

Routine patch testing with frullanolide mix: an European Environmental and Contact Dermatitis Research Group multicentre study

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Contact sensitivity to plants containing 1 or more sesquiterpene lactones (SLs) is difficult to diagnose. The mixture of SLs (SL mix) has been shown to detect only about 60% of sensitized individuals. In order to improve the diagnosis of sensitization to plants containing SLs, we have tested a mixture of frullanolides contained in *Frullania dilatata* and *Frullania tamarisci* at 3 different concentrations (0.01%, 0.033% and 0.1% in petrolatum). 8605 consecutive eczema patients in 1 North American and 15 European dermatology departments were tested with this mix, and 0.35% of positive cases to the different concentrations were found. Routine use of this mix permitted detection of only a small percentage of extra cases and did not improve the SL mix score. The frullanolide mix should therefore be restricted to investigations in particular geographical zones and/or in particular occupations.

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Sesquiterpene lactones (SLs) belong to a large and diverse group of chemicals found in several plant families. These molecules, present in 6 plant families, namely Asteraceae (or Compositae), Lauraceae, Frullaniaceae (Liverworts), Umbelliferae, Aristolochiaceae and Magnoliaceae (1–3), may cause allergic contact dermatitis.

Among the several hundreds of SL containing an α -methylene- γ -butyrolactone moiety, some have been patch tested systematically, and at

least 1 of them, alantolactone, has been shown to have a high sensitizing capacity, as evidenced by active sensitization reactions on patch testing (4). The sensitizing capacity of SL has been demonstrated in humans and animals (5), and the SL mix (SL mix), a mixture of alantolactone, costunolide and dehydrocostus lactone, has been included in the European standard series to facilitate the diagnosis of SL-induced allergic contact dermatitis (6). However, this mixture detects only about 60% of the cases of sensitization (7). Other studies (8–11) have reported variations in this percentage, which can partly be explained by phytogeographical variations (12) and the

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diversity of SL structures. An alternative preparation (7), the Compositae mix, based on a mixture of plant extracts (arnica, yarrow, tansy, German camomile and feverfew), detects a higher proportion of the cases. Dandelion and feverfew extracts, together or individually, also appear to be more useful than the SL mix (13, 14).

Several attempts have been made to improve the SL mix by adding other molecules. Thus, Orion et al. (15) included the SL parthenolide as well as the SL mix in the standard series of 2 Danish dermatology departments. Their results demonstrated that parthenolide was not a suitable supplementary screening agent compared to the SL mix, but that it may be a reasonable screening molecule on its own if the SL mix was not available.

In order to improve the diagnosis of contact dermatitis due to SLs, we have routinely used a mixture of 2 frullanolide isomers (+)- and (-)-frullanolide.

Materials and Methods

A racemic mixture of (+)- and (-)-frullanolide (Fig. 1) was synthesized (Dermatochimie, ULP, Strasbourg, France) according to the method described by Ferraz et al. (16). The racemic mixture of frullanolide was fully characterized and gave analytical data in accordance with the literature.

To test the screening value of this frullanolide mix in Europe, it was included in the standard patch test series of 1 North American and 15 European clinics. The frullanolide mix was prepared at 3 concentrations in petrolatum (pet.): 0.01%, 0.033% and 0.1%.

The frullanolide mix was applied to the back for 2 days, using Finn Chambers[®] or Van Der Bend Chambers[®] on Scanpor[®] tape (Epitest, Tuusula, Finland). Readings were taken on D2 and D3 and D4–D7, according to International Contact Dermatitis Research Group (ICDRG) guidelines. For each patient with a positive frullanolide mix reaction, a documentation sheet, recording clinical, epidemiological and patch test results for SL, Compositae mix, plant and plant extract was completed. Some patients were also patch tested with *Frullania*

dilatata and *Frullania tamarisci*, when suspected of allergic contact dermatitis from this plant.

Results

As a first step and to avoid active sensitization, only the lowest concentration of frullanolide, 0.01% pet., was tested on 2436 patients (1546 females and 890 males). 11 were positive: 4 males and 1 female had a relevant history, while in 6 females, the tests were found not relevant. 26 reactions (1.07%) were doubtful (?+), and with these weak reactions, it was difficult to distinguish between irritant and weak allergic reactions.

As a second step, 3329 patients (2021 females and 1308 males) were tested with the mixture of frullanolides at a concentration of 0.033% in pet. 7 patients were positive, 1 male (relevant) and 6 females (3 relevant and 3 non-relevant), and 37 patch test reactions were doubtful (1.10%).

In a third round, 2840 patients (1867 females and 973 males) were tested with the frullanolide mix at 0.1% pet. 12 patients were found to be positive, 4 females and 2 males being relevant and another 5 females and 1 male non-relevant.

For the 3 concentrations (0.01%, 0.033% and 0.1%), a total of 8605 patients were patch tested (Table 1). Among them, 30 patients (0.35%) were positive (22 females and 8 males) and 101 reactions were doubtful. 15 positive reactions were found relevant (7 males and 8 females), while 15 reactions were found non-relevant (14 females and 1 male). Among the 15 patients with a relevant reaction to the frullanolide mix, 12 were also positive to the SL mix.

Relevant reactions in males (7) were all related to occupational contact dermatitis: 3 woodcutters, 1 farmer and 2 foresters using firewood. Among the 8 women with relevant reactions, we found 2 florists, 5 hobby gardeners and 1 using firewood.

Discussion

These results demonstrated that:

- (1) The frullanolide mix is not a major elicitor (0.35% positive reactions in the total number of patient tested), while the SL mix detects about 1% of allergic reactions.
- (2) We did not provoke evident active sensitization using the 3 frullanolide concentrations and this could be a safe alternative to the use of the plant *Frullania* (17).
- (3) In some cases, frullanolide mix was found negative while the plant *Frullania* was positive.
- (4) 12, over the 15 patients with a relevant reaction to the frullanolide mix, were also positive to the SL mix.

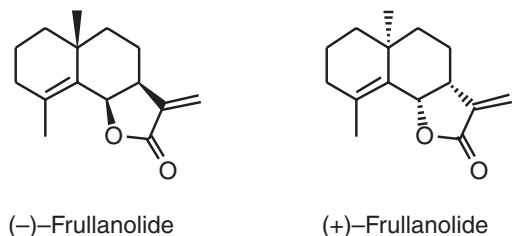


Fig. 1. Chemical structures of (+)- and (-)-frullanolide.

Table 1. Patch testing results with the frullanolide mix at 0.01%, 0.033% and 0.1% petrolatum (pet.)

Centre	Patient no.	Males/females	0.01 %		0.033 %		0.1 %		Relevant*	Non-relevant
			+	?+	+	?+	+	?+		
Malmö	838	332/506	0	0	1	0	1	0	0/1	1
Bordeaux	566	223/343	2	1	0	0	2	0	4/0	0
Barcelona	472	110/362	3	0	0	0	NT	NT	1/0	2
Bologna	335	114/221	1	0	0	0	0	1	0/0	1
Copenhagen	838	386/452	0	2	0	7	0	3	0/0	0
Oulu	482	132/350	0	2	0	8	1	0	0/0	1
Leuven	637	212/425	0	0	0	0	4	0	0/1	3
Amersham	789	265/524	0	3	0	1	0	15	0/0	0
Amsterdam	786	303/483	0	6	1	4	0	5	1/0	0
Strasbourg	1014	329/685	1	1	0	0	2	0	1/1	1
Odense	611	263/348	1	11	1	12	1	14	0/1	2
Modena	319	109/210	3	0	3	0	NT	NT	0/2	4
Louvain	461	179/282	NT	NT	1	0	1	0	0/2	0
Dortmund	407	192/215	NT	NT	0	5	NT	NT	0/0	0
San Francisco	50	20/30	NT	NT	NT	NT	0	0	0/0	0
Total	8605	3169/5436	11	26	7	37	12	38	7/8	15

*Males/females. NT = not tested.

Several parameters could explain these results. The first hypothesis could be that allergic contact dermatitis due to frullanolide present in *Frullania* is a rare event, mainly concerning foresters or people using firewood. On the other hand, we could have used too low a concentration of (+)- and (-)-frullanolide, not permitting detection of all cases of sensitization. As demonstrated in guinea-pigs, animals sensitized to the (+)-frullanolide did not cross-react to its mirror image, (-)-frullanolide, and vice versa (18). If we assume a similar response in humans, i.e. a lack of cross-reactions between the two enantiomers, (+)-frullanolide will only activate (+)-frullanolide-specific T lymphocytes. As a consequence, concentrations should be calculated with respect to each enantiomer and therefore divided by 2 (reactions to both enantiomers in humans could be explained by multiple concomitant sensitization).

The second hypothesis could be that besides frullanolide ((+)-frullanolide in *F. dilatata* (L.) Dum and (-)-frullanolide in *F. tamarisci* (L.) Dum), other allergens may be present in *Frullania* plants (Fig. 2). Cross-reactions have been demon-

strated between the SLs present in *Laurus nobilis* L. (laurel) and those of *Frullania* (18), and there can be cross-reactions with other SLs of Compositae plants, such as costunolide present in costus, isolated from *Saussurea costus* (Falc.) Lipsch.

The relevance of patch tests is sometimes difficult to establish. Thus, false-positive reactions to plants could be irritant or due to phytosanitary products, parasites and fungi. Too highly concentrated tests with chemicals can also give false-positive irritant reactions. Determination of relevance can also be difficult to establish when the patient, a farmer or florist, for example, has handled several plants of the same family or of different families, and some reactions could be due to concomitant sensitivity rather than to cross-sensitivity. Finally, plants of the same family could contain SLs, of which the chemical structure is different in the stereochemical field: there is no cross-allergy between 2 enantiomers such as the frullanolides of *F. dilatata* (L.) (Dum) and *F. tamarisci* (L.) (Dum) (19). In the same way, *Parthenium hysterophorus* L. present in North America and India contains SL, parthenin,

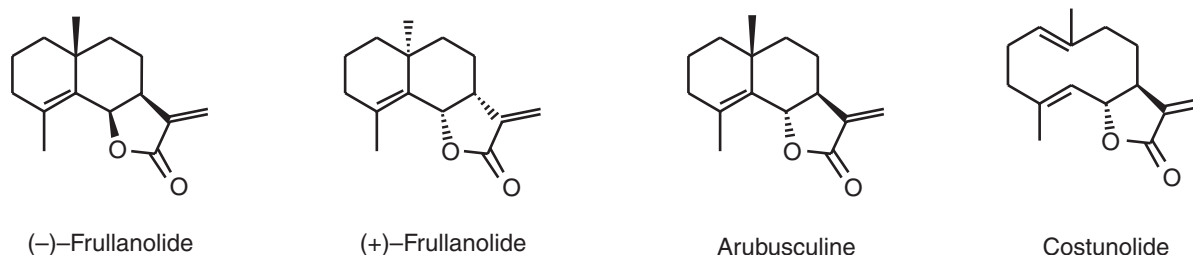


Fig. 2. Sesquiterpene lactones present in *Frullania*.

whilst the same plant in South America contains hymenin: no cross-sensitivity has been described in humans (20).

Conclusion

A frullanolide mix containing a racemic mixture of (+)- and (-)-frullanolide allowed us to detect only a small percentage of additional cases of SL sensitivity and did not represent an improvement on the SL mix. Testing with this frullanolide mix should be restricted to investigations in particular geographical areas and to particular patients exposed to *Frullania*. This frullanolide mix seems safe and not sensitizing, in contrast to patch testing with *Frullania* (17).

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