GALLOXANTHIN, A CAROTENOID FROM THE CHICKEN RETINA*

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A new carotenoid has been isolated from the chicken retina. It is proposed to call this *galloxanthin*, from *Gallus*, the generic name of the fowl, and the suffix *-xanthin*, which designates carotenoid alcohols or xanthophylls. This substance is interesting physiologically, as a new member of a series of carotenoid pigments which act as color filters for cone vision in the chicken (Wald and Zussman, 1938). It has an interest also for carotenoid chemistry, since its absorption spectrum lies in a wavelength region where—with one curious exception[1]—natural carotenoids have not previously been found.

Properties

Partition.—Galloxanthin is non-saponifiable, and displays no acidic properties. When partitioned between petrol ether and 90 per cent methanol, it goes almost entirely into the lower, alcohol layer (hypophasic). In partition between petrol ether and 80 per cent methanol, it is distributed about equally in both phases. This behavior is characteristic of xanthophylls.

Adsorption.—Galloxanthin is adsorbed very strongly from its solutions in petrol ether by calcium carbonate. This is another common xanthophyll property. The pigment when purified is adsorbed at the top of a column of calcium carbonate, and scarcely moves downward on prolonged washing with petrol ether. It does descend slowly on washing with pure benzene (C_6H_6).

In extracts of chicken retinas, galloxanthin is found mixed with astaxanthin, leaf xanthophylls, and a carotene. In the chromatogram on calcium carbonate, galloxanthin is adsorbed just below astaxanthin and above lutein. It cannot easily be separated from either pigment. All three carotenoids appear to form stereoisomeric sets (*cf.* Zechmeister, 1944), possibly as a result of extraction procedures, which overlap on the chromatogram.

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[1] A carotenoid having an absorption spectrum in the same region as that of galloxanthin has recently been found in an artificially induced mutant of the yeast *Rhodotorula rubra* (pigment "B;" Bonner, Sandoval, Tang, and Zechmeister, 1946). Apart from its spectrum this pigment has very different properties from galloxanthin.

On saponifying such mixtures, astaxanthin is autoxidized to astacene. Galloxanthin can then be separated as a yellow band just below the red band of astacene, by developing the chromatogram on calcium carbonate with pure benzene. An easier procedure is to chromatograph such a mixture out of petrol ether on powdered sugar; this holds astacene strongly, and allows galloxanthin to descend rapidly as a diffuse band, eventually to be collected in the filtrate.

Crystallization.—Attempts to crystallize this pigment have not yet succeeded. We have obtained granular precipitates repeatedly by bringing concentrated

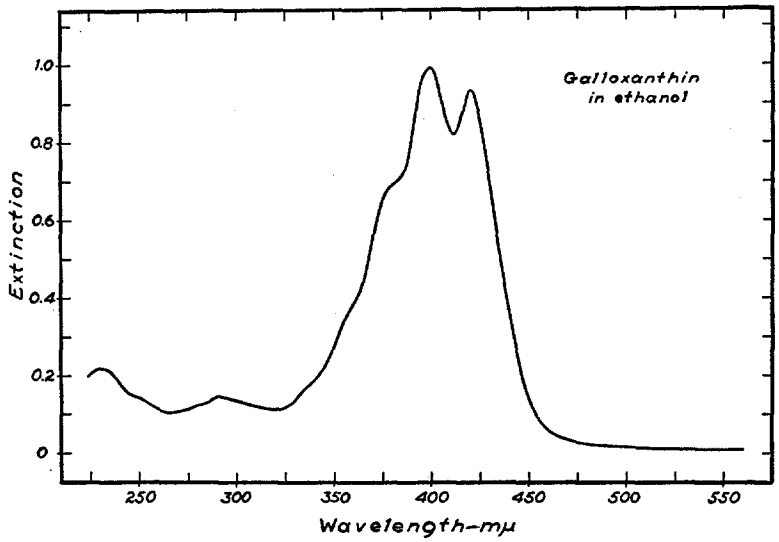

FIG. 1. Absorption spectrum of galloxanthin in solution in absolute ethanol. The absorption is plotted in terms of the extinction, log I_0/I, in which I_0 is the incident and I the transmitted intensity.

solutions in petrol ether to low temperatures or by adding water gradually to methanol or ethanol solutions in the warm and cold; but no frank crystals could be observed. It should be recognized that all this work has been done with minimal quantities of the pigment, very much less than those with which one ordinarily pursues carotenoid chemistry. This in itself has hindered many of the procedures.

Spectrum.—The spectrum of galloxanthin displays the three main bands characteristic of most carotenoids (Fig. 1). In ethanol the absorption maxima lie at about 421, 400, and 378 mμ; in hexane at 422, 401, and (380 mμ); in chloroform at 427, 407, and (387 mμ); and in carbon disulfide at about 446 and 424 mμ.

In all our preparations the central band is most prominent, but the lateral

bands appear in various relations. In some preparations the longest wavelength band is reduced to a mere inflection, while the short wavelength band comes to a pronounced peak; in others, these relations are reversed. Fig. 2 shows examples of the types of spectrum observed. From their shapes and the procedures which yielded them, it seems probable that primarily they represent mixtures of *cis-trans* stereoisomers of galloxanthin in various proportions. No systematic study of this situation has yet been made, however, and other types of change may also be involved.

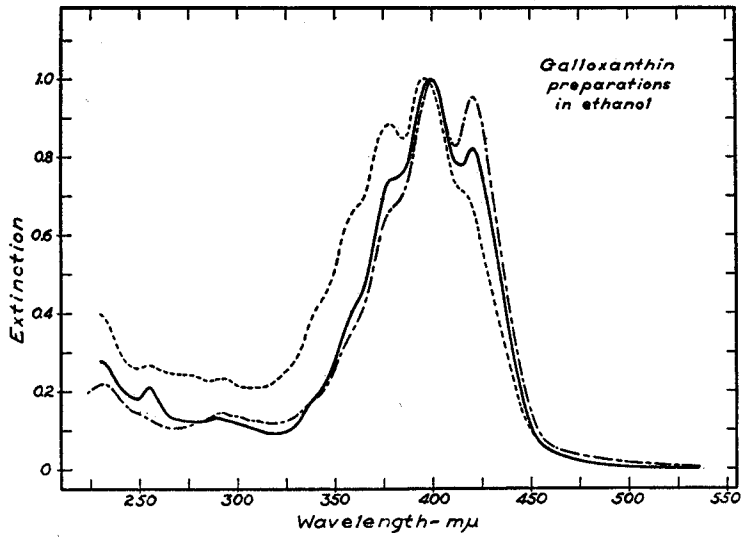

FIG. 2. Absorption spectra of three preparations of galloxanthin dissolved in absolute ethanol. These are selected to show the various forms in which the spectrum appears, probably representing primarily mixtures of *cis-trans* stereoisomers of galloxanthin in various proportions.

In addition to the main bands of galloxanthin, a small peak appears in some preparations at about 290 mμ (Figs. 1 and 2). The position of this band and its variability suggest that it is what Zechmeister has called a "*cis* peak," minimal in all-*trans* carotenoids, and prominent in those *cis* forms in which the molecule is appreciably bent. According to Zechmeister the *cis* peak almost invariably lies at 142 ± 2 mμ below the longest wavelength maximum of the all-*trans* form in hexane. In galloxanthin this would place it at about 280 mμ, slightly below the observed position. There is also in some preparations the suggestion of a small band at about 255 mμ, and one at about 230 mμ.

Galloxanthin in ultraviolet light has not been observed to fluoresce either in solution or when concentrated by adsorption on the chromatogram.

Antimony Chloride Reaction.—Galloxanthin yields the deep blue color characteristic of many carotenoids when mixed with saturated antimony trichloride in chloroform. The color develops immediately, then slowly fades as is usual in this test. The spectrum of the transient blue product, recorded within 1 minute in the Hardy photoelectric spectrophotometer, rises from about

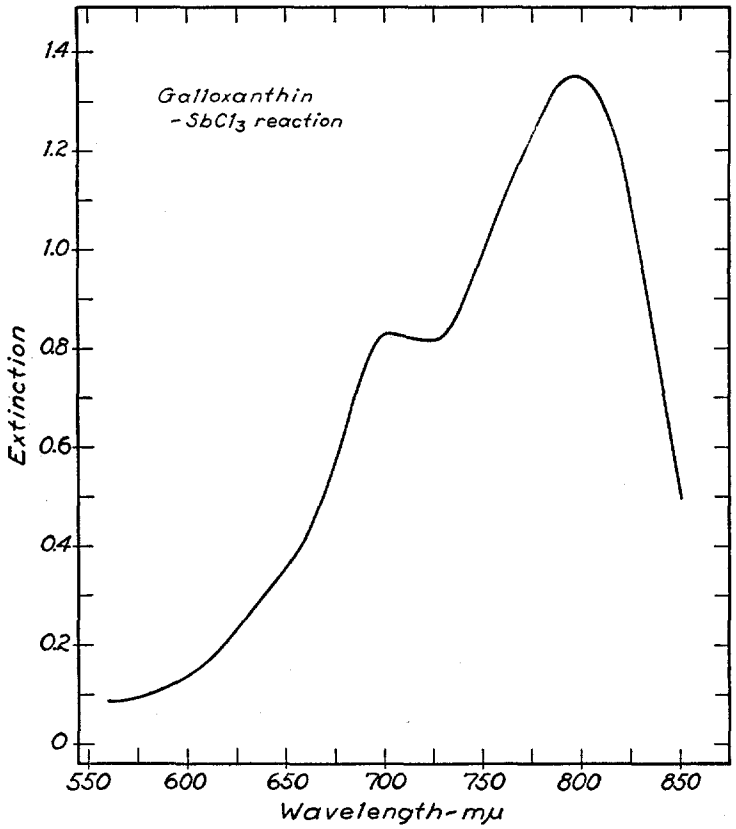

FIG. 3. Absorption spectrum of the blue product obtained by mixing a preparation of galloxanthin with antimony trichloride. The same concentration of galloxanthin in simple solution in chloroform would have an extinction at 405 mμ of 1.0.

560 mμ to an inflection at about 700 mμ, as though a maximum lay just beyond. Unfortunately this instrument does not record longer wavelengths.

We have attempted to measure the antimony chloride spectrum with the Beckman spectrophotometer, which is too slow to make this kind of measurement conveniently. Working as rapidly as possible, however, we have pieced out the spectrum in short, overlapping ranges, completing each series of measurements within 1.5 minutes after mixing the reagents. The result

with one preparation of galloxanthin is shown in Fig. 3; it is reasonably typical of all our preparations.

In the antimony chloride reaction a main absorption band is always found at 785 to 795 mμ. In various preparations the height of this band remains fairly proportional to the height of the direct spectrum. Thus the extinction in the antimony chloride test at about 790 mμ is on the average 1.35 times (range 1.17 to 1.60) as great as the extinction at 405 mμ of the same concentration of galloxanthin in chloroform. This information has been incorporated in Fig. 3, which shows the spectrum of the antimony chloride product of a preparation of galloxanthin which, in the same concentration in chloroform and in the same depth of layer, would have had an extinction at 405 mμ of 1.0.

A secondary maximum in the antimony chloride test is found at about 710 mμ. The relations between this and the main maximum vary greatly from one preparation to another. It is probable that the absorption in this region is due either to some minor modification of galloxanthin or to another type of contaminant.

DISCUSSION

Two properties of galloxanthin deserve special attention, since they have implications for its structure: (*a*) the location of its absorption spectrum; and (*b*) its extraordinary adsorbability.

The position of the main absorption spectrum of a carotenoid or synthetic polyene is determined primarily by the length of its conjugated system of double bonds (Radulescu and Barbulescu, 1931; von Euler, Karrer, Klussmann, and Morf, 1932; Hausser, Kuhn, and Smakula, 1935). Among all such substances which have been investigated, only one of known structure absorbs in the same spectral region as galloxanthin. This is the carotenoid derivative, dihydrobixin (von Euler, Karrer, Klussmann, and Morf, 1932). It possesses eight conjugated double bonds; and also two carboxyl groups in non-conjugated positions which probably have little effect upon its visible spectrum. The spectrum of dihydrobixin resembles greatly that of galloxanthin in both shape and position. It seems probable from this comparison and from the location of its spectrum relative to other carotenoids, that galloxanthin possesses eight conjugated double bonds.

It may be recalled that another retinal carotenoid, retinene$_2$, lies close in the spectrum to galloxanthin. Its single absorption band is maximal at about 405 mμ in chloroform (Wald, 1938–39). It happens that retinene spectra, however, are displaced extraordinarily far toward longer wavelengths in chloroform solution, as compared with such homopolar solvents as hexane. The absorption maximum of retinene$_2$ in hexane lies at about 374 mμ, far below the position of the main galloxanthin band in this solvent. These pigments, therefore, share only a limited coincidence in spectral location.

Other structural considerations apart, the adsorbability of carotenoids also varies with the length of conjugated system. One should, for this reason, expect that galloxanthin, with a relatively short conjugated system, might be weakly adsorbed. It has instead an extraordinarily high adsorption, stronger than that of lutein with eleven conjugated double bonds and two hydroxyl groups; and only slightly weaker than that of astaxanthin, with eleven conjugated double bonds, two keto groups probably conjugated with the polyene chain, and two hydroxyls. It is clear that the structure of galloxanthin must include other features making for strong adsorption, which more than compensate for the shortness of its conjugated system. It may for example possess more than two hydroxyl groups. There are other, subtler possibilities; witness the observation that phytofluene, apparently a hydrocarbon containing only five conjugated double bonds, displays a similarly "abnormal" adsorbability (Zechmeister and Sandoval, 1946).

Finally, something may be said of the physiological significance of galloxanthin. It occurs free in the retina, and can be extracted from the desiccated tissue by merely shaking with petrol ether. It probably is to be ranged therefore with the group of carotenoids found in free solution in the oil droplets of the retinal cones.

These pigments are so placed as to act as color filters for the individual cones. The effectiveness of galloxanthin as a filter may be judged from the fact that it is isolable from the chicken retina in such amount that if spread evenly over the whole retinal surface it would have a maximal absorption of about 50 per cent. Since its extraction and isolation necessarily entail losses, and since it probably is not distributed homogeneously over the retina, its density *in situ* may be considerably greater.

The other filter pigments of the chicken retina—astaxanthin, lutein, and the retinal carotene—absorb at considerably longer wavelengths than galloxanthin. Indeed their absorptions fall off in the very region of the violet in which the galloxanthin absorption becomes maximal. Galloxanthin is therefore in position to complement the filtering action of the other pigments; it keeps high the absorption in the violet and near ultraviolet, where it would otherwise be small.

What is the significance of such absorption? It is a reasonable view, as has been suggested many times, that the filter pigments of the chicken retina are used in color discrimination, much as three-filter systems are employed in color photography. This is therefore one possibility—that galloxanthin acts as an auxiliary to the other pigments in color differentiation. There is another function, however, for which it is more specifically fitted. It is reasonably certain that the chicken eye, like the human eye, possesses a large chromatic aberration. That is, when the eye is in focus for the middle region of the visible spectrum, it is highly myopic and hence considerably out of focus for short wavelengths (Wald and Griffin, 1947). Potentially this should

result in a considerable blurring of the retinal image. In the human eye, however, this effect is avoided by excluding most of the violet and virtually all the ultraviolet through the filtering action of the yellow lens. In the chicken the lens is not visibly colored; and here it is possible that the same removal of low wavelengths is accomplished by galloxanthin.

SUMMARY

A new carotenoid has been isolated from the chicken retina for which the name *galloxanthin* is proposed. This substance has the properties of a hydroxy carotenoid or xanthophyll. It has not yet been crystallized. On a chromatogram of calcium carbonate it is adsorbed just below astaxanthin and above lutein.

The absorption spectrum of galloxanthin lies in a region where natural carotenoids have not ordinarily been found. Its main, central absorption band falls at about 400 mμ. The position of its spectrum suggests a conjugated system of eight double bonds. This relatively short polyene structure must be reconciled with very strong adsorption affinities.

With antimony trichloride, galloxanthin yields a deep blue product, possessing a main absorption band at 785 to 795 mμ, and a secondary maximum at about 710 mμ which may not be due to galloxanthin itself.

Galloxanthin appears to be one of the carotenoid filter pigments associated with cone vision in the chicken. It may act as an auxiliary to the other filter pigments in differentiating colors; or its primary function may be to exclude violet and near ultraviolet radiations for which the eye has a large chromatic aberration.

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