REACTION PRODUCTS OF VITAMIN A ACID WITH ANTIMONY TRICHLORIDE

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It has long been known that vitamin A acid shows a purplish red color with antimony trichloride and this reaction has been used for the identification (1) and quantitative determination (2) of vitamin A acid. This reaction, however, is slower and more complex in nature than the corresponding reactions of vitamin A alcohol and vitamin A acetate. Vitamin A alcohol, A acetate, and A acid were allowed to react with SbCl$_3$ reagent, and the reaction was stopped by adding water at the stage of maximum color intensity. When the products were subjected to thin layer chromatography using polyamide powder, several spots appeared in the case of vitamin A acid, while a single spot was found in the case of vitamin A alcohol and A acetate. SbCl$_3$ reaction of vitamin A acid and the chemical property of the products were investigated by the authors.

EXPERIMENTAL

1. Samples and Reagents

Following samples and reagents were used.

Vitamin A acid — Crude vitamin A acid crystals were recrystallized twice from benzene-methanol, yielding the crystals, mp 179°.

Vitamin A Acetate — Synthesized vitamin A acetate crystal (2.8×10$^6$ I.U./g).

Vitamin A alcohol — A acetate was saponified with alkali prior to use.

SbCl$_3$ Reagent — SbCl$_3$ (chemically pure), 60 g, was dissolved in 280 ml of chloroform and 3 ml of acetic anhydride was added to the supernatant.

Ethanol Saturated with HCl — Ice-cold ethanol was saturated with HCl gas.

Acid Clay — Acid clay for vitamin D assay.

2. Apparatus

For the ultraviolet and visible spectrophotometry Hitachi Self-recording Spectrophotometer, ESP-2 type, was used.

Thin layer plate mainly used for the identification of vitamin A acid was prepared by spreading polyamide powder to a thickness of 250 μ and dried in air for 2—3 hours. Distance of development was 10.0 cm and the developing solvents were methanol and chloroform, both chemically pure preparations.

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RESULTS

1. Color Development of Vitamin A Acid after Reaction with SbCl₃ Reagent

Approximately 3 mg of A acid was dissolved in 100 ml of chloroform. To 0.3 ml of this solution was added 3 ml of SbCl₃ reagent. The visible absorption spectra at different times are shown in Fig. 1, and the absorbances at $\lambda_{\text{max}}$ against time are plotted in Fig. 2.

Maximum color intensity was attained 40 to 50 seconds after the addition of the reagent, the $\lambda_{\text{max}}$ being 573 m$\mu$. The time for maximum color intensity varied according to the conditions of reaction.

The value of $E$ at 573 m$\mu$ rapidly decreased after reaching the maximum, followed by the appearance of another peak at around 470 m$\mu$. The $E$ value of 470 m$\mu$ reached the maximum after 3 minutes, being stable for 5 minutes, followed by a gradual decrease with the appearance of a new low peak at around 530 m$\mu$. Three peaks 573, 470 and 530 m$\mu$, thus successively appeared upon addition of SbCl₃ to vitamin A acid.
2. Thin Layer Chromatography of the Reaction Products of A Acid and SbCl₃

Ten mg of A acid was dissolved in 10 ml of chloroform. To each 0.5 ml aliquot was added 5 ml of SbCl₃ reagent and the reaction was stopped immediately, 10 seconds, 45 seconds (the time for maximum $E$ at 573 m$\mu$) and 5 minutes (the time for maximum $E$ 470 m$\mu$) after the addition respectively. The chloroform layer was separated after shaking, washed with water, dried, evaporated to dryness under reduced pressure, and dissolved by adding 0.1 ml of ethanol. Two dimension thin layer chromatography was carried out on a polyamide powder plate using 2 $\mu$l each of the test solutions and methanol and chloroform as developing solvents. An ultraviolet lamp of 365 m$\mu$ was used for the detection of the spots. Fig. 3 shows the chromatogram obtained when the reaction was stopped in 45 seconds and Fig. 4 the changes of the reaction products in the course of the reaction.

![Figure 3](image-url)

**Fig. 3** Thin Layer Chromatogram of the Products of Vitamin A Acid-SbCl₃ Reaction on Polyamide Powder Plate

S, sample.

![Figure 4](image-url)

**Fig. 4** Time Course of the Changes in the Products of Vitamin A Acid-SbCl₃ Reaction as Tested on the Polyamide Powder Plate
time. As shown in Fig. 4, vitamin A acid developed a purplish red color immediately after reaction with SbCl₃, but a yellow fluorescent substance at Rf 0.50 (Y-I), a blue fluorescent substance at Rf 0.63 (B-II) and several other substances were found in the mixture after the reaction was stopped.

Y-I was already formed 1—2 seconds after the reaction, reaching the maximum value in 45 seconds, and gradually decreased thereafter. It seems to be related to the development of the maximum E value at 573 m\(\mu\) in the reaction between vitamin A acid and SbCl₃.

3. Property of the Reaction Products, Y-I and B-II

Since the attempts of completely separating Y-I and B-II by alumina chromatography, ion exchange cellulose column chromatography (I), and thin layer chromatography using silicagel G as the adsorbant failed, thin layer chromatography using polyamide powder was tried for the purpose of separation.

Ten mg of vitamin A acid was dissolved in a small amount of chloroform and after addition of 20 ml SbCl₃ reagent the whole was allowed to stand for 4 minutes. Then, 20 ml of water was added and the whole was shaken well. The chloroform layer was separated, and the solvent was evaporated to dryness. The residue was dissolved in 2 to 3 drops of benzene, which was used as the sample. Polyamide powder from which soluble substances had been removed by methanol treatment was used for chromatography using methanol as the developing solvent. Portions on the chromatogram corresponding to Y-I and B-II were removed, extracted with methanol, concentrated, dissolved in benzene again, and rechromatographed to obtain the final product.

When Y-I was allowed to react with SbCl₃, the peak was similar to the first one appeared in the reaction between vitamin A acid and SbCl₃ with the maximum absorbance at 573 m\(\mu\), though the color was more brightly violet. In the reaction of B-II with SbCl₃, the peak was similar to the second one with the maximum absorbance at 470 m\(\mu\), which appeared after addition of SbCl₃ to vitamin A acid. B-II was formed by the reaction of Y-I with SbCl₃. Table 1 and Figs. 5—7 show the properties of Y-I and B-II.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(\lambda_{\text{max}}) in UV absorption spectrum (in CHCl₃)</th>
<th>Rf on thin layer chromatogram</th>
<th>SbCl₃ reaction</th>
<th>Fluorescence (365 m(\mu))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(m\mu)</td>
<td>Methanol</td>
<td>Chloroform</td>
<td>(\lambda_{\text{max}})</td>
</tr>
<tr>
<td>Y-I</td>
<td>340, 355, 374</td>
<td>0.50</td>
<td>0.40</td>
<td>573</td>
</tr>
<tr>
<td>B-II</td>
<td>323, 341, 355</td>
<td>0.63</td>
<td>0.40</td>
<td>470</td>
</tr>
<tr>
<td>Vitamin A acid</td>
<td>368</td>
<td>0.56</td>
<td>0.58</td>
<td>573</td>
</tr>
</tbody>
</table>

*Polyamide powder was used for thin layer chromatography.
Infrared spectrum is shown in Fig. 8. It appeared that the terminal carboxyl group was present in the structural formula of Y-I, from the findings that the absorption due to a carboxyl group was seen at 1,420 and 1,700 cm\(^{-1}\) and Y-I behaved similarly to vitamin A acid in ion exchange cellulose column chromatography (ammonia-active form).
When vitamin A alcohol and A acetate were allowed to react with SbCl₃ for several seconds and the products were subjected to thin layer chromatography using polyamide powder as the adsorbent and methanol and chloroform as the developing solvents, Rf values given in Table 2 were obtained. The orange fluorescent substance with Rf 0.48 after development with methanol probably represents anhydrovitamin A (3) in view of the UV-spectrum, while the yellow fluorescent substance with Rf 0.60 following chloroform development is probably isoanhydrovitamin A.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Rf values developed with</th>
<th>Color of fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol</td>
<td>Chloroform</td>
</tr>
<tr>
<td>Vitamin A alcohol</td>
<td>0.48</td>
<td>0.60</td>
</tr>
<tr>
<td>Vitamin A acetate</td>
<td>0.74</td>
<td>0.78</td>
</tr>
<tr>
<td>Reaction product (A)</td>
<td>0.00</td>
<td>0.60</td>
</tr>
<tr>
<td>Reaction product (B)</td>
<td>0.48</td>
<td>0.80</td>
</tr>
</tbody>
</table>

4. Reaction of Vitamin A Acid with HCl

When vitamin A acid was treated with HCl or acid clay, a property similar to that seen upon SbCl₃ treatment was observed. Following experiments were therefore conducted.

Ten mg of vitamin A acid was dissolved in 10 ml each of ethanol and chloroform. To each solution was added 15 ml each of ethanol saturated with HCl and chloroform saturated with HCl. In the case of ethanol, the reaction was stopped by adding 20 ml of water after 30 seconds and extraction was immediately carried out with 50 ml of ether. Each of ether and chloroform layer was washed with water, dried, evaporated to dryness, and dissolved in benzene. A two dimension chromatography using polyamide plate and methanol and chloroform as developing solvents revealed a yellow spot at the same position as Y-I, while no spot
corresponding to B-II was detected even if the reaction time was prolonged. The property of the yellow spot isolated and purified was identical with those of Y-I. Thus, the yellow fluorescent substance produced as the result of the reaction between vitamin A acid and HCl-saturated alcohol and chloroform is concluded to be Y-I.

Although a red color developed as the result of the reaction between vitamin A acid and HCl, the spectrum of the colored solution failed to show the peak corresponding to red but revealed the close resemblance to that of Y-I.

5. Reaction of Vitamin A Acid with Acid Clay

Ten g of acid clay was moistened with benzene. To the clay was added 5 mg of vitamin A acid dissolved in 5 ml benzene. The mixture was shaken for 2–3 minutes. After removing the clay the solution was sufficiently washed with benzene to exclude free vitamin A acid, 20 ml each of water and chloroform was added and shaken. When thin layer chromatography of the chloroform layer was carried out on a polyamide plate, a yellow spot was found at the position of Y-I although the majority was vitamin A acid. This spot was isolated and was identified as Y-I. However, no B-II was found.

SUMMARY

When vitamin A acid reacted with SbCl₃ in chloroform, a purple red color having the maximum absorbance at 573 mλ developed. The maximum gradually moved to 470 mλ. Upon stopping the reaction by addition of water at the maximum color intensity at 573 mλ, a yellow fluorescent substance (I) was mainly formed. Similar treatment at the maximum color intensity at 470 mλ gave rise to a blue fluorescent substance (II).

It was revealed from the findings of thin layer chromatography, ultraviolet absorption spectrum, SbCl₃ reaction and the color of fluorescence that the substance I was changed to the substance II by the treatment with SbCl₃. Reaction of vitamin A acid with HCl or acid clay gave rise to substance I but not to substance II.

ACKNOWLEDGEMENT

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REFERENCES