Thermal Decomposition of Kitol

By

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In 1943 Embree and Shantz[1] isolated a new substance related to vitamin A from whale liver oil and called it kitol. Their kitol was glassy yellow solid, having molecular formula of $C_{40}H_{58}(OH)_2$, two hydroxyl radicals and eight double bonds.

By color reaction, absorption spectrum, analytical distillation and biological assay, they confirmed that kitol produced vitamin A by pyrolysis in molecular distillation and moreover carried on the quantitative experiment to report that 1 mole of kitol produced 1 mole of vitamin A. It was also ascertained by them that kitol ester was decomposed thermally and produced corresponding vitamin A ester.[2]

After that, Baxter and others[3] isolated kitol in crystalline form, with use of which they carried on the pyrolysis experiment, with a yield of less 1 mole of vitamin A from 1 mole of kitol.

The chemical structure of kitol is still unknown. Formation of vitamin A by thermal decomposition is an important key to infer the kitol structure, so that the authors determined to carry out the further detailed study in this respect. It must be added that this respect is very significant for the practical method of changing kitol of whale liver oil to valuable vitamin A with a good yield.

Isolation of Kitol from Whale Lvier Oil.

With hydrated methanol vitamin A and cholesterol were extracted and removed from unsaponifiable matter dissolved in petroleum ether. Then petroleum ether solution was chromatographed to concentrate kitol. This kitol, still containing a small quantity of solvent was heated in vacuo at 150° for 5 minutes to eliminate the volatile fraction completely. The absorption spectrum of kitol thus obtained is shown in Fig. 1. As seen clearly in it, there is a maximum peculiar to kitol at

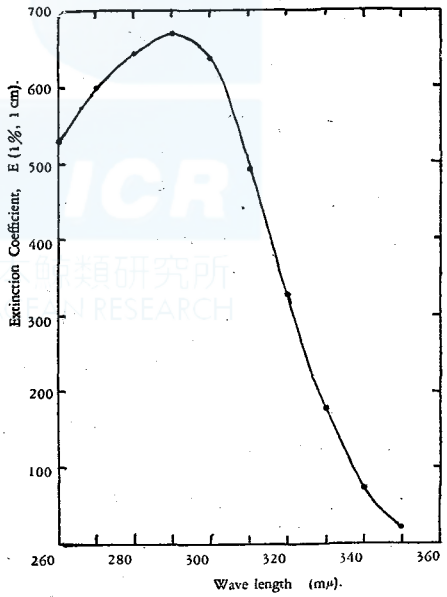

Fig. 1. Absorption spectrum of kitol.

290 mμ, the extinction coefficient E (1%, 1 cm) being 670, much higher than E (1%, 1 cm, 286 mμ)=580 obtained by Embree, being 95% of Baxter's E (1%, 1cm, 290 mμ)=707. As such an extent of purity was considered not to hinder the following experiments, this kitol was used.

Change of Kitol During Molecular Distillation.

Baxter and others[1] confirmed that 1 mole of kitol palmitate produced 0.65 mole or 0.75 mole of vitamin A palmitate by molecular distillation at 240° to 270° under the vacuum of 3μ. Previously the authors[2] observed that kitol was partly decomposed to produce vitamin A in the analytical distillation of unsaponifiable matter in whale liver oil. This time, however, the authors tried to distil kitol itself molecularly under high vacuum.

On the contrary to expectation the result was that most part of kitol was distilled by 5 minutes' heating at 220° without decomposition, but left not distilled by 1 minute's heating at 200° or 10 minutes' heating at 160°. (Figs. 2, 3 and 4)

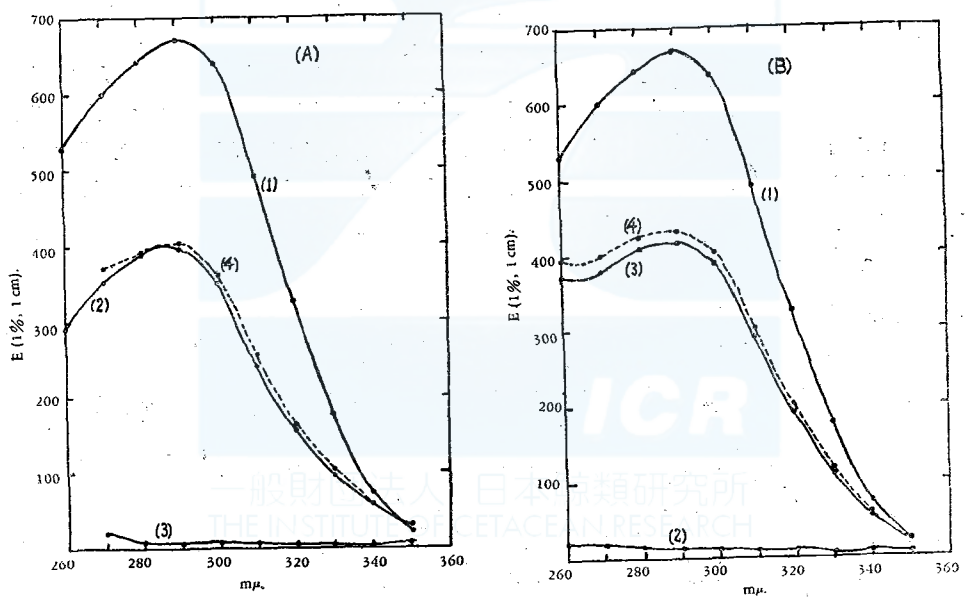

Fig. 2. Absorption spectra of molecular distillation products of kitol.
(A) 220°, 5 min. (B) 160°, 10 min. (1) kitol, (2) distillate, (3) residue, (4) total

As clearly shown in these figures, it is danger to calculate the decomposition ratio of kitol to vitamin A on the basis of the result of the experiments, because the absorption spectrum does not change so much as it shows the clear formation of vitamin A. It would be noteworthy to clarify that free kitol was

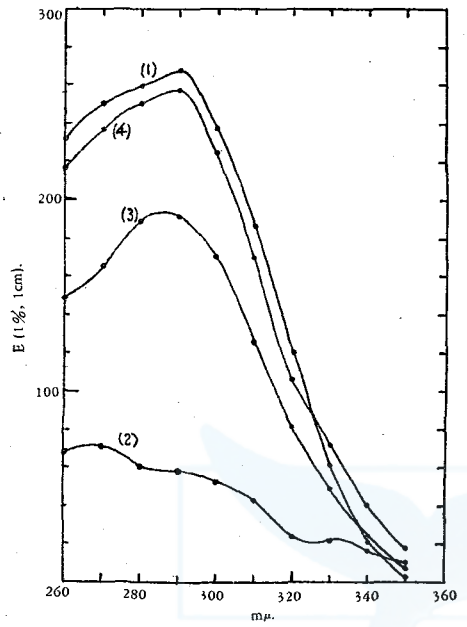

Fig. 3. Absorption spectra of molecular distillation products of kitol, 200°, 1 min, (1) kitol, (2) distillate, (3) residue, (4) total.

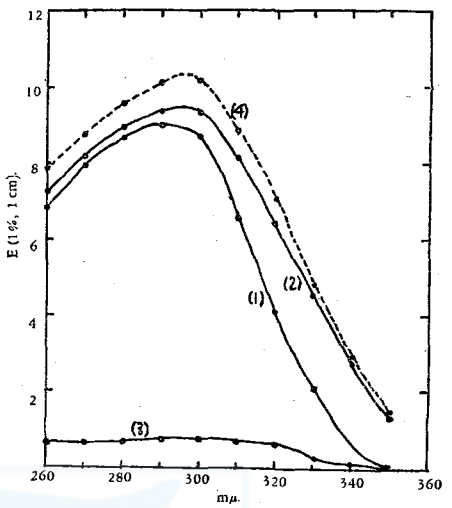

Fig. 4. Absorption spectra of molecular distillation products of kitol dissolved in the oil, 220°, 5 min., (1) kitol, (2) distillate, (3) residue, (4) total.

hardly decomposed and distilled out through molecular distillation under high vacuum and that this was true even if oil was added as solvent.

Change of Kitol Heated in Sealed Tube.

After kitol dissolved in the corn oil was heated at 220° in the slender glass tube for 1 to 16 minutes, Embree and others[1] determine the produced vitamin A by blue absorption value E (620 mμ) with antimony trichloride and remaining kitol by E (286 mμ) with vitamin A correction, on the basis of which decomposition ratio was calculated.

For the purpose of confirmation of vitamin A formation from kitol, kitol oil sealed in the glass tube with carbon dioxide was heated at 220°, with which absorption spectrum at 300 to 350 mμ was persued. The curves obtained showed clearly formation of vitamin A (Fig. 5).

The result calculated by the application of the principle of AK method which was explained in the reliminary report[1] was that in case of 8 minutes' heating 1 mole of kitol produced 1.67 mole of vitamin A.

Through the more careful experiment under the same condition change of absorption spectrum between 260 and 350 mμ was studied. The result was shown

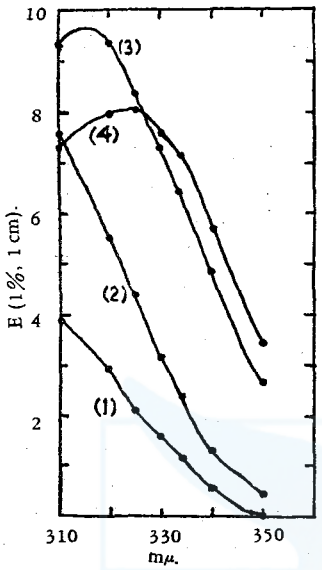

Fig. 5. Absorption spectra of decomposed kitol heated in the CO_2 sealed tube, at 220°, (1) 0 min., (2) 1 min., (3) 2min., (4) 8 min.

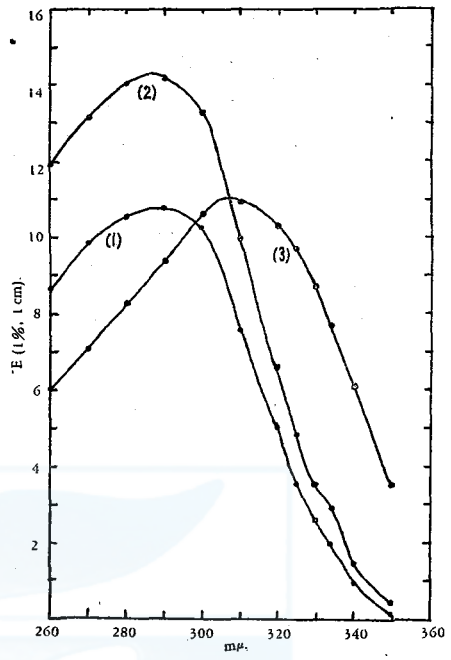

Fig. 6. Absorption spectra of decomposed kitol heated in the CO_2 sealed tube, at 220°, (1) 0 min., (2) 1 min., (2) 1 min., (3) 8 min.

in Fig. 6. The yield from 1 mole of kitol was 1.38 mole of vitamin A.

However, the experiment of heating for 1 minute showed that the potency of kitol rather increased. This problem will be discussed in the future report, for there is a necessity for further study.

Change of Kitol Heated in Vacuum Sealed Tube.

In order to improve the yield of vitamin A, the authors carried on a pyrolysis experiment about kitol under high vacuum (Fig. 7). After a thermal decomposition of kitol oil sealed under reduced pressure of 10^{-4} mm, the conclusion that 1 mole of kitol produced 1.97 mole of vitamin A was derived as expected. Also in this case the experiment of 1 minute's heating showed that vitamin A formation ratio was negative. This fact suggests us that kitol is likely to be decomposed thermally through the complicated process.

Properties of Vitamin A Formed from Kitol Decomposed by Heat.

It is still questionable whether or not vitamin A formed from kitol decomposed by heat is vitamin A in a strict sense. Perhaps it is possible that anhydro vitamin

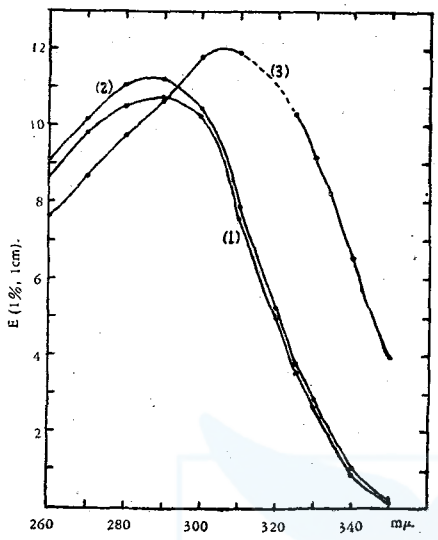

Fig. 7. Absorption spectra of decomposed kitol heated in the vacuum sealed tube, at 220°, in 10^{-4} mm, (1) 0 min, (2) 1 min., (3) 8 min.

A may be formed, too. In the present report, however, substances showing absorption spectrum similar to vitamin A which were formed from kitol decomposed, were all represented by vitamin A. The comparative color reaction between kitol oil and its thermally decomposed oil is shown in Table I.

This table shows clearly that the color reaction of thermally decomposed kitol oil quite agrees with that of vitamin A. Similarly to the preliminary report, the percentage of cis and trans vitamin A was determined, 21.2% for the former and the rest for the latter. It is interesting for the presumption of kitol structure that most of vitamin A produced from kitol is found to be trans type.

Table I

Color Reactions of Kitol before and after Thermal Decomposition

Reagent	Kitol oil	After thermal decomposition
Antimony trichloride	vivid red	blue (rather stable)
Trichloro-acetic acid	red with more kitol oil, colorless with less kitol.	azure blue
Acetic anhydride and conc. sulfuric acid	red→reddish brown	blue→purple→dark green

Experimental Part

Experiment 1. Isolation of Kitol from Whale Liver Oil.

1 kg. of Antarctic whale liver oil was saponified. After ether extraction about 200 g. of unsaponifiable matter was obtained, dissolved in 2 L of petrolem ether, extracted with about 90% methanol, until the petroleum ether layer hardly gave blue color like vitamin A with antimony trichloride reagent. The petroleum ether solution washed with methanolic potash, then with water, was dried on sodium sulfate. This petroleum solution was passed through the chromatographic column (40×4 cm) containing alumina. Then it was developed with petroleum ether saturated

with methanol, when narrow orange band appeared at the bottom. This seemed to be anhydro vitamin A. When this layer eluted, alumina was nearly colorless and showed vivid red color peculiar to kitol with antimony trichloride reagent. The adsorbent was taken out and eluted with 1L of petroleum ether and 50 cc. of methanol. This petroleum ether solution was washed with water and dried, chromatographed again. After extracting the adsorbent, the petroleum ether solution was evaporated to concentrate kitol as residue. It was heated in the micro molecular still shown in Fig. 8, at 150°, under 10^{-4} mm for 5 minutes. The residue was glassy solid of orange color, showing vivid red with chloroform solution of antimony trichloride (30%), same but lighter color with 20% chloroform solution of trichloro-acetic acid and similarly red and soon reddish brown with acetic anhydride and conc. sulfuric acid. The absorption spectrum of finally obtained kitol was shown in Fig. 1.

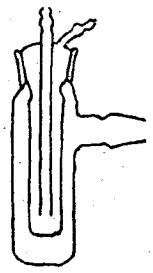

Fig. 8. Micromolecular still.

Experiment 2. Molecular Distillation of Kitol (1).

(1) Kitol obtained in the experiment 1 was heated at 220°, for 5 minutes under vacuum of 10^{-4} mm in the apparatus shwon in Fig. 8 connected with Hickman's oil diffusion pump. After reaction, evaporating surface was separated from condenser both being washed with isopropanol, the washing accurately diluted to a certain volume and the extinction was measured by Beckman spectrophotometer. Extinction coefficient was obtained by extinction divided by gram of sample per 100 cc. Thus obtained extinction coefficient was figured in Fig. 2.

(2) The result of experiment with 10 minutes' heating at 160° was also shown in Fig. 2. The sum of extinction coefficient of the distillate and the residue is far smaller than that of the sample (control). This being likely to be effected by weighing and diluting errors, the same experiment was carried on with some solvent in order to reduce errors.

Experiment 3. Molecular Distillation of Kitol (2).

(1) Evaporating benzine from the petroleum solution of kitol in vacuo, the residue gave viscous oil. Similar experiment to Exp. 1 was made with this oil. (Fig. 3).

(2) Molecular distillation of kitol oil.

Kitol and cod liver oil were distilled in order to make an experiment under the same condition as molecular distillation of natural liver oil. The cod liver oil

was the residue after 5 minutes' distillation at 150° under 10^{-4} mm in the apparatus shown in Fig. 7 with use of cod liver oil which had lost the vitamin A reaction through the preliminary heating at 200° and stirring mixed with Japanese acid clay. The kitol oil was prepared from 60 mg. of kitol obtained in the experiment 1 and 1.1 g. of the above refined cod liver oil fully mixed. This kitol oil was used in the following experiments. Moreover, since the absorption spectrum of oil itself was affected by heat, the same cod liver oil which did not contain kitol was distilled under the same condition as above. (blank test). The results are shown in Fig. 4. Heating condition was: at 220° for 5 minutes under 10^{-4} mm.

Experiment 4. Thermal Decomposition of Kitol in Carbon Dioxide Sealed Tube (1).

Kitol oil was weighed in a small test tube and the air in it was displaced by carbon dioxide. Instantly after, it was sealed and heated. When reaction was finished, sealed tube was cut off, the content dissolved in isopropanol to measure its extinction. The result was shown in Fig. 5. It was heated at 220° for 1, 2 and 8 minutes.

Now, in order to find the amount of vitamin A and kitol from this result with AK method, for convenience's sake, extinction coefficients at 330 mμ and 310 mμ were analyzed. As spectrum of pure vitamin A were used the same date as in the preliminary report, while that of kitol was based on the spectrum obtained in Exp. 1 in the present report. Hence, the following equations are obtained.

$$A(330)=1.439\ E\ (330)-0.5175\ E(310)$$
$$K(310)=1.439\ E(310)-1.218\ E(330)$$
$$A(328)=1.018\ A(330),\ K\ (290)=1.362\ K(310)$$

The equation to calculate decomposition ratio of kitol based on A (328) and K(290), was mentioned in the preliminary report.[5] The result was as follows.

Heated for	0 min.	1 min.	2 min.	8 min.
A (328)	0.265	0.635	5.79	7.225
K (290)	5.05	9.40	6.18	1.77
Mole of vitamin A per 1 mole of kitol		−0.09	−4.21	1.67

Experiment 5. Thermal Decomposition of Kitol in Carbon Dioxide Sealed Tube (2).

Almost similarly to Exp. 4, carbon dioxide displacement was made with special care and the range of the absorption spectrum was 260 to 350 mμ (Fig. 7).

AK equation was as follows:

$$A(328)=1.018\times\{1.115\ E(330)-0.2942\ E(290)\}$$
$$K(290)=1.115\ E(290)-0.4350\ E(330)$$

The result of calculation was:

Heated for	0 min.	1 min.	8 min.
A (328)	−0.248	−0.238	7.17
K (290)	10.87	14.25	6.64
mole of vitamin A per 1 mole of kitol		−0.02	1.38

Experiment 6. Thermal Decomposition of Kitol in Vacuum Sealed Tube.

Similar to Exp. 5, excepting that sample was sealed under vacuum of 10^{-4}mm. The result of calculation was as follows.

Heated for	0 min.	1 min.	8 min.
A (328)	−0.248	−0.0825	7.225
K (290)	10.87	11.24	7.89
mole of vitamin A per 1 mole of kitol		−0.361	1.97

Experiment 7. Reaction Velocity of Maleic Anhydride with Vitamin A Produced.

In the same method as the preliminary report[5] maleic anhydride reagent reacted with vitamin A obtained by 8 minutes' heating of kitol oil in carbon dioxide. After 16 hours at 25°, the recovery was 20%. Since it is known that recovery of vitamin A and neovitamin A under the same condition is respectively 3% and 83%, the percentage of neovitamin A in the sample is calculated as follows.

$$\%\ \text{of neovitamin A}=\frac{20-3}{83-3}\times 100=21.2\%$$

Namely, the vitamin A obtained by the decomposition of kitol was found to consist of 21.2% of cis type and 78.8% of trans type.

Summary

(1) Kitol was distilled nearly without decomposition, through molecular distillation under 10^{-4} mm.

(2) When kitol was heated at 220° for 8 minutes, sealed in carbon dioxide or vacuum, the absorption spectrum changed to show the formation of vitamin A. Analysis of the spectrum ascertained that 1 mole of kitol decomposed to produce 1.4 to 2.0 mole of vitamin A.

(3) Vitamin A obtained by thermal decomposition of kitol consisted of 80% of trans type and 20% of cis type.

In conclusion, the authors express their cordial thanks to Mr. T. Yamakawa and Mr. Hirao for accordance of facilities for measurement of absorption spectrum and Mr. T. Tawara for his assistance of obtaining the material and kitol extraction.

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