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C-14 OXYGENATED TAXANES FROM *TAXUS YUNNANENSIS* CELL CULTURES

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Key Word Index—*Taxus yunnanensis* Cheng et L. K. Fu; Taxaceae; cell cultures; taxanes.

Abstract—Three C-14 oxygenated taxanes were isolated from *Taxus yunnanensis* cell cultures.

INTRODUCTION

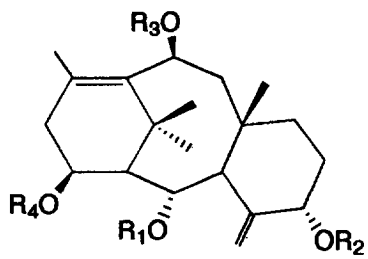
The paclitaxel(taxol®) supply crisis had at one time threatened the clinical use of this antitumour agent, first isolated from the stem bark of *Taxus brevifolia* [1]. Nowadays, the semi-synthesis is regarded as the most effective way to overcome the shortage of this drug [2]. Tissue and cell cultures, due to their success in the production of several medicinal compounds of great relevance, have increasingly been explored to meet the taxol supply. During a study on cell cultures of *T. yunnanensis*, we obtained seven taxanes oxygenated at C-14, some of which have been isolated from stem bark [3] and roots [4] of this plant. Three (**1**–**3**) are new, and we report here their structural elucidation.

RESULTS AND DISCUSSION

10β - Hydroxy - 2α,5α - diacetoxy - 14β - (2′ - methyl - 3′ - hydroxy) - butyroxy - 4(20),11 - taxadiene (**1**) was obtained as a white solid. The appearance of four methyl signals (1.97*s*, 1.72*s*, 1.18*s* and 0.84*s*) and a characteristic doublet at 2.92 ppm suggested a taxane skeleton. Two broad singlets at 5.25 and 4.81 ppm indicated the existence of C-4(20) exocyclic methylene protons. A detailed comparison of the spectral data for **1** and yunnaxan (**3**) showed an upfield shift of the signal of H-10 in **1** (δ 5.06 ppm) to 6.06 ppm in **4**. Compound **1** was thus 10-deacetyl yunnanxan.

2α,10β,14β - Trihydroxy - 5α - acetoxy - 4(20),11 - taxadiene (**2**) was also obtained as a white solid. Its FAB mass spectrum gave an adduct $[M + Na]^+$ at *m/z* 401 corresponding to a molecular weight of 378. Only one acetoxy signal was observed in the ^{13}C (169.77 and 21.78 ppm) and ^1H NMR (2.12*s*) spectra. Based on ^1H–^1H and ^{13}C–^1H COSY experiments, three methine proton signals at low field (4.13*d*, 5.08*dd* and 3.95*t*) acetoxy group at C-5. Comparison of the ^{13}C NMR data for **2** and **1** showed that C-1 resonate at a lower field (66.95 ppm) in **2** owing to the lack of the β-shielding effects of the ester group at C-2 by C-14. Treatment of **2** with acetic anhydride–pyridine at room temperature gave the known compound **5** [5].

2α - Hydroxy - 5α,10β,14β - triacetoxy - 4(20),11 - taxadiene (**3**) has a molecular formula of $C_{26}H_{38}O_7$



	R_1	R_2	R_3	R_4
				4′ 3′ 2′ 1′
1	Ac	Ac	H	Me-CH-CH-CO
				OH Me
2	H	Ac	H	H 5′
3	H	Ac	Ac	Ac
4	Ac	Ac	Ac	Me-CH-CH-CO
				OH Me
5	Ac	Ac	Ac	Ac
6	Ac	Ac	Ac	Et CO

Table 1. ^{1}H NMR spectral data for compounds **1**–**3** ($CDCl_3$, 500 MHz)

Proton	1	^{1}H–^{1}H COSY	2	3
H-1β	1.88*d* (2.4)	2β	1.92*d* (2.6)	1.96*d* (1.6)
H-2β	5.36*dd* (6.6, 2.4)	1β, 3α	4.13*dd* (6.6, 2.6)	4.09*dd* (5.7, 1.6)
H-3α	2.92*d* (6.6)	2β	2.78*d* (6.6)	2.70*d* (5.7)
H-5β	5.28*t* (2.9)	6α, 6β	5.21*t* (2.9)	5.24*t* (2.9)
H-6α	1.79*m*	5β, 7α, 7β	1.80*m*	1.80*m*
H-6β	1.79*m*	5β, 7α, 7β	1.80*m*	1.80*m*
H-7α	1.92*m*	6α, 6β, 7α	1.87*m*	1.93*m*
H-7β	1.25*m*	6α, 6β, 7α	1.18*m*	1.20*m*
H-9α	1.67*dd* (14.8, 5.6)	9β, 10α	1.64*dd* (14.8, 5.7)	1.58*dd* (14.7, 5.7)
H-9β	2.33*dd* (14.8, 11.7)	9α, 10α	2.28*dd* (14.8, 11.8)	2.25*dd* (14.7, 12.1)
H-10α	5.06*dd* (11.7, 5.6)	9α, 9β	5.08*dd* (11.8, 5.7)	6.03*dd* (12.1, 5.7)
H-13α	2.82*dd* (18.9, 9.2)	13β, 14α	2.56*m*	2.68*dd* (18.6, 9.3)
H-13β	2.37*dd* (18.9, 4.9)	13α, 14α	2.56*m*	2.57*dd* (18.6, 5.6)
H-14α	5.02*dd* (9.2, 4.9)	13α, 13β	3.95*t* (6.9)	4.84*dd* (9.3, 5.6)
Me-16	1.69*s*		1.66*s*	1.56*s*
Me-17	1.18*s*		1.27*s*	1.16*s*
Me-18	1.97*s*		1.98*s*	2.10*s*
Me-19	0.84*s*		0.88*s*	0.89*s*
H-20	4.81*brs*	5.25*brs*	5.36*brs*	5.30*brs*
	5.25*brs*	4.81*brs*	5.36*brs*	5.78*brs*
OAc	2.00*s*		2.12*s*	2.11*s*
	2.16*s*			2.07*s*
				2.04*s*
H-2′	2.40*q* (7.2)	5′		
H-3′	3.85*f* (6.3)	4′		
Me-4′	1.21*d* (6.3)	3′		
Me-5′	1.15*d* (7.2)	2′		

(FAB mass spectrometry and elemental analysis). ^{1}H and ^{13}C NMR spectra were similar to those of **1** and **5**. The fact that a double-doublet at 5.35 ppm in **5** was shifted upfield to 4.09 ppm in **3** suggested the loss of an acetyl group at C-2 in **3**, and **3** was thus assigned as 2α-hydroxy-5α,10β,14β-triacetoxy-4(20),11-taxadiene.

Comparison of the spectroscopic data for 13- and 14-oxygenated taxanes shows obvious changes of splitting pattern and coupling constants for H-13 and H-14. Futhermore, a downfield shift for C-1 is also observed in the 14-oxygenated products. In taxanes oxygenated at C-13, the C-1 methine carbon resonates at $\delta < 50$; in contrast, in C-14 oxygenated taxanes C-1 resonates at lower field (58–67 ppm) [3–6].

All C-14 oxygenated taxoids isolated by us exhibited poor cytotoxicity due to lack of the side-chain and C-4(20),5-oxetane ring. However, the content of some of these compounds, e.g. **5**, was very high (*ca* 1–2% dry wt), suggesting their potential use as raw materials for chemical conversion or biotransformation.

EXPERIMENTAL

General procedure. Mps were determined on a Boetius apparatus (uncorr.). Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. UV and recorded on a JMS-DX 300 MS spectrometer. Resin used in the experiment was produced by the Chemical Factory of Tianjin University, China. Silica gel for chromatography was purchased from Qingdao Marine Chemical Factory, China. D-101 macroporous resin was the product of Nan Kai University, China.

Plant cell cultures. Tissue and cell cultures of *T. yunnanenis* were induced from young stems and subcultured on 6,7-V medium containing 2 mg l^{-1} IAA, 1.5 mg l^{-1} 2,4-D and 0.1 mg l^{-1} kinetin. Cell cultures were obtained by transferring the callus into 6,7-V liquid medium containing same auxins and agitated by a rotary shaker at 100–120 rpm. Cultures were grown at 25°.

Extraction and isolation. Air-dried cells (120 g) were extracted with 3 × 500 ml Et_2O. The extracts were evapd *in vacuo*, further sepd on silica gel columns and plates, and eluted with a petrol–EtOAc gradient to yield **4** (0.27%), **5** (0.86%), **6** (0.16%), **7** (0.47%). The cultured liquid was passed through a column packed with D-101 macroporous resin, and the column was then washed with EtOH. The ethanolic eluates were also repeatedly chromatographed on silica gel columns and plates, and eluted with a petrol–EtOAc gradient to yield **1** (0.1 mg l^{-1}), **2** (3 mg l^{-1}), **3** (0.05 mg l^{-1}).

10β - *Hydroxy* - 2α,5α - *diacetoxy* - 14β - (2′ - *methyl* - 3′ - *hydroxy*) - *butyroxy* - 4(20),11 - *taxadiene*

Table 2. ^{13}C NMR spectral data for compounds **1–3** ($CDCl_3$, 125 MHz)

Carbon	1	^{13}C–H COSY	2	3
1	59.30	1.88*d*	66.95	63.48
2	70.60	5.36*dd*	69.45	70.21
3	41.95	2.92*d*	44.31	43.96
4	142.38		144.72	143.37
5	78.32	5.28*t*	78.57	79.08
6	28.50	1.79*m*	29.53	29.01
7	33.95	1.25*m*, 1.92*m*	34.40	34.10
8	39.66		40.30	39.68
9	47.16	1.67*dd*	47.38	44.01
10	67.33	5.06*dd*	67.51	70.22
11	138.78		138.86	135.91
12	132.20		132.39	133.70
13	39.53	2.37*dd*, 2.82*dd*	41.92	38.35
14	70.92	4.02*dd*	67.67	71.22
15	37.40		37.69	37.70
16	25.34	1.69*s*	25.35	25.97
17	32.01	1.18*s*	32.11	31.51
18	21.04	1.97*s*	21.11	20.97
19	22.55	0.84*s*	22.77	22.35
20	116.62	4.81*brs*, 5.25*brs*	117.23	116.77
$OCOCH_3$	169.70		169.77	169.58
	169.77			170.07
				171.94
$OCOCH_3$	21.87		21.78	21.88
	21.38			21.23
				21.50
1′	174.77			
2′	46.99	2.40*q*		
3′	69.48	3.85*t*		
4′	20.84	1.21*d*		
5′	13.98	1.15*d*		

(rel. int.): 559 $[M + K]^+$, EIMS *m/z* 520 $[M - H_2O]^+$ (2), 385 $[M - CH_3CH(OH)CH(CH_3)CO_2H - OH]^+$ (31), 325 $[M - CH_3CH(OH)CH(CH_3)CO_2H - CH_3CO_2H - OH]^+$ (41), 283 $[M - CH_3CH(OH)CH(CH_3)CO_2H - CH_3COOH - CH_3CO_2]^+$ (56), 265 $[M - CH_3CH(OH)CH(CH_3)CO_2H - 2 \times CH_3CO_2H - OH]^+$ (100), 249 $[M - CH_3CH(OH)CH(CH_3)CO_2H - 2 \times CH_3CO_2H - CH_3 - H_2O]^+$ (39).

2*α*,10*β*,14*β* - *Trihydroxy* - 5*α* - *acetoxy* - 4(20),11 - *taxadiene* (**2**). Mp 67–69°, $[\alpha]_D^{15}$ +39.2 (MeOH; *c* 0.13). UV λ_{max}^{MeOH} nm (log *ε*): 204 (3.29). IR ν_{max}^{KBr} cm^{-1}: 3380, 2930, 1730, 1640, 1445, 1370, 1000, 935. ^1H and ^{13}C NMR spectra: see Tables 1 and 2. FABMS *m/z* (rel. int.): 401 $[M + Na]^+$ (17).

Acetylation of **2**. Compound **2** (5 mg) was treated with 0.5 ml Ac_2O-pyridine (1:1) at room temp. for 24 hr and then diluted with H_2O. The mixt. was partitioned with $CHCl_3$, and the $CHCl_3$ layer was washed with dilute HCl, 10% $NaHCO_3$ and H_2O successively, and then dried ($MgSO_4$). The soln was then evapd to dryness to yield the triacetate of **2**, this being identical to **5**.

Compound **3**. Solid. Mp 60–61°, $[\alpha]_D^{16}$ +28.7 (EtOH, *c* 0.07). UV λ_{max}^{MeOH} nm (log *ε*): 201 (3.82). IR Elemental analysis (found: C, 67.10; H, 8.15. $C_{26}H_{38}O_7$ requires: C, 67.53, H, 8.23%).

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