Phytochemistry, Vol. 26, No. 6, pp. 1613–1615, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

FERULENOL AND ω-HYDROXYFERULENOL, TOXIC COUMARINS FROM *FERULA COMMUNIS* VAR. *GENUINA*

DRISS LAMNAOUER,* BERNARD BODO,† MARIE-THÉRÈSE MARTIN and DARIUS MOLHO

Laboratoire de Chimie appliquée aux Corps organisés, UA 401 CNRS, Muséum National d'Histoire Naturelle, 63 rue de Buffon, 75231 Paris Cedex 05, France; *Département de Pharmacie-Toxicologie, Institut Agronomique et Vétérinaire Hassan II, B.P. 6202 Rabat-Instituts, Morocco

(*Received* 16 *October* 1986)

Key Word Index—*Ferula communis* var. *genuina*; Umbelliferae; 4-hydroxycoumarin derivatives; ferulenol; ω-hydroxyferulenol.

Abstract—From the root sap of *Ferula communis*, two isoprenylated 4-hydroxycoumarins, the known ferulenol and the new ω-hydroxyferulenol, were isolated, which both show haemorrhagic action. Their structures were established from MS, IR, ^{1}H and ^{13}C NMR spectral analysis.

INTRODUCTION

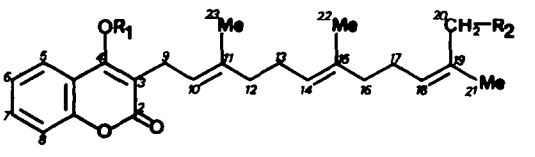
Ferula communis L. (Umbelliferae) is a perennial weed found in several Mediterranean countries. Its toxicity to livestock was recorded by such ancient authors as Pliny [1]. There are also a number of reports that the clinical signs observed in intoxicated animals after eating plant leaves can be reproduced by experimental administration of sap. The root sap of *F. communis*, on the other hand, is well known in Arabia as 'fessoukh' which is used widely in traditional medicine, especially in Morocco [1]. Previous studies [2–4] have shown that the root sap of *F. communis* from Sardinia contains an haemorrhagic substance. Further chemical investigations of this compound afforded ferulenol, the structure of which was mainly deduced from chemical degradations [5].

The present work was undertaken to examine the chemistry of root sap of *F. communis* var. *genuina* from Morocco as it appeared to be a highly toxic product. Ferulenol and another 4-hydroxycoumarin were isolated. Since spectral data for ferulenol were limited [5] these are presented here along with those of the new compound.

RESULTS AND DISCUSSION

The methylene dichloride extract of the crude sap of *F. communis* when chromatographed over silica gel afforded two major pure substances **1** and **3**. The compounds had similar IR spectra, showing the presence of a hydroxy group, conjugated ester and double bond. The EI-MS of **1** exhibited a molecular ion ($[M]^+$) at *m/z* 366 corresponding to the formula $C_{24}H_{30}O_3$. Its ^{1}H NMR spectrum showed signals for four methyl and five methylene groups linked to double bonds, three olefinic protons and four aromatic protons from an *ortho*-disubstituted benzene ring (Table 1). These data suggested that compound **1** was ferulenol. Acetylation of **1** at room temperature afforded the expected mono-acetate (**2**) by *O*-acylation of the hydroxyl on the coumarin ring. The ^{1}H NMR spectrum of **2**, was similar to that of **1**, but with an extra acetyl signal at 2.448 ppm (Table 1). The chemical shift assignments were accomplished using 2D correlation spectroscopy (COSY) technics. The observed ^{13}C NMR spectra of **1** and **2** (Table 2) agreed with the proposed structure for ferulenol. The chemical shift assignments were based on comparison with those of coumarins [6] and squalene chains [7]. The main fragmentations observed in the mass spectra of **1** and **2** are the result of the fission of the $-CH_2-CH_2-$ and $ArCH_2-CH=$ bonds to give the stabilized carbenium ions **a**, **b** and **c** (Fig. 1).

The mass spectrum of **3** displayed a molecular ion at *m/z* 382 in agreement with the formula $C_{24}H_{30}O_4$, indicating that **3** contains one more oxygen atoms than **1**. Acetylation of **3** gave a diacetyl derivative (**4**). The ^{1}H NMR spectrum of **3** was similar to that of ferulenol (**1**): it contained the same aromatic system of four protons and the same number of methylene groups and olefinic protons (Table 1). But only three methyl groups, instead of four, were observed and an additional signal (2H) appeared at 4.111 ppm, which was attributed to a $-\underline{CH_2}OH$ group. This signal was shifted up to 4.552 ppm in the spectrum of the diacetate (**4**), which moreover clearly showed the methyls of the two acetyl groups at 2.059 and 2.453 ppm. The 2D COSY allowed the total assignment of the spectrum of **4** (Table 1).



1: $R_1 = R_2 = H$
2: $R_1 = Ac$ $R_2 = H$
3: $R_1 = H$ $R_2 = OH$
4: $R_1 = Ac$ $R_2 = OCOCH_3$

†To whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data for ferulenol (1), ω-hydroxyferulenol (3) and their acetates (2) and (4)*

	1	2	3	4
5-H	7.840 *ddd* (7.5; 1.6; 0.7)	7.361 *br dd* (7.8; 1.6)	7.779 *dd* (7.9; 1.6)	7.362 *ddd* (8.0; 1.6; 0.3)
6-H	7.270 *ddd* (7.5; 7.2; 1.2)	7.260 *ddd* (7.8; 7.1; 1.1)	7.269 *ddd* (7.9; 7.3; 1.1)	7.265 *ddd* (8.0; 7.1; 1.1)
7-H	7.499 *ddd* (7.2; 8.6; 1.6)	7.507 *ddd* (7.1; 8.3; 1.6)	7.513 *ddd* (7.3; 8.3; 1.6)	7.512 *ddd* (7.1; 8.3; 1.6)
8-H	7.282 *ddd* (8.6; 1.2; 0.7)	7.345 *br dd* (8.3; 1.1)	7.304 *dd* (8.3; 1.1)	7.346 *ddd* (8.3; 1.1; 0.3)
9-H_2	3.437 *d* (7.4)	3.236 *d* (7.0)	3.422 *d* (7.0)	3.234 *d* (6.9)
10-H	5.419 *tq* (7.4; 1.3)	5.172 *tq* (7.0; 0.8)	5.392 *br t* (7.0)	5.170 *tq* (6.9; 1.3)
12-H_2, 13-H_2, 16-H_2, 17-H_2	1.9–2.2 *m*	1.9–2.1 *m*	2.0–2.2 *m*	1.9–2.2 *m*
14-H	5.075 *br t* (1.0)	5.080 *br m* (6.8)	5.067 *m*	5.080 *tq* (6.8; 1.0)
18-H	5.075 *br t* (1.0)	5.056 *br m* (6.8)	5.286 *br tq* (7.2; 1.3)	5.341 *br tq* (7.2; 1.3)
20-H_n	1.661† *br d* (1.0)	1.659† *br*	4.111 *br*	4.552 *br*
21-H_3	1.572† *br d* (1.0)	1.574† *br*	1.793 *br d* (1.3)	1.720 *br d* (1.3)
22-H_3	1.5976 *br d* (1.0)	1.574 *br*	1.614 *br d* (0.9)	1.568 *br d* (1.0)
23-H_3	1.828 *br d* (1.3)	1.753 *br*	1.835 *br*	1.754 *br d* (1.3)
R^1		OCOMe 2.448 *s*		OCOMe 2.453 *s*
R^2				OCOMe 2.059 *s*

*Measured at 250 MHz, $CDCl_3$, with TMS as int. ref.
†Assignments within the same column may be interchanged.

Comparison of the ^{13}C NMR chemical shifts of the acetates 4 and 2 (Table 2) corroborate the lack of the fourth methyl group and its replacement by an hydroxy methylene group (63.1 ppm) in 4. In fact the observed differences between the ^{1}H and ^{13}C NMR spectra of ω-acetylferulenol (2) and the diacetate (4) were consistent with the presence in the latter of a $-CH_2OAc$ group instead of a methyl group.

The mass spectrum of 3 showed the same fragment ions (a, b and c) as 1 suggesting that the location of the hydroxymethylene group must be on either the 20 or 21 position of the farnesyl chain. Unequivocal support for the structure was obtained from NOE difference measurements on the diacetyl derivative (4) which gave additional information on the stereochemistry of the farnesyl chain. When the methyl group at C-21 was irradiated, NOE were observed for the methine proton at C-18 (5%), for the methylene protons at C-20 (1%) and not for the methylene C-17. In addition irradiation of the C-20 methylene gave NOE for the C-21 methyl protons (1%) and for the C-17 methylene protons (1.5%), indicating a *Z* configuration for the C18-C19 double bond. In the same way: (i) irradiation of the C-22 methyl group gave NOE for the two methylene groups C-13 (2%) and C-16 (2%); (ii) irradiation of the C-23 methyl group gave NOE for the two methylene groups C-9 (2%) and C-12 (1%) and a very weak one for the methyl group of R^1. These results indicate an *E* configuration for the C10–C11 and C14–C15 double bonds. Compound 3 is thus ω-hydroxyferulenol.

As the NMR data were very similar for 1 and 3, they must have the same farnesyl chain configuration, thus the C10–C11 and C14–C15 double bonds of 1 are equally *E*.

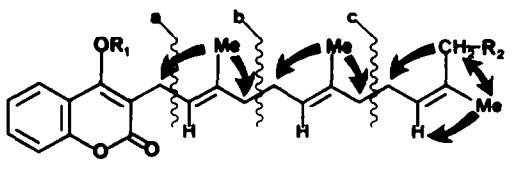


Fig. 1. (a) Main fragmentation pathway of ferulenol and derivatives (∿∿). (b) Bold arrows indicate the observed NOEs.

Table 2. ^{13}C NMR spectral data for compounds 1, 2 and 4*

	1	2	4
2-C	164.1 *s*	162.1 *s*	162.1 *s*
3-C	103.7 *s*	116.3 *s*	116.3 *s*
4-C	160.8 *s*	154.5 *s*	154.5 *s*
4a-C	116.0 *s*	119.7 *s*	119.6 *s*
5-C	122.7 *d*	118.7 *d*	118.8 *d*
6-C	123.7 *d*	124.3 *d*	124.4 *d*
7-C	131.3 *d*	131.5 *d*	131.5 *d*
8-C	116.2 *d*	116.7 *d*	116.7 *d*
8a-C	152.2 *s*	152.1 *s*	152.1 *s*
9-C	23.4 *t*	24.5 *t*	24.4 *t*
10-C	120.0 *d*	122.3 *d*	122.4 *d*
11-C	140.7 *s*	137.7 *s*	137.6 *s*
12-C	39.9† *t*	39.6 *t*	39.5 *t*
13-C	26.0 *t*	26.7 *t*	26.3† *t*
14-C	123.6 *d*	123.9 *d*	124.2 *d*
15-C	135.7 *s*	135.1 *s*	134.4 *s*
16-C	39.5† *t*	39.6 *t*	39.5 *t*
17-C	26.5 *t*	26.5 *t*	26.4† *t*
18-C	124.2 *d*	124.1 *d*	130.5 *d*
19-C	131.0 *s*	131.1 *s*	129.6 *s*
20-C	17.4 *q*	17.6 *q*	63.1 *t*
21-C	25.4 *q*	25.6 *q*	20.8 *q*
22-C	15.9 *q*	15.9 *q*	15.9 *q*
23-C	16.2 *q*	16.3 *q*	16.3 *q*
R^1CO		166.7 *s*	166.8 *s*
R^1Me		20.4 *q*	20.3 *q*
R^2CO			171.0 *s*
R^2Me			21.3 *q*

*Measured at 62.5 MHz; $CDCl_3$ with TMS as int. standard.

†Assignments within the same column may be interchanged.

EXPERIMENTAL

General. UV spectra were measured in MeOH. ^1H NMR spectra were measured in $CDCl_3$ at 250 MHz on a Bruker WM 250 and ^{13}C spectra at 20 MHz on a Bruker WP 80. MS were obtained on a Thomson-Houston THN 208 mass spectrometer using a direct inlet system, an electronic impact mode with an ionization voltage of 70 eV. Silica gel (70–230 mesh) Merck was used for CC. TLC were carried out on 0.2 mm plastic sheets silica gel 60 F_{254} and developed in: toluene–EtOAc–HCOOH, 13:6:1, CH_2Cl_2–*n*-C_6H_{12}, 19:1 or CH_2Cl_2–EtOAc, 19:1.

Plant material. Roots of *Ferula communis* L. var. *genuina* (G.G.) Burnat were collected in August 1986 in the Marrakech area at Beni Meskine's pasture. A voucher specimen was deposited in the Department of Pharmacy-Toxicology at the Institut of Agriculture and Veterinary Medicine, Rabat. The roots were cut and the milky sap that exuded, was collected and left to dry at room temp.

Isolation of ferulenol and ω-hydroxyferulenol. Dry sap (30 g) was mixed with CH_2Cl_2 (3 × 100 ml), filtered and concd to give 28.5 g of a crude viscous oil; 12 g of the oil was subjected to CC (silica gel) and eluted with first a CH_2Cl_2–EtOAc mixture with an increasing proportion of EtOAc and finally with MeOH. 8 ml fractions were collected. **1** was obtained from fractions 20–185 (2.55 g) and **3** from fractions 450–835 (2.80 g). **1** and **3** were further purified on a silica gel column eluted with CH_2Cl_2 and CH_2Cl_2–EtOAc, 19:1, respectively.

Ferulenol (**1**). Gum. MS, *m/z* (%): 366 (4) ([M]$^+$, $C_{24}H_{30}O_3$), 297 (13) (**c**), 255 (3), 230 (16), 229 (33) (**b**), 175 (100) (**a**), 136 (27), 135 (24), 123 (37), 121 (69), 109 (53); IR cm^{-1}: 3210, 2916, 1665, 1623, 1580, 1443, 1389, 1222, 1159, 1107, 947, 749, 710, 636; UV λ^{EtOH} nm: 274, 282, 307, 317.

4-Acetylferulenol (**2**). Ac_2O (1 ml) was added to a solution of **1** (100 mg) in dry pyridine (1 ml); the mixture was kept at room temp. for 3 hr, poured into H_2O and extracted with dichloromethane. The extract chromatographed on silica gel (CH_2Cl_2) yielded **2** (60 mg). Colourless gum. MS, *m/z* (%): 408 (13) ([M]$^+$), 366 (11), 365 (14), 339 (15), 321 (10), 297 (48), 255 (10), 230 (50), 229 (100), 175 (68), 136 (14), 135 (12), 123 (23), 121 (35), 109 (19); IR cm^{-1}: 2920, 2854, 1776, 1727, 1629, 1450, 1366, 1320, 1289, 1170, 1088, 1043, 902, 757, 644; UV λ^{EtOH} nm: 273, 313, 322.

ω-Hydroxyferulenol (**3**). Gum. MS, *m/z* (%): 382 (1, 5) ([M]$^+$, $C_{24}H_{30}O_4$), 364 (2), 321 (4), 297 (5) (**c**), 255 (6), 253 (5), 230 (23), 229 (26) (**b**), 175 (100) (**a**), 136 (12), 135 (52), 123 (17), 121 (93), 109 (35); IR cm^{-1}: 3324, 2922, 1676, 1616, 1566, 1493, 1448, 1223, 1099, 1051, 1001, 910, 752, 646; UV λ^{EtOH} nm: 273, 283, 307, 318.

4-Acetyl ω-acetoxyferulenol (**4**). **3** (100 mg) was acetylated and purified in the usual way to yield **4** (55 mg). MS, *m/z* (%): 466 (0.1) ([M]$^+$), 364 (1), 363 (2), 321 (2), 297 (3), 255 (7), 253 (6), 230 (98), 229 (59), 175 (78), 136 (6), 135 (30), 123 (23), 121 (100), 109 (31); IR cm^{-1}: 2923, 2854, 1779, 1729, 1632, 1447, 1366, 1234, 1170, 1088, 1040, 902, 758, 640; UV λ^{EtOH} nm: 272, 313, 322.

Acknowledgement—We thank Dr. J. P. Brouard for mass spectral measurements.

REFERENCES

1. Perrot, E. (1943–1944) *Matières premières usuelles du règne végétal*, Tome II, pp. 1647 and 1681. Masson, Paris.
2. Carboni, S. (1941) *Boll. Soc. Ital. Biol. Sper.* **16**, 544.
3. Carboni, S. (1941) *Boll. Soc. Ital. Biol. Sper.* **16**, 546.
4. Carboni, S. (1941) *Atti Soc. Toscana Sci. Nat. Pisa, Ser. A* **62**, 257.
5. Carboni, S., Malaguzzi, V. and Marzili, A. (1964) *Tetrahedron Letters* 2783.
6. Cussans, N. J. and Huckerby, T. N. (1975) *Tetrahedron* **31**, 2719.
7. Breitmaier, E. and Voelter, W. (1978) ^{13}C *NMR Spectroscopy: Methods and Applications*, 2nd edn Verlag Chemie, Weinheim.