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The Structure of Neoxanthin and the Trollein-like Carotenoid from *Euglena gracilis**

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ABSTRACT: Neoxanthin and the trollein-like carotenoid from *Euglena gracilis* were subjected to acetylation with [1-¹⁴C]acetic anhydride, treatment with acidic chloroform, and treatment with acidic ethanol in order to elucidate their structures. Results of acetylation of neoxanthin, a monoepoxy triol, demonstrate that only two hydroxyl groups can be acetylated, indicating that one of the hydroxyl groups is on a tertiary carbon atom.

Treatment of neoxanthin with acidic chloroform results in the immediate rearrangement of neoxanthin to its furanoid isomer, neochrome, and the subsequent *exo* elimination of the tertiary hydroxyl group to form the $\Delta^{5'(18')}$ derivative. In addition, *endo* elimination

occurs, leading to the formation of the $\Delta^{2',4'}$ - (or $\Delta^{3',5'(18')}$ -) monofuranoid monohydroxyl derivative. As concluded from the results of acetylation and from the course of dehydration with acidic chloroform, neoxanthin is 3,3',5'-trihydroxy-6'-hydro-5,6-epoxy- β -carotene. The course of dehydration with acidic ethanol confirms this proposed structure for neoxanthin. The trollein-like polyol carotenoid from *E. gracilis* also has only two hydroxyl groups which can be acetylated. Results of treatment with the acidic chloroform and acidic ethanol indicate that an additional hydroxyl group is on a tertiary carbon atom, but that the trollein-like carotenoid is more complicated than one with hydroxyl groups on the 3,3',5' (or 6') positions.

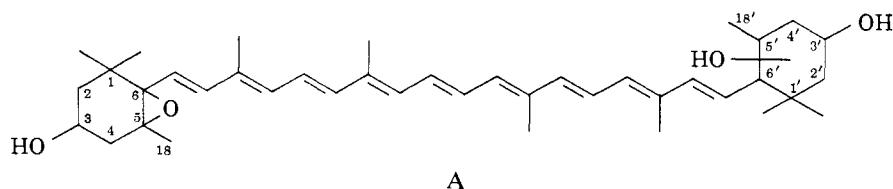
Strain (1938) first isolated neoxanthin from green leaves and proposed a $C_{40}H_{56}O_4$ formula for this pigment. From his analyses, one can conclude that, along with lutein and violaxanthin, neoxanthin is one of the major xanthophylls of green leaves. Curl and Bailey (1957) examined the effect of concentrated hydrochloric acid on ethereal solutions of neoxanthin and found that a blue color, associated with the presence of cyclic ethers (Karrer and Jucker, 1950), was formed. Based on the formation of the blue color and on the results of countercurrent distribution, they proposed a trihydroxyl 5,6-epoxide structure for this pigment, and this structure was independently supported by the

observations of Goldsmith and Krinsky (1960). The latter authors suggested that Strain's original chemical analyses could not distinguish between compounds with 56 or 58 hydrogens, and that the formula for neoxanthin might be more correctly written as $C_{40}H_{58}O_4$. The spectral similarity of neoxanthin to violaxanthin and the possibility that neoxanthin might be an intermediate in violaxanthin biosynthesis prompted Goldsmith and Krinsky (1960) to propose that neoxanthin is 3,3',5'- (or 6'-) trihydroxy-6'- (or 5'-) hydro-5,6-epoxy- β -carotene (A).

The results reported by Krinsky (1963) for the relative polarity of neoxanthin (3.24 units) were in complete accord with the epoxy triol structure. Furthermore, as evidenced by the lack of change of relative polarity after treatment with dilute acidic methanol, neoxanthin reportedly contained only nonallylic hydroxyl groups. As shown by Petrcek and Zechmeister (1956), carotenoids with allylic hydroxyl groups are

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readily converted to the corresponding methyl ether by acidic methanol.

During the course of this work Curl (1965) has found that neoxanthin will react with acidic methanol as well as with acidic acetone, in contrast with the report of Krinsky (1963). On the basis of products arising from these reactions, Curl has concluded that one of the hydroxyl groups is tertiary and located on the 5' position, as evidenced by acid-catalyzed dehydration to the 5',6'-olefin and the 4',5'-olefin 3'-methyl ether. From this he has concluded that neoxanthin is 3,3',5'-tri-hydroxy-6'-hydro-5,6-epoxy- β -carotene (I).

Trollein was first described by Curl and Bailey (1955) after isolation from orange juice. Its spectral similarity to both lutein and antheraxanthin and its lack of an ether function were pointed out by these authors. They suggested that trollein might be structurally similar to antheraxanthin with the 5,6-epoxide replaced by two hydroxyl groups, i.e., 3,3',5,6-tetrahydroxy- β -carotene. Krinsky (1963) then isolated a trollein-like carotenoid from *Euglena gracilis* as a contaminant of neoxanthin, and concluded from its relative polarity (3.0 units) that it contained three hydroxyl groups. After treatment with acidic methanol, this trollein-like compound reportedly showed no change in relative polarity, indicating the presence of only nonallylic hydroxyl groups. Curl (1965) treated trollein isolated from orange juice with acid and also observed no reaction, concluding that neither tertiary nor allylic hydroxyl groups were present.

We have independently examined the structures of neoxanthin and the trollein-like compound from *E. gracilis*. In support of Curl's results (1965), we have found from the products of acetylation and acid-catalyzed dehydration of neoxanthin that one of the hydroxyl groups is tertiary and on the 5' position. Results from acetylation and acid-catalyzed dehydration of the trollein-like compound from *E. gracilis*, however, lead us to conclude that this pigment differs from the trollein which Curl and Bailey (1955) isolated from orange juice.

Materials and Methods

Acetylation of Carotenoids with [1- 14 C]Acetic Anhydride. The procedure of Kuhn and Sørensen (1938) was followed, using [1- 14 C]acetic anhydride as the acetylating reagent for zeaxanthin, antheraxanthin, neoxanthin, and *E. gracilis* trollein. Pigment (0.4 μ mole) was dissolved in 0.5 ml of pyridine (dried over BaO), and 278 μ moles of [1- 14 C]acetic anhydride in 0.125 ml of benzene was added. The reaction flask was then flushed with nitrogen and kept in the dark at room temperature for 6 hr. The reaction was stopped by the addition of an equal volume of a 5% aqueous

NaCl solution and the pigment was transferred to diethyl ether. This solution was thoroughly washed with water to remove all traces of pyridine, dried *in vacuo* at 40°, and dissolved in petroleum ether (bp 30–60°) for column chromatography on Micro-Cel C. The mixture was resolved by development of the column with increasing concentrations of acetone in petroleum ether and elution of the resulting fractions from the extruded column. Purity was checked by thin layer chromatography on silica gel G. When necessary the fractions were rechromatographed on columns of Micro-Cel C or magnesium oxide–Celite (1:1). After evaporation of the organic solvent, radioactivity of each fraction was determined at infinite thinness in a Nuclear Chicago gas flow counter.

Reaction of Carotenoids with Acidic Chloroform. A modification of the procedure of Karrer and Leumann (1951) was employed to dehydrate carotenoids with tertiary hydroxyl groups. Acidic chloroform (0.32 N) (Jensen, 1962) was prepared by bubbling anhydrous hydrogen chloride gas into ethanol-free chloroform for 10 min. Small volumes of this acidic solution were added to the pigment dissolved in 2 ml of ethanol-free chloroform, and the mixture was kept in the dark at room temperature for 15 min. Table I shows the con-

TABLE I: Concentrations of Acid Employed for Dehydration of Carotenoids.

Carotenoid	Pigment (μ moles)	Acidic CHCl_3 (μ -moles of H^+)	Acidic Ethanol (μ moles of H^+)	Ratio (Acid: Pigment)
Neoxanthin ^a	0.1	0.6		6:1
	0.2		24.0	120:1
Trollein-like carotenoid ^b	0.05	6.0		120:1
	0.03		24.0	800:1

^a Concentration based on ϵ_{440} 13.30×10^4 l. mole⁻¹ cm⁻¹. ^b Concentration based on ϵ_{440} 13.04×10^4 l. mole⁻¹ cm⁻¹ and a molecular weight of 587.

centrations of acid and pigment employed. The reaction was terminated by the addition of a 5% solution of NaHCO_3 and the reaction mixture was transferred to diethyl ether and washed with water. The reaction products were separated by thin layer chromatography on silica gel G and were recovered by scraping both

TABLE II: Formation of [1-¹⁴C]Acetyl Derivatives of Various Xanthophylls.

Pigment	Column Fraction	Recovered (mμmoles)	cpm/mμmoles	Moles of [¹⁴ C]Acetyl Groups/mole of Carotenoid ^a	Designation
Zeaxanthin	Z-1	98.5	120.6	1.95	Diacetylzeaxanthin
	Z-2	200.4	64.2	1.04	Monoacetylzeaxanthin
	Z-3	58.0	8.5	0.14	Zeaxanthin
Antheraxanthin	A-1	158.4	122.7	1.98	Diacetylantheraxanthin
	A-2	133.2	67.0	1.08	Monoacetylantheraxanthin
	A-3	23.6	2.6	0.04	Antheraxanthin
Neoxanthin	N-1	181.0	110.1	1.78	Diacetylneoxanthin
	N-2	29.0	74.8	1.21	Monoacetylneoxanthin <i>a</i>
	N-3	145.6	61.7	1.00	Monoacetylneoxanthin <i>b</i>
	N-4	56.4	4.6	0.07	Neoxanthin
Trollein	T-1	148.7	109.1	1.76	Diacetyltrollein
	T-2	53.7	73.8	1.19	Monoacetyltrollein <i>a</i>
	T-3	80.6	63.2	1.02	Monoacetyltrollein <i>b</i>
	T-4	28.4	3.2	0.05	Trollein

^a In order to minimize the variation in the specific activity of the reagent due to spontaneous hydrolysis of the [1-¹⁴C]acetic anhydride to [1-¹⁴C]acetic acid, the reactions with all four carotenoids were started within 1 min of each other. Inasmuch as there was some hydrolysis of the reagent before it was added to the reaction mixture, the results have been normalized with respect to zeaxanthin which can form only one monoacetyl and one diacetyl derivative.

pigment and adsorbant from the glass plate and eluting the pigment with ethanol.

Reaction of Carotenoids with Acidic Ethanol. Acidic ethanol (0.12 N) was prepared by dissolving 1 ml of concentrated hydrochloric acid in 99 ml of absolute ethanol. A few drops of this solution were added to the pigment in 1.0 ml of absolute ethanol and the mixture was allowed to react in the dark at room temperature for 30 min. Table I shows the concentrations of acid and pigment employed. The reaction was terminated and the products were isolated as described in the preceding paragraph.

Relative Polarity Value. The relative polarity of each fraction was determined as described by Krinsky (1963). This value represents the total contribution of individual functional groups to the over-all polarity of a carotenoid molecule. The total polarity is determined from the partition distribution of the pigment in a petroleum ether-aqueous methanol solvent system. The individual functional groups are assigned polarity values based on a scale of 1.00 unit for the nonallylic hydroxyl group as previously described (Krinsky, 1963). Spectral characteristics of all purified fractions were determined in absolute ethanol with a Cary Model 14 spectrophotometer.

Materials. Adsorbants included silica gel G (Brinkmann Instruments), magnesium oxide (Sea Sorb 43, Fisher Scientific Co.), Celite (Johns-Manville Sales Corp.), and Micro-Cel C (Johns-Manville Sales Corp.). Antheraxanthin, zeaxanthin, neoxanthin, and trollein

were isolated from *E. gracilis* as previously described (Krinsky, 1963; Krinsky and Goldsmith, 1960; Krinsky *et al.*, 1964). [1-¹⁴C]Acetic anhydride in benzene (Calbiochem), specific activity of 5 mc/mmole, was diluted with unlabeled acetic anhydride to a specific activity of 0.144 mc/mmole.

Results

Acetylation of Carotenoids with [1-¹⁴C]Acetic Anhydride. Acetylation of carotenoids with acetic anhydride for 6 hr, followed by chromatographic separation of the reaction mixture, yields partially and completely acetylated fractions (Krinsky, 1963). By using [1-¹⁴C]acetic anhydride as the acetylating reagent and determining the specific activity of each fraction, the number of hydroxyl groups acetylated per molecule can be determined. These results are shown in Table II. The relative polarity value for each fraction is given in Table III. Each fraction is numbered in order of migration down the chromatographic column. As shown, the acetylation of both zeaxanthin (3,3'-dihydroxy-β-carotene) and antheraxanthin (3,3'-dihydroxy-5,6-epoxy-β-carotene) yield diacetylated, monoacetylated, and unreacted products. The relative polarity values obtained for these fractions agree well with the values assigned earlier to these functional groups (Krinsky, 1963). Antheraxanthin can theoretically form the 3-acetyl or 3'-acetyl derivative as the monoacetylated product. Inasmuch as only one mono-

TABLE III: Relative Polarity Values for Acetylated Xanthophyll Derivatives.

Designation	Functional Group Contribution to Relative Polarity Value							Expected Value	Found
	3-OH	3-Acetyl	5,6-Epoxy	3-OH-5,6-epoxide	3-Ac-5,6-epoxide	3,5-Di-OH	3-Ac-5-OH		
Diacetylzeaxanthin		2 (0.47)						0.94	1.13
Monoacetylzeaxanthin	1.00	0.47						1.47	1.45
Zeaxanthin	2 (1.00)							2.00	2.01
Diacetylantheraxanthin		0.47			0.71			1.18	1.22
Monoacetylantheraxanthin		0.47		1.24				1.71	1.68
Antheraxanthin	2 (1.00)		0.24					2.24	2.20
Diacetylneoxanthin					0.71		0.98	1.69	1.68
Monoacetylneoxanthin <i>a</i>	1.00		0.24				0.98	2.22	2.23
Monoacetylneoxanthin <i>b</i>	1.00		0.24				0.98	2.22	2.15
Neoxanthin	1.00		0.24			1.95		3.19	3.19
Diacetyltrollein		0.47					0.98	1.45	1.59
Monoacetyltrollein <i>a</i>	1.00						0.98	1.98	2.18
Monoacetyltrollein <i>b</i>		0.47				1.95		2.42	2.13
Trollein	1.00					1.95		2.95	2.93

acetyl fraction was observed, it is not possible to tell whether one or a mixture of both monoacetylated derivatives are present.

Following acetylation of neoxanthin, four fractions are isolated from the reaction mixture. As determined from the ratio of [^{14}C]acetyl groups to carotenoid, these fractions are diacetylneoxanthin (N-1), monoacetylneoxanthin *a* (N-2), monoacetylneoxanthin *b* (N-3), and unreacted neoxanthin (N-4). A triacetyl derivative is not formed.

The relative polarity value for the diacetyl derivative of neoxanthin is not in accord with values expected from published data (Krinsky, 1963). Recent observations, however, have led us to conclude that neighboring groups can readily influence relative polarity values by intramolecular hydrogen bonding, and that the relative polarity value for a 3-acetyl-5-hydroxyl carotenoid is 0.98 unit (B.P. Schimmer and N. I. Krinsky, unpublished observations). The relative polarity value for the diacetyl derivative of neoxanthin is thus in agreement with expected data. The two monoacetyl derivatives of neoxanthin each appear to have the 3'-acetyl-5'-hydroxyl configuration. A 3-acetyl-5,6-epoxy-3',5'-dihydroxyl configuration would result in a relative polarity of 2.65, well above the values of 2.23 and 2.15 observed with the two monoacetylneoxanthin fractions. The observed intramolecular interaction between the 3'-acetyl group and the 5'-hydroxyl group suggests that the 3'-hydroxyl and 5'-hydroxyl groups are in *cis* configuration on the cyclohexane ring in the neoxanthin molecule.

Acetylation of the trollein-like polyol carotenoid from *E. gracilis* leads to the recovery of four fractions. As determined from ^{14}C -incorporation data (Table II), these are diacetyltrollein (T-1), monoacetyltrollein *a* (T-2), monoacetyltrollein *b* (T-3), and unacetylated

trollein (T-4). The relative polarity values for these acetylated fractions (Table III) are not in agreement with those expected for the triol structure suggested by Krinsky (1963).

Reaction of Neoxanthin with Acidic Chloroform.

Reaction of neoxanthin with acidic chloroform, followed by thin layer chromatography, results in the isolation of five fractions. Table IV gives the per cent yield and relative polarity value for each fraction. The fractions are numbered in order of increasing R_F value. All the fractions recovered have a neochrome-like spectrum (λ_{max} 449, 422, and 398 in ethanol).

Fraction 1 is isolated from the origin of the thin layer plate. In addition to its neochrome-like spectrum, this fraction has a small absorption maximum at 690 $m\mu$. Upon the addition of a trace of hydrochloric acid in ethanol the 690- $m\mu$ peak is greatly enhanced and the neochrome peaks are diminished. If alkali is then added, the 690- $m\mu$ peak disappears and the resulting product exhibits a neochrome-like spectrum. Fraction 1 may be the blue complex which forms between the 5,8-epoxide of neochrome and mineral acids (Karrer, 1948). Fraction 2, as evidenced by thin layer chromatography, partition value (Table IV), and absorption spectrum, is neochrome. Fraction 3 has a relative polarity value of 2.24 units which would be expected from a derivative containing one furanoid and only two hydroxyl groups. Subsequent treatment of fraction 3 with acidic ethanol gives no reaction, thus indicating the absence of allylic hydroxyl groups (Petracek and Zechmeister, 1956). Fraction 5, as evidenced by its relative position on the thin layer plate and its relative polarity value of 1.38 units, contains one furanoid group and a single hydroxyl group.

Reaction of Neoxanthin with Acidic Ethanol. Treat-

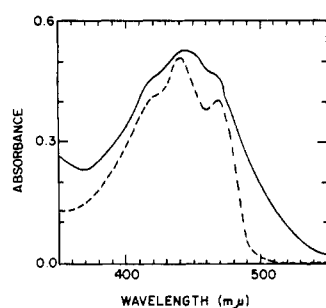


FIGURE 1: Represents the absorption spectrum of the product of dehydration of the trollein-like carotenoid from *E. gracilis*; —, in 95% ethanol; ----, after treatment with NaBH_4 .

TABLE IV: Reaction of Neoxanthin and Trollein with Acid.

Pigment	Treatment	Fraction	% Recovered ^a	Rel. Polarity
Neoxanthin	Acidic chloroform			2.92
		1	Trace	...
		2	82.0	2.99
		3	16.0	2.24
		4	Trace	...
		5	2.0	1.38
	Acidic ethanol	1	70.5	...
		2	29.5	2.26
		3	Trace	...
				3.00
Trollein-like carotenoid	Acidic chloroform	1	92.0	3.00
		2	8.0	2.30
	Acidic ethanol	1	80.0	3.00
		2	20.0	2.30

^a Recovery is based on the per cent of total recovered absorption at the λ_{max} .

ment of neoxanthin with acidic ethanol followed by thin layer chromatography results in the separation of three fractions (Table IV).

Fraction 1 is spectrally and chromatographically similar to neochrome. Fraction 2 also resembles neochrome spectrally. Its relative polarity value (2.26 units) and its chromatographic position indicate that this pigment has a furanoid group and only two hydroxyl groups.

Reaction of E. gracilis Trollein with Acidic Chloroform

or *Acidic Ethanol*. Reaction of this compound with acidic chloroform or acidic ethanol yields two fractions. Table IV gives the per cent yield and relative polarity value for each fraction.

Fraction 1, which comprises the major fraction, is identical with the starting compound as determined by spectral, partition, and chromatographic behavior. Fraction 2 has spectral properties which are unlike the original material. Fine structure is absent, and absorbancy is increased $>500 \text{ m}\mu$ (Figure 1). This fraction has a relative polarity of 2.30, indicating loss of a functional group. Treatment of this fraction in 95% ethanol with a trace of NaBH_4 results in the formation of a product with a spectrum similar to that of the starting material. The wavelength maximum and the relative polarity value for this fraction remain unchanged. Pretreatment of trollein with NaBH_4 changes neither the starting material nor the course of the reaction.

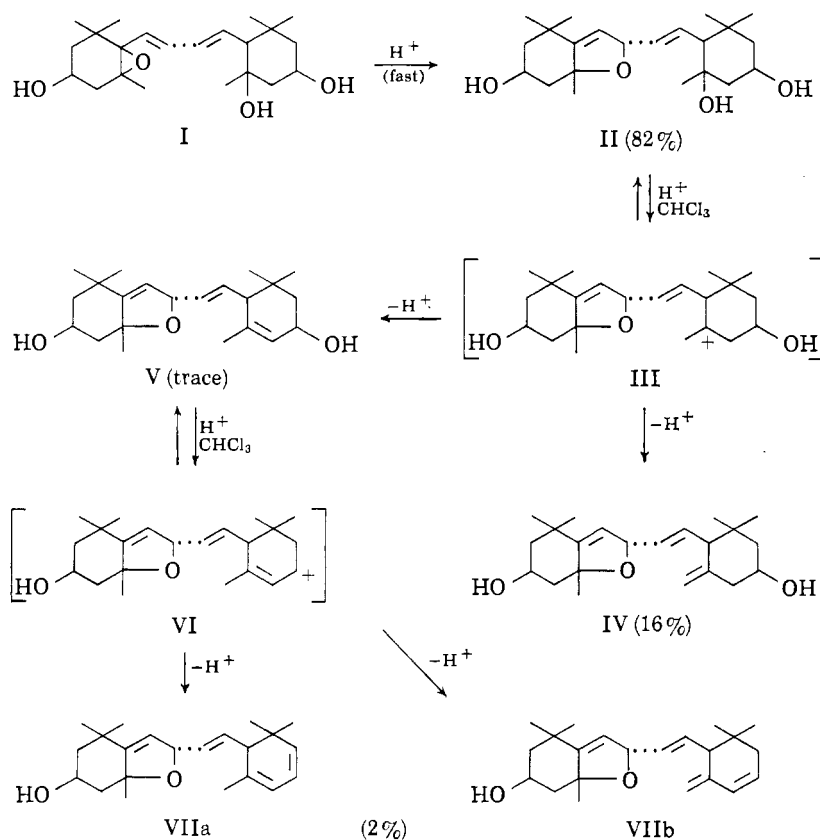
Effects of Acidic Ethanol or Acidic Chloroform on Zeaxanthin. When zeaxanthin is treated with acid under conditions similar to those employed above, no dehydration is observed. Zeaxanthin contains neither allylic nor tertiary hydroxyl groups and thus serves as a control for the acid-catalyzed dehydrations.

Discussion

Jensen (1962) has observed that the acetylation conditions described above (Methods) will esterify primary and secondary hydroxyl groups readily, but will acetylate tertiary hydroxyl groups only to a small extent ($<10\%$) and can be used to distinguish tertiary hydroxyl groups from others. Acetylation of neoxanthin, a monoepoxytriol, results in isolation of monoacetyl and diacetyl derivatives, but not of a triacetyl derivative, thus establishing the position of one hydroxyl group on a tertiary carbon atom.

Compounds containing tertiary hydroxyl groups are readily susceptible to acid-catalyzed dehydrations. Such dehydrations occur through formation of a carbonium ion intermediate and subsequent stabilization by proton elimination. The result of such a reaction is the loss of a molecule of water with the formation of a double bond. The only tertiary positions available for the hydroxyl group on the neoxanthin molecule are the 5'- and 6'-carbon atoms. An hydroxyl group on the 6'-carbon atom treated with acid would result in the formation of a carbonium ion at the 6'-carbon atom. Subsequent stabilization by proton elimination would lead to an extended chromophore. This additional conjugated double bond would result in a spectral shift of 5–7 $\text{m}\mu$ toward longer wavelengths. Such a shift was not observed. Similarly, acid treatment of a 5'-hydroxyl group could result in an extension of the chromophore only if dehydration occurred at the 5',6' position. If, however, dehydration occurred *via* a 4',5' or 5',18' elimination, the double bond would be out of conjugation and would not effect the spectrum. Furthermore, dehydration *via* a 4',5' elimination would make the 3'-hydroxyl group allylic and sus-

SCHEME I



ceptible to further reaction in the presence of acid. Our interpretation of the reaction of neoxanthin with acid chloroform is in Scheme I.

Neoxanthin (I) contains a 5,6-epoxide as well as a tertiary hydroxyl group and is immediately converted to the corresponding 5,8-furanoid by traces of acid (Karrer and Jucker, 1950). This rapid isomerization of the cyclic ether accounts for the observation that all the products isolated from the reaction of neoxanthin with acid have spectra identical with the 5,8-furanoid isomer, neochrome (II). Indeed, the first two fractions isolated from the reaction of neoxanthin with acidic chloroform are products of acid interaction with the 5,6-epoxide group. The third fraction isolated from this reaction, though, has one less hydroxyl group than neoxanthin. This fraction could have arisen from elimination of either a tertiary or an allylic hydroxyl group; however, Krinsky (1963) has already excluded the possibility of the presence of an allylic hydroxyl group. Furthermore, the product arising from treatment of neoxanthin with acidic ethanol is a result of dehydration rather than etherization as would be expected for allylic hydroxyl groups (Petracek and Zechmeister, 1956). We, therefore, conclude that the first dehydration product of neoxanthin is the result of elimination of a tertiary hydroxyl group. Inasmuch as this product does not show an increase in the length of its chromophore, as evidenced by its neochrome-like spectrum, it cannot represent a 5',6' dehydration. Furthermore, as evidenced by the absence of reaction

with acidic ethanol, this fraction does not contain any allylic hydroxyl groups and hence cannot be the 4',5' dehydration product. Fraction 3 can only be the product of dehydration of a 5'-hydroxyl group from the 5',18' position, leaving the $\Delta^{5'(18')}$ derivative (IV). The fifth fraction isolated from the reaction of neoxanthin with acidic chloroform is a monohydroxyl monofuranoid compound and is a product of the elimination of 2 molecules of water. This product would be expected if elimination of the 5'-hydroxyl group also occurred by a 4',5' dehydration (V), thus making the 3'-hydroxyl group allylic. The allylic 3'-hydroxyl group can be further dehydrated to give the $\Delta^{2',4'}$ derivative (VIIa) or the $\Delta^{3',5'(18')}$ derivative (VIIb), as described by Zechmeister and Petracek (1956) for the conversion of lutein to the deoxyluteins. Our methods do not allow us to distinguish between these two compounds. The fourth fraction, which appears only as a trace, is possibly the intermediate 4',5' dehydrated product (V). Formation of the 4',5' dehydration product with acidic ethanol would result in the rapid formation of the 3'-ethyl ether (Petracek and Zechmeister, 1956). The trace material isolated from the reaction of neoxanthin with acidic ethanol may be the 4',5'-dehydro-3'-ethyl ether.

Our results substantiate the recent observations of Curl (1965) that neoxanthin is 3,3',5'-trihydroxy-6'-hydro-5,6-epoxy- β -carotene. The major dehydration product that we obtain, however, appears to be the $\Delta^{5'(18')}$ derivative, in contrast to the $\Delta^{5'}$ derivative

obtained by Curl (1965). The difference in products may be due to the influence of the solvent systems or acid concentrations used.

Krinsky's earlier report (1963) that neoxanthin did not undergo a change in relative polarity values upon treatment with acidic methanol can be explained by the fact that Krinsky determined the relative polarity values on the complete reaction mixture. In contrast to the results obtained with concentrated acid (Curl, 1965), dilute acid as employed by Krinsky would cause only a small percentage of the neoxanthin to be dehydrated. This small amount of dehydrated products would not alter the relative polarity values of the mixture significantly.

The trollein-like carotenoid from *E. gracilis* reportedly contains three nonallylic hydroxyl groups as determined by relative polarity values (Krinsky, 1963). However, the relative polarity values of the acetylated derivatives (Table II) do not correspond to a compound having a triol structure. As observed elsewhere (B. P. Schimmer and N. I. Krinsky, unpublished observations) the relative polarity values are influenced by intramolecular hydrogen bonding. This trollein-like polyol may therefore contain more than three functional groups. Only two of the groups can be acetylated, suggesting that the others are either ethers or tertiary hydroxyl groups. Successful dehydration of this carotenoid to a pigment with a relative polarity value of 2.3 units by either acidic chloroform or acidic ethanol further supports the possibility of the presence of a tertiary hydroxyl group along with another functional group on the trollein-like pigment. This trollein-like carotenoid does not respond to acid-catalyzed dehydration in a manner expected for a triol with one of the hydroxyl groups on either the 5' or 6' position. The loss of fine structure in the spectrum of the dehydrated product (Figure 1) is usually observed when there is additional strain in one of the β -ionone rings due to either a conjugated keto group or an additional conjugated double bond in the ring (Zechmeister, 1958). These additions, however, are always accompanied by a bathochromic spectral shift. Such a shift was not observed following the dehydration of our trollein-like compound.

Bamji and Krinsky (1965), reporting on the enzymatic reduction of antheraxanthin to zeaxanthin in *E. gracilis*, have proposed that a triol intermediate such as trollein might be involved. The present investigation suggests that the trollein-like compound from *E. gracilis* does not have the required structure for the possible intermediate in this reductive reaction. More recently, Krinsky (1966) has suggested that the enzymatic reductive deepoxidation of antheraxanthin

to zeaxanthin and the light-dependent oxidative reversal form a biological cycle which could prevent lethal photosensitized oxidations from occurring in photosynthetic tissue. Neoxanthin, the only other epoxide carotenoid found in *E. gracilis*, might also take part in this protective role, in which case it would be expected to cycle with its reduced nonepoxide equivalent. Now that the structure of neoxanthin has been elucidated, a compound such as 3,3',5'-trihydroxy- β -carotene might be anticipated if neoxanthin is involved in a biological photoprotective cycle similar to the one proposed for antheraxanthin and zeaxanthin. As a basis for this study, we have investigated the chemical reductions of carotenoid epoxides and their analogy to biochemical reactions (B. P. Schimmer and N. I. Krinsky, unpublished observations).

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