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Three New Taxanes from the Roots of *Taxus yunnanensis*

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Three new taxane type diterpenoids, taxuyunnanine B, C and D were isolated together with the known taxusin, 2α-deacetoxytaxinine J and taxinine J from the roots of *Taxus yunnanensis* and their structures were elucidated by spectroscopic means.

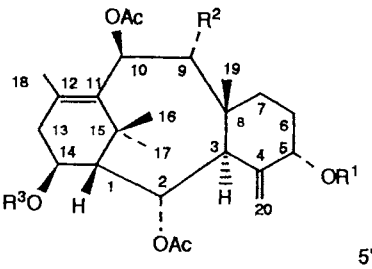
The excellent anticancer activity of taxol has prompted organic chemists to investigate *Taxus* species and this situation has led to the isolation of more than 100 natural taxanes. Recently, we reported the structure of taxuyunnanine A which was isolated from the ethereal extract of *T. yunnanensis.* This compound showed cytotoxity comparable to that of taxol.[1] Further investigation on the same extract led to the isolation of three new taxanes, taxuyunnanine B (**1**), C (**2**) and D (**3**) together with three known ones, taxusin,[2] 2α-deacetoxytaxinine J[3] and taxinine J.[4] Here we report the structural elucidation of the new compounds by spectroscopic means.

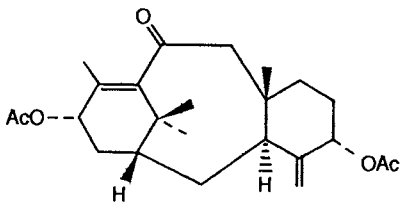
Taxuyunnanine B (**1**), $C_{33}H_{48}O_{10}$,[5] $[\alpha]_D$ +58.2° ($CHCl_3$, *c* 1.48) showed ^1H- and ^{13}C NMR signals (Tables 1 and 2) which strongly suggested that **1** was the acetate of taiwanxan (**4**)[6] or its positional isomer of the ester groups. The location of the ester groups (four acetyl groups and a 2-methylbutanoyl group) was then examined by ^1H-^{13}C long range COSY. Although a direct correlation between the carbonyl carbon of 2-methylbutanoyl group and H-14 could not be observed, the long range correlations between the carbonyl carbons of the 2-, 5-, 9- and 10-acetoxyl groups and the protons at C-2, 5, 9 and 10 were clearly demonstrated, which indicated that the group is positioned at C-14. Thus the structure of taxuyunnanine B was determined to be **1** except for the stereochemistry at C-2' which is remained unclarified.

Table 1. ^{1}H NMR data for taxuyunnanines B (1), C (2) and D (3) in $CDCl_3$ (400 MHz, δ in ppm from TMS and *J* values in Hz)

proton	1	2	3
H-1	1.93 (d, 2.4)	1.91 (br.d, 2.0)	2.01 (m)
H-2	5.43 (dd, 6.8, 2.4)	5.35 (dd, 6.4, 2.0)	1.77-1.87 (m)
H-3	2.99 (d, 6.8)	2.93 (d, 6.4)	3.08 (d, 5.9)
H-5	5.32 (br.s)	5.29 (br.s)	5.36 (br.s)
H-6a	1.85 (m)	~1.81 (m)	~1.75 (m)
H-6b	~1.75 overlap	~1.81 (m)	~1.75 (m)
H-7a	~1.72 overlap	1.24 (m)	~1.77 (m)
H-7b	~1.72 overlap	1.96 (m)	~1.29 (m)
H-9a	5.81 (d, 10.3)	~2.38 overlap	2.98 (d, 16.1)
H-9b		~1.64 overlap	2.34 (d, 16.1)
H-10	6.02 (d, 10.3)	6.06 (dd, 12.0, 5.6)	
H-13a	2.90 (dd, 19.0, 9.3)	2.82 (dd, 19.0, 9.3)	5.89 (br.t, 8.8)
H-13b	2.39 (dd, 19.0, 4.9)	~2.42 overlap	
H-14a	4.97 (dd, 9.3, 4.9)	4.99 (dd, 9.3, 4.4)	2.76 (td, 14.1, 9.8)
H-14b			1.21 (dd, 14.1, 6.3)
H_3-16	1.74 (s)	1.66 (s)	1.36 (s)
H_3-17	1.13 (s)	1.12 (s)	1.17 (s)
H_3-18	2.15 (s)	2.09 (br.s)	1.91 (d, 1.5)
H_3-19	0.86 (s)	0.84 (s)	0.80 (s)
H-20a	5.34 (s)	5.27 (s)	5.22 (s)
H-20b	4.85 (s)	4.86 (s)	4.87 (s)
2-OAc	2.024 (s) [a]	2.02 (s)	
5-OAc	2.20 (s)	2.17 (s)	2.17 (s)
9-OAc	2.020 (s) [a]		
10-OAc	2.05 (s)	2.05(s) [b]	
13-OAc			2.09 (s)
14-OAc		2.06 (s) [b]	
H-2'	2.33 (m)		
H-3'a	1.65 (m)		
H-3'b	1.46 (m)		
H_3-4'	0.89 (t, 7.6)		
H_3-5'	1.11 (d, 7.3)		

a-b) The assignments may be reversed.





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1. R^1=Ac ; R^2=OAc ; R^3= $-\overset{\overset{O}{\|}}{C}-\overset{\overset{5'\ CH_3}{|}}{CH}-CH_2CH_3$ (1' 2' 3' 4', α-MB)
2. R^1=R^3=Ac ; R^2=H
4. R^1=H ; R^2=OAc ; R^3=α-MB
5. R^1=Ac ; R^2=H ; R^3=α-MB

Table 2. ^{13}C NMR data[a] for taxuyunnanines B (1), C (2), D (3) in $CDCl_3$ (100 MHz, δ in ppm from TMS)

Carbon	1	2	3
C-1	58.85 d	58.91 d	40.03 d
C-2	69.90 d	70.52 d	29.17 t
C-3	44.20 d	42.06 d	36.74 d
C-4	141.44 s	142.28 s	145.88 s
C-5	78.25 d	78.22 d	76.06 d
C-6	28.38 t	28.86 t	28.20 t
C-7	27.30 t	33.78 t	36.65 t
C-8	44.40 s	39.65 s	39.74 s
C-9	76.74 d	43.86 t	58.69 t
C-10	72.49 d	70.06 d	205.40 s
C-11	132.75 s	135.33 s	135.08 s
C-12	137.43 s	134.73 s	149.30 s
C-13	39.70 t	39.45 t	69.93 d
C-14	69.84 d	70.52 d	32.02 t
C-15	37.00 s	37.29 s	37.55 s
C-16	25.88 q	25.40 q	27.87 q
C-17	31.58 q	31.75 q	29.83 q
C-18	21.09 q	20.92 q	14.58 q
C-19	17.36 q	22.46 q	21.34 q
C-20	118.07 t	116.93 t	113.72 t
2-AcO-Me	21.32 q	21.42 q [c]	
2-AcO-C=O	169.68 s	169.73 s [d]	
5-AcO-Me	21.93 q	21.82 q	21.73 q
5-AcO-C=O	169.77 s	169.93 s	169.88 s
9-AcO-Me	21.09 q [b]		
9-AcO-C=O	170.01 s		
10-AcO-Me	20.79 q [b]	21.42 q [c]	
10-AcO-C=O	170.06 s	170.19 s [d]	
13-AcO-Me			21.34 q
13-AcO-C=O			170.32 s
14-AcO-Me		21.45 q [c]	
14-AcO-C=O		170.01 s [d]	
C-1'	175.68 s		
C-2'	41.06 d		
C-3'	26.76 t		
C-4'	11.63 q		
C-5'	16.61 q		

a) Assignments were based on DEPT, ^{1}H-^{13}C-COSY and HMBC, and comparisons of the data with those of related compounds.

b-d) Assignments may be interchanged.

Taxuyunnanine C (**2**), $C_{28}H_{40}O_8$,[5] $[\alpha]_D$ +41.1° ($CHCl_3$, *c* 1.68), showed similar ^1H- and ^{13}C NMR spectra to those of yunnaxane (**5**)[7] except for the absence of the signals of 2-methylbutanoyl group and instead, the appearance of one more signal of the acetyl group, which suggested that all of the hydroxyl groups in **2** were acetylated. Therefore the structure of taxuyunnanine C was determined to be **2**.

Taxuyunnanine D (**3**), $C_{24}H_{34}O_5$,[5] $[\alpha]_D$ -61.0° ($CHCl_3$, *c* 0.66) contained a tetrasubstituted double bond, an exo-methylene group, a carbonyl group, and two secondary acetoxyl groups in the taxane skeleton as judged from its ^1H and ^{13}C NMR spectra (Tables 1 and 2). The locations of the two double bonds and an acetoxyl group were assigned at $\Delta^{4(20)}$ and Δ^{11}, and at 5α, respectively, based on the results of ^1H-^1H COSY spectrum and comparisons of data with those congeners. The location and stereochemistry of another acetoxyl group were assigned at C-13α by following the cross peaks [δ_H 1.91 → 5.89 → 2.76 → 1.21] in the ^1H-^1H-COSY spectrum and by the observed coupling constant (br.t, *J*=8.8 Hz) of the proton on the carbon having the acetoxyl group. The carbonyl group was positioned at C-10 on evidence of UV absorption [λ_{max} 248 nm (ε 2275)][8] and the appearance of H_2-9 signals as AB quartet [δ_H 2.34 and 2.98 (each 1H, *J*=16.1 Hz)]. Thus the structure of taxuyunnanine D was determined to be **3**. This compound is the natural taxane having the least oxygenated sites in its skelton (C-5, 10 and 13), and may help to deduce the biogenesis of natural taxanes.

The absolute stereochemistry of the new compounds were tentatively assigned as those of congeners such as yunnaxane (**5**) isolated from the same plant.[7]

The biological activities of these compounds are now under investigation.

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5) **1**: FABMS *m/z* 627 $(M+Na)^+$ and 605 $(M+1)^+$; **2** : HREIMS M. Found : 504. 2725. Calcd for $C_{28}H_{40}O_8$: 504. 2723 ; **3** : FABMS *m/z* 425 $(M+Na)^+$ and 403 $(M+1)^+$, HREIMS M. Found : 342. 2214 $(M-CH_3COOH).^+$ Calcd for $C_{22}H_{30}O_3$: 342. 2195.
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