Structure and Chemical Identity of Diadinoxanthin and the Principal Xanthophyll of *Euglena*

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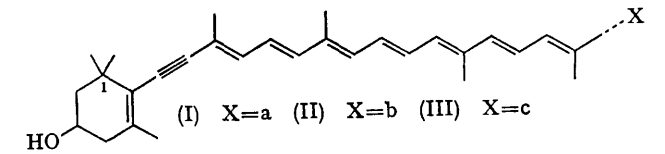
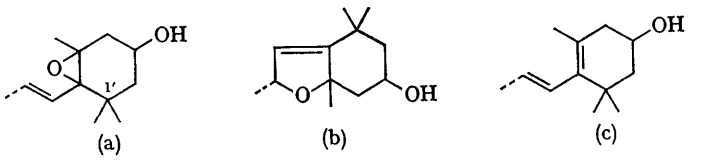
DIADINOXANTHIN, a xanthophyll of diatoms and dinoflagellates, and the principal xanthophyll of *Euglena* are now found to be identical. Preparations from the two sources are chromatographically indistinguishable and exhibit the same visible, i.r., n.m.r., and mass spectra. These observations further indicate that this xanthophyll, which we shall continue to call diadinoxanthin, is 5′,6′-epoxy-3,3′-dihydroxy-7,8-dehydro-β-carotene (I).

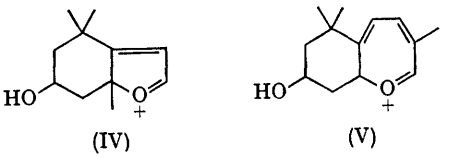
Although earlier investigations[1,2] had indicated a similarity between the xanthophyll preparations from diatoms, dinoflagellates, and *Euglena*, the *Euglena* xanthophyll has recently been misidentified as antheraxanthin,[3] the monoepoxide of zeaxanthin.[4]

Diadinoxanthin has now been isolated by chromatography[1,2] from the diatom *Nitzschia closterium* f. *minutissima* and from *Euglena gracilis*, strain Z. The chromatographic identity of the preparations was established by t.l.c., with sugar (light petroleum, b.p. 20–40°, plus 2% n-propanol), aluminium oxide G (light petroleum, acetone, n-propanol 60 : 40 : 3), and magnesia[1,2]–Celite 1 : 1, plus 13% plaster of Paris (light petroleum, acetone, n-propanol, 20 : 80 : 5). In all systems, diadinoxanthin was adsorbed between zeaxanthin and its diepoxide, violaxanthin.

Diadinoxanthin preparations from the two sources exhibited identical visible absorption spectra, λ_{max}(EtOH) 424 (sh), 448, and 478 mμ, and similar melting points, 158—162°, which varied with the rate of heating. Upon melting or with acetic acid in ethanol,[5] the xanthophyll preparations rearranged to the same furanoid form (II), λ_{max} (EtOH) 408·5 (sh), 430·5 and 458 mμ, the spectral shift, 19—20 mμ, corresponding to the isomerization of one epoxy-group.[4] I.r. maxima (KBr plates) were at 3400 (associated OH), 2962 (Me), 2936 (–CH_2), 1457 (ring –CH_2 and Me), 1380 + 1361 (>CMe_2), 1050 (sec.-OH), 962 (*trans* –CH=CH–), 1570 (conjugated –C=C), 1122, 1031, 835 (CHR=CR_2), and 705 cm.$^{-1}$. A maximum at 2175 cm.$^{-1}$, small in the spectra of the diacetate and the rearrangement product, and barely detectable in the spectrum of diadinoxanthin, could be attributed to an acetylenic group. There were no maxima characteristic of allene, C=O, ester, ether, or furanoid linkages. N.m.r. (C_5D_5N, Varian HA-100) indicated 10 methyl groups, with chemical shifts (p.p.m. downfield from hexamethylsiloxane) 0·98 (methyl attached to 1′-position), 1·12 (at positions 1,1′,5′), 1·20 (at 1), 1·85 (at 5,13,13′,9′) 1·95 (at 9) and relative intensities 1 : 3 : 1 : 4 : 1. N.m.r. also indicated an allylic methylene group, δ 2·38 (J = 5·5 Hz). These tentative assignments followed from our measurements on various carotenoids and from observations on alloxanthin.[6] Precision mass measurements (AEI MS902) of diadinoxanthin (both sources) and its rearrangement product indicated a formula $C_{40}H_{54}O_3$. The fragmentation pattern showed peaks at M 582, M − 2 (loss of H_2), M − 15 (loss of Me),[7] M − 18 (H_2O), M − 33 (Me + H_2O), M − 80 (concerted loss of C_6H_8),[8] M − 92 (toluene),[7] M − 95 (Me + C_6H_8), M − 107 (Me + toluene), and M − 146, as well as very pronounced peaks at mass numbers 181 and 221,







attributed to the pyrylium and homopyrylium[8] type ions, (IV) and (V).

The data presented above suggest formula (I) for diadinoxanthin, *i.e.*, a structure containing end groups of the violaxanthin and the alloxanthin type. Diadinoxanthin then would be the epoxide of diatoxanthin[6] (III), with which it is associated in some diatoms.[1]

Acetylation and i.r. of the xanthophyll and t.l.c. of the products given by the furanoid form upon treatment with methanolic HCl establish the presence of two hydroxy-groups, neither of which is tertiary, enolic, or allylic. The pyrylium ions indicate one hydroxy-group on each end of the molecule,[8] probably in the 3 and 3′ positions as in most natural xanthophylls.

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