

TAXOIDS FROM EUROPEAN YEW, *TAXUS BACCATA* L.

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(Received in revised form 27 June 1997)

IN HONOUR OF THE RETIREMENT OF PROFESSOR ANDRÉ CAVÉ

Key Word Index—*Taxus baccata*; Taxaceae; taxoids; 3,11-cyclotaxanes; 10-(β -hydroxybutyryl)-10-deacetylbaccatin III; cinnamoylphototaxicin II.

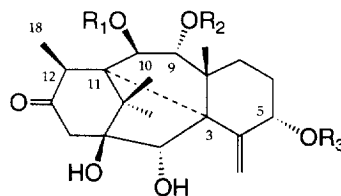
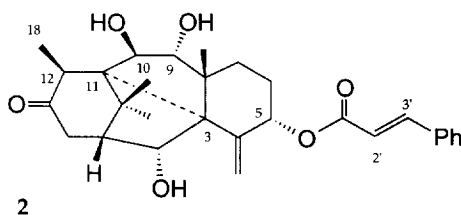
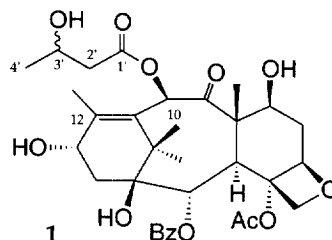
Abstract—10-(β -Hydroxybutyryl)-10-deacetylbaccatin III and 5-cinnamoylphototaxicin II were isolated from the leaves of *Taxus baccata*. Their structures were established by spectroscopic analyses. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Since the discovery of antitumour activity of paclitaxel (Taxol[®]) [1], much attention has been paid to the isolation of new taxanes from various species of *Taxus*. Our previous studies of *Taxus baccata* L. leaves resulted in the isolation and characterization of a new 11(15 $\rightarrow$ 1)*abeo*-taxane [2]. Further investigation on the same extract provided a new derivative of baccatin III, 10-(β -hydroxybutyryl)-10-deacetylbaccatin III (1), and a new 3,11-cyclotaxane, 5-cinnamoylphototaxicin II (2), in addition to the known taxoids (paclitaxel [1], 10-deacetyltaxol [3], 7- β -xiloxyl-10-deacetyltaxol [3], 10-deacetylcephalomanine [3], 5-cinnamoyltaxagifine [4], taxinine M [5], 5-cinnamoyltaxicin I [6], 5-*E*-cinnamoyl-10-acetylphototaxicin I (3) [7], 5-*Z*-cinnamoyl-10-acetylphototaxicin I (4) [7], and 5-cinnamoyl-9-acetylphototaxicin I (5) [7]. Compounds 4 and 5 were only known as semisynthetic products. We report here the structural elucidation of the two new taxoids 1 and 2.

RESULTS AND DISCUSSION

10-(β -Hydroxybutyryl)-10-deacetylbaccatin (1) was obtained as an amorphous white solid. A molecular formula of C₃₃H₄₂O₁₂ was established on the basis of its ¹³C NMR and FAB-mass spectra. IR bands at 3400, 1713 and 1642 cm⁻¹ revealed the presence of hydroxyl and carbonyl groups. Analyses of the ¹H

3, R₁ = H, R₂ = Ac, R₃ = *E*-cinnamoyl4, R₁ = H, R₂ = Ac, R₃ = *Z*-cinnamoyl5, R₁ = Ac, R₂ = H, R₃ = *E*-cinnamoyl

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Table 1. ^1H and ^{13}C NMR data for compounds **1** and **2** (500 MHz, CDCl_3 , δ values with TMS as internal standard, J in Hz)

No.	δ ^1H (1)	δ ^{13}C (1)	δ ^1H (2)	δ ^{13}C (2)
1		79.3 <i>s</i>	1.95 <i>m</i>	50.9 <i>d</i>
2	5.58 <i>d</i> (7)	75.5 <i>d</i>	5.02 <i>s br</i>	75.8 <i>d</i>
3	3.82 <i>d</i> (7)	47.3 <i>d</i>		66.6 <i>s</i>
4		81.3 <i>s</i>		141.1 <i>s</i>
5	4.95 <i>dd</i> (9.5; 2)	85.1 <i>d</i>	5.57 <i>t</i> (9)	76.2 <i>d</i>
6	2.6 <i>m</i>	36.1 <i>t</i>	2.20 <i>m</i>	19.6 <i>t</i>
	1.8 <i>m</i>		1.60 <i>m</i>	
7	4.39 <i>dd</i> (11; 7)	72.2 <i>d</i>	1.70 <i>m</i>	25.8 <i>t</i>
			1.30 <i>m</i>	
8		59.0 <i>s</i>		42.3 <i>s</i>
9		205.3 <i>s</i>	4.29 <i>d</i> (9.5)	83.9 <i>d</i>
10	6.40 <i>s</i>	76.6 <i>d</i>	4.12 <i>d</i> (9.5)	82.0 <i>d</i>
11		131.5 <i>s</i>		59.4 <i>s</i>
12		147.8 <i>s</i>	3.49 <i>c</i> (7)	52.0 <i>d</i>
13	4.79 <i>t br</i>	67.7 <i>d</i>		216.3 <i>s</i>
14	2.40 <i>m</i>	39.3 <i>t</i>	2.80 <i>d</i> (20)	38.3 <i>t</i>
			2.50 <i>dd</i> (20; 7)	
15		43.2 <i>s</i>		44.2 <i>s</i>
16	1.07 <i>s</i>	21.4 <i>c</i>	1.22 <i>s</i>	26.8 <i>c</i>
17	1.05 <i>s</i>	27.2 <i>c</i>	1.45 <i>s</i>	29.2 <i>c</i>
18	2.02 <i>s</i>	15.8 <i>c</i>	1.52 <i>d</i> (7)	16.6 <i>c</i>
19	1.65 <i>s</i>	10.1 <i>c</i>	1.43 <i>s</i>	25.2 <i>c</i>
20	4.26 <i>d</i> (8.5)	76.8 <i>t</i>	5.75 <i>s</i>	125 <i>t</i>
	4.12 <i>d</i> (8.5)		5.61 <i>s</i>	
PhCO		167.5 <i>s</i>		
Ph (i)		129.74 <i>s</i>		134.3 <i>c</i>
Ph (o)	8.10 <i>d</i> (7.5)	129.02 <i>d</i>	7.57 <i>m</i>	128.1 <i>d</i>
Ph (m)	7.48 <i>t</i> (7.5)	130.3 <i>d</i>	7.40 <i>m</i>	128.8 <i>d</i>
Ph (p)	7.60 <i>t</i> (7.5)	134.2 <i>d</i>	7.40 <i>m</i>	130.4 <i>d</i>
1'		170.8 <i>s</i>		166.0 <i>s</i>
2'	2.60 <i>m</i>	44.4 <i>t</i>	6.38 <i>d</i> (16)	117.8 <i>d</i>
	2.74 <i>dd</i> (16; 4)			
3'	4.33 <i>m</i>	65.0 <i>d</i>	7.67 <i>d</i> (16)	145.3 <i>d</i>
4'	1.29 <i>d</i> (6)	22.2 <i>c</i>		
Ac	2.28 <i>s</i>	22.9 <i>c</i>		
		171.5 <i>s</i>		

and ^{13}C NMR data (Table 1) and HMQC and COSY spectra suggested a basic skeleton of 10-deacetyl-baccatin III, together with a β -hydroxybutyryl group. The ^{13}C NMR spectra showed signals due to nine oxygenated carbons, one tetrasubstituted olefin, one monosubstituted aromatic ring, six methyl groups, one keto carbonyl group and three ester carbonyl groups. The ester group at C-10 was identified as β -hydroxybutyric acid by COSY experiment, which revealed connectivities of C-2' to C-3' and C-3' to C-4'. The stereochemistry of C-3' could not be established.

5-Cinnamoylphototaxicin II (**2**) was obtained as an amorphous white solid. A molecular formula of $\text{C}_{29}\text{H}_{36}\text{O}_6$ was established on the basis of its ^{13}C NMR and EI-mass spectra. IR bands in the regions of 3400, 1687 and 1637 cm^{-1} revealed the presence of hydroxyl and carbonyl groups. ^1H and ^{13}C NMR signals were assigned by DEPT, HMQC, COSY and

NOESY techniques, and are presented in Table 1. The NMR spectra showed signals which are different from those of the common taxoids. In particular, the C-12 methyl gave rise to a doublet in the ^1H NMR spectrum, and the ^{13}C NMR spectrum lacked the signals of the tetrasubstituted double bond and the C-3 methine, replaced by two sp^3 quaternary carbons (3 and 11) and a doublet (C-12). These spectral features are characteristic of 3,11-cyclotaxanes [8], a minor group of taxoids. Three further compounds of this type were isolated: **3**, **4** and **5**. Compounds **4** and **5** were only known as semisynthetic products, obtained by a transannular photocyclization of taxicins [7]. The ease of the photochemical reaction and the fact that these compounds were isolated from the same fraction containing their synthetic precursors, raises the question as whether the 3,11-cyclotaxanes are natural products or artefacts formed during their isolation.

EXPERIMENTAL

General. Mps: uncor. ^1H and ^{13}C NMR spectra were recorded in CDCl_3 on Bruker AMX-300 and AMX-500 spectrometers with TMS as int. standard; the NOE data and 2D ^1H - ^1H and ^{13}C - ^1H correlation spectra were obtained using standard Bruker software. FABMS: Glycerol matrix, Xe, 8 KV; Kratos MS-50. EIMS: direct inlet, 70 eV. UV: Hewlett Packard 8452A diode array spectrophotometer. IR: Midac Prospect spectrophotometer. HPLC: Waters 600 E system controller and Water 490 UV detector.

Plant material. *Taxus baccata* leaves were collected in Santiago de Compostela in March 1992.

Extraction and separation. These processes were carried out as described in our previous work [2]. 10-(β -Hydroxybutyryl)-10-deacetylbaecatin III (**1**). Reversed-phase HPLC of fr. **I-6** (Zorbax ODS, 250×20 mm; flow rate 6.0 ml min^{-1} ; UV detection at 230 nm; 7:3–0:100 H_2O – CH_3CN gradient) gave fr. **I-6a** ($R_t = 34$ min) which prep. TLC (Merck GF-254 silica gel; 24:1 CH_2Cl_2 –*iso*-PrOH) gave 2 mg of 10-(β -hydroxybutyryl)-10-deacetylbaecatin III (**1**). Amorphous white solid. FABMS m/z : 631 $[\text{M} + \text{H}]^+$. Positive EIMS m/z : 527 $[\text{M-hydroxybutyryl}]^+$. IR $\nu_{\text{max}}^{\text{CHCl}_3}$, cm^{-1} : 3400, 2922, 1713 and 1642. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$, nm: 276 and 252. ^1H and ^{13}C NMR, see Table 1. ^1H - ^1H COSY correlations (CDCl_3 , H/H): 2/3, 5/6, 6/7, 13/14, 2'/3', 3'/4'.

5-Cinnamoylphototaxicin II (**2**). Reversed-phase HPLC of fr. **I-9** (Zorbax ODS, 250×20 mm; flow rate 6.0 ml min^{-1} ; UV detection at 230 nm; 1:1–0:100 H_2O – CH_3CN gradient) gave a fr. ($R_t = 22$ min), which by prep. TLC (Merck GF-254 silica gel; 97:3 CH_2Cl_2 –*iso*-PrOH) afforded 2 mg of 5-cinnamoylphototaxicin II (**2**). Amorphous white solid. Positive EIMS m/z : 332 $[\text{M-cinnamate}]^+$. IR $\nu_{\text{max}}^{\text{CHCl}_3}$,

cm^{-1} : 3400, 1687 and 1637. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$, nm: 282. ^1H and ^{13}C NMR, see Table 1. ^1H - ^1H COSY correlations (CDCl_3 , H/H): 1/2, 1/14, 5/6, 5/6', 6/6', 6/7, 9/10, 12/18, 14/14', 2'/3'. NOESY: 1/2, 1/14, 2/16, 2/19, 5/6, 7/10, 9/16, 9/19, 10/12, 10/18, 12/20.

Acknowledgements—We are grateful to the Xunta de Galicia for financial support under projects XUGA 20904B93 and 20906B96. The plant material was kindly provided by Prof. Antonio Mouriño, the late Dr. José Luis González Mantilla and the Concello of Santiago de Compostela.

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