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SHORT COMMUNICATION

# CHLOROPHYLL c FROM *SARGASSUM FLAVICANS*— ISOLATION AND STRUCTURE

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**Abstract**—Chlorophyll c, isolated from *Sargassum flavicans*, has been purified using sephadex gels. Spectral data agree with the general structure published recently and confirm the presence of a free carboxyl group.

## INTRODUCTION

THE ESTIMATED molecular weight reported by Jeffrey[1] of chlorophyll c isolated from *Sargassum flavicans* is at variance with the recently published structure[2] of chlorophyll c obtained from the diatom *Nitzschia closterium forma minutissima* (also known as *Phaeodactylum tricornutum*).[3] Since *S. flavicans* has been shown to be free of chlorophyllase, while *Ph. tricornutum* contains a high level of this esterase,[4] this study was undertaken in order to re-examine the structure of chlorophyll c.

## RESULTS AND DISCUSSION

Chlorophyll c was extracted from the source material and freed from other pigments by chromatographing on cellulose columns and eluting with acetone–hexane. This solvent proved more effective than the *n*-propanol–hexane system commonly used for chlorophylls. A column of Sephadex LH20 in methanol was then used to remove most of the closely associated lipids. Precipitation of the eluate from $CCl_4$ solution, and crystallization of the product from tetrahydrofuran[5] or $Et_2O$ to which petroleum ether has been added, produced black crystals of chlorophyll c. The purification procedures described[5] for chlorophyll c from *Phaeodactylum tricornutum* did not remove the lipids that are associated with chlorophyll c in *Sargassum flavicans*. The use of chromatographic adsorbents, such as alumina, known to cause alterations in the structures of chlorophylls a and b, was avoided.

The purity of chlorophyll c was checked with silica gel TLC plates, using Wagner's solvent system for lipids.[6] Both chlorophyll c and phaeophytin c had an $R_f$ of approximately 0·6. However, upon treatment of phaeophytin c with diazomethane, the resulting product travelled with the solvent front, as did chlorophyll a in this solvent system.

[1] S. W. JEFFREY, *Biochem. J.* **86**, 313 (1963).
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[4] J. BARRETT and S. W. JEFFREY, *Plant Physiol.* **39**, 44 (1964).
[5] H. H. STRAIN and W. A. SVEC, in *The Chlorophylls* (edited by L. P. VERNON and G. P. SEELEY), p. 35, Academic Press, New York (1966).
[6] E. STAHL, *Thin Layer Chromatography*, p. 161, Academic Press, London (1965).

Chlorophyll c in a KBr disc showed a broad band in the i.r. region from 3700–3100 $cm^{-1}$ characteristic of a carboxyl group. The aliphatic C–H stretching bands from 3000–2850 $cm^{-1}$ were of low intensity. Two carbonyl bands were present, a more intense one at 1690 $cm^{-1}$ with two shoulders and a less intense one at 1630 $cm^{-1}$.

NMR spectra of chlorophyll c and phaeophytin c dissolved in trifluoroacetic acid were obtained. Results agree with those reported,[2] showing the absence of a side-chain esterifying the second carboxyl group. Chlorophyll c is reported to be a mixture of two compounds.[2] The mass spectrum of phaeophytin c failed to show the expected parent peaks and it was therefore treated with diazomethane to obtain more volatile derivatives. The NMR spectrum of the product dissolved in deuterochloroform now showed an additional singlet at 4·35 ppm from TMS. This peak was equivalent to three protons. It was assigned to the methyl group of the esterified acrylic acid portion of phaeophytin c.

The mass spectrum of the esterified product showed a group of moderately intense peaks having *m/e* values between 600 and 608. The molecular ion is expected to be prominent with porphyrins.[7] The expected *m/e* values for phaeophytin c monomethyl esters are 600 and 602. However, the presence of more than one component of chlorophyll c,[8] and the high temperature needed to volatilize the material, complicated the spectrum. Thus the molecular weights and ratio of the components of chlorophyll c could not be deduced.

However, the above spectral data confirm the absence of an esterifying group on the acrylic acid portion of the chlorophyll c molecule, as obtained from *S. flavicans*.

## EXPERIMENTAL

### *Purification*

The extraction and cellulose column chromatography procedures were as previously described,[1] except that 25% acetone in hexane was the eluting solvent. The eluted chlorophyll c was evaporated to dryness *in vacuo* and dissolved in MeOH–$CHCl_3$ (1:1). This solution was chromatographed on a Sephadex LH20 column prepared in methanol. The eluted chlorophyll c in methanol was mixed with an equal volume of $CCl_4$ and 10 vol. of water were added. The mixture was vigorously shaken. Residual lipids remained in the organic phase, while the chlorophyll c was filtered from the aqueous phase. It was crystallized from a mixture of tetrahydrofuran (or $Et_2O$) and petroleum ether (b.p. 40–60°).

### *Phaeophytin c and its Methylated Derivative*

Phaeophytin c was prepared by dissolving chlorophyll c in acetone and adding HCl until the solution was 1 M in HCl. The solution was kept at 4° for 2 hr, after which the precipitated phaeophytin c was filtered and dried.

A solution of phaeophytin c in tetrahydrofuran was treated with 4 moles of $CH_2N_2$ in $Et_2O$ for 5 min and the solvents were allowed to evaporate.

### *TLC*

Chlorophyll c was checked for lipid contamination using plates of silica gel G and $CHCl_3$–MeOH–$H_2O$ (65:25:4) as solvent. The lipids were visualized with iodine vapour. With contaminated samples, six lipids appeared separated from chlorophyll c.

### *Ultra-violet Spectra*

Dried purified chlorophyll c was dissolved in acetone and gave a specific extinction coefficient of 21·0 at 628 nm. Dried chlorophyll c was not readily soluble in $Et_2O$.

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[8] S. W. JEFFREY, *Biochem. Biophys. Acta* **162** (2), 271 (1968).