[COMMUNICATION NO. 35 FROM THE LABORATORIES OF DISTILLATION PRODUCTS, INC.]

Kitol, a New Provitamin A[1]

BY NORRIS D. EMBREE AND EDGAR M. SHANTZ

Whale liver oil contains considerable quantities of a substance with an absorption band having a maximum at about 290 mμ. Pritchard, *et al.*,[2] prepared a concentrate of a substance having an absorption maximum at 290 mμ, whose antimony trichloride reaction product had absorption bands at 594 and 496 mμ, which was insoluble in 83% alcohol, did not react with anhydrous hydrogen chloride, and had a biological potency of 17,900 I. U. per gram. Willstaedt and Jensen,[3] using chromatographic absorption, prepared a concentrate which had an absorption maximum at 293 mμ, whose antimony trichloride reaction product absorbed at 570 mμ, and which had a biological potency of 531,000 I. U. per gram. These reports, while somewhat contradictory, suggested the presence of a new substance with vitamin A activity. Such a possibility led us to examine whale liver oil for this new substance and for subvitamin A. The latter also has an absorption maximum at 290 mμ but has little or no biological activity.[4]

Fractionation of Whale Oil.—Two hundred grams of whale liver oil, containing 200,000[5] units of vitamin A per gram were saponified and extracted with ether in the usual manner. The unsaponifiable material (60 g.) was dissolved in 300 ml. of ethyl formate and the sterols removed by allowing them to crystallize first at −30° and again at −60°. After removal of the solvent, the sterol-free material was dissolved in corn oil residue[6] to decrease the viscosity and to give a larger volume with which to work. The oil solution was distilled in a laboratory cyclic molecular still by circulating the distilland six times at a distillation column temperature of 150°C. The vitamin A alcohol and other material of comparatively low molecular weight in the unsaponifiable fraction passed into the distillate. The residue contained a substance which had an absorption maximum at 290 mμ and gave, on reaction with antimony trichloride, a reddish color which had an absorption maximum at 428 mμ with minor maxima at 505 and 580 mμ. This substance has other properties to be described later in this article, which are interesting enough to justify giving it a specific name, and we therefore propose the term KITOL from a modification of the Greek word *Ketos* meaning whale.

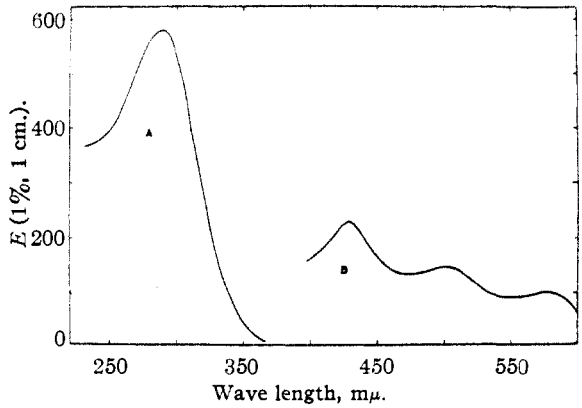


Fig. 1.—Absorption spectra of (A) kitol in ethanol and of (B) the antimony trichloride reaction product of kitol.

The residual oil solution was saponified, and the kitol appeared in the unsaponifiable fraction. This fraction was taken up in petroleum ether (Skellysolve F) and passed into an absorption column of aluminum oxide (after Brockmann). The column was developed with two liters of petroleum ether after which the bands were removed with a spatula and the adsorbed material eluted with ether and alcohol. The adsorbent at the very top of the column contained some sterols and a small amount of what appeared to be oxidized material. The rest of the column below the next fraction (kitol) contained the other substances in the unsaponifiable material, including the last traces of vitamin A which had not been removed by distillation. The kitol fraction was adsorbed twice more after which it seemed to be chromatographically pure. A final concentrate (3.50 g.) was obtained as a viscous, pale oil which at room temperature set to a yellow glassy solid. The preparation had a value of 580 for $E(1\%, 1\text{ cm.})$ (290 mμ), and its antimony trichloride reaction product had values of 228, 162, and 104 for $E(1\%, 1\text{ cm.})$ at 428, 505, and 580 mμ, respectively, for the antimony trichloride product (see Fig. 1). The addition of a small amount of acetone or alcohol greatly inhibits the formation of the antimony trichloride color while it has only a slight effect on the formation of the vitamin A blue color.

Thermal Decomposition of Kitol.—When an oil solution of kitol was subjected to molecular distillation, the kitol began to distill at around 175°. The elimination maximum occurred at 225°. The fractions containing the kitol also contained a substance giving a blue color with antimony trichloride. This color had an absorption maximum at 620 mμ like that of vitamin A. The temperature at which these fractions were collected first suggested that the "620 chromogen" was some vitamin A ester which had contaminated the kitol preparation. On redistillation of the distillates, the bulk of the 620 chromo-

(1) Presented before the Division of Biological Chemistry at the Memphis meeting of the American Chemical Society, April, 1942.

(2) H. Pritchard, H. Wilkinson, J. R. Edisbury and R. A. Morton, *Biochem. J.*, **31**, 258 (1937).

(3) H. Willstaedt and H. B. Jensen, *Nature*, **143**, 474 (1939).

(4) N. D. Embree and E. M. Shantz, THIS JOURNAL, **65**, 906 (1943).

(5) Calculated from a value of 100 for $E(1\%, 1\text{ cm.})$ (328 mμ).

(6) Refined corn oil which had had 30% of its weight removed by molecular distillation in a cyclic molecular still similar to that described by K. C. D. Hickman, *Ind. Eng. Chem.*, **29**, 968 (1937).

gen distilled at around 125°, although some also appeared again in the higher temperature distillates. The 620 chromogen that appeared in the lower temperature distillates could be distilled again at this same temperature. It was found to be formed by the decomposition of the kitol at higher temperature, and for this reason appeared in the same fractions as the kitol distillates.

Concentrates of 620 chromogen were prepared, and it was found that in addition to having the antimony trichloride blue color like vitamin A, it had an absorption band with a maximum at 328 mμ like that of vitamin A. It distributed itself between petroleum ether and 83% alcohol as did vitamin A. On treatment with $N/30$ alcoholic hydrogen chloride, the 620 chromogen was changed into a substance with three absorption bands occurring at 350, 370, and 390 mμ, a reaction paralleling the dehydration of vitamin A.

Since the physicochemical properties of the 620 chromogen were identical with those of vitamin A, a sample of this material was prepared for biological assay in the following way to ensure the absence of any natural vitamin A originally present in the liver oil. The unsaponifiable extract of 200 g. of whale liver oil was dissolved in corn oil and triglyceride constant yield oil.[7] The mixture was circulated several times in a laboratory cyclic molecular still until a temperature of 150° had been reached. The distillate contained nearly all of the vitamin A alcohol originally present, but to ensure complete removal more low-boiling constant yield oil was added and the distillation repeated up to 150°. This distillate contained only a trace of vitamin A, while none could be detected in the residue by the antimony trichloride method. The absorption spectrum of this residue (Fraction BR) was typical of kitol with a value of 25.9 for $E(1\%, 1\text{ cm.})$ (286 mμ). A bioassay of this fraction showed little or no activity (Table I).

More constant yield oil was added to residue fraction BR and this time three fractions were removed: one from 100 to 150°, another from 150 to 200°, and a third from 200 to 250°. The first fraction contained no vitamin A, the second only a trace, while the third fraction contained large amounts of the 620 chromogen along with the unchanged kitol.

Upon redistillation of the high boiling fraction, the 620 chromogen was found to distill entirely below 150°. This distillate (Fraction D-1) gave the typical vitamin A antimony trichloride blue color and had an absorption spectrum with a value of 32.1 for $E(1\%, 1\text{ cm.})$ (328 mμ). A biological assay[8] of the material was then carried out based on the following considerations. An early study[9] showed that the ratio of the biological potency to the value of $E(328\text{ m}\mu)$, or "conversion factor," ranged in value from 680 to 1810 for a large series of fish liver oils and concentrates. Later work[10] showed that many good quality fish liver oils had conversion factors around 2000. In our experience, the latter value is nearly correct for good quality fish liver oils; however, small amounts of vitamin A concentrates which have been subjected to considerable handling will have conversion factors as low as 1000. Fraction D-1 was biologically assayed by Philip L. Harris of these Laboratories and found to contain 42,000 U. S. P. units of vitamin A per gram (Table I). Although this indicates a conversion factor of only 1310, we feel that it shows that the material in Fraction D-1 with the chemical properties of vitamin A is indeed this vitamin. The weight of Fraction D-1 was 19.9% of that Fraction BR. Therefore, unless biologically active material was produced by the distillations, the potency of Fraction BR should be at least 8400 units per gram; the substantially negative assay showed that kitol is not a vitamin but a provitamin.

TABLE I

VITAMIN A ASSAY OF WHALE LIVER OIL FRACTIONS, 7 RATS PER LEVEL, U. S. P. METHOD

	Fraction BR Kitol concentrate $E(286\text{ m}\mu) = 25.9$	Fraction D-1 Pyrolytic vitamin A $E(328\text{ m}\mu) = 32.1$
Daily dose, mg.	0.167	0.028
Weight gain in 28 days	−22.5[a]	+20.4
Units per dose[b]	Much less than 1.17	About 1.17
Units per gram	Much less than 7000	About 42,000

[a] Two rats only, since the other five died before twenty-eight days. [b] A daily dose of 1.17 units (0.687 mg. of U. S. P. reference cod liver oil no. 2) gave a growth of 19.8 g.

The number of molecules of vitamin A formed by the thermal decomposition of each molecule of kitol was found by the following experiments. Six small spirals of glass tubing containing a solution of kitol in corn oil were placed in an oil-bath at 220°. After varying intervals of time, each tube was removed and immediately cooled. An ultraviolet absorption curve was run on each solution to determine the loss of kitol by the diminishing of absorption at 286 mμ. A gram of oil with $E(1\%, 1\text{ cm.})$ (286 mμ) = 1 will contain 3.0×10^{-6} mole of this substance, assuming that the pure material has a molecular weight of 575 (see next section) and a value of 580 for $E(1\%, 1$

(7) A mixture of synthetic triglycerides distilling over any desired temperature range to provide bulk for flushing the small volume of distillate from the condensing surface: J. G. Baxter, E. LeB. Gray and A. O. Tischer, *Ind. Eng. Chem.*, **29**, 1112 (1937).

(8) H. Kringstad and J. Lee, *Tids. Kjemi Bergvesen Met.*, **1**, 82 (1941), mention that certain "concentrates" of whale liver oil upon molecular distillation give rise to a material with an ultraviolet absorption band and an antimony trichloride reaction product like vitamin A, but no biological tests were mentioned.

(9) R. S. Morgan, J. R. Edisbury and R. A. Morton, *Biochem. J.*, **29**, 1645 (1935).

(10) Reviewed by J. B. Wilkie, *Ind. Eng. Chem., Anal. Ed.*, **13**, 209 (1941).

cm.) (286 mμ). The increase in absorption at 328 mμ due to vitamin A could not be accurately measured because it was masked by the stronger absorption of the kitol. Therefore, the vitamin A was measured by determining the value of $E(1\%,$ 1 cm.) (620 mμ) of the antimony trichloride reaction product. The formation of vitamin A will tend to increase the absorption at 286 mμ, however, since the vitamin absorbs in this region, and such increase must be corrected to evaluate the kitol content. Since vitamin A has a molecular weight of 286 and values[11] of 577 and 4800, respectively, for $E(1\%,$ 1 cm.) (286 mμ) and $E(1\%,$ 1 cm.) (620 mμ) ($SbCl_3$ product), a gram of oil with $E(1\%,$ 1 cm.) (620 mμ) = 1 will have a value of 0.12 for $E(1\%,$ 1 cm.) (286 mμ) and will contain 0.73×10^{-6} mole of vitamin A.

Table II shows the loss of kitol and gain of vitamin A calculated by the above relationships from successive values of $E(1\%,$ 1 cm.) (286 mμ) and $E(1\%,$ 1 cm.) (620 mμ). The decomposition of each molecule of kitol is seen to give one molecule of vitamin A.

TABLE II

THERMAL DECOMPOSITION OF KITOL

Time at 220°C., min.	E (620 mμ) ($SbCl_3$ product)	E(286 mμ)	Loss, E(286 mμ) Apparent	Loss, E(286 mμ) Cor.	Mole/g. × 10^{-6} Kitol lost	Mole/g. × 10^{-6} Vit. A gained	Ratio: Moles vit. A to kito
0	0.0	33.0	..	..	...	...	..
1	3.1	32.5	0.5	0.87	2.61	2.26	0.9
2	6.8	32.2	0.8	1.62	4.86	4.97	1.0
3	12.3	31.8	1.2	2.7	8.1	9.0	1.1
4	15.1	31.5	1.5	3.3	9.9	11.0	1.1
8	26.1	30.7	2.3	5.4	16.2	19.1	1.2
16	33.4	30.0	3.0	7.0	21.0	24.4	1.2

Other Properties of Kitol.—The concentrate of kitol with a value of 580 for $E(1\%,$ 1 cm.) (286 mμ) described earlier could not be further enriched by us and was assumed to be pure. It has a molecular weight of 575, found by averaging the values of 550 and 600 found, respectively, by cryoscopic (camphor) and ebullioscopic (carbon tetrachloride) methods.

An inaccurate preliminary pyrolysis experiment had suggested to us that two molecules of vitamin A appeared on the destruction of one molecule of kitol. We therefore postulated the empirical formula $C_{40}H_{58}(OH)_2$ (twice vitamin A, $C_{20}H_{29}OH$) for kitol. More accurate experiments such as that described in Table II have shown that only one molecule of vitamin A comes from each molecule of kitol, but otherwise the empirical formula seems to fit the observed properties quite well. The theoretical molecular weight is, therefore, 572, in good agreement with 575, the value found. The carbon hydrogen analyses (carried out by Carl Tiedcke, New York, N. Y.) of a kitol concentrate agree fairly well with the formula as the following data indicate:

	Required for $C_{40}H_{58}O_2$	Found	
Carbon, %	83.9	83.4	83.55
Hydrogen, %	10.48	10.11	10.26

Hydrogenation experiments with micro equipment[12] showed 7.85 double bonds present (assuming the molecular weight 572). We believe that eight double bonds are present since by the same method we found 4.76 double bonds instead of 5 for crystalline vitamin A and 10.6 instead of 11 for β-carotene.

The suspicion that kitol had two hydroxy groups was confirmed by the preparation of the dinitrobenzoate. Kitol (4.8 g.) was refluxed with 9.8 g. of the acid chloride in ethylene dichloride in the presence of pyridine. After twice recrystallizing, the red crystals had m. p. 200°. The elementary analyses (carried out by L. T. Hallett, Kodak Research Laboratories, Rochester, New York) of this compound are consistent with the suggested formula as the table shows:

Element	Found	Required for $(C_{40}H_{58}O_2)$-$(COC_6H_3)_2(NO_2)_4$
C	67.0	67.5
H	6.71	6.66
N	6.0	5.84
O (by diff.)	20.29	20.0

Kitol has slight optical activity: in chloroform solution a value of −1.35° was found for $(\alpha)^{25°C.}_{546.1\ m\mu}$.

Kitol is somewhat more strongly adsorbed by alumina on a chromatographic column than is vitamin A. This makes it possible to purify the kitol when it is contaminated with small amounts of the vitamin.

On distribution between petroleum ether and 83% alcohol, 75% of the kitol goes into the petroleum ether layer.

On treatment with $N/30$ alcoholic hydrogen chloride, there is no apparent change in the kitol, and the recovered material on pyrolysis yields vitamin A. When the antimony trichloride reaction product of kitol is neutralized, the extract shows an absorption somewhat like that of kitol

(11) J. G. Baxter and C. D. Robeson, THIS JOURNAL, **64**, 2411 (1942).

(12) A. N. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **12**, 705 (1940).

except that a sharper peak and a suggestion of a fine structure is shown (see Fig. 2). This material does not yield vitamin A or anhydro vitamin A on pyrolysis.

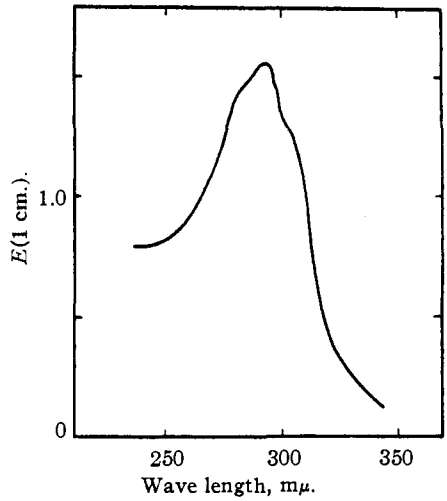


Fig. 2.—Absorption spectrum of material recovered from antimony trichloride reaction product of kitol, about 0.0027% in ethanol.

We have found small amounts of kitol in shark liver oil (commercial product) and in the oil extracted from the liver of a lamb. The liver oil from a northern pike, containing vitamin A_2, was treated by the above-mentioned procedure for concentrating kitol and showed the presence of a substance which had an absorption band at 310 mμ and whose antimony trichloride color showed an absorption band at 510 mμ. This substance on heating gave rise to a material whose antimony trichloride reaction product had an absorption band at 690 mμ. In our opinion, the 690 chromogen is vitamin A_2 and its progenitor should be called kitol$_2$.

Kitol is a provitamin A in the strict sense of the word in that kitol itself has no biological activity but when subjected to a simple physical process is transformed into vitamin A. Due to the fact that vitamin A itself is very unstable to heat, it is likely that this process, if carried out on a large scale, should be performed in a molecular still so that the vitamin A as it is formed can be removed immediately.

Summary

Kitol, a dihydric alcohol with the approximate formula $C_{40}H_{60}O_2$, has been found in considerable quantities in whale liver oil. This substance has little or no biological activity, but is transformed into vitamin A upon heating to temperatures above 200°.

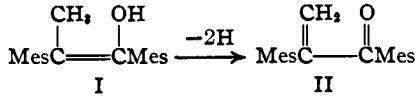
ROCHESTER, N. Y. RECEIVED NOVEMBER 23, 1942

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Vinyl Alcohols. VI.[1] 1,4-Dehydrogenation

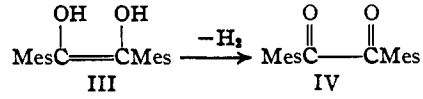
BY REYNOLD C. FUSON AND ROBERT E. FOSTER

A remarkable property of vinyl alcohols is the ability to undergo 1,4-dehydrogenation. Thus certain oxidizing agents convert 1,2-dimesityl-1-propen-1-ol (I) to mesityl α-mesitylvinyl ketone (II).[2] In this reaction the vinyl alcohols may be



I II

likened to enediols, which show a pronounced tendency to give up hydrogen and revert to the corresponding benzils. The oxidation of 1,2-dimesitylacetylene glycol (III) to mesitil (IV)[3] is an example.



III IV

It seemed probable that the ease of 1,4-dehydrogenation of a propenol such as I would prove to depend chiefly on the firmness of attachment of the hydrogen atoms of the methyl group. To test this suggestion it was planned to replace one of these hydrogen atoms by a phenyl group. The resulting vinyl alcohol would possess a benzyl radical in place of the methyl group. The benzyl radical would, of course, lose a hydrogen atom more readily than the methyl group and hence enhance the ease of 1,4-dehydrogenation.

An enol of the type in question was sought in the following way. Benzyl duryl ketone (V) was condensed with benzaldehyde, and the resulting

(1) For the preceding communication in this series see Fuson, Lindsey and Welldon, THIS JOURNAL, 64, 2888 (1942).
(2) Fuson, Byers and Rabjohn, *ibid.*, 63, 2639 (1941).
(3) Fuson and Corse, *ibid.*, 61, 975 (1939).