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J. Nat. Prod., **1994**, 57 (11), 1580-1583• DOI: 10.1021/np50113a020 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 11, pp. 1580-1583, November 1994

YUNANTAXUSIN A, A NEW 11(15→1)-*ABEO*-TAXANE FROM *TAXUS YUNNANENSIS*

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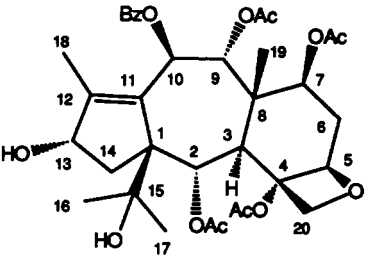
ABSTRACT.—Yunantaxusin A [**2**], an 11(15→1)*abeo*-taxane-type diterpene with an opened oxetane ring, has been isolated from the leaves and stems of *Taxus yunnanensis*. Its structure was established from its spectral data.

The diterpenoid natural product, taxol, first isolated from the bark of the Pacific Yew by Wall and his collaborators (1), is clinically active against ovarian and breast cancers (2). Because yields of taxol are very low and collection of the bark destroys the trees, a renewed interest in the isolation of related compounds from various *Taxus* species has been realized. *Taxus yunnanensis* Cheng et L.K. Fu (Taxaceae), an evergreen tree or shrub found in southern China, was reported to contain taxol in its bark (3). In the course of our continuing search for novel, potent antitumor agents (4), we have investigated the leaves and stems of this plant. This investigation has resulted in the isolation and characterization of a new *abeo*-taxane type diterpene, yunantaxusin A [**2**], along with a known *abeo*-taxane, compound **1** (5,6), and three additional known diterpenoids, 1β-hydroxybaccatin I, 10-deacetylbaccatin III, and baccatin III. We report herein on the isolation and characterization of **2**.



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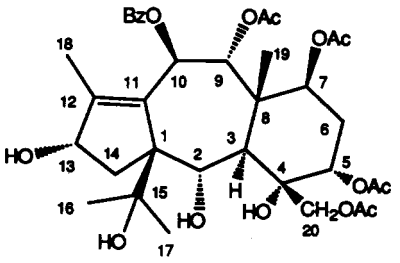

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Yunantaxusin A [**2**], colorless needles, mp 239–240°, gave a $[M-H]^-$ ion peak at *m/z* 689 in the negative fabms, which was 18 mass units greater than that of **1**. The molecular formula of **2** was established as $C_{35}H_{46}O_{14}$ by hrms. The ^{1}H-nmr spectrum of **2** (Table 1) was similar to that of **1**, showing signals due to four tertiary methyl groups (δ 1.20, 1.24, 1.36, and 2.06), assignable to the CH_3-8, $(CH_3)_2$-15, and CH_3-12 groups, respectively, four acetyl groups (δ 1.71, 2.03, 2.13, and 2.18), and a benzoyl group [δ 7.43 (2H, m), 7.56 (1H, m), and

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TABLE 1. ^{1}H-Nmr Data of Compounds **1** and **2** (in $CDCl_3$).

Proton	**1**	**2**		
	^{1}H	^{1}H	^{1}H-^{1}H COSY	nOe correlations
2	6.11, 1H, d, J=8.0 Hz	4.87, 1H, m	H-3	H-17
3	3.14, 1H, d, J=8.0 Hz	2.80, 1H, d, J=7.0 Hz	H-2	H-7
5	4.95, 1H, d, J=8.0 Hz	5.14, 1H, t, J=3.0 Hz	H_2-6	
6	1.88, 2.54, 1H each, m	1.97, 2H, m	H-5, 7	
7	5.54, 1H, t, J=8.0 Hz	5.51, 2H, dd, J=6.5 and 9.5 Hz	H_2-6	H-3, 10
9	6.14, 1H, d, J=11.0 Hz	5.97, 1H, d, J=10.5 Hz	H-10	H-2, 17
10	6.53, 1H, d, J=11.0 Hz	6.47, 1H, d, J=10.5 Hz	H-9	H-7, 18
13	4.45, 1H, d, J=7.5 Hz	4.58, 1H, m	H_2-14	
14	1.61, 2.25, 1H each, m	1.97, 1H, m 2.28, 1H, dd, J=7.5 and 14.5 Hz	H-13	
16	1.16, 3H, s	1.36, 3H, s		
17	1.06, 3H, s	1.24, 3H, s		
18	2.02, 3H, s	2.06, 3H, s		H-10
19	1.68, 3H, s	1.20, 3H, s		H-9, 20
20	4.40, 4.50 1H each, d, J=7.5 Hz	4.56, 4.73, 1H each, d, J=12 Hz		OAc-5, 20, H-19
2'	7.84, 2H, d, J=7.5 Hz	7.87, 2H, d, J=7.5 Hz	H-3'	
3'	7.41, 2H, m	7.43, 2H, m	H-2', 5'	
4'	7.54, 1H, m	7.56, 1H, m	H-3'	
OAc	1.75, 3H, s	1.71, 3H, s		
OAc	2.02, 3H, s	2.03, 3H, s		
OAc	2.08, 3H, s	2.13, 3H, s		
OAc	2.17, 3H, s	2.18, 3H, s		

7.87 (2H, d)]. The existence of a 15-hydroxy-11(15→1)-*abeo*-taxane skeleton in **2** was suggested by the presence of a downfield singlet (δ 69.3) for C-1 (6). At relatively low field, five oxygen-bearing methine signals and an oxygen-bearing methylene resonance were observed. The assignments of these resonances were established by analysis of the ^{1}H-^{1}H COSY nmr spectrum (Table 1), which indicated that **2** had the same oxygenation pattern as **1**.

The ^{13}C-nmr spectrum of **2** was different from that of **1**, especially in the region from 60 to 80 ppm (Table 2). The differences could be rationalized in terms of an opened oxetane ring moiety in **2**. This opened oxetane ring moiety, as well as the 11(15→1)-*abeo*-taxane skeleton in **2** was supported by the ^{1}H-^{13}C long-range COSY spectroscopic data. The methylene signals at δ 4.56 and 4.73 due to H_2-20 exhibited a ^{1}H-^{13}C long-range correlation with the carbonyl carbon resonance at δ 170.7, which was further coupled with the acetyl methyl signal at δ 2.13. This observation clearly indicated the presence of an acetyl group at C-20, thus confirming the presence of the opened oxetane ring moiety in **2**. The characteristic non-oxygenated carbon resonance at δ 69.3 was assignable to C-1 and was consistent with the existence of an 11(15→1)-*abeo*-taxane skeleton in **2**.

The location of the benzoyl group at C-10 was based on the observation that H-10 (δ 6.47) and the aromatic signal at δ 7.87 (2H, d, J=7.5 Hz) exhibited long-range ^{1}H-^{13}C COSY correlations with the same carbonyl carbon resonance at δ 164.2. One acetyl group was assigned at C-20 from the long-range ^{1}H-^{13}C COSY nmr spectroscopic data described above. The existence of the acetyl groups at C-7 and C-9 was easily established from the observation of analogous chemical shifts

TABLE 2. ^{13}C-Nmr Data of Compounds **1** and **2**.

Carbon	1		2	
	^{13}C	Long-range proton correlations	^{13}C	Long-range proton correlations
1	67.7 s	H-3, 10, 17	69.3 s	H-10, 16, 17
2	68.0 d		68.7 d	
3	44.0 d	H-19	43.8 d	H-5, 19
4	79.9 s	H-3, 20	75.9 s	H-3, 20
5	85.0 d	H-20	70.4 d	H-20
6	34.7 t		30.4 t	
7	70.3 d	H-9, 19	68.1 d	H-5, 19
8	43.7 s	H-9, 19	43.6 s	H-19
9	76.5 d	H-10, 19	76.4 d	H-10, 19
10	69.5 d	H-9	69.3 d	H-9
11	133.9 s	H-18	135.1 s	H-18, 2′,5′-Ar
12	151.5 s	H-18	150.2 s	H-18
13	77.0 d	H-18	77.0 d	
14	39.6 t		36.2 t	
15	75.5 s	H-16, 17	76.6 s	H-16, 17
16	27.5 q	H-17	26.8 q	H-17
17	25.2 q	H-16	27.9 q	H-16
18	11.8 q		12.0 q	
19	12.6 q		14.6 q	
20	74.9 t		64.6 t	
Benzoyl				
1′	133.3 s		129.2 s	2′,3′-Ar
2′	129.4 d		129.5 d	
3′	128.7 d		128.7 d	
4′	129.1 d		133.3 d	
COO	164.0 s	H-10, 2′-Ar	164.2 s	H-10
9-OAc	169.7 s, 20.7 q		170.2 s, 20.6 q	
7-OAc	170.3 s, 21.3 q		169.2 s, 21.3 q	
20-OAc	169.7 s, 21.5 q		170.7 s, 20.8 q	H-20
5-OAc	171.0 s, 22.3 q		171.4 s, 21.0 q	

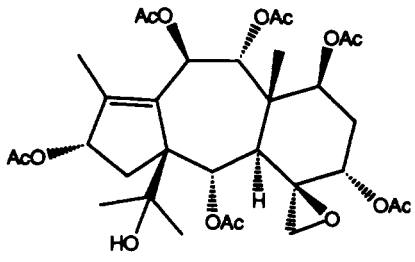
of H-7 and H-9 to those found in the ^{1}H-nmr spectrum of **1**. The remaining acetyl group was concluded to be at C-5, because the chemical shift of H-5 was almost identical with that found in 1β-hydroxybaccatin I (7).

The configurations of the hydroxy and/or ester groups at C-2, -7, -9, -10, and -13 were concluded to be α, β, α, β, and α, respectively, on the basis of analogous observed ^{1}H-nmr coupling constants to those found in **1**. The α-configuration of the C-5 acetate was easily assigned from the small coupling constant of H-5 in the ^{1}H-nmr spectrum, which was similar to that found in 1β-hydroxybaccatin I (7). The configuration of the acetoxymethyl group at C-4 was established to be β, from the observation of an nOe between the 8-CH_3 and H_2-20.

On the basis of the spectral evidence described above, the structure of **2** was thereby established.

Yunantaxusin A [**2**] is the first example of an 11(15→1)-*abeo*-taxane diterpene with an opened oxetane ring moiety obtained as a natural product. The oxetane ring at C-4 in taxane diterpenes is considered to be derived biogenetically from compounds with a 4(20)-oxirane ring through opening of the epoxide ring followed by closure to the oxetane ring (8,9). Taxane derivatives with an opened oxetane ring moiety have been isolated recently and have been considered to be precursors of taxanes with

an intact oxetane ring system (10). Compound **2** also appears to be a precursor to compound **1** and, thus, an intermediate between *abeo*-taxanes with a 4(20)-oxirane ring, such as taxuchin A [**3**] (11) and *abeo*-taxanes with an intact oxetane ring.



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EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler micro-melting point apparatus and are uncorrected. The ir spectra were recorded as KBr pellets on a Perkin-Elmer 1320 spectrophotometer. The uv spectra were measured on a Shimadzu 2101 PC spectrophotometer in absolute MeOH. Mass spectra were determined on a JEOL HX-110 mass spectrometer. ^{1}H- and ^{13}C-nmr spectra were measured on an Bruker AC-300 spectrometer with TMS as internal standard. Optical rotations were measured with a Rudolph research auto pol III polarimeter. Analytical tlc was carried out on Merck precoated Si gel F_{254} plates. Si gel H (2–25 μm, Aldrich) was used for cc under 20 pounds per square inch. Taxoids were detected by spraying with a 10% H_2SO_4 solution containing 1% $CeSO_4$, followed by heating.

PLANT MATERIAL.—The leaves and stems of *T. yunnanensis* were collected in Yunnan Province, People's Republic of China. A voucher specimen is deposited at the School of Pharmacy, Shanghai Medical University, Shanghai, People's Republic of China.

EXTRACTION AND ISOLATION.—The leaves and stems of *T. yunnanensis* (20 kg) were air-dried, ground, and extracted with EtOH. The EtOH extract was evaporated *in vacuo* to yield a dark red semi-solid. An equal amount of H_2O was added to the residue, and this solution was extracted with $CHCl_3$ five times. After evaporation of the $CHCl_3$ solution, the residue was suspended in Me_2CO-hexane (1:1). The solution was filtered and concentrated and the residue was treated with Et_2O. The Et_2O-soluble portion was filtered and concentrated to yield 262.6 g of residue. This residue was subjected to twofold chromatography on Si gel, employing a gradient of $CHCl_3$ to $CHCl_3$-MeOH (1:1) as eluent to give eight fractions according to tlc. The third fraction that eluted after baccatin III was further purified by Si gel chromatography with $CHCl_3$-MeOH (20:1) as eluent affording 45 mg of yunantaxusin A [**2**].

Yunantaxusin A [**2**].—Colorless needles (Me_2CO): mp 239–240°; $[\alpha]_D$ −52° (c=0.11, MeOH); uv (MeOH) λ max (log ε) 203 (4.26), 230 (4.10) nm; ir ν max 3490, 1740, 1270, 1230 cm^{-1}; negative fabms m/z 689 $[M-H]^-$; positive fabms m/z 713 $[M+Na]^+$; hrfabms m/z calcd for $C_{35}H_{46}O_{14}Na$ 713.2785; found m/z 713.2783; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2.

ACKNOWLEDGMENTS

The authors thank Drs. K. Mihasi and T. Fujioka, Faculty of Pharmaceutical Sciences, Fukuoka University, for mass spectral data. This investigation was supported by grant CA-17625 from the National Cancer Institute awarded to K.-H. Lee.

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Received 4 May 1994