Note

Photoactive Furocoumarins in Diseased Celery (*Apium graveolence*)

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Phytophotodermatitis has recently spread in Japan on the hands and arms of workers in farms producing celery. Several studies have reported the phytophotodermatitis in celery farmers.^{1,2)}

In 1963, Scheel *et al.*³⁾ found that this dermatitis was caused by photoactive furocoumarins produced by diseased celery infected with *Sclerotinia sclerotiorum*; this disease is called pink-rot. Essentially the same results have been reported from the United State and the European countries.⁴⁻⁶⁾

Similar photodermatitis has been found spreading in Japan. This dermatitis has recently developed on the hands and arms of farmers working with celery infected with soft-rot, but not with pink-rot. This soft-rot disease was caused by a bacterium (*Erwinia carotovora*), and spread particularly in the high summer atmospheric temperature and moisture of this country. We have reported that this dermatitis developed on 60% of celery-producing farmers, and that the causative compounds were produced by the soft-rot celery.^{7,8)} This paper describes the isolation and identification of furocoumarins from diseased celery, and their contents in the healthy and diseased plant.

Four UV-absorbing components (compounds 1–4) were purified from an extract of soft-rot diseased celery by silica gel column chromatography and silica gel thin-layer chromatography (TLC). Compounds 1, 2, 3 and 4 were identified as psoralen (1), 5-methoxypsoralen (bergapten, 2), 8-methoxypsoralen (xanthotoxin, 3) and 5,8-dimethoxypsoralen (isopimpinellin, 4) by comparing the ¹H-NMR and the mass spectra of the purified compounds with those of authentic samples.^{9,10)}

The contents of these furocoumarins in healthy and soft-rot diseased celery were measured by gas chromatography (GLC), after chromatographic separation of the components in the extract on thin-layer plates. The weight percentage compositions were calculated from the value with correction factors obtained from the chromatograms

Fig. 1. Structures of Psoralen (1), Bergapten (2), Xanthotoxin (3), Isopimpinellin (4) and Trimethylpsoralen (5).

Table I. FUROCUMARINS IN HEALTHY AND DISEASED CELERY

	Constituents (μg/g of fresh weight) in celery		
	Healthy	Soft-rot ^a	Pink-rot ^b
Psoralen (1)	tr. ^c	0.5	
Bergapten (2)	0.9	2.7	
Xanthotoxin (3)	0.0	3.3	57
Isopimpinellin (4)	0.1	3.0	
Trimethylpsoralen (5)	0.0	0.0	41
Total	1.0	9.5	

^a Infected with bacteria, *Erwinia carotovora*.

^b Infected with fungi, *Sclerotinia sclerotiorum*, as reported by Scheel.³⁾

^c <0.01, detected by TLC and GC-MS.

of standard mixtures of known furocoumarins. Although bergapten (2) and xanthotoxin (3) exhibited the same retention times (*t_R* 9.0 min) on GLC, they were separated by TLC. Each peak was identified by using the relative retention times and by GC-MS data. These results are presented in Table I.

The total amounts of the four furocoumarins were found to be 1.0 μg/g and 9.5 μg/g in fresh healthy celery and in soft-rot diseased celery, respectively. The amounts of psoralen (1) and xanthotoxin (3) were only trace or undetectable in the healthy celery. In the soft-rot celery, psoralen (1) and xanthotoxin were present in 0.5 μg/g and 3.3 μg/g, respectively. Although trimethylpsoralen (4,5',8-trimethylpsoralen, 5) has been found as a highly photoactive material in pink-rot diseased celery,³⁾ we could not detect it in either the healthy or soft-rot diseased celery.

These results indicate that the soft-rot diseased celery contained remarkably high levels of all of the furocoumarin components, although these furocoumarin levels are lower than those observed with pink-rot diseased celery.^{3,5)} An erythema resulted when rabbit skin was in contact with 0.1 μg/sq. in. of psoralen (1) and 1.5 μg/sq. in. of xanthotoxin (3).⁴⁾ Therefore, the furocoumarins in soft-rot diseased celery may be the cause of the phytophotodermatitis of celery-producing farmers in Japan.

Experimental

MS spectra were obtained with a Shimadzu GCMS-7000 spectrometer, and ^1H -NMR spectra were recorded on a Bruker AC-250 instrument, using TMS as the internal standard.

Plant materials. The healthy and diseased (soft-rot infection of *Erwinia carotovora*) celery samples were collected in Nagano prefecture in August (1987).

Isolation of furocoumarins from soft-rot celery. The soft-rot infected celery (750 g of fresh weight) was homogenized and extracted with EtOH (1 l). The extract was concentrated *in vacuo* to afford 10 g of a syrup, which was added to water (100 ml) and CHCl_3 (100 ml). After the organic layer had been separated, the CHCl_3 layer was washed with 3% NaHCO_3 and then with 2N-HCl. The neutral layer was dried over anhydrous Na_2SO_4 , and concentrated *in vacuo* to yield 4 g of a yellow paste. This paste was chromatographed on silica gel, employing benzene-ethyl acetate as the elution solvent. The fraction eluted by 4:1 benzene-ethyl acetate was rechromatographed on silica gel plate, using cyclohexane-ethyl acetate (3:1), and afforded compounds: **1** (0.2 mg), **2** (1.2 mg), **3** (2.0 mg) and **4** (1.2 mg).

Physicochemical data for compound 1. ^1H -NMR δ (CDCl_3): 8.08 (1H, d, $J=9.6$ Hz), 7.89 (1H, d, $J=2.3$ Hz), 7.86 (1H, s), 7.47 (1H, d, $J=0.7$ Hz), 6.83 (1H, dd, $J=2.3$, 0.7 Hz), 6.38 (1H, d, $J=9.6$ Hz). MS m/z : 186 (M^+ , base), 158 ($\text{M}-\text{CO}$), 130 ($\text{M}-2\times\text{CO}$).

Physicochemical data for compound 2. ^1H -NMR δ : 8.16 (1H, dd, $J=9.6$, 0.7 Hz), 7.60 (1H, d, $J=2.4$ Hz), 7.14 (1H, dd, $J=1.0$, 0.7 Hz), 7.02 (1H, dd, $J=2.4$, 1.0 Hz), 6.27 (1H, d, $J=9.6$ Hz), 4.27 (3H, s). MS m/z : 216 (M^+ , base), 201 ($\text{M}-\text{CH}_3$), 188 ($\text{M}-\text{CO}$), 173 ($\text{M}-\text{CO}-\text{CH}_3$), 145 ($\text{M}+2\times\text{CO}-\text{CH}_3$).

Physicochemical data for compound 3. ^1H -NMR δ : 7.77 (1H, d, $J=9.6$ Hz), 7.69 (1H, d, $J=2.2$ Hz), 7.35 (1H, s), 6.82 (1H, d, $J=2.2$ Hz), 6.37 (1H, d, $J=9.6$ Hz), 4.30 (3H, s). MS m/z : 216 (M^+ , base), 201 ($\text{M}-\text{CH}_3$), 188 ($\text{M}-\text{CO}$), 173 ($\text{M}-\text{CO}-\text{CH}_3$), 145 ($\text{M}-2\times\text{CO}-\text{CH}_3$).

Physicochemical data of the compound 4. ^1H -NMR δ : 8.12 (1H, d, $J=9.6$ Hz), 7.63 (1H, d, $J=2.3$ Hz), 7.01 (1H, d, $J=2.3$ Hz), 6.30 (1H, d, $J=9.6$ Hz), 4.17 (6H, s). MS m/z : 248 (M^+ , base), 231 ($\text{M}-\text{CH}_3$), 218 ($\text{M}-2\times\text{CH}_3$), 203 ($\text{M}-\text{CH}_3-\text{CO}$), 188 ($\text{M}-\text{CO}-2\times\text{CH}_3$), 160 ($\text{M}-2\times\text{CO}-2\times\text{CH}_3$).

Quantitative analysis. Gas chromatographic measure-

ments were done on a Shimadzu GC-4A with FID. The glass column (4 mm i.d. $\times$ 1 m) was packed with 3% XE-60 on Uniport HP (60/80 mesh) and was heated at 165–190°C (programmed at 3°C/min). The N_2 carrier gas flow was approximately 30 ml/min.

The healthy and diseased celery stalks (100 g) were homogenized in 500 ml of water, respectively, and extracted with EtOAc (3 $\times$ 500 ml). The combined EtOAc extracts were dried over anhydrous Na_2SO_4 , and concentrated *in vacuo* to afford a paste, which was chromatographed on silica gel by eluting with benzene-ethyl acetate solvent system to give the 10:1 benzene-ethyl acetate fraction. This fraction was chromatographed on a silica gel plate (20 $\times$ 20 cm), developing twice with CHCl_3 . Bands on the developed plates corresponding to two mixtures, psoralen (R_f 0.41) and bergapten (R_f 0.42), and xanthotoxin (R_f 0.34) and isopimpinellin (R_f 0.36), as visualized under UV light, were separated and eluted with ethyl acetate to give two mixtures of a white solid. These respective mixtures were subjected to GLC for a quantitative analysis.

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