# 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$\beta$,9$\alpha$-*p*-hydroxylbenzylidenedioxy-taxchinin C (1) and 7,13-dideacetyl-2,9,10-debenzoyl-2-tigloyl-7$\beta$,9$\alpha$-*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$\beta$,9$\alpha$-*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$\beta$,9$\alpha$-*p*-hydroxylbenzylidenedioxy-taxchinin C

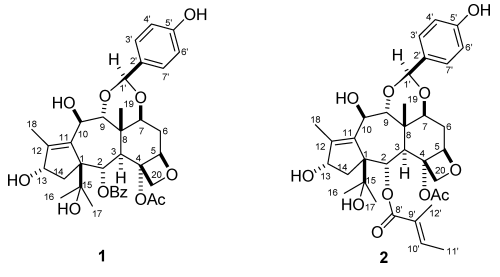
The extensive utilization of paclitaxel as an anticancer agent has stimulated interest in the analysis of the various *Taxus* species to find alternative sources of paclitaxel or related compounds with improved activity. As a result, natural taxoids are increasingly being isolated and identified.[1,2] *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$\beta$,9$\alpha$-*p*-hydroxylbenzylidenedioxy- taxchinin C (**1**) and 7,13-dideacetyl-2,9,10-debenzoyl-2-tigloyl-7$\beta$,9$\alpha$-*p*-hydroxyl benzylidenedioxy-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$\beta$,9$\alpha$-*p*-hydroxylbenzylidenedioxy groups, although a natural 4,20-*p*-hydroxyl-benzylidenedioxy 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 $C_{36}H_{42}O_{11}$ based on the positive HR-electrospray ionization (ESI)-MS (*m*/*z* 673.2644 $[M+Na]^+$, Calcd 673.2624). The $^1$H-NMR spectrum of **1** showed four characteristic methyl signals of a taxoid skeleton at $\delta_H$ 1.05, 1.06, 1.94, and 1.95, and the $^{13}$C-NMR spectrum displayed characteristic carbon resonances at $\delta_C$ 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 $\delta_C$ 80.3 (s, C-4) and 75.1 (t, C-20), along with proton signals at $\delta_H$ 4.02 (d, $J$=7.5 Hz, H-20$\alpha$) and 4.42 (d, $J$=7.5 Hz, H-20$\beta$), 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 $\delta_C$ 95.5 (d), 128.9 (d, 2×C), 115.4 (d, 2×C), 131.5 (s), and 158.5 (s), together with corresponding proton resonances at $\delta_H$ 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 ($\delta_H$ 6.15, 1H, d, $J$=7.6 Hz) and H-9 ($\delta_H$ 4.39, 1H, d, $J$=9.6 Hz) and Me-19 ($\delta_H$ 1.95, 3H, s), H-9 and Me-17 ($\delta_H$ 1.05, 3H, s), H-13 ($\delta_H$ 4.64, 1H, dd, $J$=11.6 Hz) and Me-16 ($\delta_H$ 1.06, 3H, s), H-5 ($\delta_H$ 4.96, 1H, d, $J$=8.8 Hz), and H-6a ($\delta_H$ 2.4, 1H, m), indicated that H-2, H-9, H-13, and C-20 are $\beta$-oriented, while correlations of H-3 ($\delta_H$ 3.06, 1H, d, $J$=7.6 Hz) with H-5 ($\delta_H$ 4.96, 1H, d, $J$=8.8 Hz), H-7 ($\delta_H$ 4.11, 1H, dd, $J$=9.6, 8.0 Hz), and H-10 ($\delta_H$ 5.4, 1H, dd, $J$=9.6, 4.3 Hz) confirmed the $\alpha$-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 $\alpha$-orientation of H-1′. The structure of the



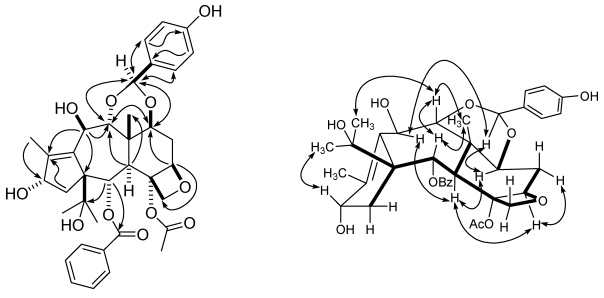



Fig. 1. Key HMBC (H ⟶ C) and ROESY Correlations (H ⟷ H) of Compound **1**

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

| No. | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_H$ mult. ($J$=Hz) | $\delta_C$ | $\delta_H$ mult. ($J$=Hz) | $\delta_C$ |
| 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$\alpha$ | 2.40 (1H, m) | 34.9 t | 1.74 (1H, m) | 34.9 t |
| 6$\beta$ | 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$\alpha$ | 1.99 (1H, dd, 14.2, 7.3) | 40.1 t | 1.85 (1H, overlap) | 40.2 t |
| 14$\beta$ | 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$\alpha$ | 4.02 (1H, d, 7.5) | 75.1 t | 4.17 (1H, d, 7.6) | 75.2 t |
| 20$\beta$ | 4.42 (1H, d, 7.5) | | 4.35 (1H, d, 7.6) | |
| OBz | — | 166.9 s | | |
| *i* | — | 129.2 s | | |
| *o* | 8.08 (2H, d, 7.4) | 130.3 d | | |
| *m* | 7.56 (2H, t, 7.5) | 129.6 d | | |
| *p* | 7.67 (1H, t, 7.5) | 134.2 d | | |
| OAc | — | 170.4 s | — | 170.3 s |
| OAc | 2.21 (3H, s) | 22.1 q | 2.13 (3H, s) | 22.0 q |
| 1′ | 6.03 (1H, s) | 95.5 d | 5.99 (1H, s) | 95.5 d |
| 2′ | — | 131.5 s | — | 131.6 s |
| 3′, 7′ | 7.40 (2H, d, 8.5) | 128.9 d | 7.37 (2H, d, 8.5) | 128.9 d |
| 4′, 6′ | 6.83 (2H, d, 8.5) | 115.4 d | 6.81 (2H, d, 8.5) | 115.4 d |
| 5′ | — | 158.5 s | — | 158.6 s |
| 8′ | | | | 168.1 s |
| 9′ | | | | 129.9 s |
| 10′ | | | 6.91 (1H, dd, 7.1, 1.3) | 138.9 d |
| 11′ | | | 1.82 (3H, s) | 14.4 q |
| 12′ | | | 1.88 (3H, s) | 14.1 q |

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

new compound is thus established as a derivative of 7,13-dideacetyl-9,10-didebenzoyltaxchinin C.[3,15)] Therefore, the structure of **1** was identified as 7,13-dideacetyl-9,10-debenzoyl-7$\beta$,9$\alpha$-*p*-hydroxylbenzylidenedioxytaxchinin C.

Compound **2**, a colorless oil, was assigned the molecular formula $C_{34}H_{44}O_{11}$ as deduced from the quasimolecular ion peak at *m*/*z* 651.2780 $[M+Na]^+$ 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 $\delta_H$ 1.03, 1.02, 1.83, and 1.90. Compound **2** had $^{1}$H- 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 $\delta_C$ 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 $\delta_H$ 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 observed cross peak between H-2 ($\delta_H$ 5.93 d, $J$=7.6 Hz) and the ester carbonyl of tiglyol ($\delta_C$ 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-tigloyl-7$\beta$,9$\alpha$-*p*-hydroxylbenzylidenedioxytaxchinin C.

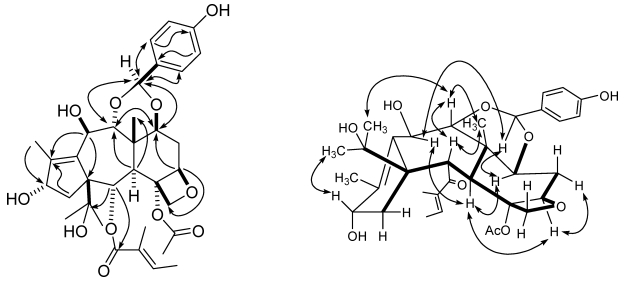


Fig. 2. Key HMBC (H ⟶ C) and ROESY Correlations (H ⟷ H) of Compound **2**

## 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. $^{1}$H- and $^{13}$C-NMR experiments were performed on a Bruker AM-400 spectrometer ($^{1}$H, 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 Sephdex 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$\beta$,9$\alpha$-*p*-hydroxylbenzylidenedioxy-taxchinin C (**1**): White powder; $[\alpha]_D^{20}$ −18.7° ($c$=0.95, $CH_3OH$); UV $\lambda_{max}$ nm (log $\varepsilon$) ($CH_3OH$): 202.00 (4.26); IR $\nu_{max}$ cm$^{-1}$ (KBr): 3428, 2975, 1715, 1617, 1521, 1451, 1369, 1270, 1109, 1150, 1109, 715; $^{1}$H- and $^{13}$C-NMR data, see Table 1; HR-ESI-MS $m/z$ 673.2644 (Calcd for $C_{36}H_{42}O_{11}Na$, 673.2624).

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

## References

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