

Identification of coumarin as the sensitizer in a patient sensitive to her own perfume but negative to the fragrance mix

V. MUTTERER¹, E. GIMÉNEZ ARNAU¹, J.-P. LEPOITTEVIN¹, J. D. JOHANSEN², P. J. FROSCH³, T. MENNÉ², K. E. ANDERSEN⁴, M. BRUZE⁵, S. C. RASTOGI⁶ AND I. R. WHITE⁷

¹Laboratoire de Dermatochimie, Université Louis Pasteur, Strasbourg, France

²Department of Dermatology, Gentofte Hospital, University of Copenhagen, Denmark

³Hautklinik, University of Witten/Herdecke, Dortmund, Germany

⁴Department of Dermatology, Odense University, Denmark

⁵Department of Occupational Dermatology, Malmö University, Sweden

⁶National Environmental Research Institute, Roskilde, Denmark

⁷St. John's Institute of Dermatology, London, England

The aim of this study was to identify the chemicals responsible for the sensitivity of a 44-year-old woman to her own perfume, but showing negative patch test results to the fragrance mix. For this purpose, the perfume concentrate from the eau de toilette was chemically fractionated. Each fraction obtained was afterwards tested on the patient using a ROAT and/or a patch test. Only 1 fraction gave a positive ROAT result. This fraction was analyzed and found to contain coumarin and ethyl vanillin. Coumarin, one of the most widely used fragrance compounds that is not present in the fragrance mix, was confirmed as being the sensitizer.

Key words: perfume contact allergy; fragrances; fragrance mix; chemical analysis; coumarin; repeated open application test; patch testing technique. © Munksgaard, 1999.

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Allergic contact dermatitis is among the more severe unwanted effects arising from the use of cosmetics. 2 of the most widely used types of cosmetic products are deodorants and perfumes (1). The majority of allergic reactions to deodorants and perfumes described in the literature can be attributed to the perfume in the product (2). A perfume may contain as many as 200 or more individual ingredients (3). This makes the diagnosis of perfume allergy by patch test procedures complicated. For over 20 years, the fragrance mix (eugenol, isoeugenol, oak moss, geraniol, hydroxycitronellal, α -amylcinnamic aldehyde, cinnamic aldehyde and cinnamic alcohol) has been used as an efficient screening agent for perfume contact allergy (4). It is estimated that this mix detects 70%–80% of all cases of fragrance sensitivity (5). In most lists of frequent allergens, the fragrance mix ranges among the "Top 5", usually number 2 after nickel sulfate. However, very few systematic investigations into the frequency of contact allergy to fragrance substances, other than the constituents of

the fragrance mix, have been performed (6, 7, 14). This means that the frequency of problems with sensitization to single fragrance materials is largely unknown.

The aim of the present study was to identify sensitizing molecules in patients sensitive to their own perfume, but with negative patch test results to the fragrance mix, by bioassay-guided chemical fractionation.

Case Report

A 44-year-old woman with no previous skin problems developed an axillary rash using a perfumed deodorant. At the same time, she started to use an eau de toilette of the same brand and developed a rash on the neck and on the trunk. The rash disappeared on treatment with topical corticosteroids for a few days. The patient started applying both products again and developed a generalized eczema. She was patch tested to the European standard series (Hermal), including the fragrance

mix 8% with 5% sorbitan sesquioleate, and showed negative results. She was also negative to series of textile and cosmetic allergens. The patient was further patch tested to the deodorant, the eau de toilette, and the individual ingredients of both. She reacted only to the products and the perfume concentrate. A repeated open application test (ROAT) (8), done in the antecubital fossa, was positive for both the deodorant and the eau de toilette.

Materials and Methods

Identification of the offending allergen in the perfume by chemical fractionation

In order to identify the chemicals responsible for the patient's sensitivity to her own perfume, but showing negative patch test results to the fragrance mix, the perfume concentrate from the eau de toilette was chemically fractionated. Chemical fractionation was afterwards combined with a ROAT of each fraction on the patient.

The perfume concentrate under study was obtained from the commercial eau de toilette by concentration under vacuum and at room temperature. It was found that the eau de toilette contained approximately 7% of perfume concentrate.

The perfume concentrate from the eau de toilette was chemically fractionated by flash column

chromatography on silica gel (MERCK; Geduran 60, 0.040–0.063 mm). The solvent for elution was a mixture of pentane and diethyl ether in a 7:3 proportion, respectively. For each fraction obtained, the solvent was removed by evaporation under vacuum.

The fractions were tested by the patient in a ROAT, using 1 drop (25 μ l) of an ethanolic solution of the test material, applied openly 3 $\times$ a day to a 4 cm² use test site, as specified in Table 1. The test concentrations of the fractions were calculated so that they corresponded to the amount present in the 2% solution of the perfume concentrate that the patient had reacted to. As the fractionation proceeded, the test concentrations were doubled to compensate for the missing compound effect of many substances present in the test preparation.

The positive fraction was further chemically fractionated by flash column chromatography on silica gel, as described above. The new fractions obtained were tested on the patient by ROAT. This procedure was followed until a ROAT-positive fraction was obtained that only contained 2 substances, the structure of which was confirmed by ¹H and ¹³C nuclear magnetic resonance spectroscopy (NMR). The offending allergen was again identified by ROAT and the reaction afterwards confirmed by a serial dilution patch test of the sub-

Table 1. Repeated open application test (ROAT) results

Test material	Concentration ^{a)} and amount used		Usage test site	Results
perfume concentrate	2.00%		elbow flexure	+ D4 ^{b),c)}
ethanol	as is		elbow flexure	–
<i>Fractionation of perfume concentrate</i>				
F1	0.48%	1.55 g	elbow flexure, left	–
F2	2.00%	0.66 g	elbow flexure, right	+ D3
F3	0.80%	1.17 g	thigh	–
<i>Fractionation of F2</i>				
F2.1	0.20%	0.47 g	elbow flexure, left	–
F2.2	2.00%	0.46 g	elbow flexure, right	–
F2.3	0.36%	0.40 g	thigh, left	–
F2.4	0.36%	0.31 g	thigh, right	?+ D7
<i>Increase of concentration</i>				
F2.1	0.80%	0.58 g	elbow flexure, left	–
F2.2	8.00%	0.42 g	elbow flexure, right	–
F2.3	1.60%	0.32 g	thigh, left	–
F2.4	1.60%	0.40 g	thigh, right	+ D2 ^{d)}
<i>Fractionation of F2.4</i>				
coumarin	1.00%	0.15 g ^{e)}	elbow flexure, left	+ D2 ^{f)}
ethyl vanillin	1.00%	0.15 g	elbow flexure, right	–

^{a)} Test material concentration in ethanol.

^{b)} D=day.

^{c)} Papules at day 2. The test was continued and at D 7 an eczematous reaction with spread to the axilla was seen.

^{d)} Reaction started at D 2. At D 7 papules and vesicles were present.

^{e)} 10 control patients did a 3-day ROAT with the same solution of coumarin and the response was negative. They had used 0.17 g of the coumarin solution on average.

^{f)} Homogeneous redness and multiple papules.

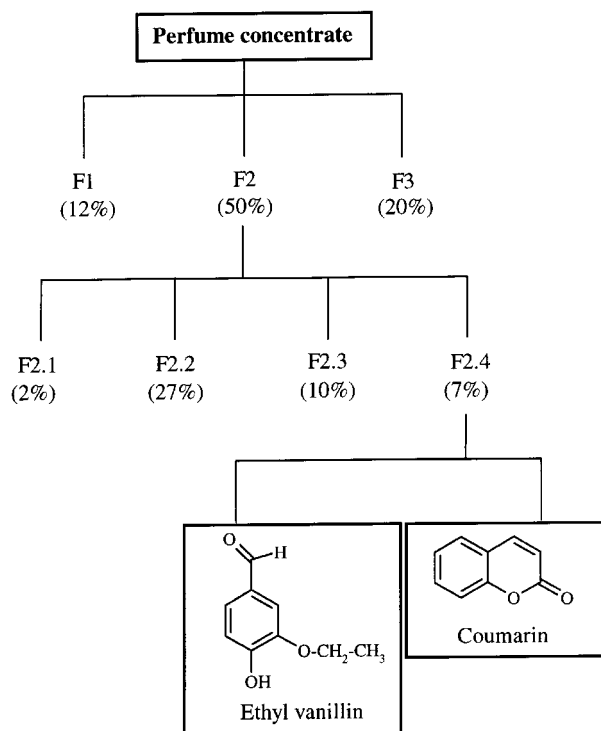


Fig. 1. Chemical fractionation of the perfume concentrate.

stance in 10%, 5% and 1%, using Finn Chambers on Scanpor tape, 2-day occlusion and reading on day (D) 3. 10 consecutive eczema patients that were patch test negative to the substance served as controls and did a 3-day ROAT with the substances in a manner similar to the patient.

Results

3 fractions (F1, F2 and F3) were initially obtained after chemical fractionation of the perfume concentrate (Fig. 1). The most important fraction was F2 that constituted 50% of the perfume concentrate. Fractions F1, F2 and F3, were tested on the patient. Only fraction F2 gave a positive response and was further chemically fractionated. 4 fractions (F2.1, F2.2, F2.3 and F2.4) were obtained and were tested on the patient. A doubtful positive reaction was shown at D 7 when fraction F2.4 was tested at a concentration of 0.36%. The reaction was confirmed to be positive at D 2 when the test material concentration was increased to 1.6%. Fraction F2.4 was analyzed and found to contain coumarin and ethyl vanillin. The patient was negative on use testing to 1% ethyl vanillin in ethanol after 1 week of application in the elbow flexure. However, the patient was positive on use testing to 1% coumarin in ethanol after 2 days of application, showing a reaction of homogeneous red-

ness and multiple papules in the elbow flexure spreading outside the area of application. The 10 control eczema patients were negative after 3 days of applying 1% coumarin to one elbow flexure. The patient was also positive at D 3 at patch testing to 10% and 5% coumarin in ethanol, but negative to ethanol.

Coumarin was present in the eau de toilette and a previous chemical analysis of the deodorant that had started the skin problems had shown that it contained 0.23% of coumarin. Based on results from a study of the perfumes most sold in Europe (9), 3 perfumes that did not contain coumarin were selected and the patient did a 14-day use test in the antecubital fossa with each perfume without any problems and still uses these perfumes.

The investigation took 2½ years and 27 consultations were needed from the onset of the disease to the identification of coumarin as the sensitizer.

Discussion

Bioassay-guided chemical fractionation allowed the identification of coumarin as the chemical responsible for sensitization in 1 patient sensitive to her own perfume but with a negative patch test result to the fragrance mix. Coumarin is one of the most widely used fragrance compounds (10). It has been found in 57% of 73 deodorants on the European market in a previous study (11). The patient's problems started with a deodorant vapospray that contained 0.23% coumarin. This is a rather high concentration compared to similar products on the market, which have been found to contain an average concentration of 0.01% coumarin (range 0.001–0.1%) (11). There is, however, no upper limit to the quantity of coumarin that may be used in cosmetics, as coumarin is not covered by any restrictions in the Code of Practice issued by the International Fragrance Research Agency (IFRA) for the manufacture of safe perfumed products. The IFRA recommendations are based on a negative maximization test carried out on 25 human volunteers (12). It has also been test negative in animals (13). Coumarin has, on the other hand, caused positive patch test reactions in 1.2–6.8% of patients tested due to suspected fragrance contact allergy (7, 14). Moreover it has also been identified as the cause of contact allergy in single cases of contact allergy to products containing the substance (15, 16). Thus coumarin is a known contact allergen and in principle the diagnosis could have been made by screening with a patch test series of fragrance allergens. The problem is that about 100 fragrance substances have been identified as contact allergens and optimal test concentrations are known for only a few of these (4). Furthermore, it

is not possible to directly relate a positive reaction on patch testing with a series of allergens to the current problem of the patient, as no information is available about the composition of marketed perfumes. A duty of declaration of all ingredients in cosmetic products has come into force from January 1997 in the European Union (17). Fragrance substances have been exempted, which not only makes the diagnosis of perfume allergy difficult but also makes it impossible to inform patients in a relevant manner about their perfume allergy.

The current case illustrates that diagnosing the specific cause of perfume allergy without relevant exposure information is possible, but requires a considerable effort by the patients and the scientists involved. Moreover the diagnosis is delayed for several years. It also shows that some perfume-allergic patients may be able to find perfumes they can tolerate if they can avoid the allergen in question.

A ROAT was used for testing the perfume fractions in the patient. This is not a standard test even though some recommendations exist (8). However, the patient had been shown to be very reactive to the perfume on ROAT and the procedure reduced the number of consultations needed to make a diagnosis. The allergic nature of the reaction to coumarin was confirmed by including controls and by patch testing with a serial dilution of coumarin.

It is necessary to use mixes of fragrance allergens to detect fragrance allergy, due to the large number of existing fragrance substances and as no information is available about the composition of perfumes used by the patients. The fragrance mix, included in the European standard patch test series, detects a large number of cases. However, no mixture can be perfect and it is estimated that possibly 30% of patients allergic to fragrances are not detected by the mix (5). Future research should be aimed at increasing the sensitivity of the mix or creating a new additional fragrance mix. In this context, coumarin, which was found to be the cause of perfume contact allergy in this study, should be included in large-scale investigations to help identify relevant new sensitizers not included in the present fragrance mix.

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Address:

Elena Giménez Arnau
Laboratoire de Dermatochimie
Clinique Dermatologique, CHU
F-67091 Strasbourg
France