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12-Hydroxyamoorastatone, a New Limonoid from *Melia azedarach* var. *Japonica*

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A chemical investigation of the stem bark of *Melia azedarach* var. *Japonica* (Meliaceae) has led to a new limonoid, 12-hydroxyamoorastatone (**1**), whose structure has been elucidated by spectroscopic measurements including 2D-NMR. The 2D-NOESY experiment on its di-*p*-bromobenzoate derivative (**1a**) has established the relative configuration of **1**.

Introduction

Melia azedarach var. *Japonica* (Meliaceae) is a large tree found commonly in southern Korea and Japan. The bark decoction of this plant has been used for intestinal worms and skin ailments in Korea.[1] As the result of an extensive study[2,3] of plants of this family, a large number of bitter principles have been isolated and classified as limonoids. As part of our continuing search for novel antitumor agents of medicinal plant origin, *Melia azedarach* var. *Japonica* was found to exhibit significant cytotoxicities against human tumor cell lines. Bioassay-directed chromatographic fractionation led to the isolation of a new cytotoxic limonoid, 12-hydroxyamoorastatone (**1**). This paper describes the isolation and structural elucidation of the new compound.

Results and Discussion

The MeOH extract of the stem bark of *M. azedarach* var. *Japonica* was fractionated by a combination of column chromatography on silica gel and LiChroprep RP-18 and finally purified by recycling preparative HPLC to give compound **1** (Figure 1).

Compound **1**, $C_{28}H_{36}O_{10}$, IR ν_{max}^{KBr} cm^{-1}: 3600-3200 (-OH), 1720br (C=O), 1242, 1057 (-OAc), 875 (furan) has resonances in its ^{1}H-NMR spectrum for three tertiary methyls (δ 0.82, 0.93 and 1.15), one acetyl (δ 2.04) and the characteristic β-substituted furan (δ 6.35, 7.30 and 7.40). These assignments were supported by its ^{13}C-NMR spectrum (Table 1), which in addition showed two ketonic carbon signals (δ 213.8 and 220.6), six oxygenated carbons (δ 65.0, 70.1, 71.4, 74.9, 78.9 and 97.3) and four quaternary carbons (δ 41.0, 42.7, 43.7 and 47.4). The 2D ^{1}H-^{1}H and ^{13}C-^{1}H COSY spectra of **1** were extensively examined to clarify the connectivity of each proton in **1**, and showed the presence of the partial structures A-C. Furthermore the methine proton at δ 4.88 (H-24) showed long-range coupling to the non-equivalent methylene proton at δ 4.11 (H-19). This result led to the partial structure D. The ^{1}H-^{1}H COSY spectrum also showed W-coupling between C-26 methyl proton (δ 1.15) and each of two methine protons at δ 3.71 (H-9) and 3.42 (H-14). The gross structure of **1** was determined by analysis of the long-range ^{13}C-^{1}H COSY (Figure 2) and by reference to the data of related limonoids, amoorastatone and 12-hydroxyamoorastatin.[4] The hydroxy group at C-1 and the acetoxy group at C-3 were axial judging from the doublet signals with J=3.7 Hz of H-1 and with J=4.1 Hz of H-3, respectively. The double doublet signal (J=14.9 and 1.8 Hz) assignable to H-5 established that the H-5 was directed anti *trans* to the H-6β.

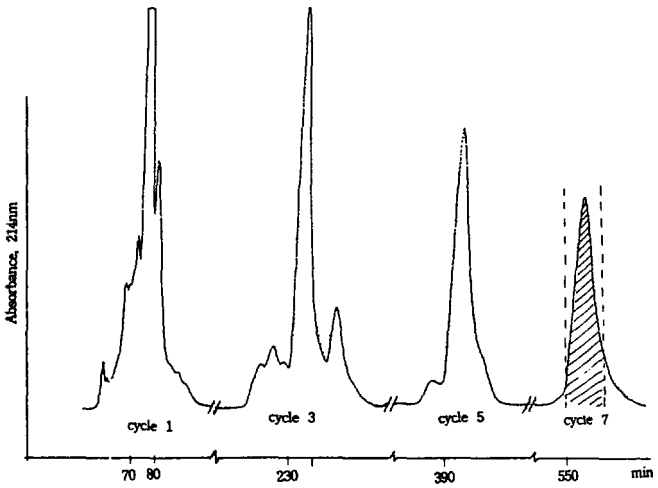


Figure 1. Recycling preparative HPLC of the compound **1**. Column: JAIGEL-GS 320 (20×500 mm), Mobile phase: MeOH, flow rate: 5ml/min.

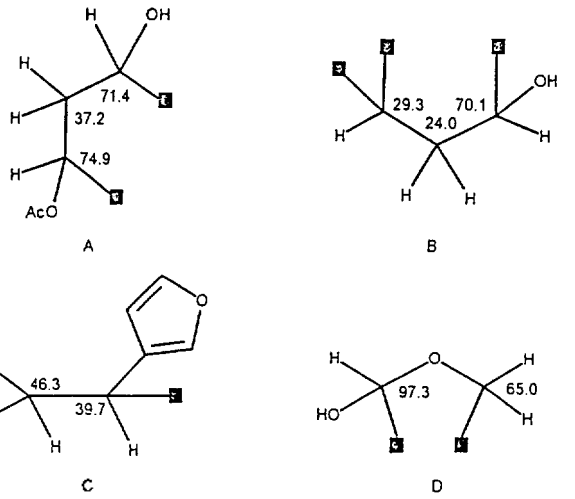


Table 1. ^{1}H and ^{13}C-NMR Data for Compound 1 (CD_3 OD)

H		C	
1	4.53d (3.7)[a]	1	71.4d[b]
2A	2.74-2.67[c]	2	37.2t
2B	1.85d (15.9)	3	74.9d
3	5.20d (4.1)	4	41.0s
5	2.78dd (14.9, 1.8)	5	29.3d
6A	2.10dt (14.9, 14.9, 1.8)	6	24.0t
6B	1.71dt (14.9, 1.8, 1.8)	7	70.1d
7	4.03br s	8	42.7s
9	3.71s	9	49.0d
12	4.22s	10	43.7s
14	3.42s	11	213.8
16A	2.74-2.67[c]	12	78.9d
16B	2.48dd (12.0, 8.6)	13	47.4s
17	3.52t(8.6)	14	59.5d
18	0.93s	15	220.6
19A	4.19d (10.5)	16	46.3t
19B	4.11d (10.5)	17	39.7d
21	7.30s	18	22.1q
22	6.35s	19	65.0t
23	7.40s	20	126.3s
24	4.88s	21	141.5d
25	0.82s	22	111.9d
26	1.15s	23	144.2d
-OAc	2.04s	24	97.3d
		25	19.9q
		26	21.1q
		-OAc	21.3q, 172.8

[a]J (Hz) in parentheses. [b]Multiplicities established by DEPT pulse sequence. [c]Not clear due to overlapping.

The relative stereochemistry of 1 was established by NOESY experiment on **1a**. Strong correlations between (i) H-12(δ 5.58) and Me-26 (δ 1.29) and (ii) H-6A (δ 2.24) and Me-26 and (iii) H-17 (δ 3.39) and Me-26 showed their *cis* relationship. Furthermore cross peak was observed between H-9 (δ 3.85) and Me-18 (δ 1.17). All other cross peaks observed in the NOESY spectrum of **1a** were well in accordance with the proposed relative stereochemistry (Figure 3).

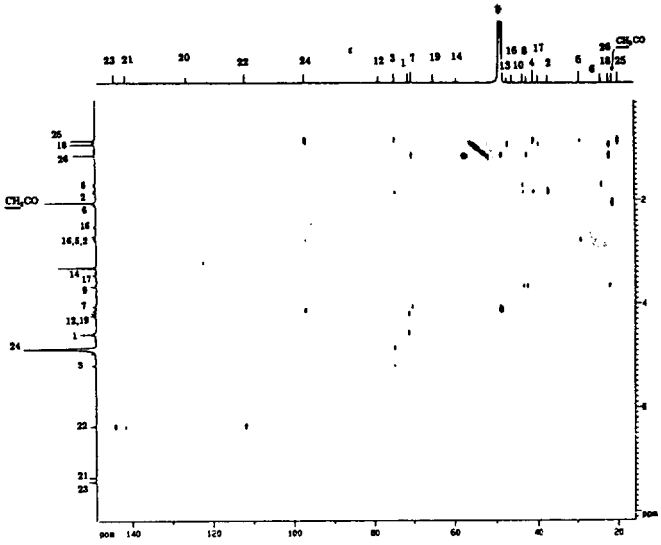


Figure 2. Long-range ^{13}C-^{1}H COSY spectrum of the compound 1. (500 MHz, CD_3OD).

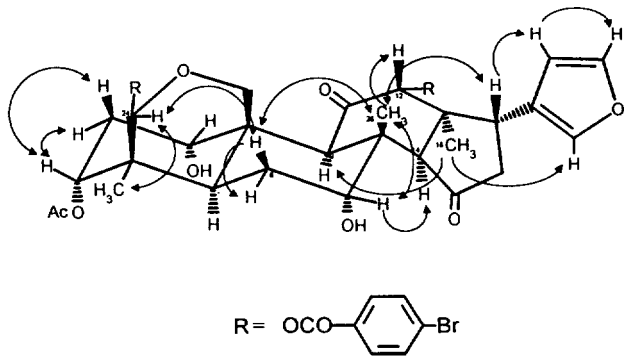
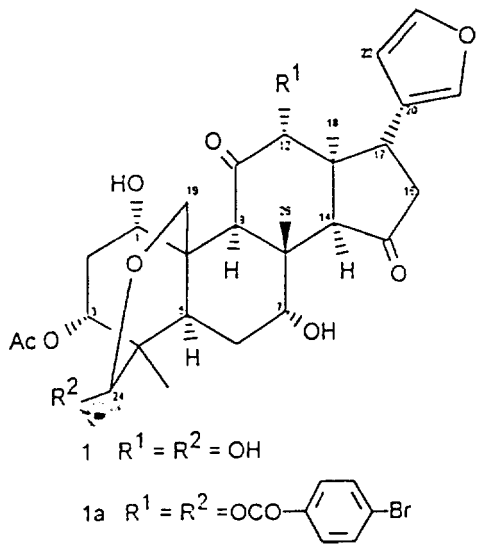


Figure 3. NOESY correlations for compound **1a**.



Experimental

Ir: KBr. Optical rotations were measured at 25℃ in

MeOH soln. NMR spectra were recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C) and chemical shifts reported relative to the residual solvent peaks (CD_3OD: 3.30 ppm, 49.0 ppm). All 2D and DEPT spectra were recorded using pulse programs supplied by Bruker. Recycling prep. HPLC was used for sepn of the mixt. The column employed was JAIGEL GS-320 (20 mm i.d.×500 mm) and solvents were HPLC grade. Detection was by UV at 214 nm.

Plant material. The stem bark of *Melia azedarach* var. *Japonica* was purchased as a commercially available product. The botanical identity was established by Prof. Ki-Hwan Pae, College of Pharmacy, Choongnam National University, Taejon. Voucher specimens of this material are deposited in our institute.

Extraction and isolation. Dried stem bark of *M. azedarach* var. *Japonica* (1.2 kg) was extracted with MeOH. The extract was partitioned between EtOAc and water. The EtOAc soluble fraction (41 g) was chromatographed on silica gel and elution started with a gradient of MeOH in CH_2Cl_2 consisting of 5 steps, containing 2, 5, 10, 50, 100% MeOH, respectively. The fraction (7.2 g) eluted by 5% MeOH-CH_2Cl_2 was rechromatographed over LiChroprep RP-18 (40-63 μm, Merck). Elution with H_2O containing increasing proportions of MeOH yielded three fractions, FO1(25% MeOH-H_2O, 0.6 g), FO2(50% MeOH-H_2O, 1.4 g), FO3(MeOH, 5.1 g). FO2 was repeatedly chromatographed over Sephadex LH-20 (MeOH) and Lobar column LiChroprep RP-18 (35% MeOH-H_2O) to give 1 (8 mg), which was finally purified by recycling prep. HPLC.

12-Hydroxyamoorastatone (1). Amorphous powder, HRMS m/z 532.2274([M], calcd for $C_{28}H_{36}O_{10}$: 532.2308). EIMS m/z (% rel.int.): 532[M]$^+$(12), 514[M-H_2O]$^+$(20), 496[M-2H_2O]$^+$(15), 472[M-AcO]$^+$(20), 454[M-AcO-H_2O]$^+$(45), 408(20), 311(20), 239(20), 163(80), 94(80). IR ν_{max}^{KBr} cm^{-1}: 3600-3200, 1720 br, 1260, 1030, 875. ^{1}H and ^{13}C-NMR spectra are listed in Table 1.

***p*-Bromobenzoylation of 1.** To a soln of **1** (3.8 mg) and 4-dimethyl amino-pyridine (38 mg) in 1 ml of dry pyridine was added *p*-bromobenzoyl chloride (11 mg). The reaction mixture was stirred at 40℃ for 48 hr, poured into ice-water, and extracted with EtOAc. The organic layer was washed with 5% HCl, 10% $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by prep. TLC (15% Acetone in CH_2Cl_2) to give di-*p*-bromobenzoate (**1a**) (1.0 mg, Rf 0.47) as amorphos powder. $[\alpha]_D^{25}$ −29.9° (MeOH; c 0.20); UV λ_{max}^{MeOH} nm (ε): 246(26300), 220(9300); IR ν_{max}^{KBr} cm^{-1}: 3500-3200, 1720 br, 1635, 1242, 1057, 875; ^{1}H-NMR(CD_3OD): δ 7.92, 7.86, 7.70, 7.66 (each 2H, d, J=8.6 Hz, Br-C_6H_4), 7.31 (1H, s, H-21), 6.33 (1H, s, H-22), 6.03 (1H, s, H-24), 5.58 (1H, s, H-12), 5.32 (1H, d, J=4.1 Hz, H-3), 4.40 (1H, d, J=10.8 Hz, H-19), 4.36 (1H, d, J=3.6 Hz, H-1), 4.20 (1H, br s, H-7), 3.85 (1H, s, H-9), 3.39 (1H, t, J=8.5 Hz, H-17), 3.35 (1H, s, H-14), 2.99 (1H, br d, J=14.6 Hz, H-5), 2.75-2.67 (2H, overlapping, H-2A and H-16A), 2.61 (1H, dd, J=12.1, 8.5 Hz, H-16B), 2.24 (1H, br t, J=14.6 Hz, H-6A), 2.06 (3H, s, -OAc), 1.91 (1H, d, J=15.8 Hz, H-2B), 1.79 (1H, dt, J=14.6, 1.8, 1.8 Hz, H-6B), 1.29 (3H, s, Me-26), 1.17 (3H, s, Me-18), 0.88 (3H, s, Me-25).

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