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DERMATITIS-INDUCING FURANOCOUMARINS ON LEAF SURFACES OF EIGHT SPECIES OF RUTACEOUS AND UMBELLIFEROUS PLANTS[1]

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Abstract—Eight species of Rutaceae or Umbelliferae, known to cause or suspected of causing photophytodermatitis, had the linear furanocoumarins psoralen, bergapten, and xanthotoxin on their leaf surfaces, in concentrations varying from 0.014 to 1800 μg/g fresh weight, equivalent to 0.17–56% of the total leaf concentration. The higher percentage generally observed for spring leaves compared to autumn leaves suggests a higher rate of transfer of these furanocoumarins to the surface in the younger leaves. Among the plants studied, *Ruta graveolens* had the highest surface concentrations of all three furanocoumarins. The relatively high effectiveness in causing dermatitis of some species with low surface concentrations may be explained by a more effective mechanism of transfer of the furanocoumarins to the skin. A role in the defense of the plant is suggested by their accumulation on the plant surface.

Key Words—Furanocoumarins, plant surface, dermatitis, Rutaceae, Umbelliferae.

INTRODUCTION

Although the existence of furanocoumarins in many species of the Rutaceae and Umbelliferae is well documented (Gray and Waterman, 1978; Murray et al., 1982; Matern et al., 1988), attempts to localize them in these plants have been

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[1] A paper based on the work reported here was presented at the Groupe Polyphénols conference, Brock University, St. Catharines, Ontario, Canada, August 16–19, 1988.

confined to entire organs, with very little attention to specific tissues (Andon and Denisova, 1974; Capalletti et al., 1984). In this laboratory, *Ruta graveolens* L. recently has been the subject of more detailed study in this context (Zobel and Brown, 1988, 1989). This species has long been notorious for inducing contact dermatitis (photophytodermatitis), which arises from the reaction of its linear furanocoumarins (psoralens, **1**) with skin DNA in the presence of UV radiation (Mitchell and Rook, 1979). High surface concentrations of these physiologically active coumarins (Zobel and Brown, 1989) can account for the marked propensity of *R. graveolens* to induce photophytodermatitis.

In the work reported here we have examined other species of the Rutaceae and Umbelliferae known to cause dermatitis (Mitchell and Rook, 1979) or suspected from anecdotal evidence of doing so, with the object of determining to what extent a substantial surface concentration of the three principal dermatitis-inducing psoralens is a general phenomenon in these families.

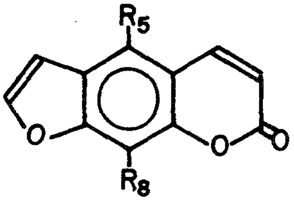
METHODS AND MATERIALS

R. graveolens was cultivated in a garden or greenhouse at Trent University, and *Heracleum lanatum* was collected from the wild state on the campus. The remaining species studied were obtained from the collection of the Royal Botanical Gardens, Hamilton, Ontario.

The procedure for removal of surface deposits by a single brief dipping of the leaves into almost boiling water (ca. 96°C, 100 ml) has recently been described (Zobel and Brown, 1988). In each case duplicate samples of ca. 10 leaves were used. Preliminary examination of the extracts was done by thin-layer chromatography on silica chromatoplates (Macherey Nagel, 0.25 mm thick) developed in ethyl acetate–isooctane, 1 : 3. This was followed by quantitative analysis of psoralens by high-performance liquid chromatography (Thompson and Brown, 1984; Zobel and Brown, 1989) on a 7.5-cm reversed-phase, 4-μm C_{18} column (Waters Nova-Pak) developed with 25% aqueous acetonitrile. Quantitation was by measurement of peak areas and reference to standard curves prepared from reference samples in our collection.

RESULTS

The three linear furanocoumarins predominating in these two families—psoralen (Scheme 1, **a**), bergapten (**b**) and xanthotoxin (**c**)—were detected on the surfaces of all five species of the Rutaceae and all three of the Umbelliferae examined in the present study (Table 1, values for duplicate samples shown in



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a $R_5 = R_8 = H$

b $R_5 = OMe$, $R_8 = H$

c $R_5 = H$, $R_8 = OMe$

SCHEME 1.

each case). However, there was a great variation in the total surface concentrations, amounting to a range of five orders of magnitude, among the different species. They were highest on young spring leaves of *R. graveolens* (ca. 1800 μg/g fresh weight) and lowest on old, autumn leaves of *Evodia danielli* (0.014). In these two plants, as well as in *Orixa japonica* and *Peucedanum officinale*, the total concentrations of the three coumarins were higher on young leaves than on old, but the converse was the case in *Phellodendron chinensis*. Owing to unavailability of material at one or the other season, we could not compare the concentrations in the spring and autumn leaves of three species. The sum of the surface concentrations on spring leaves ranged from 6.7 to 56% of the total in the leaf, and on autumn leaves from 0.17 to 37%.

The three furanocoumarins in the species investigated were found in varying amounts on the leaf surface, with xanthotoxin predominant in many cases (Table 2). The concentration of this coumarin was highest on spring leaves of *R. graveolens* (ca. 880 μg/g fresh weight), with the next highest, *O. japonica*, having ca. 100, followed by *H. lanatum* with only ca. 0.5, differences of >1000-fold. Differences of an even greater order, >10,000-fold, were observed for psoralen, with respective values of 690, 1.6, and 0.06. Bergapten was again found in the highest concentrations on *R. graveolens* (220 μg/g fresh weight), followed by *O. japonica* with 22 and *Peucedanum officinale* with 0.56, a difference of ca. 400-fold.

In only one case (Table 3) were equivalent concentrations of the three

TABLE 1. CONCENTRATIONS OF FURANOCOUMARINS ON LEAF SURFACES OF RUTACEOUS AND UMBELLIFEROUS PLANTS AS PERCENTAGE OF TOTAL LEAF CONCENTRATION

	Leaves, autumn 1987		Leaves, spring 1988	
Species	Surface conc[a]	Average % of total in leaf	Surface conc[a]	Average % of total in leaf
Ruta graveolens	705, 740	37	1850, 1800	56
Orixa japonica	18.4, 19.5	2.8	120, 128	32
Phellodendron chinesis	0.076, 0.086	3.2	0.03, 0.03	17
Evodia danielli	0.018, 0.014	9.8	0.018, 0.024	8.0
Dictamnus albus	0.020, 0.030	0.17	NA	NA
Peucedanum officinale	0.099, 0.093	1.1	0.72, 0.60	6.7
Heracleum mangazzianianum	3.8, 34.	1.9	NA	NA
Heracleum lanatum	NA[b]	NA	0.71, 0.73	8.2

[a] μg/g fresh weight.
[b] NA, no sample available.

TABLE 2. CONCENTRATION OF PSORALEN, XANTHOTOXIN, AND BERGAPTEN ON PLANT SURFACE, AND PERCENTAGE OF EACH OF TOTAL CONCENTRATION IN LEAF

	Psoralen		Xanthotoxin		Bergapten	
Species	On surface[a]	% of total[b]	On surface[a]	% of total	On surface[a]	% of total
Autumn plants						
Ruta graveolens	230, 215	33.5	435, 450	39	55, 59	33
Orixa japonica	traces	NA[c]	14.9, 14.5	3.0	4.3, 3.9	2.5
Phellodendron chinensis	0.009, 0.009	0.98	0.034, 0.030	15.6	0.039, 0.035	2.9
Evodia danielli	0.008, 0.005	8.2	0.007, 0.007	10	0.003, 0.004	20
Dictamnus albus	traces	NA	0.011, 0.010	0.13	0.03, 0.02	0.3
Peucedanum officinale	0.008, 0.007	3.0	0.070, 0.053	1.7	0.028, 0.026	0.55
Heracleum magazzianianum	1.25, 1.20	2.0	2.2, 2.05	1.9	0.28, 0.28	1.5
Spring plants						
Ruta graveolens	750, 630	54	820, 940	56	200, 285	49
Orixa japonica	1.55, 1.70	22	88, 112	31	25, 19.5	39
Phellodendron chinensis	0.01, 0.01	10	0.008, 0.015	15	<0.01	ca. 14
Evodia danielli	<0.01, <0.01	ca. 50	0.01, 0.01	5	<0.01	ca. 21
Peucedanum officinale	0.01, 0.01	2	0.09, 0.07	7	0.50, 0.63	6.6
Heracleum lanantum	0.07, 0.05	9	0.55, 0.41	7.8	0.20, 0.16	9.4

[a] μg/g fresh weight.
[b] Average value.
[c] NA, no sample available.

TABLE 3. RATIOS OF CONCENTRATIONS OF PSORALEN, BERGAPTEN, AND XANTHOTOXIN ON PLANT LEAF SURFACES[a]

	Spring leaves			Autumn leaves		
Species	P	: X	: B	P	: X	: B
Ruta graveolens	2.9	3.7	1	3.9	7.7	1
Evodia danielli	1	1	1	2.0	2.0	1
Phellodendron chinensis	3.0	1	1	0.23	0.82	1
Orixa japonica	1.2	0.02	1	0.001	3.6	1
Dictamnus albus				<0.001	1	1
Heracleum magnazzianianum	4.3	7.5	1			
Heracleum lanatum				0.33	2.7	1
Peucedanum officinale	0.29	2.2	1	0.018	0.14	1

[a] P, psoralen; X, xanthotoxin; B, bergapten.

coumarins observed, i.e., young *E. danielli* leaves. This table shows the proportions of the three, with that of bergapten taken as unity. Bergapten in the spring leaves appeared to be present in low concentrations, but in old leaves it can even predominate, as seen in *Peucedanum officinale* and *Phellodendron chinensis*.

Table 2 shows, as well as the concentration in micrograms per gram fresh weight, the concentration of each surface furanocoumarin as the percentage of its total concentration in the entire leaf. The higher percentage values were observed with spring leaves except for *Phellodendron chinensis*, where the xanthotoxin values for spring and autumn leaves were similar; *E. danielli*, where on autumn leaves the xanthotoxin values were higher; and in *Peucedanum officinale*, where the percentage of psoralen on autumn leaves was, if anything, higher than on the spring leaves. Data from Table 2 permit the inference that even the small concentrations of each coumarin on the surface are a substantial part of the total in the plant. The values in the upper part of the observed range of 0.13–56% indicate that a large part of the furanocoumarins produced within the cells is sent to the surface.

DISCUSSION

Furanocoumarins have long been known to exist in leaves of the species of Rutaceae and Umbelliferae examined here (Murray et al., 1982), but their localization had not been previously studied. In this work we have located psoralen, xanthotoxin, and bergapten on the surface of the leaves, to a substantial degree in many cases, and not only inside.

In the case of waxes, leaves can be washed with organic solvents for 10–

45 sec (Tulloch 1987; Tulloch and Hoffman, 1982) without concern about leakage, as these substances are known to exist on the surface only. However, for removal of furanocoumarins from *R. graveolens* leaves, we found organic solvents unsuitable (Zobel and Brown, 1989); methanol, for example, which is used for washing out flavonoids (Proksch et al., 1986), is well known as a tissue fixer that rapidly penetrates and denatures proteins, "fixing" the membranes. Concerned about such drastic action of methanol and acetone, we compared their ability to remove furanocoumarins with that of a very short extraction into almost boiling water. Amounts released into this last solvent were of the order of hundreds of times as high, or in some cases even higher, without leakage from the epidermal cells (Zobel and Brown, 1988), and corresponded in the present experiments to surface concentrations ranging from 0.17 to 56% of the total concentrations in the leaf. These values may still represent only minima—the smallest amounts obtainable after a single brief dipping into hot water, which at least partially removes an overcuticular layer. Further experiments are in progress to determine whether a longer dipping time will remove yet more material without leakage from the epidermal cells.

The total furanocoumarin concentration (psoralen + xanthotoxin + bergapten) differs in different species. *R. graveolens* has a remarkably high furanocoumarin concentration on the surface, even compared to the 28% of the total flavonoid concentration on the leaf surface of *Flourensia resinosa* (Wollenweber, 1986). As no mechanism for the synthesis of coumarins can exist on the surface, concentrations of all three furanocoumarins ranging between 700 and 1800 μg/g fresh weight on the surface indicate that this plant must export large amounts from the interior. The sum of the surface concentrations in *R. graveolens* was higher than that in the whole leaf of *Orixa*, which contains the next highest concentrations. Those of the three individual furanocoumarins are not equal (Table 3): xanthotoxin predominates in many cases, except for *Phellodendron* and *Peucedanum* (autumn leaves), where there is more bergapten, and in *Phellodendron* and *Orixa* (spring leaves), where the psoralen concentration is highest. The variation of proportions in autumn and spring leaves suggests developmental changes, and these variations are now being investigated in *R. graveolens*, *R. chalepensis*, and *H. lanatum* over more extended periods.

A point of importance is that even the small concentrations of furanocoumarins on the leaf surface, both individually and in total, constitute a significant percentage of the whole concentration of the plant leaf. This suggests active transport of these substances to the surface, where, as we have already discussed for *R. graveolens* (Zobel and Brown, 1989), they could play a defense role, forming a coating over the cuticular layer (see also the discussion below). Xanthotoxin is recognized as a plant toxicant (Berenbaum and Neal, 1985).

A further question of interest is why plants causing dermatitis do not contain approximately the same concentrations of furanocoumarins on the surface.

When two of the plants causing photophytodermatitis, *R. graveolens* and *H. mangazzanianum*, are compared, we note that the surface concentrations of furanocoumarins are drastically different: 1800 and 3.6 μg/g, respectively. The phenomenon could be at least partially explained by postulating different mechanisms of transferring these toxins to the skin. *R. graveolens*, having a smooth, hairless surface, could induce dermatitis when the grayish waxy cover is removed by touching. In contrast, *H. mangazzianianum* possesses erect trichomes with a very thick, strong cell wall and sharp tips that could mechanically penetrate the upper layer of the skin and carry their furanocoumarins beneath the surface. In this way smaller amounts on the surface of the plant could produce comparable levels of irritation.

Dictamnus albus seem to be more of a threat after the leaves have been crushed or when there are breaks in the skin. More studies are needed to determine if *D. albus* can induce contact dermatitis and if, at the concentration there (0.025 μg/g), it has an antibiotic function. In our laboratory, furanocoumarins in concentrations found on *R. graveolens* leaves (and even lower by a factor of 10) have been shown to play an antibacterial role, and they decreased mitoses in root tips of *Allium cepa* (A.M. Zobel and K. Mwiraria, unpublished).

In our experiment, substances other than the three furanocoumarins, exhibiting a blue-green fluorescence, were also extracted into water. As demonstrated by Cesca et al. (1986) in the case of *Daucus carota*, such a complex of various surface substances, including a furananocoumarin, can act as an insect attractant. We consider it possible that such complexes of surface compounds could serve as a defense barrier for the plant, not only at the actual surface but comparable on a microscale to the atmosphere around the surface of the Earth. For example, as furanocoumarins are readily sublimable, slow continuous sublimation under natural conditions could give such an atmosphere adjacent to the plant surface that is toxic to approaching bacteria or fungal spores. In this context we hope that workers elsewhere will be interested in identification of other compounds extracted from plant surfaces by our procedure.

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