

THE PIGMENTS OF THE ALGAE WITH SPECIAL
REFERENCE TO CERTAIN ORDERS OF THE CHLOROPHYTA

by

MILDRED RUTH PALMER

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS
in the Department of
BIOLOGY AND BOTANY

We accept this thesis as conforming to the
standard required from candidates for the
degree of MASTER OF ARTS.

Members of the Department of

Biology and Botany

THE UNIVERSITY OF BRITISH COLUMBIA

October, 1955

ABSTRACT

The chlorophyll and carotenoid pigments obtained from a number of algae were studied in gross acetone extracts. A method proposed by Richards and Thompson was used to calculate the relative proportions of chlorophylls a, b, and c, astacin and non-astacin carotenoids present. Complete absorption spectra from 350 mμ to 700 mμ were obtained for several algae, chiefly those belonging to the Chlorophyta. It was found that the presence of chlorophyll b could be detected as a small irregularity in the spectral curve at 460 mμ.

The xanthophyll pigments of twenty-eight different species of algae were investigated. Chromatographic columns were used to isolate the individual pigments. A mixture of magnesia (Micron Brand) and Hyflo Super Cel was used as the adsorbent. Ethylene chloride was used as the solvent. Fourteen different xanthophyll pigments were found.

Twenty two species of Green Algae were investigated in an attempt to show that the presence or absence of certain pigments may indicate phylogenetic relationships. Individual xanthophyll pigments appear to be of little phylogenetic significance. Groups of these pigments, however, seem to be more significant. Fairly uniform and closely related groups such as the Zygnematales and the Ulvales contain certain characteristic groups of pigments. Less uniform groups such as the Volvocales and the Cladophorales appear to lack characteristic groups of pigments.

The results agree fairly well with modern phylogenetic relationships, based on the morphology and methods of reproduction. More extensive work with more genera and species is needed before generalizations at the ordinal level can be made.

TABLE OF CONTENTS

ABSTRACT	iii
I. INTRODUCTION	1
II. ACKNOWLEDGEMENTS	3
III. REVIEW OF LITERATURE	3
A. THE CHLOROPHYLLS	3
B. THE PHYCOBILINS	6
C. THE CAROTENOIDS	7
(i) General Distribution of Carotenoids	9
(ii) Distribution of Carotenoids in the Algae	10
IV. GENERAL DESCRIPTION OF METHOD	11
V. PROCEDURE	13
A. GROSS PIGMENT EXTRACTS	13
B. CHROMATOGRAPHIC WORK	14
VI. RESULTS	16
VII. DISCUSSION	22
A. GROSS PIGMENT EXTRACTS	22
B. CHROMATOGRAPHIC WORK	25
VIII. SUMMARY AND CONCLUSIONS	29
IX. BIBLIOGRAPHY	45

THE PIGMENTS OF THE ALGAE WITH SPECIAL
REFERENCE TO CERTAIN ORDERS OF THE CHLOROPHYTA

by

MILDRED RUTH PALMER

I. INTRODUCTION

The pigments of the algae have been used for a long time in the systematics of the algae. The common names of the algal groups such as Red Algae, Brown Algae, Green Algae and Blue-green Algae are indicative of the degree of importance of pigmentation in classification. Of the three main groups of pigments - the green chlorophylls, the orange carotenoids and the blue and red phycobilins - the predominance of at least one of these is responsible for the naming of the different groups. For example, the Red Algae are red because of the predominance of the red phycobilin pigment, phycoerythrin.

The study of this problem was begun initially in order to evaluate and develop a method which might be useful in characterizing marine plankton populations on the basis of pigment analyses. As the research progressed it was finally decided to make a more complete and detailed examination of pigments other than the chlorophylls in the Green Algae (Chlorophyta) and especially in members of the morphologically diverse order Volvocales. Since it has been shown by some authors (Strain, in Smith 1951, p. 251) that the chlorophylls in the Green Algae are consistently chlorophylls a and b, it was decided to examine critically the carotenoid pigments. The

carotenoids are divided into two groups, the carotenes and the xanthophylls. Because the sample size was usually limited, it was impossible to obtain pigment extracts which had sufficient carotene in them to isolate and identify the individual carotene pigments. Hence only the xanthophyll pigments were examined in detail. Since the xanthophyll pigments are, as a group, much more variable than either the chlorophylls or the carotenes, it was hoped that this group might prove useful in comparing different groups of algae from a phylogenetic standpoint.

Many of the samples used in this investigation were obtained from natural blooms, although some unialgal cultures were used. Unialgal or pure cultures are superior to the natural populations because they do not contain other algae as contaminants. However since the size of all the samples obtained from natural blooms was relatively small, in all analyses performed the number of contaminants could be disregarded. Any contamination which may have resulted was so slight that it could not have provided sufficient pigments to be separated on the chromatographic column of the size used.

There was no attempt made to determine the relative concentrations of the different xanthophyll pigments present nor was any attempt made to compare any of the algae quantitatively with respect to these pigments under different environmental conditions.

The bibliography is not intended to be complete, particularly with respect to the older literature on the subject. It is chiefly comprised of references that are most pertinent to this study and includes the more recent and useful literature. The monograph on carotenoids by Karrer and Jucker (1950) has been most invaluable as a reference.

II. ACKNOWLEDGEMENTS

I should like to acknowledge the help and encouragement of my master's committee and particularly that of Dr. R.F. Scagel during the preparation of this thesis. I should also like to thank Dr. M. Kirsh of the Chemistry Department of the University of British Columbia for the use of his Beckman DU Spectrophotometer. Otherwise the facilities used were those of the Department of Biology and Botany at the University of British Columbia and those of the Friday Harbor Laboratories at Friday Harbor, Washington.

III. REVIEW OF LITERATURE

Although this review has resulted from the examination of a large body of literature, most of which is cited in the bibliography, it is based chiefly on a few modern, comprehensive references on the subject (Strain, 1945; Strain, in Frank and Loomis, 1949; Karrer and Jucker, 1950; Strain, in Smith, 1951; Rabinowitch, 1945 and 1955).

The pigments of the algae can be divided into three main groups - the fat soluble chlorophylls, the carotenoids and the water soluble phycobilins. The chlorophylls and carotenoids are distributed universally throughout the photosynthetic plant world. The phycobilins, however, are restricted to two algal divisions, the Cyanophyta and the Rhodophyta.

A. THE CHLOROPHYLLS

There are four different chlorophylls - chlorophyll a, b, c and d - found in algae. Chlorophylls a and b are present also in the higher plant groups and hence are the best known chlorophylls. The structural formulae for both of these chlorophylls has been worked out although

there is still some debate as to the position of the magnesium bonds and the semi-isolated double bond (Rabinowitch 1945, p. 443).

Figures 1 and 2 (p. 4) show the structural formulae for chlorophylls a and b respectively. The asterisk (*) indicates the position of the aldehyde group (-CHO) in chlorophyll b whereas in chlorophyll a there is a methyl group at the same point. This is the only difference in the formulae of the two chlorophylls.

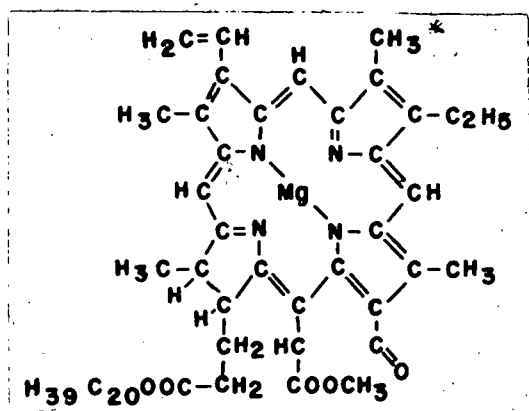


Figure 1. The structural formula of chlorophyll a (after Fisher, in Rabinowitch 1945, p. 442)

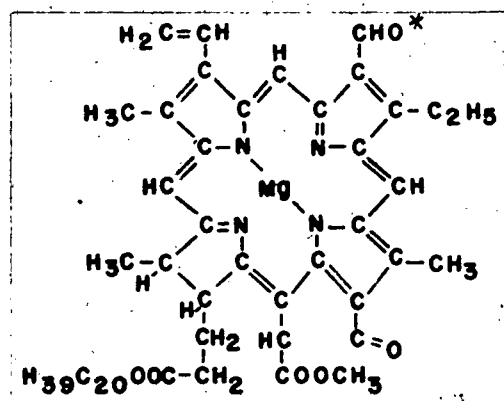


Figure 2. The structural formula of chlorophyll b (after Fischer, in Rabinowitch 1945, p. 442).

Chlorophyll a is present in all photosynthetic plants except certain of the bacteria. The latter contain a related pigment called bacteriochlorophyll. The structure of bacteriochlorophyll which is believed to catalyze photosynthesis, is given in Figure 3 (p. 4).

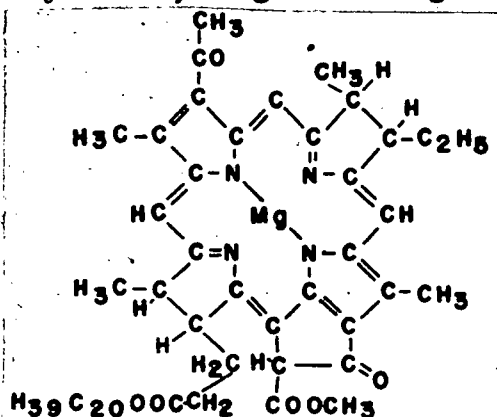


Figure 3. Structural formula of bacteriochlorophyll (after Fischer, in Rabinowitch 1945, p. 445).

Chlorophyll b is present, along with chlorophyll a, in the higher plant groups, the Chlorophyta and the Euglenophyta. The other two chlorophylls, chlorophylls c and d, are more recently isolated pigments. Chlorophyll c occurs in the Phaeophyta, the Pyrrophyta and the Chrysophyta. One alga, Vaucheria, which has been included for a long time in the Chlorophyta and is an exception to practically all the generalizations about the Green Algae, has been removed from that division and placed in the Chrysophyta because it contains chlorophyll c as the accessory chlorophyll instead of chlorophyll b.

Vaucheria is green in colour and is coenocytic, as are all the Siphonales, the group to which it was thought that Vaucheria belonged. It does not store starch, however, as do all the other Chlorophyta; nor does it contain cellulose in the cell walls, as do most of the other Green Algae. In most respects it fits very well in the Chrysophyta.

Chlorophyll c was isolated and shown to be present in the Brown Algae and the Diatoms by Strain and Manning (1942, pp. 625-636). It had been isolated before by other workers (Willstätter and Stoll, 1913) but was thought by them to be an artifact. Later Strain and Manning (1943, pp. 1-19) described another chlorophyll, chlorophyll d, which they had found in the Red Algae. The Cyanophyta or Blue-green Algae contain only chlorophyll a (Strain, in Smith 1951, p. 253).

The absorption spectrum of each of these chlorophylls is distinctive (see Figures 4, 5 and 6, p. 6). It is one of the most helpful characteristics used in the identification of the different pigments.

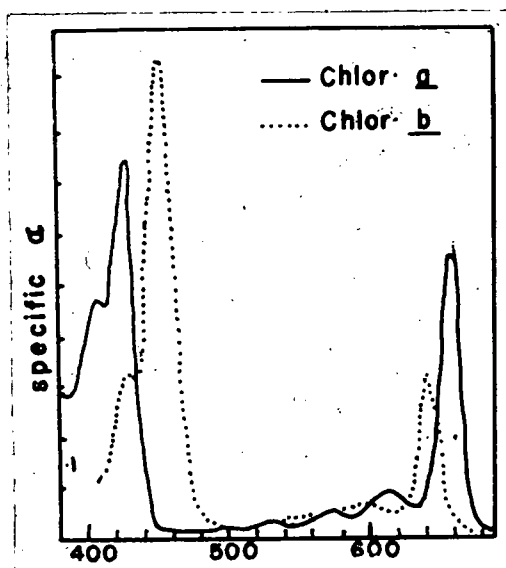


Figure 4. The absorption spectra of chlorophylls a and b in ethyl ether (after Zscheil and Comar 1941, p. 468).

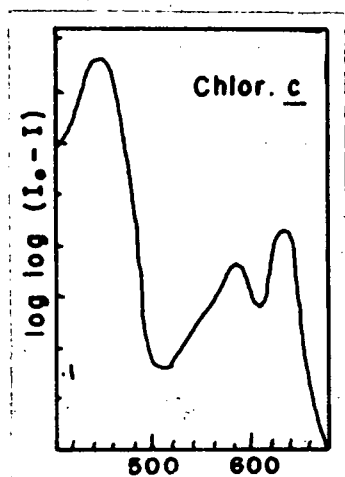


Figure 5. The absorption spectrum of chlorophyll c (after Strain and Manning 1942, p. 633)

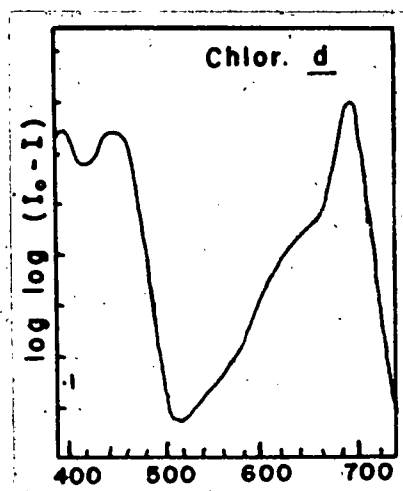


Figure 6. The absorption spectrum of chlorophyll d (after Manning and Strain 1943, p. 7).

B. THE PHYCOBILINS

The phycobilins are water soluble red and blue proteinaceous pigments found in the Red Algae (Rhodophyta) and the Blue-green Algae (Cyanophyta). Originally there were described only four different pigments, c-phycocyanin and c-phycoerythrin from the Cyanophyta and r-phycocyanin and r-phycoerythrin from the Rhodophyta. More recent study, however, has shown

that these pigments are more variable than was previously supposed (Haxo, O'h Eocha and Norris, 1955). Haxo et al found at least four different pigments related to the conventional r- and c-phycobilins.

Chemically the chromophoric group of these pigments is related to the bile pigments. This chromophoric group is attached very strongly to a protein. The pigments appear to be derived from a hypothetical pigment called bilan (Lemberg, in Rabinowitch 1945, p. 477). The structure of bilan is shown in Figure 7 (p. 7).

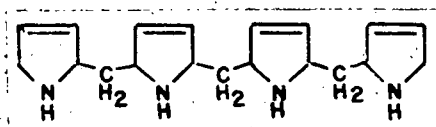


Figure 7. Structural formula of bilan (modified after Lemberg in Rabinowitch, 1945, p. 477).

C. THE CAROTENOIDS

Carotenoids are fat soluble yellow, orange and red pigments. At least eighty different carotenoids are known. They are distributed generally throughout the plant kingdom and are quite widespread throughout the animal kingdom. These pigments are composed basically of eight isoprene units ($\text{CH}_2=\text{CH}.\text{C}(\text{CH}_3)=\text{CH}_2$). It is characteristic of these compounds also that they are reversed at the centre so that the methyl groups occupy the 1:6 position at the centre rather than the 1:5 position. Lycopene, although not common in algae, is a good example of the construction of these carotenoid pigments (see Figure 8, p. 7). Of fifty carotenoid pigments whose empirical formulae are known, forty-five contain forty carbon atoms (Karrer and Jucker 1950, p. 29).

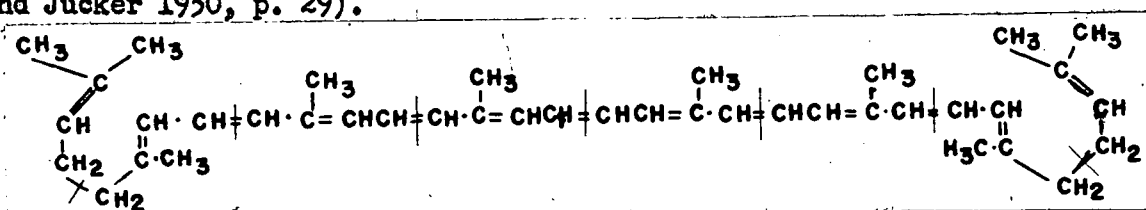


Figure 8. Structural formula of Lycopene.

The nomenclature of these pigments is rather confused, especially with regard to the term xanthophyll. In 1837 Berzelius coined the term xanthophyll for what he believed to be a single yellow pigment found in autumn leaves. Part of the pigment turned out to be the result of the presence of the same pigment as that found in carrot roots. This fraction was first called carotene but is now known to be a mixture of at least two pigments, α and β carotene. The term carotene is fairly well established as the generic name for the unsaturated hydrocarbons such as lycopene.

Willstatter and Stoll (1913) first isolated lutein, later found (Gillam and Heilbron 1935, p. 1064) to be a mixture of lutein and zeaxanthin, from egg yolk. This pigment, lutein, is the same as one of the pigments called xanthophyll by Berzelius (Rabinowitch 1945, p. 470). It is an alcohol and has many isomers. The term carotenol has been suggested to cover these pigments (Hogert, in Rabinowitch 1945, p. 471) with the main pigment of the class called luteol. Karrer and Jucker (1950) give the generic name as phytoxanthins and call the main pigment xanthophyll. Strain (in Smith 1951, p. 245) suggests xanthophyll as the generic name of the oxygen derivatives of the carotenes, and calls the chief pigment of this class lutein. None of this nomenclature is perfect. Carotenol leaves out all the oxygen derivatives which are not alcohols. The term phytoxanthins suggests that these are plant pigments only. Xanthophyll implies that the pigments occur only in leaves. The last two mentioned do include all the oxygen derivatives of the carotenes, and it is just a matter of preference which is used. The terminology suggested by Strain has been followed in this thesis.

The carotenoid pigments owe their colour properties to the large number of conjugated double bonds in the molecule. There are, as a rule,

eleven or more double bonds of which at least nine are conjugated. The individual pigments have characteristic absorption spectra. As a whole they have absorption spectra with three peaks between 400 mμ and 500 mμ, the centre one being the highest. As more double bonds enter the conjugated system, all of these peaks are displaced towards the red end of the spectrum. The addition of one double bond to the conjugated system displaces the maxima 20-22 mμ towards the red. The addition of an isolated ethylenic bond displaces the maxima 9-11 mμ toward the red. If a carbonyl group enters the conjugated system the effect is very pronounced, resulting in a shifting of the maxima about 40 mμ towards the red. These changes occur only in the terminal rings. If one ring is closed the maxima are displaced 4-5 mμ toward the blue. Introduction of a hydroxyl group has very little effect (1-2 mμ). Replacing a conjugated double bond with an epoxide group shifts the maxima 6-9 mμ towards the blue.

Most of these pigments are present as the trans-isomer but there are a few with one cis double bond. A change from the trans-isomer to the cis form shifts the maxima 3-4 mμ towards the shorter wave lengths. Cis-trans-isomerization is readily induced by treating a solution of the pigment with strong mineral acids or heat (Karrer and Jucker 1950, p. 28).

(1) General Distribution of Carotenoids

The carotenoid pigments are distributed throughout the plant kingdom. They are present in coloured bacteria, coloured fungi, etiolated seedlings and all plants containing chlorophyll. How the plants form these pigments and what use they have for them is obscure although numerous investigations have been carried out to find the answer to these problems.

In animals these carotenoids are present sometimes in the form of what might be called provitamins (Karrer and Jucker 1950, pp. 11-15). One

molecule of β carotene plus two molecules of water will give two molecules of vitamin A (l.c. pp. 11-15). The pigments of the retina also appear to be carotenoid in character (Karrer and Jucker 1950, pp. 16-17).

Carotenoids are widely distributed throughout the animal world as colouring matter. Arthropods, molluscs, echinoderms, nemertians, polychaetes, bryozoans, brachiopods, coelenterates, sponges, tunicates, fish, amphibians, reptiles, birds and mammals all contain carotenoids of one sort or another (Karrer and Jucker 1950, pp. 66-99).

(ii) Distribution of Carotenoids in the Algae

The algae as a whole contain an interesting assemblage of carotenoids. The Chlorophyta contain α and β carotene in varying amounts and up to half a dozen or more different xanthophylls. The xanthophylls are in general the same as those found in the higher plants. In addition to being found in higher plants the following are also found in the algae: lutein (usually about half the total xanthophylls present), violaxanthin, flavoxanthin, neoxanthin, zeaxanthin, and possibly some cryptoxanthin* (Strain, in Smith 1951, p. 251).

The Siphonales, a coenocytic order of the Chlorophyta, contain two carotenoids, namely siphonein and siphonaxanthin (Strain, in Smith 1951, p. 251) which have not been found in any other order of Green Algae. This order also contains α carotene as the chief carotene, in contrast to most other plants where β carotene is the most common.

The Phaeophyta and the Bacillariophyceae in the Chrysophyta contain β carotene and a number of xanthophylls found nowhere else in the plant kingdom. These pigments are fucoxanthin, which is very abundant, neofucoxanthin A and B. The Brown Algae also contain violaxanthin, flavoxanthin and neo-

*Cryptoxanthin was never found during the course of this research.

xanthin. The Diatoms (Bacillariophyceae) have diatoxanthin and diadinoxanthin. The Dinophyceae in the Pyrrophyta contain peridinin, which is the most abundant xanthophyll present, diadinoxanthin, dinoxanthin, and neodinoxanthin (Strain, in Smith 1951, p. 253), (see Table 1, p. 11).

Pigment	Phaeophyceae	Bacillariophyceae	Dinophyceae
fucoxanthin	*	*	-
neofucoxanthin A	*	*	-
neofucoxanthin B	*	*	-
violaxanthin	*	-	-
flavoxanthin	*	-	-
neoxanthin	*	-	-
diatoxanthin	-	*	-
diadinoxanthin	-	*	*
peridinin	-	-	*
dinoxanthin	-	-	*
neodinoxanthin	-	-	*

Key * present
- not present

Table 1. The distribution of xanthophyll pigments in the Phaeophyceae, the Bacillariophyceae and the Dinophyceae (modified after Strain, in Smith 1951, p. 253).

The Red Algae as a rule have only one or two xanthophylls, one of which is always lutein. The Cyanophyta (Myxophyta of some authors) contain myxoxanthin and myxoxanthophyll. Fucoxanthin has been reported in Poly-siphonia nigrescens and Callithamnion pikeanum (Carter, Heilbron and Lythgoe 1939, p. 193). Both these Red Algae are much branched, filamentous forms and are commonly covered with diatoms. Until these algae can be obtained in a diatom free state, the presence of fucoxanthin in Red Algae must be regarded as questionable.

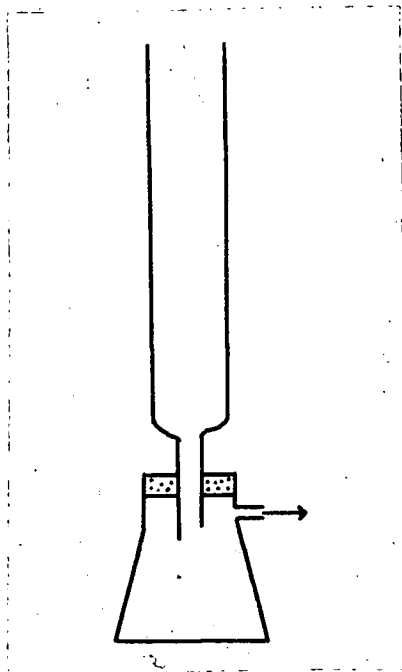
IV. GENERAL DESCRIPTION OF METHOD

Two general methods were used in the work. First was the method of Richards and Thompson (1952) for characterising plankton populations using

gross pigment extracts. This will be discussed more fully under the section dealing with procedure.

Second was the method chosen for the separation of the xanthophyll pigments. This was the chromatographic column. Because these xanthophyll pigments are so similar chemically the standard purification methods of separation using immiscible solvents followed by recrystallization are very unsatisfactory unless they are used in conjunction with a highly sensitive method such as chromatography. So far chromatography is the only method known which will permit separation of these pigments in a pure enough state to identify them.

The chromatographic column is made up of a finely divided material which is packed into a tube such as the one shown in Figure 9 (p. 12). The



adsorbing properties of the material used must be considered in selecting a suitable adsorbent. The materials to be selected as adsorbents vary greatly in their adsorption properties. Substances such as powdered sucrose or starch have weak adsorption properties whereas those such as magnesia (Micron Brand) or charcoal are the most active adsorbents.

The adsorption capacity of all adsorbents, however, depends on the solvent used. Adsorption is greatest from saturated hydrocarbons such as petroleum ether, less from cyclic

hydrocarbons and chlorinated hydrocarbons such as benzene and ethylene chloride, still less from alcohols and least of all from water.

The combination of adsorbent and solvent used is of great importance

and the choice is made through references to the literature and through the trial and error method. In this research a combination of magnesia and ethylene chloride was found most satisfactory. Powdered sugar (C & H Brand), petroleum ether and benzene were tried but did not yield good results. Sometimes a little methanol (1% to 5%) was added to the ethylene chloride to speed up the developing process.

V. PROCEDURE

The samples used for this work were collected from freshwater lakes, streams, ponds, ditches and puddles, from various marine substrata at low tide, from floats, and from unialgal cultures. The organisms were identified to genus and wherever possible to species.

A. GROSS PIGMENT EXTRACTS

As a rule a sample of not more than 50 ml. of material was required for a complete pigment analysis using the method proposed by Richards and Thompson (1952). The organisms were concentrated by filtering the sample through a millipore aerosol filter which was then dried in a vacuum dessicator. The sample was then extracted for twelve hours in the refrigerator with 5 ml. of acetone. The extract was decanted after centrifuging to remove the cells and undissolved filter.

From this extract a complete spectral analysis between wave lengths 350 mμ to 700 mμ was recorded, reading optical densities at every 10 mμ. A Beckman DU Spectrophotometer was used for these readings. Where samples containing a mixture of pigments were analysed the calculations were made according to formulae given by Richards and Thompson (1952, p. 158), to determine the relative amounts of chlorophylls a, b, c, astacin and non-astacin carotenoids present.

For those samples used only to calculate these five pigments or pigment groups, optical density readings were taken at 480 mμ, 510 mμ, 630 mμ, 645 mμ and 665 mμ. These wavelengths represent the points of least interference in the curves for chlorophylls a, b, and c, astacin and non-astacin carotenoids respectively.

B. CHROMATOGRAPHIC WORK

In preparing the sample for extraction, several different methods were used to remove all the excess water from the plants. Unicellular forms were centrifuged with a Foerst continuous flow centrifuge. This method yielded samples having a volume of from one to two millilitres of cells. Filamentous forms were washed in tap water to remove as many contaminants, both plant and animal, as possible, then squeezed dry and extracted. Samples weighed from five to ten grams when treated in this way.

The samples were extracted for two to ten hours (depending on the ease of extraction) in the dark using absolute methanol as the extractant. Methanol is a useful solvent because it is water soluble and will thus penetrate the cells of the fresh organisms and extract the soluble carotenoids and chlorophylls.

After extraction the solution was filtered and then saponified with alcoholic KOH (15 gm. KOH to 150 ml. methanol solution). Saponification makes the chlorophylls water soluble and releases any of the carotenoid pigments which are present as esters.

The extract was diluted with about 400 ml. of concentrated sodium chloride solution and the carotenoid pigments removed with ethyl ether. The ether solution was washed with distilled water (to remove the methanol) and then dried with calcium chloride. The resulting ethereal solution was then evaporated under vacuum until all of the ether was gone. The pigments

obtained were then redissolved in a small amount of ethylene chloride ($\text{CH}_2\text{ClCH}_2\text{Cl}$) and this solution was poured through a column containing a mixture of magnesia (Micron Brand) and Hyflo Super Cel in equal amounts. The column was then washed with fresh ethylene chloride. If the pigments were strongly adsorbed it was usually found better to develop the column with ethylene chloride to which 1 to 5% methanol was added. If the pigments were less strongly adsorbed, ethylene chloride alone was found satisfactory. When the pigments separated into distinct bands the column was sucked almost to dryness. Best results were obtained when the chromatographs were run under suction provided by a high vacuum pump.

At this point the column was removed from its container and the pigment bands were separated manually. The pigments were eluted with 95% ethanol and the magnesia and Hyflo Super Cel were removed by centrifuging for five minutes at about 4000 rpm.

Packing the column, according to the literature (Strain 1945, p. 41; Karrer and Jucker 1950, p. 27) is a critical feature in making a satisfactory chromatographic column. According to these authorities it must be done by pouring in about one centimeter of adsorbent at a time and tamping it down with a rod of some sort. This procedure was tried but gave unsatisfactory results. When the columns were prepared in this manner, the filtration rate was very slow, the pigments would not separate and the column could not be removed from the container.

The method finally used was as follows. Half an inch of adsorbent cotton was placed at the bottom of the container so that the material would not run right through the tube. About one centimeter of adsorbent at a time was placed in the tube and the suction flask under the column was struck on the table a few times following each addition so that the adsorbent settled

into a fairly compact column. Suction was applied to help pack the column. The final portion of adsorbent at the top of the column was tamped down with a cork on a rod. This gave a fairly firm surface on which the pigment mixture and developer could be poured. The columns were 2.5 centimeters in diameter and from 10 to 20 cm. long. The length varied directly with the number and type of pigments to be separated. The columns packed in this manner gave good results and required only about five minutes to prepare. The columns required from five to twenty minutes to develop.

Having obtained the ethanolic solution of the pigments with the procedure outlined above, a complete absorption spectrum was run on each pigment on the Beckman DU Spectrophotometer. Optical density readings were taken at 350 m μ through to about 500 m μ . When approaching the maxima, readings of optical density were taken at every 2 m μ on the scale, this being the readable limit of the Beckman Spectrophotometer between 400 m μ and 500 m μ . Between the maxima, readings were taken at every 4 m μ and near the extremities of the curves at every 10 m μ . These readings were graphed with the wave lengths as the abscissa and the optical density as the ordinate.

Identification of the pigments was based primarily on the absorption curve and the position of the maxima. The following features were also used in identifying the pigments: the shape of the absorption curve, the position and colour of the pigment on the column and the reaction of a diethyl ether solution of the pigment with 37% HCl. With some pigments the acid layer turns blue (Karrer and Jucker 1950, p. 7).

VI. RESULTS

The following table shows the results obtained using the method outlined by Richards and Thompson (1952, pp. 156-172). The numbers indicate the relative concentrations of chlorophylls a, b and c, astacin and non-

astacin carotenoids.

<u>Alga</u>	<u>Chloro- phyll a</u>	<u>Chloro- phyll b</u>	<u>Chloro- phyll c</u>	<u>Astacin</u>	<u>Non-astacin</u>
<u>CHLOROPHYTA</u>					
Order VOLVOCALES					
<u>Dunaliella</u>	5.291	.730	.593	---	3.146
<u>Chlamydomonas</u>	6.391	2.703	3.042	---	2.903
<u>Haematococcus</u>	4.784	1.991	2.120	3.602	1.497
<u>Volvox</u>	4.089	1.874	2.293	---	2.347
Order CHLOROCOCCALES					
<u>Chlorella</u>	1.796	.328	.790	---	1.626
Order ULOTRICHALES					
<u>Chaetophora</u>	1.913	.613	.116	---	.745
Order ULVALES					
<u>Ulva</u>	5.314	3.103	4.264	---	1.115
<u>CHRYSTOPHYTA</u>					
Class BACILLARIOPHYCEAE					
<u>Fragilaria</u>	4.332	.956	2.943	.106	1.915

Table 2. Relative concentrations of Chlorophyll a, b, c and astacin and non-astacin carotenoids as calculated from formulae given by Richards and Thompson (1952, p. 158).

Figures 10 to 16 show the curves obtained from spectrophotometric analyses of gross pigment extracts in 90% acetone. Some of the data in Table 1 have been obtained from these same extracts.

The following table, Table 3, contains the names (where known), the structural formulae (where known), the absorption maxima, the absorption spectra and the reactions with HCl of all the pigments studied and discussed in this thesis. The maxima, unless otherwise indicated, are given for ethanolic solutions of the pigments and those maxima from the literature, unless otherwise indicated, are from Karrer and Jucker (1950).

Table 3. The characterization of the different xanthophyll pigments found during the work for this thesis.
(continued on page 19)

<u>PIGMENT</u>	<u>MAXIMA (in mμ)</u>		<u>REACTION WITH 37% HCl</u>	<u>COMMENTS</u>
	<u>Literature</u>	<u>Found</u>		
Lutein (17, 23)*	476, 446.5, 420	476, 446, indistinct	no reaction	Forms a diffuse yellow orange band on the adsorption column which moves down the column fairly rapidly.
Violaxanthin (18, 24)	471.5, 442.5, 417.5	472, 442, 418	stable blue colouration	Forms a fairly sharp band above lutein and is orange yellow in colour.
Taraxanthin (25)	472, 443, 418	472, 442, 418	no reaction	Forms a sharp yellow band above violaxanthin.
Neoxanthin (26)	467, 437, 415	467, 437, 414	stable blue colouration	Forms a sharp, bright yellow band near the top of the column.
Flavoxanthin (19, 27 a and b)	448, 421, not given 452, 422, not given (Strain, 1938)	452, 424, 402	stable blue colouration	Forms a pale yellow band just below neoxanthin on the column.
Zeaxanthin (20, 28)	483, 451, 423.5 482, 452 (Strain, 1945) 479, 452 (Zscheile et al, 1942)	480, 451	no reaction	This pigment moves fairly rapidly down the column separating just above the lutein band. It is red orange in colour and quite distinctive.
Astacene (21, 29)	510 (in CS ₂) 500 (in pyridine) 480 (approximately in ethanol)	478	no reaction	This pigment is usually found in animals (Kuhn and Lederer, 1933, p. 488), although it is also found in <u>Euglena</u> and <u>Haematococcus</u> (Tischer, 1944). It moves rapidly through the column and the band is a bright pink.

Table 3. The characterization of the different xanthophyll pigments found during the work for this thesis (concluded on page 20).

<u>PIGMENT</u>	<u>MAXIMA (in mμ)</u>		<u>REACTION WITH 37% HCl</u>	<u>COMMENTS</u>
	<u>Literature</u>	<u>Found</u>		
Auroxanthin (22, 30 a and b)	430, 402, 382	430, 404, 383	light blue colour	This pigment is strongly adsorbed and forms a light yellow band at the top of the column. The adsorption spectrum is very similar to that recorded for auroxanthin. However, according to Karrer and Jucker (1950, p. 197) auroxanthin characteristically forms a blue colour with 15% HCl. This pigment does not. In spite of this difference it was tentatively decided to refer to this pigment obtained from <u>Stigeoclonium farotum</u> and <u>Spongomorpha coalita</u> as auroxanthin.
Unknown Xanthophyll 1 (31)		472, 451	no reaction	This pigment occurs as a diffuse red orange band above the lutein band. It separates slowly from lutein and has only been found in red algae of the Bangioideae.
Unknown Xanthophyll 2 (32)	(472,)	472, 446	no reaction	This pigment separates slowly from neoxanthin when 2% methanol is added to the ethylene chloride when developing. It forms a fairly sharp orange band and never occurs in large amounts.
Unknown Xanthophyll 3 (33)		472, 444, 420	blue colouration	Forms a yellow orange band near the middle of the column.
Unknown Xanthophyll 4 (34)		472, 445, 424	no reaction	This pigment forms an orange band below lutein on the column. It was found only in <u>Spongomorpha coalita</u> .

<u>PIGMENT</u>	<u>MAXIMA (in mμ)</u>		<u>REACTION WITH 37% HCl</u>	<u>COMMENTS</u>
	<u>Literature</u>	<u>Found</u>		
Unknown Xanthophyll 5 (35)		479, 450	no blue colour- ation but acid layer yellow.	This pigment forms a diffuse yellow orange band, very much like that of lutein, below zeaxanthin on the column.
Unknown Xanthophyll 6 (36)		477, 447, 424	-----	This pigment was only found once in this survey.

Table 3. The characterization of the different xanthophyll pigments found during the work for this thesis.

*The Figure numbers of the structural formulae and the absorption spectra respectively are given in brackets after the name of the pigment. Where only one Figure number is given it is for the absorption spectrum.

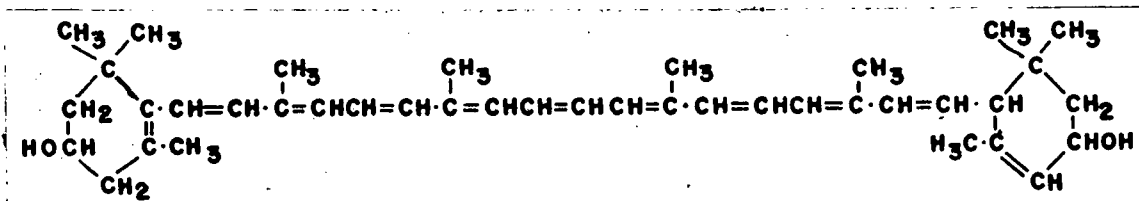


Figure 17. Structural formula of lutein.

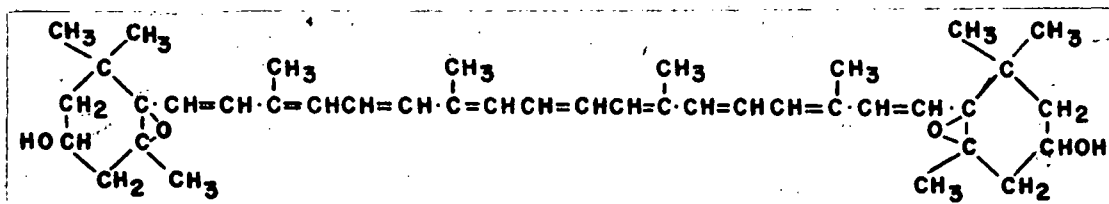


Figure 18. Structural formula of violaxanthin.

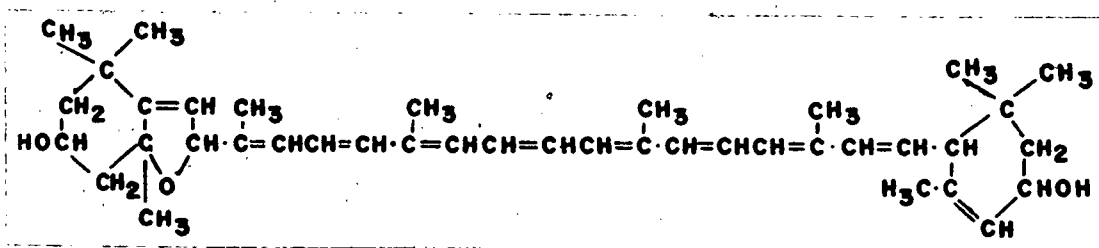


Figure 19. Structural formula of flavoxanthin.

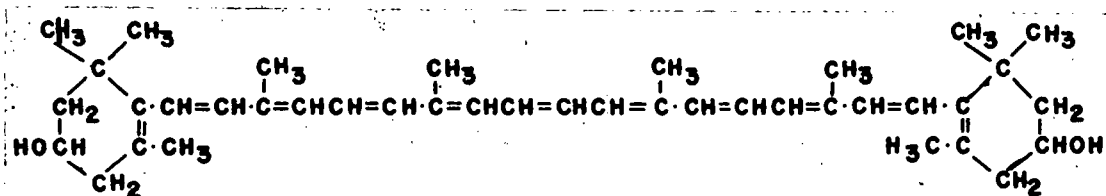


Figure 20. Structural formula of zeaxanthin.

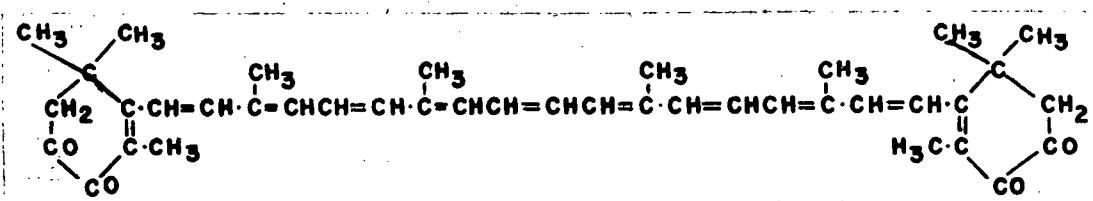


Figure 21. Structural formula of astacene.

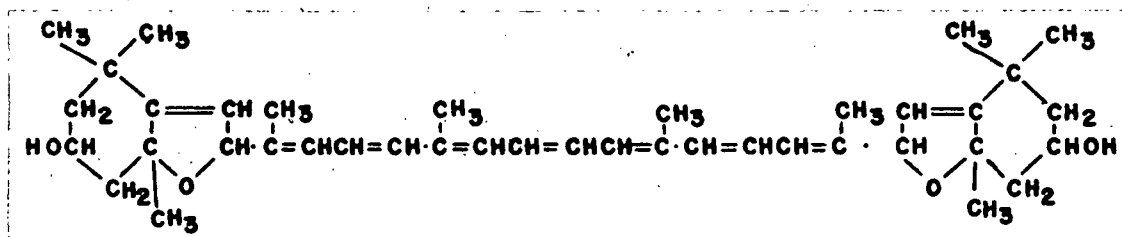


Figure 22. Structural formula of auroxanthin.

For the distribution in the algae of the fourteen pigments studied in this research and summarized in Table 3 (pp. 18-20), see Table 4 (pp. 23 and 24).

VII. DISCUSSION

A. GROSS PIGMENT EXTRACTS

The method proposed by Richards and Thompson (1952) was initially devised for the characterization of plankton populations in the ocean. Its advantage lies in the fact that only small samples (1-2 litres) of seawater are required. Diatoms (Bacillariophyceae) and Dinoflagellates (Pyrrophyta) apparently make up the major part of the phytoplankton of the sea. Since these contain only chlorophyll c as an accessory chlorophyll it can be assumed that whenever any chlorophyll b is indicated by this method there are probably some green flagellates in the nanoplankton. Since these flagellates usually disintegrate with the customary methods used at sea for preservation of plankton, it would be useful if this method could be adopted to give an indication of their presence. It was hoped that this method would be of some use in this research and thus eliminate the tedious procedure of separating the pigments individually. Unfortunately the data obtained from the few samples analysed in this way show that it is difficult to interpret the results obtained. Whenever chlorophyll b was present, as undoubtedly it is in Ulva, very high readings were obtained for chlorophyll c. It is impossible that a species of Ulva could be so contaminated with diatoms that the chlorophyll c content would be almost as high as the chlorophyll a, especially since the diatoms also contain chlorophyll a as their major chlorophyll. Hence, it appears that the presence of any chlorophyll b interferes with the chlorophyll c

Table 4. Distribution of xanthophyll pigments in the different algae examined.

ALGA	Lutein	Violaxanthin	Peraxanthin	Neoxanthin	Flavoxanthin	Zeaxanthin	Astacene	Auroxanthin	Unknown 1	Unknown 2	Unknown 3	Unknown 4	Unknown 5	Unknown 6
<u>CHLOROPHYTA</u>														
Order VOLVOCALES														
<u>Phacotus</u> sp.	-	-	*	-	-	*	-	-	-	-	*	-	-	-
<u>Lobomonas</u> pyriformis	-	*	*	-	-	*	-	-	-	-	-	-	-	-
<u>Volvox</u> sp.	*	*	-	*	-	-	-	-	-	-	-	-	-	-
Order CHLOROCOCCALES														
<u>Chlorella</u> sp.	*	*	-	*	-	-	-	-	-	-	-	-	-	*
<u>Pediastrum</u> boryanum	-	-	*	-	-	*	-	-	-	-	-	-	*	-
Order ULOTRICHALES														
<u>Stigeoclonium</u> farctum	-	-	-	-	*	*	-	*	-	-	*	-	*	*
<u>Draparnaldia</u> sp.	*	-	-	*	-	-	-	-	-	-	-	-	-	-
Order OEDOGONIALES														
<u>Oedogonium</u> sp.	-	-	-	*	-	-	-	-	-	*	*	-	-	-
Order ZYGNEATALES														
<u>Spirogyra</u> varians	-	-	*	-	*	*	-	-	-	-	-	-	*	-
<u>Spirogyra</u> sp.	*	-	-	*	-	*	-	-	-	*	-	-	-	-
<u>Zygonium</u> ericetorum	-	-	-	-	-	*	-	-	-	-	-	-	*	-
<u>Sirogonium</u> sp.	-	-	-	-	*	*	-	-	-	-	-	-	*	-
<u>Mougeotia</u> sp.	-	*	-	-	*	*	-	-	-	-	-	-	*	-
<u>Mougeotia</u> sp.	-	*	-	-	*	*	-	-	-	-	-	-	*	-
Order GLADOPHORALES														
<u>Spongomorpha</u> coalita	*	-	-	*	*	-	-	*	-	-	-	*	-	-
<u>Urospora</u> penicilliformis	*	*	-	*	*	-	-	-	-	*	-	-	-	-
Order SCHIZOGONIALES														
<u>Prasiola</u> meridionalis	*	*	-	*	-	*	-	-	-	*	-	-	-	-

ALGA	Lutein	Violaxanthin	Taraxanthin	Neoxanthin	Flavoxanthin	Zeaxanthin	Astacene	Auroxanthin	Unknown 1	Unknown 2	Unknown 3	Unknown 4	Unknown 5	Unknown 6
Order ULVALES														
<u>Enteromorpha intestinalis</u>	*	*	-	*	-	-	-	-	-	-	-	-	-	-
<u>Enteromorpha linza</u>	*	*	-	*	-	-	-	-	-	-	-	-	-	-
<u>Ulva expansa</u>	*	*	-	*	*	-	-	-	-	-	*	-	-	-
<u>Ulva sp.</u>	*	*	-	*	*	-	-	-	-	-	-	-	-	-
<u>Ulva stenophylla</u>	*	*	-	*	-	*	-	-	-	-	-	-	-	-
<u>EUGLENOPHYTA</u>														
<u>Euglena sp.</u>	*	-	-	-	-	*	*	-	-	-	-	-	-	-
<u>CHRYSOPHYTA</u>														
Class XANTHOPHYCEAE														
<u>Vaucheria sp.</u>	*	-	-	-	*	*	-	-	-	*	-	-	-	-
<u>RHODOPHYTA</u>														
Subclass BANGIOIDEAE														
<u>Bangia fucospurpurea</u>	*	-	-	-	-	-	-	-	*	-	-	-	-	-
<u>Porphyra perforata</u>	*	-	-	-	-	-	-	-	*	-	-	-	-	-
Subclass FLORIDEAE														
<u>Antithamnion pacifica</u>	*	-	-	-	-	-	-	-	-	-	-	-	-	-
<u>Iridaea heterocarpum</u>	*	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4 (concluded). Distribution of xanthophyll pigments in the different algae examined.

Key * present
- absent

reading to such an extent that the values obtained from the readings are very incorrect, even after the corrections introduced by Richards and Thompson (1952, p. 158) have been applied. And conversely, although not to such an extent, any chlorophyll c present will result in excess chlorophyll b values. It is therefore necessary to be extremely cautious in drawing conclusions from data obtained using the method proposed by Richards and Thompson.

In spite of the limitations apparent in the method, certain differences are suggested by comparing the complete absorption spectra of mixed pigment extracts. There is a distinct irregularity in the curve between 460 mμ and 470 mμ in some instances. At this point chlorophyll b has a maximum. In most of the Green Algae that were analyzed (Figures 10-13) this point showed up more or less clearly. In analyses of samples of the genus Dunaliella, however, there is no chlorophyll b maximum (Figure 14). Even from the calculation for chlorophyll b using Richards and Thompson's formulae very little chlorophyll b or c was indicated in this alga. Dunaliella is generally classified in an order, the Volvocales, of the Chlorophyta. Since the Chlorophyta generally contain chlorophyll b as the accessory chlorophyll, one would expect to find chlorophyll b in this alga. It is possible that chlorophyll b is present in very small quantities in the flagellate, but since no chromatographic study of its pigments has been made, it is impossible to say how valid this assumption may be.

B. CHROMATOGRAPHIC WORK

The Volvocales, as a group, seem to be quite variable in their pigmentation, both with respect to their chlorophylls and xanthophylls. Three genera, Phacotus, Lobomonas, and Volvox, in this group were studied with respect to their xanthophyll pigments and it was found that they were

rather diversified. Of the three genera examined each had three different xanthophylls. Phacotus and Lobomonas had two pigments in common, violaxanthin and zeaxanthin. Volvox had one pigment, taraxanthin, in common with Lobomonas, and none the same as those of Phacotus.

The Volvocales are in many respects a rather diverse group. Vegetatively they vary from unicellular bi- or quadra-flagellate forms through colonies of many shapes. The colonies may be flat- plates such as Gonium - or spherical colonies such as Pandorina, or hollow spheres such as Volvox. The reproductive cells vary from isogamy in Dunaliella, through heterogamy in many of the Chlamydomonas species, to oogamy in Volvox. It is perhaps reasonable to find that their pigments also are rather variable.

Genera which appear to be morphologically uniform and fairly closely related, such as the members of the Zygnematales, contain quite similar pigments. The Zygnematales is an order of Green Algae, which, although fairly diverse morphologically, possesses a unique method of sexual reproduction called conjugation. The order consists of two groups, the filamentous forms such as Spirogyra and Zygnema and the unicellular and colonial Desmids.

In this order four different genera, Spirogyra, Zygagonium, Sirogonium and Mougeotia, and six different species were examined. It was found that zeaxanthin was always present, and unknown xanthophyll, xanthophyll 5, was present in five out of six species and lutein was absent in the same five out of six entities. Xanthophyll 5 apparently can take the place of lutein. The absence of lutein in these organisms is of interest because, according to Strain (in Smith, 1951, pp. 251 and 253) lutein is always present in the Chlorophyta and usually as the major xanthophyll. It was lacking in Phacotus (Volvocales), Lobomonas (Volvocales), Stigeoclonium farctum (Ulotrichales) and Pediastrum boryanum (Chlorococcales).

The closely related marine genera Ulva and Enteromorpha were found to contain very similar pigments, although Ulva usually had one or two more carotenoids than the more primitive Enteromorpha. Prasiola, which is an alga having a parenchymatous organization, in appearance very much like a diminutive Ulva, grows along the seashore in the splash zone on rocks where there is a plentiful supply of seagull droppings. There has been some debate (Fritsch 1935, p. 220) concerning the order in which Prasiola should be placed. Some phycologists have placed it in the Ulotricales (Fritsch 1935, p. 217) and others have removed it to an order of its own, the Schizogoniales (Smith 1950, p. 195). A study of the pigments in Prasiola reveals that this plant contains the same three pigments (lutein, violaxanthin and neoxanthin) found in Enteromorpha, but it possesses two additional pigments (zeaxanthin and an unknown xanthophyll, xanthophyll 3) not found in any of the members of the Ulvales studied. This suggests a common origin, at least of the Ulvales and the Schizogoniales. Although these three pigments - lutein, violaxanthin and neoxanthin - are distributed quite commonly throughout the Green Algae, they occur together only in three other algae examined; namely, Volvox sp., Chlorella sp., and Urospora penicilliformis.

Figure 37 (p. 28) which illustrates a generally accepted modern phylogenetic arrangement (after Papenfuss 1951, p. 6) of the orders of the Green Algae, indicates that the Volvocales (Volvox) and the Chlorococcales (Chlorella) are in the evolutionary lines leading to the Ulvales and the Cladophorales (Urospora).

The xanthophyll pigments of these algae could be used to support the arrangement in Figure 37. However the pigments of other genera studied in these orders do not support the arrangement as well. More exhaustive study of more species and genera would have to be done before any definite conclusions about the phylogenetic relationships of these orders could be

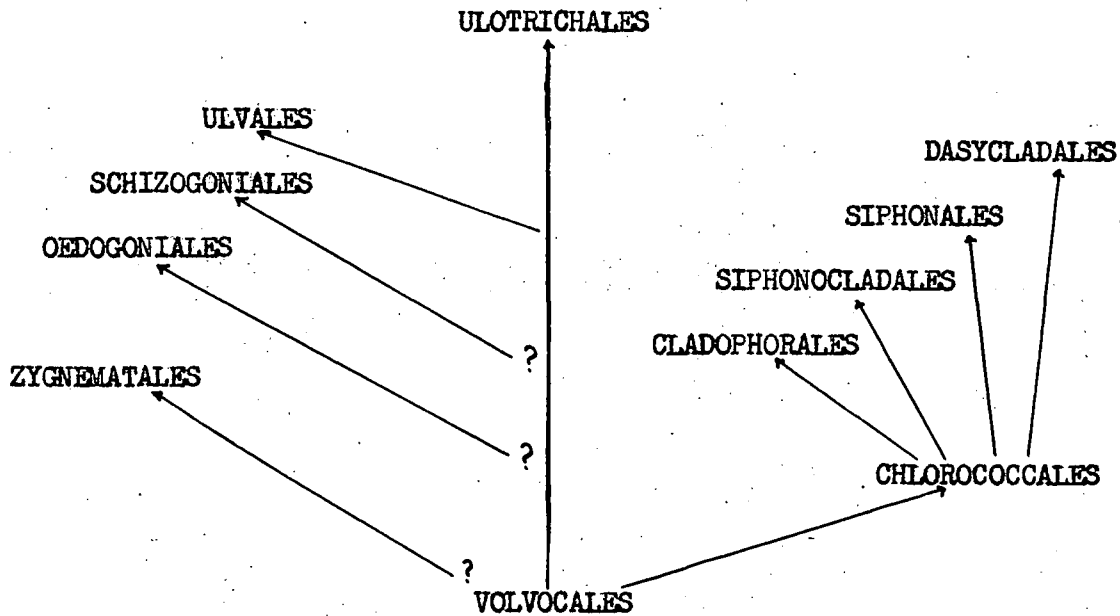


Figure 37. Diagrammatic representation of the probable inter-relationships of the orders of Green Algae (Papenfuss 1951, p. 6).

drawn at the ordinal level using the xanthophyll pigments as a criterion.

It is probable that the presence of any individual xanthophyll pigment has little phylogenetic significance. The pigments are very similar chemically and perhaps somewhat interchangeable physiologically. The differences between any two xanthophylls is sometimes not very great chemically and could possibly result from a single gene change (mutation) in the nucleus. If more were known about the function and mode of formation of the xanthophyll pigments, speculation about their phylogenetic significance would probably be more fruitful.

While the presence of a single xanthophyll has probably little phylogenetic importance, the consistent presence of two or more xanthophylls as in the case of the Ulvales and Prasiola meridionalis (Schizogoniales), is possibly significant. This is also true of the Zygnematales where zeaxanthin and Unknown xanthophyll 5 were almost always present together. However these two pigments were found together in the unrelated Pediastrum boryanum

(Chlorococcales) and Stigeoclonium farctum (Ulotrichales).

Lutein occurs commonly in the Rhodophyta and is frequently the only xanthophyll present (Strain, in Smith 1951, p. 253; Carter et al 1939, p. 102). The two members of the Bangioideae which were examined, Bangia fucospurpurea and Porphyra perforata, contained lutein and one other unidentified xanthophyll, Unknown xanthophyll 1. Unfortunately there were no other members of this group available for a comparative study.

VIII. SUMMARY AND CONCLUSIONS

The Richards and Thompson method of characterizing plankton populations by gross pigment analyses requires more thorough investigation before results obtained by this method can be interpreted with any degree of confidence. This study shows, however, that the complete absorption spectra of gross pigment extracts will frequently indicate the presence or absence of certain pigments, particularly chlorophyll b.

The carotenoid pigments of the Green Algae are surprisingly consistent considering the great variation in size, form, cell structure and the methods of reproduction found in this group. Orders such as the Volvocales, in which there is much morphological and reproductive diversity, appear to be quite variable in their pigmentation. Three genera, Phacotus, Lobomonas and Volvox, in the Volvocales were examined. Each had three different xanthophyll pigments with a total of six different xanthophylls in the group. Phacotus and Lobomonas had two xanthophyll pigments (taraxanthin and zeaxanthin) in common. Volvox had one pigment (violaxanthin) in common with Lobomonas and none the same as those of Phacotus.

The orders Zygnematales and Ulvales, in which the method of sexual reproduction is fairly uniform, were found to contain groups of two or three xanthophyll pigments fairly regularly. The Zygnematales usually contained

zeaxanthin and Unknown xanthophyll 5, plus a number of other pigments which appeared less consistently. The group of pigments characteristic of all members of the Ulvales examined consisted of lutein, violaxanthin and neoxanthin.

The presence of one xanthophyll pigment alone in an alga does not appear to have any phylogenetic significance. The consistent presence of groups of pigments appears to be more important phylogenetically.

In general the results obtained from the pigment analyses support relationships recognized on the basis of morphological and reproductive characteristics. Some phylogenetic considerations have been suggested, but these should be supported by a more exhaustive study of more species and genera before further conclusions can be drawn.

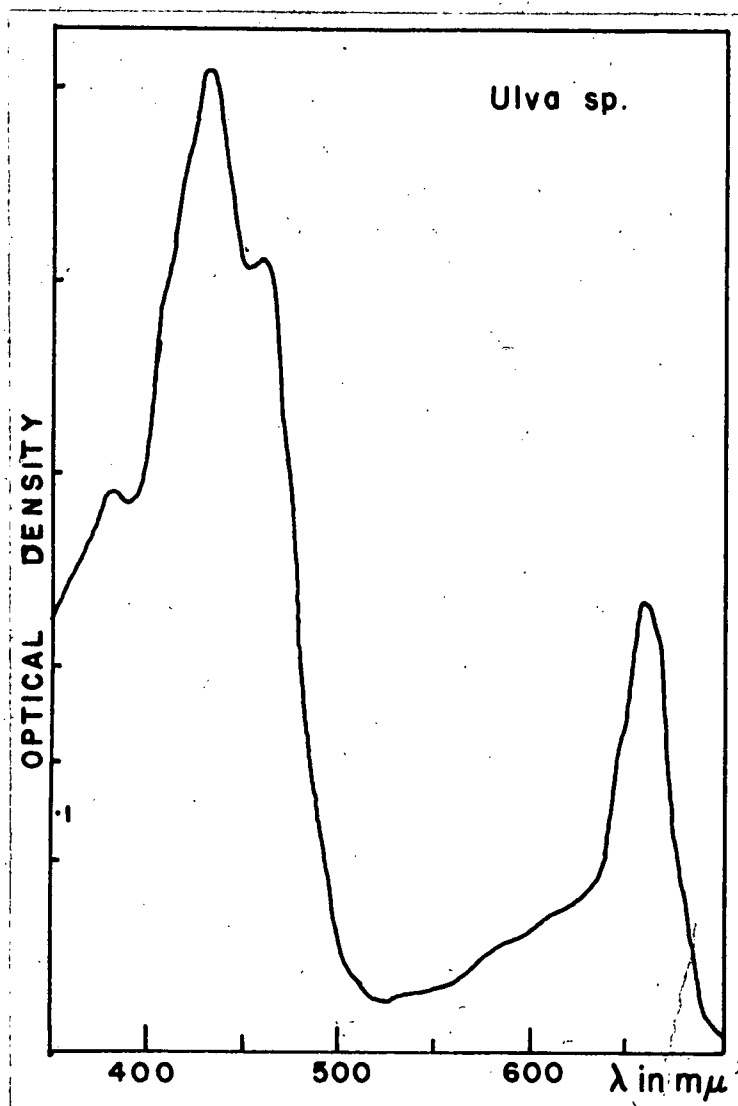


Figure 10. Absorption spectrum of a gross pigment extract of *Ulva* sp. in acetone.

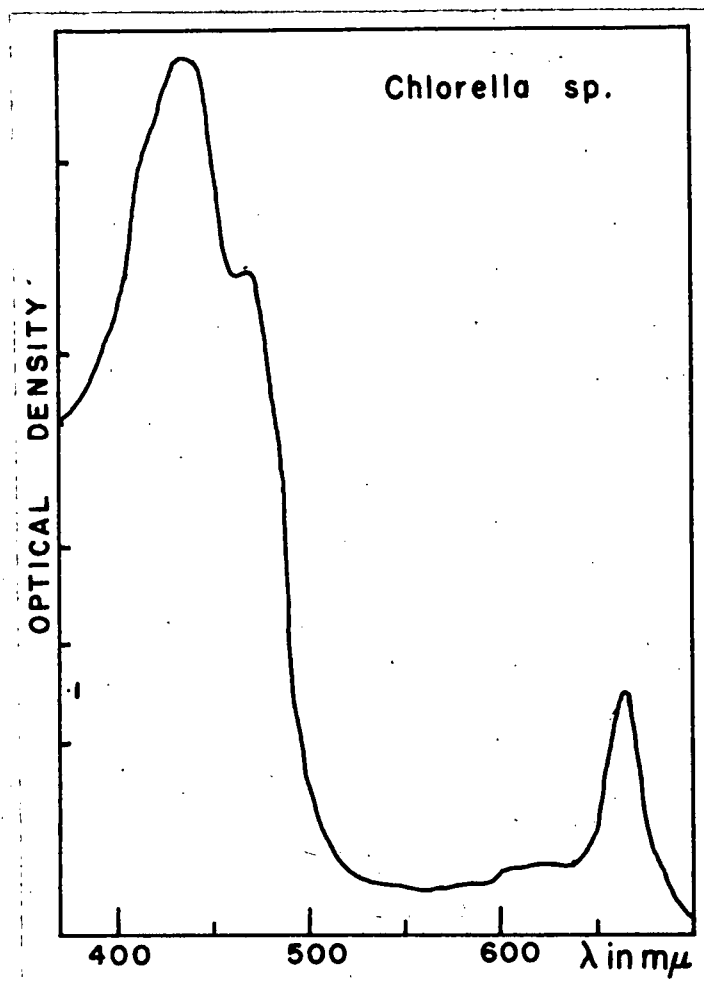


Figure 11. Absorption spectrum of a gross pigment extract of *Chlorella* sp. in acetone.

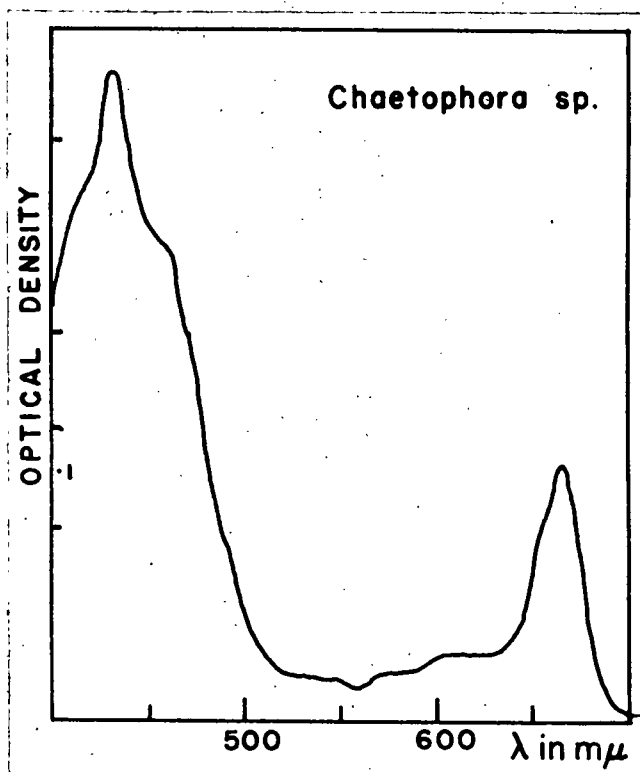


Figure 12. Absorption spectrum of a gross pigment extract of *Chaetophora* sp. in 90% acetone.

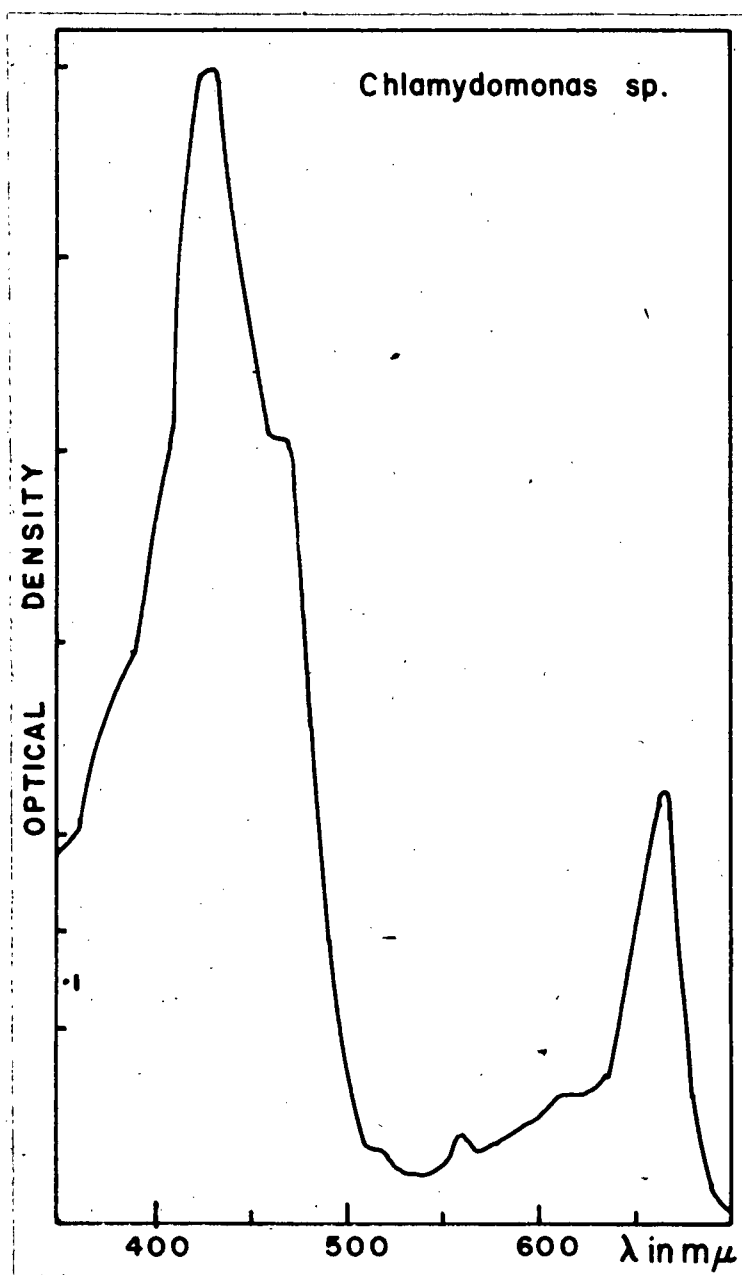


Figure 13. Absorption spectrum of a gross pigment extract of *Chlamydomonas* sp. in 90% acetone.

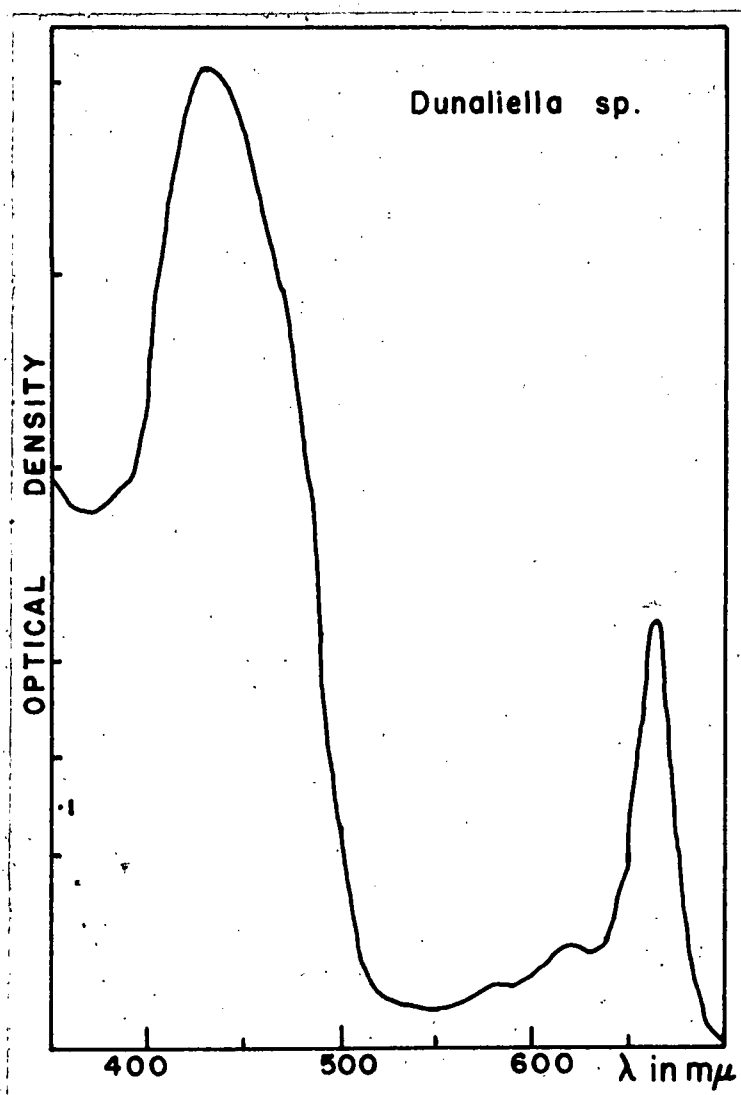


Figure 14. Absorption spectrum of a gross pigment extract of *Dunaliella* sp. in 90% acetone.

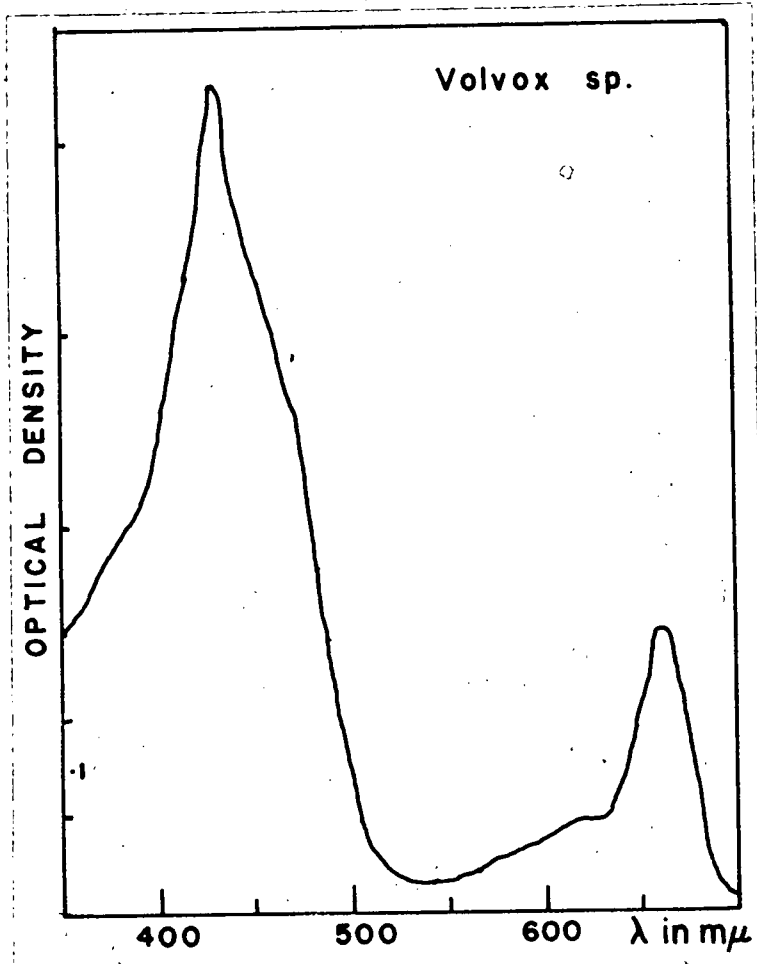


Figure 15. Absorption spectrum of a gross pigment extract of *Volvox* sp. in 90% acetone.

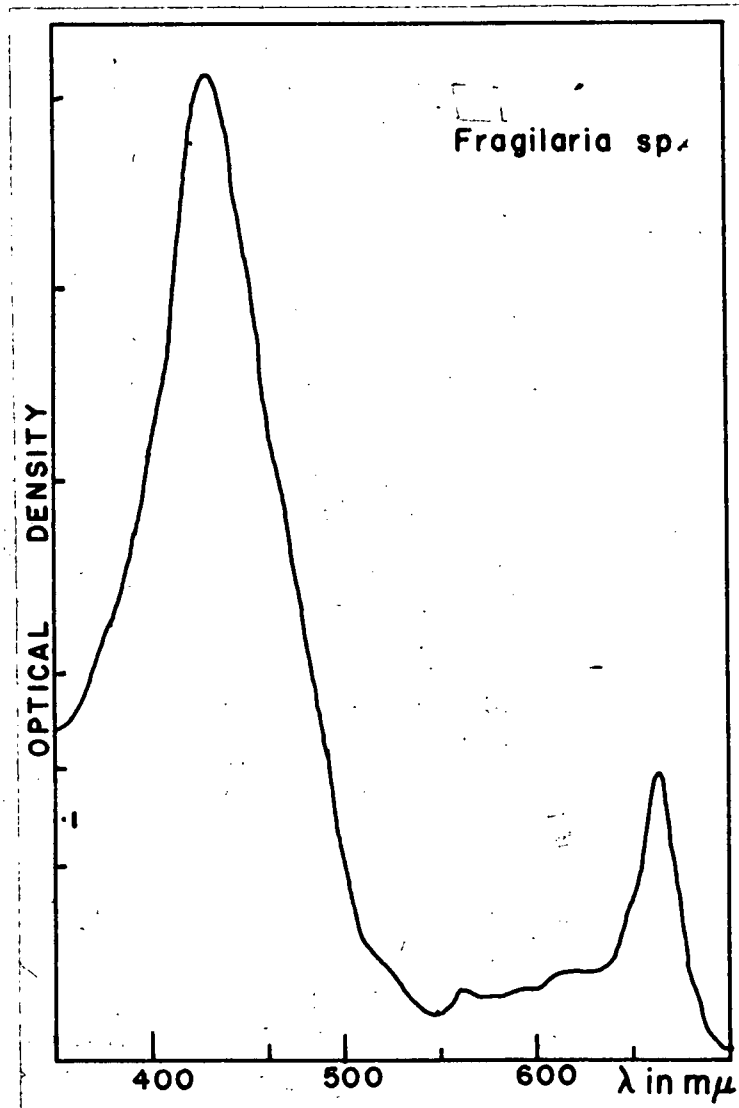


Figure 16. Absorption spectrum of a gross pigment extract of *Fragilaria* sp. in 90% acetone.

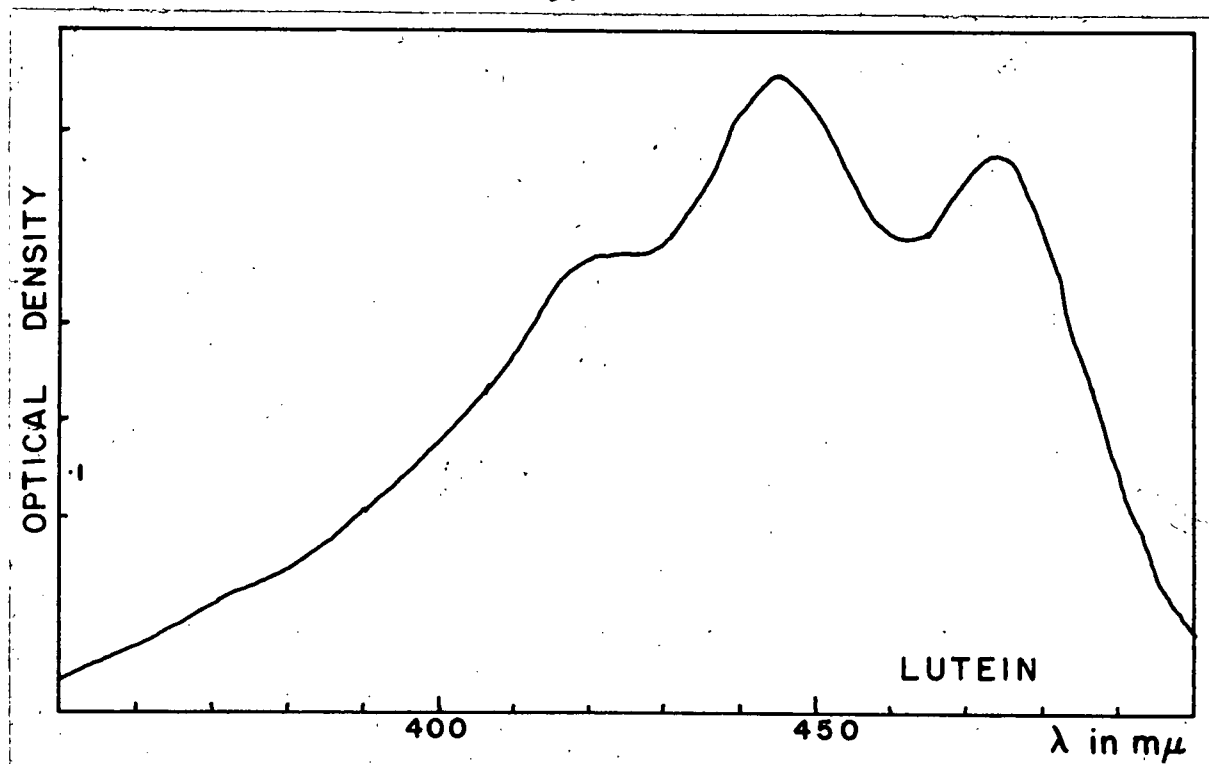


Figure 23. Absorption spectrum of Lutein in 95% ethanol.

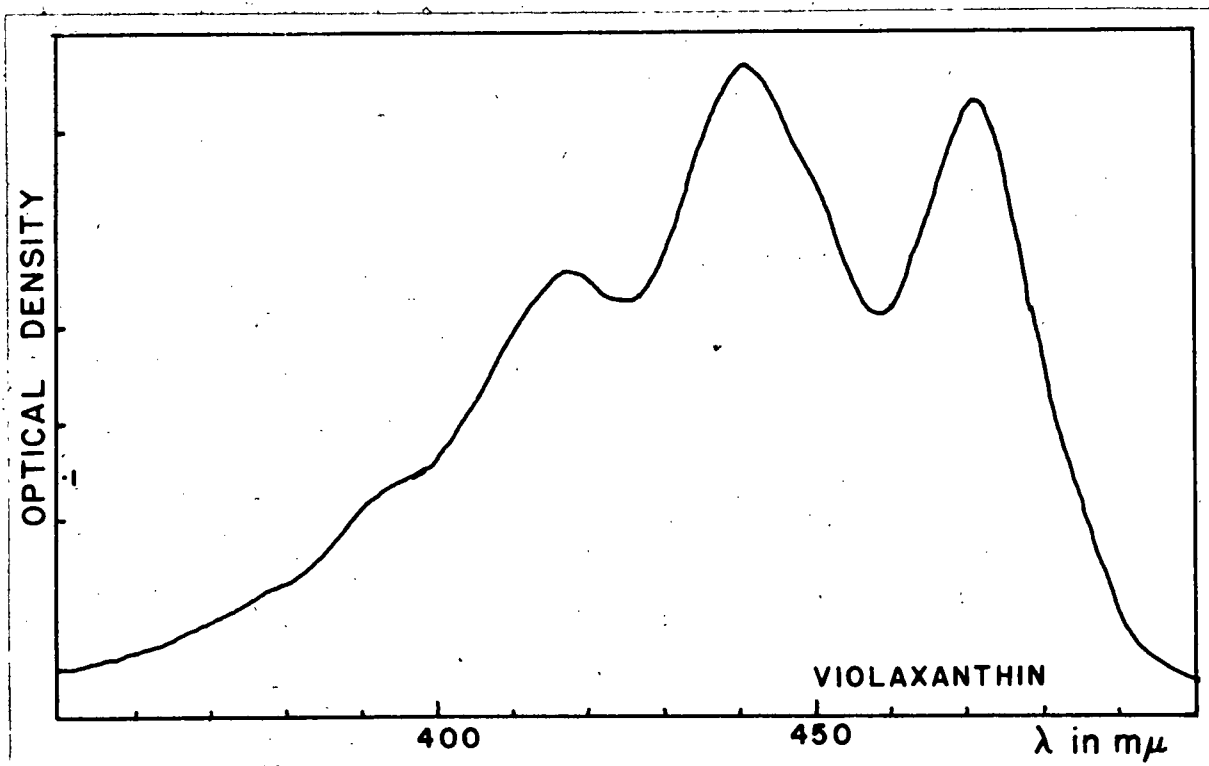


Figure 24. Absorption spectrum of Violaxanthin in 95% ethanol.

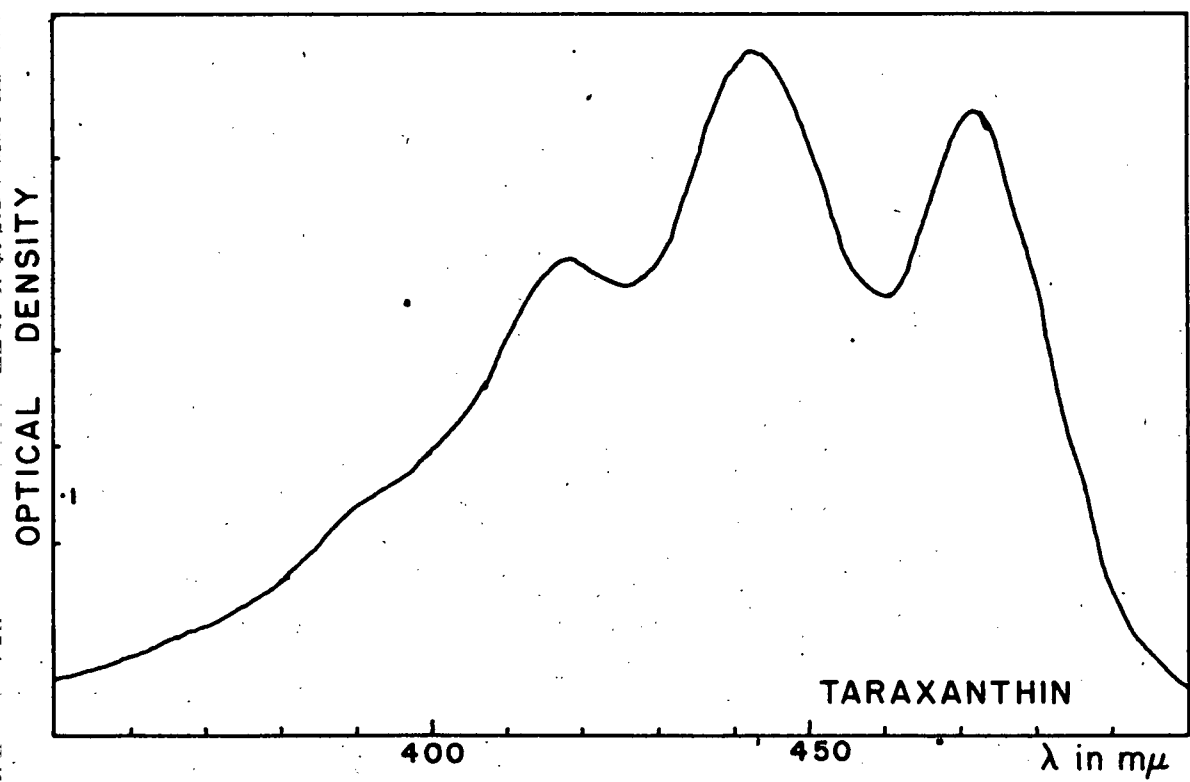


Figure 25. Absorption spectrum of Taraxanthin in 95% ethanol.

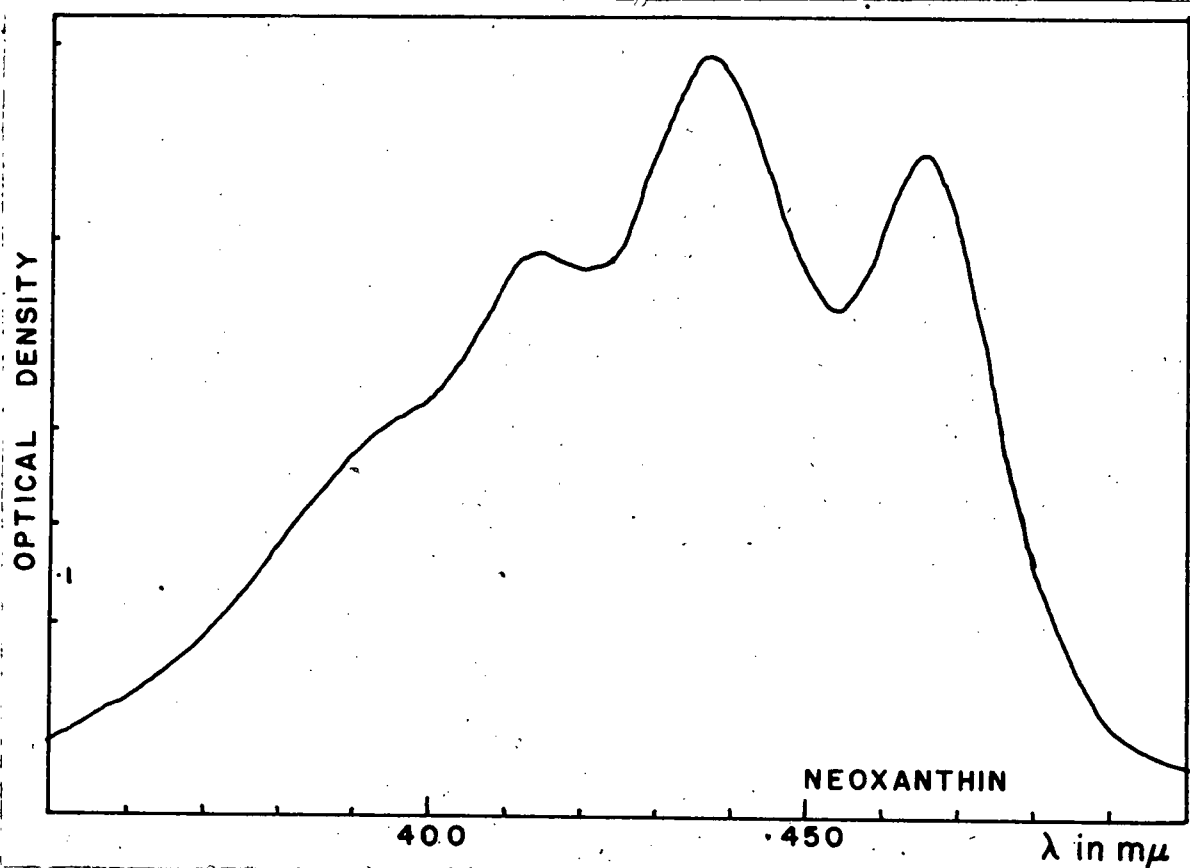


Figure 26. Absorption spectrum of Neoxanthin in 95% ethanol.

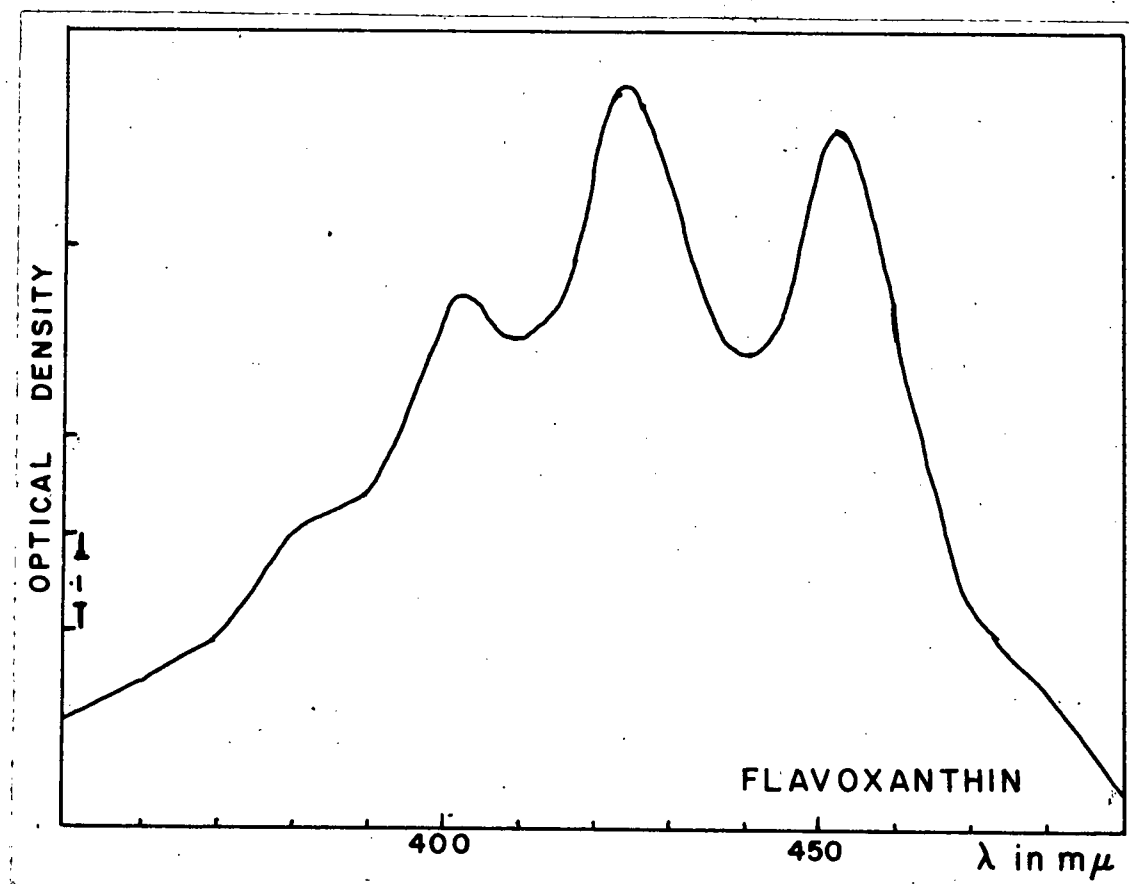


Figure 27a. Absorption spectrum of Flavoxanthin in 95% ethanol.

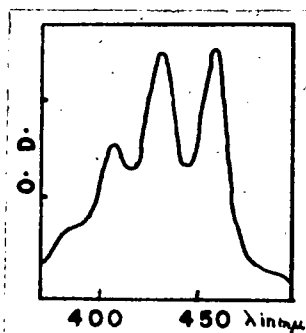


Figure 27b. Absorption spectrum of Flavoxanthin in 95% ethanol (from Karrer and Jucker 1950, p. 264).

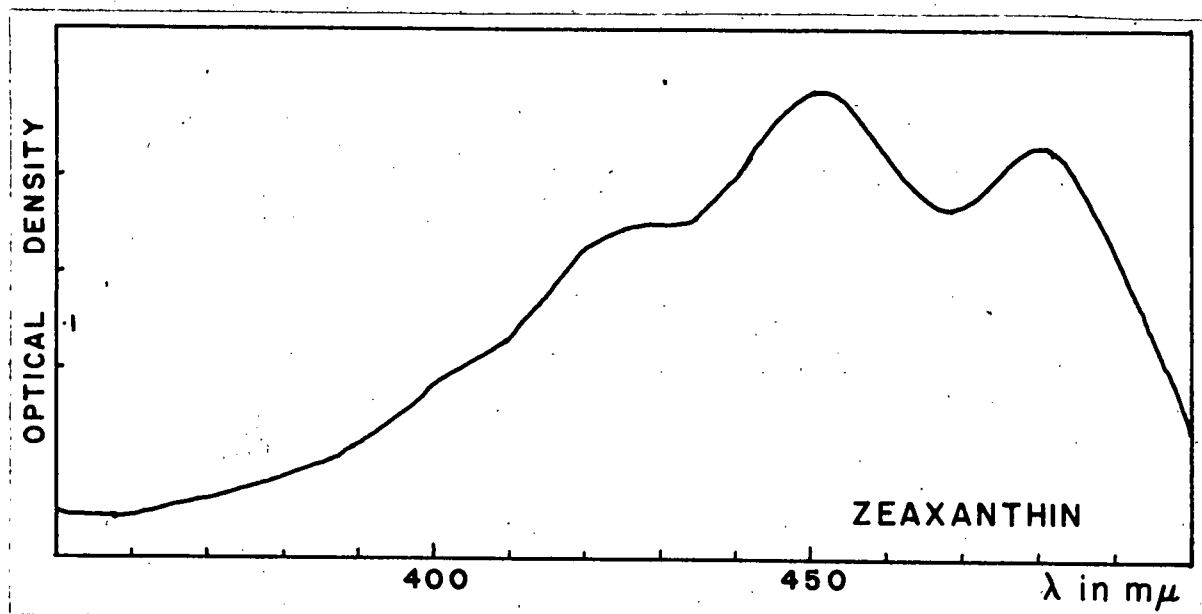


Figure 28. Absorption spectrum of Zeaxanthin in 95% ethanol.

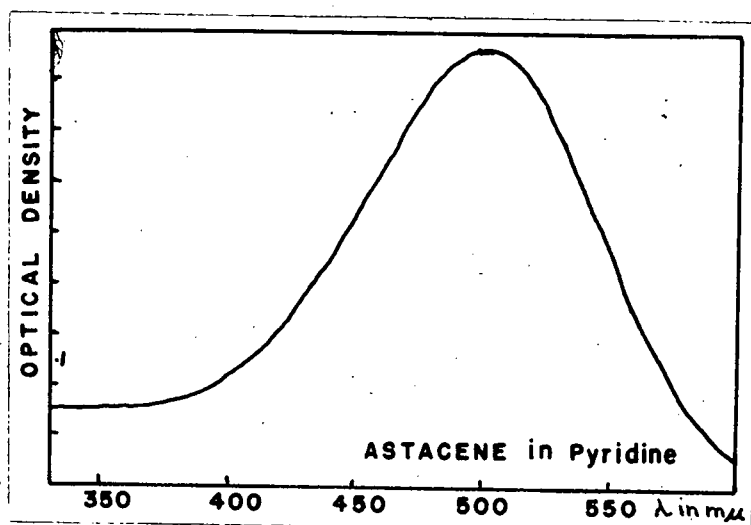


Figure 29. Absorption spectrum of Astacene in pyridine
(from Karrer and Jucker 1950, p. 265).

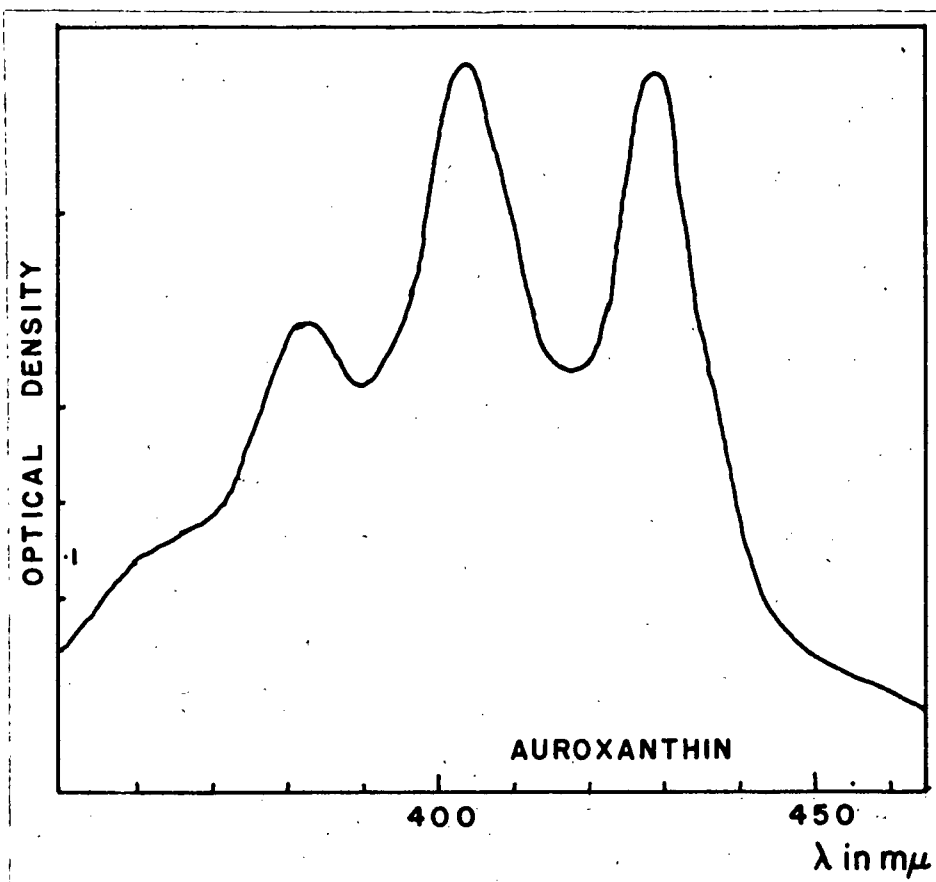


Figure 30a. Absorption spectrum of Auroxanthin in 95% ethanol.

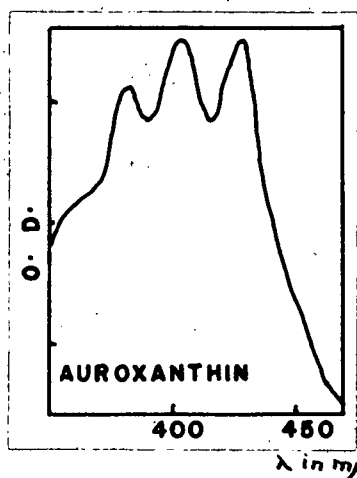


Figure 30b. Absorption spectrum of Auroxanthin in benzene (from Karrer and Jucker 1950, p. 263).

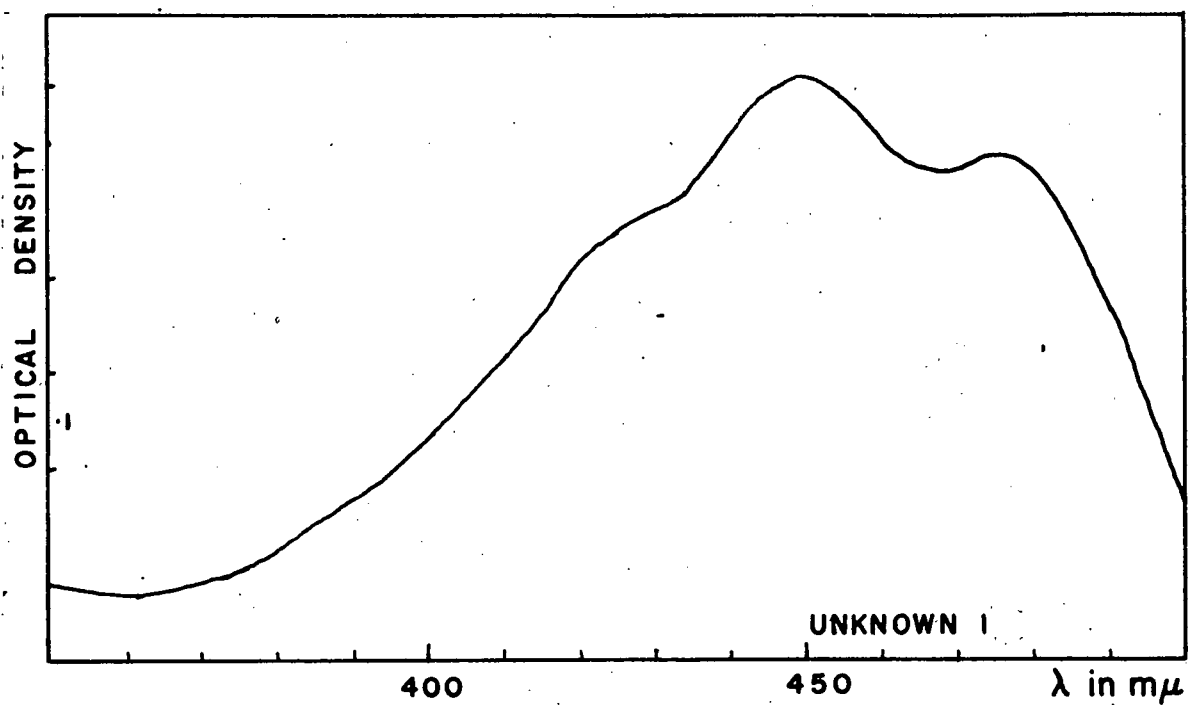


Figure 31. Absorption spectrum of Unknown Xanthophyll 1 in 95% ethanol.

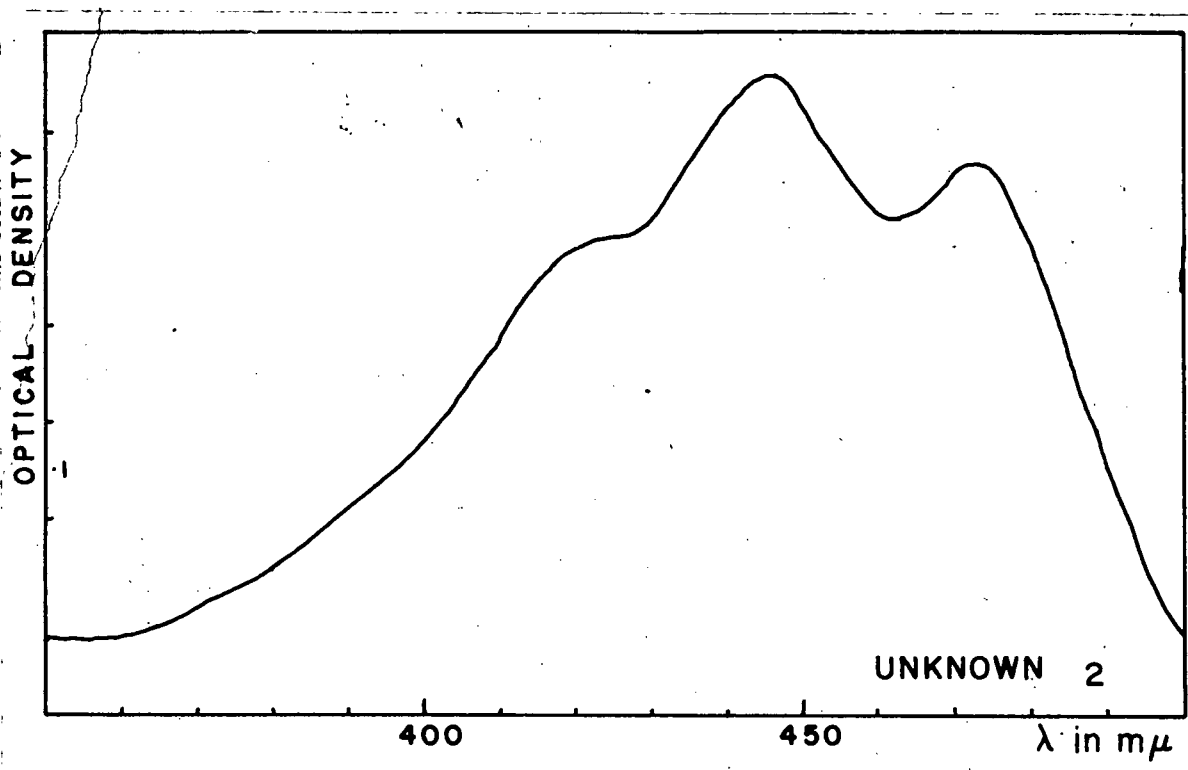


Figure 32. Absorption spectrum of Unknown Xanthophyll 2 in 95% ethanol.

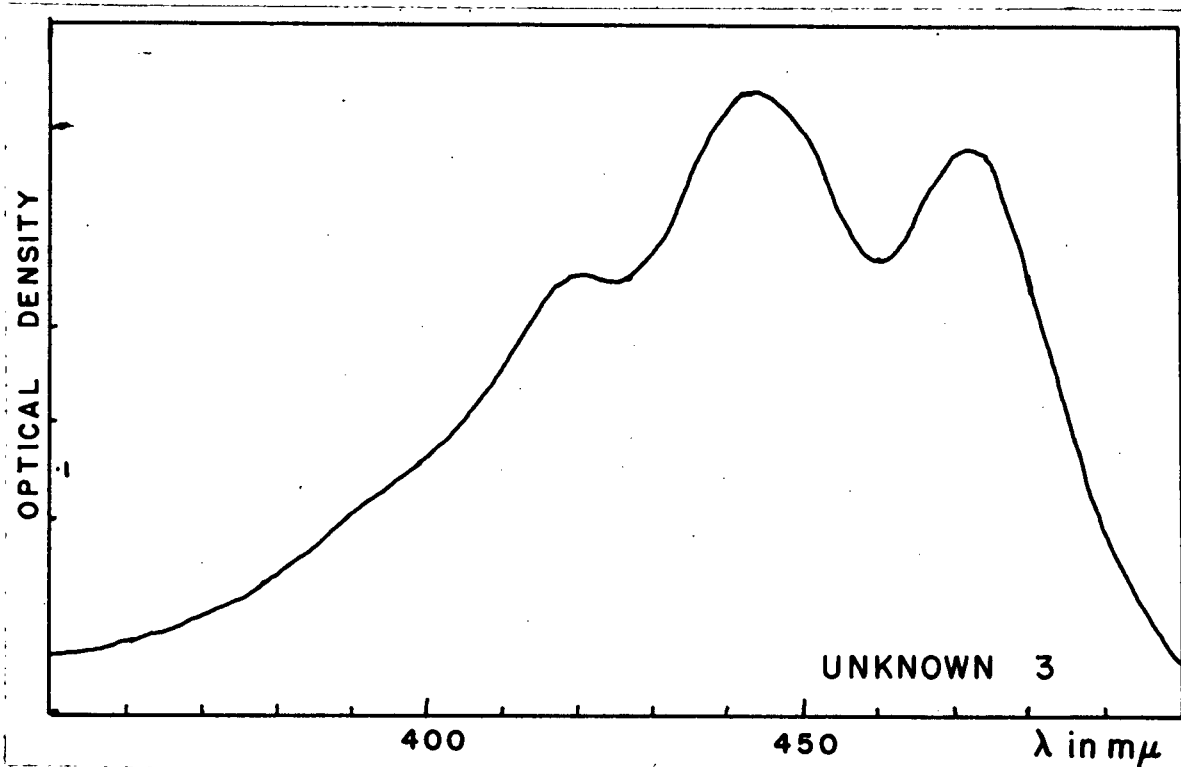


Figure 33. Absorption spectrum of Unknown Xanthophyll 3 in 95% ethanol.

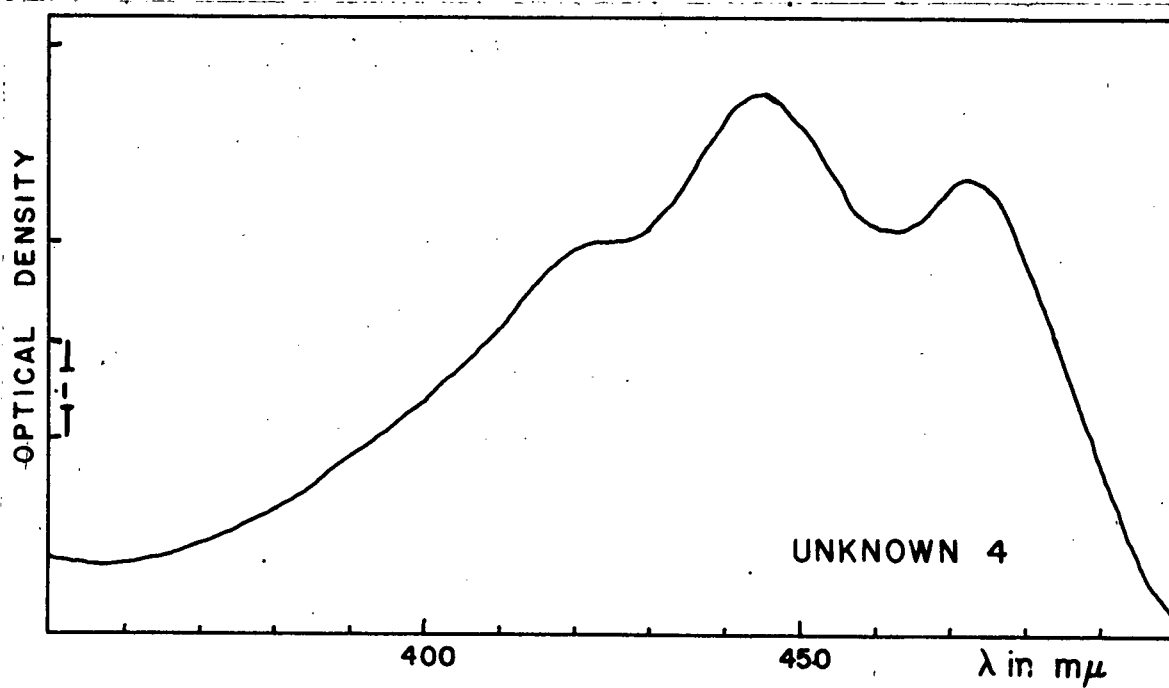


Figure 34. Absorption spectrum of Unknown Xanthophyll 4 in 95% ethanol.

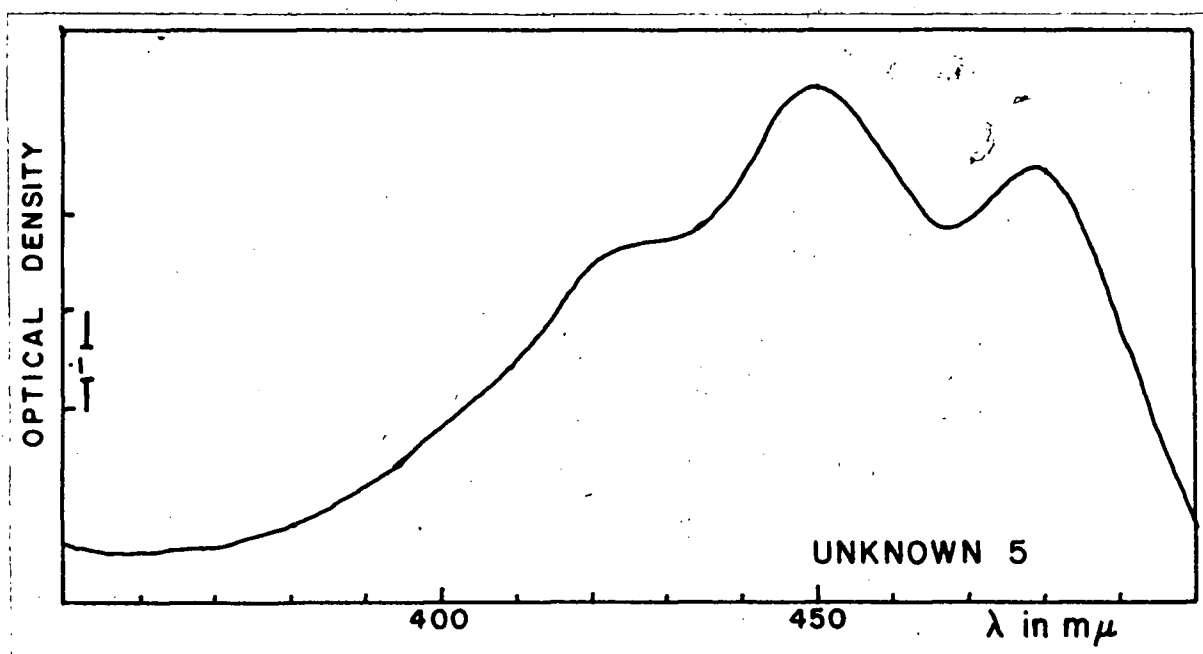


Figure 35. Absorption spectrum of Unknown Xanthophyll 5 in 95% ethanol.

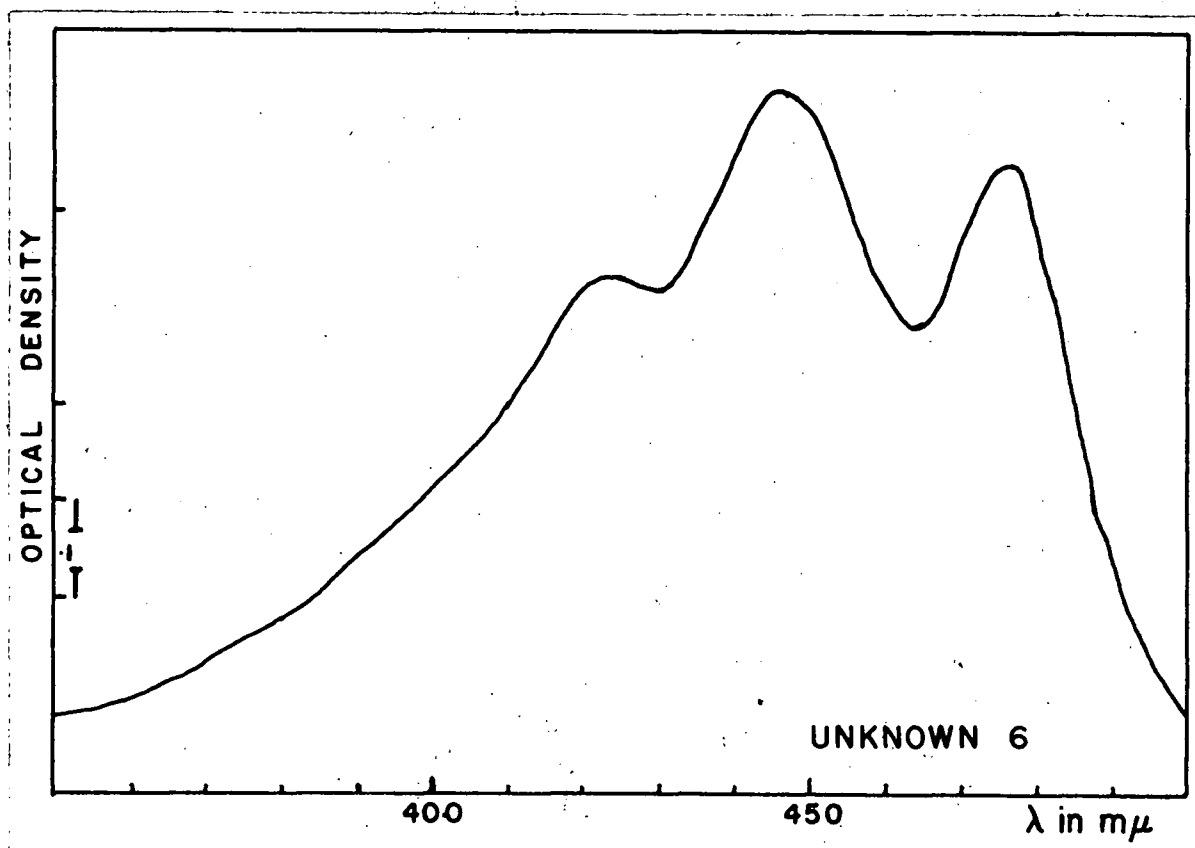


Figure 36. Absorption spectrum of Unknown Xanthophyll 6 in 95% ethanol.

IX. BIBLIOGRAPHY

Atkins, W.R.G.

1945. Autotrophic flagellates as the major constituent of the oceanic phytoplankton. *Nature* 156: 446-447.

Blinks, L.R.

1946. Photosynthetic action spectra in red algae. *Am. Jour. Bot.* 33: 836.

Bogert, M.T.

1938. Carotenoids: the polyene pigments of plants and animals. *In* Gilman, H., *Organic Chemistry* vol. II. John Wiley and Sons, Inc., New York, N.Y. 1041 pp. 98 figs.

Burr, G.O.

1941. Photosynthesis of algae and other aquatic plants. *In*, A Symposium on Hydrobiology. University of Wisconsin Press, Madison, Wisconsin. pp. 163-181.

Carter, P.W., I.M. Heilbron and B. Lythgoe.

1939. The lipochromes and the sterols of the algal classes. *Proc. Roy. Soc. London, Series B*, 128: 82-109.

Cassidy, H.G., N. Applezweig, S. Claesson, V.R. Deitz, B.J. Mair, A.J.P.

1948. Martin, S. Moore, R.L. Peck, W.A. Schroeder, L. Shedlovsky, W.H. Stein, H.C. Thomas, and L. Zechmeister. Chromatography. *Annals of the New York Acad. of Sci.* 49: 141-326.

Chase, F.M.

1941. Useful algae. *Annual Report of Smithsonian Institute*, pp. 401-452, 9 pls.

Chilchester, C.O., P.S. Wong and G. Makinney.

1954. On the biosynthesis of carotenoids. *Plant. Phys.* 29(3): 238-241.

Cook, A.H.

1945. Algal pigments and their significance. *Biol. Rev. Cambridge Phil. Soc.* 20: 115-132, 1 fig.

Dutton, H.J. and C. Juday.

1944. Chromatic adaption in relation to colour and depth distribution of fresh water phytoplankton and large aquatic plants. *Ecology* 25: 273-282.

Dutton, H.J. and W.M. Manning.

1941. Evidence for carotenoid-sensitized photosynthesis in the diatom Nitzschia closterium. *Am. Jour. Bot.* 28: 516-526.

Fox, D.L.

1937. Carotenoids and other lipoid-soluble pigments in the sea and in deep marine mud. *Proc. Nat. Acad. Sci.* 23: 295.

- Frank, J. and W.E. Loomis (eds.).
1949. Photosynthesis in Plants. Iowa State College Press. Ames, Iowa. 500 pp.
- Fritsch, F.E.
1935. The Structure and Reproduction of the Algae. Vol. 1. Cambridge University Press, London. 791 pp., 245 figs.
- Fry, B.A. and J.L. Peel (ed.).
1954. Autotrophic micro-organisms. Fourth Symposium of the Society for General Microbiology. Cambridge Univ. Press., Cambridge. 305pp.
- Gillam, A.E. and I.M. Heilbron.
1935. Vitamin A-active substances in egg yolk. Biochem. Jour. 29: 1064-1067.
- Graham, H.W.
1943. The phytoplankton. In, Biological results of the last cruise of the Carnegie. Carnegie Inst. Wash. Publ. 555, pp. 1-13. 5 figs.
1943a. Chlorophyll content of marine plankton. Sears Foundation: Jour. Marine Res. 5 (2): 153-160, 1 fig.
- Haagen-Smit, A.J., C.E.P. Jeffreys and J.G. Kirchner.
1943. Separation of carotenes from xanthophylls. Ind. Eng. Chem. Anal. Ed. 15: 179-180.
- Harvey, H.W.
1934. Measurement of phytoplankton population. Jour. Mar. Biol. Ass. United Kingdom 19: 761-763.
1942. Production of life in the sea. Biol. Rev. Cambridge Phil. Soc. 17: 221-246, 1 fig.
- Haxo, F.T. and L.R. Blinks.
1950. Photosynthetic action spectra of marine algae. Jour. Gen. Physiol. 33: 389-422.
- Haxo, F.T., C. O'h Eocha and P. Norris.
1955. Comparative studies of chromatographically separated phycoerythrins and phycocyanins. Arch. Biochem. and Biophys. 54 (1): 162-173.
- Heilbron, I.M.
1942. Some aspects of algal chemistry. Jour. Chem. Soc. 79-89.
1946. Twenty years and onwards. Chem. Eng. News 24: 1035-1039.
- Holt, A.S. and E.E. Jacobs.
1954a. Spectroscopy of plant pigments. I. Ethyl chlorophyllides a and b and their pheophorbides. Amer. Jour. Bot. 41: 710-717.
1954b. Spectroscopy of plant pigments. II. Methyl bacteriochlorophyllide and bacteriochlorophyll. Amer. Jour. Bot. 41: 718-722.

Inman, O.L.

1940. The chlorophylls and photosynthesis of thermal algae from Yellowstone National Park. Jour. Gen. Physiol. 23: 661-666.

Karrer, P.

1945. Betrachtungen zur Konstitution der Carotinoidepoxyde. Helv. Chim. Acta 28: 474-475.

Karrer, P. and E. Jucker (trans. and revised by E.A. Braude).

1950. Carotenoids. Elsevier Publishing Company Inc., New York, N.Y. 384 pp., 31 figs.

Kylin, H.

1939. Bemerkungen über die carotinoiden Farbstoffe der Algen. Kgl. Fysiograf. Sällskap. Lund, Förh. 9: 213-231.

Mandels, G.R.

1943. A quantitative study of chlorosis in *Chlorella* under conditions of sulphur deficiency. Plant Physiol. 18: 449-462, 10 figs.

Manning, W.M. and H.H. Strain.

1943. Chlorophyll *d*, a green pigment of Red Algae. Jour. Biol. Chem. 151: 1-19.

Pace, N.J.

1941. Pigments of the marine diatom *Nitzschia closterium*. Jour. Biol. Chem. 140: 483-489.

Papenfuss, G.F.

1951. Problems in the classification of marine algae. Svensk. Bot. Tidskr. 45 (1): 4-11.

Rabinowitch, E.I.

1945. Photosynthesis and Related Processes. Vol. I. Interscience Publishers Inc., New York, N.Y. 599 pp., 57 figs.
1955. Photosynthesis and Related Processes. Vol. II, 1. Interscience Publishers Inc., New York, N.Y. 489 pp., 49 figs.

Richards, F.A.

1952. The estimation and characterization of plankton populations by pigment analysis. I. The absorption spectra of some pigments occurring in diatoms, dinoflagellates, and brown algae. Jour. Mar. Res. 11 (2): 147-155.

Richards, F.A. and T.G. Thompson.

1952. The estimation and characterization of plankton populations by pigment analysis. II. A spectrophotometric method for the estimation of plankton pigments. Jour. Mar. Res. 11(2): 156-172.

Sargent, M.C.

1940. The effect of light on the development of the photosynthetic mechanism. Plant Physiol. 15: 275-290, 3 figs.

Seybold, A., K. Egle, and W. Hülsbruch.

1941. Chlorophyll- and Carotinoidbestimmungen von Süßwasseralgen. Bot. Arch. 42: 239-253.

Seybold, A. and W. Hülsbruch.

1943. Further analysis of chlorophylls and carotenoids in fresh water algae. Bot. Arch. 44: 336-341.

Smith, G.M.

1950. The Freshwater Algae of the United States. Second Edition. McGraw Hill Book Company Inc., New York, N.Y. 719 pp., 559 figs.

Smith, G.M. (ed.)

1951. Manual of Phycology. Chronica Botanica Company. Waltham, Mass. 375 pp., 48 figs.

Steele, C.C.

1937. Recent progress in determining the chemical structure of chlorophyll. Chem. Rev. 20: 1-37.

Strain, H.H.

1938. Leaf xanthophylls. Carnegie Inst. Washington Publ. No. 490, 147 pp., 23 figs.

1944. Chloroplast pigments. Ann. Rev. Biochem. 13: 591-610.

1945. Chromatographic Adsorption Analysis. Revised Reprint. Interscience Publishers Inc., New York, N.Y., 222 pp., 37 figs.

1954. Leaf Xanthophylls: the action of acids on violeoxanthin and tareoxanthin. Arch. Biochem. and Biophys. 48: 458-468.

Strain, H.H. and W.M. Manning.

1942. Chlorofucine (Chlorophyll_x), a green pigment of diatoms and Brown Algae. Jour. Biol. Chem. 144: 625-636.

1943. Pigments of Algae. In H.A. Spöehr. Annual report of the chairman of the division of biology. Carnegie Inst. Washington Year Book 42: 79-82.

Strain, H.H., W.M. Manning and G. Hardin.

1943. Chlorophyll c (chlorofucine) of diatoms and dinoflagellates. Jour. Biol. Chem. 148: 655-668.

1944. Xanthophylls and carotenes of diatoms, brown algae, dinoflagellates and sea-anemones. Biol. Bull. 86: 169-191, 6 figs.

Tischer, J.

1944. Haematococcus extracts. Zeit. physiol. Chem. 281: 143.

Willstätter, R. and A. Stoll.

1913. Untersuchungen über Chlorophyll. Berlin. 117 pp.

Zscheile, F.P. and C.L. Comar

1941. Influence of preparative procedure on the purity of chlorophyll components as shown by adsorption spectra. Bot. Gaz. 102: 463-481, 4 figs.