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Structure Determination of a Sesquiterpene Lactone Isolated from *Nouelia Insignis* Franch by 2D NMR

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Abstract: A sesquiterpene lactone was isolated from *Nouelia insignis* Franch, and its structure was elucidated (compound **1**) by 2D NMR to be $\delta\beta$, 9-dihydro-onoseriolide. Complete assignment of the ¹H and ¹³C chemical shifts for the compound was obtained. The assignments of H-1 and H-2 of the compound previously reported were found incorrect, and the correct assignments were given. The results of this study may have some practical significance for the structural elucidation of onoseriolide sesquiterpene lactones.

Key words: NMR, chemical shift assignment, onoseriolide, *Nouelia insignis* Franch

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Introduction

Nouelia insignis Franch, an endemic plant and a monotype species of the genus *Nouelia* Franch, is only confined to Sichuan and Yunnan region, especially to Panzhihua City in China^[1]. The genera *Nouelia* and *Leucomeris* (Compositae) are cytologically related. *Nouelia* may be descendant of old plants, which was developed as an ornamental plant in People's Republic of China. *Leucomeris* Kurz, a sibling genus of *Nouelia* Franch, was used as anti-inflammatory medicinal herb to cure cough and snake bite^[2,3]. The phytochemical investigation on *N. insignis* has not been reported previously. Our recent phytochemical investigation on *N. insignis* has resulted in the isolation of a sesquiterpene lactone $\delta\beta$, 9-dihydro-onoseriolide (compound **1**) for the first time. $\delta\beta$, 9-di-

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hydro-onoseriolide, a eudesmanolide with a cyclopropane ring, was only isolated from *wunderlichia mirabilis* in 1980^[4], however, there have been no studies on its NMR signals assignment by 2D NMR. Thus, in this study, the ¹H NMR and ¹³C NMR chemical shifts of this compound were assigned using both 1D NMR and 2D NMR techniques, including ¹H NMR, ¹³C NMR, HSQC, HMBC and NOESY. The present paper describes the isolation and structure elucidation of this compound, and furthermore corrected an error on the assignment of H-1 and H-2 reported in the literature.

1 Experimental

1.1 Plant material

The aerial parts of *N. insignis* were collected in Yanbian County, Panzhihua City of Sichuan Province in May 2004 and identified by Prof. Fading Fu in Chengdu Institute of Biology, the Chinese Academy of Sciences (CAS). A voucher specimen (A-182) was deposited at the Herbarium of Chengdu Institute of Biology, CAS.

1.2 Instruments

The 1D, 2D NMR spectra were recorded on a Bruker Avance 600 spectrometer with TMS as an internal standard and CDCl₃ as solvent. Mass spectrum was obtained on a Finnigan-LCQ^{DECA} mass spectrometer (ESI-MS) and a Bruker Daltonics Bio-TOF-Q mass spectrometer (HRESIMS). The ¹H and ¹³C NMR spectra were operated at 600.13 MHz and 150.92 MHz respectively, and corresponding standard pulse sequences were given in the gHSQC, and gHMBC experiments, mean while, the NOESY spectra were operated with sweep widths of 7 183.908 Hz in F_1 (¹H) dimension and 7 183.908 Hz in F_2 (¹H) dimension respectively, and the pulse intervals D1 were 1.5 s and 2.0 s, the acquisition times (N S) were 4 and 8 respectively too. The gHSQC and gHMBC spectra were recorded at the conditions with sweep widths of 7 183.908 Hz in F_1 (¹H) dimension and 31 746.031 Hz in F_2 (¹³C) dimension respectively, at the same time the gHSQC experiment was performed at pulse interval D_1 of 2.5 s, ¹³C-¹H coupling constant 140 and N S of 4. In addition, the gHMBC experiment was performed at pulse interval D_1 of 2 s, the ¹³C-¹H long-range coupling constant of 8 Hz, the ¹³C-¹H long-range coupling signals and N S of 32.

1.3 Extraction and isolation

The air-dried and powdered aerial part of *N. insignis* (4.5 kg) was macerated with 95% ethanol (25 L×3, each 7 d) at room temperature. The solvents were removed under reduced pressure to give 354 g residue, which was suspended in H₂O (1.5 L) and then partitioned successively with petroleum ether (60~90 °C, 1.5 L×4), ethyl acetate (1.5 L×8) and n-butanol (1.5 L×5) to afford corresponding fractions A (34.3 g), B (56.5 g), and C (114.5 g). Fraction A was subjected to column chromatography on MCI gel (ϕ 5×L 60 cm, 50 g) eluted with MeOH-H₂O (3 : 2, 7 : 3, 4 : 1, 9 : 1, 19 : 1, 1 : 0, V/V, each 1.0 L) to yield six subfractions A₁~A₆. Compound **1** (6 mg) was

obtained from A₁ (1.3 g) separated by column chromatography on silica gel (ϕ 2×L 25 cm, 40 g) eluted with petroleum ether-acetone (10 : 1, V/V).

2 Results and Discussion

Compound **1** was obtained as white crystal with m. p. 174~176 °C from the mixture of petroleum ether and acetone. Fourteen peaks from eighteen protons (¹H) and fifteen peaks (¹³C) were observed in the ¹H and ¹³C NMR spectra, respectively which was in accordance with its molecular formula C₁₅H₁₈O₃ derived from the quasi-molecular ion peak at *m/z* 269.099 2 [M+Na]⁺ in the HR-ESIMS. The IR peaks at ν_{\max} 3 480, 1 748, 3 055 and 895 cm⁻¹ showed the presence of hydroxyl group, carboxyl group and exo-methylene. From the molecular formula and ¹H and ¹³C NMR data (Table 1), one hydroxyl group, one lactone group (δ_{C} 173.7) and an exo-methylene (δ_{H} 5.02 brs, 4.75 brs; δ_{C} 106.4) could be concluded. Therefore, compound **1** should be a sesquiterpene lactone.

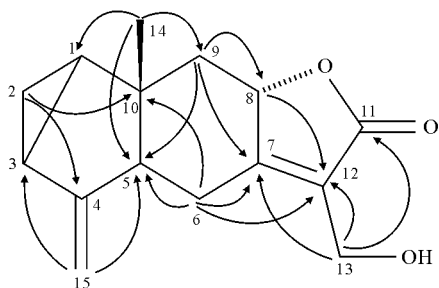


Fig. 1 The main correlation in the HMBC of compound **1**

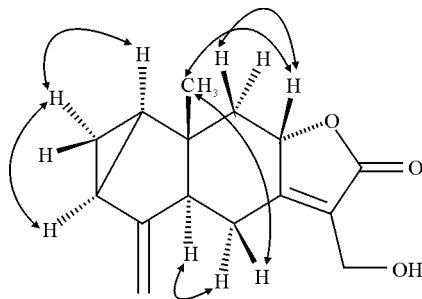


Fig. 2 The major NOE correlation of compound **1**

The ¹H NMR spectrum of compound **1** in CDCl₃ showed the signal for one methyl group at δ_{H} 0.78 (s, 3H), together with two exo-methylene protons at δ_{H} 5.02 and 4.75 (each 1H, br. s); irradiation of the broadened singlet at δ_{H} 1.97 changed the signals at δ_{H} 1.38, 0.91, 0.83 and collapsed the signals of the exo-methylene protons (H-15) to doublets, clearly indicating that these signals were those of the cyclopropane ring. In addition, the ¹³C NMR spectrum revealed one methyl signal at δ_{C} 17.3, signals of a cyclopropane ring at δ_{C} 26.8, 15.9 and 23.5, two exo-methylene carbon at δ_{C} 106.4 and 149.9, two double-bond carbon signals at δ_{C} 164.5 and 124.1, and one weak carbon signal of carbonyl group at δ_{C} 173.7, which confirm a tricyclic sesquiterpene having the lindenane carbon skeleton for compound **1**. Moreover, comparison of the ¹³C NMR of compound **1** with that of onoseriolide^[5] showed that compound **1** was obviously closely related to that of onoseriolide. However, in the ¹H NMR spectrum the presence of a double doublet at δ_{H} 5.10 (dd, $J_1 = 7.0$, $J_2 = 10.7$), which was coupled with two further double doublets, showed that the 8,9-double bond in onoseriolide was hydrogenated. All the ¹H NMR data (Table 1) fully testified this assumption. The full assignments of

proton and carbon signals and the stereochemistry of compound **1** were based on the analysis of ^1H NMR, ^{13}C NMR, HSQC, HMBC and NOESY spectra (Table 1).

Table 1 ^1H and ^{13}C NMR data of compound **1** (CDCl_3)

No.		HSQC			HMBC
C	H	δ_{H}	J/Hz	δ_{C}	
1	1	1.38	(td, $J_1=3.7$, $J_2=7.6$)	26.8	0.78 (H-14), 1.58 (H-9 β)
2	2 α	0.83	(dd, $J_1=3.7$, $J_2=8.7$)	15.9	0.78 (H-14)
	2 β	0.91	(dd, $J_1=3.7$, $J_2=8.7$)		
3	3	1.97	(br. s)	23.5	5.02, 4.75 (H-15, H-15'), 0.91, 0.83 (H-2 α , H-2 β)
4				149.9	0.91, 0.83 (H-2 α , H-2 β)
5	5	2.54	(dd, $J_1=3.0$, $J_2=13.7$)	63.9	5.02, 4.75 (H-15, H-15'), 2.11, 2.84 (H-6 α , H-6 β), 2.67, 1.58 (H-9 α , H-9 β), 0.78 (H-14)
6	6 α	2.84	(dd, $J_1=3.0$, $J_2=13.7$)	23.9	
	6 β	2.11	(t, $J=13.7$)		
7				164.5	4.40 (H-13), 2.11, 2.84 (H-6 α , H-6 β), 2.67 (H-9 α)
8	8	5.10	(dd, $J_1=7.0$, $J_2=10.7$)	80.2	2.67, 1.58 (H-9 α , H-9 β), 2.84 (H-6 β)
9	9 α	1.58	(t, $J=11.4$)	43.9	5.10 (H-8), 0.78 (H-14)
	9 β	2.67	(dd, $J_1=7.0$, $J_2=11.4$)		
10				38.4	0.78 (H-14), 0.91, 0.83 (H-2 α , H-2 β), 2.67, 1.58 (H-9 α , H-9 β), 2.11, 2.84 (H-6 α , H-6 β)
11				173.7	4.40 (H-13)
12				124.1	4.40 (H-13), 2.11, 2.84 (H-6 α , H-6 β), 5.10 (H-8)
13	13	4.40	(2H, d, $J=3.5$)	54.8	
14	14	0.78	(3H, s)	17.3	2.67, 1.58 (H-9 α , H-9 β)
15	15	5.02	(br. s)	106.4	
	15'	4.75	(br. s)		

* d: doublet; dd: doublet-doublet; td: triplet-doublet; s: singlet; br. s: broad singlet.

In the HSQC spectrum, δ_{H} 1.38 (td, $J_1=3.7$, $J_2=7.6$, H-1) was connected with δ_{C} 26.8, δ_{H} 0.91 (dd, $J_1=3.7$, $J_2=8.7$, H-2 α) and δ_{H} 0.83 (dd, $J_1=3.7$, $J_2=8.7$, H-2 β) both were connected with δ_{C} 15.9, δ_{H} 1.97 (br. s, H-3) was connected with δ_{C} 23.5; the above all also suggested the presence of the signals of a cyclopropane ring, and gave the exact assignment of each proton. However, from this experimental conclusion we found that there was an error in the attribution of H-1 and H-2 reported in the literature, in which δ_{H} 0.83 was attributed to be H-1, but it should be H-2 β , δ 0.91 and 1.38 were attributed to be H-2 α and 2 β , but they should be H-2 α and H-1. So we corrected these errors in order to afford the correct complete assignment of the ^1H and ^{13}C chemical shifts for this compound and provide some practical significance for the struc-

tural elucidation of onoseriolide sesquiterpene lactones.

In the HMBC spectrum, a cross peak of ^1H - ^{13}C long-range coupling was observed between the exo-methylene proton signals at δ 5.02 and 4.75 (H-15 and 15') and the carbon signals at δ 63.9 (C-5) and 23.5 (C-3), respectively which indicates that the exo-methylene was located at C-4 (δ 149.9). The tertiary methyl was located at C-10 from the HMBC correlations (Fig. 1) from H-14 (δ 0.78, 3H, s) to C-1 (δ 26.8), C-9 (δ 43.9) and C-5 (δ 63.9). The HMBC correlations from H-13 (δ 4.40, 2H, d, $J=3.5$) to C-7 (δ 164.5), C-12 (δ 124.1) and C-11 (δ 173.7) suggested that the hydroxyl group was at C-13. The detailed interrelations between ^1H - ^{13}C for the sesquiterpene portion are shown in Fig. 1 and Table 1.

The stereochemistry of compound **1** (Fig. 2) was revealed by the NOESY spectrum in which NOEs were observed between H-1 and H-2 α , H-3 and H-2 α . Therefore, H-1 and H-3 both were α configuration, which was also identical to that in onoseriolide reported in the literature^[5]. In the NOESY spectrum, there was correlation between H-8 and H-14 CH₃, but NOE between H-14 CH₃ and H-1 was not observed. This indicated that the stereochemistry of H-8 and H-14 CH₃ were the same but opposite to H-1, so both of them were β configuration. The detailed NOESY correlations of other protons of this sesquiterpene lactone are as shown in Fig. 2. Therefore, compound **1**, isolated from *Nouelia insignis* Franch for the first time, was determined to be 8 β , 9-dihydro-onoseriolide.

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2D NMR 对栝菊木中的一个倍半萜内酯进行结构解析

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摘 要: 通过核磁共振对一个首次从菊科单种属植物-栝菊木中分离得到的乌药烷型倍半萜内酯-8 β , 9-dihydro-onoseriolide 进行了结构解析, 通过 2D NMR (HSQC, HMBC, NOESY) 首次对该化合物的碳和氢质子信号进行全归属; 并证实其结构中的 H-1, H-2 互为颠倒, 纠正了文献报道中的错误, 该研究对乌药烷骨架倍半萜内酯的结构鉴定提供了光谱学依据.

关键词: NMR; 化学位移归属; 乌药烷型; 栝菊木

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