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Diterpenes and sesquiterpenes from the bark of *Taxus yunnanensis*

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Abstract

Two taxane-type diterpenes, 10β-acetoxy-2α,5α,7β,9α-tetrahydroxytaxa-4(20),11-dien-13-one and 2α-acetoxy-9α-benzoyloxy-5α,7β,10β,15-tetrahydroxy-11(15→1)- *abeo*taxa-4(20),11-dien-13-one, and two new drimane-type sesquiterpenes, 1β-acetoxy-7-drimen-11α-ol-12,11-lactone and 1β-acetoxy-11,12-epoxy-6-drimen-8α,11α-diol, were isolated from the bark of *Taxus yunnanensis* together with 35 known taxane-type diterpenes, a known drimane-type sesquiterpene and a known flavanone.

Keywords: *Taxus yunnanensis*; Taxaceae; Hongdoushan; Taxane-type diterpene; Drimane-type sesquiterpene

1. Introduction

The discovery of paclitaxel (Taxol®) as a potent anticancer drug from *Taxus brevifolia* has encouraged several groups all over the world to conduct research work on other *Taxus* species, in order to isolate potentially more effective paclitaxel derivatives for the treatment of various cancers or as starting materials for semi-synthesis (Baloglu and Kingston, 1999; Eisenhauer and Vermorken, 1998). As a consequence, more than 350 taxane-type diterpenes have been isolated from various *Taxus* plants, and some of them were found to possess interesting anticancer activity (Baloglu and Kingston, 1999). *Taxus yunnanensis* Cheng et L.K. Fu (Taxaceae), an evergreen tree commonly known as "Yunnan Hongdoushan" and distributed mainly in Yunnan Province of People's Republic of China (Delectis florae republicae popularis sinicae agendae academiae sinicae, 1978), is considered as a promising source of taxane-type and rearranged taxane-type diterpenes (Baloglu and Kingston, 1999). We also examined the constituents of the wood of *T. yunnanensis* and reported three new C-14 oxygenated taxane-type diterpenes, namely hongdoushans A–C (Banskota et al., 2002). Recently, we have examined constituents of the bark of the plant and isolated two new taxane-type diterpenes (**1** and **2**) and two new drimane-type sesquiterpenes (**3** and **4**), together with 35 known taxane-type diterpenes, a known drimane-type sesquiterpene and a known flavanone (Scheme 1).

2. Results and discussion

The air-dried bark of *T. yunnanensis* was extracted with refluxing MeOH to yield a MeOH extract, which was partitioned into CH_2Cl_2-soluble and CH_2Cl_2-insoluble fractions. After a series of column chromatography steps over silica gel and reversed-phase silica gel, followed by preparative TLC, the CH_2Cl_2-soluble fraction afforded four new compounds **1**–**4** together with 37 known compounds.

Compound **1** was isolated as a colorless amorphous solid with $[\alpha]_D^{25}$ +22.2° ($CHCl_3$; *c* 0.16). The molecular formula of **1** was determined to be $C_{22}H_{32}O_7$ by HRFABMS. The IR spectrum of **1** showed the absorption at 3400 and 1730 cm^{-1} corresponding to the hydroxyl and carbonyl groups, respectively. The ^1H NMR spectrum of **1** displayed signals of an acetyl methyl (δ 2.11), four tertiary methyls (δ 2.14, 1.60, 1.15, 1.11), five oxymethines (δ 6.11, 4.28, 4.23, 4.20, 4.04), an exo-olefin (δ 5.46, 5.13), two methines (δ 2.23, 3.18) and

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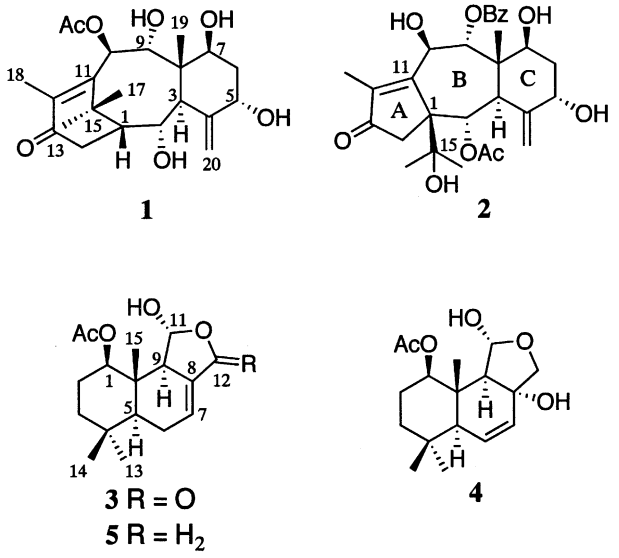

Scheme 1.

two methylenes (δ 2.70, 2.37; δ 1.88, 1.57). Beside the acetyl carbons (δ 172.1, 21.2), 20 carbon signals, including a ketone carbonyl carbon (δ 202.7) and four olefinic carbons (δ 152.7, 148.2, 138.2, 116.3), were observed in the ^{13}C NMR spectrum of **1**. These and other ^{1}H and ^{13}C NMR signals were found to be similar to those of taxuspine F (Kobayashi et al., 1995b), a known taxane isolated from the same extract, but those of **1** showed the presence of only one acetyl group instead of four in taxuspine F. Thus, **1** was considered to be a monoacetylated taxane derivative having an α,β-unsaturated ketone group at C-13 and an exo-olefin at C-4(20). This was further confirmed by the long-range correlations of H-14 and H_3-18 with the ketone carbonyl carbon (C-13) and of H_2-20 with C-3 and C-5 in the HMBC spectrum (Table 1). The location of the acetyl group was determined to be at C-10 based on the HMBC correlation between H-10 and the acetyl carbonyl carbon. Through the exhaustive analysis of the COSY, HMQC and HMBC spectra, the remaining oxymethine protons were assigned as H-2, H-5, H-7 and H-9. Accordingly, the planar structure of **1** was determined to be 10-acetoxy-2,5,7,9-tetrahydroxytaxa-4(20),11-dien-13-one. The relative stereochemistry was determined on the basis of coupling constants and NOEs observed in difference NOE experiments. Irradiation of the methyl protons at δ 1.60 (H_3-17) or at δ 1.15 (H_3-19) caused an NOE increase of H-2, and vice versa, indicating that H-2 should be β-oriented. The NOEs between H-3 and H-7 and between H-7 and H-10 revealed that H-3, H-7 and H-10 were α-oriented. Then, the coupling constants for H-7 (11.7 and 5.1 Hz) and H-5 (3.2 Hz) suggested that ring C has a chair conformation and H-5 is equatorial, i.e., β-oriented. The large coupling constant (10.0 Hz) between H-10 and H-9 together with NOE between H-7 and H-10 indicated the acetoxyl group at C-10 and the hydroxyl group at C-9 to be β- and α-oriented, respectively (Kobayashi et al., 1995b). From these data, compound **1** was determined to be 10β-acetoxy-2α,5α,7β,9α-tetrahydroxytaxa-4(20),11-dien-13-one.

Compound **2** was also isolated as a colorless amorphous solid with $[\alpha]_D^{25}$ +31.6° ($CHCl_3$; *c* 0.32) and the molecular formula $C_{29}H_{36}O_9$. The IR spectrum of **2** showed the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1700 cm^{-1}) groups. The ^{1}H NMR spectrum of **2** closely resembled that of **1** and showed an acetyl methyl, four quaternary methyls, five oxymethines, an exo-olefine, a methine and two methylenes (Table 1), but it also showed signals for a benzoyl group. The ^{13}C NMR signals of a ketone carbonyl carbon at δ 209.5 and four olefinic carbons at δ 167.7, 149.0, 145.4 and 113.9 suggested that **2** also has an α,β-unsaturated carbonyl group (C-11, C12, C13) and a C-4(20) olefin similar to those of **1**. However, the HMBC correlations of H_2-14 with C-11, C-12, C-13, C-15 and C-1 suggested that **2** should have the 11(15→1)-*abeo*taxane skeleton. This was further confirmed by the presence of an oxygen-substituted carbon at δ 76.0 (C-15) and a highly deshielded quaternary carbon at δ 64.3 (C-1) similar to those of taxacustone (Tong et al., 1995). The HMBC correlation between H-2 and the acetyl carbonyl carbon at δ 171.8 indicated the acetyl group to be located at C-2. The benzoyl and hydroxyl groups were located at C-9 and at C-10, respectively, by comparing the chemical shifts for H-9 (δ 5.40) and H-10 (δ 5.34) in $CDCl_3$ with those of 2α,7β,13α-triacetoxy-9β-benzoyloxy-11(15→1)-*abeo*taxa-4(20),11-dien-5α,10β,15-triol having a benzoyl group at C-9 (H-9: δ 5.22; H-10: δ 4.94) (Shi et al., 1998b) and taxuyunnanine U having a benzoyl group at C-10 (H-9: δ 4.46; H-10: δ 6.33) (Li et al., 2002). Most of the isolated 11(15→1)-*abeo*taxane-type diterpenes were reported to be in one of two conformations, i.e., boat/chair (type A) or chair/boat (type B) conformation with respect to rings B and C, or in equilibrium between these two conformers (Fuji et al., 1995). The ROESY correlations H_3-19/ H-2 and H_3-16/ H-2 suggested that the 2-hydroxy-2-propyl group at C-1, H_3-19 and H-2 were β-oriented (Fig. 1a). Similarly, the ROESY correlation H-3/H-7 revealed these protons to be *cis* and α-oriented. On the other hand, the small coupling constant between H-9 and H-10 (3.2 Hz in $CDCl_3$) similar to that of taxchinin G (3.7 Hz), an abeotaxane-type diterpene with similar functionality (Fuji et al., 1995), and the large coupling constant for H-5 (7.1 Hz) suggested that **2** has a type B conformation, i.e., a B-ring chair and a C-ring boat, with α-oriented H-3, H-7 and H-10 and β-oriented H-5. Thus, compound **2** was determined as 2α-acetoxy-9α-benzoyloxy-5α,7β,10β,15-tetrahydroxy-11(15→1)-*abeo*taxa-4(20),11-dien-13-one.

Compound **3** was isolated as colorless amorphous solid with $[\alpha]_D^{25}$ −46.4° ($CHCl_3$; *c* 0.65), and its mole-

cular formula was determined to be $C_{17}H_{24}O_5$ by HRFABMS. The IR spectrum of **3** showed the presence of hydroxyl (3350 cm^{-1}) and ester carbonyl (1740 cm^{-1}) groups. The ^1H NMR spectrum of **3** displayed the signals due to an acetyl methyl (δ 2.07), three quaternary methyls (δ 0.95, 0.94, 0.93), two oxymethines (δ 4.66, 5.72), a trisubstituted olefin (δ 6.86) and two methines (δ 2.58, 1.44) (Table 2). The ^{13}C NMR spectrum of **3** showed 17 signals including those for an acetyl group, suggesting that **3** is a sesquiterpene with an acetyl group. These ^1H and ^{13}C NMR data resembled those of 1β-acetoxyisodrimeninol (**5**) (Kiyota et al., 2002), a drimane-type sesquiterpene isolated from the same extract. However, presence of a signal due to an ester carbonyl carbon at δ 167.9 instead of that of a methylene carbon (C-12) in **5** suggested the presence of C-12,11-γ-lactone functionality in **3**. Thus, compound **3** was determined to be 1β-acetoxy-7-drimen-11α-ol-12,11-lactone, which was confirmed by the COSY, HMQC and HMBC spectra, together with the difference NOE experiments.

Compound **4** showed ^1H and ^{13}C NMR spectra similar to those of **3** and **5**, but they were characterized by the presence of a disubstituted olefin (δ_H 5.81, 2H; δ_C 130.0, 126.8) and an oxygen-substituted quaternary carbon (δ_C 79.0) instead of the trisubstituted olefin and a methylene in **5**. The HMBC correlations between the oxygen-substituted quaternary carbon with the olefinic protons (H-6, H-7), H_2-12 and H-9 suggested that the olefinic group of **4** should be located at C-6 instead of C-7 in **5** and that a hydroxyl group is at C-8. The large coupling constant for H-1 (11.2 Hz) suggested that it should be α-axial-oriented, while the NOEs from H-5 to H-1 and H-9 and from H-1 to H-9 in difference NOE experiment indicated α-orientation of H-1, H-5 and H-9 in chair/chair conformation of rings A and B. Inspection of a Dreiding stereomodel together with the NOE enhancements from H_3-15 to H-11 and H-12 (Fig. 1b) indicated the hydroxyl groups at C-8 and C-11 to be α-oriented. Thus, compound **4** was determined to be 1β-acetoxy-11,12-epoxy-6-drimen-8α,11α-diol.

Table 1
^1H and ^{13}C NMR data for taxane diterpenes **1** and **2**[a]

	1			**2**			
	δ_H	δ_C	HMBC[b]	δ_H	δ_H[c]	δ_C	HMBC[b]
1	2.23 *br d* (6.5)	52.8	3, 14, 16, 17			64.3	2, 3, 10, 14, 16, 17
2	4.20 *dd* (5.4, 1.0)	69.0	1, 3, 14	6.04 *d* (9.3)	6.07 *d* (9.0)	70.0	3
3	3.18 *d* (5.4)	41.9	1, 19, 20	2.81 *d* (9.3)	2.82 *d* (9.0)	47.6	19, 20
4		148.2	3			149.0	3
5	4.04 *t* (3.2)	76.0	3, 20	4.50 *dd* (9.5, 7.6)	4.66 *t* (7.1)	66.9	3, 20
6	1.88 *m*; 1.57 *m*	40.9		1.99 *m*; 1.95 *m*	2.14 *m*; 1.73 *m*	40.0	
7	4.28 *dd* (11.7, 5.1)	72.1	5, 6, 9, 19	3.61 *dd* (10.0, 8.1)	3.64 *t* (8.8)	69.6	19
8		48.6	2, 3, 7, 9, 19			46.1	2, 3, 9, 19
9	4.23 *d* (10.0)	78.9	10, 19	5.26 *br s*	5.40 *d* (3.2)	78.7	3, 10, 19
10	6.11 *d* (10.0)	76.8	9	5.26 *br s*	5.34 *d* (3.2)	70.0	
11		152.7	1, 10, 16, 17, 18			167.7	14, 18
12		138.2	10, 18			145.4	10, 14, 18
13		202.7	1, 14, 18			209.5	14, 18
14	2.70 *dd* (19.8, 6.5)	37.1	2	2.77 *d* (19.0)	2.85 *d* (18.6)	45.0	
	2.37 *d* (19.8)			2.42 *d* (19.0)	2.49 *d* (18.6)		
15		39.1	1, 10, 14, 16, 17			76.0	2, 14, 16, 17
16	1.11 *s*	37.7	17	1.15 *s*	1.25 *s*	27.6	17
17	1.60 *s*	26.2	16	0.82 *s*	0.95 *s*	28.6	16
18	2.14 *s*	14.4		1.34 *s*	1.53 *s*	9.3	
19	1.15 *s*	13.3	3, 7, 9	1.52 s	1.65 s	13.9	3, 7
20	5.46 *d* (1.2)	116.3	3	5.33 s	5.44 s	113.9	3
	5.13 *d* (1.2)			4.73 *br s*	4.74 *br s*		
OAc							
COMe	2.11 *s*	21.2		1.91 *br s*	1.98 *br s*	21.7	
CO		172.1	10, COMe			171.8	2, COMe
OBz							
7′						166.5	2, 6
1′						131.2	
2′,6′				7.80 *br d* (7.3)	7.81 *d* (7.8)	130.6	2, 3, 4, 5, 6
3′,5′				7.37 *dd* (7.8, 7.3)	7.43 *dd* (7.8, 7.3)	129.8	2, 4, 6
4′				7.51 *tt* (7.8, 1.2)	7.57 *t* (7.3)	134.6	2, 6

[a] ^1H and ^{13}C NMR spectra were measured at 400 and 100 MHz, respectively, in CD_3OD, and coupling constants (parentheses) are in Hertz.
[b] ^1H correlating with the ^{13}C resonance.
[c] Measured in $CDCl_3$.

Table 2
^1H and ^{13}C NMR spectroscopy data for sesquiterpenes **3** and **4**[a]

	3			**4**		
	δ_H	δ_C	HMBC[b]	δ_H	δ_C	HMBC[b]
1	4.66 *dd* (11.2, 4.2)	80.4	2, 15	4.78 *dd* (11.2, 4.6)	82.0	5, 15
2	1.78 *m*; 1.64 *m*	24.0		1.75 *m* (2H)	24.2	
3	1.52 *m* (2H)	39.4	2, 13, 14	1.52 *m*; 1.44 *m*	38.7	13, 14
4		32.5	5, 13, 14		32.1	5, 6, 13, 14
5	1.44 *dd* (10.5, 4.4)	48.6	3, 13, 14, 15	1.99 *br s*	51.0	6, 7, 13, 14, 15
6	2.44 *m*; 2.18 *m*	24.7	5	5.81 *br s*	130.0	5
7	6.86 *dd* (6.6, 3.6)	136.0	12	5.81 *br s*	126.8	
8		127.9			79.0	6, 7, 9, 12
9	2.58 *br s*	58.3	1, 5, 15	2.21 *br s*	68.1	1, 5, 7, 15
10		38.2	5, 15		42.5	5, 9, 15
11	5.72 *d* (5.4)	99.6		5.72 *br s*	101.9	9, 12
12		167.9		4.18 *d* (9.8); 3.78 *d* (9.8)	79.7	9, 11
13	0.93 *s*	32.3	5, 14	0.96 *s*	32.2	5, 14
14	0.95 *s*	21.2	5, 13	0.91 *s*	21.7	5, 13
15	0.94 *s*	9.6	1, 5	0.99 *s*	10.5	1, 5, 9
OAc						
1-COMe	2.07 *s*	21.4		2.08 *s*	21.5	
1-CO		171.3	1, 1-COMe		170.4	1-COMe

[a] ^1H and ^{13}C NMR spectra were measured at 400 and 100 MHz, respectively, in $CDCl_3$, and coupling constants (parentheses) are in Hertz.
[b] ^1H correlating with the ^{13}C resonance.

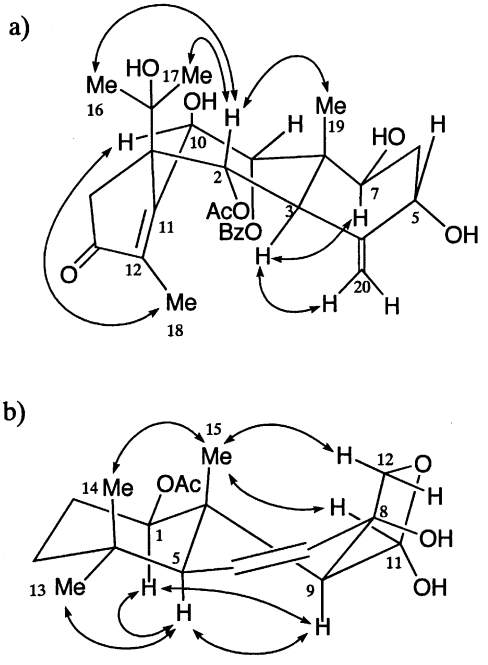

Fig.1. The ROESY correlations of a) 2α-acetoxy-9α-benzoyloxy-5α,7β,10β,15-tetrahydroxy-11(15→1)-*abeo*taxa-4(20),11-dien-13-one (**2**) and b) 1β-acetoxy-11,12-epoxy-6-drimen-8α,11α-diol (**4**).

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP-140 digital polarimeter. IR spectra were measured with a Shimadzu IR-408 spectrophotometer in $CHCl_3$ solution. NMR data were taken on a JEOL JNM LA-400 spectrometer with TMS as an internal standard. HRFABMS measurements were carried out on a JEOL JMS-700T spectrometer with glycerol as matrix. CC was performed with silica gel (Fuji Silysia, BW-820MH) or with Cosmosil $75C_{18}$-OPN (Nacalai Tesque Inc.). Analytical and preparative TLC were carried out on precoated Merck Kieselgel $60F_{254}$ and RP-$18F_{254}$ plates (0.25 or 0.50 mm thickness).

3.2. Plant material

The bark of *Taxus yunnanensis* Cheng et L.K. Fu was collected from Mt. Laojunshan by Shi Hua Yuan at an altitude of 3800 m, 100 km west of Lijiang City in Yunnan Province of People's Republic of China on October, 2000, and brought to Japan on permission of the State Pharmaceutical Administration of People's Republic of China. The identification was made by one of the authors (T. Nobukawa) and a voucher specimen (TMPW 21494) is preserved in the Museum for Materia and Medica, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

3.3. Extraction and isolation

Dried bark (3.85 kg) of *T. yunnanensis* was extracted with MeOH (20 l×3) under reflux for 2 h to afford a MeOH extract (397 g). A part of the MeOH extract (350 g) was dissolved in water (2.5 l) and extracted with CH_2Cl_2 (5 l×3) to yield a CH_2Cl_2-soluble fraction (103

g), which was subjected to silica gel cc (67×7 cm) with a $CHCl_3$–MeOH linear gradient system to give ten fractions.

Fraction 2 (17 g, 1% MeOH–$CHCl_3$ eluate) was applied to silica gel (47×4 cm) using hexane–EtOAc and then $CHCl_3$–MeOH to give seven subfractions. Subfraction 2 (832 mg) was further purified by silica gel cc and reversed-phase prep. TLC (MeOH–H_2O, 8:2) to give taxuyunnanine C (13.5 mg) (Ma et al., 1994) and taxusin (12.6 mg) (Della Casa de Marcano and Halsall, 1969). Subfraction 3 (802 mg) was also separated by cc, followed by prep. TLC (C_6H_6–EtOAc, 6:4), to give taxinine (3.1 mg) (Kurono et al., 1963), 2-deacetoxytaxinine J (2.2 mg) (Liang et al., 1988b). 2-Deacetoxytaxinine J (798 mg) was also obtained from subfraction 4 by recrystallization from hexane–EtOAc (8:2). Subfraction 5 (612 mg) was again subjected to reversed-phase silica gel with MeOH–H_2O (1:1–4:1), followed by prep. TLC (hexane-*iso*-PrOH, 3:7) to give taxa-4(20),11-dien-2α,5α,7β,9α,10β,13α-hexaacetate (16.5 mg) (Della Casa de Marcano and Halsall, 1969), yunnanxane (57.6 mg) (Chen et al., 1991) and 2-deacetoxy-7,9-dideacetyltaxinine J (3 mg) (Liang et al., 1998a). Subfraction 6 (1.4 g) was separated on reversed-phase silica gel with MeOH–H_2O (1:1–4:1), followed by prep. TLC (hexane-EtOAc, 7:3), to afford 2-deacetoxy-5-decinnamoyltaxinine J (25.6 mg) (Chattopadhyay and Sharma, 1995), 14*β*-hydroxytaxusin (34.4 mg) (Shi et al., 1998a), yunnanxane (196 mg) and taxinine B (44.6 mg) (Woods et al., 1968). Reversed-phase silica gel cc of subfraction 7 (3 g) with MeOH–H_2O (1:1–4:1), followed by normal-phase prep. TLC (hexane–*iso*-PrOH, 7:3) and reversed-phase prep. TLC (MeOH–H_2O, 8:2), to give taxinine J (23.7 mg) (Liang et al., 1998b), taxuspine F (4.1 mg) (Kobayashi et al., 1995b), 7-epitaxol (4.3 mg) (Huang et al., 1986), 5*β*,20-epoxy-1β-hydroxy-4α,7β,13α-triacetoxy-2α,9α,10β-tribenzoxytax-11-ene (5.7 mg) (Chu et al., 1992) and taxuspine D (1.5 mg) (Kobayashi et al., 1995a).

Fraction 3 (14 g, 1–2% MeOH–$CHCl_3$ eluate) was applied to silica gel (43×4 cm) with a hexane–EtOAc gradient system to give seven subfractions. Subfraction 2 (210 mg) was purified on prep. TLC (C_6H_6–EtOAc, 7:3) to give 5α-cinnamoyloxy-9α,10β,13α-triacetoxytaxa-4(20),11-diene (4.8 mg) (Yeh et al., 1988). Subfraction 3 (894 mg) was again separated by reversed-phase silica gel cc with MeOH–H_2O (1:1–4:1), followed by prep. TLC (C_6H_6–EtOAc, 6:4), to afford **3** (21.4 mg), 5,7,4′-trihydroxyflavanone (6.1 mg) (Ficarra et al., 1995), 1*β*-acetoxyisodrimeninol (8.4 mg) (Kiyota et al., 2002) and 2-deacetoxytaxinine J (440 mg). Subfraction 4 (1.9 g) was subjected to silica gel cc with hexane–EtOAc and then $CHCl_3$–MeOH, followed by prep. TLC (C_6H_6–EtOAc, 6:4), to give taxuspinanane K (52.2 mg) (Morita et al., 1998), taxuspine F (15.1 mg) and 2-deacetoxytaxinine J (29.5 mg). Subfraction 5 (2.5 g) was separated by reversed-phase silica gel cc with MeOH–H_2O (1:1–4:1), followed by prep. TLC (C_6H_6–EtOAc, 5:5), to give 2-deacetoxytaxinine J (15.9 mg), taxuspine F (4.1 mg), taxol (19.3 mg) (Wani et al., 1971), taxinine J (7.4 mg) and taxinine B (1.9 mg). Subfraction 6 (5.1 g) was applied to silica gel with hexane–EtOAc and then $CHCl_3$–MeOH, followed by reversed-phase prep. TLC (CH_3CN–CH_3OH–H_2O, 1:1:1), to give taxol (3.6 mg), taxuyunnanine (4.4 mg) (Barboni et al., 1994), 7-epitaxuyunnanine (1.5 mg) (Zhang et al., 1994), taxuspine F (1.8 mg) and taxinine J (5.4 mg). Subfraction 7 (1.5 g) was separated by reversed-phase silica gel cc with CH_3CN–CH_3OH–H_2O (1:1:2) and then CH_3CN–CH_3OH (1:1), followed by prep. TLC (hexane–EtOAc, 4:6), to give 2′β-deacetoxyaustrospicatine (27.7 mg) (Ettouati et al., 1988), decinnamoyltaxinine J (3.4 mg) (Kingston et al., 1982) and 1β-acetoxy-5α-deacetylbaccatin I (6.3 mg) (Liang et al., 1988a).

Fraction 4 (5.1 g, 2–3% MeOH–$CHCl_3$ eluate) was subjected to silica gel chromatography (35×2.8 cm) with a hexane–EtOAc gradient system to give five subfractions. Subfraction 2 (884 mg) was again separated by reversed-phase silica gel cc (MeOH–H_2O, 1:1–4:1), followed by prep. TLC (C_6H_6–EtOAc, 7:3), to give 2-deacetoxytaxinine J (54.6 mg), 2-deacetoxy-5-decinnamoyltaxinine J (5.8 mg) and taxuspinanane K (5 mg). Subfraction 3 (1.2 g) was applied to reversed-phase silica gel with CH_3CN–CH_3OH–H_2O (1:1:2–4:2:1) and purified by prep. TLC ($CHCl_3$–EtOAc, 5:5) to give **4** (5.2 mg), 5α-cinnamoyloxy-9α,10β,13α-triacetoxytaxa-4(20),11-diene (4.8 mg), taxol B (11.8 mg) (McLaughlin et al., 1981). Subfraction 4 (1.4 g) was separated by silica gel cc (1–30% MeOH–$CHCl_3$), followed by reversed-phase prep. TLC (CH_3CN–CH_3OH–H_2O, 1:1:2), to give 2′β-deacetoxyaustrospicatine (51.3 mg), 5α,13α-dihydroxy-7β,9α,10β-triacetoxy-4(20),11-taxadiene (8.7 mg) (Sun et al., 2001), 1(15→11)*abeo*-taxane (28.9 mg) (Sun et al., 2001) and 1β-acetoxy-5α-deacetylbaccatin I (6.3 mg).

Fraction 5 (5 g, 3% MeOH–$CHCl_3$ eluate) was subjected to silica gel chromatography (35×2.8 cm) using a hexane–EtOAc gradient system to afford 5 subfractions. Subfraction 2 (710 mg) was applied to reversed-phase silica gel (MeOH–H_2O, 1:1–4:1), followed by prep. TLC (hexane-*iso*-PrOH, 7:3), to give 2-deacetoxytaxinine J (27.3 mg), 2-deacetoxy-5-decinnamoyltaxinine J (3.1 mg) and 2′β-deacetoxyaustrospicatine (45.0 mg). Subfraction 3 (2 g) was again separated by reversed-phase silica gel cc (CH_3CN–CH_3OH–H_2O, 1:1:2-1:1:1) and purified by prep. TLC (hexane–$CHCl_3$–CH_3OH, 5:4:1) to give 5α,13α-dihydroxy-7β,9α,10β-triacetoxy-4(20),11-taxadiene (3.8 mg), 1β-acetoxy-5α-deacetylbaccatin I (3.5 mg), taxezopidine H (12.7 mg) (Wang et al., 1998) and 10-deacetylcephalomannine (24.5 mg) (McLaughlin et al., 1981). Subfraction 4 (1.2 g) was applied to on

reversed-phase silica gel (CH_3CN–CH_3OH–H_2O, 1:1:1) and purified by prep. TLC (hexane–Me_2CO, 8:2), to give 2′β-deacetoxyaustrospicatine (2.1 mg), 10-deacetylcephalomannine (2 mg), 10-deacetyltaxol (42.3 mg) (McLaughlin et al., 1981) and 2-deacetoxytaxinine J (15.2 mg).

Fraction 6 (2.9 g, 3–5% MeOH-$CHCl_3$ eluate) was subjected to reversed-phase silica gel chromatography (33×2.5 cm) (CH_3CN–CH_3OH–H_2O, 1:1:1) to give 4 subfractions. Subfraction 1 (255 mg) was separated by prep. TLC (hexane–$CHCl_3$–CH_3OH, 5:4:1) to give taxinine B (17.7 mg) and 2β-deacetoxyaustrospicatine (8.2 mg). Subfraction 2 (980 mg) was separated by reversed-phase silica gel cc (CH_3CN–CH_3OH–H_2O, 1:1:2–1:1:1), followed by prep. TLC ($CHCl_3$–CH_3OH, 9:1), to give taxezopidine H (7.5 mg), 5α,7β,9α,10β-tetrahydroxy-4(20),11-taxadien-13-one (7.7 mg) (Sun et al., 2001) and 10-deacetylbaccatin III (21.3 mg) (Kingston et al., 1982). Subfraction 3 (926 mg) was applied to reversed-phase silica gel (CH_3OH–H_2O, 1:1–4:1), followed by prep. TLC (hexane–$CHCl_3$–MeOH, 5:4:1), to afford 7-(β-xylosyl)cephalomannine (60.0 mg) (Sénilh et al., 1984) and 7-(β-xylosyl)taxol C (5 mg) (Sénilh et al., 1984).

Fraction 7 (3 g, 5–10% MeOH-$CHCl_3$ eluate) was separated by reversed-phase silica gel cc (15×2.5 cm) with Me_2CO–MeOH–H_2O (1:1:2–1:1:1) to give six subfractions. Subfraction 2 (786 mg) was applied to reversed-phase silica gel (MeOH–H_2O, 2:1), followed by prep. TLC (hexane–EtOAc, 2:8), to afford 7-(β-xylosyl)-10-deacetyltaxol B (8.9 mg) (Sénilh et al., 1984) and 7-(β-xylosyl)-10-deacetyltaxol (7.0 mg) (Sénilh et al., 1984). Subfraction 4 (986 mg) was further purified on reversed-phase silica gel with Me_2CO–MeOH–H_2O (1:1:3–1:1:1) to give six fractions. Among them, fractions 2 and 3 (393 mg) were again separated by reversed-phase silica gel cc (CH_3CN–CH_3OH–H_2O, 1:1:1), followed by prep. TLC (hexane–EtOAc, 2:8), to give **1** (2 mg), while fraction 6 (50.2 mg), on prep. TLC (hexane–EtOAc, 2:8), gave **2** (11.3 mg).

3.4. 10β-Acetoxy-2α,5α,7β,9α-tetrahydroxytaxa-4(20),11-dien-13-one (1)

A colorless amorphous solid; $[\alpha]_D^{25}$ +22.2° ($CHCl_3$; *c* 0.16); IR $\nu_{max}^{CHCl_3}cm^{-1}$: 3400, 1730, 1250, 1020; for ^1H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z*: 409.2188 [calcd for $C_{22}H_{33}O_7$ $(M+H)^+$, 409.2226].

3.5. 2α-Acetoxy-9α-benzoyloxy-5α,7β,10β,15-tetrahydroxy-11(15→1)-abeotaxa-4(20),11-dien-13-one (2)

A colorless amorphous solid; $[\alpha]_D^{25}$ +31.6° ($CHCl_3$; *c* 0.32); IR $\nu_{max}^{CHCl_3}cm^{-1}$: 3400, 1700, 1600, 1450, 1370, 1250; for ^1H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z*: 529.2449 [calcd for $C_{29}H_{37}O_9$ $(M+H)^+$, 529.2438].

3.6. 1β-Acetoxy-7-drimen-11α-ol-12,11-lactone (3)

A colorless amorphous solid; $[\alpha]_D^{25}$ −46.4° ($CHCl_3$; *c* 0.65); IR $\nu_{max}^{CHCl_3}cm^{-1}$: 3350, 1740, 1370, 1250, 1050; for ^1H and ^{13}C NMR spectra, see Table 2; HRFABMS *m*/*z*: 309.1696 [calcd for $C_{17}H_{25}O_5$ $(M+H)^+$, 309.1702].

3.7. 1β-Acetoxy-11,12-epoxy-6-drimen-8α,11α-diol (4)

A colorless amorphous solid; $[\alpha]_D^{25}$-16.4° ($CHCl_3$; *c* 0.32); IR $\nu_{max}^{CHCl_3}cm^{-1}$: 3400, 1730, 1370, 1240; for ^1H and ^{13}C NMR spectra, see Table 2; HRFABMS *m*/*z*: 311.1834 [calcd for $C_{17}H_{27}O_5$ $(M+H)^+$, 311.1858].

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