# NOTES

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## Diploptene: An indicator of terrigenous organic carbon in Washington coastal sediments

*Abstract*—The pentacyclic triterpene 17β(H),21β(H)-hop-22(29)-ene (diploptene) occurs in sediments throughout the Columbia River drainage basin and off the southern coast of Washington state in concentrations comparable to long-chain plantwax *n*-alkanes. The same relationship is evident for diploptene and long-chain *n*-alkanes in soils from the Willamette Valley. Microorganisms indigenous to soils and soil erosion are indicated as the biological source and physical process, respectively, for diploptene in coastal sediments. Similarity between the stable carbon isotopic composition ($\delta^{13}C_{PDB}$) of diploptene isolated from soil in the Willamette Valley ($-31.2\pm0.3$‰) and from sediments deposited throughout the Washington coastal environment ($-31.2\pm0.5$‰) supports this argument. Values of $\delta$ for diploptene in river sediments are variable and 8–17‰ lighter, indicating that an additional biological source such as methane-oxidizing bacteria makes a significant contribution to the diploptene record in river sediments. Selective biodegradation resulting from a difference in the physicochemical association within eroded particles can explain the absence of the more-$^{13}$C-depleted form of diploptene in Washington coastal sediments, but this mechanism remains unproven.

The transport of organic C to the ocean by rivers is tied closely to runoff (Meybeck 1982). The ratio of dissolved to particulate organic C (DOC:POC) varies widely between rivers, ranging from 0.1 to 0.9. An important factor governing this proportion is the topography of the drainage basin. Organic C in lowland rivers is typically dominated by DOC, whereas POC gains importance in highland rivers. Of the 0.59 Tg organic C $yr^{-1}$ exported by the Columbia River to the northeastern Pacific Ocean, 89% is DOC and 11% is POC (Dahm et al. 1981).

Riverine DOC appears to pass conservatively through estuaries (Mantoura and Woodward 1983), and its fate in the ocean is uncertain. In contrast, the fate of riverine POC is better constrained. No change of phase is required to facilitate its sedimentation from ocean waters, and the dispersion of such detrital materials is governed by the competence of water currents to transport particles away from the river mouth. Most of the POC discharged by rivers is deposited on continental shelves in close proximity to the point of input (Berner 1982).

The coastal region off Washington state receives an estimated 14.3 Tg $yr^{-1}$ of sedimentary particulate material from the Columbia River (Karlin 1980). In this region, suspended particulate material and its associated organic constituents are hydraulically sorted in the process of dispersion. As a result, distinct bands of sediment accumulate parallel to shore and extend north-northwest from the river mouth (Fig. 1). Prahl (1985) examined the composition of lignin phenol, polycyclic aromatic hydrocarbon (PAH), and aliphatic hydrocarbon mixtures contained in a grid of samples collected from these sedimentary facies. Three independent lines of evidence indicated that the composition of terrigenous organic matter deposited in Washington continental shelf and slope sediments varies spatially as a consequence of differential dispersion of

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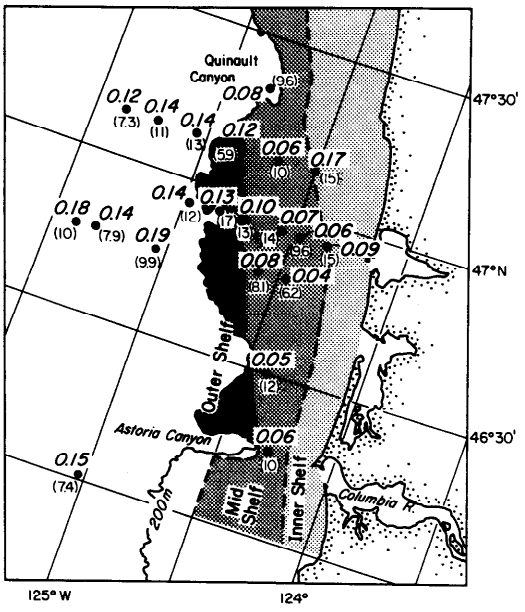


Fig. 1. Values of $D:\Sigma C_{25-31}$ (bold italic numbers) and concentrations of diploptene ($\mu g\ g^{-1}$ TOC) (in parentheses) measured in sediments throughout the southern Washington continental shelf and slope region.

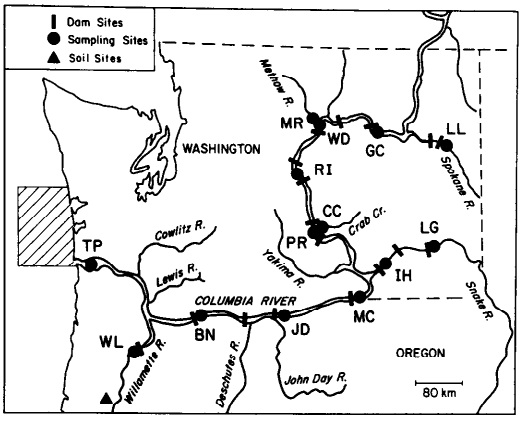


Fig. 2. Map showing where grab samples of bottom sediments were obtained for chemical analysis. The 14 locations arranged by distance upstream from the river mouth are Tongue Point (TP), Oregon City Dam (WL), Bonneville Dam (BN), John Day Dam (JD), McNary Dam (MC), Ice Harbor Dam (IH), Little Goose Dam (LG), Priest Rapids Dam (PR), Crab Creek (CC), Rock Island Dam (RI), Wells Dam (WD), Methow River (MR), Grand Coulee Dam (GC), and Long Lake Dam (LL). The boxed area adjacent to the Columbia River mouth identifies the offshore study region depicted in Fig. 1.

particles. This spatial heterogeneity complicates assessment of terrigenous organic material preserved along continental margins (Prahl and Muchlhausen 1989).

Prahl (1985) observed an offshore increase in the abundance of the $C_{30}$ pentacyclic triterpene, 17$\beta$(H),21$\beta$(H)-hop-22(29)-ene (diploptene), relative to that of an odd-carbon-predominant series of plant-wax *n*-alkanes ($C_{25}$, $C_{27}$, $C_{29}$, $C_{31}$). This distributional pattern is summarized in Fig. 1 in terms of the ratio $D:\Sigma C_{25-31}$, where D is the concentration of diploptene and $\Sigma C_{25-31}$ the summed concentrations of odd-C, long-chain *n*-alkanes. For sediments collected throughout the Columbia River basin and in particulate matter discharged at the river mouth, the concentration of diploptene was found to be roughly equal to that of individual plantwax *n*-alkanes, implying a terrestrial origin for this compound. Accordingly, the spatial pattern displayed by $D:\Sigma C_{25-31}$ values measured in Washington coastal sediments (Fig. 1) was interpreted in terms of differential dispersion of suspended particulate material introduced at the Columbia River mouth.

We here describe more recent examinations of the origin of diploptene accumulating in Washington coastal sediments and, specifically, relationships between this compound and soil organic matter in the drainage basin of the Columbia River. Venkatesan (1988) reviewed reports of the occurrence of diploptene in coastal marine sediments on a very broad geographic basis and concluded that this molecule derives largely from autochthonous sources. Results of isotopic analyses in the present study imply instead that the diploptene in Washington coastal sediments is of terrigenous origin. By analogy, origins of diploptene in other localities should be re-examined.

Grab samples of bottom sediment were collected in 1979 at 14 sites located throughout the drainage basin of the Columbia River (Fig. 2). The samples were stored frozen in clean glass bottles until hydrocarbon (this study) and lignin (Hedges et al. 1984) analysis. Further description of each sampling site can be found elsewhere (Hedges et al. 1984). Soil samples were collected in 1988–1989 at subsurface depths (3–5 cm) from three different environments

in the Willamette Valley near Corvallis, Oregon: a conifer forest, a deciduous forest, and a cultivated grassland. These samples were analyzed immediately upon collection. For purposes of environmental comparison, a fourth soil from a tropical forest in Costa Rica was obtained and analyzed by the same methods.

All samples were Soxhlet extracted (48 h) with a 50/50 mixture of benzene in methanol or hexane in acetone (250 ml). A total solvent extractable lipid (SEL) fraction was obtained by adding distilled water (50 ml) to the extract and partitioning into hexane (60 ml; 3×). The combined hexane layers were washed against a 50% saturated NaCl solution and dried over anhydrous $Na_2SO_4$. Rotary evaporation yielded an SEL residue. A hydrocarbon fraction was isolated by eluting the SEL residue with hexane (30 ml) through a column of silica gel (7 g of Kieselgel 60; 5% deactivated with water). The hydrocarbon fraction from each sample was passed through a Cu column to eliminate elemental sulfur, then taken to dryness by rotary evaporation and stored frozen in glass vials until capillary gas chromatographic (GC) analysis.

Each hydrocarbon fraction was dissolved in isooctane and analyzed on an HP5890A gas chromatograph equipped with a capillary column (DB-5; 30 m × 0.25-mm i.d.; 0.25-$\mu$m film thickness; J&W Scientific), splitless injection, temperature programing (75°–130°C at 10°C $min^{-1}$, 130°–300°C at 5°C $min^{-1}$), hydrogen carrier gas (0.7 kg $cm^{-2}$), and flame ionization detection. The *n*-alkanes, $C_{25}$, $C_{27}$, $C_{29}$, and $C_{31}$, and three pentacyclic triterpenes, diploptene (D), fern-7-ene (F), and neohop-13(18)-ene (N), were identified by comparison of retention times with those of authentic standards and were quantified by an internal-standard method with hexamethylbenzene as the GC injection standard. Comparison of electron-impact mass spectra with those of standards (70 eV, Finnigan 4000 quadrupole GC/MS with INCOS 2300 data system) confirmed the identities of D, F, and N. Replicate analyses indicated that the precision of measurement for D and $\Sigma C_{25-31}$ concentrations, and of the ratio D : $\Sigma C_{25-31}$, is $\leq 10\%$.

Hydrocarbon fractions from selected soil and sediment samples were subjected to urea adduction or $Ag^+$-impregnated silica gel column chromatography (Christie 1982) to obtain subfractions enriched in diploptene and other pentacyclic triterpenes. The stable C isotope compositions of individual molecules in these fractions were determined by isotope-ratio-monitoring gas chromatography-mass spectrometry (irmGCMS, Hayes et al. 1990). Calibration with internal standards of known isotopic composition allowed determination of $\delta^{13}C_{PDB}$ values for individual hydrocarbons with a 95% C.I. of better than $\pm 1.0$‰. All $\delta$ values refer to $^{13}C$ vs. PDB and, for simplicity in the following text, $\delta^{13}C_{PDB}$ is abbreviated to $\delta$.

Diploptene was first identified in the cuticular wax of certain ferns (Ageta et al. 1964). Other monounsaturated pentacyclic triterpenes, notably compounds of the structural class known as fernenes, were also reported as components of these plant extracts. Little attention was paid in early work to absolute concentrations in the cuticular wax or to abundances relative to more commonly encountered *n*-alkyl lipid components of cuticular waxes (Kolattukudy 1976). Such quantitative details, reported in a more recent study of rhizomes in selected ferns (Ageta and Arai 1983), showed that diploptene occurs typically at less than a fifth the abundance of fern-7-ene, the major pentacyclic triterpene. This work did not clarify whether diploptene is biosynthesized by the fern or derives from microbes intimately associated with the rhizome structure.

Bacteria have been recognized for some time as important sources of diploptene and related hopanoids. Nearly half of ~100 strains of taxonomically diverse bacteria now surveyed contained hopanoids (Rohmer et al. 1984), demonstrating the common but nonuniversal occurrence of these biochemicals in bacteria. Hopanoids are common constituents of many cyanobacteria, a wide range of gram-positive and gram-negative chemoheterotrophs, and perhaps all obligate methylotrophs and purple nonsulfur bacteria. Such compounds are atypical of archaebacteria and sulfur bacteria. The major hopanoid encountered in bacteria is a $C_{35}$ hopanetetrol (Rohmer et al. 1984). Its cellular concentration lies in

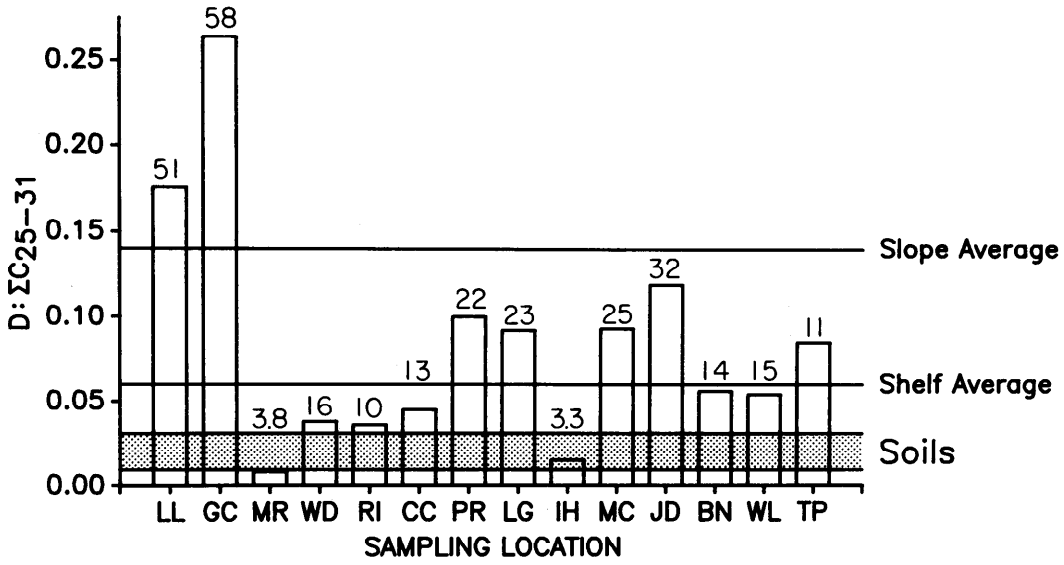


Fig. 3. Values of D: $\Sigma C_{25-31}$ measured in 14 sediments collected throughout the Columbia River basin. Codes as in Fig. 2. Average values of D: $\Sigma C_{25-31}$ for Washington continental shelf and slope sediments are indicated by horizontal lines. The range of values measured in three soils from the Willamette Valley is depicted by the shaded area. The number above each box corresponds to the diploptene concentration ($\mu g\ g^{-1}$ TOC) in the given sediment.

the range of 0.1–2 mg $g^{-1}$ dry weight of bacteria. Notably in the present context, diploptene occurs in all hopanoid-containing bacteria at ~1/20th or less of the hopanetetrol concentration.

Concentrations of diploptene in sediments throughout the Columbia River basin and Washington coastal region and in suspended particulate material discharged at the Columbia River mouth are comparable to those of the $C_{33}$ *n*-alkane (Prahl 1985). Diploptene and the $C_{33}$ *n*-alkane are also equally abundant in sediments deposited throughout Puget Sound, accumulating on the Alaskan outer continental shelf and in the southern California Bight (Venkatesan 1988 and references therein). If *n*-$C_{33}$ is a remnant of higher plantwaxes, as indicated by its association in each case with a characteristic series of long-chain, odd-predominant *n*-alkanes (Kolattukudy 1976), a terrestrial source for diploptene would appear possible in all of these coastal marine settings.

Possible terrestrial sources of diploptene are restricted to certain ferns and various genera of bacteria as previously described. No attempt was made in the work of Prahl (1985) to distinguish which of these might account for the diploptene present in Columbia River and Washington coastal sediments, though a dominant contribution from ferns would seem unlikely on the basis of mass considerations. As shown in Fig. 3, values of D: $\Sigma C_{25-31}$ measured in riverine and coastal marine sediments from the Pacific Northwest range from 0.01 to 0.25. Essentially all vascular plants including ferns biosynthesize series of long-chain ($>C_{20}$), odd-C-predominant *n*-alkanes as distinctive components of their cuticular wax (Kolattukudy 1976); however, only a few species of fern are recognized sources of diploptene. If ferns were the dominant contributing source, concentrations of diploptene as high as 1–25% of the combined concentrations of plantwax *n*-alkanes would be unexpected unless diploptene-producing ferns were the main source of vascular plant debris to the basin or diploptene was much more stable than plantwax *n*-alkanes and, consequently, enriched preferentially in early diagenetic processes. The first possibility is not supported by lignin phenol data for Columbia River sediments (Hedges et al. 1984). Alternatively, contributions of di-

Table 1. Diploptene and plantwax *n*-alkane compositions measured in various soils from temperate (Willamette Valley) and tropical (Costa Rica) environments.

| Samples | % TOC* | D† | $\Sigma C_{25-31}$† | D : $\Sigma C_{25-31}$‡ |
|---|---|---|---|---|
| Deciduous forest | 1.9 | 2.3 | 105 | 0.022 |
| <64 μm | 1.6 | 2.5 | 100 | 0.025 |
| 64–250 μm | 2.3 | 2.5 | 132 | 0.019 |
| >250 μm | 3.8 | 1.5 | 65 | 0.023 |
| Coniferous forest | 1.7 | 2.2 | 71 | 0.031 |
| Cultivated field | 0.8 | 3.0 | 333 | 0.009 |
| Tropical forest§ | 7.2 | 5.8 | 73 | 0.080 |

* Percentage of dry weight.
† Diploptene and combined $C_{25}$, $C_{27}$, $C_{29}$, $C_{31}$ plantwax *n*-alkanes; μg $g^{-1}$ TOC.
‡ Ratio of diploptene to plantwax *n*-alkane.
§ Costa Rican soil sample obtained from collection in Microbiology Department at Oregon State University.

ploptene from bacteria, in which series of long-chain odd-predominant *n*-alkanes are typically absent (Kolattukudy 1976), could account for the high D : $\Sigma C_{25-31}$ values measured in Columbia River and Washington coastal sediments.

Numerous microorganisms displaying a wide range of metabolic traits are indigenous to soils (Atlas and Bartha 1981). Representatives of the cyanobacterial genera *Anabaena, Calothrix, Nostoc,* and *Scytonema,* among others, are common microbial photoautotrophs found in soil environments. These primary producers provide fixed forms of N and organic C to the soil, thereby enhancing its fertility. Chemolithotrophic *Nitrosomonas* spp. are active in the transformation of $NH_3$ to $NO_2^-$—another process essential for the maintenance of soil fertility. Actinomycetes, such as members of the genera *Streptomyces,* make up a significant percentage (10–33%) of the bacterial biomass in many soils. These chemoheterotrophs decompose organic detritus continuously added to soils by dead and dying vegetation. Interestingly, examples of each of these bacterial genera are known to contain hopanoids (Rohmer et al. 1984). Thus, eroded soil provides a potential terrestrial source for the diploptene observed in sediments deposited throughout the Columbia River basin and off the Washington coast.

Figure 4A illustrates a typical gas chromatogram of the total hydrocarbon fractions isolated from soils collected in three different settings (a conifer forest, a deciduous forest, and a cultivated grassland) within the temperate Willamette Valley and a tropical forest setting in Costa Rica. The major components in each case are odd-C-predominant *n*-alkanes ($C_{21}$ through $C_{35}$) attributable to the cuticular wax of higher plants. The *n*-alkane series maximized at $C_{29}$ in all soils examined from the Willamette Valley as well as in all river and coastal marine sediments examined from the Pacific Northwest, and at $C_{31}$ in the Costa Rican soil. In every case, diploptene was the next most abundant compound resolved by GC. The importance of diploptene in hydrocarbon fractions is not a unique feature of the present set of soils. Ries-Kautt and Albrecht (1989) found that diploptene was the predominant pentacyclic triterpene in acidic, neutral, and basic soils. Diploptene in soils probably derives from an indigenous microbial biomass and represents a product of organisms reworking primary organic matter rather than a contribution from some macrobiological source such as ferns.

Values of D : $\Sigma C_{25-31}$ measured in the three soils from the Willamette Valley range from 0.009 to 0.031 (Table 1). Concentrations of diploptene relative to total organic C (TOC) fall within a very narrow range, 1.5–3 μg diploptene $g^{-1}$ TOC (Table 1), while those measured in Columbia River sediments (Fig. 3) are commonly an order of magnitude higher. Sediments from the Washington coastal region contain a mixture of terrestrial and marine organic carbon, the proportion of which depends on the depositional site (Hedges and Mann 1979). Concentrations of diploptene relative to the terrestrial component of TOC can be estimated from D : $\Sigma C_{25-31}$ ratios (Fig. 1). If each 280 μg of plantwax *n*-alkanes represents 1 g of terrestrial organic C (Prahl and Muehlhausen 1989), then abundances of diploptene range from 14 to 51 μg $g^{-1}$ terrestrial organic C. Thus, concentrations of diploptene are at least an order of magnitude higher in coastal sediments than in the Willamette soils. Either additional sources contribute to the diploptene pool in the riverine and coastal sediments, or a substantial fraction of soil-derived organic C is reprocessed preferentially to diploptene before the

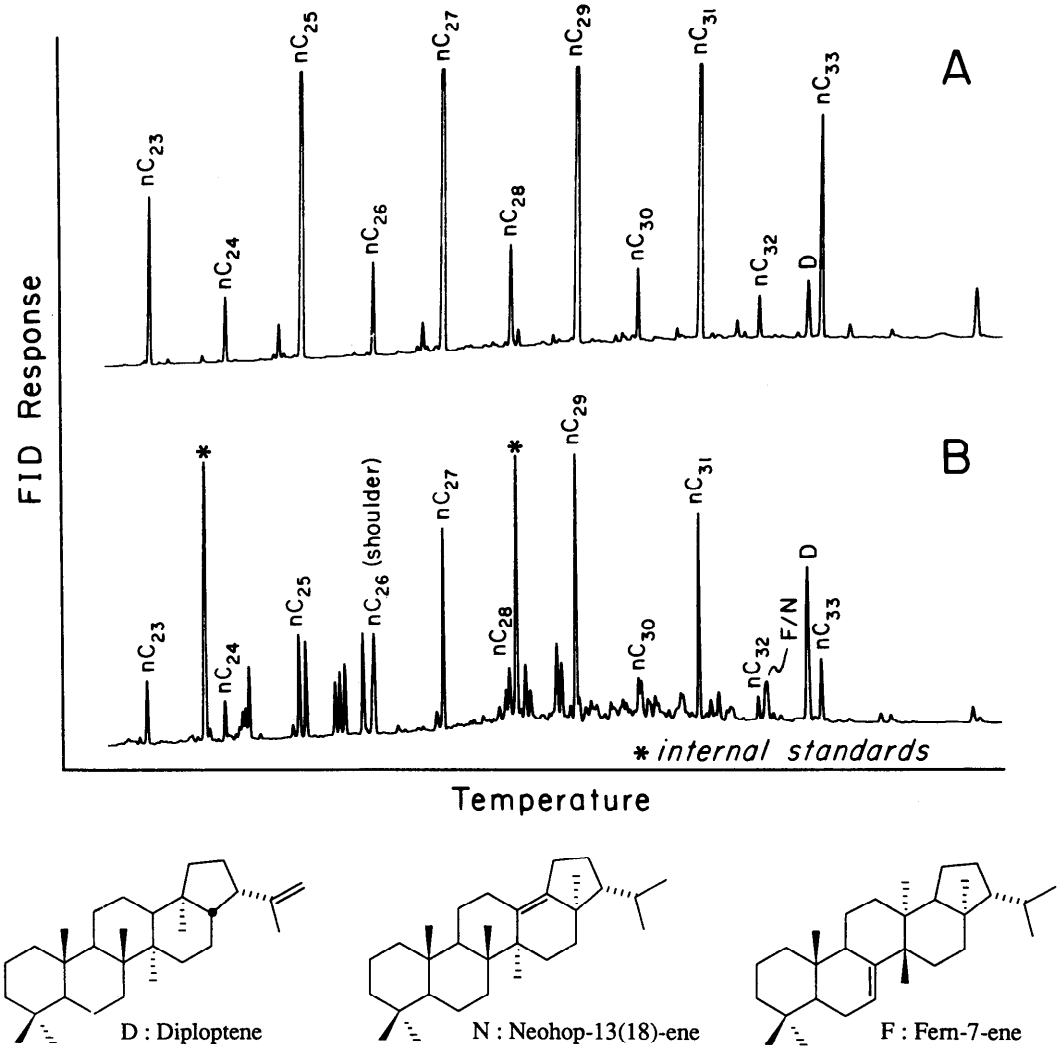


Fig. 4. Gas chromatogram of the total hydrocarbon fraction isolated (A) from deciduous forest soil collected in the Willamette Valley near Corvallis and (B) from surface sediments (0–2-cm depth) collected on the continental slope off the Washington coast. *n*-Alkanes are identified by carbon number; the three triterpenes [diploptene, neohop-13(18)-ene, fern-7-ene] are identified by the letters D, N, and F. GC conditions are defined in the text.

incorporation of soil organic material in these sediments.

The $^{13}C$ content of diploptene isolated from two Willamette soils and from four coastal sediments provides further evidence bearing on the sources of this molecule. The coastal samples include two surface sediments (0–2-cm core depth), both from ~75-m water depth on the continental shelf (46°14.9′N, 124°14.4′W; 46°49.7′N, 124°26.0′W), and two depth horizons (2–4 and 34–38 cm) of a core collected at a water depth of ~700 m on the continental slope (46°44.8′N, 125°00.7′W; Fig. 1). Ages for the two depth horizons sampled in the slope core are estimated as contemporary and ~500-yr old, respectively, based on $^{210}Pb$ accumulation rates for nearby locations (Carpenter and Peterson 1989).

Isotopic compositions of diploptene (Table 2) are essentially identical in samples of Willamette soil ($-31.2\pm0.3‰$) and

Table 2. Stable carbon isotopic composition ($\delta^{13}C$, ‰ vs. PDB) of plantwax *n*-alkanes and diploptene extracted from forest soil collected at two locations in the Willamette Valley and one location in Costa Rica and sediments collected in the Columbia River drainage basin and off the Washington coast on the continental shelf and slope.

| | *n*-$C_{25}$ | *n*-$C_{26}$ | *n*-$C_{27}$ | *n*-$C_{28}$ | *n*-$C_{29}$ | *n*-$C_{30}$ | *n*-$C_{31}$ | Diploptene |
|---|---|---|---|---|---|---|---|---|
| Willamette soil | | | | | | | | |
| No. 1 | −31.4±0.5 | −31.8±0.4 | −32.3±0.3 | −33.7±0.4 | −33.6±0.2 | −33.2±0.3 | −33.1±0.2 | −31.0±0.6 |
| No. 2 | −33.3±0.2 | −33.7±0.7 | −32.0±0.2 | −33.0±0.8 | −32.9±0.1 | −33.8±0.9 | −35.0±0.2 | −31.4±0.8 |
| Costa Rican soil | −33.8±0.4 | −33.7±0.3 | −34.3±0.2 | −34.9±0.2 | −34.0±0.4 | −35.1±0.7 | −33.7±0.5 | −33.8±0.6 |
| River sediment* | | | | | | | | |
| WL No. 1 | | | | | | | | −48.3±0.7 |
| WL No. 2 | | | | | | | | −38.9±0.2 |
| MC | −30.6±0.3 | −30.0±0.9 | −32.0±0.5 | −33.9±0.4 | −31.6±0.6 | −31.4±1.1 | −33.6±0.6 | −47.2±0.5 |
| $H_2O_2$ untreated | | | | | | | | −47.3±0.1 |
| $H_2O_2$ treated | | | | | | | | −46.9 |
| LG | | | | | | | | −44.0±0.2 |
| PR | | | | | | | | −42.0±0.2 |
| Coastal sediment† | | | | | | | | |
| Shelf No. 1 | −31.3±0.4 | −30.2±0.2 | −31.0±0.2 | −30.8±0.1 | −31.6±0.2 | −31.4±0.3 | −32.7±0.1 | −31.5±1.0 |
| Shelf No. 2 | −30.8±0.1 | −30.8±0.3 | −31.0±0.6 | −31.9±0.1 | −32.0±0.2 | −33.2±0.6 | −32.8±0.3 | −30.4±1.0 |
| Slope | | | | | | | | |
| 2–4 cm‡ | −31.2±0.2 | −29.4±0.5 | −30.7±0.2 | −30.6±0.3 | −31.6±0.2 | −30.5±0.5 | −32.1±0.2 | −31.8±0.5 |
| 2–4 cm§ | −31.1±0.1 | −31.3±0.3 | −31.6±0.2 | −32.3±0.1 | −32.3±0.0 | −32.5±0.1 | −33.0±0.1 | −31.1±0.7 |
| 34–38 cm | −30.5±0.2 | −30.1±1.0 | −30.9±0.1 | −31.1±0.7 | −31.8±0.4 | −32.1±0.1 | −32.1±0.0 | −31.0±1.6 |

* Willamette River, two sites (WL); McNary Dam (MC); Little Goose Dam (LG); Priest Rapids Dam (PR); *see Fig. 2.*
† Shelf No. 1: 46°14.9'N, 124°14.4'W; shelf No. 2: 46°49.7'N, 124°26.0'W; slope: 46°44.8'N, 125°00.7'W; *see Fig. 1.*
‡ *n*-Alkane and triterpene fraction separated by urea adduction.
§ *n*-Alkane and triterpene fraction separated by $Ag^+$-impregnated silica gel column chromatography.

all of the Washington coastal sediments ($-31.2\pm0.5$‰). Comparison also reveals concordance between the $\delta$ values for individual plantwax *n*-alkanes in the same samples from these two environments. These findings are consistent with a common source for diploptene in both settings and with the occurrence of soil organic matter as a component of TOC in coastal sediments. It is not yet known why the $\delta$ values for diploptene and plantwax *n*-alkanes in the Willamette and Costa Rican soils are so similar. The agreement may be fortuitous, but its occurrence in two disparate environments suggests otherwise. As previously discussed, diploptene in soil is biosynthesized by certain bacteria either photoautotrophically fixing $CO_2$ or metabolizing soil organic C. The $\delta$ value of TOC in the deciduous soil from the Willamette Valley is $-26.1$‰ (Eversmeyer and Prahl unpubl. data), 5‰ enriched in $^{13}C$ relative to diploptene.

Isotopic analyses of hydrocarbons in Columbia River sediments introduced a complication to the soil-erosion hypothesis. Values of $\delta$ for diploptene in river sediments are not $-31$‰ as perhaps expected from the previous discussion but are from 8 to 17‰ more depleted in $^{13}C$ (Table 2). If soils contribute diploptene to the river sediments, this contribution must be diluted by a second, isotopically light form of the same compound. Methane-oxidizing bacteria represent likely candidates for this additional diploptene source. Rohmer et al. (1984) observed hopanoids in every strain of methylotroph examined in their chemotaxonomic survey of bacteria. In freshwater environments, fermentative processes, including substantial methanogenesis, often occur near the water–sediment interface because electron acceptors other than $O_2$ are typically unavailable for use in respiratory metabolism. Diffusion of methane from sediments into the water column is one process accounting for near saturation levels of methane in many fresh waters (de Angelis and Lilley 1987 and references therein).

Hopanoids synthesized by methylotrophic bacteria using methane as their C source would be expected to display very negative $\delta$ values because biogenic methane is often extremely $^{13}C$ depleted. Values in the range $-65$ to $-85$‰ have been observed in hopanoids of presumed methylotrophic origin preserved in modern and ancient lacustrine sediments (Freeman et al. 1990; Collister et al. 1992). A variable blend of such highly $^{13}C$-depleted diploptene with the soil-derived form of diploptene could explain the range of negative $\delta$ values observed for this compound in Columbia River sediments.

If the predominant source of diploptene in Washington coastal sediments is soil organic matter and this compound has been introduced there by erosion through the Columbia River drainage, the apparent absence of the $^{13}C$-depleted form of diploptene offshore is curious. We propose that the two forms of diploptene admixed in river sediments have different physicochemical associations with eroded particles and that the more $^{13}C$-depleted form is biodegraded selectively at some point, effectively blocking its erosional transit to the offshore environment.

An attempt was made to test this hypothesis experimentally. Bulk sediment from behind McNary Dam (Fig. 2) was exposed at room temperature for 36 h to a 10% $H_2O_2$ solution. This treatment caused TOC and diploptene concentrations normalized to dry weight and D : $\Sigma C_{25-31}$ values to decrease 75, 75, and 50%, respectively. If we assume the $^{13}C$-depleted methylotrophic form of this compound was more vulnerable to biodegradation than the soil form and the $H_2O_2$ treatment provided a good chemical proxy for biodegradation, the $\delta$ value for the total diploptene extract from this sediment was expected to shift toward $-31$‰. But no appreciable change in $\delta$ value was observed as a consequence of chemical treatment (Table 2). This experimental result, although negative, does not disprove the hypothesis. Treatment with $H_2O_2$ may inadequately mimic biochemical processes that naturally degrade the two forms of diploptene presumed to be present in river sediments. Mechanisms of selective degradation have been invoked previously as reasonable explanations for other organic geochemical observations (Haddad et al. 1992; Prahl et al. 1981 and references therein).

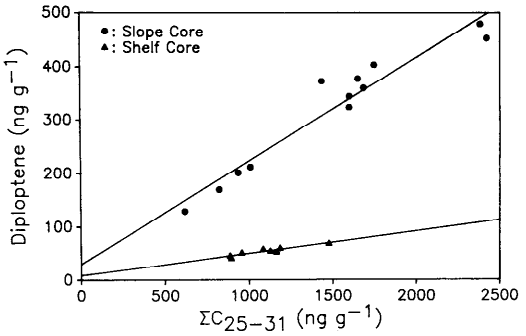


Fig. 5. Scatterplots displaying the correlation between diploptene and plantwax *n*-alkane ($\Sigma C_{25-31}$) concentrations (each per g dry sediment) measured with depth in a sediment core from the Washington continental shelf (46°49.7′N, 124°26.0′W; 73-m water depth) and slope (46°44.8′N, 125°00.7′W; 700-m water depth). The equations describing the data sets for the shelf ($D = 0.042\Sigma C_{25-31} + 8.7$, $r^2 = 0.86$, $n = 8$) and slope ($D = 0.19\Sigma C_{25-31} + 30$, $r^2 = 0.93$, $n = 12$) cores have been fitted by least-squares linear regression.

But, as in the present case, such mechanisms have never been experimentally substantiated.

Hydrocarbon fractions were also analyzed in two sediment cores from the Washington coastal region, one from the shelf (46°49.7′N, 124°26.0′W) and one from the slope (46°44.8′N, 125°00.7′W; Fig. 1). If we use $^{210}$Pb-derived sedimentation rates for nearby locations (Carpenter and Peterson 1989), the timespan represented was estimated to be ~200 yr for the shelf core and ~500 yr for the slope core. *n*-Alkanes made up the dominant component of the total hydrocarbon fractions resolved by GC (Fig. 4B), with diploptene as one of the next most abundant constituents (Prahl and Carpenter 1984). Downcore profiles for concentrations of diploptene and of long-chain *n*-alkanes ($\Sigma C_{25-31}$) are well correlated in each case, although the slopes of the regression lines differ (Fig. 5). If, as is evident from isotopic compositions (Table 2), the long-chain *n*-alkanes are predominantly of terrestrial origin, the increase of $D:\Sigma C_{25-31}$ values with distance offshore requires either that a marine component of diploptene, which happens to have the same $\delta$ value as the terrestrial diploptene, is enriched further offshore or that hydraulic sorting of land-derived particulate organic matter leads to a systematic change in the $D:\Sigma C_{25-31}$ ratio. The latter alternative, chosen on the basis of independent lines of geochemical evidence in an earlier study (Prahl 1985), is supported by the observed downcore correlations between diploptene and plantwax *n*-alkane concentrations.

We do not infer that major marine sources of diploptene are absent in Washington coastal waters, although the diploptene preserved in underlying sediments appears to be largely terrigenous. Autochthonous sources of diploptene in coastal waters are quite probable. Wakeham et al. (1984) detected diploptene as a dominant hydrocarbon in suspended particulate material collected from the oxygen minimum zone in the eastern tropical Pacific off Mexico, a region presumably receiving minimal input of terrestrial organic matter. They ascribed the presence of diploptene in the samples to an unspecified microbial source involved with geochemical cycling processes within the oxygen minimum zone. A study of the Gulf of Alaska revealed that cyanobacteria contribute significantly to total phytoplankton biomass throughout the year (Booth 1988). The importance of cyanobacteria as contributors to total primary productivity has probably been overlooked worldwide in the oceans owing to the small size ($\leq 2$ $\mu$m) of these organisms. Cyanobacterial productivity represents a potentially major autochthonous source of diploptene to marine sediments since most species surveyed to date have been shown to contain hopanoids (Rohmer et al. 1984). Booth (1988) identified species of the genus *Synechococcus* as the major cyanobacterial producers in water samples examined from the Gulf of Alaska. However, neither of the two strains of *Synechococcus* sp. surveyed by Rohmer et al. (1984) contained hopanoids. Thus, the quantitative significance of geographically widespread planktonic contributions of diploptene to marine sediments remains unclear even though evidence exists for possible marine contributions of diploptene to sediments underlying specialized regions of the ocean (e.g. Wakeham et al. 1984).

If marine sources contributed diploptene to Washington coastal sediments, the close

agreement between $\delta$ values for this compound in coastal sediments and soils would seem extremely coincidental. Moreover, isotopic compositions of other pentacyclic triterpenes [fern-7-ene and neohop-13(18)-ene] identified in the slope core (Fig. 4B) are 10–12‰ more positive than those measured for diploptene, i.e. −22.1‰ and −19.9‰ in the 2–4- and 34–38-cm intervals. Too little information exists at present to assign a specific biological source to these compounds, although a marine origin might be inferred from the isotopic results. The presence of such pentacyclic triterpenes with $\delta$ values different from those of recognized terrestrial biomarkers (i.e. plantwax *n*-alkanes) strengthens the case that the diploptene preserved in Washington coastal sediments has an allochthonous, soil origin. Evidently, any autochthonous contribution of diploptene does not survive early diagenesis and become a component of the TOC preserved in sediments from this coastal region.

Diploptene has frequently been reported as a quantitatively important hydrocarbon constituent in sediments from coastal marine environments (Venkatesan 1988). As in this case, it has appeared often but not always in association with abundant, odd-C, long-chain *n*-alkanes. The present results suggest that the diploptene preserved in other coastal marine sediments characterized by strong plantwax signatures may also derive from soil erosion and not from marine biological sources. As a result of differential particle transport (Prahl 1985), the quality of terrestrial organic C preserved in coastal marine sediments changes with distance offshore from relatively fresh, coarse-grained vascular-plant detritus to increasingly more degraded, fine-grained materials perhaps largely of soil origin. Recent work with molecular biomarkers (Prahl and Muehlhausen 1989 and references therein) now suggests that the terrestrial component of TOC-rich sediments depositing along continental margins is quantitatively more significant than indicated by earlier studies (Gearing et al. 1977 and references therein). Therefore, it seems timely to focus research attention on the identification of soil biomarkers whose systematic study would refine our knowledge of the fate of particulate forms of terrestrial organic C in the marine environment.

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# An in vivo method for the estimation of phycoerythrin concentrations in marine cyanobacteria (*Synechococcus* spp.)

*Abstract*—A new, rapid and simple method estimates phycoerythrin (PE) concentrations in natural populations of marine *Synechococcus* spp. The method is based on the observation that glycerol uncouples energy transfer between PE and other biliproteins of the *Synechococcus* phycobilisome. Under these conditions there is a precise linear relation between the intensity of PE fluorescence emission and cell PE content. The method was used to determine profiles of *Synechococcus* PE for neritic (Celtic Sea) and open-ocean (Sargasso Sea) waters. Both profiles show that cell PE increased with depth and reflect the influence that the availability of light and nutrients (nitrogen) has on the synthesis of this biliprotein.

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In the late 1970s Waterbury et al. (1979) and Johnson and Sieburth (1979) established that small coccoid cyanobacteria (*Synechococcus* spp.) make a substantial