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PRENYLATED COUMARINS AND SESQUITERPENOIDS FROM *FERULA COMMUNIS*

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Key Word Index- *Ferula communis*; Umbelliferae; ferulosis; prenylated coumarins; ferulenol; daucane esters; germacrane alcohols.

Abstract—From the latex of *Ferula communis*, two 4-hydroxycoumarin derivatives were isolated bearing a farnesylic and a 12-hydroxyfarnesylic residue, respectively, at C-3. Prenylated coumarins, which represent toxic principles of the plant, were absent in other samples, which gave, besides known compounds, a germacrane alcohol (hallohedycariol) and a daucane ester (siol *p*-hydroxybenzoate).

INTRODUCTION

Ferula communis L. is a latex-containing plant widespread in all of the Mediterranean area. The plant is reported to be toxic to livestock [1], causing an often lethal haemorrhagic disease known as 'ferulosis' or 'mal di Ferula' [1]. Ferulosis shows symptoms similar to those of poisoning from fermented sweet-clover [1], and it was suggested that *F. communis* contains anti-thrombinic coumarin derivatives [1].

3-Methyl-4-hydroxycoumarin (**1**) was first isolated from this plant [2, 3], but this compound was in fact shown to display a mild vitamin K activity [4]. An antithrombinic activity could be demonstrated only at very high doses, unlikely to be obtained in cattle upon ingestion of the plant [4]. Further studies showed that **1** was most probably an artefact, formed during the drastic conditions of isolation (dry distillation of a plant extract) from a C-3 prenylated 4-hydroxycoumarin (ferulenol), to which structure **2**, with stereochemistry at the double bonds unsettled, was assigned [5, 6].

Poisoning from *F. communis* is frequent in Sardinia, where it causes damage of considerable economic importance. It was noticed that the toxicity of *F. communis* growing in Sardinia depends on the place of collection and on the basis of biological tests a toxic and a non-toxic variety were distinguished [7, 8]. Particularly toxic appear to be the plants from the north of Sardinia and Asinara Island, which gave most of the material used for these studies.

As a part of an investigation of Sardinian plants, we have studied the constituents of two collections of *F. communis* from the north of this island.

RESULTS AND DISCUSSION

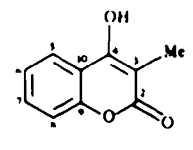
The chloroform-soluble fraction of the latex from the first collection of *F. communis* examined was made up mostly of 4-hydroxycoumarin derivatives bearing a sesquiterpene residue at C-3. The major constituents were ferulenol (**2**) [6], and the more oxygenated compound **3**, differing from **2** only in the sesquiterpene moiety, in which an allylic methyl is replaced by a hydroxymethyl group.

The stereochemistry of the farnesylic residue of ferulenol (**2**) was established by analysis of its ^{13}C NMR spectrum (Table 1), which showed three allylic methyls (C-13′, C-14′ and C-15′) shielded by γ-gauche interactions. In olefinic systems this interaction would be better referred to as *γ-cis*, since allylic groups are *peri*-planar, and

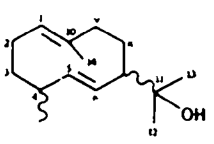
Table 1. ^{13}C NMR spectral data for compounds **2**, **3**, **8** and **11** (67.80 MHz, CDCl$_3$, TMS as reference)

	2	**3**		**8**	**11**
C-2	160.93 *s*	160.83 *s*	C-1	75.98 *d*	127.08 *d*
C-3	103.41 *s*	103.22 *s*	C-2	119.43 *d*	22.74 *t*
C-4	163.85 *s*	163.59 *s*	C-3	146.41 *s*	41.01 *t*
C-5	122.72 *d**	122.71 *d**	C-4	37.17 *t*	40.40 *d*
C-6	123.01 *d**	123.55 *d**	C-5	29.07 *t*	143.87 *d*
C-7	131.40 *d*	131.48 *d*	C-6	82.27 *s*	130.24 *d*
C-8	116.21 *d*	116.34 *d*	C-7	60.15 *s*	58.04 *d*
C-9	152.17 *s*	152.37 *s*	C-8	219.87 *s*	35.60 *t*
C-10	115.94 *s*	116.03 *s*	C-9	38.26 *t*	28.40 *t*
C-1′	23.95 *t*	23.72 *t*	C-10	51.44 *d*	131.48 *s*
C-2′	119.32 *d*	120.16 *d*	C-11	26.24 *d*	71.49 *s*
C-3′	141.26 *s*	141.60 *s*	C-12	21.01 *q*	22.62 *q*
C-4′	39.53 *t*	39.10 *t*	C-13	26.08 *q*	26.68 *q*
C-5′	25.95 *t*	25.74 *t*	C-14	18.14 *q*	16.85 *q*
C-6′	123.70 *d*†	123.70 *d*†	C-15	24.82 *q*	26.56 *q*
C-7′	135.88 *s*	135.52 *s*	C-1′	164.79 *s*	
C-8′	39.53 *t*	39.10 *t*	C-2′	122.56 *s*	
C-9′	26.49 *t*	25.74 *t*	C-3′	133.56 *d*	
C-10′	124.12 *d*†	125.82 *d*†	C-4′	131.25 *d*	
C-11′	131.11 *s*	134.46 *s*	C-5′	163.28 *s*	
C-12′	25.53 *q*	68.82 *t*	OMe	55.37 *q*	
C-13′	15.95 *q*‡	16.00 *q*‡			
C-14′	16.27 *q*‡	16.31 *q*‡			
C-15′	17.52 *q*	13.69 *q*			

*,†,‡Signals with the same symbols in the same line are interchangeable.



1



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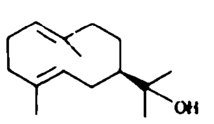

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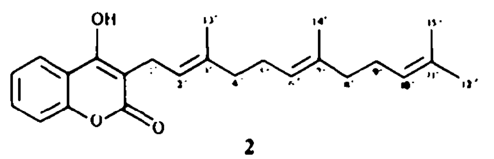

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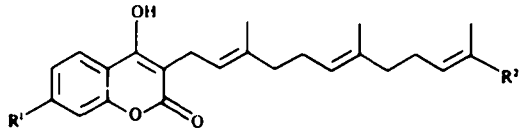
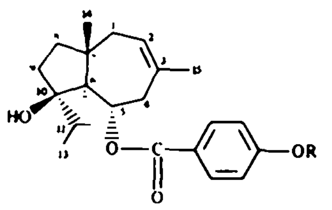
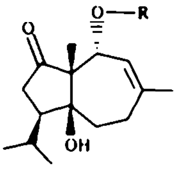
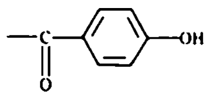
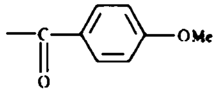


	R¹	R²
3	H	CH_2OH
4	H	CHO
13	OH	Me



	R
5	H
6	Me



	R
7	
8	
9	Ac
10	Ang

deviations from this geometry are found only in special cases, such as when bulky groups are present, or in *trans*-double bonds located in medium-sized rings (cf. the X-ray data on germacranolides). The alkyl groups resonated between 15 and 17 ppm, and one allylic methyl lacking interactions of this type resonated at 25.53 ppm [9]. This established the stereochemistry of the farnesylic residue as 2*E*,6*E*. In 3, one of the shielded allylic methyls was shifted upfield (δ 13.69), whereas the methyl resonance at δ25.53 was replaced by a triplet at 68.82, suggesting that a hydroxyl group had been introduced at C-12'.

This was further confirmed by the oxidation of 3 to the α,β-unsaturated aldehyde (4), in which the chemical shift of the aldehydic proton showed the presence of a *peri*-like interaction with a β-olefinic proton [10]. This unambiguously located the hydroxyl group at C-12'.

In the chloroform-soluble fraction of the latex of the other samples examined, coumarin derivatives were virtually absent. Cyclic sesquiterpene derivatives were isolated instead. The most abundant compound was the *O*(5)-*p*-hydroxybenzoate of jaeskeanadiol (5) [11], accompanied by minor amounts of its methyl derivative (6) [12] and the ketone (7). The latter could be thoroughly purified only after conversion to its methyl derivative (8). Except for the presence of the signals of different ester moieties, the NMR spectrum of 8 was very similar to those of the siol esters 9 and 10 [13].

The presence of a common sesquiterpene moiety in 8-10 was further confirmed by the similarity of the peculiar acylation shifts observed upon *in situ* carbamoylation with trichloroacetylisocyanate (TAI) [14] (downfield shifts for H-14 and H-12; upfield shifts for H-1 and H-2 [13]).

The absolute configuration of 8 was established by application of the allylic anysate rule [15], which showed that this chromophore had a negative absolute conformation. Since the relative configuration and conformation of siol ester are known [13], the absolute configuration of the ester from *F. communis* is that represented by formula 7.

A positive Cotton effect around 290 nm was assigned to the cyclopentanone chromophore. Since this Cotton effect is also positive in compounds 9 and 10 [13], the absolute configuration is the same in all these compounds.

The major constituent of the volatile fraction from the roots was the alcohol **11**, whose molecular formula was established as $C_{15}H_{26}O$ by mass spectrometry.

Since the ^{13}C NMR spectrum (Table 1) showed the presence of two double bonds (a trisubstituted and a disubstituted one) compound **11** is monocyclic. A prominent peak at *m/z* 59 in the mass spectrum showed the presence of a dimethylcarbinyl side-chain, further confirmed by the addition of TAI to a solution of **11** in $CDCl_3$, which resulted in a marked downfield shift of two methyl singlets. Multiplicity considerations showed that at least one of the two unconjugated double bonds had to be in a cyclic system. Since both double bonds were *trans*-substituted, a medium-sized ring was present. This was further confirmed by the presence of an anomalous UV absorption at 220 nm, which is quite typical of homoconjugated double bonds in medium-sized rings [16].

A germacrane structure (**11**) was thus assigned to this compound, with stereochemistry at C-4 and C-7 unsettled. A compound with the same constitution as **11** had already been obtained as a side-product in the course of a total synthesis of hedycariol (**12**) [17]. On account of the similarity of **11** and **12**, we propose the name allohedycariol for **11** (cf. the pairs of terpenes ocimene and alloocimene, differing only in the location of the double bonds).

Daucane esters are widespread and almost ubiquitous in plants from the genus *Ferula*. In contrast, prenylated 4-hydroxycoumarins are rare in nature. 5-Methyl-4-hydroxycoumarins prenylated at C-3 (C_{10}-coumarins)[*] have been reported from some genera of the Compositae [18], but to the best of our knowledge the only 4-hydroxycoumarins are **2** and **3** from *Ferula communis* and ammoresinol[†] (**13**) from ammoniacum, an oleo gum resin from *Dorema ammoniacum* D. Don., an Asian umbelliferous plant [19].

F. communis has been unambiguously recognized as the causative agent of severe haemorrhagic disease, which is widespread in the Mediterranean area and particularly in Sardinia since 1925 [7]. Ferulenol (**2**) displays haemorrhagic activity, and presumably represents the toxic principle of the plant. Our finding of two different chemotypes of *F. communis*, only one of which contained prenylated 4-hydroxycoumarins, confirms the early observation, based on biological tests, of the presence of two distinct varieties of this plant [7, 8]. A recent study of a Turkish collection of *F. communis* revealed a chemical pattern different from that of the plants we examined [20], suggesting that several chemotypes of *F. communis* might be present in the Mediterranean area, accounting for the contrasting data on the toxicity of this plant reported in the literature [7].

EXPERIMENTAL

Silica gel 60 (70–230 mesh) was used for CC.

Plant material. Ferula communis L. was collected near Agro di Macomer (NU) (sample A) and Agro di Oliena (NU) (sample B) in May and June, when an abundant production of latex takes place, and was identified by V.P.

Isolation of prenylated coumarins. About 10 ml of latex (obtained by spontaneous dripping from a cut stem) from sample A was diluted with NaCl soln and extracted with $CHCl_3$ to yield a gum (2.1 g). The latter was separated by CC (silica gel) using a mixture of petrol (bp 50–70°) and EtOAc as eluant. Fractions eluted with petrol–EtOAc (5:1) gave 530 mg **2**; fractions eluted with petrol–EtOAc (3:1) gave 210 mg **3**.

Ferulenol (**2**). Amorphous white powder, mp 61°; IR ν_{max}^{KBr} cm^{-1}: 3200 (*br*, OH), 1675, 1610 (coumarin), 1570, 1210, 760; UV λ_{max}^{EtOH} nm: 310, 286, 276; EIMS 70 eV (see Scheme 1), *m/z* (rel. int.): 366 $[M]^+$ ($C_{24}H_{30}O_3$) (1.2), 297 $[A]^+$ (1.8), 229 $[B]^+$ (3), 175 $[C]^+$ (8), 147 $[D]^+$ (6), 137 $[E]^+$ (7.5), 129 (30), 121 $[F]^+$ (15), 109 (100), 69 $[G]^+$ (85); FAB-MS: 368 $[M+2]^+$ (50), 69 (100); ^1H NMR ($CDCl_3$, 270 MHz, TMS as reference): δ7.76–7.22 (*m*, H-5, H-6, H-7, H-8), 5.46 (*br t*, $J_{1',2'} = 8.1$ Hz, H-2'), 5.07 (*br s*, H-6' + H-10'), 3.43 (*d*, $J_{1',2'} = 8.1$ Hz, H-1'), 1.84 (*br s* H-12'), 1.66, 1.61, 1.56 (*br s*, H-13', H-14', H-15').

12'-Hydroxyferulenol (**3**). Yellowish gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200 (*br*, OH), 1690, 1630 (coumarin), 1580, 1500, 1460, 1280, 1220, 1110; UV λ_{max}^{EtOH} nm: 310, 286, 276; EIMS *m/z* (rel. int.): no molecular ion, 297 $[A]^+$ (22), 229 $[B]^+$ (38), 187 (40), 175 $[C]^+$ (100), 135 (22), 121 $[F]^+$ (63), 81 (40), 69 $[G]^+$ (91); ^1H NMR (200 MHz, $CDCl_3$, TMS as reference): δ7.82–7.19 (H-5, H-6, H-7, H-8), 5.40 (*br m*, H-2' + H-10'), 5.07 (*br s*, H-6'), 3.98 (*br s*, H-12'), 3.38 (*d*, $J_{1',2'} = 8.1$ Hz, H-1', 1.80 (*br s*, H-15'), 1.61, 1.58 (*br s*, H-13', H-14').

Oxidation of 12'-hydroxyferulenol. A 50 mg sample of **3** was dissolved in dry CH_2Cl_2 and treated with 42 mg pyridinium chlorochromate (PCC; *ca* 1.5 M equivalent). After 5 min the reaction mixture was diluted with dry Et_2O and filtered through a short column of Florisil. Further purification by CC (5 g silica gel, petrol–EtOAc, 4:1) gave 15 mg **4** as an unstable colourless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (br, OH), 1810 (coumarin), 1785 (α,β-unsaturated aldehyde), 1640, 1500, 1280, 910; UV λ_{max}^{EtOH} nm: 310, 286, 276. EIMS *m/z* (rel. int.): 380 $[M]^+$ ($C_{24}H_{28}O_4$) (17), 362 $[M-H_2O]^+$ (28), 297 $[A]^+$ (57), 229 $[B]^+$ (57), 213 (100), 187 (50), 175 $[C]^+$ (71), 147 $[D]^+$ (29), 135 (45), 121 $[F]^+$ (88), 107 (57), 69 $[G]^+$ (45); ^1H NMR ($CDCl_3$, 270 MHz, TMS as reference): δ9.36 (*s*, H-12'), 7.76–7.21 (H-5, H-6, H-7, H-8), 6.45 (*br s*, H-10'), 5.39 (*br t*, $J_{1',2'} = 8.1$ Hz, H-2'), 5.10 (*br s*, H-6'), 3.41 (*d*, $J_{1',2'} = 8.1$ Hz, H-1'), 1.83, 1.71, 1.63 (*br s*, H-13', H-14', H-15').

Isolation of the constituents of sample B. One fresh sample of roots was chopped, causing dripping of the latex. After treating the latex and roots with $CHCl_3$, evaporation of the organic phase gave 10 g of a thick oil. The latter was chromatographed on a column of 200 g silica gel. Fractions eluted with petrol–$CHCl_3$ (3:1 gave 1.5 g **11**; those eluted with $CHCl_3$ gave 200 mg **6**, and fractions eluted with $CHCl_3$–Me_2CO (4:1) gave 2.5 g **5** and 200 mg of a mixture of **5** and **7**, which were separated after conversion to their corresponding methyl derivatives with CH_2N_2 (**6** and **8** respectively). After chromatography on 5 g silica gel, eluant petrol–EtOAc (3:1), 70 mg **8** was obtained. Known compounds were identified by comparison of their physical and spectral data with those reported in the literature [21, 22].

Allohedycariol (**11**). Colourless oil, $[\alpha]_D^{25} + 181°$ ($CHCl_3$; *c* 1.5); IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3400 (br, OH), 1670, 1660 (very weak double bonds), 1375, 980, 880; UV λ_{max}^{EtOH} (log ε) nm: 218 (3.3) (homoconjugated double bonds); EIMS 70 eV, *m/z* (rel. int.): 222 $[M]^+$ ($C_{15}H_{26}O$) (8), 204 $[M-H_2O]^+$ (8), 189 $[M-H_2O-Me]^+$ (12), 82 $[C_6H_{10}]^+$ (100), 59 $[C_3H_7O]^+$ (44); ^1H NMR (270 MHz, $CDCl_3$, TMS as reference): δ4.95–5.20 (*br m*, H-1 + H-5 + H-6), 1.53 (*br s*, H-14), 1.15 (*s*, H-12), 1.07 (*s*, H-13), 0.90 (*d*, $J_{4,15} = 6.7$ Hz, H-15); ΔTAI ($CDCl_3$): H-12: +0.34 ppm; H-13: +0.34 ppm.

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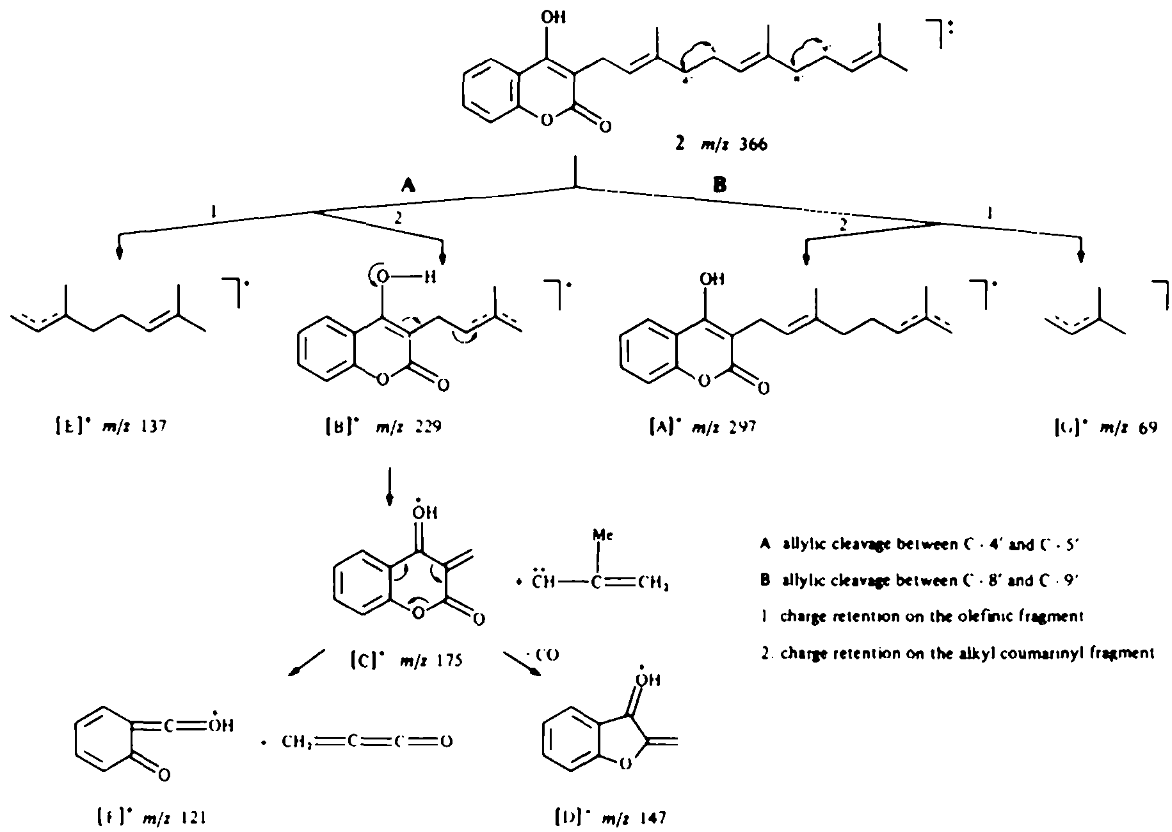
[*] Owing to the presence of the extra methyl at C-5, these compounds are probably of acetate and not of shikimate origin.

[†] The stereochemistry of the farnesylic residue of ammoresinol has been established as 2*E*,6*E* (G. Appendino and M. G. Valle, unpublished results).



Scheme 1.

Siol p-*methoxybenzoate* (8). Colourless needles from hexane, mp 127°, $[\alpha]_D^{25}$ −217° ($CHCl_3$; *c* 1.2); IR ν_{max}^{KBr} cm^{-1}: 3520 (OH), 1730 (5-membered ring ketone), 1710, 1610 (*p*-OMe benzoate), 1670 (double bond), 1170, 1100, 1000, 850, 775; UV λ_{max}^{EtOH} (log *ε*) nm: 256 (4.3), 210 (4.4); CD (MeOH): $\Delta\varepsilon_{305}$ + 1.2, $\Delta\varepsilon_{256}$ − 3.9; EIMS 70 eV, *m/z* (rel. int.): 386 $[M]^+$ ($C_{23}H_{30}O_5$) (34), 251 $[M - ArCO]^+$ (97), 233 $[M - ArCO - H_2O]^+$ (27), 135 $[ArCO]^+$ (100), 107 $[Ar]^+$ (46). ^1H NMR (200 MHz, $CDCl_3$, TMS as reference): δ7.88 (*d*), 6.90 (*d*), 3.85 (*s*, anisate), 5.72 (*br d*, $J_{1,2}$ = 7.8 Hz, H-2), 5.50 (*d*, $J_{1,2}$ = 7.8 Hz, H-1), 1.74 (*br s*, H-15), 1.12 (*d*, $J_{11,12}$ = 7.2 Hz, H-12), 1.09 (*s*, H-14), 1.00 (*d*, $J_{11,13}$ = 7.2 Hz, H-13). ΔTAI ($CDCl_3$): H-1: −0.18; H-2: −0.04; H-12: +0.08; H-14: +0.12.

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