

[CONTRIBUTION FROM THE PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION AND THE BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE]

The Carotenoids of Yellow Corn Grain¹

BY JONATHAN W. WHITE, JR., F. P. ZSCHEILE AND ARTHUR M. BRUNSON

It has only recently been recognized that the following carotenoids, other than "carotene" and "xanthophyll," often occur in the grain of yellow corn (*Zea mays* L.): zeaxanthol,² cryptoxanthol³ β -carotene, α -carotene, K carotene and neocryptoxanthol.⁴ It has been shown that the isomerization products neo- β -carotene,^{5,6} neocryptoxanthol,⁶ and neozeaxanthols A, B and C^{7,8} are present under ordinary conditions in solutions containing β -carotene, cryptoxanthol, and zeaxanthol, respectively. Nagy⁹ recently studied the carotenoid pigments of corn gluten meal and noted the formation of numerous isomeric forms, particularly of zeaxanthol, under acid conditions. The degree of isomerization of carotenoids during and after extraction from natural sources will depend upon experimental conditions. In quantitative analysis of corn grain pigments, Fraps and Kemmerer⁴ considered the presence of neocryptoxanthol. Beadle and Zscheile¹⁰ showed the necessity for inclusion of neo- β -carotene in the analysis of vegetable carotenoids.

In order to analyze solutions containing these neo-type pigments by spectroscopic methods, it is necessary that their identity and quantitative absorption spectra be known. Data given in this paper form the foundation for such a system of analysis which will be presented later.

During an investigation of the carotenoids of corn grain, several pigments were separated that had not previously been observed there.

Luteol.—Chromatography from ether on MgO-Supercel¹¹ of the saponified carotenol fraction of yellow corn showed in every case the presence of a zone immediately below the zeaxanthol zone. An ethanolic solution of the

pigment from this zone had a characteristic absorption spectrum identical with that of luteol. Absorption spectra were determined on a photoelectric spectrophotometer previously employed for pigment studies.^{10,11,12} The only other known carotenoid (except luteol esters) with an absorption spectrum of this type is α -carotene. When α -carotene and this pigment from corn grain were mixed in ether and adsorbed on magnesia, two zones were formed, indicating that the corn pigment was not α -carotene. From the above considerations it is concluded that this pigment is luteol.

Unnamed Carotene 1.—A spectrophotometric study was made of the carotene fraction of corn carotenoids, prepared from an extract by partition between hexane and 78.5% diacetone alcohol [which removed the carotenols, except cryptoxanthol] followed by partition between hexane and 92% 2-methyl-2,4-pentanediol as recommended by White and Zscheile.¹³ In addition to other deviations from the absorption spectra of the pure pigments believed to be present, it was noted that a shelf [in some preparations a maximum] was present at 4250 Å. Chromatography on magnesia (three times), followed successively by adsorption on calcium hydroxide and on Brockmann's alumina gave a product with the absorption spectrum shown in Fig. 1 as curve I. A short heating of the¹⁴ solution had no effect on the spectrum. The quantity of the compound was too small to permit the establishment of quantitative values for absorption coefficients or to permit chemical analysis. Strain¹⁵ described a carotenoid from carrots with absorption maxima at 4250 and 4000 Å. A preparation of this carotenoid was made from carrots following Strain's directions. Its absorption spectrum had maxima at 4250, 4000, 3790, and 3600 Å. and a pronounced minimum at 4150 Å.

γ -Carotene.—Investigation of the cause in corn carotene fractions of relatively high absorption on the long wave length side of the 4780 Å. maximum of β -carotene resulted in the separation of a pigment with absorption maxima [4900, 4600, and 4300 Å. in hexane] in good agreement with those of γ -carotene.¹⁶ It was adsorbed from hexane solution on magnesia above β -carotene. Quantities were too small to permit the most accurate determination of absorption values. The characteristic spectrum in hexane solution is shown in Fig. 1 as curve II.

Carotenoid with Properties Expected of a Monohydroxy- α -carotene.—The cryptoxanthol fraction of several inbred lines of yellow corn was found to produce from four to eight distinct zones on a magnesia adsorption column when absorbed from hexane and washed with ether.

(1) Studies on the Carotenoids. IV. Journal Paper Number 34 of the Purdue University Agricultural Experiment Station.

(2) P. Karrer, H. Salomon and H. Wehrli, *Helv. Chim. Acta*, **12**, 790 (1929).

(3) R. Kuhn and C. Grundmann, *Ber.*, **67**, 593 (1934).

(4) G. S. Fraps and A. R. Kemmerer, *Ind. Eng. Chem., Anal. Ed.*, **13**, 806 (1941).

(5) A. E. Gillam and M. S. El Ridi, *Biochem. J.*, **30**, 1735 (1936).

(6) L. Zechmeister and P. Tuzson, *ibid.*, **32**, 1305 (1938).

(7) L. Zechmeister and P. Tuzson, *Ber.*, **72**, 1340 (1939).

(8) L. Zechmeister, L. v. Cholnoky and A. Polgar, *ibid.*, **72**, 1678 (1939).

(9) D. Nagy, *Iowa State Coll. J. Sci.*, **15**, 89 (1940).

(10) B. W. Beadle and F. P. Zscheile, *J. Biol. Chem.*, **144**, 21 (1942).

(11) F. P. Zscheile, J. W. White, Jr., B. W. Beadle, and J. R. Roach, *Plant Physiol.*, **17**, 331 (1942).

(12) F. P. Zscheile and C. L. Comar, *Bot. Gaz.*, **102**, 463 (1941).

(13) J. W. White, Jr., and F. P. Zscheile, *THIS JOURNAL*, **64**, 1440 (1942).

(14) T. R. Hogness and V. R. Potter, *Ann. Rev. Biochem.*, **10**, 509 (1941).

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

(16) R. Kuhn and H. Brockmann, *Ber.*, **66**, 407 (1933).

In all cases but one, the zones other than those due to cryptoxanthol and to small amounts of β -carotene (not removed by the partition process) were too small to be isolated. In the case of inbred M4B¹⁷ a buff zone was found immediately below the cryptoxanthol layer. These two zones were of approximately equal thickness. After

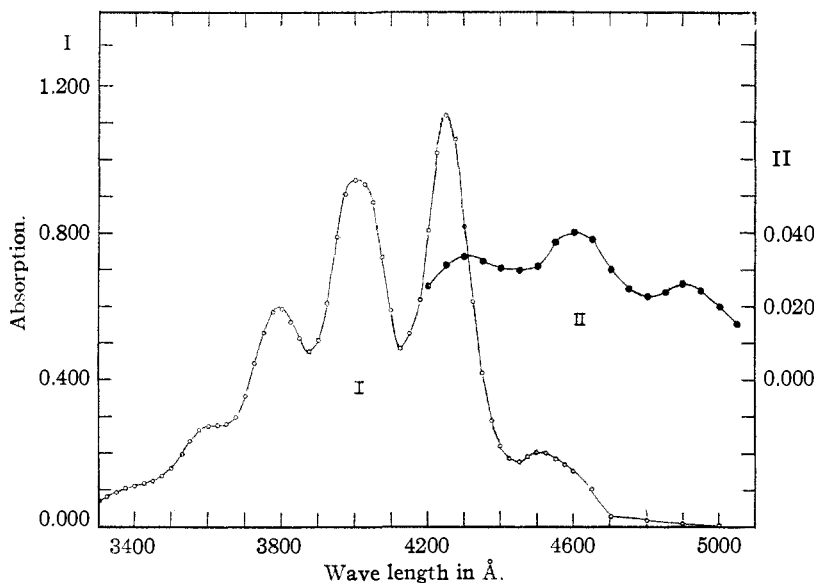


Fig. 1.—Absorption spectra in hexane: I, unnamed carotene 1; II, γ -carotene preparation from corn. Absorption = $\log_{10}(I_0/I)/l$.¹⁴

washing with ether and elution with ethanolic hexane, the characteristic absorption spectrum of the pigment in this lower zone was determined in hexane. It agreed well with that of α -carotene, having maxima at 4740, 4450 and 4250 Å., and a deep minimum at 4625 Å.

Portions of the solution were mixed with solutions of α -carotene in hexane and with luteol in ether. The resulting solutions each produced two zones when adsorbed on magnesia. The same results were obtained after the unknown compound was boiled for twenty minutes with alcoholic alkali, which indicated that it was not an ester of luteol. The mixed chromatograms showed that the compound was neither α -carotene nor luteol. In view of its occurrence in the cryptoxanthol fraction, its position of adsorption immediately below cryptoxanthol (α -carotene is adsorbed immediately below β -carotene), and its non-identity with α -carotene and luteol, it is suggested that the compound may be a monohydroxy- α -carotene. Quantities available were too small for chemical analysis.

Neocryptoxanthol.—A hexane solution of pure cryptoxanthol was heated in an amber glass flask under reflux for twenty-four hours, cooled, and adsorbed on $\text{Ca}(\text{OH})_2$ -Supercel (1:1). After washing with 20% ether in hexane, two zones were found. No oxidation products were evident. The upper one contained cryptoxanthol, the lower neocryptoxanthol. After elution the latter was reabsorbed, eluted, and made to volume. Its absorption spectrum was determined. An aliquot of this neocryptoxan-

(17) The designation of the various inbred lines is purely arbitrary and has no significance as to type of plant or chemical composition.

thol solution was evaporated to dryness *in vacuo*, made to the original volume, and its spectrum redetermined. As shown in Fig. 2, the absorption of a solution of the material after drying decreased over the range from 3800 to 4157 and increased over the range from 4157 to 5000 Å.

It is well known that the change cryptoxanthol-to-neocryptoxanthol is reversible.⁶ The absorption coefficient at 4157 Å. did not change, indicating that the absorption coefficients of cryptoxanthol and of neocryptoxanthol are identical at this wave length. This makes it possible to establish the absorption spectrum of neocryptoxanthol on a quantitative basis.¹⁸ It was necessary that the above procedure be used because the quantities of neocryptoxanthol available were too small to be weighed accurately. Also plotted in Fig. 2 is the characteristic spectrum of cryptoxanthol, recalculated from earlier data,¹⁰ placed to intersect the other two curves at 4157 Å. It will be noted that the curve of the dried preparation falls at a proportional distance between the other two curves over the entire range. Determinations of the intersection point with several preparations (intersection points, 4137, 4157, and 4177 Å. gave the quantitative absorption spectrum of neocryptoxanthol shown in Fig. 3. The maximum deviation from this curve of absorption values of six preparations is $\pm 3\%$. This curve bears a relation to cryptoxanthol which is strikingly similar to that between neo- β - and

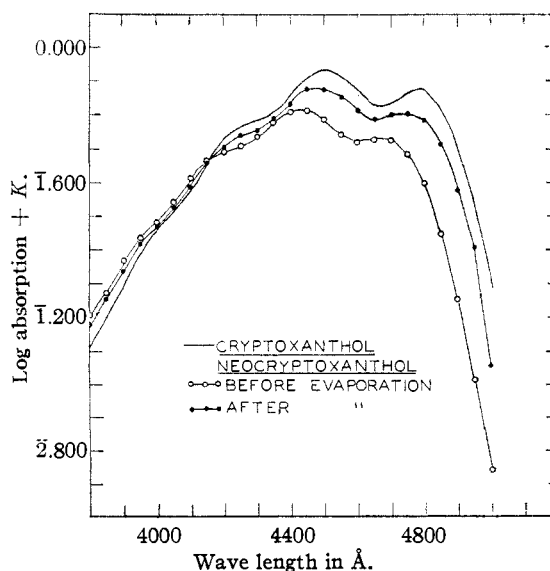


Fig. 2.—Reversion of neocryptoxanthol to cryptoxanthol in hexane solution.

(18) In the case of neo- β -carotene, this method gave results which agreed with data obtained with weighed samples.⁹

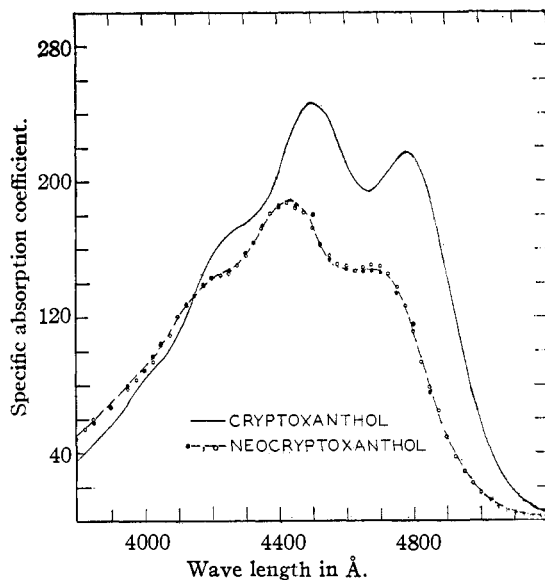


Fig. 3.—Absorption spectra of neocryptoxanthol and cryptoxanthol¹¹ in hexane.

β -carotenes.¹⁰ Table I presents absorption values for two preparations of neocryptoxanthol in hexane solution.

TABLE I
ABSORPTION VALUES OF NEOCRYPTOXANTHOL IN HEXANE SOLUTION

Wave length, Å.	Specific absorption coefficient, liters/g. cm.	
	1	2
4650 Shelf	147	148
4425 Maximum	185	187
4150-4160 Region of intersection	133-135	133-135

Neozeaxanthols.—An ethanol solution of pure zeaxanthol was heated in an amber glass flask under reflux for twenty-four hours, the pigment transferred to a 50% solution of ether in hexane, and adsorbed on Ca(OH)₂-Supercel as before. Three zones were found. Spectra of the eluted pigments were determined, and the pigment from zone 2 was identified as zeaxanthol. The other two curves had maxima shifted toward shorter wave lengths than those of zeaxanthol. Since the relation of these two neo-pigments to neozeaxanthols A, B, and C of Zechmeister and Tuzson^{7,8} was not clear, they were named neozeaxanthol I (more strongly adsorbed) and neozeaxanthol II (less strongly adsorbed). The neo-isomers were purified by further adsorption and attempts were made to apply the intersection-point method used with neocryptoxanthol. A short heating of the solutions was used instead of evaporation to dryness. When compared to the absorption curve before heating, the curve of the solution of neozeaxanthol I after heating decreased over the range 4350 to 5000 Å., increased over the range 3800 to 4200 Å., and did not change over the range 4200 to 4350 Å. Under similar treatment the absorption spectrum of neozeaxanthol II changed as follows: it increased over the range 4407 to 5000 Å., decreased over the range 3800 to 4407 Å., and did not change at 4250 Å. Adsorption showed three compounds in the solution.

In order to obtain a provisional value for the absorption spectra of these two compounds, it was assumed that the isomerization of neozeaxanthol I was to neozeaxanthol II and that of neozeaxanthol II was to zeaxanthol. It is on these assumptions that the absorption spectra of the neozeaxanthols shown in Fig. 4 were determined. These curves are only first approximations.

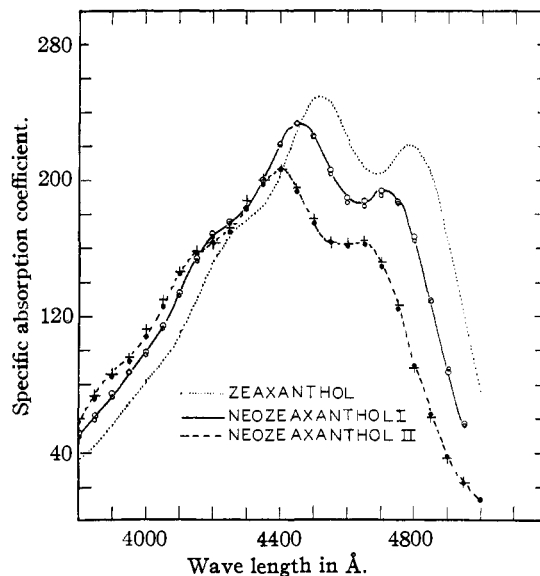


Fig. 4.—Absorption spectra of neozeaxanthols I and II in ethanol; spectrum of zeaxanthol in ethanol from earlier data.¹¹

Discussion

If the method of measurement of pigment concentration used for the determination of the carotenoids of corn is sensitive enough to detect the presence of the neocarotenoids, it is necessary that proper account be taken of their possible presence. Likewise, if the other pigments reported here are present in high enough concentration, they must be considered. Determination of the carotenoids of corn is usually made for estimation of provitamin A potency. From this point of view the provitamin A potency of neocryptoxanthol and of the carotenoid with maxima at 4250 and 4000 Å. must be determined. Fraps and Kemmerer⁴ reported neocryptoxanthol to be nearly as potent as cryptoxanthol.

In the same paper, Fraps and Kemmerer reported the presence in corn of a compound which they call K carotene and to which they assign provitamin A activity. They presented a qualitative absorption spectrum for the pigment with maxima at 4250 and 3970 Å. and a prominent shelf at 4450 to 4500 Å. Strain¹⁵ reported the isolation of a pigment from carrot roots which he termed "flavoxanthin-like carotene." Its spec-

trum had maxima at 4250 and at 4000 Å. He also noted that his product was apparently identical with a pigment isolated by van Stolk, Guilbert, and Penau in 1932. In the spectrum of this compound as separated in this Laboratory, there was no trace of a shelf or maximum at 4500 Å. The spectrum was quite similar to that of unnamed carotene 1 as shown in Fig. 1, except for the absence of the maximum at 4500 Å. This maximum is not due to contamination of unnamed carotene 1 by β -carotene since absorption at 4800 Å. is too low. No preparation of unnamed carotene 1 from corn failed to show a maximum at 4500 Å. Since Fraps and Kemmerer's⁴ curve for K carotene shows neither the 3790 Å. maximum nor the 3600 Å. shelf, it is possible that K carotene and un-named carotene 1 may not be identical.

Fraps and Kemmerer also stated that α -carotene was found in all but one of twenty-two corn varieties. The authors have never observed α -carotene in corn (six inbreds, several sweet corn varieties, and two hybrids were studied). The adsorbent used by Fraps and Kemmerer was capable of separating cryptoxanthol from neocryptoxanthol and might be expected to separate β -carotene from neo- β -carotene. However, they did not report neo- β -carotene in their corn extracts. They identified their α -carotene spectrophotometrically and by mixed chromatography. In this connection, it should be pointed out that

Gillam and El Ridi⁵ were unable to distinguish spectroscopically between α -carotene and neo- β -carotene. They found that α -carotene and neo- β -carotene formed a single zone when adsorbed on alumina from the carotene fraction of butter.¹⁹ Examination of the spectra of α -carotene and neo- β -carotene by a very sensitive photoelectric spectrophotometer^{10,11} shows that they can be differentiated spectroscopically with an instrument of sufficient accuracy. The experience of Gillam and El Ridi illustrates that failure of a mixture of two compounds to form two zones on a given adsorbent does not necessarily indicate that the compounds are identical. The writers consider it possible that the pigment identified by Fraps and Kemmerer as α -carotene might have been neo- β -carotene.

Summary

1. Luteol, γ -carotene and a compound tentatively identified as a hydroxy- α -carotene were found in yellow corn grain for the first time.
2. A compound having some properties like Fraps and Kemmerer's K carotene was also separated from yellow corn grain.
3. Preliminary quantitative absorption spectra of neocryptoxanthol and two neozeaxanthol isomers are presented.

(19) A. E. Gillam and M. S. El Ridi. *Nature*, **136**, 914 (1935).

LAFAYETTE, INDIANA

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[CONTRIBUTION FROM THE GEORGE HERBERT JONES LABORATORY OF THE UNIVERSITY OF CHICAGO]

The Malonic Ester Synthesis and Walden Inversion

BY WILLIAM E. GRIGSBY,¹ JOHN HIND, JACOB CHANLEY AND F. H. WESTHEIMER

It has been suggested recently that the reaction of alkyl halides and olefin oxides with the sodium salt of malonic ester is an example of the normal displacement reaction.^{2,3} If this is the case, the reaction should proceed with Walden inversion about the carbon atom to which the halogen (or oxygen) atom is attached. An experimental demonstration of this inversion has now been established for the reaction of cyclopentene oxide with sodium malonic ester.

In all the previously known reactions of sodium

malonic ester with an oxide—ethylene oxide,⁴ epichlorhydrin,⁴ cyclohexene oxide⁵ and others⁶—the reaction product is a lactone. With ethylene oxide, for example, the product is α -carbethoxy- γ -butyrolactone. If the reaction with cyclopentene oxide had proceeded without inversion, the product would have been the lactone of the mono-

(4) Traube and Lehmann, *Ber.*, **34**, 1971 (1901).

(5) Coffey, *Rec. trav. chim.*, **42**, 387 (1923).

(6) Kötze and Hoffman, *J. prakt. Chem.*, **110**, 101 (1925); Rothstein, *Bull. soc. chim.*, [5] **2**, 1936 (1935); Haller and Blanc, *Compt. rend.*, **142**, 1471 (1906). The reactions recorded in the literature were carried out between equimolar quantities of oxide, malonic ester, and sodium ethylate. However, repetition of the work of Traube and Lehmann, using two moles of malonic ester for each mole of sodium ethylate, showed that, in this case, excess malonic ester is without effect on the composition of the product obtained.

(1) Du Pont Fellow, 1941–1942.

(2) Hind, Dissertation, Chicago, 1939.

(3) Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, Chap. VI, see also p. 143.