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SHORT COMMUNICATION

MOLECULAR WEIGHTS AND EMPIRICAL FORMULAS OF THE XANTHOPHYLLS OF *VAUCHERIA**

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Abstract—The xanthophylls of *Vaucheria* were heteroxanthin, $C_{40}H_{56}O_4$; a partial ester or esters of vaucheriaxanthin, $C_{40}H_{56}O_5$; a dihydroxy carotenoid, $C_{40}H_{54}O_2$; and diadinoxanthin or a similar isomer, $C_{40}H_{54}O_3$.

CHROMATOGRAPHY and spectral absorption curves of the pigments from twelve species of the Heterokontae (or Xanthophyceae) revealed the same three principal xanthophylls, in addition to chlorophyll *a* and β-carotene. The algal species, collected or cultured in California about 20 years ago, were from the families Pleurochloridaceae, Vaucheriaceae, Monociliaceae and Tribonemataceae.[1] These chromatographic and spectroscopic observations were later confirmed by several investigators.[2–5] Additionally, the three xanthophylls from nine species, but not including *Vaucheria*, were reported to isomerize with acid, as typical of many epoxides.[2] The xanthophyll most strongly-adsorbed on sugar columns was, therefore, considered to be similar or identical to trollixanthin, an epoxy-trihydroxy carotenoid, and the two less-sorbed xanthophylls were reported to be antheraxanthin (zeaxanthin monoepoxide) and lutein epoxide.[2] The principal xanthophylls from *Vaucheria* and the closely-related *Botrydium* have recently been reported as a strongly-sorbed carotenoid that did not isomerize with acid, a partial ester of a new xanthophyll called vaucheriaxanthin, and antheraxanthin.[6]

Because of the difficulties in separating and identifying closely related xanthophylls, such as diadinoxanthin, antheraxanthin and lutein epoxide, especially by chromatography plus absorption properties in the visible region of the spectrum,[7] we have re-examined the xanthophylls of *Vaucheria* sp. The results confirm the presence of three xanthophylls[1–5] and a partial ester of vaucheriaxanthin,[6] but they do not support the chemical identifications.[2, 6]

The *Vaucheria* was from a natural stand collected near Argonne at the end of the season after thin ice had formed on the water. The plants were washed thoroughly in running water, scalded with boiling water, and the pigments were extracted with methanol plus light petroleum. The pigments were transferred to light petroleum and separated on large columns

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[1] H. H. STRAIN, *Annual Priestley Lectures* **32**, 51 (1958).
[2] D. M. THOMAS and T. W. GOODWIN, *J. Phycol.* **1**, 118 (1965).
[3] K. R. MATTOX and J. P. WILLIAMS, *J. Phycol.* **1**, 191 (1965).
[4] Z. SESTAK, *Preslia* **35**, 123 (1965).
[5] M. B. ALLEN, L. FRIES, T. W. GOODWIN and D. M. THOMAS, *J. Gen. Microbiol.* **34**, 259 (1964).
[6] H. KLEINIG and K. EGGER, *Z. Naturforsch.* **22b**, 868 (1967).
[7] K. AITZETMÜLLER, W. A. SVEC, J. J. KATZ and H. H. STRAIN, *Chem. Commun.* 32 (1968).

of powdered sugar.[8] The principal pigments formed zones corresponding to those described before.[1,3] A smaller zone of a xanthophyll partial ester[6] was also observed. Each pigment was eluted separately, purified by rechromatography on magnesia plus Celite,[1] eluted, and crystallized from diethyl ether by the addition of light petroleum. Molecular weights were determined with a precision mass spectrometer (AEI MS902).

The most strongly-adsorbed xanthophyll in the sugar columns had mol. wt. 600; formula $C_{40}H_{56}O_4$. The λ_{max} (EtOH) 445 and 474 nm were not altered by acetic acid in ethanol;[9] hence, although the λ_{max} were close to the earliest reported values of 447 and 476 nm[1] this xanthophyll is not identical with trollixanthin. This observation does not preclude the possible occurrence of an epoxy group whose rearrangement is blocked by other structural features (as in fucoxanthin, for example). The i.r. spectrum tentatively indicates an acetylenic triple bond, and secondary —OH, but no allene, tertiary —OH, C=O or ester groups. Comparative thin-layer chromatography indicates 3 secondary —OH groups plus an epoxy group, or 4 secondary —OH. We propose to call this pigment heteroxanthin.

The partial ester or mixture of esters from the second zone exhibited peaks at mol. wt. 794 (v. weak), 784 (strong), 766 (v. strong, but possibly 784-H_2O); λ_{max} (EtOH) 441, 469 nm. It yielded a furanoid form, with acetic acid in ethanol, λ_{max} (EtOH) 425, 453 nm. With methanolic KOH, the ester was saponified yielding a xanthophyll with mol. wt. 616; formula $C_{40}H_{56}O_5$; λ_{max} (EtOH) 441, 469 nm. With acetic acid in ethanol, this xanthophyll yielded a furanoid, λ_{max} (EtOH) 425, 453 nm. These spectral and isomerization properties of the saponified pigment correspond to those reported for vaucheriaxanthin.[6] Tentatively, the i.r spectrum indicates an allene group and both secondary and tertiary —OH groups.

The xanthophyll from the third zone exhibited mol. wt. 566; formula $C_{40}H_{54}O_2$. The λ_{max} (EtOH) 445, 473 nm were identical with the earliest values of 445 and 473 nm.[1] This pigment did not yield a furanoid form with acetic acid in ethanol; hence empirically, it may be a dihydroxy acetylenic carotenoid, several of which have been reported.[10]

The least-adsorbed xanthophyll had mol. wt. 582; formula $C_{40}H_{54}O_3$; λ_{max} (EtOH) 447, 475 nm were close to the earliest values 447·5 and 477·5.[1] It formed a furanoid, λ_{max} (EtOH) 427, 453 nm. The i.r. absorption curve was very similar to that of diadinoxanthin. Empirically this pigment is isomeric (or identical) with diadinoxanthin.[7] It cannot be an epoxide of the xanthophyll mol. wt. 566, because it has λ_{max} at longer wavelengths than this compound. It cannot be an epoxide of lutein or zeaxanthin; formulas $C_{40}H_{56}O_3$; mol. wt. 584. This *Vaucheria* pigment, "582", with three oxygen atoms, is less-sorbed on sugar than the "566" pigment, with two oxygen atoms, but on magnesia, the sequence is reversed.[1]

The principal xanthophylls of *Vaucheria* cannot be trollixanthin,[2] zeaxanthin monoepoxide[2,6] or lutein epoxide.[2] The clarification of the molecular structures of all the *Vaucheria* xanthophylls must await the preparation of larger quantities and the determination of additional properties.

[8] H. H. STRAIN and W. A. SVEC, *Advan. Chromatog.* in press.
[9] H. H. STRAIN, *Arch. Biochem. Biophys.* **48**, 458 (1958).
[10] A. K. MALLAMS, E. S. WAIGHT, B. C. L. WEEDON, D. J. CHAPMAN, F. T. HAXO, T. W. GOODWIN and D. M. THOMAS, *Chem. Commun.* 301 (1967).