Heterocycl. Commun., Vol. 17(1-2), pp. 87–91, 2011 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515/HC.2011.019

The first taxane peroxide from the rooted cuttings of *Taxus canadensis*

Zhen-Hua Pan[1], Man-Li Zhang[2], Yu-Fang Wang[2], Mei Dong[3,4], Chang-Hong Huo[2], Françoise Sauriol[5], Qing-Wen Shi[2,4,*], Hiromasa Kiyota[6,*] and Yong-Jian Zhang[1,*]

[1] Department of Pharmacology, Hebei Medical University, 361 Zhongshan East Road, 050017, Shijiazhuang, Hebei Province, P.R. China
[2] Department of Medicinal Natural Product Chemistry, School of Pharmaceutical Sciences, Hebei Medical University, Shijiazhuang, 050017, Hebei Province, P.R. China
[3] College of Basic Medicine, Hebei Medical University, Shijiazhuang, 050017, Hebei Province, P.R. China
[4] Hebei Key Laboratory of Forensic Medicine, Hebei Medical University, Shijiazhuang, 050017, Hebei Province, P.R. China
[5] Department of Chemistry, Queen's University, Kingston, Ontario, K7L 3N6, Canada
[6] Laboratory of Applied Bioorganic Chemistry, Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai 981-8555, Japan

*Corresponding authors
e-mail: shiqing-w@163.com, kiyota@biochem.tohoku.ac.jp, zhangyj@hebmu.edu.cn

Abstract

A new taxane peroxide, 4α,10β,13α-acetoxy-2α-benzoyloxy-7β,9α-epidioxy-5β,20-epoxytax-11-en-1β-ol (**1**), was isolated and characterized for the first time in rooted cuttings of the Canadian yew, *Taxus canadensis*.

Keywords: peroxide; rooted cutting; Taxaceae; Taxane; *Taxus canadensis*; yew.

Introduction

Plants from the genus *Taxus* are a rich source of biologically active diterpenoids belonging to the unique structure class of taxanes (Baloglu and Kingston, 1999; Parmer et al., 1999). Taxol® (paclitaxel) is one of the most important anti-cancer drugs currently on the market against ovarian and breast cancers and shows promising effects for a variety of other cancers, such as neck, lung, gastrointestinal and bladder. Its unique mechanism of action, limited solubility and shortage have generated worldwide interest, and extensive chemical studies have been carried out (Kingston et al., 1993; Farina, 1995; Georg et al., 1995; Baloglu and Kingston, 1999; Parmer et al., 1999; Shi and Kiyota, 2005). *Taxus canadensis* is a low trailing shrub ubiquitous to the Quebec region and its composition has been shown to be very different from other species (Dong et al., 2008). Interest in the Canadian yew has been prompted by the discovery that its needles can be a rich source of 9-dihydro-13-acetylbaccatin III (**2**), 5–7 times the amount of Taxol®, depending on the collection site (Zamir et al., 1992,1995), which is specific to this variety and has only been found as traces in the bark of *Taxus chinensis* (Zhang et al., 1992), and has been used as a starting material to semisynthesis of Taxol® and its analogues (Nikolakakis et al., 2000). Previous studies on the Canadian yew have led to the identification of more than 70 taxanes including various skeletons (Dong et al., 2008). Further investigation on rooted cutting of this plant resulted in the isolation of a taxane peroxide **1** (a 7β,9α-epidioxy derivative of **2**; Figure 1). This paper reports the isolation and identification of **1**. In order to compare the nuclear magnetic resonance (NMR) data especially to check the C-7-OH and C-9-OH functions, we also report the ^{1}H and ^{13}C data for **2** in acetone-d_6.

Results and discussion

Taxane **1** shows the molecular weight at *m/z* 669.2358 $[M+K]^+$ and 653.2595 $[M+Na]^+$ in high resolution-fast atom bombardment mass spectrum (HR-FABMS) analysis. ^{1}H and ^{13}C NMR data (Table 1 and Figure 2) and heteronuclear multiple bond correaltion spectroscopy (HMBC) spectra reveal that **1** is an analog of baccatin III including three acetyls, one benzoyl, one tetrasubstituted olefin, six oxymethines, two oxygenated quaternary carbons, and four tertiary methyl groups. Two of the methyl groups at δ_H 1.24 and 1.26 are COSY (^{1}H-^{1}H correlation spectroscopy)-correlated peaks as geminal methyls (Me-16 and Me-17). The signals at δ_H 4.14 and 4.16 (each 1H, d, *J*=8.3 Hz) and δ_C 75.7 (t) indicate the presence of an oxetane ring in the molecule. Detailed analysis of the ^{1}H-^{1}H COSY spectrum revealed the connectivities of C-2 to C-3, C-5 to C-7, C-9 to C-10, C-13 to C-14, and Me-18 to H-13. The cross-peaks of Me-16 and Me-17 to C-1, C-11, and C-15 in the HMBC experiment implied that Me-16 and Me-17 are attached to C-15; while the cross-peaks of Me-18 to C-11, C-12 and C-13 proved that Me-18 is connected to C-12. These HMBC correlations along with the ^{1}H-^{13}C long-range correlations of H-14 and H-10 to C-15 suggest the presence of a cyclohexane moiety (ring A). The cross-peaks of H-2 to C-1, H-3 to C-8, H-10 to C-15 in the HMBC spectrum are indicative of the presence of an eight-membered ring (ring B). The ^{1}H-^{13}C long-range correlations of H-3 to C-7, C-8, H-5 to C-3,

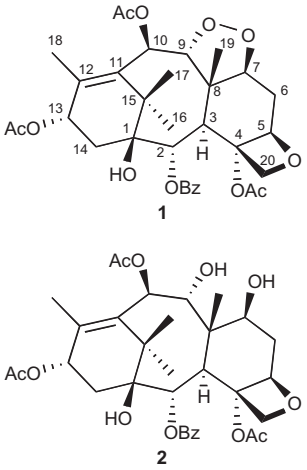

Figure 1 Structures of **1** and **2**.

C-4, C-7, and H-6 to C-8 are consistent with the presence of a cyclohexane moiety (ring C). The HMBC correlations of H-20a and H-20b to C-4 and C-5 indicate that the oxetane ring is fused to the ring C at C-4 and C-5. The H-2 proton shows the long-range correlation of the carbonyl carbon at δ_C 165.5 in the HMBC spectrum, supporting the presence of the benzoyloxy group at C-2. The H-10 proton exhibits the three-bond correlation with a carbonyl carbon at δ_C 169.5 demonstrating that an acetoxy group is attached at C-10. The chemical shift of H-13 suggests that one acetoxy group is located at C-13. The remaining acetoxy group is positioned at C-4 as implied by the chemical shift of C-4 at δ_C 81.6, like many other taxanes containing an oxetane ring (Kingston et al., 1993; Farina, 1995). Since only one hydroxy group is detected in the ^{1}H NMR spectra recorded in acetone-d_6 and $CDCl_3$, the remaining C-7-OH and C-9-OH should be accommodated by forming a C-7-C-9 peroxide. Thus, the structure of **1** was proposed to be a 7β,9α-

Table 1 ^{1}H and ^{13}C NMR data for **1** in acetone-d_6 (500 MHz for ^{1}H, 125 MHz for ^{13}C).

Position	δ_H (mult)[a]	*J* (Hz)	δ_C[b]	HMBC	NOESY[c]
1	–		**77.4**		
1-OH	3.99 (s)			1, 2, 14, 15	2,[w] 14,[w] 16,[w] 17[w]
2	5.86 (d)	6.2	73.5	1, 3, 8, 14, **165.5**	1-OH,[w] 3,[w] 9,[s] 17,[s] 19[s]
3	3.20 (d)	6.2	45.9	1, 2, 7, 8, 19, 20	2,[w] 7,[s] 10,[w] 14,[m] 18[m]
4	–		**81.6**		
5	4.93 (d)	8.1	83.6	3, 4, 7	6α,[s] 20α[m]
6α	2.53 (ddd)	14.9, 9.2, 7.6	36.5	7, 8	5,[s] 7[s]
6β	1.70 (ddd)	14.9, 10.3, 1.6			7[m]
7	4.39 (dd)	10.3, 7.6	72.1		3,[s] 6α,[s] 6β,[m] 10,[s] 18[s]
8	–		**42.1**		
9	4.62 (d)	10.6	76.6	7, 8, 10, 19	2,[s] 19[s]
10	6.17 (d)	10.6	72.6	9, 11, 12, 15, **169.5**	3,[w] 7,[s] 18[s]
11	–		**134.7**		
12	–		**139.5**		
13	6.13 (br.t)	6.9	69.3		14,[s] 16[s]
14αβ	2.3–2.4 (o.m)		35.9	12, 15, 1, 2, 13	1-OH,[w] 3,[m] 13[s]
15	–		**43.2**		
16	1.24 (s)		27.4	1, 11, 15, 17	13,[s] 17,[s] 1-OH[w]
17	1.66 (s)		22.5	1, 11, 15, 16	1-OH,[w] 2,[s] 16[s]
18	1.90 (d)	1.1	13.8	11, 12, 13	3,[m] 7,[s] 10[s]
19	1.65 (s)		12.0	3, 7, 8, 9	2,[s] 9,[s] 20β[s]
20α	4.14 (d)	8.3	75.7	3, 5	5[m]
20β	4.16 (d)	8.3		4	19[s]
Ac	2.30 (s)		22.0	**169.7**	
			169.7		
	2.17 (s)		20.2	**170.3**	
			170.3		
	2.05 (s)		20.1	**169.5**	
			169.5		
Bz*C*			**165.5**		
o-	8.10 (d)	8.2	129.7	**165.5**	
m-	7.52 (t)	7.5	128.3		
p-	7.63 (t)	7.5	133.0		

[a]Multiplicity: s, singlet; d, doublet; ddd, doublet of doublets of doublets; br, broad; t, triplet; m, mutiplet; o, overlapped. [b]The ^{13}C chemical shifts were extracted from the heteronuclear multiple quantum coherence (HMQC) experiment (±0.2 ppm). The numbers in bold represent quaternary carbons whose chemical shifts were obtained from the HMBC experiment (±0.2 ppm). [c]NOESY intensities are marked as strong (s), medium (m), or weak (w).

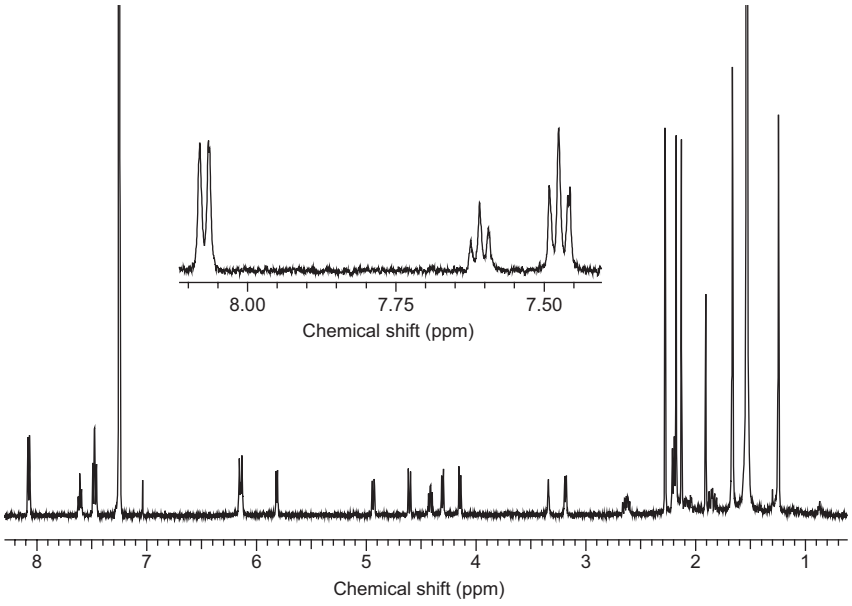

Figure 2 The ^{1}H NMR spectrum of **1**.

Table 2 ^{1}H and ^{13}C data for 9-dihydro-13α-acetylbaccatin III (**2**) in acetone-d_6 (500 MHz for ^{1}H, 125 MHz for ^{13}C).

Position	δ (H) mult	*J* (Hz)	δ (C)	HMBC	NOESY
1	–		**77.4**		
1-OH	3.88 (s)			1, 2, 14, 15	2,^{w} 14,^{w} 16,^{w} 17^{w}
2	5.79 (d)	6.0	73.4	1, 3, 8, 14, 15, **165.5**	1-OH,^{w} 3,^{w} 9,^{s} 17,^{s} 19^{s}
3	3.07 (d)	6.0	46.9	1, 2/7, 8, 19, 20	2,^{w} 7,^{s} 14,^{s} 18^{m}
4	–		**81.6**		
5	4.91 (d)	8.0	83.6	3, 4, 7	6α,^{s} 20α^{w}
6α	2.45 (ddd)	14.9, 9.1, 7.7	37.7	5, 7, 8	5,^{s} 7^{s}
6β	1.81 (o.m)				
7	4.42 (br.m)		73.4		3,^{s} 6α,^{s} 10,^{s} 18,^{s} 7-OH^{s}
7-OH	5.58 (d)	2.8			7,^{s} 19^{w}
8	–		**44.8**		
9	4.48 (br.dd)	10.4, 7.7	76.6	7/10, 8, 19	2,^{s} 17,^{s} 19,^{m} 9-OH^{m}
9-OH	5.74 (d)	7.3		8, 9	9,^{m} 10^{w}
10	6.19 (d)	10.8	72.8	9, 11, 12, 15, **169.5**	3,^{w} 7,^{s} 9-OH,^{m} 18^{s}
11	–		**135.8**		
12	–		**138.1**		
13	6.13 (br.t)		69.3		14,^{s} 16^{s}
14αβ	2.3-2.4 (o.m)		35.9		1-OH,^{w} 3,^{s} 13^{s}
15	–		**43.2**		
16	1.22 (s)		27.4	1, 11, 15, 17	13,^{s} 17,^{s} 1-OH^{w}
17	1.66 (o.s)		22.5	1, 11, 15, 16	1-OH,^{w} 2,^{s} 9,^{s} 16^{s}
18	1.92 (d)	1.3	13.8	11, 12, 13	3,^{m} 7,^{s} 10^{s}
19	1.78 (s)		12.0	3, 7, 8, 9	2,^{s} 7-OH,^{w} 9,^{s} 9-OH,^{w} 20β^{s}
20α	4.11 (d)	8.2	75.7	3, 5	5^{w}
20β	4.17 (d)	8.2		4	19^{s}
OAc	2.32 (s)		22.0	**169.7**	
			169.7		
	2.17 (s)		20.2	**170.3**	
			170.3		
	2.06 (s)		20.1	**169.5**	
			169.5		
Bz*C*				**165.5**	
o-	8.10 (d)	8.3	129.7		
m-	7.52 (t)	7.6	128.3		
p-	7.63 (t)	7.5	133.0		

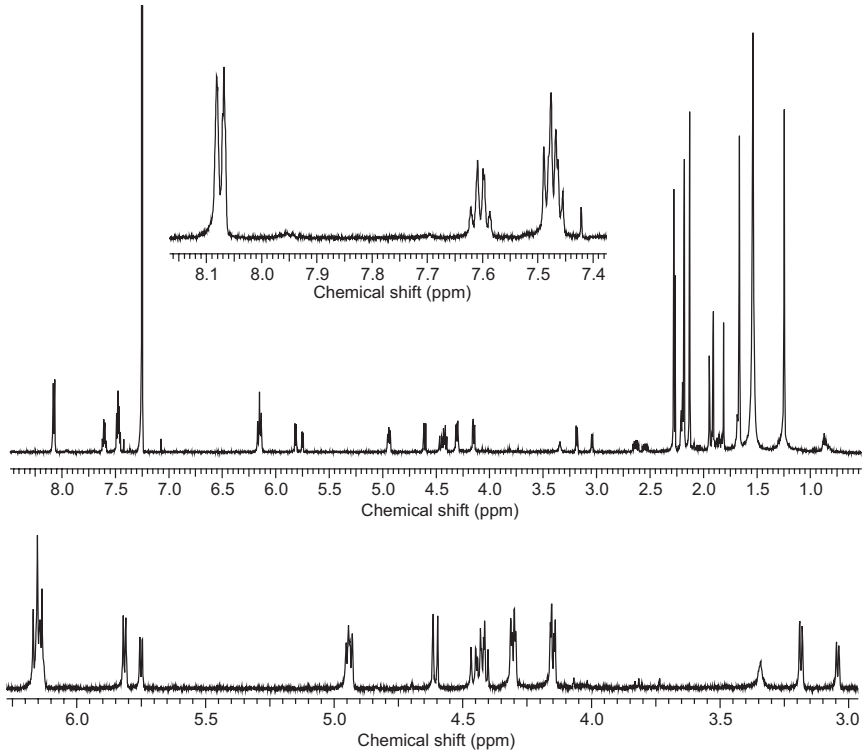

Figure 3 The ^{1}H NMR spectra of the mixture of **1** and 9-dihydro-13α-acetylbaccatin III (**2**).

epidioxy derivative of 9-dihydro-13α-acetylbaccatin III (**2**), though the HR-FABMS data of **1** showed the same molecular formula with **2**. Indeed, the ^{1}H NMR data of **1** closely resemble those of **2** (Table 2 and Figure 3) (Zamir et al., 1995) except that H-2 and H-3 show slight upfield shifts for **1**. As the original NMR data of **1** and **2** were recorded in $CDCl_3$ solution, in order to address whether C-7-OH and C-9-OH were involved in a peroxide ring, we re-run NMR data in acetone-d_6, and not C-7-OH and C-9-OH signals but the C-1-OH signal was observed for **1**. On the other hand, signals for all three free hydroxy groups were observed for **2**. This fact further substantiates our presumed structure. The relative stereochemistry of **1** was elucidated by the nuclear Overhauser effect correlated spectroscopy (NOESY) spectrum as shown in Figure 4, which is identical to that of **2**. This taxane, 4α,10β,13α-acetoxy-2α-benzoyloxy-7β,9α-epidioxy-5β,20-epoxytax-11-en-1β-ol (**1**), is the first example of a taxane peroxide.

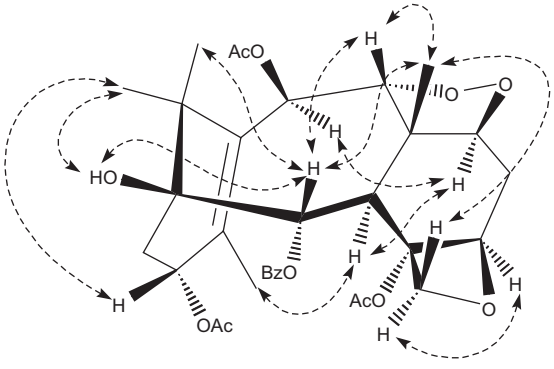

Figure 4 Relative stereochemistry of **1**. Dotted arrows denoted NOESY correlations.

Conclusion

4α,10β,13α-acetoxy-2α-benzoyloxy-7β,9α-epidioxy-5β,20-epoxytax-11-en-1β-ol (**1**), the first example of a taxane peroxide, was isolated from rooted cuttings of the Canadian yew, *Taxus canadensis*.

Experimental

General

Optical rotation: Jasco DIP-370; NMR: Bruker Avance-500; FAB-MS: Vacuum Generators ZAB-HS; flash chromatography: Silica Gel 60 (230–400 mesh, EM Science); preparative thin-layer chromatography (TLC): Silica Gel 60 F_{254} (20×20 cm×0.5 mm, EM Science); preparative HPLC: Waters Delta Prep 3000, UV 486 (210 nm), Whatman partisil 10 ODS-2 Mag-9 (9.4×250 mm).

Plant material

Rooted cuttings of *T. canadensis* Marsh. (Taxaceae) were obtained in September 2001 from Cramer Nurseries in Les Cèdres, Québec, Canada.

Extraction, isolation and characterization of 1

Air-dried rooting cuttings of *T. canadensis* were ground into a fine powder (584 g) which was then extracted with 3 l of MeOH by shaking at room temperature for 1 d in the dark. The solvent was removed and the plant material was extracted twice with MeOH and four times with a mixture of MeOH/CH_2Cl_2 (1:1). The combined organic extracts were concentrated to dryness and the residue was re-dissolved in water. Then the aqueous solution was extracted with hexane. The hexane fraction was partitioned three times with MeOH. The combined MeOH extracts were dried over anhydrous sodium sulphate, filtered and evaporated to dryness, yielding 2.0 g of a dark brown extract. The extract was re-dissolved in a minimum volume of CH_2Cl_2 and fractionated by column chromatography (180 g, bed size 4.0×33 cm). Twenty-three fractions (Fr_{1-23}) were collected after elution with successive mixtures of acetone in hexane. Fr_{1-5} to Fr_{1-7} were combined (60 mg) and applied to preparative TLC. Development with hexane/ethyl acetate (2:5) yielded crude taxane **1** (3.0 mg, R_f=0.30), which was further purified by preparative HPLC, eluted with a linear gradient of acetonitrile in water from 25% to 100% in 50 min at 3 ml/min. The material eluted at t_R=30.7 min was collected as a pure taxane **1**; amorphous solid; $[\alpha]_D^{22}$+27.5 (*c* 0.04, $CHCl_3$); for ^{1}H- and ^{13}C NMR spectral data in acetone-d_6, see Table 1; ^{1}H NMR in $CDCl_3$: δ 5.82 (1H, d, *J*=6.4 Hz, H-2), 3.19 (1H, d, *J*=6.4 Hz, H-3), 4.94 (1H, br.d, *J*=9.3 Hz, H-5), 2.63 (1H, ddd, *J*=15.2, 9.3, 7.4 Hz, H-6α), 1.85 (1H, ddd, *J*=15.1, 10.5, 1.5 Hz, H-6β), 4.42 (1H, dd, *J*=10.5, 7.4 Hz, H-7), 4.61 (1H, d, *J*=10.6 Hz, H-9), 6.15 (1H, d, *J*=10.6 Hz, H-10), 6.14 (1H, m, H-13), 2.20 (2H, d, *J*=8.4 Hz, H-14αβ), 1.25 (3H, s, Me-16), 1.67 (3H, s, Me-17), 1.91 (3H, d, *J*=1.3 Hz, Me-18), 1.67 (3H, s, Me-19), 4.31 (1H, d, *J*=8.4 Hz, H-20a), 4.15 (1H, d, *J*=8.4 Hz, 20b), 2.28 (3H, s, Ac), 2.18 (3H, s, Ac), 2.13 (3H, s, Ac), 8.08 (2H, d, *J*=8.3 Hz, Ph-*o*), 7.48 (2H, d, *J*=7.6 Hz, Ph-*m*), 7.61 (1H, t, *J*=7.5 Hz, Ph-*p*), 3.32 (1H, br.s, 1-OH); ^{13}C NMR in $CDCl_3$: δ 78.8 (C-1), 73.5 (C-2), 45.9 (C-3), 81.9 (C-4), 84.0 (C-5), 36.1 (C-6), 72.8 (C-7), 44.6 (C-8), 77.2 (C-9), 72.7 (C-10), 134.0 (C-12), 69.8 (C-13), 35.4 (C-14), 42.9 (C-15), 27.8 (C-16), 22.4 (C-17), 15.0 (C-18), 12.7 (C-19), 76.3 (C-20), 22.8 (CH_3CO), 21.2 (CH_3CO), 21.1 (CH_3CO), 169.3 (CH_3CO), 170.2 (CH_3CO), 170.3 (CH_3CO), 129.9-128.3 (Ph); HR-FABMS *m/z*: 669.2358 (calcd for $C_{33}H_{42}O_{12}K$, 669.2308), 653.2595 (calcd for $C_{33}H_{42}O_{12}Na$, 653.2569), 593.2375 (calcd for $[C_{33}H_{42}O_{12}Na–CH_3COOH]^+$, 593.2357), 533.2158 (calcd for $[C_{33}H_{42}O_{12}Na–2CH_3COOH]^+$, 533.2146), 473.1950 (calcd for $[C_{33}H_{42}O_{12}Na–3CH_3COOH]^+$, 473.1935), 411.1783 (calcd for $[C_{33}H_{42}O_{12}Na–2CH_3COOH–PhCOOH]^+$, 411.1778), 351.1574 (calcd for $[C_{33}H_{42}O_{12}Na–3CH_3COOH–PhCOOH]^+$, 351.1567).

Acknowledgements

Financial support: National Natural Science Foundation of China (81072551), Scientific Research Foundation for the Returned Overseas Chinese Scholars of Hebei Province (2006-02), Scientific Research Foundation of Hebei Province (08B032 and C2010000489), and Syngenta Ltd. (2008-Hebei Medical University-Syngenta-02).

References

Baloglu, E.; Kingston, D. G. I. The taxane diterpenoids. *J. Nat. Prod.* **1999**, *62*, 1448–1472.

Dong, M.; Zhang, M. L.; Shi, Q. W.; Gu, Y. C.; Kiyota, H. Chemical studies of *Taxus canadensis*. In *Natural Products: Chemistry, Biochemistry and Pharmacology*. Brahmachari, G., Ed. Narosa Publishing House: New Delhi, 2008; pp. 247–269.

Farina, V. The Chemistry and Pharmacology of Taxol and Its Derivatives; Elsevier: Amsterdam, 1995; Vol. 22.

Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. Taxane Anticancer Agents, Basic Science and Current Status; ACS Symposium Series: Washington DC, 1995; Vol. 583.

Kingston, D. G. I.; Molinero, A. A.; Rimoldi, J. M. The taxane diterpenods. In *Progress in the Chemistry of Organic Natural Products*. Herz, W.; Kirby, G. W.; Moore, R. E.; Steglich, W; Tamm, C. H., Eds. Springer: Wien, 1993; Vol. 61, pp. 1–206.

Nikolakakis, A.; Caron, G.; Cherestes, A.; Sauriol, F.; Mamer, O.; Zamir, L. O. New taxanes from the needles of *Taxus canadensis. Bioorgan. Med. Chem.* **2000**, *8*, 1269–1280.

Parmer, V. S.; Jha, A.; Bish, K. S.; Taneja, P.; Sight, S. K.; Kumar, A.; Poonam, J. R.; Olsen, C. E. Constituents of the yew trees. *Phytochemistry* **1999**, *50*, 1267–1304.

Shi, Q. W.; Kiyota, H. New natural taxane diterpenoids from *Taxus* species since 1999. *Chem. Biodivers.* **2005**, *2*, 1597–1623.

Zamir, L. O.; Nedea, M. E.; Belair, S.; Sauriol, F.; Mamer, O.; Jacqmain, E.; Jean, F. I.; Garneau, F. X. Taxanes isolated from *Taxus canadensis. Tetrahedron Lett.* **1992**, *33*, 5173–5176.

Zamir, L. O.; Nedea, M. E.; Zhou, Z. -H.; Belair, S.; Caron, G.; Jacqmain, E.; Jean, F. I.; Garneau, F. X.; Mamer, O. *Taxus canadensis* taxanes: structures and stereochemistry. *Can. J. Chem.* **1995**, *73*, 655–665.

Zhang, S.; Chen, W. M.; Chen, Y. H. Isolation and identification of two new taxane diterpenes from *Taxus chinensis* (Pilger) Rehd. *Yaoxue Xuebao (Acta Pharm. Sin.)* **1992**, *27*, 268–272 [*Chem. Abstr.* **1992**, *117*, 44581].

Received May 27, 2011; accepted June 14, 2011