Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 65 (2004) 891–896
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Tirucallane-type triterpenes from *Juliania adstringens*

Mitsuko Makino, Tomohiro Motegi, Yasuo Fujimoto*

College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi, Chiba 274-8555, Japan

Received 11 September 2003; received in revised form 5 December 2003

Abstract

Five tirucallane-type triterpenes were isolated along with nine known triterpenes from the bark of *Juliania adstringens*. The structures of the five triterpenes were determined by analysis of their ^{1}H and ^{13}C NMR and mass spectral data, and each compound exhibited growth inhibitory activity against leukemia cells (L-1210).

Keywords: *Juliania adstringens*; Julianiaceae; Tirucallane-type triterpenes; Cytotoxic activity

1. Introduction

J. adstringens (Julianiaceae) is a folk medicine which has been used as a preventive medicine for the cancer of digestive organs in Mexico. In our biological studies on the alcoholic extracts of the bark of this plant, the extracts were found to exhibit growth inhibitory activity against leukemia cells (L-1210). In this paper, we describe the isolation and structure elucidation of five new tirucallane-type triterpenes **1–5** along with nine known triterpenes **6–14** from dried bark of *J. adstringens*.

2. Results and discussion

The alcoholic extracts afforded nine known triterpenes, schinol (**6**) (Mulholland and Nair, 1994; Jain et al., 1995), 3β-hydroxy-masticadienolic acid (**7**) (Monaco et al., 1974), masticadienonic acid (**8**) (Mulholland and Nair, 1994), oleanolic acid (**9**) (Mahato and Kundu, 1994), oleanonic acid (**10**) (Konoike et al., 1997), 3α-hydroxy-11α,12α-epoxy-oleanane-28,13β-olide (**11**) (Ikuta and Morikawa, 1992), 3β-hydroxy-11α,12α-epoxy-oleanane-28,13β-olide (**12**) (Melek et al., 1989), β-sitosterol (**13**) and ocotillone (**14**) (Govindachari et al., 1994). These triterpenes were identified by comparison of their ^{1}H and ^{13}C NMR and mass spectral data with those described in the literature. In addition, five new triterpenes (**1–5**) were isolated from the extracts (Fig. 1).

Compound (**1**) was isolated as a colorless powder, mp 233–234 °C, $[\alpha]_D$ −29.9°(*c* 0.5, MeOH). Its MS spectrum showed the molecular ion peak at *m/z* 470.3380 (M^+) corresponding to the molecular formula $C_{30}H_{46}O_4$. The ^{1}H NMR spectrum of **1** showed the presence of five tertiary methyls (δ 0.70, 0.94, 0.98, 1.41 and 1.73), a vinyl methyl (δ 2.16) and a secondary methyl [δ 0.95 (d, $J=6.6$ Hz)] groups, an oxymethine proton [δ 3.60 (brs)] and two vinyl protons [δ 5.88 (*d*, $J=3.0$ Hz), δ 6.05 (*t*, $J=6.6$ Hz). The ^{13}C NMR DEPT spectra exhibited the presence of twenty four sp^3 carbons due to seven methyls, eight methylenes, five methines including an oxymethine (δ 75.9), four quaternary carbons, four sp^2 carbons besides carbonyl (δ 201.5) and carboxyl carbons (δ 170.3). Comparison of these spectral data with those of schinol (**6**) (Tables 1 and 2) suggested that the framework and the side chain moiety of **1** should be the same as those of **6** except for the presence of a carbonyl carbon. The UV spectrum of **1** exhibited absorption maximum at 246 nm (ε; 10,900, MeOH), suggesting the presence of an α, β-unsaturated carbonyl group. The location of this group was confirmed by analysis of its HMBC spectrum (Fig. 2). As shown in Fig. 2, the H-5 signal (δ 3.06) exhibited cross-peaks with the C-6 (δ 201.5) and the sp^2 carbon (C-7, δ 125.1) as well as having a correlation between the H-30 and the C-8 signals. These facts indicated the presence of a 6-keto-7-ene structure. The relative stereochemistry of **1** was determined by analysis of the NOESY spectrum. As shown in Fig. 3,

* Corresponding author. Tel./fax: +81-47-465-6470.
E-mail address: fujimoto@pha.nihon-u.ac.jp (Y. Fujimoto).

0031-9422/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2003.12.012

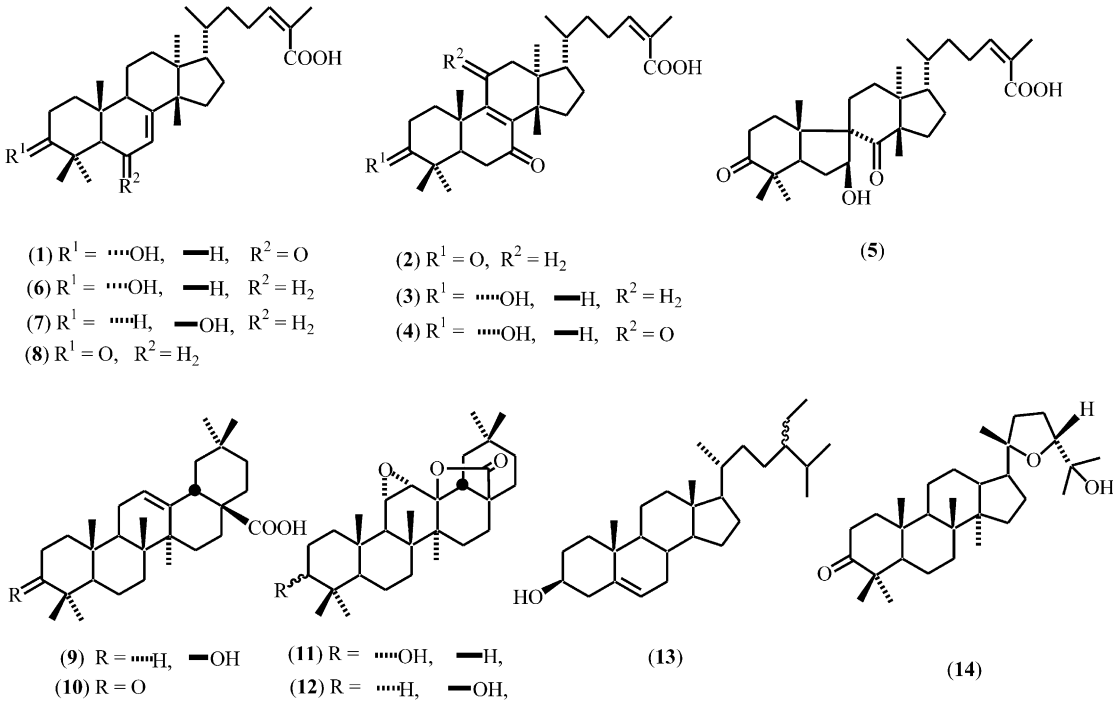

Fig. 1. Structures of **1**–**14**.

NOE correlations were observed between the following proton signals; H-19 and H-29; H-3 and H-28, H-29; H-28 and H-5; H-9 and H-5, H-18. Thus, the structure of **1** was confirmed as 3α-hydroxy-6-oxo-7, 24Z-tirucalladien-26-oic acid.

Compound **2** was isolated as a colorless powder, mp 91–93 °C, $[\alpha]_D$ −27.7° (*c* 1.0, MeOH). Its MS spectrum showed a molecular ion peak at *m*/*z* 468.3215 (M^+) corresponding to the molecular formula $C_{30}H_{44}O_4$. The UV spectrum of **2** indicated the presence of an α, β-unsaturated carbonyl group [λ_{max} (MeOH): 255 nm (ε; 10,040)]. The ^{1}H NMR spectrum of **2** showed the presence of a vinyl proton [δ, 6.07 (br. *t*, *J* = 6.6 Hz)], a secondary methyl group [δ 0.94 (δ, *J* = 6.0 Hz)] and six tertiary methyl groups [δ 0.72, 0.99, 1.09, 1.13, 1.28 and 1.92]. The ^{13}C NMR spectrum of **2** suggested the presence of two carbonyl groups (δ 197.4 and δ 214.5) and a tetra-substituted double bond (δ 139.4 and 163.9). The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1** except for a few changes, suggesting the framework and the side-chain moiety must be the same as those of **1**. The HMBC spectrum of **2** revealed cross-peaks between the proton signals due to the geminal dimethyl group at C-4 and the carbonyl carbon signal at δ 214.5, indicating presence of a carbonyl group at C-3. There are two possibilities for the position (C-7 or C-11) of the other carbonyl group. In the HMBC spectrum of **2**, the methyl proton signals at C-10 and C-14 showed long range correlations between sp^2 carbon signals at δ 163.9 and δ 139.4, respectively. Thus, the other carbonyl group is at C-7 because the β-carbon signal of the α, β-unsaturated carbonyl group generally appears at lower field than that of an α-carbon (Silverstein and Webster, 1998). The stereostructure of **2** was confirmed as shown in Fig. 1 from the results of NOE experiments.

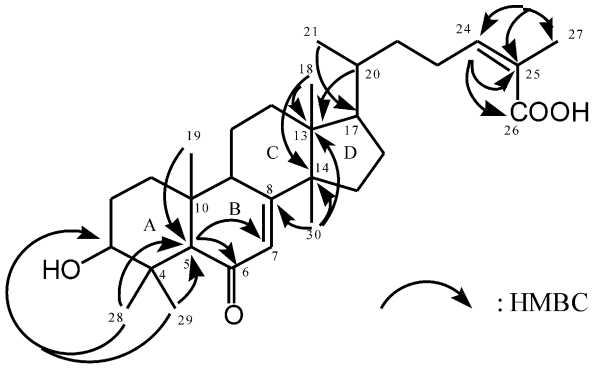

Fig. 2. Significant HMBC correlations for compound **1**.

Compound **3** was isolated as a colorless powder, mp 235–240 °C, $[\alpha]_D$ −45.1°(*c* 0.1, MeOH). Its EIMS spectrum gave a molecular ion peak at *m*/*z* 470.3400 (M^+) corresponding to molecular formula $C_{30}H_{46}O_4$. The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of **2** except for the appearance of carbon and proton signals (δ_C 74.0 and δ_H 3.63) due to an oxymethine group and loss of a carbonyl carbon signal. The HMBC spectrum of **3** showed cross-peaks between *gem*-dimethyl proton signals (δ 0.92 and 1.10, 3H each) and the oxymethine carbon resonance (δ 74.0), suggesting the presence of a hydroxyl group at C-3 position. The configuration of the hydroxyl group was assigned to the α-orientation

because the NOESY spectrum exhibited cross-peaks between the H-3 signal and both of the signals due to the *gem*-dimethyl group. Thus, the structure of **3** was represented as shown in Fig. 1.

Compound **4** was isolated as a colorless powder, mp 94–95 °C, $[\alpha]_D$ −85.7° (*c* 1.2, $CHCl_3$). Its MS spectrum gave a molecular ion peak at *m*/*z* 484.3186 (M^+) ($C_{30}H_{44}O_5$). The ^1H and ^{13}C NMR spectra of **4** suggested that the compound must be a tirucallane-type triterpene having a 3α-hydroxyl group and the same side-chain moiety as in **1**. Its UV spectrum exhibited an absorption maximum at 270 nm (ε; 6890), suggesting the presence of 1,4-dione-2-ene system. This was further confirmed by analysis of the HMBC spectrum of **4** which showed long range couplings between the following proton and carbon signals: δ 2.21 (H-5) and δ 200.5 (C-7); δ 2.46, 2.65 (H-12) and δ 202.4 (C-11); δ 1.31 (H-19) and δ 155.7 (C-9); δ 1.08 (H-30) and δ 149.9 (C-8). These facts indicated the presence of a 7,11-dioxo-8-ene structure in the molecule. The relative stereostructure of **4** was confirmed by NOE experiments as shown in Fig. 1.

Compound (**5**) was isolated as a colorless powder, mp 95–98 °C, $[\alpha]_D$ +255.1° (*c* 1.0, MeOH). Its high resolution-EIMS spectrum gave a molecular ion peak at *m*/*z* 486.3339 (M^+) corresponding to the molecular formula $C_{30}H_{46}O_5$. The ^1H and ^{13}C NMR spectra of **5** were quite similar, especially on the responses associated with rings A and D (including the side-chain moiety), to those of compound **2**. The ^{13}C NMR DEPT spectra indicated that there is an additional sp^3 quaternary carbon (δ 62.2) other than C-4, C-10, C-13, C-14 and an oxymethine carbon (δ 75.9), suggesting the presence of a secondary alcohol group in the molecule. In order to

Table 1
^1H NMR spectral data of compounds **1**–**6** (δ, ppm, 400 MHz)

Carbon	**1**[*1]	**2**[*1]	**3**[*1]	**4**[*2]	**5**[*2]	**6**[*1]
1	1.38 *m*	1.79 *m*	1.52 *m*	1.46 *m*	1.41 *m*	1.45 *m*
	2.27 *bt* (9.5)	2.17 *m*	2.24 *m*	2.24 *m*	2.41 *m*	1.98 *m*
2	1.83 *dd* (3.0, 14.0)	2.42 *m*	1.87 *dd* (3.2, 14.1)	1.67 *m*	2.32 *m*	1.84 *bd* (9.6)
	2.00 *m*	2.78 *bt* (5.9, 14.7)	2.07 *m*	2.07 *m*	2.77 *m*	2.01 *m*
3	3.60 *bs*	–	3.63 *bs*	3.51 *t* (2.6)	–	3.69 *bs*
4	–	–	–	–	–	–
5	3.06 *s*	2.14 *m*	2.60 *m*	2.21 *m*	3.25 *dd* (3.9, 6.6)	2.22 *dd* (12.5, 5.5)
6	–	2.38 *m*	2.54 *m*	2.42 *m*	1.52 *m*	1.98 *m*
	–	2.50 *m*	2.58 *m*	2.42 *m*	2.23 *m*	2.13 *m*
7	5.88 (*d*, 3.0)	–	–	–	4.36 (*t*, 6.6)	5.36 *bs*
8	–	–	–	–	–	–
9	2.95 *m*	–	–	–	–	2.50 *bd* (12.5)
10	–	–	–	–	–	–
11	1.50 *m*	2.27 *dd* (5.9, 11.3)	2.23 *m*	–	1.69 *m*	1.50 *m*
	1.66 *m*	2.36 *m*	2.44 *m*	–	2.18 *m*	1.60 *m*
12	1.60 *m*	1.80 *m*	1.30 *m*	2.46 *d* (19.1)	1.81 *m*	1.57 *m*
	1.73 *m*	1.80 *m*	1.72 *m*	2.65 *d* (19.1)	1.92 *m*	1.75 *m*
13	–	–	–	–	–	–
14	–	–	–	–	–	–
15	1.40 *m*	1.54 *m*	1.75 *m*	1.66 *m*	1.34 *m*	1.47 *m*
	1.50 *m*	2.15 *m*	2.50 *m*	2.16 *m*	1.77 *m*	1.60 *m*
16	1.26 *m*	1.38 *m*	1.38 *m*	1.38 *m*	1.28 *m*	1.32 *m*
	1.98 *m*	1.98 *m*	2.09 *m*	2.04 *m*	1.94 *m*	2.03 *m*
17	1.50 *m*	1.48 *m*	1.55 *m*	1.66 *m*	1.60 *m*	1.57 *m*
18	0.70 *s*	0.72 *s*	0.73 *s*	0.95 *s*	0.73 *s*	0.80 *s*
19	0.94 *s*	1.28 *s*	1.05 *s*	1.31 *s*	0.90 *s*	0.87 *s*
20	1.41 *m*	1.44 *m*	1.48 *m*	1.42 *m*	1.42 *m*	1.47 *m*
21	0.95 *d* (6.6)	0.94 *d* (6.0)	1.01 *d* (6.0)	0.89 *d* (6.6)	0.94 *d* (6.6)	0.97 *d* (5.8)
22	1.24 *m*	1.18 *m*	1.26 *m*	1.16 *m*	1.16 *m*	1.30 *m*
	1.65 *m*	1.56 *m*	1.67 *m*	1.56 *m*	1.50 *m*	1.68 *m*
23	2.78 *m*	2.44 *m*	2.76 *bs*	2.45 *m*	2.42 *m*	2.78 *m*
	2.87 *m*	2.60 *m*	2.89 *bs*	2.54 *m*	2.56 *m*	2.88 *m*
24	6.05 *t* (6.6)	6.07 *t* (6.6)	5.97 *t* (6.6)	6.06 *t* (7.0)	6.07 *t* (6.6)	6.06 *d* (7.3)
25	–	–	–	–	–	–
26	–	–	–	–	–	–
27	2.16 *bs*	1.92 *s*	2.16 *bs*	1.92 *bs*	1.92 *bs*	2.14 *s*
28	1.73 *s*	1.09 *s*	1.10 *s*	0.98 *s*	1.10 *s*	1.17 *s*
29	1.41 *s*	1.13 *s*	0.92 *s*	0.96 *s*	1.08 *s*	0.98 *s*
30	0.98 *s*	0.99 *s*	1.15 *s*	1.08 *s*	1.20 *s*	1.05 *s*

^1H NMR spectra were measured in C_5D_5N (*1) and $CDCl_3$ (*2).

Table 2
^{13}C NMR spectral data of compounds **1**–**6** (δ, ppm, 100 MHz)

Carbon	**1**[*1]	**2**[*1]	**3**[*1]	**4**[*2]	**5**[*2]	**6**[*1]
1	3.17	35.5	29.5	28.4	30.5	31.9
2	25.8	34.2	26.5	25.7	34.5	26.6
3	75.9	214.5	74.0	74.9	219.4	73.5
4	37.5	47.3	38.0	37.5	46.3	37.9
5	60.9	49.5	45.8	42.4	48.7	44.9
6	201.5	36.2	36.2	36.0	35.7	24.3
7	125.1	197.4	197.8	200.5	75.9	118.5
8	150.1	139.4	138.8	149.9	219.6	146.5
9	50.5	163.9	166.2	155.7	62.2	49.1
10	43.8	39.1	39.6	38.3	48.0	35.1
11	17.7	24.0	23.6	202.4	24.5	18.3
12	32.9	29.8	30.2	51.7	31.1	34.0
13	43.1	44.6	44.9	45.5	46.2	43.7
14	52.3	47.7	48.1	48.0	61.3	51.5
15	33.1	31.4	32.2	32.2	29.8	34.4
16	27.8	28.6	29.0	28.2	26.5	28.5
17	52.5	48.6	49.1	49.5	49.9	53.2
18	21.7	15.5	15.7	18.7	16.8	22.1
19	14.7	18.1	18.5	17.9	19.6	13.4
20	36.2	36.2	36.7	36.4	35.3	36.5
21	18.4	18.6	18.9	18.4	18.6	18.5
22	36.0	35.8	36.5	35.7	35.4	36.2
23	27.0	26.8	27.0	27.1	26.7	27.1
24	142.2	146.9	140.8	146.7	146.6	142.6
25	128.1	126.0	129.7	126.4	126.2	128.6
26	170.3	173.1	172.0	172.9	173.1	170.7
27	21.5	20.6	21.7	20.9	20.5	21.5
28	29.1	24.5	28.1	27.9	29.3	28.7
29	22.2	21.4	22.0	22.2	21.9	22.2
30	25.0	24.3	24.7	24.2	22.5	27.5

^{13}C NMR spectra were measured in C_5D_5N (*1) and $CDCl_3$ (*2).

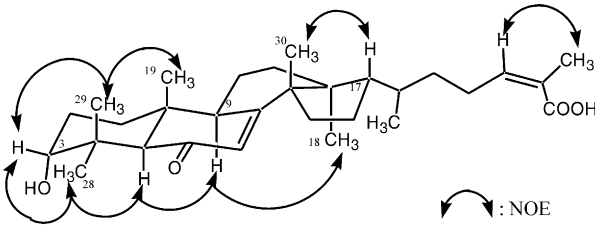

Fig. 3. Observed NOEs for compound **1**.

allocate the quaternary and oxymethine carbons, the HMBC spectrum of **5** was analyzed. As shown in Fig. 4, the methyl proton signal (δ 0.90, H-19) exhibited long-range correlations with C-1, C-5 and C-10, and the quaternary carbon (δ 62.2) signals showed cross-peaks with H-6, H-11, H-12 and the oxymethine proton (δ 4.36) signals. Furthermore, the proton signal at δ 4.36 displayed cross-peaks with the signal due to C-5 and the carbonyl carbon signal (δ 219.6, C-8) which showed correlations with the methylene proton (H-11) and methyl proton (H-30) signals. Thus **5** was represented by a spiro-structure shown in Fig. 1. The stereostructure of **5** was investigated by measurement of the NOESY spectrum. The H-5 signal (δ 3.25) exhibited a correlation with the H-7 resonance (δ 4.36) which in turn correlated

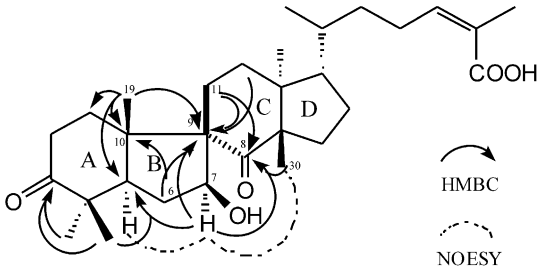

Fig. 4. HMBC and NOESY correlation for compound **5**.

with the methyl proton signal (δ 1.20) at C-14. Thus, **5** was confirmed as shown in Fig. 4.

The new compound (**2**) and known compounds, **6**, **8**, **9**, **11**, **12** and **14** showed moderate cytotoxicity against leukemia cells (L-1210: IC_{50} = 30, 20, 20, 40, 30, 20 and 20 μg/mL), respectively.

3. Experimental

3.1. General

Melting points were measured on a Yanagimoto micro melting-point apparatus (uncorr.). ^{1}H- and ^{13}C-NMR spectra were acquired on a JEOL JNM lambda-400 spectrometer in $CDCl_3$ or C_5D_5N containing TMS as internal standard, whereas MS spectra were recorded on a HITACHI M-2000 instrument. Optical rotations were measured using a JASCO DIP-360 digital polarimeter. Column chromatography was carried out on silica gel (Wakogel C-200) and Diaion HP-20 (Nippon Rensui). HPLC was conducted with a Spectra Physics SP 8800 and Sensyu SSC-3160 pump equipped with either a ERC-7520 (ERMA)-RI or HITACHI L-400-UV detectors. Silica gel 60 F254 (Merck) precoated TLC plates were used with detection carried out by spraying 10% sodium moribdene phosphorus solution followed by heating. Leukemia cells were obtained from the National Cancer Center Research Institute, Japan.

3.2. Plants material

Plant material was identified by Dr. G.O. Calderon (Herbario Nacional de Mexico, Instituto de Biologia, UNAM) and herbarium specimens have been depositied at the Instituto de Biologia, UNAM and the herbarium of the College of Pharmacy, Nihon University.

3.3. Extraction and isolation

Dried bark of *J. adstringens* (2 kg) was extracted with ethanol (4.0 l×8) under ultrasonication. The EtOH extract was concentrated under reduced pressure to give an oily material (225 g) and then 100 g of it was applied upon a Diaion HP-20 resin column, thus being eluted

successively with MeOH–H_2O (4:6, 5 l), MeOH–H_2O (7:3, 8 l), 100% MeOH (8 l) and acetone (7 l). Each was concentrated *in vacuo* to give an MeOH–H_2O (4:6) derived eluate (Fr. A, 59.2 g), a MeOH–H_2O (7:3) derived eluate (Fr. B, 38.0 g), a MeOH derived eluate (Fr. C, 16.6 g) and an acetone derived eluate (Fr. D, 20.1 g). Fr. C was subjected to silica gel CC, this being eluted successively with *n*-hexane:EtOAc = 15:1, 10:1, 5:1, 1:1, from EtOAc and MeOH to give fractions (Fr. C-1–Fr. C-8). Fr. C-3 (1310 mg) was recrystallized from EtOAc to give compound **6** (330 mg). Fr. C-6 (932 mg) was separated by normal phase HPLC (Kaseisorb LC 60-5, $10^{\phi}\times250$ mm, *n*-hexane:EtOAc = 2:1, flow rate; 3.0 ml min^{-1}). The eluate with a retention time 13–23 min was concentrated and recrystallized from CH_3CN to give compound **1** (31 mg). The mother liquor was concentrated and separated by reversed phase HPLC (Sensyu Pak PEGASIL ODS, $10^{\phi}\times250$ mm, MeOH–H_2O (85:15) at a flow rate of 3.0 ml min^{-1}) to give **2** (11 mg, *R*t = 22.4 min), **3** (5 mg, *R*t = 18.7 min), **4** (6 mg, *R*t = 12.0 min), **5** (22 mg, *R*t = 10.6 min); **7** [3 mg, *R*t was eluated at 24.3 min with a MeOH–H_2O (9:1) at a flow rate of 3.0 ml min^{-1})]. Fr. C-2 (1452 mg) was purified by normal phase HPLC (Diachroma Silica N-5 μm, $10^{\phi}\times250$ mm, *n*-hexane:EtOAc = 3:1, flow rate; 3.0 ml min^{-1}) to give **8** (132 mg, *R*t = 7.5 min), **9** (6 mg, 14.9 min), **11** (29 mg, *R*t = 15.7 min) and **12** (5 mg, *R*t = 23.3 min). Fr. D was subjected to silica gel CC and eluted successively with *n*-hexane:acetone = 50:1, 30:1, 10:1, 5:1, 1:1, from EtOAc and MeOH to give fractions (Fr. D-1–Fr. D-7). Fr. D-3 (577 mg) was purified by normal phase HPLC (Senshu Pak Silica 4251-N, $10^{\phi}\times250$ mm, *n*-hexane:EtOAc = 3:1, flow rate; 3.0 ml min^{-1}) to give **13** (83 mg, *R*t = 14.0 min) and **14** (3 mg, *R*t = 23.0 min). Fr. D-4 (2758 mg) was purified by reversed phase HPLC (CAPCELL PAK C_{18}-UG 120 Å–5μm, $10^{\phi}\times250$ mm, MeOH–H_2O (9:1) at a flow rate of 3.0 ml min^{-1}) to give **10** (46 mg, *R*t = 23.3 min).

3.3.1. 3α-Hydroxy-6-oxo-7,24Z-tirucalladien-26-oic acid (1)

Colorless powder, mp 233–234 °C; $[\alpha]_D$ −29.9° (MeOH, *c* 0.5); TLC: *R*f 0.43 (Merck, silica gel, *n*-hexane:EtOAc = 1:2), IR (KBr) cm^{-1}: 3368, 1691, 1673, 1649, UV λmax (MeOH) nm (ε): 246 (10,900), HR-EIMS (70 eV): *m*/*z* 470.3380 $(M)^+$ (calcd for $C_{30}H_{46}O_4$, requires 430.3393); for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2.

3.3.2. 3,7-Dioxo-8,24Z-tirucalladien-26-oic acid (2)

Colorless powder, mp 91–93 °C; $[\alpha]_D$ −27.7° (MeOH, *c* 1.0), TLC: *R*f 0.58 (Merck, silica gel, chloroform: acetone = 2:1), UV λmax (MeOH) nm (ε): 255 (10,040), HR-EIMS (70eV): *m*/*z* 468.3215 $(M)^+$ (calcd for $C_{30}H_{44}O_4$, requires 468.3237); for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2.

3.3.3. 3α-Hydroxy-7-oxo-8,24Z-tirucalladien-26-oic acid (3)

Colorless powder, mp 235–240 °C, $[\alpha]_D$ −45.1° (*c* 0.1, MeOH), TLC: *R*f 0.32 (Merck, silica gel, solvent; *n*-hexane:EtOAc = 1:1), λ_{max} (MeOH) nm (ε): 256 (9,970), HR-EIMS (70 eV): *m*/*z* 470.3400 $(M)^+$ (calcd for $C_{30}H_{46}O_4$, requires 470.3396); for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2.

3.3.4. 7,11-Dioxo-3α-hydroxy-8,24Z-tirucalladien-26-oic acid (4)

Yellow powder, mp 94–95 °C, $[\alpha]_D$ −85.7° (MeOH, *c* 1.0), TLC: Rf 0.24 (Merck, silica gel, *n*-hexane:EtOAc = 1:1), λ_{max} (MeOH) nm (ε): 270 (6,885), HR-EIMS (70 eV): *m*/*z* 484.3186 $(M)^+$ (calcd for $C_{30}H_{44}O_5$, requires 484.3188); for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2.

3.3.5. 3,8-Dioxo-7β-hydroxy-7,9-cycro-7,8-seco-24Z-tirucalladien-26-oic acid (5)

Colorless powder, mp 95–98 °C $[\alpha]_D$ +255.1° (MeOH, *c* 1.0), TLC: *R*f 0.34 (Merck, silica gel, *n*-hexane: EtOAc = 1:1); HR-EIMS (70 eV): *m*/*z* 486.3339 $(M)^+$ (calcd for $C_{30}H_{46}O_5$ requires 486.3345); for ^1H and ^{13}C NMR spectral data, see Tables 1 and 2.

4. Cytotoxic activity

The effects of isolated compounds on growth of leukemia cells (L-1210) were investigated as follows. Cells were suspended in RPMI 1640 medium (NISSUI) containing 10% fetal bovine serum supplemented respectively with L-glutamine and sodium carbonate. Aliquots (2 ml) of cell suspension (approximately 1×10^5 cells ml^{-1}) were transferred into vials. After addition of compounds to be tested individually at the concentration indicated, the cells were incubated under an atmosphere containing 5% CO_2 at 37 °C for 3 days and then viable cell numbers were counted. Cytotoxicity was determined by comparing viable cell numbers with that of the control.

Acknowledgements

This work was supported in part by a Grant from the Ministry of Education, Culture, Sports, Science, and Technology to promote cooperation between academia and industries.

References

Govindachari, T.R., Suresh, G., Krishna Kumari, G.N., 1994. Triterpenoids from *dysoxylum malabaricum*. Phytochemistry 37 (4), 1127–1129.

Ikuta, A., Morikawa, A., 1992. Triterpenes from *Stauntonia hexaphylla* callus tissues. Journal of Natural Products 55 (9), 1230–1233.

Jain, M.K., Yu, B.Z., Rogers, J.M., Smith, A.E., Boger, E.T.A., Ostrander, R.L., 1995. Specific competitive inhibitor of secreted phospholipase A_2 from berries of *Schinus terebinthifolius*. Phytochemistry 39 (3), 537–547.

Konoike, T., Takahashi, K., Araki, Y., Horibe, I., 1997. Practical partial synthesis of Myriceric acid A, an endothelin receptor antagonist, from Oleanolic acid. J. Org. Chem. 62, 960–966.

Mahato, S.B., Kundu, A.P., 1994. ^{13}C NMR spectra of pentacyclic triterpenoids—a compilation and some salient features. Phytochemistry 37 (6), 1517–1575.

Melek, F.R., Radwan, A.S., Ahmed, A.A., Hamman, A.A., Aboutabl, E.A., 1989. Triterpenes from *Atractylis carduus* L. Pharmazie 44, 735.

Monaco, P., Caputo, R., Palumbo, G., Mangoni, L., 1974. Triterpene components of galls on the leaves of *Pistacia terebinthus*, produced by *Pemphigus semilunarius*. Phytochemistry 13, 1992–1993.

Mulholland, D.A., Nair, J.J., 1994. Triterpenoids from *Dysoxylum Pettigrewianum*. Phytochemistry 37 (5), 1409–1411.

Silverstein, R. M., Webster, F.X., 1998. ''Spectrometric Identification of Organic Compounds'', sixth ed. (Japanese Edition, p. 224, Tokyo Kagakudojin).