Molecular Distillation of Fin Whale Liver oil

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Introduction.

The author carried on molecular distillation of fin whale liver oil, examined the distillate with special emphasis on vitamin A and tried to determine its characteristics. That is, it has been pointed out that although whale liver oil shows a vitamin A color reaction it has little biological effect. Nakamiya[1] explained it with absorption spectrum and has reported that the peak has moved from 328 mμ. Furthermore, N. D. Embree[2] reports that the peak of absorption spectrum exist at four places and called it cyclized vitamin A. It has already been discovered that this does not have any effect in accelerating growth. The absorption spectra of these are very similar to those of vitamin A itself, and another important fact is that, at 200°C, vitamin A is transformed into cyclised vitamin A type due to the presence of an unsaturated acid such as oleic acid. Therefore, it is assumed that vitamin A is cyclized by unsaturated acid in the liver oil and thus lose its growth effect. A substance called kitol was discovered recently (3), kitol, was obtained from the unsaponifiable matter in whale liver oil by molecular distillation and crystalyzing was successful (4) but its structure was not determined, although experimentally, it is just two times that of vitamin A. It resembles carotene very much and is $C_{40}H_{60}O_2$, the peak of absorption being 286 mμ. The kitol also does not possess growth accelerating effect but its color reaction is the same as that of vitamin A.

In order to solve these problems, experiment was carried on as an attempt to discover cyclized viatmin A other than vitamin A coloring substance was concentrated by molecular distillation, following which, attempts were made to obtain crystals of these, but as explained later, obtaining pure crys. tals was not successful.

No. 1. Molecular distillation of fin whale liver oil.

As explained in the introduction, molecular distillation of liver oil was carried on, but distillation of the sample was carried on as it is, without pre-treatment. That is, the object was to detect the range through which vitamin A will distil and, at the same time, to find out to what extent

molecular distillation can be used as a possible means of refining oils and fats industrially.

Molecular distillation was carried on with samples which was not deodorized or neutralized in order to determine its deodorizing and neutralizing capacity. It was intended to crystalyze the concentrated vitamin A distillate by the Hamano Method[4].

1. Sample.

Sample used was Antarctic fin whale liver oil obtained from fin whale caught by the Taiyo Fishery Co., Ltd., in 1946 and its chemical constants are as follows:

Appearance (15°C)	Yellowish white, semi-solid
Refractive Index (18°C)	1.4950
Saponification Value	178.0
Vitamin A (C. L. O. U.)	50
Iodine Value (Wijs)	141.0
Unsaponifiable matter	1.7%

Also, the vitamin A determination during the experiment was made by the Oshima method[6].

2. Apparatus.

Molecular distillation apparatus used by the author was of all-glass type and its capacity is 1.5 kg., the area of the evaporation surface is about 200 cm^2, the distance to the condensation surface 5 mm and speed of circulation is 10 c.c. per minute. Pressure in the apparatus was determined by Geisler tube and was about 10^{-2}mm Hg.

For heating, parafin oil was placed in the cylinder of the evaporation surface and heated electrically, and the distilling temperature was the temperature of the bath so it is somewhat higher than the true temperature of the oil.

3. Experimental result and observation.

600 g of the liver oil sample was charged into the above apparatus, degasified at room temperatures, and further degasified after about two hours while carrying on circulation.

The circulation was carried on while working the diffusion pump and degasification continued, temperature raised gradually and distilled. As the liver oil sample was not saponified, the distillation temperatnre was generally high and the first distillate began at 220°C. Temperature range

was divided into four parts. Chemical constants of the distillates were as follows: General Characteristic of distillates of fin whale liver oil by molecular distillation.

Distillate	Distillation Temperature (°C/10^{-2} mm Hg)	Quantity of Distillate (g)	(%)	Appearance (15°C)	n_D^{18}	Acid Value	Iodine Value	Unsaponifiable matter (%)	V. A. (C. L. O. U.)	Order
Sample		600		Yellowish white semi-solid	1.4950	3.0	141.0	1.7	50	++
(1)	−220	3	0.5	Red liquid	1.5180	43.6	124.5	5.8	133	+++ +++
(2)	220—260	32	5.3	Golden color liquid	1.5168	29.0	115.0	6.1	800	+++
(3)	260—290	64	10.7	Yellow white semi-solid	1.4995	2.1	97.5	2.8	154	+++
(4)	280—300	95	15.8	Yellow white semi-solid	1.4895	1.5	94.5	9.8	50	++
Residue		395		Light brown liquid	1.4930	1.1	130.7	1.4	3	+

In the above table, in regards to odor, it was fortunate that the original sample had considerable odor and so it was used for determining the odor of each distillate tested on eight healthy girls. Needless to say, it is difficult to indicate odor quantitatively and the result is based on general smelling senes.

Odor of residue oil was very slight, acid value low, little polymerization and it was observed that is was suitable for edible use.

Vitamin A was concentrated into this fraction. Therefore, if liver oil which has been deodorized by pre-treatment was used, it can be used as vitamin A for addition in margarine.

A greater part of the free fatty acid was distilled over at 220°C.

4. Summary.

The following conclusion was drawn from the above molecular distillation, from the point of view of refining oils and fats:

(1) Sample should be completely dehydrated and degasified.

(2) In regards to apparatus, the pump should be powerfull and circulating apparatus be absolutely secure.

(3) Neutralization is almost complete by molecular distillation.

(4) It is possible to obtain concentrated solution of vitamin A 16 times that of the sample, and if it has been deodorized by pre-treatment, it is possible to use it for addition to margarine.

(5) Odor will accumulate to the same fraction as vitamin A but since it will distill over in the second fraction after neutralization, it is believed

that the molecular weight is somewhat larger than the free acid.

(6) There is little polymerization of the residue oil so it is suiable as edible oil.

No. 2. Derivation vitamin A crystals.

Attempts were made to derive crystals from the vitamin A distillate concentrated by the method described in the previous report, by the Hamano Method[5], but pure crystals could not be obtained because the quantity of the starting sample was too small. In Hamano Method, anhydrous maleic acid is made to condense directly on vitamin A ester and crystals obtained. Next it is saponified and the acid portion of its ester is also determined.

30 g of the above concentrated liver oil is dissolved in 100 g of anhydrous benzol and anhydrous maleic acid is dissolved at the same time, carbon dioxide gas is sealed into the tube and made to react by heating at 100°C. After 48 hours, Carr-Price reaction is absolutely absent so it is assumed that condensation was completed at this point. In the inner surface of the sealed tube, a small quantity of white, amorphous crystals appeared.

After the reaction, benzol was distilled off at reduced pressure, the viscous liquid poured into 3 liters of anhydrous petroleum ether and allowed to crystalize. After standing for one day, about 0.48 g of white, powderlike crystals, were obtained at the bottom of the container. The melting point of this crystal was 189°C and since recrystalization could not be obtained at 220°C, as referred to in reference (5) it was continued to the next procedure. That is 0.48 g of the crystal was saponified with 10 c.c. of 0.1 *N* $KOH \cdot C_2H_5OH$ and acidified with HCl. At this stage, only oily substance was freed and crystalline substance could not be obtained. This oily substance was extracted with anhydrous petroleum ether and the soluble part was a white crystal with a melting point of 37°C. This is the acid portion of vitamin A, but since the quantity of the sample was too small to confirm it, it was discontinued.

The oily substance insoluble in petroleum ether was recrytalized three times with benzol-ether, but the melting points were not constant. That is, it was only observed that it began to melt at 160°C, become a brown viscous substance in the vicinity of 220°C and finished melting into reddish brown at 280°C. This is very similar so the behavior of anhydrous maleic acid mixture of carotene referred to in Nakamiya's thesis[7] observation of the experimental result.

(1) The derivation of vitamin A crystals was by Hamano's method.

(2) Anhydrous maleic acid additive was obtained, but on acidifying with HCl, only an oily substance was produced and no crystals were obtained.

(3) The melting point of the sbustance, assumed to be the petroleum ether soluble acid component, was 37°C.

(4) The petroleum ether soluble portion was recrystalized with C_6H_6·$(C_2H_5)_2O$ but the melting point was not clear and it was observed that the behavior resembled that of anhydrous maleic acid mixture of carotene.

As indicated above, the research on crystal derivation by anhydrous maleic acid mixture was unsuccessful, but it is concluded that besides vitamin A, a higher molecular substance resembling carotene, is also present in whale liver oil. Because of this, it was thought that it did not crystalize out but only formed an oily substance and also, the melting point was not clear. At present other methods for determining this is being studied.

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