# A New Quassinoid from *Ailanthus vilmoriniana*

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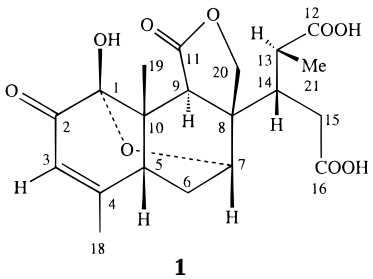
A new quassinoid, vilmorinine A (**1**), has been isolated from the cortex of *Ailanthus vilmoriniana*. The structure and conformation in the solid and solution states were elucidated by spectroscopic methods, X-ray analysis, and a computational chemical method.

The structures and antileukemic activity of various kinds of quassinoids isolated from *Eurycoma longifolia*[1−4] and *Brucea mollis*[5] (Simaroubaceae) have already been reported. During our survey of novel antileukemic compounds from higher plants, the crude extract of *Ailanthus vilmoriniana* Dode (Simaroubaceae) showed cytotoxic activity. Chromatographic purification of this extract led to the isolation of a new quassinoid, which we named vilmorinine A (**1**). This paper deals with the structure elucidation of **1** using high-field NMR methods, computational simulation study, and X-ray analysis.




**1**

The methanolic extract of 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 column chromatography ($CH_2Cl_2$−MeOH and *n*-Hex−AcOEt) followed by HPLC on ODS (MeOH−$H_2O$) to yield a new quassinoid, vilmorinine A (**1**: 0.000 33%).

Vilmorinine A (**1**), colorless needles (MeOH), mp 218−221 °C, $[\alpha]^{20}_D$ +29.3° (*c* 0.29, MeOH), showed a molecular formula of $C_{20}H_{24}O_9$, which was permitted by the HREIMS spectrum. The IR (1680 and 1760 $cm^{-1}$) and UV (242 nm) spectra indicated the presence of an α,*β*-unsaturated ketone and a *γ*-lactone. In the $^1H$ NMR spectrum, the presence of three methyl groups (*δ* 1.32, 1.65, and 1.73), an α-proton (*δ* 5.88) attached to the α,*β*-unsaturated ketone group, lactonic methylene (*δ* 4.41 and 4.73), and a proton (*δ* 5.07) attached to the oxygen-bearing carbon was observed. In the $^{13}C$ NMR spectrum, four carbonyl carbon signals (*δ* 176.22, 177.51, 178.54, and 192.57) and an acetal carbon signal (*δ* 94.90) were observed.

Using a combination of homo- and heteronuclear two-dimensional NMR techniques ($^1H-^1H$ COSY, HMQC, and HMBC spectra), complete assignments of the $^1H$ and $^{13}C$ signals of **1** were successfully performed (Table 1). HMBC correlation between H-7 and C-1, indicating an ether linkage between C-1 and C-7, was observed. On the other hand, an unusual NOE enhancement between H-5 and H-19, which is not observed in the normal picrasan skeleton, was observed, suggesting that the ring junction is *cis* for A/B. A consideration of these spectroscopic data including NOE and HMBC correlations (Figure 1) resulted in a 1,7-epoxy and 11,12-seco derivative for the structure of **1**. The relative stereostructure at C-13 and C-14 was deduced to be as in Figure 1 by NOE correlations between H-13 and H-7, between H-21 and H-15*β*, between H-14 and H-9, and between H-14 and H-13.

The structure of **1** was verified by single-crystal X-ray diffraction study, and a stereoscopic view of ORTEP drawing was shown in Figure 2.

Furthermore, to obtain the stable rotamers of side chain at C-8 in the solution state and verify the configurations at C-13 and C-14, systematic pseudo Monte Carlo simulation[6] using the MM2* force field implemented in MacroModel Bachmin (ver 4.5)[7] followed by molecular mechanics calculations were conducted. The produced conformers whose energy level was within a range of 2 kcal/mol of the global minimum were further performed by the semiempirical molecular orbital calculation using the PM3 method.[8] The 10 lowest energy conformers from the global minima are shown in Figure 3 [heavy atom RMSD = 0.48(0.26) Å]. The global minimum conformer is well satisfied with the NOE correlations as indicated above: the corresponding distances are as follows: H-13−H-7 (1.766 Å), H-21−H-15*β* (Pro-R) (2.659 Å), H-14−H-9 (2.432 Å), and H-14−H-13 (2.483 Å). Superposition of the X-ray structure and the most energetically stable conformer indicated that each structure was superimposed in fairly good agreement (heavy atom RMSD: 0.753 Å). These results indicated that the conformation in the solution state was, on the whole, homologous to that observed in the solid state.

Vilmorinine A did not show cytotoxic activity ($IC_{50}$ > 100 *μ*g/mL). The investigation of vilmorinine A in other biological assays is ongoing.

## Experimental Section

**General Experimental Procedures.** The melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. The optical rotation was measured on a JASCO DIP-4 polarimeter. The IR spectrum (KBr) was obtained on a Perkin-Elmer 1710 spectrophotometer. EI, FAB, and high-resolution mass spectra were recorded on a VG Autospec instru-

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**Table 1.** $^1$H and $^{13}$C NMR Assignments for Vilmorinine A (**1**) in Pyridine-$d_5$

| position | $^\delta$H [int; mult; *J* (Hz)] | $\delta_C$ |
|---|---|---|
| 1 | | 94.90 |
| 2 | | 192.57 |
| 3 | 5.88 (1H, br s) | 120.06 |
| 4 | | 166.35 |
| 5 | 2.51 (1H, dd, 2.3, 11.2) | 39.89 |
| 6α | 1.77 (1H, ddd, 5.4, 11.2, 14.7) | 26.11 |
| 6*β* | 2.18 (1H, dd, 2.3, 14.7) | |
| 7 | 5.07 (1H, d, 5.4) | 72.13 |
| 8 | | 49.27 |
| 9 | 3.62 (1H, s) | 46.57 |
| 10 | | 40.85 |
| 11 | | 177.51 |
| 12 | | 178.54 |
| 13 | 3.34 (1H, m) | 39.73 |
| 14 | 4.58 (1H, m) | 42.20 |
| 15α | 3.54 (1H, dd, 7.9, 16.3) | 32.90 |
| 15*β* | 2.84 (1H, dd, 2.5, 16.3) | |
| 16 | | 176.22 |
| 17 | | |
| 18 | 1.73 (3H, s) | 23.07 |
| 19 | 1.65 (3H, s) | 17.68 |
| 20α | 4.41 (1H, d, 10.9) | 70.51 |
| 20*β* | 4.73 (1H, d, 10.9) | |
| 21 | 1.32 (3H, d, 7.0) | 16.89 |

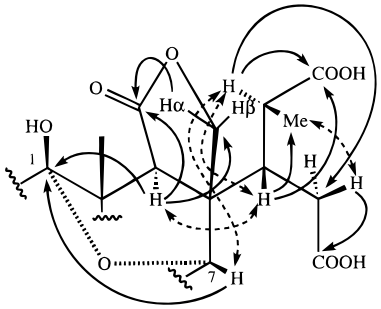


**Figure 1.** Partial structure of vilmorinine A (**1**). The arrows show $^2J_{C,H}$ and $^3J_{C,H}$ correlations in a HMBC spectrum, and the dashed arrows show NOE correlations in a phase-sensitive NOESY spectrum.

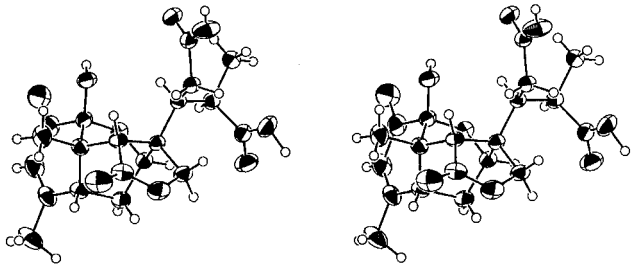

**Figure 2.** ORTEP drawing of vilmorinine A (**1**).

ment. HPLC was performed on an Inertsil PREP-ODS packed with 10 $\mu$m ODS. TLC was conducted on precoated Kieselgel 60 $F_{254}$ (Art. 5715; Merck), and the spots were detected by spraying with 10% $H_2SO_4$. $^1$H and $^{13}$C NMR spectra were run in pyridine-$d_5$ using a Bruker AM-400 instrument, with chemical shifts ($\delta$) reported in ppm. The spectra were recorded at 303 K. A phase-sensitive NOESY experiment was acquired with mixing times of 600 ms. The value of the delay to optimize one-bond correlations in the HMQC spectrum and suppress them in the HMBC spectrum was 150 ms, and the evolution delay for long-range couplings in the HMBC spectrum was set to 63 ms.

**Plant Material.** The cortex of *A. vilmoriniana* were collected from 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 & Life Science.

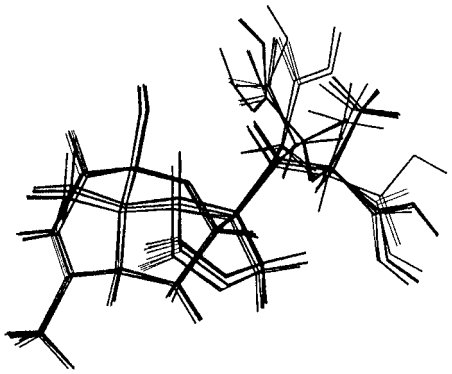

**Figure 3.** Ten lowest energy conformers of vilmorinine A, heavy atom RMSD = 0.48(0.26) Å.

**Extraction and Isolation.** The cortex of *A. vilmoriniana* (7.0 kg) were extracted with hot MeOH (30 L) three times to give a MeOH extract that was treated with $CH_2Cl_2$−$H_2O$ (1:1). The $CH_2Cl_2$-soluble fraction (95.6 g) was subjected to Si gel column chromatography using a $CH_2Cl_2$−MeOH gradient system (1:0−0:1) to separate eight fractions. The forth fraction was further subjected to Si gel column chromatography using a *n*-Hex−AcOEt gradient system (20:1−1:1) to separate seven subfractions. The last subfraction was subjected to ODS HPLC with 83% MeOH to give vilmorinine A (**1**: 23 mg, 0.000 33%).

**Vilmorinine A (1)**: colorless needles; mp 218−221 °C; $[\alpha]^{20}_D$ +29.3° (*c* 0.29, MeOH); EIMS *m*/*z* $[M]^+$ 408 (7, found $[M]^+$ 408.1420, $C_{20}H_{24}O_9$ requires 408.1427), 390 (33), 372 (75), 362 (31), 354 (63), 328 (62), 244 (93), 228 (100); IR (KBr) $\nu_{max}$ 3450 (OH), 1760 (C=O), 1680, 1560, 1420, 1380, 1180, 1130 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ 242 nm (log $\epsilon$ 3.49).

**Crystallographic Analysis of 1**. Vilmorinine A was mounted on a Mac Science MXC18 automated diffractometer with graphite-monochromated Cu Kα radiation ($\lambda$ = 1.541 78 Å) at 23 °C. The structure was determined by direct methods using the SIR program,[9] and the refinement was carried out by full-matrix least-squared methods. The molecular structures determined by these methods are illustrated in Figure 2. Crystal data are follows: molecular formula, $C_{20}H_{24}O_9$; formula weight, 408; crystal size, 1.00 × 0.10 × 0.10 mm; unit cell dimensions, $a$ = 13.437(8) Å, $b$ = 19.59(1) Å, $c$ = 7.513-(7) Å, $V$ = 1978(2) Å$^3$; crystal system, orthorhombic; space group, $P2_12_12_1$, $z$ = 4, $D_{calc}$ 1.37 g/cm$^3$; total reflections, 2256, $R$ = 0.0552, $R_w$ = 0.0733.

**Computational Methods**. Computer modeling experiments were carried out by using the MacroModel program (version 4.5) on an IRIS 4D computer (Indigo$^2$ R4400). Molecular mechanics calculations without any constraints were performed with the MM2* force field with a distance-dependent dielectric, $\epsilon = R_{ij}$. The extended cutoff distances employed were 8 Å for van der Waals, 20 Å for charge/electrostatics, and 10 Å for charge/multipole electrostatics. The obtained structures

were energy minimized by the use of the derivative convergence criteria at a value of 0.001 kJ/Å mol *in vacuo*.

The Monte Carlo calculation was carried out by using the Pseudo Monte Carlo routine. A total 5000 MC steps were performed using X-ray crystal geometry, and each of the resulting conformations was subjected to the energy-minimization calculation. Thirty-one conformers were found within a range of 2 kcal/mol of the lowest energy conformation, followed by semiempirical molecular orbital calculation with the PM3 method.

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## References and Notes

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