

CONVERSIONS OF β -CAROTENE AND SOME RELATED POLYENES
TO KETO AND HYDROXY DERIVATIVES BY MEANS OF
N-BROMOSUCCINIMIDE AND BORON TRIFLUORIDE.

Thesis by
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ABSTRACT

The reaction of N-bromosuccinimide (NBS) with β -carotene in alcohol-containing chloroform results mainly in the formation of 4-keto- β -carotene, 4,4'-diketo- β -carotene, and 4-keto-3',4'-dehydro- β -carotene. Their structures were clarified by relating them to known carotenoids through partial synthetic or degradative schemes. In the course of this work, the following new carotenoids were also prepared: 4-keto-4'-hydroxy- β -carotene, 4,4'-dihydroxy- β -carotene, 4-hydroxy-3',4'-dehydro- β -carotene and 4-keto- α -carotene.

A reaction mechanism for the conversion of carotenes to ketones is proposed, based on pertinent experiments. The infrared and visible spectra of the new compounds are discussed, and the provitamin A₂ effect of the 3,4-dehydro compounds has been demonstrated. Synthetic 4,4'-diketo- β -carotene has been identified with canthaxanthin occurring in some fungi and algae.

The investigation of the products from the hydrolysis and ethanoly-sis of BF₃-carotenoid complexes has been continued and extended: β -carotene, retro-dehydrocarotene and 3,4-dehydro- β -carotene have yielded 4-hydroxy- β -carotene. From 3,4-dehydro- α -carotene 4-hydroxy- α -carotene was obtained. retro-Disdehydrocarotene gave a new type of carotenoid, viz. 2-hydroxy-3,4-dehydro- β -carotene.

From anhydrovitamin A₁ a new isomer of vitamin A₁, viz. 4-hydroxy-axerophthene, was obtained.

A photometric method for determining the partition coefficients of carotenoids was devised and tested on thirty-two compounds.

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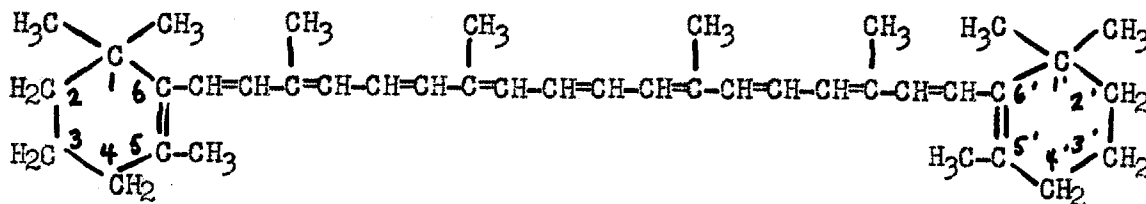
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I. THE CONVERSION OF β -CAROTENE TO SOME POLYENE KETONES.

A. THEORETICAL PART.

1. Introduction.



(I.) β -Carotene.

(The numbering system proposed by Karrer and shown above will be used throughout this Thesis). Hereafter, abbreviated formulas such as Ia will appear in place of the complete formulas.



(Ia.) β -Carotene.

The use of N-bromosuccinimide (NBS) as a dehydrogenating agent for carotenoids was introduced 10 years ago by Karrer and Rutschmann (1). By treating lycopene, $C_{40}H_{56}$, with 2 moles of NBS in carbon tetrachloride solution, they obtained "dehydrolycopene", $C_{40}H_{52}$. Zechmeister and Koe (2) extended this method in the aliphatic series by dehydrogenating the colorless polyene phytoene (3 conj. F) stepwise to phytofluene (5 conj. F), zetacarotene (7 conj. F), neurosporene (9 conj. F), and lycopene (11 conj. F).

Henbest (3) demonstrated that the β -ionylidene structure in a short polyene chain could be dehydrogenated by NBS followed by dehydrobromination of the intermediate with N-phenylmorpholine (NPM). He prepared 3,4-dehydro- β -ionone from β -ionone, Chart 1 (the intermediate bromide was not isolated).

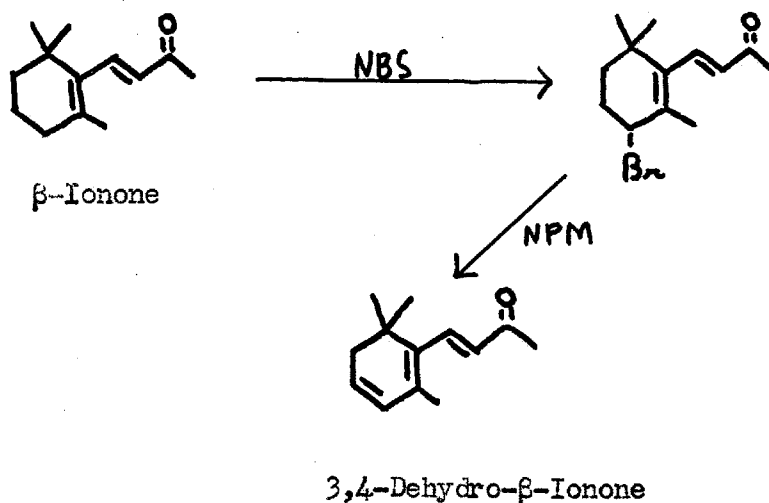


Chart 1. The Allylic Bromination and Dehydrobromination of β -Ionone.

Essentially the same procedure was employed by Farrar et al. (4) for the synthesis of vitamin A₂ starting from the methyl ester of vitamin A₁ acid (Chart 2).

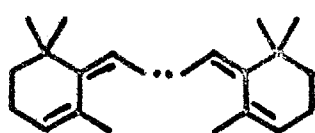


Methyl Ester of Vitamin A ₁ Acid	1. NBS 2. NPM 3. LiAlH ₄	Vitamin A ₂
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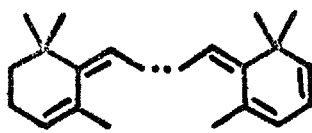
Chart 2. Synthesis of Vitamin A₂ from the Methyl Ester of Vitamin A₁ Acid.

The reaction of β -carotene (I), C₄₀H₅₆, which contains two β -ionylidene rings, with NBS was studied first by Zechmeister and Wallcave (5) and later by Zechmeister and Karmakar (6). β -Carotene

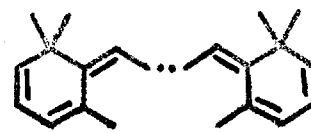
was refluxed in carbon tetrachloride with 2 moles of NBS for several hours. In this instance the use of NPM did not increase the yield of the dehydrogenation products. Under these conditions a complex pigment mixture resulted which was resolved by chromatography to yield the five crystalline hydrocarbons (XV), (II), (XVII), (XVI) and (III) shown in Chart 3. Two of these compounds, viz. retro-dehydrocarotene and anhydro-eschscholtzxanthin, had been obtained previously (7,8). The structure of retro-bisdehydrocarotene (called "bisdehydro- β -carotene" at the time of its preparation) was at first narrowed down



(XV.) retro-Dehydrocarotene



(III.) retro-Bisdehydrocarotene



(XVII.) Anhydro-eschscholtzxanthin



(XVI.) 3,4-Dehydro- β -carotene
("Dehydrocarotene II")



(II.) 3,4,3',4'-Bisdehydro- β -carotene
("Dehydrocarotene III")

Chart 3. Products from the Reaction of β -Carotene with NBS in Carbon Tetrachloride.

to two possible formulas, (II) or (III). Inhoffen and Raspé (9) soon after achieved the total synthesis of (II) whose non-identity with "bisdehydro- β -carotene" allowed only Formula (III) for the structure of this compound.

"Dehydrocarotene III", characterized by Zechmeister and Karmakar (6), was then found to be identical with Inhoffen's synthetic 3,4,3',4'-bisdehydro- β -carotene. This identification was carried out in the course of the present study and was reported on briefly (10). The 3,4-dehydro- β -carotene structure (XVI), as proposed by Zechmeister and Karmakar (6) for "dehydrocarotene II", has also been confirmed by the writer.

It should be noted with reference to the structure of the retro compounds that, along with the dehydrogenation process, the "normal" system of conjugated double bonds, originally present in β -carotene, has rearranged placing a double bond exocyclic to each end ring. The retro compounds compared to the "normal" compounds, are characterized by increased adsorbability, extensive fine structure, and the lack of a pronounced cis peak in the spectral curves of their cis forms.

The prefix retro was proposed by Oroshnik (11) in order to characterize the analogous system in the vitamin A series. The rearrangement from the "normal" to the retro system in the C₂₀ - series was postulated as taking place via the intermediate allylic carbonium ions.

This postulate is also useful in understanding the retro \longrightarrow "normal" rearrangements in the carotenoid field. Thus, both retro-dehydrocarotene (XV) and 3,4-dehydro- β -carotene (XVI) could arise from a single intermediate allylic bromo compound (XVIII) as shown in Chart 4. Further allylic bromination and dehydrobromination of the two compounds, (XV) and (XVI), with and without rearrangement, would

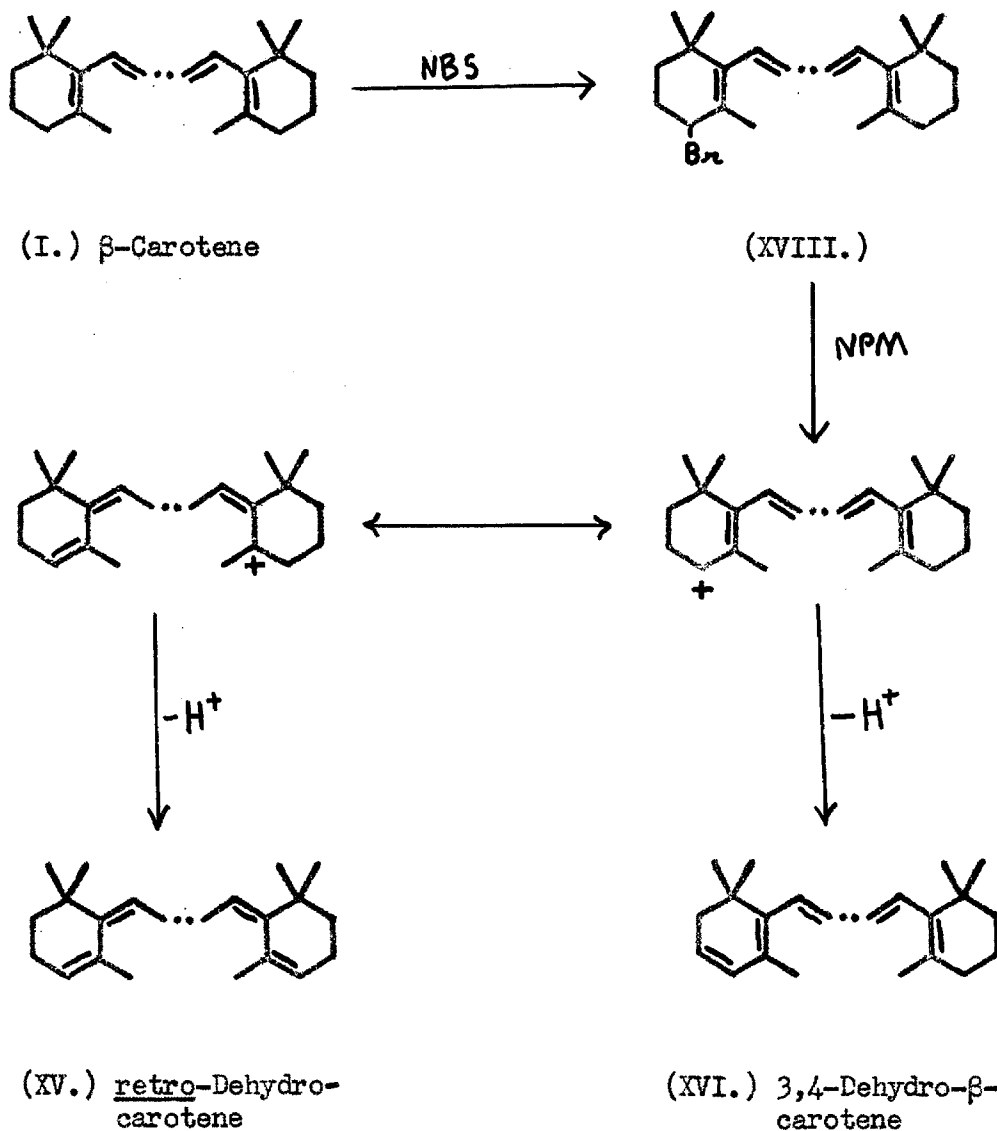


Chart 4. Mechanism of the Formation of retro and "normal" Dehydro-carotenes from β -Carotene.

lead to retro-bisdehydrocarotene (III) and 3,4,3',4'-bisdehydro- β -carotene (II), either of which could by the same scheme give rise to the last member, anhydro-eschscholtzxanthin (XVII).

The starting materials in the preparation of dehydro- β -ionone and the methyl ester of vitamin A₂ acid contain a carbonyl group at one

end of the conjugated system. This carbonyl group prevents allylic rearrangement, and consequently the "normal" conjugated system is preserved in both cases during the bromination and dehydrobromination processes (Charts 1 and 2).

It was observed by Zechmeister and Wallcave (12) that the use of NPM did not increase the yields of the products shown in Chart 4 (combined yield of the retro compounds totaled 15% of the β -carotene treated). This indicates that there were only negligible amounts of intact allylic bromo compounds present for the NPM to act upon when the reaction was stopped. This observation, along with the fact that a large fraction (25%) of β -carotene remained unreacted, suggests that in carbon tetrachloride solution the conversion with NBS begins by a slow reaction to form the allylic bromo compounds followed immediately by a faster process, viz. the dehydrobromination.

Although chloroform is not often used as a solvent in reactions involving NBS*, Farrar et al. (4) had successfully employed it for the preparation of vitamin A₂ (Chart 2). The intermediate methyl ester of 4-bromo- vitamin A₁ acid was dehydrobrominated by means of NPM without preliminary isolation.

2. Reaction of β -Carotene and NBS Dissolved in Commercial Chloroform.

When the interaction of β -carotene with NBS (molar ratio, 1:3) was investigated in commercial chloroform, it was observed that the

* In a review of NBS reactions by Djerassi (13) carbon tetrachloride was listed as the solvent in 55 NBS-olefin reactions while chloroform did not appear.

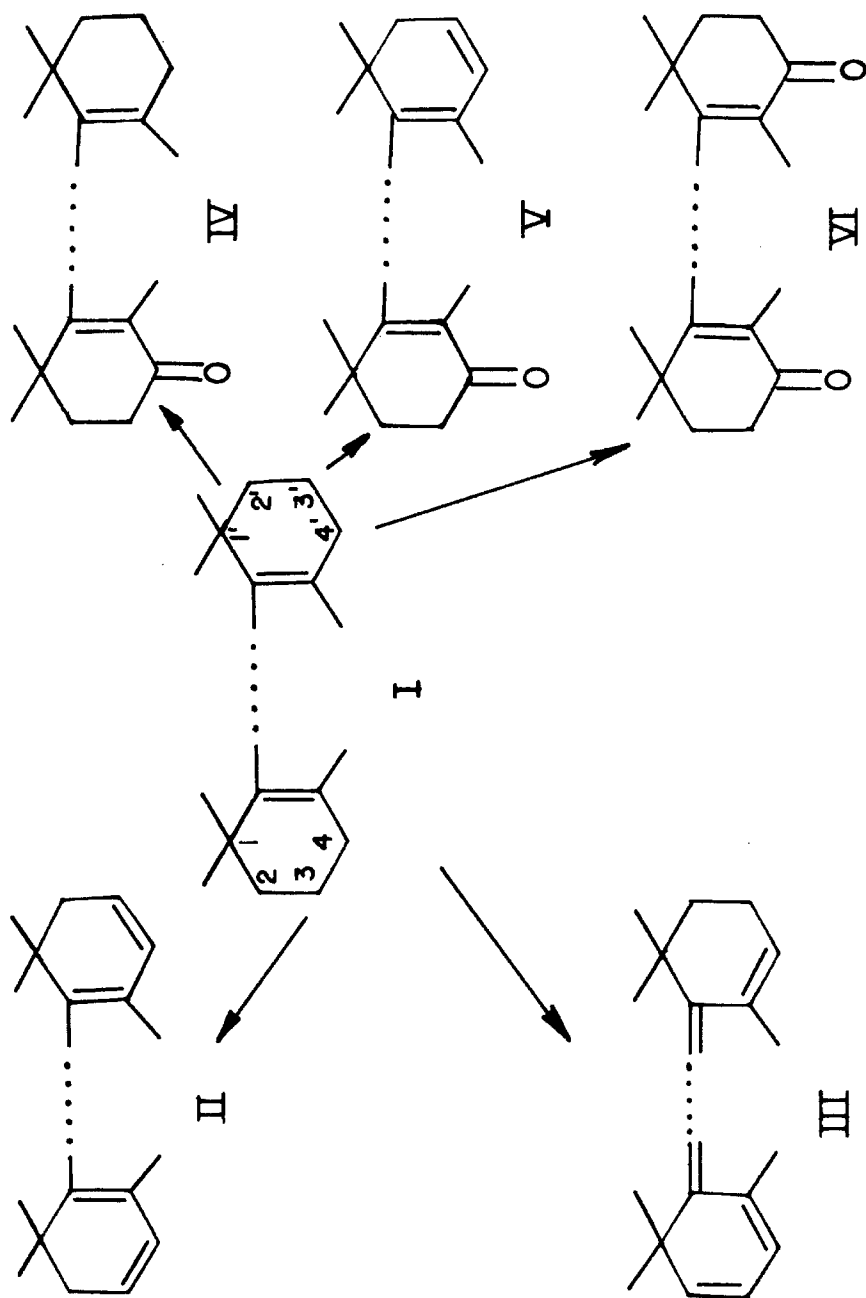
β -carotene was attacked at a much higher rate than in carbon tetrachloride. The bromination phase of the reaction (manifested by a change in the color of the solution from deep red to dark brown) was complete in 30 seconds, even at -20° , and the dehydrobromination in less than 10 minutes at the temperature of refluxing chloroform (61°). Furthermore, in sharp contrast to the reaction in carbon tetrachloride, no β -carotene remained unchanged. Actually, 60% of the β -carotene had been converted to a pigment mixture, which on chromatographic resolution, gave five crystalline compounds (Chart 5). The total yield of crystalline all-trans compounds amounted to 20-25% of the β -carotene treated. Besides minor quantities of 3,4,3',4'-bisdehydro- β -carotene (II) and retro-bisdehydrocarotene (III), the three ketones (IV), (V) and (VI) were obtained, (V) being the main product (Table 2).

Table 2.

Composition of the Reaction Product of
 β -Carotene and 3 Moles of NBS.
 (The figures indicate % of starting material and
 were obtained by photometric estimation of the respective
 stereoisomeric mixtures after chromatography)

Unchanged β -carotene (I)	0
4-Keto-3',4'-dehydro- β -carotenes (V)	40
4,4'-Diketo- β -carotenes (VI)	4
4-Keto- β -carotenes (IV)	3
<u>retro</u> -Bisdehydrocarotenes (III)	6
3,4,3',4'-Bisdehydro- β -carotenes (II)	3
Unidentified products	44

CHART 5. CONVERSION OF β -CAROTENE (I) BY N-BROMOSUCCINIMIDE
IN CHLOROFORM IN THE PRESENCE OF 1% ETHANOL.



It was established first of all that the ethanol present in commercial chloroform was responsible for the reaction being directed to the production of oxygenated pigments instead of the hydrocarbons encountered previously. Commercial chloroform, washed ethanol-free and dried by distillation over phosphorous pentoxide, acted as a medium essentially like carbon tetrachloride, except that the much higher reaction rate still obtained. From the ethanol-free chloroform retro-bisdehydrocarotene (III) was the main product; no ketones were detected. Interestingly, the ethanol content of the commercial chloroform, viz. about 1%, represents the optimum alcohol concentration for the formation of the ketones described below. A more detailed discussion of the role of ethanol is found in Section 9.

3. Characterization of the Polyene Ketones, (IV), (V) and (VI).

Elementary analysis showed the presence of two oxygen atoms in (VI) and of one in (IV) and (V) (Chart 5).

The ketone character of these compounds was demonstrated by the preparation of their oximes and 2,4-dinitrophenylhydrazones. Although hydroxylamine has been used frequently for the characterization of carotenoid ketones, the use of 2,4-dinitrophenylhydrazones has been scarcely mentioned in the literature. Yet, under the proper conditions it has given evidence of being more advantageous than hydroxylamine. Whereas the oximes are only slightly less soluble than the parent ketones, the 2,4-dinitrophenylhydrazones are much less soluble, and the derivative crystallizes out as it forms; the reaction is complete at room temperature in approximately 15 minutes, compared to several hours refluxing required for oxime formation.

In the compounds (IV)-(VI) all carbonyl groups were reduced to hydroxyl groups by means of lithium aluminum hydride, whereupon a considerable shift of the main spectral maxima towards shorter wave lengths took place. Consequently, the carbonyls had been parts of conjugated systems (14). The extent of this spectral shift amounted to 16 m μ (in hexane) for the diketone (VI) (Figs. 9 and 11) but to only 8 m μ for the monoketone (IV), whereby both extinction curves became identical with that of β -carotene.

The spectral maximum of the third ketone (V) (Fig. 4) was displaced on reduction from 468 m μ to 460 m μ (Fig. 7), and the spectrum then coincided with that of 3,4-dehydro- β -carotene (6) instead of β -carotene. This hypsochromic shift which took place in going from the ketones to the alcohols was already suggested by comparing the colors of the crystalline reduced and unreduced pigments (Figs. 24-27, p. 63).

Ganguly, Krinsky and Pinckard had used lithium aluminum hydride earlier for the reduction of $>C=O$ to $-CHOH$ in a naturally occurring carotenoid ketone, termed echinenone (4-keto- β -carotene) (15). The advantage of this reagent over the widely used aluminum isopropoxide is spectacular. Thus, a treatment of 5-10 minutes at room temperature suffices for an almost quantitative reduction of the ketone to the corresponding alcohol. Under these conditions, no trans \rightarrow cis isomerization could be detected. By contrast, the well known Meerwein-Ponndorf-Verly method required 24 hours refluxing which not only decreased the yields of the secondary alcohols, but, still worse, caused their further dehydration to hydrocarbons. Ganguly, Krinsky and

Pinckard in first attempting the reduction of echinenone (4-keto- β -carotene) by this method obtained mainly retro-dehydrocarotene (XV) which is the dehydration product of the expected alcohol, isocryptoxanthin (4-hydroxy- β -carotene) (16).

The conjugated nature of all the carbonyl groups mentioned was further confirmed for (V) and (VI) by a shift towards shorter wave lengths upon oximation (Figs. 2 and 5). Finally, the presence of a strong band at 6.04μ in the infrared spectra of all three ketones (Figs. 3, 6 and 10) pointed in the same direction.

4. Identification of Echinenone (4-Keto- β -carotene) ex β -Carotene.

The monoketone (IV) (λ_{\max} at $458 m\mu$; Fig. 1) was identified with echinenone, a pigment first isolated by Lederer (17) from sea urchins. The melting points of the ketone and of its oxime, as well as the visible and infrared spectra, were in full agreement with those of an authentic sample.*

Goodwin and Taha (18) have advanced some arguments in favor of echinenone being 4-keto- β -carotene and not 3-keto- β -carotene as had been previously suggested. More recently, Ganguly, Krinsky and Pinckard (15) have secured the 4-keto- β -carotene structure of this natural product, in agreement with the provitamin A potency as determined quantitatively by the same authors.

Thus, the present study provides for a second synthesis of echinenone, the first being the oxidation of 4-hydroxy- β -carotene to echinenone by the Oppenauer method (15).

*I am obliged to Drs. J. Ganguly, J. H. Pinckard and N. I. Krinsky for a crystalline sample of naturally occurring echinenone.

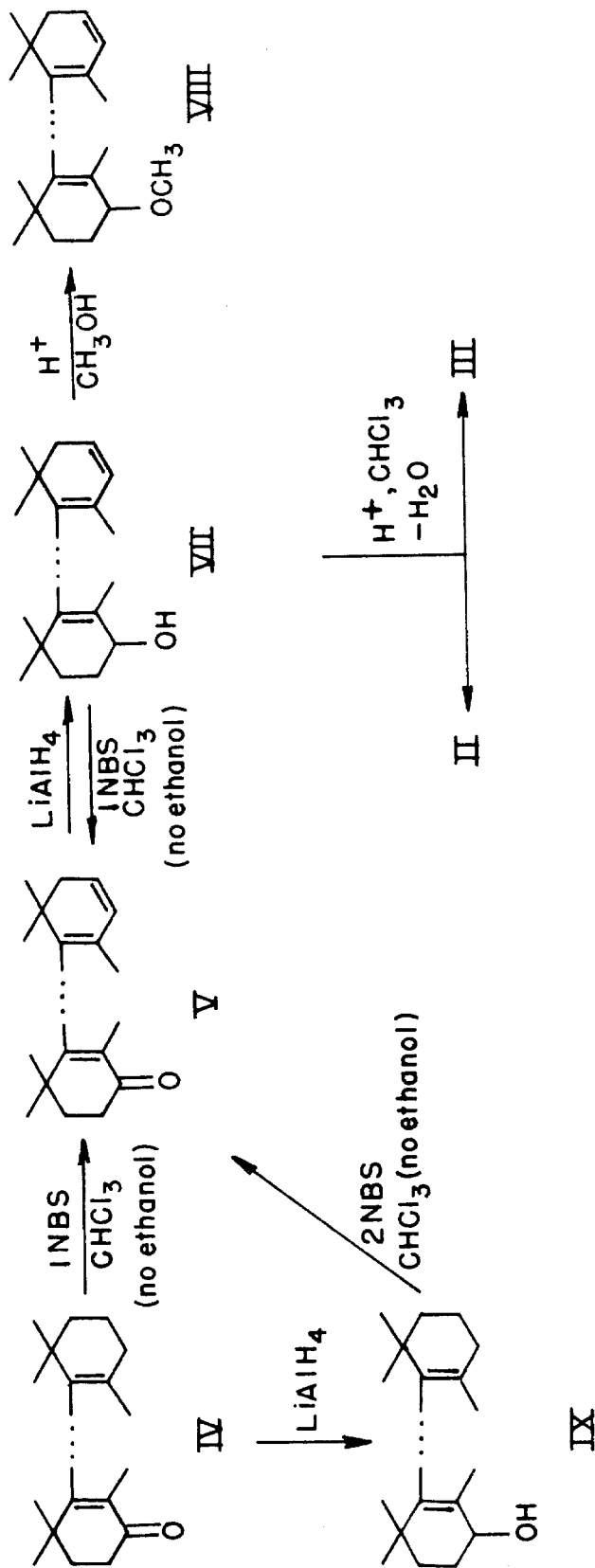
5. Structural Proof of 4-Keto-3',4'-dehydro- β -carotene.

The assignment to (V) of the 4-keto-3',4'-dehydro- β -carotene structure on the basis of spectral considerations (λ_{max} at 469 m μ ; Fig. 4) was confirmed by the partial synthesis of this compound starting from two structurally clarified substances. Furthermore, a degradative process yielded 3,4,3',4'-bisdehydro- β -carotene (II) and retro-bisdehydrocarotene (III) whose structures have also been secured. These reactions, to be discussed below, are outlined in Chart 6, p.13 .

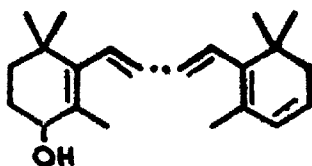
Isocryptoxanthin (4-hydroxy- β -carotene) (IX), when treated with 2 moles of NBS in ethanol-free chloroform, underwent simultaneous oxidation of its alcohol group and dehydrogenation of the non-substituted ring to give the 4-keto-3',4'-dehydro- β -carotene. Likewise, 4-keto- β -carotene (IV) led to the 3',4'-dehydro-compound when reacted with 1 mole of NBS. The position of the carbonyl group at one end of the chromophore in 4-keto- β -carotene serves to insure that no rearrangement to retro structures occurs during the dehydrobromination step; consequently, the reaction gave only a single main product (V). Likewise, only (V) was obtained as the main product starting from isocryptoxanthin. Thus one can propose that in the course of this somewhat complicated reaction the 4-carbonyl group must arise from oxidation of the alcohol before the dehydrobromination takes place in the other ring.

When the hydroxy compound (VII) obtained by the reduction of 4-keto-3',4'-dehydro- β -carotene (V) was subjected to mild acid conditions in chloroform, it suffered facile dehydration to yield two structurally clarified hydrocarbons, viz. 3,4,3',4'-bisdehydro- β -carotene (II) (λ_{max} at 471 m μ ; Fig. 16) and retro-bisdehydrocarotene

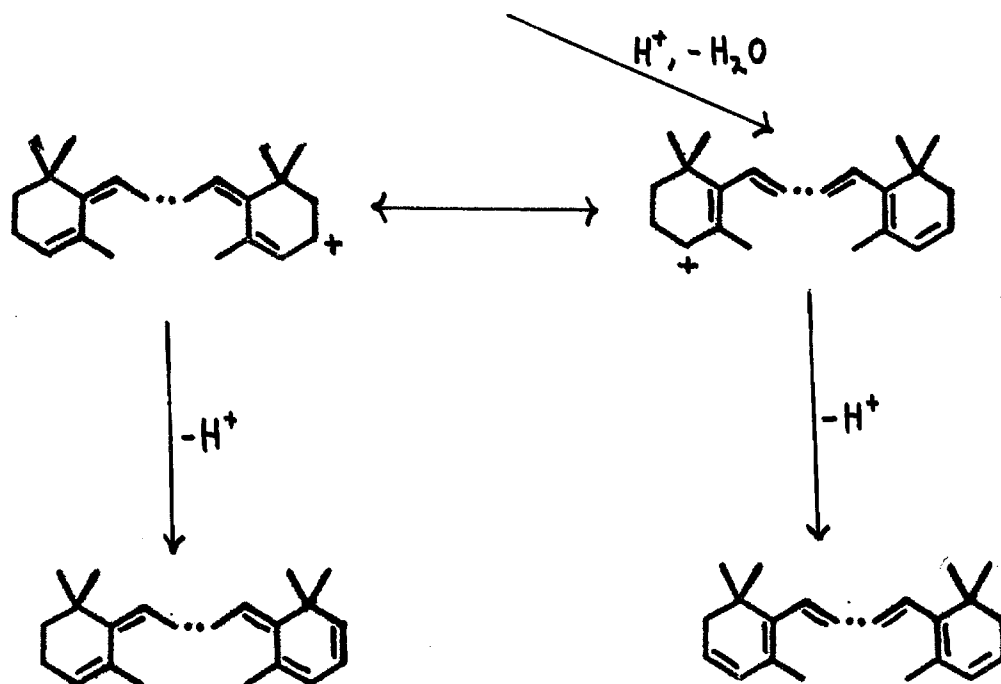
CHART 6. FORMATION AND SOME CONVERSIONS OF 4-KETO-3',4'-DEHYDRO- β -CAROTENE(V)



(III) (461, 487 and 519 mμ). In this instance, as had been shown earlier for the analogous dehydration of isocryptoxanthin (16), a retro structure resulting from an allylic rearrangement was preponderantly formed. Chart 7 outlines the mechanism of this dehydration leading to the formation of both hydrocarbons. The ratio of (II) to (III) formed was 1:20.



(VII.) 4-Hydroxy-3',4'-dehydro-β-carotene



(III.) retro-Bisdehydro-carotene.

(II.) 3,4,3',4'-Bisdehydro-β-carotene.

Chart 7. Acid Catalyzed Dehydration of 4-Hydroxy-3',4'-dehydro-β-carotene.

The allylic position of the hydroxyl group in (VII) with reference to the 5,6-double bond was substantiated by the smooth formation of its methyl ether, viz. 4-methoxy-3',4'-dehydro- β -carotene (VIII), under the influence of acid in methanol at room temperature (Chart 6). This method of preparing alkyl ethers of allylic alcohols had been used by Heilbron *et al.* but until now its application had not been extended to the C_{20} - or the C_{40} - polyene series. The reaction can be considered as a methanolysis which is catalyzed by acid. The formation of a by-product (in small yields) with a spectrum corresponding to retro-dehydrocarotene (445, 471, 502 m μ) could be rationalized by the attack of solvent (methanol) at the end of the conjugated system in the unsubstituted ring, Chart 8.

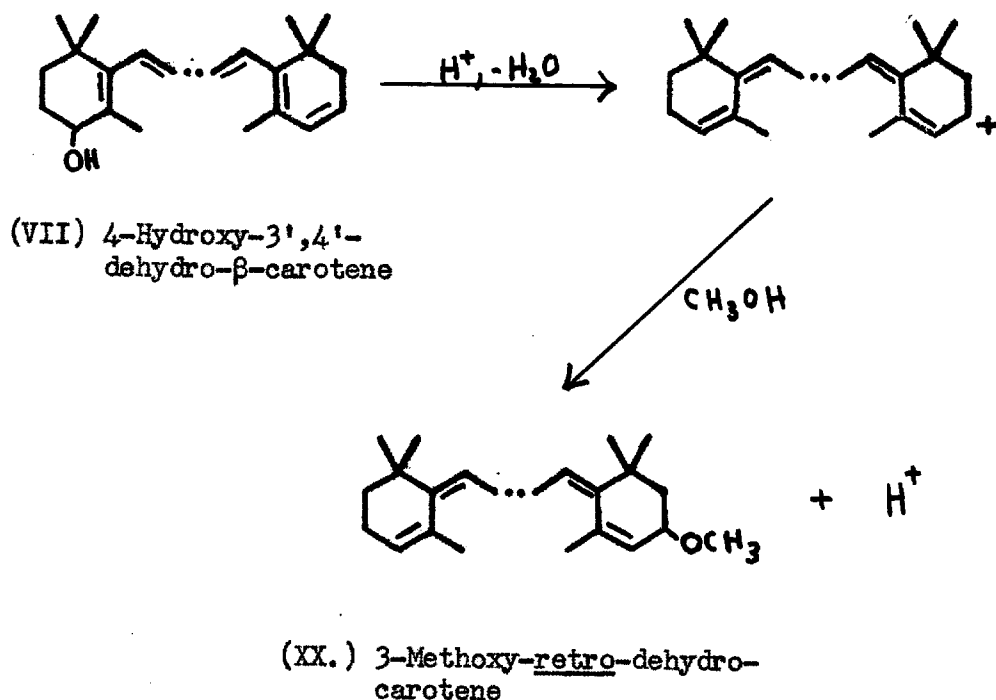
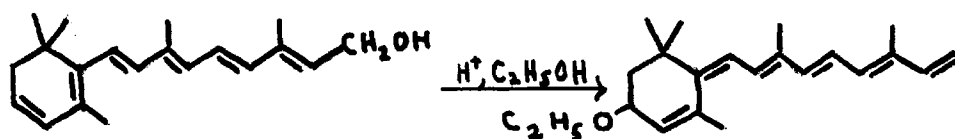


Chart 8. Formation of a retro-Product during the Methanolysis of 4-Hydroxy-3',4'-dehydro- β -carotene.

No attempt was made to secure the structure of this substance. Very recently (20) a reaction has been described which bears a close formal resemblance to the one outlined in Chart 8. Namely, 3-ethoxy-anhydrovitamin A₁ was obtained as the main product from the acid treatment of vitamin A₂ in ethanol, Chart 9.



Vitamin A₂

3-Ethoxy-anhydrovitamin A₁

Chart 9. Formation of 3-Ethoxy-anhydrovitamin A₁ from Vitamin A₂*

The identity of the spectra (and hence of the chromophores) of 4-hydroxy-3',4'-dehydro- β -carotene (VII) and "dehydrocarotene II" (XVI) prepared by Zechmeister and Karmakar (6) further supports the 3,4-dehydro- β -carotene structure assigned to this compound by these authors.

When 4-hydroxy-3',4'-dehydro- β -carotene was reacted with 1 mole of NBS, it reverted in good yield to the 4-keto compound. This reaction besides relating the hydroxyl compound again to the 4-keto-3',4'-dehydro- β -carotene, demonstrates that the allylic 2'-position must

*The course of this reaction was proposed independently by the writer (candidacy propositions, Sept. 24, 1954) to explain the anomalous results from the (attempted) dehydration of vitamin A₂ in ethanol. The product, 3-ethoxy-anhydrovitamin A₁, had previously been regarded as a hydrocarbon of uncertain structure.

be relatively resistant to an attack by NBS. This argument is strengthened by the observation that the 4-keto-3',4'-dehydro- β -carotene does not react when treated with NBS under the same conditions employed in its preparation from β -carotene. The non-reactivity of the latter compound also indicates that the 3-methylene, adjacent to the 4-carbonyl group, must be insensitive to bromine substitution from NBS.

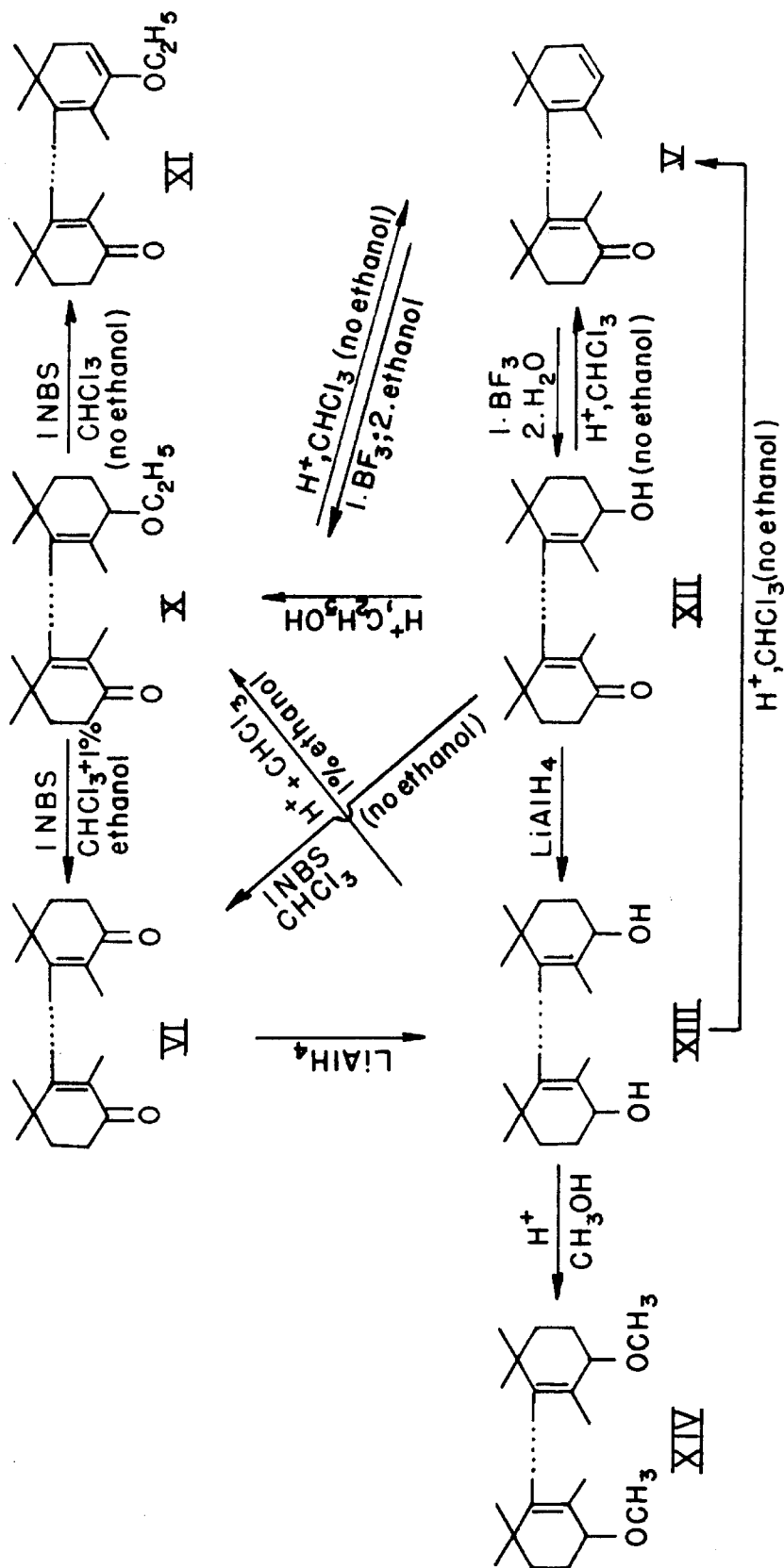
6. Structural Proof of 4,4'-Diketo- β -carotene (VI).

The conversions clarifying the structure of this diketone, as well as some other relationships of ketonic polyenes encountered in the present study, are shown in Chart 10.

The key compound in establishing these interrelationships was 4-keto-4'-hydroxy- β -carotene (XII) prepared by hydrolysis of the dark purple BF_3 complex of 4-keto-3',4'-dehydro- β -carotene (V).^{*} The position of the hydroxyl group thus formed was shown to be allylic (4'-position) by the smooth reversion under acid conditions of (XII) to 4-keto-3',4'-dehydro- β -carotene (V).

Furthermore, the spectrum of the keto-hydroxy compound was found to be identical with that of 4-keto- β -carotene (λ_{max} at 458 m μ ; Fig. 14). On the basis of these observations (XII) can only be 4-keto-4'-hydroxy- β -carotene. Its easy conversion to the corresponding ethyl ether (X) by acid catalysis in ethanol has confirmed this interpretation. This ethyl ether was also obtained directly by ethanolysis of the BF_3 - 4-keto-3',4'-dehydro- β -carotene complex (Chart 10).

^{*}The mechanism of this and other reactions of carotene- BF_3 complexes is described in Chapter II.



With the structure of (XII) verified as 4-keto-4'-hydroxy- β -carotene, the 4,4'-diketo- β -carotene structure of the diketone (VI) was firmly established, since the latter was obtained as the main product of the oxidation of 4-keto-4'-hydroxy- β -carotene by means of 1 mole of NBS. Furthermore, both the diketone (VI) and the keto-alcohol (XII) yielded the same diol (XIII) when reduced with lithium aluminum hydride. This diol showed the β -carotene spectrum (Fig. 11). It can only be interpreted as 4,4'-dihydroxy- β -carotene (XIII) and is, thus, an isomer of zeaxanthin (3,3'-dihydroxy- β -carotene) a main pigment of maize.

The allylic position of both hydroxyls in (XIII) was evidenced further by its ready conversion to the 4,4'-dimethoxy-derivative (XIV) under the influence of acid in methanol. This well crystallized compound, m.p. 154°, gained more importance when it was found to be identical with a dimethoxy- β -carotene obtained by Wallcave and Zechmeister (16) by methanolysis of the BF_3 -retro-dehydrocarotene complex. The assignment of a definite structure to their dimethoxy- β -carotene was complicated by the fact that the preparation showed 17% provitamin A activity when tested in rats (β -carotene = 100%). This biopotency, although rather weak, could not be reconciled with the 4,4'-dimethoxy structure, and it is apparent that the rat test should be repeated and perhaps revised.

The dehydration of 4,4'-dihydroxy- β -carotene under acid conditions in ethanol-free chloroform gave, unexpectedly, 4-keto-3',4'-dehydro- β -carotene (V), while in ethanol-containing chloroform, the 4-keto-4'-ethoxy-compound (X) was formed. One would rather expect the reaction

to follow one (or both) of two other courses.

The first of the two expected reactions would proceed by an initial dehydration occurring in one ring without rearrangement, to give 4-hydroxy-3',4'-dehydro- β -carotene (VII), followed by further dehydration of this compound as outlined in Chart 11 to yield the known hydrocarbons (II) and (III). However, neither of these two products were observed (their presence, however, in amounts below 1% might have escaped detection).

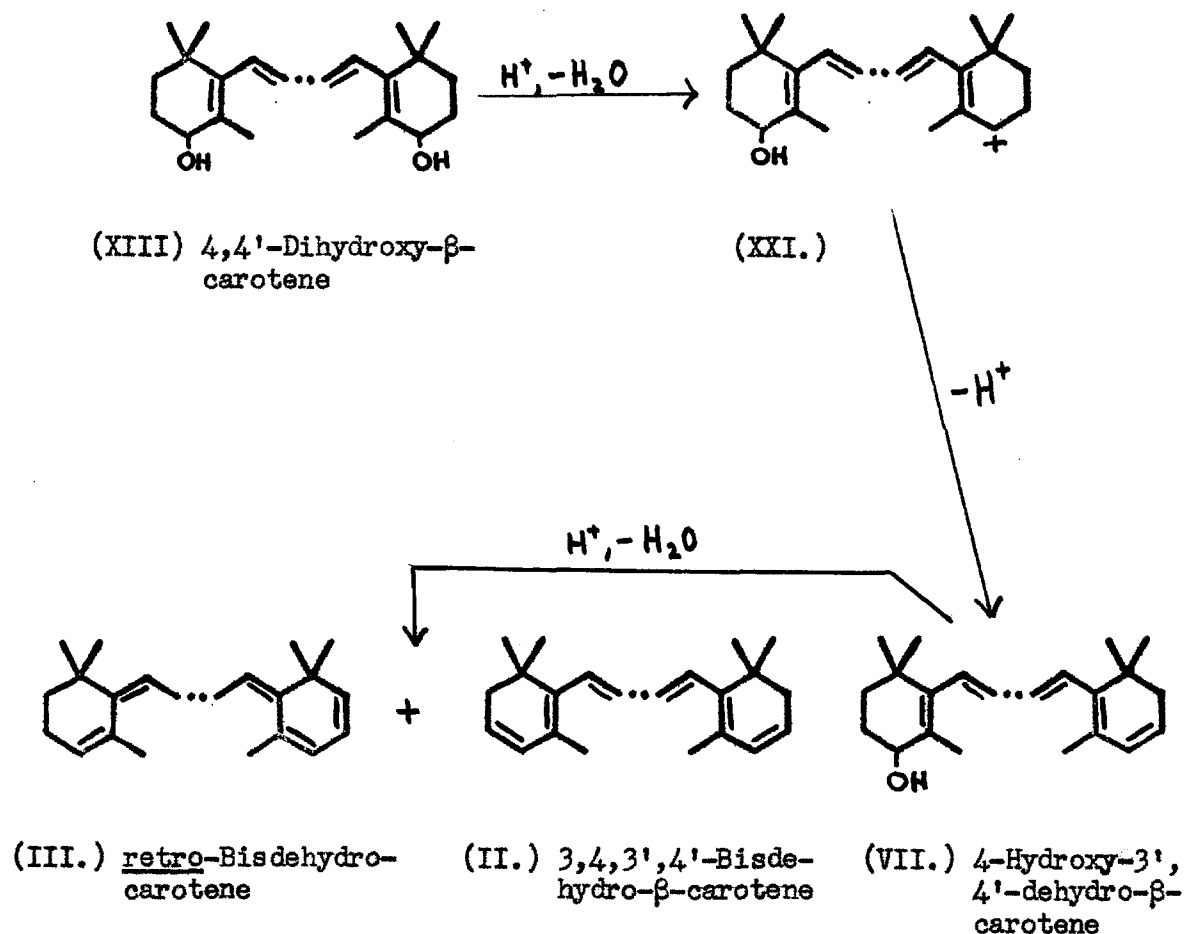


Chart 11. One Proposed Course for the Dehydration of 4,4'-Dihydroxy- β -carotene.

By the second reaction, the formation of a carbonyl in the 4-position in reactions (XIII) \longrightarrow (V) and (XIII) \longrightarrow (X) could be a priori explained by allylic rearrangement of the 4-hydroxy-4'-carbonium ion (XXI) to the retro-carbonium ion (XXII). By proton elimination (XXII) would give the retro-enol (XXIII). This enol might be expected to tautomerize to 4-keto- β -carotene (IV) (cf. Chart 12 for the sequence of these possible steps).

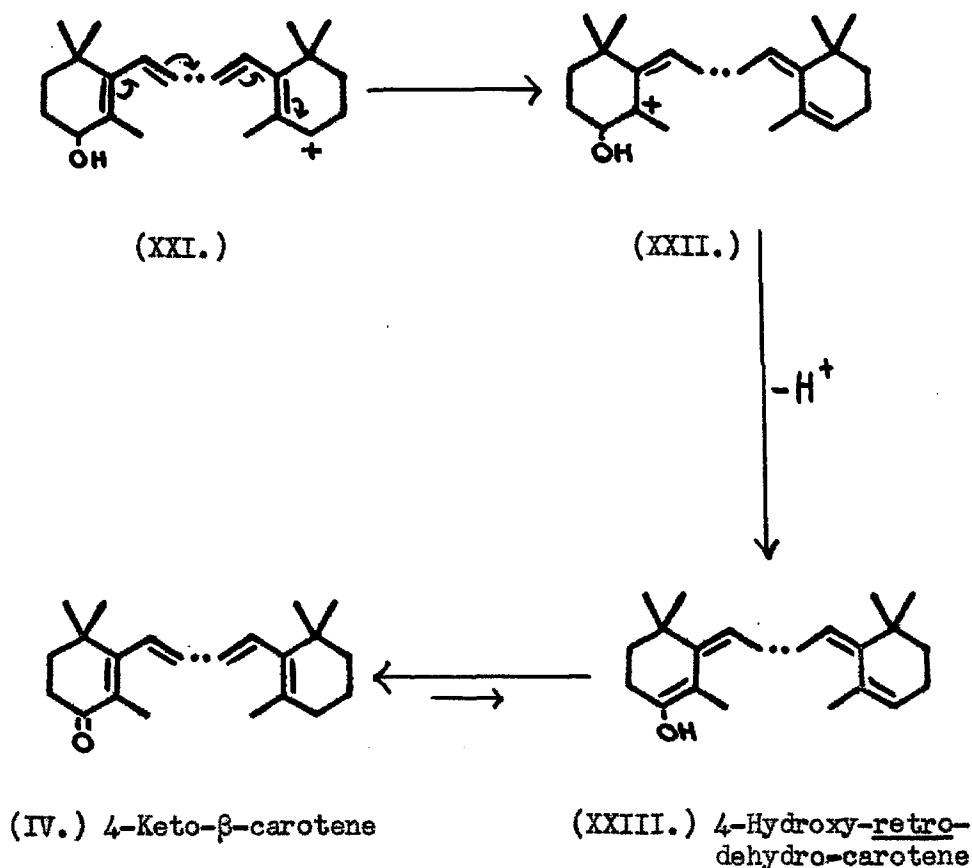
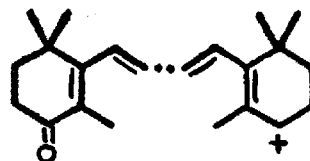


Chart 12. A Second Proposed Course for the Dehydration of 4,4'-Dihydroxy- β -Carotene.

It is plausible that at least the rearrangement of (XXI) to (XXII) does take place, since the "unrearranged" dehydration scheme (Chart 11) is apparently inoperative. However, no 4-keto- β -carotene (IV) was found in either the ethanol-containing or ethanol-free media. Neither could it have functioned as an intermediate, since it was found to be stable in both media. Therefore, the proposal outlined in Chart 12 is not entirely sufficient to explain the behavior of 4, 4'-dihydroxy- β -carotene under acid conditions.

Evidently, at some stage of the conversion, a dehydrogenation as well as a dehydration has taken place. Possibly, the carbonium ion (XXIV) is formed as an intermediate which then may give rise to either 4-keto-4'-ethoxy- β -carotene or 4-keto-3',4'-dehydro- β -carotene (V) depending on the solvent medium.



(XXIV.)

Assuming (XXIV) as an intermediate, the dehydration experiments with 4,4'-dihydroxy- β -carotene would then bear a close formal analogy to the dehydration experiments carried out with 4-keto-4'-hydroxy- β -carotene (XII \longrightarrow X, and XII \longrightarrow V) (Chart 10) since (XXIV) can certainly be considered as an intermediate in these latter reactions. It should be emphasized that the products of the acid treatment of 4,4'-dihydroxy- β -carotene and of 4-keto-4'-hydroxy- β -carotene are the same in both ethanol-containing and ethanol-free chloroform.

Finally, it must be pointed out that the 4-keto-4'-ethoxy- β -carotene obtained in the reactions described above is itself unstable in ethanol-containing chloroform, and its isolation as the main product

of the reactions (XII) \longrightarrow (X) and (XIII) \longrightarrow (X) depends on stopping the reaction soon after the formation of the ether. For example, when 4-keto-4'-hydroxy- β -carotene was subjected to acid treatment in ethanol-containing chloroform for 30 instead of 5 minutes, only the 4-keto-3',4'-dehydro- β -carotene (V) was isolated.

7. Identification of the 4,4'-Diketo- β -carotene with Canthaxanthin.

Canthaxanthin, the main carotenoid of the edible mushroom "cinnabar chanterelle" (Cantharellus cinnabarinus, Basidiomycete), was detected and obtained in crystalline form by Haxo (21). It is a neutral xanthophyll forming the main polyene pigment of the fruiting bodies in the mushroom. Later, Saperstein and Starr (22,23) isolated the same pigment from some mutant strains of Corynebacterium michiganense. They characterized canthaxanthin, secured its empirical formula ($C_{40}H_{52}O_2$) and recognized its ketone character. Furthermore, they proposed, with all reservations, a hydroxy-ketone structure for canthaxanthin containing both functional groups at the same end of the molecule.

The properties of the 4,4'-diketo compound (VI) were indicative of a possible identity with canthaxanthin, and this was substantiated by a direct comparison of our synthetic product with a sample ex Corynebacterium, kindly sent us by Drs. S. Saperstein and M. P. Starr.

This identification was based, first of all, on crystal forms, solubilities, melting points, mixed chromatogram tests, and the spectral properties of the ketone samples themselves, as well as of their reduction products. (Table 1.)

Table 1.

Comparison of Natural and Synthetic Canthaxanthin Samples

	Natural Product	Synthetic Preparation
Crystal form (from benzene-methanol)	trapezoidal prisms	trapezoidal prisms
Melting point (cor.) ^a	213°	213°
Composition (found) ^b	C, 85.31; H, 9.49	C, 85.23; H, 9.41
$E_{1\text{ cm.}}^{\text{mol.}}$ at λ_{max} 480 mμ (in benzene)	11.8×10^4	11.2×10^4
Position of λ_{max} in hexane	466 mμ ^c	466 mμ
Position of λ_{max} in ethanol	477 mμ	478 mμ
Partition, hexane: 95% methanol	slightly more in the hypophase	50:50
Provitamin A assay	negative	negative

- a These melting points were determined, under strictly identical conditions, in an electrically heated Berl block, in evacuated and sealed capillaries. Saperstein and Starr used a Fisher-Johns apparatus and found 218° for the natural product.
- b The compositions given represent, respectively, the average of two analyses reported by Saperstein and Starr, and by the present writer.
- c The value, 468 mμ was reported by Saperstein and Starr. The respective curves were identical.

Saperstein and Starr (23) had reduced canthaxanthin with aluminum isopropoxide under rather energetic conditions and obtained a new pigment (432, 457, 487 mμ, in hexane). This polyene, however, cannot represent the diol corresponding to canthaxanthin. The true diol, i.e. synthetic 4,4'-dihydroxy-β-carotene (XIII, Chart 10) shows as expected the β-carotene spectrum (479, 451 mμ). It was obtained by mild lithium aluminum hydride treatment both from natural and synthetic canthaxanthin (Figs. 13 and 11). The reduction products thus obtained were found to be identical.

The product of the aluminum isopropoxide treatment (23) cannot be clarified at the present time, but it may well represent a retro-compound with 11 conjugated double bonds, originating from some conversion other than the simple reduction of the diketone. It has been demonstrated that isocryptoxanthin, containing an allylic hydroxyl group in the 4-position, undergoes easy dehydration under the conditions of the aluminum isopropoxide reduction (15).

It is interesting to note that, besides canthaxanthin, some other naturally occurring polyene ketones, such as astaxanthin (24) and echinenone (15), contain carbonyls in the 4- (and/or 4'-) position, i. e. at that sensitive point of the β-carotene molecule which is attacked in vitro by the NBS reagent.

8. Comparison of 4,4'-Diketo-β-carotene with Aphanicin.

While investigating the polyene pigments of a fresh water algae, Aphanizomenon flos-aquae, Tischer isolated, besides other pigments, two ketones which he named aphanin and aphanicin (25). Tischer did not propose any definite structures for these pigments, but Goodwin

and Taha have pointed out that Tischer's aphanin is very likely identical with echinenone (18).

A consideration of the properties of aphanicin leads one to suspect that this compound may be identical with the synthetic 4,4'-diketo- β -carotene. It was adsorbed on alumina considerably above the aphanin zone. Tischer reported that his aphanicin is more hypophasic than aphanin when partitioned between hexane:95% methanol; even when 90% methanol was used, the hypophase was still colored. This description would also fit the 4-keto- β -carotene and 4,4'-diketo- β -carotene pair.

Although the melting point of aphanicin is 18° lower than that of our synthetic diketone, the melting points of the two oximes are in good agreement (aphanicin oxime, 241°; synthetic 4,4'-diketo- β -carotene oxime, 248°). The nitrogen value found by Tischer (3.97% N) corresponds more closely to that of a dioxime (calcd. 4.71%) than to that of a mono-oxime (calcd. 2.48%). The crystal forms of the two ketones, as obtained from benzene-methanol, are also very similar.

Unfortunately, the most essential characteristic necessary for a good comparison, i.e. the spectrum of aphanicin, as reported by Tischer is of little value, since the visual spectroscope used has severe limitations when applied to spectral curves without fine structure. Thus, only the limits of the broad absorbing band were reported with an accuracy of perhaps ± 10 m μ . Therefore, the conclusive identification of aphanicin with 4,4'-diketo- β -carotene remains to be carried out.

9. Proposed Mechanism of Ketone Formation by NBS in the Presence of Alcohols.

The reaction of NBS with polyenes in the presence of an alcohol to form conjugated ketones is, so far as is known, a new type of reaction for the NBS reagent. Accordingly, an effort was made to determine the mechanism by which the reaction occurs.

The role of the ethanol in commercial chloroform is not specific insofar as it can be replaced by methanol or benzyl alcohol (in the same molar concentrations) without decreasing the yields or ratios of the ketone polyenes (no other aliphatic alcohols were studied). Phenol and glacial acetic acid were ineffective for the formation of ketones; in their presence, the same results were obtained as with pure chloroform.

It should be stressed that the course of the reaction is very sensitive to a change in the alcohol content of the chloroform. The yields of the main product, 4-keto-3',4'-dehydro- β -carotene were compared in a series of reactions in which the ethanol content was varied from 0.1% to 10% (Table 4, p.101). The best yield was obtained using 1% (by volume) of ethanol in the chloroform. This corresponds to a 20:1 molar ratio of ethanol: β -carotene. When the concentration of alcohol was less than 1%, more of the retro-bisdehydro-carotene component appeared in the reaction product. Concentrations of alcohol in excess of 1% decreased the yield of all pigments formed. This latter phenomenon is evidence for a competing side-reaction between β -carotene and NBS, which might well involve the bromination of the double bonds to give colorless derivatives.

Some understanding of the formation of ketones from a polyene can be gained by considering the conversions 4-keto-4'-ethoxy- β -carotene (X) \longrightarrow 4,4'-diketo- β -carotene (VI) and 4-keto-4'-ethoxy-3',4'-dehydro- β -carotene (XI). As shown in Chart 10, the ethoxy group in 4-keto-4'-ethoxy- β -carotene (X) when reacted with NBS, is preserved only in the absence of alcohol; only in this instance is the 3',4'-dehydro derivative (XI) formed. However, the $-\text{OC}_2\text{H}_5$ group is replaced quantitatively by $>\text{C}=\text{O}$ in the presence of alcohol (cf. X \longrightarrow VI).

Therefore, it can be reasonably proposed that in the course of the conversion, (X) \longrightarrow (XI), in the absence of ethanol, the main effects of NBS, followed by a treatment with NPM, are, bromination in the 4-position and subsequent dehydrobromination, resulting in the appearance of the dehydrogenated ethoxy derivative (XI). The diketone is also formed in lesser amounts.

In contrast, only the diketone is formed by the action of NBS, in the presence of alcohol, on 4-keto-4'-ethoxy- β -carotene (X). Accordingly, the alcohol must cause the conversion of the 4'-brominated ethoxy compound (XXV) to the corresponding 4,4'-diethoxy derivative (XXVI); subsequently the conversion to a carbonyl from this ketal would take place (Chart 13).

The extreme ease in which α -haloethers undergo displacement reactions by alcohols to give acetals has been described in a recent review (26). The very similar intermediate (XXV) would be expected to be even more sensitive to displacement reactions because the bromoethoxyl substituted carbon occupies the allylic 4'-position, already demonstrated as a highly reactive position in similar reactions.

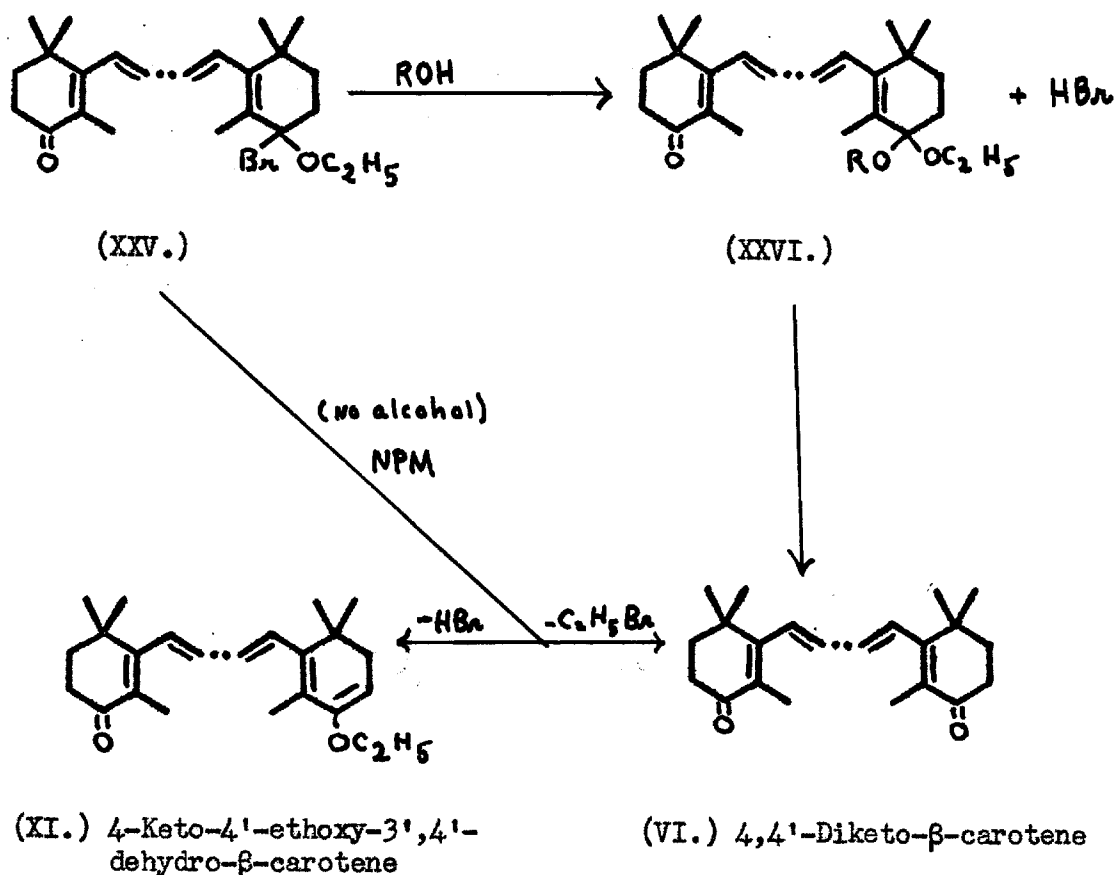


Chart 13. Conversion of the 4'-Ethoxyl Grouping to the 4'-Carbonyl by Means of NBS and Alcohol.

It is, of course, interesting that some diketone is also formed from 4-keto-4'-ethoxy- β -carotene (X) in the absence of alcohol. One might assume that C_2H_5Br is eliminated from the intermediate (XXV) to give the diketone. A similar type of reaction involving NBS has recently been described by Marvell and Joncich (27) who prepared ethyl benzoate by treating benzaldehyde diethylacetal with the NBS reagent. These authors also suggested the loss of RBr from the intermediate and the formation of a carbonyl group in the end product (Chart 14).

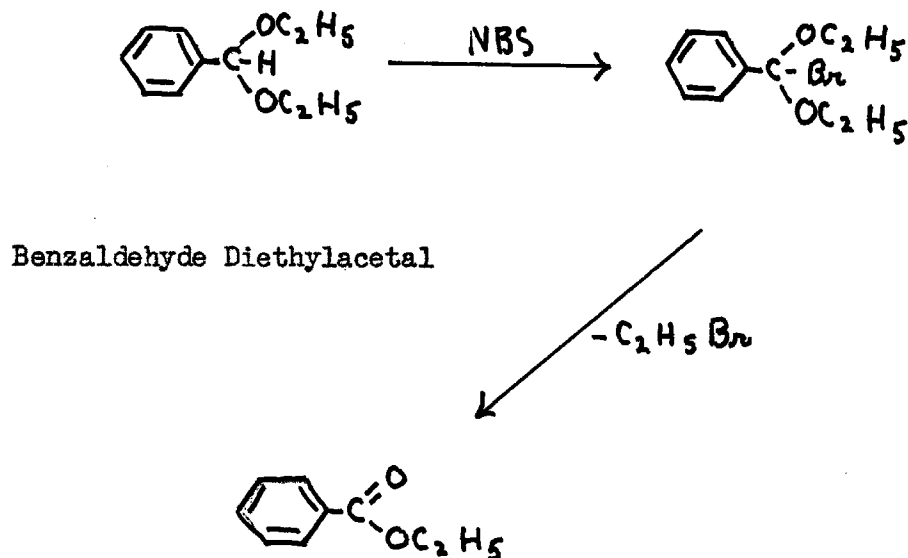
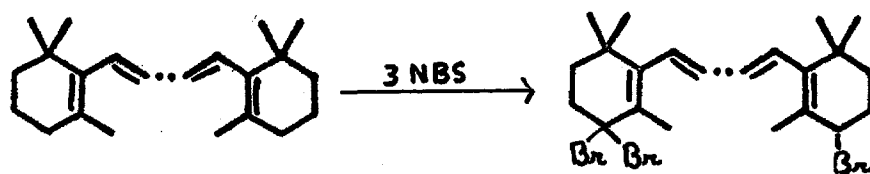


Chart 14. Preparation of Ethyl Benzoate from Benzaldehyde Diethylacetal.

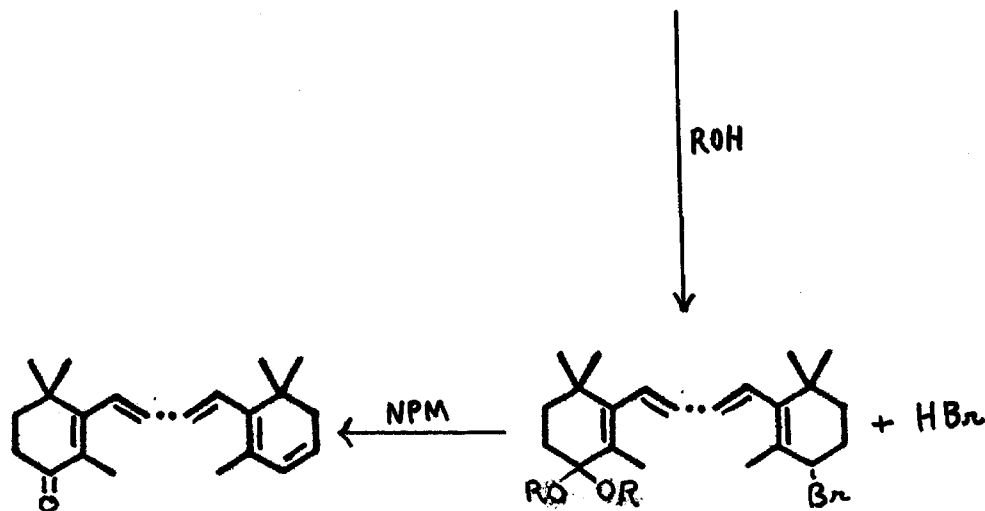
With the foregoing ideas in mind, the mechanism of the formation of the main product, viz. 4-keto-3',4'-dehydro- β -carotene from the interaction of β -carotene and 3 moles of NBS can be outlined as shown in Chart 15. The simultaneous appearance of smaller amounts of 4-keto- β -carotene and 4,4'-diketo- β -carotene can be explained by some obvious variations of this scheme.

There are, however, several other observations that should be mentioned in connection with the reaction mechanism. While the geminally dibromo-substituted 4- (or 4'-) carbon atom appears to be



(I.) β -Carotene

(XXVII.)



(V.) 4-Keto-3',4'-dehydro- β -carotene

(XXVIII.)

Chart 15. Formation of 4-Keto-3',4'-dehydro- β -carotene from β -Carotene.

very sensitive to alcohols, by contrast, the corresponding monobromo derivative must be stable, because no 4- (or 4'-) ethers have been isolated from any of the reaction mixtures obtained in the presence of alcohols.

Furthermore, in one experiment, alcohol was added to the reaction mixture of β -carotene and 3 moles of NBS in pure chloroform several minutes after the color change (red \longrightarrow brown) had indicated the formation of the brominated compounds. In this instance, the products

obtained were the same as those formed when ethanol was present from the beginning of the experiment. Consequently, the ethanol is not involved in the formation of the bromine substituted intermediates.

So far, no attempt has been made to isolate either the brominated intermediates or the (proposed) ketals.

10. Preparation of 4-Keto- α -Carotene.

The oxidation of the 4-methylene to a carbonyl group in β -carotene by the method described above has also been carried out starting with α -carotene. In this case, a 1:2 molar ratio of polyene:NBS gave good yields of the 4-keto- α -carotene, which is a new carotenoid ketone (using a 1:3 ratio caused mostly the formation of colorless products). Thus, the α -ionylidene ring of α -carotene (XXIX) (right end) must be inert to the NBS reagent under the conditions applied, and the reaction can be represented according to Chart 16 (XXIX) \longrightarrow (XXXII).

The structural assignment of 4-keto- α -carotene to (XXXII) was based on the following observations. Elementary analysis showed the presence of one oxygen and corresponded to the formula, $C_{40}H_{54}O$. The partition value (94:10) in hexane: 95% methanol was in the range for a monoketo carotenoid. Reduction of (XXXII) (λ_{max} at 452 m μ ; Fig. 18) with lithium aluminum hydride gave good yields of the corresponding alcohol (XXXIII) which showed the spectrum of α -carotene (Fig. 18). Therefore, the $>C=O$ in (XXXII) must have been part of the conjugated system. The hydroxy- α -carotene (XXXIII) has been found to be identical with 4-hydroxy- α -carotene prepared by Mr. W. V. Bush by hydrolysis of the α -carotene- BF_3 complex (unpublished).

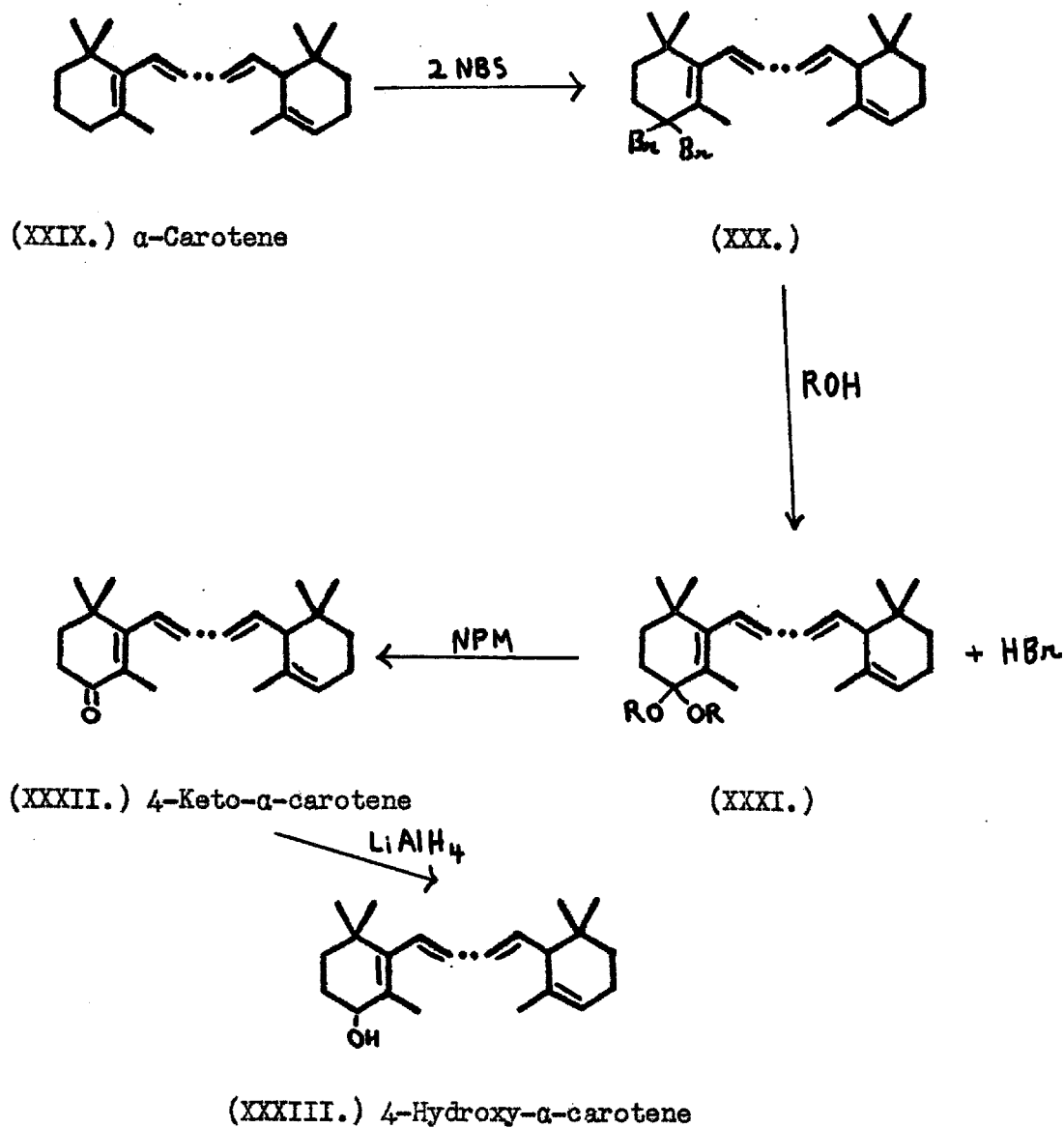


Chart 16. 4-Keto- α -carotene from α -Carotene by means of NBS in Commercial Chloroform.

11. Discussion of Spectra.

a. Visible Region

The present study has shown that when the chromophore of α - or β -carotene is lengthened either by a conjugated carbonyl group or by

a conjugated ring double bond or both, the main maximum is shifted towards longer wave lengths by definite and additive amounts. Hence, on the basis of such data as listed in Table 3, the structure of chromophores in some new carotenoid derivatives may be predicted and identified.

Table 3.

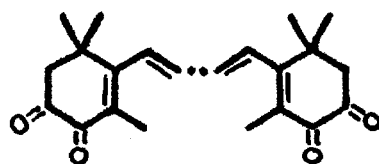
Bathochromic Effect of Conjugated Carbonyls and/or Conjugated Ring Double Bonds Attached to the End(s) of the α - or β -Carotene Chromophore.

Group(s)	Shift in $m\mu$ of λ_{max} (in hexane)	Figs.
1 Carbonyl	7	1, 14, 18
2 Carbonyls	15	9
1 Double Bond ^a	10	7, 29 (p.120)
2 Double Bonds ^a	20	16
1 Carbonyl and 1 Double Bond	19	4
1 = N-OH	5	2, 5

^a The shift of 10 $m\mu$ for one double bond and of 20 $m\mu$ for 2 double bonds was first observed by Zechmeister and Karmakar (6) for 3,4-dehydro- α -carotene, 3,4-dehydro- β -carotene, and 3,4,3',4'-bisdehydro- β -carotene ("dehydrocarotenes I-III").

Such lengthening of the conjugated system at one end substantially diminishes the extent of the fine structure in the spectral curve (Figs. 1, 7, 14, 18 and 29); when both ends are affected simultaneously, the fine structure disappears altogether (Figs., 4, 9 and 16).

The validity of these considerations seems to extend the framework of the present study. For example, it can be postulated for the spectral curve of astacene (3,4,3',4'-tetraketo- β -carotene) (XXXIV) (28, cf. p. 354) that only the 4- and 4'-carbonyls are responsible for the lack of fine structure, since 4,4'-diketo- β -carotene (VI) shows a similar effect*.



(XXXIV.) Astacene

While discussing a proposed structure of myxoxanthin, Heilbron and Lythgoe (30) made the following statement,

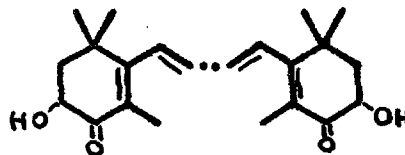
"the single-banded spectrum of such pigments is due to the simultaneous conjugation of the polar carbonyl group with two sets of unsaturated linkages"***

This statement was apparently based on the consideration of the astacene spectrum only. Subsequently, it was used as evidence in proposing tentative structures containing a $-C=C-$ cross-conjugated with the carbonyl in myxoxanthin (28, cf. p. 226) and canthaxanthin (23), whose spectral curves also showed diminished fine structure. The present study, in demonstrating that the existence of cross-conjugation is not a necessary condition for decreasing the fine structure, has invalidated the statement of Heilbron and Lythgoe.

*For this comparison, the spectral curve of the 4,4'-diketo- β -carotene was recorded in pyridine to permit a direct comparison with the published curve of astacene. The curves are qualitatively alike, but the λ_{\max} of astacene is at 500 m μ compared to 490 m μ for the 4,4'-diketo- β -carotene. Therefore the 3- and 3'-carbonyls in astacene cause a bathochromic shift of 5 m μ each.

***This statement appears italicized in the original report.

The spectral curve of astaxanthin (3,3'-dihydroxy-4,4'-diketo- β -carotene) (XXXV) (28, cf. p. 353) is identical with that of 4,4'-diketo- β -carotene (Fig. 9). The position of λ_{\max} (in hexane) of both compounds is at 466 m μ . Thus, the presence of the 3,3'-hydroxyl groups in astaxanthin has no effect on the 4,4'-carbonyls which manifests itself in the visible spectrum.



(XXXV.) Astaxanthin

Curiously, the original paper by Kuhn and Sørensen concerning astaxanthin (24) showed its spectrum, as well as two of its esters (palmitate and caprylate), in pyridine as possessing a considerable degree of fine structure with three distinct bands. Later Karrer and Würgler (31) as well as Goodwin and Srisukh (29) have published the curve of astaxanthin in hexane and carbon disulfide; in both solvents only a single broad band appears without any indication of fine structure. It is difficult to reconcile these contradictory data, since especially in the field of polyene ketones and other carotenoids the most extensive fine structure is usually observed in hexane solution. Until now, no author seems to have noticed this situation, and it is evident that the curve of astaxanthin should be retaken in pyridine.

Another point regarding the spectra of dicarbonyl compounds which has appeared recently in the literature also requires some clarification. Bohlmann (32) has remarked in a paper that "in the field of dicarbonyl compounds themselves the position of the maximum is influenced practically only by one $>C=O$ group". This statement is certainly not valid for the spectrum of 4,4'-diketo- β -carotene where it has been

shown that the bathochromic effect of the second $>C=O$ group is as large as that of the first.

b. Infrared Region.

Zechmeister and Lunde have shown that infrared spectra are useful in studying cis-trans isomerism in carotenoids and some other polyenes (33,34). The present investigation has offered an opportunity to extend the use of infrared spectra in the carotenoid field, especially to determine the presence or absence of certain functional groups. In this connection the infrared curves of the new carotenoids described above, as well as of a number of previously known carotenoids, were recorded and correlated to the respective structures.

The compounds with a conjugated carbonyl group located in the 4- or 4'-position showed a very strong band at 6.04μ (Figs. 3, 6, 10 and 15). As is well known, saturated aliphatic (or 6-membered cyclic) ketones exhibit the corresponding band at 5.83μ (35). Evidently the difference of 0.21μ is due to conjugation of the carbonyl group with the conjugated system of carbon-carbon double bonds. A single $-C=C-$ or phenyl group in conjugation with a carbonyl causes a shift of only 0.09μ (35). Thus, it appears that in the presence of a long conjugated double bond system, a further shift of 0.12μ , to the 6.04μ position, takes place.

In the β -carotenone molecule (XXXVI) we find both conjugated and unconjugated carbonyl groups, and its infrared spectrum (Fig. 19) shows very clearly the difference in the position of the two types. In this instance, the conjugated aliphatic carbonyl band is found at 6.00μ ,

at a slightly higher frequency (0.04 μ) than the conjugated 4-carbonyl band. Capsanthin (XXXVII) shows only the 6.00 μ band due to its aliphatic conjugated carbonyl. The infrared spectra of β -carotenone and capsanthin, therefore, support the structures assigned to these two carotenoid ketones (36,37).



(XXXVI.) β -Carotenone



(XXXVII.) Capsanthin

In the spectra of the 4-hydroxy compounds, prepared by reduction of the 4-ketones, several bands characteristic for the hydroxyl group appear. The first of these corresponds to the O-H stretching frequency and is located at 2.75 μ . In carbon tetrachloride it appears as a sharp, weak, band; in chloroform it is very diffuse. This particular band is found in the same position in the spectra of the 3-hydroxycarotenoids (Fig. 21).

The second characteristic band for the 4-hydroxy compounds appears at 10.05 μ (in chloroform) and can reasonably be assigned as a C-O stretching frequency (Figs. 8, 12 and 23). For 3-hydroxy carotenoids this same band is observed at 9.95 μ (in chloroform) (Fig. 22). Therefore, this difference (0.10 μ) can probably be used to differentiate between the 3- and 4- hydroxy carotenoids.

The C-O band undergoes a shift to the higher frequencies if carbon tetrachloride is used as the solvent. For the 3-hydroxy compounds it is located at 9.60 μ (Fig. 21; also shown by gazaniaxanthin, 3-hydroxy-

γ -carotene). Several 4-hydroxy carotenoids tested showed the same band at 9.75μ . For both types the intensity of this band is much greater in carbon tetrachloride than in chloroform (cf. Figs. 21 and 22).

Finally, it should be emphasized that the present study has not been primarily devoted to the infrared interpretation of structures, and hence, from this viewpoint, it is far from being complete.

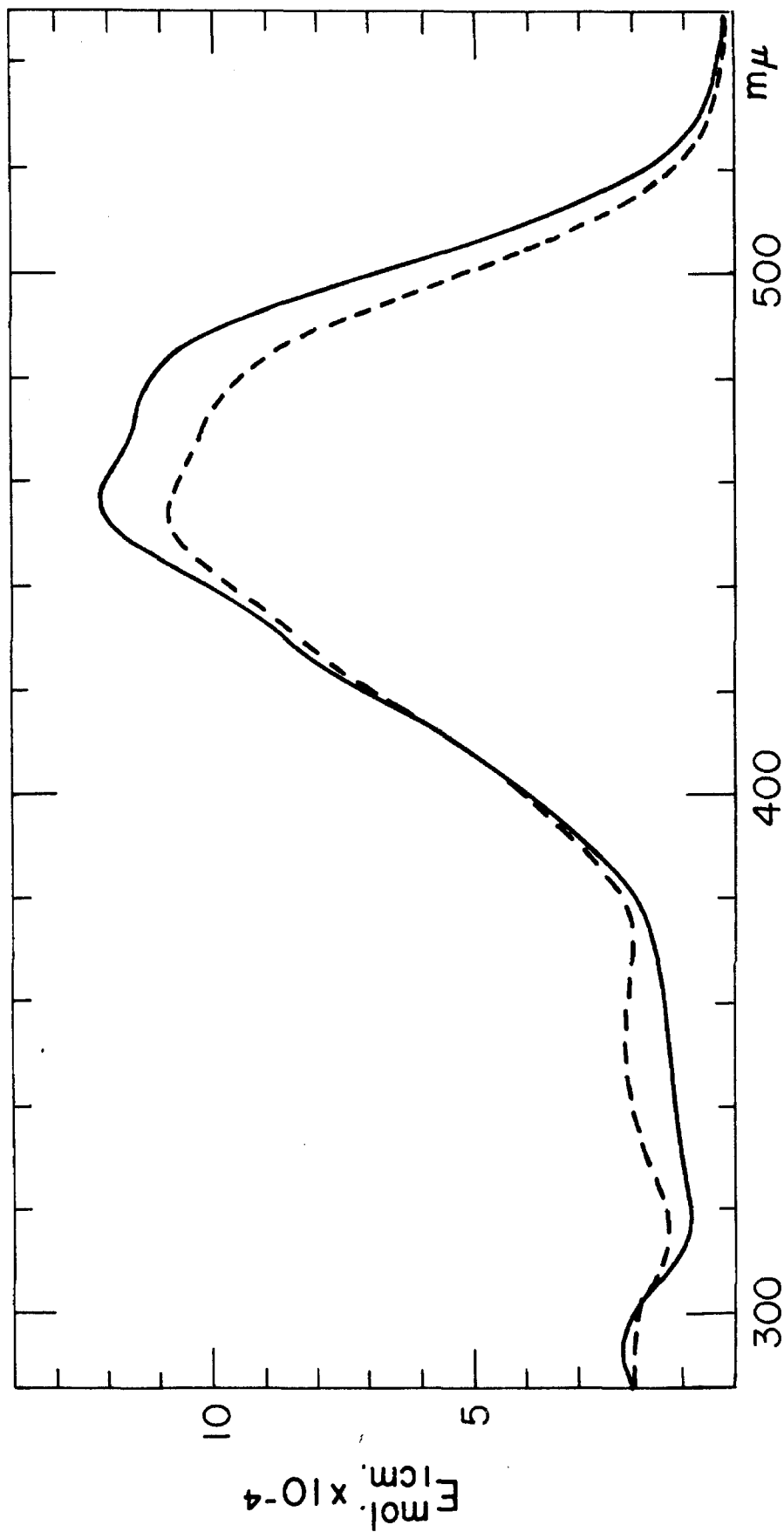


Figure 1. Molecular extinction curve in hexane of all-trans-4-keto-β-carotene: —, fresh solution; ----, after iodine catalysis in light.

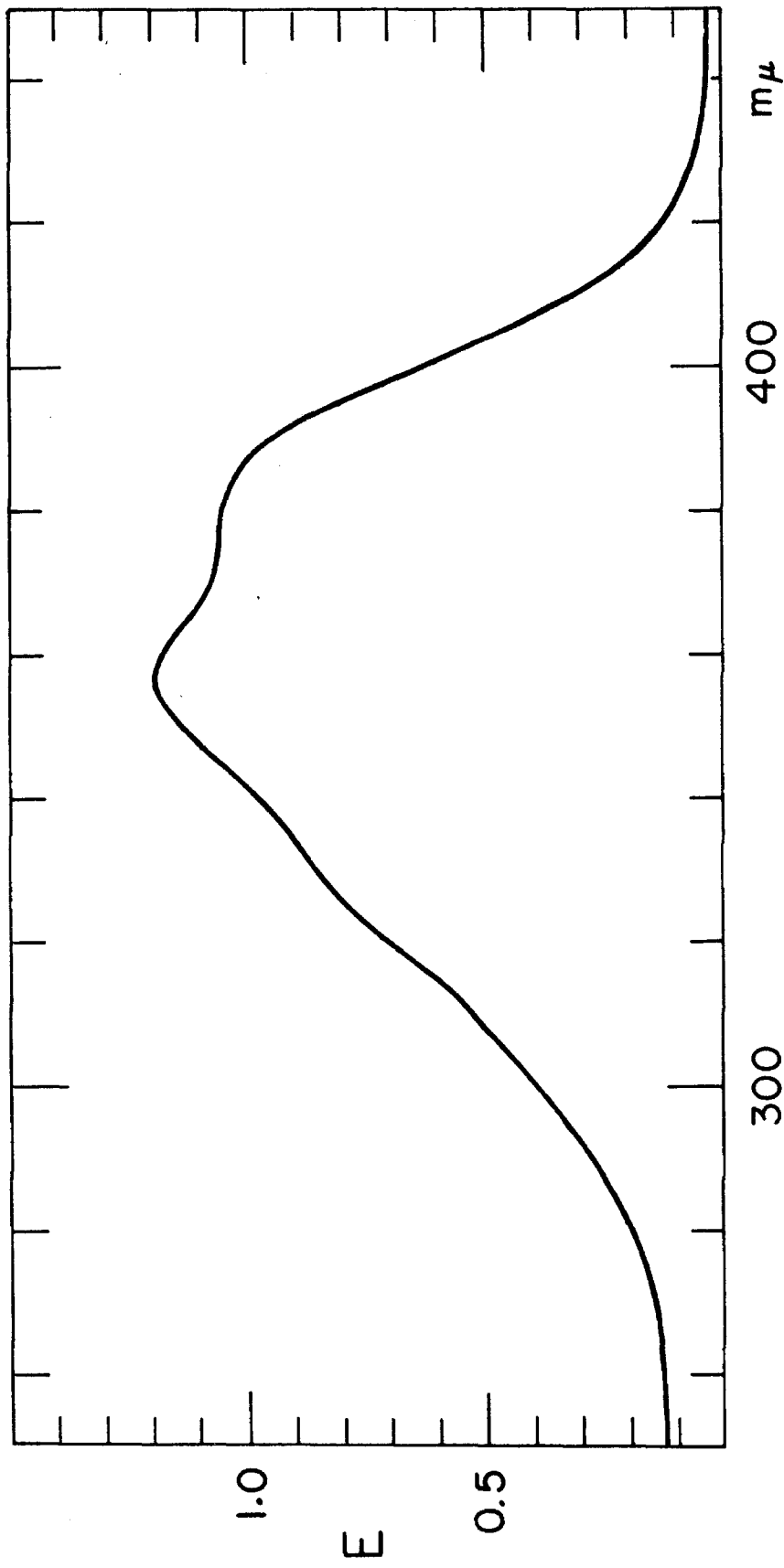


Figure 2. Extinction curve in hexane of 4-keto- β -carotene oxime.

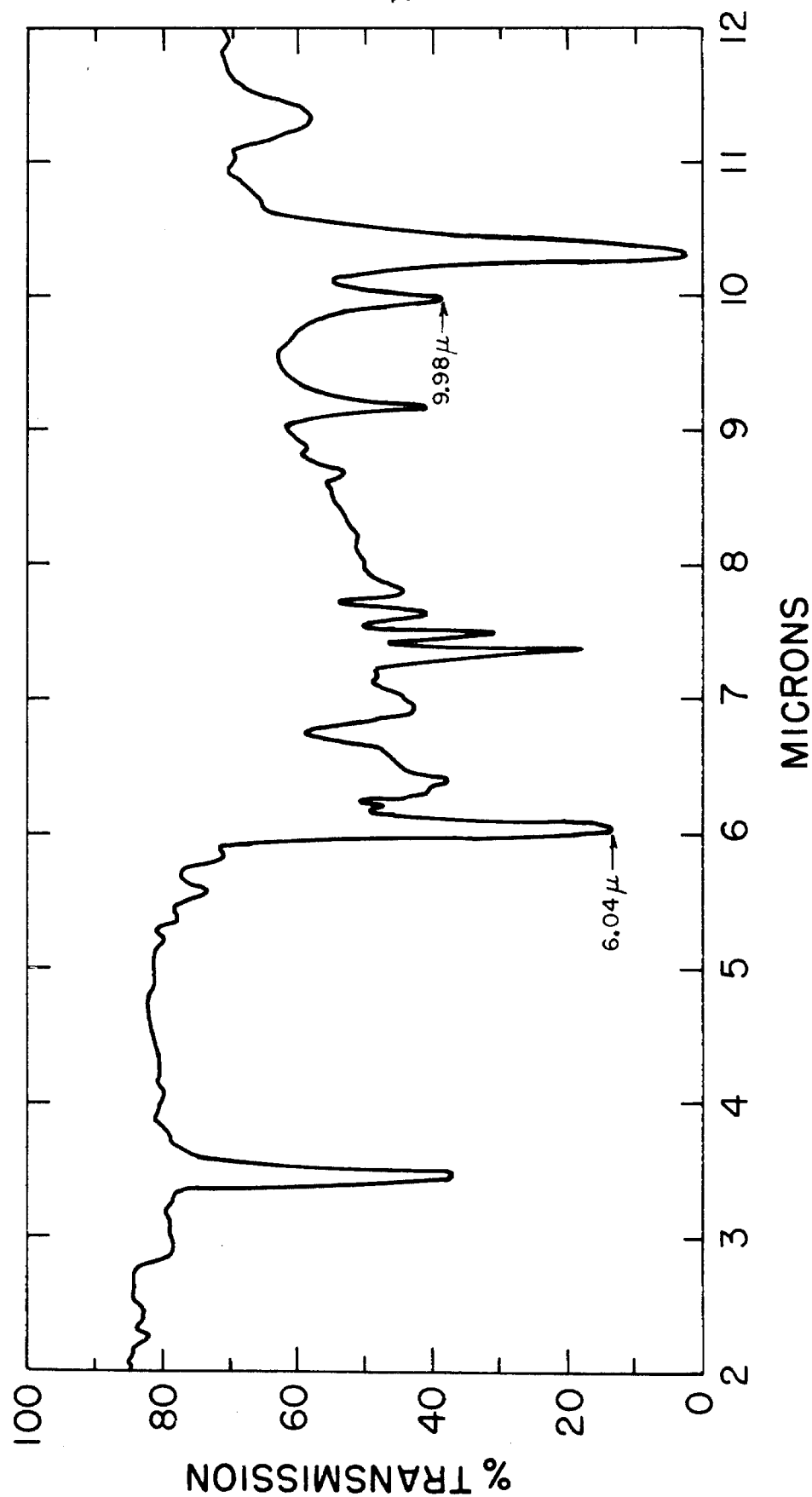


Figure 3. Infrared curve in chloroform of all-trans-4-keto- β -carotene.

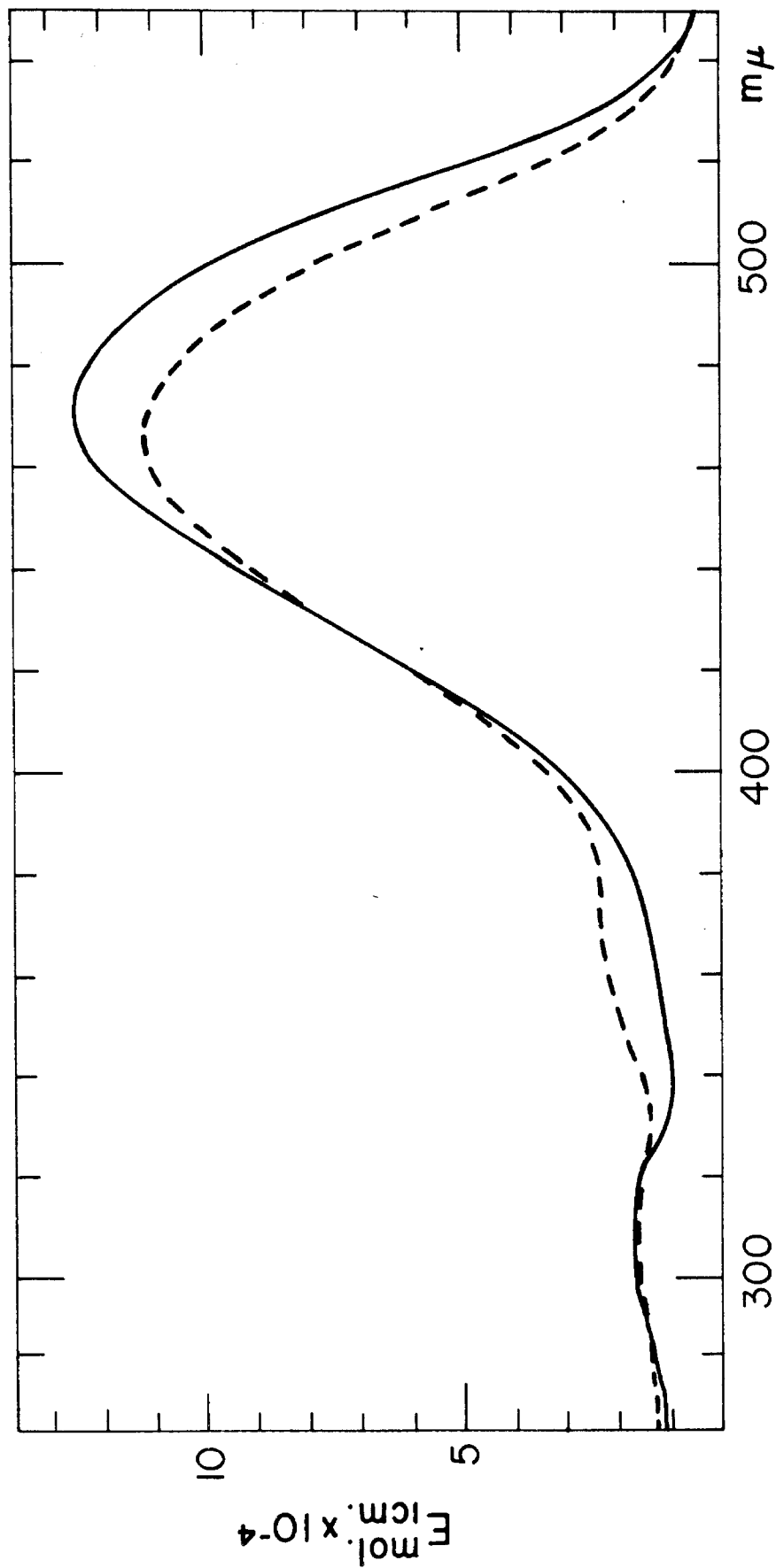


Figure 4. Molecular extinction curve in hexane of all-trans-4-keto-3',4'-dehydro-β-carotene: —, fresh solution; ----, after iodine catalysis in light.

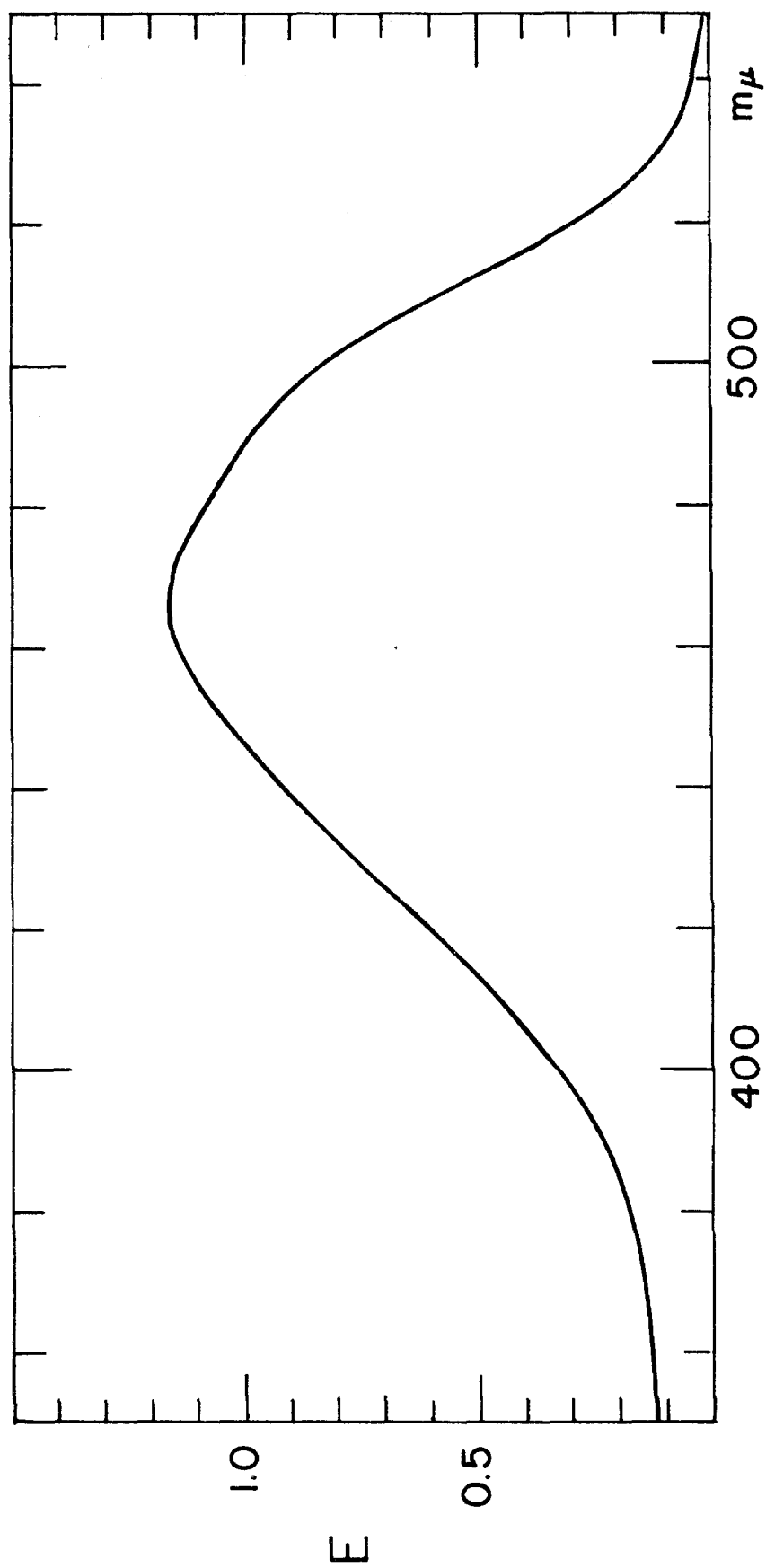


Figure 5. Extinction curve in hexane of 4-keto-3',4'-dehydro- β -carotene oxime.

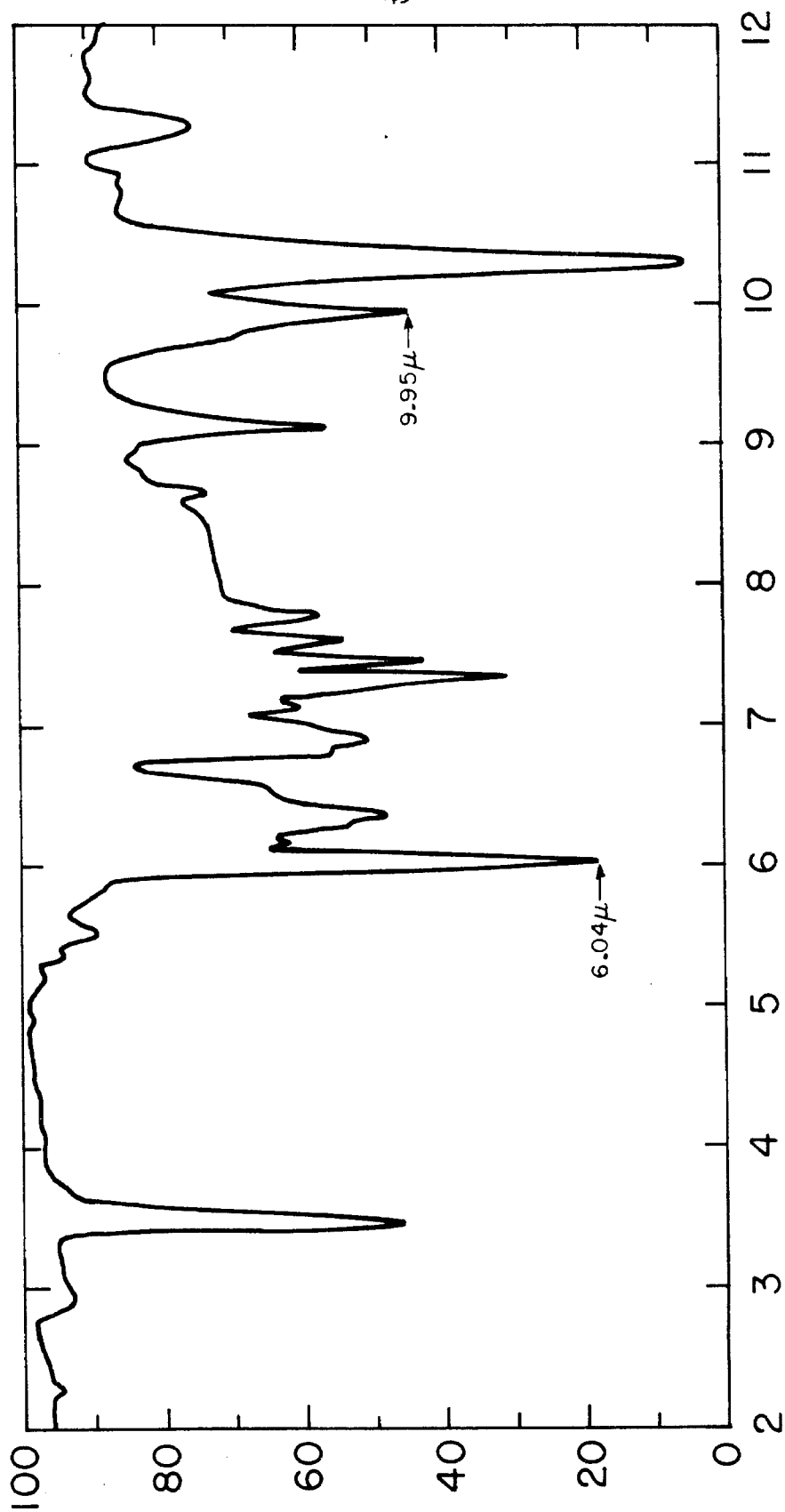


Figure 6. Infrared curve in chloroform of all-trans-4-keto-3',4'-dehydro- β -carotene.

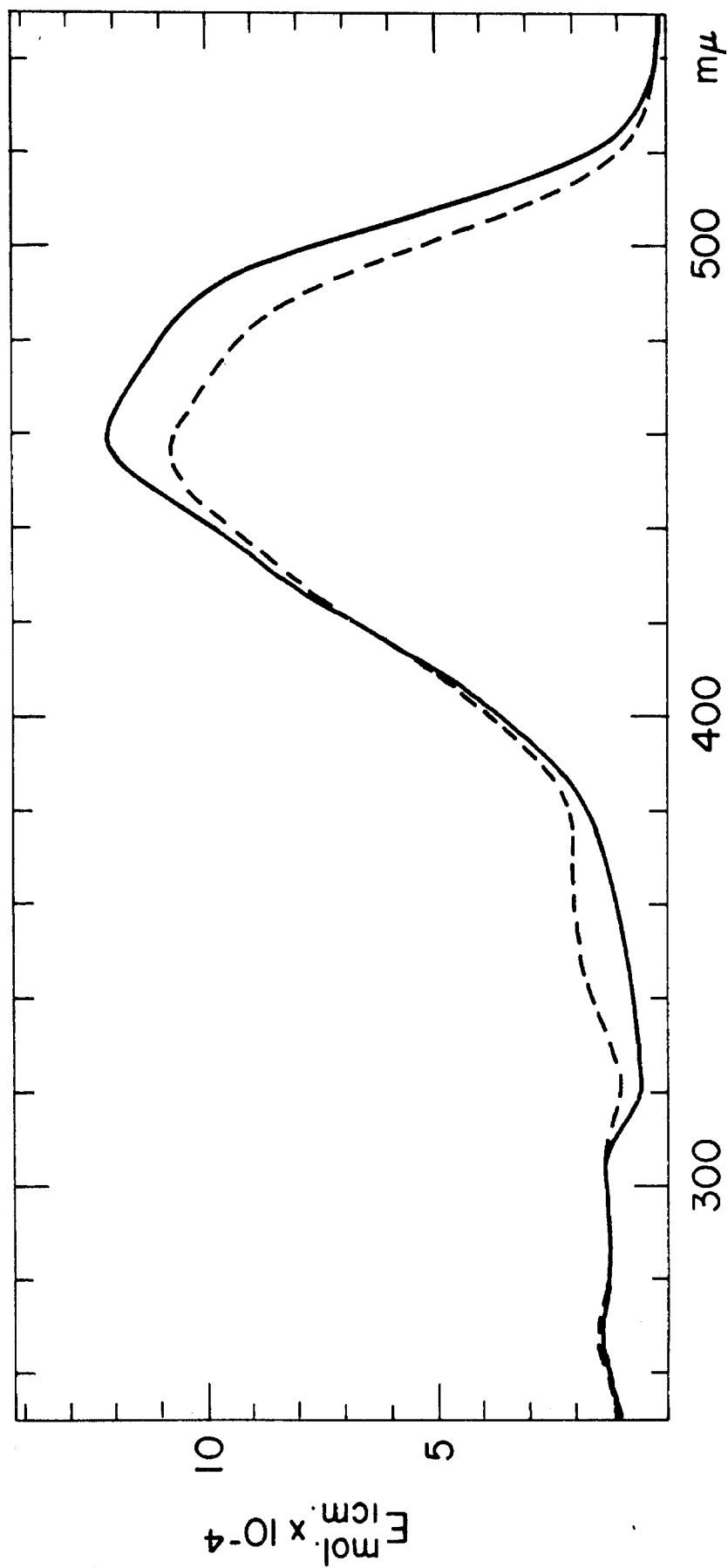


Figure 7. Molecular extinction curve in hexane of all-trans-4-hydroxy-3',4'-dehydro-β-carotene: —, fresh solution; ----, after iodine catalysis in light.

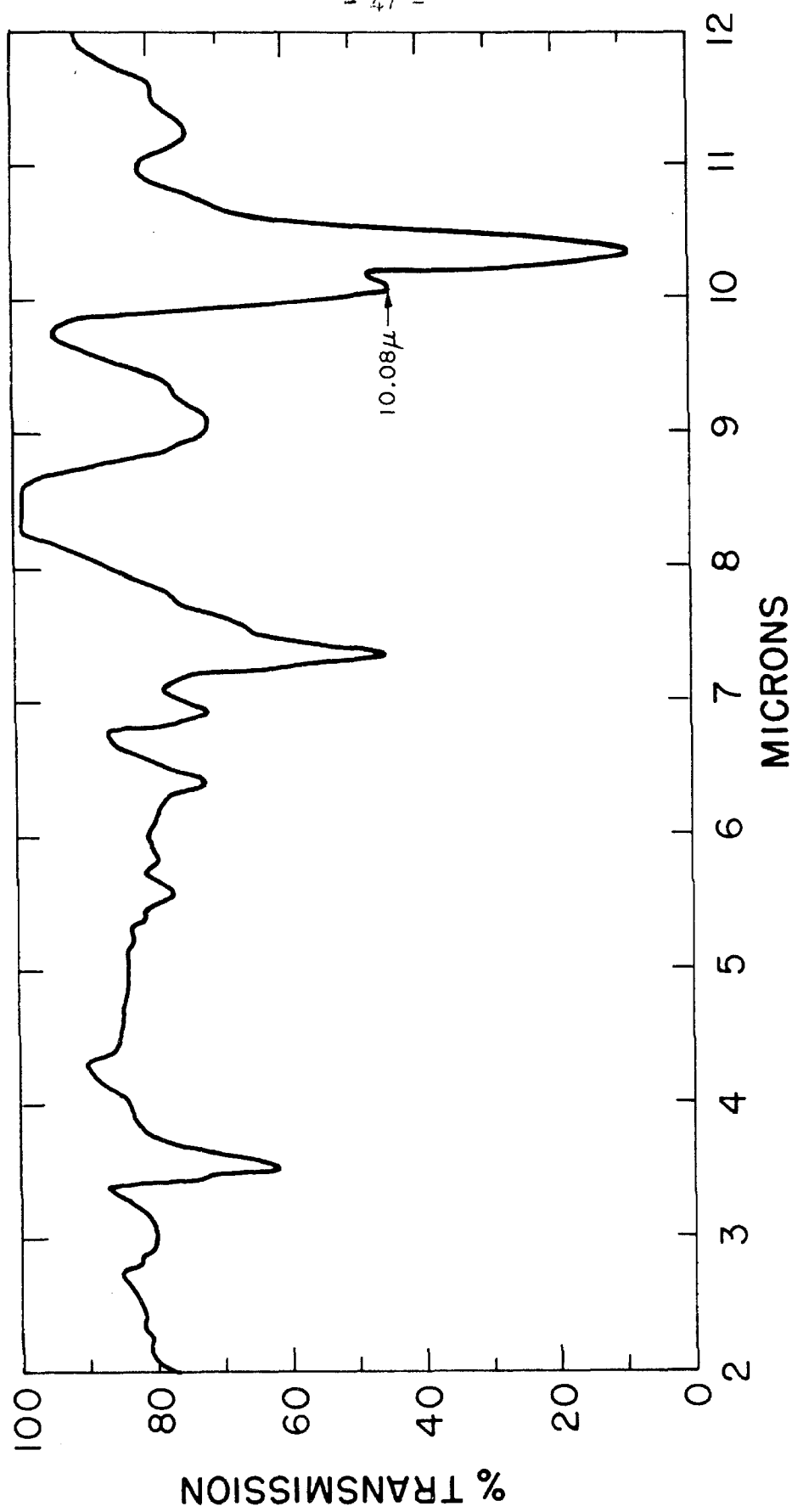


Figure 8. Infrared curve in chloroform of all-trans-4-hydroxy-3',4'-dehydro- β -carotene.

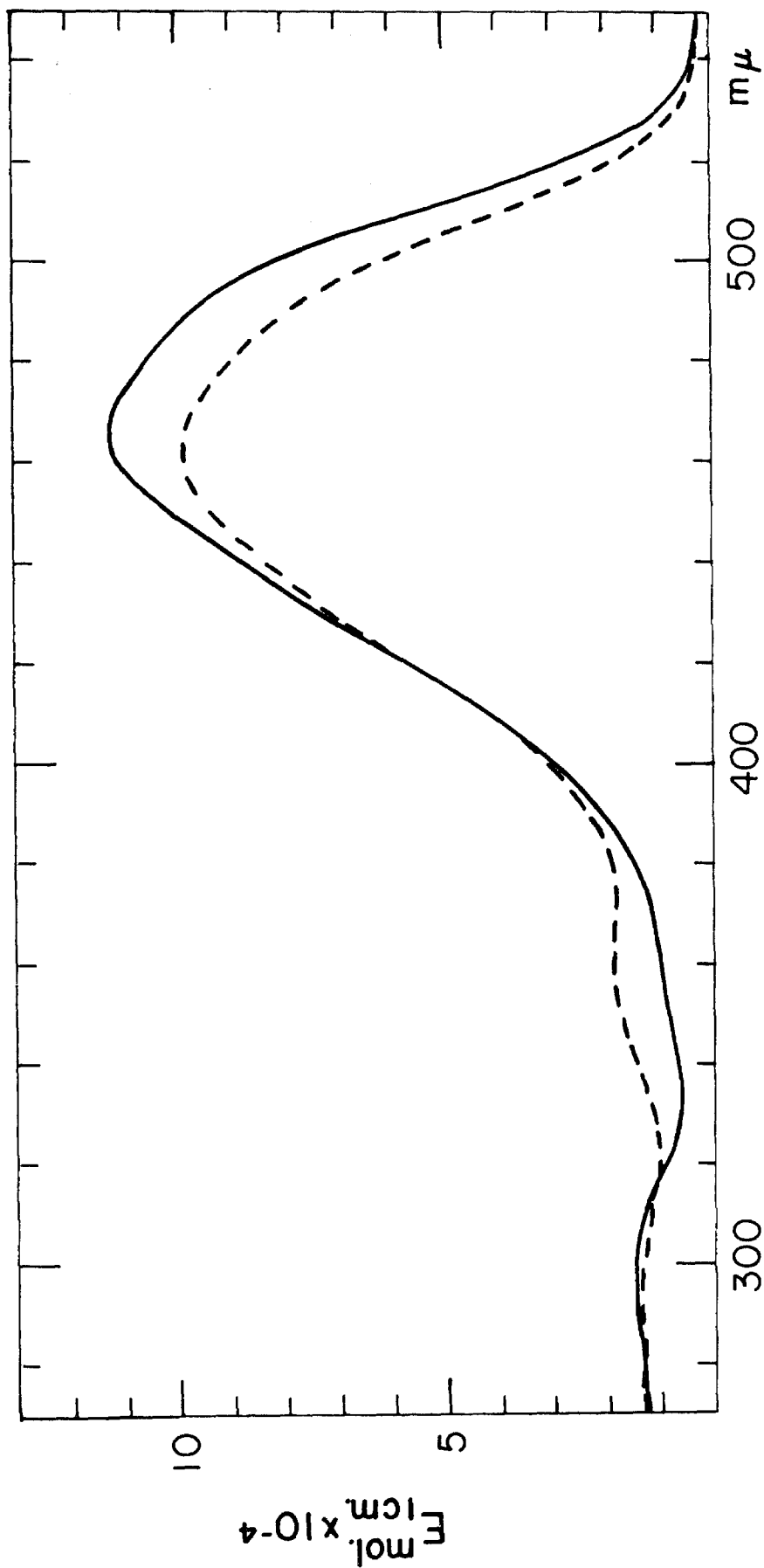


Figure 9. Molecular extinction curve in hexane of all-trans-4,4'-diketo- β -carotene: —, fresh solution; ----, after iodine catalysis in light.

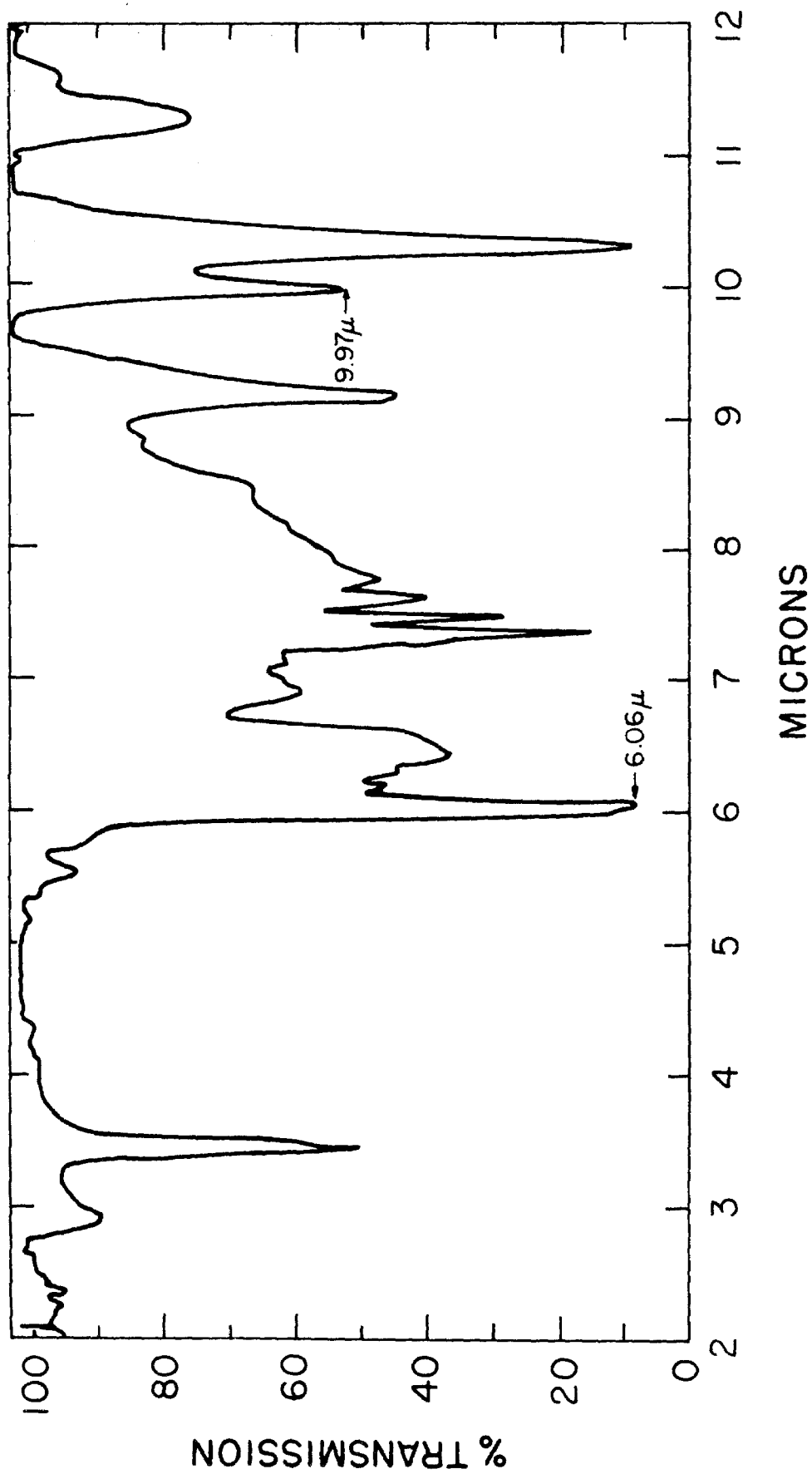


Figure 10. Infrared curve in chloroform of all-trans-4,4'-diketo- β -carotene.

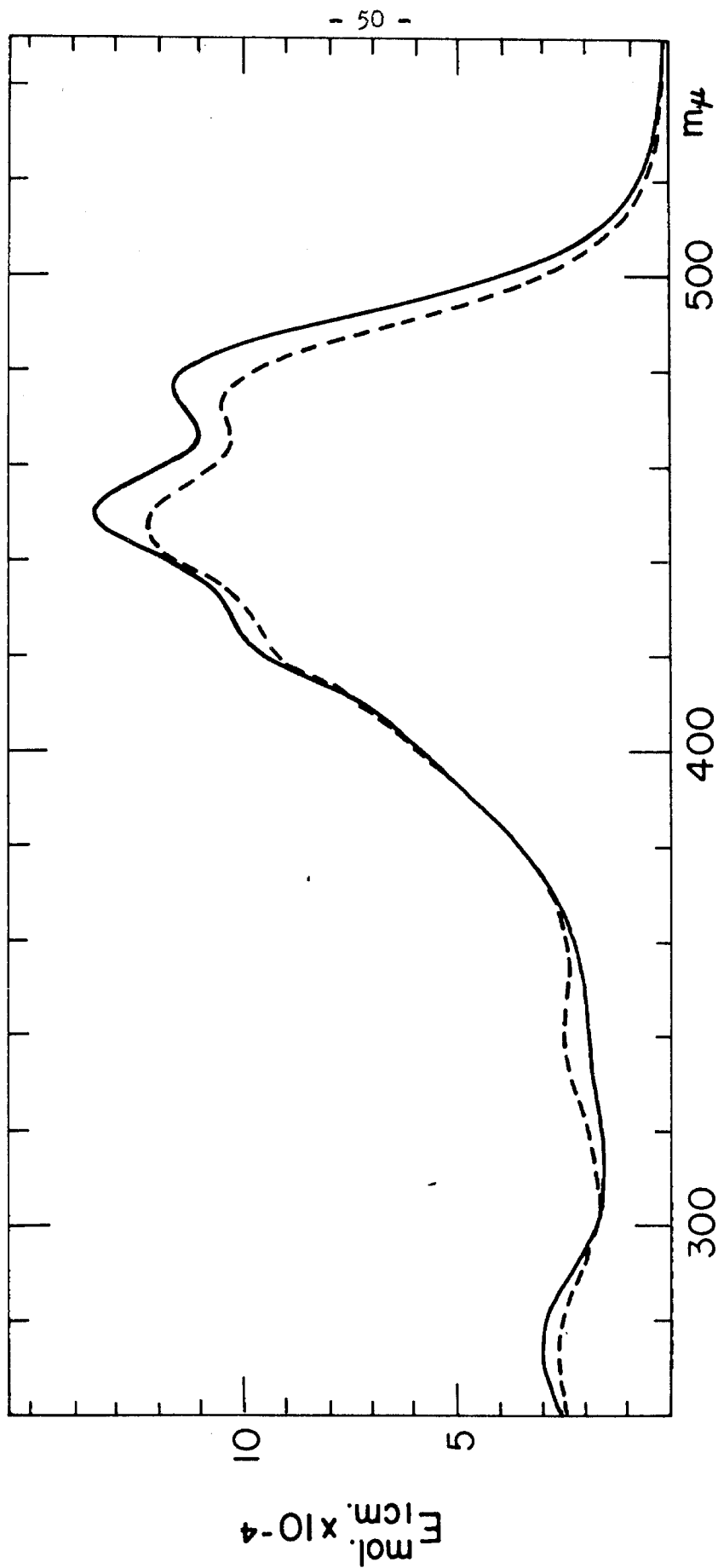


Figure 11. Molecular extinction curve in hexane of all-trans-4,4'-dihydroxy- β -carotene: —, fresh solution; ----, after iodine catalysis in light.

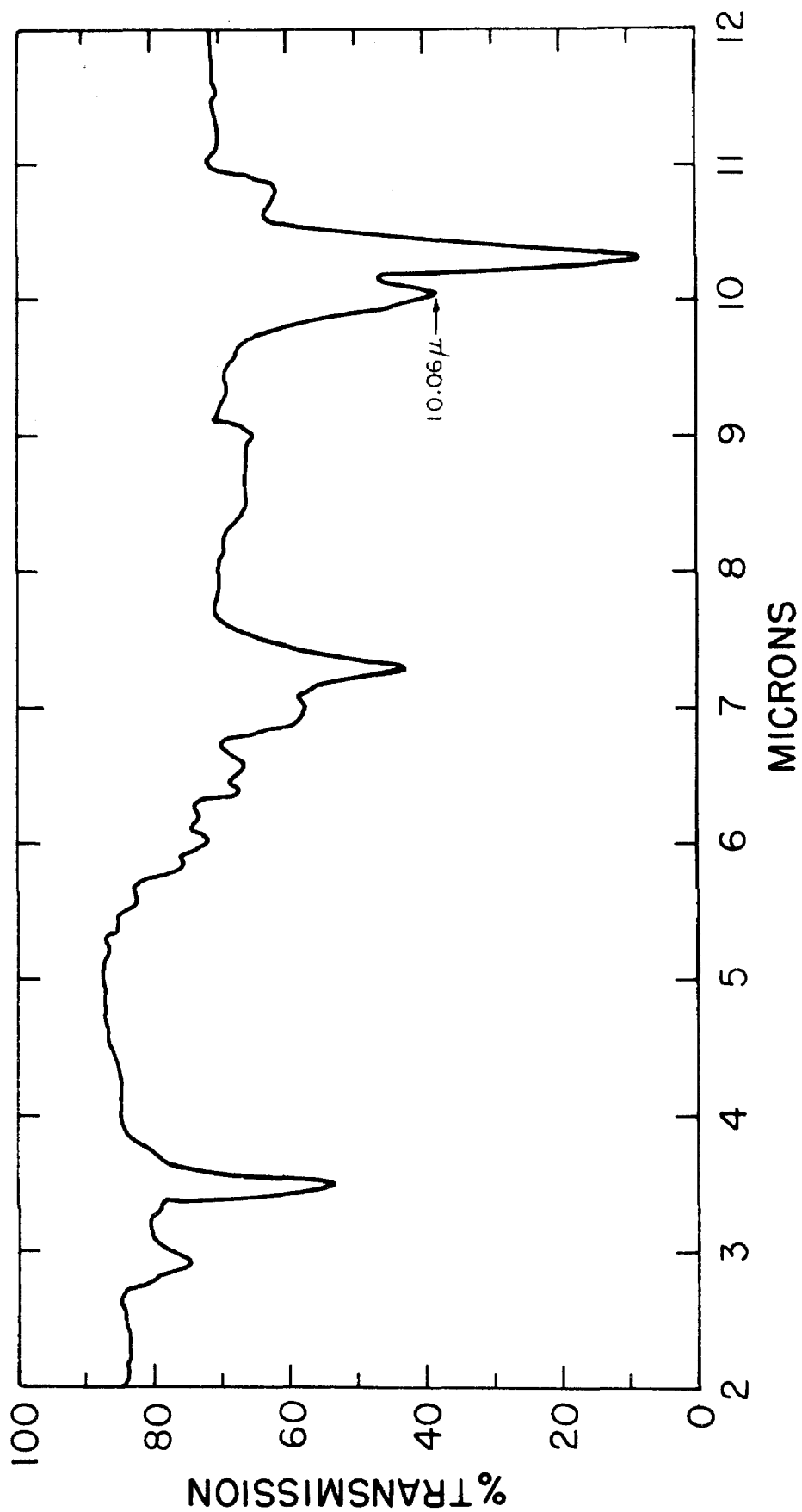


Figure 12. Infrared curve in chloroform of all-trans-4,4'-dihydroxy- β -carotene.

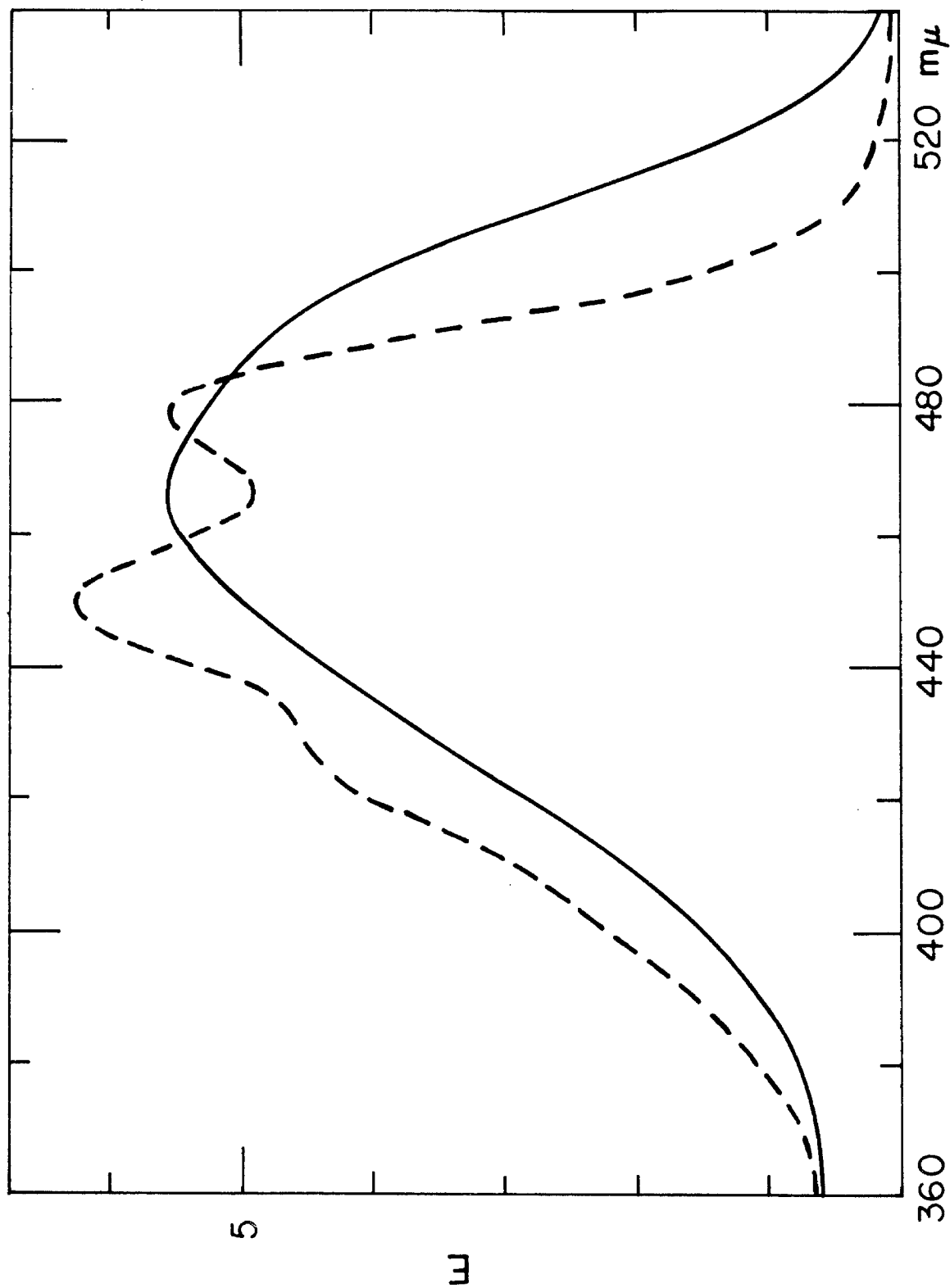


Figure 13. Extinction curve in hexane of naturally occurring 4,4'-diketo- β -carotene (—) and the lithium aluminum hydride reduction product (----).

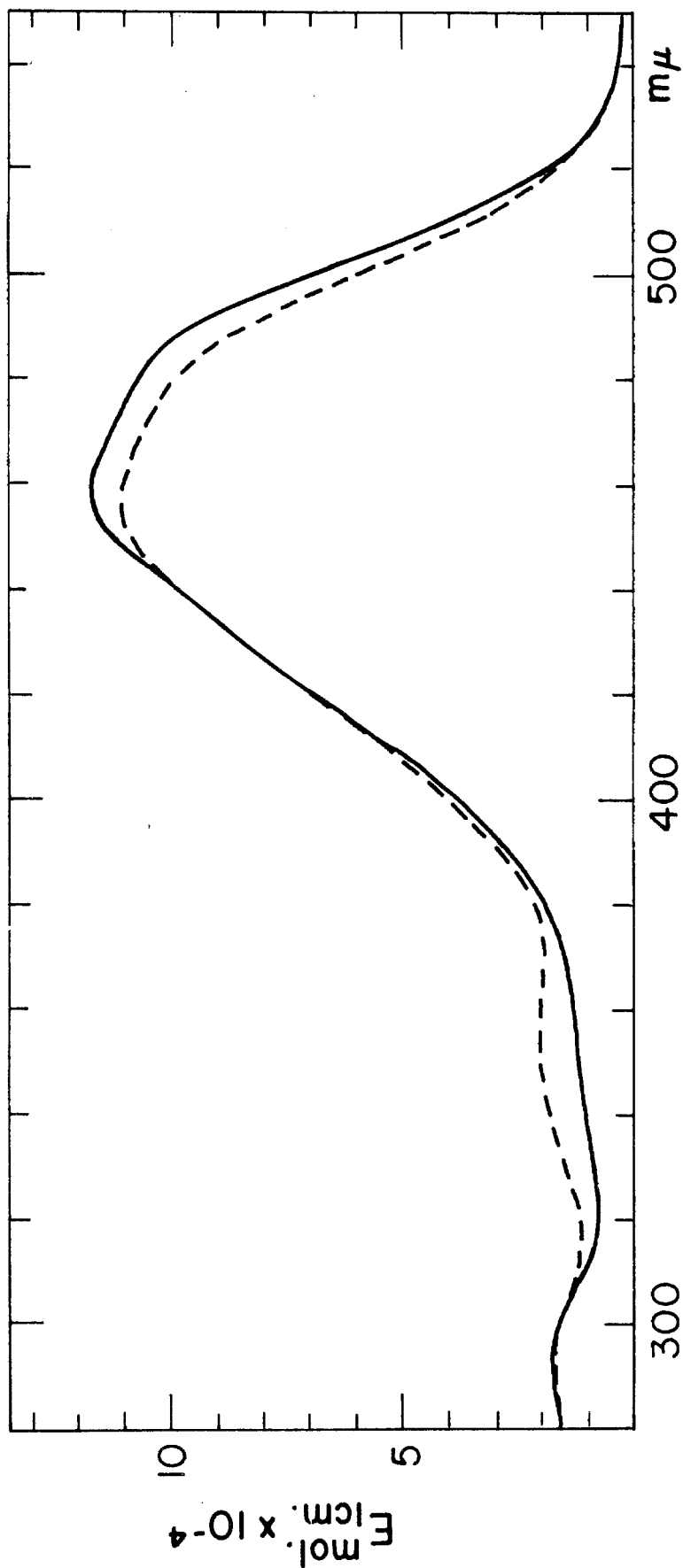


Figure 14. Molecular extinction curve in hexane of all-trans-4-keto-4'-hydroxy-β-carotene: —, fresh solution; ----, after iodine catalysis in light.

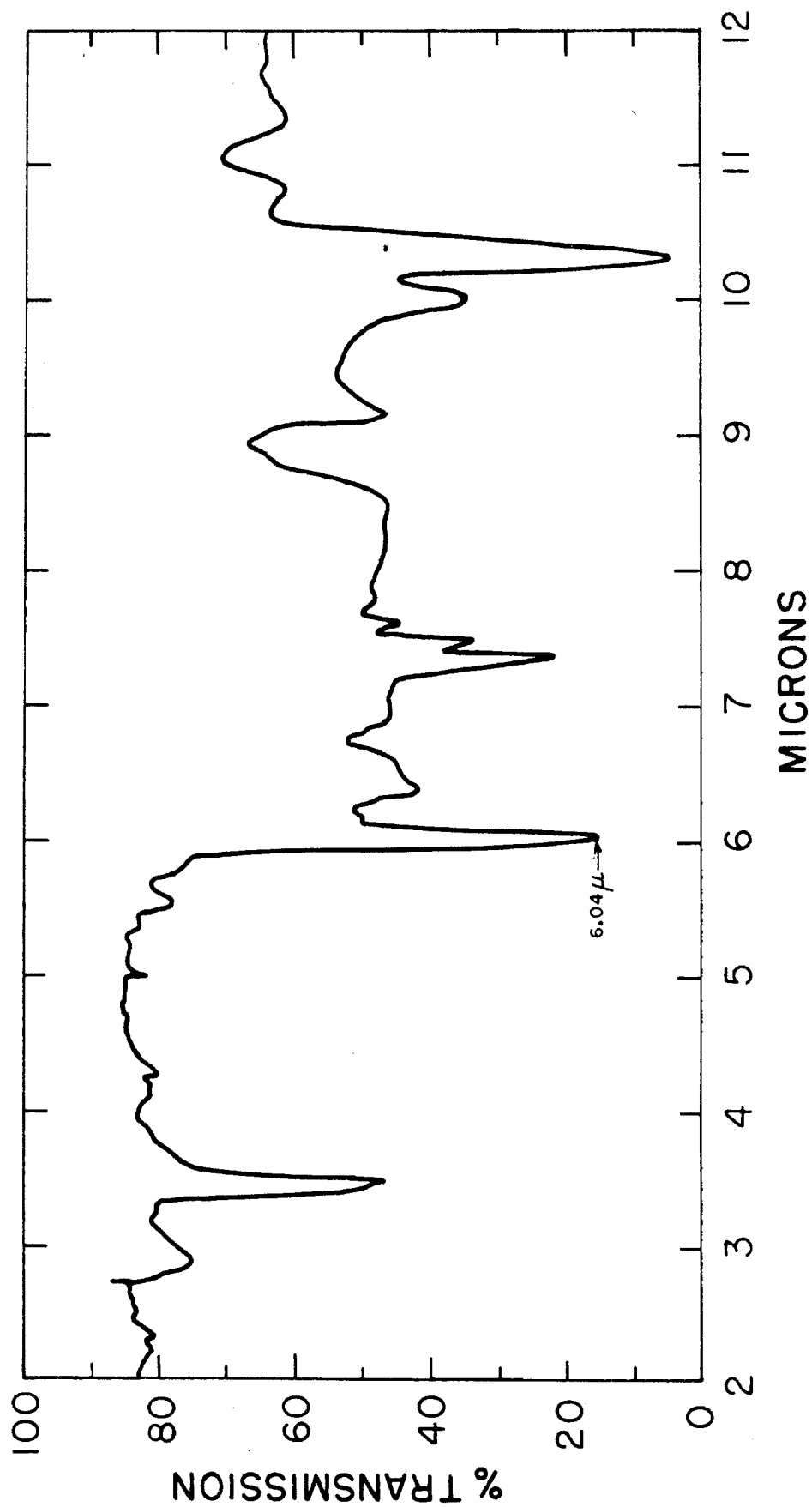


Figure 15. Infrared curve in chloroform of all-trans-4-keto-4'-hydroxy- β -carotene.

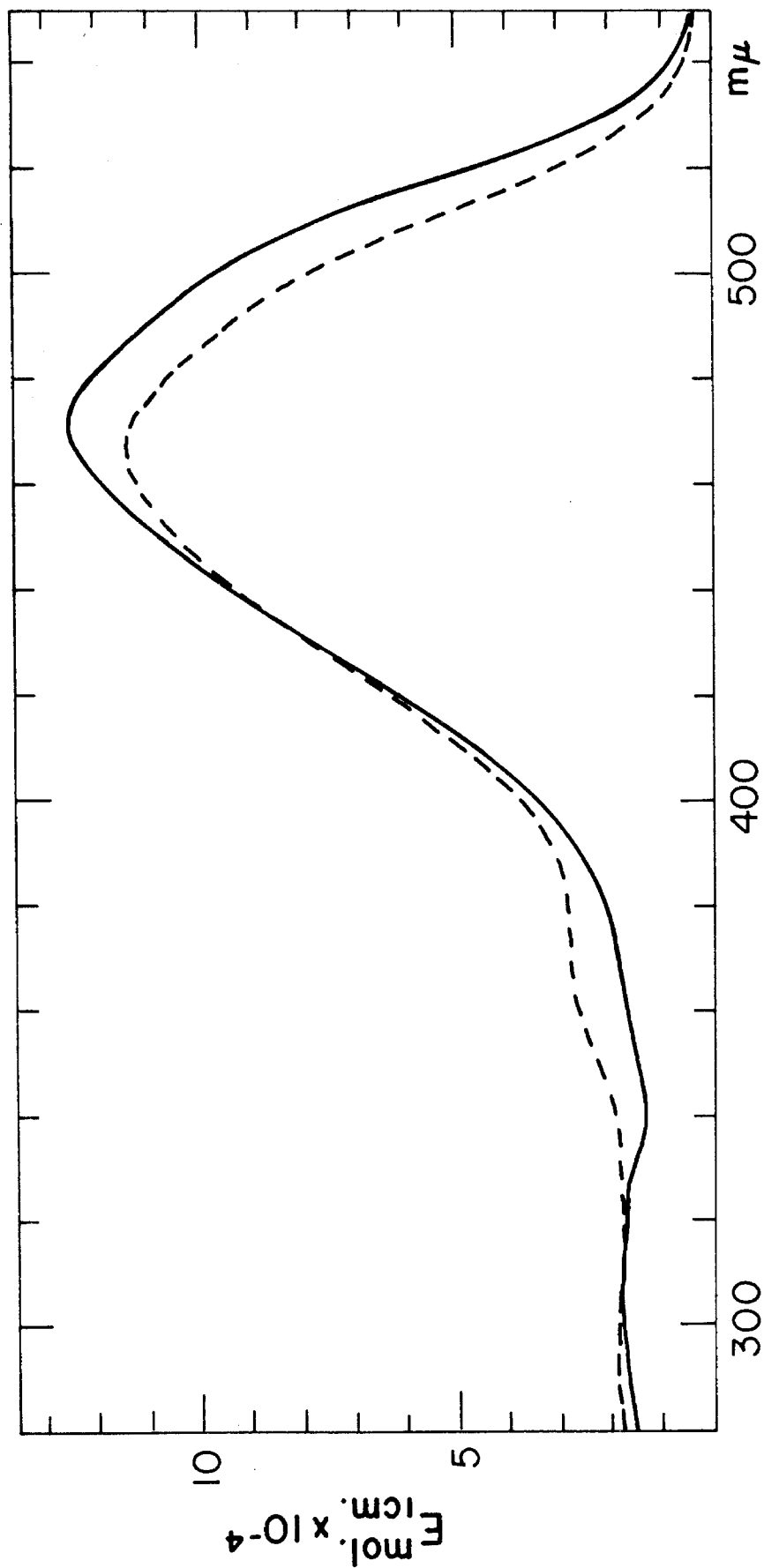


Figure 16. Molecular extinction curve in hexane of 3,4,3',4'-bisdehydro- β -carotene: —, fresh solution; ----, after iodine catalysis in light.

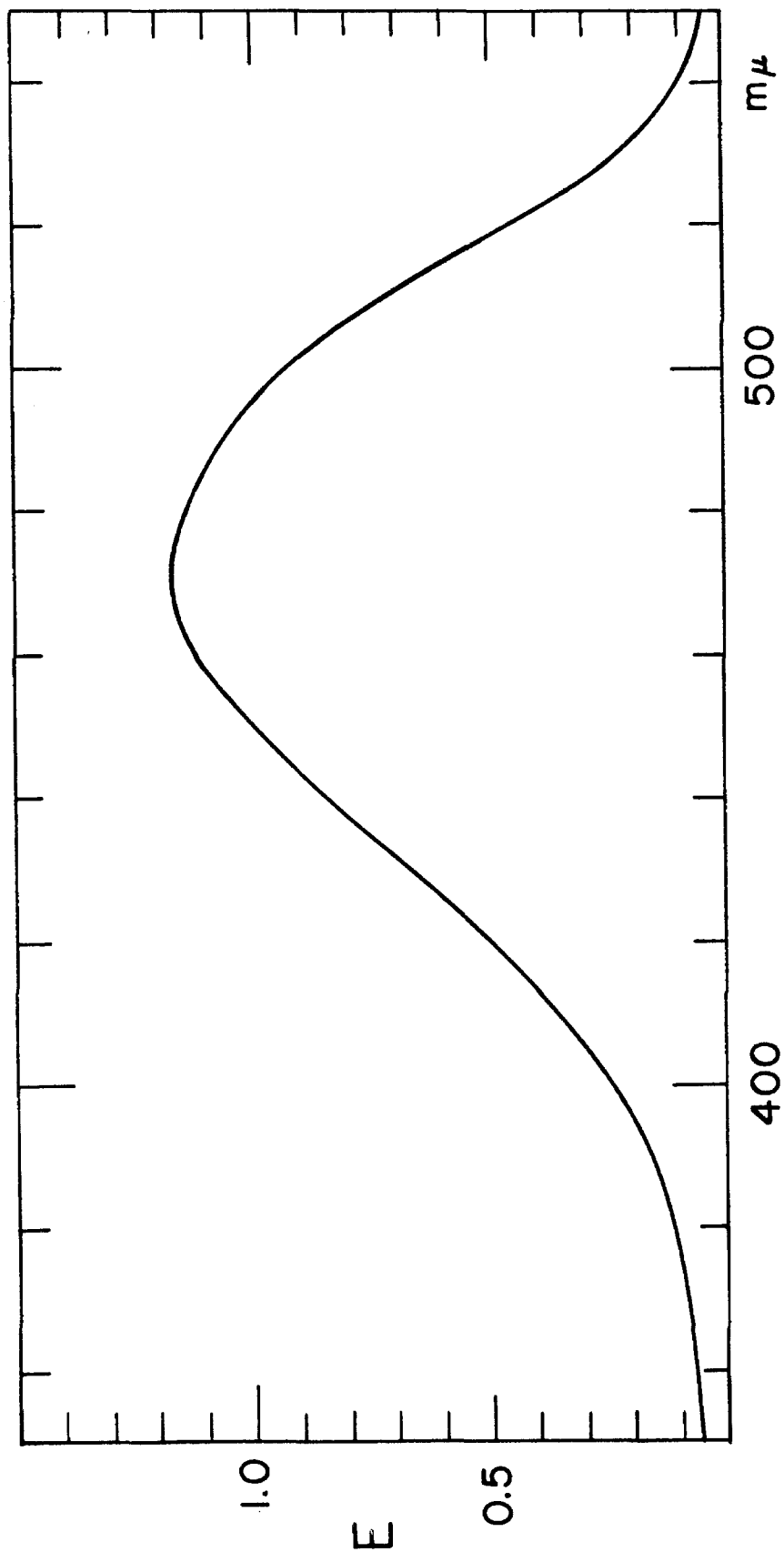


Figure 17. Extinction curve in hexane of all-trans-4-keto-4'-ethoxy-3',4'-dehydro- β -carotene.

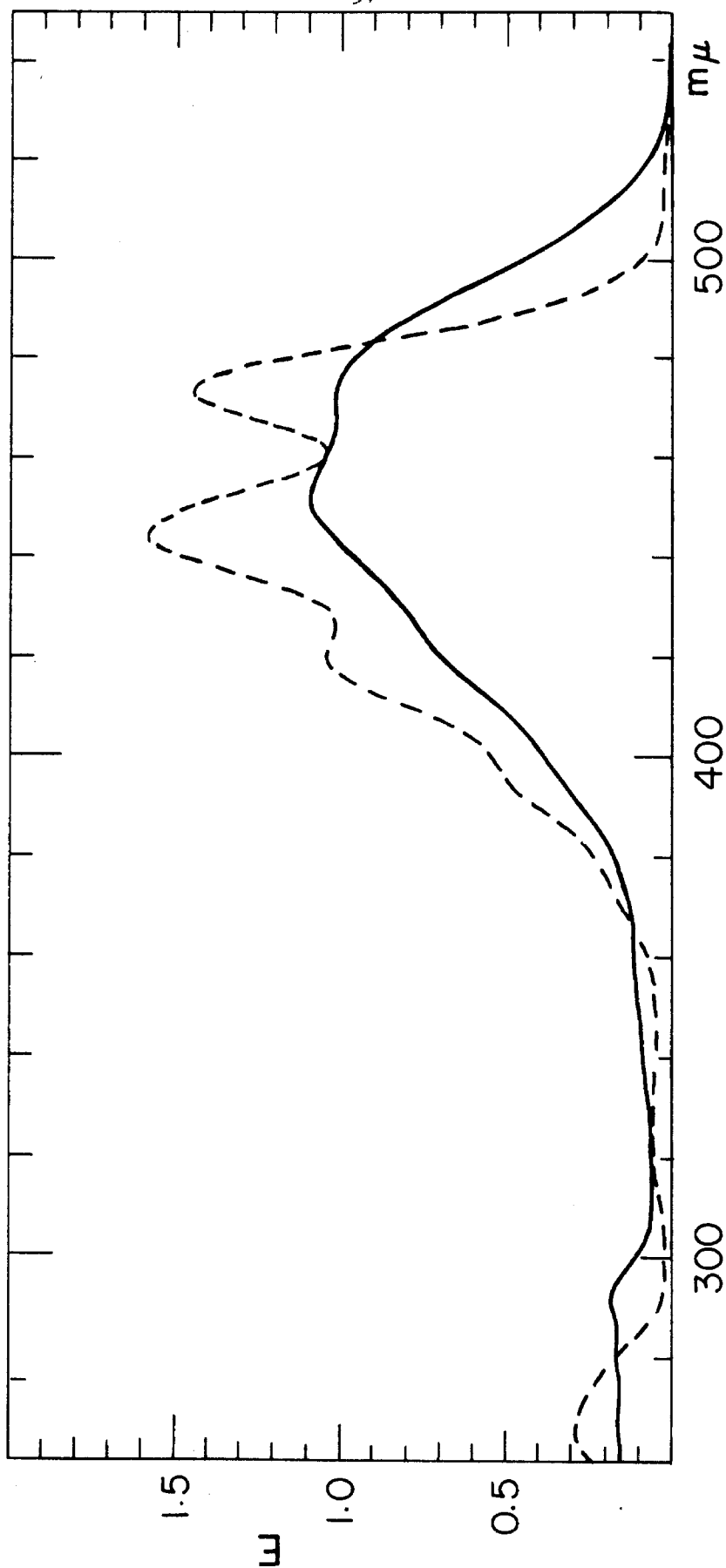


Figure 18. Extinction curve in hexane of all-trans-4-keto- α -carotene (—) and of all-trans-4-hydroxy- α -carotene (---).

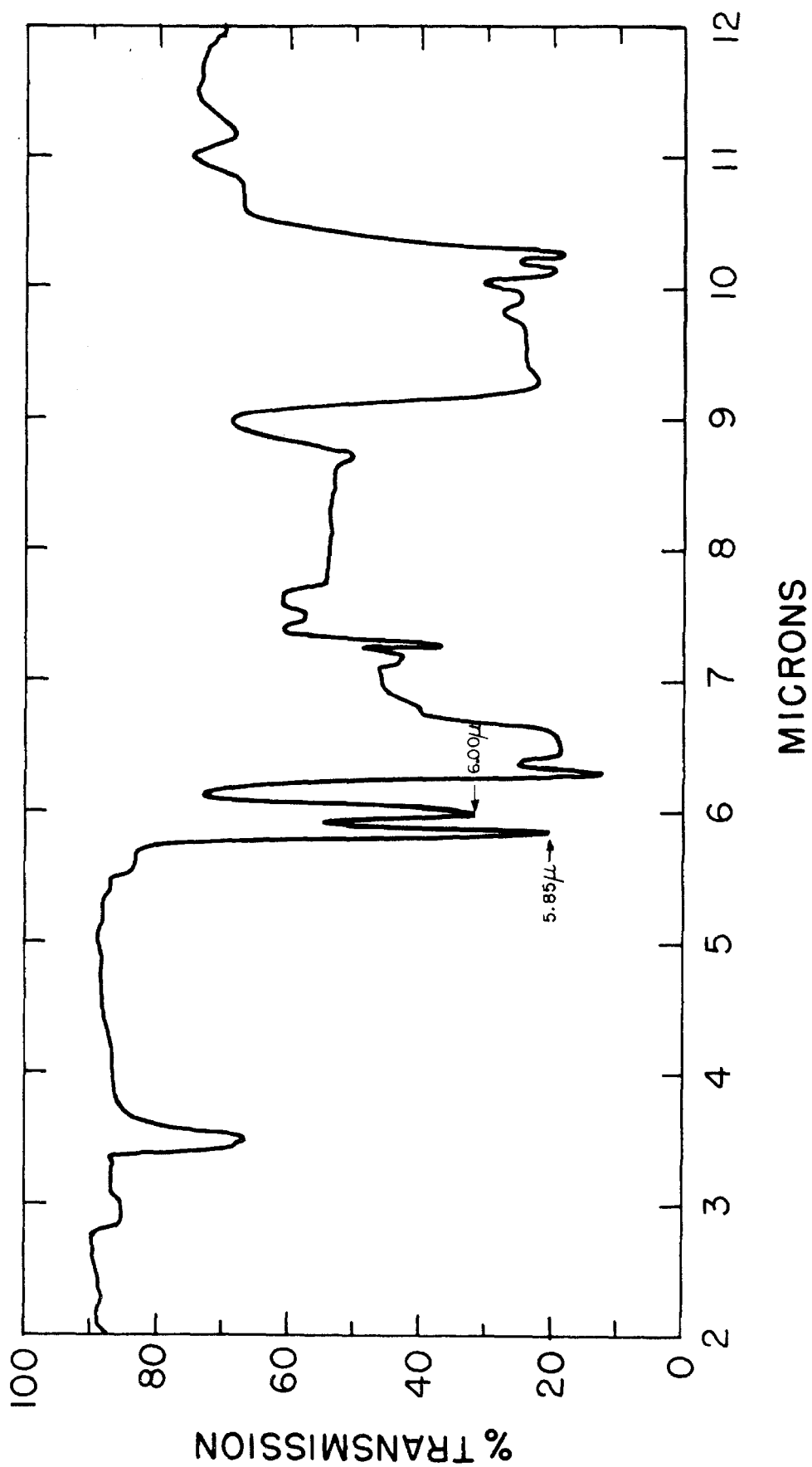


Figure 19. Infrared curve in chloroform of all-trans- β -carotenone.

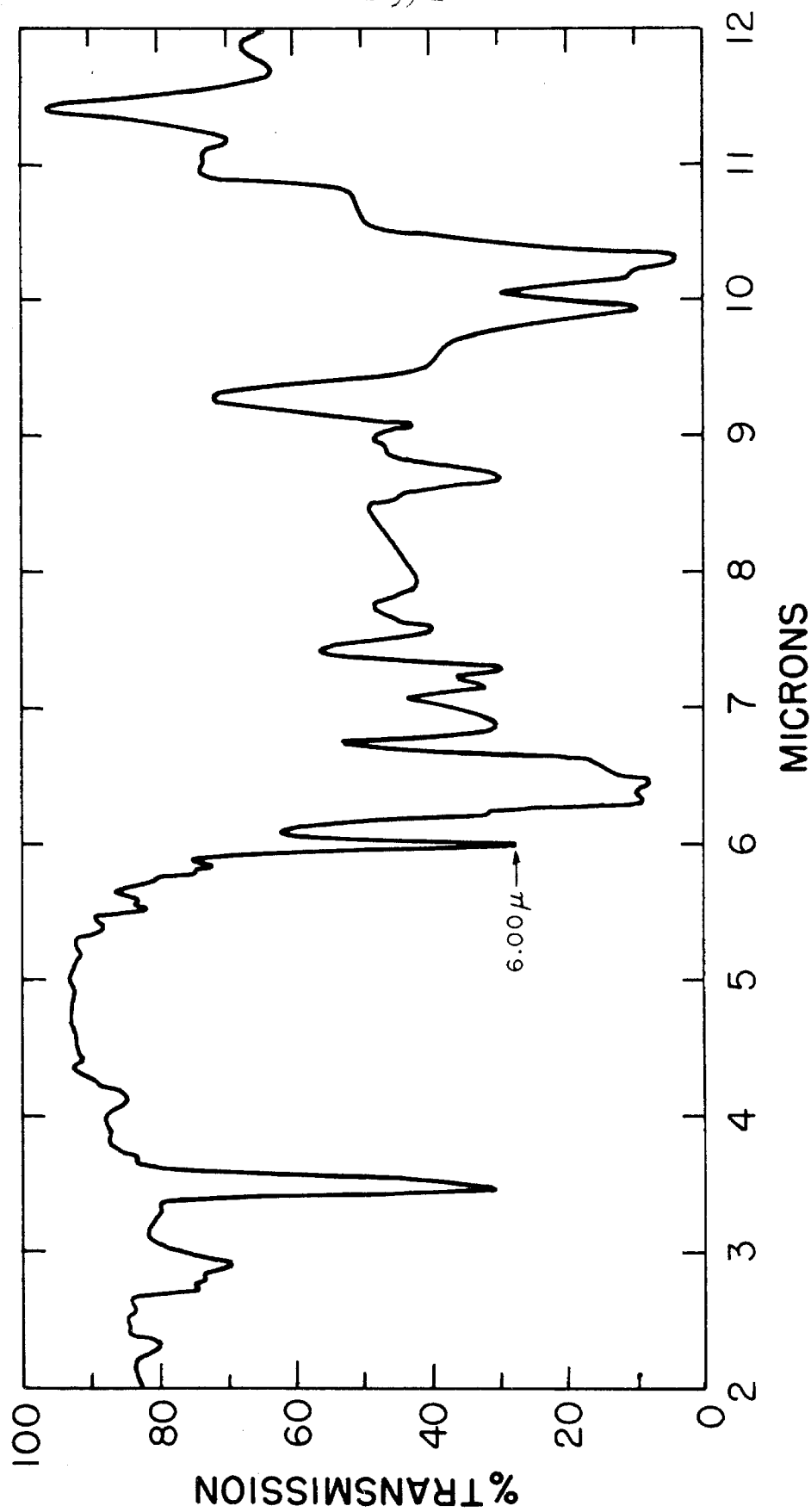


Figure 20. Infrared curve in chloroform of all-trans-capsanthin.

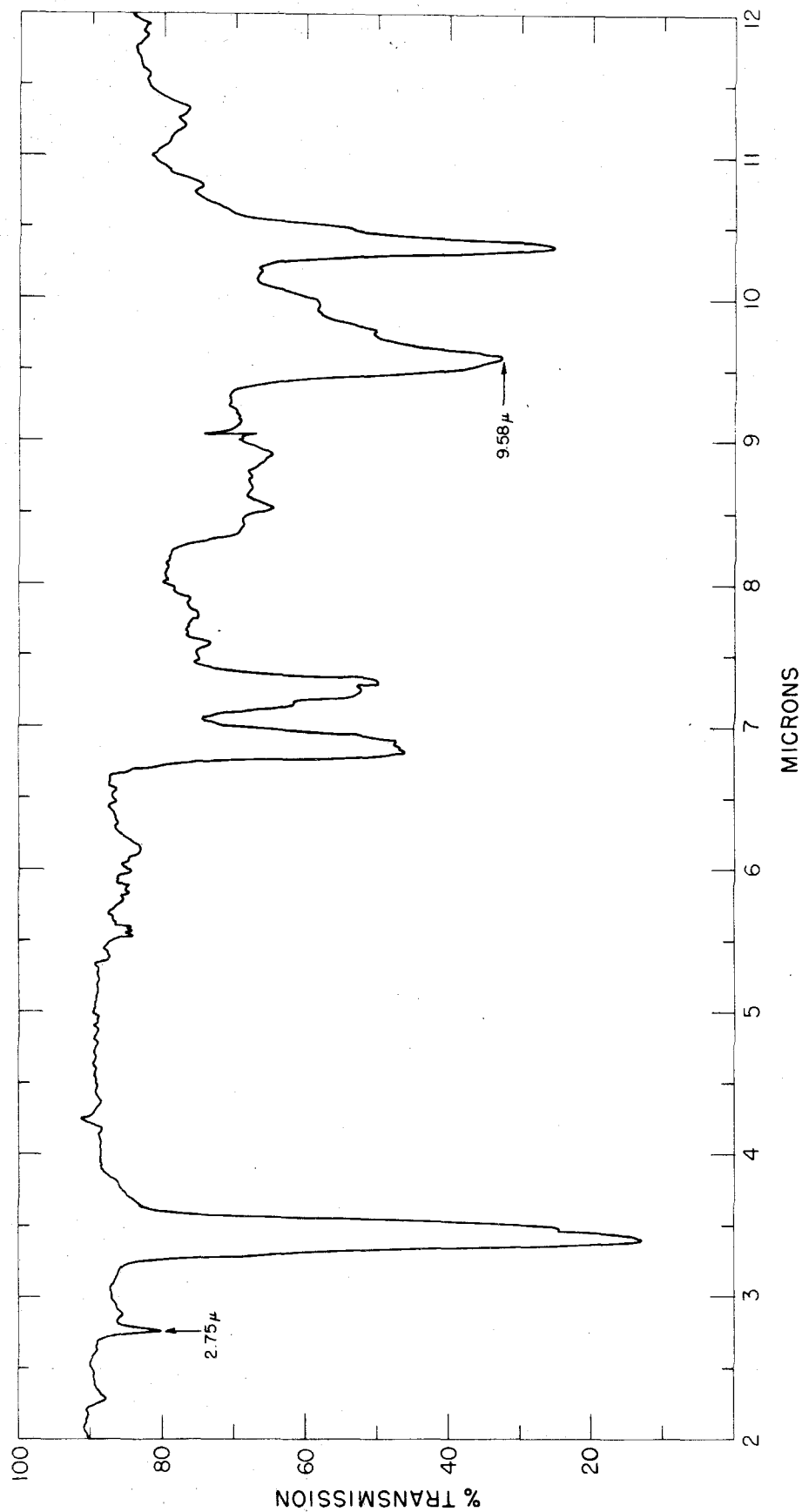


Figure 21. Infrared curve in carbon tetrachloride of all-trans-cryptoxanthin (β -hydroxy- β -carotene).

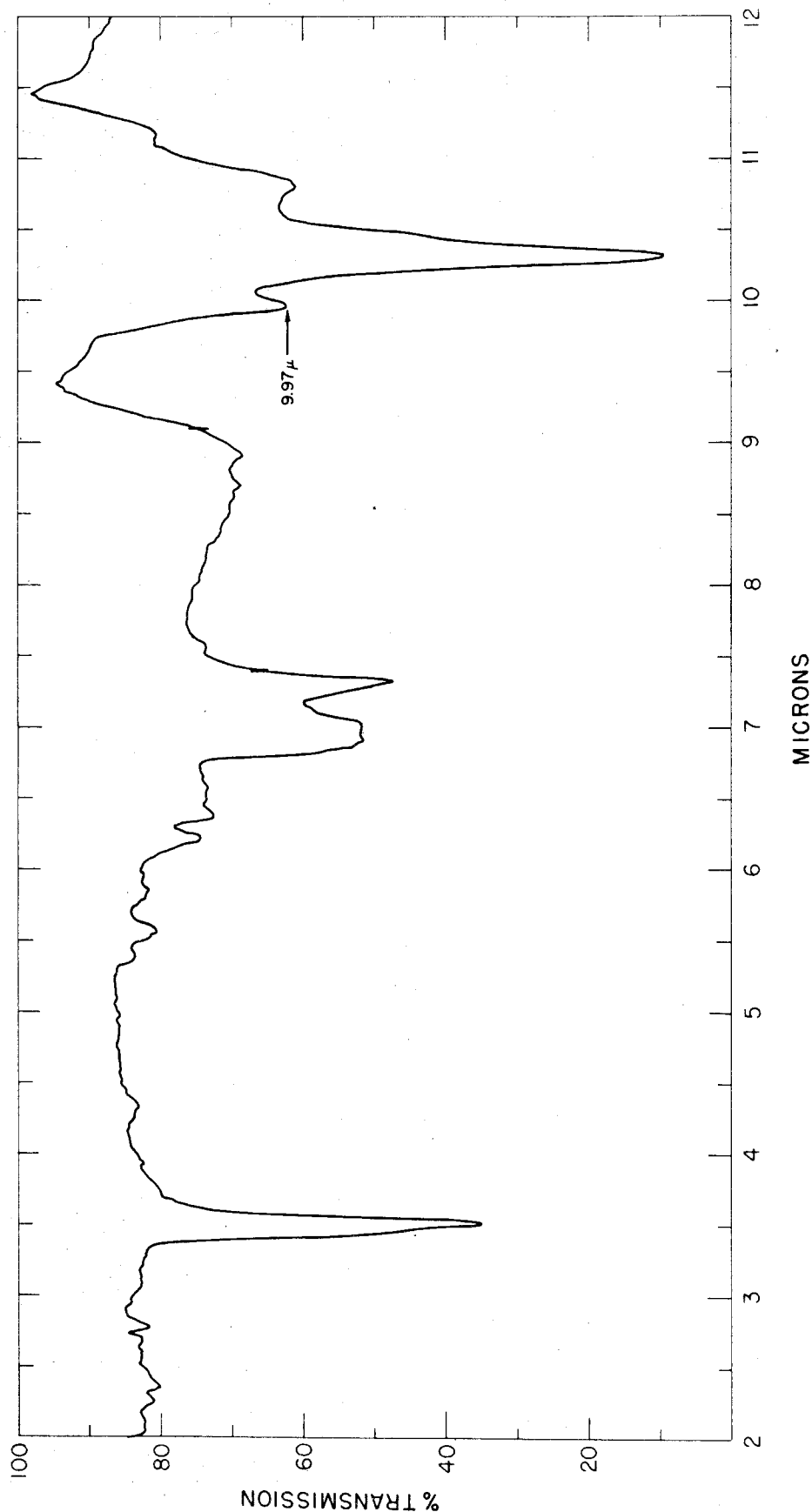


Figure 22. Infrared curve in chloroform of all-trans-cryptoxanthin
(3-hydroxy- β -carotene).

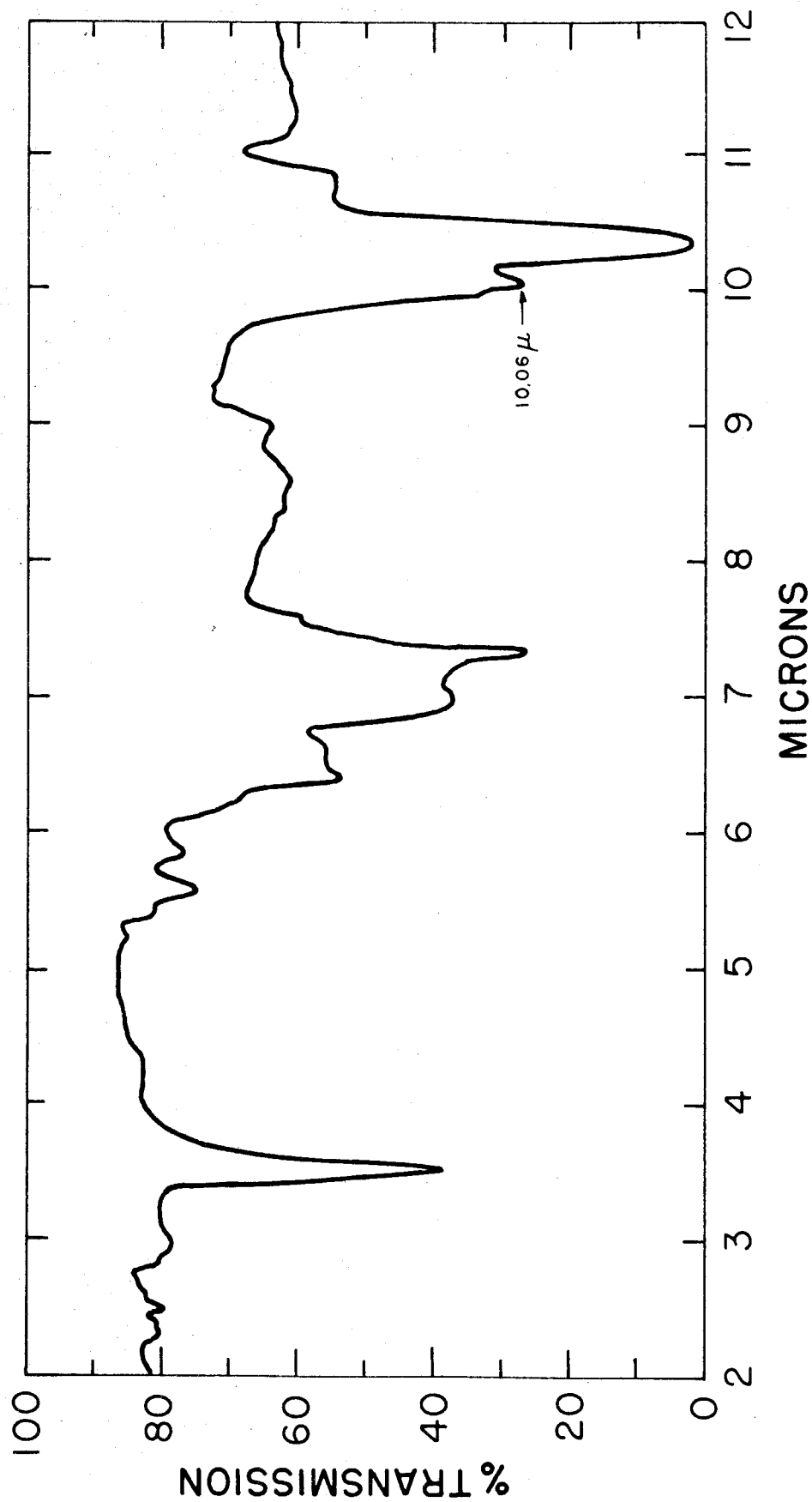
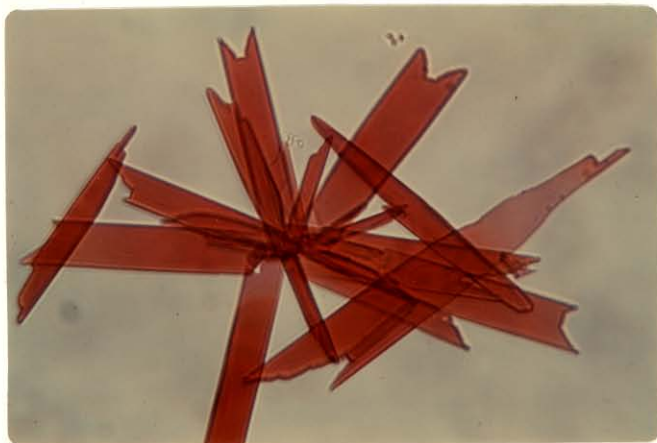



Figure 23. Infrared curve in chloroform of all-trans-isocryptoxanthin (4-hydroxy- β -carotene).



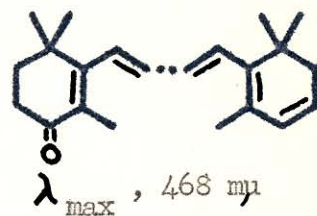
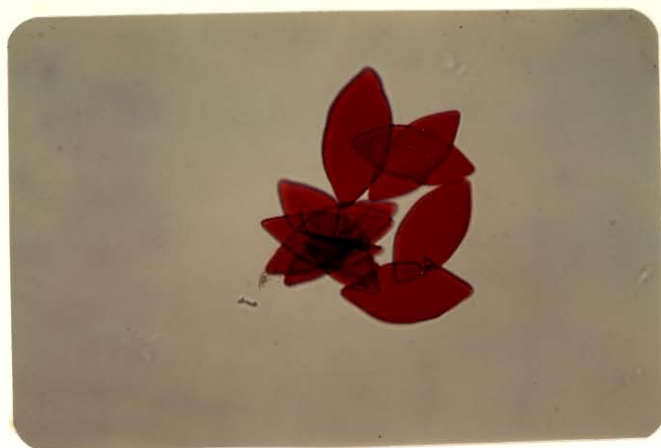
λ_{max} , 451 m μ

500X

Figure 24. β -Carotene from Methylene Chloride-Methanol*

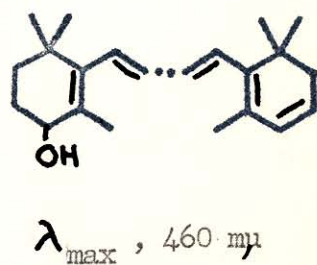
Scale 
0.1 mm

* The photomicrographs appearing in Figs. 24-28 were taken by Dr. Makio Murayama. Developing and printing was carried out by the Tricolor Laboratories, Hollywood. Transparencies were obtained from Ektachrome Type B film, from which the Ansco Color prints resulted. An 18 amp. 6 v. General Electric projection lamp was the light source operated at approximately 3000° K. A Weston film speed rating of 3 ± 1 gave satisfactory results. The color corrected optics were as follows: condenser; B & L Achromat, 8.9 mm., 1.4 N.A.; objective, B & L semi-apochromatic (Fluorite), 4 mm., 0.85 N.A.; eyepiece, E. Leitz periplan 8X. The bellows extension was adjusted with a stage micrometer so that the final image was exactly 500X.



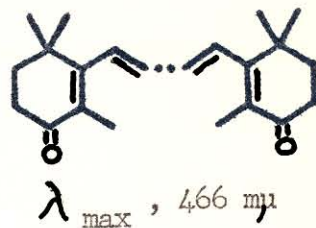
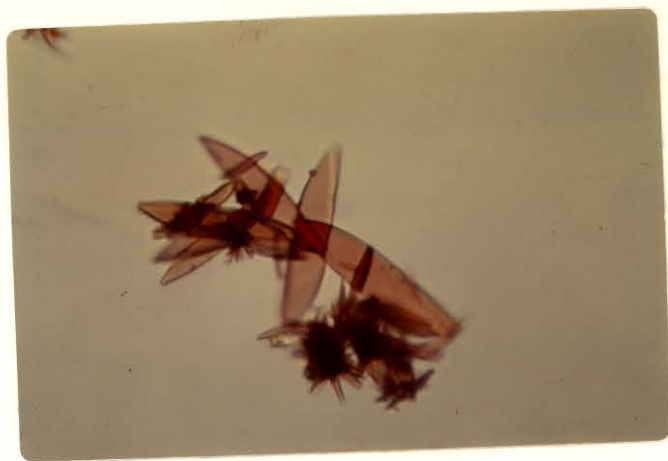
500X

Figure 25. 4-Keto-3',4'-dehydro- β -carotene from Benzene-Methanol.



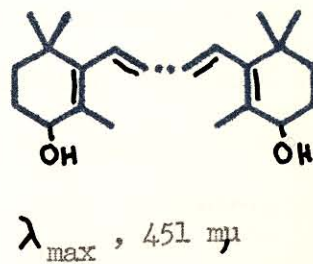
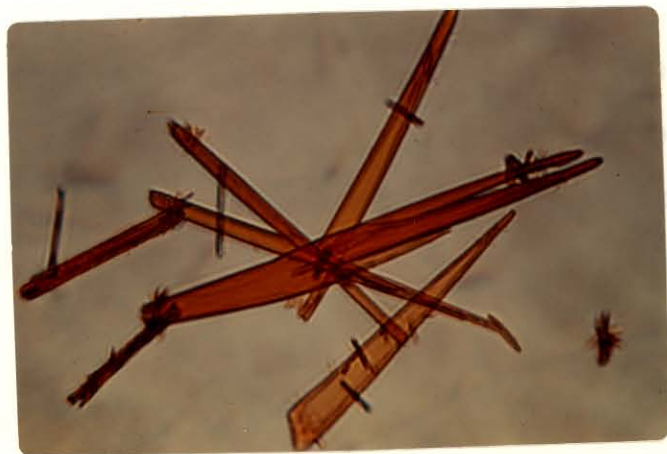
500X

Figure 26. 4-Hydroxy-3',4'-dehydro- β -carotene from Methylene Chloride-Methanol.



500X

Figure 27. 4,4'-Diketo- β -carotene from Chloroform-Ethanol.



500X

Figure 28. 4,4'-Dihydroxy- β -carotene from Chloroform-Hexane.

B. EXPERIMENTAL PART.

1. Materials and Methods.

In all of the experimental work reported in this Thesis, certain standard techniques, equipment and reagents were used. In order to eliminate undue repetition, a description of these is given in this section which applies to all the work herein, unless otherwise stated.

a. Adsorbents and Solvents.

The term lime-Celite refers to a 2:1 mixture of lime (Sierra, Superfine, U. S. Lime Products), and Celite (No. 545, Johns Mansville). The magnesia-lime-Celite was a 3:1:1 mixture of magnesia (Seasorb 43, Food, Machinery and Chemical Corporation) lime and Celite. Calcium carbonate refers to Precipitated, U.S.P. XII Heavy (Mallinckrodt) grade. Unless stated otherwise, the adsorbent used was lime:Celite and a 27 x 8 cm. column.

For chromatographic work, hexane (commercial grade), acetone (C.P. - U.S.P.), benzene (Reagent Grade) and chloroform (Reagent Grade) were used. When mixtures of these solvents were used as the developers, the figures given in these cases (e.g. hexane + 5% acetone) refer to volume percent of the second component in the mixture. Acetone was used for all elutions, unless stated otherwise; in some instances acetone-methanol mixtures were employed to elute more strongly adsorbed pigments. For crystallizations, only reagent grade solvents (except for hexane) were used.

For spectroscopic work in the ultraviolet region, a purified hexane was used. Phillip's commercial grade was treated with fuming sulfuric acid until its optical density (after washing and a final distillation) was close to that of distilled water in the region

200-300 μ . Unless otherwise stated, all spectral constants refer to this hexane. For the infrared curves, Eastman's spectral grade chloroform and carbon tetrachloride were used.

b. Methods.

Evaporations and concentrations were performed in vacuo (water aspirator) while a stream of pure dry nitrogen was bubbled through the solution. Sintered glass funnels were used for elutions, and where possible, all-glass apparatus was used for evaporations, washings, etc. Sodium sulfate was employed for the drying of the washed solutions. Washing was carried out in the Le Rosen automatic device (38).

Crystallizations, carried out exclusively by using a two solvent system, are described by naming first, the solvent in which the compound was dissolved and second, the solvent used to effect crystallization; e.g. "from benzene-methanol" means that the compound was dissolved in the minimum of warm benzene and 4-5 volumes of methanol were added dropwise at approximately 40°. Unless stated otherwise, samples for analysis were dried in an Abderhalden apparatus over phosphorous pentoxide at 50° and 1 mm. pressure. Crystalline forms were observed at 400X magnification. Melting points were taken in an electrically heated Berl block in evacuated capillaries and are corrected.

All infrared curves were taken on a Perkin-Elmer infrared spectrophotometer, Model 21 with a 1% solution of the samples contained in 1 mm. cells. Visible and ultraviolet spectra were taken on a Cary recording spectrophotometer, Model 11M.

Molecular extinctions were calculated on the basis of two independent weighings.

The test for an allylic hydroxyl group (or the alkoxyl or acetyl derivative) was carried out as follows: To a few ml. of a dilute chloroform solution of the sample in an 18 x 135 mm. test tube (approximately 3 mg./liter) was added several drops of the HCl-chloroform reagent (see below). A deepening of the color within several minutes, as compared with a blank, was considered a positive test and indicated the presence of an allylic -OH (or derivative). The color change is the result of the chromophore being lengthened by dehydration (in the case of an allylic-OH).

c. Reagents.

The N-bromosuccinimide (NBS) was obtained from Arapahoe Chemicals, Inc. and was used without further purification.

BF_3 -etherate was prepared by passing the gas (Ohio Chemical and Manufacturing Co., Cleveland) into ice-cooled ether (Reagent Grade). Upon distillation the fraction boiling from 122-125° was collected and stored in a dark brown bottle. Slightly discolored samples can be used without any decrease in the yields (16).

The HCl-chloroform reagent was prepared by saturating Merck's Reagent Grade chloroform with dry HCl gas for 10 minutes at room temperature.

Technical N-phenylmorpholine (NPM) was used entirely.

2. Large Scale Preparation of β -Carotene.

For the preparation of large amounts of β -carotene required as starting material in this investigation, the following procedure was worked out (α -carotene was also obtained). Eight grams of a commercial carotene preparation (Barnett Laboratories, Long Beach, Calif.) was dissolved in 200 ml. of warm benzene and the solution was diluted with

hexane to 1.5 liters. This solution was adsorbed on a magnesia:lime:celite filled conical percolator (45 x 22 x 8 cm.) and developed with 6 l. of benzene-hexane 1:2. (The figures on the left denote the width of zones, in mm.):

20 brown
20 pink: γ -carotene
80 interzone
200 red-orange: β -carotene
30 interzone
100 yellow: α -carotene

The β -carotene and α -carotene sections were separated by slicing them out from the top of the percolator. After elution with acetone and evaporation to dryness, the residues were crystallized from benzene-methanol or chloroform-methanol. Yield, 4 g. of β -carotene, m.p. 182-183° and 1.2 g. of α -carotene, m.p. 187-188°.

3. Reaction of β -Carotene with NBS in Ethanol-Containing Chloroform.

To a solution of 100 mg. of β -carotene and 10 ml. of chloroform (Merck's R.G., containing about 1% ethanol by volume) in a 50-ml. round-bottom flask was added rapidly, with stirring, a solution of 100 mg. of NBS in 10 ml. of the same grade of chloroform. Both solutions had been precooled to -18° in an ice-salt bath, and the temperature was held constant during the reaction. Vigorous stirring was accomplished by blowing a stream of nitrogen, through a perforated glass bulb, into the solution.

Immediately upon the addition of the NBS reagent, the deep red color of the solution changed to a dark brown. Half a minute later, 200 mg. of solid NPM was added (it dissolved immediately), and the stirring was continued for 2 more minutes. The flask was then removed from the cooling bath, and the solution refluxed on a steam bath for 15

minutes to effect dehydrobromination. Very soon after the start of refluxing the solution turned a deep red again (somewhat darker than that of the original mixture). The solution was cooled to room temperature, diluted with 40 ml. of hexane and shaken several times with 0.1 N hydrochloric acid to eliminate the NPM. The dark red, upper phase was then washed free of acid, dried, and evaporated completely. The oily residue was dissolved in 25 ml. of hexane and developed with hexane + 5% acetone:

- 5 brown
- 10 red: 4,4'-diketo- β -carotene (Zone A)
- 50 three diffuse pink zones: retro-bisdehydrocarotenes (Zone B)
- 30 deep red: all-trans-4-keto-3',4'-dehydro- β -carotene (Zone C)
- 80 six pink-orange cis isomers of above compound and 4-keto- β -carotenes (Zone D)
- 10 light orange: all-trans-3,4,3',4'-dehydro- β -carotene (Zone E)
- 15 several yellow cis isomers of above compound (no β -carotene was detected)

4. 3,4,3',4'-Bisdehydro- β -carotene (II).

The fraction corresponding to Zone E (Section 3) obtained by the treatment of 1 g. of β -carotene was rechromatographed (developer, hexane + 2% acetone):

- 150 empty section
- 15 pink-orange: all-trans-3,4,3',4'-bisdehydro- β -carotene
- 2 interzone
- 10 yellow-orange
- 3 interzone
- 5 yellow

} cis isomers of above

By photometric estimation, the sum of the 3,4,3',4'-bisdehydro- β -carotenes amounted to 10 mg., i.e. 1% of the starting material. The trans fraction was eluted, evaporated and crystallized from benzene-methanol. Yield, 4 mg. (0.4%).

Crystal Form: Macroscopically, the crystals (from benzene-methanol) appeared much deeper red than β -carotene crystals of comparable size. Under the microscope, irregular plates were observed.

Melting Point: 196-198°.

Partition Behavior: 100:0 in hexane:95% methanol.

Analysis: Calculated for $C_{40}H_{52}$: C, 90.16 ; H, 9.82.

Found : C, 89.96 ; H, 9.94.

Spectrum: λ_{\max} at 471 m μ (Fig. 16).

Molecular Extinction Coefficient: $E_{1\text{ cm.}}^{\text{mol}} = 12.7 \times 10^4$ at λ_{\max} .

Chromatographic Behavior: When developed with hexane + 2-3% acetone on lime-Celite, the bisdehydro-compound separated easily from β -carotene and occupies top position. A sample was compared with the corresponding total synthetic product received from Professor H. H. Inhoffen. The crystal form, melting points, infrared and visible spectra were found to be identical. A mixed chromatogram on lime-Celite, developed with hexane + 2% acetone, showed no separation. Our sample was also compared and identified by spectra and mixed chromatogram test with "dehydrocarotene III" first described by Karmakar and Zechmeister (6).

5. retro-Bisdehydro-carotene (III).

The pigment of Zone B obtained from 1 g. of β -carotene (Section 3) was rechromatographed (developer, hexane + 7% acetone):

20 minor pink zones	
30 deep-pink: all- <u>trans-retro</u> -bisdehydrocarotene	
25 pink-orange	} <u>cis</u> isomers of former
20 orange	
15 yellow	
10 interzone	
10 pink: 4-keto-3',4'-dehydro- β -carotene	

The 30-mm. zone was eluted, transferred to hexane and rechromatographed as described above, on two columns. The single main zone of the all-trans compound was well separated from some minor cis isomers. After elution and evaporation to dryness, it was crystallized from benzene-methanol.

Crystal Form: Long, quadrangular plates with jagged ends.

Melting Point: 205-206°.

Partition Behavior: 100:0 in hexane:95% methanol.

Analysis: Calculated for $C_{40}H_{52}$: C, 90.16 ; H, 9.84.

Found: C, 90.10 ; H, 10.03.

Spectrum: The spectrum was identical with that reported by Zechmeister and Wallcave (5) for retro-bisdehydrocarotene (519, 487, 455 mμ).

The preparation was also compared with the sample just mentioned in a mixed chromatogram test (lime:Celite; hexane + 7% acetone). No separation took place.

The combined yield of the stereoisomeric retro-bisdehydrocarotenes, as estimated photometrically, was 5.5% (β -carotene = 100%).

6. Echinenone (4-Keto- β -carotene) (IV) from β -Carotene.

Zone D (Section 3) obtained by the NBS treatment of 2 g. of β -carotene was transferred to hexane and developed on three columns using hexane + 4% acetone:

4 brown	
40 interzone	
50 pink: echinenone	
2 interzone	
50 red-orange	} <u>cis</u> -4-keto-3',4'-dehydro- β -carotenes
45 two orange zones	
10 several minor zones	
60 empty section	

The combined echinenone zones of the three columns were eluted, transferred to hexane and rechromatographed on a single magnesia-lime-Celite column using benzene-hexane (1:4) + 15% acetone as the developer. The main, brick-red zone was eluted, evaporated to dryness and crystallized from benzene-methanol. Yield, 24 mg. When the molar ratio, β -carotene:NBS, was 1:2, the corresponding crystalline yield of echinenone was 100 mg. (5%).

Crystal Form: Rectangular plates, showing a dull red color when viewed macroscopically.

Melting Point: 175-178°.

Partition Behavior: 93:7 in hexane:95% methanol.

Analysis: Calculated for $C_{40}H_{54}O$: C, 87.22 ; H, 9.88.

Found : C, 87.41 ; H, 10.05.

Spectrum: λ_{max} . at 458 m μ . The curve showed little fine structure (Fig. 1). In the infrared region, a strong carbonyl band appeared at 6.04 μ (Fig. 3).

Chromatographic Behavior: From hexane, echinenone is very strongly adsorbed on lime-Celite; however, when developed with hexane + 5% acetone it separates easily from 4-keto-3',4'-dehydro- β -carotene, the latter occupying top position. Echinenone is adsorbed below isocryptoxanthin (4-hydroxy- β -carotene) and considerably above β -carotene on lime-Celite.

A sample of this synthetic echinenone was compared with naturally occurring echinenone isolated by Ganguly et al. (15). The two samples were submitted to a mixed chromatogram test; no separation took place on lime-Celite (hexane + 4% acetone). The respective infrared spectra as well as the data given above were found to be identical.

7. Echinenone Oxime (4-Keto- β -carotene Oxime).

Twenty mg. of echinenone (ex β -carotene) was reacted with 100 mg. of hydroxylamine-HCl as described for 4-keto-3',4'-dehydro- β -carotene (Section 9). The resulting mixture was developed with benzene-hexane (1:2) on calcium carbonate-Celite (25 x 6 cm.). The main orange zone was eluted with acetone, transferred to the benzene-hexane mixture, dried, evaporated and crystallized from benzene-methanol. Yield, 7 mg.; small plates, m.p. 203-206°.

Analysis: Calculated for $C_{40}H_{55}ON$: N, 2.48 .

Found : N, 3.02 .

Spectrum: The oxime showed a maximum at 455 m μ , i.e. exactly at the wave length reported for the oxime of the natural product (15). The spectral curve showed but little fine structure (Fig. 2).

8. 4-Keto-3',4'-dehydro- β -carotene (V).

Zone C of the chromatogram described in Section 3 was eluted with acetone; the pigment was transferred to hexane by the addition of water and developed with hexane + 5% acetone. The main, bright-red zone was eluted and evaporated to dryness. The powdery residue was crystallized from benzene-methanol or from chloroform-methanol. Yield, 16 mg.

Crystal Form: The crystals obtained from benzene-methanol are shown in Fig. 25. Under the microscope, they show red-purple color; macroscopically the shiny crystals appear almost black.

Melting Point: 192-194°.

Solubility: Sparingly soluble in hexane; soluble in warm benzene; very easily soluble in chloroform; insoluble in methanol or ethanol.

Partition Behavior: 92:8 in hexane:95% methanol.

Analysis: Calculated for $C_{40}H_{52}O$: C, 87.53 ; H, 9.55.

Found : C, 87.44 ; H, 9.73.

Spectrum: λ_{\max} at 470 m μ (Fig. 4). For the infrared curve, See Fig. 6 (strong carbonyl absorption at 6.04 μ).

Molecular Extinction Coefficient: $E_{1\text{ cm.}}^{\text{mol}} = 12.6 \times 10^4$ at λ_{\max} .

Chromatographic Behavior: This pigment is strongly adsorbed on lime-Celite from hexane. It is adsorbed above echinenone and well separated from the latter upon developing with hexane + 5% acetone.

9. Oxime of 4-Keto-3',4'-dehydro- β -carotene.

Thirty mg. of the ketone and 120 mg. of hydroxylamine hydrochloride were dissolved in 5 ml. of dry pyridine. The solution was heated for 45 minutes on the steam bath, diluted with 20 ml. of benzene-hexane 1:1 and washed free of pyridine. The dark-red solution was then diluted with 50 ml. of hexane and, after drying, chromatographed on calcium carbonate-Celite (developer, hexane + 35% benzene). A pale, orange-pink zone moved rapidly down the column (probably unreacted ketone) while the main, dark-orange zone, followed by several minor pigments, migrated much more slowly. The oxime fraction, after elution, was evaporated to dryness and crystallized from benzene-methanol. Yield, 8 mg.

Melting Point: 200-201 $^{\circ}$.

Spectrum: λ_{\max} at 465 m μ (Fig. 5).

Analysis: Calculated for $C_{40}H_{53}ON$: N, 2.48.

Found: N, 2.64.

10. 2,4-Dinitrophenylhydrazone of 4-Keto-3',4'-dehydro- β -carotene.

To 13 mg. of the ketone in 3 ml. of benzene was added 6 ml. of absolute ethanol. To this mixture was added dropwise 3 ml. of the 2,4-dinitrophenylhydrazine reagent (39, there p. 171). The deep purple crystals of the derivative began to appear immediately, and within 15 minutes the process was complete. The crystalline precipitate was centrifuged and washed several times with absolute ethanol. After recrystallization from pyridine-hexane, the deep purple plates melted at 216° . Yield, 15 mg.

Analysis: Calculated for $C_{46}H_{58}O_5N_4$: C, 75.79; H, 7.74; N, 7.69.

Found: C, 75.83; H, 7.73; N, 7.68.

This substance is sparingly soluble in benzene or chloroform.

11. 4-Keto-3',4'-dehydro- β -carotene (V) from 4-Keto- β -carotene (IV).

To a solution of 14 mg. of 4-keto- β -carotene (ex β -carotene) in 10 ml. of ethanol-free chloroform (0°) was added with vigorous stirring (nitrogen stream) a precooled solution of 6.5 mg. of NBS in 10 ml. of the same grade of chloroform (1.2 moles of NBS per mole of pigment). After 2 minutes, 15 mg. of NPM was introduced into the dark brown solution. Upon refluxing for 15 more minutes, in order to effect dehydrobromination, the solution was treated as described in Section 3 (β -carotene and NBS) and the product was chromatographed (23 x 4.8 cm.; developer, benzene-hexane 1:2). Only a single main zone (deep red) of all-trans-4-keto-3',4'-dehydro- β -carotene appeared on the column, followed by a few minor cis zones. The combined yield of these stereoisomers amounted to about 50%, as estimated photometrically. The

eluate of the all-trans fraction was evaporated to dryness and crystallized from benzene-methanol; m.p. 189-190°.

Analysis: Calculated for $C_{40}H_{52}O$: C, 87.53 ; H, 9.55.

Found : C, 87.63 ; H, 9.75.

The compound did not separate in the mixed chromatogram test (lime-Celite, hexane + 5% acetone) from a sample of 4-keto-3',4'-dehydro- β -carotene prepared directly from β -carotene (Section 3). The two visible spectra taken in the region of the main band were identical.

12. 4-Keto-3',4'-dehydro- β -carotene (V) from Isocryptoxanthin (4-Hydroxy- β -carotene) (IX).

To 33 mg. of isocryptoxanthin in 15 ml. of ethanol-free chloroform (0°) was added, with stirring, 24 mg. of NBS (molar ratio of substance: reagent = 1:2) in 15 ml. of the same grade of chloroform (0°). The temperature of the reaction mixture was maintained at 0° and the vigorous stirring was continued for 1 more minute. Then 30 mg. of NPM was introduced, which changed the dark brown color to bright red immediately. The solution was refluxed for 15 minutes, treated as described above for similar conversions (cf. e.g., Section 3), and developed on a column with hexane + 5% acetone. Only a single main zone appeared, viz. that of all-trans-4-keto-3',4'-dehydro- β -carotene, besides some minor cis forms. The combined yield was 11.1 mg. (33%) as estimated photometrically. The all-trans fraction, after elution, and evaporation to dryness, was crystallized from chloroform-ethanol. Reddish-purple oval plates, m.p., 188-189°. The spectrum was identical with that of a 4-keto-3',4'-dehydro- β -carotene sample prepared directly from β -carotene (Section 3). A mixed chromatogram (lime-Celite, hexane + 5% acetone) showed no separation of the two compounds.

13. Conversion of 4-Hydroxy-3',4'-dehydro- β -carotene (VII) to the Corresponding 4-Keto Compound.

To 31 mg. of substance in 10 ml. of ethanol-free chloroform was added in a vigorous stream of nitrogen, a solution of 11 mg. of NBS (mole/mole ratio) dissolved in 10 ml. of the same grade of chloroform. The initial temperature of both solutions was 0°. The mixture turned dark brown immediately; after standing 1 minute, 20 mg. of NPM was added. Upon refluxing for 15 minutes on the steam bath, the solution was treated as described above (cf. Section 3) and, finally, developed with hexane + 5% acetone on a single column. The deep-red main zone of 4-keto-3',4'-dehydro- β -carotene and some minor cis isomers were the only ones appearing on the column. Their photometrically estimated sum amounted to 19.5 mg. (yield, 63%).

The all-trans compound was eluted and crystallized from benzene-methanol. Yield, 6 mg.; m.p., 188-190°. This preparation was identified with a sample obtained directly from β -carotene (Section 3) by melting point, partition behavior, mixed chromatogram test and spectrum.

14. Treatment of 4-Keto-3',4'-dehydro- β -carotene with NBS.

Twenty mg. of the ketone was treated with 10 mg. of NBS in 20 ml. of ethanol-free chloroform. After working up the reaction mixture as described in Section 3, 75% of the 4-keto-3',4'-dehydro- β -carotene was recovered unchanged.

15. Reduction of 4-Keto-3',4'-dehydro- β -carotene (V) to the Corresponding 4-Hydroxy Compound (VII).

A solution of 50 mg. of 4-keto-3',4'-dehydro- β -carotene in 100 ml. of 1:9 mixture of anhydrous benzene and ether was slowly added, with stirring at room temperature, to a solution of 500 mg. of lithium

aluminum hydride in 200 ml. of anhydrous ether. After standing for 15 minutes, the liquid was cooled to 0° and the excess hydride was decomposed by dropwise addition of methanol. The solution was then transferred to a separatory funnel where the gelatinous precipitate was removed from the organic phase by washing vigorously in the automatic device. (Attempts to filter the gel were unsuccessful.) The benzene-ether solution was dried and evaporated completely. The bright orange, powdery residue was dissolved in 50 ml. of benzene-hexane (1:4) and developed with hexane + 7% acetone. A main pink-orange zone appeared (90 mm.); no cis forms were observed. The pigment of the main zone was crystallized from benzene-methanol or from chloroform-ethanol. Yield, 30 mg.

Crystal Form: Macroscopically the bright orange crystals from benzene-methanol have a brilliant metallic lustre. From methylene chloride-methanol rectangular plates are observed (Fig. 26).

Melting Point: $174-175^{\circ}$.

Solubility: Sparingly soluble in hexane, easily in cold chloroform or warm benzene; slightly soluble in methanol.

Partition Behavior: 80:20 in hexane:95% methanol.

Analysis: Calculated for $C_{40}H_{54}O$: C, 87.22; H, 9.88.

Found: C, 87.33; H, 9.95.

Spectrum: λ_{\max} at 461 m μ . The wave length position of λ_{\max} and the shape of the curve were identical with those of "dehydrocarotene II" (Fig. 7). The infrared curve showed no carbonyl band (Fig. 8).

Molecular Extinction Coefficient: $E_{1\text{ cm.}}^{\text{mol.}} = 12.4 \times 10^4$ at λ_{\max} .

Chromatographic Behavior: On lime-Celite the hydroxy compound is adsorbed slightly above the parent ketone or isocryptoxanthin.

Test for Allylic Hydroxyl: Positive.

16. 4-Hydroxy-3',4'-dehydro- β -carotene Acetate.

To a solution of 60 mg. of the 4-hydroxy compound in 3 ml. of dry pyridine, 1 ml. of acetic anhydride was added and the solution was kept at 60° for 1 hour. After cooling to 20° and dilution with 60 ml. of hexane, the solution was washed free of pyridine and acetic anhydride. Finally, the hexane solution was dried and the pigment mixture developed with hexane + 2% acetone:

5 orange: unidentified
10 interzone
20 pale orange: unreacted hydroxy compound
30 interzone
30 orange: trans acetate
5 pale yellow: cis acetate

The 30-mm. fraction of the acetate was eluted, evaporated and the powdery, dull-orange residue was crystallized from benzene-methanol. Yield, 10 mg.

Crystal Form: Small irregular plates, (dull-orange).

Melting Point: 131-133°.

Partition Behavior: 96:4 in hexane:95% methanol.

Analysis: Calculated for $C_{42}H_{56}O_2$: C, 85.08 ; H, 9.52 .

Found : C, 85.04 ; H, 9.72 .

Spectrum: Identical with that of the 4-hydroxy-3',4'-dehydro- β -carotene; λ_{max} at 461 m μ .

Chromatographic Behavior: When developed with hexane + 3% acetone on lime-Celite, it is adsorbed very much below the parent hydroxy compound.

Reaction with the HCl-Chloroform Reagent: Positive.

17. 4-Methoxy-3',4'-dehydro- β -carotene (VIII).

To a solution of 25 mg. of 4-hydroxy-3',4'-dehydro- β -carotene in

5 ml. of chloroform + 25 ml. of methanol (99.5%), 10 drops of the HCl-chloroform reagent were added. The well mixed solution was then allowed to stand at room temperature for exactly 7 minutes (longer standing causes partial destruction). After dilution with 25 ml. of hexane the upper (red) phase was washed free of acid and methanol. Upon drying and complete evaporation the oily, dark-orange residue was dissolved in 15 ml. of hexane and developed with hexane + 2% acetone:

10 orange: 502, 471, 445 mμ (visual spectroscope, hexane)
10 interzone
15 pink-orange: all-trans form of the methoxy compound
10 orange: a cis isomer of above
5 several minor yellow zones
220 empty section

The all-trans pigment was crystallized from benzene-methanol.

Yield, 6 mg.

Crystal Form: Small, irregular plates.

Melting Point: 142-144°

Partition Behavior: 99:1 in hexane:95% methanol.

Analysis: Calculated for $C_{41}H_{56}O$: C, 87.17 ; H, 9.99 .

Found : C, 87.53 ; H, 10.02 .

Spectrum: As expected, it was identical with that of 4-hydroxy-3',4'-dehydro-β-carotene.

Chromatographic Behavior: When developed on lime-Celite with hexane + 2% acetone, the ether is adsorbed well below the parent alcohol.

Reaction with the HCl-Chloroform Reagent: Positive.

18. Dehydration of 4-Hydroxy-3',4'-dehydro-β-carotene (VII).

To 100 mg. of the hydroxy compound dissolved in 25 ml. of chloroform, 25 drops of the HCl-chloroform reagent were added, with stirring. The mixture was allowed to stand at room temperature for 10 minutes.

The solution was then washed once with saturated sodium bicarbonate, dried and evaporated completely. The oily, red residue was dissolved in 25 ml. of hexane and chromatographed on a 45 x 8 cm. column (developer, hexane + 5% acetone):

20 brown	
20 interzone	
40 two pale pink zones	
60 pink: all- <u>trans-retro</u> -bisdehydrocarotene	
25 pink-orange	} <u>cis</u> forms of <u>retro</u> -bisdehydrocarotene
25 pale pink	
40 yellow-orange	
150 interzone	
20 two, pale orange zones	
5 interzone	
10 orange: all- <u>trans</u> -3,4,3',4'-bisdehydro- β -carotene	
15 pale orange: a <u>cis</u> isomer of the former	

The retro-bisdehydrocarotenes totaled 54.5 mg. as estimated photometrically (yield, about 55%). The trans pigment was crystallized from benzene-methanol. Long, thin plates with jagged ends, m.p. 204-207°.

Analysis: Calculated for $C_{40}H_{52}$: C, 90.16 ; H, 9.84 .

Found : C, 90.54 ; H, 9.81 .

(corrected for 0.8% ash)

In the mixed chromatogram test, this sample did not separate from an authentic retro-bisdehydrocarotene preparation.

The spectra, taken in the visible region, were identical.

The yield of stereoisomeric 3,4,3',4'-bisdehydro- β -carotenes as estimated photometrically was 4.5 mg. (4.5%). The trans fraction was developed on lime-Celite with hexane + 3% acetone. The pigment of the main zone was crystallized from chloroform-ethanol; m.p., 195-197°. It was identified with a sample of Inhoffen and Raspe's synthetic 3,4,3',4'-bisdehydro- β -carotene by crystal form, m.p., partition behavior, and mixed chromatogram test (lime-Celite; hexane + 3% acetone).

The combined yield of the dehydration products accounted for amounted to about 60%.

19. 4-Keto-4'-hydroxy- β -carotene (XII) from 4-Keto-3',4'-dehydro- β -carotene, via the BF_3 Complex.

To 33 mg. of 4-Keto-3',4'-dehydro- β -carotene in 33 ml. of ethanol-free chloroform was added rapidly, with vigorous stirring, 3.3 ml. of BF_3 -etherate. Having shown intermediate blue and green colors, the solution turned dark purple within two minutes. It was rapidly poured, while stirring, into 400 ml. of a water-acetone (1:4) mixture. The purple complex was destroyed immediately and the solution became red-orange, rather similar in color to the starting material. Sixty milliliters of hexane was then added, the upper phase was separated, washed thoroughly in the automatic apparatus for 30 minutes, and dried. After evaporation, the dark red, oily residue was dissolved in 25 ml. of benzene-hexane (1:3) and developed with pure benzene:

10 reddish-brown
25 interzone
30 pink: all-trans-4-keto-4'-hydroxy- β -carotene
10 orange-pink: unidentified
60 interzone
10 light purple: probably unreacted starting material
125 empty section

The 30-mm. fraction was transferred to benzene, washed, dried and evaporated. The dark red, powdery residue was crystallized from chloroform-hexane. Yield, 6 mg.

Crystal Form: Diamond-shaped plates, from chloroform-hexane.

Melting Point: 164-167°

Partition Behavior: 34:66 in hexane:95% methanol

Analysis: Calculated for $\text{C}_{40}\text{H}_{54}\text{O}_2$: C, 84.75 ; H, 9.60 .

Found : C, 84.41 ; H, 9.72 .

Spectrum: The spectrum taken in the visible and ultraviolet regions was identical with that of 4-keto- β -carotene, λ_{max} at 458 m μ (Fig. 14). The infrared curve (Fig. 15) demonstrated the presence of a conjugated carbonyl (6.04 μ) and a hydroxyl group (2.90 μ).

Molecular Extinction Coefficient: $E_{1\text{ cm.}}^{\text{mol}} = 12.2 \times 10^4$ at λ_{max} .

Chromatographic Behavior: This pigment is adsorbed below 4,4'-dihydroxy- β -carotene and above 4,4'-diketo- β -carotene when developed with benzene on lime-Celite.

20. 4-Keto-4'-ethoxy- β -carotene (X).

a. From 4-Keto-4'-hydroxy- β -carotene (XII).

To a solution of 25 mg. of 4-keto-4'-hydroxy- β -carotene in 25 ml. of absolute ethanol, 5 drops of the HCl-chloroform reagent were added. The solution was then kept at room temperature for 5 minutes, whereupon, it showed mainly epiphasic behavior (hexane:95% methanol), in contrast to the behavior of the starting material. The solution was made basic by the addition of a few milliliters of saturated sodium bicarbonate, and 20 ml. of hexane was added. The red colored epiphase was washed thoroughly, dried and resolved on a column. Only one main, brick-red zone appeared (20 mm. broad). After elution and evaporation to dryness, the powdery residue was crystallized from chloroform-ethanol. Yield, 10 mg.

Melting Point: 154-156 $^{\circ}$.

Spectrum: The visible spectrum was identical with that of 4-keto- β -carotene.

Analysis: Calcd. for $\text{C}_{42}\text{H}_{58}\text{O}_2$: C, 84.79; H, 9.83; OC_2H_5 , 7.58.
Found: C, 85.02; H, 10.05; OC_2H_5 , 9.06.

Partition Behavior: 86:14 in hexane:95% methanol.

Chromatographic Behavior: On lime-Celite (developer, hexane + 6% acetone) the 4-keto-4'-ethoxy compound is adsorbed slightly above the 4-keto-3',4'-dehydro substance. On magnesia-lime-Celite an inversion occurs and the 4-keto-3',4'-dehydro- β -carotene then occupies the top position (developer, benzene-hexane 1:2).

b. From 4-Keto-3',4'-dehydro- β -carotene (V) via the BF₃ Complex.

To 50 mg. of substance in 50 ml. of chloroform (Merck's R.G.), 5 ml. of BF₃-etherate was added rapidly, with swirling, and 3 min. later the dark-purple liquid was rapidly poured into 250 ml. of absolute ethanol. The resulting red-brown solution was poured into a separatory funnel containing 125 ml. hexane. Sufficient water was added to cause separation into two phases. Saturated bicarbonate (approximately 100 ml.) was introduced to destroy the excess acid and the lower water phase was then drained off. The deep-red chloroform-hexane solution was shaken with 500 ml. of acetone; the acetone was washed out by the addition of water, and the acetone treatment was repeated again (this operation prevented a rapid discoloration of the pigment solution from a red to a dark brown on evaporation to dryness, a phenomenon which decreased the yields markedly). The solution was finally washed free of acetone, dried and evaporated to dryness. The oily residue was developed on a 50 x 8 cm. column of magnesia-lime-Celite with benzene-hexane (2:3) + 10% acetone. The main brick-red zone contained 19 mg. (38%) of the trans + cis isomers of the 4'-ethoxy compound (photometric estimation). The all-trans compound was obtained by rechromatography on lime-Celite (developer, hexane + 5% acetone). The crystalline

yield was 8 mg. (16%), m.p. 156-158°. A sample was identified with the 4'-ethoxy compound obtained under (a).

21. Reaction of 4-Keto-4'-ethoxy- β -carotene (X) with NBS, in the Absence of Ethanol. Formation of 4-Keto-4'-ethoxy-3',4'-dehydro- β -carotene (XI).

To a well-stirred solution of 40 mg. of 4-keto-4'-ethoxy- β -carotene in 20 ml. of ethanol-free chloroform (0°) was added a precooled solution of 14 mg. of NBS (mole/mole ratio), in 10 ml. of the same grade of chloroform. Fifteen seconds later, a sudden color change from deep red to dark brown was observed. Stirring was continued for 40 more seconds, whereupon 30 mg. of NPM was added. The solution was refluxed for 15 minutes and further treated as described for similar reactions (Section 3). Chromatography of the product on a 26 x 8 cm. column, developed with benzene-hexane (4:1) + 10% acetone showed the following sequence of zones:

10 brown
10 colorless
45 maroon: 4-keto-4'-ethoxy-3',4'-dehydro- β -carotene
5 interzone
45 red-orange: 4,4'-diketo- β -carotene
3 colorless
20 orange
30 pale orange
20 empty section

From the 45 mm. maroon zone, after rechromatography, was obtained 7 mg. (photometric estimation) of the 4-keto-4'-ethoxy-3',4'-dehydro- β -carotene.

Crystal Form: Deep-red, quadrangular plates.

Melting Point: 209-210°.

Partition Behavior: 44:56 in hexane:95% methanol.

Analysis: Calculated for $C_{40}H_{52}O$ (OC_2H_5): OC_2H_5 , 7.58 .

Found: OC_2H_5 , 6.51..

Spectrum: The spectral curve (Fig. 17) was practically identical with that of 4-keto-3',4'-dehydro- β -carotene.

Chromatographic Behavior: On lime:Celite this compound is adsorbed slightly above the 4,4'-diketo- β -carotene.

From the 45 mm. red-orange zone was recovered 6 mg. (photometric estimation) of the 4,4'-diketo- β -carotene, m.p. 209-212°. It was identified with an authentic sample by the mixed chromatogram, partition, and spectrum.

22. Reaction of 4-Keto-4'-ethoxy- β -carotene (X) with NBS, in the Presence of Ethanol. Formation of 4,4'-Diketo- β -carotene (VI).

In a 20 x 120 mm. pyrex test tube, a solution of 3.6 mg. of the 4-keto compound in 1 ml. of chloroform (Merck's) was cooled to 0°. To it was added 1.6 mg. of NBS in 1 ml. of the same grade of chloroform, precooled to 0°. (Stirring was accomplished by means of a nitrogen bubbler.) The dark brown color that had developed immediately on introducing the NBS disappeared within 10 seconds. Stirring was continued for 1 minute and then 20 mg. of N-phenylmorpholine was added to the deep-red solution. The mixture was heated at 50° for 15 minutes and treated further as described for similar reactions (Section 3). Chromatography was carried out on lime-Celite (20 x 4 cm.) with benzene-hexane (1:4) + 10% acetone:

5 brown
55 interzone
15 orange-red: all-trans-4,4'-diketo- β -carotene
3 interzone
12 orange: probably a cis isomer of the former
12 interzone
15 pale orange

40 several pale zones
40 empty section

The photometrically estimated yield of the all-trans diketone was 0.6 mg. A mixed chromatogram with a sample prepared directly from β -carotene (Section 25) showed no separation. The spectra in the visible region were identical.

23. Dehydration of 4-Keto-4'-hydroxy- β -carotene (XII) to 4-Keto-3',4'-dehydro- β -carotene (V).

A solution of 10 mg. of the keto-alcohol in 5 ml. of ethanol-free chloroform was treated with 15 drops of the HCl-chloroform reagent, mixed thoroughly, and allowed to stand at room temperature. The progress of the conversion was followed by checking the partition behavior of the mixture from time to time. Essentially epiphasic behavior was indicative for the completion of the dehydration process. This point was reached in about 40 minutes. The solution (slightly deeper in color than before the treatment) was diluted with 40 ml. of hexane, shaken with saturated bicarbonate, dried and evaporated completely. The hexane solution of the oily residue was developed on a 25 x 6 cm. column with hexane + 6% acetone:

4 orange
40 interzone
22 red: all-trans-4-keto-3',4'-dehydro- β -carotene
2 interzone
20 pink: probably a cis isomer of former
2 interzone
20 two pale pink zones
2 interzone
9 two pale orange zones
130 empty section

The trans zone was eluted, evaporated and crystallized from benzene-methanol. Yield, 3.5 mg., irregular plates, m.p. 189-190°. The compound was identified with the ketone prepared directly from β -carotene

(Section 3) by melting point, spectrum, partition coefficient and mixed chromatogram test.

24. Dehydration of 4-Keto-4'-hydroxy- β -carotene (XII) in the Presence of Ethanol. Formation of 4-keto-4'-ethoxy- β -carotene (X).

To a solution of 40 mg. of substance in 25 ml. of chloroform (Merck's R.G.), 25 drops of the HCl-chloroform reagent were added, dropwise, with stirring. The reaction mixture became completely epiphasic after 5 minutes, in contrast to the much longer time (30-40 minutes) required for a similar change in the partition behavior in the absence of ethanol (cf. Section 23). Excess acid was removed by shaking with bicarbonate, whereupon the solution was dried and evaporated completely. The hexane solution of the dark red, oily residue was developed on a 60 x 8 cm. column with benzene-hexane (1:1):

20 orange-red
4 pink
90 interzone
40 pale orange
40 interzone
70 red-orange: 4-keto-4'-ethoxy- β -carotene
35 scarlet: 4-keto-3',4'-dehydro- β -carotene
25 pale orange
3 interzone
25 pale orange
15 interzone
40 orange
10 yellow
30 orange
20 several minor yellow zones
130 empty section

a. 4-Keto-4'-ethoxy- β -carotene (X).

The 70-mm. zone was eluted, transferred to hexane-benzene (1:1), washed, dried and evaporated completely. Yield, 10 mg. (estimated photometrically), corresponding to 25% of the starting material. The orange colored, powdery residue was crystallized from chloroform-ethanol.

Yield, 6 mg., m.p. 154-156°.

Analysis: Calculated for $C_{42}H_{58}O_2$: C, 84.79 ; H, 9.83 .

Found : C, 84.66 ; H, 9.35 .

(Corrected for 0.8% ash.)

The spectrum was identical with that of 4-keto-4'-ethoxy- β -carotene described in Section 20. The two samples did not separate in the mixed chromatogram test (lime-Celite, hexane + 5% acetone).

b. 4-Keto-3',4'-dehydro- β -carotene (V).

The 36-mm. scarlet zone was rechromatographed on a 60 x 8 cm. column (developer, hexane + 5% acetone) in order to remove a small contamination by 4-keto-4'-ethoxy- β -carotene*. The single, bright red main zone was eluted, washed, dried and evaporated. The photometrically estimated yield was 4 mg., corresponding to 10% of the hydroxy compound treated. The deep red, powdery residue was crystallized from chloroform-ethanol; reddish purple, oval plates, m.p. 187-189°.

Analysis: Calculated for $C_{40}H_{52}O$: C, 87.53 ; H, 9.55 .

Found : C, 87.38 ; H, 9.86 .

The spectrum was identical with that of 4-keto-3',4'-dehydro- β -carotene, described in Section 8. The two samples did not separate in the mixed chromatogram test (lime-Celite, hexane + 5% acetone).

c. Dehydration of 4-keto-4'-hydroxy- β -carotene for Longer Period.

The dehydration was conducted exactly as described in (a) with the

*In some recent experiments a more advantageous separation of these two compounds has been effected by using magnesia-lime-Celite and benzene-hexane 2:3 + 10% acetone, whereby a clear interzone is observed. Interestingly, this change in the adsorption system caused an inversion of the chromatographic sequence of the two pigments.

exception that the time was lengthened from 5 to 30 minutes. In this case, only the 4-keto-3',4'-dehydro- β -carotene was isolated (50% yield, photometric estimation). None of the 4'-ethoxy compound could be detected in this experiment.

25. 4,4'-Diketo- β -carotene (VI).

Zone A (Section 3) obtained by reacting 2 g. of β -carotene with NBS was rechromatographed on two columns (developer; benzene-hexane, 3:1):

10 brown
5 minor pigment zones
30 deep red: all-trans-4,4'-diketo- β -carotene
10 orange: cis isomer of former
5 yellow: unidentified
40 three pale red, diffuse zones

The 30-mm. zone was eluted, transferred to benzene-hexane and evaporated to dryness. The powdery, red residue was crystallized from chloroform-ethanol or from benzene-methanol. Yield, 24 mg. (1.2%).

Crystal Form: Trapezoidal prisms were obtained from benzene-methanol while long plates appeared from chloroform-ethanol (Fig. 27).

Melting Point: 213-214°.

Partition Behavior: 50:50 in hexane:95% methanol.

Analysis: Calculated for $C_{40}H_{52}O_2$: C, 85.05 ; H, 9.28 .

Found : C, 85.18 ; H, 9.31 .

Spectrum: The spectral curve showed maximum extinction at 466 m μ . No fine structure appeared (Fig. 9). In ethanol, λ_{max} at 478 m μ ; in benzene, at 480 m μ . The infrared curve showed a strong carbonyl band at 6.04 μ (Fig. 10).

Molecular Extinction Coefficient: $E_{1\text{ cm.}}^{mol.} = 11.4 \times 10^4$, in hexane at 466 m μ .

Chromatographic Behavior: This compound is adsorbed considerably above echinenone or retro-bisdehydrocarotene but below zeaxanthin when developed with benzene on lime-Celite.

26. Oxime of 4,4'-Diketo- β -carotene.

Twenty milligrams of the diketo compound was treated with hydroxylamine-HCl essentially as described for the oximation of 4-keto-3',4'-dehydro- β -carotene (Section 9). The product was purified by developing on calcium carbonate-Celite with benzene. A 5 mm. orange zone near the top of the 22 x 5 cm. column was eluted with ethanol and evaporated to dryness. The residue was crystallized from pyridine-hexane, small bright-orange plates. Yield, 3 mg., m.p. 247-248°.

27. Di-2,4-Dinitrophenylhydrazone of 4,4'-Diketo- β -Carotene.

Nine milligrams of the ketone was dissolved in 5 ml. of nitrobenzene and 6 ml. of absolute ethanol was added, followed by 3 ml. of 2,4-dinitrophenylhydrazine reagent. Upon a few minutes standing, a very dark, microcrystalline precipitate began to appear. An hour later the powdery material was centrifuged, washed in the centrifuge tube several times with ethanol, and dried. Yield, 10 mg.; m.p. > 300°. Because of its extreme insolubility, the sample could not be recrystallized.

Analysis: Calculated for $C_{52}H_{58}O_8N_8$: N, 12.11 .

Found : N, 12.92 .

The corresponding, likewise sparingly soluble, mono-2,4-dinitrophenylhydrazone was obtained by dissolving 9 mg. of the ketone in 3 ml. of benzene + 6 ml. of ethanol and adding 3 ml. of the 2,4-dinitrophenylhydrazine reagent. Within several minutes the dark purple derivative began to separate and the process was complete within one-half hour. The crystals were centrifuged and washed several times with ethanol.

Feathery needles, insoluble in chloroform or pyridine, soluble in nitrobenzene; m.p. 250° (decomp.). When dissolved in nitrobenzene and treated with more reagent, the di-2,4-dinitrophenylhydrazone was obtained.

28. Conversion of 4-Keto-4'-hydroxy- β -carotene (XII) to 4, 4'-Diketo- β -carotene (VI).

To a solution of 30 mg. substance in 10 ml. of ethanol-free chloroform, kept at 0° , was added 10 mg. of NBS (molar ratio, 1:1) dissolved in 10 ml. of the same grade of chloroform. This was followed 1 minute later by the addition of 10 mg. of NPM, whereupon the solution was refluxed for 15 minutes. After a treatment as described above for similar reactions (Section 3), the product was developed with benzene. A single main zone, 60 mm. broad, appeared. Upon elution and evaporation, the dry residue was crystallized from chloroform-ethanol. Yield, 16 mg., m.p. $211-212^{\circ}$.

Analysis: Calculated for $C_{40}H_{52}O_2$: C, 85.05 ; H, 9.28 .

Found : C, 85.28 ; H, 9.51 .

This sample was identified with the diketone preparation obtained directly from β -carotene (Section 25), by melting point, spectrum, partition coefficient and mixed chromatogram test.

29. Reduction of 4,4'-Diketo- β -carotene (VI) to 4,4'-Di-hydroxy- β -carotene (XIII).

To a solution of 50 mg. of the diketone in 100 ml. of benzene-ether (1:9) 100 mg. of lithium aluminum hydride in 100 ml. of anhydrous ether was added, with swirling. After standing for 15 minutes the excess hydride was decomposed at 0° by dropwise addition of methanol. The yellow solution was washed free of the gel, dried and evaporated

completely. The bright orange, powdery residue was dissolved in 50 ml. of benzene-hexane (1:1) and developed on two columns with benzene + 5% acetone. The mixture could also be resolved using hexane + 30% chloroform. In either case, only one main, yellow zone of the trans diol appeared, which, after elution and evaporation, was crystallized from chloroform-hexane. The yield of this trans pigment was 90% (estimated photometrically).

Crystal Form: Macroscopically, the crystals appear red-orange, while under the microscope, yellow needles are observed (Fig. 28).

Melting Point: 142-145°.

Partition Behavior: 22:78 in hexane:95% methanol.

Analysis: Calculated for $C_{40}H_{56}O_2$: C, 84.44 ; H, 9.30 .

Found : C, 84.60 ; H, 9.43 .

Spectrum: The spectral curve (Fig. 11) was found to be identical with that of β -carotene. The infrared spectrum showed the absence of the carbonyl stretching band at 6.04μ (Fig. 12).

Molecular Extinction Coefficient: $E_{1 \text{ cm.}}^{\text{mol}} = 13.4 \times 10^4$ at λ_{max} .

Chromatographic Behavior: The diol is adsorbed above the parent diketone on lime-Celite or on lime-calcium carbonate-Celite when developed with benzene.

Reaction with the HCl-Chloroform Reagent: Strongly positive.

30. Reduction of 4-Keto-4'-hydroxy- β -carotene (XII) to 4,4'-Dihydroxy- β -Carotene (XIII).

Ten mg. of the keto-alcohol was reduced with lithium aluminum hydride as described in Section 29 for a similar treatment of 4,4'-diketo- β -carotene. After chromatographing the product on lime-Celite, the pigment of the single main zone was crystallized from chloroform-hexane

to give 5 mg. of 4,4'-dihydroxy- β -carotene. Orange needles, m.p. 142-143°. It was identified with the sample obtained by the reduction of the synthetic 4,4'-diketo- β -carotene (Section 29) by spectrum, partition coefficient and mixed chromatogram test.

31. 4,4'-Dimethoxy- β -carotene (VIII).

To a solution of 5 mg. of the 4,4'-dihydroxy compound in 8 ml. of methanol 1 drop of the HCl-chloroform reagent was added. After 7 minutes the reaction mixture showed epiphasic behavior in contrast to that of the starting material (hexane:95% methanol). The pigment was transferred to hexane, washed, dried and developed on a 20 x 4 cm. column with hexane + 3% acetone:

8 orange: unidentified
3 pink
30 interzone
20 orange: all-trans-4,4'-dimethoxy- β -carotene
3 interzone
10 pale orange: probably a cis isomer of the former
10 yellow
2 yellow

The 20-mm. zone was eluted, evaporated to dryness, and the orange residue (1 mg., photometrically estimated) was crystallized from benzene-methanol.

Crystal Form: Rectangular plates with split ends.

Melting Point: 153-154°.

Spectrum: The spectrum was identical with that of β -carotene.

Partition: Epiphasic (hexane:95% methanol).

This compound was found to be identical (m.p., spectrum, partition behavior) with a dimethoxy- β -carotene prepared by Wallcave and Zechmeister (16). A mixed chromatogram of the two samples showed no separation (lime-Celite, hexane + 3% acetone).

32. 4,4'-Dihydroxy- β -carotene Diacetate.

This ester was prepared from 50 mg. of the synthetic dihydroxy compound as described in Section 16 for the acetylation of 4-hydroxy-3',4'-dehydro- β -carotene. The resolution of the reaction mixture was accomplished by developing with hexane + 3% acetone:

15 orange: unreacted starting material and/or monoacetate
50 interzone
30 yellow: all-trans-4,4'-dihydroxy- β -carotene diacetate
5 pale yellow: probably a cis isomer of the former
170 empty section

The 30-mm. zone was eluted, transferred to hexane and crystallized from benzene-methanol.

Crystal Form: Stubby prisms with blunt ends.

Melting Point: 142-143°.

Partition Behavior: 86:14 in hexane:95% methanol.

Analysis: Calculated for $C_{44}H_{66}O_4$: C, 80.93 ; H, 9.26 .

Found : C, 80.49 ; H, 9.60 .

Spectrum: In the region of the main band the spectrum was identical with that of the parent dihydroxy compound.

33. Dehydration of 4,4'-Dihydroxy- β -carotene (XIII) to 4-Keto-3',4'-dehydro- β -carotene (V).

To a solution of 22 mg. of the diol in 10 ml. of ethanol-free chloroform (room temperature) 15 drops of the HCl-chloroform reagent were added dropwise, with stirring. The reaction was followed by testing the partition behavior (hexane:95% methanol). After about half an hour, the initially hypophasic behavior became epiphasic. The solution was then diluted with 30 ml. of hexane, washed with bicarbonate, dried and evaporated. The hexane solution of the dark red, oily residue was developed on lime-Celite (30 x 6 cm.) with hexane + 5% acetone:

5 brown
20 three pink zones
80 six diffuse orange zones
30 scarlet: all-trans-4-keto-3',4'-dehydro- β -carotene
5 interzone
20 orange
5 interzone } cis isomers of the former
20 pale orange
10 yellow-orange
100 empty section
(a minor pink zone passed into the filtrate)

The 30-mm. zone was eluted, transferred to hexane and developed on magnesia-lime-Celite (25 x 6 cm.) with benzene-hexane (2:3) + 10% acetone. A minor yellow pigment appeared above the main purple zone. The main zone was eluted, evaporated, and crystallized from chloroform-methanol. Reddish-purple plates, m.p. 188°. Partition behavior and the spectrum were found to be identical with those of 4-keto-3',4'-dehydro- β -carotene. A mixed chromatogram with a preparation obtained directly from β -carotene (Section 8) showed no separation on lime-Celite (hexane + 5% acetone).

34. Conversion of 4,4'-Dihydroxy- β -carotene (XIII) to 4-Keto-4'-ethoxy- β -carotene (X).

Twenty mg. of the diol in 10 ml. of chloroform (Merck's R.G.) was treated with 10 drops of the HCl-chloroform reagent. The solution showed epiphasic behavior after 10 minutes, whereupon it was shaken with bicarbonate, dried, and evaporated. The dark red, oily residue was dissolved in hexane and developed on a column with hexane + 5% acetone:

10 brown-orange
20 several orange and yellow zones
5 interzone
15 pink: all-trans-4-keto-4'-ethoxy- β -carotene
60 four, pale orange zones: probably cis isomers of the former
50 interzone
10 two minor yellow zones

The 15-mm. zone (4 mg., photometrically estimated) was eluted, transferred to hexane and evaporated to dryness. The dull red residue was crystallized from chloroform-ethanol; plates, m.p. 154-156°. The spectrum, taken in the region of the main band, was identical with that of 4-keto-4'-ethoxy- β -carotene described in Section 20. A mixed chromatogram with 4-keto-4'-ethoxy- β -carotene showed no separation (lime-Celite; hexane + 5% acetone).

35. Comparison of Synthetic 4,4'-Diketo- β -carotene (VI) with Canthaxanthin.

a. Identification of the Two Diketones.

A comparison of the physical constants, as well as some other data, is given in Table 1, p. 24. A mixed chromatogram of the natural and synthetic ketones on lime-Celite developed with benzene showed no separation, even after long development.

b. Reduction of Natural Canthaxanthin to 4,4'-Dihydroxy- β -carotene (XIII).

Approximately 0.1 mg. of the natural canthaxanthin (from the reference sample sent by Starr and Saperstein) was reduced with lithium aluminum hydride as described for the reduction of the synthetic 4,4'-diketo compound (Section 29). After chromatography, the spectrum of the sole pigment zone was taken in hexane (Fig. 13) and found to be identical with that of the synthetic 4,4'-dihydroxy- β -carotene (Fig. 11). A mixed chromatogram of the reduction products of the natural and synthetic ketones showed no separation (calcium carbonate-Celite; benzene-hexane 1:1).

The reduced canthaxanthin showed hypophasic behavior (hexane:95% methanol).

36. Conversion of α -Carotene (XXIX) to 4-Keto- α -carotene (XXXII).

α -Carotene (100 mg.) was treated with 66 mg. of NBS (molar ratio, 1:2) in ethanol-containing chloroform as described for β -carotene in Section 3. The reaction mixture was developed with hexane + 3% acetone:

4 brown
30 interzone
10 pink
4 interzone
5 orange
5 interzone
50 pink-orange: all-trans-4-keto- α -carotene
70 four diffuse orange zones: probably cis isomers of the former
15 interzone
5 orange: unreacted α -carotene

The photometrically estimated yield of the stereoisomeric 4-keto- α -carotenes was 20 mg. (corresponding to 20% of the starting material), and the yield of the all-trans form after crystallization from benzene-methanol amounted to 12 mg. (12%).

Crystal Form: Broad, orange-red slightly oval plates. Macroscopically, the crystals are much deeper in color than those of α -carotene.

Melting Point: 188-189°.

Partition Behavior: 95:5 in hexane:95% methanol.

Analysis: Calculated for $C_{40}H_{54}O$: C, 87.22 ; H, 9.88 .

Found : C, 87.12 ; H, 9.93 .

Spectrum: The curve showed but little fine structure; λ_{max} at 451 m μ (Fig. 18).

This compound was found to be identical with the oxidation product of 4-hydroxy- α -carotene obtained in our laboratory by Mr. W. V. Bush. (unpublished). This identification was based on spectra, melting points and non-separation on the column (lime-Celite, hexane + 3% acetone).

37. Reduction of 4-Keto- α -carotene (XXXII) to 4-Hydroxy- α -carotene (XXXIII).

Six milligrams of the 4-keto compound was reduced with lithium aluminum hydride in the manner described in Section 15 and the mixture was resolved on lime-Celite by developing with hexane + 5% acetone. The only main, yellow zone was eluted, evaporated and crystallized from benzene-methanol. Stubby needles; yield, 4 mg.; m.p., 176-178°.

This compound was identified with 4-hydroxy- α -carotene prepared by Mr. W. V. Bush. The spectra were identical and coincided with that of α -carotene (Fig. 18). Both samples melted at 175°. A mixed chromatogram test (lime-Celite, hexane + 4% acetone) showed no separation. In the test for allylic hydroxyl, a considerable deepening of the color was observed, although not as pronounced as with 4-hydroxy- β -carotene.

38. Factors Influencing the Interaction of β -Carotene and N-Bromosuccinimide.

a. The Effect of Ethanol in Chloroform.

β -Carotene (100 mg.) was treated with NBS (100 mg.) as described in Section 3, except that ethanol-free chloroform was used as the solvent. The reaction mixture was developed with hexane + 7% acetone on lime-Celite. The only main product observed was a mixture of stereoisomeric retro-bisdehydrocarotenes, while no 4-keto-3',4'-dehydro- β -carotene was present. The all-trans-retro-bisdehydrocarotene was identified with an authentic sample (prepared by Mr. G. Karmaker) by the spectrum and the mixed chromatogram test.

In a series of experiments, β -carotene (100 mg.) was reacted with NBS (100 mg.) under the conditions specified in Section 3, except that the ethanol content of the chloroform was varied. In each instance, the

product was chromatographed on lime-Celite and the 4-keto-3',4'-dehydro- β -carotene formed was estimated photometrically (Table 4).

Table 4.

Effect of the Ethanol Content of Chloroform in the Formation of 4-Keto-3',4'-dehydro- β -carotene.

Ethanol (% by volume) in chloroform (the first five figures refer to artificial mixtures and the last one to commercial chloroform)	% β -Carotene converted into all- <u>trans</u> -4-Keto-3',4'-de- hydro- β -carotene
0.1	7
0.7	20
<u>1.0</u>	<u>25</u>
1.4	16
10.0	5
<u>1</u>	<u>25</u>

b. Effect of Substitution of Ethanol by Other Alcohols.

When the β -carotene-NBS reaction was repeated using either 0.7% methanol or 2% benzyl alcohol in ethanol-free chloroform, the result was essentially the same as obtained with chloroform + 1% ethanol, i.e. about 25% of trans-4-keto-3',4'-dehydro- β -carotene was formed. The latter was crystallized and identified by the usual methods.

c. Effect of Glacial Acetic Acid or of Phenol.

The conversion was carried out in ethanol-free chloroform to which either 1% glacial acetic acid or 1% phenol had been added. After chromatography, retro-bisdehydrocarotene was identified as the only product; ketones were absent.

d. Effect of Ethanol Addition After the Formation of the Brominated Intermediates.

β -Carotene (100 mg.) was dissolved in 10 ml. of ethanol-free chloroform and reacted with 100 mg. of NBS in 10 ml. of the same grade of chloroform. The initial deep red color turned brown immediately. One minute after the addition of the NBS reagent, 10 ml. of an ethanol-chloroform (1:9) mixture was introduced, and the vigorous stirring was continued for one more minute. NPM was then added whereupon the solution was refluxed 15 minutes and further treated as described in Section 3. Developing on Lime-Celite with hexane + 5% acetone gave a chromatogram very similar to that observed with the initial use of commercial chloroform. The yield of the 4-keto-3',4'-dehydro- β -carotene was 13.6 mg. (estimated photometrically).

II. A STUDY OF THE BORON TRIFLUORIDE COMPLEXES OF SOME CAROTENOIDS.

A. THEORETICAL PART

1. Introduction.

It had been observed some time ago that BF_3 formed unstable, deeply colored complexes with carotenoids [Strain (40)],* and a similar phenomenon was reported by Lewis and Seaborg (41) for a second "Lewis acid", viz. BCl_3 .

However, little was known about these complexes until Zechmeister and Wallcave examined chromatographically the products resulting from the hydrolysis, or methanolysis, of the retro-dehydrocarotene- BF_3 complex. They showed that the main product from this reaction was 4-hydroxy- β -carotene (isocryptoxanthin), or its methyl ether (16). Wallcave, Zechmeister and Leemann (42) reported briefly that β -carotene also formed a dark blue BF_3 complex which produced upon methanolysis two new crystalline pigments.**

Vitamins A_1 and A_2 of the C_{20} - series also form blue complexes with another "Lewis acid", viz. SbCl_3 (43). These complexes appear somewhat more stable than those mentioned above, and the region of their maximum absorption (600-700 m μ) is often used for the quantitative determination of A_1 or A_2 in various mixtures. No decisive study has been made of the hydrolysis products of such complexes; furthermore, so far as is known, no vitamin A_1 - BF_3 complex has been reported.

*"Boron trifluoride...converted carotenoids into unstable blue pigments ...Alcohol reconverted these pigments into yellow substances that were strongly adsorbed on columns of magnesia and did not exhibit definite absorption maxima."

**The reagent was distilled BF_3 -etherate.

Considering the limited number of instances in which the products resulting from the hydrolyses of such complexes were described and structurally clarified, it was desirable to extend such studies in this interesting field.

2. 4-Hydroxy- β -carotene from β -Carotene via Its BF_3 Complex.

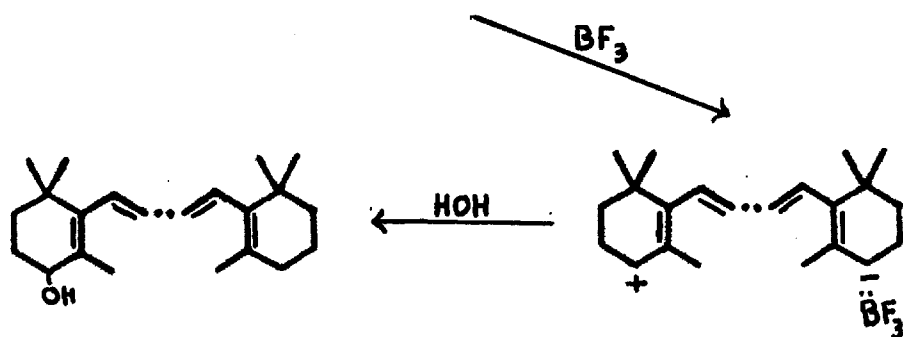
As reported earlier (42), when a solution of β -carotene in hexane is shaken for an hour with BF_3 -etherate, a dark blue complex forms in the lower BF_3 -etherate phase. On subsequent treatment with methanol two new crystalline carotenoids, Pigments "I" and "II", are formed. The visible spectra of these new carotenoids indicate that the chromophore originally present in β -carotene (11 conj. F) has been shortened to 8 and 9 conjugated double bonds respectively. The same treatment for only five minutes results mainly in a stereoisomeric mixture of β -carotenes.

In the present study it has been shown that when β -carotene is treated with BF_3 -etherate in chloroform solution (single phase) a dark blue complex forms within three minutes. Cleavage of the complex by means of an acetone-water mixture produced 4-hydroxy- β -carotene (yield of the all-trans-form, 35%).

It will be recalled that 4-hydroxy- β -carotene (isocryptoxanthin) had first been obtained by hydrolysis of the BF_3 -retro-dehydrocarotene complex (16). A mechanism was proposed by Zechmeister and Wallcave that involved essentially the α, ω -addition of water to the retro-dehydrocarotene chromophore via the carotenoid- BF_3 intermediate (Chart 17).



(XV.) retro-Dehydro-carotene



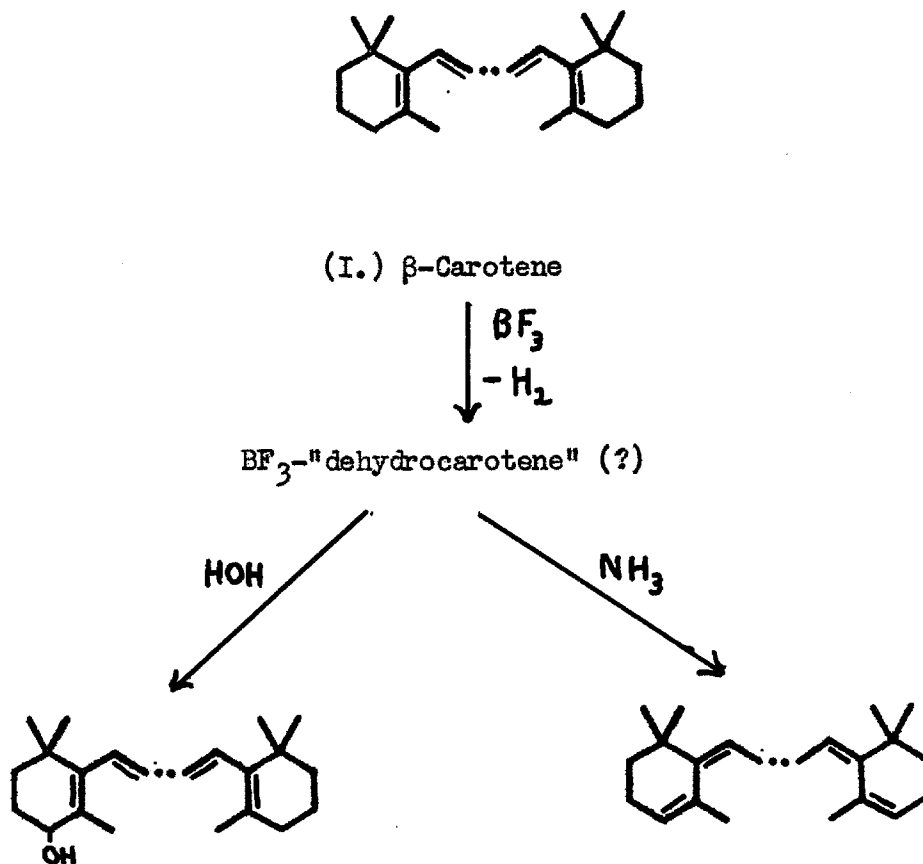
(IX.) 4-Hydroxy- β -carotene
(isocryptoxanthin)

Chart 17. Formation of 4-Hydroxy- β -carotene from retro-Dehydro-carotene (12).

In order to explain the direct formation of 4-hydroxy- β -carotene from β -carotene, employing a similar kind of hydration mechanism, one has to propose that BF_3 acts first as a dehydrogenating agent to give either retro-dehydrocarotene or 3,4-dehydro- β -carotene. Either of the latter two compounds could form a complex with BF_3 which would hydrolyze to 4-hydroxy- β -carotene (Sections 3 and 4 b).

Besides offering an efficient route for preparing 4-hydroxy- β -carotene, this reaction commands great interest because of the overall effect of replacing a hydrogen atom directly by a hydroxyl group. The process can thus be considered as a net oxidation at the 4-carbon atom.

A search of the literature did not reveal any analogous example involving BF_3 . Furthermore, since no other BF_3 -carotenoid reaction gave similar results, it can be proposed that some special feature of the β -ionylidene system in β -carotene allows its dehydrogenation by BF_3 to a "dehydrocarotene". Some indirect experimental evidence to support this view was gained by cleaving the blue complex with dry ammonia instead of water. Then the main product was retro-dehydrocarotene (XV) (Chart 18).



(IX.) 4-Hydroxy- β -carotene

(XV.) retro-Dehydrocarotene

Chart 18. Preparation of 4-Hydroxy- β -carotene and retro-Dehydrocarotene from β -Carotene.

It is also interesting that by carrying out the β -carotene complex formation in chloroform instead of hexane the overall reaction speeds up and gives a completely different set of products. In chloroform solution the Pigments "I" and "II", produced in hexane, were absent; in addition, some other different minor products appeared which have not yet been characterized.

3. 4-Hydroxy- β -carotene from retro-Dehydrocarotene via Its BF_3 Complex in Chloroform.

As mentioned above, hydrolysis of the retro-dehydrocarotene- BF_3 complex formed in hexane led to 4-hydroxy- β -carotene. When this reaction was repeated in chloroform solution, the main product was likewise 4-hydroxy- β -carotene. In this case, the change of solvent did not alter the course of the reaction as it did with β -carotene. It was observed that the formation of the retro-dehydrocarotene complex took place much more rapidly than with β -carotene.

4. Reinvestigation of Some Hydrolysis Products from 3,4-Dehydro- α - and β -carotene- BF_3 Complexes.

a. Introduction.

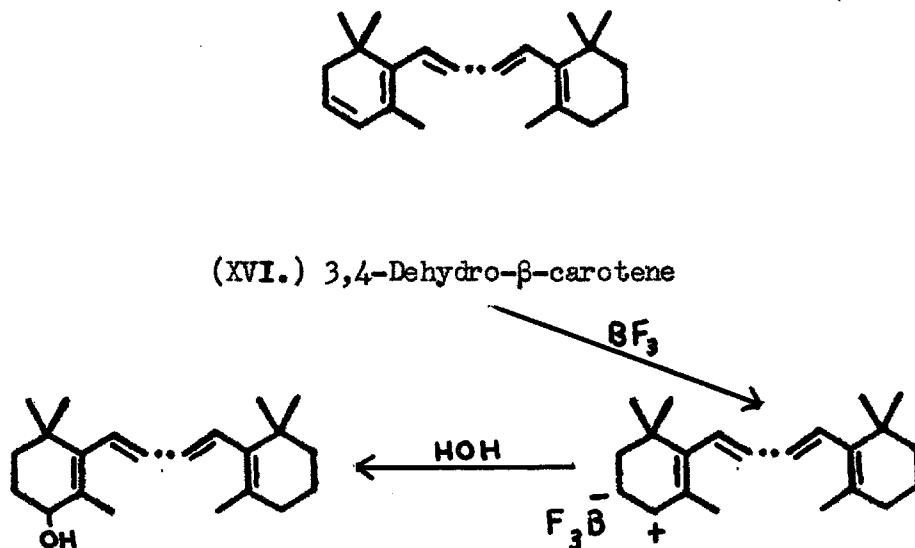
The reaction of 3,4-dehydro- α -carotene and 3,4-dehydro- β -carotene with BF_3 , followed by hydrolysis of the dark blue complexes, was carried out by Zechmeister and Karmakar using very limited amounts of substance (6). In each case, the chromophore of the hydrolysis products had reverted from that of the dehydro-compound to that of the parent compound, α - or β -carotene. These products were tentatively identified as hydrocarbons.

Since the main products resulting from such hydrolyses are usually hydroxylated, it was felt that the two conversions just mentioned should be reinvestigated.

A considerably improved method of preparing larger amounts of the dehydro-compounds from the respective α - and β -carotenes was devised (cf. Section 7); the complexes were formed and hydrolyzed essentially as described in the earlier paper.

b. 3,4-Dehydro- β -carotene.

The main product starting from 3,4-dehydro- β -carotene was identified as 4-hydroxy- β -carotene (isocryptoxanthin). The melting point, spectrum, and partition value were in excellent agreement with the same constants reported for the isocryptoxanthin obtained from retro-dehydro-carotene. Consequently, the overall reaction of 3,4-dehydro- β -carotene \longrightarrow isocryptoxanthin consists in the hydration of the 3,4-double bond as outlined in Chart 19.



(IX.) 4-Hydroxy- β -carotene

Chart 19. 4-Hydroxy- β -carotene from 3,4-Dehydro- β -carotene.

c. 3,4-Dehydro- α -carotene.

The compound obtained in good yields from 3,4-dehydro- α -carotene was also found to be an alcohol, viz. 4-hydroxy- α -carotene. Its physical constants agreed with those reported for an authentic sample prepared by the reduction of 4-keto- α -carotene (cf. Chapter IB, Section 31) or directly by hydrolysis of the α -carotene- BF_3 complex. The latter reaction has been studied in detail in our laboratory by Mr. W. V. Bush.

A reaction mechanism following the idea first expressed in Chart 19 is shown in Chart 20.

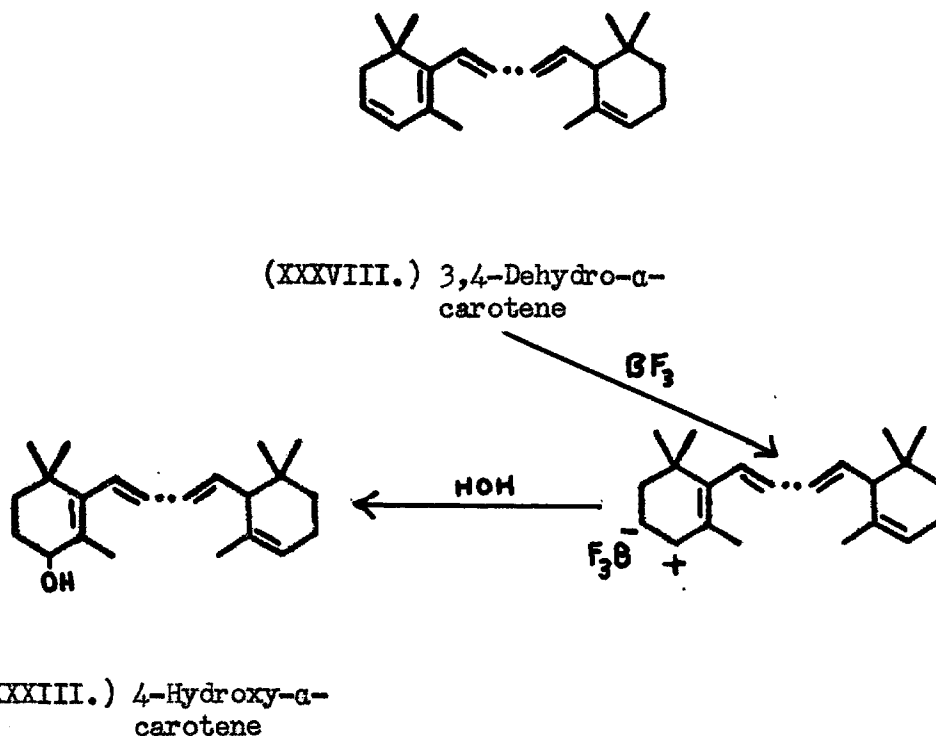
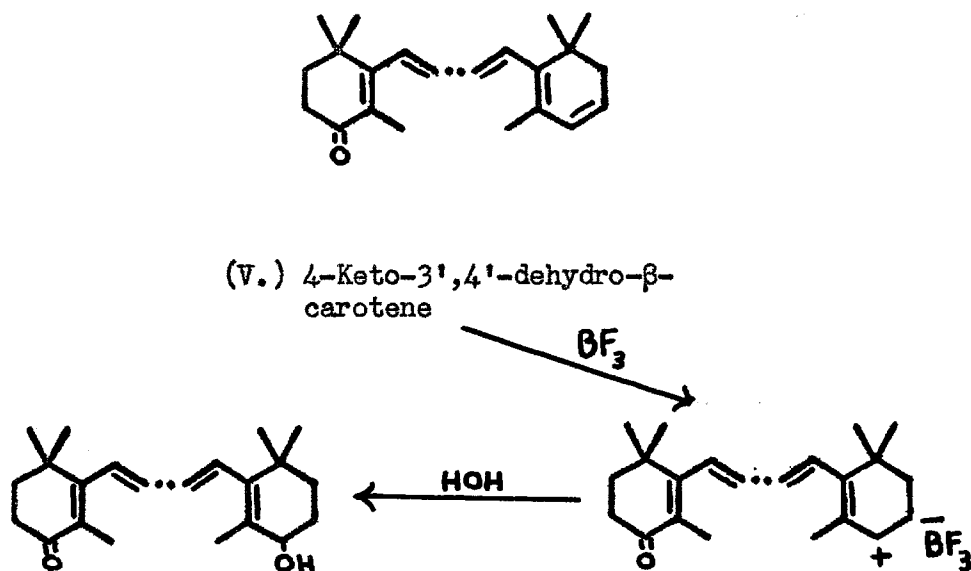


Chart 20. 4-Hydroxy- α -carotene from 3,4-Dehydro- α -carotene.

d. 4-Keto-3',4'-dehydro- β -carotene.

The BF_3 promoted hydration of the 3,4-double bond in carotenoid systems was first observed with 4-keto-3',4'-dehydro- β -carotene (V), which gave good yields of the 4'-hydroxy compound (XII) (Chart 10, p.18). Ethanolysis of the intermediate BF_3 complex led to 4-keto-4'-ethoxy- β -carotene (X) (Chart 10). In this compound the carbonyl group in one ring would probably stabilize the intermediate carbonium ion shown in Chart 21. The mechanism for the hydration is the same as that proposed for two 3,4-dehydro compounds (Chart 21).



(XII.) 4-Keto-4'-hydroxy- β -carotene

Chart 21. 4-Keto-4'-hydroxy- β -carotene from 4-Keto-3',4'-dehydro- β -carotene.

e. Discussion.

Although the main feature of these three reactions is explained by

Charts 19, 20 and 21, nonetheless, some further interesting questions can be raised.

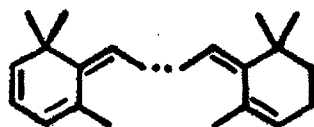
In the case of 3,4-dehydro- β -carotene (Chart 19), the saturated 4'-carbon could be attacked and transformed into a secondary alcohol group as happens in the process β -carotene \longrightarrow 4-hydroxy- β -carotene (Chart 18). Such a process would result in the formation of 4'-hydroxy-3,4-dehydro- β -carotene (\equiv 4-hydroxy-3',4'-dehydro- β -carotene). However, this compound could not be detected on chromatographing the reaction products, and its presence is doubtful.

A second question concerns the reaction outlined in Chart 20. It was observed that 4-hydroxy- α -carotene appeared as the only main product. However, one could reasonably expect that the 4',5'-double bond in the starting material (XXXVIII) would suffer hydration, since this very center is highly reactive in retro-dehydrocarotene.

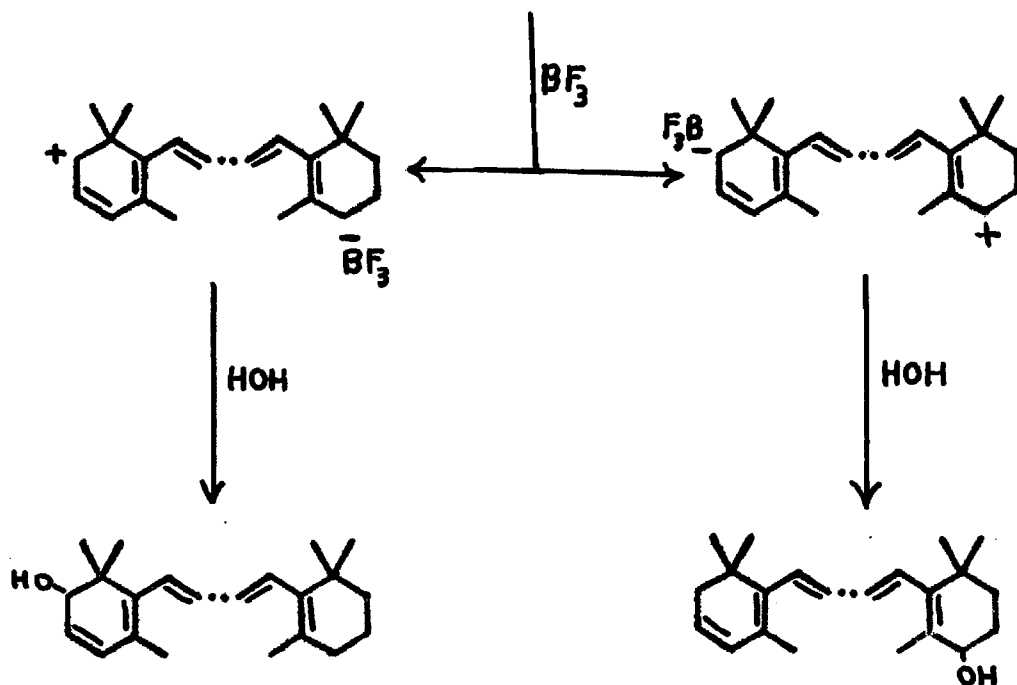
In conclusion, it appears that there is a great tendency for hydration of the 3,4-double bond when subjected to BF_3 followed by hydrolysis. Furthermore, the 3,4-hydration appears to have precedence over some other expected conversions. It will also be noted that none of the compounds containing the "normal" system of conjugated double bonds rearranged to the retro-system during the process, whereas the reverse phenomenon, retro \longrightarrow "normal", takes place very readily (cf. Chart 17).

5. 2-Hydroxy-3,4-dehydro- β -carotene from retro-Bisdehydrocarotene via Its BF_3 Complex.

Since the structure of retro-bisdehydrocarotene (III, Chart 22) had been secured, it was desirable that the study of the BF_3 complexes of retro-carotenoids should be extended to this compound.



(III.) retro-Bisdehydrocarotene



(XXXIX.) 2-Hydroxy-3,4-dehydro- β -carotene

(VII.) 4'-Hydroxy-3,4-dehydro- β -carotene

(\equiv 4-hydroxy-3',4'-dehydro- β -carotene)

Chart 22. Possible Paths for the Formation and Hydrolysis of the retro-Bisdehydrocarotene- BF_3 Complex.

Unlike retro-dehydrocarotene, compound (III.) is unsymmetrical, containing one extra ring-double bond. Therefore, hydrolysis of its

BF_3 complex could lead to either one or both of two products (XXXIX and VII, Chart 22) possessing the same structure except for the position of the hydroxyl group.

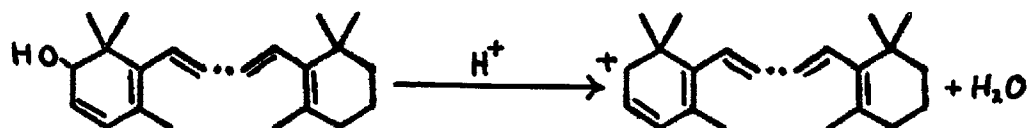
One of these proposed products, 4-hydroxy-3',4'-dehydro- β -carotene (VII) was already available from 4-keto-3',4'-dehydro- β -carotene (Chart 6, p.13).

The second product, viz., 2-hydroxy-3,4-dehydro- β -carotene (XXXIX), would represent a new carotenoid alcohol, and it would be of special interest since 2-substituted carotenoids have not yet been found in nature or prepared in the laboratory.

In pertinent experiments, the retro-bisdehydrocarotene- BF_3 complex hydrolyzed to give a single main product. After purification by chromatography, it crystallized from benzene-methanol in sturdy, square prisms which melted at 180-182°. The spectral curve (Fig. 29) was identical with that of 4-hydroxy-3',4'-dehydro- β -carotene (Fig. 7). The compound gave a strongly positive reaction with the HCl-chloroform reagent indicating an allylic hydroxyl whose presence was further confirmed by its easy conversion to an allylic ether in acidic methanol. On the basis of these observations, it was evident that the substance must either be (VII) or (XXXIX). Both melting point and crystal form pointed to a non-identity with 4-hydroxy-3',4'-dehydro- β -carotene (VII). This was also confirmed when the two pigments were submitted to the mixed chromatogram test; they were easily separable when developed with hexane + 8% acetone on lime-Celite. Since thus the structure (VII) was excluded, the new compound could only be 2-hydroxy-3,4-dehydro- β -carotene (XXXIX).

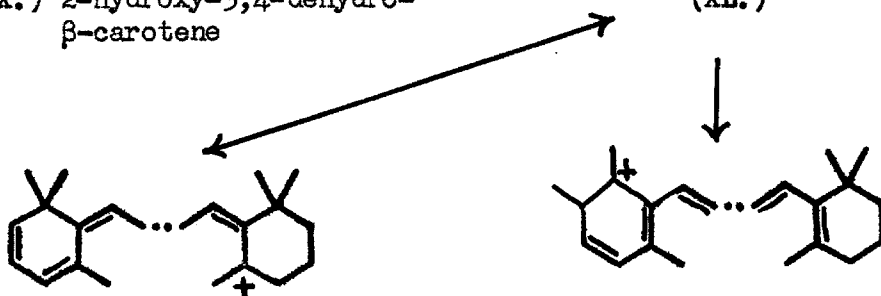
One would expect this compound to be less strongly adsorbed than the 4-hydroxy isomer, because the 2-hydroxyl group is sterically more

hindered due to the adjacent geminal dimethyl group. The Fischer-Hirschfelder models of the 2- and 4-hydroxy- β -ionylidene rings demonstrate this feature clearly. Further evidence of the more hindered nature of the 2-hydroxyl derivative is gained from its partition coefficient, i.e. 90:10 in hexane:95% methanol. It is 10% more epiphasic than the 4-hydroxy compound (80:20). This difference could also be related to the sterically screened position of the 2-hydroxyl group which would be less available for solvation by the methanol.

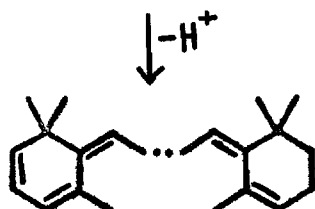


(XXXIX.) 2-Hydroxy-3,4-dehydro- β -carotene

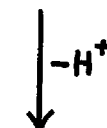
(XL.)



(LXI.)



(III.) retro-Bisdehydrocarotene



(XLII.)

Chart 23. Dehydration of 2-Hydroxy-3,4-dehydro- β -carotene.

The acid-catalyzed dehydration (in chloroform) of the 2-hydroxy compound (XXXIX) gave a 70% yield of retro-bisdehydrocarotene, which, in this case, can only arise from rearrangement of the intermediate carbonium ion (XL) to (XLI) as expressed in Chart 23.

If the "normal" carbonium ion intermediate (XL) were stable, it should participate in a Wagner-Meerwein rearrangement of the adjacent geminal dimethyl group, resulting finally in (XLII), where the ionylidene ring has been converted to a phenyl moiety. There was, however, no evidence for the formation of this compound.

The reconversion of (XXXIX) to retro-bisdehydrocarotene further supports its 2-hydroxy-3,4-dehydro- β -carotene structure.

6. Formation of a Vitamin A₁ Isomer by Hydrolysis of the Anhydrovitamin A₁-BF₃ Complex.

Since it was shown earlier (16), as well as in the present study, that the hydrolysis of two retro-carotenoid-BF₃ complexes led to the formation of allylic polyene alcohols containing a "normal" conjugated system, it seemed possible that a similar conversion could be applied to the corresponding C₂₀-series. Such a reaction would have immediate practical application by opening up a new approach to vitamin A₁ synthesis.

Previously, in all routes leading to the synthesis of vitamin A₁ a rearrangement of the "normal" to the retro system (usually occurring during the dehydration of intermediate alcohols) has been evaluated as an undesirable side-effect (44). In fact, some such routes have been abandoned because a retro-system appeared almost exclusively (45).

Thus, it was of interest to study the hydrolysis of the BF_3 complex of anhydrovitamin A_1 (XLIII) in order to determine whether or not vitamin A_1 could be recovered. On the basis of similar reactions in the carotenoid series, two products could be expected to result, viz. vitamin A_1 (XLIV) or one of its isomers, 4-hydroxy-axerophthene (XLV) (Chart 24).

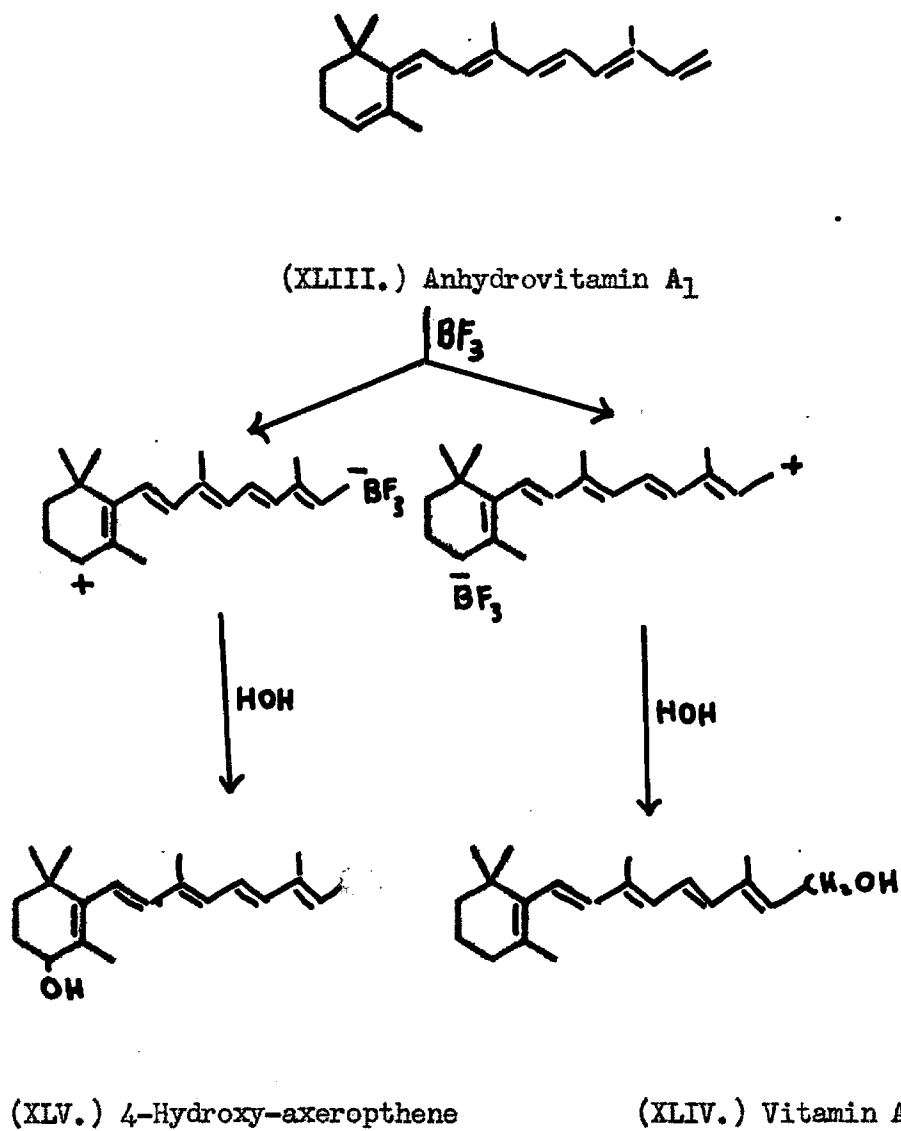


Chart 24. Two Possible Paths for the BF_3 Complex Formation of Anhydro-vitamin A_1 and Subsequent Hydrolysis.

The starting material, anhydrovitamin A₁, was prepared from crystalline vitamin A₁ by acid-catalyzed dehydration in chloroform*. The chromatographically homogenous anhydro-compound (XLIII) was used without previous crystallization for the preparation of the complex. The deep-blue anhydrovitamin A₁-BF₃ complex formed readily at 0° in chloroform. After hydrolysis of the complex, chromatography of the products and inspection of the lime-Celite column with ultraviolet light revealed the presence of a main zone which appeared very similar to that of vitamin A₁ (bright blue fluorescence). However, the adsorption behavior of this compound was very different from vitamin A₁ since on lime-Celite it developed with 5% acetone in hexane, whereas vitamin A₁ requires 15% acetone.

The spectrum of this compound in the ultraviolet region was identical with that of vitamin A₁ (λ_{max} at 325 m μ , Fig. 30). Their respective infrared spectra (Figs. 31 and 32), however, were different, especially in the C-O stretching region (9-10 μ). It was pointed out for the carotenoid series (Chapter IA, Section 9) that the wavelength position of the band mentioned varied considerably when the hydroxyl group was located on different carbon atoms. The new compound, like vitamin A₁, did show the 2.75 μ band which must be due to O-H stretching. The above considerations furnished presumptive evidence that the hydrolysis product was 4-hydroxy-axerophthene (XLV, Chart 24).

*From the dehydration of vitamin A₁ a second product was obtained which was adsorbed strongly on the column. Its spectrum (333, 348, 367 m μ) and adsorption behavior indicate that this compound may be identical with the vitamin A₁ isomer described by Cawley and Seidel (44, there p. 51). Its formation during the dehydration of vitamin A₁ hasn't been noted before although it has recently been shown that vitamin A₁ acetate under similar conditions gives the corresponding acetate (46).

Further support for this proposed structure was gained by dehydrating the compound to anhydrovitamin A₁. Hence, the hydroxyl group occupies probably the allylic position 4. The new vitamin A₁ isomer is more epiphasic (62:38 in hexane:95% methanol) than vitamin A₁ (36:64); this behavior might be expected since the compound is a secondary alcohol in contrast to vitamin A₁.

Since no vitamin A₁ was detected in the reaction mixture, it can be assumed that only one of the BF₃ -anhydrovitamin A₁ complexes shown in Chart 24 was formed as an intermediate.

It should be emphasized at this point that the results obtained in the study of this particular reaction are of a preliminary nature, especially, since the product has been prepared only on a milligram scale, and no attempt has been made to crystallize it. (The low temperature crystallization techniques generally used in the C₂₀ -series are not readily applicable to small quantities).

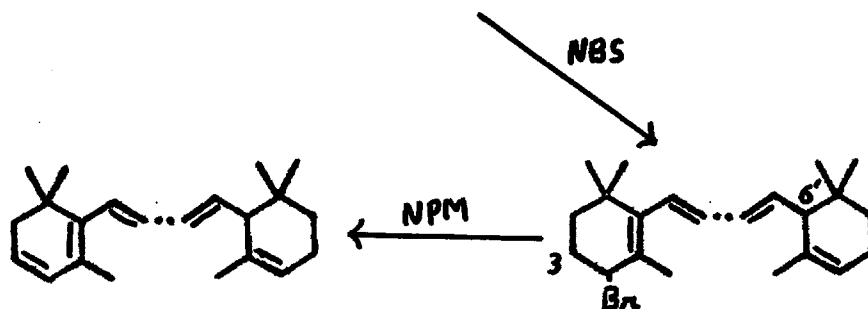
7. An Improved Method for the Preparation of 3,4-Dehydro- α - and - β -carotenes.

a. Introduction.

In Chapter I a comparative discussion was given of some bromination-dehydrobromination reactions of carotenoids with NBS in chloroform and carbon tetrachloride solutions. It was concluded that the reactions in chloroform are more easily controlled. This suggested that chloroform could well be applied to improve the yields of 3,4-dehydro- α - and - β -carotenes from α - and β -carotene as outlined in Charts 25 and 26. By the method described earlier (6), using carbon tetrachloride, 3,4-dehydro- α -carotene was obtained from α -carotene in only 3.8% yield while β -carotene gave a 1.4% yield of its 3,4-dehydro derivative. (These values refer to crystalline material).



(XXIX.) α -Carotene

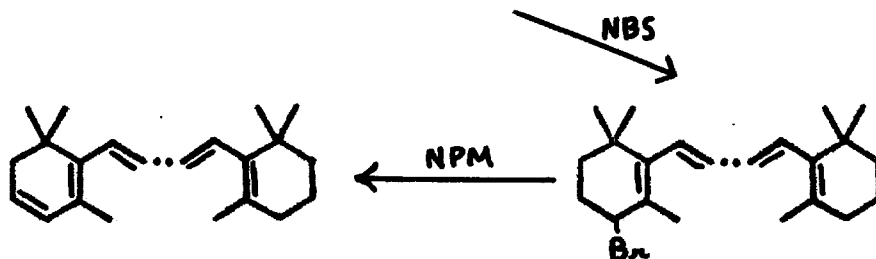


(XXXVIII.) 3,4-Dehydro- α -carotene

Chart 25. Preparation of 3,4-Dehydro- α -carotene from α -Carotene



(I.) β -Carotene



(XVI.) 3,4-Dehydro- β -carotene

Chart 26. Preparation of 3,4-Dehydro- β -carotene from β -Carotene.

In the reaction of α -carotene with NBS (Chart 25) both allylic positions, viz. 4 and 6', should be considered a priori as subject to attack by NBS. However, it had been found in the preparation of 4-keto- α -carotene (XXXII) (Chart 16, p. 33) that the 6' position was unreactive, and that the bromination occurred exclusively at C-4. In the case of the (symmetrical) β -carotene, such considerations do not arise. Hence, the 4-bromo intermediates shown in Charts 25 and 26 could be expected to appear in good yields. The course of the dehydrobromination with NPM could not be predicted; either "normal" and/or retro structures could result.

b. α -Carotene \longrightarrow 3,4-Dehydro- α -carotene.

The conversion was carried out in ethanol-free chloroform as described in Chapter I. Starting from α -carotene, approximately a 40% yield of stereoisomeric 3,4-dehydro- α -carotenes was obtained. The crystalline yield of the all-trans pigment was 14.5%. Only small amounts of other products were observed on the column, showing that no substantial rearrangement to retro-structures had taken place.

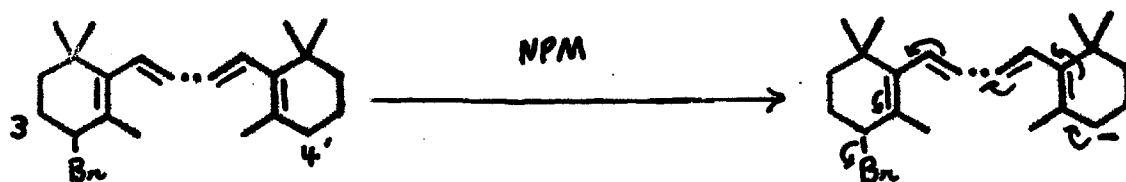
c. β -Carotene \longrightarrow 3,4-Dehydro- β -carotene.

In contrast, starting from β -carotene only 9% was converted to the stereoisomeric 3,4-dehydro mixture, and 2% could be obtained as the crystalline all-trans isomer. The top section of the column yielded mostly retro-dehydrocarotene and retro-bisdehydrocarotene (III). Their sum corresponded to 50% of the β -carotene reacted.

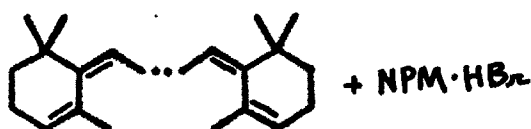
d. Discussion.

Evidently, during the dehydrobromination of 4-bromo- β -carotene (XVIII) some mechanism is operative by which the "normal" system rearranges to the retro system. This mechanism can be described as the

attack by base on a 4'-hydrogen of the brominated intermediate (XVIII) with elimination of the halogen (according to Chart 27) to give the retro compound (XV). Attack by base on the 3-hydrogen would generate, after elimination of halogen, the 3,4-dehydro- β -carotene.



(XVIII.) 4-Bromo- β -carotene



(XV.) retro-Dehydrocarotene

Chart 27. Formation of retro-Dehydrocarotene from the Intermediate 4-Bromo- β -carotene.

That a similar process does not take place with the α -carotene intermediate could possibly be attributed to the circumstance that the 6'-hydrogen atom is sterically less accessible to an attack by NPM than is the 3-hydrogen (Chart 25). The relative stability of the 6'-hydrogen atom was first suggested by Oroshnik who observed (47) in the C_{20} -series that the compounds containing an α -ionylidene ring were

much less susceptible to rearrangement than the isomeric β -ionylidene derivatives.

8. An Improved Method for the Preparation of retro-Bisdehydrocarotene from β -Carotene.

Although the interaction of β -carotene with NBS in refluxing carbon tetrachloride has served for the preparation of retro-bisdehydrocarotene in amounts sufficient for its characterization (5), this process did not lend itself to a preparative scale.

The old method involved the separation of retro-bisdehydrocarotene (and its spatial isomers) from the stereoisomeric retro-dehydrocarotenes and anhydro-eschscholtzxanthins by a tedious chromatographic process. The yield of crystalline all-trans-retro-bisdehydrocarotene (III) amounted to only 1%.

In the course of the structural work on 4-keto-3',4'-dehydro- β -carotene, the 4-keto-compound was reduced with lithium aluminum hydride in good yield to the 4-hydroxy compound. The latter was dehydrated in approximately 60% yield to the stereoisomeric retro-bisdehydrocarotenes (Chart 6, V \longrightarrow VII \longrightarrow III).

The same sequence has now been used to give a 24% yield of the stereoisomeric retro-bisdehydrocarotenes from β -carotene. The main modification of the procedure, from that given in Chart 6, consisted in utilizing the cis forms as well as the all-trans form in every step.

This was justified because the acid conditions prevailing during the last step (dehydration of the 4-hydroxy compound) resulted in the same ratio of cis-trans retro-bisdehydrocarotenes regardless of the configuration of the starting material. The yield of crystalline all-trans-retro-bisdehydrocarotene was 7%.

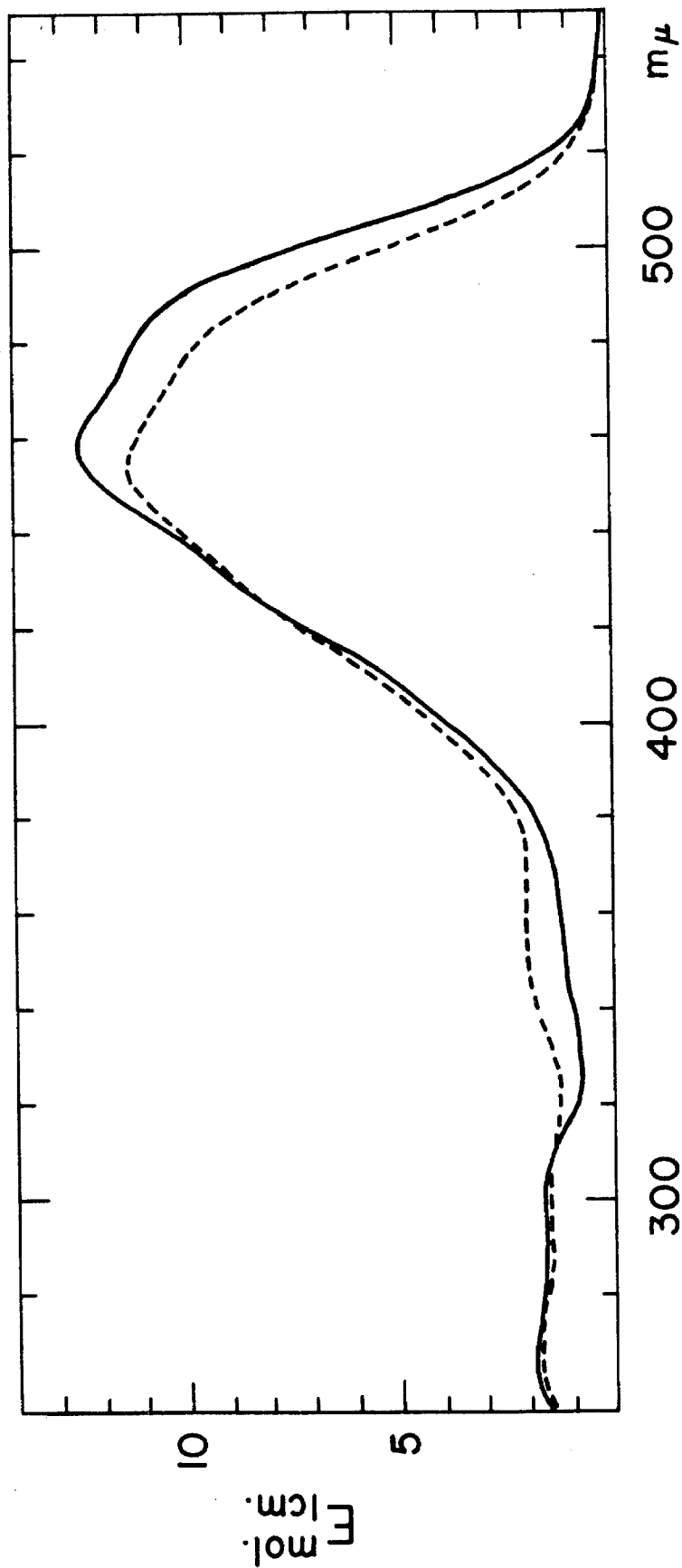


Figure 29. Molecular extinction curve in hexane of all-trans-2-hydroxy-3,4-dehydro- β -carotene: —, fresh solution; ----, after iodine catalysis in light.

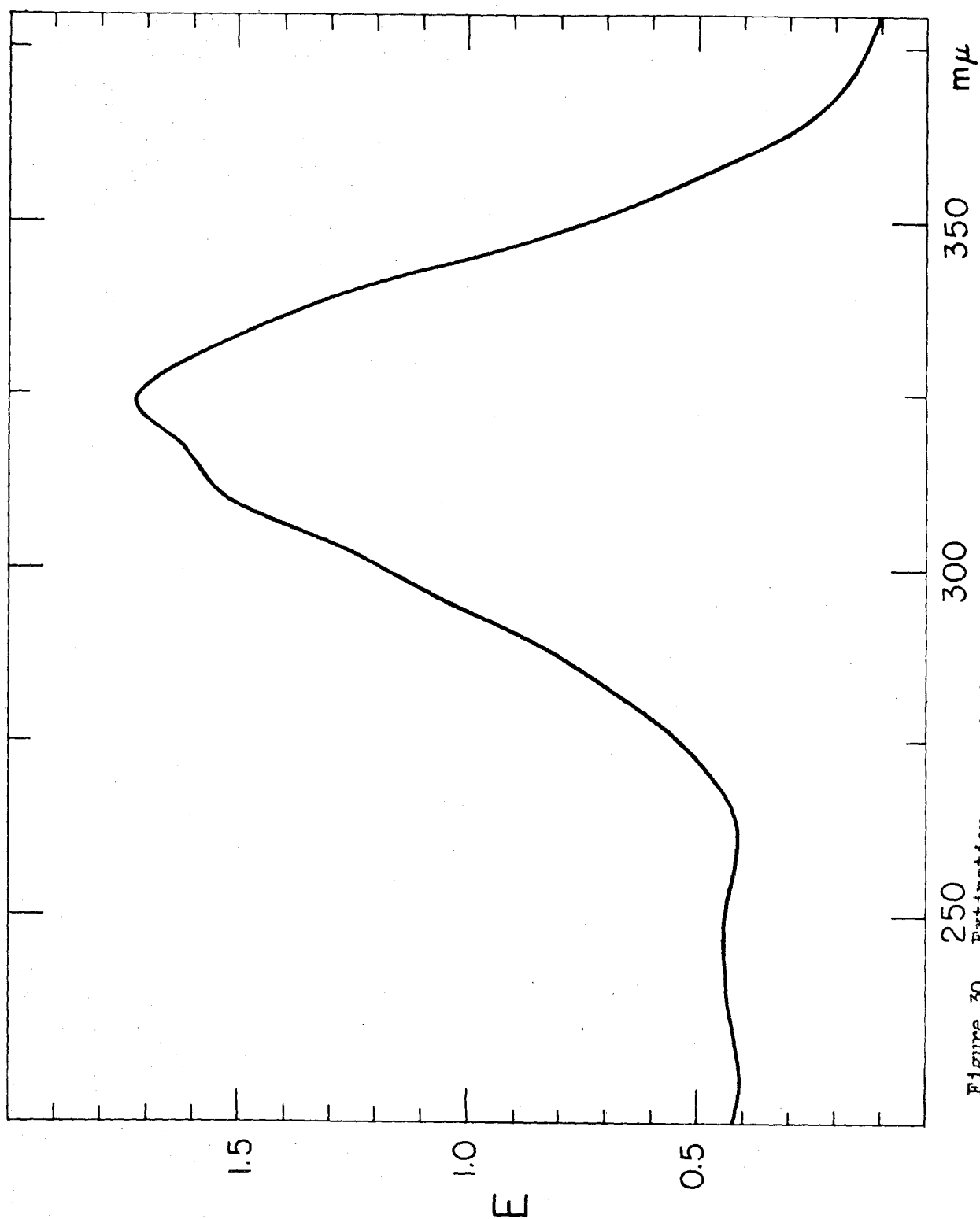


Figure 30. Extinction curve in hexane of 4-hydroxy-axerophthene.

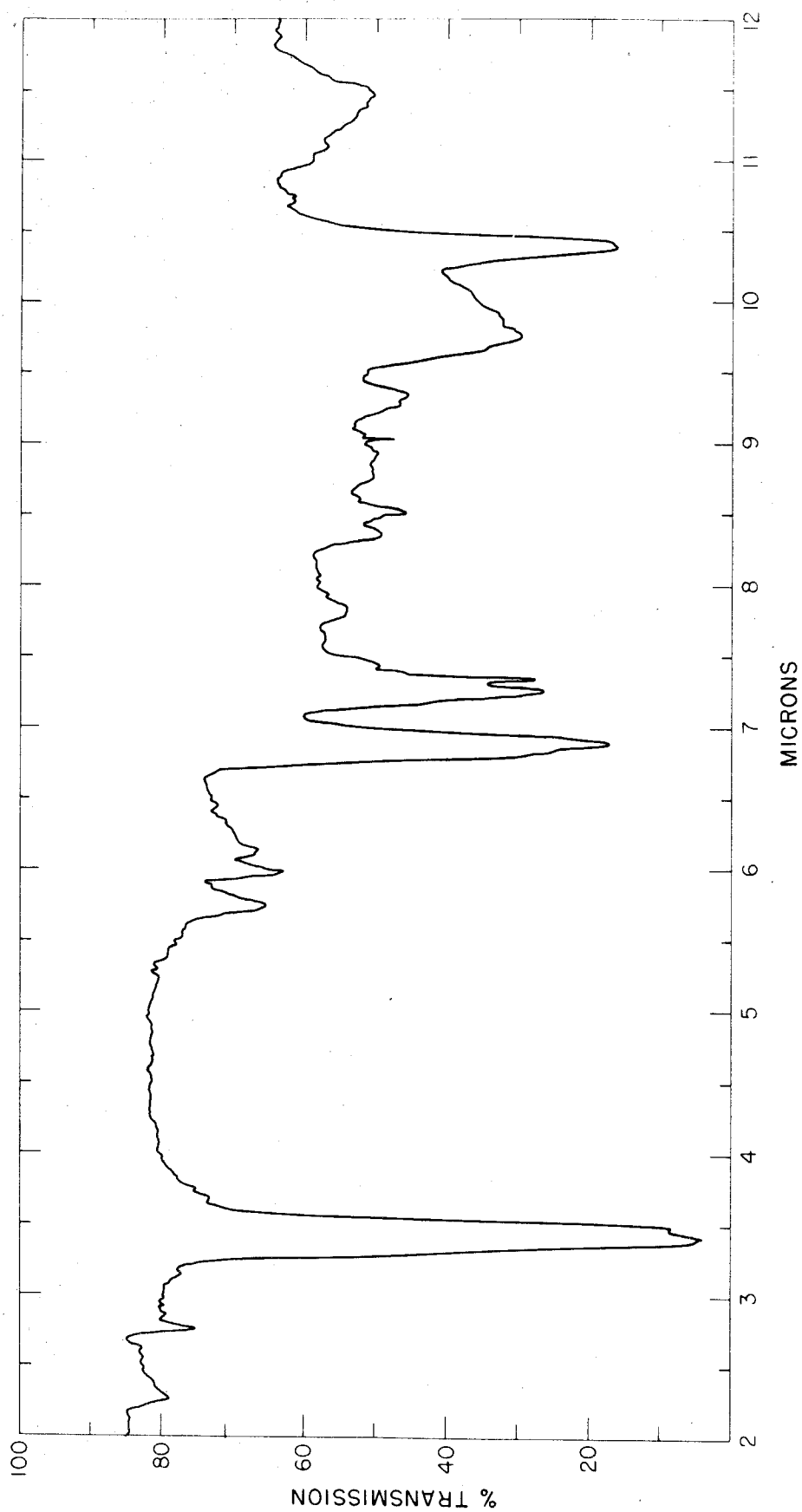


Figure 31. Infrared curve in carbon tetrachloride of 4-hydroxy-axerophthene.

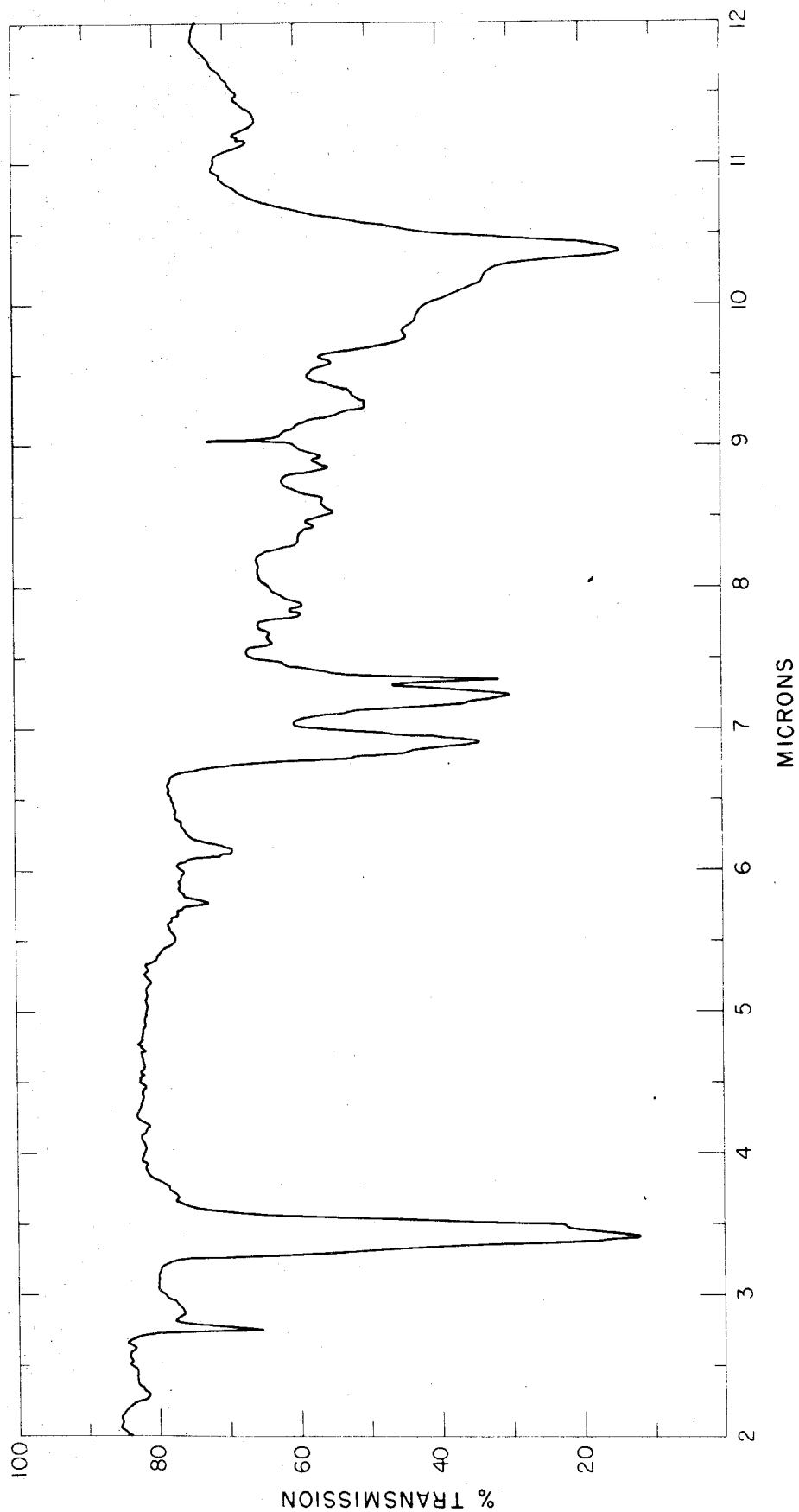


Figure 32. Infrared curve in carbon tetrachloride of vitamin A₁.

B. EXPERIMENTAL PART

1. Isocryptoxanthin (4-Hydroxy- β -carotene) from β -Carotene via the BF_3 Complex in Chloroform.

To a deep red solution of 100 mg. of β -carotene in 100 ml. of chloroform (Merck's R.G.) 10 ml. of BF_3 -etherate was added, with vigorous swirling. The solution turned green immediately, developing a deep blue color after 3 minutes, whereupon it was poured rapidly, with swirling, into a mixture of 1000 ml. of acetone and 200 ml. of water*. Hexane (200 ml.) was added and the pale orange epiphase was washed for 30 minutes, dried and evaporated completely. The orange colored residue was dissolved in 5 ml. of benzene, diluted to 50 ml. with hexane, and developed with hexane:

70 red-orange: 4-hydroxy- β -carotenes
100 interzone
30 three orange zones (450, 479 m μ)
20 interzone
10 orange (434, 458, 488 m μ)
3 interzone
10 pale yellow
30 empty section

The 70-mm. red-orange zone was rechromatographed on lime-Celite with hexane + 5% acetone as the developer:

40 empty section
50 orange: all-trans-isocryptoxanthin
30 deep yellow: cis isocryptoxanthins
45 interzone
20 two minor diffuse yellow zones
70 empty section

*In the first experiments, using a different preparation of the BF_3 -etherate, the complex was hydrolyzed by shaking the chloroform solution vigorously with 1 vol. of water (instead of acetone-water) until the blue color disappeared. With subsequent preparations of BF_3 -etherate, however, this procedure caused the orange solution (after hydrolysis) to turn dark brown during subsequent operations, resulting in lower yields. This phenomenon was prevented by the use of acetone-water as described.

The yield of the all-trans-isocryptoxanthin (determined photometrically) was 32%. After evaporation of the hexane solution, the powdery, orange residue was crystallized from chloroform-ethanol. Yield, 18 mg. (18%).

Crystal Form: Oval plates.

Melting Point: 166-168°.

Partition Behavior: 86:14 in hexane:95% methanol.

Analysis: Calculated for $C_{40}H_{56}O$: C, 86.87 ; H, 10.22 .

Found : C, 87.08 ; H, 10.44 .

Spectrum: The spectrum was identical with that reported earlier for isocryptoxanthin (and β -carotene). The infrared spectrum is shown in Fig. 23.

A mixed chromatogram with an authentic sample of isocryptoxanthin showed no separation (lime-Celite; hexane + 4% acetone).

Reaction with the HCl-Chloroform Reagent: The test for an allylic hydroxyl was positive.

2. 4-Hydroxy- β -carotene from retro-Dehydrocarotene via the BF_3 Complex in Chloroform.

The reaction was carried out with 100 mg. of all-trans-retro-dehydrocarotene as described for β -carotene but the time allowed for the complex formation was shortened to 15 seconds and the temperature of both the chloroform and the acetone-water was maintained at 0° during hydrolysis.

The pigment mixture was developed on a column with hexane + 6% acetone:

30 four pink and orange zones
45 interzone
20 yellow
5 interzone
35 orange: all-trans-4-hydroxy- β -carotene
4 interzone
15 orange
12 interzone } cis 4-hydroxy- β -carotenes
6 yellow
45 empty section

The trans-4-hydroxy- β -carotene zone was eluted, transferred to hexane, washed and dried. Yield, 13 mg. (13%). The substance crystallized as oval plates from chloroform-methanol. Yield, 7 mg. (7%). The preparation was identified with isocryptoxanthin by the partition behavior, melting point, spectrum and mixed chromatogram.

3. 4-Hydroxy- β -carotene from 3,4-Dehydro- β -carotene via the BF_3 Complex in Chloroform.

The complex formation and hydrolysis were carried out with 20 mg. of substance as described for β -carotene but the time for the complex formation was reduced to 1 minute. After chromatography, the all-trans-isocryptoxanthin (2.5 mg., photometrically estimated) was crystallized from chloroform-methanol.

Crystal Form: Oval plates.

Melting Point: 165-168°.

Partition Behavior: 86:14 in hexane:95% methanol.

Spectrum: The spectral curve was identical with that of isocryptoxanthin.

Reaction with the HCl-Chloroform Reagent: The test for allylic hydroxyl was positive.

A mixed chromatogram test with an authentic sample of isocryptoxanthin showed no separation (lime-Celite, hexane + 5% acetone).

4. 4-Hydroxy- α -carotene from 3,4-Dehydro- α -carotene via the BF_3 Complex in Chloroform.

Seventy mg. of 3,4-dehydro- α -carotene was treated with BF_3 - etherate and the complex was hydrolyzed as described for 3,4-dehydro- β -carotene. After developing on lime-Celite with hexane + 4% acetone, the main yellow-orange zone was eluted and transferred to hexane (17.5 mg. = 25%, photometric estimation). Upon evaporation, the powdery, orange residue was crystallized from benzene-methanol. Yield, 10 mg. (14%).

Crystal Form: Long, rectangular plates, mostly with broken ends.

Melting Point: 177° (sintering at 173°).

Partition Behavior: 85:15 in hexane:95% methanol.

Analysis: Calculated for $\text{C}_{40}\text{H}_{56}\text{O}$: C, 86.89 ; H, 10.21 .

Found : C, 87.01 ; H, 10.34 .

Spectrum: The curve in the visible region was identical with that of α -carotene.

Reaction with the HCl-Chloroform Reagent: The test for allylic hydroxyl was positive, but the color change was weaker than that shown by isocryptoxanthin.

No separation was observed in the mixed chromatogram test from an authentic sample of 4-hydroxy- α -carotene prepared by Mr. W. V. Bush.

5. 3,4-Dehydro- α -carotene from α -Carotene.

To a solution of 100 mg. of α -carotene in 10 ml. of ethanol-free chloroform, maintained at 0° , was added, with vigorous stirring by means of a nitrogen bubbler, a solution (0°) of NBS (33 mg.; molar ratio, 1:1) in 15 ml. of ethanol-free chloroform. After stirring for 30 seconds, 70 mg. of NPM was added to the greenish-brown solution, followed by 10

minutes' refluxing. The deep-red solution was evaporated, and the residue was dissolved in 5 ml. of benzene. Upon dilution with 40 ml. of hexane, a white precipitate appeared (probably succinimide or N-phenylmorpholine -hydrobromide) and was filtered off. The clear, red solution was then developed with hexane:

20	several orange and pink zones	
50	colorless	
30	orange: neo-3,4-dehydro- α -carotene U	
3	yellow	
20	colorless	
55	orange: all- <u>trans</u> -3,4-dehydro- α -carotene	
20	pale orange	} <u>cis</u> isomers of the former
10	yellow	
10	pale yellow	
40	empty section	

The 55-mm. zone was eluted, transferred to hexane and rechromatographed on magnesia-lime-Celite (developer, benzene-hexane 1:3). The single main zone was eluted, and transferred to hexane (yield 20 mg.; photometric estimation). The orange-red evaporation residue was crystallized from benzene-methanol; yield, 14.5 mg. = 14.5%; Rhombic plates, m.p. 190-192°. The visible spectrum was identical with that reported for 3,4-dehydro- α -carotene; λ_{\max} at 455 m μ .

6. 3,4-Dehydro- β -carotene from β -Carotene.

β -Carotene (100 mg.) was reacted with NBS (35 mg., molar ratio 1:1), and NPM as described above for α -carotene. The benzene-hexane solution of the reaction products was developed with hexane on a 30 x 8 cm. column:

10	pink	} <u>retro</u> -dehydro-carotenes and <u>retro</u> -bisdehydro-carotenes (50 mg.)
40	red-orange	
50	six orange zones	
20	pink	
10	orange	
5	colorless	
20	pink-orange: all- <u>trans</u> -3,4-dehydro- β -carotene (4.5 mg.)	
15	two yellow and pink zones: <u>cis</u> isomers of former	
10	interzone	
30	orange: unreacted β -carotene (6 mg.)	

5 interzone
40 yellow-orange: a cis isomer of β -carotene
10 pale orange
40 empty section

The all-trans-3,4-dehydro- β -carotene zone was eluted, evaporated and crystallized from benzene-methanol. Yield, 2 mg. (2%). The visible spectrum was identical with that reported for 3,4-dehydro- β -carotene; λ_{max} at 460 m μ .

7. Preparation of retro-Bisdehydrocarotene from β -Carotene.

a. Small Scale Preparation.

β -Carotene (100 mg.) was reacted with NBS and NPM as described in Chapter IB, Section 3. The solution, following the refluxing, was evaporated to a brown, pasty residue which was dissolved in 5 ml. of benzene and diluted with 75 ml. of hexane. The solution was filtered to remove a precipitate and poured into a solution of 200 mg. of lithium aluminum hydride in 200 ml. of anhydrous ether. After standing for 15 minutes, the excess hydride was decomposed with methanol. The hexane-ether solution was washed thoroughly with water, dried and evaporated completely. The hexane solution of the orange-red residue was developed with hexane + 7% acetone:

10 brown
30 interzone
30 pink: all-trans-4-hydroxy-3',4'-dehydro- β -carotene
20 orange: cis isomers of the former*
90 interzone
10 pink
80 empty section

The 30-mm. pink zone and the 20-mm. orange zone were eluted separately, transferred to hexane, and evaporated. Both residues were

*These two zones also contain some retro-bisdehydrocarotenes corresponding to Zone B of the chromatogram described in Chapter IB, Section 3.

then treated as follows: After dissolving in 20 ml. of chloroform (Merck's R.G.), 10 drops of the HCl-chloroform reagent were added with stirring. A marked deepening in the color of the red solution took place immediately. After 5 minutes standing, the chloroform solution was washed acid-free with bicarbonate and water, and evaporated to dryness. This residue was dissolved in 5 ml. of benzene, diluted to 50 ml. with hexane and developed with hexane + 8% acetone. The two chromatograms had qualitatively the same appearance:

10 light brown	
5 interzone	
10 light pink	
30 interzone	
20 pink: all- <u>trans-retro</u> -bisdehydrocarotene	(460, <u>489</u> , 518 mμ)
5 colorless	
15 orange a <u>cis retro</u> -bisdehydrocarotene	(457, <u>483</u> , 513 mμ)
5 interzone	
10 light orange	} probably <u>cis retro</u> -bisdehydrocarotenes
2 interzone	
5 light orange	

The total yield of the trans and cis retro-bisdehydrocarotenes from the two columns amounted to 24 mg. (24%, photometric estimation).

b. Large Scale Preparation.

The conversion was carried out starting from 1.6 g. of β -carotene as described under (a), with the following modifications. The pigment mixture from the hydride reduction was chromatographed on two 45 x 8 cm. percolators containing lime-Celite. The trans and cis isomers of 4-hydroxy-3',4'-dehydro- β -carotene were eluted together, transferred to hexane, washed, dried and evaporated to dryness. The dark red, oily residue (630 mg., photometric estimation) was dissolved in chloroform (500 ml.) and treated with 2 ml. of the HCl-chloroform reagent. The stereoisomeric retro-bisdehydrocarotene mixture thus obtained was resolved by chromatographing on 9 columns. The combined trans zones were

eluted, transferred to hexane, and evaporated to give a deep purple, powdery residue. Crystallization from benzene-methanol yielded 110 mg. (7%) of quadrangular plates; m.p. 202°.

The cis zones from the 9 columns were also combined. After elution, this pigment was transferred to hexane, washed, dried and evaporated to a dark red oil. The material was used without further purification for the preparation of 2-hydroxy-3,4-dehydro- β -carotene (Section 8).

8. 2-Hydroxy-3,4-dehydro- β -carotene from retro-Bisdehydro-carotene.

a. From Crystalline all-trans-retro-Bisdehydrocarotene.

To a solution of 50 mg. substance in 50 ml. of ethanol-free chloroform, kept at 0°, 5 ml. of BF₃-etherate was added, with swirling. The dark blue complex was allowed to stand for 30 sec., whereupon it was poured rapidly into a pre-cooled mixture (0°) of 600 ml. of acetone and 200 ml. of water. The dark blue color turned orange immediately. The solution was poured without delay into a 1-liter separatory funnel containing 100 ml. of hexane. Water was added, and the upper (hexane-chloroform) phase was washed thoroughly. After drying and evaporation the oily, red pigment mixture was dissolved in 50 ml. of hexane and developed with hexane + 7% acetone:

10 brown
10 interzone
20 several orange zones
30 interzone
10 pink
10 interzone
30 pink-orange: all-trans-2-hydroxy-3,4-dehydro- β -carotene
40 several orange and yellow zones: cis isomers of former
90 mainly colorless with some minor yellow zones

The 30-mm. pink-orange zone was eluted, transferred to hexane, washed and dried (Yield, 14.5 mg. or 29%; photometric estimation).

b. From the Cis Isomers of retro-Bisdehydrocarotene.

Fifty milligrams (photometric estimation) of the cis material (Section 7 b.) was treated with BF_3 and the complex was hydrolyzed as described under a. The subsequent chromatogram was qualitatively the same as that described above, but the yield of all-trans-2-hydroxy-3,4-dehydro- β -carotene was considerably lower (14%, photometric estimation).

c. Characterization of 2-Hydroxy-3,4-dehydro- β -carotene.

Forty milligrams of all-trans-2-hydroxy-3,4-dehydro- β -carotene was rechromatographed on two columns (developer, hexane-benzene 1:1). The two respective main zones were eluted with ethanol. From the combined eluates the pigment was transferred to benzene-hexane by the addition of water. After washing and drying, the solution was evaporated and the powdery, red-orange residue was crystallized from chloroform-methanol. Yield, 30 mg.

Crystal Form: Sturdy, square prisms.

Melting Point: 180-182°.

Partition Behavior: 90:10 in hexane:95% methanol.

Spectrum: The spectral curve (Fig. 29) was identical with that of 4-hydroxy-3',4'-dehydro- β -carotene, λ_{max} at 459 m μ .

Molecular Extinction Coefficient: $E_{1 \text{ cm.}}^{\text{mol}} = 12.5 \times 10^4$ at λ_{max} .

Analysis: Calculated for $\text{C}_{40}\text{H}_{54}\text{O}$: C, 87.22; H, 9.88.

Found: C, 87.36; H, 10.04.

Reaction with the HCl-Chloroform Reagent: Strongly positive.

Chromatographic Behavior: The 2-hydroxy-3,4-dehydro- β -carotene zone appeared below the 4-hydroxy-3',4'-dehydro- β -carotene, and a clear interzone was observed when a solution of the two compounds was developed on lime-Celite with hexane + 7% acetone.

9. 2-Methoxy-3,4-dehydro- β -carotene from 2-Hydroxy-3,4-dehydro- β -carotene.

Nine milligrams of the 2-hydroxy compound was dissolved in 2 ml. of chloroform and the solution was diluted with 20 ml. of abs. methanol. To this solution was added, with swirling, 5 drops of the HCl-chloroform reagent. After standing at room temperature for 3 minutes, 10 ml. of hexane was introduced and the solution was washed free of methanol and acid, dried, and evaporated. The red, oily residue was dissolved in hexane and developed on a 4 x 24 cm. column with hexane + 2% acetone:

10 two pink and orange zones
20 interzone
10 pink-orange
10 interzone
20 pale orange: a neo-U isomer of the following pigment
2 interzone
20 orange: all-trans-2-methoxy-3,4-dehydro- β -carotene
2 interzone
10 orange: cis isomer of the former
2 yellow
120 empty section

The combined yield of the stereoisomeric 2-methoxy-3,4-dehydro- β -carotenes was 5.5 mg. (60%, photometric estimation). The hexane solution of the trans isomer was evaporated and the residue was crystallized from benzene-methanol.

Crystal Form: Irregular plates.

Melting Point: 145°.

Partition Behavior: 99:1 in hexane:95% methanol.

Spectrum: The spectral curve in hexane was identical with that of the 2-hydroxy compound.

Reaction with the HCl-Chloroform Reagent: Strongly positive.

10. retro-Bisdehydrocarotene from 2-Hydroxy-3,4-dehydro- β -carotene.

Five milligrams of the 2-hydroxy compound were dehydrated in chloroform by the procedure described for 4-hydroxy-3',4'-dehydro- β -carotene, (Chapter IB, Section 18). The products, dissolved in hexane, were developed on a 24 x 4 cm. column with hexane + 8% acetone:

4 brown
60 interzone
30 pink: all-trans-retro-bisdehydrocarotene
2 colorless
30 pink-orange: cis isomer of the former
2 colorless
15 two orange zones, not well separated: cis isomers of the former
2 colorless
3 orange
90 empty section

The combined yield of the stereoisomeric retro-bisdehydrocarotenes as estimated photometrically was 4 mg. (80%). The trans isomer (518, 487, 460 m μ) did not separate on a column from an authentic sample of all-trans-retro-bisdehydrocarotene.

No 3,4,3',4'-bisdehydro- β -carotene was formed in this reaction.

11. Anhydrovitamin A₁ from Vitamin A₁.

a. Anhydrovitamin A₁.

To a solution of 200 mg. of crystalline vitamin A₁ (Distillation Products Inc.) in 50 ml. of chloroform were added, with stirring, 30 drops of the HCl-chloroform reagent. The course of the dehydration was followed by withdrawing aliquots, diluting with hexane, and recording the ultraviolet spectrum at 5 minute intervals. After 15 minutes,

the peaks typical for anhydrovitamin A₁ showed no further increase. At this point, the conversion was stopped by washing out the acid. After drying and evaporation of the chloroform solution, the oily residue was dissolved in hexane and developed on a 25 x 5 cm. alumina-lime-Celite column with hexane + 10% benzene. The main, yellow-fluorescing zone (ultraviolet light) was eluted and transferred to hexane. The spectral curve in hexane corresponded to that of anhydrovitamin A₁ (350, 369, 390 mμ). Yield, 38 mg. or 19% (photometric estimation). No attempt was made to crystallize the anhydrovitamin A₁; it was used without further purification.

b. Isomer of Vitamin A₁.

A second main zone was adsorbed very strongly to the topmost part of the column and showed intense blue fluorescence in ultraviolet light. Its spectral curve taken without further purification had maxima at 333, 348 and 367 mμ in hexane. These values correspond very closely to those reported by Cawley and Seidel (44) for an isomer of vitamin A₁ which probably has a retro system and 5 conjugated double bonds. No further effort was made to characterize this compound. *

No unreacted Vitamin A₁ could be detected on the column.

12. Formation and Hydrolysis of the Anhydrovitamin A₁ -BF₃-Complex.

Five milliliters of BF₃-etherate was added at 0° to a solution of 150 mg. of anhydrovitamin A₁ in 50 ml. of ethanol-free chloroform. The colorless solution turned bright blue immediately. After 10 seconds, the complex was hydrolyzed by pouring the solution into a mixture of 400 ml. of acetone + 100 ml. of water (0°). After the addition of 100 ml.

of hexane to the pale yellow solution, the epiphase was washed thoroughly with water, dried and evaporated. The yellow, oily residue was dissolved in hexane and developed with hexane + 5% acetone (fl. = fluorescent):

15 bright blue fl.
45 pale blue fl.
40 interzone
7 yellow fl.
40 interzone
25 blue fl.
40 interzone
5 yellow fl.
50 empty section

The 25-mm. zone was eluted with ethanol and transferred to hexane. Yield, 22 mg. or 15% (photometric estimation).

Partition Behavior: 62:38 in hexane:95% methanol.

Spectrum: The ultraviolet curve in ethanol was identical with that of vitamin A₁ (Fig. 30). The infrared spectrum is shown in Fig. 31. For infrared curve of vitamin A₁ see Fig. 32.

Chromatographic Behavior: The new compound is much less strongly adsorbed on lime-Celite than vitamin A₁. When a mixture of the two compounds was developed with hexane + 5% acetone, vitamin A₁ remained near the top of the column, while 4-hydroxy-axerophthene is easily developed.

13. Dehydration of 4-Hydroxy-axerophthene to Anhydrovitamin A₁.

To 8 mg. of the 4-hydroxy compound in 10 ml. of ethanol-free chloroform, contained in a 10 ml. Erlenmeyer flask, were added 4 drops of the HCl-chloroform reagent. During the next 5 minutes, the solution turned yellow. Hexane (20 ml.) was then used to wash the chloroform solution into a separatory funnel where the epiphase was washed with water, dried and evaporated. The oily residue was dissolved in several milliliters

of hexane and developed with the same solvent on a 2 x 20 cm. magnesia-lime-Celite column. Several pale yellow zones were held strongly at (or near) the top. Inspection of the column with ultraviolet light showed a 5-mm. broad yellow-fluorescing zone of anhydrovitamin A₁ located much below the pigment zones and well separated from some minor fluorescing zones. Its spectrum was identical with that obtained by dehydration of Vitamin A. Yield, 1.25 mg. or 15%. The two samples did not separate in the mixed chromatogram test. (Magnesia-lime-Celite; developer, hexane).

APPENDIX

A. A PHOTOMETRIC METHOD FOR DETERMINING THE PARTITION BEHAVIOR OF CAROTENOIDS.

1. Historical Development.

The behavior of some plant pigments on partition between two organic, immiscible solvents has long been used as a means of separating and characterizing these natural products. The historical development of the "partition principle" was reviewed as early as 1913 by Willstätter and Stoll in their first book on chlorophyll (48). According to these authors, G. G. Stokes wrote in 1864,

"For convenience and rapidity of manipulation, especially in the examination of very minute quantities, there is no method of separation equal to that of partition between solvents which separate after agitation...Bisulfide of carbon in conjunction with alcohol enabled the lecturer to disentangle the coloured substances which are mixed together in the green colouring-matter of leaves."

Newer investigations of plant and animal carotenoids necessitated a classification which was based largely on the partition behavior. Willstätter and Stoll classified the carotenoids as "epiphasic" or "hypophasic" according to whether they were found in the petroleum ether (epi-) or the alcohol (hypo-) phase after equilibration. For example, polyene hydrocarbons such as β -carotene or lycopene are epiphasic, while those containing two or more hydroxyl groups exhibit a preponderantly hypophasic behavior. Carotenoids containing a single hydroxyl group (e.g. cryptoxanthin) show an intermediate behavior.

Since some carotenoid alcohols occur in nature in ester form, their partition behavior can be characterized both before and after alkaline hydrolysis (change from epi- to hypophasic behavior).

2. Limitations of the Old Method.

The partition behavior of a particular carotenoid has been described in the past only in a qualitative or roughly quantitative manner. In Karrer and Jucker's monograph (28), which appeared in 1950, the carotenoids known at that time were grouped under three headings, which described their behavior in the hexane:90% methanol system, viz. epiphasic, hypophasic, and "almost equally distributed between epiphase and hypophase". Such a classification did not take advantage of the fact that the partition behavior when expressed in terms of the partition coefficient, constitutes a precise and easily reproducible physical constant.

Until the introduction of modern photometric devices for rapid concentration measurements, it was laborious to determine such constants, whereas now this can easily be accomplished using the Beckman spectrophotometer, for example.

3. Photometric Method.

The optical density of the carotenoid solution is determined photometrically in one of the two phases before and after equilibration with the second phase. The loss of extinction gives the amount of pigment which has migrated to the second phase. This operation requires 5-10 minutes, and the limit of error is approximately $\pm 1\%$. So far the partition coefficients of thirty-two carotenoids, containing various functional groups, have been determined (Table 5).

4. Discussion.

Although this study is by no means complete, some general remarks can be offered relating partition coefficient and functional groups. Mono-ketones with the carbonyl group conjugated are 93-95% epiphasic.

Table 5

Partition Coefficients of Some Carotenoids
in Hexane:95% Methanol

Compound	Functional Groups	Ratio; hexane:95% methanol.
4-Keto-3',4'-dehydro- β-carotene	one >C=O	92:8
4-Keto-β-carotene	one >C=O	93:7
4-Keto-α-carotene	one >C=O	95:5
4,4'-Diketo-β-carotene	two >C=O	50:50
β-Carotenone	four >C=O	8:92
4-Keto-4'-hydroxy-β- carotene	one >C=O, one -OH	34:66
4-Keto-4'-ethoxy- β-carotene	one >C=O, one -OC ₂ H ₅	86:14
Capsanthin	one >C=O, two -OH	4:96
Capsorubin	two >C=O, two -OH	1:99
4-Keto-4'-ethoxy- 3',4'-dehydro-β- carotene	one >C=O, one -OC ₂ H ₅ (as -C=C-OC ₂ H ₅)	44:56
4-Hydroxy-β-carotene (isocryptoxanthin)	one -OH	86:14
4-Hydroxy-α-carotene	one -OH	84:16
3-Hydroxy-β-carotene (cryptoxanthin)	one -OH	32:18
4-Hydroxy-3',4'-dehydro- β-carotene	one -OH	80:20
Desoxylutein I (3-hydroxy- 3',4'-dehydro-α- carotene)	one -OH	78:22
Zeaxanthin (3,3'- dihydroxy-β-carotene)	two -OH	15:85

Table 5 (continued)

Compound	Functional Groups	Ratio; Hexane:95% Methanol
4,4'-Dihydroxy- β -carotene	two -OH	22:78
Lutein (3,3'-dihydroxy- α -carotene)	two -OH	10:90
Gazaniaxanthin (3-hydroxy- γ -carotene)	one -OH	80:20
Vitamin A ₁ (C ₂₀ H ₂₈ O)	one -OH	36:64
4-Hydroxy-3',4'-dehydro- β -carotene acetate	one -OOCCH ₃	96:4
4,4'-Dihydroxy- β -carotene diacetate	two -OOCCH ₃	86:14
Physalagin (zeaxanthin dipalmitate)	two -OOC C ₁₅ H ₃₁	100:0
Capsanthin diacetate	two -OOCCH ₃ one >C=O	65:35
Grocetin (C ₂₀ H ₂₄ O ₄)	two -COOH	4:96
Methyl Bixin (C ₂₆ H ₃₂ O ₄)	two -COOCH ₃	26:74
4-Methoxy-3',4'-dehydro- β -carotene	one -OCH ₃	99:1
4-Ethoxy- α -carotene	one -OC ₂ H ₅	99:1
β -Carotene	none (hydrocarbon)	100:0
γ -Carotene	none (hydrocarbon)	100:0
<u>retro</u> -Dehydrocarotene	none (hydrocarbon)	99:1
<u>retro</u> -Bisdehydrocarotene	none (hydrocarbon)	100:1

Mono-hydroxy carotenoids are somewhat less epiphasic (78-86%). The xanthophylls with two hydroxy groups are much less epiphasic (10-20%), while carotenoids with three or more carbonyl or hydroxyl groups show a minimum epiphasic behavior (1-10%). All hydrocarbons studied showed 100% epiphasic character, and the methyl ethers of monohydroxy compounds approached this value (99%).

For those carotenoids which display a 90-100% hypo- or epi-phasic behavior a second partition experiment should be carried out in a system where each phase would contain 40-60% of the pigment. This can be achieved conveniently by altering the water content of the methanol phase. For example, hexane:90% methanol should then be employed for those carotenoids which have shown less than 10% epiphasic behavior in the first test.

It should be pointed out that the method is not limited to pigments, but is equally applicable to any compound which absorbs within the range of the Beckman apparatus.

5. Experimental.

a. Solvents.

The hexane and 95% methanol phases are prepared by equilibrating approximately equal volumes of commercial hexane and 95% methanol (955 ml. abs. methanol + 45 ml. dist. water) at room temperature.

b. Method.

A filtered hexane or methanol solution* (about 15 ml.) of the compound at a concentration suitable for Beckman readings is prepared.

*In general, the solvent affording higher dissolving power for the compound is chosen.

The optical density is determined at $\lambda_{\max} (E_1)$. A 5-ml. aliquot is then pipetted into a clean, dry, 10-ml. stoppered graduate cylinder, followed by 5 ml. of the other phase. The two phases are equilibrated by slowly inverting the cylinder approximately 25 times. The optical density (E_2) is then determined for the phase for which E_1 was obtained. $E_2/E_1 \times 100 = \% \text{ of epiphasic or hypophasic character (epiphasic, if } E \text{ values were determined with the hexane phase; hypophasic, if the methanol phase was used).}$

The procedure can also be used to check the purity of a sample by equilibration of the phase in which E_2 was determined with an equal volume of the other solvent. The optical density (E_3) is then determined in the first phase. If $E_3 / E_2 = E_2 / E_1$, homogeneity of the substance is indicated. If not, the sample was contaminated with a substance showing a different partition behavior.

B. PROVITAMIN A POTENCY OF SOME 3,4-DEHYDRO-CAROTENES. THE
STRUCTURE OF DESOXYLUTEIN I.

1. Vitamin A₂.

Vitamin A₂ accompanies or replaces Vitamin A₁ in the liver oils of fresh water fish. Schantz (49) isolated it in a pure form from pike livers and reported that its growth promoting activity in rats amounted to 40% of that of Vitamin A₁ (50).

The 3,4-dehydro-vitamin A₁ structure for Vitamin A₂ was first proposed by Gray and Cawley (51). Farrar et al. (4) have confirmed this structure by synthesis starting from the methyl ester of vitamin A₁ acid (see Chart 2, p. 2). More recently, Henbest (52) has reported a second synthesis starting from retinene₁. The synthetic preparation of Farrar et al. showed 30% of the Vitamin A₁ potency.

2. Provitamins A₂.

The provitamin A₁ effect of carotenoids containing at least one unsubstituted β -ionylidene ring has been well established. In a similar manner, the carotenoids with at least one unsubstituted 3,4-dehydro- β -ionylidene ring should act as provitamins A₂. It can be expected that the potency ratio provitamins A₂:A₁ will be about the same as that of Vitamin A₂:A₁. For example, 3,4,3',4'-bisdehydro- β -carotene should show 30-40% of the β -carotene potency.

The growth promoting effect (in rats) of some carotenoids prepared in the present study was kindly determined by Dr. H. J. Deuel, Jr. and Mr. A. Wells of the University of Southern California (Table 6).

3. The Structure of Desoxylutein "I".

Upon heating lutein (XLVI) in a boric acid-naphthalene melt, Zechmeister and Sease (53) were able to isolate and characterize three

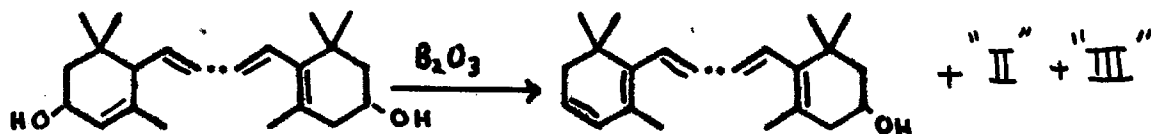
Table 6.

Biopotencies of Some Carotenoids Containing the
3,4-Dehydro- β -ionylidene Ring (Deuel and Wells).

Compound	Predicted Potency (β -carotene = 100%)	Found
3,4,3',4'-dehydro- β -carotene	30-40	45
4-keto-3',4'-dehydro- β -carotene	15-20	17
4-hydroxy-3',4'-dehydro- β -carotene	15-20	16
3-hydroxy-3,4'-dehydro- β -carotene (desoxylutein "I")	15-20	11
3,4-dehydro- α -carotene ^a	15-20	15
3,4-dehydro- β -carotene ^a	65-70	67
4,4'-diketo- β -carotene	0	0

^a These values have been reported previously by Karmakar and Zechmeister (6).

new crystalline monohydroxy carotenoids, then termed desoxyluteins
"I-III" (Chart 28).



(XLVI.) Lutein

(XLVII.) Desoxylutein "I"

Chart 28. Desoxyluteins "I-III" from Lutein.

While desoxyluteins "II" and "III" showed the α -carotene spectrum, "I" gave a curve, without a fine structure, which could not be related to any definite chromophore at that time.

Recently, three further pigments have been prepared and structurally clarified which evidently possess the same chromophore as desoxylutein "I" (λ_{\max} at 459 m μ). The first of these, 3,4-dehydro- β -carotene, was prepared by Karmakar and Zechmeister (6) while the others, 4-hydroxy-3',4'-dehydro- β -carotene (VII) and 2-hydroxy-3,4-dehydro- β -carotene (XXXIX), have resulted from the present study. On the basis of identical chromophores, it seemed probable that desoxylutein "I" should contain the 3,4-dehydro- β -carotene system of double bonds. It had been shown that the β -ionylidene ring of lutein (XLVI) had not been attacked by the dehydrating medium. Therefore, also considering the spectra, the structure of desoxylutein "I" was very likely 3-hydroxy-3',4'-dehydro- β -carotene.

Although desoxylutein "I" was originally reported to have no growth promoting effect in the rat (53), this test was conducted only to show the presence or absence of an unsubstituted β -ionylidene end grouping; hence, the weaker biopotency of the 3,4-dehydro- β -ionylidene grouping could well have been overlooked.

Therefore, it was decided to repeat the bioassay mentioned. As shown in Table 6, desoxylutein "I" showed activity (11%) which was consistent with one dehydro- β -ionylidene end grouping in the molecule. Thus the 3-hydroxy-3',4'-dehydro- β -carotene structure of desoxylutein "I" has been confirmed. It does not separate from 4-hydroxy-3',4'-dehydro- β -carotene (VII) in the lime-Celite, hexane + 7% acetone system.

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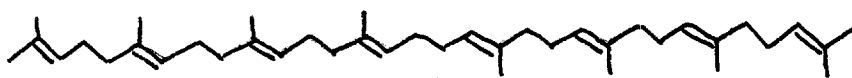
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PROPOSITIONS

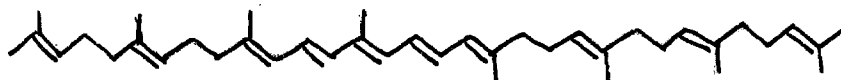
I. So far only the partial structures (conjugated system of double bonds and isopropylidene end groups) are known for the following naturally occurring C_{40} -polyenes, phytoene (3 conj. F), phytofluene (5 conj. F), zeta-carotene (7 conj. F), and neurosporene (9 conj. F). The dehydrogenation of the lower members of the series with N-bromosuccinimide gave mixtures of the higher members, whose chromophores contained only odd numbers of double bonds (1). I propose that the dehydrogenation results demonstrate that in the naturally occurring compounds mentioned, isolated double bonds must be present δ, δ to the ends of the conjugated systems and isopropylidene end groups. Furthermore, the yet unknown first member of the series, with isolated double bonds only and having a squalene-like structure, should also occur in nature (it has been synthesized and named lycopersene by Karrer). The following structures are proposed:



Lycopersene



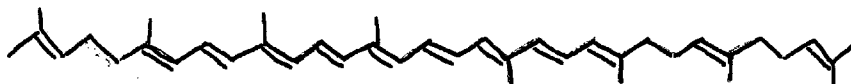
Phytoene



Phytofluene

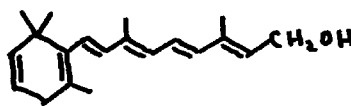


zeta-Carotene



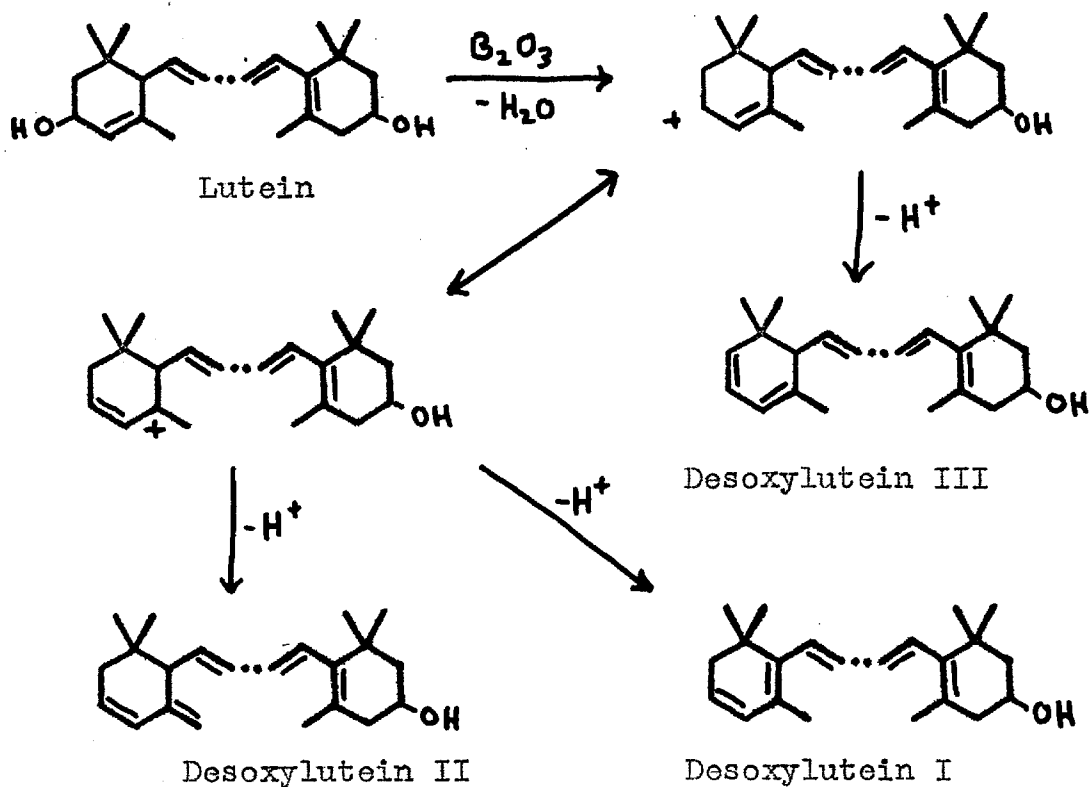
Neurosporene

2. Although many compounds closely related to vitamin A₁ have been prepared and tested for growth activity (2), The synthesis of 2,3-dehydrovitamin A₁ has not yet been reported. I propose that its biopotency will be intermediate to those of Vitamin A₁ and vitamin A₂. A method can be suggested for its synthesis starting from Vitamin A₁ acid.

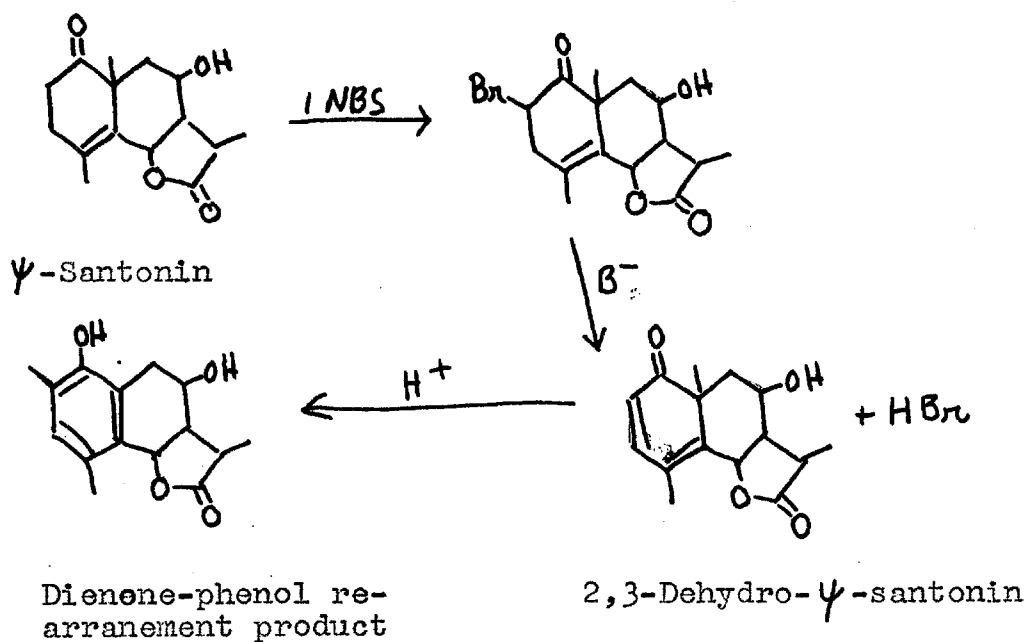


2,3-Dehydrovitamin A₁

3. The structure of desoxylutein I has been clarified in this Thesis. I propose the following structures for desoxyluteins II and III and an explanation for the formation of all three compounds during the dehydration of lutein with anhydrous boric acid (3):

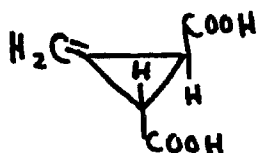


4. The clarification of the relationship of ψ -santonin (4) and β -santonin would be aided by preparing 2,3-dehydro- ψ -santonin according to the following scheme:

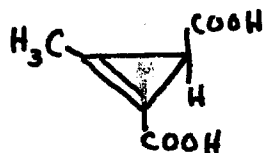


5. Very few data are available for the relative solubilities of D and L antipodes in optically active solvents. Such a body of information concerning different classes of solvents and solutes (amino acids, for example) could increase our understanding of the dissolution process and also provide a new method for the resolution of racemates by countercurrent distribution.

6. The structure of "Feist's acid" (3) has recently been questioned on the basis of infrared data which are offered in support of Structure II(5). I propose that some



I



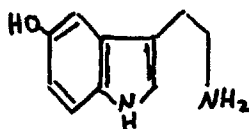
II

of the data are contradictory, and that a comparison of the infrared spectra of the acid (or the ethyl ester) in a KBr disc and in an appropriate solvent transparent in the 5-7 μ region will help to resolve the question in favor of Structure I (cf. 6).

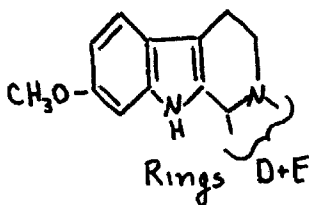
7. The physical constants reported for various preparations of cis- and trans-1,3,5-hexatriene are in poor agreement, and it is questionable whether either form has been obtained in the pure state. I propose a reinvestigation following the methods applied to the diphenylpolyenes, viz. chromatographic separation, cis-trans interconversions, and spectral studies (7,8).

8. The Le Chatelier principle might be successfully applied to the high pressure interconversion of geometrical isomers which have different molar volumes.

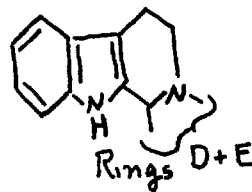
9. The action of reserpine in releasing serotonin from the central nervous system is currently being studied. (90). I propose that the closely related alkaloid canescine(10), which shows the same type of bioactivity as reserpine, be used in these studies until more is known about the metabolism of reserpine. The analysis of serotonin release is probably complicated by the presence of serotonin-like metabolic products arising from reserpine itself.



Serotonin



Reserpine



Canescine

10. I propose that 1-5 g. samples of all available crystalline organic compounds should be collected, ground to a fine powder, mixed well and repackaged in 1-g. samples. This material (trade name, Crystall-Eze) should then be made available to all laboratories for use in seeding solutions. Controversies arising from the isolation of allotropic forms should be reduced as a result. Furthermore, such a material would eliminate one excuse for chemists growing "test-tube height" beards.

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