GEOMETRICAL ISOMERS OF RETINENE

By RUTH HUBBARD, ROBERT I. GREGERMAN, AND GEORGE WALD*

(From the Biological Laboratories, Harvard University, Cambridge)

(Received for publication, September 22, 1952)

We have described experiments involving the use of five crystalline isomers of retinene: the ordinary crystalline substance known previously from the work of Ball, Goodwin, and Morton (1948); neoretinenes *a* and *b*, first isolated in our laboratory; and isoretinenes *a* and *b*, isolated in the Organic Research Laboratory of Distillation Products Industries (Hubbard and Wald, 1952; 1952–53). By all indications these substances are cis-trans isomers of one another. Ordinary crystalline retinene apparently is the all-trans isomer; while neoretinenes *a* and *b* and isoretinene *a* appear to be mono-cis forms, and isoretinene *b* a di-cis isomer.

This series of retinenes offers an unusual opportunity to examine interrelationships within a compact stereoisomeric "set." It has, however, also a special interest. According to the present theory of cis-trans isomerism in such isoprenoid structures as the carotenoids, the cis configuration occurs readily only at the double bonds adjacent to methyl groups (Pauling, 1939; Zechmeister, 1944). Retinene has two such bonds, numbered *3* and *5* in Fig. 1. Consequently only four stereoisomers of retinene are expected: the all-trans, *3*-cis, *5*-cis, and *3,5*-di-cis.

We have, however, five isomers. Specifically, we have three apparently mono-cis isomers where only two were expected; but if all these are genuine, one must look also for three di-cis forms and one tri-cis retinene—eight isomers in all where four were expected.

These substances therefore pose a dilemma. Either they are not all geometrical isomers of one another; or the theory of cis-trans isomerization in this class of compounds must be expanded. In this paper we examine the properties and interconversions of the isomeric retinenes with an eye to this problem.

* This research was supported in part by the Office of Naval Research. We have already expressed elsewhere (Hubbard and Wald, 1952–53) our great indebtedness to the Organic Research Laboratory of Distillation Products Industries of Rochester, New York, for repeated gifts of crystalline vitamin A and retinene, and for permission to use unpublished data on the characteristics of their retinene preparations.

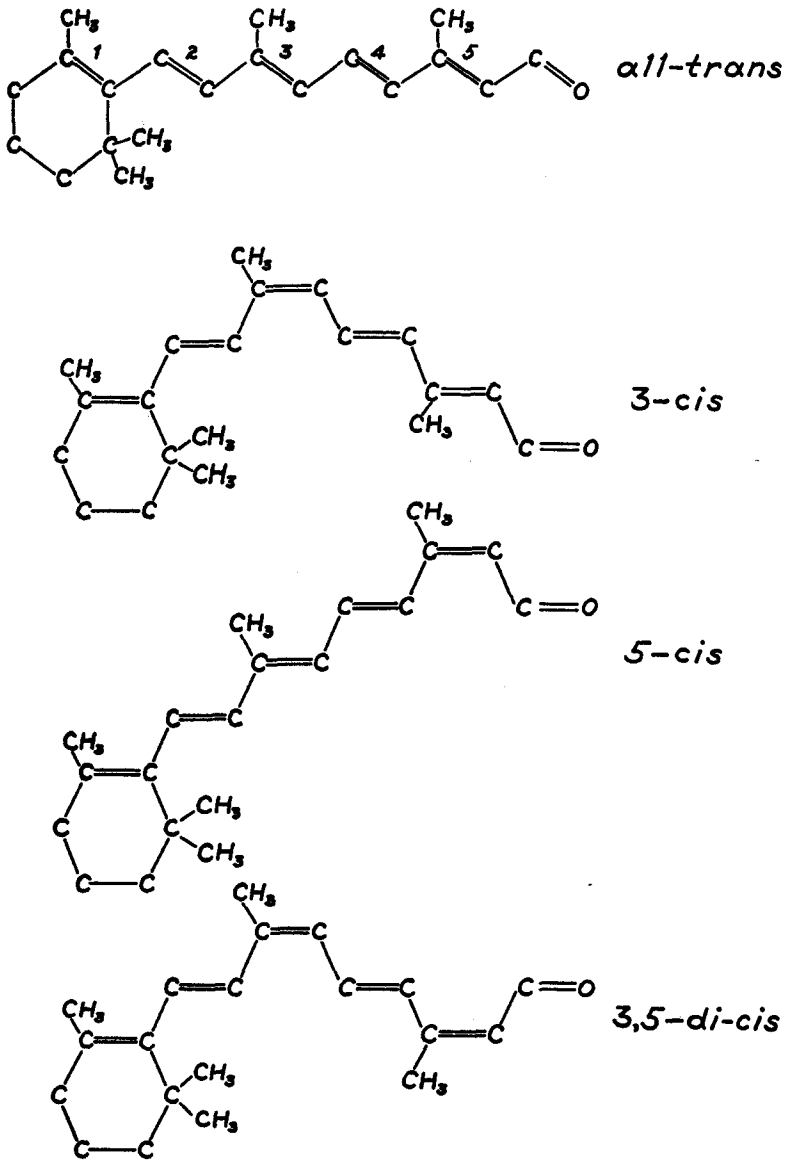

FIG. 1. Geometrical isomers of retinene. The four configurations shown are those expected to be most prevalent according to the present theory of cis-trans isomerism in this class of compounds.

Absorption Spectra

The absorption spectra of the retinene isomers in ethyl alcohol are shown in Fig. 2. The absorption constants for these and similar preparations in ethanol

are brought together in Table I. The way in which these characteristics vary in several solvents is shown in Table II.[1]

The principal absorption maxima are spaced as expected in stereoisomeric carotenoids. Zechmeister has shown with the plant carotenoids that a single cis linkage tends to shift the absorption maximum of longest wave length 4 to 6 mμ toward shorter wave lengths; two cis linkages tend to shift it about twice as far (Zechmeister, 1944). The main absorption band of all-trans retinene in ethyl alcohol is at 383 mμ; the presumptive mono-cis isomers (neoretinenes *a* and *b* and isoretinene *a*) are grouped together at 376 to 377.5

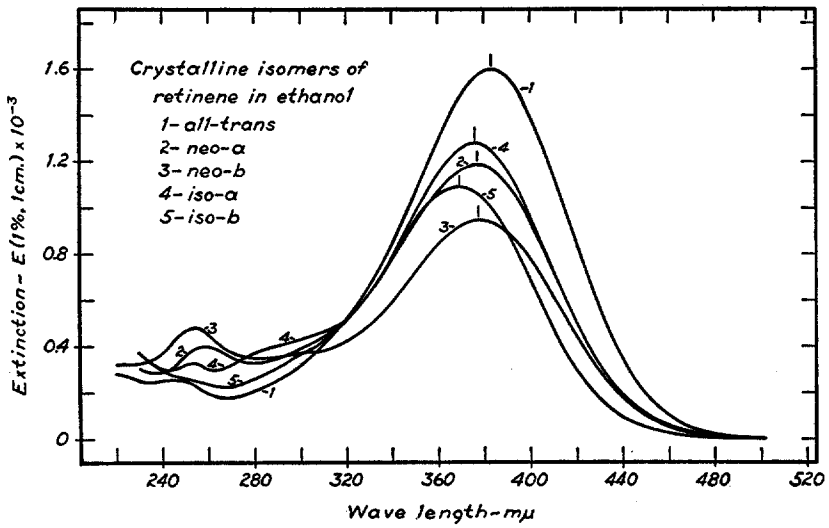

FIG. 2. Absorption spectra of crystalline retinene isomers in ethyl alcohol. The λ_{max} of each spectrum is marked with a bar. Each presumptive cis linkage shifts the λ_{max} 5.5 to 7 mμ toward shorter wave lengths.

mμ; and isoretinene *b*, the presumptive di-cis structure, lies at 369 mμ. The displacement of spectrum in ethanol appears therefore to be 5.5 to 7 mμ per cis linkage.[2]

[1] The symbol λ_{max} represents the wave length of maximum absorption. *E* (1 per cent, 1 cm.) is the extinction of a 1 per cent solution, weight by volume, measured in a layer 1 cm. in depth. It should be added that Dalvi and Morton (1951) have described a partly purified, non-crystalline preparation of what seems to be primarily neoretinene *a*. The λ_{max} of this preparation was 365 mμ in petroleum ether, 380 mμ in ethanol, and 385 mμ in chloroform. No *E* (1 per cent, 1 cm.) is reported.

[2] The retinene oximes, formed by the condensation of various stereoisomers of retinene with hydroxylamine, show similar displacements of spectrum. So, for example, all-trans retinene oxime in 1 per cent digitonin solution has λ_{max} 367 mμ; neoretinene *a* oxime, 363 mμ; and isoretinene *a* oxime, 361 mμ.

TABLE I

Crystalline Isomers of Retinene

The data assembled in this table were obtained from the paper of Ball, Goodwin, and Morton (1948); in personal communications from the Organic Research Laboratory of Distillation Products Industries; and from the work of Gregerman and Hubbard in our own laboratory. Our measurements of λ_{max} tend to run 1 to 2 mμ higher than those made at Distillation Products Industries, and are included in the table in parentheses.

Isomer	Prepared by	Absorption spectrum in ethanol		Melting point
		λ_{max}	*E (1 per cent, 1 cm.)*	
All-trans	Ball *et al.*	385.5	1400	61–62°
	Gregerman	383	1660	61–62°
	Hubbard	383	1600	
	Dist. Prod.	381 (383)	1520	
Neoretinene *a*	Gregerman	377	1190	
	Dist. Prod.	375.5 (377)	1198	75°
	Dist. Prod.	375 (377)	1235	
Neoretinene *b*	Hubbard	377.5	(900–1000)	
Isoretinene *a*	Dist. Prod.	374 (376)	1270	64.5°
Isoretinene *b*	Dist. Prod.	368 (369)	1090	

TABLE II

Absorption Characteristics of Retinene Isomers in Various Solvents

Isomer	Solvent						
	Petroleum ether		Ethyl alcohol		Chloroform		1 per cent aqueous digitonin
	λ_{max}	*E*(1 per cent, 1 cm.)	λ_{max}	*E*(1 per cent, 1 cm.)	λ_{max}	*E*(1 per cent, 1 cm.)	λ_{max}
All-trans	369	1720	383	1600	390	1520	389
Neoretinene *a*	364	1290	377	1190	385	1190	381
Neoretinene *b*	363	—	377.5	(900–1000)	386	—	384
Isoretinene *a*	362.5	1360	376	1270	384	1265	382
Isoretinene *b*	—	—	369	1090	378.5	—	375

The extinction of the long wave length band also behaves as expected within a stereoisomeric set. The all-trans isomer has the highest extinction, the cis forms ranging at lower values.

The absorption in the region 255 to 260 mμ represents what Zechmeister has called a "cis peak." Its presence is evidence of a cis linkage, and its height expresses in general the degree to which such a linkage bends the molecule.

The absorption in this region is very low in all-trans retinene, somewhat higher yet poorly defined in isoretinene *a*, still higher and well defined in neoretinene *a*, and highest of all in neoretinene *b*. In isoretinene *b* it has returned almost to the level of the all-trans isomer. If nothing more is involved in this phenomenon than the bending of the molecule, one could conclude that all-trans retinene and both isoretinenes are relatively straight, while the neoretinenes are markedly bent, *b* much more than *a*. The difficulty with so simple a view is that since we have more apparently mono-cis retinenes than the simple theory encompasses, a new theory may be needed; and this could involve new considerations which affect the height of the cis peak.

It may be significant, particularly in association with other characteristics to be discussed below, that in the ultraviolet the isoretinenes have a somewhat different type of spectrum from the other isomers. Below 240 mμ their absorption rises so as to cross the other spectra; and isoretinene *a*, though it has little absorption in the cis peak region, possesses a broad hummock of absorption in the region 270 to 310 mμ.

Antimony Chloride Reaction

All five isomers of retinene, on mixing with saturated antimony chloride solution in chloroform, yield an instantaneous blue color the absorption spectrum of which is identical for all. An example of this is shown in Fig. 3. The dominant absorption band has its λ_{max} at 666 mμ, and possesses a wide shoulder centering at about 610 mμ. There is also a small, narrow peak at 420 to 425 mμ, and between this and the main band there is a minimum at about 493 mμ.

The spectrum of the antimony chloride product is also quantitatively the same with all the isomers. The direct absorption spectra of the various isomers, measured at one absolute concentration, have very different extinctions; *E* (1 per cent, 1 cm.) varies from about 1600 in all-trans retinene to 900 to 1000 in neoretinene *b* (Fig. 2). At one absolute concentration, however, all the retinene isomers yield the same extinction in the antimony chloride test. If one wishes to have a measure of retinene concentration—and presumably also of vitamin A—that is independent of cis-trans configuration, this is the way to do it. Regardless of the stereoisomer employed, the antimony chloride test with retinene yields an *E* (1 per cent, 1 cm., 666 mμ) of 3780, with an average deviation of 140 (Table III).[3]

The obvious conclusion to be drawn from these measurements is that all the isomers of retinene yield a single product with antimony chloride. This is an extraordinary demonstration of the closeness of their relationship.

It also provides support for an argument presented several years ago by

[3] Ball, Goodwin, and Morton (1948) state the *E* (1 per cent, 1 cm., 664 mμ) of the antimony chloride product with crystalline (all-trans) retinene to be 3400.

Meunier and Vinet (1947). These workers suggested that the blue color which results when vitamin A is treated with certain acid earths, mineral acids, or antimony chloride represents an ionic state of the vitamin—a vitamin A cation. The greatly increased resonance that accompanies the ionic state is responsible for the large change in color; *i.e.*, for the shift of absorption spec-

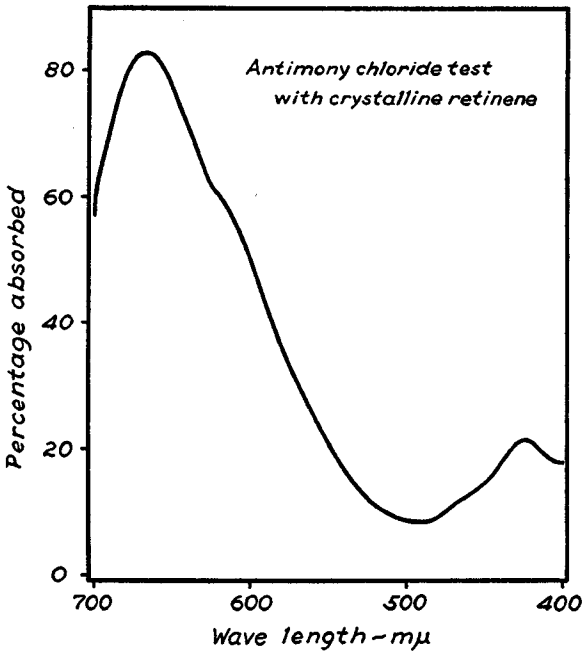

FIG. 3. Absorption spectrum of the blue product formed on mixing neoretinene *b* with antimony chloride. The curve shown was drawn by a recording photoelectric spectrophotometer in the first minute after mixing the reagents. All five isomers of retinene give the same result; the spectra for all of them vary no more than successive trials with a single isomer. In the test shown, the final mixture contained 2.01 μg. retinene per ml.; and since the extinction at 666 mμ is 0.775, the E (1 per cent, 1 cm., 666 mμ) is 3855.

trum from the ultraviolet to the red. This then is an example of the phenomenon known as halochromy—literally, color through salt formation.

The increased resonance also, according to Meunier and Vinet, forces the molecule into the all-trans configuration, which imposes least restriction on resonance. For this reason Meunier and Vinet predicted that whatever stereoisomer entered the antimony chloride reaction, a single all-trans product should result.

Our observations are in good accord with this view. To be sure, we do not

know that the antimony chloride product, in addition to being identical for all the isomers, is exclusively all-trans. It could equally well represent some constant mixture of stereoisomers, in which presumably the most stable, all-trans configuration predominates.

TABLE III

Antimony Chloride Tests with Retinene Isomers

In each test, 1 ml. of a stock solution of retinene in chloroform was mixed with 2.3 ml. of a saturated chloroform solution of antimony chloride, and the extinction at 666 mμ was measured at once. Column (1) shows the extinction of retinene in the stock solutions, measured at λ_{max}; column (2) the E (1 per cent, 1 cm.) of the various isomers (the value for isoretinene *b* is the E (1 per cent, 1 cm.) in ethanol). Column (1) divided by (2) yields the percentage concentrations of the stock solutions. Mixing with antimony chloride dilutes the concentrations 3.3 times, resulting in the final concentrations shown in (3). (4) Extinctions at 666 mμ of the antimony chloride product. These divided by the concentrations (3), and multiplied by 10,000 to bring to 1 per cent concentration, yield the values of E (1 per cent, 1 cm., 666 mμ) of column (5).

Isomer	(1) Retinene extinction E_{max}	(2) E(1 per cent, 1 cm.): retinene	(3) Concentration in $SbCl_3$ mixture	(4) E_{666}: $SbCl_3$ product	(5) E(1 per cent, 1 cm., 666 mμ): $SbCl_3$ product
			μg./ml.		
All-trans	0.685	1520	1.37	0.544	3970
Neoretinene *a*	0.673	1190	1.71	0.590	3450
Neoretinene *b*	0.618	(950)	1.97	0.757	3840
Isoretinene *a*	0.700	1265	1.68	0.628	3740
Isoretinene *b*	0.649	(1090)	1.80	0.698	3880
Average					3780 ± 140

Isomerization by Light

All the isomeric retinenes are converted by light to what seems to be the same equilibrium mixture of isomers. Light, however, also destroys retinene, as it does carotenoids generally. To hold destruction to a minimum, we irradiate for as short a time as adequate, with light from which the ultraviolet has been excluded with a Corning Noviol A filter (3389), transmitting only wave lengths longer than 410 mμ. In this way not only the amounts of radiation but the sizes of quanta employed are kept as small as possible.

Fig. 4 shows the effects of isomerization by light upon the absorption spectra of all-trans retinene and neoretinene *b*. These two isomers lie at opposite extremes in extinction of the main band and cis peak absorption, yet the spectra of their isomerization products fall very close together. The conditions of isomerization in this instance were not such as to ensure equilibrium, or the agreement might have been better. The E (1 per cent, 1 cm., 380 mμ) of

the isomerization product in ethyl alcohol is about 1100. The product of irradiating all-trans retinene reveals its content of neoretinenes *a* and *b* in the rise of the cis peak at about 250 mμ, while the appearance of a broad band of absorption in the region of 300 mμ is caused by isoretinene *a* (compare Fig. 2).

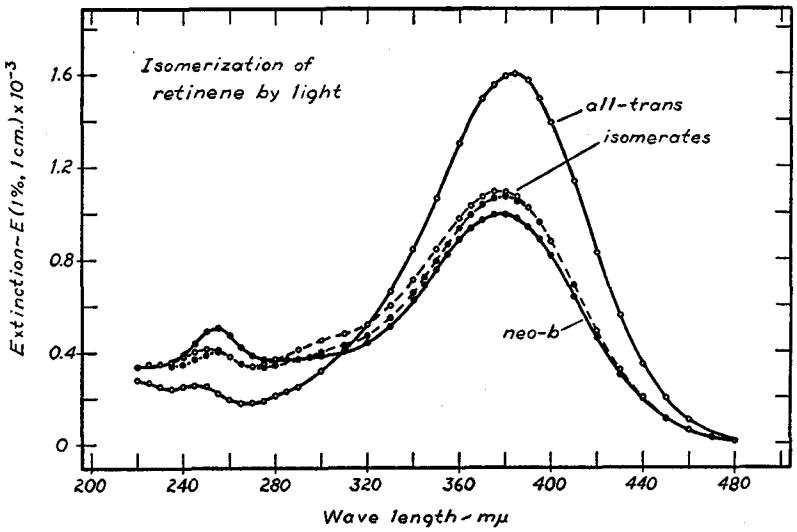

FIG. 4. The effect of exposure to light upon the spectra of all-trans retinene and neoretinene *b* in ethyl alcohol. The all-trans isomer was irradiated for 12 minutes with the light from a 160 watt micrsocope lamp filtered through Corning glasses 3389, to eliminate radiations shorter than 410 mμ, and 3966, to filter out heat radiation. The neoretinene *b* was exposed for 2 minutes to the light of the same lamp filtered through Corning glass 3060, which removes wave lengths below 380 mμ, and heat filter 3966. It is not certain that either solution had attained stereoisomeric equilibrium as the result of this treatment, yet with minor differences the spectra of the isomerization products lie very close together.

On irradiation the individual retinene isomers exhibit changes of spectrum which are in some cases so characteristic as to be useful in identifying them. On isomerization with light, the main absorption band of all-trans retinene moves 5 to 6 mμ toward shorter wave lengths; that of isoretinene *b* moves 7 to 9 mμ toward longer wave lengths; while those of all the presumptive mono-cis isomers either remain fixed or are shifted 1 to 2 mμ toward longer wave lengths. On guarded irradiation, the main absorption bands of all-trans retinene, neoretinene *a*, and isoretinene *a fall* in extinction; those of neoretinene *b* and isoretinene *b rise* in extinction; while only in neoretinene *b* is this rise in the main band accompanied by a fall in extinction of the cis peak. In all the

other isomers the extinction *rises* in the cis peak region during isomerization (*cf.* Fig. 4).

The following isomers therefore exhibit unique behavior on isomerization with light:

All-trans retinene: main absorption band shifts 5 to 6 mμ toward shorter wave lengths with large fall in extinction; large rise in extinction in cis peak region.

Neoretinene *b*: extinction of the main band rises, while that of the cis peak falls.

Isoretinene *b*: main band shifts 7 to 9 mμ toward longer wave lengths, with rise in extinction.

TABLE IV

Thermal Isomerization of Retinene

Single stereoisomers of retinene, dissolved in 1 per cent digitonin, were heated at 70°C. for 3 hours and the effects upon their absorption spectra determined. Columns (2) and (3) show the positions of the absorption maxima (λ_{max}) before and after heating; columns (4) and (5) show the maximal extinctions (E_{max}) before and after heating; and column (6) shows the percentage fall or rise in extinction.

Retinene isomer	λ_{max}		E_{max}		
	Before (2)	After (3)	Before (4)	After (5)	Percentage change (6)
	mμ	mμ			
All-trans	389	389	1.316	1.308	−0.61
Neoretinene *a*	380	381	1.260	1.240	−1.59
Neoretinene *b*	384	387	1.071	1.269	+18.5
Isoretinene *a*	381.5	381.5	1.224	1.221	−0.25
Isoretinene *b*	374	375	1.159	1.111	−4.14

Isomerization by Heat

A general procedure for stereoisomerizing carotenoids is to heat them in solution. At room temperature this is a slow process; but when a dilute solution of an all-trans carotenoid is refluxed in benzene or petroleum ether (b.p. 60–80°C.), it reaches stereoisomeric equilibrium within 15 to 60 minutes (Zechmeister, 1944, pages 281–282).

We have incubated the isomeric retinenes in 1 per cent aqueous digitonin for 3 hours at 70°C., and determined the effects upon their absorption spectra. These are summarized in Table IV. All-trans retinene and isoretinene *a* were hardly changed; neoretinene *a* fell in extinction 1.6 per cent and isoretinene *b* 4.1 per cent, each with about 1 mμ shift toward longer wave lengths. Neoretinene *b* *rose* in extinction 18.5 per cent; simultaneously its λ_{max} shifted 3 mμ toward longer wave lengths.

The most striking result of this experiment is to show that retinene isomerizes very slowly on heating in water solution. Incubation for 3 hours at 70°C. has almost no effect upon all-trans retinene or isoretinene *a*; very small effects upon neoretinene *a* and isoretinene *b*; and a fairly large effect only upon neoretinene *b*, which goes over on this treatment largely to the all-trans configuration.

The Synthesis of Rhodopsin and Isorhodopsin as an Analytical Procedure

When incubated with opsin in the dark, neoretinene *b* yields rhodopsin (λ_{max} 500 mμ), isoretinene *a* yields isorhodopsin (λ_{max} 487 mμ), and the remaining isomers of retinene are inactive (Hubbard and Wald 1952–53). This reaction can therefore be used to estimate the concentration of active isomers in mixtures and to determine their proportions.

The sensitivity of the reaction with opsin deserves some attention. Ordinarily we make this test in a total volume of 0.5 ml. in a microabsorption cell 3 mm. wide and 10 mm. deep. The incubation is carried out in the cell compartment of the Beckman spectrophotometer, and the rise of light-sensitive pigment is followed by periodic measurements of the extinction at 490 to 500 mμ. Under these circumstances 1 μg. of active retinene, incubated in the dark for an hour with excess opsin, yields light-sensitive pigment having a maximal extinction of about 0.3. This is considerably more than is needed for accurate measurement. With smaller volumes of material, smaller micro cells, and reliance upon lower extinctions, there should be no difficulty in measuring and characterizing the active retinene isomers in amounts of the order of 0.2 μg.

When neoretinene *b* and isoretinene *a* are incubated in the dark with excess opsin, a given extinction of retinene yields roughly the same extinction of rhodopsin or isorhodopsin, all extinctions measured at the appropriate absorption maxima. This result represents 100 per cent activity. The activity of any mixture of retinene isomers can therefore be expressed as the ratio, extinction of rhodopsin formed divided by extinction of retinene employed. This is not an accurate index of activity, since the various isomers of retinene have different molar extinctions; but it suffices for the present purpose. Table V shows an example of this procedure and some indication of its precision.

Interconversions of Retinene Isomers

When any of the five available isomers of retinene is irradiated with white light in digitonin solution, a mixture of isomers eventually results which appears in all cases to be identical. Incubation of this mixture with excess opsin shows that it contains 35 to 40 per cent of active isomers (*cf.* Table V). Since the light-sensitive pigment which results has its λ_{max} at 496 to 497 mμ, intermediate therefore between rhodopsin and isorhodopsin, one can conclude that both neoretinene *b* and isoretinene *a* are present, with the former predominant.

All the retinene isomers, though on irradiation they arrive eventually at the same point, do not isomerize with equal ease. They form two groups in this regard. All-trans retinene and neoretinenes *a* and *b* go over very quickly to the equilibrium mixture. The isoretinenes behave differently. Isoretinene *a* isomerizes very slowly, taking about five times as long in a given light to reach equilibrium as any of the three isomers just mentioned. Isoretinene *b* isomerizes very quickly in the light to isoretinene *a*, then this goes on very slowly to form the other isomers.

There appears therefore to be some special relation between isoretinenes *a* and *b*, and some special barrier between them and the other isomers. The barrier involves specifically the structure of isoretinene *a*; it is this that goes over only with difficulty to the other forms.

TABLE V

The Assay of Retinene with Opsin

An isomerate of retinene, produced by irradiating all-trans retinene in ethanol solution, was assayed four times, by two different observers, using two different opsin preparations. The opsin was always in excess. The amount of rhodopsin formed was measured by the difference in extinction at 500 mμ before and after bleaching (ΔE_{500}). The activity of the isomerate is computed by dividing the extinction of rhodopsin formed by the extinction of retinene in the original incubation mixture.

Observer	Opsin preparation No.	Extinction of retinene (E_{385})	Rhodopsin formed (ΔE_{500})	Activity
				per cent
R. H.	1	0.32	0.125	39.1
R. H.	2	0.47	0.186	39.6
R. I. G.	2	0.47	0.170	36.2
R. I. G.	2	0.47	0.184	39.2

From the equilibrium mixture obtained by irradiating all-trans retinene, we have isolated and crystallized neoretinene *b*, and from this have made rhodopsin. From the same isomerate of all-trans retinene we have isolated a fraction which, though it contained also some inactive retinene, owed all its activity to isoretinene *a*, from which we synthesized isorhodopsin.

From similar isomerates obtained by irradiating crystalline isoretinene *a* and neoretinene *a*, we have isolated neoretinene *b*, and from this made rhodopsin.

We have also shown that isoretinene *b*, on short irradiation yields almost exclusively isoretinene *a*, which on incubation with opsin yields isorhodopsin (Hubbard and Wald, 1952–53, Fig. 15).

Finally, it should be recalled that the bleaching of rhodopsin or isorhodopsin by light yields predominantly all-trans retinene (Hubbard and Wald, 1952–53).

These relations are summarized in part in Fig. 5. Taken together, they include pathways of conversion from each of the isomers of retinene to others, and from all of them eventually to all-trans retinene.

Status of the Retinene Isomers

It must by now be clear that the five species of retinene which we have discussed are very intimately related to one another. Their absorption spectra lie in the relations expected of geometrical isomers; they yield the same antimony chloride product; all of them on irradiation yield what seems to be the same equilibrium mixture of isomers; and they are extensively intercon-

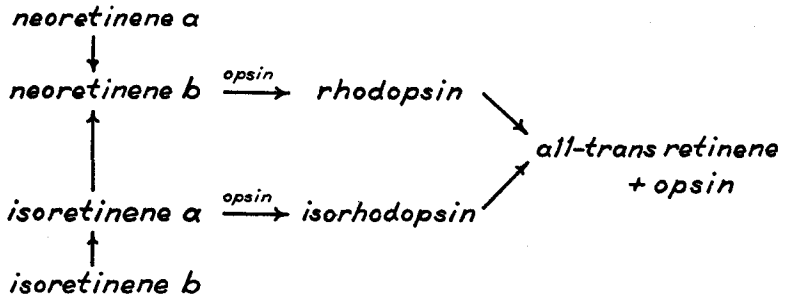

FIG. 5. Diagram of some of the demonstrated interconversions of the retinene isomers. From the product of isomerizing neoretinene *a* with light, neoretinene *b* was isolated, which on incubation in the dark with opsin formed rhodopsin. Neoretinene *b* was similarly isolated from the isomerate of isoretinene *a*, and used to synthesize rhodopsin. Isoretinene *b* on short irradiation yields almost entirely isoretinene *a*, which on incubation with opsin yields isorhodopsin. Both rhodopsin and isorhodopsin bleach in the light to a mixture of opsin and all-trans retinene.

verted by a variety of gentle treatments. There can be no doubt that these substances are isomers. The only point that needs to be considered is whether their isomerism involves something other than cis-trans configuration.

The reason for raising this question, as indicated earlier, is that we appear to have three mono-cis retinenes, where according to present theory we should find only two, the *3*-cis and *5*-cis. It appears from our molecular models of retinene that the only double bond available for a third cis linkage is *4* (*cf.* Fig. 1). A *4*-cis retinene, however, involves theoretical difficulties which may be summarized as follows:—

In such a conjugated system of alternate single and double bonds as retinene possesses, the single bonds all have some degree of double bond character. This is the result of resonance in such systems, and is the basis of their conjugation. With the resonance go other properties; the greater the resonance, the more stable is the molecule, and the further toward the red lies

its absorption spectrum. But the geometrical condition for resonance in such systems is coplanarity—all the carbon atoms of the conjugated chain must lie in the same plane. This state is achieved most completely in the all-trans configuration; and for this reason the all-trans isomer is most stable—hence most prevalent—and has the absorption spectrum of longest wave length. A cis linkage, even at the favored double bonds *3* and *5*, involves some steric hindrance between hydrogen atoms on adjacent carbons, and therefore a small departure from coplanarity. The consequent interference with resonance is responsible for the shift of spectrum 4 to 6 mμ toward shorter wave lengths, and for the lowered stability which makes cis retinenes less prevalent than the all-trans form. A cis linkage at position *4* would involve a large departure from coplanarity—at this point the molecule would not only be bent, but considerably twisted. A *4*-cis retinene is consequently expected to be highly unstable, and to have its spectrum displaced to much shorter wave lengths than the *3*- or *5*-cis isomers. Yet all our presumptive mono-cis retinenes are well represented in equilibrium mixtures, and their spectra all lie very close together.

If these theoretical difficulties can be resolved, then with three mono-cis retinenes (*3*-cis, *4*-cis, *5*-cis) we should have to consider a total of 8 geometrical isomers: the all-trans, 3 mono-cis, 3 di-cis, and one tri-cis. From this point of view all the substances we have discussed would be true geometrical isomers, and three more remain to be discovered.[4]

Another possibility has been suggested to us by Professor Robert B. Woodward of Harvard University. This is that one or more of the known retinene isomers has an α-ionone ring. This is an interesting idea, for the change from a β- to an α-ionone ring involves very nearly the same shift of absorption spectrum as the introduction of a cis linkage. Thus α- and β-apo-2-carotenal, structures which differ from α- and β-retinene only in having a longer carbon

[4] *Note Added in Proof.*—Since this discussion was written, two papers have appeared reporting the synthesis of polyenes containing "forbidden"; *i.e.*, sterically hindered—cis linkages (Oroshnik, W., Karmas, G., and Mebane, A. D., *J. Am. Chem. Soc.*, 1952, **74,** 295; and Garbers, C. F., Eugster, C. H., and Karrer, P., *Helv. Chim. Acta*, 1952, **35,** 1850). These new molecules help with one of our problems, not with the other. They show that molecules with hindered cis linkages can be much more stable than had been supposed. Yet all such molecules that have been prepared so far have very degraded absorption spectra, with low maximal extinctions, and $\lambda_{max.}$ displaced 20 to 40 mμ compared with the all-trans isomers. The spectral changes clearly indicate loss of resonance; and the fact that nevertheless these molecules are stable implies that resonance may not have as decisive effects upon stability as has been thought. Our remaining problem is to understand how such a stable molecule with a hindered cis linkage might display as little change of absorption spectrum as do our retinene isomers.

chain, differ from each other in λ_{max} by 5 to 6 mμ. The corresponding alcohols, higher homologues of vitamin A, differ in λ_{max} by 7 to 8 mμ (von Euler, Karrer, and Solmssen, 1938).

In line with this suggestion, we should think first of all of isoretinenes *a* and *b* as α-retinenes. Neither of these substances has a clearly defined cis peak, as particularly isoretinene *a* should have if it contained a single cis linkage. It will be recalled also that the isoretinenes display small peculiarities of absorption spectrum; and that isomerization experiments tend to divide this pair from the other isomers, in that isoretinene *b* readily goes to *a*, but *a* is converted only with difficulty to the remaining isomers. One might suppose that isoretinene *a* is all-trans α-retinene, and that the difficulty in isomerizing it is caused by the necessity to convert its α- to a β-ionone ring. That this change occurs at all on simple exposure to light, and that the isoretinenes yield the same antimony chloride product as the other retinenes isomers is, according to Professor Woodward, not irreconcilable with the behavior expected theoretically of α-carotenals.[5]

Such an assumption would leave the present theory of cis-trans isomerization in the carotenoids unaltered. Our five isomers might then include two α-retinenes, possibly the isoretinenes *a* and *b*; and three stereoisomers of β-retinene: all-trans; neoretinene *a*, which is probably *5*-cis (Robeson and Baxter, 1947); and neoretinene *b*, the properties of which agree very well with the *3*-cis configuration (Hubbard and Wald, 1952–53). In this view, *3,5*-di-cis retinene still remains to be discovered.

SUMMARY

Five crystalline retinenes have been isolated, which have every appearance of being cis-trans isomers of one another. They are all-trans retinene; three apparently mono-cis isomers: neoretinenes *a* and *b* and isoretinene *a*; and isoretinene *b*, an apparently di-cis isomer.

The absorption spectra of these substances display the relations expected of cis-trans isomers. The main absorption band is displaced 5.5 to 7 mμ toward shorter wave lengths for each presumptive cis linkage. Some of the presumptive cis isomers also display a cis peak at 255 to 260 mμ. All five substances yield an identical blue product on mixing with antimony chloride. All of them are converted by light to what appears to be an identical mixture of stereoisomers. Heat isomerizes them very slowly; only neoretinene *b* exhibits large changes on heating at 70°C. for 3 hours. The various isomers have been extensively interconverted by gentle procedures, and all of them have been converted to all-trans retinene.

[5] Nor need this view of the structure of the isoretinenes raise physiological problems; for we have already indicated that neither isoretinene has yet been identified *in vivo*, and isorhodopsin is not appreciably present in the retina (Hubbard and Wald, 1952–53).

The present theory of cis-trans isomerism in carotenoids predicts the existence of four stable isomers of retinene. Instead we seem to have five—specifically three mono-cis forms where two are expected. There is no doubt that all these substances are closely related isomers of one another. The only point in question is whether they differ in part by something other than cis-trans configuration. One possibility, as yet little supported by evidence, is that isomerization between β- and α-ionone rings may be involved. If, however, as seems more likely, all these substances are geometrical isomers of one another, some modification is needed in the present theory of configurational relationships in this class of compounds.

REFERENCES

Ball, S., Goodwin, T. W., and Morton, R. A., The preparation of retinene$_1$-vitamin A aldehyde, *Biochem. J.*, 1948, **42**, 516.

Dalvi, P. D., and Morton, R. A., Preparation of neovitamin A esters and neoretinene$_1$, *Biochem. J.*, 1951, **50**, 43.

von Euler, H., Karrer, P., and Solmssen, U., Homologe des Vitamins A (Axerophthols) und ein Abbauprodukt des α-Carotins, α-Apo-2-Carotinal, *Helv. Chim. Acta*, 1938, **21**, 211.

Hubbard, R., and Wald, G., Cis-trans isomers of vitamin A and retinene in vision, *Science*, 1952, **115**, 60.

Hubbard, R., and Wald, G., Cis-trans isomers of vitamin A and retinene in the rhodopsin system, *J. Gen. Physiol.*, 1952–53, **36**, 269.

Meunier, P., and Vinet, A., Chromatographie et Mésomérie, Paris, Masson et Cie, 1947, chapter 5.

Pauling, L., Recent work on the configuration and electronic structure of molecules; with some applications to natural products, *Fortschr. Chem. org. Naturstoffe*, 1939, **3**, 203.

Robeson, C. D., and Baxter, J. G., Neovitamin A, *J. Am. Chem. Soc.*, 1947, **69**, 136.

Zechmeister, L., Cis-trans isomerization and stereochemistry of carotenoids and diphenylpolyenes, *Chem. Revs.*, 1944, **34**, 267.