AN ABSTRACT OF THE THESIS OF

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Title: Use of Sediment Fractionation Techniques to Establish a Geochemical Link between Natural-occurring PAH and 3-oxytriterpenoids in Columbia River Sediments.

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Abstract approved: _____
Fredrick G. Prahl

A series of three tetracyclic aromatic hydrocarbons of molecular weight 274 have been detected in Recent sediments deposited upstream of Wells Dam on the Columbia river. These compounds represent major components of the polycyclic aromatic hydrocarbon (PAH) fraction. It has been hypothesized that these PAH are produced during early diagenesis from certain pentacyclic triterpenoid precursors (e.g. α-amyrin, ß-amyrin and lupeol) of higher vascular plant origin. The precursor assignments are based exclusively on structural similarities. A variety of 3-oxygenated pentacyclic triterpenoids (such as ß-amyrin, oleanolic and ursolic acid) have also been identified in Wells Dam sediments. Samples of unconsolidated river sediment containing this PAH series and the 3-oxygenated pentacyclic triterpenoids were separated into various fractions distinguished by size (>500 μm, 250-500 μm, 125-250 μm, 63-125 μm and <63 μm) and for particles >63 μm by density ("light" and "heavy"). These fractions were analyzed chemically in order to establish if a common particle association existed between this PAH series and the pentacyclic triterpenoids supporting a diagenetic link. Chemical analyses revealed that 50% of these tetracyclic PAH are contained in low density particles ("light") >63 μm that comprise less than 1% of total unfractionated sediment weight. These compounds are also significantly enriched in the coarsest size fraction of the sediment (≥ 500 μm). Characterization of the organic matter in the coarse and "light" fractions revealed a carbon-rich, nitrogen-depleted particle (C/N = 33.5) with a stable organic carbon isotopic composition ($\delta^{13}C$ = -26.6 ‰) within the range for terrestrial plants. Lipid analyses show a high abundance of

plantwax hydrocarbons with large CPI (C_{20-30}) values (13.0) characteristic of the surface waxes of higher plants. Lignin in Wells Dam sediments are largely undegraded as indicated by low ratios of vanillic acid to vanillin ($[Ad/Al]_v$: 0.27 to 0.40 in different particle size fractions). The proportion of nonwoody angiosperm tissues appears to increase as particle size decreases. 3-Oxygenated pentacyclic triterpenoids display distributions and enrichments within size and density fractions similar to those for the suite of PAH. Microscopic examination of the coarse and "light" fractions indicates the organic matter is primarily constituted by higher plant detritus. The 3-oxytriterpenoids present in these sediments appear to be labile and readily available for alteration. Although these natural products are the potential precursors for the MW274 PAH, their primary route of transformation appear to be in another direction. The 3-oxytriterpenoids appear to be efficiently degraded by microorganisms. Reducing microenvironments could serve to the transformation of a minimum amount of 3-oxytriterpenoids to the PAH compounds. Diploptene, a possible precursor for one of the MW274 PAH compounds shows a completely opposite distribution in the different particle size fractions to the 3-oxytriterpenoids and the MW274 PAH suite indicating a source other than vascular plant material. The particle size and density fractionation of these sediments has provided specific information demonstrating a common association of the PAH and the pentacyclic triterpenoids with vascular plant debris thus supporting their hypothesized product-precursor relationship.

Use of Sediment Fractionation Techniques to Establish a Geochemical Link between Natural-occurring PAH and 3-Oxytriterpenoids in Columbia River Sediments

by

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Con todo mi amor para
Alejandra Andrea

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INTRODUCTION

I. Naturally occurring polycyclic aromatic hydrocarbons.

Naturally occurring polycyclic aromatic hydrocarbons (PAH) are thought to be generated in several depositional environments by early diagenetic processes. Tri-, tetra- and pentacyclic hydrocarbons varying in the extent of aromatization occur widely in Recent sediments. Aromatization reactions, once thought to require long periods of time, can occur during early diagenesis of organic matter in sediments and are probably mediated by microorganisms (Biellmann et al., 1968; Greiner et al., 1976; Spyckerelle et al., 1977a; Corbet et al., 1980).

PAH derived from a variety of natural products, have been detected in lacustrine (Wakeham et al., 1980; Barnes and Barnes, 1983), deltaic (Spyckerelle et al., 1977a,b) and marine sediments (Simoneit, 1977a,b) in which they are regarded as indicators of terrestrial input. They are usually only partially aromatized and contain alkyl substituents, the location and structure of which reflect the precursor compound.

The recognition of these PAH in different depositional environments indicates a more rapid formation than was previously thought to occur. The best example of this phenomenon is perylene (**I**[1]), which has been the predominant or only PAH found in several Recent marine and freshwater sediments (Orr and Grady, 1967; Aizenshtat, 1973; LaFlamme and Hites, 1978; Wakeham et al., 1980). Retene (**II**) is another example of a short-term diagenetic product which has been found in forest soils (LaFlamme and Hites, 1978), lacustrine (Wakeham et al., 1980; Tan and Heit, 1981; Barnes and Barnes, 1983) and marine sediments (Simoneit, 1977a,b). Phenanthrene and chrysene

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[1] All chemical structures cited in the text are given in Appendix 1. Numbering conventions for major carbon skeletons discussed here are presented in Appendix 2.

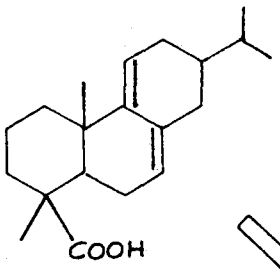
derivatives are also recognized as early diagenetic products derived from 3-oxygenated pentacyclic triterpenoids. They have been found in a French pond mud (Spyckerelle et al., 1977a), in lacustrine (Wakeham et al., 1979; Tan and Heit, 1981) and riverine sediments (Prahl, 1982). The present study focuses on the geochemistry of the phenanthrene and chrysene derivatives of molecular weight 274. Sediment fractionation techniques are used to establish whether a close product-precursor relationship exists between this series of tetracyclic aromatic hydrocarbons and 3-oxygenated pentacyclic triterpenes found in Wells Dam surficial sediments.

II. Previous studies.

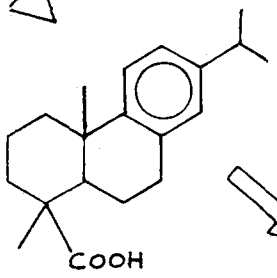
A. Retene as a diagenetic product of resin acids. PAH structurally related to diterpenoid resin-acids have been used as geochemical indicators of organic carbon inputs from resinous higher plants to a variety of Recent sediments (Simoneit, 1977b). A diagenetic relationship between 1-methyl-7-isopropyl-phenanthrene, commonly known as retene, and abietic acid (**III**) is widely accepted and accounts for the presence of retene and several other compounds structurally similar to the resin acid in certain soils and sediments (LaFlamme and Hites, 1978; Wakeham et al., 1980; Tan and Heit, 1981; Prahl, 1982; Barnes and Barnes, 1983). A simplified scheme for this diagenetic pathway is shown in Figure 1. Other diterpenoid compounds (e.g. abietene, dehydroabietin) are also part of a more generalized diagenetic scheme (Simoneit, 1986). Prahl (1982) measured abietic and dehydro-abietic acids as major components of the acid fractions isolated from Wells Dam and Methow River sediments in the Columbia River basin (Figure 4). Retene dominated the PAH mixture in these sediments, accounting for as much as 90% of the total PAH observed in the case of the Methow River (Prahl, 1982).

Diterpenoid acids with the abietane skeleton including abietic and dehydroabietic (**IV**) acids are major components of conifer resin (Langenheim, 1969; Stonecipher and Turner, 1970). The extensive coniferous forest of western Washington and the eastern Cascade Mountains (Highsmith and Kimerling, 1979) provide a plausible source of resin acids to Columbia River sediments. Marine sediments in areas receiving runoff from terrigenous vegetation such as those accumulating in the northeastern Pacific Ocean

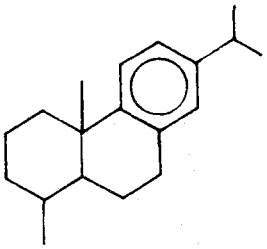
III. Abietic acid



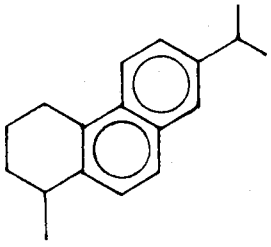
IV. Dehydroabietic acid



V. Dehydroabietin



VI. Tetrahydroretene



II. Retene

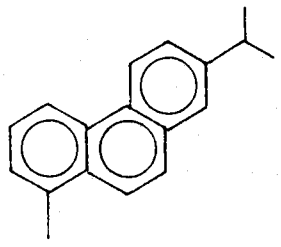


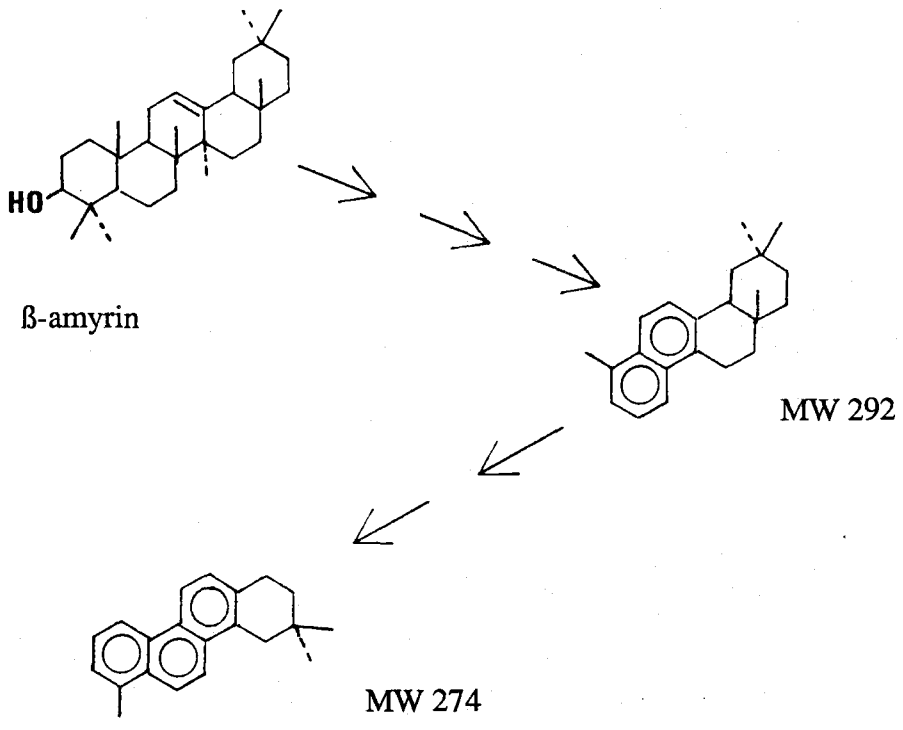
Figure 1. Simplified hypothetical degradation scheme of abietic acid to retene (after LaFlamme and Hites, 1978).

(Simoneit, 1977b; Prahl et al., 1984) and Black Sea (Simoneit, 1977a,b) also contain these diterpenoidal compounds.

The presence of the resin acid and its degradation products provides the opportunity to follow the geochemical diagenesis of these compounds. Barnes and Barnes (1983) report differences in diterpene distribution in two interlinked lacustrine basins sharing the same organic precursors. Low concentrations of aromatic diterpenoid hydrocarbons accumulating beneath oxic waters may reflect oxidation of precursor diterpenoid acids to more polar products which are not isolated in the neutral hydrocarbon fraction. The major enrichment in aromatic diterpenoid hydrocarbons in anoxic sediments suggest reducing environments are a major factor in the production and preservation of the aromatic hydrocarbon fossils of diterpenoids. In situ formation via microbial alteration of diterpenes related to abietic acid has been suggested as a source of these hydrocarbons in soils (Biellman et al., 1968) but remains speculative.

B. Diagenesis of tetracyclic aromatic hydrocarbons of molecular weight 274. In a geochemical model similar to that formulated for retene, Spyckerelle et al. (1977a,b), LaFlamme and Hites (1979) have proposed a reaction scheme linking a series of tetracyclic and pentacyclic aromatic hydrocarbons with molecular weight of 274, 292, 324 and 342 to common precursors (Figure 2). These PAH are present in a variety of different sediment environments (Spyckerelle et al., 1977a,b; LaFlamme and Hites, 1979; Wakeham et al., 1980; Tan and Heit, 1981; Prahl, 1982) and are believed to originate from the 3-oxygenated pentacyclic triterpenes α-amyrin (**VII**) and ß-amyrin (**VIII**) which occur widely in higher plants (Henderson et al., 1969; Ohmoto et al., 1970; Sainsbury, 1970; Kolattukudy, 1976). Particularly interesting for the present study is the suite of natural occurring PAH of molecular weight 274 (**IX**, **X**, **XI**) which appears widely distributed in the environment. These compounds are reported as major components of the PAH fraction in several sediments of the Columbia River basin (Prahl, 1982) and as part of the aromatic hydrocarbons in lacustrine sediments (Tan and Heit, 1981; Cranwell, 1984). Two of these compounds (**X** and **XI**) were also observed by Wakeham et al. (1980) as the most abundant components at the Aabach delta in Greifensee (Switzerland), compound **XI** is reported as the major tetrahydrochrysene in coal samples in western

Pathway 1



Pathway 2

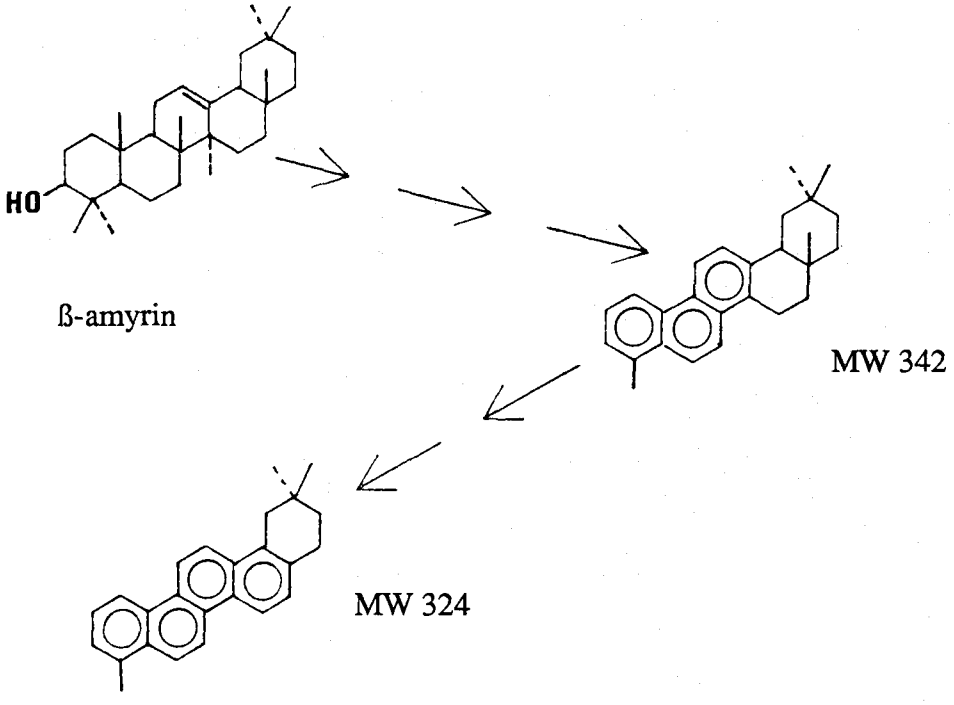


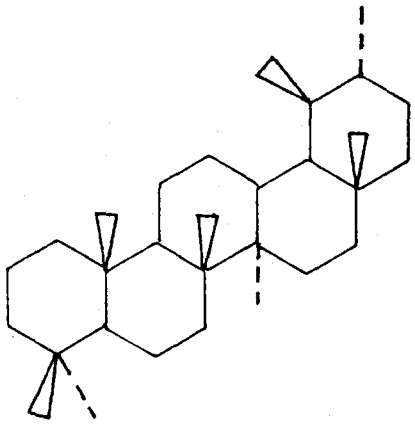
Figure 2. Hypothetical scheme for the transformation of ß-amyrin to tetra- and pentacyclic aromatic hydrocarbons.

Washington and quantifiable amounts of the 3,4,7-trimethyl isomer (**X**) (see Appendix 2c) were also present in two coals (Barrick et al., 1984); compound **XI** appears very abundant in a pond mud (Spyckerelle et al., 1977a) and as a major compound in the aromatic hydrocarbons of the Mahakam delta sediments in Borneo (Corbet et al., 1980).

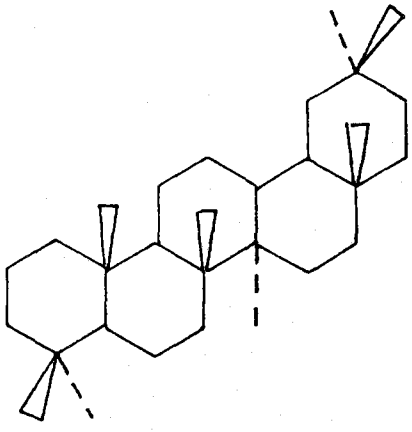
Two transformation pathways have been postulated by Spyckerelle et al., (1977a,b) and LaFlamme and Hites (1979) for converting amyrins and lupeol to aromatic hydrocarbons (Figure 2). One requires the loss of ring A, followed by succesive aromatization of rings B, C, and D, to give the tetracyclic series (hydrochrysenes and cyclopentenophenanthrene). The second pathway implies degradation is initiated by loss of the oxygen function at position 3 in the A-ring, followed by ring aromatization (beginning in ring A), leading to the ultimate formation of hydropicenes and cyclopentenochrysenes. Until recently, the degradation schemes given were conceptually convenient but there was no proof that such degradation schemes actually took place in nature. Lohmann (1988) has shown the formation of tetra- and pentacyclic monoaromatic hydrocarbons derived from lupanone. Such an origin was confirmed by incubation experiments with radiolabelled substrates. ^{14}C labels were traced from precursor to product. The triterpenoids α- and ß-amyrin have been assigned as precursors of these PAH based exclusively on structural similarity and widespread distribution of these particular isomers in vascular plants.

C. Precursors of tetracyclic aromatic hydrocarbons MW274 (3-oxygenated pentacyclic triterpenoids). Pentacyclic triterpenoids containing an oxygen function in the 3-position (refer to Appendix 2d) are widely distributed in higher plants (Kolattukudy, 1976; Glasby, 1982) where they are found in resins and plant saps in the free state and bound as fatty acylesters or glycosides (Pinder, 1960). The nonglycosidic triterpenoids are frequently found as excretions and in cuticle where they may have a protective or waterproofing function (Robinson, 1963). The pentacyclic triterpenoids are normally divided into three groups differentiated by basic ring skeletons. The three skeletal types are shown in Figure 3 and correspond to ursane, oleanane and lupane. This division is, however, somewhat arbitrary. It arose when full structures were not known and substances were related to the simplest alcohol with the same skeleton, i.e., α-amyrin, ß-amyrin and lupeol (**XII**) respectively (de Mayo,

URSANE



OLEANANE



LUPANE

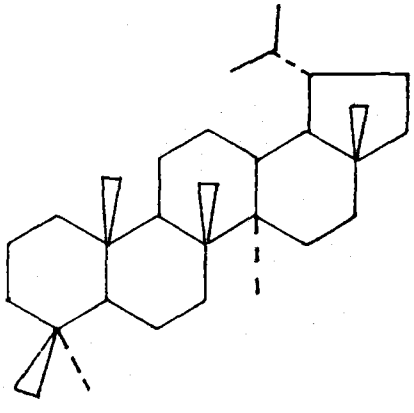


Figure 3. Major skeletal structures for pentacyclic triterpenoids derived from higher plants.

1959). Eschenmoser et al. (1955) proposed a single biosynthetic scheme for all these triterpenoids with 30 carbon atoms with squalene (**XVIII**) as progenitor. Subsequent experimental tests have confirmed this proposal (Kolattukudy, 1976 and references therein). Full structures have been elucidated and in vitro conversions from one structure into another have been accomplished (de Mayo, 1959; Pinder, 1960; Nakanishi et al., 1975). Taraxerone (**XIII**) treated with hydrochloric acid in acetic acid rearranges to ß-amyrenone (**XIV**) (Ives and O'Neill, 1958), the lupane skeleton rearranges to that of olean-13(18)-ene (**XVI**), in which the double bond occupies the most stable position (Cranwell, 1984), and the conversion of friedel-3-ene (**XVII**) under strong acidic conditions into olean-13(18)-ene is also achieved (Templeton, 1969). The acidic conditions occurring in peats and sediments may be suitable for some of these skeletal transformations to happen (Cranwell, 1984). No time scale has been established for these conversions under environmental conditions but rearranged compounds occur in shales of the Niger Delta (Ekweozor et al., 1979). Cranwell (1984) found that the distribution of triterpene ketones in the peat derived sediments of Loch Clair and Cam Loch (English Lake District) show no significant increase in relative abundance of ß-amyrenone to accompany the fall in relative abundance of taraxerone with increasing age in this sequence of sediments. This fact led Cranwell to suggest that skeletal rearrangement is unlikely in Recent sediments.

The distribution of several pentacyclic triterpenoids present in angiosperm (flowering) plants are given in Table 1. All known members of this group are oxygenated at carbon-3 (refer to structure d in Appendix 2) and occur primarily as alcohols but sometimes as ketones. They are distinguished from each other by unsaturation patterns, and additional functionality including hydroxyl groups, and frequently by carboxyl groups at the carbon-28 position. Stereochemical differences are also common distinctions in this group. The ß-amyrin is most common and occurs in all types of flowering plants. It is a pentacyclic secondary alcohol containing one site of unsaturation within the C-ring at position 12, resistant to hydrogenation (Pinder, 1960). The α-amyrin accompanies the ß-isomer in many plant resins (Pinder, 1960). In many of their chemical reactions the two compounds are quite similar. However, the double bond at position 12 in both isomers is less reactive in the α-amyrin as a result of greater steric hindrance (Pinder, 1960). Lupeol differs from the amyrins and

Table 1. 3-Oxygenated pentacyclic triterpenoids in angiosperms.

Compound name:	Source:	References:
ß-amyrin (**VIII**)	-seeds and resins of grapes and alfalfa. -major constituent of the wax of many fruits.	de Mayo, 1959; Templeton, 1969 Tulloch, 1976
α-amyrin (**VII**)	-many plant resins.	Pinder, 1960; Mahato, 1981
Lupeol (**XII**)	-the most widely distributed of all triterpenoids.	de Mayo, 1959; Glasby, 1982
Betulin (**XIX**)	-white pigment of birch bark.	Pinder, 1960
Betulinic acid (**XXIII**)	-barks.	Pinder, 1960
Oleanolic acid (**XXI**)	-clove buds, olive leaves, mistletoe and sugar beet. -grape wax. -cranberry wax.	Pinder, 1960 Radler and Horn, 1965 Croteau and Fagerson, 1971
Ursolic acid (**XXII**)	-waxy coating of apple leaves and fruits. -cranberry wax.	Silva Fernandez et al., 1964 Croteau and Fagerson, 1971
Friedelin (**XXIV**)	-tree barks. -bark of Prunus turfosa (Rosaceae) -19 species of Graminae.	Corbet et al., 1980 Sainsbury, 1970 Sainsbury, 1970
Friedelinol (**XXX**)	-often accompanies friedelin.	Sainsbury, 1970
α-amyrenone (**XXV**)	-tree barks.	Corbet et al., 1980
ß-amyrenone (**XIV**)	-tree barks.	Corbet et al., 1980
Lupenone (**XV**)	-tree barks.	Corbet et al., 1980

their derivatives in that it has a five-membered E ring bearing an isopropenyl group (Figure 3). The double bond in lupeol is easily hydrogenated (Pinder, 1960). Betulin (**XIX**) differs from lupeol in having an additional alcohol functionality at C_{28}.

Apart from those pentacylic triterpenoids already mentioned in Table 1, a large number of compounds are known which have functional groups elsewhere (e.g. Garcia-Alvarez et al., 1981), or in addition to those present above. Konoshima et al. (1981) give details about the characterization of a prosapogenin (**XX**), which corresponds to an oleanane structure attached to an oligosaccharide moiety found in Gleditsia japonica (Japanese name Saikachi), a Leguminosae widely distributed in Japan. Minocha and Tiwari (1981) report the isolation of a new triterpenoidal saponin from the roots of an spiny evergreen herb. The hydrolysis of which yielded lupeol and D-glucuronic acid.

Gymnosperm plants contain abundant phytosterols and diterpenoids but pentacyclic triterpenoids have only been found infrequently in this division of plants (Hills and Whitehead, 1966; Glasby, 1982). Angiosperm plants appear to produce simple and complex triterpenoids in abundance and considerable variety (Tulloch, 1976; Glasby, 1982). If indeed 3-oxygenated pentacyclic triterpenoids predominate in angiosperm plants, it could prove useful in the identification of specific source materials for these structures found in Recent sediments where mixed vegetation is part of the adjacent areas. Presently, very little is known about their geochemical transformations in the environment. Separation of sedimentary fractions where plant detritus occurs abundantly may help in the identification and association of pentacyclic triterpenoids to specific particles and to establish a clearer diagenetic link with structurally similar aromatic compounds observed in the same fractions.

D. Geochemical studies of particle size and density separated sediments. There have been few studies to investigate and compare the distribution of lipids within the different types of particles that comprise sediments. The few studies performed, however, have been quite revealing from a geochemical standpoint. Thompson and Eglinton (1978) showed experimentally that important information concerning the origin and geochemistry of certain aliphatic hydrocarbons and fatty acids in lacustrine sediments can be obtained from a

knowledge of how these lipids are contained within the sediment as a function of particle size. They observed that an homologous series of long chain n-alkanes (C_{21}-C_{33}) and n-fatty acids (C_{20}-C_{28}) characteristic of higher plant sources were dominant in the coarse fraction (>250 μm) of lacustrine sediments from the English Lake District. Microscopic observation of this fraction revealed an abundance of higher plant tissues from trees, reeds and grasses. A decrease in the concentration of these lipids as particle size decreased correlated with a decrease in higher plant contribution and a decrease in the CPI of n-alkanes (C_{20}-C_{30}, from 4 to 2.5). Algal debris and associated n-alkanes (n-C_{15} and n-C_{17}) were found predominantly in finer-grained sediment fractions. The low CPI n-alkane series in the clay fraction was interpreted as an indication of a different source which could be ascribed to either a fossil or a bacterial contribution.

Wade and Quinn (1979) determined the distribution of hydrocarbons and chlorinated hydrocarbons as a function of sediment depth and particle size in Narragansett Bay. They reported that most of the hydrocarbons are associated with the smaller size particles (0.3 to 45 μm) in the surface sediments from Mid-Narragansett Bay. The hydrocarbon concentration at depth showed a slightly higher content associated with the larger size particle fraction. They postulated that surface sediments receive a larger input of anthropogenic hydrocarbons than do sediments lower in the core. These hydrocarbons (e.g. petroleum) are likely to coat the sediments. Smaller particles having a greater surface area per unit volume would have a higher concentration. Lower in the core the presence of hydrocarbons was attributed mostly to the presence of terrestrial plant debris.

Prahl and Carpenter (1983) examined how PAH from different sources vary within coastal sediments as a function of both particle size and density. They reported that at least 20 to 25% of the perylene and 50% of the retene measured in whole sediments from the Washington coast were contained within coarse (>64 μm) organic carbon and lignin-rich particles of density ≤1.9 g/cc. These particles comprised less than 1% of the total sediment weight and were represented by debris primarily derived from vascular plants.

In general, these studies have shown that detrital chemicals retain a particle association indicative of their primary input. No evidence of extensive redistribution (i.e. adsorption, desorption) is observed for the natural compounds found in the sediments.

III. Present study: Objectives and Methodology.

It seems likely that the techniques of particle size and density separation of bulk sediments could be used to define how naturally occurring tetracyclic and pentacyclic aromatic hydrocarbons and 3-oxygenated triterpenes are associated with specific particles and to gain further evidence geochemically linking these compounds through product-precursor relationships. A diagenetic relationship based on more than structural similarity would seem evident if the product and likely precursors coexisted in abundance on the same sedimentary particles. The present work is focused on the suite of tetracyclic PAH with a molecular weight of 274 mass units (henceforth abbreviated MW274 PAH) and their potential pentacyclic triterpenoid precursors, i.e. α-amyrin, ß-amyrin, lupeol and derivatives thereof. Sediments collected in the Columbia River basin behind Wells Dam were examined in this study. Two specific objectives were considered during the experimental design of this research: **1)** to establish how MW274 PAH and 3-oxygenated pentacyclic triterpenoids are associated with discrete particles in the sediments and **2)** to identify the source of the particles with which the two series of compounds are associated.

A common association of the series of MW274 PAH and 3-oxygenated pentacyclic triterpenoids with specific particle carriers such as plant detritus, will strengthen the postulated hypothesis that this suite of MW274 PAH are produced during early diagenesis from 3-oxygenated pentacyclic triterpenoid precursors of a higher plant origin.

EXPERIMENTAL

I. Description of sample collection. A sample of surficial sediments from behind Wells Dam in the Columbia River, Washington (Figure 4) was collected using a small boat and a 0.025 m^2 van Veen grab sampler. The sampler had a maximum capacity of 3 liters and a maximum penetration depth of 12 cm (Hedges et al., 1984). The sample, taken at a water depth of 22 m, near the center of the reservoir, 1-3 km upstream of Wells Dam, was stored frozen in a solvent-rinsed jar until chemical analysis (Prahl, 1982) and at 4°C between replicate analyses.

II. Methods and Procedures.

Five different subsamples of the sediments collected behind Wells Dam were taken and analyzed for various geochemical properties. Two subsamples were size fractionated, one subsample was separated by density and the rest were analyzed as total ("unfractionated") sediments (Table 2).

Table 2. Subsample description.

Subsample	Code	Sampling date
1. Total sediments.	bulk 1	April 1986
2. Total sediments.	bulk 2	August 1986
3. Particle size fractionation.	size frac.-1	August 1986
4. Particle size fractionation.	size frac.-2	December 1986
5. Density separation for >63 μm particles and size separation for <63 μm particles as clays and silts.	density frac.	November 1986

A. Particle Separation Procedures.

1. Particle Size Separation. Subsamples were divided according to particle size into distinct fractions as shown in Figure 5a prior to chemical analysis.

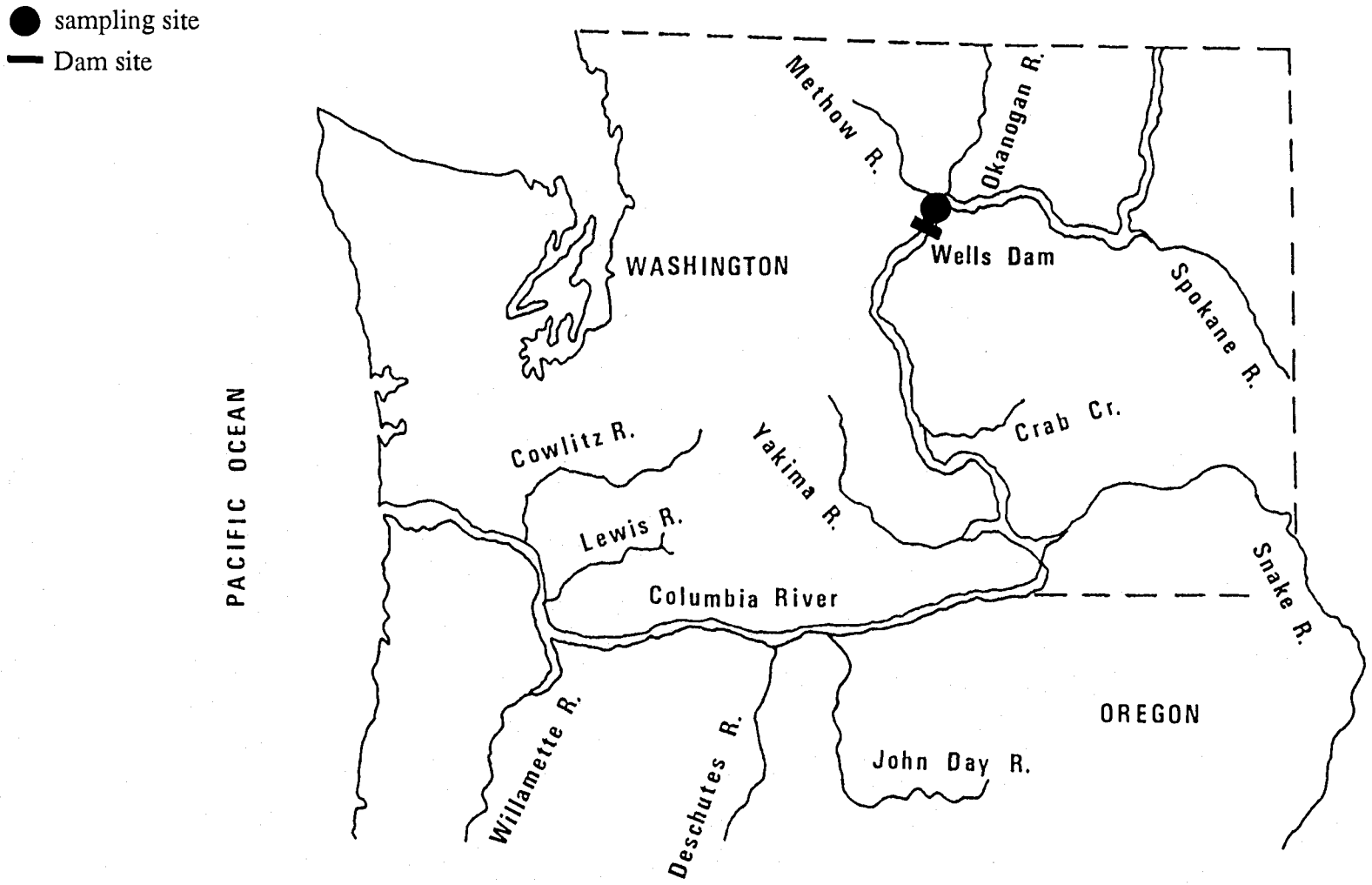
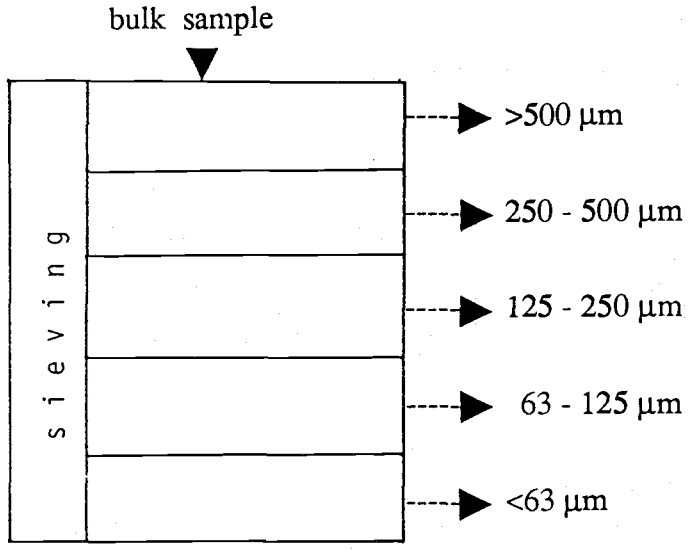


Figure 4. Location of Wells Dam sampling site in the Columbia River.

(a) Particle size fractionation.



(b) Particle density fractionation.

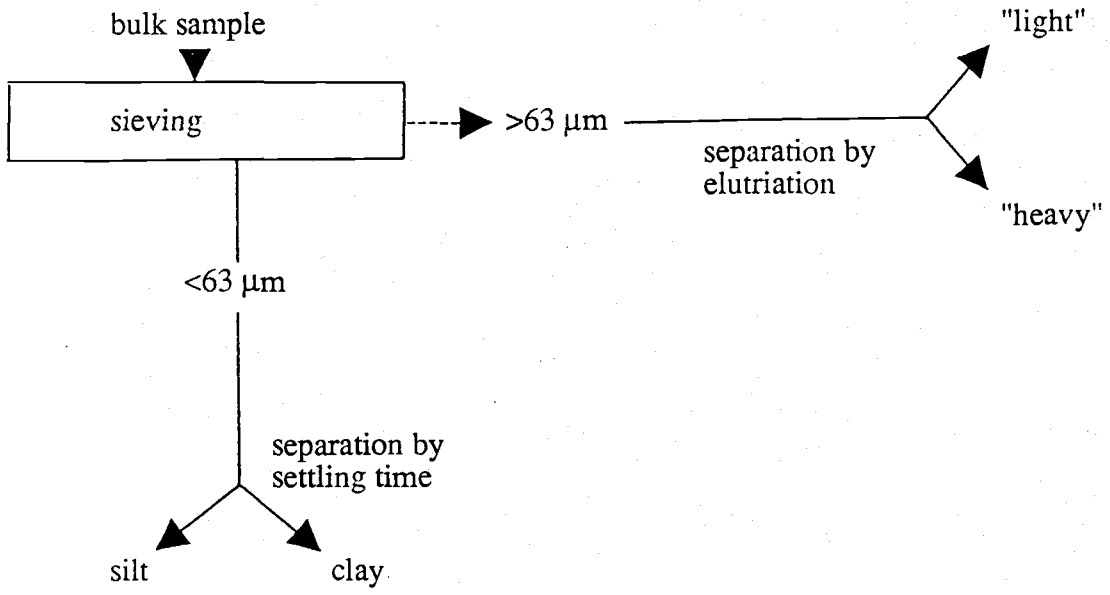


Figure 5. Schematic diagram of procedures for (**a**) particle size and (**b**) particle density fractionation of Wells Dam sediments.

Duplicate samples of the same sediment were separated into five size fractions (>500 μm, 250-500 μm, 125-250 μm, 63-125 μm and <63 μm) by wet sieving through a nest of stainless steel sieves (8-inch dia.). The bulk sediment (≈100 g wet weight) was washed through the sieves with 1.5 liters of distilled water. The <63 μm fraction was collected onto a pre-extracted GF/C Whatman glass filter (47mm dia.).

2. Particle Density Separation. Another sample (40 g wet weight) was washed through a 63 μm mesh sieve with 1.0 liter of distilled water (Figure 5b). The sediment retained by the sieve (>63 μm) was placed in a large watch glass (20 cm dia.) containing distilled water. Biogenic detritus was separated hydrodynamically from mineral material by elutriation. The two isolated materials are referred to as >63 "light" and >63 "heavy" fractions, respectively. Particles <63 μm in size were left to settle for two hours. The supernatant and the residue in the <63 μm fraction were filtered separately onto pre-extracted GF/C Whatman glass filters (47 mm dia.). These fractions are defined as clay and silt, respectively. It should be noted that no particle density fractionation was carried out on the <63 μm fraction and the division between silt and clay fractions for this experiment is based on the settling time of these particles in water.

B. Lipid Analysis.

1. Lipid Extraction and Isolation Procedures. The extraction and isolation procedures for organic solvent extractable ("free") lipids is schematized in Figure 6. Wet bulk and fractionated sediments were placed in pre-cleaned cellulose thimbles (Whatman 33x80mm) and extracted by soxhlet for 45 to 50 hours using a 50% (v/v) mixture of hexane in acetone (250 ml, B&J, high purity solvents). Total extracted lipids were partitioned into hexane (3X, 50 ml each) after addition of redistilled water (50 ml). The combined hexane fractions were washed against a 50% saturated NaCl solution and dried over anhydrous Na_2SO_4. Rotary evaporation yielded a total lipid residue. The total lipid residue of each sample was chromatographed on a column (1.1 cm dia.) of silica gel (7g, Kieselgel 60, 70-230 mesh, EM Science) packed wet in hexane. The silica gel adsorbent had been cleaned with solvents, activated for 24 hours at 220°C,

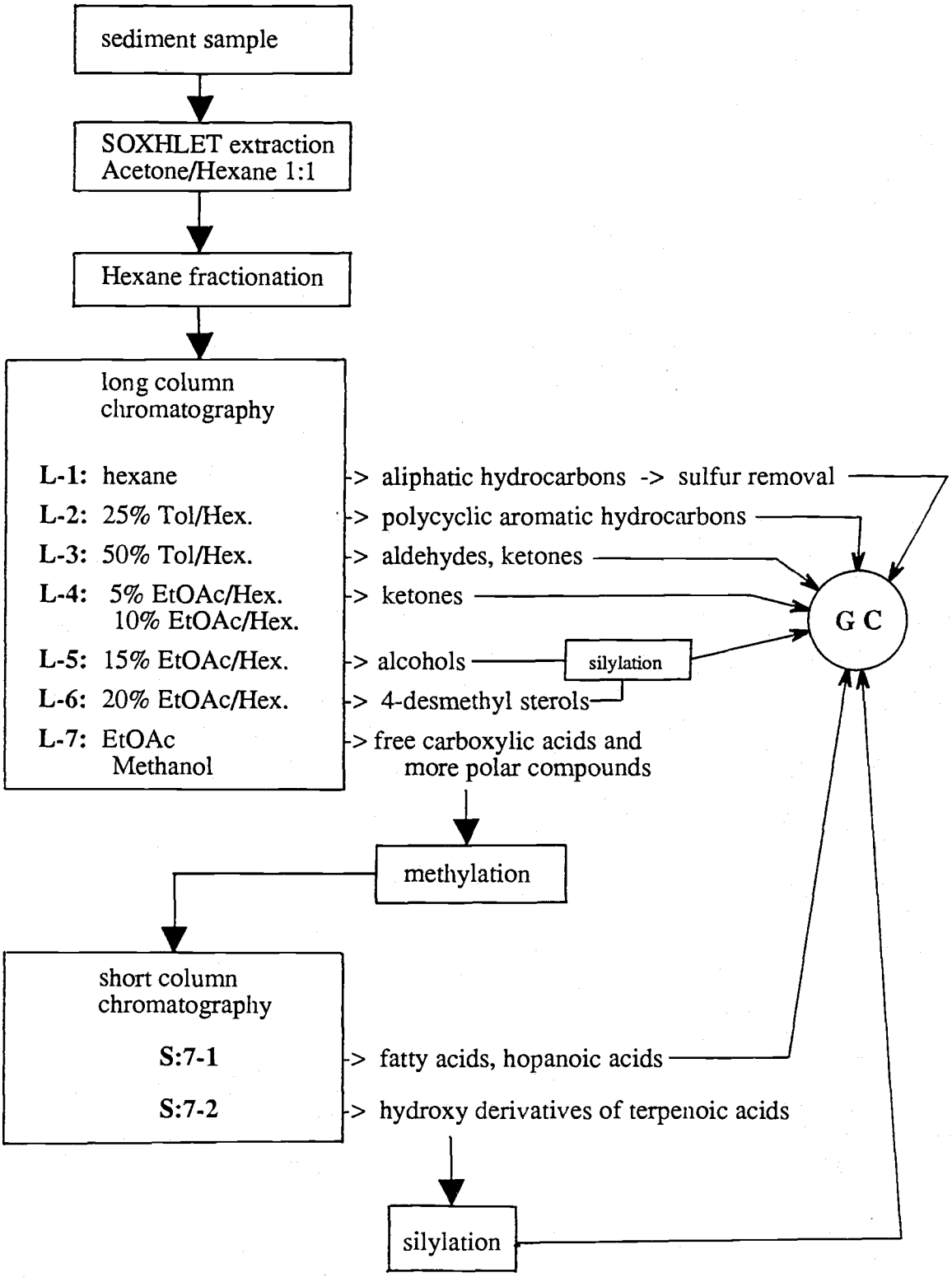


Figure 6. Schematic diagram of lipid extraction and isolation procedures.

deactivated with water (5% v/w) and stored in a vacuum dessicator prior to use.

Seven fractions of different functional classes were isolated from each sample using a series of solvents of increasing polarity: 1) 30 ml of hexane (aliphatic hydrocarbons); 2) 40 ml of 25% toluene in hexane (T/H) (PAH); 3) 40 ml of 50% T/H (n-aldehydes and ketones); 4) 20 ml each of 5 and 10% ethyl acetate in hexane (EtOAc/H) (ketones); 5) 25 ml of 15% EtOAc/H (n-alcohols); 6) 25 ml of 20% EtOAc/H (4-desmethyl sterols); 7) 15 ml of EtOAc and 10 ml of methanol (n-fatty acids, triterpenoic acids and more polar compounds). The solvent volumes defining these chromatographic fractions were established by precalibration of the silica gel with standard compounds: 3-methylheneicosane and 3-methyltricosane (alkanes), nonadecan-10-one, nonadecan-2-one, nonadecan-1-ol, and nonadecan-1-oic acid.

Acidic components in the polar seventh fraction were methylated using diazomethane (Schlenk and Gellerman, 1960) and rechromatographed on a short column of silica gel (1.4 g) using the same series of solvents but smaller volumes. Free fatty acids were analyzed by gas chromatography as fatty acid methylesters (FAME). Compounds such as n-alcohols, sterols and hydroxy derivatives of triterpenoids were converted to trimethylsilyl ethers using bis(N,O)trimethylsilyltrifluoroacetamide (BSTFA, Pierce) prior to gas chromatographic (GC) analysis.

2. Sulfur Removal. All the aliphatic hydrocarbon fractions (i.e. L-1) were concentrated to a volume of 1-2 ml and passed through an activated copper column (≈40 mesh) using 25 ml methylene chloride to remove elemental sulfur. The procedure is described by Blumer (1957).

3. Recovery efficiencies. All sediment samples were spiked with a series of standards: 3-methyltridecane, 3-methylpentadecane, 3-methylheptadecane, 3-methylheneicosane, 3-methyltricosane, nonadecan-2-one, nonadecan-10-one, nonadecan-1-ol and nonadecan-1-oic acid prior to extraction and chemical work-up in order to measure recovery efficiencies for the different functional classes. The average recoveries observed for the standards in replicate (n=16) samples are shown in Table 3.

Table 3. Recoveries for standards added to untreated wet Wells Dam sediments.

compound:	recovery (%)
3-methyltridecane	12 ±7
3-methylpentadecane	38 ±19
3-methylheptadecane	71 ±10
3-methylheneicosane	92 ±10
3-methyltricosane	94 ±11
nonadecan-2-one	48 ±4
nonadecan-10-one	49 ±4
nonadecan-1-ol	29 ±7
nonadecan-1-oic acid	25 ±8

Recovery efficiencies for 3-methyl alkanes increased with carbon number over the range C_{14} to C_{22}. High efficiencies were obtained for the greater molecular weight 3-methyl C_{21} and 3-methyl C_{23} alkanes (≈93%). Evaporative losses are the main cause for low recovery percentages for shorter carbon chain length homologues (Barrick et al., 1980). C_{19} ketones showed ≈50% recovery. Both, the alcohol and the acid standards had low recovery efficiencies (29% and 25%, respectively) which could be due to the amount of handling of these classes of compounds during column chromatography separation, split between fractions and poor derivatization. The alcohol fraction was silylated and the acid fraction methylated and silylated before injection into the GC. Although recoveries were low, these measurements had an acceptable reproducibility, making comparison among samples completely valid. Data shown in the following tables have not been corrected for recovery, therefore some caution should be exercised when interpreting absolute amounts presented elsewhere in this study.

4. Procedural blanks. A procedural blank was performed by carrying solvents through the entire procedure (from soxhlet extraction through final GC analysis) in the absence of sediments. The blank contained negligible amounts of compounds of interest. The two most commonly encountered contaminants

appeared in the L-4 fraction and were phthalate-related compounds, one of which was identified as dioctyl phthalate.

5. Gas Chromatographic (GC) analysis. Individual lipid fractions were dissolved in iso-octane and analyzed on an HP5890A gas chromatograph equipped with a fused silica capillary column (30m x 0.25mm i.d. DB-5, J&W Scientific) and flame ionization detection (FID). All analyses were performed using splitless injection, hydrogen carrier gas (10 psi head pressure) and temperature programming (75-130°C at 10°C/min, 130-300°C at 5°C/min). The injector and detector temperatures were 300°C and 325°C, respectively.

A mixture of n-alkanes (C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{28}, C_{32}, C_{36}, and C_{40}), hexamethylbenzene and 5α(H)-cholestane was run every four samples to monitor the detector response and chromatographic performance. Individual compounds were quantitated using an internal standard method. The quantity of any individual compound (X) in a sample was calculated according to the equation listed below.

$$X = X_{area} \cdot d.f. \cdot RF_{istd} \cdot RRF$$

Where:

X_{area} = GC peak area of compound of interest.

d.f. = dilution factor: (total dilution vol. for GC)/(vol. injected on GC)

RF_{istd} = response factor of the internal standard hexamethylbenzene (HMB) in the sample:

(weight of HMB_{sample}) / (area of HMB_{sample})

RRF = relative response factor of the n-alkane eluting nearest in retention time to the compound of interest, to HMB in the standard:

(weight / area of $n\text{-alkane}_{std}$) / (weight / area of HMB_{std})

The identity of individual n-alkanes, n-alcohols, and n-fatty acids was determined by comparison of their retention times with those of authentic standards.

6. GC/Mass Spectrometry. The identification of PAH and 3-oxygenated pentacyclic triterpenoids is based on interpretation and comparison with previously published mass spectra. Retention times and comparison with authentic standards was applied whenever possible. Synthetic standard

compounds such as: α-amyrin, lupeol, τ-taraxerol, friedelin, friedelinol, betulin, ursolic acid, betulinic acid and oleanolic acid were provided by Dr. Bernd Simoneit.

Mass spectra of individual compounds in the various lipid fractions were obtained on a Finnigan 4000 GC-MS system composed of a Finnigan model 9610 GC interfaced with a Finnigan model 4021 quadrupole mass spectrometer. Mass spectrometric data were acquired and processed with a Finnigan-INCOS 2300 data system. The mass spectrometer was operated in the electron impact mode (EI) using an ionization energy of 70 eV. The ionizer temperature was 270°C, the transfer line (separator oven) was at 350°C and the manifold temperature was 135°C. GC conditions were similar to those defined previously.

C. Ancillary analyses. The solvent extracted sediment samples were crushed in a solvent-rinsed ball mill and then subsampled for elemental (C,N), lignin and stable organic carbon isotopic ($\delta^{13}C$) analyses.

1. Elemental analysis. A Carlo Erba model 1106 CHN analyzer in the laboratory of Dr. J. Hedges (University of Washington) was used to determine weight percentages of organic carbon (%OC) and atomic carbon/nitrogen ratios (C/N). Solvent extracted sediment samples (without acidification to remove carbonates) were analyzed in duplicate. Because the carbonate content in these samples is negligible (Turin and Ertel, 1984), samples were analyzed without pretreatment with acid. Average reproducibilities (percent mean deviation) were ±2% and ±3%, for %OC and C/N respectively.

2. Lignin analysis. Lignin was analyzed in these samples by the CuO oxidation method described by Hedges and Ertel (1982). The phenolic products of the oxidation were quantified as their trimethylsilyl derivatives by capillary gas chromatography on a fused silica capillary column (DB-1, J&W). Analytical results for all samples are reported in the form of "lignin parameters" as defined by Hedges and Ertel (1982).

3. Stable carbon isotope analysis. Stable carbon isotope compositions of the total organic carbon in selected samples were determined by a commercial firm (Coastal Science Laboratories, Inc., Austin, Texas). The average precision of the measurement was ±0.2 per mil. All samples were treated with mineral acid prior to analysis to eliminate any interference from inorganic carbonates. The carbon isotope data are reported as δ-values, which are deviations in parts per thousand (per mil) of the $^{13}C/^{12}C$ ratios of the samples from that of the CO_2 obtained from the belemnite standard (Peedee Formation). δ-values are defined by the formula:

$$\delta^{13}C = (R/R_S - 1) \cdot 1000$$

where $R = {}^{13}C/{}^{12}C$ ratio in the sample

$R_S = {}^{13}C/{}^{12}C$ ratio in the standard (PDB).

RESULTS and DISCUSSION

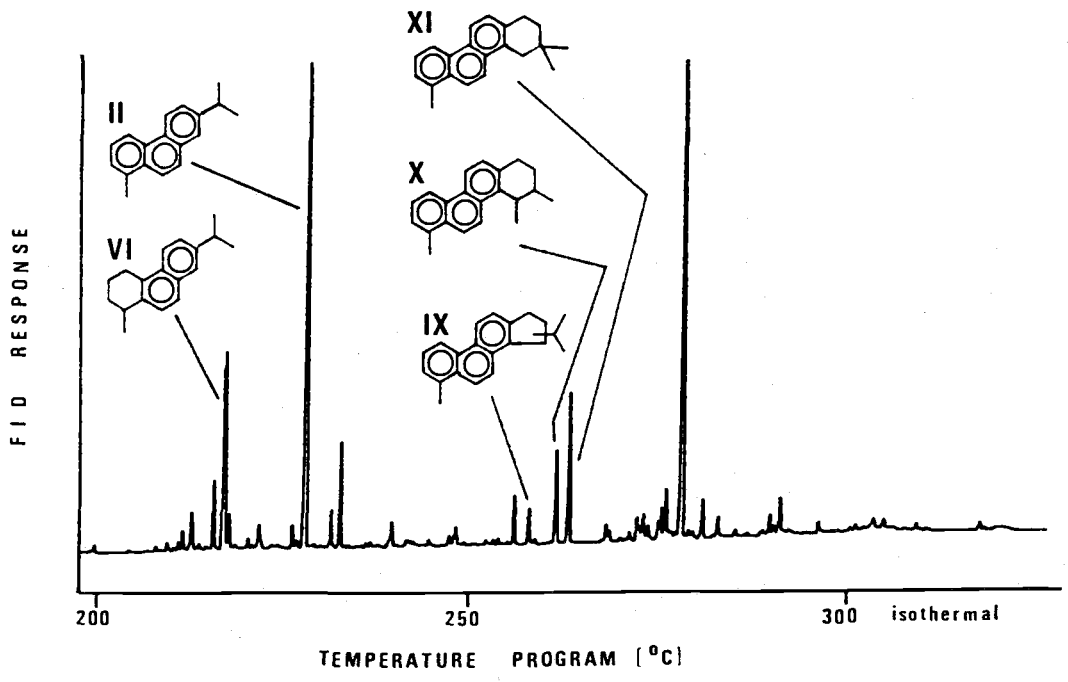
I. MW274 PAH in Wells Dam Sediments.

A. Characterization of the MW274 PAH series in whole sediments. A series of three tetracyclic aromatic hydrocarbons (**IX**, **X** and **XI**) found in sediments from Wells Dam, is identified in the chromatogram corresponding to the polycyclic aromatic hydrocarbon (PAH) fraction L-2 (Figure 7a). Compounds from this series have been observed in different sedimentary records (Spyckerelle et al., 1977a; LaFlamme and Hites, 1979; Wakeham et al., 1980; Tan and Heit, 1981; Prahl, 1982) and is believed to be produced by a similar diagenetic mechanism to that described for retene (**I**) (Simoneit, 1977a; LaFlamme and Hites, 1978; Wakeham et al., 1980; Barnes and Barnes, 1983) but from the 3-oxygenated pentacyclic triterpenoid precursors rather than resin acids. Retene and tetrahydroretene (**VI**) are also observed in Figure 7a which suggests both, the transformation of resin acids to retene and 3-oxytriterpenoids to tetracyclic aromatic hydrocarbons, could be occurring in situ or nearby and transported to this location. The present study has not been designed to describe the chemical and diagenetic processes that transform the precursors into more stable and unreactive compounds. The importance of this research stems from the detailed geochemical information obtained from one sample using sediment fractionation techniques to establish whether a product-precursor relationship between the MW274 PAH and 3-oxytriterpenoid is, in fact, plausible.

Individual components of this PAH series were identified by comparison of mass spectra with published data (LaFlamme and Hites, 1979; Wakeham et al., 1980) and correspond to 1-methyl,isopropyl-7,8-cyclopentenophenanthrene (**IX**); 3,4,7-trimethyl-1,2,3,4-tetrahydrochrysene (**X**) and 3,3,7-trimethyl-1,2,3,4-tetra-hydrochrysene (**XI**). Representative mass spectra are given in Appendix 3.

B. Efficiency and reproducibility of sediment fractionation techniques. The sediments analyzed are a complex mixture of inorganic and organic materials derived from a variety of land-based and freshwater-based sources within this

(a) Wells Dam



(b) Crab Creek

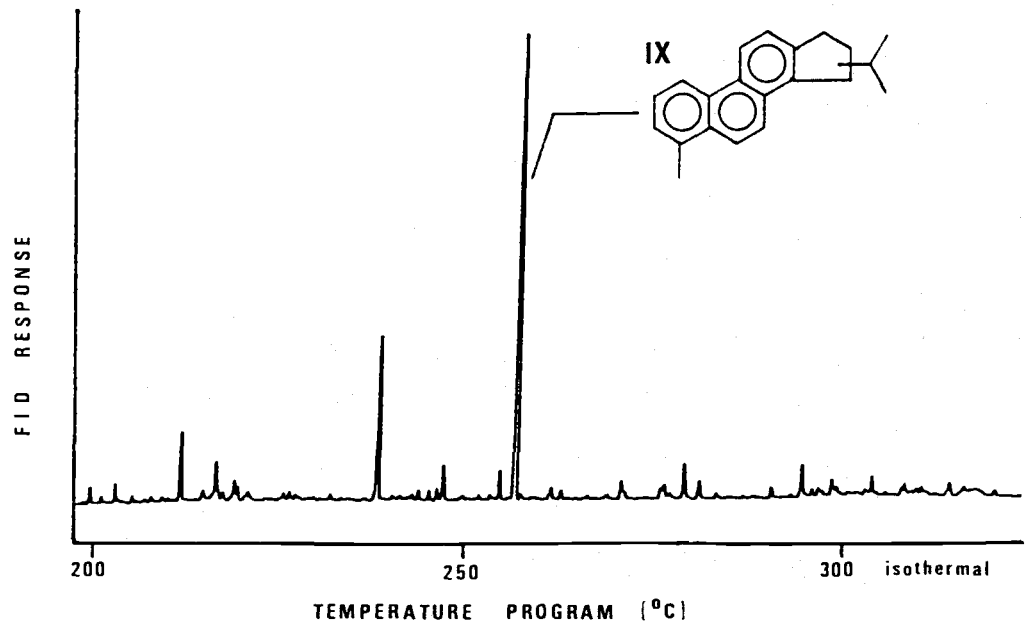


Figure 7. GC traces of the PAH fraction in bulk sediments from (a) Wells Dam and (b) Crab Creek.

region of the Columbia River basin. Sieving of these sediments allows separation of particle mixtures into distinct size fractions. The fractions thus obtained constitute a simpler subset of the whole sedimentary material. Analyses of these fractions provides resolution of the association of MW274 PAH with specific particle carriers and potentially facilitates identification of their specific geochemical origin. Sediments collected behind Wells Dam in the Columbia River were separated according to particle size and density. Assuming homogeneity in the samples, similar results for the bulk sediment concentrations, reconstructed from the fractionation experiments and actually measured in whole, unfractionated sediments would indicate that the fractionation methods used, representatively divide the whole sediment into discrete particle fractions differentiated by size and density with minimal loss or disturbance of the intrinsic content for this PAH series. "Reconstructed" bulk values are obtained from the sum of a compound concentration in each fraction from a particular experiment multiplied by the respective dry weight of each fraction divided by the total dry weight of all fractions combined. This procedure has been used in all calculations involving total reconstructed sediments, instead of applying values obtained from experiments using total bulk sediments. This treatment of the data showed average reconstructed values for MW274 PAH agree well with bulk measurements in total sediments (Figure 8, Table 4). The same procedure is used to compare measured and reconstructed values for the <63 μm fractions. The density experiment separated <63 μm particles into two size fractions: clays and silts. Reconstructed values for PAH in the density fractions are within the range of concentrations measured in replicates of <63 μm particle size fractions (Figure 8). Thus, the fractionation of the sample by particle size and density provides a reliable method to extract useful information defining the association between these PAH and their respective carrier particles.

C. PAH distribution in size fractions of sediment. Concentrations of the MW274 PAH (**IX, X, XI**) normalized to dry weight in the different particle size fractions and bulk sediments are shown in Table 4. The concentrations of these PAH normalized to dry weight give an idea of how concentrated the compounds are in each size fraction relative to the weight of that fraction independently of particle type. The size fractionation experiments (1 and 2) show that this series of PAH is 10-100X more concentrated in particles contained in the

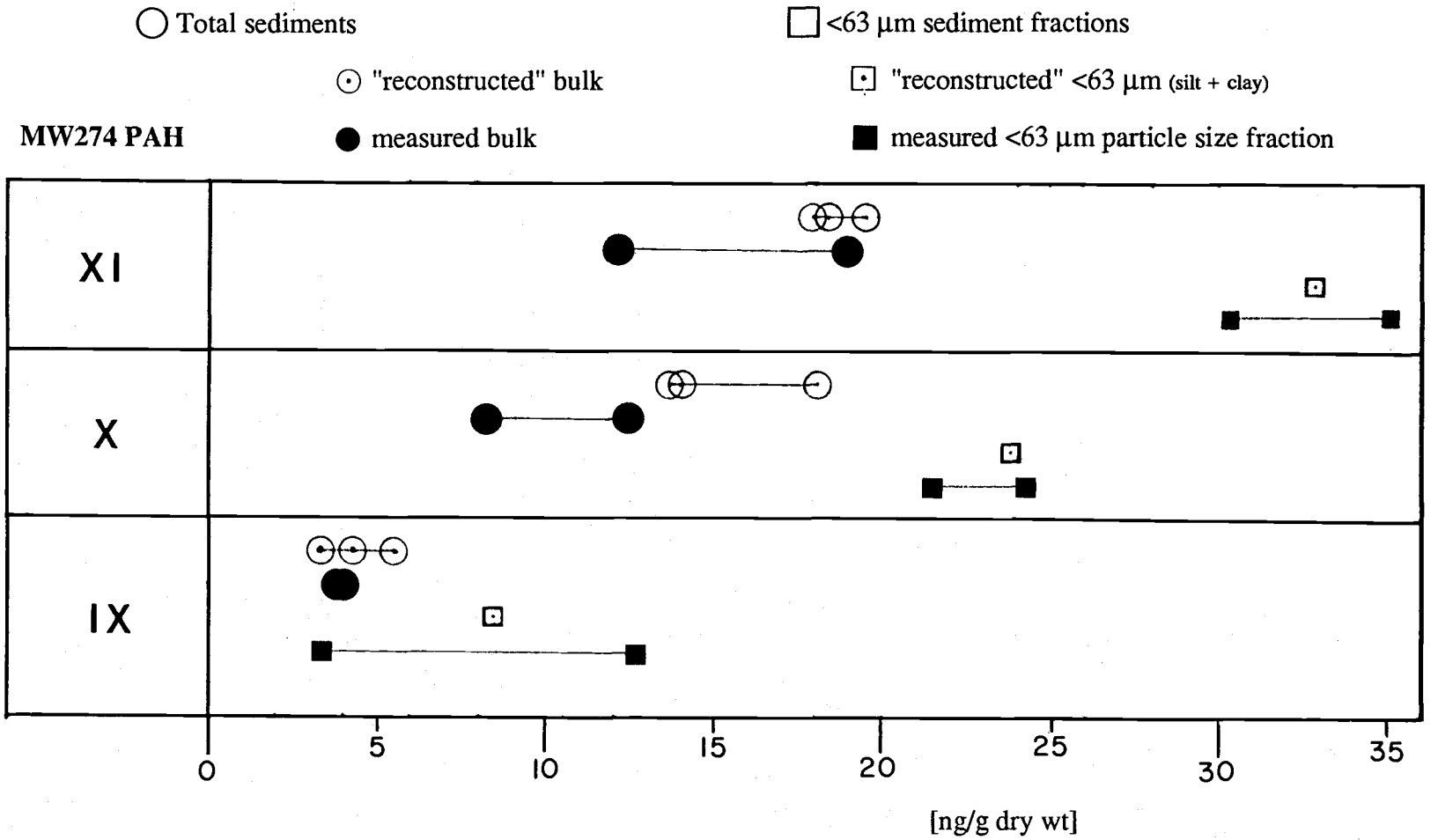


Figure 8. Comparison of MW274 PAH concentrations measured in bulk and <63 µm particle size fraction and calculated from "reconstructed" bulk and <63 µm (silt and clay) sediments.

Table 4. Weight percentages of sediment fractions and concentrations for the series of MW274 PAH IX, X and XI in total sediment and sediment fractions distinguished by size and density.

		Concentration [ng/g dry wt]		
	wt %	**IX**	**X**	**XI**
	(1)	(2)	(3)	(4)
bulk 1	100	4.0	12.5	19.0
bulk 2	100	3.9	8.3	12.2
size frac.-1				
>500 µm	0.34	192	2500	1370
250-500 µm	18.8	5.4	10.2	11.5
125-250 µm	38.7	1.2	3.0	4.1
63-125 µm	18.7	2.5	5.1	7.4
<63 µm	23.4	12.7	24.3	35.1
reconstructed	100	5.6	18.1	18.0
size frac.-2				
>500 µm	0.31	357	1300	1410
250-500 µm	19.1	4.0	8.1	13.3
125-250 µm	37.6	2.5	3.9	5.6
63-125 µm	18.3	3.6	6.7	10.1
<63 µm	24.6	3.4	21.5	30.3
reconstructed	100	4.3	13.7	18.4
density frac.				
>63µm "light"	0.72	202	1100	1430
>63µm "heavy"	75.9	-	1.2	2.0
silt	22.9	8.7	18.7	28.2
clay	0.50	-	171	251
reconstructed	100	3.4	14.0	19.5

(1): weight percent of sediment.
(2): 1-methyl,isopropyl-7,8-cyclopentenophenanthrene
(3): 3,4,7-trimethyl-1,2,3,4-tetrahydrochrysene
(4): 3,3,7-trimethyl-1,2,3,4-tetrahydrochrysene

coarse (>500 μm) fraction than the next most concentrated fractions (typically the "<63 μm" fraction). The coarse fraction represents less than half a percent of the total weight of these sediments but yet contains more than 25% of the total amount of the MW274 PAH calculated for the reconstructed sediment (Figure 9). The concentration of this series (/g dry) decreases sharply with grain size reaching its lowest concentration in the 125-250 μm fraction (Table 4). This fraction constitutes the largest weight percent of all the fractions (≈38%) but contributes on average about 10% of the total amount of MW274 PAH measured in the unfractionated sediment. These results demonstrate that this series of PAH is not associated with the average particles that constitute the major mass of the sediments. Particles highly enriched in the >500 μm fraction and comprising a minor fraction of the total sediment mass seem to be the carriers of these geochemicals.

Percentage organic carbon (%OC) values identify fractions where biogenic detritus is most highly enriched. Organic carbon measured in the different particle size fractions and bulk sediments is shown in Table 5. The coarse particles >500 μm although a minor fraction of the total weight of the sediments, represents a major component of the total organic carbon in the bulk sediments. The amount of each compound normalized to organic carbon rather than to sediment dry weight in each fraction provides an idea of how these PAH associate with the mixture of organic detritus independently of the amount of inorganic matter present in these sediments.

Enrichment factors (EF_{OC}) defined as the ratio of the concentration (/g OC) of a given compound in a sediment fraction with respect to the concentration (/g OC) in the reconstructed bulk sediment, provides a way to evaluate the association between this series of PAH and the organic detritus in the sediments. Assuming there is a constant proportion of MW274 PAH with the organic matter of its source material, variations in EF_{OC} among the different fractions would indicate the admixture of organic matter containing the MW274 PAH with organic carbon devoid of these aromatic compounds. Large differences in EF_{OC} (2-9 folds) for compounds **X** and **XI** are observed between the very coarse (>500 μm) and the middle particle size fractions (Table 5). These results reflect a different source of organic carbon devoid in PAH **X** and **XI** is present in the middle size particle range diluting their

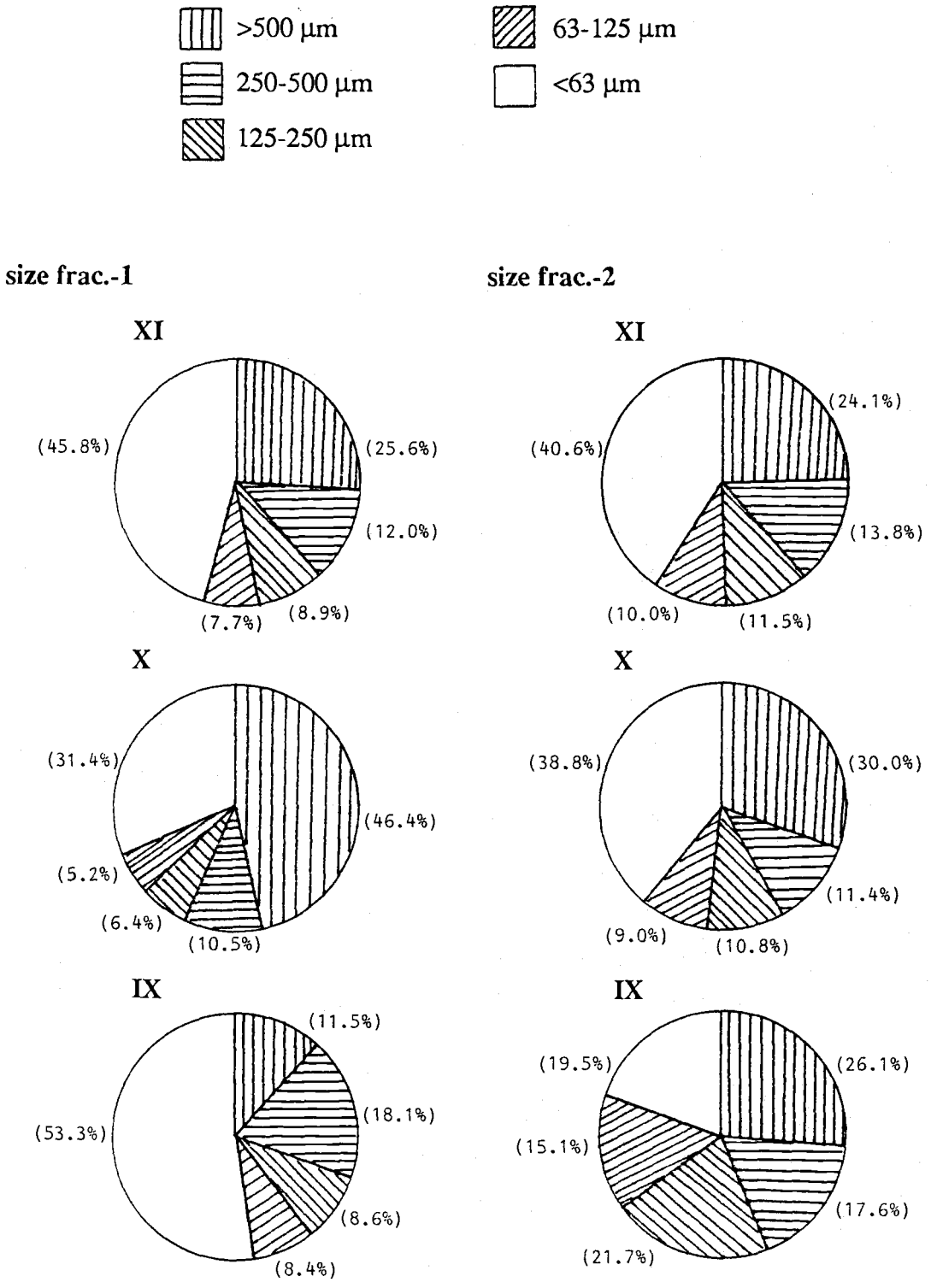


Figure 9a. Weight percentage of MW274 compounds **IX**, **X** and **XI** in the different particle size fractions relative to "reconstructed" bulk sediments.

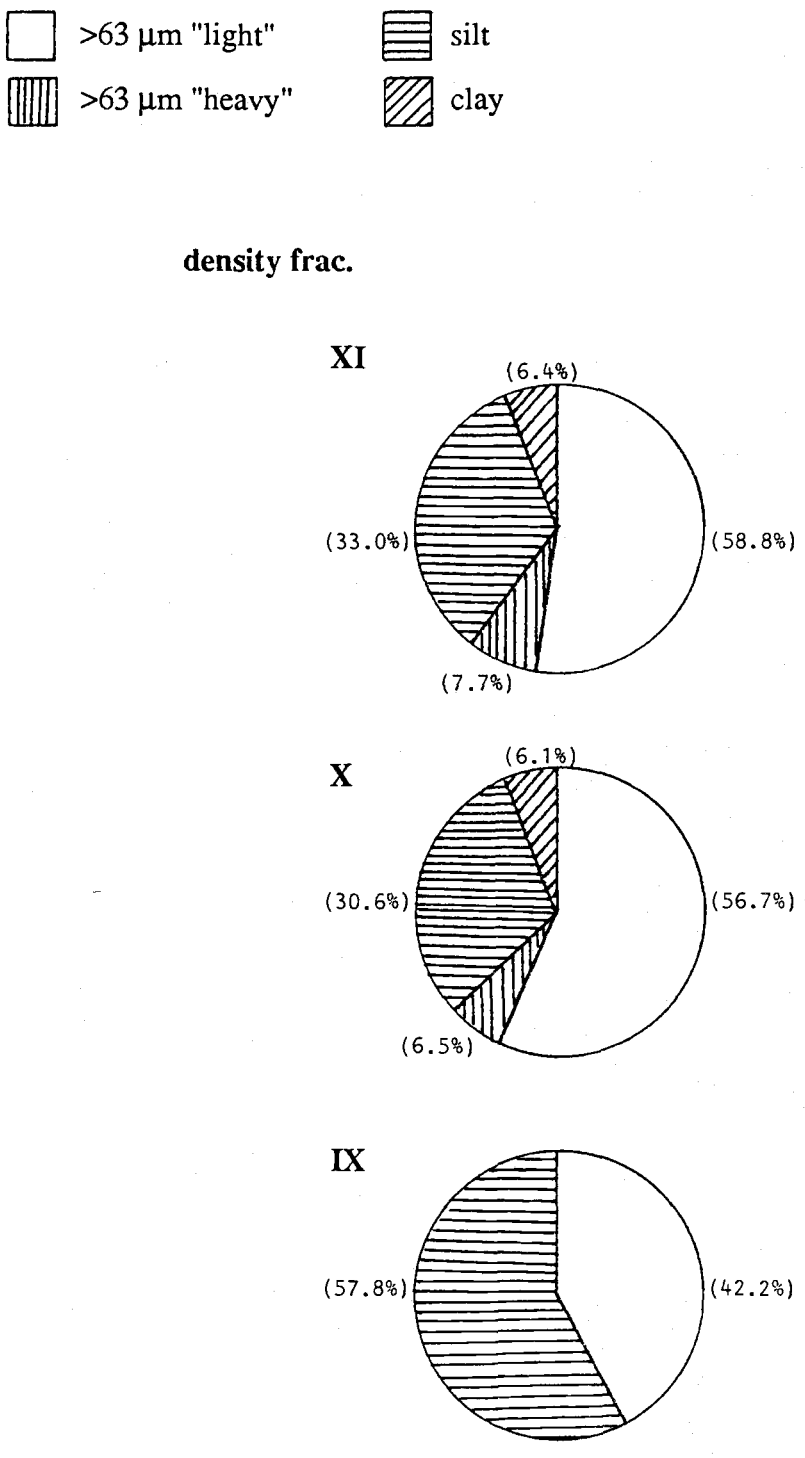


Figure 9b. Weight percentage of MW274 compounds **IX**, **X** and **XI** in the different density fractions relative to "reconstructed" bulk sediments.

Table 5. Percentage organic carbon, concentrations and enrichment factors (EFoc) for the series of MW274 PAH IX, X and XI in the different particle size and density fractions and unfractionated Wells Dam sediments.

		Concentration [µg/g OC]:			Enrichment(4):		
	% OC	IX (1)	X (2)	XI (3)	IX (1)	X (2)	XI (3)
bulk 1(5)	n.m	0.90	2.86	4.32			
bulk 2	0.40	0.99	2.10	3.09			
size frac.-1							
>500 µm	23.2	0.82	10.78	5.88	0.6	2.4	1.3
250-500 µm	0.40	1.35	2.54	2.88	1.0	0.6	0.7
125-250 µm	0.24	0.52	1.25	1.71	0.4	0.3	0.4
63-125 µm	0.31	0.81	1.64	2.39	0.6	0.4	0.5
<63 µm	0.45	2.81	5.40	7.79	2.1	1.2	1.8
reconstructed	0.41	1.36	4.42	4.38			
size frac.-2							
>500 µm	29.5	1.21	4.43	4.80	1.4	1.6	1.3
250-500 µm	0.48	0.82	1.70	2.77	1.0	0.6	0.8
125-250 µm	0.21	1.18	1.87	2.67	1.4	0.7	0.7
63-125 µm	0.29	1.23	2.32	3.47	1.4	0.8	0.9
<63 µm	0.74	0.46	2.91	4.10	0.5	1.1	1.1
reconstructed	0.50	0.86	2.74	3.69			
density frac.							
>63µm "light"	26.9	0.75	4.10	5.34	0.9	1.2	1.2
>63µm "heavy"	0.12	n.d.	1.00	1.66	-	0.3	0.4
reconstructed(5)	n.m	0.78	3.98	4.44			

(1): 1-methyl,isopropyl-7,8-cyclopentenophenanthrene.
(2): 3,4,7-trimethyl-1,2,3,4-tetrahydrochrysene.
(3): 3,3,7-trimethyl-1,2,3,4-tetrahydrochrysene.
(4): EFoc is defined as the ratio of concentrations (/g OC) in a given fraction to the reconstructed bulk.
(5): average total %OC obtained for bulk 2, size 1 and size 2 experiments (0.44%) is used to calculate concentrations for bulk 1 and reconstructed bulk for the density experiment.
n.d.: not detected
n.m.: not measured

concentration with respect to organic carbon. EF_{OC} for compound **IX** neither duplicate well in the two size fractionation experiments nor show any particular trend with particle size.

D. PAH distributions in density fractions of sediment. Result from the density fractionation experiment carried out for the >63 μm particles as described in the Experimental section provide yet another clue to PAH-particle association. The two fractions obtained using this technique ("light" and "heavy") have been separated by the hydraulic sorting of particles >63 μm using a moving field of water. This technique allows separation of discrete organic detritus from more dense mineral matrices. Organic matter dispersed and distributed throughout the entire range of particle sizes by erosional processes occurring during particle transport and deposition are viewed as a common fraction by this technique.

Concentration of the MW274 PAH per g dry in the density fractionation experiment shows that these compounds are most highly concentrated in the "light" >63 μm fraction (Table 4). This fraction represents less than 1% of the total weight of these sediments (Table 4), however, it contains approximately 50% of the total amount of these compounds in the sediments (Figure 9b). Low concentrations of the MW274 PAH series still remain in the "heavy" >63 μm fraction suggesting the separation has been incomplete. Nevertheless, this simple technique has provided an effective means of separating most of the material to which the MW274 PAH are associated into a distinctive fraction. The "heavy" >63 μm fraction contributes the major proportion (76%) to the mass of whole sediments but contains less than 8% of the total MW274 PAH (Figure 9b). This observation is in agreement with the previous conclusion from the size fractionation experiments that most of the particulate material in these sediments has no physical or geochemical relationship with these compounds.

The particle size and density fractionation experiments suggest this series of MW274 PAH is associated with low density, carbon-rich detritus. The major fraction of these compounds is concentrated in the coarsest particles sizes. Nonetheless the compounds are found dispersed throughout all sediment size fractions.

E. MW274 PAH association with carrier particles. The three compounds of the MW274 PAH suite have a common association with physically discrete particles of organic detritus ("light" >63 μm) in these sediments. These detrital particles have a high organic carbon content and separate easily from the bulk of the sediment mass when subjected to a flow of water under laboratory conditions. This observation indicates similar environmental conditions are probably required for the transformation of the individual natural products into these different PAH. This inference does not necessarily lead to the conclusion that the series of MW274 PAH are associated with the same particles within the organic detrital fractions, however. These PAH could be present, for example, in particles derived from different plant species and still show similar distributions in these sediments. Closer inspection of the distribution of each compound in the PAH series in the coarse fractions provides support that these compounds are not all contained within the same carrier particle but rather are associated with discrete particles comprising the mixture of organic detritus.

The apparent differences in EF_{OC} for compound **IX** in a given fraction when comparing replicate experiments (Table 5) suggests this compound is present in particles that may be affected by the particle size fractionation technique used. The presence of heterogeneous aggregates of minerals with organic particles will influence the distribution of the PAH in the different particle size fractions. These agglomerates were microscopically observed in several fractions after sieving. They are very unstable to abrasion and are partially broken into smaller particles when subjected to water under pressure. The variability of compound **IX** between replicates may be ascribed to this phenomenon. Compounds **X** and **XI** do not show this degree of variability between replicates. This observation can be explained in terms of different types of biogenic detritus containing compounds **IX** and the other two PAH. Prahl (1982) found 1-methyl, isopropyl-7,8-cyclopentenophenanthrene (**IX**) is the only compound of the MW274 PAH series detected in high concentration in Crab Creek sediments (Figure 7b), supporting this hypothesis. Crab Creek is a tributary that flows into the Columbia River near Priest Rapids Dam (Figure 10). Crab Creek drains exclusively the steppe region of east-central Washington. Grasses and herbaceous plants dominate in this arid drainage basin that is almost devoid of trees (Hedges et al., 1984). The precursor of compound

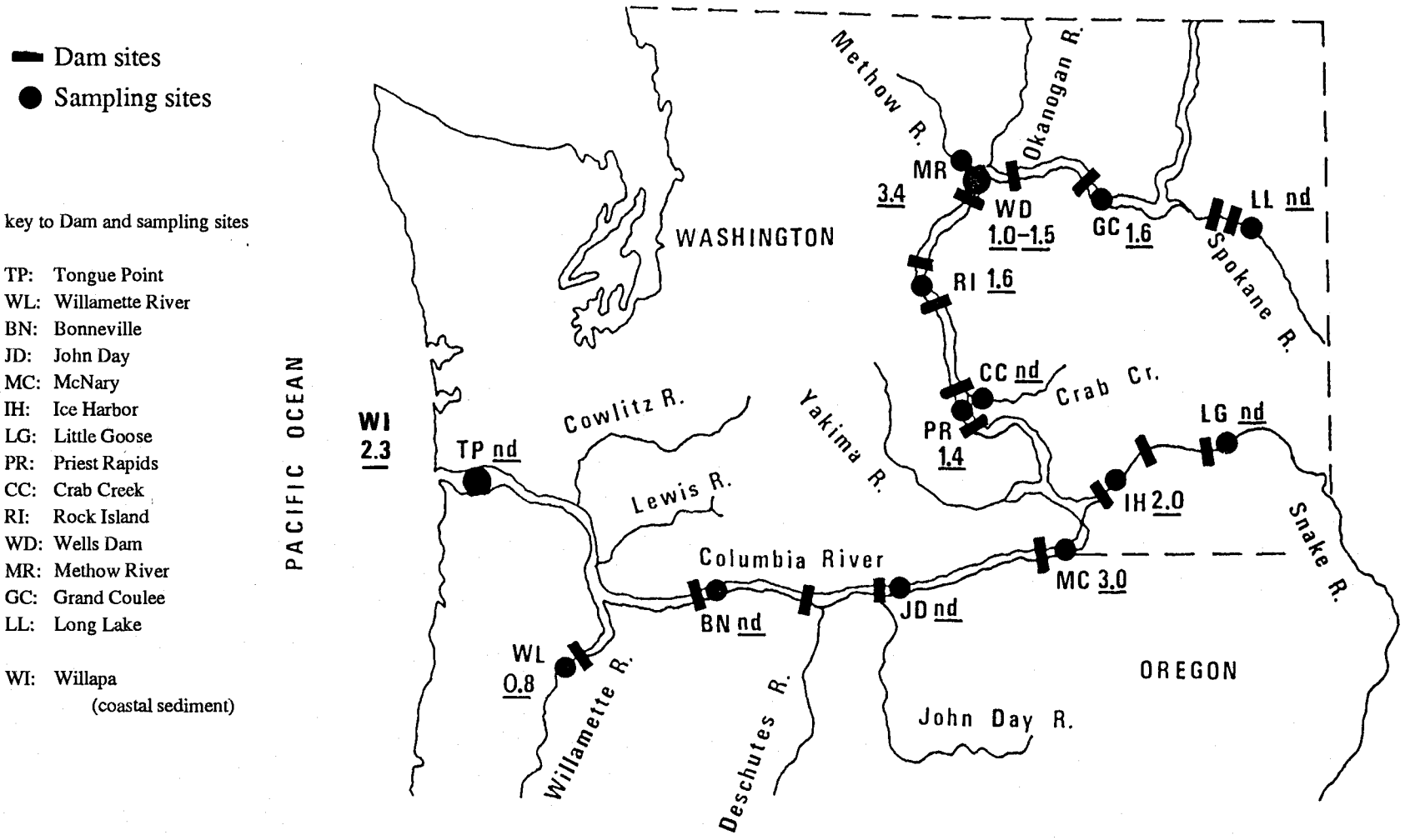


Figure 10. Ratio of MW274 PAH **XI** to **X** in different locations of the Columbia River basin (from Prahl, 1982) and the Willapa station offshore the Washington coast (Prahl, unpublished data).

IX may be present in large concentrations in this sediment. Comparative chromatograms representing the PAH fraction in Wells Dam and Crab Creek are presented in Figure 7. Differences in the variability of duplicates for compound **IX** with respect to **X** and **XI** and large differences in the distribution of these PAH in these two locations are evidence of a discrete, independent particle association for these aromatic tetracyclic compounds.

F. Relative abundance of geminal (XI) to vicinal (X) dimethyl isomers. Compounds **XI** and **X** have been related structurally to α- (**VII**) and ß- (**VIII**) amyrins, respectively. The amyrins have been reported in most classes of angiosperm plants (Henderson et al., 1969). Sometimes they are both present in the same plant, most other times only one of the two isomers appears as a principal component in plant tissues (Tulloch, 1976). Assuming these PAH as well as their precursors are contained within specific types of plant tissues, differences in the kind of plant detritus sampled will produce a variation in the relative concentrations of these PAH (Figure 10). Basically, this is a problem with the large particles, where the frequency of finding a specific type of detritus becomes little reproducible. Results obtained from replicates of the coarsest fraction (>500 μm) seem to support this assumption (Tables 4 and 5).

As particle size decreases, the MW274 PAH carrier particles are thoroughly mixed and more uniformly distributed in the less coarse particle size fractions and diluted within the PAH-devoid particles which constitute the major part of all particle size fractions ≤250 μm. This mixing provides a more homogenous distribution for the particles that contain this series of PAH in the finer sediments. The relative abundance of compounds **XI** and **X** in Wells Dam sediments approaches a value of 1.5 ±0.1 measured in 13 particle size and density fractions ≤250 μm (Figure 11). This value agrees well with those measured at other sites in the Columbia River located geographically close to Wells Dam (such as Rock Island and Grand Coulee Dams, Table 6). This homogeneity could probably result from integration of sediment sources over a comparably large drainage basin with similar vegetation. Very different ratios are observed in other locations of the Columbia River basin (such as Methow River and McNary Dam, Table 6). The Methow River, which is a small tributary that flows into the Columbia River upstream of Wells Dam, exhibits a higher

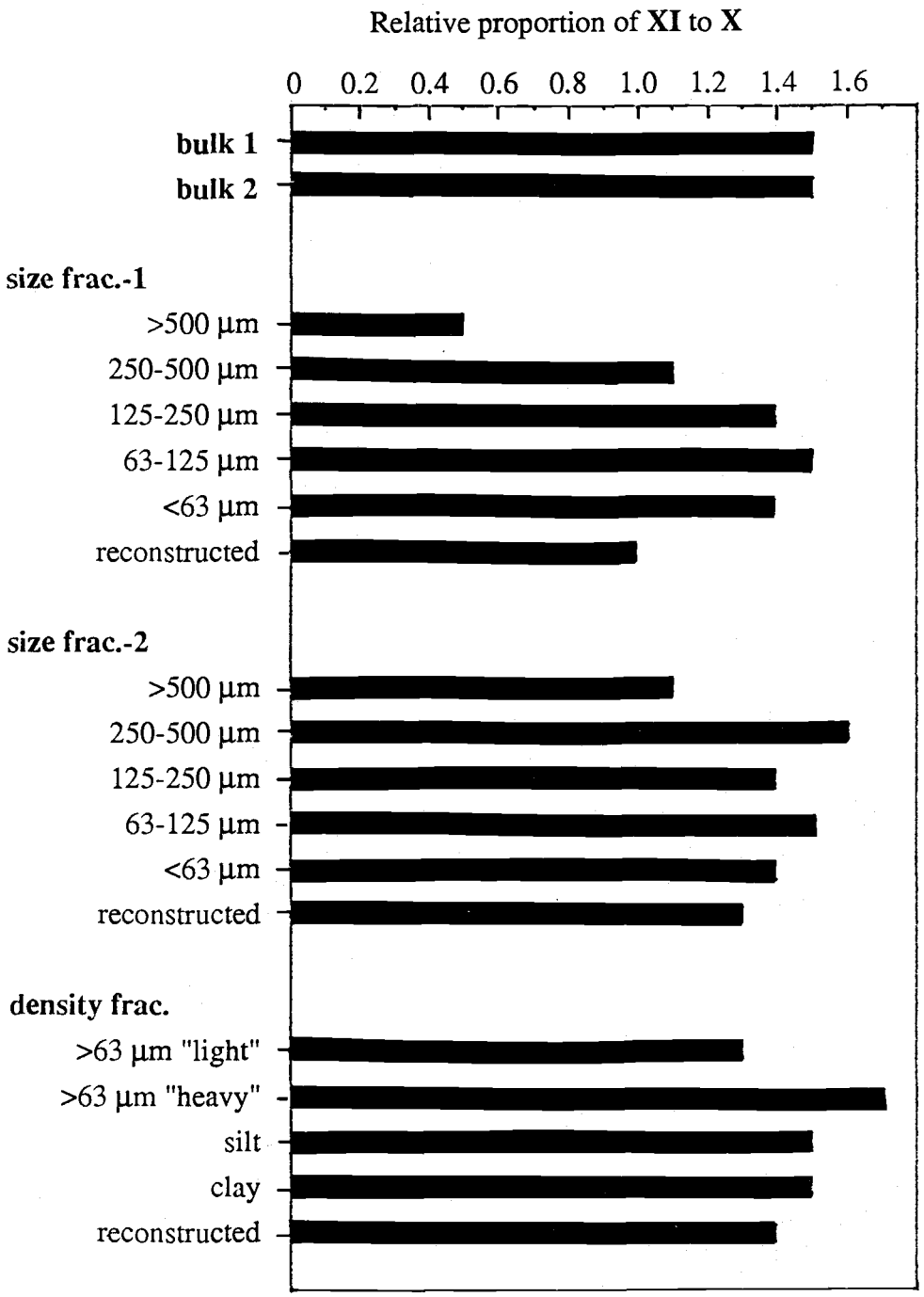


Figure 11. Relative proportion of MW274 PAH XI to X in Wells Dam total, particle size and density fractionated sediments.

proportion between these two PAH (i.e. **XI/X**=3.4). This ratio may not be very reliable due to the low concentration of compounds **X** and **XI** in sediments from this location, however Ice Harbor Dam in the Snake River shows a proportion of 2.0 and the Willamette River behind Oregon City Dam, a value of 0.8.

Table 6. Concentrations and relative abundance of geminal (**XI**) to vicinal (**X**) dimethyl substituted MW274 PAH isomers in different locations of the Columbia River basin (from Prahl, 1982) and coastal sediment (Prahl, unpublished data).

code	location(1)	concentration [ng/g dry wt.]		ratio
		X	**XI**	**XI/X**
GC:	Grand Coulee Dam	9.0	14.0	1.6
WD:	Wells Dam (2)	9.1 - 18.1	11.1 - 19.5	1.0 - 1.5
RI:	Rock Island Dam	10.9	17.5	1.6
PR:	Priest Rapids Dam	3.6	4.9	1.4
MC:	McNary Dam	8.7	26.3	3.0
IH:	Ice Harbor Dam (Snake River)	7.5	14.7	2.0
MR:	Methow River	1.0	3.4	3.4
OC:	Oregon City Dam (Willamette River)	7.3	5.7	0.8
LL:	Long Lake Dam	n.m.	n.m.	
CC:	Crab Creek	n.m.	n.m.	
LG:	Little Goose Dam	n.m.	n.m.	
JD:	John Day Dam	n.m.	n.m.	
BN:	Bonneville Dam	n.m.	n.m.	
TP:	Tongue Point	n.m.	n.m.	
Willapa (Washington coastal sediment)		5.6	12.9	2.3

(1): see Figure 10 for identification of sampling location.
(2): it includes measurements carried out in this study and Prahl, 1982.
n.d. not measured because of low concentration or GC complexity.

Concentration of suspended sediments carried by the Columbia river vary considerably throughout the year because of the seasonal contribution by tributaries (Whetten et al., 1969). However, during all seasons the Snake river

usually stands out as the major contributor of suspended sediments (Whetten et al., 1969). Sediment discharged by the Snake river into the Columbia river will play an important role in the dilution of sediments coming from the Columbia river upstream of the confluence. The MW274 PAH geochemicals will be practically undetected because of the dilution factor (Figure 10).

The presence of MW274 PAH compounds **X** and **XI** in measurable amounts in a coastal sediment sample (Table 6; Prahl, unpublished results) could be explained by transport of Columbia river sediments (Prahl and Carpenter, 1983; Prahl, 1985). It is suggested in this study that these PAH are associated with plant detritus which could carry these PAH offshore. Sorting of particles with similar hydrodynamic characteristics when discharged to the ocean will concentrate coarse plant detritus in certain areas of the continental shelf off the Washington coast (Prahl, 1985).

The relative abundance of compounds **XI** and **X** in Wells Dam sediments are similar to those obtained by Cranwell (1984) in lacustrine sediments but smaller than those reported by Wakeham et al. (1980) for Swiss lakes (6.0) and Tan and Heit (1981) for lakes in New York State's Adirondack Park (average 6.2). The extreme case is reported by Spyckerelle et al. (1977a,b) in a French pond sediment where compound **X** was not identified but compound **XI** appears to be particularly abundant (80 μg/g dry). In comparison, the >500 μm fractions in Wells Dam sediments show a concentration of 1.4 μg/g dry. On the other hand, Tissier and Saliot (1981) report compound **X** in Skagerrat marine sediments (Norwegian Sea) at a concentration of 10 ng/g dry wt., whereas, compound **XI** is barely detected. Different proportions could well be indicative of distinctive vegetation systems in each study area. The Willamette river has an extensive agricultural influence and the Methow river drain a basin with extensive coniferous forest (Hedges et al., 1984). Both show a large difference in the relative abundance of MW274 PAH **X** and **XI** (0.8 and 3.4, respectively) (Prahl, 1982). It is possible that the potential precursors for the MW274 PAH compounds are not associated with the most widespread vegetation in these regions but perhaps closely related to a minor component, such as a specific type of flowering plant. This evidence suggests that compounds **XI** and **X** are independently distributed in the different particles within the biogenic detritus. The cause of their geochemical production is obviously similar although the

source of their respective precursors could be related to different plant species represented by discrete particles within the sedimentary mixture of Wells Dam.

So far it has been shown that the MW274 PAH series is highly concentrated in particles enriched in the >500 μm and in the "light" >63 μm fractions. Similar %OC and MW274 PAH enrichments in both types of fractions strongly indicate that the particles have a similar origin. In the next section a more detailed chemical characterization of the organic detritus is presented. Defining the type of particles to which these PAH are associated provides a step forward in assessing the origin of these aromatic compounds.

II. Chemical Characterization of Particles.

Further chemical characterization of the sediment fractions separated by size and density techniques was made to try to identify what specific types of particules are contained within each fraction and to more fully describe the PAH-phase associations. This study is the first attempt to examine the association between the MW274 PAH and vascular plant debris in Recent sediments in order to test the hypothesis that this series of PAH can be produced rapidly in modern sedimentary environments. The suite of MW274 PAH could be associated with relative fresh vascular plant detritus or fossil coal fragments. Barrick et al. (1984) suggest that particulate debris from various Washington coals could account for all naturally derived PAH suites identified in Puget Sound sediments including MW274 PAH compounds **X** and **XI**. There is no direct evidence so far of non fossilized plant debris containing these PAH. Coal particles like fresh vascular plant material would behave similarly in the separation techniques employed due to roughly similar size and density characteristics (Prahl and Carpenter, 1983). Nevertheless, the fractionation is still useful to eliminate other type of particles by size or density allowing for a simpler mixture to be examined. Close chemical examination and visual inspection of the coarse particles show these sediments are rich in vascular plant debris. By visual inspection, particles of eroded coals do not appear to be a major alternate source for these PAH in Wells Dam sediments. Different types of chemical analyses carried out for each particle size fraction are presented below. Detailed information about the source of the major carbon content in the sediments is only possible using size and density fractionation techniques.

A. Organic carbon content and elemental ratios. The organic carbon content (%OC) of the "reconstructed bulk" in the fractionation experiment (0.46 ±0.06, n=2) is not significantly different from the measured value (0.40 ±0.08, n=2) in the unfractionated bulk sediments (Table 7). In early studies, Prahl and Carpenter (1983) and Hedges et al. (1984) have reported values of 0.51% and 0.53% OC, respectively, for these same bulk sediments from Wells Dam. The similarity in these results indicate the sediment can be separated into simpler fractions distinguished by size and density with minimal alteration of its

Table 7. Summary of parameters for Wells Dam particle size and density fractionated and total sediments.

	dry sed. (g)	wt %	%OC	C/N (1)	$\delta^{13}C$ (2)	CPI (3)
bulk 1	38.80	100	n.m.	n.m	n.m.	10.6
bulk 2	26.97	100	0.40	9.3	-24.9	10.2
size frac.-1						
>500 µm	0.25	0.34	23.25	n.m.	n.m.	13.0
250-500 µm	13.89	18.75	0.40	n.m.	n.m.	10.8
125-250 µm	28.70	38.74	0.24	n.m.	n.m.	9.0
63-125 µm	13.88	18.73	0.31	n.m.	n.m.	7.1
<63 µm	17.37	23.45	0.45	n.m.	n.m.	8.4
reconstructed	74.08	100	0.41			
size frac.-2						
>500 µm	0.21	0.31	29.47	33.5	-26.6	13.0
250-500 µm	12.78	19.11	0.48	11.3	-25.3	12.3
125-250 µm	25.16	37.63	0.21	6.4	-25.3	9.6
63-125 µm	12.23	18.29	0.29	8.0	-25.4	10.0
<63 µm	16.48	24.65	0.74	10.6	-25.6	7.3
reconstructed	66.86	100	0.50			
density frac.						
>63µm "light"	0.205	0.72	26.87	26.7	-26.3	10.7
>63µm "heavy"	21.63	75.88	0.12	4.1	-25.0	5.2
silt	6.52	22.88	n.m.	n.m	n.m.	8.7
clay	0.143	0.50	n.m.	n.m	n.m.	3.7
reconstructed	28.50	100				

(1): Atomic ratio

(2):

$$\delta^{13}C = \left(\frac{^{13}C/^{12}C\ (\text{sample})}{^{13}C/^{12}C\ (\text{standard})} - 1 \right) \cdot 1000$$

(3): Carbon Preference Index for n-alkanes:

$$\left(\sum_{21}^{31} \text{odds} / 2 \right) \left(1 / \sum_{20}^{30} \text{evens} + 1 / \sum_{22}^{32} \text{evens} \right)$$

n.m.:not measured

intrinsic chemical information. The percentage organic carbon in the "light" >63 μm fraction is similar to the average obtained for the >500 μm fractions from the two particle size fractionation experiments (26.9% compared to 26.4% ±4.4, n=2, respectively). The bulk organic matter in these fractions is extremely carbon-rich and nitrogen-depleted relative to the other fractions (Table 7). High measured values of C/N in the coarse and "light" fractions (33.5 and 26.7, respectively) indicate terrestrial plant tissue could make a major organic contribution. The high C/N values suggest a dominance of woody inputs in this fraction (Naiman and Sedell, 1979a). The lower C/N ratio in the "light" fraction (26.7) compared to the >500 μm fraction (33.5) can be seen as a consequence of the mixture of fresh (>500 μm) and partially degraded plant detritus (63-500 μm) (Figure 12). The density separation has left behind in the "heavy" fraction, organic material much richer in nitrogen compared to all the rest of the particle size fractions indicating a different biogenic source as the principal organic constituent in this fraction (Table 7 and Figure 12). If MW274 PAH compounds **X** and **XI** are contained in vascular plant debris, the results show these aromatic hydrocarbons are produced in the very early stages of plant tissue decomposition even before being deposited in this sedimentary environment.

B. Stable carbon isotope data ($\delta^{13}C$). Although all $\delta^{13}C$ values for organic carbon found in these fractions fall within the range reported for terrestrial plants (-23 to -27 $^{o}/_{oo}$) (Degens, 1969), values for the particle size and density fractionation experiments (Table 7) show that the coarse >500 μm and "light" >63 μm fractions are different with respect to the rest of the fractions on an isotopic basis. A near linear correlation is observed between C/N and $\delta^{13}C$ values for the different particle size and density fractions (Figure 12). Two reasons can be proposed to explain this correlation. First, the coarse particles (>500 μm) rich in plant debris, seem to represent an end member for this mixture. The finer particles (63 - 500 μm) represent mixtures of fresh plant debris and organic matter richer in nitrogen and heavier in carbon isotopes. Second, a different distribution of major biochemicals present in vascular plant debris (such as lignin, lipids, carbohydrates, proteins, etc.) occurs throughout the sediments. Lignin and lipids are nitrogen depleted and have more negative $\delta^{13}C$ values than the bulk of organic matter in plant detritus (Degens, 1969; Benner et al., 1987). The coarse fraction is enriched in lignin and solvent

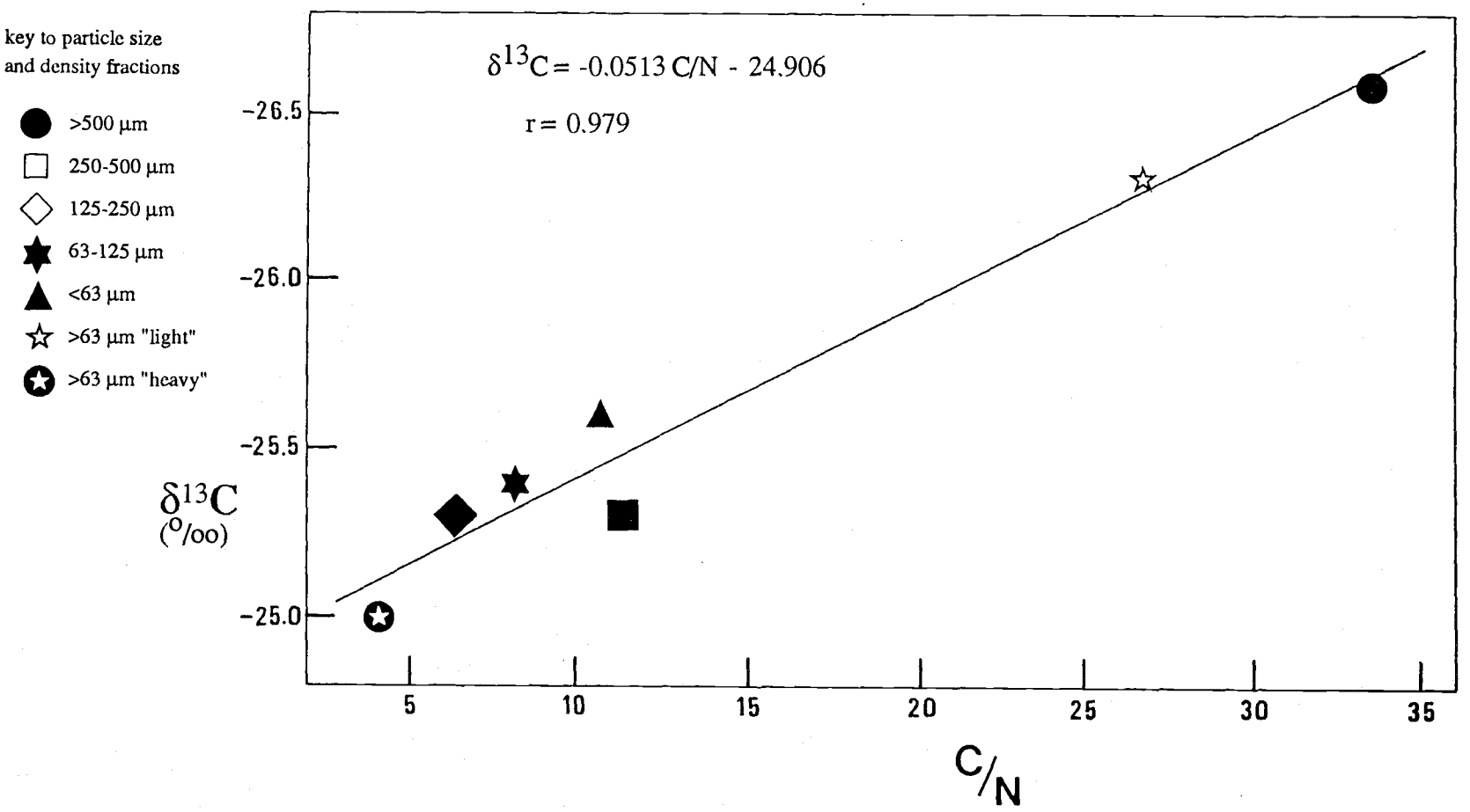


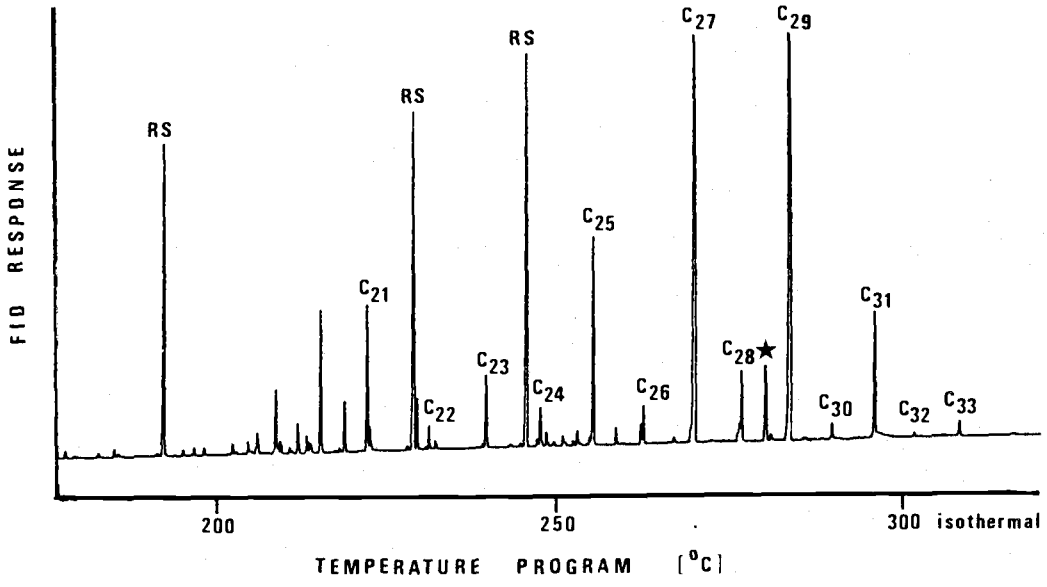
Figure 12. Relation between $\delta^{13}C$ values and C/N ratio for different particle size and density fractions in Wells Dam sediments.

extractable lipids compared to the other fractions (see discussion below). This finding is in agreement with and explains why EF_{oc} for MW274 PAH compounds **X** and **XI** are higher in the coarse (>500 μm) fraction (Table 5). It is possible that these aromatic hydrocarbons are contained in cuticular plant tissues and associated with surface waxes of vascular debris enriched in the coarse fraction of these sediments. Proteins, which have lower C/N values and less negative $\delta^{13}C$ values than lipids and lignins may be more abundant in finer particle size fractions.

C. Lipids. A series of long chain n-alkanes (20 to 32 carbons) with a strong odd/even carbon number predominance is the major constituent of the aliphatic hydrocarbon mixture isolated from the bulk, unfractionated sediment and the various particle size and density fractions (e.g. Figure 13a). The generally high odd to even carbon predominance in the range of C_{20} to C_{32} is represented by large values of the carbon preference index (CPI: 7.1-13.0) (Bray and Evans, 1961). CPI values in these fractions (Table 7) are within the range of 4-10 reported for vascular plants (Caldicott and Eglinton, 1973; Brassell et al., 1978) and in several cases even higher. The particle size fractions show a general trend toward larger CPI values in the coarse fraction. This tendency can be explained as going from pure plant debris in the >500 μm fraction to a more diverse type of biogenic detritus in the finer sediments where plant detritus can be mixed with organic matter derived from microbial activity or weathered fossil materials (Thompson and Eglinton, 1978). The presence of MW274 PAH in all particle fractions can be ascribed to the high abundance of plant detritus throughout the sediments as indicated by the predominance of plant wax hydrocarbons.

Homologous series of n-aldehydes, n-alcohols and n-carboxylic acids are also observed in these sediments (e.g. Figure 13b,c,d). The most prominent homologs for each series are C_{26}, C_{26} and C_{24} in length, respectively (Table 8). Total concentration (/g OC) of the long even carbon chains (C_{24}-C_{30}) varies with particle size similarly to the n-alkane series suggesting these series are all associated with a similar particle source (Prahl and Pinto, 1987). n-Aldehyde, n-alcohol and n-carboxylic acid homologous series account for the major portion of the GC-resolved peaks in the respective chromatographic fractions of all particle size and density fractionation experiments (Figure 13).

(a) n-alkane homologous series



(b) n-aldehyde homologous series

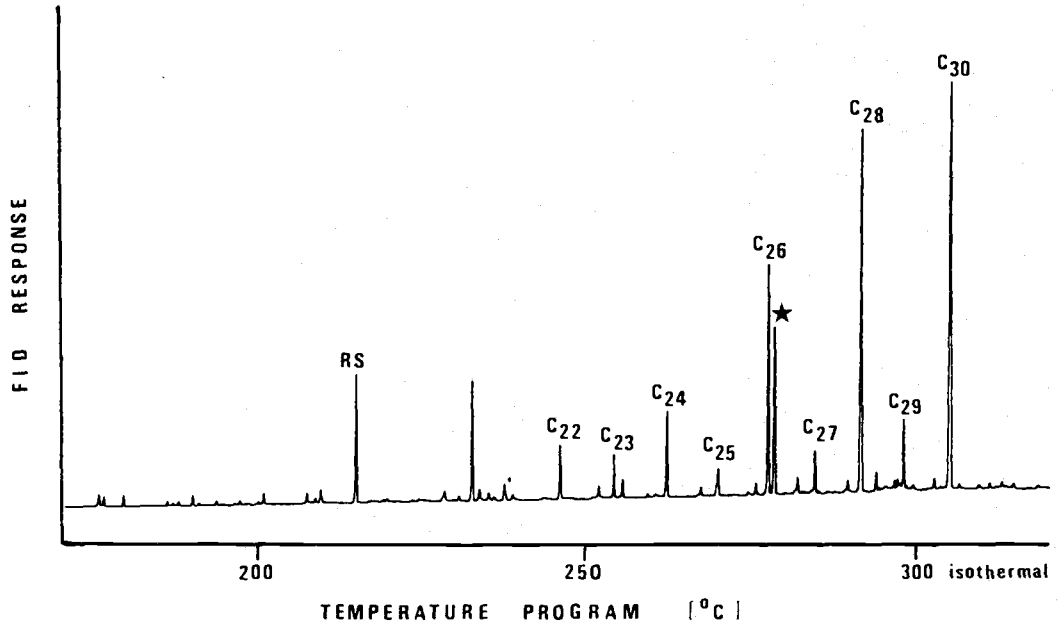
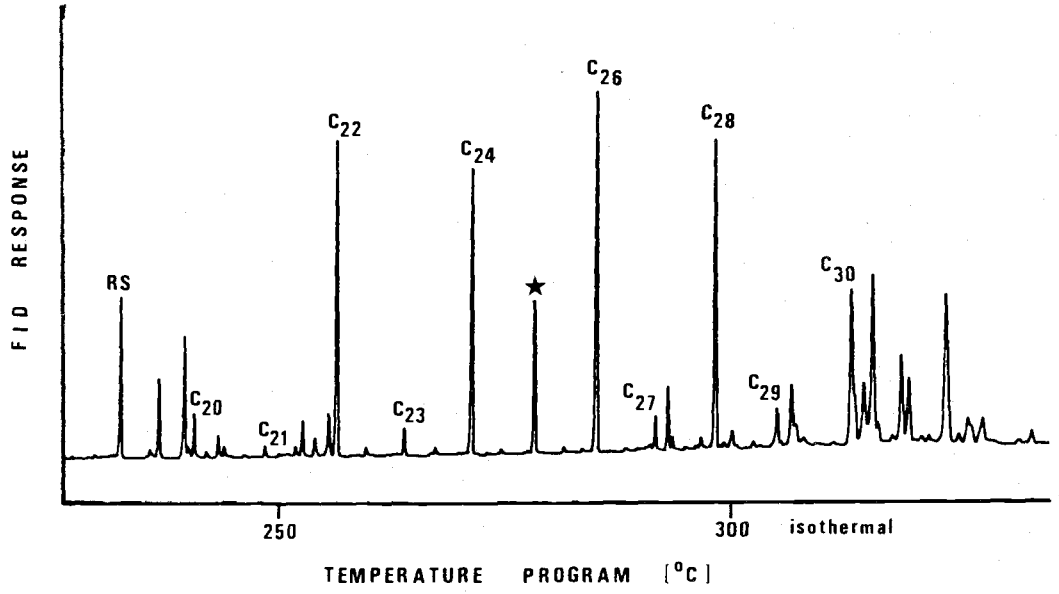


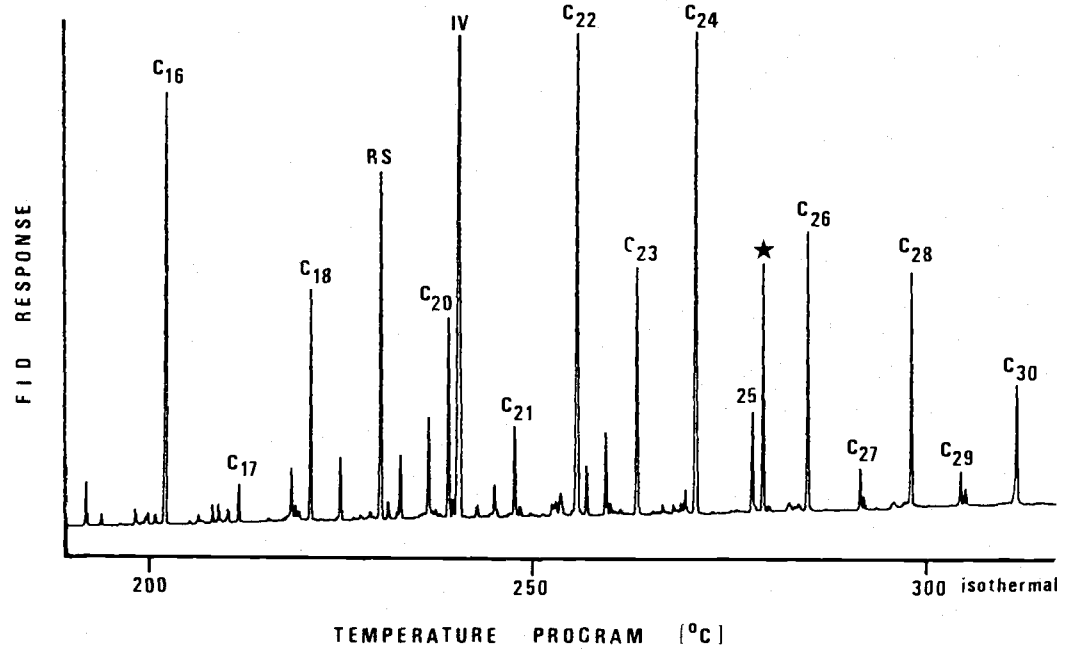
Figure 13. GC chromatograms of (**a**) n-alkane, (**b**) n-aldehyde, (**c**) n-alcohol and (**d**) n-fatty acid homologous series in the coarse (>500 μm) fraction of Wells Dam sediments. ★: internal standard. RS: recovery standard.

(c) n-alcohol homologous series



* : coelution of internal standard with C_{25} n-alcohol.

(d) n-carboxylic fatty acid homologous series



IV: dehydroabietic acid.

Table 8. Concentration (μg/g OC) of long-chain saturated aliphatic lipids in the different particle size and density fractions and unfractionated Wells Dam sediments.

(1): sum of the concentrations (μg/g OC) of C_{21}, C_{23}, C_{25}, C_{27}, C_{29} and C_{31} n-alkanes.

(2): Carbon Preference Index for n-alkanes:

$$\left(\sum_{21}^{31} \text{odds} \,/\, 2 \right) \quad \left(1 \Big/ \sum_{22}^{32} \text{evens} + 1 \,/\, \sum_{20}^{30} \text{evens} \right)$$

(3): sum of the concentrations (μg/g OC) of C_{20}, C_{22}, C_{24}, C_{26}, C_{28} and C_{30} compounds in the homologous series.

(4): Carbon Preference Index for n-aldehydes, n-alcohols and n-fatty acids:

$$\left[\left(\sum_{20}^{28} \text{evens} + \sum_{22}^{30} \text{evens} \right) \,/\, 2 \right] \left(1 \Big/ \sum_{21}^{29} \text{odds} \right)$$

(5): average total %OC obtained for bulk 2, size 1 and size 2 experiments (0.44%) is used to calculate concentrations for bulk 1 and "reconstructed" bulk for the density experiment.

(6): %OC for silt and clay fractions was not measured.

n.m.: not measured

Table 8. Concentration (μg/g OC) of long-chain saturated aliphatic lipids in the different particle size and density fractions and unfractionated Wells Dam sediments.

	n-alkanes			n-aldehydes			n-alcohols			n-fatty acids		
	$[C_{21}-C_{31}]$ (1)	C_{max}	CPI (2)	$[C_{20}-C_{30}]$ (3)	C_{max}	CPI (4)	$[C_{20}-C_{30}]$ (3)	C_{max}	CPI (4)	$[C_{20}-C_{30}]$ (3)	C_{max}	CPI (4)
bulk 1(5)	347	27	10.6	88	28	3.7	366	26	15.4	178	24	3.5
bulk 2	306	29	10.2	138	28	4.4	159	26	7.7	398	24	3.7
size frac.-1												
>500 μm	405	29	13.0	105	28	5.0	466	28	10.6	175	22	4.2
250-500 μm	224	27	10.8	46	28	2.8	303	26	8.4	120	22	4.2
125-250 μm	171	29	9.0	31	28	2.8	287	26	8.8	87	22	3.9
63-125 μm	298	27	7.1	96	28	3.9	499	26	7.2	187	22	2.9
<63 μm	451	29	8.4	148	28	3.2	881	26	8.8	127	24	3.5
reconstructed	315			87			506			134		
size frac.-2												
>500 μm	476	29	13.0	260	30	4.5	457	26	8.8	573	24,30	6.9
250-500 μm	258	29	12.3	39	28	4.0	203	26	11.4	288	24,28	7.9
125-250 μm	286	29	9.6	107	28	3.8	453	26	10.7	229	24	3.8
63-125 μm	361	29	10.0	134	28	3.7	704	26	10.9	413	24,28	3.9
<63 μm	153	29	7.3	61	28	3.2	365	26	8.5	281	24	5.7
reconstructed	275			109			401			342		
density frac.												
>63 μm "light"	713	29	10.7	154	30	5.0	546	26	6.2	729	24,30	3.8
>63 μm "heavy"	219	29	5.2	30	28	1.9	140	26	7.2	91	22	4.9
silt(6)	-	29	8.7	-	28	1.0	-	26	7.8	-	24	3.4
clay(6)	-	29	3.7	-	26	3.4	-	26	8.4	-	24	n.m.
reconstructed(5)	492			102			444			385		

These series are related to the surface wax of vascular plants (Caldicott and Eglinton, 1973; Tulloch, 1976). The homologous series appear highly enriched in the "light" >63 μm and coarse >500 μm fractions (Table 8).

These observations confirm that higher plant remains are not only a major particulate component of the coarse and "light" fractions but also they are the dominant source of the solvent extractable lipid contained in these fractions.

D. Lignin. The large presence of higher plant debris in the coarser and "light" fractions of Wells Dam sediments can be more specifically examined through an analysis of lignin. Evaluation of the lignin content of whole and fractionated samples by CuO oxidation potentially helps to characterize the type of plant tissues incorporated in the sediments and to determine if any correlation exists with the MW274 PAH compounds and specific plant types or tissues. Lignin is a phenolic polymer that occurs as a major constituent in the cell walls of vascular land plants (Sarkanen and Ludwig, 1971). Relative concentrations of lignin in the total organic fraction of individual particle size and density fractions can be estimated from the yield indicator Lambda. Lambda is defined as the total amount in mg of eight compounds belonging to three families of phenols: vanillyl (V), syringyl (S) and cinnamyl (C) phenols obtained per 100 mg of organic carbon oxidized by CuO in the sample (Hedges and Ertel, 1982). These phenols which correspond to lignin oxidation products, are well suited as source indicators for this study because they are unambiguously derived from lignin and provide a means of directly characterizing vascular plant remains. Highest lignin yields as indicated by Lambda, occur in the coarse (>500 μm) and "light" >63 μm fractions of the sediments (14.5 and 9.7 respectively, Table 9). Although these values are within the range of Lambda measured in fresh angiosperm wood (10.8-24.5) and in the upper range for fresh gymnosperm wood (4.2-13) (Hedges and Mann, 1979a), it is not possible to directly relate Lambda measured in sediments with the amount of vascular plant carbon present since Lambda is affected by: 1) vascular plant source types (some plant tissues produce higher yields of phenols under the condition of CuO oxidation than do others); 2) diagenetic transformation or loss of non-lignin carbon from vascular plant tissue (such as carbohydrates or proteins); and 3) dilution by biological sources of organic carbon which do not produce lignin, such as phytoplankton (Ertel and Hedges, 1984). A more appropriate approach to

Table 9. Summary of lignin parameters for Wells Dam particle size fractionated and bulk sediments.

	% OC	Lambda (a)	S/V (b)	C/V (c)	Va/Vh (d)	Vo/Vh (e)	Sa/Sh (f)	So/Sh (g)	cou/fer (h)
bulk	0.40	4.27	0.33	0.071	0.30	0.24	0.17	0.30	0.89
size frac.									
>500 µm	29.5	14.5	0.27	0.048	0.27	0.21	0.25	0.29	0.52
250-500 µm	0.48	4.33	0.35	0.054	0.30	0.24	0.20	0.29	0.60
125-250 µm	0.21	4.95	0.36	0.068	0.33	0.26	0.05	0.32	0.67
63-125 µm	0.29	5.07	0.43	0.082	0.34	0.25	0.21	0.29	1.00
<63 µm	0.74	2.58	0.37	0.113	0.41	0.27	0.27	0.33	1.42
reconstructed	0.50	5.74	0.32	0.064	0.31	0.23	0.21	0.30	0.77

(a): mg of vanillyl (V), syringyl (S) and cinnamyl (C) phenols per 100 mg of OC.
(b): ratio of syringyl to vanillyl phenols.
(c): ratio of cinnamyl to vanillyl phenols.
(d): ratio of vanillic acid to vanillin.
(e): ratio of acetovanillone to vanillin.
(f): ratio of syringic acid to syringealdehyde.
(g): ratio of acetosyringone to syringealdehyde.
(h): ratio of p-coumaric acid to ferulic acid.

characterize vascular plant tissues present in the sediments is using the ratios of different phenols measured in the samples.

1. Type of plant tissues in the sediments. The ratios S/V and C/V are two compositional parameters useful for characterizing the different vascular plant types and tissues that contribute to sedimentary lignin. These parameters reflect the relative levels of angiosperm and gymnosperm tissues in a mixture and of woody and non-woody tissues from either gymnosperm or angiosperm plants (Hedges and Mann, 1979a,b). Almost all gymnosperm tissues (such as pine, spruce and cedar) produce only vanillyl phenols when oxidized with CuO, although needles of Douglas fir (Pseudotsuga menziesii) have been shown to contain a small amount of syringyl phenols (Hedges and Mann, 1979a). Angiosperms (deciduous trees, grasses and flowering plants) produce syringyl phenols in addition to the vanillyl counterparts and, thus, have non-zero S/V values. The ratio C/V chemically discriminates woody from non-woody vascular plant tissue because only the lignin of the soft tissues (leaves, needles and stems) produces significant amount of the two cinnamyl phenols. Values of S/V in the particle size fractionation experiment (Table 9) systematically increase from 0.27 in the >500 μm fraction to 0.43 in the 63-125 μm fraction. This change in S/V values could represent a slight change in plant composition implying angiosperm plant detritus are relatively more important in the finer fractions compared to the coarse >500 μm fraction. Nonetheless, the low S/V values in these samples represent an overall gymnosperm-rich lignin composition (Figure 14).

In general, C/V ratios are very low indicating the lignin mixture is dominated by woody debris. Nonetheless, a clear increase in C/V values is observed in progressively finer size fractions (Table 9). These results suggest non-woody tissues may become a relatively more important component in the finer fractions. This is consistent with the idea that they are the more fragile parts of vascular plants and very likely are ground into finer particles by erosional processes (Prahl, 1985). The present data support that hypothesis.

2. Association of MW274 PAH compounds with plant detritus. More specific information about the type of plant tissues in which the MW274 PAH compounds are contained could be drawn from Table 10.

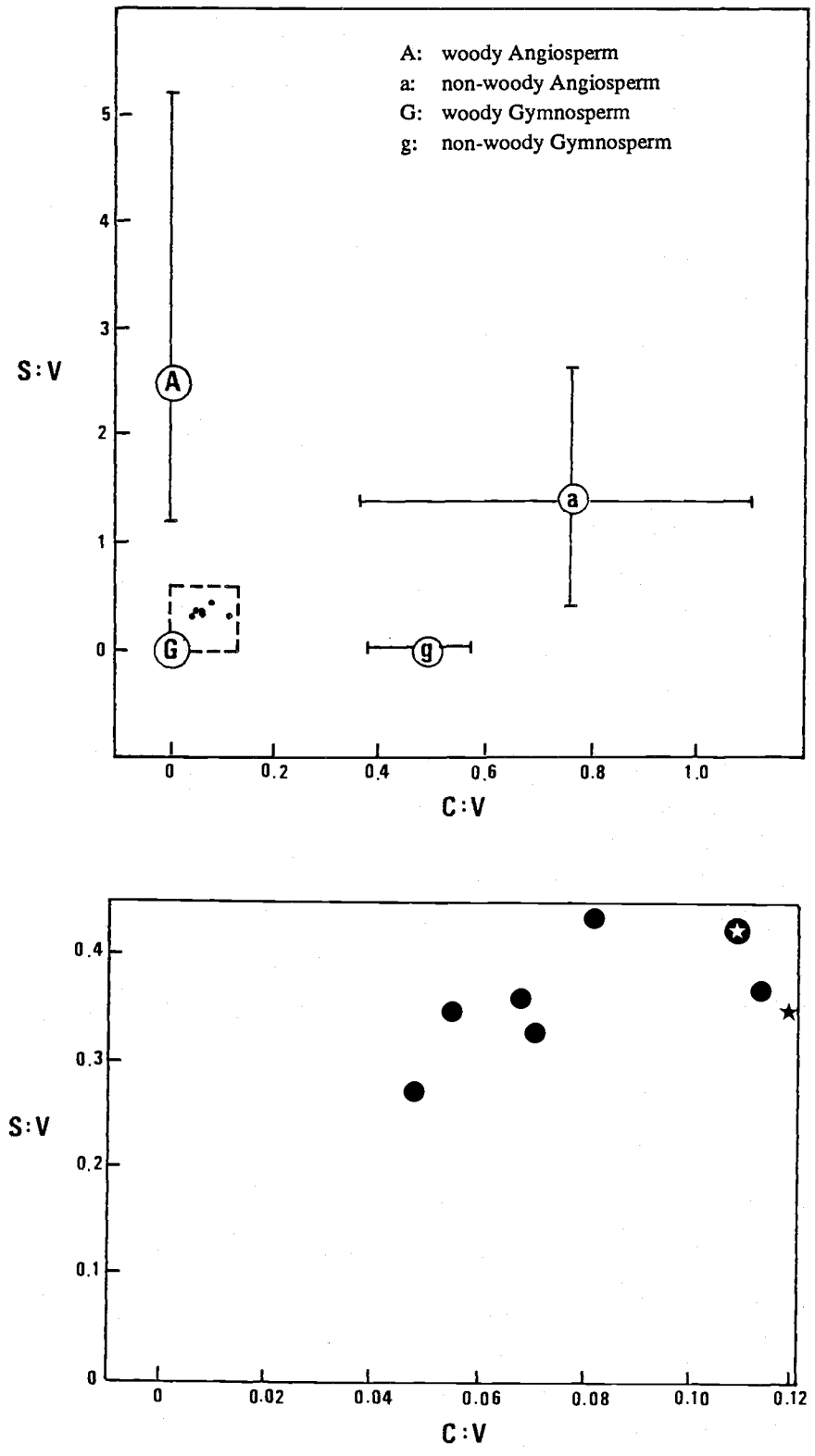


Figure 14. Compositional plot of S/V vs. C/V results from Wells Dam particle size fractionated sediments (●) and ranges around mean values for different categories of plant materials. Lower figure is an enlargement of the segmented rectangle shown in the upper figure depicting distribution of Wells Dam sediment data (●: this study; ✪ Hedges et al., 1984) and Methow river sediment (★) for comparison (Hedges et al., 1984).

Table 10. Values of lignin compositional parameters and concentrations of MW274 PAH (/g OC) measured in Wells Dam density fractionated sediments.

			PAH (ng/g OC)		
	S/V	C/V	IX	X	XI
"light" >63 μm	0.33	0.055	0.75	4.10	5.34
"heavy" >63 μm	0.17	0.167	-	1.00	1.66

S/V: ratio of syringyl to vanillyl phenols.
C/V: ratio of cinnamyl to vanillyl phenols.
IX: 1-methyl, isopropyl-7,8-cyclopentenophenanthrene.
X: 3,4,7-trimethyl-1,2,3,4 tetrahydrochrysene.
XI: 3,3,7-trimethyl-1,2,3,4 tetrahydrochrysene.

Two important assumptions are invoked for this argument. First, the precursors for the MW274 PAH are present in vascular plants and second, their transformation has occurred within the plant tissues where the MW274 PAH are still contained. The lignin compositional parameters S/V and C/V in >63 μm particles separated by density show that woody angiosperm detritus is relatively more important in the "light" compared to the "heavy" fraction. At the same time the concentration (/g OC) of the MW274 PAH series decreases by a factor of 3 to 4 from "light" to "heavy". The data suggest these aromatic hydrocarbons are not associated with the bulk part of the plant detritus which are gymnosperm-rich but contained in specific particles originated in the woody parts of angiosperms. An independent observation in the Methow River supports this contention. The sedimentary lignin composition in Wells Dam has been suggested to be strongly influenced by the introduction of sediments rich in gymnosperm woods by the Methow River and possibly the Okanogan River (Hedges et al., 1984) (Figure 4). The Methow River drains a basin with extensive coniferous forest (Highsmith and Kimerling, 1979) producing predominantly vanillyl phenols and, therefore low S/V and C/V ratios. This influence may not be important in terms of the presence of the MW274 PAH

compounds. Assuming the above argument is correct, that is the MW274 PAH are contained in woody angiosperm detritus, then the small presence of angiosperms in the Methow River may explain why the concentrations of the MW274 PAH series is a factor of 3 to 9 times lower in this sediment compared to Wells Dam sediment (Table 6; Prahl, 1982) which is likely to represent an integration of sediment sources.

III. 3-Oxygenated Pentacyclic Triterpenoids.

A. Characterization of 3-oxygenated pentacyclic triterpenoids in Wells Dam sediments. A variety of 3-oxy-pentacyclic triterpenoid derivatives have been identified in Wells Dam sediments by comparing GC retention time and mass spectral data with available reference materials, such as α-amyrin (**VII**), friedelan-3-one (also called friedelin, **XXIV**), oleanolic (**XXI**), ursolic (**XXII**) and betulinic (**XXIII**) acids. Other structures have been tentatively assigned based on interpretation and previously published mass spectra in the absence of standards, such as ß-amyrin (**VIII**) and ß-amyrenone (**XIV**). The mass spectra of these structures show one or more of the following ions: m/z 189, 203, 205, 218 and 262, characteristic of various triterpenoid skeletons (Budzikiewicz et al., 1963). The 3ß-hydroxy and 3-keto derivatives (R1: -OH and =O, respectively in Figure 15) yield fragment ✪ of m/z 218 as the base peak, which is the most characteristic fragmentation for these two classes of pentacyclic triterpenoid derivatives. The methyl ester of oleanolic acid (**XXI**,) which carries the carbomethoxy group at C-17, exhibits species ✪ at m/z 262, corresponding to 218 plus the addition of CO_2. This typical retro-Diels Alder fragmentation in ring C leading to species ✪ can thus be employed as a characteristic diagnostic tool for the presence of a Δ^{12} double bond in triterpenes of the α- and ß-amyrin class (Djerassi et al., 1962; Budzikiewicz et al., 1963; Ogunkoya, 1981). In general, the mass spectra of 3-hydroxy triterpenoid derivatives are very similar, differing in most cases only slightly in the relative intensity of some fragments. This characteristic makes individual identification difficult in the absence of standards (mass spectra data is presented in Appendix 3). Figure 16 shows the triterpene region of the gas chromatogram obtained by column chromatography in fractions L-4, L-5 and S:7-2 for different classes of compounds identified in these sediments.

1. 3-Keto derivatives. A major peak (**a1**) in the L-4 fraction has been tentatively identified as a 3-keto amyrenone (**XIV** or **XXV**) (Figure 16a). The mass spectrum shows a molecular ion of M+: 424 ($C_{30}H_{48}O$), fragment ions at m/z: 189 and 205, and the base peak at 218 indicative of a compound derived from the oleanane or ursane skeleton. Peak **a2** has been tentatively assigned to lupan-3-one (**XXVI**) by comparison with published mass spectra (Budzikiewicz et al., 1963; McEvoy, 1983). The strong M-43 ion in the mass spectrum of peak **a2**

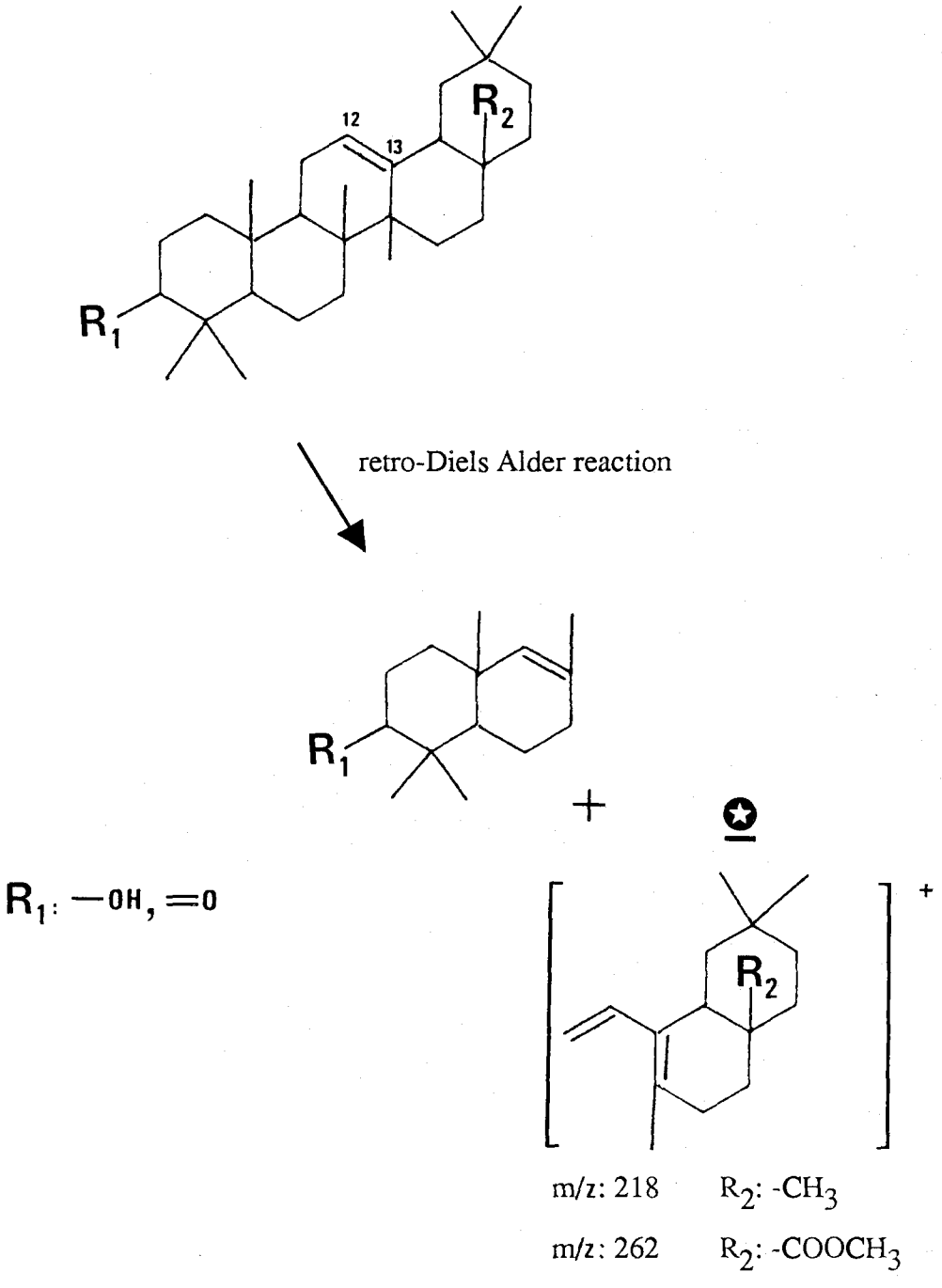
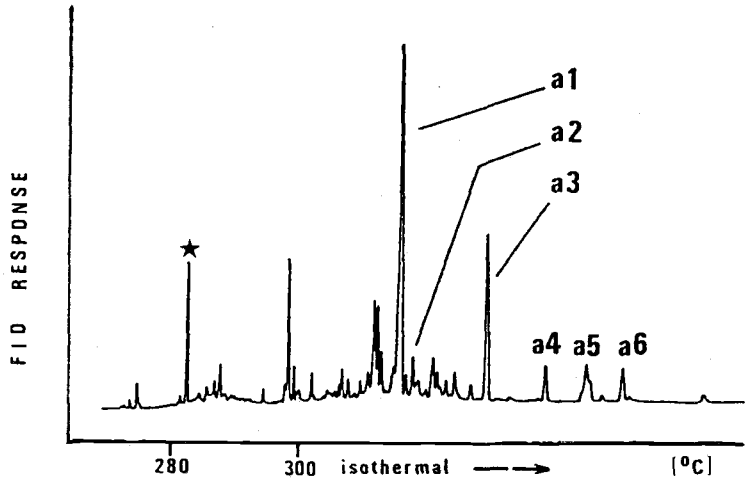
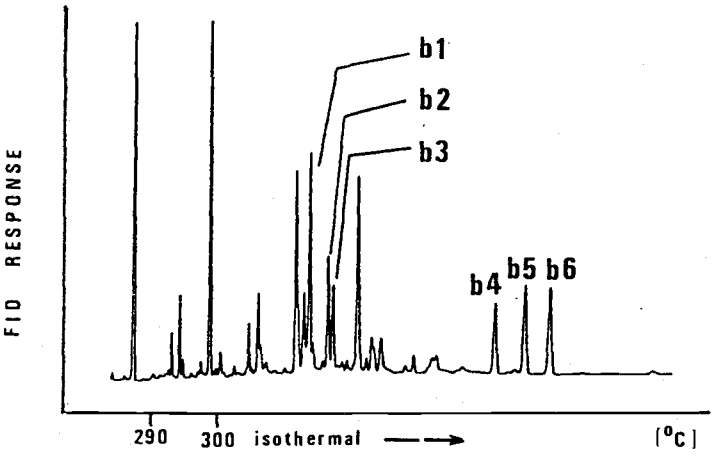


Figure 15. Characteristic fragmentation of Δ^{12} unsaturated pentacyclic triterpenoids.

(a) fraction L-4



(b) fraction L-5



(c) fraction S:7-2

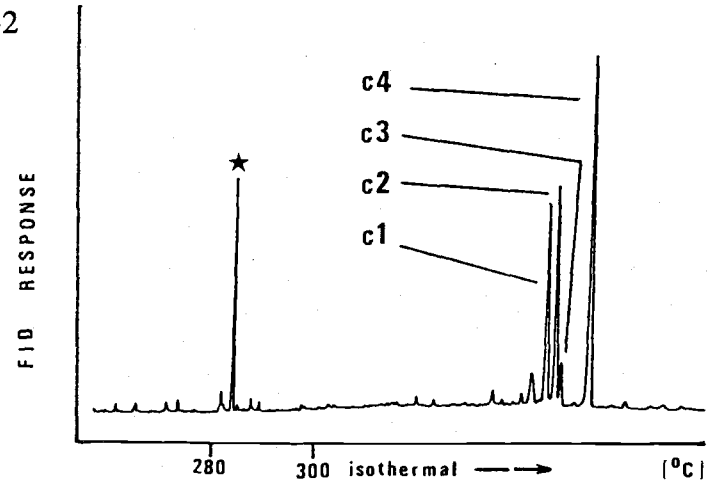


Figure 16. Pentacyclic triterpenoid region on GC traces in the >500 µm particle size fraction. (a) 3-keto derivatives. **a1**: 3-keto amyrenone, **a2**: lupan-3-one, **a3**: friedelan-3-one; **a4**, **a5**, **a6**: unknowns. (b) 3-hydroxy derivatives. **b1**: ß-amyrin, **b2**: α-amyrin, **b3**: taraxerol; **b4**, **b5**, **b6**: unknowns. (c) 3-hydroxy-17-carboxy derivatives. **c1**: unknown, **c2**: oleanolic acid, **c3**: betulinic acid, **c4**: ursolic acid. ★: internal standard.

represents the loss of an isopropyl group. Peak **a3** has been identified as friedelan-3-one (**XXIV**) by comparison with an authentic standard.

Three unknown compounds (peaks **a4**, **a5** and **a6**) appeared in the L-4 fraction of only the second particle size fractionation experiment (Figure 16a). The fragmentation pattern is almost identical amongst the three structures (Appendix 3). They all show a strong 205 fragment, characteristic of a 3-keto group in pentacyclic triterpenoids (Budzikiewicz et al., 1963). Other prominent fragments are m/z: 109, 245 and 313. A set of common fragments although lower in intensity are m/z: 407, 423, 443 and 458. When this fraction was silylated to observe any shift in retention time due to derivatization, these three peaks remained at the same retention time. This observation is evidence for no free hydroxyl groups in the molecules or if these functionalities exist, they may be difficult to derivatize because of steric hindrance. Formal identification of these compounds still remains unknown.

2. 3-Hydroxy derivatives. The presence of ß-amyrin (**VIII**) in the L-5 fraction is tentatively assigned to peak **b1** (Figure 16b). Peak **b2** has been identified as α-amyrin (**VII**) based on GC retention and comparison with an authentic standard. Mass spectrometry shows these two peaks have molecular ions consistent with pentacyclic monounsaturated alcohols (M+: 498, $C_{30}H_{50}O$-TMSi, using trimethylsilyl ether), a base peak at 218 and fragment ions at m/z: 189 and 203. The presence of a third compound (peak **b3**) with an identical molecular weight, common fragment ions to those mentioned above and fragment 189 as the base peak, suggests the presence of another pentacyclic triterpenol. Comparison with authentic standards of lupeol (**XII**) and τ-taraxerol (**XXVII**) shows that the unknown (peak **b3**) in the sediment is very similar with the latter. Differences in the proportion between two common fragments (m/z: 393 and 498) between the unknown and τ-taraxerol may be due to the position of the unsaturation. τ-taraxerol has a double bond in the 21,22 position, whereas taraxerol (**XXVIII**), its isomer, has its unsaturation in the 14,15 position. A more definitive conclusion can be reached in the presence of authentic standards.

Three compounds (peaks **b4**, **b5** and **b6**) with strong fragments at m/z: 189 and 203 and a small but characteristic fragment at m/z: 279 (3-hydroxyTMSi)

indicate the presence of three unindentified 3-hydroxyl pentacyclic triterpenoid structures (Figure 16b and Appendix 3). Their molecular weights have not been established. These compounds were not present during the first particle size separation but appeared during the density separation. It is not clear whether these structures were produced in the sediments during storage of the sample at 4°C between replicate analyses or are natural products of higher plants which are heterogeneously distributed in Wells Dam sediments. Current data is insufficient to fully characterize these structures.

3. 3-Hydroxy-17-carboxy derivatives. Two of the most abundant compounds in the S:7-2 polar fraction obtained by column chromatography (peaks **c2** and **c4**) have been identified by GC retention and comparison with authentic oleanolic (**XXI**) and ursolic (**XXII**) acids, respectively (Figure 16.c and Appendix 3). These compounds correspond to the 3-hydroxy-17-carboxy derivatives of triterpenoids belonging to the oleanane and ursane triterpenoid skeleton and are present as major lipids in these sediments. Betulinic acid (**XXIII**) has also been identified in the S:7-2 fraction (peak **c3**) by comparison with an authentic standard. Peak **c1** has not been identified. Fragments 189 and 203 although present in this structure, are minor components of the mass spectrum (see Appendix 3). Its base peak is m/z:393 which corresponds to a minor fragment in the other 3-hydroxy-17-carboxy triterpenoids.

B. Sedimentary distribution of 3-oxygenated pentacyclic triterpenoids. Sediments from Wells Dam contain compounds with the oleanane and/or ursane skeleton present as 3-keto, 3ß-hydroxy and 3ß-hydroxy-17-carboxy derivatives. The presence of these compounds in these sediments warrants a closer look to their distribution and association with the organic matter in the different particle fractions that constitute the whole sediment. The use of particle size and density fractionation techniques provided detailed information about the distribution of the MW274 PAH series and the characterization of the type of organic matter in the sedimentary fractions. The same techniques have been used to better understand the distribution of the pentacyclic triterpenoids in these sediments and have allowed us to determine that these compounds as potential precursors have similar particle specificity to what has been described for the tetracyclic MW274 PAH.

1. Sediments fractionated by particle size. The concentration of pentacyclic triterpenoids normalized to total sediment weight in the different particle size fractions and bulk sediments is presented in Table 11. The three different classes of 3-oxy pentacyclic triterpenoids show similar distributions according to grain size. They are most highly concentrated in the coarse (>500 μm) fraction ranging from 2250 to 68000 ng/g dry. Their concentrations decrease sharply in particles <500 μm in size (i.e. 1-2 orders of magnitude) and minimizes in the 125-250 μm fraction. Their concentrations increase slightly again in the fine (<63 μm) fraction. Oleanolic, ursolic and betulinic acids appear enriched in the organic particles present in the coarse fraction by a factor ranging from 2.8 to 5.4 times that observed in the reconstructed bulk sediments when concentrations are normalized to organic carbon (Table 12). Enrichments (EF_{OC}) as previously defined, correspond to the ratio of the concentration (/g OC) in each fraction with respect to the concentration (/g OC) in the reconstructed bulk. EF_{OC} are used here analogously to their application with the MW274 PAH, that is, to estimate the enrichment or depletion of these compounds normalized to organic carbon in the sedimentary fractions with respect to the bulk sediments. All fractions <500 μm in size show EF_{OC} less than the unity. Two possible explanations can account for this observation. First, the organic detritus containing these triterpenoids in the finer sediments is mixed with organic matter devoid in these compounds. Second, the pentacyclic triterpenoids are easily destroyed in the finer particles, resulting in organic matter depleted in these compounds. This second explanation suggests the 3-hydroxy-17-carboxy pentacyclic triterpenoids are labile compounds, easily altered during early diagenesis of these sediments. This argument may explain the abrupt decrease of these compounds in the coarse fraction (>500 μm) between the first and the second particle size fractionation experiments. It is possible that the presence of new structures observed in the L-4 and L-5 chromatographic fractions during the second particle size fractionation (peaks a4, a5, a6, b4, b5 and b6 in Figure 16) correspond to the transformation of 3-hydroxy-17-carboxy pentacyclic triterpenoids into 3-hydroxy and 3-keto derivatives respectively. Similar distributional features and EF_{OC} can be obtained for the other classes of pentacyclic triterpenoids that appear in Table 11.

2. >63 μm sediment fractionated by density. The 3-oxygenated pentacyclic

Table 11. 3-Oxygenated pentacyclic triterpenoid concentrations in the different particle size and density fractions and unfractionated Wells Dam sediments.

(1): MW 424 α- or ß-amyrenone
(2): MW 426 lupanone
(3): MW 426 friedelin
(4): MW 498 ß-amyrin (as TMSi ether)
(5): MW 498 α-amyrin (as TMSi ether)
(6): MW 498 taraxerol (as TMSi ether)
(7): MW 542 betulinic acid (as TMSi ether, methyl ester)
(8): MW 542 oleanolic acid (as TMSi ether, methyl ester)
(9): MW 542 ursolic acid (as TMSi ether, methyl ester)
n.d.:not detected

Table 11. (Continued)

Concentration [ng/g dry wt]

	3-keto derivatives			3ß-hydroxy derivatives			3ß-hydroxy-17-carboxy derivatives		
	XXV or XIV (1)	XXVI (2)	XXIV (3)	VIII (4)	VII (5)	XXIX (6)	XXIII (7)	XXI (8)	XXII (9)
bulk 1	250	18	84	474	240	386	211	244	522
bulk 2	190	n.d.	38	132	131	125	22	51	108
size frac.-1									
>500 µm	30200	3200	13000	12900	11300	35200	7690	41800	68000
250-500 µm	412	29	106	128	100	142	36	64	142
125-250 µm	89	7	27	171	89	59	13	29	62
63-125 µm	119	n.d.	43	138	79	73	15	36	129
<63 µm	417	n.d.	142	430	219	285	37	53	104
reconstructed	333	19	116	226	140	249	49	183	327
size frac.-1									
>500 µm	26300	6830	20300	19300	15200	5930	2250	5320	24500
250-500 µm	55	21	72	145	88	n.d.	17	19	136
125-250 µm	39	n.d.	31	76	52	n.d.	n.d.	8	29
63-125 µm	26	n.d.	32	142	79	n.d.	n.d.	14	31
<63 µm	207	n.d.	59	276	171	169	n.d.	n.d.	72
reconstructed	164	26	110	211	141	60	7.1	26	143
density frac.									
>63 "light"	12400	23500	13100	18300	20800	1670	n.d.	3330	34500
>63 "heavy"	12	n.d.	n.d.	11	8	7	n.d.	n.d.	n.d.
silt	217	n.d.	n.d.	218	157	159	n.d.	n.d.	n.d.
clay	475	n.d.	n.d.	1167	783	n.d.	n.d.	n.d.	n.d.
reconstructed	150	169	94	196	196	54	-	24	248

Table 12. Concentrations and enrichments of 3-hydroxy-17-carboxy pentacyclic triterpenoids in the different particle size and density fractions and unfractionated Wells Dam sediments.

		Concentration [µg/g OC]:			Enrichment (1):		
	%OC	XXIII (2)	XXI (3)	XXII (4)	XXIII (2)	XXI (3)	XXII (4)
bulk 1(5)	n.m.	46.3	53.5	114			
bulk 2	0.40	5.6	12.9	27.3			
size frac.-1							
>500 µm	23.25	33.1	180	292	2.8	4.0	3.7
250-500 µm	0.40	8.9	16.1	35.5	0.7	0.4	0.4
125-250 µm	0.24	5.5	12.3	25.8	0.5	0.3	0.3
63-125 µm	0.31	4.9	11.5	41.6	0.4	0.3	0.5
<63 µm	0.45	8.3	11.8	23.1	0.7	0.3	0.3
reconstructed	0.41	12.0	44.6	79.8			
size frac.-2							
>500 µm	29.47	7.6	18.1	83.1	5.4	3.6	2.9
250-500 µm	0.48	3.5	4.0	28.3	2.5	0.8	1.0
125-250 µm	0.21	n.d.	3.8	13.8	-	0.8	0.5
63-125 µm	0.29	n.d.	4.8	10.7	-	0.9	0.4
<63 µm	0.74	n.d.	n.d.	9.7	-	-	0.3
reconstructed	0.50	1.4	5.1	28.6			
density frac.							
>63µm "light"	26.87	n.d.	12.4	128			
>63µm "heavy"	0.12	n.d.	n.d.	n.d.			
silt	n.m.	n.d.	n.d.	n.d.			
clay	n.m.	n.d.	n.d.	n.d.			
reconstructed(5)	n.m.	n.d.	n.d.	n.d.			

(1): Enrichment is defined as the ratio of concentrations (/g OC) in a given fraction to the reconstructed bulk.
(2): MW 542 betulinic acid (as TMSi ether, methyl ester)
(3): MW 542 oleanolic acid (as TMSi ether, methyl ester)
(4): MW 542 ursolic acid (as TMSi ether, methyl ester)
(5): average total %OC obtained for bulk 2, size 1 and size 2 experiments (0.44%) is used to calculate concentrations for bulk 1 and reconstructed bulk for the density experiment.
n.d.: not detected
n.m.: not measured

triterpenoids found in Wells Dam sediments appear highly enriched in the "light" fraction (Table 11). Only the 3-hydroxy pentacyclic triterpenoids and the 3-keto amyrenone are also detectable in the "heavy" fraction at a very low concentration (~10 ng/g dry). The high concentration of these triterpenoids in the coarse and "light" fractions (up to 68000 ng/g dry) is clear evidence of their association with carbon-rich particles present in those two fractions where organic detritus account for most of the dry weight (see previous discussion).

C. Geochemical significance.

1. Sources for the 3-oxygenated pentacyclic triterpenoids in Wells Dam sediments. 3-oxygenated pentacyclic triterpenoids are widely distributed in vascular plants, especially in angiosperms (Glasby, 1982). The presence of large amounts of plant detritus in these sediments has been demonstrated and corroborate the origin of these compounds. Published data is lacking that describes the overall lipid composition of the cuticular waxes of dominant species in the Pacific Northwest, let alone any study about the presence of 3-oxytriterpenoids in the vegetation of this region. Recently, Standley (1988) reports the presence of triterpenoids of the lupane skeletal-type in soots from Alder wood used as fuel in residential wood combustion. Friedelin was found only in the oak soot and a variety of cyclic diterpenoids in the pine stove smoke and ambient aerosol samples. These samples were collected in suburban sections of Eugene and Corvallis, Oregon. Red Alder (Alnus rubra) and Douglas fir (Pseudotsuga menziesii) are examples of major deciduous and coniferous vegetation growing west of the Cascade Mountains in the Pacific Northwest (Highsmith and Kimerling, 1979). Debris from plants such as these is introduced throughout the drainage of the Columbia River (Hedges and Mann, 1979; Hedges et al., 1984).

2. Transformation of 3-oxygenated pentacyclic triterpenoids in Wells Dam sediments. The absence of 3-keto and 3-hydroxy-17-carboxy triterpenoids in the "heavy" >63 μm fraction and their lower concentration or undetected presence in the fine particles (<63 μm) could be an indication of their instability in Wells Dam sediments.

It was previously proposed in this study that new 3-oxytriterpenoid structures appear in the second experiment in the L-4 (peaks a4, a5, a6 in Figure 16a) and L-5 (peaks b4, b5, b6 in Figure 16b) chromatographic fractions as a consequence of the transformation of 3-hydroxy-17-carboxy derivatives (peaks c2, c3, c4 in Figure 16c). An attempt has been made in Table 13 to compare total concentrations of the 3-hydroxy-17-carboxy triterpenoids (MW 542) in fraction S:7-2 with what could be their transformation products in L-5 (peaks b4, b5 and b6 in Figure 16b). Table 13 shows there is better reproducibility of the mass balance when both classes of compounds (MW 542's and compounds b4, b5 and b6) are taken in the calculation rather than just comparing the MW 542's alone in the different experiments.

Table 13. Total concentration of oleanolic (XXI), ursolic (XXII) and betulinic (XXIII) acids (MW 542) and unidentified 3-hydroxy triterpenoids in the different particle size fractionation experiments in Wells Dam sediments.

	Total concentration [ng/g dry] of:		
Experiment:	MW 542	b4,b5,b6	MW 542 + b4,b5,b6
Size frac.-1 : August 1986	560	-	560
Density frac.:November 1986	270	370	640
Size frac.-2 :December 1986	175	225	400

MW 542: Sum of the TMSi-ether, methyl ester derivatives of oleanolic (**XXI**), betulinic (**XXIII**) and ursolic (**XXII**) acids (peaks c2, c3 and c4, respecetively, in Figure 16c).

b4,b5,b6: three unidentified 3-hydroxy triterpenoids observed in fraction L-5 (see Figure 16b).

Unpublished data we have obtained in our laboratory for the lipid composition of cuticular waxes in freshly collected leaves from Red alder and needles and pollen from Douglas-fir show no evidence of 3-oxytriterpenoids in the latter. However, the lipid analysis of alder leaves show 3-keto triterpenoids are the predominant compounds in fraction L-4 and 3-keto-carboxylic triterpenoid derivatives are more abundant than fatty acids in fraction S:7-1. It

is interesting to note the presence of 3,4-seco ring A acids derived from 3-oxygenated triterpenoids (**XXX**, **XXXI** and **XXXII**). The identification of these compounds (Appendix 3) is based exclusively on comparison with mass spectra published by Cranwell (1984).

This series of tetracyclic carboxylic acids formed by A-ring cleavage, have been found in deltaic and lacustrine sediments (Corbet et al., 1980; Cranwell, 1984) and their formation has been attributed either to microbial activity or photochemical decomposition of the 3-keto triterpenoids (Corbet et al., 1980 and references therein). Their isolation from fresh plant leaves suggest they could be products of normal photolysis of triterpene ketones in leaf waxes. Corbet et al. (1980) suggest these 3,4-seco ring-A acids are possible intermediates in the aromatization mechanisms of amyrenones as proposed by Spyckerelle et al. (1977a,b). The loss of ring-A (see structure d Appendix 2) is required to convert amyrin-like structures to the tetracyclic structures represented by the aromatic compounds **X** and **XI** (Figure 17). The presence of the 3,4-seco ring-A acids not only gives support to this postulated mechanism but also provides the ideal compound type necessary in the early diagenesis of 3-oxytriterpenoids. Red alder grows adjacent to most lower elevation streams in the heavily forested Cascade Mountains of western Oregon (Naiman and Sedell, 1979b).

3. MW274 PAH and 3-oxygenated pentacyclic triterpenoid relationships. The distribution of 3-keto, 3-hydroxy and 3-hydroxy-17-carboxy triterpenoids in the different sedimentary fractions of Wells Dam are similar to the MW274 PAH compounds **X** and **XI**. This point is shown by comparison of their EF_{OC} (Figure 18). However, particles in the finest sediments do not show enrichment of 3-oxytriterpenoids with respect to the bulk sediment as do the MW274 PAH compounds. This distributional difference suggests the presence of two different types of organic matter in which the PAH are present. One, mostly plant detritus in the coarse fractions and another, soil organic detritus in the finest sediments. Soils represent a more degraded type of organic carbon in which the 3-oxytriterpenoids have already undergone biogeochemical transformations. The high EF_{OC} for 3-oxygenated pentacyclic triterpenoids and MW274 PAH **X** and **XI** and their similar enrichments in the mid-size particle

Pathway 1

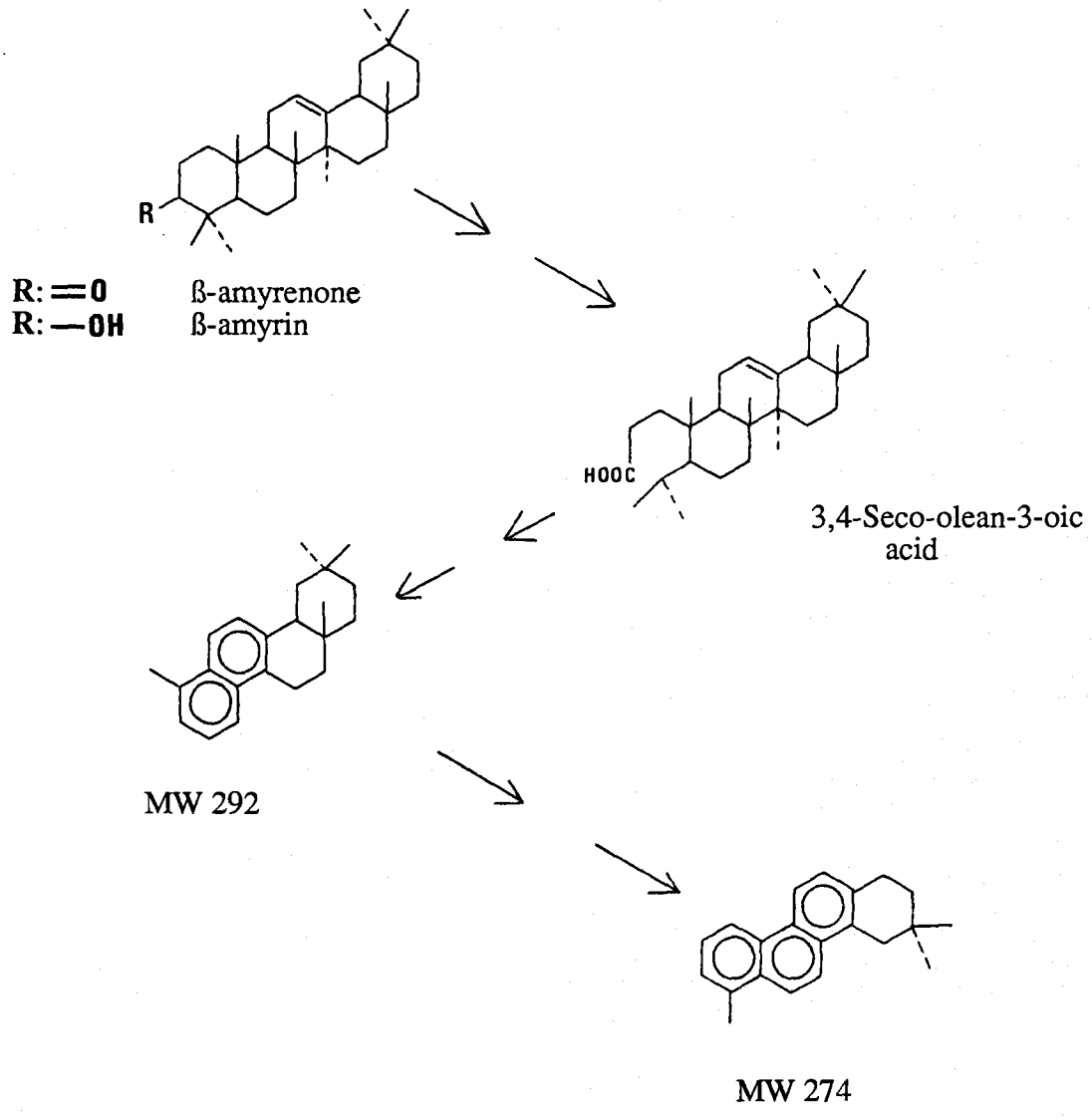


Figure 17. 3,4-Seco ring-A acid derived from 3-oxytriterpenoids found in alder leaves and possible intermediate in the aromatization mechanism of 3-hydroxy triterpenoids.

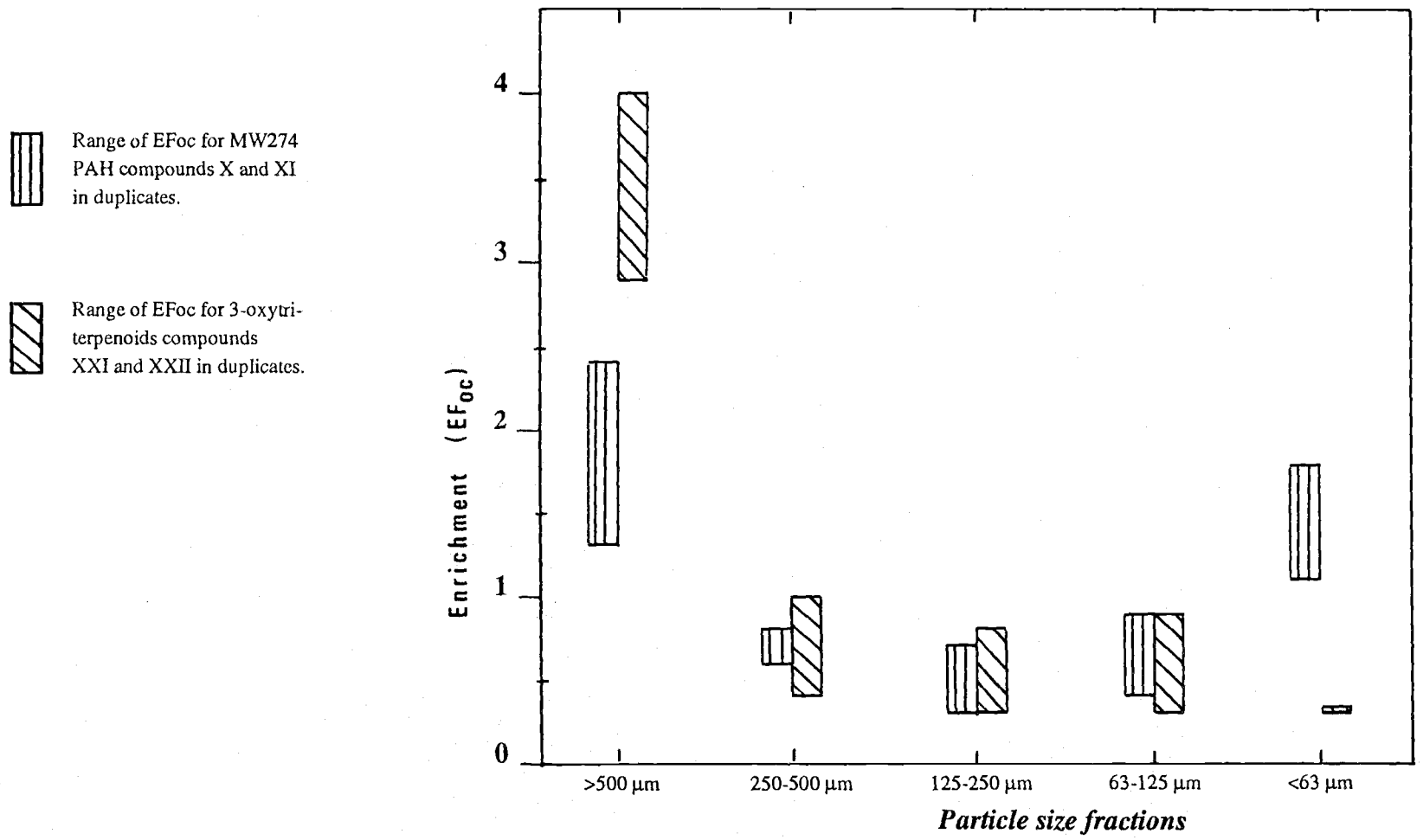


Figure 18. Enrichment factors (EFoc) for MW274 PAH X and XI and 3-oxytriterpenoid oleanolic and ursolic acids in Wells Dam particle size fractionated sediments.

fractions suggest a common particle association between these two series in Wells Dam sediments. Although these results have given experimental support to the product-precursor relationship between these series of compounds, the factors mediating early diagenesis of the natural products remains an open question.

IV. Diploptene as a Potential Precursor for MW274 Compound IX.

Until now we have seen that different series of compounds show a rather similar change in distribution with particle size (Tables 5,8,9,12). This observation could be ascribed to the dominant presence of plant detritus in these sediments. Lignin, 3-oxytriterpenoid derivatives and long chain n-alkanes are all present in vascular plants. It is also suggested in this study that the MW274 PAH suite of compounds found in these sediments is associated with plant detritus and therefore, we would expect to see a similar distribution among these series of compounds and plant detritus.

The presence of hop-22(29)-ene (also called diploptene, **XXXIII**) in the different particle size fractions show a different distribution to that observed for the 3-oxytriterpenoids, long chain n-alkanes and lignin. A systematic enrichment of diploptene (/g OC) toward finer sediments (Figure 19) suggests a different source for this geochemical.

Diploptene was first identified in ferns (Ageta et al., 1964) and subsequently Van Dorsselaer et al. (1974), Ourisson et al. (1979) and Rohmer et al. (1980, 1984) have shown diploptene and other hopanoids to be widely distributed among bacteria and cyanobacteria (blue-green algae). Some of these bacteria, such as Acetobacter and Azotobacter, are common microflora in soils.

The triterpenoid, bishomohopanoic acid (**XXXIV**) was also identified in Wells Dam sediments and shows a very similar particle size distribution to that of diploptene (Figure 19). It has been isolated from different kinds of sedimentary organic matter. It is the most abundant individual organic compound in the Messel Shale (Van Dorsselaer et al., 1974) and also an important constituent of very young sediments (Rohmer et al., 1980, 1984). A bacterial origin has been postulated to be the main source of these substances (Van Dorsselaer et al., 1974; Ourisson et al, 1979; Rohmer et al., 1980).

Suggestions of a possible product-precursor relation between diploptene and the MW274 PAH compound **IX** based on structure similarities is not supported by the distributional observations made in this experiment. Enrichment factors (EFoc) for compound **IX** showed no systematic trend with grain size and

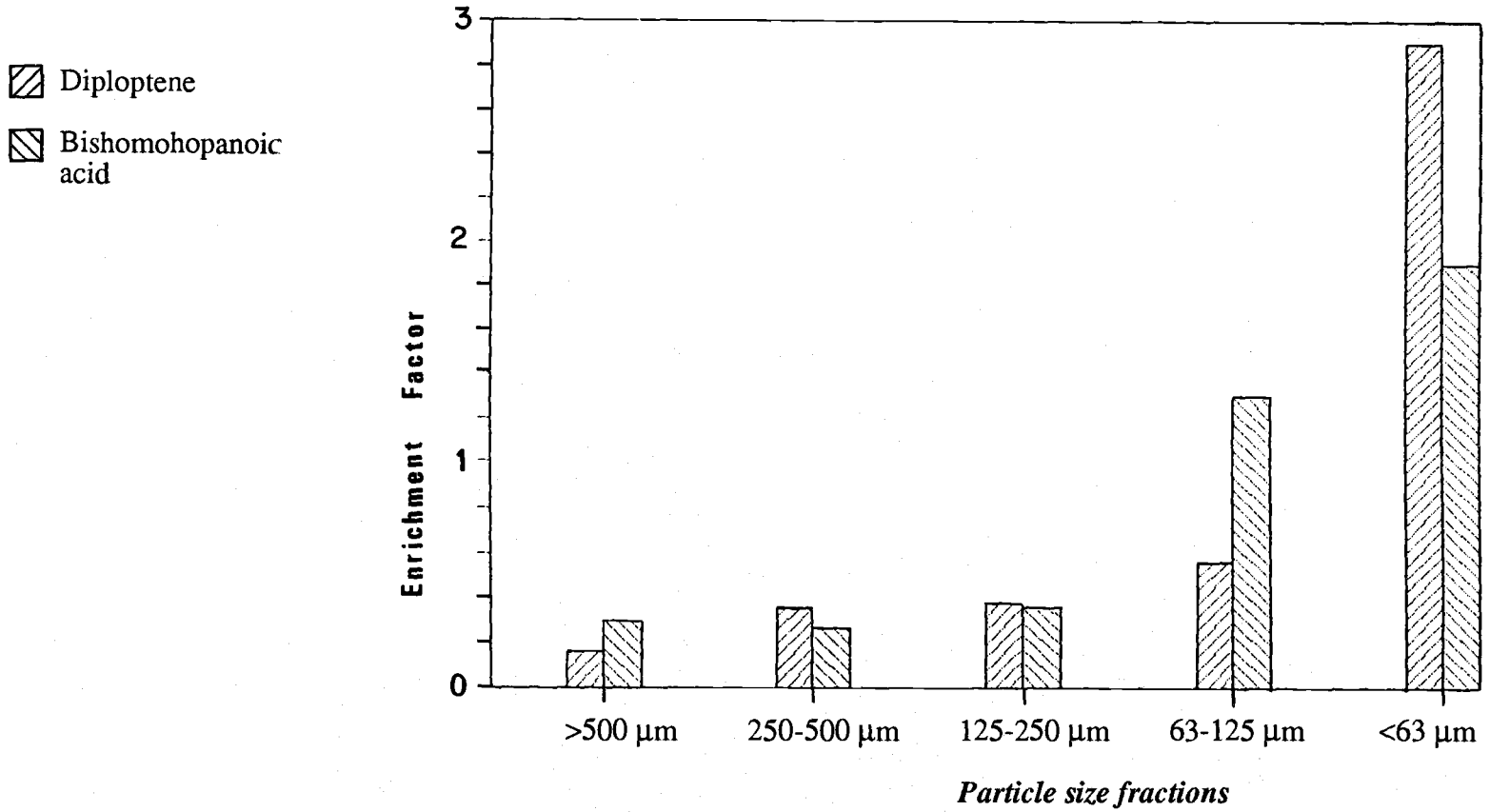


Figure 19. Enrichment factors (EFoc) for diploptene (XXXIII) and bishomohopanoic acid (XXXIV) in Wells Dam particle size fractionated sediments (Experiments1 and 2).

averaged 1.04 ±0.53 in the five fractions (Table 14). Diploptene on the other hand, displayed a clear increase in the finer size fractions (Table 14).

Table 14. Enrichment factors (EFoc)(1) for MW274 PAH compound **IX**, diploptene (**XXXIII**) and bishomohopanoic acid (**XXXIV**) in Wells Dam particle size fractionated sediments.

particle size	MW274(2) (**IX**)	Diploptene (**XXXIII**)	Bishomohopanoic acid (**XXXIV**)
>500 µm	0.6;1.4	0.16	0.29
250-500 µm	1.0;1.0	0.35	0.26
125-250 µm	0.4;1.4	0.37	0.36
63-125 µm	0.6;1.4	0.56	1.3
<63 µm	2.1;0.5	2.9	1.9

(1): Enrichment factor (EFoc) is defined as the ratio of concentration (/g OC) in a given fraction to the reconstructed bulk.
(2): duplicates (see Table 5).

These hopanoids probably remain as molecular fossils of past microbial activity in soils. These compounds have been entrapped in the humic matrix of soil particles and stabilized from destruction. The systematic enrichment (EFoc) of diploptene and bishomohopanoic acid in fine sediments suggests the presence of soil as a major contributor to organic matter increases in the finest grain size particles.

SUMMARY AND CONCLUSIONS

The particle size and density fractionation of Wells Dam sediments has shown that the natural-occurring MW274 PAH are enriched in the coarse and "light" density particle fractions of sediment. The distribution of the different MW274 PAH isomers in these sediments and other Recent sediments suggest these compounds are associated with specific particles within the sedimentary fractions and independent one from each other.

Coarse and "light" fractions, rich in organic carbon, contain a predominance of vascular plant debris. The C/N and $\delta^{13}C$ data is consistent with a predominance of organic matter derived from terrestrial plants in all fractions. The predominance of long chain n-aliphatic homologous series and high CPI (C_{20}-C_{30}) values in the coarse and "light" fractions further support the presence of large amounts of vascular plant detritus in all fractions. Microscopic observation of the coarse and "light" fractions show no evidence of coal particles, potential fossil contributors of MW274 PAH to Recent sediments (Barrick et al., 1984). The MW274 PAH series appears to be of recent diagenetic origin.

Lignin analyses show that plant detritus is mostly derived from woody gymnosperm tissues with a relative increase of non-woody angiosperm tissue in the finest size particles. Nearby areas in the basin with higher contents of woody gymnosperm tissue are devoid of the MW274 PAH compounds. It is postulated here that the MW274 PAH series is probably associated with the detritus from angiosperm plants which appear less abundant compared to gymnosperms in these sedimentary records. Values of (Ac/Al)v indicate little alteration of the lignin phenols supporting the idea of a major contribution of relatively undegraded vascular plant debris within the total organic carbon of these sediments. Although the degree of degradation appears to increase as particle size decreases.

3-Oxygenated pentacyclic triterpenoids were identified as ketone, alcohol and carboxylic acid derivatives in all fractions of Wells Dam sediments. These compounds appear most highly enriched in the coarse and "light" >63 μm

sedimentary fractions. The presence of ursolic, oleanolic and betulinic acids and α-amyrin compounds have been confirmed based on mass spectral and GC retention time comparison with authentic standards. Other possible 3-oxytriterpenoids present in these sediments are: ß-amyrin, ß-amyrenone, lupan-3-one and friedelan-3-one. The series of pentacyclic triterpenoids show similar enrichments to the MW274 PAH structures **X** and **XI** in the >63 μm particles suggesting a common association with vascular plant detritus. Enrichment factors for the MW274 PAH **IX** show no systematic trend with grain size and therefore a different source material compared to the other two PAH isomers is postulated.

Possible transformation of 3-hydroxy-17-carboxy pentacyclic triterpenoids into other types of 3-oxytriterpenoids was observed between replicate analysis of Wells Dam sediments. This fact indicates the instability of these compounds under laboratory conditions and their continuous alteration over time largely to non PAH products. The MW274 PAH compounds seem to be formed only under specialized environmental circumstances.

Diploptene, a possible precursor for compound **IX**, is present in all particle fractions but shows a distinct distribution unrelated to this structurally similar PAH. Diploptene does not seem to be intrinsic to vascular plant debris but rather associated with soil microorganisms responsible for degrading such debris. Soil organic matter is most enriched in the finest size fractions. The striking similarities in enrichments of diploptene with $_{b}$ishomohopanoic acid suggest these two compounds are both associated with the same sources; namely, soil microbes.

Future studies should include the analysis of sediments and vascular plant tissues present in the areas where active diagenetic transformations may be occurring. Examination of locations where a different proportion of the MW274 PAH compounds are present and other 3-oxytriterpenoids are present and identified will help to establish a more definitive product-precursor relationship between the PAH and their precursors.

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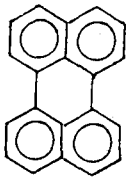
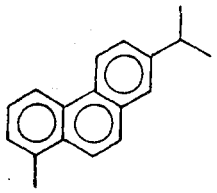
APPENDICES

APPENDIX 1

Chemical Structures Cited



I. Perylene



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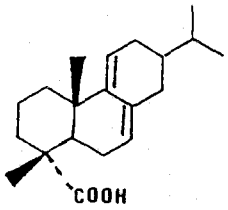
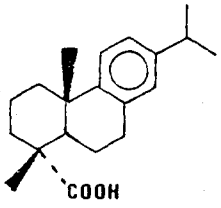
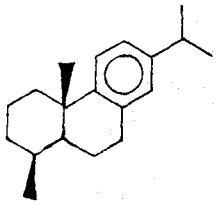


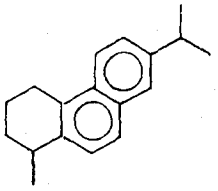
III. Abietic acid



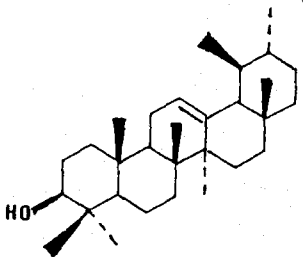
IV. Dehydroabietic acid



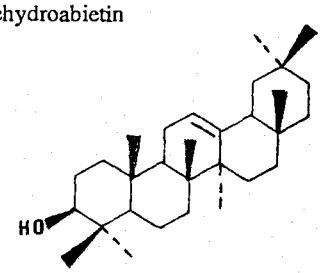
V. Dehydroabietin



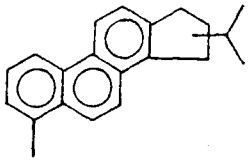
VI. Tetrahydroretene



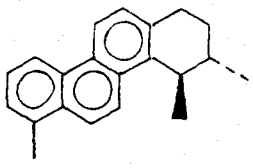
VII. α-amyrin



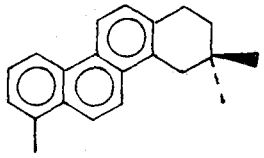
VIII. ß-amyrin



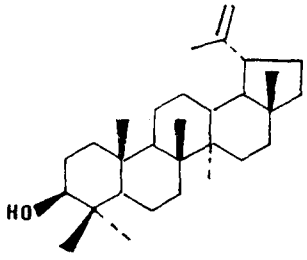
IX. 1-methyl,isopropyl-7,8-cyclopenteno phenanthrene



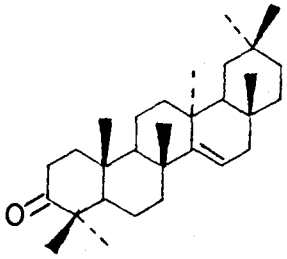
X. 3,4,7-trimethyl-1,2,3,4-tetra hydrochrysene



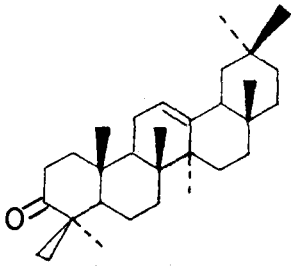
XI. 3,3,7-trimethyl-1,2,3,4-tetra hydrochrysene



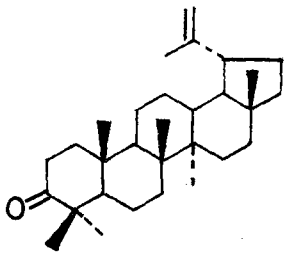
XII. Lupeol



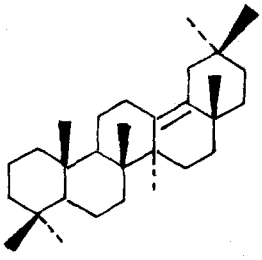
XIII. Taraxerone



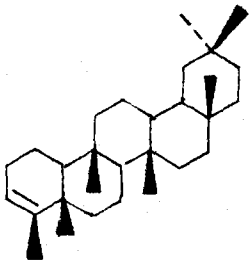
XIV. ß-amyrenone



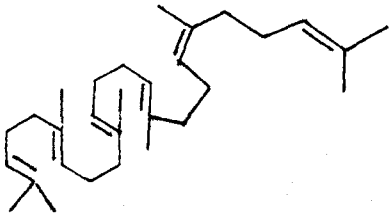
XV. Lupenone



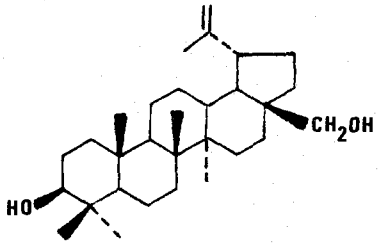
XVI. Olean-13(18)ene



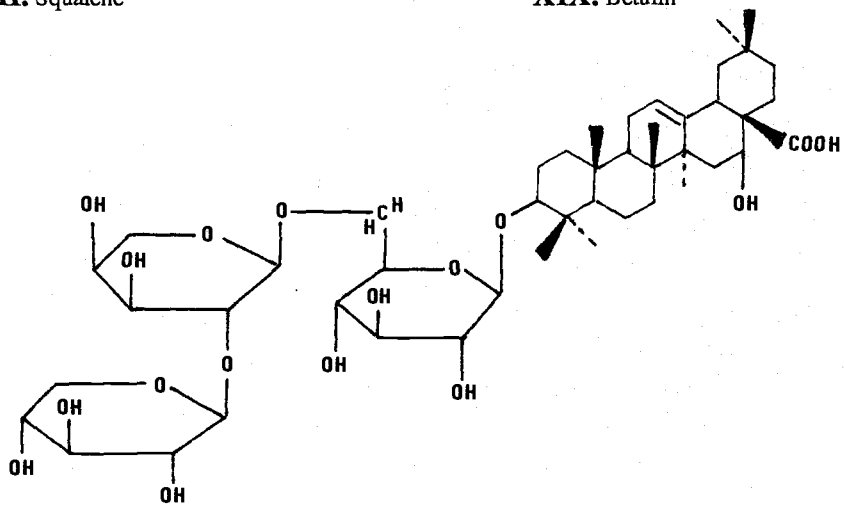
XVII. Friedel-3ene



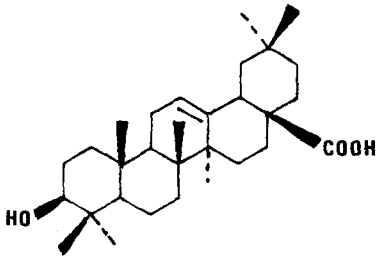
XVIII. Squalene



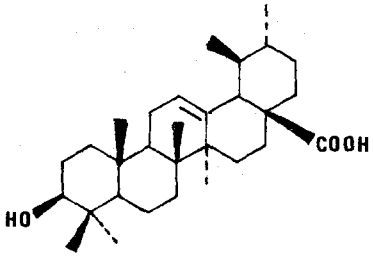
XIX. Betulin



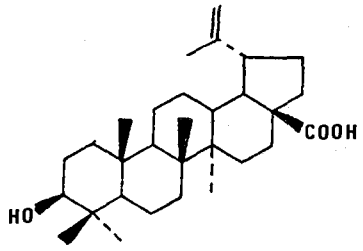
XX. Prosapogenin



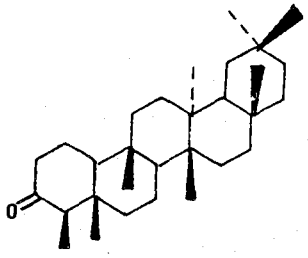
XXI. Oleanolic acid



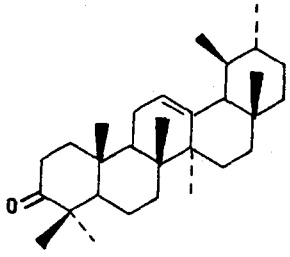
XXII. Ursolic acid



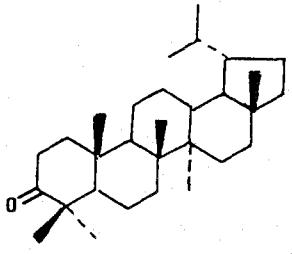
XXIII. Betulinic acid



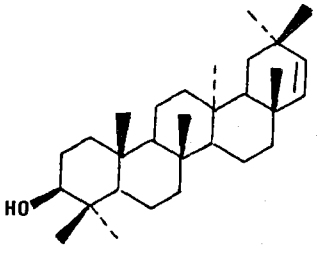
XXIV. Fredelin or friedelan-3one



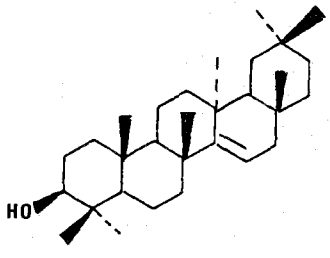
XXV. α-amyrenone



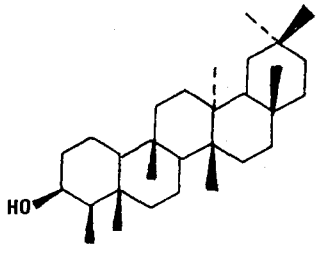
XXVI. Lupan-3one



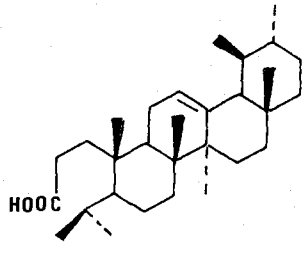
XXVII. τ-taraxerol



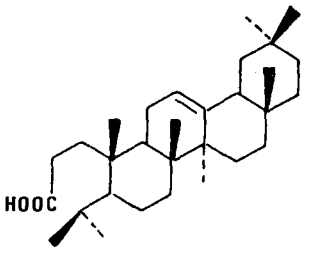
XXVIII. Taraxerol



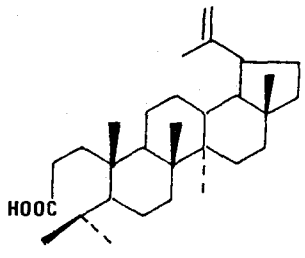
XXIX. Friedelinol



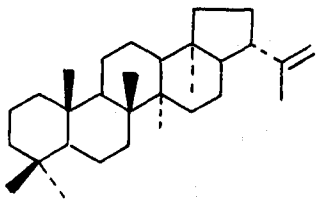
XXX. 3,4-Seco-ursol-3-oic acid



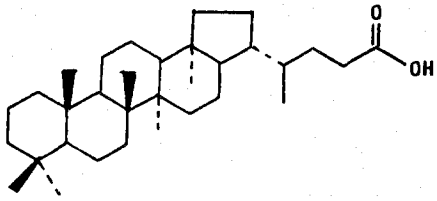
XXXI. 3,4-Seco-olean-3-oic acid



XXXII. 3,4-Seco-lupen-3-oic acid



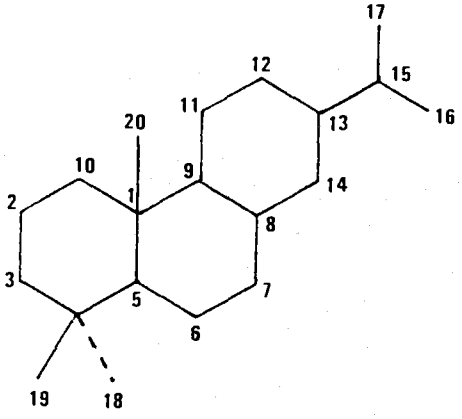
XXXIII. Diploptene



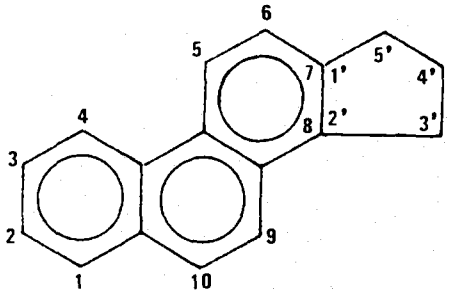
XXXIV. Bishomohopanoic acid

APPENDIX 2

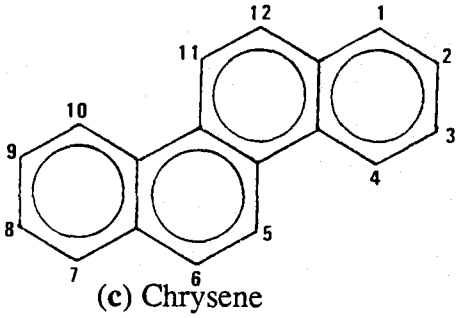
Numbering Conventions for Major Carbon Skeletons
Discussed in this Study



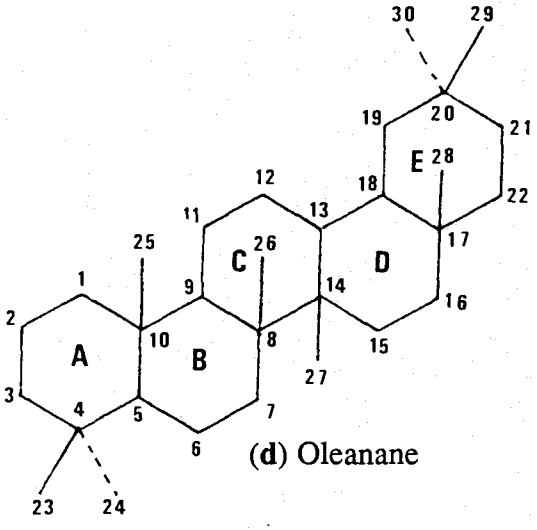
(a) Abietane



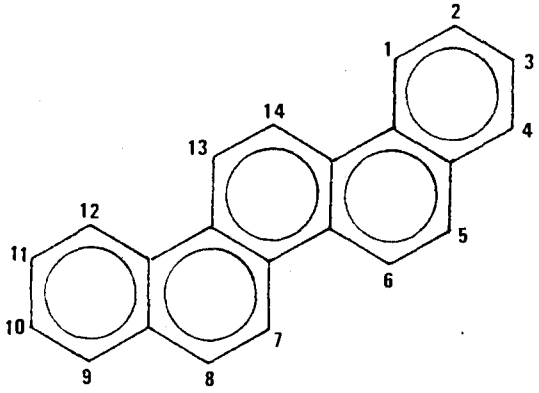
(b) 7,8-cyclopentenophenanthrene



(c) Chrysene



(d) Oleanane



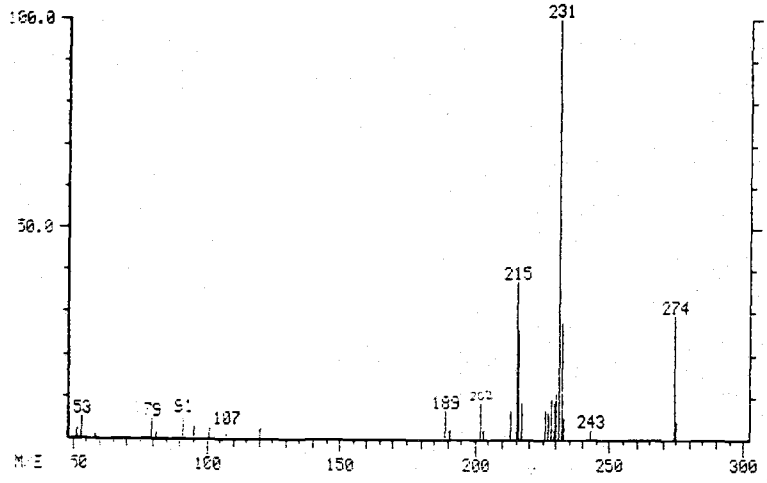
(e) Picene

APPENDIX 3

Mass Spectra of Compounds Cited in this Study

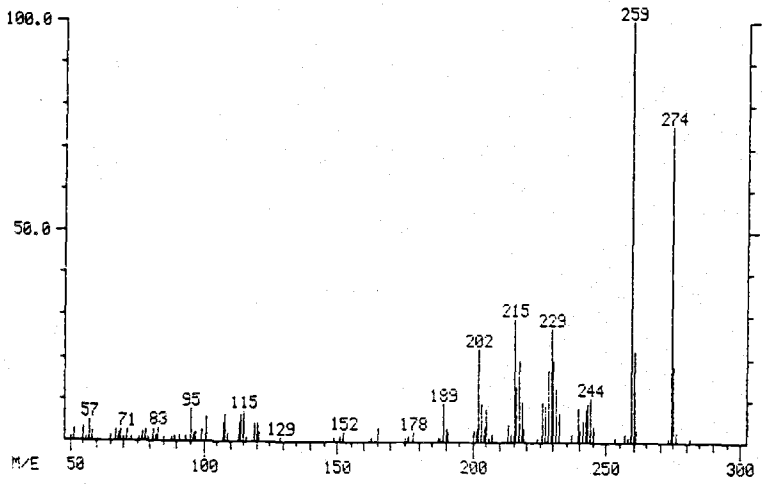
Compound **IX**

1-methyl, isopropyl-
7,8-cyclopentenophenanthrene



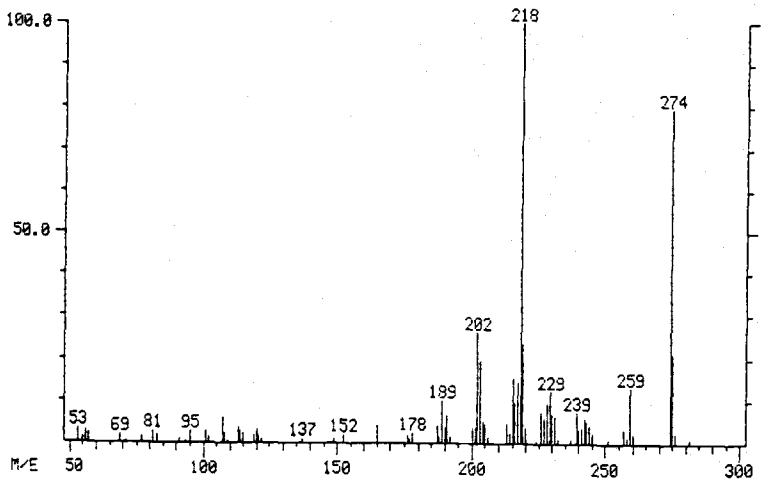
Compound **X**

3,4,7 trimethyl-
1,2,3,4-tetrahydrochrysene



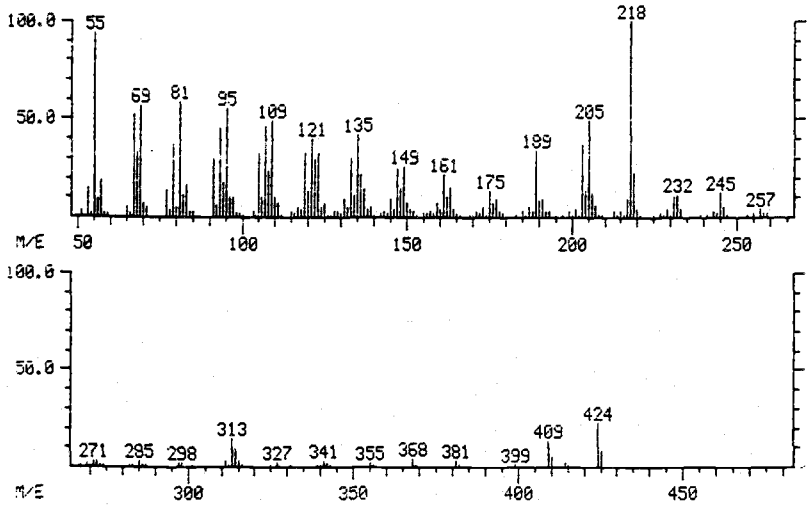
Compound **XI**

3,3,7 trimethyl-
1,2,3,4-tetrahydrochrysene



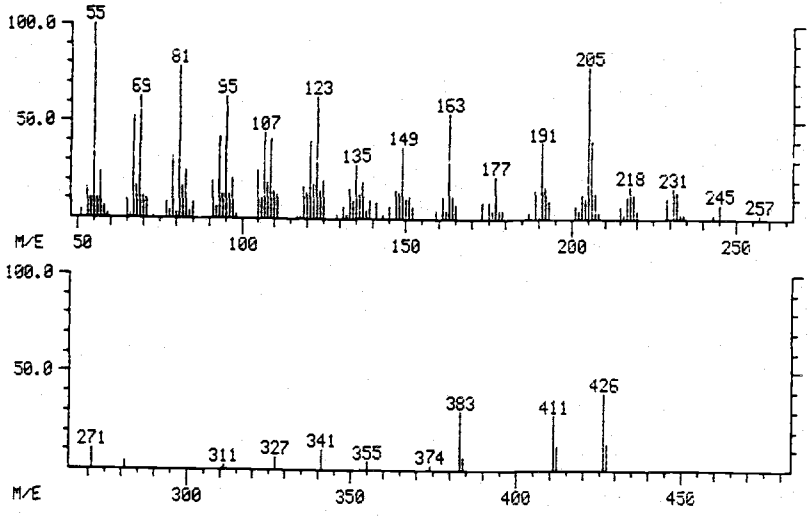
Compound **XIV**

ß-amyrenone



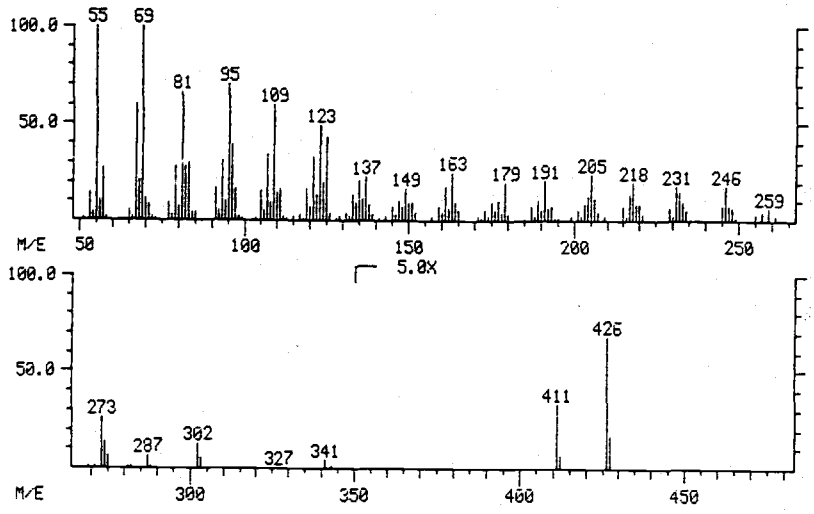
Compound **XXVI**

Lupan-3-one



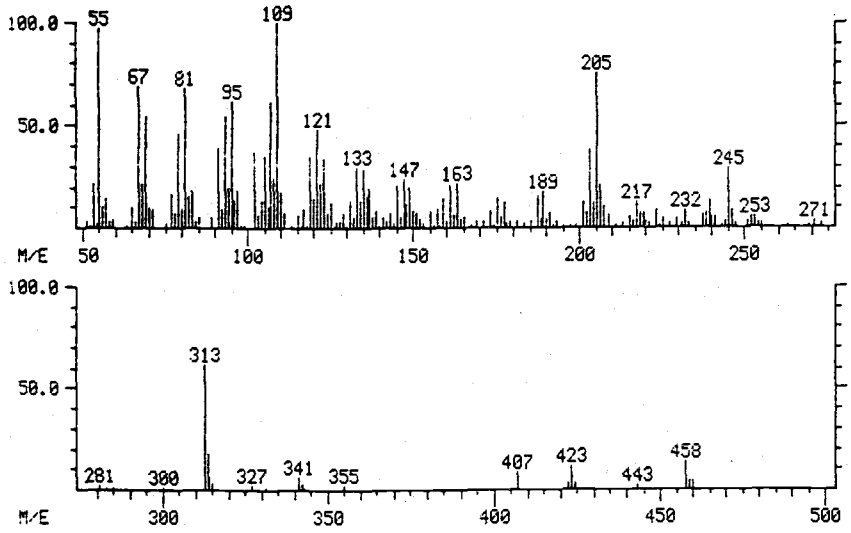
Compound **XXIV**

Friedelan-3-one



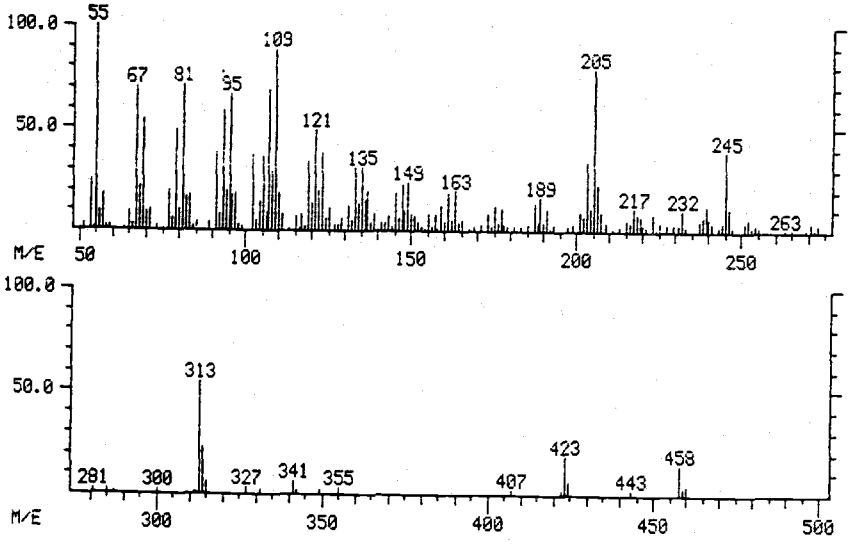
Fraction **L-4**

unidentified peak **a4**



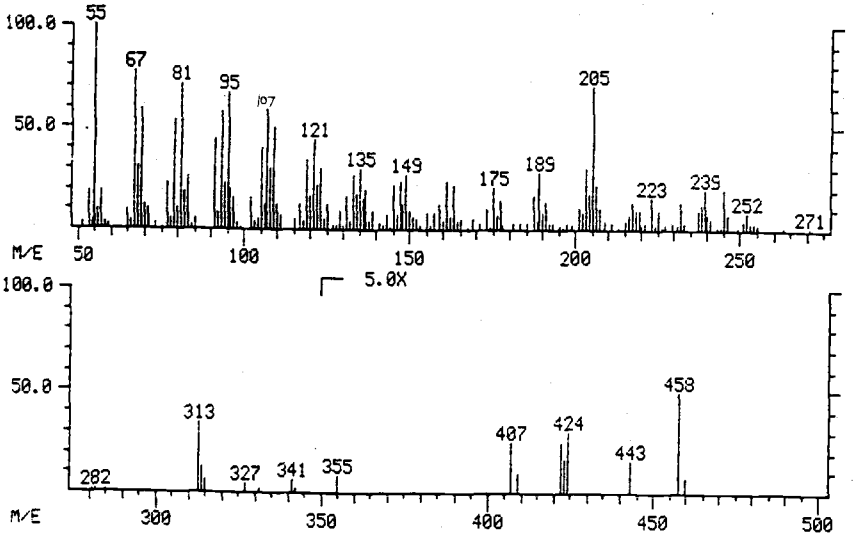
Fraction **L-4**

unidentified peak **a5**



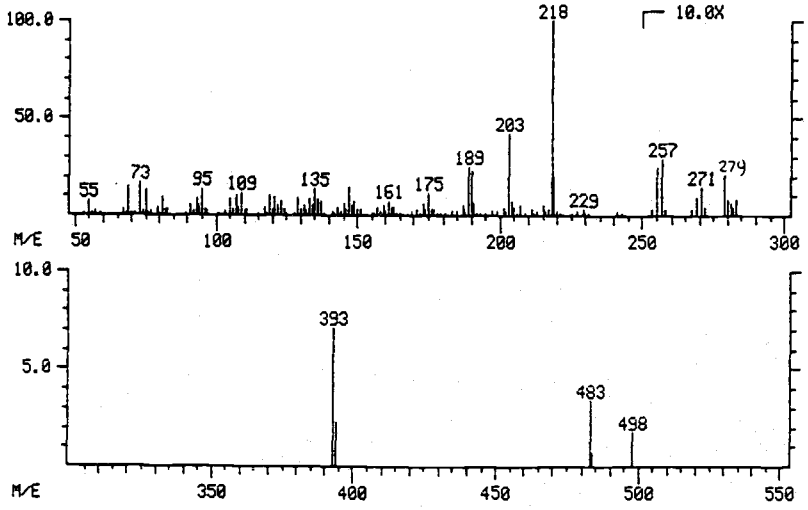
Fraction **L-4**

unidentified peak **a6**



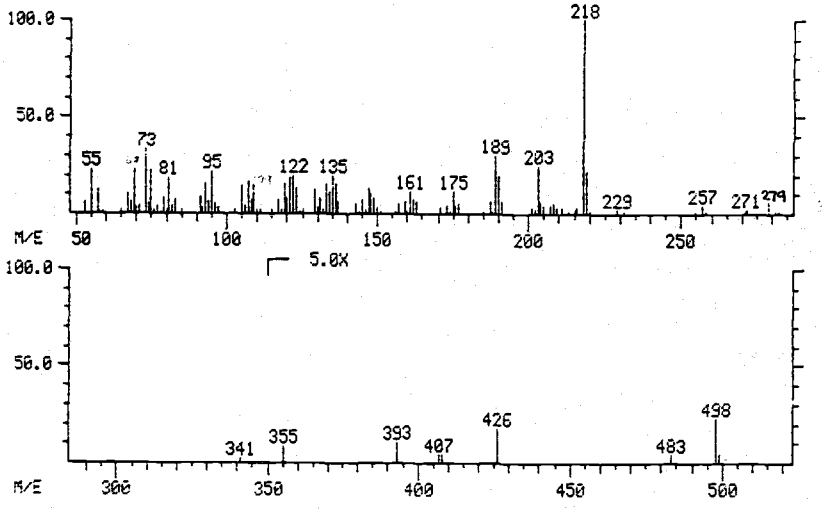
Compound **VIII**

ß-amyrin



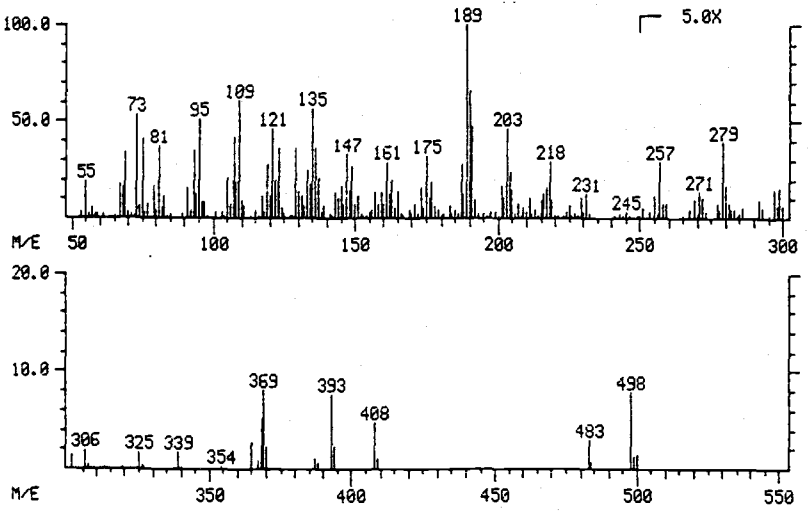
Compound **VII**

α-amyrin



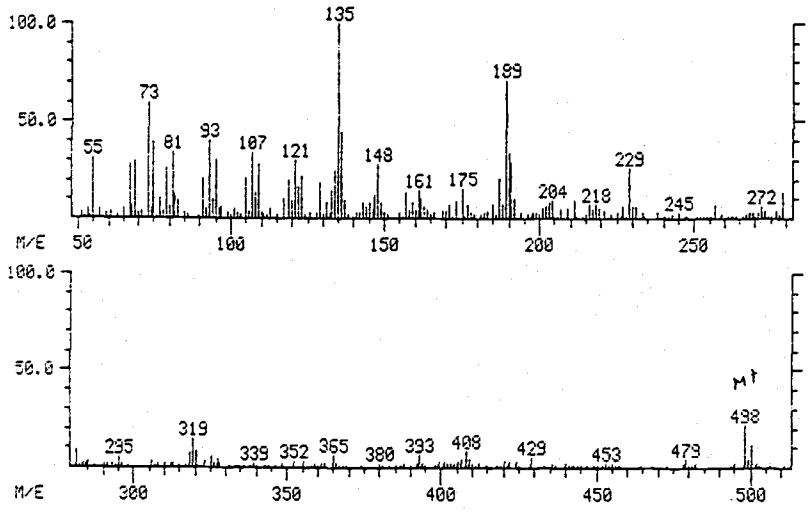
Compound **XXVIII**

Taraxerol ?



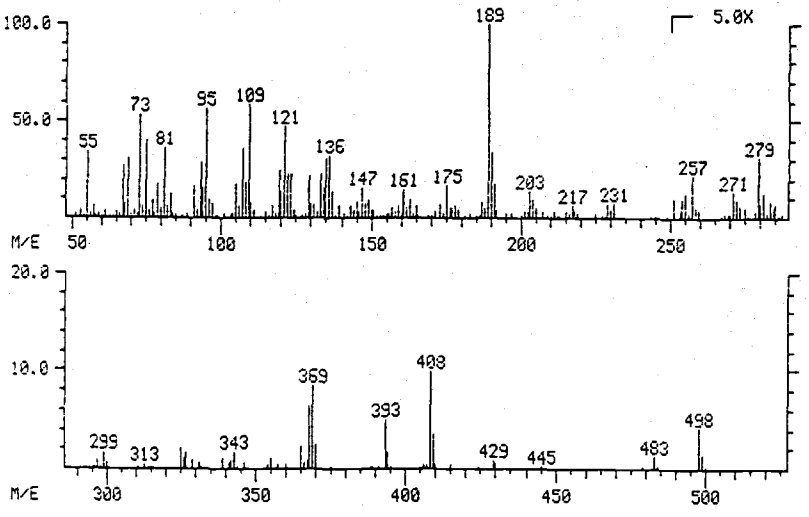
Compound **XII**

authentic standard
Lupeol



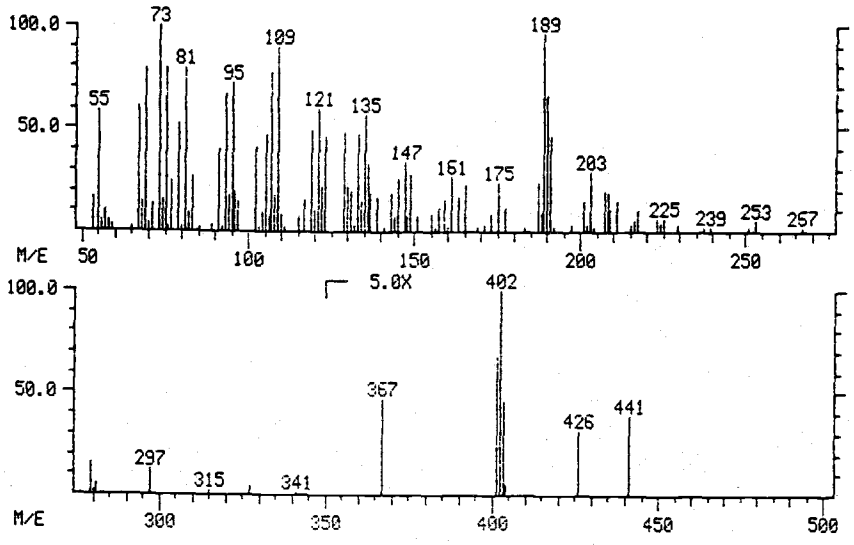
Compound **XXVII**

authentic standard
τ-taraxerol



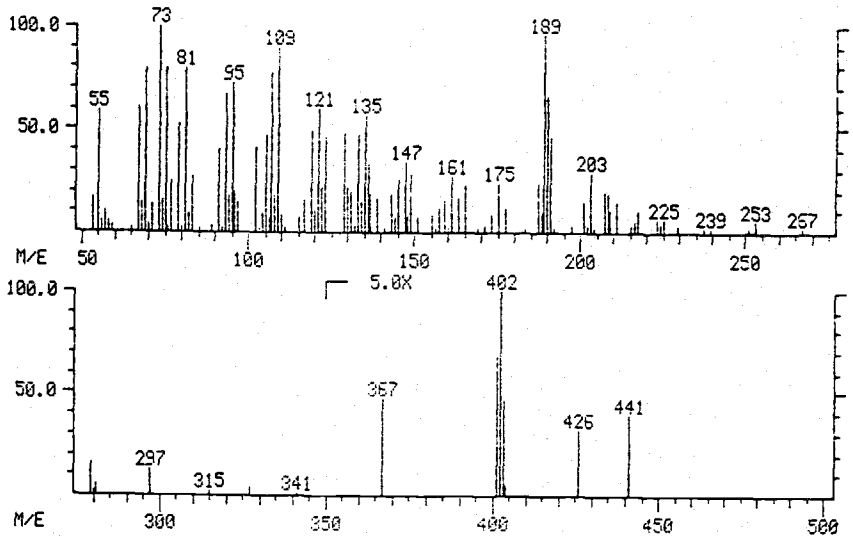
Fraction **L-5**

unidentified peak **b4**



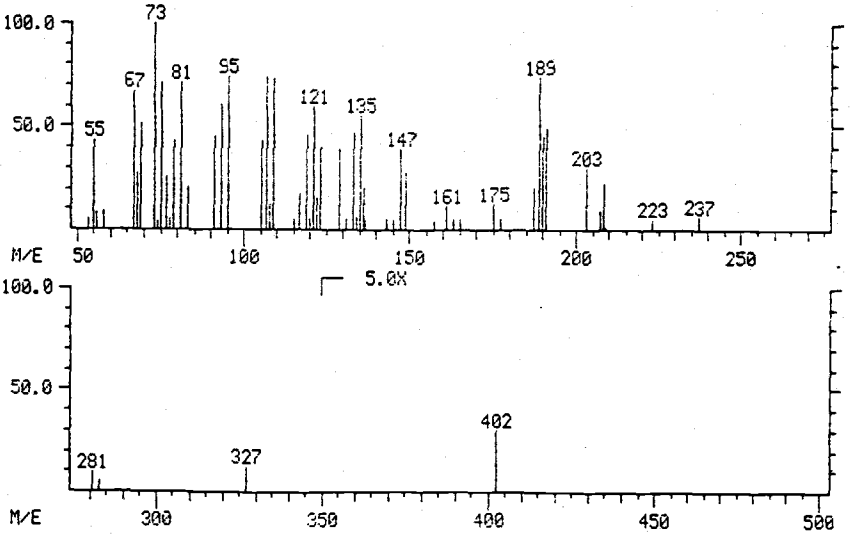
Fraction **L-5**

unidentified peak **b5**



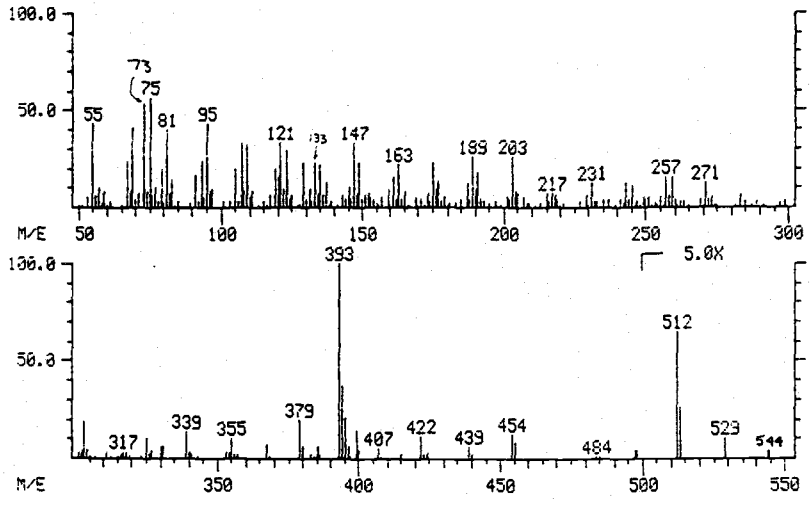
Fraction **L-5**

unidentified peak **b6**



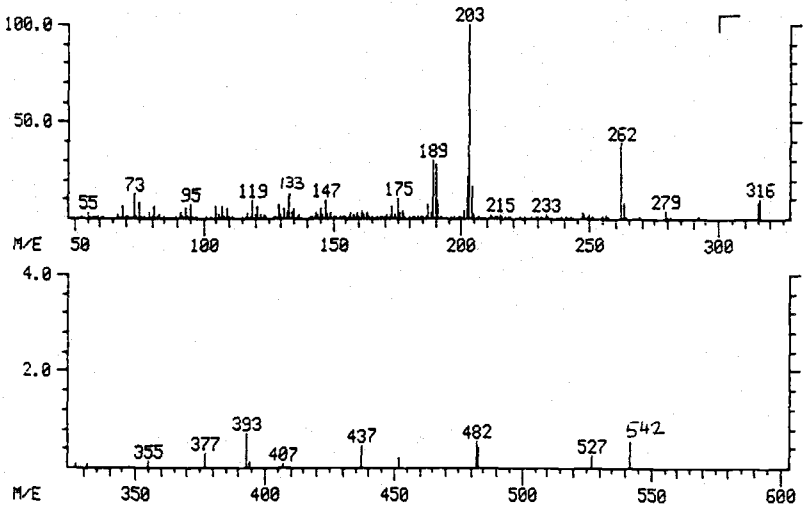
Fraction **S:7-2**

unidentified peak **c1**



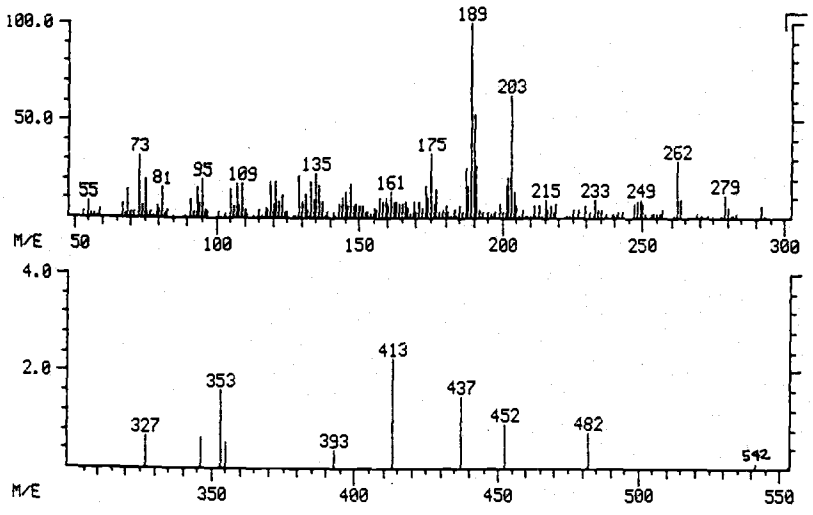
Compound **XXI**

Oleanolic acid peak **c2**



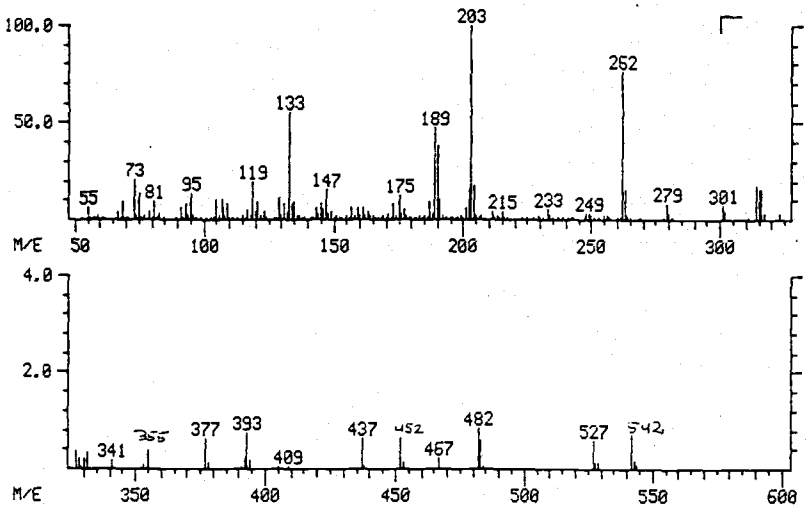
Compound **XXIII**

Betulinic acid
peak **c3**



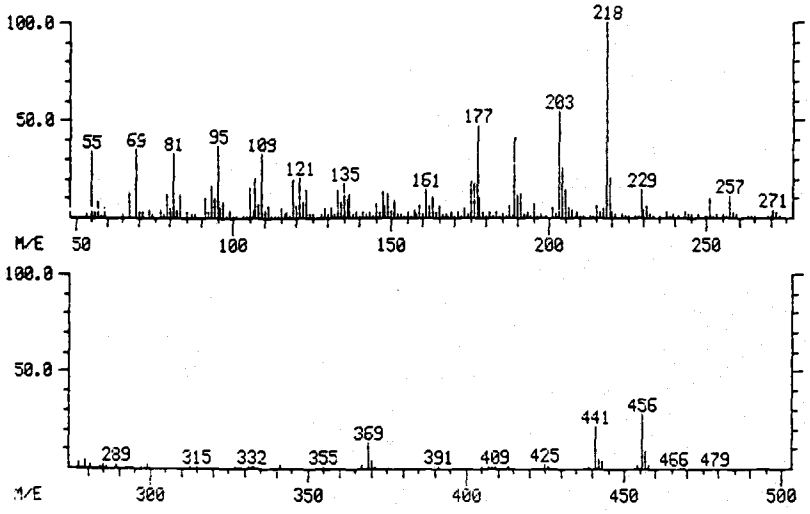
Compound **XXII**

Ursolic acid
peak **c4**



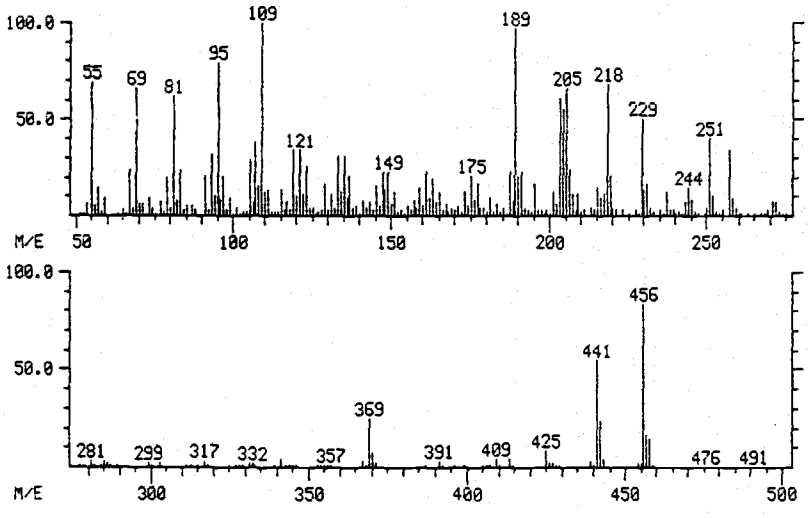
Compound **XXXI**

3,4-Seco-olean-3-oic acid



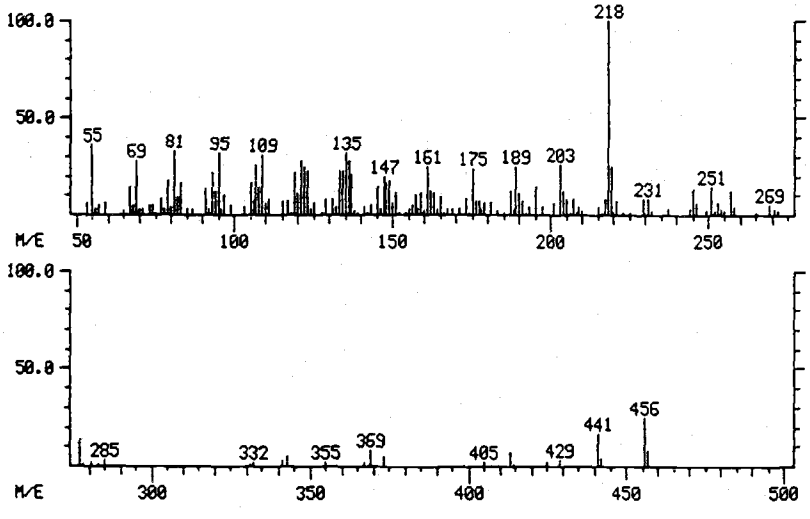
Compound **XXXII**

3,4-Seco-lupen-3-oic acid



Compound **XXX**

3,4-Seco-ursol-3-oic acid



APPENDIX 4

Distribution of retene (I), dehydroabietic acid (IV) and tetrahydroretene (IV) in Wells Dam bulk, particle size and density fractionated sediments

	I		IV		VI	
	[ng/g wt]	[µg/g OC]	[ng/g wt]	[µg/g OC]	[ng/g wt]	[µg/g OC]
bulk 1(1)	75	17.1	59	13.5		
bulk 2	127	31.8	170	42.3		
size frac.-1						
>500 µm	1190	5.1	3330	14.3		
250-500 µm	23	5.9	65	16.2		
125-250 µm	14	6.0	29	12.0		
63-125 µm	28	8.9	35	11.1		
<63 µm	66	14.7	64	14.1		
reconstructed	35	8.4	56	13.7		
size frac.-2						
>500 µm	10200	34.7	68700	233	1410	4.8
250-500 µm	55	11.4	122	25.4	9.1	1.9
125-250 µm	45	21.5	27.5	13.1	4.8	2.3
63-125 µm	41	14.1	37.5	12.9	5.3	1.8
<63 µm	68	9.2	9.7	1.3	32	4.4
reconstructed	84	16.8	259	51.7	17	3.4
density frac.						
>63 µm "light"	3010	11.2				
>63 µm "heavy"	17	14.1				
silt(2)	54	-				
clay(2)	280	-				
reconstructed(1)	48	11.0				

(1): average total %OC obtained for bulk 2, size 1 and size 2 experiments (0.44%) is used to calculate concentrations for bulk 1 and reconstructed bulk for the density experiment.

(2): %OC not measured in silt and clay fractions.

APPENDIX 5

Distribution of long chain homologous series of n-alkanes, n-aldehydes, n-alcohols and n-acids in Wells Dam bulk, particle size and density fractionated sediments

n-alkanes (ng)	bulk1	bulk2	size frac.-1 <63 (μm)	63-125 (μm)	125-250 (μm)	250-500 (μm)	>500 (μm)
17	632	383	732	114	<82	n.d.	64.5
18	210	160	220	<82	<82	<83	91.2
19	376	212	393	106	102	<91	105
20	476	213	336	132	<100	<83	78.9
21	788	3853	760	207	175	273	878
22	554	326	608	185	205	131	170
23	2220	1242	1935	564	465	439	855
24	872	730	183	421	274	286	355
25	6233	3801	3893	1575	1298	1333	2512
26	1196	621	881	446	244	256	375
27	21631	9869	10264	4441	4065	4672	8685
28	1690	752	1116	431	384	322	624
29	19683	10311	11878	4133	4300	4504	9305
30	814	528	962	256	200	158	192
31	8683	3508	6515	1905	1444	1240	1177
32	489	286	574	<123	n.d.	<124	83.6
33	1825	880	1830	477	344	295	201
34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
35	660	276	n.d.	n.d.	n.d.	n.d.	n.d.

n-alkanes (ng)	size frac.-2 <63 (μm)	63-125 (μm)	125-250 (μm)	250-500 (μm)	>500 (μm)	density frac. >63 μm "light"	"heavy"	<63 μm silt	clay
17	612	101	101	99	89	102	48	232	48
18	193	58	63	61	67	62	59	107	25
19	299	92	94	91	103	127	n.d.	145	38
20	340	107	116	91	309	134	n.d.	145	38
21	1323	426	544	691	1896	1134	n.d.	652	119
22	363	168	212	153	236	280	163	216	103
23	1080	519	576	677	738	984	300	667	174
24	569	248	339	261	352	526	231	303	122
25	2127	1462	1706	1696	2289	3650	597	1419	252
26	428	279	356	287	426	779	218	305	105
27	5361	4155	5049	5597	9219	779	218	305	105
28	536	300	329	346	830	1492	264	381	128
29	6086	4484	5441	5791	13805	17037	2061	4914	822
30	443	200	235	166	232	436	222	285	128
31	2739	1767	1790	1373	1503	3208	1153	2527	384
32	120	66	88	64	67	208	n.d.	196	75
33	822	445	421	300	245	765	363	735	131
34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
35	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

note: Weight and %OC of bulk, particle size and density fractionated sediments given in Table 7 (page 41).

n-aldehydes (ng)	bulk1	bulk2	size frac.-1 <63 (μm)	63-125 (μm)	125-250 (μm)	250-500 (μm)	>500 (μm)
20	260	144	390	123	n.d.	n.d.	54
21	373	226	433	126	133	131	119
22	866	538	1009	284	140	237	225
23	768	547	916	258	139	250	240
24	1605	1243	1352	459	255	284	592
25	795	592	132	149	141	212	626
26	4584	3936	1459	686	694	1304	3536
27	715	618	622	201	146	144	173
28	4848	4580	3581	1389	726	883	1942
29	963	832	741	169	148	156	359
30	2857	4297	1692	561	334	479	1794

n-aldehydes	size frac.-2					density frac.			
(ng)	<63 (μm)	63-125 (μm)	125-250 (μm)	250-500 (μm)	>500 (μm)	>63 μm "light"	"heavy"	<63 μm silt	clay
20	206	59	59	34	n.d.	50	30	73	22
21	252	96	124	72	266	63	53	98	31
22	606	252	322	145	582	139	132	234	59
23	596	229	276	140	449	154	96	248	n.d.
24	829	460	540	216	927	219	96	341	52
25	403	228	266	103	328	102	76	2160	34
26	2120	1458	1740	642	2675	826	180	946	110
27	409	437	442	125	709	228	52	144	43
28	2222	1770	1879	765	4423	1863	204	1074	97
29	437	171	240	78	962	604	84	202	n.d.
30	1456	743	1133	581	7461	5399	128	509	67

n-alcohols (ng)	bulk1	bulk2	size frac.-1 <63 (μm)	63-125 (μm)	125-250 (μm)	250-500 (μm)	>500 (μm)
20	2200	274	2420	434	776	379	491
21	307	90	523	613	494	422	478
22	9253	1681	9310	2414	3250	2506	3816
23	712	257	1470	284	280	194	373
24	12150	2512	12048	3683	3419	2997	2171
25	725	619	1506	534	368	294	324
26	22031	5822	20642	6903	5424	5027	7628
27	1532	648	2055	596	472	357	451
28	8799	4206	16129	5245	4536	4026	8778
29	424	1060	1630	740	446	593	700
30	8082	2436	8292	2808	2371	1890	4021

n-alcohols	size frac.-2					density frac.			
(ng)	<63 (μm)	63-125 (μm)	125-250 (μm)	250-500 (μm)	>500 (μm)	>63 μm "light"	"heavy"	<63 μm silt	clay
20	1074	408	417	391	797	508	107	376	87
21	383	118	110	129	192	137	54	126	33
22	5696	3368	2966	2170	5836	3807	674	1925	582
23	946	336	304	187	493	462	67	334	69
24	8190	4087	4023	2229	4853	4260	541	3082	383
25	1045	472	461	227	566	652	67	589	69
26	14497	7321	7326	3651	6545	8111	916	6350	616
27	1454	593	598	252	703	939	119	565	53
28	10092	6445	6035	2769	5837	7562	867	4900	605
29	1078	598	606	224	951	2147	156	660	62
30	4933	3326	3185	1222	4392	5843	525	2734	319

n-acids (ng)	bulk1	bulk2	size frac.-1 <63 (μm)	63-125 (μm)	125-250 (μm)	250-500 (μm)	>500 (μm)
20	3979	2867	1237	1144	980	805	1148
21	1510	1715	382	455	365	295	426
22	9156	10958	2129	2712	2146	2122	3369
23	3579	5032	1400	1099	788	749	1029
24	9156	14440	2435	2481	1698	1930	2926
25	1326	2558	401	700	200	309	446
26	3654	6896	1830	979	631	768	1213
27	556	995	240	328	70	50	170
28	3089	4744	1560	732	541	742	1014
29	896	566	119	n.d.	n.d.	50	150
30	1361	2491	698	n.d.	n.d.	309	418

n-acids	size frac.-2					density frac.			
(ng)	<63 (μm)	63-125 (μm)	125-250 (μm)	250-500 (μm)	>500 (μm)	>63 μm "light"	"heavy"	<63 μm silt	clay
20	1339	767	823	812	1584	1012	275	552	n.d.
21	741	638	382	388	783	1275	88	297	n.d.
22	5183	2799	2750	3408	5792	6085	767	1535	88
23	2469	1409	1277	976	2126	3040	253	690	n.d.
24	9908	3945	3603	4712	7238	9907	728	1777	108
25	1383	805	732	394	811	1867	55	311	n.d.
26	6791	2507	2034	2784	4982	5658	317	853	84
27	560	335	353	147	338	1052	49	115	n.d.
28	6585	2723	1893	3567	7165	7230	191	493	n.d.
29	341	191	236	138	311	1758	n.d.	62	n.d.
30	4510	1890	1018	2404	8690	10261	81	245	n.d.

APPENDIX 6

Distribution of diploptene (**XXXIII**) and bishomohopanoic acid (**XXXIV**) in Wells Dam bulk, particle size and density fractionated sediments

	wt%	%OC	XXXIII (ng)	XXXIV (ng)
bulk 1	100	n.m.	2037	5026
bulk 2	100	0.40	1070	3937
size frac.-1				
>500 µm	0.34	23.25	103	n.d.
250-500 µm	18.75	0.40	221	n.d.
125-250 µm	38.74	0.24	292	n.d.
63-125 µm	18.73	0.31	275	n.d.
<63 µm	23.45	0.45	2583	n.d.
reconstructed	100	0.41	3474	
size frac.-2				
>500 µm	0.31	29.47	n.d.	461
250-500 µm	19.11	0.48	n.d.	410
125-250 µm	37.63	0.21	n.d.	477
63-125 µm	18.29	0.29	n.d.	1142
<63 µm	24.65	0.74	n.d.	5986
reconstructed	100	0.50		8476
density frac.				
>63 µm "light"	0.72	26.87	103	n.d.
>63 µm "heavy"	75.88	0.12	178	n.d.
silt	22.82	n.m.	609	2044
clay	0.50	n.m.	n.d.	n.d.
reconstructed	100		890	

n.m.: not measured
n.d.: not detected