Two New Taxoids from the Leaves and Stems of *Taxus chinensis*

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Two new taxoids called 7,13-dideacetyl-9,10-debenzoyl-7 β ,9 α -*p*-hydroxylbenzylidenedioxy-taxchinin C (**1**) and 7,13-dideacetyl-2,9,10-debenzoyl-2-tigloyl-7 β ,9 α -*p*-hydroxylbenzylidenedioxy-taxchinin C (**2**) were isolated from the leaves and stems of *Taxus chinensis*. They represent the first examples of taxoids containing the 5/7/6-membered ring system with 7 β ,9 α -*p*-hydroxylbenzylidenedioxy groups, the structures of which were elucidated based on spectroscopic analyses, especially 1D and 2D NMR spectra.

Key words *Taxus chinensis*; taxoid; 7 β ,9 α -*p*-hydroxylbenzylidenedioxy-taxchinin C

The extensive utilization of paclitaxel as an anticancer agent has stimulated interest in the analysis of the various *Taxus* species to find alternative sources of paclitaxel or related compounds with improved activity. As a result, natural taxoids are increasingly being isolated and identified.^{1,2)} *Taxus chinensis* (PILGER) REHD, indigenous to China, is considered a promising source of taxoids,^{3–8)} and we have investigated the chemical constituents of *T. chinensis* in previous studies.^{9–12)}

In our continuing search for new, bioactive natural taxoids, we have reinvestigated the polar neutral fractions of the extracts from the leaves and stems of *T. chinensis* and obtained two new taxoids, 7,13-dideacetyl-9,10-debenzoyl-7 β ,9 α -*p*-hydroxylbenzylidenedioxy-taxchinin C (**1**) and 7,13-dideacetyl-2,9,10-debenzoyl-2-tigloyl-7 β ,9 α -*p*-hydroxylbenzylidenedioxy-taxchinin C (**2**). Structurally, these two new compounds are the first examples of natural taxoids containing the 5/7/6-membered ring system with 7 β ,9 α -*p*-hydroxylbenzylidenedioxy groups, although a natural 4,20-*p*-hydroxylbenzylidenedioxy taxane diterpene and several synthetic analogues have been reported.^{12–14)} In this paper, we report the isolation and structural elucidation of the two new taxoids.

Results and Discussion

Compound **1** was isolated as a white amorphous powder. Its molecular formula was determined to be C₃₆H₄₂O₁₁ based on the positive HR-electrospray ionization (ESI)-MS (*m/z* 673.2644 [M+Na]⁺, Calcd 673.2624). The ¹H-NMR spectrum of **1** showed four characteristic methyl signals of a taxoid skeleton at δ_H 1.05, 1.06, 1.94, and 1.95, and the ¹³C-NMR spectrum displayed characteristic carbon resonances at δ_C 68.4 (s, C-1), 45.1 (d, C-3), 80.3 (s, C-4), 38.4 (s, C-8), and 76.0 (s, C-15). From the above-mentioned evidence, it can be deduced that **1** is a taxoid with a 5/7/6-membered ring

system.³⁾ Moreover, the carbon resonances at δ_C 80.3 (s, C-4) and 75.1 (t, C-20), along with proton signals at δ_H 4.02 (d, *J*=7.5 Hz, H-20 α) and 4.42 (d, *J*=7.5 Hz, H-20 β), indicate the presence of an oxetane ring in this molecule.³⁾ Exhaustive comparison of the NMR data (Table 1) of **1** with those of the known 7,13-dideacetyl-9,10-debenzoyltaxchinin C,¹⁵⁾ showed strong similarities except for the presence of an additional *p*-hydroxylbenzylidenedioxy group in **1**, based on the carbon resonances at δ_C 95.5 (d), 128.9 (d, 2 $\times$ C), 115.4 (d, 2 $\times$ C), 131.5 (s), and 158.5 (s), together with corresponding proton resonances at δ_H 6.03 (1H, s), 7.40 (2H, d, *J*=8.5 Hz), and 6.83 (2H, d, *J*=8.5 Hz). The heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 1) of H-1' with C-3' and C-7', H-3' with C-5' and C-1', H-6' with C-2', and H-7' with C-1' further confirmed the presence of the *p*-hydroxylbenzylidenedioxy unit. The long-range correlations between H-1' and C-7 and C-9 in the HMBC spectrum assigned the *p*-hydroxylbenzylidenedioxy group to C-7 and C-9.

The relative stereochemistry of **1** was confirmed in a rotating frame Overhauser enhancement spectroscopy (ROESY) experiment, in which cross peaks between H-2 (δ_H 6.15, 1H, d, *J*=7.6 Hz) and H-9 (δ_H 4.39, 1H, d, *J*=9.6 Hz) and Me-19 (δ_H 1.95, 3H, s), H-9 and Me-17 (δ_H 1.05, 3H, s), H-13 (δ_H 4.64, 1H, dd, *J*=11.6 Hz) and Me-16 (δ_H 1.06, 3H, s), H-5 (δ_H 4.96, 1H, d, *J*=8.8 Hz), and H-6a (δ_H 2.4, 1H, m), indicated that H-2, H-9, H-13, and C-20 are β -oriented, while correlations of H-3 (δ_H 3.06, 1H, d, *J*=7.6 Hz) with H-5 (δ_H 4.96, 1H, d, *J*=8.8 Hz), H-7 (δ_H 4.11, 1H, dd, *J*=9.6, 8.0 Hz), and H-10 (δ_H 5.4, 1H, dd, *J*=9.6, 4.3 Hz) confirmed the α -configuration of H-3, H-5, H-7, and H-10, respectively (Fig. 1). The ROESY correlations of H-1' with H-7 and H-10 established the α -orientation of H-1'. The structure of the

Fig. 1. Key HMBC (H $\rightarrow$ C) and ROESY Correlations (H $\leftrightarrow$ H) of Compound **1**

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Table 1. ^1H - and ^{13}C -NMR Data of **1** and **2**^{a)}

No.	1		2	
	δ_{H} mult. ($J=\text{Hz}$)	δ_{C}	δ_{H} mult. ($J=\text{Hz}$)	δ_{C}
1	—	68.4 s	—	68.4 s
2	6.15 (1H, d, 7.6)	70.1 d	5.93 (1H, d, 7.6)	69.4 d
3	3.06 (1H, d, 7.6)	45.1 d	2.94 (1H, d, 7.6)	45.1 d
4	—	80.3 s	—	80.3 s
5	4.96 (1H, d, 8.8)	85.3 d	4.96 (1H, d, 8.8)	85.3 d
6 α	2.40 (1H, m)	34.9 t	1.74 (1H, m)	34.9 t
6 β	1.77 (1H, m)	—	2.37 (1H, m)	—
7	4.11 (1H, dd, 9.6, 8.0)	76.4 d	4.06 (1H, dd, 9.8, 7.9)	76.4 d
8	—	38.4 s	—	38.4 s
9	4.39 (1H, d, 9.6)	84.4 d	4.31 (1H, d, 9.6)	84.4 d
10	5.40 (1H, dd, 9.6, 4.3)	63.4 d	5.33 (1H, dd, 9.6, 5.3)	63.4 d
11	—	137.2 s	—	137.4 s
12	—	148.3 s	—	148.1 s
13	4.64 (1H, dd, 6.6, 11.6)	77.2 d	4.58 (1H, d, 3.8)	77.2 d
14 α	1.99 (1H, dd, 14.2, 7.3)	40.1 t	1.85 (1H, overlap)	40.2 t
14 β	2.23 (1H, dd, 14.2, 7.3)	—	2.11 (1H, m)	—
15	—	76.0 s	—	76.0 s
16	1.06 (3H, s)	28.2 q	1.03 (3H, s)	25.0 q
17	1.05 (3H, s)	25.0 q	1.02 (3H, s)	28.2 q
18	1.94 (3H, s)	11.5 q	1.90 (3H, s)	11.5 q
19	1.95 (3H, s)	14.2 q	1.83 (3H, s)	12.0 q
20 α	4.02 (1H, d, 7.5)	75.1 t	4.17 (1H, d, 7.6)	75.2 t
20 β	4.42 (1H, d, 7.5)	—	4.35 (1H, d, 7.6)	—
OBz	—	166.9 s	—	—
<i>i</i>	—	129.2 s	—	—
<i>o</i>	8.08 (2H, d, 7.4)	130.3 d	—	170.3 s
<i>m</i>	7.56 (2H, t, 7.5)	129.6 d	—	22.0 q
<i>p</i>	7.67 (1H, t, 7.5)	134.2 d	—	95.5 d
OAc	—	170.4 s	—	131.6 s
OAc	2.21 (3H, s)	22.1 q	2.13 (3H, s)	128.9 d
1'	6.03 (1H, s)	95.5 d	5.99 (1H, s)	115.4 d
2'	—	131.5 s	—	158.6 s
3', 7'	7.40 (2H, d, 8.5)	128.9 d	7.37 (2H, d, 8.5)	168.1 s
4', 6'	6.83 (2H, d, 8.5)	115.4 d	6.81 (2H, d, 8.5)	129.9 s
5'	—	158.5 s	—	138.9 d
8'	—	—	—	14.4 q
9'	—	—	—	14.1 q
10'	—	—	6.91 (1H, dd, 7.1, 1.3)	—
11'	—	—	1.82 (3H, s)	—
12'	—	—	1.88 (3H, s)	—

a) Recorded in acetone- d_6 at 400 MHz for ^1H , 100 Mz for ^{13}C , δ in ppm, J in Hz.

new compound is thus established as a derivative of 7,13-dideacetyl-9,10-dibenzoyltaxchinin C.^{3,15)} Therefore, the structure of **1** was identified as 7,13-dideacetyl-9,10-debenzoyl-7 β ,9 α -*p*-hydroxybenzylidenedioxytaxchinin C.

Compound **2**, a colorless oil, was assigned the molecular formula $\text{C}_{34}\text{H}_{44}\text{O}_{11}$ as deduced from the quasimolecular ion peak at m/z 651.2780 $[\text{M}+\text{Na}]^+$ in the HR-ESI-MS (positive), together with the analyses of the ^{13}C -NMR and distortionless enhancement by polarization transfer (DEPT) spectra. The NMR data (Table 1) of **2** revealed that it was also a taxane diterpene with a 5/7/6-membered ring system and an oxetane ring,³⁾ featuring four methyl signals of the taxoid skeleton at δ_{H} 1.03, 1.02, 1.83, and 1.90. Compound **2** had ^1H - and ^{13}C -NMR spectral patterns similar to those of **1**. The only difference was the presence of a tiglyol group instead of the benzoyl in **1** due to the carbon resonances at δ_{C} 168.1 (s, C-8'), 129.9 (s, C-9'), 138.9 (d, C-10'), 14.4 (q, C-11'), and 14.1 (q, C-12'), together with proton signals at δ_{H} 6.91 (dd, $J=7.1, 1.3$ Hz, H-10'), 1.82 (s, Me-11'), and 1.88 (s, Me-12'). The tiglyol group was located at C-2 based on the ob-

Fig. 2. Key HMBC ($\text{H} \rightarrow \text{C}$) and ROESY Correlations ($\text{H} \leftrightarrow \text{H}$) of Compound **2**

served cross peak between H-2 (δ_{H} 5.93 d, $J=7.6$ Hz) and the ester carbonyl of tiglyol (δ_{C} 168.1) in the HMBC experiment (Fig. 2). The relative configuration of **2** was identical to that of **1** based on the interpretation of the ROESY spectrum of **2** (Fig. 2). Consequently, the structure of **2** to be established as 7,13-dideacetyl-2,9,10-debenzoyl-2-tiglyol-7 β ,9 α -*p*-hydroxybenzylidenedioxytaxchinin C.

Experimental

General Experimental Procedures Melting points were determined on an XRC-1 micro melting point apparatus and are uncorrected. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained on a UV 2401 PC spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. ^1H - and ^{13}C -NMR experiments were performed on a Bruker AM-400 spectrometer (^1H , 400 MHz; ^{13}C , 100 MHz), while 2D NMR spectra were recorded using a Bruker DRX-500 NMR instrument. ESI-MS were taken on a Finnigan-MAT 90 instrument. HR-ESI-MS were measured with a VG Auto Spec 3000 spectrometer. Column chromatography was performed using silica gel (Qingdao Marine Chemical Inc., Qingdao, P.R. China), Lichroprep RP-18 (Merck, Darmstadt, Germany), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd.). HPLC was performed on Hewlett-Packard 1100 Series chromatographs using a Zorbax SB-C18 column (9.4×250 mm). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material The leaves and stems of *T. chinensis* (PILG.) REHD (Taxaceae) were collected in Liangshang, Sichuan Province, P.R. China, in April 2006 and identified by Prof. Lin Zhongwen. A voucher specimen (No. 200604) has been deposited in the College of Pharmacy, Dali University, P.R. China.

Extraction and Isolation The dried leaves and stems (20 kg) of *T. chinensis* were extracted three times with 95% ethanol to give a crude extract (350 g), after concentration under a vacuum. The residue was chromatographed over a silica gel column eluted with a chloroform–acetone gradient (9:1→1:1, and acetone) to give six fractions (A–E, acetone), of which three (C, D, E) (68 g) were combined and chromatographed on a DM-130 column (methanol–water, 9:1) to give a mixture of 56 g. The subfraction was chromatographed over a silica gel column eluted with a chloroform–methanol gradient (100:1→50:1) to afford four subfractions, I–IV. Subfraction II was chromatographed on Sephadex LH-20 eluted with MeOH, and a mixture of compounds **1** and **2** was obtained. The mixture was further chromatographed on a silica gel column eluted with cyclohexane–chloroform–2-propanol (6.0:3.5:0.5→5.0:4.5:0.5), and finally purified on Lichroprep RP-18 (methanol–water, 5.5:4.5) and HPLC (methanol–water, 5.0:5.0) to give compounds **1** (9.1 mg) and **2** (4.2 mg).

7,13-Dideacetyl-9,10-debenzoyl-7 β ,9 α -*p*-hydroxylbenzylidenedioxy-taxchinin C (**1**): White powder; $[\alpha]_{\text{D}}^{20}$ –18.7° (c =0.95, CH_3OH); UV λ_{max} nm (log ϵ) (CH_3OH): 202.00 (4.26); IR ν_{max} cm^{-1} (KBr): 3428, 2975, 1715,

1617, 1521, 1451, 1369, 1270, 1109, 1150, 1109, 715; ^1H - and ^{13}C -NMR data, see Table 1; HR-ESI-MS m/z 673.2644 (Calcd for $\text{C}_{36}\text{H}_{42}\text{O}_{11}\text{Na}$, 673.2624).

7,13-Dideacetyl-2,9,10-debenzoyl-2-tigloyl-7 β ,9 α -*p*-hydroxylbenzylidenedioxy-taxchinin C (**2**): Colorless oil; $[\alpha]_{\text{D}}^{20}$ –6.1° (c =0.10, CH_3OH); UV λ_{max} nm (log ϵ) (CH_3OH): 210.40 (4.38); IR ν_{max} cm^{-1} (KBr): 3440, 2973, 2928, 1707, 1630, 1520, 1369, 1254, 1168, 1104, 1070; ^1H - and ^{13}C -NMR data, see Table 1; HR-ESI-MS m/z 651.2780 (Calcd for $\text{C}_{34}\text{H}_{44}\text{O}_{11}\text{Na}$, 651.2781).

References

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