Studies on Differentiation Inducers. IV.[1] Pregnane Derivatives from Condurango Cortex

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In connection with the study of differentiation inducers from plants, the methanol extract of Condurango Cortex (bark of *Marsdenia condurango* REICH, Asclepiadaceae) was investigated to examine its differentiation-inducing activity towards mouse myeloid leukemia (M1) cell line. Six pregnane glycosides, including three new compounds, were isolated as differentiation inducers. Each of the six active glycosides has three or four deoxylated sugars which are well-known to occur in Asclepiadaceae plants. M1 cells were differentiated into phagocytic cells by these glycosides, and they were found to be more effective than their aglycones. Condurangoglycosides A (7) and C (8), having a cinnamoyl group in their aglycones, were the most potent differentiation inducers and M1 cells became phagocytic cells after 24 h treatment with these compounds.

Keywords differentiation; pregnane glycoside; *Marsdenia condurango*; condurangoside; M1 cell; Asclepiadaceae

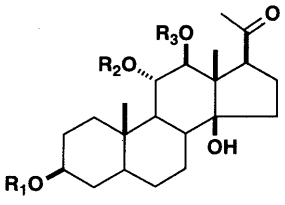
Differentiation inducers are of potential interest for the treatment of human cancers promoting the terminal differentiation of certain human tumor cells.[2–4] We have reported the differentiation-inducing activities of triterpenes, flavones and lignans. These compounds differentiated mouse myeloid leukemia (M1) cells into phagocytic cells[1,5] and some of them also induced the differentiation of human acute promyelocytic leukemia (HL-60) cells.[5] In the course of our study, the methanolic extract of Condurango Cortex (bark of *Marsdenia condurango* REICH, Asclepiadaceae) was found to have differentiation-inducing activity. The active components of the extract were investigated.

The suspension of the methanolic extract of Condurango Cortex in water was extracted with AcOEt. The water layer was passed through HP-20 and the absorbed material was eluted with 50% aqueous methanol, 75% aqueous methanol and 100% methanol, successively. The 100% methanol eluate showed differentiation-inducing activity toward M1 cells and was chromatographed on a silica-gel column and subjected high-performance liquid chromatography (HPLC) to afford active component **1** with a new pregnane glycoside **11**. Notice was taken of the existence of other minor active substances in this fraction. M1 cells were also induced to become phagocytic cells following treatment with the AcOEt layer, then the AcOEt layer was chromatographed on a silica-gel column followed by HPLC to give active compounds (**3**, **4**, **7**, **8**, **13**) with non-active pregnane glycosides (**2**, **5**, **9**, **10**, **12**). The structures of these glycosides are shown in Chart 1. Six known compounds (**7**—**10**, **12**, **13**) , which had been isolated from *Marsdenia condurango* R. previously, were identified by comparison with reported data.[6–8] The structures of the seven new compounds were deduced from spectral evidence to be condurangosides A (**1**), A_0 (**2**), B (**3**), C (**4**), B_0 (**5**), C_0 (**6**) and D_{01} (**11**).

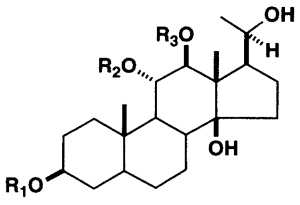
Condurangoside A (**1**), $C_{46}H_{74}O_{17}$, $[\alpha]_D$ 24.5° was obtained as an amorphous powder. ^{1}H-NMR spectrum of **1** showed two singlet methyls (δ 0.89, 0.99), three doublet methyls (δ 1.20, 1.23, 1.32), three acetyl methyls (δ 1.92, 2.07, 2.15), three methoxyl signals (δ 3.37, 3.42, 3.65) and two carbinyl protons [δ 4.69 (d, J=10 Hz), 5.18 (t, J=10 Hz)] (Table II). ^{13}C-NMR spectrum indicated that **1** was a pregnane glycoside having D-cymarose, D-oleandrose and 6-deoxy-3-*O*-methyl-D-allose(6-deoxy-3-OMe-D-allo.) as the sugar moiety (Table III). From the ^{1}H detected heteronuclear multiple bond connectivity (HMBC) spectrum of **1**, C–H long range coupling was observed between the H-1 of 6-deoxy-3-OMe-D-allo. and the C-4 of D-oleandrose, the H-1 of D-oleandrose and the C-4 of D-cymarose and the H-1 of D-cymarose and the C-3 of aglycone. In addition this sugar chain was shown to be identical to that of condurangoglycoside A (Table IV). After acid hydrolysis, compound **1** afforded a new aglycone named gagaimogenin A (**1a**). Compound **1a**, $C_{25}H_{38}O_7$, $[\alpha]_D$ 48.1°, was obtained as an amorphous powder. Deacylcondurangogenin A had been isolated by Hayashi *et al.* from aglycone mixtures of these plants,[6] and the structure of **1a** was assigned as the 11,12-diacetate of deacylcondurangogenin A by measuring its ^{1}H-, ^{13}C-NMR and HMBC spectra. These data suggested the structure of **1** to be gagaimogenin A 3-*O*-[6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1—4)-β-D-oleandropyranosyl-(1—4)-β-D-cymaropyranoside] as shown in Chart 1.

Condurangoside A_0 (**2**), $C_{52}H_{84}O_{22}$, $[\alpha]_D$ 11.4° was obtained as an amorphous powder. Acid hydrolysis of **2** gave **1a** as the aglycone moiety. From the ^{1}H- and ^{13}C-NMR spectra of **2**, the sugar moiety of **2** was found to be identical to that of condurangoglycoside A_0. Furthermore, the long-range couplings were observed by measuring the HMBC spectrum of **2** between the H-1 of D-glc and the C-4 of 6-deoxy-3-OMe-D-allo., the H-1 of 6-deoxy-3-OMe-D-allo. and the C-4 of D-oleandrose, the H-1 of D-oleandrose and the C-4 of D-cymarose and the H-1 of cymarose and the C-3 of aglycone. These data led us to conclude that the structure of **2** was gagaimogenin A 3-*O*-[β-D-glucopyranosyl-(1—4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1—4)-β-D-oleandropyranosyl-(1—4)-β-D-cymaropyranoside] as shown in Chart 1.

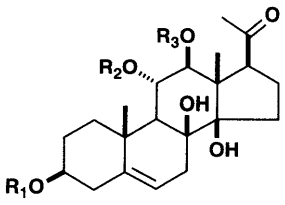
Condurangoside B (**3**), $C_{51}H_{76}O_{17}$, $[\alpha]_D$ 44.8° and



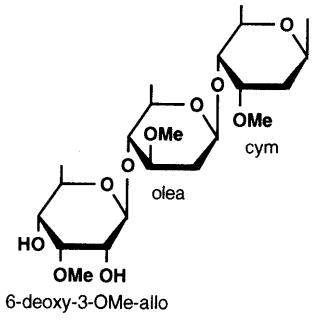
	R_1	R_2	R_3
1	SA	A	A
2	SC	A	A
3	SA	B	A
5	SC	B	A
7	SA	A	C
9	SC	A	C



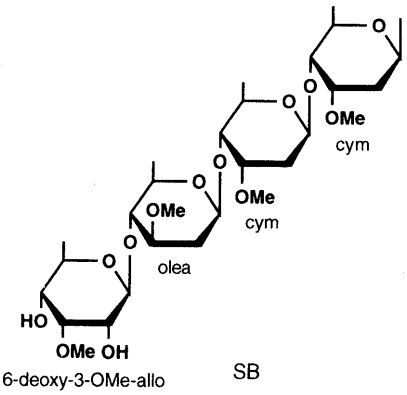
	R_1	R_2	R_3
4	SA	A	B
6	SC	A	B
8	SA	A	C
10	SC	A	C



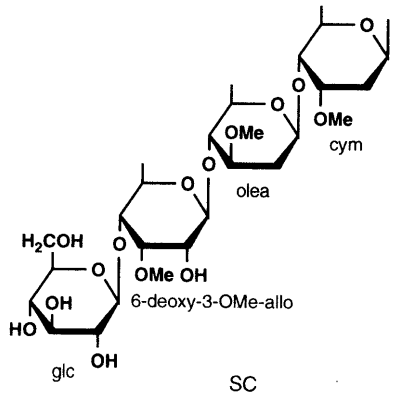
	R_1	R_2	R_3
11	SD	H	H
12	SD	A	C
13	SB	A	C



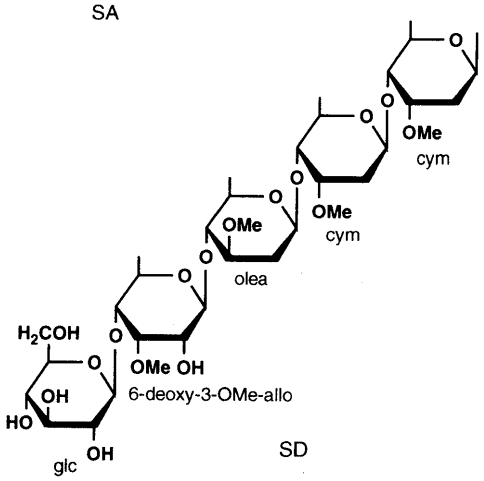
SA



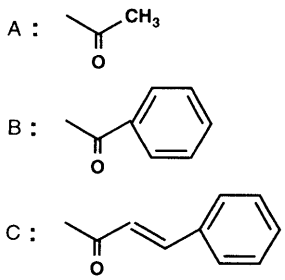
SB



SC



SD



cym, D-cymarose; olea, D-oleandrose

6-deoxy-3-OMe-allo, 6-deoxy-3-*O*-methyl-D-allose

glc, D-glucose

Chart 1

condurangoside B_0 (**5**), $C_{57}H_{86}O_{22}$, $[\alpha]_D$ 25.8 were both obtained as an amorphous powder. Acid hydrolysis of **3** and **5** gave the same new aglycone named gagaimogenin B (**3a**). Compound **3a**, $C_{30}H_{40}O_7$, $[\alpha]_D$ 31.3° was obtained as an amorphous powder and its ^{1}H-NMR spectrum showed two singlet methyls (δ 1.00, 1.09), two acetyl methyls (δ 1.74, 2.17), two carbinyl protons [δ 4.88 (d, J=11 Hz), 5.54 (t, J=11 Hz)] and benzoyl group signals [δ 7.42(2H, t, J=8 Hz), 7.57 (2H, tt, J=8, 2 Hz), 7.97 (2H, dd, J=8, 2 Hz)]. In the long-range selective proton decoupling (LSPD) spectrum of **3a**, long-range coupling was observed between the carbonyl group of benzoyl moiety (δ 166.3) and the H-11 [δ 5.54 (t, J=11 Hz)]. From these facts, the structure of **3a** was concluded to be 11-*O*-benzoyl-12-*O*-acetyl-deacylcondurangogenin A. By measuring the ^{13}C-NMR spectra of **3** and **5**, the sugar moieties of these compounds were found to be identical with those of **1** and **2**, respectively. The HMBC spectrum of these compounds showed that these structures were correct. From these data, the structure of **3** and **5** were concluded to be gagaimogenin B 3-*O*-[6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1—4)-β-D-oleandropyranosyl-(1—4)-β-D-cymaropyranoside] (**3**) and gagaimogenin B 3-*O*-[β-D-glucopyranosyl-(1—4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1—4)-β-D-oleandopyranosyl-(1—4)-β-D-cymaro pyranoside] (**5**) as shown in Chart 1.

Condurangoside C (**4**), $C_{51}H_{78}O_{17}$, $[\alpha]_D$ 34.6° and condurangoside C_0 (**6**), $C_{57}H_{88}O_{22}$, $[\alpha]_D$ 22.6° were both obtained as an amorphous powder. These two compounds gave a new aglycone named gagaimogenin C (**4a**), $C_{30}H_{42}O_7$, $[\alpha]_D$ 31.6°, as an amorphous powder on acid hydrolysis. The ^{1}H-NMR spectrum of **4a** showed two singlet methyls (δ 0.98, 1.40), a doublet methyl (δ 1.13), an acetyl methyl (δ 1.66), two carbinyl protons [δ 4.99 (d, J=10 Hz), 5.41 (d, J=10 Hz)] and benzoyl group signals [δ 7.45 (2H, t, J=8 Hz), 7.87 (1H, tt, J=8, 2 Hz), 8.03 (2H, dd, J=8, 2 Hz)]. In the LSPD spectrum of **4a**, long-range coupling was observed between the carbonyl carbon (δ 166.9) of the benzoyl moiety and the H-12 [δ 4.99 (d, J=10 Hz)]. These data suggested that the structure of **4a** was 11-*O*-acetyl-12-*O*-benzoyldeacylcondurangogenin C. Deacylcondurangogenin C (dihydrodrevogenin D) had been isolated from this plant by Sauer *et al.* and the absolute configuration of the C-20 position was confirmed from its ^{13}C-NMR chemical shifts.[9,10] From the ^{13}C-NMR spectrum of **4** and **6**, the sugar moieties of these compounds were shown to be identical to those of **1** and **2**, respectively. The HMBC spectrum of these compounds showed that these structures were correct. From these data, the structure of **4** and **6** were concluded to be gagaimogenin C 3-*O*-[6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1—4)-β-D-oleandropyranosyl-(1—4)-β-D-cymaropyranoside] (**4**) and gagaimogenin C 3-*O*-[β-D-glucopyranosyl-(1—4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1—4)-β-D-oleandropyranosyl-(1—4)-β-D-cymaropyranoside] (**6**) as shown in Chart 1.

Condurangoside D_{01} (**11**), $C_{55}H_{90}O_{24}$, $[\alpha]_D$ 22.0° was obtained as an amorphous powder. The ^{1}H-NMR spectrum of **11** showed two singlet methyls (δ 1.11, 1.37), four doublet methyls (δ 1.21, 1.22, 1.29, 1.35), an acetyl methyl (δ 2.26) and four methoxyl groups (δ 3.38, 3.44, 3.44, 3.60). In the ^{13}C-NMR spectrum of **11**, olefinic carbon signals (δ 118.6, 140.7), five anomeric carbon signals (δ 96.4, 100.4, 101.9×2, 106.6) and four methoxyl groups (δ 58.9, 58.8, 57.4, 61.7) were observed. These four methoxyl signals were characteristic for D-cymarose, D-cymarose, D-oleandrose and 6-deoxy-3-methoxy-D-allose, respectively. The chemical shifts due to the sugar moiety were basically identical to that of condurangoglycoside E_3,[8] and the same sugar structure was deduced from the HMBC spectrum of **11**. After acid hydrolysis, **11** afforded marsdenin as the aglycone moiety.[6] These data led us to conclude that the structure of **11** was marsdenin 3-*O*-[β-D-glucopyranosyl-(1—4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1—4)-β-D-oleandropyranosyl-(1—4)-β-D-cymaropyranosyl-(1—4)-β-D-cymaropyranoside].

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

Compound[a]	Conc. (μM)	Growth rate (%)	Phagocytic[b] activity
Cont.		100	−
Dex.	1	33	++
1	100	56	+
	50	44	−
2	100	67	−
	50	80	−
3	100	12	++
	50	31	+
4	100	13	++
	50	36	+
5	100	44	−
	50	67	−
6	100	41	−
	50	52	−
7	100	1	+++
	50	19	++
8	100	1	+++
	50	12	+++
9	100	60	−
	50	62	−
10	100	49	−
	50	59	−
11	100	78	−
	50	81	−
12	100	50	−
	50	54	−
13	100	0	uc
	50	12	+

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

TABLE II. ^{1}H-NMR Spectral Data of **1**—**6**, **11**

Proton no.	1	2	3	4	5	6	11
Aglycone moiety							
11	5.18 (t, J=10)	5.20 (t, J=10)	5.49 (t, J=11)	5.39 (t, J=11)	5.52 (t, J=11)	5.34 (t, J=10)	4.14 (t, J=10)
12	4.69 (d, J=10)	4.72 (d, J=10)	4.85 (d, J=11)	4.98 (d, J=11)	4.87 (d, J=11)	4.87 (d, J=10)	3.21 (d, J=10)
18	0.99	1.01	1.04	1.38	1.07	1.31	1.37
19	0.89	0.91	0.95	0.95	0.98	0.93	1.11
21	2.15	2.16	2.19	1.25 (d, J=7)	2.16	1.23 (d, J=7)	2.26
Sugar moiety							
anomeric	4.45 (dd, J=10, 2)	4.33 (d, J=7)	4.42 (dd, J=11, 2)	4.47 (dd, J=11, 2)	4.33 (d, J=7)	4.35 (d, J=7)	4.38 (d, J=7)
	4.76 (d, J=8)	4.45 (dd, J=10, 2)	4.75 (d, 8)	4.78 (d, J=8)	4.43 (dd, J=10, 2)	4.47 (dd, J=10, 2)	4.47 (d, J=10)
	4.80 (dd, J=10, 2)	4.77 (d, J=9)	4.77 (dd, J=11, 2)	4.83 (dd, J=11, 2)	4.74 (d, J=8)	4.78 (d, J=9)	4.75 (d, J=7)
		4.81 (dd, J=10, 2)			4.78 (dd, J=10, 2)	4.83 (dd, J=10, 2)	4.77 (d, J=9)
							4.85 (d, J=9)
OCH_3	3.37	3.37	3.33	3.38	3.35	3.34	3.38
	3.42	3.42	3.38	3.42	3.41	3.41	3.44×2
	3.65	3.58	3.62	3.65	3.55	3.67	3.60
6-CH_3	1.20 (d, J=6)	1.22 (d, J=6)	1.14 (d, J=7)	1.12 (d, J=7)	1.16 (d, J=7)	1.17 (d, J=6)	1.21 (d, J=6)
	1.23 (d, J=6)	1.27 (d, J=6)	1.22 (d, J=7)	1.22 (d, J=7)	1.24 (d, J=7)	1.20 (d, J=6)	1.22 (d, J=6)
	1.32 (d, J=6)	1.33 (d, J=6)	1.31 (d, J=7)	1.34 (d, J=5)	1.30 (d, J=6)	1.34 (d, J=5)	1.29 (d, J=6)
							1.35 (d, J=6)

Recorded at 270.00 or 500.0 MHz in $CDCl_3$.

Table III. ^{13}C-NMR Spectral Data of **1**—**6**, **11**

Carbon no.	**1**	**2**	**3**	**4**	**5**	**6**	**11**
Aglycone moiety							
1	37.3	37.3	37.2	37.4	37.6	37.4	39.9
2	30.4	30.4	30.3	30.5	30.3	30.5	30.4
3	76.1	76.1	76.0	76.4	76.1	76.4	78.2
4	35.4	35.4	35.4	35.7	35.6	35.7	40.9
5	44.6	44.6	44.7	45.0	44.9	45.0	140.7
6	29.4	29.4	29.4	29.6	29.5	29.6	118.6
7	28.4	28.4	28.5	28.5	28.5	28.5	37.0
8	39.8	39.8	40.2	40.3	40.3	40.3	76.2
9	50.1	50.1	50.3	50.6	50.5	50.6	50.5
10	37.9	37.9	38.1	38.2	38.4	38.2	39.5
11	71.6	71.6	72.5	72.1	72.6	72.1	71.9
12	78.3	78.4	78.3	80.5	78.4	80.5	79.1
13	54.6	54.6	54.8	54.2	54.8	54.2	56.2
14	83.8	83.8	84.0	83.9	84.0	83.6	85.9
15	33.7	33.7	33.9	33.2	34.1	33.2	36.0
16	24.2	24.2	24.3	26.8	24.4	26.8	24.6
17	58.3	58.3	58.3	53.0	58.4	53.0	59.6
18	12.4	12.4	12.4	12.5	12.5	12.5	13.0
19	11.7	11.7	11.7	12.5	11.6	12.5	17.7
20	213.4	213.4	213.4	70.4	213.7	70.4	216.1
21	31.7	31.7	31.7	23.6	31.8	23.6	32.1
Ester moiety							
11-$\underline{CH_3}CO$	21.5	21.5		21.4		21.4	
11-$CH_3\underline{CO}$	170.4	170.4		170.3		170.3	
12-$\underline{CH_3}CO$	20.8	20.8	20.6		20.5		
12-$CH_3\underline{CO}$	171.0	171.0	170.9		170.9		
CO			166.3	166.9	166.3	166.9	
C-1′			130.7	131.0	130.9	131.0	
-2′,6′			129.1	128.9	129.0	128.9	
-3′,5′			130.1	130.1	130.1	130.1	
-4′			133.7	133.4	133.6	133.4	
Sugar moiety							
1	96.0	96.0	95.9	96.2	96.1	96.2	96.4
2	37.6	37.3	37.6	37.5	37.4	37.6	37.3
3	77.9	77.9	77.8	78.0	77.9	78.0	78.1
4	82.9	83.0	82.9	82.7	82.8	82.8	83.0
5	68.9	68.9	68.8	69.0	68.9	69.0	69.0
6	18.7	18.3	18.6	18.7	18.2	18.2	18.3
OMe	58.8	58.8	58.8	58.8	58.8	58.8	58.9
1	101.9	101.9	101.9	101.7	101.7	101.7	100.4
2	38.0	38.0	38.1	38.1	38.1	38.1	37.2
3	79.3	79.3	79.2	79.3	79.4	79.4	77.7
4	83.8	83.5	83.9	83.5	83.4	83.5	83.1
5	72.0	71.9	72.0	72.1	72.2	72.2	68.9
6	18.7	18.7	18.6	18.5	18.6	18.7	18.5
OMe	57.2	57.5	57.2	57.0	57.2	57.2	58.8
1	102.0	101.9	102.0	101.9	101.8	101.9	101.9
2	73.2	72.7	73.2	73.3	72.7	72.7	37.7
3	83.5	83.3	83.4	83.6	83.4	83.4	79.3
4	74.6	83.2	74.6	74.6	83.1	83.1	83.4
5	71.0	69.5	71.0	71.1	69.6	69.6	72.0
6	18.9	18.9	18.9	18.9	18.9	18.9	18.6
OMe	62.1	61.7	62.1	62.0	61.6	61.7	57.4
1		106.6			106.5	106.5	101.9
2		75.5			75.5	75.5	72.7
3		78.4			78.3	78.4	83.3
4		72.0			72.0	72.1	83.2
5		78.4			78.2	78.2	69.5
6		63.0			63.2	63.2	18.9
OMe							61.7
1							106.6
2							75.5
3							78.4
4							72.0
5							78.4
6							63.0

Recorded at 67.8 or 125.65 MHz in C_5D_5N.

Table IV. Long-Range Connectivities by HMBC of Compound **1** in $CDCl_3$

^{1}H-signals	Cross peaks
Aglycone moiety	
0.89 (H-19)	37.6 (C-1), 37.6 (C-10), 44.6 (C-5), 49.9 (C-9)
1.00 (H-18)	54.0 (C-13), 57.1 (C-17), 77.7 (C-12), 83.7 (C-14)
4.70 (H-12)	10.7 (C-18), 54.0 (C-13), 57.1 (C-17), 71.2 (C-11), 171.0 (Me$\underline{C}$O)
5.19 (H-11)	49.9 (C-9), 77.7 (C-12), 170.5 (Me$\underline{C}$O)
Sugar moiety	
1.20 (cym-6)	68.2 (cym-5), 82.7 (cym-4)
1.23 (allo-6)	71.4 (allo-5), 72.8 (allo-4)
1.32 (olea-6)	71.2 (olea-3), 72.0 (olea-5), 79.0 (olea-4)
1.50 (olea-2)	78.6 (olea-3), 101.1 (olea-1)
1.55 (cym-2)	81.0 (cym-3), 95.6 (cym-1)
2.36 (olea-2)	78.6 (olea-3), 101.1 (olea-1)
3.20 (cym-4)	18.2 (cym-6), 68.2 (cym-5)
3.37 (olea-OMe)	78.6 (olea-3)
3.42 (allo-OMe)	77.0 (allo-3)
3.65 (cym-OMe)	81.0 (cym-3)
3.78 (allo-3)	71.4 (allo-5), 71.8 (allo-2), 72.8 (allo-4)
3.85 (cym-5)	18.2 (cym-6), 82.7 (cym-4)
4.46 (olea-1)	82.7 (cym-4)
4.78 (allo-1)	79.0 (olea-4)
4.81 (cym-1)	76.4 (C-3)

The differentiation-inducing activities of pregnane glycosides (**1**—**13**) are indicated in Table I. Six compounds (**1**, **3**, **4**, **7**, **8**, **13**) were able to differentiate M1 cells into phagocytic cells. These thirteen compounds could be classified into three groups by their aglycone moiety, that is a) deacylcondurangogenin A glycosides (**1**, **2**, **3**, **5**, **7**, **9**), b) deacylcondurangogenin C glycosides (**4**, **6**, **8**, **10**) and c) marsdenin glycosides (**11**, **12**, **13**). However, the activities of these compounds were affected by their sugar moieties rather than their aglycone moieties. The six active compounds were trisaccharides (**1**, **3**, **4**, **7**, **8**) or a tetrasaccharide (**13**) having 6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1—4)-*β*-D-oleandropyranosyl-(1—4)-*β*-D-cymaropyranoside in their structures. The seven non-active compounds (**2**, **5**, **6**, **9**—**12**) were tetrasaccharides or a pentasaccharide having *β*-D-glucose at the terminal end of their sugar moieties. In the previous paper,[1,5] we reported the inability of glucosides of triterpenes, flavones and lignans to induce the differentiation of both M1 and HL-60 cells. Glucosidation of the active compounds appeared to result in failure to induce cell differentiation.

The pregnane glycosides have acyl moieties such as acetyl, benzoyl and cinnamoyl at C-11, 12. Benzoates were more effective than acetates, but less effective than cinnamates. Furthermore, cinnamates (**7**, **8**, **13**) also showed high cytotoxicity toward M1 cells.

The M1 cells were incubated in the presence of the six pregnane glycosides (**1**, **3**, **4**, **7**, **8**, **13**) and examined for signs of cell proliferation and differentiation at intervals of 24 to 72 h (Fig. 1). The phagocytic activities of cells treated with pregnane glycosides were scarcely affected by the incubation times, while those in the group treated with dexamethasone became highly dependent on the incubation time. Cinnamates (**7**, **8**, **13**) showed high activities at a concentration of 50 μM for a 24 h incubation. The groups treated with benzoate (**3**, **4**) for 72 h were less effective

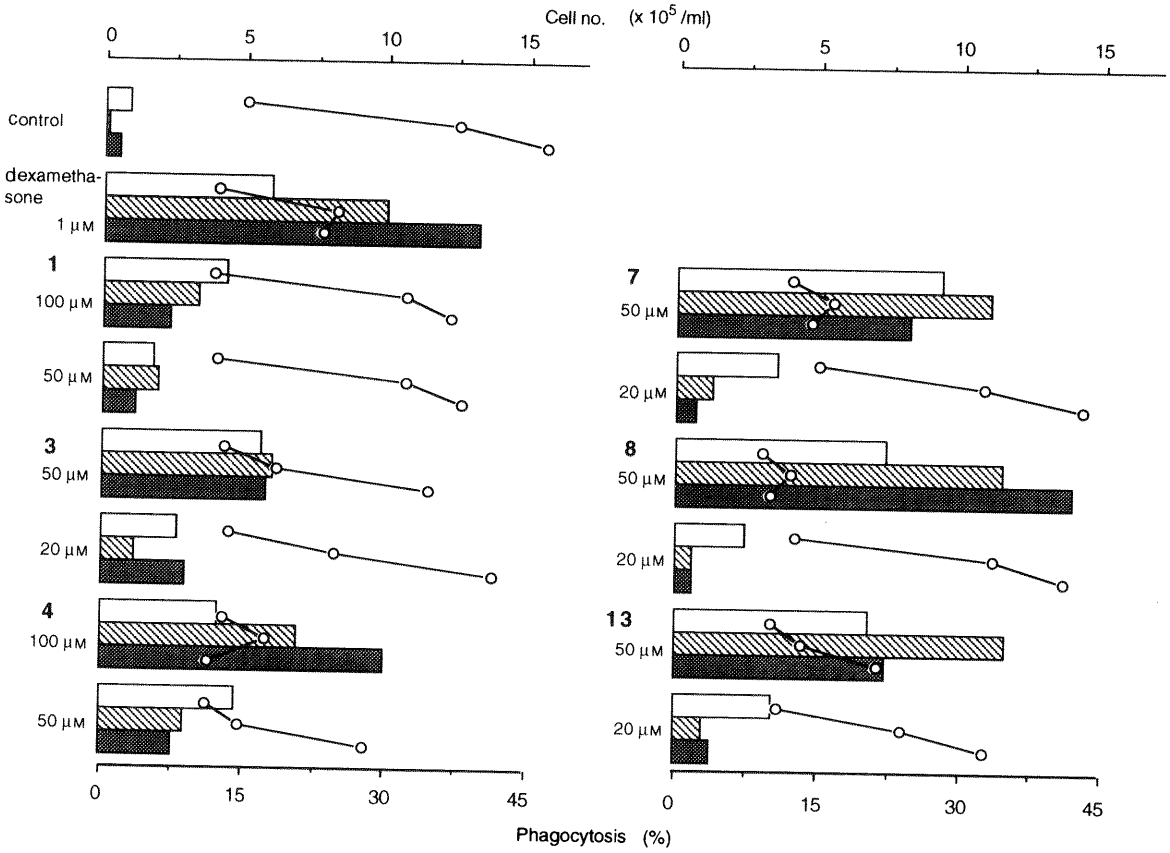


Fig. 1. Time Course of Cell Growth and Phagocytosis of M1 Cells Treated with Pregnane Glycosides from Condurango Cortex

M1 cells were incubated in the presence of the indicated compounds at various concentrations and assessed for phagocytic activity at intervals of 24 to 72 h. Each point represents the mean cell no. of at least 3 determinations. □, phagocytosis exhibited by 24 h treated groups; ▨, phagocytosis exhibited by 48 h treated groups; ■, phagocytosis exhibited by 72 h treated groups.

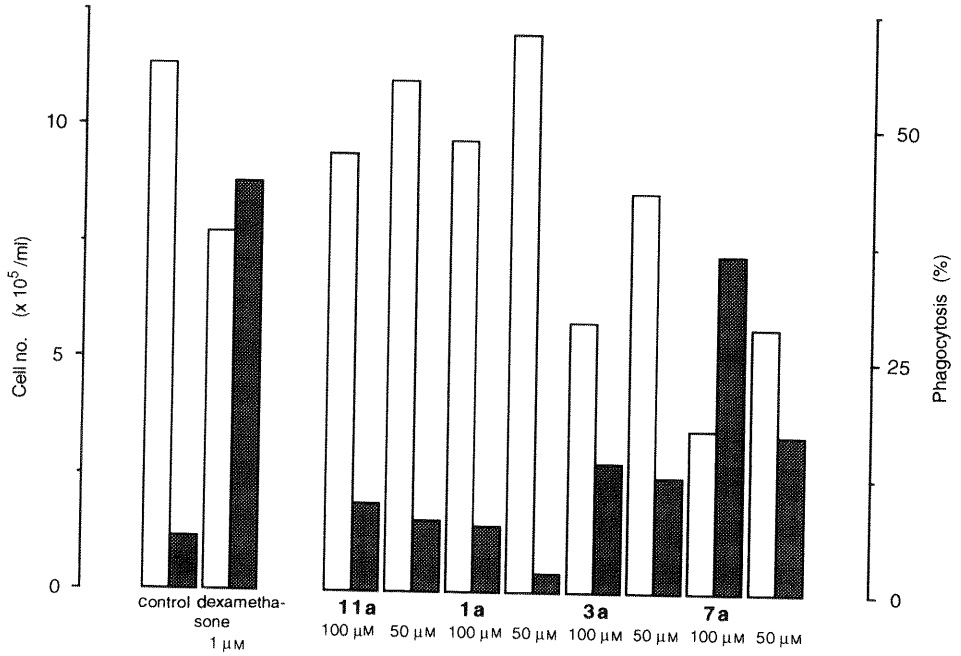


Fig. 2. Cell Growth and Phagocytosis of M1 Cells Treated with Pregnanes from Condurango Cortex

Cells were treated with various concentrations of pregnanes for 48 h. Then cell proliferation and phagocytic activity was determined as described under Experimental. □, cell number; ▨, phagocytic activity.

than those treated with cinnamate for 24 h. However, the groups treated with benzoate for 24 h were more effective than the one treated with acetate (**1**) for 72 h, as shown in Fig. 1. More hydrophobic acyl moieties led to superior differentiation-inducing activities as observed in the five compounds (**1**, **3**, **4**, **7**, **8**) which have the same sugar chain.

As shown in Fig. 2, aglycones (**1a**, **3a**, **7a**, **11a**) were tested for their differentiation-inducing activity towards M1 cells. Compounds **1a** and **11a** showed no activity even at a concentration of 100 μM. Both **3a** and **7a** were more active in inducing cell differentiation than **1a** and **11a**, but they exhibited only half the activity of their glycosides. In these compounds, the sugar chains seemed to play an important role in determining activity but the details of this are not unknown. Further investigation is required.

Experimental

General Procedure Optical rotations were measured with a JASCO DIP-360 digital polarimeter. Ultraviolet (UV) spectra were recorded on a Hitachi U3410 spectrophotometer. Mass spectra (MS) were recorded on a JEOL MS-SX 102 mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL JNM-GSX 270 and JNM-GSX 500 spectrometers (270.05, 67.8 MHz; 500.00, 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 performed on a JASCO model 800 series instrument equipped with Pro-10 Zorbax and D-ODS-7 YMC columns.

Isolation Commercially available Condurango Cortex (3 kg from Niiya in Shimizu) was extracted with hot MeOH under reflux. The extract was partitioned between AcOEt and water. The water layer (135 g) was passed through a Diaion HP-20 column. After washing the column with water, the absorbed material was eluted with 50% MeOH, 75% MeOH and 100% MeOH successively to give a brown gum (50% MeOH eluate 15 g, 75% MeOH eluate 13 g, 100% MeOH eluate 12 g). The 100% MeOH eluate was chromatographed on a silica-gel column using chloroform–MeOH as mobile phase to give fractions 1—7. From fraction 3, pregnane glycosides were isolated by HPLC: **1** (135 mg), **11** (128 mg). The AcOEt layer (204 g) was chromatographed on a silica-gel column using chloroform–MeOH as mobile phase to give fractions 1—5. From fractions 3 and 4, pregnane glycosides were isolated by HPLC: **2** (2 g), **3** (770 mg), **4** (350 mg), **5** (2.3 g), **6** (1.7 g), **7** (910 mg), **8** (910 mg), **9** (5.5 g), **10** (3.6 g), **12** (1.9 g), **13** (520 mg). Compounds **7**—**10**, **12** and **13** were identified by comparison with published data.

Condurangoside A (**1**): Colorless amorphous powder. $[\alpha]_D$ 21.5° (c=1.0, MeOH), 24.5° (c= 1.0, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 212 (3.03), 216 (2.77), 272 (2.27). *Anal.* Calcd for $C_{46}H_{74}O_{17}\cdot 3H_2O$: C, 61.45; H, 8.30. Found: C, 61.19; H, 8.27. FAB-MS m/z: 921 $[M+Na]^+$. ^{1}H-NMR: Table II and ^{13}C-NMR: Table III.

Condurangoside A_0 (**2**): Colorless amorphous powder. $[\alpha]_D$ 21.0° (c=1.0, MeOH), 11.4° (c=1.0, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 217 (3.47), 278 (3.27). *Anal.* Calcd for $C_{52}H_{84}O_{22}\cdot 3H_2O$: C, 56.00; H, 8.13. Found: C, 56.00; H, 8.06. FAB-MS m/z 1084 $[M+Na]^+$. ^{1}H-NMR: Table II and ^{13}C-NMR: Table III.

Condurangoside B (**3**): Colorless amorphous powder. $[\alpha]_D$ 31.0° (c=0.5, MeOH), 44.8° (c=1.0, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 231 (3.59), 274 (3.21), 281 (3.20). *Anal.* Calcd for $C_{51}H_{76}O_{17}\cdot H_2O$: C, 62.56; H, 8.03. Found: C, 62.80; H, 7.97. FAB-MS m/z: 984 $[M+Na]^+$. ^{1}H-NMR: Table II and ^{13}C-NMR: Table III.

Condurangoside C (**4**): Colorless amorphous powder. $[\alpha]_D$ 32.0° (c=1.0, MeOH), 34.6° (c=1.0, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 224 (4.03), 281 (3.67). *Anal.* Calcd for $C_{51}H_{78}O_{17}\cdot H_2O$: C, 62.43; H, 8.22. Found: C, 62.49; H, 8.09. FAB-MS m/z: 986 $[M+Na]^+$. ^{1}H-NMR: Table II and ^{13}C-NMR: Table III.

Condurangoside B_0 (**5**): Colorless amorphous powder. $[\alpha]_D$ 35.5° (c=1.0, MeOH), 25.8° (c=1.0, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 224 (3.97), 256 (3.58), 262 (3.59), 280 (3.61). *Anal.* Calcd for $C_{57}H_{86}O_{22}\cdot 3H_2O$: C, 58.15; H, 7.86. Found: C, 58.26; H, 7.77. FAB-MS m/z: 1146 $[M+Na]^+$. ^{1}H-NMR: Table II and ^{13}C-NMR: Table III.

Condurangoside C_0 (**6**): Colorless amorphous powder. $[\alpha]_D$ 33.5° (c=1.0, MeOH), 22.6° (c=1.0, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 223 (4.03), 279 (3.79). *Anal.* Calcd for $C_{57}H_{88}O_{22}\cdot 9/2H_2O$: C, 56.75; H, 8.10. Found: C, 56.70; H, 7.86. FAB-MS m/z: 1148 $[M+Na]^+$. ^{1}H-NMR: Table II and ^{13}C-NMR: Table III.

Condurangoside D_{01} (**11**): Colorless amorphous powder. $[\alpha]_D$ 22.0° (c=1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε): 263.5 (2.49). *Anal.* Calcd for $C_{55}H_{90}O_{24}\cdot 2H_2O$: C, 56.40; H, 8.09. Found: C, 56.12; H, 7.72. FAB-MS m/z: 1158 $[M+Na]^+$. ^{1}H-NMR: Table II and ^{13}C-NMR: Table III.

Acid Hydrolysis of Glycosides A solution of **1** (60 mg) in dioxan (15 ml) was allowed to react with 0.2 N H_2SO_4 (5 ml) at 60°C for 15 min, then H_2O (15 ml) was added and the mixture was concentrated to 20 ml. The solution was kept at 60°C for a further 30 min, and extracted with ether (20 ml). The ether layer was washed with saturated $NaHCO_3$ (5 ml × 3) and saturated NaCl (5 ml × 3), and the solvent evaporated to give a syrup, which was subjected to HPLC chromatograph on a C-8 column to give **1a** (11 mg) as an amorphous powder. Acid hydrolysis of **2**—**6** and **11** was performed as in the case of **1**. Marsdenin (**11a**) was identified by comparison with published data.

Gagaimogenin A (**1a**): Colorless amorphous powder. $[\alpha]_D$ 48.1° (c=0.4, MeOH). UV λ_{max}^{MeOH} nm (log ε): 277.5 (3.75), 216.1 (3.95). *Anal.* Calcd for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.75; H, 8.50. FAB-MS m/z: 473 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, s, H-19), 1.03 (3H, s, H-18), 1.95, 2.10 (each 3H, s, CH_3CO), 2.18 (3H, s, H-21), 3.53 (1H, tt, J=11, 5.5 Hz, H-3), 4.73 (1H, d, J=11 Hz, H-12), 5.23 (1H, t, J=11 Hz, H-11).

Gagaimogenin B (**3a**): Colorless amorphous powder. $[\alpha]_D$ 31.3° (c=0.25, MeOH). UV λ_{max}^{MeOH} nm (log ε): 230.0 (4.06), 273.3 (3.08), 281.3 (2.79). *Anal.* Calcd for $C_{30}H_{40}O_7$: C, 70.29; H, 7.86. Found: C, 70.44; H, 7.83. FAB-MS m/z: 535 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, s, H-19), 1.09 (3H, s, H-18), 1.74 (3H, s, CH_3CO), 2.17 (3H, s, H-21), 3.50 (1H, tt, J=12,6 Hz, H-3), 4.88 (1H, d, J=11 Hz, H-12), 5.54 (1H, t, J=11 Hz, H-11), 7.42 (2H, t, J=8 Hz, benzoyl), 7.57 (1H, tt, J=8, 2 Hz, benzoyl), 7.97 (2H, dd, J=8, 2 Hz, benzoyl).

Gagaimogenin C (**4a**): Colorless amorphous powder. $[\alpha]_D$ 45.4° (c=0.17, MeOH). UV λ_{max}^{MeOH} nm (log ε): 279.2 (3.92), 261.0 (3.89), 256.0 (3.88), 223.7 (4.27). *Anal.* Calcd for $C_{30}H_{42}O_7$: C, 70.01; H, 8.23. Found: C, 70.13; H, 8.21. FAB-MS m/z: 537 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, s, H-19), 1.13 (3H, d, J=7 Hz, H-21), 1.40 (3H, s, H-18), 1.66 (3H, s, CH_3CO), 3.57 (1H, tt, J=11, 5.5 Hz, H-3), 4.99 (1H, d, J=10 Hz, H-12), 5.41 (1H, t, J=10 Hz, H-11), 7.45 (2H, t, J=8 Hz, benzoyl), 7.78 (1H, tt, J=8, 2 Hz, benzoyl), 8.03 (2H, dd, J=8, 2 Hz, benzoyl).

Materials Eagle's MEM, RPMI-1640 medium, Eagle's MEM amino acids and vitamins medium were purchased from Nissui Pharmaceutical Co., Ltd. Dexamethasone was from Nakarai Chemicals, Ltd. and polystyrene latex particles were from The Dow Chemical Company.

Cell Culture M1 cells were grown in Eagle's MEM medium containing 10% heat-inactivated calf serum in a 5% CO_2 humidified atmosphere at 37°C and diluted when the cell density reached approximately 2×10^6 cells per ml.

Measurement of Phagocytosis Phagocytic activity was assayed as reported previously.[1] Cells were inoculated at a concentration of 2×10^5 cells/ml into 2 ml culture medium and incubated with 20 μl 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 serum free medium). Then the cells were washed thoroughly 3 or 4 times with phosphate buffered saline and the percentage of phagocytic cells determined.

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