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# FUROCOUMARINS IN THE CULTIVATED CARROT, *DAUCUS CAROTA*

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**Key Word Index**—*Daucus carota*; Umbelliferae; furocoumarins; psoralen; 8-methoxypsoralen; 5-methoxypsoralen; phototoxicity; photobiological assay; phytoalexins.

**Abstract**—A combination of ultrasensitive bioassay and HPLC analysis was employed to analyse the garden carrot for furocoumarins. The earlier reported negative findings were due to the relatively insensitive methods of detection. Low levels of two furocoumarins, 8-MOP and 5-MOP were detected in all parts of fresh carrot plants for the first time. Occasionally one or two other unknown photoactive compounds were also present. Diseased carrot displayed increased levels of 8-MOP and 5-MOP; psoralen was also detected. In this respect carrot is capable of a phytoalexin response similar to celery.

## INTRODUCTION

The possible occurrence of linear furocoumarins **1–3** in the cultivated carrot, *Daucus carota*, has been investigated by several authors. Crowden *et al.* [1] used seed furocoumarins as taxonomic markers in their chemotaxonomic survey of the Umbelliferae but failed to detect furocoumarins in any species of the genus *Daucus*. Only one genus out of seven genera of the tribe Dauceae investigated revealed furocoumarins. Their absence is surprising because in the subfamily Apoideae, in which the subtribe Dauceae is usually placed, furocoumarins are common. Berenbaum [2] did not detect any furocoumarins in carrot and concluded that their absence is the reason why 79% of all insects collected on carrot are generalized feeders. However Stadler and Buser [3] found small amounts of 8-methoxypsoralen (8-MOP, **3**) and 5-methoxypsoralen (5-MOP, **2**), 0.16 μg/g and 2.17 μg/g respectively, in the leaf surface wax of carrots. According to the authors, these furocoumarins, together with several other compounds present in carrot leaf surface wax, stimulate oviposition by the carrot fly.

The furocoumarins occur in several vegetables of the Umbelliferae but only recently have they been evaluated seriously in relation to man's diet. They are potent photosensitizers when activated by long wavelength (300–380 nm) UV light and form light-induced mono- or diadducts with the pyrimidine base of DNA. Thus they are photomutagenic and photocytotoxic [4]. There is sufficient risk from furocoumarins to man that medically unnecessary exposures should be avoided [5].

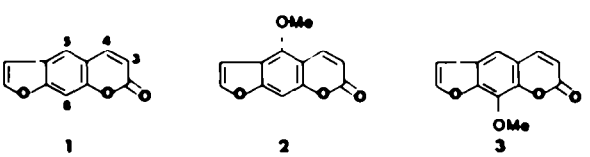
The observation of high concentrations of furocoumarins in parsnip [6] lead to the study of the furocoumarins content of garden carrots, which are more widely consumed vegetables. Carrot roots as well as foliage were analysed using a highly sensitive HPLC method for detection of subparts per million level of furocoumarins. This analysis revealed that furocoumarins were either not present or, if they were, occurred at very low levels $\leq 0.5$ ppm [7].

In spite of equivocal results relating to the presence of furocoumarins in carrots, there are several reports of dermatitis in man caused by carrots although the actual agent or agents were unknown [8, 9]. Van Dijk and Berens [10] reported carrots to have slight but definite photosensitizing activity which was, presumably, related to phytophotodermatitis.

Because we have had excellent results with an ultrasensitive bioassay for detection of furocoumarins and other photosensitizing molecules in plant and plant materials [11, 12], we decided to use it for analysis of furocoumarins in various parts of carrots. HPLC analysis was used to support and quantitate these findings.

## RESULTS AND DISCUSSION

Using our ultrasensitive bioassay it was possible to detect clearly for the first time the presence of furocoumarins in the carrot root. Various parts of carrot such as leaf surface wax, leaves, root crown, root peel and peeled root were also analysed. Levels of furocoumarins in all parts of fresh carrots were very low and not detectable by HPLC analysis (Fig. 2) but their presence was clearly detected by the bioassay (Fig. 1, $A_2$). Both 8-MOP and 5-MOP were present in all parts of fresh carrots, thus confirming and extending the observations of Stadler and Buser [3] on the occurrence of 5- and 8-MOP in carrot leaf wax. However, they occurred at the lower limit of the bioassay sensitivity which is about $1 \times 10^{-9}$ g for both standards 8-MOP and 5-MOP [11]. Furocoumarin levels in fresh carrots can be thus estimated only approximately as 0.01–0.02 μg/g (wet weight) for 8-MOP and 5-MOP. Occasionally we observed 1–2 other unknown photoactive compounds. In one sample we were




**1** **2** **3**

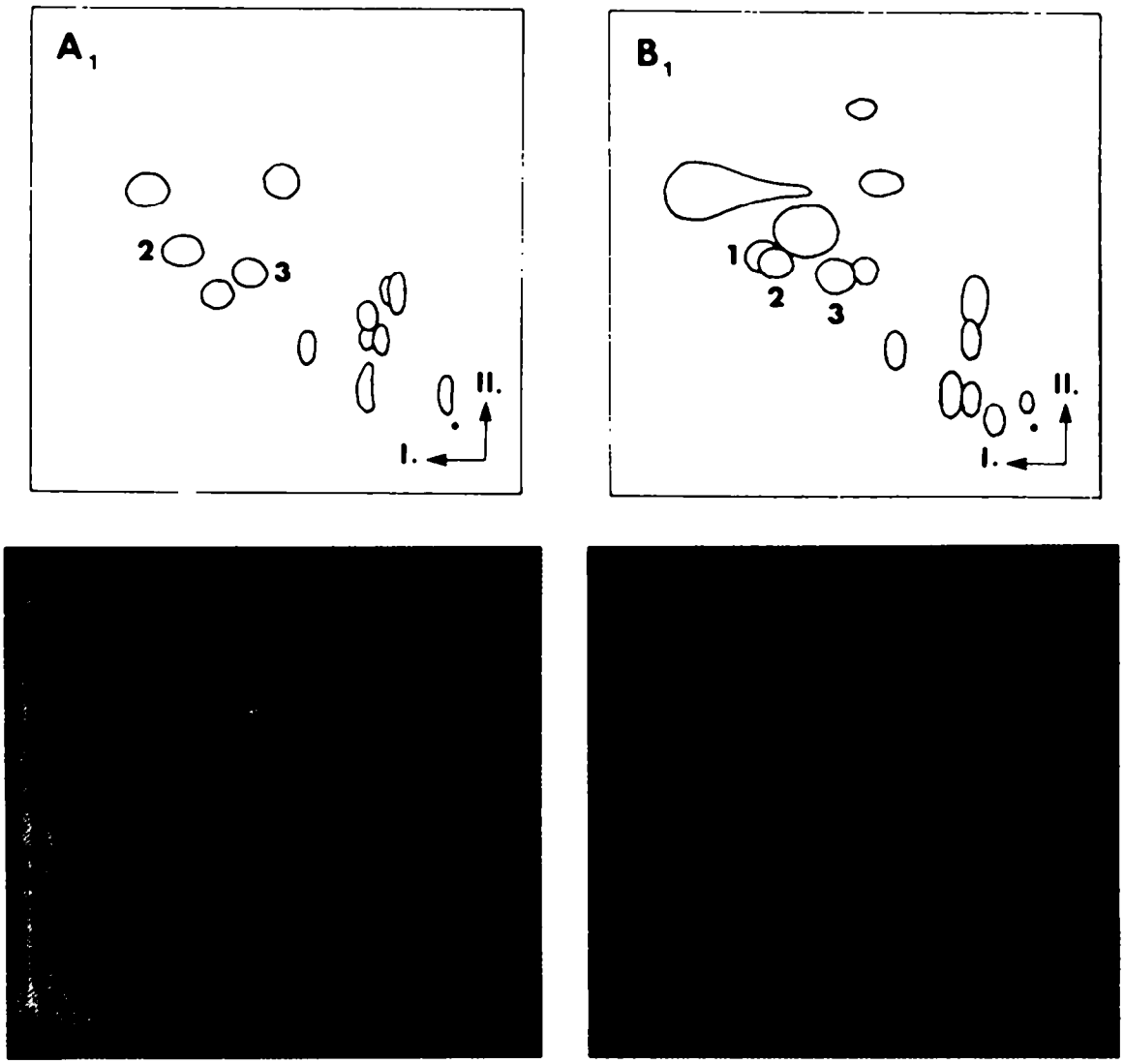



Fig. 1. Photobiological assay of fresh and diseased carrot. $A_1$ and $B_1$ represent chromatographic patterns of fresh and diseased carrot respectively as they appear when viewed with UV light (300–380 nm). $A_2$ and $B_2$ are the same chromatograms assayed photobiologically. The dark areas represent inhibition zones of bacterial growth due to the presence of photoactive furocoumarins. Sample A, fresh carrot crown, represents 510 mg (wet weight), and sample B, diseased carrot crown, 130 mg (wet weight).

able to observe phytoalexin responses in carrot. We analysed infected carrot (the fungal pathogen was not identified) and found the furocoumarin content increased to levels sufficient for HPLC analysis (Fig. 2) and also for characterization of individual compounds by mass spectra. Besides 8-MOP and 5-MOP, psoralen was also detected and the phytoalexin response pattern was very similar to patterns observed in celery [13]. Levels for furocoumarins in diseased carrots were: psoralen 0.85 μg/g, 8-MOP 1.55 μg/g wet weight. 5-MOP was also elevated (Fig. 1, $B_2$) but could not be quantified because of interfering impurities with the same retention time (Fig. 2).

Our results indicate that carrots possess biosynthetic pathways for the production of linear furocoumarins and are capable of a phytoalexin response.

## EXPERIMENTAL

*Furocoumarin standards.* The sources of furocoumarin standards have been described previously [14]. Before use, they were checked for purity by TLC, HPLC and photobiological assay and if necessary recrystallized from EtOH.

*Plant material.* Carrot plants were obtained locally from various shops. Known amounts of various carrot tissues were chopped in a blender with $H_2O$ and extracted several times with EtOAc. The samples were further processed as described in ref. [14].

*HPLC and TLC.* Reverse phase HPLC analysis was performed with a Varian MCH-10 column (4 mm × 30 cm) employing MeCN–$H_2O$ as a solvent starting at 27:75. This condition remained constant for 15 min before changing over a period of 5 min to 35:65 and remaining thus for 10 min. The flow rate was

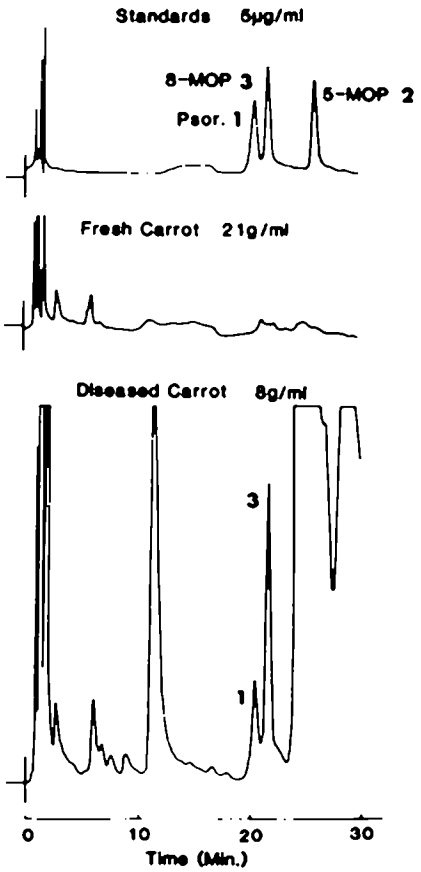



Fig. 2. Reverse phase HPLC analysis of fresh and diseased carrot. Fresh carrot represents 21 g/ml and diseased carrot 8 g/ml. For details see the text.

2 ml/min. Detection was at 254 nm and quantitation was done by peak area integration with a Hewlett–Packard 3390 A integrator. The furocoumarin standards were used at 5 μg/ml. For TLC analysis Merck precoated silica gel K 60 sheets were used (without fluorescent indicator) and two-dimensional chromatograms were developed first, with $CHCl_3$ and second, with hexane pentane–EtOAc (35:35:30). Visualization was performed with UV (300–380 nm) light.

*Photobiological assay.* This ultrasensitive photobiological assay has been described in detail in refs [11, 12]. The DNA repair deficient mutant, *Escherichia coli* $B_s$ (*rec*$^-$, *exr*$^-$, *hrc*$^-$), which is extremely sensitive to ultraviolet radiation and to chemical alkylating agents [15] was used in the soft agar overlay. Commercial nutrient agar was used as a base in square petri dishes. Dried and developed TLCs were imprinted for 30 min onto the surface of base agar prior to the soft agar overlay addition. Irradiation of petri dishes for 2 hr under UV(300–380 nm) light followed. Incubation overnight at 37° completed the bioassay. Controls without UV light irradiation were always set up simultaneously. Inhibition of bacterial growth is shown by a clear zone in the lawn of indicator bacteria.

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