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TRANSFORMATIONS OF CAROTENOIDS IN THE OCEANIC WATER COLUMN

by

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B.S., University of Rhode Island
(1977)

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1983

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Abstract

In an effort to understand the more general mechanisms and rates of pre-depositional reactions that transform organic matter, the types and relevant time scales of reactions that transform carotenoid pigments in the oceanic water column were studied.

Suspended particulate matter collected from surface waters of Buzzards Bay, Massachusetts and the Peru upwelling system has a carotenoid distribution reflecting the phytoplanktonic source of the material. The carotenoid distribution of sediment trap samples collected in these same areas was dominated by transformation products. Fucoxanthin, the primary carotenoid of marine diatoms, typically constituted 77-100% of the total fucopigments in suspended particulate material. In sediment trap samples this pigment constituted only 4-85% of the total. The remaining 15-96% of the pigments consisted of the fucoxanthin transformations products: free alcohols (2-94%), dehydrates (0-6%), and opened epoxides (0-19%).

Preliminary results suggest that carotenoid esters are hydrolyzed to free alcohols at a rate determined by the turnover of primary productivity. The dehydrated and epoxide opened intermediates of fucoxanthin represent products of transformation reactions that operate over much longer time scales (0.1-10 yrs). Dehydration and epoxide opening are not significant water column transformations, but are important in surface sediments.

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In an effort to understand the more general mechanisms and rates of pre-depositional reactions that transform organic matter, the types and relevant time scales of reactions that transform carotenoid pigments in the oceanic water column were studied.

In the present study, a model was constructed for organic matter cycling that consisted of three parts: 1) the synthesis of carotenoid pigments by phytoplankton in the euphotic zone, 2) consumption and metabolism of some fraction of these pigments by heterotrophic organisms, and 3) removal of metabolic by-products to the sediments by large particle (e.g. fecal pellet) transport. The model separates particlate matter into reservoirs according to the degradation processes that have occurred since synthesis. The goal is to sample these particulate reservoirs, determine the compositional differences between them, and construct a mechanistic pathway for the transformations that occur as material is transferred between reservoirs.

Suspended particulate matter collected in the surface waters of Buzzards Bay, Massachusetts and the Peru upwelling region has a carotenoid distribution reflecting the phytoplanktonic source of the material. The carotenoid distribution of sediment trap samples collected in these areas was dominated by transformation products. Fucoxanthin, the primary carotenoid of marine diatoms, typically constituted 77-100% of the total fucopigments in suspended particulate matter. In sediment trap samples this pigment constituted only 4-85% of the total. The remaining 15-96% of the pigments consisted of the fucoxanthin transformation products: free alcohols (2-94%), dehydrates (0-6%), and opened epoxides (0-19%).

Postulated transformation products were synthesized to determine the structure of isolated compounds. Simultaneously, iodine catalyzed photoisomerization of fucopigments was tested as a potential method for the unambiguous identification of caotenoids requiring only the nanogram amounts of material typically found in samples.

Preliminary results suggest that carotenoid esters are hydrolyzed at a rate determined by the turnover of primary productivity. The dehydrated and epoxide opened degradation products of fucoxanthin represent products of transformation reactions that operate over much longer time scales (0.1-10 yr). Dehydration and epoxide opening are not significant water column transformations, but are important in surface sediments. A transformation pathway of ester hydrolysis → dehydration → epoxide opening → further dehydration is proposed for fucoxanthin. Preliminary evidence for a parallel transformation squence for structurally similar carotenoids is also presented.

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to mom, dad, and heidi

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Recent studies of specific organic compounds in suspended particulate matter and surface sediments have demonstrated that there is a major compositional discontinuity between what is produced in the euphotic zone, and what is deposited in the surface sediments (Andersen, 1977). For example, annually some 7×10^{19}g of the carotenoid fucoxanthin is synthesized by marine phytoplankton. However there are no reports of this pigment in marine sediments. The recognition that rapidly sinking large particles contribute a major portion of the mass flux to the sea floor (McCave, 1975; Wiebe _et al._, 1976; Bishop _et al._, 1977, 1978, 1980; Honjo 1978, 1980; Rowe and Gardner, 1979; Hinga _et al._, 1979), led to the hypothesis that reactions on these particles may contribute to the discontinuity at the sediment-water interface. Organic geochemical studies of particles collected in sediment "traps" placed at depths intermediate between the sea surface and sea floor have since confirmed this hypothesis (Crisp _et al._ 1979; Wakeham _et al._, 1980; Tanoue and Handa, 1980; Lee and Cronin, 1982; Repeta and Gagosian, 1982; De Baar _et al._, 1982; Gagosian _et al._, 1982).

One of the most significant conclusions to be drawn from studies of organic compounds in sediments is that the diagenesis of biomolecules is a systematic, not random, process. Only a limited number of transformation products are formed compared to the number theoretically possible (Hunt, 1979). This concept was implicit in the earliest studies of organic geochemistry (Treibs, 1936), but has since been convincingly documented with several classes of structurally different compounds (Baker and Palmer, 1978; Ourisson _et al._, 1979; MacKenzie _et al._, 1982). The precursor/product relationship is now an established axiom in organic geochemical research, and has proved valuable in studies of petroleum geochemistry, chemical evolution, and in identifying sources of sedimentary organic matter. Perhaps the best illustration of this was the discovery of bacteriohopanetetrol in sediments prior to its discovery in *Acetobacter xylinum* (Rohmer and Ourisson, 1976)

By analogy, there is every reason to think that degradation of organic compounds in the oceanic water column proceeds as it does in the sediments, via a series of discrete transformation steps. Given this hypothesis, it is pertinent to model water column transformation reactions in order to answer questions on the rates and mechanisms of organic matter recycling, and to characterize the physical properties of the system. Answers to these questions are relevant to problems in organic matter cycling (pollutant dispersal and degradation), biodynamics (nutrition, chemotaxis, productivity controls), physical-chemical processes (adsorption, oxidation-reduction), and sedimentary geochemistry.

Gagosian and Lee (1981) have recently reviewed work on the transformation of specific organic compounds in the oceanic water column. Broadly speaking, two approaches have been taken in studying this problem: 1) the isolation and structural determination of specific transformation products that can be related to known biogenic precursors, and 2) the chemical degradation of high molecular weight transformation products into simpler molecules which can then be reassembled via model reactions. The most widely documented example of a specific precursor/product transformation in seawater is the degradation of chlorophyll-*a* to phaeopigments. Other classes of compounds have also been studied (sterols, hydrocarbons, fatty acids). However few precursor/product relations have been demonstrated, and those that have been identified are minor reactions. There is some dispute as to whether amino acid racemization occurs as an major transformation reaction or as a product of bacterial resynthesis (Lee and Bada, 1977; Bada and Hoopes, 1979). At present the status of these reactions is unclear.

The transformation of chlorophyll to phaeophytin and phaeophorbides has be studied indirectly as a measure of productivity for a number of years. As yet there has been no systematic study of the transformation mechanism, and in those studies which have been made, the structures of degradation products are poorly characterized. However, the unique structure of the chlorin macrocycle and its relative abundance in phytoplankton leaves little doubt that phaeopigments are in fact transformation products of chlorophyll.

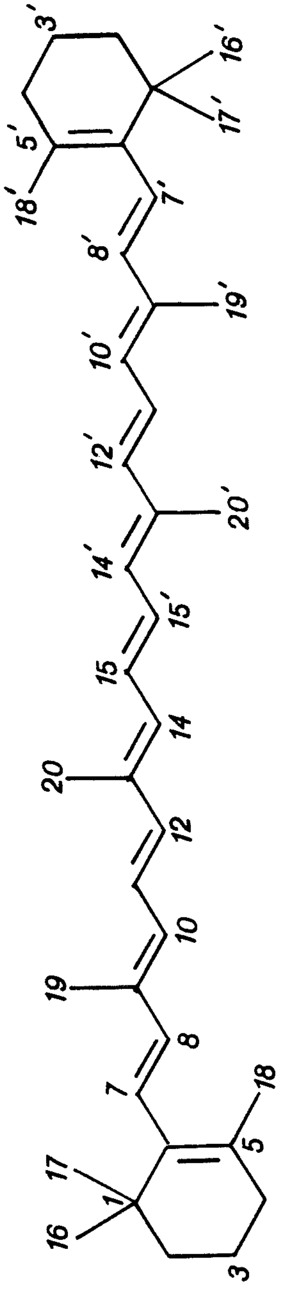
The second approach that has been used in studying the transformation of organic matter is more indirect. A significant fraction of dissolved organic carbon is bound as macromolecular polymers of simpler biogenic compounds. Chemical degradation of these polymers yield data on the relative amounts and types of subunits, which can then be used to construct hypothetical structures for a "typical" molecule (Stuermer and Harvey, 1978; Stuermer 1975). These experiments are accompanied by laboratory simulations designed to mimic potential formation reactions (Hedges, 1978). The results of these experiments are difficult to interpet. Simplification of the reaction media and the necessarily higher concentrations of reactants are geochemically unrealistic. Consequently it is difficult to draw conclusions vis-a-vis the natural environment.

The limited scope of these studies does not permit actual modelling of transformation reactions occurring in the oceanic water column. However they are useful in developing criteria for the selection of model compounds to be used as tracers. Three characteristics make water column models distinct from their sedimentary analogues: 1) the overwhelming majority of transformations in the water column are biologically mediated, 2) the water column is spatially dynamic, and 3) the time scales of interest are on the order of 10^{-2}-10^{3} yr. Therefore, inherently different sampling strategies and model compounds of different characteristics are required in experimental design.

This thesis describes an investigation of carotenoid geochemistry in order to assess the suitability of carotenoids as model compounds for the study of transformation reactions occurring in the oceanic water column. Several characteristics make these pigments attractive as potential tracers for water column processes: they are reasonably source specific (Liaaen-Jensen, 1978; 1979), they encompass a wide variety of functional groups (Straub, 1971), they are reactive over short time scales, and they are widely distributed in both photosynthetic and non-photosynthetic organisms (Liaaen-Jensen, 1978). In this study the transformations of selected pigments from marine phytoplankton and zooplankton will be investigated in order to determine the rates and mechanisms of reaction. Previous research in carotenoid marine geochemistry has been confined to sediments, and due to the differences in time scale and scope this work is not directly applicable to the present study (Louda and Baker, 1981; Watts and Maxwell, 1977; Watts _et_ _al_., 1977; Peake _et_ _al_., 1974; Fox _et_ _al_., 1944; Fox, 1937). However, the absence of phytoplankton pigments from marine sediments noted in these studies further testifies to their reactivity in the overlying water column and at the sediment-water interface.

Comprehensive reviews of carotenoid chemistry and biochemistry can be found in Goodwin (1976), Isler (1971), and in the Proceedings of the International Symposium of Carotenoid Chemistry published periodically in _Pure and Applied Chemistry_ (most recent volumes: v 47, v 51, and v 54). The carbon numbering system recommended by the IUPAC Committee on Nomenclature of Organic Chemistry and the IUPAC/IUB committee on Biochemical Nomenclature (Isler, 1971, p 857) will be used throughout the text (Figure 1). Roman numerals following trivial names (e.g. β-carotene (I)) refer to the structures given in Appendix I. Appendix II provides an alphabetical listing of trivial and IUPAC names of carotenoids discussed in the text.

Figure 1. IUPAC numbering of carotenoids.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β-Carotene (I)

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CHAPTER 2

CAROTENOID TRANSFORMATION PRODUCTS IN COASTAL MARINE WATERS; BUZZARDS BAY SUSPENDED PARTICULATE AND SEDIMENT TRAP SAMPLES.

INTRODUCTION

The study of carotenoid transformation reactions in the oceanic water column requires a detailed analysis of specific pigments. Only three such studies have been reported. Jeffrey (1974, 1976) and Neveux (1975) analyzed specific carotenoids in suspended particulate matter collected at depths of 10-100 meters and 10-500 meters respectively. Both studies found astaxanthin (II), carotene (I), diadinoxanthin (III), and fucoxanthin (IV) as major carotenoid pigments. Neveux (1975) also observed a number of unidentified yellow colored pigments and suggested they were carotenoid transformation products. However, no data were provided to support this hypothesis. Jeffrey (1976) reported only major pigments, all of which were identified as untransformed natural products. The author noted the presence of minor, unidentified pigments, but gave no further details. Jeffrey (1976) also analyzed fecal pellets collected from copepods feeding on small chain diatoms (species unidentified). Several major unidentified yellow colored pigments (presumably carotenoid transformation products) were reported. Although the evidence is circumstantial, these two studies suggest that carotenoid transformation products may be present in oceanic suspended and rapidly sinking (i.e. fecal pellets) particulate matter.

This chapter describes a detailed analysis of carotenoids and their transformation products extracted from suspended partculate matter and sediment trap material collected in Buzzards Bay, Massachusetts. The study will focus on the transformation reactions of the four quantitatively most abundant carotenoids observed by Jeffrey and Neveux: astaxanthin (from zooplanktonic crustacea), diadinoxanthin (from diatoms and dinoflagellates), fucoxanthin (from diatoms), and peridinin (V) (from dinoflagellates). These four pigments provide a good cross-section of functional groups reactive under a variety of relatively mild conditions (Liaaen-Jensen, 1971). In addition, there is some evidence from the analysis of sea urchin coelomic epithelium that heterotrophic metabolism of fucoxanthin leads to novel degradation products (Galasko et al., 1969).

EXPERIMENTAL

Sediment traps (Jannasch et al., 1980) were moored in Buzzards Bay, Massachusetts ($41^{o}32'$N, $70^{o}42'$W) 4 m below the surface in a 10 m water column. The traps were deployed for two thirty day periods (May 20 - June 21, 1980, sediment trap 2; June 21 - July 22, 1980, sediment trap 3). Seawater was collected at a depth of 1 m with a 30 ℓ Nisken bottle immediately after retrieval of sediment trap 3. Particulate matter from seawater and sediment trap samples was filtered through pre-combusted (450^{o}C, 24 hours) Gelman type AE glass fiber filters, and sonic extracted with MeOH (twice, 20 min each) and CH_2Cl_2 (once, 20 min). Extracts were concentrated to approximately 200 μℓ by vaccum rotary evaporation then separated into compound classes by gel permeation chromatography on 100Å μStyragel (Waters Associates, Milford, Mass.; USA.). The carotenoid fraction was collected, reconcentrated, then separated into individual components by high pressure liquid chromatography (HPLC) using a Waters Associates 10 μm amino (Buzzards Bay sediment trap 2) and a Spherisorb 5 μm amino column (suspended particulate matter, Buzzards Bay sediment trap 3) eluted for 45 min with a linear gradient of hexane and 0-13% MeOH/THF (20/80, v/v) at 2 ml/min. After 55 minutes, the composition of the eluant was stepped to 30% MeOH/THF in hexane to elute polyhydroxy xanthophylls. Carotenoids were detected spectroscopically at 436 nm, collected, and further analyzed by visible spectroscopy and mass spectrometry. Visible spectra were recorded on a Cary 118 dual beam scanning spectrophotometer. Mass spectra were collected on a Finnigan 3200 quadrupole mass spectrometer interfaced with an Incos (Finnigan) 2300 data system. All operations were performed at or below $20^{\circ}C$ and in low light conditions. A more detailed description of the analytical method is given in Chapter 5.

RESULTS AND DISCUSSION

Structural determination was made by comparison of HPLC retention times, absorption spectra and mass spectra with authentic standards (Chapter 4). Selective acylation of secondary alcohols with acetic anhydride in pyridine was used to establish the structure of fucoxanthinol (VI), fucoxanthinol 5'-dehydrate (VII), and isofucoxanthinol 5'-dehydrate (VIII). Tables 1, 2 and 3 present compound identification, visible and mass spectral data for Buzzards Bay suspended particulate matter and sediment trap samples 2 and 3 respectively. The analysis is limited to major carotenoid components. The relatively low sensitivity of off-line direct insertion probe mass spectrometry, and the incomplete resolution afforded by conventional packed-column HPLC, does not permit identification of minor components. In addition, the chromatographic conditions do not separate carotene isomers (e.g. α, β, γ-carotene, lycopene, etc.), and the specific isomers extant in fraction 1 of the samples are undetermined. This fraction will therefore be referred to simply as "carotene". More complete separation and mass spectral analysis of the complex carotenoid mixture must await development of on-line glass capillary HPLC-MS (Tijssen _et al._, 1981).

Buzzards Bay suspended particulate matter (BBSPM): Greater than 95% of the carotenoids isolated from BBSPM > 0.45 μm can be accounted for by five pigments: astaxanthin, fucoxanthin, peridinin, diadinoxanthin and carotene (Figure 1; Table 1). Two minor carotenoids, labelled A and B in Figure 1 are also observed. Compound A coelutes with authentic astacene (IX), an oxidation product of astaxanthin. Compound B coelutes with diadinochrome (X), the 5,8-furanoxide isomer of diadinoxanthin. These two carotenoids are not considered to be biosynthesized by marine organisms, but are often cited as analytical artifacts (Liaaen-Jensen, 1971). In the analysis of blanks spiked with authentic astaxanthin and diadinoxanthin, and of cultured phytoplankton (_Peridinium triochoidium_) of known carotenoid composition (Johansen _et al._, 1974) no degradation other than cis → trans isomerization was observed (Chapter 5, Analytical Methods). Therefore, the astacene and diadinochrome in BBSPM are indigenous to the sample. These pigments most likely originate from partially metabolized zooplankton (astacene) and phytoplankton (diadinochrome) cells.

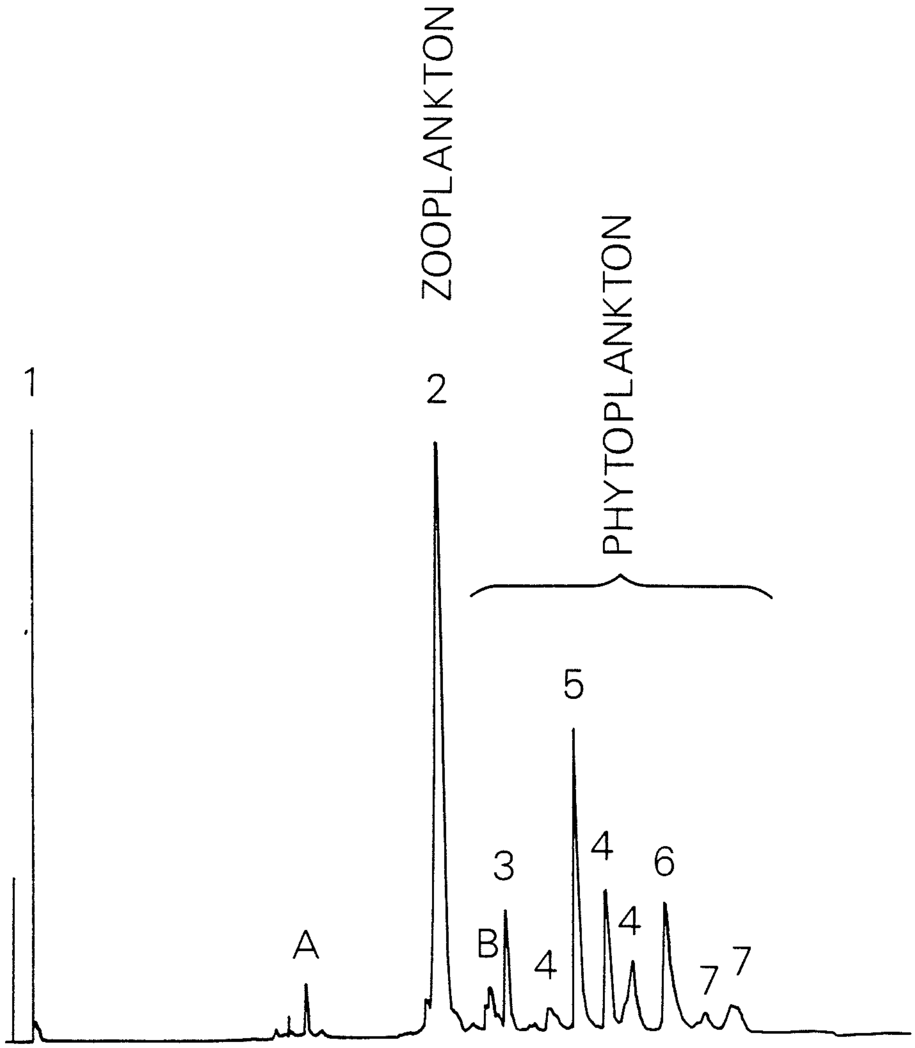
The carotenoid distribution in suspended particulate matter (Figure 1) is indicative of a mixed zooplankton/phytoplankton sample. Astaxanthin (II), carotene (I), diadinoxanthin (III), fucoxanthin (IV), peridinin (V) and their cis isomers constitute greater than 95% of the total pigments in the sample. These five pigments are widely distributed in common forms of marine zooplankton and phytoplankton. Astaxanthin is a major pigment of zooplankton crustacea (euphausiids, copepods, etc.)

Table 1. Carotenoid composition (% total carotenoids) of crustacea diatoms, dinoflagellates, and suspended particulate matter.

	Penaeus (crustacea)*	Phaeodactylum tricornatum**	Amphidinium sp.***	BBSPM	Suspended matter#
Astaxanthin	90		31	+	
Astacene			3		
B-carotene		10	3	8	+
Diadinoxanthin		22	11	9	+
Diadinochrome			3		
Fucoxanthin		68		29	+
Peridinin			84	17	+

*Tanaka *et al.*, 1976
**Abaychi and Riley, 1979
***Johansen *et al.*, 1974
# Neveux, 1975; Jeffrey 1976

Figure 1. High pressure liquid chromatogram of carotenoids extracted from Buzzards Bay suspended particulate matter: 1) carotene, 2) astaxanthin, 3) diadinoxanthin, 4) cis fucoxanthin, 5) all-trans fucoxanthin, 6) peridinin, and 7) cis peridinin. Identification of peaks A and B discussed in text. Conditions: 300 x 3.9 mm 5 μm Spherisorb amino column (slurry packed in-house), eluted for 45 min with a linear gradient of 0-13% MeOH/THF (20/80, v/v) in hexane at 2 ml/min. After 55 min the eluant was stepped to 30% MeOH/THF in hexane for an additional 15 min.


ZOOPLANKTON
PHYTOPLANKTON
1
2
A
B
3
4
5
4
4
6
7
7

(Liaaen-Jensen, 1978). Fucoxanthin and peridinin are the principal photosynthetic accessory pigments in diatoms and dinoflagellates respectively (Johansen _et al._, 1974; Jeffrey _et al._, 1975). Like astaxanthin, they comprise the bulk of carotenoid pigment (70-90%) in their respective classes of organisms (Table 1). The natural occurrence of carotene and diadinoxanthin is less precisely known. β-carotene is widely distributed in nearly all carotenoid containing marine and terrestrial plants and animals (Weedon, 1971; Goodwin, 1976; Liaaen-Jensen, 1978). Diadinoxanthin is characteristic of aquatic photosynthetic organisms, including dinoflagellates and diatoms (Liaaen-Jensen, 1978). The presence and distribution of these pigments in suspended particulate matter supports the observations of Jeffrey (1974, 1976) and Neveux (1975), and is consistent with the interpetation that the major fraction of pigmented material in the surface waters represents living organisms.

Buzzards Bay sediment trap samples (BBST): Four classes of carotenoids were isolated and identified in BBST 2 and 3: 1) fucoxanthin and related pigments, 2) diadinoxanthin and related pigments, 3) carotenoid diols, and 4) carotenes (Figures 2,3; Tables 2,3).

The carotenoid mixture of BBST-2 and BBST-3 (Figures 2, 3) is much more complex than observed for standing crop particulate matter. Approximately 60 peaks can be distinguished in the chromatogram of BBST-3, compared with only 24 peaks in the chromatogram of BBSPM. Astaxanthin, carotene, diadinoxanthin, fucoxanthin, and peridinin, which represented greater than 95% of the total carotenoids in suspended particulate matter, represent less than 8% of the total pigments in the sediment trap samples (Table 4). Astaxanthin and peridinin were not observed in either sediment trap. Fucoxanthin, diadinoxanthin, and carotene appear only as minor components.

Sediment traps are designed to collect rapidly sinking large particles not typically collected by conventional water samplers. Microscopic examination of material collected in other sediment traps (Bishop _et al._, 1978; Honjo, 1978, 1980; Staresinic, 1982; Staresinic _et al._, 1982) has demonstrated that a large fraction of the particulate matter collected originates from the primary vertical flux of material from surface waters in the form of fecal pellets, molts, carcasses, and other debris produced by heterotrophic organisms. The sediment trap samples collected for this study were deployed in a relatively shallow water column (10 m). Roman and Tenore (1978) and Roman (1978) have studied the resuspension of surface sediments at this same site in Buzzards Bay. Greater than 50% increases in particulate organic carbon and chlorophyll-_a_ were observed during tidal cycles. These studies indicate that a large portion of the material collected in the trap may originate from resuspension of surface sediments. This hypothesis is consistent with the carotenoid distribution in BBST-2 and BBST-3. The qualitative and quantitative distribution of pigments in the two sediment trap samples is virtually identical. Such a result would be expected if the traps were sampling the top 0-3 cm of sediments, where short term fluctuations in inputs and transformations would be averaged over the last 0-10 yr.

Fucoxanthin Related Pigments- Authentic all-trans fucoxanthin co-elutes with fraction 6, BBST-2 and fraction 7, BBST-3 (Figures 2, 3). Comparison of visible and mass spectra confirm the identification of these

Table 2. Compound Identification, Mass and Visible Spectral Data for Buzzards Bay Sediment Trap 2.

Fraction	$\lambda_{\max}$ nm	Major Mass Fragments
1 Carotene	666, 612, 535, 475 450, 411	549(37), 574(21), 537(15), 535(9) 523(17), 521(24)
2 Fucoxanthin 5'-dehydrate Carotenoid 3,3'-diols	690, 666, 548, 475 450, 412	641(39), 623(13), 599(45), 581(60) 471(68), 569(100), 551(99)
3 Carotenoid diols	478, 451, 421	599(11), 581(7) 565(100), 547(71), 473(8)
4 Diadinoxanthin	477, 447, 424	583(87), 565(36), 181(100)
5 Carotenoid diol	(475), 450, 425	599(19), 581(31), 563(17) 567(29) 583(31), 565(45), 547(28)
6 Fucoxanthin	476, 447, 420 (hexane)	659(51), 641(29), 599(15), 581(83) 563(35), 489(100)
7 Fucoxanthinol 5'-dehydrate	475, 450,(425)(hexane)	627(15), 599(73), 581(31), 429(100)
8 *cis*-Fucoxanthin	(470), 447,(420)	659(53), 641(45), 599(23), 581(100) 563(32), 489(61)
9 *cis*-Fucoxanthinol 5'-dehydrate	(470), 445, 418	599(61), 581(36), 429(100)
10 Fucoxanthinol		
11 Isofucoxanthinol		

Figure 2. High pressure liquid chromatograms of Buzzards Bay sediment trap sample 2. A) gradient stopped after 45 min. B) gradient stopped after 45 min, after 55 min gradient stepped to 30% MeOH/THF (20/80, v/v) in hexane. Peak numbers refer to fractions in Table 2. Conditions given in Figure 1 legend.

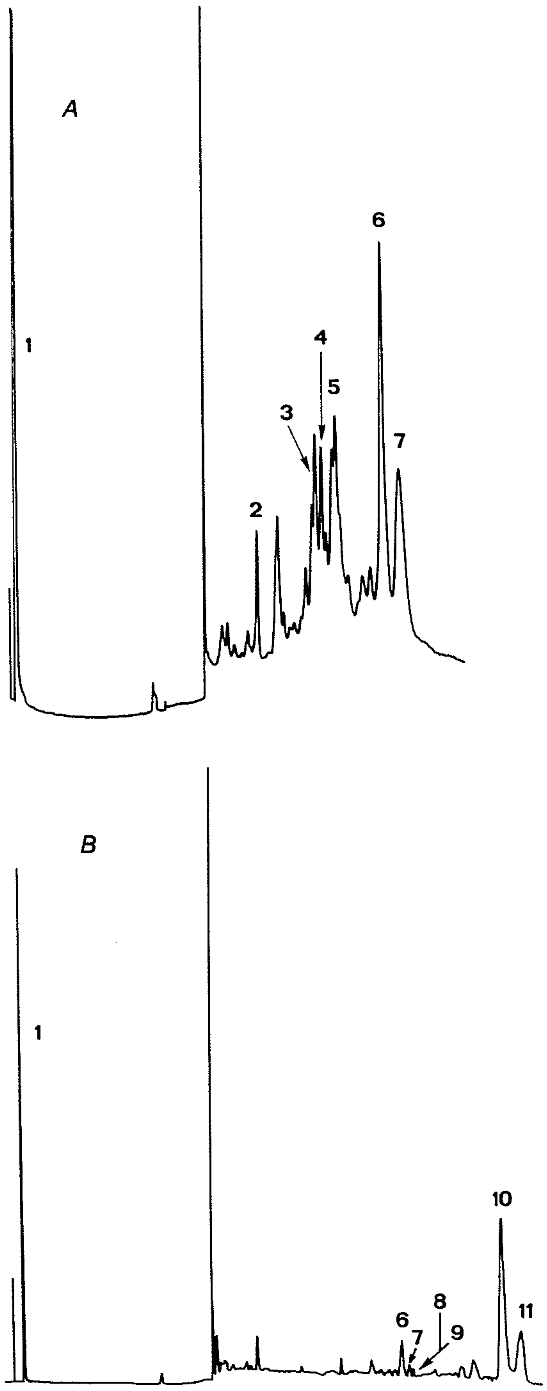

A
1
2
3
4
5
6
7
B
1
6
7
8
9
10
11

Figure 3. High pressure liquid chromatogram of Buzzards Bay sediment trap 3. Peak numbers refer to fractions in Table 3. Conditions given in Figure 1 legend.

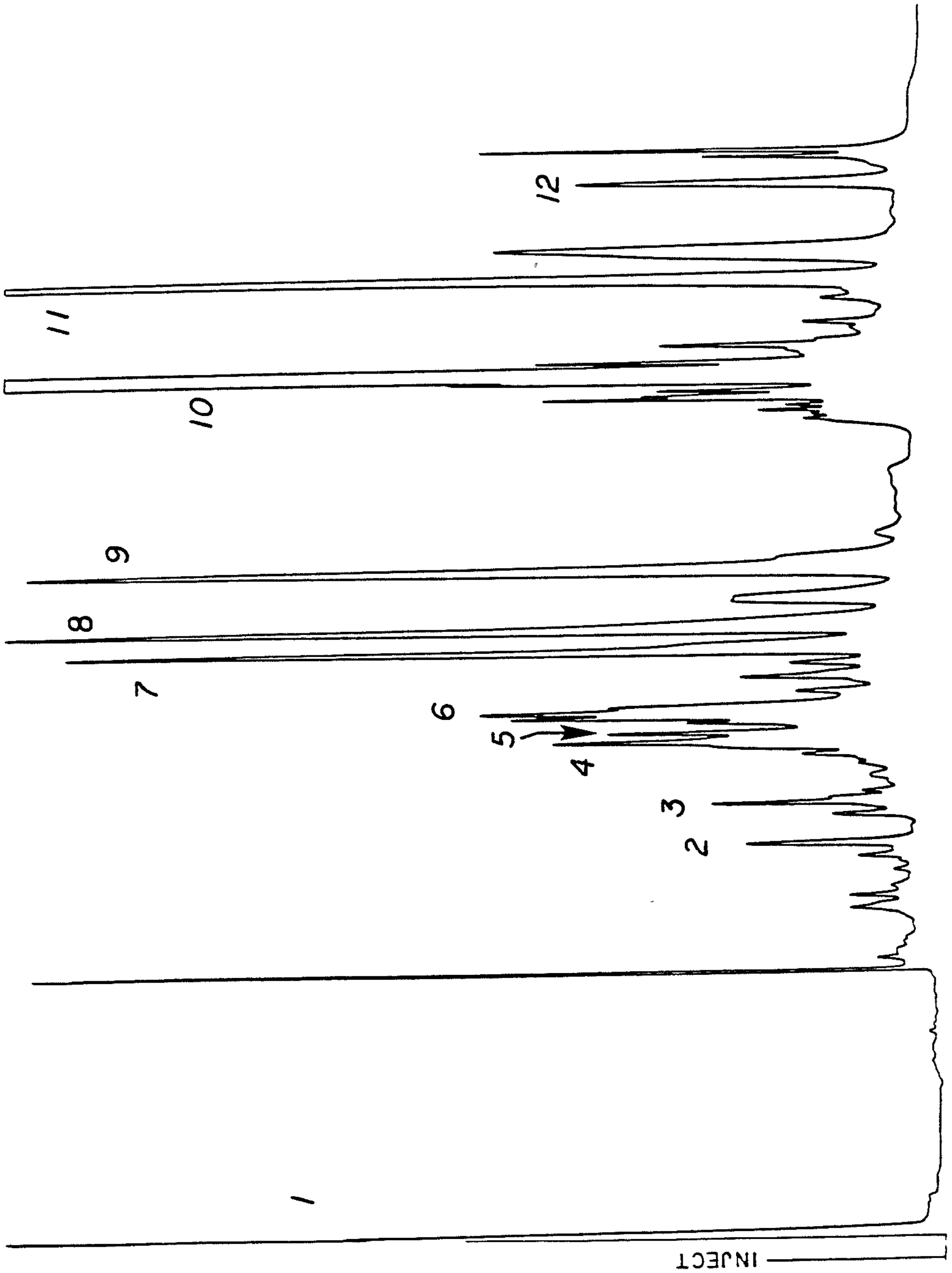

1
2
3
4
5
6
7
8
9
10
11
12
INJECT

Table 3. Compound identification, mass and visible spectral data for Buzzards Bay sediment trap 3.

Fraction		λ_{max} nm	Major Mass Fragments (% base peak)
1	Carotene	(470), 445, (425)	549(30), 537(25), 521(20), 495(10), 411(30), 409(33)
2	Fucoxanthin 5'-dehydrate		641(83), 623(25), 605(8), 599(7), 581(34), 563(28), 471(81), 429(18), 411(13)
3	Carotenoid diol (triol?) Peridinin dehydrate		583(35), 565(20) 613(11), 553(10)
4	Diadinochrome		583(26), 565(20), 181(100)
5	Diadinoxanthin		583(61), 565(35), 181(100)
6	Carotenoid diols		599(11), 583(12), 565(32), 547(22) 567(56), 549(20)
7	Fucoxanthin		659(13), 641(10), 599(19), 581(22), 563(10), 489(8), 471(3), 429(20), 197(100)
8	Fucoxanthinol 5'-dehydrate	(475), 450, (430)	627(15), 599(100), 581(60), 445(23), 429(98), 197(32)
9	Isofucoxanthinol 5'-dehydrate	475, 447, (430)	627(15), 599(100), 581(64), 445(12), 429(62), 197(45)
10	Fucoxanthinol	475, 447, 425 (Hx)	617(20), 599(95), 581(48), 563(5), 541(12), 447(88), 429(25), 197(80)
11	Isofucoxanthinol	(475), 450, (430)	617(20), 599(66), 581(43), 563(10), 541(15) 197(100)
12	Unidentified fuco-pigment		635(13), 633(11), 617(36), 599(27), 581(7) 563(5), 541(6), 525(7), 197(100)

Table 4. Quantitative distribution of fucoxanthin related carotenoids (fucopigments) in BBST-2 and BBST-3.

	%Total Fucopigments	
	BBST-2	BBST-3*
Fucoxanthin (IV)	9	5
Fucoxanthinol (VI)	61	69
Isofucoxanthinol (XII)	21	12
Fucoxanthin 5'-dehydrate (XI)	2	1
Fucoxanthinol 5'-dehydrate (VII)	6	6
Isofucoxanthinol 5'-dehydrate (VIII)	0	7
	100%	100%

*0.50 mg total pigments/gdw sediment. Determined spectroscopically on whole extract at 450nm (λ_{max}) assuming $\varepsilon = 10^5$ l/mole cm and an average molecular weight of 616 amu (Σ (MW)(X%)).

fractions as all-trans fucoxanthin (Tables 2, 3). A mass spectrum characteristic of fucoxanthin was also observed for fraction 8 of BBST-2. The visible spectrum of this compound in acetone has a single maximum at 447 nm, flanked by two shoulders at (470) and (425) nm. All-trans fucoxanthin has maxima at 449,(425)(acetone). The 2 nm difference in the principal absorption maxima is consistent with the 1-3 nm bathochromic shift observed for trans → cis isomerization (Figure 4)(Vetter *et al.*, 1971; Bernhard *et al.*, 1974; Moss and Weedon, 1976). Co-injection of fraction 8 of BBST-2 with cis/trans isomeric fucoxanthin produced by thermal equilibration of all-trans fucoxanthin confirmed the assignment of this fraction as cis-fucoxanthin isomer(s) (stereochemistry unassigned).

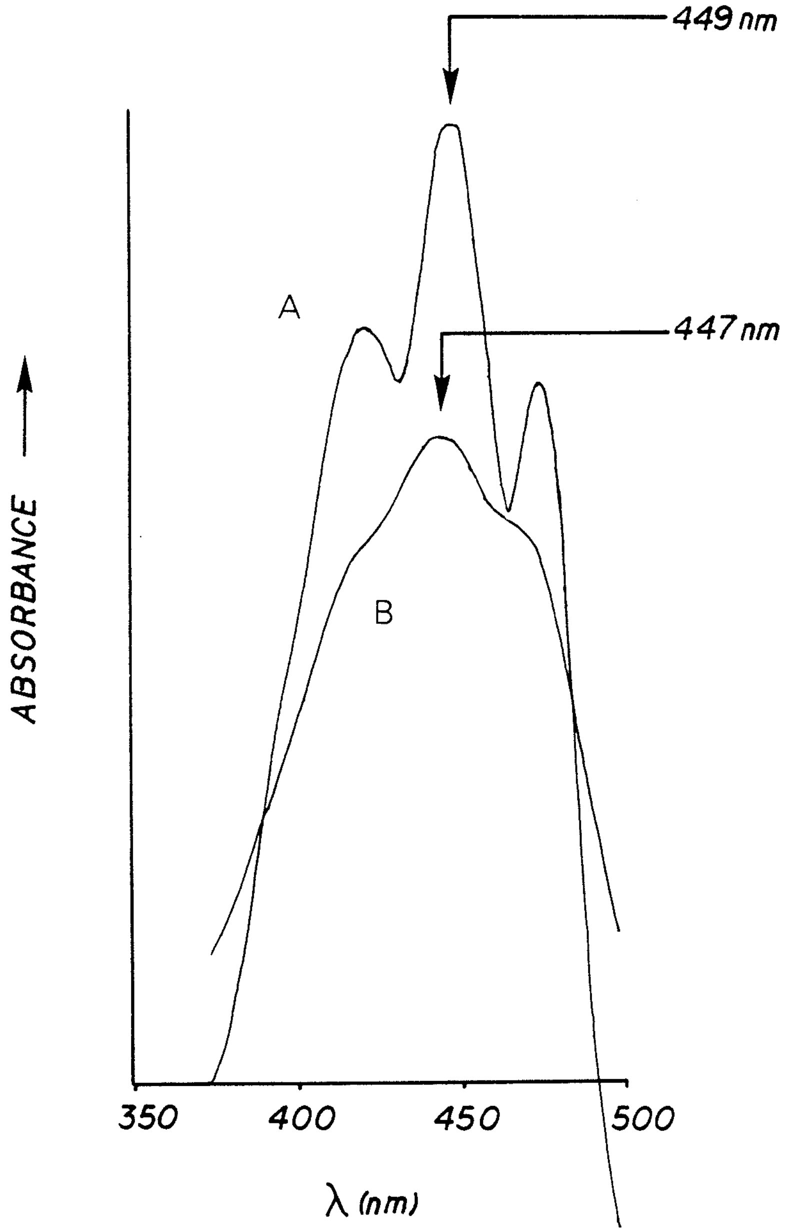
Fractions 2, 3, 5, and 7 from BBST-2 and fractions 2, 6, 8, 9, 10, 11 and 12 from BBST-3 have characteristic ions (M+1, M+1-18, M+1-60, M+1-170; *m/z* 197) which identify these fractions as fucoxanthin derived pigments (hereafter refered to as fucopigments). Mass and visible spectra support the identification of fraction 2 of BBST-2 and fraction 2 of BBST-3 as fucoxanthin 5'-dehydrate (XI), fraction 7 of BBST-2 and fraction 8 of BBST-3 as fucoxanthinol 5'-dehydrate (VII), fraction 9 of BBST-3 as a mixture of cis-fucoxanthinol 5'-dehydrate (VII) and isofucoxanthinol 5'-dehydrate (VIII), fraction 10 of BBST-3 as fucoxanthinol (VI), and fraction 11 of BBST-3 as isofucoxanthinol (XII). Coinjection of authentic fucoxanthinol (Chapter 4) confirmed the identification of fraction 10 of BBST-3 as this compound.

Fraction 12 of BBST-3 had mass fragments with *m/z* = 635, 633, 617, 599, 581, 541 and 197. Analogous fragments were observed in the mass spectra of other fuco-pigments. This fraction is considered to be a fucopigment(s), although its structure has not been determined. Complete structural elucidation will require isolation of larger quantities of this pigment for derivatization. The mass spectra of fractions 3 and 5 of BBST-2, and fraction 6 of BBST-3 include minor fragments at m/z = 599 and 581; consistent with dehydro-fucoxanthinol isomers. Fucopigments of the 5'-dehydro series have already been identified. These compounds may represent 3, 5 or 3' dehydro isomers of fucoxanthinol and isofucoxanthinol as illustrated in Figure 5.

Fucoxanthin (IV) is the major carotenoid in diatoms and some dinoflagellates, and is the major phytoplankton carotenoid in standing crop suspended particulate matter (Table 1). In BBST-2 and 3 fucoxanthin comprised only 4-7% of the total carotenoids and 5-9% of the total fucopigments (Table 4). The remaining 91-95% of the total fucopigments consisted of the fucoxanthin transformation products: fucoxanthinol, isofucoxanthinol, fucoxanthinol 5'-dehydrate, isofucoxanthinol 5'-dehydrate, and fucoxanthin 5'-dehydrate. Together these compounds consitute 82% of the total carotenoids.

Fucoxanthinol (VI) is the most abundant carotenoid in the samples (Figures 2, 3; Tables 2, 3, 4). Acylation of fraction 10 of BBST-3 with acetic anhydride in pyridine yields fucoxanthin 3-acetate (XIII) which co-elutes with authentic fucoxanthin 3-acetate synthesized from fucoxanthin (Chapter 4). Thermal isomerization of fucoxanthin 3-acetate synthesized from fraction 10 of BBST-3 produces an equilibrium mixture of cis isomers identical in both chromatographic retention time (HPLC) and relative abundance to those derived from thermal isomerization of authentic fucoxanthin 3-acetate (Table 5).

Figure 4. Visible spectra of A) BBST-2 fraction 6 (all-trans fucoxanthin) and B) BBST-2 fraction 8 (cis fucoxanthin). Spectra recorded in hexane on a Cary 118 scanning spectrophotometer.


449 nm
447 nm
A
B
ABSORBANCE
350
400
450
500
λ (nm)

The ester hydrolysis which converts fucoxanthin to fucoxanthinol can occur via two pathways; a base-catalyzed chemically mediated ester hydrolysis, or biochemical metabolism (esterase). Base-catalyzed ester hydrolysis seems unlikely as epoxide opening (formation of iso derivatives) should precede ester hydrolysis (Bonnett *et al.*, 1969; Nitsche, 1974). Figure 6 presents kinetic data from the reaction of fucoxanthin with 0.01N KOH in MeOH/H_2O (90/10). The data suggest a reaction sequence: fucoxanthin (IV) → isofucoxanthin (XIV) → isofucoxanthinol (XII). No fucoxanthinol was observed. In BBST-2 and BBST-3 fucoxanthinol and fucoxanthin, which retain the 5,6-epoxide, predominate over their opened epoxide derivatives isofucoxanthinol and isofucoxanthin. This suggests a reaction sequence of: fucoxanthin (IV) → fucoxanthinol (VI) → isofucoxanthinol (XII).

Rather than chemical ester hydrolysis, biochemical metabolism by zooplankton and other higher organisms is proposed. Fucoxanthinol has been reported as a minor component in some marine algae (Nitsche, 1974; Berger *et al.*, 1977), and is thought to be an intermediate in fucoxanthin biosynthesis (Liaaen-Jensen, 1977; 1978). Fucoxanthinol has also been found as a major fucopigment in the gut of sea urchins (Galasko *et al.* 1969; Hora *et al.*, 1970), so that it is not unreasonable to assume that other higher heterotrophs have the ability to hydrolyze fucoxanthin without rearranging the epoxide. Since resuspended surface sediments that are the source of the material collected in the sediment traps are rapidly reworked by heterotrophic organisms (Rhodes and Young, 1970), it is proposed that fucoxanthinol represents a metabolite of this heterotrophic activity.

Figure 5. Dehydration reactions of fucoxanthinol.

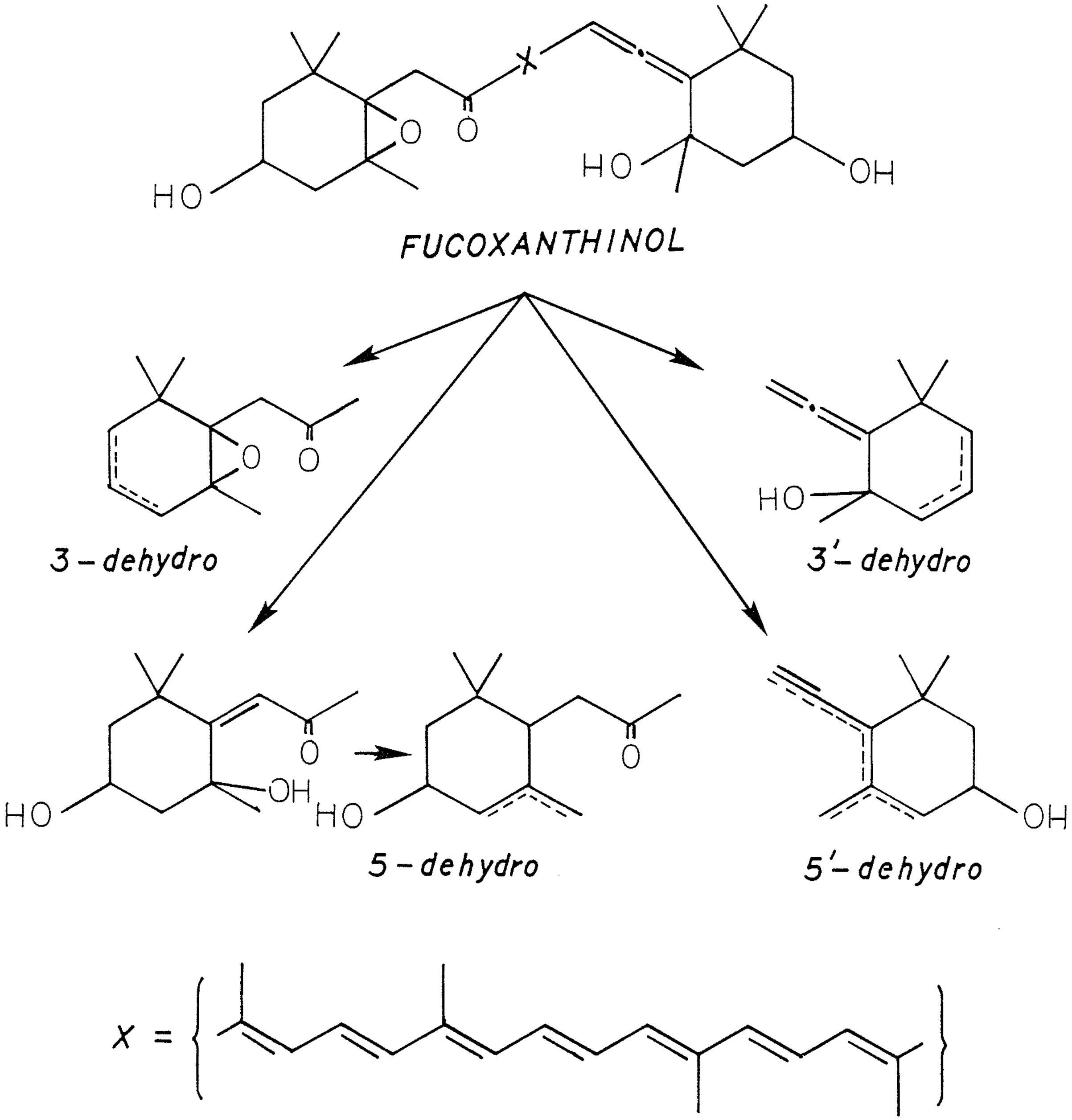

FUCOXANTHINOL
HO
O
O
HO
OH
3 – dehydro
3′– dehydro
HO
OH
HO
5 – dehydro
5′– dehydro
OH
X =

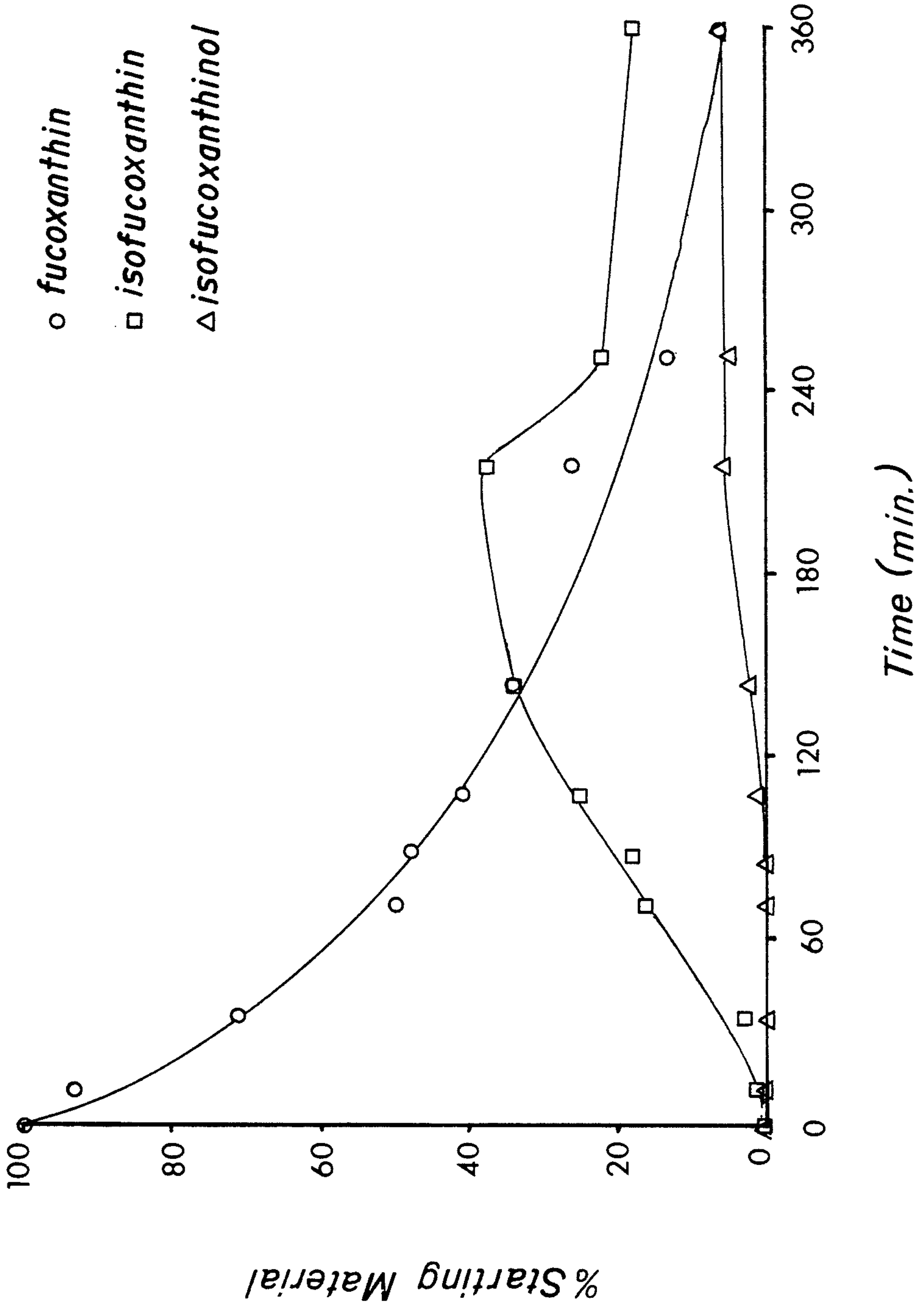
Table 5. Thermal isomerization of fucoxanthin 3'-acetate

	Retention Time (min.)*		Peak Area	
Isomer	Run 1	Run 2	Run 1	Run 2
Authentic fucoxanthin 3'-acetate (from fucoxanthin)				
cis isomer 1	0.88	0.88	0.088	0.081
cis isomer 2	0.95	0.96	0.27	0.27
all trans	1.0	1.0	1.0	1.0
cis isomer 3	1.13	1.17	0.20	0.19
Fucoxanthin 3'-acetate (from BBST-3, fraction 10)				
cis isomer 1	0.90	0.86	**	0.079
cis isomer 2	0.96	0.96	--	0.26
all trans	1.0	1.0	--	1.0
cis isomer 3	1.14	1.14	--	0.19

*300 x 3.9 mm Spherisorb 5 μm amino column eluted with 5% B (B = 20% MeOH/THF, 20/80), 95% hexane at 2 ml/min. Peak areas and retention times are normalized to the all-trans peak.

**Insufficient sample to make accurate measurements of peak area for this run.

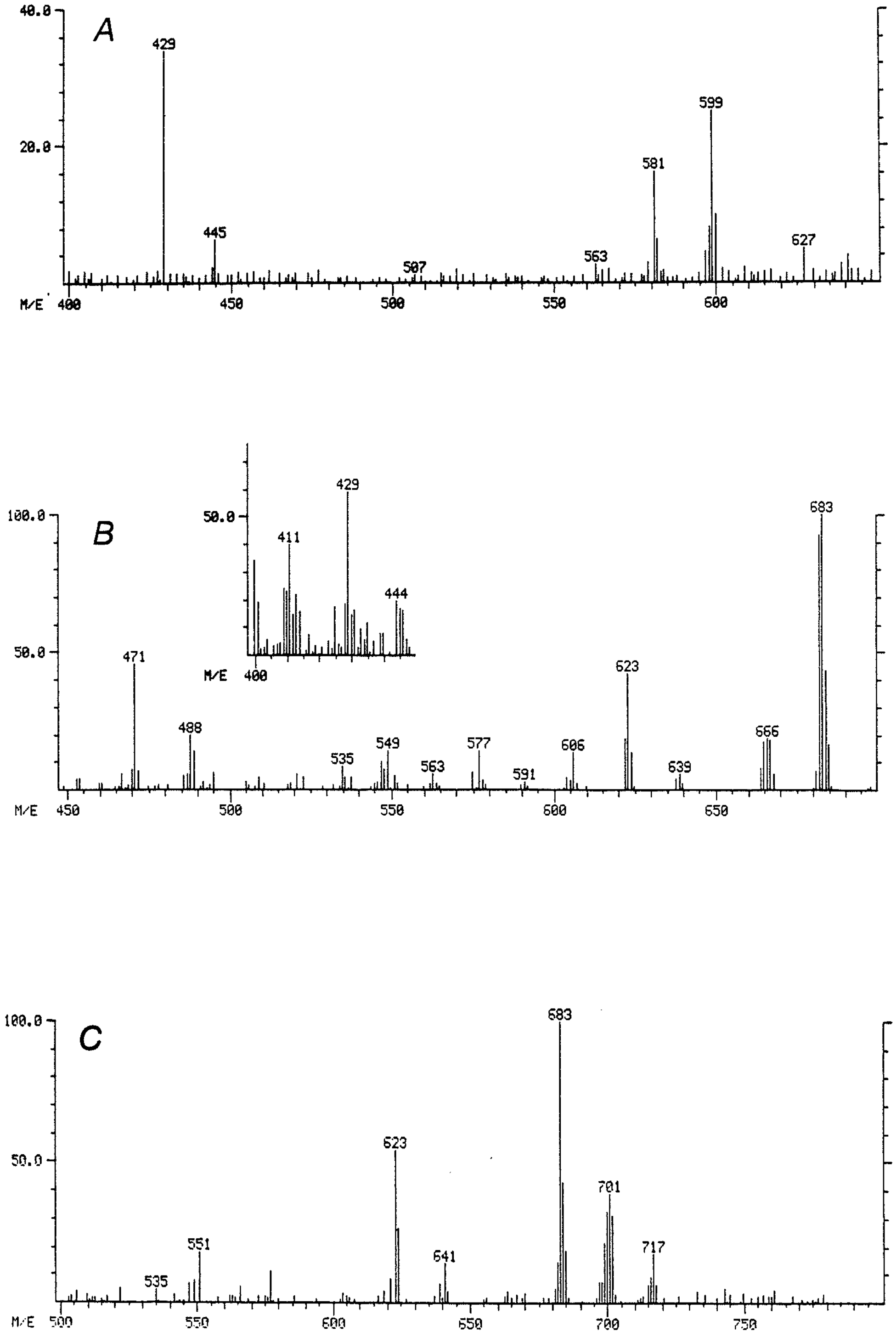
Figure 6. Kinetics of base-catalyzed epoxide opening and ester hydrolysis of fucoxanthin in 0.1mM KOH MeOH:H_2O (90:10).


○ fucoxanthin
□ isofucoxanthin
△ isofucoxanthinol
% Starting Material
100
80
60
40
20
0
Time (min.)
0
60
120
180
240
300
360

The mass spectra of fucoxanthin dehydrate (XI), fucoxanthinol dehydrate (VII), and isofucoxanthinol dehydrate (VIII) are consistent with loss of the tertiary alcohol at the 5' position (Figure 7). The mass fragment M+1-170 arises from cleavage of the 7,8 bond α to the 8-ketone. The resulting mass fragments (m/z 471, 429) correspond to a loss of a hydroxyl group at either the secondary 3' or tertiary 5' position. Acylation of the isolated fucoxanthinol dehydrate and isofucoxanthinol dehydrate with acetic anhydride in pyridine yields a diacetate. Under the conditions used in this synthesis, acetic anhydride in pyridine acylates only primary and secondary hydroxyl groups. The mass increase of 84 amu for the acylated dehydrate over the underivatized parent is consistent with the esterification of the two hydroxyl groups at the 3 and 3' position (Figure 7). Therefore dehydration of fucoxanthinol, isofucoxanthinol, and by analogy fucoxanthin proceeds with loss of the tertiary 5'-alcohol.

The site of double bond formation from acid catalyzed dehydration of the tertiary 5'-alcohol is presently a matter of debate. Egger et al. (1969) and Nitsche et al. (1969) dehydrated neoxanthin (XV) and neoxanthin diacetate (XVI) with 0.02N $HCl/CHCl_3$. Three products were formed, differing in the position of the resultant double bonds. The 5',18'-ene (Figure 8) was the major product of both reactions (60-75%), followed by the 5',6'-ene, 7',8'-yne (20-25%) and the 3',4'-, 5',18'-diene (presumably from rapid dehydration of the allylic 4',5'-ene) (0-20%) (Figure 8). In contrast, Bonnett et al. (1969) dehydrated fucoxanthin with phosphoryl chloride and reported only 4',5'-ene formation. More thorough studies of this reaction (Nitsche, 1970, 1972; Johansen and Liaaen-Jensen, 1974; Buchecker and Liaaen-Jensen, 1975) with neoxanthin (XV), neoxanthin

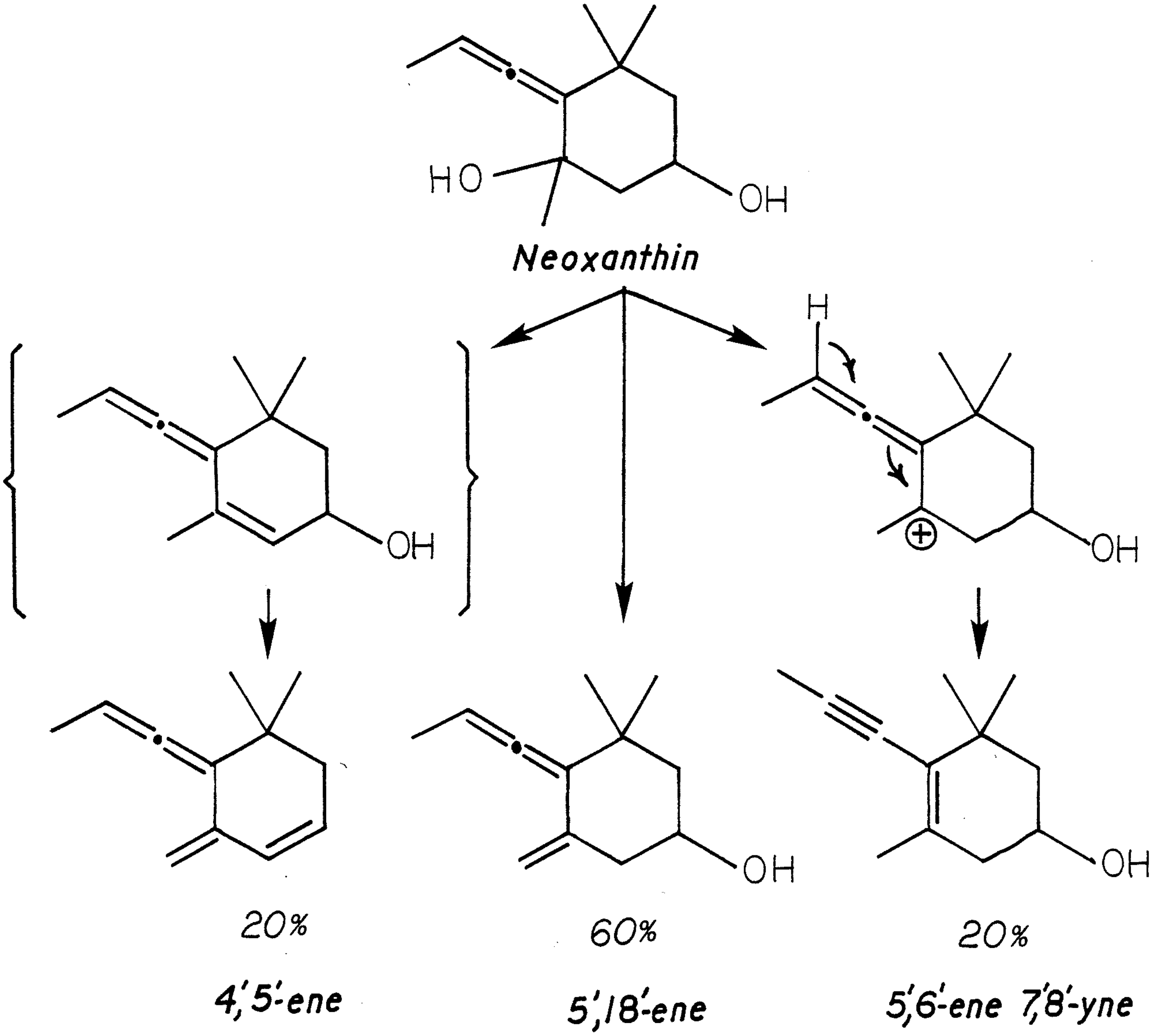
Figure 7. Chemical ionization mass spectra of A) fucoxanthinol-5'-dehydrate (BBST-3 fraction 8) (M+29 = 627, M+1= 599, M+1-18 = 581, M+1-18-18 = 563, M+1-92 = 507, M+1-154 = 445, M+1-170 = 429), B) fucoxanthinol (5?) 5'-dehydrate (BBST-3 fraction 8) acylated with acetic anhydride in pyridine (M+1 = 683, M+1-18 = 665, M+1-60 = 623, M+1-60-18 = 605, M+1-92 = 591, M+1-106 = 577, M+1-60-60 = 563, M+1-92-42 = 549, M+1-212 = 471, M+1-212-42 = 429, M+1-212-60 = 411), and C) fucoxanthinol (BBST-3 fraction 10) acylated with acetic anhydride in pyridine (M+1 = 701, M+1-18 = 683, M+1-60 = 641, M+1-60-18 = 623). Instrumental conditions: CH_4 reagent gas at 900 μm, ionization voltage 130 eV, ionization current 500 μA.


A
40.0
20.0
429
445
507
563
581
599
627
M/E 400
450
500
550
600
B
100.0
50.0
471
488
535
549
563
577
591
606
623
639
666
683
M/E 450
500
550
600
650
50.0
411
429
444
M/E 400
C
100.0
50.0
535
551
623
641
683
701
717
M/E 500
550
600
650
700
750

diacetate (XVI), fucoxanthin acetate (XIII), peridinin (V), peridinin acetate (XVII), and minulaxanthin (XVIII) dehydrated with $CHCl_3$/HCl and phosphorous oxychloride ($POCl_3$) suggested that dehydration occurs with formation of all three isomers as reported by Egger et al. (1969) and Nitsche et al. (1969). Unfortunately, yields were not reported, so the product specificity and selectivity of reagents and reaction conditions cannot be evaluated.

The site of double bond introduction for fucoxanthin 5'-dehydrate, fucoxanthinol 5'-dehydrate, and isofucoxanthinol 5'-dehydrate isolated from BBST-2 and BBST-3 was not fully established. Moss and Weedon (1976) have proposed that loss of ketene is characteristic of allylic acetate esters (Chapter 4, Figure 7). Loss of ketene (M+1-42) is not observed in mass spectra of fucoxanthin and fucoxanthin 3-acetate. However fragments corresponding to loss of ketene are observed in the mass spectra of dehydrates isolated from Buzzards Bay sediment trap samples (see M+1-212-42 = 429 for 3,3'-diacetyl-fucoxanthinol 5'-dehydrate). The data is consistent with the observation of Bonnett et al. (1969), and the double bond is tentatively assigned to the 4',5' position. However, it must be noted that the observed enhancement of the M+1-42 fragment in the mass spectra of fuco-dehydrates requires only that some of the pigment in these fractions has the double bond in the 4',5' position. Major mass fragments are also observed at M+1-60, and it cannot be determined if these fragments arise from loss of acetic acid, or loss of ketene and water. Definitive assignment of the double bond position must await synthesis of the double bond isomers and isolation of sufficient material for comparative chromatographic, IR, and ^{13}C and proton NMR studies.

Figure 8. 5'-hydroxyl dehydration of neoxanthin with 0.02 N $HCl/CHCl_3$, after Egger *et al.* (1969). Compound in parenthesis was not isolated.


HO
OH
Neoxanthin
OH
H
⊕
OH
20%
4',5'-ene
OH
60%
5',18'-ene
OH
20%
5',6'-ene 7',8'-yne

Determining the mechanism of dehydration is difficult. There are no reports of naturally occurring fucoxanthin dehydrates, and there is little evidence to favor either a biochemically or chemically mediated reaction. Chemically, an acid catalyzed dehydration seems most likely. However, substantial amounts of the unrearranged 5,6 epoxide, diadinoxanthin (III) is observed in both sediment trap samples. Under acidic conditions the 5,6 epoxide rapidly rearranges to the 5,8 furanoxide, diadinochrome (X). When reacted with acids, neoxanthin (XV) which has a 3-hydroxy-5,6-epoide (like diadinoxanthin) and a tertiary 5'-hydroxyl (like fucoxanthin), undergoes epoxide rearrangment before dehydration. If the acidic conditions necessary to dehydrate fucoxanthin existed in the samples, all the diadinoxanthin should have rearranged to the 5,8 furanoxide. Therefore, the dehydration of fucopigments is probably not a result of chemical acid catalysis, but a more site-specific metabolic reaction.

The dehydration of sterols (Rhead et al., 1971; Rubenstein et al., 1975; Dastillung and Albrecht, 1977; Gagosian and Farrington, 1978), amino acids (Bada and Hoopes, 1979), and phytol (Simoneit, 1973; De Leeuw et al., 1977), occurs in a variety of marine environments and both chemical and biochemical mechanisms have been proposed. The dehydration of stanols is structurally analogous to the dehydration of the secondary 3 or 3' hydroxyl of carotenoid alcohols. Fractions 3 and 5, BBST-2 have mass spectra consistent with the assignment of these peaks as 3, 5 or 3' dehydrates of (iso)fucoxanthinol. However, the structural assignment is not definitive. At most it can be concluded that dehydration of the 3 or 3' alcohol is a minor reaction.

The major dehydration products of fucoxanthin arise from loss of the tertiary 5'-alcohol. This suggests either that different processes are operative in the dehydration of stanols and carotenoid alcohols, or there is a general process which displays an order of biochemical reactivity (i.e. tertiary alcohols dehydrate faster than secondary alcohols). In the latter case a sequential dehydration through the 5'-monodehydro-, the 3,5' (or 3',5') didehydro- and the 3,3'5' tridehydro-derivatives should be observed. This hypothesis could be verified by an analysis of surface sediments which contain high concentrations of sterenes as in surface sediments from the Southwest African shelf and slope (Gagosian and Farrington, 1978). If sterols and carotenoids are dehydrated by the same process, these sediments should have a high concentration of carotenoid mono- and di-dehydrates.

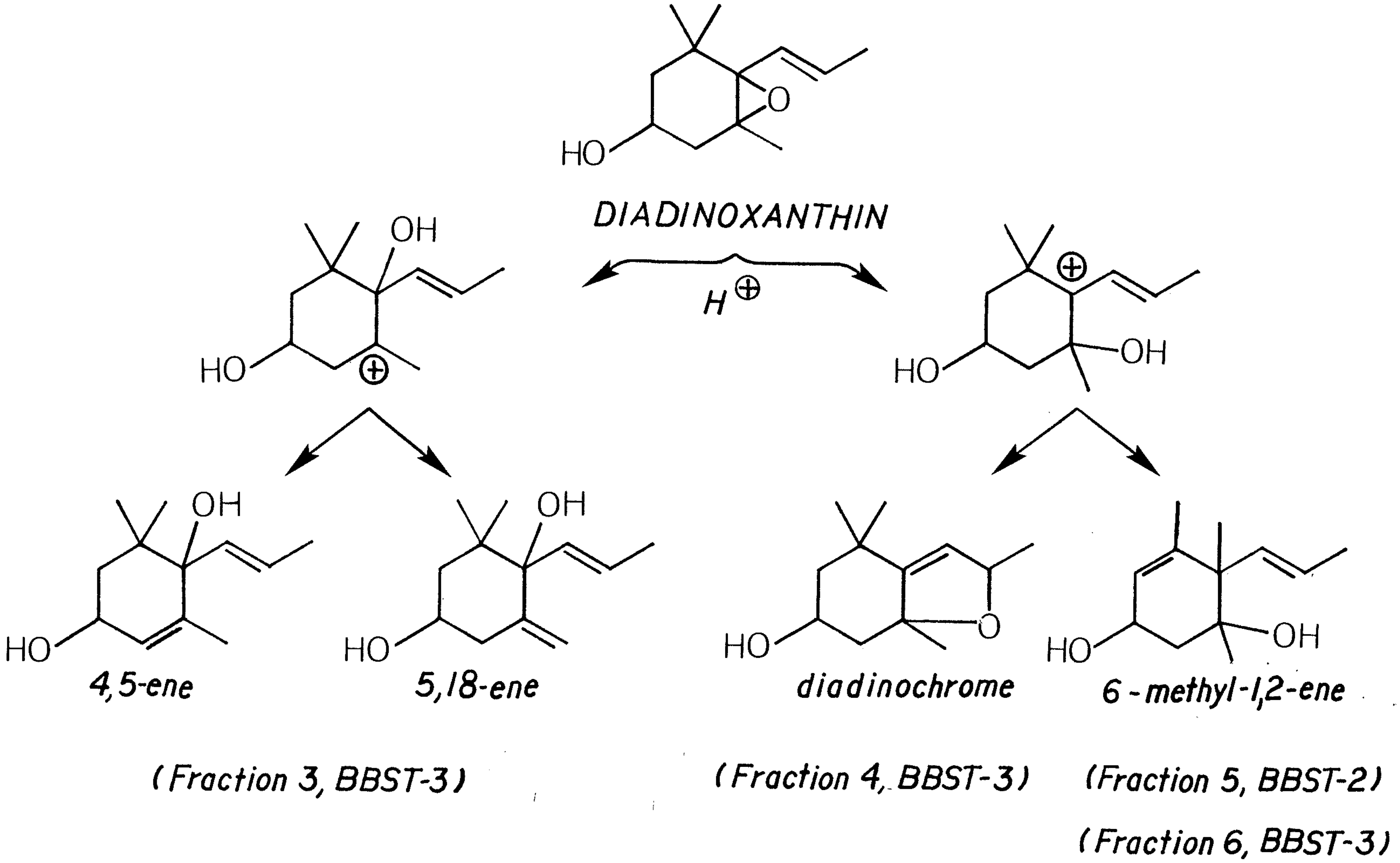
Isofucoxanthinol (XII) was the second most abundant pigment in BBST-2 and BBST-3. Isofucoxanthinol 5'-dehydrate (VIII) was also an important fucopigment in BBST-3. Bonnett _et_ _al_. (1969) observed trace quantities of isofucoxanthin and isofucoxanthinol in their analysis of _Fucus_ _vesiculosus_. The authors concluded that these compounds originate from reactions on alumina during chromatographic purification, and were not natural products. A subsequent study by Nitsche (1974) confirmed this hypothesis. Nitsche extracted 150 kg (wet weight) of _Fucus_ _vesiculosus_ and purified the carotenoid pigments by chromatography on silica. Neither isofucoxanthin nor isofucoxanthinol were isolated. In the present study no opening of the fucoxanthin 5,6 epoxide was observed in blanks spiked with fucoxanthin or in samples of Buzzards Bay suspended particulate matter. Therefore opened epoxides (iso-compounds) observed in sediment trap samples are believed to be indigenous to the samples. Glasko *et al.* (1969) did not report any isofucoxanthin or isofucoxanthinol in the coelomic epithelium of the sea urchin *Paracentrotus lividus*. Metabolism by these organisms apparently does not open the 5,6 epoxide of fucoxanthin. Griffiths and Perrot (1976) also report a fucopigment as the major carotenoid (80%) in the gut of the sea urchin *Strongylocentrotus drobachiensis*. The authors denote this pigment as isofucoxanthin. However, no structural determination was made and the pigment is probably fucoxanthinol. Since epoxide opening is a facile chemical reaction, the source of isofucoxanthinol and isofucoxanthinol 5'-dehydrate in the Buzzards Bay sediment trap samples is considered to be a chemically mediated reaction.

Diadinoxanthin, Diadinochrome, and Related Pigments- Authentic diadinoxanthin co-elutes with fraction 4 of BBST-2 and fraction 5 of BBST-3. In support of this assignment, fraction 4 of BBST-2 has absorption maxima at 477, 447, 424 (acetone) and *m/z* 583 (87%), 565 $(M+1-H_2O)$(36%), and 181(100%). Fraction 5 of BBST-3 had *m/z* 583(61%), 565(35%), and 181(100%). Authentic diadinoxanthin had λ_{max} 477, 447, 425 (acetone), and *m/z* 583 (100%), 565 (78%), 181 (100%). Baldas *et al.* (1966) have proposed that the mass fragment *m/z* 181 is characteristic of 3-hydroxy-5,6-epoxy and 3-hydroxy-5,8-furanoxides. A mechanism of 5,6 5,8 epoxide rearrangement followed by fission of the 8,9 bond was proposed.

HPLC fraction 4 of BBST-3 coelutes with authentic diadinochrome (X). Major mass fragments at *m/z* 583, 565, and 181 support this assignment. The sample was stored at -20°C for 5 months prior to analysis. Transformation of diadinoxanthin to diadinochrome is observed in suspended particulate sample extracts (MeOH) stored at -20°C for similar periods (Chapter 3). The diadinochrome observed in BBST-3 is most likely a storage artifact. This conclusion is supported by the analysis of BBST-2, which was done immediately after retrieval of the sample. No diadinochrome was observed in this sample.

Three other peaks: fraction 5 of BBST-2 and fractions 3 and 6 of BBST-3, have mass spectra with fragments characteristic of diadinoxanthin (m/z, 583, 565). Fraction 5 of BBST-2 and fraction 6 of BBST-3 have the same relative retention time and major mass fragments at m/z 583, 565 (M+1-18), and 547 (M+1-18-18). These fractions elute as a mixture of at least three compounds. Futher separations with HPLC (5 μm Spherisorb amino, eluted with mixtures of Hexane:MeOH/THF) were not successful. The mass spectrum includes a major mass fragment corresponding to M+1-18-18. Loss of two molecules of water is not typically observed in CI mass spectra of carotenoid secondary diols, but is observed for carotenoid triols which have a tertiary hydroxyl group. In addition, no prominent fragment with m/z 181 is observed in the mass spectrum of fraction 6 of BBST-3 (fraction 5 of BBST-2 was not scanned below m/z 220). Therefore this compound is not a 5,6 or 5,8 epoxide. A stucture consistent with the chromatographic and mass spectral data is the 5-hydroxy-1,2-ene derivative of diadinoxanthin (Figure 9). A mechanism of formation from diadinoxanthin is given in Figure 9. The mass spectrum of fraction 3, BBST-3 also does not include a major fragment with m/z 181. Therefore the 6-hydroxy-4,5-ene or 6-hydroxy-5,18-ene derivative of diadinoxanthin is proposed for this compound. Further characterization of these fractions must await derivatization and spectroscopic studies.

Figure 9. Reactions of diadinoxanthin in acid.


DIADINOXANTHIN
H⊕
4,5-ene
5,18-ene
diadinochrome
6-methyl-1,2-ene
(Fraction 3, BBST-3)
(Fraction 4, BBST-3)
(Fraction 5, BBST-2)
(Fraction 6, BBST-3)

Carotenoid Alcohols - HPLC fractions 2, 3 and 5 of BBST-2, and fraction 6 of BBST-3 display mass fragments characteristic of carotenoid diols (M+1; M+1-18). The pseudomolecular ion (m/z 569) of BBST-2 fraction 2 requires $C_{40}O_2H_{56}$, characteristic of the widely distributed carotenoid 3,3'-diols lutein (XIX), zeaxanthin (XX), and tunaxanthin (XXI). Lutein and/or zeaxanthin are distributed in nearly all higher plants, and many marine algae, fish, and crustaceans. Tunaxanthin is restricted in distribution to marine fish (Ronneberg, 1978; Liaaen-Jensen, 1978). Carotenoid 3,3' diols were not observed in suspended particulate matter, and it seems unlikely that marine phytoplankton and zooplankton are the source of this fraction in sediment trap material. A more probable source may be more resistant terrestrial plant material or macroscopic marine organisms such as fish. Farrington et al. (1977), Farrington and Tripp (1977), and Lee et al. (1977) report significant concentrations of terrestrial plant hydrocarbons, fatty acids, and sterols in Buzzards Bay surface sediments. An input from this source would contribute lutein, zeaxanthin, violaxanthin (XXII), and neoxanthin (XV). However, violaxanthin and neoxanthin were not observed in either sediment trap sample. Evidently terrestrial plants, which contribute a major portion of the more resistant lipids for other classes of organic compounds, do not contribute significantly to the carotenoids. Therefore, the diol observed in BBST-2 appears to have a marine source. Determination of the specific isomer of this compound is needed to more clearly establish its origin.

The mass spectrum of HPLC BBST-2 fraction 5 and BBST-3 fraction 6 includes fragments with m/z 567 and 549 characteristic of a carotenoid alcohol with a molecular formula $C_{40}H_{54}0_2$. On the basis of retention time, these fractions are most likely diols. Marine carotenoid diols with $C_{40}H_{54}0_2$ include diatoxanthin (XXIII) and monadoxanthin (XXIV). Diatoxanthin is a major pigment in many marine diatoms and dinoflagellates, however the HPLC retention time of this pigment does not correspond with fraction 5. Monadoxanthin is a minor carotenoid in algae from the class Crytophyceae. These organisms are not considered to be significant contributors to the organic matter in Buzzards Bay. In addition, monadoxanthin differs from diatoxanthin only in the position of the 5,6 double bond (retro shift in monaxanthin to the 4,5 position). Other double bond isomer pairs of this class (α , β -carotene; lutein and zeaxanthin) are not separated by the chromatographic procedure, and it is doubtful whether the technique would separate diatoxanthin from monadoxanthin. Therefore, on the basis of HPLC retention time, fraction 5 of BBST-3 is probably not monadoxanthin. Further separation and structural elucidation is necessary to fully establish the origin of this compound.

HPLC fraction 3 of BBST-2 includes carotenoids with $C_{40}0_2H_{52}$ (m/z 565). The most widely distributed carotenoid of this series is canthaxanthin (XXV); a dione. However, authentic canthaxanthin does not co-elute with this fraction. The chromatographic retention time, mass spectra, and multiple absorption maxima at 478, 451, and 421 nm are consistent only with a diol. Conjugated keto carotenoids typically display absorption spectra with no fine structure (i.e. a single absorption maximum). The visible spectra of echinenone (XXVI) (mon-one), canthaxanthin (XXV) (di-one), and astacene (IX) (tetra-one) all contain only one absorption maximum. In contrast, their hydroxyl analogues display spectra with three distinct absorption maxima (Davies, 1976; see also Figure 8, Chapter 4). The mass fragment m/z 547 is consistent with loss of water, a fragmentation not observed for carotenoid ketones. Carotenoid diols with a molecular formula $C_{40}O_2H_{52}$ are neither qualitatively nor quantitatively significant pigments (Straub, 1971). The observation of a major fraction with this composition suggests a degradation product, perhaps 3 or 3'-dehydrodiadinoxanthin. Further characterization is needed before a more complete structural assignment can be made.

Carotenes - HPLC fraction 1 of BBST-2 and fraction 1 of BBST-3 eluted as broad peaks with multiple shoulders. The mixture had visible absorption maxima at 666, 612, 535, and 411 nm characteristic of chlorophyll pigments, and 475, and 450 nm characteristic of carotenoids. Mass fragments were observed at m/z 549, 547, 537, 535, 523, 521, 495, 411, and 409 (Tables 2, 3). The mass fragment at m/z 537 corresponds to the molecular ion of carotene ($C_{40}H_{56}$, MW 536). Attempts at further separation of the mixture on silica (hexane), and RP-C18 (80/20; MeOH/H_2O) HPLC were unsuccessful.

Other Pigments - HPLC fraction 3 of BBST-3 contains mass fragments with m/z 613, 595, and 553, consistent with the assignment of this fraction as peridinin 5'-dehydrate (XXVIII). By analogy with the major series of fucoxanthin dehydrates, dehydration is expected to occur at the 5'-alcohol.

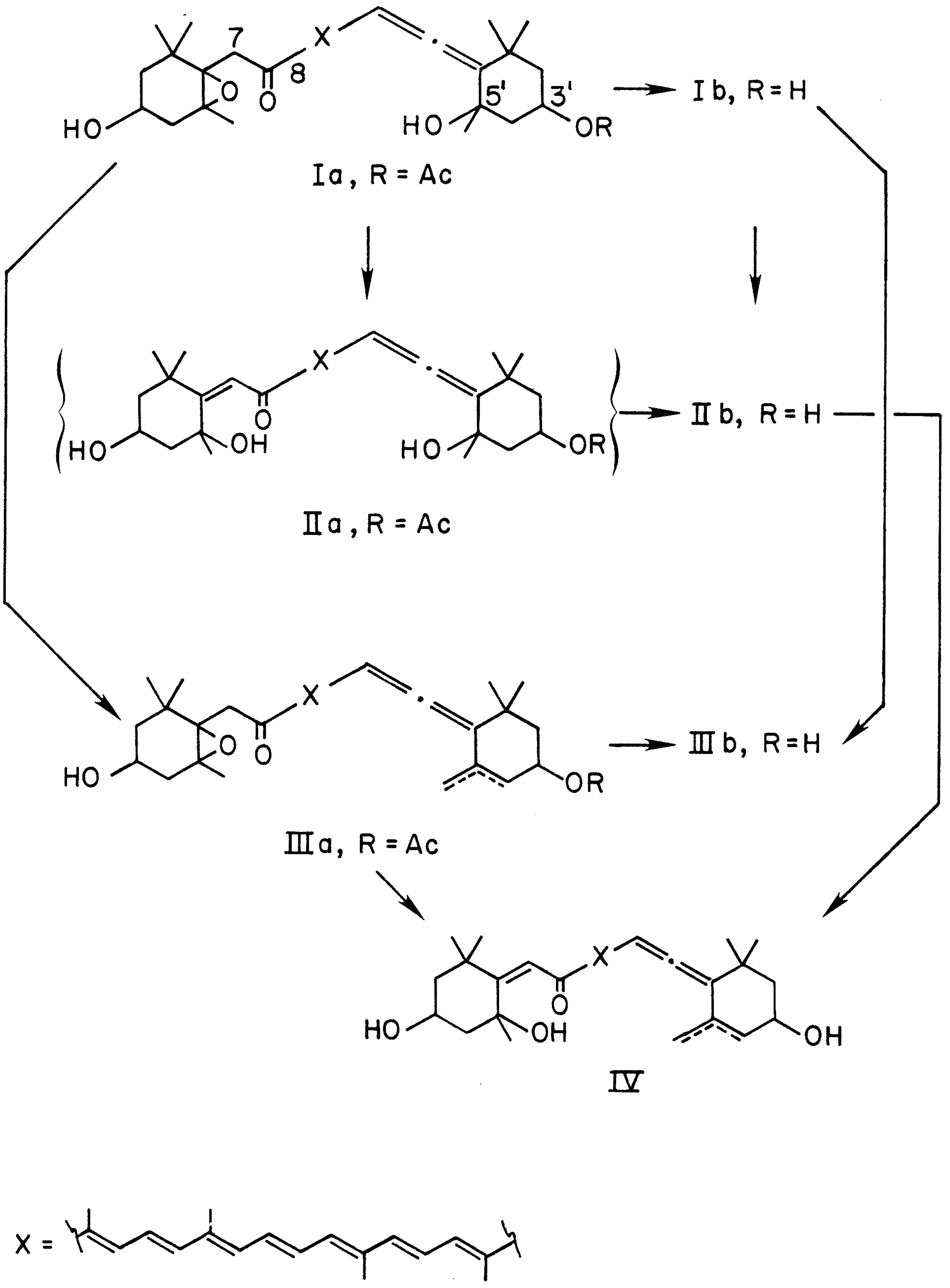
CONCLUSIONS

The carotenoid distribution in suspended particulate matter from Buzzards Bay reflects the planktonic origin of the sample. No significant concentration of transformation products were observed, which is in agreement with the result of Jeffrey (1974, 1976) and Neveux (1975). Trace quantities of the transformation products astacene (IX) and diadinochrome (X) were observed and may represent a detrital component. Analysis of suspended particulate matter collected in oceanic deep water is needed to substantiate this hypothesis.

Approximately 95% of the carotenoids isolated from sediment trap samples represent transformation products. A biochemically and chemically mediated transformation pathway for fucoxanthin (Figure 10) is proposed. In this pathway fucoxanthin is rapidly hydrolyzed to fucoxanthinol by marine heterotrophs. Fucoxanthinol is then slowly dehydrated and rearranged to isofucoxanthinol 5'-dehydrate. The distribution of fucoxanthin transformation products isolated in the sediment traps suggests that dehydration and epoxide rearrangement occur over considerably longer time scales than ester hydrolysis. Isofucoxanthin was not isolated in either trap sample, and fucoxanthin 5'-dehydrate was isolated only in trace ($\leq 1\%$) quantities.

The present set of experiments does not permit modelling of the water column transformation reactions independently of the surface sediments due to the problem of resuspension. Consequently, time scales and mechanisms cannot be clearly established. Assuming the sedimentation rate of 2.95 mm/yr determined by Farrington _et al_. (1977) and that only the bioturbated zone of 3 cm (Farrington _et al_., 1977, Rhoads, 1974) is

Figure 10. Proposed transformation pathway showing ester hydrolysis, dehydration, and epoxide opening for biochemical and chemical transformation of fucoxanthin. Isofucoxanthin (shown in parenthesis) was not isolated in this study.


7
8
O
O
X
HO
5'
3'
HO
OR
Ia, R = Ac
Ib, R = H
IIa, R = Ac
IIb, R = H
OH
IIIa, R = Ac
IIIb, R = H
IV
X =

resuspended, the maximum time of reaction for the observed transformations is 10 years.

The proposed degradation pathway for fucoxanthin also predicts that structurally similar carotenoids (peridinin (V), neoxanthin (XV), dinoxanthin (XXVII) etc.) will undergo analogous transformation reactions. The tentative assignment of fraction 3 of BBST-3 as peridinin dehydrate supports this hypothesis.

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CHAPTER 3

CAROTENOID TRANSFORMATION PRODUCTS IN THE UPWELLED WATERS OFF THE PERU COAST: SUSPENDED PARTICULATE MATTER, SEDIMENT TRAP MATERIAL, AND ZOOPLANKTON FECAL PELLET ANALYSIS.

INTRODUCTION

The relative significance, rates, and mechanisms of organic matter transformation processes in the water column has become an area of intensive study (Wiebe *et al.*, 1976; Bishop, *et al.*, 1977, 1978, 1980; Honjo, 1978, 1980; Honjo and Roman, 1978; Spencer *et al.*, 1978; Hinga *et al.*, 1979; Phaffenhofer and Knowles, 1979; Rowe and Gardner, 1979; Deuser and Ross, 1980; Deuser *et al.*, 1981). The approach to this problem, used in the present study, is to construct a model for organic matter recycling in the water column that consists of three parts: 1) the synthesis of carotenoid pigments by phytoplankton in the euphotic zone, 2) consumption of some fraction of these pigments by heterotrophic organisms, and 3) removal of metabolic by-products to the sediments by large particle transport. The model separates particulate matter into reservoirs according to the degradation processes that have occurred since synthesis. The objective of the present study is to sample these particulate reservoirs, determine the compositional differences between them, and construct a mechanistic pathway for the transformations that occur as material is transferred between reservoirs.

In practice these reservoirs are separated by particle size. Marine phytoplankton that synthesize carotenoids are typically 5-100 μm in size. These organisms are sampled as suspended particulate matter. In the model phytoplankton are consumed by heterotrophs and the solid waste products egested, in part, as rapidly sinking fecal pellets (> 100 μm). These large particles, which can be sampled with sediment traps, should reflect both the source (phytoplankton) and the process (heterotrophic metabolism) responsible for their formation.

Significant concentrations of fucoxanthin transformation products were observed in sediment trap samples collected in Buzzards Bay, Massachusetts. Three processes were suggested as responsible for the observed transformations: 1) ester hydrolysis via zooplanktonic metabolism, 2) dehydration via bacterial metabolism, and 3) epoxide opening via slow chemical degradation. In order to collect a sufficent amount of material for analysis, traps had to be deployed for a period of one month. As a result, short term (hours to days) and long term (months) transformation processes could not be distinguished. In addition, Buzzards Bay sediment trap material had two sources: the primary vertical flux of material from above the traps, and resuspended surface sediments. Consequently, transformations characteristic of water column processes could not be distinguished from transformations occurring at the sediment-water interface.

The goals of the present series of experiments are: 1) To sample the primary vertical flux of material from the surface waters without the influence of surface sediments. In this way transformation reactions characteristic of water column organic matter recycling processes can be isolated and studied independently of reactions characteristic of processes occurring at the sediment-water interface. 2) To shorten the time scale of transformation reactions sampled from the approximately 10 yr in the Buzzards Bay experiment (assuming resuspension) to 1-3 days. A shortened experiment will help to more clearly establish the time scale and sequence of transformations outlined in Figure 8, Chapter 2. 3) To couple specific processes (i.e. heterotrophic metabolism) with specific transformation reactions (i.e. ester hydrolysis). 4) To extend water column sampling to below the euphotic zone, where synthesis of phytoplankton pigments does not occur, and 5) to extend the transformation pathway proposed for fucoxanthin to other, structurally similar compounds (peridinin (V), dinoxanthin (XXVII)).

This chapter reports the results of a series of experiments conducted in the upwelling waters off the Peru coast (15°S, 75°W). Sediment trap experiments conducted in 1978 at the same location and time of year demonstrated that the particulate carbon flux at 50 meters reached values of 350 mg C/m^2day (Staresinic, 1982). Hence trap deployments of a day or less are sufficient to collect enough material for analysis. Further, values of particulate organic carbon, chlorophyll pigments, and selected classes of organic compounds showed no increase near the bottom of the water column due to sediment resuspension (Gagosian *et al.*, 1980, 1982). Therefore, resuspended material should not contribute significantly to the particles collected in the traps.

SAMPLE COLLECTION

Suspended Particulate Matter All samples were collected on the R/V Atlantis II cruise 108, leg 3 in March-April, 1981 off the Peru coast at 15°S, 75°W. Vertical profiles of suspended particulate material were collected either with a 20 ℓ glass Bodman (Gagosian *et al.*, 1979) or a 10 ℓ Nisken water sampler. Sampling locations are given in Table 1. Seawater was immediately filtered through pre-extracted

Table 1. Cast number, depth, sampling time, and location of suspended particulate matter samples collected off the Peru coast.

Cast-Depth(m)	Date	Time	Lat.(S)	Long.(W)
10-8	3/15	1512-1820	15° 27'	75° 53'
36-3	3/21	2044-2058	15° 07'	75° 36'
-5		1130-1305		
-10		1950		
-25		1315-1340		
-50		1735		
-150		1912		
44-3	3/24	1320-1345	15° 07'	75° 37'
-10				
-25				
-45				
53-5	3/25	1500	15° 02'	75° 35'
-10		1444-1500		
-20		0928-0954	15° 04'	75° 34'
76-5	3/30	1030-1047	14° 59'	75° 38'
-30				
77-5	3/30	1728-1738	15° 02'	75° 41'
91-5	4/1	0915-0945	15° 08'	75° 40'
-10		1606	15° 07'	75° 41'
-20		1244-1326		
-100		1328-1410		
-350		1445-1546	15° 08'	75° 42'
97-5	4/1	1736-1757	15° 07'	75° 41'
99-5	4/2	0955-1015	15° 06'	75° 42'
-50		1057-1115		
-200		0837-0920	15° 08'	75° 40'
104-10	4/3	1157-1212	15° 04'	75° 27'
-20				
-30				
105-10		1453-1512	15° 00'	75° 31'
-20				
-30				
106-10	4/4	0843-0908	15° 08'	75° 40'
-20				
-30				
117-1000	4/6	1134-1245	15° 25'	75° 58'

(CH_2Cl_2, 24 hr), pre-combusted (450°C, 24 hr) Gelman type AE glass fiber filters. Filters were stored at -50°C (on board cryocooler) in foil wrapped glass vials filled with methanol and capped under ultra-high purity nitrogen. Water samples were collected immediately after the deployment and recovery of the sediment traps. In this way the suspended particulate matter which serves as the ultimate source for the material collected in the sediment traps, could be monitored for its carotenoid composition throughout the period of sediment trap sampling.

Sediment Traps Moored sediment traps were constructed after the design of Staresinic (1978). Briefly, traps consisted of a 41 cm diameter PVC cylinder with a 3:1 aspect ratio atop a 45° cone with a 3/4" ball valve terminus. The traps were deployed empty, allowed to fill with surface water and to sink to pre-set depths. On recovery, traps were drained to the top of the cone, the ball valve opened, and the sample and remaining water collected for filtration. Samples (1-10 g wet weight) were immediately filtered and stored as described above. Altogether thirteen traps were deployed and recovered in this fashion. Deployment times and sampling locations are given in Table 2.

Zooplankton Fecal Pellets Photomicrographs of material collected in other trap experiments (Bishop, 1978; Honjo, 1978, 1980; Staresinic, 1982; Staresinic et al., 1982) have shown that fecal pellets from heterotrophic organisms contribute a significant fraction of the particles collected in traps. In order to obtain a fresh sample of this material, fecal pellets were collected from field cultures of zooplanktonic heterotrophs. Zooplankton were collected in a 234 μm mesh net towed 25 m below the

Table 2. Depth, sampling time, and location of sediment traps deployed off the Peru coast, March-April 1981.

Trap-Depth(m)	Depth*	Deployment : Recovery	Time**	Lat.(S)	Long.(W)
1-20	75	3/18 1116 : 3/20 1116	48	15° 03'	75°26'
2-30	80	3/18 1200 : 3/20 1028	46.5	15° 04'	75°27'
3-30† 4-50	200	3/23 0800 : 3/24 0820 3/21 1558 : 3/24 0820	24.3 64.4	15° 07'	75°38'
5-20	80	3/23 1429 : 3/26 0811	65.7	15° 04'	75°27'
6-40† 7-90	140	3/24 1822 : 3/27 0928	63.1	15° 04'	75°36'
8-7	80	3/26 0850 : 3/30 1610	104.7	15° 03'	75°28'
9-40	140	3/27 1633 : 3/30 0928	64.9	14° 59'	75°40'
11-22† 12-40	80	3/31 1155 : 4/3 0930	70.4	15° 04'	75°27'
14-40	80	4/3 1438 : 4/5 0930	42.9	15° 02'	75°32'

*depth of water column in meters.
**total deployment time in hours.
+traps deployed on same mooring line.

Table 3. Tow number, date, sampling time, and location of plankton samples collected off the Peru coast.

Sample	Date(81)	Time (local)	Latitude (S)	Longitude (W)
ZOOPLANKTON				
23-25	3/18	2117-2232	15° 10'	75° 35'
34-40	3/21	0000-0339	15° 13'	75° 32'
57-64	3/25	2240-0310	15° 14'	75° 30'
67-A, B*	3/26	2130-2234	15° 13'	75° 31'
76-80	3/29	2050-0005	15° 23'	75° 54'
88-89	4/1	1915-2110	15° 08'	75° 41'
107-108	4/5	2327-0055	15° 11'	75° 37'
ANCHOVY FECAL PELLETS**				
57-62	3/25	2240-0223	15° 14'	75° 30'

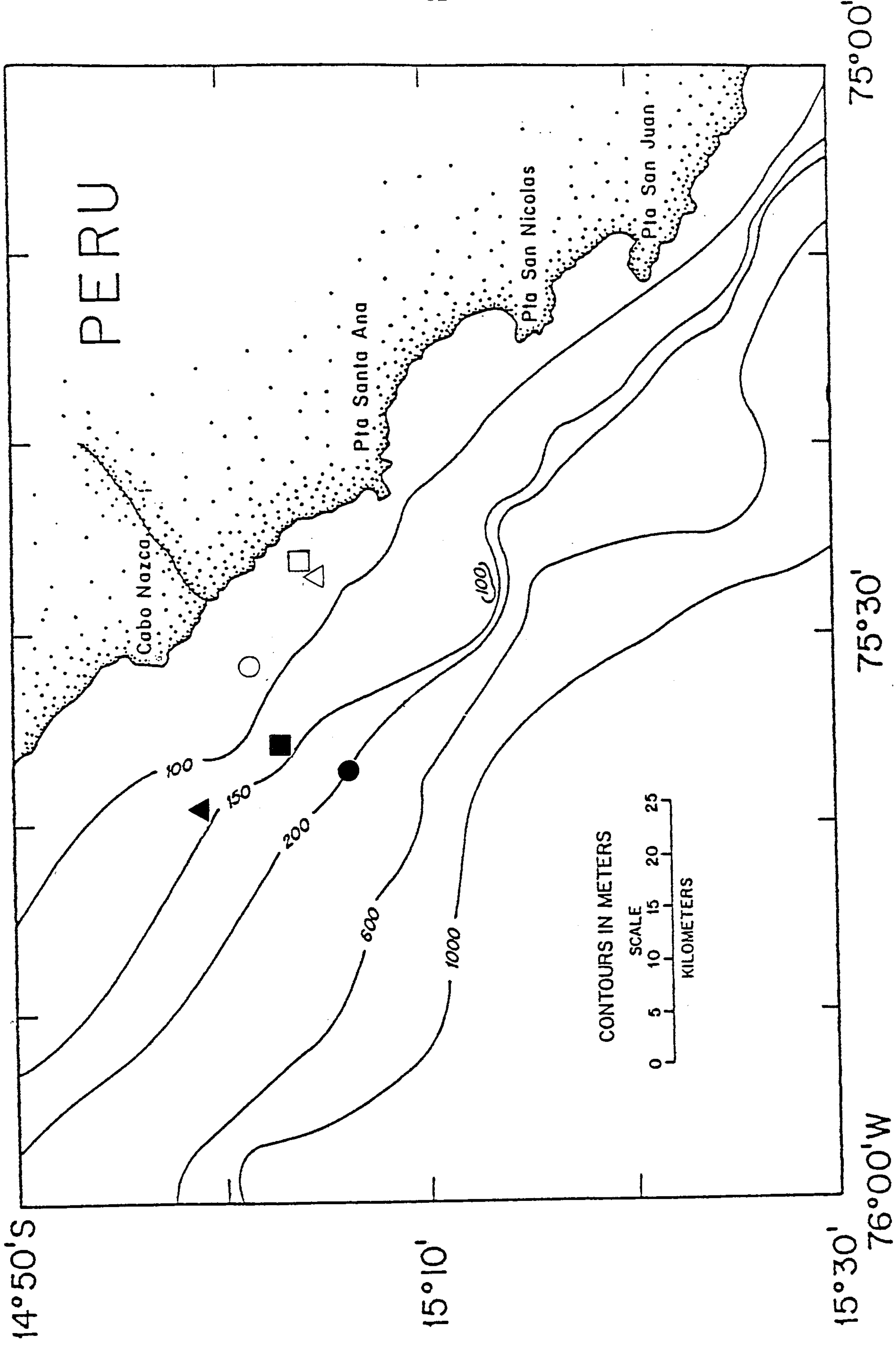
*sample split between two chambers.
**collected with zooplankton net towed at 25 m.

surface at 1-2 knots. All collections were made between 1900-0400 hr local time. Immediately after the nets were brought on board, the zooplankton were poured through a 2 mm sieve to remove small particles, and transferred to holding chambers built after the design of La Rosa (1976). Pellets were collected after 8-10 hr, filtered, and stored as described above. During one collection period (Tows 57-62) spherical green fecal pellets 2-3 mm in diameter, attributed to the anchovy *Engraulis ringens*, were collected. These pellets were separated with forceps and frozen at -20°C for future analysis. Phytoplankton were sampled with a 52 μm mesh net towed 10 m below the surface. The cod end of the net was emptied directly into a glass jar and the contents frozen at -20°C. Sampling times and locations are given in Table 3.

RESULTS

Oceanographic Setting A detailed account of the hydrographic data at 15°S, 75°W is given in Gagosian *et al.*, (1980, 1982). Underway maps of chlorophyll-a and surface temperature display features characteristic of actively upwelling systems. Cold, nutrient rich waters are upwelled near the coast and slowly advected offshore. Surface water temperatures increased from approximately 16°C at the 100 m contour to approximately 19°C at the 1000 m contour (Figure 1). Chlorophyll-*a* values increased from approximately 2 μg/l at the 100 meter contour to greater than 14 μg/l at the 400 meter contour. Offshore values decrease to approximately 2 μg/l at the 1200 m contour. Gagosian *et al.* (1980) report an average primary production value of 4 gC/m^2 day for the sampling area in February - March 1978.

Figure 1. Sediment trap locations off the coast of Peru: (□) trap 1; (△) traps 2,5,8,11,12; (●) traps 3,4; (■) traps 6,7; (▲) trap 9; (○) trap 14.


PERU
Cabo Nazca
Pta Santa Ana
Pta San Nicolas
Pta San Juan
100
150
200
600
1000
(100)
CONTOURS IN METERS
SCALE
0
5
10
15
20
25
KILOMETERS
14°50'S
15°10'
15°30'
76°00'W
75°30'
75°00'

Suspended particulate matter The concentration of diatoxanthin (XXIII), diadinoxanthin (III), fucoxanthin (IV), peridinin (V), and fucoxanthinol (VI) in suspended particulate matter collected from depths of 3-1500 m is given in Table 4. These five pigments comprised greater than 95% of the total carotenoid pigments in the samples (Figure 2). In all samples the most abundant carotenoid was fucoxanthin, a pigment characteristic of Bacillariophyceae (diatoms). Surface ($\leq$10 m) water pigment concentrations varied by nearly two orders of magnitude, from 0.12 μg/l to 11 μg/l, and correlated with chlorophyll-a (Figure 3).

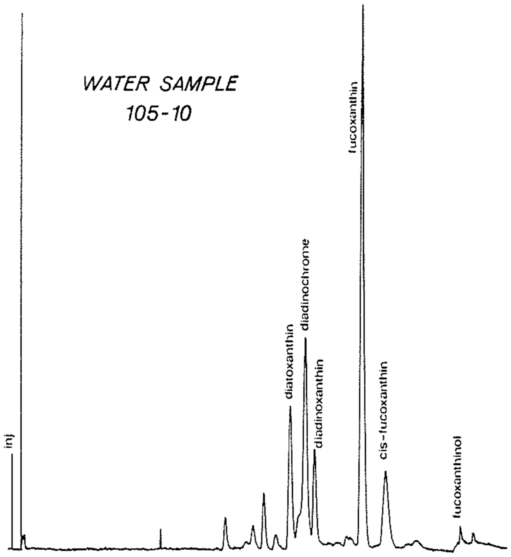
In Dinophyceae peridinin replaces fucoxanthin as the principal photosynthetic accessory pigment. The low concentrations and infrequent occurrence of this pigment in suspended particulate matter is consistent with the prior observation that dinoflagellates are relatively minor contributors to primary production in the upwelling area (Blasco, 1971; De Mendiola, 1981; Hendrickson et al., 1982). Diatoxanthin and diadinoxanthin, synthesized by both diatoms and dinoflagellates, were more frequently observed. When present, these pigments correlate well with fucoxanthin (Figure 4). The relative abundance of diatoxanthin and diadinoxanthin (10-30% of the total xanthophylls) is in accord with their abundance in cultured diatoms and dinoflagellates (See Table 1, Chapter 2; Riley and Wilson, 1967; Riley and Seger, 1969; Goodwin, 1970; Carreto and Catoggio, 1977; and references therein). These two pigments were most frequently measured in samples collected from surface waters $\leq$10 m, and in samples where the concentration of fucoxanthin was high. However, samples 44-10, 97-5, 104-10, and 104-20 do not contain any diadinoxanthin or diatoxanthin, even though concentrations of fucoxanthin in these

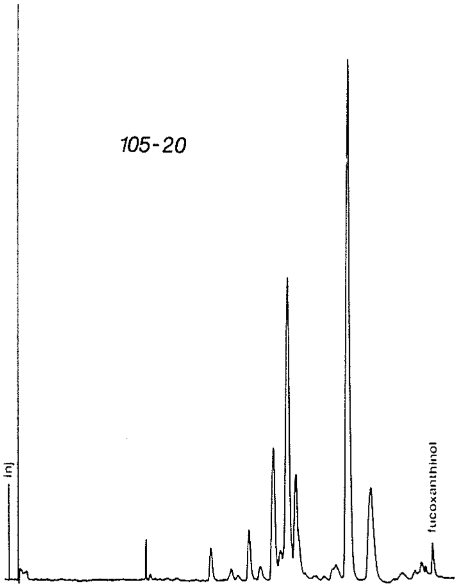
Table 4. Concentration (μg/ℓ) of phytoplankton xanthophylls is suspended particulate matter collected off the Peru coast.

Cast-Depth (m)	Diatoxanthin	Diadinoxanthin	Fucoxanthin	Peridinin	Fucoxanthinol
36-3	--	0.33	3.5	--	0.18
-5	0.02	0.03	0.19	0.11	0.01
-10	--	0.07	0.12	0.04	0.01
-25	--	0.13	0.27	0.12	0.04
-50	--	--	0.13	--	0.02
-150	--	--	0.04	--	0.01
44-3	1.5	1.8	6.2	--	0.10
-10	--	--	1.6	--	0.02
-25	--	--	0.15	--	0.01
-45	0.03	0.02	0.10	--	0.01
53-5	--	1.1	11.0	--	--
-10	0.91	1.3	6.0	0.61	0.20
-20	--	0.52	1.3	--	0.11
76-5	--	0.02	0.26	--	0.02
-30			≤ 0.002		
77-5	0.07	0.08	0.43	--	0.01
91-5	0.48	0.56	2.3	1.7	0.21
-10	0.00	0.89	3.1	1.3	0.30
-20	0.37	1.1	2.5	--	--
-100			≤ 0.001		
-350	--	--	0.02	--	0.01
97-5	--	--	1.5	--	--
99-5	0.43	0.66	1.7	--	0.29
-50	--	--	0.12	--	0.02
-200	--	--	0.02	--	0.004
104-10	--	--	0.59	--	0.01
-20	--	--	0.13	--	0.01
-30	--	--	0.28	--	0.01
105-10	0.12	0.26	0.44	--	0.01
-20	0.03	0.09	0.21	--	0.006
-30	0.07	0.01	0.27	--	0.01
106-10	1.4	2.7	5.5	--	--
-20	0.97	1.6	3.8	--	--
-30	1.2	2.8	5.8	--	--
10-1500	0.001	0.002	0.002	0.005	0.0006
117-1000			≤ 0.00005		

-- not detected (≤2 ng/l)

Figure 2. High pressure liquid chromatograms of carotenoids in suspended particulate matter samples 105-10 m, 105-20 m, and 105-30 m collected off the coast of Peru. Carotenoids separated on a 300x3.9 mm 5 μm Spherisorb amino column eluted with a 45 min linear gradient of 0-13% MeOH/THF (20/80) in hexane at 2 ml/min. After 55 min the eluant was stepped to 30% MeOH/THF in hexane for an additional 15 min.


WATER SAMPLE
105-10
inj
diatoxanthin
diadinochrome
diadinoxanthin
fucoxanthin
cis-fucoxanthin
fucoxanthinol


105-20
inj
fucoxanthinol

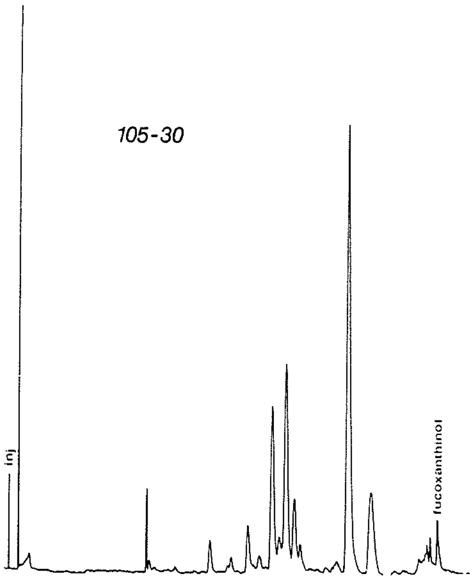

105-30
inj
fucoxanthinol

Figure 3. Correlation of fucoxanthin with chlorophyll-*a* in suspended particulate matter sampled in the euphotic zone (≤ 20 m) off the Peru coast. Chlorophyll-*a* determined fluorometrically immediately after sample collection.

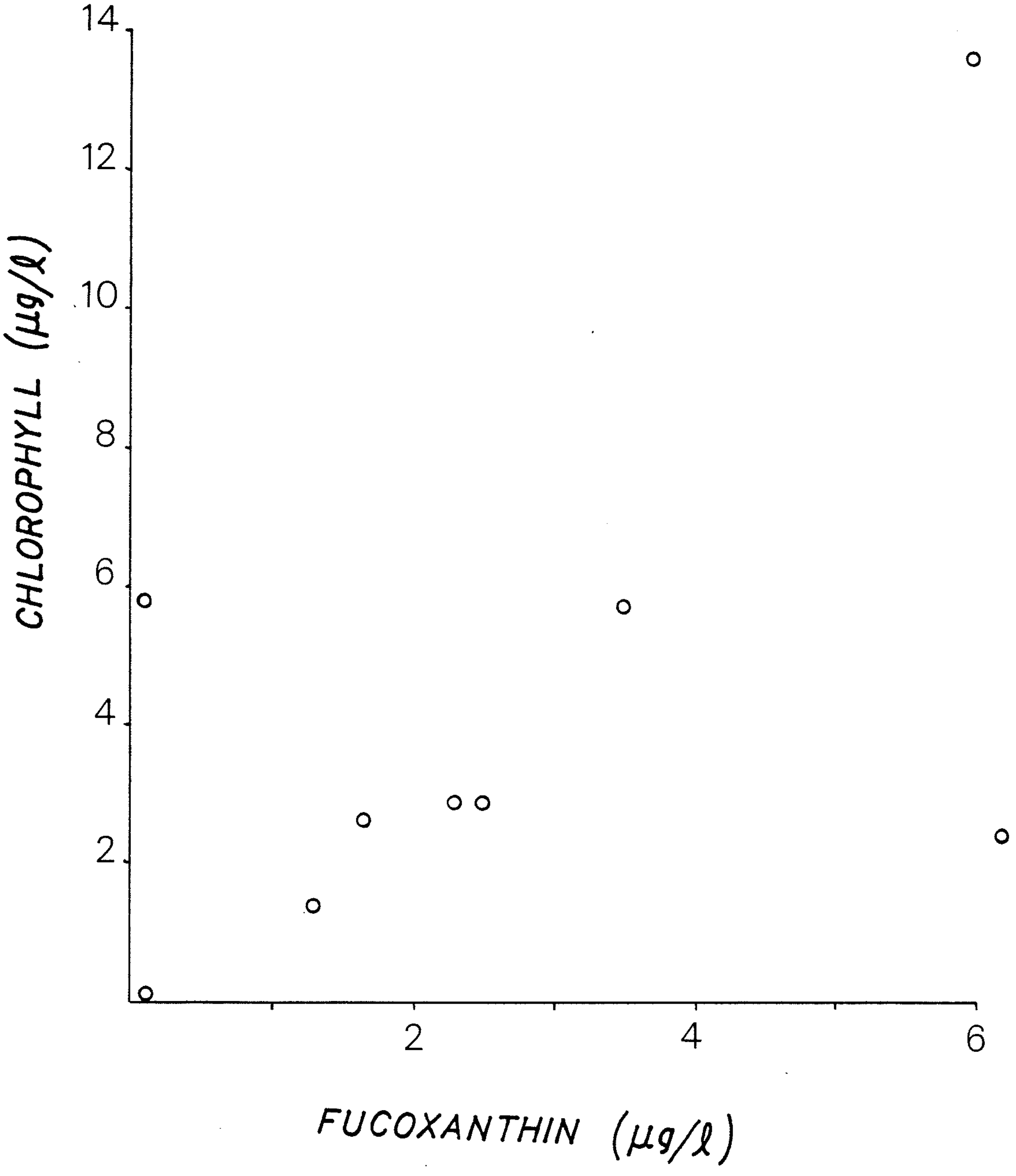

CHLOROPHYLL (μg/ℓ)
14
12
10
8
6
4
2
2
4
6
FUCOXANTHIN (μg/ℓ)

Figure 4. Correlation of fucoxanthin with diadinoxanthin in suspended particulate matter samples collected at depths ≤ 20 m. Shaded circles denote samples which contained significant contributions of peridinin. Only samples with non-zero values for diadinoxanthin are included.

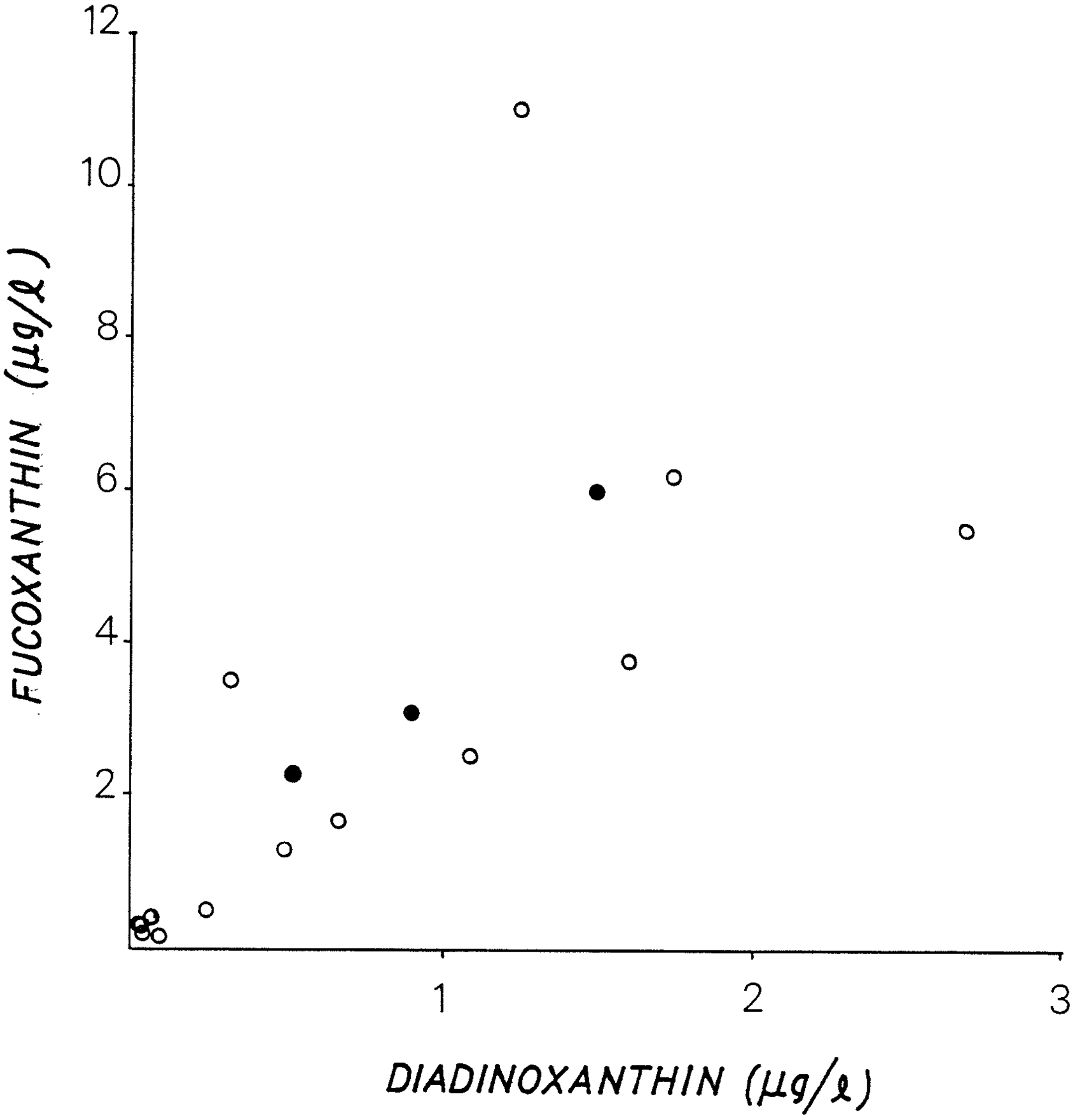
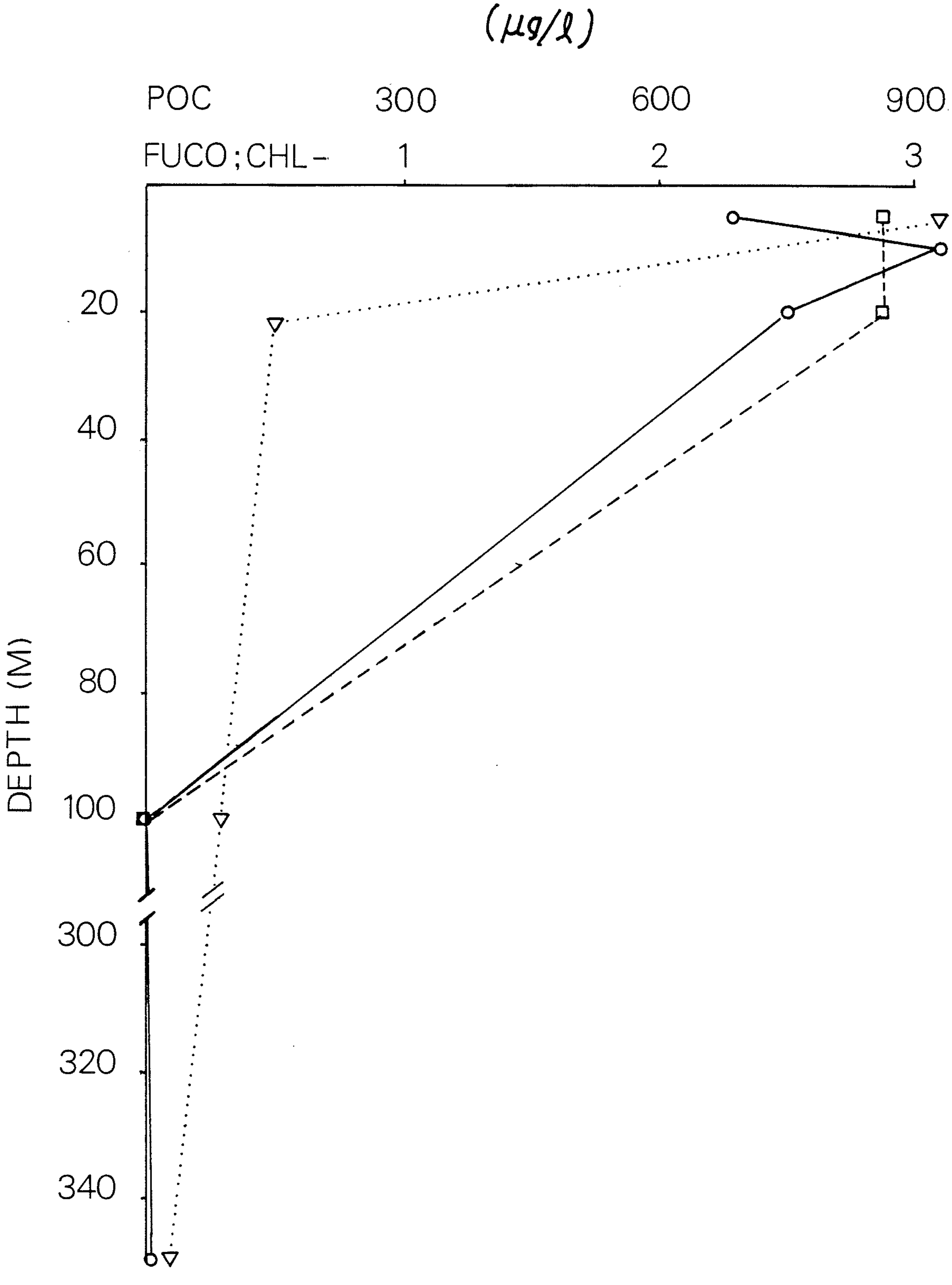

12
10
8
6
4
2
FUCOXANTHIN (µg/ℓ)
1
2
3
DIADINOXANTHIN (µg/ℓ)

Figure 5. Concentration of fucoxanthin (○—○), chlorophyll-a (□---□), and organic carbon (▽·····▽) in suspended particulate matter collected off the Peru coast as a function of depth for cast 91.


(μg/l)
POC
300
600
900
FUCO;CHL-
1
2
3
DEPTH (M)
20
40
60
80
100
300
320
340

samples is relatively high, between 0.13-1.6 μ g/l. The detection limit for individual pigments is approximately 2 ng/l. Therefore, using a minimim diadinoxanthin/fucoxanthin ratio of 0.1, diadinoxanthin should be measureable in all samples with concentrations of fucoxanthin $\geq$ 0.02 ug/l (Table 4). The patchy nature of the diadinoxanthin distribution is not an artifact of analytical sensitivity, but most likely results from variability in phytoplankton bloom conditions (Mandelli, 1969, 1972; Hagar and Stransky, 1970).

The concentration of all pigments decreases rapidly with depth, correlating particulate organic carbon (POC) and chlorophyll-a (Figure 5). The concentration of fucoxanthin in suspended particulate matter collected at depths $\geq$ 100 m ranged from 40 ng/l at 150 m (#36-150), to $\leq$ 50 pg/l (not detectable) at 1000 m (#117-1000). Approximately 2 ng/l of fucoxanthin was observed in the deepest sample collected at 1500 m.

Sediment Trap Samples Sediment trap samples 3-14 contained significant amounts of large (2-3 mm) green anchoveta fecal pellets. Trap samples 1 and 2 did not contain any recognizable fecal pellets. No zooplankton or zooplanktonic debris (molts, carcasses, fecal pellets) could be distinguished in any of the trap samples. The ratios: total carotenoids/gram dry weight of sample for sediment traps 6, 7, and 12 are in good agreement with the values from cultured marine phytoplankton (Table 5). This result supports the phytoplanktonic source of the trap material.

As part of the analytical procedure, carotenoids are separated from chlorophyll pigments by gel permeation HPLC (Chapter 5). This separation results in two fractions as monitored at 436 nm, one containing the total

Table 5. Milligrams total carotenoid per gram dry weight for sediment trap samples 6, 7, 12, phytoplankton, and zooplankton.

Trap	λ_{max}	Moles[+]	MW[++]	mg Carotenoid	mg Sample*	mg/gdw
6	455	4.2	618	0.26	133	2.0
7	453	7.4	626	0.46	105	4.4
12	455	0.88	614	0.054	35	1.5
Diatoms**						
Melosira sp.						3.3
Phaeodactylum sp.						4.8
Navicula sp.						1.2
Dinoflagellates***						
Glenodinium sp.						3.93
Amphidinium carterae						2.91
Gymnodinium splendens						1.02
Zooplankton[+++]						
Tow 88-89		24.1	596	1.44	745	1.93
Tow 99		9.5	596	0.57	360	1.58

[+] determined spectroscopically at λ_{max} assuming $\varepsilon = 10^5$. Values multiplied by 10^7.

[++] $\Sigma(\%)(MW)$, see Table 8.

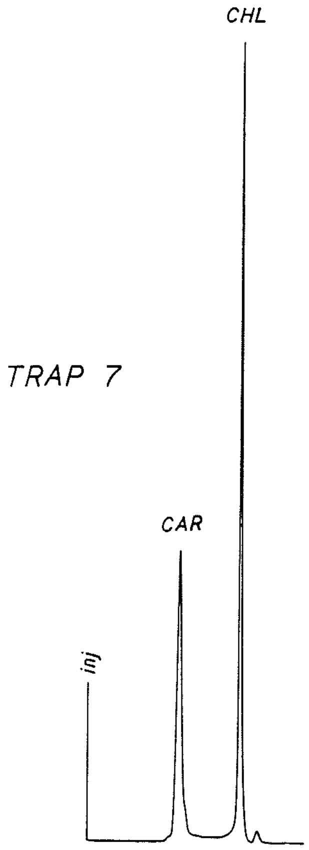
*dried overnight at 140°C.

**Hagar and Stransky, 1970

***Johansen *et al.*, 1974

[+++] Mixed sample, predominantly euphausids and copepods.

Figure 6. Gel permeation HPLC separation of total carotenoids and total chlorins in sediment trap sample 7 and fecal pellets from zooplankton collected in tow 30-40. Three (Waters Assoc.) 100 Åμ Styragel columns in series eluted with CH_2Cl_2 at 1.5 ml/min. Peaks detected at 436 nm.


CHL
TRAP 7
CAR
inj

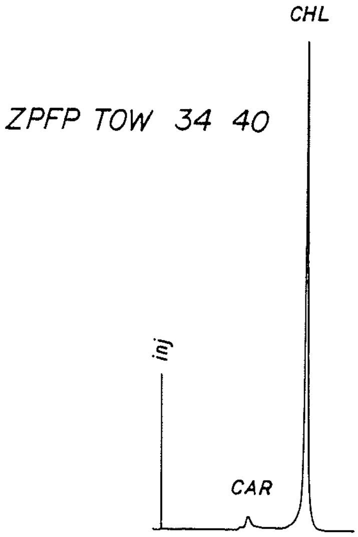

CHL
ZPFP TOW 34 40
inj
CAR

Table 6. Ratio of total carotenoids/total chlorins for suspended particulate matter, sediment trap, and zooplankton fecal pellet samples.

Sample	Car/Chl
Suspended Particulate Matter	
36-25	0.22
36-50	0.08
44-10	0.07
44-25	0.10
4-45	0.10
76-5	0.15
76-30	0.08
77-5	0.29
104-10	0.06
-20	0.07
-30	0.07
105-20	0.20
-30	0.14
	00.10 ±0.05
Sediment Traps	
2	0.32
3	0.01
4	0.13
6	0.93
7	0.37
8	0.09
11	0.05
14	0.18
	0.26 ±0.21
Zooplankton Fecal Pellets	
34-40	0.02
57-64	0.01
67a	0.04
67b	0.01
68-89	0.02
107-108	0.03
	0.02 ±0.01

carotenoids (TCa), and another containing total chlorins (TCh) (Figure 6). The ratio: area of carotenoid peak:area of chlorin peak (TCa/TCh) is directly proportional to the molar ratio of these two compound classes in the sample (Area $\propto$ [conc.]; $A_1/A_2 = (\varepsilon_1[\text{conc.}])_1/(\varepsilon 2[\text{conc.}])_2$. Changes in this ratio between suspended particulate matter and sediment trap samples indicates a selective removal of one class of pigments over the other. Table 6 presents TCa/TCh ratios for thirteen suspended particulate matter and eight sediment trap samples. Values for suspended particulate matter ranged from 0.07 to 0.29, with an average of 0.10±0.05. Sediment trap samples varied more widely, from 0.01 to 0.93, with an average of 0.26±0.21.

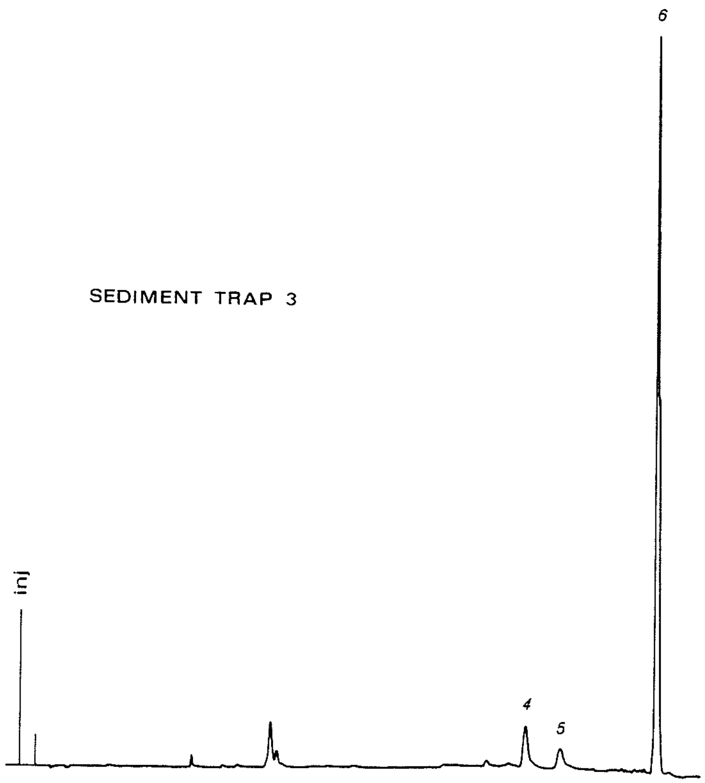
The relative abundance of diatoxanthin, diadinoxanthin, fucoxanthin and fucoxanthinol from twelve trap samples is given in Table 7. Interestingly, no peridinin was detected in any of the trap samples, even though this pigment was detected in suspended particulate matter during the deployment of traps 5, 6, 7, 11, and 12. The quantitatively most abundant pigments are fucoxanthin and fucoxanthinol. Together these pigments constitute between 65-97% of the total pigments. Individually, the concentration of each pigment varied widely, between 6-85% for fucoxanthin and 2-91% for fucoxanthinol. The inverse correlation of these two pigments is consistent with the transformation of fucoxanthin to fucoxanthinol as proposed in the previous chapter. Diatoxanthin and diadinoxanthin were measured in all trap samples in approximately the same abundance measured in suspended particulate matter samples (0-30%; Table 4). Several unidentified xanthophylls were also present in the trap

Table 7. Concentration (as % total xanthophylls*) of phytoplankton pigments in sediment trap samples.

Trap #	Diatoxanthin	Diadinoxanthin**	Fucoxanthin	Fucoxanthinol
1	9	26	63	2
2	1	10	85	4
3	2	1	6	91
4	2	6	77	14
5	0	5	79	15
6	1	8	11	80
7	3	14	38	45
8	5	5	68	22
9	3	17	37	44
11	2	10	45	42
12	2	7	17	73
14	tr	4	24	71

*% total of diatoxanthin, diadinoxanthin, fucoxanthin, and fucoxanthinol.
**calculated as the sum of diadinoxanthin and diadinochrome.

Figure 7. High pressure liquid chromatograms of sediment trap samples 3 and 9. Compound identification: 1) diatoxanthin, 2) diadinochrome, 3) diadinoxanthin, 4) all-trans fucoxanthin, 5) cis-fucoxanthin, and 6) fucoxanthinol. Conditions given in Figure 2 legend.


SEDIMENT TRAP 3

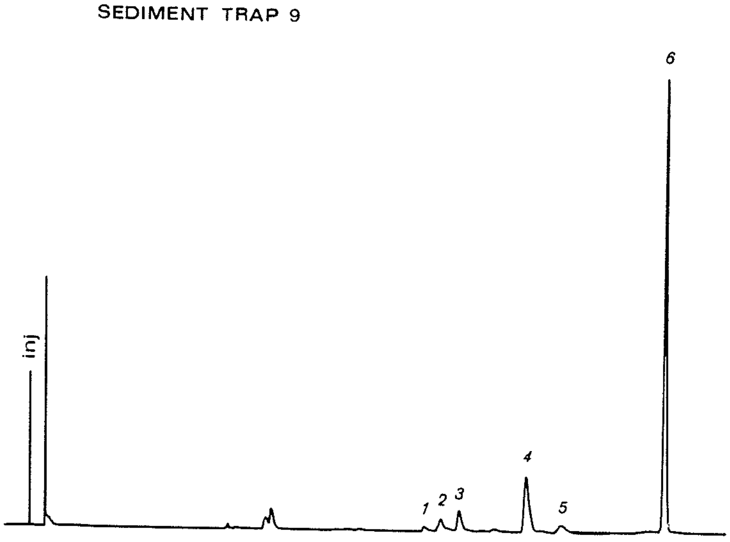

SEDIMENT TRAP 9

Figure 8. High pressure liquid chromatograms of A) zooplankton fecal pellet sample 76-80 and B) whole zooplankton from tow 107-108. Compound identifcation: 1) astacene or astaxanthin ester, 2) fucoxanthin 5'-dehydrate, 3) peridinin 5'-dehydrate, 4) astaxanthin, 5) fucoxanthin, 6) fucoxanthinol 5'-dehydrate, 7) peridinin, 8) peridininol 5'-dehydrate (?), 9) fucoxanthinol, and 10) peridininol (?). Conditions given in Figure 2 legend.

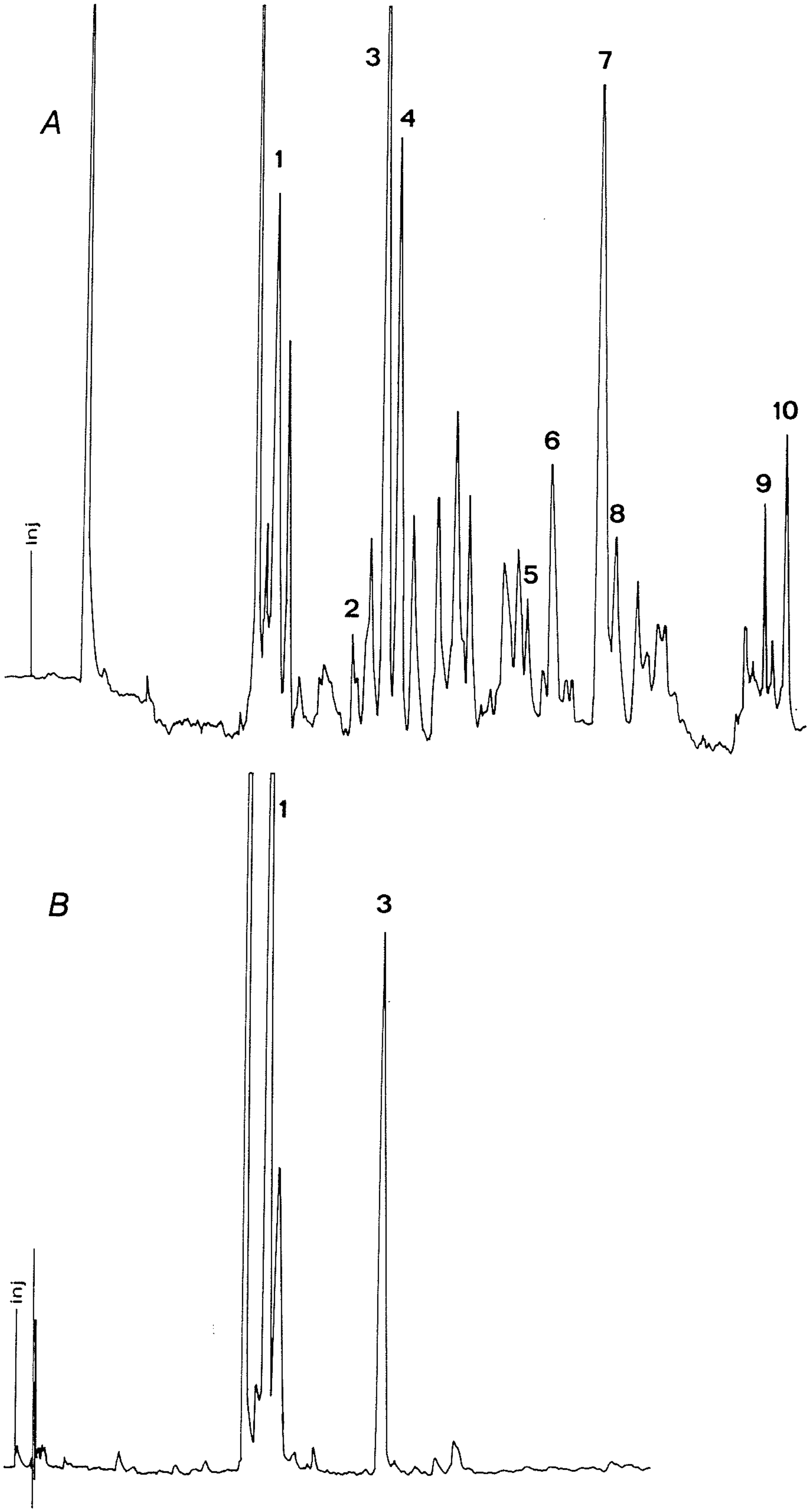

A
inj
1
2
3
4
5
6
7
8
9
10
B
inj
1
3

Table 8. Phytoplankton-derived pigments in zooplankton and anchovy fecal pellet samples.

Sample	Fucoxanthin 5'-dehydrate	Peridinin 5'-dehydrate	Diatoxanthin	Diadinoxanthin *	Fucoxanthin	Fucoxanthinol 5'-dehydrate	Peridinin	Fucoxanthinol	Peridininol
23-25	39	0	0	5	49	0	0	10	0
34-40	9	0	0	6	83	0	0	2	0
57-64	7	0	0	7	80	0	0	7	0
67a	10	0	0	2	70	+	0	19	0
67b	21	0	0	0	76	+	0	3	0
76-80	**	24	7	15	4	9	30	5	6
88-89	14	0	2	11	48	6	0	20	0
107-108	15	25	?	6	4	2	38	4	5
Anchovy	2?	0	0	2?	48	0	0	49	0

*Sum of diadinoxanthin and diadinochrome

**Fucoxanthin 5'-dehydrate not completely resolved samples, but these pigments comprise only a small percentage of the total pigments (< 5%; Figure 7).

Zooplankton Fecal Pellets Eight samples of zooplankton fecal pellets were collected. The increased complexity of the carotenoid mixture in these samples relative to suspended particulate matter and sediment trap samples is illustrated in Figure 8. Phytoplankton-derived carotenoids were characterized by high concentrations of fucoxanthin and fucoxanthin 5'-dehydrate, and low values of diatoxanthin and diadinoxanthin (Table 8).

All samples contained high concententrations of fucoxanthin 5'-dehydrate. Samples with high concentrations of fucoxanthinol and fucoxanthin 5'-dehydrate also contained significant amounts of fucoxanthinol 5'-dehydrate. Peridinin and peridinin 5'-dehydrate (XXVIII) were observed in two samples: 76-80 and 107-108. The HPLC traces of these two samples also contained a peak that is tentatively identified as peridininol (XXIX) (Figure 8). The sample size was insufficient for mass spectral confirmation, consequently the assignment of this peak as peridininol rests on liquid chromatographic evidence alone.

After completion of the pellet experiments, subsamples of zooplankton from the upper chamber of the collector were collected for analysis. The carotenoid distribution of all zooplankton samples were identical, each contained only astaxanthin and a less polar carotenoid which coelutes with astacene (Figure 8). Astaxanthin is characteristic of marine crustacea, often comprising greater than 90% of the total pigments in these organisms (Liaaen-Jensen, 1978). Astacene is a base-catalyzed oxidation product of astaxanthin, and may be an artifact of sample storage. The fraction which coelutes with astacene also has a retention time characteristic of carotenoid esters. Fatty acid esters of astaxanthin are common to marine crustacea, however no standards for these compounds were obtained and co-elution could not be demonstrated. Astaxanthin and the peak corresponding to astacene (or astaxanthin esters) were also observed in some fecal pellet samples. Microscopic examination of the fecal pellet samples immediately after collection revealed the presence of small copepods which had passed into the chamber during the course of the experiment. The presence of astaxanthin pigments in the fecal pellet samples is attributed to these organisms.

Values of total carotenoids/total chlorins for zooplankton fecal pellet samples were an order of magnitude lower than those calculated for suspended particulate matter and sediment trap samples (Figure 6; Table 6). The low values suggest selective degradation of carotenoids with respect to chlorins, and reflect the highly transformed nature of the sample.

DISCUSSION

Carotenoids as tracers of water column recycling processes

Two parameters were studied as measures of organic matter recycling processes in the water column, the ratio: fucoxanthinol/total fucopigments, and the ratio: total carotenoids/total chlorins (as determined by gel permeation HPLC). Fucoxanthinol is a by-product of heterotrophic metabolism of phytoplankton. Therefore the ratio: fucoxanthinol/total fucopigments is a measure of the relative amount of metabolized phytoplankton material in a sample, high values being indicative of highly reworked material. Values for the ratio of fucoxanthinol/total fucopigments (f/F_t)for 30 suspended particulate matter, 12 sediment trap, and 8 fecal pellet samples collected off Peru are given in Table 9.

The only report of fucoxanthinol in actively photosynthesizing marine phytoplankton was made by Berger *et al.* (1977) who measured an f/F_t ratio of 0.04 for members of the algal class Haptophyceae grown in laboratory cultures. Phytoplankton of the class Haptophyceae do not contribute significantly to the primary production in the sampling area. However Liaaen-Jensen (1977, 1978) has proposed that fucoxanthinol is an intermediate in the biosynthesis of fucoxanthin and may be present at low levels in fucoxanthin-synthesizing marine phytoplankton. The ratio of f/F_t measured by Berger *et al.* (1977) for cultured phytoplankton is in good agreement with the average ratio of 0.045 ± 0.036 measured for suspended particulate matter samples collected in the euphotic zone ($\leq$20m) off Peru.

Vertical profiles taken through the euphotic zone show a smooth increase in the ratio of f/F_t with depth (Figure 9). Suspended particulate matter samples collected at depths $\geq$50 meters had an average f/F_t ratio of 0.17 ± 0.03, a factor of four higher than measured for surface waters. The high ratio in deep water samples is indicative of a more highly metabolized standing crop of suspended particulate matter below the euphotic zone.

The presence of metabolite quantities of fucoxanthinol in diatoms and dinoflagellates could account for the low fucoxanthinol/fucopigment ratios measured in surface water samples. Alternatively, the presence of

Table 9. Fucoxanthinol/total fucopigment (f/F_t) ratios for suspended particulate matter, sediment trap, and zooplankton fecal pellet samples collected off Peru.

Suspended Particulate Matter Samples

Cast-depth	f/F_t	Cast-depth	f/F_t	Cast-depth	f/F_t
10-1500	0.23	53-5	0.00	97-5	0.00
36-3	0.05	-10	0.03	99-5	0.00
-5	0.05	-20	0.08	104-10	0.02
-10	0.08	76-5	0.07	-20	0.07
-25	0.13	-30	--	-30	0.03
-50	0.14	77-5	0.02	105-10	0.01
-150	0.20	91-5	0.11	-20	0.03
44-3	0.02	-10	0.09	-30	0.04
-10	0.01	-20	0.00	106-10	0.00
-25	0.06	-100	--	-20	0.00
-45	0.09	-350	0.33	-30	0.00

Sediment Trap Samples

Trap #	f/F_t	Trap #	f/F_T
1	0.03	7	0.54
2	0.04	8	0.24
3	0.94	9	0.54
4	0.15	11	0.48
5	0.16	12	0.81
6	0.88	14	0.75
			0.46± 0.25

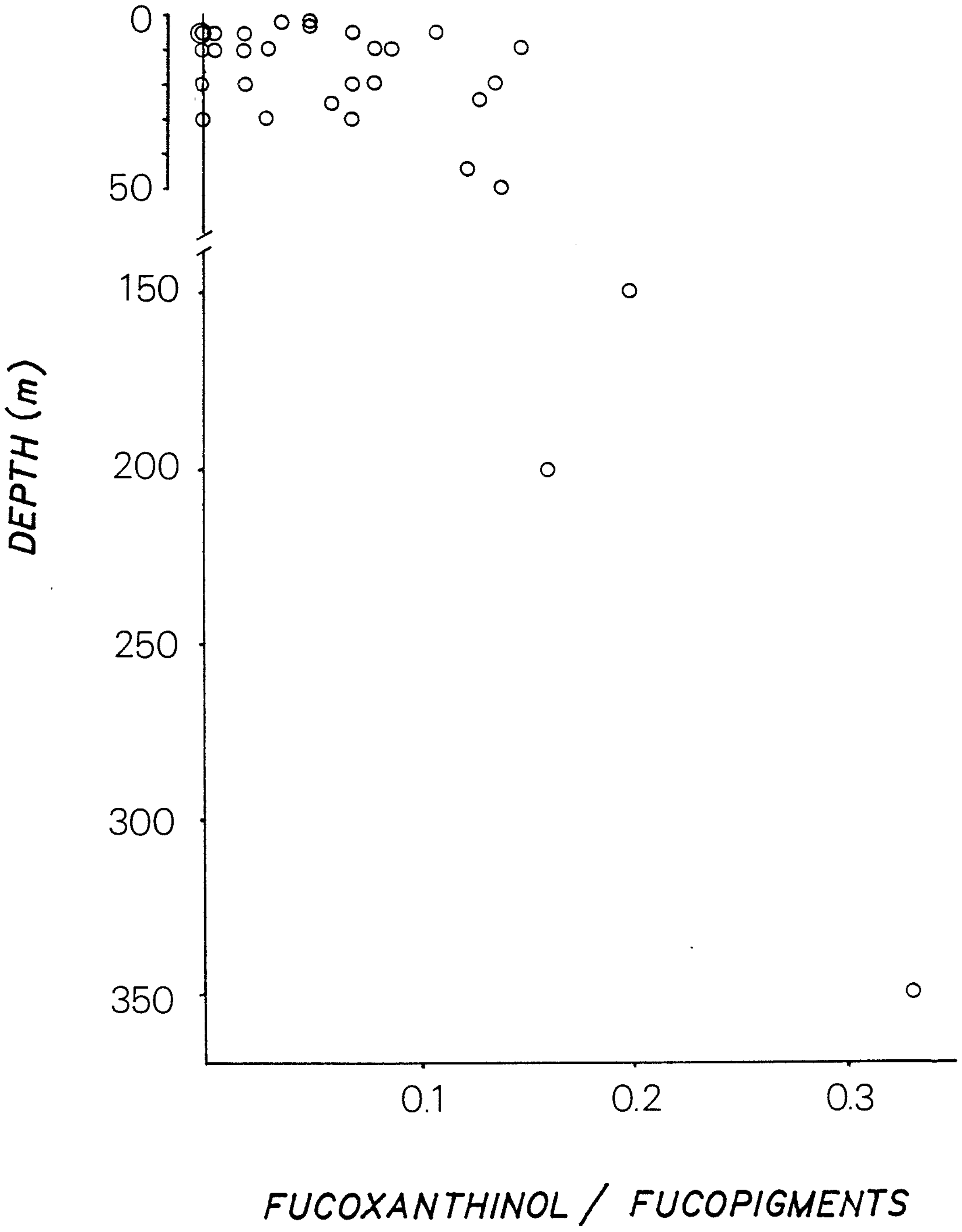
Zooplankton Fecal Pellet Samples

Tow #	f/F_t
23-25	0.10
34-40	0.02
57-64	0.08
67a	0.19
67b	0.03
76-80	0.31
88-89	0.23
107-108	0.16
	0.14± 0.08

fucoxanthinol may be attributed to the detrital background of senescent and metabolized phytoplankton cells. In the first case the concentration of fucoxanthinol should correlate with fucoxanthin. A plot of the concentration of fucoxanthinol against fucoxanthin for all samples ≤ 100 m is given in Figure 10. No correlation is observed. This result is consistent with the hypothesis that a major fraction of the fucoxanthinol in surface waters is contributed by detrital material.

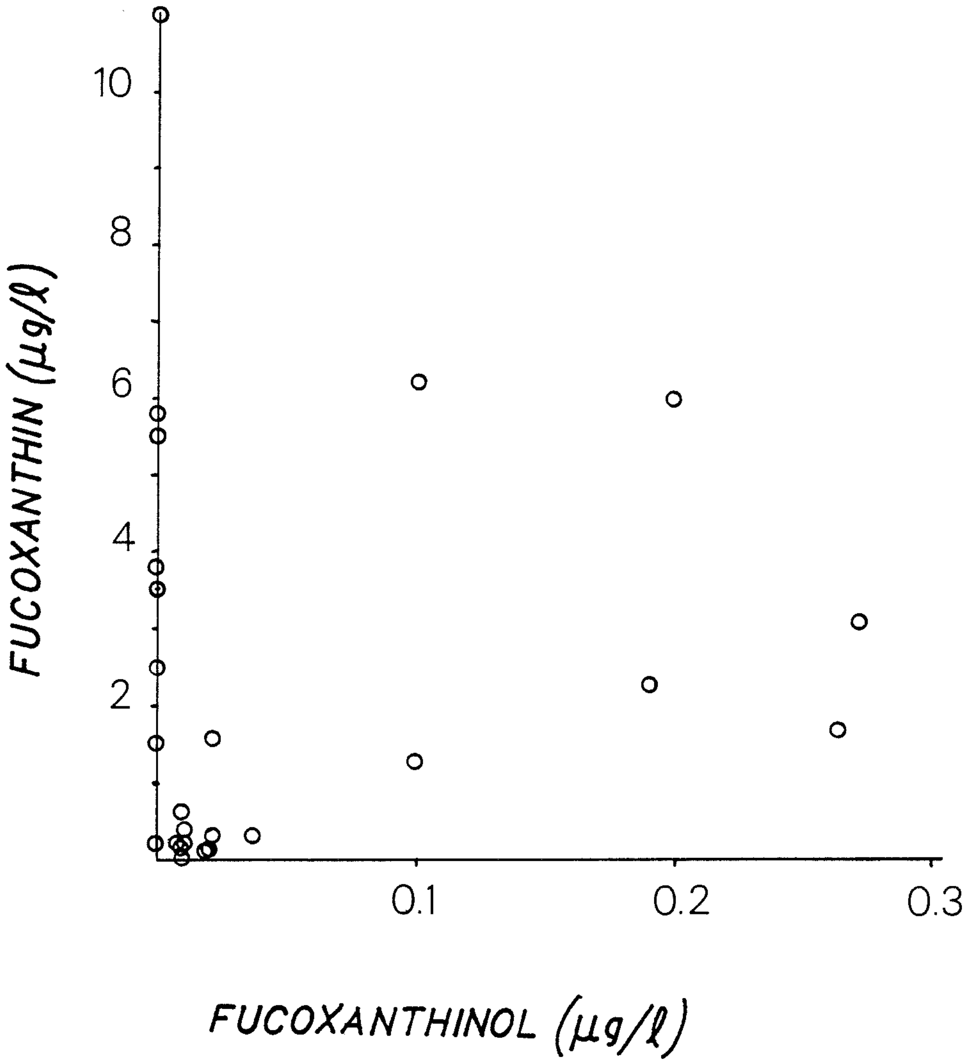
The ratio f/F_t for sediment trap samples averaged 0.46 ± 0.25, an order of magnitude higher than observed in suspended particulate matter collected from above the traps. These higher values reflect the higher percentage of metabolized material in the samples. The ratio (f/F_t) varies widely, from 0.03 for Trap 1 to 0.94 for Trap 3. The percentage of fucoxanthinol does not correlate with either trap depth or length of deployment, parameters usually associated with sample age (Lee and Cronin, 1982), but is more likely a function of the environmental and physiological factors that affect heterotrophic metabolism. Trap samples 1 and 2 were the only two samples which did not contain any recognizable fecal pellets. These traps were set nearshore in an area with characteristically low productivity (Figure 1). The absence of fecal pellets suggests that direct settling of phytoplankton is the primary source of material collected by these traps. This is reflected in the low fucoxanthinol/fucopigment ratio for these two samples (0.03 and 0.04), values more characteristic of suspended particulate matter than of other trap samples. Traps 3-14 all contained large quantitites of recognizable fecal pellets. The ratio f/F_t in these samples are a factor of 4-20

Figure 9. Increase in the ratio of fucoxanthinol/fucopigments (f/F_t) in suspended particulate matter with depth.



0
50
150
200
250
300
350
DEPTH (m)
0.1
0.2
0.3
FUCOXANTHINOL / FUCOPIGMENTS

Figure 10. Plot of fucoxanthin and fucoxanthinol concentration in suspended particulate matter collected from depths $\leq$ 100m off the Peru coast.


FUCOXANTHIN (µg/ℓ)
10
8
6
4
2
0.1
0.2
0.3
FUCOXANTHINOL (µg/ℓ)

times higher than in the samples from Traps 1 and 2.

Values for the ratio of f/F_t for zooplankton fecal pellets averaged 0.18 ± 0.11. This average is a factor of two lower than measured in sediment trap samples, but a factor of five higher than measured in surface water ($\leq$ 20 meters) suspended particulate matter. A sample of anchovy fecal pellets was also collected during Tows 57-62. The ratio of fucoxanthinol/fucopigments was 0.49, nearly identical to the average for sediment trap samples (0.46). This agreement is consistent with the visual observation that anchovy fecal pellets are the principal contributor of material to the trap.

The high concentrations of fucoxanthinol in zooplankton fecal pellets, anchovy fecal pellets, and sediment trap samples all support the hypothesis that fucoxanthin is metabolized to fucoxanthinol by heterotrophic organisms in the water column. The metabolism is not 100% efficient. Unmetabolized fucoxanthin accounts for approximately 50% of the total fucopigments in sediment trap and anchovy fecal pellet samples. Johannes and Satomi (1966) have reported the presence of intact, viable phytoplankton cells in zooplankton fecal pellets collected as part of laboratory feeding experiments. Staresinic _et al._ (1982) have reported that approximately 50% of the recognizable diatom valves in anchovy fecal pellets collected by sediment traps in the sampling area were intact and contained visible chloroplasts. These observations and the mean f/F_t ratio of 0.48 for sediment trap samples and anchovy fecal pellets collected as part of the present study suggests that approximately 50% of the carotenoids transported to the sediments in the Peru upwelling system are not degraded.

Shuman and Lorenzen (1975) have reported the quantitative degradation of chlorophyll-a to phaeophorbide-a in laboratory cultures of Calanus copepods. The study suggests the chlorin macrocycle is stable toward heterotrophic (zooplanktonic) metabolism. Conservation of chlorins would require that the ratio: total phytoplankton carotenoids/total chlorins (hereafter TPCa/TCh) will decrease or remain constant as phytoplanktonic material is metabolized. Since astaxanthin does not contribute significantly to the carotenoid mixture in either suspended particulate matter or sediment trap samples, the ratio: total carotenoids/total chlorins (TCa/TCh) for these samples is equivalent to the ratio: TPCa/TCh.

Values of TCa/TCh for suspended particulate matter and sediment trap samples averaged 0.10±0.05 and 0.26±0.21 respectively (Table 6). Due to the large scatter in the data, the factor of 2 increase in the ratio for sediment trap samples is not significant. The recycling processes that deliver phytoplanktonic material to the sediment traps do not efficiently recycle carotenoids to non carotenoid compounds (i.e. CO_2). This conclusion is supported by the average 2.6 mg total carotenoids/g dry weight measured for sediment traps 6, 7, and 12 reported in Table 5. Values of 1-5 mg total carotenoid/gram dry weight are typical of cultured diatoms and dinoflagellates (Goodwin, 1970; Johansen et al., 1974).

Included in Table 7 are TCa/TCh ratios for six zooplankton fecal pellet collections. Values are an order of magnitude lower than observed in either suspended particulate or sediment trap samples. Astaxanthin does contribute significantly to the carotenoids in some zooplankton fecal pellet samples. Therefore, the ratio of TCa/TCh measured for these samples represent maximum values for TPCa/TCh. The low ratios indicate that zooplanktonic euphausiids and copepods are highly efficient at metabolizing carotenoids to non-carotenoid products. Using the average TCa/TCh of 0.10 for suspended particulate matter, and 0.02 for zooplankton fecal pellets, an average of 1 - (0.02/0.10)= 80% of the total phytoplankton carotenoids are lost during the course of the pellet collection experiment (8-10 hours). This value is comparable to the loss of 80% of the particulate amino acids produced in the surface waters as calculated by Lee and Cronin (1982) from sediment trap data collected at this same sampling site.

Transformation reactions of carotenoid pigments

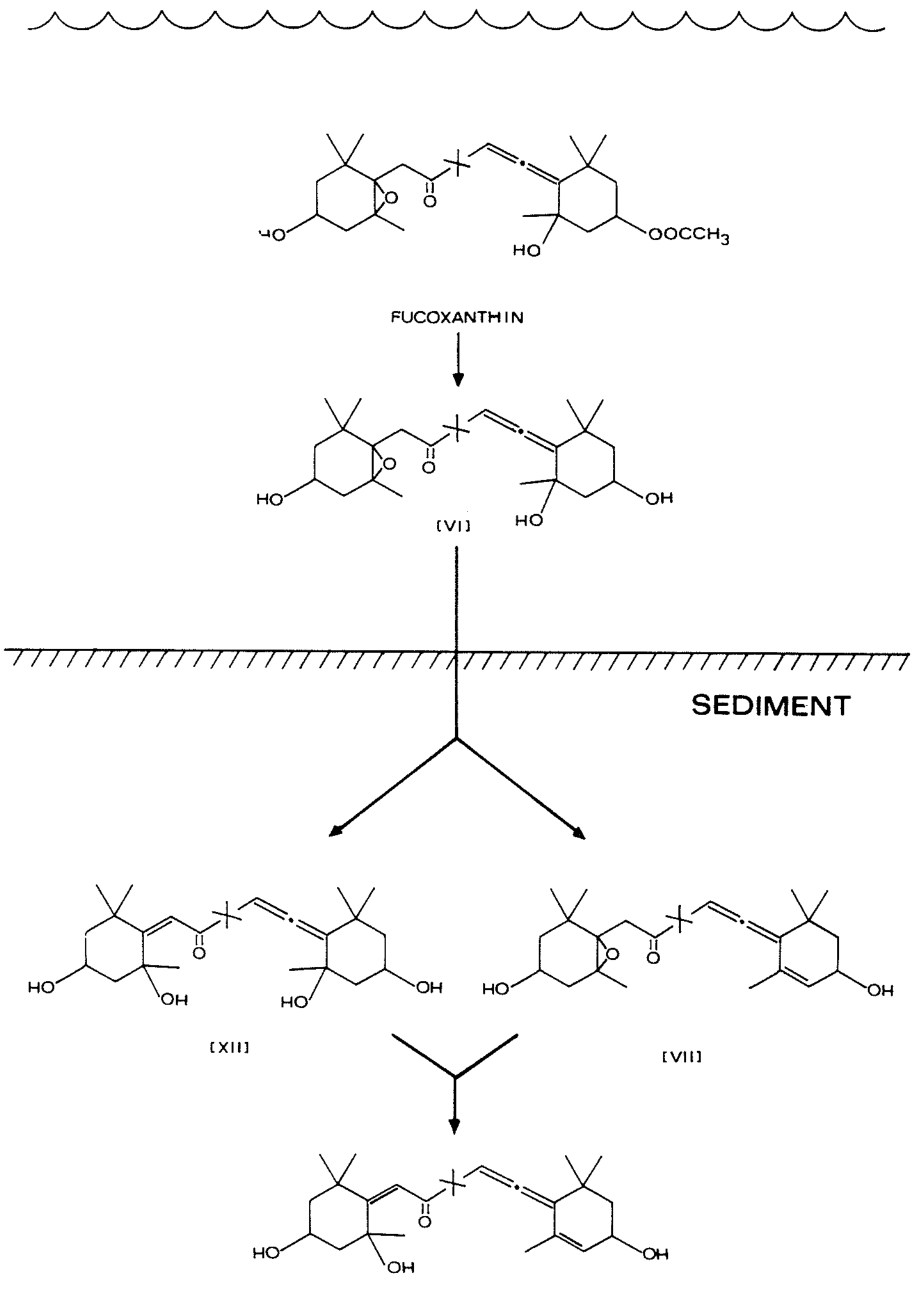
Three processes were suggested as being responsible for the fucoxanthin transformation products observed in Buzzards Bay sediment trap samples: 1) ester hydrolysis via heterotrophic metabolism, 2) dehydration via bacterial metabolism, and 3) epoxide opening via slow chemical reaction. There is a large body of evidence which documents the ester hydrolysis of chlorophyll-*a* to phaeophorbide-*a* by zooplanktonic heterotrophs (Currie, 1962; Patterson and Parsons, 1963; Barrett and Jeffrey, 1964, 1971; Moreth and Yentsch 1970; Shuman and Lorenzen, 1975; Jeffrey 1976). An analogous hydrolysis of the acetate ester in fucoxanthin was proposed by Glasko *et al*. (1969) to explain the high concentrations of fucoxanthinol isolated from the coelomic epithilieum of the sea urchin *Paracentrotus lividus* .

These studies and the results of the present set of experiments support the concept of metabolic hydrolysis of phytoplanktonic carotenoid esters to free alcohols at a rate determined by zooplankton metabolism of primary productivity. In contrast to the Buzzards Bay sediment trap samples, no significant concentrations of opened epoxides (iso-compounds) or dehydrates were observed in any of the Peru sediment trap samples. Therefore, it is proposed these transformations result from processes occurring at the sediment-water interface and/or in surface sediments.

The distribution of fucopigments in zooplankton fecal pellets was unique among the three types of samples studied in the Peru upwelling region in that significant concentrations of fuco-dehydrates were measured (Table 8). Fuco-dehydrates were also observed in Buzzards Bay sediment trap samples. Acid catalyzed chemical dehydration, and biochemically mediated enzymatic dehydration were proposed as potential formation processes. In the Buzzards Bay sediment trap samples a biochemically mediated dehydration was considered to be more favorable due to the co-existence of dehydrates with the unrearranged 5,6 epoxide diadinoxanthin. This epoxide rearranges rapidly in the presence of acids to the isomeric 5,8 furanoxide. A biochemically mediated dehydration pathway is substantiated by the coexistence of high concentrations of fuco-dehydrates and unrearranged diadinoxanthin in the fecal pellet samples from Peru. In addition, the short time scale of the collection experiments (< 10 hr), and the the fact that dehydrates were not observed in any of the Peru sediment trap samples suggests that the transformation results from a very specific biochemical process unique to the zooplanktonic fecal pellet samples.

Carotenoid metabolism by marine crustacea has not been extensively studied, however the results to date support the general principle established in studies of other higher heterotrophs that organisms

Figure 11. Proposed pathway showing ester hydrolysis of fucoxanthin (IV) to fucoxanthinol (VI) in the water column. Dehydration and epoxide opening to isofucoxanthinol 5'-dehydrate (VIII) occurs over longer time scales in the surface sediment.


HO
OOCCH$_3$
HO
FUCOXANTHIN
HO
OH
HO
[VI]
SEDIMENT
HO
OH
HO
OH
[XII]
HO
OH
[VII]
HO
OH
OH

ISOFUCOXANTHINOL 5'-DEHYDRATE

incapable of de-novo biosynthesis can only oxidize, not dehydrate, dietary carotenoids (Gilchrist and Lee, 1976; Castillo and Lenel, 1978; Castillo, 1980; Castillo et al., 1980 and references therein). On this basis, dehydrates in Buzzards Bay sediment trap samples and zooplankton fecal pellets from Peru are attributed to bacterial metabolism. Two observations support a bacterial source for the fuco-dehydrates in the fecal pellet samples. First the concentration of dehydrates does not correlate with the concentration of fucoxanthinol. The independent behavior of the two transformation products indicates the processes responsible for their formation are decoupled. Second, dehydrated fucopigments were not observed in either sediment trap samples or in anchovy fecal pellets collected off the Peru coast. It seems unlikely that metabolic dehydration would be restricted to zooplankton and not common to other higher heterotrophs, as is ester hydrolysis.

The high concentration of fuco-dehydrates and short duration of the fecal pellet collection experiments demonstrates that dehydration can occur on a time scale of <0.001 yr. Like ester hydrolysis, the rate of transformation is determined by the rate of heterotrophic metabolism of phytoplankton material. However, ester hydrolysis is characteristic of metabolism by higher heterotrophs, such as fish and zooplankton, whereas dehydration appears to be characteristic of metabolism by bacteria. Therefore, the ratio of fuco-dehydrates/total fucopigments is indicative of the fraction of bacterially metabolized material.

Opened epoxides (iso-compounds) were not detected in any suspended particulate matter, sediment trap, or fecal pellet samples. The high percentage of biologically reworked material in sediment trap and fecal pellet samples indicates that epoxide opening is not a metabolically mediated transformation. Isofucoxanthinol and Isofucoxanthinol 5'-dehydrate are the second and third most abundant carotenoids in Buzzards Bay sediment trap samples. These samples have a calculated age of ≤ 10 yr. Hence epoxide opening has a timescale T on the order: $0.01 \leq T \leq 10$ yr. In Buzzards Bay sediment trap samples the relative abundance of opened epoxides and dehydrates were approximately the same, requiring a similar time scale of formation. Therefore, bacterial dehydration of fucoxanthin in Buzzards Bay surface sediments is a factor of $10 - 10^4$ slower than in the fecal pellet collection experiments.

The transformation pathway illustrated in Figure 11 suggests that carotenoids structurally similar to fucoxanthin should undergo parallel transformation reactions. This hypothesis is confirmed by the presence of a parallel set of peridinin transformation products in fecal pellet samples 76-80 and 107-108 (Table 9). Both samples contained fucoxanthin, fucoxanthinol, and fucoxanthin 5'-dehydrate and the structural analogues peridinin, peridininol, and peridinin 5'-dehydrate.

CONCLUSIONS

The results of the Peru sediment trap experiments demonstrate that carotenoids are rapidly degraded and transformed in the oceanic water column. Specific recycling processes yield characteristic products, hence a detailed study of the carotenoid distribution in water column particulate matter can provide information on the rates, mechanisms, and efficiency of organic matter recycling processes. Carotenoid esters, synthesized by marine phytoplankton, are hydrolyzed to free alcohols by zooplankton and other higher heterotrophs at a rate determined by the turnover of primary productivity. Approximately 50% of the material collected in sediment traps is undegraded. This material is thought to supply a large fraction of the organic matter which is deposited to the sediments. Therefore, the presence of unmetabolized fucoxanthin in sediment traps and zooplankton fecal pellet samples suggests that the metabolism of phytoplanktonic material is not coincident with it's removal from the water column.

Dehydrates were significant components of zooplankton fecal pellets. However, the data suggest that dehydration and ester hydrolysis are decoupled. It does not appear that zooplankton metabolize fucoxanthin to dehydrates. The short duration of the collection experiments (< 0.001 yr), and the coexistence of unrearranged diadinoxanthin suggests that dehydrates are products of bacterial metabolism. Only trace quantities of dehydrates were measured in sediment trap samples. The primary source of material in the traps was anchovy fecal pellets. Traps were deployed for periods of 1-4 days, therefore assuming a sinking rate for anchovy fecal pellets of 1000 m/day (Staresinic, 1982; Staresinic et al., 1982), material delivered to the sediments by this mechanism will not be extensively reworked by heterotrophic microorganisms. Conversely, the lower sinking rate (10-100 m/day) and high bacterial activity associated with zooplankton fecal pellets suggests that bacterial transformation of material delivered to the sediments by this process may be significant.

Epoxide opening of fucoxanthin is not a significant water column reaction. No open epoxide (iso) transformation products were observed in any of the samples collected in the Peru upwelling experiment. As some of these samples were highly metabolized by anchovy, zooplankton, and bacteria, epoxide opening appears to be a chemically mediated transformation. The high concentrations of isofucoxanthinol and isofucoxanthinol 5'-dehydrate observed in Buzzards Bay sediment trap samples are associated with resuspended sediments. Using an average sedimentation rate of 0.3 cm/yr and depth of bioturbation of 3 cm, a time scale of 10 yr was calculated for these reactions. Since no opened epoxide isomers of either fucoxanthin or fucoxanthinol were measured in suspended particulate matter collected as deep as 1500 m, the turnover of particulate matter at this depth must occur on a relatively short time scale. A more precise understanding of the kinetics of epoxide opening in the deep sea is required before quantitative removal rates can be made.

Parallel transformations of fucoxanthin and the structurally analogous peridinin were observed in samples of zooplankton fecal pellets. Therefore the processes which transform fucoxanthin probably operate on a variety of compounds with similar functional groups. However, the reactions are specific to particular structural types; only the 5'-dehydrate of fucoxanthin was observed. At present it cannot be determined if the processes that transform carotenoids are the same which mediate similar reactions in other classes of compounds (i.e. the dehydration of sterols, hydrolysis of chlorophyll esters).

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CHAPTER 4

ISOLATION, SYNTHESIS, AND STRUCTURAL DETERMINATION OF FUCOXANTHIN AND FUCOXANTHIN TRANSFORMATION PRODUCTS.

INTRODUCTION

The diversity of naturally occurring carotenoids makes unambiguous structural identification of compounds isolated from geochemical samples a difficult, but necessary step in analysis. The problem is further complicated by the unavailability of standards and the low concentration of carotenoids in samples with respect to other lipids. This last characteristic prohibits or at least limits the use of NMR and IR spectroscopy, which require large quantities (100 μg to 10 mg) of relatively pure material.

The present chapter describes a method to identify fucoxanthin degradation products isolated from seawater particulate matter and sediments using thin layer or high pressure liquid chromatographic techniques. The approach is to synthesize proposed degradation products from fucoxanthin, treat these standards with a solution of iodine in toluene, and compare the resulting cis/trans equilibrium mixture with that derived from parallel experiments using carotenoid degradation products isolated from samples. Experiments show that the isomeric cis/trans equilibrium mixture is relatively easy to generate, yet highly specific for the degradation products (rearranged epoxides and ester hydrolysates) discussed in the previous two chapters.

EXPERIMENTAL

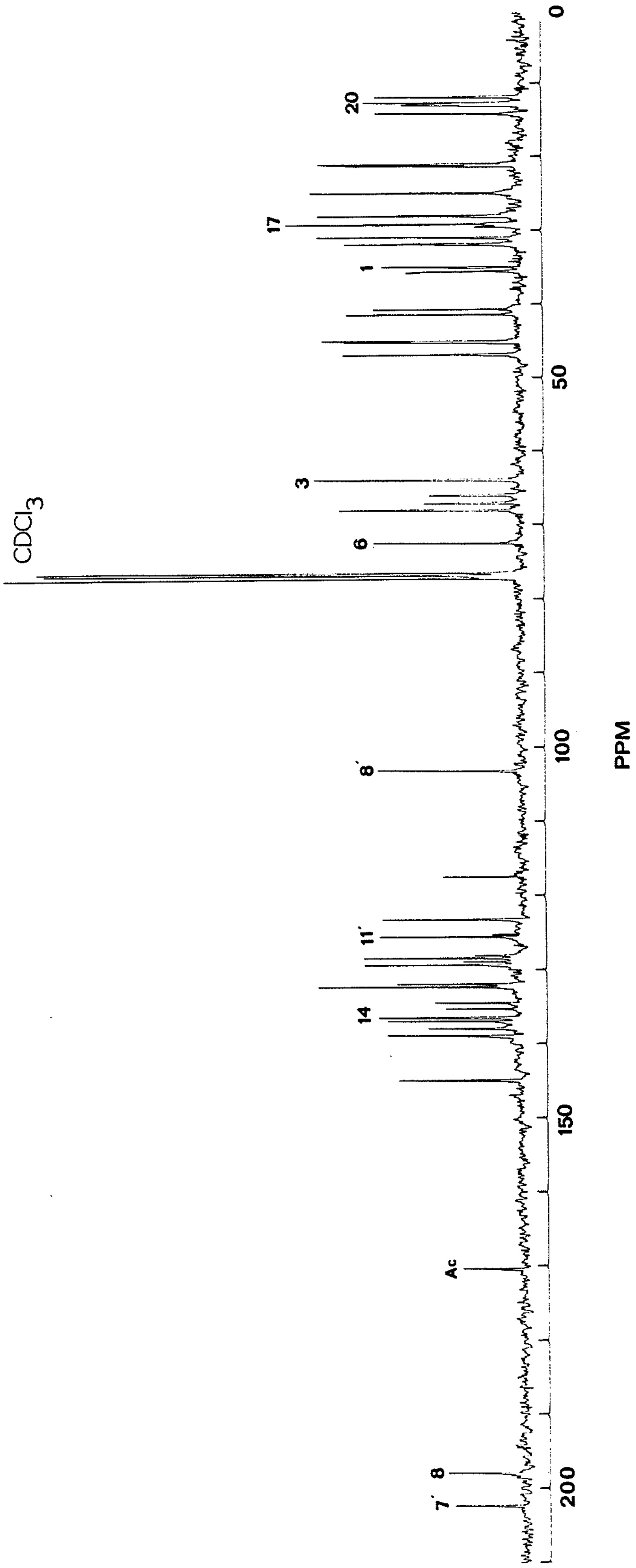
All solvents were distilled in glass (Burdick and Jackson). Unless otherwise stated, extracts were concentrated by vacuum rotary evaporation at 30°C - 40°C. Silica (60-200 mesh) for prep-column chromatography was washed with distilled water to pH 7, then activated at 150°C for 72 hr before use. Column dimensions are expressed as diameter x height. Thin layer chromatography was done on 0.20 mm silica plates pre-washed with MeOH. Unless otherwise stated all RR_f values are expressed relative to β-carotene using 20% acetone/hexane as the mobile phase. RR_f is defined as the ratio: [distance (in mm) of compound X from origin]/[distance from β-carotene to origin]. All yields are calculated from quantitative HPLC analysis of reactants and products. For internal consistency, molar extinction coefficients of $\varepsilon = 10^5$ l/mol cm at $_{max}$ was used in all yield determinations. This substitution may lead to errors of as high as ±20% (Bonnett *et al.*, 1969; Davies, 1976).

Visible spectra were recorded on a Cary 118 dual beam scanning spectrophotometer. ^{13}C-NMR spectra were recorded in $CDCl_3$ using a Brucker-270 multiple nuclei spectrometer at 67.93 MHz. ^{13}C resonance values are expressed as ppm relative to tetramethylsilane. Chemical ionization mass spectra were collected on either a Finnigan 3200 or Finnigan 4505 quadrupole mass spectrometer interfaced with an INCOS-2300 data system. Experimental details are given in Chapter 5 (Analytical Methods).

FUCOXANTHIN

i) Isolation: Fucoxanthin was isolated after the procedure of Bonnett *et al.* (1969). Briefly; Fucus sp. (Phaeophyceae) collected from the intertidal zone of Quisett Harbor, Falmouth, Massachusetts was air dried at 0^oC for three days. The dried algae (5.9 kg, dry weight) was homogenized in MeOH with a Waring blender, poured into a cotton bag, and immersed in a MeOH bath. After 24 hr, the bag was removed from the bath, allowed to drain, then re-extracted with fresh MeOH for an additional 24 hr. The dark brown MeOH extracts were combined, filtered through a bed of cotton wool, and concentrated to approximately 5% of the original volume. The MeOH concentrate was diluted with two volumes of saturated $NaCl_{(aq)}$, one volume of distilled H_2O, then extracted three times with an equal volume of Et_2O ether. The ether extracts were combined, back extracted with saturated $NaCl_{(aq)}$, concentrated, and traces of H_2O removed by repeated azeotrophic distillation with dry (5Å molecular sieve) toluene. The red brown residue was dissolved in 90% MeOH and extracted thrice with hexane to remove chlorophylls and carotenes. The MeOH layer, which contained fucoxanthin, was drawn off, concentrated to a small volume, diluted with saturated $NaCl_{(aq)}$, and extracted thrice with Et_2O ether. The ether layers were combined, evaporated to dryness, redissolved in hot toluene, and applied to a short (10cm x 5cm) column of alumina (activity grade II, neutral) packed in toluene. The column was washed with toluene to elute remaining carotenes and colorless lipids. The fraction containing fucoxanthin was eluted with 5% MeOH in toluene, evaporated to dryness, dissolved in toluene, and applied to a silica column (1.8cm x 32cm) The column was washed with toluene (2 vols.), and 10% acetone/hexane (2 vols.) to remove the remaining chlorophylls. Fucoxanthin eluted as a deep red band with 20% acetone/hexane. Fucoxanthin (IV) precipitated as deep-red needles from toluene/hexane with slow cooling to -20°C. Crystals were collected by filtration, washed with cold (-20°C) hexane, and dried under vacuum. Yield, approximately 2.8 g (0.05% dry weight) of crystalline fucoxanthin.

Figure 1. ^{13}C-NMR of fucoxanthin. Chemical shifts relative to TMS. Peak numbers refer to IUPAC carbon designations.


7'
8
Ac
14
11'
8'
CDCl3
6
3
1
17
20
200
150
100
50
0
PPM

ii) Properties of Fucoxanthin: λ_{max}(hexane) 476, 448, 426 (476, 450, 427 Bonnett et al., 1969), λ_{max}(acetone) 449, 425.

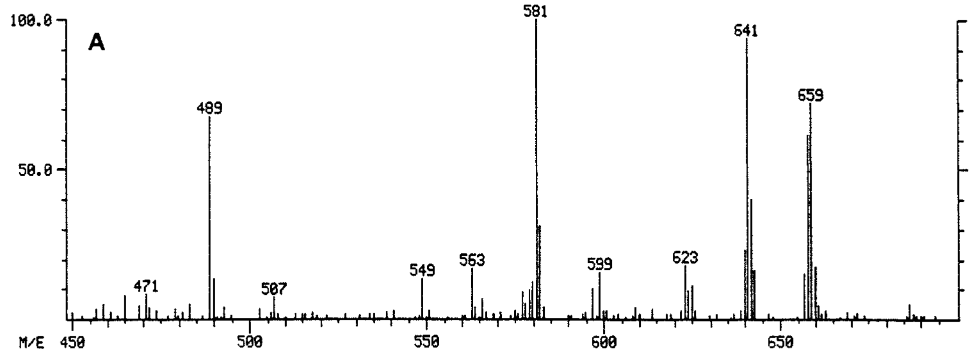
^{13}C-NMR (Figure 1) - ($CDCl_3$) δ 35.1 (C-1), 47.1 (C-2), 64.1 (C-3), 41.6 (C-4), 67.1 (C-5), 72.5 (C-6), 40.8 (C-7), 197.8 (C-8), 134.5 (C-9), 138.9 (C-10), 123.3 (C-11), 144.9 (C-12), 135.3 (C-13), 136.5 (C-14), 129.3 (C-15), 25.0 (C-16), 29.1 (C-17), 21.0 (C-18), 11.7 (C-19), 13.9 (C-20), 35.7 (C-1'), 45.3 (C-2'), 68.0 (C-3'), 45.4 (C-4'), 66.1 (C-5'), 117.5 (C-6'), 202.3 (C-7'), 103.3 (C-8'), 132.4 (C-9'), 128.4 (C-10'), 125.6 (C-11'), 137.0 (C-12'), 137.9 (C-13'), 132.0 (C-14'), 132.4 (C-15'), 31.1 (C-16'), 32.0 (C-17'), 28.1 (C-18'), 12.7 (C-19'), 12.6 (C-20'), 170.3 (C-1; acetate), 21.2 (C-2; acetate). The following assignments may be reversed (see discussion): (C-1, 1'), (C-2, 4), (C-5, 6), (C-16, 17, 18, 16', 17', 18'), (C-19, 20,19', 20'), and (C-2', 4').

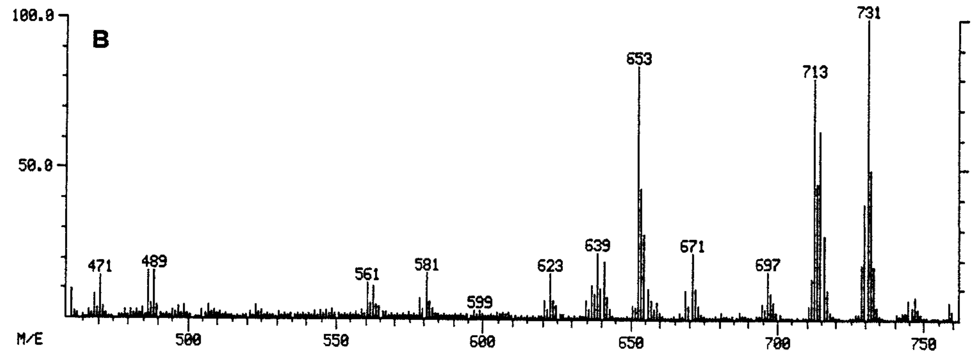
C.I.M.S. (Figure 2) - (M+1)= 659, (M+1-18)= 641, (M+1-18-18)= 623, (M+1-60)= 599, (M+1-60-18)= 581, (M+1-92)= 567, (M+1-60-18-18)= 563, (M+1-92-18)= 549, (M+1-92-60) = 507, (M+1-170)= 489, (M+1-170-18)= 471, (M+1-170-60)= 429.

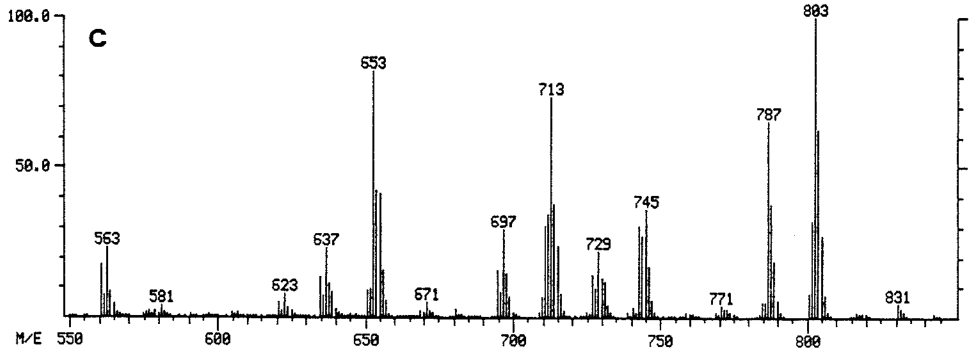
iii) Derivatization: Fucoxanthin Acetate - acylation with acetic anhydride yields a monoacetate (next section).

3-(trimethylsilyl) fucoxanthinyl ether (XXX) - Fucoxanthin (30 mg) in pyridine (1 ml) was added to 1 ml of hexamethyldisilazane (HMDS). After six hours the reaction was quenched with water and the silylated fucoxanthin extracted with CH_2Cl_2. Chromatography on silica TLC yielded two products: a major band RR_f(β-carotene) 0.59, and a very minor band which migrated with fucoxanthin (RR_f(β-carotene) 0.20). The major band was identified as the 3-silyl ether of fucoxanthin (XXX),

Figure 2. Chemical ionization mass spectra of A) fucoxanthin, B) 3-(trimethylsilyl) fucoxanthinyl ether, and C) 3,5'-bis(trimethylsilyl) fucoxanthinyl diether. Finnagan 4505 operarting conditions: methane reactant gas at 900 μm, ionization voltage 100 eV, ionization current 250 μA, conversion dynode 3 kV.


A
100.0
50.0
M/E
450
500
550
600
650
471
489
507
549
563
581
599
623
641
659


B
100.0
50.0
M/E
500
550
600
650
700
750
471
489
561
581
599
623
639
653
671
697
713
731


C
100.0
50.0
M/E
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700
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800
563
581
623
637
653
671
697
713
729
745
771
787
803
831

λ_{max}(hexane) 476, 449, 425. C.I.M.S.: (M+1)= 731, (M+1-16)= 715, (M+1-18)= 713, (M+1-18-16)= 697, (M+1-60)= 671, (M+1-60-16)= 655, (M+1-60-18)= 653, (M+1-90)= 641, (M+1-92)= 639, (M+1-60-18-16)= 637,(M+1-90-18)(M+1-92-16)= 623, (M+1-92-18)= 621, (M+1-72-60)= 599, (M+1-90-60)= 581, (M+1-90-60-16)= 563, (M+1-90-60-18)(M+1-92-60-16)= 561, (M+1-242)= 489, (M+1-242-18)= 471 (Figure 2). An aliquot of the monosilane was dissolved in two drops of THF, one drop of distilled H_2O, and 25 µl of glacial acetic acid. Reaction at room temperature for two hours yielded only one product (silica TLC) with RR_f(-carotene) identical to fucoxanthin.

3,5'-bis(trimethylsilyl) fucoxanthin diether (XXXI) - Fucoxanthin (1 mg) was dissolved in 1 ml of trimethylchlorosilane (TMCS)/pyridine (50/50, v/v). After 30 minutes the reaction was quenched by dropwise addition of water, and the pigments extracted into CH_2Cl_2. Chromatography on silica TLC gave one band, RR_f(β-carotene) = 0.99, corresponding to the 3,5'-disilyl ether (XXXI). 3,5'-*bis*(trimethylsilyl) fucoxanthinyl diether had: λ_{max}(hexane) 477, 448, 424, 400, λ_{max}(acetone) 449, 425. C.I.M.S.: (M+1)= 803, (M+1-16)=787, (M+1-16-16)= 771, (M+1-58)= 745, (M+1-60)= 743, (M+1-72)= 731, (M+1-58-16)= 729, (M+1-60-16)= 727, (M+1-90)= 713, (M+1-92)= 711, (M+1-90-16)= 697, (M+1-92-16)= 695, (M+1-72-60)= 671, (M+1-90-42-16)= 655, (M+1-90-60)= 653, (M+1-90-60-16)= 637, (M+1-92-60-16)= 635, (M+1-90-90)=623, (M+1-90-72-60)= 581, (M+1-90-90-60)= 563, (M+1-242)= 561, (M+1-242-16)= 545 (Figure 2). Silylation of fucoxanthin with trimethylsilylimidazole (TMS-Z)(Pierce 1970) and 3-(trimethylsilyl) fucoxanthinyl ether (from HMDS) with TMCS under conditions described above also yielded the 3,5'-*bis*(trimethylsilyl)

Table 1. Iodine catalyzed photoisomerization products of fucoxanthin and fucoxanthin 3-acetate. Retention times of cis isomers are normalized to the all-trans peak. Chromatographic conditions given in text.

FUCOXANTHIN

(all-trans) (ng/µl)	N*	RR$_t$									
10	6	0.36	0.48	0.74	0.78	0.87	1	1.18	1.23	1.30	1.44
5	2	0.36	0.48	0.73	0.77	0.87	1	1.18	1.23	1.31	1.44
2	2	0.36	0.48	0.73	0.77	0.86	1	1.17	1.22	1.30	1.43
1	2	0.36	0.49	0.74	0.78	0.87	1	1.18	1.24	1.31	1.44

FUCOXANTHIN 3-ACETATE

	N	RR$_t$					
from fucoxanthin	2	0.51	0.61	0.73	0.81	0.92	1
from fucoxanthinol	2	0.51	0.62	0.73	0.81	0.92	1

* number of runs. Calculated errors in retention times were within the analytical precision of the method (± 2%).

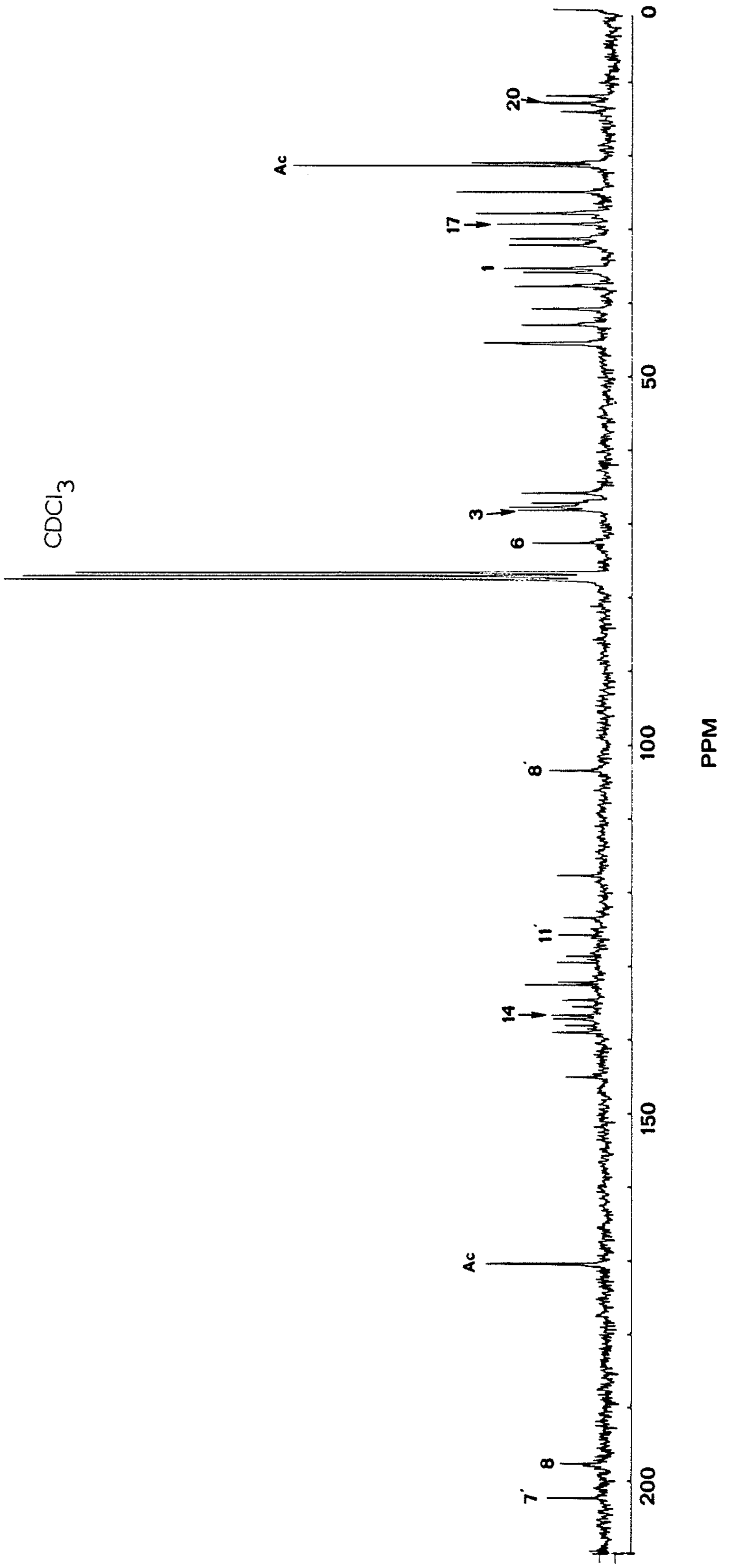
fucoxanthinyl diether. Acid hydrolysis of disilane synthesized from all methods yields fucoxanthin.

iv) Iodine Catalyzed Isomerization of Fucoxanthin: 1.07 mg of Iodine (resublimed) was dissolved in 100 ml of toluene and the solution degassed by sonication under vacuum for one hour. Aliquots (200 μl) were withdrawn and added to glass vials containing 0.2 μg, 0.4 μg, 1.0 μg, and 2.0 μg of fucoxanthin. The vials were sealed under N_2 and allowed to equilibrate in ambient (fluorescent artificial) light. After 12 hr aliquots were withdrawn for analysis. Nine cis isomers were separated by HPLC (300x3.9 mm, 5μm silica column, eluted with 12.5% B (B = MeOH/THF; 20/80, v/v) in hexane at 2 ml/min)(Table 1).

FUCOXANTHIN 3'-ACETATE

i) From Fucoxanthin: 0.1207g of (N,N)-4-dimethylaminopyridine (DMAP) was dissolved in 100 ml of pyridine. Fucoxanthin (96 mg) in 10 ml of 0.1 mM DMAP/pyridine was reacted at room temperature with 1 ml of acetic anhydride. After 15 minutes, 10 ml of CH_2Cl_2 and 10 ml of aqueous $CaCO_3$ (sat) were added to quench the reaction and neutralize excess acid. Pigments were extracted into CH_2Cl_2 and washed twice with an equal volume of aqueous $CaCO_3$(sat), and once with distilled H_2O. The extract was dried over Na_2SO_4, concentrated to a small volume, and traces of pyridine removed by repeated azeotrophic distillation with dry toluene. The crude product was purified by column chromatography on silica. The major red band was collected with the 10% acetone/hexane eluant. Chromatography on silica TLC yielded one band, RR_f (β-carotene)= 0.45. Fucoxanthin 3-acetate (XIII) crystallized as red needles from toluene/hexane. Yield, 75 mg (78%).

Figure 3. ^{13}C-NMR of fucoxanthin 3-acetate. Chemical shifts relative to TMS. Peak numbers refer to IUPAC carbon designations.


CDCl$_3$
Ac
20
17
1
3
6
8'
11'
14
Ac
8
7'
0
50
100
150
200
PPM

ii) From Fucoxanthinol: Acetic anhydride (10 μl) was added to 10 μg of fucoxanthinol (see next section for synthesis) in 100 μl of 0.1 mM DMAP/pyridine. The reaction was monitored by HPLC (20% B in hexane). Within 15 seconds of initiation, formation of fucoxanthin and fucoxanthinol 3-acetate was observed. After 20 minutes the reaction was quenched with aqueous $CaCO_3$(sat) and the product isolated as described in the previous paragraph. Approximately 12.5 μg of fucoxanthin 3-acetate (110% yield) was recovered. Iodine catalyzed photoisomerization yielded an equilibrium mixture identical to that observed in parallel experiments using fucoxanthin 3-acetate synthesized from fucoxanthin (Table 1).

iii) Properties of Fucoxanthin 3-Acetate: λ_{max}(hexane) 476, 448, 426, λ_{max}(acetone) 445. ^{13}C-NMR (Figure 3) ($CDCl_3$) 35.2 (C-1), 42.9 (C-2), 67.7 (C-3), 37.7 (C-4), 67.2 (C-5), 72.6 (C-6), 40.7 (C-7), 197.6 (C-8), 134.6 (C-9), 139.0 (C-10), 123.3 (C-11), 145.0 (C-12), 135.4 (C-13), 136.6 (C-14), 129.4 (C-15), 24.9 (C-16), 29.2 (C-17), 21.0 (C-18), 11.8 (C-19), 14.0 (C-20), 35.8 (C-1'), 45.4 (C-2'), 68.1 (C-3'), 45.5 (C-4'), 65.8 (C-5'), 117.6 (C-6'), 202.4 (C-7'), 103.4 (C-8'), 132.5 (C-9'), 128.5 (C-10'), 125.7 (C-11'), 137.1 (C-12'), 138.0 (C-13'), 132.1 (C-14'), 132.5 (C-15'), 31.2 (C-16'), 32.1 (C-17'), 27.8 (C-18'), 12.9 (C-19'), 12.7 (C-20'), 170.4 (C-1, acetate), 21.4 (C-2, acetate). C.I.M.S.: (M+1)= 701, (M+1-18)= 683, (M+1-60-18)= 623.

iv) Derivatization: Silylation - Reaction of fucoxanthin 3-acetate in pyridine with HMDS yielded no products (as determined by TLC) after 24 hr. A similar experiment using TMCS and TMS-Z (15 minutes) yielded 5'-(trimethylsilyl) fucoxanthin acetyl ether (XXXII): RR_f(β-carotene)=

0.95. λ_{max}(hexane) 478, 450, 425, λ_{max}(acetone) 445. Acid hydrolysis regenerated fucoxanthin acetate.

v) Thermal Isomerization: Fucoxanthin 3-acetate in CH_2Cl_2 was evaporated to dryness under a stream of N_2, redissolved in CH_2Cl_2, sealed under N_2, and allowed to equilibrate at room temperature overnight. HPLC analysis of the cis/trans equilibrium mixture is given in Table 5, Chapter 2, with the results of a parallel experiment using fucoxanthin 3-acetate synthesized from fucoxanthinol isolated from Buzzards Bay sediment trap #3.

vi) Iodine Catalyzed Isomerization: Approximately 2 μg of fucoxanthin 3-acetate was dissolved in 200 μl of 0.04 mM iodine/toluene (degassed). The solution was sealed under N_2, and allowed to equilibrate under ambient(fluorescent artificial) light. After 12 hr aliquots were withdrawn for analysis. Seven isomers were separated by HPLC (300x3.9 mm, 5 μm silica, eluted with 10% B in hexane)(Table 1).

FUCOXANTHINOL

i) From Fucoxanthin: Bonnett et al. (1969) synthesized fucoxanthinol by complete reduction of the 8-ketone and 3'-ester of fucoxanthin, followed by oxidation of the allylic 8-alcohol with 2,3-dichloro-5,6-dicyanoquinone (DDQ). An overall yield of 5.3% was reported. Nitsche (1974) repeated this synthesis using p-chloranil instead of DDQ to oxidize the 8-alcohol. The yield was not reported. The synthesis of Bonnett et al. (1969) is unsatisfactory for large scale preparation of fucoxanthinol; greater than 2 gm of fucoxanthin would be required to synthesize the approximately 100 mg needed for ^{13}C-NMR.

Consequently, alternative routes were explored. Reduction with NaH, $NaBH_4$, and $LiBH_4$ did not yield any measurable quantities of fucoxanthinol. Reduction with $LiAlH_4$ in ether for one hour yielded exclusively fucoxanthin-8-ol (XXXIII) and fucoxanthol (XXXIV). However, if the reaction is rapidly quenched (30 sec), a small quantity of fucoxanthinol is recovered. The presence of this intermediate suggests two competing pathways for fucoxanthol formation: fucoxanthin → fucoxanthin-8-ol → fucoxanthol, and fucoxanthin → fucoxanthinol → fucoxanthol; depending on the first reduction step. $LiAl(CH_3)_3H$ is a milder and more selective reducing agent than $LiAlH_4$, and it was felt that use of this reagent may increase the yield of fucoxanthinol (Brown, 1980).

$LiAlH_4$ (1.1097 g) was refluxed for four hours in 300 ml of dry (freshly distilled over Na metal) Et_2O. The ether solution (150 ml containing approximately 1 mmole $LiAlH_4$) was withdrawn and deactivated with 120 μl of MeOH (2.65 mmoles)(Brown and McFarlin, 1958; Brown and Deck, 1965). Fucoxanthin (400 mg) in ether (30 ml) was added to the $LiAl(CH_3)_3H$ solution. After five minutes the reaction was quenched by dropwise addition of 0.1% aqueous acetic acid (40 ml)(The reaction must be quenched with dilute acid to prevent isomerization of the 5,6 epoxide by $Al(OH)_3$. The Pigments were extracted into ether and the ether layer back-extracted twice with 0.1% acetic acid and twice with aqueous $CaCO_3$(sat), then dried over anhydrous Na_2SO_4. HPLC analysis of the product mixture yielded seven peaks (Table 2, Figure 3) including fucoxanthinol (IV) (calc. 31% yield, or 52% when corrected for unreacted

Table 2. $LiAl(CH_3)_3H$ reduction products of fucoxanthin.

Fraction		λ_{max} *	MW	Yield (%)**
1	(unknown)	427, 401, 379, 359	---	2
2	Fucoxanthin	(475), 450, 438	658	40
3,4	Fucoxanthin-8-ol*** (R,S isomers)	449, 423, 398, 378	660	14
5	Fucoxanthinol	(475), 452, (432)	616	31
6,7	Fucoxanthol*** (R,S isomers)	449, 422, 398, 379	618	15

* Spectra collected on a Carey 118 dual beam spectrophotometer. Solvent: 20% B/hexane, where B = THF:MeOH, 2:8, v/v.

** Calculated from HPLC. Corrected for differences in absorption at 436nm. Expressed as % of starting material.

*** R,S isomers can be separated on silica using 10% B in hexane. Isomeric ratios were 5:3 (fucoxanthin-8-ol) and 2:1 (fucoxanthol). Absolute stereochemistry was not assigned.

fucoxanthin)*. Fucoxanthinol was purified by repeated column chromatography (1.2 cm x 45 cm) on silica. The column was washed with 20% acetone/hexane to elute fucoxanthin and fucoxanthin-8-ol. Fucoxanthinol eluted with 30% acetone/hexane and was crystallized from hot toluene/hexane.

ii) Properties of Fucoxanthinol: RR_f(β-carotene) 0.08, λ_{max}(hexane) 477, 448, 424, λ_{max}(acetone) 447. ^{13}C-NMR (Figure 5) ($CDCl_3$) δ 35.1 (C-1), 47.1 (C-2), 64.1 (C-3), 41.6 (C-4), 67.2 (C-5), 72.9 (C-6), 40.8 (C-7), 197.9 (C-8), 134.4 (C-9), 139.0 (C-10), 123.3 (C-11), 145.0 (C-12), 135.3 (C-13), 136.6 (C-14), 130.0 (C-15), 25.0 (C-16), 29.3 (C-17), 21.1 (C-18), 11.6 (C-19), 14.0 (C-20), 35.8 (C-1'), 49.0 (C-2'), 64.1 (C-3'), 49.0 (C-4'), 66.2 (C-5'), 117.8 (C-6'), **** (C-7'), 103.2 (C-8'), 132.5 (C-9'), 128.4 (C-10'), 125.7 (C-11'), 136.9 (C-12'), 138.0 (C-13'), 132.2 (C-14'), 132.5 (C-15'), 31.2 (C-16'), 32.1 (C-17'), 28.1 (C-18'), 12.8 (C-19'), 12.7 (C-20')(**** not recorded, spectra scanned to 200 ppm). CIMS: (M+1)= 617, (M+1-18)= 599, (M+1-18-18)= 581, (M+1-92)= 525, (M+1-92-18)= 507, (M+1-170)= 447, (M+1-170-18)= 429 (Figure 6).

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* The yield of fucoxanthinol is quite sensitive to deactivation of $LiAl(CH_3)_3H$ by trace amounts of water (presumably from glassware and ether). To correct for this deactivation, each synthesis was run on a small scale to determine the precise amount of reagent needed to give maximum yields. Large scale syntheses were less susceptable to this problem as illustrated in Figure 4, which presents HPLC chromatograms from two separate syntheses (100 mg fucoxanthin each).

Figure 4. HPLC separation of $LiAl(CH_3)_3H$ reduction products of fucoxanthin from two syntheses (labelled A and B, approximately 100 mg fucoxanthin each). Peak numbers refer to fractions in Table 2. Separation conditions given in text.

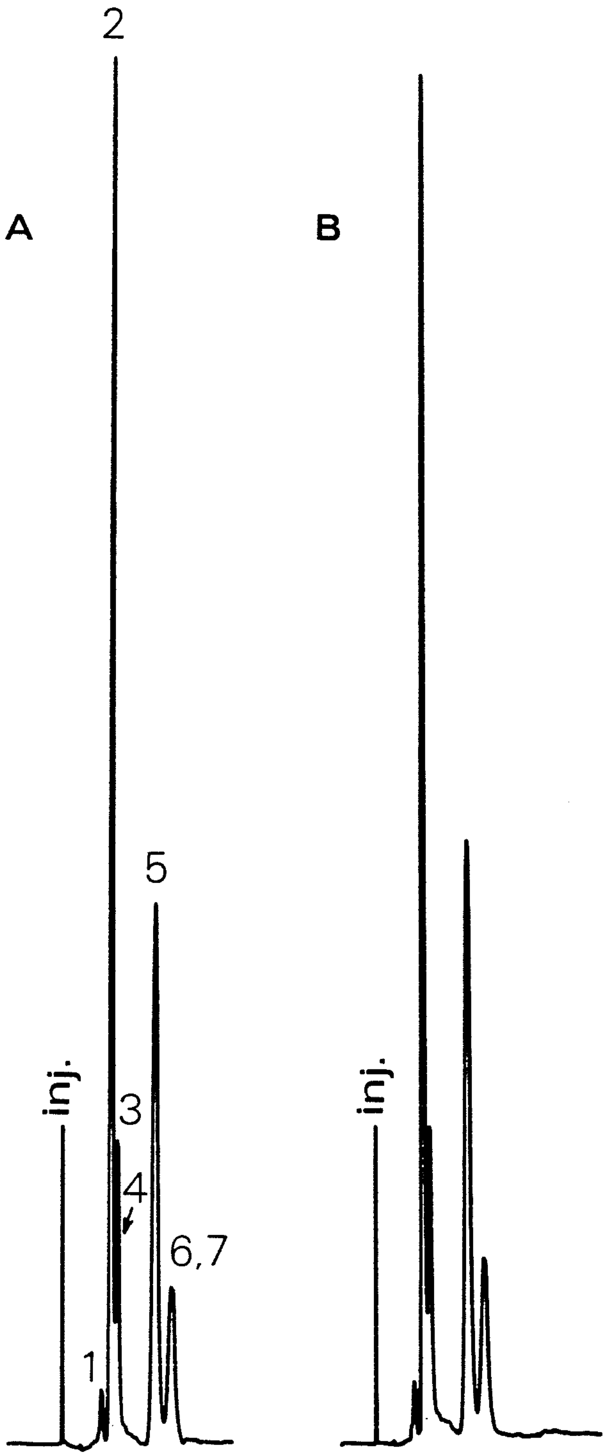

2
A
B
5
inj.
3
4
6,7
1
inj.

Figure 5. ^{13}C-NMR of fucoxanthinol. Chemical shifts relative to TMS. Peak numbers refer to IUPAC carbon designations.

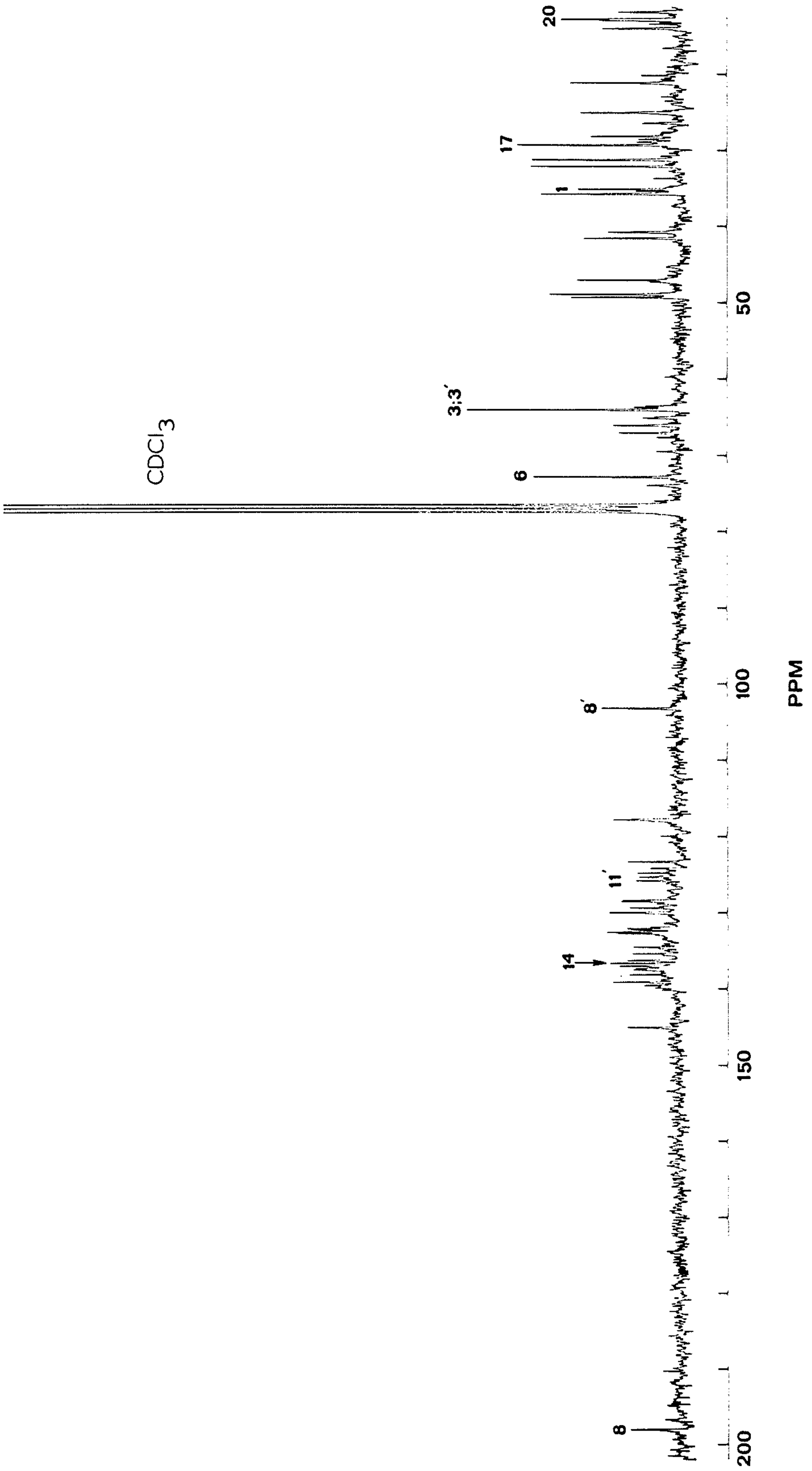

20
17
1
3,3′
6
CDCl$_3$
8′
11′
14
8
50
100
150
200
PPM

Figure 6. Chemical ionization mass spectra of A) fucoxanthinol, B) 3,3'-bis(trimethylsilyl) fucoxanthinol diether, and C) 3,3',5'-tris(trimethylsilyl) fucoxanthinyl triether. Conditions given in Figure 2 legend.

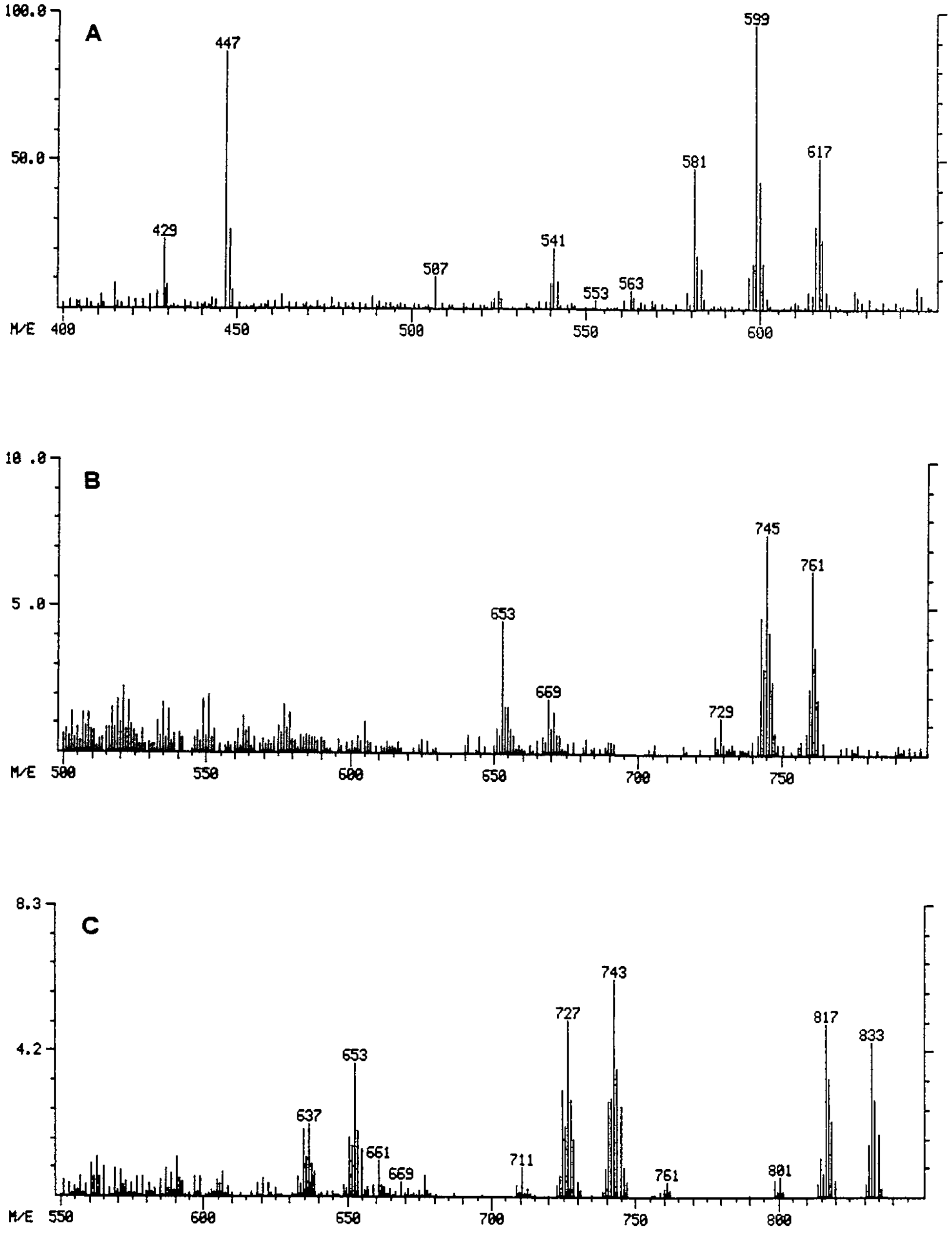

A
100.0
50.0
447
599
581
617
429
507
541
553
563
M/E
400
450
500
550
600
B
10.0
5.0
745
761
653
669
729
M/E
500
550
600
650
700
750
C
8.3
4.2
743
727
817
833
653
637
661
669
711
761
801
M/E
550
600
650
700
750
800

Table 3. Iodine catalyzed photoisomerization products of fucoxanthinol, isofucoxanthin, and isofucoxanthinol. Retention time of cis isomers are normalized to the all-trans peak. Separation conditions are given in text.

	N*	RR$_t$ (all-trans)								
Fucoxanthinol	4	0.26	0.43	0.78	1	1.24				
Isofucoxanthin-I**	4	0.18	0.22	0.30	0.36	0.85	1	1.13	1.25	1.30
Isofucoxanthin-II	2	0.19	0.25	0.31	0.37	0.87	1	1.14	1.24	1.29
Isofucoxanthinol	4	0.18	0.25	0.44	0.59	0.69	0.79	0.91	1	

* number of runs.

** I,II denote separate experiments conducted on different days.

iii) Derivatization: Fucoxanthin 3-acetate - Acylation with acetic anhydride in 0.1 mM DMAP/pyridine yields a diacetate identical in retention time (HPLC) with fucoxanthin 3-acetate (see previous section). Iodine catalyzed isomerization yields a mixture of cis/trans isomers identical in relative abundance and retention times to the mixture derived from isomerization of authentic fucoxanthin 3-acetate (Table 1).

3,3'-bis(trimethylsilyl) fucoxanthinol diether (XXXV) - Silylation with HMDS using the procedure described for fucoxanthin yielded 3,3'-bis(trimethylsilyl) fucoxanthinol diether: RR_f(β-carotene) 0.88, λ_{max}(hexane) 476, 448, 425, λ_{max}(acetone) 446. C.I.M.S.: (M+1)= 761, (M+1-16)= 745, (M+1-18)= 743, (M+1-16-16)= 729, (M+1-90)= 671, (M+1-92)= 669, (M+1-90-16)= 655, (M+1-90-18)= 653 (Figure 6).

3,3',5'-tris(trimethylsilyl) fucoxanthinol triether (XXXVI) - Silylation with TMS-Z for 10 minutes at room temperature yields the 3,3',5'-tris(trimethylsilyl) fucoxanthinol triether: RR_f(β-carotene) 0.99, λ_{max}(hexane) 476, 448, 425, λ_{max}(acetone) 446. C.I.M.S.: (M+1)= 833, (M+1-16)= 817, (M+1-16-16)= 801, (M+1-18-16)= 799, (M+1-72)= 761, (M+1-90)= 743, (M+1-92)= 741, (M+1-92-90)= 651, (M+1-90-16)= 727, (M+1-92-16)= 725, (M+1-90-16-16)= 711, (M+1-90-90)= 653, (M+1-92-90)= 651, (M+1-90-90-16)= 637, (M+1-90-92-16)= 635 (Figure 6c).

iv) Iodine Catalyzed Isomerization: Approximately 4 μg of fucoxanthinol was dissolved in 200μl of 0.04 mM iodine/toluene (degassed). The solution was sealed under N_2 and allowed to equilibrate in ambient (artificial) light. After 12 hr, the mixture was analyzed by HPLC (300x 3.9 mm, 5μm silica, eluted with 20% B in hexane). Four cis isomers were separated from all-trans fucoxanthinol (Table 3).

ISOFUCOXANTHIN

i) From Fucoxanthin: Fucoxanthin (120 mg) was dissolved in 150 ml of MeOH/H_2O (4:1 v/v) and added to an equal volume of 1 mM KOH/MeOH. After 3 hr the reaction was quenched by addition of 0.1% acetic acid (10 mls), and the products extracted into diethyl ether. Six major bands were observed on silica TLC (40% acetone/hexane)(Table 4). Isofucoxanthin was purified by repeated chromatography on silica (1.8 cm x 45 cm). The column was washed with 20% acetone/hexane to elute fractions 1 and 2 (Table 4). Isofucoxanthin (XIV) eluted with 30% acetone/hexane, and was crystallized from toluene/hexane. Isofucoxanthin cannot be separated from fucoxanthinol under the conditions used in this purification scheme. Therefore the purity of the isolated product must be confirmed by derivatization (see below).

ii) Properties of Isofucoxanthin: RR_f(β-carotene) 0.08, λ_{max}(hexane) 477, 449, 425, $\lambda_{(max)}$(acetone) 446. CIMS: (M+1)= 659, (M+1-18)= 641, (M+1-18-18)= 623, (M+1-18-18-18)= 605, (M+1-60)= 599, (M+1-60-18)= 581, (M+1-60-18-18)= 563, (M+1-92-18)= 549, (M+1-60-18-18-18)= 545, (M+1-92-60-18);(M+1-170)= 489, (M+1-170-18)= 471 (Figure 7A).

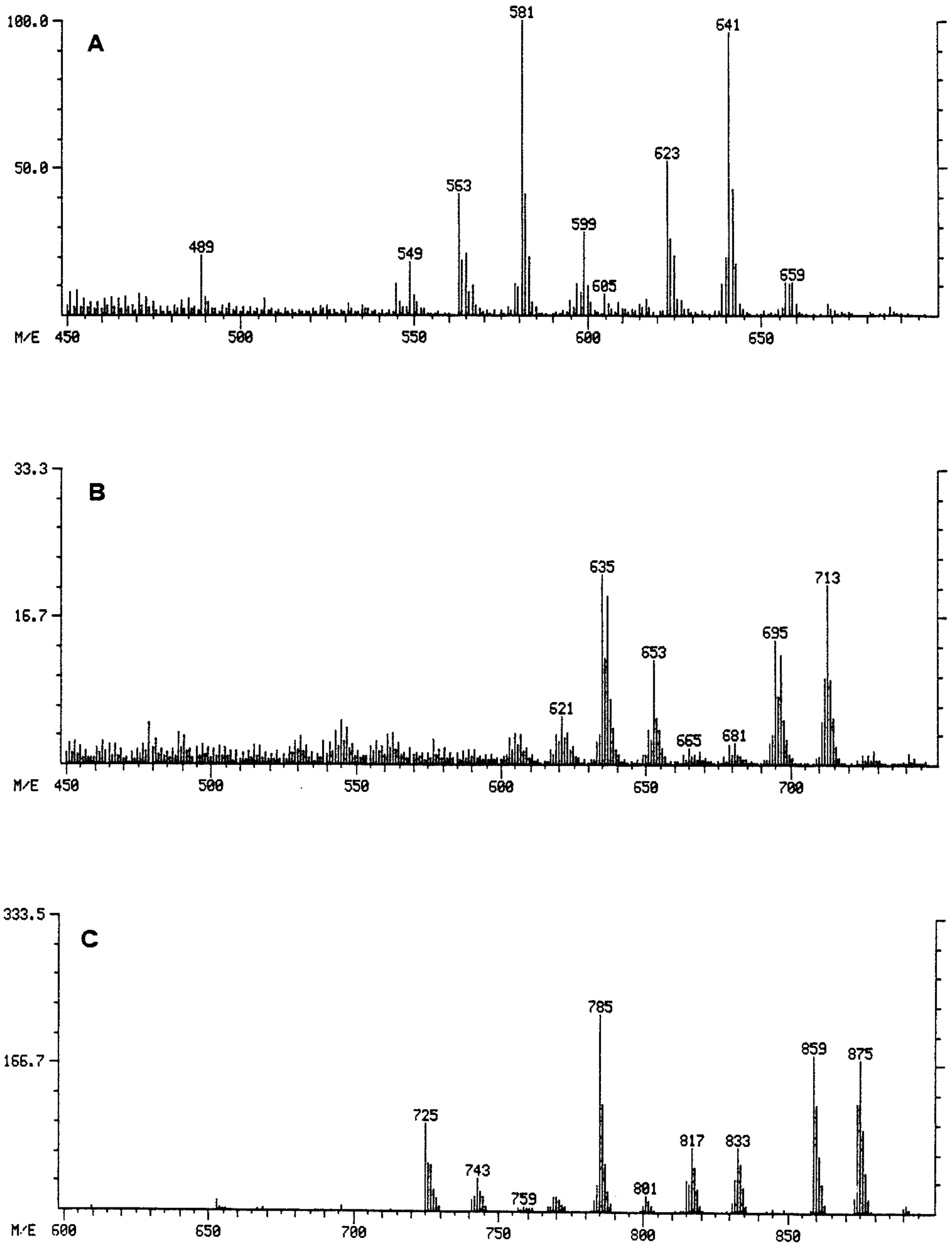
iii) Derivatization: 3-(trimethylsilyl) isofucoxanthinyl ether (XXXVII)- Silylation of isofucoxanthin with HMDS according to the procedure described for fucoxanthin yields 3-(trimethylsilyl) isofucoxanthinyl ether , RR_f(β-carotene) 0.34, λ_{max}(hexane) 476, 449, 425, λ_{max}(acetone) 447. C.I.M.S.: (M+1-18)= 713, (M+1-18-16)= 697, (M+1-18-16-16)= 681, (M+1-18-18-18)= 679, (M+1-60-18)= 653, (M+1-60-18-16)= 637, (M+1-60-18-18)= 635, (M+1-92-16)= 623, (M+1-92-18)= 621 (Figure 7B). Note the molecular ion was not detected in this spectra.

Table 4. Base hydrolysis products of fucoxanthin.

Fraction		RR_t (β -carotene)	λ_{max}*
1	green band #1	0.74	
2	fucoxanthin	0.68	449
3	green band #2	0.49	
4	isofucoxanthin	0.34	449
5	green band #3	0.24	447, 420, 398, (378)
6	isofucoxanthinol	0.17	449

*in acetone

Figure 7. Chemical ionization mass spectra of A) isofucoxanthin, B) 3-(trimethylsilyl) fucoxanthinyl ether, and C) 3,5,5'-*tris*(trimethylsilyl) fucoxanthinyl triether. Conditions given in Figure 2, legend.


A
100.0
50.0
489
549
563
581
599
605
623
641
659
M/E 450
500
550
600
650
B
33.3
16.7
621
635
653
665
681
695
713
M/E 450
500
550
600
650
700
C
333.5
166.7
725
743
759
785
801
817
833
859
875
M/E 600
650
700
750
800
850

3,5,5'-tris(trimethylsilyl) isofucoxanthinyl triether (XXXVIII) - Silylation with TMS-Z according to the procedure described for fucoxanthin yielded 3,5,5'-trisilyl-isofucoxanthin, RR_f(β-carotene) 0.97, λ_{max}(hexane) 478, 450, 427, λ_{max}(acetone) 449. C.I.M.S.: (M+1)= 875, (M+1-16)= 859, (M+1-42)= 833, (M+1-42-16), (M+1-42-16)= 817, (M+1-60)= 815, (M+1-42-16-16)= 801, (M+1-90)= 785, (M+1-90)= 785, (M+1-90-16)= 769, (M+1-90-42)= 743, (M+1-92-42)= 741, (M+1-90-60)= 725, (M+1-90-42-16-16)= 711, (M+1-90-90)= 695, (M+1-90-90-42)= 653 (Figure 7C).

iv) Iodine catalyzed photomerization: Approximately 500 ng of isofucoxanthin was dissolved in 200 μl of iodine/toluene. The solution was sealed under N_2 and allowed to equilibrate for 12 hr in ambient (fluorescent artificial) light. Eight cis isomers were separated by HPLC (300x3.9 mm, 5 μm silica, eluted with 20%B in hexane). Table 3 presents the results from two separate experiments (labelled I and II in Table 3) conducted on different days.

ISOFUCOXANTHINOL

i) From Fucoxanthin: Fucoxanthin was treated with $KOH/MeOH/H_2O$ as described in the previous section. Purification of fraction 6 (Table 4) by repeated chromatography on silica (50/50 acetone/hexane) yielded isofucoxanthinol (XII), which crystallized from hot toluene/hexane with slow cooling.

ii) Properties of Isofucoxanthinol: λ_{max} (acetone) 450. C.I.M.S.: (M+1)= 617, (M+1-18)= 599, (M+1-18-18)= 581, (M+1-18-18-18)= 563, (M+1-170)= 447, (M+1-170-18)= 429.

iii) Derivatization: 3,5,3',5'-tetrakis(trimethylsilyl) isofucoxanthinol tetraether (XXXIX) -Silylation of isofucoxanthin with TMS-Z for 15 minutes at room temperature yielded 3,5,3',5'-tetrakis (trimethylsilyl) isofucoxanthinol tetraether, RR_f(β-carotene) 0.99, λ_{max} (hexane) 450, 448, 425, λ_{max}(acetone) 446. C.I.M.S.: (M+1)= 905, (M+1-72)= 833, (M+1-72-16)= 817, (M+1-90-72)= 743, (M+1-90-72-16)= 727, (M+1-90-90)= 725, (M+1-90-90-72)= 653, (M+1-90-90-90)= 635.

iv) Iodine Catalyzed Isomerization: Approximately 500 ng of isofucoxanthinol was dissolved in 200 μl of 0.04 mM iodine/toluene (degassed). The solution was sealed under N_2 and allowed to equilibrate for 12 hr under ambient (fluorescent artificial) light. Seven cis isomers were separated by HPLC (300x3.9 mm, 5μ m silica, eluted with 20%B in hexane)(Table 3).

DISCUSSION

Visible Spectroscopy

The visible spectrum of fucoxanthin in hexane displays absorption maxima at 476, 448, and 426 nm, consistent with a conjugated nonaene chromophore (Moss and Weedon, 1976). Maxima are generally broad and lacking in fine structure, a characteristic of conjugated ketones (Vetter et al., 1971). This is particularly striking in acetone solutions, where the maximum at 476 nm appears as a shoulder to the 448 peak. Spectra recorded in hexane tend to have sharper peaks, due to the poor solvating ability of this solvent.

The solubility of fucopigments in hexane follows the order: fucoxanthin acetate > fucoxanthin > isofucoxanthin, fucoxanthinol >

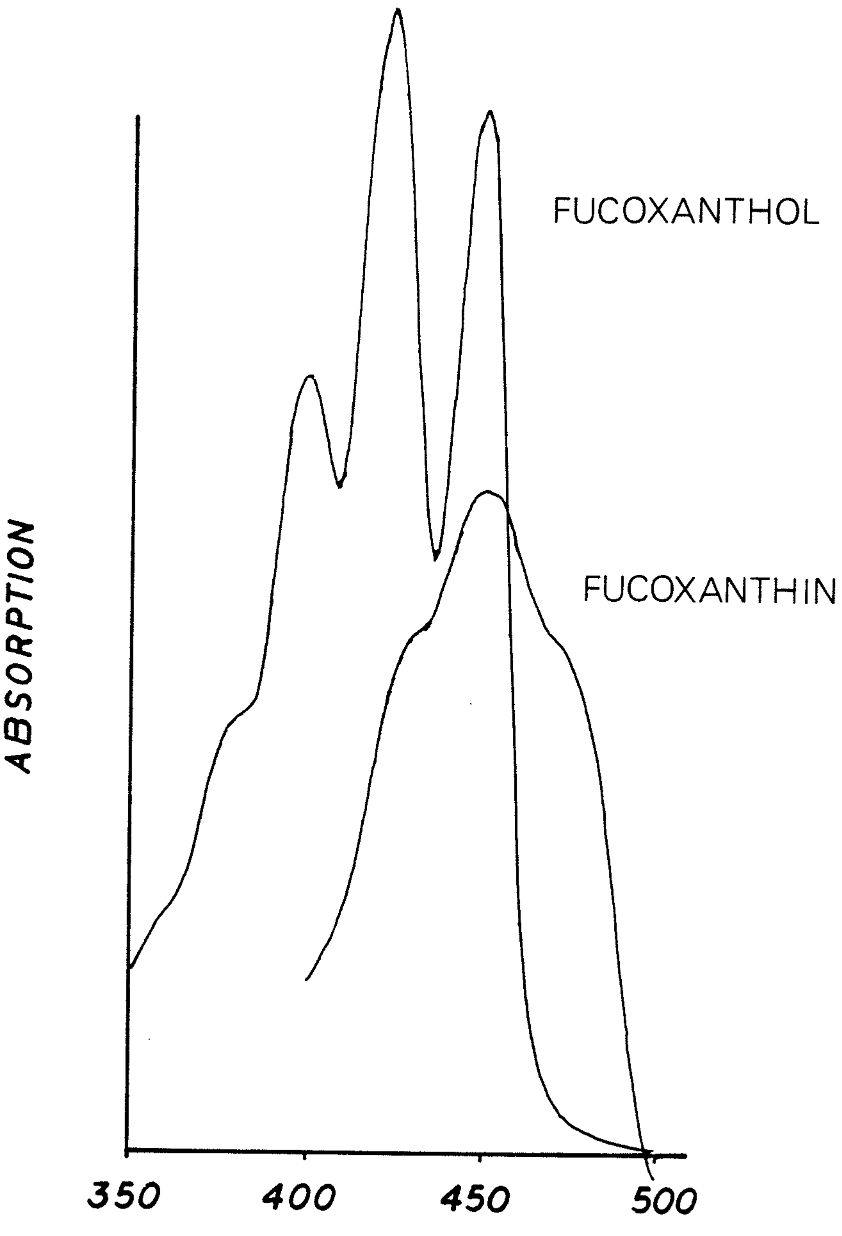
isofucoxanthinol. Isofucoxanthinol is not sufficiently soluble in hexane for accurate spectral measurements. No obvious differences in hexane solubility were noted for persilyl derivatives, although it is expected the trend will follow chromatographic retention time (RR_f).

Isomerization of the 5,6 epoxide to a tertiary 5 alcohol conjugates an additional double bond to the chromophore. A calculated 10 nm bathochromic shift in the absorption maximum is expected (Vetter _et al._, 1971). Jensen (1966) reported an absoption maxima for isofucoxanthin at 480 nm, implying a 4 nm bathochromic shift with epoxide opening (λ_{max} for fucoxanthin was not given. Therefore a value of 476 nm as measured by Bonnett _et al._ (1969) and in the present study is assumed.). No shift in the absorption maxima with epoxide opening was observed in the present study or by Bonnett _et al._ (1969). The discrepency in the results of Jensen (1966) with those of the present study and Bonnett _et al._ (1969) may be attributable to differences in calibration. Apparently the steric crowding of the 8-ketone, 5-methyl and 5-alcohol causes an out-of-plane deformation of the 7-8 bond, and significantly reduces the conjugation of the 6,7 double bond and 8-ketone. Reduction of the 8-ketone to an alcohol with $NaBH_4$ or $LiAlH_4$ results in the expected 26 nm hypsochromic shift and a significant sharpening of the absorption maxima (Figure 8; Table 1) (Liaaen and Sorenson 1959; Moss and Weedon, 1976)).

Derivatization

Selective acylation and silylation of secondary and tertiary alcohols is a sensitive method for confirming isomerization of the 5,6 epoxide (McCormick and Liaaen-Jensen, 1966). Mild acylation of secondary alcohols

Figure 8. Visible spectra of fucoxanthin and fucoxanthol in hexane:MeOH:THF (80:4:16).


FUCOXANTHOL
FUCOXANTHIN
ABSORPTION
350
400
450
500

can be accomplished with pyridine/acetic anhydride. The reaction rate and product yield can be enhanced by the addition of catalytic amounts of 4-(N,N)-dimethylaminopyridine (Fell and Lee, 1976; Holfe *et al.*, 1978). Acylation of fucoxanthinol with DMAP/pyridine acetic anhydride yielded two monoacetates (3 and 3') after only 15 sec of reaction. Typically, the reaction is complete within 10 min. DMAP has been used as a catalyst for the acylation of tertiary alcohols, for example 1-methylcyclohexanol and 1-ethynlcyclohexanol (Holfe *et al.* 1978), and potentially could acylate the tertiary 5 and 5' hydroxyl groups of fucopigments. However, no triacetate of fucoxanthinol was observed after 1 hr of reaction.

Secondary 3 and 3' alcohols can be selectively silylated with hexamethyldisilazane (HMDS)(Pierce, 1970; Knapp, 1979). This synthesis has the advantage of being reversible, therefore the presence of secondary alcohols can be determined without loss of the sample. However, silylation with this reagent is slow, (silylation of fucoxanthin is incomplete after 12 hr) so that the synthesis is not quantitative.

Rapid persilylation occurs with TCMS/HMDS in dry pyridine. Reaction is generally complete within 20 min at room temperature. Silylation at higher temperatures results in extensive bleaching, and is not recommended as a routine method. Silylated derivatives are stable on neutral silica, and can be purified by TLC, HPLC, and liquid column chromatography. Consequently, silylation may be useful as a method for obtaining highly purified pigments for NMR and mass spectrometric studies, especially from samples where co-eluting lipids are a problem.

The retention of silylated fucopigments follows the expected order of β-carotene ($RR_f = 1$) > 3,5'-*bis*(trimethylsilyl) fucoxanthinyl ether

(0.99) = 3,3',5'-tris(trimethylsilyl) fucoxanthinol triether (0.99) = 3,5,3',5'-tetrakis(trimethylsilyl) isofucoxanthinol tetraether (0.99)> 3,5,5'-tris(trimethylsilyl) isofucoxanthinyl triether (0.97) > 5'-(trimethylsilyl) fucoxanthin 3-acetyl ether (0.95) > 3,3'-bis(trimethylsilyl) fucoxanthinol diether (0.88) > 3-(trimethylsilyl) fucoxanthinyl ether (0.59) > 3-(trimethylsilyl) isofucoxanthinyl ether (0.34).

Mass spectrometry clearly shows the selectivity of derivatization using DMAP/pyridine/acetic anhydride, HMDS, and TMCS (Figures 2, 6, and 7, also Figure 7 Chapter 2). Acylation of fucoxanthin and fucoxanthinol yields exclusively fucoxanthin 3-acetate with an observed mass increase of +42 and +84 amu respectively. Silylation with HMDS yields a monosilane from fucoxanthin and isofucoxanthin (+72 amu); and a disilane from fucoxanthinol and isofucoxanthinol (+144 amu). No reaction is observed with fucoxanthin 3-acetate. Alternatively silylation with TCMS yields a disilane from fucoxanthin (+144 amu), a trisilane from isofucoxanthin and fucoxanthinol (+216 amu), and a tetrasilane from isofucoxanthinol (+288 amu).

^{13}C-NMR

Over the past decade ^{13}C-NMR spectroscopy has seen increasing application in the structural determination of carotenoids. Isotopic labelling (Vetter et al., 1971; Englert, 1975), model compounds (Bremser and Paust, 1974; Rowan and Sykes, 1974; Crombie et al., 1975; Barlow and Pattender, 1976), lanthinide shift reagents (Englert, 1975; Lienard and Thompson, 1977), and single frequency off resonance (SFOR) (Vogele et al., TABLE 5. ^{13}C-NMR Chemical Shifts of Fucoxanthin, Fucoxanthin Acetate, and Fucoxanthinol

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
FUCOXANTHIN	35.1 35.7	47.1 45.3	64.1 68.0	41.6 45.4	67.1 66.1	72.5 117.5	40.8 202.3	197.8 103.3	134.5 132.4	138.9 128.4	123.3 125.6	144.9 137.0	135.3 137.9	136.5 132.0	129.3 132.4	25.0 31.1	29.1 32.0	21.0 28.1	11.7 12.7	13.9 12.6
FUCOXANTHIN ACETATE	35.2 35.8	42.9 45.4	67.7 68.1	37.7 45.4	67.2 65.8	72.6 117.6	40.7 202.4	197.6 103.4	134.6 132.5	139.0 128.5	123.3 125.7	145.0 137.1	135.4 138.0	136.6 132.1	129.4 132.5	24.9 31.2	29.2 32.1	21.0 27.8	11.8 12.9	14.0 12.7
FUCOXANTHINOL	35.1 35.8	47.1 49.4	64.1 64.1	41.6 49.4	67.2 66.2	72.9 117.8	40.8 ***	197.9 103.2	134.4 132.5	139.0 128.4	123.3 125.7	145.0 136.9	135.3 138.0	136.6 132.2	130.0 132.5	25.0 31.3	29.3 32.1	21.1 28.1	11.6 12.8	14.0 12.7
FUCOXANTHIN*	35.2 35.8	47.3 45.4	64.2 68.2	40.6 45.5	67.2 66.2	72.6 117.6	41.8 202.4	197.9 103.4	134.5 132.6	139.2 128.5	123.4 125.7	145.1 137.1	135.4 138.1	136.7 132.2	129.5 132.6	25.0 31.3	29.2 32.2	21.5 28.2	11.9 13.0	14.0 12.7

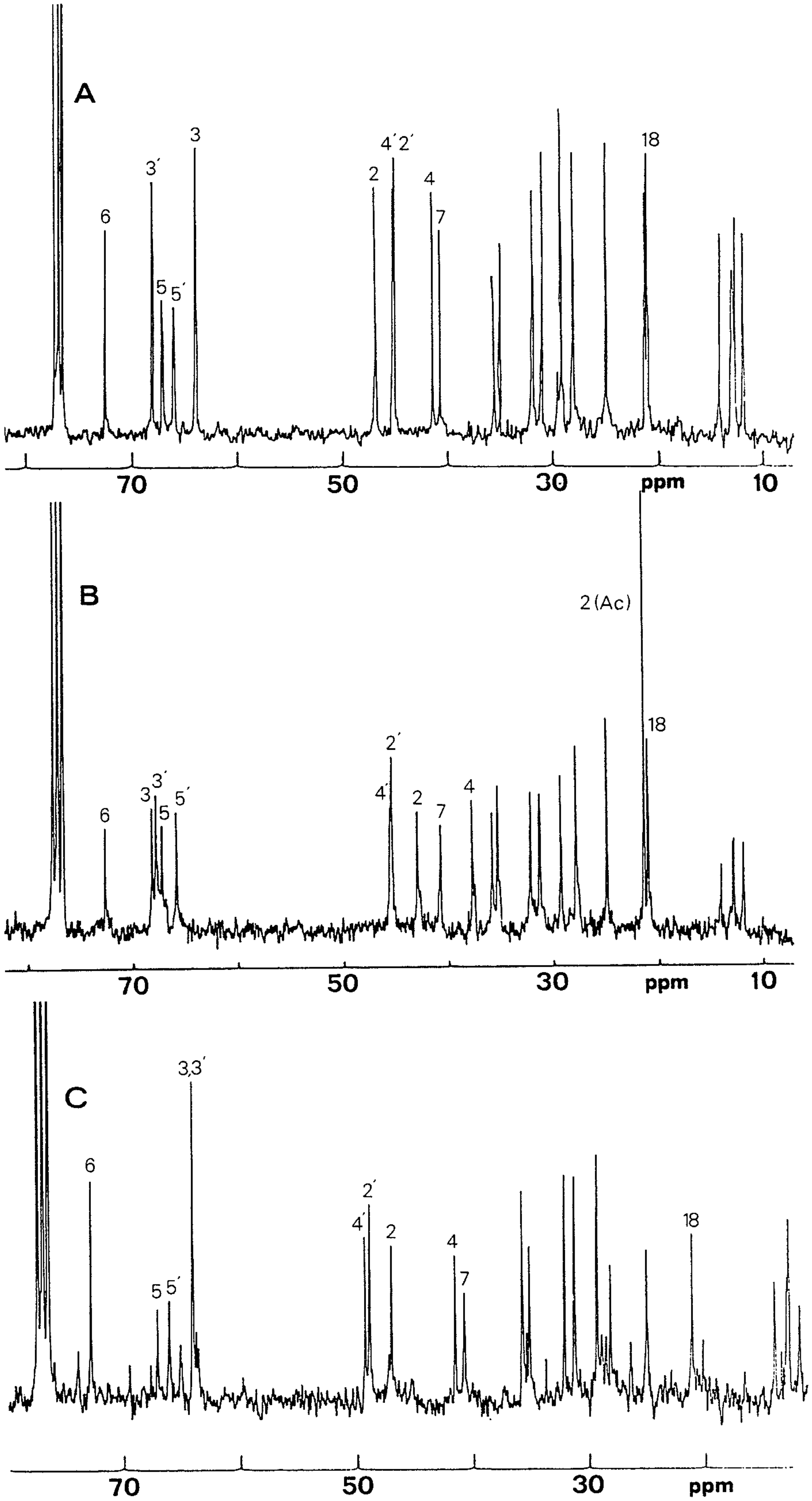
*** Spectra scanned to 200 ppm.

* Moss, 1976.

1975; Moss and Weedon, 1976) experiments have been used to assign characteristic chemical shifts. ^{13}C-NMR chemical shifts for 21 carotenoids have recently been reviewed by Moss (1976). Assignment of ring carbons is now relatively straightforward. In the present study Fourier transform broad-band ^{1}H decoupled spectra were collected for fucoxanthin and select transformation products. In general the data are in agreement with the assignments made by Moss (1976). However, the data requires revision of the assignments for C-2, C-4, C-7, C-18, C-2', C-4' and C (acetate)-1 made by Moss.

Chemical shifts for fucoxanthin, fucoxanthin acetate, and fucoxanthinol are given in Table 5. Assignments made by Moss are also included for comparison. Acylation of the C-3 hydroxyl group of fucoxanthin to form fucoxanthin 3-acetate is expected to shift the resonance frequency of the C-3 carbon downfield. Similarly, hydrolysis of the C-3' ester of fucoxanthin to form fucoxanthinol is expected to shift the resonance frequency of the C-3' carbon upfield. Second order chemical shifts of the neighboring 2, 4 and 2', 4' carbons can also be expected.

Table 5 and Figure 9 clearly show the expected chemical shifts. Fucoxanthin has C-3 and C-3' resonances at 64.1 ppm and 68.0 ppm respectively. The C-3' resonance in Fucoxanthin 3-acetate (68.0 ppm) is unchanged by acylation, however a 3.6 ppm downfield shift is observed in the C-3 resonance (67.7 ppm)(Figure 9). Additionally there is no change in the resonance frequency of the C-2' and C-4' carbons (45.5 ppm). However an approximately 4 ppm upfield shift in the resonance frequency of C-2 and C-4 is observed. The C-3, C-2, and C-4 resonances of fucoxanthin remains unchanged after $LiAl(CH_3)_3H$ reduction to fucoxanthinol (64.1, Figure 9. ^{13}C-NMR of A) fucoxanthin, B) fucoxanthin 3-acetate and C) fucoxanthinol from 10-80 ppm (TMS). Peak numbers refer to IUPAC carbon designations.


A
6
3´
5
5´
3
2
4´2´
4
7
18
70
50
30
ppm
10
B
2(Ac)
18
6
3
3´
5
5´
4´
2´
2
7
4
70
50
30
ppm
10
C
3,3´
6
5
5´
4´
2´
2
4
7
18
70
50
30
ppm

47.1, and 41.6 ppm respectively). As expected an upfield shift of 3.9 ppm, coincident with the reduction of the 3'-ester to a 3'-alcohol, is observed for the C-3' carbon resonance. A parallel downfield shift of 4 ppm to approximately 49.0 ppm is observed for C-2' and C-4' carbons.

These observations require revisions in the chemical shift assignments made by Moss (1976)(Table 5). The SFOR measurements made by Moss could not determine the absolute assignment of C-2, C-4, C-7, C-2', or C-4'. Moss assigned these carbons resonances at 47.3, 40.9, 41.8, 45.4, and 45.5 ppm respectively, but noted that the assignments may be reversed. In the synthesis of fucoxanthin 3-acetate and fucoxanthinol from fucoxanthin, only the C-7 carbon resonance should be unaffected. Of the five resonances assigned by Moss to C-2, C-4, C-7, C-2', and C-4', only the resonance at 40.8 ppm (this study, Moss's 40.9 ppm) is unchanged for fucoxanthin, fucoxanthin 3-acetate, and fucoxanthinol. Therefore reassignment of the 40.8 ppm from C-4 (Moss) to C-7 is required. In addition, the resonances at 45.3 ppm and 45.4 ppm are unchanged with the acylation of fucoxanthin but shifted approximately 4 ppm upon ester hydrolysis. Therefore these resonances are assigned to C-2' and C-4'. By elimination, the resonances at 47.1 ppm and 41.6 ppm assigned to C-2 and C-7 by Moss, must be assigned to C-2 and C-4.

Additionally, Moss assigned resonances at 21.2 ppm and 21.5 ppm to acetate C-2 (methyl) and C-18 carbons respectively. Acylation of fucoxanthin increases the intensity of the 21.4 ppm (this study) resonance, but does not affect the intensity of the 21.0 ppm (this study) signal. Conversely, the 21.4 ppm resonance is not observed in the ^{13}C-NMR of fucoxanthinol, while the resonance at 21.1 is unchanged.

Therefore, the assignments of acetate C-2 (methyl) and C-18 carbons given by Moss are reversed. The correct assignments are given in Table 5.

Mass Spectrometry

Electron impact (EI) mass spectrometry has been widely used in carotenoid studies. The fragmentation of 86 different end groups has been recently reviewed (Moss and Weedon, 1976), and in some cases the fragmentation mechanisms determined by isotopic labelling (Kjosen et al., 1971). However, conventional EIMS suffers from lack of sensitivity, and the overall complexity of the spectra. Chemical ionization mass spectrometry has been studied as a means of circumventing these problems (Carnevale et al., 1978). In general, the spectra are much simpler and display an overall greater sensitivity, especially in the high mass region. These observations were confirmed in the present study. The CI (CH_4 reagent gas) mass spectra of fucoxanthin and fucoxanthin degradation products and derivatives are characterized by intense ions at M+29 (+C_2H_5), M+1, M+1-n18(n= 1-4)(water), M+1-60 (acetate), M+1-60-n18(n= 1-3), M+1-170, M+1-170-18, and M+1-170-60 (Figure 10A, Table 6). A minor series of ions were observed at M+1-92 (toluene)-n18(n= 0-2) and M+1-106 (xylene)-18.

The most predominant series of ions in all spectra arise from loss of water. In agreement with the report by Bonnett et al., (1969) consecutive dehydration of hydroxyl groups is observed in all spectra. Hence fucoxanthin loses two, and isofucoxanthin, fucoxanthinol, fucoxanthin-8-ol, and isofucoxanthin each lose three molecules of water. Only hydroxyl groups are lost, opening of the 5,6-epoxide followed by dehydration is a minor reaction. Therefore, fucoxanthin can be distinguished from isofucoxanthin (which has the same molecular weight), on the basis of their mass spectra. However, it should be noted that the relative abundance of ions rapidly decreases with the higher dehydrates (for isofucoxanthin M+1-18= 100%, M+1-18-18= 53%, and M+1-18-18-18= 8%), so that selective derivatization remains the most straightforward and unambiguous method for determining the number of hydroxyl groups. Finally, the M+1-(3)18 and M+1-60-(3)18 ions in the mass spectra of fucoxanthin-8-ol are relatively more intense than the corresponding ions in the mass spectra of isofucoxanthin and fucoxanthinol. This suggests that loss of water from the secondary 8-allylic alcohol is a more facile reaction than the loss of the secondary 3 or 3'-ol.

Loss of acetic acid (m/z 60) is characteristic of fucoxanthin, isofucoxanthin, and fucoxanthin 3-acetate. Additionally, the M+1-60$^+$ pseudo-molecular ion gives rise to a second series of dehydrates (M+1-60-n18). The intensity of the M+1-60 ion varies considerably from approximately 50% to occasionally being absent, depending on the instrumental conditions. No ions from loss of ketene (m/z 42) are observed (Figure 10B).

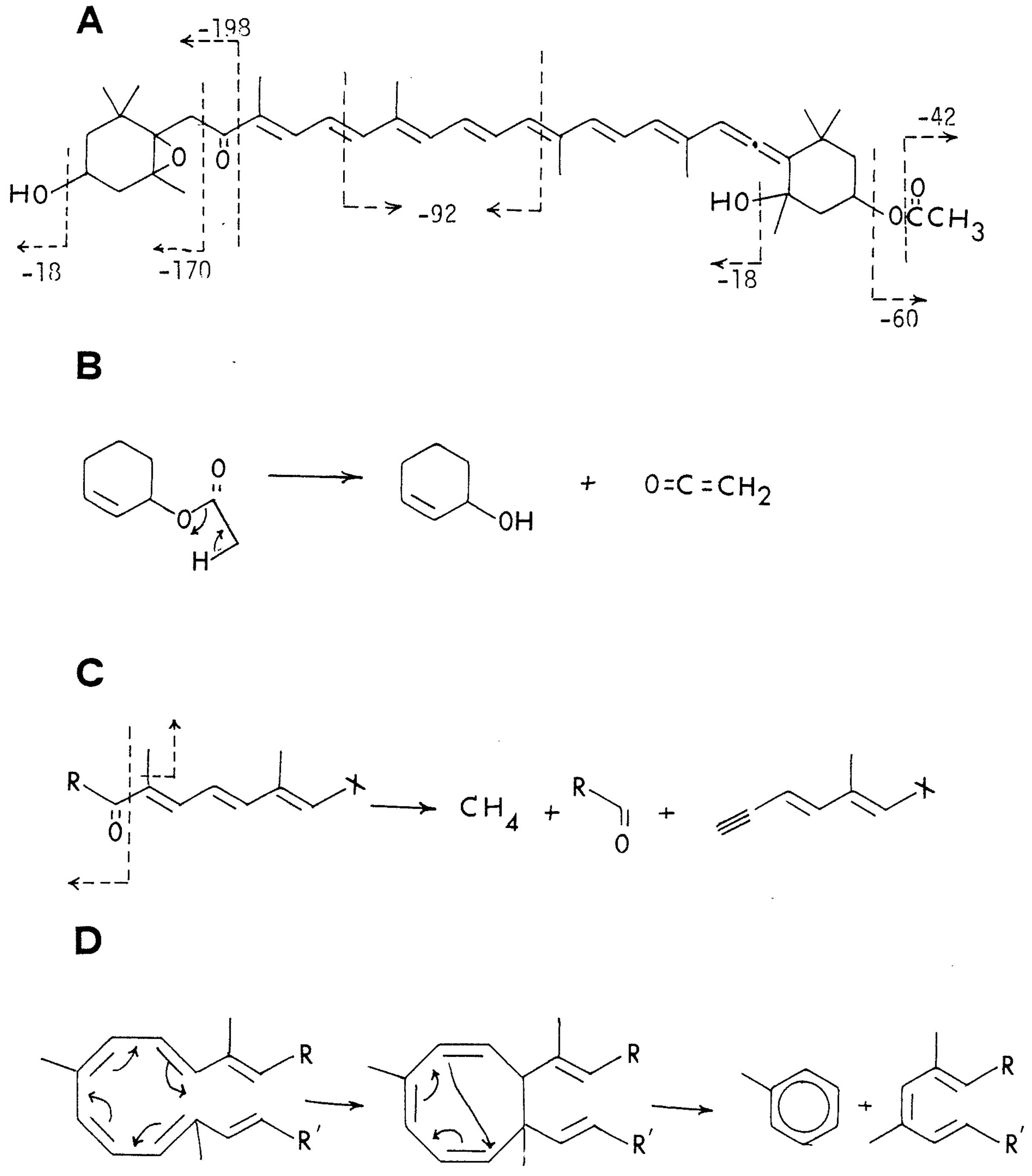
Fucoxanthin 3-acetate displays a somewhat more intense M+1 ion (relative to M+1-18) than fucoxanthin, perhaps due to the relative stability to elimination of the 3-acetyl and 3-hydroxyl group. A more intense signal at M+1-60 is also observed. No fragments are observed from loss of ketene or from loss of two molecules of acetic acid (-120 amu).

Cleavage of the 7,8 bond α to the conjugated 8-ketone gives rise to the M+1-170 series of ions. This fission produces fragments which

Table 6. Characteristic mass fragments of fucoxanthin and derivatives.

	M+1-29	M+1	M+1-n18	M+1-60	M+1-60-n18	M+1-92	M+1-92 -n18	M+1-106 -18	M+1- 170	M+1-170 -18	M+1-170 -60
Fucoxanthin	687	659	641, 623	599	581, 563	567	549	535	489	471	429
Isofucoxanthin		659	641, 623, 605	599	581, 563, 545	567	549		489	471	
Fucoxanthin-8-ol			643, 625, 607	601	583, 565, 547		551		491	473	431
Fucoxanthin 3-acetate		701	683	641	623		591	577	489		
Fucoxanthinol	645	617	599, 581, 563			525	507		447	429	
Isofucoxanthinol	645	617	599, 581, 563			525	507		447	429	

Figure 10. Fragmentation reactions of fucoxanthin: A) major C.I. fragmentation reactions, B) loss of ketene, C) fragmentation of 8,9 bond, and D) loss of toluene.


A
-198
-92
HO
O
O
-18
-170
-42
HO
O
OCCH3
-18
-60
B
O
O
H
OH
+
O=C=CH2
C
R
O
CH4
+
R
O
+
D
R
R'
R
R'
+
R
R'

separate the two end groups, and is of diagnostic value in determining the site of dehydration and derivatization. The M+1-196 ion, corresponding to cleavage of the 8,9 bond, is not observed. This is in contrast to the fragmentation patterns of the conjugated acyclic ketone in the carotenoid okenone (XL), and capsanthin (XLI), in which fragments arising from the cleavage of both sides of the ketone are observed (Enzell *et al.*, 1969, Moss and Weedon 1976, Carnevale *et al.*, 1978).

An intense fragment at m/z 197 is observed in the low mass region of all spectra. While cleavage of the 6',7' allenic bond could contribute to this ion in acetates, the appearance of m/z 197 as the base peak in the mass spectra of fucoxanthinol, fucoxanthinol dehydate, and isofucoxanthinol dehydrate argues that the contribution is small. This conclusion is confirmed by the observation of the base peak at *m/z* 239 (197+42) in the mass spectra of fucoxanthin 3-acetate. The absence of an M+1-196 ion may result from simultaneous loss of the 9-methyl group as methane (Figure 10C), to form a $M+1-196-16^+$ pseudomolecular ion, which deacylates and dehydrates to form the intense ions observed at 389 and 367 amu respectively. Sequential loss of 16 amu is not observed in normal carotenoids, but has been observed in acyclic conjugated keto-carotenoids.

Fission of the 7,8 bond is still observed after reduction of the 8-ketone to the 8-alcohol with $NaBH_4$ and $LiAlH_4$. The intensity of the M+1-170 and M+1-170-18 signals may be somewhat enhanced, although further experiments are needed to determine if the observed enhancement is real or an artifact of the instrumental conditions.

The elimination of alkyl aromatic (fragments *m/z* 92= toluene, *m/z* 106= xylene, and *m/z* 156= dimethylnapthalene) from the conjugated polyene

(Figure 10D) is perhaps the most characteristic fragmentation in EI mass spectrometry of carotenoids as a class. M+1-92 fragments were observed in moderate abundance (2-12%) for all compounds listed in Table 6. M+1-92-n18 ions were also observed, although somewhat infrequently. This is in agreement with the findings of Carnevale et al. (1978), who observed the loss of toluene and xylene, but not dimethylnapthalene from CI mass spectra of model carotenoids. In the present study no M+1-106 or M+1-156 ions were observed, however ions corresponding to M+1-106-18 were observed.

In addition to the ions discussed above, silylated derivatives display intense signals at M+1-16 (methane), M+1-72 (trimethylsilyl), and M+1-90 (trimethylsiloyl)(McCormick and Liaaen-Jensen, 1966; Nitsche, 1974; Carnevale et al., 1978). Several features of the silylated carotenoid mass spectra are of note. In contrast to their underivatized parents the persilyl derivatives of fucoxanthin and isofucoxanthin both display relatively intense fragments corresponding to M+1-42, M+1-42-n16, M+1-n90-42, M+1-92-42, and M+1-90-42-n16, where n= 1-2. Elimination of ketene appears to be more favorable than in the underivitized acetates, although loss of acetic acid still predominates. The signal from the M+1-92 fragment is also intensified. A parallel intensification of the M+1-106 fragment may occur, but this cannot be evaluated due to interference from the M+1-90-16 signal in the di, tri, and tetrasilyl ethers. However, the mass spectra of 3-silyl-fucoxanthin (only one silyl), which cannot lose M+1-90-16, does not display any fragments at M+1-106, suggesting that loss of xylene from silylated derivatives is not an important reaction.

Iodine Catalyzed Isomerization

Structural elucidation of carotenoids isolated from geochemical samples is an essential step in the reconstruction of source inputs and transformation processes. For compounds amenable to glass capillary GC-MS-computer analysis, identification has reached a high level of sophistication. However, for involatile and thermally unstable compounds not amenable to GC-MS analysis, important problems remain. This is illustrated by the only recent structural elucidation of DPEP (Quirke *et al.*, 1980). To date, identification of carotenoids isolated from lacustrine and marine sediments has relied on a combination of visible spectroscopy, chromatographic behavior, and mass spectrometry (Watts *et al.*, 1977; and references therein). While these techniques are generally satisfactory for major components corresponding to carotenoids of known structure and which can be isolated at the microgram level, they are not satisfactory for identifying minor components and transformation products. Consequently, isomerization of fucoxanthin and fucoxanthin degradation products was explored as a potential method of assisting in the unambiguous identification of compounds at the nanogram level.

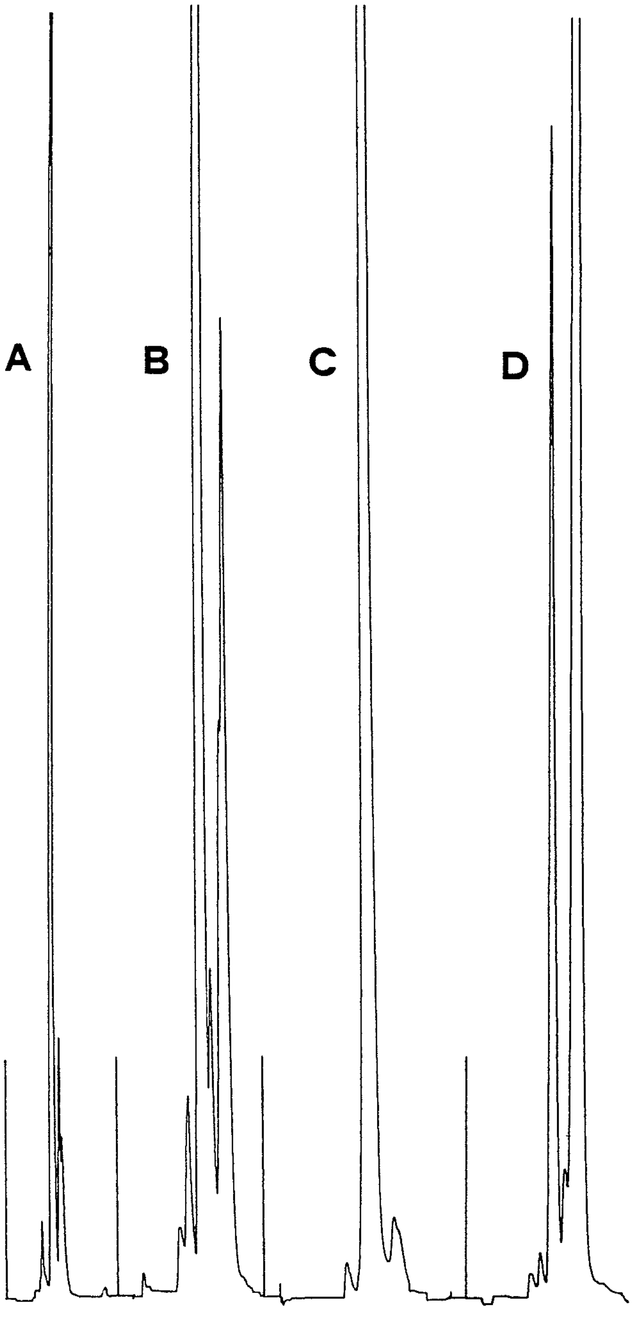
The stereomutation of carotenoids by iodine catalyzed photoisomerization has been exhaustively treated in a monograph by Zechmeister (1962). Spectroscopic properties of fucoxanthin cis isomers are discussed by Bernard *et al.*, (1974). Of relevence is the observation that complicated mixtures, with a large number of isomers, are formed upon irradiation of carotenoid/iodine solutions. The isomerization is reproducible and has the characteristics of an equilibrium mixture: it is independent of concentration, and individual isomers can be separated and

Table 7. Iodine catalyzed photoisomerization of standard fucoxanthin and fucoxanthin isolated from sediment trap experiments. Retention times of cis-isomers are normalized to the all-trans peak. Chromatographic conditions are given in the text.

Sample	RR_t (all-trans)				
Authentic fucoxanthin	0.84	0.90	1	1.15	1.41
Trap 6, all-trans	0.84	**	1	1.16	1.40
Trap 6, cis	0.84	0.89	1	1.15	1.38
Trap 7, all-trans	0.84	0.89	1	1.16	1.40

** Not resolved, appears as shoulder to the all-trans peak.

Figure 11. HPLC separation of cis/trans isomers of: A) fucoxanthin, B) isofucoxanthin, C) fucoxanthinol, and D) isofucoxanthinol produced by iodine catalyzed photoisomerization. Separation conditions: 300 x 3.9 mm 5 µm Spherisorb amino column eluted with 20% MeOH/THF (20/80) in hexane at 2 ml/min.


A
B
C
D

re-equilibrated to the original isomeric mixture. The large number of isomers generated in the stereomutation process suggests there is a low probability that two different compounds will have the same equilibrium mixture (as determined by chromatographic retention and relative abundance). Therefore, the reproducibility, concentration independence, and chromatographic resolution by contemporary HPLC make cis/trans isomerization a potentially powerful tool for identifying carotenoids isolated from geochemical samples.

The equilibrium mixture of cis/trans isomers from solutions containing 1 mg/l, 2 mg/l, 5 mg/l and 10 mg/l of fucoxanthin is given in Table 1. The data confirm the observation by Zechmeister (1962) and Bernhard _et al._, (1974) that isomerization is concentration independent and reproducible. The strikingly different isomeric mixtures of fucoxanthin, fucoxanthin 3-acetate, isofucoxanthin, fucoxanthinol, and isofucoxanthin is illustrated by the data in Tables 1, 3 and Figure 11. Under identical equilibration and chromatographic conditions fucoxanthin yielded 6, isofucoxanthin 8, fucoxanthinol 4, and isofucoxanthinol 7 cis isomers. In all mixtures, the all-trans isomer was the most abundant.

Table 7 presents the results of an experiment in which fucoxanthin isolated from Peru sediment traps #6 and #7 and standard fucoxanthin were isomerized. As in the previous set of experiments, agreement was within the expected analytical precision of the measurement (±2%). In addition, the major cis isomer of fucoxanthin (absolute stereochemistry unknown) isolated from sediment trap #6 yields an isomeric mixture identical to the all-trans fractions isolated from sediment traps #6 and #7, and standard fucoxanthin. In these experiments approximately 50 - 100 ng of pigment was analyzed. Operating at maximum sensitivity, the same measurements can be made on approximately 10 - 15 ng of material.

CONCLUSIONS

The visible spectra, ^{13}C-NMR, derivitization experiments, and mass spectrometric data are consistent with the structures of fucoxanthin (IV), isofucoxanthin (XIV), fucoxanthinol (VI), and isofucoxanthinol (XII) first proposed by Bonnett et al., (1966). Selective reduction of the fucoxanthin 3-ester with $LiAl(CH_3)_3H$ provides a simpler and higher yielding synthesis of fucoxanthinol than the $LiAlH_4$-DDQ synthesis used by Bonnett (1969).

Silylation of fucopigments with HMDS and TMS-Z provides a rapid, nondestructive method for determining the number and type of hydroxyl groups, and in determining the extent of isomerization of the 5,6-epoxide. Silylation is also useful as a means to purify carotenoids for NMR spectroscopy and mass spectrometic studies. The mass spectra of silylated fucopigments are more complex than the underivatized parents. However, interpetation is straight forward and the sensitivity is generally enhanced due to the increased volatility of these derivatives.

Iodine catalyzed photoisomerization is a promising technique for identifying carotenoids isolated from geochemical samples. The method is highly specific, non-destructive, and requires only 10-20 ng of pigment. Fucoxanthin, isofucoxanthin, fucoxanthinol, and isofucoxanthinol produce distinctly different cis/trans equilibrium mixture when isomerized under the same conditions. Fucoxanthin isomers isolated from sediment traps generate the same cis/trans equilibrium mixtures as authentic fucoxanthin.

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CHAPTER 5.

ANALYTICAL METHODS

INTRODUCTION

Carotenoids occur in sediments and suspended particulate matter as complex mixtures of biosynthetic pigments and their transformation products. Until recently, the complexity of these mixtures outstripped the techniques available for separation, precluding analysis of all but major pigments (Fox, 1937; Fox *et al.*, 1945; Jeffrey, 1974, 1976; Neveux, 1975). The introduction of high pressure liquid chromatography and mass spectrometry to non-volatile compounds have largely solved problems of separation and identification, such that quantitative analysis is now more limited by methodology (sampling, choice of chromatographic system, development of suitable internal standards, etc.) than by analytical capabilities *per se*. These developments in sophisticated analytical techniques have stimulated a renewed interest in carotenoid geochemistry. Carotenoids characteristic of specific classes of organisms have now been used to identify sources of sedimentary organic matter (Watts *et al.*, 1977; Watts and Maxwell, 1977; Hajibrahim *et al.*, 1978, Cardoso *et al.*, 1978; Griffiths *et al.*, 1978), deduce transformation pathways (Repeta and Gagosian, 1982 a,b), recognize pollution events (Griffiths and Edmondson, 1975; Griffiths *et al.*, 1969), and monitor eutrophication in Lake Zurich (Zullig, 1981).

There are numerous reports of HPLC separations of marine carotenoids (Stewart and Wheaton, 1971; Cadosch and Euguster 1974; Eskins *et al.*, 1977; Fiksdahl *et al.*, 1978; Hajibrahim *et al.*, 1978; Vecchi and Muller, 1979; Ohmacht, 1979; Eskins and Dutton, 1979; Abaychi and Riley, 1979; Paankker and Gallegraeff, 1979; Ronneberg *et al.*, 1980; Davies and

Holdsworth 1980; Schiedt et al., 1981). Schwartz and Von Elbe (1982) have reviewed HPLC analysis of plant pigments, and systematic studies of a wide variety of carotenes and xanthophylls have been reported by Fiksdahl et al., (1978) and Matus et al., (1981). In general these studies have focused on separating specific carotenoid mixtures, and have ignored the aspects of pigment degradation during analysis, reproducibility, and recovery that are fundamental to quantitative analysis.

The present chapter describes an isolation technique using cold solvent sonic extraction, gel permeation chromatography, and analytical HPLC which permits quantitative isolation of highly purified carotenoids from geochemical samples. A carotenoid not found in nature was added as an internal standard to monitor pigment degradation during sample storage and analysis. Three criteria were considered in developing the method; 1) the chromatographic system must separate the five most quantitatively important marine carotenoids (fucoxanthin, peridinin, diadinoxanthin, diatoxanthin, and astaxanthin), 2) recoveries must be quantitative at the nanogram level, and 3) carotenoids must not be transformed during analysis. Presently no published technique meets these requirements.

As in most other carotenoid analyses, the present method includes steps to extract, concentrate, and separate carotenoids from samples, and identify individual pigments on the basis of their physical properties. However the method differs from previously published techniques in several important respects. Most published methods either include the lipids extracted with carotenoids in the analysis, or remove them by methods incompatible with the recovery of many geochemically interesting pigments. For example, Watts et al., (1977) saponified sediment extracts as a means of removing lipids which interfere with mass spectrometric and HPLC analysis. Since many marine carotenoids are esters, or are unstable toward saponification (astaxanthin, fucoxanthin, peridinin) this treatment is satisfactory only for the analysis of relatively stable pigments.

The present analysis also differs in that it incorporates an internal standard to monitor recoveries and transformations which may occur as a result of the analysis. The sensitivity of modern HPLC systems has made nanogram analysis of carotenoids routine. This three to six orders of magnitude increase in sensitivity over conventional liquid column, paper, and thin layer chromatography has not been accompanied by a similar scaling of chromatographic systems. Nanogram losses of pigment are below the detection limit of conventional liquid column and thin layer chromatography, but may account for the whole sample in many geochemical analyses. Most published carotenoid HPLC analyses use silica columns. Tanaka *et al.*, (1981) have demonstrated that recovery of carotenoids from silica is not quantitative for many pigments (β-carotene (I), lutein (XIX), astaxanthin ester (III), canthaxanthin (XXV), zeaxanthin (XX), and isozeaxanthin (XLII)). In the present study astaxanthin was totally lost from samples at the 1-10 ng level using conventional packed (300 x 4.1 mm, approximately 3 grams of silica adsorbent) HPLC columns.

EXPERIMENTAL

A Waters (model 6000A pumps, 660 solvent programmer, 440 two channel detector, and U6K injector) HPLC system was used for all analysis. Pre-packed 100Å polystyrene-divinylbenzene columns for gel permeation chromatography were purchased from Waters Associates, Milford, Mass. (100Å μ Styragel). Spherisorb 5 μm amino, octadecylsilane (ODS), and silica columns for analytical HPLC separations were packed in-house using a balanced density slurry (CH_2Br_2-CH_2Cl_2) at 15,000 psi (Majors, 1972).

Particulate matter samples were sonic extracted twice in 30 ml of MeOH (20 min each) and once in 30 ml of CH_2Cl_2 (20 min). Extracts were combined, filtered through a Gelman type AE glass fiber filter, and concentrated to approximately 200 μl by vacuum rotary distillation before gel permeation chromatography. All solvents were distilled in glass (Burdick and Jackson).

Chemical ionization mass spectra were collected on either a Finnigan 3200 of 4505 quadrupole mass spectrometer interfaced with an Incos 2300 data system. Operating conditions for the Finnigan 3200 mass spectrometer were as follows: mass range 150-700 amu at 275 amu/sec, 900 μm methane reagent gas, ionization voltage 130 eV, ionization current 500 A, multiplier gain 1.5 kV, and preamplifier 10^{-7} A/V. The Finnigan 4505 mass spectrometer was operated at: mass range 150-1000 amu at 425 amu/sec, 900 μm CH_4 reagent gas, ionization voltage 100 eV, ionization current 250 μA, multiplier gain 1 kV, conversion dynode 3kV, and preamplifier 10^{-7} A/V. Unless otherwise noted, all manipulations were carried out at room temperature and under low light conditions.

RESULTS AND DISCUSSION

Gel Permeation Chromatography Separation of carotenoids from chlorophylls and other non-carotenoid lipids was considered necessary as a means of simplifying HPLC and mass spectrometric analysis. Eskins and Dutton

(1979) described a simple method of separating carotenoids from polar lipids using a short ODS column. Chlorophylls were retained with carotenoids on the ODS column and made subsequent HPLC separations more difficult. Watts _et al._ (1977) saponified sediment extracts, then partitioned carotenoids into ether. The ether solution was passed through an alumina column prior to HPLC analysis. Since astaxanthin, fucoxanthin, and peridinin are unstable towards saponification and chromatography on alumina, this method is unsatisfactory for the analysis of these pigments in recent sediments.

In principal, carotenoids can be separated from chlorophylls and most non-carotenoid lipids on the basis of their molecular size. Zwolenik (1970) reported the separation of carotenoids from chlorophylls on crosslinked polystyrene divinylbenzene gels. Highly crosslinked semi-rigid polystyrene divinylbenzene gels have been used in the separation of n-alkanes (C_1-C_{36}), aromatic hydrocarbons, alcohols (C_1-C_{16}), glycol ethers, esters (phthalates), triglycerides, quinones, phenols, acids (C_1-C_{16}), ethers (glycol), and diamines (Krishen and Tucker, 1977; Hausler _et al._ 1979; and references therein). 100Å μStyragel has an exclusion limit of approximately 1000 amu polystyrene, and an optimal separation range of between 400-100 amu polysytrene. This gel should exclude carotenoids and efficiently separate them from low molecular weight lipids. Table 1 presents retention time data for thirteen carotenoids, chlorophylls -_a_ and -_b_, and representative alkanes, aromatic hydrocarbons, wax esters, fatty acids, and sterols on two 100Å μ Styragel columns in series eluted with CH_2Cl_2 at 2 ml/min. Carotenoids elute shortly after the dead volume (approximately 10 ml) and

Table 1. Retention volumes (R_V) for carotenoids, chlorophylls, alkanes, aromatic hydrocarbons, triglyerides, fatty acids, and sterols on two 100Å μStyragel columns in series eluted with dichloromethane at 2 mls/min.

	R_V(mls)	MW
3,3'-Diols		
Fucoxanthin (IV)	11.94	658
2,2'-Dihydrolycopene (XLIII)	12.12	568
Diadinoxanthin (III)	12.22	582
Lutein (XIX)	12.28	568
Zeaxanthin (XX)	12.28	568
Ketones		
Astacene (IX)	12.06	596
canthaxanthin(XXV)	12.02	566
echinenone (XXVI)	12.38	550
4,4'-alcohols		
Isozeaxanthin (XLII)	12.40	568
Isocryptoxanthin (XLIV)	12.60	552
Carotenes		
Lycopene (XLV)	12.30	536
β-carotene (I)	12.78	536
α-carotene (XLVI)	12.74	536
Docosane (n-C_{22})	12.00	310
Tripalmitin	12.38	806
Tristearin	12.48	890
Oleic acid	13.50	282
Squalane	14.72	412
Cholesterol	16.04	386
Stigmasterol	16.04	412
Desmosterol	16.04	384
β-sitosterol	16.04	414
Campesterol	16.04	400
Napthalene	20.80	128
Benzene	21.20	78

are not well separated. Retention volume correlates with apparent molecular size, acyclic carotenoids elute before their bicyclic isomers (lycopene 12.30 ml vs. 12.76 ml for α,β-carotene; 2,2'-dihydroxylycopene 12.12 ml vs. 12.28 ml for lutein and zeaxanthin) and double bond isomers (α,β-carotene; lutein and zeaxanthin) are not separated. Within each carotenoid subclass listed in Table 4 (hydrocarbons, ketones, 4,4'-alcohols, 3,3'-diols) retention time (R_t) follows molecular weight: R_t astacene (tetra-ketone) > canthaxanthin (di-ketone) > echinenone (mono-ketone).

β-carotene was the most strongly retained carotenoid. Good separation of β-carotene from chlorophylls -a and -b, fatty acids $\leq C_{18}$, squalane, sterols, and low molecular weight aromatic hydrocarbons was achieved on two 30 cm columns connected in series (Figure 1). Separation of total carotenoids from total chlorophylls extracted from the marine dinoflagellate Peridinium tricornatum was greater than 99.7% (as measured by absorbance at 665 nm of the collected fractions).

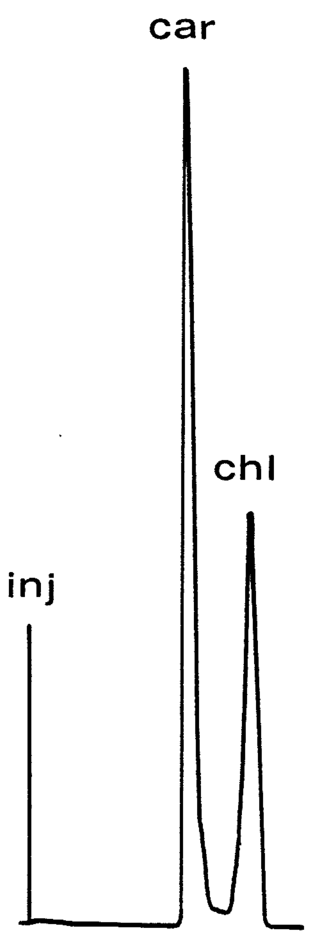
Table 2 presents data for the recovery of β-carotene (I), canthaxanthin (XXV), isozeaxanthin (XLI), lutein (XIX), and fucoxanthin (IV) after gel treatment. A standard solution of these five pigments was volumetrically split in half, one half run through the gel column, and collected. Both samples (untreated and gel treated) were taken to dryness and redissolved in equal amounts of methylene chloride. Aliquots of the untreated and gel treated standard mixtures were alternately injected onto a Waters μPorasil column eluted with 25% acetone in hexane at 4 ml/minute. Greater than 91% recovery was observed for all pigments. The gel treated sample was systematically lower than the untreated sample,

Table 2. Recovery of β-carotene, canthaxanthin, isozeaxanthin, lutein, and fucoxanthin from μ Styragel. Chromatographic conditions given in Table 1 legend.

Run	Peak Height								Mean	R*
	1	2	3	4	5	6	7	8		
Untreated										
β-carotene	180	184	199	191	218	151	167	177	183± 14	
canthaxanthin	142	155	159	173	173	124	137	148	151± 11	
isozeaxanthin	141	151	158	152	179	121	132	147	148± 12	
Lutein	162	167	174	169	191	140	144	147	162± 11	
Fucoxanthin	159	160	169	164	183	142	147	146	159± 10	
Gel Treated										
β-carotene	165	158	167	166	172	160	161	174	165± 4.4	91%
Canthaxanthin	139	136	143	140	153	137	141	152	143± 5.1	95%
Isozeaxanthin	131	127	134	131	142	128	137	143	134± 5.0	91%
Lutein	146	141	149	146	157	152	146	158	148± 4.6	91%
Fucoxanthin	150	142	151	149	159	155	149	165	153± 5.5	96%
Untreated	Normalized Peak Height									
β-carotene	128	125	126	126	122	125	126	120	125± 2.4	
Canthaxanthin	101	103	101	101	97	102	104	100	101± 2.0	
Isozeaxanthin									100	
Lutein	115	110	110	111	107	116	109	100	110± 3.2	
Fucoxanthin	113	106	107	108	102	118	111	100	108± 5.5	
Gel Treated										
β-carotene	126	124	125	127	121	125	118	122	124± 2.8	
Canthaxanthin	106	107	107	107	108	107	103	106	106± 1.4	
Isozeaxanthin									100	
Lutein	111	111	111	111	110	119	106	110	111± 3.4	
Fucoxanthin	114	112	113	114	112	121	109	115	114± 3.2	

*Recovery

Figure 1. HPLC separation of carotenoids (car) and chlorophylls (chl) extracted from Peridinium trichoidium on two 100 Å μ Styragel columns in series eluted with methylene chloride at 1.5 ml/min. UV detection at 436 nm.


car
chl
inj

suggesting the difference in recovery may be due to sample dilution rather than on-column or handling losses. This hypothesis is supported by the normalized data presented in Table 2. The only significant difference observed between the two samples was for canthaxanthin, where the range for the two samples differed by 1%.

High Pressure Liquid Chromatography Separations of phytoplankton and zooplankton pigments have been reported on reverse phase ODS (Eskins *et al.*, 1977; Daves and Holdsworth, 1980), and silica (Abaychi and Riley, 1979; Paankker and Gallegraeff, 1979). It is difficult to compare different chromatographic systems. Typically, resolution and selectivity are considered. However, these parameters are dependant on analysis time, choice of gradient, and the specific compounds being separated; such that choice of an optimal system is highly subjective. Four criteria were used in the choice of an optimal system for the present study: 1) the system must separate fucoxanthin, peridinin, diadinoxanthin, and astaxanthin, 2) recovery of pigments must be quantitative, 3) analysis time must be ≤ 1 hour, and 4) effective plates per second (N_{eff}/sec) should be maximized.

Table 3 summarizes data for three chromatographic sytems evaluated with the above criteria: an ODS column eluted with aqueous MeOH, silica eluted with hexane/acetone, and an n-propylamino (hereafter amino) column eluted with hexane/(THF/MeOH). Reverse phase separation on ODS provides good separation of fucoxanthin, peridinin, diadinoxanthin, and astaxanthin (Figure 2). Analysis time was under one hour and all pigments were quantitatively recovered. Values of N_{eff}/sec ranged from 0.53 (fucoxanthin) to 3.2 (diadinoxanthin). Comparable separations and

Table 3. Comparison of carotenoid separations on ODS, silica, and amino columns.

Column	Comments	Recovery	Analysis Time #	N_{eff}/sec
ODS***	Good separation of peridinin, fucoxaxthin, diadinoxanthin, and astaxanthin.	all pigments recovered	50	0.8:0.5:3.2:2.9
Amino**	Good separation of peridinin, fucoxanthin, diadinoxanthin, and astaxanthin.	all pigments recovered	60	1.4:2.3:5.8:8.9
Silica*	Good separation of fucoxanthin and diadinoxanthin.	astaxanthin not recovered	60	not determined

N_{eff}/sec = $(16(R_t/w)^2/R_t)$, where R_t is retention time (min) and w is peak widith at base (min). Ratios expressed as peridinin: fucoxanthin:diadinoxanthin:astaxanthin.

*** 80-100% aqueous methanol, linear 1 hour gradient at 1.5 mls/min.

** 0-13% MeOH/THF (20/80) in hexane, linear 45 minute gradient at 2 mls/min.

* 3-75% acetone in hexane, linear 20 minute gradient at 2 mls/min.

in minutes

analysis times were achieved using an amino column. However, values of N_{eff}/sec were up to a factor of 5 higher than measured on ODS.

Separation of fucoxanthin, peridinin, diadinoxanthin, and astaxanthin on silica was not successful. Astaxanthin was completely removed by the column at the 1-10 ng level. Very broad peaks could be observed with the application of 1-10 μg of astaxanthin (Figure 3). Paankker and Gallegraeff (1979) reported similar tailing of astaxanthin on silica during separation of zooplankton pigments. Tanaka _et al._ (1981) report extensive degradation of astaxanthin ester on conventional liquid columns packed with silica. Similarly, Englert and Vecchi (1980) were unable to separate astaxanthin cis/trans isomers on silica HPLC although 70 different solvent systems were tested. Apparently astaxanthin reacts irreversibly with this adsorbent.

To further test this hypothesis a comparative study was made of a conventional HPLC column (4.1mm i.d.) packed with 3 g, and a microbore column (1 mm i.d.) packed with 200 mg of Spherisorb 5 μm silica. Flow rates were adjusted to produce equivalent column volume/sec. The results are presented in Figure 3. Good recovery of approximately 10 ng of astaxanthin was achieved on the microbore columns. As in the preliminary optimization experiments, astaxanthin was not recovered from the conventional HPLC column (4.1 mm i.d.) at the 10 ng level. Experiments with astacene gave similar results. Since the distribution of astaxanthin, astaxthin esters and astacene in sediments and suspended particulate matter are of interest in the present study, and in view of the transformation of 5,6 epoxides on silica reported by Strain _et al._ (1967), further separations using silica were not pursued.

Figure 2. HPLC separation of 1) peridinin, 2) fucoxanthin, 3) astaxanthin and 4) diadinoxanthin on 5 μm Spherisorb ODS (300 x 3.9 mm, eluted with 80-100% aqueous MeOH, 1 hr linear gradient at 1.5 ml/min) and on 5 μm Spherisorb n-propyl amino (300 x 3.9 mm, eluted with a 45 min linear gradient of 0-13% MeOH/THF (20/80; v/v) in hexane at 2 ml/min). UV detection at 436 nm.

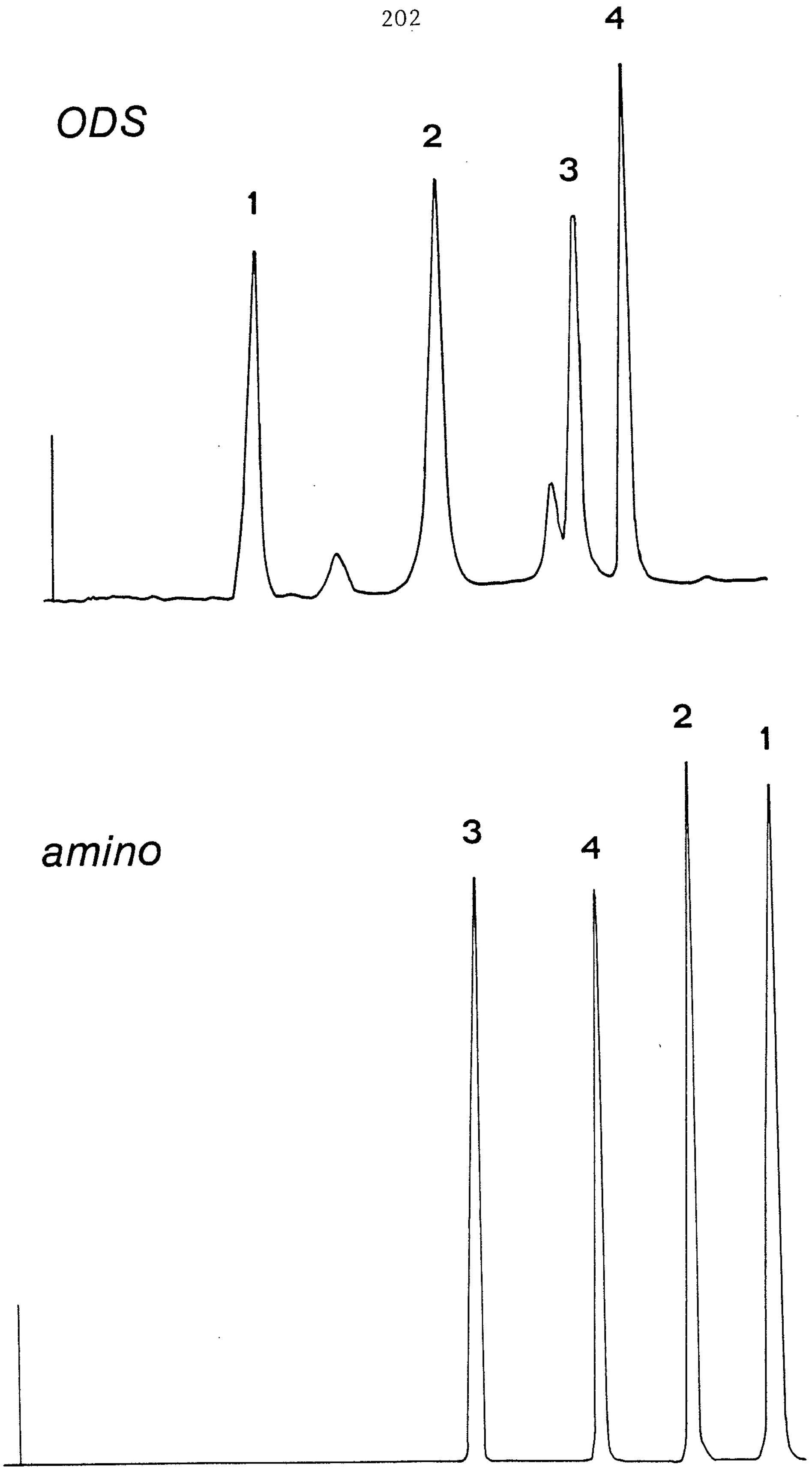
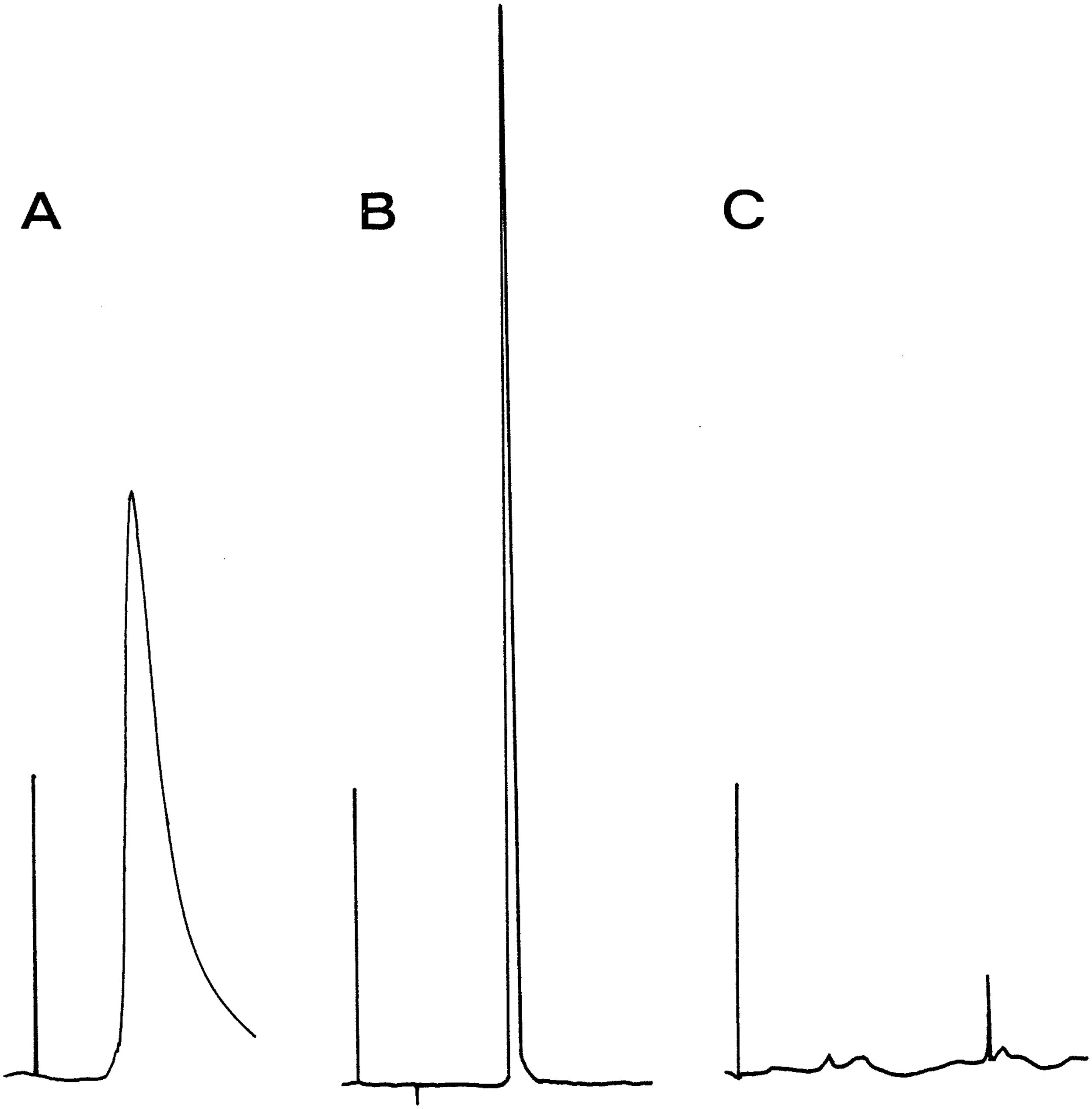

ODS
1
2
3
4
amino
3
4
2
1

Figure 3. HPLC of astaxanthin on silica column: A) Approximately 5 μg astaxanthin from a 300 x 4.1 mm 5 μm Spherisorb silica column eluted with 50/50 acetone/hexane at 2 ml/min. B) 10 ng astaxanthin from a 300x1 mm 5 μm Spherisorb silica column eluted with 35% acetone in hexane. C) 10 ng astaxanthin injected on a 300 x 4.1 mm 5 μm Spherisorb silica column eluted with 35% acetone in hexane. Flow rates adjusted to give equal column volumes/sec.


A
B
C

ODS and amino columns give equally good separation of fucoxanthin, peridinin, diadinoxanthin, and astaxanthin. Efficiencies on amino columns are higher than on ODS, affording greater resolution of complex mixtures (Figure 2). The latter technique has the added advantage that fractions can be collected in organic solvents which can be easily removed for further spectroscopic analysis. Glass lined 300 x 3.9 mm columns were packed with Spherisorb 5 μm amino using a balanced density CH_2Br_2:CH_2Cl_2 slurry at 15,000 psi. Column efficiencies of 15,000 plates/m were typically achieved. Linear gradients of 0-10%, 0-11%, 0-12%, 0-13%, 0-14%, and 0-15% MeOH/THF (20/80) in hexane were run to optimize separation and efficiency. A 45 min linear gradient of 0-13% MeOH/THF in hexane provides a good compromise of analysis time and separation. More difficult separations can be achieved by decreasing the concentration of MeOH/THF in the final solvent mixture. Retention times and peak areas were reproducible to ± 2% and ± 5% respectively. Detector response was linear for injections of 1.7-170 ng of β-carotene (least squares regression correlation coefficient 0.9999).

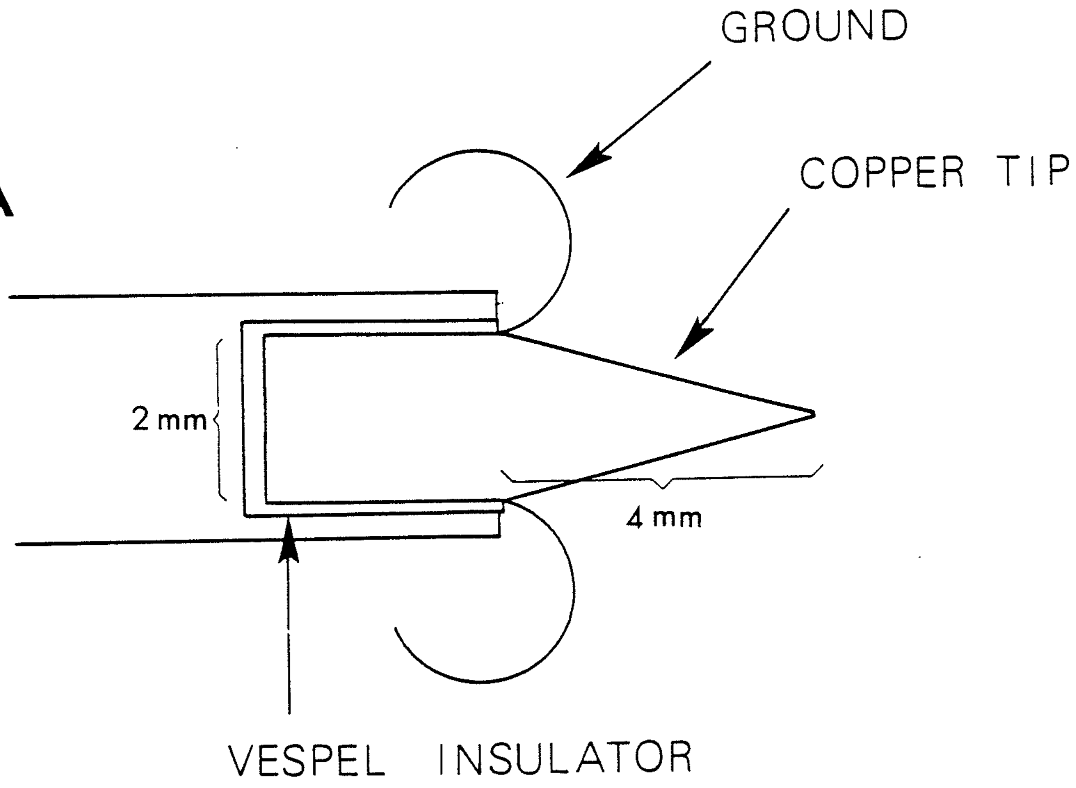
Mass Spectrometry Mass spectrometry of carotenoids has been accomplished using electron impact (EI) (Moss and Weedon, 1976), chemical ionization (CI) (Carnevale *et al.*, 1978), and field desorption (FD) (Watts *et al.*, 1975) techniques. EI mass spectrometry usually yields complex spectra with few high mass fragments (Watts, 1975). CI and FD techniques both lead to simpler spectra with enhanced abundances of high mass fragments. Field desorption has the added advantage that ionization occurs on the surface of the emitter, avoiding problems of thermal decomposition during heating to volatilize the sample. Recent advances in the mass spectrometry of nonvolatile and thermally labile molecules have focused primarily on sample ionization (Daves, 1979 and references therein). Hunt et al. (1977), Hansen and Munsen (1978), Cotter (1979), and Thenot et al. (1979) have described the use of in-beam sample introduction to obtain CI mass spectra of high molecular weight, thermally unstable lipids and nonvolatile salts. The technique has the advantage of low cost and requires only a very simple modification of the mass spectrometer.

In-beam probe studies have emphasized the importance of probe material. Ideally, the probe surface should be inert towards the sample and have good thermal conductivity for rapid heating. Hunt et al. (1977) used a tungsten wire coated with graphitized carbon. Hansen and Munsen (1978), Cotter (1979), and Thenot et al. (1979) used teflon, vespel, and glass probes respectively. Carroll et al. (1979) compared probes constructed of different materials and concluded that the composition of the probe tip was not critical. However, probes constructed from materials with poor electrical conductivity often produced spectra with erratic peaks due to localized charges on the probe suface. A siloxane coated copper tip was tested and found to give reproducible spectra comparable to those collected using teflon, vespel, or glass probes.

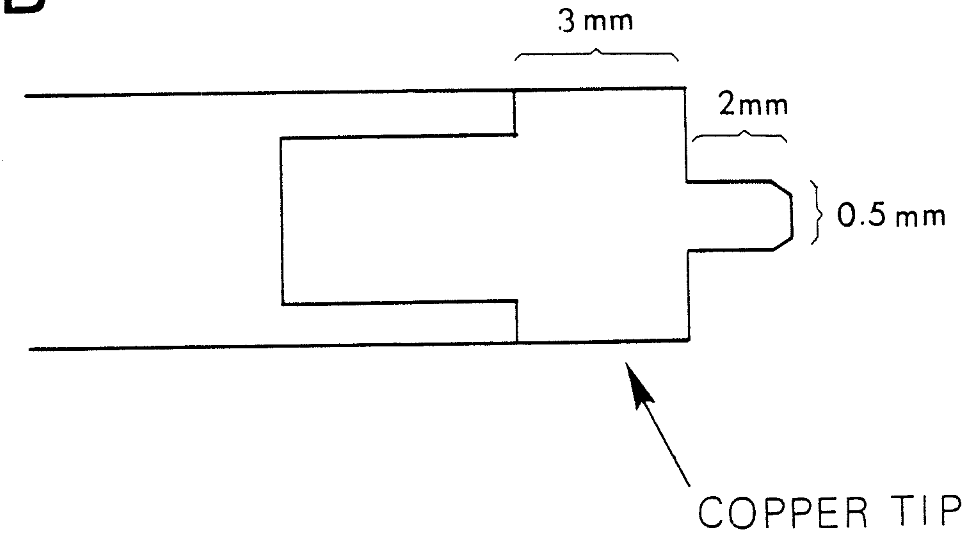
Glass and copper probes were fabricated to replace the recessed quartz sample holder of the Finnigan 3200 (Figure 4). Samples dissolved in CH_2Cl_2 were applied to the probe tip and the solvent allowed to evaporate. The probe tip was inserted into the edge of the ion beam (as monitored by a sharp change in the ion plasma), and ballistically heated to 400°C at approximately 100°C/min. Sensitivity varied with heating

Figure 4. Schematic diagram of "in beam" direct insertion probes for A) Finnigan 3200 and B) Finnigan 4505 mass spectrometers.

A


GROUND
COPPER TIP
2 mm
4 mm
VESPEL INSULATOR

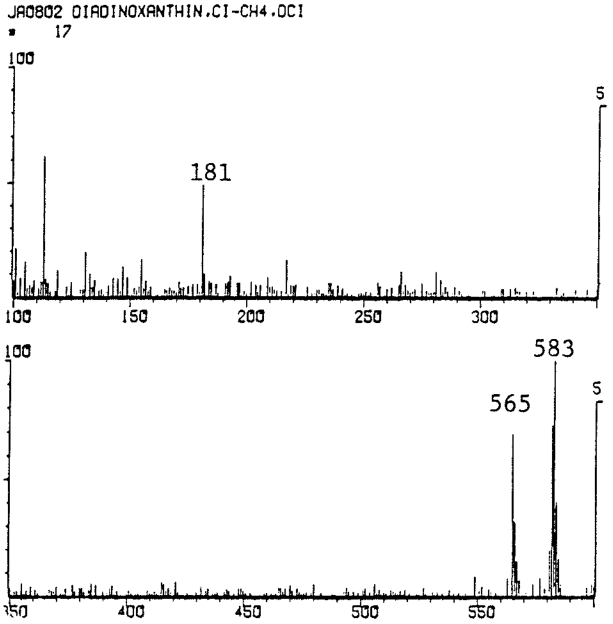
B

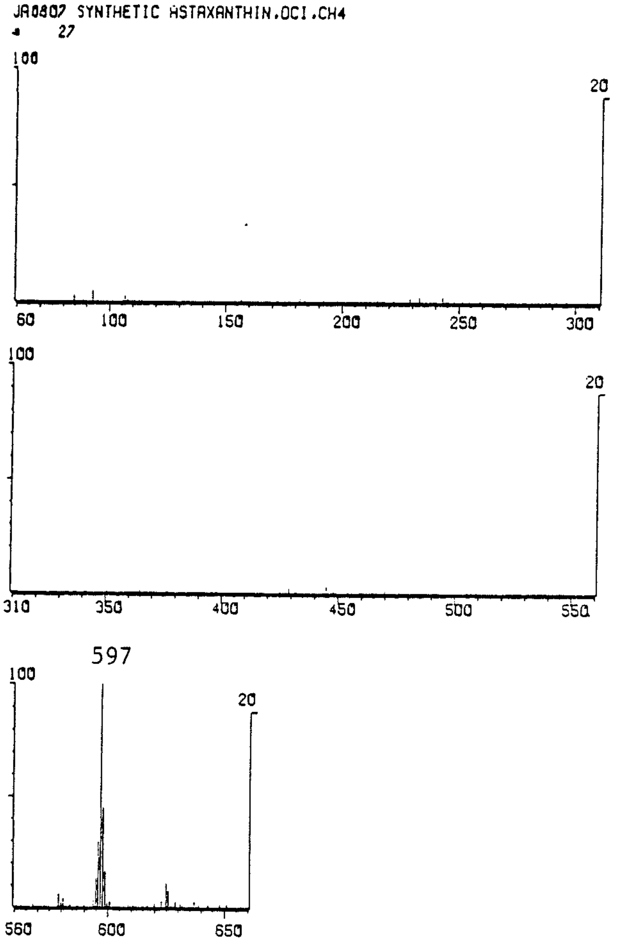

3 mm
2mm
0.5 mm
COPPER TIP

rate. Slow probe heating (20°C/min) resulted in extensive thermal decomposition of the sample and poor sensitivity. In general, larger sample loads had to be applied to the glass probe than to the copper probe to yield comparable spectra. Greater than 100 ng of β-carotene had to be applied to the glass probe for single scan detection ($S/N > 2$). Detection limits for the copper probe were 4 ng for β-carotene, 13 ng for diadinoxanthin, and 55 ng for fucoxanthin. This difference in sensitivity most likely results from the poor thermal conductivitiy and slow heating rate of the glass probe. Addition of a β-carotene carrier to the fucoxanthin standard to a final concentration ratio of 2:1, β-carotene:fucoxanthin did not lead to any measurable increase in sensitivity for fucoxanthin. Coating the copper tip with OV-101 similarly did not lead to any enhancement of sensitivity. Representative spectra of diadinoxanthin and astaxanthin are given in Figure 5.

Internal Standards Neoxanthin acetate (XVI) was used as an internal standard to monitor pigment recoveries and degradation during analysis. Neoxanthin isolated from spinach was acylated with acetic anhydride in DMAP/pyridine. After 5 min, the reaction was quenched with saturated aqueous $CaCO_3$ and the pigments extracted into CH_2Cl_2. The extract was dried over $NaSO_4$, filtered, and neoxanthin acetate purified by HPLC (amino column, 5% MeOH/THF in hexane). Neoxanthin acetate has 5,6 epoxide, 5'-hydroxy, and 3,3'-diacetyl functional groups. Therefore, this pigment can be used to monitor acid rearrangement of the 5,6-epoxide in diadinoxanthin, dehydration of the 5'-hydroxyl in fucoxanthin, and ester hydrolysis of the 3'-acetate in fucoxanthin during analysis. The resulting degradation products (5,8-furanoxide, 5'-ene, and 3'-alcohol)

Figure 5. Chemical ionization mass spectra of diadinoxanthin and astaxanthin. Conditions: Finnigan 3200 mass spectrometer, scanned from 100-700 amu at 300 amu/sec, 900 μm CH_4 reagent gas, ionization voltage 130 eV, ionization current 500 μA.


JA0802 DIADINOXANTHIN.CI-CH4.DCI
* 17
100
S
181
100
150
200
250
300
100
583
565
S
350
400
450
500
550


JA0807 SYNTHETIC ASTAXANTHIN.DCI.CH4
* 27
100
20
60
100
150
200
250
300
100
20
310
350
400
450
500
550
597
100
20
560
600
650

can be separated from the parent compound using an amino column eluted with 0-13% MeOH/THF (20/80) in hexane.

Application of method Suspended particulate matter and sediment trap samples were collected with 10 ℓ Niskin bottles off the coast of Mexico (17° 30.8' N, 109° 15.3' W). Samples were filtered through Gelman type AE glass fiber filters, and the filters stored for approximately one month at -20°C in foil wrapped glass vials filled with MeOH. Immediately before storage 0.5 ml of 5.0 μM/l (1.71 μg) neoxanthin acetate in methanol was pipetted into the samples. Blank filters in MeOH were also spiked with neoxanthin acetate and stored as described for the samples.

After returning to the lab, filters were sonic extracted in MeOH (twice, 20 min each) and CH_2Cl_2 (once, 20 min). Extracts (MeOH and CH_2Cl_2) were combined, filtered, and concentrated by vacuum rotary evaporation. Blank samples were analyzed directly by HPLC (amino column). Particulate matter samples were subjected to gel permeation chromatography prior to amino column HPLC.

Quantitative analysis of blank and sample filters showed that 93% of the neoxanthin acetate was recovered from the samples relative to the blanks (Figure 6). Epoxide rearrangement to the 5,8 furanoxide was observed for both neoxanthin acetate and diadinoxanthin in all samples. No dehydration or ester hydrolysis of neoxanthin acetate or fucoxanthin was observed in suspended particulate matter, however significant concentrations of fucoxanthinol were measured in sediment trap samples. Samples which contained fucoxanthinol did not contain neoxanthin, therefore transformation of fucoxanthin to fucoxanthinol is not a result of either sample storage or analysis. Analysis of a standard mixture of neoxanthin acetate, diadinoxanthin, fucoxanthin, astaxanthin, and peridinin showed that no transformations resulted from the method. However, if the sample is taken to dryness, equilibration with cis isomers will occur. Since no epoxide rearrangement results from the analysis, the neochrome acetate and diadinochrome measured in the samples are considered to arise from storage.

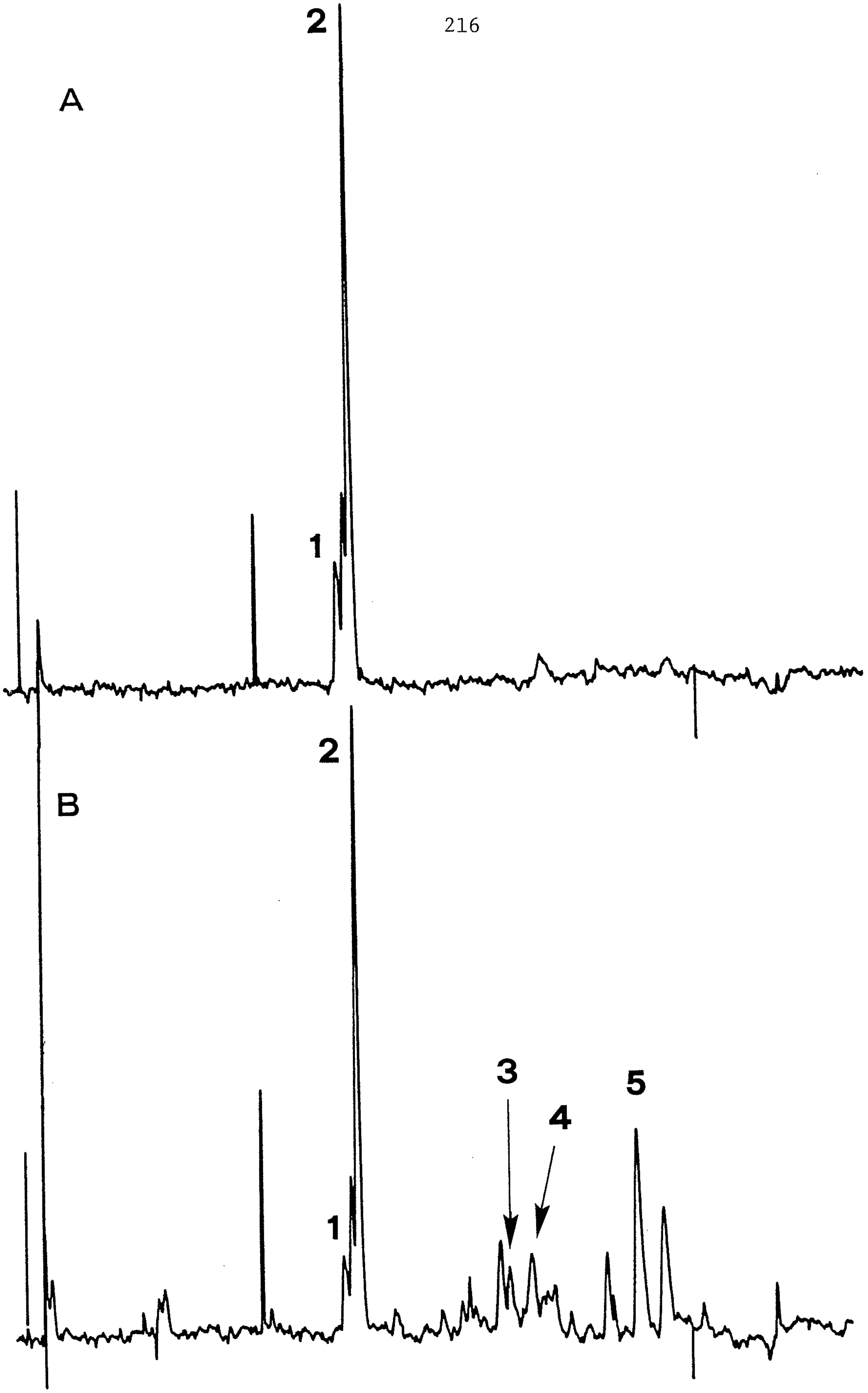
CONCLUSIONS

The carotenoid distribution in extracts from geochemical samples can be quantitatively analyzed at the nanogram level by a combination of gel permeation chromatography, bonded n-propyl amino phase analytical HPLC, and "in beam" mass spectrometric techniques. Gel permeation chromatography on 100Å polystyrene-divinylbenzene columns provides a rapid, mild, and reproducible method for separating carotenoids from chlorophylls and low molecular weight lipids. Carotenoids are almost totally excluded from the gel and elute as a single fraction.

A comparison of analytical separations on amino, silica, and ODS columns showed that good separation of fucoxanthin, peridinin, diadinoxanthin and astaxanthin is afforded by n-propyl amino columns eluted with 0-13% MeOH/THF (20/80) in hexane. Separations on silica lead to extensive loss of astaxanthin and related pigments due to irreversible adsorption onto the column. Good separations of phytoplankton and zooplankton pigments were also achieved on ODS columns eluted with aqueous MeOH, however N_{eff}/sec were up to a factor of 5 lower than measured on amino columns.

Greater than 92% recovery of neoxanthin acetate from samples and blanks was measured in replicate analysis. No transformations resulted from the analysis. However, rearrangement of 5,6 epoxides to 5,8 furanoxides was observed during storage of samples at $-20^{\circ}C$ for periods of one month.

Figure 6. Separation of carotenoids in A) blank, and B) suspended particulate matter sample collected off the coast of Mexico. The blank was prepared and stored at the same time as the sample. Compound identification: 1) neochrome acetate (XLV), 2) neoxanthin acetate (XVI), 3) diadinochrome (X), 4) diadinoxanthin (III), and 5) fucoxanthin (IV). Separation conditions given in Figure 2 legend.


A
1
2
B
1
2
3
4
5

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SUMMARY AND CONCLUSIONS

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Three classes of fucoxanthin transformation products were isolated from oceanic suspended particulate matter: free alcohols (fucoxanthinol, isofucoxanthinol, fucoxanthinol 5'-dehydrate, and isofucoxanthinol 5'-dehydrate), opened epoxides (isofucoxanthinol and isofucoxanthinol 5'-dehydrate), and 5'-dehydrates (fucoxanthin 5'-dehydrate, fucoxanthinol 5'-dehydrate, and isofucoxanthinol 5'-dehydrate). Numerous other pigments which did not coorespond to major phytoplankton or zooplankton carotenoids were also detected in sediment trap and fecal pellet samples. Mass spectrometric characterization of these pigments suggests they originate from marine phytoplankton. However, the specific structures of these pigments is as yet undertermined.

Rapid hydrolysis of fucoxanthin to fucoxanthinol by higher heterotrophs was observed in sediment trap samples collected in both Buzzards Bay and off the Peru coast. Hydrolysis of triglycerides, chlorins, and wax esters in the oceanic water column and surface sediments has been proposed to explain the distribution of free fatty acids, chlorin free acids, and alcohols in Recent sediments. It seems reasonable that hydrolysis is a quite general transformation reaction operative on diverse classes of organic esters and mediated by a wide variety of higher heterotrophs.

Schuman and Lorenzen (1975) have provided circumstantial evidence for the hydrolysis of chlorophyll-a to phaeophorbide-a by zooplanktonic herbivores. The data of the present study supports this conclusion, and suggests a need for direct experiments in which the hydrolysis of chlorophyll, carotenoid, and other organic esters can be monitored simultaneously.

5'-Dehydrates were observed in Buzzards Bay sediment trap material and fecal pellet samples. Like ester hydrolysis, dehydration may be a general transformation pathway operative on diverse classes of compounds. Dehydration products of sterols (Dastillung and Albrecht, 1977; Gagosian et. al., 1980), phytol (Simoneit, 1973; De Leeuw et. al., 1977), and amino acids (Bada and Hoopes, 1979) have been reported in a number of marine environments. However, unlike ester hydrolysis, there are no data which suggests a common transformation process. Both chemically mediated acid catalyzed and microbially mediated metabolic reactions have been proposed. An acid catalyzed chemical dehydration seems unlikely due to the coexistence of unrearranged diadinoxanthin and dehydrates in some samples. More direct evidence could come from further studies of expected peridinin, dinoxanthin, and neoxanthin dehydrates. These carotenoids contain both a 5,6 epoxide and 5'-alcohol within the same molecule.

A second, but more indirect route to establishing the mechanism of dehydration could come from simulation experiments of potentially important transformation processes. Dehydration of the tertiary 5'-alcohol can result in three products which differ in the position of double bond formation: the 4',5'-ene, 5',18'-ene, and the 5',6'-ene-7',8'-yne. Acid catalyzed chemical dehydration appears to be nonspecific, and all three isomers are observed. There are no reports of bacterial dehydration of carotenoids and the distribution of double bond isomers produced by this pathway is not known. However, studies of dehydration using enrichment cultures (Taylor et. al., 1981) should enable determination of the isomer distribution resulting from this dehydration pathway.

Another area which warrents further research is the distribution of carotenoids, especially opened epoxides of fucoxanthin, in suspended particulate matter collected below the euphotic zone, i.e the zone of synthesis for phytoplankton pigments. Unaltered phytoplankton derived carotenoids were observed in suspended particulate matter samples collected as deep as 1500 m. Assuming a fucoxanthin to organic carbon ratio of 0.005, then approximately 0.4 μg/ℓ of carbon can be accounted for by undegraded phytoplanktonic material. Using an average deep water value for POC of 5 μg/ℓ (Cauwett, 1981), some 8% of the total carbon at 1500 m is supplied by relatively undegraded phytoplankton.

Epoxide opening is thought to arise from a base catalyzed chemically mediated transformation. Assuming that the material collected in the Buzzards Bay sediment traps has an average age of 10 yr, and that the reaction follows first order kinetics, a rate constant of $k=0.139\ \mathrm{yr}^{-1}$ is calculated for this reaction. If the rate constant for epoxide opening in Buzzards Bay approximates that for the deep sea, then a residence time of less than 72 yr for the particulate matter collected at 1500 m (based on a 0.2 ng/ℓ detection limit for isofucoxanthin) is calculated. This residence time is in general agreement with the ^{14}C derived residence time measured by Williams _et. al._ (1978) for POC collected at 2000 m in the eastern North Pacific Ocean.

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APPENDIX I

Astacene (IX) 3,3'-Dihydroxy-2,3,2',3'-tetradehydro-β,β-carotene-4,4'-dione

Astaxanthin (II) 3,3'-Dihydroxy-β,β-carotene-4,4'-dione

3,3'-*bis*(Trimethylsilyl) fucoxanthinol diether (XXXV) 3,3'-*bis*(Trimethylsiloyl)-5,6-epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-8-one

3,5'-*bis*(Trimethylsilyl) fucoxanthinyl diether (XXXI) 3,5'-*bis*(Trimethylsiloyl)-5,6-epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-8-one 3'-acetate

α-Carotene (XLVI) β,ε-Carotene

β-Carotene (I) β,β-Carotene

Canthaxanthin (XXV) β,β-Carotene-4,4'-dione

Capsanthin (XVI) 3,3'-Dihydroxy-β,κ-caroten-6'-one

Diadinochrome (X) 5,8-Furanoxy-7',8'-didehydro-5,8-dihydro-β,β-carotene-3,3'-diol

Diadinoxanthin (III) 5,6-Epoxy-7',8'-didehydro-5,6-dihydro-β,β-carotene-3,3'-diol

Diatoxanthin (XXIII) 7,8-Didehydro-β,β-carotene-3,3'-diol

2,2'-Dihydrolycopene (XLIII) 2,2'-Dihydroxy-ψ,ψ-carotene

Dinoxanthin (XXVII) 5,6-epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,5',6',-tetrahydro-β,β-carotene 3'-acetate

Echinenone (XXVI) β,β-Caroten-4-one

Fucoxanthin (IV) 5,6-Epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-8-one 3'-acetate

Fucoxanthin 3-acetate (XIII) 5,6-Epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-8-one 3,3'-diacetate

Fucoxanthin 5'-dehydrate (XI) 5,6-Epoxy-3,3'-dihydroxy-6',7',18'-tridehydro-5,6,7,8,6'-pentahydro-β,β-caroten-8-one 3'-acetate

Fucoxanthinol (VI) 5,6-Epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-8-one

Fucoxanthin-8-ol (XXXIII) 5,6-Epoxy-3,8,3',5'-tetrahydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-carotene 3'-acetate

Fucoxanthinol 5'-dehydrate (XI) 5,6-Epoxy-3,3'-dihydroxy-6',7',18'-tridehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-8-one

Fucoxanthol (XXXIV) 5,6-Epoxy-3,8,3',5'-tetrahydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-carotene

Isocryptoxanthin (XLIV) β,β-Caroten-4-ol

Isofucoxanthin (XIV) 3,5,3,'5'-Tetrahydroxy-6',7'-didehydro-5,8,5',6'-tetrahydro-β,β-caroten-8-one 3'-acetate

Isofucoxanthinol (XII) 3,5,3',5'-Tetrahydroxy-6',7'-didehydro-5,8,5',6'-tetrahydro-β,β-caroten-8-one

Isofucoxanthinol 5'-dehydrate (VIII) 3,5,3',-Trihydroxy-6',7',18'-tridehydro-5,8,6'-trihydro-β,β-caroten-8-one

Isozeaxanthin (XLII) β,β-Carotene-4,4'-diol

Lutein (XIX) β,ε-Carotene-3,3'-diol

Lycopene (XLV) ψ,ψ-Carotene

Mimulaxanthin (XVIII) 6,7-Didehydro-5,6,5',6'-tetrahydro-β,β-carotene-3,5,3',5'-tetrol

Monadoxanthin (XXIV) 7,8-Didehydro-β,ε-carotene-3,3'-diol

Neochrome acetate (XLVII) 5,8-Epoxy-6',7'-didehydro-5,8,5',6'-tetrahydro-3,3',5'-trihydroxy-β,β-carotene 3,3'-diacetate

Neoxanthin (XV) 5,6-Epoxy-6',7'-didehydro-5,6,5',6'-tetrahydro-3,3',5'-trihydroxy-β,β-carotene

Neoxanthin acetate (XVI) 5,6-Epoxy-6',7'-didehydro-5,6,5',6'-tetrahydro-3,3',5'-trihydroxy-β,β-carotene 3,3'-diacetate

Okenone (XL) 1'-Methoxy-1',2'-dihydro-χ,ψ-caroten-4'-one

Peridinin (V) 5,6-Epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,5',6'-tetrahydro-10,11,20-trinor-β,β-caroten-19',11'-olide 3'-acetate

Peridinin 3-acetate (XVII) 5,6-Epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,5',6'-tetrahydro-10,11,20-trinor-β,β-caroten-19',11'-olide 3,3'-diacetate

Peridinin 5'-dehydrate (XXVIII) 5,6-Epoxy-3,3'-dihydroxy-6',7',18'-tridehydro-5,6,6'-trihydro-10,11,20-trinor-β,β-caroten-19',11'-olide 3'-acetate

Peridininol (XXIX) 5,6-Epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,5',6'-tetrahydro-10,11,20-trinor-β,β-caroten-19',11'-olide

3,5,3',5'-*Tetrakis*(Trimethylsilyl) isofucoxanthinol tetraether (XXXIX) 3,5,3',5'-*Tetrakis*(Trimethylsiloyl)-3,5,3',5'-tetrahydroxy-6',7'-didehydro-5,8,5',6'-tetrahydro-β,β-caroten-8-one

3-(Trimethylsilyl) fucoxanthinyl ether(XXX) 3-Trimethylsiloyl-5,6-epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-8-one 3'-acetate

5'-(Trimethylsilyl) fucoxanthinyl 3-acetate (XXXII) 5'-Trimethylsiloyl-5,6-epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-8-one 3,3'-diacetate

3-(Trimethylsilyl) isofucoxanthinyl ether (XXXVII) 3-Trimethylsiloyl-3,5,3,'5'-tetrahydroxy-6',7'-didehydro-5,8,5',6'-tetrahydro-β,β-caroten-8-one 3'-acetate

3,3',5'-*Tris*(trimethylsilyl) fucoxanthinol triether (XXXVI) 3,3',5'-*tris*(Trimethylsiloyl)-5,6-epoxy-3,3',5'-trihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-8-one

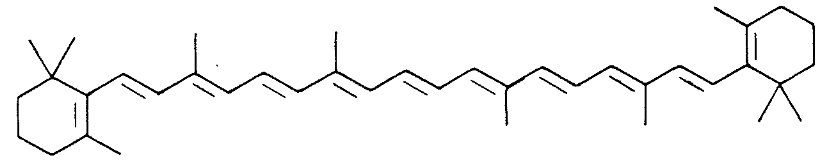
3,5,5'-*Tris*(trimethylsilyl) isofucoxanthinyl triether (XXXVIII) 3,5,5'-*tris*(Trimethylsiloyl)-3,5,3,'5'-tetrahydroxy-6',7'-didehydro-5,8,5',6'-tetrahydro-β,β-caroten-8-one 3'-acetate

Tunaxanthin (XXI) 3,3'-dihydroxy-ε,ε-carotene

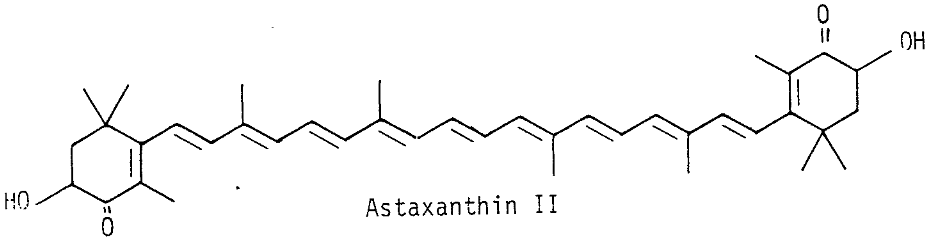
Violaxanthin (XXII) 5,6,5',6'-Diepoxy-5,6,5',6'-tetrahydro-3,3'-dihydroxy-β,β-carotene

Zeaxanthin (XX) 3,3'-dihydroxy-β,β-carotene

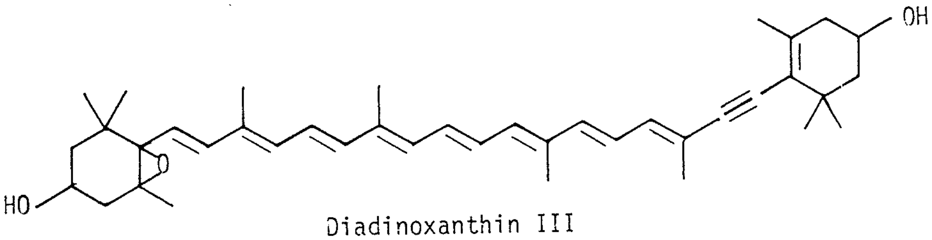
APPENDIX II



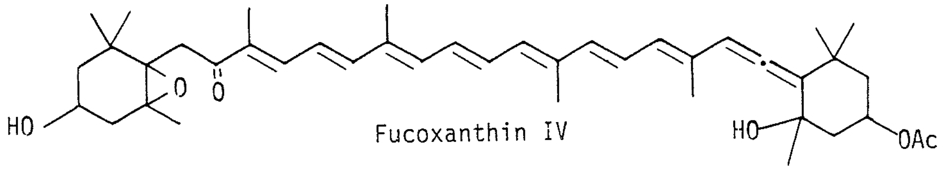
β-carotene I



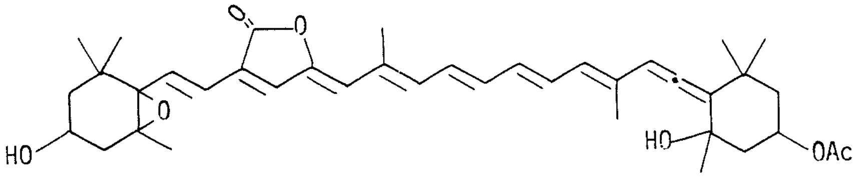
Astaxanthin II



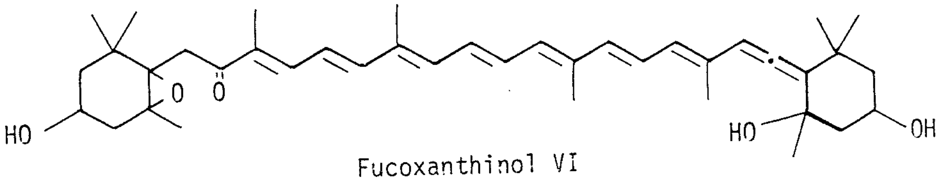
Diadinoxanthin III



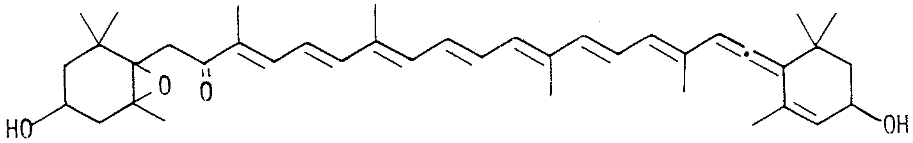
Fucoxanthin IV



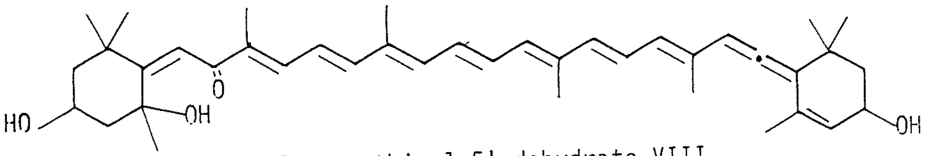
Peridinin V



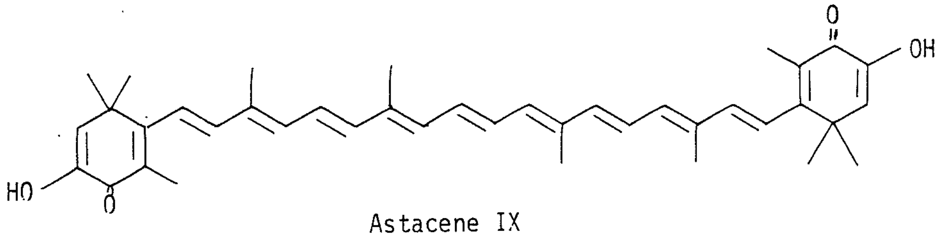
Fucoxanthinol VI



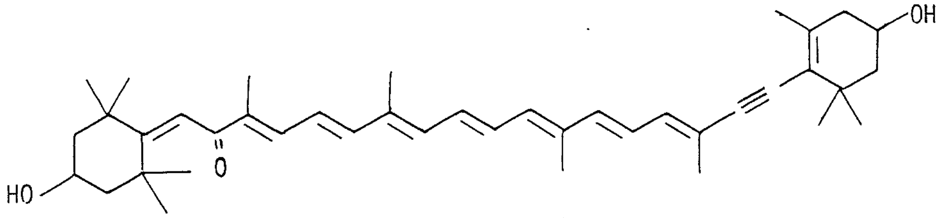
Fucoxanthinol 5'-dehydrate VII



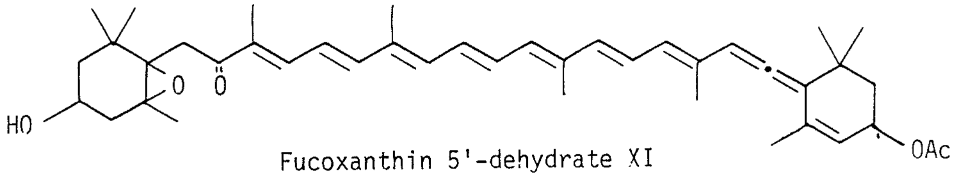
Isofucoxanthinol 5'-dehydrate VIII



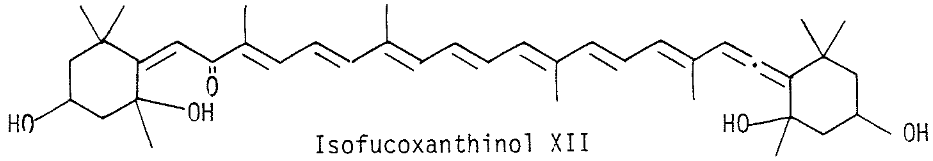
Astacene IX



Diadinochrome X



Fucoxanthin 5'-dehydrate XI



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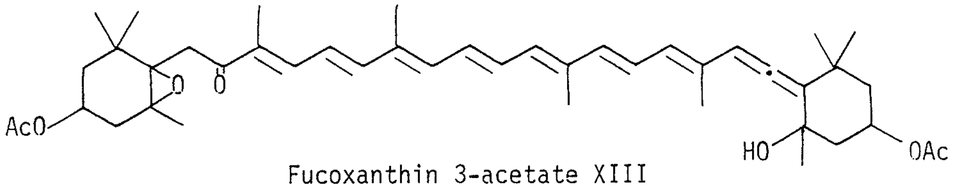
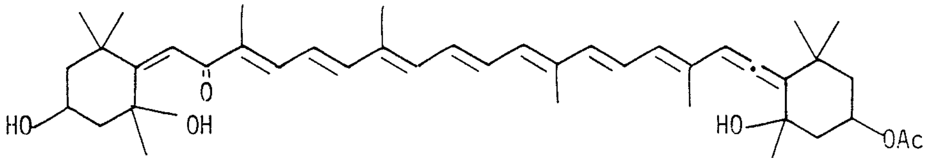
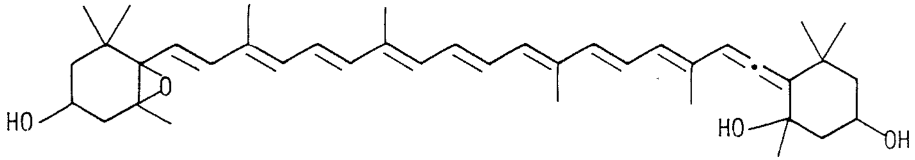

AcO
O
O
HO
OAc

Fucoxanthin 3-acetate XIII


HO
O
OH
HO
OAc

Isofucoxanthin XIV



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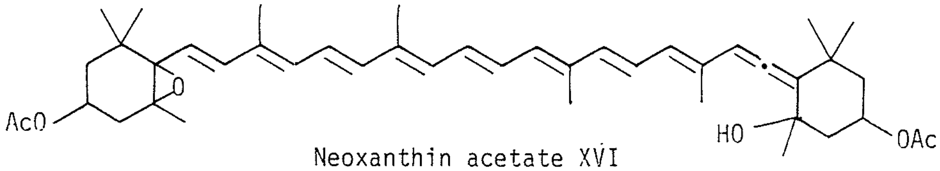
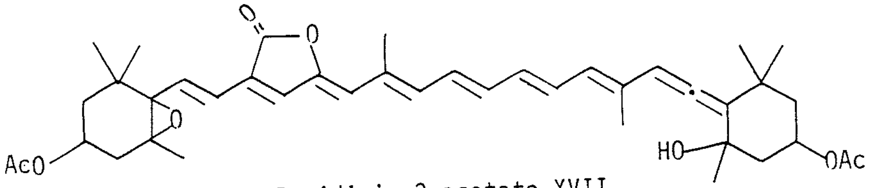


Neoxanthin acetate XVI



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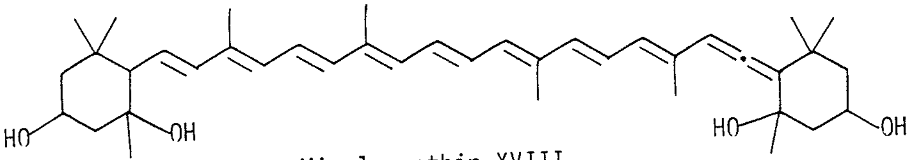
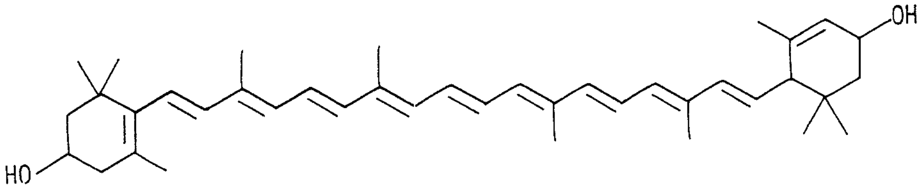
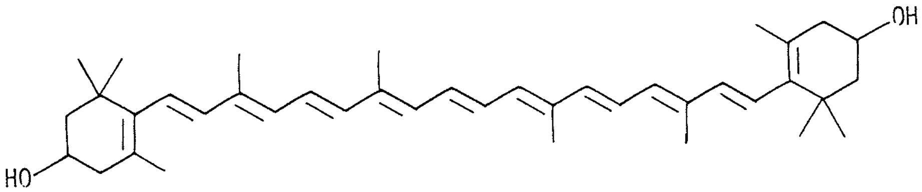


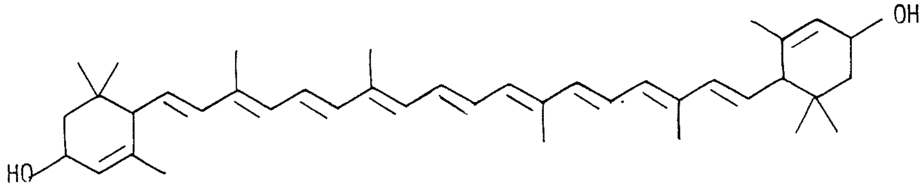
Minulaxanthin XVIII



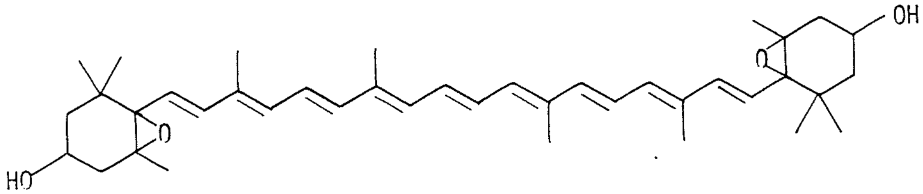
Lutein XIX



Zeaxanthin XX



Tunaxanthin XXI



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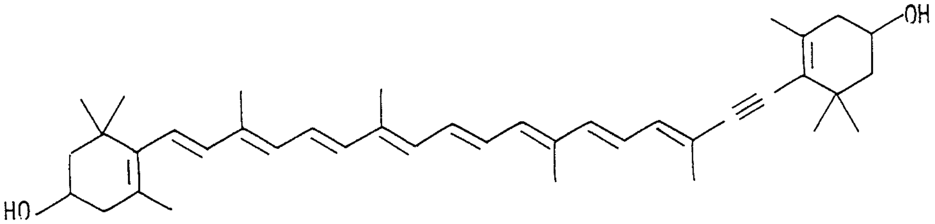

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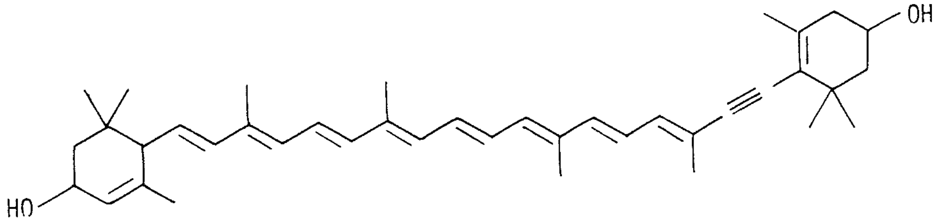
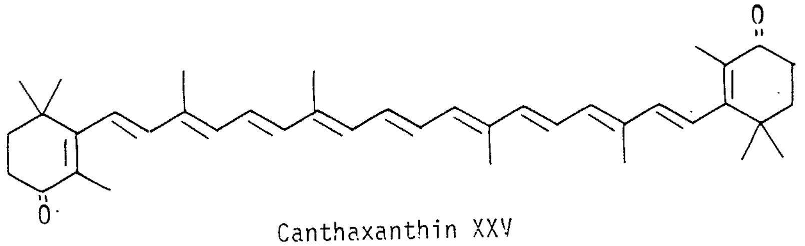
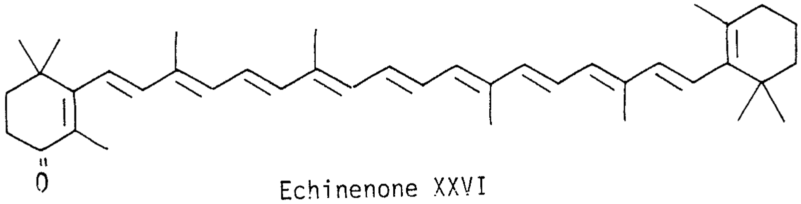


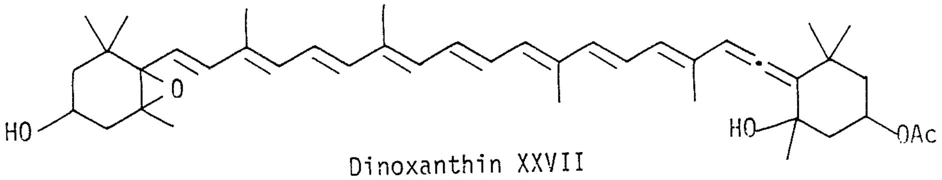
Monadoxanthin XXIV



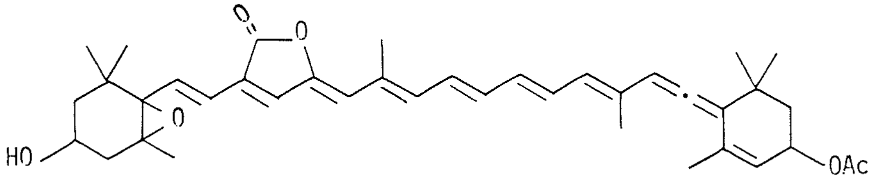
Canthaxanthin XXV



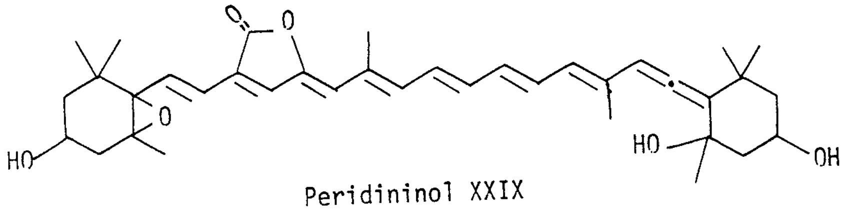
Echinenone XXVI



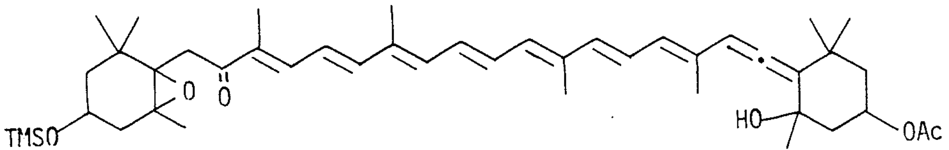
Dinoxanthin XXVII



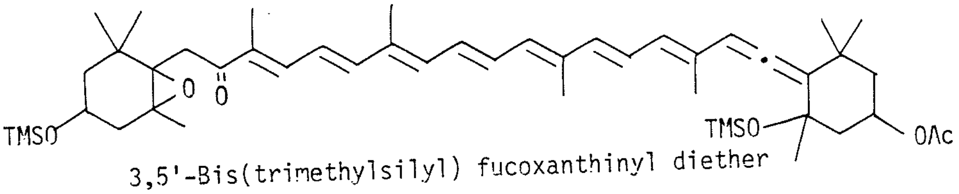
Peridinin 5'-dehydrate XXVIII



Peridininol XXIX

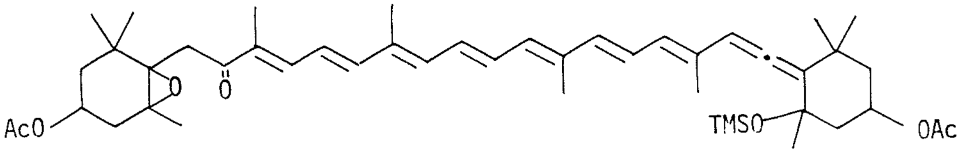

O
O
TMSO
HO
OAc

3-(Trimethylsilyl) fucoxanthinyl ether XXX

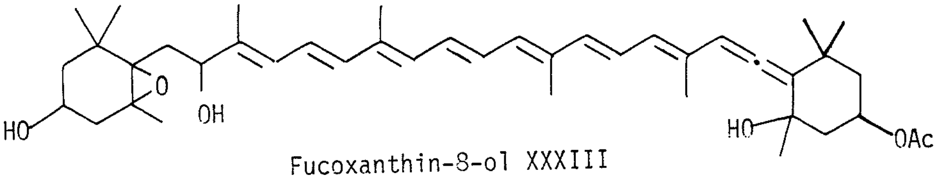

O
O
TMSO
TMSO
OAc

3,5'-Bis(trimethylsilyl) fucoxanthinyl diether

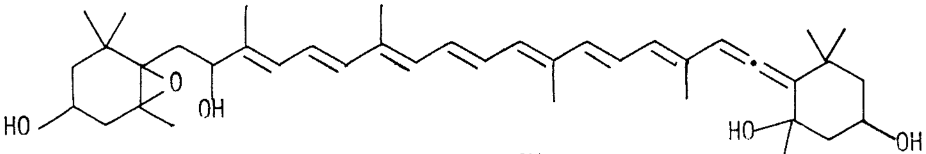
XXXI


O
O
AcO
TMSO
OAc

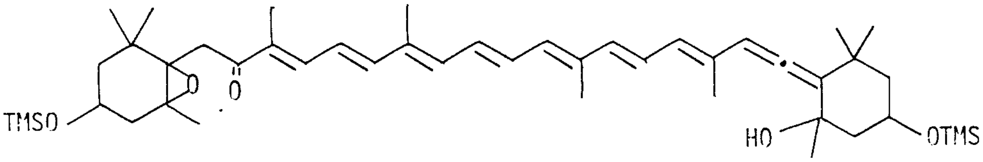
5'-(Trimethylsilyl) fucoxanthin 3-acetate XXXII


O
HO
OH
HO
OAc

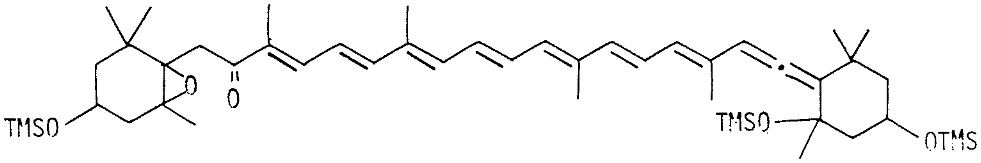
Fucoxanthin-8-ol XXXIII



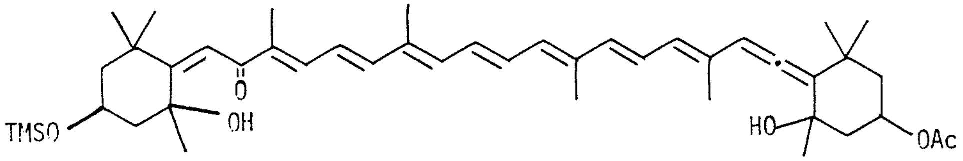
Fucoxanthol XXXIV


O
TMSO
O
HO
OTMS

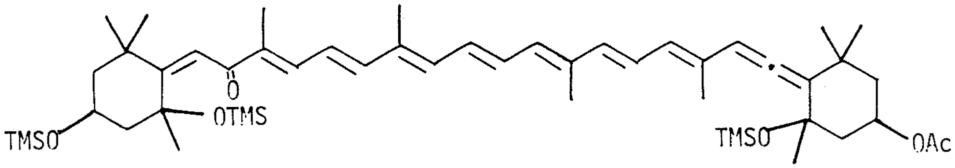
3,3'-Bis(trimethylsilyl) fucoxanthinol diether XXXV


O
TMSO
O
TMSO
OTMS

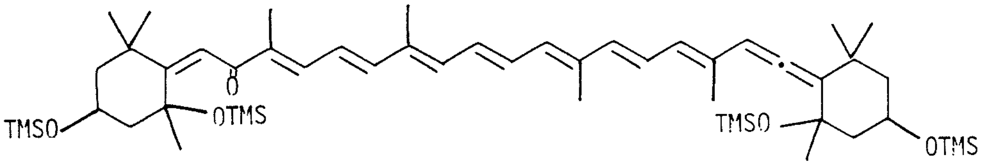
3,3',5'-Tris(trimethylsilyl) fucoxanthinol triether XXXVI


O
OH
TMSO
HO
OAc

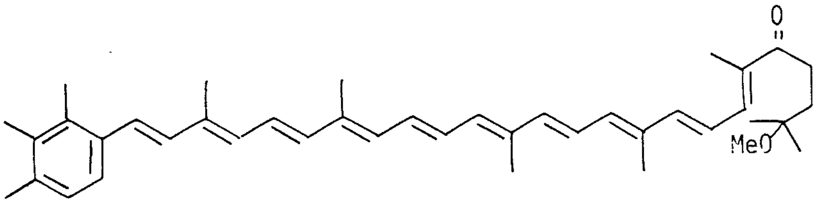
3-(trimethylsilyl) isofucoxanthinyl ether XXXVII


O
OTMS
TMSO
TMSO
OAc

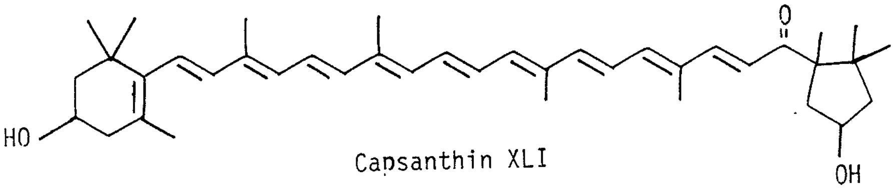
3,5,5'-Tris(trimethylsilyl) isofucoxanthinyl triether XXXVIII


O
OTMS
TMSO
TMSO
OTMS

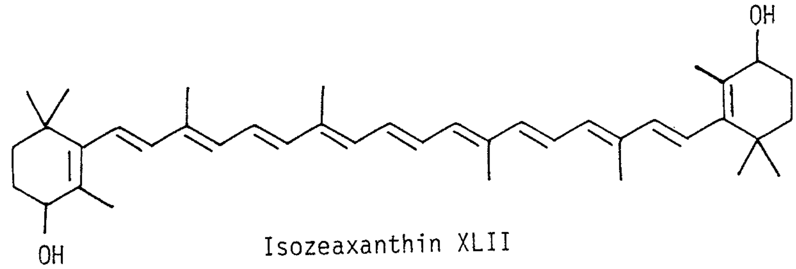
3,5,3',5'-Tetrakis(trimethylsilyl) isofucoxanthinol tetraether XXXIX



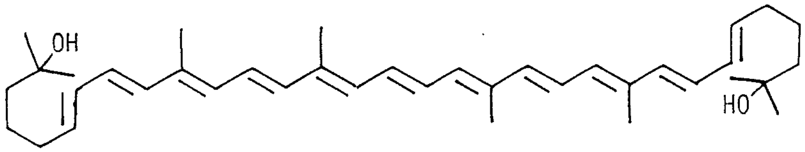
Okenone XL



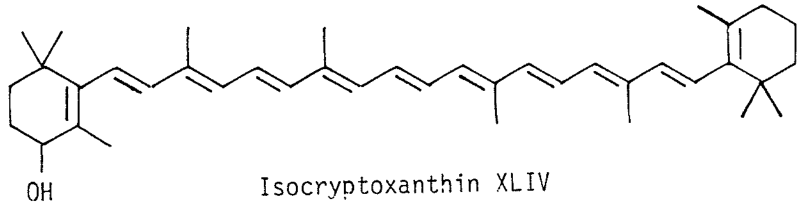
Capsanthin XLI



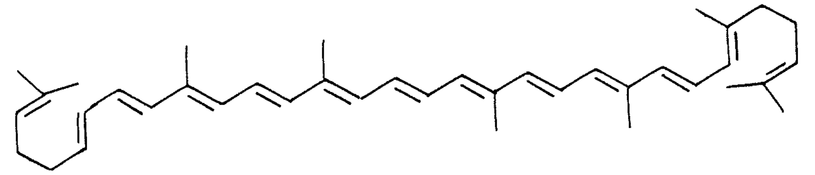
Isozeaxanthin XLII



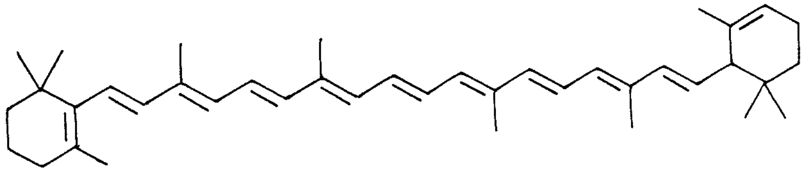
2,2'-Dihydrolycopene XLIII



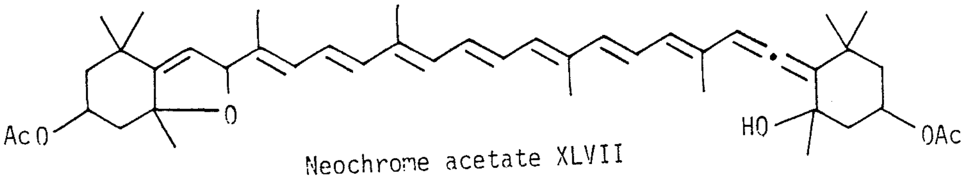
Isocryptoxanthin XLIV



Lycopene XLV



α-carotene XLVI



Neochrome acetate XLVII

BIOGRAPHICAL NOTE

The author was born on November 30, 1955 in Orange, New Jersey, to Tom and Vera Repeta. At the age of seven he moved to Rutland, Vermont. After graduating from Rutland High School, he enrolled at the University of Rhode Island and graduated in 1977 with a B.S. in chemistry. In June of 1977, the author entered the MIT/WHOI joint program in chemical oceanography.

Publications

Repeta D.J. and Gagosian R.B. (1982) Carotenoid Transformations in Coastal Marine Waters. Nature 295, 51-54.

Repeta D.J. and Gagosian R.B. (1982) Carotenoid Transformation Products in the Upwelled Waters Off the Peruvian Coast: Suspended Particulate Matter, Sediment Trap Material, and Zooplankton Fecal Pellet Analyses. In Advances in Organic Geochemistry (editor M. Bojory) J. Wiley. (in press).