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CYTOTOXIC AZADIRACHTIN-TYPE LIMONOIDS FROM *MELIA AZEDARACH*

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Key Word Index—*Melia azedarach*; Meliaceae; cytotoxicity; limonoid; azadirachtin, meliacarpinin.

Abstract—Four new limonoids, 1-tigloyl-3,20-diacetyl-11-methoxymeliacarpinin, 3-tigloyl-1,20-diacetyl-11-methoxymeliacarpinin, 1-cinnamoyl-3-hydroxy-11-methoxymeliacarpinin, and 1-deoxy-3-methacrylyl-11-methoxymeliacarpinin, together with a known limonoid, 1-cinnamoyl-3-acetyl-11-methoxymeliacarpinin, were isolated from the extract of the root bark of *Melia azedarach*. The structures were elucidated by spectroscopy.

INTRODUCTION

Limonoids have attracted much attention because of the marked insect antifeedant [1] and growth regulating properties [2], cytotoxic [3] and antiviral activities [4]. Recently, we isolated two new azadirachtin derivatives and three highly cytotoxic sendanin analogues from the ethanolic extract of the root bark of *Melia azedarach* [5]. During our continuing study of the cytotoxic limonoids from the plant, four new azadirachtin analogues, 1-tigloyl-3,20-diacetyl-11-methoxymeliacarpinin, (**1**), 3-tigloyl-1,20-diacetyl-11-methoxymeliacarpinin (**2**), 1-cinnamoyl-3-hydroxy-11-methoxymeliacarpinin (**3**), and 1-deoxy-3-methacrylyl-11-methoxymeliacarpinin (**4**), together with a known limonoid, 1-cinnamoyl-3-acetyl-11-methoxymeliacarpinin (**5**), were isolated and tested for cytotoxicity.

RESULTS AND DISCUSSION

The ethanolic extract of *M. azedarach* was suspended in water and then partitioned with dichloromethane and *n*-butanol, successively. The dichloromethane extract, the most cytotoxic part, was subjected to silica gel column chromatography eluting with a *n*-hexane–ethyl acetate gradient system (1:0–0:1). Compounds **1**, **2** and **4** were isolated from fraction K (*n*-hexane–ethyl acetate, 1:1), and compounds **3** and **5** from fraction L (ethyl acetate). Each compound was finally purified by HPLC using an ODS column.

1-Tigloyl-3,20-diacetyl-11-methoxy meliacarpinin (**1**), a colourless powder, was assigned the molecular formula $C_{37}H_{48}O_{15}$ ($[M]^+$ at *m/z* 732, mass spectrum). The ^1H and ^{13}C NMR spectra indicated the presence of a meliacarpinin skeleton with three methyl (δ 1.49, *s*, 3H; δ 1.35, *s*, 3H; δ 0.98, *s*, 3H), two methoxyl (δ 3.33, *s*, 3H; δ 3.67, *s*, 3H), two acetyl (δ 1.91, *s*, 3H; δ 2.09, *s*, 3H), one tigloyl (δ 6.90, *qq*, 1H; δ 1.76, *dd*, 3H; δ 1.81, *d*, 3H) and one hydroxyl (δ 4.14, *s*, 1H) groups and were similar to those of 1-tigloyl-3-acetyl-11-methoxymeliacarpinin [5]. However, instead of one acetyl group, as in 1-tigloyl-3-acetyl-11-methoxymeliacarpinin, there were two acetyl groups in compound **1**. One acetate group was assigned to C-3 from the cross-peak of CH_3COO/H-3 in the HMBC spectrum of **1**. The second acetyl group in **1** was assigned to C-20, because no C-20 hydroxyl-OH (δ 6.09, *s*, 1H) signal, as in 1-tigloyl-3-acetyl-11-methoxymeliacarpinin, was present in **1**. Small downfield shifts of H-17 (0.8 ppm) and H-22 (0.5 ppm) and also upfield shifts of C-17 (2.6 ppm), C-21 (3.3 ppm) and C-22 (2.5 ppm) in **1** compared with those of 1-tigloyl-3-acetyl-11-methoxymeliacarpinin supported this assignment. The 1-*O*-tigloyl group was proved from the cross-peak of C-1′/H-1 in the HMBC spectrum of **1**. The stereochemistry of **1** was deduced from NOESY correlations of Me-18/H-9, Me-18/H-17, Me-30/H-7, Me-30/H-15, Me-30/H19a, Me-29/H-3, Me-29/H-28b, Me-29/H-6, H-5/H-9, and H-7/H-21.

3-Tigloyl-1,20-acetyl-11-methoxymeliacarpinin (**2**), a colourless powder, had the same molecular formula as **1** ($[M]^+$ at *m/z* 732, EI-mass spectrum). The NMR data were similar to those of 3-tigloyl-1-acetyl-11-methoxymeliacarpinin [5]. The 20-acetate was again present. The structural difference between compounds **1**

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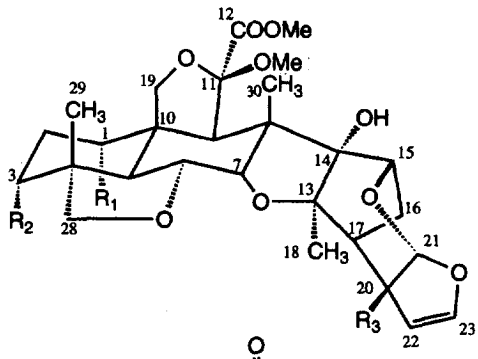
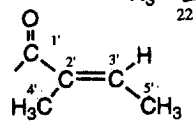
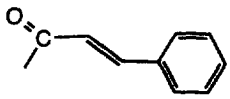


(1) R_1 = OTig, R_2 = R_3 = $OCOCH_3$
(2) R_1 = R_3 = $OCOCH_3$, R_2 = OTig
(3) R_1 = OCin, R_2 = R_3 = OH
(4) R_1 = H, R_2 = $OCOC(CH_3)=CH_2$, R_3 = OH
(5) R_1 = OCin, R_2 = $OCOCH_3$, R_3 = OH

Tig. 

Cin. 

and **2** was assumed to involve the interchange of the 1α-*O*-tigloyl and 3α-*O*-acetyl groups. The small upfield shift (0.19 ppm) of H-1β in **2** relative to 1-tigloyl-3-acetyl-11-methoxymeliacarpinin [5] supported this assignment.

1-Cinnamoyl-3-hydroxy-11-methoxymeliacarpinin (-

Table 1. ^{1}H NMR data of meliacarpin derivatives **1–4** (400 MHz, $CDCl_3$)

	1	**2**	**3**	**4**
1β	4.74 *br t* (2.6)	4.58 *br t* (3.0)	4.90 *t* (2.9)	1.35 *m*, 1.60 *m*
2α	2.17 *dt* (16.7, 2.6)	2.34 *dt* (16.8, 2.5)	2.28 *dt* (16.6, 2.6)	2.17 *m*
2β	2.06 *m*	2.05 *dt* (15.8, 3.3)	2.11 *dt* (16.6, 3.1)	1.87 *m*
3β	4.88 *br t* (2.3)	4.96 *br t* (2.6)	3.85 *br m*	5.00 *br t* (2.6)
5α	3.03 *d* (12.9)	3.21 *d* (12.4)	3.08 *d* (12.7)	2.73 *d* (12.8)
6β	3.93 *dd* (12.9, 2.7)	3.95 *dd* (12.8, 2.7)	4.00 *dd* (12.7, 2.8)	3.90 *dd* (12.8, 2.9)
7β	4.23 *d* (2.8)	4.29 *d* (2.6)	4.56 *d* (2.8)	4.52 *d* (2.7)
9α	3.47 *s*	3.59 *s*	3.61 *s*	3.16 *s*
15	4.10 *m*	4.14 *m*	4.11 *m*	4.12 *m*
16α	2.06 *m*	2.13 *m*	2.16 *m*	2.20 *m*
16β	1.80 *m*	1.85 *m*	1.86 *m*	1.89 *m*
17	2.91 *d* (4.6)	2.99 *d* (4.7)	2.16 *m*	2.17 *m*
18	1.35 *s*	1.25 *s*	1.65 *s*	1.48 *s**
19a	4.14 *d* (9.1)	4.16 *d* (9.3)	4.18 *d* (9.3)	4.06 *d* (8.6)
19b	3.84 *d* (9.1)	3.85 *d* (9.3)	3.90 *d* (9.3)	3.96 *d* (8.6)
21	5.64 *s*	5.67 *s*	5.65 *s*	5.63 *s*
22	5.38 *d* (3.0)	5.39 *d* (3.0)	4.89 *d* (2.9)	4.88 *d* (3.0)
23	6.39 *d* (3.0)	6.42 *d* (3.0)	6.38 *d* (2.9)	6.38 *d* (3.0)
28a	3.54 *d* (2.5)	3.51 *br*	4.12 *d* (6.4)	3.59 *d* (7.5)
28b	3.44 *d* (2.5)	3.51 *br*	3.61 *d* (7.6)	3.53 *d* (7.5)
29	0.98 *s*	0.99 *s*	0.95 *s*	0.98 *s*
30	1.49 *s*	1.50 *s*	1.57 *s*	1.53 *s**
3-OH			1.94 *d* (7.6)	
14-OH	4.14 *s*	4.11 *s*	4.21 *s*	4.19 *s*
20-OH			6.06 *s*	6.12 *s*
11-OMe	3.33 *s*	3.37 *s*	3.37 *s*	3.41 *s*
12-OMe	3.67*s*	3.72*s*	3.73*s*	3.81*s*
CH_3COO	1.91 *s*	1.97 *s*		
	2.09 *s*	2.03 *s*		
Tig or methacrylate				
3′	6.90 *qq* (7.1, 1.4)	6.90 *qq* (7.3, 1.7)		5.61 *t* (1.5)
				6.13 *t* (1.0)
4′	1.76 *dd* (7.7, 1.1)	1.83 *d* (1.2)		1.96 *t* (0.9)
5′	1.81 *br d* (1.2)	1.85 *d* (1.2)		
Cin				
Ph-H			7.51–7.41 *m* (5H)	
7′			7.72 *d* (16.0)	
8′			6.43 *d* (16.0)	

*Assignments may be interchanged.

3), a colourless powder showed a molecular formula of $C_{37}H_{44}O_{13}$ ($[M-COOMe]^+$ ion at *m/z* 637 in the EI-mass spectrum). The NMR spectra were similar to those of **5** [6] with three methyl (δ 1.57, *s*, 3H; δ 1.65, *s*, 3H; δ 0.95, *s*, 3H), two methoxyl (δ 3.37, *s*, 3H; δ 3.73, *s*, 3H), and one cinnamoyl (δ 7.40–7.45, *m*, 5H; δ7.72, *d*, 1H; δ 6.43, *d*, 1H) groups. The presence of 1α-*O*-cinnamoyl and 3α-hydroxyl groups was deduced from the NOE of H-7′/Me-18, H-8′/Me-18, and H-3/Me-29 and supported by small downfield shifts of 28a–H (0.5 ppm), C-4 (1.5 ppm) and C-2 (2.8 ppm) in **3**, compared with those of **5**.

1-Deoxy-3-methacrylyl-11-methoxymeliacarpinin (**4**), a colourless powder, exhibited a molecular formula of $C_{32}H_{42}O_{12}$ ($[M]^+$ at *m/z* 618, EI-mass spectrum). The NMR spectra were similar to those of 1-deoxy-3-tigloyl-11-methoxymeliacarpinin [7] except for the change of the 3α-*O*-tigloyl to a 3α-*O*-methacrylate (δ 5.61, *t*, 1H; δ 6.13, *t*, 1H; δ 1.96, *t*, 3H; δ 166.2, *s*, C-1′, 136.2, *s*, C-2′, 126.1, *t*, C-3′, 18.3, *q*, C-4′) in **4**.

The cytotoxic activity (IC_{50} values, μg ml^{-1}) of compounds (**1–5**) against P388 lymphocytic leukemia cells were 100 (**1**), 48.0 (**2**), 1.5 (**3**), 47.0 (**4**), and 10.5 (**5**), respectively. Compound **3** showed significant cytotoxic activity. However, the activity of **5**, with a C-3 acetate, decreased. Similar trends were observed with compounds **1** and **2**, that is, the cytoxic activities (IC_{50} values) of 1-tigloyl-3-acetyl-11-methoxymeliacarpinin and 1-acetyl-3-tigloyl-11-methoxymeliacarpinin were 3.2 and 3.3 μg ml^{-1}, as shown in a previous paper [5], but the cytotoxic activities of **1** and **2**, which are C-20 acetates, were almost zero. Compound **4**, with a 1-deoxy structure, showed very weak activity.

Table 2. ^{13}C NMR spectral data of compounds **1–5** (100 MHz, $CHCl_3$)

	1	**2**	**3**	**4**	**5**
1	70.2 *d*	71.1 *d*	72.2 *d*†	24.7 *t**	70.4 *d*†
2	28.2 *t*	28.0 *t*	30.8 *t*	33.4 *t**	28.0 *t*
3	70.8 *d*	70.5 *d*	70.3 *d*†	70.6 *d*	70.9 *d*†
4	42.3 *s*	42.9 *s*	43.9 *s*	42.7 *s*	42.4 *s*
5	35.1 *d*	34.8 *d*	33.9 *d*	39.6 *d*	35.0 *d*
6	71.8 *d*	71.8 *d*	71.1 *d*†	71.1 *d*	71.2 *d*†
7	82.9 *d*	83.0 *d*	83.7 *d*	84.2 *d*	83.6 *d*
8	52.0 *s*	51.9 *s*	51.5 *s*	51.3 *s*	51.4 *s*
9	48.1 *d*	48.1 *d*	47.9 *d*	54.6 *d*	47.7 *d*
10	49.9 *s*	49.9 *s*	50.2 *s*	46.1 *s*	49.9 *s*
11	106.7 *s*	107.0 *s*	106.8 *s*	106.6 *s*	106.8 *s*
12	169.1 *s*	169.5 *s*	169.2 *s*	170.0 *s*	169.3 *s*
13	93.6 *s*	93.8 *s*	94.9 *s*	94.7 *s*	95.0 *s*
14	92.9 *s*	92.7 *s*	93.2 *s*	93.0 *s*	93.2 *s*
15	82.0 *d*	82.0 *d*	81.2 *d*	81.1 *d*	81.2 *d*
16	28.9 *t*	28.9 *t*	29.7 *t*	29.7 *t*	29.7 *t*
17	48.1 *d*	48.1 *d*	50.9 *d*	50.9 *d*	50.8 *d*
18	25.9 *q*	25.3 *q*	26.4 *q*	26.3 *q*	26.4 *q*
19	70.6 *t*	70.7 *t*	70.5 *t*	71.1 *t*	70.8 *t*
20	91.8 *s*	91.8 *s*	86.3 *s*	86.2 *s*	86.3 *s*
21	105.9 *d*	106.0 *d*	109.4 *d*	109.3 *d*	109.3 *d*
22	105.5 *d*	105.5 *d*	108.1 *d*	108.0 *d*	108.0 *d*
23	146.7 *d*	146.8 *d*	145.7 *d**	145.8 *d*	145.7 *d**
28	76.1 *t*	76.1 *t*	76.6 *t*	75.5 *t*	75.6 *t*
29	18.1 *q*	18.0 *q*	18.8 *q*	18.4 *q*	18.3 *q*
30	18.1 *q*	17.8 *q*	17.7 *q*	17.3 *q*	17.7 *q*
11-OMe	52.3 *q*	52.4 *q*	52.4 *q*	52.4 *q*	52.5 *q*
12-OMe	53.1 *q*	53.1 *q*	53.3 *q*	52.8 *q*	53.3 *q*
$CH_3\underline{C}OO$	170.1 *s*	170.2 *s*			170.1 *s*
	171.4 *s*	171.3 *s*			
$\underline{C}H_3COO$	20.9 *q*	21.0 *q*			21.0 *q*
	21.4 *q*	21.2 *q*			
1′	166.7 *s*	166.8 *s*	134.0 *s*	166.2 *s*	133.9 *s*
2′	128.3 *s*	128.4 *s*	128.2 *d*	136.2 *s*	128.0 *d*
3′	138.0 *d*	138.3 *d*	129.0 *d*	126.1 *t*	129.2 *d*
4′	14.3 *q*	14.4 *q*	130.8 *d*	18.3 *q*	130.3 *d*
5′	12.1 *q*	11.9 *q*	129.0 *d*		129.2 *d*
6′			128.2 *d*		128.0 *d*
7′			146.4 *d**		145.8 *d**
8′			117.0 *d*		117.4 *d*
9′			165.6 *s*		165.6 *s*

*,†Assignments in each column may be interchanged.

EXPERIMENTAL

General. Mps uncorr. $[\alpha]_D$: Jasco DIP-4. MS: VG AutoSpec. IR: Perkin Elmer 1710. ^1H and ^{13}C NMR: Bruker AM 400 and 500 MHz at 303 KL. NOESY experiments were made with mixing time of 0.6 s and processed on a Bruker data station with an Aspect 3000 computer. Silica gel CC was carried out on Merck Kieselgel 60 (70–230 mesh) at amounts equivalent to 100 times the sample amount. MPLC was performed with a column (22 × 300 mm i.d.) packed with 40 μm silica gel or 20 μm ODS. HPLC was performed with a Hibar RT RP-18 column (20 × 250 mm i.d.) packed with 7 μm ODS. The NMR coupling constants (J) are given in Hz.

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

Extraction and isolation. The fresh root barks of *M. azedarach* (5 kg) were cut into slices and extracted 3× with 24 l. of 70% EtOH at 70°. The concd extract (241 g) was partitioned between CH_2Cl_2 and H_2O, followed by partition with *n*-BuOH and H_2O. The CH_2Cl_2-soluble fr. (56 g) was subjected to silica gel CC using a *n*-hexane–EtOAc (1:0–0:1) gradient system to give 14 fr (A–N). Fr K (1.7 g) was further chromatographed on a silica gel column eluted with *n*-hexane–Me_2CO (4:1) and purified by ODS MPLC and ODS HPLC with MeOH–H_2O or CH_3CN–H_2O eluting systems to give compounds **1** (30 mg), **2** (6 mg) and **4** (2 mg). Fr. L (15 g) was chromatographed on silica gel column eluted with CH_2Cl_2–MeOH and purified with HPLC in the same way to give compounds **3** (4.3 mg) and **5** (8 mg).

1-*Tigloyl*-3,20-*diacetyl*-11-*methoxylmeliacarpinin* (**1**). Powder, mp 150–152° (from $CHCl_3$); $[\alpha]_D$ − 3.57° ($CHCl_3$; *c* 0.6). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 1735, 1717 (sh), 1700, 1600, 1373, 1054. EI-MS *m/z*: 732 $[M]^+$ 700, 673, 640, 518. ^1H and ^{13}C NMR: Tables 1 and 2.

3-*Tigloyl*-1,20-*diacetyl*-11-*methoxylmeliacarpinin* (**2**). Powder, mp 214–216° (from $CHCl_3$); $[\alpha]_D$ +7.94° ($CHCl_3$; *c* 0.6). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470, 1735, 1720 (sh), 1700, 1650, 1615, 1600, 1373, 1060. EI-MS *m/z*: 732 $[M]^+$, 700, 673, 640, 549, 518. ^1H and ^{13}C NMR: Tables 1 and 2.

1-*Cinnamoyl*-11-*methoxymeliacarpinin* (**3**). Powder, mp 124–126° (from $CHCl_3$); $[\alpha]_D$ −2.39° ($CHCl_3$; *c* 0.2); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1740, 1710 (sh), 1630, 1600. EI-MS *m/z*: 637 $[M - COOMe]^+$. ^1H and ^{13}C NMR: Tables 1 and 2.

1-*Deoxy*-3-*methacrylyl*-11-*methoxymeliacarpinin* (**4**). Powder, mp 274–276° (from $CHCl_3$); $[\alpha]_D$ −16.3° ($CHCl_3$; *c* 0.2); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1735, 1700, 1596. EI-MS *m/z*: 618 $[M]^+$, 559, 447, 373. ^1H and ^{13}C NMR: Tables 1 and 2.

Bioassay of cytotoxic activity against P388 cells. The cytotoxic bioassay of the samples using P388 cells *in vitro* were performed by means of the MTT method [8].

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REFERENCES

1. Champagne, D. E., Koul, O., Isman, M. B., Scudder, G. G. E. and Towers, G. H. N. (1992) *Phytochemistry* **31**, 377.
2. Naqvi, S. N. H., Ahmed, S. O. and Mohammad, F. A. (1991) *Pak. J. Pharm. Sci.* **4**, 71.
3. Ahn, J.-W., Choi, S.-U. and Lee, C.-O. (1994) *Phytochemistry* **36**, 1493.
4. Andrei, G. M., Coulombie, F. C., Courreges, M. C., Detorres, R. A. and Coto, C. E. (1990) *J. Interferon Res.* **10**, 469.
5. Itokawa, H., Qiao, Z.-S., Hirobe, C. and Takeya, K. (1995) *Chem. Pharm. Bull.* **43**, 1171.
6. Nakatani, M., Arikawa, S., Okamura, H. and Iwagawa, T. (1994) *Heterocycles* **38**, 327.
7. Nakatani, M., Huang, R.-C., Okamura, H. and Iwagawa, T. (1993) *Chem. Letters* 2125.
8. Itokawa, H., Saito, K., Morita, H., Takeya, K. and Yamada, K. (1989) *Chem. Pharm. Bull.* **37**, 1619.