Studies on the Antibiotic Action of Fish Components—X.
The Antibiotic Action of Carbonyl Compound in Autoxidized Methyl Esters of Fatty Acids Derived from Shark Liver Oil

Masamichi TOYOMIZU
(Laboratory of Fisheries Chemistry, Department of Fisheries, Faculty of Agriculture, Kyushu University, Fukuoka.)

In the previous paper1), it has been described that the antibiotic action of the autoxidized shark liver oil has a close relation with the absorbancy at 530 mμ which is due to the orange-colored condensed products with thiobarbituric acid (TBA) recently used in estimating rancidity of oil.

When, however, antibiotic activities and TBA values were plotted in a figure, a strict straight line was not observed. It probably means that the antibiotic substances in the autoxidized shark liver oil are the substances colored with TBA, but that the autoxidized oil contains other substances which are colored with TBA and are not the antibiotic substances. In order to prove this by bioautography, Rf value of the antibiotic zone and those of the autoxidative products colored with TBA were investigated, and it was shown that Rf value of the antibiotic zone agrees with one of Rf values of autoxidative products.

Since carbonyl compound is colored with TBA2), the author attempted to separate carbonyl compound from the autoxidized methyl esters of fatty acids derived from shark liver oil. It has been reported that rancid flavor of oxidized oil is due to carbonyl compound, and numerous carbonyl compounds have been isolated as hydrazones from the vapor-distillates3). The author had previously attempted to separate the antibiotic substance from the autoxidized oil by distillation, but in vain, so other separation methods were employed.

**Experimental**

I. Bioautography of the antibiotic autoxidized fatty acids of shark liver oil fractionated by means of urea

The preparation employed for bioautography were obtained by the following procedure4). Fatty acids of shark liver oil were autoxidized in the presence of M/100 Cu–stearate by blowing oxygen at 100° for 1.5 hr. and then were fractionated by means of urea (antibiotic activity 2,000). Petroleum ether (b. p. 50°~60°)–acetic acid (4 : 1) was suitable as a developing solvent and Toyo No. 50 filter paper was used. After developing the chromatogram, the solvent was removed by using ABDERHALDEN’s dryer for 10 min. Then the filter paper was cut into each 0.5 cm., and each piece was laid on Peptone-HAYDUCK

Received Jan. 23, 1957
agar plate, seeded with *Debaryomyces membranaefaciens*, a strain used for the assay of the antibiotics. The agar plates were then incubated for 48 hr. at 30° and *Rf* value of the zone of growth inhibition was measured. On the other hand, the other filter paper on which chromatogram was developed was sprayed with 0.67% TBA solution in 50% acetic acid.

As shown in Fig. 1, *Rf* value of antibiotic zone agreed with that of a larger orange-colored spot sprayed with TBA reagent, and two smaller spots were present which had no connection with the antibiotic action. It means that the antibiotic substance is a substance which is colored with TBA, and that the other substances, which are colored with it but are not involved in the antibiotic action, are also present in the autoxidized fatty acids of shark liver oil.

II. Separation of carbonyl compound from the antibiotic autoxidized methyl esters of fatty acids derived from shark liver oil

The preparations employed in the study were obtained by the following procedure. Shark liver oil was saponified and then unsaponifiable matter was eliminated. After methylation and distillation, methyl esters distilled at 179°–203° (4 mm.) were obtained. Then these methyl esters were autoxidized according to the procedure described above (antibiotic activity of the autoxidized methyl esters 500).

In order to separate carbonyl compound, the autoxidized methyl esters were fractionated according to GIRARD'S method as shown in the separation chart (Fig. 2). The antibiotic substance was present only in carbonyl fraction and the antibiotic activity was 2,000, whereas non-carbonyl fraction did not show any antibiotic action. Accordingly, the antibiotic substance was concentrated in carbonyl fraction, however the yield of antibiotic activity was only 24%.

![Fig. 1. Bioautography of the antibiotic autoxidized fatty acids of shark liver oil fractionated by means of urea.](image)

![Fig. 2. Separation chart.](image)
To solve the question of the low yield, further study has been made. After treating the autoxidized methyl esters in the absence of P-reagent according to the procedure shown in the separation chart, the antibiotic activity decreased to 200. So it was concluded that the decrease of the antibiotic activity was not due to the neutralization with sodium carbonate or to boiling in ethanol, but to boiling in 10% acetic acid ethanol solution for 45 min., as shown in Table 1. In boiling the preparations in 10% acetic acid ethanol solution for 45 min., the same decrease of antibiotic activity was observed with the autoxidized fatty acids of shark liver oil as in the case of the esters, and no decrease was observed with the autoxidized shark liver oil. Therefore, stabilization of the antibiotic substance during the treatment was attempted by adding small amount of the fresh or the autoxidized shark liver oil to the autoxidized fatty acids or methyl esters, but no successful result was found as when some antioxidants were added.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antibiotic activity</th>
<th>Thermal treatment (for 45 min.)</th>
<th>Antibiotic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoxidized methyl esters of fatty acids derived from shark liver oil</td>
<td>500</td>
<td>Boiling in 10% acetic acid ethanol solution</td>
<td>200</td>
</tr>
<tr>
<td>ditto</td>
<td>500</td>
<td>After boiling in 10% acetic acid ethanol soln., neutralization with sodium carbonate</td>
<td>200</td>
</tr>
<tr>
<td>ditto</td>
<td>500</td>
<td>Boiling in ethanol</td>
<td>500</td>
</tr>
<tr>
<td>Autoxidized fatty acids derived from shark liver oil</td>
<td>500</td>
<td>Boiling in 10% acetic acid ethanol solution</td>
<td>200</td>
</tr>
<tr>
<td>Autoxidized shark liver oil</td>
<td>500</td>
<td>ditto</td>
<td>500</td>
</tr>
</tbody>
</table>

Peroxide values of the preparations before and after the thermal treatment in 10% acetic acid ethanol solution were 7.84 and 6.00 respectively, so the antibiotic action does not appear to be due to peroxide.

Carbonyl compound which was obtained by GIRARD’s method was brownish red-colored, slimy and oily substance and the yield of antibiotic activity was low. As thermal treatment which promotes further oxidation should be avoided in the present study, carbonyl compound in the autoxidized methyl esters was separated by preparing addition compound with sodium bisulfite at room temperature, and then following procedure was employed.

10 gr. of the preparations and 20 ml. of saturated bisulfite solution were poured into the flask, and after shaking the flask in a horizontal position for 30 min., the content was centrifuged and then addition compound was obtained in the layer between upper oil and lower saturated sodium bisulfite solution. After washing the addition compound with petroleum ether, it was decomposed by sulfuric acid and then carbonyl fat was extracted with ether. The yields of carbonyl fat and non-carbonyl fat were 0.75 gr. and 7.1 gr. respectively. The antibiotic activity of carbonyl fat was 4,000 and that of non-carbonyl fat could not be recognized at all as in the case of GIRARD’s method.

**Summary**

It was concluded by means of bioautography that the antibiotic substance in the antibiotic autoxidized fatty acids derived from shark liver oil was a main constituent of the
substances which were colored with thiobarbituric acid, and carbonyl compound separated from the autoxidized methyl esters inhibited microbial growth.

Acknowledgement

The author wishes to express his hearty thanks to Prof. Yukio Tomiyasu for his kind advice throughout these works.

References

1) M. Toyomizu: This Bull., 22, 368 (1956).