PERGAMON

Phytochemistry 53 (2000) 1025–1031

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Coumarins from *Ferulago capillaris* and *F. brachyloba*

Benedicto Jiménez[a], María Concepción Grande[a], Josefa Anaya[a], Pascual Torres[b], Manuel Grande[a,*]

[a]*Departamentos de Química Orgánica y de Química Farmacéutica, Universidad de Salamanca, E-37008 Salamanca, Spain*
[b]*UNED, Facultad de Ciencias, Candalix s/n, E-03202, Elche, Alicante, Spain*

Received 3 June 1999; received in revised form 16 September 1999

Dedicated to the memory of Prof. Joaquín de Pascual Teresa.

Abstract

Four new coumarins, (+)-senecioylprangol, (−)-3′-senecioyloxymarmesin, (+)-3′-hydroxyprantschimgin and (+)-2″-senecioyloxymarmesin, besides 12 known coumarins have been isolated from two *Ferulago* species. Their structures have been established by spectroscopic methods and partial synthesis. New synthetic 3′-oxocoumarins are also described. There is a remarkable difference in the contents of the most abundant coumarins found in the roots of both species: osthol and aurapten are specific to *F. capillaris* and *F. brachyloba*, respectively. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Ferulago capillaris*; *Ferulago brachyloba*; *Ferulago granatensis*; Umbelliferae; Peucedaneae; Furanocoumarins; Marmesin; Prantschimgin; Prandiol; Chemotaxonomy

1. Introduction

As a part of our phytochemical studies on the Spanish Umbelliferae (=Apiaceae), we collected near Salamanca (NWW of Spain) two *Ferulago* species similar to *F. granatensis* Boiss (Tutin et al., 1968), which have been identified as *F. brachyloba* Boiss and Reuter and *F. capillaris* (Link ex Sprengel) Coutinho. Unfortunately, some *Ferulago* species herborized from the Iberian Peninsula are not always clearly determined and a revision of this genus is now finished (García-Martín, 2000).

Previous phytochemical studies on *Ferulago* species revealed the presence of coumarins as the most frequent metabolites and they have been identified in *F. meoides* (Ognyanov & Botcheva, 1967, 1969), *F. turcomanica* (Andrianova, Sklyar & Pimenov, 1975; Serkerov, Kagramanov & Abbasov, 1976), *F. sylvatica* and *F. granatensis* (Crowden, Harborne & Heywood, 1969). Myristicine, new germacrane and aromadendrane sesquiterpenoids have also been isolated from *F. antiochia* (Miski, Moubasher & Mabry, 1990) and two flavonoids and acetylhydroquinone galactoside are reported from *Ferulago aucheri* (Doğanca, Ulubelen & Tuzlaci, 1991). More recently ferulol ester derivatives, a polyacetylene and a pyranocoumarin have been found in *F. nodosa* (Ruberto, Cannizzo, Amico, Bizzini & Piatelli, 1994) and some studies on the cytotoxic activity of *F. aucheri* (Doğanca, Gürkan, Hirlak, Tüzün & Tuzlaci, 1997) and *F. thirkeana* (Uğur, Gürkan, Körsal & Tuzlaci, 1998) have also been published. The essential oils of *F. sylvatica* (Chalchat, Garry, Gorunovic & Bogavac, 1992) and *F. trachycarpa* (Baser, Koyuncu & Vural, 1998) have also been analyzed.

In a preliminary article on the metabolites of *Ferulago* genus, we published the isolation of four coumarins from the aerial parts of a species which was tentatively identified as "*Ferulago* belonging to the

* Corresponding author. Tel.: +34-923-294400; fax: +34-923-294574.
E-mail address: mgrande@gugu.usal.es (M. Grande).

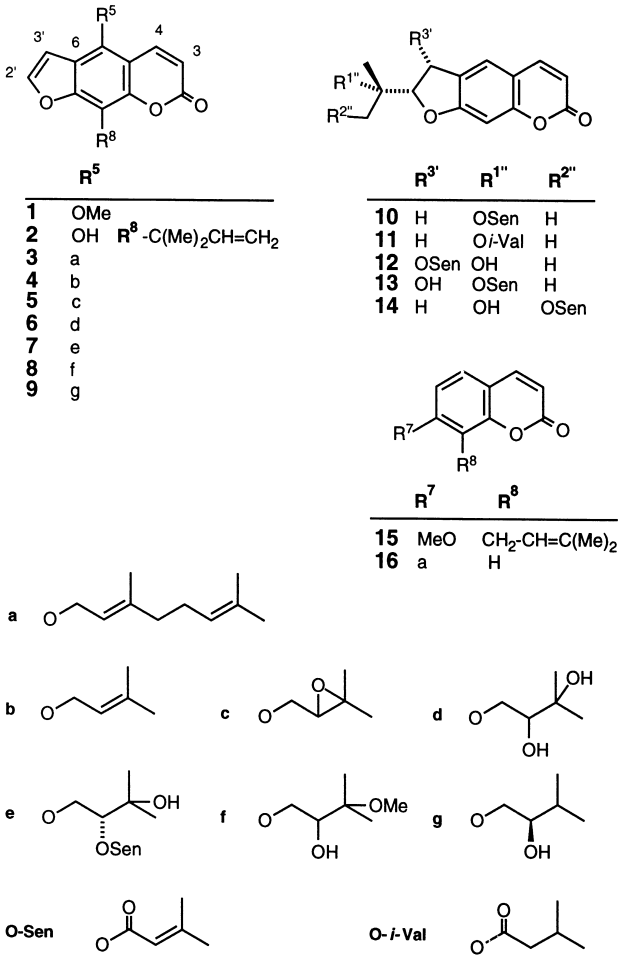
0031-9422/00/$ - see front matter
PII: S0031-9422(99)00524-5

granatensis group'' (Pascual Teresa, Jiménez, Corrales & Grande, 1979). The plant has been determined as *F. brachyloba* and we now report the isolation of five coumarins from the roots of this plant, one of them has been described in this article for the first time. We also describe here the isolation of 14 coumarins from the related species *F. capillaris*, four of them new natural products.

2. Results and discussion

From the hexane extract of *F. capillaris* (aerial parts), we have isolated seven coumarins identified as bergapten (**1**), 8-(1,1-dimethylallyl)bergaptol (**2**), bergamotin (**3**), isoimperatorin (**4**), (±)-oxypeucedanin (**5**), (−)-prantschimgin (**10**) and (−)-*iso*valerylmarmesin (**11**) (Murray, Méndez & Brown, 1982). The last coumarin was first described in our previous study on *Ferulago* sp. (Pascual Teresa et al., 1979).

The benzene extract of the roots of *F. capillaris* afforded 12 coumarins: **3**, **4**, **5**, (±)-prangol (**6**), alatol (= *tert-O*-methylprangol) (**8**), (−)-pranferol (**9**), **10**, osthol (**15**) (Murray et al., 1982) and the new coumarins **7**, **12**, **13** and **14**.



	R^5
1	OMe
2	OH
3	a
4	b
5	c
6	d
7	e
8	f
9	g

R^8 = $-C(Me)_2CH=CH_2$

	$R^{3'}$	$R^{1''}$	$R^{2''}$
10	H	OSen	H
11	H	O*i*-Val	H
12	OSen	OH	H
13	OH	OSen	H
14	H	OH	OSen

	R^7	R^8
15	MeO	CH_2-CH=C(Me)$_2$
16	a	H

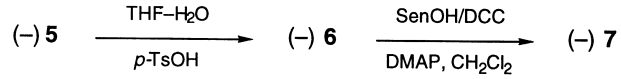
The MS (M^+ 386 amu) and ^{13}C-NMR spectra of compound **7** are in agreement with the molecular formula $C_{21}H_{22}O_7$. The IR spectrum showed hydroxyl bands (3600, 3350 cm^{-1}) and the UV spectrum revealed a characteristic absorption pattern for a 5-alkoxyfuranocoumarin (Murray et al., 1982).

The ^{1}H-NMR spectrum shows two AB systems at δ 8.08, δ 6.22 (1H, *d*, J = 9.8 Hz, H-3, H-4, respectively) and at δ 7.57, δ 6.95 (1H each, *d*, J = 2.2 Hz, H-2′, H-3′) which are characteristic of the furanocoumarin skeleton. The yellow fluorescence (under 365 nm light), the presence of a singlet proton at δ 7.13 (1H, *s*, H-8) and the deshielding of H-4, suggest for **7** the structure of a linear furanocoumarin with an alcoxyde substituent at C-5. This substituent was identified by ^{1}H-NMR spectroscopy as the $(CH_3)_2COH–CHOR–CH_2–O–$ group according to these signals: two singlets at δ 1.32 and δ 1.31 (methyl groups) and an ABM system with signals at δ 5.35 (1H, *dd*, J = 8.0, 3.2 Hz), δ 4.75 (1H, *dd*, J = 10.2, 3.2 Hz) and δ 4.55 (1H, *dd*, J = 10.2, 8.0 Hz). The remaining signals are in agreement with the presence of a senecioyl group (Grande, Aguado, Mancheño & Piera, 1986) and consequently the coumarin **7** was identified as (+)-senecioylprangol (= oxypeucedanin hydrate senecioate).

The absolute configuration of **7** was established by the synthesis of the (−)-enantiomer. (−)-Oxypeucedanin, available from *Angelica major* L. (Macías, Grande, Anaya & Grande, 1990) on hydrolysis gave (−)-prangol which was esterified with senecioic acid to yield (−)-*S* **7** and therefore, the absolute stereochemistry of (+)-**7** was established as *R* (Scheme 1).

According to the mass and ^{13}C-NMR spectral data, compounds **12** and **13** are isomeric coumarins $C_{19}H_{20}O_6$. Both have a free hydroxyl group (IR) and a senecioyloxy substituent (^{1}H-NMR). The NMR signals of **12** and **13** as compared to those of prantschimgin (**10**), suggests for these substances a dihydrofuranocoumarin skeleton with oxygenated substituents at C-3′ and C-1″. The chemical shift of H-3′ protons let us conclude that compound **12** should be 3′-senecioyloxymarmesin [δ 6.37 (1H, *d*, J = 6.2 Hz)] and **13** should be 3′-hydroxyprantschimgin [δ 5.35 (1H, *d*, J = 6.2 Hz)]. A chemical evidence for the difference between both coumarins is the behavior on treatment with Ac_2O/pyridine at room temperature: **13** easily gave an acetate while **12** remained unchanged.

An extensive study of the NMR data of **12**, includ-



Scheme 1.

ing 2D HMQC and HMBC experiments, confirmed unambiguously the constitution of these coumarins, but it was difficult to deduce the relative stereochemistry of the chiral carbon atoms from the ^{1}H-NMR data because the coupling constant between H-2′ and H-3′ (J = 6.2 Hz) does not allow to assign the relative configuration in a dihydrofuran derivative. However, under mild acidic conditions both structures are in equilibrium, as was detected by ^{1}H-NMR, which suggested a *cis* relationship between the C-2′/C-3′ substituents. To confirm this relative configuration we planned to hydrolyze the senecioate and to transform the diol into the isopropylidene acetal derivative but the hydrolysis of **12** or **13** under different conditions always produced psoralene (Scheme 2); this reactivity is also consistent with the *cis* configuration of the substituents. Lastly, both coumarins were synthesized from (−)-prantschimgin, **10** (Scheme 2).

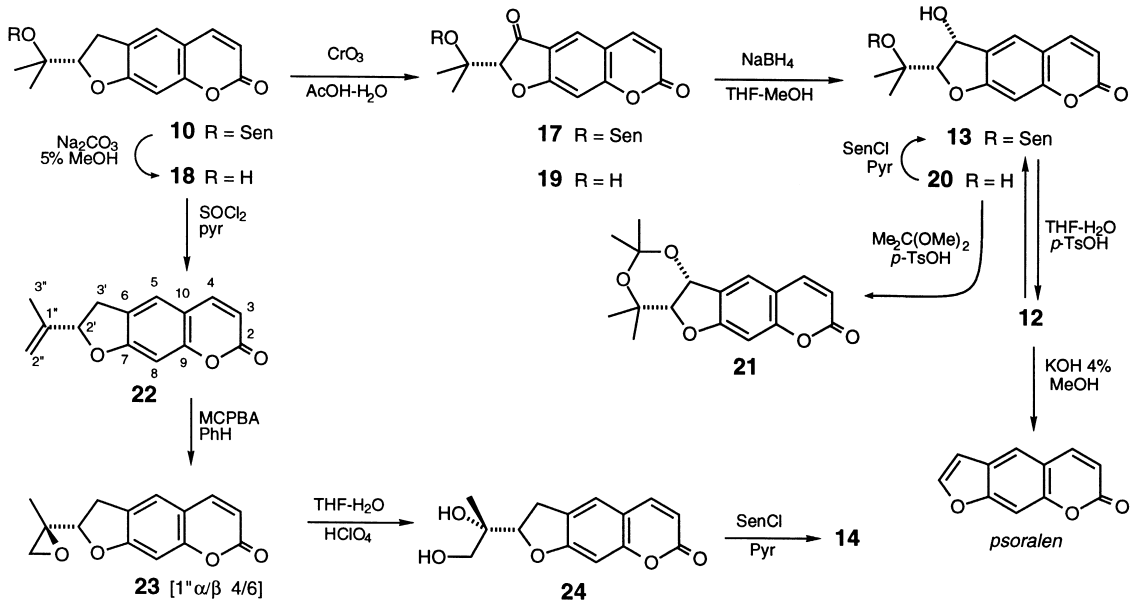
Chromic acid oxidation of **10** gave 3′-oxoprantschimgin (**17**) which was reduced with $NaBH_4$ from the less hindered face to produce the coumarin **13** as the more abundant reaction product. The spatial expansion of the senecioyloxyisopropyl group should be the responsible for the stereoselective carbonyl reduction. We observed also that during the chromatographic purification of **13** a small amount of **12** was also produced, probably by transesterification catalyzed by silica gel (Corey, Brunelle & Nicolaou, 1977; Yazawa, Tanaka & Kariyone, 1974). This transformation is also in agreement with the *cis* configuration of these coumarins.

On the other hand, benzylic oxidation of marmesin (**18**) (obtained by hydrolysis of prantschimgin (**10**) with Na_2CO_3/MeOH), gave the ketone **19** which on reduction with $NaBH_4$ was transformed into the diol **20**, that was correlated with **13** by esterification with senecioyl chloride (Scheme 2). Diol **20** easily forms the isopropylidene derivative **21**, which confirms the *cis* relationship of the substituents as well as the absolute configuration of the chirality centers according to the known absolute configuration of the starting (−)-marmesin (Harada, Hirose & Nakazaki, 1968).

Consequently, the structures (2′*S*, 3′*R*)-3′-senecioyloxymarmesin and (2′*S*, 3′*R*)-3′-hydroxyprantschimgin are proposed for (−)-**12** and (+)-**13**, respectively. The constitution of **12** has been proposed for decumbensol (Rao, Sun, Lin & Nie, 1993) but the configuration was not reported. Other related 1″-glycosides (Lemmich, Hacelaund & Thastrup, 1983) and 1″-esters as well as the diol **20** (Vilegas, Pzetti & Yariwake Vilegas, 1993) have been described previously.

Compound **14**, $C_{19}H_{20}O_6$ ($[M]^+$ m/z = 344) showed IR, UV and ^{1}H-NMR spectra that resembled those of prantschimgin (**10**), except for the presence of a free hydroxyl group, only one of the methyl singlets and a deshielded methylene group. This methylene group should be C-2″ and its chemical shift (δ 4.20, s) suggests that the primary hydroxyl group is esterified while the tertiary group is free.

The constitution of **14** as well as the stereochemistry was deduced by synthesis from (−)-marmesin as shown in Scheme 2. Dehydration of **18** with $SOCl_2$ gave *ent*-ammirin (**22**) which was epoxidized with *m*-chloroperbenzoic acid to give a diastereomeric mixture of epoxides (**23**) in a 6 : 4 ratio. The epoxides were hydrolyzed and the diols **24** esterified with sene-



Scheme 2.

cioyl chloride to give an epimeric mixture from which the main component, after purification by crystallization, was identical (spectra, mp and $[\alpha]_D$) to natural **14**.

The absolute configuration of **14** was assigned *S* for C-2′, the same as in (−)-marmesin, and *S* for C-1″ according to a previous theoretical study (Morcardó, San-Fabián & Grande, 1988). CNDO/2 and ab initio STO-3G calculations predict for the C-2′/C-1″ rotamers of **22** two energy minima which correspond to the conformations with dihedral angels (O-1′)–(C-2′)–(C-1″)═(C-2″) ca −10° (*syn* conformer) and +150° (*anti* conformer), but this last occupies a relative minimum 0.6 kcal/mol above the first one. This energy difference attests that the relative population between the *syn* and *anti* conformers at 25°C is ca 70 : 30. Assuming an attack of the peracid from the less hindered face and that the transition state for both conformers in the epoxidation process is equivalent, the major epoxide should be the β isomer. These predictions are in agreement with the experimental results: the epoxides were obtained in a β/α ratio 6 : 4 (by ^{1}H-NMR) quite similar to that of the calculated *syn*/*anti* ratio for **22**. Consequently, the major β-epoxide (1″*R*) on acid hydrolysis will give a diol with inversion of the C-1″ configuration (Hanzlik, Edelman, Michaely & Scott, 1976) and therefore the configuration of compound **14**, (+)-2″-senecioyloxymarmesin, was established as (2′*S*, 1″*S*).

Similar coumarins have been described previously such as a glycoside (Lemmich, 1995; Lemmich et al., 1983) and a C-1″ senecioate (Abyshev & Abyshev, 1983) but the C-1″ absolute configuration has not yet been determined.

As mentioned above, the benzene extract of *F. brachyloba* roots was also studied and the coumarins (±)-oxypeucedanin (**5**), (±)-prangol (**6**), (−)-prantschimgin (**10**), (+)-3′-hydroxyprantschimgin (**13**) and aurapten (**16**) were isolated.

There is a clear difference with respect to the coumarin contents of the two *Ferulago* roots. The most abundant coumarins in the roots of *F. capillaris* (about 83% of the benzene extract) are (±)-oxypeucedanin (**5**), (−)-prantschimgin (**10**) and osthol (**15**). Conversely, in the roots of *F. brachyloba* the most abundant coumarins (about 77% of the benzene extract) are (±)-oxypeucedanin (**5**), (−)-prantschimgin (**10**) and aurapten (**16**). The presence of osthol or aurapten in the roots could be used as chemotaxonomic marker to distinguish *F. capillaris* from *F. brachyloba*.

Dealkylation of marmesin to psoralene can proceed trough prandiol derivatives like **14**, but this hypothesis has recently been discarded (Stanjek, Miksch, Lueer, Matern & Boland, 1999). It has been shown that the isopropyl elimination takes place by enzyme coordination to the 1″-hydroxyl group followed by *cis* oxidation at C-3′. Accordingly, coumarins **12** or **13** could be the biogenetic precursors of the linear furanocoumarins found in the studied *Ferulago* species.

3. Experimental

3.1. General

Mps: uncorr. EIMS: 70 eV. ^{1}H- and ^{13}C-NMR: 200 or 400 and 50 or 100 MHz, respectively, with TMS as an int. std. CC Merck silica gel (0.06–0.2 mm). TLC silica gel 60 G plates. Spots were detected under UV irradiation (254 and 365 nm).

3.2. Plant material

Ferulago capillaris (Link ex Sprengel) Coutinho was collected in Villarino de los Aires, Salamanca, Spain, in April (roots) and July (aerial parts). A voucher specimen is deposited at the Herbarium of the Department of Botany, University of Salamanca (SALA 18302). The air-dried and finely grounded aerial parts (460 g) were extracted in hexane at room temperature. The extract was concentrated in vacuo (24 g) and subjected to CC on silica gel with a *n*-hexane–EtOAc mixture of increasing polarity as eluent. Apart from enriched fractions in glycerides (5 g) and β-sitosterol (400 mg), the following coumarins were isolated (TLC eluent, benzene–EtOAc 8 : 2; 365 nm fluorescence): **1** (1 g, R_f 0.50, yellow), **2** (1.7 g, R_f 0.30, dun yellow), **3** (0.5 g, R_f 0.78, yellow), **4** (2 g, R_f 0.72, yellow), **5** (0.8 g, R_f 0.43, yellow), **10** (3.7 g, R_f 0.45, blue) and **11** (5.7 g, R_f 0.43, blue). The partially dried roots of *F. capillaris* (1200 g), were extracted with benzene in a Dean–Stark apparatus. The extract (91 g) afforded after crystallization in benzene a mixture of **5** and **10** (51 g). The mother liquor was chromatographed on silica gel with *n*-hexane–EtOAc mixture of increasing polarity and the following coumarins were isolated (TLC eluent, benzene–EtOAc 7 : 3; 365 nm fluorescence): **3** (0.4 g), **4** (0.5 g), **5** (3.8 g), **6** (229 mg, R_f 0.1, yellow), **7** (30 mg, R_f 0.43, yellow), **8** (350 mg, R_f 0.33, yellow), **9** (50 mg, R_f 0.45, yellow), **10** (6 g), **12** (40 mg, R_f 0.45, blue), **13** (180 mg, R_f 0.40, blue), **14** (20 mg, R_f 0.40; blue) and **15** (17 g, R_f 0.95, blue).

The roots of *F. brachyloba* Boiss and Reuter were collected in Aldea del Obispo, Salamanca, Spain in October. A voucher specimen is deposited at the Herbarium of the Department of Botany, University of Salamanca (SALA 21302). Finely cut fresh roots (700 g) were extracted with benzene in a Dean–Stark apparatus. The extract was concentrated in vacuo (39 g), and dissolved in refluxing Et_2O. On cooling, a crystalline precipitate (23 g) containing the coumarins **5**, **10**

and **16** was separated. This crude precipitate was recrystallized in methanol to afford **5** and **10** (15 g). The soluble coumarin mixture (24 g) was subjected to several CC on silica gel and eluted with *n*-hexane–EtOAc mixture of increasing polarity: the coumarins **5** (0.5 g), **10** (2 g), **13** (0.2 g), and **16** (4.5 g, R_f 0.75, benzene–EtOAc 8 : 2, blue fluorescence) were isolated. Glycerides and a mixture of three sterols ($[M]^+$ 410, 412 and 414) were also isolated. Known compounds were identified by comparison of their spectroscopic properties with literature data.

3.3. (+)-Senecioylprangol (7)

Mp 164–165°C (MeOH); $[\alpha]_D^{20}$ +9.0 (Me_2CO; *c* 1.0); UV λ_{max}^{EtOH} nm (log ε): 220 (4.1), 250 (3.83), 258 (3.7), 267 (3.7), 309 (3.6); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600–3350, 2950, 1730, 1640, 1590, 1465, 1390, 1365, 1160, 1090, 840; ^{1}H-NMR: 6.22 (1H, *d*, *J* = 9.8 Hz, H-3); 8.08 (1H, *d*, *J* = 9.8 Hz, H-4); 7.13 (1H, *s*, H-8); 7.57 (1H, *d*, *J* = 2.2 Hz, H-2′); 6.95 (1H, *d*, *J* = 2.2 Hz, H-3′); 4.75 (1H, *dd*, *J* = 10.2, 3.2 Hz, H-2″); 4.55 (1H, *dd*, *J* = 10.2, 8.0 Hz, H-2″); 5.35 (1H, *dd*, *J* = 8.0, 3.2 Hz, H-3″); 1.32 (3H, *s*, Me-4″); 1.31 (3H, *s*, Me-5″). Sencioate: 5.70 (1H, *m*, H-2); 1.90 (3H, *d*, *J* = 0.8 Hz, Me-4); 2.19 (3H, *d*, *J* = 0.9 Hz, Me-5); ^{13}C-NMR: 161.1 (C-2), 112.6 (C-3), 139.4 (C-4), 107.6 (C-4a), 139.7 (C-5), 114.3 (C-6), 158.1 (C-7), 94.2 (C-8), 152.7 (C-8a), 147.0 (C-2′), 106.6 (C-3′), 75.7 (C-2″), 85.7 (C-3″), 72.0 (C-4″), 21.7 (C-5″), 21.7 (C-6″). Senecioate: 165.3 (C-1), 118.6 (C-2), 157.0 (C-3), 27.4 (C-4), 20.2 (C-5); EIMS m/z (rel. int.): 386 $[M]^+$ (9), 149 (63), 83 (100). *Partial synthesis*: (−)-*Oxypeucedanin*, (−)-**5**. Mp 105–106°C (MeOH); $[\alpha]_D^{20}$ −12.8 ($CHCl_3$; *c* 3.5) (220 mg, 0.77 mmol) was dissolved in THF–H_2O 3 : 1 (25 ml) and a catalytic amount of *p*-TsOH acid was added. After 2 h at room temperature, the reaction product was worked up and (−)-*prangol*, **6**, (210 mg) $[\alpha]_D^{20}$ −11 (acetone; *c* 1.5) was obtained. (−)-Prangol, (160 mg 0.5 mmol) was dissolved in CH_2Cl_2 (10 ml), and the solution was treated with senecioic acid (70 mg, 0.7 mmol), DCC (0.1 ml, 0.8 mmol) and DMAP (catalytic amount) and the mixture was stirred at room temperature for 3 h. The reaction product was purified by CC on silica gel to yield 120 mg of (−)-**7**.

3.4. (−)-(2′S, 3′R)-3′-Senecioyloxymarmesin (12)

Mp 148–149°C (*n*-hexane/CH_2Cl_2); $[\alpha]_D^{20}$ −236 ($CHCl_3$; *c* 1.3); UV λ_{max}^{EtOH} nm (log ε): 325 (3.9), 298 (3.7) sh, 222 (4.3); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600–3350, 1735, 1640, 1590, 1500, 1410, 1190, 1150, 1020, 980, 840; ^{1}H-NMR: see Table 1; ^{13}C-NMR see Table 2. EIMS *m*/*z* (rel. int.): 344 $[M]^+$ (21), 244 (14), 187 (25), 186 (60), 100 (98), 87 (57), 85 (66), 84 (32), 83 (100), 82 (57), 74 (68), 59 (81).

3.5. (+)-(2′S, 3′R)-3′-Hydroxyprantschimgin (13)

Mp 177–179°C (CCl_4); $[\alpha]_D^{20}$ +15 ($CHCl_3$; *c* 1.2); UV λ_{max}^{EtOH} nm (log ε): 326 (3.8), 258 (3.4) sh, 247 (3.5) sh, 220 (4.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600–3500, 1730, 1635, 1230, 1130, 840, 815; ^{1}H-NMR: see Table 1; ^{13}C-NMR: see Table 2; EIMS *m*/*z* (rel. int.): 344 $[M]^+$ (1), 186 (20), 149 (92), 95 (34), 85 (48), 83 (100), 69 (75), 57 (85), 55 (61), 43 (38), 41 (23). *Partial synthesis*: (−) *Prantschimgin* (**10**), (1.63 g, 5 mmol) in glacial HOAc (30 ml), was oxidized with CrO_3 (1.70 g, 17 mmol) in H_2O (3 ml) at room temperature for 3 h. The solution was diluted with H_2O (30 ml), treated with (20%) Na_2SO_3 (10 ml) and the acetic acid was removed in vacuo. The suspension was extracted with Et_2O (3 × 20 ml), dried and concentrated. The residue was chromatographed (*n*-hexane–EtOAc 8 : 2) to give **17** (1.03 g) which was purified by crystallization in MeOH.

3.6. (+)-3′-Oxoprantschimgin (17)

Mp 179–180°C (MeOH); $[\alpha]_D^{20}$ +100 ($CHCl_3$; *c* 1.7); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3020, 1750, 1730, 1640, 1590, 1500, 1400, 1370, 870, 840; ^{1}H-NMR: see Table 1; ^{13}C-NMR: see Table 2; EIMS *m*/*z* (rel. int.): 342 $[M]^+$ (0.5), 243 $[M\text{-}OSen]^+$ (15), 242 $[M\text{-}SenOH]^+$ (100), 227 (7), 214 (15), 199 (9), 171 (9), 160 (4), 115 (5), 88 (5), 69 (6).

3.7. Reduction of 17 with $NaBH_4$

To a solution of 3′-oxoprantschimgin (890 mg, 2.6 mmol) in THF : MeOH (1/1) (20 ml), $NaBH_4$ (420 mg, 11 mmol) was added in four portions. After 30 min, the excess of reagent was decomposed with THF : H_2O and (2 N) HCl was added to pH = 3, the organic solvent was removed in vacuo and the suspension was extracted with CH_2Cl_2 (3 × 40 ml). The residue after chromatography (*n*-hexane–EtOAc 7 : 3) was identical to **13**.

3.8. Psoralen

Compound **13** (145 mg) was treated with 4% KOH : MeOH (4 ml) and the reaction mixture stirred at room temperature for 3 h. After work-up in the usual way, the reaction product was subjected to CC, (*n*-hexane–EtOAc 9 : 1), to give psoralen (80 mg).

3.9. Marmesin (18)

Prantschimgin (**10**) (150 mg) was dissolved in EtOH (10 ml), added to ethanolic 4% Na_2CO_3 (20 ml) and the reaction mixture refluxed for 1 h. Then the solution was diluted with H_2O (30 ml) and the EtOH removed in vacuo. The suspension was acidified with

Table 1
^{1}H-NMR spectral data for compounds **12**, **13**, **14** and **17** (400 MHz, $CDCl_3$)

H[a]	12	13	14	17
3	6.24 *d* (9.5)	6.21 *d* (9.5)	6.21 *d* (9.5)	6.39 *d* (9.5)
4	7.61 *d* (9.5)	7.64 *d* (9.5)	7.59 *d* (9.5)	7.77 *d* (9.5)
5	7.59 *s*	7.51 *s*	7.23 *s*	7.84 *s*
8	6.86 *s*	6.81 *s*	6.72 *s*	7.07 *s*
2′	4.51 *d* (6.2)	4.66 *d* (6.2)	4.89 *dd* (9.5, 7.7)	5.24 *s*
3′	6.37 *d* (6.2)	5.35 *d* (6.2)	3.35 *dd* (16, 9.5), 3.25 *dd* (16, 7.7)	
2″	1.46 *s*	1.83 *s*	4.20 *s*	1.76 *s*
3″	1.46 *s*	1.83 *s*	1.25 *s*	1.51 *s*

[a] Other signals: OSen: 5.69 *br s* (H-2), 2.18 *br s* (Me-4), 1.90 *br s*, (Me-5).

(2 N) HCl, extracted with Et_2O, dried with Na_2SO_4 and evaporated. The residue was crystallized in $CHCl_3$ to yield *marmesin*, **18** (107 mg). Mp 189–190°C ($CHCl_3$); $[\alpha]_D^{20}$ +26 ($CHCl_3$; *c* 1.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600–3400, 2990, 1730, 1635, 1580, 1490, 1400, 1270, 1130, 910, 820; ^{1}H-NMR: 6.14 (1H, *d*, *J* = 9.5 Hz, H-3); 7.56 (1H, *d*, *J* = 9.5 Hz, H-4); 7.16 (1H, *s*, H-8); 6.66 (1H, *s*, H-5); 4.71 (1H, *t*, *J* = 9 Hz, H-3′); 3.20 (2H, *J* = 9.0 Hz, H-2′); 2.20 (1H, *s*, OH); 1.35 (3H, *s*); 1.22 (3H, *s*).

3.10. (+)-3′-Oxomarmesin (*19*)

Marmesin, **18** (683 mg, 2.8 mmol) in glacial HOAc (30 ml) was reacted with CrO_3 (1.10 g, 11 mmol) in H_2O (2 ml) and the mixture stirred at room temperature for 4 h. After work up as described above, 3′-oxomarmesin (**19**), (380 mg) was obtained. Mp 143–145°C (MeOH); $[\alpha]_D^{20}$ +67.5 ($CHCl_3$; *c* 1.3); IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 3650–3300, 1750, 1640, 1590, 1490, 1400, 1360, 1160, 1145, 860, 830; ^{1}H-NMR: 6.33 (1H, *d*, *J* = 9.8 Hz, H-3); 7.68 (1H, *d*, *J* = 9.8 Hz, H-4); 7.75 (1H, *s*, H-5); 7.10 (1H, *s*, H-8); 4.50 (1H, *s*, H-2′); 1.40 (3H, *s*); 1.31 (3H, *s*).

Table 2
^{13}C-NMR spectral data for compounds **12**, **13**, **14** and **17** (100 MHz, $CDCl_3$)

C[a]	12	13	14	17
2	160.8	160.9	161.3	160.8
3	113.0	113.0	112.4	114.6
4	143.6	143.6	143.5	143.4
4a	113.4	113.6	112.8	114.5
5	126.6	125.0	123.3	124.3
6	124.1	127.2	124.6	119.3
7	163.2	162.6	162.9	173.2
8	99.1	99.1	98.0	100.8
8a	157.1	157.0	155.6	157.0
2′	91.0	90.9	86.6	88.4
3′	71.4	71.8	29.0	196.1
1″	71.2	82.0	72.8	80.4
2″	26.6	23.5	67.5	22.3
3″	26.5	23.8	19.6	21.5

[a] Other signals: OSen: 165 *s* (C-1), 116 *d* (C-2), 159 *s* (C-3), 27 *q* (C-4), 20 *q* (C-5).

3.11. (+)-3′-Hydroxymarmesin (*20*)

To a solution of **19** (250 mg, 0.96 mmol) in MeOH (20 ml), $NaBH_4$ (180 mg, 4.8 mmol) was added in four portions. After 90 min the reaction was quenched, worked up and the crude product was purified by CC on silica gel (*n*-hexane–EtOAc 7 : 3), to give 172 mg of **20**. Mp 166–168°C (Me_2CO); $[\alpha]_D^{20}$ +24.4 ($CHCl_3$; *c* 2.8); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550–3300, 1730, 1640, 1590, 1500, 1380, 1150, 840; ^{1}H-NMR: 6.05 (1H, *d*, *J* = 9.8 Hz, H-3); 7.50 (1H, *d*, *J* = 9.8 Hz, H-4); 7.35 (1H, *s*, H-8); 6.50 (1H, *s*, H-5); 5.30 (1H, *br s*); 4.28 (1H, *br s*); 1.55 (3H, *s*); 1.50 (3H, *s*); EIMS *m/z*: (rel. int.): 262 $[M]^+$ (3.6), 186 (56), 158 (30), 130 (11.5), 102 (20), 83 (100), 69 (42), 55 (31). *Acetonide*: To a solution of **20** (62 mg, 0.24 mmol) in acetone (20 ml), 2,2-dimethoxypropane (0.5 ml, 2.3 mmol) and a catalytic amount of *p*-TsOH acid was added. After 24 h at room temperature Na_2CO_3 was added, the suspension filtered and then concentrated in vacuo to give the acetonide **21** (50 mg): Mp 166–167°C; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1630, 1578, 1440, 1400, 1385, 1185, 1125, 955, 820; ^{1}H-NMR: 6.15 (1H, *d*, *J* = 9.5 Hz, H-3); 7.52 (1H, *d*, *J* = 9.5 Hz, H-4); 7.38 (1H, *s*, H-8); 6.67 (1H, *s*, H-5); 5.50 (1H, *d*, *J* = 7.0 Hz, H-2′); 4.52 (1H, *d*, *J* = 7.0 Hz, H-3′); 1.45 (3H, *s*); 1.40 (3H, *s*); 1.35 (6H, *s*).

3.12. (+)-2″-Senecioyloxymarmesin (*14*)

Mp 100–102°C (hexane/CH_2Cl_2); $[\alpha]_D^{20}$ +28 ($CHCl_3$; *c* 0.7); UV λ_{max}^{EtOH} nm (log ε): 336 (4.3), 300 (3.8) sh, 258 (3.9) sh, 248 (3.9) sh; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–3300, 1730, 1640, 1580, 1500, 1460, 1410, 1280, 1160, 1140, 860, 830; ^{1}H-NMR: see Table 1; ^{13}C-NMR: see Table 2; EIMS *m/z* (rel. int.): 344 $[M]^+$ (23), 256 (41), 185 (35), 129 (62), 123 (33), 115 (35), 109 (36), 83 (100), 71 (70), 69 (76), 57 (75), 55 (77).

Acknowledgements

We would like to thank Dr. Felipe García-Martín for the botanical determination of the *Ferulago* species and to D. Santos Rodríguez-Andrés for his collaboration in the experimental work and to the Junta de Castilla y León (Project SA22/97) for financial support.

References

Abyshev, A. Z., & Abyshev, D. Z. (1983). Coumarin composition of *Seseli tortuosum*. *Khimiya Prirodhykh Soedinenii*, 704. (1984). *Chemical Abstracts*, *100*, 171544t.

Andrianova, V. B., Sklyar, Yu. E., & Pimenov, M. G. (1975). Coumarins of *Ferulago turcomanica* roots *Khimiya Prirodhykh Soedinenii*, *11*, 514. (1976). *Chemical Abstracts*, *84*, 27990u.

Baser, K. H. C., Koyuncu, M., & Vural, M. (1998). Composition of the essential oil of *Ferulago trachycarpa* (Fenzl) Boiss. *Journal of Essential Oil Research*, *10*, 665.

Corey, E. J., Brunelle, D. J., & Nicolaou, K. C. (1977). Translactonization route to macrocyclic lactones. *Journal of the American Chemical Society*, *99*, 7359.

Crowden, R. K., Harborne, J. B., & Heywood, V. H. (1969). Chemosystematics of the Umbelliferae — A general survey. *Phytochemistry*, *8*, 1963.

Chalchat, J. C., Garry, R. Ph., Gorunovic, M. S., & Bogavac, P. M. (1992). Composition of the essential oil of *Ferulago sylvatica* (Besser) Reichenb. (Apiaceae). *Pharmazie*, *47*, 802.

Doğanca, S., Gürkan, E., Hirlak, F., Tüzün, O. T., & Tuzlaci, E. (1997). Cytotoxicity assay of some *Ferulago aucheri* extractives using *Artemia salina* (Brine shrimp). *Fitoterapia*, *68*, 1977.

Doğanca, S., Ulubelen, A., & Tuzlaci, E. (1991). 1-Acetylhydroquinone 4-galactoside from *Ferulago aucheri*. *Phytochemistry*, *30*, 2803.

García-Martín, F. (2000) *Ferulago* Koch. In: G. Nieto Feliner & S. Jury, *Flora iberica* (vol. 10). Madrid: CSIC, in press.

Grande, M., Aguado, M. T., Mancheño, B., & Piera, F. (1986). Coumarins and ferulol esters from *Cachrys sicula*. *Phytochemistry*, *25*, 507.

Hanzlik, R. P., Edelman, M., Michaely, W. J., & Scott, G. (1976). Enzymatic hydration of [^{18}O]epoxides. Role of nucleophilic mechanisms. *Journal of the American Chemical Society*, *98*, 1952.

Harada, I., Hirose, Y., & Nakazaki, M. (1968). Absolute configuration of (+)-marmesin and (−)-hydroxytremetone. *Tetrahedron Letters*, 5463.

Lemmich, J. (1995). Monoterpene, chromone and coumarin glycosides of *Diplolophium buchananii*. *Phytochemistry*, *38*, 427.

Lemmich, J., Hacelaund, S., & Thastrup, O. (1983). Dihydrofuranocoumarin glucosides from *Angelica archangelica* and *A. silvestris*. *Phytochemistry*, *22*, 553.

Macías, M. J., Grande, M. C., Anaya, J., & Grande, M. (1990). Cumarinas de *Angelica major* Lag. Comunications to the "23 Reunión Bienal de la Real Sociedad Española de Química". Salamanca, p. 395.

Miski, M., Moubasher, H. A., & Mabry, T. J. (1990). Sesquiterpene aryl esters from *Ferulago antiochia*. *Phytochemistry*, *29*, 881.

Morcardó, F., San-Fabián, E., & Grande, M. (1988). Theoretical study of the relative stability of the rotational conformers of 2-isopropenyl-2,3-dihydrobenzofurane. *Anales de Química*, *84*, 260.

Murray, R. D. H., Méndez, J., & Brown, S. A. (1982). *The natural coumarins. Occurrence, chemistry and biochemistry*. Chichester: Wiley.

Ognyanov, I., & Botcheva, D. (1967). Natural coumarins. Part II: Felamedin (benzoyl marmesin). *Zeitschrift für Naturforschung*, *22B*, 1231.

Ognyanov, I., & Botcheva, D. Z. (1969). Natural coumarins: coumarins in *Ferulago meoides* (L.) Boiss. *Planta Medica*, *17*, 65.

Pascual Teresa, J. de, Jiménez, B., Corrales, B., & Grande, M. (1979). Cumarinas de *Ferulago* grupo *granatensis*. Isovaleril marmesina. *Anales de Química*, *75*, 175.

Rao, G., Sun, H., Lin, Z., & Nie, F. (1993). Chemical studies of traditional Chinese medicine Qian-Hu. *Tianran Chanwu Yanjiu Yu Kaifa*, *5*, 1. (1994). *Chemical Abstracts*, *121*, 53896v.

Ruberto, G., Cannizzo, S., Amico, V., Bizzini, M., & Piatelli, M. (1994). Chemical constituents of *Ferulago nodosa*. *Journal of Natural Products*, *57*, 1731.

Serkerov, S. V., Kagramanov, A., & Abbasov, R. M. (1976). Coumarins of *Ferulago turcomanica*. *Khimiya Prirodhykh Soedinenii*, *94*. (1976). *Chemical Abstracts*, *85*, 59559x.

Stanjek, V., Miksch, M., Lueer, P., Matern, U., & Boland, W. (1999). Biosynthesis of psoralen: mechanism of cytochrome P450 catalyzed oxidative bond cleavage. *Angewandte Chemie International Edition*, *38*, 400.

Tutin, T. G., Heywood, V. H., Burges, N. A., Valentine, D. H., Walters, S. M., & Webb, D. A. (1968). *Flora Europaea*, *vol. 2* (pp. 315–375). Cambridge: Cambridge University Press.

Uğur, M. S., Gürkan, E., Körsal, E. P., & Tuzlaci, E. (1998). Cytotoxicity assay and fibrinolytic evaluation of *Heracleum sphondylium* and *Ferulago thirkeana*. *Fitoterapia*, *64*, 378.

Vilegas, W., Pzetti, G. L., & Yariwake Vilegas, J. H. (1993). Coumarins from *Brosimum gaudichaudii*. *Journal of Natural Products*, *56*, 416.

Yazawa, H., Tanaka, K., & Kariyone, K. (1974). The reaction of carboxylic esters with boron tribromide. A convenient method for the synthesis of amides and transesterification. *Tetrahedron Letters*, 3995.