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STELLIFERINS A-F, NEW ANTINEOPLASTIC ISOMALABARICANE TRITERPENES FROM THE OKINAWAN MARINE SPONGE *JASPIS STELLIFERA*.

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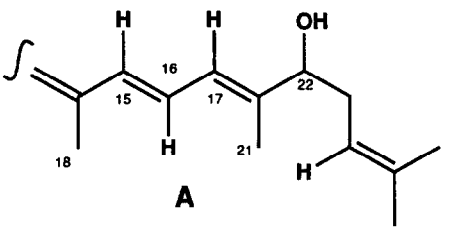
Abstract: Six new antineoplastic isomalabaricane-type triterpenes, stelliferins A-F, have been isolated from the Okinawan marine sponge *Jaspis stellifera*. The structures were established by spectroscopic analyses, espesially 1D and 2D NMR techniques. The stereostructures have been elucidated by NOESY spectra as well as chemical degradation experiments.

Terpenes and terpenoids with a variety of biological activities have been isolated from marine organisms[1]. During our continuing investigations on bioactive substances from Okinawan marine organisms[2], six new isomalabaricane triterpenes, named stelliferins A-F (1 ~ 6), with potent antineoplastic activity have been isolated from the Okinawan marine sponge *Jaspis stellifera*. Natural products possessing isomalabaricane skeleton are very rare[3], and stelliferins A-F are the second examples isolated from marine organisms.

The brown-colored sponge with internally bright yellow, *Jaspis stellifera*, was collected off Ishigaki Island, Okinawa, and kept frozen until used. The methanolic extract was partitioned between ethyl acetate and water. The ethyl acetate soluble fraction was chromatographed on a silica gel column with chloroform-methanol and hexane-ethyl acetate. The fraction eluted by hexane-ethyl acetate (6:1) was subjected to reversed-phase HPLC on ODS with methanol-water to give stelliferins A (1, 0.07% wet weight), B (2, 0.007%), and D (4, 0.006%) as colorless oils. The fraction eluted by hexane-ethyl acetate (8:1) was rechromatographed on Sephadex LH-20 column (benzene-methanol, 1:1) followed by reversed-phase HPLC separation on ODS with methanol-water (95:5) to afford stelliferins C (3, 0.004%), E (5, 0.001%), and F (6, 0.0003%).

The compound 1 {$[\alpha]^{23}_{D}$ -126° (*c* 0.54, C_6H_6)} showed a molecular ion peak at *m/z* 496, and fragment ions at 478 (M^+ - H_2O), 427 (M^+ - C_5H_9), and 357 (M^+ - $C_9H_{15}O$) in the EIMS. The molecular formula $C_{32}H_{48}O_4$ of 1 was established by HREIMS (*m/z* 496.3527, Δ -2.6 mmu). The IR spectrum revealed absorptions at 3500-3200, 1710 and 1690 cm^{-1}, which were attributed to a

hydroxy, and ester and conjugated ketone carbonyls, respectively. The ^{1}H NMR spectrum (Table 1) were complicated in the high field (2.5~1.2 ppm), and only showed 9 methyl, 4 olefinic, and 2 carbinyl protons. Three of the olefinic protons (δ 8.82, 7.00 and 6.43 in C_6D_6) were assigned to those on conjugated double bonds. The ultraviolet spectrum {λ_{max} 345 nm (ε 23000)} of 1 in ethanol indicated the presence of a trienone chromophore. A combination of the ^{1}H-^{1}H COSY[5] and homonuclear Hartman-Hahn (HOHAHA)[6] experiments allowed the partial structure of segment A. In the ^{1}H-^{1}H COSY spectrum the protons at δ 8.82 and 6.43 (labeled H-15 and H-17, respectively) showed couplings to the proton at δ 7.00 (H-16). In the HOHAHA spectrum the protons at δ 8.82 (H-15) and 6.43 (H-17) had correlations to the methyl protons at δ 1.83 (H_3-18) and 1.81 (H_3-21), respectively. In the COSY spectrum the carbinyl proton (H-22) at δ 3.95 was coupled to the methylene protons (H_2-23) at δ 2.35 and 2.24. H-22 (δ 5.10) of the acetyl derivative 5 showed lower-field shift than that of 1, indicating that a hydroxy group was attached at C-22. The H_2-23 were coupled to the proton at δ 5.22 (H-24), which was connected to the two methyl protons at δ 1.56 and 1.67 (H_3-26 and H_3-27, respectively). The connectivity between H_3-21 and H-22 was clarified by NOESY spectrum. The ^{13}C NMR data (Table 1) including DEPT experiments disclosed the presence of nine methyls (δ 12.7, 15.8, 17.1, 17.9, 20.8, 22.2, 24.8, 25.9, and 29.2), six methylenes (δ 18.4, 25.5, 33.1, 34.8, 36.8, and 38.3), four sp^3 methines including two carbinyl ones (δ 46.4, 50.1, 77.0, and 80.7), four sp^2 methines (δ 120.9, 126.7, 130.2, and 133.9), and three sp^3 (δ 35.5, 38.4, and 44.5), four sp^2 (δ 134.0, 141.5, 143.6, and 146.3) quaternary carbons other than an ester (δ 170.0) and a conjugate ketone (δ 205.0) carbonyls, thus accounting for all 32 carbons of 1. The protonated carbons were all assigned by heteronuclear multiple quantam coherence (HMQC)[7] experiments. Because the unsatuation number of 1 was eight, three rings were suggested to be included in the molecule.



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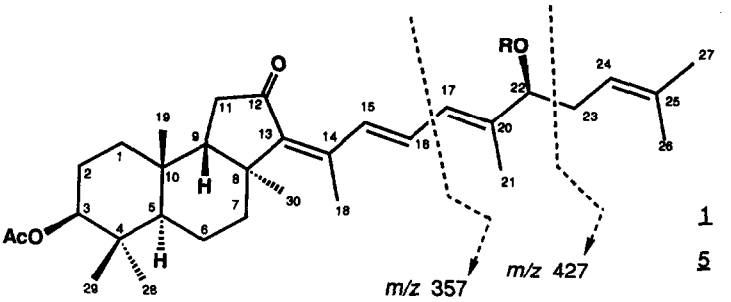
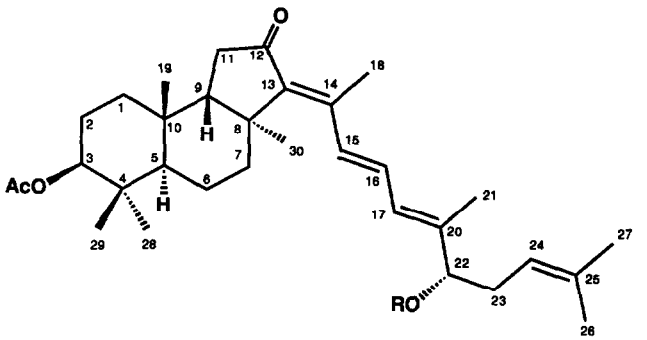
Further structure elucidation of 1 was based on the ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) technique[8] in $CDCl_3$ (Table 2). The HMBC spectrum showed connectivity from a methyl proton signal at δ 2.06 to an ester carbonyl at δ 170.8, which was shown to be coupled to an oxymethine proton at δ 4.56 (H-3), indicating that an acetoxy group is present on C-3. Both methyl proton signals at δ 0.92 and 0.89 (H_3-28 and H_3-29, respectively) were coupled to C-3, C-4 and C-5. The singlet methyl proton at δ 1.02 (H-19) was coupled to C-1, C-5, C-9 and C-10 (δ 32.9, 46.5, 50.2, and 35.4, respectively). Another singlet methyl proton at δ 1.37 (H_3-30) showed conectivities to C-7, C-8, C-9, and C-13 (corresponding to δ 38.1, 44.5, 50.2 and 145.8, respectively). In the HMBC spectrum the methyl proton (H_3-18) in the partial segment A was





1 R = H

5 R = Ac

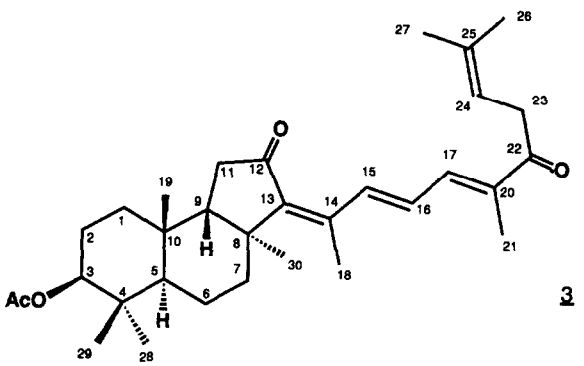
13 R = *p*-BrC_6H_4CO

2 R = H

6 R = Ac

10 R = (-)-MTPA

12 R = (+)-MTPA



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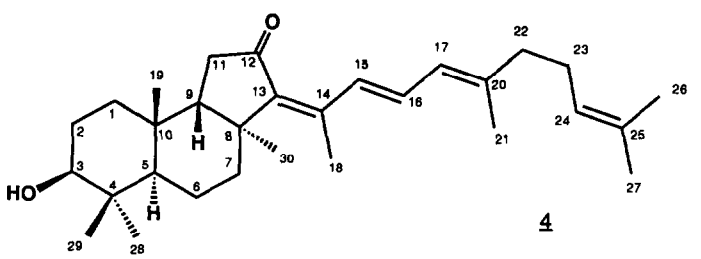

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Table 1. ^1H and ^{13}C NMR Chemical Shifts of Stelliferins A (1), B (2) and C (3) in C_6D_6[a].

	Stelliferin A (1)			Stelliferin B (2)					Stelliferin C (3)				
positn.	^1H	m.	J (Hz)	^{13}C	m[b]	^1H	m.	J (Hz)	^{13}C	m[b]	^1H	m.	J (Hz)
1.	1.24	ddd	3.9, 13.2, 16.6	33.1	t	1.27	bdd	3.9, 13.0	33.1	t	1.20	m	
	0.98	dt	2.9, 16.6			0.97	m				0.96	m	
2.	1.87	m		25.5	t	1.83	m		25.5	t	1.84	m	
	1.62	m				1.63	m				1.61	m	
3.	4.77	dd	5.0, 12.0	80.7	d	4.78	dd	4.9, 11.7	86.8	d	4.75	dd	5.0, 11.5
4.				38.4	s				38.4	s			
5.	1.59	bd	11.7	46.4	d	1.62	bd	10.9	46.8	d	1.65	m	
6.	1.45	bdd	8.8, 13.2	18.4	t	1.45	bdd	7.0, 13.8	18.4	t	1.44	m	
	1.21	btt	3.4, 13.2			1.20	m				1.14	dd	4.4, 12.9
7.	1.79	m		38.3	t	1.97	bdd	7.0, 10.0	39.6	t	1.99	m	
	1.71	dd	9.3, 13.2			1.87	bdd	4.2, 10.0			1.88	m	
8.				44.5	s				44.4	s			
9.	1.49	dd	7.3, 15.1	50.1	d	1.52	dd	5.7, 8.2	50.2	d	1.51	bd	7.7
10.				35.5	s				35.5	s			
11.	2.09	dd	7.3, 15.1	36.8	t	2.08	dd	8.2, 9.9	36.7	t	2.12	bt	7.6
	2.02	t	15.1			2.06	dd	5.7, 9.9					
12.				205.0	s				206.1	s			
13.				146.3	s				146.9	s			
14.				141.5	s				140.7	s			
15.	8.82	d	15.6	133.9	d	6.76	d	15.1	132.8	d	8.97	d	16.1
16.	7.00	dd	11.2, 15.6	130.2	d	7.02	dd	11.2, 15.1	131.8	d	6.93	dd	11.2, 15.5
17.	6.43	dd	1.0, 11.2	126.7	d	6.54	bd	11.2	126.6	d	7.31	bd	11.2
18.	1.83	d	1.0	15.8	q	2.68	s		14.7	q	1.76	s	
19.	0.74	s		22.2	q	0.73	s		22.3	q	0.73	s	
20.				143.6	s				144.5	s			
21.	1.81	s		12.7	q	1.70	d	1.0	11.8	q	2.06	d	1.0
22.	3.95	bt	6.6	77.0	d	3.99	bt	6.5	76.6	d			
23.	2.35	ddd	6.6, 7.3, 14.4	34.8	t	2.29	bt	7.2	35.0	t	3.26	bd	6.9
	2.24	ddd	6.6, 7.3, 14.4										
24.	5.22	qt	1.5, 7.3	120.9	d	5.21	qt	1.5, 7.2	120.7	d	5.59	qt	1.4, 7.7
25.				134.0	s				134.5	s			
26.	1.56	d	1.5	17.9	q	1.54	bs		18.0	q	1.57	bs	
27.	1.67	d	1.5	25.9	q	1.64	bs		26.0	q	1.66	bs	
28.	0.97	s		29.2	q	0.98	s		29.5	q	0.98	s	
29.	0.93	s		17.1	q	0.92	s		17.6	q	0.93	s	
30.	1.06	s		24.8	q	1.16	s		23.9	q	1.12	s	
AcO	1.84	s		20.8	q	1.81	s		20.7	q	1.82	s	
				170.0	s				170.0	s			

[a] δ in ppm [b] multiplicity in DEPT

Table 2. The ^1H and ^{13}C NMR Chemical Shifts of Stelliferin A (1)[a] in $CDCl_3$ and Protons to Which a Long-Range Connectivity Was Observed in the HMBC Experiment.

Position	^1H		^{13}C	m[b]	HMBC (^1H)
1	1.59	m	32.9	t	H-19
	1.40	m			
2	1.80	m	25.0	t	H-1
	1.56	m			
3	4.56	dd	80.7	d	H-5, H-28, H-29
4			38.1	s	H-3, H-5, H-28, H-29
5	1.73	m	46.5	d	H-19, H-28, H-29
6	1.48	m	18.2	t	
7	1.52	m	38.1	t	H-30
8			44.5	s	H-7, H-30
9	1.85	m	50.2	d	H-19, H-30
10			35.4	s	H-5, H-19, H-30
11	2.11	m	36.8	t	
12			206.3	t	H-11
13			145.8	s	H-15, H-18, H-30
14			142.4	s	H-15, H-16, H-18
15	7.99	d	132.1	d	H-17, H-18
16	6.84	dd	130.2	d	
17	6.28	d	126.2	d	H-15, H-16, H-21, H-22
18	2.02	s	15.9	q	H-15
19	1.02	s	22.3	q	H-5
20			143.2	s	H-21, H-22
21	1.83	s	12.6	q	H-17, H-22
22	4.10	bt	76.8	d	H-17, H-21
23	2.30	m	34.2	t	H-22
24	5.10	t	119.7	d	H-22, H-26, H-27
25			134.6	s	H-26, H-27
26	1.56	bs	17.9	q	H-27
27	1.65	bs	25.8	q	H-26
28	0.92	s	28.9	q	H-3, H-5, H-29
29	0.89	s	16.9	q	H-3, H-28
30	1.37	s	24.6	q	
3-$\underline{CH_3}CO$	2.06	s	21.1	q	
3-$CH_3\underline{CO}$			170.8	s	H-3, 3-CO$\underline{CH_3}$

[a] δ in ppm [b] multiplicity in DEPT

coupled to C-13, indicating that the segment A is attached to C-13.

The remaining carbon-carbon bonds were clarified on the basis of detailed analyses of the ^1H-^1H COSY and HMBC spectra of 1 in benzene-d_6 and informations obtained from ozonolysis product of 1. The ^1H-^1H COSY spectrum of 1 showed connectivities from H_2-1 (δ 1.24 and 0.89) to H_2-2 (δ 1.87 and 1.64), from H_2-2 to H-3 (δ 4.77), from H-5 (δ 1.71) to H_2-6 (δ 1.45 and 1.21), from H_2-6 to H_2-7 (δ 1.62 and 1.56), and from H-9 (δ 1.49) to H_2-11 (δ 2.09 and 2.02). The connectivity

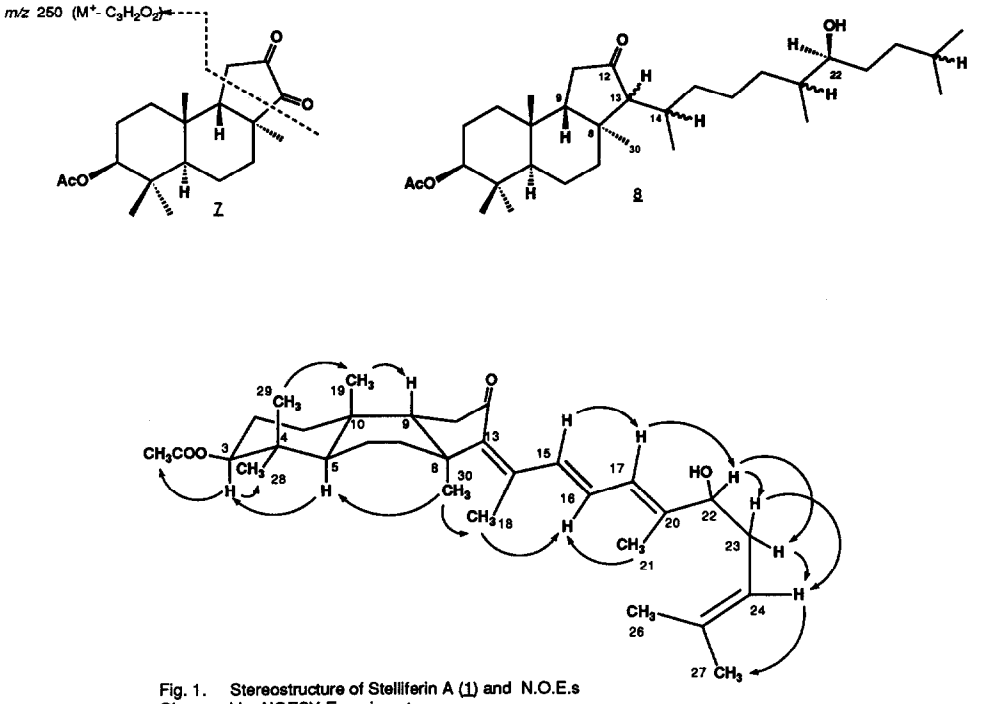

Fig. 1. Stereostructure of Stelliferin A (1) and N.O.E.s Observed by NOESY Experiments.

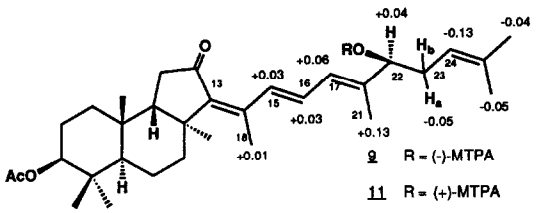

Fig. 2 ^{1}H NMR Chemical shift differences for MTPA esters:
$\Delta\delta$ (ppm) = δ {(-)-MTPA ester 9} - δ {(+)-MTPA ester 11}

between H_2-11 and C-12 was shown by the HMBC experiments. The position of ketone carbonyl group was clarified by formation of diketone 7 on treatment of 1 with ozone followed by dimethyl sulfide. The mass fragment at *m/z* 250 and IR absorption bands at 1765 and 1750 cm^{-1} of 7 indicated that 7 had a five-membered diketone functionality. Additionally, the ^{13}C NMR spectrum of the octahydro derivative 8, obtained by hydrogenation (H_2, 10% Pd-C), showed a carbon signal at δ 218.0 and an IR absorption at 1740 cm^{-1}, which were assignable to ketone carbonyl on five-member ring[9]. This observation cofirmed that the ketone carbonyl group of compound 1 was on C-12.

The relative stereostructure (Fig. 1) of 1 was elucidated by NOESY[10] experiments, chemical degradations, and from comparison with ^{13}C chemical shifts of lanostane-type triterpenes. The NOESY spectra showed the cross-peaks of H-30/H-18, H-18/H-16, H-16/H-21, and H-15/H-17, indicating 13*Z*, 15*E*, and 17*E*-configurations. The ^{13}C chemical shifts of 4β-Me (δ 18.9) and C-3 (δ 80.7) of 1 indicated the presence of 3β-acetoxy group, since 4β-Me (ca. δ 17) and C-3 (ca. δ 80) of lanostane-type triterpenes with a 3β-acetoxy group are generally higher and lower field than 4β-Me (δ 22) and C-3 (δ 78) of those with a 3α-acetoxy group, respectively[11]. A cross-peak between H-3 and H-5 shown by the NOESY spectrum suggested that 1 has 5α-hydrogen. The cross-peaks of H-5/H_3-30, H_3-19/H_3-29, and H-9/H_3-19 in NOESY spectra indicated that configrations of C-30, C-19, and H-9 were α, β, and β, respectively. The stereochemistry of 22-hydroxy group was assigned by Mosher's method using differences of ^1H-chemical shifts (Fig. 2) between (-)- and (+)-MTPA esters (9 and 11, respectively)[12]. That result (Fig. 2) revealed 22*S* [13,14].

The structures of stelliferins B (2), C (3), D (4), E (5), and F (6) were determined mainly from comparison of the ^1H NMR data with those of 1. Stelliferin B (2) {$[\alpha]^{23}_D$ -166°, (*c* 0.56, C_6H_6)} showed the same molecular and fragment ions as stelliferin A (1). The ^1H NMR spectrum of 2 (Table 1) was similar to that of 1, except for the signals for H_3-18 (δ 2.68) and H-15 (δ 6.76), which indicated that 2 was an isomer at C-13 position of stelliferin A (1). This fact was confirmed by difference NOE experiments: irradiation of H_3-30 (δ 1.16) of 2 yielded NOE (5.5 %) for H-15. The absolute configuration at C-22 was elucidated to be *S* by ^1H-chemical shift differences (Δδ; H-17: +0.02, H-21: +0.04, H-22: -0.02, H-23α: -0.13, H-24: -0.21, H-26: -0.13, H-27: -0.14) between (-)- and (+)-MTPA esters (10 and 12, respectively). Stelliferin B (2) was established to be Δ^{13}-isomer of stelliferin A.

Stelliferin C (3) {$[\alpha]^{23}_D$ -15.2°, (*c* 0.35, C_6H_6)} showed a molecular ion peak at *m/z* 494 and fragment ion at 425 (M^+-C_5H_9) and 357 (M^+-$C_9H_{13}O$) in the EIMS. The molecular formula $C_{32}H_{46}O_4$ of stelliferin C was established by HREIMS (*m/z* 494.3517, Δ -0.9 mmu). The IR spectrum showed absorptions at 1740, 1710, and 1690 cm^{-1}, which were attributed to an ester and two conjugated carbonyls, respectively. The ^1H NMR spectrum of 3 showed a doublet methylene signal at δ 3.26 (H_2-23), which was coupled to an olefin proton (δ 5.59, H-24) in the COSY spectrum, while the oxymethine proton signal for H-22, which appeared in the ^1H NMR of 1, was not observed. The ^{13}C NMR spectrum of 3 showed two conjugated carbonyls at δ 205.6 and

197.9, the former being assignable to C-12 as in that of 1, and the latter to C-22. Thus the structure of stelliferin C (3) was established to be 22-oxo form of stelliferin A (1).

Stelliferin D (4) {[α]D +201°, (*c* 0.31, C_6H_6)} showed a molecular ion peak at *m/z* 438 in the EIMS.The molecular formula $C_{30}H_{46}O_2$ of stelliferin D was established by HREIMS (*m/z* 438.3517, Δ +1.9 mmu). The ^1H NMR spectrum of 4, on comparison with that of 1, did not show the signals due to an acetyl methyl group and an oxygenated methine proton attached to C-22. The oxymethine proton on C-3 resonated at the higher field (δ 3.02) with a similar coupling pattern (*J* = 5.5 and 10.6 Hz), suggesting that a hydroxy group is present at C-3 with β-configuration. The ^{13}C NMR of 4 showed only one sp^3 oxymethine carbon, which is ascribable to C-3, and oxymethine carbon due to C-22 present in 1 was not observed, while one sp^3 methylene carbon newly appeared. From these observations the structure of stelliferin D was concluded to be (13*Z*, 15*E*, 17*E*) -3β-hydroxyisomalabarica-13, 15, 17, 24-tetraene-12-one (4).

Stelliferin E (5) {[α]D -409°, (*c* 0.13, C_6H_6)} and stelliferin F (6) {[α]D -377°, (*c* 0.005, C_6H_6)} were shown to be identical with the acetylation products of stelliferins A (1) and B (2), respectively, on the basis of the comparison of spectroscopic data. Stelliferins E (5) and F (6) were, however, quite susceptible to isomerization at C-13 position .

Stelliferins (1 ~ 6) are the second examples of isomalabaricane-type triterpenes and exhibited potent antineoplastic activities in vitro (Table 3). This is the first report in which the ^1H and ^{13}C NMR data of isomalabaricane triterpenes are fully assigned on the basis of two dimensional NMR experiments.

Table 3. Antineoplastic activities (IC_{50} values, μg/mL) of stelliferins A - F (1 ~ 6)

	L1210	KB
1	0.57	1.4
2	0.60	2.1
3	2.1	5.2
4	2.4	6.5
5 and 6	1.7	6.0

Experimental Section

General Methods. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV and IR spectra were taken on a Shimadzu UV-220 and JASCO IR Report-100 spectrometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL FX-90Q, JMN GX-270, and JEOL FX-500 spectrometers in $CDCl_3$ and C_6D_6. In $CDCl_3$ tetramethylsilane was used as internal standard for ^{1}H NMR, and δ_C 77.1 resonance of residual $CHCl_3$ was used as an internal reference for ^{13}C NMR. The resonances of residual benzene at δ_H 7.20 and δ_C 128.0 were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. EI mass spectra were obtained on JEOL JMS DX-303 spectrometer.

Collection, Extraction, and Isolation. The bright yellow-colored sponge *Jaspis stellifera* (2.6 kg) was collected off Ishigaki Island, Okinawa and kept frozen until used. The sponge (1.0 kg, wet weight) was extracted with methanol (1 L x 2). Concentration of the extract afforded 48.2 g of residue, which was dissolved in a mixture of ethyl acetate (50 mL) and water (200 mL). The aqueous layer was extracted with ethyl acetate(500 mL). The ethyl acetate solubles were concentrated under reduced pressure to give a crude residue (11.98 g), part of which (4.31 g) was chromatographed on a silica gel column (Wako gel C-300, Wako Pure Chemical, 23 x 410 mm) with chloroform/methanol (100:0~95:5). The fraction of 360-380 mL was evapolated under reduced pressure to give a residue (2.38 g). The residue was rechromatographed on a silica gel column (10 x 350 mm) with hexane/ethyl acetate (8:1 followed by 6:1). The eluate by hexane/ethyl acetate (6:1) was further purified by HPLC [YMC-Pack AM-323 ODS,Yamamura Chemical, 10 x 250 mm; flow rate, 2.5 mL/min; UV detection at 380 nm; eluent, methanol/water (93:7)] to yield stelliferins A (1, 259.5 mg, 0.07 % wet weight, R_t 14.5 min), B (2, 27.4 mg, 0.007 %, R_t 17.0 min), and D (4, 25.7 mg, 0.006 %, R_t 22.5 min). The eluate by hexane/ethyl acetate (6:1) was rechromatographed by a Sephadex LH-20 column (Pharmacia Fine Chemicals, 10 x 980 mm) with benzene/methanol (1:1) followed by HPLC [YMC-Pack AM-323 ODS, 10 x 250 mm; flow rate, 2.5 mL/min; UV detection at 380 nm; eluent, methanol/water (95:5)] to afford stelliferins C (3, 3.5 mg, 0.0009%, R_t 19.7 min), E (5, 1.3 mg, 0.0003%, R_t 22 min), and F (6, 0.3 mg, 0,00007%, R_t 23.5 min).

Stelliferin A (1). A colorless oil; $[\alpha]^{22}_D$ -126.6° (*c* 0.54, C_6H_6); IR ($CHCl_3$) 3500-3200 (br.), 2880, 2830, 1720, 1690, 1610, 1575, 1450, 1380, 1260, 1175, and 1030 cm^{-1}; UV (MeOH) 343 nm (ε 25000); EIMS *m/z* 496 (M^+), 478, 427, 409, 357, 189, 163, 149, and 135; HREIMS *m/z* 496.3527, (M^+; calcd for $C_{32}H_{48}O_4$, 496.3553).

Stelliferin B (2). A colorless oil; $[\alpha]^{23}_D$ -166° (*c* 0.56, C_6H_6); IR (neat) 3550 (br.), 3010, 2980, 2950, 2850, 1740, 1700, 1590, 1560, 1450, 1380, 1250, 1180, and 1030 cm^{-1}; UV (EtOH) 345 nm (ε 26000); EIMS *m/z* 496 (M^+), 480, 427, 409, 357, 189, 163, 135, and 43; HREIMS *m/z* 496.3511, (M^+, calcd. for $C_{32}H_{48}O_4$, 496.3553).

Stelliferin C (3). a colorless oil; $[\alpha]^{23}_{D}$ -15.2° (*c* 0.35,C_6H_6); IR (neat) 3000, 2960, 1850, 1740, 1710, 1670, 1620, 1590, 1480, 1380, 1250, 1175 and 1030 cm^{-1}; UV (EtOH) 360 (sh.) and 357 nm (ε 22000); ^{13}C NMR (C_6D_6) δ 205.6 (s), 197.9 (s), 178.0 (s), 149.0 (s), 142.8 (s), 140.9 (s), 139.0 (d), 133.0 (s), 131.4 (d), 129.1 (d), 127.2 (d), 118.3 (d), 80.6 (d), 50.0 (d), 46.8 (d), 44.5 (s), 38.3 (t), 38.1 (s), 37.8 (t), 36.8 (s), 35.5 (t), 33.1 (t), 29.2 (q), 25.7 (q), 25.5 (t), 24.5 (q), 22.2 (q), 18.4 (t), 18.1 (q), 17.1 (q), 15.6 (q), and 12.2 (q); EIMS *m/z* 494 (M^+), 479, 425, 409, 398, 357, and 43; HREIMS *m/z* 494.3405 (M^+, calcd. for $C_{32}H_{46}O_4$, 494.3396).

Stelliferin D (4). A colorless oil; $[\alpha]^{23}_{D}$ +201° (*c* 0.31, C_6H_6), IR (neat) 3450 (br.), 3000, 2980, 2940, 2860, 1690, 1630, 1580, 1550, 1450, 1380, 1270, 1200, and 1060 cm^{-1}; UV (EtOH) 350 nm (ε 20000); ^1H NMR (C_6D_6) δ 8.81 (1H, d, *J* = 15.4 Hz, H-15), 7.03 (1H, dd, *J* = 11.0 and 15.4 Hz, H-16), 6.31 (1H, br.d, *J* = 11.0 Hz, H-17), 5.20 (1H, dt, *J* = 1.5 and 7.0 Hz, H-24), 3.02 (1H, dd, *J* = 5.5 and 10.6 Hz, H-3), 2.19 ~ 2.07 (9H, m, H-7β, 11, 22, and 23), 1.90 (3H, s, H-21), 1.77 (3H, br.d, H-19), 1.72 (1H, m, H-7α), 1.69 (3H, s H-27), 1.60 (1H, m, H-5), 1.57 (3H, s, H-26), 1.45 (1H, m, H-9), 1.40 (1H, m, H-6), 1.39 (2H, m, H-2), 1.19 (1H, m, H-1), 1.16 (1H, m, H-6), 1.12 (3H, s, H-30), 1.04 (3H, s, H-28), 0.99 (1H, m, H-1), 0.80 (3H, s, H-29) and 0.72 (3H, s, H-18); ^{13}C NMR (C_6D_6) δ 204.9 (s), 146.4 (s), 144.0 (s), 141.4 (s), 137.8 (s), 132.5 (d), 131.0 (d), 125.9 (d), 117.8 (d), 78.8 (d), 50.2 (d), 46.8 (d), 44.4 (s), 40.5 (t), 39.2 (t), 38.2 (s), 36.8 (t), 35.7 (s), 33.4 (t), 29.4 (t), 29.2 (q), 27.0 (t), 25.8 (q), 24.8 (q), 22.2 (q), 17.7 (t), 17.0 (q), 16.1 (q), 15.8 (q), and 12.0 (q); EIMS *m/z* 438 (M^+), 423, 395, 381, 369, 355, 315, 207, 189, 175, 147, and 135; HREIMS *m/z* 438.3517 (M^+, calcd. for $C_{30}H_{46}O_2$, 438.3498).

Stelliferin E (5). A colorless oil ; $[\alpha]^{23}_{D}$ -409° (*c* 0.13, C_6H_6); IR (neat) 3000, 2980, 2880, 1740, 1690, 1590, 1560, 1450 (br.), 1370, 1250, 1170, and 760 cm^{-1}; UV (EtOH) 338 nm (ε 24000); ^1H NMR (C_6D_6) δ ^1H NMR (C_6D_6) δ 8.80 (1H, d, *J* = 15.4 Hz, H-15), 6.94 (1H, dd, *J* = 11.0 and 15.4 Hz, H-16), 6.57 (1H, br.d, *J* = 11.0 Hz, H-17), 5.43 (1H, t, *J* = 6.6 Hz, H-22), 5.19 (1H, tt, *J* = 1.5 and 7.0 Hz, H-24), 4.77 (1H, dd, *J* = 5.3 and 11.7 Hz, H-3), 2.50 (1H, br.dt, *J* = 7.3 and 14.4 Hz, H-23), 2.34 (1H, br.dt, *J* = 7.3 and 14.4 Hz, H-23), 2.05 (1H, dd, *J* =5.1 and 8.4 Hz, H-11), 2.02 (1H, dd, *J* = 5.1 and 11.4 Hz, H-11), 1.82 (3H, s, AcO) 1.81 (3H, s, AcO), 1.74 (3H, s, H-21), 1.70 (3H, s, H-18), 1.64 (3H, br.s, H-27), 1.55 (3H, br.s, H-26), 1.03 (3H, s, H-30), 0.97 (3H, s, H-18), 0.93 (3H, s, H-28), and 0.73 (3H, s, H-29); EIMS *m/z* 538 (M^+), 479, 470, 427, 409, 398, 357, 189, and 43; HREIMS *m/z* 538.3602 (M^+, calcd for $C_{34}H_{50}O_5$, 538.3659).

Stelliferin F (6). A colorless oil ; $[\alpha]^{23}_{D}$ -377° (*c* 0.005, C_6H_6); IR (neat) 3000, 2980, 2880, 1740, 1690, 1590, 1560, 1450 (br.), 1370, 1250, 1170, and 760 cm^{-1}; UV (EtOH) 338 nm (ε 24000);^1H NMR (C_6D_6) δ 6.94 (1H, dd, *J* = 11.0 and 15.4 Hz, H-16), 6.72 (1H, d, *J* = 15.4 Hz, H-

15), 6.57 (1H, br.d, J = 11.0 Hz, H-17), 5.43 (1H, t, J = 6.6 Hz, H-22), 5.19 (1H, tt, J = 1.5 and 7.0 Hz, H-24), 4.77 (1H, dd, J = 5.3 and 11.7 Hz, H-3), 2.63 (3H, s, H-18), 1.83 (3H, s, AcO), 1.83 (3H, s, H-21) 1.81 (3H, s, AcO), 1.64 (3H, br.s, H-27), 1.55 (3H, br.s, H-26), 1.12 (3H, s, H-30), 0.99 (3H, s, H-18), 0.91 (3H, s, H-28), and 0.71 (3H, s, H-29); EIMS *m/z* 538 (M^+), 479, 470, 427, 409, 398, 357, 189, and 43

Acetylation of Stelliferins A and B. A mixture of stelliferins A and B (1.2mg), acetic anhydride (10μL) and pyridine (100μL) was stirred at room temperature for 15h. After evaporation, the mixture was passed through a silica gel column (Wako gel C-300, 0.5 x 100 mm), eluting with hexane/ethyl acetate (10:1) to yield a mixture of monoacetates (5 and 6) of stelliferins A and B (1.0 mg 77%): IR (neat) 3000, 2980, 2880, 1740, 1690, 1590, 1560, 1450 (br.), 1370, 1250, 1170, 1030, and 760 cm-1; UV (EtOH) 338 nm (ε 22000); ^{1}H NMR of 5 (C_6D_6) δ 8.80 (1H, d, J = 15.4 Hz, H-15), 6.94 (1H, dd, J = 11.0 and 15.4 Hz, H-16), 6.57 (1H, br.d, J = 11.0 Hz, H-17), 5.43 (1H, t, J = 6.6 Hz, H-22), 5.19 (1H, tt, J = 1.5 and 7.0 Hz, H-24), 4.77 (1H, dd, J = 5.3 and 11.7 Hz, H-3), 2.50 (1H, br.dt, J = 7.3 and 14.4 Hz, H-23), 2.34 (1H, br.dt, J = 7.3 and 14.4 Hz, H-23), 2.05 (1H, dd, J =5.1 and 8.4 Hz, H-11), 2.02 (1H, dd, J = 5.1 and 11.4 Hz, H-11), 1.82 (3H, s, AcO) 1.81 (3H, s, AcO), 1.74 (3H, s, H-21), 1.70 (3H, s, H-18), 1.64 (3H, br.s, H-27), 1.55 (3H, br.s, H-26), 1.03 (3H, s, H-30), 0.97 (3H, s, H-18), 0.93 (3H, s, H-28), and 0.73 (3H, s, H-29); ^{1}H NMR of 6 (C_6D_6) δ 6.94 (1H, dd, J = 11.0 and 15.4 Hz, H-16), 6.72 (1H, d, J = 15.4 Hz, H-15), 6.57 (1H, br.d, J = 11.0 Hz, H-17), 5.43 (1H, t, J = 6.6 Hz, H-22), 5.19 (1H, tt, J = 1.5 and 7.0 Hz, H-24), 4.77 (1H, dd, J = 5.3 and 11.7 Hz, H-3), 2.63 (3H, s, H-18), 1.83 (3H, s, AcO), 1.83 (3H, s, H-21) 1.81 (3H, s, AcO), 1.64 (3H, br.s, H-27), 1.55 (3H, br.s, H-26), 1.12 (3H, s, H-30), 0.99 (3H, s, H-18), 0.91 (3H, s, H-28), and 0.71 (3H, s, H-29); EIMS *m/z* 538 (M^+), 479 (M^+-CH_3CO_2), 470, 427, 409, 390, 357, 189, and 43; HREIMS *m/z* 538.3679 (M^+, calcd for $C_{34}H_{50}O_5$, 538.3659).

Diketone 7. Stelliferin A (1, 7.9 mg) in methanol was saturated with ozone at -78°C for 20 min. After excess ozone was removed by nitrogen steam, dimethyl sulfide (20 μL) was added to the solution and kept standing at 0°C for 30 min and at room temperature overnight. The solvent was evaporated under reduced pressure and the residue was subjected to silica gel column chromatography using chloroform to afford the diketone 9 (2.3 mg, 45%). 7: ^{1}H NMR ($CDCl_3$) δ 4.57 (1H, dd, J =4.9 and 11.2 Hz), 2.47 (1H, dd, J = 7.5 and 18.1 Hz), 2.35 (1H, dd, J = 14.8 and 18.1 Hz), 2.07 (3H, s), 1.27 (3H, s), 1.08 (3H, s), 0.94 (3H, s), and 0.88 (3H, s); EIMS *m/z* 320 (M^+), 250 (M^+-$C_3H_2O_2$), 190, 175, and 43; HREIMS *m/z* 320.2001 (M^+, calcd for $C_{19}H_{28}O_4$, 320.1988) and 250.1942 (M^+-$C_3H_2O_2$, calcd for $C_{16}H_{26}O_2$, 250.1933).

Hydrogenation Derivative 8. A solution of 1 (7.3 mg) in ethyl acetate was hydrogenated over 10% Pd-C for 3h. The filtrate of the reaction mixture was concentrated under reduced

pressure and the residue was subjected to chromatography on a silica gel column (Wako gel C-300, 10 x 100 mm) with hexane/ethyl acetate (10:1) to afford the hydroganated derivative 8: IR (neat) 3550 (br.), 2980, 2950, 2880, 1740, 1730 (sh.), 1470, 1380, 1250, 1080, 1030, and 800 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.47 (1H, dd, *J* = 5.3 and 11.2 Hz, H-3), 3.37 (1H, ddd, 4.4, 8.0 and 12.5 Hz, H-22), 2.01 (1H, dd, *J* =8.1 and 18.0 Hz, H-11β), 1.98 (3H, s), 1.94 (1H, dd, *J* = 15.1 and 18.0 Hz, H-11α), 1.01 (3H, d, *J* = 6.6 Hz), 1.00 (3H, s), 0.98 (3H, d, *J* = 6.6 Hz), 0.84 (3H, s), 0.83 (3H, d, *J* = 6.6 Hz), 0.82 (3H, d, *J* = 6.6 Hz), and 0.82 (6H, s); ^{13}C NMR ($CDCl_3$) δ 218.0 (s), 171.0 (s), 80.8 (d), 71.6 (d), 51.4 (d), 47.2 (d), 42.9 (s), 38.9 (d) 38.3 (s) 38.3 (t), 38.2 (d), 36.2 (t), 35.7 (t), 35.5 (s), 35.4 (t) 33.4 (t), 31.4 (t), 30.8 (t), 29.0 (d), 28.2 (q), 25.2 (t), 25.0 (t), 23.2 (q), 21.2 (q), 21.0 (q), 18.4 (q), 18.0 (t), 16.9 (q), 15.5 (q), 14.1 (q), and 13.6 (q); EIMS, *m/z* 504 (M^+), 486 (M^+-H_2O), 471, 389, 291, and 189; HREIMS *m/z* 504.4183 (M^+, calcd for $C_{32}H_{56}O_4$, 504.4179).

Conversion of stelliferins 1 and 2 into (-)-MTPA Esters 9 and 10. Into a pyridine solution (1 mL) of the mixture (15.2 mg) of stelliferins A (1) and B (2) was added (-)-MTPA chloride (37.8 mg) and the mixture was kept standing at room temperature for 20h. After removing the solvent under reduced pressure, a part (7.8 mg) of the residue (14.3 mg) was purified by HPLC on a silica gel column (Senshu Pak Silica-1251-S, 4.6 x 250 mm) with hexane/ethyl acetate (9:1) to yeild (-)-MTPA esters 9 and 10 (3.5 and 2.2 mg, respectively).

(-)-MTPA ester 9: ^1H NMR ($CDCl_3$) δ 7.93 (1H, d, *J* = 15.4 Hz, H-15), 7.44 ~7.31 (5H, m, Ar), 6.70 (1H, dd, *J* = 11.0 and 15.4 Hz, H-16), 6.25 (1H, d, *J* = 11.0 Hz, H-17), 5.34 (1H, dd, *J* = 6.1 and 7.9 Hz, H-22), 4.84 (1H, m, H-24), 3.49 (1H, dd, *J* = 5.1 and 11.0 Hz, H-3), 3.45 (3H, s, OMe), 2.38 (1H, m, H-23a), 2.27 ~ 2.19 (1H, m, H-23b), 2.00 (3H, s, 3-CH_3CO), 1.94 (3H, s, H-18), 1.75 (3H, brs, H-21), 1.57 (3H, brs, H-26), 1.50 (3H, brs, H-27), 1.30 (3H, s, H-30), 0.95 (3H, s, H-19), 0.84 (3H, s, H-28), and 0.82 (3H, s, H-29); EIMS *m/z* 712 (M^+), 643, 583, 478, 463, 409, 398, 357, 189, and 43.

(-)-MTPA ester 10: ^1H NMR ($CDCl_3$) δ 7.44 ~ 7.30 (5H, m, Ar), 6.76 (1H, dd, *J* = 11.0 and 15.4 Hz, H-16), 6.53 (1H, d, *J* = 15.4 Hz, H-15), 6. 23 (1H, d, *J* = 11.0 Hz, H-17), 5.36 (1H, dd, *J* = 5.7 and 8.2 Hz, H-22), 4.88 (1H, m, H-24), 4.50 (1H, dd, *J* = 5.1 and 11.4 Hz, H-3), 3.45 (3H, s, OMe), 2.41 (1H, m, H-23a), 2.28 ~ 2.18 (1H, m, H-23b), 2.23 (3H, s, H-18), 2.00 (3H, s, 3-CH_3CO), 1.79 (3H, brs, H-21), 1.57 (3H, brs, H-26), 1.48 (3H, brs, H-27), 1.32 (3H, s, H-30), 0.95 (3H, s, H-19), 0.86 (3H, s, H-26), and 0.82 (3H, s, H-29); EIMS *m/z* 712 (M^+), 643, 583, 478, 463, 409, 389, 357, 189, and 43.

(+)-MTPA esters 11 and 12. Treatment of the mixture (17.1 mg) of stelliferins A (1) and B (2) with (+)-MTPA chloride (35.2 mg) and pyridine (1 mL) at room temperature for 20h followed by usual workup and purification by silica gel HPLC (the same conditions as those used for 9 and

10) furnished (+)-MTPA esters 11 (3.4 mg) and 12 (3.0 mg).

Compound 11: ^{1}H NMR ($CDCl_3$) δ 7.90 (1H, d, *J* = 15.4 Hz, H-15), 7.43 ~ 7.29 (5H, m, Ar), 6.68 (1H, dd, *J* = 11.0 and 15.4 Hz, H-16), 6.18 (1H, d, *J* = 11.0 Hz, H-17), 5.30 (1H, dd, *J* = 5.1 and 7.7 Hz, H-22), 4.97 (1H, m, H-24), 3.47 (3H, s, OMe), 2.43 (1H, m, H-23a), 2.3 ~ 2.18 (1H, m, H-23b), 2.00 (3H, s, 3-CH_3CO), 1.94 (3H, s, H-18), 1.62 (6H, brs, H-21 and H-26), 1.54 (3H, brs, H-27), 1.30 (3H, s, H-30), 0.96 (3H, s, H-19), 0.85 (3H, s, H-28), and 0.82 (3H, s, H-29); EIMS *m/z* 712 (M^+), 643, 583, 478, 463, 409, 398, 357, 189, and 43; HREIMS *m/z* 712.3961 (M^+, Calcd for $C_{42}H_{55}O_6F_3$, 712.3951).

Compound 12: ^{1}H NMR ($CDCl_3$) δ 7.51 ~ 7.35 (5H, m, Ar), 6.80 (1H, dd, *J* = 11.0 and 15.0 Hz, H-16), 6.55 (1H, d, *J* = 15.0 Hz, H-15), 6.20 (1H, d, *J* = 11.0 Hz, H-17), 5.38 (1H, dd, *J* = 5.3 and 8.2 Hz, H-22), 5.08 (1H, m, H-24), 4.57 (1H, dd, *J* = 5.1 and 11.0 Hz, H-3), 3.55 (3H, s, OMe), 2.53 (1H, m, H-23a), 2.40 ~ 2.27 (1H, m, H-23b), 2.30 (3H, s, H-18), 2.07 (3H, s, 3-CH_3CO), 1.75 (3H, brs, H-21), 1.71 (3H, brs, H-26), 1.61 (3H, brs, H-27), 1.39 (3H, s, H-30), 1.03 (3H, s, H-19), 0.94 (3H, s, H-28), and 0.90 (3H, s, H-29); EIMS *m/z* 712 (M^+), 643, 583, 478, 463, 409, 398, 357, 189, and 43.

***p*-Bromobenzoyl Derivative 13**[13]. A mixture of 1 (4.8 mg), *p*-bromobenzoyl chloride (12.2 mg), and pyridine (1 mL) was heated at 90°C for 30h. After removing the solvent under reduced pressure, the residue was passed through a silica gel cartridge (Sep Pak®, Waters Associates) with hexane/ethyl acetate (5:1) followed by HPLC on a silca gel column (Senshu Pak, 10 X 250 mm) to give the *p*-bromobenzoyl derivative 13 (3.8 mg 58%) as a yellow-colored oil. UV (EtOH) 340 (ε 22000), and 245 nm (17000); CD (EtOH) 325 (Δε +2.7), 260 (0), 250 (-3.3), and 233 nm (-5.1); ^{1}H NMR ($CDCl_3$) δ 7.90 (d, *J* = 15.0 Hz, 1H, H-15), 7.89 (d, *J* = 8.8 Hz, 2H, Ar), 7.58 (d, *J* = 8.8 Hz, 2H, Ar), 6.86 (dd, *J* = 11.0 and 15.0 Hz, 1H, H-16), 6.30 (d, *J* = 11.0 Hz, 1H, H-17), 5.40 (dd, *J* = 7.3 and 13.9 Hz, 1H, H-22), 5.08 (t, *J* = 7.0 Hz, 1H, H-24), 4.56 (dd, *J* = 5.1 and 11.0 Hz, 1H, H-3), 2.57 (m, 1H, H-23), 2.46 (m, 1H, H-23), 2.07 (s, 3H, 3-CH_3CO), 2.06 (s, 3H, H-21), 1.91 (d, *J* = 1.1 Hz, 3H, H-21), 1.68 (brs, 3H, H-26), 1.65 (brs, 3H, H-27), 1.26 (s, 3H, H-30), 1.02 (s, 3H, H-19), 0.91 (s, 3H, H-28), and 0.89 (s, 3H, H-29); EIMS *m/z* 680 (M^++2), 678 (M^+), 611, 609, 478, 409, 398, 357, 202, 200, 185, and 183; HREIMS *m/z* 680.2900 (M^++2, calcd for $C_{39}H_{51}O_5{}^{81}Br$, 680.2901).

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