

Sesquiterpene lactone mix as a diagnostic tool for Asteraceae allergic contact dermatitis: chemical explanation for its poor performance and Sesquiterpene lactone mix II as a proposed improvement

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Summary

Background. Two preparations are currently in use for the diagnosis of allergic contact dermatitis caused by Asteraceae: (i) Sesquiterpene lactone (SL) mix [three pure sesquiterpene lactones (STLs)], whose use has been questioned, owing to an insufficient rate of true-positive results; and (ii) Compositae mix, consisting of five Asteraceae extracts, which is problematic because of lack of standardization and questionable reproducibility.

Objectives. To analyse the reasons for the narrow sensitivity of SL mix from a chemoinformatic point of view, and to propose a solution by rational selection of alternative constituents for a new SL mix II covering a broader cohort of allergic patients.

Materials/methods. Structural and biological information on allergenic STLs was retrieved from databases and the literature, and molecular modelling and chemoinformatic computations were performed.

Results. An explanation for the insufficient hit rate of SL mix is that the three constituents possess extremely similar molecular structures/properties and do not represent well the structural diversity of allergenic STLs. STLs that are known as constituents of Compositae mix plants show much a wider diversity, which explains the higher positive rate.

Conclusions. On the basis of their positions in chemical property space, a new collection of STLs that more evenly cover the overall structural diversity spectrum is proposed. SL mix II is likely to detect a larger number of patients sensitized to Asteraceae.

Key words: allergic contact dermatitis; Asteraceae (Compositae); chemoinformatics; Compositae mix; molecular modelling; sesquiterpene lactones; SL mix.

Approximately 1–2% of the population in western countries suffer from plant-induced allergic contact dermatitis, an immune hypersensitivity of the delayed

type (type IV allergy) characterized by chronic lichenified eczematous eruptions that are mainly localized in the region of contact, but sometimes also generalized exanthema and skin inflammation (1–3). It has been estimated that approximately 18 900 plant species can cause phytogetic allergic contact dermatitis (1). One very important group of plants known to cause such skin reactions is the Asteraceae family (= Compositae, daisy or sunflower family), the largest family of higher plants, with more than 20 000 species. Many members of the Asteraceae contain a particular group of terpenoids, sesquiterpene lactones (STLs). Currently, about 5000 representatives of this class of natural products are known, mostly from Asteraceae (4). STLs are known

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as the essential contact allergenic principles (haptens) of Asteraceae (5). At least 80% of the known STLs contain reactive structure elements, most frequently an α -methylene- γ -lactone moiety (found in about 60% of all STLs (4)), but also other reactive centres (6), which cause their allergenicity. Such reactive centres can covalently modify skin proteins, which are thus converted into full antigens (1–3). It can be assumed that a vast number of potential STL allergens are still uncharacterized.

Contact allergy to Asteraceae occurs upon direct contact with such plants (e.g. occupational illness of gardeners) or formulations (cosmetic and pharmaceutical) containing extracts thereof, as well as after contact with tiny plant particles (pappus hairs, and partially also pollen) in the air (airborne contact dermatitis) (1–3).

For the patient, the only possibility of avoiding the recurring disease symptoms is strict avoidance of contact with the responsible material.

Unequivocal diagnosis of Asteraceae as the cause of allergic contact dermatitis is thus an important issue. However, a variety of problems exist with respect to the composition and pharmaceutical manufacture of the diagnostic preparations.

Ducombs et al. (7) developed a mixture of three chemically pure STLs (SL mix: costunolide, dehydrocostus-lactone, and alantolactone) (Fig. 1), each applied at a concentration of 0.033% in petrolatum), which was used as the test allergen of choice for patch test diagnosis for about a decade. On the basis of several multicentre studies in Europe, a remarkable number of sensitizations could be diagnosed with this standard SL mix, whereas irritant test reactions and active sensitizations were rare [for reviews, see (2, 3)]. However, it was not possible to cover all contact allergies against Asteraceae with the SL mixture. Green and Ferguson reported that only 35% of cases of Compositae allergy tested positive with SL mix (8), and Goulden and Wilkinson (9) reported a failure rate of almost 40%. This lack of efficacy was also confirmed by a multicentre study conducted by the Information Network of Departments of Dermatology in Germany (10)). According to Le Coz and Ducombs (3), only ~ 30% of all cases of sensitization can be detected with SL mix.

As an alternative test allergen the so-called Compositae mix [originally a mixture of ether extracts of five domestic Asteraceae species: *Arnica montana* (European arnica), *Matricaria recutita* (German chamomile), *Tanacetum parthenium* (feverfew), *Tanacetum vulgare* (common tansy), and *Achillea millefolium* (yarrow)] was therefore recommended and reported to identify a considerably wider cohort of patients than SL mix (10, 11).

The preparation of short ether extracts of these plants without further specification of their (exact botanical as well as geographical) origin and specifications for a standardized manufacturing protocol and chemical profile must be considered problematic from the pharmaceutical standpoint, because reproducible quality and diagnostic efficacy are not guaranteed. These problems would require very detailed studies for each of the constituent plants in order to guarantee reproducible quality and efficacy of the preparation, as the qualitative and quantitative composition is subject to an incalculable number of different influences. Because of these and further problems, the use of Compositae mix is still controversial. It is obvious that the use of a clearly defined mixture of unambiguously characterized pure compounds such as SL mix for patch testing is definitely preferable from the viewpoint of pharmaceutical quality. We have therefore addressed the question of why SL mix in its present form leads to the high false-negative rate by considering the structural and physicochemical properties of its constituents. We present a simple explanation for this apparent inefficiency, based on the high degree of structural similarity in terms of molecular recognition. Furthermore, on the grounds of chemoinformatic calculations, we propose a better selection of six STLs as constituents for a new diagnostic SL mix II.

Materials and Methods

All computations were performed with the program package Molecular Operations Environment (MOE, v. 2010.10, (12)).

The chemical structures of 4861 STLs had been retrieved from the Dictionary of Natural Products on CD Rom (v. 10:2 (13)) and combined in a database of two-dimensional (2D) structures (4). Three-dimensional (3D) molecular models of the constituents of SL mix, as well as of the STLs occurring as potential allergens in the five constituent plants of Compositae mix and of those known as constituents of allergenic Asteraceae as summarized by Hausen and Vieluf (1), were generated by performing a low-mode dynamics conformational search (standard settings) on initial 3D models with the MMFF94x forcefield and energy minimization of the lowest-energy conformer with the Austin Model 1 Hamiltonian as implemented in MOE/MOPAC. The optimized geometries were combined in a 3D molecular database.

Chemoinformatic data analysis of structural information of potentially allergenic STLs was performed by means of multivariate statistics: calculation of molecular descriptors (2D and/or 3D descriptors (12)), principal-component analysis (PCA) and graphical analysis of the results were performed with MOE.

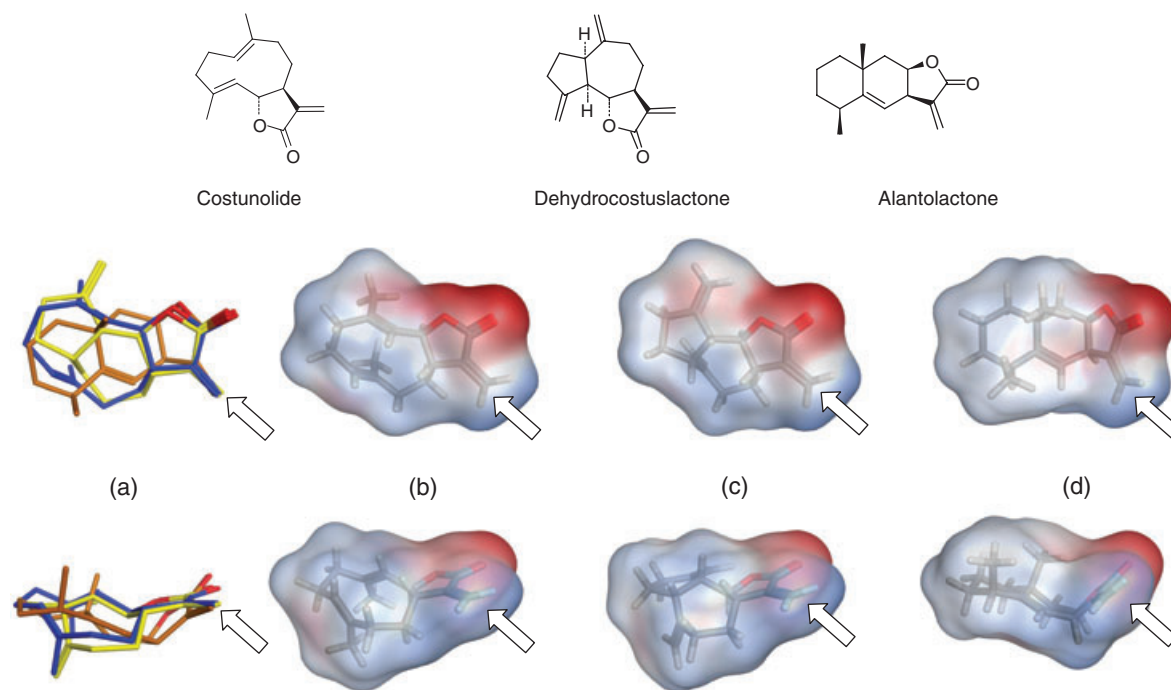


Fig. 1. Two-dimensional (2D) and three-dimensional (3D) molecular structures of the constituents included in SL mix (costunolide, dehydrocostuslactone, and alantolactone). (a–d) 3D molecular structures, shown from two different viewing angles (top and bottom row, respectively). (a) Superposed 3D structures (only carbon and oxygen shown). Blue: costunolide. Yellow: dehydrocostuslactone. Orange: alantolactone. (b–d) Molecular surface representations of the respective molecules, colour-coded according to electrostatic potential (blue, positive; red, negative). The arrows denote the reactive β -carbon atom of the α -methylene- γ -lactone site responsible for formation of full antigens.

A full list of descriptors taken into consideration and a file with 3D molecular structures are available from the corresponding author on request.

Results

In order to elicit a type IV hypersensitivity immune reaction, a fragment of a protein covalently modified by reaction with a hapten must be presented by major histocompatibility complex molecules on the surface of Langerhans cells, where it is eventually recognized by T cells, which are activated to proliferate and direct their defensive activity against this modified alien peptide. Most importantly, a subpopulation of memory cells with the same specific T cell receptor recognizing the same epitope is formed, so that a strong immune reaction is elicited in the case of renewed contact with the antigen (14). Thus, the molecular basis for T cell activity in allergic contact dermatitis is a relatively slight structural modification of a skin protein by covalent addition of a small hapten molecule. It is important to note at this point that the cross-sensitivity between two haptens must depend on the capability of T cell receptors to recognize both haptens,

that is, on the chemical similarity between them. This has been demonstrated in several instances for STLs and related haptens [for overview and illustrations, see (4); for a recent review on stereospecificity of cross-reactivity, see (15)]. Although only a few systematic studies on structure–cross-sensitivity relationships among STLs exist, it is safe to assume that a component of a diagnostic preparation will provoke a positive reaction only in a patient who has been sensitized to a structurally similar hapten.

SL mix is redundant

Figure 1 shows the structural diagrams and 3D molecular structures of the constituents of SL mix, costunolide, dehydrocostuslactone, and alantolactone. Inspection of the 3D representations and comparison of various structural and physicochemical properties (Table 1) shows that the molecular structures are very similar to each other.

In terms of molecular recognition, these three compounds would present a very similar picture to an immune receptor when presented as part of a hapten–peptide conjugate. It can hence be hypothesized, as a very likely reason for the low positive rate of SL

Table 1. Comparison of structural and physicochemical properties of the constituents of SL mix with those of 49 known allergenic sesquiterpene lactones (STLs) and with those of six STLs proposed as constituents for SL mix II

Descriptor	Costunolide	Dehydrocostuslactone	Alantolactone	Forty-nine allergenic STLs		SL mix II	
				Minimum	Maximum	Maximum	Minimum
Molecular weight	232.3	232.3	230.3	230.3	424.4	232.3	410.4
nO ^a	2	2	2	2	9	2	7
Slogp ^b	3.55	3.02	3.24	−0.13	4.34	0.95	3.55
logS ^c	−3.02	−2.97	−3.95	−3.95	−1.33	−3.71	−1.98
SMR ^d	6.85	6.62	6.63	6.62	10.52	6.80	10.52
ASA ^e	434.4	432.0	430.9	430.9	631.6	432.1	620.1
ASA+ ^f	270.8	272.2	292.5	241.0	388.4	241.0	366.6
ASA− ^g	163.7	159.8	138.4	133.8	251.6	163.7	251.6
ASA _H ^h	368.0	322.4	342.3	255.2	462.5	255.2	416.5
ASA _P ⁱ	66.4	109.6	88.6	58.1	253.8	66.4	253.8
stdim1 ^j	2.42	2.39	2.34	2.18	3.80	2.18	3.80
stdim2 ^k	1.62	1.75	1.63	1.56	2.22	1.62	2.08
stdim3 ^l	1.14	0.96	1.14	0.77	1.81	0.77	1.27
glob ^m	0.22	0.16	0.24	0.06	0.47	0.06	0.24
AM1_dipole ⁿ	5.08	4.76	4.57	1.67	9.15	3.84	7.70

^{a–n}Descriptors calculated in MOE (12) from optimized three-dimensional molecular models:

^aNumber of oxygen atoms.

^bOctanol-water partition coefficient.

^cLog of aqueous solubility.

^dMolar refractivity.

^eASA, water-accessible surface area.

^fFraction of ASA attributable to atoms with positive partial charge.

^gFraction of ASA attributable to atoms with negative partial charge.

^hFraction of ASA attributable to hydrophobic regions.

ⁱFraction of ASA attributable to polar regions.

^jStandard dimension 1 (largest molecular extension).

^kStandard dimension 2 (second largest molecular extension).

^lStandard dimension 3 (third largest molecular extension).

^mGlobularity (linear molecule = 0; perfect sphere = 1).

ⁿDipole moment calculated with the Austin Model 1 (AM1) method.

mix, that the only patients who would react to this mixture would be those who were sensitized to STLs with cross-sensitivity (and hence structural similarity) to this assembly of three very similar STLs. On the same grounds, it can be concluded that the composition is redundant; that is, a similar rate of positive diagnoses could be expected with any of the three compounds on their own.

Although animal experiments have indicated that cross-sensitivity between alantolactone and costunolide may be low (only 1 of 10 guinea pigs sensitized to the former cross-reacted to the latter) (16), it has to be noted that cross-sensitivity appears to be not absolute; that is, an individual component of cross-sensitivity (at least in part explained by the variability in T cell receptors between individuals) is likely to be involved, and this has been demonstrated for STLs in several instances. Thus, for example, cross-reactions between parthenolide (the major STL of feverfew) and SL mix have been shown to exist, but a number of patients reacted only to either one of these preparations (17).

SL mix does not represent well the entirety of potentially allergenic STLs

Among the approximately 5000 known STLs, about 4000 are potentially allergenic, as can be deduced from the presence of at least one reactive structure element capable of reacting with proteins (4). This huge number of compounds is characterized by considerable structural diversity.

Chemical diversity can be handled, measured and explained with chemoinformatic methods. A molecule can be characterized by an almost infinite number of descriptors that represent particular structural, chemical or physical properties. Many descriptors are known to be important with respect to molecular recognition phenomena (18).

If a series of n descriptors (molecular features) is extracted from a set of m molecular structures, their similarity/diversity in terms of the considered $n \times m$ descriptor matrix can be thought of as proximity/distance in an n -dimensional feature space. Principal component analysis

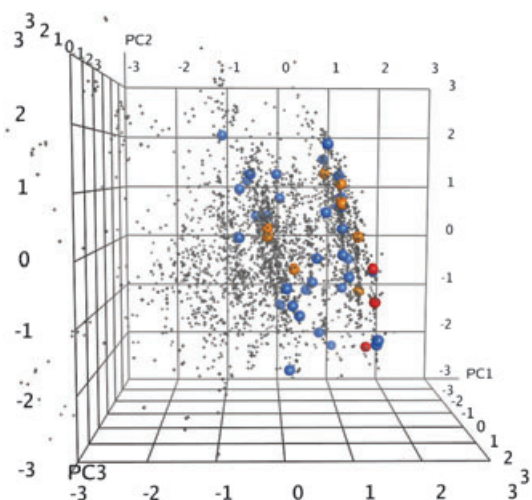


Fig. 2. Structural feature space of 3954 potentially allergenic sesquiterpene lactones (STLs). Coordinate axes represent the first three principal components (PCs) derived from 164 descriptors of two-dimensional molecular graphs, and, together, account for 63% of the total variance in structural information. Each point denotes the position of a particular compound; Red spheres: constituents of SL mix. Blue spheres: 49 known allergens extracted from the literature (1). Yellow spheres: STLs that are constituents of Compositae mix plants. Note that, owing to overlap, not all blue and yellow spheres are visible.

(PCA) then offers the possibility of mathematically reducing the complexity of this feature space to a similarity space with fewer dimensions that is more easily understandable (19).

Figure 2 shows the chemical similarity space covered by the structures of 3954 potentially reactive (i.e. potentially allergenic) STLs. The coordinate axes represent the first three principal components derived from a multi-dimensional feature space combining the information included in 164 descriptors derived from 2D molecular graphs (full list available on request). These three principal axes account for 63% of the overall variance (or information content) of the descriptor matrix, so that it can be safely assumed that the major part of information with respect to structural similarity/dissimilarity is included. Each dot represents the position of a particular STL in this chemical similarity space, such that the (Euclidean) distance of two dots is a simple relative measure of similarity/dissimilarity. The positions of costunolide, dehydrocostuslactone and alantolactone are highlighted as red spheres in Fig. 2. Obviously, the distances between them are quite small, so that only an extremely small region of the overall structural diversity of STLs is covered by these three constituents of the SL mix.

STLs that are known to occur in the five Compositae mix plants are highlighted in yellow. It is straightforward

to explain the higher rate of true-positive diagnoses obtained with this preparation than with SL mix by the higher degree of structural variance covered by these compounds. Thus, the reproducibility problems associated with Compositae mix described above might be circumvented by using a defined mixture of these pure constituents. However, the coverage of the structural diversity with respect to all potentially allergenic STLs would still not be optimal.

Figure 2 also shows the positions of 48 STLs mentioned as known constituents of Asteraceae which reportedly (1) cause allergic contact dermatitis (highlighted as blue balls). A much larger fraction of the similarity space, representative of the majority of the almost 4000 structures, is covered by these documented allergens.

We would therefore propose, on these grounds, the inclusion of a set of structurally diverse compounds covering more evenly the major regions of STL structure space in a second-generation SL mix.

SL mix does not represent well the structural diversity of known STL allergens

The structural considerations up to this point are based on descriptors that can be derived from 2D structural diagrams, that is, from flat molecular graphs that do not take stereochemistry in account. Three-dimensional information (molecular shape, charge distribution, etc.) must naturally play an important role in molecular recognition, and has been shown to be very important in STL allergy. Many STLs possess considerable numbers of asymmetric centres, and, in many cases, they are conformationally flexible molecules. This is exemplified by the observations of enantiomer-specific as well as diastereomer-specific allergic contact dermatitis (for reviews, see (4, 15)).

In order to rationally select a few representative compounds for a new diagnostic formulation, it appeared important to perform a more realistic molecular comparison that explicitly takes into account molecular properties that directly depend on 3D structure. To this end, 3D molecular models of the subset of 49 reported contact allergenic STLs were generated, and a variety of 120 descriptors dependent on 3D structure were calculated (full list available on request). A PCA was performed on this matrix of 3D descriptors. Figure 3 shows the similarity space defined by the first three principal components, which represent 65% of the total variance in the descriptor matrix. Once more, the positions of the SL mix components are shown in red, and those representing constituents of Compositae mix extracts are shown in yellow. As above, the range of diversity covered by the SL mix compounds is very small; that encompassed by the Compositae mix constituents is more representative, but still not optimal.

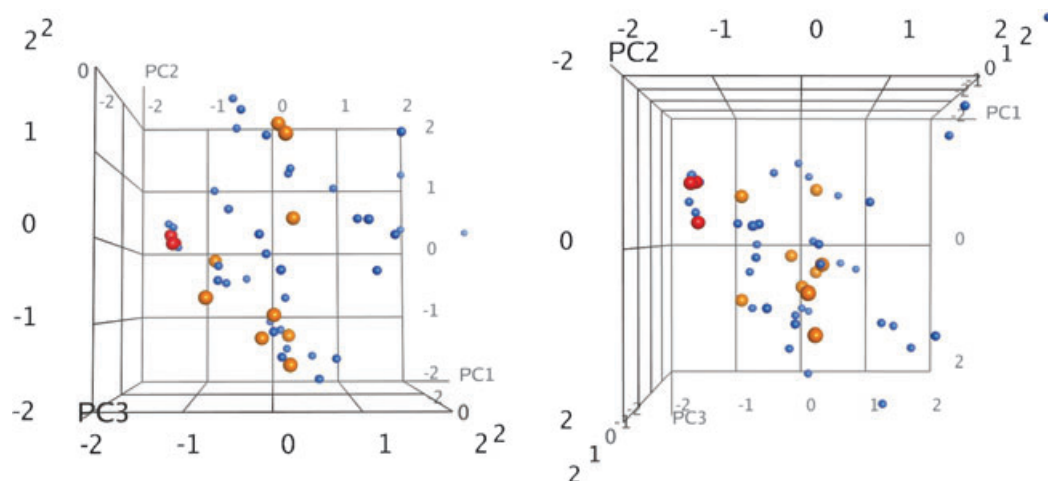


Fig. 3. Structural feature space of 49 known allergenic sesquiterpene lactones extracted from the literature (1), viewed from two different positions, along the third (left) and second (right) principal component (PC) axes, respectively. Coordinate axes represent the first three PCs derived from 120 descriptors calculated from optimized three-dimensional molecular structures; the first three components account for 36%, 19%, and 11%, respectively, of the total variance in structural information. Each sphere denotes the position of a particular compound in feature space; Red spheres: constituents of SL mix. Yellow spheres: constituents of Compositae mix plants. Note that some spheres may not be visible, owing to overlap.

Design of a more diverse and comprehensive diagnostic SL mix 2

On the basis on these calculations, a new collection of STLs covering more thoroughly the major regions of this space can be proposed. The compounds for SL mix 2 have to be chosen in such a way that the possibility of redundancy is minimized, that is, so that a minimum amount of cross-sensitivity between them and a maximum amount of cross-sensitivity with respect to all other potential allergens is to be expected on this rational basis.

Apart from this first criterion, the selection must furthermore be guided by the compounds' availability: the plant material from which each compound is to be isolated must be easily accessible, and the content of the particular compound in this plant material should be relatively high, so that it can be isolated in sufficient quantities to warrant its use in an economically feasible manner.

Moreover, in order to cover the possible range of structures in a more or less even and symmetric manner, the number of constituents in SL mix II is proposed to be increased to six.

On the basis of these considerations, five new compounds (positions highlighted in Fig. 4) were chosen that cover the major part of the overall feature space when combined with costunolide (**1**), one of the constituents of SL mix. These compounds are helenalin (**2**, from *Arnica* and *Helenium* species), artemisinin (**3**, from *Chrysanthemum* species), lactucopicrin [**4**, from *Lactuca* (lettuce) and *Taraxacum* species], nobilolide (**5**, from *Chamaemelum*

nobile, Roman chamomile), and cnicin (**6**, from *Cnicus benedictus*); for structures, see Fig. 5.

A sensitizing potential has been attributed to each of these compounds, and in all cases the plants containing them have been reported to show cross-reactivity with several other Asteraceae, so that it can be expected that each of them will contribute to the overall efficacy of such a new test allergen. Their range of molecular/physicochemical properties represents much better the overall spectrum of the 49 known allergens (compare Table 1 and Fig. 4).

Apart from costunolide, which is sufficient on its own to cover the range of many small and hydrophobic STLs, the further constituents would cover their own regions of STL structural space previously not represented in SL mix. It is noteworthy that helenalin contains two potentially allergenic structure elements (methylene lactone and a cyclopentenone moiety), and may thus possibly be considered as two allergens in one. Artemisinin is a constituent of *Chrysanthemum* species, which are among the most frequent causes of occupational contact allergy observed in gardeners. Interestingly, according to the literature, *Chrysanthemum indicum* (syn. *Dendranthema indicum*) shows a considerable number of cross-reactions with other Asteraceae (1). With respect to chemical similarity, artemisinin occupies a quite central position with respect to the most important first two principal-component axes, and is close to a variety of other STLs, so that it can be expected to show a relatively

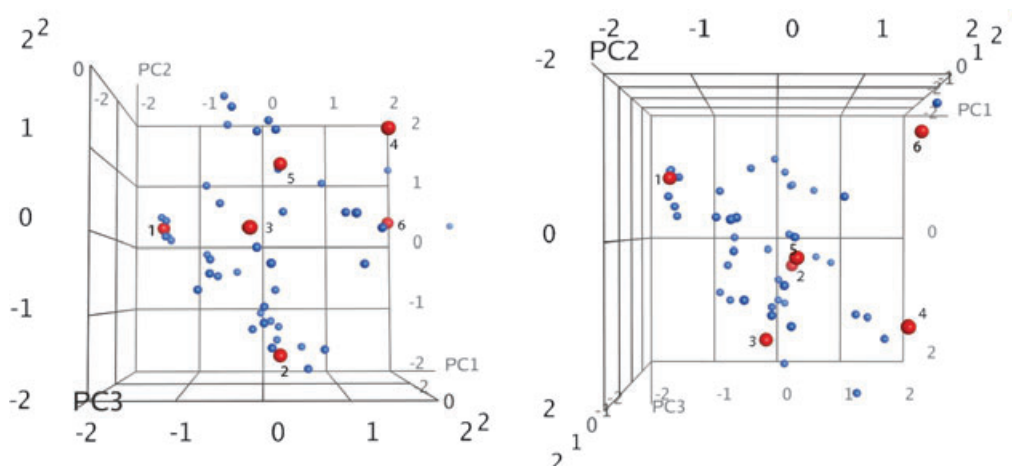


Fig. 4. Structural feature space of 49 known allergenic sesquiterpene lactones extracted from the literature (1), viewed from two different positions as in Fig. 3. Note that some spheres may not be visible, owing to overlap. Red spheres: proposed constituents of SL mix II. 1, costunolide; 2, helenalin; 3, arteglaasin A; 4, lactucopicrin; 5, nobilin; 6, cnicin. PC, principal component.

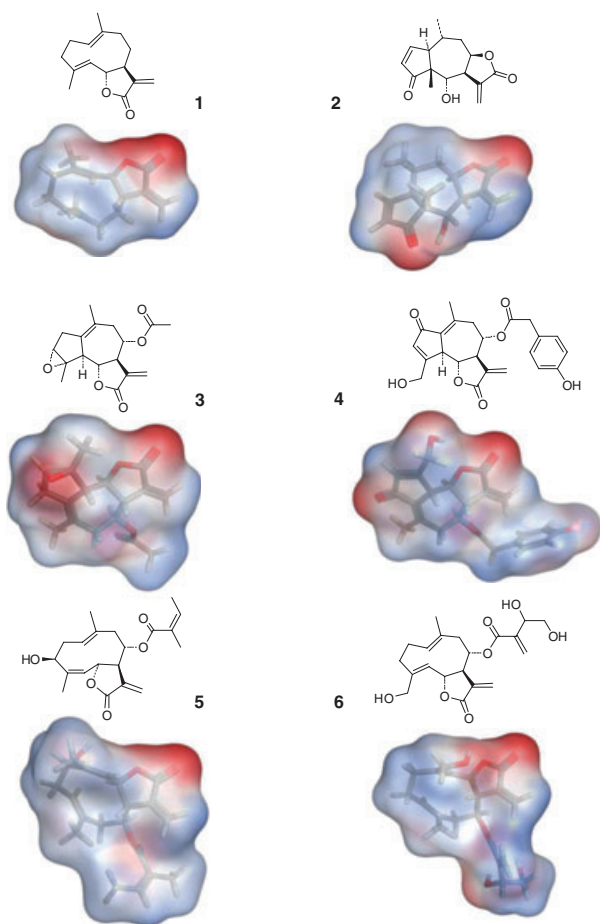


Fig. 5. Molecular structures of proposed constituents of SL mix II. 1, costunolide; 2, helenalin; 3, arteglaasin A; 4, lactucopicrin; 5, nobilin; 6, cnicin.

high rate of cross-reactions. This compound is therefore very likely to be an excellent constituent in a test preparation.

The three other compounds, lactucopicrin (**4**), nobilin (**5**), and cnicin (**6**), differ from those already mentioned by having considerably larger side chains with quite different steric and hydrophilic/hydrophobic properties. Lactucopicrin, with its aromatic ester moiety, is quite unique within the assembly of compounds under study, and occupies a part of the structural space that is not very densely populated. However, it is not known how many further, hitherto undescribed, allergens with similar properties exist (a phenylacetic acid moiety occurs in 31 compounds included in our database of 3954 potentially allergenic STLs), so that we would tend to include lactucopicrin in our proposed preparation.

Nobilin and cnicin, in spite of possessing very similar STL core structures, represent two extremes with respect to the properties of their ester groups, which constitute a considerable fraction of the total molecule. The angeloyl moiety of the former is quite hydrophobic, whereas the dihydroxyethyl-acryloyl moiety of the latter is very hydrophilic. The angeloyl group of **5** or isomeric tigloyl and seneciyl moieties occur in almost 800 of the 3954 potentially allergenic STLs, whereas diol acid side chains, such as in **6** or similar, occur less frequently, but can still be found in over 80 of the 3954 STLs. Thus, each of them would be representative of a significant group of STLs with similar side chains presented as part of the whole molecule to T cell receptors recognizing such epitopes. It is possible that ester groups are hydrolysed

in the skin by epidermal esterases, which would lead to similar core STL structures for **5** and **6**. However, it can be expected that esteric cleavage will only lead to partial decomposition. Furthermore, the α,β -unsaturated ester groups in **5** and **6** each represent an additional potentially alkylating structure element, rendering these compounds dual haptens. Thus, in both cases, reaction with the STL core or with the side chain (or with both) may occur, leading to very different epitopes, thus increasing the actual diversity of haptens in the test mixture.

The mixture thus designed is expected to reliably detect at least as many, or more, patients sensitized to Asteraceae STLs than Compositae mix. In contrast to the latter, it can be produced from chemically defined constituents, and would thus be clearly superior from the pharmaceutical standpoint. Its evaluation in clinical patch test studies is warranted, and will soon be initiated.

Conclusions

On the basis of thorough consideration of the constituents' molecular structures, it is possible to explain the inadequacy of SL mix and the higher rate of true-positive detection of patients suffering from Asteraceae allergy by Compositae mix. On this background, it is expected that the current unsatisfactory situation with two test preparations – one pharmaceutically and chemically well defined but showing poor performance, and the other showing better efficacy but suffering from numerous shortcomings with respect to pharmaceutical quality – can be overcome with the help of our newly proposed test allergen, SL mix II.

Acknowledgements

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