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Tetrahedron Letters 49 (2008) 3797–3801
Tetrahedron Letters

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

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Received 7 December 2007; revised 29 March 2008; accepted 1 April 2008
Available online 4 April 2008

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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doi:10.1016/j.tetlet.2008.04.003

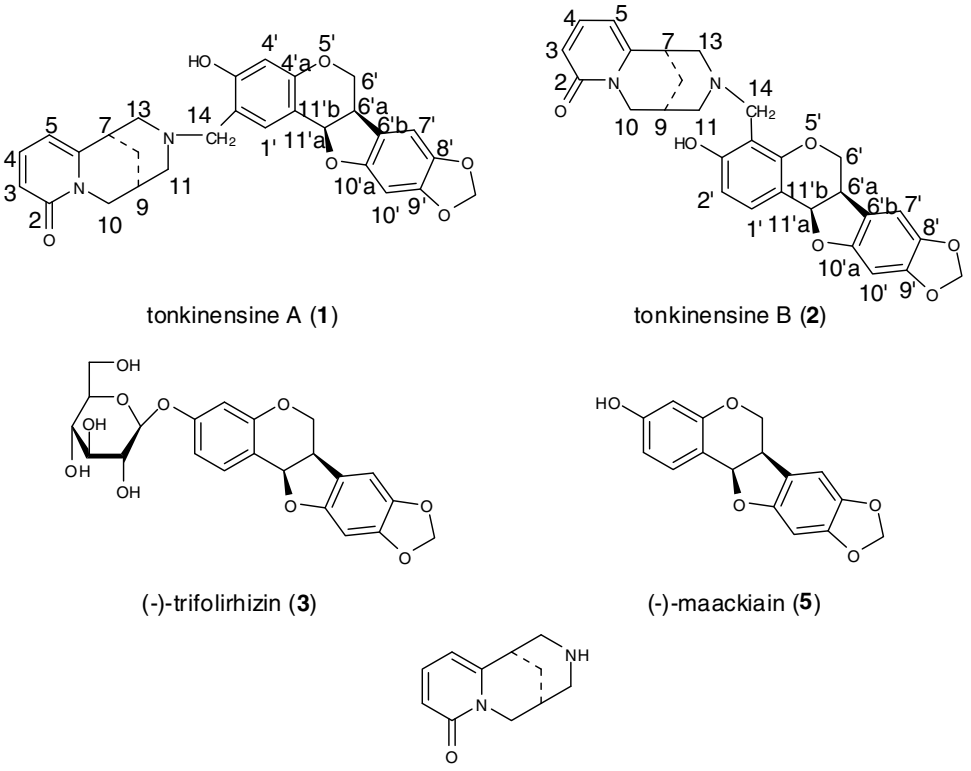


Fig. 1. Structures of tonkinensine **A** (**1**), tonkinensine **B** (**2**), (−)-trifolirhizin (**3**), (−)-cytisine (**4**), and (−)-maackiain (**5**).

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 ^1H 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 ^1H 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 ^1H 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 ^1H 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

Table 1
^{1}H and ^{13}C NMR data of **1** and **2** (in $CDCl_3$)

No.	1		2	
	δ_H (mult, *J*, Hz)[a]	δ_C[b]	δ_H (mult, *J*, Hz)[a]	δ_C[b]
2	—	163.4 s	—	163.5 s
3	6.51 (dd, 1.2, 9.2)	117.7 d	6.51 (dd, 1.2, 9.2)	117.6 d
4	7.29 (dd, 6.8, 9.2)	138.7 d	7.28 (dd, 6.8, 9.2)	138.7 d
5	5.97 (dd, 1.2, 6.8)	104.9 d	5.96 (dd, 1.2, 6.8)	105.1 d
6	—	149.4 s	—	149.3 s
7	3.05 (m)	35.1 d	3.02 (m)	35.0 d
8α	1.86 (br d, 12.8)	25.8 t	1.84 (br d, 12.8)	25.8 t
8β	1.97 (br d, 12.8)		1.96 (br d, 12.8)	
9	2.51 (br s)	27.6 d	2.50 (br s)	27.7 d
10α	4.11 (br d, 15.6)	49.5 t	4.13 (br d, 16.0)	49.5 t
10β	3.88 (dd, 6.4, 15.6)		3.89 (dd, 6.4, 16.0)	
11α	2.34 (br d, 11.6)	59.4 t	2.39 (br d, 11.2)	59.5 t
11β	3.11 (br d, 11.6)		3.10 (br d, 11.2)	
13α	2.45 (br d, 8.8)	60.7 t	2.44 (m)	60.5 t
13β	3.03 (m)		2.99 (m)	
14α	3.51 (d, 13.6)	60.9 t	3.66 (d, 14.4)	53.6 t
14β	3.61 (d, 13.6)		3.70 (d, 14.4)	
1′	7.04 (s)	130.6 d	7.21 (d, 8.4)	130.7 d
2′	—	115.2 s	6.43 (d, 8.4)	110.7 d
3′	—	159.0 s	—	159.4 s
4′	6.31 (s)	104.4 d	—	108.0 s
4′a	—	156.4 s	—	153.8 s
6′ax	3.55 (t, 11.2)	66.3 t	3.55 (t, 11.2)	66.6 t
6′eq	4.16 (dd, 4.8, 11.2)		4.18 (dd, 4.8, 11.2)	
6′a	3.39 (m)	40.2 d	3.40 (m)	40.0 d
6′b	—	118.1 s	—	118.0 s
7′	6.70 (s)	104.8 d	6.69 (s)	104.6 d
8′	—	141.7 s	—	141.6 s
9′	—	148.1 s	—	148.1 s
10′	6.41 (s)	93.7 d	6.40 (s)	93.8 d
10′a	—	154.1 s	—	154.3 s
11′a	5.38 (d, 6.4)	78.7 d	5.42 (d, 6.4)	79.0 d
11′b	—	110.7 s	—	110.6 s
Methylenedioxy	5.88 (d, 1.6)	101.3 t	5.88 (d, 1.6)	101.2 t
	5.91 (d, 1.6)		5.90 (d, 1.6)	

^{13}C multiplicities were determined by DEPT or by HSQC experiments.
[a] Recorded at 400 MHz.
[b] Recorded at 100 MHz.

IR absorptions revealed the presence of hydroxyl group (3438 cm^{-1}) and conjugated amide carbonyl (1651 cm^{-1}) functionality.

Comparison of the ^{1}H NMR and ^{13}C NMR spectra of **2** with those of **1** (Table 1) revealed that they have the different substitution patterns in ring A. The two aromatic protons at δ_H 7.21 (d, $J = 8.4$ Hz) and δ_H 6.43 (d, $J = 8.4$ Hz) showed the presence of 3′,4′-disubstituted A ring. And the ROESY correlations of $H_{6'a}$ (δ_H 3.40)/H_{6eq} (δ_H 4.18), $H_{6'a}$ (δ_H 3.40)/$H_{11'a}$ (δ_H 5.42), and $H_{6'a}$ (δ_H 3.40)/$H_{7'}$ (δ_H 6.69) implied that the rings B and C had the same pattern of junction as **1**. Furthermore, the cross-peaks of H_2-14 to C-11, C-13, C-3′, C-4′, and C-4′a in HMBC spectrum (Fig. 2) indicated that the right and left moieties were connected by a bond C(14)–C(2′). Thus, the planar structure of **2** was established as shown in Figure 1.

The relative stereochemistry of **1** and **2** was identical, as supported by their ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, ^{1}H–^{1}H COSY, ROESY spectra (Supplementary data), and the optical rotation values. The relative configuration and structure of **2** were confirmed by the X-ray crystallographic analysis (Fig. 3).[11]

A plausible biosynthetic pathway for tonkinensines **A** (**1**) and **B** (**2**) was proposed as illustrated in Scheme 1. (−)-Cytisine (**4**) and (−)-maackiain (**5**) [the aglycone of (−)-trifolirhizin (**3**)] might be the precursors for these metabolites. Formation of the 'C-14' bridge could be resulted from an oxidative coupling process in which the *N*-methyl group of (−)-*N*-methylcytisine might be oxidized to an iminium ion, and a linkage to (−)-maackiain (**5**) could occur by virtue of the phenolic group.[12]

The absolute configurations of tonkinensines **A** (**1**) and **B** (**2**) were determined by the CD spectral analysis (Fig. 4). The negative Cotton effect at 310 nm and the positive Cotton effect at 230 nm were very similar to that of (−)-cytisine (**4**) (Fig. 4), whose absolute configuration was

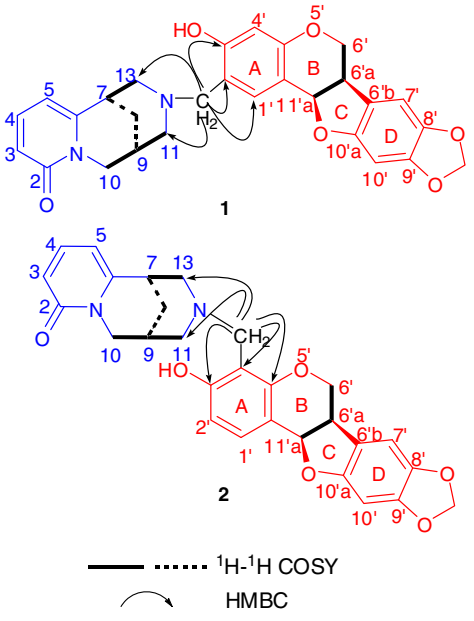


Fig. 2. Key HMBC and ^{1}H– ^{1}H COSY correlations of tonkinensines **A** (**1**) and **B** (**2**).

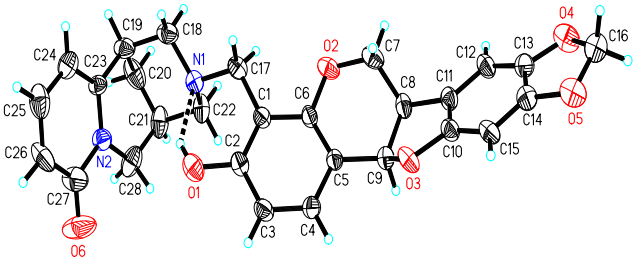


Fig. 3. X-ray structure of tonkinensine **B** (**2**).

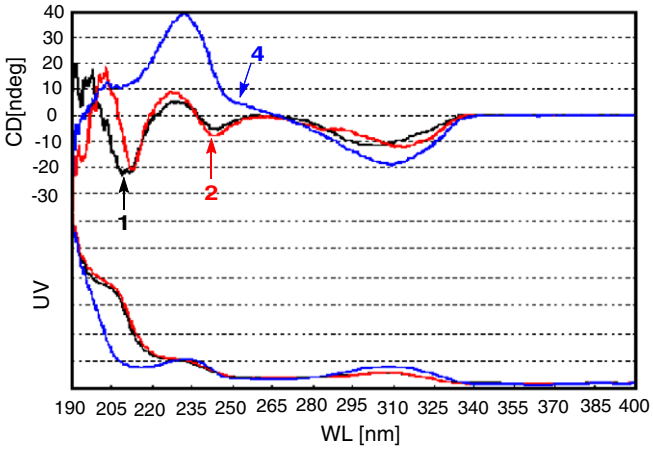
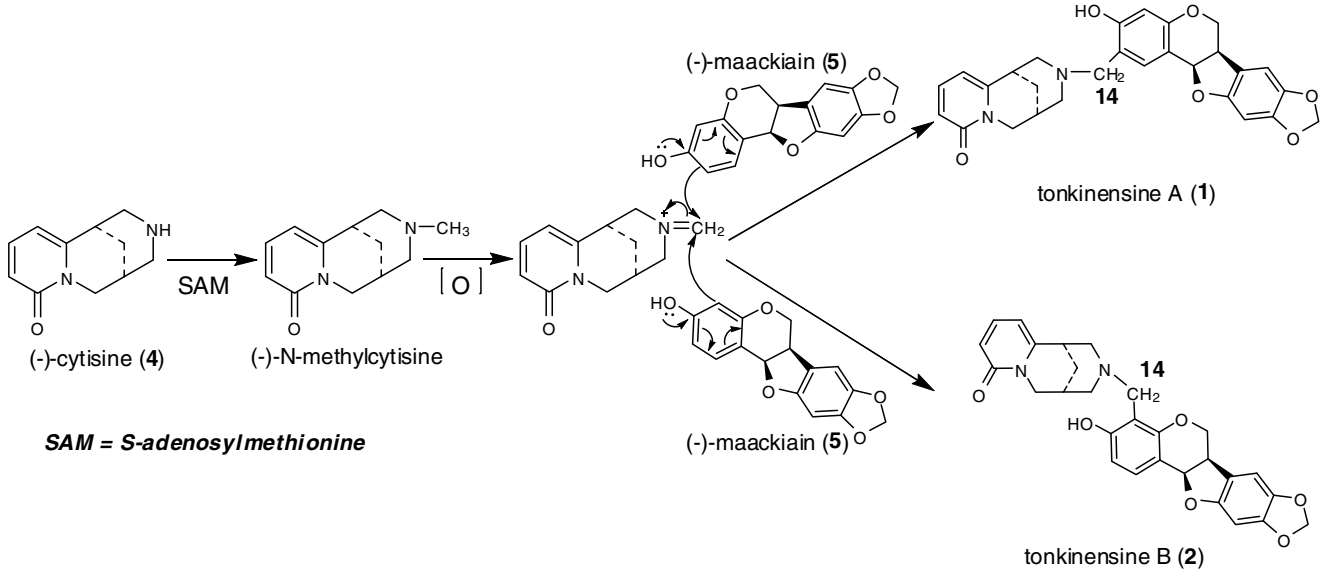


Fig. 4. CD and UV spectra of tonkinensine **A** (**1**), tonkinensine **B** (**2**), and (−)-cytisine (**4**).

assigned as 7*R*, 9*S* by CD and chemical means, suggesting that the left moiety of compound **1** and **2** also had the 7*R*, 9*S*-configuration.[13] Based on the aforementioned results, the X-ray structure of **2** (Fig. 3), and the precursors of the plausible biosynthetic pathway [(−)-cytisine (**4**) and (−)-maackiain (**5**)] obtained from this plant, the absolute configurations in the right moiety of **1** and **2** were confirmed as 6′a*R* and 11′a*R*. Thus, the absolute configurations of tonkinensines **A** (**1**) and **B** (**2**) were assigned as 7*R*, 9*S*, 6′a*R*, 11′a*R*.

Tonkinensines **A** (**1**) and **B** (**2**) were tested for their in vitro cytotoxicity against the HeLa (human cervical carcinoma) and MDA-MB-231 (human breast tumor) cell lines by using the MTT[14] method with adriamycin as a positive control ($IC_{50} = 0.405 \pm 0.003$ μM against HeLa cells and $IC_{50} = 3.37 \pm 0.05$ μM against MDA-MB-231 cells), and compound **2** showed moderate cytotoxic activity against



Scheme 1. A plausible biogenetic pathway for **1** and **2**.

the HeLa ($IC_{50} = 24.3 \pm 0.3$ μM) and MDA-MB-231 ($IC_{50} = 48.9 \pm 0.5$ μM) cell lines.

Acknowledgments

We thank National Supporting Program for TCM from Ministry of Science and Technology of China (2006 BAI 08B03-03) for financial support of this work.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.04.003.

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6. Tonkinensine **A** (**1**): colorless gums ($CHCl_3$); $[\alpha]_D^{20}$ −334 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (3.89), 231 (3.45), 308 (3.00) nm; CD (*c* 0.45, MeOH) $[\theta]_{307}$ −11.454, $[\theta]_{244}$ −5.218, $[\theta]_{228}$ +5.281; IR (KBr) ν_{max} 3431, 2922, 2852, 1649, 1547, 1473, 1460, 1134 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; positive ESIMS *m/z* (rel int) 487 $[M+H]^+$ (100); negative ESIMS *m/z* (rel int) 485 $[M-H]^-$ (100); HRESIMS *m/z* 509.1672 $[M+Na]^+$ (calcd for $C_{28}H_{26}N_2O_6Na$, 509.1689).
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10. Tonkinensine **B** (**2**): colorless crystals (in MeOH); mp 258–260 °C; $[\alpha]_D^{20}$ −327 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.85), 233 (3.51), 309 (3.18) nm; CD (*c* 0.47, MeOH) $[\theta]_{310}$ −12.076, $[\theta]_{243}$ − 7.790, $[\theta]_{227}$ +8.929; IR (KBr) ν_{max} 3438, 2918, 2848, 1651, 1547, 1475, 1460, 1146 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; positive ESIMS *m/z* (rel int) 487 $[M+H]^+$ (100); negative ESIMS *m/z* (rel int) 485 $[M-H]^-$ (100); HRESIMS *m/z* 509.1671 $[M+Na]^+$ (calcd for $C_{28}H_{26}N_2O_6Na$, 509.1689).
11. Crystallographic data for tonkinensine **B** (**2**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC-665443). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK. [fax: (+44) 1223-336-033; or email: deposit@ccdc.cam.ac.uk].
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