

Contact hypersensitivity to sesquiterpene lactones in *Chrysanthemum dermatitis*

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In six persons with contact allergy to chrysanthemum of florists, patch tests were performed with 25 sesquiterpene lactones. Positive responses were observed to 15 lactones. The most frequently encountered positive responses were to alantolactone (positive in all cases) and to arbusculin A, 8-deoxycumambrin, ambrosin, damsine and psilostachyin. The findings corroborate the assumption that an α -methylene group attached to the γ -lactone ring is an essential prerequisite for allergenic activity of sesquiterpene lactones. Pyrethrum produced a positive reaction in one of the patients, pyrethrins gave negative reactions in all. Some of the chrysanthemum patients were also hypersensitive to turpentine and colophony and to garlic, primin and tars.

Key words: chrysanthemum of florists – colophony – plant dermatitis – pyrethrum – sesquiterpene lactones – turpentine.

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The chrysanthemum is one of the most common causes of allergic contact dermatitis among florists and horticulturists (Hausen & Schulz 1973). Previously we studied nine patients with strong skin reactions to chrysanthemum of florists (*Chrysanthemum x morifolium*, Ram or *Chrysanthemum indicum* L, Compositae family of plants) and found that the highest skin reactivity was present in alcohol extracts of leaves and flowers (Bleumink et al. 1973). All cultivars assayed thus far, and obtained both from western-European and Japanese horticulturists, were found to be skin reactive. Six out of seven patients with a chrysanthemum allergy showed positive skin responses to a sesquiterpene lactone, alantolactone (Fig. 1). Two

out of four also reacted upon challenge with cumambrin A (Fig. 2, formula 11).

Sesquiterpene lactones also were found to be immunologically active in two chrysanthemum sensitive patients studied by Mitchell and his co-operators (1970, 1974).

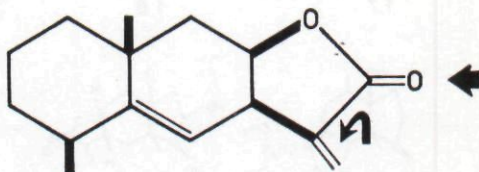


Fig. 1. Formula of alantolactone. A common feature of allergenic sesquiterpene lactones is the γ -butyrolactone ring ($\rightarrow$) with an exocyclic α -methylene group ($\curvearrowright$).

Schulz et al. (1975) performed patch tests with parthenolide and alantolactone in five patients allergic to chrysanthemum of florists. All patients showed positive tests to

parthenolide and two out of five to alantolactone. No positive reactions were found to pyrethrins (constituents with insecticidal properties, present in pyrethrum products

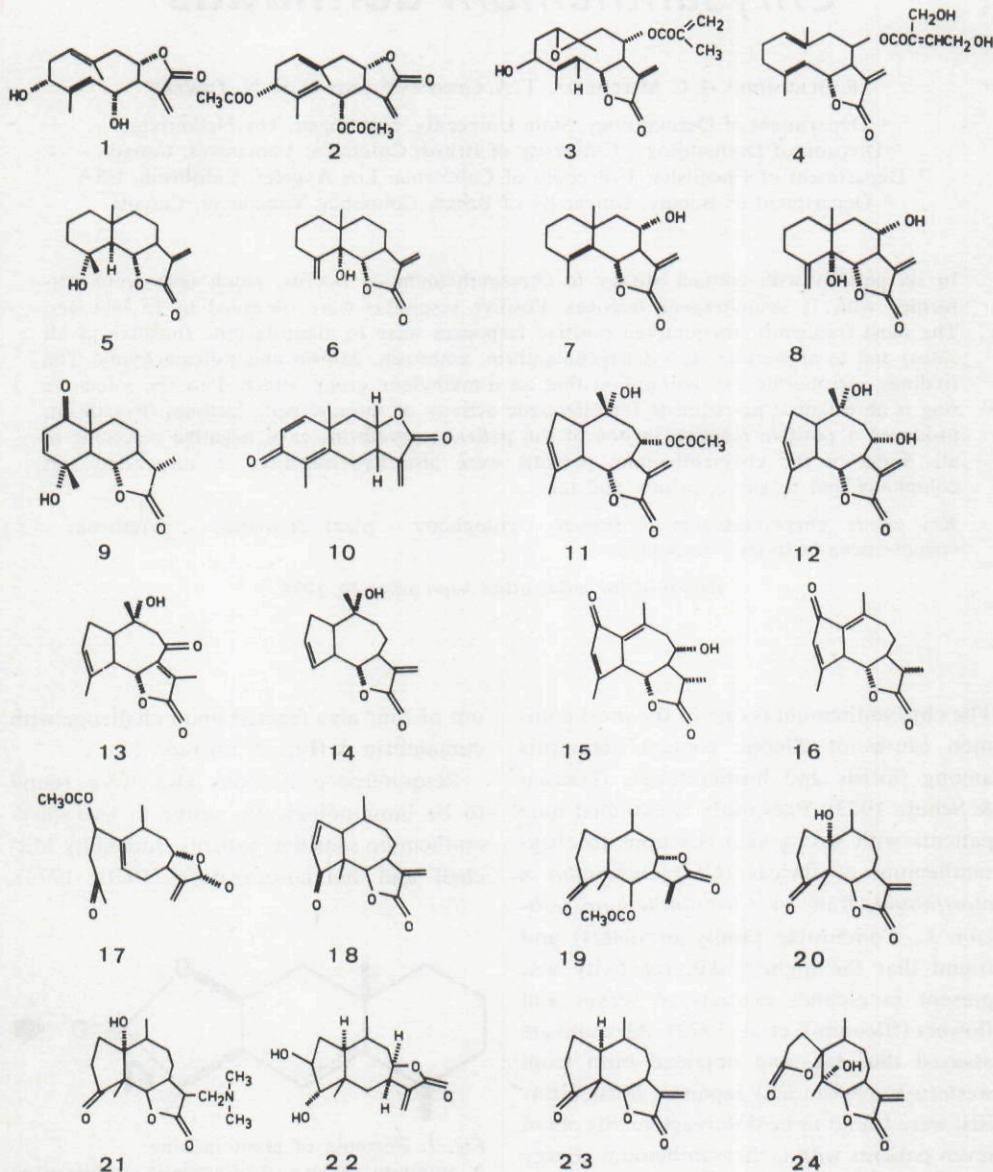


Fig. 2. Formulae of sesquiterpene lactones, tested in 6 patients allergic to chrysanthemum. The numbers correspond to those in Table 2.

and derived from certain *Chrysanthemum* species), to oil of turpentine and to a series of oxydation products of terpenes.

In this communication we report the results of patch tests with a series of 25 sesquiterpene lactones in six persons with a contact allergy to chrysanthemum. These lactones represent the different classes of potentially allergenic components which are present in many members of the Compositae family of plants (Mitchell et al. 1970, 1972b).

Materials and Methods

Patch tests were performed according to methods of the International Contact Dermatitis Research Group (Bandmann & Freigert 1973). On the patches (Scandinavian silver patch test units from van der Bend, Brielle, The Netherlands) 10 μ l of the test substances was applied. The reactions were read after 48 h by two investigators.

A total of 25 sesquiterpene lactones were assayed (Table 2 and Fig. 2). The relevant data concerning these lactones has been given earlier (Mitchell et al. 1970, 1972b). All the substances, except alantolactone, were isolated and purified in the Department of Chemistry, University of California, Los Angeles, USA and all but one proved homogeneous after extensive chemical analysis. Deacetomatricarin (Fig. 2, 16) was found to contain small amounts of an α -methylene-butyrolacton derivative. Alantolactone was obtained commercially (Sigma, St. Louis, USA) and was purified to remove iso-alantolactone by means of column chromatography after the methods described by Dupuis et al. (1974). The particular components were assayed as 0.3 % solutions in 96 % ethyl alcohol (30 μ g of the substances being applied).

In addition, patch tests were performed with a 5 % ethanolic solution of pyrethrum (Aagrunol Groningen, The Netherlands). It

Table 1. Data of patients tested with sesquiterpene lactones

Patient	Age	M/F	Occupation	Symptoms	Patch tests with mixture of chrysanthemums	Other allergens positive (besides chrysanthemums)
1.	59	M	carpenter	hand eczema	2+	various woods, bichromate, turpentine, colophony
2.	47	F	housewife	eczema of hands & face	2+	p-phenylenediamine, colophony, garlic, wood tar, coal tar
3.	44	F	housewife	eczema of face & fingers	3+	p-phenylenediamine, coal tar
4.	61	F	housewife	eczema between fingers	2+	colophony, primin, garlic, coal tar
5.	38	M	floriculturist	hand eczema (particularly between fingers)	2+	phenegan
6.	60	M	store-keeper	hand eczema	3+	turpentine, garlic, primin, colophony, balsam of Peru, wood tar

Table 2. Results of patch test with sesquiterpene lactones

		Patch test results						Presence of α -methylene group attached to lactone
		Patient						
		1	2	3	4	5	6	
Number in Fig. 2								
<i>Class Germacranolide</i>								
Chamissonin	1	—	—	—	—	—	—	Yes
Chamissonin diacetate	2	+	—	—	—	—	—	Yes
Erioflorin	3	—	—	—	—	—	—	Yes
Eupatoriopicrin	4	—	++	—	+	—	—	Yes
<i>Class Eudesmanolide</i> (= <i>Santanolide</i>)								
Arbusculin A	5	—	+	+++	+	+	++	Yes
Arbusculin C	6	—	—	+++	—	—	++	Yes
Rothin A	7	—	—	+++	—	—	++	Yes
Rothin B	8	—	—	—	—	—	—	Yes
Vulgarin	9	—	—	—	—	—	—	No
Yomogin	10	—	—	+++	—	+	—	Yes
Alantolactone	(Fig. 1)	+	++	+++	+	++	++	Yes
<i>Class Guaianolide</i>								
Cumambrin A	11	—	+	—	+	—	—	Yes
Cumambrin B	12	—	—	+++	+	—	—	Yes
Dehydrocumambrin B	13	—	—	—	—	—	—	No
8-Deoxycumambrin B	14	—	+	+++	+	++	++	Yes
Deacetylmatricarin	15	—	—	—	—	—	—	No
Deacetomatricarin ¹	16	—	—	+++	—	—	—	No
Xanthinin	17	—	—	++	—	—	—	Yes
<i>Class Pseudoguaianolide</i> (= <i>Ambrosanolide</i>)								
Ambrosin	18	—	+	+++	++	—	—	Yes
Dihydrobigelovin	19	—	—	—	—	—	—	Yes
Coronopilin	20	—	—	—	—	—	—	Yes
Derivative of coronopilin	21	—	—	—	—	—	—	No
Cumanin	22	—	—	—	+	—	—	Yes
Damsin	23	—	+	+++	++	—	—	Yes
Psilostachynin	24	—	+	+++	+	—	—	Yes

¹ Contains small amounts of α -methylene-butyrolacton derivative.

is derived from the flowers of *Chrysanthemum cinerariaefolium* Vis. and *Chrysanthemum coccineum* Willd. and is known as Dalmatian or Persian insect powder. Pyrethrins II (Pyrethrum Marketing Board, Ke-

nya) were assayed as 5 % solution in 96 % ethanol (Mitchell et al. 1972a).

All individuals were tested with a series of common contact allergens (Bandmann & Fregert 1973) and with a mixture of 10 %

ethanolic extracts of the leaves of four Dutch chrysanthemum varieties (Bleumink et al. 1973).

Tests were performed in seven patients who showed positive reactions to an extract of chrysanthemum of florists. One of the chrysanthemum reactors proved to have status eczematicus and therefore was deleted from the series. The relevant data of the remaining six patients are recorded in Table 1. In addition, tests were done in 13 controls, who were negative to a chrysanthemum extract. The ages of the controls ranged from 25 to 71 years (mean 47.5 years). They had eczema of their hands and showed one or more positive tests to common contact allergens.

Results

Table 1 records the data of the patients with chrysanthemum contact allergy. In all patients a direct relationship could be demonstrated between the occurrence of eczema and contact with chrysanthemums. Two out of six showed positive skin responses to turpentine (peroxides), four to colophony and to tars respectively, three to garlic and two to primin. Statistical analyses revealed that these overlaps of patch test reactions are highly significant ($p \leq 0.001$ with chi square tests) compared with a group of 412 persons patch-tested with the same series of common allergens in the same period the chrysanthemum patients were challenged. Pyrethrum powder elicited skin responses in one of the chrysanthemum patients and in one of the 13 controls, whereas no positive reactions to pyrethrins were noted.

A total of 25 sesquiterpene lactones were assayed, of which 15 were found to produce positive reactions in one or more patients (Table 2). The most frequently encountered positive responses were to alantolactone (positive in all patients), 8-deoxy-

cumambrin B, arbusculin A, ambrosin, damsin and psilostachynin. The pattern of reactivities found differed from patient to patient. Tests performed in controls were negative except that alantolactone elicited a one plus reaction in the same individual who responded to pyrethrum.

The results of the studies of some 50 sesquiterpene lactones (out of 260 chemically identified lactones) derived from the plants of the Compositae family have indicated that the presence of an exocyclic α -methylene group conjugated to the γ -lactone ring is a necessary condition for immunological activity by this group of compounds (Mitchell et al. 1970, 1971a, 1972b). In Table 2, therefore, a comparison is made between skin reactivity and the presence of such a group. In 20 of the sesquiterpene lactones tested, this group was present, of which 14 (70 %) were found to produce positive reactions. Cumambrin A (formula eleven in Fig. 2), cumambrin B (12) and 8-deoxycumambrin B (14) proved skin reactive, while dehydrocumambrin (13) did not give rise to skin responses in these patients. Thus, hydrogenation of the α -methylene group completely abolished the allergenicity for sensitized individuals. One out of five lactones without an α -methylene group elicited a positive reaction in one patient. This component, however, proved to contain small traces of an α -methylene-butyrolactone derivative. Therefore it cannot be excluded that the skin reactivity to deacetomatricarin (Fig. 2, 16) is caused by impurities.

Discussion

The oleoresins of related *Chrysanthemum* species have been found to contain many substances viz. terpenes such as chrysanthemone, umbellulone, carvone, α -thujone and camphor in addition to borneol β - Δ^3 -carene and sesquiterpene lactones (compare

Table 3. Sesquiterpene lactones derived from some *Chrysanthemum* species

	Compound	Botanical Source
Class Germacranolide	Chrysanolide	<i>Chrysanthemum cinerariaefolium</i>
	Parthenolide	<i>C. parthenium</i>
		<i>C. tanacetum</i>
	Pyrethrosin	<i>C. cinerariaefolium</i> <i>C. coccineum</i>
Class Eudesmanolide	Chrysanin	<i>C. cinerariaefolium</i>
	β -Cyclopyrethrosin	<i>C. cinerariaefolium</i>
	Dihydro- β -cyclopyrethrosin	<i>C. cinerariaefolium</i>
	Reynosin	<i>C. parthenium</i>
Class Guaianolide	Chrysartemin A	<i>C. parthenium</i>
	Chrysartemin B	<i>C. parthenium</i>

Table 3 and the findings of Hausen and Schulz 1973). With the exception of the lactones, these compounds are also present in many essential oils derived from plants of different plant families. Oil of turpentine, for example, contains (oxydation products of) Δ^3 -carene, pinene, menthene, limonene, α - and β -thujon: substances identical or structurally related to the above-mentioned compounds. The overlap of skin reactivities observed in the present investigation between *chrysanthemums* of florists on the one hand and turpentine and colophony on the other hand, may be attributable to the occurrence in these materials of identical or chemically related allergens. A correlation between patch tests to *chrysanthemum* and to turpentine was also observed by Baer et al. (1973). On the other hand, the investigations of Mitchell and his co-operators (1972a) indicate that terpenes do not play a major role in *chrysanthemum* allergy. Two patients who were allergic to *Chrysanthemum* species were negative to turpentine (Mitchell et al. 1971b). Similar observations were made in a German study of 5 patients allergic to *chrysanthemum* of florists (Schulz et al. 1975). Therefore, the overlap of skin reactivities observed in the present study needs further evaluation.

We suspect that, in the presence of severe patch test induced dermatitis, turpentine can occasionally produce irritant reactions which are incorrectly recorded as allergic.

In accordance with the results of Schulz et al. (1975), pyrethrins, which are the insecticidal active substances present in pyrethrum insect powder, did not give rise to patch test responses in our patients. Studies performed by Mitchell et al. (1972) indicated that the pyrethrins (esters of *chrysanthemumic acid*) did not play a significant role in pyrethrum allergy; the sesquiterpene lactone, pyrethrosin, was found to be the principal allergenic component.

In the present investigation, patch tests were performed with 25 sesquiterpene lactones representing the various classes of allergenic components of plants of the Compositae family. A total of 15 of these components proved capable of producing positive skin responses in the six patients with a contact hypersensitivity to *chrysanthemum* of florists. These findings corroborate earlier studies in which positive reactions were observed to alantolactone, cumambrin A and B, arbusculin A and B and ambrosin (Bleumink et al. 1973, Mitchell et al. 1970, Mitchell 1974, Schulz et al. 1975). In addition, individuals contact allergic to chry-

santhemums have been found to react after challenge with coronopilin, parthenolide, pyrethrosin and reynosin (Mitchell 1974, Schulz et al. 1975). Many of the sesquiterpene lactones show cross-reactivity. In this connexion the model study of Schulz et al. (1975) is of importance. They sensitized guinea-pigs with an extract of flowers and leaves of *Chrysanthemum indicum* L. and with parthenolide and alantolactone (lactones belonging to different classes).

Although the latter components are not present in chrysanthemum, animals sensitized with a chrysanthemum extract showed positive tests after challenge with alantolactone and parthenolide. Animals sensitized with parthenolide also proved to react with the extract and the chemically non-related alantolactone.

Our studies, combined with those of others, have indicated that sesquiterpene lactones play a major role in chrysanthemum allergy. Furthermore, *Chrysanthemum morifolium* (Chrysanthemum of florists) has recently been found to yield a sesquiterpene lactone (Osawa et al. 1973) which is potentially allergenic from the criteria noted above. It seems unlikely that our patients have been exposed to all the chemical compounds which showed positive reactions. The majority of the skin reactive components must be regarded as secondary allergens.

Some individuals who were sensitized to Costus Absolute derived from *Saussurea lappa* were patch-tested to a duplicate set of the lactones which have been used in the present study (Mitchell & Epstein 1974). Eight of the ten individuals showed positive patch test reactions to two or more of the lactones, most notably to 8-deoxycumambrin, arbusculin C, arbusculin A, rothin A and damsin. Only one of seven individuals was patch-tested to *Chrysanthemum morifolium*; he responded with a 3-4 + positive response.

The results of the study of Mitchell and Epstein (1974), of Mitchell (1975) and of the present study provide additional evidence for cross-reactivity of Compositae species and sesquiterpene lactones. Perhaps more important from a clinical point of view is the sustained finding that immunological studies must be complemented by a clinical history of exposure in order to provide meaningful results. The history is essential because patients may show positive patch test results to plants to which they have never been exposed. The positive reactions then indicate cross-sensitivity rather than relevance in a particular clinical situation.

In previous investigations (Mitchell et al. 1970) it was concluded that the presence of an α -methylene group attached to the γ -lactone ring (Fig. 1) is a prerequisite for allergenic activity.

The reactive α -methylene is the group by which the lactones can conjugate with proteins or cells in order to form a complete antigen capable of producing cell-mediated immune reactions.

It is known that the particular groups are reactive by Michael-type addition with nucleophilic reagents such as the SH-group of cysteine. Studies performed by Dupuis et al. (1974) showed that alantolactone can react via the α -methylene group with cysteine, with the imidazole group of histidine and with the ϵ -amino group of lysine but not with other amino acids such as arginine, serine and methionine. These properties make sesquiterpene lactones very suitable for model studies on the pathogenesis of allergic contact dermatitis in man.

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