Two New Meliacarpinins from the Roots of *Melia azedarach*

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Two new azadirachtin-type limonoids, 1-methacrylyl-3-acetyl-11-methoxymeliacarpinin (1) and 1-(2-methylpropanoyl)-3-acetyl-11-methoxymeliacarpinin (2), together with the known compounds, meliacarpinin D (3), melianin B (4) and 2 β ,3 β -dihydroxy-5 α -pregn-17(20)-(Z)-en-16-one (5), were isolated from the roots of *Melia azedarach*. The structures of 1 and 2 were elucidated by analysis of spectroscopic data and comparison of their NMR data with those of 3. Compounds 1 and 5 exhibited significant activity in the brine shrimp lethality test (BST).

Key words *Melia azedarach*; limonoid; meliacarpinin; azadirachtin; brine shrimp lethality; brine shrimp test

J. L. McLaughlin and colleagues¹⁾ have developed “the brine shrimp (*Artemia salina*) lethality test (BST)” as a convenient bioassay for screening and fractionation in the discovery and monitoring of biologically active natural products. Particularly, it attracts our attention that brine shrimp lethality has often a positive correlation to 9 KB cytotoxicity and pesticidal activity.^{2,3)} Hence, BST has the advantage of being rapid, inexpensive, and simple enough to carry out screening a number of plant extracts. During our preliminary screening of plant extracts by using the BST, the methanol extract of the roots of *Melia azedarach*^{4,5)} exhibited significant lethal activity. The bioassay-guided fractionation of the methanol extract has resulted in the isolation of the two new meliacarpinins 1 and 2 along with the previously reported meliacarpinin D (3),⁶⁾ melianin B (4)^{7,8)} and a pregnane (5).⁹⁾

In this paper, we describe the structural elucidation of the two new meliacarpinins 1 and 2 isolated from an active fraction of the root of *M. azedarach*.

The methanol extract of the root of *M. azedarach* was fractionated by silica gel chromatography. One of the active fractions eluted with CH₂Cl₂–AcOEt (2:3) was subjected to re-

peated silica gel and octadecyl silica gel (ODS) column chromatography, and finally purified by HPLC using an ODS column to yield the new compounds 1, 2 along with the known compounds, meliacarpinin D (3), melianin B (4) and 2 β ,3 β -dihydroxy-5 α -pregn-17(20)-(Z)-en-16-one (5). Identification of 3–5 was performed by spectral comparison with those reported in the literatures.^{7,9)}

Compound 1, with the molecular formula C₃₄H₄₄O₁₄ determined by its high-resolution (HR) FAB-MS at *m/z* 699 [M+Na]⁺, showed IR absorption at 3409, 1739 and 1626 cm^{−1} attributable to a hydroxyl, ester and ketone groups, respectively. The ¹H-NMR and ¹³C-NMR data (Table 1) of 1 indicated the presence of the meliacarpinin skeleton with three singlet methyls (δ_H 1.01, 1.56, 1.61), one methoxyl (δ_H 3.38; δ_C 52.5), one methyl ester (δ_H 3.74; δ_C 169.3, 53.2), one acetyl (δ_H 2.00; δ_C 170.1, 21.0) and one methacrylyl group [δ_H 1.95 (3H, br s), 5.63 (1H, m), 6.15 (1H, m); δ_C 166.0, 136.1, 126.1, 11.9] and were similar to those of meliacarpinin D (3)^{5,6)} (1-tigloyl-3-acetyl-11-methoxymeliacarpinin). All the structural units obtained from the ¹H–¹H correlation spectroscopy (COSY) and ¹H detected multiple quantum coherence (HMQC) spectra, as shown by the bold lines in Fig. 1, and seven quaternary carbons resonated at δ_C 106.9 (C-11), 94.9 (C-13), 93.3 (C-14), 86.2 (C-20), 51.2 (C-8), 49.6 (C-10) and 42.6 (C-4) corresponded well to those existing in the known meliacarpinin D (3). Additionally, the heteronuclear multi-bond correlation (HMBC) experiment, as summarized in Fig. 1, could assemble the partial units and seven quaternary carbons into the assumed meliacarpinin

Fig. 1. HMBC Correlations of 1

Bold lines indicate the partial structures inferred from COSY and HMQC, and arrows denote the correlation between protons (tail) and carbons (head) in the HMBC.

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structure. The HMBC correlation of H-1 resonated at δ_{H} 4.98 with the C-1' ester carbonyl at δ_{C} 166.0 placed the methacrylyl group on the C-1 position. The HMBC correlation between H-3 at δ_{H} 4.60 and CH_3COO at δ_{C} 170.1 proved the sole acetyl group to be linked at the C-3 position. Having the same stereochemistry with **1** as that of **3** was also supported by phase-sensitive nuclear Overhauser and exchange

Fig. 2. Relative Configuration of **1** Based on NOEs Indicated by Arrows

spectroscopy (NOESY) spectrum as shown in Fig. 2 as well as by small J values for H-1 and H-3 (Table 1). Thus compound **1** was assigned as 1-methacrylyl-3-acetyl-11-methoxymeliacarpinin.

Compound **2** had the molecular formula $\text{C}_{34}\text{H}_{46}\text{H}_{14}$ determined from HR-FAB-MS at m/z 701.2925 $[\text{M}+\text{Na}]^+$. Its ^1H - and ^{13}C -NMR spectra (Table 1) were similar to those of **1** except for the presence of a newly appeared 2-methylpropanoyl group [δ_{H} 1.16 (3H, d, $J=6.7$ Hz), 1.20 (3H, d, $J=6.7$ Hz), 2.51 (1H, qq, $J=6.7$, 6.7 Hz; δ_{C} 18.1, 18.6, 34.2, 175.8) instead of the methacrylyl group existing in **1**. The HMBC of **2** showed a cross peak between H-1 (δ_{H} 4.91) and the ester carbonyl (δ_{C} 175.8) involved in the 2-methylpropanoyl group, thereby confirming that the 2-methylpropanoyl group was located at the C-1 position. The relative stereochemistry for **2** was identical with that of **1** from the analysis of its NOESY. Thus the structure of compound **2** was determined as 1-(2-methylpropanoyl)-3-acetyl-11-methoxymeliacarpinin.

Compound **1** exhibited significant lethal activity ($\text{LC}_{50}=19 \mu\text{g/ml}$) in the BST. The other meliacarpinins **2** and **3**,

Table 1. ^1H - (600 MHz) and ^{13}C - (150 MHz) NMR (in CDCl_3) Data for Compounds **1**—**3**

	1		2		3	
	H	C	H	C	H	C
1	4.98 (dd, 2.7, 2.7)	70.7	4.91 (dd, 2.7, 2.7)	70.3	4.98 (dd, 2.7, 2.7)	70.3
2 α	2.07 (dt, 16.7, 2.7)	27.9	2.27 (dt, 17.0, 2.7)	27.8	2.34 (dt, 16.7, 2.7)	27.9
2 β	2.37 (dt, 16.7, 2.7)		2.07 (dt, 17.0, 2.7)		2.06 (dt, 16.7, 2.7)	
3	4.60 (dd, 2.7, 2.7)	71.0	4.57 (dd, 2.7, 2.7)	71.0	4.54 (dd, 2.7, 2.7)	71.07
4		42.6		42.5		42.6
5	3.08 (d, 12.9)	35.1	2.99 (d, 12.9)	34.9	3.07 (d, 12.6)	35.1
6	3.97 (dd, 12.9, 3.0)	71.1	3.94 (dd, 12.9, 2.7)	71.01	3.96 (dd, 12.6, 3.0)	71.05
7	4.53 (d, 3.0)	83.8	4.52 (d, 2.7)	83.7	4.89 (d, 3.0)	83.8
8		51.2		51.2		51.2
9	3.67 (3H, s)	47.9	3.67 (3H, s)	47.8	3.67 (3H, s)	47.9
10		49.6		49.6		49.6
11		106.9		106.91		106.9
12		169.3		169.4		169.4
13		94.9		94.9		94.8
14		93.3		93.2		93.3
15	4.14 (d, 2.5)	81.1	4.13 (d, 2.5)	81.1	4.14 (d, s)	81.1
16 β	2.22 (ddd, 13.4, 6.3, 2.5)	29.7	2.24 (ddd, 13.4, 6.0, 2.5)	29.7	2.24 (ddd, 13.4, 6.3, 2.5)	29.7
16 α	1.87 (dd, 13.4, 1.4)		1.86 (dd, 13.4, 1.4)		1.87 (dd, 13.4, 1.4)	
17	2.17 (dd, 6.3, 1.4)	50.8	2.17 (dd, 6.0, 1.4)	50.7	2.17 (dd, 6.3, 1.4)	50.8
18	1.61 (3H, s)	25.9	1.61 (3H, s)	25.9	1.59 (3H, s)	25.9
19 α	3.86 (d, 9.3)	70.6	3.85 (d, 9.3)	70.5	3.86 (d, 9.3)	70.6
19 β	4.16 (d, 9.3)		4.15 (d, 9.3)		4.16 (d, 9.3)	
20		86.2		86.2		86.2
21	5.64 (s)	109.3	5.64 (s)	109.3	5.64 (s)	109.3
22	4.89 (d, 3.0)	107.9	4.89 (d, 3.0)	107.9	4.59 (d, 3.0)	107.9
23	6.39 (d, 3.0)	145.8	6.38 (d, 3.0)	145.8	6.39 (d, 3.0)	145.8
28 α	3.62 (3H, s)	76.5	3.46 (d, 7.7)	76.5	3.55 (d, 8.0)	76.4
28 β	3.57 (d, 8.6)		3.56 (d, 7.7)		3.59 (d, 8.0)	
29	1.01 (3H, s)	18.0	0.98 (3H, s)	18.1	0.99 (3H, s)	18.0
30	1.56 (3H, s)	17.8	1.58 (3H, s)	17.7	1.56 (3H, s)	17.8
20-OH	6.14 (s)		6.16 (s)			
11-OMe	3.38 (3H, s)	52.5	3.39 (3H, s)	52.5	3.39 (s)	52.5
12-OMe	3.74 (3H, s)	53.2	3.75 (3H, s)	53.2	3.74 (3H, s)	53.2
3-AcO	2.00 (3H, s)	170.1	2.08 (3H, s)	170.2	1.99 (3H, s)	170.1
		21.0		21.2		21.1
1'		166.0		175.8		166.6
2'		136.1	2.51 (qq, 6.7, 6.7)	34.2		128.3
3'	5.63 (m)	126.1	1.20 (d, 6.7)	18.1	6.99 (qq, 7.0, 1.3)	138.2
	6.15 (m)					
4'	1.95 (3H, s)	11.9	1.16 (d, 6.7)	18.6	1.82 (dq, 7.0, 1.3)	11.9
5'					1.83 (dq, 1.3, 1.0)	14.5

however, had no lethal activity at 100 $\mu\text{g/ml}$. This result presumes that the methacrylyl group attached to the C-1 position would play an important role in having toxicity against the brine shrimp. Of all the compounds isolated from one of the active fractions the pregnane **5** was most active ($\text{LC}_{50} = 2.1 \mu\text{g/ml}$) in the BST. A number of the potent antifeedants and cytotoxic active compounds, however, have been isolated from *M. azedarach* L.¹⁰⁾ Most of the active principles belong to the azadirachtin-type tetranortriterpenoids and the sendanin-type limonoids. Therefore, our further search for other BST-active substances in the root of *M. azedarach* is in progress.

Experimental

Optical rotations were measured with a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Jasco FT-IR 5300 IR spectrophotometer. One dimensional (1D) and two dimensional (2D)-NMR spectra were recorded on a Varian Unity 600 instrument in CDCl_3 . Chemical shifts were given as δ (ppm) with tetramethylsilane (TMS) as an internal standard. MS were recorded on a JEOL AX-500 instrument.

Plant Materials The roots of *Melia azedarach* Linn. var. *Japonica* MAKINO were collected in Tokushima, Japan and a voucher specimen (1428RT) has been deposited in this institute of Tokushima Bunri University.

Extraction and Purification The MeOH extract (91 g) of the dried powdered root of *M. azedarach* was chromatographed on silica gel (Merck, 70–230 mesh) eluting with a CH_2Cl_2 –EtOAc gradient and eluant were separated into 1–6 fractions. The BST-active (100% death at 200 $\mu\text{g/ml}$) fraction 2 (3.8 g) was fractionated into 7–12 fractions by Sephadex LH-20 chromatography with MeOH. The BST-active fraction 9 (3.2 g) was subjected to a reverse phase column chromatography on Cosmosil 40C₁₈-OPN eluting with MeOH–H₂O (9:1) to give 13–19 fractions. The BST-active fraction 17 (11.6 mg) was purified by HPLC [Cosmosil 5C₁₈-AR ($\phi 10 \times 250$ mm); MeOH–H₂O (7:3; 2.0 ml min⁻¹); UV 220 nm] to give compounds **1** (4.6 mg) and **2** (5.2 mg), and meliacarpinin D (**3**) (6.2 mg). The BST-active fraction 14 (127 mg) was purified by silica gel chromatography [CHCl_3 –MeOH (97:3)] and finally by HPLC [Cosmosil 5C₁₈-AR ($\phi 10 \times 250$ mm); MeOH–H₂O (8:2; 2.0 ml min⁻¹); UV 220 nm] to give melianin B (**4**) (9 mg) and 2 β ,3 β -dihydroxy-5 α -pregn-17(20)-(Z)-en-16-one (**5**) (3 mg).

1-Methacrylyl-3-acetyl-11-methoxymeliacarpinin (**1**): Amorphous powder. $[\alpha]_D^{25} + 2.5^\circ$ ($c = 0.19$, CHCl_3). IR cm^{-1} : 3409 (OH), 1739 (C=O), 1626 (C=O). FAB-MS m/z : 699 [M+Na]⁺, 659 [M+H–18]⁺, 617 [M+H–60]⁺. HR-FAB-MS m/z : 699.2673 [M+Na]⁺ (Calcd for C₃₄H₄₄O₁₄Na: 699.2629). ¹H-NMR and ¹³C-NMR: Table 1.

1-(2-Methylpropanoyl)-3-acetyl-11-methoxymeliacarpinin (**2**): Amorphous

powder. $[\alpha]_D^{25} - 169.8^\circ$ ($c = 0.21$, CHCl_3). IR cm^{-1} : 3402 (OH), 1738 (C=O), 1624 (C=O). FAB-MS m/z : 701 [M+Na]⁺, 661 [M+H–18]⁺, 619 [M+H–60]⁺. HR-FAB-MS m/z : 701.2925 [M+Na]⁺ (Calcd for C₃₄H₄₆O₁₄Na: 701.2911). ¹H-NMR and ¹³C-NMR: Table 1.

Brine Shrimp Bioassay The bioassay was carried out according to essentially the same literature procedure.¹¹⁾ A half spoon of brine shrimp eggs (Nihon Animal Pharmaceutical Inc., Tokyo, Japan) were hatched in a container filled with air-bubbled artificial sea water which was prepared with 10 g of a commercial salt mixture (GEX Inc., Osaka, Japan) and 500 ml of distilled water. After 48 h, the phototropic shrimps were collected by pipette for bioassay. Ten shrimps were transferred to each vial, and artificial water was added to make 5 ml. To this vial each sample which was dissolved in 0.025 ml of dimethyl sulfoxide (DMSO), whereas the vial containing 0.025 ml of DMSO was regarded as a control. The surviving shrimps were counted microscopically in the stem of the pipette against a lightened background after 24 h. The percent deaths at each dose was counted as the following formula: % deaths = (the number of death shrimps – the number of half death shrimps) $\times 0.7 / 10 \times 100$.

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