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COUMARINS AND FERULOL ESTERS FROM *CACHRYS SICULA*

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Key Word Index—*Cachrys sicula*; Umbelliferae; furocoumarins; (−)-sprengelianin; ferulol esters.

Abstract—(−)-Sprengelianin, (−)-prantschimgin and other minor coumarins were isolated from the roots and/or the umbels of *Cachrys sicula*. The roots also afforded the monoterpene hydroxyaldehyde ferulol esterified to angelic, tiglic and senecioic acids. The (−)-enantiomer of sprengelianin (2′*S*) had not been reported previously. The coumarins saxalin and pabulenol and the aromatic aldehyde 2,3,4-trimethylbenzaldehyde were also identified but these substances are presumably artefacts.

INTRODUCTION

Cachrys sicula L. (= *Hippomarathrum pteroclaenum* (DC) Boiss.) is a plant endemic to the South-western Mediterranean region [1] which causes skin irritation and blistering as some of us could verify after collecting the plant material. Coumarins are commonly abundant within the species of the tribe Smyrnieae [2] and presumably these substances are responsible for the injurious action of the plant. Apart from the fungicide, antibiotic and other biological actions [3], some coumarins and psoralens in particular, can act as skin irritants for which contact of the coumarin with the humid (sweaty) skin and irradiation with UV light are essential. The contact photodermatitis produced has been attributed to the photocycloaddition between a molecule of furocoumarin and one or two pyrimidine bases of epidermal DNA [4].

In previous studies on the chemical constituents of *C. sicula* the isolation of *N,N′-o*-toluylethylenediamine [5], bergapten, isoimperatorin and isopimpinellin [6] has been reported.

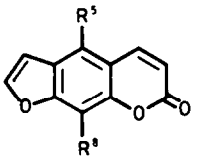
RESULTS AND DISCUSSION

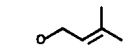
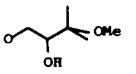
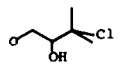
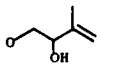
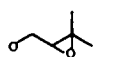
The benzene extract from the umbels of *C. sicula* (9% dry wt) was firstly chromatographed on a dry silica gel column and pure components were isolated after recrystallization or chromatography. The following coumarins were identified by spectroscopic means (^{1}H NMR, IR, UV and EI mass spectrometry) and by comparison of their physical constants with those described in the literature: isoimperatorin (**1**) [7], imperatorin (**2**) [7], bergapten (**3**) [7], xanthotoxin (**4**) [8], isopimpinellin (**5**) [8], tert-*O*-methylheraclenol (**6**) [9], (−)-prantschimgin (**10**) [7], (−)-sprengelianin (**11**) and ulopterol (**13**) [10].

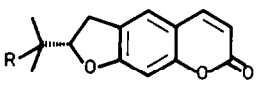
The benzene extracts from the roots (5% dry wt) afforded after chromatography, the furocoumarins imperatorin (**2**), saxalin (**7**) [9], pabulenol (**8**) [11], oxypeucedanin (**9**) [7], (−)-pranschimgin (**10**) and (−)-sprengelianin (**11**).

Only the racemic sprengelianin was previously described [12]. Alkaline hydrolysis of the laevorotatory sprengelianin isolated from *C. sicula* gave (+)-marmesin (**12**) for which the absolute configuration was known [13] and consequently, we assigned the absolute 2′*S* configuration to the natural (−)-sprengelianin. The most abundant coumarins in both umbels and the roots are (−)-prantschimgin and (−)-sprengelianin.

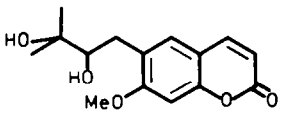
Earlier fractions of the main chromatography of the root extract afforded an aromatic aldehyde which was identified as 2,3,4-trimethylbenzaldehyde (**15**) according to IR and ^{1}H NMR data [14]. As the crude extract showed an aldehyde signal at δ9.55 clearly different from that shown by **15** (δ10.25), it was concluded that a chemical



		R^5	R^8
1	Isoimperatorin		H
2	Imperatorin	H	
3	Bergapten	OMe	H
4	Xanthotoxin	H	OMe
5	Isopimpinellin	OMe	OMe
6	t-O-Methylheraclenol	H	
7	Saxalin		H
8	Pabulenol		H
9	Oxypeucedanin		H



		R	
10	(-)-Prantschimgin		(OSen)
11	(-)-Sprengelianin		(OTgl)
12	(+)-Marmesin	OH	



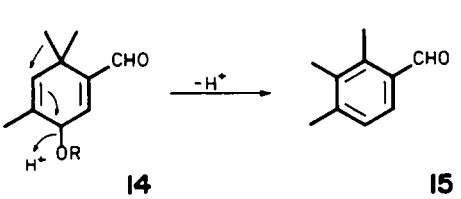
13 Ulopterol

transformation during the purification process must have happened. Careful examination of some slightly more polar fractions of the main chromatography, however, allowed us to detect and isolate the original aldehyde identified as a mixture of the senecioate, angelate and tiglate esters of ferulol (**14**) in a 9:7:4 ratio as deduced from ^{1}H NMR. The spectral data for **14** agree with those reported for other ferulol esters [15, 16].

It has been reported that acid treatment, and even distillation [16], can induce the rearrangement of ferulol esters into trimethylbenzaldehyde. In our case the conditions of the first chromatography (dichloromethane, silica gel and traces of water or HCl) could be strong enough to promote the rearrangement shown in Scheme 1.

Ferulol esters, firstly isolated from *Ferula hispanica* [14], have only been found in species of the Umbelliferae and specifically in the tribes Saniculae, Scandiceae, Smyrnieae, Ammineae and Peucedaneae–Ferulineae [15–26].

The observed rearrangement of the ferulol esters suggested to us that the minor coumarins saxaline and pabulenol could be rearranged products and not genuine metabolites of *C. sicula*. A reexamination of a fresh benzene extract from a single root allowed to confirm this hypothesis. TLC of the extract did not show any yellow fluorescent spot with R_f value corresponding to **7** or **8**, but a new spot appeared at higher R_f. The substance was purified by chromatography on silica gel and was identified as oxypeucedanin (**9**) by ^{1}H NMR. The epoxide ring



14 **15**

R = Ang, Sen, Tgl.

Scheme 1.

of **9** can be opened in the presence of HCl to give after substitution or elimination, **7** and **8**, respectively. The chlorocoumarin saxalin, reported as a natural product from other sources, could be, in some cases, also an artefact.

Triacylglycerols were also isolated from both benzene extracts. After hydrolysis and esterification with diazomethane the fatty acid methyl esters were analysed by GC. Oleic acid (umbels, 71%; roots, 44%) was the most abundant component.

EXPERIMENTAL

General. Mps are uncorr. ^{1}H NMR were measured at 60 MHz in $CDCl_3$ using TMS as int. standard. MS were measured on a quadrupole mass spectrometer, 70 eV, direct inlet probe. Analytical TLC separations were carried out using Merck silica gel-60 plates, 0.25 mm. Fluorescence, H_2SO_4–MeOH (1:3) or 5% EtOH–phosphomolybdic acid with heating for 5 min at 120° were used for spot visualization. Fluorescence of coumarins was observed under 350 nm UV light. Prep. TLC separations were carried out on Merck silica gel 60 PF_{254} plates, 20 × 20 cm, 0.75 mm, UV-visualization and EtOAc as extraction solvent. CC was performed on Merck silica gel-60, 0.063–0.2 mm.

Plant material was collected in July near Torrevieja, Alicante; and identified by Dr. A. Escarré, Department of Biology, University of Alicante.

Extraction and isolation. (a) *Aerial parts.* Air-dried and powdered immature umbels, collected after flowering (1710 g) were extracted with C_6H_6 (Soxhlet). The extract was concd *in vacuo* giving 164 g, (9.4%) of dried plant. Crude extract (7.98 g) was chromatographed on a dry silica gel column (120 cm, 259 g) using C_6H_6–EtOAc (9:1) as eluent. The column was cut into 20 fractions. Fractions 6–7 of this chromatography (1.54 g) crystallized from Et_2O to give pure **1** (420 mg). The mother liquor was concd to dryness (1.12 g) and subjected to CC on silica gel (20 g) using C_6H_6–EtOAc (7:3) as eluent to give triacylglycerols (0.73 g) which were saponified and esterified with CH_2N_2. The Me esters were analysed by GC (20% DEGS-chromosorb W AW-DMCS 80–100, 2 m × 2 mm; 190°) The main components were identified as the Me esters of oleic (71%), linoleic (23%) palmitic (6.5%) and myristic acids (0.1%). Fractions 8–10 (0.45 g) afforded after crystallization from EtOAc–MeOH pure **2** (162 mg). Recrystallizations of fraction 11 (0.76 g) from hexane–EtOAc gave pure **3** (70 mg), and fractions 12–14 (2.66 g) from C_6H_6–Et_2O gave pure **4** (150 mg). The mother liquor of this last fraction was concd to dryness and recrystallized from hexane–EtOAc allowing the isolation of pure **5** (70 mg). Fractions 15–18 (1.03 g) consisted mainly of a 1:1 mixture of **10** and **11**, identified in the extract from the roots. Fractions 19–20 of the main chromatography (0.74 g) after washing with 4% NaOH, left a residue (0.27 g) which was fractionated by silica gel CC (10 g) using mixtures of C_6H_6–EtOAc as eluent. Prep. TLC allowed the isolation of **6** (7 mg) and **13** (6 mg).

(b) *Roots.* Air-dried and cut roots (320 g) were extracted with C_6H_6 at room temp. The extract was concd *in vacuo* giving 15 g (4.7%). The crude extract, crystallized from C_6H_6, gave 4 g of a mixture containing mainly **10** and **11** in a 11:9 ratio as deduced from ^{1}H NMR. The mother liquor concd to dryness (11 g) was chromatographed on a dry silica gel column (96 cm, 120 g) using CH_2Cl_2 as eluent (300 ml). The column was cut into 17 fractions. Fraction 3 (0.269 g) of the main chromatography was washed with 5% $NaHCO_3$ and the neutral part (0.153 g) subjected to CC on silica gel (20 g) using mixtures of hexane–EtOAc as eluent to give **15** (78 mg). Fractions 4–5 (1.113 g) crystallized from Et_2O gave **2** (400 mg). The mother liquor concd to dryness (0.39 g) was

chromatographed on silica gel (20 g) with hexane–EtOAc mixtures as eluent. Prep. TLC of fractions 4–5 of the chromatography (0.124 g), eluted with C_6H_6–Et_2O (19:1), gave **14** (100 mg). Fractions 6–8 (4.02 g) contained two main compounds showing a blue fluorescence, **10** and **11**. From these fractions, by recrystallization (MeOH), pure **10** was obtained. The mother liquor of **10** concd to dryness (0.39 g), was chromatographed on silica gel (20 g), using mixtures of hexane–EtOAc as eluent. Coumarin **11** was isolated and further purified by crystallization from hexane. Fractions 12–13 (0.91 g) were washed with 5% NaOH. Liquid centrifuge chromatography (LCC) of the neutral part (0.42 g) on silica gel H (100 g, disc: ϕ 30 cm, 5 mm), hexane–Et_2O (6:4) as eluent (20 ml/min) yielded 20 fractions. Prep. TLC of fraction 7 (57 mg), C_6H_6–Et_2O as eluent, gave **7** (50 mg). Prep. TLC of fraction 6 (77 mg) from LCC, hexane–Et_2O (3:7) as eluent, gave **8** (70 mg). The acidic fraction of 12 and 13 was esterified with CH_2N_2 and analysed by GC as above. The most abundant Me esters were those of oleic (44%), palmitic (27%), palmitoleic (17%) and linoleic (9%) acids.

(−)-*Sprengelianin* (**11**). Blue fluorescence. Mp 111.5–113° (hexane); $[\alpha]_D^{23}$ −33°; $[\alpha]_{546}$ −45° ($CHCl_3$; *c* 1); UV λ_{max}^{EtOH} nm (log ε): 205 (2.32), 216 (2.26), 248 (1.62), 255 (1.58), 297 (1.76) 332 (2.12); IR ν_{max}^{KBr} cm^{-1}: 1710, 1620, 1570, 1260, 1120, 970, 820, 730, 720; ^1H NMR (60 MHz, $CDCl_3$): δ7.62 (1H, *d*, *J* = 10 Hz, H-4), 7.25 (1H, *br s*, H-5), 6.72 (1H, *br s*, H-8), 6.53 (1H, *dq*, *J* = 7 and 1.5 Hz, –HC=C), 6.18 (1H, *d*, *J* = 10 Hz, H-3), 5.05 (1H, *t*, *J* = 8 Hz, H-2'), 3.26 (2H, *d*, *J* = 8 Hz, 2H-3'), 1.62 (12H, *br s*, 4-Me); EIMS *m/z* (probe) 70 eV (rel. int.): 328 (2), 228 (1), 213 (90), 187 (8), 185 (5), 119 (28), 83 (100), 51 (41).

Hydrolysis. (−)-**11** (60 mg) was refluxed in 5% KOH–MeOH (5 ml) for 3 hr. Work-up as usual gave (+)-marmesin (**12**). Mp 189–190° (MeOH); $[\alpha]_D^{23}$ + 26.0° ($CHCl_3$; *c* 1.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600–3400, 2990, 1730, 1635, 1580, 1490, 1400, 1270, 1130, 910, 820; ^1H NMR (60 MHz, $CDCl_3$): δ7.56 (1H, *d*, *J* = 9.5 Hz, H-4), 7.16 (1H, *s*, H-5), 6.66 (1H, *s*, H-8), 6.14 (1H, *d*, *J* = 9.5 Hz, H-3), 4.71 (1H, *t*, *J* = 9 Hz, H-2'), 3.20 (2H, *d*, *J* = 9 Hz, 2H-3'), 2.20 (1H, *br s*, OH), 1.35 (3H, *s*, Me-α), 1.22 (3H, *s*, Me-α').

Ferulol esters (**14**). Oil; λ_{max}^{EtOH} nm (log ε): 223 (1.46); IR ν_{max}^{film} cm^{-1}: 2170, 1695, 1645, 1380, 1355, 1250, 1220, 1125, 1070, 940, 845, 740; ^1H NMR (60 MHz, $CDCl_3$): δ9.55 (1H, *s*, CHO), 6.95 (1H, *qq*, Tigl), 6.68 (1H, *d*, *J* = 4 Hz, H-6), 6.05 (1H, *qq*, Ang), 5.98 (1H, *br d*, *J* = 4 Hz, H-5), 5.75 (1H, *m*, Sen), 5.50 (1H, *br s*, H-3), 2.25–1.95 (9H, 3-Me), 1.75 (3H, *s*, Me-4), 1.30 (3H, *s*, Me-2), 1.24 (3H, *s*, Me-2').

2,3,4-*Trimethylbenzaldehyde* (**15**). Oil; UV λ_{max}^{EtOH} nm (log ε): 210 (2.17), 237 (1.99), 285 (sh); IR ν_{max}^{film} cm^{-1}: 1680, 1590, 1570, 1240, 1220, 812, 770; ^1H NMR (60 MHz, $CDCl_3$): δ10.25 (1H, *s*, CHO), 7.55 (1H, *d*, *J* = 8 Hz, H-2), 7.15 (1H, *d*, *J* = 8 Hz, H-3), 2.60 (3H, *s*, Me), 2.45 (3H, *s*, Me), 2.25 (3H, *s*, Me).

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