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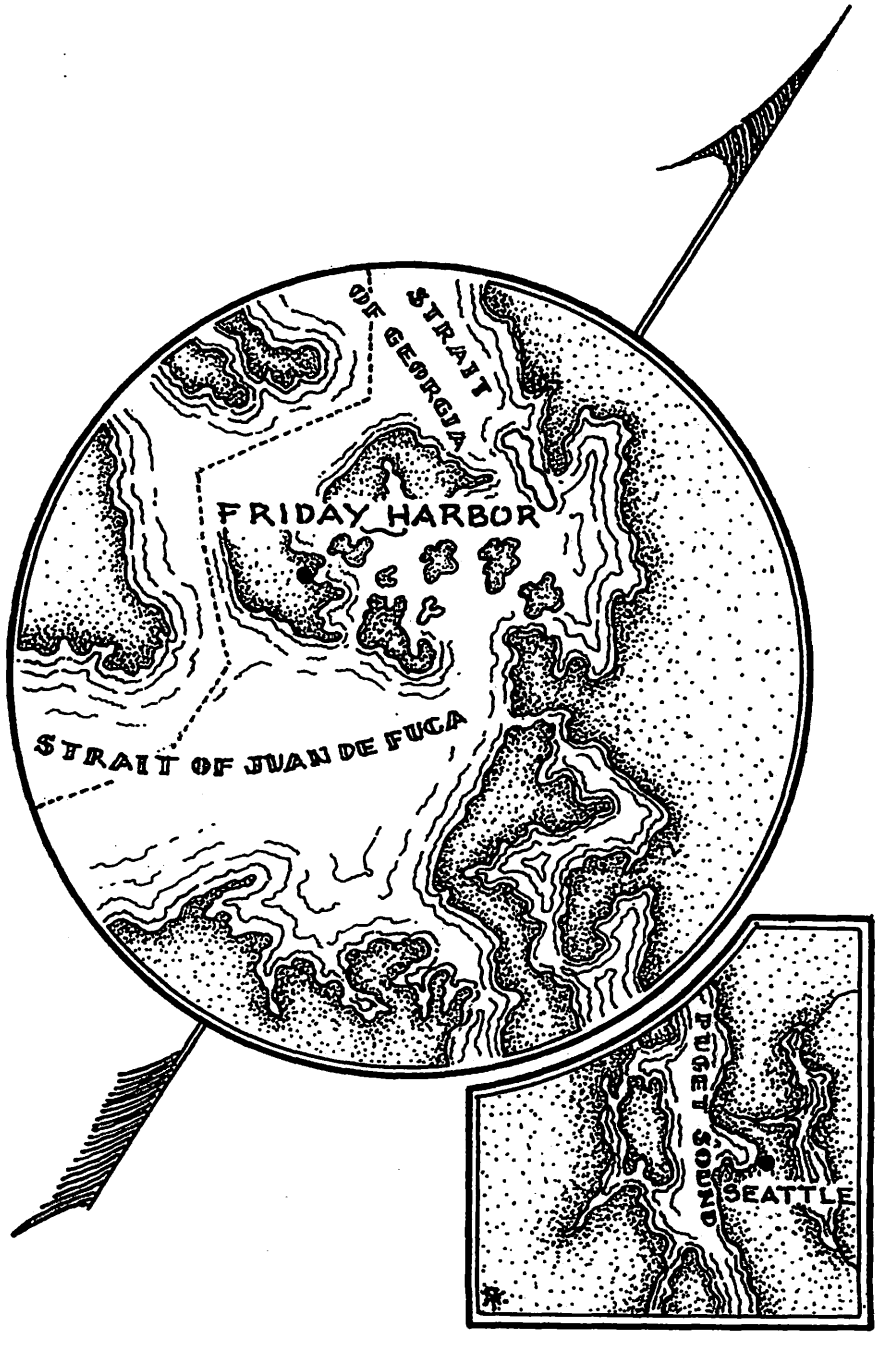
Technical Report No. 2



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THE ABSORPTION SPECTRA OF SOME PIGMENTS OCCURRING IN DIATOMS, DINOFLAGELLATES, AND BROWN ALGAE

by

Francis A. Richards and Thomas G. Thompson

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Director

The data in this report were necessary for the development of a method for estimating the chief pigments found in phytoplankton. The method was limited to semi-micro dimensions, which made the simultaneous spectrophotometric determination of the pigments in the plankton extract the most promising prospect. However, the spectrophotometric technique requires prior knowledge of the absorption spectra of the compounds involved, leading to the research resulting in the data in this report.

ABSTRACT

Chlorophylls a and c, beta carotene, neofucoxanthin A and B, fucoxanthin, diadinoxanthin and diatoxanthin are the most important pigments occurring in diatoms, and the first three are also important in dinoflagellates and brown algae. These compounds have been prepared and their absorption spectra determined over the range 350-700 $m\mu$, in 90% acetone solution. At wave lengths shorter than 400 $m\mu$ the spectra depended upon the method of preparation; investigation revealed evidence that chromatographically separable solvated molecules of otherwise identical compounds are formed during the preparation.

The compounds were prepared by solvent partition and chromatographic adsorption from extracts of diatoms and brown algae.

The absorption spectra of plant pigments are of interest in studies of photosynthesis, identification and classification of plants, analyses of solutions of the pigments (either alone or in mixtures), and in the identification and determination of the purity of pigment preparations. These compounds have characteristic spectra which vary with the solvent, so that for their spectrophotometric analysis it is necessary to know the spectra in some single solvent. There are reported herein the absorption spectra of a number of the pigments found in diatoms, dinoflagellates, and brown algae, in 90% acetone solution.

In general, solvent partition and chromatographic adsorption were used to prepare the compounds. These methods have been reported by Strain and his co-workers (8,9,10), by Pace (5) and others. Spectral data reported by previous workers, and chromatographic and chemical behavior were used as criteria of the purity and identity of the compounds. After the absorption spectra of the compounds were determined in a solvent as reported in the literature, that solvent was removed by evaporation <u>in vacuo</u> at room temperature, and the spectra then determined in 90% acetone solution. If specific absorption coefficients were available from the literature, they were used to calculate concentrations and specific absorption coefficients in 90% acetone; otherwise, relative absorption coefficients were determined and are reported, since these pigments are difficult to prepare in a sufficiently pure state in adequate quantity to determine

their specific absorption coefficients. Ninety per cent acetone was chosen because of its usefulness in the extraction of pigments from plant cells, in which it is more effective than 80% or absolute acetone.

EXPERIMENTAL

All the optical measurements were made in a Beckman Model DU Spectrophotometer, using one centimeter glass-stoppered Corex cells and a tungsten filament light source. The slit and band widths used are shown in Table 1. The "red sensitive" tube was used for measurements from 625-700 mμ, the "blue sensitive" tube for shorter wave lengths. In the range 320-399 mμ, the Corning No. 9863 Red Purple Corex filter supplied with the instrument was inserted in the light path.

Chlorophylls:

To prepare chlorophyll a, fresh fronds of the brown algae Nereocystis leutkeana were extracted with absolute methanol in the presence of magnesium carbonate. After dilution with water, most of the pigments were transferred to petroleum ether, then washed first with methanol (to remove xanthophylls), and finally with water (to remove alcohol) and dried over anhydrous sodium sulfate. The pigments in the petroleum ether solution were separated by chromatographic adsorption on a powdered sugar column, developing the chromatogram with petroleum ether containing small amounts (1-2.5%) of methanol. Chlorophyll a was collected separately as it washed through the bottom

of the column. Chlorophyll *c* remained strongly adsorbed at the top of the column, the carotenes preceded the chlorophyll *a* through the column, and xanthophylls were adsorbed between the chlorophylls *c* and *a*. The same procedure was used to prepare chlorophyll *a* from the diatom Navicula araneosum, except that a column of two parts powdered sugar to one part Johns-Manville Hyflo Super-Cel was used, and the part of the column holding chlorophyll *a* was extruded instead of eluted.

The preparation of chlorophyll *a* by the above method presented little difficulty, and the spectra of diethyl ether solutions of the product agreed well with that of chlorophyll *a* prepared by Harris and Zscheile (3, 12) by a more elaborate method.

The preparation of chlorophyll *c* by the method of Strain, et al, is considerably more difficult than that of chlorophyll *a*, and will be described in more detail. Approximately four grams of the diatom Navicula araneosum were blotted to remove excess water. One extraction with absolute and three with 80% methanol containing 0.2% dimethyl aniline removed most of the pigments. Two hundred ml of the extract were diluted with 37 ml water, and extracted with 200 ml of petroleum ether to remove the bulk of the chlorophyll *a* and carotenes. One hundred sixty ml of water, a large quantity of solid sodium chloride, and 100 ml diethyl ether were added to the alcoholic layer, and the ethereal layer discarded. Succeeding extractions were made with 75 ml portions of diethyl ether, adding sodium chloride and saturated sodium

chloride solution until the final volume was 700 ml and saturated with sodium chloride, and all the pigment was removed from the alcoholic layer. The ether layers, except the first two, were combined, washed six times with water, and dried over a small quantity of anhydrous sodium sulfate, avoiding a large excess of the salt, since it adsorbs chlorophylls from anhydrous solutions. This occasioned some difficulty, because on evaporating the ethereal solution to a small volume (in vacuum at room temperature), some water separated out. It was found necessary to stop the evaporation before any of the pigment precipitated, or to redissolve it in diethyl ether before the addition of petroleum ether. The concentrated ethereal solution was diluted at least 20 to 1 with petroleum ether, poured over a 2.7 x 25 cm column of powdered sugar (two parts) and Johns-Manville Hyflo Super-Cel (one part) and sucked through by vacuum.

The pigments, adsorbed in the top three cm, were washed with petroleum ether containing 0.2% dimethyl aniline, followed by this mixture containing 2 to 4% methanol (gradually increasing the alcoholic content of the mixture). A light yellow band was washed through the column, and then a bluish-green band finally separated from the top layer. When this band was well separated from the top light yellowish-green layer the development was stopped and the latter dug out and the chlorophyll *g* eluted with diethyl ether and a small amount of methanol.

The first formation and early development of the chromatograms always left a mixture of chlorophyll *a* and *g* in the top band. Spectro-

photometric examination of the eluted pigments showed that only upon extensive washing with petroleum ether containing up to 4% methanol was chlorophyll *a* removed from this band. When chlorophyll *a* had been removed, the adsorbed chlorophyll *c* appeared as a faintly colored, unpromising looking yellowish-green band. This apparent co-adsorption of chlorophylls *a* and *c*, not described by Strain and his co-workers, was always observed by the writer. The recovery of uncontaminated chlorophyll *c* was never accomplished, and it was necessary to correct this spectral data for the small amount of chlorophyll *a* present.

The correction for the chlorophyll *a* present in chlorophyll *c* preparations was made by calculating the concentrations of the two components (expressing the concentration of chlorophyll *c* in arbitrary units) from optical densities of the methanol solution at 665 and 635 mμ, using absorption coefficients reported by Mackinney (4) and Strain, Manning and Hardin (8, 9). Knowing the concentration of chlorophyll *a*, its contribution to the absorption was calculated over the spectral range and subtracted from the observed optical densities.

The spectrum of the mixture eluted from the top green band of a chromatogram prepared by the method of Pace (5) showed it to be chlorophylls *a* and *c*, not chlorophyll *b* as Pace assumed. Since the spectra of chlorophylls *b* and *c* are quite different, the results of his analyses of the chlorophylls of the diatom Nitzschia Closterium are probably in error as to the identity and amount of the second chlorophyll component.

Beta Carotene:

Beta carotene has been reported as the principal carotene present in marine plants (10). Although the absorption spectrum in 90% acetone has not previously been reported, those for hexane and other hydrocarbon solvents have been published by several workers (1, 2, 7, 11, 16).

A commercial preparation of beta carotene (Eimer and Amend), described by the manufacturers as xanthophyll, chlorophyll, oil and fat free, gave a blue color, characteristic of the xanthophylls (2), when shaken with 85% phosphoric acid. This reaction may have resulted from products of oxidation formed after leaving the manufacturers. The material was treated in two ways to remove these compounds. A hexane solution was (a) shaken with 90% methanol, then washed with water and dried over anhydrous sodium sulfate and (b) shaken with 85% phosphoric acid and then washed with water and dried over anhydrous sodium sulfate.

Absorption spectra of hexane solutions of the commercial carotene and of two portions treated as above differed greatly in the range 320-370 mμ. Hexane solutions of the methanol washed material showed unusually high absorptions in this range, possibly caused by methanol solvates. These preparations were not dried after the methanol washing, and the solvates apparently persisted. Ninety per cent acetone solutions of the untreated material showed a sudden increase in absorption below 350 mμ which was not observed in samples previously treated with either methanol or phosphoric acid, and evaporated to dryness under reduced pressure at room temperature.

Xanthophylls:

The spectra of 90% acetone solutions of neofucoxanthin A and B, fucoxanthin, diadinoxanthin and diatoxanthin have not been reported; the isolation and relative spectra of these compounds in methanol were reported by Strain, et al, (10). These compounds prepared by their methods from mixed diatoms collected in tide pools at Friday Harbor, Washington, showed unusually high absorptions in the spectral range 320-360 mμ. Three possible causes of these anomalies were considered: (1) Absorption by residual dimethylaniline (which shows a high absorption in this range), added to the petroleum either used to develop the chromatograms, (2) Absorption by methanol or diethyl ether solvates, and (3) Absorption by incompletely removed naturally occurring waxes observed to come through the adsorption columns while chlorophyll a was being washed through. These high absorptions could be eliminated by exhaustive drying and by avoiding dimethyl aniline, eliminating the latter possibility. To investigate the remaining possibilities, xanthophylls were prepared from diatoms by the following modified methods:

1. Elimination of dimethyl aniline.

The method of Strain, et al, was altered by omitting dimethyl aniline entirely, using powdered magnesium carbonate to prevent acidity. When no special drying procedure was used, these preparations showed high absorption at the shorter wave lengths in acetone solutions.

2. Elimination of Methanol.

Pace's acetone extraction method was used to prepare xanthophylls. To avoid contact with methanol, an attempt was made to develop the chromatogram with first acetone and then diethyl ether in petroleum ether. These mixtures resulted in a slow movement of the pigments down the column, with little or no separation of the xanthophylls, and finally the chromatogram was developed with isopropyl alcohol in petroleum ether. Ninety per cent acetone solutions of these preparations showed high absorptions at the shorter wave lengths which were not shown by ethanol solutions of similarly treated materials.

3. Exhaustive drying, to decompose possible solvates.

Ninety per cent acetone solutions of xanthophylls having unusually high absorptions in the spectral range 320-360 mμ were dried under reduced pressure at room temperature for three hours. This resulted in a marked decrease in the absorption between 320 and 400 mμ. When dried for 10 hours, there was no anomalous absorption in solutions of neofucoxanthin A and B and fucoxanthin preparations made by the method of Strain, et al, substituting powdered magnesium carbonate for dimethyl aniline.

4. Attempt to form solvates at low concentrations.

A small amount of (a) methanol and (b) diethyl ether, added to the solutions of redried material described in (3) above, resulted in too small a change in the absorption to account for the high absorptions observed before redrying.

5. Attempt to form ether solvates at high concentrations.

The pigments were again redried and redissolved in diethyl ether, this time stopping the evaporation as soon as the material appeared crystalline and dry. When redissolved in acetone, the absorbabilities were only slightly increased.

The results of (1) and (5) above show that:

(a) When an alcohol was used to develop the chromatogram, acetone solutions of the pigments had high optical densities at the shorter wave lengths unless the material was subjected to exhaustive drying.

(b) Once a preparation had been dried exhaustively, the addition of small amounts of methanol or diethyl ether to the acetone solutions did not cause unusually high optical densities.

(c) Preparations showing high optical densities in 90% acetone did not show correspondingly high densities in alcoholic solutions.

(d) The relatively non-volatile dimethyl aniline must be avoided if the spectral range under 350 mμ is to be investigated.

Solvation of leaf xanthophylls was investigated by Strain (7) and found to have some effect on the spectrum in the range 400-500 mμ. At shorter wave lengths the effect is much more pronounced. The writer's findings suggest that solvates are formed only at high concentrations (such as when being dried); they are somewhat stable in acetone, unstable in alcoholic solutions, and can be destroyed by prolonged drying in vacuum at room temperature.

In one case, readsorption of a xanthophyll fraction on a powdered sugar column resulted in the separation of three distinct bands of very similar appearance. These three bands, when eluted and subjected to three hours' drying under reduced pressure, had essentially identical spectra (that of fucoxanthin) in 90% acetone, although both acetone and ethanol solutions of the three fractions not subjected to this drying procedure had very high and inconsistent absorptions at the shorter wave lengths. Their separation on the adsorption column could be attributed to the formation of different methanol solvates of the pigment.

RESULTS

The absorption spectra of the compounds discussed above are tabulated in Tables 2 to 4. Except for chlorophyll a and beta carotene, logarithms of relative absorption coefficients, computed from observed optical densities, arbitrarily assigning the value 2.400 to the yellow maximum are given. Logarithms of specific absorption coefficients of chlorophyll a and beta carotene were calculated from concentration values determined as indicated in the tables. The Beer-Lambert law was used in the form

$$\log E = \log I_0/I - (\log L + \log c),$$

which, for a one cm light path ($L = 1$), reduces to

$$\log E = \log I_0/I - \log c.$$

Table 1

Slit and Nominal Band Widths Used in Making Absorption Measurements on the Beckman Spectrophotometer

Wave Length Range	Slit (mm)	Nominal Band Width, Millimicrons	
	90% Acetone Solutions		
320-322.4	1.8	9.0	9.4
322.5-324	1.3	6.76	6.89
325-329	0.8	4.24	4.52
330-334	0.3	1.69	1.75
335-339	0.2	1.17	1.22
340-359	0.15	.92	1.12
360-399	0.10	.75	1.05
400-700	0.04	.42	2.00
	Methanol Solutions		
320-324	0.3	1.50	1.69
325-334	0.2	1.06	1.17
335-354	0.15	.88	1.06
355-399	0.10	.71	1.05
400-409	0.05	.52	.56
410-599	0.04	.45	1.36
600-619	0.07	2.38	2.80
620-700	0.04	1.60	2.00
	Hexane Solutions		
320-324	0.3	1.50	1.59
325-334	0.2	1.06	1.17
335-349	0.15	.88	.96
350-399	0.10	.64	1.05
400-599	0.04	.42	1.36
600-700	0.07	2.38	3.50
	Ethanol Solutions		
320-322.4	0.3	1.50	1.56
322.5-334	0.2	1.04	1.17
335-349	0.15	.88	.96
350-399	0.10	.64	1.05
400-409	0.05	.52	.56
410-700	0.04	.45	2.00

Table 2

Absorption Spectra of Chlorophylls a and g

Wave Length mμ	Chlorophyll a in 90% Acetone Log $E^{1\,gm}_{1\,cm}$	Chlorophyll g in 90% acetone. Corrected for chlorophyll a content Soln 1 Log $E_{1\,cm}$	Soln 2 Log $E_{1\,cm}$	Chlorophyll g in Methanol. Corrected for chlorophyll a content Soln 1 Log E_{1cm}	Soln 2 Log E_{1cm}
320	1.449	1.449	1.528		
325	1.453	1.448	1.526		
330	1.399	1.362	1.394		
335	1.419	1.305	1.281		
340	1.403	1.283	1.279		
345	-	1.264	1.257		
350	1.464	1.241	1.245		
355	1.499	1.221	1.233		
360	1.552	1.236	1.233		
365	1.599				
370	1.648	1.262	1.257		
375	1.685				
380	1.697	1.307	1.302		
385	1.701				
390	1.701	1.361	1.352		
395	1.714				
400	1.764	1.378	1.373	1.299	1.271
405	1.829				
410	1.859	1.441	1.436	1.362	1.331
415	1.854				
420	1.849	1.572	1.573	1.461	1.434
425	1.894				
430	1.940	1.730	1.733	1.626	1.605
440	1.696	1.883	1.889	1.748	1.718
445	1.347	1.922	1.922	1.764	1.764
450	.949	1.895	1.899	1.763	1.751
455	.625				
460	.405	1.677	1.674	1.635	1.622
465	.313				
470	.276	1.216	1.202	1.342	1.317
480	.278	.732	.717	.929	.910
490	.368	.468	.441	.452	.442
500	.410	.368	.344	.237	.216
505	.414				
510	.412	.329	.295	.201	.113
515		.325	.286		
520	.417	.353	.320	.220	.192
530		.437	.415		.238

Table 2

continued

540	.591	.519	.499	.384	.352
550	.567	.545	.529	.442	.414
560	.706	.654	.645	.502	.493
570	.855	.763	.736	.580	.539
580	.942	.867	.857	.703	.685
585		.843	.834	.720	.693
590	.915	.757	.748	.684	.560
600	.986	.583	.560	.555	.560
605		.554	.541	.492	.456
610	1.144	.573	.563	.483	.442
615	1.179	.646	.637	.538	.539
620	1.172	.760	.760	.638	.618
625	1.138	.931	.914	.779	.776
630	1.076	1.015	1.024	.856	.846
631		1.021			
634				.869	
635	1.035	.985	.983	.868	.851
640	1.074	.841	.837	.821	.794
645	1.215	.642	.621	.720	.700
650	1.417	.444	.441	.547	.493
655	1.730	.268	.268		
660	1.838	.036	.043	-.383	-.461
663	1.851				
665	1.824	.020	.043		
670	1.534				
675	1.378				
680	.972				
685	.548				

Concentrations of chlorophyll g solutions computed from specific absorption coefficients reported by Zscheile (13).

Values of the relative absorption coefficient of the acetone solutions of chlorophyll g at 445 mμ arbitrarily given the same value found for the specific absorption coefficient of chlorophyll g at its maximum value (1.922 at 430 mμ). Values for chlorophyll g are thus of <u>specific</u> absorption coefficients, those for chlorophyll g are relative absorption coefficients.

Table 3

Specific Absorption Coefficients of

Beta Carotene in 90% Acetone

Wave Length	Log E $^{1gm}_{1cm}$	Wave Length	Log E $^{1gm}_{1cm}$
320	1.216	480	2.349
322.5	1.163	485	2.337
325	1.181	490	2.285
330	1.205	500	2.042
335	1.227	510	1.658
340	1.248	520	1.181
345	1.238	530	0.771
350	1.248	540	0.570
355	1.227		
360	1.253		
370	1.377		
380	1.554		
390	1.725		
400	1.906		
410	2.033		
420	2.170		
425	2.212		
430	2.251		
440	2.309		
445	2.355		
450	2.388		
452	2.400		
453	2.400		
455	2.400		
456	2.400		
460	2.385		
465	2.351		
470	2.332		
475	2.339		

Concentrations of solutions determined from aliquot samples in hexane, using specific absorption coefficients reported by Zechmeister and Polgar (11). In 90% acetone the maximum is a little lower (of 2.410) and displaced slightly toward the longer wave lengths (of 450 mμ) than in hexane solutions.

Table 4

Absorption Spectra of Diatom Xanthophylls in Ethanol and 90% Acetone Solution. Values of Log E_{1cm} given

Wave Length E_{λ}	Neofucoxanthin A		Neofucoxanthin B		Fucoxanthin		Diadinoxanthin		Diatoxanthin	
	Ethanol	90% Acetone	Ethanol	90% Acetone	Ethanol	90% Acetone	Ethanol	90% Acetone	Ethanol	90% Acetone
350	1.657	1.667	1.581	1.553	1.328	1.312	1.494	1.521	1.808	1.805
355		1.675		1.568	1.336	1.340	1.464	1.472	1.757	1.765
360	1.688	1.706	1.620	1.608	1.402	1.400	1.494	1.480	1.729	1.734
365					1.465		1.552	1.537	1.731	1.734
370	1.761	1.789	1.714	1.721	1.550	1.567	1.622	1.610	1.771	1.756
375					1.621		1.687		1.808	
380	1.868	1.892	1.833	1.846	1.700	1.726	1.753	1.748	1.879	1.846
390	1.966	1.996	1.950	1.974	1.854	1.885	1.913	1.907	1.957	1.946
400	2.081	2.110	2.070	2.099	1.994	2.027	2.022	2.027	2.070	2.066
410	2.166	2.196	2.178	2.206	2.114	2.148	2.157	2.157	2.171	2.162
420	2.244	2.279	2.251	2.283	2.216	2.252	2.239	2.258	2.266	2.264
430	2.302	2.330	2.316	2.343	2.283	2.316	2.272	2.282	2.297	2.314
440	2.352	2.381	2.359	2.392	2.346	2.377	2.365	2.376	2.354	2.353
444				2.398	2.368		2.375	2.400	2.371	
445	2.367	2.397	2.373	2.398	2.369	2.394	2.372	2.400	2.373	2.381
446	2.369	2.398	2.374	2.400	2.368	2.398	2.365	2.399	2.373	2.384
448	2.370	2.400	2.375	2.400	2.374	2.400	2.354		2.375	2.392
449	2.374	2.399	2.373	2.395		2.400				
450	2.375	2.397			2.374	2.398	2.336	2.379	2.371	2.396
451	2.374				2.374					2.400
452	2.373		2.371		2.375		2.317		2.363	2.396
455	2.369	2.379	2.367	2.385	2.373	2.387	2.285	2.330	2.343	2.388
460	2.360	2.374	2.358	2.374	2.364	2.373	2.258	2.389	2.308	2.360
465		2.366		2.361	2.358	2.366	2.272	2.389	2.288	2.329
470	2.346	2.359	2.336	2.351	2.352	2.362	2.299	2.320	2.293	2.314
472							2.300	2.328	2.317	
475					2.337		2.287	2.327	2.318	2.318
476								2.321	2.317	2.322
478										2.324
480	2.304	2.312	2.277	2.278	2.305	2.308	2.201	2.269	2.275	2.322

Table 4 (continued)

485		2.257		2.213	2.259	2.246	2.037	2.139	2.211	2.294
490	2.206	2.184	2.160	2.135	2.201	2.171	1.793	1.933	2.091	2.198
500	2.056	1.998	1.993	1.931	2.043	1.969	1.229	1.362	1.715	1.905
510	1.890	1.792	1.808	1.710	1.866	1.753	0.871	0.953	1.322	1.527
520	1.673	1.583	1.598	1.448	1.645	1.494	0.649	0.706	1.021	1.187
530	1.442	1.306	1.346	1.192	1.409	1.222	0.514	0.417	0.817	0.960
540	1.211	1.049	1.101	0.939	1.152	0.960	0.376	0.379	0.662	0.797
560	0.734	0.634	0.592	0.448	0.637	0.358	0.251	0.254	0.516	0.659

Values of Log E_{1cm} computed by arbitrarily assigning the value 2.375 to the maxima of ethanol solutions, and 2.400 in 90% acetone. The former value is close to the value found by Strain (6) for eight leaf xanthophylls in ethanol; the latter an average observed for aliquot samples in acetone solution.

Logarithms are reported, following the practice of Strain, because the shape of the plot of log E against wave length is independent of the concentration.

The coefficients given in Table 4 were determined on compounds prepared in the presence of dimethyl aniline, and therefore the data extend only to 350 $m\mu$.

DISCUSSION

The absorption spectra presented herein represent comparable data for the major pigments found in a group of plants, the diatoms, which are responsible for a very large proportion of the world's photosynthetic production of organic compounds. As is well known, the spectra of these compounds after their extraction from the plant cells may differ from their absorption in the living plants; nonetheless, the spectra of whole extracts are more instructive when one has at hand the spectra of the individual compounds. These data have been used for the spectrophotometric estimation of components of such extracts by a method to be published in a forthcoming paper.

It should be of interest to the evolutionist that chlorophyll c absorbs relatively much more blue than red light, compared with chlorophylls a and b. Thus, the diatoms, dinoflagellates, and brown algae possess a pigment admirably suited to the absorption of light of the wave lengths which penetrate deepest into the sea. Because chlorophyll c is not intensely green, and does not show up well on chromatograms, its amount is apt to be underestimated; for

this reason, it has been generally ignored by oceanographers and limnologists in studies of photosynthesis, although Strain, Manning and Hardin have come to the conclusion that "chlorofucine (Chlorophyll c) may be an important pigment in the carbon economy of nature."

The specific absorption coefficients of chlorophyll c and the diatom xanthophylls remain undetermined. Probably the magnitude of these coefficients of the xanthophylls are all very similar at their maxima, occurring around 450 mμ, since Strain (6) found but little difference in the similarly located maximum specific absorption coefficients of eight leaf xanthophylls.

Solvation effects might account for the previous identification of carotenoids which may be, in reality, differently solvated species of otherwise identical molecules. Chromatographic separation is considered prima facie evidence of the non-identity of chemical compounds, but there is no reason to reject the probability that different solvates of the same molecule can be separated chromatographically.

SUMMARY

Chlorophylls a and c, neofucoxanthin A and B, fucoxanthin, diadinoxanthin and diatoxanthin have been prepared and their absorption spectra, as well as that of beta carotene, in 90% acetone solution, have been determined and reported. Previously reported spectra of the diatom xanthophylls in ethanol have been extended into the near ultra-violet.

Evidence of the formation, chromatographic separation, and decomposition of solvated carotenoid molecular species is presented.

REFERENCES CITED

1. Baumgarten, W., Bauernfeind, J. C., and Boruff, C. S., Ind. Eng. Chem., 36, 344-347 (1944)
2. Haagen-Smit, A. J., Jeffreys, C. E. P., and Kirchner, J. G., Ind. Eng. Chem., Anal Edition 15, 179-180 (1943)
3. Harris, D. G., and Zscheile, F. P., Bot. Gaz. 104, 515-527 (1943).
4. Mackinney, G., J. Biol. Chem. 140, 315-322 (1941).
5. Pace, N., J. Biol. Chem. 140, 483-489 (1941).
6. Strain, H. H., Leaf Xanthophylls, Carnegie Institution of Washington, Publication No. 490 (1938).
7. Strain, H. H., J. Biol. Chem. 127, 191-201 (1939).
8. Strain, H. H., and Manning, W. M., J. Biol. Chem. 144, 625-636 (1942).
9. Strain, H. H., Manning, W. M., and Hardin, G., J. Biol. Chem. 148, 655-668 (1943).
10. Strain, H. H., Manning, W. M., and Hardin, G., Biol. Bull., 86, 169-191 (1944) .
11. Zechmeister, L. and Polgar, A., J. Amer. Chem. Soc. 65, 1522-1528, (1943).
12. Zscheile, F. P., Jr., Bot. Gaz., 95, 529-562 (1934).
13. Zscheile, F. P., Jr., Jour. Phys. Chem. 38, 95-102 (1934b).
14. Zscheile, F. P., Jr., and Comar, C. L., Bot. Gaz. 102, 463-481 (1941).
15. Zscheile, F. P., Jr., Comar, C. L., and Mackinney, G., Plant Physiol. 17, 666-670 (1942).
16. Zscheile, F. P., White, J. W., Jr., Beadle, B. W., and Roach, J. R., Plant Physiol. 17, 331-346 (1942).

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