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ALLERGIC CONTACT DERMATITIS TO COSTUS: REMOVAL OF HAPTENS WITH POLYMERS

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Abstract. Costus oil, an iol isolated from a Composite, *Saussurea lappa* C., used in perfumery, is responsible of numerous cases of allergic contact dermatitis (ACD). The haptens incriminated are sesquiterpene lactones, costunolide and dehydrocostuslactone. These lactones can be removed from the oil by using a polymer, aminoethyl-polystyrene, to which they become bound: the passing solution is lactone-free. A group of guinea-pigs could be successfully sensitized (Freund Complete Adjuvant Test) to costus essential oil, while another group, injected in the same way with polymer-treated essential oil (i.e. lactones) could not be sensitized. This polymer therefore provides a means to render complex plant extracts non-allergic.

Key words: α-Methylene-γ-butyrolactone haptens; Guinea pigs experimental sensitization

Since ancient times, roots of *Saussurea lappa*, Clarke (a plant of the Compositae family growing in western regions of the Himalayan mountains) have been used in the Orient for all kinds of diseases and for perfumery purpose (Arctander, 1960). On steam distillation, the dried roots yield a viscous liquid of light brown colour, having a very peculiar and very persistent odour.

Chemical investigations of Costus extracts have shown the presence of several relevant constituents (1, 3, 5, 10, 13, 16, 17, 18) and in particular two sesquiterpence lactones (16): costunolide I and dehydrocostus lactone II (2, 12, 20, 21) (Fig. 1).

Dermatologists' interest in Costus root oil started when facial contact dermatitis was reported in Japanese women. Contact sensitivity to Costus oil has been studied by Mitchell (14, 15), who attributes the sensitizing properties of the oil to costunolide and dehydrocostus lactone. It was observed that an important prerequisite for immunologic reactivity was the presence of an exocyclic methylene group conjugated to the γ-butyrolactone. Cross-sensitivity studies in Costus-sensitized patients (6, 15) showed that the presence of a conjugated methylene group was not the sole precondition for a cross reaction and that the

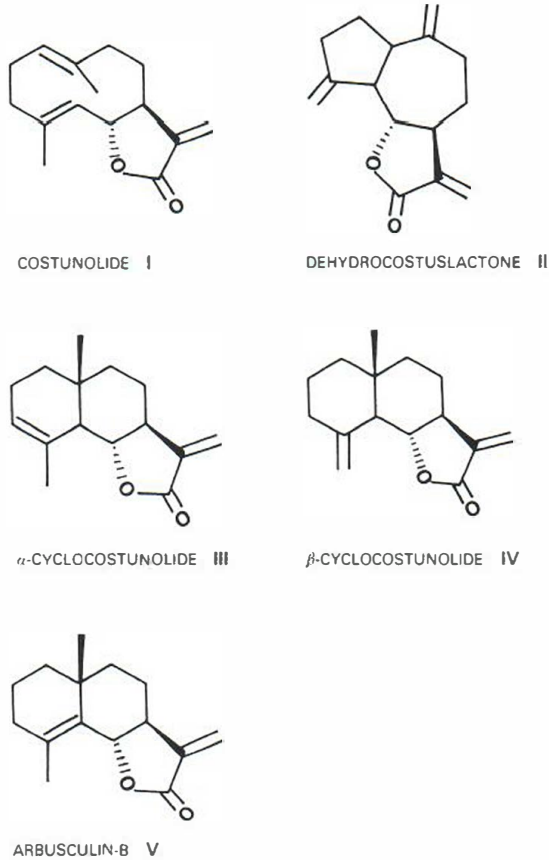
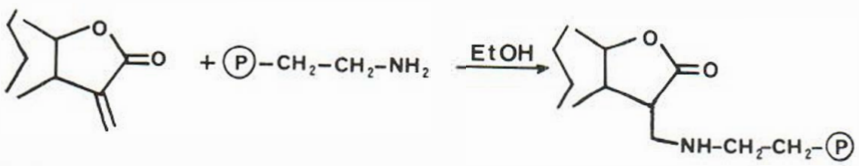


Fig. 1.



Scheme I

skeletal group of the cross-reacting lactones and substituents such as -OH or -OAc, especially at C_8 or C_6 positions, could also influence their sensitizing power.

One of the main problems in cosmetic industry is the possible sensitizing properties of the products which contain plant extracts such as Costus oil. We thought it would be extremely useful to find a way of suppressing allergic contact dermatitis (ACD) by some chemical means.

Since the mechanism of ACD to α-methylene-γ-butyrolactones is thought to proceed through the formation of a covalent bound with skin proteins, we thought that insoluble functionalized polymers such as aminoethylpolystyrene P-CH_2-CH_2-NH_2 (P = polystyrene gel) could be used to bind lactones as shown in Scheme I. Thus the filtered solution would be deprived of the lactones.

This paper describes our procedure and the study in an animal model (guinea pigs) of experimental sensitization to costus essential oil and to aminoethylpolystyrene-treated costus essential oil.

MATERIAL AND METHODS

Costus oil and costus essential oil were obtained through Roure & Bertrand, Grasse, France. Costus oil was obtained from *Saussurea lappa* C. roots by solvent (benzene)extraction. The essential oil was obtained by steam-distillation of the roots.

The essential oil (2 g) was stirred with the polymer (1 g) in ethanol solution (2.5 ml) for 3 days at room temperature. The polymer (containing the bound lactones) was filtered off, washed with ethanol; ethanol was removed and the remaining product was the 'polymer-treated essential oil'. This oil was used to sensitize group II guinea pigs (see below).

As vapour-phase chromatography showed that after one treatment with the polymer, about 1% of the initially present dehydrocostus lactone II (the main sensitizer) still remained, a second treatment identical with the first gave the polymer-treated essential oil which was used for skin-test purposes.

Guinea pig sensitization

Inbred female Himalayan spotted guinea pigs (Füllingsdorf, Switzerland) weighing 300 to 500 g were used. The animals were fed daily with vitamin C rich pellets, with lettuces and carrots for supplementation.

Sensitization was achieved by Freund's Complete Adjuvant Test (FCAT) (9). Three groups, each containing 8 animals, were used. Each animal received five intradermal injections in the nuchal region, of 0.1 ml emulsion, on each alternate day, for 10 days.

Group I received an emulsion made up of 30% crude costus essential oil in a 1 : 1 FCA–saline mixture.

Group II was injected with an emulsion of 30% polymer-treated costus essential oil in a 1 : 1 FCA–saline mixture.

Group III received an injection of a 1 : 1 FCA–saline emulsion. This group was used as FCA-treated controls.

Skin testing

Provocation was conducted on the 21st day after the beginning of sensitization, by means of open epicutaneous tests. 25 μl of various ethanol solutions was deposited on the clipped and shaved flank of the animals, on a 2 cm^2 area.

Readings were made at 24 h after skin testing. The grading scale used was: 0, no reaction; 0.5, slight erythema covering part only of the test area; 1, erythema covering all the test area; 2, erythema with infiltration and induration covering the test area; 3, intense erythema and induration spreading well beyond the test area.

The following concentrations were used: crude and polymer-treated costus essential oil, 10%; resin-bound substances, 1 & 0.3%; dehydrocostus lactone, 1%; costunolide, 1%.

Before any sensitization was carried out, irritation thresholds (primary toxicity) were determined.

RESULTS

The costus oil (or essential oil) was stirred for 3 days in the presence of aminoethylpolystyrene, the polymer was filtered off and the filtrate evaporated. We were able to recover the polymer-bound lactones through chemical treatment (4*b*). All the fractions were analysed by vapour-phase and thin-layer chromatography and pure products identified by nuclear magnetic resonance and mass spectrometry. The principle of the procedure is shown in Scheme II; the chemical composition of each recovered mixture is shown in Table I.

These results show that dehydrocostus lactone II

Table I. *Composition of lactone mixtures removed from polymer*

Polymer-removed products from	% in crude extract	Composition of mixtures				
		II	III	IV	V	Unidentified
Essential oil	13	80	8	2	–	10% unknown lactone
Oil	40	63	14	20	3	–

II: dehydrocostuslactone. III: α-cyclocostunolide. IV: β-cyclocostunolide. V: Arbusculin-B.

is the major product in both the oil and the essential oil; α- and β-cyclocostunolide (III and IV) and Arbusculin-B (V) have also been detected, especially in Costus oil, and are acidic promoted rearrangement products of costunolide I (8). Such a rearrangement occurs during the removal procedure of the bound lactones (4*a*, *b*).

To study the sensitizing power of the crude and the polymer-purified essential oil, three groups of guinea pigs were used. One was sensitized to crude costus essential oil, another to polymer-purified essential oil, and the third was used as FCA-treated controls. Results of tests are summarized in Table II.

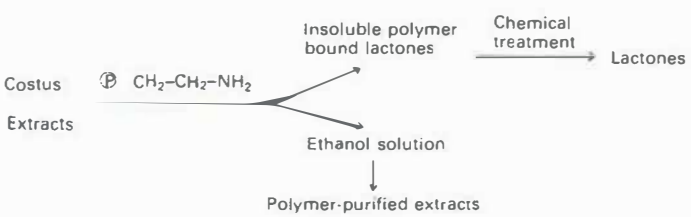
These results clearly show that (i) the animals had been sensitized to crude costus essential oil but not to polymer-treated essential oil; (ii) the polymer-removed products (essentially α-methylene-γ-butyrolactones) are allergenic; (iii) sensitized animals also prove positive to dehydrocostus lactone, but do not react to costunolide.

DISCUSSION

Comparison to the results in group I and group II showed that the polymer-treated essential oil had lost its sensitizing power. The use of insoluble aminoethylpolystyrene is thus an efficient way of rendering costus extracts non-allergic. Moreover, the products which were recovered from the polymer were shown to be essentially α-methylene-γ-butyrolactones. Although this specificity is surprising, it can be easily understood; α-methylene-γ-butyrolactones are extremely reactive products (11, 19) and react very well with amines, $R\text{-}NH_2$.

The substances removed from the polymer gave a stronger reaction than pure dehydrocostus lactone on group I sensitized guinea pigs. This could be understood if one considers that costus essential oil contains several sesquiterpene lactones and we might well be observing a polysensitization reaction. This would explain why the reaction to a single component (the major onel i.e. dehydrocostus lactone) can be weaker.

Note the weak reaction to polymer-bound substances (4/8 guinea pigs gave a weak 0.5 test), to dehydrocostus lactone (1/8 guinea pig), and to untreated essential oil (2/8 guinea pigs) in group II. This can be explained in the following manner. Group II was sensitized to costus essential oil which had been treated once with the polymer. Gas chromatography of this treated oil showed that about 1% of the initially present dehydrocostus lactone was unbound and probably induced the light sensitivity observed. A second treatment of the oil removed all traces of lactones completely.



Scheme 2. Polymer purification of Costus extracts.

Table II. *Results of open epicutaneous tests on guinea pigs*

Animals challenged with[a]	Conc. %	Guinea pigs sensitized with: Group I: Crude costus essential oil — Tests 2	1	0.5	0	Aver.	Group II: Polymer-treated essential oil[d] — Tests 2	1	0.5	0	Aver.	Group III: controls
Substances removed from polymer	1	4[b]	4	0	0	1.5	0	0	4	4	0.25	0
	0.3	0	4	3	1	0.7	0	0	1	7	0	0
Dehydrocostus lactone	1	2	5	1	0	1.2	0	0	1	7	0	0
Costunolide	1	0	0	0	8	0	0	0	0	8	0	0
Treated essential oil[c]	10	0	0	0	8	0	0	0	0	8	0	0
Non-treated essential oil	10	1	6	1	0	1.1	0	0	2	6	0	0

[a] Tests were performed using ethanol solutions. Grading scale: 0, no reaction; 0.5, slight erythema covering part only of the test area; 1, erythema covering all the test area; 2, erythema with infiltration and induration covering the test area.
[b] Number of animals with a positive test intensity 2 . . .
[c] The essential oil was treated twice with the polymer before the tests.
[d] The oil was treated once with the polymer for sensitization.

Even if only sesquiterpene lactones have been isolated and identified, this does not preclude the presence of small amounts of other sensitizers. Thus, α,β-unsaturated aldehydes such as costal or elemal have been detected (13) in costus oil. Such products are probably also bound to the polymer by the same reaction depicted in Scheme I but removed in amounts too small to be detected.

The lack of a positive reaction to costunolide is worth noting. It shows that no costunolide (or only a very small trace proportion) is present in costus essential oil, while it has been detected in costus oil. This can be explained by the relative instability of costunolide which can be transformed into other lactones through acidic treatment (8) and probably undergoes skeletal changes and degradation during steam distillation. This is evidenced for instance by the vapour-phase chromatogram where the costunolide peak is extremely broad, indicating heat decomposition in the column. Gas chromatography also shows that α- and β-cyclocostunolides are present in crude essential oil and do not derive from costunolide removal in this case.

The lack of positive reaction to costunolide also confirms the specificity observed in cross-sensitivity testing to sesquiterpene lactones. Animals sensitized to dehydrocostus lactone—a guaianolide (21)—do not react to costunolide, another α-methylene-γ-butyrolactone with a different skeleton (eudesmanolide).

CONCLUSION

The use of insoluble functionalized polystyrene is an effective way to render costus oil non-sensitizing.

Dehydrocostus lactone II (major product), α- and β-cyclocostunolides III and IV have been identified. The latter two lactones are present in small amounts in essential oil. In the oil, these two products derive from costunolide I cyclization occurring during the chemical removal of lactones from polymer.

The method described here could be generalized to other plant extracts containing α-methylene-γ-butyrolactones.

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