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C-14 OXYGENATED TAXANES FROM TAXUS YUNNANENSIS CELL CULTURES

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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 - $2\alpha,5\alpha$ - diacetoxy - 14β - (2' - methyl - 3' - hydroxy) - butyroxy - 4(20),11 - taxadiene (1) was obtained as a white solid. The appearance of four methyl signals (1.97s, 1.72s, 1.18s and 0.84s) 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 yunnaxan.

 2α ,10 β ,14 β - 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/z401 corresponding to a molecular weight of 378. Only one acetoxy signal was observed in the ¹³C (169.77 and 21.78 ppm) and ¹H NMR (2.12s) spectra. Based on ¹H-¹H and ¹³C-¹H COSY experiments, three methine proton signals at low field (4.13d, 5.08dd and 3.95t) were attributed to H-2, H-10 and H-14, and a triplet at 5.21 ppm (J = 2.9 Hz), coupled with a multiplet corresponding to two protons at 1.80 ppm, located the acetoxy group at C-5. Comparison of the ¹³C 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 C₂₆H₃₈O₇



Proton	1	¹ H ⁻¹ HCOSY	2	3
	1.88d (2.4)	2β	1.92d (2.6)	1.96d (1.6)
H-2β	5.36dd (6.6, 2.4)	1β , 3α	4.13dd (6.6, 2.6)	4.09dd (5.7, 1.6)
H-3 <i>a</i>	2.92d (6.6)	2β	2.78d (6.6)	2.70d(5.7)
H-5β	5.28t (2.9)	6α, 6β	5.21 <i>t</i> (2.9)	5.24t (2.9)
Η-6α	1.79m	$5\beta, 7\alpha, 7\beta$	1.80m	1.80m
H-6β	1.79 <i>m</i>	$5\beta, 7\alpha, 7\beta$	1.80m	1.80 <i>m</i>
Η-7α	1.92 <i>m</i>	$6\alpha, 6\beta, 7\alpha$	1.87 <i>m</i>	1.93m
H-7β	1.25m	$6\alpha, 6\beta, 7\alpha$	1.18m	1.20m
H-9α	1.67dd (14.8, 5.6)	9β , 10α	1.64dd (14.8, 5.7)	1.58dd (14.7, 5.7)
Н-9 <i>β</i>	2.33dd (14.8, 11.7)	9α , 10α	2.28dd (14.8, 11.8)	2.25dd (14.7, 12.1)
Η-10α	5.06dd (11.7, 5.6)	9α, 9β	5.08dd (11.8, 5.7)	6.03dd (12.1, 5.7)
Η-13α	2.82dd (18.9, 9.2)	13β , 14α	2.56m	2.68dd (18.6, 9.3)
H-13β	2.37dd (18.9, 4.9)	13α , 14α	2.56m	2.57dd (18.6, 5.6)
H-14α	5.02dd (9.2, 4.9)	$13\alpha, 13\beta$	3.95t (6.9)	4.84dd (9.3, 5.6)
Me-16	1.69s		1.66s	1.56s
Me-17	1.18s		1.27s	1.16s
Me-18	1.97s		1.985	2.10s
Me-19	0.84s		0.88s	0.89s
H-20	4.81 <i>brs</i>	5.25brs	5.36brs	5.30brs
	5.25brs	4.81brs	5.36brs	5.78brs
OAc	2.00s		2.12s	2.11s
	2.16s			2.07 <i>s</i>
				2.04s
H-2'	2.40q(7.2)	5'		
H-3'	3.85f(6.3)	4'		
Me-4'	1.21d(6.3)	3'		

2'

Table 1. ¹H NMR spectral data for compounds 1–3 (CDCl₃, 500 MHz)

(FAB mass spectrometry and elemental analysis). ¹H and ¹³C 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-taxa-diene.

1.15d(7.2)

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 IR spectra were recorded on Shimadzu UV-240 and Perkin-Elmer 683 spectrometers, respectively, whereas ¹H and ¹³C NMR spectra were obtained on a Bruker AM-500 apparatus. FAB and EI mass spectra were

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 Et₂O. 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 (1). Mp 227-229°, $[\alpha]_{\rm D}^{15}$ + 25.1 (MeOH, c 0.053). UV $\lambda_{\rm max}^{\rm MeOH}$ nm (log ε): 204 (4.03). IR $\nu_{\rm max}^{\rm KBr}$ cm⁻¹: 3400, 2920, 1715, 1665, 1435, 1365, 1230, 1100, 1000. ¹H and ¹³C NMR spectra: see Tables 1 and 2. FABMS m/z

Me-5'

Carbon	1	¹³ C–H COSY	2	3
1	59.30	1.88 <i>d</i>	66.95	63.48
2	70.60	5.36dd	69.45	70.21
3	41.95	2.92d	44.31	43.96
4	142.38		144.72	143.37
5	78.32	5.28t	78.57	79.08
6	28.50	1.79 <i>m</i>	29.53	29.01
7	33.95	1.25 <i>m</i> , 1.92 <i>m</i>	34.40	34.10
8	39.66		40.30	39.68
9	47.16	1.67 <i>dd</i>	47.38	44.01
10	67.33	5.06dd	67.51	70.22
11	138.78		138.86	135.91
12	132.20		132.39	133.70
13	39.53	2.37dd, 2.82dd	41.92	38.35
14	70.92	4.02 <i>dd</i>	67.67	71.22
15	37.40		37.69	37.70
16	25.34	1.69s	25.35	25.97
17	32.01	1.18s	32.11	31.51
18	21.04	1.97s	21.11	20.97
19	22.55	0.84s	22.77	22.35
20	116.62	4.81brs, 5.25brs	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 <i>q</i>		
3'	69.48	3.85t		
4'	20.84	1.21 <i>d</i>		
5'	13.98	1.15 <i>d</i>		

Table 2. ¹³C NMR spectral data for compounds 1-3 (CDCl₃, 125 MHz)

(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 $[M - CH_3CH(OH)CH(-CH_3)CO_2H - 2 \times CH_3CO_2H - (CH_3)CO_2H - 2 \times CH_3CO_2H - CH_3 - H_2O]^+$ (39).

2α,10β,14β - Trihydroxy - 5α - acetoxy - 4(20),11 taxadiene (2). Mp 67–69°, $[α]_D^{15}$ +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 ¹³C 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 Ac₂O-pyridine (1:1) at room temp. for 24 hr and then diluted with H₂O. The mixt. was partitioned with CHCl₃, and the CHCl₃ layer was washed with dilute HCl, 10% NaHCO₃ and H₂O successively, and then dried (MgSO₄). 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 ν_{max}^{KBr} cm⁻¹: 3405, 2914, 1735, 1640, 1450, 1370, 1240. ¹H and ¹³C NMR spectra: see Tables 1 and 2. FABMS m/z (rel. int.): 485 [M + Na]⁺ (4), 463 [M + H]⁺ (14).

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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