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Journal of Chromatography B, 700 (1997) 101–110

JOURNAL OF CHROMATOGRAPHY B

High-performance liquid chromatography of unmodifed rosin and its applications in contact dermatology

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Received 19 March 1997; received in revised form 2 May 1997; accepted 12 May 1997

Abstract

Rosin is a well recognised skin sensitiser and is also amongst the most common causes of occupational asthma. Due to its complex chemical composition, it is difficult to isolate its many components and this has hindered progress in the identification of the specific respiratory and contact allergens it contains. This paper reports the application of high-performance liquid chromatography and other analytical techniques to the isolation and identifcation of contact allergens in complex mixtures such as rosin. HPLC methods were developed in order to isolate as many rosin components as possible and these were then patch tested on rosin sensitive individuals. The structure of the most dermatologically active component was then determined using mass spectrometry, nuclear magnetic resonance and infrared techniques. An HPLC method has also been developed which will enable the identification of rosin in commercial products, providing a valuable tool for determining the cause of rosin contact allergy. Furthermore, mass spectral data for the common abieitic-type resin acids are compiled which were used to confirm the identification of the HPLC resin acid peaks and have not been reported previously.

Keywords: Rosin

1. Introduction

Colophony (rosin) is a natural substance obtained from various species of pine tree and used in a wide range of commercial products in both its unmodified and chemically-modified forms. The authors have previously reported on production methods, uses and health effects of rosin [1]. The chemical composition of rosin is complex, approximately 90% being resin acids and the remainder being mainly the corresponding esters, aldehydes and alcohols [2]. Of the resin acids, 90% are isomeric with abietic acid ($C_{19}H_{29}COOH$) and can be sub-divided into two main types; the abietic-types (abietic, neoabietic, levopimaric, palustric and dehydroabietic acids) with conjugated double bonds, and the pimaric types (isopimaric and pimaric acids) with non-conjugated double bonds (Fig. 1).

Three main types of rosin are produced which are differentiated by their method of extraction. Gum rosin is tapped from living trees, wood rosin is extracted from pine stumps, and tall oil rosin is obtained as a by-product in paper pulp production [3]. The global rosin production is approximately one million tonnes per year. Although all rosin types contain the same resin acids their proportions depend

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0378-4347/97/$17.00
PII S0378-4347(97)00293-4

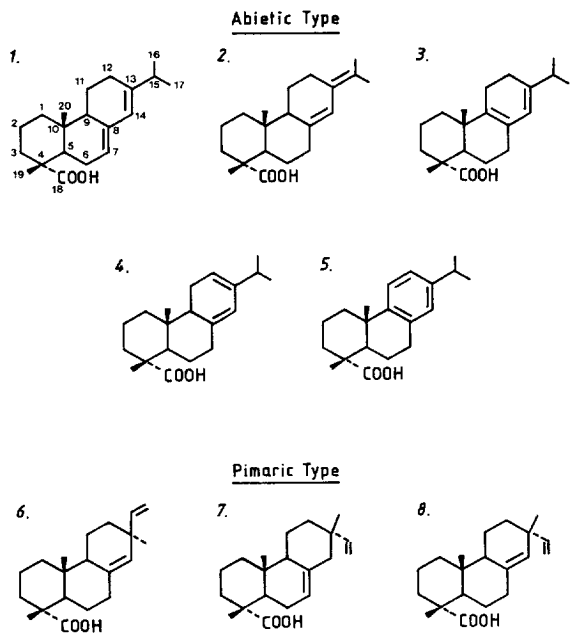

Fig. 1. Common resin acids in colophony (rosin) [1]. 1. Abietic, 2. neoabietic, 3. palustric, 4. levopimaric, 5. dehydroabietic, 6. pimaric, 7. isopimaric, 8. sandaracopimaric.

on factors such as the species of timber, locality, recovery process, handling and storage [4]. The major rosin component is abietic acid (40–50%) which is believed to be a weak contact allergen [3,5] although other studies suggest that it is not allergenic [6]. The abietic type resin acids have been reported to isomerise to abietic acid either thermally (above 175°C) or by treatment with dilute mineral acids [7]. Above 200°C abietic acid disproportionates mainly with the formation of dehydroabietic, tetrahydroabietic and dihydroabietic acids [2]. Abietic-type resin acids oxidise when exposed to air and light, producing compounds with greater allergic potential than the resin acids themselves [4,8,9].

The analysis of resin acids is usually carried out by gas chromatography (GC) after converting the resin acids to their methyl esters. The first application of GC to resin acid analysis was reported by Hudy [10] who used a column packed with butandiol succinate. Hudy suggested that resin acids may isomerise during the separation due to the acidity of the support material; difficulties in resolving the levopimarate/palustarate peaks were also reported. Subsequently, the GC characteristics of resin acids for packed columns were reported [11]. The resolution of levopimarate and palustarate was achieved with cyanosilicone stationary phases [12]. The use of capillary columns in the GC analysis of resin acids has also been reviewed [13]. Extensive mass spectral and infrared data have been compiled for the identification of resin acid methyl esters [11].

Although few data are available on the analysis of resin acids by HPLC, this technique was chosen in preference to GC for the separation and collection of rosin components prior to patch testing for several reasons including: (a) HPLC separation is carried out at room temperature thus avoiding resin acid isomerisation; (b) the higher loading capacity of HPLC columns compared to GC columns reduces the number of injections necessary to isolate sufficient individual rosin components for further analysis; (c) no resin acid methylation step is needed.

To our knowledge, HPLC separation of the common abietic type resin acids and their mass spectral data has not been reported previously. HPLC has been used mainly to separate rosin (eluting as a single peak) from other materials such as fatty acids in tall oils [14,15]. In 1981, Kamutzki and Krause [16] isolated some resin acids in rosin using a combination of two methods: (a) the addition of silver ions to the mobile phase to form Π-electron complexes with specific resin acid double bonds (this was demonstrated with levopimaric and abietic acids); (b) use of the different wavelengths of maximum absorption in the ultraviolet spectra of different isomeric resin acids. Fluorescence detection of dehydroabietic acid in an effluent from a pulping process has also been reported [17]. In 1988, Karlberg et al. [8] reported on the identification of contact allergens in Portuguese rosin using a combination of LC techniques and patch testing (described below under methodology below). The Portuguese rosin sample was fractionated initially on a glass column and the fractions collected were derivatised prior to their HPLC fractionation. The derivatised HPLC rosin fractions were then patch tested on rosin-sensitive individuals. Karlberg et al. showed that esterification did not affect the allergenicity of the rosin components [8]. This view is not shared by some workers who have shown that the allergenicity

of rosin is reduced on esterification [18,19]. More recently, an HPLC method for detection of rosin in technical products has been reported [20], which involved detection of only two (abietic and dehydroabietic acids) rosin acids.

Colophony has been associated with both occupational asthma and with contact dermatitis [3], particularly when used as a flux in soft soldering [21,22]. Until recently, the occupational health literature has contained many reported cases of occupational asthma and contact dermatitis arising from the use of colophony-based products, there has been little progress on the identification of specific allergens in rosin. One of the main reasons for this is the difficulty in separating the isomeric resin acids.

This paper reports on the application of HPLC and other analytical techniques in the isolation and identification of specific contact allergens in rosin. Both analytical and preparative HPLC methods were developed for the separation and collection of rosin components and these were then patch tested on rosin-sensitive volunteers in order to discover which of them were dermatologically active. The structures of the most dermatologically active components were then elucidated by using a combination of NMR, MS and IR techniques. Mass spectral data for the common abietic-type resin acids were compiled which enabled the identification of the isomeric resin acids which has not been reported previously.

2. Experimental

2.1. Materials

A sample of unmodified rosin (equal mixture of American, Chinese and Portuguese gum rosin) was obtained from Hermal Chemie (Kurt Herman, Hamburg, Germany). This rosin sample was a sub-sample from a batch used to prepare the commercial rosin preparation in petrolatum used for patch testing in dermatological clinics.

Resin acid standards of defined purity were obtained from Helix Biotech Scientifc (Richmond, Canada) including abietic acid (95%), neoabietic acid (99+%), dehydroabietic acid (99+%) and levopimaric acid (99+%). The abietic acid standard was purified further by passing it through a silica column, the final purity being 99%. The HPLC solvents, methanol and water (both 'HiPerSolv' grade) were purchased from BDH (Poole, UK).

2.2. High-performance liquid chromatography

The unmodified gum rosin sample was analysed initally on an analytical reversed-phase column in order to separate as many rosin components as possible including the common abietic-type resin acids. Subsequently, three preparative HPLC methods (I, II and III) were developed, enabling collection of individual rosin components. Initially the reversed-phase preparative column was intentionally overloaded in order to group the constituents of rosin into three main fractions (A, B and C) using an isocratic solvent system (HPLC method I). The three rosin fractions were then rotary evaporated in order to remove the solvent and prepared for patch testing. Two further HPLC methods (II and III) involving solvent gradients were then developed and using these the components of the three rosin main fractions were isolated and collected for further patch testing. The three preparative HPLC methods (I, II and III) involved 25, 50 and 50 repetitive injections respectively of 3.0% (w/v) rosin in methanol.

The HPLC system used, consisted of a Perkin-Elmer series 400 quaternary delivery pump and a LC-75 variable wavelength UV detector (set at 245 nm). The analytical column was a reversed-phase 5-μm Spherisorb ODS2 (250×4.6 mm I.D.) (Phase Separations, Deeside, UK) fitted with a 2-cm guard column packed with Perisorb RP18 (Anachem, Luton, UK). Preparative chromatography was done with a Perkin Elmer LiChrosorb RP-18 (10 μm, 250×16 mm I.D.) reversed-phase column. The mobile phase consisted of methanol and water. The solvent programmes for both analytical and preparative chromatography methods are shown in Fig. 2 Fig. 3, respectively. Retention times and peak areas were measured with a Hewlett–Packard integrator.

Prior to analyses, the unmodified rosin sample was dissolved in methanol (3.0% w/v) and filtered through a 0.45-μm Nylon-66 filter (Anachem, Luton, UK). The filtered solution was then stored in amber-coloured glass vials under nitrogen.

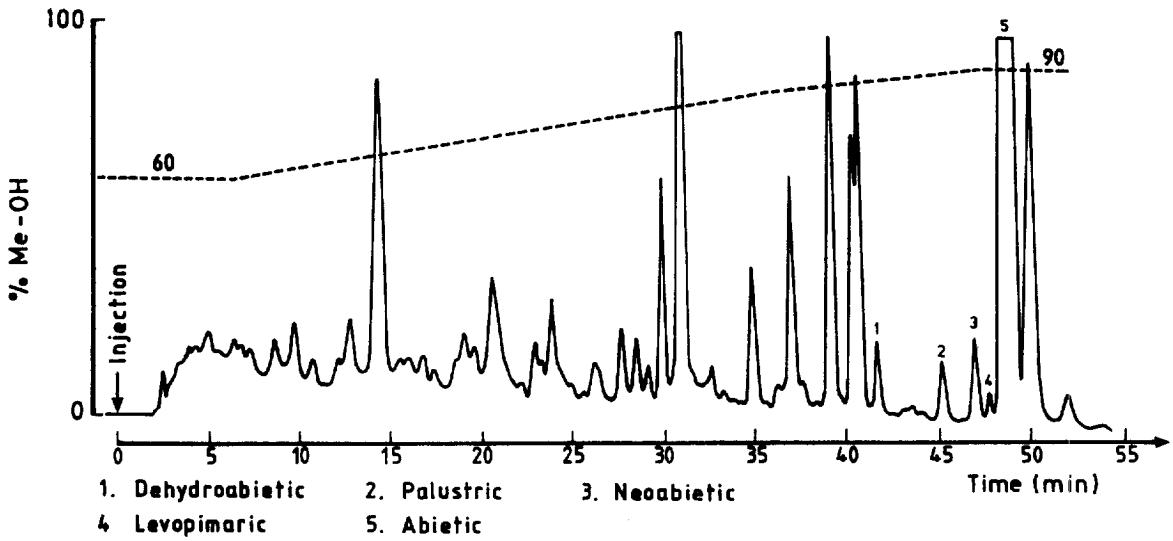

Fig. 2. Analysis of unmodified rosin under optimised HPLC conditions. Column: 5-μm Spherisorb ODS2, 250×4.6 mm I.D.; detector: UV (245 nm); mobile phase: methanol–water; flow-rate: 1.5 ml min^{-1}; sample: unmodified rosin 0.42% w/v in methanol.

2.3. Patch testing

In total, twenty patients previously diagnosed as rosin-sensitive (through patch testing with rosin in the standard series) and thirty controls were patch tested with 20 HPLC rosin components at the Skin Hospital (Birmingham, UK). The control subjects were patients who produced no reaction to rosin in the standard patch test series. Details of patch testing methodology and interpretation of results have been published elsewhere [23].

The purpose of patch testing is to detect contact allergy. It is performed by applying the suspected substance under occlusion at a suitable non-irritant concentration to normal skin and secured with adhesive tape. If an eczematous response occurs, the person is suspected of having contact allergy to the tested substance. A battery of common contact allergens (standard series) is recommended by the International Contact Dermatitis Research Group (ICDRG) for preliminary patch testing in dermatological clinics. Rosin is included in both the European and American standard series and is usually specified as an equal mixture of unmodified gum rosins (American, Portuguese and Chinese) patch tested at 20% (w/w) in petrolatum.

2.4. Spectroscopy

The dermatologically active rosin components, resin acids standards (abietic, dehydroabietic, neoabieitc, levopimaric and palustric acid) and the rosin HPLC resin acid peaks were analysed on a Kratos M5BORF mass spectrometer and the data acquired by a D555C data system. The solid samples (0.1 mg) were inserted directly via a vacuum lock. The parameters used in the chemical ionisation mode were: ion source temperature 200°C, ionisation energy 65 eV, scan range 27–844 *m/z*, scan cycle time 3.0 s, beam current 100 μA and resolution of 1500 and methane as the reagent gas.

The NMR spectra of the rosin fractions were obtained with a Jeol, GX270 high field spectrometer; CD2Cl2 solutions were used with TMS as an internal standard, under the following conditions: 100–200 scans, spectral width 4000 Hz, pulse angle 30°C and a pulse delay of 2.

The IR spectra of the dermatologically active HPLC rosin fractions were obtained with a Polaris (Mattson) IR spectrometer with a deuterated triglycine sulphate (DTGS) detector with a resolution of 4 cm^{-1}. Nujol was used as the solvent and the recording range was 4000–500 cm^{-1}.

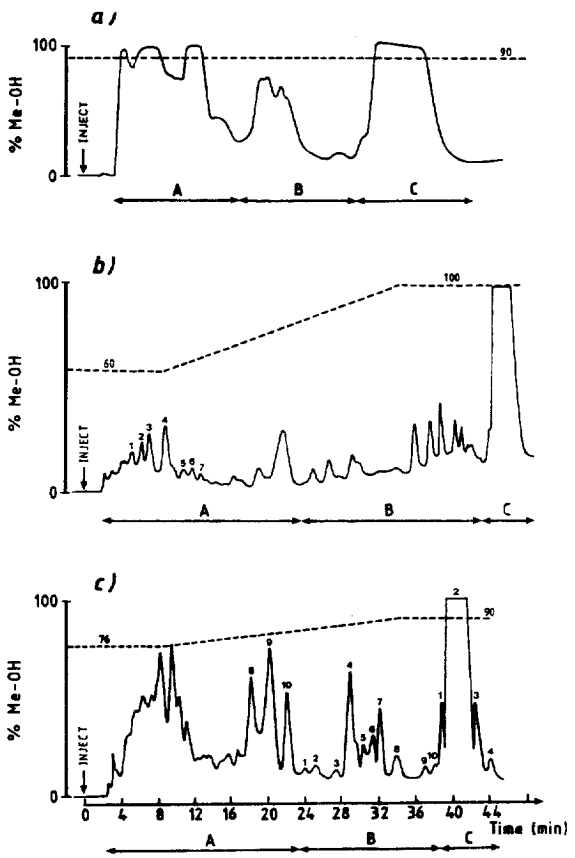

Fig. 3. Separation and collection of HPLC rosin components [23]. (a) Separation of rosin coomponents A, B and C using HPLC method I. (b) Rosin components A1–A7 using HPLC method II. (c) Rosin components A8–A10, B1–B10 and C1–C4 using HPLC method III [23]. Column: LiChrosorb RP-18, 10 μm, 250×16 mm; detector: UV (245 nm); mobile phase: methanol–water; flow-rate: 6 ml min^{-1}; sample: unmodified rosin 3% (w/v) in methanol.

3. Results

3.1. HPLC

The maximum UV absorbance for rosin (in methanol) was found to be at 245 nm. Under the optimised HPLC conditions, analysis of rosin produced at least 30 individually resolved peaks in a total analysis time of 50 min (Fig. 2). The common resin acids including dehydroabietic, palustric, neoabieitc, abietic and levopimaric acids were identified using standard resin acids as reference standards for both HPLC and mass spectral analysis. The identification of these resin acids was confirmed by mass spectral analysis of the levopimaric acid peak could not be resolved totally from the large abietic acid peak.

After initial separation of rosin into three major fractions A, B and C (HPLC method I, Fig. 3a), two further HPLC methods (II and III) were developed to isolate the components within these broad rosin fractions. HPLC method II was used to isolate the more polar components A1 to A7 (Fig. 3b), and method III was used to isolate rosin components A8 to A10, B1 to B10 and C1 to C4 (Fig. 3c). Thus a total of 24 inidividual rosin components were collected. The time taken to collect fractions from a single injection of each of the three preparative HPLC methods (I, II and III) was 34, 28 and 58 min, respectively. Patch testing 20 rosin sensitive individuals and 30 control subjects with the 24 individual rosin components showed that the most dermatologically HPLC rosin component was A9 which produced a positive reaction in 70% of rosin-sensitive individuals.

3.2. Mass spectrometry

The mass spectrum for the dermatologically active rosin fraction A9 (Fig. 4) showed that it has a relative molecular mass of 314 which is also its base peak. The fragmentation pattern is relatively simple

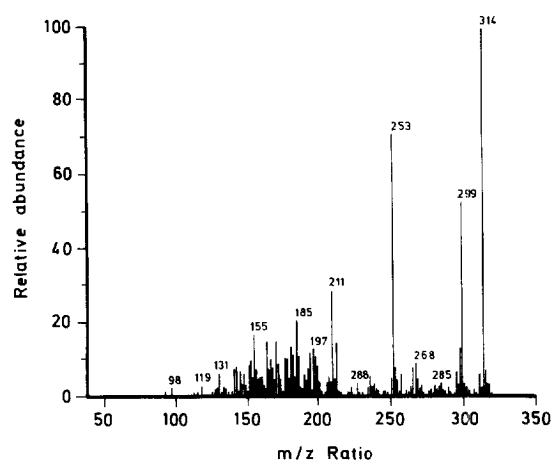

Fig. 4. Mass spectrum of rosin HPLC fraction A9.

and highly characteristic of aromatic compounds. The presence of a carboxylic acid and a ketone functional group was indicated by the loss of fragments of masses 61 (M−253) and 42 (253−211) due to CO_2H+O and CH_2O respectively. Other common fragmentations were due to loss of CH_3 groups i.e M−299 and 268−253.

Reference mass spectra for the abietic-type resin acids are shown in Fig. 5. As expected, the isomeric resin acids; abietic, levopimaric, palustric and neoabieitc produced a molecuar ion peak at *m/z* 302 and for dehydroabietic acid, the molecular ion peak occurred at *m/z* 300. The base peaks for the resin acids, however, occurred at different *m/z* values except for the abietic acid and levopimaric acids for which the molecular ion peak was the base peak. Table 1 compares the intensities (expressed as a percentage of base peak) of the fragments obtained by loss of the methyl, hydroxyl, isopropyl and carboxylic acid groups from the molecular ion peaks of the the resin acids. Dehydroabietic acid produced no fragments corresponding to the loss of the hydroxyl, isopropyl or the carboxylic acid groups from its molecular ion peak.

The NMR spectra for rosin fraction A9 was complex (Fig. 6) due to the large number of protons in different chemical environments. One of the predominant features of the spectra is the singlet and two doublets at δ 6.88, 6.96 and 7.16 ppm respectively which suggests the presence of a 1,2,4-trisubstituted benzene ring. The other important feature of the spectrum is the presence of a doublet (δ 1.21 ppm) and septet further upfield (δ 2.83 ppm), which is the characteristic pattern of an isopropyl group. The septet at δ 2.83 ppm suggests that an isopropyl group is connected to an aromatic ring. Since the protons in the isopropyl group (CH_3 and CH) are magnetically non-equivalent (diastereotropic) the methyl doublet peak under high resolution is split into two doublets centred at 81.20 and δ 1.18 pmm. The presence of two further methyl groups in the structure is indicated by singlets at δ 1.19 ppm due to CH_3-C-Ar protons and a singlet downfield at δ 1.26 ppm due to CH_3-CR_2COOH protons. Doublets at δ 2.16 ppm and δ 2.31 ppm with geminal coupling constants of 12.5 Hz were assigned to equatorial hydrogens on the six-membered ring. A third hydrogen which appears to lie under the isopropyl septet at δ 2.83 ppm was thought to be an equatorial hydrogen adjacent to a carbonyl group. The remaining nine hydrogens occur as mutiplets in the region δ 1.3–1.9 ppm. The carboxylic acid peak which usually occurs in the region δ 10–13 ppm was not visible. The OH peak for carboxylic acid is usually broad and of low intensity due to intermolecular proton exchange with water molecules present in the sample.

The IR spectrum for A9 showed prominent peaks at 2850–2500 cm^{-1} (OH) 1250 cm^{-1} (CO) vibrational bands suggesting the presence of a saturated carboxylic acid group. The bands at 1670 cm^{-1} and 1720 cm^{-1} were assigned to C=O bands of an aryl ketone and carboxylic acid respectively. An OH band at 920 cm^{-1} provided further evidence for the presence of a carboxylic acid group. Broad bands in the region 3100–3000 cm^{-1} and 1610 cm^{-1} were characteristic of an aromatic ring. Taken together, the above spectral data of rosin fraction A9 (showing the greatest dermatological activity) was consistent with the structure 7-oxodehydroabietic acid [23].

4. Discussion

Rosin is a widespread natural product which is used in many different commercial preparations and which is a well known respiratory and contact allergen. The North American Contact Dermatitis Group (NACDG) has listed over 300 products on the U.S market which contain either rosin or rosin derivatives [24]. Abietic acid is the major constituent of rosin (40–50%) and was long considered to be the main rosin allergen [25]. However, in 1985 Karlberg et al. [6] reported that in its pure form, abietic acid is not allergenic and asked the question 'what is the contact allergen in rosin?' In an attempt to answer this question, we isolated and collected rosin components using preparative HPLC. This technique allowed collection of rosin components in their natural state thus avoiding use of high temperatures, resin acid esterification and acid treatment which lead to chemical changes in rosin composition. To demonstrate the complexity of rosin, an analytical HPLC method was developed in order to separate as many rosin components as possible on a reversed-phase analytical column. Under optimised separation

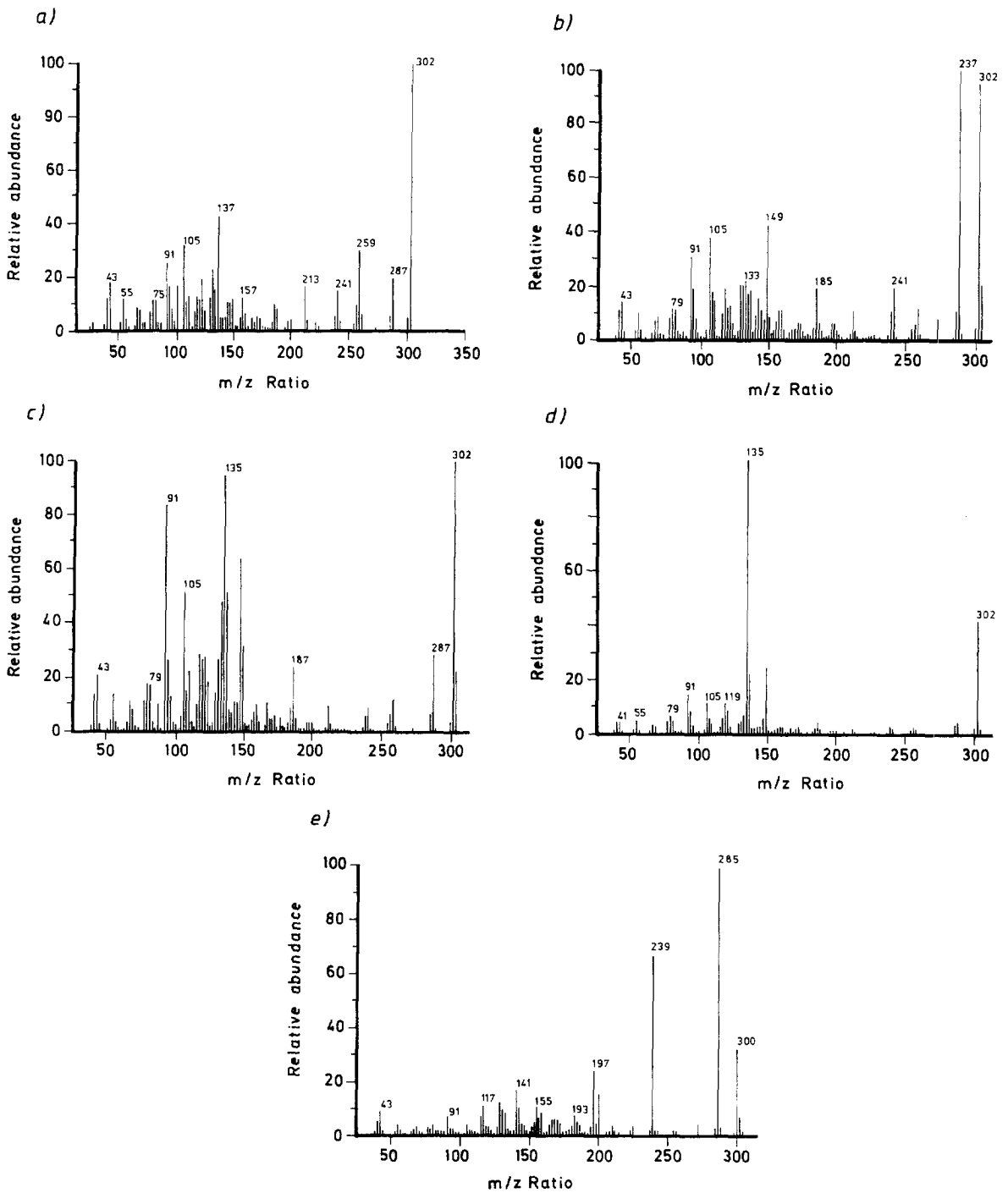

Fig. 5. Mass spectra of abietic-type resin acids. (a) Abietic acid, (b) palustric acid, (c) levopimaric acid, (d) neoabietic acid, (d) dehydroabietic acid.

Table 1
Comparison of the relative abundance of common mass fragments of resin acids

Resin acid	Intensity of base peaks (%)			
	$(M-15)^+$	$(M-17)^+$	$(M-43)^+$	$(M-45)^+$
Abietic	18.0	5.0	30.0	7.1
Levopimaric	28.6	6.5	11.7	5.7
Palustric	100.0	10.9	11.3	4.7
Dehydroabietic	100.0	0.0	0.0	0.0
Pimaric	57.4	0.0	3.5	14.4

CH_3=15, OH=17, C_3H_7=43, CO_2H=45.
The relative abundance of each fragment is the percentage intensity of the resin acid base peak.

conditions, this resulted in a chromatogram showing at least 30 resolved peaks. Of the 30 peaks, 5 were identified as the common abietic-type acids, i.e, abietic, neoabietic, palustric, dehydroabietic and levopimaric acid. The separation of these resin acids by HPLC, to our knowledge, has not been reported previously. Since rosin is used in so many different products in both occupational and non-occupational settings, it is often difficult to explain rosin allergy by reviewing the patients history [26]. Patch testing is a useful diagnostic tool in contact dermatology, however, if the specific contact allergens have not been identified then only screening materials such as the rosin mixture are used and the patient may produce a positive patch test reaction to commercial rosin product but no reaction to rosin in the standard series [27]. Furthermore, there may be difficulty in obtaining the individual components of a suspected substance and industry may be reluctant to reveal details of the exact chemical compositions of their products. In such situations the HPLC method developed to detect the common resin acids could be used to confirm the presence of rosin in commercial products. This could provide a valuable tool in determining the cause of the dermatitis so that re-exposure could be prevented or minimised.

In order to isolate the rosin components, three HPLC methods were developed. The first method was used to separate the components of colophony into three broad bands A, B and C. Patch test results for these fractions on rosin-sensitive individuals showed that all three contained allergic components.

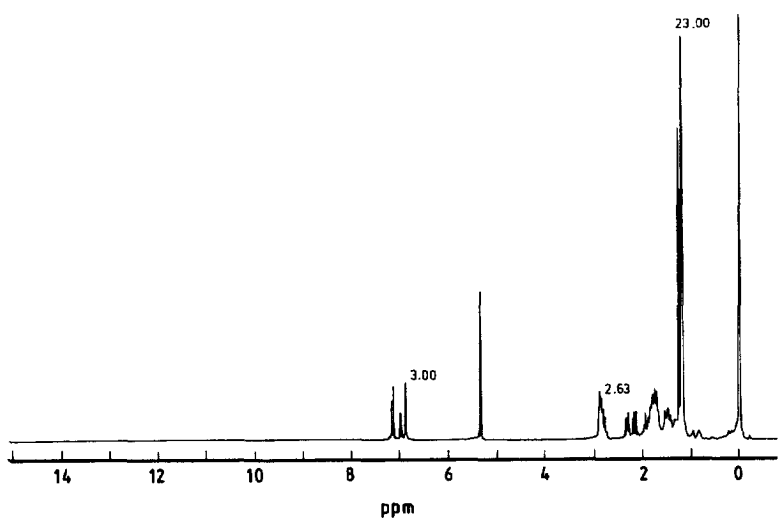

Fig. 6. NMR spectra of HPLC rosin fraction A9.

This result demonstrated the presence of more than one sensitiser in the rosin. For this reason, and because the three fractions each contained a number of components, the other HPLC preparative solvent methods developed, were used to collect individual rosin components. Patch testing with the individual rosin components on rosin-sensitive individuals showed that the most potent contact allergen in rosin was HPLC rosin fraction A9. The mass spectra for A9 suggested a molecular weight of 314, the presence of an aromatic ring, methyl and ketone functional groups. The NMR spectra suggested the presence of a 1,2,4-trisubstituted benzene ring, an isopropyl group, two further methyl groups and four methylene groups. The IR of A9 indicated the presence of a ketone group, a saturated carboxylic acid and an aromatic ring. Taken together, this evidence demonstrated that the rosin oxidation component A9 was 7-oxodehydroabietic acid which is an oxidation product of abietic acid. The proposed structure for the colophony HPLC fraction A9 is further supported by the work of Karlberg et al. [8] who isolated and identified 7-oxodehydroabieitc acid and 15-hydroxydehydroabietic acid as the main contact allergens in Portuguese colophony. For complete confirmation of the proposed structure, preparation of the authentic sample and its reference spectral data would have been valuable but this was beyond the scope of this study. 7-Oxodehydroabietic acid is an oxidation product of abietic acid, the authors have recently studied the rate of oxidation of individual resin acids and its implication in patch testing [28]. Possible mechanisms of resin acid oxidation have also been reported [2]. Because the allergens in rosin appear to be oxidation products of rosin acids, the allergic potential of rosin-based materials might be reduced by preventing this oxidation. The oxidation of resin acids is thought to involve the conjugated system of double bonds, and possible methods for minimising the tendency for air oxidation of rosin include hydrogenation, polymerisation and dehydrogenation of these double bonds.

Since 1960, the majority of rosin analyses have been carried out using esterified rosin acids, thus reference mass spectral data were only available for resin acids esters. In this study, mass spectral analysis for the common abietic-type resin acids has been compiled and was used to identify 5 resin acids. Mass spectral analysis of standard reference resin acids showed that most individual resin acids (palustric, dehydroabietic, neoabietic) have a characteristic m/z base line peak value which can be used to identify the individual resin acids. The resin acids with the same m/z value of baseline i.e abietic and levopimaric can be differentiated by the relative intensities of the main fragment ions i.e the peaks due to $(M-15)^+$ and $(M-43)^+$ ions. This information will be useful in liquid chromatography–mass spectrometry analysis of rosins.

References

[1] S. Sadhra, I.S. Foulds, C.N. Gray, D. Koh, K. Gardiner, Ann. Occ. Hyg. 38 (1989) 385.
[2] H.I. Enos, Jr., G.C. Harris, G.V. Hedrick, in: H.F. Mark, J. McKetta Jr., D.F. Othmer, A. Standen (Eds.), Kirk Othmer Encyclopedia of Chemical Technology, 2nd ed., John Wiley and Sons, New York, 1969, p. 475.
[3] S. Sadhra, Ph.D Thesis, University of Birmingham, Birmingham, 1991.
[4] A.T. Karlberg, S. Liden, Br. J. Dermatol. 113 (1985) 475.
[5] B.M. Hausen, C. Hessling, Contact Dermat. 23 (1990) 90.
[6] A.T. Karlberg, E. Bergstedt, A. Boman, K. Bohlinder, C. Liden, J. Lars, G. Nilsson, J. Wahlberg, Contact Dermat. 13 (1985) 209.
[7] N.M. Joye Jr., R.V. Lawrance, J. Am. Chem. Soc. 26 (1961) 1024.
[8] A.T. Karlberg, A. Boman, U. Hacksell, J. Hermansson, S. Jacobsson, J. Nilsson, J. Pharm. Pharmacol. 40 (1988) 42.
[9] B.M. Hausen, K. Krohn, E. Budianto, Contact Dermat. 23 (1990) 352.
[10] J.A. Hudy, Anal. Chem. 31 (1959) 1754.
[11] D.F. Zinkel, L.C. Zank, M.F. Wesolowski (Eds.), Diterpene Resin Acids, Forest Product Laboratory, U.S. Department of Agriculture, Forest Service, MD, 1971.
[12] D.F. Zinkel, C.C. Engler, J. Chromatogr. 136 (1977) 245.
[13] D. Foster, D.F. Zinkel, J. Chromatogr. 248 (1982) 89.
[14] D.F. Zinkel, L.C. Zank, Anal. Chem. 40 (1968) 1144.
[15] T.L. Chang, Anal. Chem. 40 (1968) 989.
[16] W. Kamutzki, T. Krause, Ekman Days 333 (1981) 70.
[17] D.E. Richardson, J. Chromatogr. 268 (1983) 341.
[18] E. Cronin (Ed.), Contact Dermatitis, Churchill Livingstone, Edinburgh, 1980, p. 784.
[19] J.S. Taylor, in: A.A. Fisher (Ed.), Contact Dermatis, 3rd ed., Lea and Feiger, Philiadelphia, PA, 1986, p. 644.
[20] E. Ehrin, A.T. Karlberg, Contact Dermat. 23 (1990) 59.
[21] P.S. Burge, Clin. Immun. Allergy 4 (1984) 455.
[22] I.W. Fawcett, A.J. Newman Taylor, J. Pepys, Clin. Allergy 6 (1976) 577.
[23] S. Sadhra, I.S. Foulds, C.N. Gray, Br. J. Dermatol. 134 (1996) 662.

[24] R.M. Adams (Ed.), Occupational Skin Diseases, Grunne and Stratton, New York, 1983.
[25] J. Foussereau, C. Benezra, H.I. Maibach, Occupational Contact Dermatitis. Clinical and Chemical Aspects, 1st ed., Munksgaard, Copenhagen, 1982, pp. 200.
[26] B.M. Hausen, A. Kuhlwein, K.H. Schulz, Derm. BerufUmwelt 30 (1982) 145.
[27] C. Liden, Contact Dermat. 10 (1984) 119.
[28] S. Sadhra, I.S. Foulds, C.N. Gray, Br. J. Dermatol. submitted for publication.