

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

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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 CDCl₃ 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 ¹H- and ¹³C-NMR analyses. Both diterpenoids belong to the 11(15→1)-abeo-taxane family, which has been expanding

Table 1. ¹H- and ¹³C-NMR Data for the Major Conformer of Taxchinin L (1) (CDCl₃, 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) (β) 1.81 ^{b)}	H-5, H-7, H-6β H-6α, H-7	H-5, H-6β, H-7 H-5, H-6α	34.4	CH ₂	
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) (β) 2.13 (dd, 15.1, 7.6)	H-14β, H-13 H-14α, H-13	H-3, H-14β H-13, H-14α, Me-16, Me-17	39.3	CH ₂	
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) b) 4.45	H-20b H-20a	H-20b, Me-19	75.0	CH ₂	H-3
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 ¹H-NMR, 125 MHz for ¹³C-NMR. b) Signals are not completely identified due to overlap with other signals. c, d) May be exchangeable in any column.

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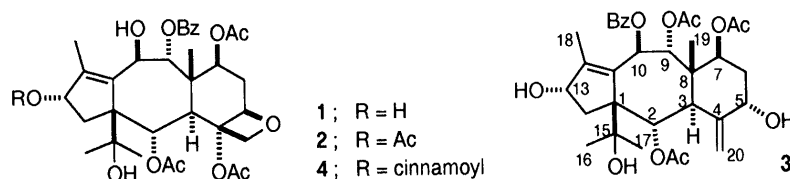


Fig. 1. Structures of Taxoids 1–4

Table 2. ^1H - 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-14 α , 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-6 α , H-6 β		84.4	CH	
6	(α) 2.39 ^{b)} (β) 2.06 ^{b)}	H-5, H-6 β , H-7 H-6 α	H-5, H-7 H-5, H-7	30.7	CH ₂	
7	5.25 (dd, 12.9, 3.7)	H-6 α , H-6 β		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-14 α , H-14 β		78.7	CH	H-14 α , Me-18
14	(α) 1.56 (dd, 15.0, 6.1) (β) 2.36 (dd, 15.1, 6.9)	H-14 β , H-13 H-14 α , H-13	H-14 β H-14 α	39.2	CH ₂	H-2
15				75.0	C	H-14 α , 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) b) 4.45 ^{b)}	H-20b H-20a		78.5	CH ₂	H-3
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 ^1H -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 ^1H -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 ^1H - 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

benzyloxy group at C-9, and two acetoxy groups at C-2 and C-7. The relative stereostructure of taxchinin L (1) was clearly elucidated by detailed two dimensional (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 ^1H - 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

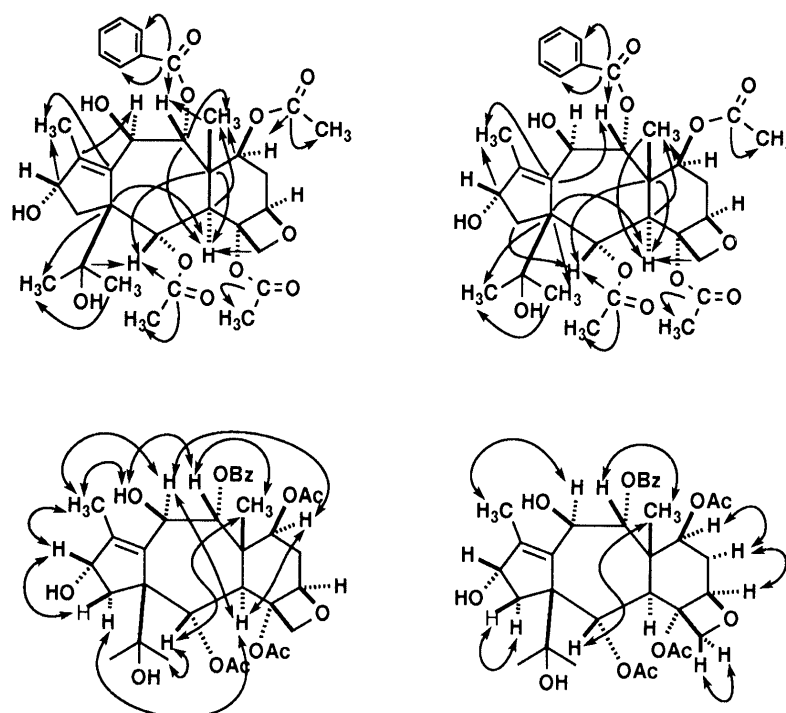


Fig. 2. Key HMBC (Upper) and ROESY (Lower) Observed for Taxchinin L (1) (Left: Major Conformer, Right: Minor Conformer)

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

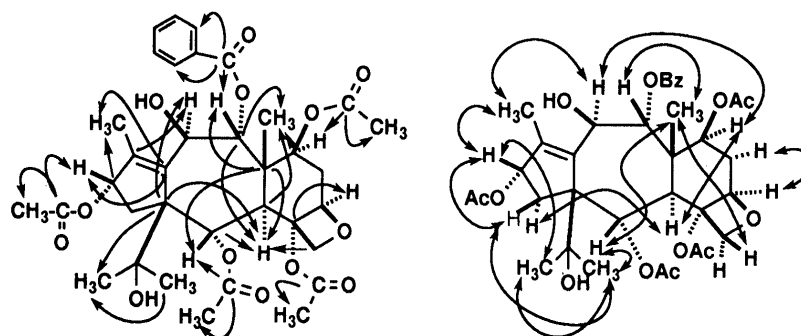
Position	δ_{H}	COSY	ROESY	δ_{C}	DEPT	HMBC
1				65.5	C	H-2, H-3, H-10, H-14 α , Me-16, Me-17
2	6.32 (d, 7.5)	H-3	Me-19	71.0	CH	H-3
3	3.20 (d, 7.6)	H-2	H-7, H-14 α	42.6	CH	H-2, H-9, Me-19
4				82.4	C	H-3, H-5, H-20b
5	5.02 (t, 8.6)	H-6 α , H-6 β	H-6 α	84.2	CH	H-6 β , H-20b
6	(α) 2.42 (ddd, 16.0, 9.0, 4.0) (β) 2.06 ^{b)}	H-5, H-7 H-5, H-6 α , H-7	H-5, H-6 β H-6 α	30.9	CH ₂	
7	5.28 (dd, 13.2, 4.2)	H-6 α , H-6 β	H-3	70.0	CH	H-6 β , 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-14 β , Me-18
12				140.7	C	H-13, H-14 β , Me-18
13	5.57 (t, 6.8)	H-14 α , H-14 β	H-14 β , Me-16, Me-18	80.9	CH	H-14 α , Me-18
14	(α) 1.71 (dd, 15.1, 6.7) (β) 2.45 (dd, 15.2, 6.9)	H-13, H-14 β H-13, H-14 α	H-3, H-14 β H-13, H-14 α , Me-17	36.2	CH ₂	H-2
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-14 β , 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) b) 4.49 (d, 7.8)	H-20b H-20a	Me-19	78.3	CH ₂	H-3
20Ac (C=O)				173.1	C	H-2, 2Ac(Me)
(Me) 2.19 (s)				22.8	CH ₃	
40Ac (C=O)				169.7	C	4Ac(Me)
(Me) 2.22 (s)				22.1 ^{b)}	CH ₃	
70Ac (C=O)				169.7	C	H-7, 7Ac(Me)
(Me) 1.91 (s)				21.0 ^{b)}	CH ₃	
130Ac (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 ^1H -NMR, 125 MHz for ^{13}C -NMR. b) Signals are not completely identified due to overlap with other signals.

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

Position	δ_{H}	COSY	ROSEY	δ_{C}	DEPT	HMBC
1				66.7	C	H-2, H-3, H-14 α , 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-14 α	44.0	CH	H-2, Me-19
4				78.7	C	H-3, H-5, H-20b
5	5.00 (d, 7.6)	H-6 α	H-6 α	84.9	CH	H-6 β , H-20a
6	(α) 2.63 (dt, 16.2, 8.0) (β) 1.83 ^{b)}	H-5, H-7, H-6 β	H-5, H-6 β H-6 α	34.6	CH ₂	
7	5.49 (dd, 8.3, 7.5)	H-6 α , H-6 β	H-3, H-6 α	70.4	CH	H-3, H-5, H-6 β , H-9, Me-19
8				43.2	C	H-2, H-3, H-6 α , 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-14 β , Me-18
13	5.62 (t, 7.4)	H-14 α , H-14 β	H-14 β , Me-16, Me-18	79.4	CH	H-14 α , Me-18
14	(α) 1.62 (dd, 14.4, 7.6) (β) 2.19 ^{b)}	H-13, H-14 β H-13, H-14 α	H-3, H-14 β H-13, H-14 α	36.5	CH ₂	H-2
15				76.5	C	H-2, H-14 α , 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-14 β , 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) b) 4.43 (d, 7.5)	H-20b H-20a		74.4	CH ₂	H-3
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 ^1H -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 ^1H -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 ^1H -NMR spectrum, it was deduced that the ratio of the two conformational isomers of **2** in CDCl_3 at -10°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 $\rightarrow$ 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 $\rightarrow$ 1)-

abeo-taxoids⁶⁻⁸⁾ 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 ¹H- and ¹³C-NMR spectra were recorded in CDCl₃ 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 H₂O and CH₂Cl₂. Concentration of the CH₂Cl₂ 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 Et₂O, mp 263–264 °C, [α]_D¹⁹ –40.0° (*c* = 0.10, CHCl₃). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3400, 2980, 2925, 1730, 1370, 1240, 1080, 1020, 720. EI-MS *m/z*: 612 (M–H₂O)⁺, 594, 570, 554, 534, 510, 495, 452, 372, 297, 240, 122, 105, 91, 77. HR-MS *m/z*: 612.2545 (M–H₂O)⁺. C₃₃H₄₀O₁₁ requires 612.2570. ¹H- and ¹³C-NMR see Tables 1 and 2.

Taxchinin M (2**)** Colorless needles from Et₂O, mp 239–242 °C, [α]_D¹⁹ –19.6° (*c* = 0.12, CHCl₃). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3425, 3050, 2925, 2850, 1740, 1650, 1600, 1450, 1370, 1280, 1250, 1030, 720. EIMS *m/z*: 654 (M–H₂O)⁺, 594, 554, 537, 494, 372, 312, 297, 252, 122, 105, 77. HR-MS *m/z*: 654.2637 (M–H₂O)⁺. C₃₅H₄₂O₁₂ requires 654.2676. ¹H- and ¹³C-NMR: Tables 3 and 4.

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