TABLE I

ALKYL ARYL THIOURETHANS, ArNHCSOR

	Thiourethan	Formula	M. p., °C.	Yield, %	%, S Calcd.	%, S Found
1	Methyl phenyl	$C_6H_5NHCSOCH_3$	92–93	63	19.17	19.16
2	Ethyl phenyl	$C_6H_5NHCSOC_2H_5$	70–72	60	17.69	17.81
3	*n*-Propyl phenyl	$C_6H_5NHCSOC_3H_7$-*n*	45–46	64	16.42	16.39
4	*i*-Propyl phenyl	$C_6H_5NHCSOC_3H_7$-*i*	84–85	75	16.42	16.51
5[a]	*n*-Butyl phenyl	$C_6H_5NHCSOC_4H_9$-*n*	51–53	50	15.32	15.46
6	*i*-Butyl phenyl	$C_6H_5NHCSOC_4H_9$-*i*	74–76	90	15.32	15.46
7[a]	*t*-Butyl phenyl[b]	$C_6H_5NHCSOC_4H_9$-*t*	86.5	31	15.32	15.01
8[a]	*n*-Amyl phenyl	$C_6H_5NHCSOC_5H_{11}$-*n*	49–50	55	14.36	14.51
9[a]	*i*-Amyl phenyl	$C_6H_5NHCSOC_5H_{11}$-*i*	44–46	80	14.36	14.38
10[a]	*n*-Heptyl phenyl	$C_6H_5NHCSOC_7H_{15}$-*n*	34	30	12.76	14.38
11[a]	*n*-Octyl phenyl	$C_6H_5NHCSOC_8H_{17}$-*n*	41–43	41	12.09	11.95
12[a]	*n*-Nonyl phenyl	$C_6H_5NHCSOC_9H_{19}$-*n*	45–47	32	11.48	11.61
13[a]	2-Phenylethyl phenyl	$C_6H_5NHCSO(CH_2)_2C_6H_5$	89.5	50	12.47	12.62
14[a]	3-Phenylpropyl phenyl	$C_6H_5NHCSO(CH_2)_3C_6H_5$	74	53	11.82	11.93
15[a]	Allyl phenyl[b]	$C_6H_5NHCSOCH_2CH{=}CH_2$	75–77	35	16.60	16.30
16	Ethyl 4-tolyl	(4)$CH_3C_6H_4NHCSOC_2H_5$	85	60		
17	Ethyl 4-nitrophenyl	(4)$NO_2C_6H_4NHCSOC_2H_5$	175	75		
18	Ethyl 2,4-dichlorophenyl	(2,4)$Cl_2C_6H_3NHCSOC_2H_5$	79	65		
19	Ethyl 4-carboxyphenyl	(4)$COOHC_6H_4NHCSOC_2H_5$	212	79		
20	Ethyl allyl	$CH_2{=}CHCH_2NHCSOC_2H_5$	B. p. 115–119 at 14 mm.			

[a] These compounds have not hitherto been reported. [b] Specific preparations given.

Summary

1. The phenyl thiourethans of a number of aliphatic alcohols have been prepared and found to be pharmacologically inactive as hypnotics.

2. Several thiourethans have been prepared for the first time by the action of isothiocyanates on sodium alcoholates. This method is of particular value where water is easily removed from the alcohol.

3. In cases where the alcohol is easily dehydrated, the water thus formed reacts with the phenyl isothiocyanate giving *sym*-diphenylthiourea instead of the anticipated alkylthiourethan.

CHAPEL HILL, N. C. RECEIVED FEBRUARY 13, 1943

[COMMUNICATION NO. 33 FROM LABORATORIES OF DISTILLATION PRODUCTS, INC.]

Anhydro ("Cyclized") Vitamin A[1]

BY EDGAR M. SHANTZ, JOHN D. CAWLEY AND NORRIS D. EMBREE

Vitamin A, when treated with anhydrous alcoholic hydrochloric acid, is changed into a substance with three absorption bands in the ultraviolet and with slight biological activity.[2] Edisbury, *et al.*,[3] first studied this reaction and noted that apparently the same substance could be obtained by breaking up with water the antimony trichloride reaction product of vitamin A. Although the reaction was first thought to be one of cyclization, the properties described below for the material indicate that the reaction is one of dehydration, and for this reason we prefer to call the product "anhydro vitamin A" instead of "cyclized vitamin A."

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

(2) N. D. Embree, *J. Biol. Chem.*, **128**, 187 (1939).

(3) J. R. Edisbury, A. E. Gillam, I. M. Heilbron and R. A. Morton, *Biochem. J.*, **26**, 1164 (1932).

Dehydration of Vitamin A.—The method described by Edisbury, *et al.*,[3] for the dehydration of vitamin A is satisfactory. A concentrate of vitamin A (in the alcohol form) is allowed to stand for fifteen minutes at room temperature in 100 parts or more of *N*/30 anhydrous alcoholic hydrogen chloride, after which the solution is neutralized, dissolved in ether and washed free of inorganic reagents. A dark orange-brown color, formed upon the addition of the acid, disappears upon neutralization. At temperatures above 30° the reaction is faster but the formation of products other than anhydro vitamin A is increased. At lower temperatures the reaction is slowed up considerably; *e. g.*, at −60° no dehydration took place in several days. An increase in the acid concentration has about the same general effect as an increase in temperature.

The material can be concentrated most simply by

chromatographing in petroleum ether solutions. In this Laboratory we have successfully used aluminum oxide (after Brockmann), "Hydralo," "Doucil," magnesium oxide, and silica gel as adsorbing agents. In all cases the anhydro vitamin A passes easily through the column and is recovered in the eluate. Preparations with $E(1\%,1\text{ cm.})$ (369 mμ) = 2500 are the most potent that we have obtained chromatographically.

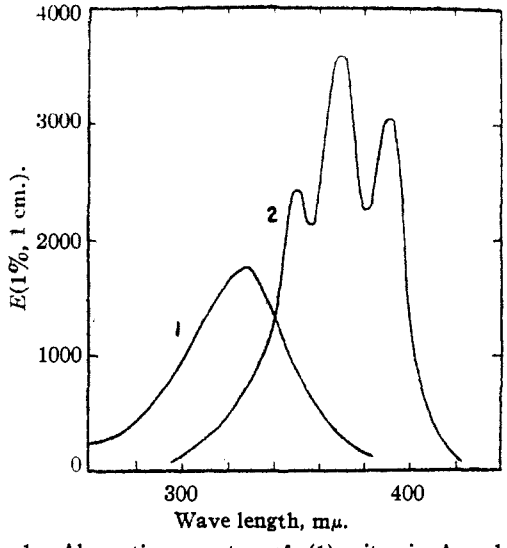


Fig. 1.—Absorption spectra of (1) vitamin A and (2) anhydro vitamin A in absolute ethyl alcohol.

Preparation of Crystalline Anhydro Vitamin A.[4]—A chromatographed concentrate of anhydro vitamin A having a value of 2200 or higher for $E(1\%,1\text{ cm.})$ (369 mμ) may be crystallized if allowed to stand for about two days at −60° at a concentration of 20% in low-boiling petroleum ether (Skellysolve F). For further purification, the supernatant liquid is decanted and the crystals rinsed with cold petroleum ether. More solvent is added and the crystals dissolved by warming up to room temperature. The solution is now allowed to crystallize again by cooling slowly at −30° for about twenty-four hours. The process can be facilitated by seeding. The crystals may now be washed and dried by decanting and rinsing as before, then spreading onto a piece of filter paper which is immediately placed in a vacuum desiccator in which they are dried overnight, preferably in a darkened cold room. The crystals are orange prisms, melting at 76–77°.

Absorption Spectra.—Three ultraviolet absorption bands[5] occur at 351, 371 and 392 mμ with values of $E(1\%,1$ cm.) of 2500, 3650, and 3180, respectively (Fig. 1). The subsidiary band at 392 mμ is always well defined, but the maximum at 351 mμ is in some samples a pronounced peak and in others only a sharp inflection. For the antimony trichloride product[6] we have found an average value for $E(1\%,1$ cm.) (620 mμ) of 5500 (Fig. 2). This value is somewhat higher than that (4800) found for vitamin A.[7]

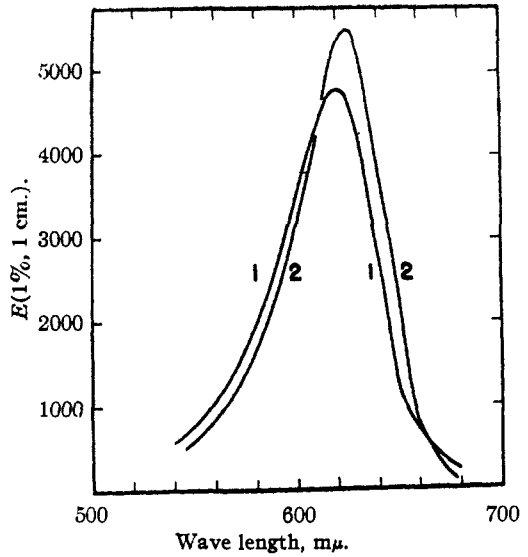


Fig. 2.—Absorption spectra of the antimony trichloride reaction products of (1) vitamin A and (2) anhydro vitamin A.

Polymerized Compounds from the Dehydration Reaction.—Increasing the concentration of vitamin in the reaction mixture to greater than 1% favors the formation of orange and red compounds. If the anhydro vitamin A is removed by molecular distillation below 150°, these products remain in the residue. However, if the distillation is carried up to 250°, they are not found in either the residue or distilled fractions. Apparently these deeply colored compounds are heat labile and have a molecular weight higher than that of vitamin A alcohol. Several of these products are formed, and they can be separated chromatographically with some difficulty. Upon adsorption, they form a complex series of bands very similar in appearance to the bands which are invariably formed immediately below the vitamin A alcohol in any chromatogram of fish liver oil unsaponifiable material.[7B] The materials eluted from these bands give a blue color at 620 mμ with antimony trichloride and have rather poorly defined ultraviolet absorption bands between 300 and 370 mμ.

Iso-anhydro Vitamin A.—Another compound, provisionally called iso-anhydro vitamin A, is formed by allowing the vitamin A to remain in contact with the acid re-

(4) The first crystals were prepared by C. D. Robeson of these laboratories and were described briefly in a paper by Baxter and Robeson presented at the Atlantic City meeting of the American Chemical Society, September, 1941.

(5) Ultraviolet absorption measurements were made with a Hilger medium quartz spectrograph equipped with a Spekker photometer. All solutions were in absolute ethyl alcohol. The extinction coefficient, or, as it is sometimes called, the percentage extinction coefficient, is represented here by the symbol $E(1\%,1$ cm.), although it is often represented by $E^{1\%}_{1\text{ cm.}}$, or E. It is defined by the equation $E(1\%,1\text{ cm.}) = (1/cd)\log I_0/I$, where I_0 = intensity of light (at the indicated wave length) entering the solution, I = intensity of the light leaving the solution, c = concentration of solution in g. per 100 ml., d = depth in cm. of the absorption cell.

(6) The absorption spectra of the antimony trichloride reaction products were measured with a Hardy recording spectrophotometer using the technique previously described.[8] The instrument was operated by E. E. Richardson of the Kodak Research Laboratories.

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

(7B) D. C. Castle, A. E. Gillam, I. M. Heilbron and H. W. Thompson, *Biochem. J.*, **28**, 1702 (1934).

agent for a prolonged period of time at room temperature. While the formation of anhydro vitamin A is complete in ten to fifteen minutes, this secondary reaction requires about twelve to sixteen hours. The substance may also be formed by treating anhydro vitamin A with hydrochloric acid or antimony trichloride.

Iso-anhydro vitamin A appears to be a single substance with an ultraviolet absorption curve similar to that of anhydro vitamin A but having its absorption bands at about 330, 350 and 370 mμ instead of 351, 371 and 392 mμ. In this new substance, as in anhydro vitamin A, the strongest band is in the middle. By following its formation on the spectrograph, it was observed that the 392 mμ band of the anhydro vitamin A slowly disappeared as the new band at 330 mμ was slowly formed. The antimony trichloride blue color (wave length of maximum 623 mμ) was similar to that of vitamin A.

Iso-anhydro vitamin A is very slightly more strongly adsorbed on alumina than is anhydro vitamin A. Its petroleum ether–83% ethanol distribution ratio is 97:3, the same as that of anhydro vitamin A. When distilled in a cyclic molecular still its elimination maximum is 10° below that of vitamin A and 10° above that of anhydro vitamin A.

The preparation and properties of one concentrate of iso-anhydro vitamin A are as follows. About one gram of a chromatographically pure concentrate of anhydro vitamin A was allowed to stand for 12 hours in 1 liter of 1/30 *N* anhydrous alcoholic hydrogen chloride. It was then diluted with water and extracted with petroleum ether (Skellysolve F). The petroleum extract was washed several times with water and dried over sodium sulfate. This solution was allowed to flow through a column of aluminum oxide (finely ground "Hydralo") followed by liberal quantities of light petroleum. The first material to be washed through was some unchanged anhydro vitamin A, followed closely by the iso-anhydro compound. This fraction was rechromatographed, and 0.398 g. of a concentrate was obtained with a value of 1320 for $E(1\%,1$ cm.) (349 mμ) and, for the antimony trichloride color, a value of 3200 for $E(1\%,1$ cm.) (620 mμ).

Carbon and Hydrogen Analyses of Anhydro Vitamin A.—Carbon and hydrogen analyses were performed on the crystalline material by L. T. Hallett of the Kodak Research Laboratories. The results, shown below, further suggested that a molecule of water had been split out of the vitamin A molecule.

	Carbon, %	Hydrogen, %
$C_{20}H_{30}O$, vitamin A alcohol or Heilbron's "cyclized" vitamin A requires	83.92	10.49
$C_{20}H_{28}$, vitamin A less H_2O requires	89.55	10.45
Anhydro vitamin A (made by Robeson)	88.44	10.46
	88.70	10.39

Zerewitinoff Determination on Anhydro Vitamin A.—Half a gram of a rechromatographed concentrate of anhydro vitamin A with $E(1\%,1$ cm.) (369 mμ) = 2400 was warmed gently for one-half hour with an excess of methylmagnesium iodide in isoamyl ether. No detectable amount of methane was evolved, indicating the absence of any hydroxyl group.

Hydrogenation of Anhydro Vitamin A.—Several samples of crystalline anhydro vitamin A were analytically reduced in a microhydrogenation apparatus[8] using absolute ethyl alcohol as a solvent and a palladinized calcium carbonate catalyst. Samples of crystals about two weeks old gave values ranging from 5.50 to 5.65 double bonds. A freshly chromatographed but uncrystallized preparation showed 5.70 double bonds. Older crystalline samples showed correspondingly lower values ranging from 5.05 to 5.27. All calculations are based on a molecular weight of 268 (vitamin A less 1 mole of water). Crystalline vitamin A alcohol when reduced under identical conditions showed 4.78 out of a theoretical 5 double bonds. We therefore feel that the values obtained strongly indicate the presence of six unsaturated bonds in the anhydro vitamin A molecule.

Stability of Anhydro Vitamin A.—Judging by the absorption spectra measured at successive time intervals anhydro vitamin A appears to be fairly stable in oil solutions, but purified concentrates and crystalline preparations degenerate quite rapidly even when stored under vacuum at −35°. If anhydro vitamin A is allowed to stand in solvents for any length of time, a pale yellow rubbery substance is deposited which appears to be a polymerization product. This is insoluble in most solvents and gives a brown color with antimony trichloride. A petroleum ether solution of anhydro vitamin A standing for forty-eight hours at room temperature deposited about a 50% yield of this material.

Distillation of Anhydro Vitamin A.—Using the technique of analytical molecular distillation,[9] the elimination maximum of anhydro vitamin A was found to be 19° below that of vitamin A alcohol. The distillations were carried out in a falling film cyclic molecular still using a triglyceride constant yield oil.

Dehydration by Alcohol Treatment of Vitamin A Ester.—In a recent communication from this Laboratory,[10] mention was made of the formation of anhydro vitamin A by the action of hot alcohols on vitamin A esters. By this method a vitamin A ester concentrate is refluxed for fifteen to twenty hours with approximately ten times its weight of a lower alkyl alcohol. The vitamin A ester breaks down into anhydro vitamin A, free fatty acid and other products. These substances may be conveniently separated from the glycerides and sterols present in the ester concentrate by distilling the refluxed material, after solvent removal, in a molecular still at a temperature not exceeding 150°. After removal of the fatty acid by alkali extraction, the anhydro vitamin A is readily separated from the other breakdown products by adsorption on alumina, tricalcium phosphate, or magnesia. Anhydro vitamin A prepared in this way is identical with that prepared by hydrochloric acid dehydration in melting point, absorption spectra and elimination maximum.

Recovery of Chromogenic Material from the Antimony Trichloride Reaction Product of Vitamin A.—Edisbury, *et al.*,[3] recovered chromogenic material from the antimony trichloride reaction product of vitamin A by treating the blue solution, which had stood for one minute, with water

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

(9) K. C. D. Hickman, *Ind. Eng. Chem.*, **29**, 968 (1937).

(10) E. LeB. Gray and J. D. Cawley, *J. Nutrition*, **23**, 301 (1942).

and extracting the mixture with ethyl ether. The extract had pronounced light absorption bands at 392, 369, 349, and 333 mμ, together with other less prominent bands. Since upon reaction with antimony trichloride the extract gave a blue color, the authors thought that some unchanged vitamin A was still present.

The chromogenic material may be recovered more advantageously in the following way. A saturated solution of antimony trichloride in chloroform is added to a chloroform solution of vitamin A. After a few seconds, an equal volume of ethyl alcohol is added and the blue color disappears. Twice the volume of petroleum ether is now added to the mixture, followed by enough 4 *N* aqueous hydrochloric acid to effect a separation of the layers. In this manner the bulky precipitate of antimony oxychloride is avoided. The petroleum ether solution is now washed with two more portions of acid, followed by water. Vitamin A solutions show no ill effects when washed with acid of this strength, if the washing is carried out rapidly. The recovered material was found to contain anhydro vitamin A. The absorption spectra showed only four bands, at 390, 370, 350 and sometimes, 332 mμ. Possibly anhydro vitamin A is an intermediate in the development of the antimony trichloride blue color.

Biological Activity of Anhydro Vitamin A.—Embree[2] reported that a concentrate of anhydro vitamin A had some biological activity but showed that all the activity could be accounted for by the probable amount of vitamin A present. But when a sample of crystalline anhydro vitamin A was bioassayed, it was found to contain about 17,500 U. S. P. units of vitamin A per gram. This activity would correspond to a contamination of about 0.4% of vitamin A, an amount not expected, but possible.

Two more preparations of anhydro vitamin A were made, one by the hydrochloric acid dehydration method, the other by the ester decomposition method, and great care was taken to prevent any contamination with vitamin A. The bioassays were carried out by Philip L. Harris and Marian R. Woodside of these Laboratories, using substantially the method of the U. S. Pharmacopoeia.[11]

The sample made by the dehydration method was found to have a potency of 15,200 units of vitamin A per gram, and the sample made by ester decomposition was found to have a potency of 16,900 units per gram. It appears, therefore, that anhydro vitamin A itself does have a small amount of vitamin A activity.

Structure of Anhydro Vitamin A.—Anhydro vitamin A is evidently a hydrocarbon. The Zerewitinoff determination renders it improbable that hydroxyl groups are present, while the elementary analysis precludes the presence of oxygen. The microhydrogenation experiments show that anhydro vitamin A contains at least five and probably six double bonds.

The absorption spectrum indicates a resonance equivalent to 6 or possibly 7 conjugated double bonds. According to Kuhn[12] a phenyl group in conjugation with an aliphatic conjugated series is equivalent to 1.5 double bonds, a double bond in a ring system is equal to 0.5 double bond in an open chain, and each methyl or other substituent group is equivalent to about 0.25 double bond. From the data of Smakula[13] on phenyl substituted unsaturated hydrocarbons, and from data on vitamin A[7] Table I was prepared giving the number of double bonds, *n*, for the optically equivalent substance, $H(CH{=}CH)_nH$. This information indicates that anhydro vitamin A has 6 or 7 double bonds.

TABLE I

Substance	*n*	$\lambda_{max.}$			$\epsilon \times 10^{-3}$		
$Ph(CH{=}CH)_5Ph$	5	328	354		41	26	
Vitamin A	5.5	328			49.8		
$Ph(CH{=}CH)_6Ph$	6	335	349	370	53.8	65.4	55.6
Anhydro vitamin A	?	351	371	392	67.0	97.8	85.2
$Ph(CH{=}CH)_7Ph$	7	353	375	396	54.3	89.8	71.3
Formula III	6.2 + (0.5?)						
IV	6.0						
V	6.2						

There does not seem to be enough information available to determine definitely the structure of anhydro vitamin A. There should, of course, be a close relation between its structure and that of vitamin A, formula I.[14] Heilbron, *et al.*,[15] suggested that "cyclized" vitamin A had the structure shown in formula II. The evidence suggesting this formula was that a 3.5% yield of 1,6-dimethylnaphthalene was obtained by the selenium dehydrogenation of a "cyclized" concentrate of vitamin A, although this same report showed that a 5.5% yield of the dimethylnaphthalene was obtained by the dehydrogenation of a sample of the same vitamin A concentrate, but which had not been "cyclized." Formula II is, at any rate, incompatible with most of the known properties of anhydro vitamin A.

Three formulas that are compatible with the properties of anhydro vitamin A are shown as formulas III, IV and V. The number of double bonds *n* for the optically equivalent substance, $H(CH{=}CH)_nH$, for these formulas are shown in Table I. To the value of *n* for formula III, the "allene" formula, an estimated value of 0.5 is added since Kuhn[16] states that cumulative double bonds have more activity than the same number of conjugated double bonds. With such a correc-

(11) U. S. Pharmacopoeia, Eleventh Decennial Revision (U. S. P. XI-1939 supplement), Mack Printing Co., Easton, Pa.

(12) R. Kuhn and C. Grundmann, *Ber.*, **70B**, 1323 (1937); R. Kuhn and A. Winterstein, *Helv. Chim. Acta*, **12**, 899 (1929).

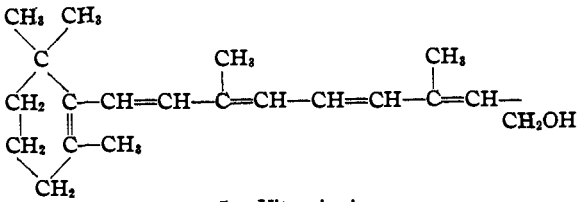
(13) A. Smakula, *Angew. Chem.*, **47**, 657 (1934); **48**, 152 (1935).

(14) P. Karrer, R. Morf and K. Schöpp, *Helv. Chim. Acta*, **14**, 1431 (1931).

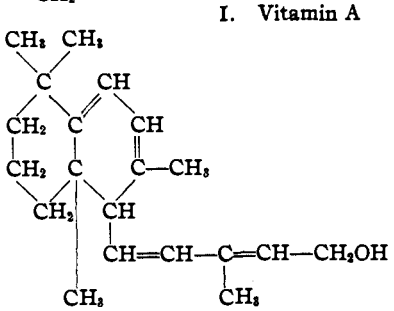
(15) I. M. Heilbron, R. A. Morton, and E. T. Webster, *Biochem. J.*, **26**, 1194 (1932).

(16) R. Kuhn and K. Wallenfels, *Ber.*, **71B**, 783 (1938).

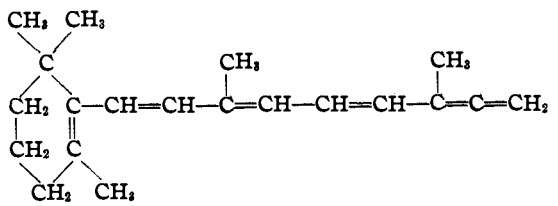
tion the optical absorption data seem to favor formula III.



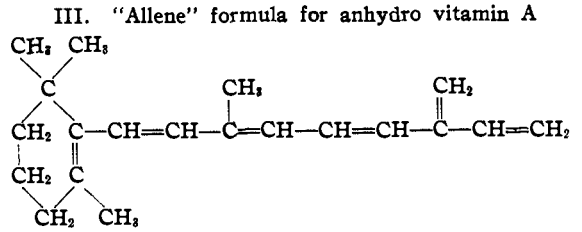
I. Vitamin A



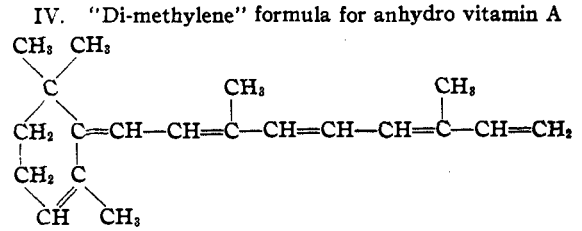
II. Heilbron's formula for "cyclized" vitamin A



III. "Allene" formula for anhydro vitamin A



IV. "Di-methylene" formula for anhydro vitamin A



V. "Methylene" formula for anhydro vitamin A

Uses of the Dehydration Reaction.—One example of the usefulness of the dehydration reaction is the method[17] for the determination of the relative amounts of vitamin A and A_2 when they are present in mixtures. Although the two vitamins cannot be separated conveniently the two anhydro or "cyclized" derivatives can be separated easily by chromatography.

Pritchard, *et al.*,[18] used the dehydration reaction to show that a fraction which they had isolated from mammalian liver oil was not ordinary vitamin A although the material had some biological vitamin A activity.

Another example is a brief study of the vitamin A in blood plasma done by us for the Memorial Hospital in connection with work its staff was doing on vitamin A in gastro-intestinal cancer.[19] Blood plasma extracts give a blue color with antimony trichloride, but they contain materials that hide the ultraviolet absorption band. It was considered desirable to obtain some other physicochemical evidence that the chromogenic material was vitamin A. The dehydration reaction seemed promising and was used in the following way. The petroleum ether extract of 100 ml. of normal human blood was furnished us by Alice T. Gorham of the Memorial Hospital. By the antimony trichloride reaction,[20] she found it to contain 75 U. S. P. units of vitamin A. The extract was saponified; the unsaponifiable fraction was then found to contain 72 units by the antimony trichloride method. The absorption spectrum (Fig. 3A) did not show the typical vitamin A band. If the calculated absorption curve (Fig. 3B) is subtracted from curve 3A, the hypothetical curve for vitamin A-free plasma extract is obtained

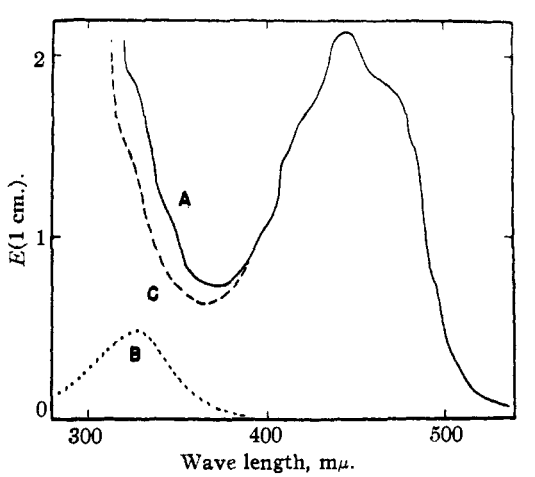


Fig. 3.—Absorption spectra of (A) blood plasma extract, unsaponifiable fraction of 100 ml. blood dissolved in 7 cc. of ethyl alcohol (1430%), (B) calculated absorption curve of vitamin A as determined by antimony trichloride color, and (C) calculated absorption curve of vitamin A-free plasma extract (curve A minus curve B).

(17) N. D. Embree and E. M. Shantz, *J. Biol. Chem.*, **132**, 619 (1940).

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

(19) J. C. Abels, A. T. Gorham, G. T. Pack and C. P. Rhoads, *J. Clin. Investigation*, **20**, 749 (1941).

(20) The blue color was measured with a photoelectric colorimeter which had been calibrated with fish liver oil distillates containing over 200,000 U. S. P. units of vitamin A per g. The potencies of the distillates were figured by multiplying the values of $E(1\%,1\text{ cm.})$ (328 mμ) by 2000.

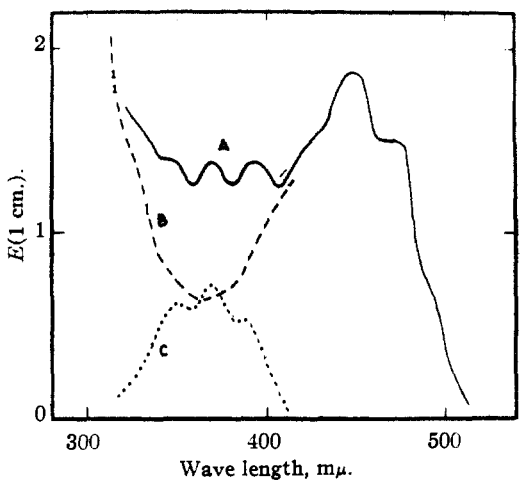


Fig. 4.—Ultraviolet absorption spectra of (A) blood plasma extract after treatment with anhydrous hydrogen chloride, (B) absorption curve of vitamin A-free plasma extract, and (C) resulting curve typical of anhydro vitamin A (curve A minus curve B).

(Fig. 3C). The saponified extract was dehydrated by treatment with $N/30$ alcoholic hydrochloric acid, giving rise to material whose absorption curve is shown in Fig. 4A. This material should not contain vitamin A, but may contain anhydro vitamin A. When the absorption curve for vitamin A-free plasma extract (4B) is subtracted from curve 4A, the resulting curve 4C is obtained. This shows the typical anhydro vitamin A bands and indicates that the original plasma extract must have contained vitamin A. As the value of $E(1\%, 1$ cm.) for the peak near 371 mμ of a crude anhydro vitamin A preparation usually is about 140–150% that of $E(1\%, 1$ cm.) (328 mμ) of the original vitamin A preparation, it is interesting to note that the values for $E(1\%, 1$ cm.) (368 mμ) on curve 4C and for $E(1\%, 1$ cm.) (328 mμ) for curve 3B are 0.00049 and 0.00035, respectively.

Summary

Anhydro (cyclized) vitamin A may be made from vitamin A by treatment with hydrochloric acid or antimony trichloride. Vitamin A in the form of its fatty acid esters is partially transformed into anhydro vitamin A by refluxing in alcohol. Crystalline anhydro vitamin A melts at 76-77° and has a value of 3650 for $E(1\%, 1$ cm.) (371 mμ). It is an unstable hydrocarbon, weakly adsorbed on most chromatographic adsorption agents and it probably has 6 double bonds and the formula $C_{20}H_{28}$. It has some biological vitamin A activity.

ROCHESTER, NEW YORK RECEIVED NOVEMBER 23, 1942

[COMMUNICATION NO. 34 FROM LABORATORIES OF DISTILLATION PRODUCTS, INC.]

A Possible New Member of the Vitamins A_1 and A_2 Group[1]

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The unsaponifiable fraction of fish liver oil contains several substances other than vitamin A which, like vitamin A, have absorption bands in the ultraviolet region and give blue colored products with antimony trichloride. The examination of the materials related to vitamin A in the unsaponifiable fraction of fish liver oil has most often been carried out by chromatographic adsorption of a petroleum ether solution of the material on a Tswett column of alumina or calcium hydroxide. The fractions with their properties that have been usually recognized are shown in Table I. The absorption maxima in the ultraviolet region (below 400 mμ) are for solutions in ethyl alcohol, while those in the visible regions are for the product obtained by the reaction of a chloroform solution of the material with a chloroform solution of antimony trichloride.

The fact that vitamins A_1 and A_2 are not readily separable by chromatographic adsorption is not necessarily a bar to the determinations of the relative proportions of each present, since, as we have already shown,[4] the "cyclized" or anhydro vitamins are readily separated chromatographically. The vitamins are usually dehydrated by treating them, in alcoholic solution, with $N/30$ dry hydrogen chloride.[5] Anhydro vitamin A_2 is quite strongly held by alumina in a Tswett column while anhydro vitamin A_1 is readily washed through with petroleum ether. Anhydro vitamins A_1 and A_2 have almost identically the same ultraviolet absorption spectrum.[4] This

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