the conclusions arrived at for the resolution of the [Co(C$_2$O$_4$)$_3$]$^{\equiv}$ ion must be altered to include this trigonal prism as well. It is obvious also that it is not possible to distinguish between these two configurations on the basis of chemical methods alone.

[illegible] and McReynolds, This Journal, **65**, 333 (1943).

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[Contribution from the Carnegie Institution of Washington, Division of Plant Biology]

A Unique Polyene Pigment of the Marine Diatom *Navicula Torquatum*

By Harold H. Strain and Winston M. Manning

Among the pigments of the colonial pennate diatom *Navicula torquatum*, we have discovered a hitherto undescribed, yellow, polyene compound. This new pigment exhibits properties characteristic of the polyene hydrocarbons or carotenes. Isomerization reactions of this pigment indicate that its chromophoric polyene group occurs in the stable or *trans* form. Its spectral absorption properties resemble those of the violaxanthins and of neoxanthin[1]; hence, all these compounds probably contain similar chromophoric structures.

In *Navicula torquatum*, this new carotenoid comprises about one-fourth to one-third of the total carotenes. It was not found in several other diatoms nor in brown algae.

Experimental

Filamentous masses of the diatom *Navicula torquatum* were collected near Half Moon Bay, California, where they were found growing epiphytically on several species of large brown algae, the common kelps of the Pacific Coast. Pigments extracted from the diatoms with alcohol were transferred to petroleum ether and adsorbed upon a column prepared from a mixture of equal parts of Micron Brand magnesium oxide No. 2641 and heat-treated siliceous earth (Filter Aid 501).[1,2] Under these conditions, the new carotenoid formed a lemon-yellow band below the orange band of β-carotene. Between these two pigment zones there appeared a small yellow zone containing an isomer of the new carotenoid. Concentration of the elutriate from the lowest yellow band yielded golden-orange crystals of the new pigment.

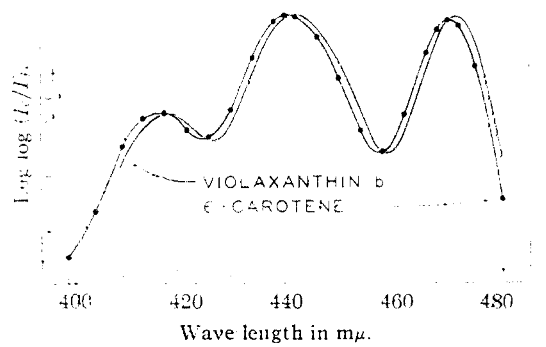

Fig. 1.—Characteristic spectral absorption curve of ε-carotene compared with that of violaxanthin b from leaves[1]; solvent, 95% ethanol.

The pigment from *Navicula* exhibited color reactions typical of polyene compounds. Crystals of this substance were turned deep blue by concentrated sulfuric acid. This acid removed the pigment from solution in chloroform and the acid layer became blue. A solution of the pigment in chloroform was also turned blue by antimony trichloride.

In respect to its solubility, adsorbability and chemical reactions, the pigment from *Navicula* behaved like a polyene hydrocarbon or carotene rather than like an ester or ether of a xanthophyll. It was but slightly soluble in methanol, yielding a pale yellow solution. It was more soluble in ethanol and quite soluble in ether and petroleum ether. Upon partition between petroleum ether and 95% methanol all but traces of the pigment dissolved in the petroleum ether layer. Treatment of the pigment with alcoholic potassium hydroxide did not alter its spectral properties, adsorbability or solubility. An ether solution of the pigment yielded no trace of blue color when treated for a week with an equal volume of concentrated hydrochloric acid. Mixed with α-carotene and adsorbed upon a column of magnesia from solution in petroleum ether, the polyene from *Navicula* formed the lower or least adsorbed band. It moved through the column about three times as fast as the α-carotene. This new pigment was not extractable from solution in petroleum ether with 85% phosphoric acid,[3] whereas all the xanthophylls and xanthophyll esters which were available for examination and which included cryptoxanthin and its *p*-nitrobenzoate were partially or wholly extractable.

When heated in *n*-propanol at 100° for three hours or when treated with iodine dissolved in petroleum ether containing pyridine, the new carotenoid yielded small quantities of a more adsorbed pigment. This product resembled the second yellow pigment observed in the extracts of the diatom. Spectral absorption curves of these pigment preparations exhibited maxima and minima which were less pronounced than those of the unheated pigment and which occurred about 5 mμ nearer the violet region of the spectrum. All these facts indicate that the new carotenoid is the stable isomer of a polyene hydrocarbon, probably a carotene, for which we suggest the provisional name ϵ-carotene.

When dissolved in the same solvents, the *Navicula* carotenoid exhibited spectral absorption maxima at wave lengths almost identical with those reported for violaxanthin b.[1, p. 73] Characteristic spectral absorption curves of these two pigments in ethanol, Fig. 1, are nearly identical in shape. They are also nearly identical in shape with the curve reported for neoxanthin,[1] although the latter is shifted about 4 mμ toward the violet region of the spectrum.

This unusual coincidence in shape of absorption curves which have absorption maxima at different wave lengths appears to be the exception rather than the rule among polyene compounds.[4] It has prompted us to compare the spectral absorption curves of the flavoxanthins of leaves[1] with the spectral curve of a "flavoxanthin-like" polyene pigment of carrots. To this end, the "flavoxanthin-like carotene," provisionally called ζ-carotene, was again prepared from carrots,[5] and its spectral absorption curve was determined in ethanol. Maxima and minima occurred at wave lengths about 22.5 mμ nearer the violet region of the spectrum than those of the flavoxanthins from leaves; yet the shapes of the curves were remarkably similar. An even closer correspondence in shape between the two curves was

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(1) H. H. Strain, "Leaf Xanthophylls," Carnegie Institution of Washington Publication No. 490, 1938.

(2) H. H. Strain, "Chromatographic Adsorption Analysis," Interscience Publishers, Inc., New York, N. Y., 1942.

(3) Haagen-Smit, Jeffreys and Kirchner, *Ind. Eng. Chem., Anal. Ed.*, **15**, 179 (1943).

(4) Hausser, *Z. tech. Physik*, **15**, 10 (1934).

(5) Strain, *J. Biol. Chem.*, **127**, 191 (1939).

found when the values of log log (I_0/I) were plotted against the frequency or reciprocal wave length. Separate isomerization experiments indicated that the "flavoxanthin-like carotene," which apparently is not identical with a similar carotene of yellow corn,[6] contains the stable or *trans* form of the polyene chromophoric group.

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(6) White, Zscheile and Brunson, THIS JOURNAL, **64**, 2603 (1942).

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RECEIVED MAY 10, 1943

COMMUNICATIONS TO THE EDITOR

α,β,γ,δ-TETRAPHENYLCHLORIN

Sir:

In 1939 Rothemund[1] described the preparation of tetraphenylporphin from pyrrole and benzaldehyde and claimed the separation of two isomers which he believed to be due to the two possible positions of the pyrrole acid hydrogen atoms. This work was continued by Aronoff and Calvin[2] in which a more complete separation was achieved by chromatographic adsorption. It was also shown that not only two but probably six different porphyrin-like substances were formed in the reaction.

Recently an examination of their spectra and a comparison with the spectra of naturally and synthetically derived porphyrins and chlorins[3] indicated that the spectra of the first two most abundant materials obtained in the tetraphenylporphin synthesis, called A (most abundant) and B (next most abundant) might be related as porphyrin to chlorin, respectively. If this be so it should be possible to convert B into A by oxidation and A into B by reduction. This we have succeeded in doing in the following manner. The spectrum of the Cu salt was used as identification since the difference there is greater and easier to observe than in the free base.

1. A sample A (several mg.) is dissolved in about 15 cc. of *n*-hexanol. Several crystals of cupric acetate are added and the solution is brought to a boil, cooled, and washed thoroughly with 6 *N* hydrochloric acid and then with water. The hexanol solution (after drying with sodium sulfate) then shows only the spectrum of the Cu salt of A, *i. e.*, a single intense band at 538 m.

2. Another sample (several mg.) of A is dissolved in 15 cc. of *n*-hexanol and several crystals (*ca.* ten-fold excess) of zinc acetate added and the solution brought to a boil. After cooling, the solution is washed with dilute acetic acid and with water and dried with sodium sulfate. This solution now shows the spectrum of the Zn salt of A which seems to consist of three bands in the following order of intensity: 555 mμ, 596 mμ and 518 mμ.

3. The solution of the A Zn salt is now boiled for two minutes and a piece of metallic sodium (5 mm.) dropped in and the boiling continued until all the sodium is dissolved or more sodium is added until the A Zn salt spectrum is completely replaced by a new one consisting of three bands 620 mμ, 600 mμ and 559 mμ. This solution shows a very powerful red fluorescence.

4. The solution from 3 is washed with water, then with 6 *N* hydrochloric acid which removes the Zn and converts the free base into the deep green hydrochloride (in the *n*-hexanol). This is then washed with water until all the hydrochloric acid is removed, leaving a solution of the free base (B) in the *n*-hexanol.

5. The solution from 4 is converted into Cu salt as described in 1 and shows two approximately equally intense bands 536 mμ and 615 mμ which is the spectrum of the B Cu salt. Operations 4 and 5 must be done as nearly in the absence of oxygen as possible.

6. Upon blowing oxygen through the solution obtained in 5 for several minutes and warming, the 615 mμ band disappears and, after a washing with water, the spectrum is that of the pure A Cu salt. The Cu salt of B obtained by chromatographing the original porphyrin preparation has also been oxidized by oxygen to give the A Cu salt.

Thus we have shown that A and B are related by oxidation and reduction and suggest that B is tetraphenylchlorin.

The work is being repeated quantitatively and will be so reported later, together with observations on the intermediate products in the oxidation and reduction.

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RECEIVED OCTOBER 13, 1943

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(1) Rothemund, THIS JOURNAL, **61**, 2912 (1939).

(2) Aronoff and Calvin, *J. Org. Chem.*, **8**, 205 (1943).

(3) Fischer-Orth, "Die Chemie des Pyrrols," Vol. 2, Part 2, 1940, Akademische Verlagsgesellschaft, Leipzig.

ROTENONE IN THE YAM BEAN (*PACHYRRHIZUS EROSUS*)

Sir:

The question of the possible occurrence of rotenone in the yam bean is of particular interest because this plant has recently been suggested[1] as a source of insecticidal material to supplement the usual rotenone-bearing insecticides. The seeds have long been known to be toxic to insects and fish, and Hwang[2] has reported the occurrence of

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(1) Hansberry and Lee, *J. Econ. Entomol.*, **36**, 351 (1943).

(2) Hwang, *Kwangsi Agr.*, **2**, 269 (1941) (in Chinese), summary in *Rev. Applied Entomol.*, **30A**, 418 (1942).