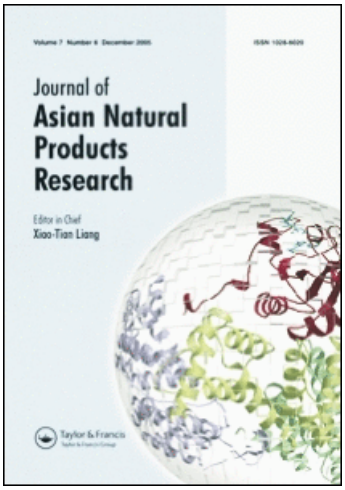

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A pair of taxoids from the needles of *Taxus canadensis*

Chun Yang[a]; Jun-Song Wang[a]; Jian-Guang Luo[a]; Ling-Yi Kong[a]
[a] Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, China

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A pair of taxoids from the needles of *Taxus canadensis*

Chun Yang, Jun-Song Wang, Jian-Guang Luo and Ling-Yi Kong*

Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China

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A pair of taxoids were isolated from the needles of *Taxus canadensis*, which were identified as 2,10-diacetyl-5(*Z*)-cinnamoylphototaxicin II (**1**), a new taxoid, and 2,10-diacetyl-5(*E*)-cinnamoylphototaxicin II (**2**), a known one, by spectroscopic methods including ^{1}H, ^{13}C NMR, HSQC, HMBC, NOESY, and mass spectra.

Keywords: *Taxus*; Taxaceae; *Taxus canadensis*; taxane; 2,10-diacetyl-5(*Z*)-cinnamoylphototaxicin II

1. Introduction

Paclitaxel (Taxol) is one of the most important anticancer drugs currently available in the market. Extensive phytochemical studies have been carried out in the past two decades, in a hope to find more effective derivatives. As a consequence, more than 500 taxane-type diterpenes have been discovered from various *Taxus* plants to date [1–5]. *Taxus canadensis* is a low-trailing shrub ubiquitous to the Quebec region in Canada, whose composition has been shown to be very different from other species [1,6,7].

In the present investigation, the isolation and structural elucidation of a new taxane, namely 2,10-diacetyl-5(*Z*)-cinnamoylphototaxicin II (**1**) and a known one, 2,10-diacetyl-5(*E*)-cinnamoylphototaxicin II (**2**), were reported.

2. Results and discussion

Compound **1** was isolated as a colorless gummy substance. HR-ESI-MS at *m/z* 587.2606 $[M+Na]^+$ revealed the molecular formula of **1** as $C_{33}H_{40}O_8$, indicating 14 degrees of double bond equivalence. Its ^{1}H NMR spectrum (Table 1) exhibited the characteristic signals of taxoid with a C-3(11) bridge [1,2]: the characteristic signal of H-3α in other taxoids at δ_H 2.57–3.73 (d, $J = 5.0$–6.0 Hz) disappeared, replaced by the characteristic signal of H-12α, usually appearing at δ_H 3.23–3.79 (q, *ca.* $J = 7.0$ Hz), together with the signal of mutually coupled Me-18 at δ_H 1.24–1.34 (d, *ca.* $J = 7.0$ Hz). The proton signals due to an exomethylene moiety were observed at δ_H 5.74 and 5.63 (each 1H, s). The presence of a *cis*-cinnamoyl moiety in **1** was revealed by the signals at δ_H 7.56 (2H, m), 7.37 (3H, m), and an AX system centered at δ_H 5.90 (1H, d, $J = 12.5$ Hz), 6.98 (1H, d, $J = 12.5$ Hz). The appearance of the signals of H-2′ and H-3′ as an AX spin system with a coupling constant of 12.5 Hz in **1**, compared with that of **2** at 15.9 Hz (Table 1), indicated that the double bond in the cinnamoyl group was of the *cis*-configuration [8],

*Corresponding author. Email: lykong@cpu.edu.cn

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Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data in $CDCl_3$ for **1** and **2**.

	1			**2**	
Position	δ_H (^{1}H mult., *J* in Hz)	δ_C (^{13}C; HSQC)	HMBC	δ_H (^{1}H mult., *J* in Hz)	δ_C (^{13}C)
1	2.13 m[a]	47.9	3	2.14 m[a]	48.0
2	6.04 d (5.1)	76.2	1, 3, 14	6.08 d (5.1)	76.7
3		66.0			66.1
4		142.7			142.8
5	5.60 t (8.5)	76.2	3, 4, 1′	5.63 t (9)	76.5
6a	2.16 m[a]	26.0		2.20 m[a]	26.3
6b	1.59 m			1.66 m[a]	
7a	2.07 m	29.7		2.01 m	29.2
7b	1.11 m		8	1.12 dt (3, 13.2)	
8		45.2			45.4
9	4.39 d (9.5)	82.7	8, 10, 19	4.43 d (9.3)	82.9
10	5.27 d (9.5)	84.5	9, 12, 15, CO-10-OAc	5.42 d (9.3)	84.4
11		58.0			58.0
12	3.43 q (7.0)	52.2	15, 18	3.58 q (7.2)	52.3
13		214.3			214.3
14a	2.58 d (20.4)	38.8	13, 15	2.62 d (20.4)	39.0
14b	2.49 dd (6.9, 20.4)		2, 13	2.50 dd (6.6, 20.4)	
15		42.7			42.8
16	1.20 s	26.7	15, 17	1.23 s	26.8
17	1.54 s	29.2	1, 15, 16	1.57 s	29.8
18	1.23 d (7.0)	15.6	11, 12, 13	1.33 d (7.2)	15.9
19	1.29 s	26.2	3, 8	1.31 s	26.4
20a	5.74 s	128.7	3, 5	5.84 s	128.8
20b	5.63 s		2	5.69 s	
2-OAc	2.04 s	21.3, 169.4	CO-2-OAc	2.08 s	21.3, 169.6
10-OAc	2.16 s	21.4, 172.5	CO-10-OAc	2.17 s	21.4, 172.6
1′		167.2			165.9
2′	5.90 d (12.5)	119.4	4′	6.38 d (15.9)	118.0
3′	6.98 d (12.5)	144.3	1′, 5′, 9′	7.67 d (15.9)	145.3
4′		135.2			134.4
5′, 9′	7.56 m[a]	129.8		7.56 m[a]	128.3
6′, 8′	7.37 m[a]	128.0		7.39 m[a]	128.9
7′	7.37 m[a]	129.2		7.39 m[a]	130.4

[a]Signal pattern unclear due to overlapping.

which was further confirmed by the observed NOESY correlation between H-2′ and H-3′ (Figure 1). In addition, the presence of two acetyl groups and one ketone group was implied by the resonances at δ_H 2.16, 2.04, and δ_C 21.4, 21.3, 172.5, 169.4, and 214.3 in the ^{1}H and ^{13}C NMR (Table 1) spectra. Detailed examination of the ^{1}H NMR spectrum of **1** showed that it exhibited different spectral features compared to regular taxanes: the disappearance of the signal of H-3α, which usually appears at δ_H 2.57–3.73 with a coupling constant in a range *ca.* $J = 5.0$–6.0 Hz, and the appearance of the methyl group at δ_H 1.23 (3H, d, $J = 7.0$ Hz). In addition, there is a quartet signal at δ_H 3.43 (1H, q, $J = 7.0$ Hz). These characteristic signals were assigned as Me-18 and H-12, respectively. The above spectral data featured that compound **1** is a 3,11-cyclotaxane [9,10]. Combined analysis

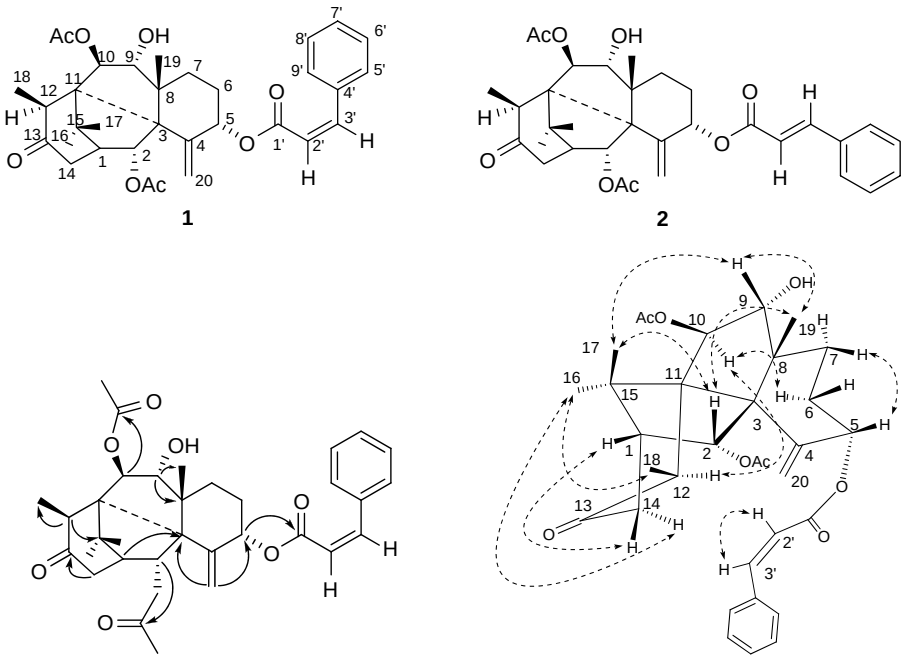

Figure 1. Stuctures of **1** and **2**, key HMBC (H → C) and NOESY (H ⇠⇢ H) correlations for **1**.

of HSQC and HMBC spectra, together with the chemical shifts and coupling constants, enabled the assignment of all functional groups on the taxane skeleton. The acetyl groups were located at C-2 and C-10 respectively, since H-2 and H-10 correlated with the carbonyl carbons of acetate at δ_C 169.4 and 172.5, respectively, in the HMBC spectrum. A pair of signals resonating at δ_H 4.39 and 5.27 with a large coupling constant ($J = 9.5$ Hz) were attributed to H-9 and H-10, substituted by a hydroxyl and an acetyl group, respectively. In the HMBC experiment, H-5 showed a three-bond correlation with the carbonyl of cinnamate (δ_C 167.2), indicating that the *cis*-cinnamoyl was at C-5. The relative stereochemistry of **1** was determined by its NOESY spectrum (Figure 1). H-2 and H-9 exhibited correlations with both Me-17 and Me-19, suggesting a β-orientation of H-2 and H-9. The cross-peak from the protons of Me-18 to the protons of Me-16 suggested that Me-18 has a β-orientation, while H-12 has an α-orientation. H-10 was α-oriented, as confirmed by its NOESY correlation with H-12 and H-6b; and H-5 was β-oriented, as shown by the correlation with H-7a. The structure of **1** was therefore characterized as 2,10-diacetyl-5(*Z*)-cinnamoylphototaxicin II.

The structural elucidation of compound **2** as 2,10-diacetyl-5(*E*)-cinnamoylphototaxicin II was made based on comparison of the NMR spectral data with those of the literature data [6].

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a JASCO P-1020 polarimeter in $CHCl_3$ solution. UV spectra were obtained on a Shimadzu UV-2450 spectrophotometer. IR (KBr) spectra were obtained on a Shimadzu FTIR-8400s spectrometer. ^1H, ^{13}C, and 2D NMR spectral data were obtained on Bruker DRX-500 spectrometer (500 and 125 MHz, respectively) with TMS as the internal standard. HR-ESI-MS was performed on Mariner ESI-TOF spectrometer.

Preparative HPLC was carried out on a Shimadzu SPD-20A instrument with one develosil ODS-UG-5 column (20 × 200 mm) coupled to UV/VIS detector at 210 nm. TLC was conducted on precoated silica gel 60 F_{254} (Qingdao Marine Chemical Co., Ltd, Qingdao, China) and detected by spraying with 10% H_2SO_4–EtOH. Column chromatography was carried out with silica gel (100–200 and 200–300 mesh; Qingdao Marine Chemical Co., Ltd).

3.2 Plant material

The needles of *T. Canadensis* were collected in September 2004 at St Jean, Quebec, Canada and authenticated by Prof. Min-Jian Qin, Department of Medicinal Plants, China Pharmaceutical University. A voucher specimen (No. 041116) is deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3 Extraction and isolation

Air-dried needles of *T. Canadensis* (8 kg) were ground and extracted with 95% ethanol four times at room temperature. The combined ethanol extracts were evaporated to dryness under vacuum, then partitioned between chloroform and water to give a dark green extract (280 g). The chloroform extract was chromatographed over a silica gel column (100–200 mesh, 1.0 kg, 10 × 15 cm) and combined according to TLC. Successive elution with gradient eluents (1, 5, 10, 20, 40, 60, and 80% ethyl acetate in cyclohexane, each 12 l) yielded seven fractions: A–G. Fraction B (17.8 g) was subjected to silica gel (200–300 mesh, 180 g, 3.5 × 25 cm) column chromatography, eluted with cyclohexane–ethyl acetate (10:1 and 6:1, each 2.5 l), to give six fractions: B_1–B_6. Fraction B_4 (3.5 g) was rechromatographed over silica gel (200–300 mesh, 180 g, 3.5 × 25 cm), eluted first with $CHCl_3$, and then with 1% MeOH in $CHCl_3$ (each 500 ml), to yield five fractions: B_4-1–B_4-5. Fraction B_4-2 (119 mg) was finally isolated by preparative HPLC to afford taxanes **1** (2.0 mg, $t_R = 37.40$ min) and **2** (7.0 mg, $t_R = 43.46$ min).

3.3.1 2,10-Diacetyl-5(Z)-cinnamoyl phototaxicin II (**1**)

A colorless gummy substance; $[\alpha]_D^{25}$ +19 ($c = 0.08$, $CHCl_3$); UV (nm) ($CHCl_3$) λ_{max} (log ε): 226 (3.45), 278 (3.67); IR (KBr) ν_{max} (cm^{-1}): 3445, 2960, 2925, 2854, 1742, 1635, 1466, 1375, 1263, 1237, 1098, 1057, 805, 770, 701; ^1H and ^{13}C NMR, HMBC spectral data ($CDCl_3$), see Table 1. HR-ESI-MS: *m/z* 587.2606 $[M+Na]^+$ (calcd for $C_{33}H_{40}O_8Na$, 587.2615).

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