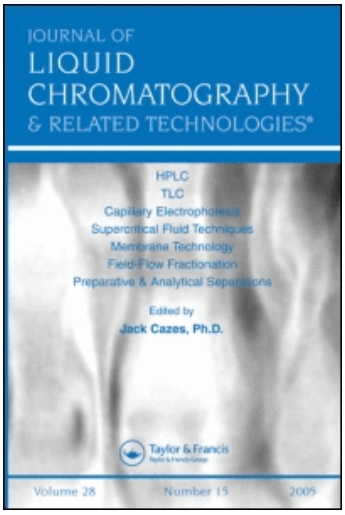

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SIMULTANEOUS HPLC QUANTIFICATION OF TWO DERMATOTOXINS, 5-METHOXYPSORALEN AND FALCARINOL, IN HEALTHY CELERY

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ABSTRACT

A method for the simultaneous quantification and separation of the dermatotoxins, falcarinol and 5-methoxypsoralen (5-MOP), in uninfected, commercially available celery is reported. Four methods of plant extraction were compared to determine the most efficient method. The analyses of crude celery extracts employed a Spectra-Physics 8800 series high performance liquid chromatograph equipped with a forward optical scanning detector. The compounds were resolved on an Alltech C-18 5 mm (25 cm x 4.6 mm ID) column preceded by a C-18 guard column. The dermatotoxins were eluted with a linear gradient of 50% methanol and water to 100% methanol in 30 minutes. The concentration of 5-MOP and falcarinol found in commercially available celery was 0.08-0.24 μg/g and 0.9-20 μg/g, respectively.

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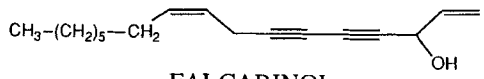
INTRODUCTION

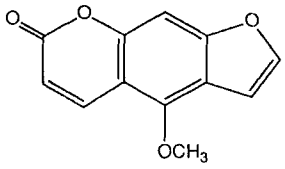
Several genera among the Araliaceae and Apiaceae families (which include many common vegetable and spices in *Hedera*, *Schefflera*, *Panax*, *Apium*, *Falcaria*, *Daucus*, *Oenanthe*, and others) have been shown to contain the polyacetylene, falcarinol (Figure 1) (1). This compound was demonstrated by Gafner *et. al.* (2), and Hansen and Boll (3) to be a potent skin sensitizer; a 0.5% topical application of falcarinol elicited severe bullous reactions in sensitized animals. The amount needed for the elicitation of this delayed hypersensitivity reaction is 0.2 μg/8 mm^2 area (2). Ultraviolet light was not required for the sensitization reaction to occur. The presence of such a potent contact allergen in a wide variety of plants leads us to believe that falcarinol sensitizations are more common than generally assumed.

Dermatitis cases due to falcarinol from English Ivy, Algerian Ivy, and Common Ivy tend to be the most generally reported (2, 3). However, no cases of falcarinol dermatitis have been attributed to celery. The majority of dermatitis cases to celery and parsnip handlers and processors are linked to the linear furanocoumarins (psoralens), which are present in these plants (5). The psoralens are potent skin sensitizers in the presence of ultraviolet light. The amount required to elicit a photosensitivity response by the linear furanocoumarin 5-MOP is 0.1 μg/8 mm^2 skin area (6). It was once believed that psoralens were only found in celery infected with the fungus *Sclerotinia sclerotiorum*; however, this was disproved when Beier *et al.* (7) and Innocenti *et al.* (8) detected psoralens in fresh uninfected celery. A study by Picardo *et al.* (9) reported that photosensitivity was not involved in one case of celery dermatitis. We suggest that this case and many cases of other non-photosensitive dermatitis common to handlers of celery may be attributable to falcarinol

The linear furanocoumarins have been shown to be both potent photosensitizing agents and to have several applications in medicine (10, 11). These compounds also exhibit phototoxicity during therapy and are suspected of being carcinogenic (10, 12).



FALCARINOL



5-METHOXYPSORALEN

FIGURE 1. Structures of dermatotoxins found in celery.

Due to the widespread occurrence of the linear furanocoumarin and polyacetylene compounds (Figure 1) in many human foods, it is important to know the amounts of toxins present in the ingested plants. Both classes of compounds have been demonstrated to be present in celery (1, 5). However, the simultaneous quantification of both classes of dermatotoxins in the same plant has not been performed previously. 5-Methoxypsoralen, the most common furanocoumarin in celery, was chosen as the a representative of the linear furanocoumarins. This study reports quantities comparable to the levels of 5-MOP found by Beier *et. al.* (7) and also determines the concentration of falcarinol in uninfected, commercially available celery.

METHODS

Chemicals: 5-Methoxypsoralen used as a standard was purchased from Sigma Chemical Company (St. Louis, MO). Falcarinol was isolated from English Ivy (*Hedera helix*) by repeated column chromatography and the purity of the sample was established by high performance liquid chromatography to be >95%. Analytical spectroscopy, nuclear magnetic resonance (NMR) and mass spectrometry (MS), confirmed the sample to

be falcarinol. All solvents used were HPLC grade and water was double distilled through a Corning Mega-pure glass distillation system (Corning, Parkersburg, WV).

Plant material: Celery cultivar Tall Utah 5270 R grown and packed in California was bought at a local market in the original carton that had been packed in a cold shipment container. All of the celery selected looked healthy, fresh, and had no signs of any disease. The leaves and bases of each celery bunch were trimmed and discarded. The stalks were cut, weighed, and extracted according to the following methods. Method 1: the extraction and work-up of frozen plant material was extracted according to the procedure of Beier *et. al.* (7). Briefly, frozen plant material was thawed, weighed and extracted with ethyl acetate. Then the crude extract was loaded onto a C-18 SepPak, and the cartridge was then eluted with 60% acetonitrile in water. The eluant was then analyzed for content of dermatotoxins. Method 2: frozen plant material was thawed and 5-g samples (6-8 replicates of each variety) were diluted with 15 ml dichloromethane and homogenized with a Brinkman Polytron homogenizer (3 x 15 ml). After homogenization, the dichloromethane extracts were taken to dryness by rotary evaporation. and the residue was dissolved in 1 ml methanol and sonicated for 1 minute. The solution was filtered through a 0.2 µm Metricel membrane filter. Method 3: 5-gram samples of fresh stalks replaced frozen material in Method 2. Method 4: dried 5-g samples were extracted with 15 ml of methanol (MEOH) using the Brinkman polytron (3X). The methanol extract was concentrated and 5 ml of water was added. The aqueous mixture was then extracted with 5 ml of ethyl ether (3X) and evaporated to dryness. The residue was then resuspended in 200 µl and filtered through a 0.2 µm Metricel membrane filter.

High Performance Liquid Chromatography: Falcarinol and 5-MOP were resolved using a Spectra-Physics 8800 system with forward optical scanning detector on an Alltech C-18 reverse phase resin column. An econosphere C-18 reverse phase column (250 mm x 4.6 mm i.d., 5mm particle size) was eluted with 50% HPLC grade Methanol and 50% double distilled water followed by a linear gradient to 100% methanol in 30 minutes. After an isocratic period of 5 minutes at 100% MeOH, a

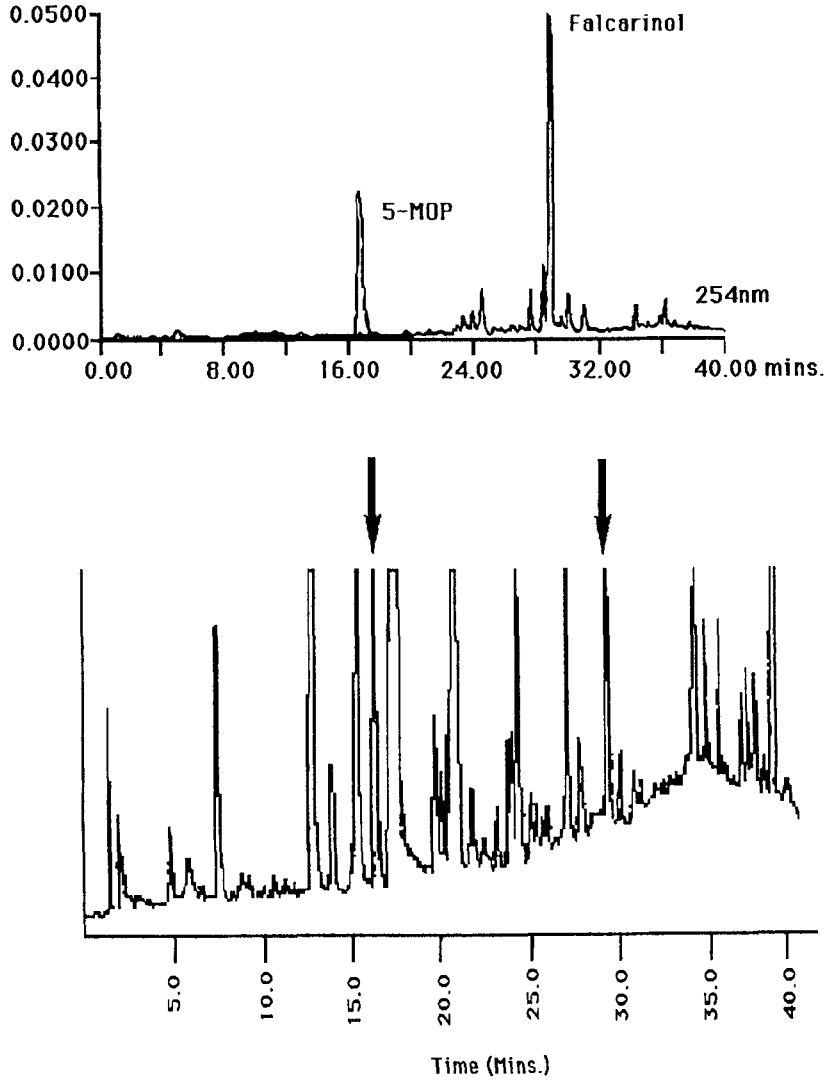


FIGURE 2. HPLC chromatograph of **A**) standards (falcarinol and 5-MOP) and **B**) of a celery extract. Arrows denote retention time of 5-MOP (16.23 min) and falcarinol (29.3 min). The absorption maxima and secondary maxima of falcarinol and 5-MOP obtained from the forward optical scanning detector of the celery samples were identical to the standards in **A**.

second linear gradient was used to return to initial conditions (50% MeOH). A flow rate of 1 ml/min was used. The UV absorbance was monitored at 254 nm. Previously isolated falcarinol and 5-MOP were used as standards in the HPLC analysis (Fig. 2). Ten µl samples were injected into the HPLC. The limit of detection for falcarinol and 5-MOP was 0.05 µg and 0.008 µg, respectively.

RESULTS AND DISCUSSION

Four methods for the extraction of celery were employed to quantitate the amounts of falcarinol, a polyacetylene, and 5-MOP, a furanocoumarin, in commercially available celery. The levels of 5-MOP in commercially available celery varied from 0.08 to 0.24 ppm whereas falcarinol was present at higher concentrations of 0.9-20 ppm (Table 1). No significant difference was observed in the amounts of dermatotoxins extracted by the different methods or material (i.e. frozen, fresh or dried). Co-injection of the falcarinol and 5-MOP standards with a plant sample confirmed the occurrence of both compounds. A representative HPLC chromatograph can be seen in Figure 2.

Although both compounds were found in the celery plant, falcarinol was not identified until Method 1 (Beier *et al.*'s method) was

TABLE 1. Content of 5-methoxypsoralen and falcarinol in healthy, commercially available celery (Utah 5260-R).

METHOD	5-MOP[1]	FALCARINOL
1	0.11 ± 0.09	(2.8 ± 1.1)[2]
2	0.09 ± 0.09	2.9 ± 1.0
3	0.12 ± 0.08	3.1 ± 1.5
4	0.10 ± 0.03	2.7 ± 1.8

[1]All values are reported in parts per million (ppm) and values represent the mean ± S.E. for groups of 6-8 replicates.
[2]Value was obtained only after rinsing C18 SEP-PAK with 100% methanol (See discussion).

modified (See Table 1-Method 1). Brier *et al.* employed a procedure that rinsed a C_{18} SEP-PAK cartridge with 60% acetonitrile (8 ml) in order to elute the furanocoumarins (7). We determined that this procedure was not sufficient to remove falcarinol from the SEP-PAK cartridge. In our workup of the sample, the SEP-PAK was washed with 100% methanol after rinsing with 60% acetonitrile. The levels of falcarinol and 5-MOP were then quantitated as described in the Methods Section.

The standard curve for the furanocoumarin and polyacetylene were generated over a concentration range of 0.008-3.0 ppm and 0.05-40 ppm, respectively. The curves for each compound had correlation coefficients of >0.992 in each case.

Our study indicates that both falcarinol and 5-MOP are present in uninfected, commercially available celery. The novel extraction procedures and analyses described in this report are rapid methods for the simultaneous quantification of both dermatotoxins. No significant differences in the levels of dermatotoxins were observed with the different extraction techniques. The amount required to elicit a photosensitivity response by the linear furanocoumarin 5-MOP is 0.1 μg/8 mm^2 skin area (6). For falcarinol, the amount needed for the elicitation of a delayed hypersensitivity reaction is 0.2 μg/8 mm^2 area (2). In the uninfected celery plant, the quantity of falcarinol is 10-fold greater than 5-MOP. Therefore, it appears likely that the polyacetylenic dermatotoxin, falcarinol, contributes to more celery dermatitis cases than has been previously reported.

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