A New Taxane Diterpene from *Taxus yunnanensis*

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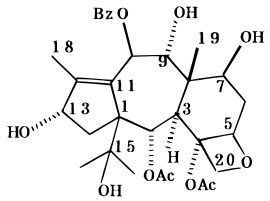
A new 11(15→1)-abeotaxane diterpene, 7,9-dideacetyltaxayuntin (**1**), and 11 known taxane diterpenes were isolated from the bark of *Taxus yunnanensis*. Their structures were determined primarily on the basis of analysis of their ^{1}H NMR, ^{13}C NMR, DEPT,^{1}H−^{1}H COSY, HMQC, HMBC, and mass spectra.

Taxus yunnanensis Cheng et L. K. Fu (Taxaceae) is an evergreen tree or shrub distributed in the wet valley area of Yunnan Province, Sichuan Province and Tibet Autonomous Region.[1] Previous studies on taxane diterpenes in the bark, leaves, and stems of *T. yunnanensis* have resulted in the isolation of over 30 taxane diterpenes.[2−11] Most of them[2,3,7,9] have the standard taxane skeleton with 6/8/6-membered rings, although eight of them[4−6,8,10,11] have the abeotaxane skeleton with 5/7/6-membered rings. We have recently isolated 12 taxane diterpenes from the bark of this plant. One of them is the new 11(15→1)-abeotaxane diterpene 7,9-dideacetyltaxayuntin (**1**). Four were identified as taxchin A, taxuchin A, baccatin VI, and baccatin IV, none of which has previously been found in this species. Taxane diterpenes which have previously been isolated from *T. yunnanensis* were 7-*epi*-10-deacetyltaxol, taxayuntin F, taxinine E, taxinine J, 1-hydroxybaccatin I, baccatin III, and taxol.



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Extraction of the bark of *T. yunnanensis* with MeOH, followed by extraction of the dried MeOH extract with CH_2Cl_2 and then solvent evaporation, gave a residue which was purified by repeated column chromatography on silica gel. Various components were crystallized with suitable solvents to give individual taxane diterpene compounds.

Compound **1** had the composition $C_{31}H_{40}O_{11}$ as deduced by a combination of FABMS, ^{13}C, DEPT, and ^{1}H NMR spectra. Its NMR spectra (Table 1) showed the presence of a taxane skeleton with four C-methyl groups (1.12, 1.17, 1.84, and 1.95 ppm), two acetoxyl methyl groups (2.01 and 2.15 ppm), four hydroxyl groups (1.80, 2.76, 3.55, and 4.18 ppm), and a benzoyloxy substituent.

Analyzing the ^{1}H−^{1}H COSY spectrum of compound **1**, a one-proton doublet signal at 6.41 ppm (J = 10.5 Hz) was correlated with a proton signal at 4.58 ppm (J = 10.5, 7.7 Hz) and assigned to H-10. The proton signal at 4.58 ppm was correlated with a hydroxyl proton signal at 4.18 ppm and assigned to H-9. A proton signal at 4.50 ppm was correlated with proton signals at 2.21 and 1.58 ppm and with the C-18 methyl proton signals at 1.95 ppm (long-range coupling) and also with a hydroxyl proton signal at 1.80 ppm. Therefore, the proton signal at 4.50 ppm is attached to C-13 (which must be hydroxylated), and proton signals at 2.21 and 1.58 ppm were assigned to H-14$\alpha\beta$. A one-proton doublet at 2.92 ppm is correlated with a proton signal at 5.94 ppm; the coupling (J = 7.7 Hz) was typical for the C-3 and C-2 protons,[12] and the chemical shift of H-2 indicated that C-2 is acylated. The chemical shifts of the characteristic proton resonances due to the oxetane moiety were virtually identical with a doublet at 4.94 ppm for H-5α, and signals for an A B system at 4.41 and 4.50 ppm for the C-20 methylene bridge.[13] Proton signals for H-6$\alpha\beta$ at 2.61 (m) and 1.75 ppm (m) were correlated with H-5 at 4.94 ppm and with the H-7 multiplet at 4.41 ppm (overlapped with one of H-20). No signals were correlated with the hydroxyl proton at 2.76 ppm.

An HMBC experiment was used to assign the quaternary carbons and the attachment of various ester functionalities. A correlation of the signal due to the benzoyl carbonyl at 165.26 ppm with that of H-10 at 6.41 ppm and the aromatic protons at 7.93 ppm *ortho* to the carbonyl group and correlation of the oxymethine at 6.41 ppm with C-1, C-11, and C-12 suggested the location of the benzoate group at C-10.[14] The two acetoxy groups were then reasonably placed at C-2 and C-4. The C-15 signal was shifted unusually downfield (δ 76.03) as compared with the corresponding signal in conventional taxane diterpenoids (δ ca. 43). This indicates that a hydroxyl group is located at C-15,[15] and the singlet resonance at 2.76 ppm must be assigned to this group. This signal for the C-1 OH group has never been observed in C-1 hydroxylated taxanes.[16] The remaining quaternary carbons at C-1, C-4, and C-8 resonated at δ 66.76, 80.33, and 43.01. No three-bond correlation between H_3-16 or H_3-17 and C-11 (HMBC experiment) was observed. All these data clearly suggested the rearranged 11(15→1)-abeotaxane skeleton[15] for compound **1**, which is a derivative of taxayuntin.[4,8]

The analysis of one- and multiple-bond ^{1}H−^{13}C correlations by HMQC and HMBC experiments allowed the unambiguous assignment of all ^{1}H and ^{13}C NMR signals and confirmed that C-2 and C-4 are acetoxylated while C-10 is benzoyloxylated.

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Table 1. ^{13}C- and ^{1}H-NMR Data for 7,9-Dideacetyltaxayuntin (**1**)

position	^{13}C	C type[a]	^{1}H[b]	$^{1}H-^{1}H$ COSY	$^{1}H-^{13}C$ COSY[c]
1	67.66	S			
2	68.17	D	5.94 (1H, d, 7.7)	C-3	X
3	44.19	D	2.92 (1H, d, 7.8)	C-2	X
4	80.33	S			
5	85.06	D	4.94 (1H, dd, 7.9, 2.3)	C-6	X
6	37.92	T	2.61 (1H, m), 1.75 (1H, m)	C-5, C-7	X
7	72.69	D	4.41 (1H, m)	C-6, C7-OH	X
			3.55, C-7-OH		
8	43.01	S			
9	78.38	D	4.58 (1H, dd, 10.5, 7.7)	C-10, C-9-OH	X
			4.18 (d, 7.7), C-9-OH		
10	71.56	D	6.41 (1H, d, 10.5)	C-9	X
11/12	135.11	S			
12/11	150.00	S			
13	77.66	D	4.50 (1H, m)	C-14, C13-OH	X
			1.80 (d, 7.5), C-13-OH	C-18	
14	39.71	T	2.21 (1H, m)	C-13	X
			1.58 (1H, m)		
15	76.03	S	2.76 (s), C-15-OH		
16	25.90	Q	1.17 (3H, s)		X
17	27.79	Q	1.12 (3H, s)		X
18	11.87	Q	1.95 (3H, s)	C-13	X
19	11.87	Q	1.84 (3H, s)		X
20	74.92	T	4.50 (1H, d, 7.9)		X
			4.41 (1H, d, 7.9)		
OAc	21.69	Q	2.01 (3H, s)		
	170.7	S			
OAc	22.0	Q	2.15 (3H, s)		
	171.2	S			
Ph−C=O	165.26	S			
q-Ph	129.6	S			
o-Ph	129.5	D	7.93 (2H, dd, 7.5, 1.5)	C-5′, C-4′	X
m-Ph	128.7	D	7.45 m	C-3′, C-5′	X
p-Ph	133.4	D	7.57 m	C-3′, C-4′	X

[a] S = C, D = CH, T = CH_2, Q = CH_3. Assignments made by DEPT technique. [b] Multiplicity and coupling constant in Hz in parentheses. [c] A X indicates that a $^{1}H-^{13}C$ correlation was observed in an HMQC spectrum.

Experimental Section

General Experimental Procedures. NMR spectra were obtained with a Bruker DRX 400 NMR spectrometer in $CDCl_3$: ^{1}H NMR (400.13 MHz), ^{13}C NMR (100.62 MHz), $^{1}H-^{1}H$ 2D COSY, $^{13}C-^{1}H$ COSY, HMQC, HMBC. The DEPT spectrum was obtained with a JEOL FX90Q spectrometer. The MS was obtained with a VG ZAB-HS MS. The UV spectrum was recorded with a Shimadzu UV-240 spectrophotometer. The melting point was determined on a thermolyne hot stage and is uncorrected.

Extraction and Isolation. Plant material was collected in Yunnan Province, People's Republic of China. A 7 kg sample of bark of *T. yunnanensis* was extracted 3× with MeOH. The MeOH extract was concentrated and diluted with H_2O. This liquid suspension was extracted with petrol (bp 60−90 °C) to remove lipid material and then 3× with CH_2Cl_2. The combined CH_2Cl_2 extracts were evaporated to dryness and yielded 75 g of crude yellow extract. The crude extract was mixed with 150 g of silica gel H and then subjected to vacuum chromatography on silica gel H, eluting with a gradient of petrol−ethyl acetate (10:0−4:6), to yield seven groups of compounds. The third, fourth, and fifth groups were further purified individually by column chromatography on silica gel with a gradient of CH_2Cl_2−acetone (100:0−100:2) or CH_2Cl_2−MeOH (100:0−100:5), and individual fractions were crystallized to give compound **1** (4.5 mg), taxchin A[17,18] (24 mg), taxuchin A[19] (1.24 g), baccatin VI[20,21] (28 mg), baccatin IV[21] (21 mg), 7-*epi*-10-deacetyltaxol[9] (42 mg), taxayuntin F[11] (324 mg), taxinine E[3] (156 mg), taxinine J[3] (28 mg), 1-hydroxybaccatin I[8] (2.34 g), baccatin III[3] (82 mg), and taxol[3] (242 mg). The known compounds **2**−**12** were identified by comparison of their MS and NMR data with literature values.

7,9-Dideacetyltaxayuntin (1). The title compound was obtained as colorless plates ($CHCl_3$): mp 266−268 °C; UV λ max (EtOH) nm 203, 228; FABMS *m/z* 589 $[MH]^+$, 467 $[MH - BzOH]^+$, 449 $[MH - BzOH - H_2O]^+$, 431 $[MH - BzOH - 2H_2O]^+$, 389 $[MH - BzOH - H_2O - AcOH]^+$, 329 $[MH - BzOH - H_2O - 2AcOH]^+$; ^{1}H and ^{13}C NMR see Table 1.

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