Allergenic Components in Modified and Unmodified Rosin

Chemical Characterization and Studies of Allergenic Activity

BY

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ABSTRACT

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Unmodified rosin (colophony) is a well-known cause of contact allergy (delayed type hypersensitivity). Rosin is obtained from coniferous trees and consists mainly of diterpenoid resin acids. Most rosin used in technical products is chemically modified.

In the common modification of rosin with maleic anhydride, the major product formed is maleopimaric acid (MPA). MPA was identified in experimental sensitization studies as a potent contact allergen. MPA is also formed when rosin is modified with fumaric acid at high temperature and with prolonged heating. The amounts of MPA in technical quality rosins modified with maleic anhydride or fumaric acid might be enough to sensitize individuals handling these rosins. The major product of the modification of rosin with fumaric acid, fumaropimaric acid (FPA), did not elicit any reactions in the animals tested.

In another common rosin modification, glycerol esterification, the major product formed was identified as glyceryl triabietate (GTA). In an experimental sensitization study none of the animals reacted to GTA. However, a minor product formed, glyceryl 1-monoabietate (GMA) showed sensitizing capacity.

The presence of new contact allergens due to the modification, together with remaining unmodified material, contributes to the risk of developing allergy from contact with these types of rosin.

A new main contact allergen in unmodified rosin was identified; 13,14(β)-epoxyabietic acid. The allergenicity of this epoxide was comparable to that of an earlier identified rosin allergen, 15-hydroperoxyabietic acid (15-HPA). The allergens were detected as their methyl esters.

Experimental sensitization and cross-reactivity of oxidation products of resin acids were studied. A pattern of cross-reactivity was observed which indicates that the hydroperoxide of abietic acid (15-HPA) may react to form a complete antigen via two different routes. One route seems to be via the formation of epoxides which then react with skin protein to form the complete antigen, and the other, via radical formation due to cleavage of the peroxide bond. The radical formed may then react with skin protein, so producing the complete antigen. Few other studies have shown results indicating the formation of several antigens from one hapten.

Key words: colophony, contact allergy, fumaropimaric acid, gas chromatography, guinea pigs, high performance liquid chromatography, maleopimaric acid, oxidation products, patch testing, resin acids, rosin, terpenes

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CONTENTS

PAPERS DISCUSSED

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals

I Karlberg A-T, Gäfvert E, Hagelthorn G, Nilsson J L G. Maleopimaric acid - a potent sensitizer in modified rosin. *Contact Dermatitis*. 1990: *22:* 193-201.

II Gäfvert E, Shao L P, Karlberg A-T, Nilsson U, Nilsson J L G. Maleopimaric acid - a contact allergen in rosin modified with fumaric acid. Submitted.

III Shao L P, Gäfvert E, Karlberg A-T, Nilsson U, Nilsson J L G. The allergenicity of glycerol esters and other esters of rosin (colophony). *Contact Dermatitis*. 1993: *28:* 229-234.

IV Gäfvert E, Shao L P, Karlberg A-T, Nilsson U, Nilsson J L G. Allergenicity of rosin (colophony) esters (II). Glyceryl monoabietate identified as contact allergen. *Contact Dermatitis*. In press.

V Gäfvert E, Nilsson U, Karlberg A-T, Magnusson K, Nilsson J L G. Rosin allergy: identification of a dehydroabietic acid peroxide with allergenic properties. *Archives of Dermatological Research*. 1992: *284:* 409-413.

VI Gäfvert E, Shao L P, Karlberg A-T, Nilsson U, Nilsson J L G. Contact allergy to resin acid hydroperoxides. Hapten binding via free radicals and epoxides. *Chemical Research in Toxicology*. In press.

ABBREVIATIONS

CCET	Cumulative contact enhancement test
15-DAP	Di -(methyl dehydroabietate-15-yl)-peroxide
FCA	Freund's complete adjuvant
FCAT	Freund's complete adjuvant test
FPA	Fumaropimaric acid
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GMA	Glyceryl-1-monoabietate
GTA	Glyceryl triabietate
15-HPA	Methyl-15-hydroperoxyabietate
15-HPDA	Methyl-15-hydroperoxydehydroabietate
HPLC	High performance liquid chromatography
IR	Infra-red spectrophotometry
LC	Liquid chromatography
MPA	Maleopimaric acid
MS-CI	Mass spectrometry with chemical ionization
MS-EI	Mass spectrometry with electron impact ionization
NMR	Nuclear magnetic resonance
Pet.	White petrolatum
TLC	Thin layer chromatography

INTRODUCTION AND BACKGROUND

Production and use of rosin

Rosin (colophony) is a resin obtained from species of the genus *Pinus*. There are three types of rosin depending on the ways of recovery - gum rosin, wood rosin and tall oil rosin. Gum rosin is obtained from living trees. The trees are tapped for oleoresin which is then distilled to obtain turpentine as the distillate and gum rosin as the distillation residue. Wood rosin is obtained from old pine stumps. An initial extract is distilled to yield wood turpentine and the wood rosin. Tall oil rosin is obtained as a by-product in the sulphate pulping of coniferous trees. The supply of pine stumps for the production of wood rosin is decreasing and today the major rosin types produced are gum rosin and tall oil rosin. (5, 33, 68, 74-76)

World production of rosin in 1988 was 1 215 000 tonnes, of which gum rosin constituted 708 000 tonnes, wood rosin 93 000 and tall oil rosin 414 000. The largest producers are China, USA, Russia, Portugal and Scandinavia. Scandinavia is the second largest producer of tall oil rosin, with in 1988 about 20%, i. e. 75000 tonnes, of the total. The major producer is the USA with 60% of the world's production. (99)

Due to its low cost and its technical properties rosin is widely used in products such as

adhesives, paint, printing ink and soldering fluxes. It is used in the sizing of paper (a process to make paper hydrophobic) and as an emulsifying agent in emulsion polymerization of synthetic rubber (17, 100, 101). Rosin and rosin components have also been used experimentally as a matrix material for controlled-release pharmaceutical preparations (81, 82, 84) The different rosin types are used interchangeably and are often chemically modified. The largest users of rosin are the paper, adhesives and printing ink industries, which together account for 66% of total production (99).

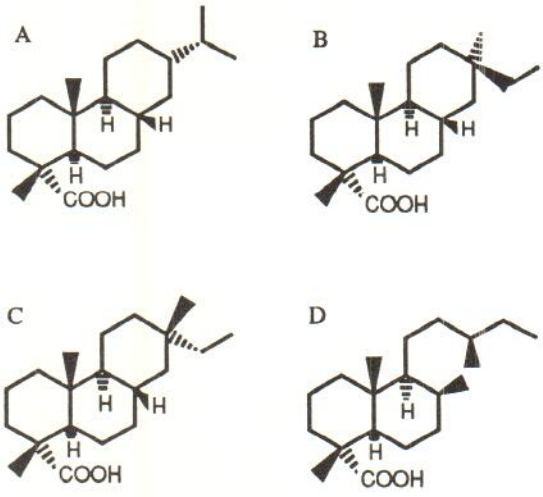
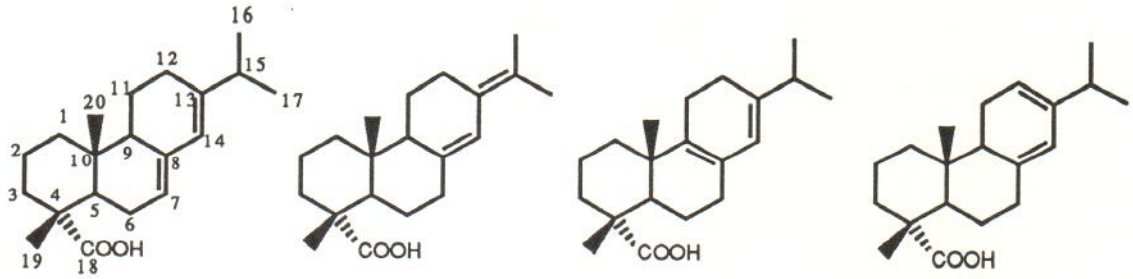


Figure 1. Resin acid classes. A. abietane type resin acids, B. pimarane type resin acids, C. isopimarane type resin acids, D. labdane type resin acids

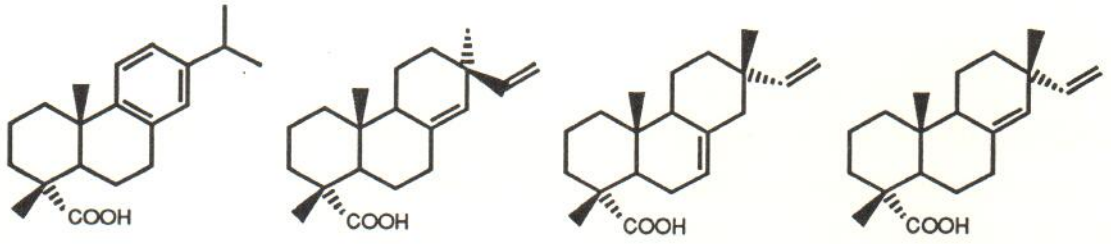
Chemical composition of rosin

Rosin is a complex mixture of diterpenoid acids, i. e. resin acids (85-95%), diterpene alcohols, aldehydes and hydrocarbons (98). Due to air exposure oxidized material is also present.

The resin acids belong to four classes with different basic skeletons; abietane, isopimarane, labdane and pimarane (Fig 1). Acids of the abietane and pimarane type structures are the most abundant. Figure 2 shows some of the most common resin acids in rosin. The numbering of the carbon atoms is from the abietane-based nomenclature shown in this figure for abietic acid. Rosin composition varies with the *Pinus* species from which the rosin is obtained, and variations may also be observed in the same species depending on growth conditions. This was observed for *Pinus merkusii* from different parts of Sumatra (14). Oleoresins from different varieties of *Pinus caribaea* also showed differences in the resin acid composition (13). The handling and storage of the rosin produced also greatly affect the composition (49, 66, 67, 110). Holmbom et al have shown typical resin acid compositions of tall oil rosin using gas chromatography-mass spectrometry (GC-MS) (49). This is shown in Table 1 together with the composition of a Portuguese gum rosin and a Chinese gum rosin analyzed by Joye and Lawrence with GC (51). The two tall oil rosin samples analyzed contain larger amounts of dehydroabietic acid than the two gum rosin samples do. This is due to oxidation-dehydration of abietane type acids in the pine chips during pulping and tall oil processing (66, 110) and to the high production temperature (49, 67).



A. Abietic acid B. Neoabietic acid C. Palustric acid D. Levopimaric acid



E. Dehydroabietic acid F. Pimaric acid G. Isopimaric acid H. Sandaracopimaric acid

Figure 2. Some of the most common resin acids in unmodified rosin (colophony)

Table 1. Relative amounts (%) of major resin acids in different rosins according to GC

Resin acid	Rosin sample[1]			
	TOR 1[2]	TOR 2[2]	PGR[3]	CGR[3]
Abietic acid	39.9	32.5	32	44
Dehydroabietic acid	30.2	37.6	5.1	4.3
Isopimaric acid	4.4	5.5	5.3	1.5
Neoabietic acid	2.4	0.8	16	15
Palustric acid	4.6	5.2	30	22
Pimaric acid	4.2	3.7	8.8	9.2
Sandaracopimaric acid	1.4	1.2	1.9	2.7

[1]TOR=tall oil rosin, PGR=Portuguese gum rosin, CGR=Chinese gum rosin.
[2]Analyzed by Holmbom et al (49).
[3]Analyzed by Joye & Lawrence (51).

Oxidation of rosin and resin acids

Rosin is easily oxidized by air. The oxidized rosin is brittle and dark in colour; properties that usually are undesirable in the technical products (76, 98). The oxidation of rosin and resin acids has been studied by several authors (25-28, 46, 54, 77, 86, 92-94). The abietane type resin acids with conjugated double bonds are more susceptible to oxidation than dehydroabietic acid and the pimaric/isopimaric type acids (98). In experimental oxidation of abietane type resin acids, the oxidation products observed include peroxy compounds, ketones, alcohols and epoxides (21, 47, 77, 92-94, 106). The oxidation of dehydroabietic acid has also been studied. Oxidation usually takes place in the benzylic positions, yielding ketones, alcohols and hydroperoxides (18, 86, 105).

Several oxidation products have been identified in rosin and wood extracts using mainly GC-MS techniques. Norin and Winell identified 15-hydroxydehydroabietic acid in common spruce (*Picea abies*) (78). Ekman identified 7-hydroxydehydroabietic acid, 15-hydroxydehydroabietic acid and 7-oxo-dehydroabietic acid in Norwegian spruce (23). 15-Hydroxy-7-oxodehydroabietic acid, 7-hydroxydehydroabietic acid and 7-oxodehydroabietic acid were isolated using liquid chromatography (LC) and identified with GC-MS in Douglas fir by Rogers and Mahood (88). Some 7-oxoabietane type resin acids were identified in tall oil rosin by Mayr using GC-MS (72). Karlberg et al isolated 7-oxodehydroabietic acid, 15-hydroxydehydroabietic acid, 15-hydroxy -7-oxodehydroabietic acid and 15-hydroperoxyabietic acid (as methyl esters) from Portuguese gum rosin using an HPLC technique (56, 57). Krohn et al isolated hydroxy and oxo compounds of dehydroabietic acid and a peroxo compound of abietic acid from French tall oil rosin using column and thin layer chromatography (64). Oxidation products of abietic and dehydroabietic acid from *Pinus massoniana* resin were isolated as their p-nitrophenyl esters by Cheung et al (12).

Modifications of rosin

Today most of the rosin used in industry is modified in different ways by causing the resin to react with other suitable chemicals. Certain desirable technical properties of the rosin can be achieved by modification. The modifications are usually interrupted when the desired technical properties have been obtained, leaving some of the rosin unmodified. The most common modfications of rosin and some products in which these modified resins are used are listed in Table 2.

Esterification of rosin is a modification process that has been used for more than 100 years. Polyhydric alcohols such as glycerol, pentaerythritol, ethylene glycol and diethylene glycol are used for the esterification. The carboxylic group in the resin acids

is bound to a tertiary carbon (carbon 4, see Figure 3). The esterification of the carboxylic group is hindered and therefore slow and requires large excesses of alcohol and high temperatures (76, 98) Esterified rosins, mainly glycerol and pentaerythritol esters, are used in adhesive tackifiers often in combination with other modifications such as disproportionation or hydrogenation (60). In rosin-based printing inks, esterified rosin or rosin both polymerized/dimerized and esterified is used. Maleic-anhydride- and fumaric-acid-modified rosins used in printing inks are often esterified (9). Glycerol esters of rosin are used in bubble gum. (38, 39, 76)

In some applications it is desirable to enhance the hydrophilic portion of rosin. This is achieved by causing the rosin to react with maleic acid, maleic anhydride or fumaric acid (dienophiles) which add to the resin acid levopimaric acid (diene) in a *Diels-Alder addition*. Levopimaric acid undergoes Diels-Alder addition at room temperature. At elevated temperatures other resin acids with conjugated double bonds are isomerized to levopimaric acid, which then reacts with the dienophile (98). Diels-Alder adducts have been analyzed in different modified rosins using GC-MS (73). This type of rosin is used in the manufacture of paper. It is added to the pulp to give the paper a smooth, hydrophobic surface (100, 101) and it is also used in rosin-based printing inks (9).

Oxidation of rosin and resin acids is often undesirable. To prevent this, the double bonds which are susceptible to oxidation can be saturated by *hydrogenation* . Total hydrogenation is achieved by reaction with hydrogen gas in the presence of a precious metal catalyst (98). Hydrogenated rosin is used in rosin-based printing inks and soldering fluxes. Salts of hydrogenated rosin are used as polymerization emulsifiers in the production of synthetic rubber (9, 38, 39, 97). Hydrogenation and subsequent esterification of rosin with glycerol or pentaerythritol are used in adhesive tackifiers (60).

Disproportionation/dehydrogenation of rosin is also a process which decreases the ability of rosin to oxidize since the amount of resin acids with conjugated double bonds is diminished in favour of dehydroabietic acid. Rosin is heated to 200-300°C most often in the presence of a catalyst such as palladium-on-carbon. (98) Disproportionated rosin is used as polymerization emulsifier in the production of synthetic rubber, for paper sizing, in adhesive tackifiers, in printing inks and as insulating material in the electronics industry (9, 17, 38, 39, 60, 101).

Polymerization/dimerization of rosin decreases its tendency to crystallize. Polymerized/dimerized rosin is used in adhesives, inks and varnishes (31). Rosin is polymerized/dimerized with an acid or a Lewis acid

catalyst. The degree of polymerization varies with the conditions (time, temperature) under which the modification is performed (76). Major products formed are dimers of resin acids, and possible structures for these have been proposed in the literature (31, 34) .

Formaldehyde modification of rosin includes several different reactions performed under different conditions where formaldehyde reacts with resin acids (98). Formaldehyde and levopimaric acid react in a Diels-Alder reaction yielding, after treatment with acid, 12-hydroxymethylabietic acid (80, 89). Abietic acid reacts with formaldehyde in a Prins reaction yielding monohydroxymethyl- and dihydroxymethyl-abietic acids (89). Formaldehyde-modified rosin is often used in paper sizing and in printing inks (9, 101).

Resin acids form sodium, potassium and ammonium *salts (resinates)* which are used as soaps and in detergents due to their amphiphilic nature. Sodium resinates are also used in paper sizing. Resinates of barium, calcium, cobalt, lead, magnesium and zinc are used in drier formulations and inks. (9, 76, 98, 101)

Table 2. The most common modifications of rosin and their uses

Modification type	Areas of use
Esterification with polyalcohols	adhesive tackifiers, printing inks, bubble gum
Diels-Alder addition of maleic acid, maleic anhydride or fumaric acid	paper size, printing inks
Hydrogenation	printing inks, soldering fluxes, polymerization emulsifiers in the production of synthetic rubber, adhesive tackifiers
Polymerization/dimerization	adhesives, printing inks, varnishes
Disproportionation/ dehydrogenation	polymerization emulsifiers in the production of synthetic rubber, paper size, adhesive tackifiers, printing inks, insulating material in electronics industry
Formaldehyde modification	paper size, printing inks
Salt formation	soap, detergents, paper size, drier formulations, printing inks

Contact allergy to rosin

Rosin is known to cause contact allergy (delayed type of hypersensitivity, type IV allergy) (1). Clinically it is among the ten most common causes of contact allergy, with many published case reports (10, 11, 16, 30, 52, 61, 62, 69, 70, 91, 107). The allergenic activity of rosin has been experimentally studied by Karlberg in her thesis "Contact Allergy to Colophony" and in subsequent articles (53, 54, 69). A series of articles on this topic has also been published by Hausen et al (38-46). Karlberg et al showed that the allergenic activity was mainly due to oxidation products of resin acids (Fig 3) (56, 57). Pure, non-oxidized abietic acid is not allergenic (55). Oxidation products were also shown by Hausen et al to possess allergenic activity (44, 46).

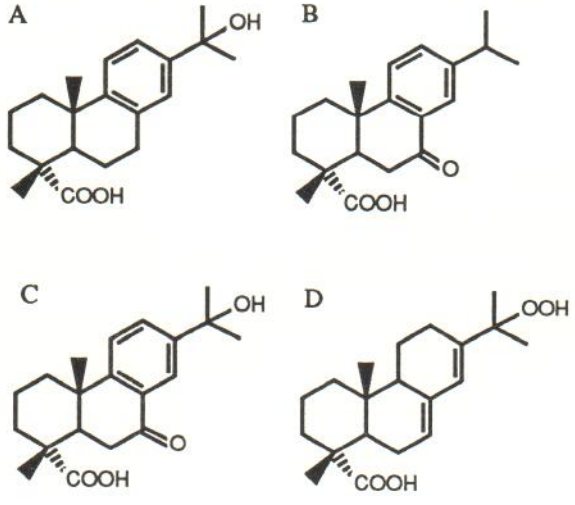


Figure 3. Oxidation products of dehydroabietic acid and abietic acid, in earlier studies identified as contact allergens in unmodified rosin. A. 15-hydroxydehydroabietic acid, B. 7-oxodehydroabietic acid, C. 15-hydroxy-7-oxodehydroabietic acid, D. 15-hydroperoxyabietic acid (15-HPA)

Karlberg et al showed in animal experiments and in humans that the allergenicity was less when the rosin was hydrogenated (58). Different types of modified rosins have been studied in experimental sensitization studies by Hausen et al (41-43, 45). The results, however, are difficult to interpret since the products tested were complex mixtures with no chemical caracterization other than what was obtained from the suppliers' information sheets.

Other biological effects of rosin

Rosin and rosin derivatives have been reported to have other biological effects than causing contact dermatitis. Several reports on the development of asthma due to exposure to rosin fumes have been published. Fawcett et al showed that rosin fumes were the cause of occupational asthma associated with soldering fluxes and hot-melt glue (29). Burge et al studied the development of asthma among electronics workers due to rosin-containing soldering fluxes (6-8). Rosin in a solder-flux wire caused contact urticaria in a printed circuit board inspector exposed to soldering fumes (87). Resin acids are toxic to fish, and LC50 values in *Daphnia pulex* were determined for dehydroabietic acid and its oxidized derivatives by Rogers & Mahood. The oxidized compounds were less toxic than dehydroabietic acid itself (88). LC50 values for dehydroabietic acid in some Scandinavian fish species were determined by Oikari (79). Dehydroabietic acid also reduced

permeability in rat renal membranes (83). HPLC and GC methods have been developed for analysing resin acids in effluents and waste water from kraft and newsprint mills since these are toxic for aquatic life (4, 85, 102, 108).

AIMS OF THE STUDY

The aims of this study were:

1) to investigate how some of the more common modifications affect the allergenicity of rosin, and

2) to extend current studies of the allergenic activity of rosin oxidation products and to study the mechanisms by which these products exert their allergenic activity.

EXPERIMENTAL PROCEDURES

Synthesis of resin-acid-derived compounds

Modified and unmodified rosins are complex mixtures of diterpenoid compounds. The modified rosins contain both modified and unmodified rosin components. Thus when a suitable synthetic route was available for the resin acid derivatives included in our studies, synthesis was preferred to time-consuming and costly isolation from the respective rosin.

Paper I: Maleopimaric acid (MPA, Fig. 4 A) was prepared by a Diels-Alder reaction between abietic acid and maleic anhydride, based on a procedure described by Ruzicka et al (90).

Paper II: Fumaropimaric acid (FPA, Fig. 4 B) was prepared by a Diels-Alder reaction between abietic acid and fumaric acid (37). A reaction-time study was performed on the Diels-Alder reaction between abietic acid and fumaric acid, which were allowed to react at 220°C for 10, 20, 60, 120 and 240 min. The relative amounts of Diels-Alder adducts from the different reactions were analyzed with GC-MS. The samples were methylated with diazomethane prior to analysis.

Papers III, IV: Glyceryl triabietate (GTA, Fig. 4 C) was prepared from abietic acid and glycerol according to procedures described by Hind et al (48). Glyceryl-1,2-diabietate ($GDA_{1,2}$ Fig. 4 E), glyceryl-1,3-diabietate ($GDA_{1,3}$ Fig. 4 F) and glyceryl-1-monoabietate (GMA, Fig. 4 D) were also obtained in the esterification of abietic acid with glycerol. A more efficient synthetic route to GMA was based on a method for esterification of abietic acid with glycol (15).

Paper VI: Methyl 13,14(α)-epoxyabietate (α-epoxyabietate, Fig. 5 A) and methyl 13,14(β)-epoxy-abietate (β-epoxyabietate, Fig. 5 B) were prepared by epoxidation of

abietic acid with *m*-chloro-perbenzoic acid following procedures described by Valverde et al (106).

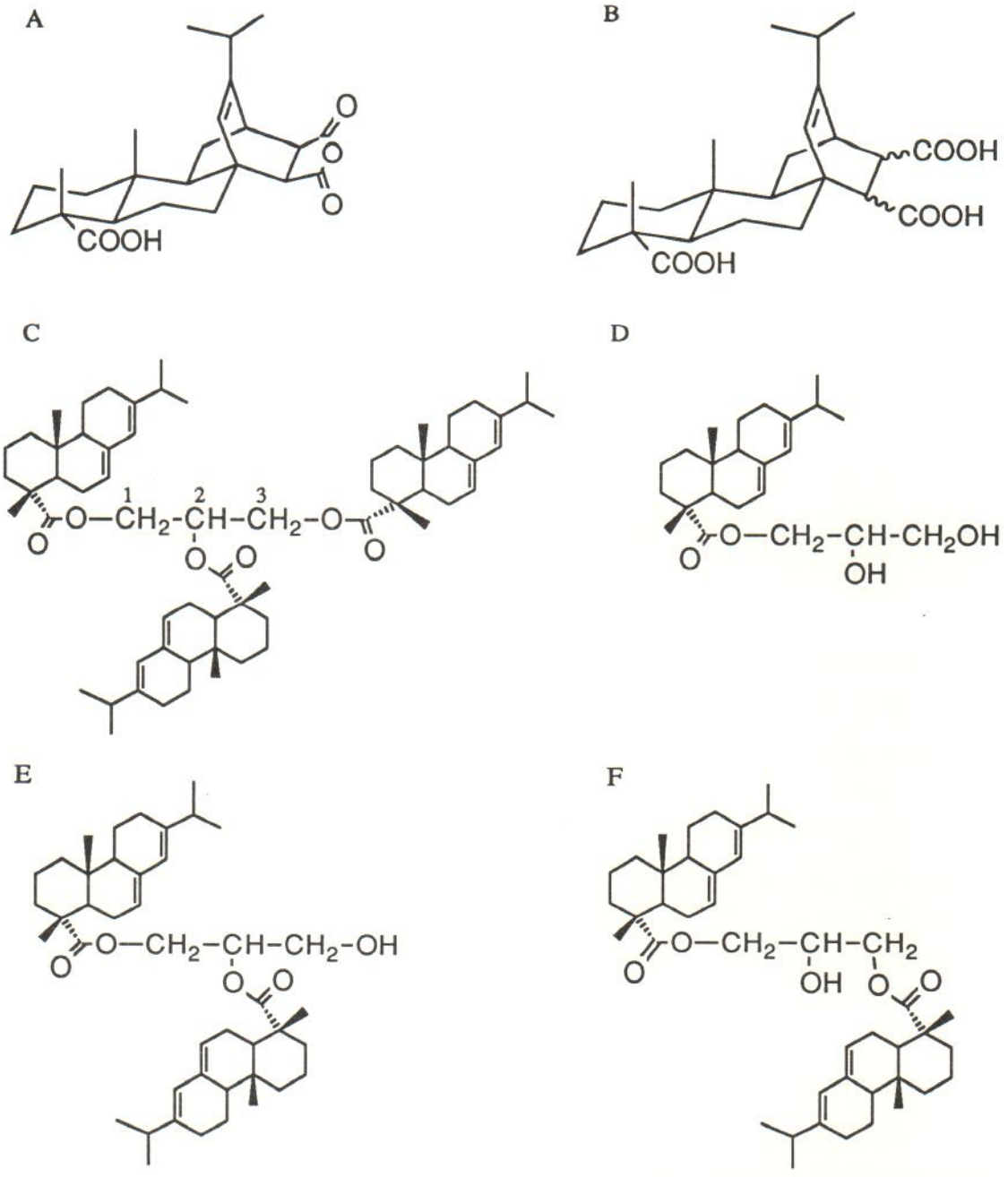


Figure 4. Structures of resin acid derivatives from modified rosins included in the study. A. endo-maleopimaric acid (MPA), B. fumaropimaric acid (FPA), C. glyceryl triabietate (GTA), D. glyceryl 1-monoabietate (GMA), E. glyceryl 1,2-diabietate ($GDA_{1,2}$), F. glyceryl 1,3-diabietate ($GDA_{1,3}$)

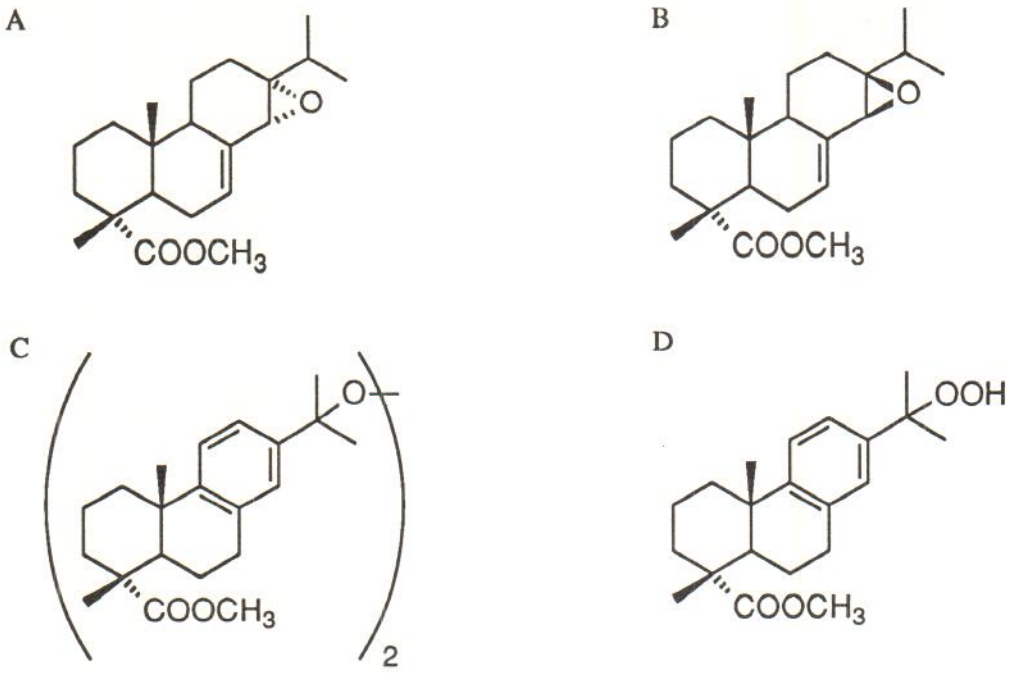


Figure 5. Structures of oxidized resin acids included in the study. A. methyl 13,14(α)-epoxyabietate (α-epoxyabietate), B. methyl 13,14(β)-epoxyabietate (β-epoxyabietate), C. di-(methyl dehydroabietate-15-yl)-peroxide (15-DAP), D. methyl 15-hydroperoxy-dehydroabietate (15-HPDA)

Isolation and detection of potential contact allergens in different rosins

The relative amounts of FPA, MPA and abietic acid were detected in commercial samples of fumaric-acid-modified rosins using GC (paper II). Synthetic FPA and MPA were used as references. The samples were methylated using diazomethane prior to analysis.

The presence of GMA and abietic acid in glycerol-esterified rosins was investigated with reversed-phase HPLC (papers III, IV). Abietic acid and synthetic GMA were used as references.

Di-(methyl dehydroabietate-15-yl)-peroxide (15-DAP, Fig. 5 C) (paper V) was isolated from Portuguese gum rosin. The rosin was esterified with diazomethane and separat ed with flash chromatography and semi-preparative HPLC. The isolated product was analyzed with ^{1}H-NMR and MS-CI. The presence of α-epoxyabietate and β-epoxyabietate (paper VI) in gum rosin and tall oil rosin was investigated with MS analysis of rosin fractions obtained by preparative TLC. The synthetic epoxides were used as references.

Sensitization and cross-reactivity studies

The experiments were carried out using female Dunkin-Hartley guinea pigs from AB Sahlins Försöksdjursfarm, Malmö, Sweden. The animals' average weight was 300-350 g at induction. They were kept on a standard diet from EWOS AB, Södertälje, Sweden and water ad libitum. The sensitization procedures used in the experiments were the Cumulative Contact Enhancement Test (CCET) and Freund's Complete Adjuvant Test (FCAT).

The results from the animal experiments were statistically analyzed using Fisher's exact test (32).

Cumulative Contact Enhancement Test (CCET)

CCET was used in the sensitization studies of MPA (paper I), GTA (paper III), GMA (paper IV), β-epoxyabietate (paper V) and FPA (paper VI). The procedure was performed according to the literature (104) but the controls were sham treated and the challenge testing was performed closed.

Induction. For induction the animals received four closed epidermal applications (each application: 2x4 cm) (days 0, 2, 7, 9) on the upper back and 2 intradermal injections of 0.1 ml of Freund's complete adjuvant (FCA) (day 7) in the same region. FCA non-specifically enhances the immune response to contact allergens. In the studies of MPA (paper I), FCA injections were omitted since MPA proved to be a potent allergen according to FCAT. Groups of 15 animals were used for the experiments. One group was a control group to which petrolatum (pet.) alone was applied.

Challenge. Closed challenge testing was performed on day 21. The different test materials in pet. were applied on the shaved flanks of the animals for 24 h using Finn Chambers®. The reactions were assessed at 48 h and 72 h after application. The minimum criterion for a positive reaction was a confluent erythema. The test concentrations were shown in pre-tests on FCA-treated animals to be non-irritating.

Freund's Complete Adjuvant Test (FCAT)

FCAT was used in the sensitization studies of MPA and in the cross-reactivity studies of 15-DAP, α- and β-epoxyabietate, 15-HPA and 15-HPDA. It was performed as described by Klecak with a slight modification according to earlier experience (3, 63). Groups of 15 animals, including one control group, were used in the experiments. In paper II, however, groups of 6 and 10 animals were used due to difficulties in isolating the test material.

Induction. On days 0, 6 and 10 the animals were given an intradermal injection on the upper back. The injections were 0.1 ml 5% (w/w) of test substance in FCA/H_2O emulsion (1:1). The control animals were given the FCA/H_2O emulsion only. *Challenge.* Closed challenge testing was performed on day 21 as described above. The test

concentrations were shown in pre-tests on FCA-treated animals to be non-irritating.

The study was approved by the local ethical committee.

Patch testing

GTA (paper III), GMA (paper IV), 15-DAP (paper V), α- and β-epoxyabietate (paper VI) and 15-HPDA (paper VI) were tested in subjects with previously verified contact allergy to rosin. Patch testing (109) was performed using Finn Chambers® on Scanpor® tape. White petrolatum (pet.) was used as the vehicle. The test material was applied and kept on for 48 h and the reactions were assessed 72 h after application. The minimum criterion for a positive reaction was erythema with infiltration. The test material was also applied on healthy subjects and shown to be non-irritating in the concentrations tested.

The study was approved by the local ethical committee.

RESULTS AND DISCUSSION

Allergenicity of maleopimaric acid(MPA) and fumaropimaric acid (FPA) (I, II)

MPA was a sensitizer both in the FCAT and in the CCET experiments. In the FCAT experiment in which the induction concentration was 3.5% (w/w) (corresponding to 9.0×10^{-2} mmol/g), significant responses were observed for all challenge concentrations applied (Table 3). A significant response was also observed when the animals were tested with a commercial maleic-anhydride-modified rosin (MA-resin) in 10% (w/w) in pet. However, the MA-resin esterified with glycerol (MA-resin ester) did not elicit any reactions in the animals. The anhydride portion of MPA is a strong electrophile and is likely to react with nucleophilic groups in proteins (-SH and $-NH_2$ groups) so producing the complete antigen. The esterification breaks up the anhydride part of MPA and the animals no longer react to this resin. Cross-reactivity was not seen to gum rosin. Rechallenge of the control animals one month later with different concentrations of MPA showed that the animals had been sensitized solely by one epidermal contact with MPA. 50-75% of the animals reacted to MPA 7.2, 3.6, 1.2 and 0.4% (w/w) in pet. (corresponding to 1.8×10^{-1}, 9.0×10^{-2}, 3.0×10^{-2} and 1.0×10^{-2} mmol/g). MPA also sensitized the animals in CCET experiments in which the intradermal FCA injections were omitted. Several induction concentrations were tested and animals induced with 7.2, 1.2 and 0.4% (w/w) in pet. (corresponding to 1.8×10^{-1}, 3.0×10^{-2} and 1.0×10^{-2} mmol/g) reacted to the highest challenge concentrations (7.2, 3.6 and 1.2% in pet.). Our data show that MPA is a potent sensitizer.

Table 3. Sensitizing potential of maleopimaric acid (MPA) in guinea pigs according to Freund's Complete Adjuvant Test (FCAT). Readings at 72 h are presented

Challenge material (in pet.)	Conc. (%)	Group I[1] (n=15) Positive reactions	p exp/co	Control group (n=15) Positive reactions
MPA	7.2	15	<0.001	0
MPA	3.6	14	<0.001	0
MPA	1.2	15	<0.001	0
MPA	0.4	12	<0.001	0
MA resin[2]	1	11	<0.001	0
MA resin ester[3]	10	0	<0.001	0
Gum rosin	10	0	NS[4]	1
Pet.		0		0

[1]Induction: MPA 3.5% (w/v) intradermally.
[2]MA resin = maleic-anhydride-modified tall oil rosin.
[3]MA resin ester = MA resin esterified with glycerol.
[4]NS = not significant

The sensitizing capacity of FPA was investigated with CCET with 7.5% (w/w) in pet. (corresponding to 1.8×10^{-1} mmol/g) as the induction concentration. FPA does not contain an electrophilic anhydride group such as MPA and we did not expect the sensitizing potential of FPA to be very high. We therefore decided to follow the original method and use FCA injections in the induction procedure to enhance the sensitivity of the test. The animals were challenged with FPA 7.5, 3.8 and 1.3% (w/w) in pet. (corresponding to 1.8×10^{-1}, 9.1×10^{-2} and 3.1×10^{-2} mmol/g). No reactions were seen in the animals, thus FPA was classified as a weak or grade-I allergen according to the classification of Magnusson & Kligman (71).

Detection of FPA, MPA and abietic acid in fumaric-acid-modification of abietic acid and rosin (II)

The synthetic FPA was shown with GC-MS to consist of two isomers (methyl esters of FPA 1 and FPA 2, MW 460) in the ratio of 1:9. These two isomers were observed in the crude reaction products of abietic acid and fumaric acid heated together for differ-

ent lengths of time. During the first 20 min no detectable amount of MPA (methyl ester of MPA, MW 414) was formed, but with prolonged heating an increasing amount of MPA was observed.

The two commercial fumaric-acid-modified rosins analyzed contained FPA as well as MPA (Table 4). FPA 1 and FPA 2 (dominating adduct) were both detected in the commercial modified tall oil rosin. However, in the modified gum rosin, MPA was the major adduct. Considerable amounts of unreacted abietic acid were detected in the rosins. The presence of MPA and unreacted rosin indicates that fumaric-acid-modified rosin may also cause contact allergy.

Table 4. The proportions of fumaropimaric acid (FPA), maleopimaric acid (MPA) and abietic acid in commercial fumaric-acid-modified rosin according to GC

Compound	RRT[2]	Samples analyzed[1]		
		FA-TOR	FA-GR	FPA (1+2)
Abietic acid	0.758	49%	83%	ND[3]
FPA 1	0.985	7%	ND	14%
FPA 2	1.000	24%	3%	86%
MPA	1.075	20%	15%	ND

1 FA-TOR = fumaric-acid-modified tall oil rosin
FA-GR = fumaric-acid-modified gum rosin
FPA (1+2) = fumaropimaric acid reference
2 RRT = retention time relative to FPA
3 ND = not detected

Two commercial maleic-anhydride-modified tall oil rosins were shown to contain 8% and 16% of MPA using GC analyses. Abietic acid was also detected in these two rosins in amounts of 33% and 24% respectively.

MPA is formed after FPA, probably through epimerization of one carboxyl group of FPA followed by spontaneous anhydride formation between the two cis carboxyl groups. The other possibility is that the reactant fumaric acid would isomerize to maleic acid, followed by loss of water to produce maleic anhydride. However, this requires the presence of acid catalysis and prolonged heating at high temperature (35). Photosensitized isomerization of fumaric acid to maleic anhydride is seen in aqueous solutions when fumaric acid is irradiated in

the presence of different photosensitizers (36). Neither strong acids nor photosensitizers were present in our reaction mixtures of neat abietic acid and fumaric acid.

Allergenicity of glyceryl triabietate (GTA) and glyceryl 1-monoabietate (GMA) (III, IV)

The main product formed in the esterification of abietic acid with glycerol was GTA - an ester between one glycerol molecule and three abietic acid molecules. GMA, an ester in which only one abietic acid molecule is esterified with glycerol, was the second largest product in the esterification. The animals that were induced with GTA 8.3% (w/w) in pet. (corresponding to $8.8x10^{-2}$ mmol/g) did not react to GTA in any of the challenge concentrations applied, i. e. 8.3, 2.8 and 0.93% (w/w) in pet. (correspond ing to $8.8x10^{-2}$, $2.9x10^{-2}$ and $9.8x10^{-3}$ mmol/g). GMA on the other hand showed some sensitizing capacity. Animals sensitized with 3.3% GMA (w/w) in pet. (corresponding to $8.8x10^{-2}$ mmol/g) showed significant responses to GMA in the two highest challenge concentrations applied, i. e. 3.3 and 1.1% (w/w) in pet. ($8.8x10^{-2}$ and $2.9x10^{-2}$ mmol/g) (Table 5). Cross-reactivity was not seen to gum rosin 10% (w/w) in pet. In the esterification of abietic acid with glycerol, two minor products were also observed: glyceryl 1,2-diabietate ($GDA_{1,2}$) and glyceryl 1,3-diabietate ($GDA_{1,3}$). These were obtained in low yield and their sensitizing potential was therefore not studied.

Animals sensitized to GMA did not react to the diabietates, and no reactions were seen to the compounds in rosin-allergic patients. The glyceryl diabietates seemed not to be rosin allergens of importance.

How GMA acts as a hapten is not clear. The abietic acid part of the molecule is still susceptible to oxidation and a hydroperoxide may be formed as for pure abietic acid (56). However, the spectral analyses showed the GMA we used in animal experiments to be a pure, non-oxidized compound. Oxidation of the abietic acid part of GMA might occur in the test preparations or on the skin. However, this is not very likely, since in animal tests, purified abietic acid does not sensitize the animals (55). An ester function may react with a nucleophilic group in protein to form a covalent bond, but generally the reactivity of an ester is low, since it is a poor electrophile (19)

Detection of GMA and abietic acid in glycerol-esterified rosins (paper IV)

Using reversed-phase HPLC according to the method developed by Ehrin & Karlberg (22), abietic acid was detected in some glycerol-esterified rosins. For reference purposes gum rosin (GR) and tall oil rosin (TOR) were also analyzed. GMA was detected using the same chromatographic parameters. The amounts of abietic acid and GMA in the rosins are shown in Table 6. Abietic acid was detected in all the rosins analyzed; GMA in 4 of 5 glycerol-esterified

rosins. The presence of abietic acid in a modified rosin or in a technical product is an indication that this rosin or product contains unmodified rosin and may cause contact allergy. However, the absence of abietic acid in a technical product may not necessarily mean that rosin has not been added. Low amounts of abietic acid in a product may be due to low amounts of rosin but may also be the result of oxidation, since abietic acid is easily oxidized. GMA is probably also easily oxidized and this must be considered when analyzing for this rosin component.

Patch testing with GMA and glycerol-esterified rosins (IV)

GTA elicited no test reactions in any of 8 rosin-allergic subjects tested (results not shown). However, GMA, which was a sensitizer in guinea pigs, did elicit reactions in 5 of 12 rosin-allergic individuals (Table 7). Two glycerol-esterified rosins both elicited a reaction in 8 of the 12. In the individuals reacting to glycerol-esterified rosin but not to GMA, the reactions are probably due to unmodified material still present in the rosins. However, in those who also reacted to GMA the reactions to the glycerol-esterified rosins may be due to GMA and/or unmodified rosin components since both GMA and unmodified material were detected with HPLC (Table 6).

Sensitization and cross-reactivity studies with oxidized resin acids (V, VI)

Di-(methyl dehydroabietate-15-yl)-peroxide (15-DAP), methyl 15-hydroperoxydehydroabietate (15-HPDA), methyl 13,14(α)-epoxyabietate (α-epoxyabietate) and methyl 13,14(β)-epoxyabietate (β-epoxyabietate) were sensitizers in guinea pigs (Fig 5 A-D). An interesting pattern of cross-reactivity was found between 15-DAP, 15-HPA, 15-HPDA and the 13,14-epoxides of abietic acid (Table 8). 15-DAP-sensitized animals reacted to 15-HPA and so did 15-HPDA-sensitized animals, but the latter did not react to β-epoxyabietate. Guinea pigs sensitized to 15-HPA showed cross-reactivity to the epoxides, but not the peroxide or hydroperoxide, of dehydroabietic acid. Animals sensitized to α- and β-epoxyabietate reacted to both epoxides and to 15-HPA. Thus 15-HPA seems to react via two different routes in the formation of the complete antigen in the skin. The cross-reactivity observed with 15-DAP and 15-HPDA may be explained by the formation of similar alkoxy radicals. Hydroperoxides and peroxides readily produce, by homolytic cleavage, alkoxy radicals (50) that can react with skin protein to form the complete antigen (Figure 6, route 1).

However, another mode of action must be involved in the cross-reactivity between 15-HPA and the epoxides of abietic acid.

Table 5. Sensitizing potential of glyceryl monoabietate (GMA) in guinea pigs according to the Cumulative Contact Enhancement Test (CCET). Readings at 72 h are presented

Challenge material (in pet.)	Conc. (%)	Group I[1] (n=15) Positive reactions	p exp/co[3]	Group II[2] (n=15) Positive reactions	p exp/co	Control group (n=15) Positive reactions
GMA	3.3	6	<0.01	2	NS[4]	0
GMA	1.1	4	<0.05	3	NS	0
GMA	0.37	1	NS	1	NS	0
Gum rosin	0.4	1	NS	8	<0.01	1
Pet.		0		0		0

[1]Induction: GMA 3.3% (w/w) epidermally.
[2]Induction: gum rosin 20% (w/w) epidermally.
[3]Significance level when exposed animals (exp) were compared to control animals (co) using Fisher's exact test.
[4]NS = not significant

Table 6. Content of abietic acid and glyceryl-1-monoabietate (GMA) in commercial glycerol-esterified rosins

Rosin[1]	Abietic acid (%)	GMA (%)
GGR	0.3	2.8
TORG	3.7	0.89
TORG 10	0.6	ND[2]
TORG/MSA	0.7	1.7
TORG/MSA 2	0.6	ND
GR	42	ND
TOR	55	ND

[1]GGR=glycerol-esterified gum rosin prepared at our laboratory
TORG=glycerol-esterified tall oil rosin, freshly prepared
TORG 10=glycerol-esterified tall oil rosin, 10 years old
TORG/MSA=maleic-modified and glycerol-esterified tall oil rosin, freshly prepared
TORG/MSA 2=maleic-modified and glycerol-esterified tall oil rosin, 2 years old
GR=Portuguese gum rosin
TOR=tall oil rosin, freshly prepared
[2]ND=not detected

Table 7. Result of patch testing with GMA and glycerol-modified rosins in patients with known contact allergy to unmodified gum rosin

Test material[1]	Test conc. (% in pet.)	Test reactions in patients (nos. 1-12)												Total positive
		1	2	3	4	5	6	7	8	9	10	11	12	
GR	5	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	12/12
TOR	10	pos.	pos.	pos.	-	pos.	-	pos.	-	-	pos.	pos.	-	7/12
GMA	5.4	pos.	-	pos.	-	-	pos.	-	-	-	pos.	-	pos.	5/12
GGR	20	pos.	-	pos.	-	-	pos.	pos.	-	pos.	pos.	pos.	pos.	8/12
TORG	20	pos.	pos.	pos.	-	-	pos.	pos.	-	-	pos.	pos.	pos.	8/12
Pet.	-	-	-	-	-	-	-	-	-	-	-	-	-	0/12

[1] GR = Portuguese gum rosin
TOR = tall oil rosin
GGR = glycerol-esterified gum rosin prepared at our laboratory
TORG = glycerol-modified tall oil rosin (technical quality)

[2] - = negative, pos. = positive

A common reaction of hydroperoxides is the epoxidation of alkenes. This may involve radical mechanisms or it may be a concerted reaction (50, 65, 95, 96). Thus, 15-HPA is able to react inter- or intramolecularly to produce epoxides of abietic acid.

Nucleophilic attack by electron-rich sites (-NH_2 and -SH groups) in skin proteins on the epoxides would covalently bind the hapten to the protein, thereby producing the antigens (Figure 6, route 2) (20).

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Table 8. Cross-reactivity pattern between hydroperoxides, peroxide and epoxides of resin acids according to Freund's complete adjuvant test (FCAT)

Challenge \ Induction	15-DAP	15-HPDA	15-HPA	α-epoxy-abietate	β-epoxy-abietate
15-DAP	+	NT	-	NT	NT
15-HPDA	NT	+	-	NT	NT
15-HPA	+	+	+	+	+
α-epoxy-abietate	NT	NT	+	+	+
β-epoxy-abietate	NT	-	+	+	+

+ = cross-reactivity observed

- = cross-reactivity not observed

NT = not tested

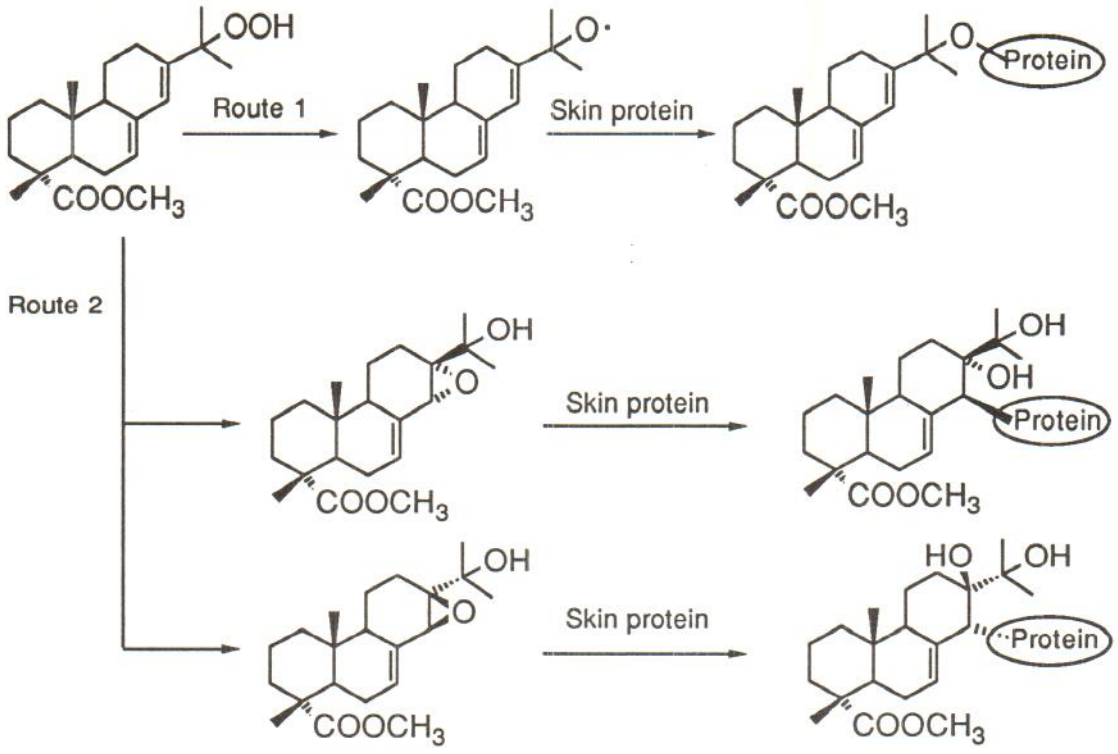

Figure 6. Two possible routes for methyl 15-hydroperoxyabietate (15-HPA) to act as a contact allergen. Route 1: generation of a free alkoxy radical which reacts with skin protein to form the complete antigen. Route 2: generation of epoxides which react with skin protein to form the complete antigen

A two-way cross-reactivity was seen between 15-HPA and the epoxides of abietic acid. This was not observed for 15-HPA, 15-DAP or 15-HPDA. Animals sensitized to 15-HPA did not react to 15-DAP or 15-HPDA significantly. This low reactivity must be further studied, but one hypothesis is that there might be a difference in the relative amounts of the two 15-HPA antigens formed. If 15-HPA produces antigens predominantly via the epoxide route, animals sensitized to 15-HPA would have more T-memory cells to this antigen than to the antigen produced via the radical mechanism. In animals sensitized to 15-HPA and exposed to 15-DAP or 15-HPDA, the T-memory cells able to bind to the radical-derived antigen formed, would be insufficient to give a macroscopic reaction. For animals sensitized to 15-DAP or 15-HPDA most of the T-memory cells are directed to an antigen formed via the radical route. When these animals are exposed to 15-HPA the few antigens formed from 15-HPA via radicals are enough to trigger off a visible test reaction

Patch testing with oxidized resin acids (V, VI)

15-DAP, 2% in pet. (0.03 mmol/g) was tested simultaneously with 15-HPA, 5% and 1% in pet. (0.14 and 0.03 mmol/g) in 7

dermatitis patients sensitive to gum rosin. None of the patients reacted to 15-DAP while 6 of 7 reacted to both concentrations of 15-HPA. Another 7 dermatitis patients sensitive to gum rosin were tested with 15-HPDA, 5%, 2.5% and 1% in pet. (0.14, 0.07 and 0.03 mmol/g) and 15-HPA, 5%, 2.5% and 1% in pet. (0.14, 0.07 and 0.03 mmol/g). None of the patients reacted to 15-HPDA while 3 reacted to 15-HPA. The low reactivity to 15-DAP and 15-HPDA may have several explanations. The patient material tested is not very large, therefore there might be other rosin-allergic individuals who are sensitive to these compounds, or the amounts of 15-DAP and 15-HPDA in rosin might be too small to induce sensitization to these particular compounds. However, reactions were observed to α- and β-epoxyabietate, 5% and 1% (0.15 and 0.03 mmol/g), when tested in twelve dermatitis patients sensitive to gum rosin. These were also tested with 15-HPA, 1% (0.03 mmol/g). The α- and β-epoxyabietates gave 3 and 5 reactions respectively, while four reactions were found to 15-HPA (Table 9). Thus the eliciting capacity of the epoxyabietates seems to be of the same magnitude as that of 15-HPA, which was earlier identified as a major rosin allergen (56).

Detection/identification of 15-DAP and 13,14-epoxides of abietic acid in rosin (V, VI)

15-DAP was isolated from gum rosin as a methyl ester and identified using ^{1}H-NMR and MS-CI with negative ion detection. The NMR spectrum was consistent with a hydroperoxide of methyl dehydroabietate but when performing MS analyses we could not observe the corresponding molecular ion. Instead, when using chemical ionization with negative ion detection we observed that the compound had the molecular weight of a peroxide between two methyl dehydroabietate molecules (MW 659) (Fig 4). The estimated amount of 15-DAP in gum rosin was 0.3%.

β-Epoxyabietate was detected in gum rosin using GC/MS(EI). Retention time and fragmentation pattern were identical with those of the synthesized reference compound. The amount was roughly estimated to 1%. β-Epoxyabietate was not detected in tall oil rosin. α-Epoxyabietate was detected neither in gum rosin nor in tall oil rosin.

Table 9. Result of patch testing with 15-HPA and epoxides of methyl abietates in patients with known contact allergy to unmodified gum rosin

Test material[1]	Test concentration (% in pet.)	Test reactions in patients (nos. 1-12)												Total positive
		1	2	3	4	5	6	7	8	9	10	11	12	
GR	5	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	12/12
15-HPA	1	pos.	pos.	pos.	pos.	-	-	-	-	-	-	-	-	4/12
α-Epoxyabietate	5	pos.	-	-	pos.	-	pos.	-	-	-	-	-	-	3/12
α-Epoxyabietate	1	pos.	-	-	-	-	-	-	-	-	-	-	-	1/12
β-Epoxyabietate	5	pos.	pos.	pos.	pos.	-	-	pos.	-	-	-	-	-	5/12
β-Epoxyabietate	1	pos.	-	pos.	-	-	-	-	-	-	-	-	-	2/12
Pet.		-	-	-	-	-	-	-	-	-	-	-	-	0/12

[1] GR = Portuguese gum rosin
15-HPA = methyl 15-hydroperoxyabietate
α-epoxyabietate = methyl 13,14(α)-epoxyabietate
β-epoxyabietate = methyl 13,14(β)-epoxyabietate

[2] - = negative, pos. = positive

GENERAL DISCUSSION

The major part of the rosin used today is modified in different ways. In our studies we have seen that some modifications created new allergens different from those in unmodified rosin. In the standard series currently used in the dermatological clinics, the screening substance for rosin allergy is unmodified rosin of the gum rosin type. A test panel with components from different types of modified rosin might prove useful in revealing additional cases of contact allergy.

Several oxidation products of resin acids have been identified as contact allergens (Figures 3, 4) (V,VI) (56, 57). These oxidation products could be considered as screening substances for contact allergy to unmodified rosin. The product would be chemically well-defined and could be analyzed for purity. However, the oxidation products identified in rosin so far have not elicited reactions in all rosin-sensitive indiviudals tested. Probably there is no major contact allergen that all rosin-sensitive individuals would react to. Individuals may develop contact allergy to different components of rosin. The different allergens in rosin may also potentiate each other. This was suggested for allergens in oxidized limonene where animals sensitized with oxidized limonene did not react to the individual oxidation products (59). Thus, at present the best screening substance for allergy to unmodified rosin seems to be the complete rosin.

Analysis of rosin allergens in different products is desirable but difficult. The analysis should be easy to perform without extensive sample pre-treatment, and the method employed should not destroy unstable contact allergens such as hydroperoxides. For heat-sensitive compounds HPLC is preferable to GC. With the GC methods published for analyses of resin acids and oxidation products of these (24) only more stable oxidation products, e. g. 15-hydroxy-, 15-hydroxy-7-oxo- and 7-oxodehydroabietic acid (Fig 3), were detected. All peroxy compounds are apparently destroyed in the gas chromatograph.

The modified rosin components now known as contact allergens are stable compounds. MPA, a contact allergen formed in the modification of rosin with maleic anhydride or fumaric acid, was detected with GC on the corresponding methyl ester (II). GMA, a contact allergen formed in the glycerol modification of rosin, was detected with reversed-phase HPLC without derivatization (IV).

The Cumulative Contact Enhancement Test (CCET) used in the sensitization studies of the modified rosin components (MPA, FPA, GTA, GMA) involves solely epidermal applications of the test substance for induction (104). CCET was chosen since it more ac-

curately reflects common exposure to rosin products. Due to the larger amount of test substance required in CCET, this test method could not be employed when studying the sensitization potential of oxidation products of resin acids which could be isolated from rosin only in small amounts. We therefore chose Freund's Complete Adjuvant Test (FCAT) which requires less test material and which we have used earlier for oxidation products in rosin (56, 57, 63).

In the present experimental sensitization studies of contact allergens from rosin we used concentrations equimolar to concentrations used in sensitization experiments for rosin allergens identified earlier (56, 57). For studying sensitization with oxidation products of resin acids, we used concentrations corresponding to those used for the hydroperoxide of methyl abietate (15-HPA, Fig. 3) (56). The induction concentration of 15-HPA was 0.14 mmol/g and challenge concentrations were 0.14 and 0.03 mmol/g. For the corresponding hydroperoxide of methyl dehydroabietate (15-HPDA, Fig. 4) and the epoxides of methyl abietate, the same concentrations for induction and challenge were used (VI, Table 8). Since the concentrations employed were equimolar, we were able to compare the results from the different experiments and conclude that 15-HPA, 15-HPDA and the epoxyabietates are sensitizers of comparable strength according to FCAT. The induction concentration of the peroxide of methyl dehydroabietate (15-DAP, Fig. 4) was 0.076 mmol/g (V). We wanted to use 0.15 mmol/g for comparison with 15-HPA but the difficulty in isolating the peroxide from rosin compelled us to lower the concentration. In view of the result (Table 8), which indicates generation of haptens via homolytic cleavage of the peroxide bond in 15-DAP, 0.076 mmol/g seems reasonable since it would give rise to 0.15 mmol/g of the radical hapten.

The rosin derivatives identified as contact allergens and detected in rosin were patch tested in rosin-allergic subjects. MPA, GMA and β-epoxyabietate, which all elicited reactions in the allergic individuals, were added to the standard test panel used at the Karolinska Hospital, Sweden. GMA was tested in 270 consecutive dermatitis patients and 8 reacted to the test substance. Five of these 8 did not react to gum rosin in the standard series. In 3 of the patients reacting to GMA, the history indicated exposure to glycerol-modified rosin. Two had leg ulcers which had been bandaged, and glycerol-modified rosin is often used in adhesives. The third patient was exposed to printing inks which, again, may contain glycerol-modified rosin. β-Epoxyabietate was tested in 437 consecutive dermatitis patients and 2 patients reacted to the material. One of these did not react to the gum rosin in the standard test. MPA has been tested in dermatitis patients, and the results from the patch testing are being evaluated.

The cross-reactivity pattern of the oxidation products of resin acids indicated that 15-HPA may react via two different routes to produce the complete antigen. The proposed routes are 1) formation of an alkoxy radical which reacts with skin protein to form the antigen and 2) formation of epoxides which react with skin protein, so producing the complete antigen. Hapten and antigen formation in the skin is difficult to study since the skin offers a heterogenous environment with complex enzyme activities. So far, the fate of contact allergens in the skin can be studied only with indirect methods such as cross-reactivity studies of chemically related substances or measurements of formed radical by radical trapping; or by isolated in vitro methods, e. g. protein binding models (2, 103). The development of in vivo or in vitro methods for studying the effect of skin metabolism on contact allergens is important for further understanding of the mechanisms of contact allergy.

CONCLUSIONS

In the commonly employed modification of rosin with maleic anhydride or fumaric acid a potent contact allergen is formed. The compound was identified as endo-maleopimaric acid (MPA), earlier described in the literature. MPA showed high sensitizing capacity in experimental sensitization studies. The major product formed in the modification of rosin with fumaric acid, fumaropimaric acid (FPA), did not elicit any reactions in the animals tested.

Glyceryl triabietate (GTA) was the major compound formed in the esterification of rosin with glycerol. In an experimental sensitization study of GTA none of the animals reacted. However, a minor compound, identified as glyceryl-1-monoabietate (GMA), showed sensitizing capacity. GMA also elicited reactions in rosin-sensitive dermatitis patients. GMA was detected in glycerol-modified rosins using HPLC.

Residual unmodified material was detected in all the modified rosins analyzed. This unmodified material may cause sensitization or, if present in low amounts, elicit reactions in individuals already allergic to rosin.

A peroxide and a hydroperoxide of dehydroabietic acid, namely di-(methyl dehydroabietate-15-yl)-peroxide (15-DAP) and methyl 15-hydroperoxydehydroabietic acid (15-HPDA), showed sensitizing capacity in animal experiments. 15-DAP was isolated from gum rosin and was also detected in tall oil rosin. Minute amounts of 15-HPDA were detected in gum rosin. 15-DAP and 15-HPDA did not elicit reactions in rosin-sensitive individuals and thus seem not to be rosin allergens of importance.

Methyl-13,14(β)-epoxyabietate (β-epoxyabietate) was identified as a new important

contact allergen in gum rosin. Another epoxide, methyl-13,14(α)-epoxyabietate (α-epoxyabietate) also showed sensitizing capacity in an experimental sensitization study. However, this epoxide was not detected in rosin.

The cross-reactivity pattern between the earlier identified rosin allergen methyl 15-hydroperoxyabietate (15-HPA) and 15-DAP, 15-HPDA and α- and β-epoxyabietate, respectively, indicates that 15-HPA reacts with skin proteins via two different routes, generating different antigens. 15-HPA may either react with skin protein as a radical obtained from homolytic cleavage of the O-O bond or via the formation of an epoxide which may react with skin proteins in a nucleophilic-electrophilic interaction.

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