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IMPROVED ISOLATION PROCEDURE FOR FUCOXANTHIN*

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Key Word Index—*Fucus serratus*; brown algae, carotenoids, fucoxanthin, peridinin, improved isolation; partition ratios.

Abstract—An improved isolation procedure for crystalline fucoxanthin from brown algae is described, involving partition and chromatography The method is applicable to peridinin. Partition ratios for fucoxanthin and peridinin are reported.

INTRODUCTION

The allenic carotenoids fucoxanthin (**1**, C_{40}-skeletal) and peridinin (**2**, C_{37}-skeletal) (Scheme 1) are the most abundant carotenoids in Nature [1, 2]. However, convenient methods for rapid isolation of these frequently requested reference pigments in gram or even mg quantities have not been published. Whereas peridinin (**2**) only is produced by microalgae, fucoxanthin (**1**) occurs in the readily available large seaweeds [1–3].

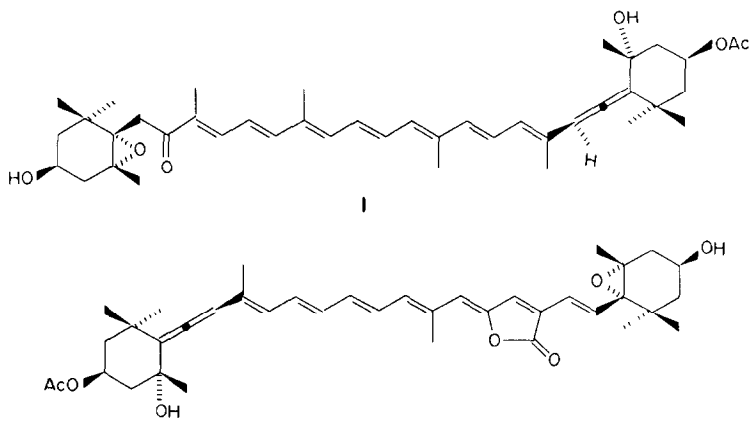
RESULTS AND DISCUSSION

Previous isolation procedures for fucoxanthin (**1**) suffered from the following disadvantages: use of fresh, brown algae [4, 5] required large volumes of solvents, grinding of fresh algae prior to drying [6–8] may initiate enzymatic reactions, time-consuming procedures result in *trans–cis* isomerization, calcium carbonate-columns [9] have low capacity and alumina used for chromatography [5, 8] must be neutral. Both fucoxanthin (**1**) and peridinin (**2**) are strongly sensitive to alkali [10, 11]. Hence saponification cannot be employed for separation from chlorophylls.

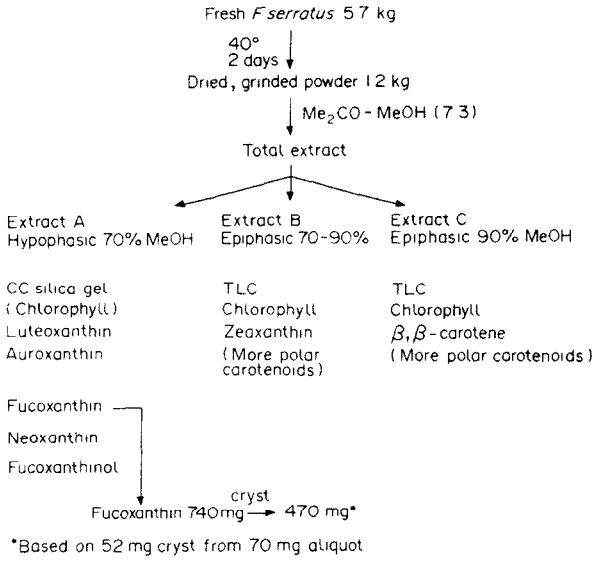
We now report an improved procedure for rapid isolation of fucoxanthin (**1**) from brown algae based on acetone–methanol extraction of carefully dried and powdered algae. Then follow partition and column chromatography, two to three times, on silica of a fucoxanthin-enriched chlorophyll and other xanthophyll-deficient extract and finally crystallization.

A flow-sheet for the isolation of fucoxanthin (**1**) from *Fucus serratus* is given in Scheme 2. The total carotenoid content of *F. serratus* was found to be *ca* 0.08% of the dried, extracted cells. Fucoxanthin (**1**) comprised *ca* 70% of the total carotenoid. From 1.2 kg fresh algae (dry matter *ca* 21%) was obtained in eight portions in total 740 mg **1** after column chromatography. The first crystallization from an aliquot containing 70 mg **1** provided 52 mg **1**, mp. 164° (evacuated tube, reported 159.5–168° [4, 5, 10, 12]), Vis λ_{max} (acetone) (420), 443 (E1% 1 cm = 1660, reported 1637–1660 in hexane [9]) and 467 nm, IR and

*Part 43 in the series 'Algal Carotenoids'. For part 42 see *Biochem Syst Ecol* 1989 (in press).



Scheme 1.



Scheme 2 Flow sheet for the isolation of fucoxanthin (1) from *Fucus serratus*

^{1}H NMR (500 MHz) data [13, 14] consistent with reported data [8, 9]. The crystallized material contained 94% all-*trans*-**1** and 2% each of the 9′-*cis*, 13-*cis* and 13′-*cis* isomers, as demonstrated by subsequent HPLC [15] and ^{1}H NMR [14]

Fucoxanthin, thus prepared, was used for characterization and identification of the individual geometrial isomers present in the iodine catalysed stereomutation mixture, as will be published elsewhere [16].

Partition coefficients [17, 18] for fucoxanthin (**1**) and peridinin (**2**, *ex Amphidinium* sp. [19]) were recorded and are reported in Fig. 1. Fucoxanthin (**1**) and peridinin (**2**) have rather similar partition behaviour, **2** being slightly more polar. The procedure outlined should therefore in principle also be useful for large scale isolation of **2** by removing chlorophylls and acetylenic carotenoids present in dinoflagellates by partition.

EXPERIMENTAL

General precautions for work with carotenoids were taken *Isolation*, see Scheme 2 *Fucus serratus* was harvested from Væreskjæret, near Trondheim, June 1988 The algae were carefully dried at 40° for 2 days with good air circulation and grinded on a Wiley mill, Model No 3 Solvents were removed from the total Me_2CO–MeOH extract (*ca* 4 l) at red pres and the residue partitioned in portions containing *ca* 100 mg **1**, between hexane (200 ml) and MeOH (200 ml), to which the calculated amount of H_2O was added, see Scheme 2 With 95% aq MeOH, three extractions with hexane was employed and with 70% aq methanol one extraction with hexane only Compound **1** was finally transferred from extract A to Et_2O and chromatographed 2–3 times on silica columns with 10–100% Me_2CO in hexane as eluent, **1** requiring 30% Me_2CO for elution Crystallization was effected from Me_2CO–hexane or CH_2Cl_2–hexane

Partition data for **1** and **2** (*ex*, *Amphidinium* sp [19]) were recorded essentially according to previous methods [17, 18] Hexane and MeOH vols (50 ml) were kept identical, disregarding the H_2O content Samples of 0 6 mg carotenoids were used

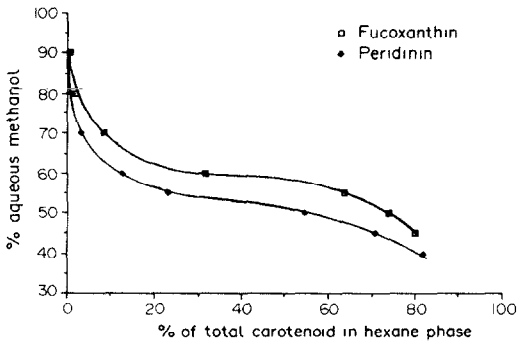


Fig 1 Relationship between percentage methanol, in the system hexane–aqueous methanol, and partition coefficient

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