Studies on Differentiation-Inducers from Arctium Fructus

Kaoru UMEHARA,[a,*] Ariko SUGAWA,[a] Masanori KUROYANAGI,[a] Akira UENO,[a] and Takao TAKI[b]

School of Pharmaceutical Sciences, University of Shizuoka,[a] 52–1 Yada, Shizuoka 422, Japan and Faculty of Medicine, Tokyo Medical and Dental University,[b] 1–5–45 Yushima, Bunkyo-ku, Tokyo 113, Japan. Received January 14, 1993

In the course of studying differentiation-inducers from plants, their isolation was performed from the methanolic extract of Arctium Fructus (the fruits of *Arctium lappa* L., Compositae), and then their phagocytic activity on differentiated mouse myeloid leukemia cells (M1) was monitered. Thirteen compounds, including five new ones, were isolated as differentiation-inducers toward M1 cells. These consisted of two lignans, eight sesquilignans and three dilignans. Arctigenin (2) was the most effective compound of all those isolated, and it induced differentiation of M1 cells at a concentration 0.5 μM. Sesquilignans were less effective than lignans and dilignans showed even weaker activity. These lignoids were inactive towards a human acute promyelocytic leukemia cell line (HL-60).

Keywords differentiation; Arctium Fructus; lignan; difference NOE spectrum; macrophage; M1 cell

A number of antiproliferative drugs and chemical compounds, such as phorbol esters, anticancer agents, vitamins and lipids have been reported to promote the terminal differentiation of certain tumor cell lines *in vitro*.[1–4] These agents showed changes in gene expression and consequent suppression of the tumor phenotype.[5] Because of these observations, differentiation inducers are of potential interest for the treatment of human cancers which would promote the terminal differentiation of certain human tumor cells.

We have studied differentiation inducers from the plant kingdom and reported the differentiation-inducing activities of triterpenes and flavones using a mouse myeloid leukemia cell line (M1) and a human acute promyelocytic leukemia cell line (HL-60).[6,7] Some triterpenes showed antiproliferative activities in these cell lines and induced cell differentiation into macrophage like cells. Among more than 200 methanolic extracts tested by us, that of Arctium Fructus (the fruits of *Arctium lappa* L., Compositae) showed a marked differentiation-inducing activity towards M1 cells. This paper describes the isolation and structural elucidation of active components from the methanolic extract of Arctium Fructus and examines the potential for differentiation-inducing activity exhibited by these compounds.

The suspension of the methanolic extract of Arctium Fructus in water was extracted with ether and the aqueous layer was then successively extracted with *n*-BuOH. The ether extract afforded two lignans and the *n*-BuOH extract afforded twelve lignans, investigated as differentiation inducers (except **3**) toward M1 cells, by repeated chromatography. The structures of these lignans are shown in Chart 1. Nine known compounds (**1**—**8** and **12**) were identified by comparison with reported data.[8–12] The structures of the five new compounds were assigned on the basis of spectral evidence as arctignan A (**9**), B (**10**), C (**11**), D (**13**) and E (**14**).

Arctignan A (**9**), $C_{30}H_{34}O_{10}$, $[\alpha]_D$ −25.3° was obtained as an amorphous powder. ^{1}H- and ^{13}C-NMR spectra of **9** showed a very similar signal pattern to that of lappaol E (**8**)[9] and suggested that these compounds should be isomers of each other differing in the position of the third phenylpropanoid unit (C-1″ to 9″). All proton signals could be assigned from their ^{1}H–^{1}H two dimensional correlated spectroscopy (^{1}H–^{1}H COSY), two dimensional J-resolved spectroscopy (2D-J) and ^{13}C–^{1}H COSY spectra. The structural distinction between **8** and **9** was achieved by measuring their nuclear Overhauser effect (NOE) spectra between H-7 and the aromatic protons. The NOEs were observed at H-2 [δ 6.70 (1H, d, J=2)] and H-6 [δ 6.62 (1H, dd, J=8, 2)] by irradiation at H-7 [δ 2.90 (1H, dd, J=14.7)], and at H-5 [δ 6.98 (1H, d, J=8)] by irradiation at H-8″ [δ 4.01 (1H, m)] in the difference NOE spectrum of **9**. These results indicated that the third phenylpropanoid unit should be attached to C-4. With regard to the absolute configuration of **9** at C-8 and C-8′, the circular dichroism (CD) spectrum showed a negative Cotton effect ($[\theta]_{234}$ −29700), as in the case of (−)-arctigenin reported by Suzuki *et al.*, confirming 8 (*R*), 8′ (*S*) configulation.[13] These data suggested that **9** is isomer of **8**, as shown in Chart 1.

Arctignan B (**10**), $C_{30}H_{32}O_{10}$, $[\alpha]_D$ 82.3° was obtained as an amorphous powder. The ^{13}C-NMR spectrum of **10** showed the presence of a γ-lactone ring (δ 178.9) and a conjugated carbonyl group (δ 199.2). Furthermore, ^{13}C-NMR signals were very similar to those of **7** except for the conjugated carbonyl signal as shown in Table III. In the ^{1}H-NMR spectrum of **10**, the H-2″ (δ 7.57) and the H-6″ (δ 7.57) were shifted downfield, compared with those of **7**, by 0.76 ppm and 0.81 ppm, respectively (Table II). In addition, one of the carbinyl methine proton signals at δ 5.11 of H-7″ observed in **7** had disappeared and the conjugated carbonyl carbon was noted to be located at C-7″. The NOE was studied at H-2 [δ 6.56 (1H, d, J=2)] and H-6 [δ 6.37 (1H, dd, J=8, 2)] by irradiation at the H-7 proton [δ 2.76 (1H, dd, J=14, 6.5)] in the difference NOE spectrum of **10**. These data suggested that the structure of arctignan B is that of **10**.

Arctignan C (**11**), $C_{30}H_{32}O_{10}$, $[\alpha]_D$ 63.8° was obtained as an amorphous powder. The ^{1}H- and ^{13}C-NMR spectra of **11** were very similar to those of **10**. The NOE was observed at H-2 [δ 6.43 (1H, d, J=2)] and H-6 [δ 6.56 (1H, d, J=2)] by irradiation at the H-7 proton [δ 2.83 (1H, dd, J=14, 5)] in the difference NOE spectrum. These results suggested that **11** is the isomer of **10** as shown in Chart 1.

Arctignan D (**13**), $C_{40}H_{44}O_{13}$, $[\alpha]_D$ −37.2° and E(**14**), $C_{40}H_{44}O_{13}$, $[\alpha]_D$ −27.0° were both obtained as an amorphous powder. The ^{13}C-NMR spectra of these compounds were very similar and exhibited twenty four aromatic carbon signals and four methoxyl carbon signals (δ 56.1, 56.1, 56.1, 56.4). This result and the molecular for-

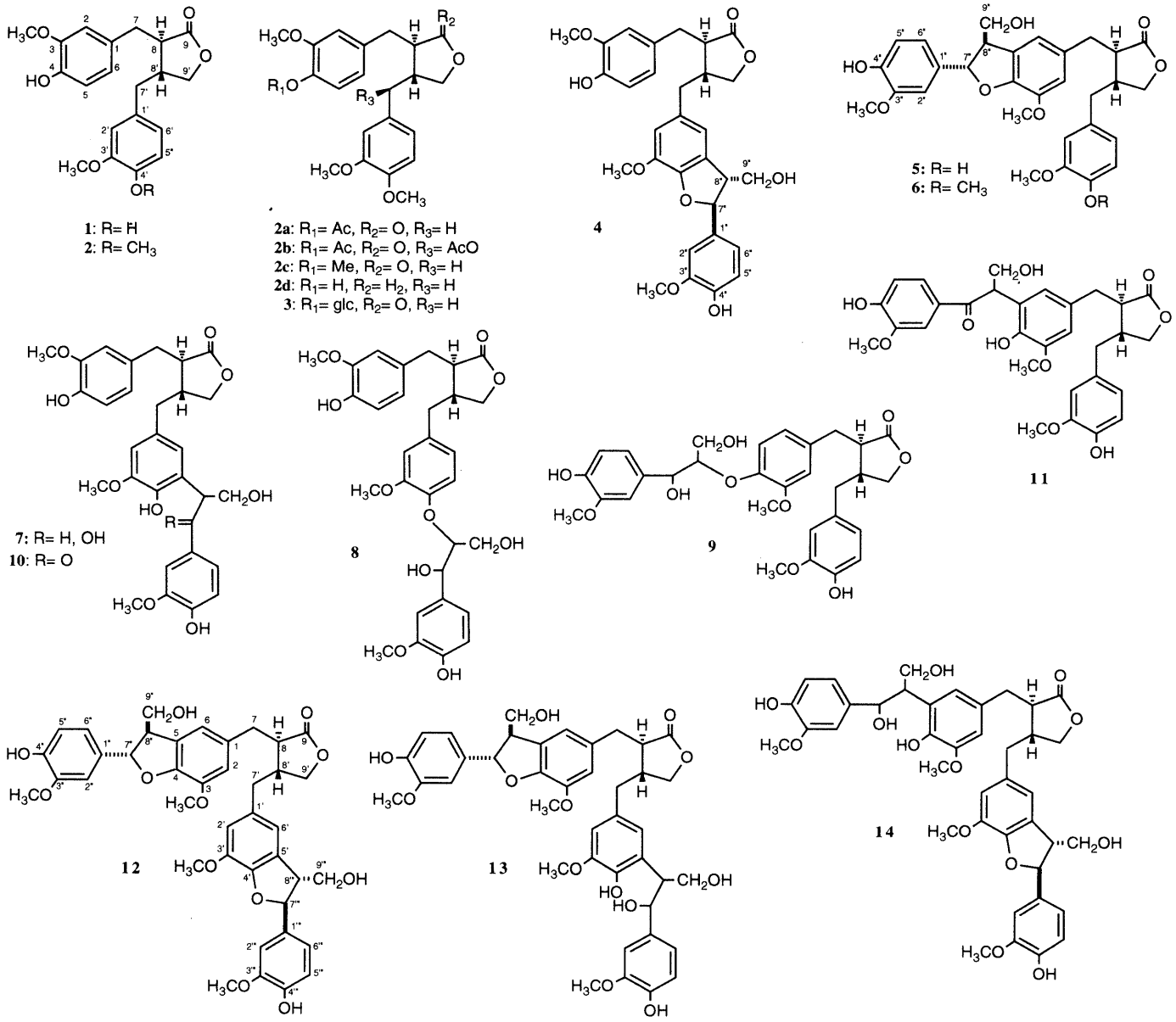

Chart 1

mula suggested that these compounds were dilignan and a compound structurally similar to lappaol F (**12**), although one of the dihydrobenzofuran rings was presumed to be opened. The ^{1}H-NMR spectrum of **13** and **14** suggested the presence of a dihydrobenzofuran ring (**13**: δ 5.44 and **14**: δ 5.41) and an opened dihydrobenzofuran ring (**13**: δ 5.11 and **14**: δ 5.11). The NOE was observed at H-2 [δ 6.65 (1H, d, J=2)] and H-6 [δ 6.53 (1H, d, J=2)] by irradiation at the H-7 proton [δ 2.85 (1H, dd, J=14, 7)] in the difference NOE spectrum of **13**. Furthermore, the NOE between H-6 and H-8″ [δ 3.54 (1H, dt, J=7, 6)] in the difference NOE spectrum led us to conclude that the structure of arctignan D was **13**. In addition, the difference NOE, recognized at H-6 [δ 6.55 (1H, d, J=2)] irradiating with H-7 [δ 2.89 (2H, d, J=6)] and H-8″ [δ 3.43 (1H, dt, J=7, 6)], suggested that the structure of arctignan E (**14**) was an isomer of **13**. The CD spectra of **13** and **14** exhibited positive Cotton effects near 290 nm. A negative Cotton effect was reported with 2(*R*),3(*R*)-dihydro-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-3-methylbenzofuran by Achenbach *et al.*[14] These data suggested that the absolute configuration of the dihydrobenzofuran ring in **13** and **14** was 7″ (*S*), and 8″ (*R*) and 7‴ (*S*) and 8‴ (*R*) respectively as shown in Chart 1.

20—50 μM of triterpenes and 5—50 μM of flavones exhibited differentiation-inducing activity towards M1 cells.[6,7] Using M1 cells and HL-60 cells, the differentiation inducing activity of lignoids was assessed in the same manner as triterpenes and flavones. The lignoids used in the assay comprised fourteen compounds isolated from Arctium Fructus and four (−)-arctignan derivatives (**2a**—**d** as shown in Chart 1). Lignans (**1**, **2**, **2a**, **2d**) exhibited differentiation-inducing activity towards M1 cells at a concentration greater than 5 μM. The 4′-Me ether of **1**(**2**) induced the conversion of more than 50% of M1 cells into phagocytic cells, even at 5 μM. Thus, the differentiation-inducing activity of **1** was enhanced by methylation of the 4′-OH group, whereas the permethyl ether of **1**(**2c**) exhibited the cytotoxicity. The glucoside of **2**(**3**) exhibited low activity but the 4-acetate of **2**(**2a**) retained activity. Although compound **2d** exhibited

TABLE I. Cell Growth and Phagocytosis of M1 Cells Treated with Lignans

Compound[a]	Conc. (μM)	Growth rate (%)	Phagocytic activity[b]	Compound[a]	Conc. (μM)	Growth rate (%)	Phagocytic activity[b]
Cont.		100	−	Cont.		100	−
Dex.	1	45	+++	Dex.	1	46	++
1	50	21	+++	**4**	100	19	++
	20	36	++		50	42	+
	10	52	+		20	100	−
	5	65	+		10	96	−
2	50	10	+++	**5**	100	19	++
	20	12	+++		50	33	++
	10	20	+++		20	67	+
	5	26	+++		10	61	−
2a	50	13	+++	**6**	100	13	++
	20	15	+++		50	31	++
	10	15	+++		20	66	−
	5	29	++		10	67	−
2c	50	29	++	**7**	100	68	+
	20	35	+		50	73	+
	10	55	+		20	98	−
	5	57	−		10	90	−
2d	50	36	+++	Cont.		100	−
	20	41	++	Dex.	1	48	+++
	10	55	++	**8**	100	42	+++
	5	64	+		50	40	+++
3	100	66	+		20	48	++
	50	66	+		10	63	++
	20	63	−	**9**	100	16	+++
	10	78	−		50	47	+++
Cont.		100	−		20	75	++
Dex.	1	58	++		10	84	++
12	100	55	+	**10**	100	38	++
	50	55	+		50	63	+
	20	78	+		20	99	−
	10	100	−		10	84	−
13	100	94	+	**11**	100	14	++
	50	100	−		50	28	+
	20	93	−		20	33	+
	10	100	−		10	56	−
14	100	97	+				
	50	96	−				
	20	100	−				
	10	100	−				

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

differentiation-inducing activity at 5 μM, the percentage of phagocytic M1 cells decreased. Sesquilignans (**4**—**11**) demonstrated differentiation-inducing activity at 50 μM. Although these eight compounds has similar structures except for the third phenylpropanoid unit (C-1‴ to C-9‴), only **8** and **9** exhibited marked differentiation-inducing activity at a 10 μM. It was noteworthy that more than 70% of M1 cells differentiated into phagocytic cells following treatment with 50 μM of **8**. The isomers **5**, **9** and **11** of **4**, **8** and **10**, showed little difference in terms of their differentiation-inducing activity. Dilignans (**12**—**14**) exhibited activity at 100 μM, however the percentage of phagocytic M1 cells was not as high.

As mentioned above, lignans such as **1**, **2**, **2a** and **2d** were the most active of all isolated in terms of induction of the cell differentiation of M1. Sesquilignans were less effective than lignans, but more effective than dilignans. The differentiation-inducing activities of lignans were not reduced by esterification of the 4-OH group, but were by etherification. M1 cells were cultured for 2 d with different doses of **2** and cell proliferation and differentiation were investigated (Fig. 1). Differentiation-inducing activity and anti-proliferative activity were observed even at concentration of 0.5 μM and the phagocytic cells increased in a dose-dependent manner. At concentrations greater than 10 μM, the percentage of phagocytic cells following treatment with **2** was equal to that of dexamethasone-treated cells. These lignoids were tested for activity using HL-60 cells and all failed to induce cell differentiation (data not shown).

Experimental

General Procedure Optical rotations were measured on a JASCO DIP-360 digital polarimeter. CD spectra were recorded on a JASCO 20A spectropolarimeter. Ultraviolet (UV) spectra were recorded on a Hitachi U3410 spectrophotometer. Mass spectra (MS) were obtained using a JEOL JNM-SX 102 mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-GSX 270 and JNM-GSX 500 spectrometer (270.05 and 67.8 MHz, 500.00 and 125.65 MHz respectively) and chemical shifts are 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) was extracted with hot MeOH under reflux. The extract was

TABLE II. ^{1}H-NMR Spectral Data of **1**—**14**

Proton No.	1	2	2d	4	5	6	7
2	6.61 (d, 2)	6.64 (d, 2)	6.56 (d, 2)	6.68 (d, 2)	6.62 (d, 2)	6.64 (d, 2)	6.65 (d, 2)
5	6.82 (d, 8)	6.82 (d, 8)	6.80 (d, 8)	6.81 (d, 8)			6.81 (d, 8)
6	6.60 (dd, 8)	6.61 (dd, 8, 2)	6.62 (dd, 8, 2)	6.61 (dd, 8, 2)	6.49 (d, 2)	6.52 (d, 2)	6.58 (dd, 8, 2)
7	2.88 (dd, 14, 7)	2.90 (dd, 14, 6.5)	2.53 (dd, 13.5, 8)	2.90 (dd, 14, 7)	2.91 (dd, 14, 6.5)	2.92 (dd, 14, 6.5)	2.87 (dd, 14, 7)
	2.94 (dd, 14, 5.5)	2.94 (dd, 14, 5)	2.61 (dd, 13.5, 6.5)	2.97 (dd, 14, 5)	2.95 (dd, 14, 5.5)	2.96 (dd, 14, 5.5)	2.94 (dd, 14, 5.5)
8	2.56 (dt, 7, 5.5)	2.57 (ddd, 8, 6.5, 5)	2.18 (m)	2.57 (ddd, 12, 7, 5)	—	2.60 (m)	2.50 (ddd, 8.5, 7, 5.5)
9			3.53 (dd, 9, 6)				
			3.91 (dd, 9, 6.5)				
2′	6.41 (d, 2)	6.46 (d, 2)	6.53 (d, 2)	6.42 (br s)	6.42 (d, 2)	6.53 (d, 2)	6.33 (d, 2)
5′	6.79 (d, 8)	6.74 (d, 8)	6.75 (d, 8)		6.80 (d, 8)	6.76 (d, 8)	
6′	6.51 (dd, 8, 2)	6.54 (dd, 8, 2)	6.59 (dd, 8, 2)	6.42 (br s)	6.54 (dd, 8, 2)	6.58 (dd, 8, 2)	6.36 (d, 2)
7′	2.53 (dd, 13.5, 8)	2.52 (dd, 13.5, 5)	2.52 (dd, 13.5, 8)	2.54 (dd, 13.5, 8)	—	2.55*	—
	2.61 (dd, 13.5, 6.5)	2.64 (dd, 13.5, 6)	2.60 (dd, 13.5, 6.5)	2.64 (dd, 13.5, 5.5)	2.63 (dd, 13.5, 7)	2.66 (dd, 13, 6)	2.56 (dd, 13.5, 5)
8′	2.47 (m)	2.48 (m)	2.18 (m)	2.49 (m)	—	2.52*	2.39 (m)
9′	3.88 (dd, 9, 7.5)	3.88 (dd, 9, 7.5)	3.53 (dd, 9, 6)	3.90 (dd, 9, 6)	3.90 (dd, 9, 6)	—	3.87 (dd, 9.5, 6.5)
	4.15 (dd, 9, 7.5)	4.13 (dd, 9, 7.5)	3.92 (dd, 9, 6.5)	4.17 (dd, 9, 7)	4.15 (dd, 9, 7)	4.15 (dd, 9, 7)	4.01 (dd, 9.5, 7.5)
2″				6.92 (d, 2)	6.92 (d, 8)	6.94 (d, 2)	6.81 (d, 2)
5″				6.87 (d, 8)	6.87 (d, 8)	6.87 (d, 8)	6.81 (d, 8)
6″				6.89 (dd, 8, 2)	6.89 (dd, 8, 2)	6.90 (dd, 8, 2)	6.76 (dd, 8, 2)
7″				5.50 (d, 7)	5.50 (d, 7)	5.53 (d, 7.5)	5.11 (d, 7)
8″				3.55 (dt, 7, 6)	3.55 (dt, 7, 6)	3.53 (dt, 7.5, 6)	3.42 (dt, 7, 6)
9″				—	—	—	—

Proton No.	8	9	10	11	12	13	14
2	6.64 (d, 2)	6.70 (d, 2)	6.56 (d, 2)	6.43 (d, 2)	6.62 (d, 2)	6.65 (d, 2)	6.65 (d, 2)
5	6.80 (d, 8)	6.98 (d, 8)	6.75 (d, 8)				
6	6.57 (dd, 8, 2)	6.62 (dd, 8, 2)	6.37 (dd, 8, 2)	6.56 (d, 2)	6.50 (d, 2)	6.53 (d, 2)	6.55 (d, 2)
7	2.87 (dd, 14, 7)	2.90 (dd, 14, 7)	2.70 (dd, 14, 5.5)	2.77 (dd, 14, 7)	2.84 (dd, 14, 7.5)	2.85 (dd, 14, 7)	2.89 (d, 6)
	2.96 (dd, 14, 5)	2.94 (dd, 14, 5.5)	2.76 (dd, 14, 6.5)	2.83 (dd, 14, 5)	2.98 (dd, 14.5, 5)	2.99 (dd, 14, 5)	2.89 (d, 6)
8	2.54 (m)	2.58 (m)	2.40 (m)	2.34*	2.55*	—	2.53*
2′	6.50 (d, 2)	6.46 (d, 2)	6.30 (d, 2)	6.42 (d, 2)	6.44 (d, 2)	6.40 (d, 2)	6.46 (d, 2)
5′	6.96 (d, 8)	6.80 (d, 8)		6.74 (d, 8)			
6′	6.51 (dd, 8, 2)	6.51 (dd, 8, 2)	6.33 (d, 2)	6.32 (dd, 8, 2)	6.55 (d, 2)	6.51 (d, 2)	6.51 (d, 2)
7′	2.53*	2.57 (dd, 13.5, 7.5)	2.38 (dd, 13.5, 8)	2.43*	—	—	2.51*
	2.62 (dd, 13.5, 6.5)	2.62 (dd, 13.5, 6)	2.49 (dd, 13.5, 6.5)	2.52*			2.63 (dd, 14, 6.5)
8′	2.48 (m)	2.47 (m)	2.31 (m)	2.32*	2.51*	—	2.44 (m)
9′	—	—	3.72 (dd, 9, 8)	3.75 (dd, 9, 7.5)	3.89 (dd, 9.5, 6)	—	—
	4.15 (dd, 9, 7.5)	4.18 (dd, 9, 7.5)	3.97 (dd, 9, 7.5)	3.98 (dd, 9, 7)	4.20 (dd, 9.5, 7)	4.11 (dd, 9, 7)	4.10 (dd, 9, 7.5)
2″	6.96 (d, 2)	6.97 (d, 2)	7.57 (d, 2)	7.54 (d, 2)	6.91 (d, 2)	6.93 (d, 2)	6.83 (d, 2)
5″	6.88 (d, 8)	6.90 (d, 8)	6.78 (d, 8)	6.76 (d, 8)	6.83 (d, 8)	6.86 (d, 8)	6.80 (d, 8)
6″	6.90 (dd, 8, 2)	6.91 (dd, 8, 2)	7.57 (dd, 8, 2)	7.55 (dd, 8, 2)	6.85 (dd, 8, 2)	6.87 (dd, 8, 2)	6.80 (dd, 8, 2)
7″	4.93 (d, 8)	4.94 (d, 8)			5.44 (d, 7)	5.44 (d, 7)	5.11 (d, 7)
8″	4.00 (m)	4.01 (m)	5.18 (dd, 8, 4.5)	5.18 (dd, 8, 4.5)	3.55 (dt, 7, 6)	3.54 (dt, 7, 6)	3.43 (dt, 7, 6)
9″	3.49 (dd, 12.5, 4)	3.50 (dd, 12.5, 4)	4.17 (dd, 11, 8)	4.19 (dd, 11, 8)	—	—	—
	3.60 (dd, 12.5, 3)	3.61 (dd, 12.5, 3.5)	—	—			
2‴					6.93 (d, 2)	6.84 (d, 2)	6.92 (d, 2)
5‴					6.84 (d, 8)	6.81 (d, 8)	6.86 (d, 8)
6‴					6.85 (dd, 8, 2)	6.78 (dd, 8, 2)	6.86 (dd, 8, 2)
7‴					5.46 (d, 7)	5.11 (d, 7)	5.41 (d, 7)
8‴					3.55 (dt, 7, 6)	3.43 (dt, 7, 6)	3.53 (dt, 7, 6)
9‴					—	—	—

Run at 270.00 MHz or 500.00 MHz in $CDCl_3$ solution. * Obscured by other signals; couplings could not be accurately determined. —, signal could not be determined.

TABLE III. ^{13}C-NMR Chemical Shifts of **1**—**14**

Carbon No.	1	2	2a	2b	2c	2d	3	4	4b	5	6
1	129.7[a]	130.3	136.5	135.8	130.4	132.9[e]	133.8	129.5	135.8	128.2	130.5[k]
2	111.5	111.8	113.3	113.1	111.4	111.1	113.4	111.8	113.2	111.2	111.4
3	146.6[b]	146.5	151.2	151.1	149.0	146.4	150.2	146.7[g]	151.2[i]	146.2	147.1
4	144.4[c]	144.4	138.7	138.6	147.9	143.9	150.4	144.2[h]	138.8	144.2[j]	145.6
5	114.3	114.0	122.6	122.5	112.5	114.2	117.6	114.3	122.6	133.0	133.0
6	122.0	121.9	121.3	121.4	121.3	121.3	122.5	122.1	121.5	117.0	117.1
7	34.5	34.3	34.6	34.3	34.3	39.1	34.9	34.5	34.3	34.7	34.6
8	46.4	46.3	46.3	43.4	46.4	46.5[f]	46.8	46.5	43.4	46.6	46.6
9	178.6	178.5	178.3	177.8	178.4	73.2	178.9	178.7	177.8	178.5	178.7
1′	129.4[a]	129.3	130.3	129.8	130.1	132.2[e]	132.1	128.5	127.7	129.8	128.2
2′	111.0	111.3	111.6	109.7	111.2	111.1	112.8	113.0	111.0	113.6	112.0
3′	146.5[b]	148.8	149.1	149.0[d]	149.0	148.8	148.9	147.0[g]	148.2	147.2	149.0
4′	144.3[c]	147.6	147.9	149.1[d]	147.9	147.3	146.5	145.6	144.5	145.6	147.9

TABLE III. (continued)

Carbon No.	1	2	2a	2b	2c	2d	3	4	4b	5	6
5′	114.0	111.5	112.0	111.2	112.0	111.9	114.7	133.0	131.4	114.6	113.2
6′	121.2	120.4	120.6	118.7	120.5	120.5	121.4	116.5	114.6	121.3	120.6
7′	38.2	37.9	38.0	75.5	38.0	39.1	38.3	38.2	75.5	38.3	38.1
8′	40.9	40.7	41.0	43.7	41.0	46.4[f]	42.0	41.0	44.0	41.3	41.2
9′	71.2	71.0	71.1	68.0	71.0	73.2	71.6	71.2	67.9	71.1	71.2
							(Glc.)				
1″							102.6	131.2	139.0	131.0	131.0[k]
2″							74.6	108.8	110.1	108.7	108.7
3″							77.7	146.7[g]	151.3[i]	146.2	146.6
4″							71.1	144.6[h]	139.8	144.5[j]	144.2
5″							77.6	114.4	122.9	114.3	114.3
6″							62.5	119.1	118.3	119.1	119.2
7″								87.8	88.1	87.9	87.9
8″								53.6	50.5	53.6	53.7
9″								64.0	64.6	64.0	64.0
OMe	55.7	55.8	55.8	55.6	55.8	55.7	56.0	55.9	55.8	55.9	55.9
	55.7	55.8	55.8	55.7	55.8	55.7	56.1	55.9	55.9	55.9	55.9
		55.8	55.8	55.8	55.8	55.8	56.4	56.1	56.2	56.1	56.0
					55.8						
AcO			20.5	20.5							
			168.7	20.8							
				168.8							
				169.6							

Carbon No.	7	8	9	10	11	12	13*	14*
1	129.6	129.4	133.8	129.7	124.0	128.4[q]	129.6[s]	126.8
2	111.7	111.6	113.1	111.7	110.6	113.1	111.0	115.6
3	146.8[l]	146.7[m]	151.2	146.6[o]	146.4[p]	147.1	147.6[t]	146.1
4	144.5	144.6	146.5[n]	144.4	141.4	145.6	144.9[u]	144.6[v]
5	114.2	114.3	120.2	114.2	129.4	132.7	134.3	136.3
6	122.0	122.0	121.3	121.9	122.2	114.3	118.7	125.4
7	34.4	34.6	34.6	34.0	34.5	34.8	35.5	35.0
8	46.3	46.6	46.5	46.3	46.5	46.6	46.9	47.1
9	178.9	178.6	178.5	178.8	178.7	178.5	179.1	179.0
1′	124.9	133.9	129.6	123.9	129.5	128.9[q]	127.0	129.0
2′	108.9	112.4	111.1	110.6	111.2	113.5	115.6	111.9
3′	147.1[l]	151.0	146.7[n]	146.5[o]	146.5[p]	147.1	146.1	147.2[w]
4′	142.8	146.4[m]	144.5	141.3	144.2	145.6	144.3[u]	144.9[v]
5′	129.2	120.6	114.3	129.2	114.6	132.9	136.2	134.3
6′	122.8	121.4	122.3	122.6	121.2	114.3	124.0	118.1
7′	38.0	38.3	38.3	38.0	37.8	38.4	38.4	38.4
8′	41.1	40.9	41.1	40.7	40.9	41.6	42.6	41.5
9′	71.2	71.2	71.3	71.0	71.1	71.2	71.5	71.6
1″	134.2	131.4	131.4	128.7	128.7	131.0[r]	130.1[s]	132.5
2″	109.9	109.4	109.3	110.0	110.6	108.8	110.3	110.8
3″	146.5[l]	146.8[m]	146.7[n]	146.9[o]	146.9[p]	146.7	148.3	148.5[x]
4″	145.1	145.6	145.6	150.6	150.6	144.2	147.1[t]	147.8[w]
5″	114.0	114.3	114.6	114.1	114.0	116.6	114.9	114.9
6″	119.3	120.1	120.6	120.4	121.1	119.2	119.9	119.6
7″	74.5	74.0	74.1	199.2	199.2	87.9	88.3	74.1
8″	50.9	89.1	89.3	47.7	47.6	53.4	54.8	52.3
9″	63.1	61.1	61.2	63.8	63.7	64.0	64.8	63.0
1‴						131.3[r]	132.3	130.3
2‴						108.8	110.9	110.4
3‴						146.7	148.3	148.3[x]
4‴						144.2	147.9[t]	147.6[w]
5‴						117.3	114.6	114.2
6‴						119.2	119.5	119.8
7‴						87.9	74.2	88.2
8‴						53.4	51.5	54.7
9‴						64.0	63.2	64.5
OMe	55.8	55.8	55.9	55.8	55.8	56.0	56.1	56.0
	55.9	55.9	55.9	55.9	55.9	56.0	56.1	56.2
	55.9	55.9	56.0	55.9	55.9	56.1	56.1	56.2
						56.1	56.4	56.4

Run at 67.8 MHz or 125.65 MHz in $CDCl_3$ or $(CD_3)_2CO$* solution. *a*—*x*) Assignment may be interchanged within each column.

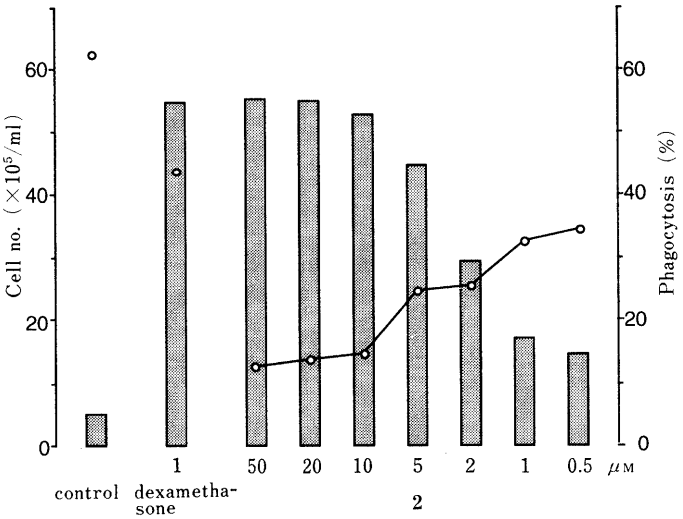

Fig. 1. Relationship between Dose and Differentiation-Inducing Activity of **2** towards M1 cells

——, cell no.; ▨, phagocytosis.

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 ext.) (1 kg) and a hydrophillic fraction (water fr.) (170 g). The ether extract (160 g) was chromatographed on a silica-gel column and HPLC was carried out to give active components **2** (3.5 g) and **6** (700 mg). From the *n*-BuOH ext., active components **1** (600 mg), **4** (1 g), **5** (500 mg), **7** (150 mg), **8** (600 mg), **9** (125 mg), **10** (1.5 g), **11** (1.4 g), **12** (600 mg), **13** (2.5 g) and **14** (2.7 g) were isolated following chromatography on silica-gel and HPLC.

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 1×10^5/ml to 2×10^6/ml in a 5% CO_2 humidified atmosphere at 37 °C. HL-60 cells were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml kanamycin and 2 mmol/l L-glutamine at 37 °C in a humidified 5% CO_5 incubator.

Materials Eagle's MEM, RPMI-1640 medium, Eagle's MEM amino acids and vitamins medium were purchased from Nissui Pharmaceutical Co., Ltd. CS and FBS were from Gibco. Antibiotics were from Meiji Seika Kaisha, Ltd. L-Glutamine was from Wako Pure Chemical Industries, Ltd. Dexamethasone was from Nakarai Chemicals, Ltd. and polystyrene latex particles were from The Dow Chemical Company.

Measurement of Phagocytosis Phagocytic activity was assayed as reported previously.[6,7] Cells were inoculated at a concentration of 2×10^5 cells/ml into 2 ml of culture medium and incubated with 20 μl of sample solution diluted with ethanol. After 48 h, the cells were washed and incubated for 4 h with a suspension of polystyrene latex particles (2 μl/ml of serum-free culture medium). Then the cells were washed thoroughly 3 or 4 times with PBS and the percentage of phagocytic cells calculated.

Arctignan A (**9**): Colorless amorphous powder. $[\alpha]_D$ −25.3° ($c=0.32$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 230 (4.43), 280 (4.02). CD ($c=6.88\times10^{-4}$, MeOH) Δε nm: −9.01 (234). *Anal.* Calcd for $C_{30}H_{34}O_{10}$ $1/2H_2O$: C, 63.93; H, 6.26. Found: C, 63.63; H, 6.24. FAB-MS *m/z*: 555 $[M+H]^+$.

Arctignan B (**10**): Colorless amorphous powder. $[\alpha]_D$ 82.3° ($c=0.84$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 228 (4.51), 281 (4.20), 309 sh (3.96). CD ($c=1.81\times10^{-4}$, MeOH) Δε nm: −9.23 (242), 5.54 (272), 6.00 (310). *Anal.* Calcd for $C_{30}H_{32}O_{10}$: C, 65.21; H, 5.84. Found: C, 64.96; H, 5.93. FAB-MS *m/z*: 553 $[M+H]^+$.

Arctignan C (**11**): Colorless amorphous powder. $[\alpha]_D$ 63.8° ($c=0.91$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 229 (4.48), 281 (4.15), 306 sh (3.88). CD ($c=1.97\times10^{-3}$, MeOH) Δε nm: −8.93 (238), 5.10 (272), 5.53 (310). *Anal.* Calcd for $C_{30}H_{32}O_{10}$ $1/2H_2O$: C, 64.16; H, 5.92. Found: C, 64.02; H, 6.04. FAB-MS *m/z*: 553 $[M+H]^+$.

Arctignan D (**13**): Colorless amorphous powder. $[\alpha]_D$ −37.2° ($c=0.82$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 229 sh (4.54), 282 (4.11). CD ($c=1.77\times10^{-3}$, MeOH) Δε nm: −10.25 (228), 1.75 (294). *Anal.* Calcd for $C_{40}H_{44}O_{13}$ $1/2H_2O$: C, 64.77; H, 6.11. Found: C, 65.01; H, 6.23. FAB-MS *m/z*: 677 $[M-3H_2O]^+$.

Arctignan E (**14**): Colorless amorphous powder. $[\alpha]_D$ −27.0° ($c=0.88$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 230 sh (4.53), 282 (4.10). CD ($c=1.92\times10^{-3}$, MeOH) Δε nm: −6.93 (230), 1.16 (298). *Anal.* Calcd for $C_{40}H_{44}O_{13}$ $1/2H_2O$: C, 64.77; H, 6.11. Found: C, 64.73; H, 6.22. FAB-MS *m/z*: 677 $[M-3H_2O]^+$.

Acetylation of 2 and 4 Both **2** (20 mg) and **4** (20 mg) were acetylated in the usual manner using acetic anhydride and pyridine to give the acetates **2a** (18 mg) and **4a** (17 mg), respectively. They were identified by comparison of ^{1}H-NMR data with reported values.[10,14] ^{13}C-NMR: Table III.

Methylation of 2 Compound 2 (5 mg) was methylated with diazomethane in the usual manner to give **2c** (5 mg). Compound **2c** was identified by comparison of ^{13}C-NMR data with reported values.[8] ^{13}C-NMR: Table III.

Acetoxylation of 2a and 4a[15] Compounds of **2a** (10 mg) and **4a** (10 mg) were dissolved in 0.5 ml AcOH and treated with an equal mass of Pb $(OAc)_4$ at 70—80 °C for 5 h. The mixture was poured into ice-cold water and extracted with Et_2O. The Et_2O soln. was washed with 10% $NaHCO_3$ soln. and then water and purified by HPLC to give **2b** (6 mg) and **4b** (5 mg) as amorphous powders. **2b** was identified by comparison of ^{1}H-NMR data with reported values.[14] ^{13}C-NMR: Table III. **4b**: Amorphous powder. $[\alpha]_D$ 46.0° ($c=0.33$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 222 sh (4.65), 280 (4.04). ^{1}H-NMR ($CDCl_3$) δ: 6.61 (1H, d, $J=2$, H-2), 6.89 (1H, d, $J=8$, H-5), 6.51 (1H, dd, $J=8$, 2, H-6), 6.61, 6.69 (each 1H, d, $J=2$, H-2′ and 6′), 5.53 (1H, d, $J=7.5$, H-7′), 7.02 (1H, d, $J=2$, H-2″), 7.02 (1H, d, $J=8$, H-5″), 6.96 (1H, dd, $J=8$, 2, H-6″), 5.59 (1H, d, $J=7$, H-7″), 2.00, 2.08, 2.26, 2.31 (each 3H, s, AcO–), 3.75, 3.82, 3.87 (each 3H, s, CH_3O–). ^{13}C-NMR: Table III.

Reduction of 2[16] Compound **2** (10 mg) was dissolved in 1.5 ml of tetrahydrofuran and treated with an equal weight of $LiAlH_4$ at room temperature for 4 h. Then the reactant was poured into ice-cold water, carefully acidified with 10% H_2SO_4. And then extracted with Et_2O. The ether extract was purified by HPLC to give **2d** (5 mg) as an amorphous powder. $[\alpha]_D$ −53.2° ($c=0.31$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 225 (4.45), 281 (3.92). ^{1}H- and ^{13}C-NMR: Tables II and III.

Acknowledgement We thank the staff of the Central Analytical Laboratory of this university for elemental analyses and measurement of MS.

References

1) S. J. Collins, F. W. Ruscetti, R. E. Gallagher, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 2458 (1978).
2) Y. Honma, K. Takenaga, T. Kasukabe, M. Hozumi, *Biochem. Biophys. Res. Commun.*, **95**, 507 (1980).
3) L. Sachs, *Nature* (London), **274**, 535 (1978).
4) K. Takeda, J. Minowada, A. Bloch, *Cancer Res.*, **42**, 5152 (1982).
5) E. Sariban, T. Mitchell, D. Kufe, *Nature* (London), **316**, 64 (1985).
6) K. Umehara, R. Takagi, M. Kuroyanagi, A. Ueno, T. Taki, Y. J. Chen, *Chem. Pharm. Bull.*, **40**, 401 (1992).
7) S. Sugiyama, K. Umehara, M. Kuroyanagi, A. Ueno, T. Taki, *Chem. Pharm. Bull.*, **41**, 714 (1993).
8) S. Nishibe, K. Okabe, S. Hisada, *Chem. Pharm. Bull.*, **29**, 2078 (1981).
9) A. Ichihara, Y. Numata, S. Kanai, S. Sakamura, *Agric. Biol. Chem.*, **41**, 1813 (1977).
10) A. Ichihara, K. Oda, Y. Numata, S. Sakamura, *Tetrahedron Lett.*, **1976**, 3961.
11) A. Ichihara, S. Kanai, Y. Nakamura, S. Sakamura, *Tetrahedron Lett.*, **1978**, 3035.
12) A. Ichihara, Y. Nakamura, H. Kawagishi, S. Sakamura, *Tetrahedron Lett.*, **1979**, 3735.
13) H. Suzuki, K. H. Lee, M. Haruna, T. Iida, K. Ito, H. C. Huang, *Phytochemistry*, **21**, 1824 (1982).
14) H. Achenbach, J. Gro, X. A. Dominguez, G. Cano, J. V. Star, L. del C. Brussolo, G. Munoz, F. Salgado, L. Lopez, *Phytochemistry*, **26**, 1159 (1987).
15) S. Nishibe, M. Chiba, A. Sakushima, S. Hisada, S. Yamanouchi, M. Takido, U. Sankawa, A. Sakakibara, *Chem. Pharm. Bull.*, **28**, 850 (1980).
16) S. Nishibe, H. Tsukamoto, S. Hisada, S. Yamanouchi, M. Takido, *Chem. Pharm. Bull.*, **29**, 2082 (1981).