inhibitors on this incorporation, have been studied and compared with the results of similar investigations carried out earlier.

2. Washed spermatozoa from buffalo and goat have been shown to incorporate radioactive amino acids into their proteins. No such incorporation was observed in cock spermatozoa.

3. The radioactivity of bull- and buffalo-spermatozoa proteins was retained on reincubation of prelabelled spermatozoa with a large excess of unlabelled amino acids for 1½ hr.

4. There was an average 50% loss of radioactivity from the proteins of bull and buffalo spermatozoa on storage at 0° for 18 hr.; under these conditions the spermatozoa are known to lose their acrosome and hyaluronidase.

5. The findings support the view that the incorporation of radioactive amino acids into the proteins of mammalian spermatozoa occurs in the acrosomal region.

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REFERENCES

Abraham, K. A. & Bhargava, P. M. (1963). *Biochem. J.* **86**, 298.
Bhargava, P. M. (1957). *Nature, Lond.*, **179**, 1120.
Bhargava, P. M., Bishop, M. W. H. & Work, T. S. (1959). *Biochem. J.* **73**, 247.
Brenner, S., Jacob, F. & Meselson, M. (1961). *Nature, Lond.*, **190**, 576.
Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M. & Yankwich, P. F. (1949). *Isotopic Carbon*, p. 317. New York: J. Wiley and Sons Inc.
Gros, F., Hiatt, H., Gilbert, W., Kurland, C. G., Rosebrough, R. W. & Watson, J. D. (1961). *Nature, Lond.*, **190**, 581.
Martin, F. & Brachet, J. (1959). *Exp. Cell Res.* **17**, 399.
Perry, E. J. (1960). *Artificial Insemination in Farm Animals*, 3rd ed. New Jersey: Rutgers University Press.
Swyer, G. I. M. (1947). *Biochem. J.* **41**, 413.

Biochem. J. (1963) **86**, 313

Purification and Properties of Chlorophyll *c* from *Sargassum flavicans*

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Chlorophyll *c* is found together with chlorophyll *a* in many types of marine algae. These include the Bacillariophyceae, Dinophyceae, Phaeophyceae and Cryptophyceae (Strain & Manning, 1942; Strain, Manning & Hardin, 1943; Haxo & Fork, 1959), and some members of the Chrysophyceae (Allen, French & Brown, 1960; Jeffrey, 1961). Although chlorophyll *c* has been obtained sufficiently free from other pigments to enable its spectrum to be determined (Smith & Benitez, 1955; Strain, 1958; Haxo & Fork, 1959) it was not obtained in the pure state. Therefore, little is known of its chemistry, and methods used for the estimation of chlorophyll *c* are indirect (Richards & Thompson, 1952). The present work describes a method which has been developed for the purification and crystallization of chlorophyll *c* from a member of the Phaeophyceae, *Sargassum flavicans*.

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EXPERIMENTAL

Materials and methods

Solvents. Magnesium-free ethanol was obtained by drying a commercial product over K_2CO_3 and distilling twice. All other solvents were analytical reagent grade, and were used without further purification.

Chromatographic adsorbents. The following were used: cellulose powder (Whatman), silicic acid (Mallinckrodt, analytical reagent), aluminium oxide for chromatography (British Drug Houses Ltd.), Hyflo Supercel (Johns Manville) and silicic acid for thin-layer chromatography (Merck).

Source of chlorophyll c. Sargassum flavicans was chosen as the source material for chlorophyll *c* because of its availability and the absence of chlorophyllase activity during extraction. This enzyme converts chlorophyll *a* into chlorophyllide *a*, which is difficult to separate from chlorophyll *c* (Jeffrey, 1961). The seaweed was collected from Port Hacking and Lake Macquarie, New South Wales, and kept in sea water until extracted.

Assay of total phosphorus. This was carried out by the method of Allen (1940).

Assay of magnesium. This was carried out by atomic-absorption spectroscopy (Walsh, 1955; Dawson & Heaton, 1961). The method is free from spectroscopic interference by other elements. Samples of chlorophyll *c* were dried at 100° in platinum crucibles and ashed at 500° for 24 hr. in a muffle furnace. The ash was dissolved in 2N-HCl (0·5 ml.) over a steam bath and was made up to 10 ml. with double-glass-distilled water. Solvent blanks were treated in a similar way. Magnesium was estimated in measured portions after the addition of strontium chloride (final concn. 1·5 mg./ml.) to correct for possible traces of aluminium, phosphate and silicate; internal magnesium standards (final concn. 0·1 and 0·2 μg./ml.) were used (B. G. Davey, personal communication).

Detection of phospholipid. Samples were analysed by chromatography on papers impregnanted with silicic acid according to the method of Marinetti & Stotz (1956), or by the technique of thin-layer chromatography in which thin films of silicic acid were dried on glass plates. The solvent used in both techniques was di-isobutyl ketone–acetic acid–water (8:5:1, by vol.), and lipids were located under u.v. light as purple or yellow fluorescent spots after staining with 0·01% Rhodamine 6G.

Extinction coefficients. These were determined on solutions of purified chlorophyll *c* as soon as this was eluted from aluminium oxide columns. Dry-weight determinations were carried out by drying down samples of this purified chlorophyll *c*. No extinction measurements were made on dried preparations because of the scarcity of crystalline material.

Absorption spectra. The absorption spectra of chlorophyll *c* solutions were measured on a Cary recording spectrophotometer (model 14). For routine extinction measurements a Unicam SP. 500 spectrophotometer was used.

Purification of chlorophyll c

Extraction of pigments. Throughout the purification procedure chlorophyll *c* solutions were always kept in the dark at −20° when extractions or chromatography were not taking place. Fresh *Sargassum* was freed from old or encrusted material and the tissue was cut into small pieces. The pigments were extracted from 500 g. lots (fresh weight) by immersing the tissue in methanol for 5–10 min., and then adding an equal volume of acetone and macerating for several minutes in a top-drive macerator. The extract was filtered through paper (Eaton–Dikeman 541) and the tissue residue re-extracted with acetone until no further pigment was released. The methanol–acetone extract (400 ml.) was mixed with diethyl ether (400 ml.) and shaken with 4 l. of 10% (w/v) NaCl to transfer the pigments to the diethyl ether layer. Acetone, methanol and water-soluble impurities were removed in the aqueous phase. The diethyl ether extracts were collected and evaporated almost to dryness in a stream of air. The residue was redissolved in the minimum volume of diethyl ether, centrifuged to remove water of condensation and stored at −20° in the dark until required for chromatography.

Separation of chlorophyll c *from chlorophyll* a *and carotenoids.* Columns (3·5 cm. internal diam. × 80 cm. long) were prepared from cellulose powder suspended in light petroleum (b.p. 60–80°). Suitable portions of the diethyl ether extract were added to the column and the pigments were separated with 0·5% (v/v) propan-1-ol in light petroleum (60–80°) as developing solvent (Strain, 1958). Carotene, chlorophyll *a* and xanthophylls were washed through the column while chlorophyll *c* remained as a light-green zone at the top. The solvent was changed to 5% (v/v) methanol in diethyl ether and just sufficient was passed through the column to concentrate the chlorophyll *c* to a narrow band without eluting it. The cellulose powder was extruded from the columns and the chlorophyll *c* zones were removed, combined and repacked as a fresh column. Chlorophyll *c* was eluted from this column with 5% (v/v) methanol in diethyl ether. The dark-green solution was evaporated almost to dryness in a stream of air and redissolved in the minimal quantity of the eluting solvent. The extract was centrifuged to remove water of condensation and stored at −20° in the dark. At this stage of the preparation (stage 1) chlorophyll *c* was free from other pigments except for traces of fucoxanthin, but was heavily contaminated with phospholipid. Six lipid fractions were present in addition to chlorophyll *c* as shown by thin-layer silicic acid chromatography.

Separation of chlorophyll c *from phospholipid*

Silicic acid chromatography. Partial separation of chlorophyll *c* from phospholipid was achieved on columns of silicic acid. Silicic acid (about 120 g.) mixed with Hyflo Supercel

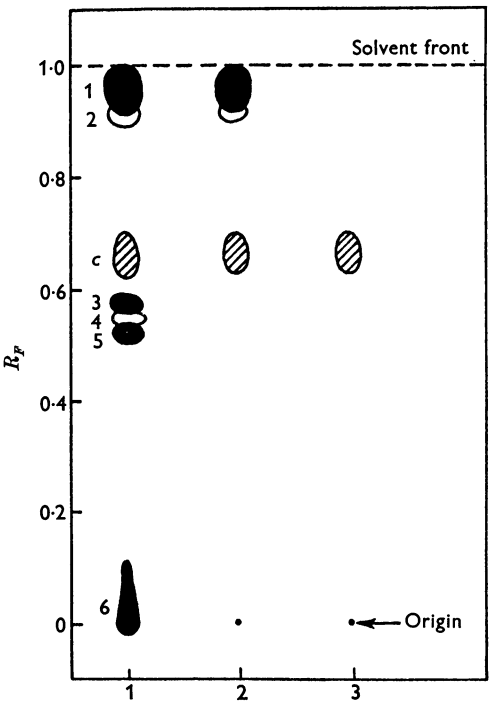

Fig. 1. Chromatogram showing lipid fractions associated with chlorophyll *c* during the purification procedure. The adsorbent was thin films of silicic acid dried on glass plates, and the solvent was di-isobutyl ketone–acetic acid–water (8:5:1, v/v/v). Lipid spots 1, 3, 5 and 6 fluoresced purple under u.v. light, spots 2 and 4 fluoresced yellow. Spot *c* corresponds to chlorophyll *c*.

(5:2, w/w) was packed as a slurry in chloroform into columns (3·5 cm. internal diam. × 45 cm. long). A suitable portion (containing about 6 mg. of chlorophyll *c*, stage 1) was placed on the column, and the chlorophyll *c* (which was not adsorbed) was washed through with 5 % (v/v) methanol in chloroform. The major part of the chlorophyll *c* travelled as a sharp dark-green band which left a small diffuse trail. Only the major dark-green band of chlorophyll *c*, which was eluted first, was collected (stage 2). The latter portion of the chlorophyll *c* band was rejected as a precaution against possible contamination with subsequent lipid fractions. The solvent flow was regulated by the application of a slight vacuum to the bottom of the column, so that the chlorophyll *c* was eluted in 20–30 min. Thin-layer chromatography on silicic acid films showed that four lipid fractions were removed by this treatment (see Fig. 1, stage 2).

Aluminium oxide chromatography. Final purification of chlorophyll *c* was achieved on columns of neutral aluminium oxide. Aluminium oxide (about 200 g.) was packed into columns (3·5 cm. internal diam. × 45 cm. long) with chloroform. Chlorophyll *c* (about 4 mg., stage 2) was applied to the top of the column where it was adsorbed as a dark-green band. Traces of carotenoid and one lipid fraction were eluted with about 500 ml. of methanol–chloroform (9:1, v/v). Elution of the lipid fraction was characterized by a marked increase in the temperature of the zone just behind the carotenoid band. The remaining lipid fraction was removed by washing with 500 ml. of ethanol–chloroform–water (5:2:1, by vol.), and again this was characterized by a warm band moving down the column. Chlorophyll *c* was finally eluted from the column with ethanol–chloroform–water (10:4:5, by vol.). The first fraction of chlorophyll *c* that was eluted was discarded as a precaution against possible contamination from traces of the preceding lipid zones. The major fraction of the chlorophyll *c* came through the column as a dark-green band and was collected. Samples of this chlorophyll *c* (stage 3) were taken immediately for determinations of magnesium, total phosphorus, extinction coefficient and dry weight. Thin-layer chromatography of this material showed no trace of lipid spots (Fig. 1).

Extinction measurements showed that the yield of chlorophyll *c* from each of the silicic acid and aluminium oxide columns was about 50 %. Therefore in a typical preparation 33 mg. of chlorophyll *c* (stage 1) yielded 18 mg. of chlorophyll *c* (stage 2) and 8·5 mg. of chlorophyll *c* (stage 3). No attempt was made to improve the yield of pure chlorophyll *c*.

Crystallization of chlorophyll c. The remainder of the chlorophyll *c* solution from stage 3 was evaporated under nitrogen until the solution changed colour and a fine copper-red precipitate appeared. A few drops of ethanol were added to redissolve the precipitate, water was added to a final concentration of about 5–10 % and the solution was left in the dark at room temperature (20°). Reddish-black crystals formed in about 24 hr. Three crystal sizes were observed. Large crystals (50–100 μ) appeared as parallel four-sided plates, minute crystals (3–5 μ) were fine hexagonal platelets, and crystals of intermediate size (10–15 μ) appeared under high power to be both hexagonal and four-sided. It is suggested that crystals of the form of a hexagonal bipyramid would show these properties. Crystallization of chlorophyll *c* could not be induced in the presence of traces of lipid. Under these conditions a copper-red amorphous precipitate was obtained on slow evaporation.

RESULTS

Purity of preparations. Table 1 shows the extinction coefficients, total phosphorus and lipid contents of a number of chlorophyll *c* preparations at

Table 1. *Analyses of some chlorophyll* c *preparations*

Experimental details are given in the text.

	$E_{1\,cm.}^{0.1\,\%}$ at 630 mμ in acetone	Total phosphorus (mg. of *P*/100 mg. of chlorophyll *c*)	Presence of lipid	Crystallization of preparations at stage 3
Preparation 1				
Stage 1	1·4	2·5	Lipids 1–6	—
Stage 2	8·3	—	Lipids 1 and 2	—
Stage 3	16·3	< 0·004	0	Crystallization not attempted
Preparation 2				
Stage 1	1·9	1·25	Lipids 1–6	—
Stage 2	7·7	0·69	Lipids 1 and 2	—
Stage 3	16·5	0	0	Crystals formed: hexagonal platelets (3–5 μ)
Preparation 3				
Stage 1	2·2	1·18	Lipids 1–6	—
Stage 2	9·9	0·10	Lipids 1 and 2	—
Stage 3	15·8	0	0	Crystals formed: parallel four-sided plates (50–100 μ)
Preparation 4				
Stage 1	1·1	2·31	Lipids 1–6	—
Stage 2	9·7	0·10	Lipids 1 and 2	—
Stage 3	15·7	0	0	Crystals formed: hexagonal bipyramids (10–15 μ)

Table 2. *Extinction in acetone of chlorophyll* c *during purification*

Experimental details are given in the text. The extinction at 630 mμ was assigned the value of 1·00 for the purposes of comparison.

Stage of purification of chlorophyll *c*	Relative extinction		
	At 630 mμ	At 580 mμ	At 450 mμ
Stages 1 and 2	1·00	0·78–0·93	9·16–10·11
Stage 3 (measured immediately after obtaining chlorophyll *c* from aluminium oxide columns)	1·00	0·87	9·30
Stage 3 (stored at −20° for > 2 hr.)	1·00	0·58–0·73	6·90–8·10

the three stages of the purification procedure. At stage 3 chlorophyll *c* showed the complete absence of lipid and phosphorus; an additional sign of purity is the ability of this chlorophyll *c* to crystallize. The *E* values of stage 3 chlorophyll *c* from preparations 3 and 4 were considered the most accurate since larger quantities (about 4 mg. dry wt.) were used for the weight determinations than in preparations 1 and 2 (0·5–1·0 mg.). The extinction coefficient, $E_{1\,cm.}^{0.1\%}$, of 15·8 at 630 mμ in acetone was used as the basis of all the extinction measurements.

Absorption spectra. Table 2 indicates that the chlorophyll *c* from stages 1 and 2 possessed similar spectra, but, after the removal of the last traces of lipid at stage 3, the extinction in the blue region of the spectrum was diminished and the maximum shifted from 450 to 442 mμ. In addition, the 630 mμ band sometimes shifted from 630 to 628 mμ, although there was no significant change in the intensity of this peak. These spectral changes occurred when the chlorophyll *c* was stored at −20° before analyses were made, but were not seen when the pure chlorophyll *c* was analysed immediately after elution from the aluminium oxide columns.

Absorption spectra of pure chlorophyll *c* (stage 3) in different solvents are shown in Fig. 2, and the absorption maxima and extinction coefficients at the maxima are given in Table 3. Absorption curves were obtained 18 hr. after the final purification on aluminium oxide. Each sample contained 5 % (v/v) of ethanol, but since the ethanol spectrum was almost identical with that of chlorophyll *c* in 100 % acetone the influence of the ethanol on the spectra was considered to be negligible. Fig. 2 shows that the absorption spectrum of chlorophyll *c* like that of chlorophyll *a* and *b* (Rabinowitch, 1951), was greatly influenced by the solvents used. Methanol depressed the absorption peaks, whereas some enhancement of the Soret band was seen with diethyl ether. In contrast with chlorophyll *a* and *b*, which have similar extinctions in 90 and 100 % acetone (Vernon, 1960), chlorophyll *c* showed a higher extinction in 90 % than in 100 % acetone.

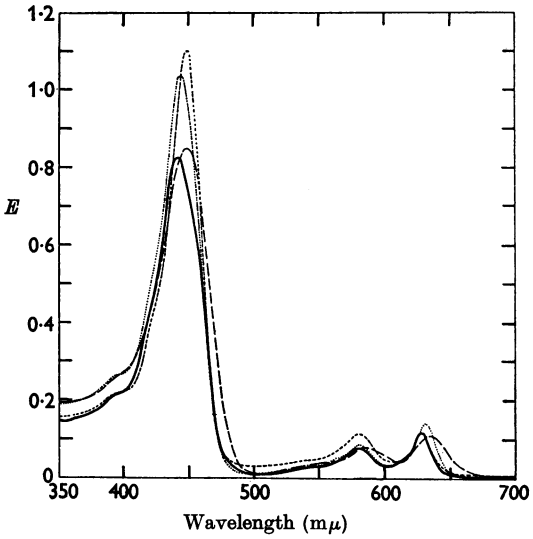

Fig. 2. Absorption spectra of chlorophyll *c* in different solvents: ——, 100 % acetone; · · · · ·, 90 % acetone; – – –, methanol; - - - - -, diethyl ether. The absorption curves were obtained 18 hr. after the final purification on aluminium oxide (see the text). Each sample was prepared by diluting 0·2 ml. of chlorophyll *c* in ethanol with 3·8 ml. of solvent (final concn. of chlorophyll *c*, 7·1 μg./ml.).

Magnesium in chlorophyll c. The mean magnesium content of six preparations of chlorophyll *c* at stage 3 was 2·31 ± 0·045 mg./100 mg. of chlorophyll *c*. The concentration of chlorophyll *c* was calculated in each case from an $E_{1\,cm.}^{0.1\%}$ value of 15·8 at 630 mμ in acetone.

Phaeophytin c. This was prepared by shaking an ethereal solution of chlorophyll *c* with 0·5 N-sulphuric acid. The absorption spectra of phaeophytin *c* and chlorophyll *c* in ethyl ether are shown in Fig. 3. Maxima were at 625, 594, 573, 530 and 430 mμ.

Solubility properties. Dry chlorophyll *c* is insoluble in diethyl ether and acetone, but is soluble in methanol, ethanol and ethyl acetate. The hydrochloric acid number (Willstätter & Stoll, 1913) of

Table 3. *Absorption maxima and extinction coefficients of pure chlorophyll* c *in different solvents*

Values are calculated on the basis of an $E^{0.1\%}_{1\,cm.}$ value of 15·8 at 630 mμ in acetone. Measurements were made 18 hr. after chlorophyll *c* was eluted from the aluminium oxide columns.

	Maxima and extinction coefficients					
Solvent	Wavelength (mμ)	$E^{0.1\%}_{1\,cm.}$	Wavelength (mμ)	$E^{0.1\%}_{1\,cm.}$	Wavelength (mμ)	$E^{0.1\%}_{1\,cm.}$
Acetone (100%)	628	15·8	580	10·7	442	115·9
Acetone (90%)	630	19·5	580	11·5	443	144·0
Diethyl ether	628	15·8	580	15·7	449	169·7
Methanol	635	15·2	585	10·7	448	119·5
Ethanol	632	16·2	582	12·7	444	123·0

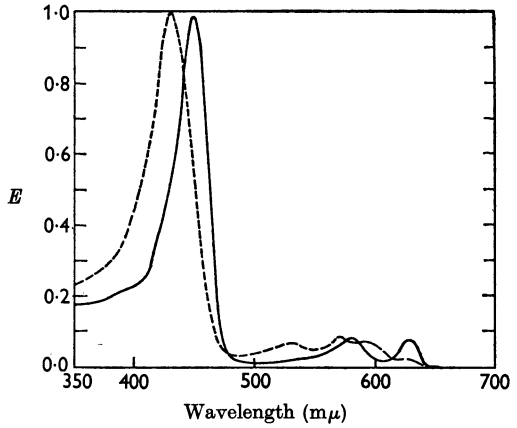

Fig. 3. Absorption spectra of chlorophyll *c* (——) and phaeophytin *c* (– – –) in diethyl ether (concn. of chlorophyll *c*, 5·7 μg./ml.).

purified chlorophyll *c* is 12, which confirms the value obtained for impure chlorophyll *c* by Granick (1949).

DISCUSSION

Chlorophyll *c* obtained from *Sargassum flavicans* showed similar spectral properties in the early stages of the purification to chlorophyll *c* obtained from other types of algae. The ratio of the blue to the red maxima of chlorophyll *c* from diatoms and dinoflagellates (Strain *et al.* 1943), from brown algae (Smith & Benitez, 1955) and from cryptomonads (Haxo & Fork, 1959) was approximately 10:1, with maxima at 633, 584 and 447 mμ in methanol, and at 626, 577 and 443 in diethyl ether (Smith & Benitez, 1955). In the present work, values of about 10:1 were obtained for the ratio of the extinctions at 450 mμ and 630 mμ for impure chlorophyll *c* (stages 1 and 2). However, with the final separation of chlorophyll *c* from lipid, a decrease in the intensity of the blue maximum and a wavelength shift from 450 to 442 mμ occurred. These spectral changes were not apparently due to alterations in the chlorophyll *c* molecule by aluminium oxide, since chlorophyll *c* which had been through aluminium oxide columns but which had not been completely freed from lipid by this process did not show the wavelength shift at the blue end of the spectrum. Whether the spectral change after removal of lipid was caused by molecular aggregation on storage at −20° or by some form of molecular rearrangement is not known at present. Brody & Brody (1961) found that aggregation of chlorophyll *a* was induced by cooling concentrated solutions to low temperatures, and that this aggregation was associated with a spectral change.

In a previous study of the pigment composition of marine algae (Jeffrey, 1961), the chlorophyll *c* concentrations were calculated from an $E^{0.1\%}_{1\,cm.}$ value of 22 at 630 mμ. This extinction coefficient was derived by Smith & Benitez (1955) from magnesium determinations on impure chlorophyll *c*, assuming a molecular weight of 893 (similar to that of chlorophyll *a*). Table 4 gives the chlorophyll *c* values recalculated on the basis of an $E^{0.1\%}_{1\,cm.}$ value of 15·8 (in acetone). The extinction at 630 mμ was used in preference to that at 450 mμ because of the stability of the extinction at the red end of the spectrum. For this reason it is recommended that determinations of chlorophyll *c* should always be based on the extinction at 628–630 mμ.

On the basis of a magnesium content of 2·31 ± 0·045 % the molecular weight of chlorophyll *c* is 1052 (range 1013–1105). This is slightly higher than the value 1020 given by Jeffrey (1962). Granick (1949) and Smith & Benitez (1955) suggested that, because chlorophyll *c* has a low hydrochloric acid number, similar to the chlorophyllides (Willstätter & Stoll, 1913), it might be phytol-free. If chlorophyll *c* is a chlorophyllide-like molecule, the molecular weight would be about 600, which is not in agreement with the present work. Chlorophyll *c* appears to have a lower magnesium content and thus a higher molecular weight than the other chlorophylls. Chlorophylls *a* and *b*, bacteriochlorophyll, *Chlorobium* chlorophyll 660 and *Chlorobium* chlorophyll 650 have magnesium contents of 2·72,

Table 4. *Chlorophyll content of some marine algae (recalculated from Jeffrey, 1961)*

$E^{0.1\%}_{1\,cm.}$ for chlorophyll *a* at 665 mμ was taken to be 84 (Smith & Benitez, 1955), and that for chlorophyll *c* at 630 mμ to be 15·8 (the present work).

	Chlorophyll content (mg./g. dry wt.)					
	Phaeodactylum tricornutum	*Skeletonema costatum*	*Nitzschia closterium*	*Isochrysis galbana*	*Sphaleromantis* sp.	*Gymnodinium* sp.
Chlorophyll *a*	5·22	0·38	3·26	2·96	2·43	0·97
Chlorophyll *c*	1·87	0·29	1·64	1·61	0·95	0·79
Total chlorophyll	7·09	0·67	4·90	4·57	3·38	1·76

2·68, 2·67, 2·92 and 2·59 % respectively (Smith & Benitez, 1955; Stanier & Smith, 1960). The relatively low magnesium content of chlorophyll *c* may indicate either that the chlorophyll *c* which crystallizes at stage 3 of the purification procedure is still impure, or that its structure differs significantly from the other chlorophylls. In this connexion it is interesting that *Chlorobium* chlorophylls 660 and 650 do not contain phytol but a C_{15} alcohol, farnesol (Rapoport & Hanlow, 1961).

SUMMARY

1. Chlorophyll *c* from *Sargassum flavicans* was purified by chromatography on cellulose, silicic acid and aluminium oxide.

2. Purified chlorophyll *c* was crystallized from aqueous ethanol in the form of hexagonal bipyramids or parallel four-sided plates.

3. The extinction coefficient, $E^{0.1\%}_{1\,cm.}$, of the purest chlorophyll *c* in acetone at 628–630 mμ was 15·8. Absorption maxima were at 628, 580 and 442 mμ with relative extinctions at the maxima of 1·0, 0·58–0·73 and 6·9–8·1 respectively.

4. The magnesium content of chlorophyll *c* was 2·31 ± 0·045 %.

5. The absorption spectrum of chlorophyll *c* was extremely sensitive to solvents, and to the presence of lipid.

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REFERENCES

Allen, M. B., French, C. S. & Brown, J. S. (1960). In *Comparative Biochemistry of Photoreactive Systems*, p. 33. Ed. by Allen, M. B. New York: Academic Press Inc.

Allen, R. J. L. (1940). *Biochem. J.* **34**, 858.

Brody, S. S. & Brody, M. (1961). *Biochim. biophys. Acta*, **54**, 495.

Dawson, J. B. & Heaton, F. W. (1961). *Biochem. J.* **80**, 99.

Granick, S. (1949). *J. biol. Chem.* **179**, 505.

Haxo, F. T. & Fork, D. C. (1959). *Nature, Lond.*, **184**, 1047.

Jeffrey, S. W. (1961). *Biochem. J.* **80**, 336.

Jeffrey, S. W. (1962). *Nature, Lond.*, **194**, 600.

Marinetti, G. V. & Stotz, E. (1956). *Biochim. biophys. Acta*, **21**, 168.

Rabinowitch, E. I. (1951). *Photosynthesis and Related Processes*, vol. 2, part I. New York: Interscience Publishers Inc.

Rapoport, H. & Hanlow, H. P. (1961). *Biochem. biophys. Res. Commun.* **6**, 134.

Richards, F. A. & Thompson, T. G. (1952). *J. Mar. Res.* **11**, 156.

Smith, J. H. C. & Benitez, A. (1955). In *Modern Methods of Plant Analysis*, vol. 4. Ed. by Paech, K. & Tracey, M. Heidelberg: Springer Verlag.

Stanier, R. Y. & Smith, J. H. C. (1960). *Biochim. biophys. Acta*, **41**, 478.

Strain, H. H. (1958). *Chloroplast Pigments and Chromatographic Analysis*, 32nd Priestley Lecture, University Park, Pennsylvania.

Strain, H. H. & Manning, W. M. (1942). *J. biol. Chem.* **144**, 625.

Strain, H. H., Manning, W. M. & Hardin, G. (1943). *J. biol. Chem.* **148**, 655.

Vernon, L. P. (1960). *Analyt. Chem.* **32**, 1144.

Walsh, A. (1955). *Spectrochim. Acta*, **7**, 108.

Willstätter, R. & Stoll, A. (1913). *Investigations on Chlorophyll*. Lancaster: Science Press Printing Co.