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DAUCANE ESTERS FROM *FERULA ARRIGONII*

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Key Word Index—*Ferula arigonii*; *Umbelliferae*; sesquiterpenoids; daucanes; sesquiterpene-coumarin ethers.

Abstract—*F. arrigonii* gave one C-10 hydroxylated and two C-2 oxygenated jaeskeanadiol esters. Sesquiterpene-coumarin ethers were also isolated. The relationship between *F. communis* and *F. arrigonii* is discussed. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Ferula communis L. is a circum-Mediterranean plant whose systematic is controversial [1, 2]. Several varieties seem to exist, but their relationship and characterisation are neither clear nor universally accepted [1, 2]. The most thoroughly investigated population of *F. communis* is that from Sardinia, where a toxic variety of the plant has long been known to exist [3]. Poisoning from *F. communis* causes a heamorrhagic syndrome known as ferulosys [4]. The economical relevance of ferulosys in livestock has spurred investigations aimed at identifying the toxic constituents of *F. communis*. In the course of these studies, it was discovered that two chemotypes of this plant grow in Sardinia, one containing heamorrhagic prenylated 4-hydroxycoumarins, and the other non-toxic daucane esters [5–8].

In this context, the recent identification of a new species of *Ferula* from Sardinia, *F. arrigonii* Bocchieri, raised considerable interest [9]. This plant is closely related to *F. communis*, and, though first observed in the island of Serpentara, it turned out to be widespread also in the southern part of Sardinia and in other small islands off its coast [9]. *F. arrigonii* was reported to contain as the major constituent 7,11-dehydrogrilactone (**1**) [10], a sesquiterpene lactone of the guaiane type whose total synthesis was recently achieved [11]. Guaianolides are common constituents of certain *Ferular* from Central Asia [12], but had never found in Mediterranean species of this genus. In the hope of finding more examples of taxonomically unique metabolites, we have now investigated samples of *F. arrigonii* from two islands off the coast of Sardinia (Serpentara, Isola dei Cavoli). Surprisingly, none of the compounds previously reported from this species could be detected. Both collections afforded instead a mixture of daucane esters and sesquiterpene-umbelliferone ethers, whose characterisation is the subject of this report.

RESULTS AND DISCUSSION

The roots of the two samples contained the same compounds, and only differed for the relative concentration of some daucane esters. The major constituent was the drimane-coumarin ester colladonin (**2a**) [13] (*ca* 0.90% on dry plant material). Several related compounds were also present which were identified as colladin (**2b**) [13], badrakemone (**2c**) [14], umbelliprenin (**3a**) [15], kataravicinol (**3b**) [16] and isosamarkandin angelate (**4**) [17, 18], as well as a mixture of daucane esters. The latter included, beside ferutidin (**5a**) [19] and lapiferin (**6**) [20], two new compounds (**5b** and **5c**). Compound **5b** was the major daucane ester from both collection (*ca* 0.60%), but the concentration of **5a** and **6** in the sample from Serpentara was about five times higher than that detected in the other sample.

The mass spectrum of **5b** showed a MW of 388, corresponding to the molecular formula $C_{23}H_{32}O_5$. Compound **5b** displayed diagnostic IR absorptions at 3557–3459 (hydroxyls), 1690 (aromatic ester carbonyl) and 1609, 1584, 1512 (aromatic ring) cm^{-1}. The ^1H NMR spectrum showed the signals of an anisoyl group (δ 7.95 and δ 6.91 AA′BB′-system; δ 3.83, *s*, –OMe) and of an oxygenated sesquiterpene moiety. Assignments of the proton signals via COSY experiments showed that **5b** was a C-2 hydroxylated deriva-

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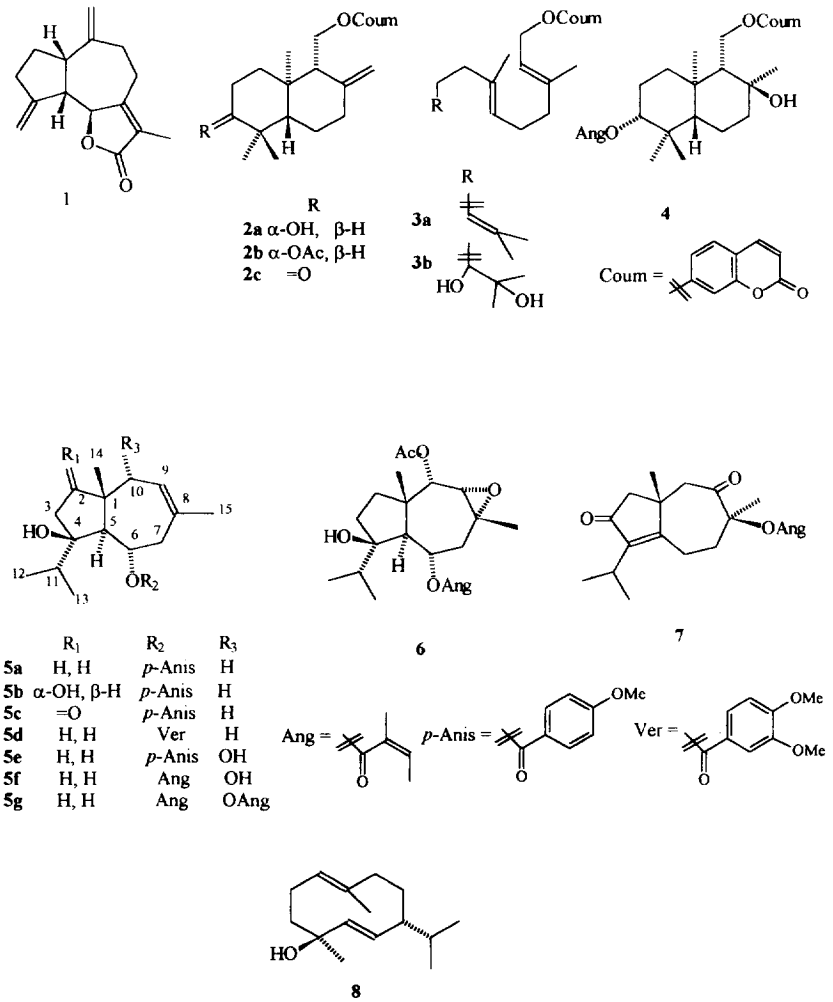

	R_1	R_2	R_3
5a	H, H	*p*-Anis	H
5b	α-OH, β-H	*p*-Anis	H
5c	=O	*p*-Anis	H
5d	H, H	Ver	H
5e	H, H	*p*-Anis	OH
5f	H, H	Ang	OH
5g	H, H	Ang	OAng

tive of ferutidin. NOE-experiments established an α-orientation for the secondary hydroxyl (NOE's H-2/H-14 and H-2/H-3β; no NOE H-2/H-5). Comparison of ^{1}H NMR spectra of **5b** and its 2-epimer [21], showed a different coupling pattern for H-2 (*d*, $J = 6.0$ Hz in **5b**, *dd*, $J = 9$ and 10 Hz in the C-2 epimer), as well as a diagnostic downfield shift of H-5 ($\Delta\delta + 0.51$) in **5b**, due to a 1,3-*cis*-relationship between H-5 and the C-2 hydroxyl. Compound **5c** ($C_{23}H_{30}O_5$, MS) was the 2-dehydroderivative of **5b**, as shown by the appearance of an extra carbonyl band in the IR spectrum (1727 cm^{-1}, cyclopentanone), the disappearance of the signal of H-2 in the ^{1}H NMR spectrum, and the replacement of the C-2 oxymethine with a carbonyl absorption at δ 219.0 in the ^{13}C NMR spectrum. The detection of a W-coupling between H-3α and the hydroxyl at C-4 ruled out an alternative structure with the keto group at C-3.

Coumarin derivatives were absent in the fruits of *F. arrigonii*, as already observed for the poisonous variety of *F. communis* [8]. The major constituent of the fruits were the daucane esters lapiferin (**6**) and ferutidin (**5a**). Jaeskeanadiol veratrate (**5d**) [22], webbiol angelate (**7**) [23], 10α-hydroxyferutidin (**5e**) [24] and the new ester **5f** were also isolated. Compound **5f** was obtained as an oil. Its ^{1}H NMR spectrum was similar to that of the bis-angelate pallinin (**5g**) [25], but only one angeloyl group was present, and H-10 resonated at higher fields (δ 3.80 in **5f**; 4.85 in **5g** [25]), showing that the 10-hydroxyl was free. Thus, compound **5f** is 10-deangeloylpallinin. The daucane ester siol anisate, a constituent of the fruits of the poisonous chemotypes of *F. communis* [8], could not be detected in the fruits of *F. arrigonii*, though it was isolated from the collection of *F. arrigonii* that afforded the guaianolide **1** [10]. The phenylpropanes laserin [26] and crocatone (=latifolone) [26] were also isolated from the fruits and the roots of *F. arrigonii*, respectively.

F. arrigonii has been reported to have a stronger and more pleasant smell than *F. communis* [9]. Indeed, large amounts of linalyl acetate (*ca* 0.20% on dry plant material) were obtained from steam distillation of the least polar fractions of our extracts. Small amounts of allohedycariol (**8**) [5, 27] were also obtained. This *ent*-germacrane alcohol is the major constituent of the oil from the non-toxic chemotype

Table 1. ^{1}H NMR spectral data of compounds **5b**, **5c** and **5f** (400 MHz, $C\underline{H}Cl_3$, *J* in Hz)

H	5b	5c	5f
2α	—	—	1.74 *br ddd* (12.0, 11.0, 10.0)
2β	3.76 *d* (6.0)	—	1.32 *ddd* (12.0, 8.5, 2.0)
3α	1.80 *br d* (16.0)	2.30 *dd* (19.5, 2.0)	1.89 *ddd* (14.0, 10.0, 2.0)
3β	1.97 *dd* (16.0, 6.0)	2.42 *d* (19.5)	1.54 *ddd* (14.0, 11.0, 8.5)
5	2.50 *d* (11.0)	2.44 *d* (10.5)	2.69 *d* (11.0)
6	5.28 *ddd* (11.0, 10.0, 3.0)	5.50 *ddd* (10.5, 9.0, 3.5)	5.24 *ddd* (11.0, 11.0, 3.5)
7α	2.55 *br dd* (14.0, 10.0)	2.56 *br dd* (15.0, 9.0)	2.70 *br ddd* (15.0, 11.0, 2.5)
7β	2.21 *dd* (14.0, 3.0)	2.43 *dd* (15.0, 3.5)	2.15 *dd* (15.0, 3.5)
9	5.53 *br dd* (8.1, 4.0)	5.57 *br d* (6.0)	5.70 *br d* (7.0)
10α	2.29 *br dd* (14.5, 4.0)	2.00 *br dd* (14.0, 6.0)	—
10β	1.92 *dd* (14.5, 8.0)	2.35 *br dd* (14.0, 6.0)	3.80 *d* (7.0)
11	1.72 *m*	1.76 *m*	1.87 *m*
12	0.96 *d* (7.0)	0.93 *d* (7.0)	0.90 *q* (6.5)
13	0.80 *d* (7.0)	0.84 *d* (7.0)	0.88 *q* (6.5)
14	1.01 *s*	1.27 *s*	1.06 *s*
15	1.79 *br s*	1.86 *br s*	1.78 *br s*
RCO–	7.95 *d* (8.0)	7.99 *d* (8.0)	6.08 *qq* (7.0, 1.5)
	6.91 *d* (8.0)	6.95 *d* (8.0)	1.97 *dq* (7.0, 1.5)
	3.83 *s*	3.88 *s*	1.85 *dq* (1.5, 1.5)
4-OH		2.50 *d* (2.0)	

of *F. communis* [5], whereas the oil of the poisonous chemotype is made up by *ent*-aristolane hydrocarbons [28].

F. communis is a prolific producer of secondary metabolites of the sesquiterpene type, which can be further elaborated by esterification with aromatic or hemiterpenoid acids (daucanes) [5, 8], etherification with umbelliferone (drimanes) [29], or prenylation with 4-hydroxycoumarin (farnesanes) [5–7]. The first two pathways have also been detected in *F. arrigonii*, though the co-occurrence of daucane esters and sesquiterpene-coumarin ethers is unprecedented in *F. communis*, and many daucane esters isolated from *F. arrigonii* have not been detected in *F. communis*. Further studies on larger populations are, however, necessary to assess the taxonomical value of these phytochemical differences, since both plants show an exceptional infraspecific variation in their secondary metabolism.

EXPERIMENTAL

CC: silica gel 70–230 mesh; HPLC: microporasil column (0.8 × 30 cm).

Plant material. F. arrigonii was collected at Serpentara (July 1996, sample A) and at Isola dei Cavoli (October 1996, sample B). Sample A consisted of roots and fruits of one single plant. Samples B of roots only. The plant material was identified by M.B. (University of Cagliari). Voucher specimens of both samples are kept at the laboratory of Torino.

Isolation of the constituents. (A) Roots (sample A as representative): the dried, powdered roots (228 g) were extracted with Me_2CO at room temp. (3 × *ca* 1 l). Removal of the solvent left 27.0 g of a dark gum, which was chromatographed on a silica gel column (45 g) eluted with a hexane–EtOAc gradient (9:1 → 5:5). Eleven major frs were collected. Frs I and II were steam-distilled and then chromatographed (silica gel, hexane–EtOAc 95:5) to give linalyl acetate (500 mg, 0.22%) and **8** (49 mg, 0.021%). Fr. III was crys-

Table 2. ^{13}C NMR spectral data of compounds **5b**, **5c**, **5f** and **7** (75 MHz, $C\underline{D}Cl_3$, assignments confirmed by HMQC/HMBC experiments)

C	5b	5c	5f	7*
1	48.2 *s*	50.7 *s*	47.6 *s*	41.2 *s*
2	80.3 *d*	219.0 *s*	36.0 *t*	48.8 *t*
3	41.6 *t*	44.1 *t*	30.9 *t*	205.3 *s*
4	85.6 *s*	80.3 *s*	86.2 *s*	144.0 *s*
5	54.6 *d*	55.5 *d*	49.2 *d*	174.6 *s*
6	70.5 *d*	69.9 *d*	70.7 *d*	21.1 *t*
7	40.6 *t*	41.3 *t*	40.2 *t*	40.4 *t*
8	133.6 *s*	133.6 *s*	136.1 *s*	85.5 *s*
9	124.6 *d*	123.3 *d*	127.1 *d*	207.2 *s*
10	35.0 *t*	35.6 *t*	72.8 *d*	42.8 *t*
11	37.0 *d*	36.5 *d*	37.1 *d*	25.3 *d*
12	18.2 *q*	18.7 *q*	18.4 *q*	20.1 *q*
13	17.7 *q*	17.8 *q*	17.4 *q*	20.5 *q*
14	20.1 *q*	19.3 *q*	20.7 *q*	30.1 *q*
15	25.8 *q*	26.9 *q*	27.5 *q*	21.4 *q*
RCO–	166.6 *s*	167.2 *s*	168.2 *s*	167.6 *s*
	122.6 *s*	122.3 *s*	127.7 *s*	127.0 *s*
	113.7 *d*	113.9 *d*	139.0 *d*	140.3 *d*
	131.6 *d*	131.8 *d*	15.7 *q*	15.9 *q*
	163.5 *s*	163.9 *s*	20.5 *q*	20.5 *q*
	55.3 *q*	56.3 *q*		

* ^{13}C NMR data on this known compound were not available in the literature [23].

tallised from Et_2O to give 592 mg (0.26%) **5a**. Fr. IV was crystallised by petrol to give 32 mg stigmasterol; the mother liquors were further chromatographed (5 g silica gel, $CHCl_3$–hexane as eluant) to give **3a** (49 mg, 0.021%) and latifolone (12 mg, 0.0053%). Fr. V and VI were crystallised from Et_2O to give **5c** (80 mg, 0.035%) and **6** (652 mg, 0.28%) respectively. Fr. VII was further purified by HPLC (hexane–EtOAc, 6:4) to give (228 mg, 0.10%). Fr. VIII was crystallised from Et_2O to give **5b** (1.39 g, 0.61%). Fr. IX was further purified by CC (20 g silica gel, hexane–EtOAc, 8:2 as eluant) to give 13 mg (0.0057%) **2c**. Fr. X was crystallised from Et_2O to give 2.1 g (0.92%) **2a**. CC of the mother liquors (hexane–EtOAc, 7:3) gave **4** (38 mg, 0.017%). Fr. XI was crystallised from Et_2O–Me_2CO to afford 228 mg (0.10%) **3b**. (**B**) Fruits: powdered fruits (228 g) were extracted with Me_2CO (2 × 1 l) at room temp. Removal of the solvent left 16.0 g of a brown oil, which was chromatographed on a silica gel column (40 g) eluted with a hexane–EtOAc gradient (95:5 → 7:3). Two major frs were collected. Fr. I was crystallised from Et_2O to give 504 mg (0.22%) **5a**. The mother liquors were further purified by CC (10 g silica gel, hexane–EtOAc, 9:1 as eluant) to give 106 mg laserin (0.046%). Fr. II was crystallised from Et_2O to give 476 mg (0.21%) **6**. The mother liquors were further purified by HPLC (hexane–EtOAc, 9:1) to give 21 mg (0.0092%) **7**, 16 mg (0.0070%) **5e**, 32 mg (0.0014%) **5d** and 40 mg **5f** (0.017%).

2α-Hydroxyferutidin (**5b**). Crystals (Et_2O), mp 83–85 °; IR ν_{max}^{KBr} cm^{-1}: 3357, 3459 (OH), 1690 (ester CO), 1609, 1584, 1512 (aromatic), 1279, 1167, 1101, 1026, 768. CI-MS (isobutane) *m/z* (rel. int.): 389 $[C_{23}H_{32}O_5]^+[M+H]^+$ (10), 371 (100).

2-Oxoferutidin (**5c**). Crystals (Et_2O), mp 123–125°; IR ν_{max}^{KBr} cm^{-1}: 3445 (OH), 1726 (cyclopentanone), 1701 (ester CO), 1607, 1580, 1614 (aromatic), 1275, 1169, 1117, 1097, 773. CI-MS (isobutane) *m/z* (rel. int.): 387 $[C_{23}H_{30}O_5]^+[M+H]^+$ (65), 369 (100).

10-Deangeloylpallinin (**5f**). Oil; IR $\nu_{max}^{Liquid\ film}$ cm^{-1}: 3460 (OH), 1710 (ester CO), 1250, 1189, 1107, 1060, 750. CI-MS (isobutane) *m/z* (rel. int.): 337 $[C_{20}H_{32}O_4]^+[M+H]^+$ (40), 319 (100).

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