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Tonkinensines **A** and **B**, two novel alkaloids from *Sophora tonkinensis*

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Abstract

Tonkinensines **A** (**1**) and **B** (**2**), two novel cytisine-type alkaloids that feature the skeleton with a linkage to pterocarpan, were isolated from the roots of *Sophora tonkinensis*. Their structures and absolute configurations were elucidated by spectroscopic methods, especially X-ray crystal diffraction and CD spectral analysis. The proposed biosynthetic pathway was also discussed. Both **1** and **2** were tested in HeLa and MDA-MB-231 tumor cell lines, and compound **2** showed moderate cytotoxic activity.

Keywords: *Sophora tonkinensis*; Quinolizidine alkaloids; Tonkinensine **A**; Tonkinensine **B**

Sophora tonkinensis (Leguminosae) is an important traditional Chinese herbal plant, namely Shan-Dou-Gen in Chinese. Its roots and rhizomes were used for the treatment of acute pharyngolaryngeal infections and sore throats.[1] Phytochemical investigations have revealed that the plant accumulated lupin alkaloids and flavones as its main constituents. Cytisine-type alkaloids are a class of natural occurring lupin alkaloids that exhibit partial agonist activity toward neuronal nicotinic acetylcholine receptors with specificity for the α4β2 subtype.[2] Currently, there is much interest in developing 'cytisine-like' nicotinic agonists for the treatment of various CNS disorders and for assisting smoking cessation.[3] Pterocarpans are isoflavonoids found in many species of Leguminosae possessing high antifungal and antibacterial activities.[4] Several pterocarpans have been reported to inhibit HIV-1 reverse transcriptase and the cytopathic effect of HIV-1 in cell cultures.[5] In this Letter, we describe the isolation, structural elucidation, postulated biogenetic formation, and biological activity of tonkinensines **A** (**1**) and **B** (**2**). To our knowledge, this is the first report of the existence of cytisine-type alkaloids that feature the skeleton with a linkage to the pterocarpan.

The air-dried and ground root materials (9 kg) were extracted with 95% EtOH to give 600 g of crude extract, which was dissolved in 5 L of H_2O to form a suspension and adjusted to pH 3 with 2 M HCl. The aqueous layer was then basified to pH 10 with 5% Na_2CO_3 and extracted with $CHCl_3$ (4000 mL × 3) to obtain 150 g of crude alkaloids. The crude alkaloids were chromatographed on a silica gel column ($CHCl_3$/MeOH, 1:0–0:1) to give six fractions 1–6. Fraction 5 (10 g) was separated on a silica gel H column ($CHCl_3$/MeOH, 50:1–5:1) to afford (−)-trifolirhizin (**3**) and (−)-cytisine (**4**) (Fig. 1). Fraction 1 (4 g) was extensively separated over silica gel H and Sephadex LH-20, and further purified on semi-preparative HPLC (Agilent 1100 pump and Agilent 1100 VWD detector, Alltima ODS column, 250 × 10 mm, CH_3OH/H_2O 73:27) to yield **1** (5 mg) and **2** (15 mg) (Fig. 1). And the precipitations (600 g) were chromatographed on a silica gel column (petroleum ether/EtOAc, 30:1–0:1) to afford (−)-maackiain (**5**).

Tonkinensine **A** (**1**),[6] a colorless gum ($[\alpha]_D^{20}$ −334 (*c* 0.11, $CHCl_3$)), showed the molecular formula of $C_{28}H_{26}N_2O_6$ as determined by HRESIMS at *m*/*z* 509.1672 $[M+Na]^+$ (calcd

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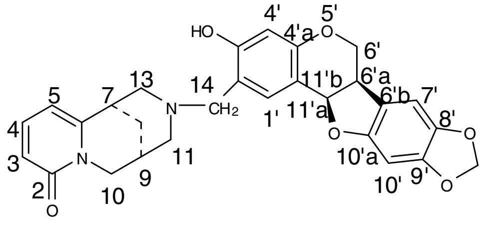
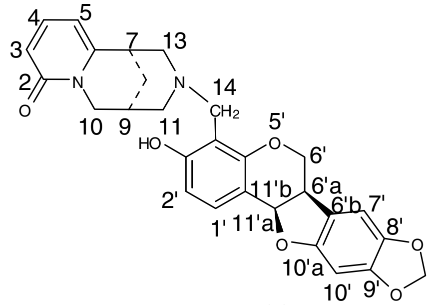
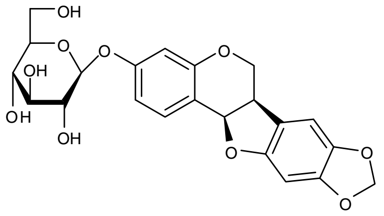


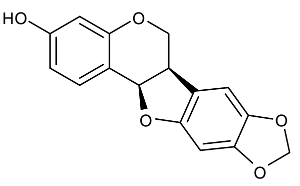
tonkinensine A (**1**)



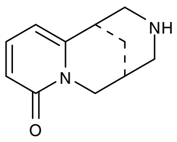
tonkinensine B (**2**)



(-)-trifolirhizin (**3**)



(-)-maackiain (**5**)



(-)-cytisine (**4**)

Fig. 1. Structures of tonkinensine **A** (**1**), tonkinensine **B** (**2**), (−)-trifolirhizin (**3**), (−)-cytisine (**4**), and (−)-maackiain (**5**).

509.1689), requiring 17 double bond equivalents. The IR absorptions revealed the presence of hydroxyl group (3431 cm^{-1}) and conjugated amide carbonyl (1649 cm^{-1}) functionality. The ^{13}C NMR and DEPT spectra resolved 28 carbon signals, which were classified by chemical shifts and HSQC spectrum as one carbonyl, nine sp^2 quaternary carbons, seven sp^2 methines, one methylenedioxy, six sp^3 methylenes, and four sp^3 methines. Among them, four methylenes (δ_C 49.5, δ_H 3.88 and 4.11; δ_C 59.4, δ_H 2.34 and 3.11; δ_C 60.7, δ_H 2.45 and 3.03; δ_C 60.9, δ_H 3.51 and 3.61) were ascribed to those bearing a nitrogen atom, while five sp^2 quaternary carbons (δ_C 141.7; δ_C 148.1; δ_C 154.1; δ_C 156.4; δ_C 159.0), one sp^3 methylenes (δ_C 68.3), and one sp^3 methines (δ_C 78.7) were assigned to those bearing oxygen atoms (Table 1).

Detailed analysis of the 2D NMR spectra of **1** revealed that it was composed of two moieties (Fig. 2). One contained four rings (rings A, B, C, and D) and the ^{1}H NMR spectrum showed signals at δ_H 4.16 (1H, dd), δ_H 3.55 (1H, m), δ_H 3.39 (1H, m), and δ_H 5.38 (1H, d), which were consistent with the presence of a pterocarpan skeleton,[7] and two sets of aromatic protons were also present for a pair of 1,2,4,5-tetrasubstituted benzenes [δ_H 7.04 (1H, s) and δ_H 6.31 (1H, s); δ_H 6.70 (1H, s) and δ_H 6.41 (1H, s)]. The ^{1}H and ^{13}C NMR data were similar to those of (−)-maackiain,[8] showing an identical pattern for the signals corresponding to rings B, C, and D. In the ROESY spectrum, the proton at δ_H 3.39 (H-6′a) showed correlations to a methylenes proton at δ_H 4.16 (H-6′eq), a methine proton at δ_H 5.38 (H-11′a), and an olefinic proton at δ_H 6.70 (H-7′). This indicated that the right moiety possessed the more stable cis-junction of rings B and C. Another moiety, an α-pyridone ring, was confirmed by the ^{1}H NMR spectrum, which showed signals at δ_H 6.51 (dd, $J = 9.2$, 1.2 Hz), δ_H 7.29 (dd, $J = 9.2$, 6.8 Hz), and δ_H 5.97 (dd, $J = 6.8$, 1.2 Hz), corresponding to H-3, H-4, and H-5, respectively. The H-10α (δ_H 4.11) and H-10β (δ_H 3.88) were also characteristic for pyridone-type quinolizidine alkaloids.[9] The ^{1}H NMR spectrum showed essentially similar signals to those of (−)-cytisine (**4**), which was previously isolated from this plant (Supplementary data). The comparison of their ^{13}C NMR spectra revealed that the signals of C-11 and C-13 were shifted downfield in the range of δ 6–7 ppm. Furthermore, in HMBC spectrum, the cross-peaks of H$_2$-14 to C-11, C-13, C-1′, C-2′, and C-3′ suggested that the right and left moieties are connected by a bond C(14)–C(2′). Thus, the basic structure of **1**, possessing an unprecedented skeleton, was established as shown in Figure 1.

Tonkinensine **B** (**2**),[10] colorless crystals (in MeOH), $[\alpha]_D^{20}$ −327 (c 0.11, CHCl$_3$), showed the molecular formula of $C_{28}H_{26}N_2O_6$ as determined by HRESIMS at m/z 509.1671 $[M+Na]^+$ (calcd 509.1689), requiring 17 double bond equivalents, which was identical to those of **1**. The