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Taxane diterpenoids from *Taxus yunnanensis* and *Taxus cuspidata*

Qing-Wen Shi[a], Takayuki Oritani[a,*], Hiromasa Kiyota[a], Ding Zhao[b]

[a]*Laboratory of Applied Bioorganic Chemistry, Division of Life Science, Graduate School of Agricultural Sciences, Tohoku University, 1-1 Tsutsumidori-Amamiya, Aoba-ku, Sendai 981-8555, Japan*

[b]*Department of Chemistry of Medicinal Natural Products, Faculty of Pharmaceutical Sciences, Hebei Medical University, 361 Zhongshan East Street, Shijiazhuang 050017, People's Republic of China*

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Abstract

Chemical examination of the seeds of the Chinese yew, *Taxus yunnanensis* Cheng et L. K. Fu and the Japanese yew, *Taxus cuspidata* Sieb et Zucc, resulted in the isolation of four taxane diterpenoids. The structures of these taxoids were established as (12α)-2α-acetoxy-5α,9α,10β-trihydroxy-3,11-cyclotax-4(20)-en-13-one; 2α,7β,13α-triacetoxy-5α,9α-dihydroxy-2(3→20)*abeo*taxa-4(20),11-dien-10-one; 9α,10β-diacetoxy-5α-cinnamoyloxytaxa-4(20),11-dien-13α-ol and the known 2α,7β,9α,10β,13-pentaacetoxy-taxa-4(20),12-diene-5α,11β-diol on the basis of spectral analysis.

Keywords: *Taxus yunnanensis* Cheng et L. K. Fu; *Taxus cuspidata* Sieb et Zucc; Taxaceae; Seeds; Taxoids; Taxane diterpenoid

1. Introduction

The clinical application of Taxol® (paclitaxel) against ovarian, breast and other carcinomas has spurred a worldwide search for a new source of this drug. Although more than 300 taxane diterpenoids have been isolated from yew trees (Parmar et al., 1999), there are still great number of new taxoids being isolated (Kobayashi et al., 1998; Kobayashi and Shigemori, 1998; Chattopadhyay et al., 1999; Gabetta et al., 1996; Fukushima et al., 1999; Yang et al., 1999; Shigemori et al., 1999), some of which show interesting activities (Yang et al., 1999; Shigemori et al., 1999). Although they represent a biorenewable source, the seeds of yew trees have received little attention (Chattopadhyay et al., 1999; Shigemori et al., 1999; Wang et al., 1997; Appendino et al., 1993; Yoshizaki et al., 1986). In previous work, we isolated three taxoids from the seeds of the Chinese yew, *Taxus yunnanensis* Cheng et L. K. Fu and the Japanese yew *Taxus cuspidata* Sieb et Zucc (Shi et al., 1999). Our further investigation of the seeds of these plants resulted in the isolation of four taxoids: a 3,11-cyclotaxane (**1**), a rearranged 2(3 → 20)*abeo*taxane (**2**), and a normal taxane (**3**) were isolated from the seeds of the Chinese yew, and a known taxane with an enol acetate moiety in ring A (**4**) (Zamir et al., 1999) was isolated from the seeds of the Japanese yew. In this communication, we would like to describe the isolation and structure elucidation of the three new compounds.

2. Results and discussion

A methanolic extract of the seeds of *T. yunnanensis* Cheng et L. K. Fu was processed as described in Section 3 to afford three new taxane diterpenoids (**1**–**3**). Compound **1** was isolated as a colorless gum with a yield of 0.00013% from the dried seeds. The IR spectrum contained a hydroxyl band centered at 3400 cm^{-1} together with acetoxy and carbonyl system bands at 1710 and 1730 cm^{-1}, which were confirmed by the signals at δ 22.2, 170.5 and 212.6 in the ^{13}C NMR

* Corresponding author. Tel.: +81-22-717-8783; fax: +81-22-717-8783.

E-mail address: oritani@biochem.tohoku.ac.jp (T. Oritani).

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spectrum. EIMS gave the ion peak at m/z 392 (M^+). HR-EIMS analysis revealed the molecular formula to be $C_{22}H_{32}O_6$ (Δ +0.5 mmu). Its ^1H NMR spectral data, shown in Table 1, showed characteristic signals of taxoids, including signals for four methyl groups at δ 1.22, 1.25, 1.43, and 1.52. Chemical shifts of the characteristic proton resonances due to an exomethylene moiety appeared at δ 5.52 and 5.68 (each ^1H, *br.s*). The connectivity of the protons at the taxane skeleton of **1** was determined by analysis of the ^1H–^1H COSY spectrum. The ^1H NMR spectrum of **1** differed from the spectra of the normal taxanes by the following features: (1) the signal due to H-3α, usually appearing at δ 2.3–3.6 ppm with a coupling constant ca. 6 Hz, was absent; (2) one of the methyl groups appeared as a doublet at δ 1.43, which showed a coupling with a quartet signal at δ 3.71 (1H, *q*, $J = 7.1$ Hz) in the ^1H–^1H COSY spectrum. These spectral features indicate that **1** was an example of a 3,11-cyclotaxane. (Appendino, 1995), a minor group of taxoids. The correlation between H-2 and C-11 in the HMBC spectrum (Fig. 1) further supported this conclusion. Assignment of the protonated carbons was done on the basis of the 2D ^{13}C–^1H correlation spectrum, whereas for the quaternary carbon resonances, the long-range coupled C–H-correlation spectrum was used. The relative stereochemistry of **1** was elucidated by the NOESY spectrum as shown in Fig. 1.

Compound **2** was obtained as a colorless gum and showed a molecular ion at m/z 492 $(M)^+$ in the EIMS spectrum. HR-EIMS analysis revealed the molecular formula of **2** was $C_{26}H_{36}O_9$ (m/z 492.2361 $[M]^+$, Δ +0.2). Absorptions at 3430, 1730, and 1680 cm^{-1} in the IR spectrum implied that **2** possessed hydroxy, ester and ketone groups, respectively. The ^1H NMR spectrum (Table 1) showed characteristic signals due to a taxoid skeleton, including four methyl (δ 0.90, 1.15, 1.42 and 1.76), and three acetyl methyl (δ 2.03, 2.07 and 2.16) groups. Detailed analysis of the ^1H–^1H COSY spectrum revealed connectivities of H-14 to H-1, H-1 to H-2, H-2 to H-20, H-5 to H-6, H-6 to H-7, H-9 to H-10 and H-13 to H-14. The spin system derived from 18-CH_3, H-13β, H-14α and H-14β was readily interpreted. The signal of 3H-appearing as a

Table 1
^1H and ^{13}C NMR spectral data of **1** and **2** (300 MHz for ^1H and 125 MHz for ^{13}C, $CDCl_3$)

Position	**1**			**2**		
	δ^1H (ppm)	*J*	$\delta^{13}C$ (ppm)	δ^1H (ppm)	*J*	$\delta^{13}C$ (ppm)
1	2.06 *br.d*	6.9	48.7	1.87 *m*		46.7
2	6.02 *d*	5.5	77.7	5.79 *br.d*	9.3	71.5
3a			66.7	2.37 *br.d*	15.4	33.3
3b				2.25 *br.d*	15.4	
4			149.2			140.1
5	4.73 *br.t*	8.9	75.7	4.32 *dd*	9.6, 14.7	67.1
6a	1.92 *m*		28.5	2.33 *dd*	5.8, 9.6	36.5
6b	1.45 *m*			1.92 *m*		
7a	1.05 *m*		30.6	5.28 *br.s*		73.4
7b	1.80 *m*					
8			45.1			45.2
9	4.27 *d*	9.4	85.0	4.60 *br.s*		77.0
10	4.20 *d*	9.4	82.6			207.5
11			59.0			141.6
12	3.71 *q*	7.1	53.7			142.2
13	5.47 *br.t*	6.4	212.6	5.34 *br.d*	9.9	67.9
14α	2.60 *br.d*	19.8	39.8	1.95 *m*		26.8
14β	2.46 *dd*	6.9, 19.8		2.69 *ddd*	7.5, 9.9, 17.4	
15			42.8			35.8
16	1.52 *s*		29.6	1.42 *s*		24.7
17	1.25 *s*		27.6	1.15 *s*		32.9
18	1.43 *d*	7.1	17.3	1.76 *br.s*		19.4
19	1.22 *s*		27.0	0.90 *s*		16.0
20a	5.68 *br.s*		125.6	5.46 *br.d*	9.3	119.8
20b	5.52 *br.s*					
2-OAc	2.05 *s*		22.2	2.16 *s*		21.5
			170.5			170.0
7-OAc				2.03 *s*		21.1
						170.0
13-OAc	1.99 *s*			2.07 *s*		21.1
						170.4

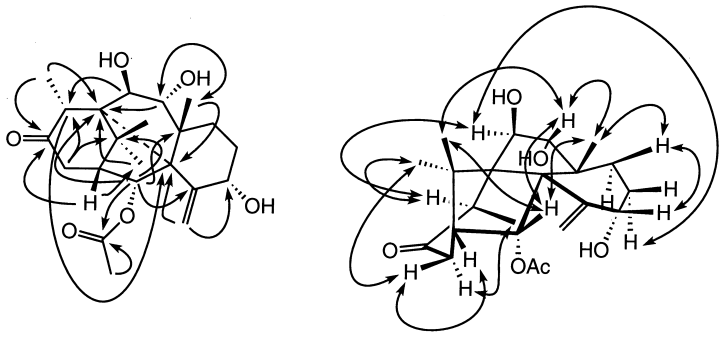

Fig. 1. ^{1}H–^{13}C long-range correlations observed in the HMBC (left); and relative stereochemistry of **1**, based on the NOESY analysis (500 MHz). Most protons are omitted for clarity.

broad singlet at δ 1.76 was assigned to 18-CH_3 based on its long-range coupling with H-13β; the broad doublet at δ 5.34 was assigned to H-13β; the multiplet at δ 1.95 and doublet of doublets of doublets δ 2.69 were assigned to the C-14 methylene protons, H-14α and H-14β, respectively, based on their geminal coupling and coupling to H-13β. The multiplet at δ 1.87, which correlated with H-14β in the ^{1}H–^{1}H COSY spectrum, was assigned to H-1β. The signal at δ 5.79, which correlated with H-1β in the ^{1}H–^{1}H COSY spectrum, was attributed to H-2β. The proton H-2β coupled with the signal at δ 5.46 (1H, *br.d*, $J = 9.3$ Hz) instead of the signals at δ 2.8–4.0 in the ^{1}H NMR spectrum is characteristic of proton of H-3α in most taxoids (Appendino, 1995; Kingston et al., 1993; Zhou and Fang, 1997). In the ^{1}H NMR spectrum, compound **2** did not show characteristic signals corresponding to an exocyclic methylene, oxetane ring or terminal methylene; however, an isolated spin system of broad doublets at δ 2.25 and 2.37 with the coupling constant $J = 15.4$ Hz was observed. These data, along with long-range correlations between H-3 and H-20, H-5 and H-20, clearly indicated that **2** had a rearranged 2(3 → 20)*abeo*taxane skeleton (Appendino, 1995), which was confirmed by HMBC and HMQC spectra. The ^{1}H and ^{13}C data of compound **2** were fully assigned on the basis of ^{1}H–^{1}H COSY, HMBC (Fig. 2) and HMQC spectra. Based on the coupling constants and NOESY spectra (Fig. 2), the protons at 2, 5, 7, 9 and 13 were assigned to β, β, α, β and β, respectively, having the same configurations as found in most natural taxoids. The relative stereochemistry of compound **2** was elucidated by means of an NOESY experiment as shown.

The structure of compound **2** is closely related to taxuspine W (Hosoyama et al., 1996; Yue et al., 1995), but its ^{1}H and ^{13}C NMR spectra showed some differences. Taxuspine W showed 10-OH signal at δ 4.20 in its ^{1}H NMR spectrum, whereas in compound **2** this signal was absent. In compound **2**, the signal of a 10-keto group resonated at δ 207.5 due to it conjugation with the C-11,12 double bond; in taxuspine W, the signal of the 9-keto appeared at δ 213.1.

Compound **3** was isolated as a colorless gum in a yield of 0.00021%. The IR spectrum of **3** exhibited typical bands at 3500 (OH), 1730 (C=O), and 1640 (C=C) cm^{-1}. HR-FABMS revealed the molecular formula to be $C_{33}H_{42}O_7$. The ^{1}H NMR spectrum had well-dispersed signals suggestive of a taxane derivative containing two acetate groups (δ 2.01 and 2.05, each 3H, *s*) and one cinnamate group (δ 6.70, ^{1}H, *d*, $J = 15.9$ Hz; δ 7.72, ^{1}H, *d*, $J = 15.9$ Hz; δ 7.39, 3H, *m*; δ 7.55, 2H, *m*). The connectivity of the protons at the taxane skeleton of **3** was determined by an analysis of the ^{1}H–^{1}H COSY spectrum. Interpretation of the ^{1}H, ^{13}C NMR and HMBC spectra permitted

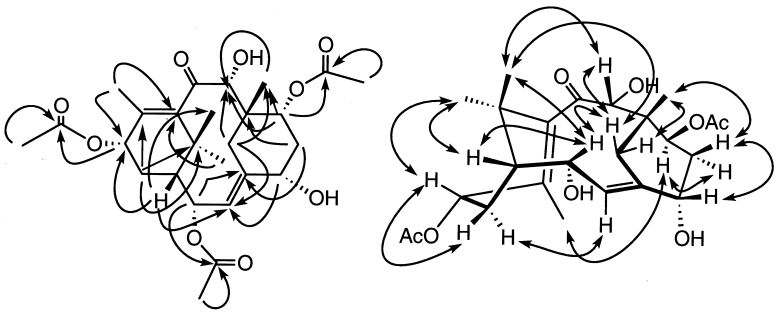

Fig. 2. ^{1}H–^{13}C long-range correlations observed in the HMBC (left), and relative stereochemistry of **2**, based on the NOESY analysis (500 MHz).

the positional assignment of functional groups. Long-range correlations of H-10 to C-15, H-16 and H-17 to C-11 and H-3 to C-1, C-9, C-19 and C-20 in the HMBC spectrum indicated that **3** possessed a 6/8/6-membered ring system. The isolated spin system comprising doublets at δ 5.81 and 6.07 were attributed to H-9β and 10α, respectively, with a large vicinal coupling ($J = 10.4$ Hz) indicating a *trans*-oriented configuration. ^{1}H NMR signals at δ 4.91 (1H, *br.s*), 5.26 (1H, *br.s*), and 3.16 (1H, *br.d*, $J = 5.7$ Hz) were characteristic of an exocyclic methylene and the C-3 ring junction proton in a taxa-4(20),11-diene (Appendino, 1995; Zhou and Fang, 1997), respectively. The structure of **3** was, therefore, assigned as 9α,10β-diacetoxy-5α-cinnamoyloxytaxa-4(20),11-dien-13α-ol. The relative stereochemistry of **3** was deduced from the NOESY experiment and the coupling constants. The presence of a cinnamate group at the α-position of C-5 was shown by the cross peaks between H-2′ and 18-CH_3 and between H-5 and H-20.

Compound **4** was identified on the basis of extensive analysis of spectral data to be 2α,7β,9α,10β,13-pentaacetoxy-taxa-4(20),12-diene-5α,11β-diol, previously isolated from *T. canadensis* (Zamir et al., 1999). Taxane diterpenoids of this type, exhibiting an enol acetate moiety in ring A, have been previously reported as natural (Shigemori et al., 1999; Kobayashi et al., 1996a, 1996b) and as synthetic (Johnson et al., 1997; Kelly et al., 1996) products.

Compound **1** is a new 12α-methyl member of a smaller group of 3,11-cyclotaxanes, wherein the saturated C-11,12 double bond makes the 18-CH_3 shift upfield and become a doublet. At the same time, the H-12 resonance at ca. δ 3.7 appears as a quartet, and the H-9β resonance appears further downfield than that of H-10α. Compound **2** was a rare rearranged 2(3 → 20)*abeo*taxane with a 9α-OH and 10-one moiety; this is the first report of such a compound. Compound **4** is a taxane with an enol acetate moiety in ring A; in this kind of taxoid, H-9β and H-10α have a relatively smaller coupling constant (ca. 4.9 Hz).

3. Experimental

3.1. General

Optical rotations were recorded on a Horiba SEPA-300 digital polarimeter. IR spectra were obtained on a Jasco IR-810 instrument. MS were measured on a Jeol JMS-700 spectrometer using EI and FAB modes. ^{1}H and ^{13}C NMR spectra were obtained with a Varian Unity Inova 500 and Varian GEMINI 2000/300 spectrometers operating at 500 and 300 MHz for ^{1}H, 125 MHz for ^{13}C with $CDCl_3$ as solvent at 20°C. Chemical shifts of the taxanes are reported in δ (ppm) using TMS as an internal standard. Open column chromatography was performed using Merck silica gel 60 (100–200 mesh). Thin layer chromatography was carried out with the precoated Merck silica gel 60 F_{254} plates. Preparative TLC was performed using the same type of plates but with 0.85 mm thickness (dried for 24 h at room temperature and activated for 4 h at 120°C). The spots were detected under UV (254 nm) and/or by spraying with 10% sulfuric acid and then heating on a hot plate.

3.2. Plant material

The seeds of *T. yunnanensis* were collected in Congteng country, Yunnan Province, in the southwest of China, in October 1995. The botanical identification was made by Prof. J.H. Wang, School of Pharmaceutical Science, Hebei Medical University, People's Republic of China. The seeds of *T. cuspidata* Sieb et Zucc were collected in Toyama Prefecture of Japan, in October 1998. The botanical identification was made by Prof. Takashi Oritani (Toyama Prefectural University). The voucher specimen have been deposited in our laboratory at Graduate School of Agricultural Science, Tohoku University, Japan. The plant material was stored at 5°C.

3.3. Extraction and isolation

Air dried seeds (2.2 kg) of *T. yunnanensis* Cheng et L. K. Fu were crushed and extracted with hexane three times at room temperature to remove the major part of the neutral solubles which were not investigated further. The resulting residue was extracted three times with methanol (MeOH); the combined MeOH extracts were evaporated in vacuo to afford a residue (135 g). This residue was diluted with water and extracted five times with EtOAc (85 g). The combined EtOAc layer was further extracted with 5% HCl. After

neutralization, the aqueous layer was extracted three times with EtOAc. The combined EtOAc extract, upon evaporation, yielded 8.8 g of a yellowish syrup, which was subjected to column chromatography (CC), eluted with hexane–ethyl acetate (2:1, 1:1, 1:2, 1:4). Twelve fractions were obtained and each was evaporated to dryness in vacuo. Fractions 5 (900 mg), 6 (750 mg) and 7 (450 mg) were further separated by preparative thin layer chromatography (TLC) repeatedly with different developing solvent ($CHCl_3$–MeOH, hexane–EtOAc, hexane–acetone), and finally compounds **1** (3 mg), **2** (2 mg) and **3** (4.8 mg) were separated in pure form.

Air dried and crushed seeds (0.9 kg) of *T. cuspidata* Sieb et Zucc were extracted with hexane three times at room temperature to remove most of the undesired neutral components. The remaining plant material was extracted twice with methanol (MeOH), and the MeOH extracts were pooled, condensed and partitioned between EtOAc and water. The EtOAc layer, after being condensed to a residue (25 g) under reduced pressure, was adsorbed on 30 g of silica gel and subjected to silica gel (300 g) column chromatography. The column was eluted with hexane–acetone (4:1, 3:1, 2:1, 1:1 and 1:2, each 2000 ml) into 11 fractions. Fraction 9 (1550 mg) was repeatedly separated and purified by means of preparative TLC of silica gel with hexane–acetone (3:2), hexane–EtOAc (3:5), and $CHCl_3$–MeOH (100:5) as the solvent systems, which finally afforded compound **4** (4 mg) in pure form.

*3.4. (12α)-2α-Acetoxy-5α,9α,10β-trihydroxy-3,11-cyclotax-4(20)-en-13-one (**1**)*

Gum, $[\alpha]_D^{24}$ -17° ($CHCl_3$, *c* 0.01); IR (film, $CHCl_3$) ν_{max}: 3400, 2930, 1730, 1710, 1370, 1240, 1050, 910 and 730 cm^{-1}; ^1H and ^{13}C NMR spectral data: see Table 1; EIMS: *m/z*: 392 ($[M]^+$), 332 ($[M - AcOH]^+$), 256 and 43; HR-EIMS: 392.2204 (calculated for $C_{22}H_{32}O_6$, 392.2197).

*3.5. 2α,7β,13α-Triacetoxy,5α,9α-dihydroxy-2(3 → 20)abeotaxa-4(20),11-dien-10-one (**2**)*

Colorless gum, $[\alpha]_D^{24}$ -13° ($CHCl_3$, *c* 0.01); IR ν_{max} ($CHCl_3$, film): 3430, 3010, 2920, 1730, 1680, 1370, 1240, 1130 and 760 cm^{-1}; ^1H and ^{13}C NMR spectral data: see Table 1; EIMS *m/z*: 492 ($[M]^+$), 432 ($[M - AcOH]^+$), 372 ($[M - 2AcOH]^+$), 312 ($[M - 3AcOH]^+$), 284 ($[M - 3AcOH–H_2O]^+$), 283, 121, 105 and 43; HR-EIMS *m/z*: 492.2361 (calculated for $C_{26}H_{36}O_9$, 492.2357).

*3.6. 9α,10β-Diacetoxy-5α-cinnamoyloxytaxa-4(20),11-dien-13αol (**3**)*

Colorless gum, $[\alpha]_D^{24}$ -13° ($CHCl_3$, *c* 0.03); IR ν_{max} ($CHCl_3$, film): 3500, 3010, 2950, 2930, 1730, 1700, 1640, 1580, 1450, 1370, 1250, 1110 and 750 cm^{-1}; ^1H NMR spectral data (300 MHz, $CDCl_3$): 1.78 (1H, *m*, H-1), 1.80 (2H, *m*, H-2), 3.16 (1H, *br.d*, $J = 5.7$ Hz, H-3), 5.48 (1H, *br.s*, H-5), 1.99 (1H, *m*, H-6a), 1.78 (1H, *m*, H-6b), 1.80 (1H, *m*, H-7a), 1.73 (1H, *m*, H-7b), 5.81 (1H, *d*, $J = 10.4$ Hz, H-9), 6.07 (1H, *d*, $J = 10.4$ Hz, H-10), 4.50 (1H, *m*, H-13), 1.15 (1H, *dd*, $J = 5.5$, 15.1 Hz, H-14α), 2.85 (1H, *m*, H-14β), 1.25 (3H, *s*, 16-CH_3), 0.97 (3H, *s*, 17-CH_3), 2.30 (3H, *s*, 18-CH_3), 0.76 (3H, *s*, 19-CH_3), 5.26 (1H, *br.s*, H-20a), 4.91 (1H, *br.s*, H-20b), 2.05 (3H, *s*, 9-$COCH_3$), 2.01 (3H, *s*, 10-$OCOCH_3$), 6.70 (1H, *d*, $J = 15.9$ Hz, H-2′), 7.72 (1H, *d*, $J = 15.9$ Hz, H-3′), 7.55 (2H, *m*, H-5′), 7.39 (3H, *m*, H-6′,7′); ^{13}C NMR spectral data (125 MHz, $CDCl_3$): 40.6 (C-1), 28.5 (C-2), 39.2 (C-3), 149.2 (C-4), 77.6 (C-5), 28.1 (C-6), 30.4 (C-7), 43.8 (C-8), 78.2 (C-9), 73.9 (C-10), 135.1 (C-11), 142.0 (C-12), 68.9 (C-13), 37.4 (C-14), 39.5 (C-15), 27.1 (C-16), 32.7 (C-17), 16.8 (C-18), 18.2 (C-19), 114.9 (C-20), 21.9, 171.2 (9-OAc), 21.6, 170.8 (10-OAc), 167.1 (C-1′), 118.7 (C-2′), 146.3 (C-3′), 135.2 (C-4′), 129.7 (C-5′), 128.9 (C-6′), 131.0 (C-7′); NOESY correlations ($CDCl_3$, H/H): 19/9, 17/7, 17/13, 18/10, 18/2′, 20/5; FABMS *m/z*: 589 ($[M + K]^+$), 561, 547, 529, 517, 429, 333, 205 and 43; HR-FABMS *m/z*: 589.2562 (calculated for $C_{33}H_{42}O_7K$, 589.2565).

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References

Appendino, G., 1995. The structural elucidation of taxoids. In: Farina, V. (Ed.), The Chemistry and Pharmacology of Taxol and its Derivative. Pharmacochemistry library, vol. 22. Elsevier, Amsterdam, pp. 55–102 (Chapter 2).

Appendino, G., Tagliapietra, S., Ozen, H.C., Gariboldi, P., Gabetta, B., Bombardelli, E., 1993. Taxanes from the seeds of *Taxus baccata*. J. Nat. Prod. 56, 514–520.

Chattopadhyay, S.K., Tripathi, V., Sharma, R.P., Shawl, A.S., Joshi,

B.S., Roy, R., 1999. A brevifoliol analogue from the Himalayan yew *Taxus wallichiana*. Phytochemistry 50, 131–133.

Fukushima, M., Takeda, J., Fukamiya, N., Okano, M., Tagahara, K., Zhang, S.X., Zhang, D.C., Lee, K.H., 1999. A new taxoid, 19-acetoxytaxagifine, from *Taxus chinesis*. J. Nat. Prod. 62, 140–142.

Gabetta, B., Fuzzati, N., Orsini, P., Peterlongo, F., Appendino, G., Velde, V., 1996. Paclitaxel analogues from *Taxus* x media cv. Hicksii. J. Nat. Prod. 62, 219–223.

Hosoyama, H., Inubushi, A., Katsui, T., Shigemori, H., Kobayashi, J., 1996. Taxuspines U, V, and W, new taxane and related diterpenoids from the Japanese yew *Taxus cuspidata*. Tetrahedron 52, 13145–13150.

Johnson, R.A., Dobrowolski, P.J., Nidy, E.G., Gebhard, I., Qualls, S.J., Wicniski, N.A., Kelly, R.C., Pitts, T.W., Lopes, N.M., Dekoning, T.F., Speaker, S.J., Weed, S.D., McGovren, J.P., Chidester, C.G., 1997. 12,13-Isotaxanes — synthesis of new potent analogs and X-ray crystallographic confirmation of structure. J. Med. Chem. 40, 2810–2812.

Kelly, R.C., Wicnienski, N.A., Gebhard, I., Qualls, S.J., Han, F., Dobrowolski, P.J., Nidy, E.G., Johnson, R.A., 1996. 12,13-Isobaccatin III. Taxane enol esters (12,13-isotaxanes). J. Am. Chem. Soc. 118, 919–920.

Kingston, D.G.I., Molinero, A.A., Rimoldi, J.M., 1993. The taxane diterpenoids. In: Herz, W., Kirby, G.W., Moore, R.E., Steglich, W., Tamm, C.H. (Eds.), Progress in the Chemistry of Organic Natural Products, vol. 61. Springer, New York, pp. 1–206.

Kobayashi, J., Hosoyama, H., Katsui, T., Yoshida, N., Shigemori, H., 1996a. Taxuspines N, O, and P, new taxoids from Japanese yew *Taxus cuspidata*. Tetrahedron 52, 5391–5396.

Kobayashi, J., Hosoyama, H., Shigemori, H., Koiso, Y., Iwasaki, S., 1996b. Taxuspine D, a new taxane diterpene from *Taxus cuspidata* with potent inhibitory activity against Ca^{2+}-induced depolymerization of microtubules. Experientia 51, 592–595.

Kobayashi, J., Hosoyama, H., Wang, X.X., Shigemori, H., Sudo, Y., Tsuruo, T., 1998. Modulation of multidrug resistance by taxuspine C and other taxoids from Japanese yew. Bioorg. Med. Chem. Lett. 8, 1555–1558.

Kobayashi, J., Shigemori, H., 1998. Bioactive taxoids from Japanese yew *Taxus cuspidata* and taxol biosynthesis. Heterocycles 47, 1111–1133.

Parmar, V.S., Jha, A., Bisht, K.S., Taneja, P., Singh, S.K., Kumar, A., Rajne Jain, D., Olsen, C.E., 1999. Constituents of the yew trees. Phytochemistry 50, 1267–1304.

Shigemori, H., Sakurai, C.A., Hosoyama, H., Kobayashi, A., Kajiyama, S., Kobayashi, J., 1999. Taxezopidines J, K, and L, new taxoid from *Taxus cuspidata* inhibiting Ca^{2+}-induced depolymerization of microtubules. Tetrahedron 55, 2553–2558.

Shi, Q.W., Oritani, T., Sugiyama, T., Zhao, D., Murakami, R., Wei, H.Q., 1999. Six new taxane diterpenoids from the seeds of *Taxus chinensis* var. *mairei* and *Taxus yunnanensis*. J. Nat. Prod. 62, 1114–1118.

Wang, X., Shigemori, H., Kobayashi, J., 1997. Taxezopidine A, a novel taxoid from seeds of Japanese yew *Taxus cuspidata*. Tetrahedron Lett. 38, 7587–7588.

Yang, S.J., Fang, J.M., Cheng, Y.S., 1999. *Abeo*-taxanes from *Taxus mairei*. Phytochemistry 50, 127–130.

Yoshizaki, F., Madarame, M., Takahashi, C., Hisamichi, S., 1986. Principal constituents of the seeds of Japanese yew (*Taxus cuspidata*). Shoyakugaku Zashi 40, 429–433.

Yue, Q., Fang, Q.C., Liang, X.T., He, C.H., 1995. Taxayuntin E and F: two taxanes from leaves and stems of *Taxus yunnanensis*. Phytochemistry 39, 871–873.

Zamir, L.O., Zhang, J., Wu, J., Sauriol, F., Mamer, O., 1999. Five novel taxanes from *Taxus canadensis*. J Nat. Prod. 62, 1268–1273.

Zhou, J.Y., Fang, Q.C., 1997. The ^{1}H-NMR features of natural taxane diterpenoid compounds. Acta Botanica Sinica 39, 467–476.