THE SYNTHESIS FROM VITAMIN A_1 OF "RETINENE$_1$" AND OF A NEW 545 mμ CHROMOGEN YIELDING LIGHT-SENSITIVE PRODUCTS*

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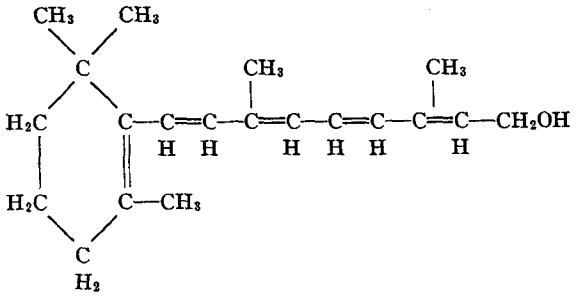
I

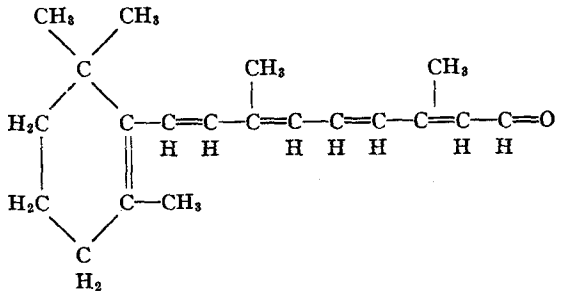
INTRODUCTION

The bleaching of rhodopsin in the retina liberates the yellow carotenoid retinene$_1$, which is subsequently converted to vitamin A_1 (Wald, 1935–36 *a*, *b*). Retinene$_1$ is characterized by an absorption maximum in chloroform solution at 387 mμ (365 mμ in hexane); and yields when mixed with antimony chloride a deep blue product possessing an absorption maximum in the red, at 664 mμ.

Recently R. A. Morton and his coworkers at the University of Liverpool have converted vitamin A_1 synthetically into a product which is comparable in absorp-



Vitamin A_1, $C_{20}H_{29}OH$



Vitamin A_1-aldehyde, $C_{20}H_{28}O$

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tion spectrum and antimony chloride reaction with retinene$_1$ (Ball, Goodwin, and Morton, 1946). They believe this to be vitamin A_1-aldehyde. Morton (1944) had suggested this structure for retinene$_1$ earlier on theoretical grounds.

The presence of an aldehyde group in the synthetic product is substantiated by the preparation of a crystalline 2,4-dinitrophenylhydrazone, and by the formation of a silver mirror with Tollens's reagent (ammoniacal silver nitrate containing a trace of alkali). It is not yet certain, however, that this is the only departure from the structure of vitamin A_1; nor do the properties of Morton's product conform wholly with those reported for vitamin A_1-aldehyde synthesized by other means (Hunter and Hawkins, 1944; Van Dorp and Arens, 1947). The relations between the synthetic substance and retinene preparations from the retina are also not yet entirely clear.

Nevertheless Morton's contribution represents a signal advance. One can be confident that the synthetic product is closely related to if not identical with retinene$_1$. I shall refer to it below as "retinene$_1$," allowing the quotation marks to express such reservations as remain concerning its identity with the natural product.

In the simple procedure devised by Ball, Goodwin, and Morton, a petroleum ether solution of vitamin A_1 is let stand in the cold over solid manganese dioxide for 3 to 4 days, in the course of which most of it is transformed into "retinene$_1$."

On repeating this experiment I found that a basic element in the process is the powerful adsorption of vitamin A_1 by manganese dioxide. Unless the vitamin is present in excess, all of it is adsorbed out of solution; and no "retinene$_1$" ever takes its place. If after a time one elutes the manganese dioxide with ethanol in petroleum ether, one finds not "retinene$_1$" but a new substance which yields with antimony chloride a wine-colored product, with an absorption maximum at 545 mμ. I shall refer to this hereafter as the 545 mμ chromogen.

The further study of these reactions led to a new procedure, by which "retinene$_1$" or the 545 mμ chromogen can be synthesized at will from vitamin A_1 in a matter of minutes. This may be characterized as a controlled *chromatographic oxidation.* In the present paper this process is described, spectral properties of natural retinene$_1$ are compared with those of the synthetic product, and a first account is given of the 545 mμ chromogen. From the latter substance colored products are obtained which strikingly resemble in some of their properties the visual photopigment rhodopsin, itself a natural derivative of vitamin A_1.

II

The Reaction

On examining the original procedure of Ball *et al.*, in which a petroleum ether solution of vitamin A_1 is exposed to solid manganese dioxide, I found the course

of the reaction to be as follows: Manganese dioxide adsorbs vitamin A_1 very strongly, transforming it rapidly to "retinene$_1$." The latter is much less strongly adsorbed, and so is displaced from the manganese dioxide surface by new vitamin A_1 as rapidly as it is formed. Hence one observes that it replaces vitamin A_1 in the supernatant solution. This happens, however, only in the presence of excess vitamin A_1. If no excess is present, the "retinene$_1$" remains adsorbed and is further transformed to the 545 mμ chromogen. Even when excess vitamin A_1 has been employed, and "retinene$_1$" appears in the supernatant, the final charge of vitamin A_1 on the manganese dioxide surface is retained to form the 545 mμ chromogen; and elution of the manganese dioxide at the end of the process yields this substance alone.

Having recognized this situation, I found that the entire procedure can be recast in chromatographic form. The manganese dioxide is packed in the familiar type of chromatographic column. A solution of vitamin A_1 in petroleum ether is poured in at the top, and the desired product is drawn off in the filtrate. Whether this is "retinene$_1$" or the 545 mμ chromogen depends only on whether, with a given amount of vitamin A_1, one uses a short or a long column.

For example, if one begins with a solution of 10 mg. of crystalline vitamin A_1 alcohol in petroleum ether, and about 0.6 gm. of manganese dioxide packed in a column about 1 cm. long and 1 cm. wide, one can pour the vitamin A_1 solution in at the top, wash with further petroleum ether, and within a few minutes draw off under light suction a concentrated solution of "retinene$_1$" as filtrate.

Alternatively, if one uses a longer column (*ca.* 2.5 gm. manganese dioxide in a column about 4 cm. long and 1 cm. wide) the vitamin A_1 is entirely adsorbed, and no carotenoid appears in the filtrate even on prolonged washing with petroleum ether. After washing for 20 to 30 minutes, elution of the column with 5 per cent ethanol in petroleum ether yields a bright yellow solution of the 545 mμ chromogen.

The sequence of the reactions can be demonstrated very simply. One can prepare "retinene$_1$" on a short column of manganese dioxide, then readsorb this on a second column of manganese dioxide. On elution of the latter one obtains the 545 mμ chromogen alone.

Under the circumstances described, the separation of products is virtually complete. The "retinene$_1$" contains little or no admixture of 545 mμ chromogen, the 545 mμ chromogen no observable "retinene$_1$." On elution of the 545 mμ chromogen one does obtain relatively small quantities of other substances, all of which possess absorption bands in chloroform maximal at 360 to 370 mμ, and all of which yield with antimony chloride purple or violet products with absorption bands maximal at 552 to 560 mμ. These are being investigated further.

III

"Retinene$_1$"

The proposal that natural retinene$_1$ is vitamin A_1-aldehyde rests at present primarily on its similarity with the synthetic product in spectrum and antimony chloride reaction. In deference to the observations of Morton's group on the latter substance, still largely unpublished, I should like to do no more in the present section than to confirm the extraordinary correspondences in spectrum between the natural and synthetic substances; and to raise one problem which involves a possible lack of complete identity between them.

Absorption spectra of preparations of the synthetic product and of retinene$_1$ from cattle retinas, both dissolved in chloroform, are shown in Fig. 1. The synthetic material had been partly purified by chromatographic adsorption on calcium carbonate, the cattle retinene$_1$ by adsorption on magnesium oxide. Both preparations have virtually identical spectra, with absorption maxima at about 387 mμ.

The retinenes exhibit peculiar displacements of spectrum with change of solvent, the structural implications of which are discussed below. The natural and synthetic substances maintain a close correspondence of spectrum in all the solvents which I have tried. In both types of preparation, the absorption maxima lie at about 380 mμ in absolute ethanol and at about 365 mμ in hexane.

The spectrum of the blue product obtained by mixing retinene$_1$ with antimony chloride reagent is also shown in Fig. 1. Practically identical spectra are obtained from the synthetic product and natural retinene$_1$, in this case from the squid retina. In both instances the absorption is maximal at 664 to 666 mμ, with a broad inflection in the region 610 to 615 mμ.

In the antimony chloride test with natural retinene$_1$, the density of color rises slowly to a maximum about 90 seconds after mixing the reagents (room temperatures), then slowly falls. The synthetic product displays the same type of behavior, at least to a first approximation.

In preparations of synthetic "retinene$_1$," the ratio of the extinction of the antimony chloride product at about 660 mμ to the extinction of the chloroform solution at 387 mμ, for equivalent concentrations of "retinene$_1$" measured in the same depth of layer, was 1.65–1.68. In a preparation of natural retinene$_1$ from cattle retinas the corresponding ratio was 1.69. (In these experiments the antimony chloride extinction was measured in the Pulfrich photometer (Zeiss), using the S66.6 filter. A measurement of the extinction at 664 mμ made in monochromatic light would have yielded a somewhat higher value, and hence also higher values of the above ratios—actually about 2.9.)

These correspondences are so close that one is tempted to conclude without more hesitation that the synthetic substance is identical with natural retinene$_1$. There are, however, several problems which remain to be clarified, among which I should like to mention one.

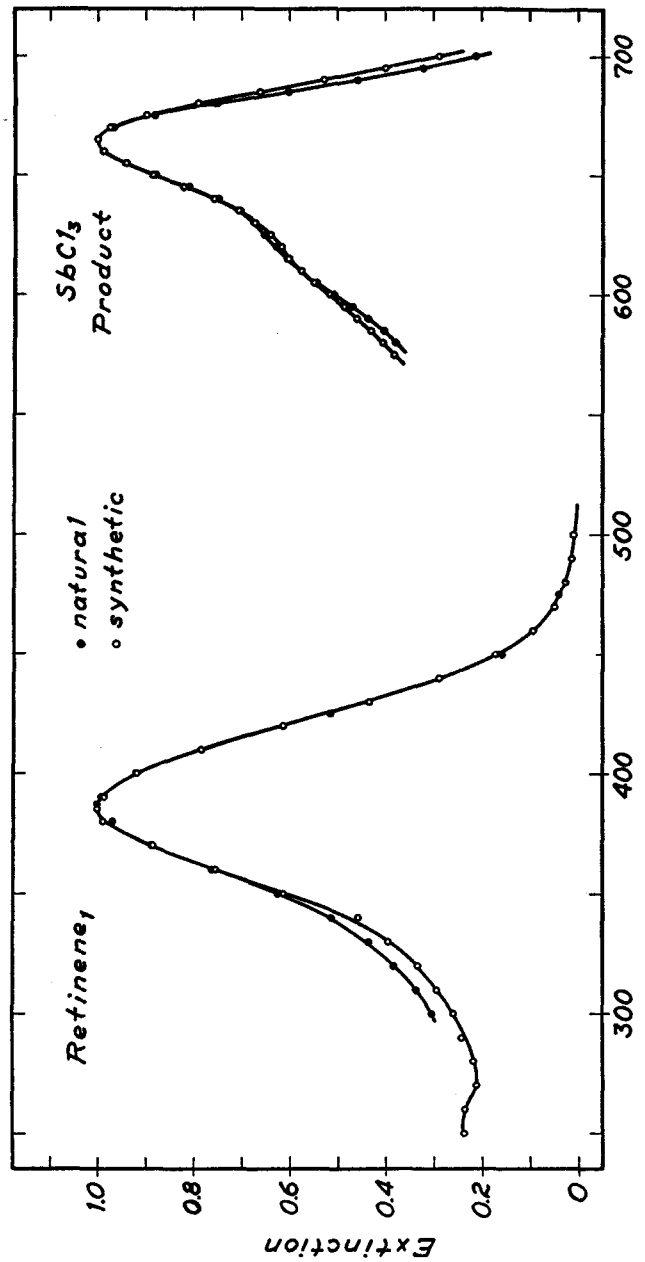


FIG. 1. Comparison of natural retinene$_1$ with the synthetic product. Absorption spectra of cattle retinene$_1$ in chloroform, and of the blue antimony chloride product of squid retinene$_1$, compared with similar preparations of the synthetic substance. The absorption is plotted as extinction or optical density, log I_o/I, in which I_o is the incident and I the transmitted intensity.

Some time ago I found that natural preparations of retinene$_1$ behave as pH indicators, going from relative colorlessness in alkaline solution to deep yellow in acid, with a corresponding shift in absorption spectrum toward longer wavelengths in acid solution (Wald, 1937–38, pages 810–811). This is the primary basis of similar changes in the product of bleaching rhodopsin in solution (Chase, 1935–36); a product which has for this reason been called "indicator yellow" (Lythgoe, 1937), and which I believe to be a retinene$_1$-protein (Wald, 1937–38, page 813). Comparable changes in color with pH are observed in whole retinas (Wald, 1936–37, pages 50–51).

Unpurified preparations of natural retinene$_1$ dissolved in 1 per cent aqueous digitonin shift markedly in absorption spectrum with pH. The absorption maximum lies at about 380 mμ at pH 7, about 385 mμ at pH 4, and about 366 mμ at pH 9.4. The maximum extinction is also highest in alkaline solution, the spectrum growing lower and broader as the solution is acidified.

I have expressed earlier my doubt that these changes are necessarily properties of retinene itself, though I saw no better alternative at the time than to ascribe them to this substance (Wald, 1937–38, page 810). Synthetic "retinene$_1$" however does not change at all in spectrum with pH. What is stranger, neither does natural retinene$_1$ following adsorption on aluminum oxide. We are exploring these relationships further.

IV

The 545 mμ Chromogen

Spectrum.—The spectrum of the 545 mμ chromogen in chloroform is shown in Fig. 2. The main absorption band lies in the near ultraviolet at about 380 mμ, and a secondary maximum appears at about 290 mμ.

With change of solvent the spectrum undergoes the same peculiar type of displacement as is found in the retinenes. This is shown in Fig. 3. In absolute ethanol the band maxima lie at about 376 and 290 mμ, in hexane at about 361 and 277 mμ.

When mixed with antimony chloride reagent this substance yields an immediate deep purplish red product, the spectrum of which also is shown in Fig. 2. It possesses the main maximum at about 545 mμ by which the substance is temporarily designated. A secondary maximum appears at about 328 mμ. The latter lies very close to the position of the absorption band of vitamin A_1 in chloroform. It probably means that the antimony chloride product includes a conjugated polyene system of the same length as is found in vitamin A_1 (5 conjugated double bonds) in addition to the more extended system responsible for the main band.

The 545 mμ chromogen does not fluoresce, either in solution, or in the adsorbed condition.

Acid-Base Properties.—From the mode of formation of the 545 mμ chromogen

one might suppose it to be a higher oxidation product of vitamin A_1 than the aldehyde, perhaps therefore vitamin A_1 acid. Its properties, however, do not

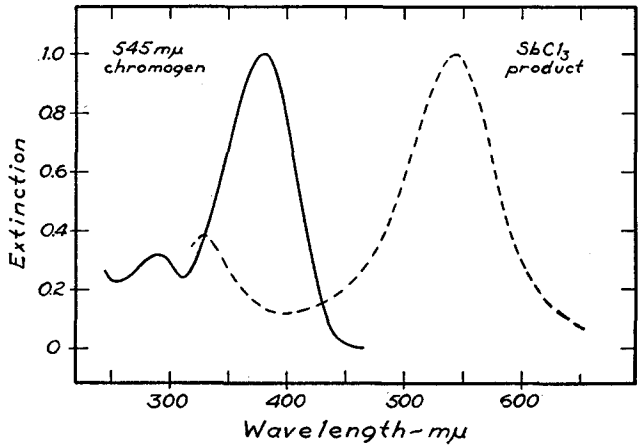


FIG. 2. Absorption spectra of the 545 mμ chromogen in chloroform, and of its wine-colored product with antimony chloride.

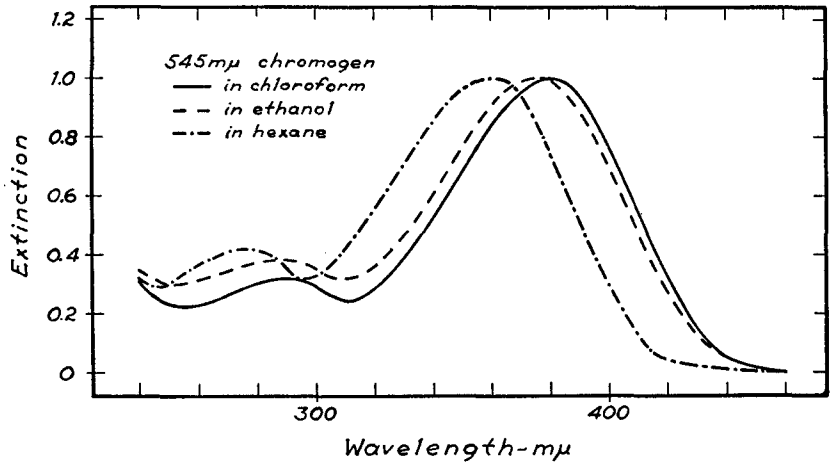


FIG. 3. Absorption spectra of the 545 mμ chromogen in chloroform, ethanol, and hexane. The spectrum displays a peculiarly large displacement between hexane and polar solvents, due apparently to the presence of a conjugated carbonyl group.

agree with those reported for vitamin A_1 acid (Arens and Van Dorp, 1946; Karrer, Jucker, and Schick, 1946); nor does it display any acidic character whatever.

On the contrary this substance behaves in solubility like a weak base, com-

parable in this with a nitro- or chloroaniline. It is taken up strongly by mineral acids with the formation of highly colored products. Concentrated sulfuric acid removes it completely from hexane solution, forming a salmon-colored product with an absorption maximum at about 520 mμ. Concentrated hydrochloric acid (37 per cent) also extracts it completely from hexane, forming an unstable orange-red product with a maximum at about 500 mμ. Dilutions of hydrochloric acid down to about 27 per cent still extract perceptible color from hexane solutions of the pigment. Still higher dilutions of acid remain uncolored, but a purplish red material forms in the acid-hexane interface.

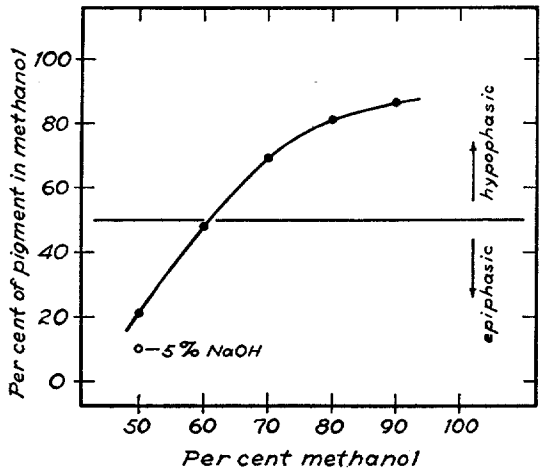


FIG. 4. Partition of the 545 mμ chromogen between petroleum ether and various concentrations of aqueous methanol. The extinctions of pigment in both layers have been added together, and the percentage of the total extinction found in the methanol layer is plotted as ordinate.

The 545 mμ chromogen shares this mildly basic character, as also the formation of highly colored products with mineral acids, with a number of carotenoid pigments, all of which represent higher levels of oxidation than the dihydroxy-carotenoids: flavoxanthin, $C_{40}H_{56}O_3$; capsanthin, $C_{40}H_{58}O_3$; capsorubin, $C_{40}H_{60}O_4$; violaxanthin, $C_{40}H_{56}O_4$; azafrin, $C_{27}H_{38}O_4$; fucoxanthin, $C_{40}H_{56}O_6$. This is a first indication, therefore, that the 545 mμ chromogen may be a poly-oxy-carotenoid.

Partition.—The 545 mμ chromogen is extraordinarily hypophasic. In partition between petroleum ether and various dilutions of methanol, it shows very great tendency to enter the methanol layer. The quantitative results of a graded series of such partitions are shown in Fig. 4. In each case the pigment was distributed by violent shaking between equal volumes of petroleum ether

and aqueous methanol. The amounts of pigment entering each layer were determined by measuring the maximal extinctions, at 360 mμ in petroleum ether, and at 375 to 385 mμ in the various concentrations of methanol. The figure shows the percentages of total pigment entering the methanol layer.

These measurements show that the 545 mμ chromogen is hypophasic even with 65 per cent methanol. It distributes about equally between petroleum ether and 60 per cent methanol. With 50 per cent methanol about 20 per cent still enters the methanol layer; and this fraction is approximately halved with 5 per cent sodium hydroxide in 50 per cent methanol, showing the absence of acidic character.

The 545 mμ chromogen therefore is as highly hypophasic as the most hypophasic of the carotenoid alcohols. In its partition behavior it resembles fucoxanthin, a C_{40} carotenoid possessing 4 to 5 hydroxyl groups, and probably 1 or 2 carbonyls.

Adsorption.—Like the higher carotenoid alcohols also, the 545 mμ chromogen is strongly adsorbed on calcium carbonate. On columns of this substance it is adsorbed close to the top as a rather diffuse yet homogeneous yellow zone, which moves downward extremely slowly on prolonged washing with petroleum ether. With mixtures of petroleum ether and benzene (C_6H_6) 1:1 to 1:3, the pigment moves at moderate speed down the column, becoming increasingly diffuse, yet remaining a single zone.

Photosensitive Derivatives.—Carotenoids—including the retinenes and vitamins A—quite generally form with antimony chloride highly unstable colored products which fade rapidly. The wine-colored product formed by the 545 mμ chromogen, however, is quite stable, at least for a period of hours.

This product displays a curious and I believe significant property. *It is highly photosensitive.* In intense light it bleaches in 1 to 2 minutes to an almost colorless condition.

The course of such a bleaching experiment is recorded in Fig. 5. The antimony chloride product is originally stable in darkness. On exposure to a moderately bright light, it bleaches partially (solid lines). It appears to recover in part in subsequent periods in darkness (broken lines). It is clear from this experiment that the antimony chloride product displays a high order of light sensitivity.

The colored products which the 545 mμ chromogen yields with sulfuric and hydrochloric acids are also markedly photosensitive. The salmon-pink sulfuric acid product bleaches to yellow-orange, the maximum shifting from 520 mμ toward shorter wavelengths. The orange-red hydrochloric acid product, as has been remarked, is highly unstable even in darkness; but on exposure to sunlight it bleaches with enormously increased rapidity, losing virtually all its color within about 15 seconds.

V

General Considerations

Chromatographic Reactions.—The process which has been described for converting vitamin A_1 to "retinene$_1$" or to the 545 mμ chromogen is of considerable general interest. In such a chromatographic process, one is dealing with a highly oriented type of reaction. The solid, in this case manganese dioxide, is

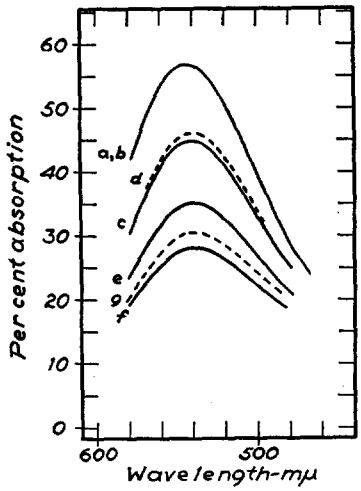


FIG. 5. Bleaching of the 545 mμ antimony chloride product in moderate light. The spectrum of this solution was measured immediately after mixing the reagents (*a*). After 7 minutes in darkness it was found unchanged (*b*). It was irradiated for 30 seconds with a 60 watt tungsten lamp at a distance of about 9 inches (*c*). Left dark for 10 minutes longer, the absorption *rose* to (*d*). It was re-irradiated as before for 1 minute (*e*), then for 1 minute longer (*f*). Left in darkness for another 32 minutes, the spectrum again *rose* to (*g*). The curves shown are tracings of spectra measured with the recording photoelectric spectrophotometer of Hardy.

at once adsorbent and reagent. The adsorbed molecules are attached to it at one or more specific points, and it is presumably at these that the reaction occurs.

One may expect from such an arrangement a high degree of specificity, direction, and control, mimicking on occasion the character of an enzymic process. The products may be expected to be more restricted in number and on occasion different in kind from those obtained in comparable reactions in free solution. From this viewpoint the use of solid adsorbents which react with their adsorbates deserves careful systematic investigation.

It is an instance of the extraordinary insight of Michael Tswett, the founder of chromatography, to have foreseen such a development. In his 1906 paper

he remarks: For special purposes, however, one will turn just to chemically effective adsorbents (hydrolyzing, reducing, oxidizing).

Structure of the 545 Mμ Chromogen.—Determination of the detailed structure of this substance must await its study by degradation, synthesis, and the assay of specific groups. One can gain some insight into its general nature, however, from its mode of origin and the properties already described.

This product of the mild oxidation of vitamin A_1 probably also contains twenty carbon atoms. The displacement of its absorption spectrum toward the red from that of vitamin A_1 indicates a longer conjugated system. For the reasons which follow, it seems probable that this is achieved by adding to the polyene structure of vitamin A_1 a conjugated carbonyl group.

The evidence that "retinene$_1$," the precursor of the 545 mμ chromogen, contains an aldehydic carbonyl (Ball, Goodwin, and Morton, 1946) provides a

TABLE I

Refractive indices and dipole moments of pentane and hexane (which together effectively constitute petroleum ether), absolute ethanol, and chloroform.

Solvent	Refractive index, n_D	Temperature	Dipole moment
		°C.	*Debye units*
Pentane	1.358	15.7	0
Hexane	1.375	20	0
Ethanol	1.361	20	1.696
Chloroform	1.446	20	1.18

first indication of this. But this matter can be approached independently through spectroscopic data, which reflect not only upon the structure of the 545 mμ chromogen but on that of retinene$_1$.

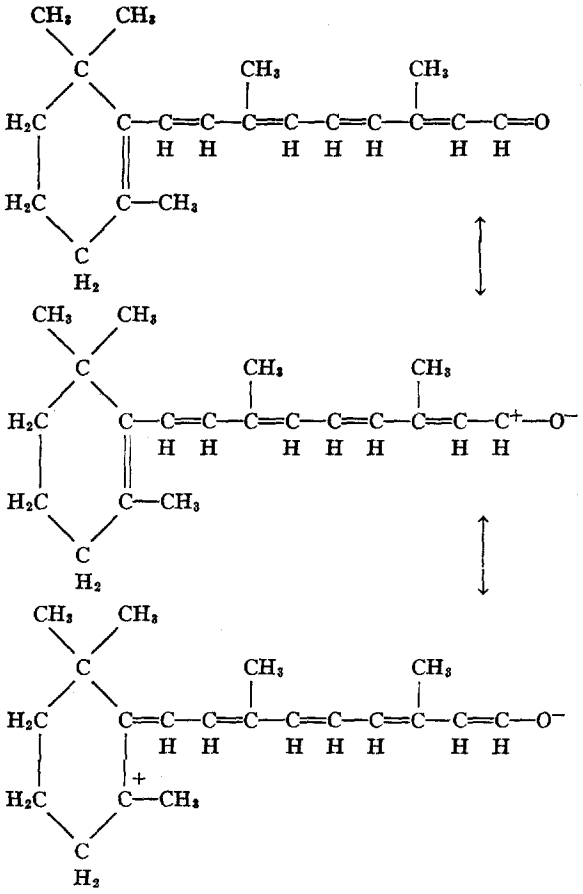
The spectra of substances in solution ordinarily are displaced toward longer wavelengths with increase in refractive index of the solvent (Kundt's rule). This is true also of carotenoids and synthetic polyenes. On this basis the spectrum should shift little or not at all when a carotenoid is transferred from hexane or petroleum ether to ethanol, which has an almost equal refractive index; but should be displaced markedly on transferring to the much more highly refringent chloroform (Table I).

This is the type of displacement observed in the great majority of carotenoids. Between hexane and ethanol the spectrum is shifted only 0 to 2 mμ, between either of these solvents and chloroform 9 to 15 mμ (Table II).

The vitamins A (A_1 and A_2) behave also in this manner. The retinenes, however, present an entirely different type of relation. In them there is an abnormally large shift of spectrum between hexane and chloroform (about 20 mμ); and the spectrum in ethanol lies close to that in chloroform. Among

carotenoids of known structure this peculiar relation is found also in rhodoxanthin. One finds it again in the 545 mμ chromogen (Fig. 3 above; Table II).

Its significance appears to be as follows. Rhodoxanthin ($C_{40}H_{50}O_2$) possesses two carbonyl groups in conjugation with its polyene system. According to Morton, "retinene$_1$" also possesses a conjugated carbonyl group. The carbonyl radical is highly polar, and the positive charge on the carbon atom tends to be transmitted down the length of the polyene chain. Such polyene carbonyl compounds therefore exist as resonance hybrids of a number of alternative structural arrangements. In the case of vitamin A_1-aldehyde, for example, the limiting states may be diagrammed as follows:—



Such resonance hybrids exhibit a special shift of absorption spectrum toward the red.[1] Thus, while the addition of an ordinary ethylene group (—CH =

[1] A statement of this general relation and references can be found in Pauling (1944), pages 281 and 431.

CH—) to a conjugated polyene chain shifts the spectrum about 20 to 25 mμ toward the red, the addition of a conjugated carbonyl shifts it about 30 to 40 mμ (compare for example the spectra of α- and β-apo-2-carotinols with those of the corresponding aldehydes (von Euler, Karrer, and Solmssen, 1938)). Such a large shift in spectrum (40 mμ in hexane) is found in retinene$_1$ as compared with vitamin A_1; and a slightly smaller but comparable shift (35 mμ in hexane) is found in the 545 mμ chromogen compared with vitamin A_1.

This special effect of the carbonyl group is enhanced in solvents which are themselves polar. In these one finds *superimposed* upon the spectral changes

TABLE II

Absorption maxima of carotenoids in hexane (or petroleum ether), ethanol, and chloroform showing the displacement of spectrum in a few representative carotenoids, and the special position which such a carbonyl-containing carotenoid as rhodoxanthin shares with retinene$_1$ and the 545 mμ chromogen. When the spectrum has multiple bands, that of longest wavelength is given. The column II − I shows the shift of spectrum in mμ between hexane and ethanol, the column III − II that between chloroform and ethanol.

Carotenoid	I Hexane	II Ethanol	II − I	III Chloroform	III − II
α-Carotene	478	476	−2	485	9
β-Carotene	482	482	0	497	15
Cryptoxanthin	484	486	2	497	11
Lutein	476	476	0	488	12
Violaxanthin	472	472	0	484	12
Vitamin A_1	325	325	0	332	7
Rhodoxanthin	524	538	14	546	8
Retinene$_1$	365	380	15	387	7
545 mμ chromogen	360	376	16	380	4

which go with refractive index of the solvent a special effect correlated with the dipole moment of the solvent. While hexane and ethanol are very similar in refractive index, in dipole moment ethanol comes close to chloroform, and is far removed from the homopolar hexane or pentane (Table I).

In carotenoids which contain a conjugated carbonyl group, therefore, the displacement of spectrum between hexane and chloroform is abnormally large; and the spectrum in ethanol tends to approach that in chloroform. This type of behavior in the retinenes and the 545 mμ chromogen is *prima facie* evidence of the presence of such a conjugated carbonyl group.[2]

[2] It has been noted a number of times that carotenoids and synthetic polyenes which contain carbonyl groups in conjugation with the polyene system exhibit peculiarly large changes in color or spectrum on transfers between hexane and alcohol (Zechmeister and von Cholnoky, 1935; Hausser, Kuhn, Smakula, and Hoffer, 1935; Zechmeister and Tuzson, 1936).

The further properties of the 545 mμ chromogen indicate the presence of hydroxyl groups. Its highly hypophasic character in partition between petroleum ether and aqueous methanol, its strong adsorption on calcium carbonate, and its mildly basic character and production of colored products with mineral acids, all range it among the higher carotenoid alcohols. Its behavior in all these situations is comparable, for example, with that of fucoxanthin, a C_{40} carotenoid containing 4 to 5 hydroxyl groups and probably 1 or 2 carbonyls.

What is decisive in such structures is probably the ratio of polar groups to the length of hydrocarbon chain. From this viewpoint the similarity in behavior to fucoxanthin would suggest in such a C_{20} carotenoid as the 545 mμ chromogen the presence of two hydroxyls. This inference is consistent with the observation that in those properties which seem to depend upon hydroxyl groups, the 545 mμ chromogen clearly surpasses vitamin A_1, which possesses a single hydroxyl.

Considerations such as these can never provide more than a guide to further study; but the net result of this argument is that there is good reason to believe the 545 mμ chromogen to be a hydroxy-carbonyl derivative of vitamin A_1, which probably contains two hydroxyls in addition to one carbonyl group.

Relation to Visual Photopigments.—From the viewpoint of visual chemistry, it is obviously a stimulating observation that from vitamin A_1, the natural precursor of rhodopsin, one can obtain synthetic derivatives which bear some resemblance to the photosensitive pigments of the retina in spectrum, and which are themselves light-sensitive. Such are the products of the action of antimony chloride or mineral acids upon the 545 mμ chromogen. They have a more general status, which I shall discuss in a later paper.[3]

I think it very probable that in such products one approaches closely the fundamental structures of the visual photopigments; and that the essential process in their formation is the union of two such molecules as the 545 mμ chromogen—or indeed the retinenes—with the loss of water.[4] This matter also must be reserved for a later paper.

[3] *Note added in proof.*—After this was written I found that the blue products of the action of antimony chloride on retinene$_1$ (natural or synthetic) and on vitamin A_1 also are light-sensitive. These products break down even in the dark, that from retinene$_1$ slowly, that from vitamin A_1 rapidly. In both cases light greatly speeds the dissipation of the blue color. This action of light on the vitamin A_1-antimony chloride product has been reported earlier by Caldwell and Parrish (*J. Biol. Chem.*, 1945, **158,** 181). As these authors note, in using the antimony chloride reaction for the quantitative estimation of vitamin A_1, the photosensitivity of the blue color makes it important to use a photometer or colorimeter which is sparing of light. This precaution must also be extended to retinene$_1$.

[4] Meunier and Vinet (1945) have suggested that a yellow pigment obtainable from vitamin A_1 on chromatographing on calcium hydroxide or on certain acid earths, or on treatment with antimony chloride, is di-vitamin A_1 ether, the product of condensing two molecules of vitamin A_1 with elimination of water.

SUMMARY

Ball, Goodwin, and Morton (1946) have reported that vitamin A_1 in contact with solid manganese dioxide is transformed slowly into a substance which displays spectroscopic properties of retinene$_1$. The latter is known to be the precursor of vitamin A_1 in the rhodopsin cycle of the retinal rods. The synthetic product is here referred to as "retinene$_1$."

In the present experiments this observation is confirmed. The procedure is recast in the form of a chromatographic oxidation. Manganese dioxide is packed in a column, vitamin A_1 solution poured in at the top, and the product drawn off in the filtrate. Depending upon the proportions of manganese dioxide and vitamin A_1, the product is either "retinene$_1$," or a new substance which yields with antimony chloride a wine-red product with maximal absorption at 545 mμ (545 mμ chromogen). This procedure is an example of a potentially important class of chromatographic reactions.

The synthetic "retinene$_1$" is virtually identical with the natural substance in absorption spectrum and antimony chloride reaction. It lacks the pH indicator properties of crude natural retinene$_1$.

The 545 mμ chromogen possesses absorption maxima at *380* and 290 mμ in chloroform; at *376* and 290 mμ in ethanol; and at *361* and 277 mμ in hexane. It is non-fluorescent. It has no acidic character, but on the contrary is mildly basic, being extracted from hexane by sulfuric or hydrochloric acids to form orange-red products. In partition between petroleum ether and aqueous methanol it is highly hypophasic. It is adsorbed strongly on calcium carbonate.

Certain peculiarities in spectral behavior indicate the presence of a carbonyl group in the 545 mμ chromogen, and support Morton's proposal that such a group occurs in retinene$_1$. Other properties of the 545 mμ chromogen indicate hydroxyl groups. This substance therefore appears to be a hydroxy-carbonyl derivative of vitamin A_1.

The red products which the 545 mμ chromogen forms with antimony chloride or with sulfuric or hydrochloric acids are all markedly light-sensitive. They appear to be formed by the condensation of two molecules with loss of water; and to bear a close generic relation to the prosthetic groups of the visual photopigments.

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