A Rapid Method for the Separate Determination of Vitamin A and Kitol in the Whale-liver Oil

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Spectrophotometric determination of vitamin A in the whale-liver oil is usually interfered by the existence of kitol.[1] The method to determine vitamin A by the color reaction with glyceroldichlorohydrine has been proposed to reject the interference of kitol,[2] but there remained unknown how to determine kitol in the co-existence of vitamin A.

The author has found that the simple method of semimicro molecular distillation is useful to separate vitamin A from kitol. The method was used to separate quantitatively the free cholesterol from the ester one in the blood plasm,[3] but no example has been applied to liver oil constituents. It can be naturally assumed that both vitamin A and kitol exist in the liver oil in state of ester form, then the distilling temperature differs each other so much that each compound can be separated by molecular distillation. The paper describes the fundamental experiment in detail to perform the newly found method.

Experimental Part

1. Distillation at 200° and Recovery of Vitamin A.—Semimicro molecular distillation apparatus[4] consisting of semimicro molecular still and Hickman's oil diffusion pump was used. The evaporation surface was 2.5 cm. diameter, and the distance between evaporator and condenser was 0.8 cm., the condensing surface being cooled by water current. Samples were liver oils of pollack, salmon shark, hammerhead shark, mixed shark (mixture of several kinds of shark-liver), and whales A and B, all being commercially prepared.

Just 0.120 cc. of the sample was micropipetted, from which the film thickness, one of the important factors in molecular distillation, was calculated as 0.25 mm. At first, the temperature of oil bath was kept constant at 200° and the distillation time was changed from 2 min. to 40 min. After each distillation, the condensing surface was taken out and washed with chloroform to recover the condensed vitamin A into an aliquot, which was treated by antimony trichloride reagent to measure the recovered vitamin A, the cod-liver oil unit being conveniently calculated for the weight of original sample 111.7 mg.

(calculated from mean specific gravity multiplied by 0.120) (Table 1).

Table 1. Recovery of Vitamin A in Distillation at 200° (C.L.O.U.)

Distillation time min.	Liver oil of:					
	Pollack	Salmon shark	Shark, mixed	Hammerhead shark	Whale A	Whale B
2	4.04	—	—	23.0	99.0	—
5	6.10	9.0	6.30	33.7	140	80.0
12	7.10	11.0	8.10	42.7	191	105
22	7.10	11.0	8.13	44.2	215	130
40	—	—	—	45.2	240	—
Original	7.25	11.2	9.04	45.7	206	108
Ratio*	100	100	100	104	113	124

* Ratio ≡ R 200° (22/12): that is, % of recovered vitamin A after 22 min. for that after 12 min.

Color reaction of the original liver oil of mixed shark, hammerhead shark and whale considerably differed from the standard color, so that the ratio of recovered vitamin A after 22 min. for that after 12 min. expressed in percentage was tentatively introduced, which divided liver oils into two groups. To one group with the ratio of 100% belonged the liver oil of pollack, salmon shark, and mixed shark, and to the other group with the ratio more than 100% belonged the liver oil of hammerhead shark, mixed shark, and whale, the former containing vitamin A ester, while the latter containing kitol ester in addition to vitamin A ester. Also Table 1 shows that about 15 minutes' heating is necessary to recover vitamin A completely by molecular distillation at 200°.

2. Distillation for 15 minutes and Recovery of Vitamin A.— Secondly, the distilling time was kept constant for 15 min. and the temperature was changed from 150° to 250° (Table 2).

In order to discriminate the liver oil containing kitol from that free from kitol the ratio of the recovered vitamin A at 230° for that at 200° during distillation for 15 min., expressed in percentage, was introduced. The ratio in case of the whale-liver oil is about 130%, while those of pollack and salmon shark-liver oils are about 100%. The difference 30% is attributed to that the former contains much amount of kitol. Moreover, the color test of the residual oil was fonnd to be useful to check the presence of kitol, blue color representing vitamin A, and red color representing kitol. The recovery of vitamin A at

Table 2. Recovery of Vitamin A in Distillation for 15 min. (C.L.O.U.)

Distillation temperature °C	Liver oil of:					
	Pollack	Salmon shark	Shark, mixed	Hammerhead shark	Whale A	Whale B
150	—	—	1.27	—	55.1	—
180	—	8.0	4.71	28.3	141	84.6
200	7.30	11.2	8.14	44.8	206	114
230	7.21	11.4	9.67	53.3	269	147
250	—	—	9.19	53.9	240	157
Color reaction of the residue with antimony trichloride reagent						
150	—	—	blue	—	bluish violet	—
180	—	blue	blue	blue	bluish violet	bluish violet
200	pale blue	pale bluish violet	pale reddish violet	red	red	red
230	colorless	colorless	pale red	pale red	red	red
250	—	—	pale red	pale red	blue	blue
Ratio*	99	102	119	119	131	129

* Ratio ≡ R 15 min. (230/200): that is, % of recovered vitamin A at 230° for that at 200°.

250° of whale-liver oil was less than that at 230° because a portion of the condensed vitamin A oil dropped to the residue, which was clearly proved by that the color test of the residual oil was blue.

3. Change of Absorption Spectrum during Distillation.— In the separation method before mentioned the distilled vitamin A was stable at condensing surface cooled with water, but the stability of remaining kitol was unknown. It was proved by absorption spectrum that kitol

Table 3. Extinction Coefficient of the Whale-liver Oil before and after Distillation, E (1%, 1 cm.)

Wave length, mμ	Original liver oil	Distillation, 200°, 15 min.			Distillation, 250°, 15 min.		
		Distillate	Residue	Total	Distillate	Residue	Total
310	53.2	30.4	25.40	55.8	46.6	12.80	59.4
320	52.4	34.5	19.02	53.5	53.1	9.14	62.2
325	50.8	35.85	15.55	51.4	55.0	7.05	62.1
328	49.0	35.85	13.85	49.7	54.9	6.10	61.0
330	47.5	35.25	12.75	48.0	54.1	5.52	59.6
334	43.5	32.9	10.65	43.6	50.5	4.44	54.9
340	35.85	28.65	7.38	36.0	43.6	2.74	46.3
350	22.9	19.52	3.96	23.5	29.45	1.14	30.6

did not decompose during distillation at 200° for 15 min. Precisely weighed sample of the whale-liver oil was placed in a small dish on the evaporating surface and was distilled for 15 min. at 200° and 250°. After distillation, the absorption spectra of distillate and residue were measured in isopropanol by Beckman spectrophotometer, while the absorption spectrum of the original sample was independently measured. Each extinction coefficient was calculated from the total weight of the sample.

As shown in Table 3, in case of the distillation at 200° and for 15 min., the total extinction coefficient of the distillate and residue at each wave length after distillation was almost equal to that of the original sample, on the other hand the difference clearly appeared in case of the distillation at 250° and for 15 min. Consequently, it was proved that both vitamin A and kitol did not change during the separation process by molecular distillation at 200° and for 15 min. Separated vitamin A and kitol can be determined by colorimetric or spectrophotometric method as usual. Simple colorimetric method to determine kitol will be reported by the author in future.

Summary

It was found that when the whale-liver oil or other fish-liver oils containing kitol in addition to vitamin A was distilled in a semimicro molecular still at 200°, for 15 min., and with the film-thickness of 0.25 mm., all vitamin A ester distilled on the condenser and kitol ester remained in the residue without decomposition.

References

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