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Animal Carotenoids. 9.* On the Absolute Configuration of Astaxanthin and Actinioerythrin

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Dedicated to Professor Dr. techn. Nils Andreas Sørensen at the occasion of his 65th birthday December 8, 1974

The use of chiroptical methods for determination of the absolute configuration of astaxanthin (*1*) is discussed.

Chemical conversion of astaxanthin (*1*) diester into zeaxanthin (*7*) failed.

Consideration of the CD-spectrum of the tetrol *10*, obtained by $LiAlH_4$-reduction of astaxanthin (*1*) diester, led to the (3*S*,3′*S*)-assignment for astaxanthin (*1a*).

Chiroptical properties of actinioerythrin (*13*) are reported.

The constitution of astaxanthin (*1*) was elucidated by Kuhn and Sørensen [1] in 1938. Astaxanthin (*1*) is converted to astacene (*2*) under alkaline conditions in the presence of oxygen,[1] Scheme 1. The structure *1* of astaxanthin has subsequently been confirmed by Davis and Weedon [2] by partial synthesis of astacene (*2*) from canthaxanthin (*3*). However, the absolute configuration at 3,3′-positions has not yet been determined.

From feeding experiments with (^{14}C)-lutein it has been claimed that astaxanthin (*1*) in goldfish (*Carassius auratius*) is formed biogenetically from lutein.[3] Lutein, first considered to have (3*R*,3′*S*,6′*R*)-configuration,[4] was later demonstrated to possess (3*R*,3′*R*,6′*R*)-configuration (*4*).[5–7] If astaxanthin (*1*) were synthesized *in vivo* from lutein (*4*) without epimerization on the enzyme, astaxanthin should have 3*S*,3′*R*-configuration and be achiral. However, astaxanthin *ex Hommarus gammarus* (from crustacyanin) is optically active.[8a] Its biosynthesis in goldfish, therefore, calls for further attention.

From its chiroptical properties astaxanthin cannot be bound to positive centers of proteins

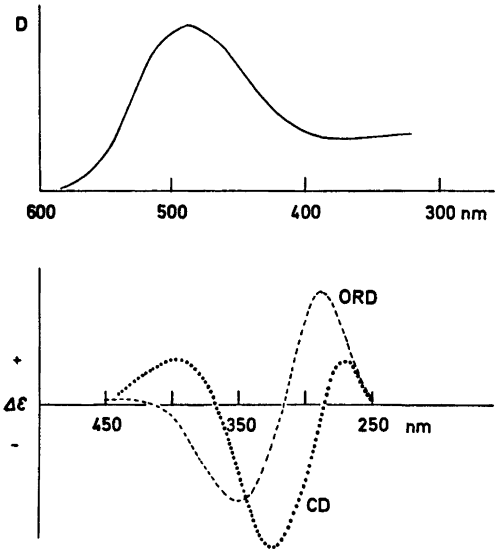

Fig. 1. Electronic absorption spectrum (—— top), CD-spectrum (· · ·, bottom) and ORD-spectrum (– – –, bottom; calculated from the CD-spectrum) of astaxanthin (*1*) *ex Hommarus gammarus* in chloroform solution.

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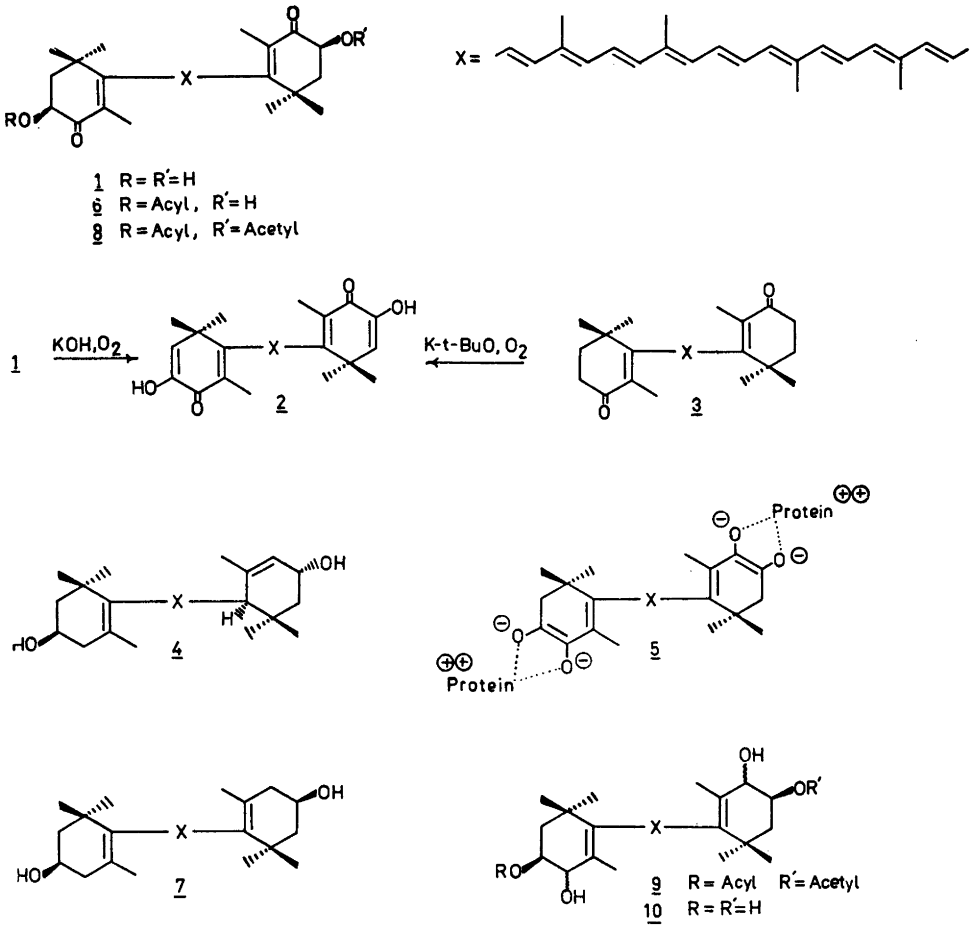


Scheme 1.

in lobster eggs as a bis-dianion (*5*),[1] because mere extraction with organic solvents cannot produce chirality of the carotenoid. The optical activity of astaxanthin *ex* ovoverdin is recently confirmed.[8b]

In order to establish the absolute configuration at the two chiral centers of astaxanthin (*1*) the use of chiroptical properties was considered. CD and ORD spectra of carotenoids have been investigated by several groups (*cf.*, *e.g.*, Refs. 8 – 10) but are theoretically not well understood.

Klyne and his colleagues [8a] have, quite successfully, developed an empirical rule which states that the two chiral end groups of a carotenoid contribute independently to the optical activity, so that contributions for these end groups are additive. This procedure is probably justified only for absorption bands connected with excitations of "partial chromophores" but not for the CD within the main band of all-*trans* carotenoids. However, due to the intense absorption of carotenoids in the latter region only few ORD/CD measurements are available for the visible region.[9,11]

Mills' rule [12,13] has also successfully been applied for determining the stereochemistry of 2- and 3-hydroxy-β-cyclogeranyl rings in carotenoids.[14,15] Recent work in our laboratories has further indicated that the preferred conformation of a chiral β-cyclogeneranyl ring is indeed of decisive importance for the CD spectra of carotenoids regardless of the type of substituents on the cyclohexene ring when dealing with half-chair conformations.[7]

RESULTS AND DISCUSSION

The ORD curve of a concentrated solution of astaxanthin (*1*) *ex* lobster has been reported by Buchwald and Jencks [16] for the 600 – 400 nm region and of astaxanthin *ex Hommarus gammarus* and of astaxanthin diacetate *ex Hommarus gammarus* and *ex Halocynthia papillosa* by Bartlett *et al.*[8a] in the 400 – 213 nm region. No stereochemical conclusions have been drawn from these data.

Fig. 1 shows the CD-spectrum of astaxanthin (*1*) *ex Hommarus gammarus* recorded in the 420 – 250 nm region. The ORD spectrum (450 – 250 nm) calculated [17] from this CD spectrum is consistent with data reported previously by Bartlett *et al.*[8a] In the 450 – 400 nm region their ORD curve deviates somewhat from that reported by Buchwald and Jencks.[16] In concentrated solution in the 600 – 400 nm region Buchwald and Jencks [16] probably measured some artefacts. The CD spectrum of astaxanthin monoester (*6*) *ex Haematococcus pluvialis*.[18] is shown in Fig. 2. In spite of the different solvents used, the CD spectra of *1* and *6* are very similar, thus supporting earlier reports [19] that individual chiral carotenoids have the same absolute configuration regardless of the biological source.

In general, the absolute configuration of a cyclohexenone can be derived from its CD spectrum in the range of the $n\rightarrow\pi^*$ band under the restrictions that this band can be unequivocally identified in the CD spectrum, and that a safe prediction can be made for the preferred conformation of the cyclohexenone ring.[20] Unfortunately, at least the first problem cannot be solved, because for astaxanthin (*1*) the CD band corresponding to the $n\rightarrow\pi^*$ transition cannot be identified. For simple conjugated oligo-enones, the $n\rightarrow\pi^*$ band appears at longest wavelengths corresponding to the transition of lowest energy. Calculations by Moore [21] have shown, however, that commencing with a polyene aldehyde of five carbon-carbon double bonds, the first $\pi\rightarrow\pi^*$ band should appear at longer wavelengths than the corresponding $n\rightarrow\pi^*$ band. In this connection recent work on a steriodal trienone has revealed that the $n\rightarrow\pi^*$ band occurs at *ca.* 360 – 380 nm and the $\pi\rightarrow\pi^*$ band at 345 nm.[22] With this short chromophore the separation of the two bands is rather small and distinctly visible only in unpolar solvents. In the case of astaxanthin (*1*), one would expect the $n\rightarrow\pi^*$ band to appear in the visible range where the anisotropy factor $g' = \Delta\varepsilon/\varepsilon$ is too small to give reliable CD data. Below 400 nm the CD spectrum of astaxanthin (*1*) resembles somewhat that of zeaxanthin (*7*), Scheme 1, which contains no carbonyl groups. A hypsochromic shift of the CD spectrum of *7* compared to that of *1*, is parallelled by a similar shift of the $\pi\rightarrow\pi^*$ band in the visible region of the corresponding isotropic electron absorption spectra. Such resemblance can, however, not be taken as proof for the same absolute configuration at the chiral centers C-3 and C-3′ without sufficient reference data.

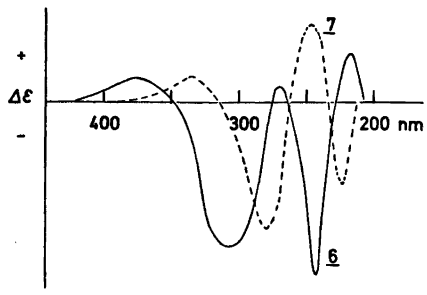


Fig. 2. CD-spectra of astaxanthin monoester (*6*, ——) and zeaxanthin (*7*, - - -) in EPA 5:5:2 solution.

For further work astaxanthin monoester (*6*) *ex Haematococcus pluvialis* [18] was used. All attempts to convert *6 via* its monoacetate *8* into zeaxanthin diester (or its enantiomer) failed.

These included the reduction of the two carbonyl groups of *8* to the tetrol-diester *9* with $NaBH_4$ followed by (i) SO_3 pyridine complex treatment and $LiAlH_4$-reduction,[23] (ii) formation of the *p*-toluene sulfonate [24] or methane sulfonate [25] derivative and subsequent $LiAlH_4$-reduction, (iii) replacement of hydroxyl by bromide followed by $LiAlH_4$-reduction.[26]

A second approach directed towards reduction of the keto groups of *6 via* N-derivatives, studied with canthaxanthin (*3*) as a model compound, was also unsuccessful. Thus, canthaxanthin (*3*) provided no hydrazone, phenylhydrazone or tosylhydrazone, but gave an oxime and a semicarbazone. Reduction of the semicarbazone under strongly alkaline conditions [24] failed. Previous attempts along the same lines have also been unsuccessful.[27]

Since a direct correlation with natural zea-

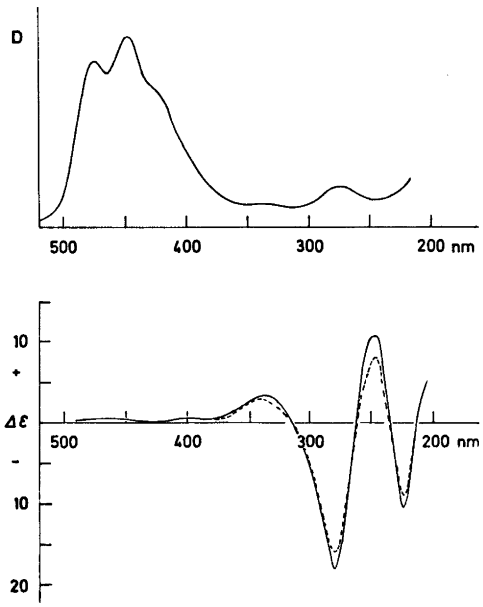


Fig. 3. Electronic absorption spectrum of the tetrol *10* (——, top), and CD-spectra of tetrol *10* (——, bottom) and zeaxanthin (*7*, – – –, bottom) *ex Flexithrix* strain QQ [30] in EPA 5:5:2 solution.

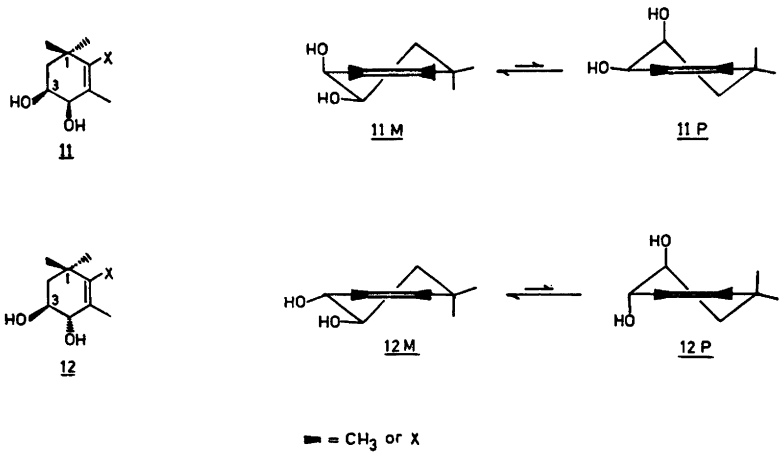
xanthin (*7*), known to have 3*R*,3′*R*-configuration [28,29] could not be achieved, chiroptical data of the tetrol *10* (obtained by $LiAlH_4$-reduction of *8*) was used to solve the stereochemistry of astaxanthin (*1*). The CD spectrum of the tetrol *10* together with that of zeaxanthin (*7*) *ex Flexithrix* sp.[30] with corrected [7] Δε-value is given in Fig. 3. The CD curves of *10* and *7* are very similar in shapes and signs of the individual Cotton effects; rotational strengths of the tetrol *10* being slightly higher than those reported for zeaxanthin (*7*).[7,8,31] By the following arguments this result is taken to prove the 3*S*,3′*S*-configuration of astaxanthin (*1*).

It is well known [32] that reduction by $LiAlH_4$ or $NaBH_4$ of a cyclohexanone preferentially leads to the equatorial alcohol if the ketone is unhindered, whereas, in the contrary case, the axial alcohol prevails. In the case of a cyclohexenone usually a mixture of epimers is formed,[33] especially if the ring is not rigid. Reduction of *8* was therefore expected to yield a mixture of three diastereomeric tetrols.

A series of attempts to isolate individual diastereomers of the tetrol *10* failed, thus also precluding the possible use of hydrogen bonding studies by IR. The systems investigated included that in which the diastereomeric tetrols derived from actinioerythrin (*13*) could be separated [34] and the one where Bodea and coworkers [35] claimed successful separation of $NaBH_4$-reduced astaxanthin.

However, separation of the tetrol *10* into individual diastereomers is not essential for our argumentation.

Assuming 3*S*,3′*S*-configuration for astaxanthin (*1*), the *cis* diol end group *11* will have 3*S*,4*R*-configuration and the *trans* diol end group *12* the 3*S*,4*S*-configuration, Scheme 2. Of the two half-chair conformations *12M* and *12P* associated with the *trans* diol *12*, *12M* will be



Scheme 2.

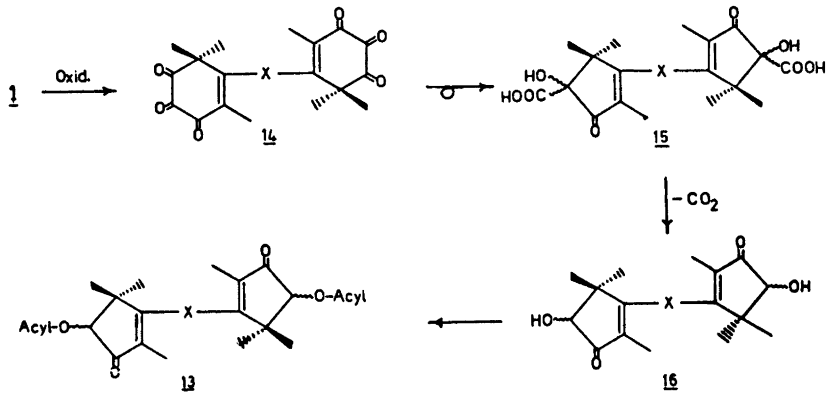
preferred because there is only one (1:3-Me:H) interaction as opposed to one (1:3-OH:Me) and one (1:3-OH:H) interaction present in the *12P*-conformation. In the case of the *cis* diol *11*, the *M*-helical conformation *11M* will also be preferred because the *P*-conformation contains a (1:3-OH:Me) interaction which is thought to be more severe than the sum of a (1:3-OH:H) and a (1:3-Me:H) interaction, *cf.* Ref. 36, especially in EPA solution where solvation will take place. Zeaxanthin (*7*), whose end group has the same configuration for the 3-OH-group, is also expected to have the ring predominantly in the *M*-conformation to avoid such a (1:3-OH:Me) interaction; this is consistent with ^{1}H NMR evidence.[37,38] The preponderance for the *M*-conformation of the cyclohexene half-chair of these end groups (*viz.* monohydroxylated end group of *7*, and *cis* and *trans* dihydroxylated *11* and *12* of *10*) will be greatest for the trans diol *12* end group and smallest for the *cis* diol end group *11*.

As already mentioned, there is reason to assume [10] that the signs of the CD bands are determined solely by the helicity of the (half-chair) conformation of the cyclohexene ring. This is consistent with another rule proposed [39] for the CD of conjugated dienes and enones, which states that the axial substituents (including hydrogen) in allylic position to the diene or α-position to the conjugated ketone determine the sign of the Cotton effects, *cf.* Ref. 40. On the basis of this hypothesis and the conformational analysis given above, the CD of *7*, *11*, and *12* should thus be mainly determined by the configuration at C-3 (or C-3′). As the signs and positions of the Cotton effects of *7* and the tetrol *10* are exactly the same, we conclude, therefore, that the configuration at C-3 and C-3′ of *10* and thus also of astaxanthin (*1*) is *S*.

Considering now the rotational strength observed for zeaxanthin (*7*) and the tetrol *10*, Fig. 3. From the arguments advanced above, complex hydride reduction resulting in a preponderance of products with the *cis* diol end group *11* would be expected to result in a $\Delta\varepsilon$-value for the tetrol *10* lower than for zeaxanthin (*7*). Preponderance of products with the *trans* diol end group *12* would, on the other hand, result in higher $\Delta\varepsilon$-values relative to zeaxanthin (*7*). The actual values observed ($\Delta\varepsilon = -14.8$ for *7*, *versus* $\Delta\varepsilon = -18.0$ for *10*) only allows the tentative conclusion that more of the *trans* diol end group *12* was formed than of the *cis* diol end group *11*.

Independent support for the stereochemical assignment of astaxanthin (*1*) may be possible through partial synthesis of the 3,3′-dimethyl ether of the tetrol *10* from optically active zeaxanthin (*7*) dimethyl ether for CD comparison with *10*. The route utilized by Surmatis and Thommen [41] for the synthesis of astaxanthin (*1*) dimethyl ether is one possible approach.

Another carotenoid with unknown stereochemistry is actinioerythrin (*13*, Scheme 3), which is a naturally occurring diester of a bis-cyclopentenone-α-ketol [34] with the unique 2,2′-bis-nor-carotenoid structure. It has been suggested that *13* is formed *in vivo* from astaxanthin (*1*) [42] by oxidation to *14* (Scheme 3), benzilic acid rearrangement to *15*, decarboxylation to the bis-α-ketol *16*, and finally esterification. As the



Scheme 3.

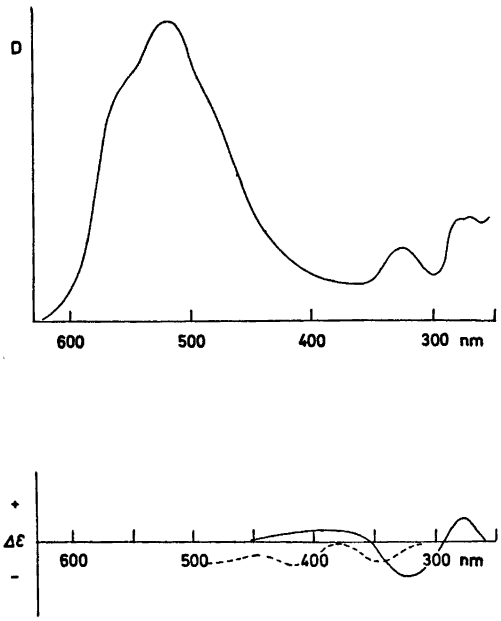


Fig. 4. Electronic absorption spectrum (——, top) of actinioerythrin (*13*), CD-spectrum (——, bottom) and ORD-spectrum (- - -, bottom; calculated from the CD-spectrum) of actinioerythrin (*13*) in chloroform solution.

prochiral intermediate *14* may be involved, the absolute configuration of the OR-group of *13* must not necessarily, from biosynthetic considerations, have the same streochemistry as that of astaxanthin (*1*). On the other hand, optical activity of *13* does not contradict such a biogenetic pathway since a chiral enzyme can stereospecifically react either with the *re*- or the *si*-side of the ring of *14*. Actinioerythrin *13* is indeed chiral as evidenced by its CD spectrum, Fig. 4. The ORD spectrum calculated [17] therefrom is also given in Fig. 4. In the case of *13*, a negative CD within the main absorption band in the visible region was observed. Because of high noise and a small anisotropy factor no quantitative values can be given, however. Due to the difficulties encountered in the interpretation of the CD of cyclopentenones [20] no conclusions about the absolute streochemistry of actinioerythrin (*13*) can be drawn at present, except that the two chiral centers of this molecule must have the same chirality.

EXPERIMENTAL

Materials. Astaxanthin *ex Hommarus gammarus* and astaxanthin monoester *ex Haematococcus pluvialis* were obtained from the collection of Professor N. A. Sørensen, and astaxanthin *ex* lobster eggs provided by Hoffmann-La Roche, Basel. Actinioerythrin was left from a previous investigation.[24]

Methods. Methods used were those commonly employed in the Trondheim laboratory.[24] CD spectra were recorded in Copenhagen in EPA (ether:isopentane:ethanol, 5:5:2) solution with a Roussel-Jouan Dichrographe II.

Acetylation of astaxanthin monoester (6). *6* (0.5 mg) in dry pyridine (2 ml) was treated with acetic anhydride (0.2 ml) for 6 h at 25 °C to yield quantitatively *8*; λ_{max} 485 nm ($CHCl_3$), 474 nm (MeOH); $R_F = 0.5$, silica gel G plate developed with 20 % APE (acetone in petroleum ether).

$LiAlH_4$ reduction of 8. *8* (0.5 mg) in dry ether (10 ml) was treated with $LiAlH_4$ at 25 °C for 3 min (representative experiment). After extractive isolation with $CHCl_3$, *10* was isolated and purified by TLC on silica gel G plates developed with APE. The tetrol *10* had the following properties: λ_{max} (430), 453, 480 nm (acetone), 451 nm (MeOH); *m/e* 600 (M), 582 (M − 18), 564 (M − 36), 546 (M − 54), 508 (M − 92) and 494 (M − 106); $R_F = 0.35$, silica gel G plates developed with 40 % APE.

Attempted separation of diastereomeric tetrols 10. Attempted separation of the diastereomeric tetrols *10* on kieselguhr paper, cellulose plates, cellulose columns, acetylated polyamide columns and MgO columns failed. On the latter column *10* failed to move with 10 % acetone in benzene; Nicoara *et al.*[25] reported successful separation of *10* in this system.

$NaBH_4$ reduction of 8. *8* (0.5 mg) in wet MeOH (10 ml) was treated with $NaBH_4$ at 0 °C for 5 min. After extractive isolation *9* was obtained in quantitative yield; λ_{max} (430), 453, 480 nm (acetone); 451 nm (MeOH); $R_F = 0.4$, silica gel G plates developed with 25 % APE.

Reactions of 9. *9* (0.1 mg) was treated for 20 h at 25 °C with SO_3-pyridine (10 mg) in dry THF (2 ml);[23] no sulfate ester was detected upon TLC analysis. Treatment of *9* (0.1 mg) with excess *p*-toluene sulfonyl chloride in ether or pyridine gave no ester. Treatment of *9* in dry pyridine with methane sulfonyl chloride resulted in degradation of the pigment. Attempted formation of the 4,4′-dibromo derivative of *9* by treatment with NBS-$(CH_3)_2S$ in CH_2Cl_2 [26] failed.

Reactions with canthaxanthin (3). *3* when treated with hydrazine hydrochloride, phenylhydrazine hydrochloride, or tosylhydrazine in MeOH or pyridine solvents failed to yield the corresponding hydrazones even at elevated temperatures. *3* smoothly formed the oxime and semicarbazone under standard conditions. The latter when treated with KOMe in dimethyl

sulfoxide [24] failed to yield the reduced product when treated at 100 °C in a sealed tube under N_2 for 30 min.

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