

Isolation and Structure Elucidation of a Flavanone, a Flavanone Glycoside and Vomifoliol from *Echiochilon Fruticosum* Growing in Tunisia

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Abstract: A flavanone and a flavanone glycoside, together with vomifoliol, have been isolated for the first time from the aerial parts of the plant *Echiochilon fruticosum* and identified. Their structures were established on the basis of spectroscopic measurements, mainly 2D NMR using COSY, HMQC and HMBC experiments.

Keywords: Flavanone, heteroside, vomifoliol, *Echiochilon fruticosum*, NMR.

Introduction

This research is a contribution to the chemical study of some plants growing in Tunisia [1], with the objective of discovering new natural products with potential biological activity. We have chosen for this purpose the plant *Echiochilon fruticosum* (Boraginaceae). Only some botanical and agronomical studies have been carried out in this plant, and, to the best of our knowledge, so far it has not been the subject of any phytochemical investigations. *Echiochilon fruticosum* may be considered one of four important herbs found in the northern desert of Egypt [2]. The ecological relationships of

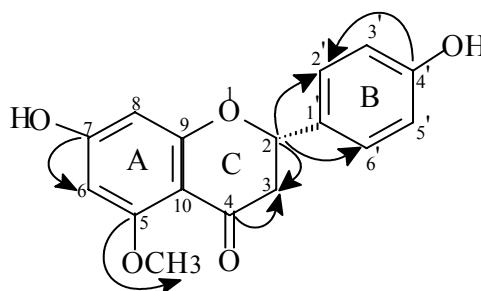
this plant in siliceous sand deposits north of Wadi El-Natrum (Egypt) were studied and it showed in particular a high correlation with soil salinity [3]. The nutritive value of *Echiochilon fruticosum* from the western Mediterranean desert of Egypt has been investigated and the possibility of its use as forage for domestic animals (mainly sheep and goats) was demonstrated [4]. We note that in Tunisia, this species is widespread in the region of Chott Meriem (Sousse, Tunisia) and in the desert, where it is used as grazing for animals [5-6]. We report here the isolation and the structure elucidation of a flavanone, naringenin 5-methyl ether (**1**), a flavanone glycoside, pinocembrin-7-glycoside (**2**) and vomifoliol (**3**) from the ethyl acetate aerial part extract of *Echiochilon fruticosum*. The structures were established on the basis of 2D NMR spectroscopic experiments and by comparison of the NMR data with literature values [7-10].

Results and Discussion

Compound 1

A molecular formula of $C_{16}H_{14}O_5$ was obtained from the ESMS $[MH^+]$ at m/z 287 and from the ^{13}C -NMR analysis. Furthermore, the IR spectrum revealed absorptions attributable to free hydroxyl (3366 cm^{-1}) and ketone (1642 cm^{-1}) groups. An examination of its NMR data (Table 1) and a comparison with the literature [7-8] suggested that compound **1** was a flavanone. Thus, its 1H -NMR spectrum revealed characteristic resonances of aromatic protons such as $H_{2',6'}$ (δ 7.35, d, $J=8.4\text{ Hz}$), $H_{3',5'}$ (δ 6.90, d, $J=8.4\text{ Hz}$), H_8 (δ 6.02, s, 1H) and H_6 (δ 6.09, s, 1H). A typical methoxyl signal at δ 3.82 ppm was also observed. The multiplicities and the weak coupling constants of H_6 and H_8 were in agreement with the existence of the hydroxyl group at C_7 (δ 162.9). The presence in the 1H -NMR spectrum of signals at δ 5.38 (1H, dd, $J=13, 2.9\text{ Hz}$), δ 3.12 (1H, dd, $J=17.1, 2.9\text{ Hz}$) and δ 2.85 (1H, dd, $J=17.1, 13.1\text{ Hz}$), in conjunction with the ^{13}C -NMR signals at δ 78.6, and 129.9 ppm, inferred from HMQC and HMBC spectra (Table 1), pointed to the presence of an $-O-CH-CH_2-CO-$ system in the C-ring.

Figure 1. Heteronuclear multiple-bond correlations for **1**.
Arrows point from carbon to proton.



This conclusion was reinforced by the peak correlating signals at δ 3.12 and 5.38 ppm observed in the ^1H - ^1H COSY spectrum. Other heteronuclear correlations deduced from the HMBC spectrum were also in agreement with the proposed structure (Figure 1). Elucidation of the absolute configuration at C_2 was based on the values of the coupling constants with the methylenic protons $\text{H}_{3\alpha,\beta}$. ($J_{\text{ax-ax}}=13.1$ and $J_{\text{ax-eq}}=2.9$ Hz). The close similarity of the H_2 chemical shifts (Table I) with those of the literature [7-8] thus confirmed the *S*- configuration of C_2 .

Table 1. ^1H - and ^{13}C -NMR spectral data for compounds **1**, **2** and **3**.

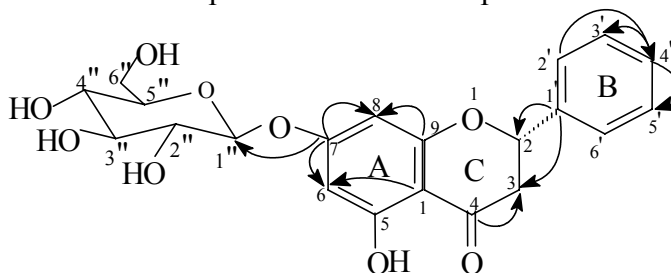
Position	Compound 1		Compound 2		Compound 3	
	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H
1					78.9	
2	78.6	5.38; dd ; 13.1 ; 2.9	79.3	5.48; dd ; 2.9 ; 13.1	41.0	
3a	52.9	3.12; dd; 2.9; 17.1	43.2	2.86; dd; 17.1; 2.9	49.6	2.25 (a); d; 16.9
3b		2.85; dd; 17.1; 13.1		3.12; dd; 17.1; 13.1		2.45 (b); d; 16.9
4	196.4		196.5		195.5	
5	168.0		162.9		127.9	5.90; m
6	93.8	6.09; d; 2.17	97.0	6.21; d; 2.1	162.2	
7	162.9		165.6		129.0	5.81; m
8	96.3	6.02; d; 2.3	95.8	6.23; d; 2.1	135.7	5.84; m
9	164.0		162.8		68.1	4.41; m
10	102.0		105.0		23.7	1.29; d; 6.4
11					24.0	1.01; s
12					23.0	1.08; s
13					18.8	1.89; s
1'	129.9		138.3			
2'	127.7	7.35; d; 8.4	126.0	7.48; d; 7.5		
3'	115.4	6.90; d; 2.3	129.6	7.41; m		
4'	156.4		128.6	7.39; m		
5'	115.4	6.90; d; 8.4	129.6	7.41; m		
6'	127.7	5.35; d; 8.4	126.0	7.48; d; 7.5		
1''			101.0	4.97; d; 7.1		
2''						
3''						
4''						
5''						
6''a			61.1	3.74; dd		
6''b			61.1	3.87; dd		

Compound 2

A molecular formula of $C_{21}H_{22}O_9$ was deduced from the ES MS $[MNa^+]$ at m/z 441. The IR spectrum exhibited absorptions corresponding to hydroxyl (3321 cm^{-1}) and ketone (1652 cm^{-1}) groups. The comparison of the ^1H -NMR spectrum with that of **1** (Table I) permitted us to note on the one hand, the presence of all proton signals of **1** with the exception of that of the methoxyl group at 3.82 ppm, and on the other, one additional signal at δ 7.39 (m, $H_{4'}$). This observation suggests that the aglycone is pinocembrin. This hypothesis was confirmed on the basis of the correlation observed between the $H_{4'}$ proton and C_2 (δ 127.7) in the HMBC spectrum (Figure 2). The identification of the sugar as β -D-glycopyranoside was easily deduced from the analysis of the ^1H - ^1H COSY spectrum using the anomeric proton as a starting point. The attachment of a glucose unit at C_7 (δ 165.6 ppm) was apparent from the heteronuclear H_1-C_7 HMBC connectivity (Figure 2). As in compound **1**, the absolute configuration of C_2 was deduced from the comparison of H_2 chemical shifts of **2** with the reported literature values [7-8].

Figure 2. Heteronuclear multiple-bond correlations for **2**.

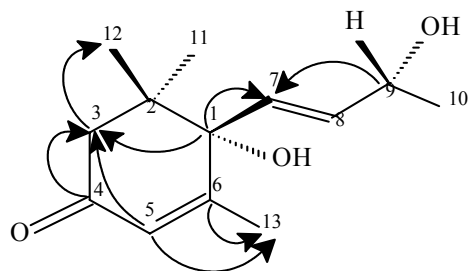
Arrows point from carbon to proton



Compound 3

The ESMS of this compound showed a molecular ion $[MNa^+]$ at m/z 247, suggesting a molecular formula of $C_{13}H_{20}O_3$. The comparison of the corresponding NMR data (Table 1) with those reported in literature [9-10] led to its identification as vomifoliol. The use of some 2D NMR experiments has permitted the confirmation of this structure. The cyclohexenone structure was confirmed by the observation in the HMBC spectrum of correlations between the AB system H_{3ab} (δ 2.25, δ 2.45, $J_1=J_2=16.9\text{ Hz}$) and C_4 (δ 195.5), C_1 (δ 78.9) and C_5 (δ 127.9). The location of the two high field methyl groups CH_3 -11 and CH_3 -12 at C_2 was ascertained by the detection of the $H_{3a,b}$ - C_{11} and $H_{3a,b}$ - C_{12} correlations. The position of the side chain on C_1 of the cyclohexenone ring is confirmed by the observation in the HMBC spectrum of the H_7 - C_1 correlation. The COSY spectrum revealing correlations between the olefinic signal H_7 (δ 5.81) with the other olefinic proton H_8 (δ 5.84) which in turn had a cross peak with H_9 (δ 4.41) permitted us to confirm the structure of this side chain. All these NMR data together with the mass spectrum suggested the structure of vomifoliol reported for compound **3**.

Figure 3. Heteronuclear multiple-bond correlations for **3**.
Arrows point from carbon to proton.



Conclusions

Echiochilon fruticosum, which has not been previously chemically investigated, was found to contain three known compounds, namely a flavanone: naringenin 5-methyl ether (**1**), a flavanone glycoside: pinocembrin glycoside (**2**) and a cyclohexenone derivative: vomifoliol (**3**). Their structures were established on the basis of 2D NMR experiments. With regards to possible biologically interesting properties of these compounds, it is well known that some flavanones have distinct taste properties, being bitter, sweet or bittersweet. Furthermore, some of them, such as naringenin, are reported to have inhibitory effects on microorganisms, whereas, the corresponding glycosides appeared to be inactive [11]. Vomifoliol has been reported to play an important role as an endogenous regulator of the stomatal aperture [10].

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Experimental

General

Mass spectra were obtained with a Micromass spectrometer (Q-ToF Micro) linked to an ESI source. ^1H -, ^{13}C - and 2D-NMR spectra of **1** were recorded in CDCl_3 with a Bruker NMR-300 spectrometer, operating at 300 and 75 MHz, respectively. The ^1H -, ^{13}C - and 2D-NMR spectra of **2** and **3** were obtained in a mixture of CDCl_3 and CD_3OD (compound **2**) and CDCl_3 (compound **3**) on a Bruker Avance AM-400 spectrometer. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz.

Plant material

The aerial parts of *Echiochilon fruticosum* were harvested in March 2000 in Chott Meriem, Sousse (Tunisia). A herbarium specimen of this plant was deposited in the herbarium of the Laboratoire de Biologie Végétale et Botanique, Ecole Supérieure d'Horticulture et d'Elevage de Chott Mériem, Université du Centre, Sousse, Tunisia.

Extraction

Dried and finely powdered *Echiochilon fruticosum* aerial parts (1200 g) were extracted with methanol in a Soxhlet apparatus during 7 days. The crude residue (150 g) obtained after filtration and evaporation of the solvent was dissolved in water then extracted successively with petroleum ether, EtOAc and BuOH, yielding 86 g, 10 g and 24 g fractions, respectively.

Isolation of pure compounds 1-3

The ethyl acetate extract (10g) was resolved by silica gel column chromatography (SDS, ref. 2100027, petroleum ether, AcOEt, methanol gradients) so that ten main fractions were collected. Fraction 1 was in turn chromatographed over silica gel with CHCl_3 to provide 10 subfractions. Subfraction 6 (120 mg) was rechromatographed on silica gel into 65 fractions using as eluent a 9.8:0.2 $\text{CHCl}_3/\text{MeOH}$ mixture. The combined fractions 31 to 47 (55 mg) were further purified on a silica gel column to give compound **1** as an oily substance (26 mg); $[\alpha]_{\text{D}}^{22} -14$ ($C = 0.17$; CHCl_3), $R_f = 0.5$ ($\text{CHCl}_3/\text{MeOH}$ 9.5:0.5); IR (KBr) $\nu_{\text{max}} \text{ cm}^{-1}$ 3366 (br OH), 1642 (C=O); for ^1H - and ^{13}C -NMR data see Table I.

From the ten main fractions indicated above, fraction 5 was divided into 5 subfractions on a silica gel column using CHCl_3 as eluant. The most polar one was further purified on a silica gel column using $\text{CHCl}_3/\text{MeOH}$ gradients to provide compound **2** as a white solid (1.5 mg); $[\alpha]_{\text{D}}^{22} -35$ ($C = 0.01$; MeOH), $R_f = 0.5$ ($\text{CHCl}_3/\text{MeOH}$ 8.5:1.5); IR (KBr) $\nu_{\text{max}} \text{ cm}^{-1}$ 3321 (br OH), 1652 (C=O); for ^1H - and ^{13}C -NMR data see Table I.

Fraction 3 (1g) was passed through an RP18 reverse phase column using water/methanol to provide ten subfractions. The subfraction with medium polarity (15 mg) was further resolved on a silica gel column using a gradient of $\text{CHCl}_3/\text{MeOH}$ as eluent to afford 4 subfractions; the third one (3 mg) was finally purified on a preparative plate eluted with a 9:1 $\text{CHCl}_3/\text{MeOH}$ mixture to give compound **3** as a white solid (1 mg); $[\alpha]_{\text{D}}^{22} +41$ ($C = 0.01$; CHCl_3), $R_f = 0.5$ ($\text{CHCl}_3/\text{MeOH}$ 9:1); IR (KBr) $\nu_{\text{max}} \text{ cm}^{-1}$ 3430 (br OH), 1657 (C=O); for ^1H - and ^{13}C -NMR data see Table I.

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Sample availability: Samples of compounds **1** (6 mg), **2** (1 mg) and **3** (0.8 mg) are available from the authors.