Three New Oxetane-Ring-Containing Taxoids from *Taxus chinensis*

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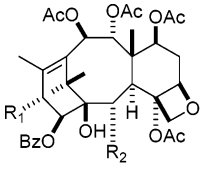
Three new 14β-benzoyloxy taxoids containing an oxetane ring, namely, 14β-benzoyloxybaccatin IV (**1**), 14β-benzoyloxy-13-deacetylbaccatin IV (**2**), and 14β-benzoyloxy-2-deacetylbaccatin VI (**3**), have been isolated from the leaves and stems of *Taxus chinensis*. Their structures were elucidated on the basis of 1D and 2D NMR spectroscopic data.

Reports on the phytochemistry, semisynthesis, and biosynthesis of paclitaxel and related taxoids have proliferated in recent years.[1−3] Although paclitaxel and its semisynthetic analogue docetaxel have exhibited significant clinical effects, these drugs often result in a number of side effects and multidrug resistance (MDR).[4] As part of a study on the constituents of *Taxus* species, we have investigated the constituents of *Taxus chinensis*, collected in Sichuan Province of mainland China. From extracts of the leaves and stems of *T. chinensis*, three new oxetane-ring-containing 14β-benzoyloxy taxoids have been isolated, namely, 14β-benzoyloxybaccatin IV (**1**), 14β-benzoyloxy-13-deacetylbaccatin IV (**2**), and 14β-benzoyloxy-2-deacetylbaccatin VI (**3**). The structures of these new compounds were elucidated on the basis of spectroscopic data analysis. Only a few taxanes functionalized at C-14 have been reported in previous research work,[5,6] and the availability of C-14 oxygenated taxoids with an oxetane functionality has great pharmacological potential, allowing the synthesis of additional oxygenated derivatives of paclitaxel.



1 R_1=OAc, R_2=OAc
2 R_1=OH, R_2=OAc
3 R_1=OAc, R_2=OH

Compound **1** was obtained as colorless prisms (MeOH). The molecular formula $C_{39}H_{48}O_{16}$ was deduced from its positive FABMS and 1D NMR spectral data and confirmed by HRFABMS (*m/z* 773.3023 $[M + H]^+$, calcd 773.3021). Its IR spectrum showed the presence of hydroxyl (3462 cm^{-1}) and ester carbonyl (1745 cm^{-1}) groups. The ^1H NMR and ^{13}C NMR spectral data of **1** indicated the presence of six acetyl groups, one benzoyloxy group, two olefinic carbons, four methyl carbons, two methylenes (including one oxygenated methylene), eight methines (seven of which were oxygenated), and four quaternary carbons (including two oxygenated ones). These spectral data suggested that **1** has a basic taxane skeleton. The 1D and 2D NMR data obtained for **1** allowed the assignments of all proton and carbon signals. The observation of the characteristic signals at δ 78.3 (C, C-1), 47.6 (CH, C-3), 46.7 (C, C-8), 136.5 (C, C-11), 138.6 (C, C-12), and 45.3 (C, C-15) indicated that **1** is a 6/8/6 ring-system taxoid.[7] The presence of an oxetane ring was determined from characteristic proton signals at δ 4.53 (1H, d, J = 7.9 Hz, H-20a), 4.18 (1H, d, J = 7.9 Hz, H-20b), and 4.99 (1H, d, J = 9.0 Hz, H-5) and characteristic carbon signals at δ 81.7 (C, C-4), 84.3 (CH, C-5), and 76.6 (CH_2, C-20).[8] In addition, six oxygenated methine proton signals appeared at δ 5.77 (1H, d, J = 6.0 Hz, H-2), 5.57 (1H, dd, J = 9.7, 7.8 Hz, H-7), 6.05 (1H, d, J = 11.3 Hz, H-9), 6.18 (1H, d, J = 11.3 Hz, H-10), 6.36 (1H, d, J = 6.3 Hz, H-13), and 5.71 (1H, d, J = 6.3 Hz, H-14), while six oxygenated methine carbon signals appeared at δ 71.7 (CH, C-2), 72.5 (CH, C-7), 73.4 (CH, C-9), 71.3 (CH, C-10), 75.4 (CH, C-13), and 72.7 (CH, C-14), which revealed that C-2, C-7, C-9, C-10, C-13, and C-14 carbons were functionalized by oxygenated groups. The locations of the functional groups were determined unambiguously by analysis of the C−H long-range correlations in the HMBC spectrum of **1**. From cross-peaks observed between H-2, H-7, H-9, H-10, and H-13 and ester carbonyl signals, this suggested the five acetoxyl groups were at C-2, C-7, C-9, C-10, and C-13. Moreover, the location of a benzoate group at C-14 was confirmed by the HMBC correlation from H-14 at δ 5.71 (1H, d, J = 6.3 Hz) to the benzoyl carbonyl at δ 165.7. The remaining acetyl group was assigned at C-4 due to the relative downfield shift of C-4 to δ 81.70, similar to baccatin IV.[9] Finally, the relative stereochemistry of **1** was determined by analysis of the NOESY spectrum (Figure 1). The NOESY correlations observed between H-2/H-9 and H-2/Me-17, H-3/H-7, H-5/H-6α, H-9/Me-17, H-10/H-7, H-13/Me-16, and H-3/H-14 indicated that H-2, H-9, and H-13 were β-oriented, while H-3, H-7, H-10, and H-14 were α-oriented. Thus, the structure of **1** was established as 14β-benzoyloxybaccatin IV.

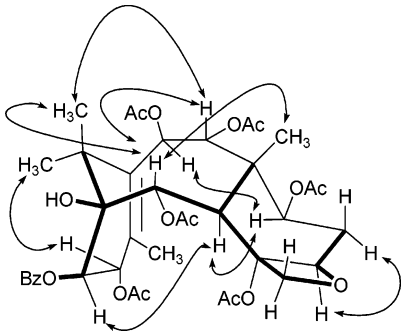


Figure 1. Key ROESY correlations of **1**.

Compound **2** was isolated as colorless needles (acetone−petroleum). The molecular formula $C_{37}H_{46}O_{15}$ was confirmed by HRFABMS (*m/z* 731.3269 $[M + H]^+$, calcd

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Table 1. ^{13}C and ^{1}H NMR Data (acetone-d_6) of Compounds **1**−**3**[a]

	1		**2**		**3**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	78.3 s		77.5 s		78.5 s	
2	71.7 d	5.77 (1H, d, 6.0)	72.9 d	5.72 (1H, d, 6.0)	75.3 d	4.17 (1H, dd, 9.3, 6.0)
3	47.6 d	3.18 (1H, d, 6.0)	47.7 d	3.22 (1H, d, 6.0)	47.9 d	2.95 (1H, d, 6.0)
4	81.7 s		81.3 s		83.0 s	
5	84.3 d	4.99 (1H, d, 9.0)	84.3 d	4.97 (1H, d, 6.2)	83.7 d	4.97 (1H, d, 8.2)
6	35.4 t	2.15 (1H, m)	35.4 t	2.45 (1H, m)	35.5 t	2.44 (1H, m)
		1.75 (1H, m)		1.75 (1H, m)		1.81 (1H, m)
7	72.5 d	5.57 (1H,dd, 9.7, 7.8)	72.7 d	5.61 (1H, dd, 9.8, 7.7)	72.8 d	5.53 (1H, dd, 9.8, 7.8)
8	46.7 s		46.5 s		46.6 s	
9	73.4 d	6.05 (1H, d, 11.3)	75.4 d	6.02 (1H, d, 11.4)	75.4 d	5.90 (1H, d, 11.4)
10	71.3 d	6.18 (1H, d, 11.3)	71.6 d	6.21 (1H, d, 11.4)	71.3 d	6.14 (1H, d, 11.4)
11	136.5 s		134.5 s		136.8 s	
12	138.6 s		143.5 s		138.0 s	
13	75.4 d	6.36 (1H, d, 6.3)	75.5 d	5.00 (1H, d, 5.8)	74.2 d	6.46 (1H, dd, 6.7, 1.6)
14	72.7 d	5.71 (1H, d, 6.3)	76.5 d	5.68 (1H, d, 5.8)	73.6 d	5.17 (1H, d, 6.7)
15	45.3 s		43.3 s		43.7 s	
16	28.4 q	1.43 (3H, s)	28.5 q	1.68 (3H, s)	28.6 q	1.23 (3H, s)
17	24.0 q	1.75 (3H, s)	23.8 q	1.31 (3H, s)	23.9 q	1.66 (3H, s)
18	15.2 q	2.01 (3H, s)	15.8 q	2.18 (3H, s)	15.1 q	2.01 (3H, s)
19	12.9 q	1.58 (3H, s)	12.9 q	1.56 (3H, s)	12.9 q	1.62 (3H, s)
20	76.6 t	4.53 (1H, d, 7.9)	75.5 t	4.51 (1H, d, 7.9)	77.9 t	4.62 (1H, d, 8.8)
		4.18 (1H, d, 7.9)		4.15 (1H, d, 7.9)		4.55 (1H, d, 8.8)
OCOPh	165.7 s		166.0 s		167.8 s	
i	131.1 s		131.7 s		134.3 s	
o	130.6 d	8.05 (2H, d, 8.0)	130.6 d	8.05 (2H, d, 8.0)	130.6 d	8.04 (2H, d, 8.3)
m	129.3 d	7.50 (2H, t, 7.8)	129.2 d	7.50 (2H, t, 8.0)	129.4 d	7.53 (2H, t, 8.0)
p	133.7 d	7.62 (1H, t, 7.4)	133.7 d	7.62 (1H, t, 7.4)	134.4 d	7.66 (1H, t, 7.5)
OAc	171.4 s		171.3 s		171.0 s	
OAc	171.1 s		171.1 s		170.9 s	
OAc	171.0 s		170.8 s		170.6 s	
OAc	170.9 s		170.2 s		170.3 s	
OAc	170.7 s		169.3 s		169.4 s	
OAc	170.3 s					
OAc	23.0 q	2.15 (3H, s)	23.0 q	2.20 (3H, s)	22.8 q	2.23 (3H, s)
OAc	21.4 q	2.12 (3H, s)	21.4 q	2.18 (3H, s)	21.4 q	2.13 (3H, s)
OAc	21.4 q	2.11 (3H, s)	21.4 q	2.11 (3H, s)	20.9 q	2.11 (3H, s)
OAc	21.4 q	2.02 (3H, s)	20.9 q	1.99 (3H, s)	20.8 q	2.08 (3H, s)
OAc	21.0 q	1.99 (3H, s)	20.8 q	1.98 (3H, s)	20.8 q	2.06 (3H, s)
OAc	20.8 q	1.97 (3H, s)				

[a] Assignments were made using HMQC and HMBC techniques.

731.2912). Its IR spectrum showed the presence of hydroxyl (3481 cm^{-1}) and ester carbonyl (1741 cm^{-1}) groups. The NMR spectral data of **2** were very similar to those of **1** except for the presence of one hydroxy group at C-13 and the absence of an acetoxy group. The upfield shift of H-13β from δ 6.36 (1H, d, J = 6.3 Hz) in **1** to δ 5.00 (1H, d, J = 5.8 Hz) in **2** confirmed that the hydroxy group at C-13 in **2** replaced an acetoxy group at C-13 in **1**. The relative stereochemistry of **2** was established by its NOESY spectrum. The NOESY correlations observed between H-2/H-9 and H-2/Me-17, H-3/H-7, H-5/H-6α, H-9/Me-17, H-10/H-7, H-13/Me-16, and H-3/H-14 indicated that H-2, H-9, and H-13 were β-oriented, while H-3, H-7, H-10, and H-14 were α-oriented. Therefore, compound **2** was elucidated as 14β-benzoyloxy-13-deacetylbaccatin IV.

Compound **3**, colorless lamellar crystals (acetone), showed an $[M + 1]^+$ ion peak at m/z 731.2911 (calcd 731.2915) in its positive FABMS, consistent with the molecular formula ($C_{37}H_{46}O_{15}$), which was confirmed by its ^{13}C NMR spectrum. Analysis of the ^{1}H and ^{13}C NMR data indicated that compound **3** is a derivative of baccatin VI.[10] On comparison of the ^{13}C NMR spectrum of **3** with that of **1** (Table 1), it was revealed that **3** differs from **1** only by a hydroxyl group at C-2 in **3** replacing an acetoxyl group at C-2 in **1**. The relative stereochemistry of OH-2 was α-oriented as determined by the NOE correlations between H-2β (δ 4.17, 1H, dd, J = 6.0, 9.3 Hz) with Me-19 (δ 2.01, 3H, s), H-9β (δ 5.90, 1H, d, J = 11.4 Hz), and Me-17 (δ1.66, 3H, s) in the NOESY spectrum. Consequently, compound **3** was elucidated as 14β-benzoyloxybaccatin VI.

Experimental Section

General Experimental Procedures. Melting points were determined on an XRC-1 micro melting point apparatus and are uncorrected. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained on a UV 2401 PC spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. ^{1}H and ^{13}C NMR experiments were performed on a Bruker AM-400 spectrometer, while 2D NMR spectra were recorded using a Bruker DRX-500 NMR instrument. FABMS and HRFABMS were taken on a VG Auto Spec-3000 or on a Finnigan MAT 90 instrument. Column chromatography was performed on silica gel (Qingdao Marine Chemical Inc. China), Lichroprep RP-18 (Merck, Darmstadt, Germany), and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd.). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The leaves and stems of *Taxus chinensis* (Taxaceae) were collected in Sichuan Province of China in March 2000 and identified by Prof. Lin Zhongwen. A voucher specimen (No. 20012) has been deposited at the Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. The dried leaves and stems (15 kg) of *Taxus chinensis* were extracted three times with 95% ethanol to give a crude extract after concentrating under a

vacuum. The residue was dissolved with MeOH−H_2O (9:1) and divided into MeOH-soluble and -insoluble parts. The MeOH-soluble part was further extracted with chloroform to give 250 g of extract. This was chromatographed over a silica gel column employing solvents of increasing polarity (petroleum−EtOAc, 9:1−2:8, and acetone) to give 10 fractions, of which three fractions (16.5 g) (petroleum−EtOAc, 6:4; 5:5; 4:6) were further chromatographed over a silica gel column eluted by $CHCl_3$−MeOH (100:1−50:1) to afford fractions 1−23 (5.9 g) and 24−32 (10 g). Fractions 1−23 were combined and chromatographed on a silica gel column eluted with cyclohexane−$CHCl_3$−2-propanol (5.0:4.5:0.5) to give compound **1** (5 mg). Fractions 24−32 were combined and chromatographed on Sephdex LH-20, eluted with MeOH−H_2O (5:5) to MeOH−H_2O (1:9). From the MeOH−H_2O (6:4) fraction, a mixture of compounds **2** and **3** was obtained. The mixture was purified employing Lichroprep RP-18 eluted by MeOH−H_2O (5.5:4.5) and HPLC by MeOH−H_2O (5.0:5.0) to give compounds **2** (10 mg) and **3** (16 mg).

Compound 1: colorless prisms crystals (MeOH); mp 270−272 °C; $[\alpha]_D^{15}$ +38.8° (*c* 0.31, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 220.2 (3.34), 240.6 (3.99), 275.8 (3.05) nm; IR (KBr) ν_{max} 3462, 1745, 1437, 1374, 1228, 1118, 1027, 714 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; positive FABMS *m/z* 773 $[M + H]^+$ (24), 713 (100), 654 (7), 577 (7), 106 (22), 78 (5); HRFABMS *m/z* 773.3023 $[M + H]^+$ (calcd for $C_{39}H_{49}O_{16}$, 773.3021).

Compound 2: colorless needle crystals (acetone−petroleum); mp 252−253 °C; $[\alpha]_D^{19}$ +32.5° (*c* 0.123, CH_3COCH_3); UV (MeOH) λ_{max} (log ϵ) 226.8 (4.05), 272.2 (2.96) nm; IR (KBr) ν_{max} 3742, 3481, 1941, 1741, 1634, 1373, 1252, 1231, 1115, 1028, 717 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; positive FABMS *m/z* 731 $[M + H]^+$ (32), 713 (53), 671 (100), 447 (13), 105 (49), 83 (37); HRFABMS *m/z* 731.3269 $[M + H]^+$ (calcd for $C_{37}H_{47}O_{15}$, 731.2912).

Compound 3: colorless lamellar crystals (acetone); mp 241−243 °C; $[\alpha]_D^{16}$ +9.4° (*c* 0.57, MeOH); UV (MeOH) λ_{max} (log ϵ) 228.4 (4.22), 274.4 (2.94) nm; IR (KBr) ν_{max} 3443 (OH), 1740 (ester C═O), 1636, 1437, 1374, 1250, 1106, 713 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; positive FABMS *m/z* 731 $[M + H]^+$ (32), 713 (53), 671 (100), 447 (13), 105 (49), 83 (37); HRFABMS *m/z* 731.2911 $[M + H]^+$ (calcd for $C_{37}H_{47}O_{15}$, 731.2915).

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