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PSORALEN AND OTHER LINEAR FUROCOUMARINS AS PHYTOALEXINS IN CELERY

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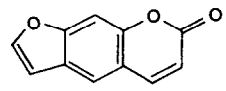
Key Word Index—*Apium graveolens*; Umbelliferae; celery; phytoalexin; linear furocoumarins; psoralen; bergapten; xanthotoxin; isopimpinellin; biosynthesis; photosensitization; celery dermatitis.

Abstract—A linear furocoumarin phytoalexin response was observed in stressed celery (*Apium graveolens*). Heretofore, linear furocoumarins found in diseased celery were thought to be mycotoxins. This is the first documentation of psoralen, bergapten and isopimpinellin being phytoalexins. Phytoalexin response was initiated by general elicitors including copper sulfate, UV light and cold. The distribution of linear furocoumarins (psoralen, bergapten, xanthotoxin and isopimpinellin) was temperature-dependent, and studies with $CuSO_4$ showed that onset of the phytoalexin response was concentration-dependent.

INTRODUCTION

It is well known that the linear furocoumarins xanthotoxin (**3**) [1–3] and bergapten (**2**) [3] can be isolated from celery infected with *Sclerotinia sclerotiorum*, and that the levels of linear furocoumarins in diseased celery are sufficient to cause skin disorders (photosensitization) in celery handlers and processors [2]. Earlier studies [4, 5] have shown linear furocoumarins to occur in healthy celery. Other investigators [2, 3] have observed these compounds only in *S. sclerotiorum* infected plants, while Yu [6] observed the stimulated production of xanthotoxin and 4,8,5′-trimethylpsoralen in celery treated with *Sclerotium rolfsii*, *Rhizoctonia solani*, *Erwinia aroideae* and 5% NaCl. Therefore, the existing literature is unclear about the origin of linear furocoumarins in infected celery. These compounds could not be isolated from the disease organism *S. sclerotiorum* [3, 6], and it has been pointed out that the relationship between celery and *S. sclerotiorum* is poorly understood [2].

In a recent report [5], we have shown the presence of four linear furocoumarins, psoralen (**1**), bergapten (**2**), xanthotoxin (**3**) and isopimpinellin (**4**) in healthy samples of three celery cultivars grown in the U.S.A.; however, we were unable to find 4,8,5′-trimethylpsoralen. The biological activity of these compounds is extremely diverse because of their interactions with DNA. Linear furocoumarins are known to be phototoxic to insects [7]. The combination of UV light and each of compounds **1**–**4** is antibacterial, while **1** and **3** are antibacterial without UV light [8]. Mixtures of furocoumarins containing **4** [9], and the single compounds **1** [10] and **3** [11, 12] are known to be antifungal.



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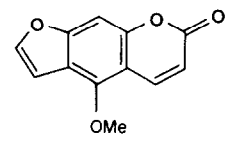

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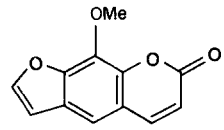

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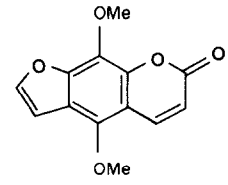

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The biosynthesis of alkoxy-furocoumarins proceeds through at least two different pathways. The first and most often encountered route is through hydroxylation of psoralen with subsequent alkylation [13]. The other proceeds through hydroxylation of marmesin [14]. When the biosynthesis proceeds through hydroxylation of psoralen, psoralen is considered a transient precursor to the alkoxy-furocoumarins [13]. This is borne out by the low or non-detectable levels of psoralen previously observed in *Apium graveolens* and *Petroselinum sativum* [4]. We have also observed low levels of psoralen in three cultivars of fresh, healthy celery [5].

We now present experimental evidence to show that celery (*Apium graveolens*) produces linear furocoumarins as phytoalexins. Psoralen is also shown to be a major linear furocoumarin constituent of copper sulfate treated celery that is subsequently maintained at low temperature.

RESULTS AND DISCUSSION

Commercial celery (*Apium graveolens*) var. 5270-R was

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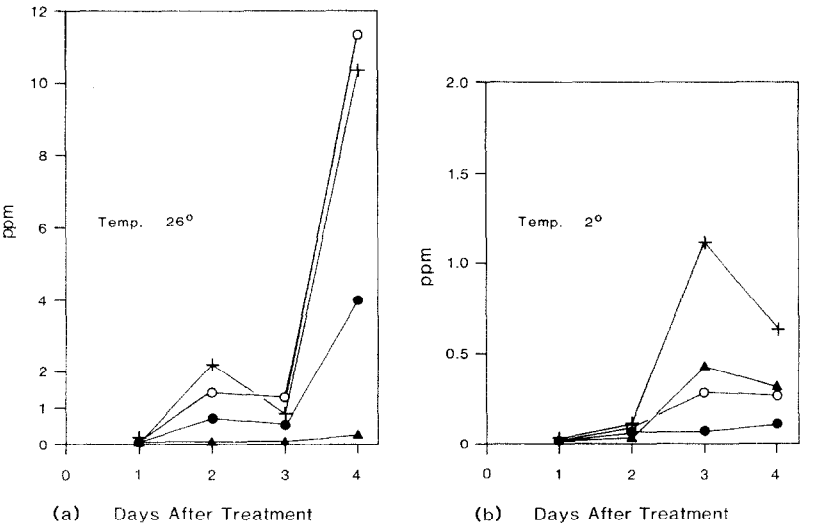

Fig. 1. Distribution of psoralen (▲), bergapten (○), xanthotoxin (+) and isopimpinellin (●) over a 4 day period in $CuSO_4$ treated celery cv 5270-R at (a) a temperature of 26° and (b) a temperature of 2°.

treated in a variety of ways (Cu^{2+}, cold, UV light) known to elicit phytoalexin responses [15–17]. Plant material (petioles) was extracted with ether. The extracts were quantitated by HPLC [5], which was also used to isolate individual compounds for further spectral studies. The mass spectra of the four linear furocoumarins found in celery, psoralen (**1**), bergapten (**2**), xanthotoxin (**3**) and isopimpinellin (**4**) were each consistent with published data [18].

Celery was treated with UV light, cold and sodium hypochlorite resulting in increased levels of linear furocoumarins of 3-, 9- and 2-fold over controls respectively (Table 1). The concentration of the four compounds was determined at 24 hr intervals after treatment with $CuSO_4$ (Fig. 1). Xanthotoxin is apparently the most abundant linear furocoumarin in healthy celery [5], and this was confirmed early in the experiment. After 3 days of incubation at a temperature of 26°, bergapten and xanthotoxin levels increased the most, while psoralen remained a transient species. However, at 2°, psoralen increased at day 3 to a level above bergapten and isopimpinellin.

The effect of elicitor concentration was evaluated by observing the levels of bergapten and xanthotoxin at 72 hr after treating with various concentrations of $CuSO_4$ (Fig. 2). When plotting the relative percentage of each compound (the relative percentages of bergapten were obtained by dividing each value for bergapten by the value

Table 1. Total linear furocoumarin content and the increase over control values after a 72 hr incubation of stressed 5270-R celery

Elicitor	Total linear furocoumarins (ppm) Sample	Control	Increase over controls
UV Light	7.4	2.2	3.4
Cold	2.2	0.25	8.8
Chlorox	4.9	2.2	2.2

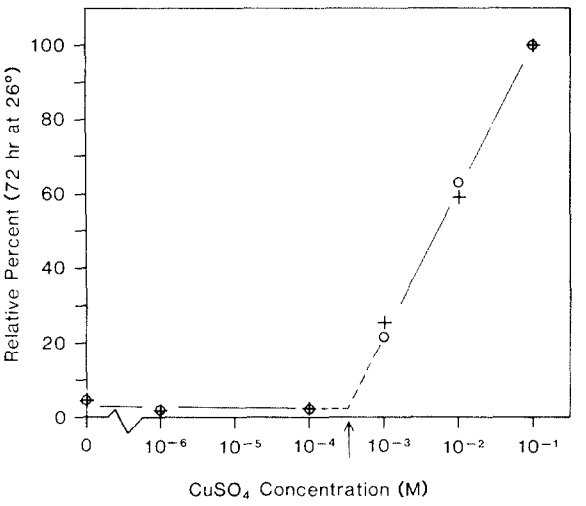

Fig. 2. Relative percentage of bergapten (+) and xanthotoxin (○) measured after 72 hr in celery cv 5270-R treated with different concentrations of $CuSO_4$ for a 0.5 hr period.

observed with a $CuSO_4$ concentration of 1×10^{-1} M; the same procedure was used for xanthotoxin), two coincident straight lines were observed. These lines intersected at approximately 5×10^{-4} M $CuSO_4$. Therefore, it appears that a definite threshold for phytoalexin elicitation exists, along with a linear response with respect to $CuSO_4$ concentrations greater than 5×10^{-4} M.

Although xanthotoxin [1–3] and bergapten [3] have been isolated from celery infected with *S. sclerotiorum*, this is the first report that conclusively establishes xanthotoxin, bergapten, isopimpinellin and psoralen as phytoalexins in celery. Previously, linear furocoumarins were thought to be mycotoxins in diseased celery [19, 20]. This is apparently the first report of bergapten, iso-

pimpinellin and psoralen being observed as phytoalexins in any plant species. Xanthotoxin has previously been reported as a phytoalexin in parsnip root (*Pastinaca sativa*) [21]. It is interesting that the levels of psoralen, the presumed biosynthetic precursor of the other linear furocoumarins observed in celery, rose significantly in the cold (with respect to the alkoxy-furocoumarins) after onset of the phytoalexin response. This is probably due to a decreased rate of biosynthetic transformation of psoralen to other alkoxy-furocoumarins in the cold conditions.

EXPERIMENTAL

Chemicals. The authentic linear furocoumarins used in this study were obtained from the following sources: bergapten (Polysciences, Inc., Warrington, PA; purified by passage through silica SEP-PACs in $CHCl_3$ and recrystallized from MeOH), xanthotoxin (Biochemical Laboratories, Redondo Beach, CA), psoralen (generously provided by M. A. Pathak, Harvard Medical School, Boston, MA) and isopimpinellin (isolated from *Ammi majus* [18]).

Identification. The four linear furocoumarins (psoralen, bergapten, xanthotoxin and isopimpinellin) observed in celery were identified by their MS on a Varian/MAT CH-7 magnetic scan spectrometer. These spectra were identical to those of the authentic furocoumarins [18].

Plant material. A. graveolens cv 5270-R was grown and packed in California and shipped to Texas. The celery was obtained at a Texas supermarket and taken directly from the original shipping carton.

Analysis. All samples were analysed for linear furocoumarins by HPLC according to the procedure of Beier *et al.* [5].

Treatment of plant material. In all cases, the plant material was washed with H_2O and the tops trimmed prior to treatment. After treatment, the plant material was sealed in plastic bags until completion of each treatment period, the plant material was then finely chopped with a food processor and immediately extracted with ether [5].

UV. The petioles were placed under a combination of long and short wave UV light in a Chromato-Vue light box (Ultra-Violet Products, Inc., San Gabriel, CA) at room temp. for 1 hr. After irradiation the petioles were kept at 26° for 72 hr in the dark, and then analysed. Untreated controls were kept in the dark at 26° and analysed in the identical manner.

Sodium hypochlorite. The petioles were immersed in a 1:50 dilution of a commercially obtained 5.25% aq. soln of NaOCl for 20 min (this method was used to observe the effects of sterilizing the plant) and kept in room lighting conditions. The petioles were processed after an incubation of 72 hr at 26°. The controls were immersed in H_2O for 20 min, incubated and processed in like manner.

Cold. The petioles were placed in a freezer (−15°) for 70 min. The cold treated samples and controls were held in room lighting conditions at 26° and analysed after 72 hr.

Copper sulfate. The petioles were immersed in a soln of 9×10^{-3} M $CuSO_4$ for 0.5 hr at room temp. The petioles were held in room lighting conditions and 26° and analysed at 24-hr intervals up to 96 hr after treatment (Fig. 1). The petioles were immersed in varying concns of $CuSO_4$ (1×10^{-1}, 1×10^{-2}, 1×10^{-3}, 1×10^{-4} and 1×10^{-6} M) for 0.5 hr at room temp. These samples were held in room lighting conditions at 26° for 72 hr and analysed (Fig. 2). The controls in both cases were immersed in distilled water for 0.5 hr at room temp., and analysed at the same time interval as the samples.

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