Structures of Taxchinins L and M, Two New Diterpenoids from *Taxus chinensis* var. *Mairei*

Kiyoshi TANAKA,[a] Kaoru FUJI,*[,a] Toshio YOKOI,[b] Tetsuro SHINGU,[b] Bo LI,[c] and Handong SUN[c]

Institute for Chemical Research, Kyoto University,[a] Uji 611, Japan, Faculty of Pharmaceutical Sciences, Kobe Gakuin University,[b] Nishi-ku, Kobe 651–21, Japan, and Kunming Institute of Botany, Academia Sinica,[c] Kunming 650204, Yunnan, China. Received April 15, 1996; accepted May 13, 1996

The structures of two new diterpenoids, taxchinin L (1) and taxchinin M (2), from the leaves and stems of *Taxus chinensis* var. *mairei* were elucidated by means of NMR spectroscopy. These diterpenoids have an 11(15→1)-*abeo*-taxane skeleton and exist as an equilibrium mixture of two conformers in $CDCl_3$ at an ambient temperature.

Key words diterpenoid; structure elucidation; *Taxus chinensis* var. *mairei*; NMR; *abeo*-taxane skeleton; conformation

The discovery of the promising anticancer agent taxol has stimulated an extensive search for better plant sources and improved analogues of this type of drug.[1,2] To date over 100 diterpenoids have been isolated from various *Taxus* species.[3] As a part of our ongoing studies on new taxoids with antitumor activities,[4–8] we have isolated two new pseudotaxoid diterpenoids, taxchinins L (**1**) and M (**2**), from the leaves and stems of *Taxus chinensis* var. *mairei*. collected at Yunnan, China. Their structures were elucidated by spectral means, mainly based on ^{1}H- and ^{13}C-NMR analyses. Both diterpenoids belong to the 11(15→1)-*abeo*-taxane family, which has been expanding

Table 1. ^{1}H- and ^{13}C-NMR Data for the Major Conformer of Taxchinin L (**1**) ($CDCl_3$, δ in ppm from TMS, at −10 °C)[a]

Position	δ_H	COSY	ROESY	δ_C	DEPT	HMBC
1				66.4	C	H-3, H-10, H-14α, 15-OH, Me-17
2	5.95 (d, 7.3)	H-3	Me-17, Me-19	68.5	CH	H-3, H-14α
3	3.15 (d, 7.4)	H-2	H-7, H-10, H-14α	43.0	CH	Me-19
4				79.4	C	H-3, H-5, H-6α, H-20b
5	4.94 (d, 6.2)	H-6α	H-6α, H-6β	85.4	CH	H-6α, H-7, H-20a
6	(α) 2.61 (ddd, 16.0, 8.8, 6.5)	H-5, H-7, H-6β	H-5, H-6β, H-7	34.4	CH_2	
	(β) 1.81[b]	H-6α, H-7	H-5, H-6α			
7	5.46 (dd, 8.6, 5.6)	H-6α, H-6β	H-3, H-6α, H-10	69.9	CH	H-5, H-6α, H-6β, H-9, Me-19
8				43.4	C	H-2, H-3, H-6, H-7, H-9, Me-19
9	5.97 (d, 10.1)	H-10	Me-19, 10-OH, 15-OH	80.8	CH	H-3, H-7, H-10, Me-19
10	4.69 (t, 10.3)	H-9, 10-OH	H-3, H-7, Me-18	66.7	CH	H-9, 10-OH
11				137.5	C	H-10, H-13, H-14β, Me-18
12				146.1	C	H-10, H-13, H-14β, Me-18
13	4.45[b]	H-14α, H-14β		77.5	CH	H-14α, Me-18
14	(α) 1.43 (dd, 15.0, 6.9)	H-14β, H-13	H-3, H-14β	39.3	CH_2	
	(β) 2.13 (dd, 15.1, 7.6)	H-14α, H-13	H-13, H-14α, Me-16, Me-17			
15				76.3	C	H-2, H-14α, H-14β, Me-16, Me-17
16	1.02 (s)		H-13, H-14β, 15-OH, Me-17	27.3	CH_3	Me-17
17	1.22 (s)		H-2, H-14β, 15-OH, Me-16	25.1	CH_3	Me-16
18	1.96 (s)		H-10, H-13, 10-OH	11.4	CH_3	
19	1.73 (s)		H-2, H-9, H-20a	14.0	CH_3	H-3, H-7, H-9
20	a) 4.53 (d, 7.4)	H-20b	H-20b, Me-19	75.0	CH_2	H-3
	b) 4.45	H-20a				
10-OH	4.28 (d, 10.3)	H-10	H-7, H-9, Me-18			
15-OH	4.03 (br s)		H-9, Me-16, Me-17			
2OAc (C=O)				170.7	C	H-2, 2Ac(Me)
(Me)	2.05 (s)[c]			21.9[d]	CH_3	
4OAc (C=O)				171.7	C	4Ac(Me)
(Me)	2.17 (s)			22.5	CH_3	
7OAc (C=O)				170.7	C	H-7, 7Ac(Me)
(Me)	1.79 (s)[c]			21.7[d]	CH_3	
9OBz (C=O)				167.7	C	H-9, H-2′, H-6′
Bz (1′)				130.0	C	
Bz (2′, 6′)	7.99 (d-like)	Bz3′, 5′		129.7	CH	H-4′
Bz (3′, 5′)	7.45 (t-like)	Bz2′, 6′, Bz4′		128.3	CH	
Bz (4′)	7.57 (t-like)	Bz3′, 5′		133.1	CH	H-2′, H-6′

a) 500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR. *b*) Signals are not completely identified due to overlap with other signals. *c, d*) May be exchangeable in any column.

* To whom correspondence should be addressed.

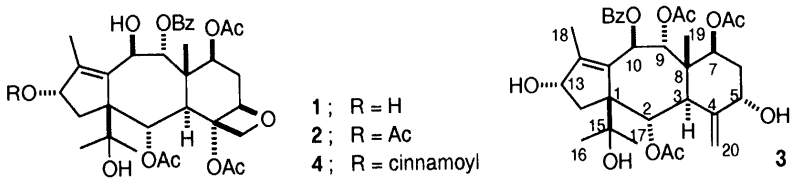

Fig. 1. Structures of Taxoids **1**—**4**

Table 2. ^{1}H- and ^{13}C-NMR Data for the Minor Conformer of Taxchinin L (**1**) ($CDCl_3$, δ in ppm from TMS, at $-10\,°C$)[a]

Position	δ_H	COSY	ROESY	δ_C	DEPT	HMBC
1				65.1	C	H-3, H-14α, Me-16, Me-17
2	6.31 (d, 7.6)	H-3	Me-19	70.9	CH	H-3
3	3.29 (d, 7.5)	H-2		42.1	CH	Me-19
4				83.1	C	H-3, H-5
5	4.98 (dd, 8.5, 8.1)	H-6α, H-6β		84.4	CH	
6	(α) 2.39[b]	H-5, H-6β, H-7	H-5, H-7	30.7	CH_2	
	(β) 2.06[b]	H-6α	H-5, H-7			
7	5.25 (dd, 12.9, 3.7)	H-6α, H-6β		70.0	CH	Me-19
8				44.4	C	H-2, H-3, Me-19
9	5.20 (d, 4.5)	H-10		76.0	CH	
10	4.90 (d, 4.3)	H-9	Me-18	68.0	CH	
11				135.5	C	H-9, Me-18
12				144.3	C	Me-18
13	4.45[b]	H-14α, H-14β		78.7	CH	H-14α, Me-18
14	(α) 1.56 (dd, 15.0, 6.1)	H-14β, H-13	H-14β	39.2	CH_2	H-2
	(β) 2.36 (dd, 15.1, 6.9)	H-14α, H-13	H-14α			
15				75.0	C	H-14α, Me-16, Me-17
16	0.91 (s)			30.0	CH_3	Me-17
17	1.25 (s)			27.9	CH_3	Me-16
18	1.29 (s)			13.1	CH_3	
19	1.76 (s)		H-2, H-9	14.3	CH_3	H-3
20	a) 4.76 (d, 8.0)	H-20b		78.5	CH_2	H-3
	b) 4.45[b]	H-20a				
2OAc (C=O)				172.5	C	H-2, 2Ac(Me)
(Me)	2.18 (s)			22.8	CH_3	
4OAc (C=O)				171.7	C	4Ac(Me)
(Me)	2.28 (s)			22.5	CH_3	
7OAc (C=O)				169.6	C	7Ac(Me)
(Me)	1.88 (s)			20.9	CH_3	
9OBz (C=O)				165.2	C	H-9, H-2′, H-6′
Bz (1′)				129.0	C	
Bz (2′, 6′)	8.08 (d-like)	Bz3′, 5′		129.7	CH	H-3′, H-5′
Bz (3′, 5′)	7.42 (t-like)	Bz2′, 6′, Bz4′		128.5	CH	
Bz (4′)	7.57 (t-like)	Bz3′, 5′		133.4	CH	

a) 500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR. *b*) Signals are not completely identified due to overlap with other signals.

rapidly since our first report on the structure of taxchinin A (**3**) in 1992.[4] In contrast to the fixed skeletal conformation of ordinary taxoids, the 11(15→1)-*abeo*-taxoids generally show a characteristic slow equilibrium between two or more conformational isomers in solution.[8]

Taxchinin L (**1**) has mp 263—264 °C, and its ^{1}H-NMR spectrum in $CDCl_3$ at an ambient temperature showed broad signals, which sharpened upon cooling to below 0 °C. At −10 °C, two distinct sets of resonances appeared. The ^{1}H- and ^{13}C-NMR spectra of taxchinin L (**1**) are very similar to those of taxchinin J (**4**).[7,8] The difference is the absence of one cinnamoyl group at C-13 in **1**, which was confirmed by the upfield shift of the H-13 signal [δ 4.45 (1H, overlapped, major and minor conformers)] (Tables 1 and 2). The heteronuclear multiple bond connectivity (HMBC) spectrum established the location of the benzoyloxy group at C-9, and two acetoxyls at C-2 and C-7. The relative stereostructure of taxchinin L (**1**) was clearly elucidated by detailed two dimensinal (2D) NMR spectral analysis and the principal rotating frame Overhauser enhancement spectroscopy (ROESY) pattern is shown in Fig. 2. Careful inspection of NMR spectra suggested that in $CDCl_3$ at −10 °C, taxchinin L (**1**) exists as an equilibrium mixture of two conformational isomers with boat/half-boat : chair/half-chair conformations of the B/C rings in a ratio of 81 : 19.

Taxchinin M (**2**), mp 239—242 °C, also exhibited NMR spectroscopic behavior characteristic of an 11(15→1)-*abeo*-taxane diterpenoid due to conformational equilibrium in a solution. Its ^{1}H- and ^{13}C-NMR spectra at −10 °C are quite similar to those of taxchinin L (**1**) and taxchinin J (**4**).[8] The difference is the presence of an acetyl

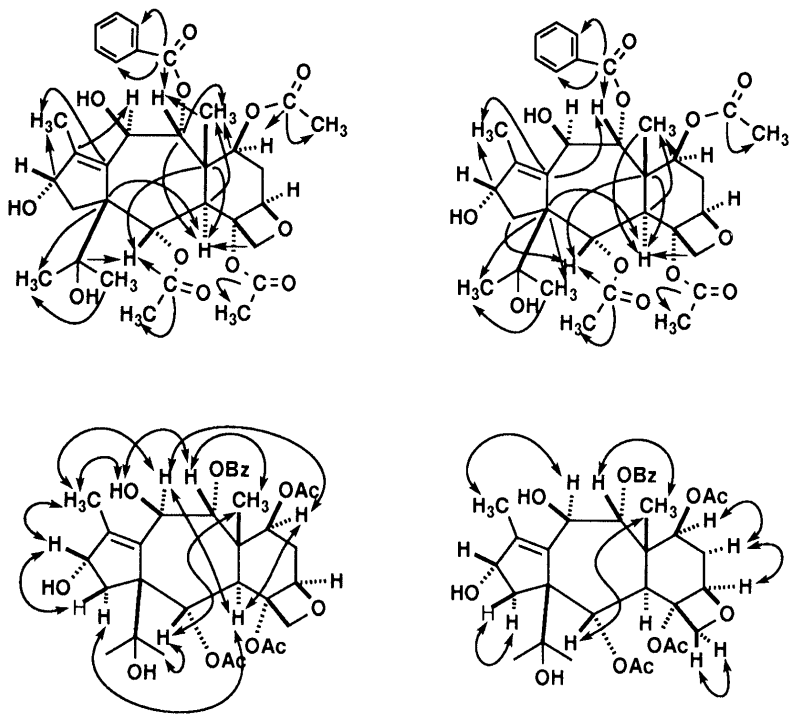

Fig. 2. Key HMBC (Upper) and ROESY (Lower) Observed for Taxchinin L (**1**) (Left: Major Conformer, Right: Minor Conformer)

Table 3. ^{1}H- and ^{13}C-NMR Data for Conformer A of Taxchinin M (**2**) ($CDCl_3$, δ in ppm from TMS, at $-10\,°C$)[a]

Position	δ_H	COSY	ROESY	δ_C	DEPT	HMBC
1				65.5	C	H-2, H-3, H-10, H-14α, Me-16, Me-17
2	6.32 (d, 7.5)	H-3	Me-19	71.0	CH	H-3
3	3.20 (d, 7.6)	H-2	H-7, H-14α	42.6	CH	H-2, H-9, Me-19
4				82.4	C	H-3, H-5, H-20b
5	5.02 (t, 8.6)	H-6α, H-6β	H-6α	84.2	CH	H-6β, H-20b
6	(α) 2.42 (ddd, 16.0, 9.0, 4.0)	H-5, H-7	H-5, H-6β	30.9	CH_2	
	(β) 2.06[b]	H-5, H-6α, H-7	H-6α			
7	5.28 (dd, 13.2, 4.2)	H-6α, H-6β	H-3	70.0	CH	H-6β, Me-19
8				44.4	C	H-2, H-3, H-10, Me-19
9	5.26 (d, 4.6)	H-10	H-10, Me-19	75.2	CH	H-3
10	4.93 (d, 4.5)	H-9	H-9, Me-18	67.9	CH	H-9
11				137.8	C	H-9, H-13, H-14β, Me-18
12				140.7	C	H-13, H-14β, Me-18
13	5.57 (t, 6.8)	H-14α, H-14β	H-14β, Me-16, Me-18	80.9	CH	H-14α, Me-18
14	(α) 1.71 (dd, 15.1, 6.7)	H-13, H-14β	H-3, H-14β	36.2	CH_2	H-2
	(β) 2.45 (dd, 15.2, 6.9)	H-13, H-14α	H-13, H-14α, Me-17			
15				75.3	C	Me-16, Me-17
16	0.98 (s)		H-13, Me-17	30.4	CH_3	Me-17
17	1.30 (s)		H-14β, Me-16	28.0	CH_3	Me-16
18	1.22 (s)		H-10, H-13	13.3	CH_3	
19	1.79 (s)		H-2, H-9, H-20a	13.9	CH_3	H-3
20	a) 4.71 (d, 7.8)	H-20b	Me-19	78.3	CH_2	H-3
	b) 4.49 (d, 7.8)	H-20a				
2OAc (C=O)				173.1	C	H-2, 2Ac(Me)
(Me)	2.19 (s)			22.8	CH_3	
4OAc (C=O)				169.7	C	4Ac(Me)
(Me)	2.22 (s)			22.1[b]	CH_3	
7OAc (C=O)				169.7	C	H-7, 7Ac(Me)
(Me)	1.91 (s)			21.0[b]	CH_3	
13OAc (C=O)				170.8	C	H-13, 13Ac(Me)
(Me)	2.14 (s)			21.3	CH_3	
9OBz (C=O)				164.9	C	H-9, H-2′, H-6′
Bz (1′)				129.2	C	
Bz (2′, 6′)	8.12 (d-like)	Bz3′, 5′		129.9	CH	
Bz (3′, 5′)	7.46 (t-like)	Bz2′, 6′, Bz4′		128.3	CH	
Bz (4′)	7.57 (m)	Bz3′, 5′		133.4	CH	

a) 500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR. *b*) Signals are not completely identified due to overlap with other signals.

Table 4. ^{1}H- and ^{13}C-NMR Data for Conformer B of Taxchinin M (**2**) ($CDCl_3$, δ in ppm from TMS, at $-10\,^\circ C$)[a]

Position	δ_H	COSY	ROSEY	δ_C	DEPT	HMBC
1				66.7	C	H-2, H-3, H-14α, Me-16, Me-17
2	6.03 (d, 7.4)	H-3	Me-17, Me-19	68.2	CH	H-3
3	2.97 (d, 7.4)	H-2	H-7, H-14α	44.0	CH	H-2, Me-19
4				78.7	C	H-3, H-5, H-20b
5	5.00 (d, 7.6)	H-6α	H-6α	84.9	CH	H-6β, H-20a
6	(α) 2.63 (dt, 16.2, 8.0)	H-5, H-7, H-6β	H-5, H-6β	34.6	CH_2	
	(β) 1.83[b]		H-6α			
7	5.49 (dd, 8.3, 7.5)	H-6α, H-6β	H-3, H-6α	70.4	CH	H-3, H-5, H-6β, H-9, Me-19
8				43.2	C	H-2, H-3, H-6α, H-9, Me-19
9	6.01 (d, 10.8)	H-10	Me-19	80.5	CH	Me-19
10	4.75 (t, 10.8)	H-9, 10-OH	H-7, Me-18	66.3	CH	H-9
11				139.5	C	H-13, Me-18
12				142.7	C	H-13, H-14β, Me-18
13	5.62 (t, 7.4)	H-14α, H-14β	H-14β, Me-16, Me-18	79.4	CH	H-14α, Me-18
14	(α) 1.62 (dd, 14.4, 7.6)	H-13, H-14β	H-3, H-14β	36.5	CH_2	H-2
	(β) 2.19[b]	H-13, H-14α	H-13, H-14α			
15				76.5	C	H-2, H-14α, Me-16, Me-17
16	1.12 (s)		H-13, Me-17	27.7	CH_3	Me-17
17	1.25 (s)		H-9, H-14β, Me-16	25.4	CH_3	Me-16
18	1.88 (s)		H-10, H-13	11.4	CH_3	
19	1.75 (s)		H-2, H-9, H-20b	13.3	CH_3	H-3, H-7
20	a) 4.48 (d, 7.6)	H-20b		74.4	CH_2	H-3
	b) 4.43 (d, 7.5)	H-20a				
2OAc (C=O)				170.7	C	H-2, 2Ac(Me)
(Me)	2.04 (s)			21.7[b]	CH_3	
4OAc (C=O)				169.1	C	4Ac(Me)
(Me)	2.12 (s)			22.1	CH_3	
7OAc (C=O)				170.7	C	H-7, 7Ac(Me)
(Me)	1.81 (s)			22.0[b]	CH_3	
13OAc (C=O)				171.0	C	H-13, 13Ac(Me)
(Me)	2.14 (s)			21.1	CH_3	
9OBz (C=O)				168.0	C	H-9, H-2′, H-6′
Bz (1′)				130.1	C	
Bz (2′, 6′)	7.99 (d-like)	Bz3′, 5′		129.7	CH	
Bz (3′, 5′)	7.46 (t-like)	Bz2′, 6′, Bz4′		128.2	CH	
Bz (4′)	7.54 (m)	Bz3′, 5′		133.1	CH	

a) 500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR. b) Signals are not completely identified due to overlap with other signals.

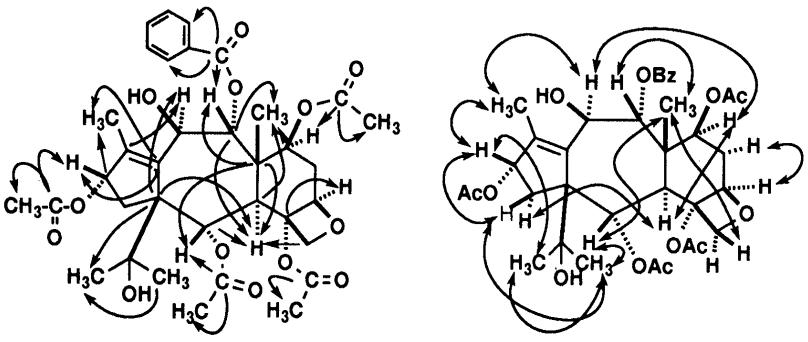

Fig. 3. Key HMBC (Left) and ROESY (Right) Observed for the Conformer A (Boat/Half-Boat for B/C Ring) of Taxchinin M (**2**)

group at the C-13 oxygen in **2**, which was proven by the ^{1}H-NMR signal at δ 5.57 (t, J=6.8 Hz, H-13 for conformer A) and δ 5.62 (t, J=7.4 Hz, H-13 for conformer B) (Tables 3 and 4). Further, the HMBC spectrum of **2** revealed the location of one benzoyloxy group at C-9, and three acetoxyls at C-2, C-7 and C-13. The crucial ROESY pattern is depicted in Fig. 3 and such 2D NMR spectral analysis established the relative stereochemistry of **2**. From detailed analysis of the coupling constants in the ^{1}H-NMR spectrum, it was deduced that the ratio of the two conformational isomers of **2** in $CDCl_3$ at $-10\,^\circ C$ is approximately 1 : 1 [boat/half-boat (conformer A): chair/half-chair (conformer B) for the B/C rings).

Taxchinins L (**1**) and M (**2**) both belong to the 11(15→1)-*abeo*-taxoid group, and more than forty members of this class have been identified so far.[8,9] We previously demonstrated that the acylation pattern and bulkiness at C-10 bias the equilibrium in solution, so the absence of the acyl group at C-10 in both diterpenoids, **1** and **2**, is considered to be responsible for their slow conformational equilibrium. The present study and previous conformational studies on related 11(15→1)-

abeo-taxoids[6-8] indicate that the major conformer with a free hydroxyl at C-13 tends to adopt a boat/half-boat conformation of the B/C rings, in which the C-9 and -10 substituents both occupy equatorial positions.

Experimental

General Experimental Procedures The general procedures used were as previously described.[5] The ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ using a Bruker ARX-500 instrument.

Plant Material and Isolation *T. chinensis* var. *mairei* was collected in September 1990 at Yunnan, China and the plant was identified by Prof. Yue Zhongsu, Kunming Institute of Botany, China. Dried and ground stems and leaves (50 kg) were extracted with MeOH to afford 1.9 kg of the crude extract, which was partitioned between H_2O and CH_2Cl_2. Concentration of the CH_2Cl_2 layer gave 440 g of the extract. Column chromatography on silica gel gave 76 mg of taxchinin L (**1**) in 1.3×10^{-4} % yield and 10 mg of taxchinin M (**2**) in 2×10^{-5} % yield.

Taxchinin L (1) Colorless needles from Et_2O, mp 263—264 °C. $[\alpha]_D^{19}$ −40.0° (c=0.10, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400, 2980, 2925, 1730, 1370, 1240, 1080, 1020, 720. EI-MS m/z: 612 $(M-H_2O)^+$, 594, 570, 554, 534, 510, 495, 452, 372, 297, 240, 122, 105, 91, 77. HR-MS m/z: 612.2545 $(M-H_2O)^+$. $C_{33}H_{40}O_{11}$ requires 612.2570. ^{1}H- and ^{13}C-NMR see Tables 1 and 2.

Taxchinin M (2) Colorless needles from Et_2O, mp 239—242 °C, $[\alpha]_D^{19}$ −19.6° (c=0.12, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3425, 3050, 2925, 2850, 1740, 1650, 1600, 1450, 1370, 1280, 1250, 1030, 720. EIMS m/z: 654 $(M-H_2O)^+$, 594, 554, 537, 494, 372, 312, 297, 252, 122, 105, 77. HR-MS m/z: 654.2637 $(M-H_2O)^+$. $C_{35}H_{42}O_{12}$ requires 654.2676. ^{1}H- and ^{13}C-NMR: Tables 3 and 4.

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