ON THE NATURE OF THE ECZEMATOGENIC COMPONENT
OF OXIDIZED $\Delta^2$-CARENE*

Medical part:
SVEN HELLERSTRÖM, M.D., AND NILS THYRESSON, M.D.,
Chemical part:
SVEN-GÖSTA BLOHM, B.A. AND GUNNAR WIDMARK, PH.D.

In the experience of both painters and physicians in Sweden, French turpentine has a conspicuously lesser tendency to cause dermatitis, than has Swedish turpentine. Prompted by these observations Hellerström (1) was able to show that the greater sensitizing power of Swedish turpentine was directly or indirectly due to the $\Delta^2$-carene content. French turpentine does not contain any $\Delta^2$-carene. Investigations by Danbolt and Burckhardt (2), Burckhardt and Schaaf (3), and by Hellerström (1) confirmed that stored turpentine causes more severe dermatitis than does freshly distilled turpentine. Storing in air is conducive to the formation of oxidation products; such products occur both in Swedish turpentine and French balsam turpentine that have been stored in air, and the $\Delta^2$-carene in Swedish turpentine is rather more rapidly oxidized than $\alpha$-pinene, which is the chief constituent of French turpentine. Hellerström and Lundén (4) assumed that these circumstances had some significance in the causation of eczema. They subsequently (4) found that the eczematogenic component in turpentine apparently is not attached to the pure hydrocarbons, terpenes C$_{15}$H$_{26}$, but to products formed by their oxidation. In a later paper (Hellerström, Thyresson, Blohm, Widmark (5)) a technic was presented that made possible concentration of the eczematogenically active component. This technic was based on the principle of displacement adsorption—a variant of column chromatography—and a characteristic breaking down of the test substance into an active and an inactive part was possible in a series of different constituents of turpentine (carene, sylvestrene, $\alpha$-pinene, limonene). In so doing, the eczematogenically active component was invariably concentrated at the end of the sample that traversed, by displacement with ethyl alcohol, a column filled with silica gel. For a detailed description of the experimental technic, vide Blohm (6). All experiments were performed on a microscale with samples amounting to a drop or so.

In this paper we shall deal first with the attempts that were made to concentrate the active component directly. However, as will be clear from the following these failed owing to the unstable character of the component. An indirect method was therefore employed with a view to elucidating the component’s nature as far as possible. These latter experiments, which comprised a series of physicochemical investigations into the characteristics of $\Delta^2$-carene during various stages of oxidation, will only be summarized here. A detailed report of the entire pro-

* From the Department of Dermatology, Karolinska Institutet and Department of Organic Chemistry, University of Stockholm, Sweden.

Received for publication October 1, 1954.
procedure, etc. is beyond the scope of this paper, and will shortly be published in a purely chemical journal (Blohm, Widmark (7)).

**SELECTION OF WORKING MATERIAL**

Analysis by displacement adsorption gave, as reported earlier (5) characteristic adsorptograms for a series of different constituents of turpentine. The identification level itself (recorded by refractive index) showed the presence of inhomogeneities. A characteristic peak occurred, especially at the end of the sample level (figs. 1 and 2). It should be observed, however, that only in respect of fig. 2 was this peak found to carry an active factor; in fig. 1 it is quite inactive. This study suggests that the “peak” is not an unequivocal phenomenon, but may be composed of a series of constituents, all of them probably having a higher refractive index, and always a greater affinity to SiO₂, than the mass of the sample itself, and not all of them being eczematogenically active.

To ensure experimental uniformity it was therefore essential to have a terpene showing a regular and homogeneous refractive index level on analysis by displacement adsorption. This method of analysis accordingly served also as an indicator of the degree of purity of the preparation. On experimental purification of various terpenes that were available, only an American Δ³-carene (Fisher, Goldblatt (8)) was found to meet with these requirements. That substance was therefore taken as the basic material for further investigations. However, only very limited supplies of this highly purified terpene were available, and it was therefore necessary to conduct the experiments on a microscale.

**DIRECT ATTEMPTS TO ISOLATE AN ACTIVE COMPONENT**

The current method for separation of constituents in liquid phase consists in distillation. Preliminary tests showed, however, that this procedure was impracticable here. If, for example, an active, oxidized Δ³-carene was distilled,
TABLE I

<table>
<thead>
<tr>
<th>Time, Mins.</th>
<th>$n^2_{D}$</th>
<th>Vol. µl</th>
<th>Patch Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.4970</td>
<td>5</td>
<td>++</td>
</tr>
<tr>
<td>15</td>
<td>1.4964</td>
<td>5</td>
<td>++ (+)</td>
</tr>
<tr>
<td>60</td>
<td>1.4970</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>

neither distillate nor residue showed any activity. Thermolysis of the required constituent had occurred. To obtain some idea of the velocity of decomposition, oxidized carene was placed in a series of scaled ampules, which were then heated at 150°C (the boiling point of $\Delta^3$-carene at normal pressure is about 172°C) for varying lengths of time.

As will be seen from table I, the activity disappeared immediately. An interesting observation was that, when the ampules were opened, those that had been heated were under a considerable pressure of oxygen. For this reason the active constituent was probably to be sought among thermolabile $\Delta^3$-carene-oxygen compounds.

By means of adsorption on a microscale, concentration of the eczematogenically active factor had succeeded. However, the amounts of concentrated product obtained were of the order of only one-thousandth of a milliliter. For a direct and more extensive investigation of the active factor, these amounts were much

![Fig. 3](image-url)

![Fig. 4](image-url)

Figs. 3 and 4. Attempt to isolate eczematogenic component of oxidized highly purified $\Delta^3$-carene by displacement adsorption in macroscale. Fig. 3: Adsorptogram in microscale (40 µl) showing the eczematogenic factor concentrated to the peak. Fig. 4: Corresponding sample on macroscale (3.000 µl); the eczematogenic factor destroyed.
too small. It seemed plausible, therefore, when distillation had been found impracticable, to try concentration by displacement adsorption on a semimicro- or macroscale, and in that way to obtain larger quantities of active fractions for further investigation. For this experiment, a few milliliters of highly purified carene were oxidized to a suitable oxidation level; i.e., the amount of active peak was at a maximum (examined on microscale with a 40 μl sample), but no polymerization had yet begun (see description below of the behavior of Δ3-carene under oxidation). An existing polymerization constituent, due to its high viscosity, definitely prevented percolation through the longer column required by the macroscale.

The Δ3-carene which had accordingly been oxidized was then separated in a column 570 mm high and 7 mm wide. For the microtest, the corresponding dimensions of the column were 250 and 1.4 mm respectively. The adsorptograms obtained are shown in figs. 3 and 4.

Worthy of note is that such an extremely high “peak” was obtained in macroscale. To all appearances substantial concentration of the active factor had occurred. Skin tests showed, however, no activity whatsoever, in any of the peak fractions obtained in the microtest; the only positive reaction (one-plus and two-plus) was obtained in respect of the first fraction. Comparison of the adsorptograms in figs. 3 and 4 shows that a shift of the identification level occurred on macroseparation, demonstrating that the first few fractions did not consist of pure hydrocarbon alone. The cause of this surprising result is evidently to be sought in the great instability of the active factor. Detailed study of the process of decomposition which has not yet been completed, suggests that there are a number of factors to be considered. Thus the temperature, the reaction time with the adsorbent, and the increase in concentration which the active product undergoes, all have some influence. Measurements (by thermoelement) showed that the increase of temperature was 10 degrees or so higher in macroseparation than was the case with the microscale. Due to the greater dimensions of the column on the macroscale, the duration of contact between sample and silica gel was also increased. Finally, as regards the increase in concentration, the aforementioned indirect studies of the course of oxidation show that when the active component has reached a certain concentration it is transformed spontaneously into other products.

INDIRECT INVESTIGATION OF THE NATURE OF THE ACTIVE FACTOR

Direct isolation of the active factor, as will be clear from the foregoing, did not give any positive results, although some information was obtained as to the nature of the factor sought. The active constituent was an oxidation product of terpene; further, it was thermolabile and could not be concentrated by displacement adsorption on a macroscale. Since, however, an unequivocal concentration had occurred on microscale, it was decided to study the various conditions, chemical and physicochemical, that might be used to interpret the process that caused peak formation in the microadsorptogram, i.e., the occurrence of the active factor.
Under well-defined conditions a highly purified terpene, Δ3-carene, was made to take up known amounts of oxygen. At frequent intervals small samples were removed from the reaction mixture. These samples were immediately subjected to analysis by a number of independent chemical and physicochemical methods, such as displacement adsorption on a microscale, viscometry, iodometric titration, and measurement of the refractive index. The reason why displacement adsorption was used has already been discussed. The study of the viscosity was

Fig. 5. Δ3-carene + oxygen. Velocity of reaction.

Fig. 6. Oxidized Δ3-carene. Relation between oxygen added (on the abscissa) and oxygen content calculated from iodine titration (on the ordinate).

Fig. 7. The increase in viscosity in the reaction of Δ3-carene and oxygen.

Fig. 8. Microadsorptograms of Δ3-carene with different oxygen contents.

Fig. 9. The increase in refractive index in the reaction of Δ3-carene and oxygen.
designed to secure a measure of the degree of polymerization; turpentine, as is
known, becomes increasingly viscous when exposed to atmospheric oxygen. The
number of reactive groups that might easily oxidize iodide to iodine without the
latter added to double bonds in the terpene was studied by iodometric titration.
Finally, as regards the refractive index measurements, it was hoped that these
would show if further conversion would take place.

The experimental results are summarized in figs. 5–9. A detailed report on the
experimental methods and data will be presented, as mentioned above, in the
chemical literature.

From figs. 5, 6, 7 and 8, where the ordinate shows the amount of oxygen taken
up per unit of \( \Delta^3 \)-carene, and the abscissa the individually measured values, it
will be seen that the uptake of oxygen by \( \Delta^3 \)-carene has a discontinuous course.
The initial phase is characterised by a relatively delayed rate of oxygen uptake
(fig. 5); the relation between the equivalent amount of oxygen liberated from

![Fig. 10. Relation between micro adsorbed, highly oxidized \( \Delta^3 \)-carene (partly poly-
merized) and skin test.](image)

the oxidized carene, by iodometric titration, and that added at oxidation of the
carene (fig. 6); an extremely slight increase in viscosity relative to the original
\( \Delta^3 \)-carene (fig. 7); and, lastly, a marked peak at the end of the displacement ad-
sorptogram (fig. 8).

The refractive index curve (fig. 9) shows no conspicuous variations; it rises
steadily throughout the investigated phase of oxidation.

This initial phase terminates at an oxygen concentration of about 5 ml \( O_2 
per gm \( \Delta^3 \)-carene and is followed by a reaction resulting in the formation of poly-
merization products; the viscosity then rises markedly (fig. 7) and the velocity
of oxidation increases in relation to the initial phase (fig. 5). The relation be-
tween the equivalent amount of oxygen liberated by iodometric titration and
the amount added is broken (fig. 6). The displacement adsorption analysis (fig. 8)
serves to illustrate the change that has occurred in the course of the reaction.
The terminal peak of the curve broadens increasingly and a new constituent
appears with a low affinity to the adsorbent (it is concentrated in the first few
fractions emerging) and a very high refraction index. In the manual work on the
analyses it was found, moreover, to be highly viscous, in contrast to the other fractions.

Experimental skin tests on turpentine hypersensitive patients (figs. 2 and 10) show that the eczematogenically active constituent is attached only to the "peak" and not to the component appearing first, whether it consists of pure Δ⁵-carene (fig. 2) or polymerization products (fig. 10). Comparison with figs. 2 and 10 demonstrates, too, that the effect of the eczematogenic components is not dependent on the presence of polymerization products.

From the above comparison of the purely chemical and physicochemical indirect investigations of the course of oxidation, and the results of the skin tests, it is evident that the eczematogenic factor is related to the process occurring in the initial phase of oxidation. The structure of the active factor cannot be fully elucidated until that component has been directly isolated and broken down. The indirect investigation reported in the foregoing discussion nevertheless lends a high degree of probability to the following conclusions. The viscosity measurements and the behavior on displacement adsorption analysis indicate that the active factor is monomolecular. From the marked relation on iodometric titration, between oxygen found and oxygen added, with the circumstance that of two mols of oxygen supplied one mol was liberated, it is clear that one —O—O—H group (hydroperoxide group) per molecule of Δ⁵-carene is present. The eczematogenically active factor occurring on contact between Δ⁵-carene and oxygen, would accordingly be a monomolecular Δ⁵-carene-hydroperoxide. This assumption is also supported by experience of its thermolability and instability in high concentrations (vide the experiments with direct isolation of the active factor.

An idea of the activity of this eczematogenic factor is provided by Table II. The amount of test fluid used in the experiment was 5 μl, and the amount of active constituent was computed from a comparison of the refraction index of the sample and the corresponding oxygen content. It emerges that an incipient cutaneous reaction was already obtained with an amount of about 20·10⁻⁶ gm, and that a full (three-plus) reaction was produced by about 300·10⁻⁶ gm active constituent.

**SUMMARY**

It had earlier been shown that dermatitis due to turpentine is caused not by the hydrocarbons but by oxidation products. By means of displacement adsorp-
tion on a microscale (*ad modum* Blohm), concentration of an eczematogenic factor had been accomplished with a series of different terpenes of varying degrees of purity.

On the basis of these facts further experiments were conducted with a view to elucidating the nature of the active factor. For this purpose a highly purified, well-defined Δ⁴-carene (a characteristic constituent of Swedish turpentine) was taken as the specimen fluid. Attempts to isolate an active factor directly from oxidized Δ⁴-carene (by distillation) gave negative results due to the thermolability of the product sought. It was found, moreover, that although concentration on a microscale had succeeded, the unstable nature of the factor made concentration on a macroscale impossible.

Since direct isolation of the component sought had failed, an indirect method of investigation was resorted to. To a well-defined, highly purified Δ⁴-carene were added, step by step, known amounts of oxygen, and the various stages of oxidation were studied by means of independent chemical and physicochemical methods. Skin tests made parallel therewith suggested that formation of eczematogenically active factors was related solely to the initial phase of oxidation. Judging by data from the chemical investigation of the Δ⁴-carene–oxygen reaction—which are only touched upon here and will shortly be presented in the chemical literature—the eczematogenic factor is probably formed from a monomolecular hydroperoxide of Δ⁴-carene. Definite verification of this, and elucidation of the molecular structure, will not be possible, of course, until direct isolation has succeeded. To that end the experiments are proceeding. From the theoretically calculated molecular weight of the active factor it is found that amounts of about 20 μ will produce positive cutaneous reactions.

REFERENCES