Fasciculic Acids A, B and C as Calmodulin Antagonists from the Mushroom *Naematoloma fasciculare*

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Three new fasciculol esters, fasciculic acids A (1), B (2) and C (3), having potent calmodulin antagonistic activity were isolated from the toxic mushroom *Naematoloma fasciculare* (Fr.) KARST. Their structures were elucidated on the basis of spectral and chemical evidence.

Keywords Basidiomycetes; *Naematoloma fasciculare*; fasciculol ester; fasciculic acid A; fasciculic acid B; fasciculic acid C; calmodulin antagonist; W-7

Ca^{2+} is involved in many regulatory processes including excitation–contraction coupling, excitation–secretion coupling, ionic permeability of membranes, and cell growth and differentiation. Calmodulin, a Ca^{2+}-binding protein, serves as a major intracellular Ca^{2+} receptor and plays a pivotal role in many cellular functions.[1] Recently, fasciculols B, C, and F have been isolated as calmodulin inhibitors from *Naematoloma* (*N.*) *fasciculare* (Fr.) KARST (Japanese name: nigakuritake).[2] During the course of our screening of bioactive constituents from mushrooms (basidiomycetes), three new fasciculol esters, fasciculic acids A (**1**), B (**2**) and C (**3**), having potent calmodulin antagonistic activity were isolated from the same mushroom along with fasciculols C (**4**), D (**5**) and E (**6**).[3-5] In this paper, we describe the isolation, structural elucidation and the calmodulin-antagonistic activity of fasciculic acids.

The fruiting bodies were extracted with methanol and the extract was separated into ethyl acetate, *n*-butanol and water-soluble portions. Chromatographies of the ethyl acetate and *n*-butanol fractions led to the isolation of fasciculic acids.

Fasciculic acid A (**1**), mp 177—179 °C, $[\alpha]_D^{27}$ +8.3° (c = 1.0, MeOH), obtained as colorless needles (from $CHCl_3$–MeOH), showed the molecular ion peak at m/z 620 in the field desorption mass spectrum (FD-MS). The molecular formula $C_{36}H_{60}O_8$ was deduced from the above mass, ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectral data (Tables I and II). The infrared (IR) spectrum of **1** showed absorptions at 3400 and 1720 cm^{-1} due to hydroxyl, ester and/or carboxyl groups, respectively. ^{13}C-NMR (δ 172.0, s) and the formation of a monomethyl ester (**7**) by treatment with diazomethane supported the presence of a carboxyl group. The ^{1}H-NMR spectrum of **1** showed methyl signals as seven sharp singlets (δ 0.73, 0.90, 1.10, 1.14, 1.22, 1.52, 1.55) and a sharp doublet (δ 1.03, J = 5.0 Hz) indicating the presence of seven tertiary methyl groups and a secondary methyl group. Carbinyl hydrogen signals were observed as two doublets (δ 3.51, J = 10.6 Hz, δ 3.77, J = 8.5 Hz) and a triple doublet (δ 5.53, J = 10.6, 4.2 Hz) implying two hydroxyl methine groups and an acyloxyl methine group. Furthermore, the signal at δ 1.79 assignable to a tertiary methyl group attached to the carbon atom bearing a hydroxyl group and signals of two methylene groups at δ 3.12 (1H, d, J = 12.0 Hz), 3.16 (2H, s) and 3.18 (1H, d, J = 12.0 Hz) implied the presence of a 3-hydroxy-3-methylglutarate moiety. This is also indicated by the ^{13}C-NMR spectrum of **1** which showed signals of a methyl group (δ 28.85), two methylene groups (δ 46.29,

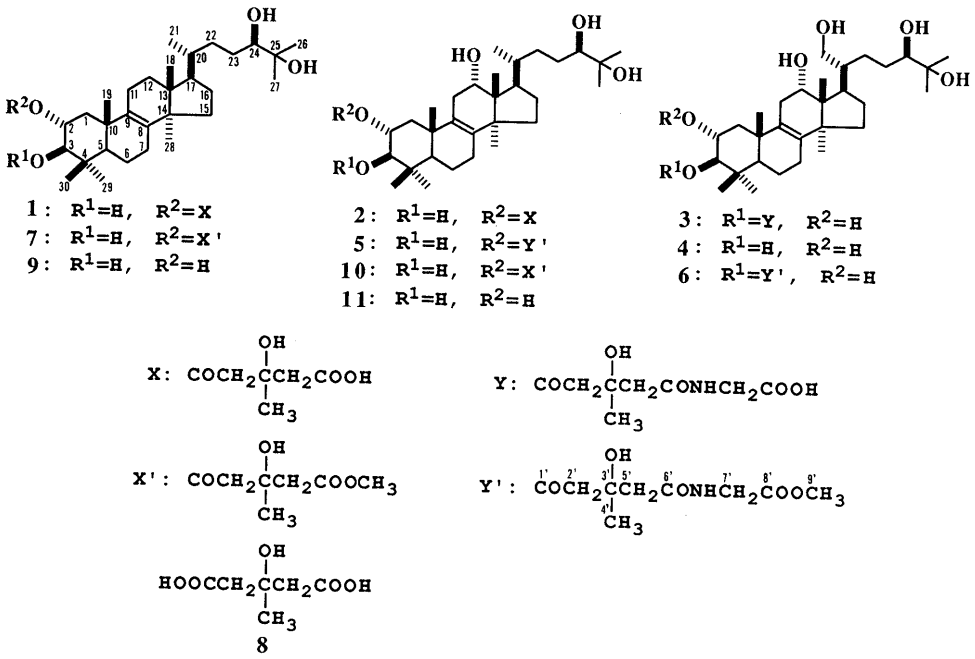

Fig. 1

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46.91), an oxygenated quaternary carbon (δ 70.18), an ester and a carboxyl group (δ 171.63, 176.65). The above spectroscopic data suggested that fasciculic acid A (**1**) is constructed from fasciculol A (**9**)[6] and 3-hydroxy-3-methylglutaric acid (**8**). On alkaline hydrolysis, **1** afforded two compounds which were identified as **8** and **9** on the basis of spectral and chemical evidence. In the ^{1}H-NMR spectrum, a triple doublet signal at δ 4.16 (H-2) in **9** was observed at δ 5.53 in **1**. This downfield shift was assumed to be an acylation shift. The acylation shift was also observed in the ^{13}C-NMR spectrum. Namely, signals for C-1, C-2 and C-3 of **9** at δ 44.77, 68.99 and 83.53 were shifted by -3.39, $+4.82$ and -3.88 ppm, respectively, in the spectrum of **1**. Therefore, the ester linkage should be at the C-2 hydroxyl group. From the results described above, the structure of **1** was concluded to be 2-*O*-(3-hydroxy-3-methylglutaryl) fasciculol A (**1**) as shown in Fig. 1.

TABLE I. ^{1}H-NMR Spectral Data for Fasciculic Acids A (**1**), B (**2**) and C(**3**)[a]

Position	1	2	3
18	0.73 (3H, s)	0.76 (3H, s)	0.73 (3H, s)
19, 28, 29, 30	0.90, 1.10, 1.14, 1.22 (3H, s, each)	1.14, 1.18, 1.25, 1.47 (3H, s, each)	1.00, 1.02, 1.10, 1.38 (3H, s, each)
21	1.03 (3H, d, J=5.0)	1.35 (3H, d, J=5.0)	4.35 (2H, m)
26, 27	1.52, 1.55 (3H, s, each)	1.50, 1.53 (3H, s, each)	1.53, 1.56 (3H, s, each)
2	5.53 (1H, td, J=10.6, 4.2)	5.55 (1H, td, J=11.0, 4.5)	4.21 (1H, td, J=10.0, 4.5)
3	3.51 (1H, d, J=10.6)	3.50 (1H, d, J=11.0)	5.05 (1H, d, J=10.0)
12		4.27 (1H, br d, J=7.0)	4.30 (1H, br s)
24	3.77 (1H, br d, J=8.5)	3.82 (1H, br d, J=7.0)	3.90 (1H, br d, J=7.5)
2′	3.12 (1H, d, J=12.0) 3.18 (1H, d, J=12.0)	3.12 (1H, d, J=13.5) 3.16 (1H, d, J=13.5)	3.13 (1H, d, J=13.5) 3.21 (1H, d, J=13.5)
4′	1.79 (3H, s)	1.80 (3H, s)	1.81 (3H, s)
5′	3.16 (2H, s)	3.11 (2H, s)	3.07 (1H, d, J=12.5) 3.15 (1H, d, J=12.5)
7′			4.13 (2H, br s)

a) Spectra were taken in pyridine-d_5.

Fasciculic acid B (**2**), $C_{36}H_{60}O_9$, mp 98—103 °C, $[\alpha]_D^{26}$ +19.3° (c=1.0, MeOH), an amorphous powder, showed IR absorptions at 3425 and 1720 cm^{-1} assignable to hydroxyl, ester and/or carboxyl groups, respectively. The presence of a carboxyl group was also supported by the methylation of **2** with diazomethane giving a monomethyl ester (**10**). As shown in Tables I and II, the ^{1}H- and ^{13}C-NMR spectra of **2** closely resembled those of **1**, and besides signals corresponding to those for **1**, the proton signal at δ 4.27 (1H, d, J=7.0 Hz) and the carbon signal at δ 71.92 due to an extra hydroxyl methine group were observed in those of **2**. Thus, **2** was supposed to be 2-*O*-(3-hydroxy-3-methylglutaryl) fasciculol B. In fact, alkaline hydrolysis of **2** afforded two compounds which were identified as fasciculol B (**11**)[4] and **8** on the basis of chemical and spectral evidence.

Fasciculic acid C (**3**), $C_{38}H_{63}O_9$, mp 141—144 °C, $[\alpha]_D^{28}$ +36.3° (c=1.0, MeOH), was obtained as a pale yellow amorphous powder. Its ^{1}H-NMR spectrum was quite similar to that of fasciculol E (**6**) except that the methyl signal (δ 3.60, 3H, s) due to the ester in **6** is absent in the ^{1}H-NMR spectrum of **3**. When treated with diazomethane, **3** was readily methylated to give a monomethyl ester whose physico-chemical and spectral data were in good agreement with those of **6**. Thus, the structure of **3** is shown to be 3-*O*-[(*N*-glycyl-3-hydroxy-3-methylglutaryl)] fasciculol C as illustrated in Fig. 1.

Fasciculols C (**4**), D (**5**) and E (**6**) were also obtained after chromatography of the ethyl acetate-soluble portion. Their structures were established by comparison of physicochemical and spectral data with reported values.

The compounds listed in Table III were tested for their ability to inhibit the calmodulin-sensitive and calmodulin-insensitive PDE's from bovine heart. Among them, fasciculic acid B (**2**) exhibited the strongest inhibitory activity on both of the PDE's. Fasciculic acid A (**1**) inhibited calmodulin-sensitive PDE activity (IC_{50}, 10 μM) without affecting calmodulin-insensitive PDE, suggesting that this

TABLE II. ^{13}C-NMR Spectral Data for Fasciculic Acids A (**1**), B (**2**) and C (**3**)[a,b]

Position	1	2	3	Position	1	2	3
1	41.38 (t)	41.24 (t)	40.00 (t)	21	18.93 (q)	17.98 (q)	61.15 (t)
2	73.81 (d)	73.79 (d)	66.89 (d)	22	34.04 (t)	34.56 (t)	33.02 (t)
3	79.65 (d)	79.64 (d)	79.71 (d)	23	28.81 (t)	29.00 (t)	29.73 (t)
4	40.06 (s)	40.04 (s)	39.11 (s)	24	78.98 (d)	79.02 (d)	78.96 (d)
5	50.95 (d)	50.61 (d)	50.67 (d)	25	72.67 (s)	72.69 (s)	72.70 (s)
6	18.53 (t)	18.58 (t)	18.51 (t)	26	26.16 (q)	26.17 (q)	26.11 (q)
7	28.48 (t)	28.32 (t)	28.11 (t)	27	25.77 (q)	25.68 (q)	25.78 (q)
8	134.30 (s)	132.99 (s)	133.62 (s)	28	24.35 (q)	25.20 (q)	24.18 (q)
9	134.69 (s)	135.31 (s)	134.88 (s)	29	28.39 (q)	28.32 (q)	28.73 (q)
10	38.49 (s)	38.37 (s)	38.20 (s)	30	17.23 (q)	17.23 (q)	17.96 (q)
11	21.39 (t)	26.52 (t)	26.62 (t)	1′	171.63 (s)	171.60 (s)	171.80 (s)
12	26.50 (t)	71.92 (d)	73.12 (d)	2′	46.29 (t)	46.33 (t)	44.34 (t)
13	44.74 (s)	49.95 (s)	50.55 (s)	3′	70.18 (s)	70.19 (s)	70.96 (s)
14	50.01 (s)	49.88 (s)	50.23 (s)	4′	28.85 (q)	28.84 (q)	28.92 (q)
15	31.16 (t)	34.16 (t)	33.02 (t)	5′	46.91 (t)	46.91 (t)	44.34 (t)
16	31.05 (t)	32.54 (t)	32.28 (t)	6′	176.65 (s)	174.44 (s)	172.59 (s)
17	50.49 (d)	43.38 (d)	44.01 (d)	7′			47.12 (t)
18	15.96 (q)	16.68 (q)	17.14 (q)	8′			176.50 (s)
19	20.24 (q)	20.10 (q)	20.25 (q)				
20	36.60 (d)	36.76 (d)	38.56 (d)				

a) Spectra were taken in pyridine-d_5. The chemical shifts have been assigned by comparison with the reported ^{13}C-NMR data of fasciculols B, C, D and F.[5] b) Multiplicities were determined from DEPT data.

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TABLE III. The 50%-Inhibitory Concentrations of Fasciculic Acids A (**1**), B (**2**) and C (**3**) for cAMP Phosphodiesterase (μM)

Test compounds	Calmodulin-sensitive activity	Total activity
Fasciculic acid A (**1**)	10	>100
Fasciculic acid B (**2**)	6	15
Fasciculic acid C (**3**)	60	>100
Fasciculol C (**4**)	43	95
Fasciculol D (**5**)	29	89
Fasciculol E (**6**)	>100	>100
W-7	65	>100

compound is a specific calmodulin antagonist and is about 10 times more potent than W-7, a well known calmodulin antagonist.[7] Fasciculic acid C (**3**), fasciculol C (**4**) and fasciculol D (**5**) also inhibited PDE activities but their selectivities were low.

Experimental

Melting points were determined on a Yanagimoto micro hot plate and are uncorrected. IR spectra were recorded on a JASCO A-100S infrared spectrometer and ultraviolet (UV) spectra on a Hitachi 323 spectrometer. Optical rotation values were measured on a JASCO DIP-340 polarimeter. ^{1}H-NMR spectra were recorded on JEOL GX-500 (500 MHz), JEOL JNM-FX-100 (100 MHz) and JEOL JNM-PMX-60 (60 MHz) spectrometers, and ^{13}C-NMR spectra on a JEOL GX-500 (125 MHz) using tetramethylsilane (TMS) as an internal standard. Chemical shifts are shown in δ (ppm) and multiplicities are given as follows: singlet = s, doublet = d, triplet = t, multiplet = m and broad = br. Coupling constants (J) are shown in Hz. Electron impact (EI)- and FD-MS were taken on Hitachi M-52 and JEOL DX-303 spectrometers, respectively. Thin layer chromatography (TLC) analyses were performed on Kieselgel 60F_{254} (Merck) and spots were detected under UV irradiation (254 nm) and by heating on a hot plate after spraying Ehrlich and anisaldehyde–sulfuric acid reagents.

Isolation Procedure The fruiting bodies of *N. fasciculare* (1.1 kg) collected at Miyagi Prefecture in 1986, were extracted with MeOH (3.0 l) at room temperature for 3 d. After removal of the solvent, the residue was dissolved in H_2O (150 ml) and then extracted twice with AcOEt (150 ml) and *n*-BuOH (100 ml) successively. The AcOEt and *n*-BuOH fractions were each concentrated under reduced pressure to give a gummy syrup (12.3 and 4.7 g). The AcOEt extract (11.3 g) was chromatographed on silica gel (100 g: 3.5 cm i.d. × 21.5 cm) using $CHCl_3$–AcOEt and $CHCl_3$–MeOH as eluents. Fractions were further purified by silica gel, alumina and Florisil chromatographies to afford fasciculic acids A (**1**, 284 mg), B (**2**, 119 mg) and C (**3**, 371 mg), and fasciculols C (**4**, 226 mg), D (**5**, 338 mg) and E (**6**, 2.3 g). The *n*-BuOH extract (4.0 g) was fractionated by column chromatographies on silica gel and Florisil to give fasciculic acids A (32 mg), B (35 mg) and C (421 mg).

Fasciculic Acid A (**1**): TLC, *Rf* 0.21 ($CHCl_3$:MeOH:H_2O = 40 : 10 : 1). IR ν_{max}^{KBr} cm^{-1}: 3600—2600 (OH), 3400 (OH), 2950, 2875, 1720 (C=O). UV λ_{max}^{MeOH} nm (log ε): 201.6 (4.24). FD-MS m/z: 620 (M^+). EI-MS m/z: 517 ($M^+ - C_4H_7O_3$), 161 ($C_6H_9O_5$), 145 ($C_6H_9O_4$). ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Fasciculic Acid B (**2**): TLC, *Rf* 0.14 ($CHCl_3$:MeOH:H_2O = 40 : 10 : 1). IR ν_{max}^{KBr} cm^{-1}: 3600—2600 (OH), 3425 (OH), 2950, 2875, 1720 (C=O). UV λ_{max}^{MeOH} nm (log ε): 202.1 (3.90). FD-MS m/z: 636 (M^+). EI-MS m/z: 523 ($M^+ - C_4H_7O_3$), 161 ($C_6H_9O_5$), 145 ($C_6H_9O_4$). ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Fasciculic Acid C (**3**): TLC, *Rf* 0.06 ($CHCl_3$:MeOH:H_2O = 40 : 10 : 1). IR ν_{max}^{KBr} cm^{-1}: 3600—2600 (OH and NH), 3350 (OH), 2950, 2880, 1720 (C=O), 1640 (CONH). UV λ_{max}^{MeOH} nm (log ε): 201.6 (4.13). FD-MS m/z: 710 [$(M+H)^+$]. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Fasciculol C (**4**): An amorphous powder, mp 185—186 °C (lit. 244 °C), $[\alpha]_D^{28}$ +68.8° (c = 1.0, MeOH, lit. +67.5°). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 2950, 2875. UV λ_{max}^{MeOH} nm (log ε): 203.3 (3.77). FD-MS m/z: 509 [$(M+H)^+$]. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.73 (3H, s, CH_3), 1.15 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.30 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.53 (3H, s, CH_3), 3.40 (1H, d, J = 11.0, H-3), 3.85 (1H, br d, J = 8.0, H-24), 4.15 (2H, br s, H-21), 4.15 (1H, br s, H-2), 4.33 (1H, br d, J = 7.0, H-12).

Fasciculol D (**5**): An amorphous powder, mp 91—94 °C (lit. 95—97 °C), $[\alpha]_D^{28}$ +9.1° (c = 1.0, MeOH, lit. +14.1°). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH and NH), 2950, 2875, 1730 (C=O), 1650 (CONH). UV λ_{max}^{MeOH} nm (log ε): 202.3 (3.94). FD-MS m/z: 708 [$(M+H)^+$]. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.60 (3H, s, CH_3), 0.94 (3H, s, CH_3), 1.04 (3H, d, J = 5.0, H-21), 1.08 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.23 (3H, s, CH_3), 1.45 (3H, s, CH_3), 2.63 and 2.67 (1H, d, J = 12.5, H-2′, each), 2.72 and 2.75 (1H, d, J = 12.5, H-5′, each), 3.23 (1H, d, J = 11.3, H-3), 3.34 (1H, br s, H-24), 3.78 (3H, s, OCH_3), 4.01 (1H, br d, J = 6.3, H-12), 4.05 (2H, br s, H-7′), 5.07 (1H, td, J = 11.2, 4.5, H-2), 7.25 (1H, br s, NH).

Fasciculol E (**6**): An amorphous powder, mp 96—98 °C (lit. 105—106 °C), $[\alpha]_D^{29}$ +32.2° (c = 1.0, MeOH, lit. +30.6°). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH and NH), 2950, 2875, 1730 (C=O), 1650 (CONH). UV λ_{max}^{MeOH} nm (log ε): 202.7 (3.96). FD-MS m/z: 724 [$(M+H)^+$]. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.73 (3H, s, CH_3), 1.03 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.37 (3H, s, CH_3), 1.52 (3H, s, CH_3), 1.53 (3H, s, CH_3), 1.83 (3H, s, CH_3), 3.03 and 3.08 (1H, d, J = 12.5, H-5′, each), 3.12 and 3.18 (1H, d, J = 12.5, H-2′, each), 3.60 (3H, s, OCH_3), 3.85 (1H, br d, J = 8.3, H-24), 4.12 (2H, br s, H-7′), 4.20 (1H, br t, J = 11.5, H-2), 4.25 (1H, dd, J = 17.0, 6.0, H-21), 4.29 (1H, m, H-12), 4.31 (1H, dd, J = 17.0 6.0, H-21), 5.10 (1H, d, J = 11.5, H-3), 9.25 (1H, br s, NH).

Methylation of Fasciculic Acid A (1) An excess of diazomethane in Et_2O was added to a solution of **1** (31.1 mg) in MeOH (1.0 ml), and the solution was left to stand at 0 °C for 10 min. The reaction mixture was concentrated under reduced pressure, and then the residue was chromatographed on silica gel ($CHCl_3$–AcOEt) to afford the monomethyl ester (**7**, 26.1 mg) as colorless needles (from Et_2O), mp 167—169 °C. IR ν_{max}^{KBr} cm^{-1}: 3475 (OH), 2950, 1725 (C=O). FD-MS m/z: 635 [$(M+H)^+$]. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 0.69 (3H, s, CH_3), 0.89 (6H, s, 2 × CH_3), 1.08 (3H, d, J = 4.0, H-21), 1.16 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.41 (6H, s, 2 × CH_3), 1.63 (3H, s, CH_3), 2.54 and 2.70 (1H, d, J = 12.2, H-2′, each)), 2.63 (2H, s, H-5′), 3.09—3.40 (2H, m, H-3 and H-24), 3.71 (3H, s, OCH_3), 4.14 (1H, s, OH), 5.03 (1H, td, J = 11.4, 4.0, H-2).

Methylation of Fasciculic Acid B (2) Methylation of **2** (22.0 mg) was carried out in the same way as for **1** to give the monomethyl ester (**10**, 16.7 mg) as colorless needles (from Et_2O), mp 65—71 °C. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2950, 2875, 1725 (C=O). FD-MS m/z: 651 [$(M+H)^+$]. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.53 (3H, s, CH_3), 0.77 (3H, s, CH_3), 0.94 (3H, d, J = 6.2, H-21), 0.97 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.03 (6H, s, 2 × CH_3), 1.31 (3H, s, CH_3), 2.49 and 2.54 (1H, d, J = 14.1, H-2′, each), 2.57 and 2.62 (1H, d, J = 14.1, H-5′, each), 3.06 (1H, d, J = 11.0, H-3), 3.30 (1H, dd, J = 9.1, 6.0, H-24), 3.31 (3H, s, OCH_3), 3.84 (1H, br t, J = 6.0, H-12), 4.00 (1H, s, OH), 4.17 (1H, d, J = 6.0, OH), 4.30 (1H, d, J = 4.0, OH), 4.84 (1H, td, J = 11.0, 4.0, H-2).

Methylation of Fasciculic Acid C (3) **3** (30.0 mg) was methylated in the same way as for **1** to afford the monomethyl ester (26.1 mg) as an amorphous powder. The spectral and physico-chemical data were identical with those of **6**.

Hydrolysis of Fasciculic Acid A (1) A solution of 2% NaOH (2.5 ml) was added to a solution of **1** (62.0 mg) in EtOH (2.5 ml) at 0 °C. After stirring at room temperature for 30 min, the reaction mixture was diluted with H_2O (2.5 ml) and then extracted with Et_2O (10 ml) twice. The Et_2O layer was concentrated under reduced pressure followed by chromatography on alumina ($CHCl_3$: MeOH = 9 : 1) to give **9** (41.1 mg) as colorless needles (from Et_2O), mp 203—205 °C (lit. 209 °C). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2950, 2875. EI-MS m/z: 476 (M^+), 443 ($M^+ - H_2O - CH_3$), 425 ($M^+ - 2H_2O - CH_3$). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.78 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.05 (3H, d, J = 5.0, H-21), 1.13 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.28 (3H, s, CH_3), 1.54 (3H, s, CH_3), 1.57 (3H, s, CH_3), 3.41 (1H, d, J = 11.0, H-3), 3.80 (1H, br s, H-24), 4.16 (1H, td, J = 11.0, 4.2, H-2), 5.50 (1H, br s, OH), 5.78 (1H, s, OH), 5.89 (1H, br s, OH), 6.10 (1H, br s, OH). ^{13}C-NMR (125 MHz, pyridine-d_5) δ: 16.01 (q, C-18), 17.43 (q, C-30), 18.70 (t, C-6), 18.95 (q, C-21), 20.63 (q, C-19), 21.51 (t, C-11), 24.40 (q, C-28), 25.85 (q, C-27), 26.14 (q, C-26), 26.66 (t, C-12), 28.53 (t, C-7), 28.88 (t, C-23), 29.11 (q, C-29), 31.10 (t, C-16), 31.25 (t, C-15), 34.13 (t, C-22), 36.70 (d, C-20), 38.43 (s, C-10), 39.69 (s, C-4), 44.77 (t, C-1), 44.84 (s, C-13), 50.05 (s, C-14), 50.86 (d, C-17), 50.99 (d, C-5), 68.99 (d, C-2), 72.70 (s, C-25), 79.05 (d, C-24), 83.53 (d, C-3), 134.38 (s, C-8), 134.95 (s, C-9). The H_2O layer was neutralized with Amberlite IR-120B, and then the solvent was removed under reduced pressure followed by crystallization from MeOH to give **8** (14.7 mg) as colorless needles, mp 96—99 °C (lit. 99—102 °C). IR ν_{max}^{KBr} cm^{-1}: 3700—2400 (OH), 1705 (C=O). FD-MS m/z: 163 [$(M+H)^+$]. ^{1}H-NMR (60 MHz, CD_3OD) δ: 1.40 (3H, s, CH_3), 2.62 (4H, s, 2 × CH_2).

Hydrolysis of Fasciculic Acid B (2) Hydrolysis of **2** (68.0 mg) was

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carried out in the same way as described for **1** to afford **11** and **8** (39.6 and 14.3 mg, respectively). **11**: an amorphous powder, mp 214—215 °C (lit. 235 °C). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2950, 2875. EI-MS *m/z*: 492 (M^+), 459 ($M^+ - H_2O - CH_3$), 441 ($M^+ - 2H_2O - CH_3$). ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.78 (3H, s, CH_3), 1.15 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.28 (3H, s, CH_3), 1.35 (3H, d, J=5.1, H-21), 1.46 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.53 (3H, s, CH_3), 3.40 (1H, d, J=9.6, H-3), 3.80 (1H, br s, H-24), 4.16 (1H, ddd, J=10.9, 9.6, 4.0, H-2), 4.27 (1H, dd, J=7.7, 4.5, H-12), 5.48 (1H, br s, OH), 5.60 (1H, d, J=3.8, OH), 5.82 (1H, s, OH), 5.78 (1H, s, OH), 6.10 (1H, s, OH).

Assay for Calmodulin-Inhibitory Activity The calmodulin-antagonistic activity was calculated from the difference of the cyclic adenosine monophosphate (AMP) phosphodiesterase (PED) activities in the presence and absence of calmodulin. The incubation mixture contained, in a final volume of 1.0 ml, 40 mM Tris–HCl buffer (pH 7.4), 2.0 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.01 unit of calmodulin-deficient PDE from bovine heart (Sigma) and 1.0 mM cyclic AMP in the presence or absence of 1.0 mM ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA). The mixture was preincubated in the absence of test compounds and cyclic AMP at 30 °C for 5 min, followed by the addition of a test compound and further preincubation for 5 min. The reaction was started by the addition of cyclic AMP, and stopped by adding one-tenth volume of cold 55% trichloroacetic acid. The amount of phosphate liberated during the 20 min incubation (30 °C) was determined as reported by Martin and Doty.[8] Each experiment was repeated three times.

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