Analytical Distillation of Vitamin A in the Whale Liver Oil

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1. Introduction.

The presence of ordinary vitamin A and a vitamin A possessing different characteristics has been confirmed through research by Willstaedt and Jensen[1] and Nakamiya, et al[2] but recently, it has been reported by Baxter, et al[3] that kitol, a kind of provitamin A has been isolated from the unsaponifiable matter in whale liver oil, which has twice the molecular weight of vitamin A and which can be decomposed by heat into vitamin A. The authors carried on an analysis of vitamin A in the unsaponifiable matter of the Antartic whale liver oil (it is not certain whether the whale was a fin or blue whale but it is certain that it was from one of the two) by molecular distillation.

2. Analytical Distillation of Unsaponifiable Matter in Tunny Liver Oil.

First, in order to obtain an analytical distillation curve of a standard vitamin A, unsaponifiable matter of tunny liver oil, which is rich in ordinary vitamin A was used, to which was added mixed glycerides obtained by synthesis, as controlled yield oil[4] (abbreviated to C. Y. O.) and the result indicated in Table 1. was obtained. Graphically, it is as shown in Fig. 1. According to this graph, elimination maximum (abbreviated to E. M.) appears in the vicinity of 185°C and it is assumed that this is ordinary vitamin A.

Experiment 1. Synthesis of C. Y. O.

Coconut oil fatty acid is supplemented with acetic acid, butyric acid, and capric acid and equi-molar ratio mixture of fatty acid of the various carbon atom numbers were prepared, glycerine added and esterified in the presence of decalin. The mixed glycerides thus obtained were distilled in a cyclic still and divided into various fractions through a range of 130—280°C. Next, equal quantity of the various fractions were mixid and used as C. Y. O. and residue above 280°C used as R. O. (residue oil). Temperature determination was made in the middle of the heating oil bath within the evaporating cylinder. During the process, the bath was well stirred so it is assumed that it is approximately the temperature of the evaporating sur-

face. Also, the temperature of the residue oil which flowed down the heating cylinder and dropped from the bottom was also determined, from which it was discovered that the value was approximately 60°C lower than that of the oil bath. Vacuum was 10^{-2}—10^{-3}mm. and the distance between the evaporating surface and condensing surface was 0.5 cm. Temperature was raised every 10°C and at each range, distillate for each cycle was removed. These conditions were used for all analytical distillation explained hereinafter.

Experiment 2. Analytical Distillation of Unsaponifiable Matter in Tunny Liver Oil.

Analytical distillation was carried on with 76 g. of oil of 102 liver oil unit, obtained by adding 35 g. of C. Y. O. and 35 g. of R. O. to unsaponifiable matter from 25 g. of tunny liver oil for injection purpose (400 liver

Table 1. Analytical Distillation of Unsaponifiable Matter in Tunny Liver Oil

No.	Temp. (°C)	Distillate (g)	Liver Oil Unit	Liver Oil Unit × Weight	% of Recovered Vitamin A
1	130	2.4	103	248	3.2
2	140	2.1	109	233	3.0
3	150	2.5	310	788	10.2
4	160	1.8	400	729	9.4
5	170	1.8	585	1060	13.7
6	180	2.8	598	1643	21.2
7	190	2.0	462	903	11.2
8	200	2.5	341	852	11.0
9	210	2.6	202	533	6.4
10	220	1.9	143	270	3.5
11	230	1.2	130	156	2.0
12	240	1.2	65	77	1.0
13	250	1.2	45	52	0.7
14	260	1.1	64	93	0.9
15	270	1.7	55	91	1.2

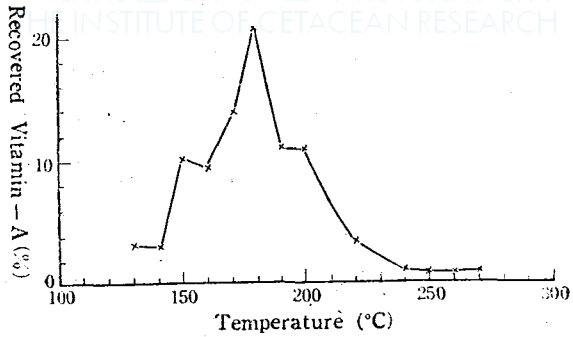


Fig. 1. Analytical Distillation Curve of Unsaponifiable Matter in Tunny Liver Oil.

oil unit). Oil distilling over at each 10°C (1 cycle) was taken, its weight and liver oil unit determined and the percentage of recovered vitamin A was calculated by the following formula:

% of recovered vitamin A=[(liver oil unit × weight of each distillate)/(liver oil unit × weight of oil before distillation)] × 100%.

Shown graphically, the experimental result is as indicated in Fig. 1.

3. Analytical Distillation of Unsaponifiable Matter in Whale Liver Oil.

Analytical distillation was carried on with unsaponifiable matter of Antarctic whale liver oil under the entirely similar conditions as in the case of tunny liver oil explained above. The result is as indicated in Fig. 2. As can be clearly seen from the graph, E. M. of No. 1. is in the vicinity of 180°C and entirely coincides with the E. M. as in the case of tunny liver oil. That is, there is no mistake that this is vitamin A. Next, in the case of whale liver oil, there is a tendency of a second E. M. to appear, but in the case of Fig. 2., since the same condition as in Fig. 1. was followed strictly, the distillate of C. Y. O. above 280°C was small and the distillation was stopped here. Thus, in order to obtain the second E. M., C. Y. O. and R. O. above 280°C was newly added and distilled. As a result of this, the second E. M., as can be clearly seen in Fig. 3., appeared. The first E. M. was in the vicinity of 185°C, the same as in the previous case, so that there is no question, but the shape of the second E. M. is somewhat vague, a shape which is formed by several peaks of single substance overlapping and its temperature ranges from 270—290°C. According to Hickman[5] natural vitamin A ester has E. M. at a temperature of about 90°C higher than free vitamin A, and β-carotene has an E. M. at a temperature about 30°C higher.

E. M. of kitol itself, comparable to that of vitamin A, could not be observed but from the fact that its molecular weight is twice that of vitamin A, it can be assumed that it has an E. M. somewhat near that of β-carotene. However, kitol decomposes into vitamin A with heat so that to what extent the transformation into vitamin A took place during the analytical distillation still remains a problem.

Experiment 3. Analytical Distillation of Unsaponifiable Matter in Whale Liver Oil.

(1) The whale liver oil sample was obtained during whaling in the Antarctic Ocean and was informed as blue whale liver oil. Its general

characteristic is as follows:

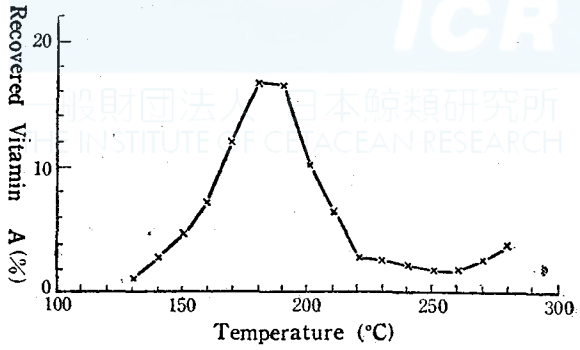
Specific gravity d_4^{17} 0.9230, refractive index n_D^{17} 1.4842, acid value 0, saponification value 166, unsaponifiable matter 11.5%, liver oil unit 312.

Unsaponifiable matter was obtained from 50g. of this liver oil and 80g. of oil of liver oil unit 66 was obtained by adding 35g. of C. Y. O. and 35g. of R. O., the same as before and analytical distillation carried on under the same condition as before. (Table 2 and Fig. 2)

Table 2. Analytical Distillation of Unsaponifiable Matter in Whale Liver Oil (1)

Number	Temp. (°C)	Distillate (g)	Liver Oil Unit	Liver Oil Unit × Weight	% of Recovered Vitamin A
1	130	1.3	38	50	1.0
2	140	2.2	62	136	2.6
3	150	2.2	111	242	4.6
4	160	1.8	212	382	9.3
5	170	2.2	297	651	12.4
6	180	2.6	341	885	16.8
7	190	2.6	333	865	16.5
8	200	2.7	205	552	10.5
9	210	2.6	179	358	6.3
10	220	1.5	106	159	3.0
11	230	2.4	59	140	2.7
12	240	1.7	69	117	2.2
13	250	1.3	72	94	1.8
14	260	1.3	78	100	1.9
15	270	1.7	75	127	2.4
16	280	2.1	95	200	3.8

Fig. 2. Analytical Distillation Curve of Unsaponifiable Matter in Whale Liver Oil (1)



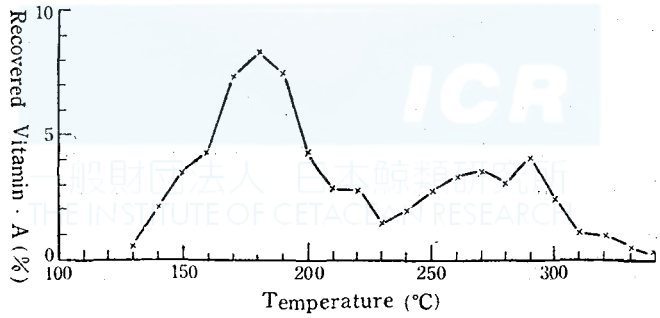
(2) 20 g. of new sample of the same liver oil as before was taken, unsaponifiable matter obtained from this, and to 240 g. of oil (liver oil unit 188) obtained by adding 35 g. of C. Y. O., 35 g. of R. O. and a further 60 g. of R. O. (C. Y. O. above 280°C) and 70 g of molecular distillation residue of

oil-shark liver oil and analytical distillation carried on (Table 3 and Fig. 3).

Table 3. Analytical Distillation of Unsaponifiable Matter in Whale Liver Oil (2)

Number	Temp. (°C)	Distillate (g)	Liver Oil Unit	Liver Oil Unit × Weight	% of Recovered Vitamin A
1	130	0.4	53	19	0.5
2	140	1.2	67	78	2.1
3	150	2.3	61	136	3.6
4	160	1.6	103	162	4.3
5	170	1.9	140	270	7.4
6	180	2.3	138	319	8.4
7	190	2.6	110	284	7.5
8	200	2.8	57	162	4.3
9	210	2.0	55	110	2.9
10	220	3.2	33	105	2.8
11	230	3.4	17	58	1.5
12	240	3.6	21	74	2.0
13	250	4.8	22	105	2.8
14	260	8.2	30	128	3.4
15	270	5.0	28	141	3.7
16	280	3.6	34	122	3.2
17	290	6.4	25	159	4.2
18	300	5.2	19	99	2.6
19	310	5.4	9	46	1.2
20	320	5.9	6	36	1.0
21	330	6.3	3	17	0.5
22	340	7.2	2	11	0.3

Fig. 3. Analytical distillation curve of unsaponifiable matter in whale liver oil (2)



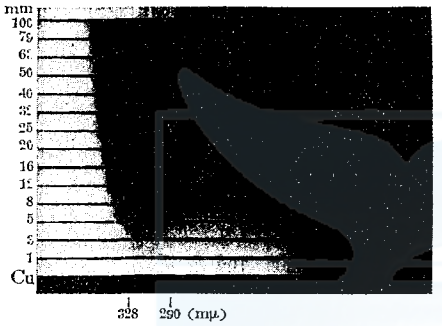
4. Absorption Spectrum of Analytical Distillate of Unsaponifiable Matter in Whale Liver Oil.

As a means of accertaining whether or not the two E. M. 's obtained as a result of analytical distillation of unsaponifiable matter in whale liver oil are due to vitamin A and kitol, absorprion spectrum of its E. M. distillate was taken. As a result of this, the first E. M. distillate showed a

typical vitamin A absorpition (Fig. 4) but in the second E. M. distillate, absorption was between the maximum absorption of vitamin A, 328 mμ, to the maximum absorption of kitol, 290 mμ, and somewhat stronger on the 290 mμ side (Fig. 5). An explanation for this may be that kitol was present in this whale liver oil and a part of it was decomposed by heat during analytical distillation. (Fig. 4 and 5).

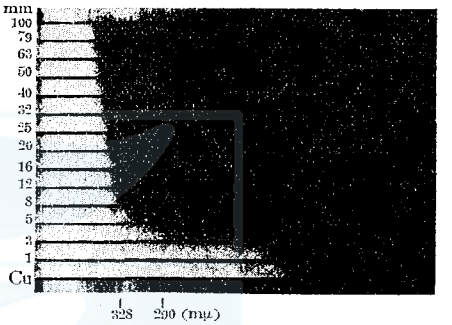
Experiment 4. Absorption Spectrum of the Distillate of Analytical Distillation of Unsaponifiable Matter in Whale Liver Oil.

Fig. 4



Molecular Distillate at 180°C of Whale Liver Oil Unsaponifiable.

Fig. 5



Molecular Distillate at 280°C of Whale Liver Oil Unsaponifiable.

From the various distillations of (2) of Experiment 3, distillate of two E. M., 180°C and 280°C were taken as representative ones, a fixed quantity of the unsaponifiable matter after saponification was extracted with ether and absorption spectrum of this ether solution photographed. Concentrations appropriate for vitamin A in the 180°C distillate was 1/20,000 mol and 175,000 mol for the 280°C distillate. In the former, absorption appeared at 345, 328, 310, 300 and 290 mμ, being the strongest. In the latter, many vague absorption appeared from 328 mμ to 290 mμ and it was noticed that it was somewhat stronger on the 290 mμ than on the 328 mμ side.

5. Conclusion.

As a result of molecular distillation of unsaponifiable matter in whale liver oil, it was observed that the elimination maxima appeared at two places, 185°C and 270—290°C. The first maximum coincided with the maximum of unsaponiable matter in tunny liver oil and is ordinary vitamin A. The second, as observed from the absorption spectrum, absorption occured from 290 mμ, the absorption maximum of kitol, to 328 mμ, absorption maximum of vitamin A. Therefore, it is conculuded that kitol is present in whale liver oil and a part of it decomposed by heat to vitamin A

during analytical distillation.

References

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3) F. B. Clough, H. M. Kascher, C. D. Robeson, J. G. Baxter, *Science*, **105**. 436 (1947); N. D. Embree, E. M. Shantz, *J. Am. Chem. Soc.*, **65**, 910 (1943).
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5) K. C. D. Hickman, *Chem. Rev.*, **34**, 95 (1944).