whereupon the T band formed again. This transfer was carried out three more times with the same results. In another experiment the beta-carotene was eluted before its transfer to a new column. The same phenomenon was observed. In a third experiment the beta-carotene solution was introduced on the column and washed with chloroform until the two bands were 30–40 mm apart. The vacuum was released for 10 to 15 minutes. Development was resumed until the main band moved another 40 mm and the vacuum again released. Four separate bands of the T-pigment were produced on the same column by this method. It appears that the conversion goes on continuously if the T pigment is constantly removed.

Part of the chloroform was purified by washing successively with concentrated sulfuric acid, dilute sodium hydroxide and water. The dried (potassium carbonate) and distilled chloroform did not effect a separation. Experiments were carried out to ascertain whether the purified chloroform could regain its separatory power. Irradiation of the solution in an open vessel by a Mazda S–4 mercury vapor lamp from a distance of 22 mm for 30 minutes had no effect. The addition of as little as 0.2 per cent. methanol restored the resolving power of the chloroform. Ethanol appeared less effective; acetone, water or hydrochloric acid were of no value. The addition of the alcohols to skellysolve B did not improve the resolving power of skellysolve B.

Previous workers[1,2] judged similar phenomena to be due to isomerization. However, pigment T does not resemble the beta-carotene isomers[3] reported to form above beta-carotene on the column. The present authors believe that under the conditions encountered beta-carotene may undergo isomerization or some other spontaneous change. The chloroform-methanol solution separates the newly formed compound on the column by shifting the adsorption affinity of the compounds. The authors are not familiar with any previous reports demonstrating such a rapid change without the use of specific activation. Hence these brief observations are reported to facilitate further investigation. Specific experiments to establish the reversibility of this change were not carried out.

Resemblance of the absorption spectrum of the T pigment to a combined absorption curve pigment band C3a[4] separated from the carotene fraction of yellow corn, and beta-carotene is observed. The absorption curves of beta-carotene (dotted line) and of pigment C3a (dashed line) are presented as background in Fig. 1. Chromatographically, pigments C3a and T are identical by mixed adsorption on activated alumina

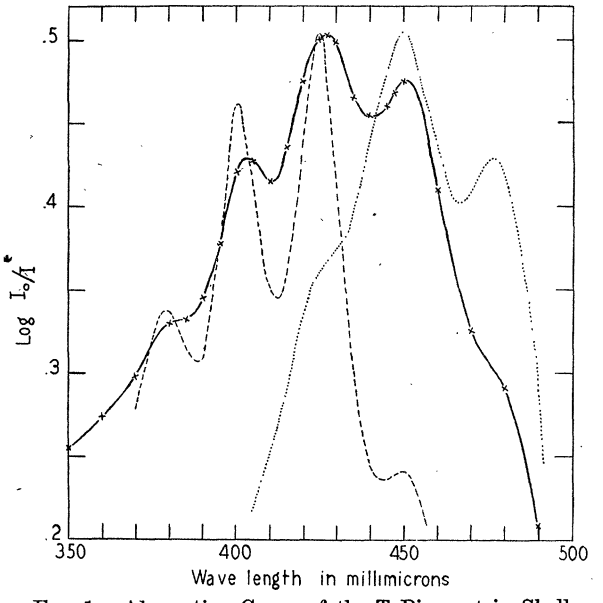

FIG. 1. Absorption Curve of the T Pigment in Skellysolve B. —x—x—x = Pigment T; - - - - - - - - = Pigment C3a; ..... = Beta-Carotene.

with non-treated chloroform. Spectrophotometrically, however, this identity is not confirmed.

Since pigment C3a is derived from the carotene fraction of corn its formation during the life of the corn plant, or during the extraction and saponification process may be postulated, in which case it may represent a final stage, whereas pigment T represents an intermediate one and in the stage of transformation. If pigment T is a mixture of beta-carotene and pigment C3a, a separation should have occurred by the adsorption procedure.

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ON THE OCCURRENCE OF A FLUORESCING POLYENE WITH A CHARACTERISTIC SPECTRUM

IT has been frequently observed by members of our group,[1] as well as by other authors,[2] that when some carotenoid containing plant extracts were

[1] L. Zechmeister and A. Polgár, *Jour. Biol. Chem.*, 140: 1, 1941; L. Zechmeister and R. B. Escue, *Proc. Nat. Acad. Sci.*, 27: 528, 1941; L. Zechmeister and W. A. Schroeder, *Jour. Biol. Chem.*, 144: 315, 1942; A. L. LeRosen and L. Zechmeister, *Arch. Biochem.*, 1: 17, 1942.
[2] *Cf.*, for example, H. H. Strain, "Leaf Xanthophylls," Carnegie Institution of Washington, No. 490 (1938), pp. 41 and 123.

chromatographed, certain sections of the column or the filtrate showed fluorescence in ultraviolet light.

It was now found that some of these faintly colored (or colorless) substances can be separated and accumulated in well-defined zones on a calcium hydroxide column. Among others one of the compounds mentioned which shows an intense, somewhat greenish fluorescence in hexane or alcohol solution possesses a characteristic spectral curve. In hexane three sharp maxima appear in the near ultra-violet region, *viz.*, at 367,.*348* and 332 mμ (Fig. 1). Similar is the extinction curve of the alcohol solution, with sharp maxima at 368, *349* and 332–3 mμ. The

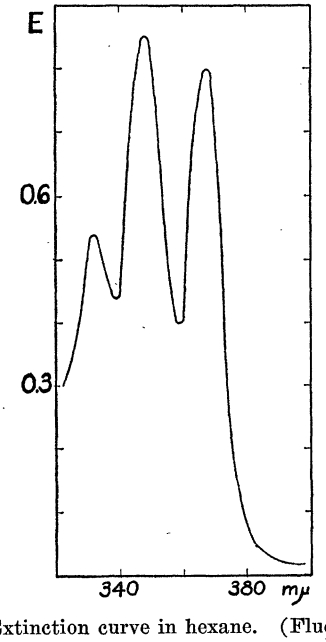

FIG. 1. Extinction curve in hexane. (Fluorescing compound *ex* tomatoes).

highest maximum is located at about 20 mμ longer wave-length than that of vitamin A and differs by a few millimicrons only from that of vitamin A_2.

The curve as represented in Fig. 1 shows a fine structure in the fundamental band, in contrast to the corresponding curves of the two vitamins mentioned. It is, however, very similar to the extinction curve[3] of anhydro vitamin A ("cyclized" vitamin A),[4] the alcoholic solution of which shows maxima at 392, *371* and 351 mμ. Nearly identical in wave-lengths are the maxima of "iso-anhydro vitamin A" (about 370, *350* and 330 mμ)[3] and of our compound.

The compound under discussion is epiphasic when partitioned between hexane and 90 per cent. methanol. In the mixed-chromatogram test it is adsorbed much below the vitamin A, a little below the β-carotene and in the region of the α-carotene zone. The polyene structure of this compound follows from the nature of its spectrum, from the positive Carr-Price reaction and from its behavior toward iodine. When a hexane solution is catalyzed with iodine and illuminated at room temperature, a subsequent chromatogram shows substantial amounts of isomerization products. Much less is the extent of spontaneous isomerization. The isomers form well-defined zones below the unchanged portion on the Tswett column which fluoresce in ultraviolet light.

Our compound seems to be widespread in nature, since its extinction curve was frequently observed with extract fractions of very different starting materials. It was first obtained when some flowers, belonging to various families, were investigated, *viz.*, *Bignonia sp.*, *Tecomaria capensis*, *Gelsemium sempervirens* and *Gazania rigens*. The green leaves of wild oat (*Avena sp.*) tested contained only a very small quantity of the compound. The latter could possibly play some part in our nutrition, since it occurs in relatively marked amounts in carrots as well as in fresh or canned tomatoes, and in orange pulp and peel.

To the authors' knowledge well-characterized polyenes of the indicated type have hitherto not been found in the vegetable kingdom. However, it is possible that a fraction of our vitamin A supply may originate from certain plant products whose molecules contain a much shorter conjugated system than the carotene chromophore.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A 100 KV ELECTRON MICROSCOPE

IN the first few years of electron microscopy, development and research was entirely carried out by university laboratories. Soon, however, the commercial value of this new instrument was discovered, and the industrial development started, first abroad and then

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