FIRST EXAMPLE OF 11,12-EPOXYTAXANE-GLUCOSIDE FROM THE NEEDLES OF TAXUS CUSPIDATA

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Abstract: A new taxane-glucoside was isolated from the needles of *Taxus cuspidata*. The structure was established as 2α,9α,10β-triacetoxy-11,12-epoxytax-4(20)-en-13-one-5α-*O*-β-D-glucopyranoside (**1**) on the basis of 1D- and 2D spectral analyses.

Introduction

Novel mechanism, limited source and the clinical use of Taxol® (paclitaxel) against breast and ovarian cancers has spurred a worldwide thoroughly phytochemical study of *Taxus* species. Although more than 400 taxanes have been reported (1), the biosynthesis of taxol still unclear and there are still new taxanes waiting to be isolated (2). *Taxus cuspidata* is one of the most extensively investigated yews, more than 120 taxanes has been isolated from this species (3). As a continuation of our research on plants of *Taxus* genus (4), we re-examined the needles of *T. cuspidata* and isolated a new taxane-glycoside with 11,12-epoxy ring. Its structure was elucidated on the basis of the spectral evidences.

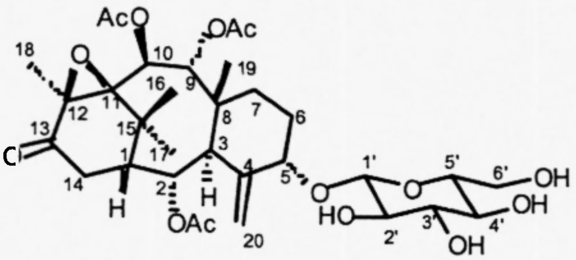

Figure-1 : A new epoxytaxane isolated from *T. cuspidata*.

Results and Discussion

Compound **1** (**Figure 1**) was isolated as amorphous powder from methanol extracts of *T. cuspidata* needles, $[\alpha]_D^{22}$ +49

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(*c* 0.07, MeOH). Its molecular formula was determined to be $C_{32}H_{46}O_{14}$ from combined analysis of the high-resolution FAB-MS (*m/z* 693.2523 $[M+K]^+$) and ^{13}C NMR spectral data, in which 32 carbon resonances were observed. The ^1H- and ^{13}C NMR spectra of compound 1 (**Table** 1) exhibited characteristic signals due to a taxane derivative including four tertiary methyl groups at δ_H 0.74 (3H, s, Me-16), 1.77 (3H, s, Me-17), 1.88 (3H, s, Me-18), and 0.95 (3H, s, Me-19), two of them were COSY-correlated peaks as geminal methyls (Me-16 and Me-17); three acetoxy groups resonated at δ_H 2.05, δ_C 20.3, 168.7; δ_H 2.03, δ_C 19.6, 169.3 and δ_H 2.01, δ_C 19.6, 168.6 respectively; an *exo*-cyclic methylene group at δ_H 5.33 (1H, br.s, H-20a), 5.21 (1H, br.s, H-20b), and δ_C 117.4 (C-20), 143.0 (C-4); C-3 ring junction proton at δ_H 3.25 (1H, d, J=5.5 Hz, H-3); and a ketone at δ_C 208.4. The ^1H NMR and HMQC spectra of 1 showed that a total of 42 protons were attached to carbons, indicating the presence of four hydroxyl groups. HMBC correlation of H_3-18 to ketonic carbon at δ_C 208.4 revealed that the ketone was located at C-13. The chemical shift and splitting patterns of H-14α and H-14β further supported the presence of the ketone group was located at C-13: H-14α and H-14β displayed a large geminal coupling constants at relative downfield region (δ_H 2.57, dd, J = 19.3, 9.0 Hz, H-14β; 2.71, d, J = 19.3 Hz, H-14α). Using H-14 α and H-14 β as reference, the spin system from H-14 βa/H-14 β to H-1 to H-2 to H-3 as well as long-range correlations of H-3 to H-20a/20b to H-5 and H-5 to H-6 to H-7 were readily interpreted from the ^1H-^1H COSY spectrum. A set of isolated AB system resonated at δ_H 5.99, which showed long-range HMBC correlations with C-7, C-8 and C-19, and 5.36, which exhibited two and three bond proton-carbon correlations with C-11, C-12 and C-15, were assigned to H-9 and H-10 (each 1H, d, J = 10.9 Hz), respectively. The chemical shifts of H-2, H-9 and H-10 suggested that three acetyl groups were attached to C-2, C-9 and C-10; this conclusion was further confirmed by HMBC experimental results, H-2, H-9 and H-10 displayed long-range correlations with corresponding carbonyl carbons. The remaining oxygenated proton signal at δ_H 4.30 (1H, br.s), which showed long-range correlation with H-20b in the ^1H-^1H COSY spectrum, was attributed to H-5. Additionally, an anomeric carbon signal at δ_C [illegible]s five oxygenated carbon signals between δ_C 61.9 and 77.6, and six hydrogen signals between δ_H 3.18 and 4.39 indicated the existence of one sugar moiety. The chemical shift, multiplicity, and coupling constant values indicated the presence of a glucopyranosyl unit as reported in the literatures (5-7). The coupling constant value of the anomeric proton H-1′ (J = 7.9 Hz) demonstrated the β-conformation of the glycosidic bond (8). Therefore, it was concluded that the sugar unit exhibited a β-glucosidic linkage to the aglycon. The HMBC correlation of H-1′ to C-5 provided a key evidence for connection of the glucosyl moiety to C-5. The ^1H-^1H COSY experiments allowed the sequential assignment of all the proton resonances within sugar residue, starting from the anomeric proton signal at δ_H 4.39. Additionally, the ^{13}C NMR spectrum of 1 showed two oxygenated tertiary carbon signals at δ_C 64.1 and 59.7 and these two signals were assigned to C-11 and C-12, respectively, from the HMBC spectrum. Judging from the ^{13}C NMR spectrum and molecular formula, only one oxygen atom and one double-bond equivalent were left as required by the molecular composition, thus the presence of an epoxide at C-11 and C-12 was suggested. This conclusion was in good agreement with observed the chemical shift of H-10, unusual up-field shift of H-10 was caused by the magnetic anisotropy effect of 11,12-epoxy ring. H-10 in 11,12-epoxide taxoids is similar to H-5 in the taxoids with C-4(20) oxirane ring, both of them dramatically shifted to up-field comparing with corresponding protons in the taxoids with C-4(20),11-didouble bonds (9,10). HMBC correlations of H-10/C-15, H_3-16/C-11, H_3-17/C-11 as well as H-2/C-8, H-3/C-19, H-3/C-20, H_3-19/C-3 implied that compound 1 had a 6/8/6-membered ring system. The assignments of the chemical shifts of all the carbons were secured with the help of HMQC and HMBC experiments. The relative stereochemistry of 1 was established from analysis of the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) data, chemical shifts and their coupling constants. The coupling constant between H-9 and H-10 (J = 10.9 Hz) and observed NOESY correlations of H-2/H-9, H-9/H_3-17 established a chair-boat conformation for ring-B, which was the typical conformation of natural taxoids. The β-orientation of H-2 and H-9 were assigned by NOESY correlation of H-2/H_3-17, H-19/H-9, and H-9/H_3-17. The α-orientation of H-10 was applied by the observation of NOESY correlation of H-10/H_3-18. H-5 was supposed as an α-orientation from the viewpoint of biosynthesis as in the most natural taxoids (1). The β-orientation of the epoxide group at C-11 and C-12 was established by NOESY correlation of H_3-18/H-3 and H-10. The up-field chemical shift of H_3-16 due to the presence of C-11,12 epoxide near H_3-16 and γ-effect between C-16 methyl group and 11,12-epoxide also suggested that epoxide group had a β-orientation (**Figure 2**). Taking all these spectral data into account, the structure of 1 was elucidated unequivocally to be 2α,9α,10β-triacetoxy-11,12-epoxytax-4(20)-en-13-one-5α-*O*-β-D-glucopyranoside.

Although several taxane-xyloside have been isolated from different yews since twenty years ago (1), the first taxane-glycoside had not been reported until 1995 (5). Recently several taxane-glycosides were isolated from different *Taxus* species (6-7). Compound 1 is the first example of taxane-glycoside with an 11,12-epoxide ring isolated so far from *Taxus* species (1,5,11).

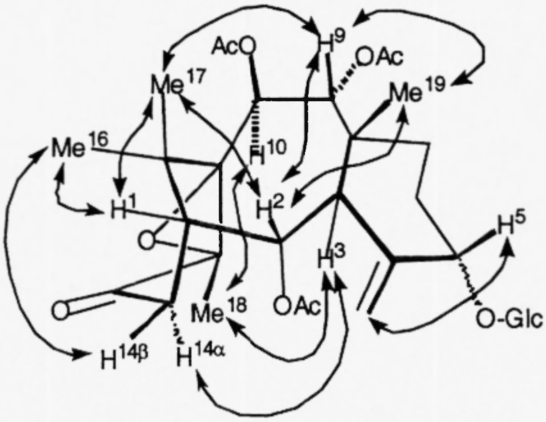

Figure-2 : The arrows showed selected NOE correlations of 1.

Acknowledgements

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Table-1 : ^{1}H- and ^{13}C NMR data of 1 (500 MHz for ^{1}H, 125 MHz for ^{13}C, acetone-d_6)

Position	δH, (mult,^{a} *J* value)	δC^{b}	HMBC (H→C)	NOESY
1	1.99 (o.m)	51.5		
2	5.78 (dd, 5.5, 1.7 Hz)	70.1	8, 14, 15, 168.7	1,^{w} 9,^{s} 17,^{s}19^{m}
3	3.25 (d, 5.5 Hz)	42.3	1, 2, 8, 19, 20	14a,^{m} 18^{m}
4	--	143.0		
5	4.30 (br.s)	83.2		20a,^{s} G-1'^{s}
6a 6b	1.98 (m) 1.76 (m)	28.8		
7a 7b	1.88 (m) 1.72 (m)	26.6		
8	--	43.2		
9	5.99 (d, 10.9 Hz)	76.3	7, 8, 10, 19, 169.3	2,^{s} 17,^{s} 19^{m}
10	5.36 (d, 10.9 Hz)	71.3	9, 11, 12, 15, 168.6	18^{s}
11	--	64.1		
12	--	59.7		
13	--	208.4		
14α 14β	2.71 (d, 19.3 Hz) 2.57 (dd, 19.3, 9.0 Hz)	38.1	1, 2, 13, 15 1, 2, 12, 13	3,^{m} 14b^{s} 1,^{w} 14a,^{s} 16^{w}
15	--	37.8		
16	0.74 (s)	28.3	1, 11, 15, 17-Me	1,^{w} 14b,^{w} 17^{s}
17	1.77 (s)	24.7	1, 11, 15, 16-Me	2,^{s} 9,^{s} 16^{s}
18	1.88 (s)	14.7	11, 12, 13	3,^{m} 10^{s}
19	0.95 (s)	17.5	3, 7, 8, 9	2,^{s} 9,^{s} 6b/7b^{s}
20a 20b	5.33 (s) 5.21 (s)	117.4	3, 4, 5 5	5,^{s} 20b^{s} 20a^{s}
2-OAc	2.05 (s)	20.3 168.7	168.7	
9-OAc	2.03 (s)	19.6 169.3	169.3	
10-OAc	2.01 (s)	19.6 168.6	168.6	
G-1'	4.39 (d, 7.9 Hz)	101.5	5	G-5',^{s} 5^{s}
G-2'	3.18 (o.t)	73.5		
G-3'	3.34 (o.m)	77.6		
G-4'	3.32 (o.m)	70.7		
G-5'	3.26 (o.m)	76.9		
G-6'a G-6'b	3.82 (d, 12.0 Hz) 3.63 (d, 12.0 Hz)	61.9		

^{a} Mult. multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; br, broad; m, multiplet; o. overlapping. The precision of the coupling constants is ± 0.5 Hz. ^{b} The ^{13}C chemical shifts were extracted from the HSQC and HMBC experiments (±0.2 ppm).

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