ 0.68 (3H, s, 10-Me), 1.25 (3H, s, 4-Me), 1.28 (3H, s, 8-Me), 1.57 (1H, t, *J* = 10.7 Hz, H-9), 1.70 (3H, s, 14-Me), 1.82 (3H, d, *J* = 1.0 Hz, 18-Me), 3.35 (3H, s, 4-COOMe), 4.13 (1H, dd, *J* = 2.2 and 4.4 Hz, H-3), 6.64 (1H, dd, *J* = 1.0 and 11.2 Hz, H-17), 6.78 (1H, dd, *J* = 11.2 and 15.1 Hz, H-16), 8.89 (1H, d, *J* = 15.1 Hz, H-15), and 9.30 (1H, s, H-19); EIMS *m/z* 428 (M^+) and 369 ($M-CO_2CH_3)^+$; HREIMS *m/z* 428.2554 (M^+, calcd for $C_{26}H_{36}O_5$, 428.2562). **Compound 11.** $[\alpha]_D^{19}$ -85° (*c* 0.14, C_6H_6); IR (neat) ν_{max} 3500, 1710, and 1670 cm^{-1}; UV (EtOH) λ_{max} 251 (ε 9000), 344 (34000), and 359 nm (sh); ^1H NMR (C_6D_6) δ 0.68 (3H, s, 10-Me), 1.39 (3H, s, 4-Me), 1.46 (3H, s, 8-Me), 1.56 (1H, t, *J* = 10.7 Hz, H-9), 1.77 (3H, d, *J* = 1.0 Hz, 18-Me), 2.49 (3H, s, 14-Me), 3.35 (3H, s, 4-COOMe), 4.13 (1H, dd, *J* = 2.2 and 4.4 Hz, H-3), 6.52 (1H, dd, *J* = 1.0 and 11.2 Hz, H-17), 6.76 (1H, dd, *J* = 11.2 and 15.1 Hz, H-16), 6.86 (1H, d, *J* = 15.1 Hz, H-15), and 9.47 (1H, s, H-19); EIMS *m/z* 428 (M^+) and 369 ($M-CO_2CH_3)^+$; HREIMS *m/z* 428.2557 (M^+, calcd for $C_{26}H_{36}O_5$, 428.2562).

Methyl Esters (12 and 13) of Jaspiferals E (5) and F (6). The mixture of jaspiferals E and F (**5** and **6**, 7.0 mg) was treated with CH_2N_2 by the same procedure as described above, and then the residue was purified by a silica gel column (hexane/EtOAc, 4:1) followed by reversed phase HPLC [Develosil Ph-5, MeOH/H_2O (65:35)] to afford **12** (1.8 mg, t_R 15.2 min) and **13** (1.2 mg, t_R 14 min) as pale yellow oil. **Compound 12.** $[\alpha]_D^{18}$ -51° (*c* 0.18, C_6H_6); IR (neat) ν_{max} 3500, 1730, 1700, and 1680 cm^{-1}; UV (EtOH) λ_{max} 219 (ε 9000) and 304 nm (34000); ^1H NMR (C_6D_6) δ 0.66 (3H, s, 10-Me), 1.12 (3H, s, 4-Me), 1.23 (3H, s, 8-Me), 1.47 (3H, s, 14-Me), 1.57 (1H, t, *J* = 10.7 Hz, H-9), 3.35 (3H, s, 4-COOMe), 4.09 (1H, brs, H-3), 6.28 (1H, dd, *J* = 7.6 and 16.1 Hz, H-16), 9.06 (1H, d, *J* = 16.1 Hz, H-15), and 9.68 (1H, d, *J* = 7.6 Hz, H-17); EIMS *m/z* 388 (M^+); HREIMS *m/z* 388.2238 (M^+, calcd for $C_{23}H_{32}O_5$, 388.2250). **Compound 13.** $[\alpha]_D^{18}$ -116° (*c* 0.12, C_6H_6); IR (neat) ν_{max} 3500, 1730, 1700, and 1680 cm^{-1}; UV (EtOH) λ_{max} 251 (ε 9000), 344 (34000), and 359 nm (sh); ^1H NMR (C_6D_6) δ 0.64 (3H, s, 10-Me), 1.19 (3H, s, 4-Me), 1.25 (3H, s, 8-Me), 1.57 (1H, t, *J* = 10.7 Hz, H-9), 2.25 (3H, s, 14-Me), 3.34 (3H, s, 4-COOMe), 4.09 (1H, brs, H-3), 6.27 (1H, dd, *J* = 7.3 and 15.7 Hz, H-16), 7.19 (1H, d, *J* = 15.7 Hz, H-15), and 9.53 (1H, d, *J* = 7.3 Hz, H-17); EIMS *m/z* 388 (M^+); HREIMS *m/z* 388.2271 (M^+, calcd for $C_{23}H_{32}O_5$, 388.2250).

Methyl Ester (14) of Jaspiferal G (7). Jaspiferal G (**7**, 2.2 mg) was treated with CH_2N_2 by the same procedure as described above, and then the residue was purified by a silica gel column (hexane/EtOAc, 4:1) to afford **14** (1.8 mg, t_R 11.2 min) as colorless oil. **Compound 14.** $[\alpha]_D^{18}$ -82° (*c* 0.18, C_6H_6); IR (neat) ν_{max} 3500, 1730, 1700, and 1680 cm^{-1}; UV (EtOH) λ_{max} 205 (ε 7000) and 266 nm (12000); ^1H NMR (C_6D_6) δ 0.60 (3H, s, 10-Me), 1.21 (3H, s, 4-Me), 1.42 (3H, s, 8-Me), 1.57 (1H, t, *J* = 10.7 Hz, H-9), 2.32 (3H, s, 14-Me), 3.35 (3H, s, 4-COOMe), 4.09 (1H, brs, H-3), and 10.35 (1H, s, H-15); EIMS *m/z* 362 (M^+) and 301 ($M-CO_2CH_3)^+$; HREIMS *m/z* 362.2112 (M^+, calcd for $C_{21}H_{30}O_5$, 362.2094).

α-Diketone Compound (18). The mixture of compounds **8** and **9** (1.0 mg) in EtOH (100 μL) was treated with O_3 at -78 °C for 1 min, and then excess O_3 was removed by N_2 gas. To the reaction mixture at -78 °C was added dimethyl sulfide (2 μL), and stirring was continued for 10 h at room temperature. After solvent was evaporated, the residue was subjected to a silica gel column (hexane/EtOAc, 4:1) to afford the diketone (**18**, 0.6 mg). Each methyl ester (the mixture of **10** and **11**, the mixture of **12** and **13**, and **14**, each 2.0 mg) was treated with O_3 by the same procedure as described above to afford **18**. **Compound 18.** colorless oil; $[\alpha]_D^{19}$ -23° (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3400, 1765, 1750, and 1715 cm^{-1}; ^1H NMR (C_6D_6) δ 0.48 (3H, s, 10-Me), 0.63 (1H, m, H-1), 0.72 (1H, m, H-5), 0.89 (3H, s, 4-Me), 1.10 (3H, s, 8-Me), 1.36 (1H, m, H-6), 1.15 (1H, dd, *J* = 5.7 and 13.4 Hz, H-9), 1.37 (1H, m, H-2), 1.42 (1H, m, H-1), 1.55 (1H, m, H-7), 1.67 (1H, brdd, *J* = 9.3 and 13.2 Hz, H-6), 1.78 (1H, m, H-11), 1.83 (1H, m, H-11), 2.06 (1H, brdd, *J* = 7.9 and 13.4 Hz, H-9), 2.14 (1H, brd, *J* = 12.8 Hz, H-2), 3.31 (3H, s, OMe), and 4.02 (1H, brs, H-3); EIMS *m/z* 322 (M^+); HREIMS *m/z* 322.1765 (M^+, calcd for $C_{18}H_{26}O_5$, 322.1781).

(*S*)-(-)-MTPA Ester (19) of Jaspiferal A Methyl Ester (8). To a CH_2Cl_2 solution (500 μL) of the methyl ester **8** (2.3 mg), 4-dimethylaminopyridine (20.0 mg), and (*S*)-(-)-2-methoxy-2-phenyl-trifluoromethylacetic acid (MTPA, 20.0 mg), dicyclohexylcarbodiimide (20.0 mg) was added at room temperature, and stirring was continued for 30 min. After evaporation of solvent, the residue was passed through a silica gel column (hexane/EtOAc, 9:1) to afford the (*S*)-MTPA ester (**19**, 2.5 mg): yellowish oil; $[\alpha]_D^{22}$ -123° (*c* 0.25, C_6H_6); IR (neat) ν_{max} 3450, 1710, and 1670 cm^{-1}; UV (EtOH) λ_{max} 275 (ε 9400), 374 (47000), and 388 nm (sh); ^1H NMR ($CDCl_3$) δ 0.81 (3H, s, 10-Me), 1.10 (3H, s, 4-Me), 1.18 (1H, m, H-1β), 1.18 (3H, s, 8-Me), 1.54 (1H, m, H-1α), 1.75 (2H, m, H_2-6), 1.78 (1H, m, H-9), 1.92 (1H, m, H-2α), 2.02 (3H, br.s, 18-Me), 2.03 (3H, s, 14-Me), 2.07 (3H, m, H-5 and H_2-7), 2.12 (1H, m, H-11α), 2.16 (1H, m, H-11β), 2.25 (1H, m, H-2β), 3.50 (3H, OMe), 3.66 (3H, s, 4-COOMe), 5.65 (1H, dd, *J* = 2.2 and 4.3 Hz, H-3), 6.22 (1H, dd, *J* = 7.8 and 15.1 Hz, H-20), 6.65 (1H, br.d, *J* = 10.2 Hz, H-17), 6.92 (1H, dd, *J* = 11.2 and 15.4 Hz, H-16), 7.18 (1H, d, *J* = 15.1 Hz, H-19), 7.35 ~ 7.55 (5H, m, Ph), 8.23 (1H, d, *J* = 15.4 Hz, H-15), and 9.61 (1H, d, *J* = 7.8 Hz, H-21); EIMS *m/z* 670 (M^+) and 652 $(M-H_2O)^+$; HREIMS *m/z* 670.3145 (M^+, calcd for $C_{38}H_{45}O_7F_3$, 670.3119).

(*R*)-(+)-MTPA Ester (20) of Jaspiferal A Methyl Ester (8). The methyl ester **8** (2.2 mg) was treated with (*R*)-(+)-MTPA (20.0 mg) by the same procedure as described above to afford the (*R*)-MTPA ester (**20**, 2.2 mg): yellowish oil; $[\alpha]_D^{22}$ -19° (*c* 0.22, C_6H_6); IR (neat) ν_{max} 3450, 1710, and 1670 cm^{-1}; UV (EtOH) λ_{max} 276 (ε 9900), 374 (53000), and 388 nm (sh); ^1H NMR ($CDCl_3$) δ 0.79 (3H, s, 10-Me), 1.04 (3H, s, 8-Me), 1.15 (1H, m, H-1β), 1.22 (3H, s, 4-Me), 1.36 (1H, m, H-1α), 1.72 (2H, m, H_2-6), 1.77 (1H, m, H-9), 1.86 (1H, m, H-2α), 2.01 (3H, m, H_2-7), 2.02 (3H, br.s, 18-Me), 2.03 (3H, s, 14-Me), 2.02 (1H, m, H-11α), 2.15 (1H, m, H-2β), 2.16 (1H, m, H-11β), 2.20 (1H, m, H-5), 3.58 (3H, OMe), 3.67 (3H, s, 4-COOMe), 5.55 (1H, dd, *J* = 2.2 and 4.3 Hz, H-3), 6.22 (1H, dd, *J* = 7.8 and 15.1 Hz, H-20), 6.65 (1H, br.d, *J* = 10.2 Hz, H-17), 6.92 (1H, dd, *J* = 11.2 and 15.4 Hz, H-16), 7.18 (1H, d, *J* = 15.1 Hz, H-19), 7.35 ~ 7.55 (5H, m, Ph), 8.23 (1H, d, *J* = 15.4 Hz, H-15), and 9.61 (1H, d, *J* = 7.8 Hz, H-21); EIMS *m/z* 670 (M^+) and 652 $(M-H_2O)^+$; HREIMS *m/z* 670.3123 (M^+, calcd for $C_{38}H_{45}O_7F_3$, 670.3119).

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