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Malabanones A and B, novel nortriterpenoids from *Ailanthus malabarica* DC

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Abstract—Novel octanor- and nonanor-triterpenoids, malabanones A (**1**) and B (**2**), which incorporate a unique tricyclo[4.3.1.0^{1,6}]decane unit in the structure, were isolated from the stem bark of *Ailanthus malabarica* DC. Their structures were elucidated by the analysis of spectral data. Compounds **1** and **2** were considered to be biosynthesized from ailanthol (**3**), which was also isolated from this plant.

Ailanthus malabarica DC (Simaroubaceae) is a large tree distributed in India and Indo-China, and is regarded as an important medicinal plant useful for the treatment of dysentery, dyspepsia, febrifuge and bronchitis.[1,2] From this plant, a cycloapotirucallane triterpenoid ailanthol (**3**), which possesses a unique tricyclo[4.3.1.0^{1,6}]decane structure, has been isolated.[3] In the present study, from this plant, we isolated two novel nortriterpenoids malabanones A (**1**) and B (**2**), both relating to **3** in structure. Compounds **1** and **2** are unusual octanor- and nonanor-triterpenoids, respectively, with no sidechain on ring D.

Ground stem bark of *A. malabarica* collected in Malaysia was extracted successively with hexane, CH_2Cl_2 and MeOH. The CH_2Cl_2 extract was placed over a Si gel column and eluted with $CHCl_3$ containing an increasing amount of MeOH. Further purification by MPLC (Si gel and RP-18) and HPLC (ODS) afforded **1** (0.00013%), **2** (0.00015%) and **3** (0.035%). Compound **3** was identified as ailanthol by comparing its spectral data with reported ones.[3]

Malabanone A (**1**) was obtained as amorphous powder. Its molecular formula was determined to be $C_{22}H_{30}O_3$ by the $[M-H_2O]^+$ ion peak at *m/z* 324.2061 (calcd 324.2089 for $C_{22}H_{28}O_2$) in the HREIMS. Its ^{1}H NMR spectrum showed the presence of four tertiary methyl groups (δ 1.10, 1.14,

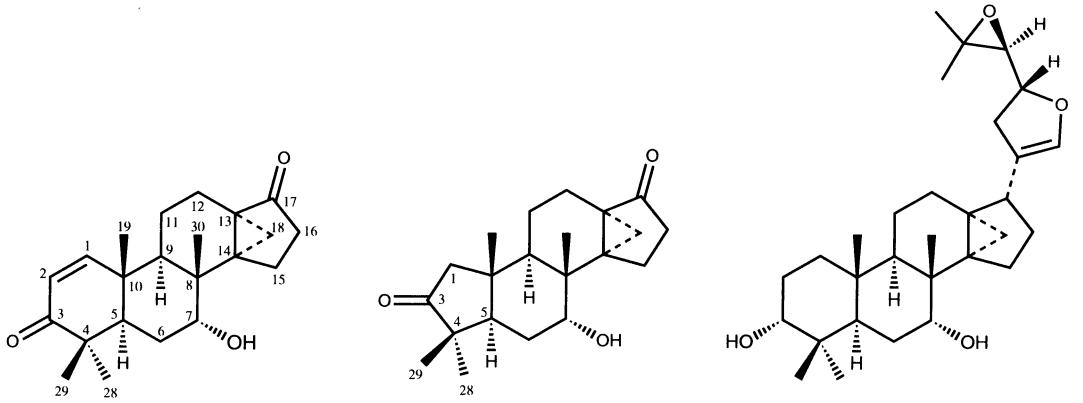

Figure 1.

Keywords: malabanones A and B; nortriterpenoids; *Ailanthus malabarica*.
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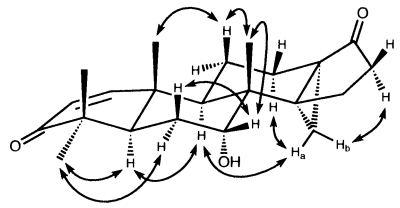

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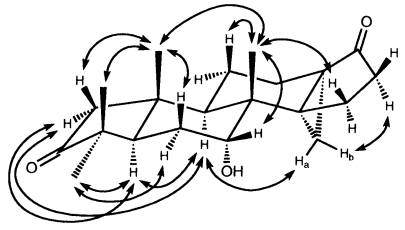

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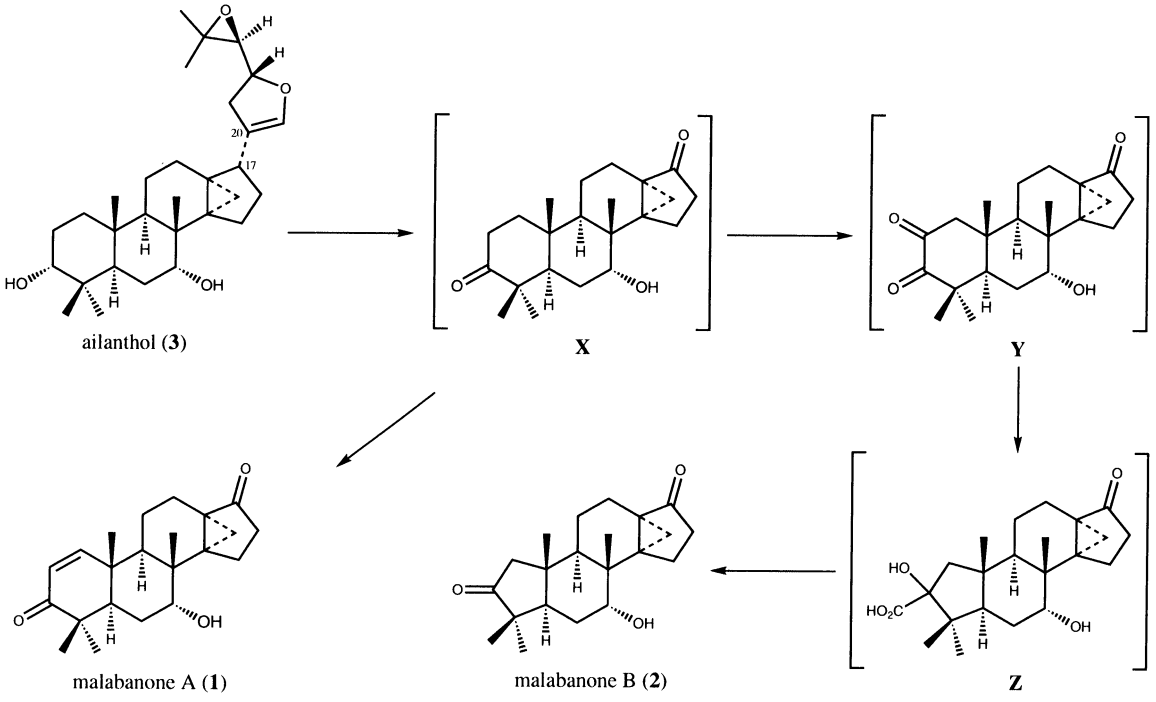
Figure 2. Selected NOESY correlations for malabanones A (**1**) and B (**2**).

1.16 and 1.17), a cyclopropane ring (δ 1.31 and 1.60, both d, J=5.0 Hz) and two olefinic protons conjugated with a carbonyl group (δ 5.84 and 7.04, both d, J=10.2 Hz). IR spectrum suggested the presence of a hydroxyl group (3502 cm^{-1}) and a conjugated ketone (1668 cm^{-1}) which was also supported by the characteristic UV absorption at 224 nm (log ϵ 4.19). Analysis of the ^{13}C NMR and HMBC spectra suggested the presence of two ketone groups at C-3 and C-17, a hydroxyl group at C-7, and a cyclopropane ring consisting of C-13, C-14 and C-18. The stereochemistry of **1** was determined by the analysis of NOESY spectrum (Fig. 2). Correlations between H-5 and H-9, H_3-19 and H-11_β, H_3-30 and H-11_β, H-9 and H-18_a, and H-12_α and H-18_a revealed that the A/B and B/C ring junctures were both in *trans* relations and that the cyclopropane ring was in α-orientation. The correlation noted between H_3-30 and H-7, and the small J-value (2.2 Hz) between H-7 and H-6_β revealed that the C-7 hydroxyl group was in an axially-oriented α-configuration. From these observations, malabanone A was determined to have structure **1** shown in Fig. 1.

Malabanone B (**2**) was obtained as amorphous powder. Its molecular formula was determined to be $C_{21}H_{30}O_3$ by HREIMS. Comparison of the ^1H and ^{13}C NMR spectra of **2** with those of **1** showed that **2** had the same B, C, D and E rings as **1** and that, accordingly, the structural differences between the two compounds resided in the A ring. Analysis of the ^{13}C NMR and HMBC spectra revealed that the A ring of **2** had a substituted cyclopentanone structure. The presence of a non-conjugated cyclopentanone was also supported by the IR absorption at 1733 cm^{-1}. The HMBC correlations between C-3 and H-1_α, H-1_β, H_3-29 and H_3-28 suggested that the ketone group was present at C-3. The NOESY correlations between H-5 and H-9, H_3-19 and H_3-30, and H-6_β and H_3-19 showed that the A/B ring juncture of **2** was in *trans* relation (Fig. 2). From these observations, malabanone B was determined to have structure **2** shown in Fig. 1.

Although a large number of triterpenes have been isolated from natural sources, nortriterpenoids with no side chain on ring D are very few.[4] Further, malabanones A (**1**) and B (**2**) possess a unique and unusual tricyclo[4.3.1.$0^{1,6}$]decane structure.[3,5,6] Since compounds **1**–**3**, all possessing the same B-C-D-E ring structure, were isolated from the same plant source, some biosynthetic relations may be suggested among them. A possible biogenetic pathway from ailanthol (**3**) to malabanones A (**1**) and B (**2**) is proposed in Scheme 1. Both oxidative scission of the C_{17}–C_{20} bond and oxidation



Scheme 1. A possible biosynthetic scheme for malabanones A (**1**) and B (**2**) from ailanthol (**3**).

of the C-3 hydroxyl group of **3** take place to produce a diketo intermediate **X**. Dehydrogenation of **X** affords malabanone A (**1**), whereas further oxidation of **X** produces a 2,3,17-triketo intermediate **Y**, which undergoes a benzilic acid-type rearrangement to produce an α-hydroxy acid **Z**.[7] By successive oxidative decarboxylation, **Z** affords malabanone B (**2**).

Malabanones A (**1**) and B (**2**) showed a weak cytotoxic activity on P-388 murine leukemia cells with IC_{50} values of 16 and 38 μg/mL, respectively, and ailanthol (**3**) a moderate activity with an IC_{50} value of 4.2 μg/mL.

1. Experimental

1.1. General

Optical rotations were measured on a Jasco DIP-360 digital polarimeter. UV spectra were taken on a Hitachi 557 spectrophotometer. IR spectra were measured on a Perkin–Elmer 1710 spectrophotometer. NMR spectra were measured on Bruker DRX-500 and DPX-400 spectrometers. Mass spectra were obtained on a VG AutoSpec E spectrometer. Prep. MPLC was performed on a Kusano C.I.G. system equipped with a Kusano KU 331 UV detector (at 220 nm) and a Labo System RI-98 RI detector. HPLC was performed on a Shimadzu LC-6AD system equipped with a SPD-10A UV detector (at 220 nm) and a reversed-phase column, Wakosil-II 5C18HG Prep (5 μm, 20×250 mm), using mixed solvent systems of MeOH/H_2O at a flow rate of 5 mL/min.

1.2. Plant material

Ailanthus malabarica DC was collected in the Penang Botanical Garden, Malaysia in January, 1997. It was authenticated by comparison with a voucher specimen previously deposited at School of Pharmaceutical Sciences, University of Science Malaysia, Minden, Penang, Malaysia.

1.3. Extraction and isolation

Dried and ground stem bark of *A. malabarica* (1.5 kg) was extracted successively with hot hexane, CH_2Cl_2 and MeOH at boiling temperature. The solvent of the CH_2Cl_2 extract was removed in vacuo and the residue (65 g) was chromatographed over Si gel (Merck 70–230 mesh, 1.3 kg) with $CHCl_3$ containing an increasing amount of MeOH. A total of nine fractions were collected. The fourth fraction (6.00 g) was separated by MPLC (Si gel, 50 μm, 40×500 mm) with hexane/$CHCl_3$/MeOH (6:3:1) to give five fractions. The second fraction (2.26 g) was separated by HPLC using MeOH/H_2O (90:10) to afford compound **3** (520.2 mg, 0.035%, t_R=39.2 min). The third fraction (1.13 g) of the above mentioned MPLC was separated by MPLC (RP-18, 40–63 μm, 22×300 mm) using MeOH/H_2O (85:15) to afford eight fractions. The fourth fraction (23.6 mg) was

Table 1. ^1H and ^{13}C NMR chemical shifts assignments for malabanones A (**1**) and B (**2**) in $CDCl_3$

	1[a]		**2**[b]	
Position	δ_C	δ_H	δ_C	δ_H
1	159.0	7.04 (d, 10.2)	55.8	α 1.94 (dq, 15.5, 1.1) β 2.20 (d, 15.5)
2	125.7	5.84 (d, 10.2)		
3	205.0		223.4	
4	44.3		45.5	
5	43.8	2.39 (dd, 12.0, 3.4)	50.0	2.30 (dd, 12.8, 3.1)
6	25.5	α 1.76 (dt, 14.1, 3.4) β 1.80 (ddd, 14.1, 12.0, 2.2)	25.0	α 1.74 (dt, 13.8, 3.1) β 1.82 (ddd, 13.8, 12.8, 2.4)
7	72.7	3.98 (br s)	73.2	4.01 (br s)
8	39.9		40.2	
9	39.1	1.46 (dd, 12.5, 1.8)	43.2	1.55 (m)
10	39.3		41.0	
11	17.1	α 1.38 (m) β 1.52 (m)	18.8	α 1.15 (m) β 1.42 (m)
12	20.6	α 2.53 (dd, 14.3, 7.5) β 1.62 (m)	19.8	α 2.44 (dd, 14.7, 7.8) β 1.62 (m)
13	34.3		34.5	
14	42.2		42.0	
15	22.2	α 1.87 (m) β 2.16 (m)	22.4	α 1.87 (m) β 2.20 (m)
16	32.6	α 2.21 (m) β 2.11 (m)	32.5	α 2.23 (m) β 2.10 (m)
17	214.5		214.7	
18	27.0	a 1.60 (d, 5.0) b 1.31 (d, 5.0)	27.0	a 1.65 (d, 4.9) b 1.32 (d, 4.9)
19	19.6	1.14 (s)	17.9	0.91 (d, 1.1)
28	27.6	1.16 (s)	27.3	1.05 (s)
29	21.4	1.10 (s)	21.1	0.99 (s)
30	20.8	1.17 (s)	19.6	1.14 (s)

Chemical shifts are reported in ppm relative to residual C*H*Cl_3 resonance at 7.26 ppm for ^1H NMR and *C*DCl_3 resonance at 77.03 ppm for ^{13}C NMR. Multiplicity and *J*-values in Hz are given in parentheses.

[a] The spectra were obtained at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR.

[b] The spectra were obtained at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR.

separated by HPLC using MeOH/H_2O (50:50) to afford compounds **1** (2.0 mg, 0.00013%, t_R=157.8 min) and **2** (2.3 mg, 0.00015%, t_R=162.0 min).

1.3.1. Malabanone A (1). Amorphous powder; $[\alpha]_D$=−15° (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max}nm (log ϵ): 224 (4.19); IR (film) $\nu_{max}cm^{-1}$: 3502, 2944, 1708, 1668, 1457, 1386, 1052; ^1H and ^{13}C NMR: refer to Table 1; HMBC correlations: H-1 (C-3, C-5, C-10), H-2 (C-4, C-10), H-5 (C-1, C-4, C-6, C-7, C-10, C-19, C-28, C-29), H-6_α (C-5, C-7, C-10), H-6_β (C-5, C-10), H-7 (C-5, C-6, C-9, C-30), H-9 (C-1, C-5, C-8, C-10, C-11, C-14, C-19, C-30), H-11_α (C-9, C-12), H-11_β (C-9, C-12, C-13), H-12_α (C-9, C-11, C-13, C-17, C-18), H-12_β (C-11, C-13, C-14, C-18), H-15_α (C-13, C-14, C-16, C-17, C-18), H-15_β (C-14, C-18), H-16_α (C-15, C-17), H-16_β (C-13, C-14, C-15, C-17), H-18_a (C-8, C-12, C-13, C-14, C-15, C-17), H-18_b (C-8, C-12, C-13, C-14, C-15, C-17), H_3-19 (C-1, C-5, C-9, C-10), H_3-28 (C-3, C-4, C-5, C-29), H_3-29 (C-3, C-4, C-5, C-28), H_3-30 (C-7, C-8, C-9, C-14); EIMS *m/z* (%): 342 (M^+, 7), 325 (24), 324 (100), 311 (39); HREIMS calcd for $C_{22}H_{28}O_2$ $[M-H_2O]^+$ 324.2089, found 324.2061.

1.3.2. Malabanone B (2). Amorphous powder; $[\alpha]_D$=+60° (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max}nm (log ϵ): 210 (3.68), 276 (2.31); IR (film) $\nu_{max}cm^{-1}$: 3475, 2938, 1733, 1714, 1456, 1386, 1058; ^1H and ^{13}C NMR: refer to Table 1; HMBC correlations: H-1_α (C-3, C-9, C-10, C-19), H-1_β (C-3, C-4, C-5, C-10, C-19), H-5 (C-4, C-6, C-7, C-9, C-10, C-19, C-28, C-29), H-6_α (C-5, C-7, C-8, C-10), H-6_β (C-5, C-10), H-7 (C-5, C-9), H-9 (C-8, C-10, C-11, C-12, C-19, C-30), H-11_α (C-8, C-13), H-11_β (C-12), H-12_α (C-9, C-11, C-13, C-17, C-18), H-12_β (C-11, C-13, C-14, C-18), H-15_α (C-13, C-14, C-16, C-17, C-18), H-15_β (C-14, C-17, C-18), H-16_α (C-15, C-17), H-16_β (C-14, C-15, C-17), H-18_a (C-8, C-12, C-13, C-14, C-15, C-17), H-18_b (C-8, C-12, C-13, C-14, C-15, C-17), H_3-19 (C-1, C-5, C-9, C-10), H_3-28 (C-3, C-4, C-5, C-29), H_3-29 (C-3, C-4, C-5, C-28), H_3-30 (C-7, C-8, C-14); EIMS *m/z* (%): 330 (M^+, 15), 312 (100), 300 (57), 163 (55), 105 (52), 91 (57), 41 (66); HREIMS calcd for $C_{21}H_{30}O_3$ 330.2195, found 330.2207.

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