Cell Differentiation-Inducing Diterpenes from *Andrographis paniculata* NEES

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The methanol extract of the aerial part of *Andrographis paniculata* NEES showed potent cell differentiation-inducing activity on mouse myeloid leukemia (M1) cells. From the ethyl acetate-soluble fraction of the methanol extract, six new diterpenoids of *ent*-labdane type, 14-*epi*-andrographolide (3), isoandrographolide (4), 14-deoxy-12-methoxyandrographolide (7), 12-*epi*-14-deoxy-12-methoxyandrographolide (8), 14-deoxy-12-hydroxyandrographolide (9) and 14-deoxy-11-hydroxyandrographolide (10) as well as two new diterpene glucosides, 14-deoxy-11, 12-didehydroandrographi-side (12) and 6′-acetylneoandrographolide (14), and four new diterpene dimers, bisandrograpolides A (15), B (16), C (17) and D (18), were isolated along with six known compounds. The structures of the diterpenoids were determined by means of spectral methods. Some of these compounds showed potent cell differentiation-inducing activity towards M1 cells.

Keywords *Andrographis paniculata*; Acanthaceae; *ent*-labdane; cell differentiation-inducing activity; andrographolide; diterpene dimer

Although much attention has been given to cell differentiation inducers as new types of anti-tumor agent, only a few studies have been reported on differentiation inducers from plant sources.[1,2] Therefore, we have searched for naturally occurring substances which induce differentiation of leukemia cells. For screening of the differentiation-inducing activity, we used an assay of the inducibility of phagocytosis of polystyrene latex particles by mouse myeloid leukemia (M1) cells.[3] We have reported some triterpenoids, isolated from Caryophylli Flos[4] and *Panax ginseng*, flavonoids[5] from *Citrus* spp. lignanes[6] from Arctii Fructus and steroids[7] from *Physalis alchechengi* as differentiation inducers. Of the many kinds of tested extracts, the methanol extract of the aerial part of *Andrographis paniculata* NEES (Acanthaceae) showed a very bitter taste and had potent differentiation-inducing activity on M1 cells. *A. paniculata* is widely used in traditional medicine in India, southeast Asia and China. From the aerial parts of this plant, androglapholide[8–11] has been isolated as a bitter substance together with several diterpenoids and diterpene glucoside,[11–14] and from its rhizomes several kinds of flavonoids have been isolated.[15] During investigation of the differentiation inducers of *A. paniculata*, we obtained six new diterpenoids with the *ent*-labdane skeleton, 14-*epi*-andrographolide (**3**), isoandrographolide (**4**), 14-deoxy-12-methoxyandrographolide (**7**), 12-*epi*-14-deoxy-12-methoxyandrographolide (**8**), 14-deoxy-12-hydroxyandrographolide (**9**) and 14-deoxy-11-hydroxyandrographolide (**10**) as well as two new diterpene glucosides, 14-deoxy-11,12-didehydroandrographiside (**12**) and 6′-acetylneoandrographolide (**14**), and four new dimers of diterpenoids, bisandrographolide A (**15**), B (**16**), C (**17**) and D (**18**), together with six known compounds, andrographolide (**1**), andrographiside (**2**),[13] 14-deoxyandrographolide (**5**),[16] deoxyandrographiside (**6**),[11,13,14] 14-deoxy-11,12-didehydroandrographolide (**11**)[16] and neoandrograpolide (**13**).[16] This paper deals with the isolation, structure elucidation and differentiation inducing activity of these diterpenoids.

The methanol extract of the aerial parts of the plant was partitioned between ethyl acetate and water. The ethyl acetate layer showed significant differentiation-inducing activity against M1 cells. The ethyl acetate layer was repeatedly chromatographed on a silica gel column and further purified by high-performance liquid chromatography (HPLC) using a reversed-phase column to give eighteen compounds.

Andrographolide (**1**), andrographiside (**2**), 14-deoxyandrographolide (**5**), deoxyandrographolide (**6**), 14-deoxy-11,12-didehydroandrographolide (**11**) and neoandrographolide (**13**) were identified by comparison of their spectral data with reported data. The assignments of the proton nuclear magnetic resonance (^{1}H-NMR) (Table I) and carbon-thirteen nuclear magnetic resonance (^{13}C-NMR) (Table II) data of **1**, **6**, **11**, **13** were unambiguously made on the basis of the ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) and ^{13}C–^{1}H shift correlation spectroscopy (^{13}C–^{1}H COSY). Our assignments of the carbon chemical shifts of **1** showed that the assignments of **1** reported by Abeysekera *et al.*[17] should be revised.

14-*epi*-Andrographolide (**3**) was isolated as colorless plates, mp 225—228 °C, $[\alpha]_D$ −87.8°. The fast atom bombardment mass spectrum (FAB-MS) showed the pseudo molecular ion, $[M+H]^+$, at *m/z* 351, which corresponds to the molecular formula $C_{20}H_{30}O_5$. The infrared (IR) spectrum showed the presence of hydroxyl (3398 cm^{-1}), α,β-unsaturated-γ-lactone (1727, 1673 cm^{-1}) and *exo*-methylene (905 cm^{-1}) groups. The ^{1}H-NMR and ^{13}C-NMR spectra of **3** were closely related to those of **1**, except for the carbon signals at C-13, C-15, C-16 (Tables I and II). These data suggested that **3** was the 14-epimer of **1**.

Isoandrographolide (**4**) was isolated as colorless plates, mp 209—211 °C, $[\alpha]_D$ −47.2°. The FAB-MS of **4** showed

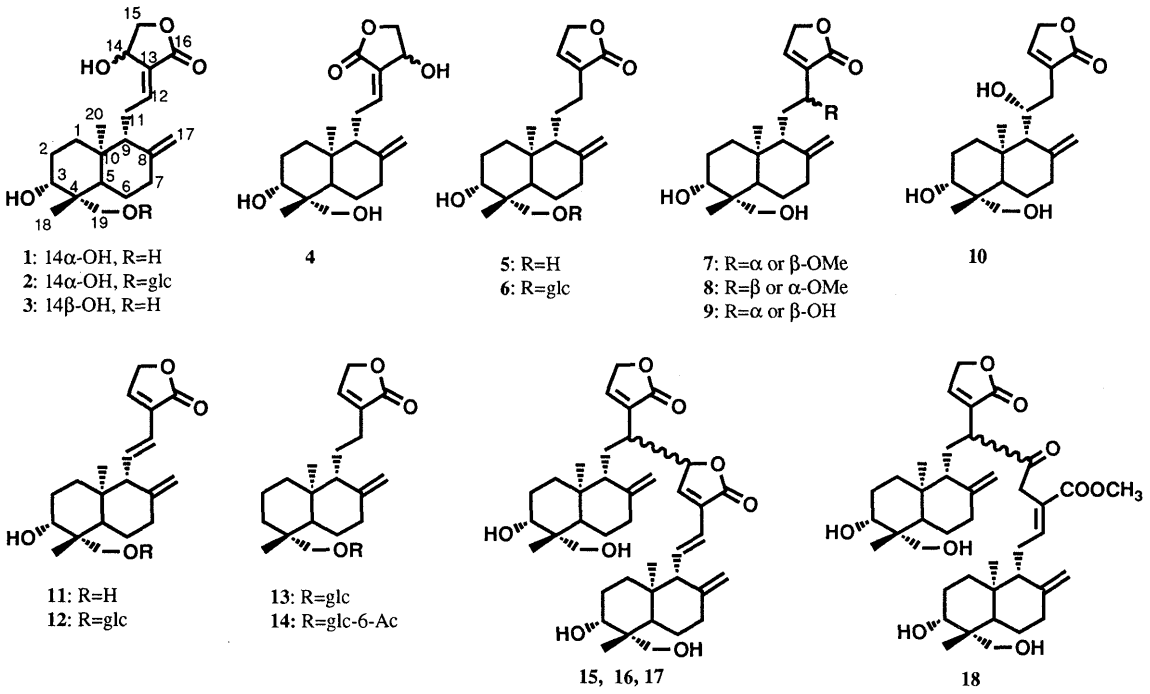

Chart 1

the pseudo molecular ion, $[M+H]^+$ at m/z 351, which corresponds to the molecular formula $C_{20}H_{30}O_5$. The IR spectrum of **4** showed the presence of hydroxyl, α,β-unsaturated-γ-lactone and *exo*-methylene groups. The ^{1}H-NMR and ^{13}C-NMR spectra were similar to those of **1** (Tables I and II). Comparison of the ^{1}H-NMR and ^{13}C-NMR data of **4** with those of **1** showed that the H_2-11 signal was shifted downfield by 0.27 and 0.40 ppm, the H-12 signal was shifted upfield by 0.46 ppm, and the C-14 signal was shifted downfield by 3.0 ppm. These data indicated that **4** was the geometric isomer of **1** or **3** at the $\Delta_{12,13}$ bond. In order to confirm this, a difference nuclear Overhauser effect (NOE) experiment was carried out. When H-12 of **1** and **4** was irradiated, NOE was not observed at the signal due to H-14 in **1**, but was observed at the signal due to H-14 in **4**. Accordingly, the structure of isoandrographolide could be represented as **4**. The absolute configuration at C-14 could not be established.

14-Deoxy-12-methoxyandrographolide (**7**) was isolated as colorless plates, mp 148—150 °C, $[\alpha]_D$ −71.0°. The FAB-MS of **7** showed the pseudo molecular ion, $[M+H]^+$, at m/z 365, which corresponds to the molecular formula $C_{21}H_{32}O_5$. The IR spectrum of **7** showed the presence of hydroxyl and α,β-unsaturated-γ-lactone groups. The ^{1}H-NMR spectrum of **7** showed a signal due to a methoxyl group at δ 3.27 (3H, s), and a signal due to a proton attached to the carbon having the methoxyl group at d 4.28 (1H, br d, J=4.3 Hz). Other signals in the ^{1}H-NMR spectrum were similar to those of **5** (Table I). The location of the methoxyl group on **5** was determined from the ^{13}C–^{1}H COSY and ^{1}H–^{1}H COSY results shown by arrows in Fig. 1. These correlations from H-5 to H-15 verified that the methoxyl group was located at C-12 of **5**. A comparison of the ^{13}C-NMR data of **7** with those of **5** showed that the signal of the carbon at C-12 (α position) was shifted downfield by 50.4 ppm, that of the carbon at C-11 (β position) was shifted downfield by 8.3 ppm, and that of the carbon at C-9 (γ position) was shifted upfield by 4.5 ppm. Therefore, the structure of **7** was elucidated to be 14-deoxy-12-methoxyandrographolide.

12-*epi*-14-Deoxy-12-methoxyandrographolide (**8**) was isolated as colorless needles, mp 202—203 °C, $[\alpha]_D$ +16.1°. The FAB-MS of **8** showed the pseudo molecular ion, $[M+H]^+$, at m/z 365, which corresponds to the molecular formula $C_{21}H_{32}O_5$. The IR spectrum of **8** showed the presence of hydroxyl, α,β-unsaturated-γ-lactone and *exo*-methylene groups. The ^{1}H-NMR and ^{13}C-NMR spectra of **8** (Tables I, II) were closely related to those observed for **7** except for a signal due to H-12 (δ 4.33, 1H, dd, J=4.5, 8.5 Hz). These data suggested that **8** was the 12-epimer of **7**. The absolute configuration at C-12 of **7** and **8** could not be established. 14-Deoxy-12-methoxyandrographolide has been isolated by Fujita *et al.*,[11] but the melting point of this compound (121—124 °C) was much lower than that of **7** or **8**. The ^{13}C-NMR spectrum, the optical rotation and the configuration at C-12 of this compound have not been reported. Thus, this compound was assumed to be a mixture of **7** and **8**, or one of them.

14-Deoxy-12-hydroxyandrographolide (**9**) was isolated as an amorphous powder, $[\alpha]_D$ −9.7°. The FAB-MS of **9** showed the pseudo molecular ion, $[M+H]^+$, at m/z 351, which corresponds to the molecular formula $C_{20}H_{30}O_5$. The IR spectrum of **9** showed the presence of hydroxyl, α,β-unsaturated-γ-lactone and *exo*-methylene groups. The ^{1}H-NMR spectrum of **9** (Table I) was similar to those of **7** and **8** with the exception of the signal due to the methoxyl group. A comparison of the ^{13}C-NMR data of **9** with those of **7** and **8** showed that the carbon signal at

TABLE I. ^{1}H-NMR Chemical Shifts (δ) of Diterpenoids (C_5D_5N)

	1	2	3	4	5	6	7
H-3	3.6[a]	3.52 (dd, J=4.0, 11.6)	3.6[a]	3.6[a]	3.6[a]	3.49 (dd, J=4.0, 11.3)	3.6[a]
9	1.9[a]					1.6[a]	2.24 (d, J=11.0)
11	2.71 (br t, J=6.4)	2.73 (t, J=7.0)	2.71 (m)	2.98 (ddd, J=7.0, 11, 16)	1.7[a]	1.6[a]	1.8[a]
12	7.16 (td, J=6.1, 1.9)	7.17 (td, J=6.7, 1.6)	7.17 (td, J=6.5, 1.6)	6.70 (br t, J=7)	2.52 (br t, J=15)	2.52 (br t, J=13)	4.28 (br d, J=10.5)
					2.19 (m)	2.19 (m)	
14	5.40 (br s)	5.32 (br s)	5.39 (br s)	5.04 (br s)	7.19 (t, J=1.5)	7.16 (br t, J=1.5)	7.48 (br s)
15	4.50 (dd, J=10.0, 2.5)	4.46 (dd, J=10.0, 2.6)	4.50 (dd, J=10.5, 2.0)	4.38 (dd, J=10, 3.0)	4.73 (d, J=1.5)	4.73 (br s)	4.88 (br s)
	4.60 (dd, J=10.0, 6.0)	4.58 (dd, J=10.0, 5.9)	4.59 (dd, J=10.5, 6.0)	4.51 (dd, J=10, 6.5)			
17	4.84 (br s)	4.81 (br s)	4.84 (br s)	4.70 (br s)	4.74 (br s)	4.73 (br s)	5.02 (s)
	4.86 (br s)	4.85 (br s)	4.86 (br s)	4.87 (br s)	4.94 (br s)	4.92 (br s)	5.21 (s)
18	1.49 (s)	1.45 (s)	1.49 (s)	1.48 (s)	1.49 (s)	1.44 (s)	1.47 (s)
19	3.6[a]	3.85 (d, J=10.5)	3.61 (d, J=10.5)	3.6[a]	3.6[a]	3.84 (d, J=10.3)	3.61 (d, J=10.5)
	4.43 (d, J=10.5)	4.61 (d, J=10.5)	4.43 (d, J=10.5)	4.43 (d, J=11.0)	4.44 (d, J=11.0)	4.61 (d, J=10.3)	4.44 (d, J=11.0)
20	0.65 (s)	0.84 (s)	0.66 (s)	0.71 (s)	0.73 (s)	0.83 (s)	0.68 (s)
–OMe							3.27 (s)
–OAc							
glc-1		4.79 (d, J=7.8)				4.79 (d, J=7.8)	
2		3.95 (t, J=7.8)				3.95 (t, J=7.8)	
3		4.1[a]				4.1[a]	
4		4.1[a]				4.1[a]	
5		3.88 (m)				3.88 (m)	
6		4.29 (dd, J=5.4, 11.6)				4.29 (dd, J=5.1, 11.6)	
		4.46[a] (dd, J=2.4, 11.6)				4.45 (dd, J=2.5, 11.6)	

	8	9	10	11	12	13	14
H-3	3.54 (dd, J=5.5, 11.3)	3.5[a]	3.61 (dd, J=4.0, 11.0)	3.6[a]	3.54 (dd, J=4.5, 12.0)	0.9[a]	
						2.13 (br t, J=13)	
9			1.77 (br s)			1.6[a]	
11			4.80 (dd, J=9, 5)	7.15 (dd, J=10.3, 15.8)	7.17 (dd, J=10.3, 15.5)	1.6[a]	
						1.7[a]	
12	4.33 (dd, J=4.5, 8.5)	5.03 (br t, J=6)	2.77 (dd, J=5, 14)	6.26 (d, J=15.5)	6.25 (d, J=15.5)	2.2[a]	
			3.15 (dd, J=9, 14)			2.50 (tm, J=13)	
14	7.56 (br s)	7.56 (br s)	7.33 (br s)	7.32 (br t)	7.30 (br s)	7.15 (br t)	7.16 (s)
15	4.86 br s)	4.79 (d, J=1.5)	4.69 (d, J=17.5)	4.79 (br s)	4.78 (br s)	4.72 (d, J=1.5)	4.73 (s)
			4.60 (d, J=17.5)				
17	4.81 (s)	4.91 (br s)	5.11 (s)	4.74 (br d)	4.73 (br d)	4.70 (s)	
	4.91 (s)	4.96 (br s)	5.77 (s)	4.86 (br d)	4.83 (br d)	4.89 (s)	
18	1.47 (s)	1.43 (s)	1.48 (s)	1.51 (s)	1.47 (s)	1.17 (s)	1.14 (s)
19	3.59 (d, J=10.5)	3.6[a]	3.68 (d, J=11.0)	3.64 (d, J=11.0)	3.91 (d, J=10.5)	3.48 (d, J=9.5)	3.44 (d, J=9.5)
	4.43 (d, J=10.5)	4.53 (d, J=11.0)	4.44 (d, J=11.0)	4.47 (d, J=11.0)	4.66 (d, J=10.5)	4.32 (d, J=9.5)	4.32 (d, J=9.5)
20	0.68 (s)	0.73 (s)	1.37 (s)	0.87 (s)	1.05 (s)	0.63 (s)	0.73 (s)
–OMe	3.26 (s)						
–OAc							1.98 (s)
glc-1					4.83 (d, J=7.5)	4.82 (d, J=7.5)	4.75 (d, J=7.5)
2					4.01 (d, J=7.5)	4.03 (d, J=7.5)	4.18 (t, J=8.5)
3					4.2[a]	4.2[a]	4.0[a]
4					4.2[a]	4.2[a]	4.0[a]
5					3.90 (m)	3.95 (m)	3.63 (m)
6					4.35 (dd, J=5.5, 11.5)	4.38 (dd, J=5.5, 12.0)	4.9[a]
					4.51 (dd, J=3.0, 11.5)	4.54 (dd, J=3.0, 12.0)	

a) Obscured by overlapping with other signals.

TABLE II. ^{13}C-NMR Chemical Shifts (δ) of Diterpenoids (C_5D_5N)

	1	2	3	4	5	6	7	8	9	10	11	11a	12	13	14	19
C-1	37.3	37.9	37.3	37.2	37.3	37.8	37.2	37.2	37.3	39.7[a]	38.7	38.3	39.3	39.0	39.0	37.0
2	29.0	29.2	29.0	28.9	29.0	29.2	29.0	29.0	29.0	29.0	28.8	24.1[b]	29.1	19.3	19.2	25.8
3	79.8	79.1	80.0	79.8	80.0	79.1	79.9	79.9	79.9	80.0	80.1	80.0	79.2	36.4	36.1	79.7
4	43.2	43.3	43.3	43.2	43.3	43.3	43.2	43.2	43.2	43.3	43.3	41.3	43.3	39.8	39.8	41.2
5	55.3	55.7	55.2	55.3	55.6	55.8	55.4	55.3	55.5	56.4	54.7	54.7	55.0	56.2	56.1	55.2
6	24.3	25.0	24.4	24.4	24.7	25.3	24.5	24.6	24.6	24.9	23.6	23.7[b]	24.6	24.7	24.6	24.5
7	38.1	38.4	38.3	38.2	38.6	38.8	38.7	38.5	38.6	40.1[a]	37.0	36.7	37.2	38.7	38.7	37.8
8	147.9	148.2	148.2	148.3	148.0	148.2	148.0	149.1	149.3	147.6	149.2	147.9	149.6	148.1	148.1	145.4[c]
9	56.3	56.6	57.0	56.7	56.5	56.6	52.0	52.1	52.4	60.3	61.7	61.7	61.9	56.6	56.6	56.0
10	39.1	39.5	39.4	39.3	39.5	39.7	39.0	39.5	39.6	37.2	39.0	38.6	39.3	38.6	38.5	39.0
11	25.0	25.1	24.4	23.8	22.4	22.4	30.7	29.4	31.9	67.5	135.6	135.6	135.9	22.0	22.1	25.8
12	147.0	146.9	146.8	148.1	25.0	25.0	75.4	75.7	65.9	34.1	121.9	121.4	121.8	24.9	25.0	146.4[c]
13	130.2	130.1	132.7	129.8	134.2	134.2	135.4	134.1	138.2	132.0	128.8	129.2	128.8	134.1	134.1	125.8
14	66.0	66.0	66.2	69.0	145.2	145.2	146.8	148.8	146.8	146.0	145.1	143.2	145.0	145.3	145.3	104.9[c]
15	75.4	75.2	71.3	74.3	70.5	70.5	71.1	70.8	70.7	70.6	70.3	69.5	70.3	70.6	70.5	143.8[c]
16	170.7	170.6	168.1	169.9	174.4	174.4	173.5	173.2	173.6	174.9	172.8	172.1	172.8	174.6	174.6	167.8
17	108.8	108.5	108.4	108.1	107.1	107.0	108.3	107.2	107.1	110.6	108.7	109.2	108.5	106.9	107.0	108.7
18	23.7	24.4	23.6	23.7	23.6	24.4	23.6	23.5	23.6	23.7	23.6	22.7	24.4	28.1	27.9	22.6
19	64.1	72.0	64.1	64.1	64.2	72.0	64.2	64.1	64.2	64.2	64.2	64.8	72.3	72.5	72.0	64.6
20	15.2	14.8	15.3	15.2	15.3	15.0	15.5	15.3	15.4	17.4	16.0	15.2	15.5	16.6	15.5	14.5
glc-1		105.4				105.4							105.5	105.5	104.8	
2		74.9				74.8							74.9	75.3	75.0	
3		78.7				78.7							78.7	78.7	78.4	
4		71.8				71.8							71.6	71.7	71.6	
5		78.4				78.4							78.6	78.4	75.1	
6		62.8				62.8							62.6	62.8	64.8	
–OMe							75.9	56.7								
C=O												170.5		170.7		170.4
												170.8				170.8
CH_3												21.0		20.7		21.0
												21.2				21.1

a—*c*) The assignments may be interchanged in the same column. Spectra of **11a** and **19** were measured in $CDCl_3$ solution.

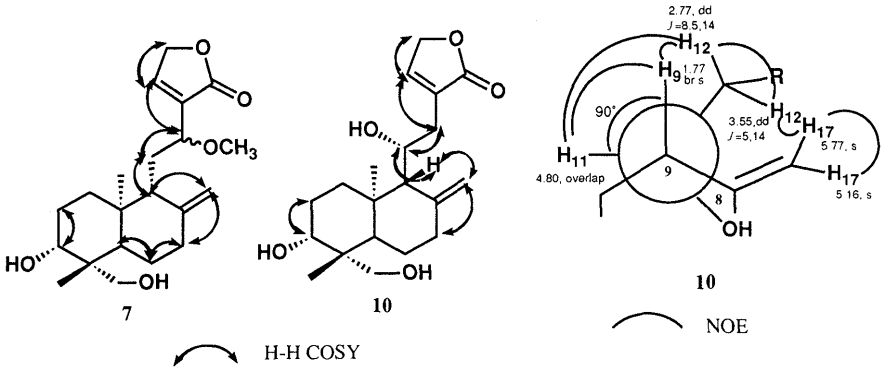

Fig. 1. ^{1}H–^{1}H COSY Spectra of **7** and **10**, and NOE Spectrum of **10**

C-12 (α position) was shifted upfield by *ca.* 10 ppm and the carbon signal at C-11 (β position) was shifted downfield by 1—4 ppm (Table II). Therefore, the structure of **9** was concluded to be 14-deoxy-12-hydroxyandrographolide. The absolute configuration at C-12 of **9** was not established.

14-Deoxy-11-hydroxyandrographolide (**10**) was isolated as an amorphous powder, $[\alpha]_D$ +6.3°. The FAB-MS of **10** showed the pseudo molecular ion, $[M+H]^+$, at *m/z* 351, which corresponds to the molecular formula $C_{20}H_{30}O_5$. The ^{1}H-NMR spectrum of **10** (Table I) was similar to those of **9**. A comparison of the ^{1}H-NMR data of **10** with those of **9** showed that the H_2-17 signal was shifted downfield to δ 5.11 and δ 5.77, the H-20 signal was shifted downfield by 0.64 ppm, and the H-14 signal was shifted upfield by 0.23 ppm. These facts indicated that **10** was a 11-hydroxyl derivative of **5**. This was confirmed by the ^{1}H–^{1}H COSY results, as shown by arrows in Fig. 1. The ^{13}C-NMR data (Table II) supported the structure **10**. In order to determine the absolute configuration at C-11 of **10**, the NOE difference spectrum was examined. Upon irradiation at H-9, H-12 and H-17, NOEs were observed between H-9 and H-12$_a$ (δ 2.77), H-12$_b$ (δ 3.55) and H-17$_a$ (δ 5.77) and H-17$_b$ (δ 5.11) and H-7 (δ 2.35). In the ^{1}H-NMR spectrum of **10**, H-9 appeared as a broad singlet, which indicated that the dihedral angle between H-9 and H-11 must be near 90°. These facts suggested the absolute configuration at C-11 to be *R* as shown in Fig.

1. Therefore, the structure of **10** was concluded to be 14-deoxy-11(*R*)-hydroxyandrographolide.

14-Deoxy-11,12-didehydroandrographiside (**12**), $[\alpha]_D$ +18.0°, was isolated as an amorphous powder. The FAB-MS of **12** showed the pseudo molecular ion, $[M+1]^+$, at *m/z* 495, which corresponds to the molecular formula $C_{26}H_{38}O_9$. The IR spectrum of **12** showed the presence of hydroxyl, α,β-unsaturated-γ-lactone and *exo*-methylene groups. The ^{1}H-NMR and ^{13}C-NMR spectra (Tables I, II) of **12** showed the signals due to a β-D-glucopyranosyl group [δ 4.83 (1H, d, J=7.5 Hz, anomeric) and δ 105.5, 78.7, 78.6, 74.9, 71.6 and 62.6. Other signals in the ^{1}H-NMR and ^{13}C-NMR spectra were similar to those of **11** having a 1,2-disubstituted *trans* olefin. The ^{13}C-NMR signal of C-19 of **12** was shifted downfield by 8.1 ppm compared with that of **11**. From these data, the structure of 14-deoxy-11,12-didehydroandrographiside (**12**) was concluded to be 19-glucosyl-14-deoxy-11,12-didehydroandrographolide.

6′-Acetylneoandrographolide (**14**) was isolated as an amorphous powder. The FAB-MS of **14** showed the pseudo molecular ion, $[M+H]^+$, at *m/z* 523, which corresponds to the molecular formula $C_{28}H_{42}O_9$. The IR spectrum of **14** showed the presence of hydroxyl, α,β-unsaturated-γ-lactone and ester groups. The ^{1}H-NMR and ^{13}C-NMR spectra (Tables I and II) of **14** showed the signals due to an acetyl group [δ 1.98 (s) and δ 20.7, 170.7]. Other signals in the ^{1}H-NMR and ^{13}C-NMR spectra were similar to those of **13**. In the ^{13}C-NMR spectrum of **14**, the carbon signal of C-5′ was shifted upfield by 3.3 ppm and that of C-6′ was shifted downfield by 2.0 ppm compared with those of **13** indicating that the hydroxyl group at C-6′ was acylated. From these data, the structure **14** was assigned to 6′-acetylneoandrographolide.

Bisandrographolide A (**15**), $[\alpha]_D$ +1.6°, was isolated as an amorphous powder. The FAB-MS of **15** showed the pseudo molecular ion, $[M+H]^+$, at *m/z* 665, which corresponds to the molecular formula $C_{40}H_{56}O_8$. The ^{1}H-NMR spectrum of **15** showed four singlet signals due to tertiary methyl groups at δ 0.57, 0.81, 1.18 and 1.24, respectively. The ^{13}C-NMR spectrum of **15** showed forty signals. These data suggested that **15** was a dimer of diterpenoids. The ^{1}H-NMR spectrum of **15** further showed signals due to two secondary and two primary hydroxyl groups [δ 3.40 (dd, J=10, 3.5 Hz), 3.3 (overlapped), 4.18 (d, J=11 Hz), 4.10 (d, J=11 Hz) 3.32 (d, J=11 Hz), 3.24 (d, J=11 Hz) (each 1H)], two exocyclic methylenes [δ 4.82, 4.77, 4.48, 4.39 (each 1H, s)], two olefinic protons [δ 7.35, 7.06 (both 1H, s)] on the endocyclic α,β-unsaturated γ-lactone, a methylene group and a methine group [δ 4.83 (2H, s), 5.14 (1H, m)] attached to oxygen of the lactone group, and a *trans*-disubstituted double bond [δ 6.82 (1H, dd, J=16, 10 Hz), 6.08 (1H, d, J=16 Hz)]. The above data and the ^{13}C-NMR data (Table IV) indicated the presence of two sets of AB rings with the bicarbocyclic skeleton and substitution pattern of the congeners of **1**, two endocyclic α,β-unsaturated γ-lactones, and one *trans*-disubstituted double bond. The assignments of carbons and protons were made on the basis of the ^{13}C–^{1}H COSY, ^{1}H–^{1}H COSY, and correlation spectroscopy *via* long range coupling (COLOC) spectrum. The ^{1}H–^{1}H COSY and COLOC results are indicated by arrows in Fig. 2. From these correlations and the above data, bisandrographolide A was concluded to be a dimer between C-12 and C-15′ of andrographolide derivatives, and the structure was concluded to be **15** (in Fig. 2). The ^{13}C-NMR data of **15** were consistent with the structure **15**.

Bisandrographolide B (**16**), $[\alpha]_D$ −8.9°, and bisandrographolide C (**17**), $[\alpha]_D$ −74.5° were isolated as amorphous powders. The FAB-MS of **16** and **17** showed the same pseudo molecular ion $[M+H]^+$, at *m/z* 665, which corresponds to $C_{40}H_{56}O_8$, the same as that of **15**. The ^{1}H-NMR and ^{13}C-NMR spectra of **16** and **17** (Tables III and IV) corresponded closely to those of **15**. The ^{1}H–^{1}H COSY spectrum of **16** and **17** showed the same correlations that were observed for **15**. These data suggested that **16** and **17** were the stereo isomers of **15** at C-12 or C-15′. The absolute configurations at C-12 and C-15′ of **15**, **16** and **17** could not be established.

Bisandrographolide D (**18**), $[\alpha]_D$ −40.1°, was isolated as an amorphous powder. The FAB-MS of **18** showed the pseudo molecular ion, $[M+H]^+$, at *m/z* 697, which corresponds to the molecular formula $C_{41}H_{60}O_9$. The ^{1}H-NMR spectrum of **18** showed four singlets (each 3H) due to tertiary methyl groups at δ 0.61, 0.64, 1.21, 1.24 and a methoxyl group at δ 3.67. The ^{13}C-NMR spectrum of **18** showed forty-one signals including a methoxyl group (δ 52.0). These data suggested that **18** was a dimer of diterpenoids having a methoxyl group. The ^{1}H-NMR spectrum of **18** further showed signals due to two primary and two secondary hydroxyl groups [δ 3.3 (4H, overlapped), 4.16 (d, J=11 Hz), 4.16 (d, J=11 Hz) (both 1H)], two exocyclic methylenes [δ 4.96, 4.83, 4.76, 4.36 (each 1H, s)], an olefinic proton [δ 7.25 (1H, br s)] on the endocyclic α,β-unsaturated-γ-lactone group, a methylene group [δ 4.81 (2H, br s)] attached to oxygen of the lactone group, a methylene group [δ 3.63 (d, J=17.5 Hz), 3.36 (d, J=17.5 Hz) (both 1H)], a methine group [δ 3.90 (br d, J=11 Hz)], and a trisubstituted double bond [δ 6.86 (1H, t, J=6 Hz)]. The above data and the ^{13}C-NMR data (Table IV) indicated the presence of two sets of AB rings with the bicarbocyclic skeleton and substitution pattern of the congeners of **1**, one endocyclic α,β-unsaturated γ-lactone, a tri-substituted double bond, a methylene connected to two tertiary carbons, a methyl ester (δ 167.2, 52.0), and a carbonyl group (δ 206.3). The assignments of carbons and protons were made on the basis of the ^{13}C–^{1}H COSY, ^{1}H–^{1}H COSY, and COLOC spectra. The ^{1}H–^{1}H COSY and COLOC results were shown by arrows in Fig. 2. From these correlations and the above data, bisandrographolide D was assumed to be **18**. In order to determine the geometry at $\Delta_{12',13'}$, a differentiating NOE spectral experiment was conducted. When the signal due to H-12′ (δ 6.86) was irradiated, NOE was not observed at the signal due to H-14′, but was observed at the signals due to H-11′ (δ 2.23, 2.1), H-17′ (δ 4.36) and a carbomethoxy group. Thus, the configuration at C-12′ and C-13′ was supposed to be *E*. The ^{13}C-NMR data were consistent with the structure **18**. On the basis of these results, bisandrographolide D can be represented

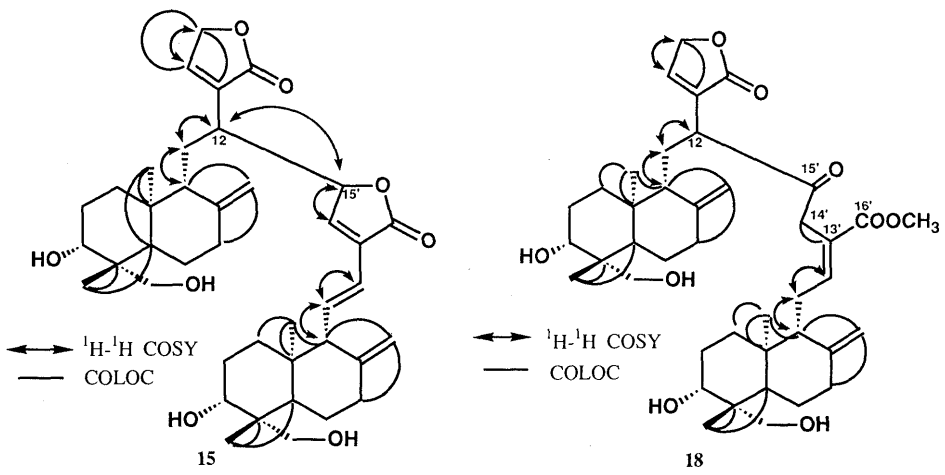

Fig. 2. ^{1}H–^{1}H COSY and COLOC Spectra of **15** and **18**

TABLE III. ^{1}H-NMR Chemical Shifts (δ) of Diterpene Dimers

	15	16	17	18
H-3	3.40 (dd, J=10.0, 3.5)	3.6[a]	3.6[a]	3.3[a]
9	1.6[a]		2.0[a]	1.57 (d, J=12.0)
11	1.47 (br d, J=13.5)	2.22 (br t, J=15, 4)	1.74 (m)	1.64 (td, J=2, 13)
	1.8[a]		2.22 (m)	2.12 (d, J=12.5)
12	2.93 (m)	3.40 (dm, J=9.0)	3.38 (br d, J=10.0)	3.90 (br t, J=11.0)
14	7.35 (s)	7.67 (d, J=1.5)	7.63 (br s)	7.25 (br s)
15	4.83 (s)	4.77 (dd, J=18, 2)	4.68 (d, J=19.0)	4.81 (br s)
		4.87 (d, J=18.0)	4.82 (d, J=18.0)	
17	4.39 (s), 4.82 (s)	4.67 (s), 4.92 (s)	4.90 (s), 4.60 (s)	4.76 (s), 4.96 (s)
18	1.18 (s)	1.40 (s)	1.47 (s)	1.21 (s)
19	3.24 (d, J=11.0)	3.6[a]	3.6[a]	3.3[a]
	4.10 (d, J=11.0)	4.45 (d, J=11.0)	4.43 (d, J=10.8)	4.16 (d, J=11.0)
20	0.57 (s)	0.67 (s)	0.68 (s)	0.61 (s)
3′	3.35[a]	3.52 (dd, J=10.0, 5.5)	3.6[a]	3.3[a]
9′	2.32 (d, J=10.0)	2.4[a]	2.3[a]	1.8[a]
11′	6.82 (dd, J=10.0, 16.0)	7.19 (dd, J=10.5, 16.0)	7.13 (dd, J=9.7, 15.7)	2.1[a], 2.23 (dq, J=17.0, 1.5)
12′	6.08 (d, J=16.0)	6.27 (d, J=16.0)	6.26 (d, J=15.7)	6.86 (br t)
14′	7.06 (s)	7.66 (br s)	7.52 (br s)	3.36 (d, J=17.5)
				3.63 (d, J=17.5)
15′	5.14 (m)	5.61 (br d, J=5.0)	5.56 (br s)	
17′	4.48 (s), 4.77 (s)	4.77 (s), 4.81 (s)	4.72 (s), 4.79 (s)	4.36 (s), 4.83 (s)
18′	1.24 (s)	1.50 (s)	1.49 (s)	1.24 (s)
19′	4.18 (d, J=12.0)	3.6[a]	3.6[a]	3.3[a]
	3.32 (d, J=12.0)	4.45 (d, J=11.0)	4.45 (d, J=10.8)	4.16 (d, J=11.0)
20′	0.81 (s)	0.87 (s)	0.85 (s)	0.64 (s)
-OMe				3.67 (s)

a) Obscured by overlapping with other signals. Spectra of **15** and **18** were measured in $CDCl_3$ solution. Spectra of **16** and **17** were measured in C_5D_5N solution.

as **18**. The absolute configuration at C-12 could not be established.

These diterpenoids isolated from *A. paniculata* have the same carbon skeleton as that of andrographolide (**1**). A hypothetical biosynthetic pathway to these compounds is shown in Figs. 3 and 4. Andrographolide (**1**), which is considered to be a common intermediate for the compounds isolated in this study is presumably derived from **5** through epoxidation, followed by cleavage of the epoxide ring. Compound **1** in turn forms a 12-cation intermediate through elimination of the hydroxyl group and double bond migration. The 12-cation intermediate may be converted into compounds **3**, **4**, **7**, **8**, **9** and **11** as shown in Fig. 4. On the other hand, compound **10** may be derived *via* oxidation from **11**. The diterpene dimers, **15**, **16**, **17** and **18** are thought to be derived from the 12-cation intermediate and the enol lactone derivative (**19**), which was assumed to be derived from **1** by dehydration. In practice, **7**, **8** and **11** were derived from **1** by treatment with thionyl chloride in methanol solution, and the enol lactone diacetate (**19a**) was derived from **1** in pyridine and acetic anhydride in a short reaction time. These chemical reactions support the hypothetical biosynthetic pathways in the plant.

These diterpenoides isolated from *A. paniculata* were tested for cell differentiation-inducing activity against M1 cells. The activity was determined by observation of the inducibility of phagocytosis of M1 cells accoding to the reported method.[7] Some of these compound showed the potent phagocytosis and growth-inhibitory activities against the M1 cells. The inducibility and cytostatic activity of the eight monomeric diterpenoids, **1**, **3**, **4**, **5**, **7**, **8**, **9** and **10**, towards M1 cells are shown in Fig. 5A. Of these monomeric compounds, **7** showed moderate cell differentiation activity, and the other seven compounds showed potent activity. Compounds **1**, **3** and **4** showed a phagocytic ratio of more than 30% at the concentration of 5×10^{-6} M. The diterpene dimers, **15**, **16**, **17** and **18**, were also examined (Fig. 5B). Compounds **15**, **16** and **17** showed potent activities. The activity of these dimers was more potent than that of the monomers. The dimer **18** showed no induction of phagocytosis, but was growth-inhibitory. This should be attributed to cleavage of one of the two α,β-unsaturated-γ-lactone moieties. The glucoside derivatives, **2**, **6**, **12**, **13** and **14** showed week activities of phagocytosis induction and growth inhibition, though the acetyl derivative, **14**, was relatively potent (Fig. 5C). From these results, it was supposed that α,β-unsaturated-γ-lactones having an *exo*-olefin group or diene group conjugated with the lactone carbonyl are effective inducers of phagocytosis.

Experimental

Melting points were determined with a Yanagimoto micro melting

TABLE IV. ^{13}C-NMR Chemical Shifts (δ) of Diterpene Dimers

	15	16	17	18		15	16	17	18
C-1	36.7	37.1	37.2	36.5	C-1′	38.3	38.7[d]	38.6	37.0
2	28.1	29.0	29.0	28.3[g]	2′	28.1	28.8	28.8	28.2[g]
3	80.3	79.7[b]	79.8[f]	80.4	3′	80.6	80.0[b]	80.0[f]	80.4
4	42.8	43.3[c]	43.3	42.8	4′	42.8	43.2[c]	43.3	42.8
5	55.6	55.1	55.5	55.4[h]	5′	54.6	54.7	54.7	55.2[h]
6	24.0	24.5	24.6	23.8[i]	6′	23.0	23.7	23.7	24.0[i]
7	38.1	38.5[d]	38.4	38.1	7′	36.6	36.9	36.9	37.9
8	147.0	147.7	147.9	147.1[j]	8′	148.0	150.0	150	146.3[j]
9	55.1	53.8	53.9	53.9	9′	61.7	61.7	61.7	55.9
10	39.5	39.4	29.6	39.2	10′	38.6	39.0	38.9	38.9
11	23.0	25.1	25.8	26.4	11′	136.9	136.6	136.3	24.6
12	38.4	38.8[d]	37.6	45.9	12′	120.8	121.7	121.7	147.5
13	134.2	131.5	132.4	132.8	13′	130.1	130.2	130.0	124.6
14	148.3	149.1	149.1	146.5	14′	145.0	146.7	147.3	43.1
15	70.6	71.1	71.6	70.8	15′	81.1	82.5	80.4	206.3
16	173.5	174.2	174.3	173.4	16′	171.7	172.0	172.2	167.2
17	108.1	107.5	107.8	108.4	17′	109.1	108.7	108.8	108.8
18	22.9	23.6	23.6	22.9	18′	22.8	23.6	23.6	22.9
19	64.1[a]	64.1[e]	64.2	64.2	19′	64.2[a]	64.2[e]	64.2	64.2
20	14.9	15.2	15.3	15.4	20′	22.8	16.0	16.0	15.1
									52.0

a—j) Assignments may be interchanged in the same column. Spectra of **15** and **18** were measured in $CDCl_3$ solution. Spectra of **16** and **17** were measured in C_5D_5N solution.

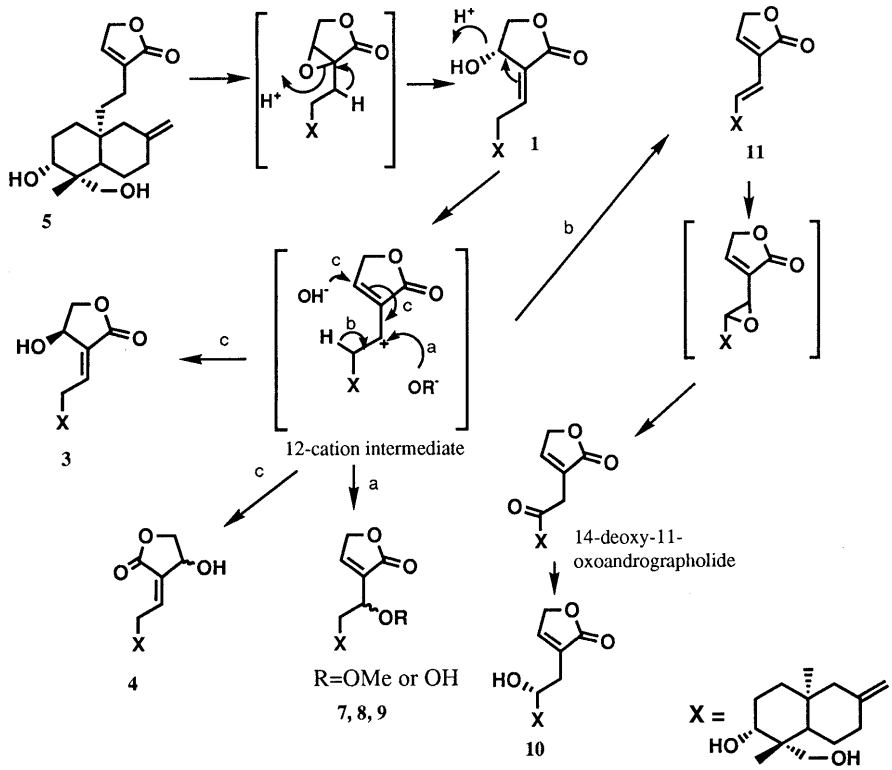

Fig. 3. Hypothetical Biogenetic Scheme for the Diterpenoids

point apparatus and are uncorrected. IR spectra were recorded with a JASCO IR700 spectrometer. Ultraviolet (UV) spectra were taken with a Hitachi U3410 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained with JEOL GSX-270 and GSX-500 FT NMR spectrometers, and chemical shifts are given in ppm from tetramethylsilane as an internal standard. FAB-MS was recorded on a JEOL JMX-SX102 mass spectrometer using glycerol as a matrix. Optical rotations were measured with a JASCO DIP-360 digital polarimeter at 24 °C. Kieselgel 60 (Merck) was used for column chromatography. Analytical and preparative HPLC were carried out on a YMC R-ODS-7, SH343-7 S-7 120A ODS packed column, and Shiseido Capcell pack C_8, C_{18}.

Extraction and Isolation The aerial parts (5 kg) of *Andrographis paniculata* NEES cultivated at the Izu medicinal plant research station, National Institute of Hygienic Sciences, were collected in October and extracted with hot MeOH. The concentrated extract was fractionated between ethyl acetate and water. The ethyl acetate layer was concentrated *in vacuo*, and crude **1** was precipitatedas crystals. It was collected by filtration, and recrystallized from MeOH to give **1** (100 g). The filtrate

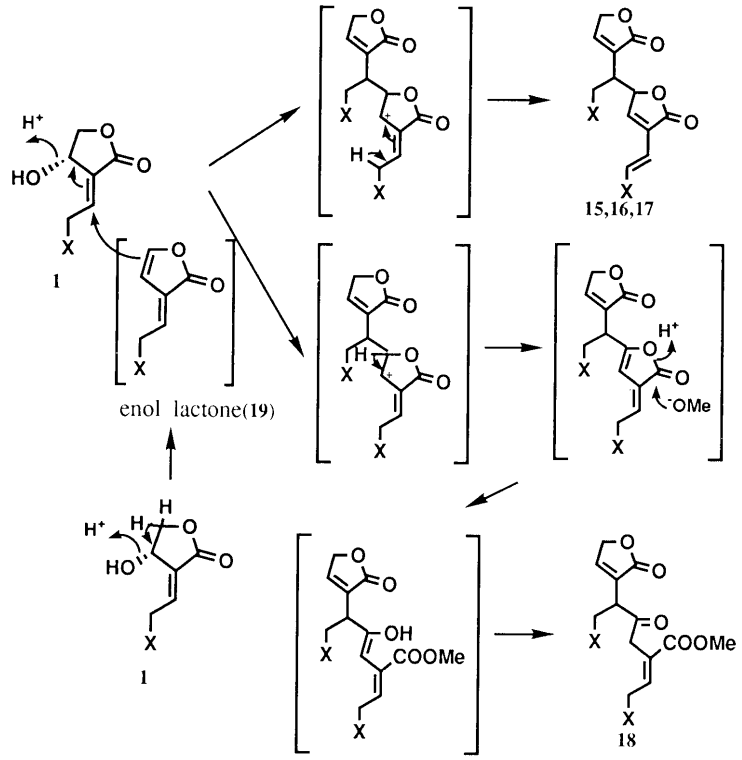

Fig. 4. Hypothetical Biogenetic Scheme for the Diterpenoid Dimers

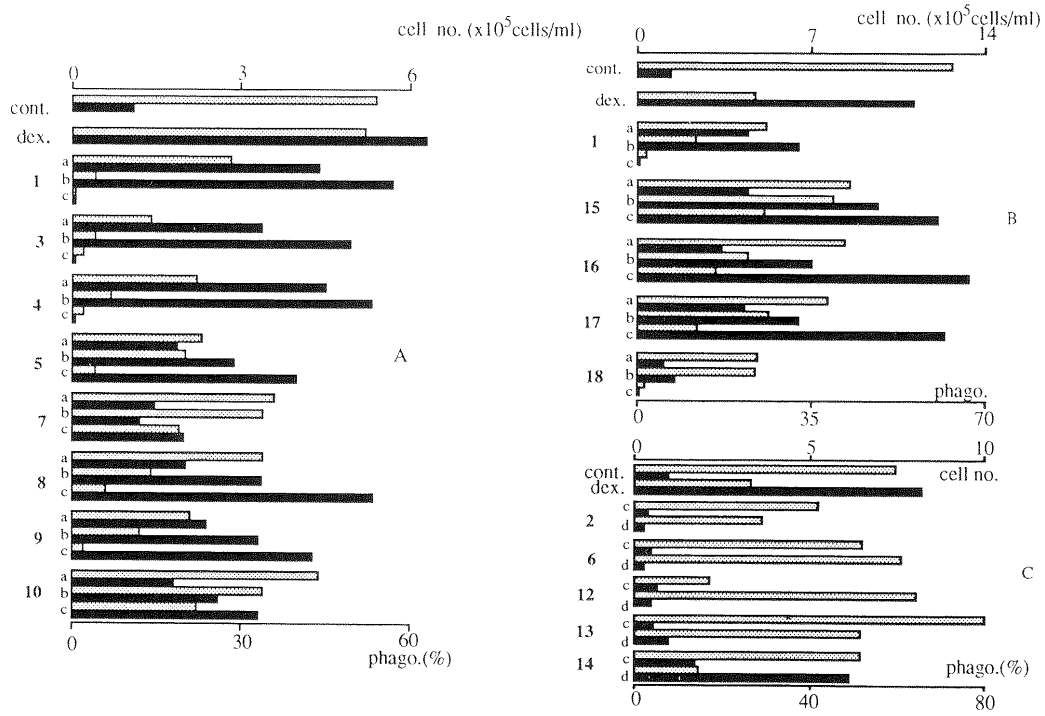

Fig. 5. Effect of Diterpenoids on Cell Growth and Induction of Phagocytic Activity

A, monomeric diterpenoids; B, diterpene glucosides; C, dimers. Concentration: a) 5×10^{-6} M, b) 1.7×10^{-5} M, c) 5×10^{-5} M, d) 1×10^{-4} M. Dexamethasone was used as a positive control (1×10^{-6} M). ▨, cell number; ■, phagocytosis.

(240 g) was chromatographed on a silica gel column with a $CHCl_3$–MeOH gradient solvent system to give many fractions, which were combined into fractions A—G based on the TLC patterns. Fraction B (70 g) was subjected to chromatography on a silica gel column with a hexane EtOAc gradient solvent system to give crude **11** (30 g). Crude **11** was recrystallized from EtOAc hexane to give **11** (18 g). Fraction C (12 g) was repeatedly chromatographed on semi preparative HPLC to give **14** (5 mg), **15** (30 mg), **16** (25 mg), **17** (45 mg) and **18** (10 mg). Fraction D (4 g) was recrystallized from MeOH to afford **5** (40 mg). The mother liquor of **5** was subjected to preparative HPLC to give **7** (80 mg) and **8**

(60 mg). Fraction E (40 g) was filtered to give **1** (12 g). The filtrate was repeatedly subjected to preparative HPLC to give **3** (15 mg), **4** (15 mg), **9** (20 mg) and **10** (3 mg). Fraction F (15 g) was rechromatographed on a silica gel column and recrystallized from EtOAc–MeOH to afford **13** (5 g). Fraction G (25 g) was subjected to rechromatography on a silica gel column to give crude **2** (5 g). Crude **2** was recrystallized from MeOH to give **2** (2 g). The less polar fraction obtained from fraction H (5 g) was subjected to preparative HPLC to give **6** (80 mg) and **12** (15 mg).

Andrographolide (1) Colorless plates. mp 230—239 °C (from MeOH). $[\alpha]_D$ −112.7° (c=0.53, MeOH). UV λ^{MeOH} nm (ε): 223.2 (13000). IR (KBr): 3414, 1727, 1673, 905 cm^{-1}. FAB-MS m/z: 351 $[M+H]^+$ $C_{20}H_{31}O_5$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

Andrographiside (2) Colorless plates. mp 201—203 °C (from MeOH). $[\alpha]_D$ −87.9° (c=1.01, MeOH). UV λ^{MeOH} nm (ε): 223.4 (11500). IR (KBr): 3406, 1727, 1674, 889 cm^{-1}. FAB-MS m/z: 513 $[M+H]^+$ $C_{26}H_{41}O_{10}$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

14-*epi*-Andrographolide (3) Colorless plates. mp 225—228 °C (from MeOH). $[\alpha]_D$ −87.8° (c=0.45, MeOH). UV λ^{MeOH} nm (ε): 220.0 (12000). IR (KBr): 3398, 1727, 1673, 905 cm^{-1}. FAB-MS m/z: 351 $[M+H]^+$ $C_{20}H_{31}O_5$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

Isoandrographolide (4) Colorless plates. mp 209—211 °C (from MeOH). $[\alpha]_D$ −47.2° (c=0.55, MeOH). UV λ^{MeOH} nm (ε): 220.0 (8000). IR (KBr): 3332, 1751, 1675, 897 cm^{-1}. *Anal.* Calcd for $C_{20}H_{30}O_5 \cdot 1/2H_2O$: C, 66.83; H, 8.69. Found: C, 66.69; H, 8.87. FAB-MS m/z: 351 $[M+H]^+$ $C_{20}H_{31}O_5$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

14-Deoxyandrographolide (5) Colorless plates. mp 176—177 °C (from MeOH). $[\alpha]_D$ −30.7° (c=0.95, MeOH). UV λ^{MeOH} nm (ε): no absorption near 220 nm. IR (KBr): 3280, 1753, 905 cm^{-1}. FAB-MS m/z: 335 $[M+H]^+$ $C_{20}H_{31}O_4$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

Deoxyandrographiside (6) Colorless plates. mp 200—202 °C (from MeOH). $[\alpha]_D$ −38.5° (c=0.33, MeOH). UV λ^{MeOH} nm (ε): no absorption near 220 nm. IR (KBr): 3384, 1747, 898 cm^{-1}. FAB-MS m/z: 497 $[M+H]^+$ $C_{26}H_{41}O_9$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

14-Deoxy-12-methoxyandrographolide (7) Colorless plates. mp 148—150 °C (from MeOH). $[\alpha]_D$ −71.0° (c=1.04, MeOH). UV λ^{MeOH} nm (ε): no absorption near 220 nm. IR (KBr): 3382, 1744 cm^{-1}. *Anal.* Calcd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 68.88; H, 8.93. FAB-MS m/z: 365 $[M+H]^+$ $C_{21}H_{33}O_5$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

12-*epi*-14-Deoxy-12-methoxyandrographolide (8) Colorless needles. mp 202—203 °C (from MeOH). $[\alpha]_D$ +16.1° (c=0.28, MeOH). UV λ^{MeOH} nm (ε): no absorption near 220 nm. IR (KBr): 3412, 1746, 890 cm^{-1}. *Anal.* Calcd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 68.95; H, 8.89. FAB-MS m/z: 365 $[M+H]^+$ $C_{21}H_{33}O_5$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

14-Deoxy-12-hydroxyandrographolide (9) Amorphous powder. $[\alpha]_D$ −9.7° (c=0.39, MeOH). UV λ^{MeOH} nm (ε): no absorption near 220 nm. IR (KBr): 3402, 1746, 891 cm^{-1}. *Anal.* Calcd for $C_{20}H_{30}O_5 \cdot 1/2H_2O$: C, 66.83; H, 8.69. Found: C, 66.77; H, 8.72. FAB-MS m/z: 351 $[M+H]^+$ $C_{20}H_{31}O_5$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

14-Deoxy-11-hydroxyandrographolide (10) Amorphous powder. $[\alpha]_D$ +6.3° (c=0.55, MeOH). FAB-MS m/z: 351 $[M+H]^+$ $C_{20}H_{31}O_5$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

14-Deoxy-11,12-dihydroandrographolide (11) Colorless needles. mp 203—204 °C (from MeOH). $[\alpha]_D$ +5.4° (c=1.02, MeOH). UV λ^{MeOH} nm (ε): 250.2 (10000). IR (KBr): 3422, 1741, 883 cm^{-1}. FAB-MS m/z: 333 $[M+H]^+$ $C_{20}H_{29}O_4$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

14-Deoxy-11,12-dihydroandrographiside (12) Amorphous powder. $[\alpha]_D$ +18.0° (c=0.23, MeOH). UV λ^{MeOH} nm (ε): 250.7 (7800). IR (KBr): 3424, 1749, 891 cm^{-1}. FAB-MS m/z: 495 $[M+H]^+$ $C_{26}H_{39}O_9$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

Neoandrographolide (13) Colorless plates. mp 201—203 °C (from MeOH). $[\alpha]_D$ −40.0° (c=1.11, MeOH). UV λ^{MeOH} nm (ε): no absorption near 220 nm. IR (KBr): 3446, 1749, 891 cm^{-1}. FAB-MS m/z: 481 $[M+H]^+$ $C_{26}H_{41}O_8$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

6′-Acetylneoandrographolide (14) Amorphous powder. IR (KBr): 3432, 1748 cm^{-1}. FAB-MS m/z: 523 $[M+H]^+$ $C_{28}H_{43}O_9$. ^{1}H-NMR (Table I). ^{13}C-NMR (Table II).

Bisandrographolide A (15) Amorphous powder. $[\alpha]_D$ +1.6° (c=1.02, MeOH). *Anal.* Calcd for $C_{40}H_{56}O_8 \cdot 1/2H_2O$: C, 70.35; H, 8.56. Found: C, 69.92; H, 8.43. FAB-MS m/z: 665 $[M+H]^+$ $C_{40}H_{57}O_8$. ^{1}H-NMR (Table III). ^{13}C-NMR (Table IV).

Bisandrographolide B (16) Amorphous powder. $[\alpha]_D$ −8.9° (c=0.47, MeOH). *Anal.* Calcd for $C_{40}H_{56}O_8 \cdot 1/2H_2O$: C, 71.29; H, 8.53. Found: C, 71.24; H, 8.60. FAB-MS m/z: 665 $(M+H)^+$ $C_{40}H_{57}O_8$. ^{1}H-NMR (Table III). ^{13}C-NMR (Table IV).

Bisandrographolide C (17) Amorphous powder. $[\alpha]_D$ −74.5° (c=1.14, MeOH). *Anal.* Calcd for $C_{40}H_{56}O_8 \cdot H_2O$: C, 70.35; H, 8.56. Found: C, 70.62; H, 8.70. FAB-MS m/z: 665 $[M+H]^+$ $C_{40}H_{57}O_8$. ^{1}H-NMR (Table III). ^{13}C-NMR (Table IV).

Bisandrographolide D (18) Amorphous powder. $[\alpha]_D$ −40.1° (c=0.41, MeOH). *Anal.* Calcd for $C_{41}H_{60}O_9 \cdot 1/2H_2O$: C, 69.76; H, 8.71. Found: C, 69.65; H, 8.90. FAB-MS m/z: 697 $[M+H]^+$ $C_{41}H_{61}O_9$. ^{1}H-NMR (Table III). ^{13}C-NMR (Table IV).

Derivation of 14-Deoxy-12-methoxyandrographolide (7), 12-*epi*-14-Deoxy-12-methoxyandrographolide (8) and 14-Deoxy-11,12-dihydroandrographolide (11) from Andrographolide (1) Andrographolide (**1**) (500 mg) was dissolved in a solution of methanol (100 ml) and $SOCl_2$ (5 ml), and the solution was left stand at room temperature overnight. Excess H_2O was added to the reaction solution and extracted with EtOAc. The EtOAc extract was washed with dilute $NaHCO_3$ aqueous solution and H_2O, successively, and concentrated under *vacuo*. The residue was subjected to HPLC to give **7** (97.8 mg), **8** (16.7 mg) and **11** (8.7 mg), which were identical with the natural compounds in terms of ^{1}H-NMR spectrum, and HPLC and TLC behavior.

Acetylation of Andrographolide (1) i) Andrographolide (**1**) (200 mg) was dissolved in a solution of pyridine (2 ml) and Ac_2O (1 ml). The solution was left to stand at room temperature for 1 h, then poured into ice-cold water to give a gum. The gum was purified by preparative TLC to yield the enol lactone diacetate (**19a**) (100 mg) as an amorphous solid. ^{1}H-NMR ($CDCl_3$): 4.58 (1H, dd, J=4.5, 12.0 Hz, H-3), 6.67 (1H, t, J=7.0 Hz, H-12), 6.35 (1H, dd, J=3.3, 0.8 Hz), 6.98 (1H, dd, J=4.8, 1.8 Hz), 4.43, 4.86 (each 1H, s, H-17), 1.01 (3H, s, H-18), 4.09, 4.35 (each 1H, d, J=12.0 Hz, H-19), 0.78 (3H, s, H-20), 2.02 (6H, s, Ac). ^{13}C-NMR (Table II).

ii) Andrographolide (**1**) (200 mg) was dissolved in a solution of pyridine (2 ml) and Ac_2O (1 ml). The solution was left to stand at room temperature for 27 h, then poured into ice-cold water to give a gum, which was purified by preparative TLC and HPLC to yield the diacetate (**11a**) (15 mg) of **11**. ^{1}H-NMR ($CDCl_3$): 4.57 (1H, dd, J=1.6, 10.5 Hz, H-3), 6.99 (1H, dd, J=10.0, 16.0 Hz, H-11), 6.12 (1H, d, J=16.0 Hz, H-12), 7.16 (1H, t, J=1.9 Hz, H-14), 4.80 (1H, s, H-15), 4.79, 4.81 (each 1H, s, H-17), 1.03 (3H, s, H-18), 4.14, 4.36 (each 1H, d, J=12.0, H-19), 0.89 (3H, s, H-20), 2.03 (3H, s, Ac), 2.04 (3H, s, Ac). ^{13}C-NMR (Table II).

Measurement of Phagocytosis Phagocytic activity was tested as described in the previous paper[7] according to Hayashi's method.[18] The M1 cells were inoculated at a concentration of 2×10^5 cells ml^{-1} into 2 ml of culture medium with 20 μl of sample solution diluted with EtOH. After 48 h the cells were washed and incubated for 4 h with a suspension of polystyrene latex particles (2 μl ml^{-1} of serum-free culture medium). Then the cells were washed thoroughly 4 times with phosphate-buffered saline (PBS) and the percentage of phagocytic cells was calculated.

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