M−60, M−80. Furanoid rearrangement of *4a* gave two products with electronic spectra, mass spectra, and R_F-values (co-chromatography) as *5a* and *5b* described above. Separate acetylation of the furanoid products *5a* and *5b* gave 60 % conversion to the corresponding acetates, inseparable in our chromatographic systems and from *5a,b* derived from *1* in a special reaction discussed in the following paper.[8] On this basis it is concluded that the natural epoxide has structure *4a* (*cis*). It deserves comment that natural violaxanthin (5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol), has a *trans* relationship between the 5,6-epoxy bridge and the 3-hydroxy substituent.[4,7] However, the present epoxide *4a* and violaxanthin both have the same axial/equatorial relationship between the hydroxy substituent and the polyene chain, see Scheme 2 for alternative conformations A and B of *4a*.

The second naturally occurring epoxide *7* exhibited λ_{max} (ether) 423, 443, and 472 nm consistent with data reported for the mono-epoxide of β,β-carotene [9] and mass spectrum like *4a* except an (M−56−18) peak. Acetylation of *7* provided a monoacetate with unchanged electronic spectrum and mass spectrum as for *4a*-monoacetate with no significant RDA-fragmentation. Furanoid rearrangement of *7* gave two products both with λ_{max} (methanol) 404, 423, and 445 nm and *m/e* 568 (M), M−16, M−80, 221, inseparable from two furanoid products obtained from β,β-caroten-2-ol (*2*) in the reaction discussed in the following paper.[8] From the stereochemistry observed for the corresponding reaction of β,ε-caroten-2-ol (*1*) [8] and by analogy with the natural epoxide *4a*, the stereochemistry of *7a* (*cis*) is considered likely for the second epoxide *7* isolated from *T. iolithius*.

Methods commonly employed in this laboratory were used. Experimental details are given elsewhere.[10] The epoxides *4a* and *7* were readily separated from *1*, *2*, and *3* on magnesium oxide columns (benzene). R_F-values (Schleicher & Schüll No. 287 circular, kieselguhr paper, 1 % acetone in petroleum ether) were: *4a* (0.56), *4b* (0.35), *4a*-acetate (0.88), *5a* and *5b* (0.48 and 0.18), *5c* and *5d* (0.33 and 0.18), *6a* and *6b* (0.72), *7* (0.53), *7*-acetate (0.84), furanoid *7* (0.22 and 0.55), and furanoid *7*-acetates (0.74). Purification for mass spectrometry was achieved by TLC on kieselgel, 20 % acetone in petroleum ether).

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Algal Carotenoids. XII.* Chemical Reactions of Carotenoids with 2-Hydroxylated β-Rings

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Spectroscopic evidence alone was sufficient for the structural elucidation of the first carotenoids with 2-hydroxylated β-rings, namely β,ε-caroten-2-ol (*1a*), β,β-caroten-2-ol (*2a*), and β,β-carotene-2,2′-diol (*3*), Scheme 1, from the green alga *Trentepohlia iolithus*.[1,2] We now report a chemical characterization of carotenoids possessing this end group (*1a* and *2a*).

Models reveal steric hindrance of the 2-hydroxy-substituent of a β-ring. Lower reactivity than for analogous 3-hydroxy carotenoids was therefore predicted.

Standard acetylation [3] of β,β-caroten-2-ol (*2a*) was slower than for β,β-caroten-3-ol (*4a*): 50 % and 100 % conversion, respectively, to the corresponding acetates *2b* and *4b* after 3.5 h, see Fig. 1. The 2-hydroxy compound

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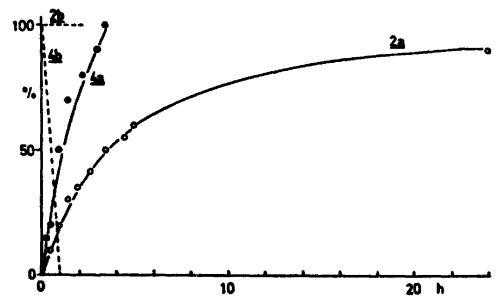


Fig. 1. Relative rates of acetylation of β,β-caroten-2-ol (*2a*), β,β-caroten-3-ol (*4a*), and relative rates of hydrolysis of β,β-carotenyl-2-acetate (*2b*) and β,β-carotenyl-3-acetate (*4a*).[17]

(*2a*) was chromatographically less strongly adsorbed than the 3-hydroxy analogue *3a* in agreement with the larger shielding of the hydroxy group in *2a*. However, no separation of the acetates *2b* and *4b* was achieved.

β,β-Caroten-2-yl acetate (*2b*) crystallized as red prisms, m.p. 81–83 °C, with predicted spectral properties (visible, IR, ^{1}H NMR). Alkali treatment of the acetates *2b* and *4b* (5 % KOH, −25 °C) resulted in complete hydrolysis of *4b* after 50 min when *2b* was not effected, see Fig. 1. However, *2b* was completely hydrolyzed after 60 min at 20 °C.

The low rate of acetylation was also observed for *1a*, which provided the acetate *1b* in 85 % yield after 16 h.

No difference in the rate of silylation of the 2-hydroxy compound *2a* and the 3-hydroxy compound *4a* was observed under standard conditions[3] at −30 °C. The trimethylsilyl ether *2c* (Scheme 1) was less polar than *4c*.

Comparative methylation of the 2-hydroxy compounds *1a* and *2a* and the 3,3′-dihydroxy compound *5a* (=zeaxanthin) was attempted by various modifications of Kuhn's method.[4–7] Abnormal products[8] were obtained using silver oxide;[4] *2a* also gave an abnormal product[8] with barium oxide.[5] By the CH_3I/BaO/DMF/DMSO modification[6,7] *1a* gave the methyl ether *1d*[8] and *2a* provided the methyl ether *2d*.[8] More satisfactory was methylation with methyl iodide and sodium hydride.[9,8] The 2-hydroxy compound *1a* gave the methyl ether *1d* (60 % of recovered carotenoid; 72 % total recovery) under conditions where the 3,3′-diol (*5a*) was quantitatively converted to the dimethyl ether *5d* (85 %) and monomethyl ether *5e* (15 %).[8] The methyl ether *1d* had *m/e* 566=M, M−15, M−56 (RDA-fragmentation of ε-ring[10]), M−92, M−106, M−158. All methyl ethers exhibited the same visible absorption as the parent alcohols.

The results discussed demonstrate the lower reactivity of the sterically hindered 2-hydroxy group in *1a* and *2a* than of the corresponding 3-hydroxy group in *4a* and *5a* as to acetylation, hydrolysis of the acetates and methylation. Differences in the rate of silylation, fast for both categories, were not established. Together with the higher R_F-values for the 2-hydroxy compounds their low rate of reaction may serve to distinguish carotenoids with 2-hydroxylated and 3-hydroxylated β-rings on the micro scale.

The green/blue colour reaction of the 2-hydroxy compounds *1a*, *2a*, and *3a* with hydrochloric acid,[10,1] rationalized by the isolation of a furanoid product of *1a* on treatment with 0.01 M HCl in chloroform-methanol,[11] prompted further investigation on the reaction leading to the partly characterized furanoxides.

The structures of the furanoid products obtained from the mono-ols *1a* and *2a* will first be discussed, Scheme 2. Under conditions to be described below two furanoxides *6a* and *6b* were obtained from *1a* and two furanoxides *7a* and *7b* from *2a*.

The furanoxides *6a* and *6b* were found identical (R_F-values, electronic and mass spectra as well as slow acetylation to the same acetates) with the furanoxides obtained from the so-called *cis* 5,6-epoxide (*cis* relationship between the epoxy and hydroxy substituent) *8* of *1a*. The location of the hydroxy group and the stereochemistry, including the C-8 epimeric nature of the two furanoxides follow therefrom. It is pointed out that the reaction X (Scheme 2) leading from *1a* to the furanoxides *6a*+*6b* must be stereospecific at C-5.

The furanoxides *7a* and *7b* from *2a* were found identical by the criteria mentioned above with the C-8 epimeric furanoid rearrangement products of natural 5,6-epoxy-5,6-dihydro-β,β-caroten-2-ol (*9*), tentatively considered to be a *cis* epoxide.[11] This would imply the same stereospecificity at C-5 in the reaction X leading from *2a* to *7a*+*7b*.

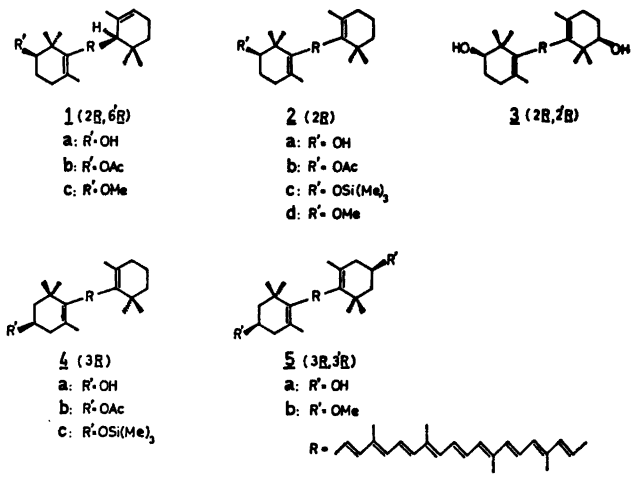
The nature of reaction X will now be considered. The presence of minor epoxides[11] as contaminants would offer an easy explanation. However, this possibility is ruled out from the ready separation of *1a* and *2a* from their 5,6-epoxides,[11] and from the yields obtained in reaction X (pigment recovery 15–30 %; furanoid products comprising 20–60 % of the recovered carotenoids).

A series of experiments designed to define the reaction are summarized:

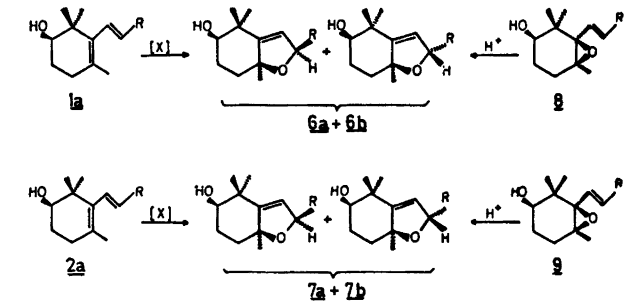
(a) Contrary to the previous assumption[1] hydrogen chloride is not the essential factor X, which is present in certain qualities of chloroform (Baker *p.a.* and a technical quality DAB 7, but not Merck *p.a.*) when chromatographed on alumina and stored at least 20 h before use.

(b) The reaction, formally requiring addition of an oxygen atom, was not prevented under presumed anaerobic conditions.

(c) No evidence for dichlorocarbene being involved was obtained. Thus treatment of *2a* with dichlorocarbene[12] gave no furanoid products, but two other products, one corre-



Scheme 1.



Scheme 2.

sponding to $C_{41}H_{56}Cl_2O$ (formal addition of dichlorocarbene) from mass spectral data.

(d) A free hydroxy group in 2-position is essential for the reaction. Thus no furanoid products were obtained from the acetate *1b* or the methyl ether *1d* with the 2-hydroxy function blocked. Moreover, no furanoid products were obtained from the 3,3'-dihydroxy compound *5a* or from β,β-carotene.

A free radical reaction of a phosgene peroxide complex,[13–16] formed on storage of chloroform after removal of the stabilizer, with participation of the 2-hydroxy group, providing the *cis* epoxide, subsequently rearranging to the furanoxides by traces of acids, or directly to the C_8-epimeric furanoxides, is considered.

Experimental details are given elsewhere.[17] R_F-Values (Schleicher & Schüll No. 287 circular, kieselguhr paper, percentage figure indicates acetone in petroleum ether) were: *1a* (2 %, 0.71), *1b* (1 %, 0.87), *1c* (1 %, 0.86), *2a* (2 %, 0.63), *2b* (1 %, 0.90), *2c* (0 %, 0.83), *2d* (2 %, 0.93), *4a* (1 %, 0.51), *4b* (1 %, 0.90), *4c* (0 %, 0.78), *5a* (10 %, 0.40), *5b*,[8] *6a*+*6b* (1 %, 0.48+0.18), *7a*-acetate = *7b*-acetate (1 %, 0.74), *7a*+*7b* (1 %, 0.55+0.22), *8* (1 %, 0.56), and *9* (1 %, 0.53).

Recommended procedure for preparation of furanoxides: To the 2-hydroxy carotenoid (0.05 – 1 mg) dissolved in *p.a.* chloroform (5 ml) is added Baker *p.a.* chloroform, chromatographed on Merck neutral alumina, activity grade 1, and stored under ordinary atmosphere for at least 20 h, (0.1 – 0.2 ml). A green colour spontaneously develops, turning yellow on ordinary work-up after a few minutes with ether and water.

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