THE ESTIMATION AND CHARACTERIZATION OF PLANKTON POPULATIONS BY PIGMENT ANALYSES

I. THE ABSORPTION SPECTRA OF SOME PIGMENTS OCCURRING IN DIATOMS, DINOFLAGELLATES, AND BROWN ALGAE[1]

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ABSTRACT

The spectra of chlorophyll c, beta carotene, neofucoxanthin A, neofucoxanthin B, fucoxanthin, diadinoxanthin and diatoxanthin in 90% acetone solutions are reported. These constants are necessary for the simultaneous spectrophotometric determination of the major plankton pigments described.

INTRODUCTION

There are reported herein the absorption spectra of 90% acetone solutions of a number of the pigments found in diatoms, dinoflagellates, and brown algae. These constants were determined as a necessary preliminary in the development of a spectrophotometric method for the simultaneous determination of several pigments found in acetone extracts of plant and animal materials. Solvent partition and chromatographic adsorption were used to prepare the compounds. The methods have been reported by Strain and his co-workers (4, 5, 6, 7), by Pace (3), 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 spectrum of a compound was determined in a solvent as reported in the literature, that solvent was removed by evaporation *in vacuo* at room temperature, and the spectrum was 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; otherwise, relative absorption coeffi-

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cients were determined and are reported here. Ninety per cent acetone was chosen because of its greater effectiveness than 80% or absolute acetone in the extraction of pigments from plant cells.

EXPERIMENTAL

Absorbencies were measured 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 I. Tube and filter selections were in accordance with the manufacturer's recommendations.

Chlorophylls. To prepare chlorophyll *a*, fresh fronds of the brown alga *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; they were then washed first with methanol (to remove xanthophylls) and finally with water (to remove alcohol), after which they were 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.

The spectrum of chlorophyll *a* prepared by the above method agreed well with that of chlorophyll *a* prepared by Harris and Zscheile (2, 9) with a more elaborate method.

Chlorophyll *c* was prepared by the chromatographic method of Strain, *et. al.* (5, 6) from the diatom *Navicula araneosum.* The first formation and early development of the chromatograms always left a mixture of chlorophylls *a* and *c* in the top band. Spectrophotometric examination of the eluted pigments showed that chlorophyll *a* was removed from this band only upon extensive washing with petroleum ether containing up to 4% methanol. This apparent coadsorption of chlorophylls *a* and *c*, not described by Strain and his co-workers, was always observed by the writer.

The spectrum of the mixture eluted from the top green band of a chromatogram prepared by the method of Pace (3) showed that it was also 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.

It should be noted that Strain and Manning (5) found somewhat different spectra for methanol solutions of chlorophyll *c* prepared by solvent partition and by chromatographic adsorption. Acetone solu-

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

Wave Length Range $m\mu$	*Slit (mm)*	*Nominal Band Width* $m\mu$	
	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

tions of chlorophyll *c* prepared by solvent partition have not been studied.

Beta Carotene. A commercial preparation of beta carotene (Eimer and Amend) was used to determine the 90% acetone spectrum of this compound. Although it was described by the manufacturers as xanthophyll, chlorophyll, oil and fat free, the commercial preparation

TABLE II. Absorption Spectra of Chlorophylls *a* and *c*

Wave Length $m\mu$	*Chlorophyll* a *in 90% Acetone* Log E $\frac{1\text{ gm}}{1\text{ cm}}$	*Chlorophyll* c *in 90% acetone. Corrected for chlorophyll* a *content* Log E 1 cm *Soln.* 1	Log E 1 cm *Soln.* 2	*Chlorophyll* c *in Methanol. Corrected for chlorophyll* a *content* Log E 1 cm *Soln.* 1	Log E 1 cm *Soln.* 2
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	.718	.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
540	.591	.519	.499	.384	.352

TABLE II—(*continued*)

Wave Length $m\mu$	*Chlorophyll* a *in 90% Acetone* Log E $\frac{1\text{ gm}}{1\text{ cm}}$	*Chlorophyll* c *in 90% acetone. Corrected for chlorophyll* a *content* Log E $\frac{}{1\text{ cm}}$ *Soln.* 1	*Soln.* 2	*Chlorophyll* c *in Methanol. Corrected for chlorophyll* a *content* Log E $\frac{}{1\text{ cm}}$ *Soln.* 1	*Soln.* 2
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 *a* solutions computed from specific absorption coefficients reported by Zscheile (10).

The relative absorption coefficients of the acetone solutions of chlorophyll *c* at 445 mμ are arbitrarily given the value 1.922 at 430 mμ. Values for chlorophyll *a* are *specific* absorption coefficients, those for chlorophyll *c* are relative absorption coefficients.

gave a blue color, characteristic of the xanthophylls (1) when shaken (in hexane solution) with 85% phosphoric acid. In 90% acetone solution it showed a sudden rise in absorbency in the range 320 to 350 mμ. To purify the preparation it was dissolved in hexane, shaken with 90% methanol, washed with water, and dried over anhydrous

TABLE III. Specific Absorption Coefficients of Beta Carotene in 90% Acetone

Wave Length	Log $E\,\frac{1\text{ gm}}{1\text{ cm}}$	*Wave Length*	Log $E\,\frac{1\text{ gm}}{1\text{ cm}}$
320	1.216	455	2.400
322.5	1.163	456	2.400
325	1.181	460	2.385
330	1.205	465	2.351
335	1.227	470	2.332
340	1.248	475	2.339
345	1.238	480	2.349
350	1.248	485	2.337
355	1.227	490	2.285
360	1.253	500	2.042
370	1.377	510	1.658
380	1.554	520	1.181
390	1.725	530	0.771
400	1.906	540	0.570
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		

Concentrations of solutions determined from aliquot samples in hexane, using specific absorption coefficients reported by Zechmeister and Polgar (8). In 90% acetone the maximum is a little lower (cf. 2.410) and displaced slightly toward the longer wave lengths (cf. 450 $m\mu$) than in hexane solutions.

sodium sulfate. Hexane solutions of beta carotene thus treated also showed unusually high absorbencies in the range 320–370 mμ, but if the solvent were removed and the carotene exhaustively dried under reduced pressure at room temperature, the absorbencies in this range showed no great increases. These observations suggest the formation of methanol solvates which persist in hexane and acetone solution but which can be broken down by drying *in vacuo*. Similar observations were made on fresh xanthophyll preparations.

Xanthophylls. Neofucoxanthin A and B, fucoxanthin, diadinoxanthin and diatoxanthin were prepared by the methods of Strain, *et al.* (7) from mixed diatoms. Their spectra in ethanol and 90% acetone solutions over the range 350 to 560 mμ are reported in Table IV. In the range 320 to 350 mμ, the spectra were found to be inconsistent and to vary with the treatment of the material, as was the case with beta carotene (see above).

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

Wave Length mμ	*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
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 (4) for eight leaf xanthophylls in ethanol; the latter is an average observed for aliquot samples in acetone solution.

RESULTS

The absorption spectra of the compounds discussed above are tabulated in Tables II to IV. Except for chlorophyll *a* and beta carotene, logarithms of the relative absorption coefficients, computed from observed absorbencies, 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 that were determined as indicated in the tables. The Beer-Bouquer law was used in the form

$$\log E = \log D - (\log L + \log c) ,$$

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

$$\log E = \log D - \log c .$$

D is the absorbency, $\log I_0/I$. 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 IV were determined on compounds prepared in the presence of dimethyl aniline, and therefore the data extend only to 350 mμ.

DISCUSSION

The absorption spectra presented herein represent comparable data for the major pigments found in the diatoms, a group of plants which is responsible for a very large proportion of the world's photosynthetic production of organic compounds. These data have been used for the spectrophotometric estimation of the components of 90% acetone extracts of mixed plankton by a method published in a paper by Richards with Thompson which appears earlier in this issue.

It should be of interest to the evolutionist that chlorophyll *c* absorbs relatively much more blue than red light as 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, 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 (6) have come to the conclusion that "chlorofucine (Chlorophyll *c*) may be an important pigment in the carbon economy of nature."

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.

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