

Contact allergy to the active ingredients of Bioban P 1487

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Bioban P 1487, consisting of the 2 active ingredients 4-(2-nitrobutyl)-morpholine (M) and 4,4'-(2-ethyl-2-nitro-1,3-propanediyl)-bis morpholine (DM), is a preservative recommended for metal working fluids. The aims of this study were to assess the frequency of contact allergy to Bioban P 1487, when added to a standard patch test series, as well as to test the individual active ingredients in patients sensitized to Bioban P 1487. Of the patients consecutively tested, 1.5% reacted positively to Bioban P 1487. Positive test reactions were noted in only 4 of 12 Bioban P 1487-sensitized patients tested with the individual active ingredients. Both active ingredients are contact sensitizers, but in most patients with this contact allergy it remains difficult to identify the sensitizing hapten.

Key words: allergic contact dermatitis; Bioban P 1487; CAS 1854-23-5; CAS 2224-44-4; delayed hypersensitivity; 4,4'-(2-ethyl-2-nitro-1,3-propanediyl)-bis morpholine; high-performance liquid chromatography; metal working fluids; 4-(2-nitrobutyl)-morpholine; preservatives; occupational; engineering industry. © Munksgaard, 1996.

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Bioban P 1487, consisting of 2 active ingredients, is a preservative recommended for metal-working fluids. It was introduced on the Swedish market in 1981 and contact allergy to it was first reported by Dahlquist in 1984 (1), followed by other reports (2-4). The purpose of this study was to assess the frequency of contact allergy to Bioban P 1487, when added to a standard patch test series, as well as to test the active ingredients in patients sensitized to Bioban P 1487.

Materials and Methods

Substances

Bioban P 1487, containing a mixture of 4-(2-nitrobutyl)-morpholine (CAS no 2224-44-4) (M) 70%, 4,4'-(2-ethyl-2-nitro-1,3-propanediyl)-bis morpholine (CAS no 1854-23-5) (DM) 20%, and inert ingredients 10% (5), was kindly supplied by the manufacturer (Angus Chemie GMBH, Germany). Another name for DM is 4,4'-(2-ethyl-2-nitrotri-methylene)-dimorpholine. The structures of the 2 active ingredients are shown in Fig. 1.

Patch testing

Bioban P 1487 in ethanol/water 50/50 v/v at 0.5% w/v was used for patch testing 1102 consecutive

dermatitis patients. 12 patients with positive test reactions to Bioban P 1487 were patch tested with the 2 individual active components obtained by the high-performance liquid chromatography and gel permeation chromatography technique described below. The substances were dissolved in ethanol (99.5%) and patch tested in serial dilutions of equi-

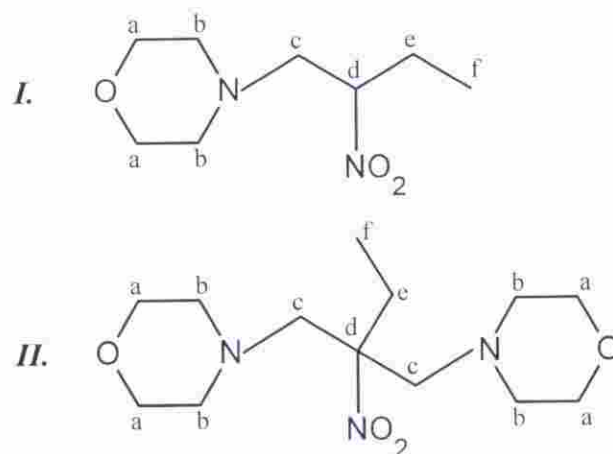


Fig. 1. (I): Structure of 4-(2-nitrobutyl)-morpholine. (II): Structure of 4,4'-(2-ethyl-2-nitro-1,3-propanediyl)bis morpholine. Letters in figure refer to hydrogen and carbon atoms and their positions in the NMR chromatograms.

Table 1. Results of patch testing in 12 Bioban P 1487-sensitive patients tested with equimolar concentrations of the active ingredients of Bioban P 1487: M=4-(2-nitrobutyl)-morpholine; DM=4,4'-(2-ethyl-2-nitro-1,3-propanediyl)-bis morpholine

Substance/ Ethanol	% w/v	Patient				
		1	2	3	4	5-12
M	0.50	+	+	-	+	-
	0.089	+	-	-	-	-
	0.016	+	-	-	-	-
	0.0028	+	-	-	-	-
	0.0005	+	-	-	-	-
	0.000089	+	-	-	-	-
	0.000016	-	-	-	-	-
DM	0.76	+	+	+	-	-
	0.14	+	+	-	-	-
	0.024	+	-	-	-	-
	0.0043	+	-	-	-	-
	0.00076	-	-	-	-	-

+: positive test reaction interpreted as allergic; -: negative test reaction.

molar concentrations starting at $0.03 \text{ mole} \cdot \text{l}^{-1}$ (Table 1). A volume of $25 \mu\text{l}$ of the test solution was used on each patch unit. The AI-test technique (Astra Agency, Sweden) was used for testing, and the patches were removed after 2 days. The tests were read after 1 more day, according to the criteria of the ICDRG.

High-performance liquid chromatography (HPLC)

For preparative applications, a fully automatic HPLC system with sample collection was used. This system could also be run manually. The automatic process was controlled by an F-AV electronic process control unit (Film and AV, Sweden). The solvent delivery system consisted of an LDC Gradient Master and 2 LDC ConstaMetric III pumps (Laboratory Data Control, LDC, USA) with a maximum flow rate of 22.5 ml/min each. The injector was pneumatic, Rheodyne model 7010 A (Rheodyne, USA), and samples were collected by a pneumatic 6-position valve, Rheodyne model 5011 A. The UV-detector, LDC SpectroMonitor III, was operated at 270 nm . The columns ($11 \text{ mm i.d.} \cdot 100 \text{ mm}$ semipreparative and $4 \text{ mm i.d.} \cdot 200 \text{ mm}$ analytical) were packed with Nucleosil C₁₈, $5 \mu\text{m}$ (Macherey-Nagel & Co., Germany).

Gel permeation chromatography (GPC)

A column ($24 \text{ mm i.d.} \cdot 360 \text{ mm}$) packed with Bio-Beads S-X8 (200–400 mesh, Bio Rad laboratories, USA) was used. The eluent was tetrahydrofuran (THF) (Janssen Chimica, Belgium) with a flow rate of 1.0 ml/min . THF was always freshly distilled before use. The eluent was monitored at 280 nm by a UV detector (Uvicord S, LKB, Sweden).

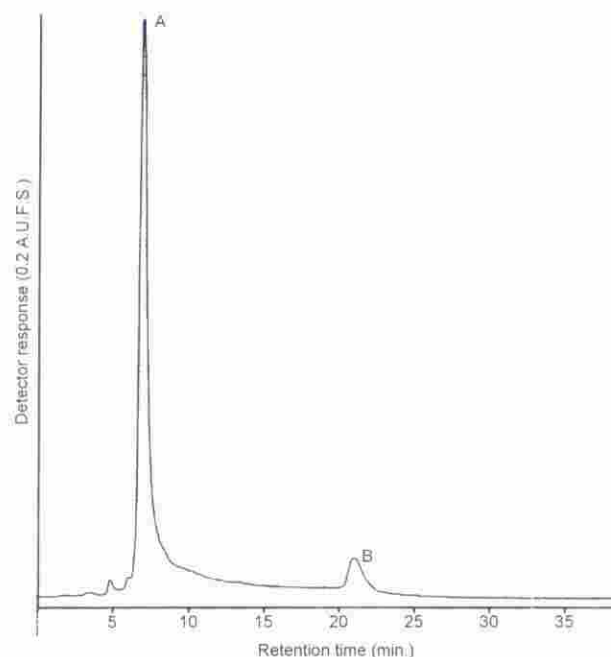


Fig. 2. HPLC chromatogram of Bioban P 1487. Conditions: eluent, methanol/water 50/50 v/v; flow rate, 1.0 ml/min ; sample size, $20 \mu\text{l}$.

Spectra

Gas chromatography/mass spectrometry (GC-MS) was performed on a Finnigan 4021 mass spectrometer, and an OV-101 fused silica capillary column (25 m) was used to obtain the mass spectrum of substance A. A Jeol JMS-SX 102 mass spectrometer was used with direct inlet to obtain a mass spectrum of substance B. Electron impact ionization and chemical ionization were used. Nuclear magnetic resonance (NMR) spectra of substance A and substance B were obtained with a 300 MHz Varian XL 300 NMR Spectrometer and a Bruker ARX, 500 MHz NMR spectrometer, respectively. The ^1H and the ^{13}C NMR-spectra were recorded from samples dissolved in CDCl_3 and with tetramethylsilane as an internal reference ($\delta = 0.00 \text{ ppm}$).

Isolation of active ingredients

Bioban P 1487 consists of 2 active ingredients. HPLC analysis of Bioban P 1487 suggested that peak A and peak B corresponded to the active ingredients (Fig. 2). Bioban P 1487 at $3.0\% \text{ v/v}$ in methanol/water $40/60 \text{ v/v}$ was separated into 3 fractions, using the gradient HPLC system and the semipreparative column. Fraction II corresponded to peak A (substance A) and fraction III to peak B (substance B).

Fractions II and III were evaporated in vacuum

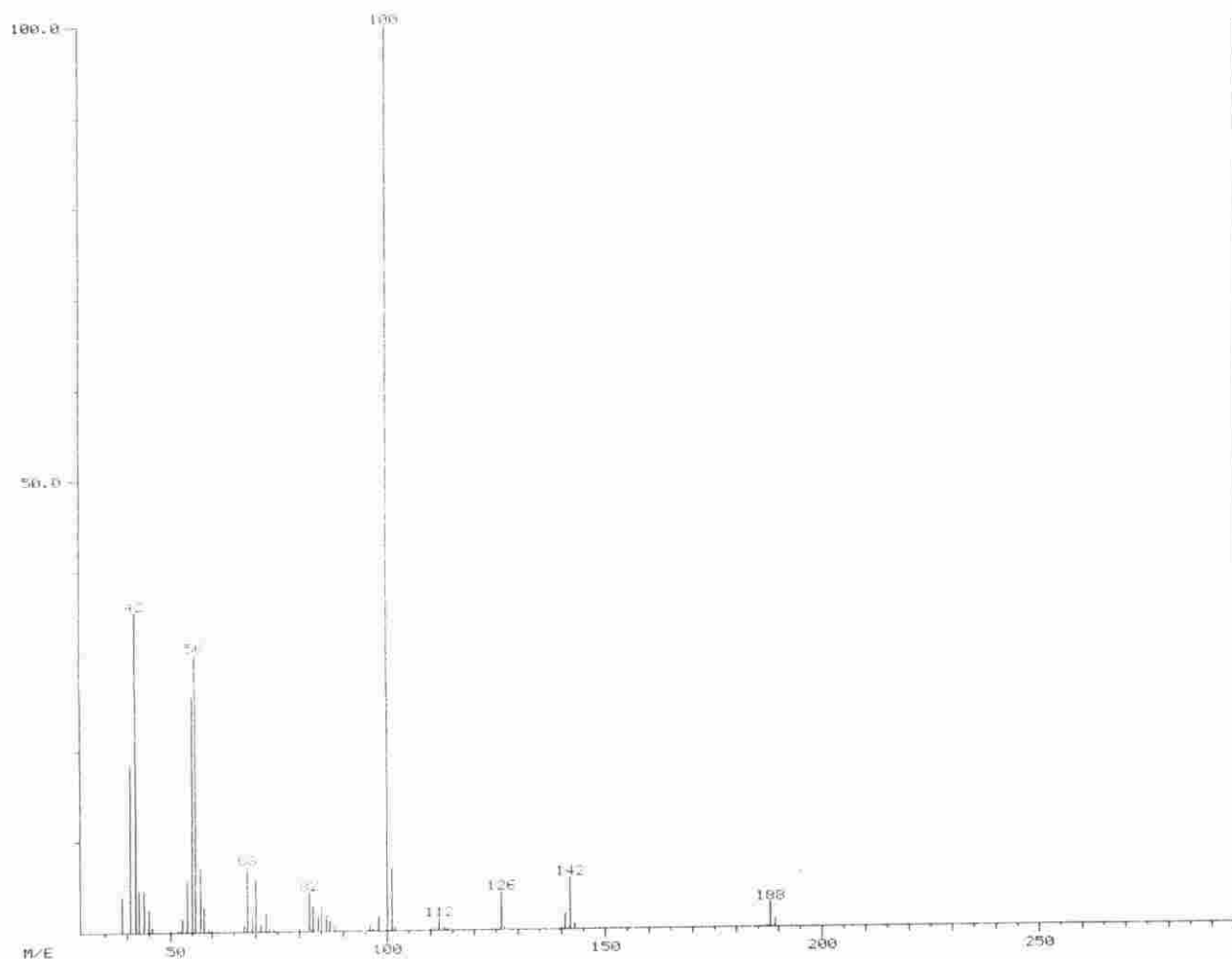


Fig. 3. Mass spectrum of 4-(2-nitrobutyl)-morpholine (GC-inlet, 70 eV).

(30°C) and the residues were analysed with the analytical HPLC system. Fraction II seemed to be of high purity, so substance A was analysed by MS and $^1\text{H-NMR}$. The chromatogram of fraction III showed a small amount of the expected substance B and an almost equal amount of substance A. The occurrence of substance A in fraction III could not be explained by difficulties in separating the 2 compounds in the separation process.

The presence of substance A in fraction III could have been because of instability of the compound during the separation and evaporation processes. Therefore, we separated the Bioban P 1487 mixture using a GPC column with THF as eluent. The fractions were evaporated in vacuum (30°C), analysed with the analytical HPLC system and compared to the previous analytical results. Substance B of high purity was obtained in this way. The problems of obtaining pure substance B from HPLC isolation were further investigated. Pure B from GPC isolation was dissolved in methanol/water (60/40) at a concentration of 10 mg/ml. The mixture was allowed to

stand at room temperature and was analysed by HPLC. Degradation of substance B occurred, forming a peak in the HPLC diagram that corresponded to substance A. After 3 h, the concentration of substance B had decreased to 50% of its initial concentration, and after 16 h only 15% remained. It was concluded that the presence of water was the probable cause of degradation of substance B. During HPLC separation, the substance is in constant contact with water and this could explain our earlier findings. The stability of substance B during GPC separation could be explained by the water-free environment. Theoretically, DM in contact with water could degrade to M, morpholine and formaldehyde, forming a chemical equilibrium. This possibility was strongly indicated by adding equimolar amounts of morpholine and formaldehyde to Bioban P 1487. When the mixture was analysed, peak A had decreased while peak B had increased, indicating transformation of substance A to substance B. Our findings indicate that the balance between M and DM is unstable and dependent

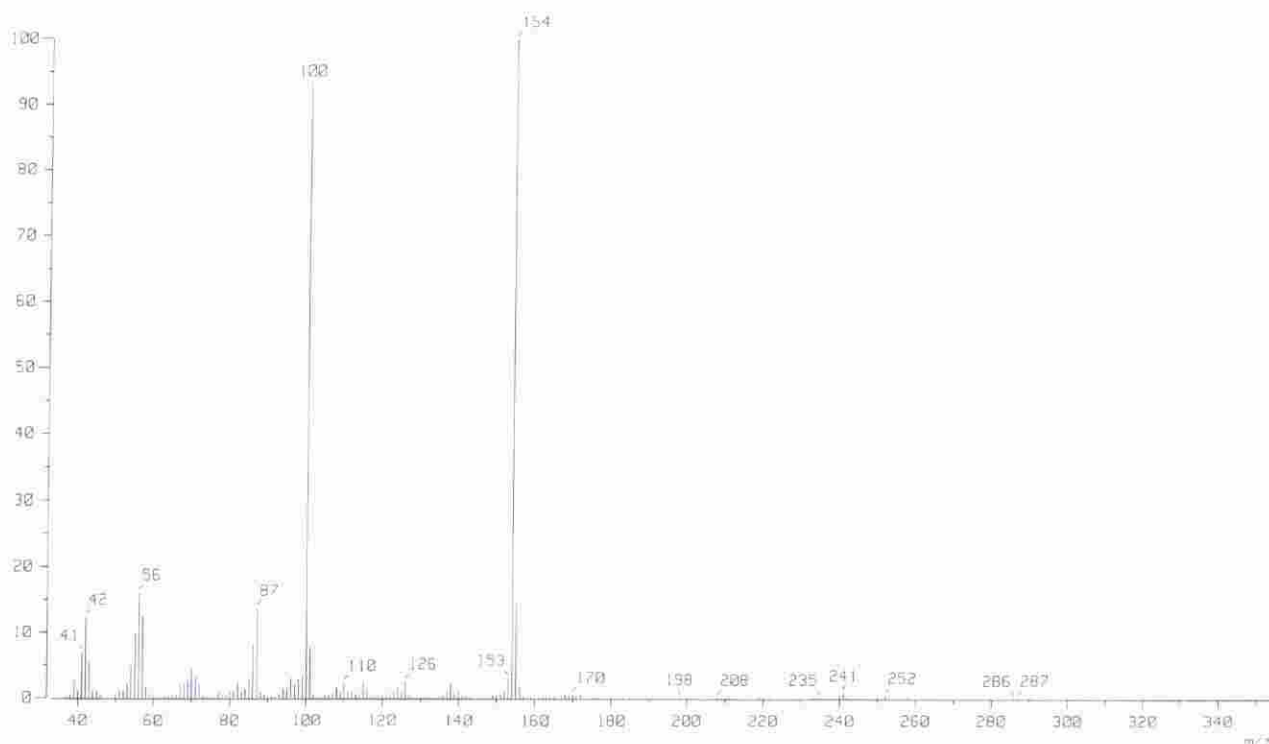


Fig. 4. Mass spectrum of 4,4'-(2-ethyl-2-nitro-1,3-propanediyl)-bis morpholine (probe, 70 eV)

on the amount of water in the environment. Substance B from GPC separation was analysed with ^1H -NMR, ^{13}C -NMR and MS.

Identification of substance A

The MS exhibits a molecular ion peak at m/e 188 consistent with the molecular weight of M. Loss of a nitro group from M gives a peak at m/e 142 and loss of a nitropropyl group gives a peak at m/e 100 (Fig. 3).

^1H -NMR (CDCl_3): δ =

- 0.94 ppm (3H_f , $J=7.1$ Hz, triplet);
- 1.72 ppm (1H_{e2} , $J_{e2-f}=7.1$ Hz, $J_{e2-e1}=15$ Hz, $J_{e2-d}=3.5$ Hz);
- 1.88 ppm (1H_{e1} , $J_{e1-e2}=15$ Hz, $J_{e1-f}=7.1$ Hz, $J_{e1-d}=2.4$ Hz);
- 2.35 ppm (2H_{b2} , doublet split by a-protons);
- 2.47 ppm (1H_{c2} , $J_{c2-d}=3.5$ Hz, $J_{c2-e1}=14$ Hz, doublet split by d-protons);
- 2.54 ppm (2H_{b1} , doublet split by a-protons);
- 2.96 ppm (1H_{e1} , $J_{e1-d}=11$ Hz, $J_{e1-e2}=14$ Hz, doublet split by d-protons);
- 3.62 ppm (4H_a , multiplet);
- 4.57 ppm (1H_d , multiplet);

Identification of substance B

The MS exhibits a small molecular ion peak at m/e 287 consistent with the molecular weight of

DM. This molecular weight was confirmed with MS, using chemical ionization with NH_3 as reaction gas, giving a $M+1$ peak at m/e 288 with a relative peak height of 100%. Loss of a nitro group from DM gives a peak at m/e 241, loss of a CH_2 morpholine group gives a peak at m/e 187, rearrangement involving loss of a hydrogen atom. A nitro and a CH_2 -morpholine group gives a peak at m/e 154 and the $(\text{CH}_2\text{-morpholine})^+$ -ion gives a peak at m/e 100 (Fig. 4). ^1H -NMR (CDCl_3): δ =0.87 ppm (3H_f , $J_{f-e}=7.4$ Hz, triplet); 2.04 ppm (2H_e , $J_{e-f}=7.4$ Hz, quartet); 2.47 ppm (8H_b , multiplet); 2.86 ppm (2H_c , $J_{c2-e1}=14.3$ Hz, AB quartet); 3.61 ppm (8H_a , multiplet); ^{13}C -NMR (CDCl_3): δ = C_f 8.13 ppm; C_e 25.28 ppm; C_b 55.07 ppm; C_c 60.17 ppm; C_a 67.14 ppm; C_d 94.66 ppm.

Results

A positive reaction to Bioban P 1487 was noted in 17 out of 1102 (1.5%) consecutive dermatitis patients patch tested with the augmented standard series. When retesting the patients the same result was obtained. Table 1 shows the results of patch testing with serial dilutions of M and DM. Positive reactions were noted in only 4 out of 12 patients. 2 of the patients had positive reactions to both M and DM. 1 patient showed positive reactions down to 0.000089% w/v for M and 0.0043% w/v for DM. No irritant reactions were noted to any of the ac-

tive ingredients. 20 controls tested negatively to M at 0.5% w/v and DM at 0.76% w/v. The highest possible contaminations of M and DM with each other were 0.19% and 0.24% w/w, respectively.

Discussion

Besides giving the frequency of contact allergy in consecutively patch tested dermatitis patients, patch testing with Bioban P 1487 in the standard series might have been expected to help identify other sources and uses of Bioban P 1487 in addition to metalworking fluids. A contact allergy rate of 1.5% in consecutively tested dermatitis patients was unexpectedly high, and only 1 of these 17 patients with positive reactions to Bioban P 1487 had been exposed to metalworking fluids. However, no other exposure source could be traced for those 16 patients without exposure to metalworking fluids. Interpretations other than Bioban P 1487 being the primary sensitizer may therefore explain the high rate. As Bioban P 1487 is a formaldehyde releaser, positive test reactions to Bioban P 1487 might indicate formaldehyde allergy. However, no patient showed a simultaneous positive patch test reaction to formaldehyde. The active ingredients are both morpholine derivatives and other morpholine derivatives are, for example, used as rubber chemicals. One morpholine derivative is present in the MBT mix in the standard test series, but none of the Bioban P 1487-sensitive patients were positive to MBT mix.

Both active ingredients of Bioban P 1487 were demonstrated to be sensitizers but surprisingly few patients tested positively. There might be various explanations for this result. Differences in bioavailability between when the active ingredients were tested separately and when they were present in Bioban P 1487 could be one explanation, though the individual active ingredients were tested at higher concentrations. Despite these higher concentrations, only 4 out of 12 patients showed positive reactions to the ingredients. A sensitizing contaminant in Bioban P 1487, separated into a fraction not tested, is a 2nd possible explanation. Compound allergy, an additive or a synergistic reaction when M and DM are tested together in Bioban P 1487, might also explain the negative test results.

From patch test results in humans (Table 1), it is difficult to draw any conclusion as to the sensitizing capacity of the 2 ingredients. M is present at a higher concentration in Bioban P 1487 than DM. Furthermore, this difference may be even greater, as DM appears to decompose into M in aqueous solutions, e.g., water-based metalworking fluids. However, a recent sensitization study in the guinea

pig demonstrated M to be a moderate sensitizer and DM to be a strong one (6). Therefore, from the dermatological point of view, the initiative of the manufacturer to reduce the concentration of DM in Bioban P 1487 from 20% to 5% is to be welcomed, as it probably will diminish the risk of sensitization.

This study shows that contact allergy to Bioban P 1487 is not uncommon in consecutively patch tested dermatitis patients. Although Bioban P 1487 is used in water-based metalworking fluids, the preservative is not stable in aqueous solutions. Therefore, petrolatum, rather than water, should be used as the vehicle for patch testing purposes, until all the sensitizers deriving from Bioban P 1487 have been identified (7). When a patient is exposed to a metalworking fluid preserved with Bioban P 1487, patch testing is recommended with the metalworking fluid both freshly prepared and used. Both active ingredients are sensitizers, but in most patients with this contact allergy it remains difficult to identify the sensitizing hapten.

Acknowledgement

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