[CONTRIBUTION FROM THE CARNEGIE INSTITUTION OF WASHINGTON, DIVISION OF PLANT BIOLOGY]

Carotene. X. A Comparison of Absorption Spectra Measurements on α-Carotene, β-Carotene and Lycopene

BY JAMES H. C. SMITH

In a previous paper, measurements of the absorption spectra of crystalline carotenes obtained from a number of different kinds of leaves have been reported.[1] The absorption curves of the different samples of leaf carotenes were not identical but were shown to approximate the curve for beta-carotene. The variations were attributed either to differences in the structures of the carotenes or to the presence of impurities.

That small amounts of impurities were present in these carotenes has been shown to be true by adsorption experiments. While the presence of these impurities may have accounted partially for the differences observed, a part of the differences there noted should undoubtedly be ascribed to uncertainties inherent in the spectrographic method employed. Because of these uncertainties it was impossible to establish whether or not the absorption spectra of the beta-carotenes from different sources are the same. For this reason and for this purpose a photoelectric spectrophotometer has been constructed which possesses the required precision.

By means of this spectrophotometer the absorption spectra of a number of samples of carefully purified beta-carotene from different kinds of leaves have been measured and have been found to be identical. These absorption spectra also agree with that of beta-carotene prepared from carrot roots by adsorption. The identity of these absorption spectra combined with the identity of other physical properties of these carotenes makes it appear probable that the beta-carotenes examined are structurally the same.

In the previous publication the absorption spectra of alpha-carotene and beta-carotene, dissolved in ethanol, were also reported. These along with the spectrum of lycopene have now been determined in other solvents by means of the more accurate photoelectric spectrophotometric method. It is hoped that a report of the results obtained may aid in establishing the correct spectroscopic constants of these pigments.

The necessity for correct data has been demonstrated many times both in chemical and in physiological investigations. One particular instance, which is of importance, is the specification of the positions of the absorption bands of pure beta-carotene to be used as a standard for vitamin A by the Committee of the Health Organization of the League of Nations on the Standardization of Vitamin Preparations. No recommendation was made as to the method of observation to be used.[2] Since the observed positions of the maxima vary significantly depending on the methods of observation, as will be shown in the experimental part, the Committee should have specified also the methods of measurement.

The values of the absorption coefficients of solutions of these pigments in 20% ether in ethanol (Fig. 1) were found to agree very well with the results recently published by Miller, Mackinney and Zscheile.[3] The only significant differences noted were at the short wave lengths in the case of lycopene and at the maxima of absorption of alpha-carotene. The close agreement of these results with those obtained by the use of a different instrument and of different materials strengthens the confidence that may be placed in these data.

It should be added that the results obtained for beta-carotene are very near those reported by McNicholas[4] for "pure carotin," thus indicating that the carotene sample which he measured approximated pure beta-carotene. The values obtained for lycopene are in essential agreement with the earlier results found by Matlack and Sando.[5]

A comparison of the results found in this investigation with those reported by Smith and Milner[1] shows that the absorption curves of alpha-carotene and beta-carotene in 95% ethanol and in 20% ether in ethanol are approximately the same.

One interesting outcome of the measurements on the absorption coefficients of alpha-carotene and beta-carotene obtained from carrot roots by

(1) James H. C. Smith and H. W. Milner, *J. Biol. Chem.*, **104**, 437 (1934).

(2) Commission report of the Committee of the Health Organization of the League of Nations on Standardization of Vitamin Preparations, *Quart. Bull. of the Health Organization of the League of Nations*, **3**, 483 (1934).

(3) Elmer S. Miller, G. Mackinney and F. P. Zscheile, Jr., *Plant Physiol.*, **10**, 375 (1935).

(4) H. J. McNicholas, *Bur. Standards J. Research*, **7**, 171 (1931).

(5) M. B. Matlack and Charles E. Sando, *J. Biol. Chem.*, **104**, 407 (1934).

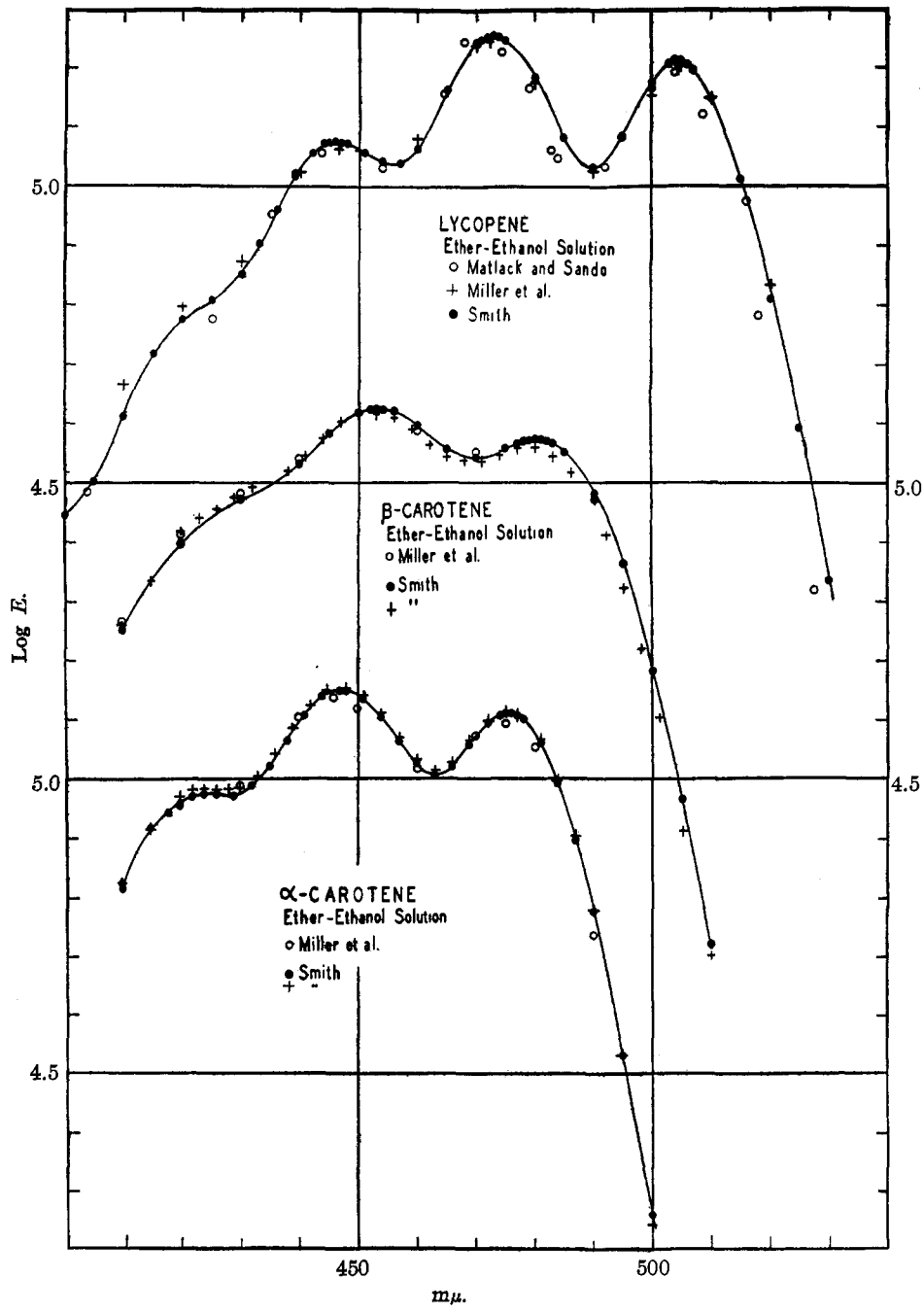

Fig. 1.—Absorption spectra of lycopene, beta-carotene and alpha-carotene. Solvent, 20% ether in ethanol. The values of log E for lycopene and alpha-carotene are shown on the left side of the drawing, those for beta-carotene on the right.

means of adsorption on magnesium oxide, is the progressive shift in the absorption curves brought about by successive crystallizations. In Fig. 1 this is shown for beta-carotene. The curve marked with plus signs was measured on a sample which was eluted from the adsorption column, recovered and crystallized. The final measurements, designated by black circles, were determined on the same sample after it had been recrystallized several times. A sample obtained in an intervening crystallization possessed absorption constants intermediate between those reported. This observation indicates that adsorption alone may not purify a pigment completely.

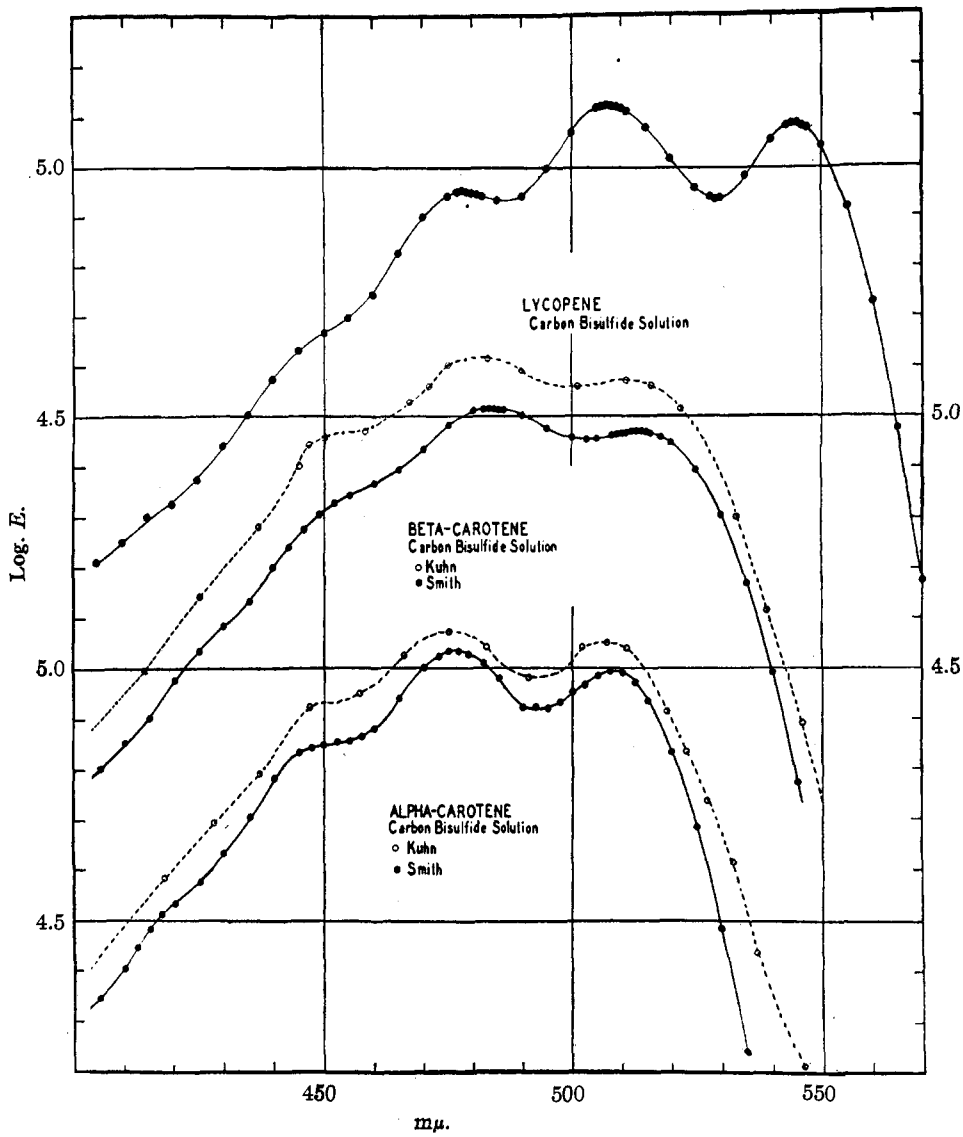

Fig. 2.—Absorption spectra of lycopene, beta-carotene and alpha-carotene. Solvent, carbon bisulfide. The values of log E for lycopene and alpha-carotene are shown on the left side of the drawing, those for beta-carotene on the right.

Measurements of the absorption coefficients of these three pigments in carbon bisulfide solution (Fig. 2) have yielded results which are considerably different from those reported in the literature. The values for alpha-carotene and beta-carotene are approximately 9 and 23% lower, respectively, than the corresponding results reported by Kuhn.[6] The maximum absorption coefficient of lycopene in carbon bisulfide found in these experiments is about 16% higher than that given by Smakula.[7]

(6) Richard Kuhn, "Chemistry at the Centenary (1931) Meeting of the British Association for the Advancement of Science," W. Heffer and Sons, Ltd., Cambridge, England, 1932, p. 110.

(7) Alexander Smakula, *Z. angew. Chem.*, **47**, 657 (1934).

Thus far it has been impossible to find the causes for these differences. The results obtained in this investigation appear to differ by a constant factor from those found by Kuhn because the curves of log E *vs.* wave length show an almost constant displacement throughout their length. It seems improbable that the carotenes especially could contain enough impurities to account for such large differences since the analytical data and other physical properties agree so well. Other causes such as oxidation or adsorption on the walls of the volumetric apparatus used in the preparation of the solutions were shown by separate experiments to be improbable also.

It is very often necessary to compare the spectrophotometric results obtained in different laboratories. For this reason any spectrophotometric apparatus which is to be used for a series of measurements should be described in some detail. Indication as to its reliability should also be given.

Inasmuch as results have already been reported[8,9] and it is anticipated that other results will follow which have been measured by means of this apparatus, a short description of the apparatus seems desirable.

A diagram of the spectrophotometric apparatus is shown in Fig. 3. Light from a 32 c. p. automobile headlight L, collimated by lens l_2, passed through the camera shutter S_1 and was focused by lens l_1 onto the slit O_1 of the predispersion monochromator A (a small Bausch and Lomb wave length spectrometer). The partially monochromatized light passing from slit O_2 was focused onto slit O_3 of monochromator B (Gaertner wave length spectrometer L230). The highly monochromatized beam emerging from slit O_4 passed through either of the absorption cells C_1 or C_2 which could be placed in the light path by means of a sliding carriage. These cells were supported in a light tight container C. The whole beam of light was collected by the photoelectric cell P (General Electric cell PJ-15). The current generated was amplified and measured. Neutral filters of different opacities were mounted at f in order to vary the light intensities entering the monochromator.

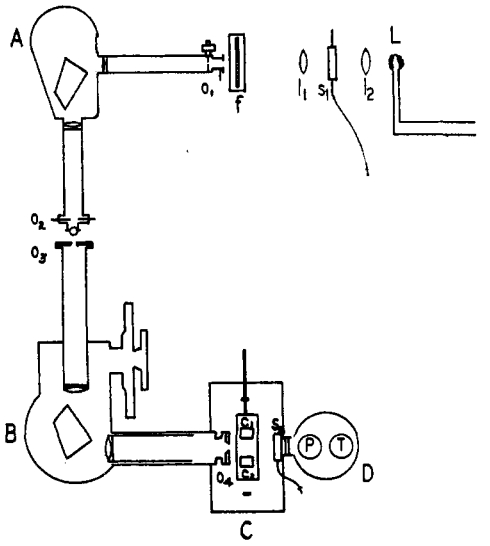

Fig. 3.—Arrangement of spectrophotometric apparatus.

The slits on monochromator B were of the bilateral type (Gaertner L162b). Both slits were set at 0.07 mm. as measured by the scale on the slits.

The absorption cells were made completely of glass, the plane parallel end-plates being fused to the barrel of the tube. The inside dimensions of the cells were 2.00 cm. long and 1.4 cm. in diameter. A side arm served for filling and emptying the cells.

The photoelectric current was amplified by means of the electrical circuit in Fig. 4. The photoelectric current was passed through the high resistance R_1 (5×10^{10} ohms). The small potential set up across this resistance was impressed on the grid g of the amplification tube (General Electric Co. FP-54). The amplified current thus induced was measured by means of galvanometer G. The sensitivity of the galvanometer was controlled by the Ayrton shunt S.

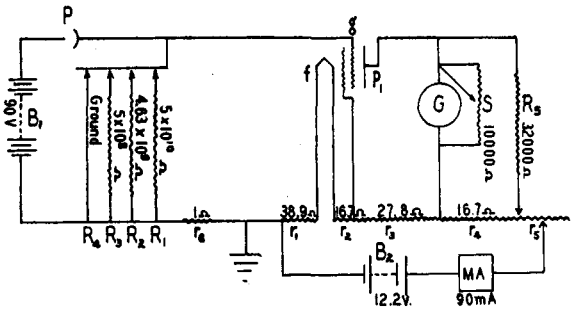

Fig. 4.—Diagram of amplification circuit.

The current necessary to operate the amplification apparatus was furnished by storage cells B_2 (*ca.* 12.2 v.). The current flowing through the circuit was controlled by the variable resistance r_5 and kept at approximately 90 milliamperes. The dark current from the photoelectric cell was neutralized by the current flowing through the circuit R_5-G-r_4 and was regulated by the variable resistance r_4.

Known auxiliary voltages could be impressed on resistance r_6 (1 ohm) in order to determine the voltage sensitivity of the system.

The photoelectric cell P, the amplification tube T and the resistances R_1-R_5 were all housed in a cylindrical brass container D (Fig. 3). In order to increase the steadiness of the amplification circuit the air in the container was kept dry by means of calcium chloride. The connection between the photoelectric cell and the grid of the amplification tube was short and rigid which was also conducive to steadiness.

The galvanometer (Leeds and Northrup No. 2280) was placed 3.3 meters from the scale. One mm. deflection of the galvanometer corresponded to 0.00003 volt. Theoretically it should have been possible to detect photoelectric currents of the order of 6×10^{-16} amperes. Actually this was not the case because of the unsteadiness of the system. The difficulties caused by the unsteadiness of the system were obviated by operating the galvanometer at 0.1 sensitivity.

The galvanometer scale was graduated in logarithms of centimeters deflection rather than in centimeters. This made it possible to obtain the log $1/T$ by subtracting the deflection of the galvanometer with the solution in the light path from the deflection with the solvent so placed. The scale was movable so as to allow small adjustments in zero to be made.

Readings were taken in the following manner. The light was shut off with shutter S_1 and after one minute the zero taken. The solution was interposed and after thirty

(8) C. B. van Niel and James H. C. Smith, *Arch. Mikrobiol.*, **6**, 219 (1935).

(9) G. Mackinney, *J. Biol. Chem.*, **111**, 75 (1935).

seconds the deflection of the galvanometer was read. The solvent was then introduced and the galvanometer deflection again read after thirty seconds. After the light had been cut off for one minute the cycle was repeated at another wave length.

If lower amplification was desired it was possible to substitute the other resistances R_2-R_3 for R_1 by means of a rotary switch operated from outside the brass container D. In case it was necessary to determine the dark current of the cell the circuit could be grounded through the wire R_4.[10]

The apparatus was tested in a number of ways to show that correct measurements would be obtained by its use.

(1) The amplification circuit was tested to see if the throw of the galvanometer was proportional to the voltage applied between the filament and the grid of the amplification tube. For this purpose auxiliary currents were passed through resistance r_6. The voltages produced were measured by means of a potentiometer (Leeds and Northrup, Type K). These potentials were correlated with the observed deflections of the galvanometer. Direct proportionality was found. This showed that the amplification was linear.

(2) Two methods were employed to test the proportionality of the deflection of the galvanometer to the intensity of the light incident on the photoelectric cell.

(a) The first method was that described by P. A. Leighton and W. G. Leighton.[11] If a constant transmission ratio for a given absorbing medium is found for different light intensities, it may be shown that the apparatus gives a response directly proportional to the light intensity. Constant transmission ratios at different light intensities were obtained. This showed that the apparatus responded directly proportionally to the incident light intensity. The data are shown in Table I.

(b) Next the apparatus was shown to give a response directly proportional to the incident light intensity by the interposition of screens of known transmissions between lenses l_1 and l_2. These screens were prepared by perforating thin metal sheets of known area with holes of known diameter. The holes were equally distributed over the area of the plate.

TABLE I

PROPORTIONALITY OF RESPONSE TO LIGHT INTENSITY. WAVE LENGTH 530 mμ

Galvanometer deflections Solution	Solvent	Transmissions
132	223	0.5919
264	446	.5919
320	540	.5926

Good agreement was obtained between the observed and calculated transmissions (Table II).

(3) The wave length drum on the monochromator B was calibrated against lines from the mercury arc. The calibration was correct at all wave lengths employed within ±1 Å. except at 5460.7 Å., where the deviation was 2.7 Å.

TABLE II

COMPARISON OF TRANSMISSIONS OBSERVED WITH PHOTOELECTRIC APPARATUS WITH THE TRANSMISSIONS CALCULATED FOR SCREENS

Transmissions calculated from dimensions	Transmissions observed
1.00	1.00
0.69	0.674
.500	.504
.353	.351
.241	.240
.177	.171
.124	.120
.074	.073

(4) The wave length range of the monochromatic beam used for absorption measurements was determined at different wave lengths. With the slits O_3 and O_4 set at 0.07 mm. the effective widths of the bands[12] transmitted were 3.5 Å. at 4358 Å. and 8 Å. units at 5461 Å.

(5) The beam emerging from O_4 was examined for "false light." When illuminated with white light the large monochromator A, used alone, transmitted a considerable amount of "false light." When the small predispersion monochromator A was inserted in the light path this "false light" was eliminated. This was demonstrated by viewing the emergent beam by means of a hand spectroscope. A further test of the monochromatic quality of the light consisted in comparing the transmission of the same solution determined with monochromatic light of the same wave length obtained first from the automobile headlight by means of the two monochromators and second from the mercury arc. The fact that the transmissions measured were identical showed the effectiveness of the monochromatization of the radiation from the headlight.

(6) To determine whether the apparatus gave correct over-all results, measurements were made of the absorption coefficients of a solution composed of an equal molecular mixture of copper sulfate and potassium chromate in 2 *N* ammonium hydroxide. This solution has been recommended as a standard by Weigert.[13] The results (Fig. 5) are in complete agreement with those obtained by von Halban and Siedentopf[14] and differ from those reported by Weigert[13] at only a few of the shorter wave lengths. This demonstrated the reliability of the apparatus.

There is considerable need for standard solutions to be used as references in absorption spectroscopy. If concordant data were available on the absorption coefficients of a series of solutions, the task of calibrating an apparatus for absorptions spectroscopy would be considerably lessened.

(10) The amplification circuit was designed by Dr. Theodore Dunham, Jr. [*Phys. Rev.*, **44**, 329 (1933); *Carnegie Inst. Wash. Y. B.*, **32**, 143 (1933)] of the Mount Wilson Observatory of the Carnegie Institution of Washington. The container with the installation of the photoelectric cell of the amplification tube and of the resistances were made under Dr. Dunham's supervision and we want to express our greatest appreciation for his valuable assistance.

(11) P. A. Leighton and W. G. Leighton, *J. Phys. Chem.*, **36**, 1890 (1932).

(12) The effective widths of the bands were obtained by taking the difference between the two wave lengths at which the galvanometer deflections were half of the maximum deflection caused by monochromatic radiation from a mercury arc.

(13) Fritz Weigert, *Ber.*, **49**, 1513 (1916).

(14) H. von Halban and K. Siedentopf, *Z. physik. Chem.*, **100**, 221 (1922).

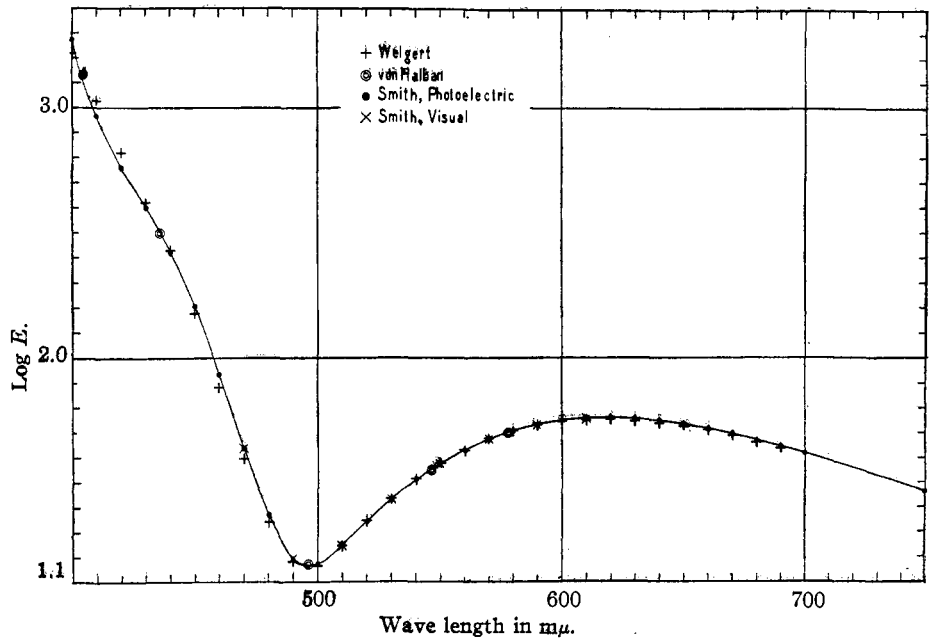

Fig. 5.—Absorption spectrum of copper sulfate + potassium chromate dissolved in 2 *N* ammonium hydroxide.

One perplexing aspect of the problem of the absorption spectroscopy of the carotenoid pigments has been the variance of the wave lengths reported for the absorption maxima of these pigments. This has been true particularly for beta-carotene. The positions reported for the absorption maximum farthest toward the red vary by almost 100 Å. This variation can be traced to the different methods used for observation.

The extreme difference exists between the data obtained by spectrophotometric methods and by visual spectroscopic observations in which a copper ammonium filter has been included in the light path. The latter method has been commonly used in Europe. Kuhn[6] has investigated the causes for this difference and has ascribed this discrepancy to an optic-physiologic effect.

Inasmuch as the measurements reported by Kuhn were made with two different types of instruments, a prism spectrophotometer and a grating spectroscope, it seems probable that the difference he found might be due to the different types of instruments used. Therefore this matter was investigated in our laboratory by means of a prism spectroscope and prism spectrophotometers. The results (Table III) corroborate those found by Kuhn and demonstrate that the effect is not caused by the difference in types of dispersing instruments.

The results in Table III show that: all photometric measurements give the same values; visual observations with the spectroscope alone show a slight shift to longer wave lengths of the positions of the absorption maxima furthest toward the red; visual observations by means of the spectroscope supplemented with the copper ammonium filter give the large shift noted by Kuhn.

TABLE III

POSITIONS OF THE ABSORPTION MAXIMA OF ALPHA-CAROTENE AND OF BETA-CAROTENE DISSOLVED IN CARBON BISULFIDE

		Alpha-carotene		Beta-carotene	
1	Spectroscope + filter	478.9	512.8	488.1	522.3
2	Spectroscope alone	478.1	508.8	485.9	516.8
3	Spectrophotometer + filter (visual)	476.5	507.2	485.1	513.3
4	Spectrophotometer (visual)	476.6	507.4	483.4	512.5
5	Photoelectric spectrophotometer	476	507.5	484	513

The cause for the difference observed in the position of the long wave length maximum of beta-carotene has been sought in the complex light intensity effects inherent in the visual spectroscopic observations. An evaluation of these effects (see Fig. 6) has explained many of the difficulties encountered in the visual spectroscopic determination of the absorption maxima of beta-carotene.

In the spectroscopic examination of absorption spectra, the eye is used as a photometer to estimate the relative intensity of adjacent portions of

the spectrum. Assuming that perception can be treated as an accurate physical indicator, the physiological response can be calculated from known data. The relative physiological response to a single wave length (I_λ) will be equal to the product of the relative luminosity ($L_\lambda = E_\lambda V_\lambda$) of the light source times the transmittancy (T_λ) of the various media through which the light of indicated wave length passes. This may be expressed by the equation

$$I_\lambda = I_S L_\lambda T_\lambda$$

where I_S is a standard intensity equal for all wave lengths, E_λ the relative emission intensity of the light source, and V_λ the visibility of the particular wave length of light used. The equation given can be transformed into a form more useful for obtaining the desired results graphically.

$$\log \frac{I_S}{I_\lambda} = \log \frac{1}{L_\lambda} + \log \frac{1}{T_\lambda}$$

The curve log $1/L$ (Curve 2) is calculated from data on visibility taken from the "International Critical Tables"[15] and from data on relative emissivity of an incandescent tungsten lamp (gas filled) given by Luckiesch.[16]

The values of log $1/T$ for a solution of beta-carotene in carbon bisulfide, obtained photometrically, are plotted against wave length in Curve 3. When these values are corrected for the luminosity of the source, Curve 4 is obtained. From this curve it is obvious why it has been difficult to measure the absorption maxima of beta-carotene by means of visual spectroscopic observations. While apparent maxima are observed experimentally, the positions differ from those calculated. This is due probably to a psychological effect produced by the variations in intensities at those wave lengths.

A theoretical curve in which the absorption of the copper ammonium filter (Curve 1) has been included (Curve 5) shows the advantage of introducing this filter. The maxima become well defined, and it is for this reason that it has received such extensive use in European laboratories. The position of the principal absorption maximum is approximately the same as when determined photometrically. The position of the long wave length maximum is considerably displaced toward the red. These calculations confirm the observations and also give a reasonable explanation of the effects produced with the copper ammonium filter.

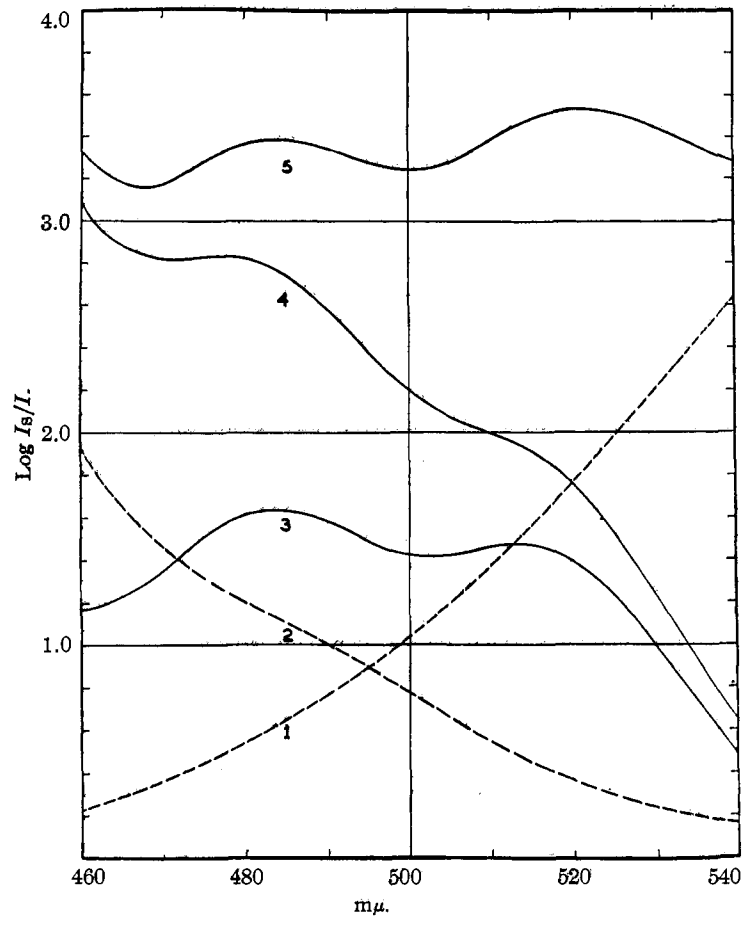

Fig. 6.—A comparison of the positions of maximum absorption of beta-carotene obtained under different conditions: Curve 1, absorption of copper ammonium filter; Curve 2, luminosity curve of light source; Curve 3, absorption of beta-carotene (carbon bisulfide solution) determined photoelectrically; Curve 4, visual absorption of beta-carotene, calculated (sum of curves 2 and 3); Curve 5, visual absorption of beta-carotene with copper ammonium filter (sum of curves 1, 2 and 3).

It should be noted that spectroscopic measurements will be affected by the magnitude of the absorption of the solution used, the spectral distribution of the light from the light source, the visibility of the observer, and by the shape of the absorption maxima involved. The position of a sharp maximum, such as that possessed by alpha-carotene, will be affected much less than the position of a flat maximum such as that of beta-carotene.

(15) "International Critical Tables," Vol. V, p. 436.

(16) M. Luckiesch, "Color and its Applications," D. Van Nostrand Co., New York City, 1921, p. 21.

From these results it can readily be seen that the specification of the Committee of the Health Organization of the League of Nations on the Standardization of Vitamin Preparations for the positions of the absorption maxima for pure beta-carotene (519 and 485.5 mμ in carbon bisulfide solution) to be used as a standard for vitamin A means very little unless the method of measurement is specified as well. Since the spectrophotometric methods have definite theoretical advantages and give fixed values for the positions of the maxima, 513 and 484 mμ ± 1.5 mμ (carbon bisulfide solution), it appears that this method should receive serious consideration by the Committee for recommendation as the standard method of observation.

Materials Used

1. Standard Solution According to Weigert.—Copper sulfate pentahydrate (Merck reagent) was recrystallized and dried in the air. Analysis showed it to have the proper composition.

Potassium chromate (Merck highest purity) was used without recrystallization. This material was shown by analysis to be pure.

The ammonium hydroxide was made by dissolving ammonia gas in distilled water. The ammonia gas was obtained by boiling a concentrated solution of ammonium hydroxide (ammonium hydroxide, C. P. Baker analyzed). The stock solution thus prepared was diluted to 2 N × 0.9971.

A 0.1 M solution of each of these salts was prepared in 2 N ammonia. To obtain the solutions for absorption measurements, equal volumes of the two solutions were combined and the mixture diluted to the desired concentration with 2 N ammonia.

2. Solutions of the Carotenoids

Alpha-Carotene.—The alpha-carotene used in these experiments was separated from carrot-root carotene by adsorption on magnesium oxide by Dr. H. H. Strain. The properties of the sample, before further purification, have been reported.[17] Before use in these absorption measurements the sample was recrystallized from a mixture of methanol and dioxane. This material melted at 187° (corr.). Elementary analyses showed the following composition.

Anal. Calcd. for $C_{40}H_{56}$: C, 89.48; H, 10.52. Found: C, 89.15, 89.24; H, 10.58, 10.52.

The absorption coefficients determined on this material are designated by crosses in Fig. 1 and by black circles in Fig. 2.

A second sample of alpha-carotene, originally from the same separation as the sample just described, was recrystallized twice from carbon bisulfide by the addition of absolute ethanol and once from dioxane and absolute ethanol. This sample melted at 187° (corr.). The absorption coefficients found for this carotene are marked by black circles in Fig. 1.

(17) H. H. Strain, *J. Biol. Chem.*, **105**, 528 (1934).

Beta-carotene.—The beta-carotene was obtained from carrot-root carotene by fractional adsorption on an adsorption column. The absorption coefficients of this material, already described,[17] are marked with crosses in Fig. 1. After two recrystallizations from carbon bisulfide by addition of absolute ethanol and three from dioxane and methanol, the absorption coefficients of the carotene were measured. The values are given in Figs. 1 and 2. The beta-carotene melted at 184° (corr.).

Lycopene.—The lycopene sample was isolated from canned tomato puree. The tomato pulp was filtered from the juice and dehydrated with methanol. The dehydrated pulp was treated with carbon bisulfide and the extract concentrated. The lycopene was precipitated from the concentrate by the addition of absolute ethanol. After another precipitation from chloroform solution with ethanol it was crystallized from petroleum ether (b. p. 65–70°). At this stage it was stored in the refrigerator for two years in an evacuated ampoule. During storage a considerable amount of material insoluble in carbon bisulfide was formed. The remaining lycopene, however, was purified just before use by six crystallizations from carbon bisulfide by the addition of ethanol. The melting point was 174.0–174.5° (corr.). The absorption curves are shown in Figs. 1 and 2. The absorption coefficients of this lycopene were slightly higher than those obtained with lycopene freshly prepared from rose hips.

Solvents.—The carbon bisulfide was always redistilled a short time before use. Between experiments it was stored in a dark place to lessen the coloration caused by exposure to light.

When ether was required it was always freshly distilled from sodium.

The absolute ethanol used was fractionated from commercial C. P. absolute ethanol shortly before use.

Preparation of Solutions.—The solutions of the pigments in carbon bisulfide were prepared by dissolving the pigment in 49.99 cc. of carbon bisulfide and diluting 3.009 cc. of this solution to 24.93 cc. The samples of the pigments (0.4–0.8 mg.) were weighed on a micro-balance.

The solutions in 20% ether in ethanol were prepared by dissolving the pigment sample in 10.00 cc. of ether and diluting to 49.99 cc. with ethanol. 3.009 cc. of this solution was diluted to 24.93 cc. with an ethanol solution containing 20.0 cc. of ether in 100.00 cc. of solution.

Calculation

All calculations of the absorption coefficients recorded in this paper were made according to the formula

$$E = (1/Lc) \log_{10} (I_0/I)$$

where I_0 and I are respectively the transmissions of L cm. of solvent and of solution, the concentration of which is c moles per liter.

The author wishes to thank Dr. H. A. Spoehr, Dr. H. H. Strain, Dr. Gordon Mackinney, and Dr. Wesley G. Leighton for their suggestions and aid in many matters concerning this research and also to express his appreciation of the advice and assistance rendered in the early stages of the develop-

ment of this method by Dr. J. C. Clark and Dr. Harold Mestre, formerly of Stanford University.

Summary

By means of a new photoelectric spectrophotometric apparatus the absorption spectra of highly purified alpha-carotene, beta-carotene and lycopene dissolved in 20% ether in ethanol and in carbon bisulfide have been determined. The absorption coefficients found agree well with the results obtained previously for the ether–ethanol solutions but differ considerably from those reported for carbon bisulfide solutions. The causes for the differences in carbon bisulfide solutions are not apparent.

An explanation has been offered for the variations in positions of the absorption maxima of alpha-carotene and beta-carotene determined spectrophotometrically and spectroscopically, supplemented with a copper ammonium filter. The cause probably lies in the superposition of the absorption of the filter on that of the pigment. Because of the variations possible in the latter method of measurement due to changes in concentrations of the solutions, only the positions of the absorption maxima determined photometrically should be reported.

STANFORD UNIVERSITY, CALIF.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF BROWN UNIVERSITY]

Properties of Electrolytic Solutions. XVII. The Conductance of Some Salts in Benzene and Dioxane

BY WILLIAM F. LUDER AND PHILIP B. KRAUS[1] WITH CHARLES A. KRAUS AND RAYMOND M. FUOSS

In a previous paper of this series,[2] data were presented which demonstrated the existence of simple binary equilibria for 1-1 salts in benzene, a non-polar solvent. This paper contains data for silver perchlorate, tetrabutylammonium acetate and tetrabutylammonium perchlorate in benzene at 25°, for tetraisoamylammonium iodide in benzene at 60° and for tetrabutylammonium acetate and perchlorate in dioxane at 25°. All of these salts show minima in the equivalent conductance at small concentrations, below which the conductance rises in approximate agreement with the limiting form of the law of mass action

$$c\Lambda^2 = \text{const.}$$

which is valid when association to ion pairs is nearly complete.

I. Materials, Apparatus and Method

Solvents.—Benzene was purified as described by Fuoss and Kraus;[2] m. p. 5.4°, specific conductance 3–10 × 10^{-15} mho. Dioxane was purified by the method of Kraus and Vingee;[3] m. p. 11.65°, specific conductance 2–6 × 10^{-15} mho.

Salts.—The tetraisoamylammonium iodide was the same as that used by Fuoss and Kraus.[2] Silver perchlorate was made by dissolving silver oxide (from silver nitrate and potassium hydroxide) in 60% perchloric acid. After evaporation to incipient crystallization, the salt was dried at 115°, dissolved in benzene and filtered and the solution was then evaporated to dryness under reduced pressure. The product apparently contained some impurity, possibly benzene, according to the analyses: found 51.31, 51.17, 50.16% Ag; calculated, 52.03%. Erratic conductance results were obtained with silver perchlorate which was recrystallized from water, and it was only after it had been treated with benzene and filtered from a small amount of insoluble residue that reproducible values were obtained. Tetrabutylammonium iodide was made by the method of Cox, Kraus and Fuoss,[4] m. p. 142° (This is 2° lower than Cox's value.) The perchlorate was made from the iodide by metathesis with silver perchlorate in 95% alcohol; after standing overnight to coagulate colloidal silver iodide, the solution was filtered and allowed to evaporate at room temperatures. The product was recrystallized from anhydrous ethyl acetate (6 cc. of ethyl acetate per gram of salt, 90% yield on first crop); m. p. 207°. Tetrabutylammonium acetate was made by neutralizing with acetic acid an alcoholic solution of the hydroxide (from the iodide and silver oxide). After room temperature evaporation in a desiccator containing first calcium chloride and subsequently phosphorus pentoxide, the salt was twice recrystallized from benzene (50 cc. benzene per gram of salt; 95% yield on first crop); m. p. 116°.

Apparatus and Procedure.—The electrical equipment was that used by Fuoss and Kraus.[2] The cells were of the Erlenmeyer type described by Kraus and Fuoss;[5] the cell constants were 0.1811, 0.02027 and 0.01743. The procedure was essentially that described by Kraus and Fuoss:[4] concentrated solutions from weight burets were

(1) The data for tetraisoamylammonium iodide at 60° and for silver perchlorate at 25° are contained in a thesis, submitted by Philip B. Kraus in partial fulfilment of the requirements for the degree of Master of Science in the Graduate School of Brown University, June, 1933.

(2) Fuoss and Kraus, THIS JOURNAL, **55**, 3614 (1933).

(3) Kraus and Vingee, *ibid.*, **56**, 511 (1934).

(4) Cox, Kraus and Fuoss, *Trans. Faraday Soc.*, **31**, 749 (1935).

(5) Kraus and Fuoss, THIS JOURNAL, **55**, 21 (1933).