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A TAXANE-11,12-OXIDE FROM *TAXUS YUNNANENSIS*

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Key Word Index—*Taxus yunnanensis*; Taxaceae; taxoid; taxol; decinnamoyl-taxinine B-11,12-oxide.

Abstract—A new taxoid, decinnamoyl-taxinine B-11,12-oxide, was isolated from the leaves and stems of *Taxus yunnanensis*. This is the first example of an 11,12-epoxy taxoid isolated from the yew tree. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Over the last two decades, paclitaxel (Taxol®) has attracted much attention and is currently considered as one of the most important cancer chemotherapeutic agents [1]. However, large-scale clinical usage of taxol has been hampered by its limited supply. Although the total and semi-syntheses of taxol have recently been reported [2–5], the supply of the drug still depends on natural resources, currently the barks of several species of *Taxus*, which are very slow-growing evergreens. Because of the very low yields of taxol (below 0.01%) at the expense of destructive bark consumption in a large scale, there is great interest in the isolation of taxol and related compounds from renewable resources, such as leaves and stems. In previous work, we reported the isolation of several new taxane diterpenoids from leaves and stems of *Taxus yunnanensis* indigenous to China [6–8]. Further investigation has led to isolation of a unique 11,12-epoxy taxoid named decinnamoyl-taxinine B-11,12-oxide (**1**), together with taxol, cephalomannine, 10-deacetyl-7-epitaxol, 10-deacetyl-7-*epi*-cephalomannine, baccatin III, taxagifine, 1-hydroxybaccatin I, 5-decinnamoyl-taxinine J, baccatin VI and taxacin.

RESULTS AND DISCUSSION

Compound **1** gave a $[M + K]^+$ ion peak at *m/z* 689 in the FAB mass spectrum and the molecular formula $C_{28}H_{38}O_{11}$ was established by analysis of the ^{13}C NMR and HR-FAB mass spectral data. In addition to the four methyl signals typical of taxane derivatives, the signals of four acetates were also present in the NMR spectrum (Table 1). Two characteristic ^1H NMR signals at δ 5.35 and 5.01 (broad singlets) and ^{13}C NMR signals at δ 143.3(*s*) and 117.5(*t*) suggested the presence of an exomethylene unit. A distinct AB pattern at δ 2.30 and 2.64 (J_{AB} = 19.9 Hz, H-14), as part of a three-spin system, indicated the existence of a keto group at C-13, which was further confirmed by the presence of a carbon signal at δ 208.4. This downfield chemical shift suggested that the C-13 carbonyl was not conjugated. The ^{13}C NMR spectrum of compound **1** did not show the signals of C-11 and C-12 in the olefinic region; these signals were replaced by those of two quaternary carbons at δ 59.9 and 64.6, showing that the endocyclic double bond was saturated and further suggesting the presence of an epoxide group at C-11, C-12. Five oxygen-bearing methine signals at relatively low field were observed in the ^1H NMR. The assignments of these resonances were established by analysis of ^1H–^1H COSY and ^1H–^{13}C COSY. The ^1H NMR spectrum of compound **1** was similar to that of taxinine B(**2**)[9], except for the absence of the cinnamoyl signals and the upfield ($\Delta\delta$ 1.04) of the H-5β shift. These changes indicated that the C-5 hydroxyl was free. By exclusion of C-5, the four acetate groups were attached to four available sites at C-2, C-7, C-9 and C-10.

The configuration of the oxygenated groups at C-2, C-5, C-7, C-9 and C-10 was established by analogy. The ^1H NMR coupling pattern was similar to that of taxinine B. NOE showed that the predominant conformation of the eight-membered ring was boat–chair. The β-orientation of the epoxide group at C-11, C-12 was established from the observation of weak NOE responses of 10-Hα and 14-Hα elicited by CH_3-18. The chemical shift of CH_3-17 was unusually upfield at δ 0.81, which suggested that it was located in the

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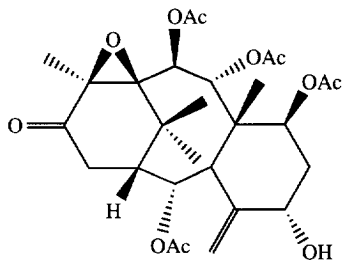

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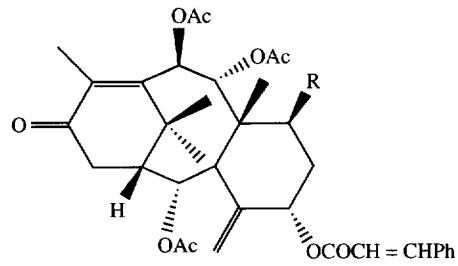


2 R = OAc

3 R = H

shielding region of the oxirane, thus confirming the β-configuration.

On the basis of the spectral evidence described above, the structure of compound **1** was established as decinnamoyl-taxinine B-11,12-oxide(**1**) (Fig. 1). It is the first example of an 11,12-epoxy taxoid isolated from the yew tree.

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compound **1**

Position	^{1}H*	^{13}C†
1β	1.99 *m*	51.67 (*d*)
2β	5.78 *dd* (5.9, 1.8)	68.97 (*d*)
3α	3.27 *d* (5.9)	40.30 (*d*)
4	–	143.30 (*s*)
5β	4.34 *t* (3.0)	75.24 (*d*)
6α	2.00 *m*	37.00 (*t*)
6β	1.70 *m*	
7α	5.66 *dd* (11.6, 5.4)	69.09 (*d*)
8	–	47.03 (*s*)
9β	5.43 *d* (11.2)	75.68 (*d*)
10α	6.02 *d* (11.2)	71.42 (*d*)
11	–	59.90 (*s*)
12	–	64.56 (*s*)
13	–	208.35 (*s*)
14α‡	2.30 *d* (19.9)	38.16 (*t*)
14β‡	2.64 *dd* (19.9, 8.8)	
15	–	38.55 (*s*)
16-CH_3	1.83 *s*	25.43 (*q*)
17-CH_3	0.81 *s*	29.17 (*q*)
18-CH_3	2.07 *s*	14.60 (*q*)
19-CH_3	1.03 *s*	13.24 (*q*)
20	5.35 *s*	117.54 (*t*)
	5.01 *s*	
$COCH_3$	2.02 *s*	20.58 (*q*)
	2.02 *s*	20.72 (*q*)
	2.02 *s*	21.15 (*q*)
	2.03 *s*	21.37 (*q*)
$COCH_3$	–	168.74 (*s*)
		168.11 (*s*)
		169.53 (*s*)
		169.59 (*s*)

*Multiplicity and coupling constants in Hz in parentheses.

†*s* = C, *d* = CH, *t* = CH_2, *q* = Me, assignments were made by DEPT.

‡Assignment based on NOE.

The $\Delta^{11,12}$-double bond of taxanes is normally unaffected by catalytic hydrogenation, ozonolysis or osmylation [1]. In order to determine whether the unique C-11(12) oxirane can be generated from epoxidation of the usual $\Delta^{11,12}$-double bond of taxoids, we explored the epoxidation with taxinine (**3**), referring to the synthesis of taxane 11,12-oxides [10,11]. Treatment of taxinine with H_2O_2/NaOH gave two hydrolysis products **4** and **5**. Conversely, treatment of taxinine with *m*-chloroperbenzoic acid gave a single reaction product (**6**) whose 4(20) epoxide has a stereochemistry (α) that differs from the natural congeners. The α-stereochemistry of the oxirane of compound **6** was established by the observation of an NOE between H-20a and the 19-methyl, whereas no NOE between H-14α and H-20α could be detected (Table 2).

EXPERIMENTAL

General. NMR were obtained on a Bruker AM 500 specrometer in $CDCl_3$ at room temp. ^{1}H chemical shifts are recorded in δ from int. TMS and ^{13}C shifts are based on the $CHCl_3$ signal at δ 77.0.

Plant material. Taxus yunnanensis Cheng et L. K. Fu was collected in March 1993 in Yang-bi County, Yunnan Province, China and identified by Prof. Yu-Heng Chen. A voucher specimen is kept at the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences.

Extraction and isolation. Dried leaves and stems (25 kg) were extracted with EtOH to yield 1.5 kg of

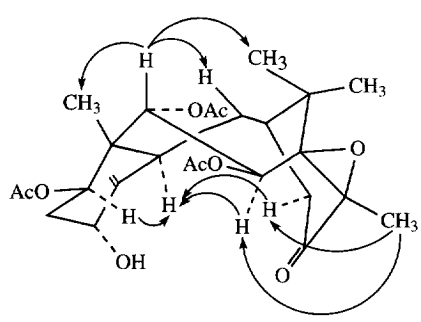


Fig. 1. Comformation of compound **1**.

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **4–6**

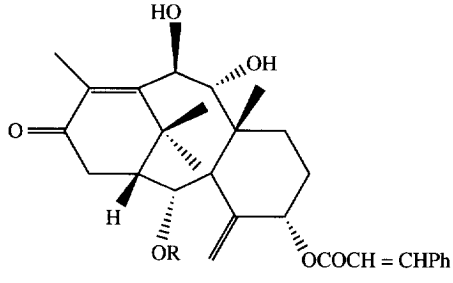
	4		**5**	**6**	
Position	^{1}H*	^{13}C	^{1}H*	^{1}H*	NOE
1	2.16 *dd* (7.00, 2.00)	37.61	2.36 *dd* (6.84, 2.19)	Overlapped	
2	5.52 *dd* (6.23, 1.90)	69.83	4.21 *brs*	5.48 *d* (3.59)	9β, 16, 19-Me
3	3.37 *d* (6.05)	43.27	3.22 *d* (6.45)	3.30 *d* (4.15)	
4	–	142.56	–		
5	5.34 *brs*	73.49	5.32 *brs*	4.36 *brd*	
6†	1.97 *m*	28.57	1.97 *m*	1.81 *m*	
7†	1.83 *m*	36.17	1.78 *m*	1.73 *m*	
8	–	38.04	–		
9	4.90 *d* (8.52)	77.89	4.87 *d* (9.19)	5.89 *d* (10.2)	
10	4.20 *dd* (9.15, 3.14)	78.71	4.08 *d* (9.13)	6.02 *d* (10.2)	
11	–	134.66	–		
12	–	155.17	–		
13	–	199.74	–		
14α	2.43 *d* (20.0)	48.93	2.24 *d* (20.0)	2.76 *d* (20.0)	
14β	2.84 *dd* (20.0, 6.89)		2.85 *dd* (20.0, 6.98)	2.90 *dd* (20.0, 6.63)	
15	–	44.51	–		
16-Me	1.54 *s*	26.30	1.64 *s*	1.74 *s*	
17-Me	1.24 *s*	25.37	1.25 *s*	1.15 *s*	
18-Me	2.07 *s*	17.73	2.10 *s*	2.10 *s*	
19-Me	1.12 *s*	14.12	1.15 *s*	0.99 *s*	9β, 2β, 20β
20	5.32 *s*	118.13	5.43 *s*	2.56 *d* (4.9)	20β, 5β
	4.85 *s*		5.38 *s*	2.92 *d* (4.9)	20α
1′	–	166.36	–		
2′	6.45 *d* (16.0)	116.60	6.40 *d* (16.0)	6.52 *d* (1.60)	
3′	7.65 *d* (16.0)	145.53	7.64 *d* (16.0)	7.66 *d* (16.0)	
1″		135.82			
2″,6″	7.76 *d* (7.16)	128.94	7.75 *d* (7.64)	7.77 *d*	
3″,5″	7.43 *m*	128.47	7.43 *m*	7.43 *m*	
4″		130.29			
$COCH_3$	2.14 *s*	21.43		2.29 *s*	
		169.67		2.04 *s* × 2	

*Coupling constants in H in parentheses.
†Overlapped by other signals.

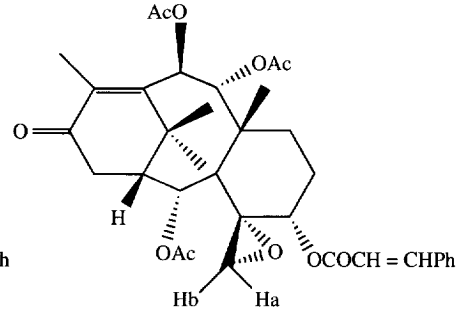
crude extract, which was dissolved in an equivalent amount of H_2O and extracted with petrol, CH_2Cl_2 and EtOAc successively. Part (*ca* 106 g) of the CH_2Cl_2 fr. was separated by vacuum chromatography [petrol, cyclohexane–Me_2CO (1:1) and Me_2CO]. Concn of the cyclohexane–Me_2CO (1:1) fr. under red. pres. gave 84 g of residue, which was then subjected to dry CC on silica gel using MeOH–CH_2Cl_2 (1:40) as eluent; 10 frs were collected. Fr. 8 was rechromatographed on silica gel and RP-18 CC to give compound **1** (3 mg).

Decinnamoyl-taxinine B-11,12-oxide (**1**). Needles, mp 160° (decomp.). FAB-MS, *m/z*: $[M+K]^+$589, $[M+H]^+$551; HRFAB-MS, *m/z*: $[M+H]^+$ 551.2590 (calcd for $C_{28}H_{39}O_{11}$: 551.2492).

Treatment of taxinine with H_2O_2/NaOH. Taxinine (**3**) (100 mg, 0.165 mmol) was dissolved in MeOH (*ca*



4 R = Ac
5 R = H



6

1 ml) and Me_2CO (*ca* 1.5 ml), and H_2O_2 (30%, 19 μl) and NaOH (1N, 20 μl) were added. After stirring for 8 hr at room temp., the reaction mixt. was worked-up to concn under red. pres. The residue was subjected to CC on silica gel (200–300 mesh) ($CHCl_3$–MeOH, 40:1) and then rechromatographed on RP-18 (MeOH–H_2O, 7:3 ~ 4:1) to obtain compounds **4** and **5**.

Treatment of taxinine with m-chloroperbenzoic acid. Taxinine (**3**) (100 mg, 0.165 mmol) was dissolved in CH_2Cl_2 (*ca* 3 ml) and *m*-chloroperbenzoic acid (50%, 113.9 mg, 0.33 mmol) and NaOAc (54 mg, 0.65 mmol) were added. After being stirred for 20 hr at room temp. the reaction mixt. was worked-up by washing with satd solns of $NaHCO_3$ and NaCl and drying over Na_2SO_4. A powder of compound **6** (78 mg, yield 76.0%) was obtained by chromatography.

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