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# IDENTIFICATION OF XANTHOPHYLLS KI AND KIS OF THE PRASINOPHYCEAE AS SIPHONEIN AND SIPHONAXANTHIN

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**Abstract**—Previous preliminary results had indicated that xanthophylls KI and KIS of the Prasinophyceae might be identical with the xanthophylls siphonein and siphonaxanthin respectively, found in the siphonalean green algae. NMR studies of xanthophyll KIS indicate that it is in fact identical with siphonaxanthin. The fatty acids to which the latter is esterified when in the form of xanthophyll KI differ from those found in the siphonein of *Codium fragile*.

## INTRODUCTION

THE PRASINOPHYCEAE[1] are a rather ill-defined group of green monads possessing one, two or four flagella. Cell walls are absent, but the cell membrane is generally covered by scales or a theca. The flagella are usually covered with scales. The pigment composition of many of these organisms has been described.[2–8] The majority show relatively normal chlorophycean-like pigment compositions,[7] but certain organisms (notably certain species of *Pyramimonas*, *Heteromastix* and *Asteromonas*)[7] show pigment patterns resembling the normal chlorophycean pattern but lacking the xanthophyll lutein. Another major xanthophyll is apparent in these flagellates; this is the orange ketonic xanthophyll, xanthophyll KI.[6] Chlorophylls *a* and *b* are present in all these flagellates but they also accumulate small amounts of a protochlorophyll-like pigment, magnesium 2,4-divinylphaeoporphyrin $a_5$ monomethyl ester.[2]

The xanthophyll KI, which can be isolated from unsaponified pigment extracts of the organisms, is converted to the more polar xanthophyll KIS upon saponification and is presumably esterified. Xanthophylls KI and KIS showed the same spectral and chromatographic properties as those of siphonein and siphonaxanthin respectively.[6] The latter generally constitute the major xanthophyll fraction of the siphonalean green algae.

The present paper presents further evidence that xanthophyll KIS and siphonaxanthin are identical and that xanthophyll KI, like the siphonein of the Siphonales, is siphonaxanthin esterified with fatty acids.

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[2] T. R. RICKETTS, *Phytochem.* **5**, 223 (1966).
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## RESULTS

The NMR studies of xanthophyll KIS showed signals of appropriate intensities in positions identical with those obtained with the siphonaxanthin of *Codium fragile*.[9] These were at: 5·53, 6·52, 8·03, 8·09, 8·39, 8·51, 9·01, 9·06 and 9·14$\tau$. It was therefore concluded that xanthophylls KIS and siphonaxanthin were identical. The structure of siphonaxanthin has been established as 3,3′,19-trihydroxy-7,8-dihydro-8-oxo-α-carotene.[9,10]

Xanthophyll KI showed similar signals to those obtained with xanthophyll KIS, the main difference being in the absence of a signal at 5·53 $\tau$ and the presence of a signal, of similar magnitude, at 4·90 $\tau$. This result was to be expected upon esterification of the primary hydroxyl group at C19.[11] The results of GLC of the methyl esters of the fatty acids released upon saponification of xanthophyll KI are shown in Table 1. The fatty acids have only been

TABLE 1. THE GLC RETENTION VOLUMES AND APPROXIMATE RELATIVE ABUNDANCES OF THE METHYL ESTERS OF THE FATTY ACIDS DERIVED FROM XANTHOPHYLL KI OF *Heteromastix longifilis*

| Retention volume (relative to palmitate) | Approximate relative abundance (%)* | Tentative identity† |
|---|---|---|
| 0·10 | 0·1 | C8 |
| 0·45 | 0·5 | C13 |
| 0·54 | 0·7 | ?C13 unsat. |
| 0·70 | 12·5 | C14 |
| 0·80 | 11·3 | C15 |
| 1·00 | 34·6 | C16 |
| 1·12 | 29·4 | C16:1 |
| 1·32 | 1·8 | ?C16 unsat. or C17 |
| 1·42 | 2·1 | C18 |
| 1·59 | 4·5 | C18:1 |
| 1·90 | 1·3 | C18:2 |
| 2·20 | 0·7 | C20 |
| 2·35 | 0·5 | ? |

* Calculated from the areas of the peaks.
† Retention volumes of standards relative to palmitate were kindly provided by Dr. J. F. Peberdy.

tentatively identified. It can be seen that xanthophyll KI, like the siphonein of *Codium fragile*[9] is siphonaxanthin esterified with a variety of fatty acids. These are neither qualitatively nor quantitatively identical with those fatty acids obtained from the siphonein of *Codium fragile*. The siphonein from *Caulerpa prolifera* is said to be esterified only with lauric acid.[12]

## DISCUSSION

The finding that the structures of xanthophyll KIS and siphonaxanthin are identical could indicate that there is some systematic relationship between the Prasinophyceae and

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the Siphonales. There are however considerable differences between the overall pigment patterns in the two groups. The majority of the Prasinophyceae show a relatively normal chlorophycean-type pigment pattern.[7] The small number of organisms in this group which possess xanthophyll KI (esterified siphonaxanthin) as the major carotenoid also contain a xanthophyll which has a related structure but which is more polar (xanthophyll K2). They lack lutein and β-carotene predominates over α-carotene.[7] In contrast to this, in the Siphonales, α-carotene usually predominates over β-carotene, lutein is present and xanthophyll K2 is absent. Siphonein and siphonaxanthin together generally constitute the largest carotenoid fraction.[13,14] Thus, it appears that any systematic relationship is probably fairly distant.

The structure of siphonaxanthin is basically that of lutein but is more complex, having ketone and primary hydroxyl groups in the conjugated side chain linking the two ring systems.[9,10] The majority of algal xanthophylls have an unmodified conjugated chain linking the two ring systems.[15] It thus seems probable that organisms possessing siphonaxanthin are more advanced in an evolutionary sense (at least as far as carotenoid synthesis is concerned) than those possessing only lutein. This idea conflicts with morphological views of the Prasinophyceae which regard them as being relatively primitive green algae.[16]

It should be noted that a xanthophyll, 3,3′,19-trihydroxy-α-carotene (loroxanthin), has been reported in several green algae.[11,17–21] The pigments called ? trollein, isolated from some Prasinophyceae,[6,7] are probably also identical with this xanthophyll. The latter differs from siphonaxanthin in structure only in the lack of a ketone group at C8 and in the presence of only one hydrogen atom at C7.[9–11] Thus, it could be a biosynthetic intermediate in the production of siphonaxanthin.

Several algal groups possess fucoxanthin as the main xanthophyll. This, like siphonaxanthin, possesses a ketone group at C8 (in addition to an allene grouping at C7′, which is also present in the same place in neoxanthin).[15] It is tempting to speculate that there may be some evolutionary relationship between the algal groups forming fucoxanthin and those forming siphonaxanthin. One should however note that the structures of fucoxanthin and neoxanthin are very similar, the main structural difference being in the possession of a C8 ketone group by fucoxanthin. One might therefore query why no fucoxanthin is formed in the Prasinophyceae and in the Siphonales, as systems capable of introducing a ketone group at C8 are obviously present in both groups. Neoxanthin is also present in both groups and it seems not unreasonable to suppose (in view of their close structural similarity) that fucoxanthin could be formed via neoxanthin. However, neoxanthin has yet to be detected in fucoxanthin forming organisms.

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## EXPERIMENTAL

*Materials*

As described in Ricketts.[9]

*Cultures*

150 l. of culture of *Heteromastix longifilis* Butcher (Plymouth Collection No. 58) were grown in Erdschrieber medium in 1·5 l. vol. The inoculum consisted of 20 ml per flask of a unialgal bacteria-containing culture of *Heteromastix longifilis*. The cultures were incubated at 14° with a regimen of 16 hr light (150 lm/ft$^2$) and 8 hr darkness per 24 hr. They were harvested towards the end of the logarithmic phase of growth by centrifugation using a Sharples centrifuge with a clarifier rotor (flow rate 450 ml/min; approximately 23,000 rev/min) in two approximately equal volumes. The pigments were extracted from the cells by successive extractions with 90%, v/v acetone–water (to completion) and centrifugation of the extracts between each extraction. The acetone extracts were then mixed with $Et_2O$ and diluted with saturated salt solution until two phases separated. The ethereal layer was removed. All the pigments were extracted from the aqueous phase with ether and then the ethereal phases were washed with water to remove acetone and to reduce the ethereal volume. The ethereal extracts were then evaporated to dryness in $N_2$ at low temperature in the dark and stored at −20° in $N_2$ in the dark until required for chromatography.

*Chromatography and Purity*

Pure xanthophyll KI was isolated by methods similar to those described in Ricketts.[9] The yield was calculated to be approximately 1 mg (assuming $E^{1\%}_{1\,cm} = 2500$ in ethanol, which is likely to be higher than the true extinction coefficient since part of the mass is due to esterified fatty acids. Many ketonic xanthophylls have extinction coefficients which are much below 2500). Thus the actual recovery may be in the region of 2 mg.

*Saponification. NMR Studies and GLC*

The conversion of xanthophyll KI to xanthopyll KIS by saponification, the recovery of the freed fatty acids from the hydrolysate and their conversion to methyl esters, and the NMR studies of the xanthophylls (in $CDCl_3$, using tetramethylsilane as an internal standard) were carried out in Ricketts;[9] part of the spectrum (3–8$\tau$) was repeated 192 times and a computed spectrum for this region determined using the CAT analogue computor. A complete repeated spectrum after this operation indicated little, if any, destruction during the repeated runs.

For GLC the methyl esters of the fatty acids were dissolved in a small amount (*ca.* 100 $\mu$l) of petrol (b.p. 40–60°) and aliquots chromatographed on 2-m columns of diethylene glycol succinate on HMDS Chromosorb W., 80–100 mesh, 20:80, w/w, in a Perkin–Elmer F11 dual column gas chromatograph with a flame ionization detector. Operational details were as follows: injection block temperature 220°; temperature programme 150–190° at 4°/min; sensitivity $5 \times 10^2$; air 30 lb/in$^2$; hydrogen 14 lb/in$^2$, chart speed 5 mm/min; balanced columns were used with nitrogen as the carrier gas using flow rates of about 25 ml/min

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