Structure of Heteroxanthin, a Unique Xanthophyll from the Xanthophyceae (Heterokontae)

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Summary Heteroxanthin has been shown to be 3,5,6,3'-tetrahydroxy-5,6-dihydro-7',8'-didehydro-β,β-carotene.

HETEROXANTHIN, a unique xanthophyll of molecular mass 600 and empirical formula $C_{40}H_{56}O_4$, occurs in all species of the xanthophyceae examined so far.[1,2] It is often accompanied by a partial ester of vaucheriaxanthin.[1-3] The nonesterified vaucheriaxanthin has a mass of 616 and an empirical formula of $C_{40}H_{56}O_5$.[1] In the Heterokontae, heteroxanthin always occurs with diatoxanthin and diadinoxanthin,[1-3] but it is not found with these two latter pigments in other organisms such as diatoms.[4-6]

With unsaponified extracts of the Heterokontae, and with columns of powdered sugar, heteroxanthin forms the topmost yellow zone (above the partial ester of vaucheriaxanthin). With the saponified extracts, however, the vaucheriaxanthin forms the topmost zone. Both heteroxanthin and vaucheriaxanthin partial ester are more strongly sorbed than the trihydroxy-epoxy-analogue, neoxanthin. Because powdered sugar attracts primarily the OH groups of xanthophyll molecules,[5,7] heteroxanthin probably contains four OH groups.

From their recorded spectra and their strong adsorbability, both heteroxanthin and vaucheriaxanthin could be regarded as neoxanthin-like xanthophylls.[1] From current observations, however, heteroxanthin is, chromophorically, like diadinoxanthin; vaucheriaxanthin is like neoxanthin. Observations with n.m.r., i.r., and mass spectrometry (m.s.) establish that heteroxanthin is a hydrated diadinoxanthin, namely, 3,5,6,3'-tetrahydroxy-5,6-dihydro-7',8'- didehydro-β,β-carotene(I).[3]

Isolated by chromatography and carefully purified by rechromatography and crystallization, heteroxanthin exhibits λ_{max}(EtOH) 422—424 (sh), 448, and 478 nm and a curve identical with that of diadinoxanthin(II).[8] No significant shift of the absorption maxima was produced by traces of iodine (all *trans*-double bonds) or by dil. HCl in EtOH [no isomerization of an epoxy-group to a furanoid group, as in vaucheriaxanthin, diadinoxanthin(II), and neoxanthin].

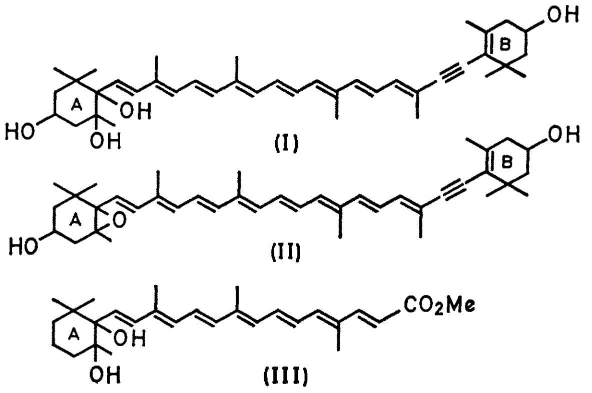
I.r. (KBr pellet) maxima were: 3400 (assoc. -OH), 3030, 2960, 2930, 2860 (CH, CH_2, CH_3), 1565 (C=C), 1450 (ring CH_2), 1380—1360 (*gem* CH_3), 1115, 1050, 1030 (sec. -OH), 965 (*trans* ·CH:CH·), 900, 865, and 830 cm^{-1} (·CH:C<), and only a weak band at 2170 cm^{-1} (C$\equiv$C). The usual band at 1150 cm^{-1} due to tertiary -OH at C-5, as in neoxanthin and vaucheriaxanthin, is known to be absent or very weak in other carotenoids, as fucoxanthin[9] and azafrin methyl ester (III).[10]

N.m.r. values were: τ 8·06, 8·15, 8·43, 8·51, 8·79, and 8·87 (C_5D_5N, Me_6Si_2, Varian HA-100) with relative intensity about 1 : 4 : 1 : 1 : 1 : 2 and τ 8·05, 8·08, 8·12, 8·79, 8·85, 8·87, 8·90, and 9·18 ($CDCl_3$, Me_6Si_2) with relative intensity about 1 : 3 : 1 : 1 : 1 : 1 : 1 : 1. These values correspond to those for half molecules, as in diadinoxanthin (IIB)[3,8] and azafrin (IIIA).[9,11]

Mass spectrometry indicates that heteroxanthin contains: two labile -OH groups; three -OH groups at one end of the molecule with only one at the other end; a structure condensable to pyrylium- and homopyrylium-type ions,[8,12] and two -OH groups at positions 5 and 6. In high-resolution (AEI MS902 with data acquisition and analysis system), m.s. showed $M - 18$, H_2O; $M - 36$, $2H_2O$; $M - 92$, C_7H_8, toluene; $M - 110$, $H_2O + C_7H_8$; $M - 143$, $C_8H_{15}O_2$; $M - 186$, $C_{10}H_{17}O_3$; $M - 199$, $C_{11}H_{19}O_3$; $M - 204$, $H_2O + C_{10}H_{17}O_3$; $M - 226$, $C_{13}H_{22}O_3$; and m/e 181, $C_{11}H_{17}O_2$; and 221, $C_{14}H_{21}O_2$.

M.s. indications follow from high yields of $M - H_2O$ and $M - 2H_2O$, from the several fragments with three oxygen atoms (-OH groups) and with hydrogen composition corresponding to a saturated terminal ring (IA), and from typical hydroxy-pyrylium and hydroxy-homopyrylium type ions, m/e 181 and 221, usually obtained from 3-hydroxy-5,6-epoxides (IIA).[8,12] The corresponding ions without the -OH (m/e 165 and 205) are obtained from unsubstituted 5,6-epoxides (carotene epoxides)[12] and also from 5,6-dihydroxy-carotenoids (IIIA) as we infer from m/e 205 from azafrin.[13] The $M - 143$, $C_8H_{15}O_2$, corresponds to the hydroxy-derivative of the prominent $M - 127$, $C_8H_{15}O$ fragment typical of 5,6-dihydroxy-rings (IIIA) as in azafrin and azafrin methyl ester.[13]

M.s. showed a notable absence of $M - 106$, C_8H_{10}, xylene and $M - 124$, $H_2O + C_8H_{10}$, as with azafrin and azafrin methyl ester (III).[13] The loss of xylene from the C-8 to C-13 or C-9 to C-14 portions of the chain may be blocked by the substituents at C-6 on one end and by the triple bond at the other.[14]



(I)

(II)

(III)

Heteroxanthin did not form methyl ethers (MeOH–HCl) (hence no allylic -OH). It formed a diacetate (Ac_2O in pyridine overnight), and according to Kleinig and Egger only one of the two remaining -OH groups could be silylated,[2] as is also true of the two tertiary -OH groups of azafrin.[15] In m.s. the acetate readily lost $C_2H_4O_2$, and also formed a fragment with one $C_2H_3O_2$ group and two additional oxygen atoms, as expected with the 3-acetate of a 3,5,6-trihydroxy-, saturated end-group (IA): $M - 60$, $C_2H_4O_2$; $M - 120$, $2C_2H_4O_2$; $M - 241$, $C_{13}H_{21}O_4$.

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[1] H. H. Strain, W. A. Svec, K. Aitzetmüller, M. Grandolfo, and J. J. Katz, *Phytochemistry*, 1968, **7**, 1417.
[2] H. Kleinig and K. Egger, *Z. Naturforsch.*, 1967, **22**b, 868.
[3] H. H. Strain, F. L. Benton, M. C. Grandolfo, K. Aitzetmüller, W. A. Svec, and J. J. Katz, *Phytochemistry*, in the press.
[4] H. H. Strain, W. M. Manning, and G. Hardin, *Biol. Bull.*, 1944, **86**, 169.
[5] H. H. Strain, *Ann. Priestley Lectures*, 1958, **32**.
[6] H. H. Strain and W. A. Svec, *Adv. Chromatog.*, 1969, **8**, 119.
[7] H. H. Strain, *J. Amer. Chem. Soc.*, 1948, **70**, 588.
[8] K. Aitzetmüller, W. A. Svec, J. J. Katz, and H. H. Strain, *Chem. Comm.*, 1968, 32.
[9] S. P. Singh, S. Bhattacharji, and A. B. Sen, *J. Indian Chem. Soc.*, 1963, **40**, 925.
[10] H. Müller and P. Karrer, *Helv. Chim. Acta*, 1965, **48**, 291.
[11] M. S. Barber, J. B. Davis, L. M. Jackman, and B. C. L. Weedon, *J. Chem. Soc.*, 1960, 2870.
[12] J. Baldas, Q. N. Porter, L. Cholnoky, J. Szabolcs, and B. C. L. Weedon, *Chem. Comm.*, 1966, 852.
[13] C. R. Enzell, G. W. Francis, and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1969, **23**, 727.
[14] K. Aitzetmüller, H. H. Strain, W. A. Svec, M. C. Grandolfo, and J. J. Katz, *Phytochemistry*, 1969, **8**, 1761.
[15] A. McCormick and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1966, **20**, 1989.