Cytotoxic Limonoids and Tetranortriterpenoids from *Melia azedarach*

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The ethanolic extract of the root bark of *Melia azedarach* exhibited cytotoxic activity against lymphocytic leukemia P388 cell lines *in vitro*. Systematic fractionation of the extract monitored by cytotoxic bioassay led to the isolation of two new azadirachtin-type limonoids, 1-tigloyl-3-acetyl-11-methoxymeliacarpinin (1) and 1-acetyl-3-tigloyl-11-methoxymeliacarpinin (2), together with three highly cytotoxic sendanin-type limonoids, 29-isobutylsendanin (3), 12-hydroxyamoorastin (4) and 29-deacetylsendanin (5). The acetylated derivatives of 4 also underwent cytotoxic bioassay.

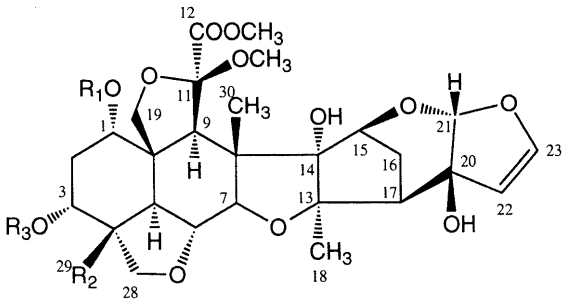
Key words *Melia azedarach*; cytotoxity; limonoid; azadirachtin; sendanin

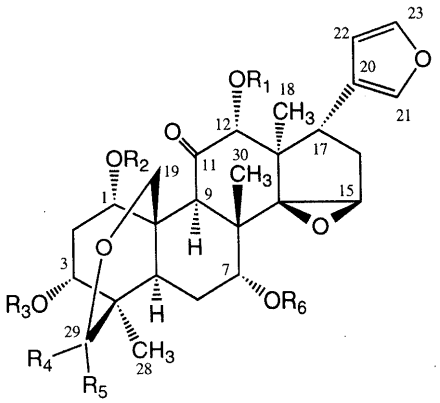
Melia azedarach L. (Meliaceae) is a widely distributed tree, whose barks, fruit and leaves have been traditionally used as anthelmintics in China[1] and to cure malaria, fevers and venereal diseases in Africa.[2] During our preliminary screening test for antitumour agents from plants, the ethanolic extract of the root bark of *M. azedarach* exhibited significant cytotoxic activity against P388 cells *in vitro*. In this paper, we describe the isolation, structural elucidation and cytotoxic activity of some of the effective principles from this plant.

Results and Discussion

An aqueous solution of the ethanolic extract prepared from *M. azedarach* was partitioned with dichloromethane and *n*-butanol, successively. The corresponding extracts, CH_2Cl_2, *n*-BuOH and H_2O, were obtained by concentration under reduced pressure. When each extract was subjected to cytotoxic bioassay against P388 cells, the activity was found to be concentrated in the dichloromethane extract. The cytotoxic extract was subjected to silica-gel column chromatography to furnish fractions A—N obtained by eluting with an *n*-hexane–ethyl acetate gradient system. Further repeated HPLC of the active fraction L using an octadecyl silica (ODS) column gave compounds **1**—**5** monitored with a bioassay-directed isolation procedure as shown in Table 3. Compounds **1** and **2** were novel substances, while **3**—**5** were known and respectively confirmed to be 29-isobutylsendanin,[3] 12-hydroxyamoorastin and 29-deacetylsendanin[4-6] following comparison of their spectral and physical data with that in the literature.

1-Tigloyl-3-acetyl-11-methoxymeliacarpinin (**1**) obtained as colourless crystals, mp 165—167 °C and $[\alpha]_D$ −12.6°, had the molecular formula $C_{35}H_{46}O_{14}$ from high resolution-electron impact ionization mass spectrometry (HR-EIMS). NMR indicated the presence of two methoxyl (δ 3.35 and 3.70, each 3H, s), one acetyl (δ 1.94, 3H, s) and one tigloyl (δ 6.90, 1H, qq; δ 1.78, 3H, dd; δ 1.83,



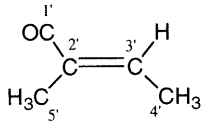


(**1**) R_1=Tig, R_2=CH_3, R_3=$COCH_3$

(**1a**) R_1=Tig, R_2=$COOCH_3$, R_3=$COCH_3$

(**2**) R_1=$COCH_3$, R_2=CH_3, R_3=Tig

Tig=Tigloyl



(**3**) R_1=R_3=$COCH_3$, R_2=R_5=R_6=H, R_4=Isobutyryl

(**4**) R_1=R_2=R_6=H, R_3=$COCH_3$, R_4=OH, R_5=H or R_4=H, R_5=OH

(**5**) R_2=R_6=H, R_1=R_3=$COCH_3$, R_4=OH, R_5=H or R_4=H, R_5=OH

(**4a**) R_2=R_5=R_6=H, R_1=R_3=$COCH_3$, R_4=$OCOCH_3$

(**4b**) R_5=R_6=H, R_1=R_2=R_3=$COCH_3$, R_4=$OCOCH_3$

(**4c**) R_2=R_5=H, R_1=R_3=R_6=$COCH_3$, R_4=$OCOCH_3$

(**4d**) R_5=H, R_1=R_2=R_3=R_6=$COCH_3$, R_4=$OCOCH_3$

Chart 1

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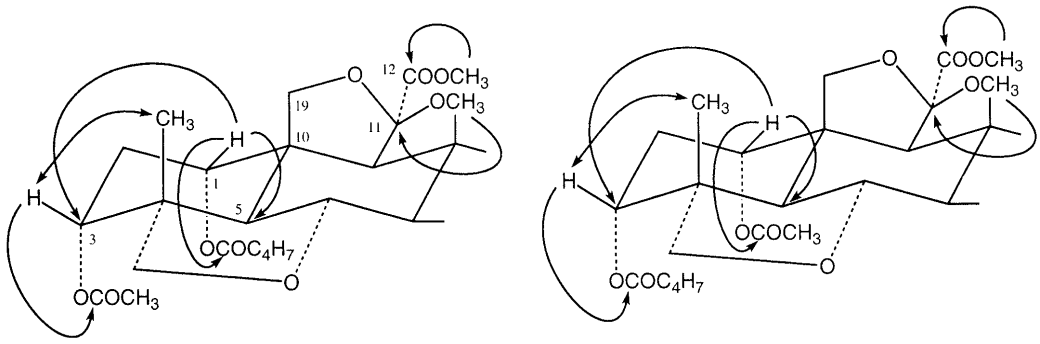

Fig. 1. HMBC (→) and NOESY (↔) Correlations in Partial Structures of **1** and **2**

Table 1. ^{1}H-NMR Data of Limonoids from *Melia azedarach* at 400 MHz ($CDCl_3$, δ, J=Hz)

	1	2	4[a)]	5	4a	4b	4c	4d
1	4.77 br t (2.7)	4.58 br t (2.8)	4.56 d, 4.63 d (3.2)	4.25 br m, 4.32 br m	4.28 br m	5.16 d (3.5)	4.26 d (4.9)	5.15 d (3.2)
2	2.14, 2.08	2.33, 2.05		2.83 dt (17, 4.8)	2.81 dt (16, 4.6)	2.72 dt (17, 4.6)	2.80 dt (16, 4.6)	2.71 dt (16, 4.2)
3	4.90 br t (3.1)	4.96 br t (2.8)	4.91 d, 5.55 d (3.3)	4.88 d, 5.34 d (4.2)	5.27 d (2.8)	5.37 d (3.2)	5.24 d (2.8)	5.38 d (2.8)
5	3.00 d (12.7)	3.06 d (12.8)	2.95 m	2.63 m	2.73 dd (14, 4.1)	2.88 dd (14, 4.2)	2.57 dd (14, 4.4)	2.71 dd (13, 4.2)
6	3.94 dd (12.7, 2.8)	3.93 dd (12.8, 2.8)						
7	4.49 d (2.8)	4.52 d (2.8)	3.71 br m	3.59 br m, 3.63 br m	3.67 br m	3.69 br m	4.78 br t (2.3)	4.81 br t (2.4)
9	3.53 s	3.66 s	4.17 s	4.58 s	4.61 s	4.30 s	4.62 s	4.34 s
12			4.89 s	5.31 s	5.27 s	5.40 s	5.24 s	5.31 s
15	4.11 m	4.12 m	3.80 s	3.75 s	3.75 s	3.73 s	3.60 s	3.76 s
16	2.06, 1.85	2.19, 1.86	2.20 q (6.4)	2.21 dq (6.4, 0.7)	2.23 dq (6.4, 0.7)	2.24 dq (6.4, 0.7)	2.22 dq (6.3, 0.7)	2.23 dq (6.2, 0.8)
17	2.12	2.16	3.04 dd (9.7, 4.6)	2.96 dd (11.2, 6.2)	2.98 dd (11, 6.2)	2.97 dd (11, 6.2)	2.96 dd (11, 6.3)	2.96 dd (11, 6.0)
18	1.48 s	1.60 s	1.39 s, 1.40 s	1.31 s, 1.32 s	1.32 s	1.26 s	1.29 s	1.24 s
19a	4.15 d (9.3)	4.15 d (9.3)	4.33 d, 4.55 d (13)	4.16 d, 4.30 d (12)	4.30 d (13)	4.29 d (13)	4.30 d (13)	4.32 d (13)
19b	3.87 d (9.3)	3.85 d (9.3)	4.43 d, 4.75 d (13)	4.27 d, 4.49 d (12)	4.33 d (13)	4.35 d (13)	4.34 d (13)	4.36 d (13)
21	5.62 s	5.63 s	7.21	7.12	7.13	7.12	7.12	7.12
22	4.85 d (2.9)	4.88 d (2.9)	6.65	6.13	6.15	6.10	6.14	6.10
23	6.36 d (2.9)	6.37 d (2.9)	7.31 t (1.6)	7.32 t (1.6)	7.33 t (1.6)	7.33 t (1.6)	7.34 t (1.6)	7.34 t (1.6)
28a	3.57 d (3.0)	3.58 d (7.9)	0.94 s, 0.96 s	0.87 s, 0.88 s	0.82 s	0.82 s	0.79 s	0.82 s
28b	3.55 d (3.0)	3.52 d (7.9)						
29Me	0.98 s	0.98 s	4.89 s, 5.04 s	4.78 s, 4.87 s	5.79 s	5.80 s	5.76 s	5.80 s
30Me	1.53 s	1.55 s	1.17 s, 1.24 s	1.14 s, 1.17 s	1.16 s	1.16 s	1.22 s	1.24 s
1-OH				2.30 d, 2.35 d (7.0)	2.30 br d (7.2)		2.35 br d (7.2)	
14-OH	4.26 s	4.12 s						
20-OH	6.09 s	6.13 s						
11-OMe	3.35 s	3.37 s						
12-OMe	3.70 s	3.73 s						
OAc	1.94 s	1.98 s	1.84 s, 1.85 s	1.97 s, 1.98 s	1.94 s	1.96 s	1.98 s	1.99 s
				2.08 s, 2.09 s	2.11 s	1.98 s	2.07 s	2.00 s
					2.11 s	2.04 s	2.11 s	2.02 s
						2.12 s	2.18 s	2.12 s
Tigloyl								2.20 s
3′	6.90 qq (7.1, 1.4)	6.90 qq (7.1, 1.4)						
4′	1.78 dd (7.1, 1.1)	1.81 dd (7.1, 1.1)						
5′	1.83 d (1.1)	1.82 d (1.1)						

a) Measured at $CDCl_3$ + 20% pyridine-d_5.

3H, d) group similar to those of 1-tigloyl-3-acetyl-11-methoxyazadirachtin (**1a**).[7,8] However, instead of the three methoxyl, five methyl and four carbonyl (δ 170.1, 173.3, 169.7 and 166.8) groups in **1a**, two methoxyl, six methyl and three carbonyl (δ 170.1, 169.0 and 166.6) groups were observed in compound **1**. This suggested that one of the methoxycarbonyl groups in **1a** was replaced by the methyl group in **1**. The deduction that a methyl group (δ 0.98, 3H, s) was attached to the position C-4 in **1** was also supported by the movement upfield (0.5 ppm) of the chemical shifts due to the 3β, 6β and 28β positional protons in comparison with **1a**. This was also supported by the cross-peak between the 3β-proton and the 29-methyl protons in the nuclear Overhauser effect spectroscopy (NOESY) spectrum. The position of the acetyl and tigloyl groups was confirmed by the ^{1}H–^{13}C long-range correlation of the heteronuclear multiple bond connectivity (HMBC) spectrum (Fig. 1). From above results, compound **1** was determined to be 1-tigloyl-3-acetyl-11-methoxymeliacarpinin as shown in Chart 1.

1-Acetyl-3-tigloyl-11-methoxymeliacarpinin (**2**), colourless crystals, mp 149—151 °C, $[\alpha]_D$ +5.8°, had the same molecular formula $C_{35}H_{46}O_{14}$ as **1** from EI-MS and ^{13}C-NMR spectral data suggesting the presence of two methoxyl, one acetyl and one tigloyl group to be similar to those of **1**. The structural difference between compounds **1** and **2** was assumed that the 1α-*O*-tigloyl and 3α-*O*-acetyl groups in **1** were interchanged in **2**. This assumption was confirmed by the ^{1}H–^{13}C long-range correlation of the HMBC spectrum (Fig. 1). Therefore, compound **2** was

Table 2. ^{13}C-NMR Data of Limonoids from *Melia azedarach* (100 MHz, δ, $CDCl_3$)

	1	2	3	4[a]	5	4a	4b	4c	4d
1	70.2 d	71.1 d	70.1 d	69.1, 69.2 d	70.3 d	70.1 d	72.3 d	69.9 d	72.2 d
2	28.3 t	28.0 t	35.0 t	35.5, 36.2 t	35.4, 36.2 t	35.1 t	33.5 t	35.0 t	33.5 t
3	70.7 d	70.4 d	73.6 d	73.4, 76.3 d	73.6, 76.3 d	73.7 d	72.8 d	73.7 d	73.0 d
4	42.4 s	42.7 s	39.5 s	39.5, 39.6 s	40.0, 40.1 s	39.3 s	38.9 s	39.2 s	38.9 s
5	35.1 d	35.1 d	28.0 d	25.3, 27.7 d	25.5, 27.9 d	28.0 d	29.3 d	28.8 d	30.0 d
6	71.1 d	71.1 d	25.8 t	25.0, 27.0 t	25.5, 27.4 t	25.8 t	26.2 t	22.8 t	23.1 t
7	83.2 d	83.8 d	70.4 d	69.4 d	70.5 d	70.4 d	70.3 d	72.8 d	72.8 d
8	51.3 s	51.2 s	42.6 s	41.8, 41.9 s	42.4, 42.5 s	42.6 s	42.9 s	41.3 s	41.6 s
9	47.9 d	47.9 d	48.4 d	47.6, 47.8 d	48.4, 48.7 d	48.4 d	48.1 d	49.4 d	49.0 d
10	49.8 s	49.7 s	41.5 s	41.2 s	41.8 s	41.6 s	39.9 s	41.5 s	39.8 s
11	106.7 s	107.0 s	206.7 s	213.6, 213.7 s	206.8 s	206.6 s	206.0 s	205.5 s	204.7 s
12	169.0 s	169.4 s	78.6 d	78.3, 78.4 d	78.5, 78.7 d	78.6 d	77.6 d	78.6 d	77.5 d
13	94.9 s	94.9 s	46.0 s	45.7, 45.8 s	45.7, 45.8 s	46.0 s	45.6 s	46.1 s	45.6 s
14	93.0 s	93.3 s	72.0 s	72.7, 72.9 s	72.1, 72.3 s	72.0 s	71.5 s	71.4 s	71.3 s
15	81.2 d	81.1 d	58.5 d	58.0 d	58.7 d	58.5 d	58.0 d	58.0 d	57.8 d
16	29.6 t	29.7 t	33.6 t	32.4 t	33.6 t	33.6 t	32.1 t	33.6 t	31.8 t
17	50.7 d	50.8 d	38.4 d	38.5 d	38.2 d	38.4 d	38.6 d	38.4 d	38.4 d
18	26.5 q	25.9 q	20.7 q	20.4, 20.5 q	20.8 q	20.7 q	20.7 q	20.7 q	20.7 q
19	70.5 t	70.7 t	64.7 t	58.1, 63.7 t	58.7, 63.9 t	64.8 t	64.4 t	64.6 t	64.1 t
20	86.2 s	86.3 s	122.5 s	123.5, 123.6 s	122.5, 122.6 s	122.5 s	122.4 s	122.2 s	122.2 s
21	109.2 d	109.3 d	142.5 d	141.1 d	142.4 d	142.5 d	142.6 d	142.6 d	142.7 d
22	108.0 d	107.9 d	111.9 d	112.3 d	111.9 d	111.9 d	111.7 d	111.8 d	111.7 d
23	145.7 d	145.8 d	140.7 d	139.8 d	140.7 d	140.7 d	140.6 d	140.7 d	140.7 d
28	76.5 q	76.6 q	15.8 q	13.7 q	15.6 q	15.8 q	15.8 q	15.7 q	15.8 q
29	18.3 t	18.1 t	94.4 d	95.2, 95.5 d	96.1, 96.4 d	94.5 d	94.4 d	94.4 d	94.4 d
30	17.8 q	17.7 q	19.4 q	18.1, 19.0 q	18.5, 19.6 q	19.3 q	19.3 d	19.4 q	19.6 q
COOMe	170.1 s	170.1 s							168.9 s
							169.1 s	169.5 s	169.1 s
						169.8 s	169.8 s	169.6 s	169.5 s
			169.9 s	169.4, 169.6 s	170.1 s	169.9 s	169.9 s	169.8 s	169.7 s
			170.4 s		170.6 s	170.4 s	170.1 s	170.4 s	169.9 s
$COCH_3$	21.0 q	21.0 q	22.3 q	22.1, 22.3 q	22.5, 22.8 q	22.4 q	22.1 q	22.2 q	22.0 q
						21.1 q	21.1 q	21.0 q	21.1 q
									21.1 q
							21.3 q	21.3 q	21.3 q
			21.5 q		21.4, 21.5 q	21.5 q	21.4 q	21.3 q	21.3 q
OCH_3	53.2 q	53.8 q							
	52.4 q	53.1 q							
Isobutyryl or tigloyl									
1′	166.6 s	166.7 s	175.7 s						
2′	128.5 s	128.4 s	34.2 d						
3′	137.9 d	138.1 d	18.6 q						
4′	14.4 q	14.4 q	18.9 q						
5′	12.1 q	11.9 q							

a) Measured in $CDCl_3$ + 20% pyridine-d_5.

established to be 1-acetyl-3-tigloyl-11-methoxymeliacarpinin as shown in Chart 1.

To investigate the relationship between structures and activities, various acetylated derivatives of **4** were prepared. Acetylation of **4** was carried out in the usual way using acetic anhydride and pyridine to give sendanin (**4a**), 1-acetylsendanin (**4b**), 7-acetylsendanin (**4c**) and 1,7-diacetylsendanin (**4d**). The structure of **4a** was confirmed to be sendanin by comparing its physical and spectral data with that in the literature.[9] Both **4b** and **4c** had the same molecular formula $C_{34}H_{42}O_{13}$ from HRMS and their NMR spectra were similar to those of **4a** except for one additional acetyl group. The 1β- and 7β-positional proton chemical shifts, δ 4.28 and 3.67 in **4a**, were shifted downfield to δ 5.16 in **4b** and to δ 4.78 in **4c**, respectively. Consequently, **4b** and **4c** were shown to be 1-acetylsendanin and 7-acetylsendanin, respectively; **4d** was also established as 1,7-diacetylsendanin.

Compounds **4** and **5** are present as a mixture of two 29-positional epimers in solution. Normally, the chemical shift of 3β-H appears more downfield in the *exo*-configuration of 29-OR than in the *endo*-configuration, since the 29-positional alcoholic oxygen located in a quasi-1,3-diaxial direction exerts a marked deshielding effect on 3β-H.[10] In 29-deacetylsendanin (**5**), the chemical shifts of the 3β-H in the *exo*- and *endo*-configurations of 29-OH are observed at δ 5.34 and 4.88 ($CDCl_3$) respectively, while in **4** they are at δ 5.55 and 4.91 ($CDCl_3$ and 20% pyridine-d_5). In sendanin with an *exo*-configuration of 29-OAc, it appears at δ 5.25 ($CDCl_3$).[10] Comparing the chemical shifts of 3β-H in **4a**, **4b**, **4c** and **4d** with the above data, the four acetylated derivatives of **4** should be due to the *exo*-configuration of the 29-acetyl group. This assumption was confirmed by the NOESY experiment on **4c**, which showed a clear correlation between 29-H and 6β-H as well as 19β-H. The fact that only the

Table 3. Cytotoxic Activities against P388 Cells *in Vitro*

	Yield (g)	IC_{50} (μg/ml)		Yield (g)	IC_{50} (μg/ml)
Ethanolic extract	241	1.7	Fractions		
Partitions			J (5:5)[a]	2.64	3.0
CH_2Cl_2 extract	56	0.17	K (5:5)[a]	1.86	0.07
n-BuOH extract	148	50	L (0:10)[a]	15.1	<0.1
H_2O extract	35	100	M (5:5)[b]	12.8	6.5
Fractions			N (5:5)[b]	2.83	6.0
A (9:1)[a]	1.13	6.0	Isolated compounds		
B (9:1)[a]	0.40	7.9	**1**	0.054	3.2
C (9:1)[a]	0.83	3.0	**2**	0.033	3.3
D (8:2)[a]	2.12	3.0	**3**	0.018	0.034
E (8:2)[a]	2.43	4.2	**4**	0.40	0.090
F (8:2)[a]	3.37	1.3	**5**	0.30	0.026
G (7:3)[a]	2.11	2.4	**4a**	—	0.078
H (7:3)[a]	2.96	2.5	**4b**	—	0.44
I (5:5)[a]	2.49	3.0	**4c**	—	0.55
			4d	—	>10

a) Eluted with *n*-hexane–EtOAc. *b*) Eluted with EtOAc–MeOH.

exo-configuration existed after acetylation suggested that the 29-OH in the *exo*-configuration could be more easily acetylated than one in the *endo*-configuration, by converting the *endo*-type gradually to the *exo*-type during the acetylation process.

The cytotoxic activity of compounds **1**—**5** and **4a**—**4d** against P388 lymphocytic leukemia cells are shown in Table 3. Three sendanin-type limonoids, **3**, **4** and **5**, isolated from fr. L exhibited very strong cytotoxic activity against P388 cells *in vitro*, however, from the unremarkable cytotoxic increase for the limonoids from fr. L, it was assumed that the cytotoxic activity of fr. L was due to synergism between their sendanin-type limonoids. Also, acetylation of the 1α- or 7α-OH of compound **4** decreased the cytotoxic activity. In particular, when both the 1α- and 7α-OH of **4** were acetylated, the cytotoxity was almost lost. In addition, azadirachtin-type limonoids, **1** and **2**, also exhibited significant cytotoxic activity, but to a lesser degree than the sendanin-type limonoids except for **4d**. The azadirachtin-type compounds are interest because of their remarkable inhibition of insect feeding and ecdysis inhibiting activity,[11,12] but their cytotoxic activity has not been reported until now.

Experimental

General Procedure Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. $[\alpha]_D$: JASCO DIP-4. MS: VG AutoSpec. IR: Perkin Elmer 1710. ^{1}H- and ^{13}C-NMR: Bruker AM 400 and 500 MHz at 303 K. NOESY experiments were carried out with a mixing time of 0.6 s and processed on a Bruker data station with an Aspect 3000 computer. Silica-gel column chromatography was carried out on Merck Kieselgel 60 (70—230 mesh) using amounts equivalent to 100 times the sample. Medium pressure liquid chromatography (MPLC) was performed on a column (22 mm i.d. × 300 mm) packed with 20 μm silica-gel or 20 μm ODS. Final purification was made by HPLC using a Hibar RT RP-18 column (20 mm i.d. × 250 mm) packed with 7 μm ODS. The NMR coupling constants (J) are given in Hz.

Plant Material Fresh root bark of *M. azedarach* L. was collected at Jiangsu, China in 1993. The species was identified by Professor Zhi-Yu Zhang (Second Military Medical University, Shanghai, China). A reference specimen has been deposited in Herbarium of the Tokyo College of Pharmacy.

Extraction and Isolation The fresh root bark of *M. azedarach* (5 kg) was cut into slices and extracted three times with 24 l 70% EtOH at 70 °C. The concentrated extract (241 g) was partitioned between CH_2Cl_2 and H_2O, then between *n*-butanol and H_2O. The CH_2Cl_2 soluble fraction (56 g) was subjected to silica-gel column chromatography using *n*-hexane–EtOAc (1:0—0:1) as an eluting system to give fourteen fractions (frs. A—N). Fraction L (15 g), one of the most active fractions, was further chromatographed on a silica-gel column and eluted with CH_2Cl_2–MeOH (60:1—10:1). Then the fractions eluted by CH_2Cl_2–MeOH, (60:1) and (30:1), were further subjected to ODS MPLC and purified using ODS HPLC with MeOH/H_2O or MeCN/H_2O solvent systems to give compounds **1** (54 mg), **2** (33 mg), **3** (18 mg), **4** (400 mg) and **5** (300 mg).

1-Tigloyl-3-acetyl-11-methoxymeliacarpinin (1) Colourless crystals, mp 165—167 °C (from acetone), $[\alpha]_D$ −12.6° ($CHCl_3$; c=0.5); IR ν_{max} ($CHCl_3$) cm^{-1}: 3240, 1742, 1707, 1624; EI-MS m/z: 690 [M$^+$], 658, 631, 575, 519; HRMS m/z: Found 690.2865, required for $C_{35}H_{46}O_{14}$ 690.2887. ^{1}H- and ^{13}C-NMR spectra are listed in Tables 1 and 2.

1-Acetyl-3-tigloyl-11-methoxymeliacarpinin (2) Colourless crystals, mp 149—151 °C (from acetone), $[\alpha]_D$ +5.8° ($CHCl_3$; c=0.2); IR ν_{max} ($CHCl_3$) cm^{-1}: 3450, 1742, 1705, 1650, 1630; EI-MS m/z: 690 [M$^+$], 658, 631, 575, 519, 477; ^{1}H- and ^{13}C-NMR spectra are listed in Tables 1 and 2.

The structures of 29-isobutylsendanin (**3**),[3] 12-hydroxyamoorastin (**4**) and 29-deacetylsendanin (**5**)[4-6] were confirmed by comparing their physical and spectral data with that in the literature.

Acetylation of 4 12-Hydroxyamoorastin (**4**, 50 mg) was acetylated with 2 ml Ac_2O–pyridine (1:1) for 16 h at room temperature. Then toluene was added and the reaction mixture concentrated under reduced pressure. The residual material was subjected to ODS HPLC using a MeCN–H_2O (1:1) solvent system to give four acetylated derivatives, sendanin (**4a**, 13.5 mg), 1-acetylsendanin (**4b**, 17.5 mg), 7-acetylsendanin (**4c**, 14.5 mg) and 1,7-diacetylsendanin (**4d**, 5 mg). The structure of sendanin (**4a**) was elucidated by comparing the physical and spectral data with that in the literature.[9]

1-Acetylsendanin (4b) Colourless crystals, mp 158—160 °C (from acetone), $[\alpha]_D$ −8.6° ($CHCl_3$, c=0.1); IR ν_{max} ($CHCl_3$) cm^{-1}: 1740, 1720 (sh), 1600; HRMS m/z: Found 658.2608, required for $C_{34}H_{42}O_{13}$ 658.2625. ^{1}H- and ^{13}C-NMR spectra are listed in Tables 1 and 2.

7-Acetylsendanin (4c) Colourless crystals, mp 150—152 °C (from acetone), $[\alpha]_D$ −15.6° ($CHCl_3$, c=0.1); IR ν_{max} ($CHCl_3$) cm^{-1}: 1740, 1600; HRMS m/z: Found 658.2629, required for $C_{34}H_{42}O_{13}$ 658.2625. ^{1}H- and ^{13}C-NMR spectra are listed in Tables 1 and 2.

1,7-Diacetylsendanin (4d) Colourless crystals, mp 253—255 °C (from acetone), $[\alpha]_D$ −13.0° ($CHCl_3$, c=0.9); IR ν_{max} ($CHCl_3$) cm^{-1}: 1740, 1720 (sh), 1600; EI-MS m/z: 700 [M$^+$]. ^{1}H- and ^{13}C-NMR spectra are listed in Tables 1 and 2.

Bioassay of Cytotoxic Activity against P388 Cells MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay was performed in a 96-well plate.[13] The assay is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to give a blue formazan product which can be measured spectrophotometrically. Mouse P388 leukemia cells (2×10^4 cells/ml) were inoculated in each well with 100 μl/ml RPMI-1640 medium (Nissui Pharm. Co., Ltd.) supplemented with 5% fetal calf serum (Mitsubishi Chemical Industry Co., Ltd.) and kanamycin (100 μg/ml) at 37 °C in a humidified atmosphere of 5% CO_2. Various drug concentrations (10 μl) were added to the cultures at day 1 after transplantation. At day 3, 20 μl MTT solution (5 mg/ml) per well was added to each cultured medium. After a further 4 h of incubation, 100 μl 10% sodium dodecyl sulfate–0.01 N HCl solution was added to each well and the formazan crystals in each well were dissolved by stirring with a pipette. The optical density measurements were made using a microplate reader (Tohso MPR-A4*i*) at two wavelengths (550 and 700 nm). In all these experiments, 3 replicate wells were used to determine each data point.

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