Taxodikaloids A and B, Two Dimeric Abietane-Type Diterpenoids from *Taxodium ascendens* Possessing an Oxazoline Ring Linkage

Xing-Hao Huang,[†,‡] Ling-Xue Tao,[§] Chang-Qiang Ke,[†] Chunping Tang,[†] Hai-Yan Zhang,[§] Yang Ye,[†,∥] Li-Gen Lin,[*,⊥] and Sheng Yao[*,†]

[†]State Key Laboratory of Drug Research & Natural Products Chemistry Department, Shanghai Institute of Materia Medica, and [§]CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China

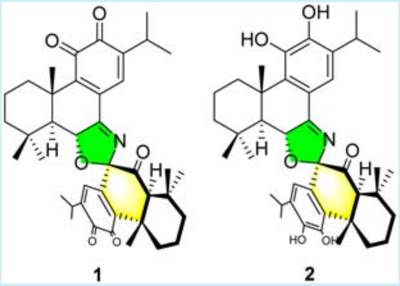
[‡]University of Chinese Academy of Sciences, No. 19A Yuquan Road, Beijing 100049, China

[∥]School of Life Science and Technology, ShanghaiTech University, Shanghai 201203, China

[⊥]State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macau, China

Supporting Information

ABSTRACT: Two dimeric diterpenoids, taxodikaloids A (**1**) and B (**2**), have been identified from the seeds of *Taxodium ascendens*. The diterpenoid structures were established on the basis of comprehensive spectroscopic analysis, and the absolute configuration of taxodikaloid A (**1**) was further confirmed by single-crystal X-ray diffraction. Both structures feature an unprecedented oxazoline ring linkage connecting two abietane diterpenoid monomers. A plausible biosynthetic pathway for compounds **1** and **2** is proposed. Both compounds show potential neuroprotective activity against Aβ_{25-35}-induced damage in SH-SY5Y cells.



Terpenoids are one of the most important classes of secondary metabolites found in the family Cupressaceae, in particular the diterpenoids, which have captured much attention in recent years due to their diverse bioactivities.[1−3] Interestingly, diterpenoids of specific types can form novel dimeric skeletons via different connecting methods. Previous investigations have revealed around 22 diterpenoid dimers with different linkages.[4] For example, taiwaniadducts B−I, obtained from *Taiwania cryptomerioides*, are products derived from an abietane and a labdane diterpene through a [4 + 2]- or [5 + 2]-cycloaddition reaction.[5,6] Formosadimer A and calocedimer C, isolated from *Calocedrus macrolepis var. formosana,* are dimers of two abietane moieties connected via one ether bridge.[7,8] Formosaninol and sugikurojin are also dimeric abietane diterpenes, but with two ether bridges, isolated from *Calocedrus macrolepis var. formosana* and *Cryptomeria japonica*, respectively.[9,10] In our previous work, two dimeric diterpenes containing a novel 2,3-dihydrofuranyl linkage between two abietane halves were identified from *Cunninghamia lanceolata.*[11]

Taxodium ascendens (Cupressaceae), introduced as an afforestation tree, grows widely in the Yangtze River Basin, China. Chemical investigations of this species have resulted in the identification of abietane-type diterpenoids, lignans, and triterpenoids.[12−14] In our ongoing efforts in the search for new scaffolds, the seeds of *T. ascendens* were thoroughly investigated, which led to the discovery of two dimeric abietane-type diterpenoids, taxodikaloids A (**1**) and B (**2**). These diterpenoids possess an unprecedented oxazoline ring linkage between two monomers. Herein, we describe the isolation and structural elucidation of these compounds and their neuroprotective effect against Aβ_{25-35}-induced damage in SH-SY5Y cells. A biosynthetic pathway for these two compounds is also proposed.

The air-dried seeds of *T. ascendens* (11 kg) were ground and extracted with 95% ethanol three times (7 days each). The pooled extracts were concentrated under reduced pressure to yield a residue (1.8 kg) that was then suspended in hot water (5 L) and extracted with petroleum ether (PE) and EtOAc successively, yielding a PE (380 g) and an EtOAc (150 g) fraction. The EtOAc fraction was further separated by column chromatography (CC) over MCI gel, silica gel, Sephadex LH-20 gel, and preparative HPLC to give compounds **1** (5.3 mg) and **2** (8.6 mg) (Figure 1).

Taxodikaloid A (**1**) was obtained as green needle-shaped crystals. Its molecular formula, $C_{40}H_{49}NO_6$, was deduced by the ion peak at m/z 639.3564 $[M]^+$ in the HR EI-MS, which indicated 17 degrees of unsaturation. The IR spectrum suggested the existence of carbonyl group(s) (1739 cm^{-1}). The ^{13}C and DEPT NMR spectra of **1** showed 40 resonances, ascribed to 10 methyls, 6 methylenes, 7 methines (five sp^3 and two sp^2), and 17 quaternary carbons (Table 1). The ^1H NMR spectrum showed signals for two aromatic protons [δ_H 7.33 (d, J = 1.2 Hz, H-14) and 6.38 (d, J = 1.2 Hz, H-14′)], four secondary methyls [δ_H 1.09

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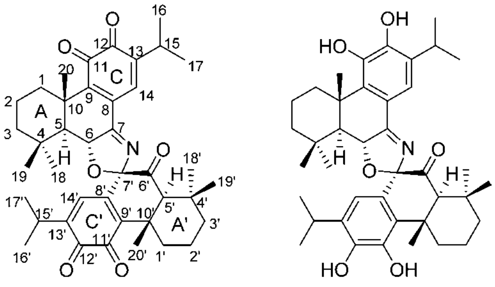


1 2

Figure 1. Structures of taxodikaloids A (**1**) and B (**2**).

(d, $J = 6.8$ Hz, H_3-16), 1.10 (d, $J = 6.8$ Hz, H_3-17), 1.15 (d, $J = 6.9$ Hz, H_3-16′), and 1.17 (d, $J = 6.9$ Hz, H_3-17′)], six tertiary methyls [δ_H 1.10 (s, H_3-18), 1.21 (s, H_3-19), 1.48 (s, H_3-20), 0.94 (s, H_3-18′), 1.34 (s, H_3-19′), and 1.36 (s, H_3-20′)], one oxygenated methine proton [δ_H 5.52 (d, $J = 13.2$ Hz, H-6)], and two septet protons [δ_H 2.96 (dtd, $J = 13.9, 6.8, 1.2$ Hz, H-15) and 2.92 (dtd, $J = 13.9, 6.9, 1.2$ Hz, H-15′)] (Table 1). The ^{1}H−^{1}H COSY correlations of two methyls (δ_H 1.09, 1.10) with one septet proton (δ_H 2.96), and the other two methyls (δ_H 1.17, 1.15) with the other septet proton (δ_H 2.92) indicated the presence of two isopropyl groups in the structure of **1** (Figure 2). The ^{1}H and ^{13}C NMR data of **1** resemble two sets of signals of 11,12-dioxoabieta-8,13-diene.[15] All this evidence suggested that **1** might contain two units of abietane-type diterpenes.

The linkage of the two diterpene units was deduced by detailed analysis of the ^{1}H−^{1}H COSY, HSQC, and HMBC spectra (Figure 2). In the first unit, the doublet proton at δ_H 1.60 was assigned as H-5 based on the HMBC correlations from this proton to C-4 (δ_C 34.2) and C-10 (δ_C 42.8). The ^{1}H−^{1}H COSY correlation from the oxygenated methine proton (δ_H 5.52) to H-5 led to the assignment of H-6. The low-field carbon at δ_C 174.4 was inferred as C-7 by the HMBC correlations from H-5, H-6, and H-14 to this carbon. In the second unit, the singlet proton at δ_H 3.11 was assigned as H-5′ by the HMBC correlations of this proton to C-4′ and C-10′. The HMBC correlations from H-5′ to the carbonyl carbon at δ_C 202.7, as well as from H-5′ and H-14′ to the quaternary carbon at δ_C 108.14, allowed the assignment of C-6′ and C-7′, respectively. Considering the two 11,12-dioxoabieta-8,13-diene units and the C-6′ carbonyl group occupy 15 of the 17 degrees of unsaturation in **1**, the remaining two degrees of unsaturation might be ascribed to the linkage between the two diterpenoid moieties. The ^{13}C NMR shift of C-7′ is typical of a ketal carbon which could be a spiro carbon participating in the formation of a new ring fragment. Thus, the extra nitrogen atom and oxygen atom in the molecule were

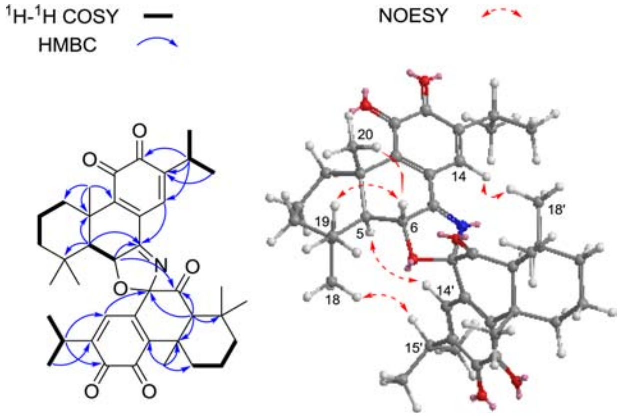


Figure 2. ^{1}H−^{1}H COSY, key HMBC, and NOESY correlations of compound **1**.

Table 1. ^{1}H and ^{13}C NMR Data (δ in ppm, J in Hz) for Compounds 1 and 2 in $CDCl_3$

	1					**2**					
no.	δ_H (J, Hz)[a]	δ_C[c]	no.	δ_H (J, Hz)	δ_C	no.	δ_H (J, Hz)[b]	δ_C[d]	no.	δ_H (J, Hz)	δ_C
1	1.23 m 2.77 m	35.5	1′	1.41 m 2.81 m	35.9	1	1.76 m 3.00 m	36.9	1′	1.67 m 3.21 m	36.9
2	1.59 m 1.72 m	18.6	2′	1.59 m 1.72 m	18.6	2	1.60 m 1.75 m	19.3	2′	1.63 m 1.77 m	19.2
3	1.27 m 1.54 m	41.5	3′	1.20 m 1.43 m	41.6	3	1.33 m 1.55 m	40.9	3′	1.23 m 1.31 m	42.0
4		34.2	4′		32.5	4		34.0	4′		33.0
5	1.60 d (13.3)	56.5	5′	3.11 s	62.2	5	1.91 d (13.0)	55.5	5′	3.48 s	61.6
6	5.52 d (13.2)	86.0	6′		202.7	6	5.40 d (12.9)	84.4	6′		207.5
7		174.4	7′		108.1	7		172.2	7′		108.8
8		142.5	8′		133.3	8		130.2	8′		119.5
9		152.3	9′		147.0	9		136.7	9′		132.8
10		42.8	10′		44.5	10		42.0	10′		45.8
11		181.5	11′		182.3	11		141.9	11′		141.9
12		180.2	12′		180.5	12		145.0	12′		140.9
13		149.4	13′		148.2	13		132.6	13′		132.7
14	7.33 d (1.2)	131.0	14′	6.38 d (1.2)	132.5	14	7.56 s	116.8	14′	6.74 s	117.7
15	2.96 dtd (13.9, 6.8, 1.2)	28.0	15′	2.92 dtd (13.9, 6.9, 1.2)	28.0	15	2.99 m	27.7	15′	2.95 m	27.6
16	1.09 d (6.8)	21.7	16′	1.15 d (6.9)	21.4	16	1.18 d (6.8)	22.8	16′	1.16 d (6.8)	22.5
17	1.10 d (6.8)	21.4	17′	1.17 d (6.9)	21.5	17	1.26 d (6.8)	23.4	17′	1.19 d (6.8)	22.8
18	1.10 s	35.4	18′	0.94 s	32.6	18	1.17 s	35.3	18′	0.93 s	32.2
19	1.21 s	22.4	19′	1.34 s	22.0	19	1.24 s	22.0	19′	1.39 s	22.6
20	1.48 s	21.1	20′	1.36 s	22.0	20	1.50 s	21.7	20′	1.41 s	21.4

[a] Data measured at 600 MHz. [b] Data measured at 500 MHz. [c] Data measured at 150 MHz. [d] Data measured at 125 MHz.

assigned to a C-7/C-7′ and C-6/C-7′ bridge, respectively, to form an oxazoline ring. The HMBC correlation between H-6 and C-6′ further confirmed the linkage. Thus, the gross structure of taxodikaloid A was determined.

The relative configuration of **1** was established through analysis of NOESY spectrum (as shown in Figure 2) and coupling constants analysis. The strong correlations between H-6 and both H_3-19 and H_3-20, along with the coupling constant of H-5/H-6 (J = 13.3 Hz), revealed that H-6 is β-oriented. The NOE correlations between H-5 and H-14′, as well as between H-14 and H_3-18′, suggested that rings A and C′ and rings C and A′, are spatially close. Thus, the configuration of the spiro C-7′ was assigned as R*. The structure and absolute configuration (5S,6R,10S,5′S,7′R,10′S) of compound **1** were further confirmed by single-crystal X-ray diffraction analysis [Cu Kα, at 296 K; Flack parameter: 0.21 (8); CCDC 1519934] (Figure 3).

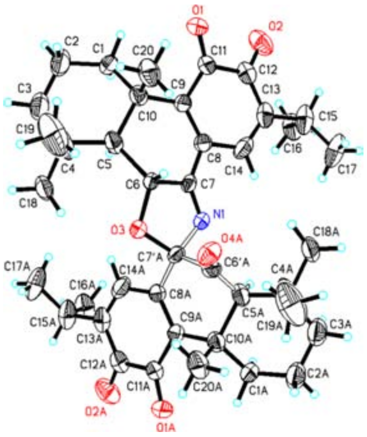


Figure 3. ORTEP drawing of **1**.

Taxodikaloid B (**2**) was isolated as a dark green, amorphous powder. The ion peak at m/z 644.3946 $[M + H]^+$ in the HR ESI-MS indicated that **2** has a molecular formula of $C_{40}H_{53}NO_6$, with 15 degrees of unsaturation. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**, except that the four carbonyl carbons in **1** are replaced by four olefinic quaternary carbons in **2** (Table 1). This evidence revealed the presence of catechol rings C and C′ in **2**, rather than o-quinone rings as in **1**. ^1H−^1H COSY, HSQC, and HMBC data allowed full construction of the planar structure of compound **2** (Figure 4). Next, a ROESY experiment was used to determine the relative configuration of **2**. The ROE correlations of H-6/H_3-20, H-6/H_3-19, H-5/H-14′, and H-14/H_3-18′ indicated that compound **2** maintains the same relative configuration as **1** (Figure 4). Similar Cotton effects in the ECD spectra of compounds **1** and **2** further confirmed that the absolute configuration of these two compounds is identical (Figure 5). Thus, the structure of compound **2** was determined.

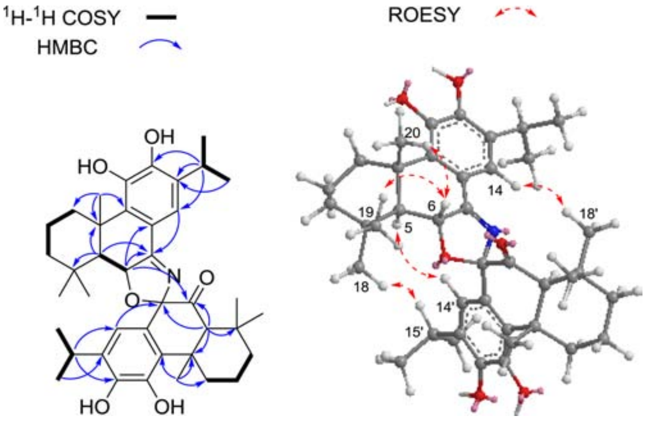


Figure 4. ^1H−^1H COSY, key HMBC, and ROESY correlations of compound **2**.

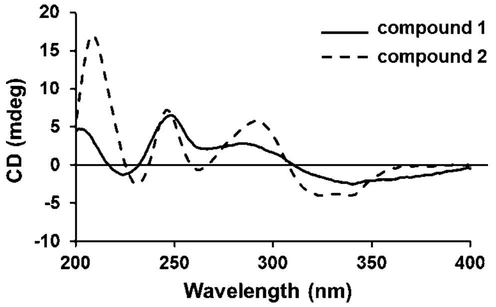
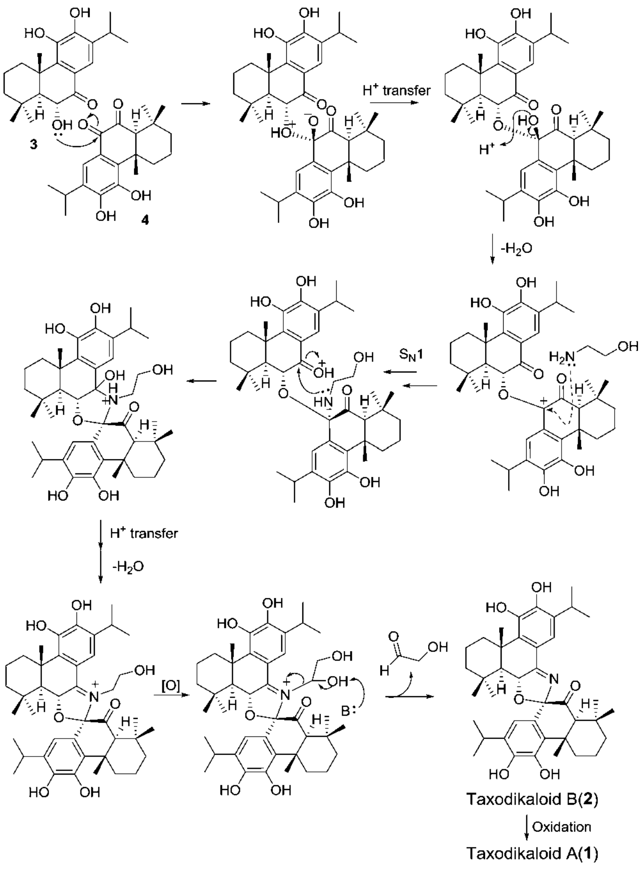


Figure 5. ECD spectra of compounds **1** and **2**.

Since the nitrogen atom in most natural products originates from amino acids such as serine and alanine,[16] a plausible biogenetic pathway for compounds **1** and **2** from two known compounds (**3** and **4**), which were also obtained in this study, is proposed (Scheme 1). The decarboxylation product of serine, 2-aminoethanol, could participate in the process. The nucleophilic hydroxyl group in 6-hydroxydemethylcryptojaponol (**3**)[17] might attack the carbonyl group in (4aS,10aS)-1,1,4a-trimethyl-5,6-

Scheme 1. Proposed Biosynthetic Pathway for Taxodikaloids A (1) and B (2)



bis(oxidanyl)-7-propan-2-yl-2,3,4,4a,10a-hexahydrophenanthrene-9,10-dione (**4**), followed by a hydrogen transfer, to form a hemiketal. In the existence of Lewis acids, a carbenium ion could form by the leaving of H_2O. Subsequently, 2-aminoethanol attacks the carbenium ion through an S_N1 reaction. The nucleophilic nitrogen atom further attacks the carbonyl group in the cryptojaponol fragment, thereby building the oxazoline ring. After one step of oxidation, the 2-hydroxyacetyl side chain is cleaved in the existence of base to form compound **2** which, after further oxidation steps, leads to the formation of compound **1**.

Extracellular deposits of amyloid β (Aβ) protein is one of the main pathological hallmarks of Alzheimer's disease (AD), and the Aβ cascade hypothesis is one of the dominant hypotheses for AD etiology.[18,19] Aβ has been generally adopted as an inducer of neuronal injury to study the protective effects and mechanism of action of new pharmacotherapies for AD.[20,21] Aβ_{25-35}, a synthetic peptide that possesses the same β-sheet structure and exhibits large fibrils, possesses most of the physical and biological properties of full length-Aβ[22] and is often used to study the neuroprotective effects of various compounds predicted to modulate Aβ toxicity in vitro.[23,24] Compounds **1** and **2** were evaluated for neuroprotective activity against Aβ_{25-35}-induced damage in SH-SY5Y cells. Results showed that Aβ_{25-35} caused a reduction of cell viability to 62.45% and compounds **1** and **2** (at 10 μM) improved the cell viability to 78.15% and 78.88%, respectively (Table S2).

To the best of our knowledge, taxodikaloids A and B are the first examples of dimeric abietane-type diterpenoids containing an oxazoline ring identified from a natural source. The oxazoline ring, constructed by concomitant formation of an oxygen and a nitrogen bridge between C-6 and C-7 of one abietane moiety and C-7 of the other, represents a new way to connect two terpene halves. The nitrogen atom introduced into the novel skeleton is thought to originate from amino acids, which are regarded as the nitrogen source for many natural products (e.g., diterpene alkaloids such as caesanines A–D).[25] Our findings expand the linkage types of dimeric diterpenoids and further offer new targets for synthetic chemists.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03597.

> Experimental procedures and spectroscopic data for all new compounds; general experimental procedures; 1D and 2D NMR data; HR ESI-MS, IR; and UV spectra of the new compounds; crystallographic information for compound **1** (PDF)
>
> X-ray data for compound **1** (CIF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: ligenl@umac.mo.
*E-mail: yaosheng@simm.ac.cn.

ORCID

Yang Ye: 0000-0003-1316-5915

Notes

The authors declare no competing financial interest.

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