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Foeniculoxin, a New Phytotoxic Geranylhydroquinone from Phomopsis foeniculi

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Abstract: Phomopsis foeniculi, the causal agent of fennel stem necrosis, when grown on liquid culture produced toxic metabolites. One of the more abundant phytotoxic metabolites, named foeniculoxin, was isolated and characterized using spectroscopic and chemical methods as 2-(6,7-dihydroxy-7-methyl-3-methylen-1-ynyl)-hydroquinone, a new geranylhydroquinone which is moderatly toxic on host and non-host plants.

INTRODUCTION

Recently, near Florence (Italy), a disease of fennel [*Foeniculum vulgare* subsp. *vulgare* var. *vulgare* (Mill.) Thell. and var. *dulce* (Mill.) Thell.] was found to cause the necrosis of stems, leaves and inflorescences leading to a marked decrease in fruit production. A fungal pathogen, *Phomopsis foeniculi*, was isolated from infected tissues. This pathogen has already been reported on the same host in France[1]. Further studies ascertained the ability of the fungus, when grown in liquid culture, to produce toxins that might be involved in pathogenesis[2].

As fennel fruits are economically important in the phytotherapy and food industry, a research was planned to select lines of fennel resistant to the disease using the toxins produced *in vitro* by *P. foeniculi*.

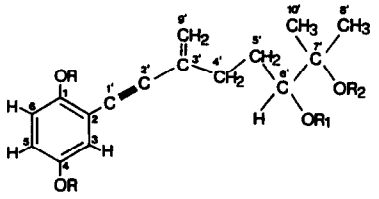
This paper describes both the isolation and the chemical and biological characterization of one of the more abundant phytotoxins produced by *P. foeniculi*, named foeniculoxin, which is a new monosubstituted geranylhydroquinone with an unusual isoprenyl side chain.

RESULTS AND DISCUSSION

The phytotoxic organic extract (EtOAc) of culture filtrates of *P. foeniculi* was purified by a combination of column and TLC chromatography, as described in detail in Experimental, to yield foeniculoxin (1) as a homogeneous oily compound withstanding crystallization.

When assayed at a concentration of $3.6\ 10^{-3}$ M, foeniculoxin reduced root growth of the germinating seeds of both fennel and tomato while it induced necrosis on tobacco leaves and wilting and/or isolated necrotic spots on the leaves of tomato cuttings.

Foeniculoxin has a molecular formula of $C_{16}H_{20}O_4$ for a total of seven unsaturations. In fact, its IR spectrum showed bands characteristic of hydroxy, aromatic, olefinic and geminal methyl groups and a weak band typical of an alkyne group substituted with two similar mass groups[3]. The UV absorptions indicated the presence of a *p*-hydroquinone residue, whose conjugation was probably extended; in fact, the expected blue-

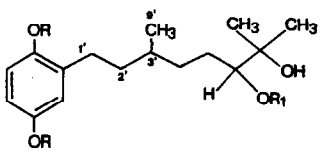


1 R=R1=R2=H

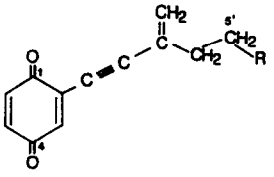
2 R=R1=Ac , R2=H

3 R=Me R1=R2=H

4 R=H R1+R2=Me2C



5a,b R = R1=H 6a,b R = R1=Ac



7 R=–CH(OH)–CH(OH)CH(CH3)2

8 R=CHO

shift of the maxima from 328 and 276-265 to 305 and <220 nm in alkaline solution were respectively observed[4]. These structural features were confirmed by an accurate examination of the ^1H NMR spectrum of foeniculoxin (Table 1).

In fact, three aromatic protons (H-3, H-5 and H-6), representing an ABC system typical of a 1,2,4-trisubstituted benzene ring, were present. As **1** gave a positive test with 1% $FeCl_3$ solution, the two substituents on C-1 and C-4 were probably two phenol hydroxy groups, while that on C-2 was an alkyl C-10 side chain containing the remaining three unsaturations and the other two oxygen atoms. As expected, when the same spectrum was recorded in $DMSO_{d6}$ four D_2O exchangeable hydroxy groups were observed, namely two singlets, typical of phenol protons, at δ 9.15 and 8.85[5] and the signals of two alcohol protons[5], resonating, respectively, as a singlet at δ 4.10 (HOC-7') and a doublet at δ 4.40 (HOC-6'), the latter being coupled with the geminal proton that in turn appeared as a complex multiplet at δ 3.20. This proton (H-6'), a double doublet (J=10.7 and 1.7 Hz) present at δ 3.35 when the spectrum was recorded in CD_3OD (Table 1), in the 2D $^1H,^1H$ NMR spectrum (COSY)[6] coupled with the multiplets present at δ 2.00 and 1.55, attributed to a vicinal methylene group (H_2C-5'), that in turn correlated with another methylene group appearing as two multiplets at δ 2.50 (H-4'A) and 2.34 (H-4'B). In addition the protons of H_2C-4' showed a typical allylic coupling[7] with the protons of a terminal disubstituted olefinic group resonating as a broad doublet (J=1.9 Hz) and a broad singlet at δ 5.39 and 5.33, respectively. Considering its multiplicity, H-6' should be only further bonded to a quaternary carbon probably bearing the two methyl groups appearing at δ 1.18 and 1.16 and the remaining tertiary hydroxy group. From these results a 5,6-dihydroxy-6-methylheptenyl residue was deduced as a partial structure of the side chain that was confirmed by the consistent chemical shift values observed for the corresponding carbons in the ^{13}C NMR spectrum and for the correlations evidenced in the 2D $^{13}C,^1H$ spectrum (Table 1)[6]. Moreover, the ^{13}C NMR spectrum showed the presence of two singlets typical of a disubstituted alkyne group at δ 87.0 (C-1') and 94.3 (C-2')[6,8]. The latter account for the remaining two unsaturations, therefore, the C-10 side chain was a 6,7-dihydroxy-7-methyl-3-methylen-1-ynyl residue with a typical geranyl carbon skeleton. In addition, the ^{13}C- NMR spectrum showed the presence of two singlets of the hydroxylated hydroquinone carbons at δ 152.3 (C-1) and 151.9 (C-4), three doublets of the corresponding secondary carbons at δ 119.4 (C-3), 118.2 (C-5) and 117.2 (C-6) and the singlet at δ 133.3 (C-2) due to the quaternary carbon bearing the isoprenyl side chain[6,8]. These partial structures were confirmed by the correlations and the multiplicities observed in the 2D $^{13}C,^1H$ long-range and in the gated decoupled spectra,

respectively, carried out on **1** and shown in detail in Table 1.

Table 1. ^{1}H and ^{13}C NMR (CD_3OD) of foeniculoxin (**1**). The chemical shifts are in δ-values (ppm)

C*	δ	m+	Hδ*	m	*J* (Hz)	2D ^{13}C, ^{1}H LRC#	^{13}C-GD§
1	152.3	*s*				6.67	*t*
2	133.3	*s*					
3	119.4	*d*	6.70	*d*	2.9		
4	151.9	*s*				6.67	
5	118.2	*d*	6.62	*dd*	8.8, 2.9		
6	117.2	*d*	6.67	*d*	8.8		
1'	87.0	*s*				6.70	*d*
2'	94.3	*s*				5.39, 5.33	*t*
3'	112.5	*s*					
4'A	35.5	*t*	2.50	*m*			
4'B			2.34	*m*			
5'A	31.1	*t*	2.00	*m*			
5'B			1.55	*m*			
6'	78.5	*d*	3.35	*dd*	10.7, 1.7	2.50, 1.55, 1.18, 1.16	
7'	73.8	*s*				3.35, 1.18, 1.16	
8'[a]	25.4	*q*	1.18	*s* (3H)			
9'A	121.3	*t*	5.39	*br d*	1.9		
9'B			5.33	*br s*			
10'[a]	25.3	*q*	1.16	*s* (3H)			

*2D ^{1}H,^{1}H and ^{13}C,^{1}H experiments delineated the correlation of all protons and the corresponding carbons in **1**; +multiplicities were determined by DEPT spectrum; #long-range correlation spectrum; §gated decoupled spectrum; [a]these attributions may be reversed.

From these data, foeniculoxin turned out to be a new geranyl hydroquinone and may be formulated as 2-(6,7-dihydroxy-7-methyl-3- methylen-1-ynyl)-hydroquinone (**1**).

The phytotoxin structure was supported by the peaks observed in the EI and FAB mass spectra of **1** (see Experimental). As expected, in EI MS, the molecular ion (*m/z* 276), losing in succession H_2O, MeCHMe and CO residues[5], generated the ions at *m/z* 258, 215 and 187, respectively. By other two alternative fragmentation pathways the molecular ion, losing in succession H_2O, Me, H_2O or H_2O and MeCOHMe moieties[5], produced the ion at *m/z* 258, 243, 225 or the ion at *m/z* 199, respectively. Moreover, the molecular ion by a similar Mc-Lafferty rearrangment yielded through loss of a $MeCOCOH(Me)_2$ molecule the ion at *m/z* 174 ($C_{11}H_{10}O_2$, base peak). Finally, the ion due to the loss of H_2O from the alkynyl hydroquinone residue was observed at *m/z* 115, as well as that of the MeCOHMe residue recorded at *m/z* 59. In FAB MS, the pseudomolecular ion $[M+H]^+$ at *m/z* 277 losing in succession two H_2O molecules generated the ions at *m/z* 259 and 241.

The foeniculoxin structure was confirmed by preparing some key derivatives, whose spectrocopic data were all consistent (Experimental).

The presence of four hydroxy groups in **1**, one of which could not be acetylated, was confirmed by converting foeniculoxin into the corresponding 1,4,6'-*O,O',O"*-triacetylderivative **2** by the usual reaction with Ac_2O and pyridine, and the phenolic nature of two of them was supported through toxin transformation into

the 1,4-*O,O'*-dimethylether **3** by treatment of **1** with ethereal diazomethane. The latter derivative led to the confirmation of the substituent pattern of the aromatic ring by a series of NOE-difference spectra[6]. In fact, by irradiation of MeO-1 at 3.83 only the *ortho*-located H-6 at δ 6.79 was affected, while irradiating the MeO-4 at δ 3.76 the two *ortho*-located protons H-3 and H-5 at δ 6.94 and 6.84, respectively, exhibited similar NOE effects. The presence in **1** of a disubstituted triple bond conjugated with a terminal disubstituted olefinic group was ascertained through their complete saturation obtained by catalytic hydrogenation of the toxin. Although the two reduced diastereomeric derivatives **5a** and **5b**, as well as their corresponding 1,4,6'-*O,O',O''*-triacetylderivatives **6a** and **6b** could not be separated using normal chromatographic methods, their spectroscopic data were fully consistent with the expected structures and with that of **1**. The presence in **1** of a 1,2-diol system on C-6' and C-7' of the side chain was first confirmed by converting the toxin into the corresponding 6',7'-*O,O'*-isopropylidene derivative **4** by the usual acid catalyzed reaction with dry acetone. Finally, this glycol as well as the hydroquinone nature of **1** justified its easy $NaIO_4$ oxidation. As expected, foeniculoxin was converted into the corresponding *p*-benzoquinone derivative **7** and the related aldehyde **8** arose from the oxidative cleavage of the glycol system. In conclusion, the terpenoid hydroquinone structure of foeniculoxin seems satisfactorily demonstrated and very different from that related to toxic betaenones[9] hypothesized for the unpure phytotoxic metabolite previously isolated from culture filtrates of the same fungus by G. Hunault *et al.*[2]. The terpenoid hydroquinones are a well known family of natural substances closely related to the corresponding *p*-benzoquinones into which they are easily oxidized. Terpenoid hydroquinones, specially *p*-benzoquinone ones, which have very important biological activities[10,11], are common as natural plant, animal and microrganism (bacteria and fungi) metabolites[10-13]. To our knowledge the geranylhydroquinones isolated from *Cordia alliodora*[14] and then from *C. elaeagnoides*[15] are the terpenoid hydroquinone compounds closest to foeniculoxin.

EXPERIMENTAL

General

Optical rotations: CH_3OH; IR and UV: neat and MeCN, respectively; ^1H and ^{13}C NMR spectra: CD_3OD, unless noted, at 400 and or 270 MHz and 100 and/or 67.92 MHz, respectively, using the same solvent as the internal standard. Carbon multiplicities were determined by DEPT (Distortionless Enhancement by Polarization Transfer) spectra[6]. DEPT, NOEDS, COSY (Correlated Spectroscopy), 2D heteronuclear and long range heteronuclear chemical shift correlation and gated decoupled experiments were performed using Bruker standard microprograms; EI and FAB MS: 70 eV and glycerol/thioglycerol with Xe atom at 9.5 kV, respectively. Analytical and preparative TLC: silica gel (Merck, Kieselgel 60 F_{254}, 0.25 and 0.50 mm, respectively) or on reverse phase (Whatman, KC 18 F_{254}, 0.20 mm) plates. Column chromatography: silica gel (Merck, Kieselgel 60, 0.063-0.20 mm); solvent systems: (A) $CHCl_3$-*iso*-PrOH (5.7:1); (B) H_2O-EtOH (1.5:1); (C) $CHCl_3$-*iso*-PrOH (32.3:1); (D) $CHCl_3$-*iso*-PrOH (9:1); (E) $CHCl_3$-*iso*-PrOH (19:1).

Production, extraction and purification of foeniculoxin (1)

Single spore cultures of *P. foeniculi*, freshly reisolated from artificially infected fennel, were grown on potato-dextrose-agar at 25° and used to inoculate 1 l Erlenmeyer flasks containing 200 ml of modified Czapek-Dox medium (Saccharose 30 g; K_2HPO_4 1 g; $MgSO_4x7H_2O$ 0.5g; KCl 0.5 g; $FeSO_4x7H_2O$ 0.01 g; *L*-Asparagine 2g; yeast extract 2 g; H_2O 1 l; pH 6.7). The cultures were incubated at 25° for 18 days in shaken culture (100 rev./min), then filtered and lyophilized. The lyophilized material (5.6 g) obtained from the culture filtrates (1.5 l) was resuspended in distilled H_2O (0.75 l, pH 6.5-7.0) and extracted with EtOAc (4x500 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated under reduced pressure. The brown oily residue (400 mg), with a high phytotoxic activity, was fractionated by column chromatography eluted with solvent system A to yield 12 groups of homogeneous fractions. The pooled fractions between groups 4-7 and 11-12 showed phytotoxic activity. The residues (20.5, 34.2 and 22.6 mg, respectively) left from groups 5-7,

containing the main metabolite (R_f 0.42 and 0.62 by TLC on silica gel, eluent A and on reverse phase, eluent B, respectively) were combined and further fractionated by preparative TLC (silica gel, eluent A) producing a crude metabolite (43.7 mg). The latter was finally purified by preparative TLC on reverse phase (eluent B) yielding foeniculoxin (**1**) as a homogeneous oily compound (17.7 mg, 12 mg/l) withstanding crystallization.

Biological methods

Each sample was dissolved in a minute amount of MeOH and brought up to the required concentration with distilled H_2O. Phytotoxic activity of foeniculoxin was tested at $3.6x10^{-3}$ M on tobacco leaves, on tomato seeds and cuttings and on fennel seedlings using the leaf mesophyll infiltration method[16] and/or the absorption method[17,18].

Foeniculoxin (1)

Foeniculoxin (**1**) had: $[\alpha]^{25}D$ +9.0 (*c* 0.10); UV λmax nm (log ε): 328 (3.83), 276 (4.03), 265 (4.09); alkaline solution: 305 (3.95), <220; IR νmax cm^{-1}: 3265 (OH), 2205 (C≡C); 1610 (C=C) 1559, 1496, 1449 (Ar C=C), 1361 (gem-CH_3); ^1H and ^{13}C NMR spectra: Table 1; EI MS, *m/z* (rel. int.): 276 $[M]^+$ (6), 258 $[M-H_2O]^+$ (26), 243 $[M-H_2O-Me]^+$ (19), 225 $[M-2xH_2O-Me]^+$ (8), 215 $[M-H_2O-MeCHMe]^+$ (16), 199 $[M-H_2O-(Me)_2COH]^+$ (17), 187 $[M-H_2O-(Me)_2CH-CO]^+$ (35), 174 [M-MeCOCOH(Me)$_2$, $C_{11}H_{10}O_2]^+$ (100), 173 $[M-CH_2CH(OH)COH(Me)_2$, $C_{11}H_9O_2]^+$ (36), 137 (34), 115 $[Ar(OH)_2C{\equiv}C-H_2O]^+$ (25), 103 (7), 91 (10), 77 (11), 59 [MeCOHMe, C_3H_7O]+ (41); FAB MS *m/z* (rel. int.) 277 $[M+H]^+$ (100), 259 $[M+H-H_2O]^+$ (95), 241 $[M+H-2xH_2O]^+$ (16).

1,4,6'-O,O',O"-Triacetylfoeniculoxin (2)

Foeniculoxin (**1**, 4.5 mg) was acetylated with pyridine (300 μl) and Ac_2O (300 μl) at room temperature overnight. The oily residue left by the reaction work-up was purified by preparative TLC (silica gel, eluent C) to give **2** as a homogeneous compound (3.8 mg). The spectroscopic data of **2** differed from those of **1** in the following: IR νmax cm^{-1}: 1769 (PhOAc), 1733 (ROAc); ^1H NMR ($CDCl_3$), δ: 7.25 (1H, *dd*, *J*=8.3 and 2.8 Hz, H-5), 7.08 (2H, *m*, H-3 and H-6), 4.85 (1H, *dd*, *J*=10.4 and 3.5 Hz, H-6'), 2.34, 2.30 and 2.13 (3H each, *s*, three MeCO groups); ^{13}C NMR ($CDCl_3$), δ: 171.2, 169.0 and 169.7 (*s*, three MeCO groups), 21.1, 21.1 and 20.8 (*q*, three MeCO groups); EI MS *m/z* (rel. int.): 402 $[M]^+$ (3), 384 $[M-H_2O]^+$ (6), 360 $[M-CH_2CO]^+$ (15), 343 $[M-AcO]^+$ (22), 342 $[M-AcOH]^+$ (100), 327 $[M-AcOH-Me]^+$ (6), 301 $[M-AcO-CH_2CO]^+$ (15), 300 $[M-AcOH-CH_2CO]^+$ (53), 282 $[M-2xAcOH]^+$ (31), 267 $[M-2xAcOH-Me]^+$ (25), 258 $[M-AcOH-2xCH_2CO]^+$ (92), 241 $[M-AcO-CH_2CO-AcOH]^+$ (55), 240 $[M-2xAcOH-CH_2CO]^+$ (80), 225 $[M-2xAcOH-CH_2CO-Me]^+$ (80), 212 $[M-2xAcOH-CH_2CO-CO]^+$ (71), 200 $[M-AcOH-2xCH_2CO-Me_2CO]^+$ (96), 199 $[M-2xAcOH-CH_2CO-Me_2COH]^+$ (65).

1,4-O,O'-Dimethylfoeniculoxin (3)

To a solution of foeniculoxin (**1**, 9.5 mg) in MeOH (4.5 ml) ethereal CH_2N_2 (4.5 ml) was added. The mixture was allowed to stand at room temperature for 12 h and then evaporated under a stream of nitrogen. Purification of the residue by preparative TLC (silica gel, eluent D) produced **3** as a pure oil (4.6 mg). The spectroscopic data of **3** differed from those of **1** in the following: IR νmax cm^{-1}: 1132 (Ar-O-R); ^1H NMR ($CDCl_3$), δ: 3.83 and 3.76 (3H each, *s*, MeO-1 and MeO-4, respectively); ^{13}C NMR ($CDCl_3$), δ: 56.4 and 55.8 (*q*, two MeO groups); EI MS *m/z* (rel. int.): 304 $[M]^+$ (31), 286 $[M-H_2O]^+$ (2), 273 $[M-MeO]^+$ (2), 272 $[M-MeOH]^+$ (3), 271 $[M-H_2O-Me]^+$ (14), 255 $[M-H_2O-MeO]^+$ (9), 245 $[M-MeO-CO]^+$ (12), 202 [M-MeCOCOH(Me)$_2$, $C_{13}H_{14}O_2]^+$ (100), 201 $[M-CH_2CH(OH)COH(Me)_2$, $C_{13}H_{13}O_2]^+$ (23); FAB MS *m/z* (rel. int.): 305 $[M+H]^+$ (100), 287 $[M+H-H_2O]^+$ (58).

6',7'-O,O'-Isopropylidenefoeniculoxin (4)

Foeniculoxin (**1**, 8 mg) in dry Me_2CO (12 ml) was stirred with dry $CuSO_4$ (300 mg) under reflux for 5 h.

The mixture was filtered and evaporated to give an oily residue which was purified by preparative TLC (silica gel, eluent D) to yield **4** as a homogeneous oil (3.1 mg). The spectroscopic data of **4** differed from those of **1** in the following: IR νmax cm^{-1}: 1456 (acetal-CH_3), 1220 (C-O-C-O); ^1H NMR ($CDCl_3$), δ: 3.71 (1H, *dd*, *J*=9.5 and 3.2 Hz, H-6'), 1.44 and 1.34 (3H each, *s*, $\underline{Me}_2C$); ^{13}C NMR ($CDCl_3$), δ: 106.6 (*s*, $Me_2\underline{C}$), 82.2 (*d*, C-6'), 80.2 (*s*, C-7'), 28.1 and 26.9 (*q*, $\underline{Me}_2C$); EI MS *m/z* (rel. int.): 316 $[M]^+$ (3), 301 $[M\text{-}Me]^+$ (28), 258 $[M\text{-}Me_2CO]^+$ (59), 243 $[M\text{-}Me\text{-}Me_2CO]^+$ (25), 225 $[M\text{-}Me\text{-}Me_2CO\text{-}H_2O]^+$ (13), 215 $[M\text{-}Me_2CO\text{-}MeCHMe]^+$ (25), 199 $[M\text{-}Me_2CO\text{-}MeCOHMe]^+$ (49), 187 $[M\text{-}Me_2CO\text{-}MeCHMe\text{-}CO]^+$ (58).

Catalytic hydrogenation of foeniculoxin

Foeniculoxin (**1**, 12.5 mg) in MeOH (4.0 ml) was added to a presaturated 5% Pd/C (8.0 mg) suspension in the same solvent (4.0 ml) and hydrogenated at room temperature and atmospheric pressure under stirring. After 12 h the reaction was stopped by filtration, evaporated under reduced pressure and the residue purified by two successive preparative TLC steps (silica gel, eluent A) to give a mixture of diastereomeric hexahydro derivatives **5a** and **5b** (7.1 mg) which could not be separated using normal or reverse phase chromatography. **5a** and **5b** had: UV λmax nm (log ε): 293 (3.52); alkaline solution: 250 (3.77), which are in agreement with the data reported for suitable substituted hydroquinones[4]; IR νmax cm^{-1}: 3333 (OH), 1507 , 1457 (Ar C=C), 1377 (gem- CH_3); EI MS *m/z* (rel. int.): 282 $[M]^+$ (2), 264 $[M\text{-}H_2O]^+$ (17), 246 $[M\text{-}2xH_2O]^+$ (4), 232 $[M\text{-}2xH_2O\text{-}CH_2]^+$ (2), 224 $[M\text{-}Me_2CO]^+$ (10), 203 $[M\text{-}2xH_2O\text{-}\ CH_2\text{-}HCO]^+$ (18), 189 $[M\text{-}2xH_2O\text{-}HCO\text{-}2xCH_2]^+$ (7), 175 $[M\text{-}2xH_2O\text{-}HCO\text{-}3xCH_2]^+$ (29), 163 $[Ar(OH)_2CHCH{=}CHMe, C_{10}H_{11}O_2]^+$ (16), 136 $[Ar(OH)_2\text{-}CH{=}CH_2, C_8H_8O_2]^+$ (23), 130 (100), 123 $[Ar(OH)_2\text{-}CH_2, C_7H_7O_2]^+$ (65), 109 $[Ar(OH)_2, C_6H_5O_2]$+ (9), 91 (36); FAB MS *m/z* (rel. int.): 283 $[M+H]^+$ (23), 282 $[M]^+$ (30), 265 $[M+H\text{-}H_2O]^+$ (100); ^1H NMR spectrum differed from that of **1** in the following signal systems, **5a**, δ: 3.22 (1H, *br d*, *J*=9.8 Hz, H-6'), 1.50 (1H, *m*, H-3'), 1.15 (6H, *s*, Me-8' and Me-10'), 0.97 (3H, *d*, *J*=6.4 Hz, Me- 9'); **5b**, δ 3.25 (1H, *br d*, *J*=9.8 and 1.7 Hz, H-6'), 1.50 (1H, *m*, H-3'), 1.13 and 1.12 (6H, *s*, Me-8' and Me-10'), 0.96 (3H, *d*, *J*=6.0 Hz, Me-9'); ^{13}C NMR spectrum differed from that of **1** in the following signal systems, **5a**, δ: 38.1 (*t*, C-1'), 33.9 (*d*, C-3'), 28.7 (*t*, C-2'), 20.3 (*q*, Me-9'); **5b**, δ: 38.6 (*t*, C-1'), 33.9 (*d*, C-3') 28.7 (*t*, C-2'), 20.0 (*q*, Me-9').

Acetylation of the hexahydroderivatives 5a and 5b

The hexahydroderivatives **5a** and **5b** (in mixture, 5.6 mg) were converted into the corresponding triacetyl derivatives **6a** and **6b** using the same method described above to obtain **2** from **1**. The oily residue left by the reaction work-up was purified using preparative TLC (silica gel, eluent C) to give the triacetyl derivatives **6a** and **6b** as oily mixture (4.6 mg) which could not be separated by normal chromatographic methods. **6a** and **6b** had UV λmax nm (log ε) 270 (2.80), 264 (2.83) in agreement with data reported for suitable substituted hydroquinones[4]. Their spectroscopic data differed from those of **5a** and **5b** in the following: IR νmax cm^{-1}: 1763 (PhOAc), 1733 (ROAc); EI MS *m/z* (rel. int.): 366 $[M\text{-}CH_2CO]^+$ (3), 348 $[M\text{-}AcOH]^+$ (5), 324 $[M\text{-}2xCH_2CO]^+$ (3), 306 $[M\text{-}CH_2CO\text{-}AcOH]^+$ (5), 264 $[M\text{-}2xCH_2CO\text{-}AcOH]^+$ (30), 246 $[M\text{-}CH_2CO\text{-}2xAcOH]^+$ (25), 206 $[M\text{-}2xCH_2CO\text{-}AcOH\text{-}Me_2CO]^+$ (22), 123 (100); FAB MS *m/z* (rel. int.): 409 $[M+H]^+$ (100), 349 $[M+H\text{-}AcOH]^+$ (86), 307 $[M+H\text{-}\ AcOH\text{-}CH_2CO]^+$ (32); ^1H NMR (C_6D_6) **6a**, δ: 7.11 (1H, *d*, *J*=2.6 Hz, H-3), 7.01 (1H, *d*, *J*=8.7 Hz, H-6), 6.94 (1H, *dd*, *J*=8.7 and 2.6 Hz, H-5), 4.97 (1H, *dd*, *J*=10.0 and 2.1 Hz, H-6'), 2.00, 1.84 and 1.80 (3H each, *s*, three $\underline{Me}CO$) and **6b**, δ: 7.08 (1H, *d*, *J*=2.6 Hz, H-3), 7.00 (1H, *d*, *J*=8.7 Hz, H-6), 6.93 (1H, *dd*, *J*=8.7 and 2.6 Hz, H-5) , 4.98 (1H, dd, *J*=12.8 and 5.1 Hz, H-6'), 1.97, 1.83, and 1.80 (3H, each, *s*, $\underline{Me}CO$); ^{13}C NMR ($CDCl_3$) **6a** and **6b** three double lines at δ: 171.2, 169.4 and 169.4 (*s*, three $Me\underline{C}O$) and 21.2, 21.2 and 20.9 (*q*, three $\underline{Me}CO$).

Oxidation of foeniculoxin with $NaIO_4$

To a solution of foeniculoxin (12.5 mg) dissolved in MeOH (16 ml) was added $NaIO_4$ (12.5 mg) in H_2O (5 ml). The reaction was left at room temperature under stirring in the dark, and after 1 h was stopped with cold Me_2CO (5 ml) and ethylene glycol (1.0 ml). After 15 min, the mixture was extracted with CH_2Cl_2 (4x15

ml), dried (Na_2SO_4) and evaporated under reduced pressure. The orange residue was purified by preparative TLC (silica gel, eluent E) to yield the two *p*-benzoquinone derivatives **7** and **8** as orange and yellow oily compounds (1.4 and 1.0 mg), respectively. **7**, in agreement with the data reported in literature for suitable substituted *p*-benzoquinones[3,4,10],had: UV λmax nm (log ε): 325 (3.5), 263 (3.92) and IR νmax cm^{-1}: 3364 (OH), 2208 (C≡C), 1676 and 1658 (quinone C=O), 1577 (C=C), 1373 (gem-CH_3); ^1H and ^{13}C NMR spectra ($CDCl_3$), assigned in agreement to literature data[6,8] differed from that of **1** in the following signal systems: ^1H NMR, δ 6.87-6.78 (3H, quinones), ^{13}C NMR, δ: 187.9 (*s*, C-1), 187.7 (*s*, C-4), 136.7 (*d*, C-5 and C-6), 135.2 (*d*, C-3), 131.0 (*s*, C-2); EI MS, in agreement to the behaviour described for *p*-benzoquinones[19], *m/z* (rel. int): 276 $[M+2H]^+$ (3), 258 $[M+2H-H_2O]^+$ (7), 243 $[M-2H-H_2O-Me]^+$ (7), 225 $[M+2H-2xH_2O-Me]^+$ (4), 215 $[M+2H-H_2O-MeCHMe]^+$ (11), 199 $[M+2H-H_2O-(Me)_2COH]^+$ (11), 187 $[M+2H-H_2O-(Me)_2CH-CO]^+$ (12), 174 $[M+2H-MeCOCOH(Me)_2$, $C_{11}H_{10}O_2]^+$ (25), 147 (9), 137 (11), 115 $[Ar(OH)_2C{\equiv}C-H_2O]^+$ (25), 97 (40), 73 (100). The aldehyde **8**, in agreement with data reported for suitable substituted quinones[3,4,10] and in respect to **7** had: IR νmax cm^{-1}: 1733 (HCO); ^1H NMR ($CDCl_3$) δ: 9.84 (1H, *br s*, HCO-6'), 2.78 (2H, *br t*, *J*=7.0, H-4'), 2.61 (2H, *br t*, *J*=7.0 Hz, H-5'); EI MS, in agreement with the behaviour of *p*-benzoquinones[19], *m/z* (rel. int.): 213 $[M-H]^+$ (4), 185 $[M-H-CO]^+$ (6), 167 $[M-H-CO-H_2O]^+$ (18), 150 $[M-H-CO-H_2O-OH]^+$ (11), 149 $[M-H-CO-2xH_2O]^+$ (98), 123 $[M-H-CO-2xH_2O-HC{\equiv}CH]^+$ (13), 97 $[M-H-CO-2xH_2O-2xHC{\equiv}CH]^+$ (37), 83 (50), 71 (48), 69 (100).

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