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ALLERGENS IN SESAME OIL CONTACT DERMATITIS

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Abstract. In 13 patients with contact allergy to sesame oil, studies were undertaken to elucidate the nature of the allergens. Sesamol, sesamin and sesamolin were identified in crude and purified (pharmaceutical) sesame oil. Patch tests showed 8 of the 13 patients to be positive to sesamol and 12 to sesamolin and sesamin. Patch tests with the pure substances on thin-layer sheets were inconclusive as to any difference between these substances. Group allergy to several substances related to sesamol could not be clearly demonstrated.

Key words: Sesame oil; Allergens; Contact dermatitis; Patch tests; Chromatography

Recently we described 15 patients with contact hypersensitivity to sesame oil. They were elderly people, mainly female, from a series of 98 patients with leg ulcers and eczema. All had been treated for many years with Linimentum Zinci Oxydi Oleosum (Neth. P.) or other sesame oil containing preparations (2). In an other series of 84 patients with leg ulcers, 15 appeared to be allergic to sesame oil (4). As was shown, the sensitizing factors could be located in the unsaponifiable part of the oil (2). This fraction constitutes about 2% of the crude oil and contains sesamol, sesamolin and sesamin (1). (Fig. 1). Sesame oil used for pharmaceutical purposes contains about 0.002% sesamol and sesamolin and about 0.27% sesamin (3). Sesamol is an antioxidant; it is for this reason that sesame oil is relatively stable. Sesamol and sesamolin are used, for example, as a synergist in pyrethrin containing insecticides. Our investigations were concentrated on these three substances, each one a possible allergen in sesame oil contact dermatitis.

MATERIAL AND METHODS

Crude sesame oil was obtained from the "Golden Wonder" factory at Zwolle, the Netherlands. The purified pharmaceutical oil is prepared by them for the pharmaceutical retailers. The sesamol used was No. 18032 from the catalogue "Rare and fine chemicals" K & K, Plainview, New York, USA. A sample of sesamin was kindly presented to us by Dr J. A. Durden, South Charleston, USA.

1. Preparation

Sesamolin and sesamin were prepared in two different ways.

(*a*) Crude sesame oil, which contains much more sesamin and sesamolin than the purified oil, was extracted with an equal portion of methanol at 40°C. The solvent was evaporated and the residue treated with pentane, in which sesamin and sesamolin do not dissolve.

The two substances were separated by thin-layer chromatography according to Stahl (7). Location: UV light 254 nm.

(*b*) The crude oil was dissolved in a mixture of hexane/ether 95 : 5, and led over a silica gel column. The triglyceride fraction was eluted with the same mixture. Then the polar lipids, sesamin and sesamolin were eluted with hexane/ether 50 : 50. The eluate was evaporated and the residue treated with hexane, in which sesamin and sesamolin do not dissolve. They were isolated by preparative thin-layer chromatography, on silica gel plates, with benzene/acetone 97: 3 as solvent.

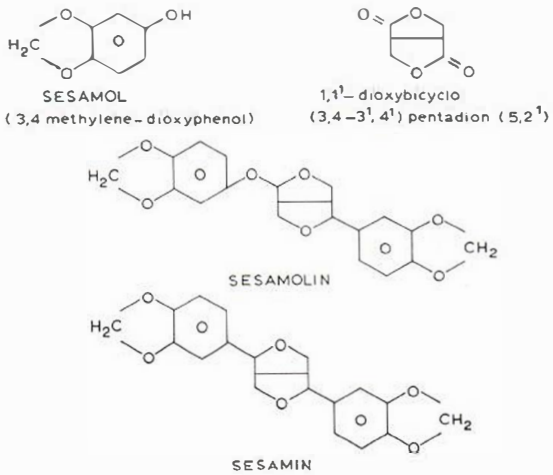

Fig. 1. Structure of sesamol, sesamin, sesamolin and 1,1¹-dioxybicyclo (3,4-3¹,4¹) pentadion (5,2¹).

Table I. *Substances, concentration and vehicles standard patch tested in patients*

MEK = methyl-ethyl-ketone

No.	Substance	Concentration, %	Vehicles used	No. of patients tested
1	Sesamol	5	MEK, ethanol, petrolatum	13
2	Sesamin	5	MEK, petrolatum	13
3	Sesamolin	5	MEK, petrolatum	13
4	1,1[1]-dioxybicyclo (3,4-3[1],4[1])-pentadion (5,2[1])	5	MEK, petrolatum	13
5	1,1[1]-Dioxybicyclo (3,4-3[1],4[1])-pentadion (2,5[1])	5	MEK, petrolatum	13
6	3,4 Methyl-dioxy-cinnamic acid	5	30 % DMSO in ol. olivae	13
7	3,4 Methyl-dioxy-phenyl-propyl-carbonyl-ethyl ether	5	MEK	13
8	3,4 Methyl-dioxy-phenyl-propyl-carbonyl ether	5	MEK	12
9	3,4 Methyl-dioxy-cinnamide	5	30 % DMSO in ol. olivae	13
10	3,4 Methyl-dioxy-oxystyryl-ethyl-ketone-malonester	5	MEK	6
11	Methylpiperonylate	5	MEK	12
12	Piperonyl-ethylether	5	MEK	12
13	Piperonyl-cyclonene	5	MEK	12
14	Piperonyl-methylketone	5	MEK	12
15	Piperonylic acid	5	30 % DMSO in ol. olivae	13
16	Piperonylbutoxide	10	MEK	13
17	Piperonal	5	MEK	12
18	3,4 Methyl-dioxystyryl-ethylketone	5	MEK	13
19	Dihydrosafrol	5	MEK	13

2. Identification

Sesamol was identified in the crude and purified sesame oil with the colour reaction according to Baudouin (1, 6). The two substances isolated from crude sesame oil as described in 1*a* and 1*b* were identified as sesamin and sesamolin, and their presence in the purified sesame oil was proved in three ways.

(*a*) Thin-layer chromatography: The layer used was silica gel 60 F 254 (Merck).

Solvent: benzene/acetone 97.5 : 2.5. Location: spray with concentrated sulphuric acid, heating for 5 minutes at 110°C (7).

(*b*) Gas chromatography: With the thin-layer chromatography methods used it was not possible to separate sesamin from sesamolin. A flame ionisation detector was used, and five different liquid phases were tried on crude and purified oil preparations (10 % carbowax 20M, 3 % OV-1, 1 % SE-30,3, 8 % SE-30, 1 % OV-17) at temperatures from 180° to 240°C.

(*c*) Mass spectrometry: Sesamin and sesamolin prepared according to method 1*b* were used.

3. Patch tests

Thirteen patients were available for patch testing. Patch tests were performed according to the standards of the International Contact Dermatitis Research Group. Sesamol, sesamolin and sesamin were tested in different vehicles. Moreover 1,1[1]-dioxybicyclo (3,4-3[1],4[1])-pentadion (5,2[1]), 1,1[1]-dioxybicyclo (3,4-3[1],4[1])-pentadion (2,5[1]), possibly representing the middle group of sesamol and sesamin, and in addition several substances chemically related to sesamol were tested (Table I).

As the sesamolin and sesamin isolated by method 1*b* were about 5–10 % contaminated with each other, a patch test series was devised in analogy to the technique used by Verspijck Mijnssen (8).

On Bakerflex silica gel 1B-F plates 250 μg of the samples was applied.

Solvent: benzene/acetone 97.5 : 2.5. Location under UV light 254 nm. The spots and controls (solvent) were cut into pieces 2.0 × 1.5 cm.

After wetting with one drop of MEK (methyl-ethyl-ketone), these pieces were applied to the skin and covered with a standard test patch. Several triglycerides prepared on thin layer sheets were also tested.

Substances tested in this way are listed in Table II.

Table II. *Substances tested on thin layer sheets*

No.	Substance	No. of patients
20	Sesamol	8
21	Sesamin	12
22	Sesamolin	12
23	1,1[1]-dioxybicyclo (3,4-3[1],4[1]) pentadion (5,2[1])	8
24	Glycerol tripalmitate	8
25	Glycerol trioleate	8
26	Glycerol tristearate	8
27	Trilinoleine	8
28	Solvet (control)	8

RESULTS

With the colour reaction according to Baudouin, sesamol was identified in the purified oil.

The Rf values obtained with method 2*a* were: sesamin 0.36, colour: purple, and sesamolin 0.53, colour: green after spraying.

The same Rf values were found for: sesamin from Dr Durden; sesamin, prepared from crude oil according to methods 1*a* and 1*b*; sesamin, prepared from pharmaceutical oil according to method 1*b*. The same Rf values were also found for sesamolin, i.e. sesamolin, prepared from crude oil according to methods 1*a* and 1*b* and sesamolin prepared from pharmaceutical oil according to method 1*b*.

In each stage of the gas chromatographic procedures, after injection of a mixture of sesamin and sesamolin, a single peak appeared, with the same retention time as produced by sesamin from Dr Durden. The same peak was obtained with material isolated from purified oil, prepared according to method 1*b*. From these results we may conclude that either sesamin or sesamolin or both were present in the injected solutions. The results of the mass-spectrometric investigations showed the samples to contain two different substances with molecular weights in agreement with those of sesamin and sesamolin. Moreover the results did not disagree with the chemical structures of sesamin and sesamolin. Sesamolin appeared to be contaminated with sesamin to about 5–10%.

From these results it seemed reasonable to conclude that sesamin and sesamolin were isolated from the crude sesame oil, and that the pharmaceutical oil also contained these substances.

The number of patients tested with each substance is listed in tables I and II. Negative patch tests in all patients were obtained with substances 5, 10, 11, 12, 14, 16, 17, 18, 19, 24, 25, 26, 27 and with 28 (control). The positive results are listed in Table III. In 16 control subjects tested, no positive reactions were found except in 8 who showed one or more doubtful reactions to the substances dissolved in DMSO containing Ol. olivae (nos. 6, 9 and 15).

DISCUSSION

The results indicate that sesamol, sesamin and sesamolin are present in the purified, pharmaceutical oil. Sesamin and sesamolin, which have almost identical molecules, are the main substances involved in allergic contact dermatitis to sesame oil. Only one patient of 13 was negative to both. As the sesamin concentration in the purified oil is about one hundred times that of sesamolin, sesamin may possibly be the main inciting allergen of both, assuming an almost equal sensitizing capacity. Sesamol produced only 8 positive patch tests in the 13 patients. In patient no. 6, sesamol was the only reacting substance. This may suggest that sesamol is immunochemically different from sesamin and sesamolin. Some patients seem to be sensitized either to sesamol or to the group of sesamin and sesamolin, others react to all three substances. Statistical evidence is lacking, however. Because the structures of the three substances are so much alike we thought it useful to perform the thin-layer-sheet tests. The 250 micrograms used was in our preliminary experience the greatest possible amount to yield a complete separation of the substances on the thin layer. Preliminary patch tests with these sheets gave encouraging results. As, unfortunately, the stronger reacting patients were used in these preliminary experiments, too few of the remaining patients showed clear-cut positive allergic reactions. The reading of these patch tests was another difficulty that was encountered. As the sheets have a rather sharp edge, unexpected forms of papular reactions were seen, for example rings, reflecting the boundaries of the sheets, were rather frequently observed. These reactions were noted as dubious positive and are not listed in Table III. Some patients were tested with scrapings from the sheets, and showed hopeful results. More experience with this technique may eventually yield another useful test method in patch testing small quantities of pure allergens.

1,1' dioxybicyclo (3,4–3',4') pentadion (5,2') and the (5',2) form were tested because they might represent the "middle-group" of sesamin and sesamolin (Fig. 1). In only 2 patients were positive reactions found.

The substances 6 to 19 of the test series were chosen because part of their structure very much resembles sesamol, in fact positions 1 and 6 of the benzene group are occupied in these substances by chains of different composition and length. To substance 6, five patients and to substances 9 and 13, four patients showed positive reactions. This may well be due to the composition of the vehicle which contained 30% DMSO, because 8 of our 16 controls showed dubious positive reactions with just these three substances. Cross-reactivity for this group, of which some members are more or less daily

Table III. *Positive patch tests after 48 hours in 13 patients with sesame oil allergy*

1 = +, 2 = + +, 3 = + + +, N = not tested, — = negative

No.	Substance	Patient no.												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1	Sesamol	3	2	2	—	3	2	—	—	—	—	3	2	3
2	Sesamin	1	2	2	2	2	—	1	2	3	1	1	2	1
3	Sesamolin	2	2	3	2	1	—	3	2	3	1	3	2	2
4	1,1[1]-Dioxybicyclo (3,4-3[1],4[1]) pentadion (5,2[1])	—	—	—	—	—	—	—	—	—	—	3	—	—
6	3,4 Methyl-dioxy-cinnamic acid	2	2	1	—	—	—	1	—	2	—	—	—	—
7	3,4 Methyl-dioxy-phenyl-propyl-carbonyl ethyl ether	—	1	—	—	—	—	—	—	—	—	—	—	—
8	3,4 Methyl-dioxy-phenyl-propyl-carbinol-ether	—	1	—	—	—	—	—	—	N	—	—	—	—
9	3,5 Methyl-dioxy-cinnamide	—	2	—	—	—	—	—	1	—	—	—	—	—
13	Piperonyl cyclonene	—	1	—	—	—	—	—	—	N	—	—	—	—
15	Piperonylic acid	1	2	2	—	—	—	—	—	—	—	—	2	—
20	Sesamol (thin layer)	2	—	—	—	2	—	—	—	N	N	N	N	N
21	Sesamin (thin layer)	1	3	—	1	—	—	3	—	N	—	3	2	3
22	Sesamolin (thin layer)	—	2	—	—	—	—	3	—	N	—	3	—	3
23	1,1[1]-Dioxybicyclo (3,4-3[1],4[1]) pentadon (5,2[1]) (thin layer)	—	—	1	—	—	—	—	—	N	N	N	N	N

encountered, such as for example piperonyl-butoxyde and piperonal, is therefore not assumed.

All of the about 30 patients in the Netherlands with a proven contact allergy to sesame oil have leg ulcers, most of them also having stasis eczema. Frequent and prolonged application of sesame oil-containing pastes must have sensitized these patients. The naturally occurring conservants in sesame oil apparently have their disadvantages, especially in patients with stasis eczema in whom contact allergy to locally applied therapeutics is known to occur very frequently (4, 5). Why contact dermatitis caused by sesame oil was only recently encountered is difficult to say. The retailers assured us that during the last 10–15 years no difference in fabrication and composition of sesame oil has occurred. In the Netherlands, this widely used medicament has probably been unjustly regarded as a very safe ointment constituent, which proves that no "indifferent" drug is completely harmless.

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REFERENCES

1. De Graaf, W. C., Schoorl, N. & Wielen, P. van der: Commentaar op de Nederlandsche Pharmacopee, 5th ed. Oosthoek's Uitgeversmij., Utrecht, 1929.
2. Dijk, E. van, Neering, H. & Vitányi, B. E. J.: Contact hypersensitivity to sesame oil in patients with leg ulcers and eczema. Acta Dermatovener (Stockholm) *53:* 133, 1973.
3. Fujunura, K. & Toyama, Y.: Changes in sesamol, sesamolin and sesamin contents of sesame oil in the course of extraction and refining processes. Abura Kogayu *7:* 31, 1958. (Chem. Abstr. 1958, no. 114446).
4. Malten, K. E., Kuiper, J. P., van der Staak, W. B. J. M.: Contact allergic investigations in 100 patients with ulcus cruris. Dermatologica *147:* 241, 1973.
5. Rudzki, E. & Kleniewska, C.: Kontaktallergie auf einige Lokaltherapeutika und Konservierungsmittel. Dermatologica *143:* 36, 1971.
6. Specifications for the Quality Control of Pharmaceutical Preparations. 2nd International pharmacopoeia, p. 401 W. H. O. Geneva 1967.
7. Stahl, E.: Dünnschicht-Chromatographie. 2nd ed. Springer-Verlag, Berlin, 1967.
8. Verspijck Mijnssen, G. A. W.: Patch testing with paper chromatograms. Dermatologica *134:* 379, 1967.

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