Two New Lignans from *Taxus yunnanensis*†

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The phytochemical investigation on the ethanolic extracts of the barks of *Taxus yunnanensis* (Taxaceae) led to the isolation of two new *neo*-lignans, named taxuyunins A (1) and B (2), along with the known diterpenolignan, brevitaxin (3). Their structures were elucidated on the basis of spectroscopic and chemical evidences. Compound 1 is a *neo*-lignan xyloside while compound 2 belongs to the rare *neo*-lignan possessing a ramified C_3 side chain.

Keywords *Taxus yunnanensis*, Taxaceae, *neo*-lignans, taxuyunin A, taxuyunin B

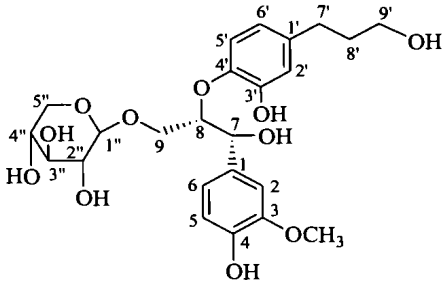
Introduction

Plants belonging to the genus *Taxus* (the yew trees) are known to produce taxane diterpenoids (taxoids) especially the famous anti-cancer paclitaxel (*Taxol*®).[1] In addition to the predominant taxoids, they also yield non-taxane compounds such as non-taxane diterpenoids, steroids, lignans, flavonoids, sugar derivatives and so on.[2-5] A series of new and known taxoids have been reported in our previous phytochemical work[6-16] on the roots and barks of *Taxus yunnenansis*, Cheng et L. K. Fu, a member of the genus *Taxus* and an evergreen tree endemic to Yunnan of China. Very recently, two new *neo*-lignans named taxuyunins A (**1**) and B (**2**), together with the known diterpenolignan, brevitaxin (**3**) (Scheme 1), were isolated and identified from the ethanolic extracts of the barks of *T. yunnanensis*. In this paper these new compounds are isolated and their structures are elucidated.

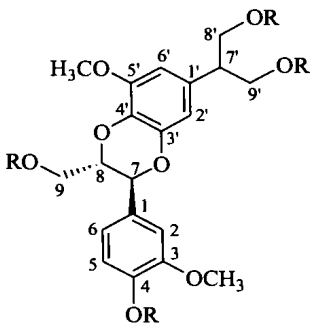
Results and discussion

Taxuyunin A (**1**) was isolated as white amorphous powders. Its negative FABMS showed $[M-H]^-$ at *m/z* 495 while positive FABMS displayed $[M+H]^+$ at *m/z* 497, indicating its molecular weight as 496, consistent with a molecular formula of $C_{24}H_{32}O_{11}$, which was confirmed by HRFABMS (found *m/z* 497.2061 $[M+H]^+$, cal-

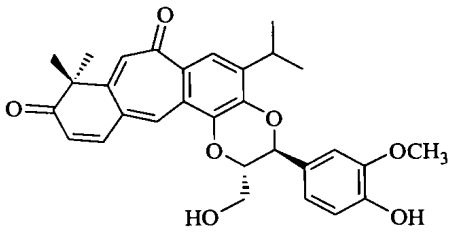
Scheme 1



1



2 R = H
2a R = Ac



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cd 497.2023). Its ^1H and ^{13}C NMR spectra (Tables 1, 2) indicated the presence of a methoxyl group [δ_H 3.80 (3H, s), δ_C 56.2 (q)] and a xylosyl moiety [δ_H 4.23 (d, J = 7.5 Hz, 1H, H-1″), 3.23 (t, J = 7.5 Hz, 1H, H-2″), 3.29—3.31 (m, 1H, H-3″), 3.45—3.51 (m, 1H, H-4″), 3.79—3.82 (m, 1H, H-5″a), 3.15 (t, J = 10.4 Hz, 1H, H-5″b), and δ_C 105.4 (d, C-1″), 75.0 (d, C-2″), 77.7 (d, C-3″), 71.2 (d, C-4″), 66.9 (t, C-5″)]. Besides, two 1,3,4- trisubstituted phenyl groups, two methlenes, two oxygen-bearing methylenes and two oxygen-bearing methines were also observed. These spectral features suggested that compound **1** was a mono-methylated derivative of a lignan xyloside. 2D NMR (^1H-^1H COSY, HMQC, HMBC and ROESY) analysis (Table 3) of **1** indicated that the aglycone of **1** was identical with a previously known *neo*-lignan, 3-methoxy-8,4′-oxyneoligna-3′,4,7,9,9′-pentol.[17] In the HMBC spectrum, the ^1H-^{13}C long-range correlations between C-9 [δ 68.8 (t)] and the anomeric H-1″ [δ 4.23 (d, J = 7.5 Hz, 1H)] and the anomeric C-1″ [δ 105.4 (d)] and H_2-9 [δ 3.76 (d, J = 4.6 Hz, 2H)] allowed the assignment of the xylosyl group at C-9. The relatively small coupling constant between H-7 and H-8 (J = 5.7 Hz) suggested an erythro-configuration in the glycerol chain.[17] Compound **1** thus was formulated as *rel*-(7*R*,8*S*)-3-methoxy-9-xylosyl-8,4′-oxyneoligna-4,7,3′,9′-tetrol (Scheme 1), and named taxuyunin A.

Table 1 ^1H NMR data (δ) of compounds **1**, **2** and **2a**

Proton	**1**[a,d]	**2**[b]	**2a**[c]
H-2	7.02 (d, 1H, J = 2.0 Hz)	7.56 (s, 1H)	7.33 (s, 1H)
H-5	6.74 (d, 1H, J = 8.1 Hz)	7.35—7.37 (overlap, 1H)	7.29 (d, 1H, J = 8.1 Hz)
H-6	6.86 (dd, 1H, J = 2.0, 8.1 Hz)	7.35—7.37 (m, 1H)	7.16—7.20 (m, 1H)
H-7	4.97 (d, 1H, J = 5.7 Hz)	5.43 (d, 1H, J = 8.1 Hz)	5.11 (d, 1H, J = 7.8 Hz)
H-8	4.22—4.26 (m, 1H)	4.29—4.34 (m, 1H)	4.44—4.49 (m, 1H)
H-9	3.76 (d, 2H, J = 4.6 Hz)	4.22 (d, 1H, J = 12.4 Hz)	4.50 (dd, 1H, J = 3.0, 12.0 Hz)
		3.88 (dd, 1H, J = 3.2, 12.5 Hz)	4.18 (dd, 1H, J = 4.3, 12.2 Hz)
H-2′	6.68 (d, 1H, J = 2.2 Hz)	7.05 (1H, s)	6.94 (s, 1H)
H-5′	6.87 (d, 1H, J = 8.3 Hz)	—	—
H-6′	6.55 (dd, 1H, J = 2.2, 8.3 Hz)	6.87 (s, 1H)	6.76 (s, 1H)
H-7′	2.54 (t, 2H, J = 7.7 Hz)	3.44 (qui, 1H, J = 6.3 Hz)	3.46 (qui, 1H, J = 6.7 Hz)
H-8′	1.73—1.78 (m, 2H)	4.43—4.48 (m, 1H)	4.55—4.59 (m, 2H)
		4.33—4.38 (m, 1H)	
H-9′	3.53 (t, 2H, J = 6.8 Hz)	4.43—4.48 (overlap, 1H)	4.55—4.59 (2H, overlap)
		4.33—4.38 (overlap, 1H)	
3-OCH_3	3.80 (s, 3H)	3.73 (s, 3H)	3.75 (s, 3H)
5′-OCH_3	—	3.77 (s, 3H)	3.76 (s, 3H)
OAc	—	—	2.26 (s, 3H, 4-OAc)
			1.98 (s, 6H)
			1.94 (s, 3H)

[a] Recorded at 500 MHz in CD_3OD. [b] Recorded at 400 MHz in pyridine-d_5. [c] Recorded at 500 Hz in pyridine-d_5. [d] Signals of the xylosyl moiety: δ_H 4.23 (d, J = 7.5 Hz, 1H, H-1″), 3.23 (t, J = 7.5 Hz, 1H, H-2″), 3.29—3.31 (m, 1H, H-3″), 3.45—3.51 (m, 1H, H-4″), 3.79—3.82 (m, 1H, H-5″a), 3.15 (t, J = 10.4 Hz, 1H, H-5″b).

Table 2 ^{13}C NMR data (δ) of compounds **1** and **2**

Carbon	**1**[a,c]	**2**[b]	Carbon	**1**[a,c]	**2**[b]
C-1	133.8 (s)	126.2 (s)	C-1′	138.5 (s)	134.9 (s)
C-2	111.7 (d)	112.3 (d)	C-2′	117.2 (d)	110.1 (d)
C-3	149.0 (s)	148.8 (s)	C-3′	148.8 (s)	145.3 (s)
C-4	147.2 (s)	148.8 (s)	C-4′	145.5 (s)	128.9 (s)
C-5	115.9 (d)	116.6 (d)	C-5′	118.6 (d)	149.6 (s)
C-6	120.6 (d)	121.6 (d)	C-6′	120.6 (d)	106.2 (d)
C-7	73.8 (d)	77.0 (d)	C-7′	32.5 (t)	52.0 (d)
C-8	85.5 (d)	79.8 (d)	C-8′	35.5 (t)	65.0 (t)
C-9	68.8 (t)	61.5 (t)	C-9′	62.3 (t)	65.0 (t)
3-OCH_3	56.2 (q)	56.0 (q)	5′-OCH_3		56.0 (q)

[a] Recorded at 125 MHz in CD_3OD. [b] Recorded at 100 MHz in pyridine-d_5. [c] Signals of the xylosyl moiety: δ_C 105.4 (d, C-1″), 75.0 (d, C-2″), 77.7 (d, C-3″), 71.2 (d, C-4″), 66.9 (t, C-5″).

Table 3 2D NMR data of compounds **1** and **2** (**1** in CD_3OD and **2** in pyridine-d_5)

Proton	1		2		
	COSY	HMBC	COSY	ROESY	HMBC
H-2	H-6	C-1,4,6,7	H-6	H-7,8,3-OCH_3	C-1,3,4,6,7
H-5	H-6	C-1,3,4	H-6		C-1,4
H-6	H-2,5	C-2,5,7	H-2,5	H-7,8	C-1,2,4
H-7	H-8	C-1,2,6,8,9	H-8	H-2,6,9	C-1,2,6,8
H-8	H-7,9	C-1,7,4′	H-7,9	H-2,6,9	C-7
H-9	H-8	C-7,8,1″	H-8	H-7	C-7,8
H-2′	H-6′	C-3′,4′,6′,7′	H-6′	H-7′,8′,9′	C-3′,4′,6′,7′
H-5′	H-6′	C-1′,3′,4′			
H-6′	H-2′,5′	C-2′,4′,7′	H-2′	H-7′,8′,9′,5′-OCH_3	C-2′,4′,5′,7′
H-7′	H-8′	C-1′,2′,6′,8′,9′	H-8′,9′	H-2′,6′,8′,9′	C-1′,2′,6′,8′,9′
H-8′	H-7′,9′	C-1′,7′,9′	H-7′	H-2′,6′,7′	C-1′,7′,9′
H-9′	H-8′	C-7′,8′	H-7′	H-2′,6′,7′	C-1′,7′,8′
H-1″	H-2″	C-9,3″,5″			
H-2″	H-1″,3″	C-1″,3″			
H-3″	H-2″,4″	C-2″,4″			
H-4″	H-3″,5″	C-3″,5″			
H-5″	H-4″	C-1″,3″,4″			
3-OCH_3		C-3		H-2	C-3
5′-OCH_3				H-6′	C-5′

Taxuyunin B (**2**) was obtained as white needles, m.p. 169 ℃. EIMS displayed $[M]^+$ at *m/z* 392, suggesting a molecular formula of $C_{20}H_{24}O_8$ based on HREIMS (found *m/z* 392.1468, calcd 392.1471) and its ^1H and ^{13}C NMR data (Tables 1, 2). It contained two methoxy groups from the NMR signals at δ_H 3.73, 3.77 (each 3H, s); δ_C 56.0 (q, 2 × C). The NMR spectra of **2** showed the presence of three oxymethylenes, three methines including two oxygen-containing ones, and twelve aromatic carbons arising from a 1,3,4-trisubstituted and a 1,3,4,5-tetrasubstituted phenyl groups. The 2D NMR spectra (Table 3) revealed that **2** was another lignan consists of two partial structures, 1-(3-methoxy-4-hydroxyphenyl)-propane-1,2,3-triol and 2-(3,4-dihydroxy-5-methoxyphenyl)-propane-1,3-diol. The former structure unit was similar to that of **1** while the latter one was different from the other unit of **1** due to the C_3 side chain in **2** was ramified. A pair of two-proton multiplet signals at δ_H 4.43—4.48 (H-8′a and H-9′a) and 4.33—4.38 (H-8′b and H-9′b) simultaneously showed ^1H-^1H correlations with a methine signal at δ_H 3.44 (qui, J = 6.3 Hz, 1H, H-7′), and HMBC cross peaks with carbon signals at δ_C 52.0 (d, H-7′) and 65.0 (t, 2 × C, C-8′ and C-9′), which confirmed the existence of the isopropane-1,3-diol moiety.

The molecular formula of **2**, corresponding to 9 degrees of unsaturation, suggested further loss of one H_2O between 7-OH and 3′-OH in **2**, and thus an 1,4-dioxane ring as occurred in compound **3** was formed to link the above-described structure units. Treatment of **2** with Ac_2O/pyridine (1∶1) gave the tetra-acetylated derivative **2a**. ^1H NMR spectrum of **2a** demonstrated three aliphatic acetyls at δ_H 1.94 (s, 3H) and 1.98 (s, 6H) and an aromatic acetyl at δ_H 2.26 (s, 3H). HMBC spectrum of **2a** established the four acetyls to be at C-9, C-8′ and C-9′ respectively, which further supported the above deduction.

In the ROESY spectrum (Table 3) of **2**, correlations between H-7 and H_2-9, H-8 and H-2, H-8 and H-6 were clearly shown, which together with the consideration of the large coupling constant between H-7 and H-8 (J = 8.1 Hz), assigned the *trans* stereochemistry between H-7 and H-8. Accordingly, compound **2** was identified as *rel*-(7*S*, 8*S*)-3, 5′-dimethoxy-7, 3′-epoxy-8, 4′-oxyneoligna-4, 9, 8′,9′-tetrol (Scheme 1), and named taxuyunin B.

Although an irregular phenylpropanoid, 2-(3-methoxy-4-hydroxyphenyl)-propane-1,3-diol, which is very similar to the partial structure of compound **2**, has been isolated from *Apollonias barbujana*[18] and *Juniperus phcenicea*[19] by different research groups, compound **2** is hitherto an unprecedented example of natural *neo*-lignan bearing a ramified C_3 side chain.

Through comparison of the EIMS and ^1H and ^{13}C NMR data of **3** with those values reported in the literatures,[20,21] compound **3** was identified as the known diterpenolignan, brevitaxin (Scheme 1).

Experimental

General procedures

Melting point was performed on an XRC-1 micromelting point apparatus and was uncorrected. 1D and 2D NMR experiments were carried out either on a Bruker AM-400 or DRX-500 spectrometer. Chemical shifts (δ) were given with reference to the solvent signals. EIMS and HREIMS

were taken on a VG Auto Spec-3000 or a Finnigan MAT 90 instrument. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectral data were obtained on a UV 210A spectrometer. Optical rotations were carried out on a HORIBA SEPA-300 High Sensitive Polarimeter or Perkin-Elmer model 241 Polarimeter. Column chromatogrphy was realized either on silica gel (200—300 mesh, Qingdao Marine Chemical Inc., China), or Lichroprep RP_{18} gel (40—63 μm, Merck, Darmstadt, Germany). Fractions were monitored by TLC on silica gel and spots were visualized by heating plates sprayed with 10% H_2SO_4 in EtOH.

Planta material

The bark of *Taxus yunnanensis*, Cheng et L. K. Fu (Taxaceae) was collected in Lijiang Prefecture of Yunnan Province of China. A voucher specimen (No. YAF-97-18) has been deposited at the Yunnan Academy of Forestry, Kunming, China.

Extraction and isolation

Dried bark (50 kg) was milled and extracted by maceration in EtOH for one week, the extract was concentrated *in vacuo* to a syrup, diluted with H_2O and partitioned with $CHCl_3$. The $CHCl_3$ layer was evaporated *in vacuo* to afford a residue (500 g), which was absorbed on 800 g of Si gel and chromatographed on a pre-packed (2 kg) silica gel column. Gradient elution was accomplished with $CHCl_3$-Me_2CO (10:0, 9:1, 8:2, 7:3, 0:10, *V*:*V*). From the $CHCl_3$-Me_2CO/9:1 (*V*:*V*) eluate, compound **3** (25 mg) was crystallized directly. The $CHCl_3$-Me_2CO/7:3 (*V*:*V*) eluate (7.2 g) was rechromatographed on silica gel column (150 g), eluting with $CHCl_3$-*i*-PrOH (9:1, *V*:*V*) to afford 12 fractions. Fractions 8—12 were combined (4.0 g) and further chromatographied over silica gel (150 g) eluting with $CHCl_3$-MeOH (7:1, *V*:*V*), and over RP_{18} silica gel (100 g) eluting with MeOH-H_2O (1:1, *V*:*V*) to yield compounds **1** (30 mg) and **2** (8 mg).

Taxuyunin A (**1**) Colorless amorphous solid, $[\alpha]_D^{28.5}$ + 58.33 (*c* 0.15, CH_3OH); ^1H NMR data see Table 1; ^{13}C NMR data see Table 2; IR (KBr) ν: 3449, 2967, 2876, 1653, 1447, 1381, 1364, 1336, 1283, 1262, 1234, 1143, 1103, 1056, 1080, 1026, 912, 882, 846, 686, 616, 535 cm^{-1}; Negative FABMS *m/z* (%): 495 $[M-H]^-$, 369 (6), 339 (6), 327 (10), 325 (10), 311 (7), 297 (2), 277 (20), 184 (100), 166 (32), 152 (18), 138 (3), 127 (4), 110 (4), 92 (76), 89 (7), 60 (12); Positive FABMS *m/z* (%): 497 [$(M+H)^+$, 27], 479 (43), 465 (4), 447 (7), 430 (10), 347 (29), 329 (18), 299 (15), 264 (100), 248 (10), 212 (83), 194 (41), 163 (69), 115 (4), 69 (30); Positive HRFABMS calcd for $C_{24}H_{32}O_{11}$ 497.2023 $[M+H]^+$, found 497.2061.

Taxuyunin B (**2**) White needles, m.p. 169 ℃, $[\alpha]_D^{15.6}$ + 5.62 (*c* 0.40, CH_3OH); UV (MeOH) λ_{max} (log ε): 280 (4.1), 231 (4.7), 208.5 (5.2) nm; ^1H NMR data see Table 1; ^{13}C NMR data see Table 2; IR (KBr) ν: 3479, 2946, 2895, 1603, 1516, 1460, 1370, 1279, 1226, 1162, 1123, 1064, 1035, 997, 903, 864, 825, 781, 765, 726, 652 cm^{-1}; EIMS (70 eV) *m/z* (%): 392 ($[M]^+$, 55), 374 (14), 360 (3), 341 (7), 297 (7), 271 (3), 255 (10), 225 (22), 214 (30), 193 (7), 180 (95), 166 (39), 149 (60), 137 (100), 124 (73), 105 (49), 91 (58), 77 (52), 65 (38), 55 (50); HREIMS calcd for $C_{20}H_{24}O_8$ 392.1471 $[M]^+$, found 392.1468.

Acetylation of **2** A solution of **2** (2 mg) was dissolved in 0.3 mL pyridine, then treated with 0.3 mL Ac_2O for 24 h at room temperature. Usual work-up of the reaction mixture provided 3 mg **2a**. 4,9,8′,9′-Tetraacetyl-taxuyunin B (**2a**): white amorphous solid, $C_{28}H_{32}O_{12}$; ^1H NMR data see Table 1; EIMS (70 eV) *m/z* (%): 560 ($[M]^+$, 93), 518 (16), 500 (100), 458 (33), 398 (34), 309 (18), 249 (28), 238 (21), 222 (86), 179 (34), 151 (23), 131 (31), 91 (26).

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