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Insignin A, A Novel C_{21}-Steroidal Aglycone from *Biondia insignis*

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Abstract: Insignin A, a new C_{21}-steroidal aglycone having the rare 15,16-*seco* pregnane skeleton was isolated from the acidic hydrolysis part of the 95% EtOH extract of *Biondia insignis*. It's structure was identified to be 15R,16-epoxy-3β,14β,16β-trihydroxy-15,16-secopregn-5-ene-20-one based on the spectral data.

Keywords: Asclepiadaceae, *Biondia insignis*, C_{21}-steroidal aglycone, insignin A.

The genus *Biondia* having six species is exclusively distributed in China. But none of them was investigated in the aspect of phytochemistry before this work. From the view of plant taxonomy, the genus *Biondia* in the Trib. Asclepiadeae is close to the genus *Cynanchum*, and the plants of the latter mainly contain C_{21}-steroids. In order to investigate whether the plants of the genus *Biondia* have C_{21}-steroidal compounds and what kind of skeleton they may have, the whole plant of *Biondia insignis* Tsiang (5.9 kg)[1] collected in Ma'erkang County, Sichuan Province was extracted with 95% EtOH. The alcoholic extract (460g) was separated into the petroleum ether and chloroform parts. The $CHCl_3$ portion (350g) was refluxed with 600mL of 0.5% H_2SO_4 EtOH solution for 1h to yield 70g of crude aglycones. The crude aglycones were chromatographically isolated over Si gel and reversed-phase C_{18} Si gel to afford two C_{21}-steroidal aglycones. One was a known 13,14/14,15-*diseco* C_{21}-steroid, glaucogenin C[2] and the other was a new aglycone named insignin A (**1**) which had a novel 15,16-*seco* C_{21}-steroidal skeleton. Here we presented the structure elucidation for **1** with the aid of 1D and 2D NMR data.

Compound **1** was determined to have the molecular formula $C_{21}H_{32}O_5$ by HREIMS analysis (*m/z* 364.2240 [M^+], calcd: 364.2250, Δ1.0mmu) and its unsaturation degree was six. The Lieberman-Burchard test on **1** showed positive reaction, indicating a C_{21}-steroid. The IR spectral data revealed the presence of hydroxyls (3429, 3385 and 3261cm^{-1}), carbonyl (1693cm^{-1}) and ether or acetal (1060, 1047 and 1027cm^{-1}). The ^{13}C NMR and DEPT spectra (see **Table 1**) showed that **1** had three methyls, seven methylenes, six methines and five quaternary carbons, revealing a pregnane backbone with a double bond at C5/C6 and a ketone at C20. In the HMBC, the acetyl methyl group at δ 2.31 (H21) had ^1H-^{13}C long-range correlations with C20 at δ 214.0 (s) and

C17 at δ 59.8 (s), and the angular methyl group at δ 1.20 (s) correlated with the carbons at δ 33.5 (C12), 37.9 (C13), 71.7 (C14) and 59.8 (C17), indicating a hydroxyl substitution at C14 rather than C17. The proton at δ 5.58 (H15) showed ^{1}H-^{13}C long-range correlations with carbon at δ 37.5 (C8), 37.9 (C13), 71.7 (C14) and 54.9 (C16), locating the hemiacetal at C15. The proton signals at δ 3.67 (d, 12.4) and 4.84 (dd, 4.2, 12.4) attributed to an oxygen-substituted methylene (C16) had ^{1}H-^{13}C long-range correlations with C15 and C20, and C13, C15, C17 and C20, respectively, further supporting the above conclusion and establishing the 15,16-*seco* of ring-D. Compared to purpnigenin[3], the chemical shifts for the C13 and C14 of **1** were 10.5 and 13.5ppm, respectively, lower than those in ref. 3. Moreover, the ^{13}C NMR data for C16, an oxygen-substituted methylene was about 10ppm lower than that for normal hydroxyl methyl groups such as the ones in glucose and glycerol. The extraordinary upfield shifts of C13, C14 and C16 indicated that all of them were shielded by their corresponding environment axial substitutions, revealing both 14- and 15-hydroxyl groups at axial positions (**Figure 1**). In purpnigenin[3], the ring-C was in a boat-conformation but in **1** it was chair-conformation, therefore, 4.3ppm of γ-shielding effects of 14-hydroxyl onto C10 was observed, further supporting the 14-hydroxyl at the axial position. The 4.2Hz of coupling constants of H17 indicated an "*ae*" coupling between H17 (equatorial) and H16β (axial), signing the acetyl at the α-position of C17. The NOE effects between the protons at δ 5.58 (H15) and 2.55 (H7β), δ 2.67 (H17β) and 1.20 (H18), and δ 6.52 (HO-14) and 1.20 (H18) confirmed the sterochemistry of C14, C15 and C17. Therefore, the structure of **1** was determined to be as shown in **Figure 1**.

Insignin A was one of the members of the *seco*-pregnane family consisted of four structure-types represented by glaucogenins A[4], B[4, 5], C[2] and D[5], and neocynapanogenin A[6] (13,14/14,15-*diseco*); atratogenins A and B[7] (14,15-*seco*); gracigenin[8] (8,14-*seco*) and insignin A (15,16-*seco*). The isolations of 13,14/14,15-*diseco*- and 14,15-*seco*-pregnanes resulted in the establishment of the genus *Vincetoxicum* from *Cynanchum.* The identification of gracigenin supported that *Adelostemma gracillimum* (used to be classified as *C. saccatum*[8]) was different from *Cynanchum* sp. as well. Therefore, the finding of insignin A from *B. insignis* provided chemical evidence to support the independence of the genus *Biondia* in the present taxonomy system.

Figure 1. The structure and the stereoview of **1**.

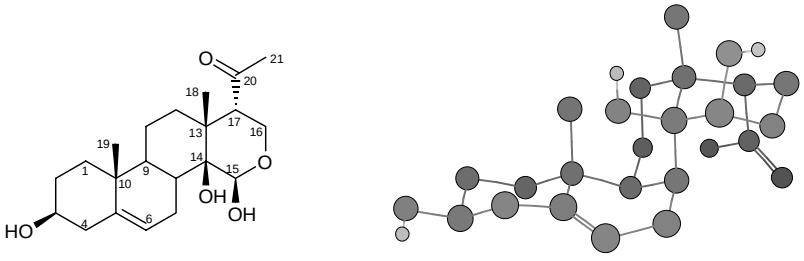

Table 1. The ^{1}H, ^{13}C NMR Assignments and ^{1}H-^{13}C Long-Range Correlation (HMBC) Data for **1**[a].

position	^{13}C	^{1}H[b]	HMBC
1α	38.1 (t)	1.22 (m)	C3, C5, C10, C19
1β		1.89 (dt, 3.2, 13.2)	
2β	32.6 (t)	1.85 (m)	C1, C3, C4, C10
2α		2.24 (m)	
3a	71.3 (d)	3.80 (m)	/
4	43.4 (t)	2.61 (m, 2H)	C2, C5, C6, C10
5	141.0 (s)	/	/
6	122.1 (d)	5.50 (d, 4.8)	C4, C7, C10
7β	25.6 (t)	2.55 (m)	C5, C6, C8, C9, C14
7α		2.61 (m)	
8α	37.5 (d)	2.27 (m)	C7, C9, C10, C11, C14, C15
9α	44.9 (d)	2.29 (m)	C8, C10, C11
10	37.8 (s)[c]	/	/
11	19.9 (t)	1.62-1.65 (m, 2H)	C8, C9, C10, C12, C13
12β	33.5 (t)	1.23 (m)	
12α		2.97 (dt, 6.4, 12.0)	C11, C13
13	37.9 (s)[c]	/	/
14	71.7 (s)	6.52 (s, HO-14β)	C8, C9 (weak), C13, C14
15α	98.1 (d)	5.58 (d, 4.1)	C8, C13, C14, C16
		8.31 (d, 4.1, HO-15β)	C14, C15
16α	54.9 (t)	3.67 (d, 12.4)	C15, C20
16β		4.84 (dd, 4.2, 12.4)	C13, C15, C17, C20
17β	59.8 (d)	2.67 (d, 4.2)	C12, C13, C14, C16, C20
18	19.5 (q)	1.20 (s, 3H)	C12, C13, C14, C17
19	19.4 (q)	1.07 (s, 3H)	C1, C5, C9, C10
20	214.0 (s)	/	/
21	31.7 (q)	2.31 (s, 3H)	C17, C20

[a] ^{1}H, ^{13}C NMR and HMBC spectra were obtained at 500MHz,125MHz and 500MHz, and recorded in C_5D_5N at room temperature, respectively.
[b] Coupling constants are presented in Hz. Unless otherwise indicated, all proton signals integrate to 1H.
[c] Data at each column were interchangeable.

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