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A new norlignan from *Taxodium ascendens*

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ABSTRACT

A new norlignan, (2*R*,3*R*,4*S*,5*S*)-2,4-bis(4-hydroxyphenyl)-3,5-dihydroxy-tetrahydropyran (**1**), together with 9 known compounds were isolated from the branches and leaves of *Taxodium ascendens*. Their structures were mainly determined on the basis of MS, IR, 1D and 2D NMR spectral evidences. Methanol extract showed inhibitory activity on carbonic anhydrase II with an IC_{50} value of 4.27 μg/ml, acetone extract and methanol extract inhibited activity of cathepsin B with IC_{50} values of 2.12 and 3.71 μg/ml, respectively.

1. Introduction

Taxodium ascendens Brongn belongs to Taxodiaceae and distributes naturally in the south-east of North America [1], which can grow up to 25 m high. Several diterpenes and C-32 triterpenes have been reportedly isolated from its leaves [2,3]. As part of our systematic investigations on chemical and bioactive constituents from Taxodiaceae plants, we carried out extensive chemical studies on the branches and leaves of *T. ascendens*, and obtained ten phenolics including a new norlignan (**1**) and nine known compounds (**2**–**10**). Meanwhile, the petroleum, acetone, methanol extracts, and compounds **1**–**10** were tested on carbonic anhydrase II and cathepsin B bioassays. Results indicated that the methanol extract showed inhibitory activity on carbonic anhydrase II with an IC_{50} value of 4.27 μg/ml, acetone and methanol extracts inhibited the activity of cathepsin B with IC_{50} values of 2.12 and 3.71 μg/ml respectively. In this paper, the isolation and structural elucidation of these compounds are reported.

2. Experimental

2.1. Generals

Optical rotations: Horiba SEAP-300. IR: Bio-Red FTS-135. UV: 2401PC. NMR: Bruker AM-400 and DRX-500. MS: VG Autospec-3000.

2.2. Plant

Branches and leaves of *T. ascendens* were collected in the Kunming Botanic Garden, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, PR China, in November 2003. A voucher specimen (No. 0040503) was deposited in the herbarium of Kunming Institute of Botany.

2.3. Extraction and isolation

The dried and powdered branches and leaves (11.5 kg) were respectively extracted with acetone and then with methanol at room temperature for three times. The extracts were concentrated under reduced pressure and afforded 220 g of acetone extract and 210 g of methanol extract. After degreasing by petroleum, the acetone extract (111 g) was purified by CC (2.3 kg SiO_2; $CHCl_3$/MeOH mixtures of increasing polarity), giving fractions (Fr.) 1–10. Fr.4 eluted

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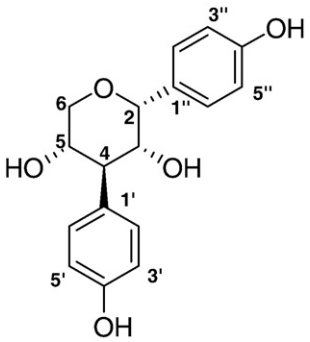

Fig. 1. The structure of compound **1**.

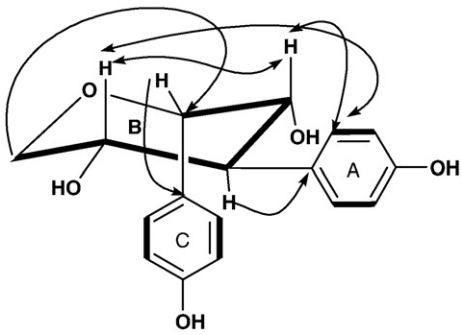

Fig. 2. The key 1H–1H COSY, HMBC and ROESY correlation of **1**.

with $CHCl_3/CH_3OH$ 12:1 to afford **4** (37 mg) and **6** (16 mg). Fr.5 was subjected to repeated CC (SiO_2; $CHCl_3/(CH_3)_2CO$ 1.5:1, $CHCl_3/CH_3OH$ 9:1; Gel Sephadex LH-20; CH_3OH/H_2O 6.5:3.5) to yield **1** (2 mg), **2** (101 mg) and **3** (95 mg). Fr.6 was subjected to repeated CC (SiO_2; $CHCl_3/CH_3OH$ 8.5:1.5; Gel Sephadex LH-20; CH_3OH/H_2O 6.5:3.5) to yield **8** (76 mg) and **5** (33 mg). Fr.7 was subjected to repeated CC (SiO_2; $CHCl_3/CH_3OH$ 8:2) to yield **7** (780 mg). The methanol extract (210 g) was purified by CC (2.4 kg SiO_2; $CHCl_3$/MeOH mixtures of increasing polarity) to give 6 Frs. Fr.3 was subjected to repeated CC (SiO_2; $CHCl_3/CH_3OH$ 5:1; Gel Sephadex LH-20; CH_3OH/H_2O 1:1) to yield **9** (61 mg) and **10** (33 mg).

Compound**1** (Fig. 1), (2*R*,3*R*,4*S*,5*S*)-2,4-bis(4-hydroxyphenyl)-3,5-dihydroxy-tetrahydropyran, colourless liquid. $[\alpha]_D^{17} = +94.2$ ($c = 0.17$, MeOH). UV (MeOH): 202.0 (4.54), 226.6 (4.31), 276.6 (3.65). IR (KBr): 3421, 2073, 1614, 1448, 1242, 1223, 827, 565, 535. For the ^1H and ^{13}C NMR spectral data see Table 1. HRTOFMS *m/z*: 301.1089 $[M-H]^-$. Calcd. for $C_{17}H_{17}O_5$ 301.1075. FAB$^-$MS *m/z*: 301 $[M-H]^-$.

3. Results and discussion

Compound **1** possessed the molecular formula of $C_{17}H_{18}O_5$ by HRTOFMS ($[M-H]^-$ at *m/z* 301.1089, calcd. 301.1075), which was confirmed by ^{13}C and DEPT NMR spectra. The IR spectrum of **1** showed absorption bands for OH (3421 cm^{-1}) and C=C groups (1614 cm^{-1}). Its UV spectrum revealed the presence of aromatic (Ph) groups (202, 227, and 277 nm). The ^1H and ^{13}C spectra (see Table 1) showed the presence of two *p*-OH substituted aromatic groups (A and C) and five other carbon atoms, which suggests that compound **1** might have a norligan skeleton.

In the 1H–1H COSY (see Table 1), the correlations between proton signals H-6, H-5, H-4, H-3 and H-2 indicated the presence of –(O)CH_2–CH (OH)–CH–CH (OH)–CH(O)– moiety (Fig. 2).

In the HMBC (see Table 1), the correlation between H-6 and C-2 indicated the presence of ring B. The correlations between H-4 and C-1′, H-2 and C-1″ suggested that ring A is linked with ring B through C-1′ and C-4, and ring B is linked with ring C through C-1″ and C-2 (Fig. 2). Thus, the structure of **1** was established as 2,4-bis(4-hydroxyphenyl)-3,5-dihydroxy-tetrahydropyran.

The structure of compound **1** was very similar with trimethylsequirin E. The coupling constant of H-6 (see Table 1) was almost the same as trimethylsequirin E ($J_{8,9\ ax} = 10.0$ Hz, $J_{8,9eq} = 4.0$ Hz) [4]. At the same time, the absolute configuration of C-5 in all naturally occurring norlignans was reported to be *S* [4,13], thus the absolute configuration of C-5 was determined to be *S*. In the ROESY (see Table 1), H-5 showed cross-peaks with H-3, which indicated that these two protons were both *a*-configuration and the absolute configuration of C-3 was *R*. Similarly, the correlations between H-3, H-5 and H-2′, 6′ were observed, which indicated that the absolute configuration of C-4 was *S*. Meanwhile, the coupling constituent of H-2 (4.2 Hz) and H-3 (5.5 Hz and 4.4) showed the two protons were both *a*-configurations, which was confirmed by the appearance of cross-peaks between H-2

Table 1
^1H and ^{13}C NMR data of **1** (500 MHz and 100 MHz, CD_3OD, *J* in Hz, δ in ppm).

C	δ_H	δ_C	H–H COSY	HMBC (H→C)	ROESY
1′		127.5			
2′, 6′	7.24 (d, 8.5)	127.6	3′,5′	3′,4,4′,5′	3,5
3′, 5′	6.73 (d, 8.5)	116.0	2′,6′	1′,2′,4′,6′	
4′		157.3			
4	3.48 (t, 6.4)	51.5	3,5	1′,2,2′,3,6,6′	3,5
5	4.58 (m)	84.0	4,6	1′,3,4	2′,3,6′
6	3.57 (dd, 11.7, 5.4), 3.53 (dd, 11.7, 3.7)	63.1	5	2,4,5	
1″		134.4			
2″, 6″	7.21 (d, 8.5)	132.5	3″,5″	2,3″,4″,5″	
3″, 5″	6.77 (d, 8.5)	116.2	2″,6″	1″,2″,4″,6″	
4″		157.9			
2	4.97 (d, 4.2)	88.1	3	1″,2″,3,4,6,6″	
3	4.29 (dd, 5.5, 4.4)	81.6	2,4	1″,2,5	2′, 5, 6′

and H-2″, 6″, and the absolute configuration of C-2 was *R*. Thus, the structure of **1** was finally determined as (2*R*,3*R*,4*S*,5*S*)-2,4-bis(4-hydroxyphenyl)-3,5-dihydroxy-tetrahydropyran, named taxodascendin, the first norlignan whose C-3 on ring B was substituted by a hydroxy.

Nine known compounds **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9** and **10** were respectively determined as: sequosemperverin B (**2**) [4], agatharesinol (**3**) [5], cryptoresinol (**4**) [6], 4-[3-hydroxy-2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]propyl]-2-methoxyphenyl-1-O-β-D-glucopyranside (**5**) [7] (7′*S*,8′*R*)-4,7′-epoxy-3,3′-dimethoxy-4,9,3′,4′,9′-lignanepentol (**6**) [8,9], (7′*R*, 8′*S*)-4,7′-epoxy-3,3′-dimethoxy-4,9,3′,4′,9′-lignanpentol-4′-*O*-*β*-*D*-glucopyranside (**7**) [10], (2*R*,3*S*)-3,3′,4′,5,7-pentahydroxyflavane (**8**) [3], 1,2-dimethoxyphenyl-4-*O*-*β*-*D*-glucoside (**9**) [11] and 1,2,3-trimethoxyphenyl-5-*O*-*β*-*D*-glucoside (**10**) [12].

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fitote.2009.05.001.

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