

Contact allergy to 5,5'-di-tert-butyl-2,2'-dihydroxy-(hydroxymethyl)-dibenzyl ethers, sensitizers in p-tert-butylphenol-formaldehyde resin

ERIK ZIMERSON AND MAGNUS BRUZE

Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden

Allergy to p-tert-butylphenol-formaldehyde resin (PTBP-F-R) is not rare. This resin consists of a large number of substances, most of which are still unknown. More knowledge about the sensitizers in the resin is a good basis for development of diagnosis, treatment and prevention. The aim of this investigation was to study allergens in PTBP-F-R by isolation of some medium molecular weight substances from the resin and patch testing these in individuals hypersensitive to PTBP-F-R. 2 isolated substances were shown to be allergens in PTBP-F-R, 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether and 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether. 13 patients hypersensitive to PTBP-F-R were patch tested with serial dilutions of 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether and 12 of them reacted positively. 12 patients hypersensitive to PTBP-F-R were patch tested with serial dilutions of 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether and 11 of them reacted positively. Positive patch test reactions were seen down to $0.0000025 \text{ mmole} \times 1^{-1}$ ($\approx 0.01 \text{ ppm}$) for both 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether and 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether in the most sensitive patient. HPLC analysis of 2 PTBP-F-Rs showed the presence of 1.0–1.7% w/w 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether and 0.75–0.90% w/w 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether in the resins.

Key words: allergic contact dermatitis; p-tert-butylphenol-formaldehyde resin; PTBP-F-R, 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether; 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether; CAS 35851-08-2. © Munksgaard, 2000.

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Contact allergy to p-tert-butylphenol-formaldehyde resin (PTBP-F-R) is not rare (1–4). One of the first reports on allergy to the resin was published in 1958 by Malten, who described occupational eczema due to p-tert-butylphenol (PTBP) in a shoe adhesive (5). Still today, a frequent difficulty for patients with PTBP-F-R allergy is to find shoes that they can tolerate. The resin consists of a large number of substances, the vast majority of which are not chemically defined. More knowledge about the sensitizers in the resin could contribute to improved diagnosis and prevention.

In recent years, we have isolated and identified several low molecular weight allergens from PTBP-F-R (6–8). The substances described in these investigations are all monomeric components of the resin. The isolation has been done

by dividing PTBP-F-R into fractions by different chemical separation methods. The fractions have been patch tested in patients hypersensitive to PTBP-F-R, and the substances in fractions giving positive patch test reactions have been separated further and patch tested again. This has been repeated until we have found individual chemical substances responsible for elicitation of the positive patch test reactions. These investigations have, however, indicated the presence of other potent allergens in PTBP-F-R. The purpose of this study was to investigate further some medium molecular weight allergens, in a fraction of PTBP-F-R thought to contain mainly dimeric components. This study is a part of a larger investigation aiming at isolation and identification of the main sensitizers in PTBP-F-R.

Materials and Methods

Substances

The following substances were used: methanol, acetonitrile, acetone, tetrahydrofurane (THF), and ethyl acetate in pro analysi quality manufactured by Lab-Scan (Ireland); sodium sulfate anhydrous, sodium chloride and hydrochloric acid were manufactured by Acros Organics (Belgium); aluminium oxide (activated, neutral) from Merck (Germany); ethanol 99.5% from Kemetyl AB (Sweden); THF was always passed through a column packed with aluminium oxide followed by distillation to remove peroxides before it was used. 2,6-dimethylol p-tert-butylphenol (2,6-MPTBP) and 2-methylol p-tert-butylphenol (2-MPTBP) were synthesized at our department, according to a method described by Agatha & Schubert (9) but with some modification.

Resins

The 2 PTBP-F-Rs used were Schenectady-Midland resin SP134 (PTBP-F-R 1), which is a resol resin obtained from Chemotechnique (Sweden), and PTBP-F-R Alresen PA 103 (PTBP-F-R 2), another resol resin obtained from Vianova Resins GmbH (Germany).

Gel permeation chromatography (GPC)

GPC is a chromatographic method that separates chemical compounds according to their molecular size. In an earlier investigation we separated the substances in PTBP-F-R into different fractions by using GPC, and patch tested these fractions in patients hypersensitive to PTBP-F-R (8). We detected several GPC fractions containing allergens. In the present paper we describe the investigation of allergens in 1 of these GPC fractions, GPC fraction 5. This GPC fraction is described in that earlier investigation.

Extraction of low molecular weight components from the resin

PTBP-F-R 1 (13.0 g) was dissolved in acetonitrile (325 ml). After addition of 650 ml methanol/water (70/30 v/v) and 3.9 ml of a saturated solution of sodium chloride the mixture was filtered and the solution obtained evaporated under vacuum (30°C) to a final volume of around 200 ml. This mixture was extracted with ethyl acetate (3×50 ml). The combined ethyl acetate phases were dried

by the addition of sodium sulfate, filtered and then evaporated under vacuum (30°C). The oily residue was dissolved in a mixture of 325 ml acetonitrile and 650 ml methanol/water (70/30 v/v). If the solution was turbid, methanol was added until a clear solution was obtained. The components of this solution were separated by chromatographic methods described below.

High-pressure liquid chromatography (HPLC)

Semipreparative HPLC-system 1 was performed using a solvent delivery system consisting of an LDC Gradient Master and 2 LDC Constametric III pumps (Laboratory Data Control, USA). The automatic process was controlled by an F-AV electronic process control unit (Film och AV, Sweden). The injector, allowing an injection volume of 10 ml, was a Rheodyne 7010 (Rheodyne, USA) equipped with an electric actuator (Film och AV). Samples were collected by a pneumatic 6-position valve, Rheodyne model 5011 A (Rheodyne). The process was monitored using an LKB Uvicord S UV-detector (LKB, Sweden) operating at a wavelength of 280 nm and an F-AV computer system (Film och AV). The column (22 mm i.d.×300 mm) was packed with 5 µm Nucleosil C₁₈ (Macherey-Nagel & Co., Germany).

Semipreparative HPLC-system 2 was performed using a solvent delivery system consisting of a LDC Gradient Master and 1 LDC Constametric III pump and 1 LDC Constametric I pump (Laboratory Data Control). The automatic process was controlled by an F-AV electronic process control unit (Film och AV). The injector, allowing an injection volume of 1 ml, was a Rheodyne 7010 (Rheodyne) equipped with a pneumatic actuator (Rheodyne). Samples were collected by a pneumatic 6-position valve, Rheodyne model 5011 A (Rheodyne). The process was monitored using a LDC SpectroMonitor III UV-detector (Laboratory Data Control) operating at a wavelength of 280 nm. The column (11 mm i.d.×100 mm) was packed with 5 µm Nucleosil C₁₈ (Macherey-Nagel & Co.). This system was used for purification and analysis of IX and X. Flow rate 7–8 ml/min. Linear gradient elution with eluent A methanol/water 50/50 v/v and eluent B methanol 100% was used; 42% B to 100% B in 10 min for IX, and 46% B to 100% B in 10 min for X.

Analytical HPLC was performed using a Varian 5000 (Varian, U.S.A.) pump system equipped with a Rheodyne 7010 injector (Rheodyne) with a 20 µl loop and a column (4.6 mm i.d.×250 mm) packed with 5 µm Nucleosil C₁₈ (Macherey-Nagel & Co.). The built in UV-100 detector (Varian) was operated at a wavelength of 280 nm.

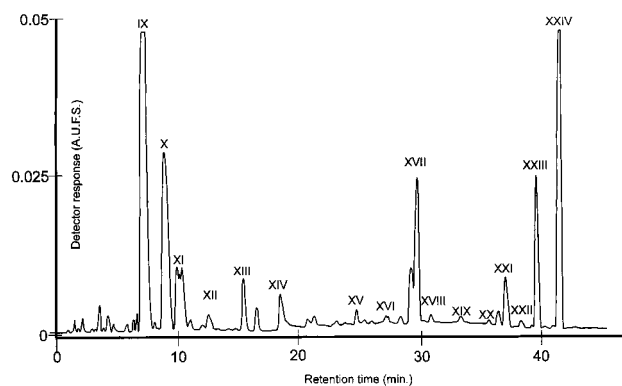


Fig. 1. HPLC chromatogram showing the separation of the components in GPC fraction 5 by analytical HPLC. Flow rate 1.0 ml/min. Linear gradient elution with eluent A methanol/water 65/35 v/v and eluent B methanol 100%; 53% B to 100% B in 36 min.

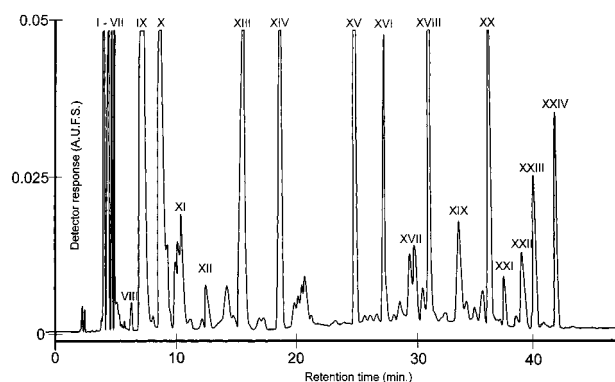


Fig. 2. HPLC chromatogram showing the separation of the components in an extract from PTBP-F-R by analytical HPLC. Flow rate 1.0 ml/min. Linear gradient elution with eluent A methanol/water 65/35 v/v and eluent B methanol 100%; 53% B to 100% B in 36 min.

Investigation of components in extract from PTBP-F-R and in GPC fraction 5

Analytical HPLC was used to separate the components in the extract and in GPC fraction 5. The chromatogram of GPC fraction 5 is shown in Fig. 1 and the chromatogram of the extract is shown in Fig. 2. The main peaks that these chromatograms have in common are marked with roman numerals IX–XXIV. The numerals I–VII refer to monomeric substances described elsewhere (8).

Isolation of compounds in extract from PTBP-F-R

HPLC-system 1 was used to isolate fractions from the extract of PTBP-F-R 1 described above. An HPLC chromatogram from the separation of substances in the extract is shown in Fig. 3. The roman numerals refer to the corresponding peaks in

Fig. 1 and Fig. 2. The peaks marked IX and X were collected as 2 fractions, which were evaporated under vacuum (30°C) and the residues dissolved in methanol/water (70/30 v/v), giving 3% w/v solutions. These were purified further using HPLC system 2. The fractions collected were evaporated under vacuum (30°C) until almost all the methanol was evaporated, the residue was extracted with ethyl acetate, the ethyl acetate phase was collected and dried by the addition of anhydrous sodium sulfate, filtered and evaporated to dryness. The residues were identified and used for preparation of patch test solutions as described below.

Nuclear magnetic resonance spectrometry (NMR)

^1H -spectra were recorded from samples of IX and X dissolved in CDCl_3 or acetone- d_6 using a Bruker ARX, 500 MHz NMR spectrometer. Tetramethylsilane was used as an internal reference ($\delta = 0.00$ ppm).

Mass spectrometry (MS)

A Jeol JMS-SX 102 mass spectrometer was used with direct inlet to obtain a mass spectrum of IX and X. Chemical ionization with xenon was used and sodium iodide was added to the sample.

Chemical structures of IX and X

IX. The ^1H -NMR-spectrum indicates aromatic and aliphatic hydroxyl groups, 1 type of tert-butyl group and 1 type of aromatic ring system with 2 hydrogen atoms. There are also signals characteristic for hydroxymethyl groups and/or benzyl ethers. The MS shows a molecular weight of 402,

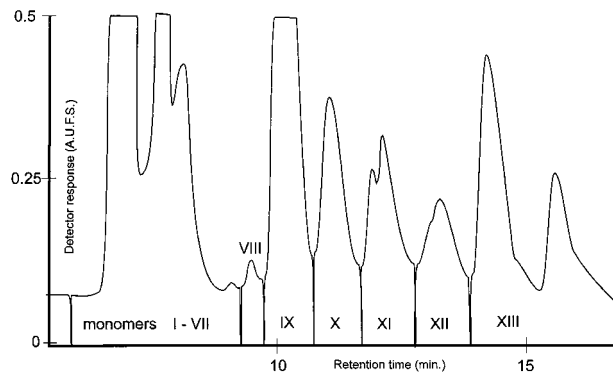


Fig. 3. HPLC chromatogram showing the separation of the components in an extract from PTBP-F-R by semipreparative HPLC. Flow rate 15 ml/min. Linear gradient elution with eluent A methanol/water 50/50 v/v and eluent B methanol 100%; 60% B to 100% B in 20 min.

which is consistent with a PTBP-F-R dimer. The dimer must be symmetric according to the NMR data. This leads to the conclusion that this substance is built up by 2 molecules of p-tert-butylphenol connected by a methylene ether bridge in the ortho positions and substituted on each p-tert-butylphenol unit also in ortho position with a hydroxymethyl (methylol) group. IX should thus be 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether (Fig. 4). Further support for the positions of the substituents was obtained by synthesis of 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether from 2,6-MPTBP. 100 mg 2,6-MPTBP was dissolved in 1.0 ml of THF and 1 drop (approximately 20 μ l) of conc. HCl. Investigations of the reaction mixture by analytical HPLC revealed a primary reaction product with the same retention time as IX. This product was isolated by semipreparative HPLC-system 2 and gave a substance with the same ^1H -NMR data as was obtained with IX. This confirmed that IX was 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether.

^1H -NMR (CDCl_3): δ 1.27 (s, 18H, t-butyl), 2.40 (s (broad), 2H, $-\text{CH}_2-\text{OH}$), 4.74 (s, 4H, $-\text{CH}_2-\text{O}-\text{CH}_2-$), 4.80 (s, 4H, $-\text{CH}_2-\text{OH}$), 7.08 (d, 2H, $J=2.45$ Hz, ArH), 7.11 (d, 2H, $J=2.42$ Hz, ArH), 7.80 (s, 2H, ArOH); MS (probe, FAB+Na) m/e (relative intensity): 425 ($\text{M}+23$) $^+$ (100).

X. The ^1H -NMR-spectrum indicates 2 different aromatic hydroxyl groups and 1 type of aliphatic

hydroxyl group, 2 different tert-butyl groups and 2 types of aromatic ring systems with 2 and 3 hydrogen atoms, respectively. There are also signals characteristic for hydroxymethyl groups and/or benzyl ethers. The MS shows a molecular weight of 372, which is consistent with a PTBP-F-R dimer. This leads to the conclusion that this substance is built up by 2 molecules of p-tert-butylphenol connected by a methylene ether bridge in the ortho positions and substituted on 1 p-tert-butylphenol unit also in ortho position with a hydroxymethyl (methylol) group. X should thus be 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether (Fig. 4). Further support for the positions of the substituents was obtained by synthesis of 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether from 2,6-MPTBP and 2-MPTBP. 50 mg 2,6-MPTBP and 50 mg 2-MPTBP were dissolved in 1.0 ml of THF and 1 drop (approximately 20 μ l) of conc. HCl was added. Investigations of the reaction mixture by analytical HPLC revealed reaction products with the same retention times as IX and X. The product with the same retention time as X was isolated by semipreparative HPLC system 2 and gave a substance with the same ^1H -NMR data as those obtained with X. This confirmed that X was 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether.

^1H -NMR (acetone- d_6): δ 1.269 (s, 9H, t-butyl), 1.274 (s, 9H, t-butyl), 2.40 (s (broad), 2H, $-\text{CH}_2-\text{OH}$), 4.69 (s, 2H, $-\text{CH}_2-\text{O}-\text{CH}_2-$), 4.70 (s, 2H, $-\text{CH}_2-\text{O}-\text{CH}_2-$), 4.76 (s, 2H, $-\text{CH}_2-\text{OH}$), 6.80 (d, 1H, $J=8.6$ Hz, ArH), 7.19 (d o d, 1H, $J_1=8.6$ Hz, $J_2=2.9$ Hz, ArH), 7.23 (s, 2H, ArH), 7.34 (d, 1H, $J=2.9$ Hz, ArH), 8.23 (s, 1H, ArOH), 8.28 (s, 1H, ArOH); MS (probe, FAB+Na) m/e (relative intensity): 395 ($\text{M}+23$) $^+$ (100).

Patch test with serial dilutions of IX and X

A concentration of 1.0% w/v ($25 \text{ mmol} \times 10^{-1}$) of IX was chosen as a starting point for the preparation of test solutions. An equimolar concentration was chosen for X, 0.93% w/v. From these solutions 5 or 7 10-fold serial dilutions were made in acetone.

13 patients with contact allergy to PTBP-F-R detected by routine patch testing at our department were patch tested in this study. All 13 patients were patch tested with IX, but only 12 of them with X due to lack of patch test space in 1 of them. The test solutions were patch tested with the Finn Chamber technique using Scanpor as adhesive and with 15 μ l of the test solution on each patch unit. The patches remained on the back for 48 h and the reading was performed 24 h after removal. To reduce the suffering from many strong patch test

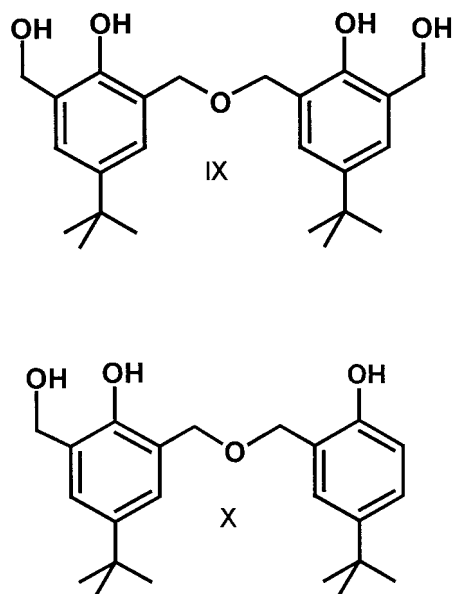


Fig. 4. Chemical structures of the substances used for patch testing; 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether (IX) and 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether (X).

Table 1. Patch test reactions to acetone solutions of 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether (IX) and 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether (X) in 13 patients hypersensitive to PTBP-F-R

Substance mmol $\times 10^{-1}$ (% v/W)	Patient												
	1	2	3	4	5	6	7	8	9	10	11	12	13
IX 2.5 (1.0%)	NT	+	+	+	NT	+	NT	+	NT	—	+	+	+
0.25	+	+	+	+	NT	+	+	+	+		+	+	+
0.025	+	+	+	+	+	+	+	+	+		+	+	+
0.0025	+	—	+	+	+	+	+	+	+		+	—	+
0.00025	+		+	—	—	+	+	+	+		—		—
0.000025	+		—			—	+	+	+				
0.0000025	—						NT	+	—				
0.00000025							NT	—					
X 2.5 (0.93%)	NT	+	+	+	NT	+	NT	+	NT	—	NT	+	+
0.25	+	+	+	+	NT	+	+	+	+		NT	+	+
0.025	+	+	+	+	+	+	+	+	+		NT	+	+
0.0025	+	+	+	+	+	+	+	+	+		NT	+	+
0.00025	+	—	+	—	+	+	+	+	—		NT	—	+
0.000025	—		—		—	—	+	+			NT		—
0.0000025							NT	+			NT		
0.00000025							NT	—			NT		

+: positive patch test reaction of an allergic nature; —: negative patch test reaction; NT: not tested.

reactions among our patients, we avoided patch testing with the most concentrated solutions in patients from whom we expected strong patch test reactions. This study was approved by the Ethical Committee of Lund University.

Purity of the test substances IX and X

Investigations were made with analytical HPLC. The substances were dissolved in methanol at a concentration of 10 mg/ml and these solutions were used for the analyses.

Analysis of IX and X in 2 PTBP-F-Rs

Freshly prepared solutions of the resins (5.0% w/v in ethanol) were made. These samples were diluted 5 $\times$ with methanol/water and injected into the semipreparative HPLC system 2. An injection volume of 200 μ l was used.

Results

The components of PTBP-F-R had been separated by GPC into 13 different fractions that had been patch tested in 5 patients hypersensitive to PTBP-F-R. As the patch testing was done with serial dilutions we could compare the test responses to the different fractions. The patients reacted most strongly to GPC fraction 5.

By using HPLC, we were able to isolate and purify 2 substances from PTBP-F-R, which were main components in GPC fraction 5. These substances, IX and X, were identified using data obtained from MS and NMR analysis. Substance IX was identified as 5,5'-di-tert-butyl-2,2'-dihydroxy-

3,3'-dihydroxymethyl-dibenzyl ether and substance X as 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether. The chemical structures of these compounds are shown in Fig. 4.

The results of patch testing with serial dilutions of IX and X are shown in Table 1. 12 of 13 patients hypersensitive to PTBP-F-R reacted positively to IX and 11 of 12 reacted positively to X. 1 individual patient tested negatively to both substances. Most patients showed a strong reactivity to the test substances, with positive reactions down to a concentration of around 0.01 ppm for both IX and X in the most sensitive patient.

HPLC investigation of the purity of IX and X showed no impurities above an estimated detection limit of 0.1% w/w (Table 2).

None out of 3 dermatitis controls reacted positively to IX or X in patch test solutions of 0.025 and 0.0025 mmol $\times 10^{-1}$, respectively. Comparisons between the test and control groups give significant differences for IX ($p=0.0071$) and for X ($p=0.0088$).

HPLC analyses showed that PTBP-F-R 1 con-

Table 2. HPLC investigation of the purity of test substances, 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether (IX) and 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether (X)

Test substance	Contaminant (% w/w)		
	IX	X	Other contaminants
IX	NA	<0.1	<0.1*
X	<0.1	NA	<0.1*

*Estimated detection limit for UV-absorbing contaminants other than IX and X. NA: not applicable.

tained 1.7% w/w IX and 0.90% w/w X, while PTBP-F-R 2 contained 1.0% w/w IX and 0.75% w/w X.

Discussion

To avoid relapses of allergic contact dermatitis it is necessary for sensitized subjects not only to avoid the sensitizer itself but also cross-reacting substances. This implies that the chemical identity of the actual allergens is known. Regarding complex products such as different resins this knowledge is very limited. In this paper we focus on isolation of allergens in PTBP-F-R by patch testing chromatographic fractions and/or pure substances from the resin in individuals hypersensitive to PTBP-F-R. The sensitizing capacity and the cross-reaction patterns of the allergens can then be investigated in animals. Knowledge obtained in this way of the main sensitizers in PTBP-F-R is a requirement for the development of efficient chemical analytical methods, which can be used to trace the allergens in the environment.

Isolation of substances from PTBP-F-R, followed by patch testing them in patients hypersensitive to PTBP-F-R, is an effective method for finding the main allergens in these complex products. In a previous report, we found indications of potent allergens in a medium molecular weight fraction isolated from PTBP-F-R by GPC, which is a chromatographic method that separates chemicals in a mixture on the basis of their molecular size (8). GPC fraction no 5, which gave positive patch test reactions in most patients and to which the patients showed the highest reactivity (8), was used as a starting point for isolation of allergens in this investigation. To facilitate the isolation of substances an extraction method was developed by which directly from the resin we could extract a fraction where we found the same main substances as in GPC fraction 5. This was shown by HPLC analysis of the extract and GPC fraction 5. The HPLC chromatograms are shown in Fig. 1 and 2. We concentrated our efforts on isolation of the substances represented by the peaks marked IX and X in the chromatograms, as these are main components in GPC-fraction 5. These substances were isolated and purified using semipreparative HPLC.

The isolated substance IX was shown to be 5,5'-di-tert-butyl-2,2'-dihydroxy-3,3'-dihydroxymethyl-dibenzyl ether. Another name for this substance is 3,3'-[oxybis(methylene)]bis[5-(1,1-dimethylethyl)-2-hydroxy-benzenemethanol]. Substance X was shown to be 5,5'-di-tert-butyl-2,2'-dihydroxy-3-hydroxymethyl-dibenzyl ether.

Patch testing with serial dilutions of IX and X

revealed a very high reactivity to both substances. The most sensitive patient reacted to IX down to 0.0000010% w/v (≈ 0.01 ppm) and to X down to 0.0000009% w/v (≈ 0.01 ppm).

The contact allergies to these substances are in all probability genuine since none of 3 controls reacted positively when patch tested with IX and X, respectively. Furthermore, the chemical investigations indicated that the test substances were of high purity and the high reactivity among the patients makes it highly unlikely that any impurity could have influenced the observed test results.

1 patient did not react positively when patch tested with IX and X. This patient was earlier tested with the GPC fractions and did not react to GPC fraction 5 (containing IX and X) at that time (8) but to other GPC fractions containing mainly monomeric substances. Patch testing with p-tert-butylcatechol (PTBC), shown to be an allergen in PTBP-F-R, was also negative in this patient. This indicates the presence of other important allergens in PTBP-F-R besides PTBC, IX and X.

IX and X are dimers in PTBP-F-R. In earlier investigations we isolated 2 dimers from P-F-R, 4,4'-dihydroxy-3-hydroxymethyl-diphenyl methane and 4,4'-dihydroxy-3,3'-dihydroxymethyl-diphenyl methane (10). Patch testing with these 2 dimers in serial dilutions showed that they were potent allergens. Investigations in the guinea pig confirmed these results and showed that 4,4'-dihydroxy-3,3'-dihydroxymethyl-diphenyl methane was a strong and 4,4'-dihydroxy-3-hydroxymethyl-diphenyl methane a moderate sensitizer (11). 4,4'-dihydroxy-3,3'-dihydroxymethyl-diphenyl methane also induced several cross-reactions in the guinea pig. IX and X seem to be equally strong allergens as the dimers from P-F-R but their sensitizing capacities and cross-reaction patterns need to be investigated in the guinea pig.

IX and X are chemically related to the dimers from P-F-R, although some structural differences exist. The connecting bridge between the monomeric units consists of a methylene group ($-\text{CH}_2-$) in the P-F-R dimers but is a methylene ether group ($-\text{CH}_2-\text{O}-\text{CH}_2-$) in the PTBP-F-R dimers. As the methylene groups in IX and X are attached to an aromatic ring these compounds are of the benzyl ether type. A reason behind the difference in connecting bridges could be the steric hindrance brought about by the bulky tert-butyl groups, which makes it more difficult for the monomeric units to get close to each other in the PTBP-F-R dimers. It is, however, known that methylene ether bridges can be converted into methylene bridges by heating in a process where formaldehyde is released (12). The presence of the p-tert-butyl groups in the PTBP-F-R dimers and a different orien-

tation in space of the phenolic hydroxyl groups with regard to the methylene and methylene ether bridges are other differences. Otherwise the dimers of PTBP-F-R and P-F-R are of about the same molecular size and have the same type of reactive groups or centres, each of which consists of a hydroxymethyl (methylol) group in ortho position to a phenolic hydroxyl group.

In earlier attempts to investigate allergens in PTBP-F-R, IX has been described as a part of a synthetic patch test preparation without any ability to evoke positive patch test reactions when tested in 5 patients hypersensitive to PTBP-F-R at a concentration of 5% (9). In that investigation, however, no analytical data were provided for the identification of the synthesized product claimed to contain IX. Besides in this report, IX has been described in a paper concerning the properties of polychloroprene adhesives (13). No reference to X has been found in the scientific literature.

In this paper, we report 2 new allergens in PTBP-F-R, which are candidates for the title of being main allergens in the resin. However, before any certain conclusions can be drawn, more information on sensitizing capacity and cross-reaction patterns from sensitization studies in the guinea pig is needed.

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References

1. Tarvainen K. Analysis of patients with allergic patch test reaction to a plastics and glue series. *Contact Dermatitis* 1995; 32: 346–351.
2. Handley J, Todd D, Bingham A, Corbett R, Burrows D. Allergic contact dermatitis from para-tertiary-butylphenol-formaldehyde resin (PTBP-F-R) in Northern Ireland. *Contact Dermatitis* 1993; 29: 144–146.
3. Mancuso G, Reggiani M, Berdondini R M. Occupational dermatitis in shoemakers. *Contact Dermatitis* 1996; 34: 17–22.
4. Marques C, Goncalo M, Goncalo S (1994) Sensitivity to para-tertiary-butylphenol-formaldehyde resin in Portugal. *Contact Dermatitis* 1994; 30: 300–301.
5. Malten K E. Occupational eczema due to parateritary butylphenol in shoe adhesive. *Dermatologica* 1958; 117: 103–109.
6. Zimerson E, Bruze M, Goossens A. Simultaneous p-tert-butylphenol-formaldehyde resin and p-tert-butylcatechol contact allergies in man and sensitizing capacities of p-tert-butylphenol and p-tert-butylcatechol in the guinea pig. *J Occup and Environ Med* 1999; 41: 23–28.
7. Zimerson E, Bruze M. Demonstration of the contact sensitizer p-tert-butylcatechol in p-tert-butylphenol-formaldehyde resin. *Am J Contact Dermat* 1999; 10: 2–6.
8. Zimerson E, Bruze M. Low molecular weight contact allergens in p-tert-butylphenol-formaldehyde resins. In manuscript form.
9. Agatha G, Schubert H. Untersuchungen zur Allergen-Identifizierung bei der p-tertiären Butylphenol-Formaldehydharz-Allergie. *Dermatol Monatschr* 1979; 165: 337–345.
10. Bruze M, Fregert S, Persson L, Zimerson E. Contact allergy to 4,4'-dihydroxy-(hydroxymethyl)-diphenyl methanes; sensitizers in a phenol-formaldehyde resin. *J Invest Dermatol* 1986; 87: 617–623.
11. Bruze M. Sensitizing capacity of 4,4'-dihydroxy-(hydroxymethyl)-diphenyl methanes in the guinea pig. *Acta Derm Venereol* 1986; 66: 110–116.
12. Saunders K J. *Organic polymer chemistry*. London: Chapman and Hall, 1973: 293–294.
13. Tanno T, Shibuya I, Mukouyama Y. *Weyerhaeuser Sci Symp* (1980), 1979; 2: 231–253.

Address:

Erik Zimerson
Department of Occupational and Environmental Dermatology
Malmö University Hospital
S-205 02 Malmö
Sweden