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THE PRESENCE OF PHYTOL IN BROWN AND BLUE-GREEN ALGAE AND ITS RELATIONSHIP TO EVOLUTION

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Abstract—Phytol was identified in the chlorophyll-a-containing *Fucus vesiculosus* and *Phormidium luridum* by nuclear magnetic resonance, infrared, and mass spectroscopy, and by thin-layer chromatography. It was not accompanied by significant amounts of related isopentenoid alcohols. The development of highly precise control of the isopentenoid pathway leading to phytol and its esterification to chlorophyll is therefore indicated at an early evolutionary stage which seems to be related to the development of the porphyrin pathway but not to the development of chloroplastic and other major subcellular membranes.

INTRODUCTION

RECENTLY it has become evident that the degree of polymerization of the isopentenoid unit is not highly controlled in fungi and bacteria. Thus, the principal ubiquinones of *Saccharomyces cerevisiae*, *Escherichia coli*, and *Rhodospirillum rubrum* have chain lengths, respectively, of 6, 8, and 10 C_5-units.[1] This raises the possibility that control of chain length in the pathway leading to the phytol portion of chlorophyll might similarly be variable in photosynthetic organisms. However, except for one case (the occurrence of farnesol in *Chlorobium* chlorophylls[2, 3]), the alcohol portion of chlorophyll has been found to be phytol as first discovered by Willstätter.[4, 5] Both Willstätter's extensive data[5] and those of others deal principally with higher plants. Less data is available among lower organisms, but bacteriochlorophyll-a[3] and three genera of Chlorophyta[5–7] (*Ulva*, *Scenedesmus*, and *Chlorella*), have been reported to contain phytol. Since the probability of finding variations in chain length are seemingly greater lower in the evolutionary scale, we have extended the examination of isopentenoid alcohols in algae to include representatives of the Phaeophyta (*Fucus vesiculosus*) and Cyanophyta (*Phormidium luridum*).

RESULTS

Both *Fucus vesiculosus* and *Phormidium luridum* yielded a nearly colorless oil, 60 mg/100 g of air-dried material and 66 mg/100 g of wet cells, respectively, in the phytol region of an alumina chromatogram. In both cases there was no major distribution of molecular weights

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[1] G. D. DAVES, JR., R. F. MURACA, J. S. WHITTIEK, P. FRUS and K. FOLKERS, *Biochem.* **6**, 2861 (1967).
[2] H. RAPOPORT and H. P. HAMLOW, *Biochem. Biophys. Res. Commun.* **6**, 134 (1961).
[3] A. S. HOLT, D. W. HUGHES, H. J. KENDE and J. W. PURDIE, *Plant Cell Physiol.* **4**, 49 (1963).
[4] R. WILLSTÄTTER, O. SCHUFFLI and E. W. MEYER, *Ann. Alen.* **418**, 121 (1918).
[5] R. WILLSTÄTTER, F. HOCHEDER and E. HUG, *Ann. Alen.* **371**, 1 (1909).
[6] I. IWATA, H. NAKATA, M. MIZUSHIMA and Y. SAKURAI, *Agr. Biol. Chem.* (*Tokyo*) **25**, 319 (1961).
[7] I. IWATA and Y. SAKURAI, *Agr. Biol. Chem.* (*Tokyo*) **27**, 253 (1963).

as determined either by TLC or mass spectrometry. The molecular weight by the latter technique was 296, and in both techniques as well as by i.r. spectrometry and NMR spectroscopy, the two samples clearly had the same structure as an authentic sample of phytol obtained from Mann Research Laboratories.

The TLC procedure used is known[10–12] to separate isopentenoid alcohols as a function of molecular weight and number of double bonds. The coincidence of the spots (R_f 0·67) from the algae with that of standard phytol (Mann Research Laboratories) was precise, and there was less than *ca.* 1 per cent of other material visible. The mass spectral data of the two samples and the standard showed expected fragmentation at *m/e* 296 (parent peak), 278 (M less H_2O), 263 (M less H_2O less CH_3), 253 (M less C_3H_7), 252 (M less C_3H_7 less H), 250 (M less H_2O less C_2H_4), 249 (M less H_2O less C_2H_5), 196 (M less $CH_3—CH_2—C(CH_3)═CH—CH_2OH$), 126 ($CH_2═CH—CH_2—C(CH_3)═CH—CH_2—OH$), and 111 (the latter less CH_3). The i.r. spectra showed the presence of OH and C═C and the "finger print" of the two samples was the same as that of our authentic sample (Fig. 1). While the *Fucus* sample showed very little if any impurity, the *Phormidium* sample was contaminated by a phthalate (λ_{max} 5·72, 7·75, 7·84, 8·90 μ and *m/e* 149 and 167) which was unfortunately introduced from solvents. Most of it was removed by chromatography, but some of the tailing edge of the elution band remained in the phytol. The authentic sample also showed a small amount of carboxyl impurity.

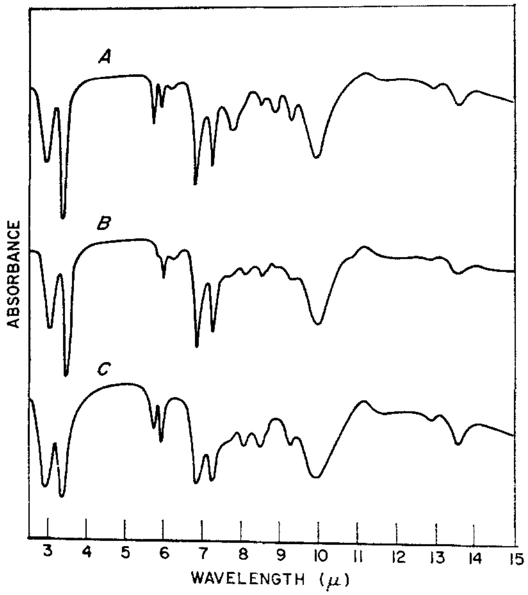

FIG. 1. I.r. SPECTRA OF PHYTOL SAMPLES OBTAINED FROM (A) *Phormidium luridum*, (B) *Fucus vesiculosus*, AND (C) MANN RESEARCH LABORATORIES.

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[8] W. A. KRATZ and J. MYERS, *Am. J. Botany* 42, 282 (1955).

[9] J. BIGGINS, *Plant Physiol.* 42, 1442 (1967).

[10] P. J. DUNPHY, J. D. KERR, J. F. PENNOCK and K. J. WHITTLE, *Chem. & Ind.* 1549 (1966).

[11] A. R. WELLBURN and F. W. HEMMING, *Phytochem.* 5, 969 (1966).

[12] H. K. MANGOLD, in *Thin-Layer Chromatography* (edited by E. STAHL), pp. 137–181, Springer-Verlag, New York (1965).

The NMR spectra showed the expected doublet at 4·01 ppm for the two C-1 protons adjacent to H and O, a triplet at 5·35 ppm for the vinyl H on C-2, as well as the expected CH, CH_2, and CH_3 peaks which were characteristic of phytol and in the same relative intensities. Of these the peak at 1·61 ppm with an integrated intensity relative to the doublet at 4·01 the same as in standard phytol confirmed the presence of one methyl group attached to C=C which in turn confirms the presence of one double bond. For reasons which are not clear, the ratios in the algal and standard samples were closer to 2/1 than to the theoretical 3/2. This is probably an instrumental error, but in any case it is not characteristic of the algal phytol.

DISCUSSION

The data unequivocally demonstrate the presence of phytol in representatives of both brown and blue–green algae. It was not accompanied in measurable quantities by analogs of phytol with either different chain lengths or different numbers or kinds of double bonds. The phytol observed is presumably esterified to chlorophyll. The porphyrin part of the photosynthetic mechanism in both kinds of algae used has previously been identified as chlorophyll-a.[13] Since blue–green algae are procaryots, the presence of phytol in *Phormidium luridum* indicates that the phytol pathway evolved earlier than did the evolution of the kind of subcellular membranes characteristic of eucaryots. The same conclusion can be drawn about the chlorophyll pathway. Furthermore, the pathway leading finally to phytol seems to have arisen parallel with or later than the evolution of the porphyrin pathway, for the following reasons. The normal sequence of events in chlorophyll biosynthesis[13, 14] is elaboration of the porphyrin structure *per se*, introduction of magnesium ion, closure of the ketonic ring, reduction of C-7,8, and finally esterification with phytol. Throughout all but the last of these conversions the propionic acid moiety is present and in principle is available for esterification. If phytol had evolved earlier than porphyrins, one might have expected to see it introduced early in the porphyrin pathway, but this is not the case. In fact, the very earliest part of the chlorophyll pathway in which phytol is observable is at the protochlorophillide stage. This is similar to the usual pattern except in reversal of the final reduction of C-7,8 and esterification. That a position not earlier than this in the chlorophyll pathway is utilized (which we are suggesting is for evolutionary reasons) for phytol's introduction is further indicated by the fact that chlorophyll-c, which is reported[13] to resemble the C-7,8 unsaturated protochlorophillide-a (rather than chlorophyll-a), is not found in nature esterified at all.

EXPERIMENTAL

Fucus vesiculosus was collected on the Atlantic shore of New Jersey. *Phormidium luridum* var. *olivaceae* Boresh was grown at the University of Pennsylvania on Medium C (an alkaline salt mixture) of Kratz and Meyers[8] through the kindness of Dr. John Biggins. The gas phase was air supplemented with 4% CO_2. Further details are to be found elsewhere.[9] A pressed cake of washed, whole cells (100 g) of the blue–green algae or 100 g of dried macerated brown algae was extracted in a Soxhlet with acetone for several hours beyond the time required for extraction of chlorophyll. The acetone was removed under reduced pressure, and the residue was dried under high vacuum at 50°. To this was added 200 ml of 10% ethanolic KOH, and the mixture after 16 hr at room temperature was refluxed for 1 hr. It was then diluted with 600 ml of water and and extracted into petrol. ether (b.p. 30–60°) (5 × 200 ml). The dried extract was evaporated to dryness under reduced pressure and the colored oil so obtained was chromatographed on 70 g of alumina (Woelm, Alupharm

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[13] L. Bogorad in *Chemistry and Biochemistry of Plant Pigments* (edited by T. W. Goodwin), pp. 29–70, Academic Press, New York (1965).

[14] L. Bogorad and R. F. Troxler, in *Biogenesis of Natural Compounds* (edited by P. Bernfeld), 2nd Edition, pp. 261–272, Pergamon Press, Oxford (1967).

Chemicals), activated at 110° overnight and deactivated with 3% of water. Fractions of 20 ml each were taken using 100 ml each of the following solvents (ratios by volume): hexane, hexane–benzene (3/1), hexane–benzene (1/1), hexane–benzene (1/3), benzene, benzene–ether (1/1), ether, ether–methanol (1/1), and methanol. Fractions 7 and 8 contained colored material which was probably carotenoid, but it was not investigated further. Fractions 26 and 27 contained the oil identified as phytol. Subsequent fractions contained sterol. The phytol was rechromatographed prior to determination of physical constants.

NMR spectra were measured on a Varian A-60 instrument by Mrs. Maria Lalevic. Mass spectrometry was carried out by Morgan Schaffer Corp. of Quebec, Canada. The TLC procedure (Kieselguhr impregnated with paraffin oil; acetone–H_2O:4/1) was essentially as previously described.[10] I.r. spectra were obtained on a Perkin-Elmer Infracord as a thin film on NaCl.

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