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IMPORTANCE OF PURE CHEMICALS IN INVESTIGATIONS OF CROSS SENSITIVITY

Cross sensitization among halogen salicylaldehydes

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Investigations of cross sensitization are mostly performed with chemicals of non-controlled purity. Thus contaminating chemicals may be responsible for observed allergic reactions and reported cross sensitization patterns should be regarded with caution. In this study an unexpected cross-reaction pattern is shown to be caused by a contaminating chemical.

Material and Methods

A woman aged 65 developed contact dermatitis after use of an antifungal ointment[1] containing 1 % of 3,5-dibromosalicylaldehyde. She had previously used this ointment without any eczematous reaction. One day after treating a nasal furuncle she developed a dermatitis with papules, vesicles and oedema of the nose, cheeks and periorbital region. Patch testing was performed with a routine series of substances and with the suspected ointment. She showed weak reactions to cinnamon, coal-tar, wood tars and a mixture of perfumes. The ointment gave a vesiculous reaction. When tested with the constitutents of the ointment she reacted only to 3,5-dibromosalicylaldehyde. Patch testing was then carried out during the following three months with substances chemically related to this substance (see nos. 1–12 in Table 1).

All substances were used in a concentration of 0.5 % in alcohol. Al-test,[2] designed by Fregert (6), and Leucoplast®[3] adhesive plaster were used.

The test substance was applied on the back for 48 hours. The reaction was read some hours after the adhesive tape had been removed and after a further 24 hours. A reaction with infiltration and/or vesicles or papules was recorded as positive.

In order to ascertain that the test reactions were not of a primary toxic nature, all substances giving positive reactions were tested in ten control subjects. All these controls gave negative reactions.

Gas-liquid chromatography was carried out on all substances giving positive reactions.

For the *separation procedure* a Perkin-Elmer gas chromatograph 880 was used. The column material was made of 2 % polyphenyl ether OS 124 on Chromosorb G (AW-DMCC, 80–100 mesh). The column was 200 cm long. The temperature was 160°C and the gas flow 30 ml per minute. Samples of 1 μl (5 % in ethanol) were injected.

For the *preparation procedure* of pure

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[1] Funguentan®, AB Recip, Stockholm, Sweden.

[2] IMECO (Astra Agency Co.) AB, Stockholm, Sweden.

[3] Bayersdorf, Federal Republic of Germany.

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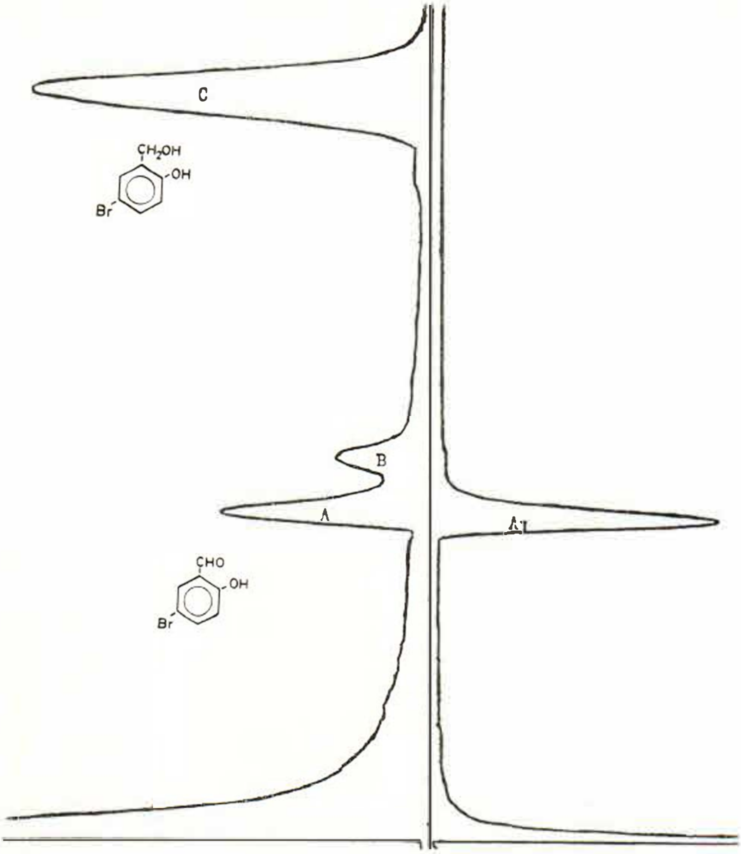


Fig. 1. Gas chromatogram. Right side: 5-bromosalicylaldehyde (conc. 10 × bromsalizol). Left side: Bromsalizol indicated as 5-bromosalicylalcohol. Peak A corresponds to peak A_1 i.e. bromosalicylaldehyde. Peak B corresponds to a substance of unknown structure. Peak A + B gave aldehyde reaction and positive patch test reaction. Peak C corresponds to 5-bromosalicylalcohol; gave no aldehyde reaction and negative patch test reaction.

5-bromosalicylaldehyde[4] and the substances corresponding to other peaks than the head peak a Perkin-Elmer Vapor Fractometer Model 116 E, provided with Termistor detector, was used. The column was the same as used for the separation procedure. Helium was used as carrier gas. Samples of 0.2 ml (5 % in ethanol) were injected. The compounds were trapped in glass tubes refrigerated at −30°C.

Gas chromatogram of bromsalizol[5] (No. 12) showed three different peaks (Fig. 1). Peak A corresponded to that of 5-bromosalicylaldehyde (No. 2). The compound corresponding to peak B was not identified. The head peak C corresponded to the main substance bromsalizol (No. 12).

The compounds corresponding to peak A + B (Fig. 1) of bromsalizol (No. 12) and that corresponding to head peak C were isolated. The other substances that gave positive reactions had no contaminants recorded with the method used.

Aldehyde identification test was carried out with o-dianisidine on all the test substances (2). All aldehydes (Nos. 1–9) as well as non-purified bromsalizol (No. 12) containing 3,5-bromosalicylaldehyde as a contaminant gave positive reactions. The reaction of the compound corresponding

[4] Eastman Organic Chemicals, USA.
[5] Hynson, Westcoss & Dunning, Inc., USA.

to peaks A + B (Fig. 1) of bromsalizol was positive. The head peak C gave no such reaction.

Investigation of Cross Sensitivity

The structural formula of the antimycotic substance 3,5-dibromosalicylaldehyde (3,5-dibromo-2-hydroxy benzaldehyde) is given in Table 1 (No. 1).

The importance of the presence and position of the *aldehyde group*, the *hydroxyl group* and *halogen atoms* was studied by patch testing with eleven chemically related compounds. The results are given in Table 1. In addition to 3,5-dibromosalicylaldehyde four pure substances gave positive reactions.

The bromine atoms (in 3- and 5-position) are not essential for the allergic capacity, which is clear from the positive test reactions to substances Nos. 2 and 3.[6]

Compounds with the halogen iodine or chlorine in 3- and 5-position (Nos. 4 and 5)[7] and chlorine in 5-position gave positive reactions. Thus the presence of different halogens did not seem to influence the allergenic capacity. The gas chromatogram did not indicate any presence of salicylaldehyde which might have explained the reaction.

Patch tests with benzaldehyde (No. 6)[8] and phenol (No. 7)[8] gave negative reactions. Both the aldehyde and hydroxyl groups are thus necessary.

The aldehyde and hydroxyl groups should be in orto-position since the corresponding meta- and para-compounds gave negative test results Nos. 8, 9).[9]

The aldehyde group is essential since this could not be exchanged by the corresponding alcohol or carboxyl groups (Nos. 10, 11).[10]

The drug bromsalizol (bromosaligenin) —used for various arthritides (7)—gave a positive patch test reaction. This was not expected, since salicylalcohol gave a negative reaction. However, gas chromatogram of this drug showed three peaks (Fig. 1). Among the small peaks A and B one corresponded to bromosalicylaldehyde. A preparation of the substances gave a positive aldehyde reaction. Patch testing with the substances from peaks A + B gave strong positive skin reactions but the substance from the head peak C (5-bromosalicylalcohol) gave a negative reaction. Thus, the contaminating 5-bromosalicylaldehyde in bromsalizol was responsible for the positive patch test reaction to bromsalizol.

Discussion

The dermatitis under investigation was clearly related to the use of an antimycotic ointment containing 3,5-dibromosalicylaldehyde and contact allergy was demonstrated by patch testing. The patient showed sensitivity to four other compounds chemically related to 3,5-dibromosalicylaldehyde. In addition to the aromatic nucleus the structural requirements for cross sensitization between the compounds studied are the following:

1. An aldehyde and a hydroxyl group in orto-position.
2. A halogen in 3- and/or 5-position may be present or not.

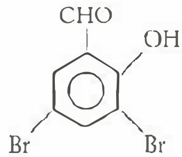
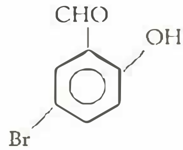
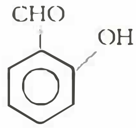
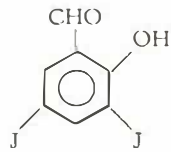
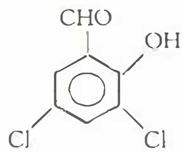
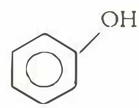
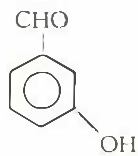
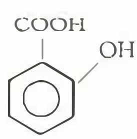
Contact allergy to salicylaldehyde is previously reported (3, 5). It does not seem to be common since among 516 routine patch tested ecezmatous patients in this clinic only one positive recation was found (1).

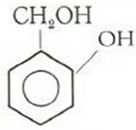
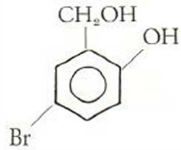
The aldehyde test with o-dianisidine indicated that bromsalizol (an alcohol) contained an aldehyde. The gas chromatogram showed that 5-bromosalicylaldehyde and another contaminant were present. The 5-bromosalicylaldehyde might therefore be considered the cause of the unexpected positive patch test reaction to bromsalizol.

It is well known that not only industrial

[6] Kebo AB, Sweden.
[7] Kindly synthetized by AB Recip, Sweden.
[8] Kebo AB, Sweden.
[9] Light & Co. Ltd., England.
[10] Schuchardt, West Germany.

Table 1. *Patch test reactions*

No.	Substance	Formula	Reaction
1	3,5-dibromosalicylaldehyde, 3,5-dibromo-2-hydroxybenzaldehyde		+
2	5-bromosalicylaldehyde		+
3	salicylaldehyde, 2-hydroxybenzaldehyde		+
4	3,5-diiodinesalicylaldehyde		+
5	3,5-dichlorosalicylaldehyde		+
6	benzaldehyde		–
7	phenol		–
8	3-hydroxybenzaldehyde		–
9	4-hydroxybenzaldehyde		–
10	salicylic acid, 2-hydroxybenzoic acid		–

No.	Substance	Formula	Reaction
11	salicyl alcohol, 2-hydroxybenzylalcohol, saligenin		–
12	5-bromosalicyl alcohol, bromosaligenin, bromsalizol*		–

* Commercial bromsalizol gave positive reaction.

chemicals but also those marketed as "pro analysi" very often are impure (4).

Nevertheless, most earlier cross sensitization studies have been performed with substances poorly defined with regard to purity, and false conclusions may have been drawn. Our findings show that studies on cross sensitization patterns should only be undertaken with substances carefully checked for purity.

SUMMARY

A patient with allergic contact dermatitis due to an antimycotic ointment containing 3,5-dibromosalicylaldehyde was studied with a view to elucidate the cross sensitivity pattern. The structural requirement was an aldehyde and a hydroxyl group in orto-position, while halogens were of no importance. Unexpectedly one of the substances gave a positive reaction which could be explained by a contaminant. It is stressed that the use of pure test substances is an absolute prerequisite in cross sensitization investigations.

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