HETEROCYCLES, Vol. 63, No. 9, 2004, pp. 2101 - 2109
Received, 23rd June, 2004, Accepted, 5th August, 2004, Published online, 6th August, 2004

TWO SESQUITERPENE-COUMARINS FROM THE ROOTS OF *FERULA MARMARICA*

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Abstract - Two new sesquiterpene-coumarin conjugates designated foetidin acetate and foetidone were isolated from the roots of *Ferula marmarica*. The structures of the sesquiterpene-coumarins have been fully characterized by spectroscopic methods, among which foetidin acetate was confirmed by single-crystal X-Ray crystallography. Absolute configuration of both new compounds was established by CD.

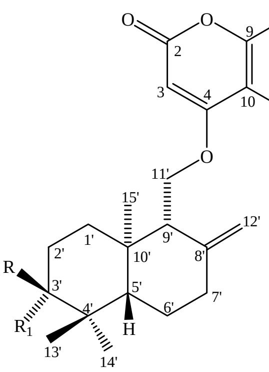
Plants of the genus *Ferula* (family Apiaceae) are long-lived plants, which are widespread throughout the Mediterranean to Central Asia. The gum resins, which are obtained by incision of the roots of several species, are used as spices and drugs in many countries and the resins have been reported to be stomachic, vermifuge and carminative disorders.[1] Recently, it has been reported that the sesquiterpene-coumarins from *Ferula fukanensis* have nitric oxide production inhibitory effects.[2] The genus *Ferula* is well documented as a good source of secondary metabolites including biologically active compounds such as sesquiterpenes,[3] sulfide derivatives,[4] and prenylated benzoic acid,[5] as well as sesquiterpene-coumarins. A recent review shows us that more than 100 sesquiterpene-coumarins have been isolated from the genus *Ferula*.[6] As part of our ongoing studies of plants of the genus *Ferula*,[7-10] we investigated on the constituents of *Ferula marmarica*, an Egyptain beach plant. A previous investigation of the roots of *F. marmarica* has revealed the presence of a sesquiterpene-coumarin named as marmaricin.[11] According to the review, all of the sesquiuterpene-coumarins from the genus *Ferula* were derived from umbelliferone (7-hydroxycoumarin)[6] except for foetidin.[12] In most sesquiterpene-coumarins, a sesquiterpene moiety usually attached to C-7 of the coumarin nucleus. Foetidin was isolated from the roots of *Ferula assa-foetida*, and it was the first example of naturally occurring 4-hydroxycoumarin with no additional groups at the coumarin moeity. To the best of our knowledge, up to present, there are three natural 4-hydroxycoumarin derivatives beside foetidin. They are: *O*-(2-oxo-2*H*-chromen-4-yl)-D-glucopyranuronic acid was isolated from the urine of dog,[13] setarin, a 4-propenoxycoumarin, was isolated from the leaves of *Setaria italica*,[14] and 4-hydroxycoumarin was isolated from *Ruta graveolens*.[15] The present paper deals with the isolation and structural elucidation of two naturally occurring sesequiterpene-coumarins, represented to 4-hydroxy derivatives from the roots of *Ferula marmarica*.

RESULTS AND DISSCUSTION

A combination of size exclusion chromatography (Sephadex LH-20) and HPLC of the chloroform extract of the dried roots of *Ferula marmarica*, resulted in the isolation of two 4-*O*-sesquiterpene-coumarins.

Compound (**1**), was obtained as an amorphous powder. HRFABMS showed a $[M+H]^+$ ion peak at *m/z* 425.2333 (calcd 425.2328) corresponding to a molecular formula $C_{26}H_{32}O_5$. The proposed structure is in accordance with IR absorptions at 3400, 1690 and 1620 cm^{-1} for hydroxyl, carbonyl and aromatic functional groups and UV absorptions maxima at 270 and 306 nm typical for 4-alkoxycoumarin.[16] The spectral data of **1** suggested a rare skeleton type in comparison with that common in the genus *Ferula*. ^1H NMR spectrum displayed a pattern of aromatic protons for a coumarin nucleus substituted only at C-4 and a pattern for sesquiterpene unit. A singlet proton at δ_H 5.63 (H-3), together with two triplet-doublet protons at δ_H 7.44 and 7.16 (*td*, *J*= 8.5, 1.3 Hz, H-6 and 7) and two double-doublet protons at δ_H 7.20 and

7.65 (H-5 and 8), were the typical pattern of a coumarin with an alkoxy group at C-4.[12] Four tertiary methyls at δ_H 1.97 (3H), 0.90 (3H), and 0.87 (6H), two methylene protons at δ_H 4.22 (2H), two broad singlets at δ_H 4.83 (1H) and 4.44 (1H) for an exo-double bond, and a double-doublet methine proton at δ_H 4.48 (1H), in the upfield region were characteristic of a 5-hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-yl acetate moiety. The ^{13}C NMR spectrum of compound (**1**) exhibited 26 nonequivalent carbon atoms, among which were detected nine carbon atoms of a coumarin nucleus and the other 17 carbons for an acetyl group and a sesquiterpene unit. The assignment of the carbon signals was established by DEPT and HMQC experiments. The DEPT experiment of **1** classified four methyls, four aliphatic methylenes, an olefinic carbon (δ_C 107.8), one primary hydroxylated carbon, one secondary hydroxylated carbon, and seven methines (see Table 1). In addition, the signal at δ_C 170.6 was assigned to the carbonyl carbon of the acetate group. Seven quaternary carbons were observed. The ^{1}H-^{1}H COSY spectrum showed that the methine proton at δ_H 4.48 (H-3') is coupled to the upfield methylene protons at δ_H 1.68 (H-2'$_{eq}$) and 1.58 (H-2'$_{axi}$) and the methylene protons at δ_H 4.22 (H_2-11') are also coupled to the methane proton at δ_H 2.24 (H-9'). The location of the *O*-acetoxyl group of **1** was further confirmed by the 2D NMR spectrum. In the HMBC spectrum, the methine signal at δ_H 4.48 (H-3') showed ^3J correlations with the carbon signals at δ_C 16.4 (C-14'), 28.0 (C-13'), and 170.6 (acetyl carbonyl carbon) and ^2J correlation with 37.8 (C-4'). The proton signals at δ_H 1.68 and 1.58 (2'-H_2) had long-rang correlations with the carbon signals at 36.7 (C-1'), 79.9 (C-3'), 37.8 (C-4'), and 38.4 (C-10'), implying that the acetyl group attached to C-3'. Two methyls, H_3-13' and H_3-14', showed HMBC correlations to the quaternary carbon signal at δ_C 37.8 (C-4'), and methine carbon signals at δ_C 79.9 (C-3'), and 54.1 (C-5'). Furthermore, the methylene protons at δ_H 4.22 (H_2-11') showed an HMBC correlation to an olefinic carbon at δ_C 165.3 (C-4), indicating that the sesquiterpene unit is attached to the C-4 of the coumarin moiety *via* an ether linkage. Although the coupling constants suggested the stereochemistry of H-3', H-5', H-9', and H-11', the NOESY experiment supported the proposed stereochemistry of **1** (Figure 1). The relative configuration of compound (**1**) was also established by X-Ray analysis (Figure 2). Thus, compound (**1**) was determined as formula (**1**) and given a name, foetidin acetate.



1: R = H, R_1 = OAc
2: R + R_1 = O

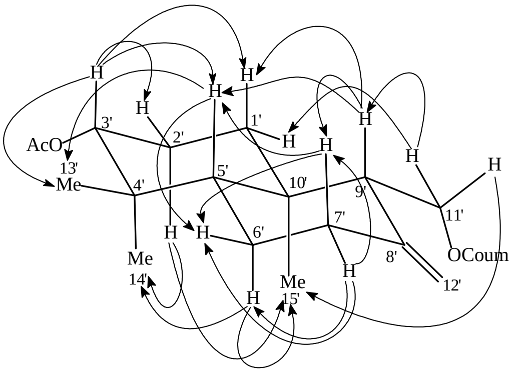


Figure 1. Selective NOESY Correlatiions of **1**.

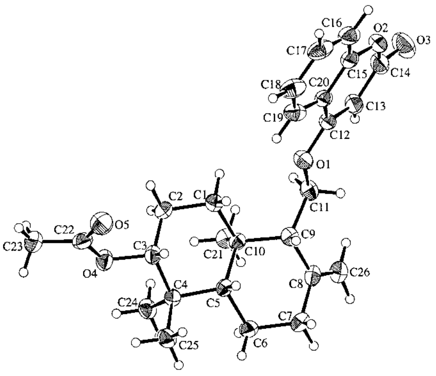


Figure 2. ORTEP drawing of **1**.

The molecular formula of compound (**2**) was confirmed to be $C_{24}H_{28}O_4$ by HRFABMS at *m/z* 381.2068 (calcd for $[M+H]^+$ ion, 381.2066). The IR (3735, 1726, 1622 cm^{-1}) and UV spectra of **2** almost identical to those of **1**. The structure of **2** was proved to be a bicylic sesquiterpene-coumarin from ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC and HMBC spectrometry. The ^{1}H and ^{13}C NMR spectral data are shown in Table 1. The ^{13}C NMR and DEPT spectral data showed 24 carbons including 3 methyls, 6 methylenes, 7 methines and 8 quaternary carbon atoms. Extensive NMR spectroscopic experiments (^{1}H–^{1}H COSY, HMQC and HMBC) indicated that **2** resembled **1**. The main difference between **1** and **2** was an appearance of a ketonic carbon signal at δ_C 215.6, which was assigned to C-3’ instead of the absence of the acetyl methyl signal of **1**. The HMBC correlations between the methylene protons (H_2-2’) and ^{13}C-NMR signals at δ_C 38.7 (C-1’), 215.6 (C-3’), 48.0 (C-4’), and 37.1 (C-10’) further confirmed the designed structure. The HMBC correlations of the methyls at δ_H 1.11 (H_3-13’) and 1.04 (H_3-14’) with ^{13}C-NMR signals at δ_C 215.6 (C-3’) and 48.0 (C-4’). The HMBC correlations between the protons at δ_H 1.83 and 2.06 (H_2-1) with ^{13}C-NMR signals at δ_C 215.6 (C-3’), 55.2 (C-5’), 53.8 (C-9’), and 34.5 (C-2’) confirmed that a ketone should be attached to C-3’. The NOESY experiment supported the proposed stereochemistry. H-2’$_{axi}$ showed cross-peaks with H-14’$_{axi}$, H-15’$_{axi}$, and H-1’$_{eq}$, whereas H-5’$_{axi}$ exhibited cross-peaks with H-13’$_{eq,}$ H-6’$_{eq}$, and H-9’$_{axi}$. H-9’$_{axi}$ also revealed cross-peaks with H-1’$_{axi}$ and H-7’$_{axi}$. Furthermore, H-11’$_{eq}$ showed cross-peaks with H-15’$_{axi}$ and H-1’$_{eq}$. Thus, the compound (**2**) was as the structure and given the name, foetidone.

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds (**1**) and (**2**) (^{1}H-NMR at 500 MHz, ^{13}C-NMR at 125 MHz, in $CDCl_3$)

	1		**2**	
	δ_H (J in Hz, mult.)	δ_C (multiplicity)[a]	δ_H (J in Hz mult.)	δ_C (multiplicity)[a]
2		162.5 (C)		162.8(C)
3	5.63 (*s*)	90.1 (CH)	5.68 (*s*)	90.5 (CH)
4		165.3 (C)		165.5 (C)
5	7.65 (8.5, 1.3 *dd*)	122.9 (CH)	7.70 (8.5, 1.3 *dd*)	123.1 (CH)
6	7.16 (8.5, 1.3 *td*)	123.7 (CH)	7.20 (8.5, 1.3 *td*)	124.0 (CH)
7	7.44 (8.5, 1.3 *td*)	132.1 (CH)	7.53 (8.5, 1.3 *td*)	132.5 (CH)
8	7.20 (8.5, 1.3 *dd*)	116.4 (C)	7.28 (8.5, 1.3 *dd*)	116.8 (C)
9		153.0(C)		153.3 (C)
10		115.5(C)		115.7 (C)
$1'_{axi}$	1.48 (14, 12, 7, 2.4 *dddd*)		1.83 (14,8 *td*)	
		36.7 (CH_2)		38.7 (CH_2)
$1'_{eq}$	1.68 (*m*)		2.06 (14, 11, 8, 2 *dddd*)	
$2'_{axi}$	1.58 (15.2, 14, 12, 2.4 *dddd*)		2.66 (14, 11, 8 *ddd*)	
		23.8 (CH_2)		34.5 (CH_2)
$2'_{eq}$	1.68 (14, 4 *dq*)		2.36 (*m*)	
$3'_{axi}$	4.48 (15.2, 4 *dd*)	79.9 (CH)		215.6 (C)
4'		37.8 (C)		48.0 (C)
$5'_{axi}$	1.22 (12.4, 5 *dd*)	54.1 (CH)	1.65 (12, 6 *dd*)	55.2(CH)
$6'_{axi}$	1.38 (16, 12, 12, 3.5 *dddd*)		1.57 (12, 8 *dd*)	
		23.0 (CH_2)		24.4 (CH_2)
$6'_{eq}$	1.68 (*m*)		1.72 (11 *dq*)	
$7'_{axi}$	2.06 (13, 11, 12, 3.5 *dddd*)		2.13 (12, 8 *td*)	
		37.0 (CH_2)		37.8 (CH_2)
$7'_{eq}$	2.40 (13, 3.6 *dq*)		2.50 (12 *dq*)	
8'		145.5 (C)		145.2 (C)
$9'_{axi}$	2.24 (6.4 *t*)	54.1 (CH)	2.36 (*m*)	53.8(CH)
10'		38.4 (C)		37.1 (C)
$11'_{eq}$	4.22 (6.8, 6.4 *br dd*)	66.4 (CH_2)	4.32 (5.2 *br d*)	66.6(CH_2)
$12'_a$	4.83 (*br s*)		4.95 (*br s*)	
		107.8 (CH_2)		108.7 (CH_2)
$12'_b$	4.44 (*br s*)		4.56 (*br s*)	
$13'_{eq}$	0.90 (*s*)	28.0 (CH_3)	1.11 (*s*)	25.8 (CH_3)
$14'_{axi}$	0.87(*s*)	16.4 (CH_3)	1.04 (*s*)	21.9 (CH_3)
$15'_{axi}$	0.87(*s*)	15.1 (CH_3)	1.04 (*s*)	14.8 (CH_3)
OAc	1.97(*s*)	21.0 (CH_3)		
		170.6 (C)		

[a] Multiplicity was determined by DEPT experiments

The absolute configurations of the new compounds were derived from the CD spectra based on a previous extensive study on the absolute stereochemistry of the related compounds,[17,18] in which the most useful

CD band suitable for assignment of absolute configurations for bicyclic sesquiterpene coumarin ethers is the short Cotton effect at ca. 205 nm (π to π* for exe-methylene). The absolute configuration of new compounds (**1**) and (**2**) turned out to be 5’*S*,10’*R* for the substituted 3’-ketodecaline structure in comparison with Δε values of the known compounds, acetylpectachol B[17] and pectanone.[18] The negative Cotton effects in the CD spectrum of **1** with Δε= -3.4 at 216 nm was clearly indicating a 5’*S*, 10’*R* 8-methylene-*trans*-decaline system. The value of ∣Δε∣ >10 at 216 nm is consistent with an axial CH_2OAr at C-9’ whereas equarorial CH_2OAr gives ∣Δε∣ <5. Moreover, a slight positve Cotton effects at around 300 nm due to the carbonyl n-π* transition of cyclohexanone A ring of **2** was observed, whereas no Cotton effect at this region was observed in the CD spectrum of **1**. Thus, the absolute configuration of **2** can be proved as 5’*S*, 9’*R*, 10’*R* based on the negative sign of the short wavelength transition at 203 nm, Δε= -3.1.

EXPERIMENTAL

General Method

Optical rotations were measured with a *JASCO P-1020* digital polarimeter. The UV spectra were obtained on a *Hitachi 200-20* spectrophotometer, and IR spectra were measured on a *Hitachi 260-30* spectrophotometer. CD spectra were measured on a *JASCO J-810* circular dichroism spectrometer. NMR (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR, using $CDCl_3$ as solvents for measurement) spectra were obtained on a Varian NMR spectrometer (*Unity Plus 400* and *Unity INOVA-500*) or a Bruker AMX-400 NMR spectrometer. Low-resolution EI-MS were collected on a *Quattro* GC/MS spectrometer having a direct inlet system. High-resolution FAB-MS were collected on a *Finnigan/Thermo Quest MAT 95XL* spectrometer. JASCO *PU-986* pumps and *UV-970* UV-VIS detector were used in a HPLC system. Hypersil ODS 5 μm (250 ×4.6 mm i.d.) and preparative ODS 5 μm (250 × 21.2 mm i.d.) columns were applied.

Plant Material

The roots of *Ferula marmarica* were collected in Jan, 2001 from north beach, Agibaa beach, Matrouh, Egypt. Voucher specimens are deposited in the herbarium of the Department of Botany, National Research Center, El-Tahrir St., Dokki, Cairo, Egypt.

Extraction and Isolation

Dried and coarsely powdered resins (250 g) were extracted with $CHCl_3$ (1 L) at rt for 24 h. The extract (12.3 g) was subjected to flash chromatography over silica gel, with hexane containing an increasing amount of EtOAc. Fr. A (600 mg) was further purified on silica gel column chromatography (hexane–EtOAc, 1:1), and the resulted fractions (250 mg) was applied to a Sephadex LH-20 column eluted with (hexane–EtOAc, 1:2) to give foetidone (**2,** 50 mg). Fr. B (320 mg) was subjected to silica gel chromatography (Hexane-$CHCl_3$, 1:3) then over Sephadex LH-20 (hexane–EtOAc, 1:2) and finally was purified by HPLC, resverse phase (MeOH-H_2O, 80:20) to give foetidin acetate (**1**, 33 mg), which was crystallized from hexane-EtOAc 10:1.

Foetidin acetate (1)

Amorphous solid; $[\alpha]_D$ -26.2° (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) : 300 (3.78), 276 (3.59), 265 (3.59), 204 nm (3.11); CD (MeOH) λ_{max} ($\Delta\varepsilon$)=216 (-3.4); IR (film) ν_{max} 3500, 1690, 1620 cm^{-1}; EIMS *m/z* (rel. int.) 424 [M]+ (100), 262 $[M\text{-coumarin}]^+$ (4), 162 $[M\text{-sesquiterpene}]^+$ (60); ^1H and ^{13}C NMR spectral data see Table 1. HRFABMS *m/z* : $[M+1]^+$ 425.2333 (calcd 425.2328, Δ= -0.4 mmu)

Foetidone (2)

Amorphous solid; $[\alpha]_D$ +122.2° (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) : 302 (4.58), 275 (4.28), 265 (4.28), 204 nm (3.68); CD (MeOH) λ_{max} ($\Delta\varepsilon$)=203 (-3.1); IR (film) ν_{max} 3735, 1726, 1622 cm^{-1}; EIMS *m/z* (rel. int.) 380 [M]+ (100), 218 $[M\text{-coumarin}]^+$ (30), 162 [M-sesquiterpene]+ (100); ^1H and ^{13}C NMR spectral data see Table 1. HRFABMS *m/z* : $[M+1]^+$ 381.2068 (calcd 381.2066, Δ= -1.1 mmu).

Crystallographic Analysis of 1

The reflection data were collected on a Mac Science DIP 2030 imaging plate area detector with mono-chromated Mo-Kα radiation (λ=0.71069 Å) with ω-techniques to a maximum 2θ value of 52.0° at rt (25±1°C). Of the 4605 reflections which were collected, 2625 were unique. The structure was solved by direct methods SIR 92,[19] and expanded using Fourier techniques.[20] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of the full-matrix least squares refinement was based on 2546 observed reflections ($I>1.00\sigma(I)$). The structure was finally refined to R=0.059 (R_w=0.064). The crystal data are: *a*=9.944 (2) Å, *b*=10.230 (2) Å, *c*=22.138(3) Å, *V*=2252.0 (6) $Å^3$, space group $P2_12_12_1$, Z=4, Dc=0.606 g/cm^3.

ACKNOWLEDGEMENTS

This investigation was supported by a grant from the Committee on Chinese Medicine and Pharmacy, Department of Health, Taiwan, R.O.C.

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