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Crystalline Vitamin A[1]

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The Holmes and Corbet Crystals

Holmes and Corbet[2] crystallized the vitamin A in ishinagi liver oil from methyl alcohol as yellow needles, melting at 7.5–8°. The authors[3] crystallized the vitamin A in ling cod and other fish liver oils from ethyl formate as yellow prisms, melting at 63–64° (hereafter called 64° crystals) (Fig. 1). Because the Holmes and Corbet crystals had different properties from ours we have prepared and examined specimens of the crystals melting at 7° (hereafter called 7° crystals).[4] This examination indicated that the 7° crystals are not pure vitamin A, but contain methyl alcohol. Crystalline vitamin A melts at 64°. The evidence for these conclusions and certain properties of crystalline vitamin A are given in this paper.

The 7° crystals were prepared from the liver oil of the California jewfish (*Stereolepis gigas*). This oil is similar to the liver oil of the Japanese ishinagi (*Stereolepis ishinagi*), used by Holmes and Corbet. The method of isolation was similar to that of Holmes and Corbet except that the vitamin A esters were concentrated by molecular distillation of the fish liver oil prior to saponification and crystallization from methyl alcohol.

The moist crystals were dried at a temperature gradually rising from −30 to −5° in a system continuously evacuated by a high vacuum condensation pump (limiting vacuum = 0.001 mm.). The rate of drying was determined by measuring the vapor pressure in the system with a Pirani type gage. The pressure decreased steadily during six hours to a value of 0.008 mm., then remained substantially constant for sixteen hours at −5°. The crystals were then examined. They were needle-like in structure (Fig. 2), resembling those of Holmes and Corbet, but melted less sharply at 7–10°.

The following observations were made:

(1) The 7° crystals retained methyl alcohol. This was determined by drying a sample in a stream of nitrogen at 50° and finding the loss in weight. The percentage of alcohol found (10%) corresponded to one mole of methyl alcohol per mole of vitamin A. Since, however, the samples used in the determination were small, this percentage was considered to be only approximate. The 64° crystals lost no weight when they were similarly dried.

(2) The 64 and 7° crystals were interconvertible. When the 7° crystals were cooled in ethyl formate solution, 64° crystals separated. When a solution of the 64° crystals in methyl alcohol was cooled 7° crystals were deposited.

(3) The 64 and 7° crystals gave the same crystalline vitamin A acetate (m. p. 57–58°) and β-naphthoate (m. p. 74–75°). Also, the extinction coefficient of the 7° crystals ($E^{1\%}_{1cm.}$ 328 mμ = 1720, corrected for methyl alcohol present) was nearly the same as that of the 64° crystals ($E^{1\%}_{1cm.}$ 328 mμ = 1750).

It was concluded from these data that the 64° crystals represent crystalline vitamin A. The 7° crystals, which appeared to be similar to those made by Holmes and Corbet, were vitamin A containing methyl alcohol.

Evidence was noted indicating that the methyl alcohol in the 7° crystals was not merely entrained: (1) Most of the alcohol in the moist 7° crystals, as previously mentioned, was removed readily under high vacuum but the last 10% could not be removed in this way. (2) The 7° crystals had a different crystalline structure than crystalline vitamin A (when the latter was crystallized from methyl alcohol) and they separated from solution more rapidly and in greater yield (see next section). (3) Specimens of the 7° crystals prepared independently by Holmes and Corbet,[2] by Mead[5] and by us melted at substantially the same temperature, and hence probably contained similar quantities of methyl alcohol, although they were crystallized and dried by different procedures. It seems improbable that this would have occurred if the methyl alcohol had merely been held by entrainment.

The question as to whether the 7° crystals represent a true molecular compound containing

(1) Presented in part before the Division of Biological Chemistry of the American Chemical Society, Atlantic City meeting, September, 1941.

(2) Holmes and Corbet, THIS JOURNAL, 59, 2042 (1937).

(3) Baxter and Robeson, *Science*, 92, 203 (1940).

(4) At the Atlantic City meeting the 64 and 7° crystals were identified as α- and β-vitamin A. This nomenclature has been discarded.

(5) Mead, *Biochem. J.*, 33, 589 (1939).

TABLE I

CRYSTALLIZATION OF VITAMIN A CONCENTRATE ($E^{1\%}_{1cm.}$ 328 mμ = 1550) FROM METHYL ALCOHOL, ETHYL FORMATE, PROPYLENE OXIDE AND PETROLEUM ETHER (B. P. 30–65°)

Concentration of vitamin A (g./100 cc. solution) = 20%.

Crystn.	Solvent	Seeded with cryst.	Type of crystal separating	Crystn. temp., in deg.	% yield of vit. A[a]
1	MeOH	Vit. A	Vit. A	−35	18
2	MeOH	Vit. A (MeOH)	Vit. A (MeOH)	−35	62
3	MeOH	Vit. A (MeOH) or no seeding	Vit. A (MeOH)	−70	81
4	Et. Form.	Vit. A, vit. A (Et. Form.[b]) or no seeding	Vit. A	−35	40
5	Et. Form.	Vit. A	Vit. A[c]	−70	74
6	Et. Form.	Vit. A (Et. Form.) or no seeding	Vit. A (Et. Form.)	−70	60
7	Prop. ox.	Vit. A	No crystn.	−35	..
8	Prop. ox.	No seeding	Vit. A	−70	20
9	Pet. eth.	No seeding	Vit. A	−35	20
10	Pet. eth.	No seeding	Vit. A	−70	45

[a] Yield figures obtained after evaporating solvent from crystals in stream of nitrogen at 50°. [b] These seed crystals dissolved. [c] The solution was crystallized, but not filtered, at −35 and −55° before being crystallized and filtered at −70°.

fixed proportions of vitamin A and methyl alcohol was not examined.

Crystallization of Vitamin A.—In the further study of the affinity of vitamin A for various solvents, a rich concentrate ($E^{1\%}_{1cm.}$ 328 mμ = 1550) was crystallized from methyl alcohol, ethyl formate, petroleum ether (b. p. 30–65°), and propylene oxide, at temperatures of approximately −35 and −70°. The solutions were usually seeded to prevent supersaturation and were allowed to crystallize for as long as two weeks, in certain cases, to attain equilibrium. The nature of the crystals formed and the yields obtained are shown in Table I.

These experiments indicated that vitamin A has an affinity for ethyl formate as well as for methyl alcohol. In the table the notations "vitamin A (MeOH)" and "vitamin A (Et Form.)" mean that the designated crystals contained methyl alcohol and ethyl formate which could not be removed completely under high vacuum without melting the crystals. The notation does not imply that a molecular compound existed nor that a molecular proportion of solvent was retained by the crystals.

Vitamin A (MeOH) separated in Crystallizations (Crystns.) 2 and 3, *i. e.*, when a 20% solution of a vitamin A concentrate in methyl alcohol was cooled to −35° and seeded with vitamin A (MeOH) crystals, or cooled to −70° without seeding. In Crystn. 1, vitamin A separated. Thus, either vitamin A or vitamin A (MeOH) could be crystallized from methyl alcohol.

Vitamin A (Et Form.) crystallized from ethyl formate at −70° (Crystn. 6) unless the solution was cooled gradually to −70° in the presence of vitamin A crystals. Then vitamin A crystallized (Crystn. 5). Only vitamin A could be crystallized from ethyl formate at −35° (Crystn. 4).

Since vitamin A (Et Form.), like vitamin A (MeOH), separated from solution more rapidly than vitamin A, concentrates from distilled and saponified fish liver oils were first crystallized in this form at −70° to remove the bulk of the impurities in the non-saponifiable matter. Vitamin A was then obtained by recrystallizing the concentrate from ethyl formate at −35°.

Vitamin A (Et Form.) was only briefly examined. Even after filtration under pressure the preparations contained as much as 80% adsorbed and entrained ethyl formate. After fifteen hours of continuous evacuation at −35° (final vapor pressure = 0.009 mm.) one sample melted at −4 to 2° and contained 12% ethyl formate. Another preparation contained 10% ethyl formate and melted at 7–10°. The percentages are again only approximate.

Solvent-free vitamin A crystallized from propylene oxide or petroleum ether (Crystns. 7–10). Thus, it appears that only the more polar solvents have an affinity for vitamin A. The hydroxyl group of the vitamin is evidently responsible for this property because esters of vitamin A, such as the acetate, crystallized from methyl alcohol or ethyl formate without solvent of crystallization.

Fig. 1.—Crystalline vitamin A, m. p. 63–64°, × 14. (Photomicrographs, Figs. 1 and 2, courtesy of R. P. Loveland, Eastman Kodak Company, Research Laboratories.)

Fig. 2.—Crystals of vitamin A(MeOH), m. p. 7–10°, ×48.

Properties of Crystalline Vitamin A

Extinction Coefficient at 328 mμ and 622 mμ.—Nine recent preparations of crystalline vitamin A have had an average extinction coefficient at the absorption maximum of 1750 ± 21 in absolute ethyl alcohol[6] (unless otherwise stated all extinction coefficients reported in this paper were determined in this solvent). One of these preparations had an extinction coefficient at the absorption maximum of 1780 (average of 3 determinations) (Fig. 3). Dr. F. P. Zscheile and R. L. Henry of Purdue University kindly assayed this latter preparation and also obtained the value 1780 at the maximum. They have recently reported spectrographic data for other preparations of crystalline vitamin A.[7]

The spectrographic measurements did not support the value of $E^{1\%}_{1cm.}$ 325 mμ = 1880 for pure vitamin A, suggested as the probable value by Morton.[8]

The position of the absorption maximum of vitamin A varied from 326–328 mμ in ethyl alcohol with our instrument. Zscheile and Henry, using an instrument capable of greater precision, found the maximum in ethyl alcohol to be at 324 mμ. Thus, the wave length now assigned to the vitamin A maximum (328 mμ) may be incorrect. It has, however, seemed advisable to determine the extinction coefficients reported in this paper at the absorption maximum and to report them as at 328 mμ unless the maximum lay outside the range 326–328 mμ.

In petroleum ether (b. p. 30–65°) and ethyl formate the average extinction coefficients of vitamin A at 328 mμ were 1760 and 1715, respectively. These values were not considered significantly different from that in ethyl alcohol. In cyclohexane and chloroform the extinction coefficients were 1550 and 1260 at 328 mμ and 333 mμ, respectively. These values seemed to be significantly lower than the value in ethyl alcohol.

We believe that certain solvents such as cyclohexane are able to repress the absorption of vitamin A. The suggestion of Adamson and Evers[9] that the extinction coefficient of vitamin A is higher in ethyl alcohol than in cyclohexane because complex formation occurs in the former does not seem probable in view of the high extinction coefficient of vitamin A in petroleum ether.

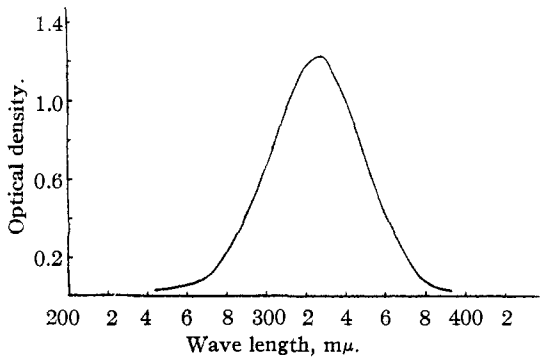

Fig. 3.—Spectrophotometric curve of crystalline vitamin A in ethyl alcohol ($E_1^{1\%}_{cm.}$ 328 mμ = 1780).

The extinction coefficient of solutions of vitamin A in ethyl alcohol was unchanged after two days of storage in amber bottles at room temperature. Thus, we were unable to confirm the observation of Darby, quoted by Holmes and Corbet.[2] that the extinction coefficient of highly purified vitamin A preparations rapidly decreases in ethyl alcohol solution. The solutions in ethyl alcohol were stable also to radiation from the spark used with the spectrograph even when exposures of 160 sec., four times the normal exposure, were given. Zscheile and Henry reported a similar finding.[7] However, the extinction coefficient of solutions of vitamin A in cyclohexane and chloroform did decrease from 2 to 6% during storage for twenty-four hours at room temperature.

The extinction coefficient of the antimony trichloride blue color of vitamin A at 622 mμ was 4800. This is the average of five determinations on vitamin A with $E^{1\%}_{1cm.}$ 328 mμ = 1750 and agrees well with the value 4700 previously reported[8] from measurements on other vitamin A preparations. In a typical determination 1 cc. of a vitamin A solution in chloroform (0.001576 g./100 cc.) was treated with 10 cc. of a chloroform solution of antimony trichloride (saturated at 20°). The percentage transmission at 622 mμ of the transitory blue color was measured within three seconds with a Hardy recording spectrophotometer.[10]

From our work the value $E^{1\%}_{1cm.}$ 617 mμ = 6000, suggested as being probable by Morton,[8] appears to be too high.

(6) Mr. G. Wait and assistants of this Laboratory made the spectrographic measurements using a Hilger quartz spectrograph, model E-498, with a Spekker ultraviolet photometer. The light source was a tungsten–steel spark.

(7) Zscheile and Henry, *Ind. Eng. Chem., Anal. Ed.*, **14**, 422 (1942).

(8) Morton, *Ann. Rev. Biochem.*, **XI**, 368 (1942).

(9) Adamson and Evers, *Analyst*, **66**, 106 (1941).

(10) We are indebted to Mr. E. Richardson, Eastman Kodak Company Research Laboratories, for measuring the absorption spectra.

The "L" Value.—Vitamin A can be assayed rapidly by measuring the intensity of its antimony trichloride blue color with certain photoelectric colorimeters, such as the Evelyn. This instrument uses sharply filtered rather than monochromatic light. Therefore, the results are expressed in terms of a quantity L rather than E. L is analogous to E, the extinction, and the L value ($L^{1\%}_{1cm.}$ 622 mμ) is analogous to $E^{1\%}_{1cm.}$ 622 mμ. Dann and Evelyn[11] found that L was proportional to the vitamin A concentration for galvanometer readings (G) between 20 and 70. Thus, in this range the L value was nearly constant. Koehn and Sherman,[12] however, found that L was not proportional to the vitamin A concentration over this range and the L value was consequently not constant. In these experiments pure vitamin A was not used. Both groups of workers used the non-saponifiable matter from fish liver oils, possibly containing oxidized vitamin A.

The relation between L and the vitamin A concentration is of practical as well as theoretical interest. For this reason the L value of crystalline vitamin A was measured at concentrations corresponding to galvanometer readings from 20–70 (Table IIa). The L value of crystalline vitamin A acetate was measured in the same way to determine the effect of esterification on the L value of vitamin A (Table IIb).

TABLE II

VARIATION OF THE L VALUE ($L^{1\%}_{1cm.}$ 622 mμ) WITH CONCENTRATION OF (A) VITAMIN A AND (B) VITAMIN A ACETATE

Concn. (g./100 cc. × 10^4)	Galvanometer reading, average, cor.	$L^{1\%}_{1cm.}$ 622 mμ
a. Vitamin A ($E^{1\%}_{1cm.}$ 328 mμ = 1740)		
1.73	74^2	3880
2.60	64^0	3920
3.46	55^2	3875
4.33	47^3	3895
5.19	41^3	3840
6.06	36^2	3800
6.93	31^0	3860
7.79	28^0	3730
8.66	24^2	3705
9.35	21^3	3725
10.39	18^1	3735
b. Vitamin A acetate ($E^{1\%}_{1cm.}$ 328 mμ = 1470)		
1.63	77^1	3600
2.61	66^2	3565
3.26	59^2	3635
4.89	46^2	3565
5.22	44^2	3540
6.52	36^0	3575
8.15	28^2	3510
9.78	22^3	3455
11.41	17^3	3455
13.04	14^2	3375

The procedure was similar to that of Dann and Evelyn. Samples of vitamin A (0.0433 g.) and vitamin A acetate (0.0815 g.) were dissolved in chloroform (50 cc.) to prepare stock solutions. From 1–9 cc. of these stock solutions were further diluted to give solutions with the desired vitamin A concentration. Portions of these solutions (1 cc.) were treated with 10 cc. of a solution of antimony trichloride in chloroform, saturated at 20°. Duplicate readings were taken at each dilution (maximum deviation = 1 (G) unit) and the values of G were averaged.

A linear relation did not exist between L and the concentration of vitamin A or vitamin A acetate for galvanometer readings between 20 and 70. A nearly linear relation did exist between G = 30–70. In this range vitamin A ($E^{1\%}_{1cm.}$ 328 mμ = 1740) had an average L value of 3865 ± 39. Vitamin A acetate ($E^{1\%}_{1cm.}$ 328 mμ = 1470) had an average L value of 3570 ± 40.

From the standard deviations it was calculated that 21 out of 22 observations of the L value of vitamin A or vitamin A acetate would lie within ± 2.2% of the average value, for galvanometer readings taken between G = 30–70. This error was doubled if the range G = 20–70 was used.

Our purest vitamin A ($E^{1\%}_{1cm.}$ 328 mμ = 1780) had an L value of 3990. The equivalent L value of vitamin A calculated from the L value of vitamin A acetate was 4090. It thus appears that the L value of vitamin A is about 4000 and that the blue color of one ester of vitamin A had a greater equivalent absorption than vitamin A itself.

The following relations were determined between the extinction coefficients of vitamin A ($E^{1\%}_{1cm.}$ 328 mμ = 1740) and vitamin A acetate ($E^{1\%}_{1cm.}$ 328 mμ = 1470) and the average L values

Vitamin A: $E^{1\%}_{1cm.}$ 328 mμ = L value × (0.45 ± 0.005).

Vitamin A acetate: $E^{1\%}_{1cm.}$ 328 mμ = L value × (0.41 ± 0.005)

The value for the conversion factor of vitamin A lies between the value of 0.41 ± 0.05 reported by Dann and Evelyn and the value 0.50 ± 0.01 reported by McFarland and Sutherland.[13]

Other Physical and Chemical Constants.—The *molecular weight* of vitamin A, determined

(11) Dann and Evelyn, *Biochem. J.*, **32**, 1008 (1938).

(12) Koehn and Sherman, *J. Biol. Chem.*, **132**, 527 (1940).

(13) McFarland and Sutherland, *Can. J. Research*, **16**, 421 (1938).

by a micro modification of the Menzies–Wright ebullioscopic method in ethyl alcohol, was 263.[14] The calculated value for $C_{20}H_{30}O$ was 286. The *index of refraction* was $n^{22}D$ 1.6410. In this determination the refractive indices of 20–70% solutions of vitamin A in refined mineral oil were measured with an Abbe refractometer. These indices when plotted against the vitamin A concentration gave a straight line which could be accurately extrapolated to 100% vitamin A. The *acetyl value*, determined by the method of West, Hoaglund and Curtis,[15] was 149. The calculated value was 150 mg. CH_3CO per gram. The *iodine value* determined by the method of von Mikusch and Frazier[16] was 390. The theoretical value was 444. A 500% excess of 0.4 *N* Hanus solution was used for a reaction period of three hours at 0°.[17] The *elimination maximum* was 125°, one degree below that of the reference dye, Celanthrene Red, added to the solution before distillation. Glyceride constant yield oil was used.[18]

Biological Potency

Crystalline vitamin A was found to have a provisional biological potency of 4,300,000 U. S. P. XI units per gram. This value is about 30% higher than that suggested as being probable by Morton.[8] The factor for converting the average extinction coefficient at 328 mμ of the preparations bioassayed to U. S. P. XI units was 2460.

This conversion factor of 2460 is higher than the value of 2000 widely accepted as the conversion factor of vitamin A in fish liver oils. If confirmed, it means that fish liver oils contain substances (or a substance) absorbing at 328 mμ which are either biologically less active than crystalline vitamin A or are biologically inactive.

Baxter, *et al.*,[19] have reported evidence tending to show that these substances may have vitamin A activity. However, we have not been successful in separating them from vitamin A by chromatographic adsorption. Robinson[20] has suggested that these substances absorbing at 328 mμ are oxidized vitamin A which has no biological activity. His experimental results, however, do not appear to establish this explanation. A significant discrepancy did not appear between spectrographic and biological assays made on his oxidized vitamin A preparations until the 328 mμ band of vitamin A had been badly deformed and shifted toward the ultraviolet.

The value of 4,300,000 U. S. P. XI units per gram for crystalline vitamin A was obtained as the average of eight biological assays (a total of 160 rats used) made by Dr. P. L. Harris of this Laboratory. They will be reported in detail elsewhere. Vitamin A was also assayed by H. J. Cannon of the Laboratory of Vitamin Technology, Chicago. From his results an average biological potency of 4,700,000 U. S. P. XI units per gram was calculated. For the 7° crystals Harris found a biological potency of 3,900,000 U. S. P. XI units per gram, corrected for the methyl alcohol present. Only two assays were performed, however, so the biological potency was not established as accurately as for vitamin A itself.

Experimental

Preparation of Crystalline Vitamin A

A. Distillation of Fish Liver Oils.—Cryst. vitamin A was prepared from shark liver ($E^{1\%}_{1cm.}$ 328 mμ = 100), ling cod liver ($E^{1\%}_{1cm.}$ 328 mμ = 129), California jewfish liver ($E^{1\%}_{1cm.}$ 328 mμ = 317) and halibut viscera ($E^{1\%}_{1cm.}$ 328 mμ = 72). oils.

A cyclic molecular still, similar to that described by Hickman[18] was used. The vitamin A esters distilled from 180–220° at a pressure of 0.003 mm. The distillates were light yellow oils without fishy taste or odor. Fractions with an extinction coefficient at 328 mμ of 400 or greater were combined for saponification. From 50–70% of the initial vitamin A was obtained at this potency.

B. Saponification.—A vitamin A ester concentrate (67 g., $E^{1\%}_{1cm.}$ 328 mμ = 539) from distilled ling cod liver oil was saturated with nitrogen by bubbling commercial gas through it for ten minutes. Then 190 cc. of 2 *N* alcoholic potassium hydroxide, similarly saturated with nitrogen, was added. Saponification was effected by refluxing the mixture for thirty minutes in an atmosphere of nitrogen. The mixture was then diluted with freshly boiled and cooled water and extracted with reagent grade ethyl ether. The extract was washed with 10% aqueous potassium carbonate, with water, and dried. The ether was distilled under reduced pressure.

The free vitamin A concentrate (28.6 g., $E^{1\%}_{1cm.}$ 328 mμ = 1260) was a red viscous oil which could be directly crystallized from ethyl formate. However, vitamin A of greater purity was obtained by first distilling the saponified concentrate.

C. Redistillation.—The saponified concentrate from (B) was dissolved in two volumes of corn oil residue and

(14) We wish to thank Dr. L. T. Hallett, Eastman Kodak Company Research Laboratories, for the microanalysis.

(15) West, Hoaglund, and Curtis, *J. Biol. Chem.*, **104**, 627 (1934).

(16) von Mikusch and Frazier, *Ind. Eng. Chem., Anal. Ed.*, **13**, 782 (1941).

(17) The determination was kindly done by Mr. E. S. Barnitz of this Laboratory.

(18) Hickman, *Ind. Eng. Chem.*, **29**, 968 (1937); Embree, *ibid.*, **29**, 975 (1937).

(19) Baxter, Harris, Hickman and Robeson, *J. Biol. Chem.*, **141**, 991 (1941).

(20) Robinson, *Biochem. J.*, **32**, 807 (1938).

distilled in a cyclic molecular still. Fractions were taken at 10°-intervals from 90–160° with two cycles at each temperature. Vitamin A distilled principally from 105–135° as an odorless, orange oil. The distillate was viscous and the condenser wall had to be warmed to cause the distillate to flow into the receiver. Fractions with an extinction coefficient of 1400 or greater were combined for crystallization (17.5 g., $E^{1\%}_{1cm.}$ 328 mμ = 1440).

D. Crystallization of Vitamin A (Ethyl Formate).—The vitamin A concentrate from (C) was dissolved in ethyl formate (70 cc.), cooled to −35° for eighteen hours, seeded with cholesterol and then cooled to −55° for eight hours. The solids which separated (1.1 g.) were filtered in a Büchner funnel cooled with dry-ice. The filtrate deposited no solids when cooled at −70° for eighteen hours so the flask was scratched at intervals to promote crystallization. In three hours crystallization began and was complete in three days. The crystals were filtered and washed in a modified Büchner funnel, cooled with dry-ice (see Filtration and Drying). Much entrained solvent was pressed out with dental dam. After distillation of ethyl formate under reduced pressure a vitamin A concentrate was obtained (9.2 g., $E^{1\%}_{1cm.}$ 328 mμ = 1670).

E. Crystalline Vitamin A.—The vitamin A concentrate from (D) was dissolved in ethyl formate (40 cc.) and stored at −35° for eighteen hours during which time the flask was repeatedly scratched. Vitamin A began to separate in large prismatic crystals during this period and crystallization was allowed to continue for an additional twenty-four hours. The crystals were filtered in the modified Büchner funnel and dried.

The first crop of crystals weighed 1.67 g. By concentrating the filtrate and seeding it at −35° an additional 1.06 g. was obtained. The yield was further increased by 1.32 g. by cooling the filtrate from the second crop to −70° for five days and recrystallizing the vitamin A at −35°. The average $E^{1\%}_{1cm.}$ 328 mμ value of the three crops of crystals was 1720. Thus, the yield of crystalline vitamin A was approximately 15% of that present in the fish liver oil. After two crystallizations from ethyl formate vitamin A melted at 63–64° and had $E^{1\%}_{1cm.}$ 328 mμ = 1750.

Anal. Calcd. for $C_{20}H_{30}O$: C, 83.84; H, 10.56. Found: C, 83.90, 83.70; H, 10.30, 10.40.[14]

An alternative crystallization procedure gave an over-all yield of 25%. The solution of (D) in ethyl formate (40 cc.) was crystallized at −35, −55, and finally at −70°. The crystals were not filtered until crystallization was completed at −70°. Under these conditions vitamin A and not vitamin A (ethyl formate) separated. The extinction coefficient of the crystals at 328 mμ was 1710 but more small crystals with inferior keeping quality were present than when the other procedure was used.

Vitamin A was sealed in glass ampoules, under vacuum, and stored at −35°. Under these conditions the extinction coefficient and the biological potency were unchanged after four to six months.

Filtration and Drying.—A modified Büchner funnel was constructed to filter vitamin A under nitrogen pressure. A circular steel ring, 0.5" wide, was provided for the mouth of the funnel (75 mm. inside diameter). This was held in place by tie rods connected to a similar steel ring fitted to the throat of the funnel. A steel cover was provided with a hose connection to admit nitrogen. A pressure-tight union between ring and cover was obtained with a rubber gasket and six bolts attached to the periphery of the metal ring. These bolts fitted through holes in the cover. Excess solvent in the crystals was removed by pressure from a piece of dental dam fitted between gasket and cover. The funnel was cooled by a removable insulated jacket filled with dry-ice.

To dry the crystals the cover was tightened and the funnel was evacuated by connecting its outlet to an oil pump. Evacuation for fifteen to twenty hours at a temperature gradually rising from −60 to 5° was usually necessary. Filtrations and drying were done in a cold room at 5°.

Refrigeration.—Three temperatures were used most frequently, −35, −55, and −70°. An ice-cream cooling cabinet maintained a temperature of −35° ± 5°. A wooden box insulated with cork, and cooled with dry-ice served to keep a temperature near −55°. Vessels were cooled to −70 ± 10° by packing them in powdered dry-ice and storing them in the insulated cabinet.

Solvents.—Eastman Kodak Co. ethyl formate was filtered through anhydrous potassium carbonate to remove formic acid and was then fractionally distilled. Solvents such as propylene oxide, petroleum ether (b. p. 30–65°), and methyl alcohol were Eastman grade and were used without further purification.

Summary

Crystalline vitamin A has been isolated from shark liver, ling cod liver, California jewfish liver and halibut viscera oils as yellow prisms, melting at 63–64°. Evidence was obtained indicating that the crystalline vitamin A of Holmes and Corbet (m. p. 7.5–8°) contained methyl alcohol.

Crystalline vitamin A had a provisional biological potency of 4,300,000 U. S. P. XI units per gram. Its conversion factor, 2460, was higher than the conversion factor of vitamin A in fish liver oils.

The extinction coefficient of crystalline vitamin A at 328 mμ was 1780. The extinction coefficient of the vitamin A antimony trichloride blue color at 622 mμ was 4800. The L value ($L^{1\%}_{1cm.}$ 622 mμ), measured with an Evelyn photoelectric colorimeter, was 3990. L was a nearly linear function of the vitamin A concentration for galvanometer readings (G) between 30 and 70.

The molecular weight, the elimination maximum, the index of refraction, the acetyl value, and the iodine value of crystalline vitamin A were determined. These confirmed the formula for vitamin A proposed by Karrer.

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