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ABSOLUTE CONFIGURATION OF ESCHSCHOLTZXANTHIN*

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Key Word Index—*Eschscholtzia californica*; Papaveraceae; carotenoid; eschscholtzxanthin; all-*trans* (3*S*,3′*S*)-4′,5′-didehydro-4,5′-*retro*-β,β-carotene-3,3′-diol; partial synthesis from (3*R*,3′*R*)-zeaxanthin; absolute configuration.

Abstract—The chirality of eschscholtzxanthin (all-*trans* (3*S*,3′S)-4′,5′-didehydro-4,5′-*retro*-β,β-carotene-3,3′-diol) at 3,3′ was assigned from the CD correlation of the natural material and the semi-synthetic carotenoid prepared by (NBS-dehydrogenation of natural zeaxanthin ((3*R*,3′*R*)-β,β-carotene-3,3′-diol). The $\Delta^{6(6')}$-*trans* configuration followed from ^{1}H NMR evidence, including nuclear Overhauser experiments with rhodoxanthin, retrodehydrocarotene (4′,5′-didehydro-4,5′-*retro*-β,β-carotene) and smaller *retro* model compounds revealing a general preference for the Δ^6-*trans* configuration in *retro* compounds. Biosynthetic considerations are made.

INTRODUCTION

Eschscholtzxanthin is a representative of naturally occurring *retro* carotenoids with shifted double bonds in the polyene chain [1] and has two chiral centra (3,3′). First isolated by Strain [2] from the Californian yellow poppy *Eschscholtzia californica*, eschscholtzxanthin was assigned the constitution **4** (stereochemistry not defined) by Karrer and Leumann [3]. Entschel and Karrer [4] later prepared **4** from natural physalien (**2**, zeaxanthin dipalmitate) by NBS dehydrogenation, followed by alkaline hydrolysis, cf. Scheme 1. Natural and semi-synthetic eschscholtzxanthin (**4**) had identical visible light absorption spectra, gave no melting point depression and both exhibited a positive $[\alpha]_{645}$-value.

The absolute configuration of zeaxanthin (**1** = (3*R*,3′*R*)-β,β-carotene-3,3′-diol) has since been established [5–7]. For unequivocal determination of the chirality at 3,3′ of eschscholtzxanthin (**4**) CD correlation of the natural and semisynthetic compound was required. Moreover, the configuration of the $\Delta^{6(6')}$-double bonds was not previously established.

RESULTS AND DISCUSSION

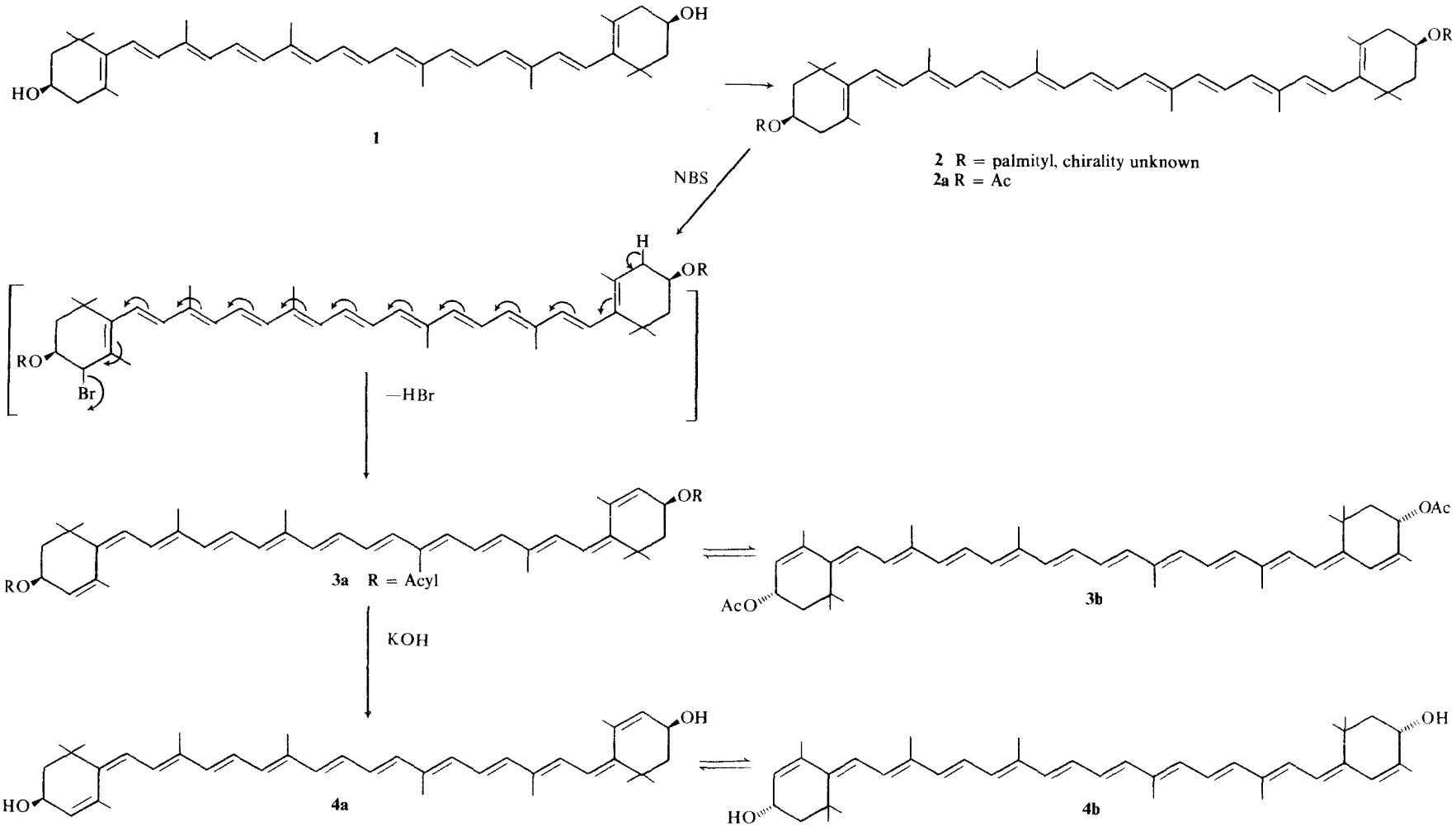
Natural zeaxanthin (**1**) ex *Flavobacterium* sp. was converted to the diacetate **2a** by the standard acetylation procedure [8]. Treatment with NBS, followed by dehydrobromination with morpholine gave eschscholtzxanthin diacetate (**3**), subsequently hydrolysed to the free diol. **4**. The physical properties of semi-synthetic eschscholtzxanthin (**4**) including electronic, ^{1}H NMR and mass spectra were consistent with those of the natural material [2–4, 9, 10].

The CD spectra of natural and semi-synthetic eschscholtzxanthin are shown in Fig. 1. The obvious agreement of the characteristic and remarkably strong Cotton effect leaves no doubt about the same absolute configuration at 3/3′ of the natural and semi-synthetic sample. The assumed mechanism of NBS-dehydrogenation involves allylic bromination in 4-position [11–14] followed by elimination of hydrogen bromide with rearrangement to the *retro* structure (Scheme 1). The allylic bromide has not been isolated except for compounds with shorter conjugated systems, e.g. ψ-ionone [15], but the mechanism involves no change of configuration at 3,3′.

The identical CD and ^{1}H NMR spectra of natural and semi-synthetic eschscholtzxanthin further demonstrate the same geometry of the polyene chain. *Cis* double bonds are known to influence the Cotton effect drastically [5, 16]. Regarding the configuration of the $\Delta^{6(6')}$-double bonds, both *cis* (**3a**, **4a**) and *trans* (**3b**, **4b**) configurations (Scheme 1), must be considered.

For the *retro* diketone rhodoxanthin Mayer *et al.* [17] have favoured $\Delta^{6(6')}$-*trans* configuration (**5b**, Scheme 2) on the basis of ^{1}H NMR data. The presence of Δ^6-*trans* (major) and Δ^6-*cis* (minor) isomers in solutions of compounds with *retro* or modified *retro* end-groups was recently concluded from the frequent observation of extra signals in their ^{1}H NMR spectra [10]. Particularly indicative were the signals of the ring methyl groups, the protons of which were found to be differently shielded in the Δ^6-*trans* and -*cis* isomers. It was assumed in all these cases that the major component with the signal(s) of the *gem.* dimethyl groups at C-1 at lower field and the signal of the olefinic methyl group at C-5 at higher field should have Δ^6-*trans*. Inspection of molecular models (see also Fig. 2) reveals that in this isomer a strong steric interaction takes place between the hydrogen at C-8 and those of the equatorially oriented methyl group at C-1. In the Δ^6-*cis* isomer on the other hand, a similar interaction of H-8 and the hydrogens of the methyl group at

*Part 12 in the series "Carotenoids of higher Plants". For Part 11 see Bodea, C., Andrewes, A. G., Borch, G. and Liaaen-Jensen, S. (1978) *Phytochemistry* **17**, 2037.


OH
HO
1
OR
RO
2 R = palmityl, chirality unknown
2a R = Ac
NBS
H
OR
RO
Br
—HBr
OR
RO
3a R = Acyl
OAc
AcO
3b
KOH
OH
HO
4a
OH
HO
4b

Scheme 1

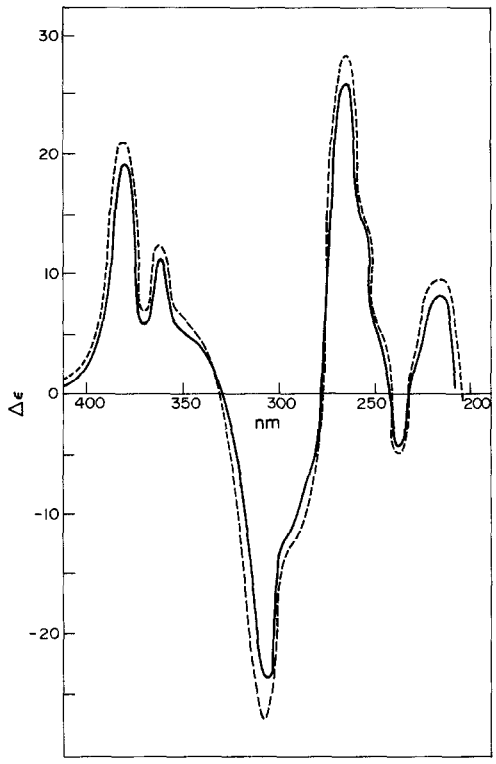


Fig. 1. CD spectra in EPA (diethyl ether, isopentane, ethanol 5:5:2) solution of —— natural eschscholtzxanthin (**4b**) and - - - - semi-synthetic eschscholtzxanthin (**4b**).

C-5 is present. The assignment of the Δ^6-*trans* structure to the preferred isomer of *retro* compounds on the basis of the observed ^{1}H NMR chemical shifts of the C-1 and C-5 methyl groups may thus be rationalized in terms of the expected anisotropy effects of the Δ^8 double bond and the different steric interactions in the two isomers. This interpretation was now fully confirmed by a series of nuclear Overhauser (NO) experiments with appropriate

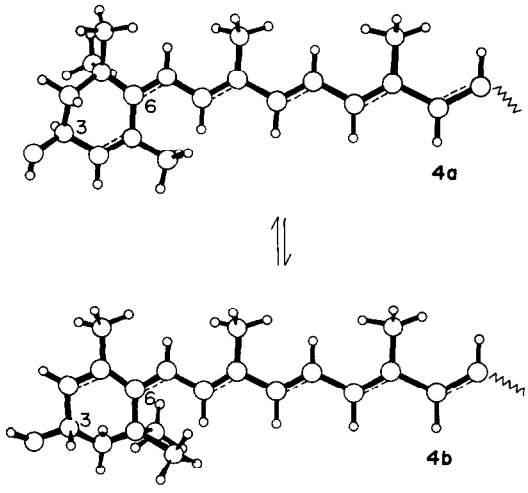


Fig. 2. Presumed conformations of Δ^6-*cis* eschscholtzxanthin (**4a**) and the preferred Δ^6-*trans* eschscholtzxanthin (**4b**).

retro model compounds of three types (Scheme 2):
(A) 3-keto derivatives: rhodoxanthin (**5**) and the model compounds **6a** and **6b**;
(B) compounds without oxygen-substituted ring: anhydrovitamin A (**7**), *retro*dehydrocarotene (**8**) and *retro*-vitamin A acetate (**9**);
(C) 3-acetoxy derivatives: model compounds **10a** and **10b**.

NO experiments with an available sample of synthetic rhodoxanthin (**5**) [18] cited in the Experimental confirmed the previous assignment [17] of the main isomer (90%) as Δ^6-*trans* (**5b**) and the minor isomer (10%) as Δ^6-*cis* (**5a**).

Since the olefinic range of the spectrum of rhodoxanthin (**5**) is not well suited for NO experiments due to the strongly overlapping signals, additional experiments were carried out with a sample consisting of a mixture of the two model compounds **6a** and **6b** [19]. From these experiments unequivocal signal assignments for both the Δ^6-*cis* (**6a**, *ca* 30%) and the Δ^6-*trans* (**6b**, *ca* 70%) isomers were obtained. The chemical shifts of both isomers (see Scheme 2) are in good agreement with those assigned to the corresponding isomers of rhodoxanthin (**5a** and **5b**).

The observation that the Δ^6-*trans* isomer is generally preferred and that in this isomer the C-1 methyl protons are deshielded relative to the Δ^6-*cis* isomer, whereas the reversed order is observed for the methyl group at C-5, is obviously a general trend for *retro* compounds as confirmed below by further studies of compounds of category B and C (Scheme 2).

NO-experiments with anhydrovitamin A (**7**) now clearly proved that this compound also possesses a Δ^6-*trans* configuration in solution as was previously assumed [10]. In contrast to several other *retro* compounds anhydrovitamin A seems to exist practically solely as Δ^6-*trans*.

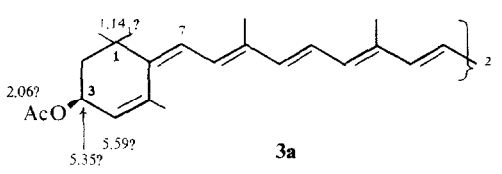
The preferred occurrence in an available sample [20] of Δ^6-*trans* *retro*dehydrocarotene (**8b**, *ca* 75%) relative to the Δ^6-*cis* isomer (**8a**, *ca* 25%), here established by NO experiments, also fits into this pattern.

*Retro*vitamin A acetate (**9**) was also previously assumed to possess a Δ^6-*trans* structure [10]. The present examination, however, revealed that stored solutions may contain at least four isomers, two of which with Δ^6-*trans* (70% together of total) and two with Δ^6-*cis* configuration (30% of total: see Experimental).

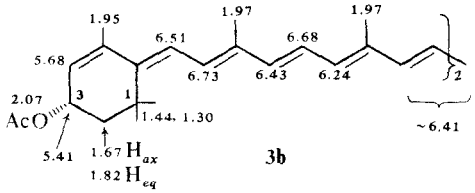
Although we were unable to obtain clear results from a series of NO experiments with eschscholtzxanthin diacetate (**3**) due to the complexity of the olefinic range of the spectrum and the lack of sufficient material, the following observations and chemical shift arguments strongly militate in favour of a preferred Δ^6-*trans* configuration (**3b**) for this compound.

On the basis of the discussion above the assignment of Δ^6-*trans* or Δ^6-*cis* to eschscholtzxanthin or its diacetate should be possible if the relevant chemical shifts of the end groups of both isomers were unequivocally known. Unfortunately, however, this is not the case. Although the ^{1}H NMR spectra of eschscholtzxanthin (**4**) did show minor extra peaks at 1.10 ppm (one of the C-1 methyl groups?) and 5.68 ppm (H-4?) as one would expect for a Δ^6-*cis* isomer (**4a**), a positive identification of this isomer was not possible.

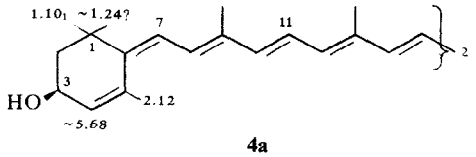
Correspondingly, the spectra of the diacetate **3** had small extra peaks at 1.14 ppm and 5.58 ppm, the expected shift range for the Δ^6-*cis* isomer. As above, most other



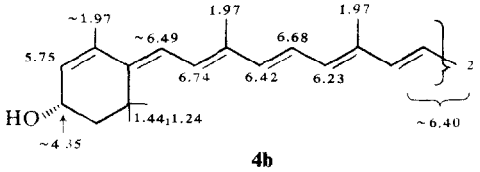
3a



3b

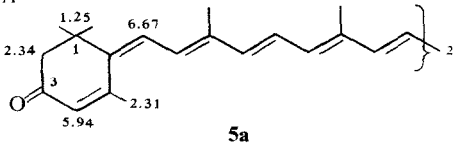


4a

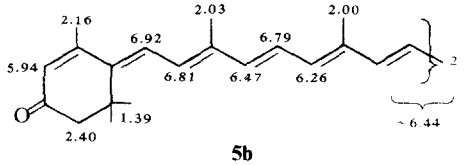


4b

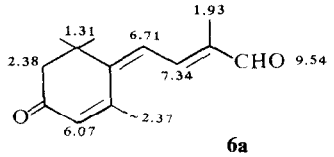
A



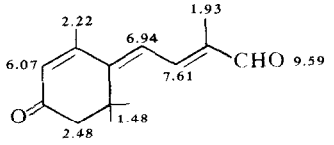
5a



5b

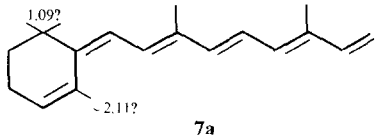


6a

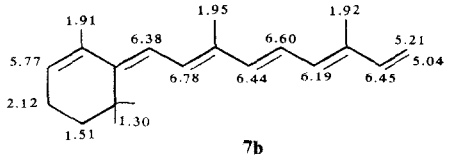


6b

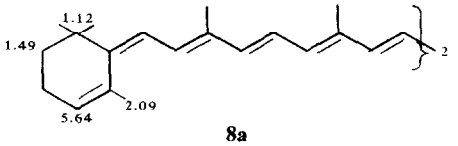
B



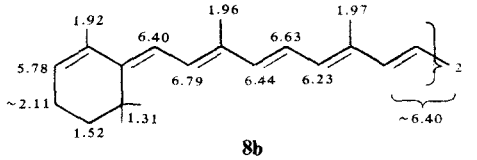
7a



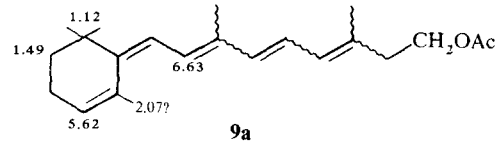
7b



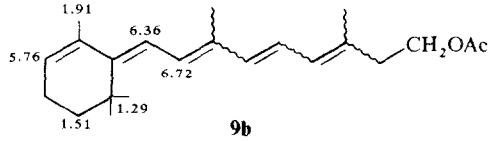
8a



8b

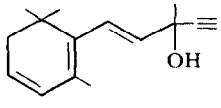


9a



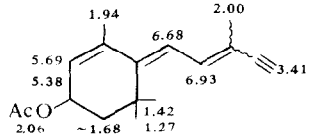
9b

C

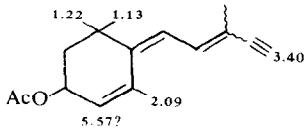


11

AcOH

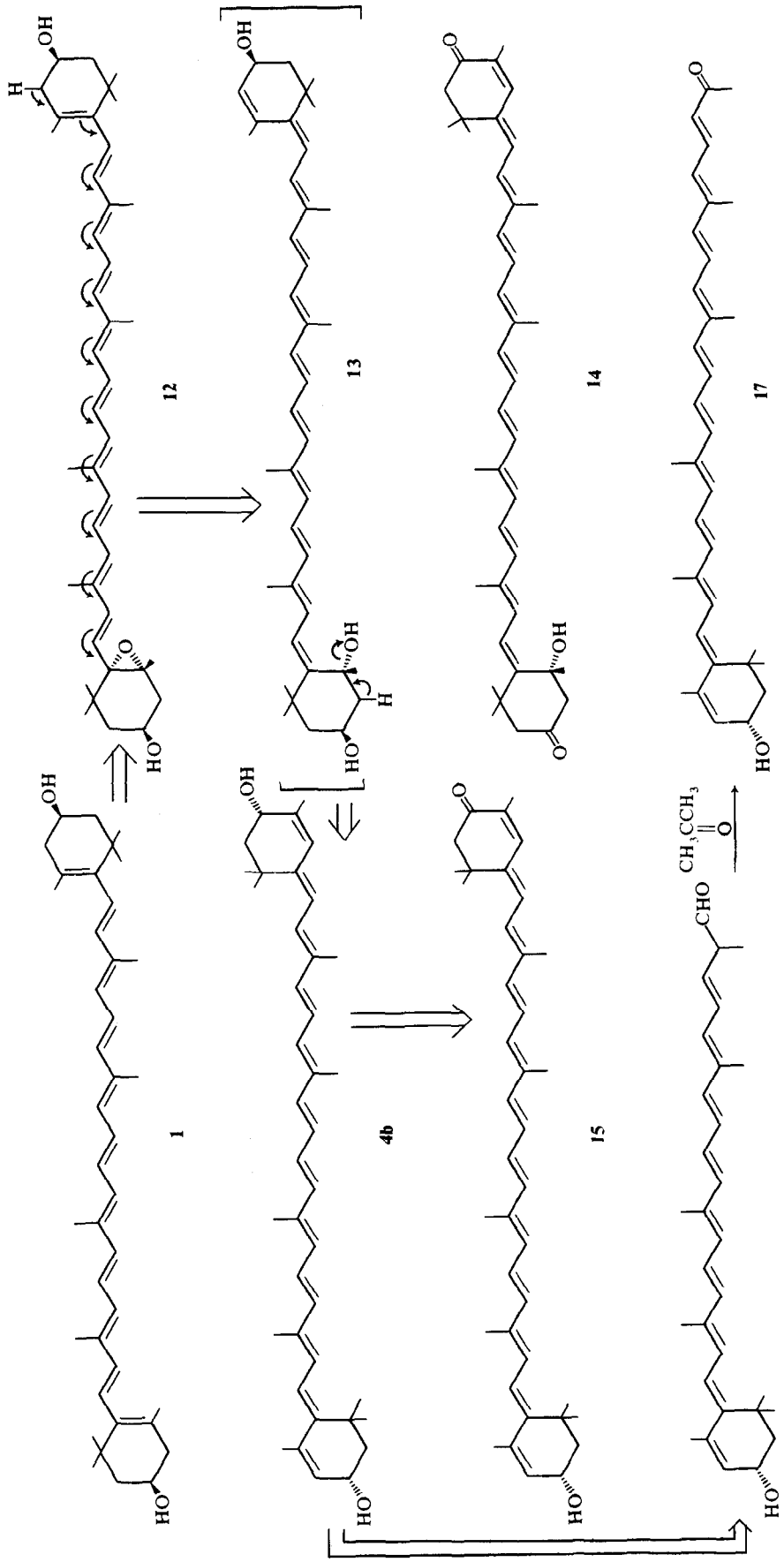


10b



10a

Scheme 2



Scheme 3

signals of the minor component were hidden by the comparatively strong signals of the main isomer.

A reliable prediction of the expected chemical shift values for the signals of the end groups of the Δ^6-*trans* (**3a**) and Δ^6-*cis* (**3b**) isomers of eschscholtzxanthin diacetate was, however, possible by comparison with the data for the acetoxy model compounds **10a** and **10b** obtained from **11** [21]. Although the configuration of Δ^8 was not known, the data (Scheme 2) can only be interpreted by the assumption that the main component should have the Δ^6-*trans* structure (**10b**) and the minor component a Δ^6-*cis* structure (**10a**). From the observed chemical shifts of the main isomer **10b** which closely agree with those of eschscholtzxanthin diacetate we conclude that the latter must have the Δ^6-*trans* structure **3b**. As with other *retro* compounds we consider the possibility of a moderate isomerization Δ^6-*trans-cis* in solution as most likely.

Computer-plots of the most favoured conformations of the two isomers, both with quasi-equatorial C-3 substituents, are shown in Fig. 2. For the Δ^6-*trans* isomer **3b** the assumed conformation is in agreement with the observed spin couplings (see Experimental).

Biosynthetic considerations

Our finding that the chirality of eschscholtzxanthin (**4b**) is the same as that of zeaxanthin (**1**) at positions 3,3′ supports the suggestion made earlier by Eugster and Karrer [3] that zeaxanthin (**1**) is a biosynthetic precursor of eschscholtzxanthin (**4b**). Their suggestion was based on the presence of zeaxanthin in *E. californica* and the partial synthesis of eschscholtzxanthin from zeaxanthin dipalmitate. Williams *et al.* [22], partly from incorporation studies with labelled mevalonate, suggested a biosynthetic route to eschscholtzxanthin (**4b**) from zeaxanthin (**1**) via antheraxanthin (**12**) [23] and the hypothetic triol **13** as in Scheme 3 (stereochemistry disregarded). This is consistent with the chirality at 3(3′) for **1**, **12** and **4b** and would predict the configuration at C-5 for the unknown intermediate **13**. If loniceraxanthin [24] were an alternative product of **13**, the configuration **14** of loniceraxanthin may be postulated.

Definition of the stereochemistry of eschscholtzxanthin (**4b**) also deduces the stereochemistry of eschscholtzxanthrone [25], likely to be an oxidation product **15** of **4b**. If correct, the constitution suggested for tangeraxanthin [26] suggests that it is an artefact [27, 28] **17** formed from the apocarotenal **16**, a plausible metabolic product of eschscholtzxanthin (**4b**).

EXPERIMENTAL

Materials and methods. These were as commonly employed in the Norwegian Laboratory [29]. Instruments used were as specified elsewhere [29]. ^{1}H NMR spectra were recorded at 60 MHz (compounds **10a** and **10b**), 100 MHz (natural eschscholtzxanthin), 90 MHz (ref. compounds **6a** and **5b**) or 270 MHz (all other compounds). All the spectra were run in $CDCl_3$ as the solvent. NO experiments were done at 90 MHz (CW and FT-mode: sample **6**) or 270 MHz (FT-mode) with carefully degassed solns (3 to 5 pump-freeze-thaw cycles). For calculation of molecular models a computer program was used.

Zeaxanthin diacetate (**2a**). Zeaxanthin (**1**, 500 mg ex *Flavobacterium* sp.) was submitted to standard acetylation [8]; yield 518 mg **2a** (90%); $\lambda_{max}^{Me_2O}$ nm: 427, 451 and 479; NMR ($CDCl_3$): δ 1.07 (12H, *s*, *gem.* dimethyl), 1.74 (6H, *s*, CH_3-18,18′), 1.98 (12H, *s*, in-chain CH_3), 2.05 (6H, *s*, CH_3COO), 5.8–6.4 (14H, *m*, olefinic): MS *m/e*: 652 (M), 592 (M-60), 562 (M-92), 546 (M-106), 494 (M-158) and 457 (M-195).

Semi-synthetic eschscholtzxanthin diacetate (**3b**). To a stirred, chilled (−18°) soln of zeaxanthin diacetate (**2a**, 500 mg) in $CHCl_3$ (5 ml, dried and stabilized with 1% EtOH) was added *N*-bromosuccinimide (175 mg). After 30 sec *N*-ethylmorpholine (0.5 ml) was added. The mixture was stirred at 25° for 2 min and then refluxed for 15 min. Extraction with Et_2O and chromatography gave **3b**.

Semi-synthetic eschscholtzxanthin (**4b**). Standard saponification [8] of the diacetate **3b**, isolation with Et_2O, CC (Si gel, increasing amounts of Me_2CO in petrol) followed by TLC (Si gel, 0.2 mm), provided **4a**, yield 48 mg (8.4% overall yield from **1**) after crystallization 22 mg. Semi-synthetic **4a** had λ_{max}^{hexane} nm 442, 472 and 502 $\lambda_{max}^{Me_2CO}$ nm: 445, 474 and 504; NMR ($CDCl_3$): δ 1.11 (*s*, one *gem.* CH_3 in **4a**?), 1.24 and 1.44 (*s*, *gem.* diCH_3), 1.97 (CH_3, *s*, 18,18′,19,19′,20,20′), 5.75 (*br* H-4) and 6-7 (olefinic H's). From the intensities of the δ 1.11/1.44 signals the content of **4a** was estimated to *ca* 15%; MS *m/e* 566 (M), 506 (M-56), 493 (M-79), 474 (M-92), 460 (M-106), 427 (M-139) and 412 (M-152); CD, Fig. 1.

Semi-synthetic **4a** was inseparable from natural **4a** on TLC (Si gel) and Schleicher & Schüll No. 287, 10% Me_2CO in petrol (= S & S 287, 10% APE) R_f = 0.15

Natural eschscholtzxanthin (**4b**) ex *Eschscholtzia californica*, available from a previous study [2] with λ_{max} as above: CD, Fig. 1, R_f as above, autoxidized readily. A previously recorded [9] ^{1}H NMR spectrum ($CDCl_3$) had δ 1.10 (*s*, one *gem.* CH_3 in **4a**?), 1.24 and 1.44 (*s*, *gem.* diCH_3 in **4b**), 1.97 (*s*, CH_3 18, 18′, 19,19′, 20,20′), *ca* 4.35 (*br*, H-3), 5.75 (*br* H-4) in **4b**. For further shifts see Scheme 2. The content of **4b** was estimated to *ca* 15%.

Eschscholtzxanthin diacetate (**3b**), *ca* 5 mg, ex natural **4b**, available from a previous study [2] was more stable and suitable for NMR experiments: R_f = 0.64 (S & S 287, 10% APE); λ_{max} as for **4b** and ($CHCl_3$) 454, 480 and 512 nm; ^{1}H NMR δ ($CDCl_3$) fresh soln: 1.30 and 1.44 (*s*, *gem.* diCH_3), 1.82 (*dd*, H-2,2′$_{ax}$; J = 12 Hz, 10 Hz), 1.67 (*dd* H-2,2′$_{eq}$; J = 12 Hz, 5 Hz), 1.95 (CH_3 18,18′), 1.97 (CH_3 19,19′ and 20,20′), 2.07 (Ac), 5.41 (*br*, H-3,3′*ax.*), 5.68 (H-4,4′), 6.24 (*d*, H-12,12′), $J_{11,12}$ = 11.5 Hz), *ca* 6.41 (*m*, H-14,14′ and H-15,15′), 6.43 (*d*, H-10,10′), 6.51 (H-7,7′; $J_{7,8}$ = 12.5 Hz); 6.68 (*dd*, H-11,11′; $J_{10,11}$ = 14.8 Hz), 6.73 (*d*, H-8,8′; $J_{7,8}$ = 12.5 Hz). MS *m/e*: 650 (M), 590 (M-60), 530 (M-60-60), 516, 43.

^{1}H NMR studies of model compounds. Rhodoxanthin (**5**). As revealed by the ^{1}H NMR spectrum, an available sample of synthetic rhodoxanthin [18] consisted of *ca* 90% of a main and *ca* 10% of a minor isomer. Irradiation of the signal of the C-5 methyl protons at 2.16 ppm of the main isomer resulted in a sharpening of the signal of H-4 at 5.94 ppm and, at the same time, in an increase of its integral of *ca* 25%. In addition, the signal of H-7 which appeared as a doublet at lowest field of all the olefinic protons also showed an NO enhancement of 13%, proving that the main isomer should have the Δ^6-*trans* structure **5b**. The chemical shifts for both isomers **5a** and **5b** as far as they were discernible are given in Scheme 2. The assignments were in all cases confirmed by various decoupling experiments.

Model compounds **6a** *and* **6b**. A mixture of **6a** and **6b** [19] was used. Irradiation of the signal of the C-1 methyl groups at 1.48 ppm of the main isomer (*ca* 70%) resulted in a *ca* 30% NO enhancement of the signal of H-8 at 7.61 ppm. Similarly, irradiation of the methyl protons at C-5 at 2.22 ppm gave an NO enhancement of the signals of H-5 and H-7 of the main component of more than 20 and 21% respectively. This allows the unequivocal assignment of the Δ^6-*trans* structure **6b** to the main component having the protons of the *gem.* dimethyl group less shielded than in **6a** with Δ^6-*cis*. Here, irradiation at 2.37 ppm of the C-5 methyl protons of the minor component resulted in an NO enhancement of *ca* 10% of the H-8 signal at 7.34 ppm in accordance with Δ^6-*cis* (**6a**).

Anhydrovitamin A (**7**). The ^{1}H NMR spectrum is given in Fig. 19 of ref. [10]. Irradiation of the *gem.* methyl protons at 1.30 ppm resulted in a NO enhancement of the signal of H-8 at 6.78 ppm of *ca* 20%. The assignment of H-8 was unequivocally

confirmed by decoupling experiments. Irradiation of the overlapping signals at *ca* 1.93 ppm of the methyl protons at C-5, C-9 and C-13 gave an increase of the signal intensities of H-4 (20%), H-7 (25%), H-11 (22%) and H-15a (~10%) in accordance with a planar all-*trans* structure **7b**. The existence of a few % of Δ^6-*cis* (**7a**) could only be presumed from the observation of a small extra peak at 1.09 ppm tentitatively attributed to the *gem.* dimethyl protons of this isomer.

Retro*dehydrocarotene* (**8**). The ^{1}H NMR spectrum of a sample obtained as a side product from the oxidation of β-carotene to canthaxanthin [20] revealed the presence of the two isomers, here in an approximate ratio of 3:1. The assignment of Δ^6-*trans* to the main component was likewise based on NO experiments. Irradiation of the signal of the C-1 methyl groups at 1.31 ppm resulted in a *ca* 18% enhancement of the H-8 doublet at 6.79 ppm proving the close steric approach of the two types of hydrogen and hence the Δ^6-*trans* structure **8b** for the main component. The signal of the C-1 methyl group of the Δ^6-*cis* isomer is located at 1.12 ppm, i.e. again at slightly higher field than in the *trans* compound (see Scheme 2).

Retro*vitamin A acetate* (**9**). The ^{1}H NMR of stored solns revealed the presence of at least 4 isomers, two of which with Δ^6-*trans* had their *gem.* dimethyl protons absorbing very closely together at *ca* 1.29 ppm (70% together); the two other isomers with Δ^6-*cis* at *ca* 1.12 ppm (30%).

Model compounds **10a** and **10b**. These were obtained from **11** as a mixture of both isomers in a ratio 3:1 [21] as revealed by the ^{1}H NMR spectrum (see Scheme 2).

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