A STUDY OF THE BORON TRIFLUORIDE COMPLEXES OF SOME CAROTENOID HYDROCARBONS AND THE PRODUCTS OF THEIR HYDROLYTIC AND ALCOHOLYTIC CLEAVAGE.

Thesis by

Warren V. Bush

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ABSTRACT

Formation of a deeply-colored α-carotene - boron trifluoride complex, by means of boron trifluoride etherate, and subsequent hydrolysis of the complex result mainly in the formation of 4-hydroxy-α-carotene, indicating that, under *the conditions applied, only the β-ionone end of the complex* is reactive; ethanolysis gave the corresponding ethoxy derivative. The structures of these two compounds and some of the other reaction products were clarified. On this basis the structure of the BF_3-complex has also been established, and a mechanism for its hydrolysis and alcoholysis was postulated. The length of the resonating system within the proposed complex is consistent with the observed deep blue color.

Lycopene and γ-carotene were studied along similar lines. The observed formation of 5,6-dihydroxy-5,6-dihydrolycopene by hydrolysis of the lycopene complex can be explained by means of a structure and a hydrolysis mechanism both similar to those of the α-carotene complex.

The formation of 4-hydroxy-γ-carotene by hydrolysis of the γ-carotene complex is in accordance with an analogous structure and hydrolysis mechanism. The β-ionone end of the γ-carotene complex appears to be more reactive than the ψ-ionone end.

The *cis-trans* isomerization of 4-hydroxy-, 4-keto-, and 4-ethoxy-α-carotene as well as 5,6-dihydroxy-5,6-dihydrolycopene was studied.

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I. α-CAROTENE AND BORON TRIFLUORIDE ETHERATE

A. THEORETICAL PART

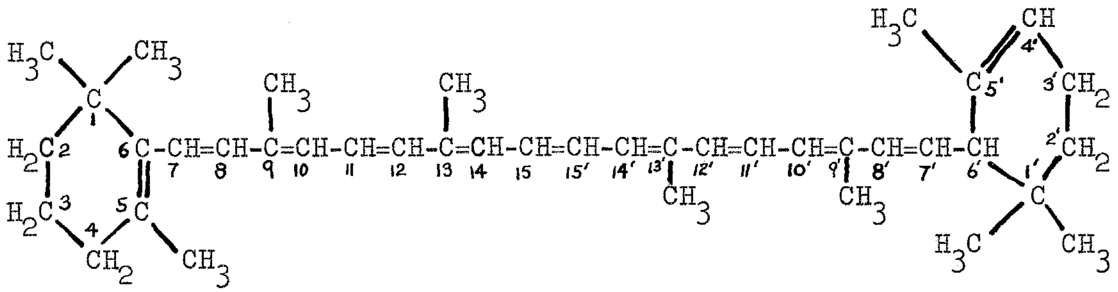
1. Introduction.

Carotenoid complexes are not uncommon: some carotenoids occur in the vegetable and animal kingdoms complexed with proteins, for example. At least one such carotenoid-protein complex has been found in visual pigments and plays an important part in retinal processes (1).

In vitro, carotenoids form deeply colored complexes with some mineral acids and Lewis acids such as antimony trichloride or boron trifluoride. Some of these complexes are relatively stable: the dark blue complexes of carotenes or vitamin A with anhydrous antimony trichloride in chloroform (Carr-Price reagent) are in use for the quantitative evaluation of vitamin A preparations. Strain observed the conversion of α- and β-carotenes into unstable blue pigments by boron trifluoride (2).

Boron trifluoride complexes with carotenoids, on hydrolytic or other cleavage, yield a variety of products; some of the polyenes thus produced are structurally similar to certain natural carotenoids. An example of the in vitro formation of a compound similar to a natural product is that of isocryptoxanthin (4-hydroxy-β-carotene), which was prepared from β-carotene via the BF_3 complex by Petracek (3,4).

The present investigation was undertaken in order to gain some insight into the nature of boron trifluoride-carotenoid complexes, and into the mechanism and products of the hydrolytic cleavage of those complexes. As the first subject, α-carotene (I) was chosen. As is well known, it is isomeric with the previously studied β-carotene (II), but has an unsymmetrical structure containing an "α-ionone" and a "β-ionone" end group (see the formula).



"β-Ionone ring"　　　　"α-Ionone ring"

(I.) α-Carotene.

(Ia.) α-Carotene.

(II.) β-Carotene.

The numbering system shown was proposed by Karrer, and will appear throughout this Thesis; for convenience, however, abbreviated formulas such as (Ia) and (II) will also be used. In all carotenoids which have only one "β-ionone" ring, unprimed numbers will refer to this ring.

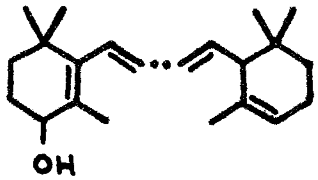
Wallcave (5,6) found that, in hexane solution, the β-carotene-boron trifluoride complex, after a formation period of a few minutes, gave rise on hydrolysis mostly to some cis isomers of β-carotene; by changing the solvent to chloroform, however, Petracek was able to oxidize the β-carotene molecule in good yields to isocryptoxanthin (4-hydroxy-β-carotene).

Chloroform was used for the present study, since the primary purpose was to achieve not stereoisomerization, but chemical conversions of the starting material.

2. Hydrolysis of the α-Carotene - Boron Trifluoride Complex.

4-Hydroxy-α-carotene. - The main product of this hydrolysis was found to be 4-hydroxy-α-carotene (III). The yield of this compound, including some of its cis forms, corresponded to about 30 per cent of the original α-carotene.

A composition of $C_{40}H_{56}O$ was indicated by elementary analysis.



(III.) 4-Hydroxy-α-carotene.

The reactions used to secure the structure of 4-hydroxy-α-carotene are summarized in Chart 1 (p. 6) and are listed below under (a.) to (g.).

(a.) The most important of these conversions was an oxidation by N-bromosuccinimide ("NBS"), a reagent whose effects on several carotenoids were studied in detail by Petracek (7), and also by Wallcave (8), Karmakar (9), and Koe (10).

4-Hydroxy-α-carotene (III, maxima in hexane, 474, <u>445</u>, 421 mμ) was oxidized by NBS in alcohol-free chloroform to a ketone (IV, λ_{max} = 452 mμ), which was shown by its partition behavior and its formation of only a <u>mono</u>-2,4-dinitrophenylhydrazone (V) to be a mono-ketone. Moreover, the increase of the wavelength of maximum absorption and the loss of fine structure in the visible spectrum indicated that the keto group was in conjugation with the main chromophore (11). Evidently, the carbonyl group was formed by oxidation of a hydroxyl group allylic to the main chromophore, <u>i.e.</u>, located in the 4-position; the ketone is thus 4-keto-α-carotene (IV).

(b.) By means of NBS oxidation in R. G. chloroform, α-carotene itself (I) was converted to 4-keto-α-carotene, which was found to be identical with the NBS oxidation product of 4-hydroxy-α-carotene. (A 2:1 mole ratio of NBS to α-carotene was used.)

(c.) α-Carotene was oxidized by NBS (1:1 mole ratio) in alcohol-free chloroform to 3,4-dehydro-α-carotene (VI) which was shown to be identical with the compound formed by acid dehydration of 4-hydroxy-α-carotene. Karrer (12) has pointed out that only 4-hydroxy carotenoids (<u>i.e.</u>, allylic carotenoid alcohols, as distinguished from 3-hydroxy or non-allylic alcohols) are dehydrated by dilute anhydrous HCl in chloroform ("acid chloroform reagent"): for example, the natural product cryptoxanthin (3-hydroxy-β-carotene) is <u>not</u> dehydrated by this reagent.

(d.) The oxidation, 4-hydroxy-α-carotene → 4-keto-α-carotene, was reversed by reduction of the ketone with lithium aluminum hydride.

(e.) 4-Hydroxy-α-carotene could be converted easily to the 4-ethoxy compound (VII, identical with that formed by ethanolysis of the α-carotene - boron trifluoride complex) by addition of a trace of the acid chloroform reagent to an absolute ethanol solution of the carotenoid alcohol. This process was reversed by adding a trace of acid to the carotenoid ethyl ether, dissolved in a water - acetone mixture.

(f.) A mono-acetate (VIII) was formed by adding acetyl chloride to 4-hydroxy-α-carotene in pyridine solution; cold 20 per cent methanolic KOH was used to hydrolyze the ester back to 4-hydroxy-α-carotene.

(g.) Both 4-ethoxy-α-carotene and 4-hydroxy-α-carotene acetate were easily converted to 3,4-dehydro-α-carotene by means of the acid chloroform reagent.

All these reactions, together with the presence of a peak at 2.76μ in the infrared spectrum (Fig. 2, p. 34), prove that there is (at least) one hydroxyl group present, in the allylic (4-) position. The observed lack of vitamin A potency further confirms the presence of a substituent in the β-ionone ring.

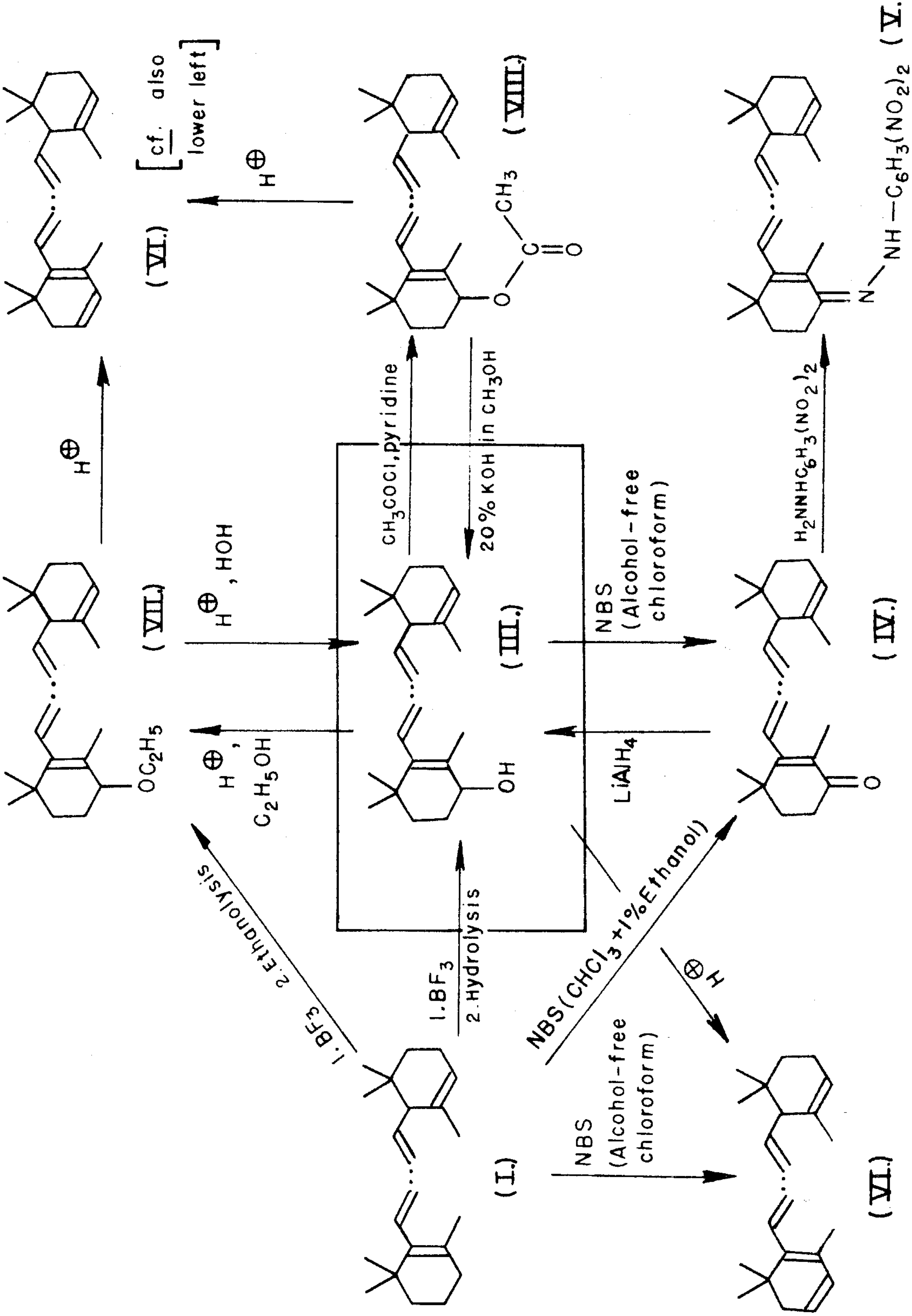


Chart 1. Proof of the 4-Hydroxy-α-carotene Structure.

The spectrum taken in the visible region (Fig. 1, p. 33) shows that the α-carotene chromophore was recovered intact.

Evidence that the 4-hydroxy substitution is the only modification of the α-carotene molecule is altogether as positive as the determination of the presence of the hydroxyl group (a.-d.):

(a.) The partition ratio of 4-hydroxy-α-carotene (84:16) between hexane and 95 per cent methanol is equal to that observed for other naturally occurring and synthetic carotenoid monohydric alcohols with no other substitution (13).

(b.) Allylic dehydration by means of the acid chloroform reagent gives a hydrocarbon, 3,4-dehydro-α-carotene.

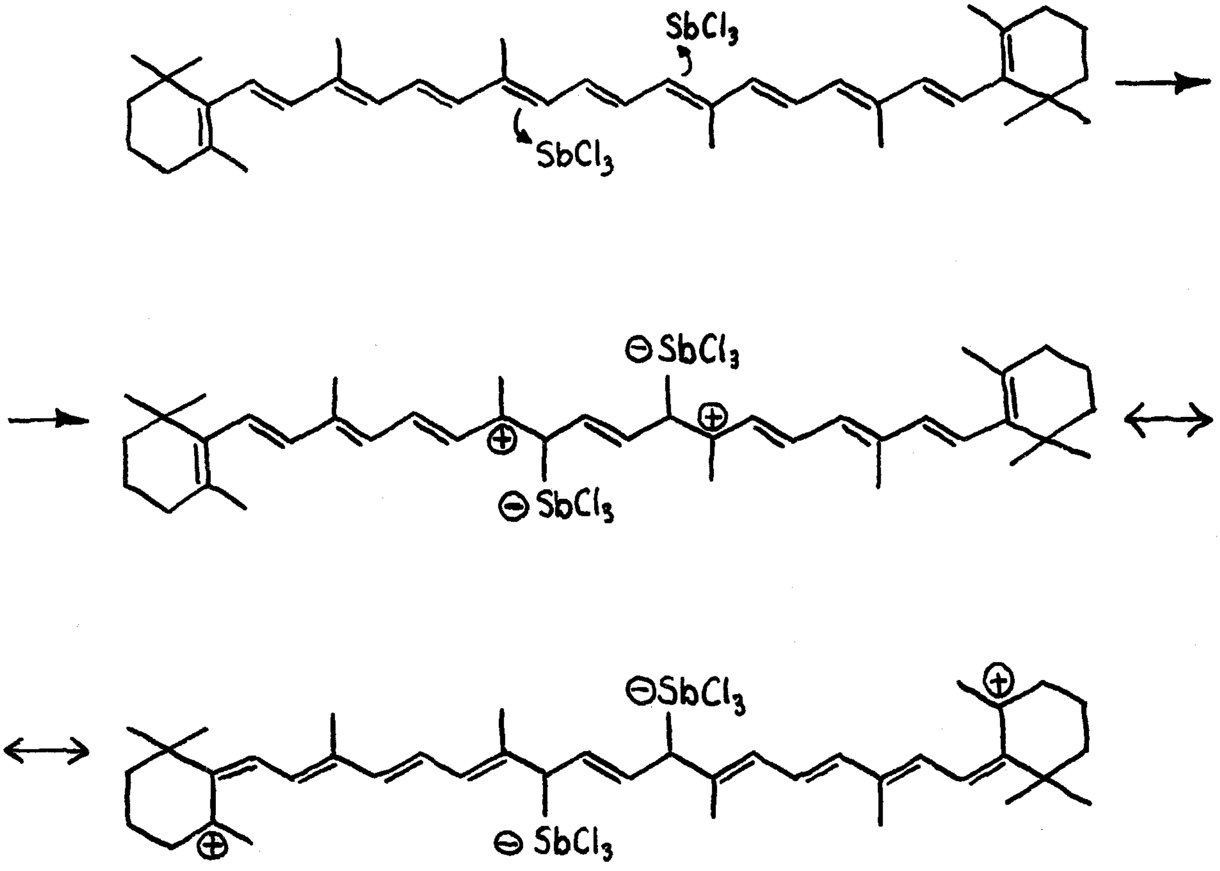
(c.) Oxidation of 4-hydroxy-α-carotene by NBS gives a mono-ketone (4-keto-α-carotene) which, from its partition ratio appears to be otherwise unsubstituted.

(d.) Two conversion products of 4-hydroxy-α-carotene formed from the BF_3 complex are identical with compounds produced by direct NBS oxidation of α-carotene. It is therefore believed that the isolated double bond in the α-ionone ring has not been altered during the course of the BF_3 reaction.

Formation of 4-Hydroxy-α-carotene. - The most important result of forming and hydrolyzing the α-carotene - boron trifluoride complex is thus seen to be oxidation of the 4-carbon (oxidation state of -2 in the hydrocarbon, α-carotene, to oxidation state 0 in the alcohol, 4-hydroxy-α-carotene). During the course of the reaction, a pair of electrons must be removed from the α-carotene molecule or from the α-carotene -

boron trifluoride complex. One possible route to this oxidation will now be presented, along with evidence to support the hypothesis.

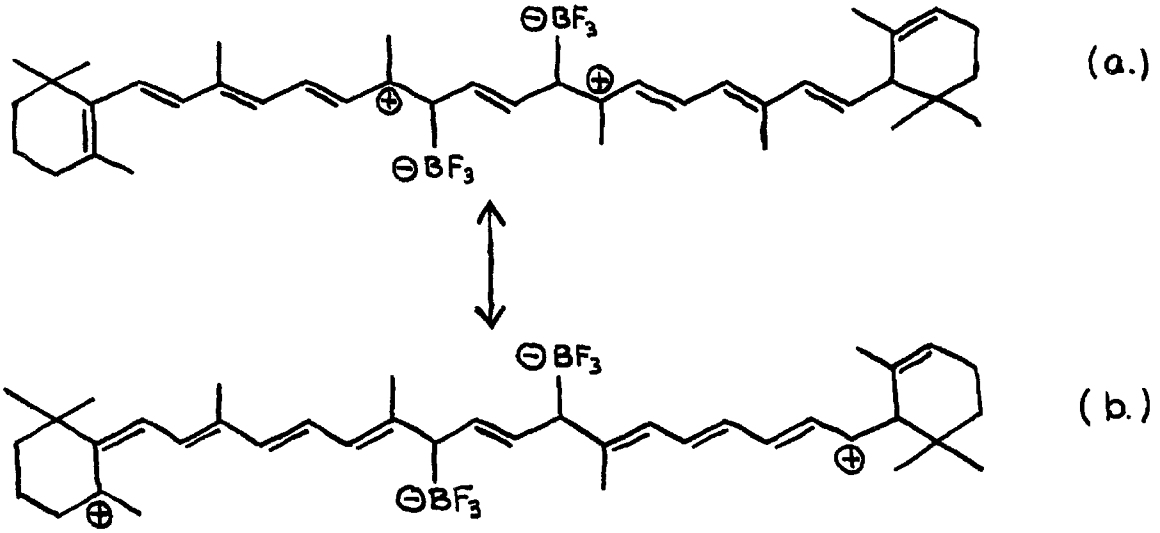
Meunier (14) has indicated that, for the β-carotene - antimony trichloride complex, the dark blue color is due to a resonating double carbonium ion, in which the effective chromophore consists in reality of two chromophores, each involving four conjugated double bonds. The limiting formulas proposed by Meunier are:



(IX.) Limiting Formulas for the β-Carotene - SbCl$_3$ Complex.

Later careful measurements by Brüggemann, Krauss, and Tiews (15) have shown that the complex of β-carotene with antimony pentachloride does indeed contain two molecules of Lewis acid to one molecule of β-carotene. Moreover, the same authors showed that the β-carotene complexes with $FeCl_3$, $AsCl_5$, $SbCl_5$, and $AlCl_3$ all contain the same chromophore, hence probably the same resonating systems.

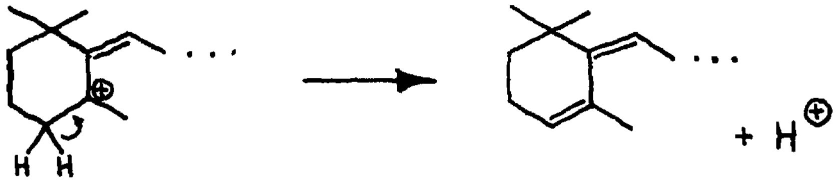
According to a similar scheme, the limiting formulas of the resonating systems in the α-carotene - boron trifluoride complex would be (X a. and b.):



(X.) Limiting Formulas for the α-Carotene - BF_3 Complex.

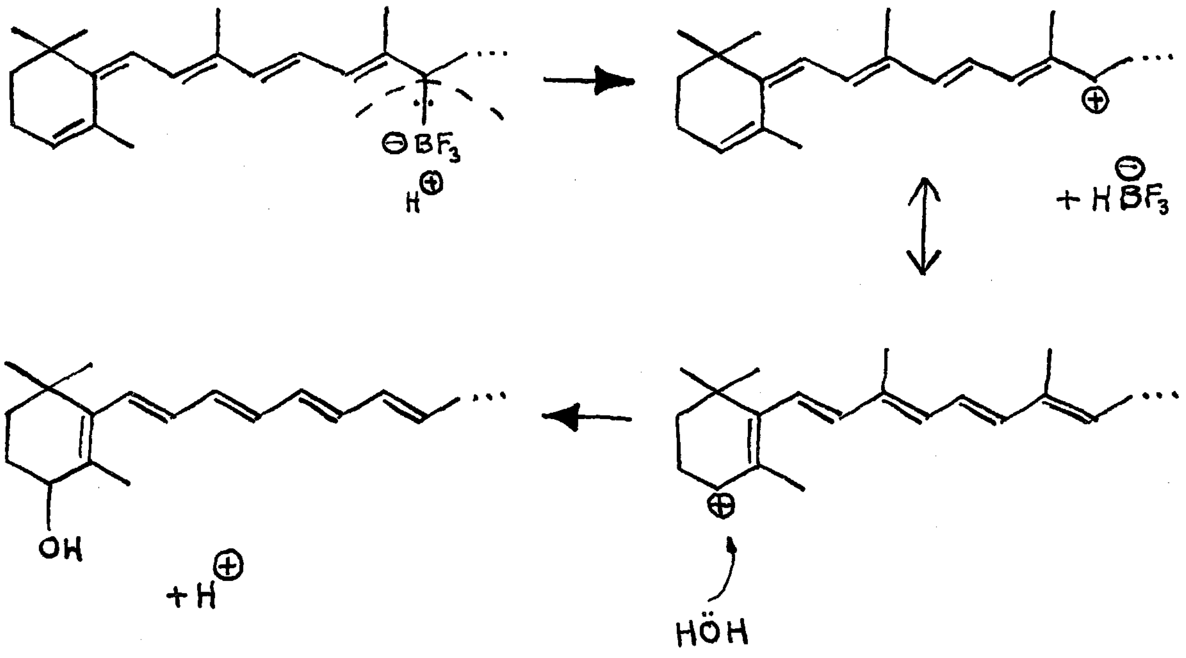
Examination of these limiting formulas shows that one of the resonating systems indeed involves four conjugated double bonds, but that the other one is only three conjugated double bonds "long," and according to Meunier would involve absorption of light at about 540 mμ, i.e., in the middle of the "green" region of the visible spectrum. The boron trifluoride complex of α-carotene is indeed observed to be more "purple" (*i.e.*, more "red") than the corresponding β-carotene complex, which is a deep "royal blue."

The "β-ionone" end of the α-carotene molecule, at which reaction is observed to occur, will first be considered. The first step in cleavage of the complex, according to Meunier, is the removal of a proton from the 4-carbon, at the left end of limiting formula (Xb.). The pair of electrons thus liberated forms a 4,5 double bond:

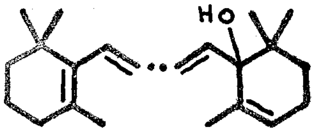


The 4-carbon has now passed from a formal oxidation state of -2 to -1, but the 14-carbon has a formal oxidation state of -2 instead of its normal -1; hence, no overall oxidation has yet occurred. One method of removing the necessary two electrons would be by combination of a proton (from the water added to the reaction mixture) with the $^{\ominus}BF_3$ portion of the complex, simultaneously with the removal of $^{\ominus}BF_3H$ from the complex. The $HBF_3^{\ominus}$ ion is known to exist in the case of $Na^{\oplus}\ HBF_3^{\ominus}$ (16); it is probably very short-lived in the reaction mixture under discussion, if indeed it exists at all as such. Other species which could remove a pair of electrons, such as the hydride ion, $H^{\ominus}$, or a $BF_3^{(2-)}$ ion, could be proposed, but their use here instead of $HBF_3^{\ominus}$ would suffer the same disadvantage of low probability.

Assuming the loss of an $HBF_3^{\ominus}$ ion, a 14-carbonium ion would result, which could transfer its charge by resonance to the 4-carbon, there to be neutralized by combination with water and subsequent loss of a proton:

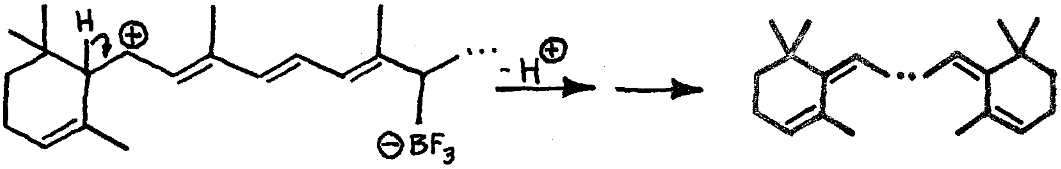


Steric hindrance by the gem-dimethyl group at the 1'-position seems to prevent any attack at the "α-ionone" end of the α-carotene molecule. One of the methyl groups in the 1- or 1'-position is actually above and the other below the plane of the paper; neither is in the plane of the paper as shown in the formulas. A model shows that the 6- and 6'-positions are, by reason of the neighboring methyl and gem-dimethyl groups, virtually inaccessible. Allylic substitution of a hydroxyl group at the "α-ionone" end of the molecule would result in the still unknown 6'-hydroxy-α-carotene (XI); however, no substance having the properties which would be expected for such a compound has been isolated from the reaction mixture.



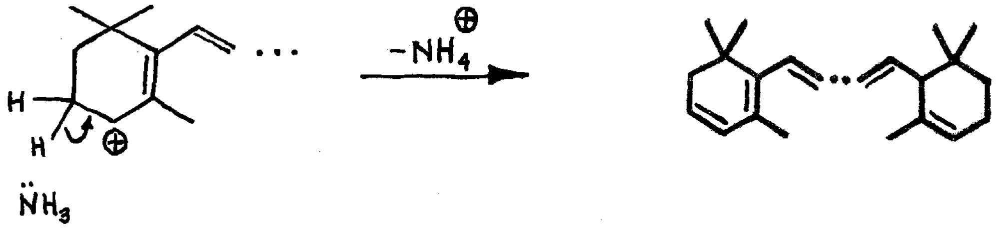
(XI.) 6'-Hydroxy-α-carotene.

Additional evidence of the difficulty of even removing a proton from the 6'-position is the fact that ammonolysis of the α-carotene - boron trifluoride complex gave rise only to 3,4-dehydro-α-carotene (VI); no retro-dehydrocarotene (XII) was observed although it could be predicted to occur in the mixture on the basis of the following scheme:



"α-Ionone" ring. (XII.) retro-Dehydrocarotene.

The observed 3,4-dehydro-α-carotene could be formed according to the following scheme (only the final steps are shown; the initial steps would be similar to those just described for the formation of 4-hydroxy-α-carotene):



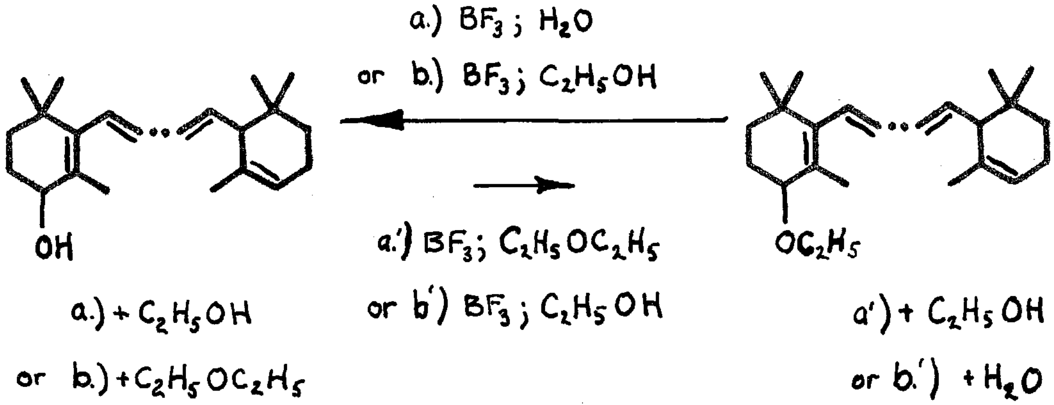
"β-Ionone" ring. (VI.) 3,4-Dehydro-α-carotene.

It is interesting to note that no 4-amino-α-carotene was found among the ammonolysis products.

The observations just described constitute additional evidence for the existence of two "independent" resonating systems in the carotene - boron trifluoride complex. The "half-complex" at the "β-ionone" end seems to be fairly stable: the positive charge is on a tertiary carbon in each of the formal limiting states of formula (X), p. 9 ; moreover, this "half-complex" leads to well-defined reactions. The "half-complex" at the "α-ionone" end of the α-carotene molecule, on the other hand, would be somewhat less stable because the positive charge must be placed on a secondary carbon in one of the limiting formulas (X b.), and would be less likely to lead to reaction because of the presence of the gem-dimethyl group on the ring in the 1'-position.

4-Ethoxy-α-carotene. - About 10 per cent of the initial α-carotene was recovered in the form of 4-ethoxy-α-carotene (and some of its cis isomers). This compound appears to be formed in a manner similar to that already described for 4-hydroxy-α-carotene, with the ethoxyl group derived from the approximately 1 per cent ethanol present as a stabilizer in R. G. chloroform. Careful de-alcoholation of the chloroform immediately prior to use increased the yield of 4-hydroxy-α-carotene to nearly 40 per cent, and decreased the yield of 4-ethoxy-α-carotene to a minimum of 0.8 per cent.

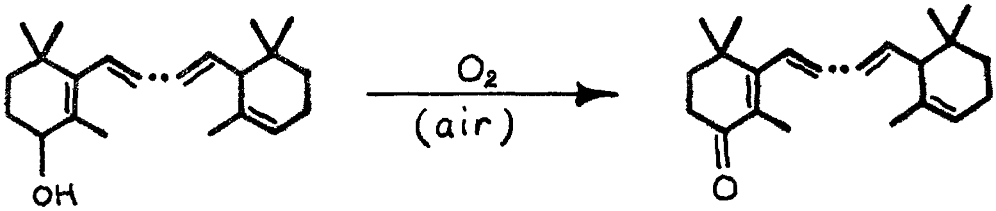
Regardless of the measures taken to exclude alcohol from the reaction mixture, about 0.8 per cent of the α-carotene was converted to 4-ethoxy-α-carotene in each experiment. It is believed that, under the influence of the BF_3, trans-etherification takes place (17) between the 4-hydroxy-α-carotene and the ethyl ether added in the form of boron trifluoride etherate:



Alexander (18) gave an explanation of such acid catalyzed ether cleavage and formation reactions. Conductivity measurements have shown that pure boron trifluoride etherate is about 0.07 per cent ionized to $(C_2H_5)^{\oplus}$ and $(BF_3OC_2H_5)^{\ominus}$ at 25° (19).

4-Keto-α-carotene. - In the course of the experiments on stereoisomerization of 4-hydroxy- and 4-ethoxy-α-carotene, it was found that, in those solutions which were shaken frequently (for example, during irradiation), small amounts of the carotenoid alcohol or ether were converted to the 4-keto-compound. Bubbling air through a chloroform solution of 4-hydroxy-α-carotene gave about a 10 per cent conversion of the alcohol to the ketone before extensive autoxidative destruction occurred.

It is believed that the 4-keto-α-carotene observed among the cleavage products of the α-carotene - boron trifluoride complex is not itself a primary product, but rather that it is formed by air oxidation of the 4-hydroxy- and 4-ethoxy-compounds during the period of violent shaking of the reaction mixture, after adding water to the complex.

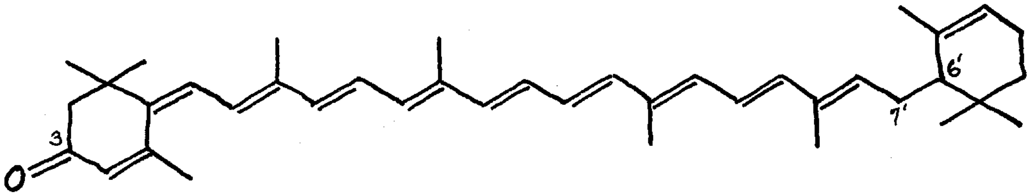


Unchanged α-Carotene. - It is possible that the recovered α-carotene was indeed uncomplexed during the entire course of the reaction. However, calculated on the basis of 2 moles of BF_3 to 1 mole of α-carotene, an approximately 200-fold excess of boron trifluoride was used in the complex formation. Since the complex is easily formed and relatively stable, it is probable that all of the α-carotene was present as the boron trifluoride complex before addition of water to the reaction mixture and that the 1 per cent α-carotene was recovered by simple removal of the attached BF_3 molecules from the complex shown in formula (X), p. 9.

"Unidentified Pigment I." - This compound has a visible spectrum similar to that of lycopene (XIII, an aliphatic hydrocarbon, $C_{40}H_{56}$, with eleven conjugated and two isolated double bonds), except that the fine structure is not as pronounced as in the lycopene spectrum. The partition behavior of "unidentified pigment I" strongly suggests a ketone, and the relatively weak adsorption (compared to lycopene) on lime-Celite indicates that the cyclic end groups are still present, or at least that the isopropylidene double bonds of lycopene are absent.

(XIII.) Lycopene.

A still unknown compound which would be expected to have the properties observed for "unidentified pigment I" would be 3-keto-6',7'-dihydro-retro-dehydrocarotene (XIV), containing eleven conjugated double bonds, including a carbonyl group:



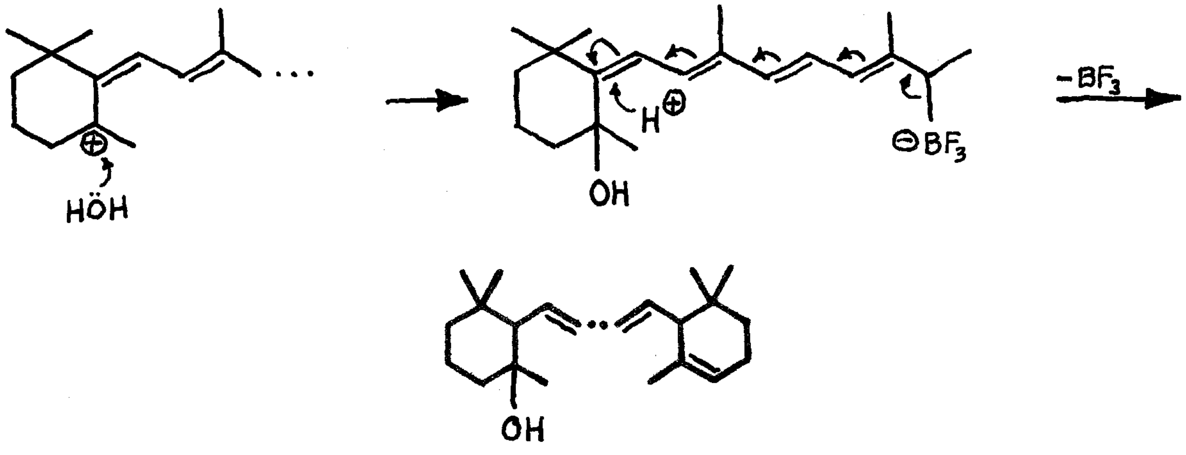
(XIV.) 3-Keto-6',7'-dihydro-retro-dehydrocarotene.

It is to be noted that a "retro" (exocyclic double bond) structure (20) is proposed for this compound. As Petracek has pointed out (4), the retro structure is characterized by stronger adsorbability and extensive fine structure in the spectral curve, when compared to the corresponding "normal" isomer, *e.g.*, 3,4-dehydro-β-carotene, λ_{max} in hexane = 462 mμ (9); retro-dehydrocarotene, λ_{max} in hexane = 471 mμ (21). A carbonyl group in conjugation with the carbon-carbon chromophore has the effect of decreasing fine structure in the spectral curve, and increasing the wavelength of maximum absorption, as can be seen by comparing the curves of 4-hydroxy-α-carotene (Fig. 1, p. 33) and 4-keto-α-carotene (Fig. 5, p. 37). The combination of the retro structure and the conjugated carbonyl group in the proposed compound could result in the observed moderate fine structure, with a higher value for λ_{max} than that observed for "normal" 4-keto-α-carotene.

Unfortunately, too little of this compound was obtained accurately to determine its structure by further tests.

"Unidentified Pigment II." - This compound, like the other three "unidentified pigments," was obtained in amounts too small for a complete structure determination; however, its observed properties give an excellent "preview" of what its correct structure may be. The partition coefficient could support either a ketone (13) or a hindered alcohol character (3). The infrared spectrum (Fig. 14, p. 46) shows absorption in the region of 2.75μ, supporting the presence of an alcohol group. The negative acid chloroform test shows that either this group is not allylic to the main chromophore or that, for some other reason, dehydration cannot occur. The visible and ultraviolet spectrum (Fig. 13, p. 45) indicates a nine-conjugated-double-bond chromophore (22).

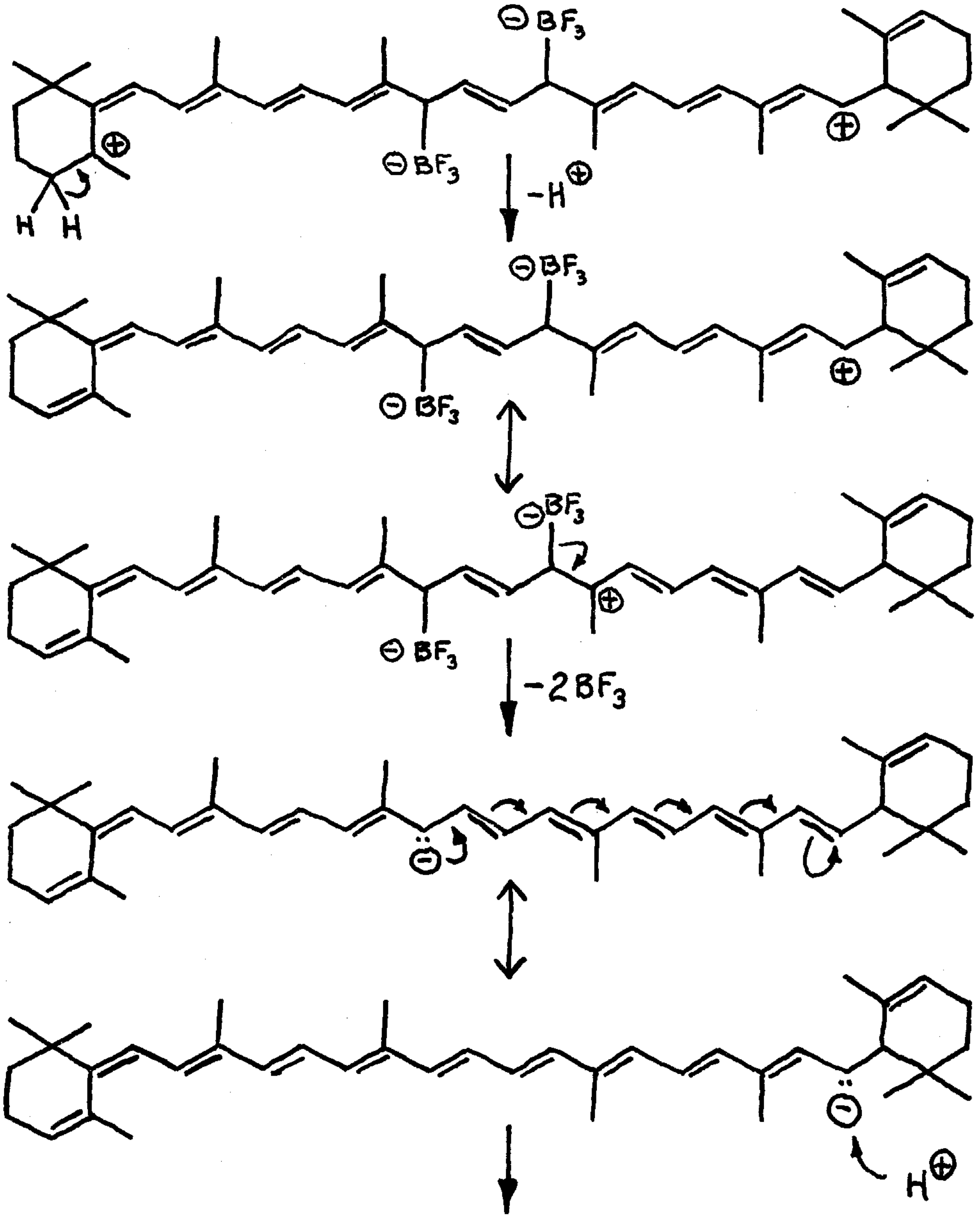
A compound which would be predicted to have all of the observed properties is the still unknown 5-hydroxy-6-hydro-α-carotene (XV), which could arise according to the scheme indicated below:



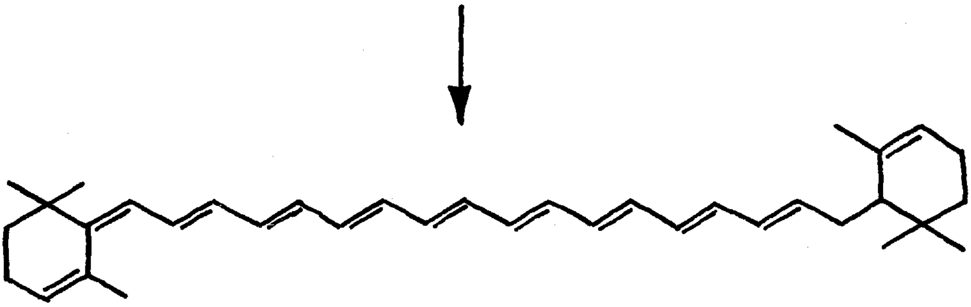
(XV.) 5-Hydroxy-6-hydro-α-carotene.

"<u>Unidentified Pigment III</u>." - This hydrocarbon has an unusual combination of properties: its ultraviolet and visible spectral curve shows a higher wavelength of maximum absorption (Fig. 15, p.47) than α-carotene (λ_{max} in hexane = 452 mμ instead of 445 mμ for α-carotene), and yet this pigment is adsorbed below α-carotene on lime-Celite. (It was found not to be a cis isomer of α-carotene.)

An unknown compound which would be predicted to have the observed properties would be 6',7'-dihydro-retro-dehydrocarotene (XVI), which could arise as follows:



(Xb)



(XVI.) 6',7'-Dihydro-*retro*-dehydrocarotene.

The proton could have added, of course, to the first of the two resonating forms of the anion, giving rise to a carotenoid hydrocarbon containing two independent, approximately half-length chromophores. Several compounds were isolated from the fluorescent filtrate of the chromatogram of the hydrolytic cleavage mixture which, from the ultraviolet spectra, appeared to have two independent chromophores of four to six double bonds in length.

"*Unidentified Pigment IV*." - On the basis of its properties, this compound is a hydrocarbon with eight conjugated double bonds. Such a compound could arise either by a scheme similar to that proposed for "unidentified pigment III," or by hydrogenation of two of the double bonds in α-carotene, perhaps *via* the $HBF_3^{\ominus}$ ion mentioned on p. 10 , which is known to reduce crystal violet and malachite green (16).

3. Alcoholysis of the α-Carotene - Boron Trifluoride Complex.

The main products of methanolysis and ethanolysis of this complex were, as expected, 4-methoxy- and 4-ethoxy-α-carotene, respectively. The yields of both compounds, including *cis* isomers, were about 40 per cent, similar to the yield of 4-hydroxy-α-carotene prepared by hydrolysis of the complex in alcohol-free chloroform.

The mechanism for the alcoholysis of the complex is probably the same as that proposed for hydrolysis (p. 7). It is interesting to note in this connection that "unidentified pigment III" (p. 18) was found among the alcoholysis and ammonolysis products.

The rate of ethanolysis, seems to be considerably greater than the rate of dehydrogenation by ammonolysis: unless the chloroform used to dissolve both the α-carotene complex and the anhydrous ammonia (in ammonolysis experiments) was entirely freed of ethanol, good yields of 4-ethoxy-α-carotene were obtained. 3,4-Dehydro-α-carotene appeared only under completely water- and alcohol-free conditions.

4. Stereoisomerization of 4-Hydroxy-α-carotene and Some Derivatives.

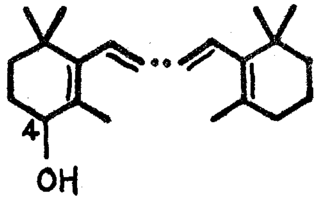
Stereoisomeric studies of the hydrocarbon β-carotene (23) and its naturally occurring 3-hydroxy derivative cryptoxanthin (24) have shown (with some minor differences in quantitative distribution ratios, for which the reader is referred to the papers mentioned) two or three general similarities.

Stereoisomerization by light, when iodine-catalyzed, leads to formation from each compound of at least one isomer which is adsorbed above the all-trans isomer, and which exhibits a relatively low extinction value in the cis-peak region (25, 26, 27). Several cis isomers are formed which are adsorbed below the all-trans compound and which have a relatively high cis-peak. (As is well known, a high cis-peak is believed to be due to a central or near-central cis configuration, leading to a "V"-shaped or "bent" molecule (25).)

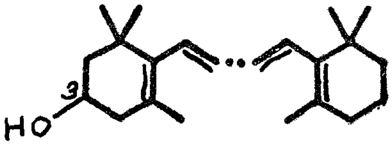
Melting crystals is a stereoisomerization process which usually yields a complex mixture of stereoisomers and destruction products; the fused reaction mixture may contain certain stereoisomers not produced by any other treatment.

A study of the stereoisomerization of α-carotene (28) showed that it behaves similarly to β-carotene.

There is no known, naturally-occurring, mono-hydroxy derivative of α-carotene. Until now, no stereoisomerization study in the α-carotene series similar to that of cryptoxanthin in the β-carotene series has been possible. However, the preparation of 4-hydroxy-α-carotene has now permitted such a parallel study. Although 4-hydroxy-α-carotene is, strictly speaking, analogous to isocryptoxanthin (XVII, 4-hydroxy-β-carotene) rather than cryptoxanthin (XVIII, 3-hydroxy-β-carotene), examination of the visible and ultraviolet spectrum shows that the allylic hydroxyl group in 4-hydroxy-α-carotene has negligible effect on the chromophore.



(XVII.) Isocryptoxanthin.



(XVIII.) Cryptoxanthin.

Exposure of 4-hydroxy-α-carotene, in dilute hexane solution, for two hours to direct sunlight, diffuse sunlight ("open shade"), or to strong incandescent lamp irradiation resulted in very low yields of <u>cis</u> isomers, as did refluxing in hexane for one-half hour (Tables 3 and 4, pp.26 and 27).

The isomers A - D produced by these treatments were adsorbed <u>below</u> the all-<u>trans</u> compound.

Iodine catalysis, however, produced the neo U and V isomers, adsorbed above the all-<u>trans</u> form, in addition to A - D (Table 1, p. 25). The 48 per cent of the starting material unaccounted for was probably destroyed by autoxidation.

Melting crystals, as in the previously mentioned studies of α-carotene, β-carotene, and cryptoxanthin, resulted in a much higher degree of rearrangement to <u>cis</u> forms than any other method used (Table 2, p. 26). No new isomer was created by fusion. Total 4-hydroxy-α-carotene recovered was higher than the total of 4-hydroxy- and 4-keto-α-carotene in the iodine catalysis experiments, probably because of less autoxidative destruction in the fusion experiments.

4-Ethoxy-α-carotene gave, on iodine catalysis and on fusion, results analogous to those for the 4-hydroxy-compound, with the exception that no neo D isomer was found (Tables 5 and 6, p. 28).

In the experiments with 4-keto-α-carotene (Tables 7 and 8, p. 29) again, a more extensive rearrangement to cis forms by fusion than by iodine catalysis was noted. However, 4-keto-α-carotene seems to be more resistant to oxidative destruction than the 4-hydroxy- compound.

The molar extinction curves of the various cis isomers obtained (Figs. 3,4,7,10, and 11, pp. 35 to 43) show that, as in the α-carotene, β-carotene, and cryptoxanthin sets, those isomers adsorbed above the all-trans isomer have very low cis-peaks, while those adsorbed below the all-trans zone show fairly strong cis-peaks.

Some properties of the stereoisomeric 4-hydroxy-, 4-ethoxy-, and 4-keto-α-carotenes discussed in this Section are listed in Tables 9 to 11 (pp. 30 to 32).

Table 1.

An Example of Product Ratios for Iodine-catalyzed Stereoisomerization of 4-Hydroxy-α-carotene

Stereo-isomer	% of Recovered 4-Hydroxy-α-carotene	% of Starting Material*
Neo V	13	4
Neo U	<1	<1
All-*trans*	62	20
Neo A	21	7
Neo B	<1	<1
Neo C	3	1
Neo D	<1	<1

*During this and other analogous stereoisomerization experiments, considerable autoxidation occurred. One of the main products of this irreversible process was 4-keto-α-carotene, which in turn was partly stereoisomerized. In the experiment described above 17% of the starting material was recovered as 4-keto-α-carotene, with the stereoisomeric distribution ratio, neo U:all-*trans*:neo A:neo B = 6:66:15:13.

Table 2.

An Example of Product Ratios for Fusion Stereoisomerization of 4-Hydroxy-α-carotene

Stereo-isomer	% of Recovered 4-Hydroxy-α-carotene	% of Starting Material*
Neo V	8	5
Neo U	2	1
All-*trans*	38	22
Neo A	12	7
Neo B	10	6
Neo C	25	15
Neo D	5	3

*In contrast to stereoisomerizations by other methods, fusion stereoisomerizations gave no noticeable amount of 4-keto-α-carotene.

Table 3.

Summary of Product Ratios for Stereoisomerization of 4-Hydroxy-α-carotene by Photoflood Irradiation

Stereo-isomer	% of Recovered 4-Hydroxy-α-carotene	% of Starting Material*
All-*trans*	97	82
Neo A	1	1
Neo B	1	1
Neo C	<1	<1
Neo D	<1	<1

*4% of the starting material was autoxidized to 4-keto-α-carotene.

Table 4.

Summary of Product Ratios for Stereoisomerizations of 4-Hydroxy-α-carotene

Stereo-isomer	% of Recovered 4-Hydroxy-α-carotene	% of Starting Material*
a. By Refluxing in Darkness:		
All-*trans*	95	75
Neo A	2	1
Neo B	2	2
Neo C	1	1
b. By Standing in Diffuse Daylight:		
All-*trans*	99	81
Neo A	<1	<1
Neo B	<1	<1
Neo C	<1	<1
c. By Standing in Direct Sunlight:		
All-*trans*	94	72
Neo A	3	2
Neo B	2	2
Neo C	1	1

*6% of the starting material was autoxidized to 4-keto-α-carotene in a.; 7% in b.; and 5% in c.

Table 5.

An Example of Product Ratios for Iodine-catalyzed Stereoisomerization of 4-Ethoxy-α-carotene

Stereo-isomer	% of Recovered 4-Ethoxy-α-carotene	% of Starting Material*
Neo V	13	8
Neo U	1	1
All-*trans*	61	35
Neo A	11	6
Neo B	8	5
Neo C	6	3

*3% of the starting material was autoxidized to 4-keto-α-carotene.

Table 6.

Summary of Product Ratios for Fusion Stereoisomerization of 4-Ethoxy-α-carotene

Stereo-isomer	% of Recovered 4-Ethoxy-α-carotene	% of Starting Material
Neo V	7	4
Neo U	2	2
All-*trans*	51	32
Neo A	17	10
Neo B	15	9
Neo C	8	5

Table 7.

An Example of Product Ratios for Iodine-catalyzed Stereoisomerization of 4-Keto-α-carotene

Stereo-isomer	% of Recovered 4-Keto-α-carotene	% of Starting Material
Neo U	11	9
All-*trans*	52	42
Neo A	19	15
Neo B	12	10
Neo C	6	4

Table 8.

Summary of Product Ratios for Fusion Stereoisomerization of 4-Keto-α-carotene

Stereo-isomer	% of Recovered 4-Keto-α-carotene	% of Starting Material
Neo U	7	3
All-*trans*	40	19
Neo A	22	11
Neo B	16	7
Neo C	15	7

Table 9.

Stereoisomeric 4-Hydroxy-α-carotenes

(listed in the sequence of decreasing adsorption affinities)

Isomer	Maxima in Hexane (*cf*. curves pp. 33 - 36)	$E_{1\ cm.}^{mol.}$ (hexane)*	Partition Ratio	Allylic Test (Acid $CHCl_3$)
Neo V	468, 443, 420 mμ	13.0 x 10^4	85:15	+
Neo U	464, 436, 416	12.7	85:15	+
All-*trans*	474, 445, 421	14.5	84:16	+ (fastest)
Neo A	467, 438, 416	13.2	85:15	+
Neo B	466, 439, 416	12.2	85:15	+
Neo C	468, 439, 418	12.2	85:15	+
Neo D	465, 437, 415	12.6	85:15	+
Iodine equilibrium mixture	469, 441, (420, shoulder)	12.2	80:20	+ (slow)

*In Tables 9-11,

$$E_{1\ cm.}^{mol.}(\text{cis isomer}) = E_{1\ cm.}^{mol.}(\text{all-trans}) \times \frac{(\text{maximum extinction of cis isomer})}{(\text{maximum extinction of all-trans})},$$

in solutions of identical concentration.

Table 10.

Stereoisomeric 4-Ethoxy-α-carotenes

(listed in the sequence of decreasing adsorption affinities)

Isomer	Maxima in Hexane (*cf.* curves pp. 40 to 43)	$E_{1\ cm.}^{mol.}$ (hexane)*	Partition Ratio	Allylic Test (Acid $CHCl_3$)
Neo V	468, 439, 417 mμ	15.7 x 10^4	99:1	+
Neo U	464, 436, 413	14.2	99:1	+
All-*trans*	473, 444, 420	14.3	99:1	+
Neo A	465, 438, 414	12.3	99:1	+
Neo B	466, 438, 416	12.0	99:1	+
Neo C	470, 442, 419	11.3	99:1	+
Iodine equilibrium mixture	469, 441, (420,shoulder)	13.4	98:2	+

* *Cf.* Table 9.

Table 11.

Stereoisomeric 4-Keto-α-carotenes

(listed in the sequence of decreasing adsorption affinities)

Isomer	λ_{max} in Hexane (*cf.* curves pp. 37 to 39)	$E^{mol.}_{1\ cm.}$ (hexane)*	Partition Ratio	Allylic Test (Acid $CHCl_3$)
Neo U	446 mμ	11.8 x 10^4	92:8	-
All-*trans*	452	12.6	91:9	-
Neo A	444	10.7	91:9	-
Neo B	442	9.6	91:9	-
Neo C	440	9.6	91:9	-
Iodine equilibrium mixture	448	11.1	90:10	-

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* *Cf.* Table 9.

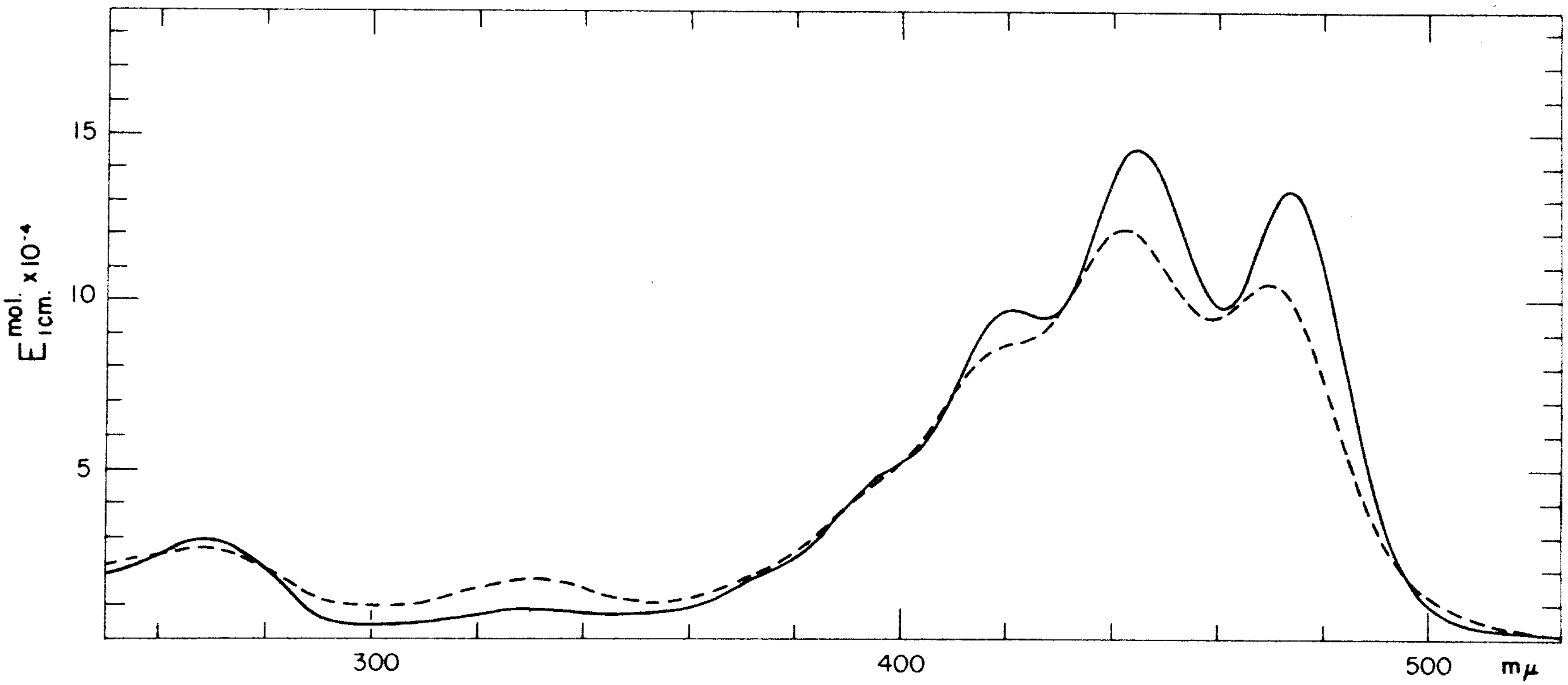


Fig. 1. Molecular Extinction Curve of 4-Hydroxy-α-carotene in the Visible and Ultraviolet Regions (in Hexane): ———, fresh solution of the all-*trans* compound; and - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

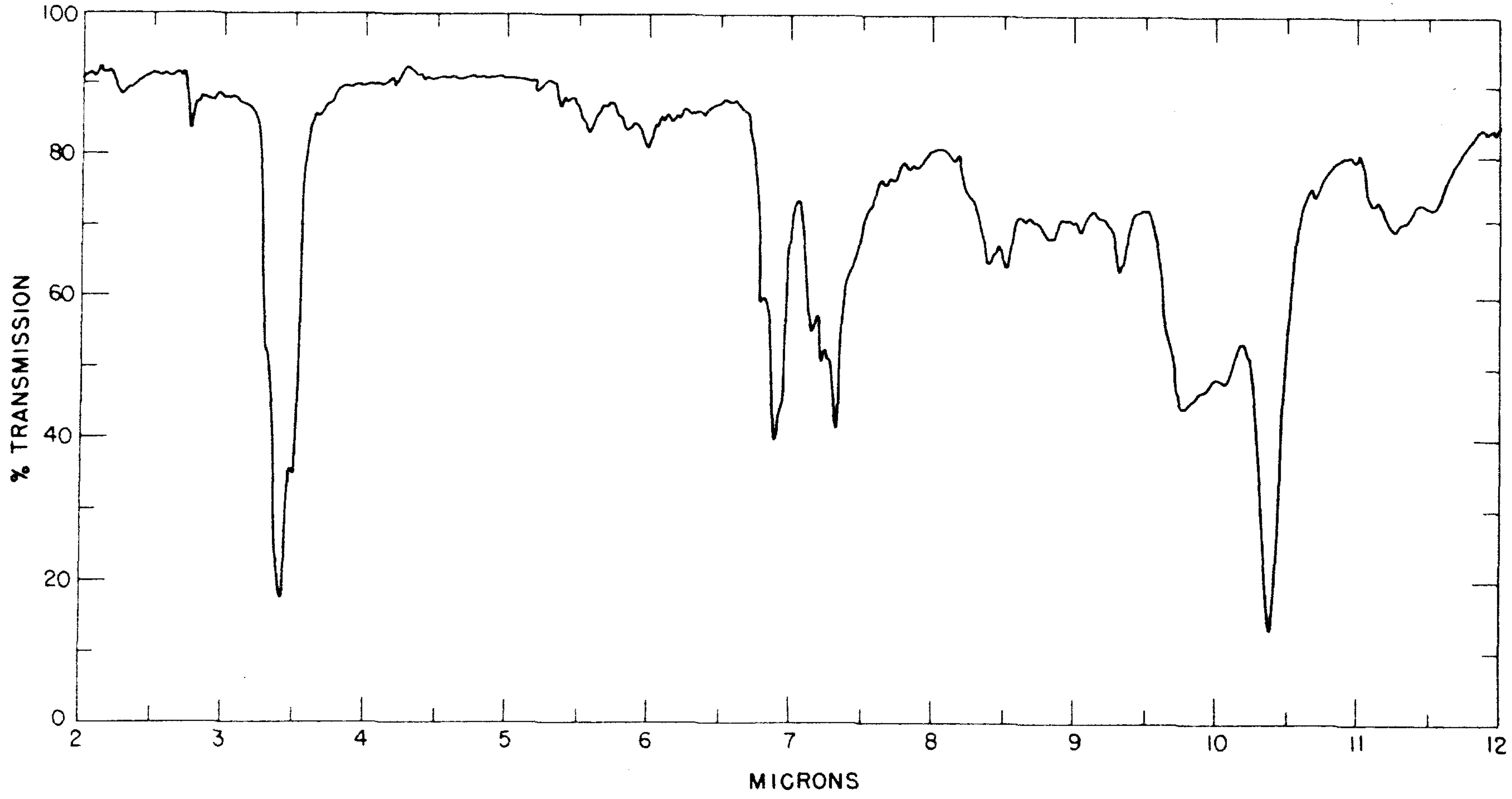


Fig. 2. Infrared Spectrum of All-*trans*-4-hydroxy-α-carotene in Carbon Tetrachloride.

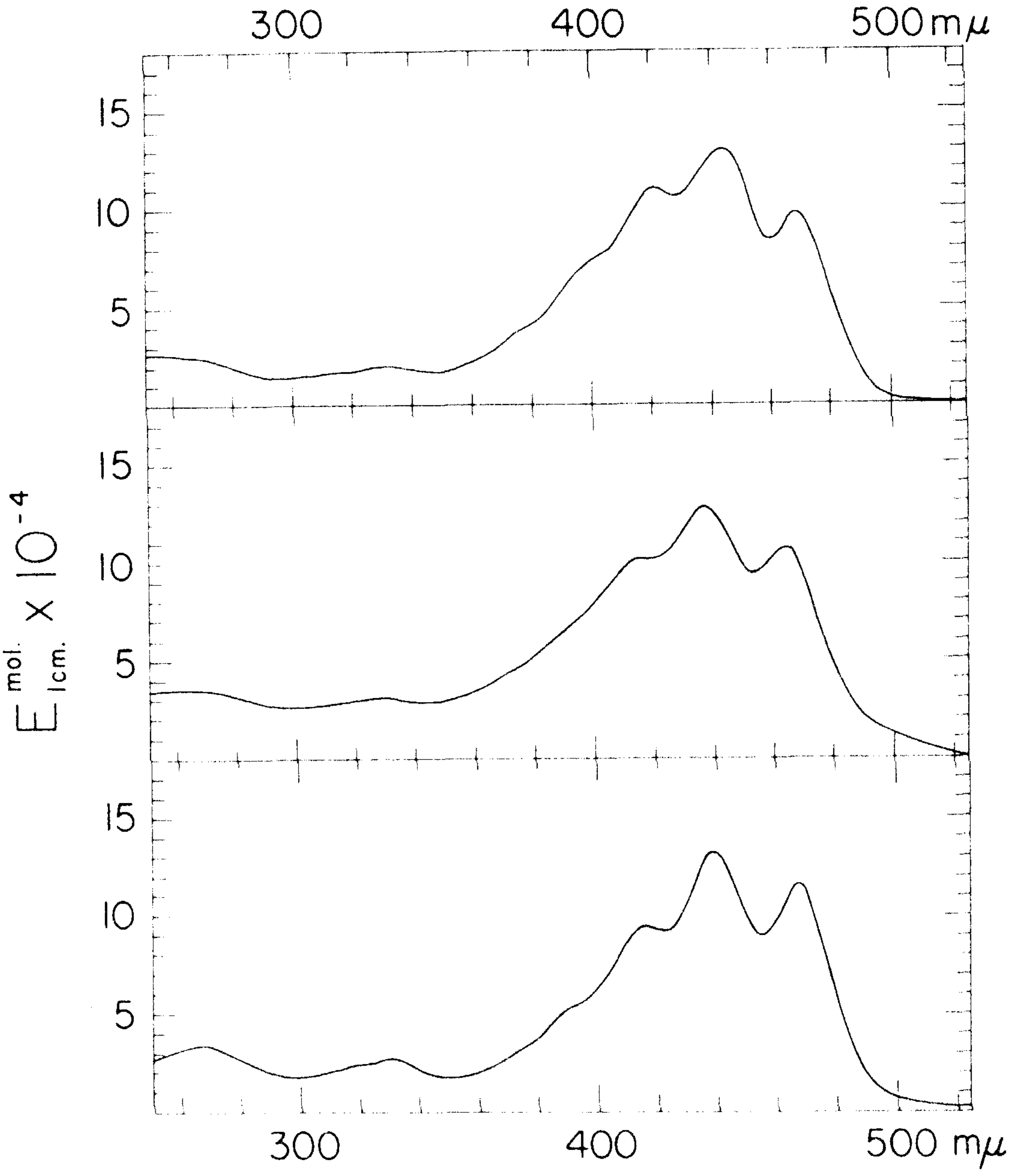


Fig. 3. Molecular Extinction Curves of Neo-4-hydroxy-α-carotenes V, U, and A (in Hexane). Top, neo V; middle, neo U; and bottom, neo A.

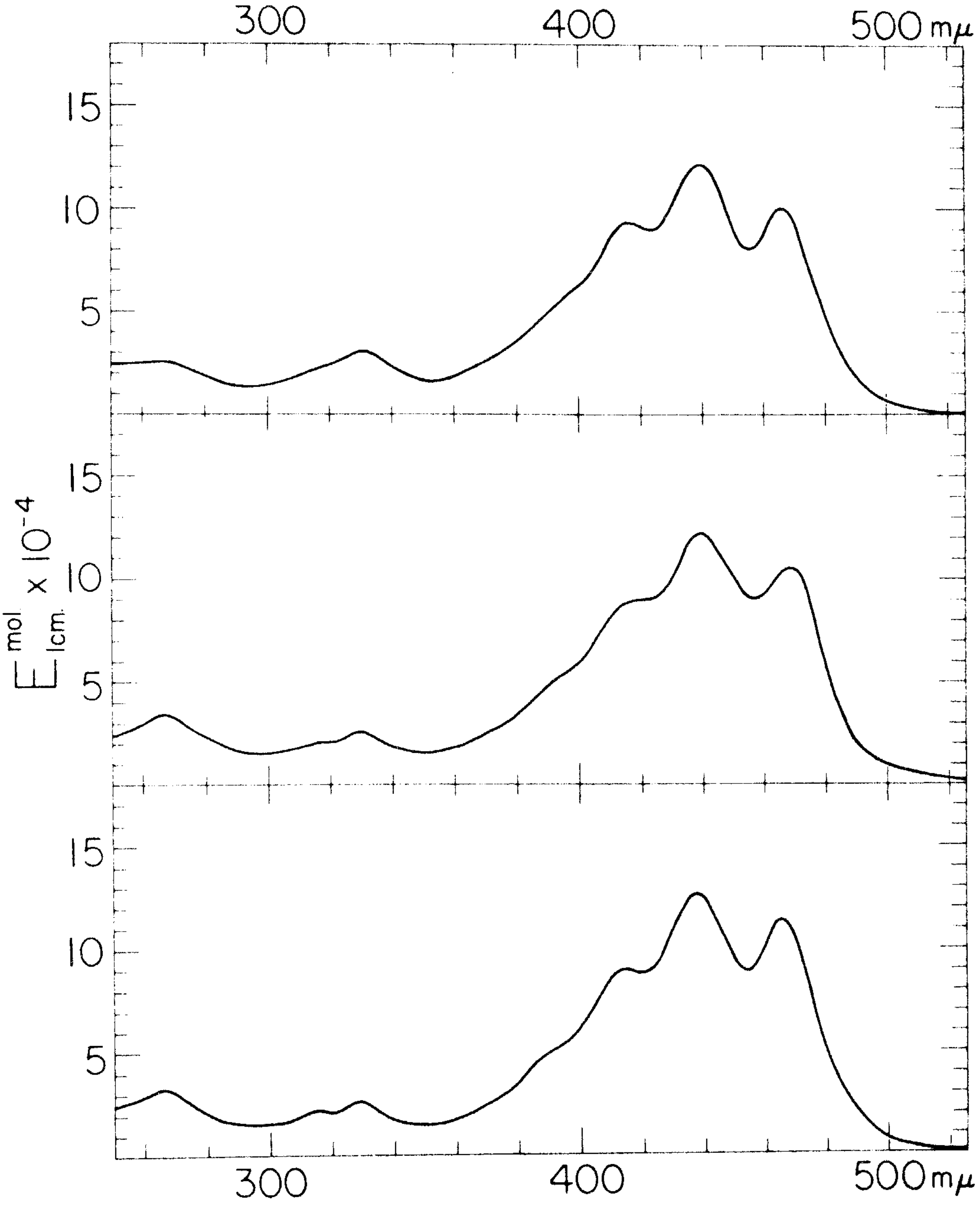


Fig. 4. Molecular Extinction Curves of Neo-4-hydroxy-α-carotenes B, C, and D (in Hexane). Top, neo B; middle, neo C; and bottom, neo D.

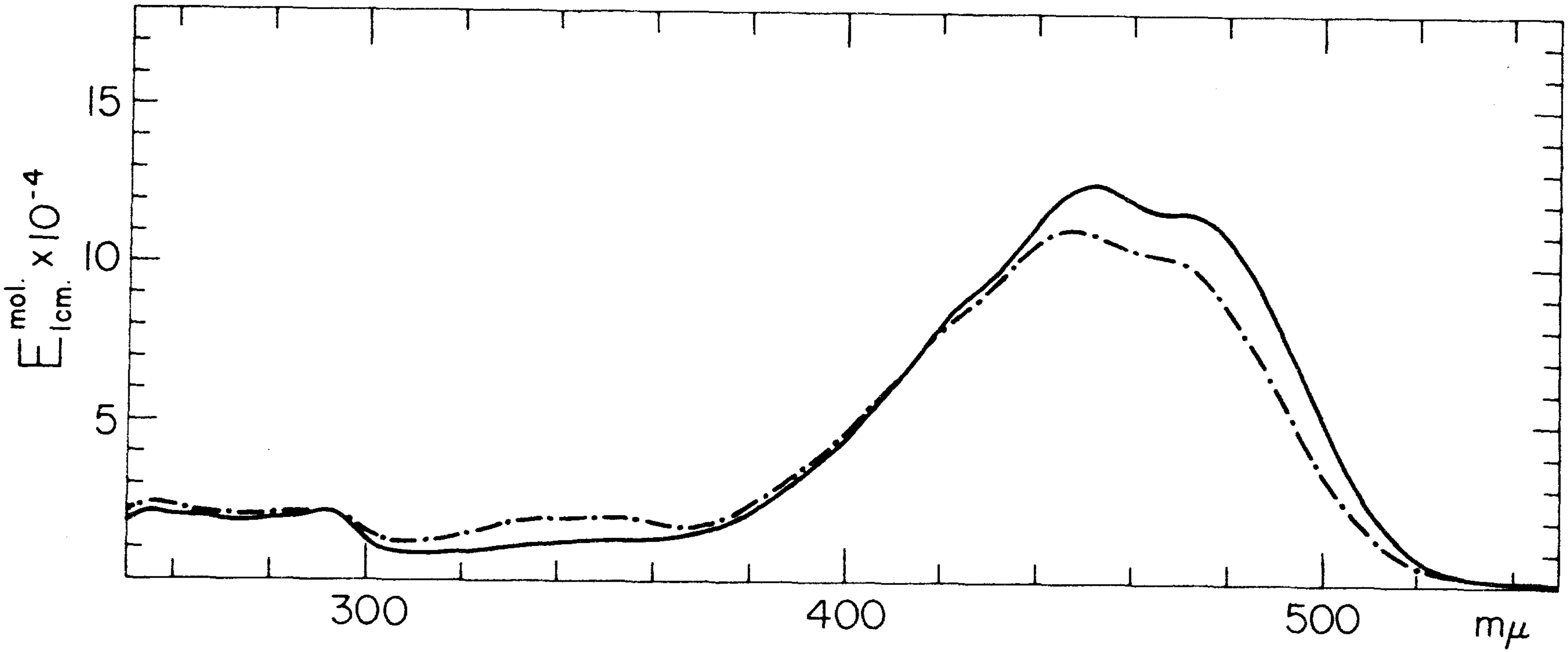


Fig. 5. Molecular Extinction Curve of 4-Keto-α-carotene in the Visible and Ultraviolet Regions (in Hexane): ———, fresh solution of the all-*trans* compound; and -·-·-, iodine-catalyzed stereoisomeric equilibrium mixture.

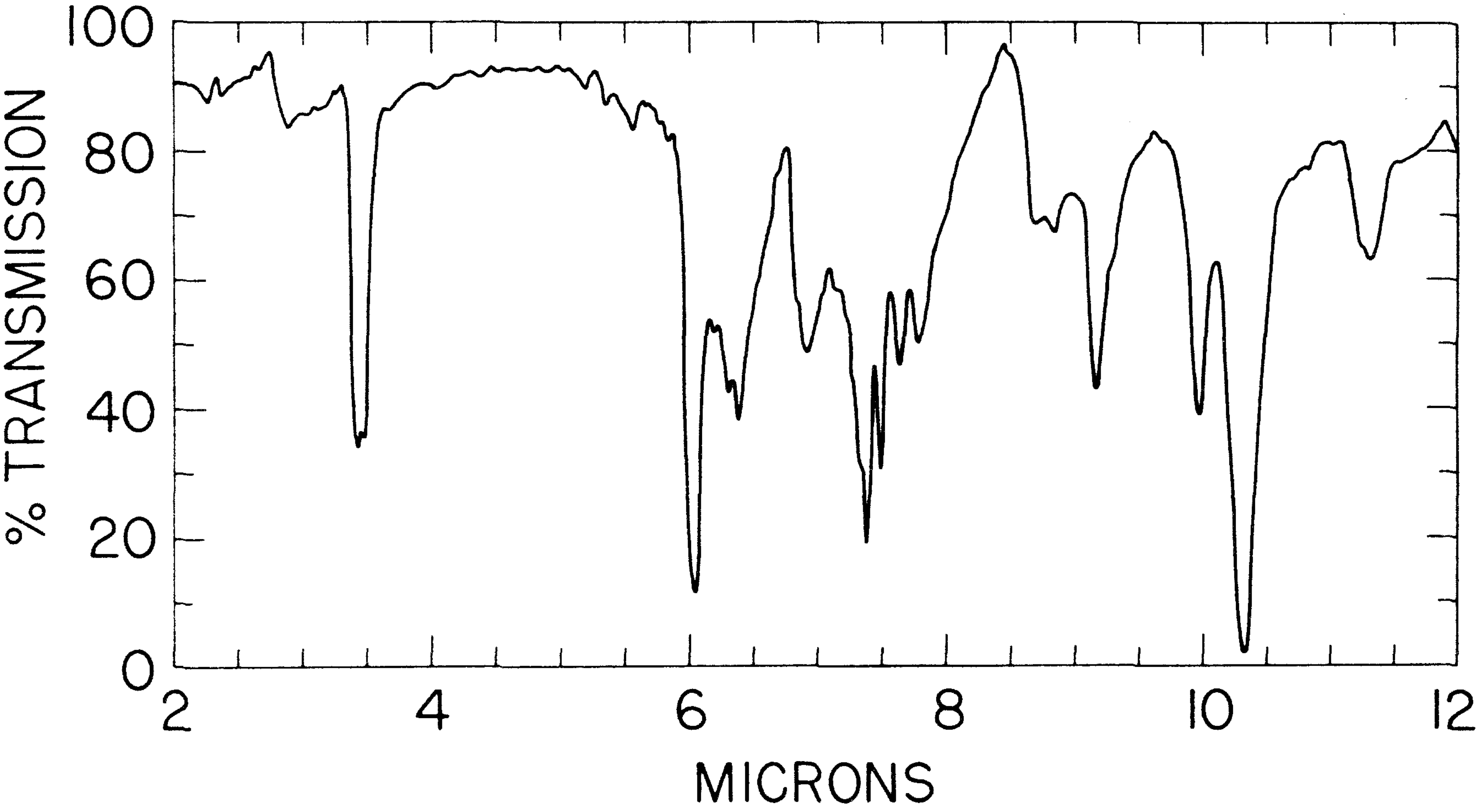


Fig. 6. Infrared Spectrum of All-*trans*-4-keto-α-carotene in Chloroform.

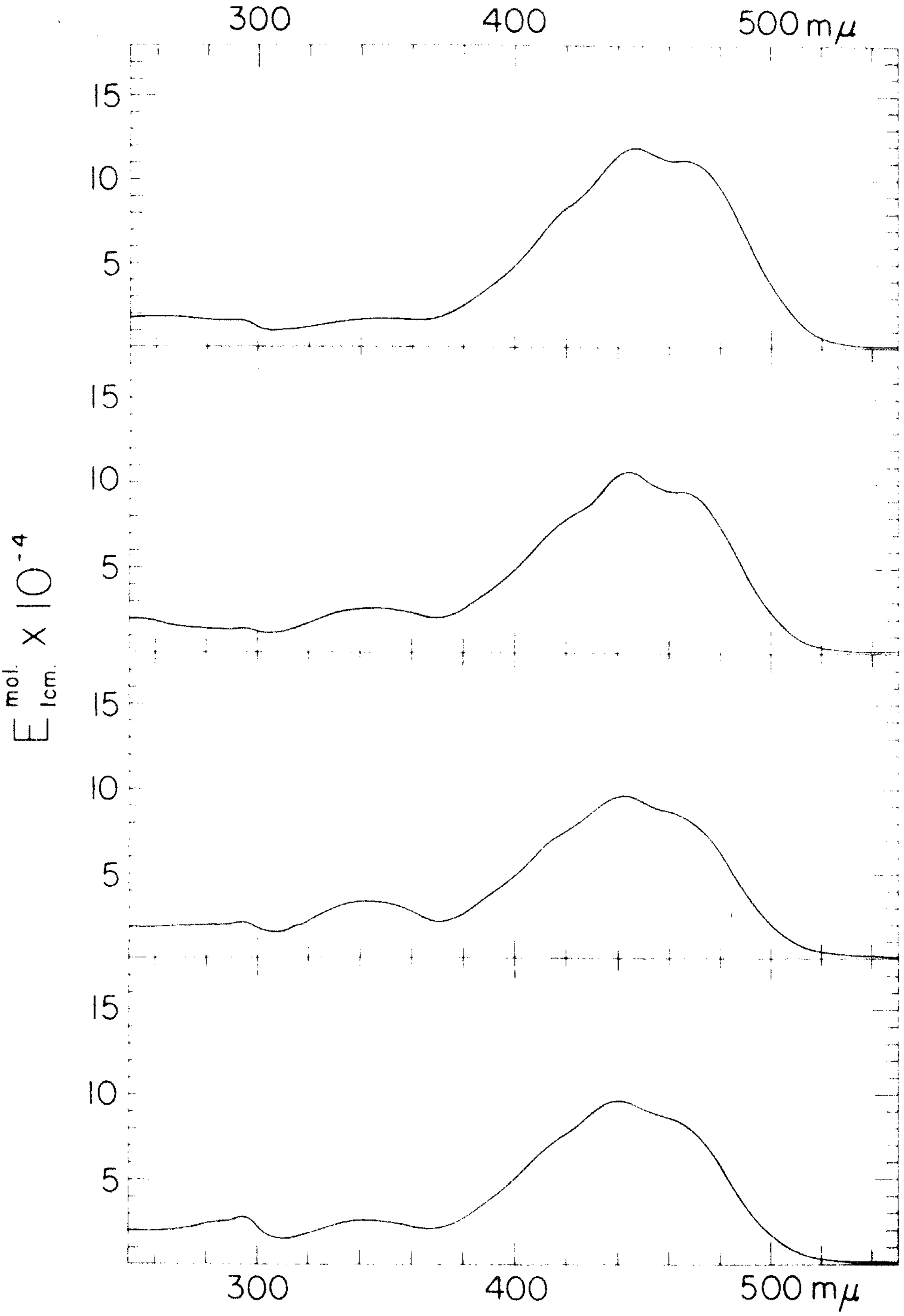


Fig. 7. Molecular Extinction Curves of Neo 4-Keto-α-carotenes U, A, B, and C (in Hexane). Top to bottom: neo U, neo A, neo B, and neo C.

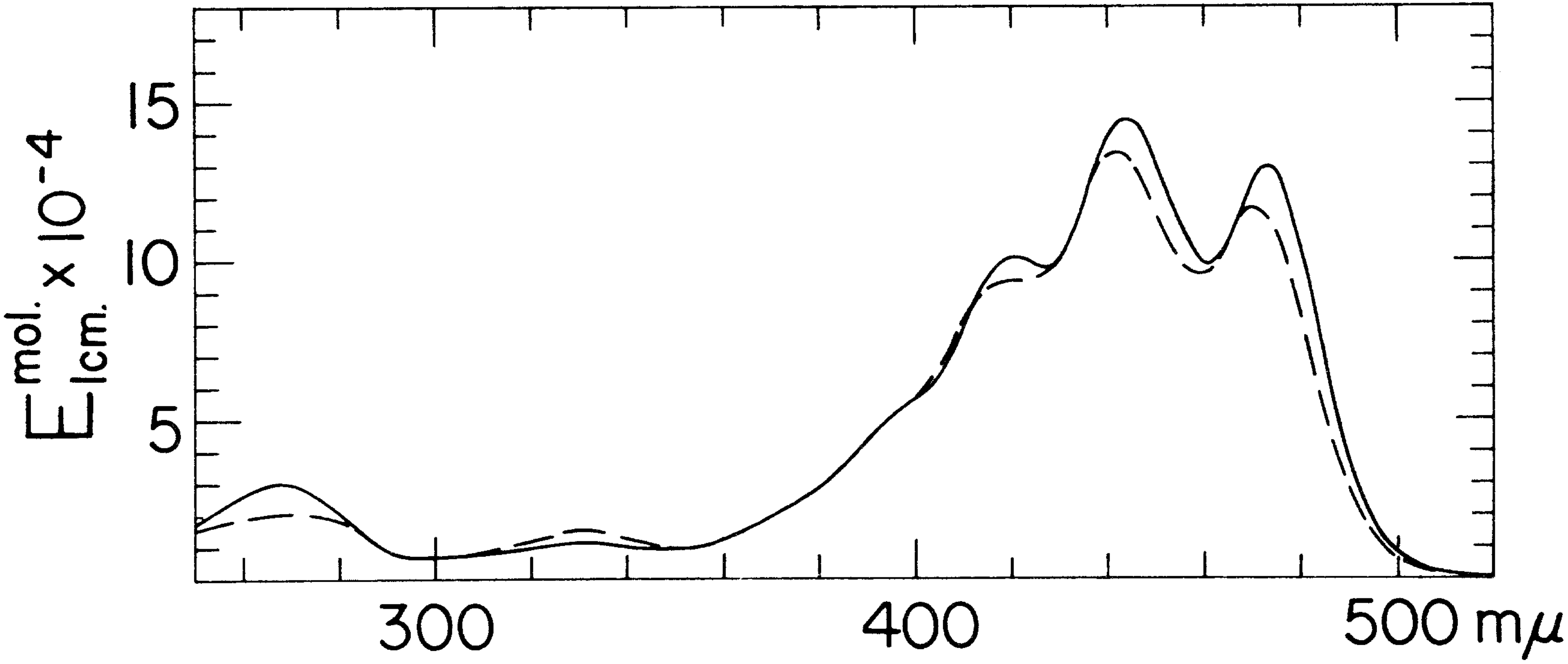


Fig. 8. Molecular Extinction Curve of 4-Ethoxy-α-carotene in the Visible and Ultraviolet Regions (in Hexane): ———, fresh solution of the all-*trans* compound; and - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

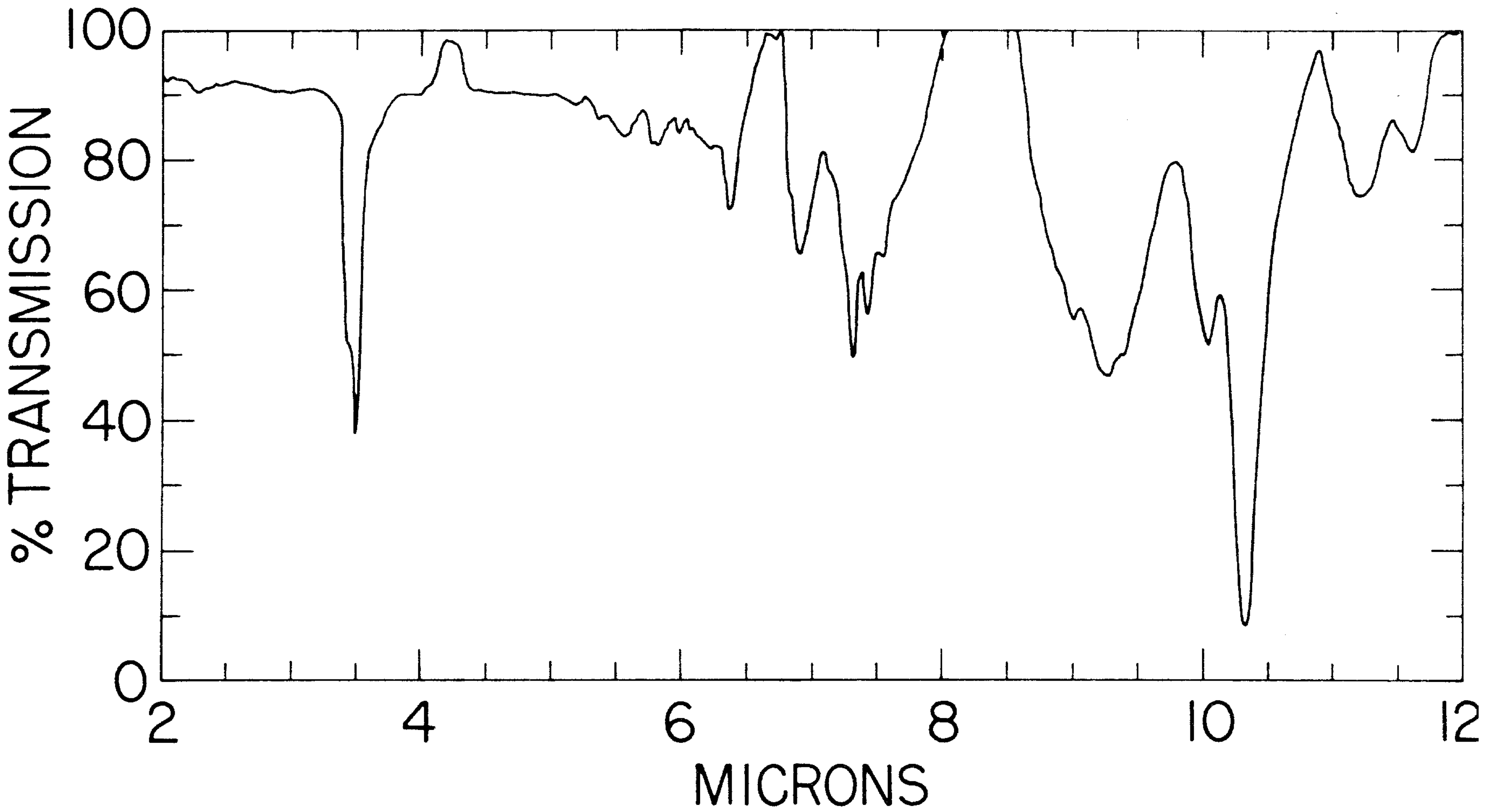


Fig. 9. Infrared Spectrum of All-*trans*-4-ethoxy-α-carotene in Chloroform.

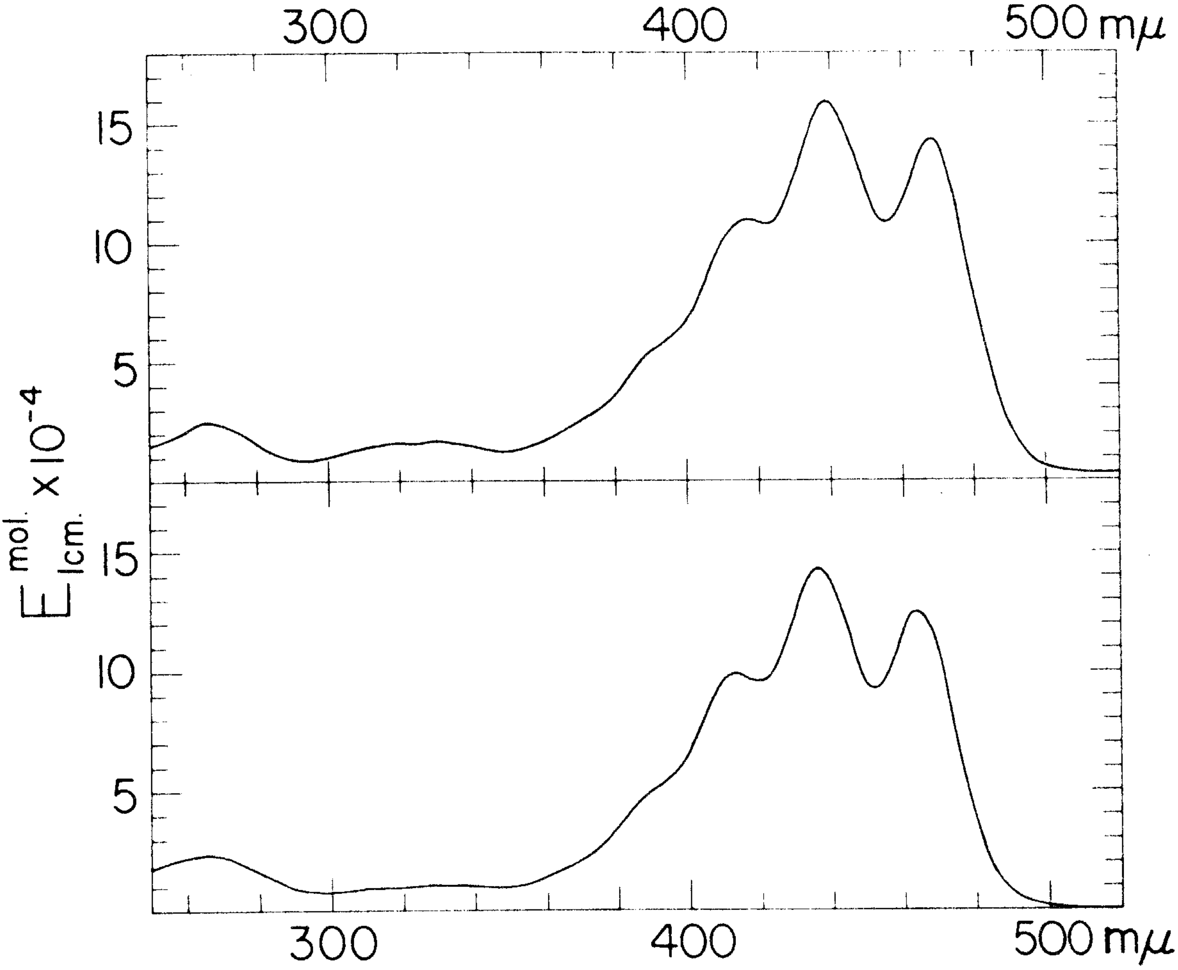


Fig. 10. Molecular Extinction Curves of Neo-4-ethoxy-α-carotenes V (top) and U (bottom) (in Hexane).

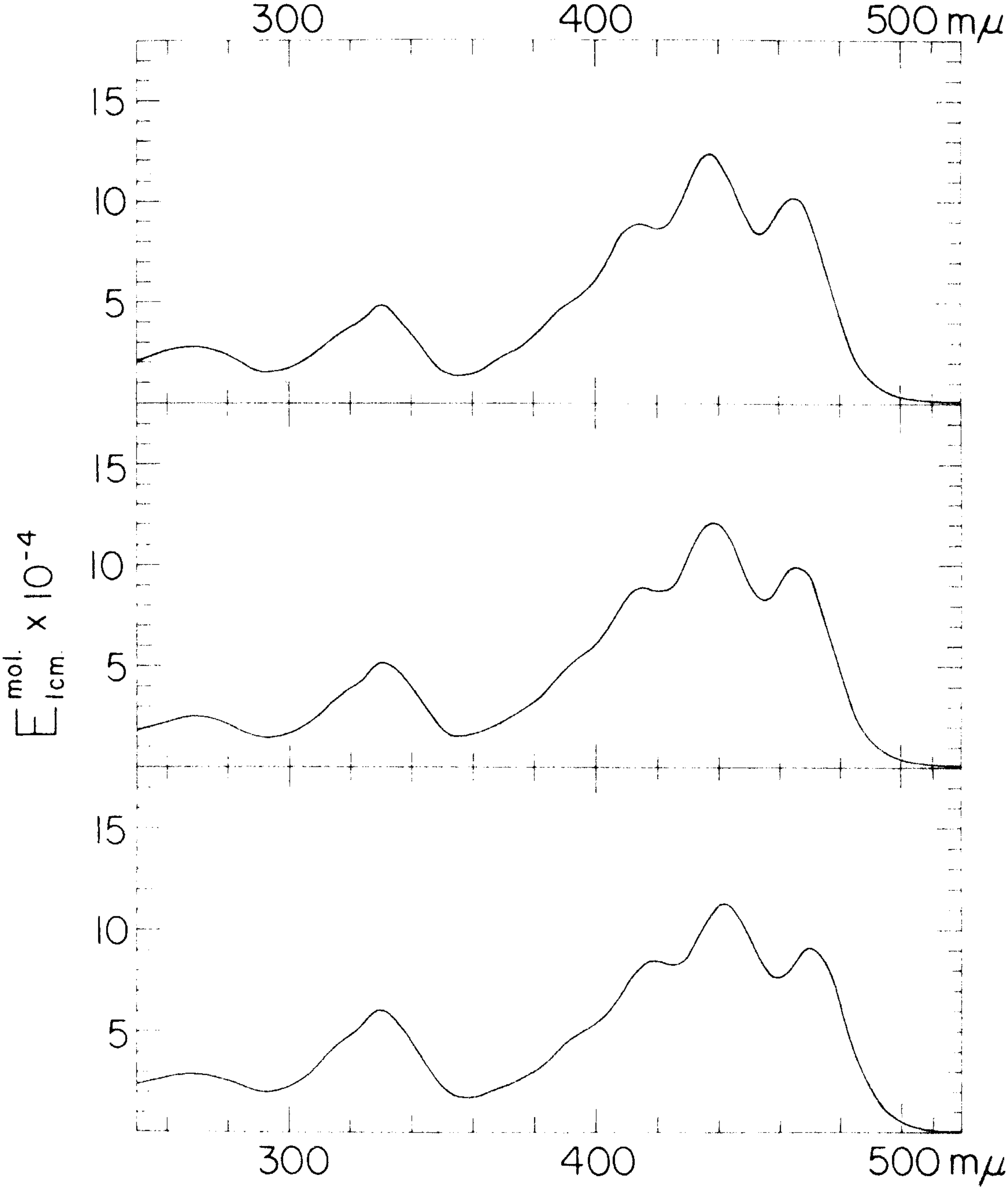


Fig. 11. Molecular Extinction Curves in Hexane of Neo-4-ethoxy-α-carotenes A (top), B (middle), and C (bottom).

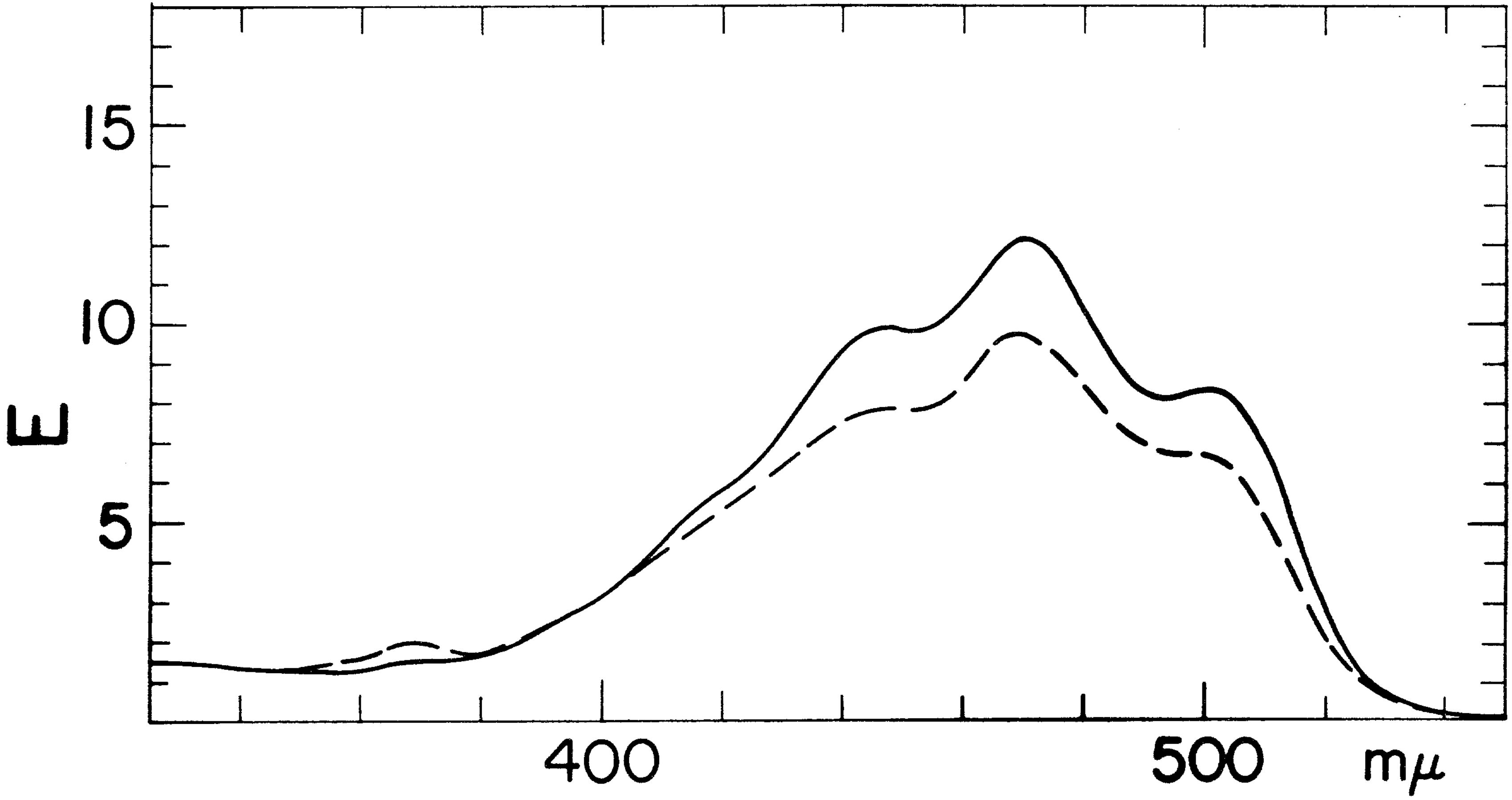


Fig. 12. Extinction Curve in Hexane of "Unidentified Pigment I": ——, fresh solution of the all-*trans* compound; and - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

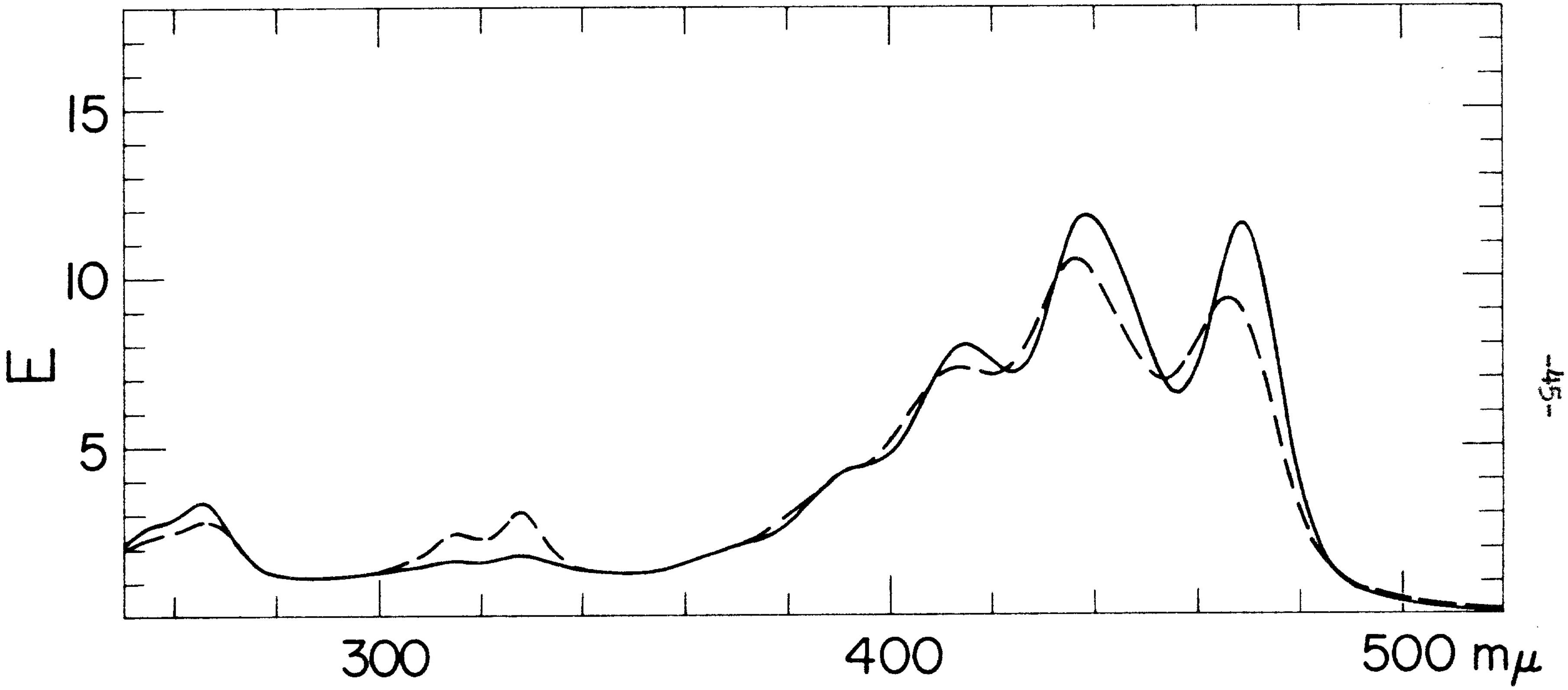


Fig. 13. Extinction Curve in Hexane of "Unidentified Pigment II": ——, fresh solution of the all-trans compound; - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

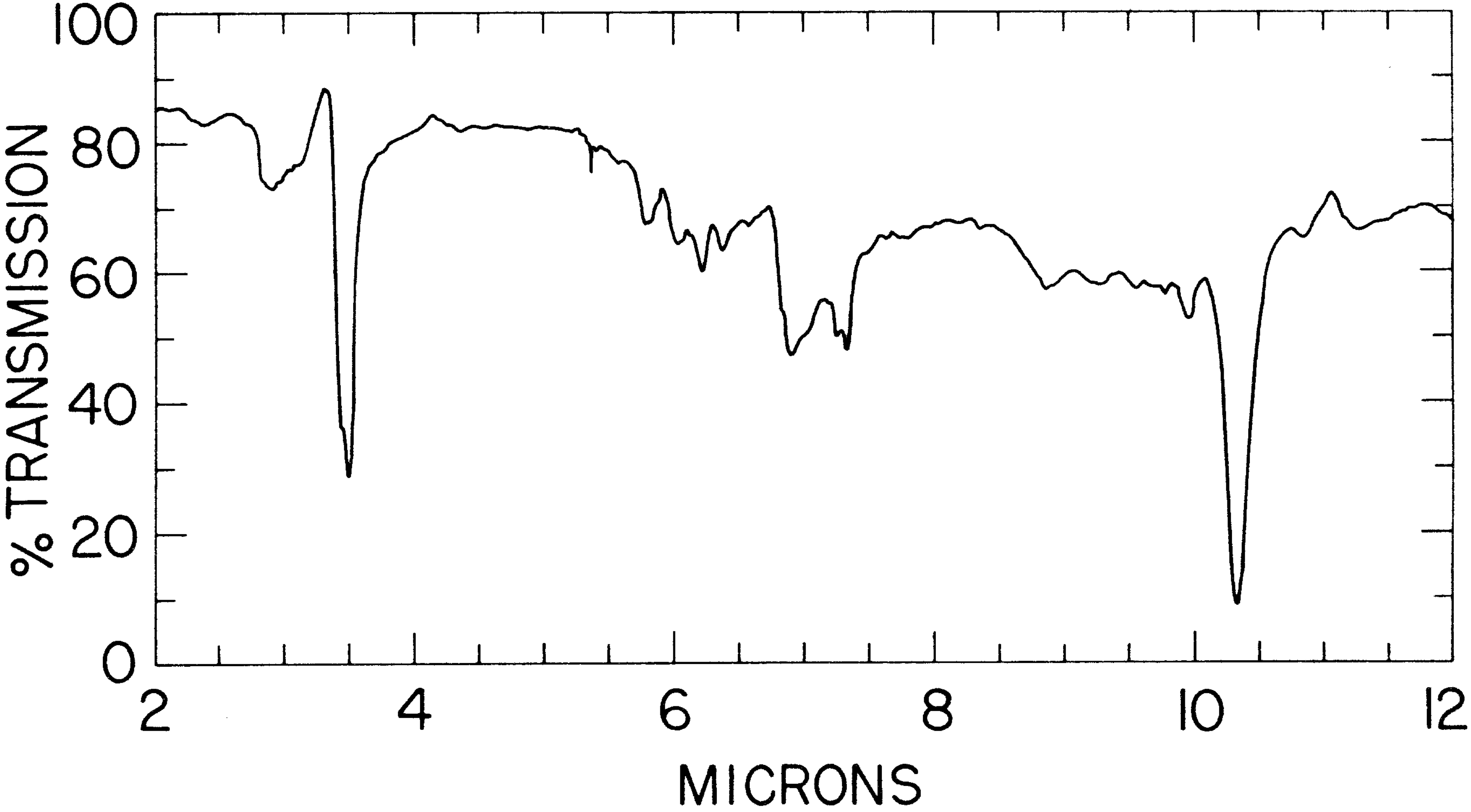


Fig. 14. Infrared Spectrum of All-*trans*-"Unidentified Pigment II" in Chloroform.

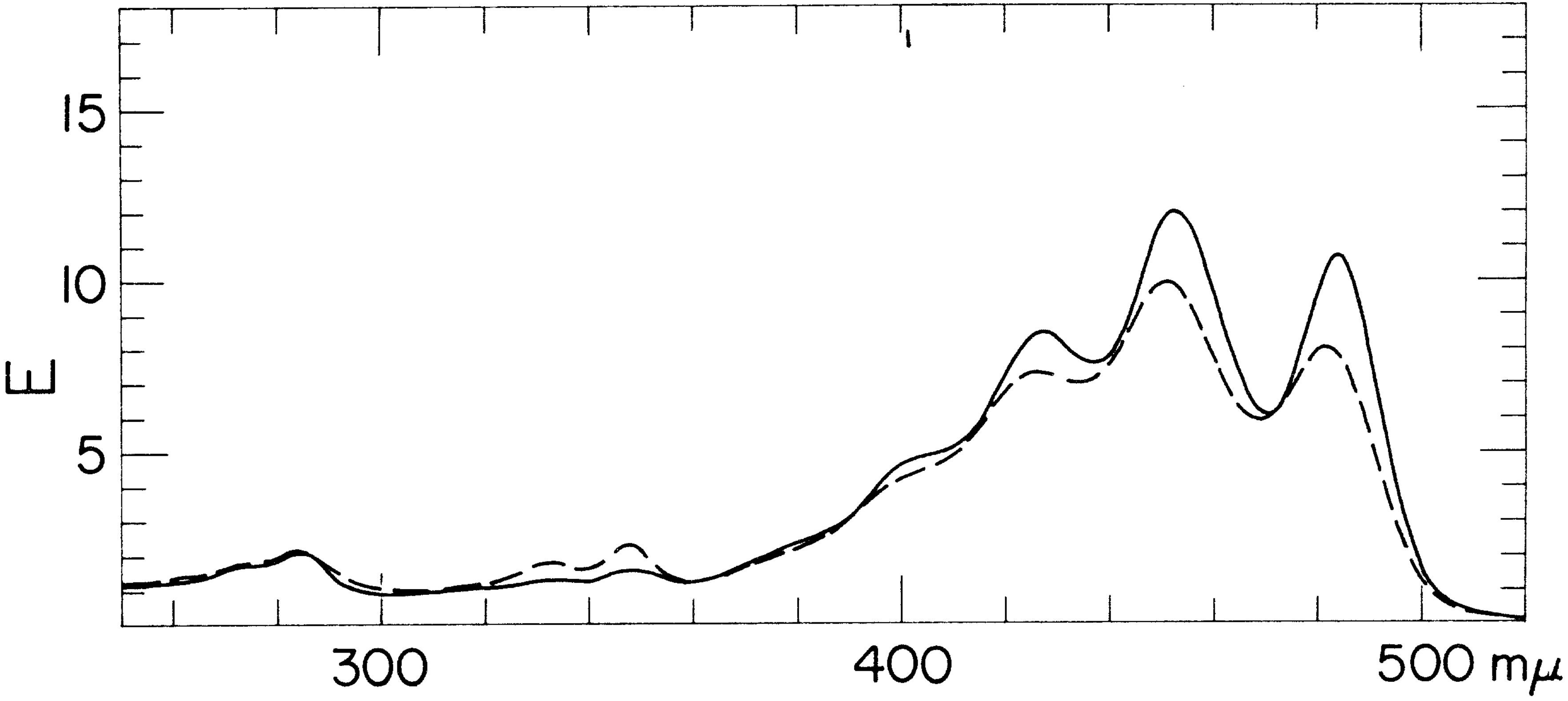


Fig. 15. Extinction Curve in Hexane of "Unidentified Pigment III": ———, fresh solution of the all-trans compound; and - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

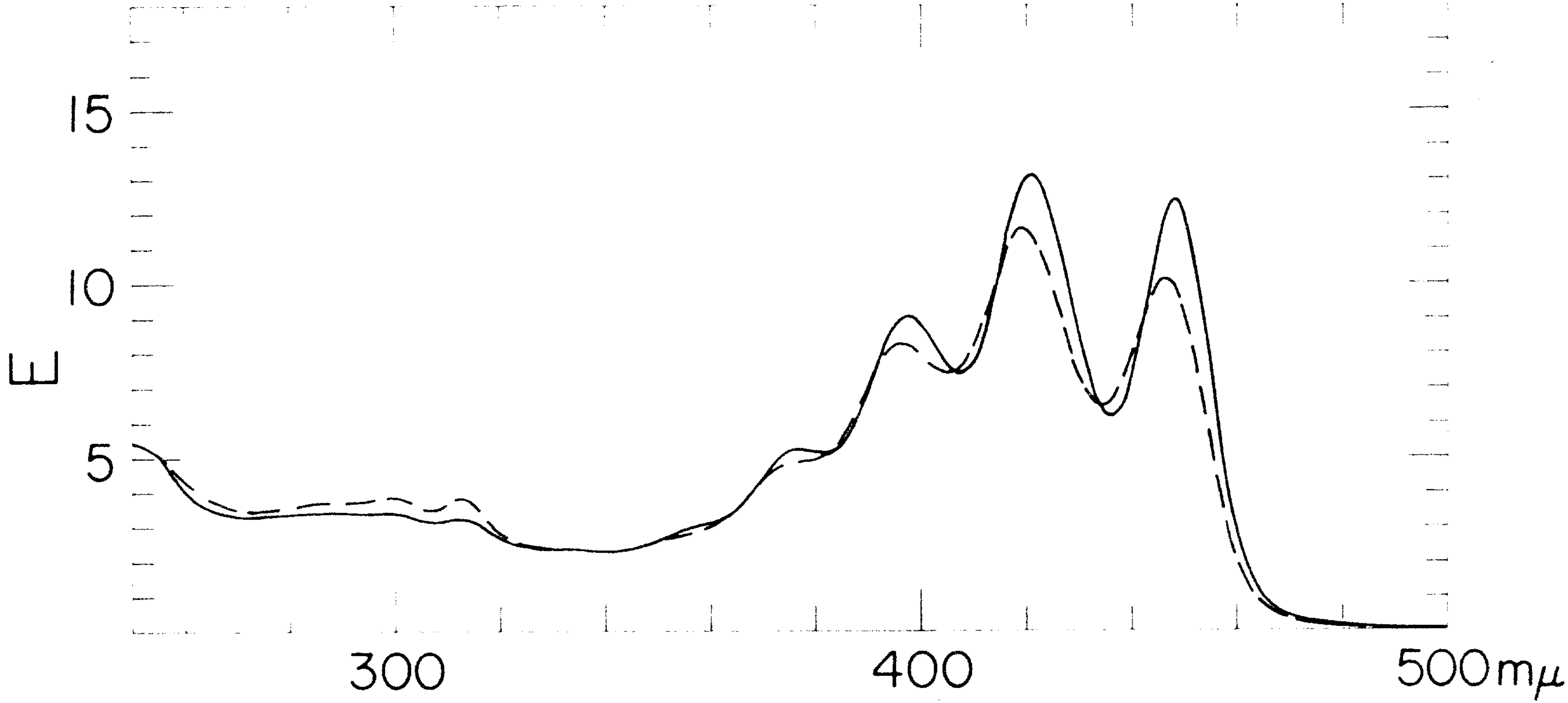


Fig. 16. Extinction Curve in Hexane of "Unidentified Pigment IV": ———, fresh solution of the all-*trans* compound; and - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

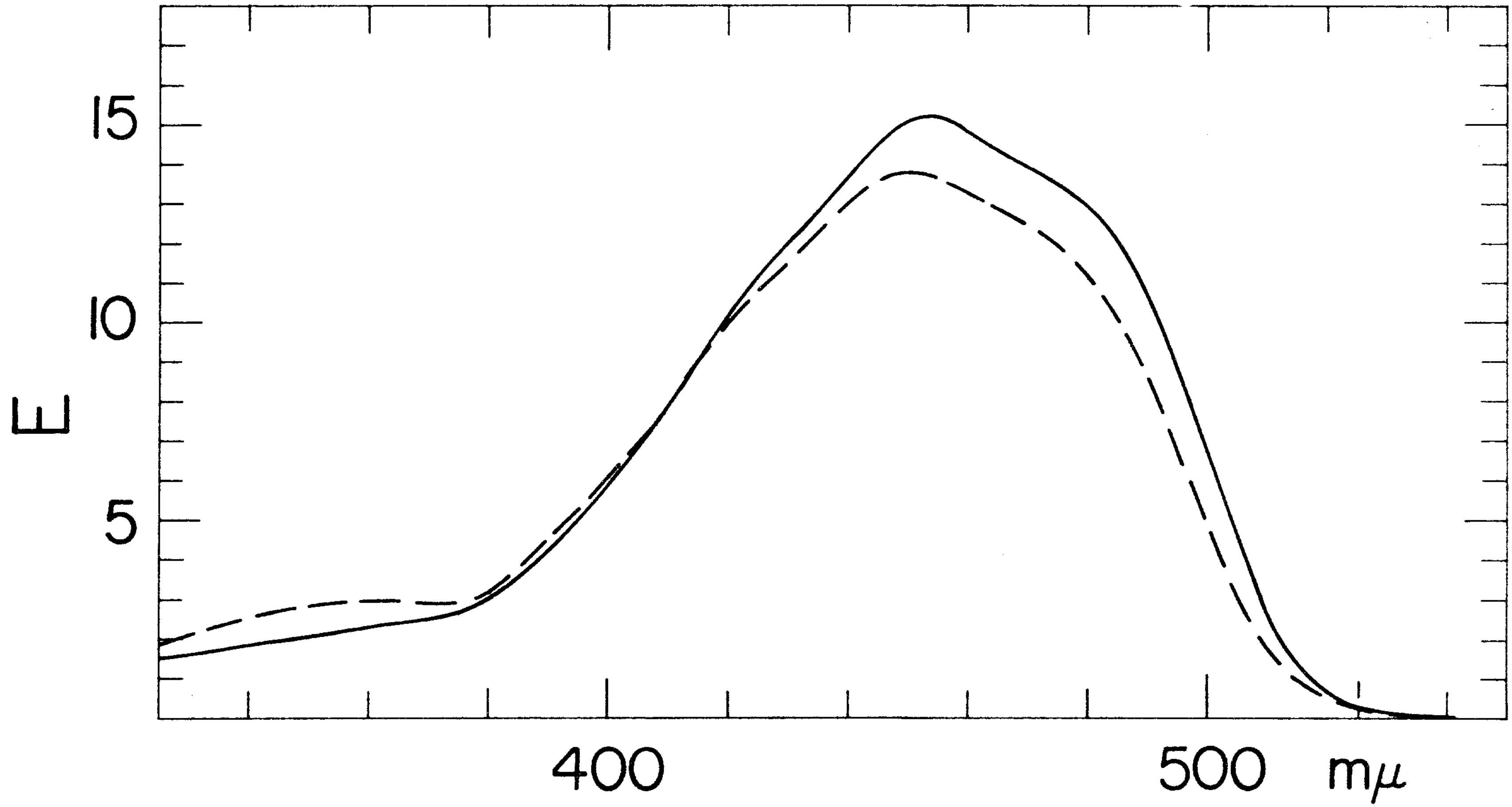


Fig. 17. Extinction Curve in Hexane of 3,4-Dehydro-α-carotene: ———, fresh solution of the all-*trans* compound; and - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

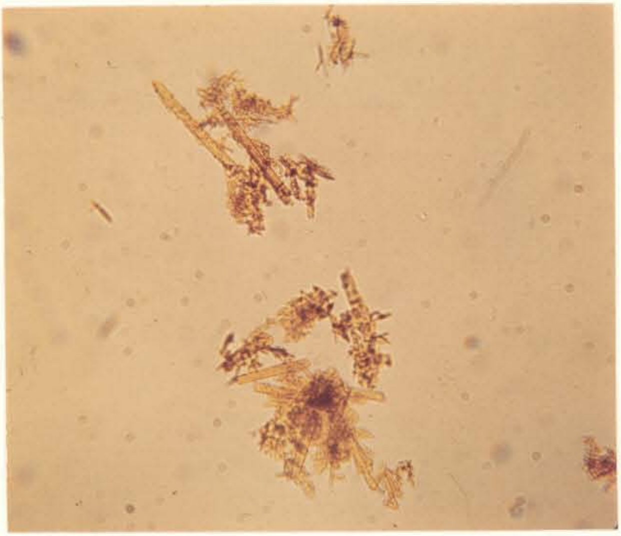


Fig. 18. 4-Hydroxy-α-carotene.

Scale: 0.1 mm.

Figs. 18 - 22. Photomicrographs. These photomicrographs were taken using a Bausch & Lomb Model HA microscope with 43x achromatic objective (0.65 N.A.), 10x Huygenian eyepiece, and 1.25 N.A. Abbe Condenser. The light source was a G. E. 115 v. projection lamp, operated at 130 v. The camera was a Zeiss Ikon Contaflex I, with microscope adapter; film, Kodachrome Type A. Total magnification after photographic processing: 250x.

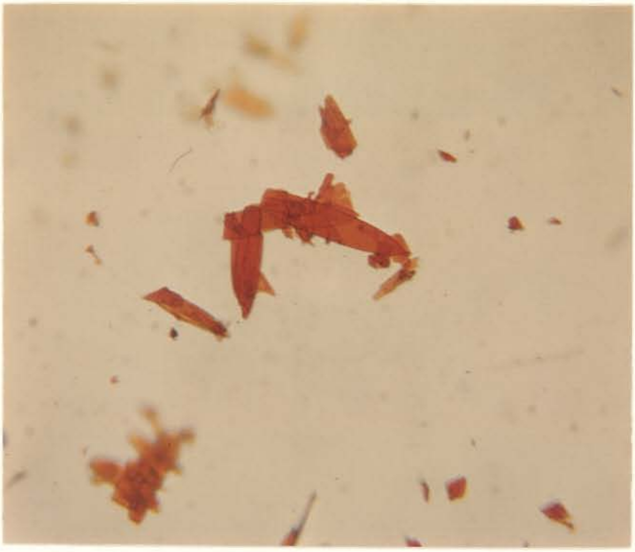


Fig. 19. 4-Keto-α-carotene.

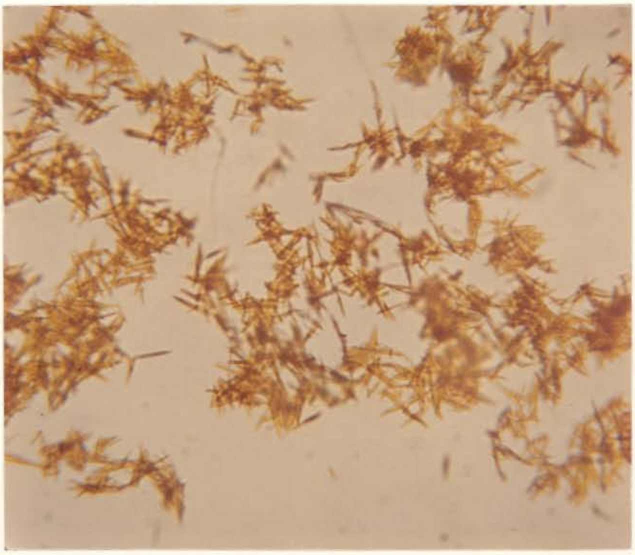


Fig. 20. 4-Ethoxy-α-carotene.

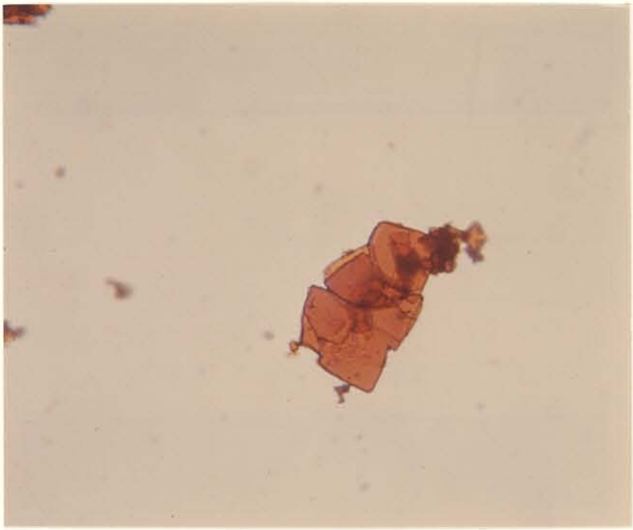


Fig. 21. 3,4-Dehydro-α-carotene.

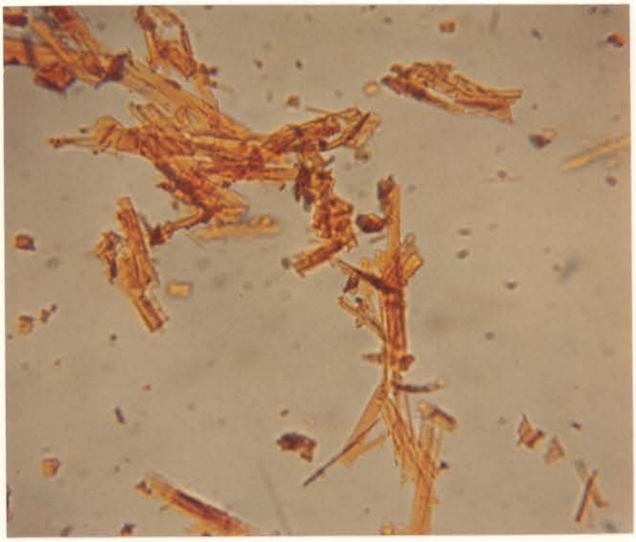


Fig. 22. 5,6-Dihydroxy-5,6-dihydrolycopene.

B. EXPERIMENTAL PART

1. Materials and Methods.

In the isolation and characterization of the hydrolysis and alcoholysis products of the boron trifluoride - α-carotene complex, some materials, equipment, and techniques were used frequently enough to warrant their description here. This description applies to all experiments conducted in the course of the present study unless mention is made to the contrary.

Adsorbents. - In most instances, calcium hydroxide (Sierra Hydrated Lime; Superfine, United States Lime Products Corp., Los Angeles, California) was used as the adsorbent, mixed with 1/2 part (by weight) of Celite (No. 545; Johns-Manville Co.). This adsorbent will be referred to as "lime-Celite."

Other adsorbents used included magnesium oxide ("Sea Sorb" 43; Food Machinery and Chemical Corp., San Jose, California), and aluminum oxide (Alorco, Grade F; Aluminum Ore Co., East Saint Louis, Illinois, -80 mesh, reground to -200 mesh), in mixtures with Celite and sometimes also with lime.

Solvents. - For development of chromatograms, hexane (Phillips Petroleum Co., commercial grade) was used, either as sold or mixed with certain percentages by volume of acetone (C. P., U. S. P.). The elution of chromatographic zones was accomplished with acetone on sintered glass funnels. Benzene used for crystallization was Reagent Grade, and the methanol used for the same purpose was commercial grade, either as obtained (referred to as "methanol") or mixed with 5 per cent water by volume ("95 per cent methanol").

Reagent Grade chloroform, C. P. acetone, distilled water, and a saturated solution of Reagent Grade sodium bicarbonate in distilled water were used in the complexing and hydrolysis reactions.

The "optical" hexane used in the visible and ultraviolet spectral regions was prepared by treating Phillips commercial grade hexane repeatedly with fuming sulfuric acid (65 per cent free SO_3) until the optical density of the hexane was approximately equal to that of water. The hexane was then distilled and stored in brown bottles. Eastman Spectro Grade (Distillation Products Industries, Rochester, New York) chloroform and carbon tetrachloride were used as solvents for determining infrared spectra.

Reagents. - Boron trifluoride etherate was prepared essentially according to the method of Hennion et al. (17). 1 lb. of boron trifluoride gas (Ohio Chemical and Manufacturing Co., Cleveland, Ohio) was dissolved in 1 lb. of anhydrous ether (Reagent Grade) while stirring and cooling in an ice bath. The mixture was distilled through a short column packed with 5-mm. glass beads; the fraction boiling at 124-125° (cor.) was collected and stored in a well-closed brown glass-stoppered bottle. All-glass apparatus was used, and glass and silicone stopcock grease (Dow Corning Corp., Midland, Michigan) and carborundum boiling chips were the only substances to come into contact with the boron trifluoride etherate.

The N-bromosuccinimide was manufactured by Arapahoe Chemicals, Inc., Boulder, Colorado.

α-Carotene, $C_{40}H_{56}$, was prepared by chromatographic resolution of commercial carotene (Barnett Laboratories, Long Beach, California).

Spectra. - Visible and ultraviolet spectra were obtained using a Cary Recording Spectrophotometer, Model 11M, with 10 millimeter cells. For preliminary visual observations a Loewe-Schumm Evaluating Grating Spectroscope (Zeiss) was used. Infrared spectra were recorded in a Perkin-Elmer Infrared Spectrophotometer, Model 21, as 1 per cent solutions in 1.0 millimeter sodium chloride cells, using a sodium chloride prism.

The molar extinction values given represent the average of results based on two independent weighings.

Chromatographic Columns. - Cylindrical, 45 x 4.5 cm. columns were used in most experiments; columns of other dimensions will be noted where appropriate.

Other Remarks. - Evaporations were carried out under aspirator vacuum, in a water bath kept at 55°; agitation was supplied by a slow stream of nitrogen.

Hexane and chloroform solutions were washed acetone-free in the automatic LeRosen apparatus (29) and dried over anhydrous sodium sulfate (Reagent Grade).

All crystallizations were accomplished in a two-solvent system. The sample was first dissolved in a minimum amount of warm (40°) benzene, and then the second solvent (methanol or 95 per cent methanol) was added cautiously, dropwise, running down the warm glass wall, while the solution was being rapidly stirred. The temperature was maintained at 40° until crystallization was practically complete, then kept at 4° (in the refrigerator) for about a day. The crystals were centrifuged, washed with a small amount of the second solvent, centrifuged again, and dried for two hours *in vacuo* in an Abderhalden apparatus at the temperature of refluxing acetone.

Melting points were taken in an electrically heated Berl block, in evacuated and sealed capillaries, and have been corrected.

Partition coefficients were determined by the method of Petracek (13) and refer to the hexane - 95 per cent methanol system.

Fluorescence was observed in the light of a portable ultraviolet lamp (Photoflood bulb No. 1 with molded Corning light filter No. 5840) or of a mercury arc lamp, the glass envelope of which acted as a filter to eliminate most of the visible light (G. E. Mazda No. B-H4).

Iodine Catalysis. - After the extinction of the original solution had been measured, approximately 1 per cent of the pigment weight of iodine (1 drop of a 0.1 per cent solution in optical hexane) was added to the solution while still in the 3-ml. spectrophotometer cell. The solution was then subjected to ten minutes of illumination as described on p. 88 .

The solution of iodine in hexane was of such a concentration that 1 drop of it was necessary, resulting in a volume change of only about 1 per cent in the spectrophotometer cell.

Test for Allylic Hydroxyl or Alkoxyl Group. - To a few milliliters of a dilute chloroform solution of the compound (3 mg. per liter) in a test tube (18 x 150 mm.) was added 5 drops of a saturated solution of dry HCl gas in chloroform. Marked deepening of the color within a few minutes, when compared to an untreated parallel sample, was considered to be a positive test, confirmed when necessary by chromatography and spectrophotometric readings.

Microanalyses were performed by Dr. A. Elek, Los Angeles, Calif., by Mr. G. Swinehart, formerly of the California Institute of Technology, (Professor A. J. Haagen-Smit's Laboratory), and by Microchemical Specialties Co., Berkeley, Calif.

Bioassays were carried out by the late Dr. H. J. Deuel, Jr. and by Mr. A. Wells, both of the University of Southern California, Los Angeles.

2. Formation and Hydrolysis of the α-Carotene - Boron Trifluoride Complex.

A total of 3 grams of α-carotene, in thirty 100-mg. portions, was treated with boron trifluoride etherate according to the following unit process scheme:

a.) 100 mg. of α-carotene was dissolved in 100 ml. of chloroform. The solution was not cooled below room temperature.

b.) 10 ml. of boron trifluoride etherate was added with stirring.

c.) The complexing reaction was allowed to proceed for 120 to 150 seconds, i. e., exactly 60 seconds after the solution had turned from a brownish green to a clear, deep blue.

d.) The solution was poured rapidly, with thorough agitation, into a mixture of 20 ml. water and 200 ml. acetone, which had been previously cooled to 0°. The original red-orange color of the α-carotene was restored within about 20 seconds.

e.) The acetone - chloroform mixture was poured into a 1-liter separatory funnel containing 100 ml. of saturated sodium bicarbonate solution and 150 ml. of iced water. The liquid was thoroughly shaken, allowing the carbon dioxide formed to escape at frequent intervals.

f.) When no further pressure increase was noted, the two layers were allowed to separate, and the lower (chloroform) layer was poured into an equal volume of acetone, shaken well, and washed acetone-free. (The washing was considered to be complete when the volume of the hypophase had been reduced to 100 ml.)

g.) The deep red-orange chloroform solution (hypophase) was dried and evaporated completely.

h.) The residue was dissolved in about 25 ml. of hexane and chromatographed on lime-Celite, using approximately 750 ml. of hexane as developer; development required 3/4 of an hour. The sequence of zones, from top to bottom, was as follows (the figures on the left denote the width of the zones in mm.; dashed lines indicate the locations at which the column was cut):

42	orange, with narrow brown bands at the top	Zone R
3	red	
9	yellow	
26	light yellow	
- - - -	- - - - - - - -	- - - -
5	colorless interzone	
10	light yellow	
- - - -	- - - - - - - -	- - - -
32	yellow	Zone Q
- - - -	- - - - - - - -	- - - -
3	light yellow	
10	pale yellow	
10	pale yellow	
18	faint pink	
12	pale orange	
5	colorless interzone	
10	very pale orange	
5	colorless interzone	
- - - -	- - - - - - - -	- - - -
27	yellow	Zone T
- - - -	- - - - - - - -	- - - -
23	pale yellow	
10	colorless interzone	
10	very pale yellow	
- - - -	- - - - - - - -	- - - -

30	yellow	Zone B
18	pale yellow	
14	colorless interzone	
28	pale green	Zone G
	Remainder of column, colorless	
	Filtrate, pale green with blue-grey fluorescence in ultraviolet light	

The lettered zones were cut out and eluted with acetone.

i.) The pigments of the respective zones were transferred to hexane by the addition of water; the hexane solutions were washed acetone-free and dried.

j.) The solution originating from the top zones, collectively designated as Zone R, was evaporated to about 25 ml. and developed on lime-Celite with about 750 ml. of 4 per cent acetone in hexane. Development was stopped when the following sequence was obtained (after about 3/4 of an hour):

16	light brown	
2	colorless interzone	
26	several faint yellow and orange zones	
22	nearly colorless interzone	
50	yellow	Zone A
8	nearly colorless interzone	
82	orange-yellow	Zone C
3	pink	Zone D
6	yellow	
25	orange-yellow	

13 red	Zone E
4 orange	
26 light yellow	
10 pink	
17 pale yellow	
14 pale pink	
10 nearly colorless interzone	
16 yellow	Zone H
24 yellow	
Remainder of column, colorless	

The lettered zones were cut out and eluted with acetone, then transferred to hexane; the hexane solutions were washed acetone-free and dried.

k.) The lettered pigment solutions (in hexane) were stored in the refrigerator in filled, stoppered volumetric flasks until the thirty separate portions of each zone, originating from the thirty 100-mg. unit columns, could be combined.

3. Characterization of the Fractions.

4-Hydroxy-α-carotene. - The combined hexane solutions of the thirty Zones C were evaporated to 150 ml., whereupon some red crystals appeared; these were redissolved by diluting the solution to 300 ml. with hexane. This pigment solution was rechromatographed on lime-Celite on two 60 x 8 cm. columns, using about 3 liters of 3 per cent acetone in hexane to develop each column. The following sequence was obtained after four hours' development:

30 several tan zones
60 colorless interzone
30 pale yellow (neo-4-hydroxy-α-carotene V, etc.)
60 colorless interzone
250 orange (all-*trans*-4-hydroxy-α-carotene)
50 yellow (neo-4-hydroxy-α-carotene A, etc.)
30 red
60 pink
30 colorless

The neo-4-hydroxy-α-carotene V, all-*trans*-4-hydroxy-α-carotene, neo-4-hydroxy-α-carotene A, and the combined red and pink bottom zones were cut out separately, eluted, transferred to hexane, washed, and dried. The neo-4-hydroxy-α-carotene V fraction was eventually combined with Zone A, since Zone A was believed to contain at least one *cis*-4-hydroxy-α-carotene on the basis of visual spectroscopic observations. The neo-4-hydroxy-α-carotene A was combined with Zone D, and the combined red and pink zones with Zone E, for the same reason. The solution of all-*trans*-4-hydroxy-α-carotene was evaporated to dryness and crystallized from benzene-95 percent methanol. Yield: 630 mg. = 21 per cent of the starting material (estimated photometrically). The crystals obtained weighed 285 mg. = 9.5 per cent of the α-carotene complexed.

Properties of All-*trans*-4-hydroxy-α-carotene:

Spectrum: Wavelengths of maxima 474, 445, 421 $m\mu$; $E^{mol.}_{1\ cm.} = 14.5 \times 10^4$ at 445 $m\mu$ (in hexane). The ultraviolet and infrared curves appear on pp. 33 and 34 .

Crystal Form: Large rectangular orange plates with "split" or "splintered" ends (like a wooden plank). (Photomicrograph on p. 50.)

Melting Point: 177-178°.

Partition Behavior: 84:16.

Chromatographic Behavior: Develops with 3 to 4 per cent acetone in hexane on lime-Celite; it is adsorbed above the 4-keto-α-carotene zone.

Reaction with Acid Chloroform: Gives a positive reaction, but the color change is not as pronounced as in the case of isocryptoxanthin.

Analysis: (two independent samples)

Calcd. for $C_{40}H_{56}O$:	C, 86.89;	H, 10.21.
Found:	C, 86.42;	H, 10.17.
	86.88	11.10.

Provitamin A Activity in the Rat: None.

Stereoisomers of 4-Hydroxy-α-carotene. - The hexane solutions of the thirty Zones A (p. 60) were combined with the two portions of neo-4-hydroxy-α-carotene V, etc. resulting from the rechromatographing of Zone C; the total mixture was evaporated to 100 ml., then chromatographed on a single column, using about 1 liter of 4 per cent acetone in hexane as developer:

70 mostly colorless
70 pale yellow (neo-4-hydroxy-α-carotene V)
60 colorless interzone
18 pale yellow (neo-4-hydroxy-α-carotene U)
54 colorless interzone
58 yellow (all-*trans*-4-hydroxy-α-carotene)
120 mostly colorless; several very narrow, faint yellow zones (neo-4-hydroxy-α-carotenes A, B, etc.)

The neo-4-hydroxy-α-carotenes A, B, etc. were eventually combined with Zone D; the all-*trans*-4-hydroxy-α-carotene was crystallized and added to the substance obtained from Zone C (p. 61).

Attempts were made to crystallize neo-4-hydroxy-α-carotenes V and U from hexane - absolute ethanol, hot methanol, benzene - methanol, and from benzene - 95 per cent methanol systems without success. The properties reported in Table 9 (p. 30) refer to pure, chromatographically homogeneous preparations.

Hexane solutions of the thirty Zones D were combined with the portions of neo-4-hydroxy-α-carotenes A, B, etc. resulting from the rechromatographing of Zones C and A; the total solution was evaporated to 100 ml. and developed on a single column, using about 1 liter of 4 per cent acetone in hexane. After one and a half hours the following sequence was observed:

8 brown: oxidative destruction products
59 colorless interzone
8 yellow (neo-4-hydroxy-α-carotene V)
8 colorless interzone
7 faint yellow (neo-4-hydroxy-α-carotene U)
28 colorless interzone

32 yellow (all-*trans*-4-hydroxy-α-carotene)
8 colorless interzone
18 light yellow (neo-4-hydroxy-α-carotene A)
7 colorless interzone
10 light yellow (neo-4-hydroxy-α-carotene B)
9 very light pink
12 colorless interzone
32 yellow (neo-4-hydroxy-α-carotene C)
32 colorless interzone
13 faint yellow (neo-4-hydroxy-α-carotene D)
46 colorless interzone
16 pink
25 very faint yellow
72 colorless

Filtrate, several very faint yellow, orange,
and pink zones

Unsuccessful attempts were made to crystallize neo-4-hydroxy-α-carotenes A, B, C, and D; however, the properties listed in Table 9 (p. 30) refer to chromatographically pure and homogeneous substances.

All isomers were identified as members of the stereoisomeric 4-hydroxy-α-carotene set by the method described on p. 87.

4-Keto-α-carotene. - *The hexane solutions of the thirty* zones E (p. 61) were combined, evaporated to 50 ml., and chromatographed on two columns, using about 750 ml. of 3 per cent acetone in hexane to develop each chromatogram:

24 colorless
6 yellow
26 colorless interzone
14 faint pink
27 colorless interzone
12 yellow
32 colorless interzone
39 light orange

22 pink
27 orange
43 red ("unidentified pigment I")
18 yellow
13 pinkish orange
77 red (all-trans-4-keto-α-carotene)
10 yellow
19 colorless interzone
41 orange

Filtrate: three minor orange zones

The 4-keto-α-carotene was crystallized from benzene-methanol. Photometric estimate of yield: 19.3 mg. = 0.7 per cent of the original α-carotene; the crystals weighed 14.6 mg. = 0.5 per cent.

Properties of 4-Keto-α-carotene:

Spectrum: Ultraviolet and infrared spectral curves are reproduced on pages 37 and 38. λ_{max} = 452 mμ; $E_{1\ cm.}^{mol.}$ = 12.6×10^4 at 452 mμ (in hexane).

Crystal Form: Salmon-pink "toy boat"-shaped platelets ex benzene-methanol. (Photomicrograph on p. 51.)

Melting Point: 188-189°.

Partition Behavior: 91:9.

Chromatographic Behavior: Develops with 3 - 4 per cent acetone in hexane; it is adsorbed on lime-Celite below 4-hydroxy-α-carotene but above 4-ethoxy-α-carotene and considerably above α- and β-carotenes; it is adsorbed above 3,4-dehydro-α-carotene. In a mixed chromatogram test it did not separate from 4-keto-α-carotene produced by oxidation of α-carotene with N-bromosuccinimide.

Reaction with Acid Chloroform: Negative.

Analysis: Calcd. for $C_{40}H_{54}O$: C, 87.21; H, 9.88.

Found: C, 87.35; H, 10.83 (corrected for 1.4 per cent ash).

Provitamin A Activity in the Rat: None.

Formation of 2,4-Dinitrophenylhydrazone: A crystalline derivative can be prepared (p. 83).

"Unidentified Pigment I." - The solution of this pigment resulting from rechromatographing Zone E was evaporated to dryness and the residue was crystallized from benzene-methanol. Photometric estimate of yield: 8 mg. = 0.3 per cent. Weight of the crystals, 4 mg. = 0.1 per cent.

Properties of "Unidentified Pigment I":

Spectrum: The ultraviolet curve will be found on p. 44; maxima in hexane: 503, 472, 442 mμ.

Crystal Form: Small, deep red oval platelets (from benzene-methanol).

Melting Point: 184-186°.

Partition Behavior: 94:6.

Chromatographic Behavior: Adsorbed somewhat above 4-keto-α-carotene but below 4-hydroxy-α-carotene. On lime-Celite it is adsorbed much more weakly than retro-dehydro-β-carotene.

Reaction with Acid Chloroform: Negative.

4-Ethoxy-α-carotene. - The hexane solution originating from the combined thirty zones Q (*cf*. p. 59) was evaporated to 100 ml. and developed on an 8 x 60 cm. column with about 2 liters of hexane. The following sequence was observed:

10	several tan zones, probably due to destruction
55	colorless
10	very pale yellow
135	colorless
155	yellow (all-*trans*-4-ethoxy-α-carotene)
25	light yellow (*neo*-4-ethoxy-α-carotene A)
5	colorless interzone
25	pale yellow (neo-4-ethoxy-α-carotene B)
20	pale yellow (neo-4-ethoxy-α-carotene C)

Remainder of column and filtrate, colorless

The all-*trans*-4-ethoxy-α-carotene was crystallized from benzene-methanol. Yield: 87 mg. = about 3 per cent (estimated photometrically). The crystals weighed 69 mg. = 2.3 per cent of the starting material (α-carotene).

Properties of All-trans-4-ethoxy-α-carotene:

Spectra: Wavelengths of maximum extinction 473, *444*, 420 mμ; $E^{\text{mol.}}_{1\text{ cm.}} = 14.3 \times 10^4$ at 444 mμ (in hexane). The ultraviolet and infrared spectral curves appear on pp. 40 and 41.

Crystal Form: Clusters of oval or pin-shaped, dull yellow-orange platelets (*ex* benzene-methanol). (Photomicrograph on p. 51.)

Melting Point: 176-177°.

Partition Behavior: 99:1.

Chromatographic Behavior: Develops with hexane on lime-Celite; is adsorbed above α-carotene and even somewhat above β-carotene but below 4-keto-α-carotene. Does not separate from 4-ethoxy-α-carotene obtained by ethanolysis of the α-carotene - boron trifluoride complex.

Reaction with Acid Chloroform: Gives a positive reaction; the color change is rapid and at least as pronounced as that of isocryptoxanthin; it is more marked than in the case of 4-hydroxy-α-carotene.

Analysis: Calcd. for $C_{42}H_{60}O$: C, 86.83; H, 10.41
Found: C, 86.68; H, 10.13 (corrected for 2.0 per cent ash).
Calcd. for 1 C_2H_5O- group: 7.75 per cent
Found: 7.62.

Provitamin A Activity in the Rat: None.

Unsuccessful efforts were made to crystallize neo-4-ethoxy-α-carotenes A, B, and C. Photometrically estimated yields: Neo A, 21.9 mg. = 0.7 per cent; Neo B, 16.5 mg. = 0.5 per cent; Neo C, 3.7 mg. = 0.1 per cent. The properties listed in Table 10(p. 31) refer to chromatographically homogeneous compounds.

All stereoisomers were identified as belonging to the set of 4-ethoxy-α-carotene by the method described on p. 87 .

Unchanged α-Carotene (*Recovery*). - The thirty zones T were combined, evaporated to 25 ml., and developed on a 4.5 x 25 cm. lime-Celite column with about 400 ml. of hexane. Only a single main zone was observed, the pigment of which was crystallized from benzene-methanol. Yield (recovery of unchanged α-carotene): 2.7 mg. = about 1 per cent (estimated photometrically). Weight of crystals: 2.3 mg. = 0.8 per cent.

Analysis: Calcd. for $C_{40}H_{56}$: C, 89.49; H. 10.52
Found: C, 89.69; H, 10.74.

The thus recovered pigment was shown to be all-*trans*-α-carotene by comparison of its properties with those of an authentic sample (Table 12, p. 71).

"*Unidentified Pigment II.*" - The combined thirty zones H were evaporated to 25 ml., then developed on a column with 2 per cent acetone in hexane:

114 colorless
28 pink
19 colorless
59 yellow ("unidentified pigment II, neo U")
15 colorless
33 yellow ("unidentified pigment II," all-*trans*)
9 pink
47 yellow ("unidentified pigment II, neo A")
26 pink-orange
31 orange-yellow
15 faint yellow

Remainder of column, and filtrate, colorless

Table 12

Comparison of α-Carotene with the Pigment Recovered from Zones T

	α-Carotene *ex* carrots	α-Carotene *ex* Zone T
Partition behavior in hexane - 95 per cent methanol	100:0	100:0
Melting Point	187-188°	185-187°
Maxima (in hexane):		
before iodine catalysis	475, 445, 420 mμ	474, 444, 420 mμ
after iodine catalysis	470, 442, (417)	470, 442, (417)
Reaction with acid chloroform	negative	negative
Crystal form	yellow-orange prisms	yellow-orange prisms
Chromatographic behavior	no separation in mixed chromatogram test	

The zones of "unidentified pigment II" ("neo U," all-*trans*, and "neo A") were each rechromatographed on lime-Celite as above. A single main zone appeared in each case. Only the "all-*trans*" isomer could be crystallized. Total (combined) yield, estimated photometrically, about 10 mg. = 0.3 per cent; yield of crystalline all-*trans* compound, 3 mg. = 0.1 per cent.

Properties of "Unidentified Pigment II":

Spectra: The ultraviolet and infrared spectral curves will be found on pp. 45 and 46. Maxima in hexane: all-*trans*, 469, 438, 415 mμ; "neo U," 467, 437, 416; "neo A," 462, 433, 413; iodine equilibrium mixture, 466, 436, 414 mμ.

Crystal Form: Small, irregular clusters of orange-yellow platelets.

Melting Point: 177-178° (sinters at 173°).

Partition Behavior: 94:6.

Chromatographic Behavior: Adsorbed below 4-keto-α-carotene but slightly above 4-ethoxy-α-carotene on lime-Celite. Adsorbed considerably below neurosporene.

Reaction with Acid Chloroform: Negative.

"Unidentified Pigment III." - The combined hexane solutions of the thirty zones B were evaporated to 25 ml. and developed for 1 hour on a magnesia-lime-Celite column 3:1:1 with about 1 liter of 3 per cent acetone in hexane. The 60 cm. broad main zone contained about 15 mg. (0.5 per cent yield)of this uncrystallizable pigment.

Properties of "Unidentified Pigment III":

Spectrum: The ultraviolet curve will be found on p. 47.

Maxima in hexane: 484, 452, 427 mμ.

Partition Behavior: 100:0.

Chromatographic Behavior: Adsorbed below α-carotene on lime-Celite.

Reaction with Acid Chloroform: Negative.

"Unidentified Pigment IV." - The hexane solution of the thirty zones G was evaporated to 50 ml. and developed on magnesia-lime-Celite 3:1:1 with about 1200 ml. of 1 per cent acetone in hexane:

140	complex series of minor zones
66	pale yellow-green
71	yellow-green ("unidentified pigment IV, neo U")
62	yellow-green ("unidentified pigment IV," all-*trans*)
90	colorless interzone
3	bright yellow

Remainder of column, and filtrate:
colorless, with slight bluish-grey fluorescence in ultraviolet light

The "unidentified pigments IV," *viz*., "neo U," and all-*trans*, were each rechromatographed on a single 3.5 x 20 cm. column. Neither isomer could be crystallized, but the properties listed below refer to the chromatographically homogeneous all-*trans* substance. The compounds were proved to be stereoisomers of each other. Yield (photometric estimate): about 30 mg. (1 per cent) of each isomer.

Properties of "Unidentified Pigment IV":

Spectrum: The ultraviolet curve will be found on p. 48.

Maxima in hexane: 448, *421*, 398, 376 mμ.

Partition Behavior: 100:0.

Chromatographic Behavior: Adsorbed much below α-carotene both on lime-Celite and on magnesia-lime-Celite 3:1:1.

Reaction with Acid Chloroform: Negative.

4. *Alcoholysis of the α-Carotene - Boron Trifluoride Complex*.

The complex of 100 mg. of α-carotene, prepared as described on p. 50, was cleaved by the addition, with rapid swirling, of 200 ml. of absolute *ethanol* which had been precooled to 4°. The resulting solution was washed, dried, evaporated, and chromatographed in the same manner as was the product of hydrolysis (pp. 58 to 60). Developing the reaction product on lime-Celite with about 1 liter of hexane took 1 hour:

34	several pink and tan zones
12	yellow
41	colorless interzone
56	yellow (*cis* isomers of 4-ethoxy-α-carotene)
39	colorless interzone
118	yellow (all-*trans*-4-ethoxy-α-carotene)
35	light yellow (*cis* isomers of 4-ethoxy-α-carotene)
7	pink-orange
12	yellow (unchanged α-carotene)
10	colorless interzone
24	yellow (unidentified pigment III)

Remainder of column: colorless

Filtrate: light yellow, with bright green fluorescence in ultraviolet light

From photometric measurements, the yield of all-trans-4-ethoxy-α-carotene was found to be 16.0 mg. = 16 per cent of the reacted α-carotene. The crystals obtained from benzene-methanol weighed 9.9 mg. = 9.9 per cent. The sum of the cis isomers was 24 mg. (photometric estimate), hence the total yield of the conversion, α-carotene → 4-ethoxy-α-carotene, amounted to 40 per cent. Only very little (0.9 per cent) α-carotene remained unchanged.

The boron trifluoride complex of a second 100-mg. portion of α-carotene was cleaved with 200 ml. of pre-cooled absolute methanol. The resulting chromatogram was similar to that obtained on ethanolysis. The combined yield of the 4-methoxy-α-carotene formed (including cis isomers) was 40 per cent, and about 10 per cent was isolated in the form of all-trans-4-methoxy-α-carotene crystals. The recovered unchanged α-carotene amounted to 1 per cent.

Properties of 4-Methoxy-α-carotene:

Spectrum in the UV and visible regions: Very similar to that of α-carotene, 4-hydroxy-α-carotene, or 4-ethoxy-α-carotene. Maxima in hexane: 474, 445, 421 mμ.

Crystal Form: Oval, dull yellow-orange platelets (from benzene-methanol).

Melting Point: 170-171°.

Partition Behavior: 99:1.

Chromatographic Behavior: Adsorbed on lime-Celite below 4-keto-α-carotene but above α-carotene and slightly above 4-ethoxy-α-carotene.

Reaction with Acid Chloroform: Positive, comparable in speed to that of isocryptoxanthin.

Analysis: Calcd. for 1 CH_3O- group: 5.47 per cent

Found: 4.91.

5. Ammonolysis of the α-Carotene - Boron Trifluoride Complex.

Preliminary experiments in which R. G. chloroform was used as a solvent during complex formation gave very high yields of 4-ethoxy-α-carotene. Therefore, the chloroform was dealcoholated and dehydrated, immediately prior to use, by gravity filtration through a 3.5 x 18 cm. column of highly activated alumina (Woelm, basic, activity I) (30).

90 mg. of α-carotene was dissolved in 100 ml. of alcohol-free chloroform, and 9 ml. of boron trifluoride etherate was added. The mixture was swirled rapidly and allowed to stand at room temperature for exactly 2 minutes. The complex was broken by careful addition of 40 ml. of a saturated solution of anhydrous ammonia in alcohol-free chloroform; the red carotenoid color was thus restored. A greenish-white precipitate formed immediately and could not be dissolved by further addition of ammoniacal chloroform.

The liquid was washed, dried, evaporated, and redissolved in hexane. Recovery of pigment (calcd. as "α-carotene") was slightly less than 2.5 per cent. The mixture was developed on a 4.5 x 24 cm. lime-Celite column in about 20 minutes with 250 ml. of hexane:

4	several brown zones
8	violet-brown
21	orange-brown
18	pale yellow-orange
15	yellow
10	very pale orange
19	very pale yellow
12	light orange
5	pale yellow
15	colorless interzone
20	orange (3,4-dehydro-α-carotene)
29	yellow (unchanged α-carotene)
12	colorless interzone
20	yellow-orange (unidentified pigment III)
8	yellow

Remainder of column: colorless
Filtrate: pale green, with strong greenish-grey fluorescence in ultraviolet light

The 3,4-dehydro-α-carotene was identified by its spectrum and by non-separation from authentic 3,4-dehydro-α-carotene (from dehydrogenation of α-carotene by N-bromosuccinimide, p. 79), in a mixed chromatogram test. Yield: 2 mg. = 2.2 per cent of the starting material or about 9 per cent of the recovered pigment. Recovery of unchanged α-carotene: 3 mg. = 3.3 per cent of the starting material or 13 per cent of the recovered pigment. *retro*-Dehydrocarotene was absent.

6. *Conversions of 4-Hydroxy-α-carotene and Some Derivatives.*

a. *3,4-Dehydro-α-carotene ex 4-Hydroxy-α-carotene: Dehydration of 4-Hydroxy-α-carotene.* - 15 mg. of 4-hydroxy-α-carotene was dissolved in 20 ml. of R. G. chloroform and

10 drops of the acid chloroform reagent was introduced. After 10 minutes' standing, 50 ml. of hexane was added and the solution was washed, first with 100 ml. of saturated sodium bicarbonate, then with water for 15 minutes in the LeRosen apparatus. The dry evaporation residue was dissolved in 25 ml. of hexane and developed with about 800 ml. of hexane:

14	several yellow and tan zones	
6	pale pink	
50	colorless interzone	
20	pale yellow	
23	colorless interzone	
47	pale orange	
60	light yellow (4-ethoxy-α-carotene)	
18	pale yellow	
22	pale yellow	
68	orange (3,4-dehydro-α-carotene)	
22	pale orange	(*cis* forms of the above)
39	pale orange	
16	pale orange	

Remainder of column: colorless
Filtrate: colorless

Photometrically established yield of 3,4-dehydro-α-carotene: 6 mg. of all-*trans* (= 40 per cent) and 2.8 mg. of *cis* forms (= 19 per cent); the crystals of all-*trans*-3,4-dehydro-α-carotene (from benzene-methanol) weighed 3.2 mg. = 21 per cent. The 4-ethoxy-α-carotene, identified by its spectrum and by non-separation in a mixed chromatogram test from an authentic sample (obtained by ethanolysis of the α-carotene - boron trifluoride complex), was photometrically estimated as 2.0 mg. = 13 per cent.

b. *3,4-Dehydro-α-carotene ex 4-Ethoxy-α-carotene and 4-Methoxy-α-carotene.* - 15 mg. of 4-ethoxy-α-carotene was dissolved in 20 ml. of R. G. chloroform and 10 drops of acid chloroform reagent was added. After 10 minutes, the mixture was worked up as described for dehydration on p. 77. The yields of 3,4-dehydro-α-carotene and 4-ethoxy-α-carotene were the same as in the case of dehydration.

15 mg. of 4-methoxy-α-carotene, dealcoholated in the same manner, gave similar yields of 3,4-dehydro-α-carotene and 4-*ethoxy*-α-carotene.

c. *3,4-Dehydro-α-carotene ex α-Carotene and N-Bromosuccinimide.* - 200 mg. of α-carotene was dissolved in 20 ml. of R. G. chloroform; and 66 mg. (1:1 mole ratio) of N-bromosuccinimide ("NBS") was dissolved separately in 20 ml. of chloroform. Both solutions were cooled to -17°. While the carotene solution was being violently agitated with a stream of nitrogen, the NBS solution was added quickly. The reaction was allowed to continue for exactly 1 minute (not longer), then 132 mg. of N-phenyl-morpholine (NPM) crystals was added and the agitation was continued for 1 more minute.

After the nitrogen stream was stopped, the reaction mixture was removed from the ice - salt bath and heated under reflux for 15 minutes. The solution was washed first with 150 ml. of saturated sodium bicarbonate and then continuously with water for 15 minutes. After drying and complete removal of the chloroform, the residue was dissolved in 100 ml. of hexane and developed on two 8 x 30 cm. lime-Celite columns with about 600 ml. of 1 per cent acetone in hexane for each column:

15	several tan zones
13	pink
9	colorless interzone
10	pale pink
9	colorless interzone
21	pink (4-keto-α-carotene)
14	pale orange
20	pale orange
22	colorless interzone
109	orange (3,4-dehydro-α-carotene)
27	yellow (*cis* isomers of 3,4-dehydro-α-carotene)

Remainder of column, and filtrate: colorless

Yield: 94.2 mg. of all-*trans*-3,4-dehydro-α-carotene = 47 per cent. The crystals (from benzene - methanol) weighed 44.2 mg. = 22 per cent.

The photometrically established yield of 4-keto-α-carotene was 19 mg. = 9.5 per cent.

Properties of All-trans-3,4-dehydro-α-carotene:

Spectrum: The ultraviolet curve will be found on p. 49 .

λ_{max} in hexane: 454 mμ.

Crystal Form: Dull, brownish-orange, nearly rectangular plates (from benzene - methanol). (Photomicrograph on p. 52 .)

Melting Point: 191-192°.

Partition Behavior: 100:0.

Chromatographic Behavior: Adsorbed above α-carotene but below β-carotene on lime-Celite. The sample did not separate in the mixed-chromatogram test, from Karmakar's "dehydrocarotene I" (9).

Reaction with Acid Chloroform: Negative.

d. *4-Keto-α-carotene ex α-Carotene and N-Bromosuccinimide.* - A solution of 200 mg. of α-carotene in 20 ml. *of R. G. chloroform was treated as described above for the* preparation of 3,4-dehydro-α-carotene (p. 79), except that 132 mg. of NBS and 264 mg. of NPM were used (2:1 mole ratio of NBS to α-carotene, with correspondingly increased amount of NPM). Each chromatogram was developed with about 750 ml. of 3 per cent acetone in hexane:

15	several brown and tan zones
26	pink
9	colorless interzone
20	pink
19	colorless interzone
43	pink (4-keto-α-carotene)
28	light orange
40	light orange
22	colorless interzone
25	orange (*3,4-dehydro-α-carotene*)
10	yellow

Remainder of column, and filtrate: colorless

Yields: 4-keto-α-carotene, 67.2 mg. = 34 per cent of the starting material; 3,4-dehydro-α-carotene, 30.5 mg. = 15 per cent. The crystals of 4-keto-α-carotene (from benzene - methanol) weighed 17 mg. = 8.5 per cent.

e. Oxidation of 4-Hydroxy-α-carotene to 4-Keto-α-carotene with N-Bromosuccinimide. - 50 mg. of 4-hydroxy-α-carotene was dissolved in 15 ml. of R. G. chloroform which had been made alcohol-free immediately prior to use by washing with water for 20 minutes and then distilling in darkness over P_2O_5. 16 mg. of NBS was dissolved in a separate 15-ml. portion of alcohol-free chloroform. The two solutions were cooled to 0°, and the NBS solution was added to the carotene solution while the latter was being violently agitated by a stream of nitrogen. After 1 minute, 33 mg. of NPM was added, and agitation continued for 2 minutes longer. *The mixture was then heated under reflux for 15 minutes,* washed, etc., as in the two NBS oxidations described on pp. 79 and 81. The resulting mixture was developed with 750 ml. of 4 per cent acetone in hexane on a single column:

105	several very pale zones
20	pale violet
20	colorless interzone
90	pale salmon-pink
22	colorless interzone
97	salmon-pink (4-keto-α-carotene)
32	yellow
42	yellow

Remainder of column, and filtrate: colorless

The 4-keto-α-carotene was rechromatographed on a 3.5 x 32 cm. column with 3 per cent acetone in hexane as developer.

Yield: 12.4 mg. = 25 per cent (photometric). The crystals (from benzene - methanol) weighed 9.2 mg. = 18 per cent.

f. 2,4-Dinitrophenylhydrazone of 4-Keto-α-carotene. - 9 mg. of 4-keto-α-carotene was dissolved in 2 ml. of benzene (R. G.) in a centrifuge cone and 4 ml. of absolute ethanol was added cautiously, with stirring. To the clear solution was added 2 ml. of the 2,4-dinitrophenylhydrazine reagent described by Shriner and Fuson (31). After half an hour's standing, the microcrystals were centrifuged, (mother liquor, intensely colored), washed once with a few ml. of absolute ethanol and twice with hexane, then dried for two hours at the temperature of refluxing acetone in an Abderhalden apparatus. Thus, 9.8 mg. of crystals, m.p. 216-217°, was obtained. Yield: 81 per cent, based on 4-keto-α-carotene. The following analysis shows that the compound is a derivative of a mono-ketone.

Calcd. for $C_{46}H_{58}N_4O_4$:	N, 7.67 per cent.
Found:	N, 7.19 per cent.

g. Reduction of 4-Keto-α-carotene to 4-Hydroxy-α-carotene. - 25 mg. of 4-keto-α-carotene was dissolved in 50 ml. of 10 per cent benzene in anhydrous ether; simultaneously 100 mg. of lithium aluminum hydride was suspended in 100 ml. of anhydrous ether. Both liquids were cooled to 0°, and the carotenoid solution was poured quickly into the hydride suspension with rapid swirling. The mixture was allowed to stand at 0° for 15 minutes; the excess lithium aluminum hydride was then decomposed by cautious, dropwise addition of methanol which had been pre-cooled to 0°.

The mixture, containing much gelatinous precipitate, was washed vigorously in the LeRosen apparatus until the effluent had a pH of 6-7 (about 20 minutes). The resulting solution was dried and developed on a column with 4 per cent acetone in hexane. Only a single main yellow zone appeared, containing 4-hydroxy-α-carotene. Yield: 22.1 mg. = 88 per cent (photometric); the crystals weighed 16 mg. = 64 per cent. In a mixed chromatogram test this substance did not separate from an authentic 4-hydroxy-α-carotene sample prepared by hydrolysis of the α-carotene - boron trifluoride complex.

h. <u>Etherification of 4-Hydroxy-α-carotene</u>. - Two 25-mg. portions of 4-hydroxy-α-carotene were dissolved in 2 x 5 ml. of R. G. chloroform. To one was added 25 ml. of absolute <u>ethanol</u> and 10 drops of the acid chloroform reagent, and to the other, 25 ml. of absolute <u>methanol</u> and 10 drops of acid chloroform. After 5 minutes each solution was diluted with 25 ml. of hexane and washed once with 50 ml. of saturated sodium bicarbonate and then with water, continuously, for 20 minutes. After drying, each solution was developed on a single column with 2 per cent acetone in hexane. The upper, minor yellow zone contained unchanged

4-hydroxy-α-carotene, and the lower, main yellow zone was shown to be the desired ether, by partition coefficient test, spectrum, reaction with acid chloroform, and mixed chromatography with the corresponding ether resulting from alcoholysis of the α-carotene - boron trifluoride complex.

Yields: 4-methoxy-α-carotene, 13.6 mg. = 54 per cent; 4-ethoxy-α-carotene, 9 mg. = 36 per cent.

Both 4-methoxy-α-carotene and 4-ethoxy-α-carotene could be converted into 4-hydroxy-α-carotene in about 40 per cent yield by the procedure just described, using water instead of absolute alcohol and adding just enough acetone to produce a single-phase mixture.

i. Acetylation of 4-Hydroxy-α-carotene. - The method of acetylation was essentially that used by Zechmeister and von Cholnoky (32) for capsanthin, slightly modified for a mono-alcohol. To a solution of 25 mg. of 4-hydroxy-α-carotene in 0.3 ml. of anhydrous pyridine, 2 drops of freshly distilled C. P. acetyl chloride were added; the pasty mixture was stirred and kept at room temperature for 1/2 hour. 1.7 ml. of absolute methanol was then added. Colorless crystals of the pyridine - acetyl chloride complex dissolved rapidly while the 4-acetoxy-α-carotene slowly crystallized out, forming long, needle-like, dark red prisms. The crystals were washed twice with methanol, dried and weighed. Yield: 20.6 mg. = 82 per cent.

Properties of 4-Acetoxy-α-carotene:

Spectrum: Similar to those of α-carotene and 4-hydroxy-, 4-ethoxy-, and 4-methoxy-α-carotene. Maxima in hexane: 474, 444, 421 mμ.

Crystal Form: Long, thin, deep red-orange prisms (like needles), (from pyridine - methanol).

Melting Point: 159-161°.

Partition Behavior: 99:1.

Hydrolysis: 5 mg. of the acetate was dissolved in 25 ml. of hexane and shaken with 25 ml. of 20 per cent methanolic potassium hydroxide for 12 hours; development of the product on a 3.5 x 20 cm. column with 4 per cent acetone in hexane gave a single main zone. Its pigment had a partition ratio of 83:17 and did not separate from 4-hydroxy-α-carotene in a mixed chromatogram test.

Chromatographic Behavior: Adsorbed on lime-Celite below 4-keto-α-carotene but above 4-ethoxy-α-carotene. (Some hydrolysis occurs on chromatography with basic adsorbents if the solvents and adsorbents are not completely anhydrous.)

Reaction with Acid Chloroform: Positive, similar to that of 4-ethoxy-α-carotene, p. 69.

Analysis: Calcd. for $C_{42}H_{58}O_2$: C, 84.80; H, 9.83
Found: C, 85.23; H, 10.28.

7. Stereoisomerization of 4-Hydroxy-α-carotene and Some Derivatives.

General Remarks.

Molar extinction curves of the various *cis* - *trans* isomers obtained in the following study will be found on pages 33 to 43. To be considered as a member of a stereoisomeric set, an isomer had to qualify in both of the following tests:

1.) Comparison of its ultraviolet spectrum before and after iodine catalysis with corresponding spectra of the all-*trans* form. The two iodine equilibrium curves must be identical.

2.) Mixed chromatography of a fresh solution of the crystalline all-*trans* sample with the main pigment zone obtained by chromatographing the iodine-catalyzed equilibrium mixture of the *cis* isomer. There must be no separation.

Immediately prior to determining the ultraviolet spectrum of each isomer, the sample was carefully rechromatographed on a small column, and any minor zones found were cut out and discarded. The amount of each stereoisomer recovered was determined indirectly, by measuring the absorbance of the iodine-catalyzed equilibrium mixture at λ_{max}. The molar extinction coefficients for the iodine-equilibrium mixtures were estimated for each compound from measurement of the extinction at λ_{max} of the iodine-catalyzed equilibrium mixture from two independently weighed samples of the all-trans compound. In the following experiments, these values were used:

$E_{1\ cm.}^{mol.}$ (I_2-equil.) of 4-hydroxy-α-carotene = 12.2×10^4

" 4-keto-α-carotene = 11.1×10^4

" 4-ethoxy-α-carotene = 13.4×10^4.

Each member of a stereoisomeric set, resulting from one method of isomerization, was identified with the corresponding zone resulting from other rearrangement methods by comparison of spectra and by mixed chromatography.

4-Hydroxy-α-carotene.

a. Iodine-catalyzed Stereoisomerization.

25.2 mg. of crystalline all-trans-4-hydroxy-α-carotene was dissolved in 100 ml. of hexane in a Pyrex volumetric flask, and approximately 0.3 mg., i.e. about 1 per cent of the pigment weight, of iodine (as a 0.7 mg./ml. hexane solution) was added. The contents of the flask were thoroughly mixed. The flask was then placed on a white support at 60 cm. distance from two parallel 3500° Mazda fluorescent lamps (40 watts; 120 cm. long, white and yellowish) and illuminated for 2 hours. The flask was shaken gently every 10 minutes; average ambient temperature was 26°.

Photometric estimations at λ_{max} of the iodine-equilibrium mixture, made before and after the isomerization, showed a total pigment recovery of 85 per cent (calculated as 4-hydroxy-α-carotene).

The solution was developed on a single column for 75 minutes with about 700 ml. of 4 per cent acetone in hexane:

19	several minor tan zones
16	colorless interzone
28	yellow (neo-4-hydroxy-α-carotene V)
18	colorless interzone
9	very pale yellow (neo-4-hydroxy-α-carotene U)
53	orange-yellow (unchanged all-*trans*-4-hydroxy-α-carotene)
14	yellow (neo-4-hydroxy-α-carotene A)
11	yellow (neo-4-hydroxy-α-carotene B)
15	orange (neo-4-keto-α-carotene U)
12	yellow (neo-4-hydroxy-α-carotene C)
8	yellow (neo-4-hydroxy-α-carotene D)
9	colorless interzone
80	pink-orange (all-*trans*-4-keto-α-carotene)
34	colorless interzone
42	orange (neo-4-keto-α-carotene A)
24	colorless interzone
27	orange (neo-4-keto-α-carotene B)

Remainder of column: colorless
Filtrate: pale yellow, with grey fluorescence in ultraviolet light

The individual zones were cut out, eluted, transferred to hexane, washed, and dried; each substance was identified as belonging either to the 4-hydroxy- or to the 4-keto-α-carotene set by the methods previously described. Similar operations were performed in each of the following experiments (no further mention of these operations will be made).

An example of the relative amounts of the stereoisomers formed will be found in Table 1, p. 25. Repetitions of this experiment gave relative values of distribution ratios within 5 per cent of those given in Table 1.

b. *Stereoisomerization by Fusion (Melting Crystals).*

15.6 mg. of all-*trans*-4-hydroxy-α-carotene was weighed into a closed-end, 7-mm. O. D. glass tube and sealed at a pressure of less than 0.01 mm. of mercury. The tube was then immersed in a wax bath at 200° for 90 seconds and then plunged into an ice water bath. The tube was opened and its contents, now a red-brown glassy substance, was, with the initial aid of 5 drops of R. G. chloroform, dissolved in 100 ml. of hexane. Photometric estimation of this material indicated a 92 per cent pigment recovery (calculated as 4-hydroxy-α-carotene).

The substance was developed on one column, for just under 2 hours with about 1 liter of 4 per cent acetone in hexane. Development was stopped when a colorless interzone of at least 3 mm. width had appeared between each zone:

8	several minor tan zones
52	colorless interzone
30	yellow (neo-4-hydroxy-α-carotene V)
12	colorless interzone
26	very pale yellow (neo-4-hydroxy-α-carotene U)
22	colorless interzone
67	yellow (unchanged all-<u>trans</u>-4-hydroxy-α-carotene)
8	colorless interzone
20	yellow (neo-4-hydroxy-α-carotene A)
5	colorless interzone
17	yellow (neo-4-hydroxy-α-carotene B)
8	colorless interzone
32	light yellow (neo-4-hydroxy-α-carotene C)
3	nearly colorless interzone
11	light greenish yellow
3	nearly colorless interzone
13	yellow (neo-4-hydroxy-α-carotene D)

Remainder of column: colorless
Filtrate: pale yellow, with greenish-grey fluorescence

An example of the relative amounts of stereoisomers formed will be found in Table 2, p. 26. An independent duplicate of this experiment gave the same relative product ratios within ± 10 per cent.

c. Stereoisomerization by Refluxing in Darkness.

22.7 mg. of all-*trans*-4-hydroxy-α-carotene was dissolved in 100 ml. of hexane and the solution was refluxed in darkness for 1/2 hour. During refluxing, a slow stream of nitrogen was bubbled through to provide gentle agitation and to prevent excessive autoxidation. Photometric estimations before and after refluxing showed 99 per cent pigment recovery.

The solution was developed on one column with about 800 ml. of 4 per cent acetone in hexane within one and a half hours:

12	several minor tan zones
29	colorless
30	pale yellow
25	colorless interzone
134	orange-yellow (unchanged all-*trans*-4-hydroxy-α-carotene)
6	yellow (neo-4-hydroxy-α-carotene A)
10	yellow (neo-4-hydroxy-α-carotene B)
10	yellow (neo-4-hydroxy-α-carotene C)
11	colorless interzone
83	pink-orange (all-*trans*-4-keto-α-carotene)

Remainder of column: colorless
Filtrate: light yellow, with weak bluish-grey fluorescence

The combined neos A, B, and C were rechromatographed on a 3.5 x 20 cm. column; after 20 minutes of development with about 200 ml. of 4 per cent acetone in hexane, an excellent separation of the three isomers was achieved.

After identification, the amount of each isomer was estimated photometrically. The results are summarized in Table 4, p. 27.

d. Stereoisomerization by Standing in Diffuse Daylight.

25.4 mg. of all-*trans*-4-hydroxy-α-carotene was dissolved in 100 ml. of hexane in a Pyrex volumetric flask that was placed on a white support, outdoors, in open shade. Illumination was allowed to proceed for two hours (11 a.m. to 1 p.m.): the average ambient air temperature was 19°, and the final solution temperature was also 19°.

During illumination, the incident light energy was measured with a General Electric Model DW-68 photoelectric exposure meter. The instrument was held horizontally and pointed at each cardinal compass point, and also vertically. The average incident light intensity in lumens per square foot was: North, 4000; East, 1600; South, 800; West, 1600; Vertical, 6000.

Photometric estimations before and after illumination indicated 98 per cent pigment recovery.

The mixture was chromatographed on one column. Except that the top pale yellow zone was absent and that there were 2 - 3 mm. differences in the breadth of the zones, the observations were as described in Part c., above. A summary of the results of this experiment is presented in Table 4, p. 27.

e. Stereoisomerization by Standing in Direct Sunlight.

25.0 mg. of all-*trans*-4-hydroxy-α-carotene was dissolved in 100 ml. of hexane in a Pyrex volumetric flask and the flask was placed on a white support, outdoors, in direct sunlight. Illumination was allowed to proceed for 2 hours (11 a.m. to 1 p.m.). The average ambient air temperature was 21°, and the final solution temperature was 35°.

The average incident light intensity in lumens per square foot was: North, 12,400; East, 8,000; South 25,600; West, 10,400; Vertical, 14,800.

Photometric estimations before and after illumination showed a 96 per cent pigment recovery.

The mixture was chromatographed on a single column. With the exceptions that the top pale yellow zone was absent and that there were 2 - 3 mm. variations in the breadth of the zones, the procedure and observations were as described in Part c., above. A summary of the results of this experiment is presented in Table 4, p. 27.

f. <u>Stereoisomerization by Irradiation with Photoflood Bulbs</u>.

25.3 mg. of all-<u>trans</u>-4-hydroxy-α-carotene was dissolved in 100 ml. of hexane in a Pyrex volumetric flask which was then placed in the center of an equilateral triangle formed of three General Electric No. 1 Photoflood bulbs, each placed so that the minimum distance from the circumference of the flask to the circumference of the bulb was 10 ± 0.5 cm. The flask was illuminated for 2 hours. The average ambient air temperature 28°, and the final solution temperature was 31°; an electric fan was used to provide cooling.

Photometric estimations made before and after irradiation indicated 94 per cent recovery of pigment. The solution was chromatographed on a single column; procedures and observations were similar to those in Part c., above, with the exceptions that there were minor variations of 2 - 3 mm. in the width of the zones, and that a trace of neo-4-hydroxy-α-carotene D appeared on the second chromatogram (of the combined minor zones). A summary of the products and their ratios will be found in Table 3, p. 26.

4-Ethoxy-α-carotene.

a. Iodine-catalyzed Stereoisomerization.

8.7 mg. of all-*trans*-4-ethoxy-α-carotene was dissolved in 50 ml. of hexane in a Pyrex volumetric flask and treated in the manner described for 4-hydroxy-α-carotene, p. 88.

Photometric estimations made before and after the illumination showed a pigment recovery of 87 per cent.

The solution was chromatographed on a single column; development was accomplished in about 1 hour with approximately 500 ml. of 1 per cent acetone in hexane:

17	several minor tan zones
7	very pale pink
22	colorless interzone
18	pale pink (all-*trans*-4-keto-α-carotene)
85	colorless interzone
27	pale yellow (neo-4-ethoxy-α-carotene V)
4	colorless interzone
7	very pale yellow (neo-4-ethoxy-α-carotene U)
65	colorless interzone
74	yellow (all-*trans*-4-ethoxy-α-carotene)
17	colorless interzone
15	pale yellow (neo-4-ethoxy-α-carotene A)

8 colorless interzone
11 pale yellow (neo-4-ethoxy-α-carotene B)
6 colorless interzone
8 pale yellow (neo-4-ethoxy-α-carotene C)

Remainder of column: colorless
Filtrate: pale yellow, with grey fluorescence

An example of the stereoisomeric ratios will be found in Table 5, p. 28. Repetitions of this experiment gave relative values of distribution ratios within ± 5 per cent of those given in Table 5.

b. *Stereoisomerization by Fusion (Melting Crystals).*

10.0 mg. of all-*trans*-4-ethoxy-α-carotene was fused in a manner similar to that described for 4-hydroxy-α-carotene, p. 89. Pigment recovery, 87 per cent.

The substance was developed on one column for 1 hour with about 500 ml. of 1 per cent acetone in hexane:

9 several minor tan zones
115 colorless
28 light yellow (neo-4-ethoxy-α-carotene V)
6 colorless interzone
10 very pale yellow (neo-4-ethoxy-α-carotene U)
61 colorless interzone
79 yellow (all-*trans*-4-ethoxy-α-carotene)
24 colorless interzone
18 pale yellow (neo-4-ethoxy-α-carotene A)
13 nearly colorless interzone
15 pale yellow (neo-4-ethoxy-α-carotene B)
11 nearly colorless interzone
18 very pale yellow (neo-4-ethoxy-α-carotene C)

Remainder of column: colorless
Filtrate: pale yellow-green, with slight bluish fluorescence in ultraviolet light

A summary of the relative amounts of isomers formed will be found in Table 6, p. 28.

4-Keto-α-carotene.

a. Iodine-catalyzed Stereoiosmerization.

12.7 mg. of crystalline all-*trans*-4-keto-α-carotene was dissolved in 50 ml. of hexane in a Pyrex volumetric flask and treated in the manner described for 4-hydroxy-α-carotene, p. 88.

Photometric estimations indicated 93 per cent pigment recovery.

The mixture was developed on a single column with about 500 ml. of 3 per cent acetone in hexane for 1 hour:

11	several minor tan zones
69	colorless
42	light pink (neo-4-keto-α-carotene U)
29	colorless interzone
78	pink (all-*trans*-4-keto-α-carotene)
37	colorless interzone
34	pink-orange (neo-4-keto-α-carotene A)
21	colorless interzone
18	pink-orange (neo-4-keto-α-carotene B)
5	colorless interzone
9	pink-orange (neo-4-keto-α-carotene C)

Remainder of column: colorless
Filtrate: colorless, with blue fluorescence

An example of the relative amounts of isomers formed is given in Table 7, p. 29; repetition of this experiment gave the same product ratios within ± 5 per cent.

b. Stereoisomerization by Fusion (Melting Crystals).

9.8 mg. of all-*trans*-4-keto-α-carotene was fused in the manner described for 4-hydroxy-α-carotene, p.89.

Photometric estimation showed a 66 per cent pigment recovery.

The solution was developed on a single column, with 500 ml. of 3 per cent acetone in hexane in about 1 hour:

10	several minor tan zones
61	colorless
25	pink (neo-4-keto-α-carotene U)
38	colorless interzone
75	pink (all-*trans*-4-keto-α-carotene)
49	colorless interzone
44	pink-orange (neo-4-keto-α-carotene A)
35	colorless interzone
18	orange (neo-4-keto-α-carotene B)
9	colorless interzone
15	orange (neo-4-keto-α-carotene C)

Remainder of column: colorless
Filtrate: light yellow-green, with grey fluorescence

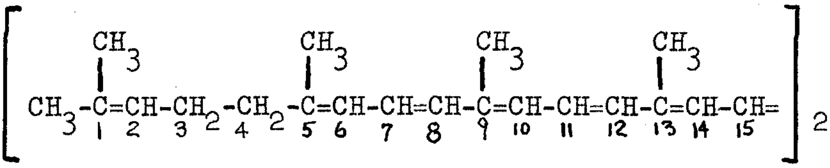
A summary of the relative amounts of isomers formed will be found in Table 8, p. 29.

II. LYCOPENE AND BORON TRIFLUORIDE ETHERATE

A. THEORETICAL PART

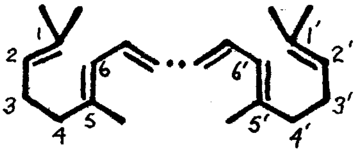
1. Introduction.

The second carotenoid chosen for the present study was the aliphatic hydrocarbon, lycopene (XIII, XIIIa), $C_{40}H_{56}$.



$$\left[CH_3-\overset{CH_3}{\overset{|}{\underset{1}{C}}}=\underset{2}{CH}-\underset{3}{CH_2}-\underset{4}{CH_2}-\overset{CH_3}{\overset{|}{\underset{5}{C}}}=\underset{6}{CH}-\underset{7}{CH}=\underset{8}{CH}-\overset{CH_3}{\overset{|}{\underset{9}{C}}}=\underset{10}{CH}-\underset{11}{CH}=\underset{12}{CH}-\overset{CH_3}{\overset{|}{\underset{13}{C}}}=\underset{14}{CH}-\underset{15}{CH}= \right]_2$$

(XIII.) Lycopene.

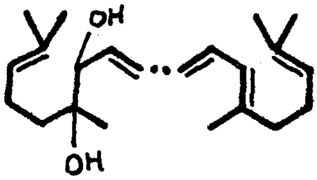


(XIIIa.) Lycopene formula, showing the structural relation between lycopene and β-carotene.

Lycopene is known to give an unstable blue color with antimony trichloride in chloroform (33), but no mention of the interaction of boron trifluoride and lycopene was found in the literature.

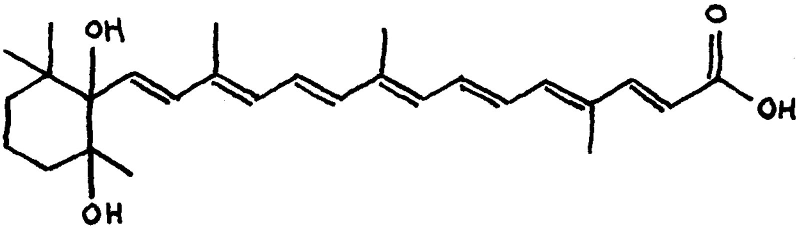
2. Hydrolysis of the Lycopene - Boron Trifluoride Complex.

5,6-Dihydroxy-5,6-dihydrolycopene (XIX) is the principal product of this hydrolysis.



(XIX.) 5,6-Dihydroxy-5,6-dihydrolycopene.

Although rare, the 5,6-dihydroxy-5,6-dihydro-structure is not unknown in natural products. Thus, azafrin, extracted from the roots of some South American plants for use as a fat coloring matter (34), has the following structure:



(XX.) Azafrin.

The spectrum of our 5,6-dihydroxy-5,6-dihydrolycopene shows the presence of 10 conjugated double bonds (22). Evidently, its chromophore is identical with that of Compound "G," formed by catalytic hydrogenation of lycopene (35). The infrared spectrum shows absorption at 2.78μ, indicating the presence of at least one alcohol group; and the analyses confirmed the presence of two hydroxyls, as did the formation and hydrolysis of a diacetate (XXI).

The reactions used to secure the structures of the hydrolysis products of the lycopene complex appear in Chart 2 (p. 101). The conversions of particular interest for the 5,6-dihydroxy-5,6-dihydrolycopene structure follow:

(a.) The formation of lycopene mono-epoxide (XXII) and its subsequent hydrolysis to 5,6-dihydroxy-5,6-dihydrolycopene (XIX) indicate the presence of adjacent hydroxyl groups. Since lycopene epoxide does not occur in the literature, it is believed that this is the first synthesis of an aliphatic epoxide in the carotenoid series.

(b.) Additional evidence of the presence of adjacent ("α-glycolic") hydroxyl groups is the formation of an acetonide (XXIII) that could be hydrolyzed back to the original diol.

(c.) The formation of a mono-ether (XXIV, 5-hydroxy-6-methoxy-5,6-dihydrolycopene) <u>via</u> the potassium salt intermediate shows the presence of two different kinds of hydroxyl groups: that -OH which does not react, is probably tertiary, or hindered, or both; the other one may represent a secondary or an unhindered tertiary alcohol group.

When, during the formation of the 5-hydroxy-6-methoxy-compound, the reaction mixture was heated to 100° instead of 60°, much pigment was destroyed; the yield of 5-hydroxy-6-methoxy-5,6-dihydrolycopene was considerably reduced, but a mono-ether with a lycopene-chromophore was isolated in about 1 per cent yield. This compound is adsorbed on lime-Celite above lycopene but below lycoxanthin; it is believed

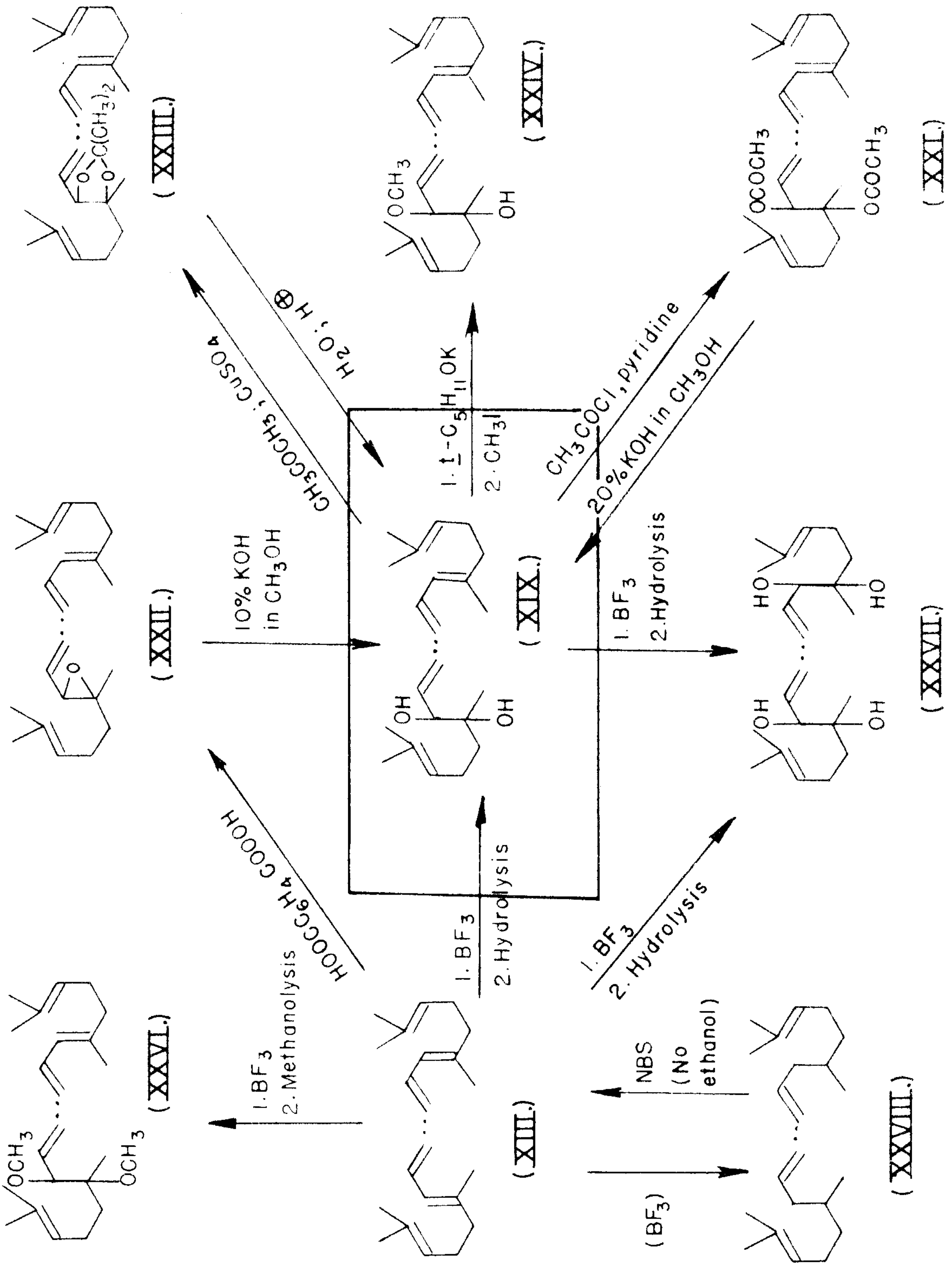
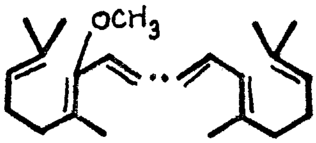


Chart 2. Some Conversions Proving the Structure of 5,6-Dihydroxy-5,6-dihydrolycopene.

to be 6-methoxylycopene (XXV), formed by dehydration of 5-hydroxy-6-methoxy-5,6-dihydrolycopene by the influence of the strong base.



(XXV.) 6-Methoxylycopene.

(d.) Methanolysis of the lycopene - boron trifluoride complex gave 5,6-dimethoxy-5,6-dihydrolycopene (XXVI), different from the dihydroxy- and mono-hydroxy-mono-methoxy-compounds in partition and chromatographic behavior, but having the same chromophore.

Formation of 5,6-Dihydroxy-5,6-dihydrolycopene. - Considering a mechanism similar, in the initial steps, to that proposed for the hydrolysis of the -carotene - BF_3 complex (p. 10), 5,6-dihydroxy-5,6-dihydrolycopene probably arises according to Chart 3 (p. 103). Again it was necessary to propose an oxidation of the carotenoid molecule (elimination of electrons) by some step such as removal of a hydride ion, or the $HBF_3^{\ominus}$ ion, as shown in the Chart.

Unchanged Lycopene. - A rather large amount of unchanged lycopene was recovered (compared to the α-carotene experiments), since, in order to prevent excessive destruction and obtain the maximum yield of 5,6-dihydroxy-5,6-dihydrolycopene, it was necessary to add water to the reaction mixture before all the lycopene was complexed.

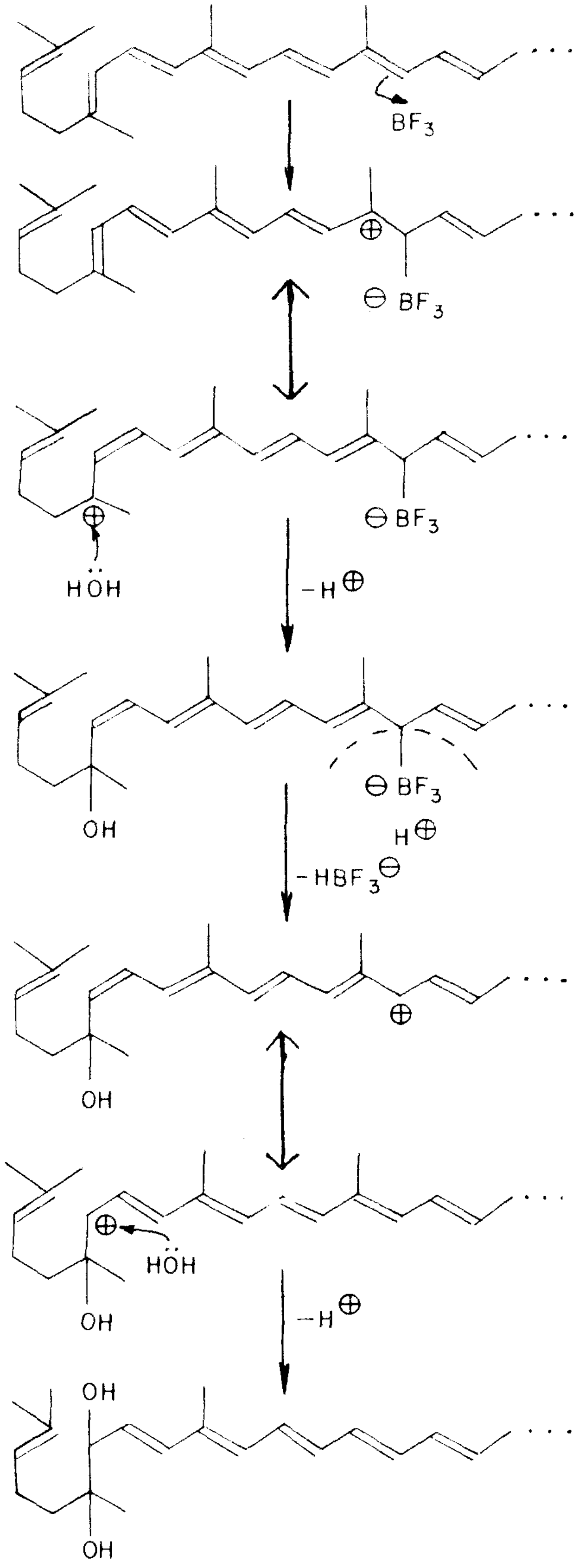


Chart 3. Proposed Formation of 5,6-Dihydroxy-5,6-dihydrolycopene from Lycopene.

5,6,5',6'-Tetrahydroxy-5,6,5',6'-tetrahydrolycopene (XXVII). - The spectrum of this compound indicates the presence of a 9-double-bond chromophore, similar to Lijinsky's Compound "F" (35); and the partition behavior as well as the infrared spectrum strongly support a polyhydroxy structure. The formation of a tetra-ol from the lycopene - boron trifluoride complex probably results from simultaneous hydrolysis at both ends of a complex involving two molecules of BF_3 and one lycopene, similar to the 2:1 complexes proposed for Lewis acids and α- or β-carotene (p. 8).

The tetra-ol could also be prepared by hydrolyzing the BF_3 complex of 5,6-dihydroxy-5,6-dihydrolycopene.

5,6,5',6'(?)-Tetrahydrolycopene (XXVIII). - This compound seems to result from a hydrogenation process during the hydrolysis of the complex, in a manner similar to that proposed for the formation of the hydrogenated α-carotene derivatives (p. 20). The spectrum, similar to that of Lijinsky's Compound "F" (35), indicates 9 conjugated double bonds; the partition behavior is that of a hydrocarbon. Although it could not be proved that this compound is a symmetrically hydrogenated lycopene derivative (i.e., saturated at the 5,6 and 5',6' double bonds), the ready crystallizability and the conversion to lycopene by NBS do favor the proposed structure.

3. Stereoisomerization of 5,6-Dihydroxy-5,6-dihydrolycopene.

All-*trans*-lycopene, when rearranged by the usual methods, yields only two main *cis* isomers, neos A and B, adsorbed below the all-*trans* isomer (25). In contrast, 5,6-dihydroxy-5,6-dihydrolycopene can be converted into at least 4 different *cis* forms, one of which is located above the all-*trans* compound on the column.

Some properties of the various stereoisomers are listed in Table 16, p. 108; the spectral curves will be found in Figs. 23 and 25, pp. 109 and 111; and the ratios of *cis* - *trans* isomers observed upon various stereoisomerization treatments appear in Tables 13-15 (pp. 106 - 107).

Table 13

Product Ratios for Iodine-Catalyzed Stereoisomerization of 5,6-Dihydroxy-5,6-dihydrolycopene

Stereoisomer	Per cent of Recovered 5,6-Dihydroxy-5,6-dihydrolycopene	Per cent of Starting Material
Neo U	11	8
All-*trans*	51	34
Neo A	12	8
Neo B	17	12
Neo C	9	6

Table 14

Product Ratios for Fusion Stereoisomerization of 5,6-Dihydroxy-5,6-dihydrolycopene

Stereoisomer	Per cent of Recovered 5,6-Dihydroxy-5,6-dihydrolycopene	Per cent of Starting Material
Neo U	12	10
All-*trans*	50	42
Neo A	15	12
Neo B	22	18
Neo C	1	<1

Table 15

Product Ratios for Stereoisomerizations of 5,6-Dihydroxy-5,6-dihydrolycopene

Stereoisomer	Per cent of Recovered 5,6-Dihydroxy-5,6-dihydrolycopene	Per cent of Starting Material
a. By Refluxing in Darkness:		
All-*trans*	96	85
Neo *A*	1	1
Neo B	2	2
Neo C	1	<1
b. By Standing in Direct Sunlight:		
All-*trans*	93	79
Neo *A*	2	2
Neo B	4	3
Neo C	1	1
c. By Standing in Diffuse Daylight:		
All-*trans*	99	92
Neo *A*	<1	<1
Neo B	<1	<1
Neo C	<1	<1
d. By Photoflood Irradiation:		
Neo U	2	2
All-*trans*	94	78
Neo *A*	1	1
Neo B	2	2
Neo C	1	1

Table 16

Stereoisomeric 5,6-Dihydroxy-5,6-dihydrolycopenes

Isomer	Maxima in Hexane (cf. curves pp. 109 to 111)	$E^{mol.}_{1\ cm.}$ (hexane)*	Partition Ratio**	Allylic Test (Acid $CHCl_3$)
Neo U	481, <u>452</u>, 430 mμ	16.0 x 10^4	46:54	-
All-<u>trans</u>	488, <u>456</u>, 431	16.1	45:55	-
Neo A	479, <u>450</u>, 426	14.2	45:55	-
Neo B	480, <u>450</u>, 426	14.5	46:54	-
Neo C	479, <u>450</u>, 428	13.6	46:54	-
Iodine equilibrium mixture	481, <u>452</u>, 429	14.2	47:53	-

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* *Cf*. Table 9.

** In hexane - 95 per cent methanol.

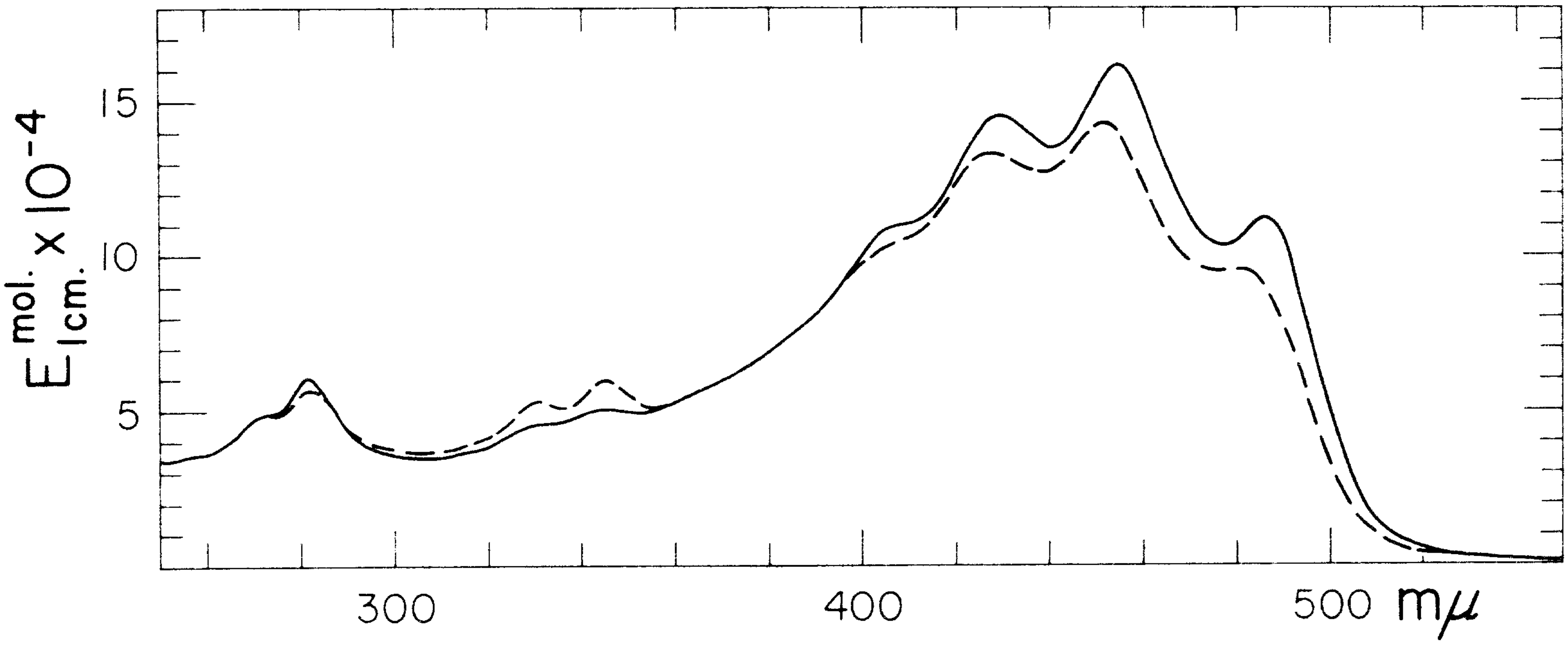


Fig. 23. Molecular Extinction Curve of 5,6-Dihydroxy-5,6-dihydrolycopene in the Visible and Ultraviolet Regions (in Hexane): ———, fresh solution of the all-*trans* compound; and - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

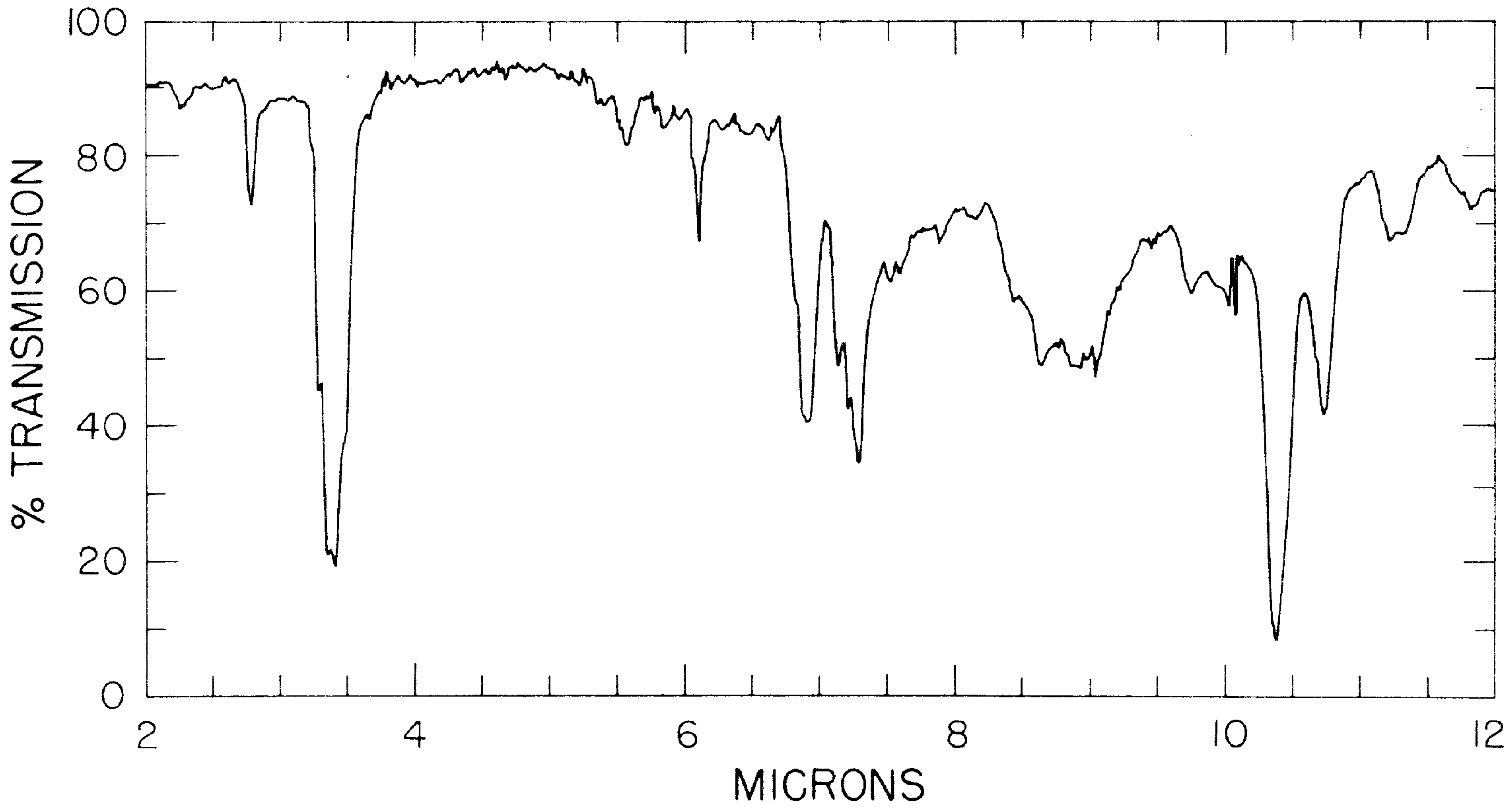


Fig. 24. Infrared Spectrum of 5,6-Dihydroxy-5,6-dihydrolycopene in Carbon Tetrachloride.

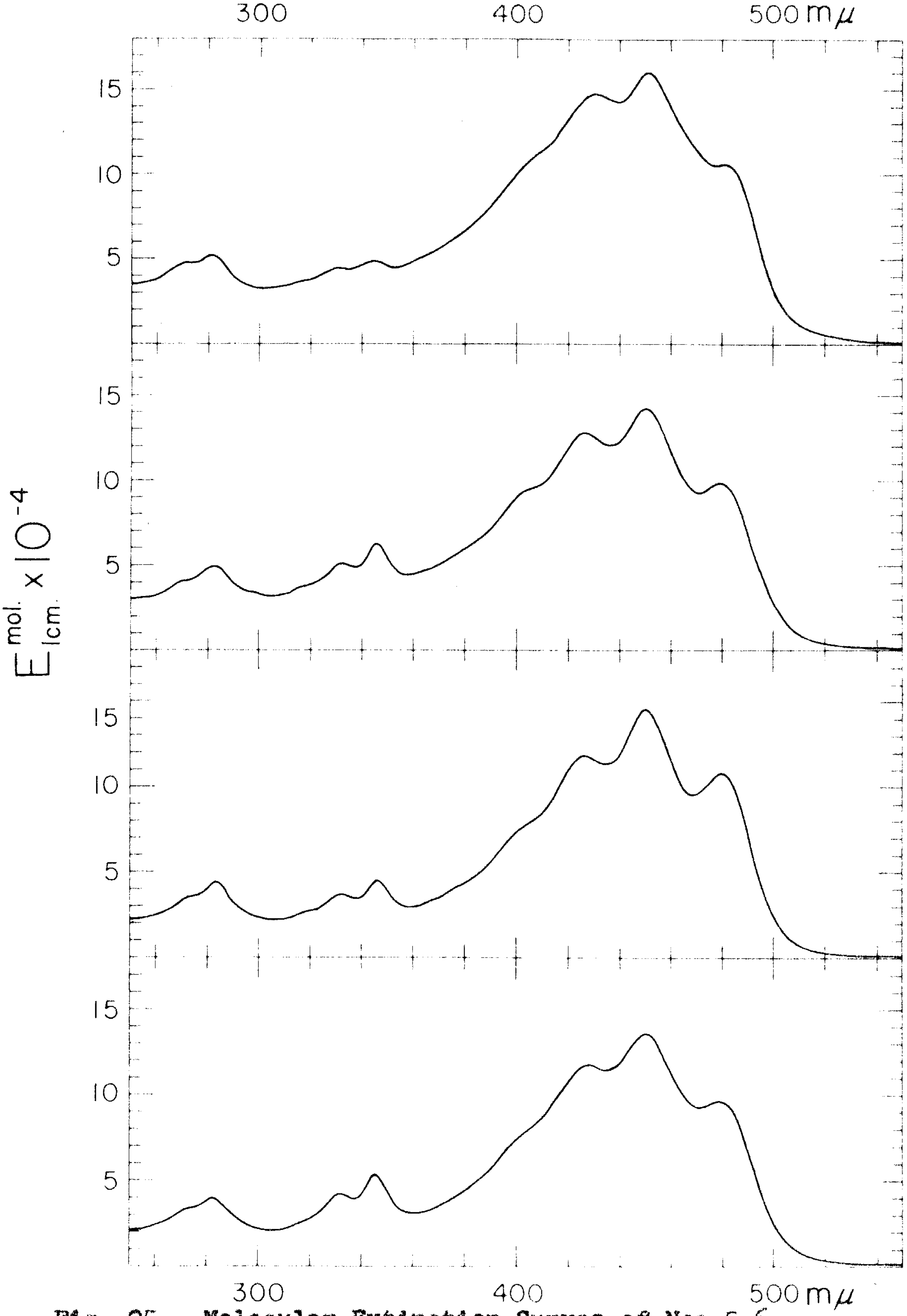


Fig. 25. Molecular Extinction Curves of Neo 5,6-Dihydroxy-5,6-dihydrolycopenes U, A, B, and C (in Hexane). Top to bottom: neo U, neo A, neo B, and neo C.

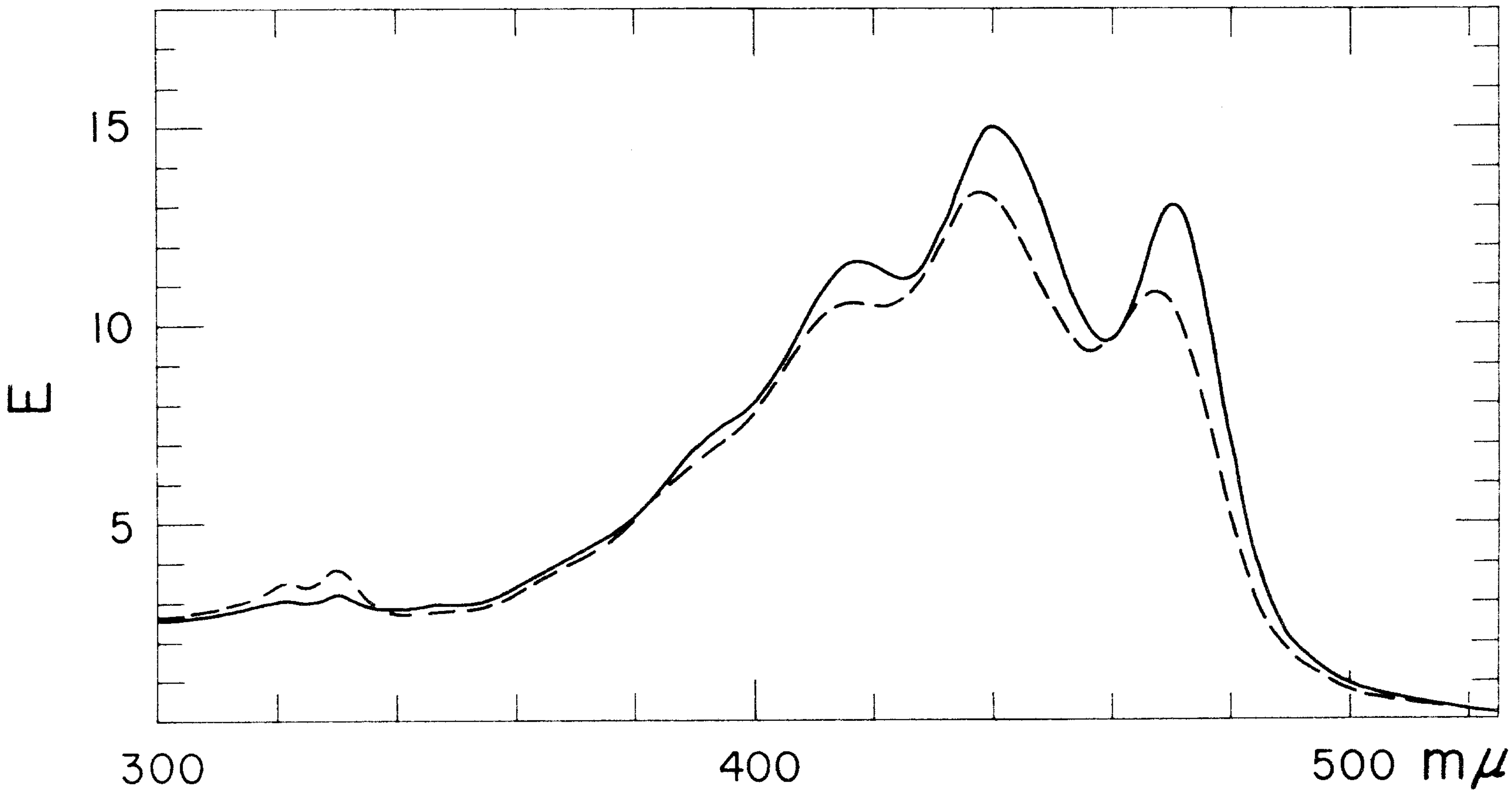


Fig. 26. Extinction Curve in Hexane of 5,6,5',6'-Tetrahydroxy-5,6,5',6'-tetrahydrolycopene: ———, fresh solution of the all-<u>trans</u> compound; and - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

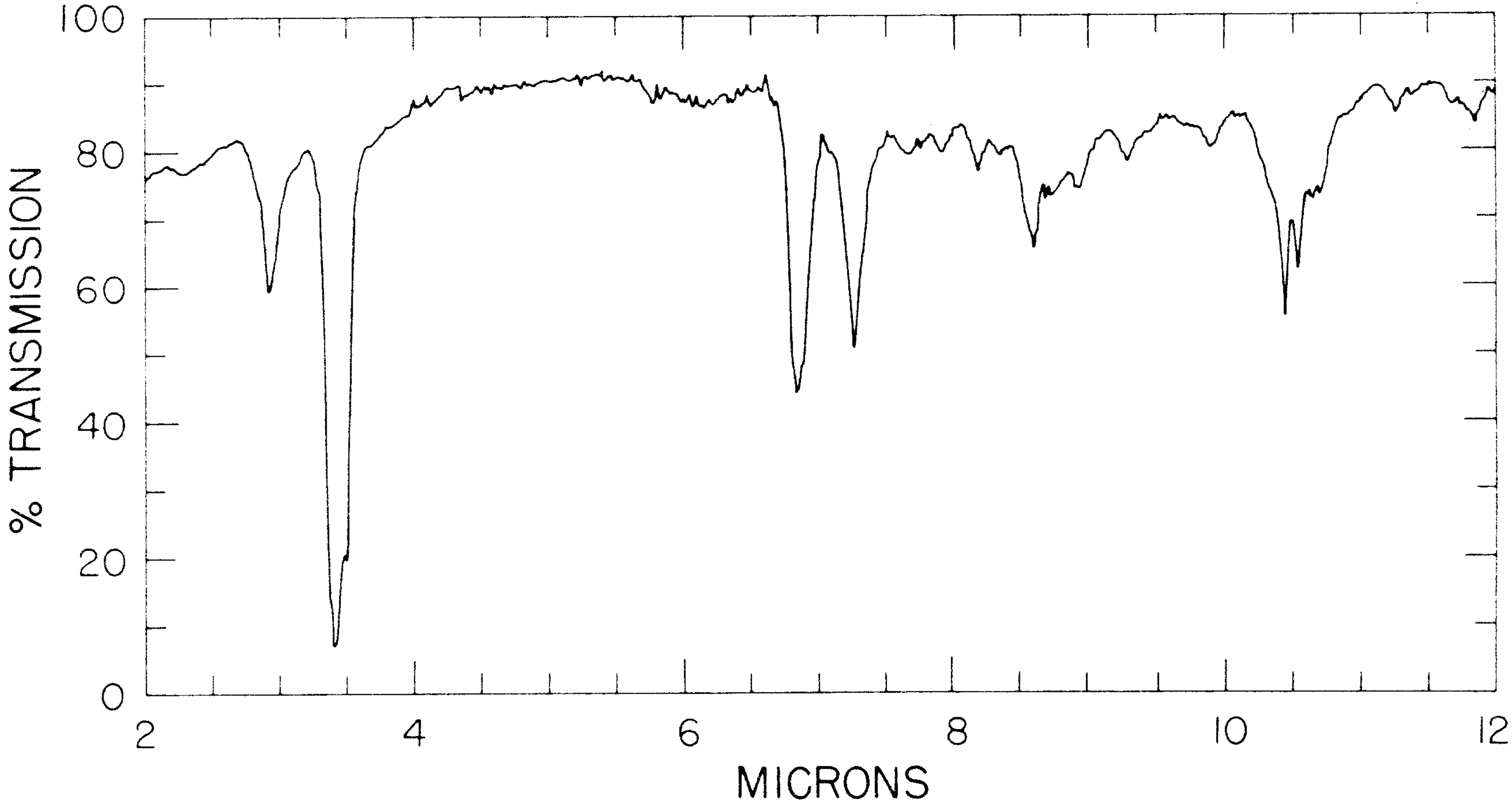


Fig. 27. Infrared Spectrum of 5,6,5',6'-Tetrahydroxy-5,6,5',6'-tetrahydrolycopene, as a Nujol mull.

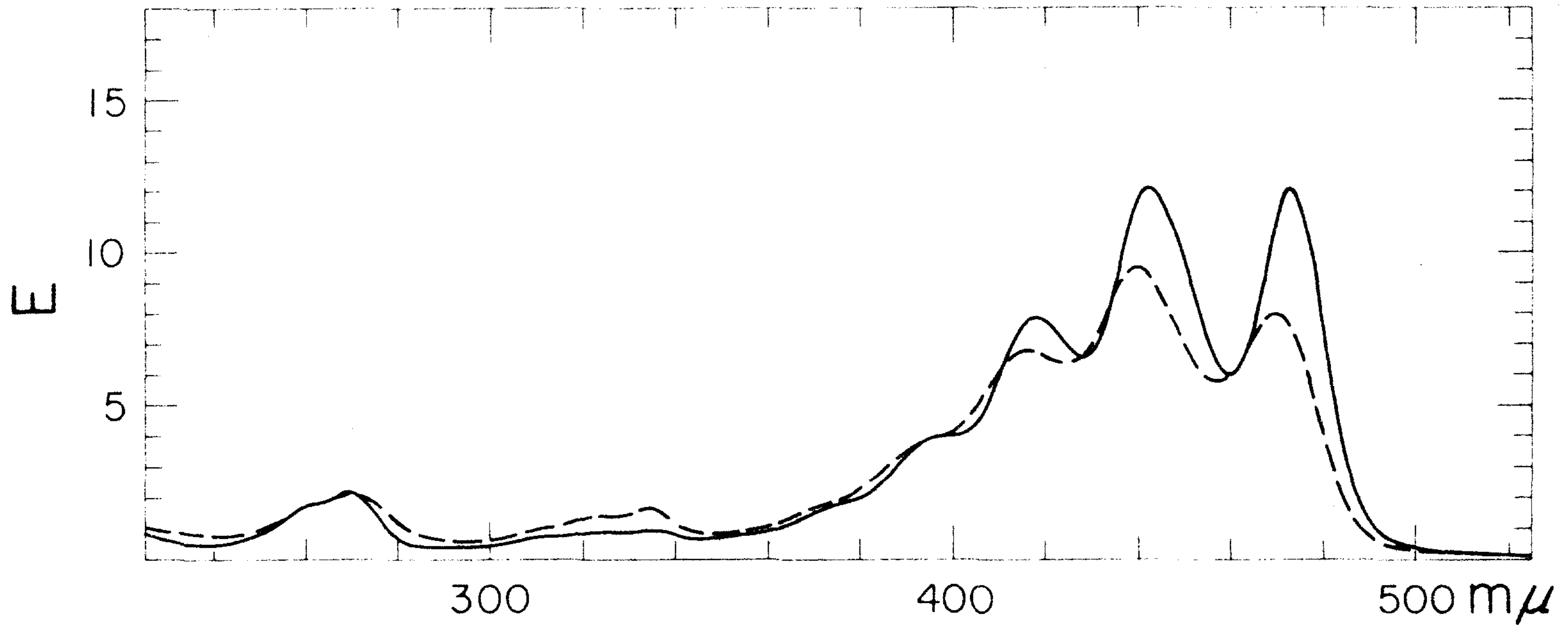


Fig. 28. Extinction Curve in Hexane of 5,6,5',6'(?)-Tetrahydrolycopene: ———, fresh solution of the all-*trans* compound; and - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

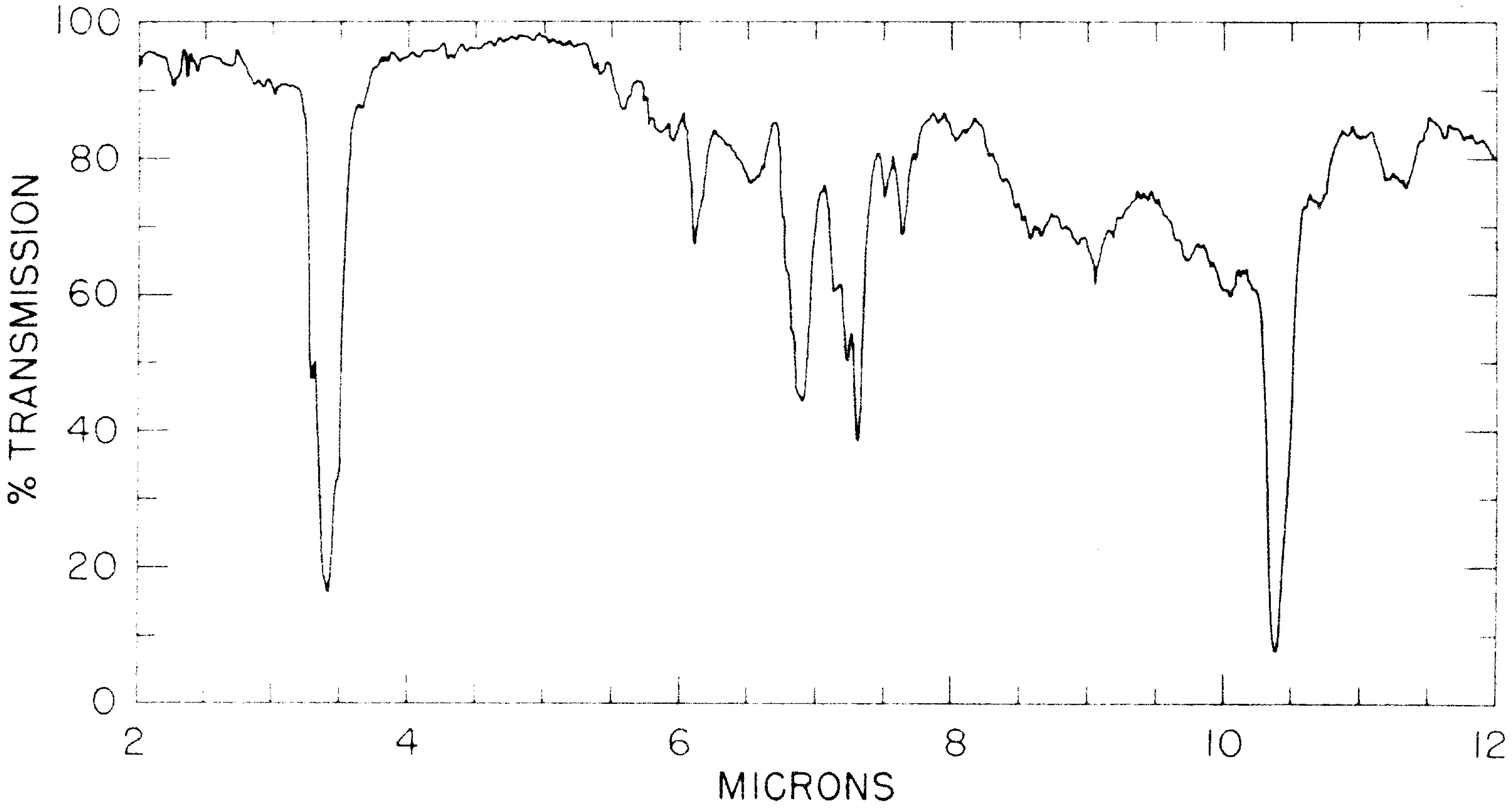


Fig. 29. Infrared Spectrum of 5,6,5',6'(?)-Tetrahydrolycopene in Carbon Tetrachloride.

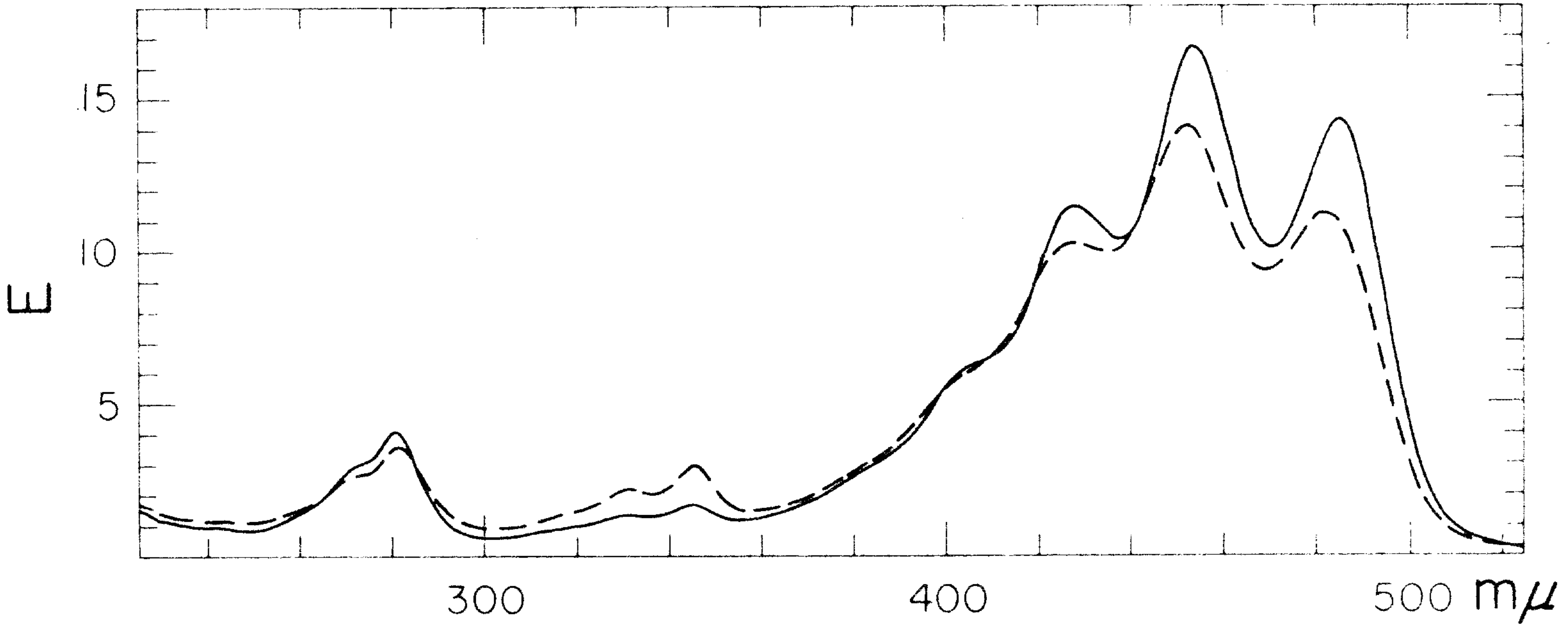


Fig. 30. Extinction Curve in Hexane of 5-Hydroxy-6-methoxy-5,6-dihydrolycopene: ———, fresh solution of the all-*trans* compound; and - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

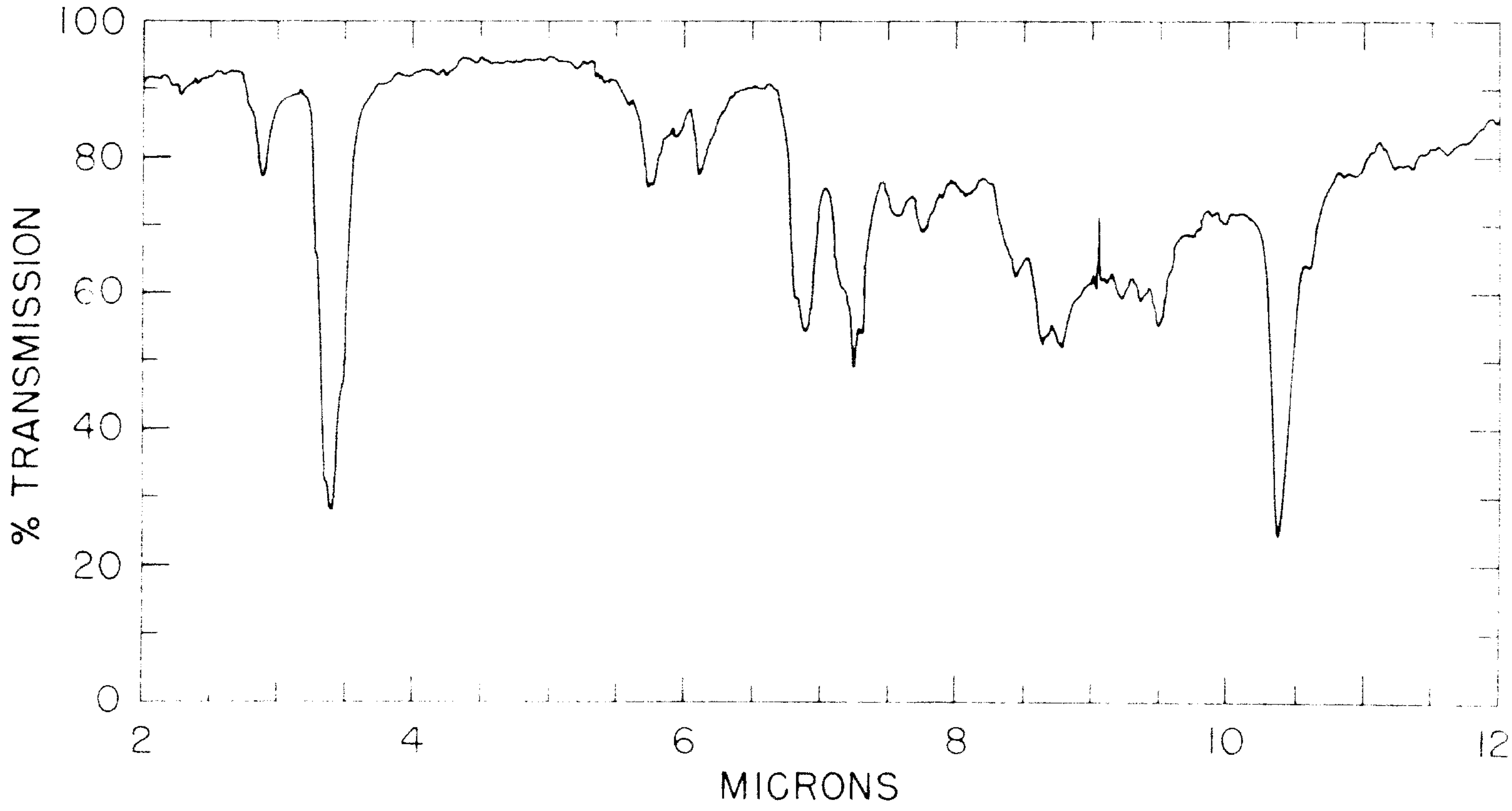


Fig. 31. Infrared Spectrum of 5-Hydroxy-6-methoxy-5,6-dihydrolycopene in Carbon Tetrachloride.

B. EXPERIMENTAL PART

1. Materials and Methods.

Most of the materials, apparatus, and techniques used in this study of the lycopene series were the same as those described in Chapter I. The principal changes are listed below, and apply to all experiments in this Section unless mention is made to the contrary.

Adsorbents. - In most instances, the adsorbent was the lime-Celite mixture (p. 53); it is to be understood when none is noted. Other adsorbents included zinc carbonate (powdered technical, Harshaw Scientific Div. of Harshaw Chemical Co., Los Angeles, Calif.) in mixtures with Celite.

Solvents. - Hexane mixed with technical chloroform in various volume ratios was used for development of chromatograms. (Acetone - hexane mixtures could not be used, since the high proportions of acetone necessary to migrate the pigments prevented a clear separation of the zones.) For elution, acetone was used. The complexing and cleavage processes were carried out in alcohol-free chloroform, prepared by washing continuously 1.5 liters of R. G. chloroform with water for 1 hour, then drying it with phosphorus pentoxide and distilling over P_2O_5. These operations were carried out in *quasi*-darkness. Alcohol-free acetone was obtained by heating under reflux for 4 hours a suspension of 8 g. of potassium permanganate in 4 liters of C. P. acetone.

Twenty grams of anhydrous potassium carbonate was then added, and the solution was filtered and distilled.

Lycopene was prepared from commercial tomato paste according to Sandoval and Zechmeister (36).

Partition behavior was determined by the method of Petracek (13) in the systems, hexane - 95 per cent, -90 per cent, or -85 per cent methanol.

Chromatographic Columns. - Cylindrical 28 x 8 cm. columns were used in most instances; this size is to be understood unless stated otherwise.

2. Formation and Hydrolysis of the Lycopene - Boron Trifluoride Complex.

A total of 8 grams of lycopene, divided into eighty 100-mg. portions, was treated as follows:

a.) 100 mg. of lycopene was dissolved in 50 ml. of alcohol-free chloroform; the solution was cooled to 0°.

b.) 3 ml. of boron trifluoride etherate (20°) was added with brisk agitation in an ice bath.

c.) The complexing was allowed to continue in the cold for only 30 seconds, even though the deep blue color of the complex did not fully develop in that time. (Longer complexing time resulted in extensive destruction of pigment.)

d.) The greenish-brown solution was poured quickly, with thorough mixing, into a pre-cooled mixture of 180 ml. of alcohol-free acetone and 20 ml. of saturated sodium bicarbonate solution. After about 10 seconds, the original orange-brown carotenoid color was restored, and the mixture was allowed to assume room temperature.

e.) Twenty such units were combined (corresponding to 2 g. of lycopene in 1 l. of chloroform) and washed in the continuous apparatus, in darkness, for 2 hours.

f.) The then deep red solution was dried, evaporated to 100 ml., and diluted to 1 l. with hexane. Photometric estimation at this point showed 51 per cent pigment recovery, calculated as lycopene.

g.) The solution was developed on 20 columns with about 1 l. of chloroform - hexane 2:3 for each:

16	several brown zones	
30	several very pale yellow and pink zones	
14	light yellow	(Zone 0)
70	many pale orange and pink zones	
10	colorless interzone	
46	orange	(Zone I)
24	yellow	(Zone II)
21	pink	(Zone III)
24	several orange zones	(Zone IV)

Remainder of column: colorless
Filtrate: contained one 8-cm. broad, greenish-yellow zone (Zone V), and showed strong grey-blue fluorescence in ultraviolet light

h.) The pigments of the respective zones were eluted and transferred to chloroform - hexane 2:3 by addition of water. Since the organic phase had a density only slightly less than that of water, its volume was doubled by addition of hexane prior to thorough washing.

i.) The solutions 0 - V were dried and stored in the refrigerator until all the separate portions of each zone could be combined.

3. Characterization of the Fractions.

5,6-Dihydroxy-5,6-dihydrolycopene. - The 80 Zones I were combined and evaporated to 500 ml. Development on four columns required about 1 l. of chloroform - hexane 2:3 for each:

108	several narrow, pale, yellow and tan zones, with colorless interzones
11	colorless interzone
80	bright orange (5,6-dihydroxy-5,6-dihydrolycopene)
22	yellow (cis isomers of the above)
27	colorless interzone
10	orange

Remainder of column: colorless
Filtrate: pale yellow

After elution, transfer into chloroform - hexane 2:3, and evaporation, the 5,6-dihydroxy-5,6-dihydrolycopene was crystallized from benzene - 95 per cent methanol; the cis isomers were combined with Zone II. Photometrically established yield of all-trans substance: 604 mg. = 7.5 per cent of the 8 g. of lycopene used; the crystals weighed 337 mg. = 4.2 per cent.

Properties of 5,6-Dihydroxy-5,6-dihydrolycopene:

Spectrum: Wavelengths of extinction maxima, 488, 456, 431 mμ. At λ_{max}, $E^{\text{mol.}}_{1\text{ cm.}} = 16.1 \times 10^4$ (in hexane). The ultraviolet and infrared curves appear on pp. 109 and 110.

Crystal Form: Long, thin, needle-like, orange prisms (from benzene - 95 per cent methanol). (Photomicrograph on p. 52 .)

Melting Point: 170-171°.

Partition Behavior: 45:55 in hexane - 95 per cent methanol; 76:24 in hexane - 90 per cent methanol.

Chromatographic Behavior: Adsorbed on lime-Celite above lycopene but below lycoxanthin (compared with an authentic lycoxanthin sample originating from *Solanum dulcamara* berries).

Reaction with Acid Chloroform: Negative.

Analysis:	Calcd for $C_{40}H_{58}O_2$:	C, 84.15; H, 10.24
	Found:	C, 84.20; H, 10.56
		83.92; 10.14.

These results refer to two independent samples; each was corrected for 1.7 per cent ash which could not be removed even by thirty-fold washing of benzene solutions with conductivity water.

Calcd. number of -OH groups:	2.0
Found (Zerevitinov):	2.1.

Cis Isomers of 5,6-Dihydroxy-5,6-dihydrolycopene. - The 80 Zones II and the *cis* isomers obtained from chromatographing Zone I were combined and evaporated to 100 ml., then diluted to 1 l. with hexane and developed on four columns with 1 l. of chloroform - hexane 1:2 for each column:

34 several tan zones
18 colorless interzone
9 orange (all-*trans*-5,6-dihydroxy-5,6-dihydrolycopene)
45 colorless interzone
44 orange (*cis*-5,6-dihydroxy-5,6-dihydrolycopene)
8 colorless interzone
8 orange (*cis*-5,6-dihydroxy-5,6-dihydrolycopene)
8 orange (*cis*-5,6-dihydroxy-5,6-dihydrolycopene)
8 orange (*cis*-5,6-dihydroxy-5,6-dihydrolycopene)
12 pink
15 light yellow
8 colorless interzone
10 bright orange

Remainder of column, and filtrate: colorless

The sum of the all-*trans* and *cis* isomers thus isolated was 296 mg. = 3.7 per cent of the lycopene. None of the *cis* isomers could be crystallized, despite the ready crystallizability of the all-*trans* form. The crystals of the all-*trans* compound (from benzene - 95 per cent methanol) weighed 20 mg.

Unchanged Lycopene (*Recovery*). - The combined Zones III were evaporated to 100 ml. and diluted to 1 l. with hexane. Development on 8 columns with 1 l. of chloroform - hexane 3:7 for each gave a single main pink zone, the pigment of which was crystallized from benzene - methanol and identified with lycopene *ex* tomatoes by the properties listed in Table 17, p. 124. Recovery: 1.126 g. = 14.1 per cent.

The combined Zones IV were rechromatographed in the same way. The main orange zone contained 288 mg. of neo-lycopene A = 3.6 per cent of the starting material. A very narrow, pale orange zone found much below neo-lycopene A

Table 17

Comparison of the Pigment Recovered from Zones III with Tomato Lycopene

	Lycopene *ex* Tomatoes	Lycopene *ex* Zones III
Partition behavior in hexane - 95 per cent methanol	100:0	100:0
Melting point	173-174°	172-174°
	(no depression of mixed melting point)	
Maxima in hexane		
before iodine catalysis	504, 472, 443	503, 472, 444 mμ
after iodine catalysis	498, 467, 444	498, 467, 443
Reaction with acid chloroform	Negative	Negative
Crystal form (from benzene - methanol)	Long, deep red prisms	Long, needle-like, red prisms
Chromatographic behavior	No separation in mixed chromatogram tests on lime-Celite or on zinc carbonate-Celite 4:1.	

contained about 2 mg. (0.03 per cent) of a pigment which had the same ultraviolet spectrum as, and did not separate from in the mixed chromatogram test, the acetonide of 5,6-dihydroxy-5,6-dihydrolycopene (p. 132). This pigment was not detected when dioxan was substituted for acetone in the hydrolysis of the complex.

5,6,5',6'-Tetrahydroxy-5,6,5',6'-tetrahydrolycopene. - The combined Zones 0 were evaporated to 100 ml. and developed on a single column with 2 l. of chloroform - hexane 1:1. The main light yellow zone contained about 10 mg. (0.1 per cent yield) of a pigment that was so soluble in methanol that benzene - methanol systems could not be used for crystallization; 2.1 mg. of crystals were obtained from chloroform - hexane.

Properties of 5,6,5',6'-Tetrahydroxy-5,6,5',6'-tetrahydrolycopene:

Spectrum: Maxima in hexane 470, 440, 417 mμ. The ultraviolet and infrared curves appear on pp. 112 and 113.

Crystal Form: Very small, nearly rectangular, yellow-orange prisms, arranged in clusters radiating from a center (from chloroform - hexane).

Melting Point: 144-147°, sinters at 140°.

Partition Behavior: 0:100 in hexane - 95 per cent methanol;
4:96 in hexane - 90 per cent methanol;
8:92 in hexane - 85 per cent methanol.

Chromatographic Behavior: Very strongly adsorbed on lime-Celite; found above lycoxanthin.

Reaction with Acid Chloroform: Negative.

5,6,5',6'(?)-Tetrahydrolycopene. - The filtrates from the 80 chromatograms on p. 120(containing Zone V) were combined, evaporated to 1 l., and developed on 10 columns with 1 l. of hexane for each. The main yellow zone was crystallized from benzene - methanol. Yield: 9.8 mg. = 1.2 per cent (photometric estimate); the crystals weighed 2.0 mg. (0.3 per cent).

Properties of 5,6,5',6'(?)-Tetrahydrolycopene:

Spectrum: Maxima in hexane, 473, 441, 418 mμ. The ultraviolet and infrared curves appear on pp. 114 and 115.

Crystal Form: Long, hair-like, light yellow needles, occasionally radiating from a single point to form a "fur ball" (from benzene - methanol).

Melting Point: 137-139°, sinters at 135°.

Partition Behavior: 100:0 in hexane - 95 per cent methanol.

Chromatographic Behavior: Develops with hexane on lime-Celite; is adsorbed below α-carotene.

Reaction with Acid Chloroform: Negative.

4. Methanolysis of the Lycopene - Boron Trifluoride Complex.

Using a sequence of operation similar to that described for the hydrolysis (p. 119), 250 mg. of lycopene, in two

125-mg. portions, was treated with BF_3 etherate (15 ml. each), and the resulting complex was cleaved with absolute methanol (600 ml. each, at 0°). The product was developed on a single 100 x 8 cm. lime-Celite column with 4 l. of chloroform - hexane 1:9 for 6 hours:

10	brown
125	several red and orange zones (lycopene and *cis* isomers)
47	colorless interzone
118	bright yellow-orange (all-*trans*-5,6-dimethoxy-5,6-dihydrolycopene)
165	yellow *cis* forms of 5,6-dimethoxy-5,6-dihydrolycopene)

Remainder of column: several minor yellow zones
Filtrate: light yellow, with green fluorescence in ultraviolet light

The photometrically estimated, unchanged lycopene, including some *cis* forms, was 33.2 mg. = 13.3 per cent of the starting material. The yield of all-*trans*-5,6-dimethoxy-5,6-dihydrolycopene amounted to 33.6 mg. = 13.4 per cent; an additional 31.5 mg. = 12.6 per cent was recovered in the form of *cis* isomers. Crystals obtained (benzene - 95 per cent methanol) from the all-*trans* fraction weighed 20 mg. = 8 per cent of the original lycopene.

Properties of 5,6-Dimethoxy-5,6-dihydrolycopene:

Spectrum: The ultraviolet spectrum is very similar to that of the 5,6-dihydroxy-compound. Maxima in hexane 487, *456*, 430 mμ.

Crystal Form: Long, flat, orange-yellow prisms, often in clusters radiating from a central point (from benzene -

95 per cent methanol).

Melting Point: 153-154°.

Partition Behavior: 98:2 in hexane - 95 per cent methanol.

Chromatographic Behavior: Adsorbed on lime-Celite below neolycopene A, but above β-carotene.

Reaction with Acid Chloroform: Negative.

Analysis: Calcd. for two CH_3O- groups: 10.32 per cent
Found: 9.33.

5. Some Conversions of 5,6-Dihydroxy-5,6-dihydrolycopene and Its Derivatives.

a. Formation of 5,6,5',6'-Tetrahydroxy-5,6,5'6'-tetrahydrolycopene from 5,6-Dihydroxy-5,6-dihydrolycopene via the Boron Trifluoride Complex. - Complexing 25 mg. of 5,6-dihydroxy-5,6-dihydrolycopene as described for lycopene, and hydrolyzing the complex at room temperature, yielded a complex pigment mixture. Development on a 24 x 4.5 cm. lime-Celite column with 1 l. of chloroform - hexane 2:3 took about 75 minutes. Much destruction of pigment was noticed, but the single main yellow zone contained about 2 mg. of pigment identical with 5,6,5',6'-tetrahydroxy-5,6,5',6'-tetrahydrolycopene (p. 125).

b. Formation of Lycopene from 5,6,5'6'(?)-Tetrahydrolycopene by Means of N-Bromosuccinimide. - 15 mg. of 5,6,5',6'(?)-tetrahydrolycopene was treated with NBS (1:1 mole ratio) as described for 3,4-dehydro-α-carotene on

p. 79 . Development on a 20 x 3.5 cm. lime-Celite column with 400 ml. of chloroform - hexane 1:5 yielded one 8-mm. broad red zone and several orange zones of similar width below it. The red zone contained 0.1 mg. of lycopene (yield, 0.7 per cent), and the orange pigments were stereoisomers of a 10-conjugated-double-bond hydrocarbon (1 mg. = 7 per cent).

c. Etherification of 5,6-Dihydroxy-5,6-dihydrolycopene: Preparation of 5-Hydroxy-6-methoxy-5,6-dihydrolycopene. - The directions given here were adapted from the procedure of Karrer and Takahashi (37) for the etherification of zeaxanthin. 50 mg. of 5,6-dihydroxy-5,6-dihydrolycopene was dissolved in 15 ml. of boiling, dry toluene. 25 mg. of potassium was dissolved in 0.5 ml. of *tert*-amyl alcohol and diluted to 1 ml. with boiling dry toluene. The two solutions were combined, whereupon the mixture quickly turned from red-orange to dark brown. The mixture was kept at 60° for 15 min., then cooled to 0° in an ice bath. After addition of 2 ml. of methyl iodide, the solution was warmed to 60° and kept there for an hour. Then 60 ml. of hexane was added, and the solution was washed continuously with water until the hypophase was no longer basic (about 20 min.).

The product was evaporated to dryness and dissolved in 100 ml. of hexane, with the initial aid of a few drops of chloroform. Development on a 24 x 4.5 cm. lime-Celite column required about 500 ml. of chloroform - hexane 1:5:

5	brown-orange
7	orange
15	orange (5,6-dihydroxy-5,6-dihydrolycopene)
12	orange
67	yellow-orange (all-*trans*-5-hydroxy-6-methoxy-5,6-dihydrolycopene)
54	yellow (*cis* forms of 5-hydroxy-6-methoxy-5,6-dihydrolycopene)
27	colorless interzone
10	very pale yellow

Remainder of column: colorless
Filtrate: very pale yellow; no fluorescence in ultraviolet light

The amount of all-*trans*-5-hydroxy-6-methoxy-5,6-dihydrolycopene was photometrically estimated as 18 mg. = 36 per cent conversion; an additional 10 mg. (20 per cent) was recovered in the form of *cis* isomers. The all-*trans* crystals obtained from benzene - methanol weighed 12 mg. (= 24 per cent).

Properties of 5-Hydroxy-6-methoxy-5,6-dihydrolycopene:

Spectra: Maxima in hexane 486, *455*, 430 mμ. The ultraviolet and infrared curves appear on pp. 116 and 117.

Crystal Form: Thin, nearly rectangular, pinkish-orange plates (from benzene - methanol).

Melting Point: 146-147°.

Partition Behavior: 84:16 in hexane - 95 per cent methanol.

Chromatographic Behavior: Adsorbed on lime-Celite above 5,6-dimethoxy-5,6-dihydrolycopene but just below lycopene.

Reaction with Acid Chloroform: Negative.

d. Preparation of Lycopene Mono-epoxide. - To 100 mg. of lycopene, dissolved with some warming in 400 ml. of absolute ether, was added 100 mg. of perphthalic acid (200 per cent excess) dissolved in 200 ml. of absolute ether. The mixture was allowed to stand for one week in the dark at room temperature and was shaken twice daily during that time. After a week, lycopene crystals were filtered off. The filtrate was washed with 2 x 100 ml. of saturated sodium bicarbonate solution and evaporated completely. The residue was dissolved in hexane and developed for 1 hour on a 24 x 4.5 cm. column with 400 ml. of chloroform - hexane 1:5:

16	brown
5	very pale pink
5	colorless
94	pink-orange (lycopene)
15	yellow-orange
4	yellow-orange
10	colorless
12	very pale orange (lycopene mono-epoxide)

Remainder of column, and filtrate: colorless

The yield of lycopene mono-epoxide was photometrically estimated to be 1.5 mg. = 1.5 per cent. Total lycopene recovered unchanged, 80 mg. = 80 per cent.

Properties of Lycopene Mono-epoxide:

Spectrum: The spectrum in the ultraviolet and visible regions is similar to those of 5,6-dihydroxy- and 5,6-dimethoxy-5,6-dihydrolycopene. Maxima in hexane, 487, 455, 428 mμ.

Partition Behavior: 99:1 in hexane - 95 per cent methanol.

Chromatographic Behavior: Adsorbed on lime-Celite slightly above 5,6-dimethoxy-5,6-dihydrolycopene.

Reaction with Acid Chloroform: Negative.

Hydrolysis: One mg. of lycopene mono-epoxide in 20 ml. of hexane was shaken with 10 ml. of 10 per cent methanolic KOH for 24 hours. The epiphase was then washed free of base (20 minutes in the LeRosen apparatus) and developed on a 20 x 3.5 cm. lime-Celite column with 400 ml. of chloroform - hexane 3:7 for one and a half hours. The main pale orange zone contained 0.2 mg. (photometric estimate) of a pigment which was shown by mixed chromatography, partition behavior, and spectrum to be identical with 5,6-dihydroxy-5,6-dihydrolycopene prepared by hydrolysis of the lycopene - boron trifluoride complex.

e. Formation of 5,6-Dihydroxy-5,6-dihydrolycopene Acetonide. - To 25 mg. of 5,6-dihydroxy-5,6-dihydrolycopene, dissolved in 25 ml. of anhydrous benzene, 25 ml. of anhydrous acetone and 50 mg. of anhydrous copper sulfate were added. The solution was shaken in the dark, continuously for 16 hours, washed acetone-free, and evaporated to dryness. The residue, redissolved in hexane, was developed on a 20 x 3.5 cm. lime-Celite column with 500 ml. of chloroform - hexane 1:9. The main orange zone and two minor orange zones (just below) contained unchanged starting material and *cis* isomers, total 22 mg. The minor yellow zone near the bottom of the column contained 0.6 mg. (2.4 per cent conversion) of the desired acetonide.

Properties of 5,6-Dihydroxy-5,6-dihydrolycopene Acetonide:

Spectrum: Similar to 5,6-dihydroxy- and 5,6-dimethoxy-5,6-dihydrolycopene; maxima in hexane, 486, 455, 429 mμ.

Partition Behavior: 97:3 in hexane - 95 per cent methanol.

Chromatographic Behavior: Adsorbed very slightly above 5,6-dimethoxy-5,6-dihydrolycopene on lime-Celite.

Reaction with Acid Chloroform: Negative.

Hydrolysis: To 0.5 mg. of the acetonide in 10 ml. of absolute ethanol was added 1 ml. of 1 N HCl, and the mixture was shaken for 4 hours; 25 ml. of hexane and 25 ml. of acetone were then introduced, and the epiphase was washed acetone- and alcohol-free. A chromatogram of the product on a 18 x 1.8 cm. lime-Celite column, developed with 100 ml. of chloroform - hexane 3:7 (1 hour), showed one main orange zone, which contained 0.2 mg. of 5,6-dihydroxy-5,6-dihydrolycopene.

f. Formation of 5,6-Dihydroxy-5,6-dihydrolycopene Diacetate. - 9 mg. of 5,6-dihydroxy-5,6-dihydrolycopene was dissolved in 0.3 ml. of anhydrous pyridine in a centrifuge cone, and 10 drops of acetyl chloride (C. P.) were added cautiously, one at a time, while stirring and cooling in an ice bath. The mixture was then heated on a steam bath under reflux for 1 hour. The contents of the centrifuge cone were transferred to a separatory funnel by rinsing the cone successively with 50 ml. of hexane, 50 ml. of acetone, and 3 x 5 ml. of absolute methanol. The pigment was transferred to hexane and washed continuously for 30 min. Development with 250 ml. of chloroform - hexane 1:5 on a 20 x 3.5 cm. column of zinc carbonate - Celite 6:1 gave, after one and a half hours, two main orange zones. The upper one contained unreacted 5,6-dihydroxy-5,6-dihydrolycopene (3 mg.) and the lower one, 3 mg. of 5,6-dihydroxy-5,6-dihydrolycopene diacetate.

Properties of 5,6-Dihydroxy-5,6-dihydrolycopene Diacetate:

Spectrum: Similar to those of 5,6-dihydroxy- and 5,6-dimethoxy-5,6-dihydrolycopene. Maxima in hexane, 487, 455, 428 mμ.

Partition Behavior: 89:11 in hexane - 95 per cent methanol.

Chromatographic Behavior: Adsorbed on zinc carbonate - Celite 6:1 below 5-hydroxy-6-methoxy-5,6-dihydrolycopene but above 5,6-dimethoxy-5,6-dihydrolycopene.

Reaction with Acid Chloroform: Negative.

Hydrolysis: 2 mg. of the diacetate was hydrolyzed as described for 4-acetoxy-α-carotene (p. 86). The chromatogram, developed on lime-Celite (20 x 3.5 cm) with 250 ml. of chloroform - hexane 3:7, showed a single main orange zone which contained 1.5 mg. of 5,6-dihydroxy-5,6-dihydrolycopene.

6. Stereoisomerization of 5,6-Dihydroxy-5,6-dihydrolycopene.

The materials and procedures used were similar to those described for 4-hydroxy-α-carotene on pp. 87 to 94, except that 600 ml. of chloroform - hexane 3:7 was used to develop the chromatograms in about 1 hour. $E^{mol.}_{1\ cm.}$ of the iodine-catalyzed equilibrium mixture = 14.2×10^4 (in hexane).

a. Iodine-catalyzed Stereoisomerization.

From 24.8 mg. of all-*trans* starting material, the photometrically estimated pigment recovery was 92 per cent:

24	several brown zones
10	colorless interzone
28	orange (neo U)
5	colorless interzone
13	orange
60	nearly colorless interzone
117	orange (all-*trans*)
21	light yellow
24	colorless interzone
30	orange (neo A)
23	colorless interzone
39	orange (neo B)
12	colorless interzone
22	orange (neo C)

Remainder of column, and filtrate: colorless

A summary of the relative amounts of the stereoisomers formed will be found in Table 13, p. 106.

b. Stereoisomerization by Fusion (Melting Crystals).

The photometric estimate showed a pigment recovery of 94 per cent of the 14.3 mg. treated. The column was similar to that described above, except that all pigment zones were somewhat paler, and the filtrate had a pale yellow fluorescence in ultraviolet light. A summary of the relative amounts of isomers formed will be found in Table 14, p. 106.

c. Stereoisomerization by Refluxing in Darkness.

The pigment recovery was 99 per cent of the original 24.0 mg. The chromatogram showed one main zone (20 cm.) containing unchanged all-*trans* pigment, and three pale zones below it, containing neos A, B, and C. The ratios of the isomers formed are shown in Table 15, p. 107.

d. Stereoisomerization by Standing in Direct Sunlight.

The average incident light intensity was: N, 10,400 lumens per square foot; E, 8000; S, 28,000; W, 14,400; and V, 32,000. The average ambient air temperature was 24°, and the final solution temperature was 35°. The starting material weighed 22.6 mg., and the photometrically estimated pigment recovery was 94 per cent. The chromatogram was similar to that described in Part c., above. The stereoisomeric ratios are summarized in Table 15, p. 107.

e. Stereoisomerization by Standing in Diffuse Daylight.

The all-*trans* starting material (23.5 mg.) was exposed to light of the average intensity N, 3600 lumens per square foot; E, 1600; S, 800; W, 2400; and V, 10,400. The average air temperature and the final solution temperature were 24°. The recovered pigment (99 per cent, photometric estimate) gave a chromatogram similar to that described in Part c. The stereoisomeric ratios are summarized in Table 15, p. 107 .

f. Stereoisomerization by Irradiation with Photoflood Bulbs.

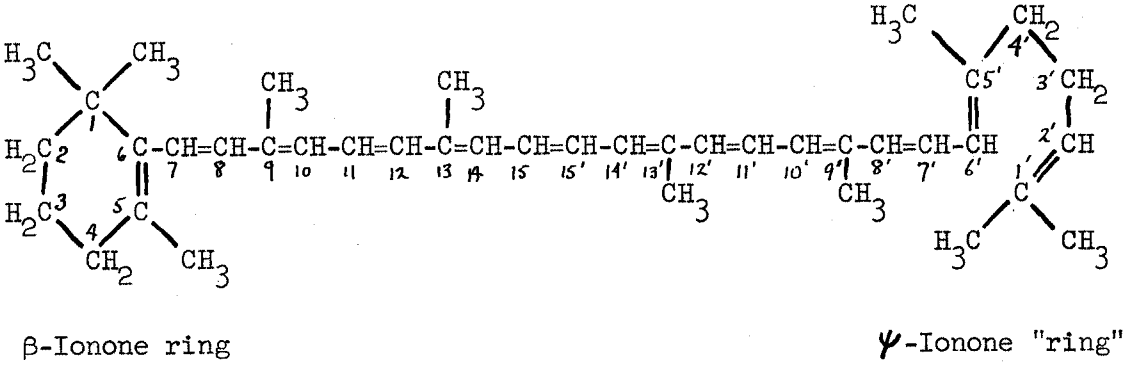
The average ambient air temperature was 29°, and the final solution temperature was 35°. Pigment recovery: 93 per cent of the original 22.2 mg. The chromatogram was similar to that described in Part c., except that the neo U isomer appeared as a minor zone above the all-*trans* compound. The stereoisomeric ratios are summarized in Table 15, p. 107.

III. γ-CAROTENE AND BORON TRIFLUORIDE ETHERATE

A. THEORETICAL PART

1. Introduction.

The third complex to be investigated was that of γ-carotene, $C_{40}H_{56}$ (XXIX):



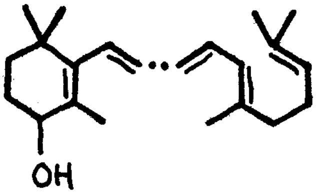
(XXIX.) γ-Carotene.

(XXIXa.) γ-Carotene.

The formula shows that one end of the γ-carotene molecule is cyclized to a "β-ionone ring," while the other end is aliphatic, as in lycopene. Thus, two types of hydrolysis products could be expected from the γ-carotene - boron trifluoride complex: one series similar to the lycopene products and the other involving substitution on the β-ionone ring.

2. Hydrolysis Products of the γ-Carotene - Boron Trifluoride Complex.

4-Hydroxy-γ-carotene (XXX) was the "main" product isolated from a very complicated pigment mixture. Its partition ratio and an absorption band at 2.78μ gave strong indication that this compound is a monohydric alcohol. The reactions used to characterize 4-hydroxy-γ-carotene are summarized in Chart 4 (p. 140) and described below (a.-d.).



(XXX.) 4-Hydroxy-γ-carotene.

(a.) Dehydration by the acid chloroform reagent proves the allylic position of the -OH group (12). Absence of fine structure in the visible spectrum of the dehydration product shows that the hydroxyl group is attached to the β-ionone ring, since 3,4-dehydro-γ-carotene (XXXI) would not be expected to exhibit extensive fine structure (4). In contrast, 3',4'-dehydro-γ-carotene (XXXII), which would be formed from dehydration of the (unknown) 4'-hydroxy-γ-carotene, would show fine structure.

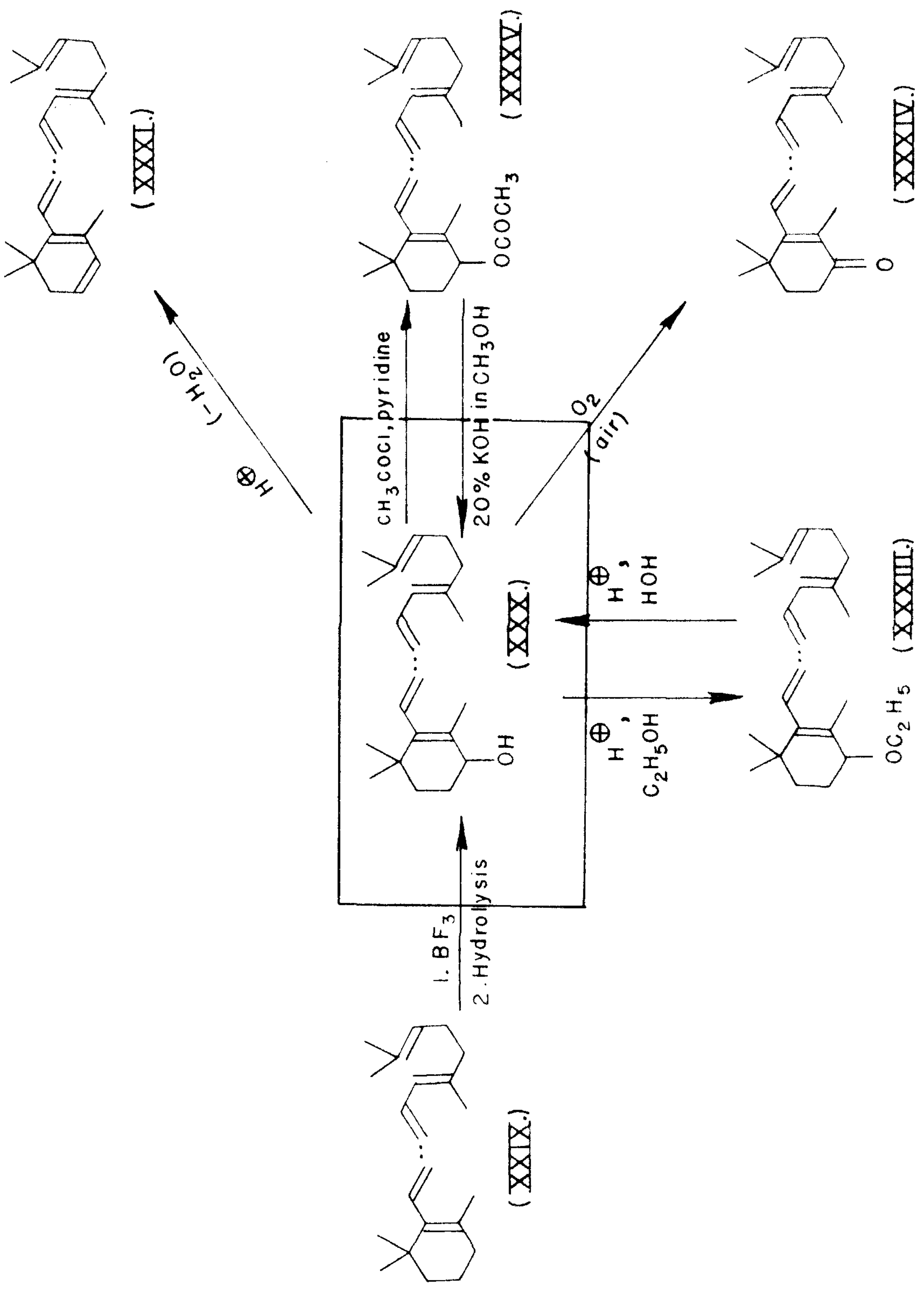


Chart 4. Conversions Demonstrating the 4-Hydroxy-γ-carotene Structure.

(XXXI.) 3,4-Dehydro-γ-carotene

(No fine structure expected.)

(XXXII.) 3',4'-Dehydro-γ-carotene.

(Extensive fine structure expected.)

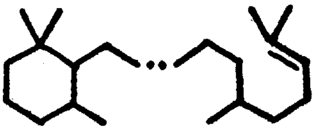
(b.) The formation and hydrolysis of an ether (XXXIII) simply by acid catalysis also shows that the hydroxyl group is allylic to the main chromophore.

(c.) 4-Hydroxy-γ-carotene was oxidized by air to 4-keto-γ-carotene (XXXIV). The ketone had a longer wavelength maximum than the hydroxy compound, indicating that the carbonyl group formed was in conjugation with the main chromophore.

(d.) The formation and hydrolysis of 4-acetoxy-γ-carotene (XXXV) represent pertinent evidence.

Formation of 4-Hydroxy-γ-carotene undoubtedly occurred by a mechanism similar to that which resulted in the formation of 4-hydroxy-α-carotene (p. 10). A second parallel to the behavior of α-carotene can also be made: namely, that only the β-ionone ring is substituted. Other parallels to the lycopene and α-carotene series, such as the hydrogenation of the starting material, can be seen in the minor products listed below.

5,6,7,8,5',6',7',8'(?)-Octahydro-γ-carotene (XXXVI) is probably produced in a manner similar to the reduced lycopene and α-carotene derivatives described above. From its partition it is seen to be a hydrocarbon while the visible spectrum indicates the presence of a 7-double-bond chromophore. However, the chromophore could not be proved to be symmetrically located; neither could the absence of an isolated double bond (e.g., in the 5,6-position) be demonstrated.



(XXXVI.) 5,6,7,8,5',6',7',8'-Octahydro-γ-carotene.

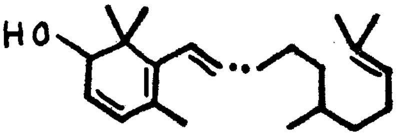
5,6,5',6'-Tetrahydro-γ-carotene (XXXVII). - The intense fine structure in the spectrum supports the absence of a ring double bond (both β-carotene and lycopene have 11 conjugated double bonds, but the spectral curve of β-carotene shows much less fine structure than does that of lycopene).

(XXXVII.) 5,6,5',6'-Tetrahydro-γ-carotene.

5',6'-Dihydro-γ-carotene (XXXVIII). - Its spectrum is almost identical to that of α-carotene; hence the chromophore is believed to consist of 10 conjugated double bonds, one of which is located in the β-ionone ring.

(XXXVIII.) 5',6'-Dihydro-γ-carotene.

2-Hydroxy-3,4-dehydro-5',6',7',8'-tetrahydro-γ-carotene (XXXIX) gives a positive allylic test and shows the partition behavior of a hindered alcohol (13). The spectrum displays the lack of fine structure characteristic of a 3,4-dehydro-compound (or a conjugated ketone); and the position of the λ_{max} indicates 10 conjugated double bonds. Petracek (4) has explained the formation of 2-hydroxy-3,4-dehydro-β-carotene from *retro*-bisdehydro-β-carotene and described the properties of the 2-hydroxy compound. Probably, the 2-hydroxy-3,4-dehydro-structure is obtained *via* dehydra-



(XXXIX.) 2-Hydroxy-3,4-dehydro-5',6',7',8'-tetrahydro-γ-carotene.

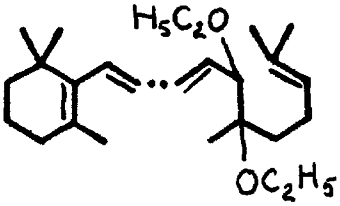
tion of the 4-hydroxy derivative to the 3,4-dehydro-structure, and, during the 5 - 10 seconds required for the hydrolysis of the γ-carotene - BF_3 complex, the 3,4-dehydro compound is re-complexed and undergoes a second allylic substitution, to give the 2-hydroxy-3,4-dehydro-structure. Hydrogenation of the aliphatic end of the molecule occurs as described for lycopene (p. 104).

Unchanged γ-carotene was identified with an authentic sample by spectrum, partition behavior, melting point, and mixed chromatogram test.

5,6-Dihydro-γ-carotene (XL) is a hydrocarbon with a spectrum nearly identical to that of *5,6-dihydroxy-5,6-dihydrolycopene*. It is believed that the 5,6 double bond of γ-carotene has been hydrogenated, resulting in a 10-double-bond chromophore that is located entirely in the aliphatic portion of the molecule.

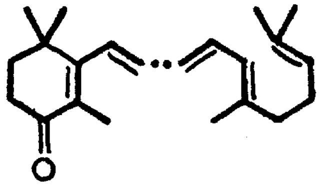
(XL.) 5,6-Dihydro-γ-carotene.

5',6'-Diethoxy(?)-5',6'-dihydro-γ-carotene (XLI) shows nearly the α-carotene spectrum; hence the 5',6' double bond is here missing. This compound is not identical to any known α-carotene derivative, however. The partition ratio is characteristic of a di-ether, and the proposed ethoxyl groups may have originated from a trace of ethanol present in the acetone used during hydrolysis of the complex. A second possibility is that this compound would represent the acetonide of the still unknown 5',6'-dihydroxy-5',6'-dihydro-γ-carotene.



(XLI.) 5',6'-Diethoxy-5',6'-dihydro-γ-carotene.

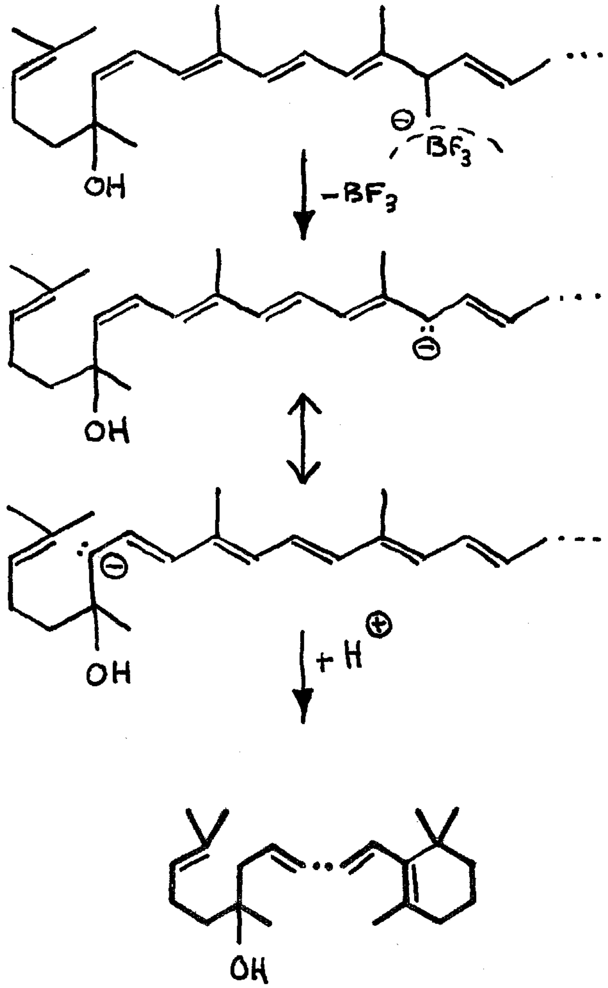
<u>4-Keto-γ-carotene</u> (XXXIV) was identified by its spectrum, partition behavior, preparation by oxidation of 4-hydroxy-γ-carotene, and a peak at 6.00 μ. It is believed that this compound is an autoxidation product of 4-hydroxy-γ-carotene, rather than a primary product of the hydrolysis (*cf*. 4-keto-α-carotene, p. 15).



(XXXIV.) 4-Keto-γ-carotene.

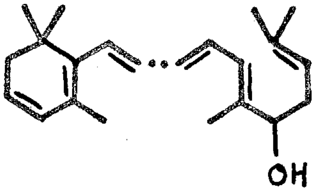
<u>5'-Hydroxy(?)-5',6'-dihydro-γ-carotene</u> (<u>XLII</u>). - The similarity of the spectrum of this compound to that of α-carotene shows that the two have identical chromophores. A peak at 2.77μ and the partition ratio indicate the

presence of a single "hindered" hydroxyl group, although an additional ethoxyl group, for example, is not excluded. The formation of this compound may have taken place *via* some initial stages (not shown here, *cf.* p. 103) similar to the formation of 5,6-dihydroxy-5,6-dihydrolycopene.



(XLII.) 5'-Hydroxy-5',6'-dihydro-γ-carotene.

4'-Hydroxy-3,4-dehydro-γ-carotene (XLIII). - The length of the chromophore of this compound was indicated by the spectrum. The presence and allylic nature of the hydroxyl group were shown by the infrared curve and the positive allylic test. A 2-position for the hydroxyl group would be in contradiction with the partition ratio (13); the 4'-position is the only location for the hydroxyl group consistent with all observed properties of the compound. This is the only known example of allylic substitution of a hydroxyl group at the aliphatic end of either γ-carotene or lycopene.



(XLIII.) 4'-Hydroxy-3,4-dehydro-γ-carotene.

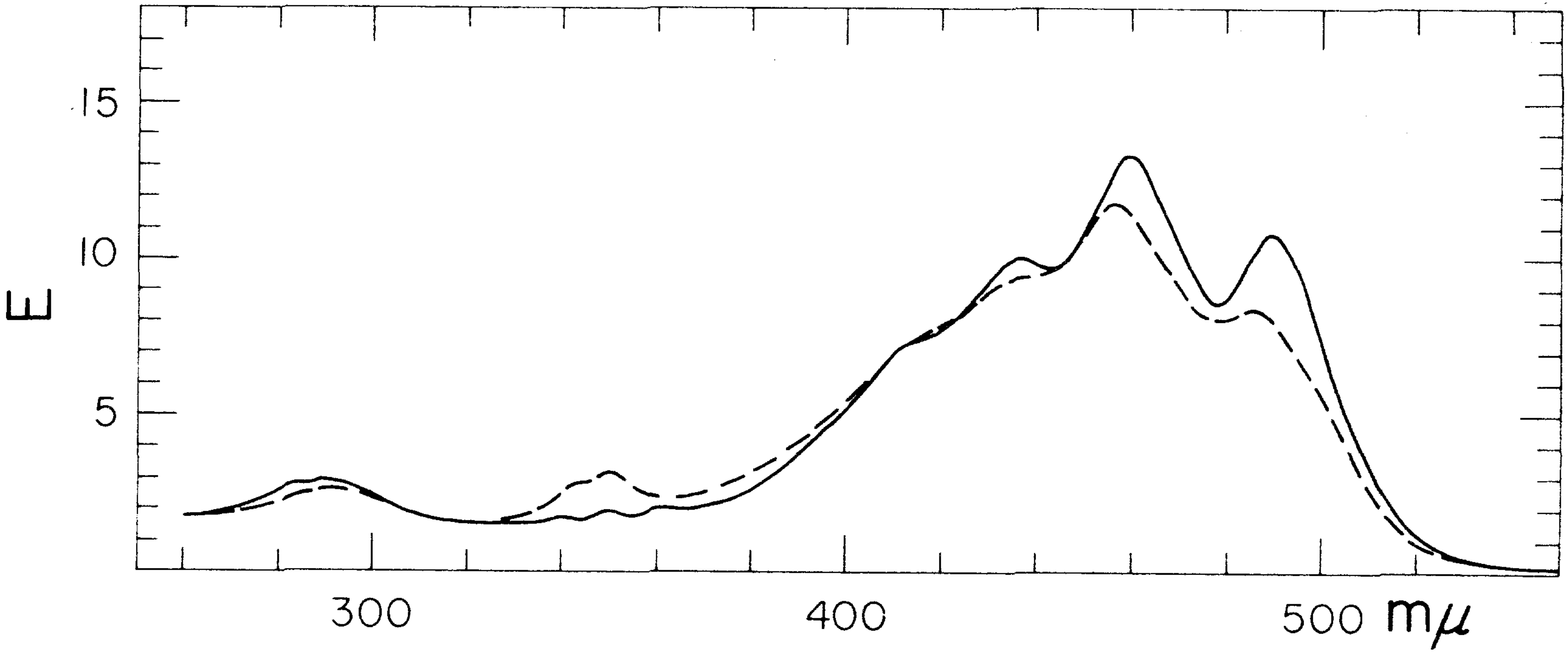


Fig. 32. **Extinction Curve in Hexane of 4-Hydroxy-γ-carotene: ——, fresh solution** of the all-<u>trans</u> compound; and - - -, iodine-catalyzed stereoisomeric equilibrium mixture.

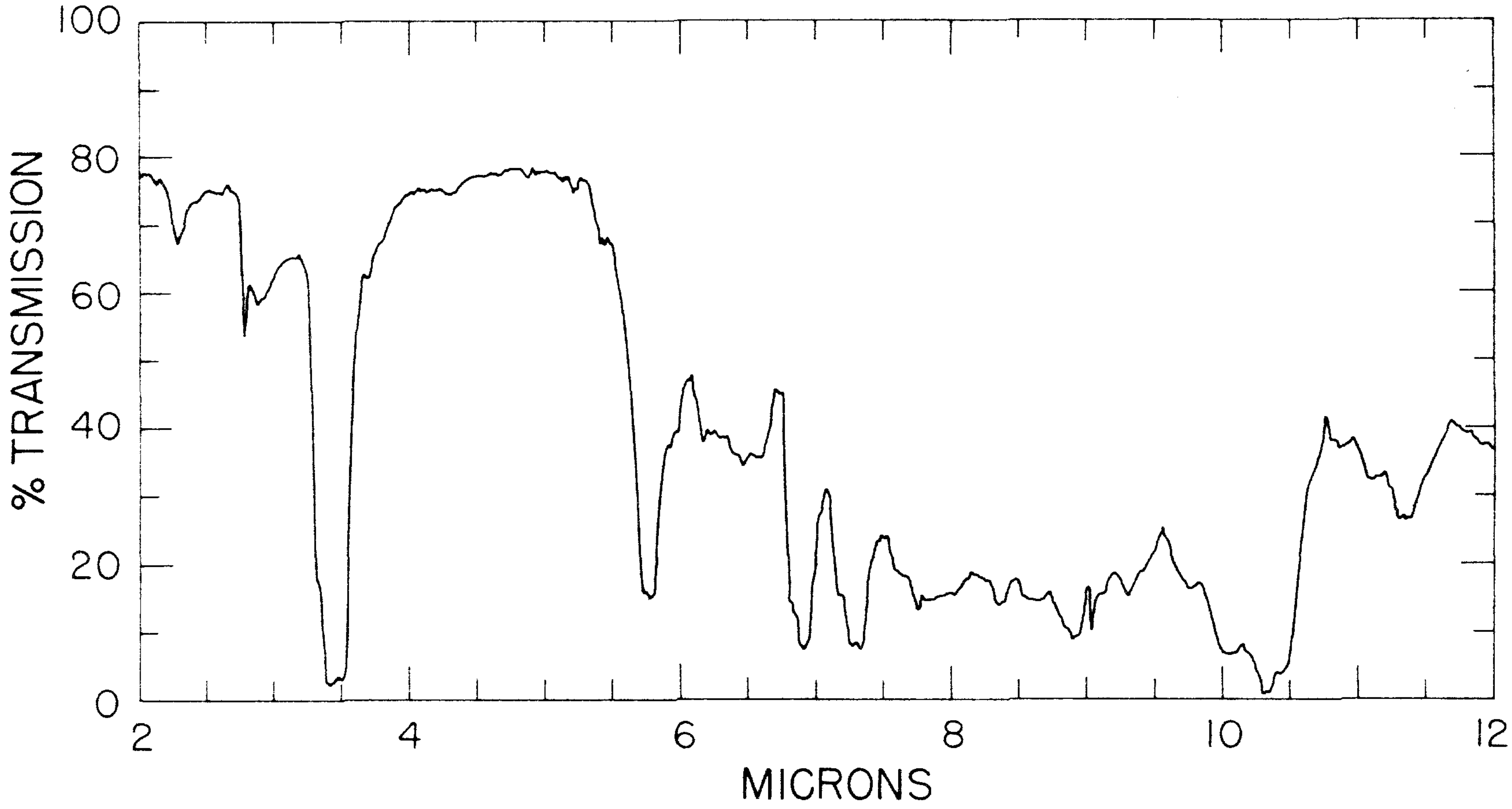


Fig. 33. Infrared Spectrum of 4-Hydroxy-γ-carotene in Carbon Tetrachloride.

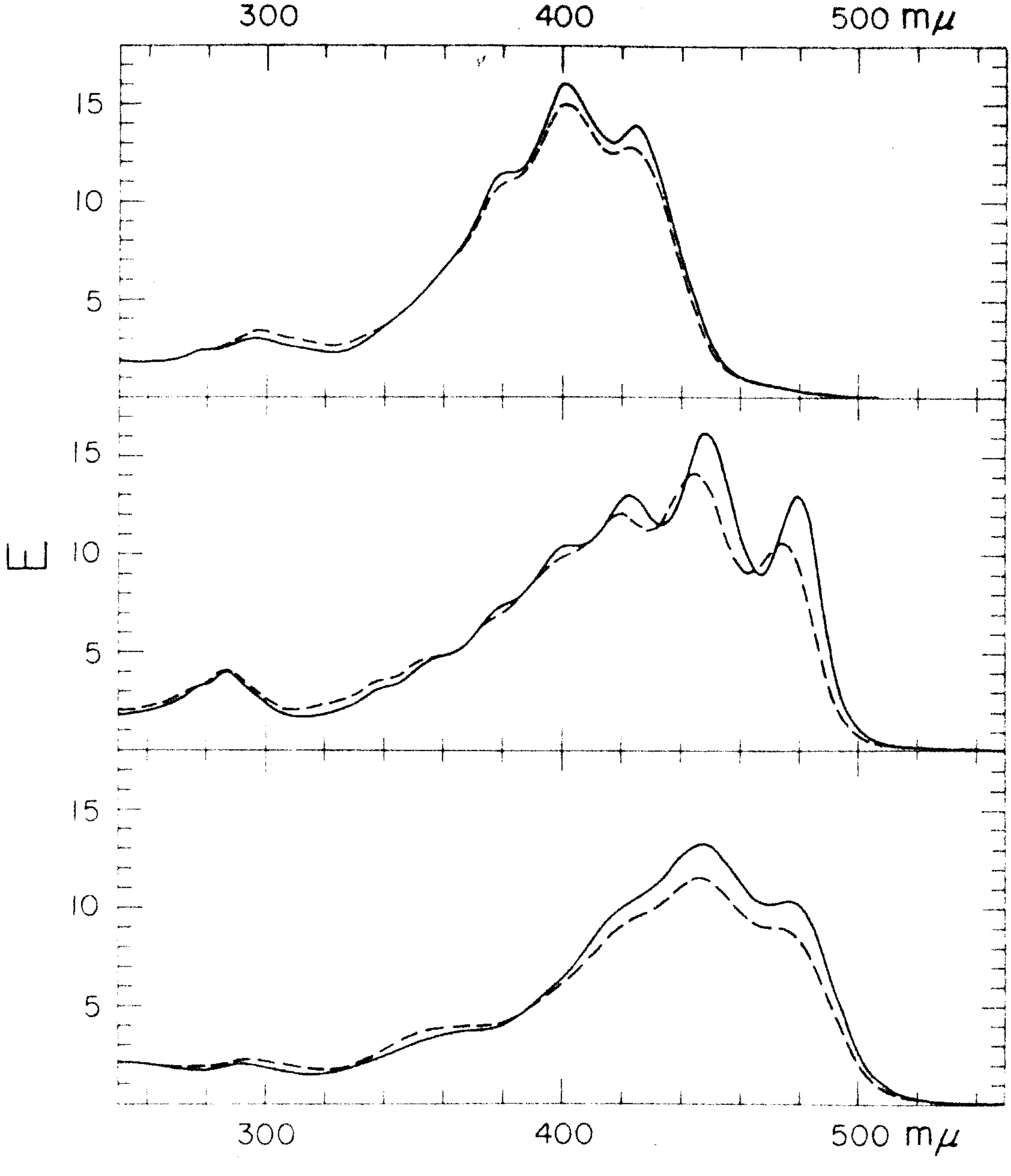


Fig. 34. Extinction Curves in Hexane of 5,6,7,8,5',6',7',8'(?)-Octahydro-γ-carotene (top), 5,6,5',6'-Tetrahydro-γ-carotene (middle), and 5',6'-Dihydro-γ-carotene (bottom): ———, fresh solution of the respective all-trans compounds; and - - -, iodine-catalyzed stereoisomeric equilibrium mixtures.

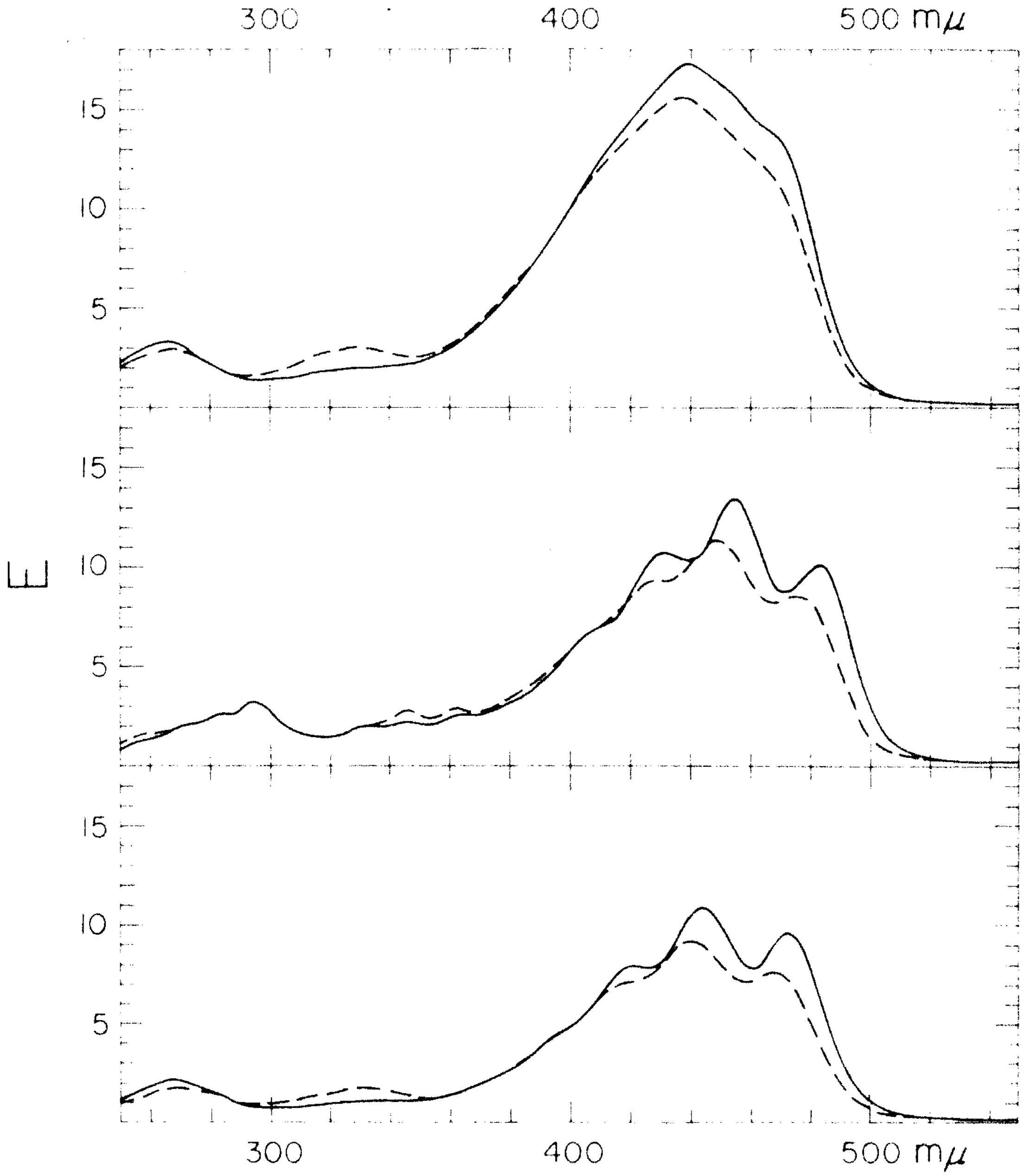


Fig. 35. Extinction Curves in Hexane of 2-Hydroxy-3,4-dehydro-5',6',7',8'-tetrahydro-γ-carotene (top), 5,6-Dihydro-γ-carotene (middle), and 5',6'-Diethoxy(?)-5',6'-dihydro-γ-carotene (bottom): ———, fresh solution of the respective all-*trans* compounds; and - - -, iodine-catalyzed stereoisomeric equilibrium mixtures.

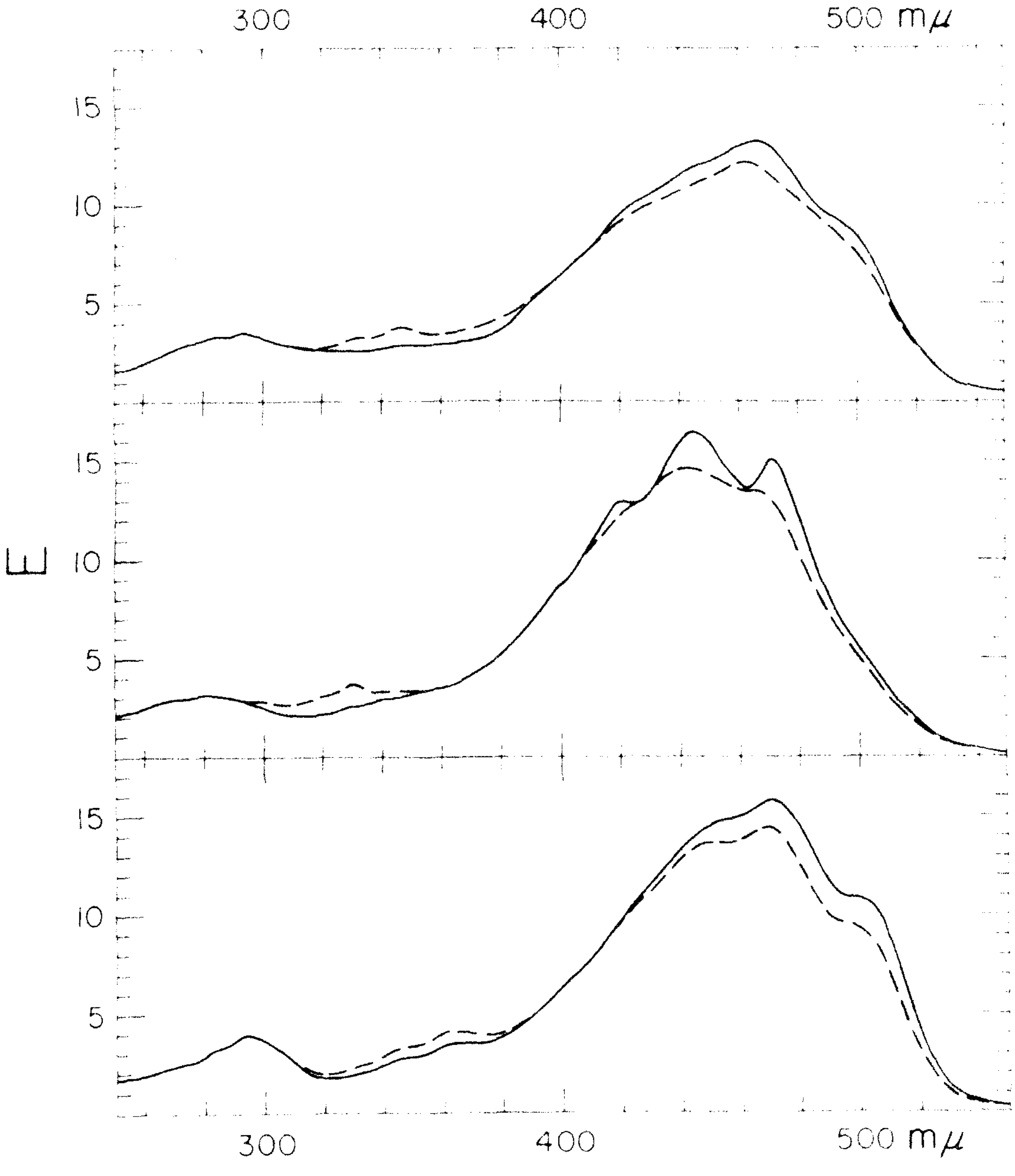


Fig. 36. Extinction Curves in Hexane of 4-Keto-γ-carotene (top), 5'-Hydroxy(?)-5',6'-dihydro-γ-carotene (middle), and 4'-Hydroxy-3,4-dehydro-γ-carotene (bottom): ———, fresh solution of the respective all-trans compounds; and - - -, iodine-catalyzed equilibrium mixtures.

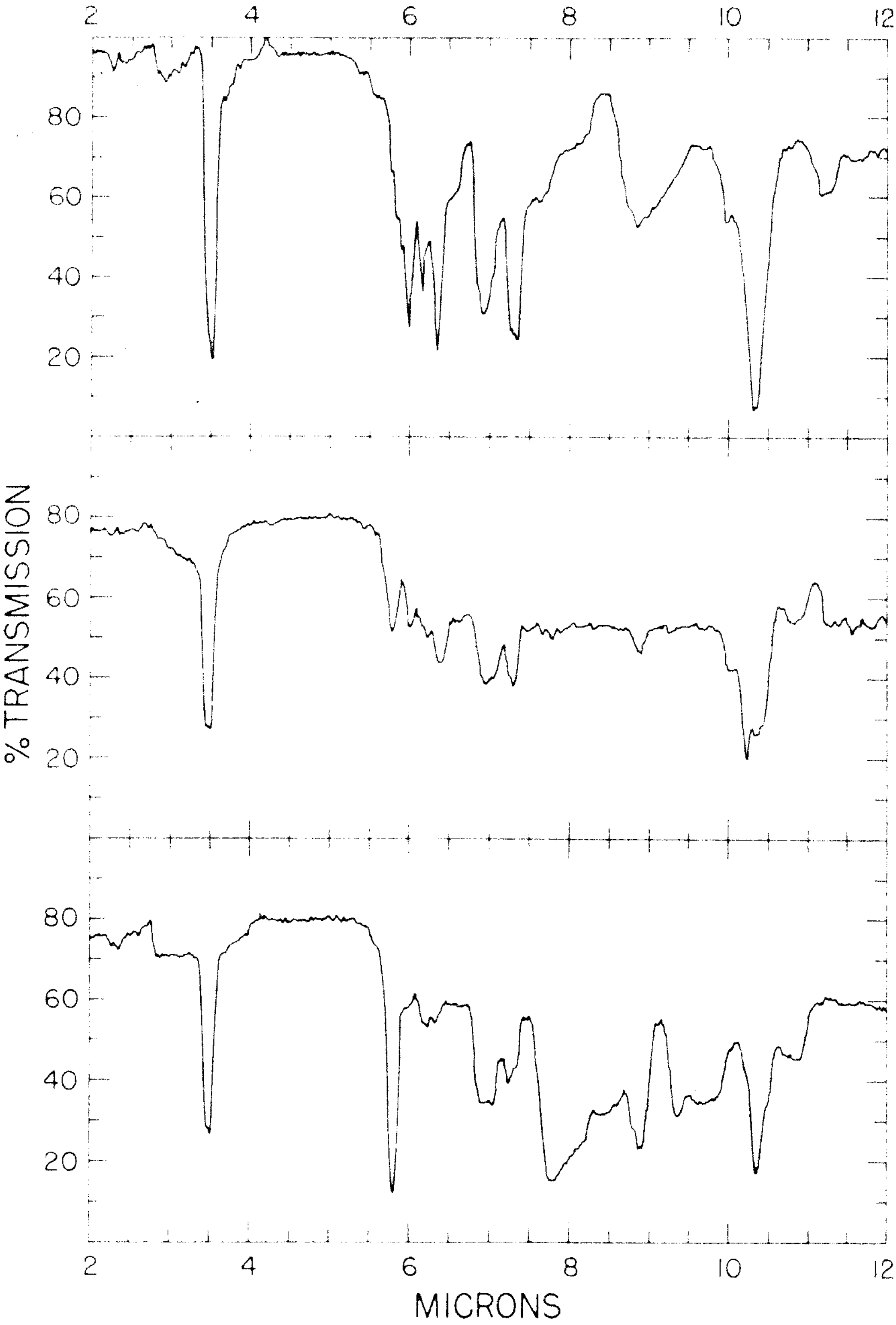


Fig. 37. Infrared Spectra in Chloroform of 5,6,7,8,-5',6',7',8'(?)-Octahydro-γ-carotene (top), 5,6,5',6'-Tetrahydro-γ-carotene (middle), and 5',6'-Dihydro-γ-carotene (bottom).

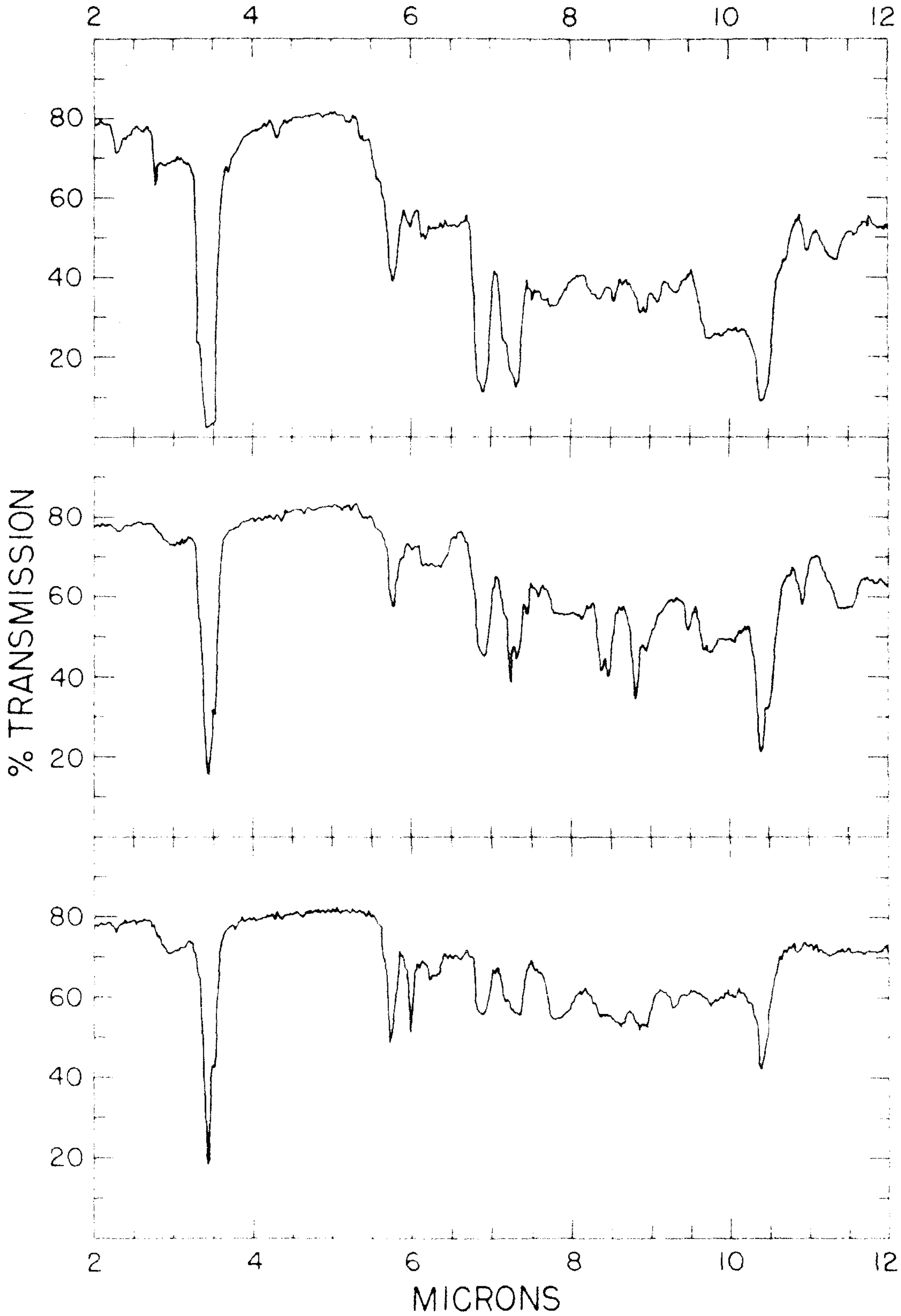


Fig. 38. Infrared Spectra in Carbon Tetrachloride of 2-Hydroxy-3,4-dehydro-5',6',7',8'-tetrahydro-γ-carotene (top), 5',6'-Diethoxy(?)-5',6'-dihydro-γ-carotene (middle), and 4-Keto-γ-carotene (bottom).

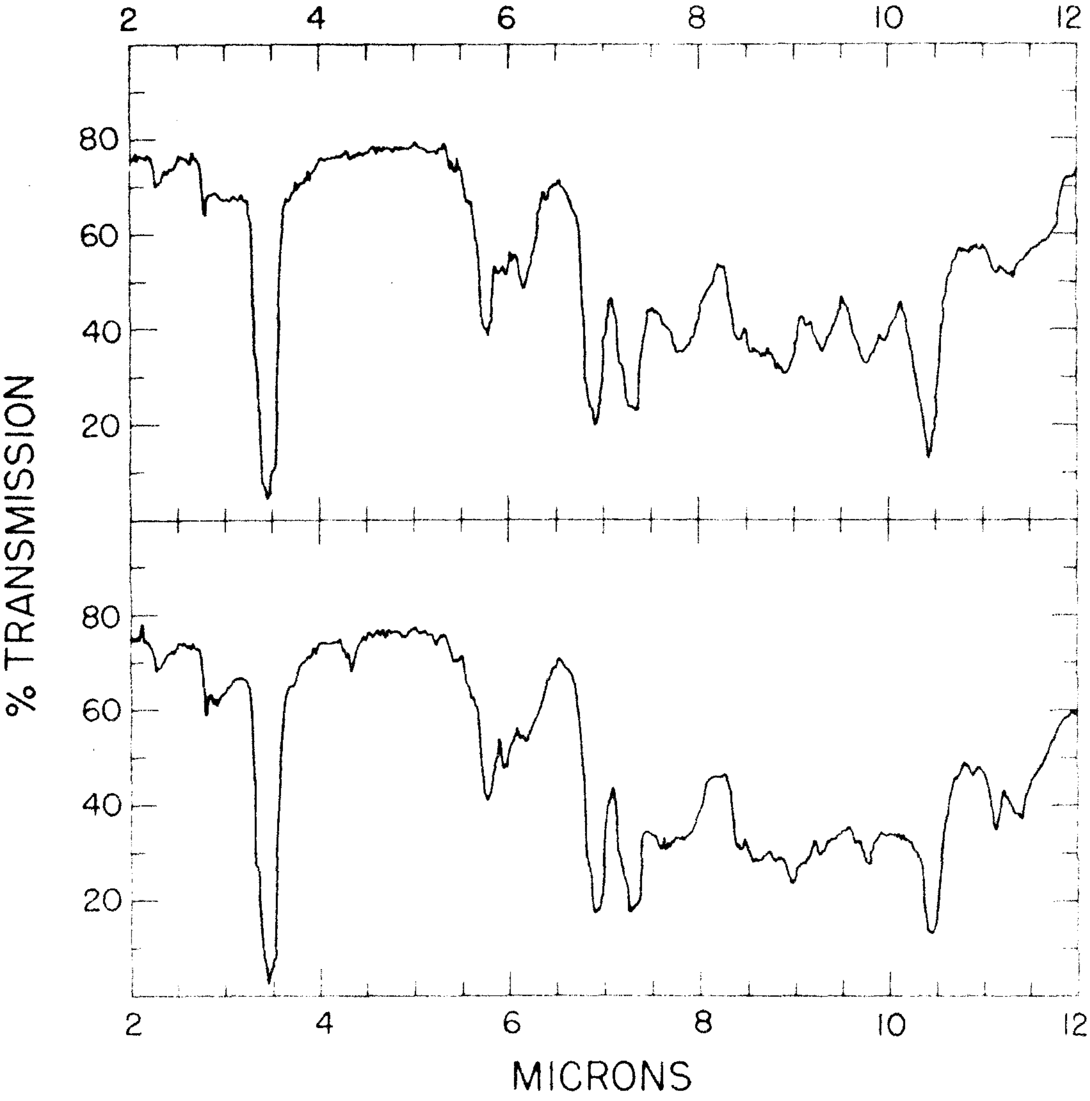


Fig. 39. Infrared Spectra in Carbon Tetrachloride of 5'-Hydroxy(?)-5',6'-dihydro-γ-carotene (top) and 4'-Hydroxy-3,4-dehydro-γ-carotene (bottom).

B. EXPERIMENTAL PART

1. Materials and Methods.

γ-Carotene. - The γ-carotene sample was prepared from pro-γ-carotene obtained from *Pyracantha angustifolia* berries according to Zechmeister and Pinckard (38). The poly*cis* compound was converted to all-*trans*-γ-carotene by iodine catalysis and subsequent chromatography. About 25 mg. of pro-γ-carotene was dissolved in 100 ml. of hexane in a Pyrex volumetric flask. After addition of 0.25 mg. of iodine, the solution was illuminated for two hours under the fluorescent lamp described on p. 88.

Following illumination, three such portions were combined and developed with 1 l. of 3 per cent acetone in hexane on a 30 x 8 cm. lime-Celite column. The pigment of the main salmon-pink zone, all-*trans*-γ-carotene, was crystallized from benzene - methanol, and the minor zones (mostly *cis* isomers) were collected and catalyzed again with iodine. Total yield, 250 mg. of all-*trans*-γ-carotene, m. p. 131.5 - 133.5° (39).

All other materials and procedures have been described in the α-carotene Chapter of this Thesis.

2. Formation and Hydrolysis of the γ-Carotene - Boron Trifluoride Complex.

A total of 250 mg. of all-*trans*-γ-carotene, in ten portions, was complexed and hydrolyzed at room temperature as follows:

a.) 25 mg. of γ-carotene was dissolved in 25 ml. of alcohol-free chloroform.

b.) 1 ml. of boron trifluoride etherate was added with vigorous agitation, and the complexing was allowed to proceed for exactly 120 seconds. The solution was then a dark blue-green, not yet a pure blue.

c.) The solution was poured into a mixture of 10 ml. of saturated sodium bicarbonate solution, 10 ml. of distilled water, and 75 ml. of C. P. acetone. The red carotenoid color was restored within 5 to 10 seconds.

d.) The hydrolysis product was then diluted to 200 ml. with hexane and allowed to stand until all ten portions had been similarly treated.

e.) The pigment of the ten units combined was transferred to hexane by careful addition of water; the epiphase was washed acetone-free, dried, and evaporated completely. The photometrically estimated pigment recovery, calculated as γ-carotene, was 99 mg., or about 40 per cent of the starting material.

f.) The residue was dissolved in 100 ml. of hexane (with the initial aid of a few drops of chloroform) and developed on four 45 x 4.5 cm. columns with 500 ml. of 4 per cent acetone in hexane to each (about 75 min.):

5	tan	(Top Zones)
10	bright orange	
7	purple	
10	dull orange	
7	pale purple	
22	pale orange	
31	pale orange	
24	pale pink	(Zone E)
12	colorless interzone	
33	pale yellow-orange	(Zone D)
38	nearly colorless interzone	
77	several poorly separated pale yellow and orange zones (cis isomers of Zones A - E)	
20	colorless interzone	
19	orange	(Zone C)
10	colorless interzone	
15	yellow	(Zone B)
30	nearly colorless, with pale yellow fluorescence in ultraviolet light	
12	yellow-green	(Zone A)
27	pale yellow-green, with yellow-grey fluorescence	

Remainder of column, and filtrate (10 ml.): colorless, with no fluorescence

The four portions each of Zones A - E were combined, eluted with acetone, transferred to hexane, and washed acetone-free.

The combined "Top Zones" were eluted with ethanol, transferred to hexane, and washed ethanol-free; they contained 47.5 mg. of pigment (19 per cent of the starting material), calcd. as γ-carotene.

g.) The combined "Top Zones" were developed for two and a half hours on two 45 x 4.5 cm. columns with 1 l. of acetone - hexane 1:9 for each:

14	tan	
14	pink	(Zone M)
17	orange	(Zone L)
20	orange	(Zone K)

15	pink	(Zone J)
16	yellow	(Zone I)
35	nearly colorless interzone	
20	orange	(Zone H)
54	nearly colorless interzone	
20	yellow	(Zone G)
23	nearly colorless interzone	
24	yellow	(Zone F)

Remainder of column: five evenly spaced, narrow, pale yellow zones
Filtrate: pale yellow

The two portions of each lettered zone were combined, eluted with acetone - ethanol 1:1, transferred to hexane, washed acetone-free, and dried.

3. Characterization of the Fractions.

Each of the lettered zones was rechromatographed on a single 20 x 3.5 cm. lime-Celite column with the developers recorded under the individual compounds. Each chromatogram showed a single main zone separated from any minor zones present by colorless interzones at least 5 mm. wide.

Ultraviolet and infrared spectra of the individual compounds will be found on pp. 148 to 155; the curves were recorded using chromatographically pure and homogeneous material in all cases when crystals were not available; Zones A - D, F, I, K, and M have not crystallized. The yields reported below are photometric estimates, unless otherwise stated. Some properties of the fractions are listed below, in the order of increasing adsorption affinities.

Zone A (*5,6,7,8,5',6',7',8'(?)-Octahydro-γ-carotene*):

Yield: 1.7 mg. = 0.7 per cent of the original γ-carotene.

Chromatographic Behavior: Develops with hexane; is adsorbed below α-carotene.

Partition Behavior: 100:0 in hexane - 95 per cent methanol.

Reaction with Acid Chloroform: Negative.

Zone B (*5,6,5',6'-Tetrahydro-γ-carotene*):

Yield: 3.1 mg. = 1.3 per cent.

Chromatographic Behavior: Develops with hexane; is adsorbed slightly above β-carotene.

Partition Behavior: 100:0 in hexane - 95 per cent methanol.

Reaction with Acid Chloroform: Negative.

Zone C (*5',6'-Dihydro-γ-carotene*):

Yield: 2.3 mg. = 0.9 per cent.

Chromatographic Behavior: Develops with hexane; is adsorbed above β- but below γ-carotene.

Partition Behavior: 100:0 in hexane - 95 per cent methanol.

Reaction with Acid Chloroform: Negative.

Zone D (*2-Hydroxy-3,4-dehydro-5',6',7',8'-tetrahydro-γ-carotene*):

Yield: 4 mg. = 1.6 per cent.

Chromatographic Behavior: Develops with 2 per cent acetone in hexane; is adsorbed slightly below γ-carotene.

Partition Behavior: 90:10 in hexane - 95 per cent methanol
98:2 in hexane - 90 per cent methanol.

Reaction with Acid Chloroform: Positive.

Zone E (Unchanged γ-Carotene):

Yield (Recovery): 1.7 mg. = 0.7 per cent.

Chromatographic Behavior: Develops slowly with 2 per cent acetone in hexane; does not separate from an untreated sample of the starting material in a mixed chromatogram test.

Crystal Form: Salmon-colored needles, often occurring in clusters (from benzene - methanol).

Melting Point: 133-134°.

Partition Behavior: 100:0 in hexane - 95 per cent methanol.

Reaction with Acid Chloroform: Negative.

Zone F (5,6-Dihydro-γ-carotene):

Yield: 0.5 mg. = 0.2 per cent.

Chromatographic Behavior: Develops easily with 5 per cent acetone in hexane; is adsorbed slightly above γ-carotene.

Partition Behavior: 100:0 in hexane - 95 per cent methanol.

Reaction with Acid Chloroform: Negative.

Zone G (5',6'-Diethoxy(?)-5',6'-dihydro-γ-carotene):

Yield: 1.3 mg. = 0.5 per cent.

Chromatographic Behavior: Develops with 5 per cent acetone in hexane; is adsorbed above 4-ethoxy-α-carotene and γ-carotene, but below 4-keto-γ-carotene.

Partition Behavior: 96:4 in hexane - 95 per cent methanol.

Reaction with Acid Chloroform: Negative.

Zone H (4-Keto-γ-carotene):

Yield: 1.2 mg. = 0.5 per cent.

Chromatographic Behavior: Develops slowly with 5 per cent acetone in hexane; is adsorbed above γ-carotene and 4-keto-β-carotene but below 4-hydroxy-γ-carotene.

Crystal Form: Dark red rhomboidal platelets (from benzene - methanol).

Melting Point: 140-142°.

Partition Behavior: 89:11 in hexane - 95 per cent methanol
98:2 in hexane - 90 per cent methanol.

Reaction with Acid Chloroform: Negative.

Zone I (*cis* Forms of 5'-Hydroxy(?)-5',6'-dihydro-γ-carotene):

This zone contained a total of 2 mg. (0.8 per cent yield) of pigments which were shown, by the usual methods (*cf*. p. 87), to be (uncrystallizable) *cis* isomers of 5'-hydroxy(?)-5',6'-dihydro-γ-carotene.

Zone J (All-*trans*-5'-hydroxy(?)-5',6'-dihydro-γ-carotene):

Yield: 2.3 mg. = 0.9 per cent.

Chromatographic Behavior: Develops slowly with acetone - hexane 1:9; is adsorbed above isocryptoxanthin (4-hydroxy-β-carotene) but below 4-hydroxy-γ-carotene.

Crystal Form: Long, straw-yellow needles, clustered into "furry balls" (from benzene - 95 per cent methanol).

Melting Point: 136-138°.

Partition Behavior: 87:13 in hexane - 95 per cent methanol
97:3 in hexane - 90 per cent methanol.

Reaction with Acid Chloroform: Negative.

Zone K (*cis* Forms of 4-Hydroxy-γ-carotene):

This Zone contained a total of 3.7 mg. (1.5 per cent yield) of pigments which were shown to be *cis* 4-hydroxy-γ-carotenes.

Zone L (All-trans-4-hydroxy-γ-carotene):

Yield: 5.9 mg. = 2.4 per cent.

Chromatographic Behavior: Develops very slowly with acetone - hexane 1:9; is adsorbed above isocryptoxanthin (4-hydroxy-β-carotene), but below lycopene.

Crystal Form: Nearly rectangular, deep orange plates, many with jagged or broken ends (from benzene - methanol). The crystals weighed 0.5 mg. (0.2 per cent yield).

Melting Point: 144-145°.

Partition Behavior: 82:18 in hexane - 95 per cent methanol
92:8 in hexane - 90 per cent methanol.

Reaction with Acid Chloroform: Positive; the color change is immediate and pronounced. The spectrum of the dehydrated product showed a broad maximum at 467 mμ but no fine structure in the visible region.

Acetylation: To a solution of 2 mg. of 4-hydroxy-γ-carotene in 1 drop of anhydrous pyridine 1 drop of acetyl chloride (C. P.) was added. After standing for an hour, first 10 ml. of methanol and then 10 ml. of hexane were added. The pigment was transferred to hexane and developed with the same solvent on a 18 x 1.8 cm. column. The upper zone contained unesterified 4-hydroxy-γ-carotene (0.1 mg.), and the lower one contained 1.4 mg. of 4-acetoxy-γ-carotene. Its spectrum is very similar to that of γ-carotene and 4-hydroxy-γ-carotene. The partition ratio of the 4-acetoxy-compound is 95:5 in hexane - 95 per cent methanol. The acetate can be hydrolyzed to 4-hydroxy-γ-carotene by shaking a dilute hexane solution with 1 vol. of 20 per cent methanolic KOH.

Etherification: 2 mg. of 4-hydroxy-γ-carotene was dissolved in 5 ml. of absolute ethanol, and 3 drops of the acid chloroform reagent was introduced. After standing for 12 hours, the pigment was transferred to 10 ml. of hexane and developed on a 18 x 1.8 cm. column with 2 per cent acetone in hexane. The top orange zone contained unreacted 4-hydroxy-γ-carotene, 0.2 mg., and the lower, main orange zone contained 0.9 mg. of 4-ethoxy-γ-carotene. The spectrum of the latter is very similar to that of γ-carotene. The partition ratio of the ether is 99:1 in hexane - 95 per cent methanol. The 4-ethoxy-compound was hydrolyzed to 4-hydroxy-γ-carotene by the method just described for preparation of the ether, but using water - acetone 1:4 in place of absolute ethanol.

Oxidation: 1 mg. of 4-hydroxy-γ-carotene was dissolved in 5 ml. of R. G. Chloroform and air was bubbled through for 1 hour. The pigment was then transferred to hexane and developed with 5 per cent acetone in hexane on a 18 x 1.8 cm. column; the upper zone (orange) contained 4-hydroxy-γ-carotene, and the lower one (pink) contained 0.1 mg. of 4-keto-γ-carotene (10 per cent conversion).

Zone M (*4'-Hydroxy-3,4-dehydro-γ-carotene*):

Yield: 2.3 mg. = 0.9 per cent.

Chromatographic Behavior: Develops very slowly with acetone - hexane 1:9; is adsorbed above 4-hydroxy-γ-carotene.

Partition Behavior: 83:17 in hexane - 95 per cent methanol

95:5 in hexane - 90 per cent methanol.

Reaction with Acid Chloroform: Positive.

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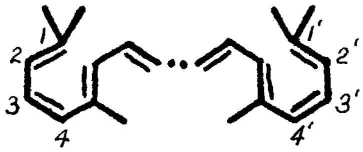
PROPOSITIONS

1. The tomato pigment lycopene is proposed as an indicator for the detection and estimation of atmospheric oxidants (smog). Lycopene would have the same or greater sensitivity to oxidants and the same ease of photometric estimation as, for example, the phenolphthalein used by Professor Haagen-Smit (1). The polyene pigment would have the additional advantage that formation of acetone could be used to estimate the atmospheric ozone concentration as well as the total oxidants, without the need for a separate ozone test material, such as rubber (2).

2. According to Karrer, the dehydrogenation of lycopene by means of N-bromosuccinimide in carbon tetrachloride solution yields "dehydrolycopene," which crystallizes directly from the reaction mixture (3). A chromatographic analysis of the mother liquor of such crystals, recently conducted in this laboratory by the present author, showed that not only a single but at least three "dehydrolycopenes" are formed, one of which was adsorbed below lycopene on zinc carbonate - Celite or on lime - Celite.

Furthermore, oxidation of lycopene by NBS in R. G. chloroform (stabilized with ethanol), a procedure known to give ketones in the β- and α-carotene series (4,5), yielded only hydrocarbons in this instance, viz., the same dehydrolycopenes (but in different ratios) as were formed by Karrer's oxidation in alcohol-free CCl_4.

a.) The following structures and names are proposed for the three dehydrogenated lycopenes observed, listed below (with lycopene, included for reference) in the order of decreasing adsorption affinity:



Bisdehydrolycopene

(identical with the product termed by Karrer "dehydrolycopene").

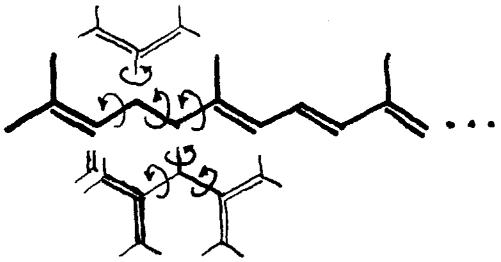
Monodehydrolycopene.

(3,4-Dehydrolycopene.)

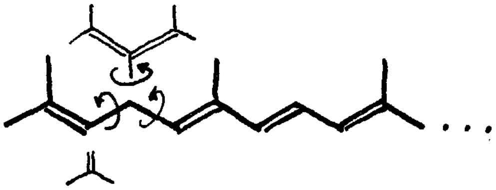
Lycopene.

"retro"-Dehydrolycopene.

b.) The reason that *retro*-dehydrolycopene is less strongly adsorbed than lycopene, despite having one more double bond in the chromophore (twelve), is proposed as follows: Because of free rotation about the three single bonds which separate each of the two isolated double bonds from the main chromophore, they can be placed in the lycopene molecule in a greater potential volume than in *retro*-dehydrolycopene, in which the separation from the main chromophore is by only two single bonds. The lycopene molecule can, therefore, more readily adapt its shape to the variety of shapes of adsorbing surfaces encountered by the molecules in the course of the migration down the column than can the molecule of the *retro*-compound. The sketch below illustrates the greater "space-filling" capability of the isolated double bonds in lycopene than in *retro*-dehydrolycopene.

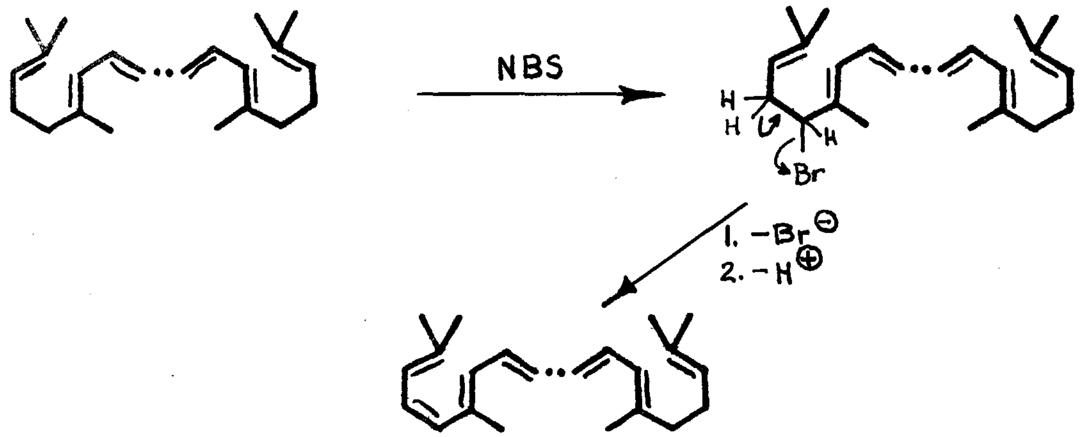


Lycopene.

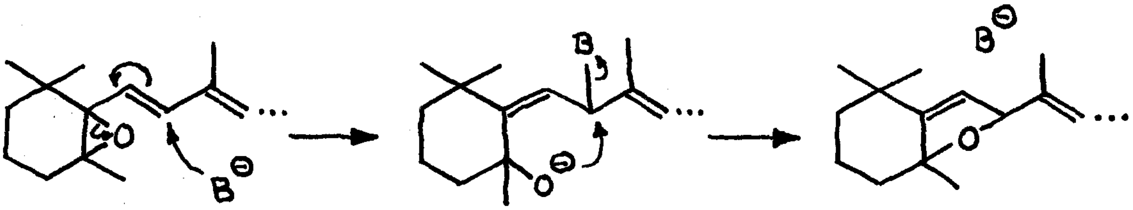


***retro*-Dehydrolycopene.**

c.) It is proposed that the non-formation of any keto derivative from lycopene, under conditions known to produce ketones from α- and β-carotenes, is due to the fact that the hydrogen atoms attached to the 3-carbon are allylic to the isolated double bond (this not being the case for the 3-position of, e.g., β-carotene), and hence can take part in a spontaneous dehydrobromination as illustrated below for the formation of monodehydrolycopene:



3. The acid-catalyzed conversion of carotenoid epoxides to furanoid oxides has been described (6,7); the reaction is highly sensitive to minute traces of acid. However, it has been observed by Dr. K. Tsukida in our laboratory that adsorption of β-carotene epoxides on highly activated, strongly basic alumina is an effective method of converting such epoxides to furanoid oxides. The following mechanism is proposed for the base-catalyzed conversion ($B^{\ominus}$ = a strongly basic anion):



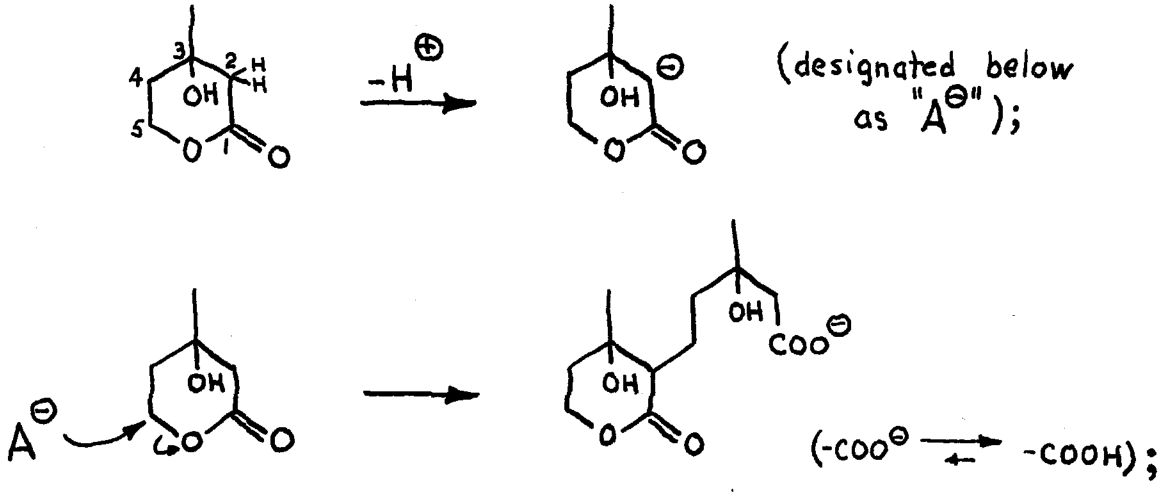
4. Changing the hardness and other properties of polyethylene by the use of ionizing radiations to cross-link polymer chains (8) has led to the discovery of many new applications of the modified polymers and to an expanded market for polyethylene. It is proposed that a new class of polyethylenes could be created by the effect of ultrasonic waves in the megacycle range (piezoelectric transducers). Ultrasonic vibrations are known to accelerate the polymerization of styrene (9), and acrylonitrile has been shown to polymerize under the influence of free radicals formed by such vibrations (10).

5. A survey of the literature revealed only a single mention of telomerization of C_2F_4 with primary or secondary alcohols (11). It is proposed that a high molecular weight, fluorine-rich telomer of C_2F_4, with methanol as telogen, would have nearly the same properties as those of poly(tetrafluoroethylene), although perhaps chemical resistance will slightly decrease. The advantage of the proposed telomer would be that it could be dyed on the surface by means of ordinary dyestuffs. Such materials would find use wherever

colored "Teflon" would be desirable, e.g., in color-coded insulation of wires. By varying the oxygen content, a more resistant or a more easily colorable telomer could be prepared, depending on the desired use of the material.

6. An acetate - replacing factor for lactobacilli has been discovered in Merck's laboratories and proved to be β,δ-dihydroxy-β-methylvaleric acid (12). Under the influence of the enzymes in yeast or rat liver extracts (13,14), this acid is known to condense, by formation of a bond between the 2-carbon of one molecule and the 5-carbon of another, to *yield squalene, among other products.*

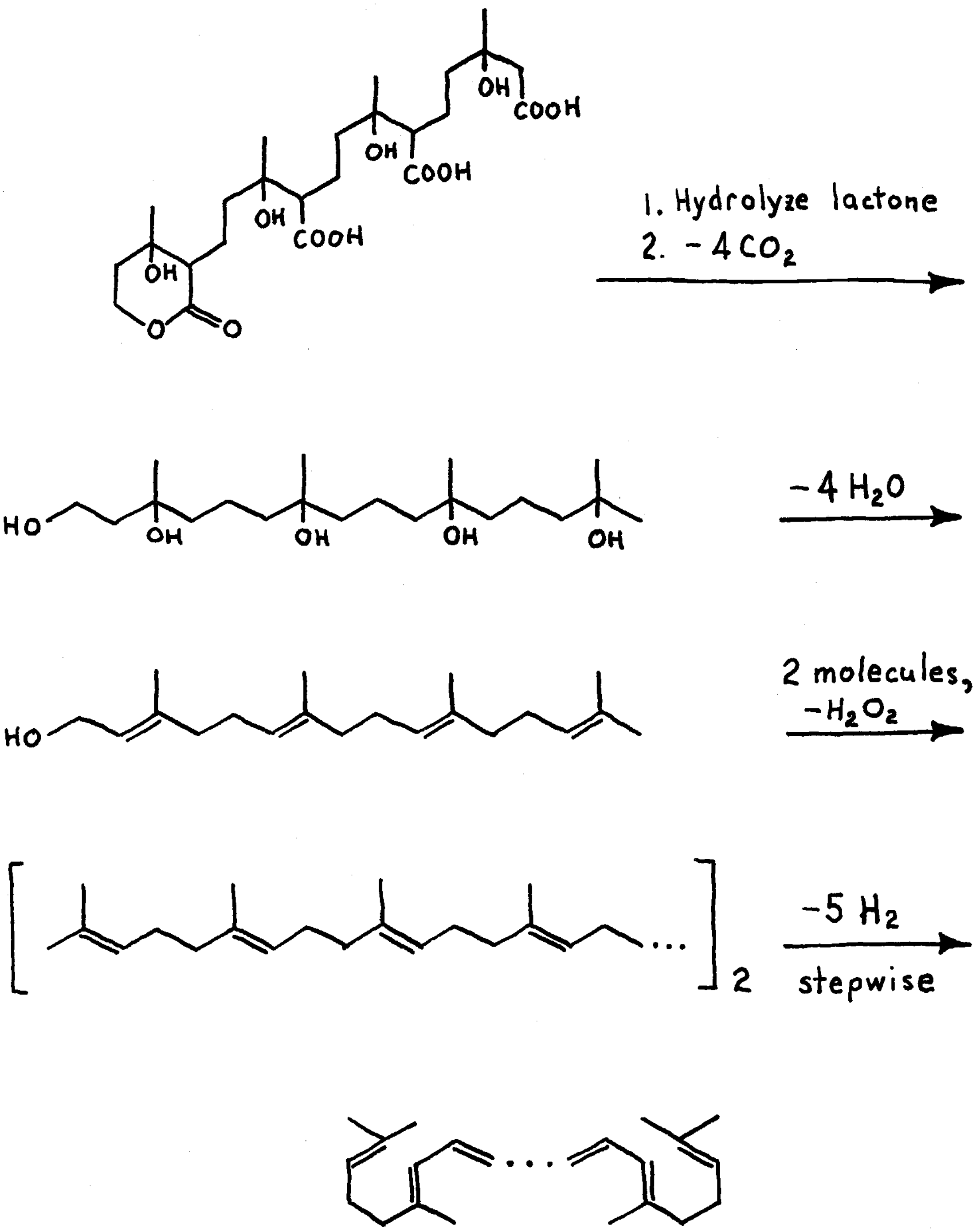
It is proposed that β,δ-dihydroxy-β-methylvaleric acid (in the form of its δ-lactone) is a precursor of lycopene and other aliphatic carotenoids in plants, according to the following enzyme-catalyzed sequence of conversions:



(designated below as "$A^{\ominus}$");

($-COO^{\ominus} \rightleftharpoons -COOH$);

Repeat addition of $A^{\ominus}$ twice more →


OH
COOH
OH
COOH
OH
COOH
OH
O
O
1. Hydrolyze lactone
2. - 4 CO₂
HO
OH
OH
OH
OH
- 4 H₂O
HO
2 molecules,
-H₂O₂
2
-5 H₂
stepwise

7. A continuous chromatographic process is proposed which could be operated using commercially available (e.g., from Dorr-Oliver, Inc., Stamford, Conn.) equipment with a single modification, i.e., the attachment of dual (or multiple) doctor knives to a rotary drum filter. The latter would be programmed as shown in Fig. A, p. 177; an outline flow diagram of the entire process appears in Fig. B (p. 178).

8. It is proposed that ultrasonic waves of the amplitude and frequency range commonly produced by magnetostrictive transducers could be used to cure "dimension lumber" (12 in. x 12 in. and larger cross section) faster than by conventional kiln-drying methods, and without the danger of loss by "case-hardening" and subsequent splitting of the lumber (15).

9. As is well known, competent experts are developing an afterburner or a similar device to promote complete combustion of automobile and truck exhaust gases. In public health interest it is proposed that, following such development, a federal law should be enacted prohibiting the operation of any automobile or truck not equipped with such a device. Penalty for violation should not be less severe than that now imposed for manslaughter.

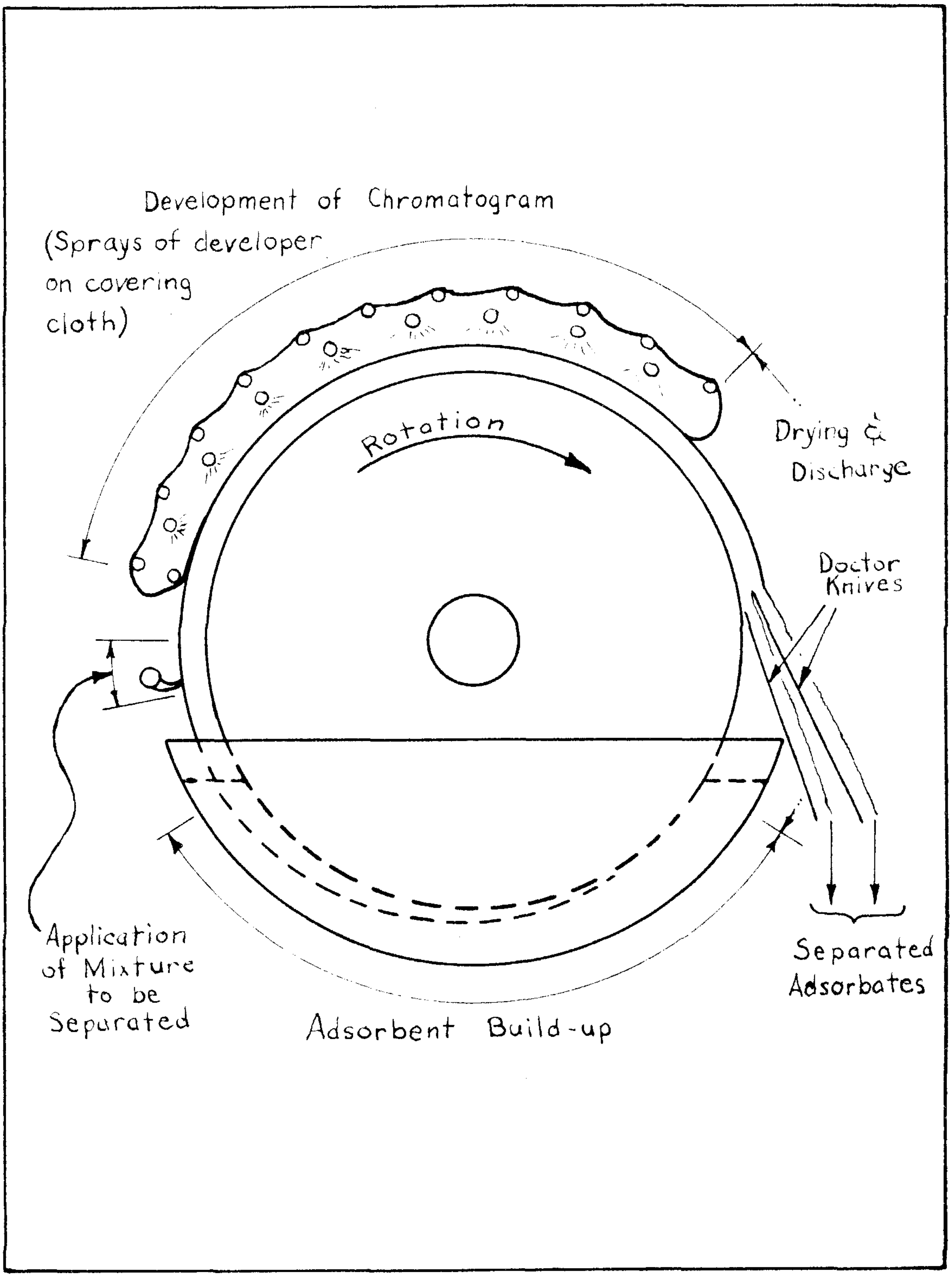


Fig. A. Programming of Drum Filter for Proposed Continuous Chromatographic Resolution Process.

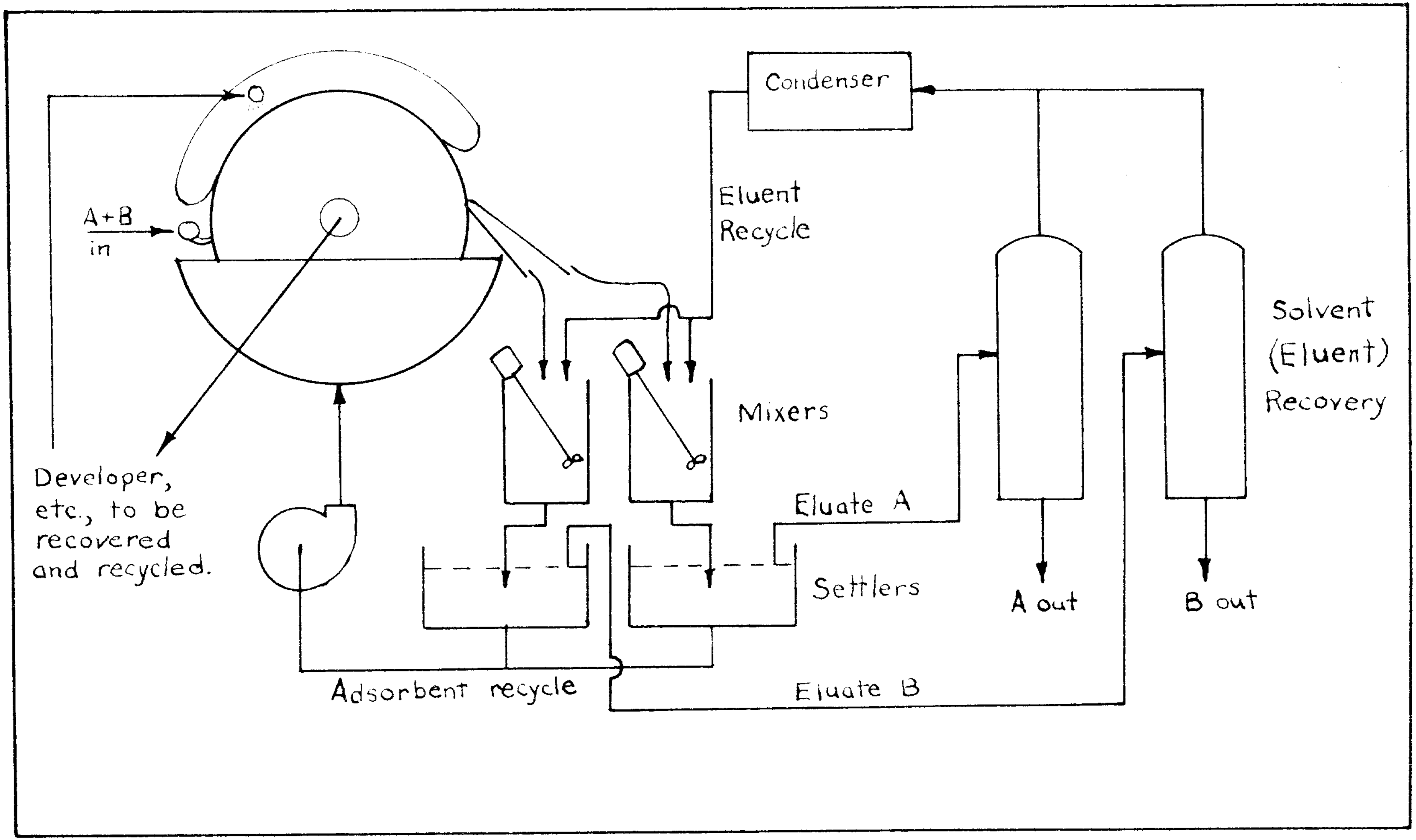


Fig. B. Outline Flow Diagram for Proposed Continuous Chromatographic Resolution Process.

10. It is proposed that the graduate teaching assistant "manpower pool" would be more efficiently used and would result in a higher quality of instruction if the following schedule was adopted:

a.) During the first year, each graduate student would meet all the requirements imposed as a consequence of the placement examinations. The graduate students would be permitted, in addition, to register for research or courses to satisfy some of the minor requirements. (The total registration would be limited to 45 units per term.)

b.) During their second and subsequent years of residence, the graduate teaching assistants would be divided into three groups: each group to teach full time for a single term, with the remaining time to be devoted to research, minor subject, and if necessary, completion of the placement requirements.

c.) If, unexpectedly, the teaching manpower supply should exceed demand, it is proposed that selected graduate students be permitted to receive outside teaching appointments (limited to one term) at local high schools, junior colleges, etc.

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