Carotenoid pigments
Recent sediments
Diatomaceous sediment
Peruvian shelf
Namibian shelf

Pigments caroténoïdes
Sédiments récents
Sédiment à diatomées
Plateau continental péruvien
Plateau continental namibien

Novel carotenoid pigments in organic rich sediments from the Peru continental shelf

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ABSTRACT

A study was made of carotenoid pigments present in surface sediments sampled from the Peru continental shelf (12°01.8'S, 77°29.3'W). Two "novel" carotenoids which have been reported from Namibian Shelf sediments were shown to be present in the Peruvian samples.

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RÉSUMÉ

Nouveaux caroténoïdes dans les sédiments du plateau continental péruvien

Les pigments caroténoïdes ont été étudiés dans les sédiments superficiels du plateau continental péruvien (12°01, 8'S, 77°29, 3'W). Deux « nouveaux » caroténoïdes, signalés antérieurement dans les sédiments du plateau namibien, y ont été trouvés.

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INTRODUCTION

Carotenoids are widely distributed, being found in all photosynthetic and some non-photosynthetic organisms, and many have been shown to be source specific (Liaaen-Jensen, 1978; 1979). Although they are a rather labile group of natural products, some carotenoids have been shown to survive in sediments for relatively short geological time periods under favourable reducing conditions (*e.g.* Watts, Maxwell, 1977; Cardoso *et al.*, 1978). They may, therefore, provide useful markers of biological input in certain sedimentary environments.

A study of the carotenoid content of a Namibian Shelf sediment has revealed the presence of two previously unknown carotenoid structures as major pigment components (Tibbetts, 1980; Tibbetts, Maxwell, in prep.). The origin of these compounds is unclear at present; they do not occur in any of the diatom species so far investigated and diatoms are believed to be overwhelmingly the major biological input to the sediments studied.

This paper presents a carotenoid analysis of an interfacial sediment from the Peruvian continental shelf. The results are compared with those from the Namibian sediments (Tibbetts, 1980; Tibbetts, Maxwell, in prep.), to investigate the possible presence of these "novel" carotenoids in the Peruvian sediments.

MATERIAL AND METHODS

Sampling and extraction

1) A sediment core was collected, using a precleaned, stainless steel box corer (Peters *et al.*, 1980) from a site on the Peruvian continental shelf (water depth 145 m; 12°01.8'S; 77°29.3'W; Fig. 1). The interfacial sediment layer (1-2 mm) was removed immediately using a broad-bladed stainless steel spatula, placed in chloroform/methanol (2 : 1 v/v) and stored under nitrogen in the dark at −20°C.

At the laboratory the sediment in chloroform/methanol was sonicated for one hour, then allowed to stand at room temperature in the dark, under nitrogen, for several hours. After centrifugation the surpernatant solvent was removed and washed with 0.05 M KCl

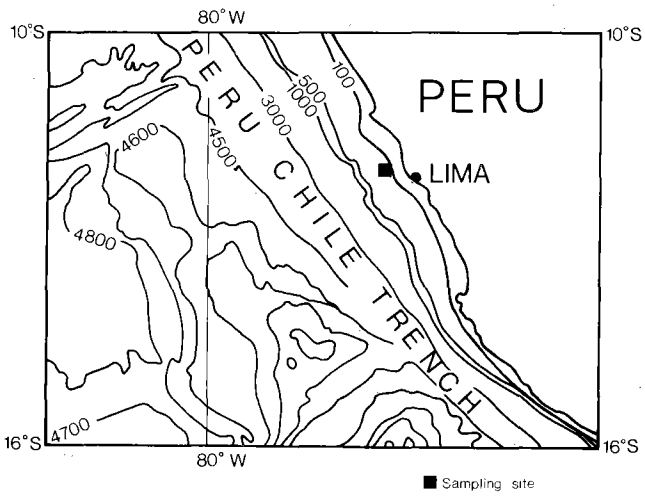


Figure 1
Location of sampling site on Peruvian Shelf.

to give a two-phase system (Folch *et al.*, 1957). The chloroform layer was removed, taken to dryness by vacuum rotary evaporation at 25°C, and redissolved in chloroform. This extract contained most of the unbound lipids in the sediment, including the carotenoids, most of which are fat soluble (Moss, Weedon, 1976). An aliquot was dried, redissolved in acetone, and stored for carotenoid analysis.

2) A vertical subcore was taken in a precleaned butyric liner and deep frozen prior to extraction.

The surface (1-2 cm) sediment layer was removed into isopropanol/hexane (4: 1, 300 ml) and sonicated under nitrogen for 30 minutes. The solvent was decanted and the extraction repeated twice. Solvent fractions were combined, centrifuged (4 000 rpm, 10 min.) and reduced in volume. Water (200 ml) was added and the sample extracted into diethyl ether (2 × 100 ml) and ethyl acetate (100 ml). The volume of solvent was reduced to provide the total organic extract (TOE) for HPLC and TLC.

Extraction (2) was performed to provide bulk material for the extraction of carotenoids for mass spectrometric analysis. Chloroform, which can contain traces of HCl, may cause alteration of some carotenoids, but both extraction techniques used gave similar results for the carotenoids studied here.

HPLC analysis

The lipid extract was analysed directly for carotenoids by normal phase HPLC, based on a method developed by Hajibrahim *et al.* (1978) using silica columns (Partisil 5 μm irregular). The equipment comprised two solvent delivery pumps (waters M6000A), a solvent programmer (waters 720) and uv/vis spectrophotometer (waters 450) with a 8 μl flow-cell, as a detector. Samples were introduced on to the column via a loop injector (waters U6K). HPLC grade solvents (Rathburn Chemicals), degassed using helium, were used throughout. Separations were made using a concave gradient (curve 7 on the waters 720 programme) of acetone (2-75%) in hexane, over 30 min. at a flow rate of 1 ml/min. The absorbance of the eluant was measured at 451 nm. After each run, the solvent programme was reversed over 10 min. and then kept at initial conditions for at least 5 min. before the next injection.

A reversed phase system was also used, based on the method reported by Braumann and Horst Grimme (1981) using a μ Bondapak C18 column and detection at 451 nm. Separations were made using a linear gradient of acetonitrile (75)/methanol (25; 75-100%) in water at a flow rate of 1.5 ml/min. for 20 min. then maintained at 100% for a further 15 min. After each run the gradient was reversed and kept at initial conditions for at least 5 min. before the next injection.

Isolation and clean up of carotenoids for further analysis

Semi-preparative HPLC was carried out using a 5 μm silica Partisil column (25 cm × 8 mm i.d.) and normal phase gradient elution as for the Partisil analytical column, but with increased flow rates to maintain a similar pressure across the column.

Identification of carotenoid components was achieved using co-elution of known standards under the two different solvent conditions, stop-flow visible scanning and mass spectrometry (EI) of isolated fractions from semi-preparative HPLC and TLC.

Alkaline hydrolysis (saponification) in carotenoid analysis is a convenient way of removing chlorin pigments, but can affect certain labile carotenoids like fucoxanthin and astaxanthin. However, saponification of the total lipid extract greatly improves the conditions for isolation of one of the novel carotenoids (Tibbetts, 1980).

Saponification was performed by drying a sample of the lipid extract, redissolving in a minimum of ethanol (10-20 ml) and the addition of 10% of that volume of potassium hydroxide solution (aq. 60% w/v). This was left overnight, at ambient, in the dark, then distilled water (200 ml) added, followed by extraction into diethyl ether (2 × 100 ml) and ethyl acetate (100 ml) to give the total neutral fraction (TNF).

Preparative thin layer chromatography (TLC) was performed on 250 μm silica coated plates. The plates were cleaned using acetone and the top 2 cm layer removed. They were reactivated (120° *ca* 30 min.) and allowed to cool in a dessicator prior to use. The neutral fraction was separated into functional classes on the silica plates using methylene chloride/ethyl acetate (4: 1). A further TLC stage, using 40% acetone in hexane as a developer, isolated the individual novel carotenoids. Bands were removed from the plates and eluted with acetone under nitrogen.

Visible spectra were recorded on a Beckman DB-G scanning spectrophotometer. The samples were dissolved in acetone and recordings were made in the range 300-700 nm.

Mass spectrometry analysis

Mass spectra were obtained using a VG Analytical ZAB-HF and recorded on UV sensitive chart paper. The mass spectrometer was operated in the electron impact (EI) mode with an ion source temperature of 200°C, using a filament current of 1 mA and an ionisation voltage of 70 eV. The instrument was scanned over the range 700-20 amu, with an exponential down scan, at a rate of 10 sec./decade.

The carotenoids were inserted into the source in a glass capillary on a direct insertion probe, which was then slowly heated by the source to *ca* 150°C. Thereafter it was heated to *ca* 350°C at *ca* 50°C/min.

RESULTS

The HPLC trace of the total organic extract from the Peruvian sediment is shown in Figure 2. Certain pigments have been tentatively identified by co-elution with standards (under two different solvent conditions) and visible spectra. The least polar peak on the HPLC trace of the Peru extract represents the carotene component. In the Walvis extract this was shown to comprise β-carotene (Tibbetts, 1980).

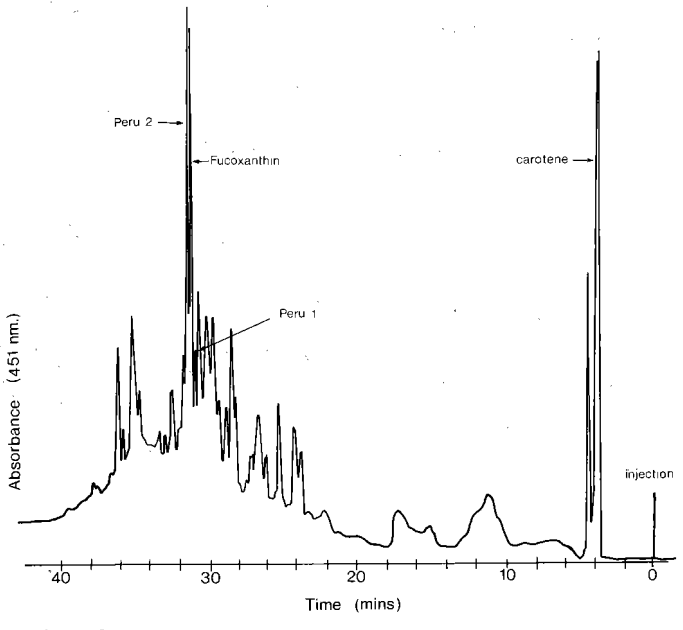


Figure 2
Peruvian Shelf (0-2 *mm*). *Total organic extract.*

Two unknown carotenoids are apparent at the peak positions marked Peru 1 and Peru 2. Their relative retention times were similar to those of the two unknown carotenoids (Walvis 1 and Walvis 2) reported by Tibbetts (1980) from the Namibian Shelf (22°35.0'S; 13°45.0'E; water depth 127 m). Further evidence for their presence in the Peruvian sediments is presented in Figure 3 which shows a trace of the total neutral fraction after saponification of the total lipid extract. The peak assigned to Peru 1 remains unaffected in the neutral fraction which agrees with data for Walvis 1

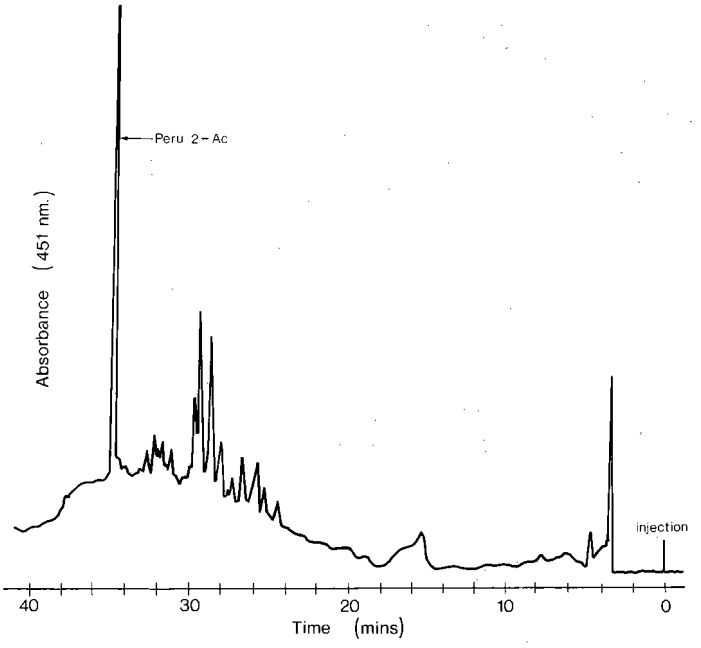


Figure 3
Peruvian Shelf (0-2 *mm*). *Total neutral fraction after saponification.*

(Tibbetts, 1980). Peru 2, however, is modified producing a new peak which is observed later in the chromatogram. Similarly, Walvis 2, on saponification, loses an acetyl group forming a hydroxyl group, thus increasing its retention time (Tibbetts, 1980; Tibbetts, Maxwell, in prep.).

Isolation of Peru 1 and Peru 2 from the neutral fraction was then carried out. Preparative TLC showed Peru 2 to represent a major amount of the carotenoid component of the sediment and the increased retention time for Peru 2-Ac greatly facilitated the isolation and purification of this compound. The visible spectrum (Table) shows good agreement with that for Walvis 2-Ac.

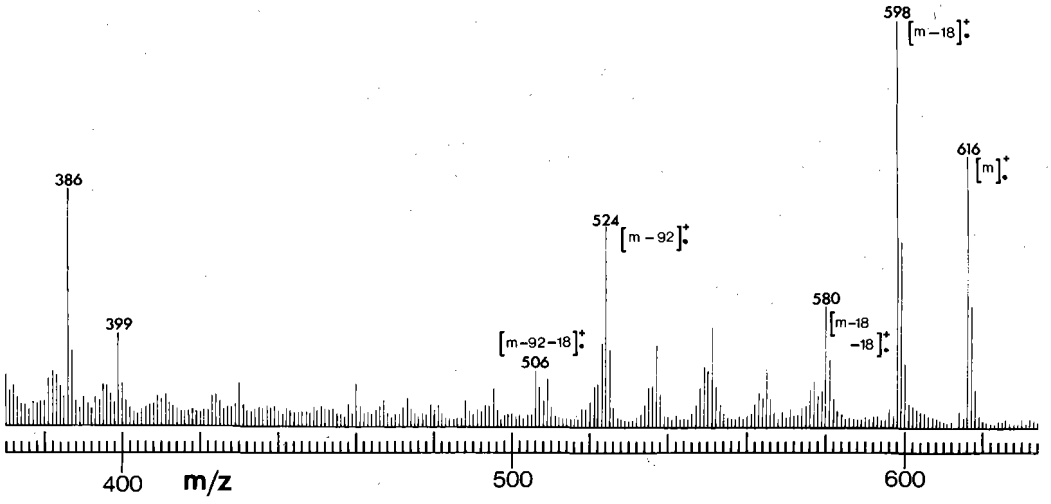
Mass spectrometry results

Mass spectrometry of the Peru 2-Ac produced a spectrum (Fig. 4) which showed close agreement with that reported by Tibbetts (1980) and Tibbetts and Maxwell (in prep.) for Walvis 2-Ac. The heaviest ion (m/z 616)

Table
Visible spectra of "novel" carotenoids from the Namibian Shelf and the Peruvian Shelf.

Sample			λ max	(in acetone)	n.m.
Walvis 1 (Tibbetts, 1980)			427	447	471
Peru 1 (This work)			425	445	472
Walvis 2 (Tibbetts, 1980)		398	420	446	
Peru 2 (This work)		398	420	447	
Walvis 2-Ac (Tibbetts, 1980)	(377)	398	420	447	
Peru 2-Ac (This work)	(377)	396	420	447	

Figure 4
The E.I. mass spectrum of Peru 2-Ac (after saponification).



is thought (Tibbetts, 1980; Tibbetts, Maxwell, in prep.) to be the molecular ion of the carotenoid. The cleavage of an alcohol group produces a loss of H_2O (m/z 18) resulting in a base peak of m/z 598. Other ions present include m/z 580 ($[M-18-18]^+$) and m/z 524 due to the loss of toluene from the molecule. The ions m/z 399 and m/z 386 seen in the Peru 2-Ac spectrum (Fig. 5) were also detected in the spectrum of Walvis 2-Ac, but their significance is unknown.

The other peaks seen in the Peru 2-Ac spectrum can all be accounted for by background hydrocarbon contamination, for which this spectrum has not been corrected.

CONCLUSIONS

Our conclusion is that the unknown carotenoid Peru 2 is identical to the carotenoid Walvis 2, reported by Tibbetts (1980) in a Namibian Shelf sediment.

Some structures for Walvis 2-Ac were suggested by Tibbetts (1980) after extensive experimental study which included a range of spectrometric techniques (EI-MS, CI-MS, N.M.R., I.R. UV/vis). These structures are reported elsewhere (Tibbetts, Maxwell, in prep.).

Walvis 1 and Peru 1 may also be identical, but there is insufficient evidence for complete confirmation, as Peru 1 represents only a minor component of the sediment carotenoids.

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