Phytochemistry, 1973, Vol. 12, pp. 2481 to 2482. Pergamon Press. Printed in England.

β,β-CAROTENE-2,3,3′-TRIOL: A NEW CAROTENOID IN *ANACYSTIS NIDULANS**

ROBERT L. SMALLIDGE and FORREST W. QUACKENBUSH
Department of Biochemistry, Purdue University, West Lafayette, IN 47907, U.S.A.

(*Received* 28 *March* 1973. *Accepted* 19 *April* 1973)

Key Word Index—*Anacystis nidulans*; Cyanophyta; blue-green algae; carotenoids; β,β-carotene-2,3,3′-triol; 2,3,3′-trihydroxy-β-carotene.

PRELIMINARY examination of the carotenoid pigments of *Anacystis nidulans* (Kratz and Allen, Culture Collection of Algae, Dept. of Botany, Indiana University No. 625) showed the presence of β-carotene, zeaxanthin and some unidentified xanthophylls.[1] Further examination of the most polar of these xanthophylls has revealed other properties that distinguish it from any presently known pigment.

Isolation

Cultures grown 2–4 days at 40° under aeration in bright incandescent light were harvested by centrifugation and extracted repeatedly with acetone. After removal of solvent under reduced pressure, the residue, either with or without saponification, was chromatographed on magnesia. Acetone–hexane eluted the carotene fractions and acetone–hexane–ethanol separated the more polar pigment from the other xanthophylls. This pigment, after re-chromatographing on magnesia and on Microcel-*C* was crystallized from $CHCl_3$–MeOH (1:3). It constituted about 5% of the total carotenoids present in the alga. Crystalline or freshly chromatographed samples were used for physical and chemical tests.

Properties of the New Xantliophyll

Chromatogtaphy of the unknown pigment (I) on kieselguhr-impregnated paper (S & S. 287)[2] with acetone-hexane (1:19) gave R_f 0·10; zeaxanthin, 0·25; lutein, 0·29. TLC of I on calcium hydroxide–silica gel G (1:1) gave R_f 0·10; zeaxanthin 0·28. Absorption maxima in EtOH; I, 450, 478 nm; zeaxanthin, 451, 478 nm; lutein 446, 474 nm. In EtOH and other solvents the topography of visible absorption curve of I was indistinguishable from that of zeaxanthin. IR in dil. CCl_4: I showed maximum at 3400 cm^{-1}, zeaxanthin no maximum although both had maxima at 3620 cm^{-1}; this is suggestive of vicinal hydroxy groups. IR of I in KBr showed no absorption at 2167 or 1923 cm^{-1} indicative of absence of acetylenic and allenic structures. Chemical treatments: I gave no reaction on oxidation with Chloranil–I_2, no reaction on dehydration with HCl–$CHCl_3$ or methylation with dry HCl in $CHCl_3$–MeOH, in contrast to positive reactions with lutein. $LiAlH_4$ treatment did not increase polarity. $CuSO_4$–acetone treatment[3] produced the less polar acetonide. Molecular ions

* Journal Paper No. 5078 AES, Purdue University.

[1] KARUNAKARAN, M. E. (1968) Ph.D. Thesis, Purdue Univ., *Diss. Anstr.* B 1968 29 (3) 869, *Univ. Microfilms*, Ann Arbor, Michigan 68–12, 572.
[2] JENSEN, A. and LIAAEN-JENSEN, S. (1959) *Acta Chem. Scand.* **13**, 1863.
[3] MCCLOSKEY, J. A. and MCCLELLAND, M. (1965) *J. Am. Chem. Soc.* **87**, 5090.

by MS: original cpd (I) *m/e* 584 (zeaxanthin and lutein, 568); mass characteristics of the various ions were consistent with those from compounds with 3-hydroxy-β-end groups, and with those predictable for 2,3-dihydroxy-β-end groups. Acetonide (II) *m/e*, 624. These data are consistent, in all respects, with the proposed structure, β,β-carotene-2,3,3′-triol. Our new compound extends knowledge of the family of cyclic 2-hydroxy carotenes, the first of which were obtained recently from a green alga.[4]

[4] KJOESEN, H., ARPIN, N. and LIAAEN-JENSEN, S. (1972) *Acta Chem. Scand.* **26**, 3053.