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# PHOTOACTIVE FUROCOUMARINS IN FRUITS OF SOME UMBELLIFERS

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**Key Word Index**—Umbelliferae; fruits; photoactive furocoumarins; HPLC analysis; photobiological assay; quantification; psoralen; 5-methoxypsoralen; 8-methoxypsoralen; isopimpinellin; isoimperatorin; imperatorin; oxypeucedanin; sphondin; chemotaxonomy.

**Abstract**—Fruits of *Anethum graveolens*, *Angelica archangelica*, *Anthriscus cerefolium*, *Apium graveolens*, *Carum carvi*, *Coriandrum sativum*, *Cuminum cyminum*, *Daucus carota*, *Foeniculum vulgare*, *Heracleum sphondylium*, *Levisticum officinale*, *Pastinaca sativa*, *Petroselinum crispum* and *Pimpinella anisum* were analysed for photoactive furocoumarins using HPLC analysis and photobiological bioassay. These sensitive methods revealed furocoumarins not previously detected.

## INTRODUCTION

The Umbelliferae is the family richest in species containing furocoumarins [1, 2]. Some furocoumarins such as psoralen (**1**), 5-methoxypsoralen (5-MOP, **2**) and 8-methoxypsoralen (8-MOP, **3**) are potent photosensitizers when activated by near-UV light (300–380 nm). They intercalate readily into DNA and form light-induced mono- or di-adducts with pyrimidine bases. Thus they are phototoxic, mutagenic and photocarcinogenic. Severe dermatitis can result after contact with furocoumarin-containing plants in the presence of sunlight [3]. Consumption of furocoumarins may also present some toxicological risk to man. In PUVA (psoralen-UVA light) photochemotherapy for psoriasis about 20 mg of 8-MOP is administered daily. This treatment is, however, recognized by the World Health Organization to be causally related to human skin cancer [4]. Various parts of many umbelliferous plants have been utilized by man for centuries [5]. In recent years there has been dietary concern about the furocoumarin content of umbelliferous vegetables such as celery [6], parsnip [7] and carrot [8, 9].

It is known, however, that the highest concentration of these molecules is found in the fruits. Quite a few umbelliferous fruits, and the oils obtained from them, are widely used for flavouring in foods and beverages. Some are used in perfumery. The fruits of hogweed, *Heracleum sphondylium* L., are made into an alcoholic beverage in some European and Asian areas and used for a liqueur in France [5]. The medicinal value of umbelliferous fruits, once highly prized, is less important today.

Only a few umbelliferous fruits have been recently analysed in detail and the photoactive furocoumarins quantified [10, 11]. Using HPLC analysis [12] and an ultrasensitive bioassay [13], we report in this paper on the concentration of photoactive furocoumarins in the umbelliferous fruits most frequently used by man.

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## RESULTS AND DISCUSSION

The concentrations of photoactive furocoumarins **1**–**10** in various umbelliferous fruits are presented in Table 1. The plant species are arranged according to their furocoumarin level. The ultrasensitive bioassay was used for the detection of photoactive furocoumarins [13]. The lower limits of the bioassay detection for compounds such as imperatorin (**6**) and sphondin (**10**) were not previously known and have been established. They are imperatorin $1 \times 10^{-7}$ g and sphondin $5 \times 10^{-9}$ g. Also included in the table is isopimpinellin (**4**), which is considered non-phototoxic [3] but its phototoxicity has been demonstrated by Ivie [14] and Kavli *et al.* [10]. The phototoxicity of isopimpinellin is weak, however, and cannot be detected by the bioassay even at a concentration of $4 \times 10^{-5}$ g.

The fruits of *Angelica archangelica* L. had the highest content of total photoactive furocoumarins, approximately 1.29% dry weight. The total coumarin content in ripening fruits was reported to reach up to 2.59% then to decrease to 1.46% in ripe fruits [15]. All five furocoumarins detected in our sample were reported previously [2]. We failed to detect angelicin (**9**), also reported in fruits of angelica [16].

The fruits of parsnip, *Pastinaca sativa* L., had considerably lower amounts of photoactive furocoumarins (*ca* 0.06–0.19% dry weight) than angelica. There is obviously great variation in furocoumarin content and composition in various parsnip cultivars. Orlov and Sirenko [17] found the total furocoumarin content in fruits of 20 varieties of cultivated parsnip to range from 0.12 to 2.63% dry weight. 8-MOP was the major component in eleven varieties, imperatorin in eight varieties and 5-MOP in one variety. Berenbaum [11] reported the average furocoumarin content in ripe wild parsnip seed to be approximately 1% of the dry weight, almost three times greater than that of the seed of the cultivar. In her study, all seven cultivars and the majority of individual wild plants had imperatorin as a major component. Although we detected the same furocoumarins as Berenbaum [11], there was a great variation in concentra-

Table 1. Photoactive furocoumarin concentrations in various umbelliferous fruits (values are in μg/g fruit)

| Plant* | Compound† | | | | | | | | Unknown photoactive compounds |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 10 | |
| Angelica | | | | | | | | | |
| Herb[a] | | 3477.00 | 427.30 | 128.20 | 3852.00 | 5030.00 | — | — | — |
| Parsnip | | | | | | | | | |
| Hollow Crown[b] | — | 429.76 | 642.10 | 205.16 | — | 355.76 | — | 7.61 | 2 |
| All American[c] | — | 402.94 | 682.14 | 108.90 | — | 758.60 | — | 3.14 | 2 |
| Harris Model[c] | - | 213.48 | 169.98 | 91.44 | — | 161.16 | — | 3.01 | 2 |
| Hogweed | | | | | | | | | |
| Wild[d] | | 136.20 | 25.53 | 8.00 | – | 35.76 | — | ‡ | 4 |
| Parsley | | | | | | | | | |
| Champion Moss Curled[b] | 1.43§ | 12.44§ | ‡,§ | | 5.62§ | 1.38§ | 25.71§ | — | |
| Single Hardy Italian[b] | 1.33§ | 10.50§ | ‡,§ | — | 5.00§ | 5.92§ | 21.41§ | — | — |
| Green Velvet[e] | 9.72§ | 9.22§ | 5.37§ | | 3.52§ | 4.09§ | 6.64§ | - | |
| Clivi[e] | | 5.59§ | — | — | ‡,§ | 0.69§ | 10.82§ | — | — |
| Hamburg Rooted[f] | | ‖,§ | ‖,§ | — | — | — | — | — | — |
| Celeriac | | | | | | | | | |
| Giant Smooth Prague[f] | | 11.16 | | ‡ | — | – | — | | |
| Giant Smooth Prague[g] | - | 16.93 | | ‡ | | | | | |
| Celery | | | | | | | | | |
| Paris Golden[h] | | 2.30 | — | ‡ | — | — | — | - | |
| Utah Tall Green[g] | — | 6.68 | - | ‡ | | | — | — | — |
| Lovage | | | | | | | | | |
| Herb[i] | 3.18§ | 6.38 | ‡ | | - | 12.82§ | — | — | 3 |
| Fennel | | | | | | | | | |
| Vegetable[f] | ‡ | 5.24 | ‖,§ | ‡,§ | - | 2.80§ | - | — | 1 |
| Herb[a] | ‡ | ‡ | ‖,§ | ‡,§ | | ‡,§ | — | — | |
| Anise | | | | | | | | | |
| Herb[a] | — | ‖ | ‖,§ | | | - | — | | 2 |
| Coriander | | | | | | | | | |
| Herb[c] | | ‖ | ‖,§ | - | — | — | | | 2 |
| Dill | | | | | | | | | |
| Herb[b] | — | ‖ | ‖,§ | — | — | - | | — | |
| Caraway | | | | | | | | | |
| Herb[f] | — | ‖,§ | ‖,§ | – | – | - | | — | |
| Carrot | | | | | | | | | |
| Danvers Half Long[g] | - | ‖,§ | ‖,§ | – | - | - | — | | — |
| Mokum F1 Hybrid[e] | | ‖,§ | ‖,§ | | | — | | — | - |
| Gold Pak[g] | — | ‖,§ | ‖,§ | — | — | | — | - | — |
| Kundulus[e] | | ‖,§ | ‖,§ | - | | — | | - | |
| Nantes[g] | — | ‖,§ | ‖,§ | — | — | | — | – | - |
| Chantenay[g] | — | ‖,§ | ‖,§ | — | - | - | — | | |
| Cumin | | | | | | | | | |
| Herb[a] | — | - | — | — | - | — | - | - | — |
| Chervil | | | | | | | | | |
| Herb[i] | — | — | - | - | — | | — | - | — |

*Superscripts indicate source: a, Suffolk Herbs, Organic Farmers & Growers Ltd., Suffolk, U.K.; b, A.E. McKenzie Co., Ltd., Brandon, Manitoba, Canada; c, Pacific Northwest Seed Co. Inc., Vernon, B.C., Canada; d, Oxford, U.K.; e, Thompson & Morgan Ltd., Ipswich, U.K.; f, Buckerfield's Ltd., Vancouver, B.C., Canada; g, Island Seed Co. Ltd., Victoria, B.C., Canada; h, Ontario Seed Co. Ltd., Waterloo, Ontario, Canada; i, Suttons Seeds Ltd., Torquay, U.K.

†See formulae.

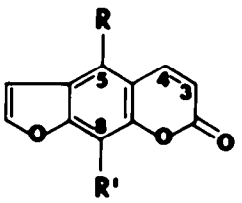
‡Approximately 0.5 μg/g (HPLC detection limit = 0.1 μg/g).

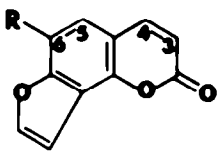
§New information.

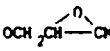
‖Approximately 0.005 μg/g (bioassay detection limit = 0.001 μg/g for 5-MOP and 8-MOP).

tion of the individual compounds in our samples. Two weakly active unknown photoactive compounds were also present.

The fruits of hogweed, *Heracleum sphondylium* L., possessed 5-MOP as a major component. All identified furocoumarins seen in our sample have been reported previously [2]. We were unable to identify one photoactive compound which was present in moderate concentration. There were also trace amounts of three other photoactive compounds. Angelicin and psoralen, rep-





| COMPOUND | R | R' |
|---|---|---|
| 1 Psoralen | H | H |
| 2 5-MOP | OMe | H |
| 3 8-MOP | H | OMe |
| 4 Isopimpinellin | OMe | OMe |
| 5 Isoimperatorin | $OCH_2CH{=}CMe_2$ | H |
| 6 Imperatorin | H | $OCH_2CH{=}CMe_2$ |
| 7 Oxypeucedanin |  | H |
| 8 Oxypeucedanin hydrate | $OCH_2CHOH{-}COHMe_2$ | H |

| COMPOUND | R |
|---|---|
| 9 Angelicin | H |
| 10 Sphondin | OMe |

orted by Karlsen *et al.* [18] and by Mandenova *et al.* [19], could not be detected in our sample.

The fruits of parsley, *Petroselinum crispum* L., cultivars grown for leaves, had a total furocoumarin content of approximately 0.003% of the dry weight. The major component was oxypeucedanin; other furocoumarins detected were 5-MOP, 8-MOP, isoimperatorin (**5**) and imperatorin (**6**). The fruits also contained a small amount of psoralen, normally a relatively rare component in umbelliferous fruits. According to Murray *et al.* [2], only some species of *Heracleum*, *Foeniculum*, *Prangos* and *Trachyspermum* contain psoralen in the fruit. The fruits of the cultivar Hamburg Rooted had an exceptionally low furocoumarin content. Kato *et al.* [20] reported a compound they identified as heraclenol from parsley fruits. From their NMR data it is obvious that this compound was in fact oxypeucedanin hydrate (**8**) which originated from oxypeucedanin (by the opening of the epoxide ring [2]) during the hydrolysis in 2 N hydrochloric acid.

The fruits of celery and celeriac, *Apium graveolens* L. var. *dulce* (Miller) Pers. and *A. graveolens* L., var. *rapaceum* (Miller) Gaud., respectively, showed a similar pattern, 5-MOP being slightly higher in celeriac. Garg *et al.* [21] isolated isoimperatorin as well as 5-MOP and isopimpinellin from a large sample (4 kg) of celery fruits.

The fruit of lovage, *Levisticum officinale* Koch, contained imperatorin as a major component and small amounts of 5-MOP and psoralen. The bioassay also confirmed the presence of 8-MOP. Naves [22] and Dauksha and Denisova [23] isolated 5-MOP from lovage fruits. Psoralen was characterized together with 5-MOP by Karlsen *et al.* [24] as being present in the root.

The fruits of two varieties of fennel, *Foeniculum vulgare* Miller, were analysed. The fruits of vegetable fennel, *F. vulgare* Miller subsp. *dulce* (DC.) Bertol., contained small amounts of 5-MOP and imperatorin and traces ( $< 0.5\ \mu g/g$) of psoralen and isopimpinellin. There was also one unknown, weakly photoactive compound. The fruits of the herb, *F. vulgare* Miller subsp. *vulgare*, had the same furocoumarins but only in trace amounts. 5-MOP and psoralen have been reported in the fruits of fennel by Mendez and Poceiro (in Murray *et al.* [3], unpublished results).

The fruits of anise, *Pimpinella anisum* L., coriander, *Coriandrum sativum* L., dill, *Anethum graveolens* L., caraway, *Carum carvi* L., and carrot, *Daucus carota* L., had no detectable furocoumarins by HPLC analysis. However, using the ultrasensitive bioassay, we were able to detect traces of 5-MOP and 8-MOP in all of them. Both compounds were at the limits of the detection ($1 \times 10^{-9}$ g [13]) and their levels in the fruits could be estimated only approximately as $< 0.005\ \mu g/g$. The fruits of anise also had two other unknown, slightly photoactive compounds. 5-MOP has been reported from anise fruits by Kartnig and Scholz [25]. The fruits of coriander had two additional weakly photoactive compounds, one unknown and the other probably imperatorin. Kartnig [26] reported the presence of 5-MOP in coriander fruits. 5-MOP was also found in dill fruits by Kartnig [26] and Dranik and Prokopenko [27]. There is no reference to the presence of the furocoumarins in the caraway fruits or in any other parts of the plant. Small amounts of 5-MOP and 8-MOP were detected recently in leaf surface wax [28] and in leaf and root of the carrot [9].

The fruits of cumin, *Cuminum cyminum* L., and chervil, *Anthriscus cerefolium* (L.) Hoffm., did not contain any photoactive furocoumarins, as shown by both HPLC analysis and bioassay. There are no reports of photoactive furocoumarins in any part of these plants.

The total concentration of photoactive furocoumarins is extremely high in the fruits of angelica and parsnip. It reaches over 12 mg/g in angelica and over 1 mg/g on average in parsnip. The furocoumarin content in other analysed umbelliferous fruits is much lower or negligible.

In nature many umbelliferous fruits containing seeds remain in the soil for several months before germination; the presence of furocoumarins could be related to their possible role as antibacterial, antifungal, allelopathic agents or seed dormancy regulators [29]. The most common compounds found in umbelliferous fruits are 5-MOP and 8-MOP, both of which are strongly photoactive.

Seed furocoumarins were used by Crowden *et al.* [30] as taxonomic markers in their chemotaxonomic survey of Umbelliferae. From a chemosystematic point of view, the absence of a certain furocoumarin is as important as a positive detection. However, many compounds can occur in the plant in amounts which are below the detection limits of the analytical methods. The introduction of a new, more sensitive analytical method for the detection of furocoumarins enabled us to re-evaluate earlier observations. New information on the presence of various furocoumarins in umbelliferous fruits are presented in Table 1. The presence of 8-MOP and 5-MOP in carrot leaf wax, leaves and root [9, 28] was also confirmed in carrot fruits. From a taxonomic point of view, this new finding suggests the close relationship of *Daucus* with other members of subfamily Apioideae, where furocoumarins are common. This is also true for the caraway which belongs to the tribe Apieae but no previous record on the presence of furocoumarins in caraway fruits exists. The fruits of only two species had no detectable furocoumarins by our analysis. Chervil belongs to the tribe Scandiceae and cumin to the tribe Apieae; furocoumarins are common in both tribes. It is possible that the sample analysed was too small for detection.

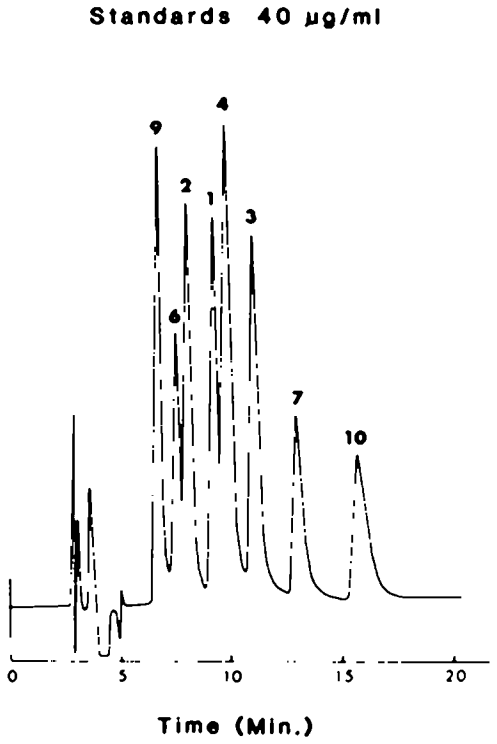



Fig. 1. Separation of furocoumarin standards by normal phase HPLC. The injection volume was 20 µl. See text for full details of HPLC conditions. 1 = Psoralen, 2 = 5-MOP, 3 = 8-MOP, 4 = isopimpinellin, 6 = imperatorin, 7 = oxypeucedanin, 9 = angelicin, 10 = sphondin.

## EXPERIMENTAL

*Furocoumarin standards.* Psoralen was purchased from Upjohn Co., Kalamazoo, MI, U.S.A. 8-MOP was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. 5-MOP was a gift from Gerot Pharmazeutika, Vienna, Austria. Angelicin was synthesized in our laboratories. Imperatorin was obtained from Roth Chemical Co., Carl Roth KG, Karlsruhe, F.R.G. Isopimpinellin was a gift from Dr. J. P. Kutney, Department of Chemistry, University of British Columbia, Vancouver. Isoimperatorin was isolated from the roots of *Angelica dawsonii*, sphondin from the roots of *Heracleum lanatum*, and oxypeucedanin from the leaves of *Petroselinum crispum* [31]. The identity of the last three compounds was established by NMR and MS.

*Plant material.* Fruits were purchased locally and from the U.K. Fruits of *Heracleum sphondylium* were collected in Oxford, U.K. Depending on the furocoumarin content expected, samples of 50, 100 and 500 mg of fruits were extracted twice with 50 ml EtOAc for 24 hr. This extraction was sufficient since there were no furocoumarins in subsequent extracts as determined by HPLC and bioassay. The combined extracts were evaporated, dissolved in 0.5 ml $CHCl_3$, filtered through a 0.5 µm filter and directly analysed by HPLC and TLC.

*HPLC and TLC.* All solvents were HPLC grade, purchased from Burdick and Jackson Laboratories, U.S.A. A Varian 5000 HPLC system equipped with a Rheodyne 7125 loop injector was used with detection by UV at 254 nm. Data processing was done by peak area on a Hewlett-Packard 3390 A integrator. A Varian Si-5 Column (4 mm × 30 cm) was used for HPLC analysis employing the method of ref. [11] with a slight modification. The solvent was cyclohexane–*iso*-$Pr_2O$ *n*-amyl alcohol (15:4:0.5) with a flow rate of 1 ml/min, except for isoimperatorin which was separated with 15% EtOAc in cyclohexane with a flow rate of 1 ml/min. Detection was at 254 nm and $AT = 0.16$ auf. Standards, dissolved in $CHCl_3$, were used at concns of 5, 10 and 20 µg/ml. See Fig. 1 for separation of standards. For TLC analysis, Merck precoated silica gel K 60 sheets (without fluorescent indicator) were used and two-dimensional chromatograms were developed, the first with $CHCl_3$ and second with hexane–pentane–EtOAc (7:7:6). Visualization was performed with UV (300–380 nm) light.

*Photobiological assay.* This ultrasensitive photobiological assay has been described in detail in refs. [9, 13, 32]. The DNA repair deficient mutant, *Escherichia coli Bs*-1 (*rec* +, *exr* −, *hrc* −), which is extremely sensitive to UV radiation and to chemical alkylating agents [33], was used as a test micro-organism.

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