

The role of sesquiterpene lactones in contact hypersensitivity to some North and South American species of feverfew (*Parthenium* - Compositae)

ELOY RODRÍGUEZ¹, W. L. EPSTEIN² AND J. C. MITCHELL³

¹School of Biological Sciences, University of California, Irvine, California

²Department of Dermatology, University of California Medical Center, San Francisco, California

³Division of Dermatology, The University of British Columbia, Vancouver, Canada

Sixteen taxa of the genus *Parthenium* from North and South America and 20 sesquiterpene lactones derived from plants of the Compositae family were used for patch testing six volunteers who were known to be contact sensitive to plants and lactones of this family. The patterns of cross-sensitivity and structure-activity relationships of the allergenic lactones are evaluated and the clinical and ecological relevance is discussed.

Key words: Compositae – contact hypersensitivity – *Parthenium* – sesquiterpene lactones.

Received for publication February 7, 1977

This paper continues a series of studies of contact hypersensitivity to sesquiterpene lactones of plant species of the Compositae (Chrysanthemum) family. The purposes of the studies have been to evaluate molecular structure and immunochemical requisites of this group of compounds for allergic contact dermatitis and to evaluate cross-sensitivity patterns in relation to clinical experience and ecological relevance.

Numerous skeletal-types of sesquiterpene lactones (a class of C₁₅ terpenoids isolated from plant taxa of the Compositae and some liverworts, *Frullania*) are known to cause allergic eczematous contact dermatitis (Mitchell et al. 1971, 1975a, b; Mitchell & Epstein 1974; Bleumink et al. 1976).

Members of the subtribe *Ambrosiinae* (which include *Ambrosia*, *Iva*, *Hymenoclea*, *Dicoria*, *Xanthium* and *Parthenium*) are of particular interest since many species produce copious amounts of lactones (in some cases up to 4 % of their dry leaf weight). The species *Parthenium hysterophorus*, recently introduced into India from the Americas, is a major cause of contact dermatitis in adult males (Lonkar et al. 1974). The trichomes (plant hairs) contain lactones (Rodríguez et al. 1976).

Current interest in *P. hysterophorus* and its allergenic lactones suggested a detailed chemical and clinical study of 16 species of *Parthenium* which produce three types of sesquiterpene lactones.

This study was supported by the Medical Research Council, Ottawa, Canada, Grant MT-2164 and by the Skin Disease Research Foundation.

Table 1. Pattern of cross-sensitivity to extracts of 16 taxa of *Parthenium*

Genus <i>Parthenium</i> Plant species	Patch test reactions						Total
	Patients						
	1	2	3	4	5	6	
<i>P. schottii</i>	1+	—	3+	—	3+	—	3/6
<i>P. tomentosum</i>	—	—	2+	—	1-2+	—	2/6
<i>P. tomentosum</i> var. <i>stramonium</i>	2+	1-2+	3-4+	—	4+	1+	5/6
<i>P. fruticosum</i> var. <i>trilobatum</i>	—	—	2+	—	3-4+	—	2/6
<i>P. cineraceum</i>	—	—	3+	—	4+	—	2/6
<i>P. rollinsianum</i>	—	—	—	—	—	—	0/6
<i>P. incanum</i>	1-2+	—	3+	—	3+	—	3/6
<i>P. integrifolium</i>	—	—	—	—	4+	—	1/6
<i>P. integrifolium</i> var. <i>auriculatum</i>	—	—	3+	—	4+	—	2/6
<i>P. confertum</i>	1+	—	2-3+	2+	4+	—	4/6
<i>P. confertum</i> var. <i>lyratum</i>	—	—	2+	—	3+	—	2/6
var. <i>divaricatum</i>	—	—	2+	—	2-3+	—	2/6
var. <i>microcephalum</i>	NT	—	1+	2+	4+	—	3/5
<i>P. densipilum</i>	1+	—	3+	1-2+	4+	—	4/6
<i>P. hysterothorus</i>	—	—	3+	—	4+	—	2/6
<i>P. glomeratum</i>	—	—	3-4+	1+	4+	—	3/6
Total positive	5	1	14	4	15	1	40+

Material and Methods

Twenty-five grams of dried leaves for each taxon of *Parthenium* (refer to Table 1) were ground into fine pieces and extracted separately with 250 ml of acetone. The extracts were shaken at 20°C for at least 24 h and then filtered. The resulting green or brown-yellow solutions were concentrated in a vacuum rotatory and evaporated to 25 ml (100 %). For patch testing the solutions were diluted 1:10 with acetone. Individual sesquiterpene lactones from *Parthenium* were prepared in petrolatum (.01 % w/w) according to the method outlined in Mitchell et al. (1971).

Extraction and isolation of sesquiterpene lactones from *Parthenium* taxa

Extraction of bulk, dried leaf material and subsequent isolation and purification of sesquiterpene lactones for nmr, ir and ms analyses were carried out according to the following procedure.

a. One hundred grams of dried leaf material were pulverized in a disc mill or Waring® blender. Approximately 500 ml of technical grade chloroform were added and the mixture allowed to stand overnight.

b. The chloroform slurry was filtered and the dark-green filtrate was evaporated to dryness under water pump vacuum. The thick, tar-like slurry was subsequently dissolved in 95 % ethanol (250 ml). If the thick syrup did not readily dissolve, the solution was heated on a steam bath for 5 min and allowed to cool to room temperature. Then 250 ml of 4 % aqueous lead acetate solution was added to precipitate the more polar components.

c. The aqueous cloudy solution was next filtered through a celite pad (diatomaceous earth) and the filtrate concentrated *in vacuo* until water and oily droplets remained. The mixture was extracted three times with chloroform (75 ml) and the extracts were dried over anhydrous magnesium sulfate.

The terpene-containing solution was next filtered and evaporated under water pump vacuum.

d. The crude plant extract containing sesquiterpene lactones and in some cases triterpenes and highly methylated flavonoids, was dried *in vacuo*, and the weight of the crude syrup determined. A proton magnetic resonance (pmr) spectrum was recorded for the crude syrup. The number of lactones in the crude syrup was determined by thin-layer chromatography (TLC) using 0.25 mm silical gel-G plates developed in two

solvent systems, benzene-acetone (4:1;v/v) and/or chloroform-ether (5:1;v/v). After 20 min, the TLC plates were developed in an iodine chamber or sprayed with aqueous 5 % KMnO_4 . As many as 2-7 spots were detected in most syrups, with the major constituent(s) darkly stained.

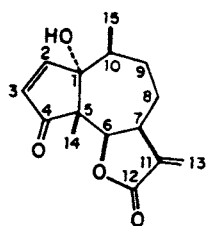
Results

Clinical findings

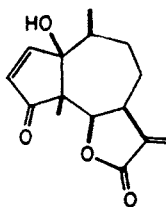
Six male volunteers previously sensitized to Costus Absolute, a perfume material (de-

Table 2. Sesquiterpene lactones used for patch testing

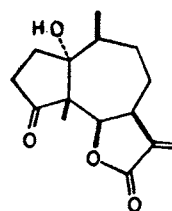
Sesquiterpene lactones of <i>Parthenium</i> species	Patch test reactions						Total
	Individual						
	1	2	3	4	5	6	
Parthenin	-	-	-	NT	-	-	0/5
Hymenin	-	-	-	-	2+	-	1/6
Coronopilin	-	-	-	-	3-4+	-	1/6
Damsin	1+	1+	3+	3+	4+	1+	6/6
Ambrosin	-	-	3+	-	4+	-	2/6
Confertin	-	-	3+	-	4+	-	2/6
Conchosin-A	-	NT	-	NT	1+	-	1/4
Conchosin-B	NT	NT	-	NT	2+	-	1/3
Conchosin-C+D	NT	NT	-	NT	2+	-	1/3
Tetraneurin-A	-	NT	2+	NT	3+	-	2/4
Tetraneurin-B	-	NT	-	NT	-	-	0/4
Tetraneurin-C	-	NT	-	NT	3+	-	1/4
Tetraneurin-D	-	NT	-	NT	-	-	0/4
Tetraneurin-F	NT	NT	-	NT	-	-	0/3
Ligulatin-B	-	NT	-	NT	-	-	0/4
Ligulatin-C	NT	NT	-	NT	-	NT	0/2
Oaxacin	-	NT	3+	NT	3+	-	2/4
Stramonin-A	-	-	-	NT	-	-	0/5
Stramonin-B	-	-	-	NT	-	-	0/5
Tomentosin	NT	NT	-	NT	2-3+	NT	1/2
Purified Costus lactone fraction 0.01 %	4+	4+	4+	4+	4+	4+	6/6
Total positive	2	2	6	2	13	2	27
Costus Absolute – lowest dilution to which positive reactions were observed	0.001 %				Individual 1		
	0.001 %				Individual 2		
	0.0001 %				Individual 3		
	0.001 %				Individual 4		
	0.00001 %				Individual 5		
	0.0001 %				Individual 6		



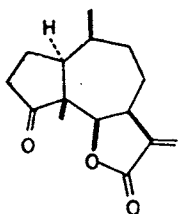
Parthenin



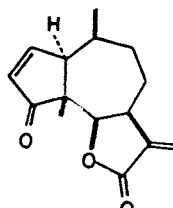
Hymenin



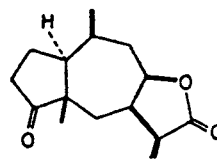
Coronopilin



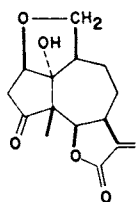
Damsin



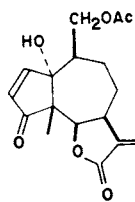
Ambrosin



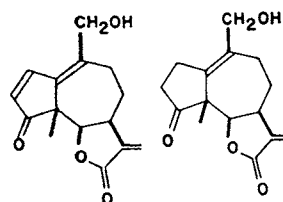
Confertin



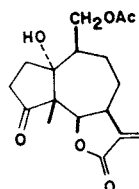
Conchosin-A



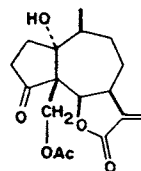
Conchosin-B



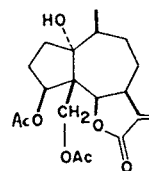
Conchosin-C & -D



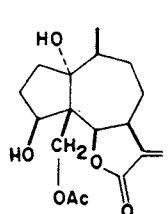
Tetraneurin-A



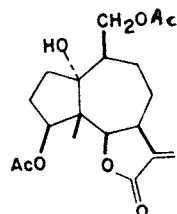
Tetraneurin-B



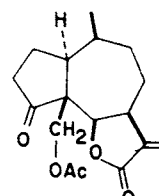
Tetraneurin-C



Tetraneurin-D



Tetraneurin-F



Ligulatin-B

Fig. 1. Structures of sesquiterpene lactones from *Parthenium* used in patch testing.

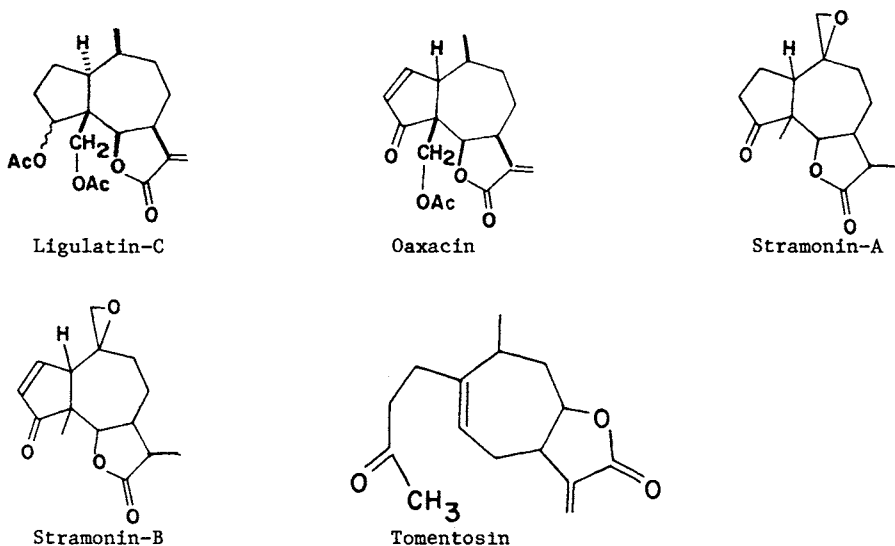


Fig. 1. (cont.).

rived from *Saussurea lappa*, Compositae) served as subjects. Similarly sensitized individuals were found in a previous study (Mitchell & Epstein 1974) to be contact sensitive to sesquiterpene lactones derived from plants of the Compositae family and to some of the plants of the family. The present panel was tested to further evaluate cross-sensitivity between lactones and *Parthenium* species.

Patch test methods and materials

Patch tests were carried out using plant acetone extracts and .01 % petrolatum preparations of pure sesquiterpene lactones applied under occlusion for 48 h.

Results of testing to plant extracts

The pattern of contact sensitivity to extracts of 16 *Parthenium* plants is shown in Table 1. Some positive reactions to 15 of the 16 plant extracts were observed in most of the sensitized individuals.

Only *P. rollinsianum*, a Mexican desert shrub not known to contain sesquiterpene

lactones, produced negative patch test reactions in all six individuals tested. The results suggest that individuals who are sensitized to lactones will react to many plants of the genus *Parthenium*.

Patch testing to sesquiterpene lactones

In Table 2, patch test reactions to sesquiterpene lactones are listed for the six individuals. Individual 5 who was most highly sensitive (at minimum dilution of 0.00001 %), reacted to most of the lactones. Twelve lactones produced positive reactions in some of the individuals who were tested. Eight lactones produced negative reactions in all the individuals who were tested.

The structures of sesquiterpene lactones used for patch testing are presented in Fig. 1 with their distribution in those plant taxa extracted tabulated in Table 3.

Discussion

The volunteers in this study were primarily sensitized to Costus Absolute and they were

Table 3. Distribution of sesquiterpene lactones in taxa of *Parthenium* used for patch testing•

Taxa**	Sesquiterpene lactone(s) present
<i>P. schottii</i>	coronopilin confertin tetraeurin-B tetraeurin-D
<i>P. tomentosum</i>	ligulatin-B ligulatin-C oaxacin tomentosin
<i>P. tomentosum</i> var. <i>stramonium</i>	stramonin-A & -B
<i>P. fruticosum</i> var. <i>trilobatum</i>	tetraeurin-C tetraeurin-D tetraeurin-E ivalbatin acetate***
<i>P. cineraceum</i>	ligulatin-B ligulatin-C
<i>P. rollinsianum</i>	none
<i>P. incanum</i>	coronopilin
<i>P. integrifolium</i>	tetraeurin-C tetraeurin-E
<i>P. integrifolium</i> var. <i>auriculatum</i>	tetraeurin-C tetraeurin-E
<i>P. confertum</i>	damsin
<i>P. confertum</i> var. <i>lyratum</i>	tetraeurin-A tetraeurin-E
<i>P. confertum</i> var. <i>divaricatum</i>	conchosin-A conchosin-B conchosin-C & -D
<i>P. confertum</i> var. <i>microcephalum</i>	hymenin
<i>P. densipilum</i>	damsin
<i>P. hystrophorus</i>	parthenin ambrosin
<i>P. glomeratum</i>	hysterin*** damsin

* Structure elucidation studies have been published elsewhere (Rodríguez et al. 1971; Rodríguez 1975)

** Refer to Rodríguez (1975) for collection sites and vouchers of all taxa investigated

*** Insufficient amounts of compound available for patch testing.

exquisitely sensitive to the purified costus lactone fraction, which contains at least two germacranolides, constunolide and dihydro-costunolide. As is usual, their patterns of cross reactivity with *Parthenium* plants were highly individualistic. Thus, two subjects reacted to virtually all plant extracts, while two reacted to 4 or 5 extracts and two reacted to only one plant extract, *P. tomen-*

tosum var. *stramonium* from Sinaloa, Mexico. This small tree contains stramonin A to E (Rodríguez 1975). Unfortunately only stramonin A and B (Fig. 1) were available for patch testing and the negative responses in the five subjects tested were expected because they lack the methylene side grouping thought to be required for skin test reactivity to sesquiterpene lactones (Mitchell

1975a). The correlation between plant extract positivity and chemical reactivity, however, proved to be quite good. All four reactors to *P. confertum* extract also gave positive reactions to damsine which is the main lactone in that Mexican plant. There were only 8 instances out of 35 positive extract reactions (excluding *P. tomentosum* var. *stramonium*) in which the subject failed to react to at least one of the chemicals in that plant. The eight failures were verified by repeated patch testing, so that an artefact of testing probably does not account for the negative results. Although many possibilities exist for the lack of fit, no obvious explanation crowds the foreground. Also there were two subjects (2 and 6) who were sensitive to damsine but did not react to any plant extracts containing the chemical (*P. confertum*, *P. densipilum* and *P. glomeratum*). These false positives also remain unexplained.

The scourge of India, *P. hysterophorus*, produced only two positive reactions, in the most reactive subjects (3 and 5). Both subjects reacted to ambrosin but not to parthenin, the two active lactones in this weed, although parthenin appears to be the major sensitizer in *P. hysterophorus* in India (Lonkar et al. 1974). The only difference between ambrosin and parthenin is the substitution in ambrosin of a hydrogen at C₁ for the hydroxyl group. Hymenin, the C-1 hydroxy stereoisomer of parthenin was positive in only one of the six subjects, while the extract of *P. confertum* var. *microcephalum* which contains hymenin gave positive reactions in three of the five patients.

Damsine, which gave positive responses in all six subjects in this study also gave positive reactions in five of the 10 most sensitive subjects tested in an earlier costus cross-sensitivity study (Mitchell & Epstein 1974). The structure of damsine differs from ambrosin only in the lack of a double bond between C₂ and C₃, so that perturbations at

C₁, C₂ and C₃ of the cyclopentanone ring (5-membered ring) may play an important role in determining cross reactivity just as the ethylene side grouping at C₁₁-C₁₃ (Fig. 1). Further structure-activity relationships of sesquiterpene lactones will be needed to elucidate this point.

Some other biological activities of sesquiterpene lactones, including cytotoxicity, depend primarily on a lactonic exo-cyclic methylene group (Rodriguez et al. 1976) and the current findings confirm the importance of this group as a necessity for immunologic activity. However, more subtle and so far obscure requirements which are repeatedly evident, require further investigation. Other parts of the molecule presumably play a role for allergenicity in addition to the role of the lactone methylene group for carrier protein attachment.

Ecological aspects

The current findings re-emphasize the clinical importance of cross-sensitivity patterns between this group of plants and derived compounds. It has become clear that if a patient shows a positive patch test reaction to a plant of this family or to a lactone of this family, the clinical significance of the findings can only be assessed by reference to the clinical history. Thus, patients may react to plants or chemicals to which they have never been exposed. Plants which are remote geographically can produce positive reactions in such patients. Thus, such positive reactions may be immunologically relevant but ecologically irrelevant. Thus, an individual who was experimentally sensitized to a perfume material of India (*Costus*, *Saussurea*) may thereby be contact sensitive to ragweed of North America (*Ambrosia*), to chrysanthemum of florists, to *Parthenium* the weed scourge of India, and to artichoke (*Cynara*) chicory (*Cichorium*) and lettuce (*Lactuca*) of the kitchen.

Chrysanthemum of florists contains a

sensitizing lactone arteglin A, in common with a weed, *Artemisia douglasiana*. Costunolide, which is a sensitizer in the perfume material Costus Absolute, is also derived from a garden plant named Maiden's Ruin, *Artemisia balchanorum*, from a weed *Hymenoclea monogyra* and a common horticultural plant *Cosmos hybridus* of the Compositae family, from an industrial timber tree, *Liriodendron tulipifera* of the Magnoliaceae family and from a liverwort *Frullania* of the Bryophytes. The perfume material oil of Champaca (*Michelia*, Magnoliaceae) which produced positive patch test reactions in Compositae-sesquiterpene lactone-sensitive patients shares with ragweed (*Ambrosia*) the known allergenic lactone parthenolide and cross-reacts with *Magnolia* (Magnoliaceae), with chamomile (*Mataricaria*, Compositae) and with laurel oil (*Laurus nobilis*, Lauraceae) (Mitchell 1975b).

The distribution patterns of contact dermatitis from these diverse sources are rather similar and can resemble photodermatitis and atopic dermatitis (Lonkar et al. 1974, Storrs et al. 1976).

In this maze of differential diagnosis and of immunochemistry, involving some 600 plants of at least six plant families and their products including perfumes and flavorings, a clinical and ecological history for relevant exposure is essential.

References

- Bleumink, E., Mitchell, J. C., Geissman, T. A. & Towers, G. H. N. (1976) Contact hypersensitivity to sesquiterpene lactones in *Chrysanthemum* dermatitis. *Contact Dermatitis* 2, 81-88.
- Lonkar, A., Mitchell, J. C. & Calnan, C. D. (1974) Contact dermatitis from *Parthenium hysterophorus*. *Transactions of the St. John's Hospital Dermatological Society* 60, 43-53.
- Mitchell, J. C., Roy, A. K., Dupuis, G. & Towers, G. H. N. (1971) Allergic contact dermatitis from ragweeds (*Ambrosia* species). *Archives of Dermatology* 104, 73-76.
- Mitchell, J. C. (1975a) Contact allergy from plants. In *Recent Advances in Phytochemistry*, Vol. 9, ed. Runeckles, V. C. New York: Plenum Press.
- Mitchell, J. C. (1975b) Biochemical basis of geographic ecology. *International Journal of Dermatology* Part 1, 14, 239-250; Part 2, 14, 301-321.
- Mitchell, J. C. & Epstein, W. L. (1974) Contact hypersensitivity to a perfume material, Costus Absolute. The role of sesquiterpene lactones. *Archives of Dermatology* 110, 871-873.
- Rodríguez, E., Yoshioka, H. & Mabry, T. J. (1971) The sesquiterpene lactone chemistry of the genus *Parthenium* (Compositae). *Phytochemistry* 10, 1145-1154.
- Rodríguez, E. (1975) The chemistry and distribution of sesquiterpene lactones and flavonoids in *Parthenium* (Compositae) systematic and ecological implications. Ph. D. Thesis. University of Texas, Austin.
- Rodríguez, E., Dillon, M. L., Mabry, T. J., Mitchell, J. C. & Towers, G. H. N. (1976) Dermatologically active sesquiterpene lactones in trichomes of *Parthenium hysterophorus* L. (Compositae). *Experientia* 32, 236-237.
- Rodríguez, E., Towers, G. H. N. & Mitchell, J. C. (1976) Biological activities of sesquiterpene lactones. *Phytochemistry* 15, 1573-1580.
- Storrs, F., Mitchell, J. C. & Rasmussen, J. E. (1976) Contact hypersensitivity to liverwort and the Compositae family of plants. *Cutis* 18, 781-686.

Address:

Dr. E. Rodríguez
School of Biological Sciences
University of California
Irvine
California 92717
USA