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NOVEL DITERPENOIDS FROM *TAXUS CHINENSIS*

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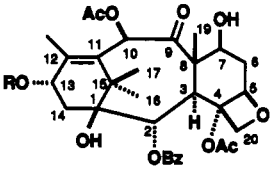
ABSTRACT.—Five new diterpenoids, taxchinins A [**3**], B[**4**], and C [**5**], 19-hydroxy-7-*epi*-baccatin III [**6**], and 10-deacetyl-10-oxobaccatin V [**7**], have been isolated from the needles and stems of *Taxus chinensis* together with twelve known compounds including taxol [**1**]. These structures were elucidated and identified by spectroscopic techniques. A single X-ray crystallographic analysis of taxchinin A [**3**] determined its unique 5/7/6-membered ring system. Taxchinins B [**4**] and C [**5**] were shown to have the same ring system.

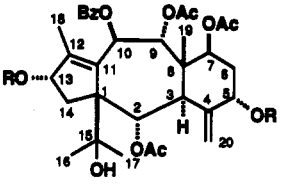
Taxol [**1**], a highly functional diterpene isolated in 1971 (1) from the yew tree, *Taxus brevifolia* (Taxaceae), is currently considered the most exciting lead in cancer chemotherapy (2,3). It is currently in phase III clinical trials in the U.S. (4–7) and has also now been approved for clinical use in ovarian cancer by the FDA. In spite of the encouraging spectrum of activity, progress in developing taxol as a drug has been relatively slow, largely because of the lack of an abundant supply and difficulties in formulation (8). Some aspects of taxoids (9–11), such as their promising antitumor activity against different cancers, unique structural features, unusual biogenesis, and novel mode of action, stimulated the search for new related diterpenes having similar activity from the widely distributed species of the family Taxaceae (12–20) throughout the world. At the same time, several research groups have been involved in attempts of total synthesis of taxol (21–23) as well as partial synthesis (24–26) from abundant but inactive taxanes to meet the needs for clinical use. We report here the isolation and structure determination of five new diterpenoids together with eleven known congeners and one lignan from *Taxus chinensis* (Pilgre) Rehd. A part of this work has appeared in preliminary form (27). *Taxus chinensis* is an evergreen tree which grows in China, and from which the isolation of some taxoids has already been reported by a Chinese research group (28–30).

RESULTS AND DISCUSSION

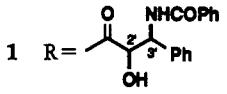
Besides the five new diterpenoids **3–7** described below, eleven known congeners along with a lignan were isolated from MeOH extracts of needles and stems of *T. chinensis* collected at Yunnan province in China, in the yields shown in the experimental section. Known compounds are taxol [**1**] (1), cephalomannine (31), 7-*epi*-10-deacetyltaxol (17), baccatin III [**2**] (32), 10-deacetylbaccatin III (32), 19-hydroxybaccatin III (17), 10-deacetylbaccatin V (31), baccatin IV (32), baccatin VI (32), taxagifine (33), 1-acetoxy-5-deacetylbaccatin I (34), and one lignan, α-conidendrin (35).

Taxchinin A [**3**] was determined to have a molecular formula of $C_{33}H_{42}O_{11}$ by analysis of the ^{13}C-nmr and fabms data. The ^{1}H-nmr signals at δ 4.60 and 5.19 (each 1H, brs) and those in the ^{13}C nmr at δ 152.9 (tert-C) and 113.0 (CH_2) suggest the presence of an exomethylene moiety. The presence of a benzoate was verified by the observation





3 R=H
8 R=Ac



1 R=

2 R=H

of ^{1}H-nmr signals at δ 7.86 (2H, d, J=7.3 Hz), 7.55 (1H, m), and 7.42 (2H, m). Both ^{1}H- and ^{13}C-nmr spectra revealed three *O*-acetyl groups. Furthermore, it was shown by the DEPT and CH-COSY spectra that seven sp^3 carbons bearing an oxygen functional group exist in the molecule, and four of them are responsible for the carbons carrying *O*-acyl groups and one for a carbon of a tertiary alcohol. The presence of two remaining secondary alcohols was confirmed by the downfield chemical shift of the methines from δ 4.34 (1H, brs, H-5) and 4.47 (1H, brt, J=6.3 Hz, H-13) to 5.27 and 5.56, respectively, on acetylation with Ac_2O/pyridine (Table 1). The benzoyl carbonyl signal at δ 164.3 had a correlation with the H-10 and aromatic protons ortho to the carbonyl group in a ^{13}C-^{1}H long range COSY spectrum (36), which established the location of the benzoate at C-10. Consequently, the three acetoxyls must be at the positions C-2, C-7, and C-9. Detailed investigation of its ^{13}C-^{1}H long range COSY spectrum (Table 1) revealed that the C-15, bearing geminal dimethyl groups, was unusually shifted downfield (δ 75.6) as compared with that of conventional taxane diterpenoids (δ ca. 43) (37–40). This indicates that a hydroxyl group is located at C-15. These considerations lead to the possibility of a molecule with a novel 5/7/6-ring system, which accounts well for the ^{1}H- and ^{13}C-nmr spectra observed. Thus, the unusual downfield shifts for the carbons of ring A in taxchinin A [**3**] (Table 1) are attributable to an increase in ring strain. A single crystal X-ray diffraction analysis (27) of taxchinin A [**3**] was carried out to confirm the structure deduced from the spectral data.

The eims of taxchinin B [**4**] showed a peak at *m/z* 784 corresponding to $[M-H_2O]^+$. The hrms gave an ion at *m/z* 784.3075 (calcd for $C_{44}H_{48}O_{13}$, 784.3094). The signals at δ 4.40, 4.50 (each 1H, d, J=7.7 Hz) in ^{1}H nmr and δ 74.7 (CH_2) in ^{13}C nmr indicated the presence of an oxetane ring in the molecule. The comparison of ^{1}H- and ^{13}C-nmr spectra of taxchinin B [**4**] (Tables 2 and 3) with those of taxchinin A [**3**] led to the conclusion that taxchinin B [**4**] contains a ring system similar to that of taxchinin A [**3**] except for the presence of an oxetane ring. The signals at δ 68.2 (tert-C) and 75.6 (tert-C) in ^{13}C nmr could be assigned to the C-1 and C-15, respectively, by DEPT, HETCOR, and ^{13}C-^{1}H long range COSY spectra. Judging from the ^{13}C-nmr and DEPT spectra, the presence of nine sp^3 carbons bearing oxygens was suggested; seven of these are accounted for by carbons carrying *O*-acyl groups, one by CH_2O of the oxetane, and the last one as the carbon of a tertiary alcohol. The existence of four acetyl and one benzoyl groups as well as one cinnamoyl group was revealed by both ^{1}H- and ^{13}C-nmr spectra. The protons of the benzoyl and cinnamoyl groups appeared at δ 7.87 (2H, d, J=7.4 Hz), 7.43 (3H, m), 7.56 (5H, m), 7.80 (1H, d, J=16 Hz), and 6.47 (1H, d, J=16 Hz). Additionally, uv absorption maxima at 218, 223, and 280 nm (log ε 4.23, 4.24, and 4.23, respectively) also supports the presence of a cinnamoyl group. The prominent fragment peaks in the mass spectrum of taxchinin B [**4**] at *m/z* 131 [C_9H_7O] and 105 [C_7H_5O] are characteristic for loss of cinnamoyl and benzoyl groups. In order to determine the location of ester

TABLE 1. ^{1}H- and ^{13}C-nmr Data for **3** and **8** ($CDCl_3$, δ in ppm from TMS).

Position	Compound								
	3						**8**		
	δ_C[a]	DEPT	^{13}C-^{1}H LRCOSY[b]	δ_H[c]	COSY	NOESY	δ_C[d]	DEPT	δ_H[e]
1	68.5	C	Me-16, Me-17, H-3, H-10				68.7[f]	C	
2	67.5	CH	H-3	6.07 (d, 9.2)	H-3	Me-17, Me-19	67.5	CH	6.05 (brd, 9.9)
3	42.2	CH	Me-19 Hα-20	3.37 (d, 9.2)	H-2	H-7	43.1	CH	3.20 (d, 9.4)
4	143.7	C	H-3				140.1	C	
5	74.5	CH	Hβ-20	4.34 (brs)	Hβ-6	Hβ-20	75.8[g]	CH	5.27 (brs)
6	37.2	CH_2		2.00 (α) (m) 1.80 (β) (m)	Hβ-6, H-7 H-5, Hα-6, H-7		34.8	CH_2	2.00 (m) 2.00 (m)
7	69.3	CH		5.49 (dd, 5, 11)	Hα, Hβ-6	H-3, H-10	68.8[f]	CH	5.47 (dd, 5, 13)
8	45.2	C	Me-19, H-3, H-2				44.9	C	
9	76.1	CH	Me-19, H-10	6.02 (d, 10.6)	H-10	Me-19	75.9[g]	CH	6.05 (brd, 9.9)
10	69.6	CH	H-9	6.56 (d, 10.6)	H-9	H-7, Me-18	68.9[f]	CH	6.66 (d, 10.8)
11	133.3	C	Me-18				135.5	C	
12	152.9	C	Hα,β-14				148.9	C	
13	77.0	CH	Me-18	4.47 (brt, 6.3)	Hα,Hβ-14	Me-16, Me-18	78.9	CH	5.56 (brt, 7)
14	40.6	CH_2	Hα-14	2.34 (α) (dd, 7, 15) 2.00 (β) (m)	H-13, Hβ-14 H-13, Hα-14		37.8	CH_2	2.50 (dd, 7.6, 15) 2.00 (m)
15	75.6	C	Me-16, Me-17, 2H-14, H-2				75.5	C	
16	25.6	Me	Me-17	1.10 (s)		H-13	25.4	Me	1.15 (s)
17	27.7	Me	Me-16	1.19 (s)		H-2	27.6	Me	1.21 (s)
18	12.2	Me	Me-18	2.11 (s)		H-10, H-13	11.9	Me	2.12 (s)

TABLE 1. Continued.

Position	Compound								
	3						8		
	δ_C[a]	DEPT	^{13}C-^{1}H LRCOSY[b]	δ_H[c]	COSY	NOESY	δ_C[d]	DEPT	δ_H[e]
19	13.4	Me	Me-19, H-3, H-9	1.04 (s)		H-2, H-9	13.3	Me	1.05 (s)
20	113.0	CH_2		4.60 (α) (brs)	Hβ-20	Hβ-20	115.4	CH_2	4.68 (brs)
				5.19 (β) (brs)	Hα-20	Hα-20, H-5			5.27 (brs)
2-OAc C=O	171.3	C	H-2, Me (2-OAc)				171.7[h]	C	
Me	21.4	Me		2.00 (s)			21.2[i]	Me	1.98 (s)
7-OAc C=O	170.1	C	7-OAc				170.9[h]	C	
Me	21.8	Me		2.06 (s)			21.7[i]	Me	2.04 (s)
9-OAc C=O	169.6	C	H-9, Me (9-OAc)				170.2[h]	C	
Me	20.7	Me		1.75 (s)			20.6[i]	Me	1.72 (s)
5-OAc C=O							169.8[h]	C	
Me							21.0[i]	Me	1.23 (s)
13-OAc C=O							169.8[h]	C	
Me							21.5[i]	Me	1.08 (s)
OBz C=O	164.3	C	H-10, *o*-Ph				164.5	C	
i-Ph	129.1	C					129.0	C	
o-Ph	129.4	CH		7.86 (d, 7.3)	*m*-Ph	*m*-Ph	129.7	CH	7.85 (d, 7.3)
m-Ph	128.7	CH		7.42 (m)	*o*-Ph, *p*-Ph	*o*-Ph, *p*-Ph	129.0	CH	7.41 (m)
p-Ph	133.0	CH		7.55 (m)	*m*-Ph	*m*-Ph	133.7	CH	7.55 (m)

[a]100 MHz.
[b]Long Range COSY.
[c]400 MHz.
[d]50 MHz.
[e]200 MHz.
[f,g,h,i]Assignments may be interchangeable in the vertical column.

TABLE 2. ^{1}H-nmr Data[a] for **4** and **5** (400 MHz, $CDCl_3$).

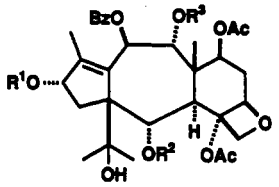
Proton	Compound				
	4		**5**		
	δ_H	COSY	δ_H	COSY	NOESY
H-2	6.18 (d, 7.9)	H-3	6.55 (d, 7.3)	H-3	15-OH, Me-16, Me-19
H-3	3.04 (d, 7.8)	H-2	3.18 (d, 7.7)	H-2	H-7
H-5	5.01 (d, 7.6)	Hα-6	4.97 (d, 7.3)	Hα-6	Hα-6, Hα-20
Hα-6	2.60 (m)	H-5, Hβ-6, H-7	2.70 (m)	H-5, Hα-6, H-7	H-5
Hβ-6	1.91 (dd, 8.3, 15.4)	Hα-6, H-7		Hα-6, H-7	
H-7	5.62 (t, 8.3)	Hα,β-6	5.67 (t, 8.1)	Hα,β-6	H-3, H-10
H-9	6.22 (d, 10.9)	H-10	6.56 (d, 11.3)	H-10	H-10, Me-16, Me-19
H-10	6.62 (d, 10.9)	H-9	6.77 (d, 11.0)	H-9	H-7, H-9, Me-18
H-13	5.64 (t, 6.7)	Hα,β-14, Me-18	5.71 (t, 7.7)	Hα,β-14	Me-18, Me-17
Hα-14	2.46 (m)	H-13, Hβ-14	2.45 (dd, 7.3, 14.3)	H-13, Hβ-14	
Me-16	1.23 (s)		1.21 (s)		H-2, H-9, 15-OH
Me-17	1.21 (s)		1.21 (s)		H-13, 15-OH
Me-18	2.02 (d, 1.8)	H-13	1.97 (s)		H-10, H-13
Me-19	1.68 (s)		1.87 (s)		Hβ-20
Hα-20	4.40 (d, 7.7)	Hβ-20	4.19 (d, 7.7)	Hβ-20	H-5, Hβ-20
Hβ-20	4.50 (d, 7.7)	Hα-20	4.51 (d, 7.7)	Hα-20	Hα-20, Me-19
*C*Hα=CHβ	6.47 (d, 16.0)	Hβ			
*C*Hα=*C*Hβ	7.80 (d, 16.0)	Hα			
15-OH	2.72 (s)		2.62 (s)		Me-16, Me-17, H-2
Ph-H	7.87 (d, 7.3)		8.04 (d, 7.3)		
	7.43 (m)		7.75 (d, 7.3)		
	7.56 (m)		7.63 (m)		
			7.49 (m)		
			7.35 (m)		
			7.22 (m)		
2-OAc	2.01 (s)				
4-OAc	2.01 (s)		2.17 (s)		
7-OAc	2.10 (s)		2.17 (s)		
9-OAc	1.76 (s)		1.83 (s)		

[a]δ in ppm from TMS.

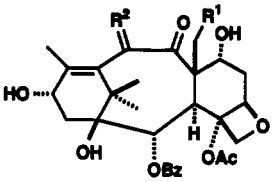
TABLE 3. ^{13}C-nmr Data for **4** and **5** ($CDCl_3$).

Carbon	Compound				
	4[a]			**5**[b]	
	δC	DEPT	^{13}C-^{1}H LRCOSY	δC	DEPT
C-1	68.2	C	H-10, Hα-14, Me-16, Me-17	68.1	C
C-2	67.9	CH	H-3	68.4	CH
C-3	44.7	CH	H-5, Me-19, Hα-20, Hβ-20	44.4	CH
C-4	79.4	C	H-3, H-5, Hβ-20	79.1	C
C-5	84.7	CH	H-5, Hβ-6, Hα-20	84.8	CH
C-6	34.8	CH_2	Hα-6, H-7	34.8	CH_2
C-7	70.6	CH	H-3, H-5, Hβ-6, H-9, Me-19	70.6	CH
C-8	43.7	C	H-2, H-3, Hα-6, H-9, H-10, Me-19	43.8	C
C-9	76.4	CH	H-10, Me-19	77.5	CH
C-10	68.6	CH	H-9	68.5	CH
C-11	135.9	C	Me-18, H-13	136.4	C
C-12	148.1	C	Me-18	148.0	C
C-13	79.1	CH	Me-18	78.8	CH
C-14	37.3	CH_2		36.7	CH_2
C-15	75.6	C	15-OH, H-2, Hα,β-14, Me-16, Me-17	75.8	C
C-16	27.7	Me	Me-16	27.7	Me
C-17	25.4	Me	15-OH, Me-17	25.4	Me
C-18	12.1	Me	Me-18	11.8	Me
C-19	12.5	Me	H-7, Me-19	13.1	Me
C-20	74.7	CH_2	H-3, Hα-20	74.6	CH_2
2-OAc C=O	170.3	C	H-2, Me (2-OAc)		
Me	21.6	Me			
4-OAc C=O	169.2	C	Me (4-OAc)	170.5[c]	C
Me	22.0	Me		21.6[d]	Me
7-OAc C=O	169.8	C	H-7, Me (7-OAc)	169.4[c]	C
Me	21.4	Me		21.0[d]	Me
9-OAC C=O	169.8	C	H-9, Me (9-OAc)		
Me	20.6	Me			
13-OAc C=O				171.0[c]	C
Me				21.9[d]	Me
OBz C=O	164.1	C	H-10, *o*-Ph (OBz)	164.7	C
				166.8	C
				166.1	C
Ph	134.1	C		130.1	C
	129.1	C		129.6	C
	133.4	CH		129.1	C
	130.6	CH		133.7	CH
	129.5	CH		133.2	CH
	129.0	CH		133.1	CH
	128.7	CH		129.9	2×CH
	128.2	CH		129.4	CH
				128.8	CH
				128.5	CH
				128.2	CH
COCHα=CHβ	117.3	CH	Hα, Hβ		
COCHα=CHβ	146.0	CH	Hβ		
COCHα=CHβ	166.5	C	H-13, CHα=CHβ		

[a]Measured at 100 MHz.
[b]Measured at 50.3 MHz.
[c,d]Assignments may be interchanged.



4 R^1=cinnamoyl, R^2=R^3=Ac
5 R^1=Ac, R^2=R^3=Bz



6 R^1=OH, R^2=β-OAc, α-H
7 R^1=H, R^2=O

groups, a ^{13}C-^1H long range COSY experiment was performed (Table 3). A correlation of the signal due to the benzoyl carbonyl at δ 164.1 with that of H-10 at δ 6.61 and the aromatic protons at δ 7.86 ortho to the carbonyl group suggested the location of the benzoyl group at the position of C-10. Furthermore, the cinnamoyl carbonyl signal at δ 166.5 had correlations with that of H-13 at δ 5.62 and those of two vinyl protons of the cinnamoyl group at δ 7.80 and 6.47, which established the location of the cinnamate at C-13. Consequently, the four acetoxyl groups must be at positions C-2, -4, -7, and -9. The stereochemistry of taxchinin B [**4**] was elucidated by a NOESY experiment (Figure 1). An examination of the NOESY data indicated a very close stereochemical relationship to taxchinin A [**3**], with a β orientation of ring D as in other known taxanes (37–40). Thus, ^1H- and ^{13}C-nmr signals of taxchinin B were totally assigned by 2D nmr techniques (HH-COSY, CH-COSY, NOESY, and long range CH-COSY). Therefore, the structure of taxchinin B is formulated as **4**.

Taxchinin C [**5**] showed an ion in the fabms at *m/z* 839 corresponding to $[M+H]^+$, and hrms exhibited an ion at *m/z* 716.2806 corresponding to $[M-PhCOOH]^+$ (calcd for $C_{40}H_{44}O_{12}$, 716.2831). Therefore, taxchinin C [**5**] has a mol wt of 838 with a composition of $C_{47}H_{50}O_{14}$. Both the ^1H- and ^{13}C-nmr spectra of taxchinin C [**5**] (Tables 2 and 3) are similar to those of taxchinin B [**4**] except for the signals due to the ester groups. Thus, the main difference is derived from the presence of three benzoyl and three acetyl groups in taxchinin C [**5**]. The downfield shift of the signals for H-2, -3, -9, and -10 (Tables 2 and 3) in its ^1H-nmr spectrum suggested the location of three benzoates at positions C-2, -9, and -10, which was supported by an observation of the downfield shift of the signals for C-2 and C-9 and an upfield shift of C-13 in its ^{13}C-nmr spectrum (Table 3). As a result, it was deduced that the three acetoxyls were located at C -4, -7, and -13. The DEPT and the other 2D nmr techniques, including ^1H-^1H COSY, HETCOR, and NOESY, were undertaken to confirm the structure proposed for taxchinin C [**5**].

It turned out that taxchinins A [**3**], B [**4**], and C [**5**] have a novel 5/7/6-ring system, which is a rearranged carbon skeleton of the known taxoids such as taxol. The same rearranged carbon skeleton has only appeared in the literature as the reaction products of taxol [**1**] in treatment with electrophilic reagents such as methanesulfonyl chloride and acetyl chloride (11,41) or of 10-deacetylbaccatin III [**2**] with organic acid (21,42).

Compound **6** showed ions at *m/z* 603, 625, and 695 in fabms, which correspond to

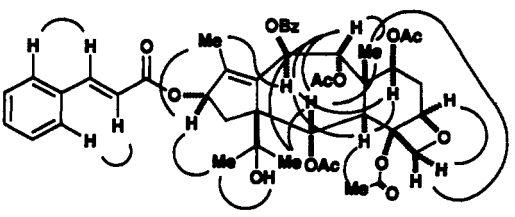


FIGURE 1. Key nOe's observed for taxchinin B [**4**].

TABLE 4. ^{1}H- and ^{13}C-nmr Data for **6** and **7** ($CDCl_3$, δ in ppm from TMS).

Position	Compound						
	6				**7**		
	δ_H[a]	COSY	δ_C[b]	DEPT	δ_H[c]	δ_C[d]	DEPT
1			79.3	C		79.2	C
2	6.26 (d, 7.7)	H-3	74.7	CH	5.80 (d, 7.3)	74.9	CH
3	4.00 (d, 7.3)	H-2	40.8	CH	4.07 (d, 7.3)	39.6	CH
4			81.6	C		81.3	C
5	4.98 (dd, 2.8, 9.4)	H-6	82.7	CH	4.92 (brt, 5.9)	82.7	CH
6	2.20 (m)	H-5, H-7	35.5	CH_2		35.3	CH_2
7	3.86 (ddd, 1.8, 5.1, 11.7)	H-6, 7-αOH	72.4	CH	3.82 (brd, 12)	77.3	CH
8			61.3	C		57.4	C
9			210.0	C		208.9	C
10	6.86 (s)		78.8	CH		189.5	C
11			132.3	C		140.9	C
12			144.5	C		146.6	C
13	4.83 (m)	H-14, Me-18, 13-αOH	68.0	CH	4.92 (brt, 5.9)	67.7	CH
14	2.20 (m)	H-13	37.9	CH_2		38.7	CH_2
15			42.3	C		39.8	C
16	1.26 (s)		22.5	Me	1.08 (s)	26.2	Me
17	1.07 (s)		26.6	Me	1.08 (s)	22.4	Me
18	1.99 (d, 1.5)	H-13	15.9	Me	1.70 (s)	14.8	Me
19	4.48 (α) (dd, 5.9, 12.5) 4.24 (β) (dd, 7.9, 12.3)	Hβ-19, 19-OH Hα-19, 19-OH	62.9	CH_2	1.96 (s)	14.4	Me
20	4.45 (d, 8.6) 4.37 (d, 8.6)	Hβ-20 Hα-20	77.9	CH_2	4.42 (d, 8.5) 4.30 (d, 8.5)	77.4	CH_2
OAc			176.2	C		172.8	C
	2.22 (s)		20.9	Me		22.0	Me

TABLE 4. Continued.

Position	Compound						
	6				7		
	δ_H[a]	COSY	δ_C[b]	DEPT	δ_H[c]	δ_C[d]	DEPT
OAc			172.8	C			
	2.37 (s)		20.1	Me			
i-Ph			129.7	C		129.4	C
o-Ph	8.12	*m*-Ph	130.4	CH	8.12	130.3	CH
	(dd, 1.5, 7.0)				(dd, 1.6, 7.8)		
m-Ph	7.49 (m)	*o*-Ph, *p*-Ph	128.9	CH	7.50 (m)	129.0	CH
p-Ph	7.62 (m)	*m*-Ph	133.9	CH	7.64 (m)	134.2	CH
PhCO			169.8	C		167.4	C
7-αOH	4.94 (d, 11.4)	H-7					
13-αOH	2.07 (d, 4.0)	H-13					
19-OH	2.95	Hα,β-19					
	(dd, 5.9, 8.1)						

[a]400 MHz.
[b]100 MHz, assigned by HETCOR and DEPT.
[c]200 MHz.
[d]50 MHz, assigned by HETCOR and DEPT.

$[M+H]^+$, $[M+Na]^+$ and $[M+glycerin+H]^+$, respectively. The mol wt of 602 with a composition of $C_{31}H_{38}O_{12}$ was suggested from an ion at 584.2249 for $[M-H_2O]^+$ (calcd for $C_{31}H_{36}O_{11}$, 584.2257) in the hrms. The structural determination of **6** was greatly simplified by a direct comparison of its mass and ^1H- and ^{13}C-nmr spectral data (Table 4) with those of 19-hydroxybaccatin III. The eims of **6** was identical with that of 19-hydroxybaccatin III (17), whereas the major difference between their ^1H-nmr spectra appeared as an upfield shift of the signal due to H-7 to δ 3.86 (ddd) and a downfield shift of H-10 to 6.86 (s) for compound **6**. This observation is characteristic of the stereochemical relationship between the 7β-OH and 7α-OH epimers in another series of taxoids (17,43). An observation of the upfield shift of the signals for C-3 and C -5 as well as the downfiield shift for C-9 is consistent with the structure of the compound **6** as a 7-*epi*-taxane (38). The structure of compound **6** was thus deduced to be 7-*epi*-19-hydroxybaccatin III.

Compound **7** showed characteristic signals at δ 189.5 and 208.9 in the ^{13}C-nmr spectrum, which suggested an α-dicarbonyl structure at C-9 and C-10. The ^1H-nmr signal at δ 3.82 (1H, brd, J=12 Hz, H-7) indicated an α orientation of the 7-OH (17,38,43). Both the ^{13}C- and ^1H-nmr spectra of **7** (Table 4) are quite similar to those of 10-deacetyl-10-oxo-7-*epi*-taxol (43), except for the absence of the side chain at the C-13 position. Therefore, the structure of the compound **7** was formulated as 10-deacetyl-10-oxobaccatin V.

Taxol is the only plant-produced antimitotic agent (44) known to promote the assembly of microtubules and inhibit the tubulin disassembly process (45,46). A good correlation between the inhibition of tubulin asssembly and cytotoxicity has been well documented (26). Kingston and coworkers (41) reported that the ring-contracted taxol derivative showed comparable activity to taxol in tublin disassembly assay. The ability of the new diterpenes **3–7** to affect microtubule assembly was examined according to the method of Lataste *et al.* (46). These compounds had little effect (ID_{50}>100 μM) compared with that of taxol [**1**] (ID_{50} 0.29 μM).

In summary, we investigated the diterpenoid constituents in *T. chinensis* and isolated five new diterpenoids with twelve known components. Three of the new diterpenoids isolated in the present study have a novel 5/7/6-membered ring system. Natural occurrence of this type of terpenoid is hitherto unknown, and the compounds might provide useful information about the biogenesis of the taxane-type skeletons.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on Yanagimoto melting point apparatus and are uncorrected. Ir spectra were recorded with JASCO IR-810 spectrometer and uv spectra with a Shimadzu UV-220 spectrophotometer. Mass spectra were measured with a JMS-DX 300 MS spectrometer, and optical rotations were taken on a HORIBA SEPA-200 polarimeter. ^1H- and ^{13}C-nmr spectra were recorded using Varian Gemini-200, JEOL JNM-GZ 400, or Bruker AM-400 instruments. Analytical hplc was carried out on a JASCO Triroter instrument using a CHEMCOSORB 7DPh column (4.6×300 mm) with detection at 227 nm and using MeOH-H_2O-MeCN (20:41:39) as eluent. A JAI LC-908 model recycle HPLC was employed for preparative hplc by using a direct connection of JAIGEL 1H and 2H columns (each 20×600 mm). Cc was performed with Si gel 60 (150–325 mesh), spherical cosmic 75C18 Si gel, or Sephadex LH-20, and preparative tlc was carried out on E. Merck Si gel 60 F254 plates (0.5 mm).

PLANT MATERIAL.—*T. chinensis* was collected in November 1990 at Yunnan, China. The plant was identified by Prof. Yue Zhongsu, Kunming Institute of Botany, China, and a voucher specimen is on deposit at the same Institute.

EXTRACTION AND ISOLATION.—Dried and ground stems and needles (60 kg) were extracted with MeOH to afford 3.3 kg of the crude extract, which was diluted with H_2O and partitioned against CH_2Cl_2 to yield 850 g of the extract. The CH_2Cl_2 extract was dissolved in Et_2O and successively washed with H_2O, 5% $NaHCO_3$ aqueous solution, 1 N HCl, and H_2O. Concentration of the Et_2O layer under reduced pressure

gave 321 g of the residue as a mixture of neutral materials, which was then subjected to cc on Si gel. The gradient elution of the column with a solvent system of *n*-hexane/Me_2CO provided 43 fractions. Isolation and purification by repeated chromatography, including preparative hplc, tlc, and cc, and recrystallization from an appropriate solvent furnished 17 pure compounds. Taxchinins B [**4**], C [**5**] and taxagifine were isolated from fractions 19–20 in yields of 8.1×10^{-4}, 5.5×10^{-4}, and 8.3×10^{-4}%, respectively, from the dried plant material (60 kg). Baccatin VI (5×10^{-5}%) and 10-deacetylbaccatin V (4.38×10^{-3}%) were obtained from fractions 22–24. From fractions 25–26, 10 deacetyl-10-oxobaccatin V [**7**], baccatin IV, 1-acetoxy-5-deacetylbaccatin I, baccatin III [**2**], and α-conidendrin were isolated in yields of 5×10^{-4}, 1.6×10^{-4}, 7×10^{-4}, 6.2×10^{-4}, and 2.5×10^{-4}%, respectively. Taxchinin A [**3**] (4.7×10^{-3}%), taxol [**1**] (1.1×10^{-4}%), cephalomannine (8×10^{-5}%), 7-*epi*-10-deacetyltaxol (6×10^{-5}%), 7-*epi*-19-hydroxybaccatin III [**6**] (1.1×10^{-4}), and 19-hydroxybaccatin III (1.0×10^{-4}%) were obtained from fractions 27–31.

Taxchinin A [**3**].—Colorless plates from Et_2O: mp 208–210°, $[\alpha]^{19}D$ −34.62 (*c*=0.875, CH_2Cl_2). *Anal.* found C 64.38, H 6.97; calcd for $C_{33}H_{42}O_{11}$, C 64.50, H 6.84. Ir ($CHCl_3$) ν max 3550, 3350, 3025, 2975, 1720, 1650, 1360, 1240, 1060, 1020, 700 cm^{-1}; fabms *m/z* $[M+K]^+$ 653, $[M+Na]^+$ 637, $[M+H]^+$ 615, $[M-Me]^+$ 599, $[M-OH]^+$ 597; eims *m/z* $[M-H_2O]^+$ 596, $[M-2H_2O]^+$ 578, $[M-H_2O-HOAc]^+$ 518, 478, 358, 254, 236, 122, 105 (base peak), 91, 77; hrms *m/z* 578.2522 (calcd for $C_{33}H_{38}O_9$, 578.2516); ^1H and ^{13}C nmr see Table 1.

ACETYLATION OF TAXCHININ A [**3**] TO THE DIACETATE **8**.—A mixture of 47 mg of taxchinin A [**3**] and 0.5 ml each of pyridine and Ac_2O was allowed to stand at room temperature overnight. Usual workup gave the residue, which was purified by preparative tlc [CH_2Cl_2-Me_2CO (95:5)] to yield 51 mg of taxchinin A diacetate [**8**] in 96% yield. Mp 186–188°; fabms *m/z* $[M+H]^+$ 699; eims *m/z* 580, 520, 460, 398, 236, 122, 105, 77; ^1H and ^{13}C nmr see Table 1.

Taxchinin B [**4**].—Colorless needles from MeOH: mp 176–178°; $[\alpha]^{20}D$ +7.40 (*c*=0.405, CH_2Cl_2). *Anal.* found C 65.79, H 6.49; calcd for $C_{44}H_{50}O_{14}$, C 65.84, H 6.23. Uv (EtOH) λ max (log ε) 218 (4.23), 223 (4.24), 2.80 (4.23) nm; ir (KBr) ν max 3450, 3060, 2975, 1740, 1640, 1370, 1240, 1160, 1030, 720 cm^{-1}; eims *m/z* $[M-H_2O]^+$ 784, $[M-HOAc]^+$ 724, $[M-PhCO_2H]^+$ 680, $[M-PhCO-PhCH{=}CHCO_2H]^+$ 549, 356, 252, 219, [cinnamic acid]$^+$ 148, [cinnamoyl]$^+$ 131, $[PhCO_2H]^+$ 122, $[PhCO]^+$ 105, 91, 77; hrms *m/z* 784.3075 (calcd for $C_{44}H_{48}O_{13}$, 784.3094), 680.2834 (calcd for $C_{37}H_{44}O_{12}$, 680.2833), 549.2346 (calcd for $C_{28}H_{37}O_{11}$, 549.2336); ^1H nmr see Table 2; ^{13}C nmr see Table 3; ^{13}C-^1H Long Range COSY see Table 3; NOESY see Figure 1.

Taxchinin C [**5**].—Colorless needles from *n*-hexane/Me_2CO, mp 212–214°; $[\alpha]^{20}D$ −45.6 (*c*=3.5, CH_2Cl_2). *Anal.* found C 67.59, H 6.17; calcd for $C_{47}H_{50}O_{14}$, C 67.30, H 5.97. Ir (KBr) ν max 3575, 3450, 3075, 2925, 1740, 1650, 1600, 1450, 1380, 1270, 1240, 1100, 700 cm^{-1}; eims *m/z* $[M-H_2O]^+$ 820, $[M-HOAc-H_2O]^+$ 760, $[M-PhCO_2H]^+$ 716, 598, 537, 476, 416, 356, 252, 210, $[PhCO_2H]^+$ 122, $[PhCO]^+$ 105, 91, 77, 60; fabms *m/z* $[M+H]^+$ 839; hrms *m/z* 716.2806 (calcd for $C_{40}H_{44}O_{12}$, 716.2831); ^1H nmr, COSY, and NOESY see Table 2; ^{13}C nmr see Table 3.

7-epi-19-Hydroxybaccatin III [**6**].—Colorless needles from Et_2O: mp 263–265°; $[\alpha]^{19}D$ −105.2 (*c*=0.135, $CHCl_3$). *Anal.* found C 61.28, H 6.54; calcd for $C_{31}H_{38}O_{12}$, C 61.79, H 6.31. Ir (KBr) ν max 3420, 3075, 2950, 1720, 1650, 1380, 1260, 1110, 1080, 1030, 720 cm^{-1}; eims *m/z* $[M-H_2]^+$ 600, $[M-H_2O]^+$ 584, $[M-2H_2O]^+$ 566, $[M-OAc]^+$ 543, $[M-OAc-H_2O]^+$ 525, 402, 342, 253, 191, 122, [base peak, PhCO]$^+$ 105, 91, 77; fabms *m/z* $[M+glycerin+H]^+$ 695, $[M+Na]^+$ 625, $[M+H]^+$ 603; hrms *m/z* 584.2249 (calcd for $C_{31}H_{36}O_{11}$, 584.2257); ^1H nmr, ^{13}C nmr, and COSY see Table 4.

10-Deacetyl-10-oxobaccatin V [**7**].—Colorless needles from Et_2O/*n*-hexane: mp 170–172°; $[\alpha]^{19}D$ −100.25 (c=0.40, $CHCl_3$); ir (KBr) ν max 3420, 3010, 2980, 2940, 1720, 1710, 1260, 1100, 1060, 1040 cm^{-1}; eims *m/z* $[M]^+$ 542, 524, 452, 420, 392, 314, 262, 217, 122, 105 (base peak), 91, 77; hrms *m/z* 542.2154 (calcd for $C_{29}H_{34}O_{10}$, 542.2152); ^1H and ^{13}C nmr see Table 4.

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