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THE PREPARATION AND ABSORPTION SPECTRA OF FIVE PURE CAROTENOID PIGMENTS[1, 2]

F. P. ZSCHEILE, JONATHAN W. WHITE, JR., B. W. BEADLE, AND J. R. ROACH

(WITH FOUR FIGURES)

Introduction

For several years one of the objectives of this laboratory has been the development of a satisfactory spectroscopic method of analysis of simple extracts of biological materials for each of the principal carotenoid pigments. Efforts are being made to devise methods by which several pigments in a single extract may be analyzed with as little manipulation as possible. The degree of simplicity will depend upon the number and nature of the pigments present and the ease of extraction, as well as other factors which may vary according to the material.

The work reported here includes the isolation and purification of five of the more commonly occurring carotenoids, the accurate determination of their absorption spectra, and the effect of variation of the spectral region isolated on the numerical values of the absorption coefficients. These spectra are expected to provide the fundamental constants of a system of quantitative spectroscopic analysis which will include all of these pigments. The pigments studied were alpha-carotene, beta-carotene, cryptoxanthol, luteol, and zeaxanthol. Three carotenol esters were also isolated. Similar studies on other pigments are expected to follow.

The isolation and purification of these two carotenes has been discussed in considerable detail (**12, 15, 17**). The most authoritative and detailed discussions of the carotenols are those of STRAIN (**16**) and ZECHMEISTER (**19**). Comparison shows that in most cases agreement with their results is good; however, a few improvements in preparative technique are reported here.

[1] Studies on the Carotenoids 1: The first of a group of papers on their spectrometry and chemistry.

[2] Joint contribution from the Purdue Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

The usual method for isolation of these pigments has been chromatography of the unsaponifiable fraction of plant extracts followed by repeated crystallization. Recrystallization of the carotenoids is usually accomplished by the addition of a liquid in which the pigment is only slightly soluble to a concentrated solution of the pigment.

Methods

The instability of the carotenoids toward heat, oxygen, and light was recognized. While in solution, the pigments were kept at refrigerator temperature in the dark and under an atmosphere of nitrogen as much as possible. Crystalline preparations were dried at room temperature *in vacuo* and stored during study in an easily accessible vacuum chamber kept in darkness at 0° C. at a pressure of about 15 μ of mercury (measured by a "Tru-Vac" gauge). Pigments were stored for longer periods in evacuated glass tubes in a refrigerator.

Manipulation of solutions was as rapid as possible and in a nitrogen atmosphere whenever feasible. Solutions were never heated above 40° C. Reduction in volume was accomplished by evaporation under reduced pressure. The possibility of alteration of the pigments by residues from cleaning of glassware by acid dichromate solution was eliminated by the use of a trisodium phosphate rinsing solution.

The magnesium oxide (Micron Brand no. 2641, California Chemical Co.) suggested by STRAIN (**15**) was used, diluted by one part of "Hyflo Super Cel" (Johns-Manville). Before use, the columns were flushed by a current of nitrogen. Water jackets were used to keep the column temperature below 10° C.

PURIFICATION OF SOLVENTS

All solvents used were purified as described below, distilled in all-Pyrex apparatus with non-lubricated joints, and stored in darkness.

Petroleum ether. A commercial grade of solvent was exhaustively treated with alkaline potassium permanganate solution and redistilled.

Hexane. The hexane used in this work was obtained from a commercial grade of petroleum ether with a labelled boiling range of 65° to 67° C. This was treated exhaustively with alkaline permanganate and redistilled. Only the portion which distilled between 65° and 67° C. was used.

Ethanol. Commercial 99.5 per cent. ethyl alcohol was refluxed with zinc dust and solid sodium hydroxide for one hour, then distilled.

Carbon disulphide. The "Analytical Reagent" grade was redistilled shortly before use.

Methanol. Commercial synthetic methanol was treated in the same manner as was the ethanol.

Ether. Commercial ether was redistilled after standing for a week over solid sodium hydroxide.

Chloroform. The "Analytical Reagent" grade was redistilled shortly before use.

Purification of pigments

ALPHA-CAROTENE.—The alpha-carotene was obtained from commercial samples of carrot root "crystalline carotene" (Nutritional Research Assoc., Inc.), which consisted of a mixture of alpha- and beta-carotenes, together with various impurities. The preliminary purification was accomplished by the use of chromatographic adsorption on magnesium oxide. The pigment mixture was dissolved in a minimum amount of petroleum ether (40°–60° C.) and adsorbed on the column. After development by thorough washing with petroleum ether, the zones containing alpha- and beta-carotenes were rather clearly defined and separated from the impurities. The column was removed by sections, and the alpha-carotene eluted from the adsorbent with 5 per cent. ethanol in petroleum ether. The ethanol was washed from the petroleum ether and the pigment adsorbed on a second column. This adsorption process was repeated four times, until there was no visible impurity on the column.

The alpha-carotene solution in petroleum ether (34°–40° C.) from the last column was passed through a fine sintered-glass filter to remove dust and other insoluble contaminants and evaporated to dryness. The pigment was taken up in a minimum amount of carbon disulphide, and crystallized by the addition of two volumes of ethanol. The crystals were removed by filtration through a Berlin crucible in an atmosphere of nitrogen. After being washed with a small volume of cold ethanol, the crystals were transferred to a glass capsule and dried for 24 hours. Four crystallizations were necessary to obtain a product having absorption coefficients which remained constant after an additional crystallization.

BETA-CAROTENE.—The beta-carotene was purified from the same source and with the same technique as described above for alpha-carotene. After the fourth crystallization, no increase in the specific absorption coefficients occurred.

ZEAXANTHOL.—This pigment is the principal carotenol of corn grain (**19**). It also occurs in *Capsicum annuum* (**19**), *Physalis alkekengi* (**19**) and in small quantities in leaves (**16**).

Yellow corn grain and two varieties of *Capsicum annuum,* ripe pimento peppers and commercial powdered paprika, were investigated as possible sources by methods similar to those given below. Only traces of zeaxanthol were obtained in these cases; therefore the red calyx of the Physalis plant was examined.

It has been observed in this laboratory that the use of dichloroethane as

a solvent in chromatographic adsorption, as suggested by STRAIN (**16**), resulted in columns that filtered and dried slowly. The zones tended to run together if attempts were made to separate them before the column was dry. This solvent is somewhat difficult to remove *in vacuo* at room temperature. Adsorption of the pigments from it was such that long columns were necessary and 12 to 30 hours were at times needed to complete development of a column. It was observed that diethyl ether was a very good solvent for chromatography of carotenols on magnesium oxide. It has nearly as high solvent powers for the pigments as dichloroethane, is easily purified to the necessary extent, and is readily removed by evaporation *in vacuo*. When ether is used as the solvent, the zones may be separated almost immediately after the level of the ether used for washing reaches the top of the column. The carotenes are not adsorbed from ether; most carotenols are adsorbed from ether to nearly the same extent as are the carotenes from petroleum ether; cryptoxanthol is adsorbed weakly. Ether was used in all subsequent adsorption columns for carotenols. Ethanol or ethanol-ether solutions were used to elute the carotenols. Traces of alcohol in the ether used for chromatography must be avoided.

Physalis calyx (217 gm.) was used in the first experiment. The dry ground material was extracted with low-boiling (34°–40° C.) petroleum ether. The solvent was evaporated under reduced pressure and the residual deep red oil was saponified 3 days at room temperature. A preliminary adsorption yielded 5 zones. The two which were most likely to contain zeaxanthol were combined and readsorbed. A zone was separated which, after another adsorption, yielded 68.6 mg. of crude zeaxanthol, m. p. 194–196° C. Three recrystallizations from chloroform and methanol gave a product that melted at 206.6° C. All melting points were determined on a microscope hot stage described by ZSCHEILE and WHITE (**22**).

A second lot of Physalis (215 gm.) was ground, extracted, and saponified as above. Behavior of the pigments on subsequent adsorption from ether indicated that the saponification was incomplete. After the adsorption column had been thoroughly washed with ether, 3 zones remained adsorbed, the lowest of which continued into the filtrate.

Zone 1 (at the top) was eluted, transferred to sufficient chloroform to make a saturated solution, and 2½ volumes of methanol were added slowly. Zeaxanthol (28 mg.) crystallized in glittering plates that melted at 201.2° C. Two recrystallizations from chloroform-methanol raised the melting point to 206° C.

The pigment from zone 2 was crystallized as above. A yield of 95 mg. was obtained (m. p. 143° C.). Recrystallization did not raise the melting point. The absorption spectrum was of the beta-carotene type. The specific absorption coefficient at 4525 Å in hexane was 171. The specific absorption

coefficient calculated for zeaxanthol monopalmitate is 174. KARRER and SCHLIENTZ (**5**) reported a preparation of zeaxanthol monopalmitate to melt at 148° C. A sample (6.8 mg.) of the pigment was hydrolyzed and about 1.5 mg. of zeaxanthol was obtained (m. p. 206.4° C.). These results indicate a monoester of zeaxanthol.

The pigment from zone 3 was transferred to petroleum ether and readsorbed. The pigment from the uppermost zone of this column was crystallized from chloroform-methanol. A product (704 mg.) that melted at 97.5° C. was obtained. This corresponds to physalien, which ZECHMEISTER reported to melt at 98.5° to 99.5° C. (**19**) and to be zeaxanthol dipalmitate.

Another sample of physalien (twice recrystallized, m. p. 100.6° C., 121 mg.) was saponified in ether at room temperature. After washing with water, the solution was evaporated to dryness under reduced pressure. The residue (48.6 mg.) melted at 206.5° C. The recrystallized product weighed 38 mg., melted at 208.0° C., and its specific absorption coefficient at 4525 Å in ethanol was 232. The substance was dried at 84° C. at a pressure of 15 μ of mercury for 3 hours. This raised the absorption coefficient to 249 and indicated that 6.6 per cent. of the original weight was solvent.

CRYPTOXANTHOL.—This compound is the principal vitamin A precursor in corn grain (**7**).

The cryptoxanthol was isolated from miscellaneous residues from the isolation of zeaxanthol from Physalis described above. These fractions were combined and adsorbed from ether on magnesium oxide. Six zones, including the filtrate, were found. The uppermost was discarded since it probably contained impurities and oxidation products. Zeaxanthol was isolated from the next zone. The third zone was small and was not examined. The fourth zone yielded small amounts of luteol. The fifth zone was not contiguous with the fourth and yielded 10 mg. of cryptoxanthol, which melted at 167.3° C. Recrystallization from chloroform by addition of three volumes of ethanol raised the value to 169° C. The pigment had an absorption spectrum of the beta-carotene type. The sixth zone (filtrate), light in color, was discarded.

During the separation of the zeaxanthol esters previously described, a zone separated below the physalien zone. Attempts to crystallize the pigment at 5°–10° C. produced deep red crystals, which melted to a red oil at room temperature. Saponification of this oil yielded a pigment melting at 167° C. which was identified as cryptoxanthol. The low-melting solid, therefore, may have been a cryptoxanthol ester, in consideration of its position on the adsorption column and its product after saponification. All of the cryptoxanthol preparations obtained were combined and recrystallized three times. Weight 8.6 mg., m. p. 165.6° C.

LUTEOL.—Luteol is the principal carotenol of leaves (**15**). Corn seed-

lings were used as a source for the isolation. The ground tissue, dried below 45° C., was extracted successively with petroleum ether, ether, methanol, and acetone. The pigments were transferred to ether and saponified at room temperature for 48 hours. The ether solution was washed with water and concentrated. Waxy material was removed on a chilled filter.

The carotenol pigments in the ether solution were chromatographically separated from the carotenes. The adsorption process was repeated until

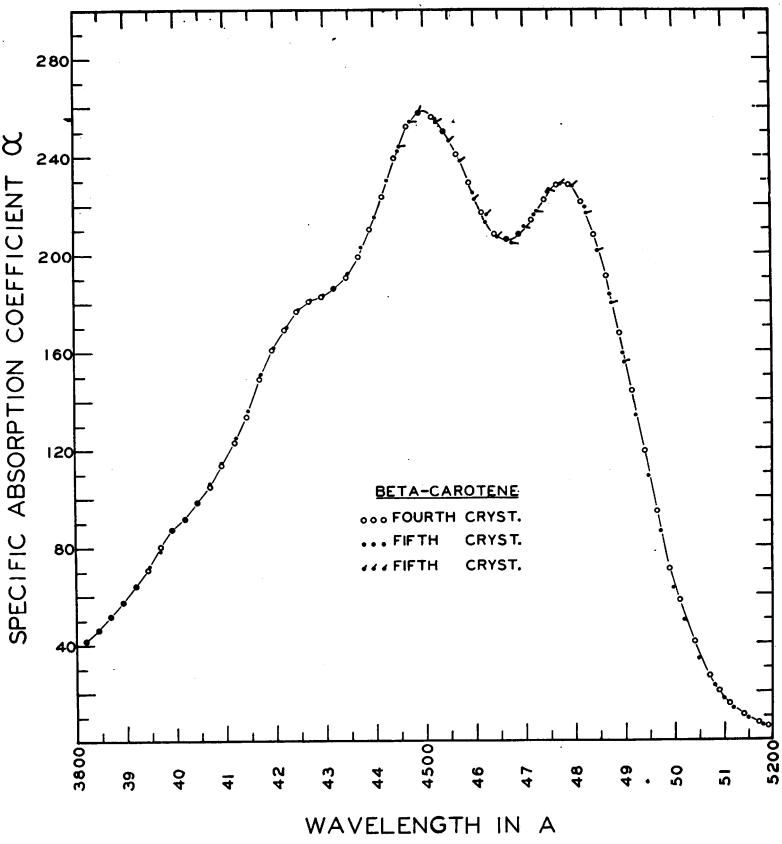

FIG. 1. Absorption spectrum of beta-carotene in hexane solution.

only the luteol band appeared. The eluate was then washed free from ethanol and the remaining ether solution evaporated to dryness. The pigment was taken up in a minimum amount of warm carbon disulphide. To this solution was added about 1 ml. of ethanol for every 10 mg. of luteol present. The solution was warmed and nitrogen was blown onto the surface of the solution to evaporate carbon disulphide until crystals just started to form. At this point the solution was warmed slightly to dissolve all of the

luteol. The solution then remained at 0° C. under nitrogen. After standing 24 hours the crystals were filtered and the product recrystallized three times (m. p. 150.8° C.). Luteol crystallized very easily from chloroform and ethanol but was found to be unstable in this solution, as noted by STRAIN (**16**).

When luteol was crystallized from carbon disulphide-ethanol solution, approximately 10 per cent. of solvent was retained. This is in agreement with STRAIN'S work (**16**). It is necessary to heat luteol at 84° C. for 4 hours under a high vacuum to remove this solvent. Almost identical absorption curves were obtained for a luteol sample heated 4 hours and for one heated 8 hours.

A sample from alfalfa leaves was obtained by chromatography and recrystallization of a commercial "xanthophyll" preparation (American Chlorophyll Co.).

SPECTROSCOPIC METHODS

The absorption spectra of the pigments were determined by means of a new photoelectric spectrophotometer recently employed for similar measurements on the chlorophyll components (**21**). In principle this instrument is similar to that described by HOGNESS, ZSCHEILE, and SIDWELL (**3**). The improved instrument will be described in detail at a later time. It employs a large Müller-Hilger Universal Double Monochromator with crystal quartz optics. A Mazda incandescent filament lamp was the source of radiation. ZSCHEILE and COMAR (**21**) reported the precision error of measurement of $\log_{10}\frac{I_o}{I}$ values by this instrument to be $\pm$ 0.5 per cent. for $\log_{10}\frac{I_o}{I}$ values of 0.200–0.800, with total precision errors, including weight and volumetric technique, not in excess of $\pm$ 0.6 per cent.

For these measurements, all three slits were of uniform width with the exit slit 6 mm. in height. For all pigments except zeaxanthol, the slits were 0.08 to 0.06 mm. in width; they isolated spectral regions of 8.4 to 18 Å over the region studied (3800–5200 Å). The widths of the spectral regions isolated were calculated according to the method of HOGNESS, ZSCHEILE, and SIDWELL (**3**). In the region of maximum absorption, the regions isolated were approximately 12 to 16 Å. The two curves for zeaxanthol were obtained with slits from 0.05 to 0.15 mm. The regions isolated were thus 5 to 44 Å. In the region of maximum absorption, the regions isolated were approximately 10 to 30 Å. Measurements were made at intervals of 20 or 25 Å.

The absorption spectra reported here are expressed in terms of the specific absorption coefficient, α:

$$\alpha = \frac{\log_{10}\frac{I_o}{I}}{cl}$$

I_o = intensity of radiant energy transmitted by solvent-filled cell.
I = intensity of radiant energy transmitted by solution-filled cell.
c = concentration in grams per liter.
l = thickness of solution layer in centimeters.

The concentration is expressed in grams per liter because it is expected that these spectra will be used for quantitative analysis of the pigments. The cell lengths and concentrations were adjusted to keep the $\log_{10} \frac{I_o}{I}$ values between 0.200 and 0.800.

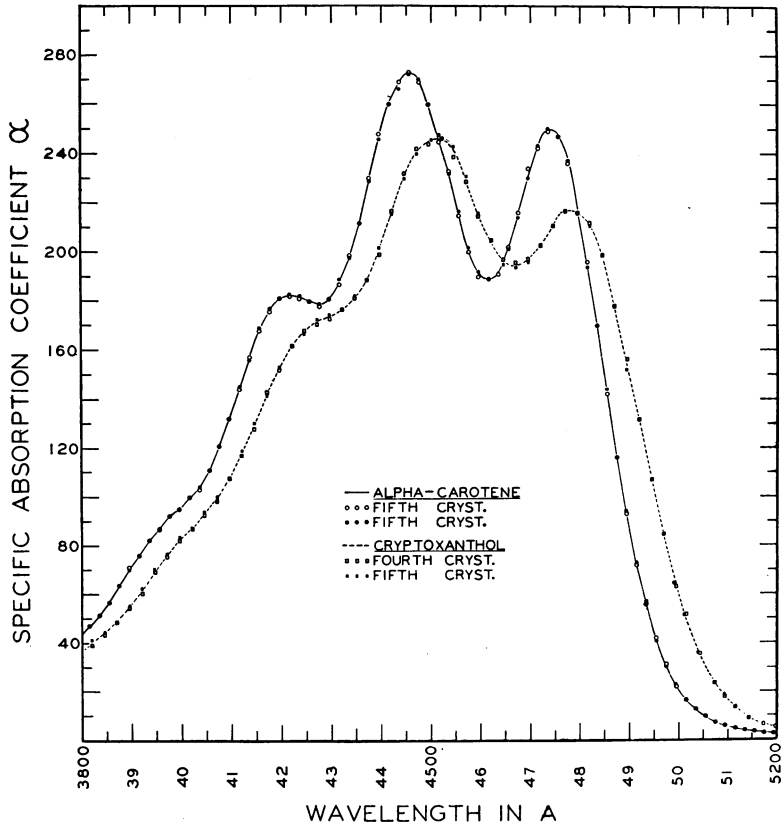

FIG. 2. Absorption spectra of alpha-carotene and cryptoxanthol in hexane solution.

ABSORPTION SPECTRA

Figure 1 presents absorption data for beta-carotene in hexane solution. To illustrate the overall precision of the entire experimental procedure, three entirely separate sets of spectrophotometric observations are presented for three weighed samples from two different crystallizations. Observations as well as weighings were made by two different observers.

In figure 2 are the curves for alpha-carotene and cryptoxanthol in hexane solution. Two sets of data are included for each pigment. Two different operators obtained the data for alpha-carotene.

Similar data for luteol and zeaxanthol in ethanol solution are presented in figure 3. Each set of data for zeaxanthol represents a different crystal-

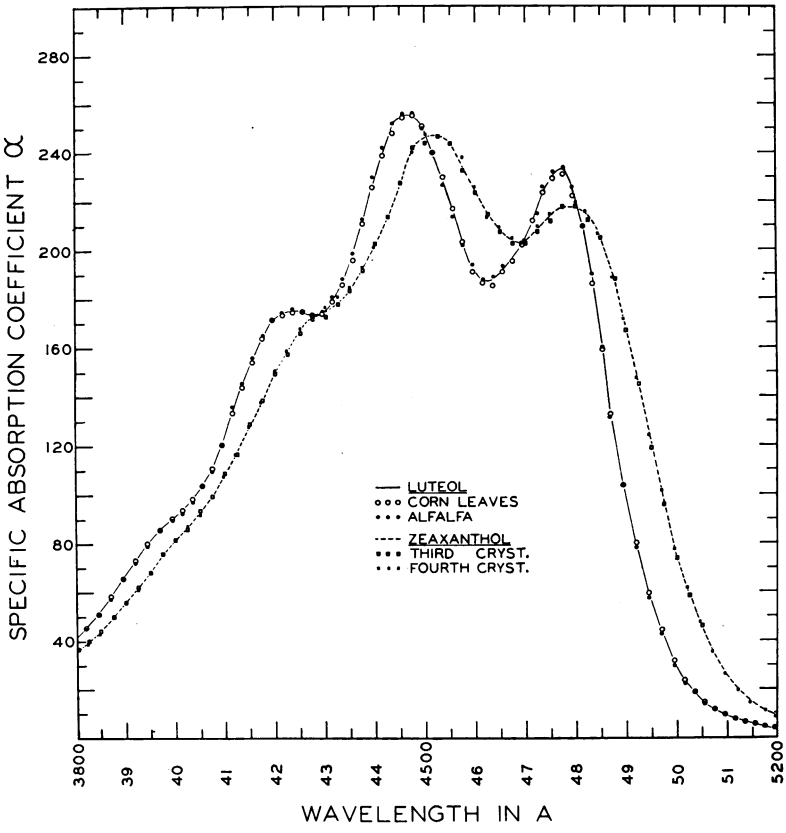

FIG. 3. Absorption spectra of luteol and zeaxanthol in ethanol solution.

lization. In the case of luteol, the samples for each set of data were from a different source of material, corn and alfalfa leaves. These curves are in excellent agreement with the absorption spectrum of luteol isolated from barley leaves as reported by STRAIN (**16**).

Tables I and II present the numerical values of absorption coefficients at maxima and minima.

EFFECT OF SLIT WIDTH AND TESTS FOR SCATTERED RADIATION

A further study was made of the effect of slit width on the absorption values obtained for beta-carotene so that direct comparison could be made

TABLE I

ABSORPTION VALUES OF CAROTENOIDS IN HEXANE SOLUTION

	ALPHA-CAROTENE		BETA-CAROTENE		CRYPTOXANTHOL	
	λ (Å)	α	λ (Å)	α	λ (Å)	α
Maxima	4220	182.0*	.....	.....	.....	.....
	.....	183.0	.....	.....	.....	.....
	4460	273.0	4500	257.5	4515	246.0
	.....	272.0	.....	257.5	.....	246.5
	.....	.....	.....	258.2	.....	.....
	4740	249.0	4780	228.2	4780	216.0
	.....	249.0	.....	228.2	.....	217.0
	.....	.....	.....	228.2	.....	.....
Minima	4280	178.0	.....	.....	.....	.....
	.....	179.0	.....	.....	.....	.....
	4615	189.0	4665	206.0	4670	194.0
	.....	189.0	.....	205.8	.....	196.2
	.....	.....	.....	206.0	.....	.....

* Values reported in tables I and II are for different samples corresponding to the curves shown in figures 1, 2, and 3.

with the results of MILLER (**11**) on this subject. MILLER found that the values of absorption coefficients for beta-carotene were extremely sensitive to slit widths. In figure 4, a comparison is made between MILLER'S results obtained with a single monochromator with glass optics and our results obtained with a double monochromator with quartz optics. Our experiments covered a much wider set of slit conditions than did those of MILLER and strikingly different results were obtained. Our measurements were made when slits varied in width from 0.015 to 0.90 mm. corresponding to a

TABLE II

ABSORPTION VALUES OF CAROTENOIDS IN ETHANOL SOLUTION

	LUTEOL		ZEAXANTHOL	
	λ (Å)	α	λ (Å)	α
Maxima	4230–4250	175.0	.....	.....
	.....	175.8	.....	.....
	4465	255.0	4520	247.0
	.....	256.0	.....	248.0
	4775	231.0	4790	220.0
	.....	234.5	.....	218.0
Minima	4280	173.7	.....	.....
	.....	174.2	.....	.....
	4625	185.7	4690	204.0
	.....	188.0	.....	203.0

spectral region isolated of 3 to 225 Å. Results were those which might be expected from the nature of the absorption curve and from the assumption that no scattered radiation was present from other regions of the spectrum. Absorption values decreased at the maxima and increased at the minima when the spectral region isolated became sufficiently great. These changes were relatively small and dependent upon the average absorption over the

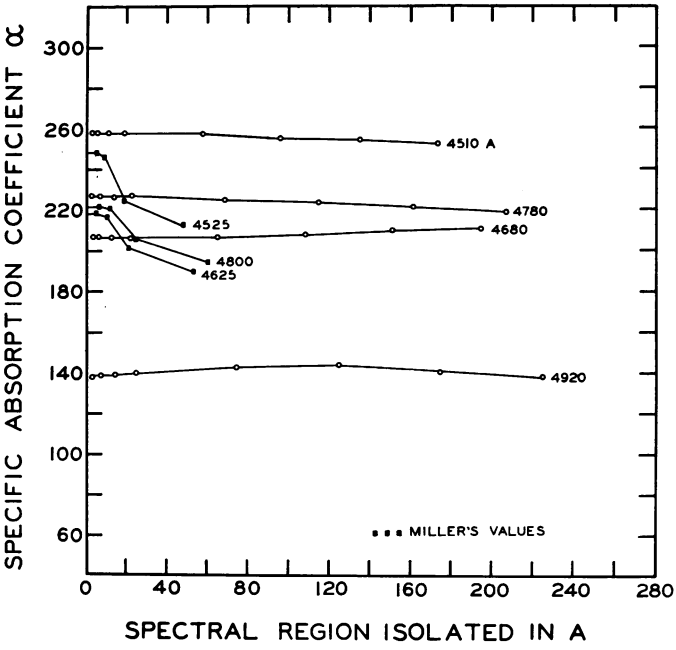

FIG. 4. Effect of width of spectral region isolated on the specific absorption coefficient of beta-carotene at selected wavelengths.

region considered. It is evident from figure 4 that constant absorption values were obtained under the following conditions:

4500 Å	(maximum)	slits	0.015–.30 mm.	spectral region	3–58 Å
4665 Å	(minimum)	"	0.015–.50 mm.	" "	3–108 Å
4780 Å	(maximum)	"	0.015–.30 mm.	" "	3–69 Å
4920 Å	(slope)	"	0.015–.06 mm.	" "	3–14 Å

The data of MILLER are calculated directly from his data for beta-carotene [fig. 5 (**11**)] and from the region isolated as given by him for his monochromator [table III (**11**)].

Since the 4358 Å line from the mercury spectrum is the only well-isolated line in this region which would permit an extensive study of slit widths without inclusion of other lines, it was the only one studied. Absorption coefficients of beta-carotene solutions at 4358Å remained constant over a

slit width range of 0.06–0.50 mm. when the incandescent lamp was replaced by a mercury arc source.

Absorption measurements were made with an incandescent source at 4500, 4665, and 4780 Å when the following glass filters were placed in front of the entrance slit of the monochromator: Jena BG7 (absorbs from 6400 Å to the infra-red limit of the photocell), Corning 385 (absorbs below 3700 Å), Corning 585 (absorbs from 5000 to 6900 Å), and all 3 filters in series. No variation was observed from the values obtained without a filter.

Discussion

The absorption spectra of carotenoids in several solvents were studied before the final choice of solvent was made. The ether-ethanol mixture that has been used considerably as a solvent for carotenoids (**11, 14**) was found to produce less resolution of the principal bands of the carotenes (lower ratios of the maxima to the minima) than was the case for hexane. Moreover, absorption curves for hexane solutions of alpha- and beta-carotenes diverge appreciably more on the slopes toward the red than is the case for ether-ethanol. This is of considerable importance from the analytical viewpoint. Ether-ethanol has the further disadvantage of being a mixed solvent and this factor would make it very difficult to employ in certain types of analytical procedure. The curve of zeaxanthol in ether-ethanol is very close to that reported here for the ethanol solution.

Several hydrocarbon solvents were used for observations of the beta-carotene spectrum. Compared with results in hexane, the maxima for heptane solutions are shifted approximately 25 Å toward the red. With petroleum ether fractions distilling over a range of 20° C., we were unable to obtain reproducible spectroscopic results. This difficulty is attributed to lack of uniformity in the composition of the solvent. CARTER and GILLAM (**1**) have found that the position of the maxima of beta-carotene varies with the boiling range of the petroleum ether solvent. Very minor differences between results in hexane and pure 2,2,4-trimethylpentane ("iso-octane") were found for both alpha- and beta-carotenes. Hexane is an excellent solvent from all viewpoints. It is equal to "iso-octane" from the spectroscopic and analytical viewpoints and has the further advantage of being available at a comparatively low price. We consider it well adapted to routine analytical work.

The numerical values given by MILLER (**11**) for the width of spectral regions isolated at various wavelengths are approximately twice the results that would be calculated by his method from the dispersion data given by HOGNESS, ZSCHEILE, and SIDWELL and by the manufacturer (**3**) for the same type of monochromator. His values (plotted in figure 4) are 1½ times as great as would be obtained if calculated by the method employed here.

It is apparent from the results shown in figure 4 that the double monochromator is far superior, insofar as scattered radiation is concerned, to the single monochromator as employed by MILLER. The presence of scattered radiation from another region of the spectrum would explain his results obtained with different slit widths. The results with the 4358 Å mercury line and with previously filtered incandescent radiation are further evidence

TABLE III

SPECIFIC ABSORPTION COEFFICIENTS AT HIGHEST MAXIMUM

SOLVENT	ALPHA-CAROTENE	BETA-CAROTENE	CRYPTO-XANTHOL	LUTEOL	ZEA-XANTHOL
Hexane	270 (13)* 273 (6) 272	275 (13) 302 (6) 258	246		
20 per cent. ether in ethanol	263 (14) 258 (11) 271 (18)	249 (11) 251 (14) 255 (18)		258 (16, p. 82)	
Carbon disulphide	202 (14) 218 (6)	194 (14) 194 (9) 243 (6)	204 (16, p. 106)	216 (8) 191 (16, p. 79)	223 (8) 200 (13) 185 (16, p. 106)
Chloroform		220 (2)		180 (2) 238 (16, p. 80)	149 (2)
Petroleum ether (b.p. 55–65° C.)	279 (17)	253 (17)			
Ether		208 (4)		248 (4) 260 (16, p. 82)	
Ethanol			247 (16, p. 105)	221 (13) 254 (16, p. 84) 255	249 (16, p. 102) 236 (13) 248

* Most of these values were approximated from graphs in the papers quoted. The values without reference numbers are averages of those presented in this paper.

that scattered radiation is negligible under the conditions of measurement employed in this laboratory.

It is evident that all of the spectra discussed are of one of two types, those possessed by carotenoids of either the alpha-carotene or the beta-carotene type of molecular structure. It should be possible to analyze a mixture quantitatively for each of any two compounds having such different types of spectra. Wavelengths at which their absorption values are widely different may be employed for simultaneous equations, the solution of which

will give both the total concentration and that of each component. Wavelengths at which the absorption curves cross or coincide may be employed for total concentration (**10, 20**). These applications will be developed in later papers. It will be observed that the spectrum of cryptoxanthol is quite similar to that of beta-carotene. The former is lower by a nearly constant factor. The percentage composition of pure mixtures of these two pigments cannot be determined by present spectrophotometric methods, since in no region do the absorption curves separate from each other by more than 5 per cent.

The values of the absorption coefficients at the highest maxima, as obtained by workers in this field, are assembled in table III. These values have been recalculated in terms of specific absorption coefficients to facilitate comparison. It will be seen that the values obtained in this investigation are in fair agreement with the majority of the others.

Summary

1. The following carotenoids were isolated and purified by chromatographic and crystallization methods accompanied by spectroscopic control: alpha-carotene, beta-carotene, cryptoxanthol, luteol, and zeaxanthol.

2. The absorption spectrum of each was determined from 3800 to 5200 Å by a photoelectric spectrophotometric method.

3. Absorption coefficients were found to be relatively insensitive to changes in slit width, except in regions where absorption values change rapidly with wavelength. Experiments with mercury arc radiation and filters also indicate that scattered radiation is negligible.

4. The absorption values given are considered suitable for a spectroscopic basis of analysis for individual carotenoids.

5. These values are compared to those obtained by other workers. Differences due to solvent are discussed.

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PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION
LAFAYETTE, INDIANA

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