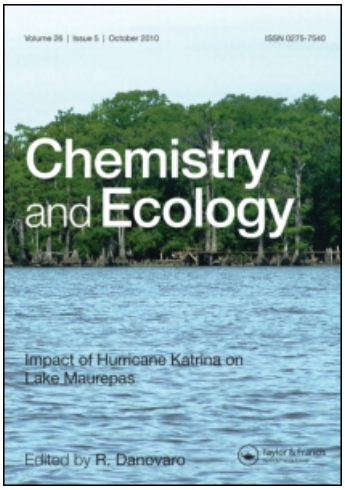

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Possible Origins of a Novel Carotenoid in Sediments from two Organic-rich Sites

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To cite this Article Ridout, P. S. , Morris, R. J. and Tibbetts, P. J. C.(1986) 'Possible Origins of a Novel Carotenoid in Sediments from two Organic-rich Sites', Chemistry and Ecology, 2: 2, 73 — 88
To link to this Article: DOI: 10.1080/02757548608070824
URL: http://dx.doi.org/10.1080/02757548608070824

Chemistry in Ecology, 1986, Vol. 2, pp. 73–88
0275–7540/86/0202/0073 $18.50/0

Printed in Great Britain

Possible Origins of a Novel Carotenoid in Sediments from two Organic-rich Sites

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(*Received March 3, 1984; in final form August 23, 1984*)

Samples of Recent sediment taken from box core samples from the Peru Continental Shelf (12°01.8'S; 77°29.3'W) and the Namibian Shelf (22°35.0'S; 13°45.0'E) were examined for pigments. Two 'novel' carotenoids, previously reported from the Namibian Shelf were identified, one (Peru 2/Walvis 2) as a major component at both sites. The sediments at these sites represent organic-rich diatomaceous oozes formed as a result of high primary productivity.

Comparison of the sediment chemistry of the sites suggests that the novel carotenoid Peru 2/Walvis 2 may have its origin in a previously uncharacterised biological source, although the possibility that it is a transformation product of fucoxanthin cannot be ruled out.

INTRODUCTION

Studies of the organic-rich oozes which occur on the continental shelves and slopes off Namibia and Peru suggest that they are derived directly from the rapid sedimentation of phytoplankton blooms (Morris and Calvert, 1977; Cronin and Morris, 1982; Poutanen and Morris, 1983). Both are sites of intense seasonal upwelling (Hart and Currie, 1960; Gunther, 1936) which results in periods of exceptionally high primary

productivity (Steeman-Nielsen and Jensen, 1957; Ryther *et al.*, 1970), the dense phytoplankton blooms which occur being apparently dominated by diatoms (Hart and Currie, 1960; Kollmer, 1963; Diester-Haass and Schrader, 1979; Schuette and Schrader, 1981; Neaverson, 1934; Saidova, 1971; Jouse, 1972; De Vries and Schrader, 1981).

At first sight both environments would appear to be ideally suited for geochemical studies aimed at relating the composition of sediments to biological sources of input, providing as they do a very simple direct relationship between planktonic production and sediment formation. Minerologically the oozes are dominated by opaline silica (Morris and Calvert, 1977; Poutanen and Morris, 1983; Smith *et al.*, 1982); providing confirmation of the large diatom input. The observation of many diatoms in the near-surface sediments, some with intact chloroplasts (Smith *et al.*, 1982, 1983c) and the presence of large amounts of recognisable phytoplankton pigments (Brongersma-Sanders, 1951; Morris and Calvert, 1977; Cronin and Morris, 1982; Poutanen and Morris, 1983) fatty acids (Morris and Calvert, 1977; Volkman *et al.*, 1982; Smith *et al.*, 1983a, b) and sterols (Wardroper *et al.*, 1978; Smith *et al.*, 1982, 1983c), is further evidence of rapid sedimentation and burial of virtually intact phytoplankton cells.

The detailed steroid geochemistry of the Namibian (Smith *et al.*, 1982) and Peruvian (Smith *et al.*, 1983c) shelf oozes indicates that we cannot explain the organic input to these sediments merely in terms of diatom blooms. The 4-methyl sterol, dinosterol, is found to be a major sterol component of both sediments. Dinosterol has been found to be the major sterol in a number of dinoflagellate species (Shimizu *et al.*, 1976; Withers *et al.*, 1978; Alam *et al.*, 1979) and, to date, appears to be unique to this group of organisms. It has, in fact, been regarded as a dinoflagellate "marker" in geochemical studies (Boon *et al.*, 1979). However, no dinoflagellate remains, such as cysts, were observed during microscopic examinations of the oozes. The conclusion from these studies was that there could be major, as yet uncharacterized, biological inputs to the Namibian and Peruvian shelf sediments (Smith *et al.*, 1982, 1983a).

Carotenoids have a wide distribution and some have been shown to survive in sediments for relatively short geological time periods under reducing conditions (Watts and Maxwell, 1977; Cardoso *et al.*, 1978). As many carotenoids are source specific (Liaaen-Jensen, 1978, 1979) they may represent useful markers in some sedimentary environments. Tibbetts (1980) has shown two previously unknown carotenoid structures (Walvis 1 and Walvis 2) to be present in Namibian Shelf sediment

as major pigment components. The presence of these compounds in Peruvian Shelf sediments has been the subject of a preliminary note (Ridout *et al.*, 1984) with one (Peru 2) representing a major pigment component. The origin of these compounds at two diatomaceous-rich sites is unclear at present, as they do not occur in any diatom species so far investigated.

A core from the Namibian Shelf and one from the Peruvian Shelf have been subjected to a wide range of inorganic and organic chemical analyses (Wardroper *et al.*, 1978; Cronin and Morris, 1982; Smith *et al.*, 1982; Poutanen and Morris, 1983; Smith *et al.*, 1983a, 1983b, 1983c; Ridout *et al.*, 1984), the intention being to build up as complete a picture as possible concerning the sedimentary environment at these two sites.

This paper compares a further carotenoid analysis of the Namibian Shelf sediment with more detailed analyses of the carotenoids in the Peruvian Shelf sediment. It is hoped that this work will help evaluate the use of carotenoids as indicators of biological source and add directly to our understanding of biological productivity in the Namibian and Peruvian upwelling areas.

MATERIALS AND METHODS

A fully detailed description of sampling and extraction has been previously reported (Ridout *et al.*, 1984).

Some carotenoids can produce transformation products very easily, so a number of precautions were taken to minimise carotenoid decomposition during sample work up, storage and analysis. All procedures, where possible, were carried out in low light conditions, under an inert atmosphere (white spot grade nitrogen). Large solvent volumes were reduced using low temperature ($<30°C$) vacuum rotary evaporation, and small volumes were reduced under a stream of nitrogen at room temperature. Some sediment sub-cores were extracted, immediately after sampling, into chloroform/methanol (2:1, v/v) and stored at $-20°C$ under nitrogen. Other sub-samples from the cores were immediately frozen under N_2 at $-20°C$; these samples were later extracted with both the chloroform/methanol system and an isopropanol/hexane (4:1, v/v) system (Ridout *et al.*, 1984). Other control experiments involved the extraction of fresh phytoplankton material, the component carotenoids being studied for any transformations with storage time. All the methods

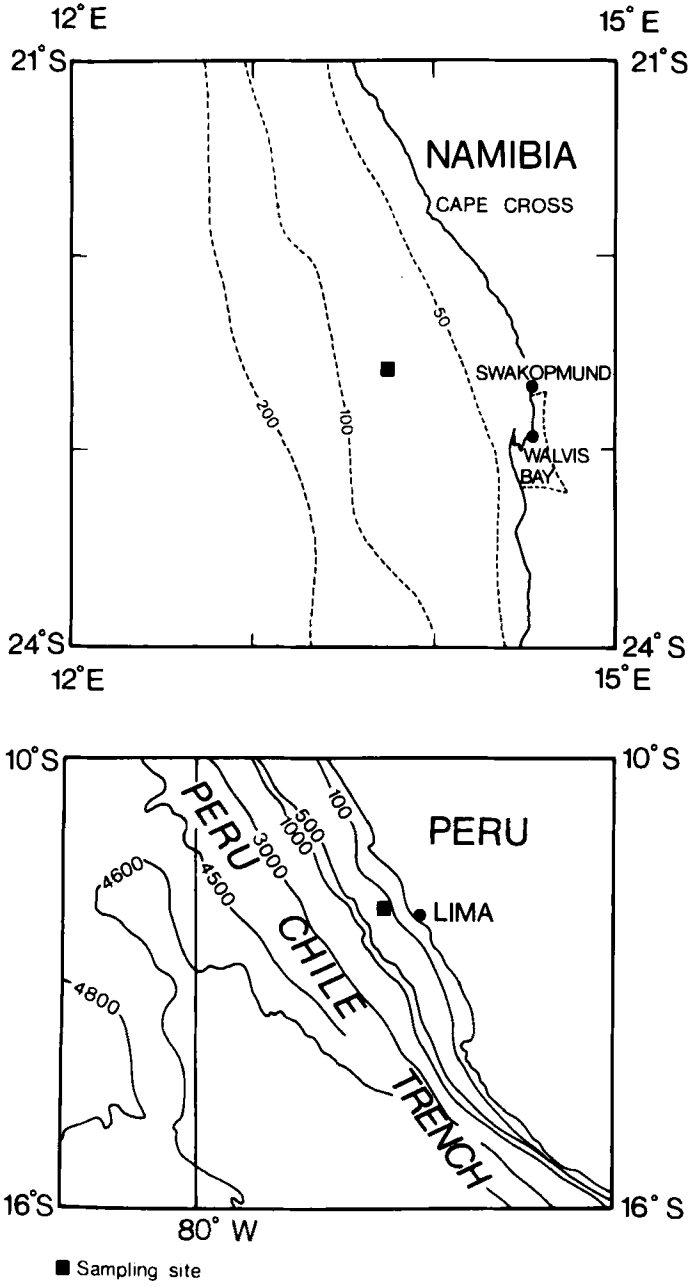

FIGURE 1 Location of sampling sites on the Namibian Shelf and the Peruvian Shelf.

of storage and extraction gave consistent results for the carotenoids studied here.

The interfacial layer (1–2 mm) was taken from a box core sample of a Peruvian Shelf sediment (12°01.8'S; 77°29.3'W. Water depth 145 m) (Figure 1) and extracted into chloroform/methanol (2:1, v/v) (Folch *et al.*, 1957). The extract was dried and redissolved in acetone for storage. A sample of sediment from the Namibian Shelf (22°35.0'S; 13°45.0'E. Water depth 127 m) (Figure 1) was taken (13–28 cm) and extracted using chloroform/methanol (2:1 v/v) (Folch *et al.*, 1957). The extract was dried and redissolved in acetone for storage.

ANALYSIS BY HPLC

Normal phase HPLC was carried out based on a system reported by Hajibrahim *et al.* (1978). Separations were made on a silica column (Partisil 5 μm irregular) using a concave gradient (curve 7 on the Waters 720 programme) of acetone (2–75%) in hexane for 30 minutes at 1 ml/min. Conditions were reversed over 10 minutes, after each run, then held at initial conditions before the next injection. Absorbance was measured at 451 nm.

A reserved phase system was used, based on the method reported by Mantoura and Llewellyn (1983) using an octadecyl-silane bonded silica column (5 μm ODS Hypersil). The method utilised an ion pairing reagent, "P", which comprised tetrabutyl ammonium acetate (1.5 g) and ammonium acetate (7.7 g) made up to 100 ml with distilled water. The mobile phases were solvent A which comprised "P": water: methanol (10:10:80) and solvent B which comprised acetone: methanol (20:80). Separations were made on a linear gradient from 100% A to 100% B in 10 min (flow rate 1.8–2.5 ml/min) followed by an isocratic hold at 100% B (flow rate 2.5–3.2 ml/min) over 12 mins. The system was reversed over 5 minutes and held at initial conditions for a further 5 minutes before the next injection. Each sample was mixed with the ion pairing reagent, solution "P" (3:1, sample: "P") and allowed to stand for 5 minutes before injection on to the column. Absorbance measurements were made at 451 nm. Fluorescence detection was performed with excitation at 440 nm and emission measured at >600 nm.

The equipment used comprised two solvent delivery pumps (Waters Ass. M6000A), a solvent programmer (Waters Ass. 720), a loop injector (Waters Ass. U6K), a uv/vis scanning spectrophotometer (Waters Ass.

450) and a fluorescence detector (Laboratory Data Control, fluoromonitor III).

Identification was achieved using co-injection of known standards, coinjection of algal extracts of known composition, and visible spectroscopic scanning.

RESULTS

The normal phase HPLC trace of the Peruvian sediment extract (Figure 2) shows the positions of the "novel" carotenoids, Peru 1 and Peru 2. A similar analysis of the Namibian Shelf sediment (Figure 3) shows the

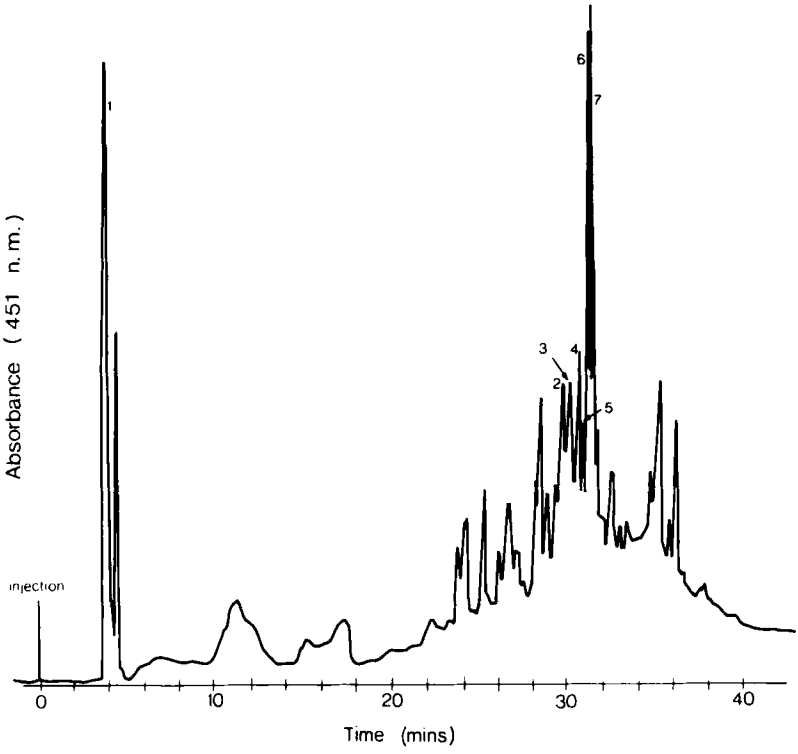

FIGURE 2 Normal-phase HPLC trace of a total organic extract from the Peruvian Shelf showing absorbance (λ = 451 nm). Peak identities: (1) Carotene; (2) Lutein; (3) Zeaxanthin; (4) Diatoxanthin; (5) Peru-1; (6) Fucoxanthin; (7) Peru-2.

presence of Walvis 1 and Walvis 2 as reported by Tibbetts (1980) in the same relative positions as Peru 1 and Peru 2. The reversed phase HPLC trace of the Peru sediment extract (Figure 4) shows an improved separation of Peru 2 and fucoxanthin, although their expected relative positions are reversed. The Peru 2 peak on the reverse phase system was shown to co-elute with Peru 2 on the normal phase system. It was also shown to have a similar visible spectrum to Peru 2 which had been previously isolated (Ridout *et al.*, 1984). Evidence for the identical structure of Walvis 2 and Peru 2 using mass spectrometry has been previously reported (Ridout *et al.*, 1984). Peru 1 was not identified using reverse phase. The HPLC traces provide additional information regarding some of the other pigments present at both sites. The peak labelled carotene in the Peruvian sediment was shown in the Namibian sediment to comprise β-carotene (Tibbetts, 1980). Its presence is not unexpected as it is so wide-spread in nature (Weedon, 1971). Canthaxanthin was tentatively identified by coinjection, but only in the Peruvian

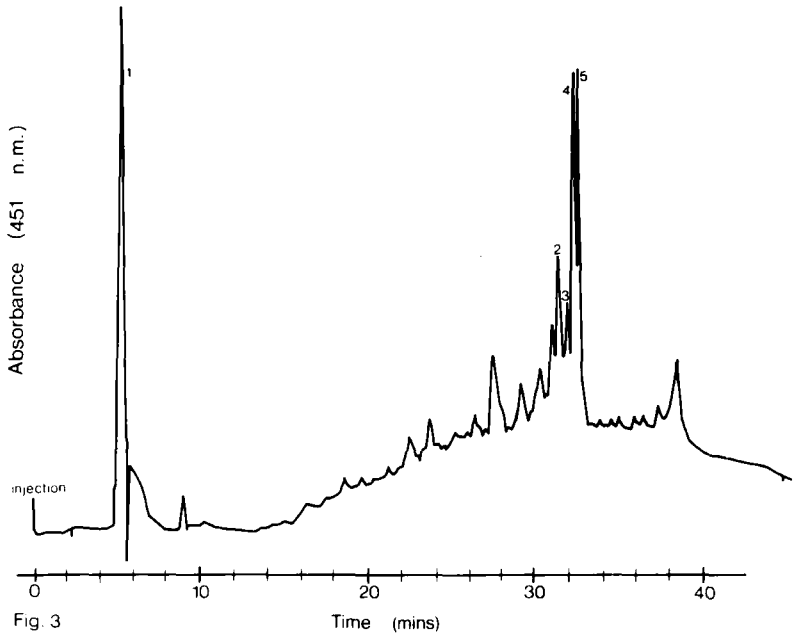

FIGURE 3 Normal-phase HPLC trace of a total organic extract from the Namibian Shelf showing absorbance (λ = 451 nm). Peak identities: (1) β-carotene; (2) Diatoxanthin; (3) Walvis-1; (4) Fucoxanthin; (5) Walvis-2.

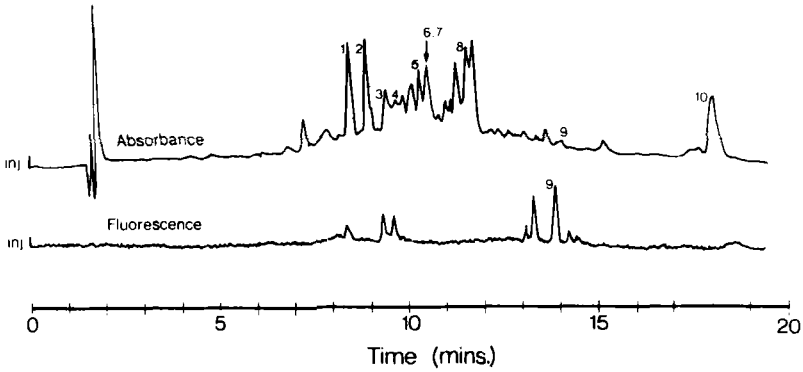

FIGURE 4 Reverse-phase, ion-pair HPLC traces of a total organic extract from the Peruvian Shelf showing absorbance (λ451 nm) and fluorescence (λEM = 440 nm; λEX > 600 nm). Peak identities: (1) Fucoxanthin; (2) Peru-2; (3) Astaxanthin; (4) Diadinoxanthin; (5) Diatoxanthin; (6) Zeaxanthin; (7) Lutein; (8) Canthaxanthin; (9) Chlorophyll "a"; (10) Carotene.

extract suggesting some input from zooplankton at that site. The absorbance peak labelled astaxanthin in the Peruvian extract (Figure 4) has been increased as a result of the co-elution of a chloropigment which shows up on the fluorescence trace. The astaxanthin component, therefore, is relatively minor at the Peruvian site, and was not detected in the Namibian sediment which indicates a small contribution to the sediments from crustaceans. Astaxanthin has been reported to become irreversibly absorbed on silica columns (Tanaka *et al.*, 1981) but the minor amounts indicated on normal phase HPLC were confirmed by reverse phase HPLC. Zeaxanthin and lutein could not be resolved using the reverse phase system (Figure 4); however, the lutein component was separated on normal phase (Figure 2). This showed some lutein to be present at the Peruvian site which suggests a possible input from higher plants, although it should be noted that lutein does occur in some algal classes. Diatoxanthin, diadinoxanthin and fucoxanthin are all major components of diatoms so their presence is in agreement with other evidence for a large diatomaceous input to the sediments at both sites.

DISCUSSION

It was the conclusion of Ridout *et al.* (1984) that Peru 2 and Walvis 2

were identical compounds and it was evident that they each represented a major carotenoid component of the Peruvian and Namibian sediments. These findings are fully supported by the additional chromatographic data reported here. Peru 1 and Walvis 1 represent only minor carotenoid components, so further discussion is confined to Peru 2 and Walvis 2.

The Peruvian Shelf, like the Namibian Shelf, is an area of high, primary productivity, with sediments comprising mainly an organic-rich diatomaceous ooze, but with the greater terrestrial contribution (Poutanen and Morris, 1983). The sedimentation rate in this area is thought to be very high. Gagosian *et al.* (1983) quote 1–2 cm/yr based on sediment trap data, whilst ^{210}Pb (Henrichs and Farrington, 1984; Koide and Goldberg, 1982) and $^{228}Th/^{232}Th$ (Koide and Goldberg, 1982) data suggests rates between 0.34 cm/yr and 1.3 cm/yr for near-surface sediments.

The origin of unknown carotenoid 2 in the Peruvian and Namibian sediment is not clear, but suggestions of the more probable origins are discussed below.

Sediment diagenesis

The Peruvian interfacial sediment studied was thought to be less than one year old, yet Walvis 2 was found in the Namibian sediment which was up to 100 years old (Morris, unpublished). If the explanation for the unknown carotenoid is in sediment diagenesis then a very rapid specific structural alteration must have occurred to the primary carotenoid just after sedimentation in both areas, the subsequent products being stable. On the basis of the sterol/stanol ratio (Smith *et al.*, 1983b, c) diagenetic changes in the interfacial Peru sediment to the planktonic detrital matter would appear to be very small. However, some carotenoids are extremely labile and rapid alteration in the sediment/water interface cannot be ruled out.

Bacterial action in the water column

Unfortunately an analysis for bacteria marker compounds was not performed on the Namibian sediment and no estimate of the importance of bacterial activity can be made. However, fatty acid analysis of other Walvis Bay sediments (Boon *et al.*, 1975, 1977) showed some bacterial action to have occurred.

A detailed fatty acid analysis of the Peruvian interfacial sediment showed that fatty acids normally taken as indicators of bacterial activity (i.e. iso and anteiso acids) made only a small contribution to the total (< 15% of the total fatty acids) (Smith *et al.*, 1983b, c). The conclusion was that the original phytoplanktonic input to the sediment had not been significantly altered by bacterial action. Thus, unless a very specific bacterial-mediated alteration to the carotenoid has occurred in the water column, this seems an unlikely source for a major part of the sediment carotenoids.

Major events such as jelly-fish "blooms"

Large numbers of jelly-fish have been observed in the Walvis area (Morris, unpublished) and the discovery of gorgosterol (a common sterol in coelenterates) as a major component in the sediment by Wardroper *et al.* (1978) led workers to suggest that jelly-fish make a significant contribution to the sediment lipids. However, this sterol has since been identified in dinoflagellates which live as zooanthellae in certain coelenterates (Steudler *et al.*, 1977; Withers *et al.*, 1979; Kokke *et al.*, 1981).

Only low levels of gorgosterol were found in the Peru interfacial sediment (Smith *et al.*, 1983a) and this may reflect the absence in the area of the large jelly-fish blooms previously found in Walvis Bay (Wardroper *et al.*, 1978; Smith *et al.*, 1982). Thus jelly-fish blooms must be an unlikely origin for this unknown carotenoid, which represents a major sediment pigment component in both areas.

Alteration of Fucoxanthin (a major diatom carotenoid) by herbivorous consumption

Known secondary production in the Walvis area seems unlikely to be able to utilise more than a small fraction of the primary production (Hart and Currie, 1960; Kollmer, 1963; Unteraberacher, 1964). Calanoid copepods were the most important planktonic crustaceans reported. Being rich in wax esters (Morris and Culkin, 1976, and references therein) their importance in the water column can also be judged by the levels of fatty alcohols in the sediment. Although fatty alcohols are certainly present (Morris and Calvert, 1977) they are at relatively low levels (< 10% of total sediment fatty acids). Hence in terms of relative biomass, zooplankton are minor biological components of the water

column and it seems most unlikely that their grazing activities could be responsible for supplying the major xanthophylls to the sediment.

Calanoid copepods are known to be minor components of the biomass in the Peruvian upwelling area (Ryther *et al.*, 1970). Their abundance may be judged by the relative levels of *n*-alcohols in the underlying sediments (see earlier discussion). Smith *et al.* (1983b) reports *n*-alcohols to be present at $<5\%$ of the total fatty acid levels in the interfacial sediment studied here. Thus, as for the Namibian sediment, it appears that the relative abundance of zooplankton in the area is small and would not account for the occurrence of a major unknown carotenoid. Further support is given by the low amount of astaxanthin in the sediment; a characteristic component of zooplankton crustacea (Liaaen-Jensen, 1978). Also, observations of the sediment interface showed it to comprise mainly phytoplankton cells with little evidence of faecal pellets or other secondary material.

The mineralogical composition of the sediments indicated that at both sites, opaline silica was a major component (25–85% dry wt.). Therefore, although the zooplankton input may not be small in absolute terms, its relative influence is minor as a result of the extremely large input of diatoms.

However, the low abundance of markers for heterotrophic organisms does not rule out the possibility of significant recycling of primary organic matter. Repeta and Gagosian (1982) reported, in their sediment trap samples, up to 95% of the phytoplankton fucoxanthin was metabolised, with fucoxanthinol as a major transformation product. The site studied in their work showed anchovy faecal pellets to be present in abundance, whereas there was no such evidence at the site reported here. Saponification (alkaline hydrolysis) of the total organical extracts resulted in Peru 2/Walvis 2 losing an acetyl group and forming an hydroxyl group (Ridout *et al.*, 1984; Tibbetts, 1980). This modified compound, Peru 2-Ac/Walvis 2-Ac, has the same mass spectrum as that of fucoxanthinol. However, the visible spectrum of Peru 2-Ac/Walvis 2-Ac (λmax 420) (Figure 5) indicates that the main conjugated system in the molecule contains one less double bond than fucoxanthinol (λmax 452 nm).

A major input from a primary uncharacterised source

This was certainly the conclusion of Smith *et al.* (1982) for the Namibian Shelf, when the "chemical indicator" of dinoflagellates (dinosterol) was

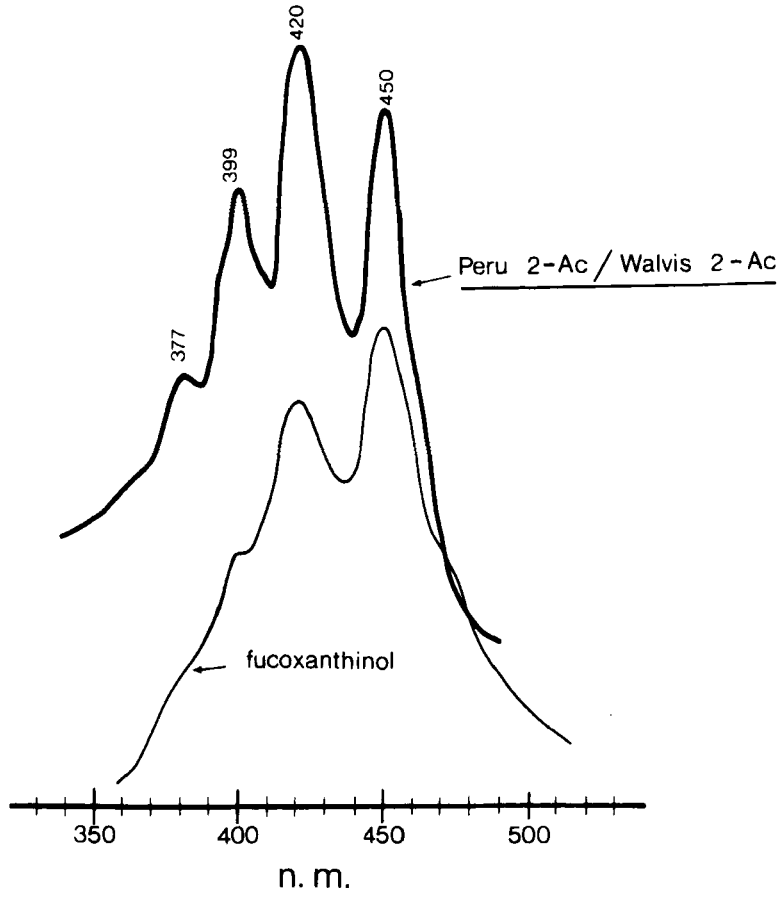

FIGURE 5 Visible spectra, in ethanol, of Peru 2-Ac/Walvis 2-Ac and fucoxanthinol.

found to be the major sterol in the sediment without any trace of their physical remains. Normally dinoflagellates form highly resistant cysts at some stage of their life cycle and the presence of these cysts in the sediments is evidence of their occurrence in the overlying water column. Possibly there are some species which do not form these cysts which are specific to these waters, but dinoflagellates do not appear to represent a significant component of the diatom-rich sediment studied at this Walvis Bay site. The suggestion that nannoplankton may make a major contribution to these sediments (Smith *et al.*, 1982) seems more plausible. They would leave no fossil record and would be either missed

or destroyed by conventional phytoplankton nets. It is only recently with improvements in sampling techniques that their importance in the primary producing biomass is being recognised (e.g. Jeffrey and Hallegraeff, 1980; Hallegraeff, 1981).

The presence of dinosterol as a major sediment sterol and the absence of recognisable dinoflagellate cysts in the Peruvian interfacial sediment led Smith *et al.* (1983) to postulate the existence of a major uncharacterised biological contribution to the sediments. Such a biological input could, of course, also be responsible for contributing large amounts of, as yet, uncharacterised pigment to the sediments.

CONCLUSION

This work discusses the discovery of a major unknown carotenoid in a Peruvian Shelf interfacial sediment (Ridout *et al.*, 1984), which is identical to a compound previously reported by Tibbetts (1980) and Tibbetts and Maxwell (in prep.) in a Namibian Shelf sediment. Its occurrence in two separate environments, we believe, allows a better assessment of its likely origin.

We cannot exclude the possibility that the novel carotenoid, Peru 2, is a transformation product from the diatom carotenoid fucoxanthin. The extreme lability of some carotenoids may allow some transformation products to be formed before significant changes in the polyunsaturated lipid geochemistry is seen. In addition, specific bacterial-mediated transformations to the primary carotenoids in the water column cannot be ruled out. It should, however, be remembered that the accumulation of sediments at the sites studied on both the Peruvian and Namibian Shelves are believed to occur as a result of the rapid flux of large pulses of phytoplankton material in a very good state of preservation.

On the basis of the existing evidence, we believe that Peru 2 and Walvis 2 may have an origin in the so far uncharacterised biological source postulated by Smith *et al.* (1982, 1983a) in order to explain some puzzling aspects of the steroid geochemistry of the Namibian and Peruvian sediments. This source may be found in the nannoplankton which are known to contain various types of small flagellates including non-thecate dinoflagellates (Hallegraeff, 1981). Clearly a priority for future investigations is a better understanding of the taxonomy and chemical composition of this group of primary producers.

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