Studies on Differentiation Inducers. VI.[1] Lignan Derivatives from Arctium Fructus. (2)

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In the previous paper, we reported the differentiation inducing activities of lignoids from Arctium Fructus (the fruits of *Arctium lappa* L., Compositae) against mouse myeloid leukemia cells (M1). We reinvestigated the active components of this extract and isolated three new dilignans. Furthermore, structure modifications were carried out using the most active lignan (arctigenin, 1) and its structure–activity relationship was investigated. Its aliphatic esters were more effective in inducing the differentiation of M1 cells than its aromatic esters. Especially, *n*-decanoate, which was the most active derivative, induced more than half of the M1 cells into phagocytic cells at a concentration of 2 μM.

Key words differentiation; Arctium Fructus; lignan; M1 cell; macrophage

Mouse myeloid leukemia cells (M1) are induced into mature macrophage-like cells by proteins, vitamins and other compounds.[2] By using M1 cells, we investigated low moleculer weight differentiation inducers from the plant kingdom and reported the activities of triterpenes, flavones, lignoids and steroids from Caryophylli Flos, *Citrus* Species, Arctium Fructus and Asclepiadaceae plants, respectively.[1,3,4] In searching for more active inducers of the cell differentiation, we reinvestigated the extract of Arctium Fructus, and structural modifications of the active compounds were carried out.

A suspension of the methanolic extract of Arctium Fructus in water was extracted with ether. The aqueous layer was extracted with *n*-BuOH. The *n*-BuOH layer was chromatographed on silica gel and high performance liquid chromatography (HPLC) to afford new active components **3**—**5** named arctignan F(**3**), G(**4**) and H(**5**), with some known lignoids (Chart 1).

Arctignan F (**3**), $C_{40}H_{42}O_{13}$, $[\alpha]_D$ +82.9° was obtained as an amorphous powder. The ^{13}C-NMR spectrum of **3** was similar to that of lappaol F (**6**, dilignan) except for the conjugated carbonyl signal (Table 4). In the ^{1}H-NMR spectrum of **3**, a doublet signal (δ 5.48, J=8 Hz) of H-7‴ which was observed in **6** had disappeared, and the conjugated carbonyl carbon was noted to be located at C-7‴ (Table 3). Nuclear Overhauser effects (NOE) were observed at H-2 [δ 6.58 (1H, d, J=2 Hz)] and H-6 [δ 6.55 (1H, d, J=2 Hz)] by irradiation at the H-7 proton [δ 2.79 (1H, dd, J=14, 7 Hz)], and at H-6, H-2″ [δ 6.92 (1H, d, J=2 Hz)] and H-6″ [δ 6.90 (1H, dd, J=8, 2 Hz)] by irradiation at the H-8″ [δ 3.59 (1H, dt, J=7, 6 Hz)]. The absolute configurations at C-8 and C-8′ of **3** were confirmed as 8(R) and 8′(R) by a negative Cotton effect ($[\theta]_{242}$ −11600) in the circular dichroism (CD) spectrum of this compound.[5] From these data, we concluded that the structure of arctignan F is **3**.

Arctignan G (**4**), $C_{40}H_{44}O_{13}$, $[\alpha]_D$ +81.7° and arctignan H (**5**), $C_{40}H_{44}O_{13}$, $[\alpha]_D$ +145.2° were obtained as amorphous powder. The ^{13}C-NMR spectra of these compounds were very similar and exhibited 24 aromatic carbon signals and four methoxyl carbon signals (Table 4). These results suggested that these compounds were dilignan and isomers of each other, differing in the position of the third and fourth phenylpropanoid units (C″-1 to C″-9 and C‴-1 to C‴-9). The structural distinction between **4** and **5** was achieved by measuring their NOE spectra between H-7 and the aromatic protons. In the difference NOE spectrum of **4**, NOEs were observed at H-2 [δ 6.75 (1H, d, J=2 Hz)] and H-6 [δ 6.66 (1H, dd, J=8, 2 Hz)] by irradiation at H-7 [δ 2.92 (1H, dd, J=14, 7 Hz)]. In the spectrum of **5**, NOEs were observed at H-2 [δ 6.65 (1H, d, J=2 Hz)] and H-6 [δ 6.25 (1H, d, J=2 Hz)] by irradiation at H-7 [δ 2.89 (1H, dd, J=14, 6 Hz)]. The absolute configurations at C-8 and C-8′ of **4** and **5** were confirmed from their CD spectra as 8(R), 8′(R).[5] From these results, the structure of arctignan G is **4** and that of arctignan H is **5**.

Differentiation inducing activities of lignoids were tested using mouse a myeloid M1 cell line (Table 1). Lignan (**1**) showed differentiation inducing activities at the concentration of 10 μM against M1 cells. Compound **2a** (acetate of **2**) induced M1 cells to phagocytic cells at a concentration of greater than 20 μM, whereas **2** did not show any activity.[3b] As we reported previously, pregnane glycosides having a deoxylated-sugar chain showed activities against M1 cells, but those having glucose at the terminal in their sugar chain were all inactive.[4] The hydrophobicity of compounds seems to have an important role to in determining their differentiation inducing activity. In the case of dilignans (**3**—**6**), **4** and **5** showed activities at 50—100 μM, whereas compounds **3** and **6** showed only slight activity at those concentrations. Two active sesquilignans, lappaol E and arctignan A (an isomer of lappaol E), were observed to possess the highest differentiation inducing activities of the sesquilignans we tested, and they have an oxygen-linked phenylpropanoid unit (C-1″ to 9″).[3c] Both **4** and **5** have an oxygen-linked phenylpropanoid unit (C-1″ to 9″ for **4** and C-1‴ to 9‴ for **5**), and the importance of this moiety in the structures was recognized.

To investigate more potent differentiation inducers, structural modifications of **1** were carried out. As shown

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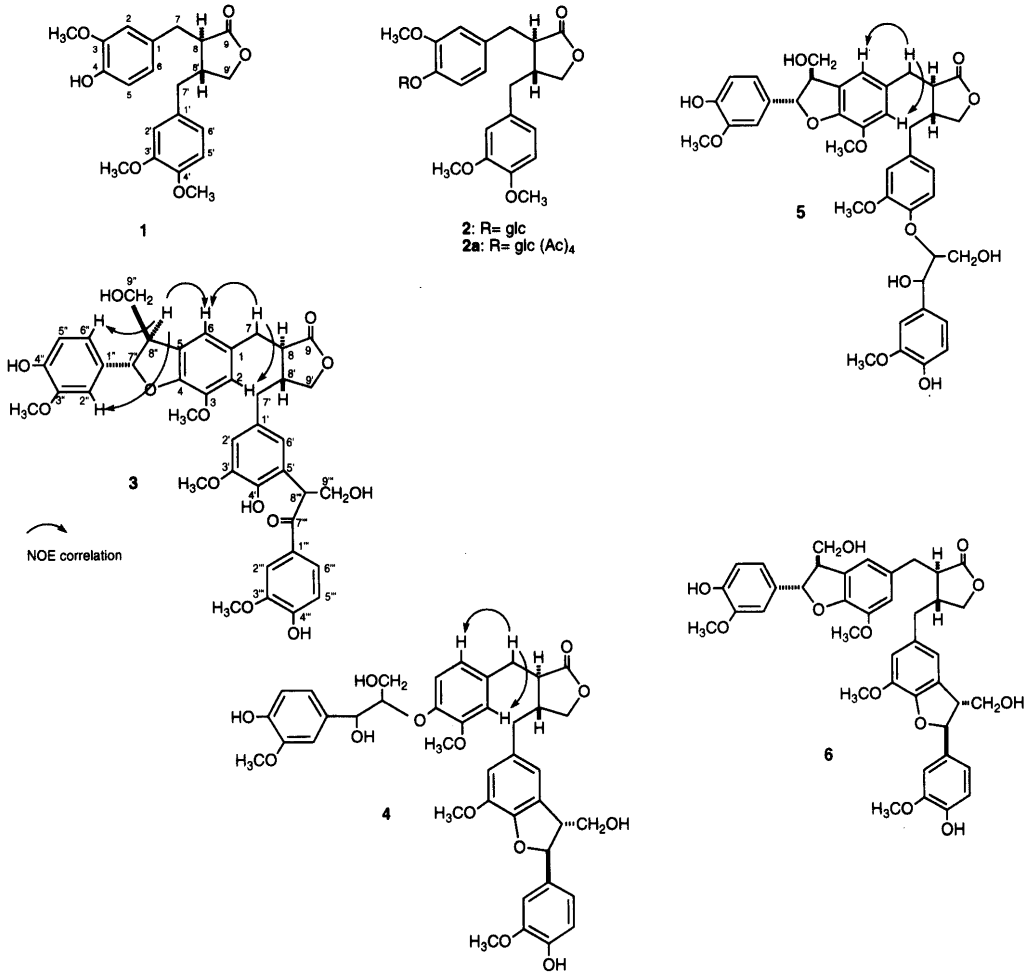


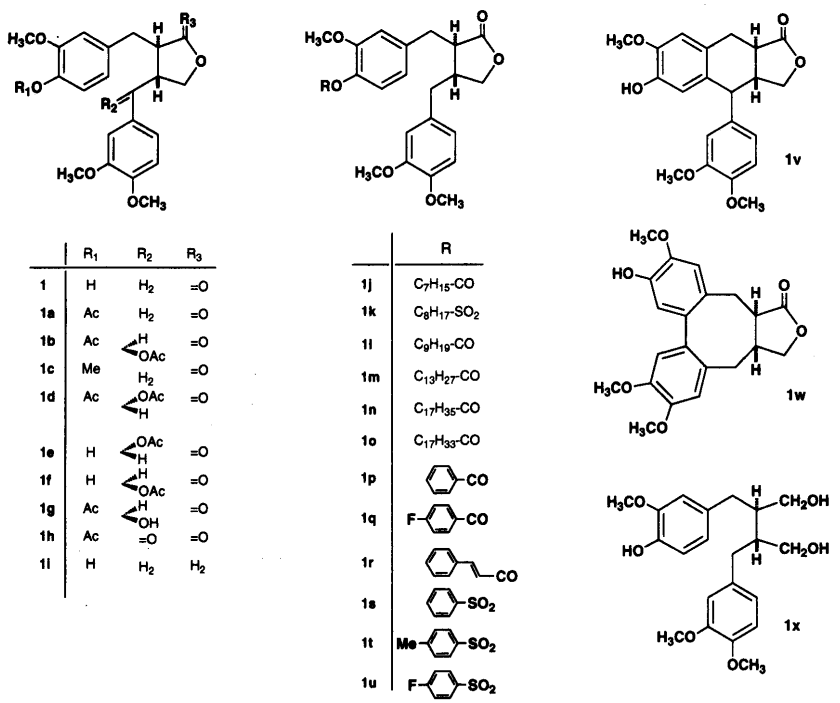
Chart 1

Table 1. Cell Growth and Phagocytosis of M1 Cells Treated with Lignoids

Compound[a]	Conc. (μM)	Growth rate (%)	Phagocytic[b] activity	Compound[a]	Conc. (μM)	Growth rate (%)	Phagocytic[b] activity
Cont.		100	−	Cont.		100	−
Dex.	1	69	+++	Dex.	1	68	+++
1	100	9	++	**3**	100	17	++
	50	21	+++		50	56	++
	20	19	++		20	67	+
	10	40	++		10	74	+
2	100	65	+	**4**	100	27	+++
	50	69	+		50	55	++
	20	72	−		20	67	+
	10	76	+		10	77	++
2a	100	0	uc	**5**	100	41	+++
	50	10	++		50	52	+++
	20	44	++		20	76	++
	10	74	+		10	83	++
				6	100	12	uc
					50	41	++
					20	44	++
					10	100	++

a) Cont., control; Dex., dexamethasone. *b*) +, >10%; ++, >25%; +++, >50%; uc, uncountable.

in Table 2, **1a** exhibited differentiation inducing activities nearly equal to that of **1**, but **1c** did not.[3c] Much attention was paid to the activity of esters of **1**; many derivatives were tested for their activities using M1 cells. Among the aromatic esters, fluorides (**1q**, **1u**) weakened the differentiation inducing activities, whereas sulfonates (**1s**—**1u**) showed poor anti-proliferative effects. Aliphatic esters (**1j**—**1o**) exhibited higher activities than the aromatic esters (**1p**—**1u**). In particular, decanoate (**1l**) was the most active compound, and many M1 cells were induced into



	R_1	R_2	R_3
1	H	H_2	=O
1a	Ac	H_2	=O
1b	Ac	H, OAc	=O
1c	Me	H_2	=O
1d	Ac	OAc, H	=O
1e	H	OAc, H	=O
1f	H	H, OAc	=O
1g	Ac	H, OH	=O
1h	Ac	=O	=O
1i	H	H_2	H_2

	R
1j	C_7H_{15}-CO
1k	C_8H_{17}-SO_2
1l	C_9H_{19}-CO
1m	$C_{13}H_{27}$-CO
1n	$C_{17}H_{35}$-CO
1o	$C_{17}H_{33}$-CO
1p	Ph-CO
1q	F-Ph-CO
1r	Ph-CH=CH-CO
1s	Ph-SO_2
1t	Me-Ph-SO_2
1u	F-Ph-SO_2

Chart 2

Table 2. Cell Growth and Phagocytosis of M1 Cells Treated with Lignoids

Compd.[a]	Conc. (μM)	Growth rate (%)	Phagocytic[b] activity	Compd.[a]	Conc. (μM)	Growth rate (%)	Phagocytic[b] activity	Compd.[a]	Conc. (μM)	Growth rate (%)	Phagocytic[b] activity
Cont.		100	−	Cont.		100	−	Cont.		100	−
Dex.	1	43	+++	Dex.	1	48	+++	Dex.	1	47	+++
1	50	18	+++	**1j**	20	16	+++	**1**	20	27	+++
	5	26	+++		2	61	++		2	64	++
1a	50	15	+++	**1k**	20	19	++	**1p**	20	25	++
	5	33	++		2	55	+		2	62	+
1b	50	67	−	**1l**	20	10	+++	**1q**	20	20	+
	5	79	−		2	59	+++		2	57	+
1c	50	28	++	**1m**	20	22	++	**1r**	20	19	++
	5	63	−		2	61	+		2	65	+
1d	50	65	−	**1n**	20	18	++	**1s**	20	46	+
	5	83	−		2	65	+		2	87	−
1e	50	70	+	**1o**	20	25	++	**1t**	20	80	+
	5	82	−		2	80	−		2	83	+
1f	50	68	+					**1u**	20	59	+
	5	84	−						2	75	+
1g	50	76	−					Cont.		100	−
	5	90	−					Dex.	1	69	+++
1h	50	69	+					**1v**	50	74	+
	5	78	−						10	88	−
1i	50	23	+++					**1w**	50	75	++
	5	58	+						10	100	+
								1x	50	65	−
									10	100	−

a) Cont., control; Dex., dexamethasone. b) +, >10%; ++, >25%; +++, >50%; uc, uncountable.

phagocytic cells with it.

All of the lignoids we isolated from Arctium Fructus have a γ-lactone ring in their structures. Two well-known antitumor lignans, podophyllotoxin and steganacin,[6] have unique ring systems in addition to the γ-lactone ring in their structures. In seeking means to estimate the biological function of the ring in cell differentiation, structure modifications of the ring were performed. As shown in Table 2, the γ-lactone ring appears to have an important role in inducing cell differentiation, while both **1i** and **1x** showed poor activities compared with **1**. Compounds **1v** and **1w** have unique ring systems similar to those of podophyllotoxin and steganacin, respectively. These two showed lower activities against M1 cells than **1**, as recognized in Table 2. From these results, the cyclohexene skeleton of **1v** and the cyclooctadiene skeleton of **1w** showed negative effects for the activities. 7′-Oxygenations of **1** were considered to be an unfavored method for

Table 3. ^{1}H-NMR Spectral Data (δ in ppm)

Proton No.	3	4	5	6
2	6.58 (d, 2)	6.75 (d, 2)	6.65 (d, 2)	6.64 (d, 2)
5		7.00 (d, 8)		
6	6.55 (d, 2)	6.66 (dd, 8, 2)	6.25 (d, 2)	6.51 (d, 2)
7	2.79 (dd, 14, 7)	2.92 (dd, 14, 7)	2.89 (dd, 14, 6)	2.88 (dd, 14, 7)
	2.85 (dd, 14, 5)	3.01 (dd, 14, 5)	2.92 (dd, 14, 5)	3.02 (dd, 14, 5)
8	2.43 (m)	2.57 (m)	2.53 (m)	2.58 (m)
2′	6.38 (d, 2)	6.34 (d, 2)	6.57 (d, 2)	6.46 (d, 2)
5′			7.02 (d, 8)	
6′	6.40 (d, 2)	6.46 (d, 2)	6.60 (dd, 8, 2)	6.57 (d, 2)
7′	2.35 (dd, 13, 8)	2.59 (dd, 14, 7)	2.63 (m)	2.45 (dd, 14, 6)
	2.48 (dd, 13, 8)	2.66 (dd, 14, 6)		2.63 (dd, 14, 6)
8′	2.43 (m)	2.48 (m)	2.48 (m)	2.56 (m)
9′	3.72 (dd, 9, 8)	3.93 (dd, 9, 7)	3.89 (dd, 9, 8)	3.93 (dd, 9, 7)
	3.95 (dd, 9, 7)	4.23 (dd, 9, 8)	4.22 (dd, 9, 7)	4.22 (dd, 9, 7)
2″	6.92 (d, 2)	6.95 (d, 2)	6.93 (d, 2)	6.94 (d, 2)
5″	6.87 (d, 8)	6.88 (d, 8)	6.88 (d, 8)	6.86 (d, 8)
6″	6.90 (dd, 8, 2)	6.89 (dd, 8, 2)	6.89 (dd, 8, 2)	6.88 (dd, 8, 2)
7″	5.48 (d, 7)	4.89 (d, 8)	5.49 (d, 7)	5.47 (d, 8)
8″	3.59 (dt, 7, 6)	4.04 (m)	3.53 (dd, 13, 6)	3.57 (dt, 7, 6)
9″	—	3.50 (dd, 12, 4)	—	—
		3.60 (dd, 12, 5)		
2‴	7.56 (d, 2)	6.93 (d, 2)	6.94 (d, 2)	6.95 (d, 2)
5‴	6.80 (d, 8)	6.88 (d, 8)	6.88 (d, 8)	6.89 (d, 8)
6‴	7.55 (dd, 8, 2)	6.89 (dd, 8, 2)	6.89 (dd, 8, 2)	6.88 (dd, 8, 2)
7‴		5.47 (d, 7)	4.04 (m)	5.48 (d, 7)
8‴	5.18 (dd, 8, 5)	3.54 (dd, 13, 6)	3.51 (dd, 12, 5)	3.57 (dt, 7, 6)
9‴	—	—	3.61 (dd, 12, 3)	—

Run at 270.00 MHz or 500.00 MHz in $CDCl_3$. —, signal could not be determined.

Table 4. ^{13}C-NMR Spectral Data (δ in ppm)

Carbon number	3	4	5	6
1	130.6	133.8	128.3	128.4
2	112.1	113.0	114.4	112.8
3	147.9	151.1	147.2	147.2
4	144.7	146.7	145.7	145.6
5	134.3	120.2	133.1	132.6
6	118.5	122.5	113.3	114.3
7	34.8	34.6	34.6	34.8
8	46.8	46.5	46.7	46.6
9	178.8	178.6	178.7	178.8
1′	130.0	128.9	134.1	128.9
2′	115.4	114.5	112.8	113.2
3′	147.0	147.1	151.1	147.2
4′	142.6	145.8	146.7	145.7
5′	124.2	132.9	120.6	132.8
6′	123.9	113.2	121.8	114.3
7′	38.2	38.1	38.6	38.4
8′	41.9	41.1	41.1	41.3
9′	71.1	71.4	71.3	71.3
1″	131.8	131.5	131.5	131.0
2″	111.2	114.5	109.0	108.8
3″	148.1	146.8	144.4	146.7
4″	147.7	145.7	146.7	144.3
5″	115.2	109.4	117.2	116.6
6″	119.3	120.2	119.1	119.3
7″	88.2	74.1	53.6	88.0
8″	54.7	89.1	88.2	53.5
9″	64.7	61.4	61.5	64.0
1‴	129.9	131.2	130.9	131.4
2‴	110.1	108.9	114.4	108.9
3‴	148.3	144.3	146.7	146.7
4‴	152.0	146.7	145.7	144.2
5‴	114.5	116.5	109.4	117.3
6‴	120.7	119.3	120.2	119.4
7‴	198.3	88.3	74.2	88.0
8‴	48.4	53.6	89.3	53.4
9‴	63.9	64.4	64.6	64.0
OMe	56.0	56.1	56.0	56.0
	56.0	56.1	56.1	56.0
	56.0	56.1	56.1	56.1
	56.2	56.2	56.2	56.1

Run at 67.8 MHz or 125.65 MHz in $CDCl_3$ solution.

structural modifications since such derivatives (**1b, 1d—1h**) scarcely exhibited the activities.

Experimental

General Procedure CD spectra were recorded on a JASCO 20A spectropolarimeter. Ultraviolet (UV) spectra were recorded on a Hitachi U3410 spectrometer. Mass spectra (MS) were obtained using a JEOL JMS-SX 102 mass spectorometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL JNM-GSX 270 and JNM-GSX 500 spectrometers (270.05 and 67.8 MHz, 500.00 and 125.65 MHz, respectively), and chemical shifts were given in δ (ppm) with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). HPLC was carried out on a JASCO model 800 series instrument using Pro-10 Zorbax and D-ODS-7 YMC columns.

Isolation Commercially available Arctium Fructus (10 kg from Niiya in Shimizu, Shizuoka prefecture) was extracted with hot MeOH under reflux. The extract was concentrated under reduced pressure and then partitioned between Et_2O and water. The water phase was successively partitioned between *n*-BuOH and water to give a lipophillic fraction (*n*-BuOH fr.) (830 g) and a hydrophillic fraction (water fr.) (170 g). From the *n*-BuOH fr., **1** (56 g), **2** (155 mg), **3** (11 mg), **4** (5 mg), **5** (2 mg), **6** (226 mg), matairesinol (665 mg), lappaol A (202 mg), lappaol B (1.7 g), isolappaol A (651 mg), and lappaol C (21 mg) were isolated by subsequent chromatography on silica gel and HPLC.

Arctignan F (**3**): Colorless amorphous powder. $[\alpha]_D$ +82.9° (c=0.63, MeOH). UV λ_{max}^{MeOH} (log ε) 227.7 (3.63), 281.4 (4.26), 303.7 (3.99). CD ($c=5.94\times10^{-5}$, MeOH) $\Delta\varepsilon$ (nm): 4.12 (229), −3.51 (242), 4.39 (273), 4.27 (310). *Anal.* Calcd for $C_{40}H_{42}O_{13}\cdot1/2H_2O$: C, 64.94; H, 5.86. Found: C, 64.87; H, 5.77. FAB-MS m/z: 730 $[M]^+$, 731 $[M+H]^+$.

Arctignan G (**4**): Yellow amorphous powder. $[\alpha]_D$ +81.7° (c= 0.10, MeOH). UV λ_{max}^{MeOH} nm (log ε) 231.0 (4.52), 281.1 (4.13). CD ($c=1.51\times10^{-5}$, MeOH) $\Delta\varepsilon$ (nm): −6.21 (232), 14.6 (246), 10.6 (294), 4.4 (310). *Anal.* Calcd for $C_{40}H_{44}O_{13}$: C, 65.57; H, 6.01. Found: C, 65.42; H, 5.88. FAB-MS m/z: 733 $[M+H]^+$.

Arctignan H (**5**): Colorless amorphous powder. $[\alpha]_D$ +145.2° (c= 0.06, MeOH). UV λ_{max}^{MeOH} nm (log ε) 228.3 (4.65), 282.4 (4.30). CD ($c=3.59\times10^{-5}$, MeOH) $\Delta\varepsilon$ (nm): −6.76 (233), 2.03 (248), 1.36 (293). *Anal.* Calcd for $C_{40}H_{42}O_{13}\cdot1/2H_2O$: C, 64.94; H, 5.86. Found: C, 64.82; H, 5.80. FAB-MS m/z: 755 $[M+Na]^+$, 756 $[M+Na+H]^+$.

Acetylation of 1 and 2 Both **1** (900 mg) and **2** (20 mg) were acetylated in the usual manner using acetic anhydride and pyridine to give the acetates **1a** (1000 mg) and **2a** (25 mg). They were identified by direct comparison with authentic samples.

Acetoxylation of 1a Compound **1a** (800 mg) was dissolved in AcOH and treated with $Pb(OAc)_4$ at 70—80 °C for 5 h. The mixture was poured into ice-cold water and extracted with AcOEt. The AcOEt soln. was washed with 10% $NaHCO_3$ and water. AcOEt was removed *in vacuo*. The crude product was purified by HPLC to give **1b** (330 mg), **1d** (85 mg), **1e** (12 mg), **1f** (3 mg), **1g** (15 mg), **1h** (47 mg). **1b** was identified by direct comparison with authentic sample. **1d**: Amorphous powder. $[\alpha]_D$ −9.6° (c=1.41, MeOH). UV λ_{max}^{MeOH} nm (log ε): 224 (4.23), 279 (3.78). FAB-MS m/z: 473 $[M+H]^+$. **1e**: Amorphous powder. $[\alpha]_D$ 32.3° (c=1.10, MeOH). UV λ_{max}^{MeOH} nm (log ε): 231 (4.25), 281 (3.84). FAB-MS m/z: 431 $[M+H]^+$. **1f**: Amorphous powder. $[\alpha]_D$ −6.7° (c=0.33, MeOH). UV λ_{max}^{MeOH} nm (log ε): 232 (4.21), 281 (3.78). FAB-MS m/z: 431 $[M+H]^+$. **1g**: Amorphous powder. $[\alpha]_D$ 13.4° (c=0.37, MeOH). UV λ_{max}^{MeOH} nm (log ε): 223 (4.21), 279 (3.77). FAB-MS m/z: 431 $[M+H]^+$. **1h**: Amorphous powder. $[\alpha]_D$ 5.9° (c=0.68, MeOH). UV λ_{max}^{MeOH} nm (log ε): 227 (4.36), 278 (4.14), 309 (3.96). FAB-MS m/z: 429 $[M+H]^+$.

Esterification (I) of 1 Compound **1** (50 mg) was dissolved in 1 ml trimethylamine and treated with an equal amount of *n*-octanoyl chlorides at room temperature overnight. The reactant was purified by HPLC to give **1j** (10 mg) as a light yellowish oil. $[\alpha]_D$ −17.4° (c=0.95, MeOH). UV λ_{max}^{MeOH} nm (log ε): 222 (4.35), 280 (3.92). FAB-MS m/z: 499

$[M+H]^+$. Esterification with *n*-octylsulfonyl chloride was performed in the same manner. **1k** (7 mg): light yellowish oil. $[\alpha]_D$ −16.2° ($c=0.71$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 222 (4.30), 280 (3.74). FAB-MS *m/z*: 548 $[M+H]^+$.

Esterification (II) of 1 Compound **1** (50 mg) was dissolved in 1 ml pyridine and treated with an equal amount of *n*-decanoyl chloride at room temperature overnight. The reactant was purified by HPLC to give **1l** (58 mg) as a colorless oil. $[\alpha]_D$ −16.2° ($c=0.68$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 222 (4.25), 280 (3.79). FAB-MS *m/z*: 528 $[M+H]^+$. Other esters were prepared as in the case of **1l**. **1m** (45 mg): Amorphous powder. $[\alpha]_D$ −16.3° ($c=1.26$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 222sh (4.65), 280 (4.04). FAB-MS *m/z*: 584 $[M+H]^+$. **1n** (85 mg): Amorphous powder. $[\alpha]_D$ −10.8° ($c=0.83$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 222 (4.38), 280 (3.94). FAB-MS *m/z*: 640 $[M+H]^+$. **1o** (45 mg): Amorphous powder. $[\alpha]_D$ −10.9° ($c=0.64$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 203 (4.88), 222 (4.31), 279 (3.81). FAB-MS *m/z*: 638 $[M+H]^+$. **1p** (57 mg): Amorphous powder. $[\alpha]_D$ −12.9° ($c=0.97$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 228 (4.36), 277 (3.94). FAB-MS *m/z*: 477 $[M+H]^+$. **1q** (60 mg): Amorphous powder. $[\alpha]_D$ −9.9° ($c=1.01$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 203 (5.02), 229 (4.53), 277 (3.89). FAB-MS *m/z*: 497 $[M+H]^+$. **1r** (61 mg): Amorphous powder. $[\alpha]_D$ −7.3° ($c=0.89$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 217 (4.56), 223 (4.53), 282 (4.59). FAB-MS *m/z*: 503 $[M+H]^+$. **1s** (41 mg): Amorphous powder. $[\alpha]_D$ −34.5° ($c=1.02$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 209 (4.64), 218 (4.43), 276 (3.83). FAB-MS *m/z*: 513 $[M+H]^+$. **1t** (59 mg): Amorphous powder. $[\alpha]_D$ −32.5° ($c=1.19$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 204 (4.91), 225 (4.50), 277 (3.85). FAB-MS *m/z*: 527 $[M+H]^+$. **1u** (66 mg): Amorphous powder. $[\alpha]_D$ −37.4° ($c=0.99$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 219 (4.41), 278 (3.78). FAB-MS *m/z*: 546 $[M+H]^+$.

Oxidation (I) of 1b[7] Compound **1b** (70 mg) was dissolved in 2 ml of the solution (tetrahydrofuran (THF): conc. HCl = 20 : 1) and stirred for 2 d at room temperature. The mixture was diluted with water and extracted with ethyl acetate. Purification by HPLC afforded a conidendrin monomethyl ether (**1v**) (8 mg). **1v**: Colorless amorphous powder. $[\alpha]_D$ −4.03° ($c=0.12$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 229.8 (4.49), 282.6 (4.13). CD ($c=6.58\times10^{-5}$, MeOH) $\Delta\varepsilon$ (nm): 1.85 (239), 3.91 (271), −10.1 (288). FAB-MS *m/z*: 370 $[M]^+$.

Oxidation (II) of 1[8] In a 50 ml two-necked round bottom flask with an inlet for N_2 was placed 45 mg (0.26 mol) of RuO_2, $2H_2O$ in dry CH_2Cl_2 (2.5 ml), trifluoroacetic acid (TFA) (5 ml) and trifluoroacetic anhydride (TFAA) (2.5 ml). Then, **1** (0.1 g) in CH_2Cl_2 (2.5 ml) was added dropwise at 0 °C and immediately thereafter BF_3–Et_2O (0.3 ml). The flask was immersed in an ultrasound bath (water) thermostated at 18 °C (±2 °C), and the mixture was stirred for 7 h. The suspension was treated at 0 °C with 5% $NaHCO_3$. The aqueous layer was decanted and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated NaCl solution and dried over Na_2SO_4. After filtration, the solution was evaporated and purified by HPLC to give **1w** (14 mg). **1w**: $[\alpha]_D$ +208.3° ($c=0.16$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 248.3 (4.17), 281.8 (4.12). CD ($c=6.76\times10^{-5}$, MeOH) $\Delta\varepsilon$ (nm): 67.3 (232), 23.3 (250), 7.18 (290). FAB-MS *m/z*: 370 $[M]^+$.

Reduction of 1[3c] Compound **1** (600 mg) was dissolved in tetrahydrofuran (20 ml) and treated with $LiAlH_4$ (600 mg) at room temperature for 4 h. Then, the reactant was poured into ice-cold water, carefully acidified with 10% H_2SO_4, and extracted with Et_2O. The ether extract was purified by HPLC to give **1x** (350 mg) as an amorphous powder. $[\alpha]_D$ −13.4° ($c=0.65$, MeOH). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 222 (4.24), 282 (3.90).

Cell Culture M1 cells were grown in Eagle's MEM medium containing 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml kanamycin and 2 mmol/l L-glutamine in 10% heat-inactivated calf serum (CS) over the range of 1×10^5/ml to 2×10^6/ml in a 5% CO_2 humidified atmosphere at 37 °C.

Materials Eagle's MEM, Eagle's amino acids and vitamins medium were purchased from Nissui Pharmaceutical Co., Ltd. CS was from Gibco. Antibiotics were from Meiji Seika Kaisha, Ltd. L-Glutamine was from Wako Pure Chemical Industries, Ltd. Dexamethasone was from Nakalai Chemicals, Ltd. Polystyrene latex particles were from The Dow Chemical Company. *n*-Octanoyl chloride, *n*-octyl sulfonyl chloride, *n*-decanoyl chloride, *n*-tetradecanoyl chloride, *n*-octadecanoyl chloride, *cis*-9-octadecenyl chloride and benzene sulfonyl chloride were from Tokyo Kasei Kogyo, Ltd. Benzoyl chloride, cinnamoyl chloride, tosyl chloride, *p*-fluorobenzoyl chloride and *p*-fluorobenzene sulfonyl chloride were from Sigma Chemical Co.

Measurement of Phagocytosis Phagocytic activity was assayed as reported previously.[3c]

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