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A taxane with a novel 9α,13α-oxygen bridge from *Taxus cuspidata* needles

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Abstract

A novel taxane with an unprecedented hemiacetal ring between C-13 and C-9 was isolated from the needles of *Taxus cuspidata*. The structure was characterized as (12αH)-2α,10β-diacetoxy-5α-cinnamoyloxy-9α,13α-epoxytax-4(20)-ene-11β,13β-diol (**1**). This is the first example of a natural taxane with a C-13 and C-9 oxygen bridge to form an unusual 6/8/6/6-membered ring system.

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1. Introduction

Many taxane-type diterpenoids have been found in various yew trees[1,2] and some of them exhibited interesting biological activities.[3–5] *Taxus cuspidata* (Taxaceae) is low trailing shrub or tall tree ubiquitous to the northeastern region of People's Republic of China, Korea, Japan and Quebec region of Canada. The composition has been extensively invested.[6–8] Although more than 200 taxanes were isolated from the needles, stem, bark, and seeds of *T. cuspidata*, there are still new taxoids waiting isolation and structural elucidation. The isolation of new and minor taxanes is important in the context of the plant biosynthesis and metabolism of these compounds.[9,10] As a continuation of our ongoing program about this plant,[11,12] we have now isolated a novel metabolite (**1**)[†] featured an unprecedented hemiacetal oxygen bridge between C-13 and C-9 not previously encountered in the taxane metabolites from *Taxus* species including *T. cuspidata*. In this Letter, we report the structure elucidation of this new taxane.

The molecular composition of **1** was established from combined analysis of high-resolution FABMS and ^{13}C NMR spectrum as $C_{33}H_{42}O_9$, which suggested the presence of thirteen degrees of unsaturation. The NMR spectral data of **1** is shown in Table 1. The ^1H NMR spectrum exhibited the three-proton signals due to the four methyl groups at δ_H 0.90, 1.20, 1.18, and 1.47, two of the methyl signals at δ_H 1.20 and δ_H 1.47 were COSY-correlated peaks as geminal methyls. The extraction of this metabolite from *Taxus cuspidata* and the four methyl groups might suggest it to be a taxane analogue.[13,14] Two acetyl groups were observed at δ_H 2.09 and 1.87 as confirmed by ^{13}C NMR signals at δ_C 21.1, 171.4 and δ_C 21.3, 170.7. The proton signals due to the cinnamoyl group were observed at δ_H 7.60 (2H, m), 7.39 (3H, m), and an AB system centered at δ_H 6.49 (1H, d, $J = 16.0$ Hz) and δ_H 7.74 (1H, d, $J = 16.0$ Hz) indicating the (*E*)-geometry of the double bond. This was further confirmed by UV absorption at 278 nm, which we used in the HPLC analysis (see Fig. 1).

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[†] Metabolite **1**. $[\alpha]_D^{22}$ +42 (*c* 0.1, $CHCl_3$). HRFABMS *m*/*z* 621.24649 $[M+K]^+$ (calcd for $C_{33}H_{42}O_9K$, 621.24659).

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Table 1
The ^{1}H and ^{13}C NMR data of taxane **1** (500 MHz for ^{1}H, 125 MHz for ^{13}C in $CDCl_3$)

Position	$\delta^{1}H$ (mult)[a]	J (Hz)	$\delta^{13}C$[b]	HMBC	NOESY[c]
1	2.00 (br d)	9.2	48.7	3, 11, 13, 15, 16	2^{m}, 14α^{m}, 16^{s}
2	5.50 (d)	11.8	71.5	3, 14, 15, 170.7	1^{m}, 20b^{s}, 17^{s}, 19^{m}
3	3.43 (d)	11.8	43.5	2, 4, 8, 19, 20	7α^{s}, 14α^{s}
4			**145.1**		
5	5.34 (t)	2.6	75.5		6^{s}, 20a^{s}
6	1.89 (m)		27.6		5^{s}, 7β^{s}, 19^{s}
7α	2.48 (td)	13.7, 13.7, 6.6	37.9	6, 8	3^{s}, 7β^{s}, 9^{s}
7β	1.03 (br ddd)	13.7, 4.5, 2.1			6^{s}, 7α^{s}, 9^{s}
8			**43.9**		
9	4.24 (d)	10.0	79.0	3, 7, 8, 10, 11, 13	7α^{s}, 7β^{s}, 10^{s}, 19^{m}
10	5.65 (d)	10.0	73.5	9, 11, 12, 15, 171.4	9^{s}, 18^{s}
11			**75.0**		
12	2.37 (q)	6.8	44.1	11, 13, 18	16^{s}, 18^{s}
13			**99.3**		
13-OH	2.64 (s)			13, 14	18^{w}
14α	2.28 (br d)	15.0	33.7	1, 2, 12, 13, 15	1^{m}, 3^{s}, 14β^{s}
14β	2.11 (o, m)			1, 2, 13	14α^{s}, 16^{s}
15			**40.7**		
16	1.20 (s)		29.1	1, 11, 15, 17-Me	1^{s}, 12^{s}, 14β^{s}, 17^{s}, 13-OH^{w}
17	1.47 (s)		22.6	1, 11, 15, 16-Me	2^{s}, 16^{s}, 19^{m}
18	1.18 (d)	6.8	6.5	11, 12, 13	10^{s}, 12^{s}
19	0.90 (s)		16.2	3, 8, 9	2^{m}, 6^{s}, 9^{m}, 17^{m}, 20b^{w}
20a	5.22 (s)		112.1	3, 4, 5	5^{s}, 20b^{s}
20b	4.62 (s)			3, 4, 5	2^{s}, 19^{w}, 20a^{s}
2-OAc	1.87 (s)		21.3	170.7	
			170.7		
10-OAc	2.09 (s)		21.1	171.4	
			171.4		
1′			**166.0**		
2′	6.49 (d)	16.0	118.7	1′, Ph-*i*	
3′	7.74 (d)	16.0	144.6	1′, 2′, Ph-*o*	
Ph-*i*			**134.5**		
Ph-*o*	7.60 (m)		128.2	3′, Ph-*p*	
Ph-*m*	7.39 (m)		129.0		
Ph-*p*	7.39 (m)		130.4		

[a] Mutiplicity: s, singlet; d, doublet; t, triplet; m, mutiplet; o, overlapped; br, broad.

[b] The ^{13}C chemical shifts were extracted from the HMQC experiment (±0.2 ppm). The numbers in bold character represent quaternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm).

[c] NOESY intensities are marked as strong (s), medium (m), or weak (w).

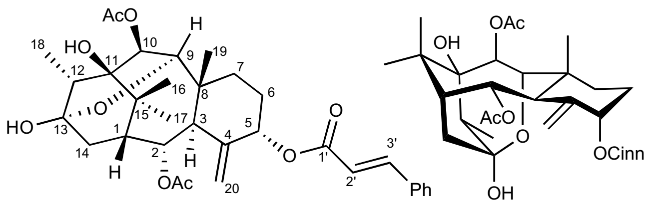

Fig. 1. The structure of **1**.

The connectivities of the protons of **1** were determined by analysis of the ^{1}H–^{1}H COSY spectrum. Interpretation of ^{1}H, ^{13}C NMR, and HMBC spectral data permitted the positional assignment of functional groups. In the ^{1}H NMR spectrum, **1** disclosed four protons attached to oxygenated carbons (two acetoxy, one cinnamoyloxy, and one hydroxy groups). A pair of AB systems resonating at δ_H 5.65 and δ_H 4.24 with a large coupling constant ($J = 10.0$ Hz) were attributed to H-10 and H-9, whose chemical shifts required an acyl and an un-acyl oxygen group to be attached to C-10 and C-9, respectively.[13,14] The 18-methyl resonated as a doublet at δ_H 1.18 (3H, d, $J = 6.8$ Hz) indicated that the C-11,12 double bond was saturated.[13,14] The signal resonating at δ_H 2.37 (1H, q, $J = 6.8$ Hz), which showed long-range H–C correlations with C-11, C-13, and C-18 in the HMBC spectrum and exhibited H–H correlation with CH_3-18 in the ^{1}H–^{1}H COSY spectrum, was attributed to H-12. Carbon-11 resonated at δ_C 75.0, which was correlated by H-9, Me-16 and Me-17, implying that a hydroxy group bonded to C-11. The signals at δ_H 5.22 (1H, s), 4.62 (1H, s) in the ^{1}H NMR spectrum, which correlated with C-3, C-4, and C-5 in the HMBC spectrum, and the signals at δ_C 112.1 and δ_C 145.1 in the ^{13}C NMR spectrum were the characteristic signals of exocyclic methylene group in taxane family.[13,14] The signal at δ_H 3.43 (1H, d, $J = 11.8$ Hz), which correlated with C-4, C-19, and C-20, was attributed to C-3 ring junction proton in a taxa-4(20),11-diene analogue.[13,14] Using H-3 as the reference, the signals of H-1, H-2, and

H-14 were confirmed by the ^{1}H–^{1}H COSY spectrum. The chemical shift of H-2 at δ_H 5.50 (1H, d, $J = 11.8$ Hz) suggested that an acetoxy group attached to C-2. This was verified by the long-range ^{1}H–^{13}C correlation of H-2 with a carbonyl carbon at δ_C 170.7 in the HMBC spectrum. The signal at δ_H 5.34 (1H, t, $J = 2.6$ Hz) was assigned to H-5 judging from ^{1}H–^{1}H COSY systems of H-5, H-6, and H-7 and HMBC correlations. The cinnamoyloxy group was supposed to attach at C-5 as indicated by the chemical shift of H-5 as in the most natural taxane analogues,[1,2] although we did not get solid proof from H-5 to C-1′ in the HMBC spectrum due to scarcity of the sample. In the ^{1}H and ^{13}C NMR spectra of **1**, we did not observe the characteristic signal of H-13 and C-13 as in the other taxanes; meanwhile, without additional double bond and ketone group were observed in the ^{1}H and ^{13}C NMR spectra.[13,14] Taking into account that the molecular formula of **1** required the accommodation of a further unsaturation, its core was configured with another ring. An important aspect of the analysis was the HMBC correlation of the signal H-9 (δ_H 4.24) with C-13 (δ_C 99.3) (Table 1), suggesting a hemiacetal moiety at C-13 with C-9 oxygen group.[15–17] Thus, the structure of **1** was rigorously characterized as (12αH)-2α,10β-diacetoxy-5α-cinnamoyloxy-9α,13α-epoxy-tax-4(20)-ene-11β,13β-diol.

The relative stereochemistry of **1** (Fig. 2) was elucidated from analysis of the NOESY experiment, the chemical shifts and their coupling constants. The coupling constant between H-9 and H-10 ($J = 10$ Hz) and observed NOESY correlations of H-2/H-19, H-2/H-17, H-9/H-19 established a crown-like conformation for ring-B. The β-orientation of H-2 and H-9 was assigned by NOESY correlation of H-2/H-17, H-19/H-2, and H-9/H-19. The α-orientation of H-10 was applied by the observation of NOESY correlations of H-10/H-18 and H-10/H-16. The unusual large vicinal coupling of H-2β and H-3α ($J = 11.8$ Hz) in **1** is indicative of a large dihedral angle between these two protons. Inspection of a model showed that the angle between H-2β and H-3α was nearly 180°, while H-1β and H-2β have 90° dihedral angle without a coupling. Although H-9β and H-10α are *trans*-oriented, these are almost eclipsed in the crown conformation with a smaller dihedral angle; as a result, H-9β and H-10α exhibited a strong NOE and a large coupling constant.

Although more than 500 natural taxanes have been reported to date,[1,2,18] **1** is unique in that having an ether-bridged hemiacetal ring between C-13 and C-9. The first taxane with a hemiacetal ring between C-13 and C-17, taxezopidine A, had been isolated from the seeds of this plant.[15] Taxezopidine A, which may be derived biogenetically from taxinine A, has a double bond between C-11 and C-12 as in the most taxanes.[1,2] From a biogenetic point of view, **1** should be considered to arise from taxinine (the most abundant metabolite in yews), the double bond between C-11 and C-12 was saturated by adding a molecular of water to generate **1**. It is noted that **1** has a crown-like backbone conformation similar to that of usual taxanes consisting 6/8/6-membered ring system, although **1** contains an additional tetrahydropyran ring between C-9 and C-13.

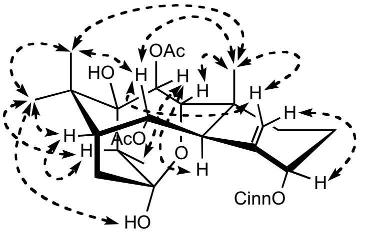

Fig. 2. Relative stereochemistry of **1**. Dashed arrows denote the key NOE correlations.

Taxane **1** did not show cytotoxicity against human breast cancer MCF-7 cell line.

2. Experimental

2.1. General

Optical rotation, JASCO DIP-370; NMR, Bruker Avance-500; FAB-MS, a Vacuum Generators ZAB-HS; flash chromatography, Silica gel 60 (230–400 mesh EM Science); semi-preparative HPLC, Waters Delta Prep, UV 486 detector (210 nm), and Whatman partisil 10 ODS-2 Mag-9 (9.4 × 250 mm).

2.2. Extraction and isolation

Fresh leaves of the Japanese yew, *Taxus cuspidata* (2.2 kg) collected in Toyama Prefecture in 1997, Japan, were dried in a well-ventilated dark room and immersed in two portions of MeOH for every 1 week at ambient temperature. After MeOH was removed in vacuo, the residue was dissolved in EtOAc and fractionated by weak acid and weak alkaline solution in order. The neutral fraction (21 g) was dissolved in acetone, absorbed onto 25 g Silica gel, and loaded onto 540 g opened silica gel column chromatography. Gradient elution with acetone–hexane system (30–80%) afforded forty 500 mL fractions including 10 major fractions, designated as $\mathrm{Fr_{A\text{-}J}}$. Column chromatography of $\mathrm{Fr_H}$ and $\mathrm{Fr_I}$, using hexane–EtOAc (2:3 to 1:5) as mobile phase, afforded fifteen subfractions, $\mathrm{Fr_{H\text{-}1}}$ to $\mathrm{Fr_{H\text{-}15}}$. Subfractions $\mathrm{Fr_{H\text{-}9}}$ and $\mathrm{Fr_{H\text{-}10}}$ were pooled (87 mg) on their TLC properties and re-chromatographed over silica gel (20 g) stepwise eluting with a hexane–acetone (2:3 and 1:3) gradient to yield 5 subfractions ($\mathrm{Fr_{H\text{-}9\text{-}1}}$ to $\mathrm{Fr_{H\text{-}9\text{-}5}}$). The fraction $\mathrm{Fr_{H\text{-}9\text{-}3}}$ (15 mg) was further applied to a semi-preparative HPLC. Elution with a linear gradient of MeCN in H_2O from 25% to 100% in 50 min at 3 mL/min furnished **1** (2.3 mg, $t_R = 25.18$ min).

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