CCXIV. SPECTROMETRIC MEASUREMENTS ON VARIOUS CAROTENOIDS.

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In a previous investigation involving the spectrophotometric determination of carotene and xanthophyll in grass extracts [Gillam *et al.*, 1933] it was found that the determination of the blue colour with antimony trichloride (intensity of absorption at 585–590 $m\mu$) gave higher values than were to be expected from the carotenoid content as determined by the yellow colour. It was considered possible that the discrepancy might be due to the presence of small amounts of carotenoids, differing from carotene and xanthophyll in being much more strongly chromogenic towards antimony trichloride. In addition to direct search for the disturbing factor, it was decided to determine the intensity of blue colour developed with antimony trichloride and various known carotenoids, with a view to finding such a highly chromogenic substance as has been postulated. As such a substance is not yet forthcoming, the present communication serves to record the quantitative visible and ultraviolet absorption spectroscopic data in chloroform, and visible data in chloroformic antimony trichloride, for such pure carotenoids as have been available, with a view to their subsequent use in the spectrometric determination of these pigments.

The determinations were made photographically for the most part, using a Hilger E 3 quartz spectrograph and either a Spekker or a sector photometer, but absorption in the visible was also determined on a Hilger-Nutting visual spectrophotometer (Table I). The examination of the antimony trichloride blue colours was also made with this instrument, using the technique previously applied in the case of vitamin A [Heilbron *et al.*, 1931]. The antimony trichloride reagent was made up as a saturated solution in alcohol-free chloroform, one drop of acetic anhydride being usually added in each test to render the solutions optically clear. The concentrations were so arranged that the ratio of antimony trichloride to pigment solution was always more than 10 to 1 and, when the colours were transient, the values recorded were the mean of many separate mixings with the intensity measurements following immediately.

Euler *et al.* [1932] have recorded the visible absorption spectra of a number of carotenoids in both chloroform and antimony trichloride solution, but intensities were not measured.

Through the kindness of Prof. Chibnall and Dr Pollard, of the Imperial College of Science, I was able to examine a pure specimen of β -carotene from cocksfoot grass. This had no detectable optical activity, and m.p. 182° (corr.). Its intensity of absorption was some 10% higher than that of the best sample previously examined [Gillam *et al.*, 1933]. The new value of $E_{1\text{cm.}}^{1\%}$, 2200, in chloroform solution, must therefore be adopted for future spectrophotometric carotene determinations. In light petroleum (b.p. 60–80°) the corresponding value was 2500.

The typical carotenoids, carotene and lutein exhibit absorption spectra consisting of a group of bands in the visible spectrum, a single band at 330–350 $m\mu$

Table I. Absorption spectra of carotenoids in chloroform.

Substance	Formula	Absorption maxima* in $m\mu$					Intensity of absorption at the main visible maximum		References to some other recent absorption spectra determinations
		493	463	436	—	348	280	—	
β -Carotene (<i>ex</i> cocksfoot grass)	$C_{40}H_{56}$	—	—	—	—	—	—	—	McNicholas [1931], Pummerer and Rebmann [1928], Kuhn and Lederer [1931], Schertz [1923]
<i>iso</i> Carotene	"	512	483	453	(426)	389	367	317	Gillam <i>et al.</i> [1932], Kuhn and Lederer [1932]
Lycopene	"	513	480	451	371	(353)	302	(290)	Pummerer <i>et al.</i> [1929]
Lutein (<i>ex Tagetes</i>)	$C_{40}H_{58}O_2$	485	453	428	(407)	340	275	—	McNicholas [1931], Schertz [1925]
Lutein (<i>ex</i> nettles)	"	489	456	428	—	340	273	—	Kuhn and Smakula [1931], Gillam <i>et al.</i> [1933]
Zeaxanthin	"	490	460	(430)	—	348	279	—	Kuhn and Smakula [1931]
Violaxanthin	$C_{40}H_{58}O_4$	485	454	427	—	335	268	—	Kuhn and Winterstein [1931]
Fucoxanthin† (<i>ex Fucus vesiculosus</i>)	$C_{40}H_{56}O_6$	(490)	458	—	—	331	269	—	Euler <i>et al.</i> [1932]
Azafurin methyl ester	$C_{38}H_{46}O_4$	450	425	(402)	312	—	—	—	Kuhn and Deutsch [1933]
Astacene (<i>ex</i> lobsters)	$C_{40}H_{58}O_4$	500	—	—	—	—	260	—	Fabre and Lederer [1934], Kuhn and Lederer [1933]

* Inflections in brackets.

 $E_{1\text{ cm}}^{1\%} = \log I_0/I$ for a 1 cm. layer of a 1% solution (I_0 = intensity of incident light), (I = intensity of transmitted light). ϵ molar = $\log I_0/I \div (c \times d)$ where c = the molar concentration, d = thickness in cm.

† Cf. Heilbron and Phipers [1935].

and another at 270–280 $m\mu$ (Fig. 1). Lycopene (Fig. 2) follows the same general outline, but differs in that the two simple ultraviolet bands are each resolved into two components. The resolution is only shown easily when a light source with a continuous spectrum is used (*e.g.* an under-water tungsten spark or a hydrogen tube). The curves recorded by Pummerer, *et al.* [1929], do not show this resolution (solvent *cyclohexane*).

Fig. 1. Lutein (*ex Tagetes*) in CHCl_3 .

Fig. 2. Lycopene in CHCl_3 .

Fig. 3. Zeaxanthin in CHCl_3 .

Fig. 4. — Violaxanthin in CHCl_3 ; ---- Viola-xanthin in CHCl_3 after partial decomposition by ultraviolet rays.

Violaxanthin in dilute solution in chloroform appears to be more stable to light and air (in glass vessels) than carotene or lutein. On the other hand, its chloroform solution is very sensitive to short-wave ultraviolet rays. This was made evident in determining the ultraviolet absorption spectrum of the solution, when the radiations from an iron-nickel arc, even at a distance of 5 feet, were sufficient to change the colour of the solution from yellow to green in about 30 minutes. By irradiation of a dilute solution (about 0.001 %) with a quartz mercury vapour lamp, it was observed that:

(a) In a quartz vessel, the solution rapidly turns green, then bluish green and finally colourless.

(b) The blue solution, which with the yellow of the unchanged violaxanthin presumably gives the green colour, exhibits two narrow but intense absorption bands with maxima at 411 and 438 $m\mu$ (Fig. 4).

(c) In glass apparatus the solution is unchanged in colour even with exposures up to 30 minutes at 8 in. from the lamp. The ultraviolet rays which bring about the photochemical change must therefore be of shorter wave-length than 325 $m\mu$, the approximate short-wave limit of transmission of glass.

Table II. *The colour and absorption spectra of the antimony trichloride solutions of the carotenoids.*

Substance	Colour	Absorption spectra	Intensity of absorption $E_{1\text{ cm.}}^{1\%}$			Effect of adding 7-methylindole before the SbCl ₃ None
			615 $m\mu$	585 $m\mu$	420	
β -Carotene (<i>ex</i> cocksfoot grass)	Slate-blue	A single band at 590 $m\mu$	—	—	—	None
<i>iso</i> Carotene	Slate-blue	Single band at 583–587 $m\mu$	—	—	370	—
Lycopene	Blue	Strong end-absorption in the red, weak inflection near 585 $m\mu$	260	200	—	—
Lutein (<i>ex Tagetes</i>)	Blue (forms slowly)	Clear band at 617 $m\mu$ and inflection 580–590 $m\mu$, strong absorption in the red beyond 700 $m\mu$	315	230	—	No 617 $m\mu$ band. Bands at 585 and 545 $m\mu$
Lutein (<i>ex</i> nettles)	Blue (forms slowly)	Clear band at 619 $m\mu$ and inflection 580–590 $m\mu$; strong absorption in the red	360	230	—	
Zeaxanthin	Blue	Bands at 621 and 587 $m\mu$; the latter forms first and fades rapidly	370	330	—	Single band 583 $m\mu$ does not now fade rapidly
Violaxanthin	Blue (immediate; contrast lutein)	Clear band at 585 $m\mu$, ill defined one at 615 $m\mu$; the first decreases rapidly whilst the latter increases	340	350	—	—
Fucoxanthin (<i>ex Fucus vesiculosus</i>)	Blue	End-absorption in the red only	290	190	—	None
Astacene (<i>ex</i> lobsters)	Transient violet-blue	Broad indefinite band near 590 $m\mu$	—	1000 (At 590 $m\mu$)	—	None
Azafrin methyl ester	Deep royal blue	End-absorption in the red; broad but definite band at 595–600 $m\mu$	—	650 (At 596 $m\mu$)	—	—
*Vitamin A	Deep blue	Clear maximum 617–620 $m\mu$; masked maximum 580–583 $m\mu$	5000	2600	—	Single band at 583 $m\mu$

* Data by Heilbron and Morton cited by Carr and Jewell [1933].

The colour change depends upon the decomposition of the chloroform by the short-wave ultraviolet rays with the production of hydrogen chloride, which then produces the blue colour with the violaxanthin. As might be expected, the blue colour reverts to yellow on the addition of alkali. These observations suggest a variation of the test for violaxanthin, in which it gives a blue colour with 25 % hydrochloric acid, the variation being to produce the colour in the chloroform solution by exposure to short-wave ultraviolet rays (mercury vapour lamp).

The blue colour obtained by the action of antimony trichloride on lutein has been examined by Euler *et al.* [1932] and by Goldhammer and Kuen [1933], both of whom record only one absorption maximum at $586m\mu$. Under the experimental conditions used in this investigation the absorption band characteristic of the blue solution was found to lie at $617m\mu$ with only a subsidiary or masked maximum near $586m\mu$ and strong absorption in the red. These maxima were obtained with four different crystalline samples of lutein from various sources and were also typical of the xanthophyll fractions of several species of grass [Gillam *et al.*, 1933]. The visual determination of the wave-length maxima is supported by the intensity measurements (Table II).

Fig. 5. Fucoxanthin (*ex Fucus vesiculosus*) in CHCl_3 .

Fig. 6. Azafrin methyl ester in CHCl_3 .

Fig. 7. Astacene in chloroform.

It is interesting to note that the three hydroxy-polyenes, vitamin A, lutein and zeaxanthin, give colours with antimony trichloride, all of which exhibit an absorption band at $617\text{--}620m\mu$ and another near $585m\mu$. The similarity is only qualitative since vitamin A shows by far the strongest absorption bands, being some fifteen times as chromogenic as either of the two xanthophylls. It has been shown that the addition of 7-methylindole to a vitamin A extract

before the antimony trichloride inhibits the formation of the 617–620 $m\mu$ band completely, leaving only a maximum at 583 $m\mu$ [Emmerie *et al.*, 1931; Morton, 1932]. The two xanthophylls behave in a similar manner after this treatment, lutein showing two maxima at 585 and 545 $m\mu$ and zeaxanthin showing only one at 583 $m\mu$.

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