posed to be in non-crystalline regions of the polymers. Furthermore, the total polar group concentration changes gradually and continuously in a given series. Hence, the percentage taken up should not be so sensitive a function of composition as I, the spacing in the crystallites. This is confirmed by the data of Table I or by comparison of Figs. 16 and 17.

Acknowledgment.—We are grateful to Dr. B. S. Biggs and Mr. R. H. Erickson, who prepared many of the polymers, especially to Mr. N. R. Pape, who gave valuable assistance in the X-ray studies, and to Mr. J. H. Heiss, Jr., for the determinations of Young's modulus and moisture sorption.

Summary

Thirty-one linear polyamides and copolyamides of varying crystal structures and concentrations of polar linkages along the chains have been studied as fibers and as polycrystalline sections by X-ray diffraction. Also, the elastic modulus and moisture sorption were determined on typical samples of the polymers.

The series represents a range of solid polymers from soft to porcelain-like in properties, with several-fold variation in Young's modulus. The polar linkages which join the paraffin sections of the base units together in the long chains associate in adjacent macromolecules to form hydrogen-bonded dipole layers. This interaction is supposed to govern the physical properties of the solids. By this concept it was possible to interpret systematically the melting points, hardness, elastic modulus and moisture sorption of the solids in terms of the concentration, separation, population and perfection of the dipole layers. Disorder introduced by copolymerization in which dipoles were shifted randomly along the chains altered the average dipole layer separations and also replaced polar groups in the layers by hydrocarbon chain sections. Such disorder caused marked softening of the solids. It likewise caused the X-ray identity periods along the chains to vary with composition of the copolyamides in a novel fashion. Periods were found in the copolymers both larger and smaller than those which arise from any simple polyamides made from the same base units.

Macromolecular solids containing some crystalline regularity may apparently be treated as defect systems in which, nevertheless, relatively simple factors such as the position and organization of interacting polar groups govern physical properties.

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[COMMUNICATION NO. 38 FROM THE LABORATORIES OF DISTILLATION PRODUCTS, INC.]

Crystalline Aliphatic Esters of Vitamin A[1]

BY JAMES G. BAXTER AND CHARLES D. ROBESON

This Laboratory is engaged in the preparation of the pure growth promoting factors in fish liver oils. As part of this program three crystalline aliphatic esters of vitamin A have been prepared: vitamin A acetate, vitamin A palmitate, and divitamin A succinate. Since in fish liver oils vitamin A is esterified with aliphatic acids, such as palmitic,[2] these crystalline esters are suitable for the study of vitamin A as it occurs naturally. Vitamin A β-naphthoate, a previously prepared crystalline ester,[3] was also made to compare it with the aliphatic esters. It had the undesirable property that the aromatic nucleus contributed extraneous absorption at 328 mμ.

This paper is concerned with these properties of the esters: (a) their resistance to atmospheric oxidation, (b) their extinction coefficients at 328 mμ and of their antimony trichloride blue colors at 620 mμ, (c) their biological potency compared with that of crystalline vitamin A. • Part (c) is a preliminary report of bioassays made by Dr. P. L. Harris of this Laboratory.

The esters were made by esterifying crystalline vitamin A[4] with the appropriate acid halide. The yields of ester were sharply reduced when potent vitamin A concentrates ($E^{1\%}_{1cm.}$ 328 mμ = 1200 or greater) were esterified instead of crystalline vitamin A.

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

(2) Tischer, *J. Biol. Chem.*, **125**, 475 (1938).

(3) Hamano, *Sci. Pap. Inst. phys. chem. Res.*, Tokyo, **28**, 69 (1935); Mead, *Biochem. J.*, **33**, 589 (1939).

(4) Baxter and Robeson, THIS JOURNAL, **64**, 2411 (1942).

Resistance to Atmospheric Oxidation.—In crystalline form, vitamin A acetate was the most stable of the esters prepared. The stability test consisted of exposing thin layers of the crystals to air at 5° in darkness. The percentage decomposition at various times was determined by the percentage drop in the extinction coefficient at 328 mμ (Table I).

TABLE I

RELATIVE RESISTANCE OF CRYSTALLINE VITAMIN A ESTERS TO ATMOSPHERIC OXIDATION AT 5°

Vitamin A	% of initial $E^{1\%}_{1\,cm.}$ (328 mμ) value after weeks 1	2	7	16
Acetate			97	88
β-Naphthoate			95	40
Palmitate	89	73		
Succinate	84	68		

The superior stability of vitamin A acetate may be partly due to the crystal size (Fig. 1).[5] The large prisms expose a smaller area to the action of air, per unit amount of vitamin A, than do the smaller prismatic crystals of di-vitamin A succinate or the plate-like crystals of vitamin A palmitate and vitamin A β-naphthoate (Fig. 2, 3, 4). The excellent stability of vitamin A β-naphthoate cannot be attributed to the size of its crystals. Possibly they have less tendency to adsorb air than vitamin A palmitate and succinate.

All the esters could be stored in evacuated, sealed glass tubes at −35° for long periods of time without change in the extinction coefficient. Thus, the extinction coefficient of vitamin A palmitate at 328 mμ was unchanged after nine months of storage under these conditions. The tubes were evacuated with a high vacuum condensation pump for four hours before they were sealed off. This removed most of the air adsorbed on the surface of the crystals.

Extinction Coefficients at 328 and 620 mμ.—The extinction coefficients of the esters at 328 mμ, in ethyl alcohol, are given in Table 2A.[6] The extinction coefficient of the vitamin A obtained by saponifying the esters is also compared with the equivalent extinction coefficient of vitamin A calculated from the coefficients of the esters. It appears that the equivalent absorption of vitamin A at 328 mμ is slightly depressed in the fatty acid esters. A marked depression in the equivalent absorption of vitamin A was observed in di-vitamin A succinate and a smaller one was observed in vitamin A β-naphthoate.

The extinction coefficient and melting point obtained for vitamin A β-naphthoate ($E^{1\%}_{1\,cm.}$ 328 mμ = 1090, m. p. 74–75°) were lower than the values reported by Mead ($E^{1\%}_{1\,cm.}$ 328 mμ = 1180, m. p. 78°).[3] Our value for the melting point agreed more closely with the value of 76° determined by Hamano. However, through the kindness of Dr. Mead we were able to examine a sample of his vitamin A β-naphthoate. We found an $E^{1\%}_{1\,cm.}$ (328 mμ) value of 1000 and a melting point of 74–75°. Hence, the β-naphthoate crystals made in the two laboratories appear to be equally pure. Probably the values of the constants were not the same because the experimental technique was different.

The extinction coefficients at 620 mμ of the antimony trichloride blue colors of the esters are given in Table 2B.[7] The equivalent extinc-

TABLE II

EXTINCTION COEFFICIENTS OF (A) THE VITAMIN A ESTERS AT 328 mμ, IN ETHYL ALCOHOL, (B) THE ANTIMONY TRICHLORIDE BLUE COLORS OF THE VITAMIN A ESTERS AT 620 mμ

Vitamin A	$E^{1\%}_{1\,cm.}$ (328 mμ)	Equiv. $E^{1\%}_{1\,cm.}$ (328 mμ) calcd. for vit. A	$E^{1\%}_{1\,cm.}$ (328 mμ) for vit. A by saponification of esters[a]
	(A)		
Ester:			
Acetate	1510	1730	1710
Palmitate	940	1720	1700
Succinate	1240	1420	1700
β-Naphthoate	1090	1640[b]	1700
Alcohol:	1780[4]		
Vitamin A	$E^{1\%}_{1\,cm.}$ (620 mμ)	Equiv. $E^{1\%}_{1\,cm.}$ (620 mμ) calcd. for vit. A	
	(B)		
Ester:			
Acetate	4580	5250	
Palmitate	2535	4640	
β-Naphthoate	2940	4520	
Succinate	4450	5090	
Alcohol:	4800[4]		

[a] Amber glassware was used during saponification and ether extraction to prevent decomposition of vitamin A by light. The estimations were kindly done by H. Rawlings of this Laboratory. [b] In calculating this from $E^{1\%}_{1\,cm.}$ (328 mμ) for vitamin A β-naphthoate, a correction was needed for the absorption of the naphthoate radical at 328 mμ. The correction used was the molecular extinction coefficient of ethyl β-naphthoate at 328 mμ. This we found to be 1058.

(5) Photomicrographs, Figs. 1, 2, 3, 4, courtesy of Mr. R. P. Loveland, Eastman Kodak Company, Research Laboratories.

(6) Spectrographic measurements were made with a Hilger quartz spectrograph, model E-498, with a Spekker ultraviolet photometer. The light source was a tungsten-steel spark.

(7) We are indebted to Mr. E. Richardson, Eastman Kodak Company, Research Laboratories for measuring the absorption spectra. A Hardy recording visual spectrophotometer was used.

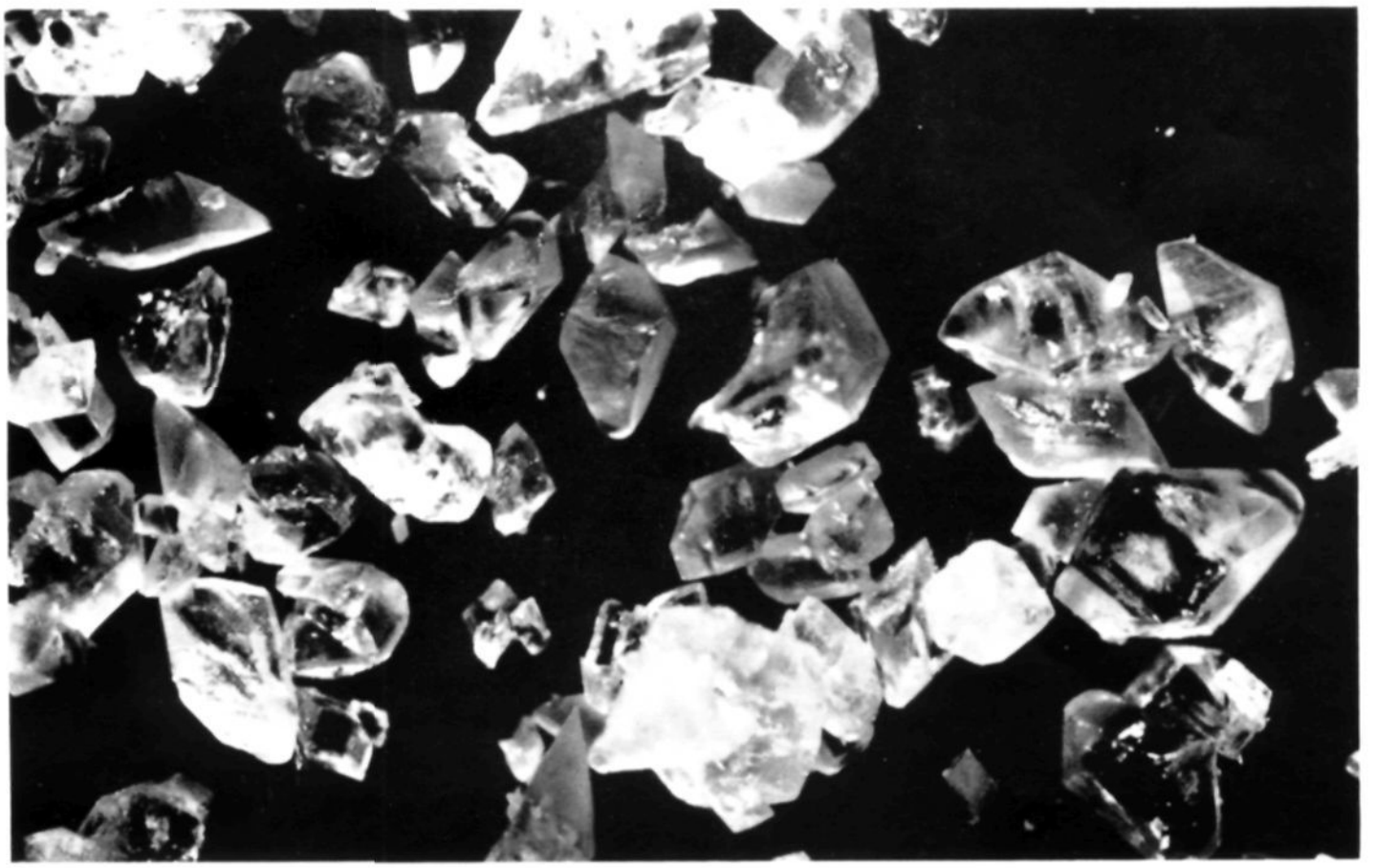

Fig. 1.—Vitamin A acetate, 30×.

Fig. 2.—Di-vitamin A succinate, 30×.

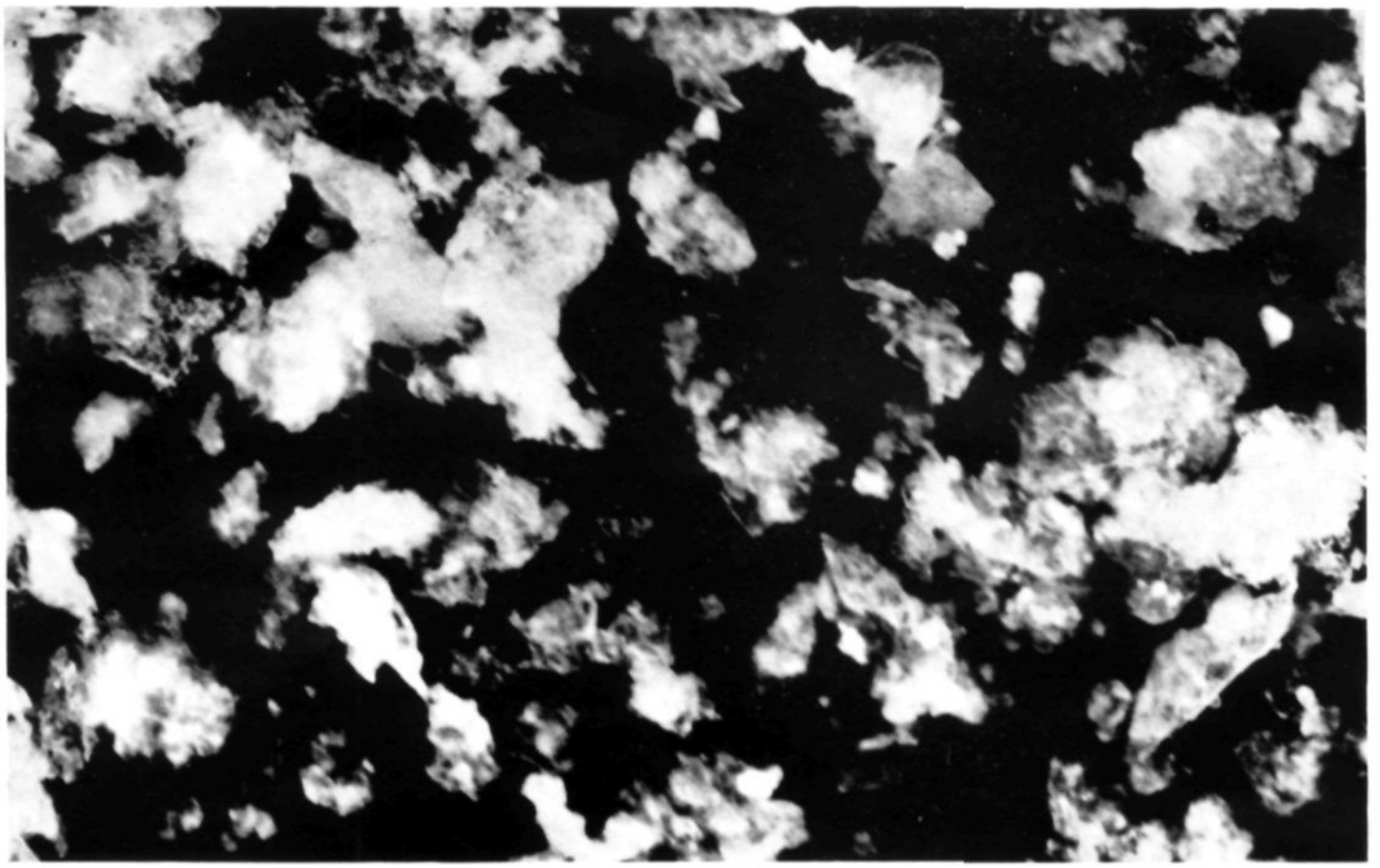

Fig. 3.—Vitamin A palmitate, 40×.

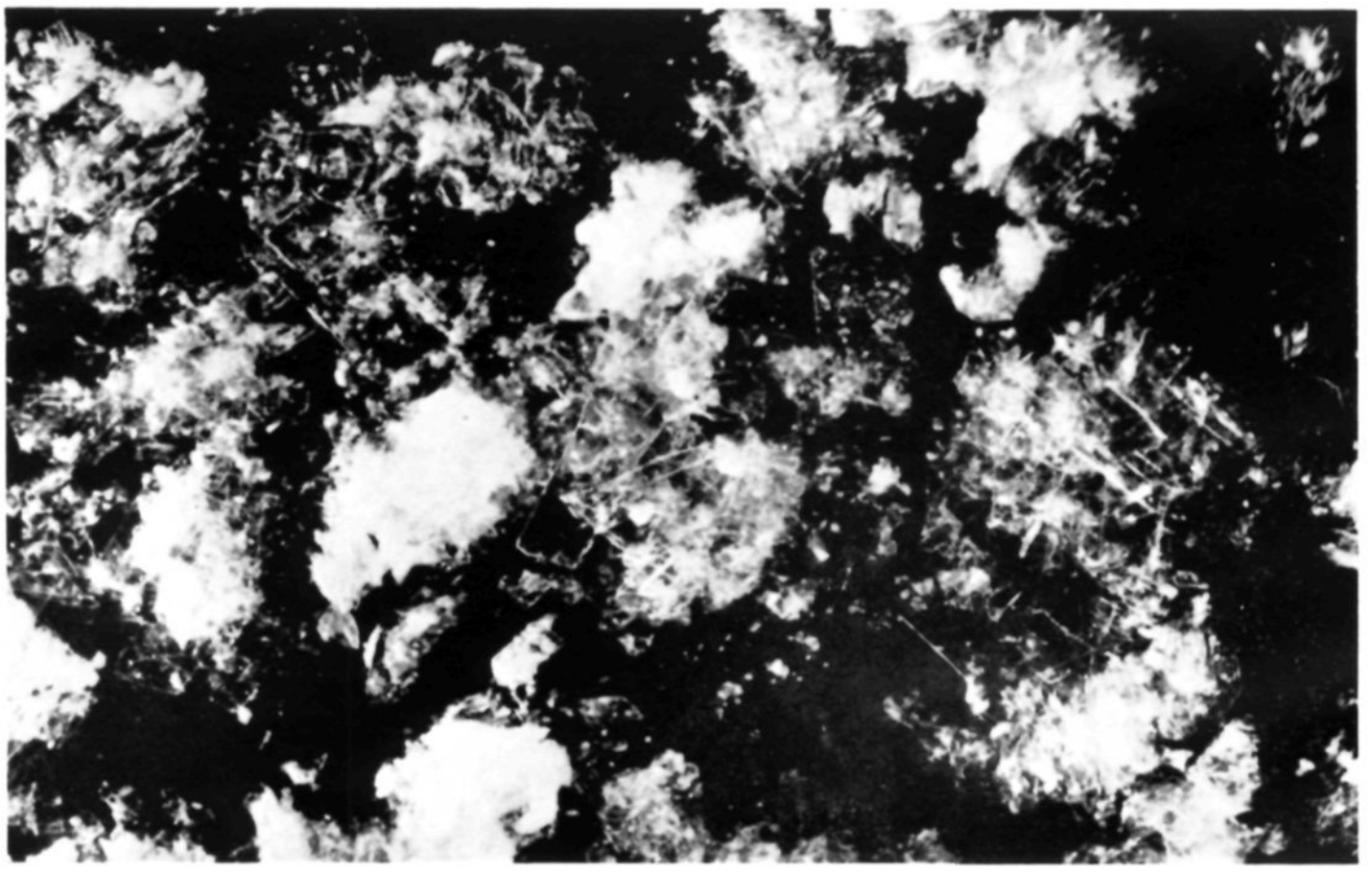

Fig. 4.—Vitamin A β-naphthoate, 24×.

tion coefficients of vitamin A calculated from the extinction coefficients of the esters are also reported. On an equivalent basis vitamin A acetate had a greater $E^{1\%}_{1\,cm.}$ (620 mμ) value than the other vitamin A esters prepared. The equivalent extinction coefficient of the acetate at 620 mμ was also greater than that of free vitamin A.

The method used to determine the $E^{1\%}_{1\,cm.}$ (620 mμ) values has been described.[4]

Biological Potency.—The crystalline aliphatic esters of vitamin A had substantially the same equivalent biological potency as crystalline vitamin A (Table III). The conversion factors of the esters (biological potency/$E^{1\%}_{1\,cm.}$ 328 mμ) were also substantially the same as that of vitamin A. The high conversion factor of di-vitamin A succinate was partly due to the low equivalent extinction coefficient of vitamin A in this ester. The factor for vitamin A β-naphthoate was significantly higher than that of the aliphatic esters. The reason for this is not yet clear.

This finding that certain aliphatic esters of vitamin A have the same equivalent biological potency as vitamin A agrees with the work of Braude, *et al.*[8] These workers found that a natural vitamin A ester concentrate prepared by the molecular distillation of a fish liver oil had the same conversion factor as a vitamin A concentrate prepared by the molecular distillation of a saponified fish liver oil. However, the conversion factor of the vitamin A esters in undistilled fish liver oils has usually been greater than that of the free vitamin A prepared from the oils by saponification.[9] This difference was probably due to the presence of impurities in the oils which absorbed at 328 mμ but which had lower biological potencies than vitamin A. Baxter and Robeson[4] and Braude, *et al.*,[8] review what is known of these substances.

At present it is difficult to compare conversion factors measured in different laboratories because the bioassay procedures are not sufficiently uniform. These procedures may not give the same potency for a pure vitamin A compound. Thus, the biological potency of vitamin A β-naphthoate determined in this Laboratory (four assays gave a mean of 3,440,000 U. S. P. XI units per g.) was over 50% higher than the value found by Underhill and Coward (2,225,000 I. U. per g.).[10] It is in establishing such uniform bioassay procedures in different laboratories that we believe the new crystalline esters should be most useful.

TABLE III

COMPARISON OF BIOLOGICAL POTENCY OF VITAMIN A ESTERS AND VITAMIN A

Vitamin A	No. assays	Total no. of rats	Mean biological potency (U. S. P. XI units/g. × 10^{-6})	Equiv. biological potency of vitamin A (U. S. P. XI units/g. × 10^{-6})	Conversion factor: biological potency / $E^{1\%}_{1\,cm.}$ (328 mμ)
Acetate	9	90	3.52	4.04 ± 0.23	2350 ± 133
Palmitate	8	80	2.31	4.23 ± .31	2520 ± 183
Succinate	1	24	3.14	3.59	2630
β-Naphthoate	4	40	3.44	5.29 ± .24	3160 ± 145
Alcohol	8	160	4.30[a]	4.30 ± .39	2460 ± 227

The biological potencies assigned to the vitamin A esters in this paper are provisional values which may be changed slightly in the forthcoming paper by Harris in which the assays are described in detail. H. J. Cannon of the Laboratory of Vitamin Technology, Chicago, independently performed biological assays on solutions of the crystalline compounds (except di-vitamin A succinate) in refined cottonseed oil. These solutions were identified only by code numbers. The biological potencies calculated from his results lay, in each case, within the standard deviations found by Harris.

Experimental

Vitamin A Acetate.—Crystalline vitamin A (4.5 g., 0.016 mole) was dissolved in a mixture of ethylene chloride (25 cc.) and pyridine (5 cc.) and cooled to 10°. A solution of acetyl chloride (1.4 g., 0.018 mole) in ethylene chloride (25 cc.) was slowly added. After standing for two hours at room temperature, protected from light, the mixture was poured into 0.5 *N* aqueous sulfuric acid and extracted with ether. The extract was successively washed with 0.5 *N* sulfuric acid, water, 10% potassium carbonate solution, and water. After distillation of ether under reduced pressure, the residue (5.0 g.) was crystallized from methyl alcohol (50 cc.) at 5°. Recrystallization gave pale yellow prismatic crystals of vitamin A acetate which were filtered and dried under suction in a funnel of the type previously described.[4] The yield was 75% of the theoretical. The acetate melted at 57–58° and had an elimination maximum of 132.5° from petroleum constant yield oil compared with 121° for the standard reference dye, Celanthrene Red.

Anal. Calcd. for $C_{22}H_{32}O_2$: C, 80.48; H, 9.76. Found: C, 80.34; H, 9.86.

Vitamin A Palmitate.—Vitamin A (3.5 g., 0.012 mole) was dissolved in a mixture of ethylene chloride (35 cc.) and pyridine (5.0 cc.) and cooled to −15°. Palmityl chloride (3.7 g., 0.013 mole) was added slowly with shaking. After standing for three hours at room temperature, the reaction mixture was extracted as for vita-

(8) Braude, Foot, Henry, Kon, Thompson and Mead, *Biochem. J.*, **35**, 693 (1941).

(9) Emmett and Bird, *J. Biol. Chem.*, **119**, xxxi (1937); Hickman, *J. Biol. Chem.*, **128**, xliii (1939); Moll and Reid, *Hoppe-Seyl. Z.*, **260**, 9 (1939).

(10) Underhill and Coward, *Biochem. J.*, **33**, 594 (1939).

min A acetate, except that after the carbonate washes the ether solution was washed three times with 0.5 *N* sodium hydroxide. After removal of ether the palmitate was obtained as a yellow oil (6.02 g.). This oil was dissolved in petroleum ether (100 cc., b. p. 30–65°) and extracted five times with 83% ethyl alcohol to remove unesterified vitamin A. The recovered palmitate (5.1 g., $E^{1\%}_{1\ cm.}$ 328 mμ = 861) still contained traces of palmitic acid (acid value = 5). This was removed by crystallization from propylene oxide (165 cc.) at −30° for two hours. The acid was filtered (0.19 g.) and the filtrate was cooled slowly, by lagging to −30°. In eighteen hours vitamin A palmitate crystallized as thin yellow plates which were filtered and dried under vacuum. The yield (3.1 g.) was 48% of the theoretical. After recrystallization from propylene oxide the palmitate had m. p. 27–28°. Its elimination maximum from glyceride constant yield oil was 213° (Celanthrene Red 126°).

Anal. Calcd. for $C_{36}H_{60}O_2$: C, 82.45; H, 11.45. Found: C, 82.11; H, 11.52.

The preparation of crystalline vitamin A palmitate was troublesome because the ester frequently separated in amorphous form. This form appeared to be more soluble in propylene oxide than the crystals and separated from solution more slowly. Decomposition of vitamin A during esterification did not cause the formation of amorphous palmitate. It separated frequently from crude preparations with high $E^{1\%}_{1\ cm.}$ (328 mμ) values.

To prevent the formation of amorphous palmitate certain changes in the preparative method were made. Pyridine was replaced by quinoline; ethylene chloride was replaced by chloroform. The esterification was performed in a system from which oxygen was rigorously excluded. The solutions were both rapidly cooled and slowly cooled during the crystallization step. However, these changes were ineffective.

It was then thought that the amorphous ester might be a geometrical isomer of the crystalline ester. Therefore, catalysts known to isomerize carotenoids were employed. Solutions of the amorphous ester in propylene oxide were treated with traces of iodine and organic bases. The solutions were exposed to sunlight. Unsuccessful attempts were then made to crystallize the ester samples. At present the experimental conditions necessary to ensure the preparation of crystalline palmitate are not known.

Di-vitamin A Succinate.—Vitamin A (2 g., 0.007 mole) was dissolved in a mixture of ethylene chloride (10 cc.) and pyridine (2 cc.) and cooled to 0°. A solution of succinyl chloride (0.55 g., 0.0035 mole) in ethylene chloride (10 cc.) was slowly added, with shaking, and the mixture was allowed to stand one hour at room temperature. The extraction was performed as for vitamin A acetate.

The crude product, a red oil (2.4 g.) was crystallized from ethyl formate (10 cc.), then recrystallized from ethyl formate (15 cc.), at −35°. The crystals were yellow prisms, m. p. 76–77°. The yield was 46% of the theoretical. The elimination maximum was above 250° from glyceride constant yield oil.

Anal. Calcd. for $C_{44}H_{62}O_4$: C, 80.73; H. 9.48. Found: C, 80.26; H, 9.56.

Vitamin A β-Naphthoate.—This was obtained by a procedure which gave a better yield than that reported by Mead.[8] Vitamin A (4.5 g., 0.016 mole) was dissolved in ethylene dichloride (25 cc.) and pyridine (5 cc.). β-Naphthoyl chloride (m. p. 51–52°, 3 g., 0.016 mole) in ethylene dichloride (25 cc.) was added at 25° and the mixture was allowed to stand for four hours at room temperature. The ester was extracted as for vitamin A palmitate. It consisted of a viscous yellow oil (7 g.). This was crystallized from absolute ethyl alcohol (150 cc.) at 5° (yield 58% of theoretical). The ester consisted of delicate, yellow plates which melted at 73–74°. After one recrystallization the m. p. was 74–75°. Repeated crystallizations did not raise the m. p. further.

Anal. Calcd. for $C_{31}H_{36}O_2$: C, 84.53; H, 8.18. Found: C, 84.90; H, 8.13.

Summary

1. Three crystalline esters of vitamin A have been prepared by the esterification of crystalline vitamin A. These esters are: vitamin A acetate, vitamin A palmitate, and di-vitamin A succinate.

2. The extinction coefficients of the esters at 328 mμ and of the antimony trichloride blue colors at 620 mμ have been determined.

3. The biological potency of the esters, adjusted for differences in the molecular weights, was the same as that of crystalline vitamin A.

4. Vitamin A acetate was the most resistant of the crystalline preparations to atmospheric oxidation. It appears to be the most stable crystalline ester of vitamin A yet prepared.

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