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UV-MEDIATED ANTIBIOTIC ACTIVITY OF SOME COMPOSITAE SPECIES

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Abstract—Roots, leaves, and flowers of 80 species of Compositae were tested for phototoxic activity against *Candida albicans*. Many genera showed activity, especially in the roots. No active genera were found in the tribe Cichorieae. Phototoxic compounds were isolated from *Chrysanthemum leucanthemum* florets and *Cirsium arvense* roots. Chemotaxonomic evidence plus preliminary chemical data suggests that the compounds are polyacetylenic in nature. Unlike other phototoxic compounds, these are inactive against human skin.

INTRODUCTION

Certain furanocoumarins, such as psoralen, when applied to human skin cause damage in the presence of long-wave UV light (320–370 nm), but not in the dark [1]. This phototoxicity has been well examined with plants and with chemicals obtained from the families Rutaceae, Leguminosae and Umbelliferae (and a few others), which are rich in furanocoumarins [2]. A simple test for phototoxic activity involves placing a small part of the test plant on an agar plate spread with the yeast, *Candida albicans* [3]. Plant materials which cause a halo of dead cells around them after incubation under UV, but not after dark incubation, are termed phototoxic.

Daniels examined a number of plants for phototoxicity towards *Candida* and found that the fruit of marigold (*Tagetes*) gave a positive test [3]. We were curious about this because furanocoumarins have never been reported from any of the Compositae which contains several important horticultural species, many of which are known to contain cutaneous allergens [4]. The effects of sunlight have been reported to aggravate dermatitis from such plants [5]. We have, therefore, screened members of this family for phototoxic effects on *Candida* and carried out preliminary experiments on guinea-pig and human skin.

RESULTS AND DISCUSSION

Many composite species were found to possess phototoxic activity in the *Candida* test (Table 1), in many cases the roots being the most active. The only tribe of the Compositae that showed no phototoxic activity in any of the species tested was the Cichorieae. *Achillea millifolium* (Anthemideae) has been reported to evoke phytophotodermatitis [2] on dubious chemical evidence. This activity could not be confirmed experimentally [6] and the plant was only marginally active in our test. Since furanocoumarins are not known from Compositae, two active plants were selected for further analysis; *Chrysanthemum leucanthemum*, in which the immature disc florets showed significant activity (this activity was localized in the immature achene, and was exhibited only in sliced or bruised achenes) and *Cirsium arvense*, the roots of which have been recommended as a wild food [7].

The phototoxic fractions from *Chrysanthemum leucanthemum* floret extracts were chromatographed and tested against *Candida*. The areas of growth inhibition correlated with two UV-absorbing spots. The UV spectrum of the more highly phototoxic compound suggested that it was a polyacetylene (Fig. 1). This compound, when scraped, eluted and re-run, yielded another

Table 1. Phototoxic activity to *Candida albicans* of some Compositae species

Species	Collection Date	Place*	Activity in lvs	stm	rt	fl, ach
Anthemideae						
Achillea millefolium L. ssp. *lanulosa* (Nutt.) Piper	July 27	Vancouver	–	–	–	–
	Aug 21	Kamloops	–	–	±	–
Anacyclus depressus Ball. (5370-175-73)	Sept 27	Vancouver	–	–	–	nt
Anthemis cotula L.	July 9	Vancouver	–	–	–	–
Artemisia frigida Willd.	Aug 21	Kamloops	–	–	–	–
A. sp. (Bot. Gar.)	Aug 14	Vancouver	–	–	–	–
Chrysanthemum leucanthemum L.	July 4	Vancouver	–	–	–	+ imm
	July 9	Vancouver	–	–	–	+ imm
ssp. *montanum* (6120-192-73)	Sept 27	Vancouver	–	–	–	nt
C. maximum Ramond. (?)	July 22	Vancouver	–	–	–	+ imm
Matricaria matricariodes (Less.) Porter	July 9	Vancouver	–	–	–	+ imm
	July 30	Vancouver	anti	–	–	–
Santolina chaemycyparissus L. (7329-117-72)	Sept 27	Vancouver	–	–	+	nt
Tanacetum vulgare L.	July 23	Vancouver	–	–	–	–
	July 30	Vancouver	nt	nt	nt	–
Astereae						
Chrysopsis villosa Nutt.	July 5	Kamloops	–	–	–	–
Erigeron filifolius Nutt.	July 5	Kamloops	–	–	+	–
E. compositus Pursh. (4406-52-72)	Sept 27	Vancouver	–	–	anti	–
E. linearis (Hook.) Piper	July 5	Kamloops	–	–	+	–
E. strigosus Muhl var. *strigosus*	Sept 10	Vancouver	+	–	+, anti	anti, +
E. sp.	July 5	Kamloops	–	–	+	–
E. sp.	July 22	Vancouver	–	–	–	–
E. sp.	July 30	Elk Ridge	–	nt	+	–
E. sp.	Aug 21	Kamloops	–	–	–	–
E. sp.	Aug 21	Kamloops	–	–	+	–
Chrysothamnus nauseosus (Pall.) Britt. var. *albicaulis* (Nutt.) Rydb.	Aug 21	Kamloops	–	–	–	–
Grindelia integrifolia DC var. *macrophylla* (Greene) Cronq. (dried root inactive)	Sept 4	Kamloops	–	–	+	–
Olearia haastii Hook. (5880-055-73)	Sept 27	Vancouver	–	–	–	nt
Solidago canadensis L. var. *subserrata* (DC.) Cronq.	Aug 25	Vancouver	–	nt	–	–
S. sp.	July 22	Vancouver	–	–	–	–
Cichorieae						
Agoseris aurantiaca (Hook.) Greene	July 30	Mt. Seymour	–	–	–	–
A. sp.	Sept 4	Kamloops	–	–	–	–
Cichorium intybus L.	Aug 25	Vancouver	–	–	–	–
Crepis atrabarba Heller	July 5	Kamloops	–	–	–	–
Hieracium aurantiacum L.	July 22	Vancouver	–	–	–	–
H. albiflorum Hook.	July 30	Elk Ridge	–	nt	–	–
H. gracile Hook.	July 30	Elk Ridge	–	nt	–	–
	Aug 25	Vancouver	–	–	–	–
H. umbellatum L.	July 30	Elk Ridge	–	nt	–	–
Hypochaeris radicata L.	July 27	Vancouver	–	–	–	–
Lactuca sativa	Aug 25	Vancouver	–	–	–	nt
Lactuca (endive)	Aug 25	Vancouver	–	–	–	–
Lapsana communis L.	July 23	Vancouver	–	–	–	–
Sonchus asper (L.) Hill	Aug 25	Vancouver	–	–	–	–
Taraxacum officinale Weber	Aug 25	Vancouver	–	nt	–	nt
Cynareae						
Arctium minus (Hill.) Bernh.	Aug 21	Kamloops	–	–	–	–
Carduus nutans L.	Aug 13	Virginia	–	–	–	–
Centaurea diffusa Lam.	Aug 13	Virginia	+	–	+	–
C. maculosa Lam.	July 22	Vancouver	–	–	+	–
	Aug 13	Virginia	+	–	+	–

Table 1 (*cont'd*)

Species	Collection Date	Collection Place*	Activity in lvs	Activity in stm	Activity in rt	Activity in fl, ach
C. nigra L.	Aug 13	Virginia	+	+	+	+, anti
Cirsium arvense (L.) Scop. var *horridum* Wimm. & Grab.	July 14	Vancouver	–	–	+	–
	Aug 21	Kamloops	–	–	+	–
C. vulgare (Savi) Tenore	July 22	Vancouver	–	–	+	–
Eupatorieae						
Eupatorium fistulosum Barratt.	Aug 13	Virginia	–	–	–	–
Helenieae						
Eriophyllum lanatum (Pursh) Forbes. var. *integrifolium* (Hook) Smiley	July 30	Elk Ridge	–	–	+	–
(7708-156-73)	Sept 27	Vancouver	–	–	+, anti	–
Helenium autumnale L. var *grandiflorum* (Nutt.) T. & G. (cult.)	Aug 25	Vancouver	nt	nt	nt	–
Tagetes minutus L. (dried)			+	+	nt	†
Gaillardia aristata Pursh.	July 5	Kamloops	–	–	+	–
	Aug 21	Kamloops	–	–	–	–
(cult)	Aug 13	Vancouver	–	–	–	–
Heliantheae						
Bidens amplissima Greene	Sept 29	Vancouver	–	–	+	–
Dahlia variabilis	Aug 25	Vancouver	+	–	+	+ imm
Galinsoga ciliata (Raf.) Blake	Aug 30	Vancouver	–	–	–	–
Helianthus annuus L. (cult)	Aug 29	Vancouver	–	–	–	–
(wild)	Sept 4	Kamloops	–	–	–	–
H. tuberosus L.	Aug 25	Vancouver	–	–	–	nt
Heliopsis helianthoides (L.) Sweet	Aug 13	Virginia	–	+	+, anti	+
Rudbeckia hirta L.	Aug 13	Virginia	–	+	+, anti	+
R. laciniata L.	Aug 13	Virginia	–	+	+	+
Madia glomerata Hook.	Aug 23	Vancouver	–	nt	+	+ (achenes)
(dried)	Aug 25	Vancouver	–	–	–	–
Inuleae						
Anaphalis margaritacea (L) B. & H.	July 22	Vancouver	–	–	–	–
Antennaria rosea (*A. microphylla* Rydb.)	July 5	Kamloops	–	–	–	–
Gnaphalium microcephalum Nutt.	July 22	Vancouver	–	–	–	–
G. uliginosum L.	Aug 31	Vancouver	–	–	–	–
Inula orientalis (3642-143-72)	Aug 13	Vancouver	–	–	+	–
Leontopodium alpinum Cass. (5543-175-73)	Sept 27	Vancouver	–	–	–	nt
Mutisieae						
Gerbera nivea Sch. Bip. (5538-175-73)	Sept 27	Vancouver	–	–	–	nt
Senecioneae						
Adenocaulon bicolor Hook.	July 30	Elk Ridge	–	–	–	–
Arnica cordifolia Hook.	July 5	Kamloops	–	–	–	–
A. latifolia Bong.	July 30	Elk Ridge	–	nt	+	–
	Aug 25	Mt. Seymour	–	–	+	+
A. millis Hook.	July 30	Elk Ridge	–	nt	+	–
(7932-167-74)	Sept 27	Vancouver	–	–	+, anti	nt
A. sororia Greene	July 5	Kamloops	–	–	–	–
Doronicum austriacum Jacq. 3976-148-72	Sept 27	Vancouver	–	–	–	nt
Senecio integerrimun Nutt.	July 5	Kamloops	–	–	–	–
S. vulgaris L.	July 23	Vancouver	–	–	–	–
Vernonieae						
Vernonia novaboracensis Willd.	Aug 13	Virginia	–	–	–	–
Stokesia laevis (Hill) Greene (3648-134-72)	Aug 13	Vancouver	–	–	–	–

* *Vancouver* indicates collection sites within urban Vancouver, Canada. *Mt. Seymour* and *Elk Ridge* are alpine sites in the Fraser Valley, British Columbia, Canada. The numbers following some plant names indicate that these plants were grown in the Botanical Garden, University of British Columbia, under these acquisition numbers. Other plants were collected near Kamloops, B.C. and near Radford, Virginia, U.S.A.

† Brown fls +; yellow fls –; achenes +, with pappus esp active.

Abbreviations used: lvs, leaves; stm, stem; rt, root; fl, floret parts; ach, achene; imm, immature achene; + = positive; – = negative; nt, not tested; anti, antibiotic activity.

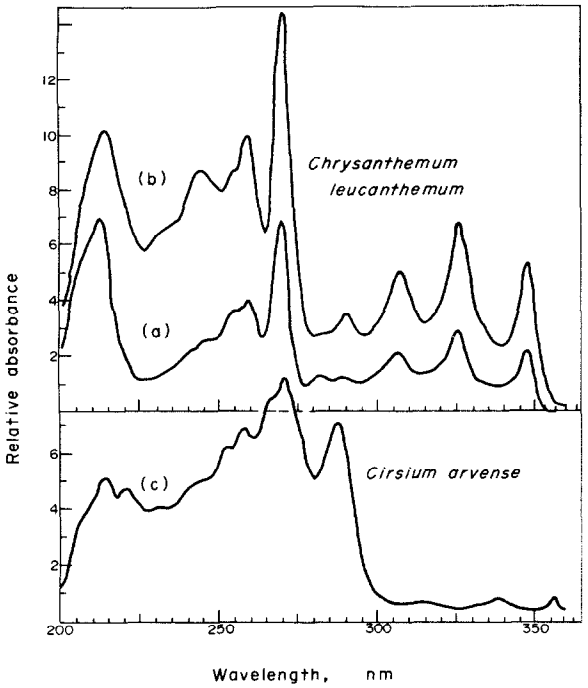

Fig. 1. Absorption spectra of phototoxic compounds from *Chrysanthemum* and *Cirsium*: (a) and (b)—major and minor phototoxic compounds, respectively, from *Chrysanthemum leucanthemum* florets; (c)—compound from *Cirsium arvense* roots.

phototoxic compound, similar to the less toxic compound noted in the original chromatograms. The spectrum of this compound was very similar to that of the first (Fig. 1). When crystallization of the more highly toxic compound was attempted, dark brown crystals were obtained, presumably of a breakdown product since there was considerable loss in activity. The phototoxic fraction from *Cirsium* roots showed a single phototoxic, UV-absorbing compound (Fig. 1) when the chromatograms were tested on *Candida* plates.

The circumstantial evidence of the UV spectra and decomposition to coloured insoluble compounds leads us to suspect that the phototoxic compounds from the two plants are polyacetylenes. The UV spectrum of the first compound from *Chrysanthemum* is very similar to that of $H_3C[C\equiv C]_3$–$[CH=CH]_2$–$(CH_2)_3$–OAc, and a congener $H_3C[C\equiv C]_3$–$[CH=CH]_2$–$(CH_2)_2$–OAc is recorded in leaves of *Chrysanthemum* [8]. Since the Compositae are rich in such compounds, it is also possible that most of the other phototoxic compounds are also polyacetylenic. It may be noted that the tribe Cichorieae, poor in such compounds [8], is also poor in phototoxic representatives.

However, a comparison of the list of photoactive species (Table 1) and of those reported to contain acetylenic compounds [8] reveals no direct correlation. It seems unlikely, for example, that the sole acetylene listed from *Cirsium arvense*, $H_2C[C\equiv C]_5$–$CH=CH_2$, is the active compound, since it has also been found in the inactive *Doronicum austriacum*. As in many other cases, the picture is probably confused by biochemical races and differences in composition or concentration at the time of testing.

The mechanism of phototoxic action of these compounds is unknown, but does seem to be different to that of the polyene antibiotics. Sterols are effective in 'protecting' fungi from the effects of these antibiotics, which interfere with membrane structure [9]. However, when plates of nutrient agar were coated with cholesterol (10 $\mu g/cm^2$) before the addition of *Candida*, there did not seem to be any protection of the yeast from the phototoxic activity. The acetylenes were without activity when applied to human or guinea-pig skin. The *Cirsium* compound caused reddening of guinea-pig skin, but only in the dark, suggesting that it was destroyed in the light. Their phototoxic action is probably different from that of the furanocoumarins, which complex with the thymine of skin DNA, and cause damage in the presence of light of 320–370 nm [1]. These experiments point out that the *Candida* test cannot be used by itself alone to distinguish compounds phototoxic to human skin [10], although it may point the way to other interesting biological phenomena or perhaps to new antibiotics.

EXPERIMENTAL

In vitro *assay*. Pieces of rinsed plant material were placed on agar plates of Sabaroud's medium which had been spread with *Candida albicans* (UBC 53) according to the method of Daniels [3]. Care was taken to ensure that cut edges were present on all leaves, flower parts bruised or cut, tap roots sliced and fibrous root fragments bruised. The plates were incubated at room temp. under a Westinghouse UV lamp (F 15T8-BL; maximum light output at 350 nm) for at least 12 hr. A duplicate plate was incubated in the dark at room temp. Plants which caused a greater area of clearing after light incubation than after dark were termed phototoxic. Those plants which caused the killing of *Candida* in both light and dark were termed antibiotic.

Investigations of the phototoxic compounds. Chrysanthemum leucanthemum. Disc florets (100 g) were frozen with solid CO_2, and ground to a powder with a mortar and pestle. The powder was allowed to thaw at 4° in 300 ml of EtOH. The material was filtered and solids re-ground in 100 ml more EtOH, then in 200 ml Et_2O. Filtrates were combined, filtered and evapd to a tar. Tar was triturated in 2 5-ml aliquots of petrol (65–100°). The solid residue was found to be inactive. 1·5 ml of the extract was separated on a column of neutral alumina (activity grade 1) plus 10% Celite developed with 3% Me_2CO in petrol. A band with strong UV absorption (366 nm) showed phototoxic activity. Several other fractions showed antibiotic activity. The UV absorbing band was concentrated and applied to Eastman Chromagram sheets (Si gel without fluorescent indicator). The strips were developed in 3 solvent systems; system I, C_6H_6–Me_2CO, 9:1; system II, toluene–petrol 1:1; and system III, petrol–Me_2CO, 9:1. The UV absorbing areas were marked with pencil, and each series of separated compounds was cut from the sheet and cut longitudinally. One half of each strip was placed face down on a *Candida* plate under UV, and the other strip was placed on a plate incubated in the dark. Major areas of clearing appeared around spots of R_f 0·65, 0·45 and 0·45 in systems I, II and III, respectively. Minor clearing appeared at R_f 0·82, 0·75 and 0·94 in systems I, II and III respectively.

Cirsium arvense. *C. arvense* roots (245 g) were washed and ground with hot EtOH. The hot extract was evaporated to dryness and tarry residue triturated with petrol (65–100). The material remaining was taken up in H_2O, and extracted continuously for 4 hr with Et_2O. The Et_2O extract was taken to dryness and dissolved in petrol. The petrol solns were pooled and chromatographed on a Si gel–Celite column as above. Phototoxic activity was present in several fractions, but when petrol alone was used as eluant, most of the activity came off in a single yellow UV absorbing band. This band was chromatographed on Si gel chromagrams and tested on *Candida* plates.

Experiments with mammalian skin. The 2 compounds from *Chrysanthemum* and the one from *Cirsium*, in concentrations active against *Candida*, were applied in duplicate to the shaved back of white guinea pigs. Half the spots were masked while the skin of the animals was exposed to UV radiation for 45 min (Westinghouse No. F 15T8 BL) at a distance of 18–20 cm. The compound from *Chrysanthemum* was applied to the skin of the back of 3 white adult human males (10–50 μl of a soln with an OD 269 of 17). Immediately after application, and 24 hr after application in a 2nd expt, the skin was exposed to light from 6 Westinghouse lamps (20 W F 2 OT12) at 39 cm for 1 hr.

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REFERENCES

1. Pathak, M. A. and Kramer, D. M. (1969) *Biochim. Biophys. Acta* **195,** 197.
2. Pathak, M. A., Daniels, F. and Fitzpatrick, T. B. (1962) *J. Invest. Dermatol.* **39,** 225.
3. Daniels, F. (1965) *J. Invest. Dermatol.* **44,** 259.
4. Mitchell, J. C., Fritig, B., Singh, B. and Towers, G. H. N. (1970) *J. Invest. Dermatol.* **54,** 233.
5. Lonkar, A., Mitchell, J. C. and Calnan, C. D. (1974) *Trans. St. John's Hospital Derm. Soc.* **60,** 43.
6. Van Dijk, E. and Berrens, L. (1964) *Dermatologica (Basal)* **129,** 321.
7. Clark, L. J. (1973) *Wild Flowers of British Columbia,* Gray's Sidney, B.C., Canada.
8. Bohlmann, F., Burkhardt, T. and Zdero, C. (1973) *Naturally Occurring Acetylenes.* Academic Press, London.
9. Fowlks, E. R., Leben, C. and Snell, J. F. (1967) *Phytopathology* **51,** 246.
10. Muller, R. and Mitchell, J. C. (1971) *J. Invest. Dermatol.* **39,** 340.