The rearrangement undoubtedly takes place through an intermediate O-acetyl base, and is much more rapid in the Ψ-ephedrine derivative than in the one having the configuration of ephedrine. The speed of rearrangement is affected by the hydrogen ion concentration, and is practically instantaneous at a sufficiently high *p*H.

WASHINGTON, D. C. RECEIVED AUGUST 26, 1946

[COMMUNICATION NO. 91 FROM THE LABORATORIES OF DISTILLATION PRODUCTS, INC.]

Neovitamin A[1]

BY CHARLES D. ROBESON AND JAMES G. BAXTER

It was previously reported that a portion of the vitamin A in the unsaponifiable matter of fish liver oils could not be crystallized.[2] This so-called "noncrystallizable vitamin A" has been further studied and from it has been isolated in crystalline form a previously unrecognized geometric isomer of vitamin A, which we shall call "neovitamin A." Our work indicates that neovitamin A differs from vitamin A only in the spacial configuration about the double bond nearest the hydroxyl group.

The new isomer constitutes about 35% of the total vitamin present in a number of the common fish liver oils, so that its physical and chemical properties, and especially its biological potency, are of commercial as well as theoretical interest. This paper is concerned with these properties, with the method of isolation, and with the structure of the newly recognized vitamin. A preliminary report on the work has already been published.[3]

Isolation of Crystalline Neovitamin A.—Some of the preliminary steps in the isolation work have previously been described.[4] The source material was shark liver oil. This was distilled in a cyclic molecular still to concentrate the neovitamin A and vitamin A esters. The nonsaponifiable matter was prepared from the concentrate and redistilled to further concentrate the two vitamins. The major amount of the vitamin A was separated from this distillate by crystallization from ethyl formate at −70°. After removal of solvent from the filtrate an orange oil was obtained, from which substantially all the remaining vitamin A was removed by selective adsorption. Finally, the neovitamin A concentrate was esterified with phenylazobenzoyl chloride and the ester crystallized. This ester (m. p. 94–96°, Fig. 1) melted higher than the corresponding vitamin A ester (m. p. 79–80°, Fig. 2).

Saponification of the ester yielded neovitamin A as an oil which crystallized from a solution in ethyl formate at −35° as pale yellow needles (m. p. 58–60°). The crystals (Fig. 3) were markedly different in appearance than the yellow prisms of vitamin A (m. p. 62–64°, Fig. 4) and a mixed melting point determination showed a depression.

The crystalline anthraquinone β-carboxylate esters of neovitamin A and vitamin A were prepared and found to differ. The neo ester was red (m. p. 134–136°) while the vitamin A derivative was yellow (m. p. 121–122°). Hamano[5] and Mead[6] obtained red crystals (m. p. 118°) and yellow crystals (m. p. 123–124°) which they considered to be polymorphic forms of the same compound. It now seems probable that their red crystals, which melted 16–18° lower than ours, were a mixture of the neovitamin A and vitamin A esters, while their yellow crystals were pure vitamin A anthraquinonecarboxylate.

Physical and Chemical Properties of Neovitamin A

Ultraviolet Absorption Spectrum.—Neovitamin A has an absorption curve similar in shape to that of vitamin A but the position of the maximum (328 mμ) is slightly different from that of vitamin A (324–5 mμ, Fig. 5). An average value of $E^{1\%}_{1\,cm.}$ (328 mμ) = 1645 for neovitamin A was found (5 preparations).[7]

Infrared Spectrum.—The infrared transmission spectrum of neovitamin A is compared with that of vitamin A in Fig. 6. The curves are almost identical, with slight differences occurring in the position of the bands at 9.25 and 8.00 microns.[8]

Atmospheric Oxidation.—Neovitamin A was slightly more resistant to atmospheric oxidation than vitamin A when dissolved in refined cottonseed oil at a concentration of 20,000 U. S. P. units per gram. The palmitic esters of the two compounds were equally stable in refined cottonseed oil at a concentration of 90,000 units per gram. These conclusions were based on data obtained by uniformly exposing the oil solutions to air at 55° in an oven. A rocking device and glass rocker tubes provided, respectively, the agitation

(1) Presented in part at the Atlantic City Meeting of the American Chemical Society, Atlantic City, New Jersey, April 11, 1946.

(2) J. G. Baxter, P. L. Harris, K. C. D. Hickman and C. D. Robeson, *J. Biol. Chem.*, **141**, 991 (1941).

(3) C. D. Robeson and J. G. Baxter, *Nature*, **155**, 300 (1945).

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

(5) S. Hamano, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, **32**, 44 (1937).

(6) T. H. Mead, *Biochem. J.*, **33**, 589 (1939).

(7) Mr. G. Wait and assistants of this Laboratory made the measurements using a Beckman spectrophotometer.

(8) The infrared transmission spectra were obtained by Dr. S. F. Kapff of this Laboratory, using a Perkin-Elmer infrared spectrometer, Model 12-A.

Fig. 1.—Neovitamin A *p*-phenylazobenzoate, × 15.[a]

Fig. 2.—Vitamin A *p*-phenylazobenzoate, × 15.

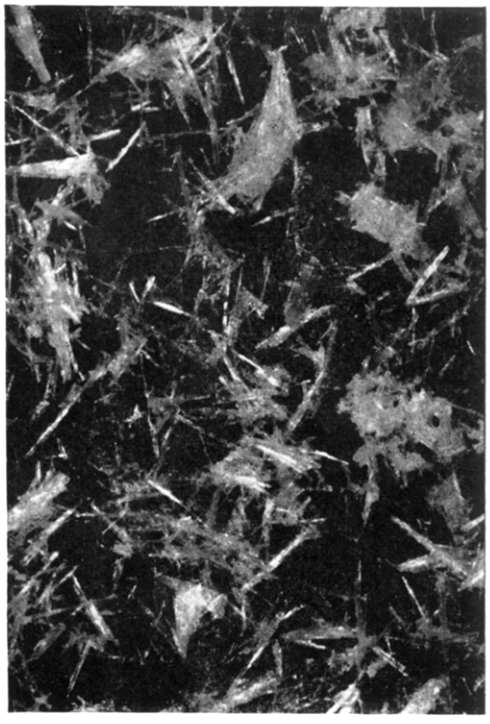

Fig. 3.—Neovitamin A, × 15.

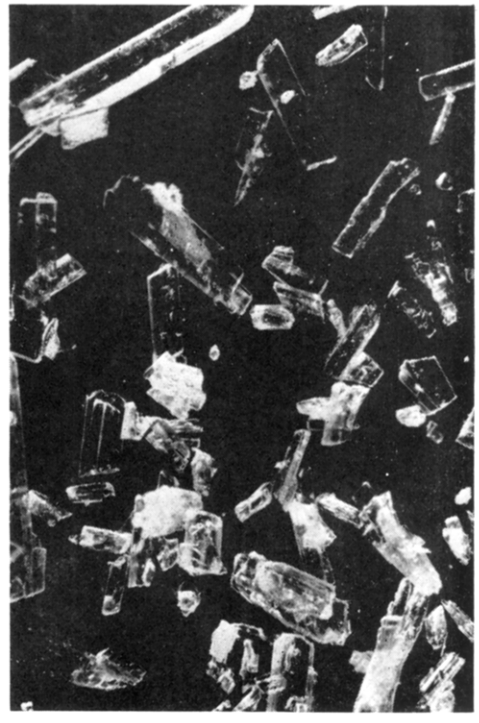

Fig. 4.—Vitamin A, × 10.

[a] Photomicrographs, Figs. 1, 2 3 and 4, courtesy of R. P. Loveland, Eastman Kodak Company, Research Laboratories.

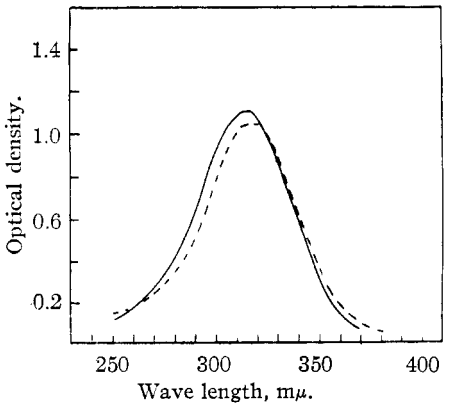

Fig. 5.—Spectrophotometric curves of: neovitamin A, ----($E_{1\,cm.}^{1\%}$ 328 mμ = 1645) and vitamin A, —($E_{1\,cm.}^{1\%}$ 325 mμ = 1750).

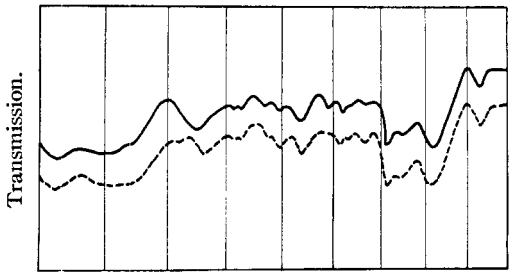

Fig. 6.—Infrared transmission curves of neovitamin A, - - - -, and vitamin A, —. The percentage transmission is not given as the ordinate because this axis was shifted to permit comparison of the curves. These were, however, plotted from percentage transmission data.

and the containers for the samples. At suitable intervals the potencies were determined by the antimony trichloride method (Table I).

TABLE I

STABILITY OF VITAMIN A AND NEOVITAMIN A

	Sample	% of original potency after exposure to air at 55° for: 12 hours	20 hours
1	Solution of vitamin A in refined cottonseed oil (20,000 U. S. P. units/gram)	79	58
2	Solution of neovitamin A in refined cottonseed oil (20,000 U. S. P. units/gram)	87	71
3	Solution of vitamin A palmitate in refined cottonseed oil (90,000, U. S. P. units/gram)	92	72
4	Solution of neovitamin A palmitate in refined cottonseed oil (90,000 U. S. P. units/gram)	92	75

Dehydration.—Treatment of neovitamin A with a solution of hydrochloric acid in ethanol yielded the anhydro compound which appeared to be identical with anhydro vitamin A.[9] The melting points of the two crystalline compounds (76–77°) were the same and no melting point depression was observed for a mixture. Furthermore, the ultraviolet absorption curves were identical.

The rate of dehydration for the new isomer was found to be considerably slower than that for vitamin A (Fig. 7). The reaction was followed by measurement of the increased absorption at 392 mμ due to the formation of anhydro vitamin A.

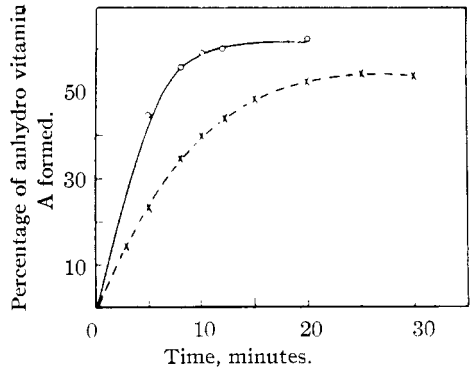

Fig. 7.—Dehydration at 25° of neovitamin A, - - - -, and vitamin A, —, with alcoholic hydrochloric acid.

At one time the neovitamin A content of fish liver oils was determined by a procedure based on the differing rates of dehydration of the two isomers. This has since been discarded in favor of a method employing maleic anhydride.

Reaction with Maleic Anhydride.—Maleic anhydride reacts relatively rapidly with the conjugated double bonds in vitamin A to form an adduct[10] which gives no blue color with antimony trichloride. Neovitamin A and its esters react at a much slower rate. For example, after reaction of neovitamin A palmitate with maleic anhydride in benzene solution for sixteen hours approximately 90% of the vitamin was recovered unchanged as measured by the blue color reaction. The recovery of vitamin A palmitate under the same conditions was only 5% (Fig. 8).

This reaction formed the basis of a method of assaying neovitamin A in fish liver oils and concentrates. A sample of the oil or concentrate was treated with maleic anhydride in benzene for sixteen hours at 25°. The percentage recovery of vitamin was then determined. From this value and the known values for pure neovitamin A and vitamin A palmitates under the same conditions, the percentage of each isomer in the sample could be calculated. Alternatively, but less conveniently, the assay could be performed on the unsaponifiable matter from the sample, using neovitamin A and vitamin A as standards.

(9) E. M. Shantz, J. D. Cawley and N. D. Embree, THIS JOURNAL, **65**, 901 (1943).

(10) K. Kawakami, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, **26**, 77 (1935).

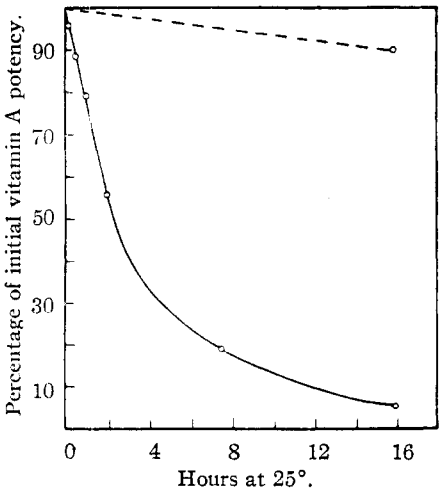

Fig. 8.—Reaction of neovitamin A palmitate, - - - -, and vitamin A palmitate, —, with maleic anhydride.

It is evident from the data in Table II that neovitamin A constitutes from 30 to 40% of the total vitamin A in a large number of fish liver oils.

TABLE II

OCCURRENCE OF NEOVITAMIN A IN FISH LIVER OILS AND DISTILLATES

	Sample	Percentage of total vitamin A as neo isomer
1	U. S. P. Reference Cod Liver Oil no. 3	39
2	Distilled vitamin A concentrates	39, 35
3	Distillate from dogfish liver oil	39
4	Dogfish liver oil	36
5	Soupfin shark liver oil	37
6	Distillate from soupfin shark liver oil	33
7	Unsaponifiable matter from soupfin shark liver oil distillate	34
8	Halibut liver oil	32
9	California jewfish liver oil	34

Isomerization.—Chemical interconversion of neovitamin A and vitamin A was accomplished through the anthraquinonecarboxylate esters by treatment with a trace of iodine in benzene solution. Assays by the maleic anhydride method showed that neovitamin A anthraquinonecarboxylate was converted to the extent of about 70% to the corresponding vitamin A ester in two hours at 25°. The pure vitamin A ester was prepared from the mixture by crystallization and identified by its melting point.

Under the same conditions vitamin A anthraquinonecarboxylate was converted to the neo ester in approximately a 30% yield. Pure neovitamin A ester could not be obtained from the mixture, however, due to co-crystallization of the two forms. Chemical agents appeared to form isomerization mixtures consisting predominantly of the vitamin A ester regardless of which isomer was used.

Biological Properties

The biological potency of neovitamin A is of interest because the recent work of Deuel and coworkers[11] has shown that the provitamin A activity of carotenoids is influenced to a considerable degree by their stereochemical configuration. Preliminary biological assays on neovitamin A, carried out by the Biological Department of this Laboratory, have shown that its potency is substantially the same as that of vitamin A. The assays were done by the standard U. S. P. growth method modified to include three dosage levels for the standard as well as for the test substances. The assay results will be reported in detail elsewhere.

This equivalence appears to be due to interconversion *in vivo* of the isomers as shown by the following experiment. Equal amounts of neovitamin A and vitamin A (158,000 units) were fed over a period of two weeks to rats previously depleted of the vitamin. The animals were then sacrificed and the oil recovered from the livers. Assays showed that 82% of the vitamin found in the livers of the rats receiving neovitamin A was present as vitamin A. However, only 11% of the vitamin found in the livers of the animals receiving vitamin A was found to be in the neo form. Thus, the rat was able to convert neovitamin A to vitamin A, which explains why the two vitamins have similar biological potencies.

The small proportion of neovitamin A in the mixed vitamin A's of rat liver oil may be due to a lack of need for the neo isomer by the animal which forthwith converts it to vitamin A.

Structure

The stereochemistry of the carotenoids has been clarified greatly by the work of Pauling,[12] and Zechmeister and co-workers.[13,14] According to their views, the natural carotenoids usually have a *trans*-configuration about each double bond. A *cis*-configuration is considered to be possible at certain double bonds which are denoted as being "stereochemically effective." In vitamin A only the double bonds numbered *1* and *2* (Fig. 9) are considered stereochemically effective, so that only four geometrical isomers can be expected to exist—the *trans–trans*, *trans–cis*,

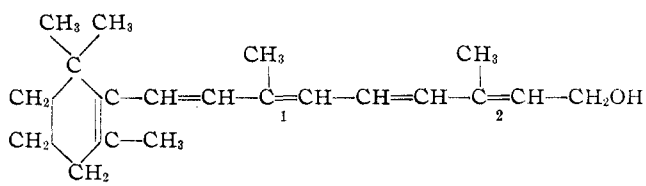

Fig. 9.—Stereochemically effective double bonds in vitamin A.

(11) H. J. Deuel, Jr., E. R. Meserve, A. Sandoval and L. Zechmeister, *Arch. Biochem.*, **10**, 491 (1946).

(12) L. Pauling, *Fortschr. Chem. Organ. Naturstoffe*, **3**, 203 (1939).

(13) L. Zechmeister, A. L. LeRosen, F. W. Went and L. Pauling, *Proc. Nat. Acad. Sci.*, **27**, 468 (1941).

(14) L. Zechmeister, *Chem. Rev.*, **34**, 267 (1944).

cis–trans, and *cis–cis*. The problem is to determine which configuration represents neovitamin A and which vitamin A.

The difference in wave length of 3 mμ between the absorption maxima of vitamin A and neovitamin A indicates, from the work of Zechmeister and co-workers, that the two compounds differ in the configuration of the methyl group and hydrogenation about only one double bond. The first clue as to the position of this double bond was the finding that on dehydration with alcoholic hydrochloric acid, vitamin A and neovitamin A give anhydro vitamin A but at different rates. Since the best evidence indicates that anhydro vitamin A is formed from vitamin A by the elimination of water from the hydroxyl group and an adjacent hydrogen atom (9), it was concluded that the isomers differ in configuration only at double bond *2*.

The available evidence, based on analogy, favors a *cis-* structure for neovitamin A and a *trans-* structure for vitamin A about this double bond. This evidence is summarized in Table III.

TABLE III

EVIDENCE FOR *cis-* STRUCTURE FOR NEOVITAMIN A

Property	Usual behavior of *cis-* isomer	Behavior of: Neovitamin A	Vitamin A
Melting point	Lower than *trans-*[a]	58–60°	62–64°
Extinction coefficient at absorption maximum	Lower than *trans-*[b]	1645 (328 mμ)	1740 (325 mμ)
Addition of maleic anhydride	Slower than *trans-*[c]	Slow	Rapid

[a] Ref. 14. [b] H. P. Koch, *Chem. Ind.*, 273–275 (1942). [c] R. S. Morrell, *et al.*, *Trans. Faraday Soc.*, **38**, 362–366 (1942).

The *trans* structure for vitamin A is further supported by the fact that the catalytic interconversion studies previously described indicate that it is the more stable form of the two isomers. In general it has been found[14] that iodine catalysis of carotenoid compounds produces a preponderant amount of the more stable all *trans* form.

It is of interest that the absorption maximum of neovitamin A is located at a longer wave length than that for vitamin A since among the carotenoids, *cis* isomers have so far been found to absorb at shorter wave lengths than the corresponding all *trans* compounds. We have no explanation for this apparent anomaly at present.

No unequivocal evidence is available to establish whether the configuration about double bond *1* is *trans* or *cis* but we shall tentatively assume that it is *trans* in both isomers because this is the common configuration for the naturally occurring carotenoids.

We thus tentatively assign to neovitamin A and vitamin A the *trans–cis*, and *trans–trans* configurations, respectively, about double bonds *1* and *2*.

Experimental Part

Isolation of Crystalline Neovitamin A

Preparation of Mixed Vitamin A Concentrate.—A concentrate of vitamin A and neovitamin A was prepared by the method previously described for making crystalline vitamin A.[4] A shark liver oil (1000 g., $E^{1\%}_{1\,cm.}$ (327 mμ) = 130) was distilled in a cyclic molecular still giving fractions distilling from 180–230° at 0.003 mm. which contained the mixed vitamin A and neovitamin A esters in concentrated form.

The most potent fractions were combined and the resulting concentrate (99 g., $E^{1\%}_{1\,cm.}$ (327 mμ) = 547) was saponified with 2 *N* alcoholic potassium hydroxide. The unsaponifiable fraction (41 g., $E^{1\%}_{1\,cm.}$ (327 mμ) = 1260) was dissolved in two volumes of corn oil residue and distilled. The fractions distilling from 105–135° constituted a mixed vitamin A concentrate (22.3 g., $E^{1\%}_{1\,cm.}$ (327 mμ) = 1500).

Concentration of Neovitamin A.—The concentrate was dissolved in ethyl formate (200 cc.) and cooled to −55° for fifteen hours. The solids which separated (0.5 g.) were filtered in a cooled Büchner funnel and the filtrate was cooled to −70°. The flask was scratched at intervals to promote crystallization of the vitamin A. After forty-eight hours the crystals were filtered at −70° and washed with cold ethyl formate. The filtrate was distilled under reduced pressure, yielding a residue (11.2 g., $E^{1\%}_{1\,cm.}$ (327 mμ) = 1350) which consisted of a concentrate of neovitamin A, still containing some vitamin A.

Purification of Neovitamin A by Selective Adsorption.—The residue (11.2 g.) was adsorbed from petroleum ether solution (100 cc., b. p. 30–65°) on a column (5 cm. × 25 cm.) of sodium aluminum silicate (225 g.).[15] The column was washed with more petroleum ether (1 liter). The filtrate contained a small amount (0.7 g.) of unadsorbed impurities including anhydro vitamin A. The yellow zone which occupied the top 15 cm. of the column was removed in sections, each of which was eluted with ethyl ether. The least strongly adsorbed section (bottom 2 cm.) yielded concentrate I (2.7 g., $E^{1\%}_{1\,cm.}$ (328 mμ) = 1410) which was found to contain neovitamin A and vitamin A in the ratio of 88:12. The ratio of neovitamin A to vitamin A decreased progressively from the lower to the upper sections of the zone.

Preparation of *p*-Phenylazobenzoate of Neovitamin A.—*p*-Phenylazobenzoyl chloride (2.35 g.) in methylene chloride (15 cc.) was added slowly to neovitamin A concentrate I (2.7 g.) in methylene chloride (15 cc.) and pyridine (3 cc.). After standing for five hours at room temperature, water (1 cc.) was added and the reaction mixture warmed to 50° to hydrolyze excess acid chloride. The reaction mixture was then poured into 5% hydrochloric acid (100 cc.) and extracted with ether (150 cc.). The ether extract was washed with 5% hydrochloric acid to remove pyridine, 0.5 *N* potassium hydroxide, and finally with water. After drying over sodium sulfate the solvent was evaporated, yielding a red viscous oil (4.25 g.). Petroleum ether (25 cc.) was added and the solution filtered to remove the azoic anhydride with which the product was contaminated. The petroleum ether filtrate and washings were cooled to −35° which caused crystallization of the *p*-phenylazobenzoate of neovitamin A (1.6 g.).

After two recrystallizations of the ester from a petroleum ether–acetone mixture at 25°, the orange crystals (0.7 g., II) were dried under vacuum. They consisted of feathery needles, m. p. 94–96°, $E^{1\%}_{1\,cm.}$ (330 mμ) = 1460. A depression of melting point occurred when mixed with a sample of vitamin A *p*-phenylazobenzoate (m. p. 79–80°) prepared by the same procedure.

Preparation of Crystalline Neovitamin A.—The *p*-phenylazobenzoate (0.5 g.) was dissolved in 5 cc. of boil-

(15) Trade name, Doucil (American Doucil Company, 121 South 3rd Street, Philadelphia, Pennsylvania).

ing alcohol and 2 cc. of 4 *N* alcoholic potassium hydroxide was added. The mixture was refluxed for fifteen minutes and poured into water (25 cc.). The ether extract was washed with 0.5 *N* potassium hydroxide and water, dried over anhydrous sodium sulfate, filtered and the ether evaporated under a stream of nitrogen. The residue of neovitamin A was a viscous yellow oil (0.29 g.) which was crystallized from ethyl formate (1 cc.) at −35°. The pale yellow needles of neovitamin A were filtered and dried under vacuum; m. p. 58–60°; $E^{1\%}_{1\text{ cm.}}$ (328 mμ) = 1645. When mixed with vitamin A (m. p. 62–64°) a depression in the melting point to approximately 54° was observed.

Anal. Calcd. for $C_{20}H_{30}O$: C, 83.84; H, 10.56. Found: C, 83.67; H, 10.31.

Other Esters.—The acetate and palmitate of neovitamin A were prepared by the procedure previously reported for the corresponding esters of vitamin A.[16] The esters, after purification by adsorption, had $E^{1\%}_{1\text{ cm.}}$ (328 mμ) = 1410 and 810, respectively. Attempts to crystallize these esters were unsuccessful.

Esters of neovitamin A and vitamin A were prepared with anthraquinone β-carboxyl chloride by essentially the same method described for the preparation of the *p*-phenylazobenzoates. After two crystallizations from methyl acetate the red crystals of neovitamin A anthraquinone-β-carboxylate melted at 134–136°. The vitamin A ester crystallized from acetone or methyl acetate as yellow plates melting at 121–122°.

Dehydration of Neovitamin A.—Anhydro neovitamin A was prepared by the procedure previously described for anhydro vitamin A.[9] The crystals melted at 76–77° and exhibited no melting point depression when mixed with a sample of anhydro vitamin A. The two anhydro compounds were, therefore, judged to have the same constitution.

The rate of dehydration was studied by measuring the increase in extinction coefficient at 392 mμ at intervals after adding hydrochloric acid in ethanol (10 cc., 0.06 *N*) to ethanol solutions (20 cc., approximately 0.0006 g./100 cc.) of neovitamin A and vitamin A. The measurements were made on a Beckman spectrophotometer. The percentage of anhydro vitamin A formed (Fig. 7) was calculated by the formula

$$\%\text{ anhydro vitamin A} = E^{1\%}_{1\text{ cm.}}\ (392\ \text{m}\mu)/3180$$

where $E^{1\%}_{1\text{ cm.}}$ (392 mμ) was the extinction coefficient for the samples at any given time, and 3180 was the value for pure anhydro vitamin A. The formula used is not strictly correct because no correction for the extinction coefficient at 392 mμ, due to the unchanged neovitamin A or vitamin A, was made. However, this value was so small in comparison with the observed values that the error introduced by its omission was negligible.

Analytical Method for Neovitamin A with Maleic Anhydride.—A sample of the oil or concentrate to be analyzed was dissolved in benzene and an aliquot of 5 cc. containing approximately 1250 total units of mixed vitamin A was pipetted into a 10-cc. amber volumetric flask. The flask was filled to the mark with maleic anhydride solution (10 g. maleic anhydride per 100 cc. benzene). The mixture, after shaking, was stored at 25° for sixteen hours. An aliquot (1 cc.) was then removed and diluted to a proper concentration for assay by the antimony trichloride method. A sample of the stock solution which had not been treated with the maleic anhydride solution was also assayed by the same method. From these values the percentage recovery of vitamin was calculated.

A similar procedure using neovitamin A palmitate and vitamin A palmitate showed recoveries of 90 and 5%, respectively, after sixteen hours (Fig. 8). The acetates of neovitamin A and vitamin A showed recoveries after sixteen hours identical with those of the corresponding palmitic esters.

(16) J. G. Baxter and C. D. Robeson, THIS JOURNAL, **64**, 2407 (1942).

Thus the percentage of neovitamin A in the sample (if in esterified form) could be calculated by the formula

$$\%\text{ neovitamin A} = \frac{R - R_1}{R_2 - R_1} \times 100$$

where R = recovery of vitamin in the test sample, R_1 = 5 and R_2 = 90.

Unesterified neovitamin A and vitamin A showed recoveries of 83 and 3%, respectively, after sixteen hours. Therefore, when assaying concentrates prepared by saponification these values were substituted for R_2 and R_1.

Varying the concentration of the neovitamin A or vitamin A palmitates in the reaction over the range from 1000 to 10,000 units per 5 cc. aliquot changed the recovery by less than 5%. Reducing the concentration of maleic anhydride in the reagent to 5 g. per 100 cc. resulted in a 10% recovery of vitamin A. When the reaction was carried out at 5°, the recoveries were found to be 98% for neovitamin A palmitate and 22% for vitamin A palmitate after sixteen hours.

Isomerization Experiments.—Crystals of neovitamin A anthraquinone-β-carboxylate (0.2 g., $E^{1\%}_{1\text{ cm.}}$ 333 mμ = 1020, m. p. 134–135°) were dissolved in benzene (50 cc.) containing 0.2 mg. of iodine. The solution was allowed to stand at 25° for two hours exposed to daylight. The solution was then filtered through a column of powdered sodium thiosulfate to remove the iodine and the solvent was evaporated in a stream of nitrogen. The residue assayed 29% neo isomer and was crystallized from methyl acetate (1 cc.) at −35°. The crystals were orange and melted at 114–120°. One recrystallization at room temperature yielded yellow crystals of melting point 120–122° which showed no melting point depression when mixed with crystals of vitamin A anthraquinone β-carboxylate.

Under the same conditions vitamin A anthraquinone-β-carboxylate ($E^{1\%}_{1\text{ cm.}}$ 330 mμ = 1065, m. p. 121–122°) yielded a product assaying for 31% of the neo isomer. Crystallization from methyl acetate yielded a mixture of red and yellow crystals from which the pure neo ester was not prepared.

Summary

1. A naturally occurring geometrical isomer of vitamin A has been isolated in crystalline form from soupfin shark liver oil. This isomer, which we have called neovitamin A, has been found to be present also in cod, dogfish, halibut and California jewfish liver oils to the extent of approximately 35% of the total vitamin A.

2. Evidence is presented indicating that the two isomers differ in the spacial configuration about the double bond nearest the hydroxyl group. Neovitamin A is postulated tentatively to have the *cis* configuration about this bond while vitamin A has the *trans* structure.

3. Certain physical properties of neovitamin A and vitamin A, such as their ultraviolet absorption spectra and infrared spectra, are compared. Chemical properties which are compared include the relative rate of oxidation in air, rate of dehydration with mineral acids to form anhydro vitamin A, and rate of addition of maleic anhydride. The marked difference in the reactivity of the isomers toward this last reagent is the basis of an analytical method for determining the percentage of neovitamin A in the mixed vitamin A's in fish liver oils.

4. The biological potencies of neovitamin A and vitamin A as measured by the U.S.P. growth

method in rats proved to be identical. An explanation for this was found in the ability of the rat to convert neovitamin A to vitamin A.

5. Catalytic interconversion of the anthraquinone carboxylate esters of the two vitamins was accomplished *in vitro* by the action of iodine in benzene solution.

ROCHESTER 13, NEW YORK RECEIVED AUGUST 28, 1946

[CONTRIBUTION FROM THE PROCTER AND GAMBLE CO.]

The Phase Nature of Beta Sodium Palmitate

BY R. H. FERGUSON, F. B. ROSEVEAR AND H. NORDSIECK

Introduction

In an earlier paper[1] evidence was presented to show that solid sodium soaps could be described in terms of four distinct crystalline structures, each identifiable with the aid of one or two carefully selected X-ray short spacings. At the same time[2] certain minor variations were recognized among patterns of the beta structure, depending upon whether "anhydrous" palmitate, hydrous palmitate or commercial soap was involved. Similar minor effects in the omega structure were noted in a companion paper,[3] and the variations in beta palmitate were localized in the narrow composition range below about 3% water.

In the present contribution, which extends the earlier X-ray evidence and supplements it with vapor pressure and microscopic results, it is shown that the foregoing spacing variations in beta sodium palmitate are not indicative of new phases, but arise from the existence of a continuous beta solid solution phase which, at room temperature, extends from almost anhydrous soap to about 2.5% water.

The four phase concept outlined in references 1 and 3, while not intended to rule out possible additional phases, is nevertheless regarded by some as an oversimplification. In this connection certain of the above variations in X-ray patterns have recently been rediscovered and used, along with other variations of comparable magnitude, as evidence for the existence of definite soap hydrates. This brings to ten the number of proposed crystalline phases in the sodium soaps.[4,5]

In showing that two, and possibly three, of these phases are simply members of one solid solution series, our evidence, while confined to the beta structure, suggests that a similar close relationship may exist among other patterns bearing marked resemblance to each other.

Experimental

A. Materials and Methods.—The sodium palmitate used in this work is characterized in reference 1. With the exception of a few points below 2.2% water, specifically noted below, all experimental points were obtained on soap crystallized by cooling homogeneous melt, usually neat soap, from above the "Tc" curve.

For samples below about 5% moisture, the starting material was prepared as follows: 20-g. samples of 20–25% water content were held in sealed glass tubes at 100° with occasional shaking until homogeneous. After spontaneous cooling (100 to 30° in thirty to sixty minutes), the contents of each tube were ground, given a preliminary drying in air of not less than 30% humidity, stored several days in closed jars to equalize moisture, and then analyzed to ensure a moisture content of at least 3%. The X-ray spacings of each batch were identical with those of the original soap crystallized from melt.

For points above 5% moisture, portions of the crystallized melt were placed directly in the vapor pressure tubes.

Per cent. moisture was determined from the loss in weight resulting from one hour of holding at 150°. Values are reproducible to ±0.2%.

Further experimental details are given in connection with the corresponding results.

B. X-Ray Results.—Figure 1 presents graphically, for a series of hydrous sodium palmitate samples, the four strongest short spacings as a function of per cent. water. It is evident that from about 2.5% water up to at least 95% water, the spacings are constant at about 2.77, 3.16, 3.90 and 4.29 Å., whereas below 2.5% water each of the four varies continuously, the smaller spacing increasing to about 2.84 Å. and the three larger spacings decreasing to about 3.07, 3.75 and 4.17 Å., respectively, at 0.2% water. Attention has been focused on the three smaller spacings since the fourth spacing is complex and less closely measurable; it is included because it shows the same type of behavior as the other three.

Table I supplements Fig. 1 by presenting data for the entire pattern at each of several representative moisture contents in and beyond the range of pattern variability. Study of this table shows that, except for the four reflections plotted in Fig. 1, the rest of the spacings are practically constant. It is especially noteworthy that the long spacing is constant even in the moisture range where the short spacings of Fig. 1 vary continuously.

X-Ray patterns were obtained by techniques previously described,[1,6] all samples being sealed in thin-walled Pyrex capillaries. Estimated accuracy of short spacings is about ±0.02 Å., of long spacings ±0.5 Å. Most of the data of Fig. 1 were obtained at 5 cm. sample-to-film distance, but

(1) R. H. Ferguson, F. B. Rosevear and R. C. Stillman, *Ind. Eng. Chem.*, **35**, 1005–1012 (1943).

(2) Note *b* in Table II and note *a* in Table V of ref. 1.

(3) R. H. Ferguson, *Oil & Soap*, **21**, 6–9 (1944).

(4) M. J. Buerger, L. B. Smith, F. V. Ryer and J. E. Spike, Jr., *Proc. Nat. Acad. Sci. U. S.*, **31**, 226–233 (1945).

(5) K. W. Gardiner, M. J. Buerger and L. B. Smith, *J. Phys. Chem.*, **49**, 417–428 (1945).

(6) R. H. Ferguson and H. Nordsieck, *Ind. Eng. Chem.*, **36**, 748–752 (1944).