A New Taxane from the Hard Wood of *Taxus cuspidata*

Shu-Hong Yu[a], Man-li Zhang[a], Yu-Fang Wang[a], Chang-Hong Huo[a], Yu-Cheng Gu[a,b], and Qing-Wen Shi[a]

[a] School of Pharmaceutical Sciences, Hebei Medical University, 361 Zhongshan East Road, 050017, Shijiazhuang, Hebei Province, P. R. China
[b] Syngenta Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, U. K.

Reprint requests to Dr. Qingwen Shi. E-mail: shiqingwen@hebmu.edu.cn

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A new taxoid metabolite with an unusual double bond between C-13 and C-14 was isolated from the methanol extract of the hard wood of *Taxus cuspidata*. The structure was established as 2α,5α,7β,9α,10β,13β-hexaacetoxy-11β-hydroxyl-19β-benzoxytaxa-4(20),13-dien-12,16-epoxide (**1**), named 5,13-diacetyltaxinine M-13-enol, on the basis of spectral analysis including ^{1}H NMR, ^{13}C NMR, HMQC, HMBC, NOESY and confirmed by HR-FAB-MS.

Key words: Taxus cuspidata, Yew, Taxaceae, Hard Wood, Taxanes

Introduction

Taxus species have attracted a great deal of attention since paclitaxel (Taxol®), an anticancer drug used to treat ovarian, breast and lung cancers [1], was first isolated from the bark of *Taxus brevifolia* [2]. Since then, a large number of taxane diterpenoids have been isolated from various *Taxus* species [3 – 6]. *Taxus cuspidata*, an evergreen tree, is one of the most extensively studied yew, and more than 130 new taxanes have been reported [7 – 9]. As a continuation of our phytochemical study on *T. cuspidate* [10 – 14], we investigated the hard wood of *T. cuspidata* and isolated a new taxinine M-type taxane with a rare double bond between C-13 and C-14. We report herein the structure elucidation of this new compound (Fig. 1).

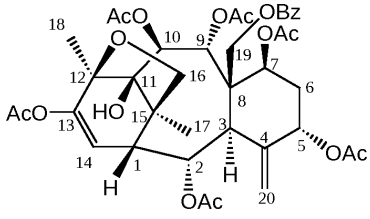

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Fig. 1. The structure of 5,13-diacetyltaxinine M-13-enol (**1**).

Results and Discussion

Compound **1** was isolated as a colorless amorphous powder from the methanol extract of *T. cuspidata* hard wood. The molecular composition of **1**, $C_{39}H_{46}O_{16}$, was derived by analysis of positive high-resolution FAB-MS at *m/z* = 809.2419 $[M+K]^+$ and was further substantiated by the ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR spectral data of **1** are summarized in Table 1. The ^{1}H NMR spectrum disclosed well-dispersed characteristic signals of taxanoids [15, 16] including three-proton signals due to the two tertiary methyl groups at δ_H = 1.29, and 1.13. Six acetyl groups were observed between δ_H = 2.02 and 2.21, and were further confirmed by corresponding ^{13}C NMR signals at δ_C = 168.0, 168.1, 169.8, 169.9, 170.2, and 172.5. A benzoyl group was also observed in both ^{1}H and ^{13}C NMR spectra: δ_H = 8.15 (2H, d, *J* = 7.6 Hz), 7.49 (2H, t, *J* = 7.6 Hz), 7.59 (1H, t, *J* = 7.6 Hz), and δ_C = 166.7 for the carbonyl of the benzoyl group. A pair of singlets at δ_H = 5.51 (1H, s) and 4.77 (1H, s) in the ^{1}H NMR spectrum were the characteristic signals of taxane with an *exo*-double bond at C-4 [15, 16]. The ^{13}C NMR spectrum of **1** revealed signals due to eight primary, four secondary, thirteen tertiary, and fourteen quaternary carbons. Of them, 17 sp^2-hybridized and 9 sp^3-hybridized carbon atoms are connected to oxygens judging from their chemical shifts. These carbons carried 45 hydrogens, indicating that the last hydrogen from the molecular formula was accommodated in a hydroxyl group. Indeed, a broad singlet signal was observed at δ_H = 4.00, which exhibited long rang correlations with C-10, C-11, C-12, C-13, and C-15 in the HMBC experiment. This observation suggested that this hydroxyl group was attached to C-11. To clarify the intermolecular connectivities, the HMBC ex-

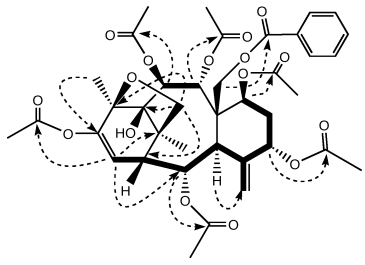

Fig. 2. Dotted arrows indicate the selected key HMBC correlations (H→C) of **1**. Connectivities (bold bones) were established by the ^{1}H-^{1}H-COSY correlations. Most protons are omitted for clarity.

periment and ^{1}H-^{1}H-COSY spectra were performed (Fig. 2). Full assignments of the proton and carbon signals were secured by ^{1}H-^{1}H-COSY, HSQC, and HMBC spectra. Detailed analysis of the HMBC correlations of H-18 to C-11, C-12, and C-13 confirmed that Me-18 was attached at C-12. In the HMBC spectrum, the cross-peaks of H-16 and H-17 to C-1, C-11, and C-15 indicated that C-16 and C-17 were connected at C-15. ^{1}H-^{13}C long-range correlations between H-1 and C-11, C-13, C-14, and C-15, H-16, H-17 and C-1 and C-11, and between H-14 and C-1, C-12, C-13, and C-15 indicated the presence of a cyclohexane moiety (ring A). The cross-peaks of H-2 to C-3, C-8, H-3 to C-1, C-2, and C-8, H-10 to C-11, and C-15, H-1 to C-2, C-3, C-11, and C-15, H-9 to C-8 and C-11 in the HMBC spectrum suggested the presence of an eight-membered ring (ring B). Long-range coupling of H-19 to C-3, C-7, C-8 and C-9 implied that Me-19 was located at C-8. ^{1}H-^{13}C long-range correlations between H-3 to C-4, C-5, and C-8, H-7 to C-6, C-8 were indicative of the presence of a cyclohexane moiety (ring C). Both H-16a and H-16b showed three-bond correlations with C-12 in the HMBC map indicating that a new ring was formed through an oxygen. This conclusion was further supported by the chemical shifts of C-12 (δ_C = 86.9) and C-16 (δ_C = 82.0) as well as the chemical shifts and coupling constants of H-16a and H-16b (δ_H =3.76, 1H, d, J = 8.1 Hz, H-16a; δ_H = 3.49, 1H, d, J = 8.1 Hz, H-16b) [17 – 19]. From the ^{1}H-^{1}H-COSY spectrum, it was possible to differentiate four discrete spin systems. A diagnostic signal at δ_H = 3.47 typical for H-3α [15, 16] showed correlations with H-2 and H-20b in the ^{1}H-^{1}H-COSY NMR map and exhibited HMBC correlations with C-1, C-2, C-4, C-5, C-7, C-8, C-9, C-19, and C-20. Using H-3 as a starting point, the spin system from H-3 to H-14 through H-2 and H-1 and the spin system from H-5 to H-7 through H-6a and H-6b were established. H-3 and H-5 also showed long-range correlations with H-20b and H-20a, respectively. Other three pairs of doublets were attributed to H-9 (1H, d, J = 3.0 Hz) and H-10 (1H, d, J = 3.0 Hz), and to the two geminal oxygenated methylenes (H-16 and H-19). These signals are the typical features of taxinine M-type taxane [17 – 19]. H-16 and H-19 resonated as a pair of doublets with relatively large coupling constants. The C-19-oxygenated methylene had a larger coupling constant than the C-16-oxygenated methylene because the latter is accommodated in a ring. H-2 resonated downfield as a broad doublet with a large coupling constant between H-3 and H-2 (J = 10.3 Hz). As there is no double bond at position C-11,12 and a new ring formed between C-12 and C-16, the chemical shifts of H-9 and H-10 are very close with a very small coupling constant. The peculiar conformation of ring B in this class of taxanes is required by the presence of the C-12, C-16 oxygen bridge.

According to the chemical shifts and HMBC correlations, five acetoxy groups were attached at C-2, C-5, C-7, C-9, and C-10, and the benzoyl group was positioned at C-19. The remaining acetoxy group (δ_C = 168.0), therefore, must be located at C-13, and this was in agreement with the rather downfield chemical shift of C-13 (δ_C = 152.2). The relative stereochemistry of **1** was defined on the basis of the NOESY spectrum and the coupling constants as well as by comparison with related compounds [15 – 19]. On the basis of the above arguments, the structure of **1** was characterized unequivocally as 2α,5α,7β,9α,10β,13β-hexaacetoxy-11β-hydroxyl-19β-benzoxytaxa-4(20),13-dien-12,16-epoxide (**1**), named 5,13-diacetyltaxinine M-13-enol, as depicted in Fig. 1.

Compound **1** is a new taxinine M-type diterpenoid with a rare double bond instead a ketone group at C-13 as in all the other taxinine M-type taxanes. It is the first example of such taxane isolated from *Taxus sp.* [3 – 5], although more than 15 taxanine M-type taxoids have been reported from *Taxus* plants since the first one was reported in 1981 [19].

Compound **1** did not exhibit potential *in vitro* cytotoxicity screening against the human breast cancer MCF-7 and ovary cancer HAC-2 cell lines.

Experimental Section

General

Optical rotation values were recorded on a Jasco DIP-370 digital polarimeter. All NMR data were obtained at r. t.

Table 1. The ^{1}H and ^{13}C NMR data of **1** in $CDCl_3$ (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR).

Position	δ_H (mult)	J (Hz)	^{1}H-^{1}H-COSY	δ_C	HMBC	NOESY
1	2.64 (d)	3.9 (br.d)	2, 14	54.8	2, 3,11, 13, 14, 15, 16	2, 14
2	6.10 (br.d)	10.3	1, 3	69.9	169.9, 3, 8, 14	1, 3, 16b
3	3.47 (d)	10.3	2, 20b	41.4	1, 2, 4, 5, 7, 8, 9, 19, 20	2, 10
4	–			141.1		
5	5.30 (br. s)		6a, 6b, 20a	74.3	170.2, 3, 4, 6, 7, 20	6a, 6b
6a	2.28 (m)		5, 6b, 7	36.2	4, 5, 7, 8	5, 6b, 7
6b	1.67 (m)		5, 6a, 7		7	5, 6a, 7
7	5.43 (dd)	10.8, 6.3	6a, 6b	68.8	168.1, 6, 8, 9, 19	6a, 6b
8	–			49.8		
9	5.38 (d)	3.0	10	70.2	172.5, 7, 8, 11,19	10, 17
10	6.07 (d)	3.0	9	64.6	169.8, 8, 9, 11, 12, 15	9
11	–			78.8		
12	–			86.9		
13	–			152.2		
14	5.84 (d)	3.9	1	112.2	168.0, 1, 2, 3, 12, 13, 15, 18	1
15	–			48.1		
16a	3.76 (d)	8.1	16b	82.0	1, 15, 17	10
16b	3.49 (d)	8.1	16a		1, 11, 15	2
17	1.29 (s)			17.0	1, 2, 11, 15, 16	9
18	1.13 (s)			12.5	11, 12, 13	
19a	5.15 (d)	12.3	19b	61.4	166.7, 3, 7, 8, 9	19b
19b	4.38 (d)	12.3	19a		166.7, 3, 7, 8, 9, 10	19a
20a	5.51 (s)		5, 20b	115.6	3, 4, 5, 6, 8	20b
20b	4.77 (s)		3, 20a		3, 4, 5, 8	20a
2-OAc	2.02 (s)			21.0[a]	169.9	
				169.9		
5-OAc	2.21 (s)			21.1[a]	170.2	
				170.2		
7-OAc	2.02 (s)			21.2[a]	168.1	
				168.1		
9-OAc	2.14 (s)			21.3[a]	172.5	
				172.5		
10-OAc	2.09 (s)			20.9[a]	169.8	
				169.8		
13-OAc	2.14 (s)			20.8[a]	168.0	
				168.0		
Bz C=O	–			166.7		
i				128.9		
o	8.15 (d)	7.6		130.0	166.7, *o*, *i*, *m*, *p*	*m*
m	7.49 (t)	7.6		128.6	166.7, *o*, *i*, *m*	*o*, *p*
p	7.59 (t)	7.6		133.6	*o*, *m*	*m*
OH	4.00 (br. s)				10, 11, 12, 13, 15	

[a] These signals were exchangeable.

on a Varian-300 spectrometer. Positive ion Fast Atom Bombardment Mass Spectra (FAB-MS) were obtained with a Vacuum Generators ZAB-HS instrument. Flash chromatography was performed on silica gel 60 (230 – 400 mesh EM Science). Thin-layer chromatography was conducted on silica gel 60 F_{254} pre-coated TLC plates (0.25 mm or 0.5 mm, EM Science). The compounds were visualized on TLC plates with 10 % sulfuric acid in ethanol and heating on a hot plate. Na_2SO_4 was the drying agent used in all work-up procedures. Analytical HPLC was performed on a Waters 600 FHU delivery system coupled to a PDA 996 detector. Preparative HPLC was carried out on a Waters Delta Prep 3000 instrument coupled to a UV 486 tunable absorbance detector set at 227 nm (Waters). Analytical HPLC was performed with two Whatman partisil 10 ODS-2 analytical columns (4.6×250 mm^2) in series. Preparative HPLC was performed with a partisil 10 ODS-2 MAG-20 preparative column (22×500 mm^2). The products were eluted with a 50 min linear gradient of acetonitrile (25 to 100 %) in water at a flow rate of 18 mL min^{-1}. Data were registered with MILLENIUM software (Waters). All the reagents and solvents were of the best available commercial quality and were used without further purification.

Plant material

The hard wood of *Taxus cuspidata* was collected in the fall of 2000 in the Toyama Prefecture in the north-west of Japan. The botanical identification was made by Professor T. Oritani of Toyama Prefectural University, Toyama, Japan. Several voucher specimens have been deposited in the Laboratory of Applied Bioorganic Chemistry, Graduate School of Agricultural Sciences, Tohoku University, Japan (no. NMC-2000-10-1).

Extraction and isolation

The air-dried hard wood (4500 g) of *T. cuspidata* was chipped and submerged in MeOH for one week at r. t. The methanolic extract was decanted and concentrated *in vacuo*; a dark-brown tar was obtained. The residue was diluted with brine and then extracted with $CHCl_3$. The $CHCl_3$-soluble portion was evaporated under reduced pressure to give a residue (40 g), which was loaded on to a silica gel column and eluted with increasing polarity of a mixed solvent (hexane-acetone). Fractions were pooled on the basis of their TLC to give 16 main fractions (Fr. 1 – 16). Fraction 5 was applied to further chromatographic separations using stepwise elution (hexane-ethyl acetate) to give 6 sub-fractions (Fr. 5 – 1 to Fr. 5 – 6) according to TLC. Sub-fraction Fr. 5 – 3 was subjected to reverse-phase preparative HPLC, the material eluted at 44.17 min was collected, dried and further purified by preparative TLC using hexane-ethyl acetate as mobile phase. The preparative TLC plate was cut into small strips under UV. The compounds were carefully removed by scraping off the silica gel and then were exhaustively extracted with acetone. After solvent evaporation, the residue afforded compound **1** (4.5 mg).

*2α,5α,7β,9α,10β,13β-Hexaacetoxy-11β-hydroxyl-19β-benzoxytaxa-4(20),13-dien-12,16-epoxide (**1**)*

Amorphous gum; $[\alpha]_D^{22} = -14^\circ$ (c = 0.20, $CHCl_3$); ^1H and ^{13}C NMR, HMBC, and NOESY spectral data, see Table 1. HRMS ((+)-FAB): *m/z* = 809.2419 (calcd. 809.2423 for $C_{39}H_{46}O_{16}K$, $[M+K]^+$).

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[1] W. K. Murphy, F. V. Fossella, R. J. Winn, D. M. Shin, H. E. Hines, H. M. Gross, E. Davilla, J. Leimert, H. Dhingra, M. N. Raber, I. H. Krakoff, W. K. Hong, *J. National Cancer Institute* **1993**, *85*, 384 – 388.
[2] M. C. Wan, H. L. Taylor, M. E. Wall, P. Coggon, A. T. McPhail, *J. Am. Chem. Soc*. **1971**, *93*, 2325 – 2327.
[3] E. Baloglu, D. G. I. Kingston, *J. Nat. Prod.* **1999**, *62*, 1448 – 1427.
[4] V. S. Parmar, A. Jha, K. S. Bisht, P. Taneja, S. K. Singh, A. Kumar, Poonam, R. Jain, C. E. Olsen, *Phytochemistry* **1999**, *50*, 1267 – 1304.
[5] G. Appendino, *Nat. Prod. Rep*. **1995**, *12*, 349 – 360.
[6] D. G. I. Kingston, P. G. Jagtap, H. Yuan, L. Samala, in *Progress in the Chemistry of Organic Natural Products, Vol. 84*, (Eds.: W. Herz, H. Falk, G. W. Kirby), Springer, Wien, **2002**, p. 53.
[7] J. Kobayashi, H. Shigemori, *Heterocycles* **1998**, *47*, 111 – 112.
[8] J. Kobayashi, H. Shigemori, *Med. Res. Rev.* **2002**, *22*, 305 – 328.
[9] H. Shigemori, J. Kobayashi, *J. Nat. Prod.* **2004**, *67*, 245 – 256.
[10] Q. W. Shi, T. Oritani, T. Sugiyama, T. Horiguchi, R. Murakami, D. Zhao, T. Oritani, *Tetrahedron* **1999**, *63*, 8365 – 8367.
[11] R. Murakami, Q. W. Shi, T. O. Oritani, *Phytochemistry* **1999**, *52*, 1577 – 1580.
[12] Q. W. Shi, T. Oritani, D. Zhao, R. Murakami, T. Oritani, *Planta Med*. **2000**, *66*, 294 – 299.
[13] M. L. Zhang, Q. W. Shi, M. Dong, Y. F. Wang, C. H. Huo, Y. C. Gu, B. Cong, H. Kiyota, *Tetrahedron Lett.* **2008**, *49*, 1180 – 1183.
[14] C. H. Huo, X. H. Su, X. Li, X. P. Zhang, C. F. Li, Y. F. Wang, Q. W. Shi, H. Kiyota, *Magn. Reson. Chem.* **2007**, *45*, 527 – 530.
[15] D. G. I. Kingston, A. A. Molinero, G. M. Rimoldi, in *Progress in the Chemistry of Organic Natural Products, Vol. 61*, (Eds.: W. Herz, R. E. Moore, G. W. Kirby, W. Steglich, C. Tamm), Springer, Wien, **1993**, p. 1.
[16] G. Appendino, in *The Chemistry and Pharmacology of Taxol and Its Derivatives, Vol. 22*, (Ed.: V. Farina), Elsevier Science, Amsterdam, **1995**, pp. 1 – 53 and pp. 55 – 101.
[17] X. X. Wang, H. Shigemori, J. Kobayashi, *Tetrahedron* **1996**, *52*, 12159 – 12164.
[18] L. Barboni, P. Gariboldi, E. Torregiani, G. Appendino, M. Varese, B. Gabetta, E. Bombardelli, *J. Nat. Prod*. **1995**, *58*, 934 – 939.
[19] G. Chauviere, D. Guenard, C. Pascard, F. Picot, P. Potier, T. Prange, *J. Chem. Soc., Chem. Comm.* **1982**, 495 – 496.