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COUMARINS FROM *PEUCEDANUM OSTRUTHIUM*

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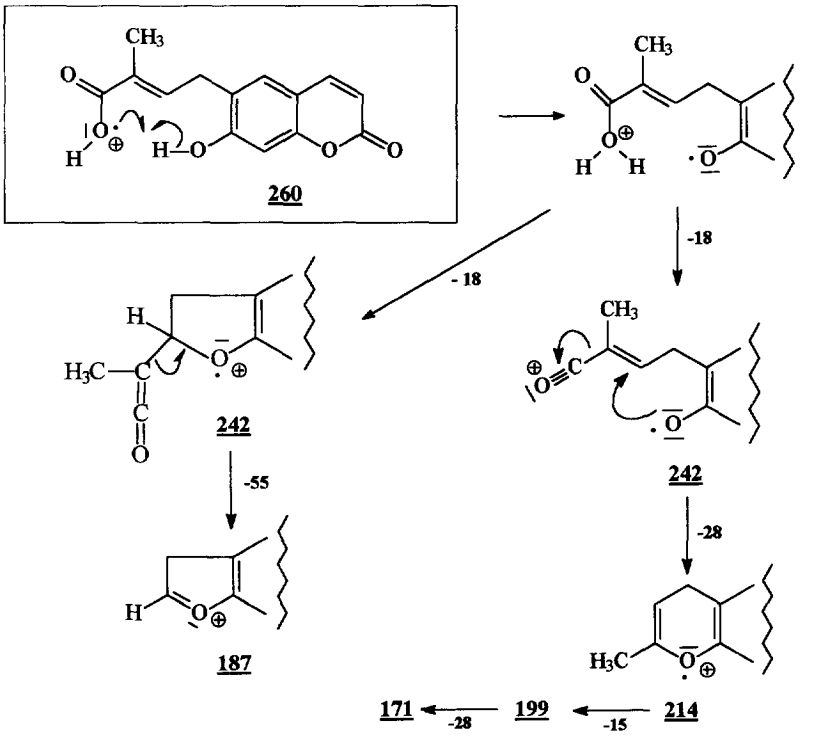
Key Word Index—*Peucedanum ostruthium*; roots; coumarins; 6-(3-carboxybut-2-enyl)-7-hydroxycoumarin; 3′-acetate of oxypeucedanin hydrate.

Abstract—Two new coumarins, 6-(3-carboxybut-2-enyl)-7-hydroxycoumarin and the 3′-acetate of oxypeucedanin hydrate, were isolated from the roots of *Peucedanum ostruthium*, and their structures were established mainly by mass spectrometry and 2D NMR techniques. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Peucedanum ostruthium (L.) Koch has been used since ancient times in folk medicine against various diseases. Based on a screening programme of medicinal plants for their possible antiphlogistic activity, we have observed that aqueous extracts of roots of *P. ostruthium* significantly inhibited at a dose of 0.03 mg kg^{-1} p.o. the carrageenan induced raw paw oedema. A corresponding phytochemical study resulted in the isolation of two new coumarins, 6-(3-carboxybut-2-enyl)-7-hydroxycoumarin (**1**) as active principle and the 3′-acetate of oxypeucedanin hydrate (**2**), along with known coumarins [1–3]. Hitherto, the type of prenylated coumarin (**1**), containing a free carboxyl group, has only been reported in *Evodia vitiflora* [4].



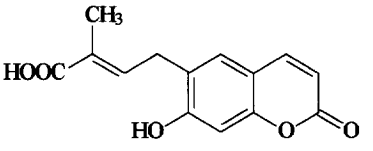
Scheme 1. EI mass spectral fragmentation pattern of compound **1**.

RESULTS AND DISCUSSION

In order to clarify the structures of **1** and **2**, mass spectrometric examinations were carried out first. The mass spectrum of **1** showed a molecular ion of *m/z* 260 as well as fragments at *m/z* 242, 187, 199 and 171 from which we can derive the fragmentation pattern shown in Scheme 1. Compound **1** was reacted with diazomethane and the resulting methylated derivative was identified by means of GC–mass spectral analysis as a dimethyl derivative. The mass spectrum of **2** (showed ions at *m/z* (rel. int.) 346 $[M]^+$ (27), 202 (100), 174 (24), 146 (27) and 145 (24); the ion of *m/z* 202 appeared as the base peak and corresponded to a psoralen hydroxylated at positions C-5. This ion is formed by splitting off the acylated side chain as a neutral molecule and can be interpreted as a McLafferty rearrangement where a hydrogen atom on the side chain is transferred to the ether oxygen atom.

The structures of **1** and **2** derived by the mass spectra are in accordance with the results of the ^{1}H NMR investigations. By means of the ^{1}H–^{1}H COSY spectra, it was possible to characterize the spin systems of **1** and **2** and to assign all proton resonances. The lactone-ring protons of **1** and **2** each form an AX-two-spin system, the coupling constants of which reflect *cis*-arrangements of the double bond protons. Due to the long-range coupling via five bonds of H-4 with H-8, the signal of the H-4 proton appears as a doublet–doublet signal. The two furan-ring protons of **2** also form a two-spin system whose small coupling constant is typical of furan rings. H-a shows also a long-range coupling via five bonds to the H-8 proton resulting in a doublet–doublet signal for the H-a proton. An analogous multiplicity results for the H-8 proton (Table 1).

The position of the acetyl residue in the prenyl side chain of **2** was deduced from the chemical shifts of the protons of the prenyl residue. The proton on the C′-2 carbon atom was shifted to low field in the synthetically accessible 2′-*O*-acetyl and diacetyl derivative of the oxypeucedanin (δ 5.36 and 5.33) [5], but this could be observed with the 3′-*O*-acetyl derivative **2**. The



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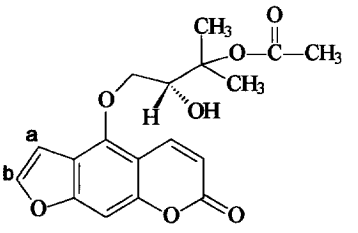

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Table 1. ^{1}H NMR spectral data for compounds **1** and **2** (500 MHz in MeOH-d_4)

Proton	δ (^{1}H)	δ (^{1}H)*	Couplings† (Hz)
1			
H-3	6.09 *d*		$^3J_{H\text{-}3,H\text{-}4} = 9.5$
H-4	7.79 *d*		$^3J_{H\text{-}4,H\text{-}3} = 9.5$
H-5	7.31 *d*		
H-8	6.67 *s*		
H_2-1′	3.44 *d*		$^3J_{H\text{-}1',H\text{-}2'} = 7.5$
H-2′	6.59 *m*		$^3J_{H\text{-}2',H\text{-}1'} = 7.5$
CH_3	1.90 *s*		
2			
H-3	6.26 *d*	6.25	$^3J_{H\text{-}3,H\text{-}4} = 9.8$
H-4	8.37 *dd*	8.39	$^5J_{H\text{-}4,H\text{-}8} = 0.7$
H-8	7.18 *dd*	7.15	$^5J_{H\text{-}8,H\text{-}a} = 1.0$
H-a	7.12 *dd*	7.13	$^3J_{H\text{-}a,H\text{-}b} = 2.4$
H-b	7.72 *d*	7.71	$^3J_{H\text{-}b,H\text{-}a} = 2.4$
H-1′	4.85 *dd*	4.70	$^2J_{H\text{-}1'(gem.)} = 9.9$
	4.45 *dd*	4.36	$^3J_{H\text{-}1',H\text{-}2} = 2.6, 7.9$
H-2′	4.00 *dd*	3.88	$^3J_{H\text{-}2',H\text{-}1'} = 2.6, 7.9$
Me	1.66 *s*	1.26	
	1.61 *s*	1.21	
	2.03		

*H-NMR data oxypeucedanin hydrate.

†$\Delta J_{H,H} = 0.1$ Hz.

acetoxy residue in position C-3′, however, caused a clear low field shift of the signals of the terminal methyl groups.

EXPERIMENTAL

General. NMR: Bruker AMX-500 (^{1}H frequency: 500.13 MHz), 5-mm reverse probe head, solvent: MeOH-d_4, temp. 303 K. The MeOH signal was used as int. standard (^{1}H: δ 3.3). 90° pulse: ^{1}H: 9.8 μsec. COSY: 45° mixing pulse. MS: Finigan MAT 8500, EI, 70 eVf. GC–MS: Finigan MAT 312 system with MAT-SS-300 data system, EI, 70 eV and Varian 3700, 30 m × 0.3 cm DB-1 fused-silica column; H_2 was used as carrier gas; temp. programme 80–300° (3°C min^{-1}).

Plant material. Air-dried roots (2 kg) of *P. ostruthium* were obtained from Fa. E. Ritzberger, Linz, Austria. The plant material was authenticated by microscopy and TLC. A voucher specimen (Sch.D./91) is deposited at the Institute of Pharmacognosy at Graz.

Isolation of compound **1**. Roots were extracted with 15% EtOH at room temp, the extract was concd *in vacuo* and perforated with petrol and Et_2O. The remaining aq. layer was subjected to CC (Polyamide, H_2O–MeOH gradient). Frs of 500 ml were collected and monitored by TLC. Frs 92–100 were combined and compounds sepd by prep. TLC. Final purification was carried out on Sephadex LH-20 with MeOH to give 2.5 mg **1**. Needles from MeOH, mp 257–260°.

Isolation of compound **2**. Roots (500 g) were extracted with MeOH at room temp. The MeOH soln was evapd to dryness *in vacuo*. The residue was suspended in H_2O and extracted with petrol. The petrol layer was concd to give a ppt. of needles containing a mixt. of **2**, oxypeucedanin, isoimperatorin and oxypeucedanin hydrate. Further purification of the mixt. by prep. TLC on silica gel GF_{254} using $CHCl_3$–MeOH–H_2O (80:20:21) afforded 3 mg **2**. Needles from MeOH, mp 116–119°.

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