Acta Derm Venereol (Stockh) 1983; 63: 308–314

Contact Allergy to Parthenolide in *Tanacetum parthenium* (L.) Schulz-Bip. (Feverfew, Asteraceae) and Cross-reactions to Related Sesquiterpene Lactone Containing Compositae Species*

B. M. HAUSEN[1] and P. E. OSMUNDSEN[2]

[1]*Department of Dermatology, University Hospital, Hamburg, West Germany and* [2]*Skin Clinic, Brønshøj, Denmark*

Hausen B, Osmundsen PE. Contact allergy to parthenolide in *Tanacetum parthenium* (L.) Schulz-Bip. (Feverfew, Asteraceae) and cross-reactions to related sesquiterpene lactone containing Compositae species. Acta Derm Venereol (Stockh) 1983; 63: 308–314.

A case of specific, delayed hypersensitivity induced by repeated contact with a wild form of feverfew (*Tanacetum parthenium*) is reported. In the flowers investigated the content of the responsible contact allergen parthenolide—a potent sesquiterpene lactone—appeared to be 10 times greater (0.6–0.9 %) than in earlier years. Guinea pig experiments confirm the strong sensitizing potency of this Compositae species. Cross-reactions were elicited with 11 of 21 mostly Compositae plants containing chemically related sesquiterpene lactones. The strongest reactions were elicited by tansy, yarrow (milfoil), marguerite, aster, sunflower, laurel and Frullania. Structure elucidation by X-ray crystallographic analysis established the precise molecule configuration of parthenolide, although the lattice parameters of the isolated compound were not in accordance with those published in the literature. *Key words: Compositae allergy; Sensitizing experiments in guinea pigs; Parthenolide; Sesquiterpene lactones; Allergen content; Cross-reactions; X-ray analysis.* (Received October 29, 1982.)

BM. Hausen, Universitätshautklinik, Martinistr. 52, D-2000 Hamburg 20, Fed. Rep. of Germany.

Allergic contact dermatitis caused by plants usually results from frequent handling of ornametal flowers, as it affects florists, gardeners and nursery workers. Non-occupationally induced hypersensitivity may also develop in individuals who often handle plants, such as housewives and hobby gardeners, especially when they are very fond of their home-grown specimens. The most rarely seen cases are those observed after accidental contact with wild allergenic plants or weeds in abandoned gardens. One such rare case is reported here.

CASE REPORT

A 63-year-old retired foreman had suffered since 1975 from recurrent dermatitis on his hands and forearms, most marked on the dorsal side, and on his face, mainly around the eyes. Routine patch testing was negative in 1977. Patch tests were repeated in 1978 with the routine test series in which Frullania had—accidentally—been included. He showed a strong reaction (+++) to Frullania *per se*, while all the other tests remained negative. The patient reported that he has a little garden,where he spent most of his time. Due to the response to Frullania, patch testing was now performed with 19 plants from his garden. These had been selected by the patient, as it was he who most often came into contact with them. These tests proved negative, too. Since the exzema was not seasonal and since path testing with several relevant plants had proved negative,the positive reaction to Frullania seemed unable to explain his skin lesions.

* Part IV of the series: The sensitizing capacity of Compositae plants.

In the following years his dermatitis became more intense. In November 1978 he suffered from a particularly severe attack after having removed a weed from his garden which had spread too much. He presented a small sample of it, regretting that he had forgotten to mention it and to bring it for patch testing in 1978. A leaf elicited a strong patch test reaction. Some years ago the patient had taken this plant for his garden while on holiday in Sweden. The family liked the plant very much and it had been allowed to spread all over the garden until this year.

Botanical examination of a small specimen pulled out of the snow in late November 1981 revealed its identity as feverfew (*Tanacetum parthenium* (L.) Schulz-Bip.) of the Compositae (=Asteraceae) family (Fig. 1). The patient was advised to have the weed completely eradicated from his garden (which was done by others). However, the plant had spread to his neighbour's garden. When we needed some material for chemical examination the patient collected several specimens from his neighbour's garden and promptly suffered—although he had been warned not to do it himself—a severe recurrence of his dermatitis.

MATERIAL AND METHODS

Patch testing with feverfew was done using an ether extract of the entire plant, incorporated into petrolatum (1%), and the main constituent of feverfew, the sesquiterpene lactone parthenolide, incorporated into petrolatum (0.1%). Patch testing was performed with ether extracts of 19 members of the Compositae family known to contain sesquiterpene lactones of related structures (all in petrolatum). The Finnchamber® method and fixation with Scanpor® was used, readings being taken at 24 h, 48 h and 72 hours. Extracts of laurel and reindeer moss were included in the series as they too are known to contain sesquiterpene lactones. All patch test results were recorded on photographic slides.

Table I. *Patch test results and cross-reactions in the feverfew-sensitive patient*

All plant extracts have previously been tested in at least 50 control persons (vehicle: white petrolatum)

Name	Botanical name	Concentration, %	24 h	48 h	72 h
Feverfew	*Tanacetum parthenium* (L.) Schulz-Bip.	1	+++	++++	–
Parthenolide	–	0.1	++	+++	–
Arnica	*Arnica montana* L.	0.5	0	0	0
Aster	*Aster sp.* (cultivar)	10	++	+++	+++
Mugwort	*Artemisia vulgaris* L.	10	0	0	0
Hemp Agrimony	*Eupatorium cannabinum* L.	2.5	+	+	+
Marguerite	*Leucanthemum vulgare* Lam.	1	+	++	++
Reindeer moss	*Cladonia alpestris* (L.) Rabenh.	3	0	0	0
Frullania	*Frullania dilatata* (L.) Dum.	1	++	+++	+++
Gallant Soldier	*Galinsoga parviflora* Cav.	0.5	0	0	0
Common Daisy	*Bellis perennis* L.	10	0	0	0
Autumnal Hawkbit	*Leontodon autumnalis* L.	2.5	0	0	0
Common Dandelion	*Taraxacum officinale* Wiggers	2.5	0	0	0
Laurel	*Laurus nobilis* L.	2	++	++	++
Scentless Mayweed	*Matricaria maritima* L.	10	0	0	0
Tansy	*Tanacetum vulgare* L.	1	++	+++	+++
Pot Marigold	*Calendula officinalis* L.	10	0	0	0
Yarrow, Milfoil	*Achillea millefolium* L.	1	++	++	++
Salad, Cabbage lettuce	*Lactuca sativa* L. var. *capitata* L.	10	0	0	0
Common Sunflower	*Helianthus annuus* L.	5	++	++	++
Wild Camomile	*Chamomilla recutita* (L.) Rausch.	2.5	++	++	++
Wormwood	*Artemisia absinthium* L.	10	++	++	++
Youth and Age	*Zinnia elegans* Jacq.	10	++	++	++

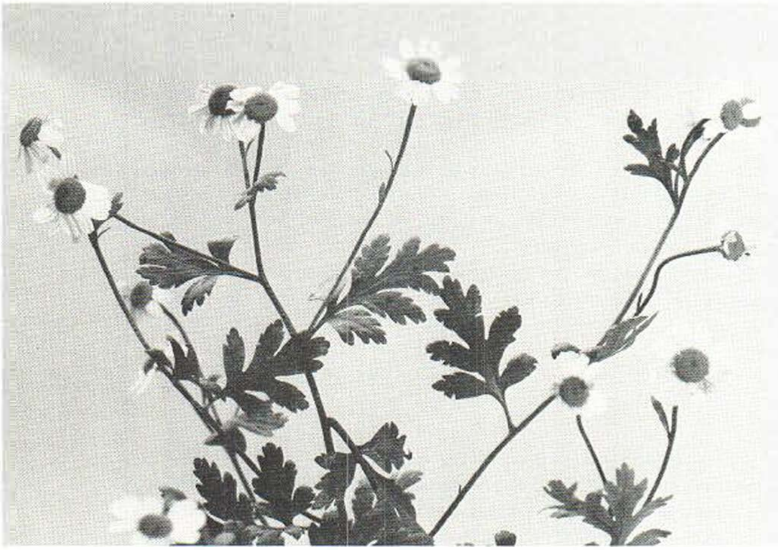

Fig. 1. Feverfew—*Tanacetum parthenium* (L.) Schulz-Bip.

The patch test reactions were evaluated according to the system proposed by Rook, Wilkinson & Ebling (12): 0 = negative, ?+ = doubtful reaction, + = weak reaction (non-vesicular), ++ = strong reaction (oedematous or vesicular) +++ = extreme reaction.

Experimental sensitization was carried-out in 10 female Albino guinea pigs of the Pirbright white-strain (350–400 g) using ether extracts of feverfew (in acetone; 10%) provided by the Botanical Gardens, Hamburg. The open epicutaneous method was applied and the procedure continued over 9 days. At this stage all animals had reached a +++reaction. Challenge was performed 11 days later with three dilutions of the raw extract (10%, 3%, 1%) and two of parthenolide (0.1%, 0.03%), isolated from the extract. Further details wil be given in a forthcoming publication (3).

In a second step 10 guinea pigs were sensitized with 45 mg of pure parthenolide using the

Table II. *Results of the sensitisation experiments with feverfew extracts and its main constituent parthenolide*

Material	No. of animals	Concentration, %	24 h				48 h				72 h			
			++	+	(+)	0	++	+	(+)	0	++	+	(+)	0
Open epicutaneous method														
Feverfew extract	10	10	10	–	–	–	4	6	–	–	1	5	4	–
Feverfew extract	10	3	6	4	–	–	5	5	–	–	4	4	2	–
Feverfew extract	10	1	4	5	1	–	3	6	1	–	1	4	4	1
Parthenolide	10	0.1	–	5	5	–	–	4	5	1	–	–	8	2
Parthenolide	10	0.03	–	–	2	8	–	–	2	8	–	–	–	10
FCA-method														
Parthenolide	10	0.1	10	–	–	–	9	1	–	–	8	2	–	
Parthenolide	10	0.03	5	4	1	–	7	3	–	–	3	4	3	–
Parthneolide	10	0.01	–	4	6	–	–	7	3	–	–	3	6	1

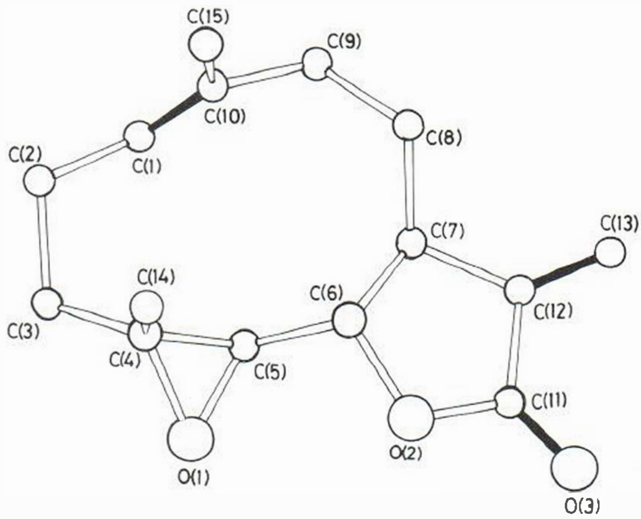

Fig. 2. ORTEP-drawing of parthenolide obtained by X-ray crystallographic analysis with atom numbering but without H atoms. Double bindings are marked black.

FCA-method. Intradermal injections with 15 mg parthenolide emulsified in 3 ml of Freund's complete adjuvant and 3 ml distilled water were given in the shoulder area from the left to the right paw (5 injections à 0.1 ml) on day 1, 5 and 9. Control animals were treated with FCA alone and equal amounts of distilled water.

In order to determine the yield of its constituent parthenolide (after cultivation in various soils) and to compare it with that of the plant material returned to its wild state and found 100 km north of Hamburg, the weed from the patient's garden was grown in the Botanical Gardens, Hamburg, and also in the garden of the present author (P. E. O.). The plant material (605 g) was extracted with peroxide free ether for 5 min. After evaporation of the solvent, the extract (yield: 13.7 g) was divided into two parts, of which one was used for the patch testing and animal sensitization experiments. The other part served for chemical examination, isolation, quantitative determination and structural elucidation of the main allergen.

500 mg of the ether extract was separated on 64 preparative TLC plates (thin-layer-chromatography) using the solvent system chloroform – methanol (100 : 1), running time: 75 min. The margin of one plate was sprayed with a solution of equal amounts of H_2SO_4 and H_2O, a reagent fairly specific for a large group of sesquiterpene lactones, and heated to 200°C for 90 sec. The compound corresponding to that turning purple by this treatment was scraped from the remaining plates and removed from the silica gel by elution with ether and acetone. The amounts of the constitutent so obtained served for patch tests on the patient and on the animals as well as for comparison with parthenolide and X-ray analysis. A part of the collected compound was dissolved, after further TLC purification, in 1 ml benzine (40/60) to which 3-4 drops of ether were added, and allowed to crystallize in a refrigerator at −20°C. A crystal of sufficient size was selected and used for X-ray crystallographic analysis. The structure was solved by direct methods using MULTAN 80 (8).

The fresh weight of the feverfew plant material deriving from different locations was as follows: garden of the patient's neighbour: 11.1 g; author's garden (P. E. O.): 8.2 g; feverfew found 100 km north of Hamburg: 210 g.

RESULTS

As shown in Table I the patient reacted to the feverfew extract with an almost extreme reaction, and a very strong reaction to its main constituent, which was later determined as parthenolide. Eleven of the 21 tested sesquiterpene lactone-containing plant extracts

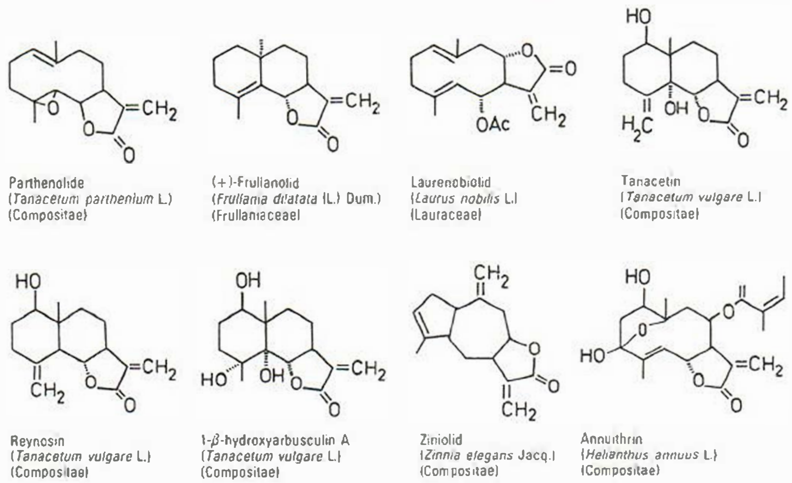

Fig. 3. Formulae of parthenolide and structurally related sesquiterpene lactones occurring in the Compositae and other species to which the feverfew-sensitive patient cross-reacted.

cross-reacted. Strong to strongest responses were obtained with Frullania, tansy, camomile, sunflower, aster, marguerite, laurel, yarrow and youth and age. Moderate reactions were seen with hemp agriomony and wormwood, while arnica, mugwort, reindeer moss, cabbage lettuce and some other Compositae species remained negative.

The sensitization experiments with an extract of feverfew as well as with the pure parthenolide were successful in all guinea pigs. The results (Table II) demonstrate the high sensitizing potential of feverfew and of parthenolide (toxic limit for parthenolide: 1%).

The ether extract of feverfew from the Botanical Gardens, Hamburg, yielded 0.63% of parthenolide. The feverfew specimens from the garden of the patient's neighbour (same soil as in the patient's garden) contained 0.59% of parthenolide, whereas the feverfew plants grown in the author's garden contained 0.21%. The flowers collected 100 km north of Hamburg,where they had returned to their wild state, revealed an amount of nearly 1% (0.91%). TLC separation of the ether extract yielded two further consitutents which were stained by the sesquiterpene lactone reagent. However, as they occurred in only trace amounts, no further attention was payed to their isolation and testing.

Comparison by TLC of the isolated main constituent of our feverfew material with authentic parthenolide and investigation of its further physical properties (IR, UV, melting point) revealed that the two substances were identical. However the lattice parameters of our compound were not in accordance with those published by Quick & Rogers in 1976 (9). Therefore X-ray crystallographic analysis was continued with one of the largest crystals obtained after repeated TLC purification and renewed crystallization from acetone, on the assumption that the constituent would not be parthenolide itself, but an enantiomer there of. However, the final elucidation of the structure (Fig. 2) clearly revealed that the molecule displayed the same configuration as demonstrated in two independent molecules of parthenolide by the same authors (9). Details will be published elsewhere.

DISCUSSION

Cases of allergic contact dermatitis due to *Tanacetum parthenium* (L.) Schulz-Bip. are very rare (5, 7, 10), especially in northern Europe. The flower is not native to Middle Europe and has no significance as an oramental plant on the market. However, it has been cultivated widely in private gardens, from where it has spread abundantly to the open country, returning to its wild state. While wild feverfew preferred special soils in North

Germany and Denmark in the past and was thus restricted to particular regions, we now observed that not only this plant but several related Compositae species grew more rankly and abundantly in 1981 and especially in 1982 than in many years before. This may explain the reported case and two further cases observed late this year in the Department of Dermatology in Hamburg (6).

Recently another case has been observed in connection with an attempt to introduce a new ornamental form of *Tanacetum parthenium* onto the flower markets under the misleading name of "camomile". In accordance with all previous and present cases, parthenolide was revealed to be the only contact sensitizer in this report, too (2).

European specimens yield parthenolide as the main sesquiterpene lactone constituent of feverfew (4, 14). Its *eliciting* capacity has already been established by Mitchell et al. (7) in 1971, who obtained extremely strong reactions (++++) to 1% concentrations in their patient. Experimentally the high *sensitizing* potency of parthenolide was already proven in guinea pigs by the open epicutaneous method in 1975 by Schulz et al. (13), who suspected it to be a constituent of *Chrysanthemum indicum* L. (chrysanthemum of the florists) at that time (which subsequently proved not to be true). However, sensitizing experiments with ether extracts of feverfew have not been performed so far.

Besides its wide abundance this year (1982), feverfew showed a remarkably high content of parthenolide. While Herout in 1959 (4) and Soucek et al. (14) in 1961 found 0.06% in their specimens, we obtained more than 10 times as much in our plant material. Possibly, this explains why our patient suffered particularly from his dermatitis in 1981 and 1982. Older ether extracts, from 1978, left in our refrigerator, only yielded about 0.1%. Furthermore, these findings demonstrate the essential influence of different growing conditions (e.g. soil, localization, weather, season, etc.) on the content of a contact allergen in a known sensitizing plant. In particular, the influence of origin becomes evident if one compares our results with those from abroad. Romo et al. (11) found the eudesmanolide santamarin (0.12%) and traces of three further sesquiterpene lactones (but no parthenolide) in Mexican specimens of *Tanacetum parthenium*.

As previous patch tests with relevant plants from the patient's garden had proved negative, the present responses to differet extracts of Compositae species must be regarded as cross-reactions. The results obtained corroborate that cross-reactivities exist mainly between those species botanically closely related and containing chemically related sesquiterpene lactones (Fig. 3). Concerning the reactions to marguerite, aster and tansy, it must be kept in mind that these species formerly belonged to the same genus and a previous synonym for *Tanacetum parthenium* Schulz-Bip. has been *Chrysanthemum parthenium* (L.) Bernh.

As far as the immunological process in allergic contact hypersensitivity is understood, the specifically sensitized T-lymphocytes detect primarily the α-methylene-γ-lactone group of the sesquiterpene lactone ("immunological requisite"), while structural differences between basic skeletons, e.g. guaianolide, eudesmanolide or germacranolide, seem to play only a secondary role; cross-reactions may be inhibited or reduced by steric hindrance through a substituent at C-positions in the vicinity of the methylene group.

Accordingly, comparison with two previous studies reveals that at least five Compositae species cross-react in *Chrysanthemum*- or *Tanacetum*-sensitive patients more frequently than others, namely tansy (*Tanacetum vulgare* L.), yarrow (milfoil) (*Achillea millefolium* L.), sunflower (*Helianthus annuus* L.), camomile (*Chamomilla recutita* (L.) Rausch.: syn. = *Matricaria chamomilla* L.) and aster (*Aster* sp.) (1, 2). These observations strengthen the assumption that the sensitizing constituents of the cultivated aster and the common camomile—which are still unknown—may be sesquiterpene lactones with structures closely related to those listed in Fig. 3.

We have advised our patient to avoid the feverfew and all the related Compositae plants in his environmet, even though they did not cross-react. It has been recognized that contact hypersensitivity to a weak sensitizer (e.g. another Compositae plant) will develop much more readily on the basis of a pre-existing contact allergy to a strong sensitizer, e.g. feverfew. Besides that the cross-reactions have shown that the patient must avoid contact with all those species of *other* plant families that are known to contain sesquiterpene lactones, e.g. Frullania and laurel, to prevent further relapses.

ACKNOWLEDGEMENTS

We are indebted to Dr H. Nothdurft, Botanical Gardens of Hamburg, for the botanical identification of feverfew specimens and for the supply of sufficient plant material. Prof. Dr W. Herz, Tallahassee, Florida, kindly provided parthenolide. We wish to thank H. H. Bartsch, Institute of Mineralogy, University of Hamburg, for the X-ray analysis. This study was supported in part by a grant from Deutsche Forschungsgemeinschaft, Bonn, West-Germany.

REFERENCES

1. Hausen BM. The sensitizing capacity of Compositae plants. III. Test results and cross-reactions in Compositae-sensitive patients. Dermatologica (Basel) *159*: 1, 1979.
2. — Berufsbedingte Kontaktallergie auf Mutterkraut (*Tanacetum parthenium* (L.) Schulz-Bip.; Asteraceae). Dermatol Beruf Umwelt *29*: 18, 1981.
3. Hausen BM, Nothdurft H. The sensitizing capacity of Compositae plants. V. Results of guinea pig sensitization experiments with plant extracts used in cosmetics, folk medicine and otherwise [in preparation].
4. Herout V, Soucek M, Sorm F. Parthenolide, another sesquiterpene lactone with a few-membered ring. Chem & Ind 1069, 1959.
5. Maiden JH. On some plants which cause inflammation or irritation of the skin. Agric Gaz New South Wales *20*: 1073, 1909.
6. Mensing H, Kimmig W, Hausen BM. Air-borne contact dermatitis due to feverfew (*Tanacetum parthenium*) [in prepration].
7. Mitchell JC, Geissman TA, Dupuis G, Towers, GHN. Allergic contact dermatitis caused by *Artemisia* and *Chrysanthemum* species. J Invest Dermatol *56*: 98, 1971.
8. MULTAN 80 (Main P, Fiske SJ, Hull SE, Lessinger L, Germain G, Declerq, JP, Woolfson, MM). A system of computer programs for the automatic solution of crystal structures form X-ray diffraction data. Department of Physics, University of York, England, and Louvain, Belgium.
9. Quick A, Rogers D. Crystal and molecular structure of parthenolide (4,5-Epoxygermacra-1(10), 11(13)-dien-12,6-lactone). J Chem Soc P II: 465, 1976.
10. Robertson WD, Mitchell JC. Allergic contact and photodermatitis. Can Med Assoc J *97*: 380, 1967.
11. Romo J, Romo de Vivar A, Trevino R, Joseph-Nathan P, Diaz E. Constituents of *Artemisia* and *Chrysanthemum* species. Phytochem *9*: 1615, 2970.
12. Rook A, Wilkinson DS, Ebling FJG. Textbook of Dermatology, vol. 1:425, 3rd ed. Blackwell, Oxford, 1979.
13. Schulz KH, Hausen BM, Wallhöfer L, Schmidt-Löffler P. Chrysanthemen-Allergie, II. Experimentelle Untersuchungen zur Identifizierung der Allergene. Arch Dermatol Forsch *251*: 235, 1975.
14. Soucek, M, Herout, V, Sorm F. On terpenes. CXVIII: Constitution of parthenolide. Coll Czech Chem Comm *26*: 803, 1961.