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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5868–5871

Taxodistines A and B, abietane-type diterpenes from *Taxodium distichum*

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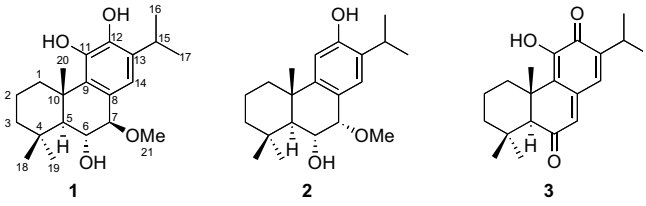
Received 11 May 2007; revised 19 July 2007; accepted 7 August 2007
Available online 11 August 2007

Abstract—Two new abietane-type diterpenes, taxodistines A (**1**) and B (**2**), have been isolated by the guidance of inhibitory effect of tubulin polymerization from the fruits of *Taxodium distichum* and the structures were elucidated by using 2D NMR data. Taxodistine B (**2**) showed inhibition of tubulin polymerization.

Antimitotic agents interact with microtubule formed by the self-association of the α,β-tubulin heterodimers and are of interest for the insights they can provide into the roles of microtubules as well as their potential activity in the treatment of cancer diseases.[1] In recent years, structurally diverse antimitotic agents such as paclitaxel and vinblastine have been isolated from higher plants. Paclitaxel is potent inhibitor of cell proliferation and arrests cells in mitosis, but in contrast to vinblastine, promotes the polymerization of purified tubulin, causing stabilization and bundling of microtubules.[1] Recently much effort has been directed to the isolation and synthesis of new antimitotic drugs that target the tubulin/microtubule system and display efficacy against drug-refractory carcinomas.[2]

During our search for bioactive compounds targeting the tubulin/microtubules from medicinal plants,[3] we found that the extract from the fruits of *Taxodium distichum* (Taxodiaceae) remarkably inhibits polymerization of tubulin. Several diterpenes such as taxodione (**3**) and taxodone from *T. distichum* have been known to show potent cytotoxic activity.[4] Our efforts on identifying new natural products that target tubulin resulted in the isolation of two new abietane-type diterpenes, taxodistines A (**1**) and B (**2**), from the fruits of *T. distichum*. This paper describes the structure elucidation of **1** and **2** on the basis of spectroscopic data as well as inhibitory effects on tubulin assembly.



1 **2** **3**

Structures of Taxodistines A (1) and B (2).

The fruits of *T. distichum* were extracted with MeOH, and the extract was partitioned between CH_2Cl_2 and H_2O. CH_2Cl_2-soluble materials were subjected to SiO_2 columns (CH_2Cl_2/MeOH and Hexane/EtOAc/MeOH/0.5% TFA) and then an ODS column (MeOH/H_2O) to afford taxodistines A (**1**, 0.4% yield) and B (**2**, 0.02% yield) together with taxodione (**3**, 0.3% yield).[5]

Taxodistine A {**1**, $[\alpha]_D^{22} + 81°$ (*c* 0.9, $CHCl_3$)} was revealed to have the molecular formula $C_{21}H_{32}O_4$, by HRESITOFMS [*m/z* 371.2210 $(M + Na)^+$, Δ +1.2 mmu]. The UV absorption at 318 nm and 282 nm indicated the presence of the benzene ring. IR absorption implied the presence of hydroxyl (3450 cm^{-1}) group. The ^1H and ^{13}C NMR data (Table 1) suggested the presence of one sp^2 methine, five sp^2 quaternary carbons, three sp^3 methylenes, four sp^3 methines, two

Keywords: Abietane-type diterpene; Taxodistine A; Taxodistine B; Tubulin.
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doi:10.1016/j.bmcl.2007.08.012

Table 1. ^{1}H [δ_H (J, Hz)] and ^{13}C [δ_C] NMR data of taxodistines A (**1**) and B (**2**) in $CDCl_3$ at 300 K

Compound	**1**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1a	1.38 (1H, ddd, 13.3, 11.0, 4.8)	36.9	1.43 (1H, ddd, 12.4, 11.9, 3.1)	39.1
1b	2.99 (1H, ddd, 13.3, 5.4, 4.8)		2.08 (1H, brd, 11.9)	
2a	1.28 (1H, m)	19.0	1.61 (1H, m)	18.9
2b	1.68 (1H, m)		1.76 (1H, m)	
3a	1.28 (1H, m)	42.4	1.23 (1H, m)	42.7
3b	1.47 (1H, m)		1.48 (1H, m)	
4		33.7		33.9
5	1.51 (1H, d, 11.2)	53.2	1.25 (1H, m)	52.9
6	4.23 (1H, dd, 11.2, 8.7)	68.8	4.61 (1H, dd, 10.0, 2.8)	86.1
7	4.48 (1H, d, 8.7)	85.9	4.69 (1H, d, 2.8)	81.9
8		126.5		125.1
9		133.6		149.7
10		41.6		38.5
11		141.9	6.63 (1H, s)	110.0
12		139.9		153.0
13		132.1		131.6
14	6.81 (1H, s)	115.7	7.11 (1H, s)	128.7
15	3.03 (1H, m)	27.3	3.15 (1H, m)	26.8
16	1.25 (3H, d, 7.4)	22.6	1.25 (3H, d, 7.4)	22.6
17	1.26 (3H, d, 7.4)	22.7	1.26 (3H, d, 7.4)	22.6
18	1.23 (3H, s)	35.8	1.04 (3H, s)	34.9
19	1.20 (3H, s)	23.2	1.16 (3H, s)	22.4
20	1.44 (3H, s)	21.8	1.30 (3H, s)	25.1
21	3.24 (3H, s)	53.0	3.35 (3H, s)	54.9

sp^3 quaternary carbons, and six methyl groups. Among them, two sp^3 methines (δ_C 68.8 and 85.9), two sp^2 quaternary carbons (δ_C 141.9 and 139.9), and one methyl (δ_C 53.0) were substituted to the oxygen atom.

Partial structures **a**–**c** (C-1–C-3, C-5–C-7, and C-15–C-17) were deduced from detailed analyses of 2D NMR data (^{1}H–^{1}H COSY) of **1** (Fig. 1). The HMBC cross-peaks of H_3-19 to C-3, C-4, C-5, and C-18 indicated

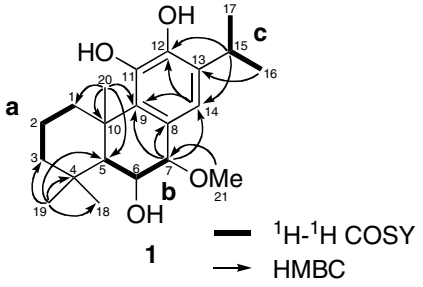

Figure 1. Selected 2D NMR correlations for taxodistine A (**1**).

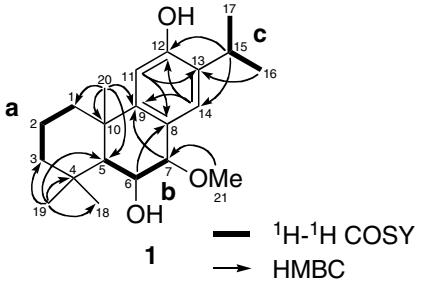

Figure 3. Selected 2D NMR correlations for taxodistine B (**2**).

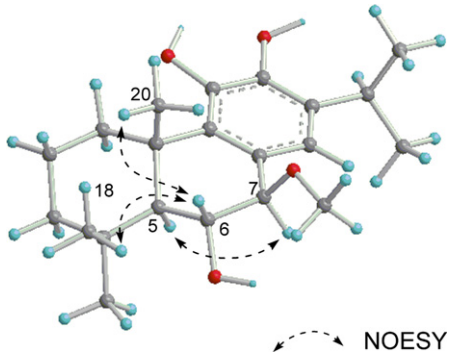

Figure 2. Selected NOESY correlations and relative stereochemistry for taxodistine A (**1**).

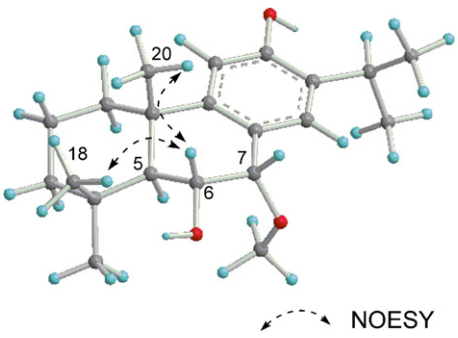

Figure 4. Selected NOESY correlations and relative stereochemistry for taxodistine B (**2**).

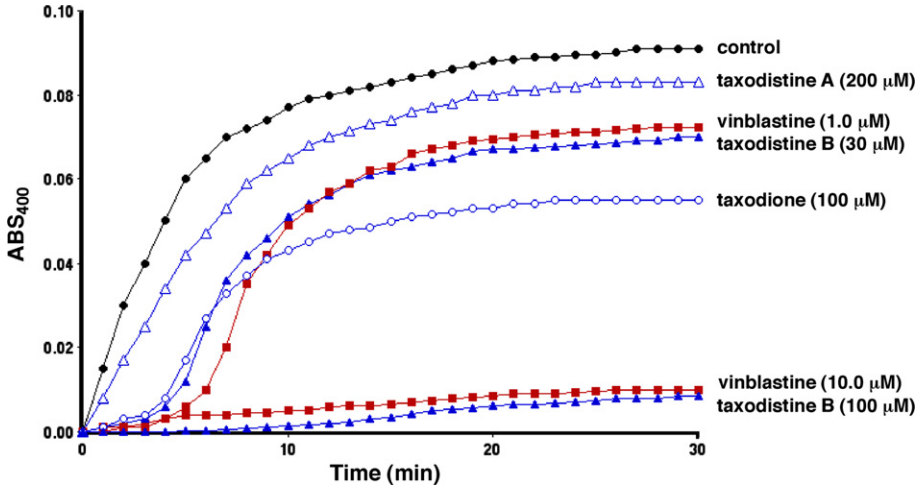

Figure 5. Inhibitory effects of taxodistines A and B, and vinblastine on the polymerization of tubulin protein. Various concentrations of diterpenes were mixed with tubulin protein (1.0 mg/mL) at 0 °C and incubated at 37 °C. The absorbance at 400 nm was measured.

the connection among C-3, C-5, C-18, and C-19 through C-4. HMBC correlations for H_3-20 to C-1, C-5, C-9, and C-10 indicated connection among C-1, C-5, C-9, and C-20 through C-10. On the other hands, HMBC correlations for H-7 to C-8, C-9, and C-14 supported the connectivity of the benzene ring (C-8, C-9, and C-11–C-14) and C-7. And the connection between C-13 and C-15 was deduced from HMBC correlations of H_3-16 to C-13, and H-15 to C-12 and C-14. Furthermore, the presence of a methoxy group at C-7 was elucidated by the HMBC correlation from H_3-21 to C-7. Thus, the gross structure of taxodistine A was assigned as **1**.

The relative stereostructure of **1** as shown in computer generated 3D drawing (Fig. 2) was deduced from cross-peaks observed in the NOESY spectrum and ^3J coupling constants. The NOESY correlation of H-6/H_3-18 and H_3-20 indicated β-orientation of H-6, CH_3-18, and CH_3-20. The coupling constant, $^3J_{H5/H6}$ = 11.2 Hz, indicated *anti* conformation between H-5 and H-6. And the coupling constant, $^3J_{H6/H7}$ = 8.7 Hz indicated *anti* conformation between H-6 and H-7. The α-configuration of both H-5 and H-7 was supported by the NOESY cross-peak between H-5 and H-7. Thus, the relative stereochemistry of **1** was assigned as shown in Figure 2.

Taxodistine B {**2**, $[\alpha]_D^{22} + 63°$ (*c* 0.5, $CHCl_3$)} was revealed to have the molecular formula $C_{21}H_{32}O_3$, by HRESITOFMS [*m*/*z* 300.2107 $(M\text{-}MeOH)^+$, Δ +1.8 mmu]. The UV absorption at 278 nm indicated the presence of the benzene ring. IR absorption implied the presence of hydroxyl (3420 cm^{-1}) group. The ^1H and ^{13}C NMR data (Table 1) suggested that **2** had the same abietane-skeleton as that of **1**, except for the presence of an aromatic proton at C-11 (δ_H 6.63, δ_C 110.0) (Fig. 3). The relative stereochemistry of taxodistine B (**2**) was deduced by NOESY spectrum and ^3J coupling constants. The configuration of β-oriented H-6, CH_3-18, and CH_3-20 was supported by the NOESY correlation of H-6/H_3-18 and H_3-20. The coupling constant, $^3J_{H5/H6}$ = 10.0 Hz indicated *anti* conformation between H-5 and H-6. And the coupling constant, $^3J_{H6/H7}$ = 2.8 Hz indicated *gauche* conformation between H-6 and H-7. Thus, the relative stereochemistry of **2** was assigned as 11-deoxy-7-*epi*-taxodistine A (Fig. 4).

Taxodistines A (**1**) and B (**2**) showed cytotoxicity against murine lymphoma P388 cells at IC_{50} 0.43 and 6.5 μg/mL, respectively. The effect of taxodistines A (**1**) and B (**2**) was examined against polymerization of microtubules.[6] Microtubule polymerization and depolymerization were monitored by the increase and decrease in turbidity. The results are summarized in Figure 5 as the changes in the relative absorbance at 400 nm. After 30 min, polymerization of microtubules was inhibited 90% by 100 μM taxodistine B (**2**). Taxodistine A (**1**) did not inhibit the polymerization process at higher concentration (200 μM), while taxodione (**3**) showed 45% inhibition at 100 μM.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and grants from the Research Foundation for Pharmaceutical Sciences and The Open Research Project. The authors thank Prof. Koichi Takaya, Tokyo University of Pharmacy & Life Science, for cytotoxicity assay.

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6. Microtubule assembly was monitored spectroscopically by using a spectrophotometer equipped with a thermostatically regulated liquid circulator. The temperature was held at 37 °C and changes in turbidity were monitored at 400 nm. For the drug–protein studies, drugs were dissolved in 1% DMSO concentration. The turbidity changes were monitored throughout the incubation time.