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QUASSINOIDS FROM *AILANTHUS VILMORINIANA*

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Key Word Index—*Ailanthus vilmoriniana*; Simaroubaceae; quassinoid; vilmorinine; spectroscopic analysis.

Abstract—Five new quassinoids, named vilmorinines B–F, have been isolated from the cortex of *Ailanthus vilmoriniana*. Their structures were established by various spectroscopic methods.

INTRODUCTION

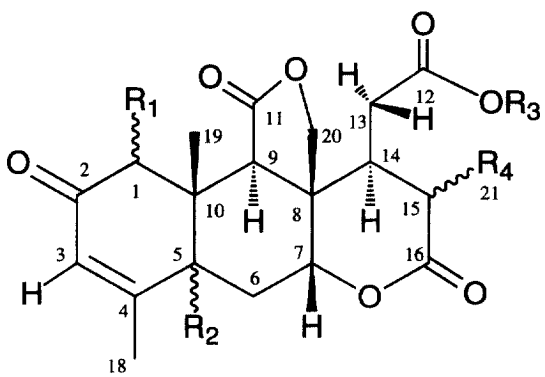
In the course of a search for new antitumour substances from higher plants [1], especially Simaroubaceae [2–6], the crude extract of *Ailanthus vilmoriniana* showed cytotoxic activity against P388 leukaemia cells. In a previous study [7], a new quassinoid named vilmorinine A was obtained from this plant. Further investigation led us to isolate five novel quassinoids, vilmorinines B (**1**)–F (**5**). In this paper, their structural elucidation is reported.

RESULTS AND DISCUSSION

The methanolic extract prepared from the cortex of *A. vilmoriniana* was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-soluble material was subjected to silica gel CC (CH_2Cl_2–MeOH) to give eight fractions. Further purification of each fraction furnished five new quassinoids, vilmorinines B (**1**)–F (**5**).

Vilmorinine B (**1**) showed the partial structures of an α,β-unsaturated carbonyl group, two lactone groups and one ester carbonyl group (IR, UV and ^{13}C NMR). Further, the proton signals of Me-19, Me-21, Me-18, OMe, H-3(olefinic) and H_2-20 were observed. From the observed data, and the ^1H-^1H and ^1H-^{13}C long range coupling correlations (Fig. 2), **1** was characterized as the C_{11}—C_{12} bond-cleaved quassinoid shown in Fig. 1. The stereochemistry of **1** was established by NOESY as shown in Fig. 3.

Vilmorinine C (**2**) gave IR, UV, MS and NMR spectral data similar to those of **1**, but no OMe signal was observed in the NMR spectrum of **2** and the $[M]^+$ peak was 14 amu less than that of **1**. These findings



	R_1	R_2	R_3	R_4
1	βOH	αH	Me	βMe
2	βOH	αH	H	βMe
3	αOH	αH	H	βMe
4	βOH	βH	H	βMe
5	βOH	βH	H	αMe

Fig. 1. Quassinoids isolated from *Ailanthus vilmoriniana*.

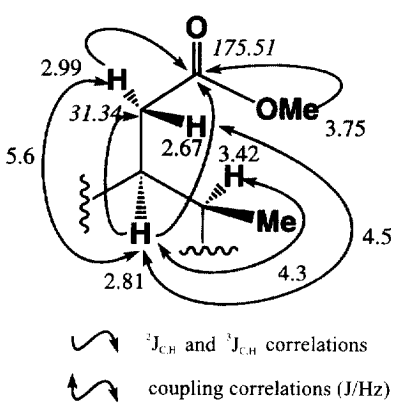


Fig. 2. Partial structure of vilmorinine B (**1**).

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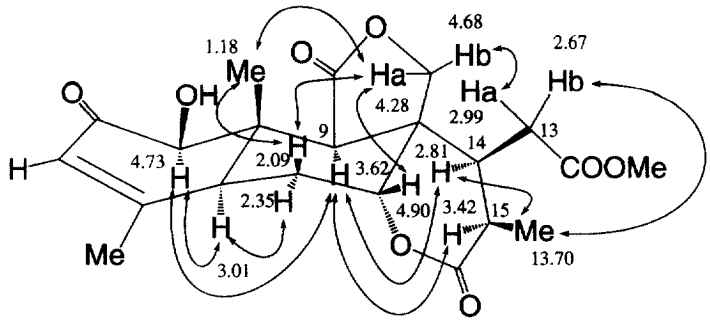
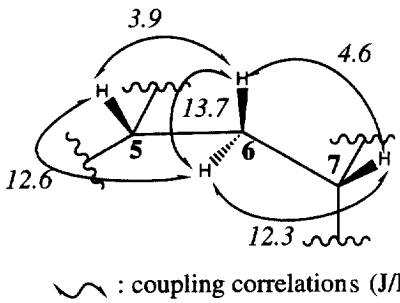


Fig. 3. NOE correlations of vilmorinine B (**1**).



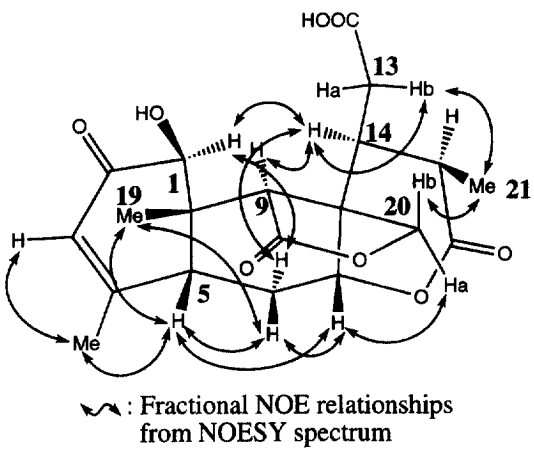


Fig. 4. ^{1}H-^{1}H Coupling and NOE correlations of vilmorinine E (**4**).

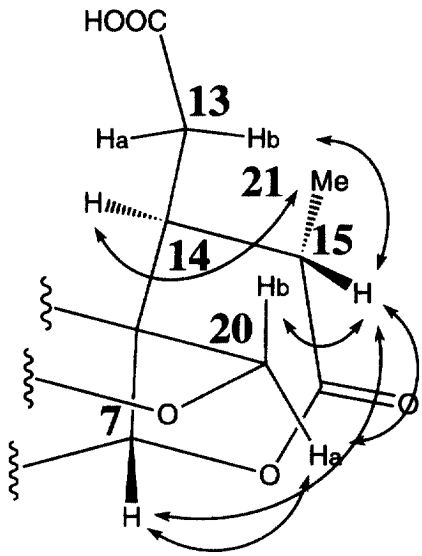


Fig. 5. Partial NOE correlations of vilmorinine F (**5**).

show that vilmorinine C (**2**) has the structure as shown in Fig. 1.

Vilmorinine D (**3**) gave the same $[M]^+$ peak as **2**. IR, UV, MS and NMR spectral data were similar to those of **2**, but the C-1 chemical shift of **3** was shifted more upfield (8.6 ppm) than that of **2**. Consequently, vilmorinine D (**3**) was determined to be the 1-epimer of **2** (Fig. 1).

Vilmorinine E (**4**) also gave the same $[M]^+$ peak as **2**. Its IR, UV, MS and NMR spectral data were similar to those of **2**, but the NOE correlations between Me-19 and H-5, Me-19 and H-6β, H-1 and H-6α, H-1 and H-14 and H-14 and H-6α (Fig. 4) suggested that vilmorinine E (**4**) was the 5-epimer of **2**.

Vilmorinine F (**5**) also had the same $[M]^+$ peak as **2**. Its IR, UV, MS and NMR spectral data were similar to those of **4**, but the NOESY spectrum of **5** did not show the NOE correlations between Me-21 and H-13β, and H-21 and Hb-20 which were observed in vilmorinines B (**1**)–E (**4**). Vilmorinine F (**5**) was confirmed to be the 15-epimer of **4** by NOESY (Fig. 5).

Vilmorinines A (**1**)–F (**5**) are probably biosynthesized by lactonization between 7-OH and 12-COOH of a quassinoid such as vilmorinine A [7] which is formed by oxidative cleavage of the C—11/C—12 bond of a quassinoid such as chapparine [8].

EXPERIMENTAL

General

M.p.s: uncorr; ^{1}H and ^{13}C NMR: pyridine-d_5 with TMS as int. standard, Bruker AM400 or AM500. ^{13}C Multiplicities were determined by the DEPT pulse sequence. 2D-NMR: NOESY, HMQC, HMBC; EI-MS (70 eV) and FAB-MS (8 kV, glycerol): VG Auto-Spec E or Finnigan MAT TSQ-700; IR: KBr or $CHCl_3$; UV: MeOH; Prep. HPLC: 10 μm ODS col-

Table 1. ^{13}C- and ^{1}H-NMR chemical shifts for vilmorinines B (**1**)–F (**5**)

C/H	Vilmorinine B (**1**)		Vilmorinine C (**2**)		Vilmorinine D (**3**)		Vilmorinine E (**4**)		Vilmorinine F (**5**)	
	δ_C	δ_H mult. (J/Hz)	δ_C	δ_H mult. (J/Hz)	δ_C	δ_H mult. (J/Hz)	δ_C	δ_H mult. (J/Hz)	δ_C	δ_H mult. (J/Hz)
1	84.65 *d*	4.73 *s*	84.58 *d*	4.78 *s*	76.00 *d*	4.57 *s*	75.22 *d*	4.98 *s*	74.82 *d*	5.03 *s*
2	197.49 *s*		197.50 *s*		196.92 *s*		198.09 *s*		198.28 *s*	
3	126.88 *d*	6.15 *br s*	126.82 *d*	6.13 *br s*	125.55 *d*	6.10 *br s*	124.88 *d*	6.00 *s*	124.82 *d*	6.03 *s*
4	160.30 *s*		160.49 *s*		159.93 *s*		160.87 *s*		161.03 *d*	
5	40.87 *d*	3.01 *br d* (12.5)	40.96 *d*	3.08 *br d* (12.6)	34.62 *d*	3.35 *br d* (13.7)	47.19 *d*	2.61 *dd* (12.6, 3.9)	47.33 *d*	2.61 *dd* (12.6, 4.2)
6	25.97 *t*	2.35 (α) *ddd* (15.6, 2.4, 2.4) 2.09 (β) *ddd* (15.6, 12.5, 2.4)	25.99 *t*	2.36 (α) *br d* (15.0) 2.10 (β) *ddd* (15.0, 12.6, 2.4)	26.12 *t*	2.40 (α) *ddd* (15.0, 2.7, 2.7) 2.10 (β) *ddd* (15.0, 13.7, 2.9)	30.82 *t*	2.04 (α) *ddd* (13.7, 12.6, 12.3) 2.33 (β) *ddd* (13.7, 4.6, 3.9)	31.51 *t*	2.10 (α) *ddd* (13.5, 12.7, 12.7) 2.34 (β) *ddd* (13.7, 4.4, 4.2)
7	75.64 *d*	4.90 *br s*	75.75 *d*	4.97 *br s*	76.38 *d*	4.98 *br s*	79.51 *d*	4.80 *dd* (12.3, 4.6)	79.09 *d*	4.78 *dd* (12.2, 4.4)
8	45.73 *s*		45.80 *s*		44.92 *s*		46.24 *s*		30.00 *s*	
9	54.25 *d*	3.62 *s*	54.26 *d*	3.82 *s*	48.30 *d*	4.23 *s*	45.66 *d*	3.88 *br s*	46.22 *d*	4.01 *s*
10	44.99 *s*		44.99 *s*		42.34 *s*		42.95 *s*		42.82 *s*	
11	172.72 *s*		176.01 *s*		176.69 *s*		174.69 *s*		175.15 *s*	
12	175.51 *s*		174.83 *s*		174.94 *s*		174.37 *s*		170.00 *s*	
13	31.34 *t*	2.99 (a) *dd* (17.1, 4.5) 2.67 (b) *dd* (17.1, 5.6)	31.98 *t*	3.02 (a) *dd* (17.2, 4.7) 2.78 (b) *dd* (17.1, 5.0)	32.16 *t*	3.77 (a) *dd* (16.9, 4.7) 3.03 (b) *dd* (16.9, 4.6)	31.44 *t*	3.84 (a) *m* 2.96 (b) *dd* (16.4, 12.4)	34.51 *t*	3.62 (a) *m* 2.76 (b) *dd* (12.5, 8.8)
14	40.57 *d*	2.81 *ddd* (5.6, 4.5, 4.3)	40.56 *d*	2.95 *m*	41.61 *d*	3.03 *m*	32.17 *d*	3.80 *m*	35.05 *d*	3.58 *m*
15	36.90 *d*	3.42 *dq* (6.5, 4.3)	31.34 *d*	3.47 *m*	36.94 *d*	3.42 *dqd* (6.8, 3.9)	36.66 *d*	3.71 *m*	40.75 *d*	2.72 *m*
16	172.23 *s*		172.49 *s*		172.34 *s*		172.35 *s*		171.47 *s*	
18	22.15 *q*	1.77 *s*	22.16 *q*	1.77 *s*	22.16 *q*	1.74 *s*	22.31 *q*	1.84 *s*	22.38 *q*	1.84 *s*
19	10.70 *q*	1.18 *s*	10.66 *q*	1.18 *s*	14.82 *q*	1.17 *s*	18.14 *q*	1.68 *s*	17.98 *q*	1.67 *s*
20	69.41 *t*	4.28 (a) *d* (10.6) 4.68 (b) *d* (10.6)	69.67 *t*	4.32 (a) *d* (10.5) 4.83 (b) *d* (10.5)	72.81 *t*	4.37 (a) *d* (10.4) 4.90 (b) *d* (10.4)	67.45 *t*	4.53 (a) *d* (9.0) 4.41 (b) *d* (9.0)	67.27 *t*	4.63 (a) *d* (9.1) 4.33 (b) *d* (9.1)
21	13.70 *q*	1.30 *d* (6.5)	13.86 *q*	1.42 *d* (6.8)	13.74 *q*	1.38 *d* (6.8)	14.90 *q*	1.41 *d* (7.9)	15.17 *q*	1.64 *d* (6.8)
OMe	54.25 *q*	3.75 *s*								

Measurements were performed on pyridine-d_5 at 400 MHz.
^{13}C Multiplicities were established by each DEPT pulse sequence.

umn; MPLC: 20 μm ODS column; Kieselgel 60; Anal. TLC: silica gel 60F_{254} and RP-18 F_{254} (0.25 mm) precoated plates. Spot detection: UV light at 254 nm and/or spraying with 10% H_2SO_4.

Plant material

The cortices of *Ailanthus vilmoriniana* were collected at Emeishan, Sichuan Province, People's Republic of China, in 1994. The botanical identification was made by Dr Zhi-Sheng Qiao, Department of Pharmacognosy, College of Pharmacy, Second Military Medical University, Shanghai, China. A voucher specimen has been deposited in the herbarium of Tokyo University of Pharmacy and Life Science.

Extraction and isolation

The cortices of *A. vilmoriniana* (7.0 kg) were extracted with MeOH (30 l) three times. The MeOH extract (864 g) was partitioned between CH_2Cl_2 and H_2O. 95 g of CH_2Cl_2-soluble fraction (170 g) was subjected to silica gel CC using a CH_2Cl_2–MeOH (1:0–0:1) gradient system to give 8 frs.

The fourth fraction (78 g) was applied to a silica gel column chromatography using a *n*-hexane–EtOAc (20:1–1:1) gradient system to furnish 7 frs. The last fraction was applied to ODS MPLC and HPLC (MeOH–H_2O or MeCN–H_2O solvent system) to give vilmorinines C (**2**, 22.1 mg), D (**3**, 7.7 mg), E (**4**, 26.1 mg) and F (**5**, 2.3 mg).

The fifth fraction (23 g) was subjected to ODS MPLC using MeOH–H_2O (9:11) solvent system to give 6 frs. The first fraction was applied to silica gel MPLC using a *n*-hexane–EtOAc (20:1–1:1) gradient system to give vilmorinine B (**1**, 45.8 mg).

Vilmorinine B (**1**). Colourless amorphous powder, m.p. 178–181°, $[\alpha]_D$ +40° (*c* 0.35, MeOH). UV λ_{max} (MeOH) nm (log ε): 238 (3.6); IR ν^{CHCl_3} cm^{-1}: 3465, 1770, 1741, 1682, 1263, 1024; FAB-MS *m/z*: 407 ($[M+H]^+$, Calcd for $C_{21}H_{27}O_8$: 407.1705, Found: 407.1709); ^1H and ^{13}C NMR (pyridine-d_5): Table 1.

Vilmorinine C (**2**). Colourless needles, m.p. 222–224°, $[\alpha]_D$ +14° (*c* 0.25, MeOH). UV λ_{max} (MeOH) nm (log ε): 238 (3.7); IR ν^{KBr} cm^{-1}: 3400, 1740, 1680, 1260, 1200; FAB-MS *m/z*: 393 ($[M+H]^+$, Calcd for $C_{20}H_{25}O_8$: 393.1549, Found: 393.1520); ^1H and ^{13}C NMR (pyridine-d_5): Table 1.

Vilmorinine D (**3**). Colourless needles, m.p. 238–240°, $[\alpha]_D$ −51° (*c* 0.25, MeOH). UV λ_{max} (MeOH) nm (log ε): 240 (3.8); IR ν^{KBr} cm^{-1}: 3450, 1740, 1730, 1680, 1260, 1220; FAB-MS *m/z*: 393 ($[M+H]^+$, Calcd for $C_{20}H_{25}O_8$: 393.1549, Found: 393.1535); ^1H and ^{13}C NMR (pyridine-d_5): Table 1.

Vilmorinine E (**4**). Colourless needles, m.p. 184–186°, $[\alpha]_D$ −34° (*c* 0.06, MeOH). UV λ_{max} (MeOH) nm (log ε): 237 (3.8); IR ν^{KBr} cm^{-1}: 3450, 1750, 1680, 1220; FAB-MS *m/z*: 393 ($[M+H]^+$, Calcd for $C_{20}H_{25}O_8$: 393.1549, Found: 393.1554); ^1H and ^{13}C NMR (pyridine-d_5): Table 1.

Vilmorinine F (**5**). Colourless needles, m.p. 251–254°, $[\alpha]_D$ +67° (*c* 0.02, MeOH). UV λ_{max} (MeOH) nm (log ε): 238 (3.7); IR ν^{KBr} cm^{-1}: 3400, 1740, 1680, 1260, 1200; FAB-MS *m/z*: 393 ($[M+H]^+$, Calcd for $C_{20}H_{25}O_8$: 393.1549, Found: 393.1547); ^1H and ^{13}C NMR (pyridine-d_5): Table 1.

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