CAROTENOIDS

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AUTHOR'S FOREWORD

The first monograph on carotenoids was written in 1922 by L. S. PALMER (*Carotenoids and Related Pigments*, New York). In view of the state of the subject at the time, this author could say relatively little about the chemistry of these natural pigments and his work therefore consisted mainly of a summary of the knowledge then available concerning the distribution and the biological significance of the carotenoids.

In 1934, L. ZECHMEISTER'S excellent book *Carotinoide* (Berlin) appeared, in which the great advances which had been made in the chemistry of these polyene pigments during the period 1927–1934 were described. Since then, the unravelling of the chemical nature of the carotenoids has further advanced and progress has also been made in the elucidation of their biological significance. A great deal of material has thus accumulated during a relatively short period.

It was the desire to sift and collate the extensive literature on carotenoids which led to the writing of the present monograph on this class of natural pigments. Special attention has been paid not only to the chemistry but also to the distribution and biological significance of the carotenoids. It is hoped that the numerous tables will help to clarify the relationships between the different pigments.

P. KARRER, E. JUCKER

ZÜRICH, August 1948

TRANSLATOR'S FOREWORD

In the present English edition of Professor P. KARRER's and Dr E. JUCKER's book, a number of corrections have been made, and a certain amount of new material, covering some of the more important investigations published since the appearance of the Swiss edition, has been added.

I am indebted to my wife for assistance in preparing the translation, and to Dr B. C. L. WEEDON for help in checking the proofs.

E. A. BRAUDE

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GENERAL PART

Introduction

The term *carotenoids* refers to a group of pigments, yellow to red in colour, which are widely distributed in the vegetable and animal kingdoms, and are distinguished by the following features: they are generally composed of isoprene residues, usually eight, arranged in such a way that in the middle of the molecule two methyl groups are present in 1:6 positions, while all other side-chain methyl groups occupy 1:5 positions. The general structure of the carotenoids is of the aliphatic or aliphatic-alicyclic type and their chromophoric systems contain numerous conjugated carbon-carbon double bonds.

All carotenoids are soluble in fats and lipoids; the term *lipochromes* is derived from this property. The only water-soluble carotenoids are those which, owing to the presence of acidic groups (e.g. carboxyl or enol groups), are able to form water-soluble alkali salts, or have acquired lyophilic properties by esterification with sugar residues (e.g. in crocin).

In view of their general chemical structure, carotenoids may be regarded as a sub-group of the polyene pigments. However, the latter also include pigments not composed of isoprene residues, but containing an unbranched aliphatic chain of conjugated double bonds (e.g. the diphenylpolyenes).

A revised nomenclature for carotenoids has recently been proposed*, but in the present monograph the individual pigments are mostly referred to by the names given to them by their discoverers.

The great interest which the carotenoids have aroused during the last twenty years is conditioned not only by their interesting chemical structure but also by their biological and physiological importance. Several of these pigments are pro-vitamins of vitamin A and thus play an essential part in the animal and human organism. Their significance in the vegetable kingdom has so far been less thoroughly investigated but there can be little doubt that here also they fulfil important functions.

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* The Nomenclature of the Carotenoid Pigments (Report of the Committee on Biochemical Nomenclature of the National Research Council, accepted by the Nomenclature, Spelling and Pronunciation Committee of the American Chemical Society — Chem. Eng. News *24* (1946) 1235. — Report of the 'Commissions de Réforme de la Nomenclature de Chimie organique et de Chimie biologique'. London, July 1947).

As the following chapters will show, between 70 and 80 carotenoids have been found in nature up to the present time. They can all be related to one parent substance, lycopene. By means of simple chemical changes such as cyclisation, double bond migration, partial hydrogenation, introduction of hydroxyl-, keto-, or methoxyl-groups, or introduction of an oxygen bridge, etc., the whole range of pigments can be derived from this parent substance. Their visible light absorption comprises a range of about 300 mμ (ca. 400–700 mμ). The carotenoids thus represent one of the most striking examples of the manifold variation of a parent substance by the vegetable and animal cell.

CHAPTER I

Mode of occurrence of carotenoids in plants and animals
Detection and estimation of carotenoid pigments

1. MODE OF OCCURRENCE IN PLANTS

Relatively little information is available regarding the mode of occurrence of carotenoids in plants. In view of their non-polar nature, the majority of natural polyene pigments are insoluble in water and do not normally occur dissolved in the cell fluid. An exception is provided by crocetin which occurs in the cell fluid in the form of its water-soluble gentiobiose ester, crocin. Water solubility can be conferred not only by esterification with sugars, as in the case of crocin, but also by combination with proteins. Esterification can occur with the carotenoid carboxylic acids (e.g. crocetin, bixin and azafrin), while combination with proteins has so far been observed mainly with polyene pigments (e.g. astacene) occurring in animals. (Compare MENKE[1]).

The majority of vegetable carotenoids occur in the chromatophores. They seldom occur crystalline, but are usually present in colloidal suspension in the cell lipoids or in admixture with solid or semi-solid fats. MENKE[1] has recently found that certain carotenoids in the plastids are combined with proteins; this is in accord with the observations of JUNGE[2] regarding the mode of occurrence of carotenoids in animals. According to GOLDOWSKI and PODOLSKAJA[3], the carotenoids of the sunflower seeds occur in the aqueous and not in the oily phase. This is in contrast to the findings of SAVELLIS[4], according to whom the carotenoids are present in separate lipoid droplets in the chloroplasts. It will be clear from these partly contradictory results that this question has not yet been fully clarified. For further data and examples, reference should be made to the original literature[5].

2. MODE OF OCCURRENCE IN ANIMALS

Many attempts have been made in recent years to determine the fate of carotenoids taken up with the vegetable food by the animal organism. It has

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been found that part of the pigments is excreted unchanged, while the remainder is absorbed. The absorbed carotenoids are either deposited in the fat tissues, nerve tissues, inner organs, etc., or converted into other substances which often fulfil important physiological functions in the animal organism (e.g. vitamin A). In the animal organism, the carotenoids either occur dissolved in fats or combined with protein in the aqueous phase. Colloidal solutions are also observed[6].

A typical example of a carotenoid-protein complex is the astaxanthin proteid, ovoverdin. This water-soluble chromoproteid occurs, for example, in the green eggs of the lobster and in many other crustacea (cf. p. 230). Astaxanthin occurs as the fat-soluble carboxylic acid ester in the red hypodermis of the lobster, and the retina of the chicken also contains at least two different esters of this pigment[7].

JUNGE[8] has recently carried out investigations on the pigments of insects and has observed that many of these appear to be carotenoid-protein complexes. Thus, phytoxanthins (e.g. xanthophyll) as well as epiphasic carotenoids (e.g. β-carotene) have been found to be constituents of such chromoproteids.

The mode of occurrence of carotenoids in blood serum is also of importance. The present view is that the carotenoids here occur in the aqueous phase combined with lipoids and proteins[9].

VON EULER and ADLER[10] have established the occurrence of carotene in the retina. According to experiments by BRUNNER and collaborators[11], the pigment here occurs in the colloidal state.

3. DETECTION AND ESTIMATION

In order to establish the presence of carotenoids in natural sources, the dried materials (e.g. leaves or blossoms) are treated with certain reagents (e.g. concentrated sulphuric acid), which produce characteristic colourations. According to MOLISCH[12], the polyene pigments are best detected by first destroying the surrounding substances, e.g. fats, and only then applying the colour tests. In practice, the material is first treated with concentrated aqueous alcoholic alkali, which dissolves the fats and sets free the carotenoids. At the same time, the phytoxanthin esters* are hydrolysed and the phytoxanthins are liberated. In this way, crystalline carotenoids are often obtained and can be recognised under the microscope. Their presence can also be shown by colour reactions. Recently, however, it has become increasingly usual to isolate the carotenoids first and to characterise them subsequently. For this purpose the micromethod of KUHN and BROCKMANN[13] is often employed. It must be emphasised,

* In nature, phytoxanthins often occur esterified as colour waxes, e.g. physalien, helenien.

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however, that for the complete identification of a carotenoid it is necessary to carry out a chromatographic purification and to isolate the pigment in the crystalline state.

4. COLOUR REACTIONS

The polyene pigments are well known to give blue or violet solutions with a variety of strong acids such as concentrated sulphuric acid, hydrochloric acid, perchloric acid, trichloracetic acid, and with acid chlorides, such as antimony trichloride or arsenic trichloride. These colourations, although not specific, can be used as qualitative tests[14].

(a). Reaction with concentrated sulphuric acid: This reaction is carried out by carefully forming a layer of concentrated sulphuric acid under an ethereal solution of the pigment. The sulphuric acid layer acquires an intense dark blue to blue-violet or, occasionally, greenish-blue colour which disappears on the addition of water[15].

(b). Other strong acids: Fuming nitric acid produces a transient blue colouration. A number of observations have also been published recently regarding the blue colouration produced by concentrated aqueous hydrochloric acid. It appears that the following carotenoids colour concentrated aqueous hydrochloric acid blue:

(i). Aldehydes, e.g. β-citraurin, β-apo-2-carotenal.

(ii). Some carotenoids containing several hydroxyl groups, e.g. fucoxanthin, azafrin.

(iii). Carotenoid epoxides and their furanoid transformation products, e.g. violaxanthin, auroxanthin, xanthophyll epoxide, flavoxanthin, β-carotene diepoxide, aurochrome.

In practice, the hydrochloric acid reaction is carried out in the following way: The pigment is dissolved in a little ether and concentrated aqueous hydrochloric acid is added to the solution. After shaking, the acid layer is coloured blue. With a number of hydrocarbon epoxides, such as α-carotene monoepoxide, the blue colouration is very weak and only persists for a short time. For further details, reference should be made to the description of this reaction in the sections on individual carotenoids.

(c). Antimony trichloride in chloroform solution (Carr-Price reagent):

Similarly to vitamin A, carotenoids give dark blue colourations with the Carr-Price reagent[16]. These blue colourations often have characteristic absorption maxima, and can be used for the quantitative estimation of carotenoids[17].

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5. SPECTROSCOPY

An important part of the characterisation of a carotenoid is the determination of its absorption maxima. (With regard to the relationships between constitution and colour, and extinction curves, cf. page 53). On examining the solution of a carotenoid, usually in carbon disulphide or petroleum ether, in a spectrometer, two or three sharp absorption bands can usually be observed. Their positions can be accurately determined (approximately to within 0.5 mμ) and represent the wavelengths of the absorption maxima. These data are characteristic for each carotenoid and together with other physical constants are used for its identification. (Detailed light absorption data will be quoted in the description of the individual pigments in later sections). By means of the photographic method of determining solution spectra, as developed, for instance, by VON HALBAN, KORTÜM and SZIGETI[18], or by other suitable means, the complete absorption curves can also be determined[19].

6. COLORIMETRY

There are numerous methods for the colorimetric determination of a carotenoid, all of which have in common the comparison of an unknown quantity of the pigment with a standard solution. Potassium dichromate[20], azobenzene[21], bixin[22] and β-carotene[23] have been used as standard substances. Instruments not requiring standard solutions have also been employed[24]. It is necessary to separate the carotenoids before their colorimetric determination, otherwise misleading results are obtained.

7. FLUORESCENCE SPECTRA

Following the determination of the fluorescence spectra of different diphenylpolyenes by HAUSSER and collaborators[25], DHÉRÉ[26] examined vitamin A, β-carotene and lycopene at —180° in this respect. The determination of fluorescence spectra has not, however, found general application.

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