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On the occurrence and origins of hopanoids in the Chesapeake Bay (Maryland, virginia)

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DEDICATION

This dissertation is dedicated to my mother in appreciation for her continued encouragement and support throughout my education.

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ABSTRACT

Analyses of surface sediment samples from the Chesapeake Bay and sediment cores from the James and the Potomac River showed that pentacyclic triterpenoids of the hopanoid skeleton were ubiquitously present in all samples. The hopanoids have been identified and quantified by gas chromatographic retention data obtained on SE-52 stationary phase, and mass spectral comparisons with the branched/cyclic fraction of a Lorraine coal extract as well as published data from authentic standards. Hopanoid acids are of extended 17β(H),21β(H)-structure, ranging from C_{31} to C_{33}. The 17β(H),21β(H)-bishomohopanoic acid (C_{32}) is always the major acid found in the samples. All acids were present as a single epimer (22R). The 17α(H),21β(H)-hopane series is predominant in all the samples, with lesser amounts of the 17β(H),21β(H)-hopane series and some hopenes also present. The extended 17α(H),21β(H)-hopanes ($>C_{31}$) are found as mixtures of the 22R and 22S diastereomers. This indicates that there is a significant input of fossil hopanes into the Chesapeake Bay. Generally, high concentrations were found at river-mouth stations and in the northern Bay areas associated with industrial activities and intense urban development. These results are consistent with an anthropogenic source for the aromatic hydrocarbons present in the samples.

Fossil hopanes appear to derive from a variety of sources including coal, crude oil, refined motor oil, asphalt particles and street dust. A comparison of hopanoid distributions in Bay sediments with possible source materials suggests that motor oil, asphalt particles and street dust are potentially important sources of fossil hopanes to the Bay. There is evidence that the input of hopanoids to surface soils is related to highway usage. These source materials and the associated fossil hopanes are reaching the Bay via natural and urban runoff, either directly or via river transport. Final accumulation in Bay sediments is evident from the elevated concentrations of fossil hopanes at river-mouth stations. These accumulations indicate that rivers are important sources of fossil hopanes to the Bay.

An anomaly in the S/R ratio of the 17α(H),21β(H)-homohopane (C_{31}) in many sediment samples from the Bay is interpreted as evidence of a microbially induced

isomerization of 17β(H),21β(H)-C_{31} hopane (R) to 17α(H),21β(H)-C_{31} hopane (R). Indirect evidence suggests that decarboxylation of hopanoid acids to form 17β(H),21β(H)-hopanes of one carbon number less than the parent compound is a major reaction in the early diagenesis of hopanoids. Isomerization of 17β(H),21β(H)-C_{32} hopanoid acid to 17α(H),21β(H)-C_{32} hopanoid acid is suspected to have occurred in previously deposited estuarine sediments.

ON THE OCCURRENCE AND ORIGINS

OF

HOPANOIDS IN THE CHESAPEAKE BAY

I. INTRODUCTION

Pentacyclic triterpenoids of the hopanoid skeleton (Figure 1) are widely distributed both in the biosphere and the geosphere. While the 17β(H),21β(H) hopane series is exclusively present in biological material, especially in microorganisms, the 17α(H),21β(H) hopane series has not yet been detected in living organisms. During the diagenesis process, the natural 17β(H),21β(H) hopanes undergo isomerization at position 17 and 22 to yield the more stable 17α(H),21β(H) hopanes and mixtures of 22R and 22S diastereomers (Ensminger et al., 1973; Van Dorsselaer et al., 1975). However, the biological precursors are often preserved in fossil materials. This has allowed them to be used as biological markers. A detailed analysis of hopanoids in a sediment may reveal to some extent the origin of the sedimentary organic matter, as well as chemical reactions occurring in the depositional environment.

The Chesapeake Bay, including its tributary tidal drainage area, represents the largest estuarine system on the U.S. Atlantic Coast. Like other estuaries, it is being rapidly filled with sediments; sediments from rivers, from shore erosion, from the remains of organisms that inhabit it, and from the sea. A few studies have been conducted on distributions of hydrocarbons in sediments from the Chesapeake Bay and its subestuaries (Walker et al., 1975 a&b; DeVoe and Voll, 1980; Voudrias, 1981; Bieri et al., 1982 a&b; Lu, 1982). The only study in the Bay that includes hopanoids in sediments was made by Voudrias (1981) who studied the impact of marinas on three Eastern Virginia estuarine

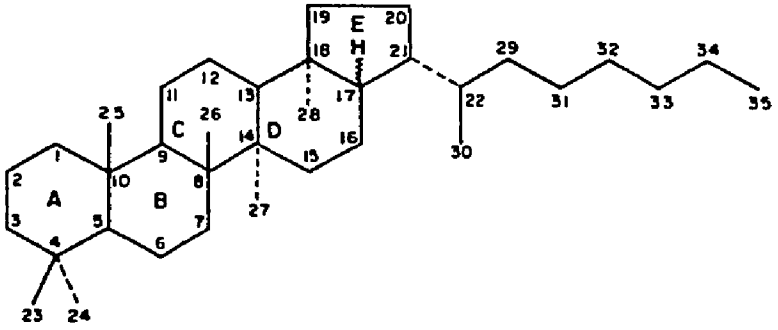


Figure 1. Designations of carbon atoms and rings for the hopanoid structure. The H at position 17 cis to the side-chain at position 21 for the 17α(H), 21β(H)-hopane series and trans for the 17β(H), 21β(H)-hopane series.

creeks. He reported that sediments from creeks with marinas contained significantly higher levels of aromatic and aliphatic hydrocarbons than did the control samples. All the samples analyzed by GC/MS have shown evidence of 17α(H),21β(H) hopanes, which were attributed to petroleum contamination from the marinas. No attempt was made to identify the biogenic hopanes in the samples.

A great deal of work has been done at the University of Strasbourg, France and the University of Bristol, England regarding the organic geochemistry of hopanoid molecules. These have resulted in a detailed knowledge of early and late diagenetic pathways of these molecules. However, very little is known concerning sources and distributions of hopanoids in the Chesapeake Bay. This is unfortunate as hopanoids are known to be widespread and geochemically interesting components of organic matter in nature (Ourisson et al., 1979 and references cited therein). The objectives of this research were:

1. To investigate the types and the abundances of hopanoid molecules in surface sediments from the Chesapeake Bay. An attempt was made to characterize the compounds qualitatively by combined gas chromatography/mass spectrometry (GC/MS) as well as quantitatively by gas chromatography.

2. To assess the possible sources of fossil hopanes in Bay sediments and their probable modes of transportation into the Bay. This information will contribute to our understanding of the extent of petroleum pollution in the Chesapeake Bay and its tributaries.

3. To investigate evidence for hopanoid transformations in the estuarine environment of the Chesapeake Bay. The study of hopanoid molecules in the sediments of the Chesapeake Bay should provide new

information on early recent organic diagenesis in our estuarine system, if it exists.

II. LITERATURE REVIEW

The review reported in this chapter covers occurrences of hopaniods in the biosphere and geosphere, including some diagenetic mechanisms. Details on the probable biochemical function and on the evolutionary significance of hopanoids have been discussed by Ourisson et al. (1979). Structures of compounds are given in Figure A1 in the Appendix.

A. Biosynthesis of Pentacyclic Triterpenoids

Biosynthesis of aliphatic hydrocarbons by both terrestrial and marine organisms is a well established fact. Chibnall and co-workers reported as early as 1934 that terrestrial plants produce almost exclusively n-paraffins with an odd number of carbon atoms. Eglinton and Hamilton (1963) found that odd-numbered paraffins in the range from C_{23} to C_{33} predominated in plants. In many instances, only one or two odd-numbered paraffins were dominant. Alcohols and acids occurred with mainly even-numbered carbon chains from C_{24} to C_{36}. Even-numbered paraffin chains and odd-numbered acids and alcohols were also present, but in very small amounts (Waldron et al., 1961). Marine phytoplankton synthesize smaller odd-carbon paraffins of length C_{15}, C_{17}, C_{19} and C_{21} (Clark and Blumer, 1967), whereas in marsh grasses carbon chains from C_{21} to C_{29} predominate (Burns and Teal, 1973). Bacterial lipids have

been found to contain equal amounts of even-numbered carbon and odd-numbered carbon n-alkanes (Davis, 1968), although in several species the C_{17} chain length is dominant (Blumer, 1967; Han and Calvin, 1969).

Several branched alkanes have also been found in organisms, i.e., pristane in some fishes and zooplankton (Blumer, 1967), phytoplankton and other microorganisms (Calvin, 1969); phytane in bacteria (Han and Calvin, 1969).

Squalene (I) is the most common triterpene formed by polymerization of the 5-carbon isoprene unit. Squalene occurs in most marine organisms, although only the higher trophic levels appear to accumulate it in substantial amounts. For example, copepod lipids contain 0.01 percent squalene, whereas the lipids of basking shark livers may contain more than 20 percent squalene (Blumer, 1967).

The role of squalene as an intermediate in the biosynthesis of steroids and triterpenoids has been described in the biochemical literature. Cyclization of squalene epoxide (II) in biological system leads to the production of triterpenoid alcohols, ketones and sterols; cyclization of squalene itself can also occur and produce triterpenoid alkenes or alcohols, i.e., diplopterol (XIV), hopene-b (III), fernene (IV), and tetrahymanol (V) (Nes and McKean, 1977). Bird et al. (1971 a&b) showed the formation of sterols, squalene and hop-22(29)-ene (diploptene) (XV) by the bacterium Methylococcus capsulatus grown in a mineral salt medium with methane as sole carbon source.

The biosynthesis of bactereohopane polyols (XXXII) has been proposed by Forster et al. (1973) to proceed in two different ways: (a) the cyclization of an acyclic C_{35} precursor followed by hydroxylations or (b) the attachment of a C_5 building block to a preformed pentacyclic system either at position 22 or 29. Position 22 was preferred since 22-hydroxyhopane (XIV) was simultaneously present in the lipid of Acetobacter xylinum (Forster et al., 1973). However, Rohmer (cited in Van Dorsselear, 1975) has shown that the C_5 building block is attached to the hopane structure at position 29 rather than position 22 as proposed by Forster et al. (1973).

The work to date has clearly revealed that several ferns, some mosses and lichens, and many microorganisms belonging to widely separated taxonomic groups, contain these triterpene derivatives (Ourisson et al., 1979; and references cited therein). In general, animals appear to be unable to synthesize triterpenes, but manufacture instead tetracyclic systems like cholesterol, derived from lanosterol. Tetrahymanol, isolated by Mallory et al. (1963) from a protozoan, has been related to gammacerane (VI) and noted as the first pentacyclic triterpenoid alcohol isolated from an animal.

So far, the major hopanoids present in living organisms have been identified as either 3-oxygenated hopanoids or 3-desoxy hopanoid alkenes and alcohols. Saturated hydrocarbons of the hopane skeleton have not been found at present in significant amounts in biological material, except in the case of the thermophilic bacteria Bacillus acidocaldarius where traces of 17β(H),21β(H) hopane (XVIII-c) and of a corresponding C_{31} hopane (XIX-a) have been detected (De Rosa et al., 1973).

B. Geochemical Occurrence of Polycyclic Triterpenoids

Polycyclic triterpanes have been found in recent and ancient sediments by many investigators. More than 100 different derivatives of hopane have been isolated from geological samples of varied origin (Ourisson et al., 1979). Ruhemann and Raud, in 1932, extracted Central German brown coal, and isolated the triterpenes betulin (VII) and allobetulin (VIII) plus other compounds with thirty carbon atoms that remained unidentified. A detailed review of earlier works dealing with triterpenes in fossil material was published by Bergmann (1963).

A report on the isolation and mass spectral identification of individual pentacyclic triterpenoid compounds was first published by Burlingame et al. (1965), who isolated the C_{27}, C_{28} and C_{29} steranes and a triterpane (lupane) (IX) from extracts of the Colorado Green River Formation of Eocene age (50 million years). A year later, Hills and Whitehead (1966) isolated three pentacyclic triterpanes from a Nigerian crude oil. Eglinton and co-workers (Murphy et al., 1967; Hennerson et al., 1968) documented the presence of cholestane (XII-a), ergostane (XII-b), stigmastane (XII-c), gammacerane, hopane and lupane in the Green River shale. The triterpene alcohol, isoarborinol (X), has been found in the Messel oil shale of Germany (Albrecht and Ourisson, 1969). Mattern et al. (1970) reported the presence of arborinone and friedelin (XI) in Messel shale. Kimble et al. (1974) detected an intact triterpene, hop-17(21)-ene (XVII) and a minor isomer of 30-normoretane in Messel oil shale.

Arpino (1973) studied the Bouxwiller and Me´nat Eocene shales and reported the presence of a 17β(H),21β(H) hopane series ranging from C_{27}

to C_{32} (XVIII a-c; XIX a&b), with only one diastereomer at position 22 in the higher homologs. Ensminger et al. (1975) reported that the major triterpenoids in Messel oil shale were 17β(H),21β(H) C_{29} and C_{31}. Hop-17(21)-ene (XVII) was the main triterpene found in the alkene fraction. They also identified C_{27}, C_{29}, C_{30} and C_{31} compounds of the 17α(H),21β(H) hopane series (XXII a-c; XXIII-a) as major components of the branched and cyclic alkane fraction in the Lorraine coal field (France). The 17α(H),21β(H) C_{31} hopane (XXIII-a) was present as an approximately 1:1 mixture of 22R and 22S diastereomers.

In most geological samples and crude oils, degraded triterpanes containing 27 and 29, but not 28 carbon atoms were found to be present (Kimble et al., 1974; Ensminger et al., 1975; Arpino, 1973). This has been rationalized in analogy to the observed absence of a C_{17}-isoprenoid hydrocarbon in fossil fuels because of the low probability of cleavage of two carbon-carbon bonds located at the same carbon atom (C-22) of the hopane side chain. Recently, a C_{28} member of the hopane family, 17α(H),18α(H),21β(H)-28,30-bisnorhopane (XXIV) was reported in a Monterey shale from offshore Santa Barbara, California and in a related California crude oil (Seifert et al., 1978), in a core sample from DSDP Leg 47A (Cornford et al., 1979), and in a North Sea core sample and various North Sea crude oils (Grantham et al., 1980).

The majority of triterpanes usually are less abundant than the n-alkanes in the same samples (Van Dorsselaer et al., 1974). The C_{28}-triterpane (XXIV), however, is an exception in so far as it has been found in relatively large amounts in the Monterey shale (Seifert et al.,

1978) and in the core sample from DSDP Leg 47A (Cornford et al., 1979). Bjoroy and Rullkotter (1980) reported a C_{27}-triterpane, tentatively identified as 25,28,30-trisnormoretane (XXV), as the major saturated hydrocarbon of a Jurassic shale from the Norwegian continental shelf. The unusually high concentrations of these two nuclear demethylated triterpanes and their uneven distributions are beleived to relate to specific diagenetic conditions (Rullkotter et al., 1982).

A C_{32} hopanoid acid (XXVII-b) was discovered in large quantities in Messel oil shale (Ensminger et al., 1973). These authors also identified bisnorhopanoic acid (C_{28}) (XXVI-a) in a 120 million-year-old marine cretaceous shale from near the coast of Gaboon. Van Dorsselaer (1975) examined several recent and ancient sediments for hopanoids. Various triterpanes of the 17α(H),21β(H)-hopane series (C_{27}-C_{35}) (XXII a-c; XXIII a-e) and of the βα-hopane (moretane) series (C_{29}-C_{31}) (XX a&b; XXI-a) were identified. In addition, 17β(H),21β(H) C_{31}, C_{32} and C_{33} hopanoid acids (XXVII a-c) were also found to be present. Ensminger (1977) reported the presence of ββ-C_{31} to C_{35} hopanoid acids, with C_{32} as the major acid in sedimentary rocks from the Toarcian Basin, France. In addition, βα- and αβ-C_{31} to C_{33} hopanoid acids (XXIX a-c; XXXI a-c) were also identified.

Schmitter et al. (1978) were the first to report the presence of hopanoid acids in crude oils. The αβ-C_{32} hopanoid acid was found to be the most abundant acid in all the samples examined. The absence of the

C_{29} hopanoid acid parallels the absence or the low abundance of the C_{28}-hopane in most crude oils. Both probably have the same origin in crude oils (Schmitter et al., 1978). In correspondence to hopanoid hydrocarbons, ββ - and βα-isomers of hopanoid acids decrease in abundance while the αβ-isomers increase with increasing maturity of sediments (Ensminger, 1977; Schmitter et al., 1978). However, isomerization of the acids, at positions 17, 21 and 22, is much slower than that of the hydrocarbons (Ensminger, 1977).

In addition to being identified in mature sediments and crude oils, hopanoid hydrocarbons and acids were also found in lake sediments (Eglinton et al., 1975; Brooks et al., 1976; Romher et al., 1980), estuarine sediments (Barrick and Hedges, 1981; Voudrias, 1981), coastal sediments (Bieri et al., 1978; Simoneit et al., 1979; Simoneit and Kaplan, 1980), and deep sea sediments (Dastillung, 1976; Simoneit, 1977&1978; Boon et al., 1978; Barnes et al., 1979; Brassell et al., 1980; Schorno, 1980). Besides acids and hydrocarbons, triterpenoid alcohols, ketones and aldehydes of the extended hopane series have also been identified in many recent and ancient sediments (Dastillung, 1976; Dastillung et al., 1980 a&b).

C. Diagenesis of Triterpenoids

1. Bacterial diagenesis:

Several reviews have been written on the biological degradation of hydrocarbons. Generally , isoalkanes are degraded at lower rates than the corresponding n-alkanes, and cycloalkanes are degraded slower than

isoalkanes (Jobson et al., 1972). However, Hill (1969) reported rapid growth of bacteria on cycloalkanes.

Rubinstein et al. (1977) reported that steranes and terpanes were unaffected by bacteria in laboratory degradation experiments and in natural fossil fuels. Reed (1977) studied the molecular composition of weathered petroleum and found that cycloalkanes were not readily utilized by petroleum-oxidizing microbes. Tricyclic and tetracyclic alkylated diterpanes were also unaffected whereas the series from norhopane through tetrakisnorhopane was interpreted to be the product of the bacterial degradation of the hopane molecule. Seifert and Moldowan (1979) found diasteranes and tricyclic terpanes to survive heavy biodegradation so well they can be used as source fingerprints in biodegraded oils. The transformation of hopanes to Ring A/B demethylated hopanes was observed by Rullkotter and Wendisch (1982) who found that degradation of the 17α(H),21β(H) hopane series proceeded from the high molecular weight end, whereas in steranes the degradation started from the lower molecular weight end (C_{27}). Preferential degradation of the 20R-diastereomers was observed for the C_{28} and C_{29} steranes. The opening of ring C of 17α(H),21β(H) hopanes as a further biodegradation step was also reported (Rullkotter and Wendisch, 1982).

Microbial methylation reactions were found to occur at C-24 in the side chain during the biosynthesis of sterols (Lederer, 1969). Thus, Ensminger et al. (1972) proposed that the C_{31} triterpane present in Messel oil shale originally entered the sediment as a 3-desoxy C_{30} triterpene (e.g.diploptene) and was subsequently microbiologically

methylated. On the other hand, triterpanes with less carbon atoms were thought to be the degradation products of C_{30} precursors.

Modifications of hopanoids by biological oxidation or dehydrogenation have been reported for lichens (Huneck, 1971), fungi (Tsuda et al., 1967; Ejiri and Shibata, 1974) and for bacteria (Rohmer and Curisson, 1976). Ensminger (1974) suggested that hopanoid acids in geological samples resulted from a microbial oxidation of natural triterpenoids such as diploptene.

The recent discovery of bacteriohopane polyols has led Rohmer et al. (1980) to postulate that these compounds are the precursors for all hopanoids from C_{30} to C_{35} found in recent muds from two small ponds near Strasbourg, France. Degradation of the side-chain and isomerization at C-17, leading to 17α(H),21β(H) hopanoids, were suggested to occur in situ either by microorganisms or by abiotic reactions.

2. Chemical diagenesis:

Chemical diagenesis involves a large number of chemical reactions which are most important in the upper meters of sediment, at temperatures up to about 50 °C. Many of these reactions result in the total destruction of the primary compound, or in the formation of condensed geopolymers (e.g. kerogen, humic and fulvic acids), but some result in slow, subtle changes at the molecular level.

Laboratory studies do not reproduce actual conditions that produce chemical changes in organic matter. In general, temperature is viewed as the most influential variable while many other parameters are held constant. Thus, Hoering and Abelson (1962) showed that heating recent

and ancient sediments or the kerogen from sediments at temperatures between 185-400 ^{o}C, produced significant amounts of hydrocarbons similar to petroleum. Jurg and Eisma (1964) thermally altered behenic acid, (C22:0), which was adsorbed on bentonite clay and observed that the major hydrocarbon found was the C_{21} alkane, accompanied by small amounts of other n-alkanes. They suggested that decarboxylation of the acid is the major reaction, but that cracking may occur in addition. Hence, the considerable amounts of fatty acids contributed to sediments may be the source of some of the hydrocarbons abundant in ancient sediments.

Laboratory experiments (Rhead et al., 1971; Steel et al., 1972; Sieskind et al., 1979) in which sterols were heated for varying lengths of time at different temperatures under reducing condition show that the major compounds which were formed were steranes and sterenes corresponding to the original carbon number range of unaltered steroids. Sieskind and co-workers (1979) hypothesized that the transformations are catalysed by superacid sites which are present in kaolinite and montmorillonite. These experiments have demonstrated possible pathways for the conversion of biolipids into hydrocarbons, some of which are widespread in ancient sediments and fossil fuels.

Artificial petroleum generation by pyrolysis of kerogen or bitumen from a sediment at temperatures between 375 to 550 ^{o}C has shown that 17α(H)-C_{27} and 17α(H),21β(H)-C_{29} hopanes increased in concentration at the expense of the C_{30} hopane (Gallegos, 1975; Seifert, 1978). The hopane/moretane and 17α(H)/17β(H)-C_{27} hopane ratios were observed to increase with maturity of the kerogen (Seifert, 1978). This is

consistent with the generally observed loss of 17β(H),21β(H) configuration with increased thermal stress (Van Dorsselaer et al., 1977).

Ensminger (1977) conducted artificial maturation experiments with diploptene adsorbed on clay and reported that diploptene is readily isomerized into various hopenes, e.g., Hop-17(21)-ene and Hop-13(18)-ene (Hopene-II), at room temperature under mild conditions. Reduction of diploptene into ββ-C_{30} hopane was also observed under a hydrogen atmosphere and in the presence of clay minerals (Ensminger, 1977).

Mild oxidation of the polyhydroxyhopane has been shown to generate 17β(H),21β(H)-C_{30}, C_{31} and C_{32} hopanoid acids with the C_{32} acid as the dominant product (Rohmer, cited in Van Dorsselaer, 1975).

Other stereochemical changes have been shown to occur during the maturation process of hopanoids in a sediment (Ensminger et al., 1973; Van Dorsselaer, 1975; Ensminger, 1977). These are:

1) isomerization at position 22 of extended 17α(H),21β(H) hopanes leading to a mixture of 22R and 22S diastereomers.

2) disappearance of the less stable 17β(H),21β(H) hopanes due to isomerization at position 17 or 21 into 17α(H),21β(H) hopanes or 17β(H),21α(H) hopanes.

It was suggested that isomerization reactions of 17β(H) to 17α(H) hopanes are probably controlled by acid sites in kerogen.

III. EXPERIMENTAL METHODS

A. Area of Study

The area under study was the Chesapeake Bay and two of its subestuaries, the James and the Potomac. In general, the salinity of the water in the Bay varies from near zero at the head of the Bay to nearly that of sea water at its mouth. Gravity acting upon the density difference between fresh water and sea water gives rise to a characteristic two-layer estuarine circulation pattern (Schubel et al., 1976). Sources of sediments to the Bay are external, marginal, and internal. On a system wide-basis, the external sources are predominant, and the rivers account for the vast majority of this input. Eight major rivers flow into the Chesapeake Bay. Of these the Susquehanna River is the largest, with a mean yearly discharge that accounts for nearly 50 % of the total freshwater input to the Chesapeake Bay estuarine system, and for more than 85 % of the total freshwater input to the Bay above the mouth of the Potomac (Folger, 1972). The characteristic mode of sediment transport into and within the estuarine portions of the Bay is as suspended load (Schubel, 1976). The distribution and transportation of suspended sediment in the main body of the Bay were discussed by Schubel and Biggs (cited in Schubel, 1976).

The James River is the southernmost major tributary of the Chesapeake Bay and the largest river in Virginia. It is approximately 400 miles in length, with a total drainage area of over 10,000 square miles. The James is tidal from Hampton Roads to Richmond, a distance of

about 100 miles with an average tidal range of 3 feet. The average depth of the tidal portion of the James is approximately 20 feet. The salinity intrusion normally extends upstream to the vicinity of Jamestown Island, about 40 miles from the mouth.

The Potomac, with a long-term average discharge of about 310 cubic meter/sec, is the second largest river entering the Bay, accounting for approximately 19 % of the freshwater input. Colonial Beach is located, on the south shore, about 35 nautical miles upstream from the mouth. The bottom sediments consist of firm muds and clays of moderate to high compaction. The average water depth is 20 feet, and the average salinity at the surface is from 6 to 10 ppt., depending on the season. About 50 % of the total sediment load entering the Potomac comes from the drainage basin above the Point of Rocks (60 miles upstream of Washington D.C.). Direct erosion of the banks of the estuary is considered to be very small (Lippson et al., 1979).

Clay Bank is located on the east shore of the lower York River in Gloucester County, Virginia. The area is scarcely populated with marsh land covering a considerable part of the area. The only major highway in the area is U.S. Route 17. Two asphalt-surfaced roads (Route 614 and Route 616) connect the Clay Bank area to U.S. Route 17. Route 708 is a dirt road that leads to the York River.

B. Sampling Methods and Locations

1. Chesapeake Bay sediments, Md. & Va.: Chesapeake Bay sediment samples were obtained with a Smith-McIntyre grab. The grab samples were collected by Mr. P. deFur in the Fall of 1979, during the second cruise of the VIMS-EPA Chesapeake Bay program for the analysis of toxic substances. Twenty-two subsamples were taken for separate analyses reported in this study. Sediments were immediately placed in wide mouth glass jars with Teflon lined caps and kept in an ice box until return to the laboratory. They were then frozen in the jars and stored until analysis. Location of the sampling stations are shown in Figure 2.

2. James River sediments, Va.: Eight core samples were taken with a modified gravity-type corer having a diameter of 2 inches, in August, 1979. Cores of sediments were obtained up to 70 cm in length. In addition, a six-foot core (VC-14) with a 3-3/8 inch inside diameter corer was obtained from Mr. R. Gammish (James River Maintenance Dredging Demonstration Project). This core was recovered in November 1980 using a vibratory coring device. All cores, contained in their plastic core liners, were stored vertically and immediately sectioned upon returning to the laboratory. The sectioned samples were placed in solvent-rinsed jars and subsequently stored in a freezer until analysis. Location of sampling stations are shown in Figure 3.

3. Potomac River sediments, Va: A piston core was collected in Colonial Beach (CB-002) from the Potomac River by Mr. R. Gammish in 1980 during the VIMS Sand Inventory cruise. Following retrieval, the core was stored vertically until returned to the laboratory for sectioning. The plastic core barrel was split longitudinally and the sediment which had been in contact with the barrel was carefully scraped away with a

Figure 2. Location of sampling stations in the Chesapeake Bay estuary.

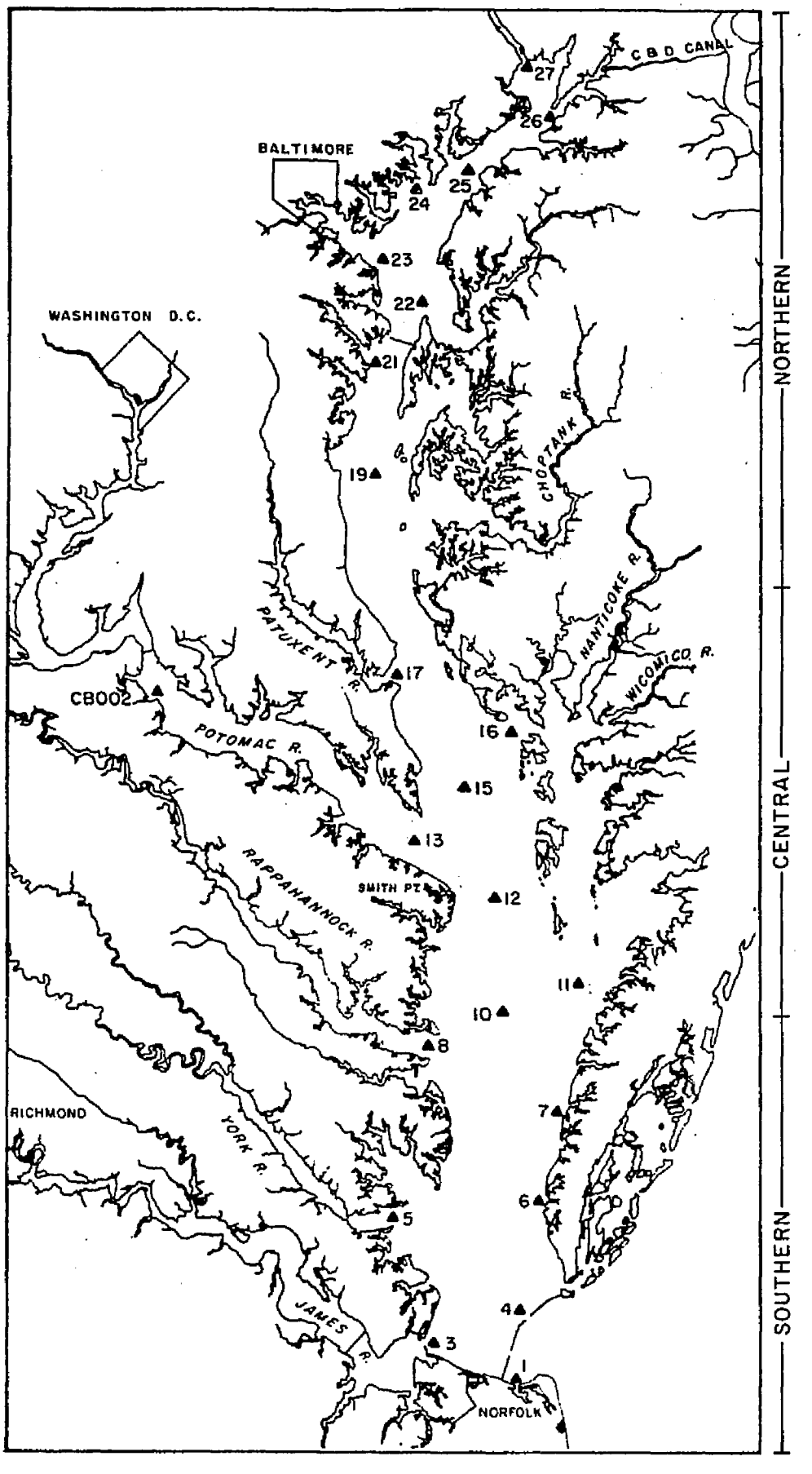
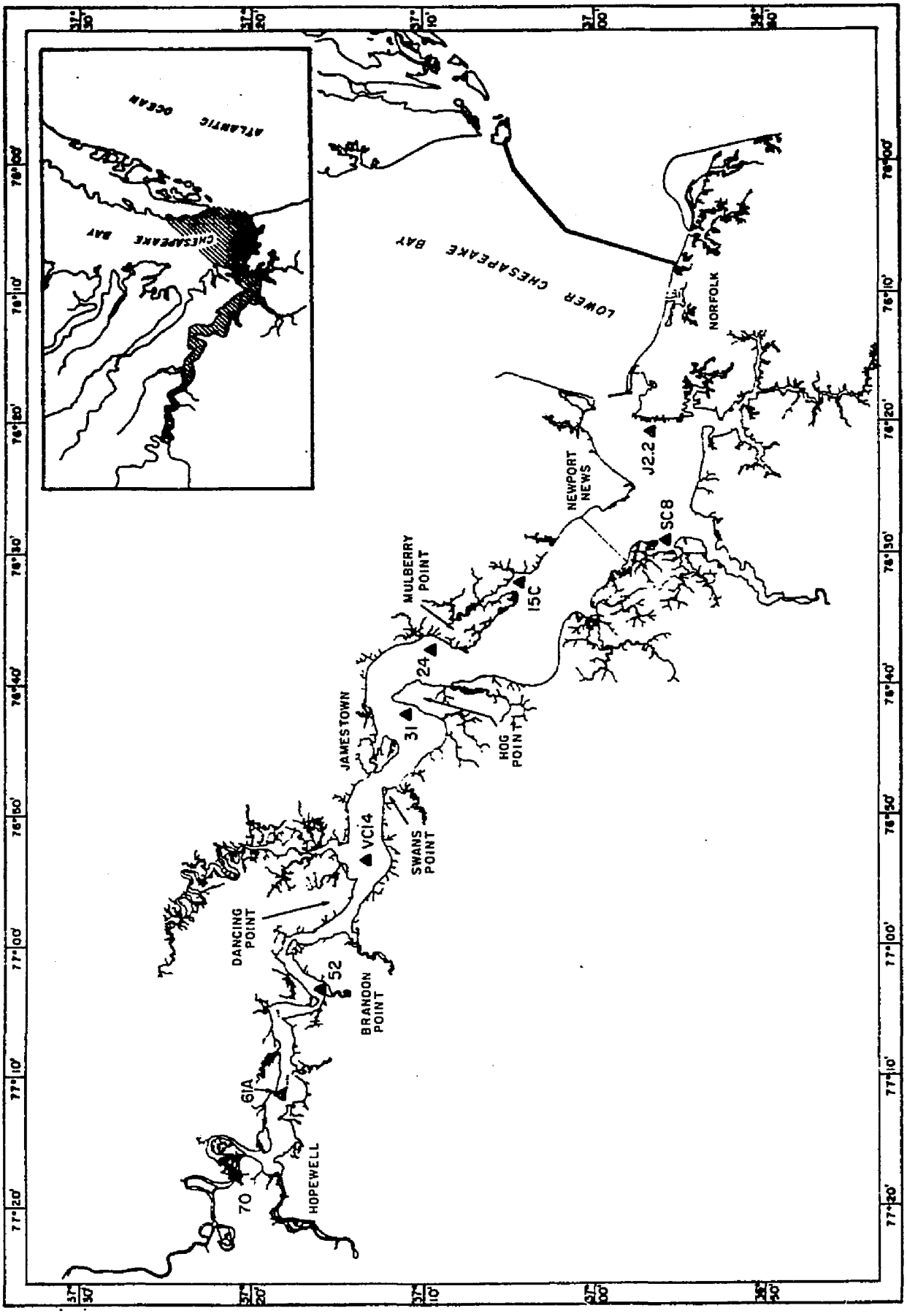

C B D CANAL
BALTIMORE
WASHINGTON D.C.
CHOPTANK R.
PATUXENT R.
NANTICOKE R.
WICOMICO R.
CB002
POTOMAC R.
RAPPAHANNOCK R.
SMITH PT.
RICHMOND
YORK R.
JAMES R.
NORFOLK
27
26
25
24
23
22
21
19
17
16
15
13
12
11
10
8
7
6
5
4
3
1
NORTHERN
CENTRAL
SOUTHERN

Figure 3. Location of sampling stations in the James River estuary.


ATLANTIC OCEAN
CHESAPEAKE BAY
LOWER CHESAPEAKE BAY
NORFOLK
NEWPORT NEWS
J2.2
SC8
15C
MULBERRY POINT
24
31
JAMESTOWN
HOG POINT
VC14
SWANS POINT
DANCING POINT
52
BRANDON POINT
61A
70
HOPEWELL
37°30'
37°20'
37°10'
37°00'
76°00'
76°10'
76°20'
76°30'
76°40'
76°50'
77°00'
77°10'
77°20'

spatula before measured sections were cut and placed in solvent-rinsed jars. Samples were stored frozen in the jars until analysis. Location of the sampling station is shown in Figure 4.

4. Roadside soil, Va.: Two surface soil samples were collected with a solvent-rinsed shovel near cement-surfaced streets in Newport News (Jefferson Avenue) and Virginia Beach (Atlantic Avenue). One sample was collected near an asphalt-surfaced street in Norfolk (Granby Street). The samples were placed in solvent-rinsed jars and stored in a freezer until analysis.

5. Clay Bank soil, Va.: Surface soil was collected with a solvent-rinsed shovel at a site located 7 miles from VIMS, 4 miles off U.S. Route 17. Two samples (708-1 and 708-2) were collected from this location. In addition, one sample (17-1) was collected near U.S. Route 17 (asphalt-surfaced highway) and one sample (614-1) was collected 4 miles away from U.S. Route 17 (near Route 614). See Figure 5 for sampling station locations.

In addition, four unused and one used motor oil from four different manufacturers, an asphalt sample, a coal sample and a crude oil sample were analyzed for hopanoids. The motor oil was the 10W-30 type made by Mobil (MO-1), Sears (MO-2), K-mart (MO-3) and Vavoline (MO-4). The asphalt sample was collected from a parking lot in front of the Gloucester Point U.S. Post Office. The coal sample was taken from an old coal mine that is no longer in operation, located about 15 miles west of Richmond, Virginia. A Libyan crude oil was obtained from a collection of crude oils from the Virginia Institute of Marine Science.

Figure 4. Location of station CB-002 on the Potomac River.

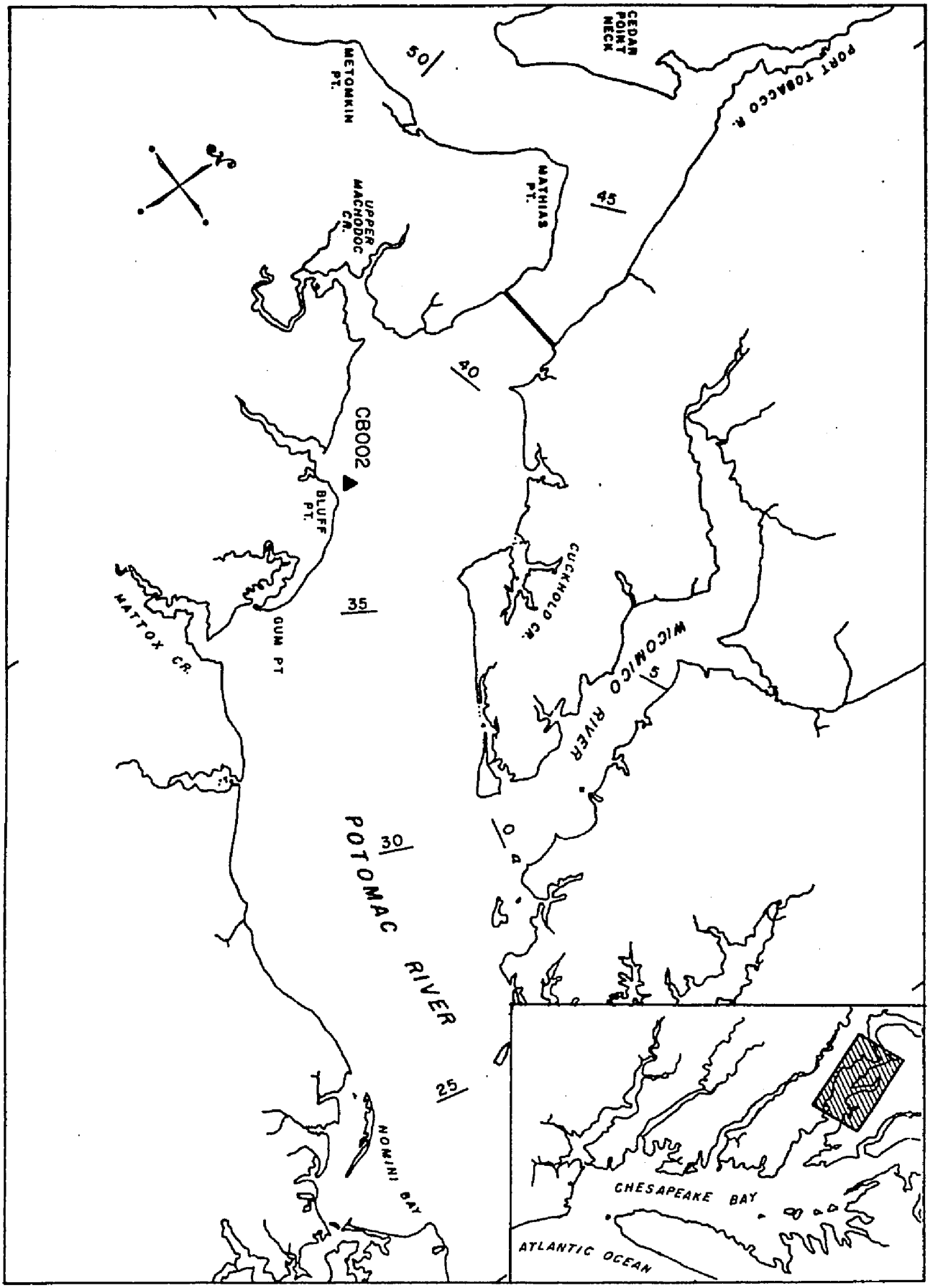
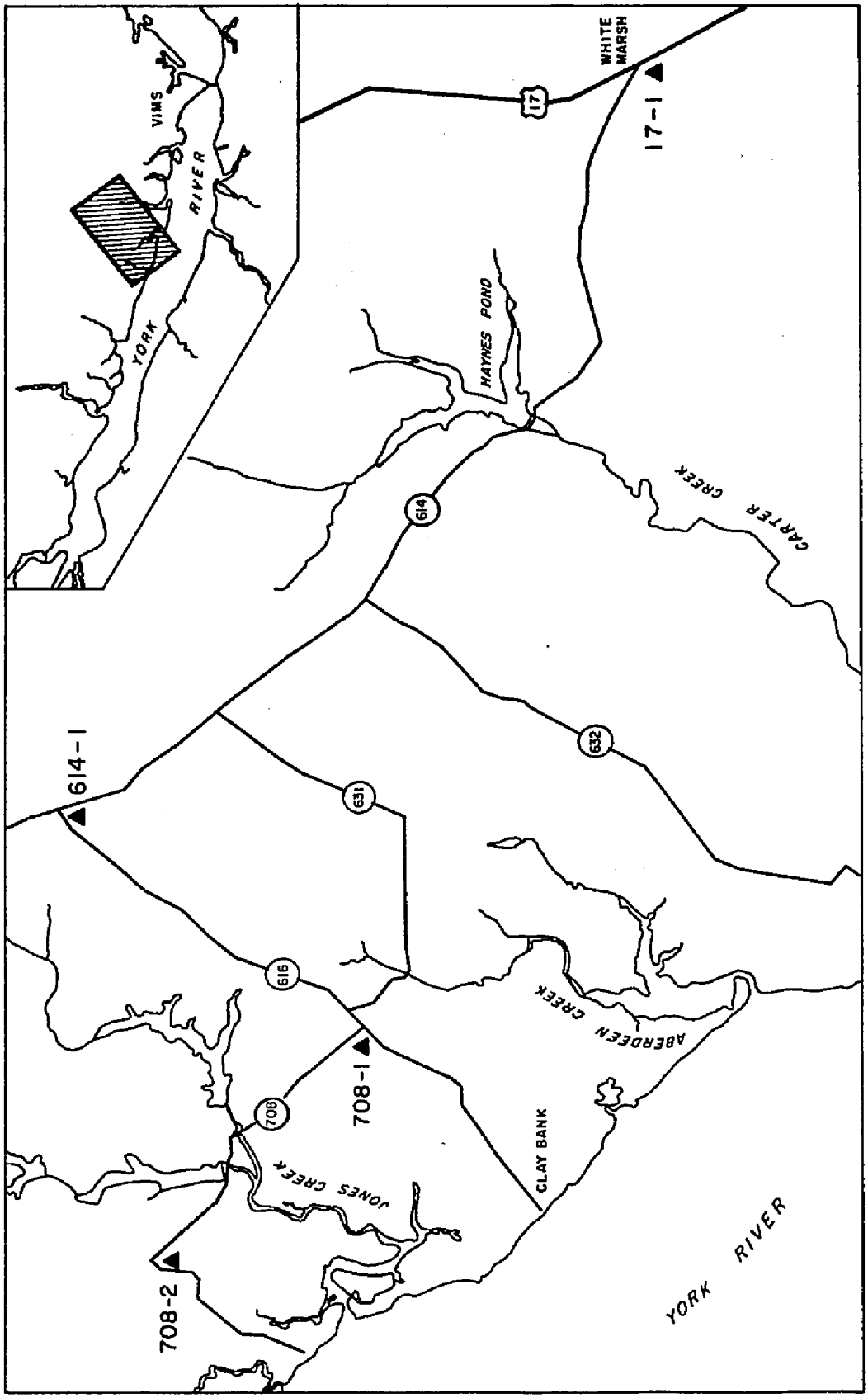

METOMKIN PT.
CEDAR POINT NECK
PORT TOBACCO R.
MATHIAS PT.
UPPER MACHODOC CR.
50
45
40
CB002
BLUFF PT.
35
MATTOX CR.
GUM PT
CUCKHOLD CR.
WICOMICO RIVER
5
POTOMAC RIVER
30
25
NOMINI BAY
CHESAPEAKE BAY
ATLANTIC OCEAN

Figure 5. Location of surface soil sampling stations at Clay Bank.


VIMS
RIVER
YORK
WHITE MARSH
17
17-1
HAYNES POND
614
CARTER CREEK
632
631
614-1
616
708
708-1
ABERDEEN CREEK
JONES CREEK
CLAY BANK
708-2
YORK RIVER

C. Analytical Procedures

Analytical procedures for the analysis of hopanoid acids and hydrocarbons in sediments, soils, motor oil, asphalt, and coal were identical with the exception of the sample pretreatment and extraction steps. Hopanoid acids were analyzed only in the sediment samples. A flow-chart for the procedure, representing the analysis of sediments, is presented in Figure 6.

To minimize the possibility of contamination, only the purest solvents available were used for the analysis (Burdick and Jackson, distilled-in-glass). Frequent blanks were performed to determine background levels. All glassware was first washed with hot soapy water, soaked overnight in 20 % Chem-solv solution and then rinsed several times with hot tap water and de-ionized water. This was followed by washing with 1:1 hydrochloric acid and rinsing with de-ionized water and acetone. Finally, the glassware was oven dried at 180-200 ^{o}C and stored in a dust free container or wrapped with clean aluminum foil. Before use, the glassware was rinsed again with acetone, methanol, toluene and finally with the solvent to be used for the analysis.

1. Sample preparation and extraction: Immediately prior to analysis, the sediment and soil samples were thawed, placed in pre-cleaned stainless steel trays and then freeze-dried in a Virtis 10-MR-TR freeze-drier for about 24 hours. The freeze-dried sediments were homogenized with a mortar and pestle, transferred to clean Teflon-capped jars and kept in a freezer until extraction. Sediments of 30 to 50 g dry weight

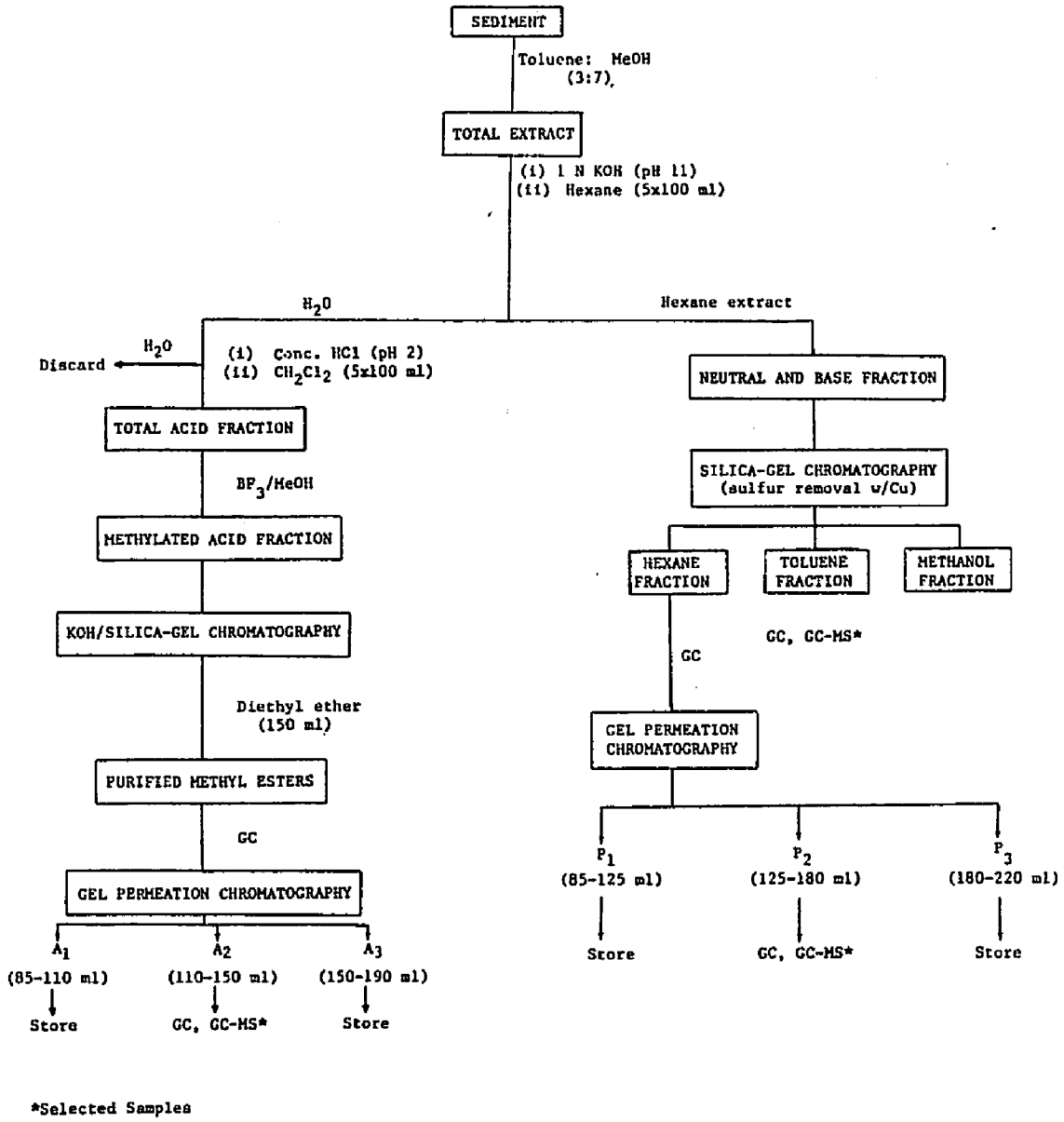


Figure 6. Flowchart of analytical procedure.

were spiked with internal standards (2-methyl octadecane, 1,1'-binaphthyl, cholestane and 5β-cholanic acid) and then Soxhlet-extracted with 300 ml toluene:methanol (3:7) azeotropic mixture for 48 hours. Subsequently, they were reduced to approximately 50 ml by rotary evaporation, taken up into water (100 ml) and adjusted to about pH 11 by addition of 1N KOH.

The Richmond coal sample was homogenized with a mortar and pestle before extraction. About 10 g of the coal sample was Soxhlet-extracted with a 3:7 toluene:methanol azeotropic mixture for 48 hours. The extract was reduced in volume and then further separated by silica-gel chromatography for subsequent analysis.

A piece of asphalt (900 g) was ultrasonicated with 1 liter of hexane for 45 minutes. The hexane soluble fraction was evaporated to dryness yielding an organic material of about 2 grams. This residue was taken up in 10 ml of hexane and stored in the freezer prior to silica-gel chromatography and subsequent analysis.

One gram of each motor oil and of the crude oil was dissolved in 5 ml of pentane and then reduced in volume by a gentle stream of nitrogen gas to about 1 ml (only the soluble part of crude oil was used for the analysis, the asphaltene was discarded). The extract was taken up in 2 ml hexane and stored in the freezer prior to silica-gel chromatography and subsequent analysis.

2. Lipid separation:

Neutral and basic compounds were obtained by partitioning from the alkaline extract into hexane (5x100 ml). After rotary evaporation to 3

ml, the hexane extract was transferred to a 15 ml centrifuge tube and evaporated gently under a nitrogen stream to 1 ml.

a. Silica-gel chromatography: Silica-gel (Bio-Sil, 100-200 mesh) was Soxhlet-extracted with hexane for 24 hours, then allowed to air dry. This pre-extracted silica-gel was activated by heating at 220 ^{o}C for 16 hours. Meanwhile, activated copper was prepared according to the method of Blumer (1957).

The silica-gel column (10x300 mm) with coarse glass frit at the bottom was rinsed with hexane. Fifteen grams of activated silica-gel were slurried with 50 ml of hexane, transferred to the column and the solvent was allowed to drain. The gel was settled by a vibrator to a height of 17.5 cm. Activated copper powder 1.5 cm thick was then applied on top of the column to remove any sulfur present in the samples. The column was washed with 50 ml of hexane with a solvent flow rate of about 2 ml per minute.

The neutral fraction was quantitatively transferred onto the column with a disposable glass pipette and eluted as follows: 20 ml hexane, 35 ml 40/60 (V/V) hexane/toluene and 50 ml methanol. Three fractions were collected, one for each eluting solvent. The first 5 ml of the hexane fraction was discarded. The hexane and toluene fractions were analyzed by gas-liquid chromatography, after being evaporated under nitrogen to 0.2 ml. The methanol fraction was kept refrigerated.

b. Gas chromatography of hexane and toluene fractions: The hexane fraction, containing mostly aliphatic hydrocarbons, and the toluene

fraction, containing mostly aromatics, were analyzed using a 45 m by 0.25 mm i.d. SE-52 WCOT glass capillary column prepared in this laboratory. Residual solvent was removed and both fractions were dissolved in 0.2 ml toluene before injecting in the splitless mode at 75 ^{o}C. Temperature was programmed from 75 to 300 ^{o}C at 6 ^{o}C/min using high purity helium as the carrier gas. A Varian 3700 gas chromatograph with a flame ionization detector (FID) was used. The FID detector was maintained at 280 ^{o}C and the injection port temperature was 275 ^{o}C .

Individual hydrocarbons were quantified relative to internal reference standards. 2-Methyloctadecane (2-MOD) was used as an internal standard for the hexane fraction and 1,1'-binaphthyl as an internal standard for the toluene fraction. Peak areas for each chromatogram were computed with a HP 3352B Laboratory Data System.

c. Gel permeation chromatography: The hexane fractions were further separated by gel permeation chromatography (GPC AutoPrep 1001, Analytical Biochemistry Laboratory, Inc.) in order to isolate hopanoid molecules from the more abundant n-alkanes. Gel permeation chromatography was employed instead of the conventional molecular sieve (O'Connor et al., 1962) method used by other investigators. Gel permeation chromatography was found to be easier to work with and less time consuming than the molecular sieve method. However, gel permeation chromatography does have the disadvantage of distorting the n-alkane chromatogram. High-molecular weight n-alkanes (> C_{17}) are fractionated in the P1 fraction whereas the small n-alkanes are retained in the P2 fraction (Figure 6). No fractionation of hopanes was observed.

The gel permeation column was prepared as follows: Ninety grams of Bio-Bead S-X8 gel (Bio-Rad Laboratories) were weighed into a beaker, covered with 400 ml of methylene chloride and allowed to stand overnight. This allowed the resin time to swell. The gel was then slurried into a silicone-treated glass chromatographic column (25X600 mm) under vacuum, and compressed to a height of 50 cm. The column was installed in the GPC AutoPrep 1001 and washed with about 1 liter of methylene chloride taking care to insure that all air was completely eluted from the GPC column prior to use.

The performance of GPC for the separation of hopanes was first checked by passing a standard solution containing n-alkanes (n-C_{11} to n-C_{32}), branched alkanes (pristane and 2-methyloctadecane) and cyclic alkanes (n-decylcyclohexane, androstane and cholestane) through the column. Cholestane was used as an indicator for the elution of hopanoids from the column. The perfomance of GPC for separating cholestane is shown in Table A1 in the Appendix. The elution volume from 125-180 ml was established as the hopane fraction. The elution volume from 90-125 ml contained mainly high molecular weight n-alkanes and some branched alkanes.

Extracts from samples were applied to the column in 3 ml methylene chloride, and elution was carried out with the same solvent. The flow of fluids through the column is from bottom to top. The eluent flow rate was maintained at 7.0 ml/min. The operational pressure was approximately 5 psi/ft of column. Three fractions were collected: elution volumes of 85-125 ml, 125-180 ml and 180-220 ml respectively. The first 85 ml were discarded. All fractions were concentrated first

by rotary evaporation, and then with a stream of dry nitrogen to about 0.2 ml. The second fraction, containing pentacyclic triterpanes, was further analyzed by gas chromatography and GC/MS after changing the solvent to 0.2 ml toluene.

d. Gas chromatography of triterpanes: Triterpanes were analyzed by gas chromatography, using the same conditions as previously described. Samples were injected splitless at 75 ^{o}C, heated to 200 ^{o}C at 20 ^{o}C/min, held isothermal at 200 ^{o}C for 1 minute and then temperature programmed from 200 to 300 ^{o}C at 2 ^{o}C/min. All the hopane isomers were eluted in the second temperature-programmed region. Samples were also co-injected with n-C_{28} and n-C_{36} so that Kovats indices of the hopanoids could be calculated.

The concentrations of the triterpanes were determined using the internal standard, cholestane, added to the samples before extraction. For samples of coal, asphalt, motor oil and crude oil where cholestane was not spiked into the samples before silica-gel chromatography, the concentrations of hopanoids were determined by coinjection of the samples with a known volume of cholestane. The calculation of peak areas and the corresponding concentrations was accomplished by a HP 3352B Laboratory Data System interfaced with the GC.

3. Analysis of hopanoid acids:

Following partitioning of the neutral compounds, the aqueous solution was acidified with concentrated HCl to pH 2. The acidic compounds were isolated by partitioning into methylene chloride (5x100

ml). Prior to their analysis, hopanoid acids must be converted to volatile derivatives. Methyl esters are the preferred derivatives because they are volatile, easily formed, and stable. Hence, the acid fraction was rotary evaporated to a few milliliters, the residue quantitatively transferred to a 15 ml tube and the solution evaporated to dryness with a stream of dry nitrogen. The methyl esters were then prepared by adding 1-2 ml of a methanol-boron trifluoride solution (14 % BF_3 in methanol; Alltech, Arlington Heights, Ill., U.S.A.) to the tube and heating at 70 ^{o}C for one hour. The esters were extracted with diethyl ether (5x3 ml) after addition of 5 ml of water. The methyl ester solution was concentrated to 1 ml with a stream of dry nitrogen. The esters were further purified by KOH-coated silica-gel chromatography.

a. KOH/Silica-gel chromatography: This procedure, modified from McCarthy and Duthie (1962), is summarized below. The isopropanol-KOH was prepared according to the method of Keeney (1956). The isopropanol-KOH solution should contain approximately 50 mg KOH per ml. One hundred grams of silica-gel (Bio-Rad Laboratory), 100 mesh, was rinsed two or three times with diethyl ether and permitted to dry in air.

Five grams of this silica-gel were weighed into a small beaker. To this, 10 ml of isopropanol-KOH and 30 ml of diethyl ether were added. After standing 5 minutes, the silica-gel was slurried into a chromatography column (10x30 mm) and washed with 100 ml of diethyl ether. A solvent flow rate of 5 ml per minute was satisfactory.

The sample was quantitatively transferred onto the column with a disposable glass pipette. The methyl esters were eluted in one fraction with 150 ml of diethyl ether and concentrated by rotary evaporation. The diethyl ether is easily removed under vacuum so one must be careful not to overheat the water bath, otherwise bumping occurs and the sample is lost.

b. Gas chromatography of methyl esters: The purified methyl esters were injected splitless into a glass capillary column after the solvent was changed to 0.2 ml toluene. GC conditions were the same as described before for the hexane and toluene fractions.

c. Gel permeation chromatography: Following analysis of the methyl esters, the remaining methyl ester solution was further separated by gel permeation chromatography. The performance of GPC in separating hopanoid acids from fatty acids was based on the separation of cholanic acid methyl ester. The result of this analysis is shown in Table A2. An elution volume of 110-150 ml was established as hopanoid acid methyl ester fraction.

Three fractions were collected: 85-110 ml, 110-150 ml and 150-190 ml. The first 85 ml were discarded. All three fractions were concentrated by rotary evaporation and then with a stream of dry nitrogen to about 0.2 ml. The second fraction which contains hopanoid acid methyl esters was further analyzed by gas chromatography and GC/MS after changing the solvent to 0.2 ml toluene.

d. Gas chromatography of hopanoid acid methyl esters: The hopanoid acid methyl esters were separated by gas chromatography using a glass capillary column (45 m x 0.25 mm i.d., SE-52) with helium as carrier. Sample runs were temperature programmed from 75 ^{o}C to 250 ^{o}C at 20 ^{o}C/min and held isothermal for 1 minute, then programmed again from 250 to 300 ^{o}C at 2 ^{o}C/min. Helium flow was 3.0 ml/min. The injection port temperature was 275 ^{o}C and the detector temperature was 280 ^{o}C.

4. Gas chromatography-Mass spectrometry (GC/MS):

Molecular characterizations of individual acids and hydrocarbons were made on selected sediment samples by GC/MS using a DuPont model 21-492-B mass spectrometer interfaced to a Varian model 2700 gas chromatograph modified for wall coated glass capillary columns (SE-52, 30 m length, 0.25 mm i.d.). The same GC temperature program as previously described was used for these analyses.

The effluent of the GC column was transferred to the mass spectrometer source directly via a 0.12 mm i.d. glass capillary, heated to 370 ^{o}C. The ion source temperature was approximately 275 ^{o}C. Electrons of 70 eV in an electron impact source formed the fragments observed in the mass spectra. The scan rate was 1 sec/decade for the mass range 51-517 or 69-617 AMU. The mass spectrometric data were acquired and processed using a Dupont Model 21-094B data system. Background spectra were also recorded. Fragment masses were calibrated with a perfluoroalkane mixture (PCR Product No.1233). All reported

spectra were corrected for background and normalized to the most intense fragment ion above m/e 50.

5. Total organic carbon:

Total organic carbon concentrations of an acid-treated aliquot from each sediment sample were measured with a Leco Carbon Analyzer. A 0.5 g sample was treated for 12 hours with 1:1 HCl, washed with de-ionized water, filtered and dried at 60 ^{o}C. The sample was then weighed in a Leco carbon-free crucible and measured for organic carbon content in a Leco Analyzer after ignition to 3000 ^{o}F. in an induction furnace.

The readings obtained for each carbon determination, along with temperature-pressure correction factors, were used to calculate the percent of each carbon fraction in the sample. Precision of these measurements was $\pm$ 5% for all samples analyzed.

6. Compound identification:

n-Alkanes, pristane, and perylene were identified by comparison of the GC retention times and mass spectra of selected samples with authentic standards, or by retention times alone once the Kovats Indices (KI) or Aromatic Retention Indices (ARI) had been established for a particular GC column. Since no authentic standards of hopanoid molecules were available, some identifications were based on a comparison of sample mass spectra with those published in the literature (Van Dorsselaer, 1975; Dastillung, 1976; Ensminger, 1977). In addition, Kovats Indices for identified compounds were calculated and used for identification of the other samples (Table A3). Other identifications

were based on a branched/cyclic fraction of a Lorraine coal extract (kindly provided to Dr. R. H. Bieri by Dr. P. Albrecht, Universite Louis Pasteur de Strasbourg, France).

Mass spectra and GC elution patterns of hopanoid acids published by Van Dorsselaer (1975) and Ensminger (1977) were used for the identification of the acids.

The mass spectra of hopane derivatives are distinctive in that the spectra are characterized by an abundant ion at m/e 191. They are the most commonly recognized triterpane spectra to date in geological samples (Kimble et al., 1974). The two main fragments (m/e 191 and m/e 148+R) are characteristics which vary with stereochemistry of the compound (Figure 7). However for compounds with the same stereochemistry, the relative intensities of these two main fragments are characteristic of the particular structure.

Fragment ions m/e 191, 149, 177, 205, 219, 233, 247, and 261 were used for the identification of hopanoid hydrocarbons (Van Dorsselaer, 1975; Ensminger, 1977).

Hopanoid acid methyl esters were identified by fragment ions m/e 191, 249, 263, and 277 (Van Dorsselear, 1975).

ββ-, βα-, and αβ-hopane series were recognized as follows:

ββ-hopane series: m/e 191 < m/e 148+R

βα-hopane series: m/e 191 ~ m/e 148+R

αβ-hopane series: m/e 191 > m/e 148+R

(Kimble et al., 1974; Ensminger, 1974).

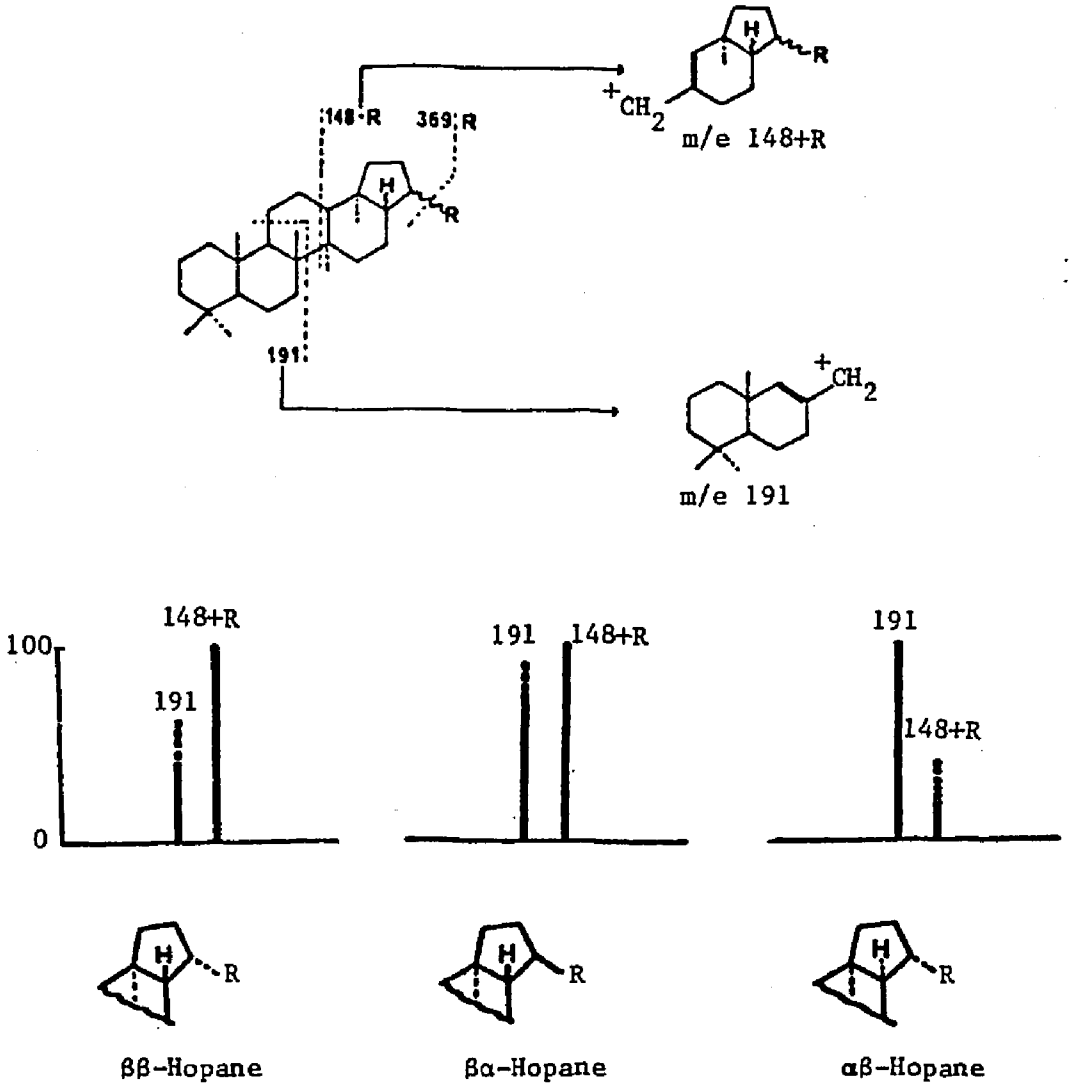


Figure 7. Major mass spectrometric fragmentations of hopanoid molecules under electron impact.

R and S epimers were identified according to Ensminger (1977), the first eluting epimer is 22S and the last is 22R.

D. Evaluation of Methodology

Since individual hopanoid standards were not available, quantitative results reported here were based on cholestane and 5β-cholanic acid. The same two compounds served to evaluate the extraction and isolation of hopanes and hopanoid acids. In addition, 2-methyloctadecane (2-MOD) and 1,1′-binaphthyl were used as the recovery standards for the hexane and toluene fractions. Five subsamples of a pre-extracted, homogenized sea-sand were spiked with 50 microliters each of cholestane, 5β-cholanic acid, 2-methyloctadecane and 1,1′-binaphthyl (1000 ng/μl). The samples were processed in the same manner as those for the sediment samples. The overall recovery data for these compounds is shown in Table 1. Precision values for the GC analysis of hopanoid hydrocarbons were obtained from injection of the branched and cyclic alkane fraction from a Lorraine coal extract. The extract, 1.0 ml, was spiked with cholestane, 50 μg, before injection. The concentrations of 17α(H),21β(H) hopanes with 27-32 carbons in the extract were calculated relative to the cholestane concentration. It is necessary to point out that the hopane concentrations reported here are not the original concentrations present in the Lorraine coal standard since the coal extract accidentally dried during storage of the vial in the freezer. Hexane was added to redissolve the residue and 100 μl of the solution were taken and used as GC retention standard. The results are presented in Table 2.

Since quantitation of all components is based on the appropriate internal standards spiked into the samples before extraction, the recovery data presented in Table 1 is meant to show that the extraction and isolation procedures used herein produce acceptable yields.

TABLE 1

Overall compound recovery.

Compound	Amount Added	Percent Recovery Mean ±%S.E.
2- Methyloctadecane	50 µg	83±8
Cholestane	50 µg	66±12
1,1'-Binaphthyl	20 µg	79±7
Cholanic acid methyl ester	50 µg	53±15

TABLE 2

Precision of GC analysis for hopanes*.

Compound	Replicates 1	2	3	4	5	6	Mean ±%S.E.
C_{27}	17.2	17.0	17.1	16.9	17.3	17.2	17.1±0.4
C_{29}	27.4	28.1	28.5	26.8	27.6	28.1	27.8±0.9
C_{30}	25.1	25.2	26.1	25.1	25.4	25.4	25.4±0.6
C_{31}:S	13.9	14.4	15.0	14.4	14.7	15.0	14.6±1.2
R	9.0	9.4	9.6	9.5	9.4	9.5	9.4±1.0
C_{32}:S	9.4	9.8	10.9	10.7	10.0	10.1	10.2±2.3
R	4.8	5.2	5.9	5.8	5.6	5.6	5.5±3.1

*Assumes all compounds have the same GC response as that of cholestane which is 770 μV.sec/ng.

Reproducibility of the method employed is more important than the compound recovery yield. Precision of this methodology, based on 5 subsamples of sea-sand, was ±12 % for cholestane and ±15 % for cholanic acid methyl ester (precision is reported as ± % standard error from the mean). Recovery for cholestane was found to be 66 % and for cholanic acid methyl ester was 53 %. Precision of the GC analyses, based on 6 GC injections of the branched and cyclic alkane fraction from the Lorraine coal extract, was ±2 % (standard error of the mean) for quantifiable hopanes. The error can be assumed to be somewhat larger for the higher molecular weight compounds than for the compounds close to the standard (Table 2).

IV. RESULTS

Twenty two surface sediment samples from the Chesapeake Bay were examined for the types and distribution of hopanoid molecules. An effort was made to present quantitative as well as qualitative results. All concentrations were normalized to organic carbon to account for variations in sediment composition from station to station. For purposes of general discussion, the samples are divided into 3 groups; Southern Bay, Central Bay and Northern Bay samples (Figure 2). Compound structures are given in the parenthesis and shown in Figure A1 in the Appendix.

Hopanoid hydrocarbons were identified in all samples from the Bay. Concentrations varied considerably, but all samples showed measurable quantities of hopanoid hydrocarbons (Table 3). Typical gas chromatograms of hopanoid hydrocarbons, from stations 21 and 27, are shown in Figure 8.

The hopanoids were dominated by the 17α(H),21β(H) hopane series ranging from C_{27} to C_{35} (XXII a-c; XXIII a-e). The extended hopanes, C_{31}- C_{35} were present as mixtures of 22S and 22R diastereomers. 17β(H),21β(H) Hopanes ranging from C_{27} to C_{32} (XVIII a-c; XIX-a&b) were also present, but at lower concentrations. Only a single epimer at position 22 of the 17β(H),21β(H)-C_{31} hopane, commonly assumed to be 22R (Ensminger, 1974), was present. 17β(H),21α(H) C_{29} and C_{30} hopanes (XX

TABLE 3

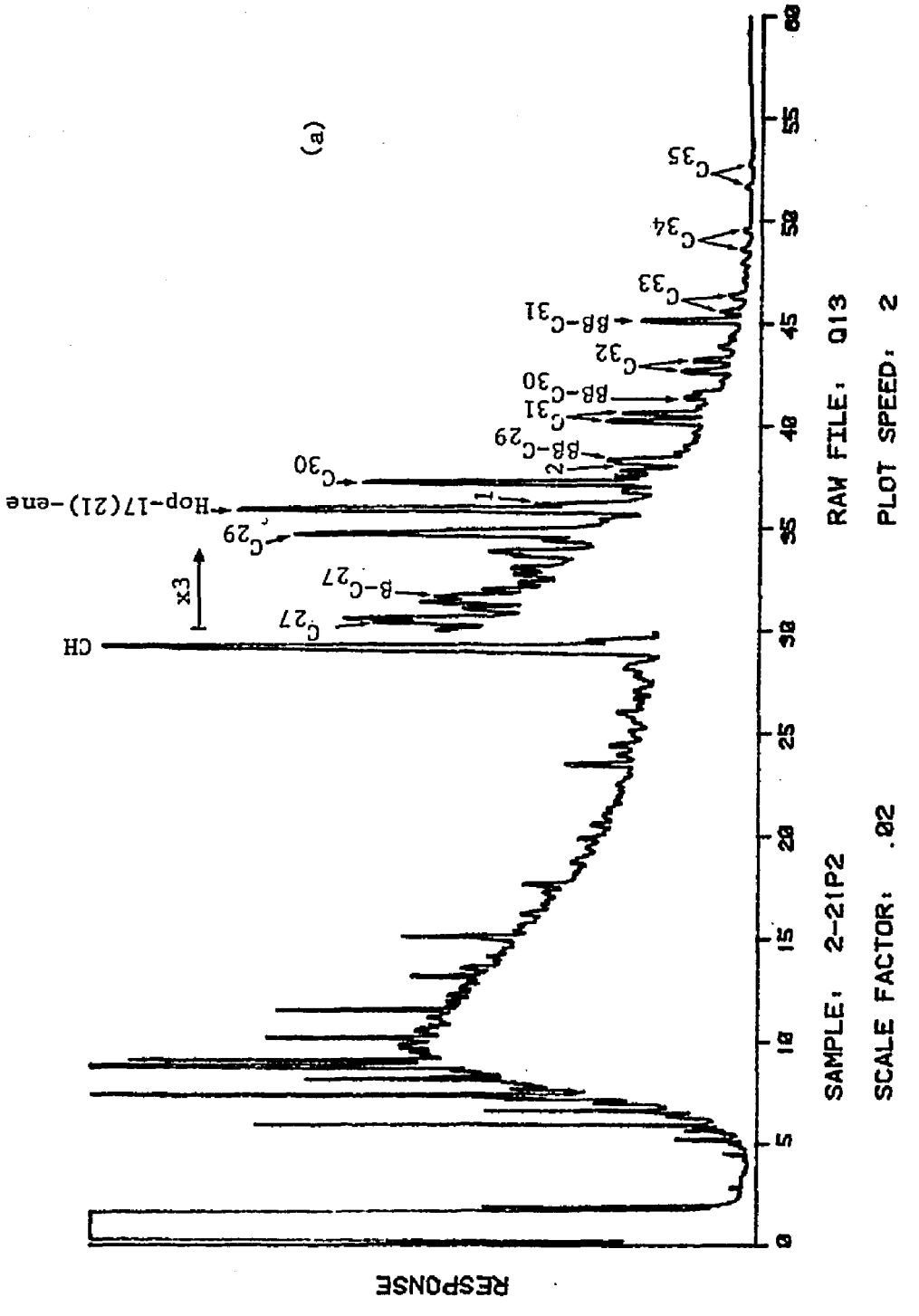
Hopanoid hydrocarbons in sediments from the Chesapeake Bay (μg/g OC).

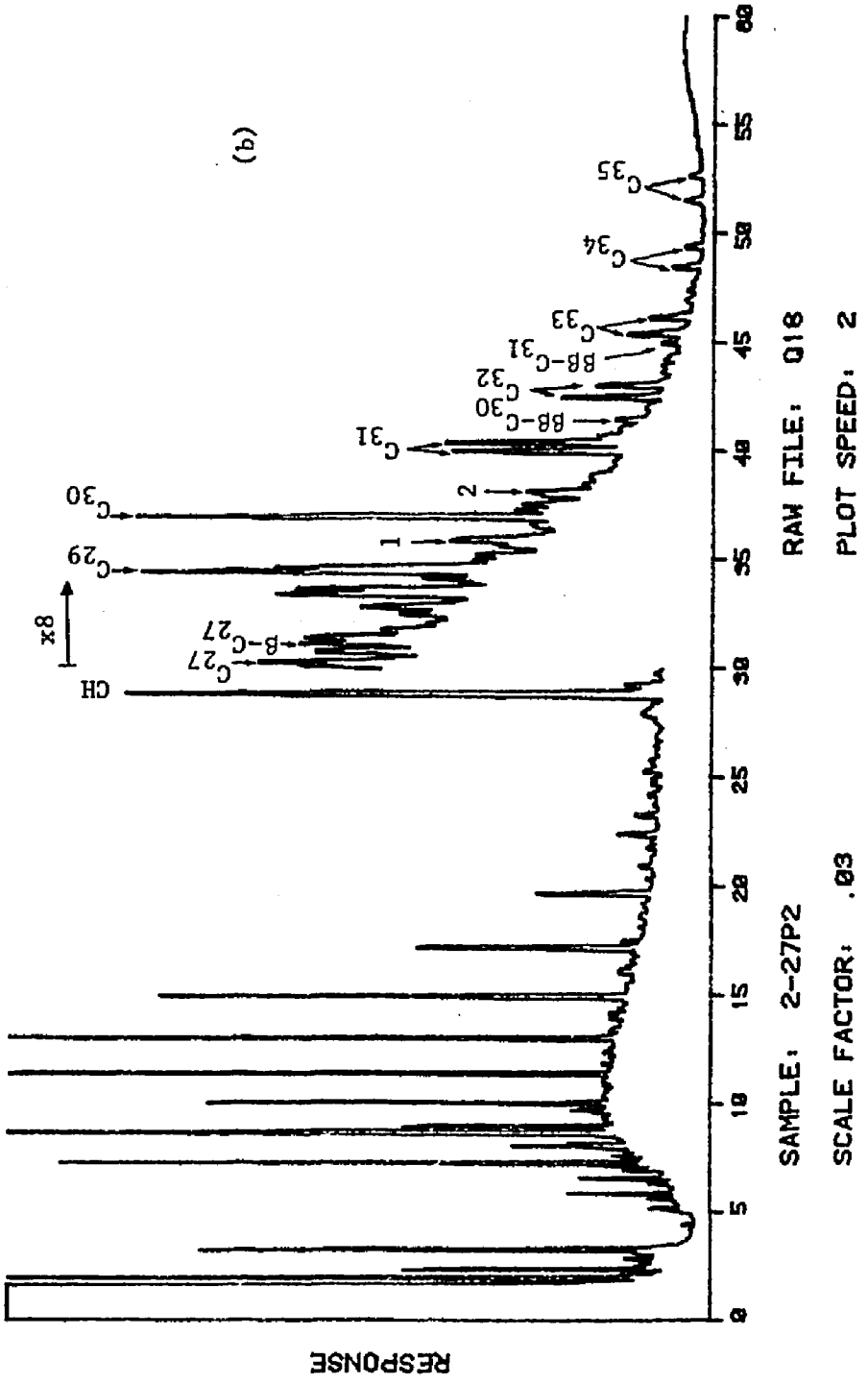
	Station	17α-C_{27}	β-C_{27}	αβ-C_{29}	Hop-(17)21-ene	* βα-C_{29}	αβ-C_{30}	* ββ-C_{29}	αβ-C_{31} S	αβ-C_{31} R	ββ-C_{30}	αβ-C_{32} S	αβ-C_{32} R	ββ-C_{31}	αβ-C_{33} S	αβ-C_{33} R	αβ-C_{34} S	αβ-C_{34} R	ββ-C_{32}	αβ-C_{35} S	αβ-C_{35} R	* βα-C_{30}
Southern Bay	1	N.D.	N.D.	N.D.	0.92	N.D.	2.0	N.D.	0.86	0.67	N.D.	N.D.	N.D.	0.51	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	3	3.6	3.6	9.1	1.1	0.18	8.0	1.6	3.0	3.0	0.74	1.7	1.3	1.6	0.92	0.53	0.39	0.28	0.57	0.34	0.28	1.5
	4	N.D.	1.4	1.6	2.8	N.D.	0.86	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	5	2.4	3.3	9.5	6.7	2.0	4.5	1.2	1.6	1.9	0.74	0.85	0.67	2.5	0.33	0.28	0.17	0.14	0.33	0.16	0.13	0.76
	6	N.D.	1.4	1.2	3.0	N.D.	0.81	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	7	N.D.	1.2	3.0	N.D.	N.D.	1.9	N.D.	0.77	1.0	N.D.	N.D.	N.D.	2.1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	8	12	3.8	7.3	11.7	N.D.	3.2	1.1	1.5	1.6	0.38	0.54	0.46	2.0	0.28	0.21	0.10	tr	0.22	tr	tr	0.80
	10	6.7	2.2	7.1	9.3	1.7	3.3	0.65	1.7	1.7	N.D.	0.68	0.56	2.2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.83
Central Bay	11	3.7	1.6	5.2	8.4	1.1	2.0	0.36	1.0	1.0	0.24	N.D.	N.D.	2.1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.62
	12	4.2	1.3	3.8	6.6	0.90	1.6	N.D.	0.82	0.80	N.D.	N.D.	N.D.	1.3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	13	2.0	2.7	7.5	10.1	1.9	4.1	1.1	1.8	2.6	0.61	0.89	0.70	3.0	0.50	0.38	0.21	0.18	0.37	0.19	0.18	1.2
	15	11	1.9	7.9	14.1	N.D.	3.0	0.87	1.3	1.5	0.33	0.51	0.41	1.8	0.29	0.20	0.10	0.06	0.31	0.08	0.07	0.85
	16	0.30	2.0	4.1	8.1	0.80	1.2	0.51	0.68	0.74	0.39	0.25	0.19	2.2	N.D.	N.D.	N.D.	N.D.	0.75	N.D.	N.D.	0.72
	17	7.7	2.6	8.1	19.7	1.6	4.1	0.44	1.7	1.9	0.40	0.71	0.55	3.0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.4
	19	8.6	N.D.	6.6	12.1	N.D.	3.6	0.24	1.7	1.2	0.23	1.9	1.5	2.8	0.78	0.64	N.D.	N.D.	N.D.	N.D.	N.D.	0.34
	21	2.1	0.33	5.4	6.8	2.0	4.9	0.89	1.7	1.6	0.58	0.98	0.75	2.3	0.56	0.44	0.23	0.17	0.20	0.32	0.30	0.87
Northern Bay	22	3.6	3.0	7.7	5.4	2.6	7.1	0.95	2.4	1.8	0.20	1.2	0.88	1.5	0.62	0.45	0.23	0.20	0.07	0.20	0.17	0.79
	23	3.2	1.7	9.2	4.2	3.6	9.4	1.2	3.7	2.8	0.93	2.1	1.8	2.6	1.2	0.88	0.68	0.42	0.25	0.52	0.48	1.4
	24	N.D.	1.5	2.7	9.7	N.D.	5.2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.8	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	25	3.6	1.9	6.6	7.8	N.D.	3.9	2.2	2.7	1.8	0.71	1.2	0.93	2.0	0.53	0.44	0.18	0.14	0.18	tr	tr	0.30
	26	3.3	1.7	3.9	10.2	1.9	7.0	0.60	3.0	1.8	N.D.	1.6	1.1	3.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.3
	27	2.9	1.6	7.3	0.20	2.2	8.4	0.20	4.1	4.2	0.63	2.5	1.9	0.42	1.6	1.2	0.77	0.82	N.D.	0.62	0.60	1.1

N.D.:Not detected
tr:trace
*: Upper limit of compound concentration; see discussion

Figure 8. Representative gas chromatograms of hopanes extracted from the Chesapeake Bay sediments

a) station 21
b) station 27
CH: cholestane (spike), C_{27}-C_{35}: αβ-hopanes,
1: βα-C_{29} hopane, 2: βα-C_{30} hopane.


(a)
RESPONSE
CH
x3
C_{27}
B-C_{27}
C_{29}
Hop-17(21)-ene
C_{30}
BB-C_{29}
C_{31}
BB-C_{30}
C_{32}
BB-C_{31}
C_{33}
C_{34}
C_{35}
SAMPLE: 2-21P2
SCALE FACTOR: .02
RAW FILE: Q13
PLOT SPEED: 2


(a)
RESPONSE
SAMPLE: 2-27P2
SCALE FACTOR: .03
RAW FILE: 018
PLOT SPEED: 2
CH
x8
C_{27}
B-C_{27}
C_{29}
C_{30}
1
2
C_{31}
BB-C_{30}
C_{32}
BB-C_{31}
C_{33}
C_{34}
C_{35}

a&b) were identified in many samples. Hop-17(21)-ene (XVII) was the only hopene found in the Bay samples, with concentrations ranging from 0.20 to 20 μg/g OC (Table 3).

The composition and the concentrations of hopanoid acids in surface sediments of the Chesapeake Bay are shown in Table 4. Figure 9 presents typical gas chromatograms of hopanoid acid methyl esters from stations 24 and 26. Identified hopanoid acids were dominated by 17β(H),21β(H) isomers, ranging from C_{31} to C_{33} (XXVII a-c) (Figure 9). All acids were present as a single epimer (22R). The 17β(H),21β(H)-bishomohopanoic acid (C_{32} acid) (XXVII-b) was the most prominent hopanoid acid in all samples analyzed. Only a trace amount of this acid was present at station 23. No indications of hopanoid acids were found at station 27. 17β(H),21α(H) and 17α(H),21β(H) hopanoid acids were tentatively identified in many samples from the Bay by their mass spectra and GC elution pattern (Boon et al., 1978).

The areal distribution of total aliphatic hydrocarbons in Bay samples is shown in Figure 10. The typical n-alkane distribution lies within the C_{21} to C_{35} range, with C_{27} to C_{31} being the dominant members and C_{29} or C_{31} being the most frequently encountered maximum (Figure 11). They showed a strong odd-carbon-number preference (CPI = 2.7 - 4.6; Table 5). An exception is station 27 with a CPI of 1.1. n-Alkane concentrations ranged from 10 to 80 % (w/w) of the total aliphatic hydrocarbons in the same samples (Table 5). For most samples examined here, the unresolved complex mixture (UCM) was unimodal, centered in the range 2900-3000 (Kovats Index) and extending from 1600 to 3500. Bimodal distributions centered at 1600-1700 and 2800-2900 were observed for some

TABLE 4

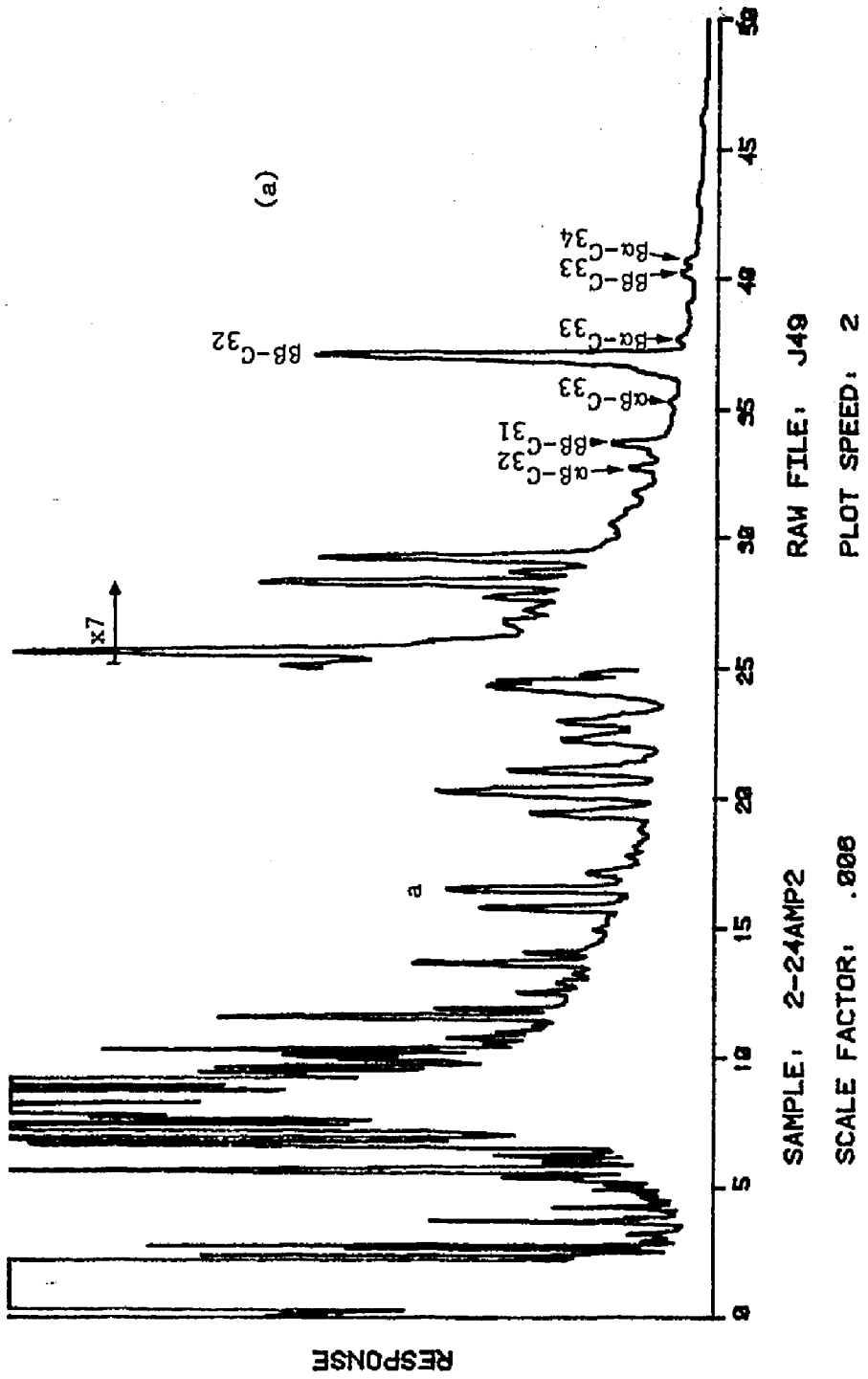
Hopanoid acids identified in surface sediments from the Chesapeake Bay (μg/g OC).

	Station	αβ-C_{32}	ββ-C_{31}	αβ-C_{33}	ββ-C_{32}	βα-C_{33}	αβ-C_{34}	ββ-C_{33}	βα-C_{34}	Total
Southern Bay	1	-	-	-	2.0	-	-	-	-	2.0
	3	-	-	-	5.6	-	-	-	-	5.6
	4	-	-	-	2.7	-	-	-	-	2.7
	5	-	-	-	13.2	-	-	-	-	13.2
	6	-	-	-	15.2	-	-	-	-	15.2
	7	-	-	-	24.3	-	-	-	-	24.3
	8	1.3	0.7	0.4	16.0	0.9	-	-	-	19.3
	10	-	2.2	-	34.5	-	-	-	-	36.7
Central Bay	11	1.5	2.7	-	27.2	1.0	-	0.7	-	33.1
	12	-	-	-	19.9	-	-	-	-	19.9
	13	-	-	-	12.5	-	-	-	-	12.5
	15	0.9	-	0.6	12.4	-	-	-	-	13.9
	16	-	-	-	20.6	-	-	-	-	20.6
	17	3.2	2.2	0.8	32.4	1.5	-	0.7	-	40.8
	19	-	-	-	10.6	-	-	-	-	10.6
	21	-	-	-	10.2	-	-	-	-	10.2
Northern Bay	22	1.9	1.5	2.1	17.5	-	-	-	-	23.0
	23	-	-	-	trace	-	-	-	-	trace
	24	2.0	3.5	0.4	44.8	0.9	-	1.4	1.0	54.0
	25	-	0.6	-	8.8	-	-	-	-	9.4
	26	9.7	13.1	1.8	94.2	2.0	-	4.6	2.3	127.7
	27	-	-	-	-	-	-	-	-	-

-: Not detected

Figure 9. Representative gas chromatograms of hopanoid acid methyl esters from the Chesapeake Bay sediments

a) station 24
b) station 26
a: cholanic acid methyl ester (spike).


RESPONSE
(a)
x7
αβ-C32
ββ-C31
αβ-C33
ββ-C32
βα-C33
ββ-C33
βα-C34
a
0
5
10
15
20
25
30
35
40
45
50
SAMPLE: 2-24AMP2
RAW FILE: J49
SCALE FACTOR: .008
PLOT SPEED: 2

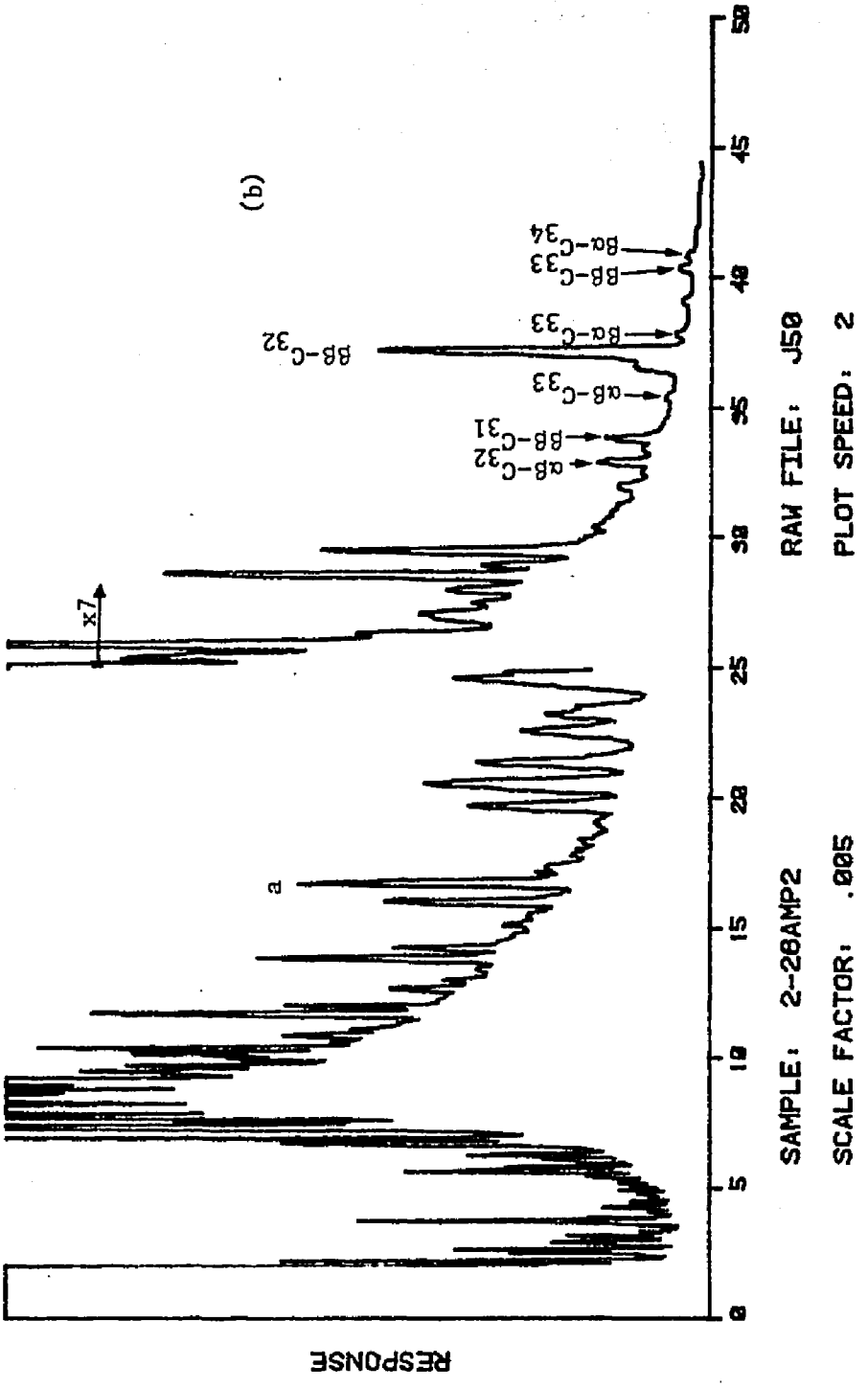

(b)
RESPONSE
SAMPLE: 2-28AMP2
SCALE FACTOR: .005
RAW FILE: J50
PLOT SPEED: 2
x7
a
αβ-C32
ββ-C31
αβ-C33
ββ-C32
βα-C33
ββ-C33
βα-C34

Figure 10. Distribution of total aliphatic hydrocarbons in surface sediments from the Chesapeake Bay.

TOTAL ALIPHATICS

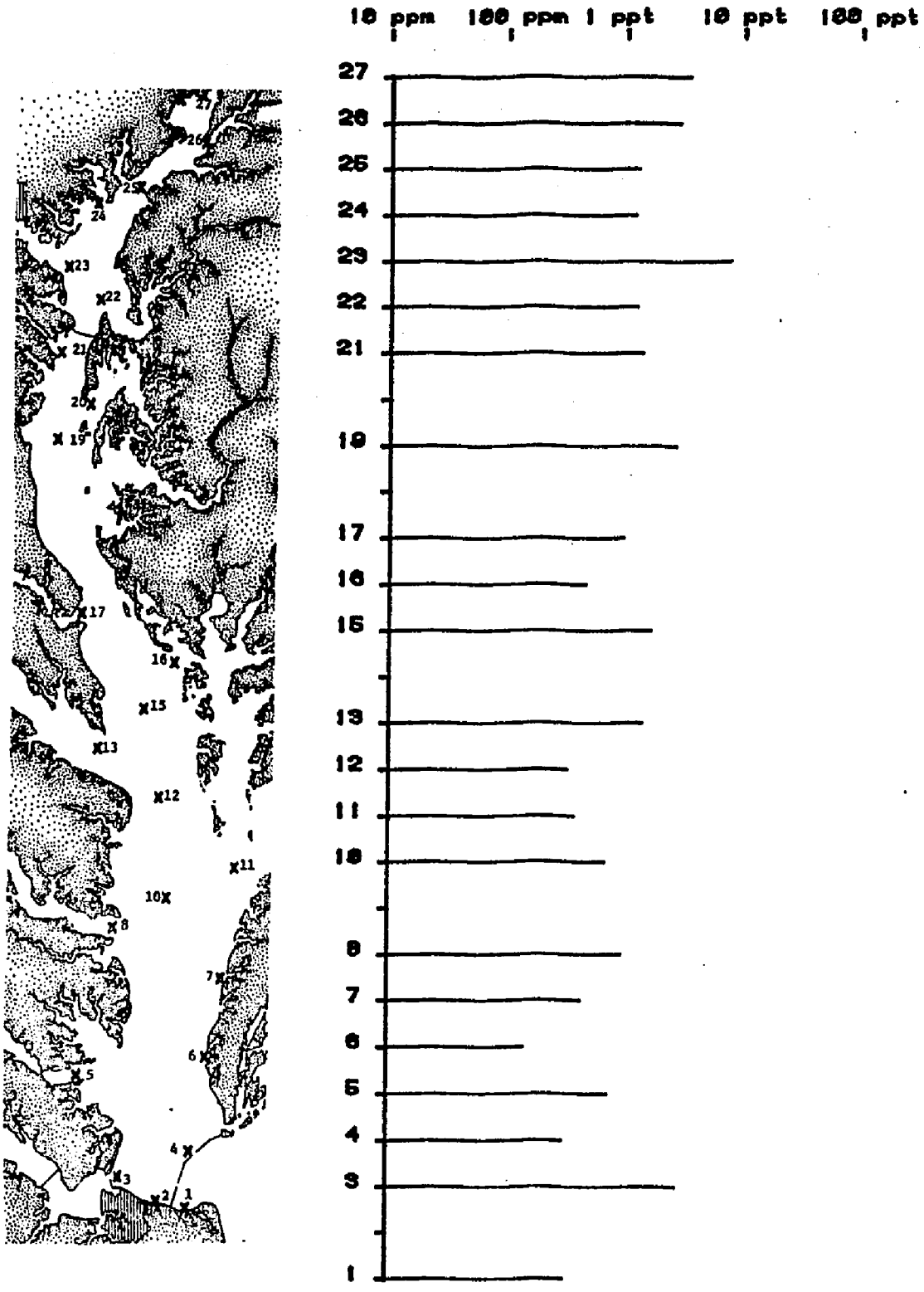
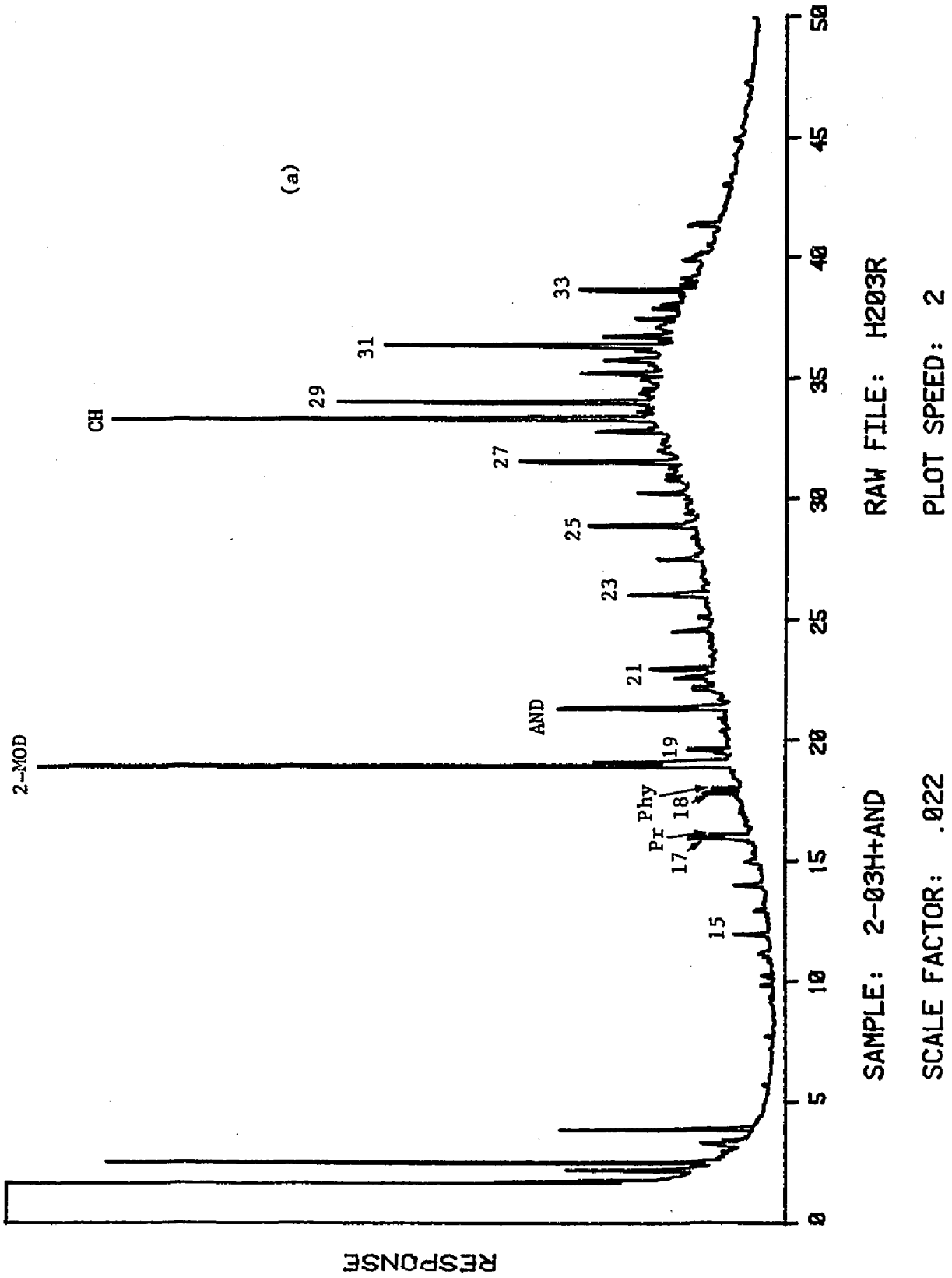


Figure 11. Representative gas chromatograms of total alkane fractions from the Chesapeake Bay sediments

a) station 3
b) station 23
N-alkanes are numbered according to number of carbons in compound, Pr: pristane, Phy: phytane, AND: androstane (co-injected standard), 2-MOD: 2-methyloctadecane (spike), CH: cholestane (spike).


(a)
2-MOD
AND
CH
Pr
Phy
15
17
18
19
21
23
25
27
29
31
33
0
5
10
15
20
25
30
35
40
45
50
RESPONSE
SAMPLE: 2-03H+AND
RAW FILE: H203R
SCALE FACTOR: .022
PLOT SPEED: 2

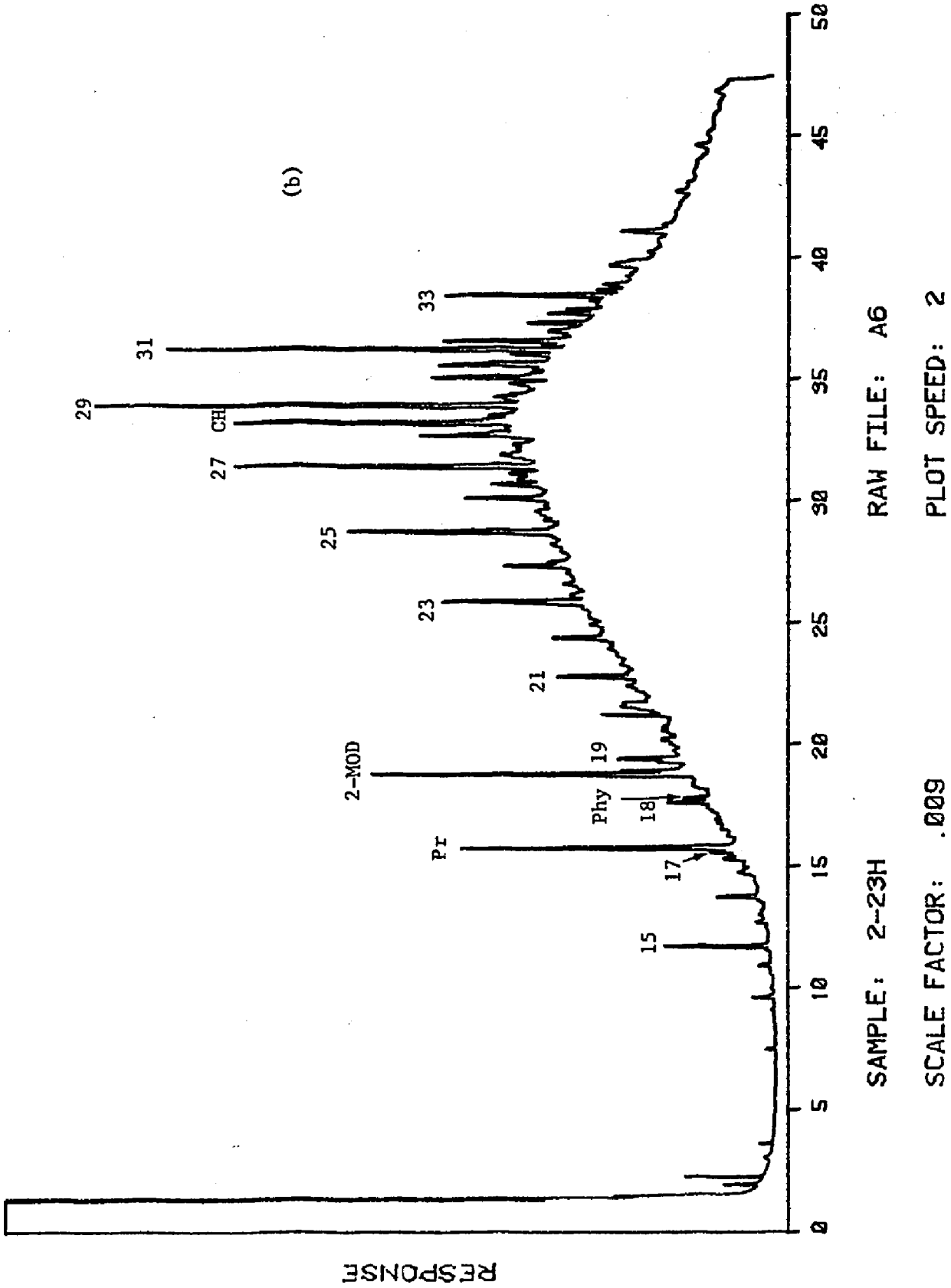

(b)
RESPONSE
0
5
10
15
20
25
30
35
40
45
50
15
17
Pr
18
Phy
19
2-MOD
21
23
25
27
CH
29
31
33
SAMPLE: 2-23H
RAW FILE: A6
SCALE FACTOR: .009
PLOT SPEED: 2

TABLE 5

Hydrocarbon concentrations for surface sediments from Chesapeake Bay (µg/g OC).

		Aliphatic Fraction						Aromatic Fraction			
	Station	Total n-Alkanes	Total Resolved	Total Aliphatics	CPI[a]	UCM	n-Alkanes / Total Aliphatics	Total Resolved	Total Aromatics	UCM	% OC
Southern Bay	1	144	326	326	2.7	0	0.44	94	94	0	0.36
	3	203	519	2855	3.2	2,336	0.11	232	386	154	1.02
	4	148	312	312	N.D.	0	0.47	25	25	0	0.15
	5	181	246	760	4.1	514	0.24	99	319	220	1.74
	6	106	148	148	N.D.	0	0.72	41	41	0	0.25
	7	232	438	438	4.6	0	0.78	34	34	0	0.43
	8	163	404	985	4.2	581	0.27	45	56	11	2.04
	10	303	424	711	4.2	287	0.43	47	87	40	0.39
Central Bay	11	205	276	388	4.5	112	0.53	62	82	20	0.47
	12	165	308	338	3.9	30	0.49	38	38	0	0.25
	13	260	453	1459	4.2	1,006	0.18	132	229	97	2.59
	15	331	545	1410	4.1	865	0.19	81	194	113	2.93
	16	212	317	477	4.7	160	0.44	48	90	42	1.19
	17	222	396	1005	4.1	609	0.22	79	182	103	2.70
	19	615	1832	2756	3.9	924	0.22	807	933	126	1.00
	21	181	283	1414	3.8	1,131	0.13	230	447	217	2.80
Northern Bay	22	173	226	1259	3.4	1,033	0.14	196	636	440	3.19
	23	620	1231	7757	3.5	6,526	0.08	362	1100	738	3.59
	24	398	518	1226	4.4	708	0.32	159	213	54	3.25
	25	457	648	1313	3.0	665	0.35	454	619	165	3.46
	26	943	1088	2854	4.3	1,766	0.33	182	317	135	3.03
	27	1375	1636	3510	1.1	1,874	0.39	20200	23065	2,865	1.08

[a]: Carbon Preference Index from C_{24} to C_{32}

N.D.: Not determined

of the Central and Southern Bay samples (stations 3, 5, 13 and 15) and for the Northern Bay station 25. The UCM was high in samples from river-mouth stations and in the Northern Bay samples. Station 19 also showed a large UCM (Table 5).

Total aromatic hydrocarbon concentrations in the Bay samples exhibited the same trends as those of total aliphatic hydrocarbon concentrations. Generally, high concentrations were found in samples at the mouths of major rivers and in the Northern Bay area (Figure 12; Table 5). The UCM of the aromatic fraction was also high in samples at river-mouth stations, in all of the Northern Bay samples and at station 19 (Table 5).

Table 3 shows that 17α(H),21β(H) hopanes are the most abundant hopanes in the Bay samples. An attempt was made to determine the origins of these 17α(H),21β(H) hopanes. Potential sources of hopanes that are in common use and contain fossil, fully maturated hopanes were investigated. These included a regional bituminous coal, motor oils and a crude oil. The compositions and abundances of these hopanoids are shown in Table 6. Hopanoid acids were not analyzed in any of these possible source materials.

Major hopanoids found in the Richmond coal sample were of the 17α(H),21β(H)-hopane series, ranging from C_{27} to C_{32} (XXII a-c; XXIII a&b). The extended hopanes were present as diastereomers, 22R and 22S. 17β(H),21α(H)-C_{29} and C_{30} hopanes (XX a&b) were also present, but not the 17β(H),21β(H)-hopane series (XVIII and XIX). No hopene was found in the coal sample.

Figure 12. Distribution of total aromatic hydrocarbons in surface sediments from the Chesapeake Bay.

TOTAL AROMATICS

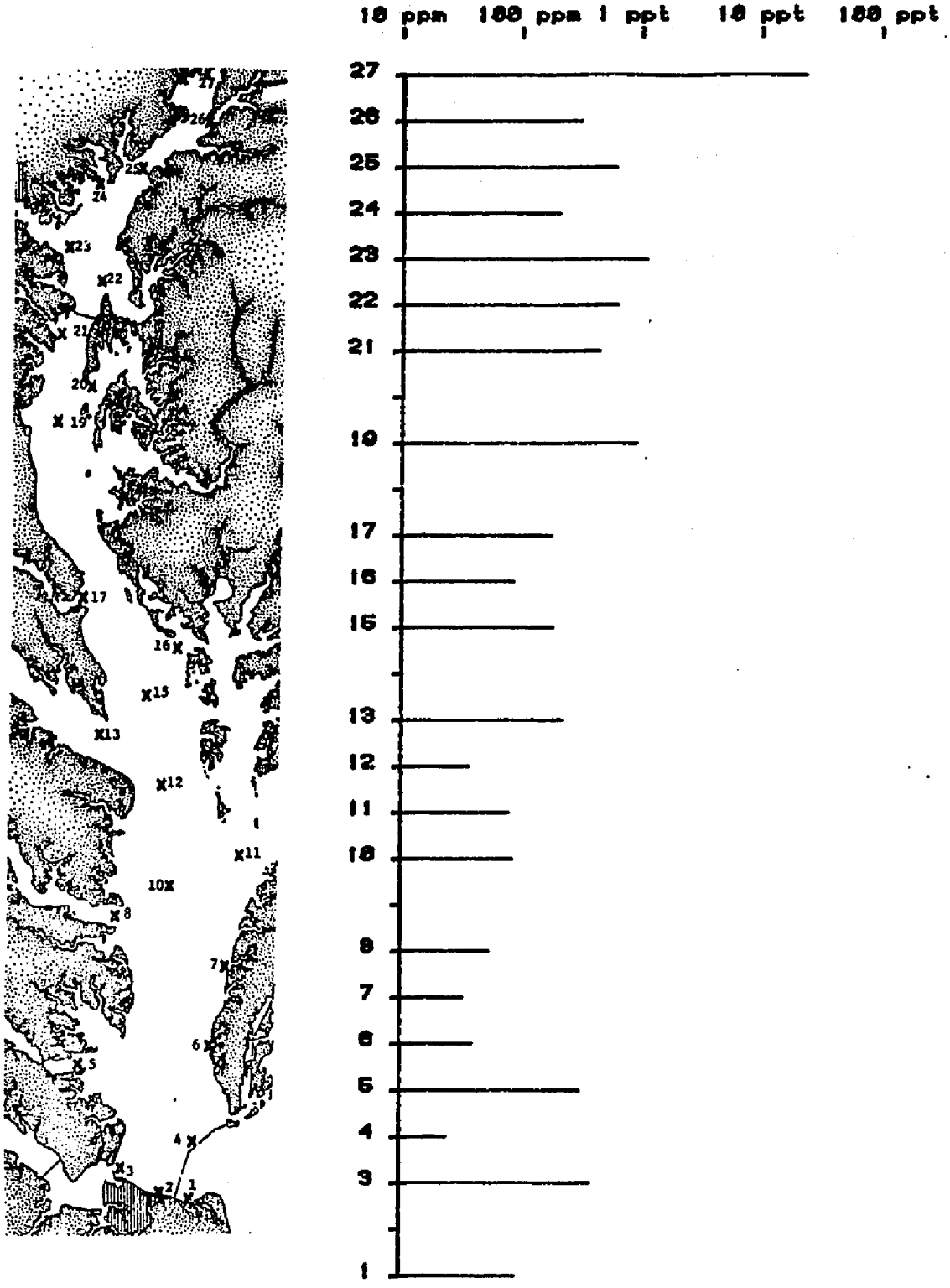


TABLE 6

Hopanoid compositions in possible source materials (μg/g).

Sample	17α-C_{27}	αβ-C_{29}	βα-C_{29}	αβ-C_{30}	βα-C_{30}	αβ-C_{31}		αβ-C_{32}		αβ-C_{33}		αβ-C_{34}		αβ-C_{35}		% OC
						S	R	S	R	S	R	S	R	S	R	
Coal*	104	245	36	201	51	64	104	22	15	tr	tr	nd	nd	nd	nd	75.6
Asphalt	287	536	200	563	106	113	86	43	37	12	9	tr	tr	nd	nd	ND
Libyan Crude	268	403	113	474	53	176	97	98	62	38	24	27	15	tr	tr	ND
MO-1	156	519	42	387	27	273	175	128	77	118	80	42	25	26	12	ND
MO-2	121	344	31	298	15	134	79	74	50	55	37	31	19	22	12	ND
MO-3	148	394	40	374	20	213	140	137	82	100	59	58	39	48	22	ND
MO-4	129	340	36	343	18	199	142	132	99	102	60	52	37	47	28	ND
UM-1	145	489	50	346	17	258	158	120	73	115	74	42	24	27	13	ND

MO: unused motor oil
UM: used motor oil
* : concentration in ng/g OC
tr: trace
nd: not detected
ND: not determined

Hopane concentrations in a Libyan crude oil are shown in Table 6. The gas chromatogram is shown in Figure 13. The hopanoids consist of 17α(H),21β(H)-hopanes (XXII and XXIII), which dominated over the 17β(H),21α(H)-hopane series. The 17β(H),21β(H)-hopane series again was absent and so was hopene. Extended 17α(H),21β(H) hopanes were present as mixtures of 22S and 22R diastereomers. The S/R ratios for these extended 17α(H),21β(H) hopanes (C_{31}- C_{34}) were found to be between 1.6 to 1.8 (Table 7).

Typical gas chromatograms of four unused and one used motor oil are shown in Figure 14. Hopanes in the unused motor oil (MO-1) and the used motor oil of the same brand (UM-1) were found to be similar in their composition and concentration (Figure 14 a&b; Table 6). In fact, all motor oil samples were very similar in this respect. The dominant hopanes present were those of the 17α(H),21β(H) configuration ranging from C_{27} to C_{35}. C_{29} and C_{30} hopanes were the major hopanes found in all samples. Extended hopanes were present as mixtures of 22S and 22R diastereomers. The S/R ratios for extended 17α(H),21β(H) hopanes (C_{31}-C_{35}) in motor oils ranged from 1.4 to 2.2 (Table 7). Some 17β(H),21α(H)-C_{29} and C_{30}hopanes (XX a&b) were also found, but at much lower concentrations. No traces of 17β(H),21β(H)-hopanes (XVIII and XIX) and hopene were detected in motor oil samples. The hopane composition of motor oils is very similar to that of the crude oil, probably because motor oils are distillate fractions of petroleum.

Figure 13. Gas chromatogram of hopanes extracted from Libyan crude oil

CH: cholestane (co-injected standard),
C_{27}-C_{34}: αβ-hopanes.

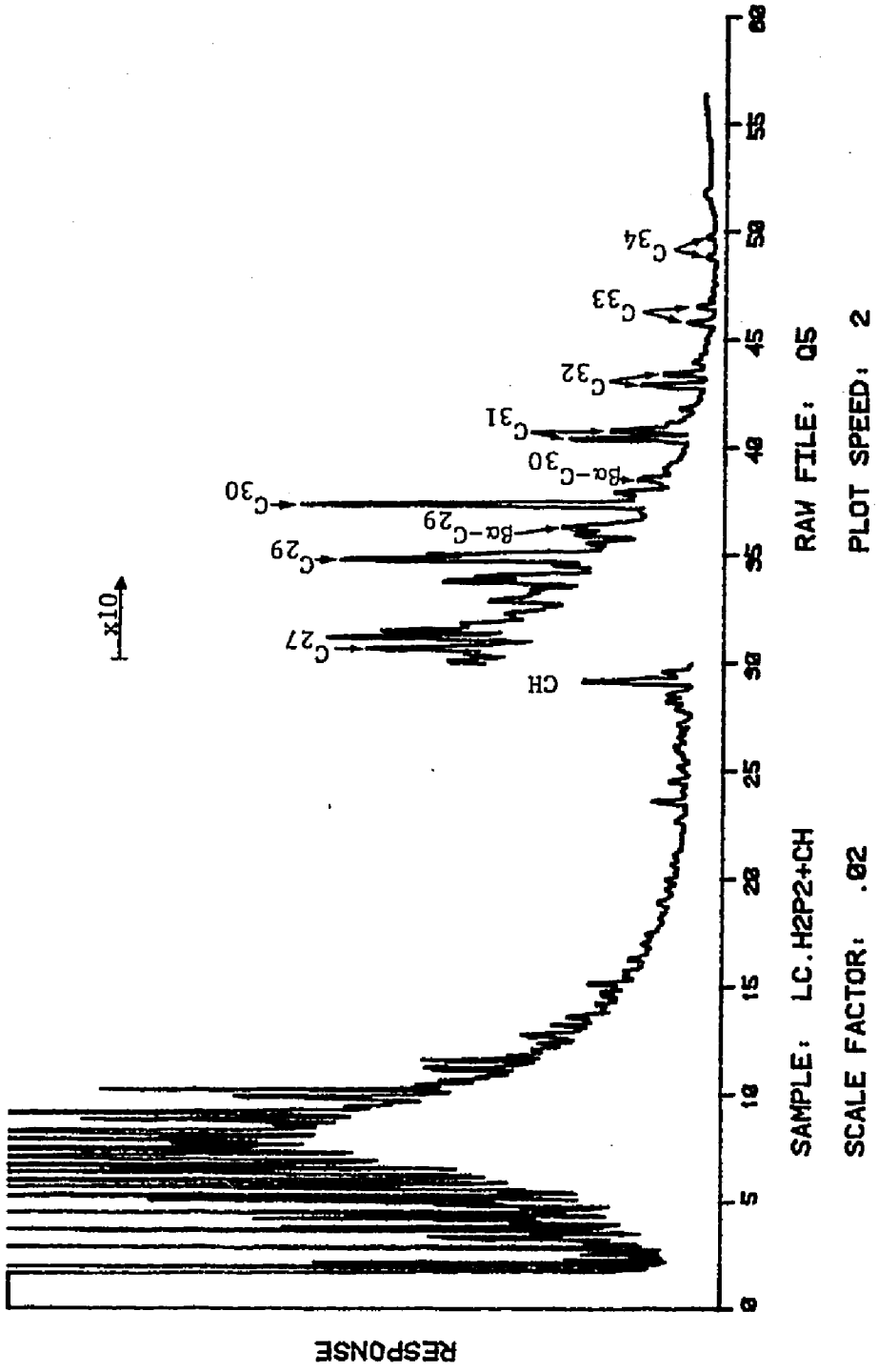

RESPONSE
x10
CH
C_{27}
C_{29}
$Ba-C_{29}$
C_{30}
$Ba-C_{30}$
C_{31}
C_{32}
C_{33}
C_{34}
0
5
10
15
20
25
30
35
40
45
50
55
60
SAMPLE: LC.H2P2+CH
RAW FILE: Q5
SCALE FACTOR: .02
PLOT SPEED: 2

TABLE 7

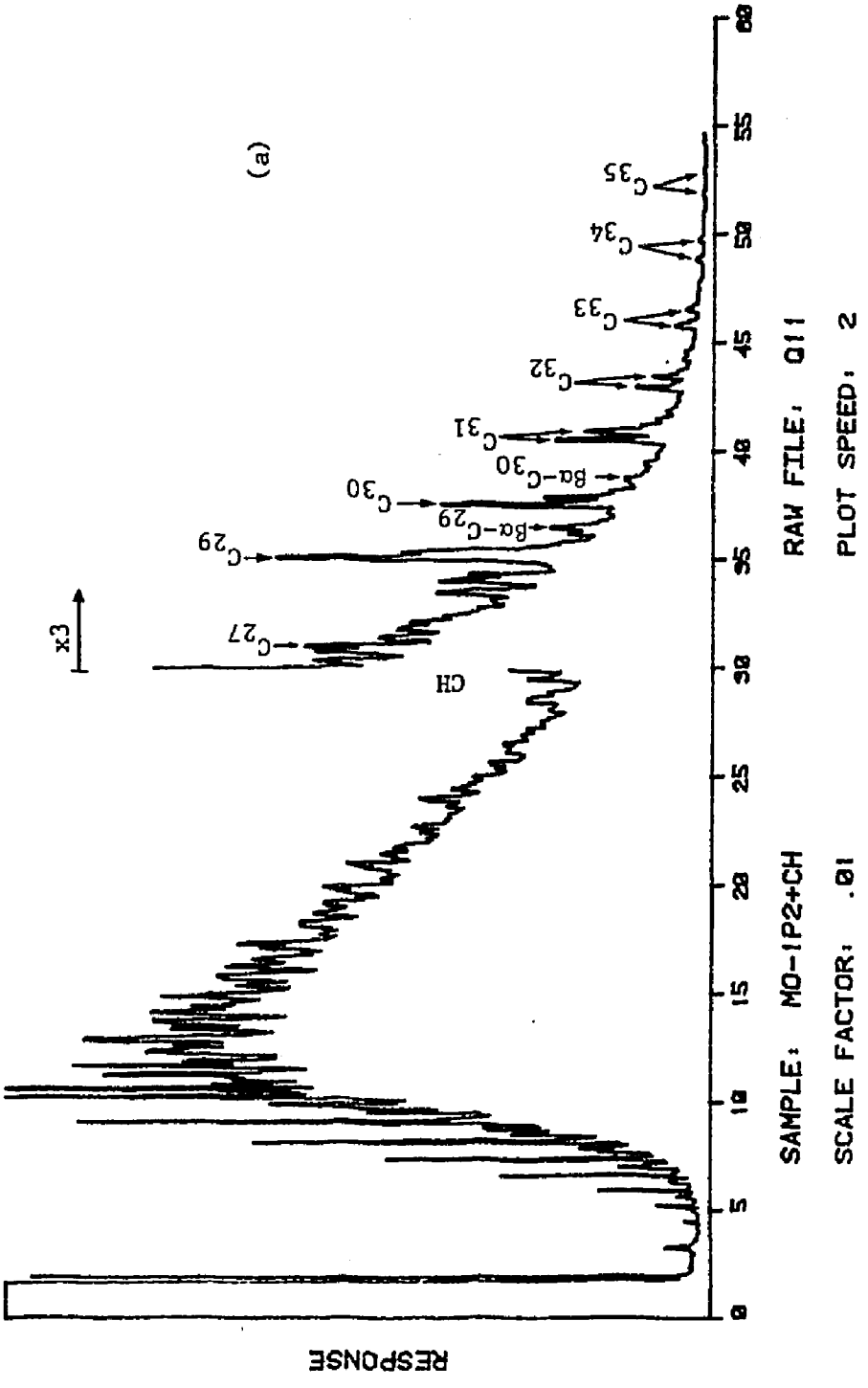
Stereomeric ratios for hopanoids in possible source materials and soil samples.

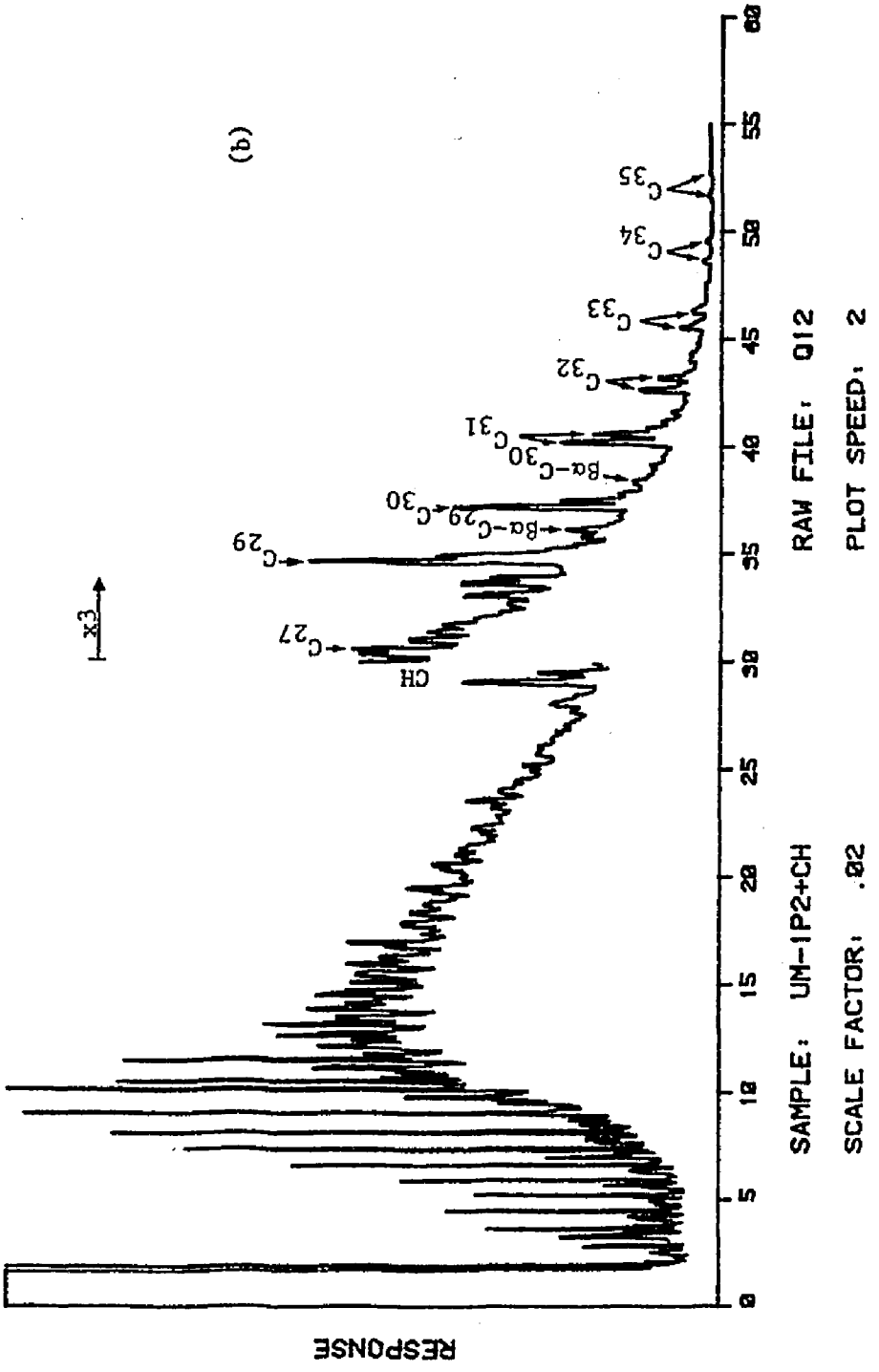
	S:R ($\alpha\beta$)					$\beta\alpha$:$\alpha\beta$	
	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}	C_{29}	C_{30}
Coal	1.3	1.5	N.D.	N.D.	N.D.	0.15	0.25
Asphalt	1.3	1.2	1.3	N.D.	N.D.	0.37	0.19
Libyan crude	1.8	1.6	1.6	1.8	N.D.	0.28	0.11
MO-1	1.6	1.7	1.5	1.7	2.2	0.08	0.07
MO-2	1.7	1.5	1.5	1.6	1.8	0.09	0.05
MO-3	1.5	1.7	1.7	1.5	2.2	0.10	0.05
MO-4	1.4	1.4	1.7	1.4	1.7	0.11	0.05
UM-1	1.6	1.6	1.6	1.8	2.1	0.10	0.05
Surface soils							
Norfolk (N-1)	1.6	1.3	1.1	1.4	1.6	0.33	0.25
Virginia Beach (VB-1)	1.3	1.7	2.2	1.8	2.0	0.31	0.20
Newport News (NN-1)	1.4	1.7	1.9	2.0	1.4	0.30	0.18
White Marsh (17-1)	1.3	1.6	1.4	1.7	2.0	0.42	0.21

N.D.: not determined
MO: unused motor oil
UM: used motor oil

Figure 14. Representative gas chromatograms of hopanes extracted from motor oil

a) unused motor oil
b) used motor oil
CH: cholestane (co-injected standard),
C_{27}-C_{35}: αβ-hopanes.


(a)
RESPONSE
0
5
10
15
20
25
30
35
40
45
50
55
60
SAMPLE: MO-1P2+CH
SCALE FACTOR: .01
RAW FILE: Q11
PLOT SPEED: 2
x3
CH
C_{27}
C_{29}
Ba-C_{29}
C_{30}
Ba-C_{30}
C_{31}
C_{32}
C_{33}
C_{34}
C_{35}


(a)
RESPONSE
SAMPLE: UM-1P2+CH
SCALE FACTOR: .02
RAW FILE: Q12
PLOT SPEED: 2
0
5
10
15
20
25
30
35
40
45
50
55
60
x3
CH
C_{27}
C_{29}
Ba-C_{29}-C_{30}
Ba-C_{30}
C_{31}
C_{32}
C_{33}
C_{34}
C_{35}

Since highways are known contributors of aromatic pollutants such as the PAH's (Waibel, 1976; Giger and Schaffner, 1977; Wakeham et al., 1980) it was logical to analyze samples related to highway use. Hopane concentrations in one asphalt sample were investigated and found to be the highest of all the source materials analyzed. Figure 15 presents the composition of hopanoids in the asphalt sample. 17α(H),21β(H) Hopanes were most prominent and ranged from C_{27} to C_{34}, with the extended hopanes present as mixtures of 22S and 22R diastereomers (Figure 15; Table 6). The S/R ratios were found to be 1.2 - 1.3 for the asphalt sample (Table 7). 17β(H),21α(H)-Hopanes were present at lower concentrations, whereas no 17β(H),21β(H)-hopanes and hopene could be detected. The UCM of the asphalt extract exhibited a bimodal distribution and was different from the UCM of the crude oil and motor oil extracts.

The concentrations and composition of hopanoids in four roadside soil or dust samples are presented in Table 8. The hopanoids in these samples were similar to those found in mature source materials such as crude oil, motor oil and asphalt. 17α(H),21β(H) Hopanes were dominant over the 17β(H),21α(H) hopanes, and no indication of 17β(H),21β(H) hopanes or hopene was observed in the soil samples. Extended 17α(H),21β(H) hopanes were present as mixtures of 22S and 22R diastereomers. Sample 17-1, collected near U.S. Route 17, showed the highest hopane concentrations. The other three surface soil samples contained approximately equal concentrations of hopanoids. A gas chromatogram of hopanoids from a surface soil sample is shown in Figure 16.

Figure 15. Gas chromatogram of hopanes extracted from asphalt
CH: cholestane (co-injected standard),
C_{27}-C_{34}: αβ-hopanes.

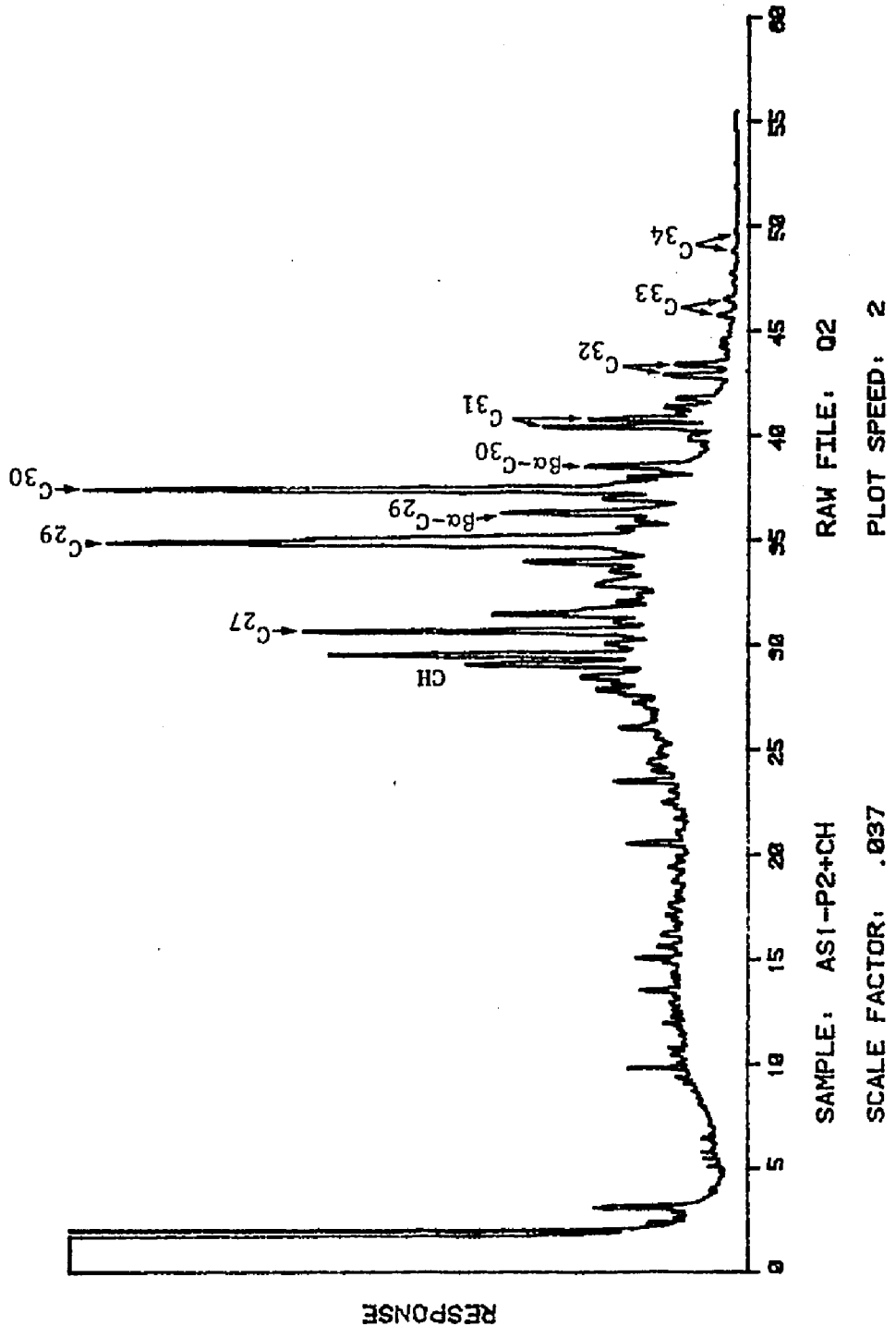

RESPONSE
0
5
10
15
20
25
30
35
40
45
50
55
60
SAMPLE: AS1-P2+CH
SCALE FACTOR: .037
RAW FILE: 02
PLOT SPEED: 2
C_{34}
C_{33}
C_{32}
C_{31}
$\beta\alpha$-C_{30}
$\beta\alpha$-C_{29}
C_{30}
C_{29}
C_{27}
CH

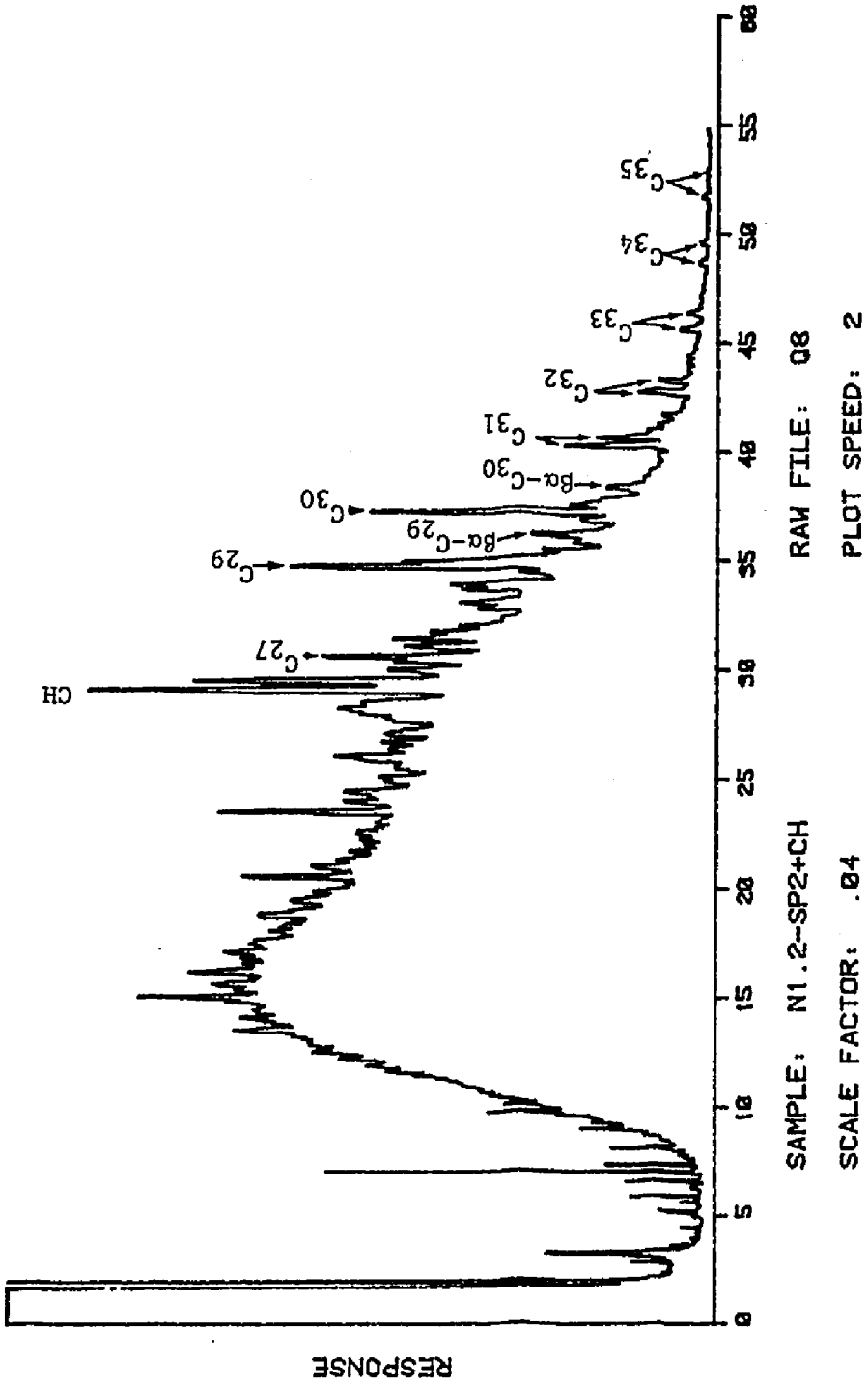
TABLE 8

Hopanoid concentrations in surface soil samples (µg/g OC).

Sample	17α-C_{27}	αβ-C_{29}	βα-C_{29}	αβ-C_{30}	βα-C_{30}	αβ-C_{31}		αβ-C_{32}		αβ-C_{33}		αβ-C_{34}		αβ-C_{35}		% OC
						S	R	S	R	S	R	S	R	S	R	
N-1 Norfolk	107	177	58	147	37	55	34	27	21	18	16	13	9	11	7	0.85
VB-1 Virginia Beach	66	127	40	109	22	82	61	40	23	20	9	9	5	6	3	0.83
NN-1 Newport News	63	122	36	104	19	89	64	50	30	30	16	12	6	10	7	0.72
17-1 White Marsh	223	317	132	298	62	123	92	37	23	15	11	5	3	2	1	1.63

Figure 16. Representative gas chromatograms of hopanes extracted from surface soil sample (Norfolk)

CH: cholestane (co-injected standard),
C_{27}-C_{35}: αβ-hopanes.


RESPONSE
0
5
10
15
20
25
30
35
40
45
50
55
60
CH
C_{27}
C_{29}
$\beta\alpha$-C_{29}
C_{30}
$\beta\alpha$-C_{30}
C_{31}
C_{32}
C_{33}
C_{34}
C_{35}
SAMPLE: N1.2-SP2+CH
SCALE FACTOR: .04
RAW FILE: 08
PLOT SPEED: 2

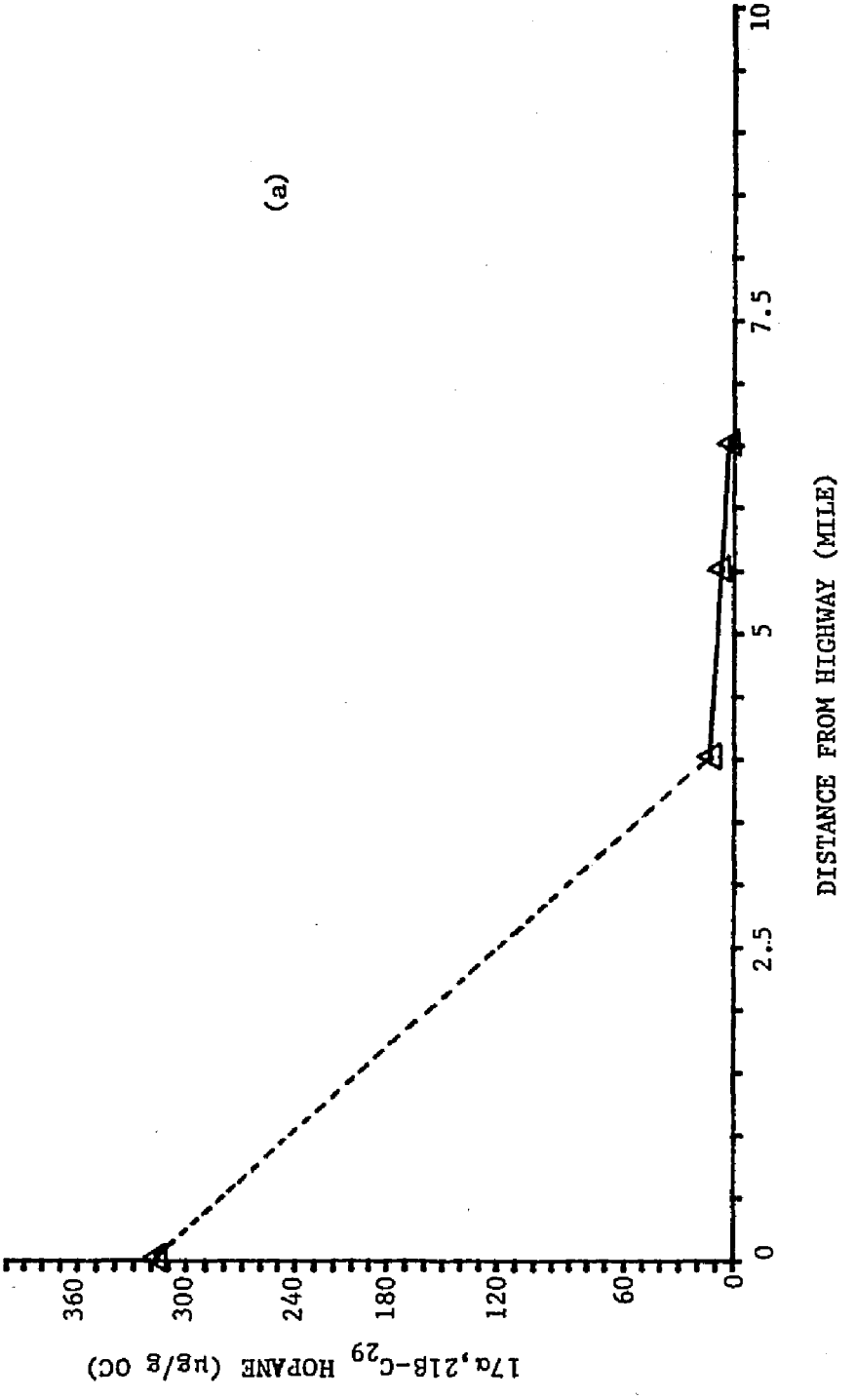
An input of fossil contaminants from automotive traffic is suggested by Figure 17 a&b, which shows concentrations of 17α(H),21β(H)-C_{29} and C_{30} hopanes in soil samples collected at four different locations away from U.S. Route 17 (Table 9).

Finally, because the rivers flowing into the Bay carry eroded sedimentary material, a contribution from eroded sedimentary rock detritus also must be investigated. Since such material is unlikely to be mature in a geological sense, it is necessary to analyze core samples in order to distinguish between such an input and recent diagenesis of biogenic hopanoids.

A core sample (CB-002) taken from Colonial Beach on the Potomac River was analyzed for hopanoid hydrocarbons and acids. Individual hopanoid hydrocarbon concentrations are shown in Table 10. Gas chromatograms of hopanes at this core location are shown in Figure 18. 17α(H),21β(H) Hopanes ranging from C_{27} to C_{35} were recognized in the top sediment layer. The extended hopanes were present as mixtures of 22S and 22R diastereomers. At the lower depths, 17β(H),21β(H)-hopanes (XVIII and XIX) were the dominant series, with 17β(H),21β(H)-homohopane (C_{31}) (XIX-a) as the major compound. Only a single epimer (22R) was present. 17α(H),21β(H) Hopanes (XXII and XXIII) were more abundant than 17β(H),21α(H)-hopanes (XX). Extended 17α(H),21β(H)-C_{31} hopanes were present as a mixture of diastereomers whereas only one of the diastereomers of αβ-C_{32} hopane was found in the subsurface samples, as identified by the Kovats index (KI = 3289). Hop-17(21)-ene (XVII) was the major compound in these samples. Diploptene (XV) was detected in

Figure 17. Concentrations of hopanoids vs distance away from highway

a) $\alpha\beta$-C_{29} hopane
b) $\alpha\beta$-C_{30} hopane


17α,21β-C29 HOPANE (μg/g OC)
360
300
240
180
120
60
0
0
2.5
5
7.5
10
DISTANCE FROM HIGHWAY (MILE)

(a)

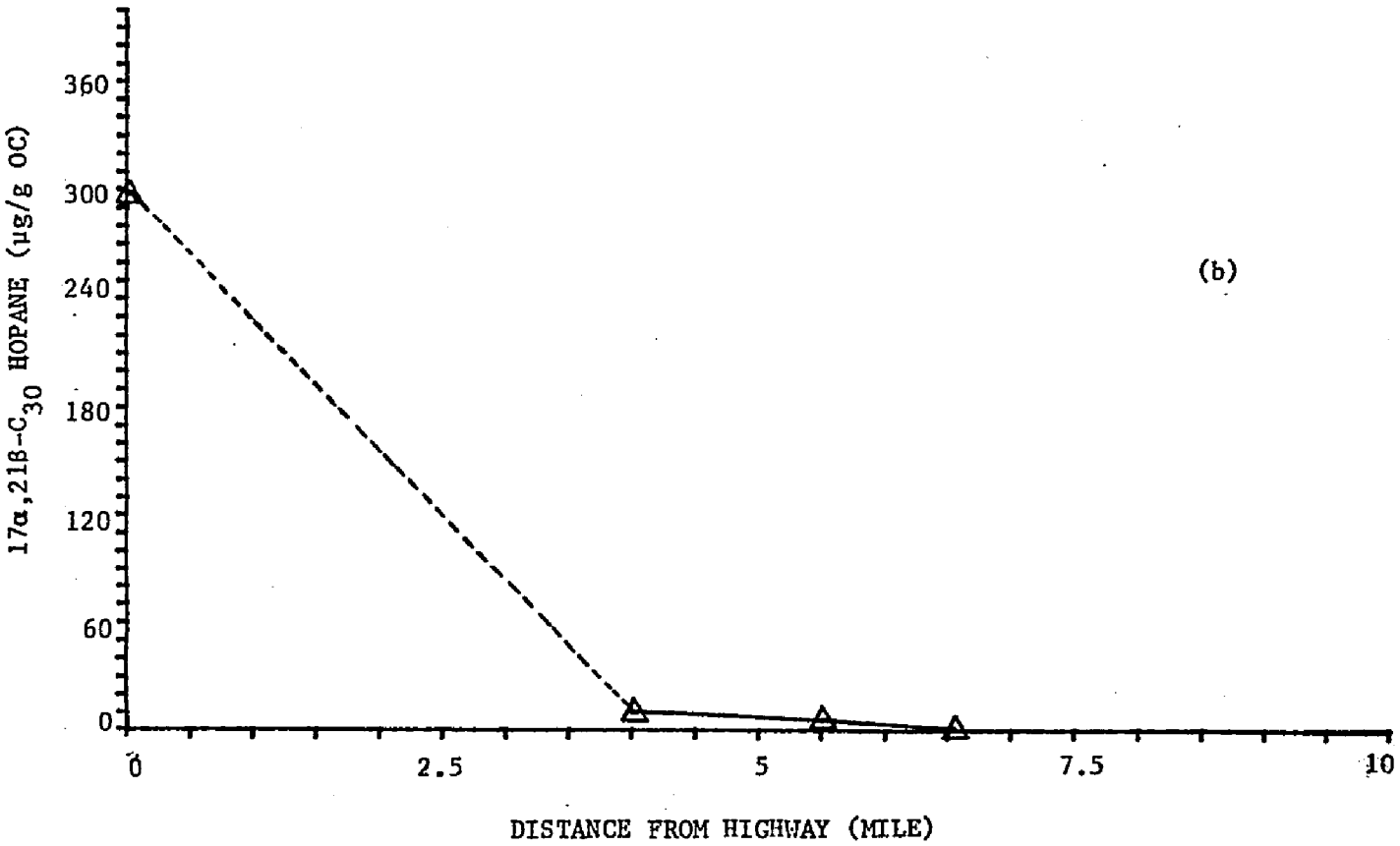

360
300
240
180
120
60
0
17α,21β-C30 HOPANE (µg/g OC)
0
2.5
5
7.5
10
DISTANCE FROM HIGHWAY (MILE)
(b)

TABLE 9

Hopanoid concentrations in surface soils vs. distance away from the highway (μg/g OC).

Distance from Highway (mile)	17α-C_{27}	αβ-C_{29}	βα-C_{29}	αβ-C_{30}	βα-C_{30}	αβ-C_{31}		αβ-C_{32}		αβ-C_{33}		αβ-C_{34}		αβ-C_{35}		% OC
						S	R	S	R	S	R	S	R	S	R	
0	223	317	132	298	62	123	92	37	23	15	11	5	3	2	1	1.63
4	3	13	4	10	2	8	5	4	2	2	2	2	1	1	0.5	0.83
5.5	2	7	3	6	2	4	3	2	2	2	1	1	0.5	1	0.5	0.20
6.5	0.3	2	0.8	1	0.2	3	3	2	1	1	1	0.8	0.7	0.7	0.5	0.60

TABLE 10

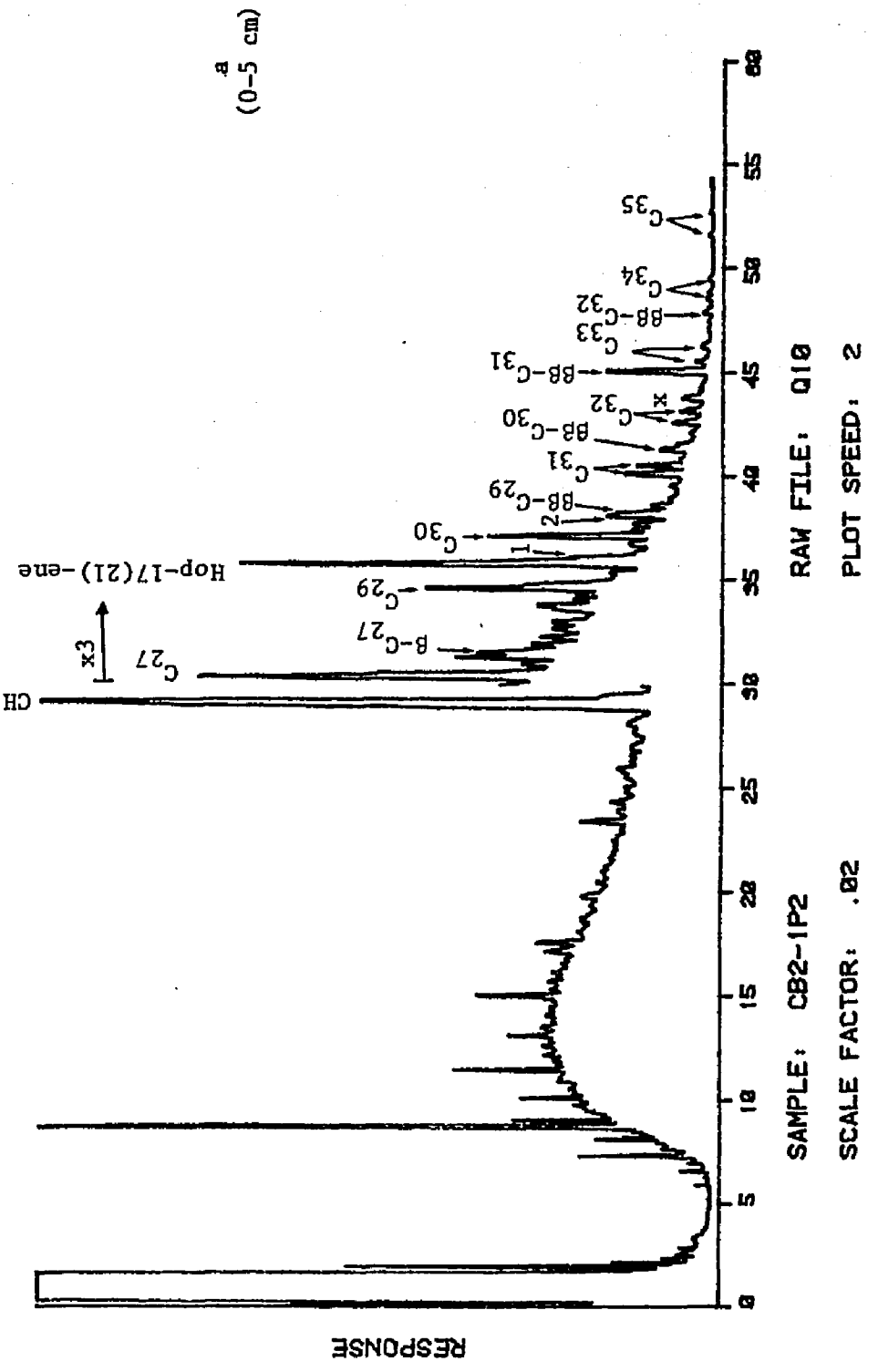
Hopanoid hydrocarbons in sediments from station CB-002 (µg/g OC).

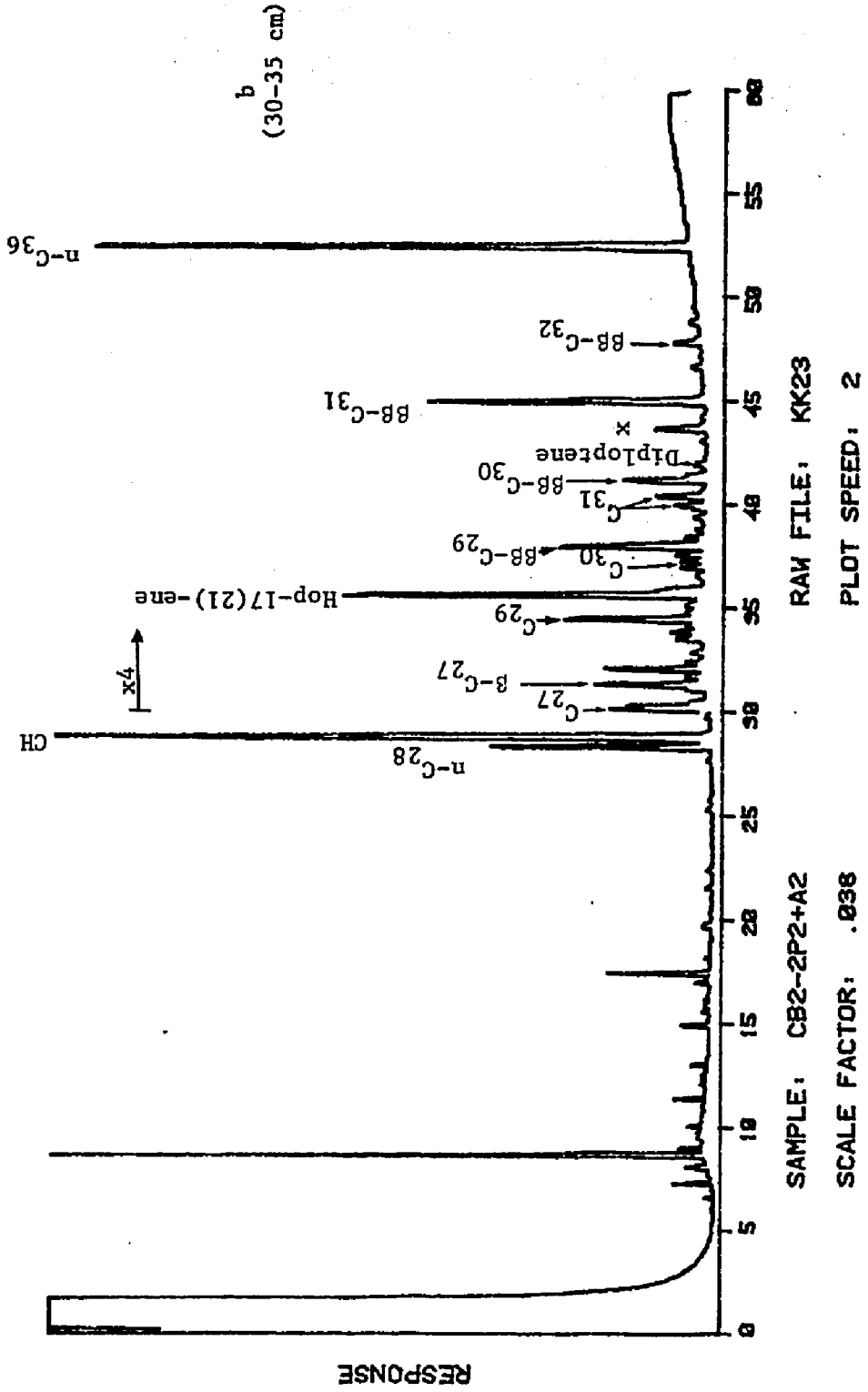
Depth (cm)	17α-C_{27}	17β-C_{27}	αβ-C_{29}	Hop-17 (21)-ene	βα-C_{29}	αβ-C_{30}	βα-C_{30}	ββ-C_{29}	αβ-C_{31} S	αβ-C_{31} R	ββ-C_{30}	Diploptene	αβ-C_{32} S	αβ-C_{32} R	ββ-C_{31}	αβ-C_{33} S	αβ-C_{33} R	αβ-C_{34} S	αβ-C_{34} R	ββ-C_{32}	αβ-C_{35} S	αβ-C_{35} R
0- 5	11	3.7	11	16	2.8	6.4	1.3	2.2	2.4	1.8	1.4	0.20	1.1	0.92	5.2	0.60	0.46	0.23	tr	0.37	tr	tr
30-35	1.8	3.1	3.2	8.3	0.98	0.50	0.72	4.7	0.91	1.2	2.4	0.30	n.d.	tr	6.7	n.d.	n.d.	n.d.	n.d.	0.68	n.d.	n.d.
60- 65	0.11	2.0	4.2	7.2	1.1	0.45	0.78	5.1	0.90	1.2	2.4	0.40	n.d	tr	6.8	n.d.	n.d.	n.d.	n.d.	0.63	n.d.	n.d.
90- 95	0.74	2.3	4.7	7.6	1.2	0.44	0.68	5.6	1.1	1.3	2.5	0.40	n.d.	0.15	6.8	n.d.	n.d.	n.d.	n.d.	0.78	n.d.	n.d.
120-125	0.45	2.9	4.8	7.5	1.2	0.47	0.87	5.9	1.0	1.2	2.6	0.43	n.d.	tr	7.1	n.d.	n.d.	n.d.	n.d.	0.68	n.d.	n.d.
150-155	0.46	2.4	5.0	7.5	1.2	0.42	0.83	5.5	1.0	1.1	2.4	0.48	n.d.	0.14	6.5	n.d.	n.d.	n.d.	n.d.	0.68	n.d.	n.d.

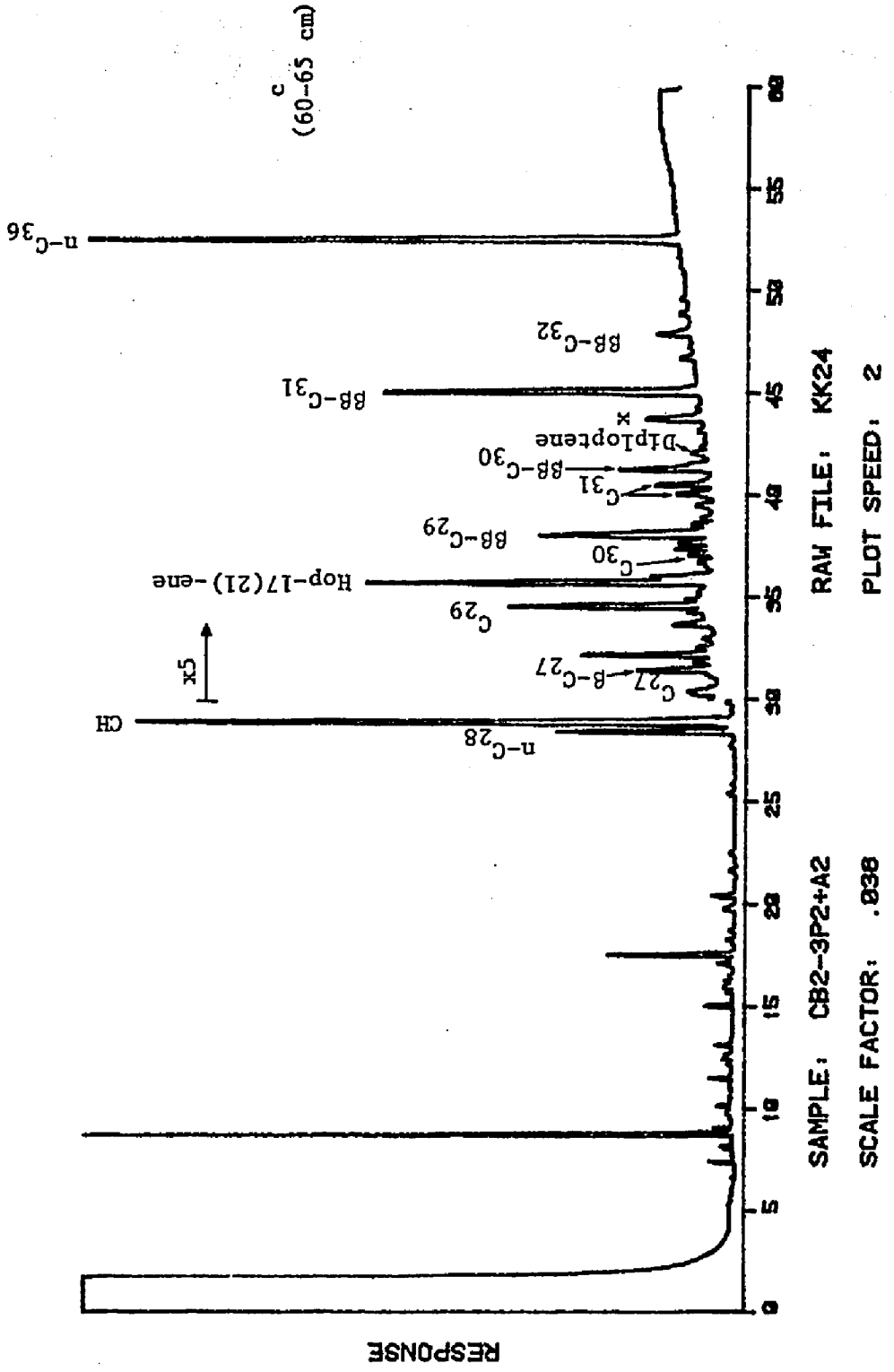
n.d.: not detected
tr: trace

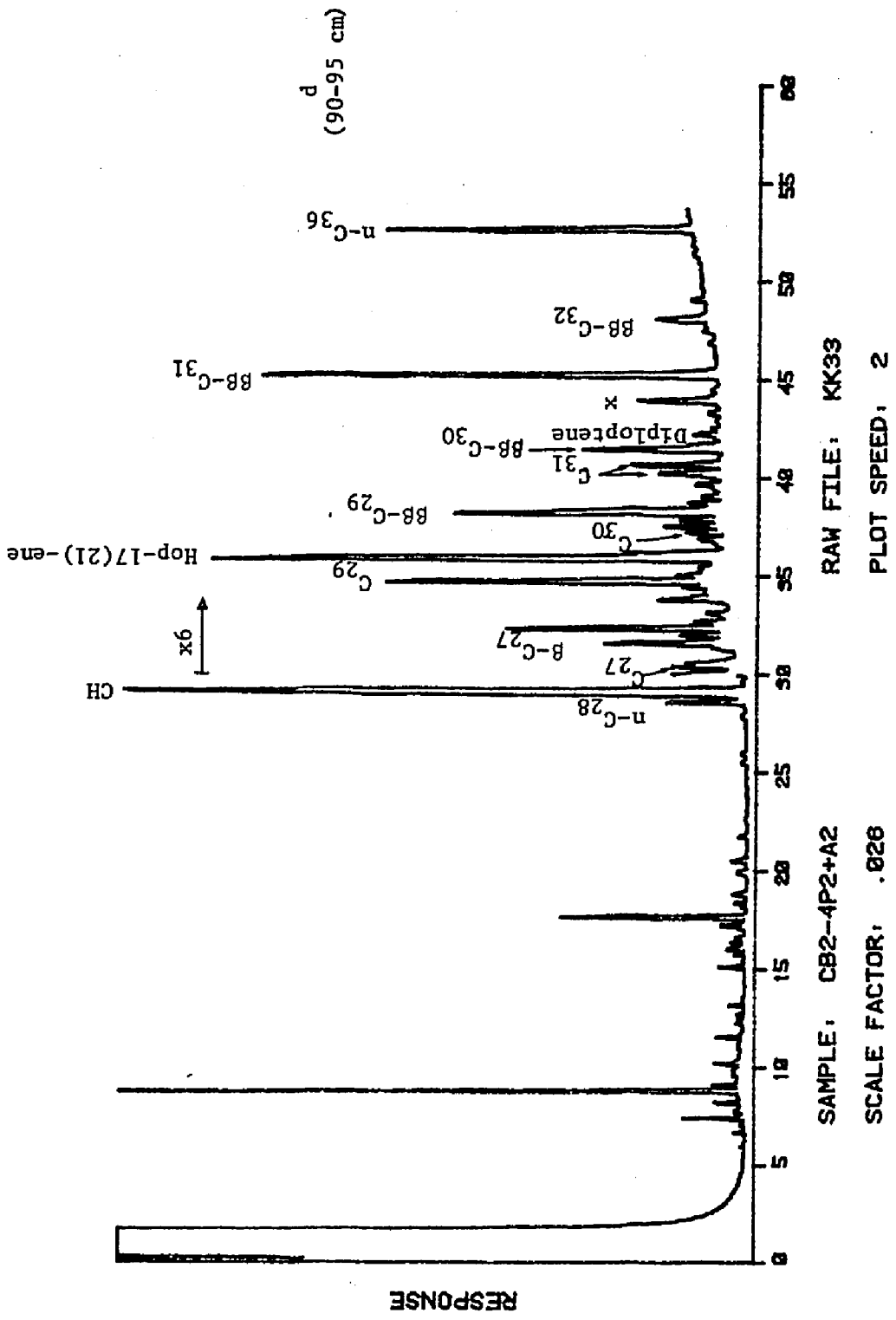
Figure 18. Gas chromatograms of hopanes extracted from Colonial Beach sediment core (CB-002) a-f.

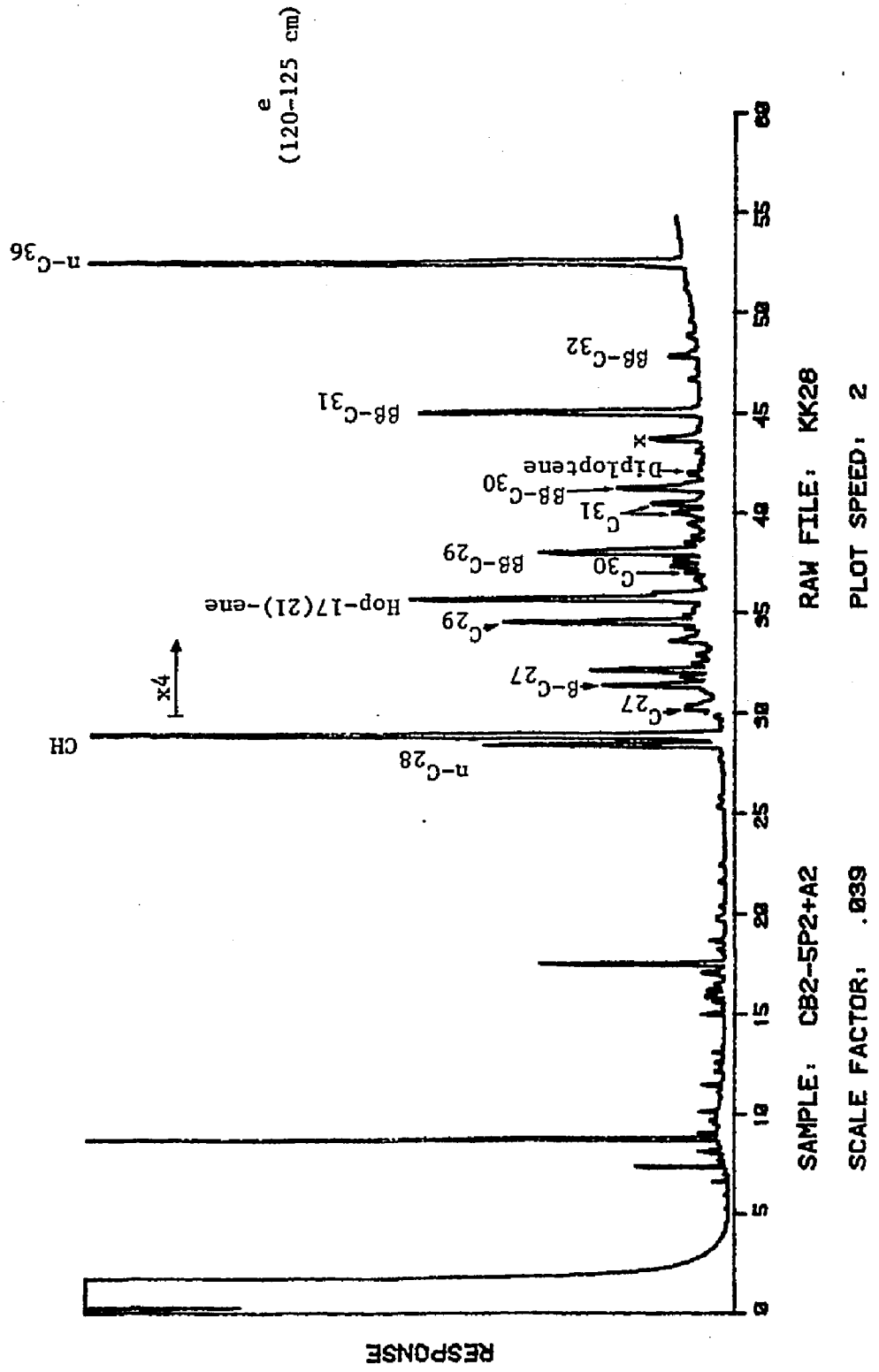
$n\text{-}C_{28}$, $n\text{-}C_{36}$: co-injected standards, CH: cholestane (spike), C_{27}-C_{35}: αβ-hopanes, 1: $\beta\alpha\text{-}C_{29}$ hopane, 2: $\beta\alpha\text{-}C_{30}$ hopane, x: ββ-homohop-29(31)-ene (tentatively identified).

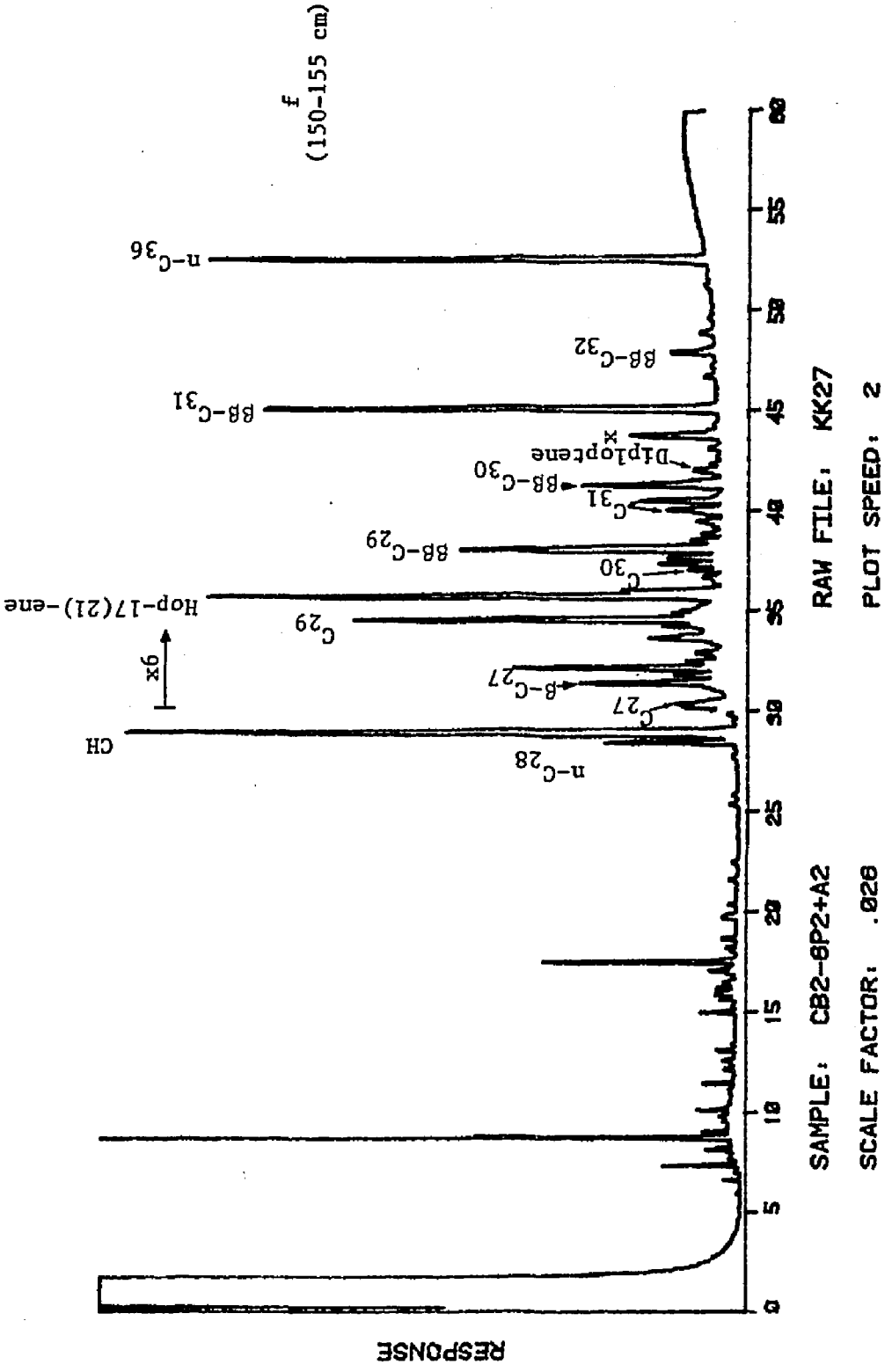

(0-5 cm)a
RESPONSE
SAMPLE: CB2-1P2
SCALE FACTOR: .02
RAW FILE: Q10
PLOT SPEED: 2
CH
C27
x3
Hop-17(21)-ene
C29
B-C27
C30
1
2
BB-C29
C31
BB-C30
C32
x
BB-C31
C33
BB-C32
C34
C35


b
(30-35 cm)
n-C36
BB-C32
BB-C31
x
Diploptene
C31
BB-C30
C30
BB-C29
Hop-17(21)-ene
C29
C27
β-C27
x4
CH
n-C28
RESPONSE
0
5
10
15
20
25
30
35
40
45
50
55
60
SAMPLE: CB2-2P2+A2
RAW FILE: KK23
SCALE FACTOR: .038
PLOT SPEED: 2


RESPONSE
SAMPLE: CB2-3P2+A2
SCALE FACTOR: .838
RAW FILE: KK24
PLOT SPEED: 2
0
5
10
15
20
25
30
35
40
45
50
55
60
n-C36
BB-C32
BB-C31
Diploptene
C31
BB-C30
C30
BB-C29
Hop-17(21)-ene
C29
C27-B-C27
5x
CH
n-C28
(60-65 cm)
c


RESPONSE
SAMPLE: CB2-4P2+A2
SCALE FACTOR: .026
RAW FILE: KK33
PLOT SPEED: 2
(90-95 cm) p
n-C36
ββ-C32
ββ-C31
x
Diploptene
C31
ββ-C30
ββ-C29
C30
Hop-17(21)-ene
C29
9x
β-C27
C27
n-C28
CH


e
(120-125 cm)
n-C36
BB-C32
BB-C31
x
Diaplopterene
BB-C30
C31
BB-C29
C30
Hop-17(21)-ene
C29
B-C27
C27
x4
CH
n-C28
RESPONSE
0
5
10
15
20
25
30
35
40
45
50
55
60
SAMPLE: CB2-5P2+A2
RAW FILE: KK28
SCALE FACTOR: .039
PLOT SPEED: 2


f
(150-155 cm)
n-C36
ββ-C32
ββ-C31
Diploptene
x
C31
ββ-C30
C30
ββ-C29
Hop-17(21)-ene
C29
9x
C27
β-C27
n-C28
CH
RESPONSE
0
5
10
15
20
25
30
35
40
45
50
55
60
SAMPLE: CB2-6P2+A2
RAW FILE: KK27
SCALE FACTOR: .028
PLOT SPEED: 2

all sections of this core, but its concentration was small compared to hop-17(21)-ene (about 1 - 7 % of hop-17(21)-ene: see Table 10). ββ-homohop-29(31)-ene was tentatively identified in all sections of the core by comparison of the mass spectra with that published by Van Dorsselaer (1975).

Hop-17(21)-ene, 17α(H),21β(H)-C_{29} hopane, (XXII-b) and 17α(H),21β(H)-C_{30} hopane (XXII-c) were highest at the surface, being present in concentrations of 16, 11 and 6 μg/g OC, respectively. The concentrations decreased rapidly in the upper 30 cm and remained essentially constant at the lower depths (Figure 19). 17β(H),21β(H)-C_{30} and C_{31} hopanes increased in concentrations between the surface and the 30 cm depth and then also remained constant throughout the rest of the core (Figure 19).

Figure 20 presents gas chromatograms of the methyl esters of hopanoid acids extracted from the Colonial Beach sediments. Eight hopanoid acids were identified in the surface sample. All samples contained hopanoid acids ranging from C_{31} to C_{33}. The series has the 17β(H),21β(H) stereochemistry (XXVI and XXVII) in all cases, with only one epimer (22R) present. 17β(H),21β(H)-C_{32} hopanoid acid (XXVII-b) was the major acid in all samples (Table 11). 17α(H),21β(H) Hopanoid acids and 17β(H),21α(H) hopanoid acids were tentatively identified in some sections of the core (Table 11) by their mass spectra and GC elution pattern. Only one epimer (22R) of these acids was present. The vertical distributions of the 17β(H),21β(H) acids are shown in Figure

Figure 19. Vertical distribution of hopanoid hydrocarbons in the sediment core at station CB-002.

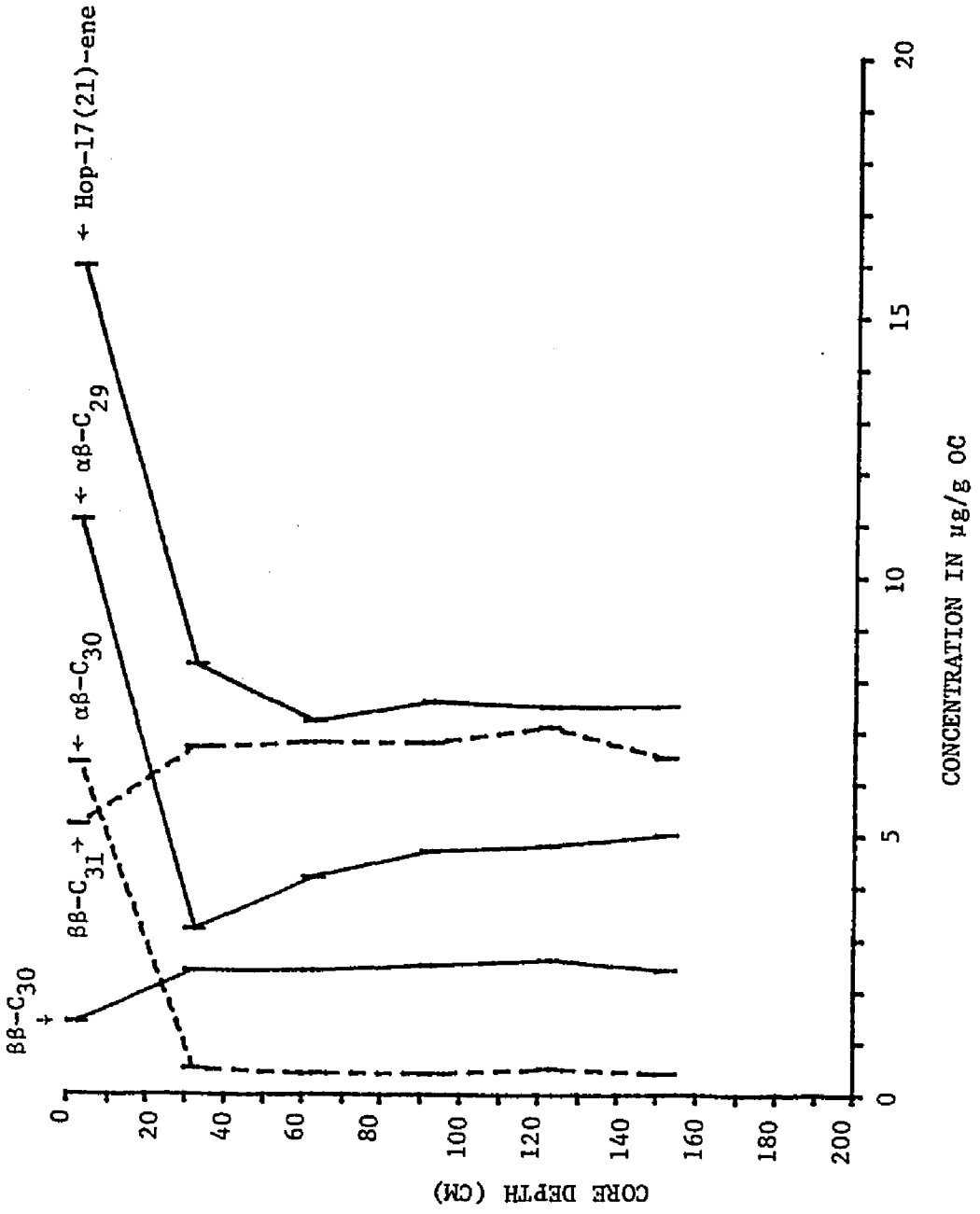
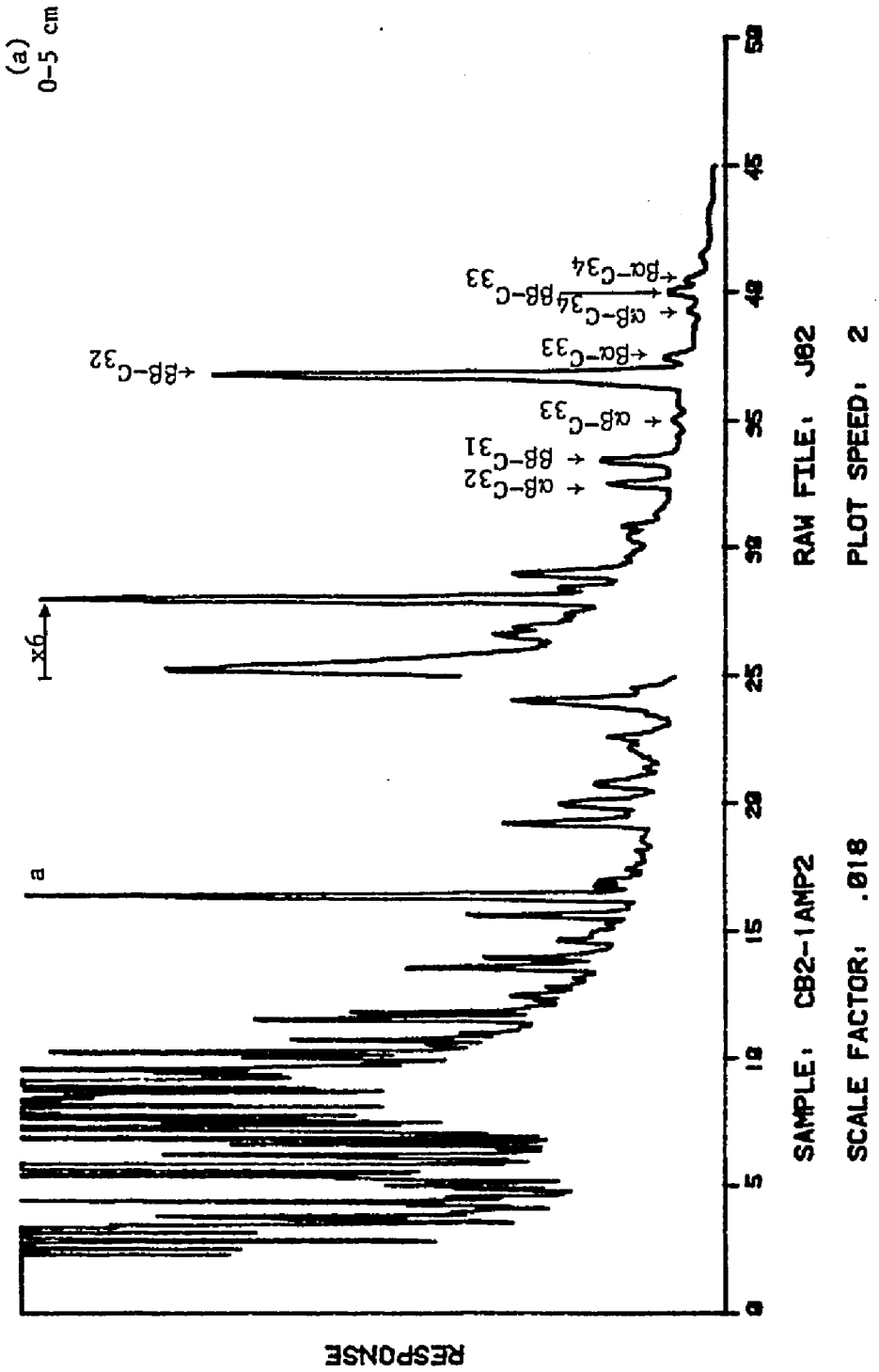
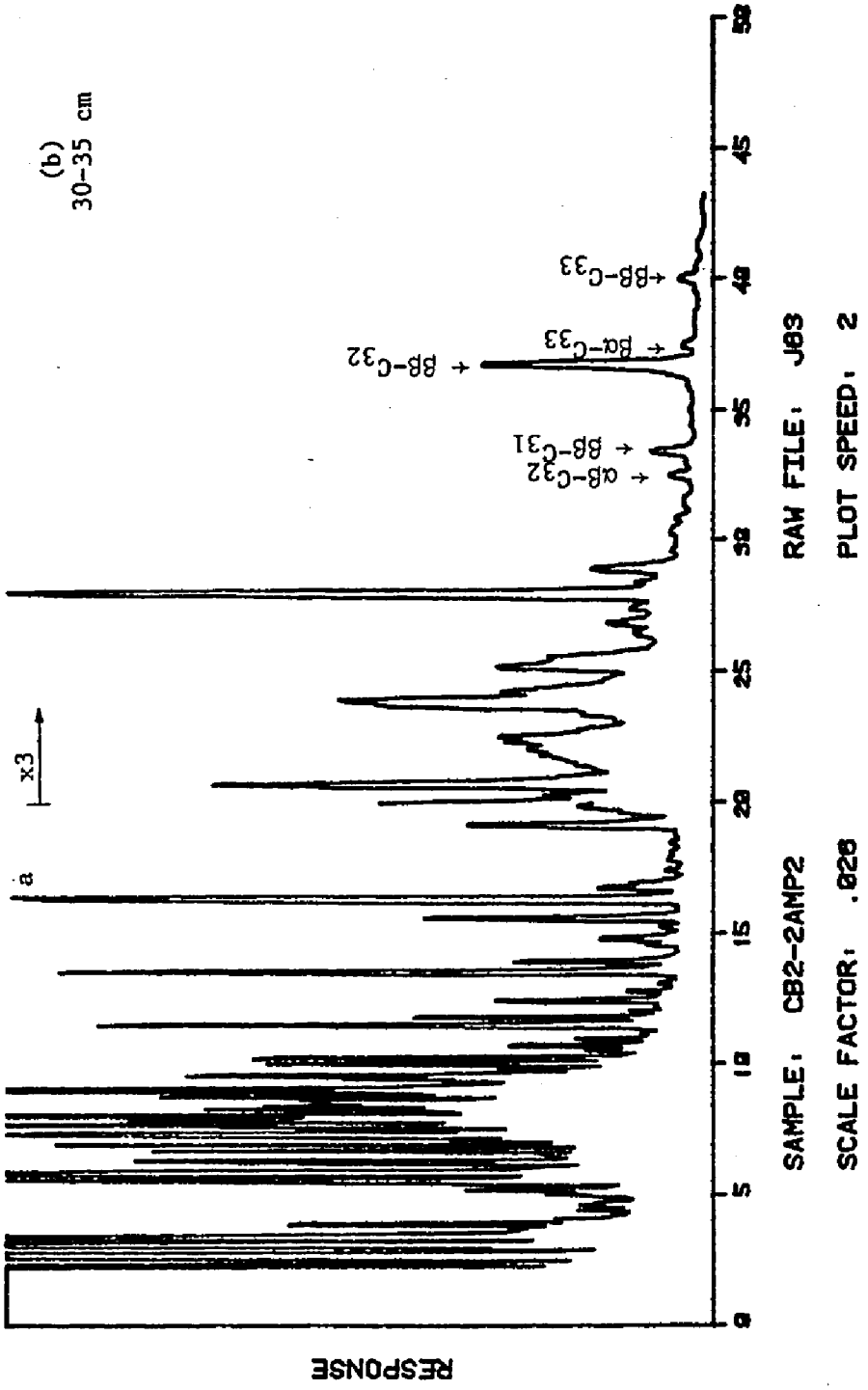
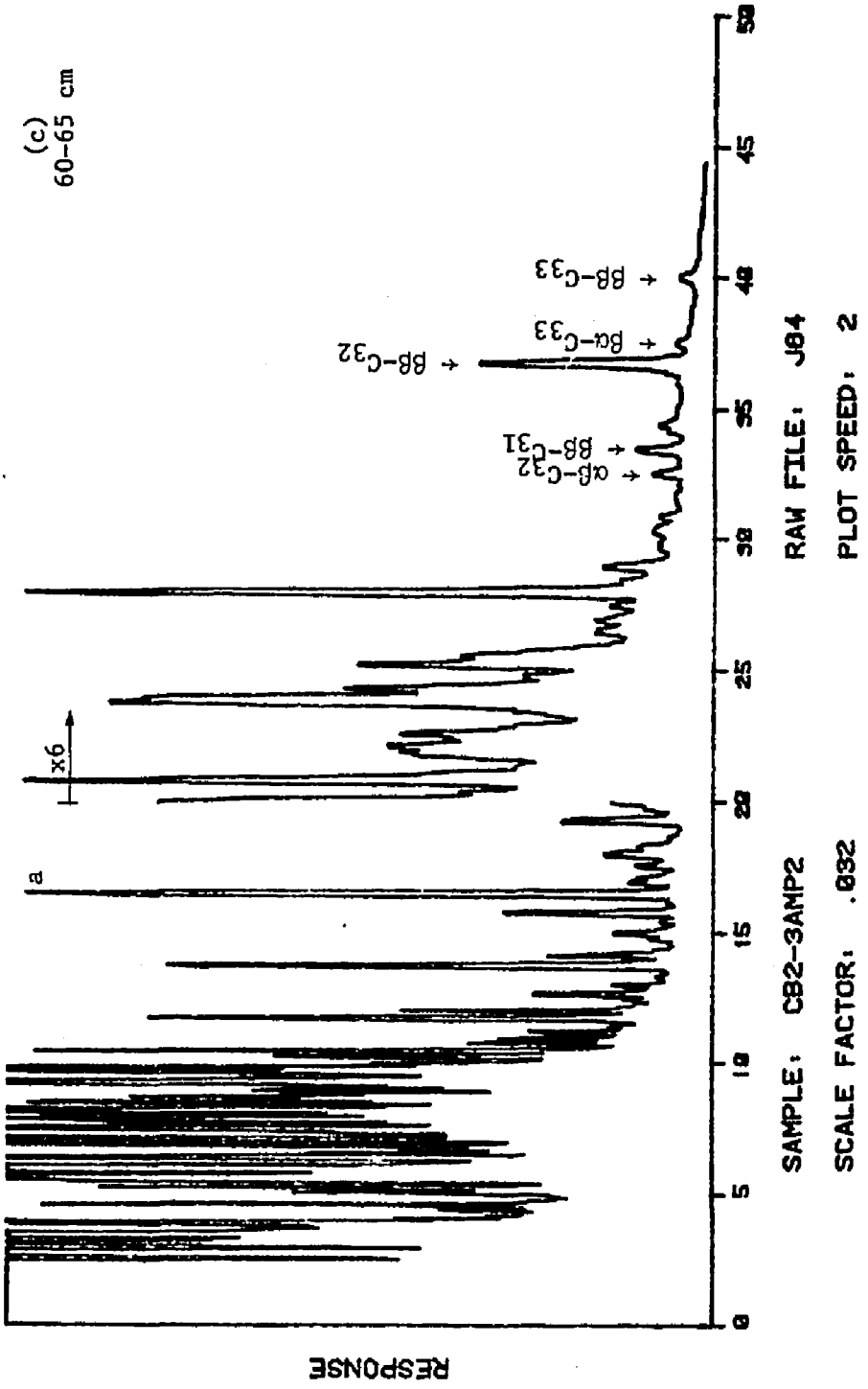

ββ-C30
ββ-C31
αβ-C30
αβ-C29
Hop-17(21)-ene
0
20
40
60
80
100
120
140
160
180
200
CORE DEPTH (CM)
0
5
10
15
20
CONCENTRATION IN µg/g OC

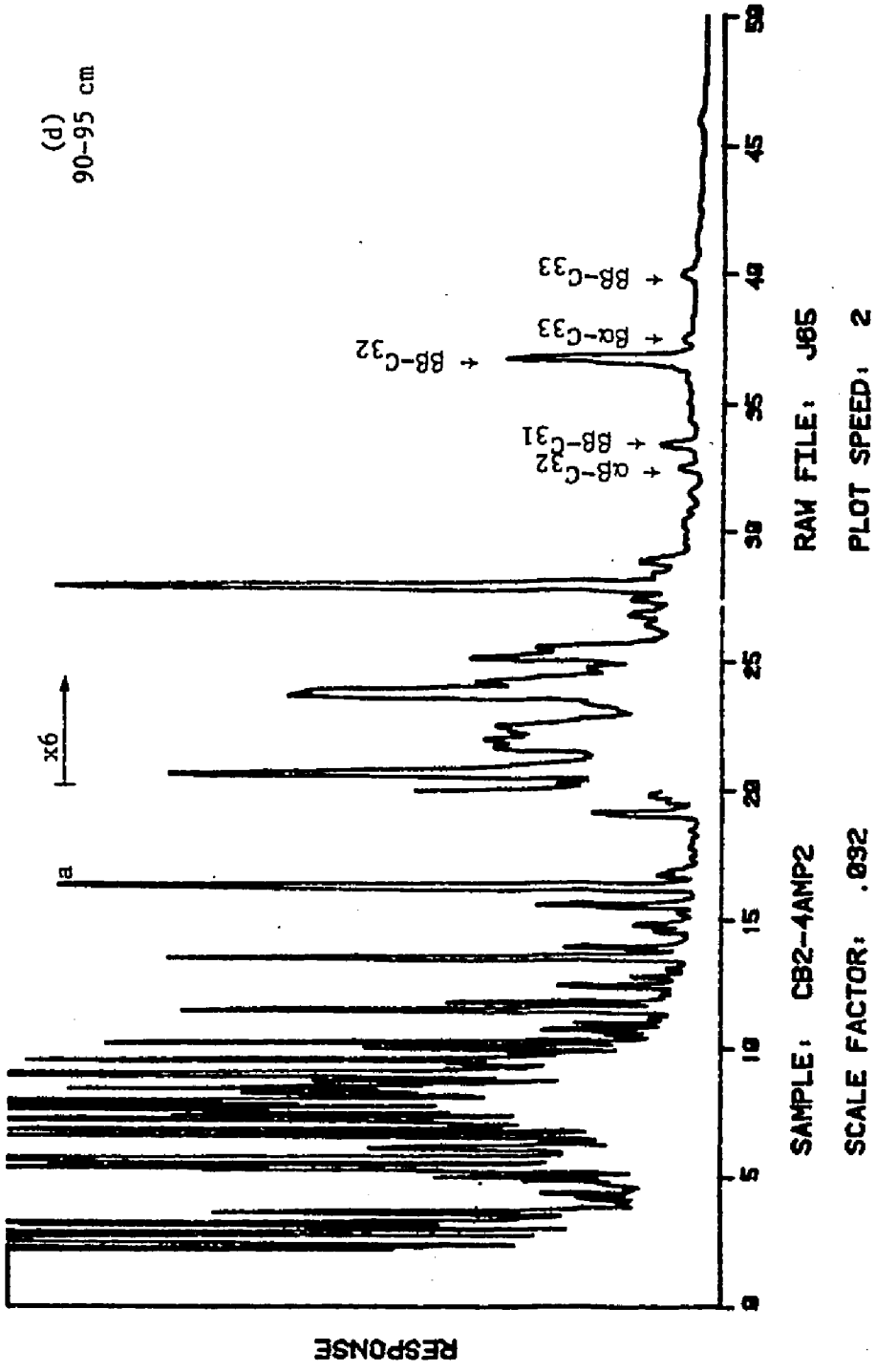
Figure 20. Gas chromatograms of hopanoid acid methyl esters in the sediment core at station CB-002 (a-f)

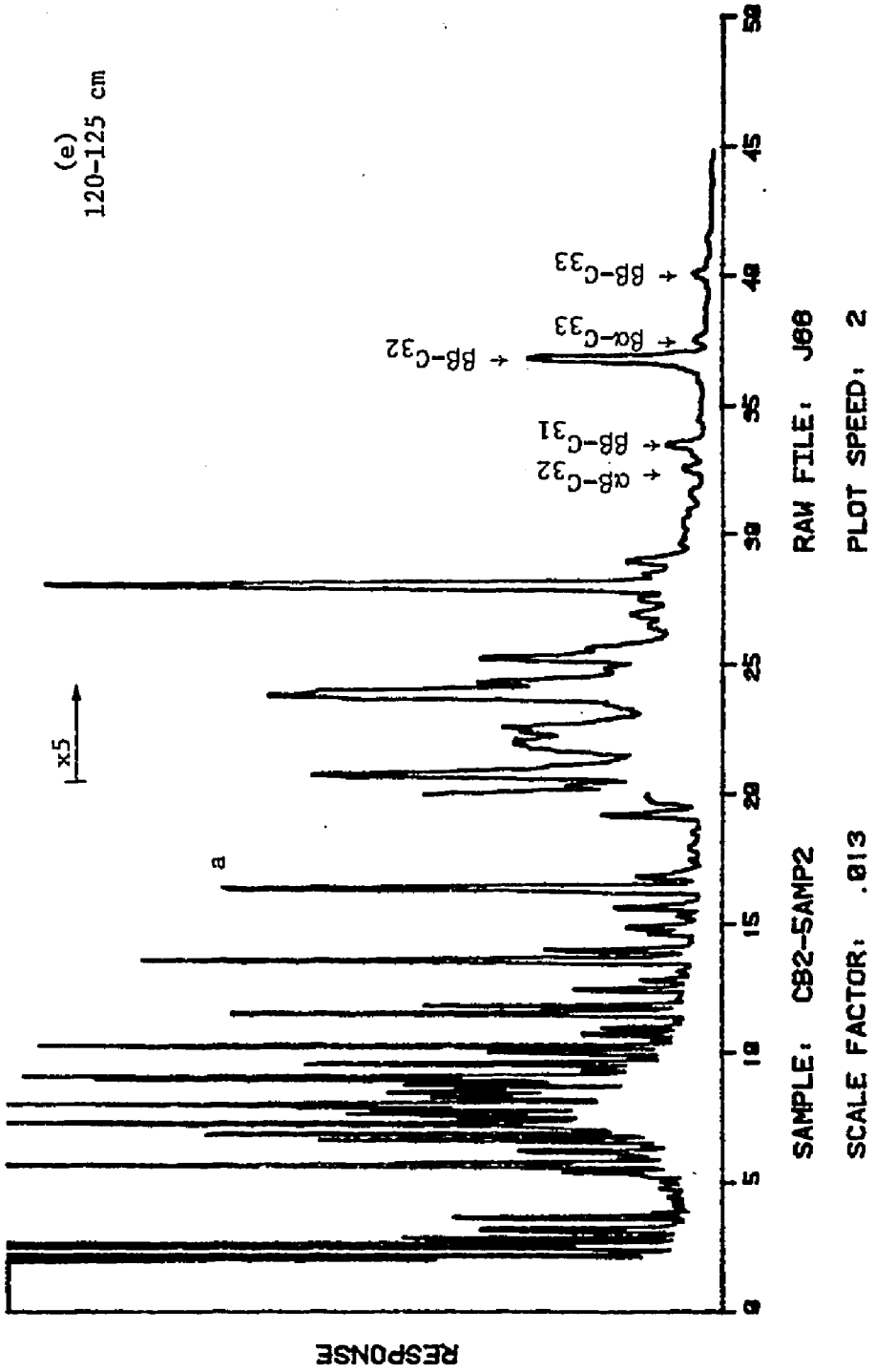
a: cholanic acid methyl ester (spike).


(a)
0-5 cm
RESPONSE
SAMPLE: CB2-1AMP2
SCALE FACTOR: .018
RAW FILE: J82
PLOT SPEED: 2
a
gx
αβ-C32
ββ-C31
αβ-C33
ββ-C32
βα-C33
αβ-C34
ββ-C33
βα-C34


(b)
30-35 cm
x3
a
αβ-C32
ββ-C31
ββ-C32
βα-C33
ββ-C33
RESPONSE
SAMPLE: CB2-2AMP2
RAW FILE: J83
SCALE FACTOR: .028
PLOT SPEED: 2


(c)
60-65 cm
a
x6
← ββ-C33
← βα-C33
← ββ-C32
← αβ-C32
← ββ-C31
RESPONSE
0
5
10
15
20
25
30
35
40
45
50
SAMPLE: CB2-3AMP2
RAW FILE: J84
SCALE FACTOR: .032
PLOT SPEED: 2


(d)
90-95 cm
RESPONSE
a
x6
+ αβ-C32
+ ββ-C31
+ ββ-C32
+ βα-C33
+ ββ-C33
0
5
10
15
20
25
30
35
40
45
50
SAMPLE: CB2-4AMP2
RAW FILE: J85
SCALE FACTOR: .032
PLOT SPEED: 2


(e)
120-125 cm
x5
a
← αβ-C32
← ββ-C31
← ββ-C32
← βα-C33
← ββ-C33
RESPONSE
SAMPLE: CB2-SAMP2
RAW FILE: J08
SCALE FACTOR: .813
PLOT SPEED: 2

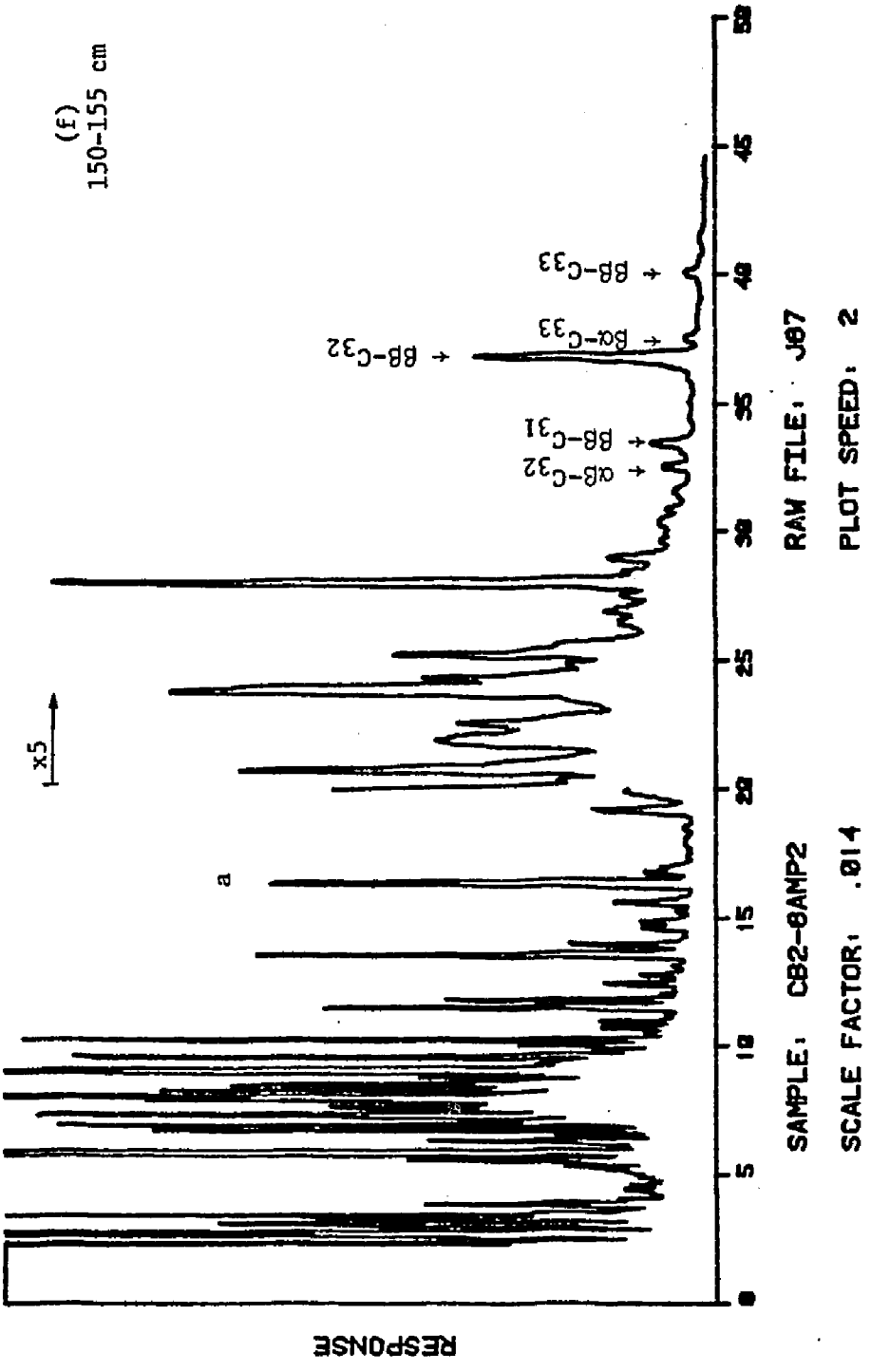

(f)
150-155 cm
x5
a
+ αβ-C32
+ ββ-C31
+ ββ-C32
+ βα-C33
+ ββ-C33
RESPONSE
SAMPLE: CB2-8AMP2
SCALE FACTOR: .014
RAW FILE: J07
PLOT SPEED: 2

TABLE 11

Hopanoid acids identified in sediments from core CB 002 (µg/g OC).

Depth	αβ-C_{32}	ββ-C_{31}	αβ-C_{33}	ββ-C_{32}	βα-C_{33}	αβ-C_{34}	ββ-C_{33}	βα-C_{34}	Total
0- 5	18.4	25.3	3.0	198.0	10.8	3.0	12.1	6.7	278.2
30- 35	1.7	3.2	N.D.	20.2	11.0	N.D.	1.4	N.D.	37.5
60- 65	0.6	1.1	N.D.	6.3	tr	N.D.	0.5	N.D.	8.5
90- 95	0.6	1.0	N.D.	6.0	0.3	N.D.	0.5	N.D.	7.8
120-125	0.8	2.0	N.D.	9.7	0.5	N.D.	0.7	N.D.	13.7
150-155	1.0	1.2	N.D.	10.3	0.5	N.D.	0.8	N.D.	13.8

N.D.: Not detected
tr: trace

21. The concentrations of all acids decreased rapidly between the surface and a depth of 30 cm.

Figure 22 presents gas chromatograms of the hexane fraction from station CB-002. The total aliphatic hydrocarbon content in the surface sediment was 3430 µg/g OC and decreased to less than 500 µg/g OC at lower depths (Table 12). n-Alkanes in the surface sample reflected a predominantly terrestrial plant wax input as evidenced by their odd-even distribution and a maximum at n-C_{29} (Eglinton and Hamilton, 1963). Algal residues generally yield hydrocarbon chains in the range from 16 to 19 carbons (Han and Calvin, 1969; Blumer et al., 1971). Such hydrocarbons represented only a minor fraction in the sample.

The distributions of n-alkanes in the deeper samples also indicated a predominantly terrigenous (higher plant wax) origin. However, n-alkanes in the deeper samples exhibit high concentrations of n-C_{23} and n-C_{24}, which might suggest an origin from microbially altered algal detritus (Cranwell, 1973; Johnson and Calder, 1973).

Unresolved complex mixtures (UCM) were present in the surface layer and, to a lesser extent, in the deepest section of the core (Table 12). Pristane was not detected in all sections of the core, whereas low concentrations of phytane were present in the first two sections.

Total aromatic hydrocarbons also showed a drop in concentration from the surface value of 887 µg/g OC to 170 µg/g OC at a depth of 30 cm. A gradual increase in concentration with depth was observed between 60 to 150 cm. Perylene was the major PAH in all samples from core CB-002 and was found to increase with depth (Table 12). A large UCM in the aromatic fraction of the surface sample was observed (Table 12).

Figure 21. Vertical distribution of hopanoid acids in the sediment core at station CB-002.

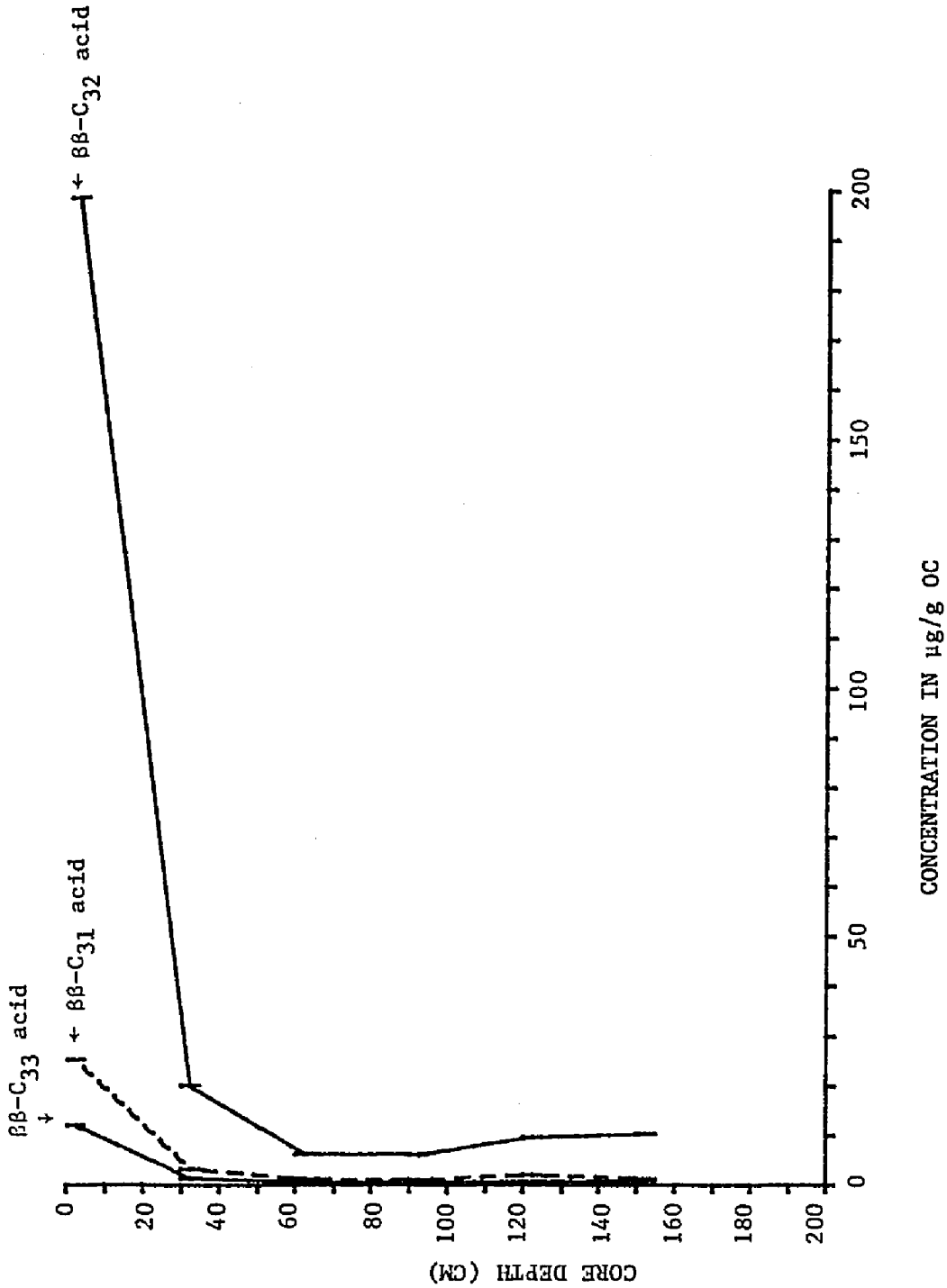
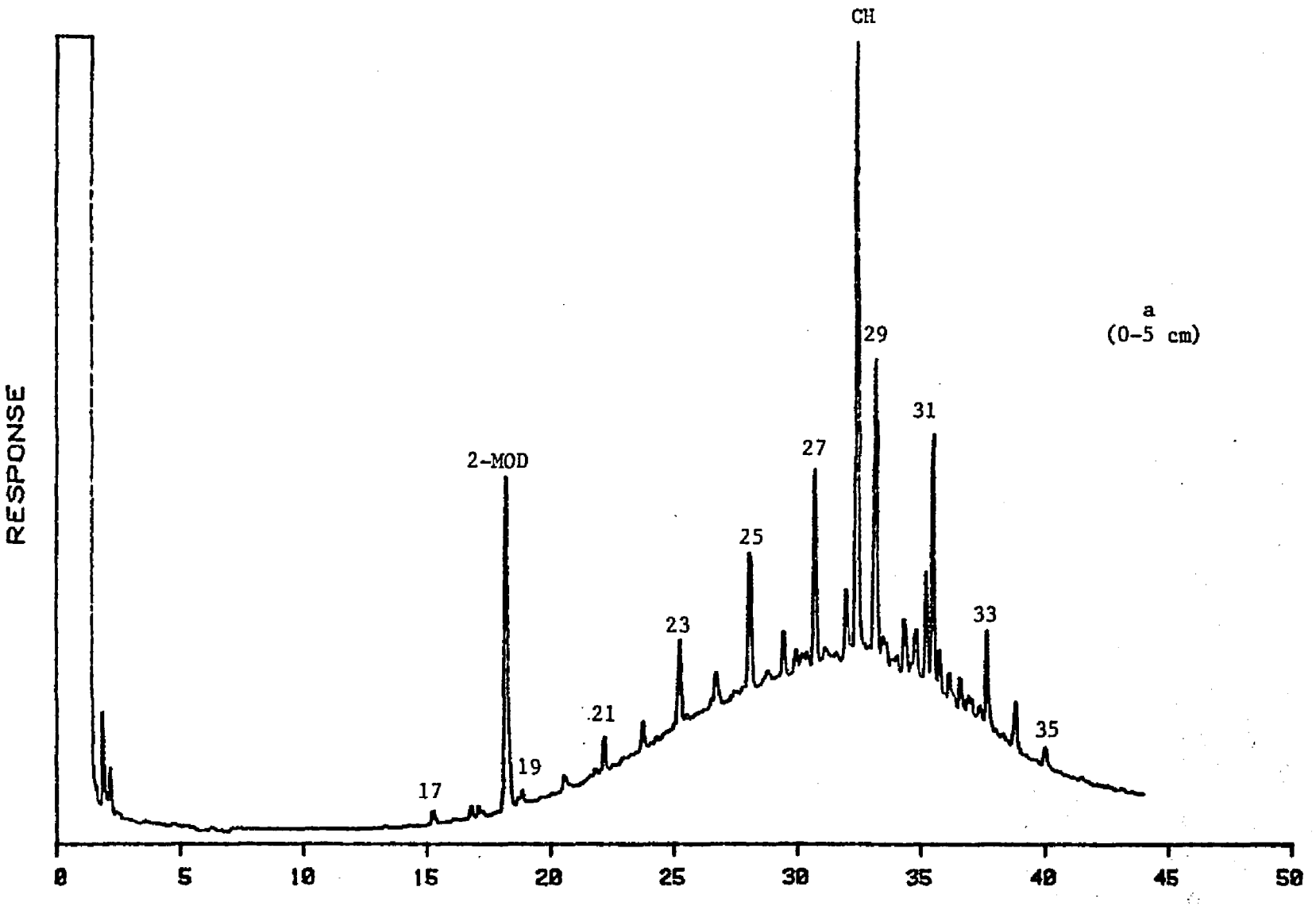

ββ-C32 acid
ββ-C31 acid
ββ-C33 acid
CORE DEPTH (CM)
CONCENTRATION IN µg/g OC
0
20
40
60
80
100
120
140
160
180
200
0
50
100
150
200

Figure 22. Gas chromatograms of hexane fractions in the sediment core at station CB-002 (a-f)

N-alkanes are numbered according to number of carbons in compound, 2-MOD: 2-methyloctadecane (spike), CH: cholestane (spike).

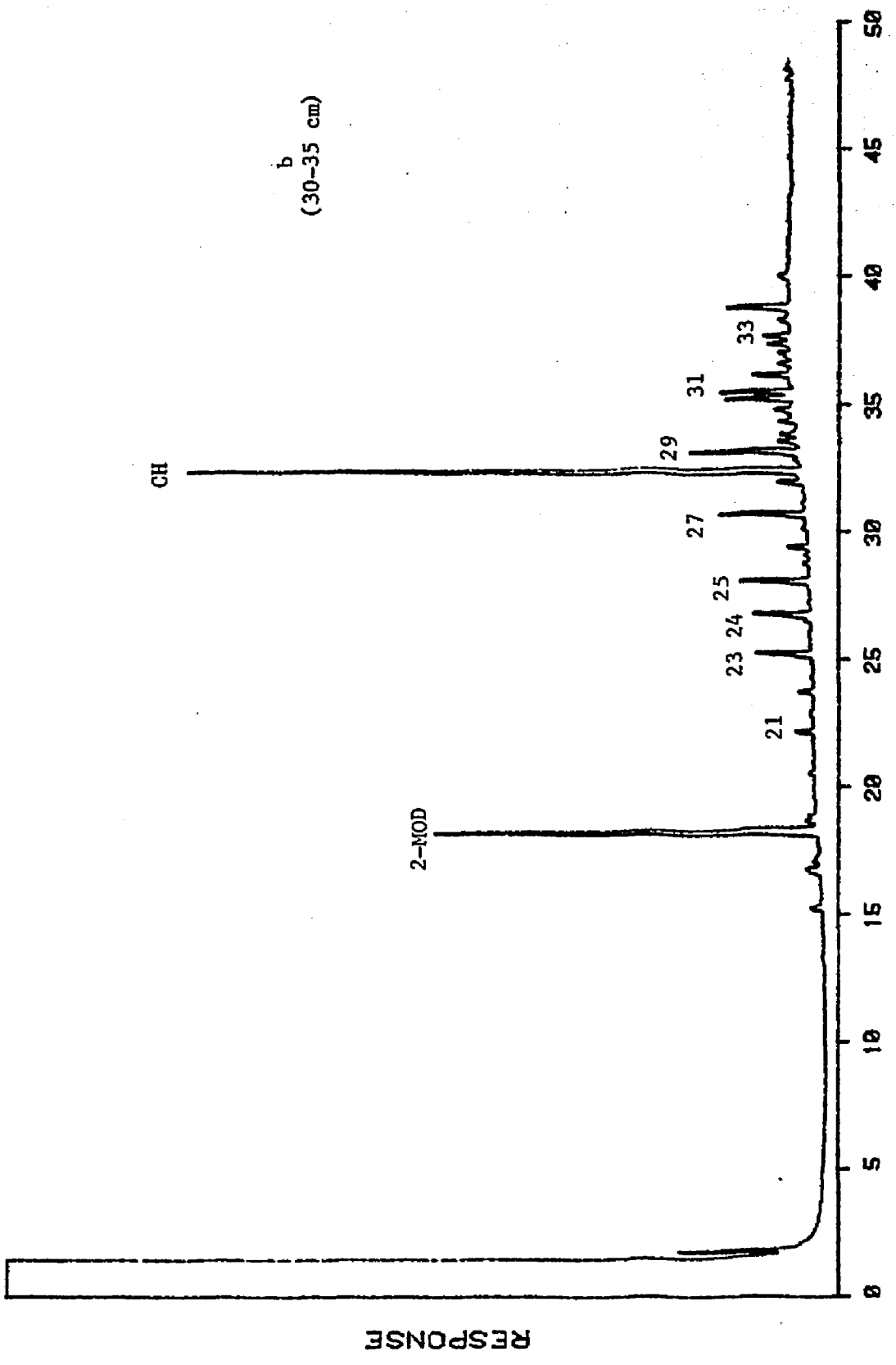


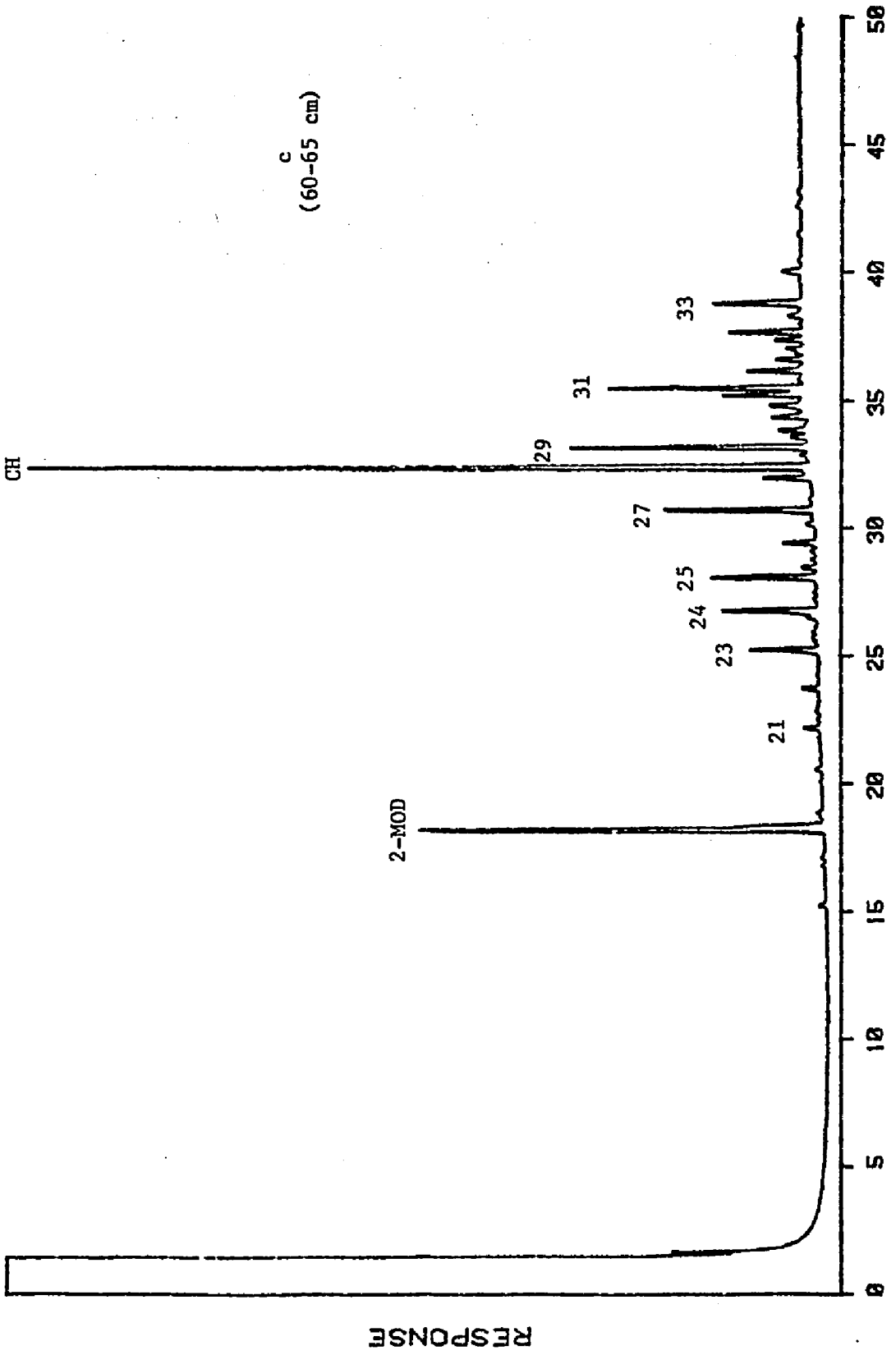
SAMPLE: CB2-1H

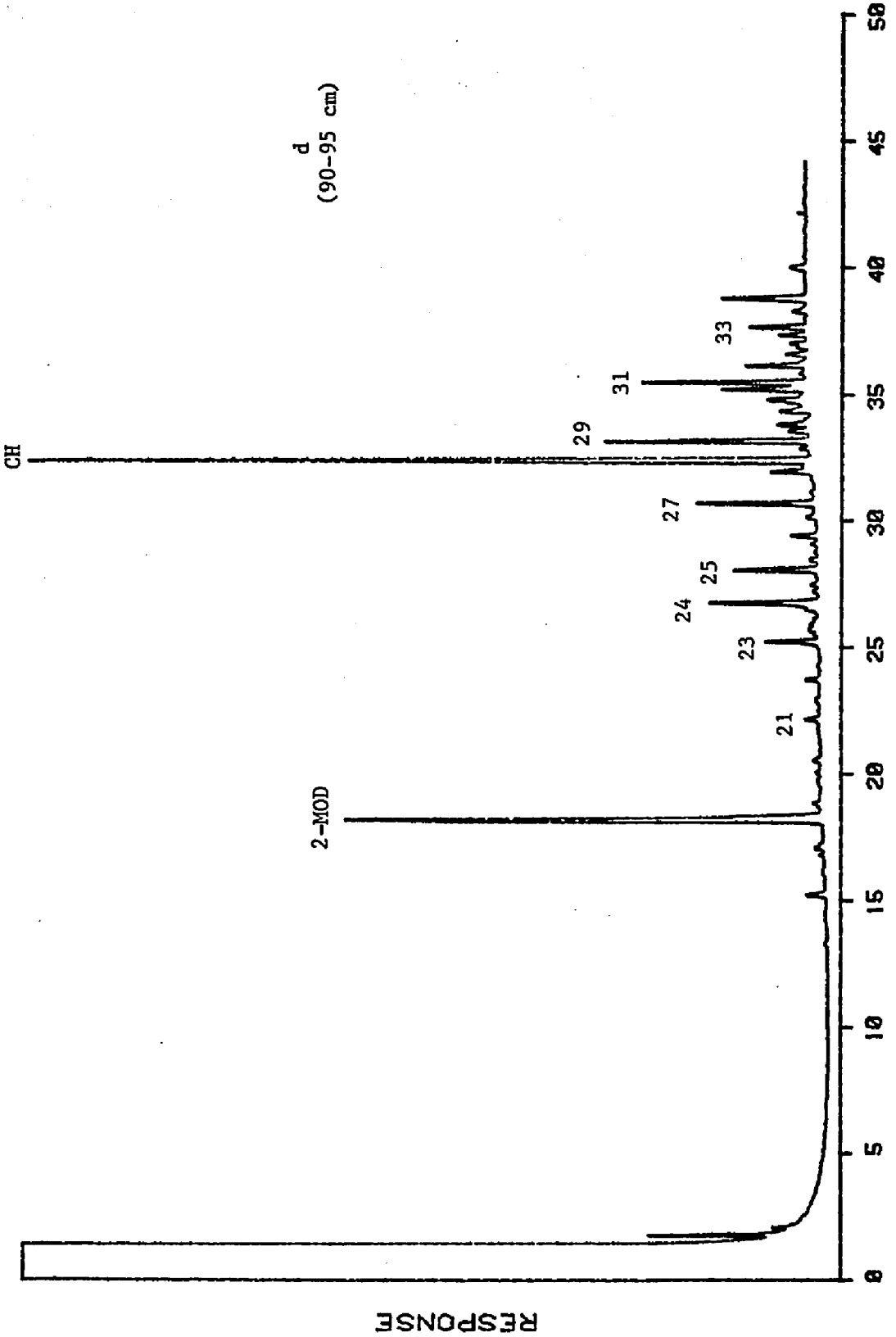
RAW FILE: J2

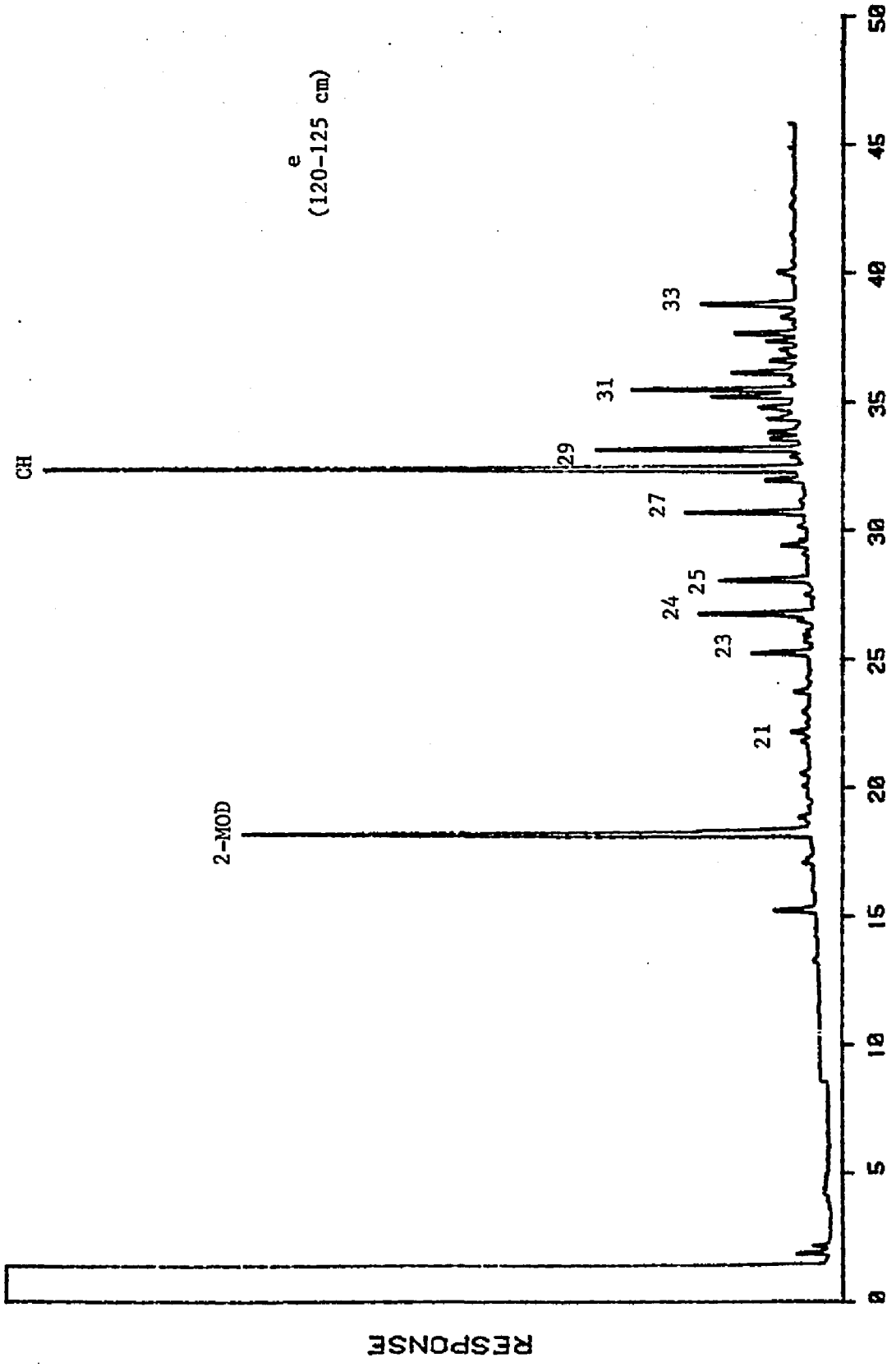
SCALE FACTOR: .014

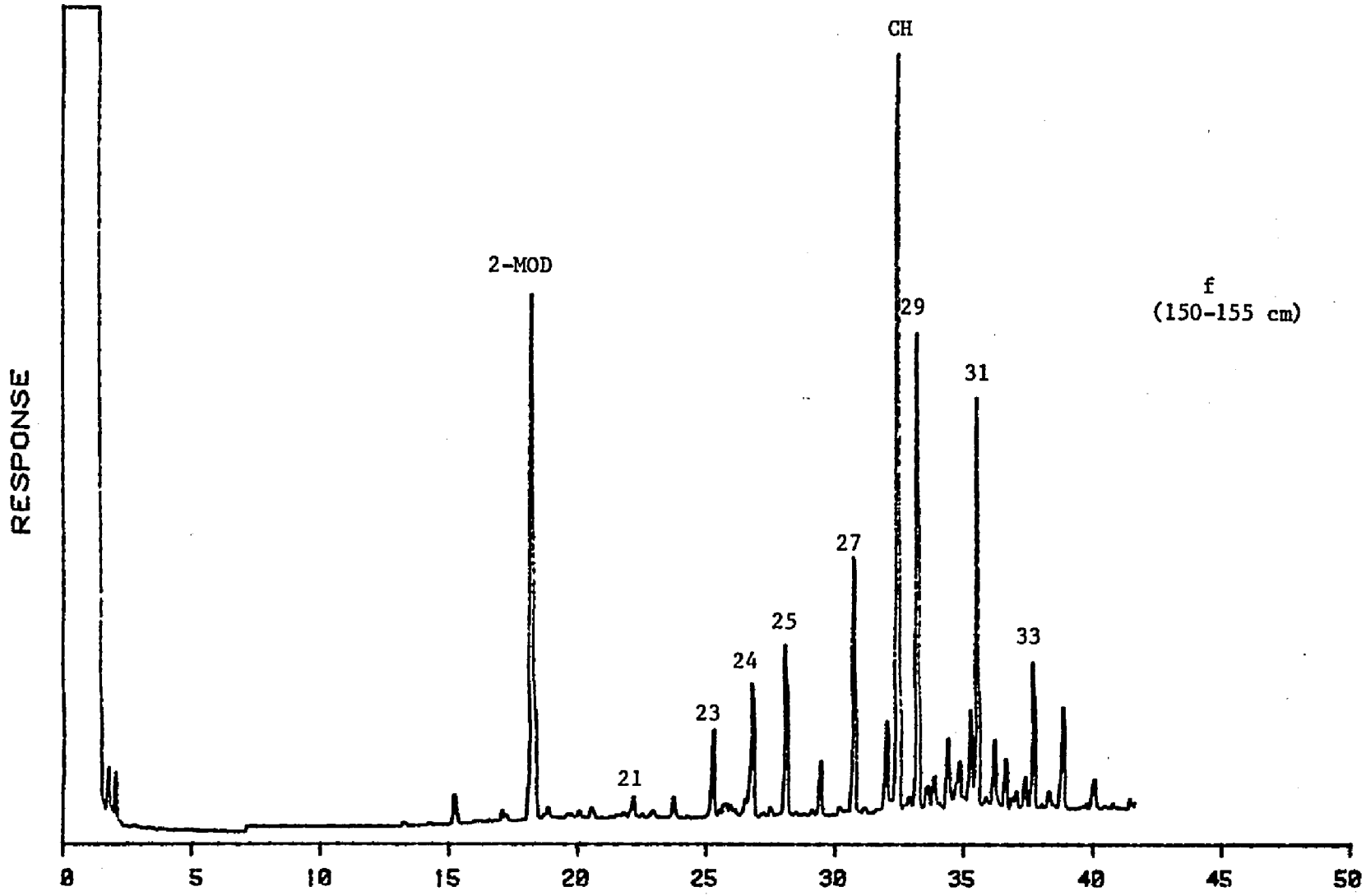
PLOT SPEED: 2


RESPONSE
0
5
10
15
20
25
30
35
40
45
50
b
(30-35 cm)
CH
2-MOD
21
23
24
25
27
29
31
33
SAMPLE: CB2-2H
RAW FILE: J3
SCALE FACTOR: .022
PLOT SPEED: 2


c
(60-65 cm)
CH
2-MOD
21
23
24
25
27
29
31
33
0
5
10
15
20
25
30
35
40
45
50
RESPONSE
SAMPLE: CB2-3H
RAW FILE: J4
SCALE FACTOR: .021
PLOT SPEED: 2


d
(90-95 cm)
CH
2-MOD
21
23
24
25
27
29
31
33
0
5
10
15
20
25
30
35
40
45
50
RESPONSE
SAMPLE: CB2-4H
RAW FILE: J5
SCALE FACTOR: .019
PLOT SPEED: 2


RESPONSE
0
5
10
15
20
25
30
35
40
45
50
CH
2-MOD
21
23
24
25
27
29
31
33
e
(120-125 cm)
SAMPLE: CB2-5H
RAW FILE: J6
SCALE FACTOR: .018
PLOT SPEED: 2



SAMPLE: CB2-6H

RAW FILE: J7

SCALE FACTOR: .014

PLOT SPEED: 2

TABLE 12

Hydrocarbon concentrations in sediments from Colonial Beach, Potomac River (µg/g OC).

	Aliphatic Fraction						Aromatic Fraction				
Depth (cm)	Total n-Alkanes	Total Resolved	Total Aliphatics	CPI[a]	UCM	n-Alkanes / Total Aliphatics	Total Resolved	Total Aromatics	UCM	Perylene	% OC
0- 5	465	725	3429	4.6	2,704	0.14	160	887	727	8.7	1.53
30- 35	223	373	373	6.1	0	0.60	96	170	74	48	1.77
60- 65	228	392	392	5.4	0	0.58	93	133	40	50	1.44
90- 95	169	296	296	5.6	0	0.57	113	149	36	59	1.40
120-125	145	273	273	5.4	0	0.53	154	196	42	96	1.42
150-155	276	392	441	5.4	49	0.63	175	219	44	107	1.49

[a]: Carbon Preference Index from C_{24} to C_{32}

N.D.: Not determined

In the James River, nine core samples were analyzed for hopanoids. The hopanoids in these sediments were again dominated by degraded and extended 17α(H),21β(H) hopanes (XXII a-c; XXIII a-e) (Table 13). Both the 22S and 22R diastereomers were present. 17β(H),21β(H) Hopanes ranging from C_{27} to C_{31} were encountered in many samples. 17β(H),21β(H)-C_{32} Hopane was found only in two subsurface samples from station VC-14 (Table 13). Hop-17(21)-ene and diploptene were detected in many of the James River cores. Figure 23 presents gas chromatograms of hopanoid hydrocarbons in surface sediments of the James, stations 52 and 61A.

The composition and the concentrations of hopanoid acids in sediments from the James are shown in Table 14. Identified hopanoid acids were dominated by the extended 17β(H),21β(H) hopanoid acids, ranging from C_{31} to C_{33} (XXVII a-c) (Figure 24). A single epimer at position 22 (22R) was present for all these acids. The 17β(H),21β(H)-C_{32} hopanoid acid (XXVII-b) was the dominant acid in all samples. The highest hopanoid acid concentrations in the surface sediments were found at station 70 and the lowest at station 61A.

Individual hopanoids, like those of total aliphatics and total aromatics (Table 15; Figure 25), show no obvious trend with depth in these cores. Although the concentrations vary with depth, they do so erratically (Figure 26). This is consistent with Pb-210 data (Table 16) for station 15C and those reported by Goldberg et al. (1978) which indicate that these sediments have been disturbed by bioturbation or physical mixing. The Pb-210 data for the sediment core from the Colonial Beach (CB-002) are also presented in Table 16. A summary of hydrocarbon and hopanoid acid analyses for surface sediments from the

TABLE 13

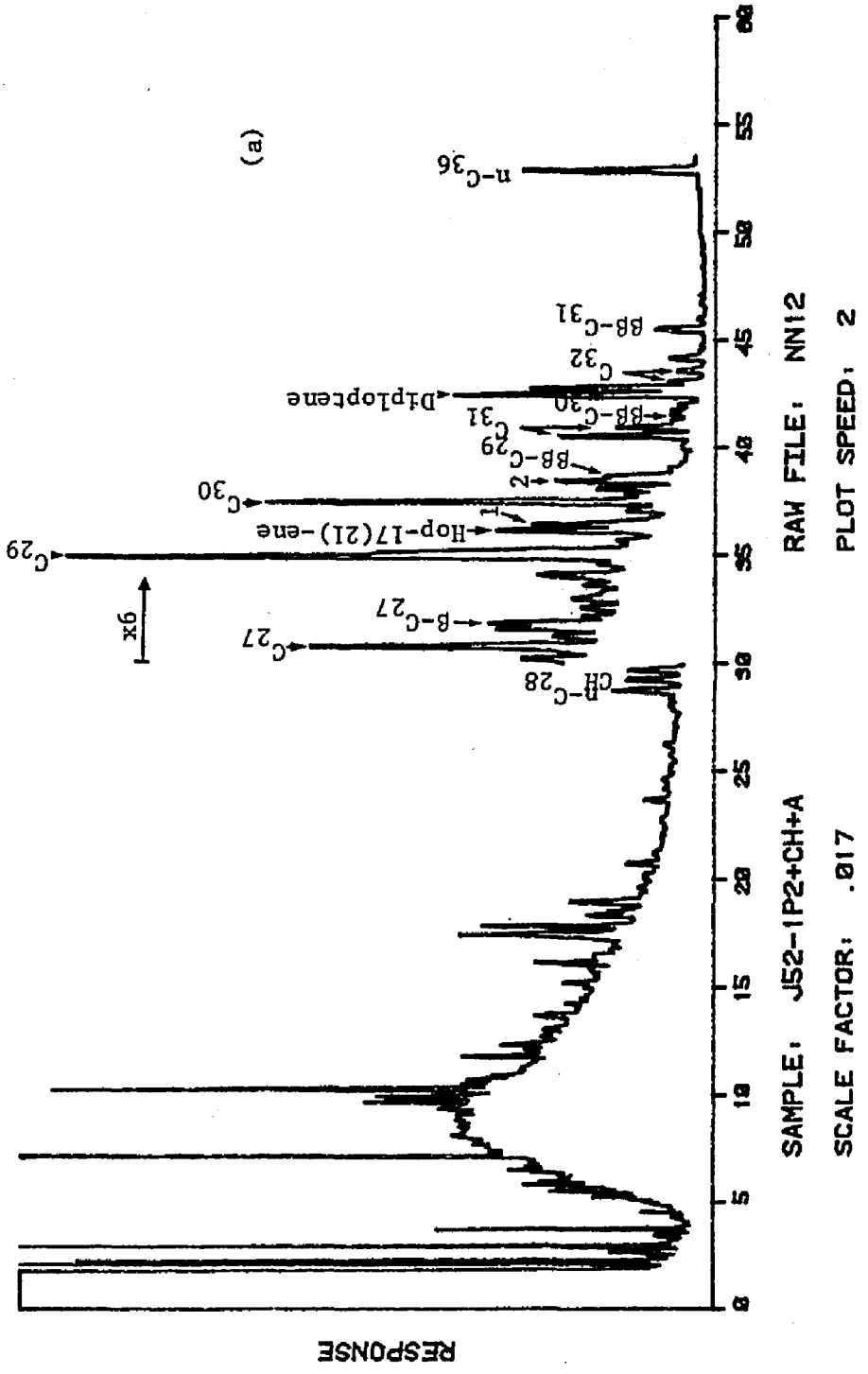
Hopanoid hydrocarbons in sediment cores from the James River (μg/g OC).

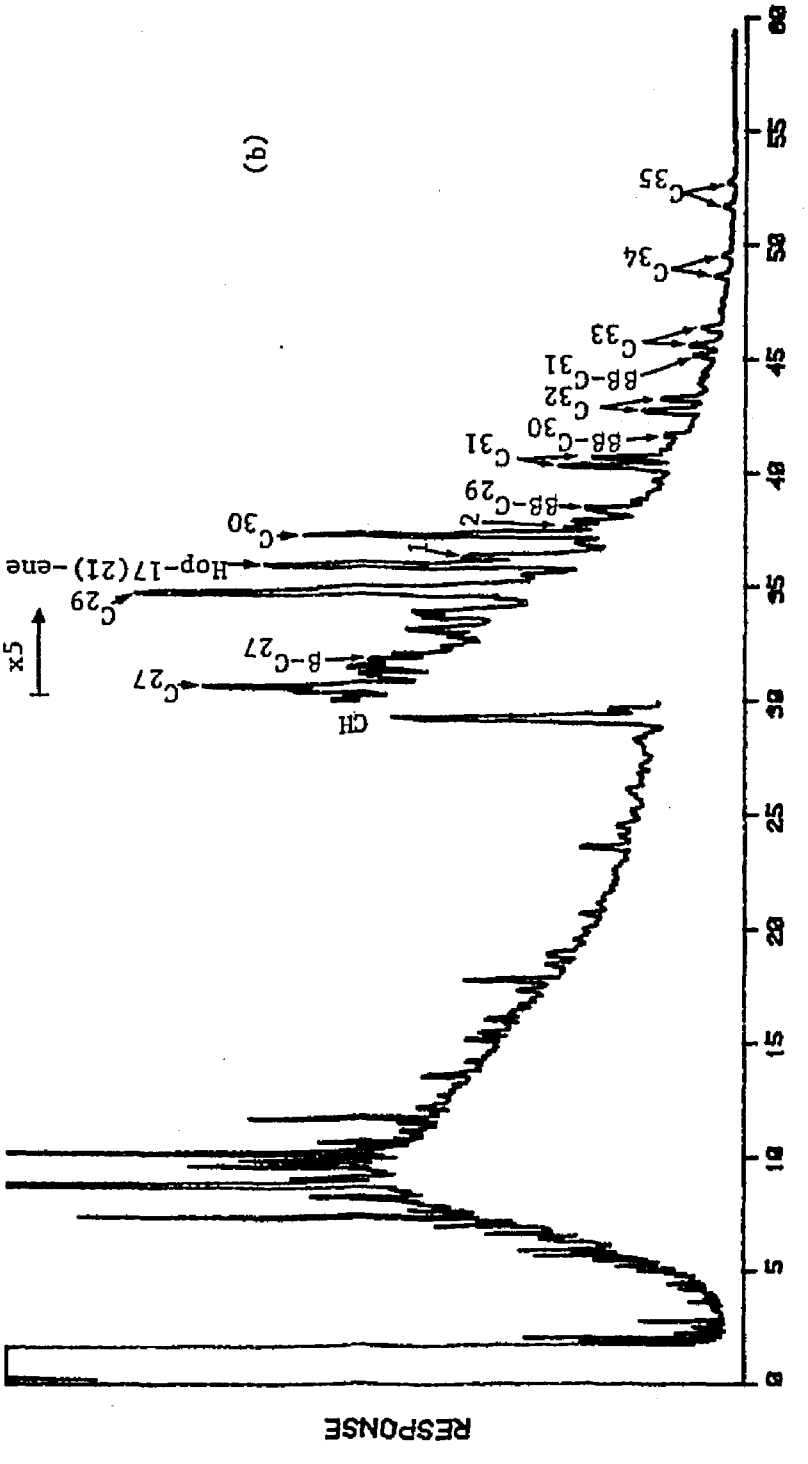
-: not detected
tr: trace
*: upper limit of compound concentration; see discussion.

Station	Depth (cm)	17α-C_{27}	17β-C_{27}	αβ-C_{29}	Hop(17) 21-ene	Ba*-C_{29}	αβ-C_{30}	Ba*-C_{30}	ββ-C_{29}	ββ-C_{31} (S)	ββ-C_{31} (R)	ββ-C_{30}	Diploptene	ββ-C_{32} (S)	ββ-C_{32} (R)	ββ-C_{31}	ββ-C_{33} (S)	ββ-C_{33} (R)	ββ-C_{34} (S)	ββ-C_{34} (R)	ββ-C_{32}	ββ-C_{35} (S)	ββ-C_{35} (R)
Station 70	0- 10	5.0	2.1	7.5	9.1	-	6.4	0.56	1.1	2.6	1.9	0.16	-	1.4	1.1	0.30	0.80	0.60	0.34	0.18	-	0.41	0.40
	20- 30	6.1	3.7	13	11	3.9	12	1.1	2.2	4.7	3.4	0.36	-	2.3	1.8	0.66	1.5	1.1	0.76	0.62	-	0.70	0.42
	40- 50	10	4.4	20	5.2	6.5	19	0.72	3.2	6.3	4.5	0.23	-	3.0	2.7	0.41	1.8	1.3	0.84	0.50	-	0.77	0.54
	50- 60	3.0	3.4	16	5.0	5.1	13	1.3	0.74	4.7	3.6	-	-	2.3	1.9	0.20	1.3	0.95	0.56	0.38	-	0.44	0.33
Station 61A	0- 10	6.7	1.7	17	7.1	4.2	6.4	1.1	1.7	2.6	1.8	-	-	1.4	1.1	0.59	0.83	0.62	0.44	0.24	0.04	0.44	0.35
	20- 30	8.9	2.3	29	28	-	11	1.9	3.0	4.8	2.6	-	-	2.5	1.9	0.90	1.6	1.1	0.8	0.41	-	0.84	0.53
	40- 50	11	3.5	26	8.0	4.9	15	1.7	3.5	5.4	4.1	0.38	-	2.9	2.5	0.57	1.8	1.3	0.97	0.60	0.07	0.93	0.65
	50- 60	10	2.5	23	7.0	4.7	12	1.6	0.54	4.2	3.3	-	-	2.2	1.8	0.44	1.4	0.99	0.72	0.46	0.06	0.74	0.63
Station 52	0- 10	8.1	3.5	15	4.2	3.9	10	1.6	2.9	3.1	1.4	0.40	5.9	0.79	0.9	1.2	-	-	-	-	-	-	-
	20- 30	11	4.7	20	4.1	5.7	13	1.8	3.5	3.6	2.3	0.70	13	0.91	0.74	1.4	-	-	-	-	-	-	-
	40- 50	15	5.5	28	7.7	7.6	21	2.2	6.0	5.2	3.5	0.66	5.9	1.5	1.2	1.3	0.28	0.23	-	-	-	-	-
	60- 70	18	5.3	32	7.6	8.9	24	1.9	6.2	5.0	3.6	0.76	4.8	1.4	1.2	1.9	0.22	0.20	-	-	-	-	-
Station VC-14	0- 5	0.26	3.1	4.3	8.1	-	0.37	1.2	4.5	0.74	0.73	1.2	-	-	-	4.6	-	-	-	-	0.45	-	-
	30- 35	0.19	3.2	4.0	7.2	-	0.18	1.0	4.0	0.64	0.73	1.8	0.27	-	-	5.0	-	-	-	-	0.41	-	-
	60- 65	0.16	3.1	3.3	7.2	-	0.12	0.91	2.5	0.50	0.62	1.0	-	-	-	3.8	-	-	-	-	tr	-	-
	90- 95	0.16	2.4	2.9	6.2	-	0.20	0.78	3.0	0.50	0.56	1.3	0.18	-	-	3.5	-	-	-	-	0.30	-	-
	120-125	0.33	3.4	3.8	8.6	-	0.28	1.1	4.3	0.58	0.82	1.9	0.26	-	-	5.2	-	-	-	-	0.47	-	-
	150-155	0.10	3.7	3.8	9.0	-	0.31	1.1	4.4	0.48	0.86	1.9	0.22	-	-	5.0	-	-	-	-	0.43	-	-
	180-185	0.39	3.4	4.0	8.7	0.78	0.37	1.1	4.3	0.68	0.77	1.7	0.29	-	-	4.6	-	-	-	-	0.43	-	-
Station 31	0- 10	3.6	4.4	13	1.8	3.5	7.8	1.3	5.0	2.6	2.2	1.0	6.2	1.1	0.70	2.6	0.32	0.18	-	-	-	-	-
	10- 20	6.5	6.6	11	2.0	3.6	7.6	1.4	5.3	1.8	1.2	1.0	0.30	1.0	0.42	3.2	-	-	-	-	-	-	-
	30- 40	6.4	4.4	11	-	4.5	10	0.7	2.3	2.2	1.7	0.50	-	0.92	0.61	2.0	0.21	0.14	-	-	-	-	-
	60- 70	1.8	3.1	5.6	1.3	1.4	2.5	0.8	2.5	0.80	0.25	0.45	0.46	-	-	1.6	-	-	-	-	-	-	-
Station 24	0- 10	3.9	2.1	7.6	1.3	2.2	6.3	0.60	1.3	1.6	1.0	0.40	1.0	0.53	0.41	1.8	-	-	-	-	-	-	-
	10- 20	9.4	7.3	23	3.6	8.1	20	0.79	3.5	6.3	5.4	1.7	6.1	2.9	2.1	4.3	0.90	0.63	0.41	0.24	-	-	-
	20- 30	16	9.4	31	1.9	12	29	1.0	3.0	7.7	6.7	1.3	-	3.8	2.8	3.0	1.3	0.90	0.50	0.35	-	-	-
	30- 40	6.5	4.6	16	2.7	4.9	12	0.82	3.6	3.7	3.7	1.1	-	1.3	0.91	2.8	0.30	0.14	-	-	-	-	-
	40- 50	3.4	2.3	6.8	1.1	2.0	5.9	0.52	1.2	1.7	1.7	0.23	0.30	0.62	0.45	1.6	-	-	-	-	-	-	-
	50- 60	6.4	3.3	13	-	3.5	10	0.93	1.4	2.3	1.6	0.35	-	0.83	0.62	1.6	-	-	-	-	-	-	-
Station 15C	0- 10	6.7	3.6	23	6.6	5.3	13	2.2	2.6	4.7	4.1	1.4	-	2.4	2.0	3.1	1.3	0.96	0.71	0.36	0.45	0.63	0.52
	20- 30	8.8	3.4	20	4.2	6.5	14	2.4	2.4	4.5	3.3	0.71	-	2.5	2.0	3.3	1.4	0.93	0.62	0.33	0.42	0.40	0.26
	40- 50	12	3.2	21	3.0	5.1	13	1.9	2.5	3.9	2.6	0.56	-	2.1	1.7	3.1	1.3	0.88	0.45	0.30	0.36	0.44	0.35
	60- 70	7.8	3.1	18	8.9	7.4	19	4.2	2.6	6.2	4.2	0.97	-	3.6	3.0	4.7	2.2	1.6	1.0	0.91	0.51	0.82	0.58
Station SCB	0- 10	6.3	5.0	16	5.6	6.1	15	-	0.79	5.7	3.7	0.74	-	2.9	2.2	4.1	1.6	1.4	tr	tr	-	tr	tr
	20- 30	4.4	1.3	15	2.5	4.6	10	1.1	3.8	3.5	2.8	-	-	1.7	1.4	3.0	0.79	tr	tr	tr	-	tr	tr
	40- 50	-	2.9	5.0	2.0	-	1.9	-	-	-	-	-	-	-	-	3.3	-	-	-	-	-	-	-
Station J2.2	0- 10	6.3	0.22	20	9.1	3.9	11	-	-	3.7	2.9	0.22	-	2.2	1.7	2.6	1.2	1.0	0.57	0.27	-	0.61	0.44
	20- 30	-	1.3	26	10	6.4	15	0.30	0.30	5.5	3.8	-	-	3.1	2.3	3.4	1.7	1.3	0.71	0.45	-	0.72	0.53
	40- 50	8.3	4.0	23	13	6.5	16	1.8	3.6	4.4	4.2	0.44	-	3.1	2.3	3.1	1.8	1.3	0.73	0.54	-	0.80	0.58
	50- 60	12	2.6	22	9.0	5.3	14	1.6	2.0	4	4	0.64	-	2.5	2.1	3.8	1.5	1.0	0.58	0.48	-	0.62	0.32
	60- 70	-	-	7.6	3.8	2.4	1.6	0.64	3.0	-	2	0.93	-	-	-	4.7	-	-	-	-	-	-	-

Figure 23. Representative gas chromatograms of hopanes extracted from the James River surface sediments

a) station 52
b) station 61A
CH: cholestane (spike), C_{27}-C_{35}: αβ-hopanes,
1: βα-C_{29} hopane, 2: βα-C_{30} hopane.


(a)
RESPONSE
SAMPLE: J52-1P2+CH+A
SCALE FACTOR: .817
RAW FILE: NN12
PLOT SPEED: 2
0
5
10
15
20
25
30
35
40
45
50
55
60
n-C36
ββ-C31
C32
Diplopterene
ββ-C30
C31
ββ-C29
2
C30
1
Hop-17(21)-ene
C29
9x
β-C27
C27
αβ-C28


RESPONSE
SAMPLE: 81A-1P2
SCALE FACTOR: .01
RAW FILE: 018
PLOT SPEED: 2
0
5
10
15
20
25
30
35
40
45
50
55
60
C_{35}
C_{34}
C_{33}
BB-C_{31}
C_{32}
BB-C_{30}
C_{31}
BB-C_{29}
C_{30}
Hop-17(21)-ene
C_{29}
B-C_{27}
C_{27}
5x
CH

(b)

TABLE 14

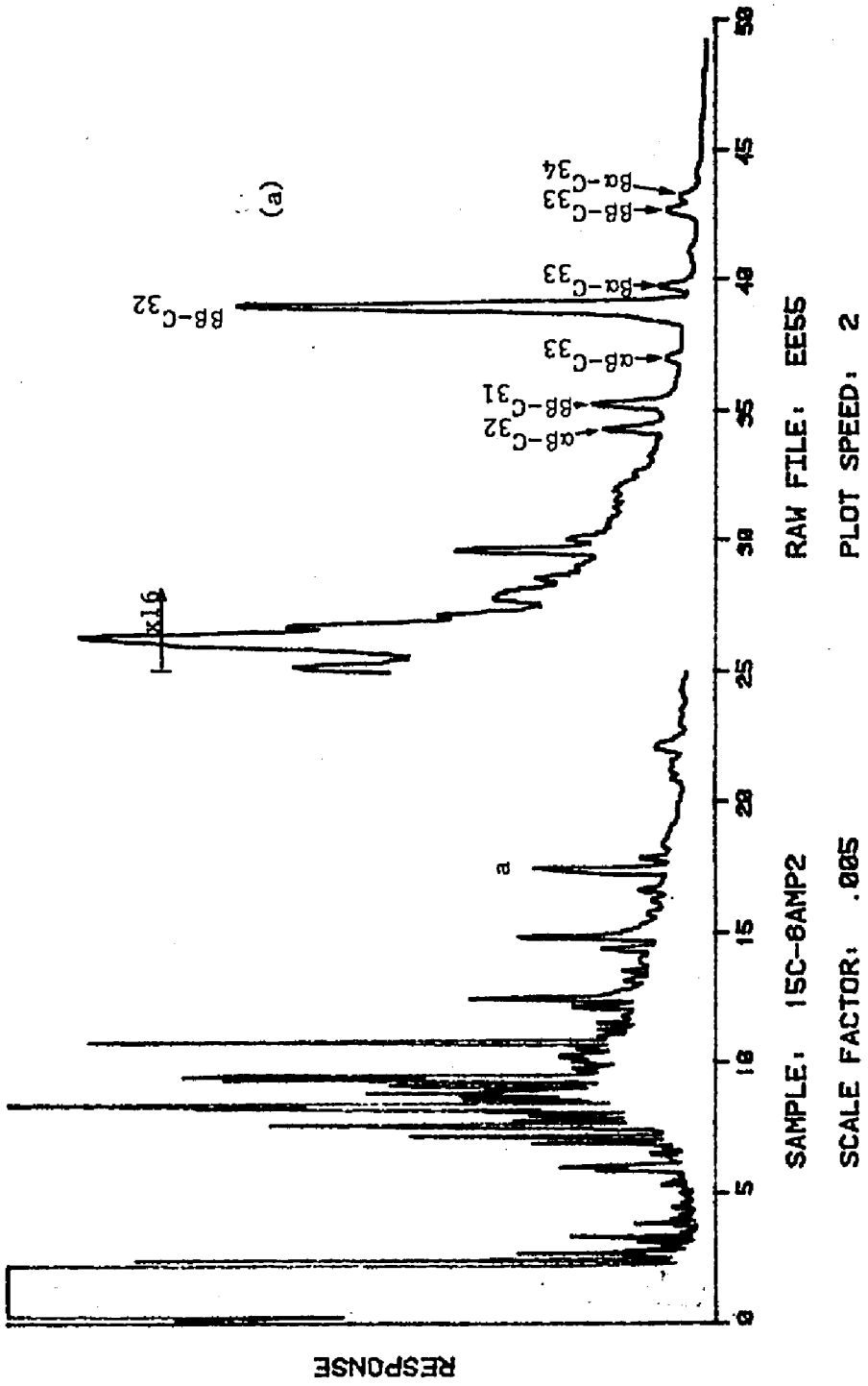
Hopanoid acids identified in the James sediments (μg/g OC).

	Depth	αβ-C_{32}	ββ-C_{31}	αβ-C_{33}	ββ-C_{32}	βα-C_{33}	αβ-C_{34}	ββ-C_{33}	βα-C_{34}	Total
Station 70	0-10	4.0	5.7	4.8	29.0	-	-	-	-	43.5
	10-30	2.0	0.6	-	7.2	-	-	-	-	9.8
	40-50	6.1	2.8	0.3	27.1	0.6	-	0.3	0.3	37.5
	50-60	3.8	1.5	0.2	13.9	tr	-	tr	tr	19.4
Station 61A	0-10	-	-	-	2.5	-	-	-	-	2.5
	20-30	-	-	-	10.7	-	-	-	-	10.7
	40-50	1.6	0.4	-	5.5	-	-	-	-	7.5
	50-60	5.5	2.5	0.5	27.1	-	-	-	-	35.6
Station VC14	0- 5	1.1	2.3	-	12.8	0.8	-	1.0	-	18
	30- 35	0.6	1.3	-	7.9	0.3	-	0.4	-	10.5
	60- 65	0.4	1.2	-	6.3	-	-	0.3	-	18.2
	90- 95	0.4	0.9	-	5.2	0.3	-	0.3	-	7.1
	120-125	0.6	1.9	-	12.2	0.7	-	1.3	-	16.7
	150-155	0.8	2.6	-	15.8	1.0	-	1.5	0.4	22.1
	180-185	0.7	2.1	-	13.8	0.8	-	1.1	0.4	18.9
Station 15C	0-10	-	-	-	4.2	-	-	-	-	4.2
	20-30	-	3.6	-	10.9	1.3	-	0.6	-	16.4
	40-50	2.4	3.1	0.8	25.9	1.5	-	3.0	-	36.7
	50-60	1.8	2.6	0.6	20.9	1.1	-	1.5	0.9	29.4
	60-70	1.8	2.4	0.5	13.1	0.7	-	0.8	0.4	19.7
Station SC8	0-10	1.8	3.0	-	15.2	-	-	-	-	20.0
	20-30	1.4	2.8	-	7.7	0.9	-	-	-	12.8
	40-50	0.6	1.0	-	5.2	-	-	-	-	6.8
	50-60	-	2.2	-	8.5	-	-	-	-	10.7
Station J2.2	0-10	0.9	1.1	0.2	10.1	0.3	-	0.2	-	11.2
	20-30	-	1.3	-	12.0	-	-	-	-	13.3
	40-50	-	-	-	6.3	-	-	-	-	6.3
	50-60	-	-	-	2.6	-	-	-	-	2.6
	60-70	-	0.7	-	4.8	-	-	-	-	5.5

- : Not detected
tr: Trace

Figure 24. Representative gas chromatograms of hopanoid acid methyl esters from the James River sediments

a) station 15C (60-70 cm)
b) station J2.2 (0-10 cm)
a: cholanic acid methyl ester (spike).


RESPONSE
SAMPLE: 15C-8AMP2
SCALE FACTOR: .005
RAW FILE: EE55
PLOT SPEED: 2
(a)
x16
αβ-C32
ββ-C31
αβ-C33
ββ-C32
βα-C33
ββ-C33
βα-C34

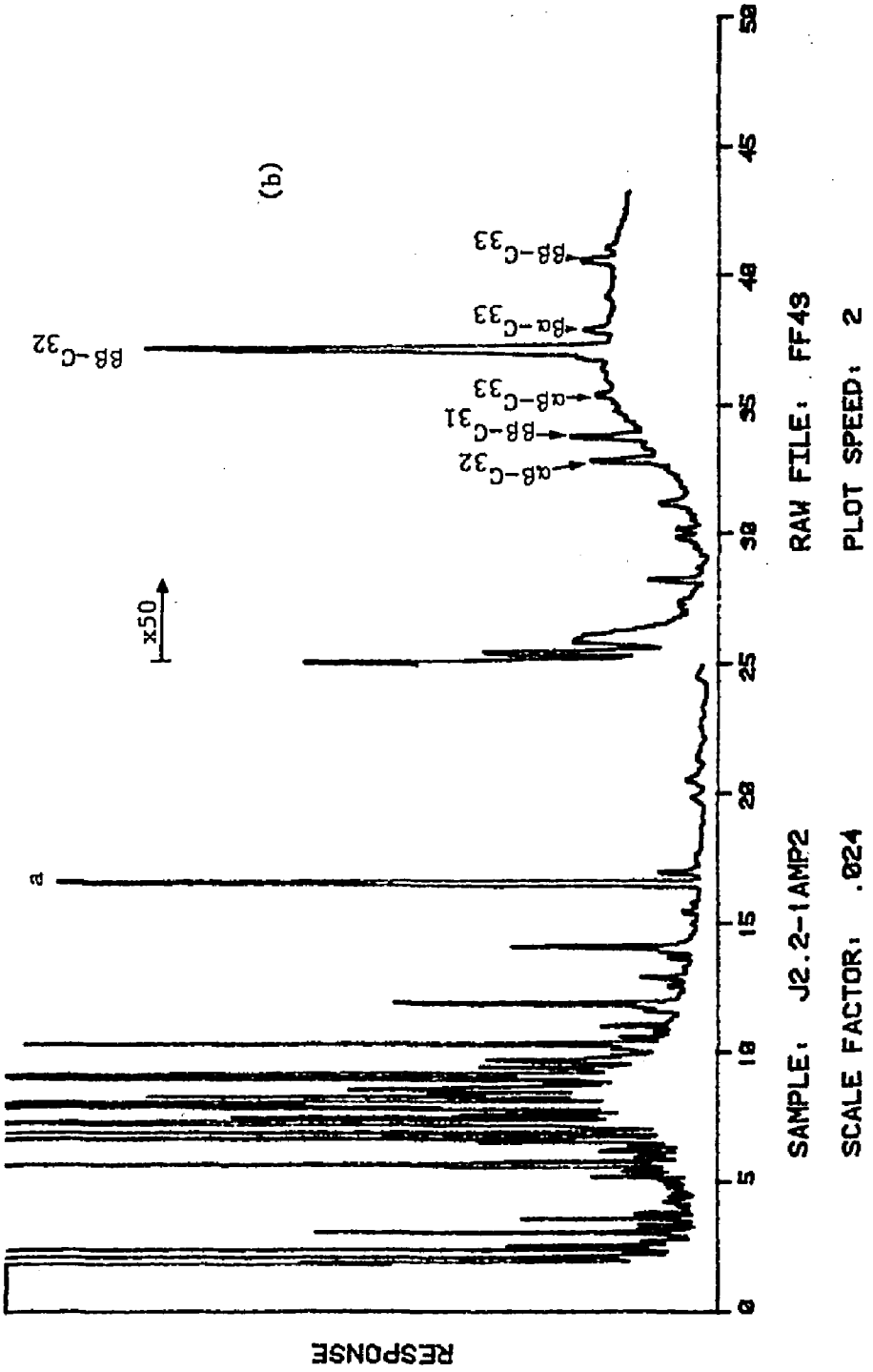

(b)
a
x50
αβ-C32
ββ-C31
αβ-C33
βα-C33
ββ-C33
ββ-C32
RESPONSE
0
5
10
15
20
25
30
35
40
45
50
SAMPLE: J2.2-1AMP2
RAW FILE: FF43
SCALE FACTOR: .024
PLOT SPEED: 2

TABLE 15

Hydrocarbon concentrations in sediments from James River (µg/g OC).

Station/Depth (cm)	Aliphatic Fraction						Aromatic Fraction				
	Total n-Alkanes	Total Resolved	Total Aliphatics	CPI[a] 24-32	UCM	n-Alkanes / Total Aliphatics	Total Resolved	Total Aromatics	UCM	Perylene	% OC
70/0-10	715	1116	4352	5.8	3236	0.16	278	896	618	7.3	4.45
70/20-30	948	1781	5957	4.9	4176	0.16	361	1525	1164	15	3.48
70/40-50	679	1305	6372	3.7	5067	0.11	543	2240	1697	12	4.12
70/50-60	489	714	11877	2.6	11163	0.04	493	2319	1826	5.0	4.41
61A/0-10	306	366	2013	5.2	1647	0.15	353	806	453	18	2.82
61A/20-30	750	958	5077	5.2	4119	0.15	456	1625	1169	15	2.70
61A/40-50	470	902	4149	4.1	3247	0.11	574	2084	1510	28	2.93
61A/50-60	442	966	6279	4.3	5313	0.07	605	2012	1407	32	2.59
52/0-10	394	547	2606	5.1	2059	0.15	115	299	184	10	3.59
52/20-30	920	1353	6765	4.8	5412	0.14	149	410	261	21	3.78
52/40-50	1104	2300	10036	4.2	7736	0.11	138	492	354	11	3.90
52/60-70	808	1154	10100	3.2	8946	0.08	108	648	540	8.7	4.31
VC14/0-5	205	284	349	3.5	65	0.59	149	230	81	59	1.18
VC14/30-35	113	179	197	3.1	18	0.57	147	169	22	74	1.71
VC14/60-65	176	251	1757	3.4	1506	0.10	129	143	14	56	0.98
VC14/90-95	94	136	313	3.5	177	0.30	81	100	19	41	2.06
VC14/120-125	88	107	294	3.3	187	0.30	127	155	28	63	1.39
VC14/150-155	291	395	458	4.9	63	0.64	132	168	36	67	1.65
VC14/180-185	131	199	275	3.5	76	0.48	119	146	27	61	1.50
31/0-10	408	637	3060	4.6	2426	0.13	69	386	317	6.5	3.48
31/10-20	394	525	2560	4.7	2035	0.15	76	388	312	9.2	3.05
31/30-40	330	487	1650	4.1	1163	0.20	111	289	178	18	2.93
31/60-70	106	138	623	4.2	485	0.17	123	418	295	12	2.45

TABLE 15 (Continued).

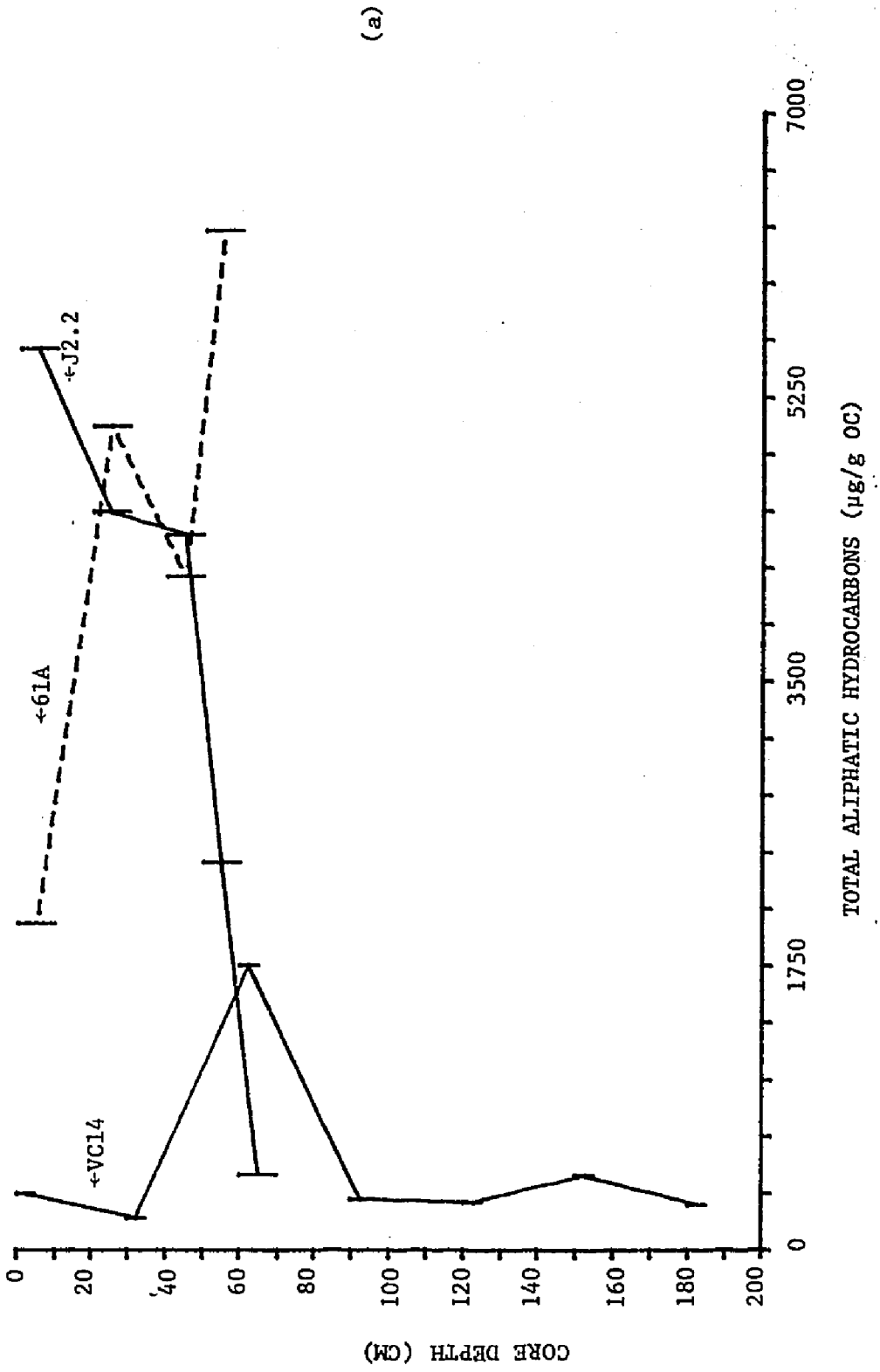
Station/Depth (cm)	Aliphatic Fraction						Aromatic Fraction				
	Total n-Alkanes	Total Resolved	Total Aliphatics	CPI[a] 24-32	UCM	n-Alkanes / Total Aliphatics	Total Resolved	Total Aromatics	UCM	Perylene	% OC
24/0-10	410	700	3140	4.0	2440	0.13	112	582	470	7.7	2.78
24/10-20	442	896	5520	4.1	4624	0.08	85	221	136	11	3.02
24/20-30	460	616	4570	3.8	3954	0.10	120	528	408	14	2.90
24/30-40	335	461	3330	4.0	2869	0.10	123	443	320	7.7	2.85
24/40-50	350	474	2920	3.3	2446	0.12	166	1112	946	11	2.45
24/50-60	213	272	1523	3.2	1251	0.14	202	869	667	22	2.52
15C/0-10	465	727	3635	4.0	2908	0.13	130	780	650	9	2.32
15C/20-30	781	1116	6651	3.3	5535	0.12	81	189	108	3	2.31
15C/40-50	538	703	5132	3.2	4429	0.10	122	764	642	7	2.67
15C/50-60	688	1000	9000	3.0	8000	0.08	178	1026	848	8	2.43
15C/60-70	589	1288	10175	3.2	8887	0.06	212	969	757	16	2.41
SC8/0-10	293	409	2290	3.4	1881	0.13	179	256	77	4.3	1.10
SC8/20-30	237	325	1332	3.5	1007	0.18	209	318	109	0.60	1.21
SC8/40-50	238	351	597	3.9	246	0.40	100	100	0	1.6	1.05
SC8/50-60	339	479	1629	4.4	1150	0.21	102	102	0	1.9	0.93
J2.2/0-10	364	942	5558	3.0	4616	0.06	153	918	765	5.0	1.57
J2.2/20-30	328	598	4547	2.7	3949	0.07	186	850	664	8.0	1.18
J2.2/40-50	364	639	4409	3.5	3770	0.08	139	957	818	6.8	1.26
J2.2/50-60	249	419	2388	3.7	1969	0.10	203	1170	967	16	1.28
J2.2/60-70	248	466	466	4.1	0	0.53	92	123	31	16	1.06

[a]: Carbon Preference Index from C_{24} to C_{32}

N.D.: Not determined

Figure 25. Vertical distributions of hydrocarbons in three selected sediment cores from the James River

a) total aliphatic hydrocarbons
b) total aromatic hydrocarbons.


(a)
+J2.2
+61A
+VC14
0
20
40
60
80
100
120
140
160
180
200
CORE DEPTH (CM)
0
1750
3500
5250
7000
TOTAL ALIPHATIC HYDROCARBONS (µg/g OC)

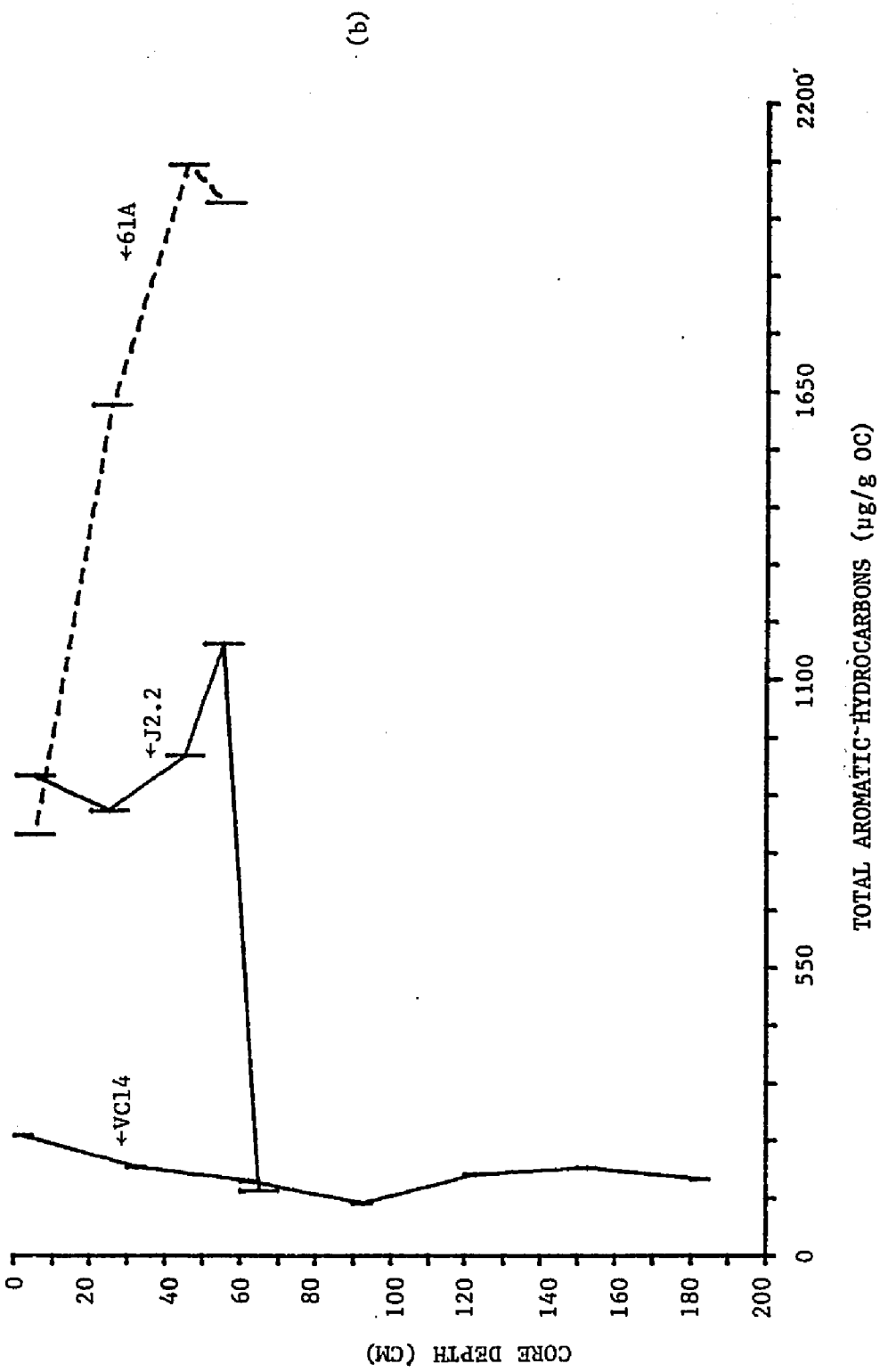
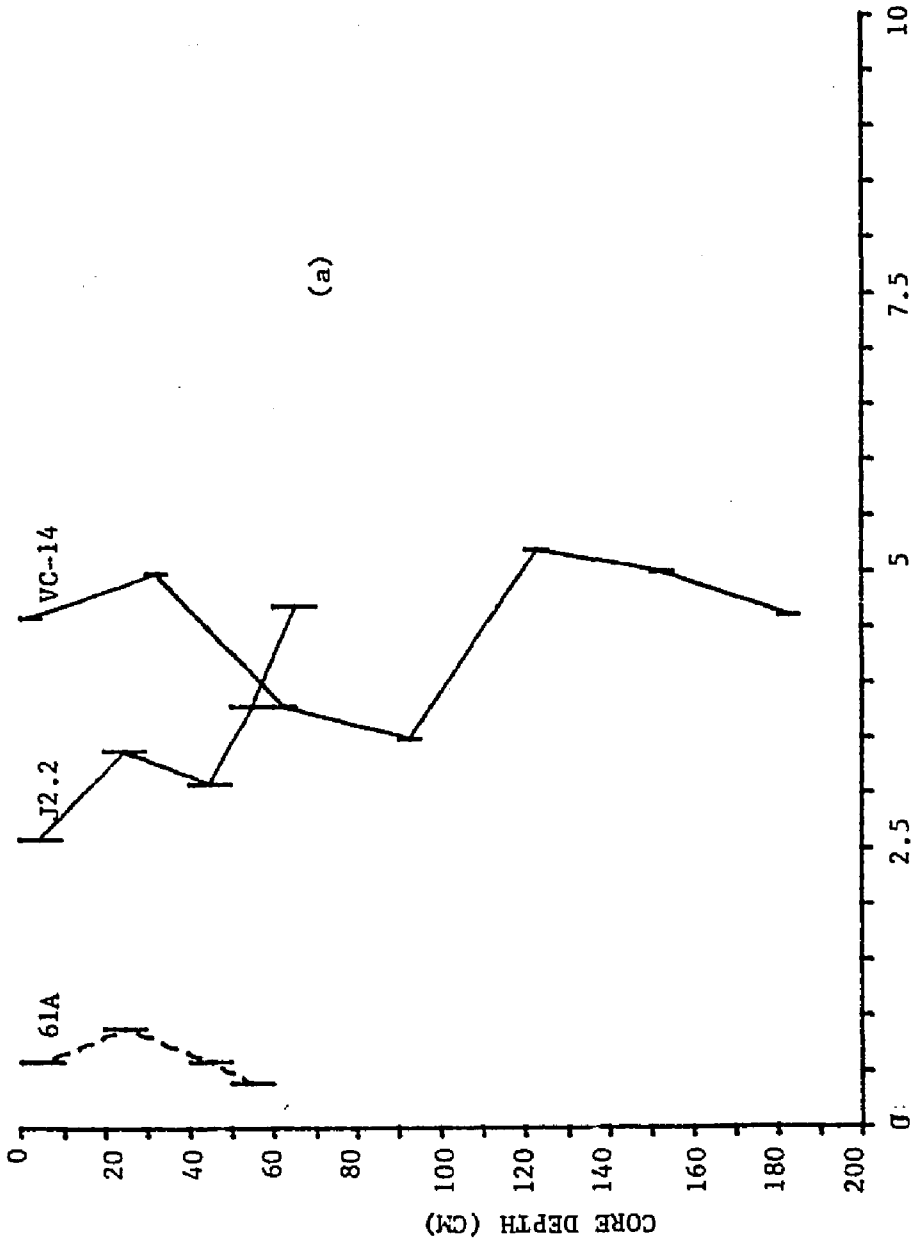

(b)
←61A
←J2.2
←VC14
0
20
40
60
80
100
120
140
160
180
200
CORE DEPTH (CM)
0
550
1100
1650
2200
TOTAL AROMATIC HYDROCARBONS (µg/g OC)

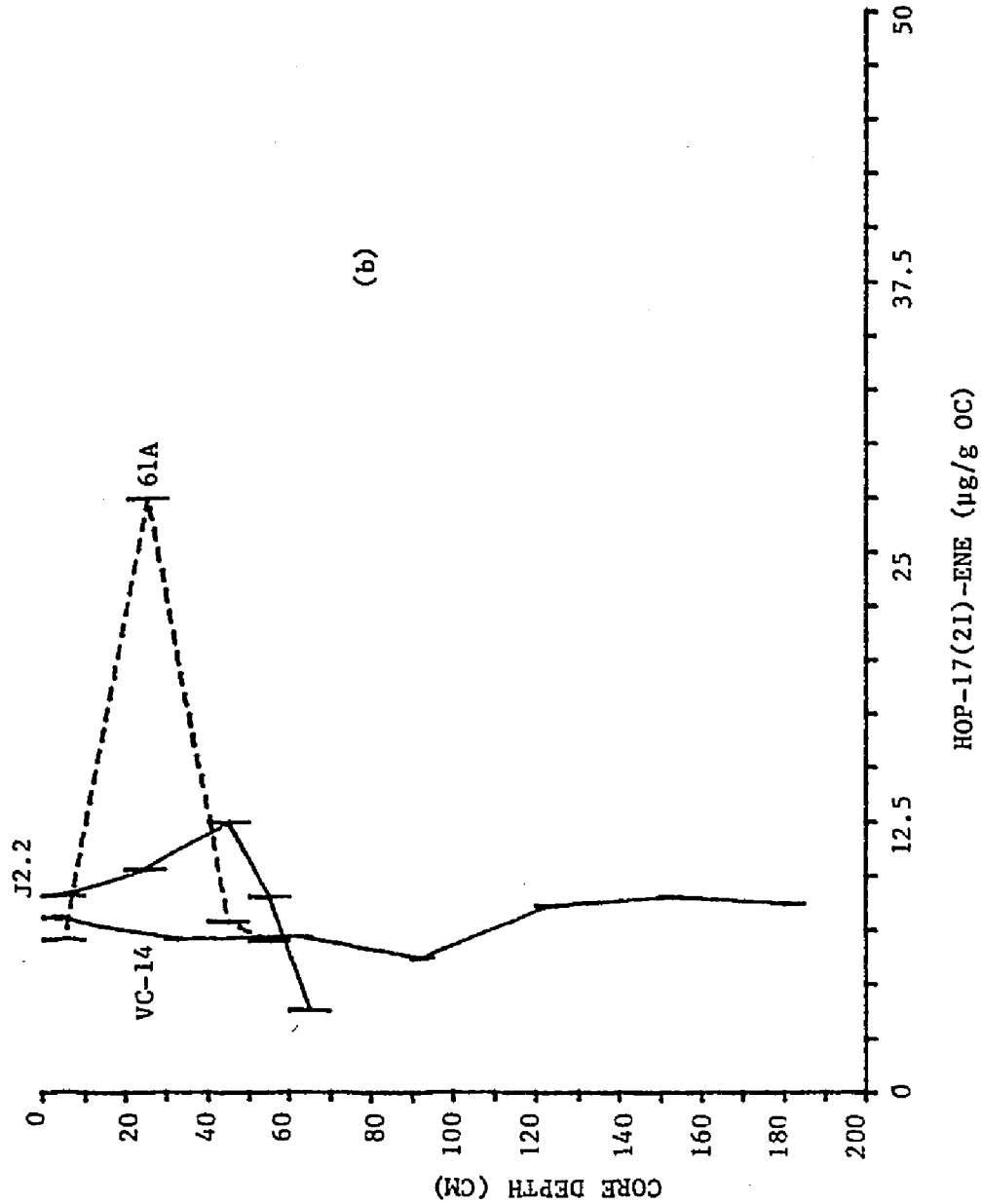
Figure 26. Vertical distributions of hopanoids in three selected sediment cores from the James River

a) $\beta\beta$-C_{31} hopane

b) hop-17(21)-ene

c) $\beta\beta$-C_{32} hopanoid acid.


(a)
VC-14
J2.2
61A
CORE DEPTH (CM)
0
20
40
60
80
100
120
140
160
180
200
0
2.5
5
7.5
10
17β,21β-C31 HOPANE (μg/g OC)


(b)
J2.2
61A
VC-14
CORE DEPTH (CM)
0
20
40
60
80
100
120
140
160
180
200
0
12.5
25
37.5
50
HOP-17(21)-ENE (µg/g OC)

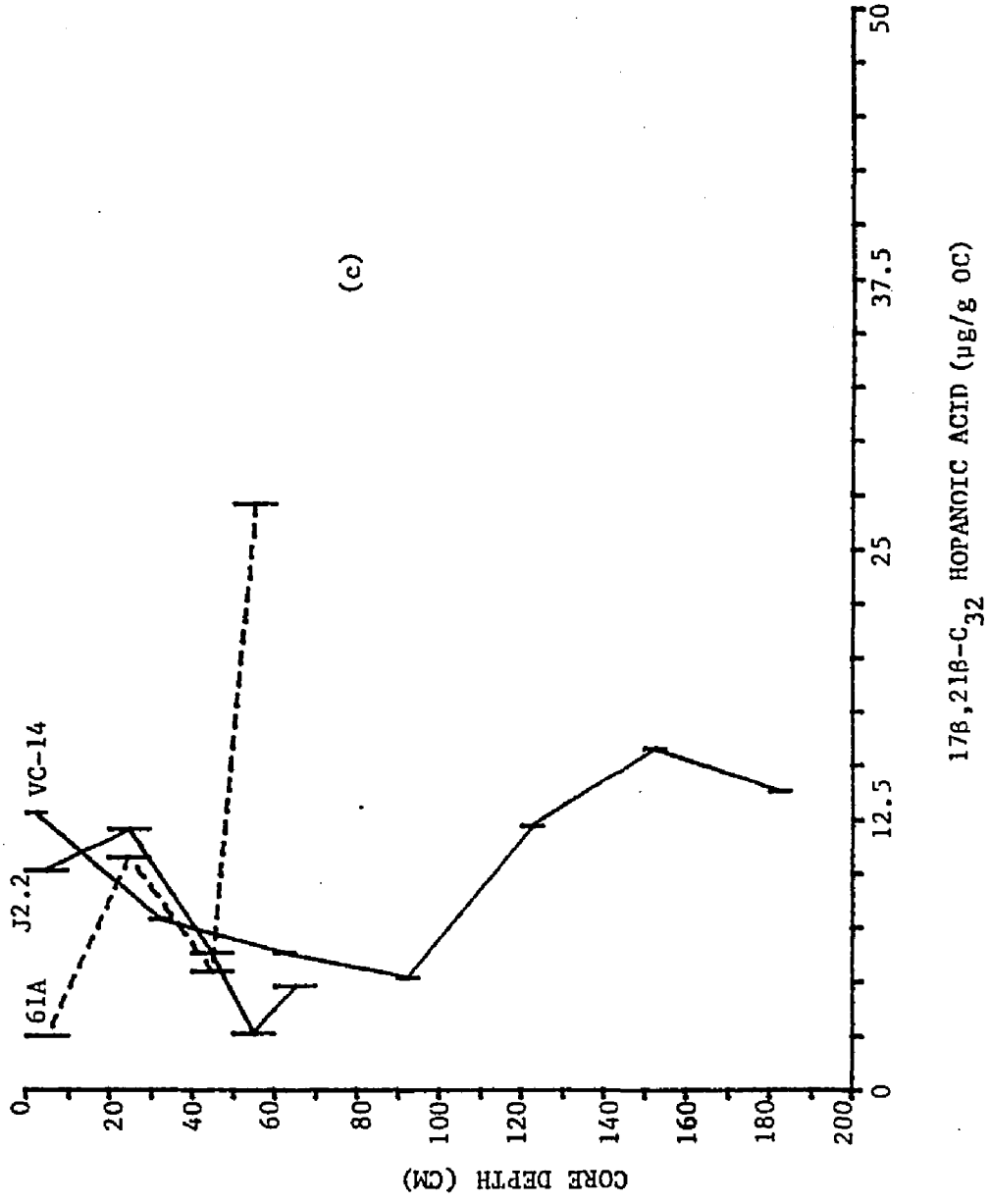

(c)
VC-14
J2.2
61A
0
20
40
60
80
100
120
140
160
180
200
CORE DEPTH (CM)
0
12.5
25
37.5
50
17β,21β-C32 HOPANOIC ACID (μg/g OC)

TABLE 16

^{210}Pb activity in sediment cores from Colonial Beach and the James River*

Station	Depth (cm)	Activity (cpm)
CB002 (Colonial Beach)	0- 5	1.9±0.2
	30- 35	0.74±0.07
	60- 65	1.23±0.17
	90- 95	1.24±0.11
	150-155	1.18±0.21
15C (James River)	0- 10	2.5±0.25
	20- 30	2.4±0.2
	40- 50	2.7±0.2
	60- 70	2.6±0.2

*: ^{210}Pb analysis was performed by Mr. M. Koide, Scripps Institution of Oceanography, San Diego, California.

Chesapeake Bay is shown in Table 17, and that for the James is shown in Table 18.

TABLE 17

Summary of hydrocarbon and hopanoid acid analyses for sediments from the Chesapeake Bay.

	Station	Aliphatic Hydrocarbons		Aromatic Hydrocarbons		Hopanoid Hydrocarbons			Hopanoid Acids	
		Resolved/Total	CPI[a]	Total[b]	Resolved/Total	Total αβ[b]	Total ββ[b]	Hop-17(21)-ene[b]	ββ-C_{32}[b]	Total[b]
Southern Bay	1	1	2.7	94	1	3.5	0.51	0.92	2.0	2.0
	* 3	0.2	3.2	390	0.6	32	8.1	1.1	5.6	5.6
	4	1	-	25	1	2.4	1.4	2.8	2.7	2.7
	* 5	0.3	4.1	320	0.3	23	8.0	6.7	13	13
	6	1	-	41	1	2.0	1.4	3.0	15	15
	7	1	4.6	34	1	6.7	3.3	n.d.	24	24
	* 8	0.4	4.2	56	0.8	34	7.5	12	16	19
	10	0.6	4.2	87	0.5	22	5.1	9.3	35	37
Central Bay	11	0.7	4.5	82	0.8	13	4.3	8.4	27	33
	12	0.9	3.9	38	1	24	2.6	6.6	20	20
	* 13	0.3	4.2	230	0.6	45	7.8	10	13	13
	15	0.3	4.1	190	0.4	27	5.2	14	12	14
	16	0.7	4.7	90	0.5	7.5	5.9	8.1	21	21
	* 17	0.4	4.1	180	0.4	25	6.5	20	32	41
	19	0.7	3.9	930	0.9	27	3.2	12	11	11
	* 21	0.2	3.8	450	0.5	19	4.3	6.8	10	10
Northern Bay	22	0.2	3.4	640	0.3	25	5.8	5.4	18	23
	* 23	0.2	3.5	1,100	0.3	36	6.7	4.2	tr	tr
	24	0.4	4.4	210	0.7	7.9	4.3	9.7	45	54
	25	0.5	3.0	620	0.7	22	7.1	7.8	8.8	9.4
	* 26	0.4	4.3	320	0.6	22	5.7	10	94	128
	* 27	0.5	1.1	23,065	0.9	36	2.8	0.20	n.d.	n.d.

tr: trace
n.d.: not detected
-: not determined

a: Carbon Preference Index from C_{24} to C_{32}
b: concentrations in µg/g OC
* stations at mouth of major rivers

TABLE 18

Summary of hydrocarbon and hopanoid acid analyses in surface sediments from the James River.

	Aliphatic Hydrocarbons		Aromatic Hydrocarbons		Hopanoid				
					Hyrocarbons			Acids	
Station	Resolved/Total	CPI[a]	Total[b]	Resolved/Total	Total αβ[b]	Total ββ[b]	Hop-17(21)-ene[b]	ββ-C_{32}[b]	Total[b]
70	0.3	5.8	896	0.3	29	3.7	9.1	29	44
61A	0.2	5.2	806	0.4	40	4.0	7.1	2.5	2.5
52	0.2	5.1	299	0.4	39	14	4.2	N.D.	N.D.
VC14	0.8	3.5	230	0.7	6.4	14	8.1	13	18
31	0.2	4.6	386	0.2	32	19	1.8	N.D.	N.D.
24	0.2	4.0	582	0.2	21	7	1.3	N.D.	N.D.
15C	0.2	4.0	780	0.2	60	11	6.6	4.2	4.2
SC8	0.2	3.4	256	0.7	55	11	5.6	15	20
J2-2	0.2	3.0	918	0.2	52	3.0	9.1	10	11

N.D.: Not Determined

[a]: Carbon Preference Index from C_{24} to C_{32}

[b]: Concentrations in μg/g OC

V. DISCUSSION

Analyses of hopanoids in surface sediments from the Chesapeake Bay clearly indicate 17α(H),21β(H) hopanes as dominant compounds (Table 3). αβ-Hopanes are known to be abundant in fossil materials. So far, they have never been identified in living organisms. Geological evidence as well as laboratory experiments suggest that αβ-hopanes are thermodynamically more stable than their ββ - counterparts and derive from the latter via an acid catalyzed isomerization during maturation (Ensminger et al., 1973; Van Dorsselaer; 1974). The recent finding of small amounts of αβ-hopanes in uncontaminated recent muds from two ponds near Strasbourg, France (Rohmer et al., 1980), however, suggests that αβ-hopanes can also evolve from microbially induced isomerization reactions. There is a wide range of sedimentary material between recent and fossil strata that all contain varying amounts of αβ-hopanes.

In order to understand the origin of hopanoids in Chesapeake Bay sediments one cannot just concentrate on αβ-hopanes. One must discuss as a first step their relation to all other hopanoids present in the samples and compare this information to known characteristic hopanoid distributions in fully maturated, unmaturated and recent sedimentary environments. These characteristic distributions can be summarized as follows:

Fully maturated sources of hopanoids always contain only the more stable 17α(H),21β(H)-hopanes ranging from C_{27} to C_{35} with possibly traces of 17β(H),21α(H)-hopanes, whereas 17β(H),21β(H)-hopanes are absent. However, trace amounts of 17β(H)-C_{27} may be present in maturated source rocks and petroleum. The extended 17α(H),21β(H)-hopanes (C_{31}- C_{35}) are always present as mixtures of 22S and 22R diastereomers. Seifert and Moldowan (1980) determined the S/R ratio for crude oils and bitumen to be in the range of 1.4 to 1.6, measured with baseline GC resolution. Other indicators of maturity are the decreasing (17β,21α)/(17α,21β) hopane and (17β/17α)-C_{27} hopane ratios (Seifert, 1978). The (17β,21α)/(17α,21β) hopane ratios are typically < 6 % and no more than 15 % in petroleum and in bitumen from oil producing formations (Seifert and Moldowan, 1980). Analyses of hopanoids in several mature source materials, for example one crude oil and five motor oil samples (Table 6) confirm these observations. Although the S/R ratios for extended αβ-hopanes (C_{31} to C_{35}) in the analyzed source materials range from 1.3 to 2.2 (Table 7), as determined from GC peak areas, the high ratios in general were determined on peaks of low concentration (C_{35}) and thus are not as reliable.

Trace amounts of hopanoid acids may be present in petroleum with αβ-, ββ- and βα- configurations (Schmitter et al., 1978). The presence of ββ-hopanoid acids in maturated hopanoid samples indicates that isomerization of acids is slower than that of the hydrocarbons (Ensminger, 1977). Again, extended 17α(H),21β(H) and 17β(H),21α(H) hopanoid acids (C_{30}- C_{34}) are present as mixtures of two epimers at C-22

indicating high degree of maturation. The S/R ratio for extended hopanoid acids, however, is always less than that of hydrocarbons in the same sample (Ensminger, 1977).

Recent input, on the other hand, consists mainly of hopanes of the 17β(H),21β(H) structure type in the range between C_{27} to C_{32}. Concentrationwise, these ββ-hopanes may be overpowered by 17β(H),21β(H) hopanoid acids and by hop-17(21)-ene or diploptene. The bishomohopanoic acid (C_{32}) (XXXIII) commonly is dominant in recent sediments (Eglinton et al., 1975; Boon et al., 1978; Simoneit et al., 1979; Simoneit and Kaplan, 1980). At present, bacteriohopane polyols (XXXII) are believed to be the primary precursors of these natural hopanoids (Rohmer et al., 1980). In the study of hopanoids in recent sediments from two ponds near Strasbourg, France; Rohmer et al. (1980) isolated a suite of intermediate compounds with C_{27} and C_{29}- C_{35} carbon atoms with several functional groups, i.e., acids and alcohols which are thought to be the oxidation products of autochthonous bacteriohopane polyols. Hopanoids of the αβ- configuration may be present in trace amounts in recent sediments (Rohmer et al., 1980).

In more evolved sediments, the whole series of hopanes and hopanoid acids ranging from those which are characteristic of recent sediments to those of maturated fossil hopanes may be encountered. αβ-Hopanes usually are predominant over the βα- and ββ-hopanes. Extended αβ-hopanes again consist of mixtures of 22S and 22R epimers. However, the S/R ratios are usually less than one. Hop-17(21)-ene has been found in many immature sediments and shales (Van Dorsselaer, 1975; Ensminger, 1977). Hopanoid acids in sedimentary deposits, ranging from C_{31} to C_{33}

with ββ-, αβ- and/or βα- configuration, are present in higher concentrations than in petroleum. However, only one epimer of the extended αβ- and βα- configurations is generally found in immature sediments (Ensminger, 1977).

The presence of hopanoid molecules in the Bay samples and the question of their origin will now be viewed in the light of these criteria, as well as with other information that is available from the data.

First, we note that highest concentrations of 17α(H),21β(H)-hopanes are found in samples from river mouths (Figure 27; Table 17). This can be interpreted as evidence that these rivers are sources of αβ-hopanes, especially since the amount of organic carbon, to which the concentrations are normalized, parallels the concentration trends (Table 5). Additional support for this conclusion will come from a discussion of the James River samples.

Hopanoid acids of αβ-configuration were identified in about 1/3 of the samples. Unlike the αβ-hopanes, they are not clearly more concentrated at the mouths of rivers (Figure 28; Table 4). There is only one case where a relative concentration maximum coincides with the presence of a river (the Patuxent), and this occurrence may be a coincidence. In addition to αβ-hopanoid acids, a few sediment samples also contain small amounts of βα-hopanoid acids. In both cases, only one epimer at C_{22} was present, which indicates that these αβ- and βα-hopanoid acids are unrelated to the fully maturated material that may provide the αβ-hopanes. The additional fact that ββ-C_{32} hopanoid acid is the major triterpenoid acid in all samples and is 10 to 20 times more

Figure 27. Distribution of total fossil hopanes in surface sediments from the Chesapeake Bay.

TOTAL FOSSIL HOPANES

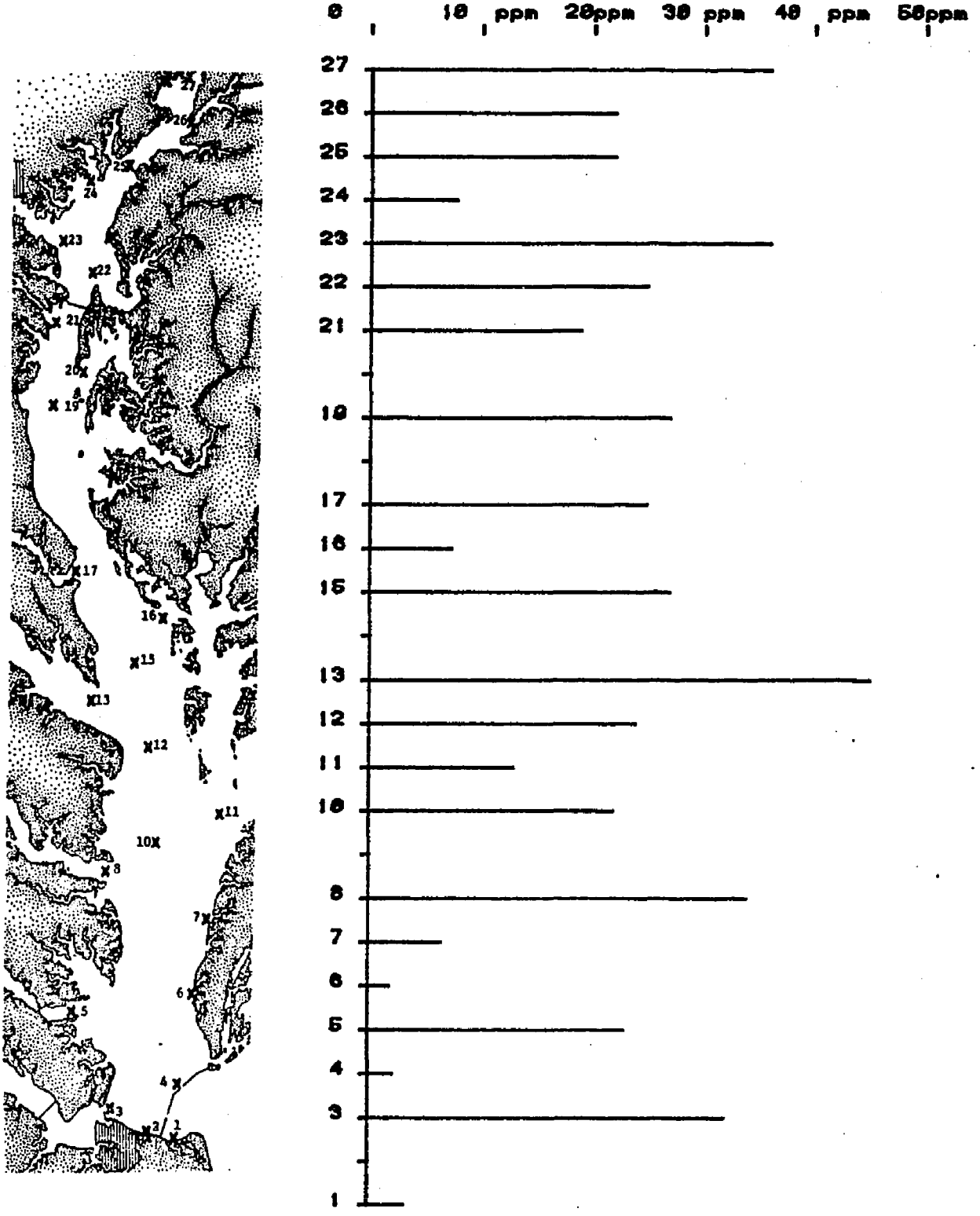
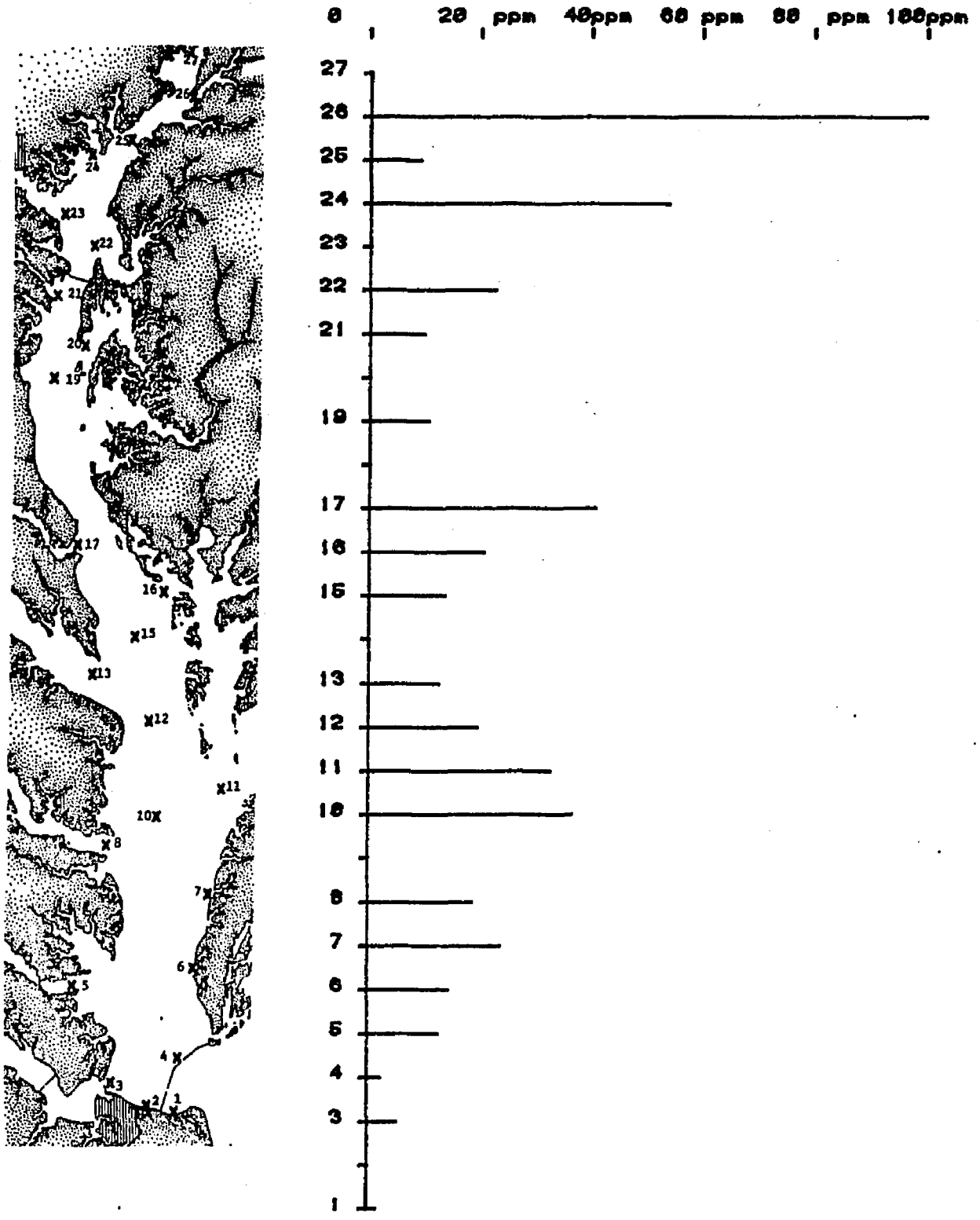


Figure 28. Distribution of total hopanoid acids in surface sediments from the Chesapeake Bay.


TOTAL HOPANOID ACIDS
0
20 ppm
40ppm
60 ppm
80 ppm
100ppm
27
26
25
24
23
22
21
19
17
16
15
13
12
11
10
8
7
6
5
4
3
1

concentrated than the corresponding $\alpha\beta$-C_{32} acid (where the latter is available for such a comparison) points in the same direction.

The absence of fully maturated hopanoid acids and the dominant presence of $\alpha\beta$-hopanes, two facts that seem to contradict each other, can be reconciled if one can identify a maturated source of hopanes that is devoid of hopanoid acids or contains the latter in only trace amounts. Petroleum is known to be such a source (Seifert, 1975) and as a consequence, hopane-containing derivatives such as motor oils, some asphalts and bunker C oils will also have this characteristic. Maturated coal and sedimentary rocks are likely to contain substantial amounts of acids (Ensminger, 1977).

For a petroleum related origin (including oils and oil contaminated soils) $\alpha\beta$-hopanes from C_{27} to C_{35} should be present, with epimer ratios S/R ~ 1.4 for extended hopanes. The data in Tables 3 and 19 clearly do not conform to these expectations, indicating other than fully maturated $\alpha\beta$-hopane components to be substantial. With few exceptions, the full range of $\alpha\beta$-hopanes is only found in river mouth samples. In most cases, this is a sensitivity problem, since the concentrations decrease with increasing carbon number. Most of the disagreement is in the S/R ratios for homohopane (C_{31}), which deviate substantially from those in oil. Two explanations can be offered: the lower ratios are the result of an admixture of immature sedimentary detritus with C_{31} S/R ratios close to or less than unity and/or they are a consequence of the addition of pure R isomer. Sedimentary detritus from the erosion of immature rock formations, unless its C_{31} S/R ratio

TABLE 19

Stereomeric ratios for hopanoids in surface sediments from the Chesapeake Bay.

		αβ:ββ		βα:αβ		S:R (αβ)					
	Station	C_{32} acid	*C_{27}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}	Average $C_{32} + C_{33}$
Southern Bay	1	N.D.	N.D.	N.D.	N.D.	1.3	N.D.	N.D.	N.D.	N.D.	N.D.
	3	N.D.	1.0	0.20	0.19	1.0	1.3	1.7	1.4	1.2	1.5
	4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	5	N.D.	0.73	0.21	0.17	0.84	1.3	1.2	1.2	1.2	1.3
	6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	7	N.D.	N.D.	N.D.	N.D.	0.77	N.D.	N.D.	N.D.	N.D.	N.D.
	8	0.08	3.2	N.D.	0.25	0.94	1.2	1.3	N.D.	N.D.	1.3
	10	N.D.	3.0	0.24	0.25	1.0	1.2	N.D.	N.D.	N.D.	N.D.
Central Bay	11	0.06	2.3	0.21	0.31	1.0	N.D.	N.D.	N.D.	N.D.	N.D.
	12	N.D.	3.2	0.24	N.D.	1.0	N.D.	N.D.	N.D.	N.D.	N.D.
	13	N.D.	0.74	0.25	0.29	0.69	1.3	1.3	1.2	1.1	1.3
	15	0.07	5.8	N.D.	0.28	0.87	1.2	1.5	1.7	1.1	1.4
	16	N.D.	0.15	0.20	0.60	0.92	1.3	N.D.	N.D.	N.D.	N.D.
	17	0.10	3.0	0.20	0.34	0.89	1.3	N.D.	N.D.	N.D.	N.D.
	19	N.D.	N.D.	N.D.	0.09	1.4	1.3	1.2	N.D.	N.D.	1.3
	21	N.D.	6.4	0.37	0.18	1.1	1.3	1.3	1.4	1.1	1.3
Northern Bay	22	0.11	1.2	0.34	0.11	1.3	1.4	1.4	1.2	1.2	1.4
	23	N.D.	1.9	0.39	0.15	1.3	1.2	1.4	1.6	1.1	1.3
	24	0.04	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	25	N.D.	1.9	N.D.	0.08	1.5	1.3	1.2	1.3	N.D.	1.3
	26	0.10	1.9	0.49	0.19	1.7	1.5	N.D.	N.D.	N.D.	N.D.
	27	N.D.	1.8	0.30	0.13	0.98	1.3	1.3	0.94	1.0	1.3

N.D.: Not determined.
*: α/β

is close to zero, would require relatively large amounts of material to be added to significantly affect the ratio of homohopane epimers in the sediments. Similar to the observations on αβ-hopanes, hopanoid acids should be higher in samples collected at river mouths. This, however, has been found not to be a pronounced effect as mentioned earlier. The more likely possibility would then be the admixture of pure R isomer. This demands that αβ-homohopane is generated via some rapid diagenetic process from recent hopanoid input. Decarboxylation of the abundant ββ -bishomohopanoic acid, followed by in situ isomerization of ββ-homohopane to αβ-homohopane would provide such a mechanism. The Huttenheim and Robertsau muds (Rohmer et al., 1980) suggest that these processes are possible under proper environmental conditions, which include generally reducing conditions and microbiological isomerization as a fast process to replace acid catalysis for the ββ- to αβ-transition. Since C_{31} and C_{33} hopanoid acids are present in much lower concentrations (Table 4), only the C_{31} S/R ratio is affected, although analogue reactions would also occur for the ββ-C_{31} and ββ-C_{33} acids. It is interesting to note that the C_{31} S/R ratio in soil samples (Table 7) do not show this dilution effect.

Substantial disagreement with expectations also exists for the (17β,21α)/(17α,21β) C_{29} and C_{30} hopane ratios. Instead of ratios that typically are <6 % in crude oils and bitumen (Seifert and Moldowan, 1980) these ratios, where they have been determined in Bay samples, with few exceptions are >20 %. Such a result would require a source of βα-hopanes in the sediments other than that from petroleum products.

However, since (17β,21α)/(17α,21β) hopane ratios for Libyan crude, Richmond coal and asphalt are also unreasonably high (Table 6), one is tempted to suspect that the peaks identified as βα-C_{29} and C_{30} hopanes in addition contain something else. In sediment samples, superimpositions of βα-C_{29} hopane with hop-17(21)-ene and βα-C_{30} hopane with ββ-C_{29} hopane (Figure 8 a&b) also create some resolution problem in integration of these compounds which could have caused the observed discrepancies. Although hop-17(21)-ene and ββ-C_{29} hopane are not supposed to be present in crude oil, Figure 14 shows that there are some other compounds superimposed with the βα-hopanes. The (17β/17α)-C_{27} ratio is not usable as an indicator for the input of fossil hopanes to recent sediments because of the high contribution of recent 17β(H)-C_{27}.

Although the distribution of αβ-hopanes in sediment samples deviates in some aspects from the characteristics of maturated material, these discrepancies can be understood to result from an admixture of recent hopanes and petroleum and its derivatives. For example, the ratio of resolvable to total aliphatic hydrocarbon concentration is an indicator for the presence of petroleum, even though the CPI may indicate biosynthesized material. Aromatic hydrocarbons, in general, are not biosynthesized and where they are present, they are likely the result of some polluting event. This situation is somewhat complicated by the fact that there are two major types of sources of aromatic hydrocarbons: one that originates from geochemical processes and another that is synthesized in high temperature environments (combustion). Only

the former relates to hopanes. However, in a first approximation, the resolvable to total aromatic hydrocarbon concentration ratio again can be used as an indicator of petroleum. Table 17 shows that both indicators can be taken as supportive evidence that these samples contain petroleum or petroleum related products.

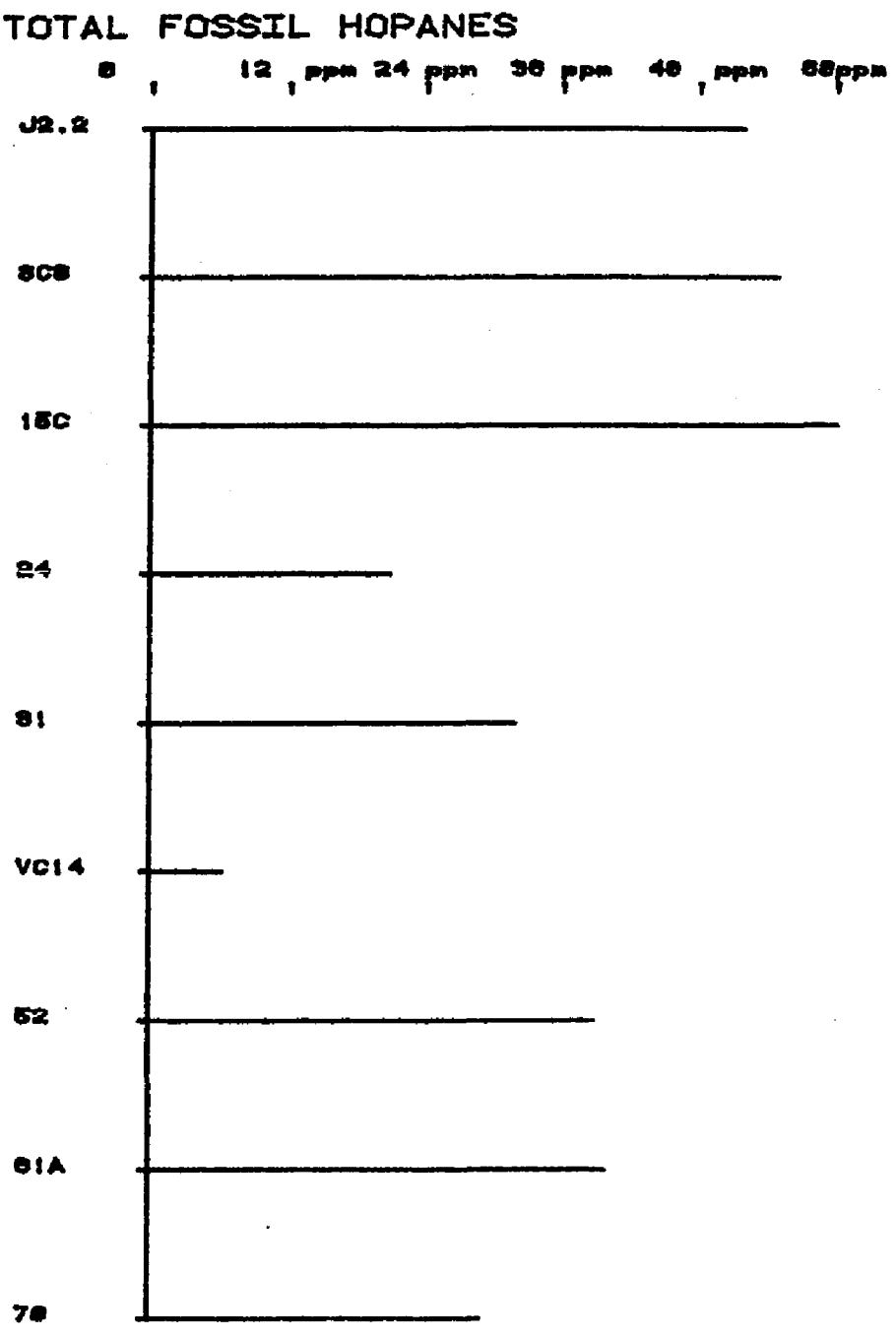
Petroleum in the Chesapeake Bay is likely to originate from boating activities, runoff from land and introduction via sewage treatment plant outfalls. Four possible types of contributors of 17α(H),21β(H) hopanes to Chesapeake Bay sediments have been examined and are shown in Table 6. Of these, motor oil is an important potential source, as it is being used in large quantities in automobile and marine engines. Hopanoids from motor oils can enter the Bay in different ways. Boats with outboard motors use a mixture of gasoline and oil as fuel and most of the oil is discharged with the exhaust into the water. Used motor oils from automobiles, although it is illegal, may be discarded into the environment and reach the Bay via runoff, or it may be dumped into a drainage system, from where it will eventually reach the Bay. Motor oils could then provide a concentrated source of hopanes to recent sediments. In rural areas, used motor oils have been commonly sprayed on dirt roads and private driveways to act as a binder for street dust. The practice has probably not yet been abandoned altogether. Again, such practices will add fossil hopanes to surface soils and in the end contribute fossil molecules to Bay sediments via natural runoff from land.

The significance of asphalt abrasion from highways as an important contributor to the PAH content of lake sediments has been shown by

Wakeham et al. (1980). Asphalt particles and street dust could therefore also be contributors of fossil hopanes to the Bay. There is a concentration gradient of hopanes in soil samples collected at different distances away from a major highway (U.S. Route 17) (Figure 17), suggesting that the input of hopanoids to surface soils is related to highway usage. Hopanoids in roadside soils or dusts collected off asphalt-surfaced highways and cement-surfaced streets show similar concentrations and composition (Table 8). The bimodal UCM in Figure 16 may reflect a superimposition of asphalt abrasion products and oil exhaust from motor vehicles in samples collected off asphalt-surfaced highways. For samples collected near cement-surfaced streets, it is conceivable that the biodegradation and weathering of motor oils would produce a hopanoid composition similar to that found in asphalt. Alternatively, these cement-surfaced streets could have been paved with asphalt before they were reconstructed. The probable mode of transport for street dust and the associated fossil hopanes to the Bay is via stormwater runoff, followed by transport as river and stream particulate matter. Although eolian transport is also possible, it is probably short range.

Table 18 clearly shows that the James River is an important source of αβ-hopanes into the Bay. Surface sediments from the James River (Table 13) contain αβ-hopanes as dominant compounds. However, no evidence of a concentration gradient towards the mouth of the River was observed (Figure 29). This either indicates that a dominant fossil hopane source is absent or that currents and dredging operations exert a homogenizing influence. The fact that James River cores are relatively homogeneous in terms of their Pb-210 activities (Goldberg et al., 1978;

Figure 29. Distribution of total fossil hopanes in surface sediments from the James River.


TOTAL FOSSIL HOPANES
0
12 ppm
24 ppm
36 ppm
48 ppm
60ppm
J2.2
8C8
15C
24
81
VC14
52
61A
70

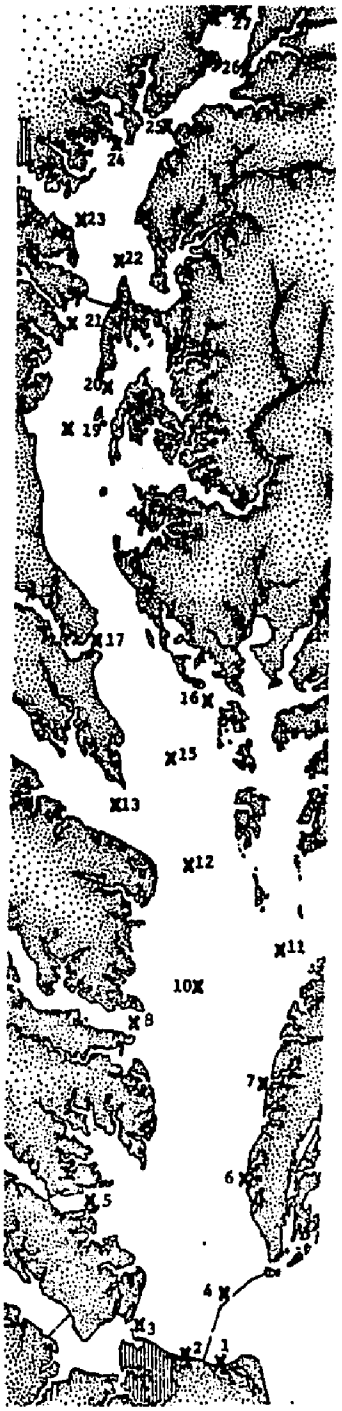
M. Koide, personal communication; present study) indeed suggests that sediment mixing is common. As a result, the James River cores cannot be used for a diagenetic analysis of individual hopanoids. In general, the total αβ-hopane concentrations in the James River surface sediments are higher than those in the Bay, except for the river mouth stations (Tables 17 and 18). All but one sample (VC-14) exhibit low resolvable to total aliphatic and total aromatic hydrocarbon concentration ratios (Table 18), which again indicates petroleum input.

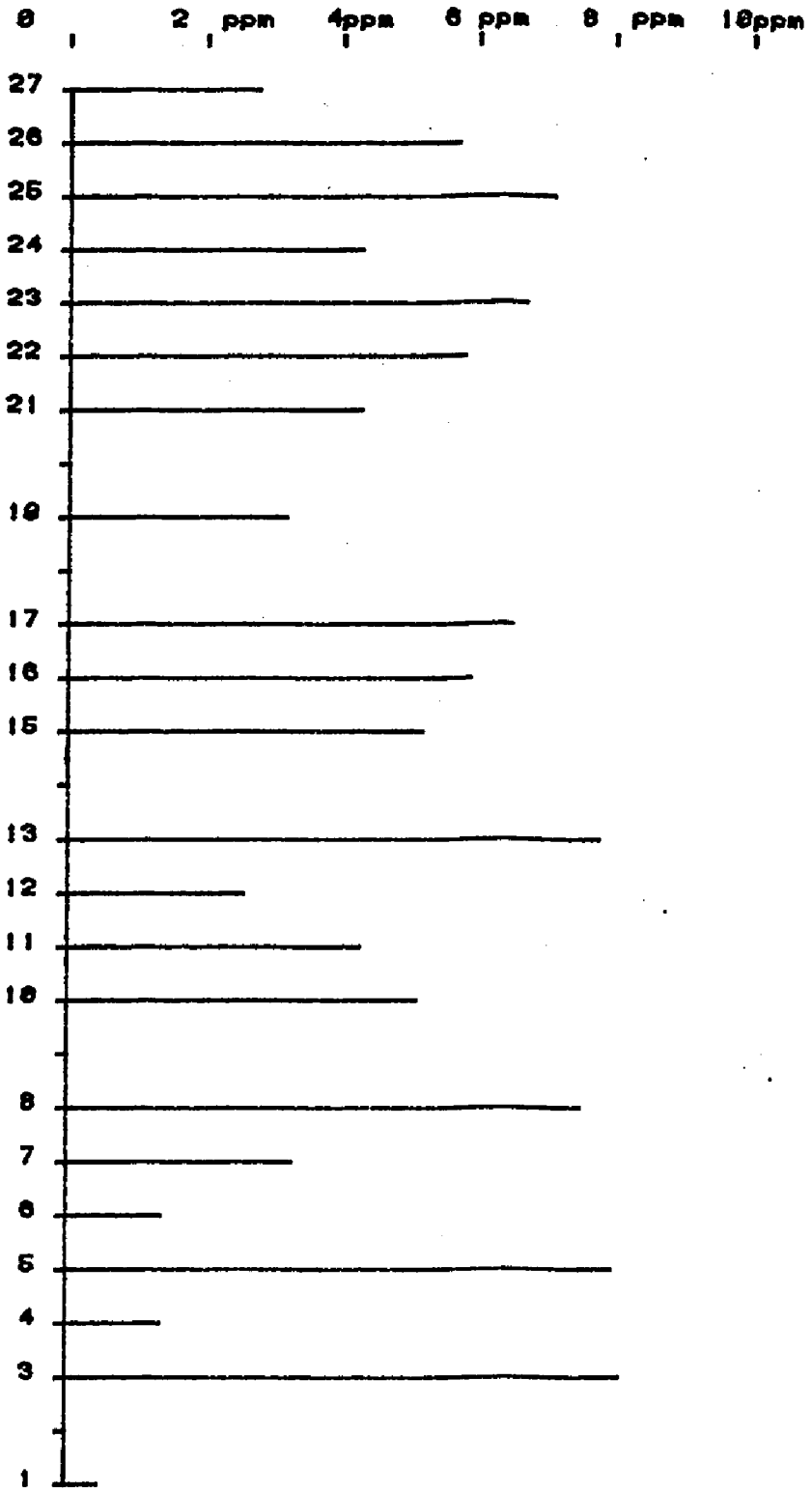
In addition to αβ-hopanes in Bay sediments, Tables 3 and 17 show hopanes of 17β(H),21β(H) configuration to also be quite abundant, lagging by only a factor of 3 to 5 behind the αβ-hopanes in most samples (Table 17). Furthermore, there are substantial amounts of 17β(H),21β(H) acids and hop-17(21)-ene present, suggesting contribution from recent biosynthesis. With all these hopanoids present in the Chesapeake Bay sediment extracts, it is clear that one is looking at a considerably more complex situation than Dastillung and Albrecht (1976) in their "La Rochelle" sediment. Accordingly, the use of fossil (αβ-) hopanes as test molecules for oil pollution under such circumstances loses some of the simplicity that was an attractive aspect of their paper.

The ββ-hopane distributions in Bay samples resemble those of the αβ-hopanes in so far as high concentrations, in general, are found in samples from river mouths (Figure 30; Table 17). This, again, suggests that rivers are sources of ββ-hopanes, although the ββ-hopanes are of natural origin rather than contaminants as the fossil αβ-hopanes are. A contribution of ββ-hopanes, hopenes and ββ-hopanoid acids from the

Figure 30. Distribution of total biogenic hopanes in surface sediments from the Chesapeake Bay.

TOTAL BIOGENIC HOPANES





weathering of sedimentary rocks is possible but the random distribution of hopanoid acids already mentioned argues against it.

In the discussion of extended αβ- epimer ratios, it was concluded that first a decarboxylation and subsequent isomerization would provide enough R epimer of αβ-homohopane to change the S/R ratio to a value less than the analog fossil values determined for the C_{32} and C_{33} hopanes. If this hypothesis is correct, then it should also fit the distribution of ββ-hopanes in the Bay samples.

According to Rohmer et al. (1980), ββ-hopanes in the marine environment in most cases do not have a biotic origin, but derive from biogenic precursors by diagenetic reactions. The most common precursor, bacteriohopane tetrol, has been found to disappear rapidly, to form mainly ββ-bishomohopanoic acid (C_{32}), together with lesser amounts of ββ-C_{31} and ββ-C_{33} hopanoid acids (Van Dorsselaer, 1975). In a generally reducing environment (such as that provided by anoxic sediments), decarboxylation has been shown to progress at low temperatures via acylate radical formation (Cooper and Bray, 1963). The data on ββ-hopanes clearly show ββ-homohopane (R) to be more abundant than either ββ-hopane or ββ-bishomohopane. Since ββ-C_{32} hopanoid acid is the major acid in these sediments, the assumption of a decarboxylation step for the formation of ββ-homohopane is logical. Further assuming that a proportional amount of ββ-hopane has been formed from decarboxylation of the ββ-C_{31} acid, one finds that the precursor/product ratio is too high by approximately a factor of two as compared to the ratio of ββ-C_{31} hopane/ ββ-C_{32} acid in the same

samples. This is not unreasonable, since ββ-hopane has diploptene, hop-17(21)-ene and diplopterol as additional precursors (Ensminger, 1977), and these, like the bacteriohopane tetrol are known to be abundantly produced by bacteria and blue-green algae. Hop-17(21)-ene also is easily formed by isomerization of diploptene (Ensminger, 1977).

An interesting observation is made in the sample from the mouth of the Patapsco River (Station 23). This sample contains hop-17(21)-ene and ββ-hopanes, but only a trace amount of ββ-bishomohopanoic acid. This area receives industrial as well as domestic input from the City of Baltimore and at the time of sampling (Fall, 1979) may have lacked the conditions necessary to oxidize the sidechain of bacteriohopane tetrol to ββ-hopanoid acids. An alternative will be that decarboxylation of the acids at this station is so rapid that all the acids transform into ββ-hopanes. That this is not a permanent problem, however, can be seen from the fact that ββ-C_{31} is also present and is the most abundant ββ-hopane in the sample.

Hop-17(21)-ene in surface sediments can be derived: (1) directly from bacteria and incorporated into the sediments or (2) from isomerization of diploptene at the sediment-water interface. Since diploptene was not detected in any of the Chesapeake Bay samples, the hop-17(21)-ene in the samples either derived from bacteria or isomerization of diploptene in these samples is so rapid that all of diploptene is converted to hop- 17(21)-ene. According to Ensminger (1977), isomerization of diploptene occurs in a mild acidic environment (reducing environment). Considering that decarboxylation is responsible for the formation of ββ-C_{31} hopane as previously discussed,

isomerization of dipolptene in the same sediment samples can also be expected. However, direct contributions of hop-17(21)-ene from bacteria cannot be neglected.

It is well known that these natural hopanoids are very unstable and tend to transform to the more stable hopanoid compounds. Some transformations that may occur in recent sediments are the isomerization at position 17 of the ββ-hopanoids to their more stable, αβ-hopanoid counterparts (Eglinton et al., 1975; Rohmer et al., 1980) and the isomerization of diploptene to hop-17(21)-ene (Ensminger, 1977) as mentioned above. Isomerization of the ββ-C_{31} to αβ-C_{31} hopane is believed to occur in many surface sediment samples from the Bay as discussed earlier. Similar to the observations in Bay samples, evidence of isomerization of ββ-C_{31} hopane can also be seen in the record from a sediment core taken from Colonial Beach (CB-002). The S/R ratio of the αβ-C_{31} hopane in the surface sample is characteristic of that from fossil materials whereas the ratio in all subsurface samples is less than one (Table 20). There appears to be evidence of an isomerization of ββ-C_{32} acid to αβ-C_{32} acid in these core sediments. However, such transformation does not correspond with the nature of hopanoid acid isomerization which is known to be rather slow compared to that of the hydrocarbons (Ensminger, 1977). The constant ratios of ββ-C_{32}/ αβ-C_{32} acids may be viewed as an indication that isomerization of the acid has already occurred in previously deposited estuarine sediments. The presence of higher concentrations of hop-17(21)-ene relative to

TABLE 20

Stereomeric ratios for hopanoid hydrocarbons and acids at station CB002.

Depth, Cm	Hydrocarbons αβ:ββ					Hydrocarbons S:R			Acids αβ:ββ	
	*C_{27}	C_{29}	C_{30}	C_{31}	C_{32}	C_{31}	C_{32}	C_{33}	C_{32}	C_{33}
0- 5	0.92	5.0	4.6	0.81	5.5	1.3	1.2	1.3	0.09	0.25
30- 35	0.58	0.68	0.21	0.31	N.D.	0.76	N.D.	N.D.	0.08	N.D.
60- 65	0.04	0.82	0.19	0.31	N.D.	0.75	N.D.	N.D.	0.09	N.D.
90- 95	0.33	0.84	0.17	0.35	0.19	0.85	N.D.	N.D.	0.10	N.D.
120-125	0.22	0.81	0.18	0.31	N.D.	0.83	N.D.	N.D.	0.08	N.D.
150-155	0.32	0.91	0.17	0.32	0.21	0.91	N.D.	N.D.	0.10	N.D.

N.D.: Not determined
*: α/β

diploptene (Table 10) and the isomerization of $\beta\beta$-C_{31} to $\alpha\beta$-C_{31} hopane, suggest an acidic sedimentary environment as discussed by Ensminger (1977) and Van Dorsselear et al. (1977). However, the absence of $\beta\beta$-C_{33} to C_{35} hopanes suggests that oxidative cleavage of the sidechain of bacteriohopane tetrol rather than reduction of the hydroxyl groups and subsequent demethylation was the major mechanism at the sediment-water interface. The non-continuous deposition at this core location, as seen from the Pb-210 data (Table 16) which show an older layer of sediment at the 30-35 cm level sandwiched between the surface and deeper sediments (M. Koide, personal communication), suggests that it would be very difficult to conclusively assess diagenetic effects for these core samples. The vertical distribution of hopanoid molecules at station CB-002 (Tables 10 and 11) clearly confirms the Pb-210 observations. All components were highest at the surface, decreased rapidly in the upper 30 cm and remained reasonably constant throughout the core. The decrease in Pb-210 activity between the 0-5 cm and the 30-35 cm samples would correspond to a sedimentation rate of 1.1 cm/year, which is in good agreement with data obtained nearby (Knebel et al., 1980).

VI. CONCLUSIONS

Conclusions from this study are summarized as follows:

1. Hopanoid molecules have been found to be ubiquitously present in all samples from the Chesapeake Bay and its two subestuaries, at concentrations that vary with sampling locations. In general, concentrations are higher at river mouth stations and in the Northern Bay area than at stations near the Eastern shore and in the Central and Southern Bay.

2. The dominant hopanoids are hopanes of 17α(H),21β(H) structure and range from C_{27} to C_{35}, with extended hopanes generally present as mixtures of 22S and 22R diastereomers. The presence of these fossil hopanes in recent sediments can readily be attributed to anthropogenic inputs. The 17β(H),21β(H) hopanes and hop-17(21)-ene in Bay samples are associated with recent biogenic input. Their contributions in general are lower than those of the fossil hopanes.

3. A comparison of fossil hopanes in Bay sediments and several source materials suggests that motor oils, asphalt particles and street dust are potential and possibly major present-day sources for the 17α(H),21β(H) hopanes in the Bay. These source materials and the associated fossil hopanoids can reach the Bay via natural runoff from

land during rain storms, sewage outfalls, and transport by rivers if they are not introduced directly. Motor oil may be directly deposited into the Bay by boating and marine transport activities.

4. Rivers appear to be important sources of fossil hopanes to the Bay as evidenced by higher concentrations of fossil hopanoids at river mouths relative to Bay sediments. A number of surface sediments from the James River contain comparable hopanoid concentrations. The elevated concentrations of fossil hopanes at river-mouth stations generally correlate with industrial and urban growth in the region surrounding each river, thereby indicating an anthropogenic origin for these fossil 17α(H),21β(H) hopanes.

5. Indirect evidence suggests that the mode of hopanoid transformation in the Chesapeake Bay estuary is mainly by decarboxylation of hopanoid acids, leading to ββ-hopanes of the one carbon number less than the acid, after the acids were formed by oxidation of the bacteriohopane polyols side-chain. This indicates an oxidizing environment in the water column and at the water/sediment interface. Evidence for a reducing environment is provided by the decarboxylation of hopanoid acids and of the isomerization from diploptene to hop-17(21)-ene.

6. Evidence has also been presented for early, probably microbially induced diagenesis, by the isomerization of ββ-C_{31} hopane

(R) to $\alpha\beta$-C_{31} hopane (R) in many surface sediment samples from the Chesapeake Bay.

The study of hopanoid molecules has shown that there is a significant anthropogenic input of fossil fuel-related products into the Chesapeake Bay estuary. Evidence has also been presented as to early diagenetic transformations of natural hopanoid molecules. Hopanoid molecules have proved to be quite valuable in adding to our knowledge of estuarine organic geochemistry.

APPENDIX

TABLE A1

Gel permeation chromotography of standard compounds (hexane fraction).

Compound	Abundances of compounds in each elution volume (ml)										
	0-90	90-100	100-110	110-115	115-120	120-125	125-130	130-135	135-140	140-180	180-220
$n\text{-}C_{11}$,$n\text{-}C_{12}$	-	-	-	-	-	-	-	-	+	+++	-
$n\text{-}C_{13}$,$n\text{-}C_{14}$	-	-	-	-	-	-	-	+	++	+++	-
$n\text{-}C_{15}$,$n\text{-}C_{16}$	-	-	-	-	-	tr	+	++	+++	+	-
$n\text{-}C_{17}$,$n\text{-}C_{18}$	-	-	-	-	-	+	+++	++	+	tr	-
$n\text{-}C_{19}$,$n\text{-}C_{20}$	-	-	-	-	+	+++	+++	++	tr	-	-
$n\text{-}C_{21}$,$n\text{-}C_{22}$,$n\text{-}C_{23}$	-	-	-	+	++	+++	+	tr	-	-	-
$n\text{-}C_{24}$,$n\text{-}C_{25}$	-	-	+	+++	++	+	tr	-	-	-	-
$n\text{-}C_{26}$,$n\text{-}C_{28}$	-	-	+	+++	++	+	tr	-	-	-	-
$n\text{-}C_{30}$,$n\text{-}C_{32}$	-	+	++	++	tr	-	-	-	-	-	-
Pristane	-	-	-	-	+	++	++	-	tr	-	-
2-Methyloctadecane	-	-	-	-	+	++	+++	++	tr	-	-
n-Decylcyclohexane	-	-	-	-	-	-	-	tr	+	+++	-
Androstane	-	-	-	-	-	-	-	+	++	+++	-
Cholestane	-	-	-	-	-	-	+	++	++	++	-

- : not present
+ : present in measurable amount
tr: trace amount

TABLE A2

Gel permeation chromatography of methyl ester (acid fraction).

Compound	Abundances of compounds in each elution volume (ml)										
	0-90	90-100	100-110	110-115	115-120	120-125	125-130	130-135	135-140	140-180	180-220
C_{25}	-	+	++	tr	-	-	-	-	-	-	-
C_{26}	-	+	++	tr	-	-	-	-	-	-	-
C_{27}	-	+	++	-	-	-	-	-	-	-	-
C_{28}	-	++	+	-	-	-	-	-	-	-	-
C_{30}	-	++	+	-	-	-	-	-	-	-	-
Cholanic acid	-	-	-	tr	+	+++	++	++	-	-	-

- : not present
+ : present in measurable amount
tr: trace amount

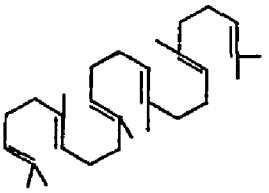
TABLE A3

Kovats indices of hopanoid hydrocarbons.

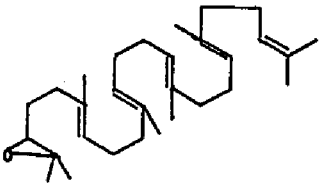
Compound	Kovats Index (mean ±%S.E.)	n
5α-Cholestane	2815±0.01	72 (Internal Std.)
17α-C_{27}	2864±0.02	53
17β-C_{27}	2901±0.01	53
αβ-C_{29}	3002±0.01	52
Hop-17(21)-ene	3041±0.01	52
βα-C_{29}	3051±0.02	72
αβ-C_{30}	3087±0.02	72
βα-C_{30}	3108±0.01	50
ββ-C_{29}	3120±0.01	50
αβ-C_{31}(S)	3189±0.02	72
αβ-C_{31}(R)	3201±0.02	72
ββ-C_{30}	3228±0.01	30
Diploptene	3254±0.01	30
αβ-C_{32}(S)	3270±0.02	56
αβ-C_{32}(R)	3289±0.02	56
ββ-C_{31}	3352±0.01	67
αβ-C_{33}(S)	3367±0.02	42
αβ-C_{33}(R)	3390±0.02	42
ββ-C_{32}	3445±0.02	26
αβ-C_{34}(S)	3466±0.02	29
αβ-C_{34}(R)	3496±0.02	29
αβ-C_{35}(S)	3567±0.02	24
αβ-C_{35}(R)	3600	24

ββ:17β(H), 21β(H)
βα:17β(H), 21α(H)
αβ:17α(H), 17β(H)

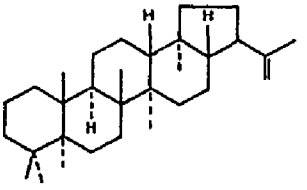
Figure A1. Structure of compounds.



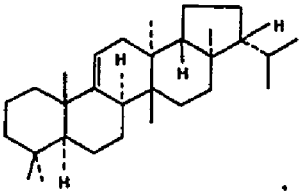
I. Squalene



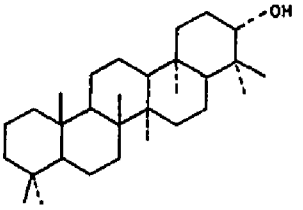
II. Squalene epoxide



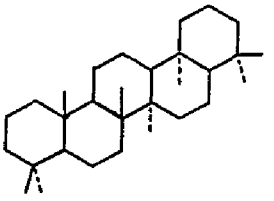
III. Hopene-b



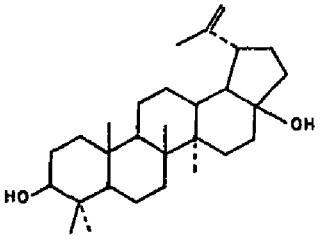
IV. Fernene



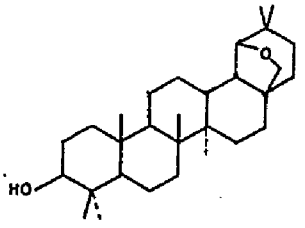
V. Tetrahymanol



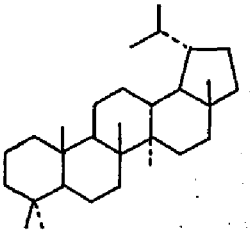
VI. Gammacerane



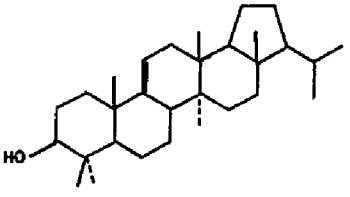
VII. Betulin



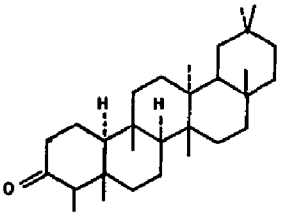
VIII. Allobetulin



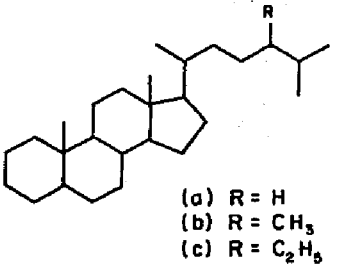
IX. Lupane



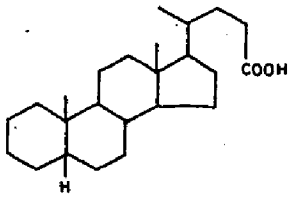
X. Isoarbarinol



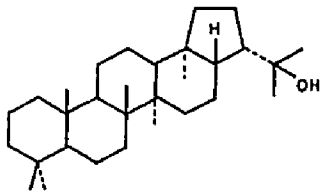
XI. Friedelin



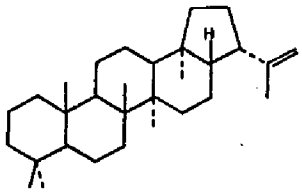
XII. Steranes



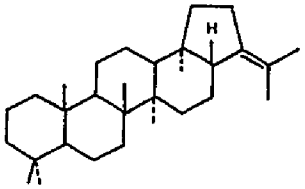
XIII. 5β - cholanic acid



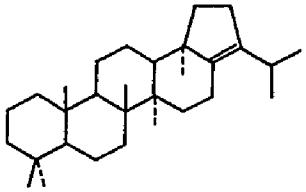
XIV. 22-hydroxyhopane (Diplopterol)



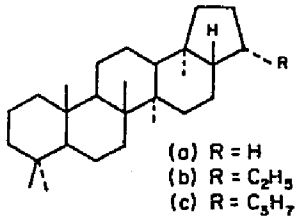
XV. Diploptene (Hop-22(29)-ene)



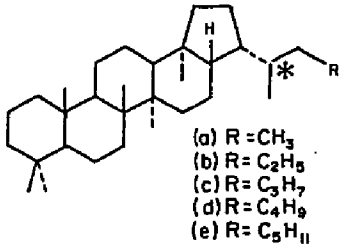
XVI. Hop-21(22)-ene



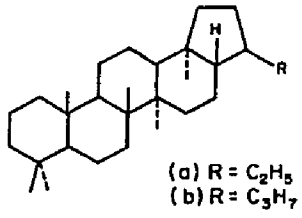
XVII. Hop-17(21)-ene



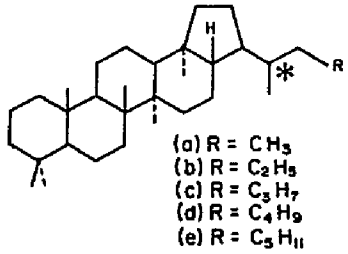
XVIII. 17β(H), 21β(H)-hopanes



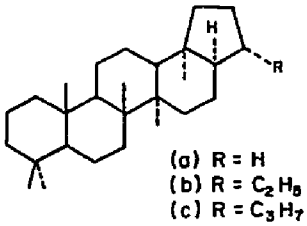
XIX. Extended 17β(H), 21β(H)-hopanes



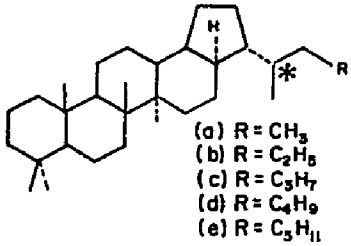
XX. 17β(H), 21α(H)-hopanes



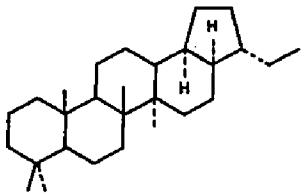
XXI. Extended 17β(H), 21α(H)-hopanes



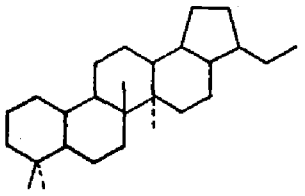
XXII. 17α(H), 21β(H)-hopanes



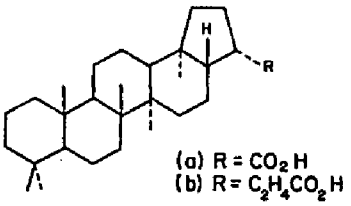
XXIII. Extended 17α(H), 21β(H)-hopanes



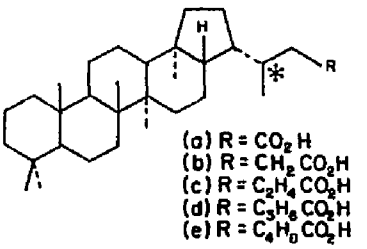
XXIV. 17α(H), 18α(H), 21β(H)-28,30-bisnorhopane



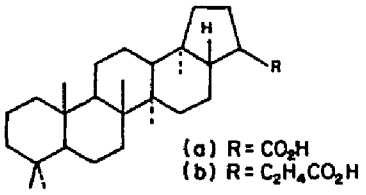
XXV. 25, 28, 30-trisnormoretane



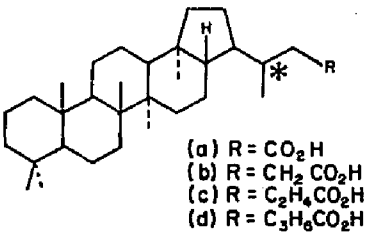
XXVI. 17β (H), 21β (H)-hopanoic acids



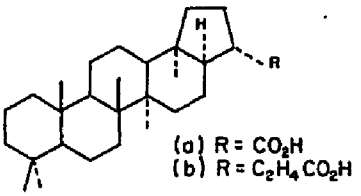
XXVII. Extended 17β(H), 21β(H) - hopanoic acids



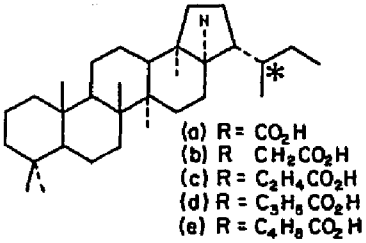
XXVIII. 17β(H), 21α(H)-hopanoic acids



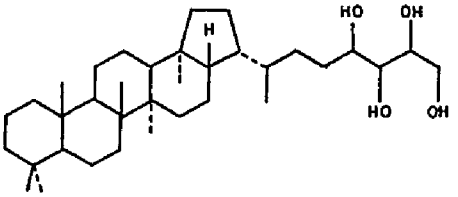
XXIX. Extended 17β(H), 21α(H)- hopanoic acids



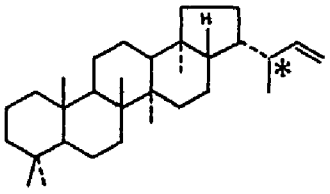
XXX. 17α (H), 21β(H)-hopanoic acids



XXXI. Extended 17α (H), 21β(H) - hopanoic acids



XXXII. Bacteriohopane polyols



XXXIII. 17β(H), 21β(H)-homohop-29 (31)-ene

Figure A2. Gas chromatogram of branched/cyclic alkane fraction from the Lorraine coal extract

n-C_{28}, n-C_{36}: co-injected standards, CH: cholestane (co-injected standard), C_{27}-C_{33}: αβ-hopanes.

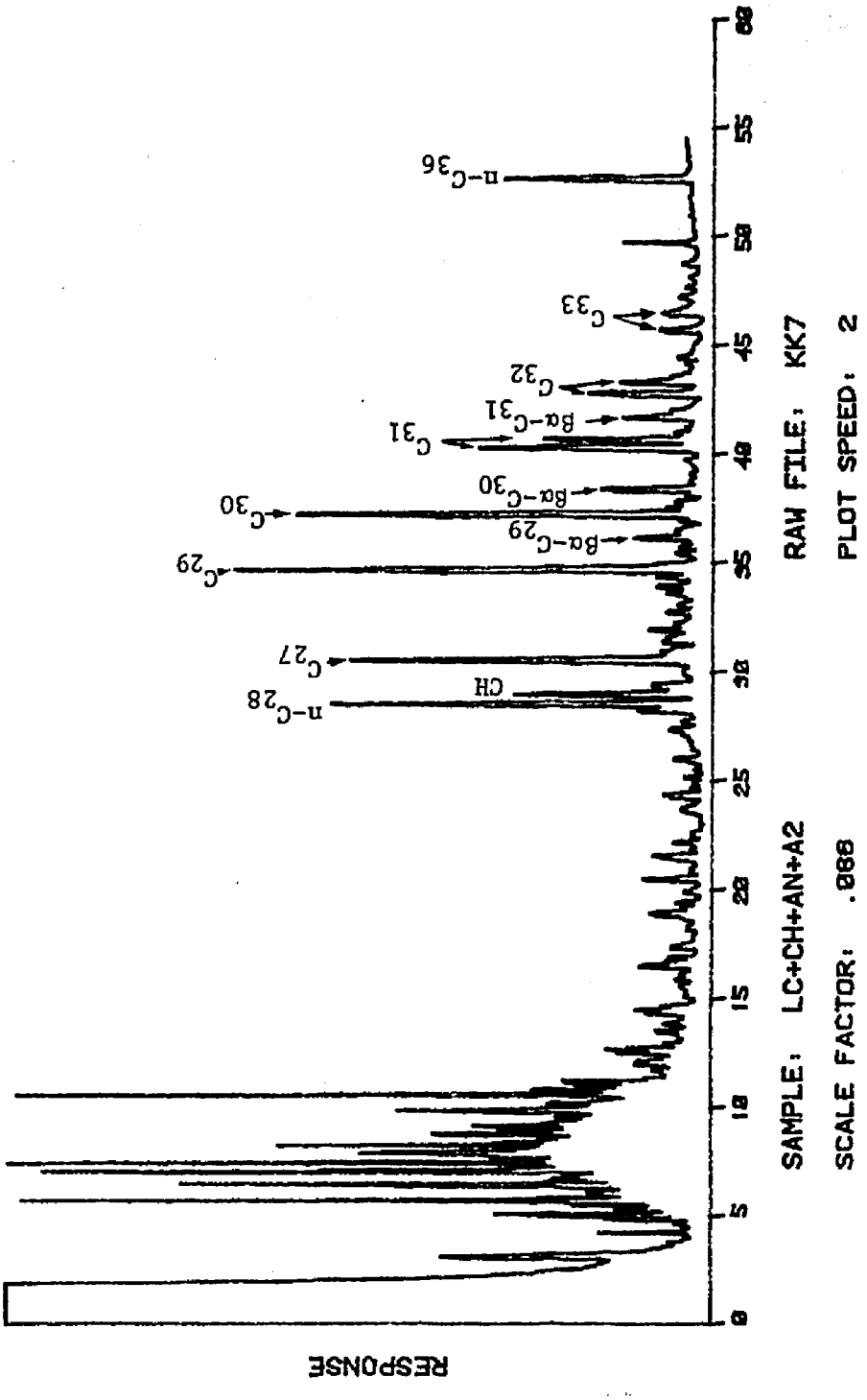

RESPONSE
0
5
10
15
20
25
30
35
40
45
50
55
60
n-C28
CH
C27
C29
Ba-C29
C30
Ba-C30
C31
Ba-C31
C32
C33
n-C36
SAMPLE: LC+CH+AN+A2
SCALE FACTOR: .088
RAW FILE: KK7
PLOT SPEED: 2

Figure A3. Mass chromatograms (m/e 191) of hopanoids.

A. Branched/cyclic alkane fraction from the Lorraine coal extract.
B. Hopane fraction from a sediment core extract, station CB-002 (120-125 cm).
C. Hopanoid acid fraction from a sediment core extract, station CB-002 (120-125 cm).

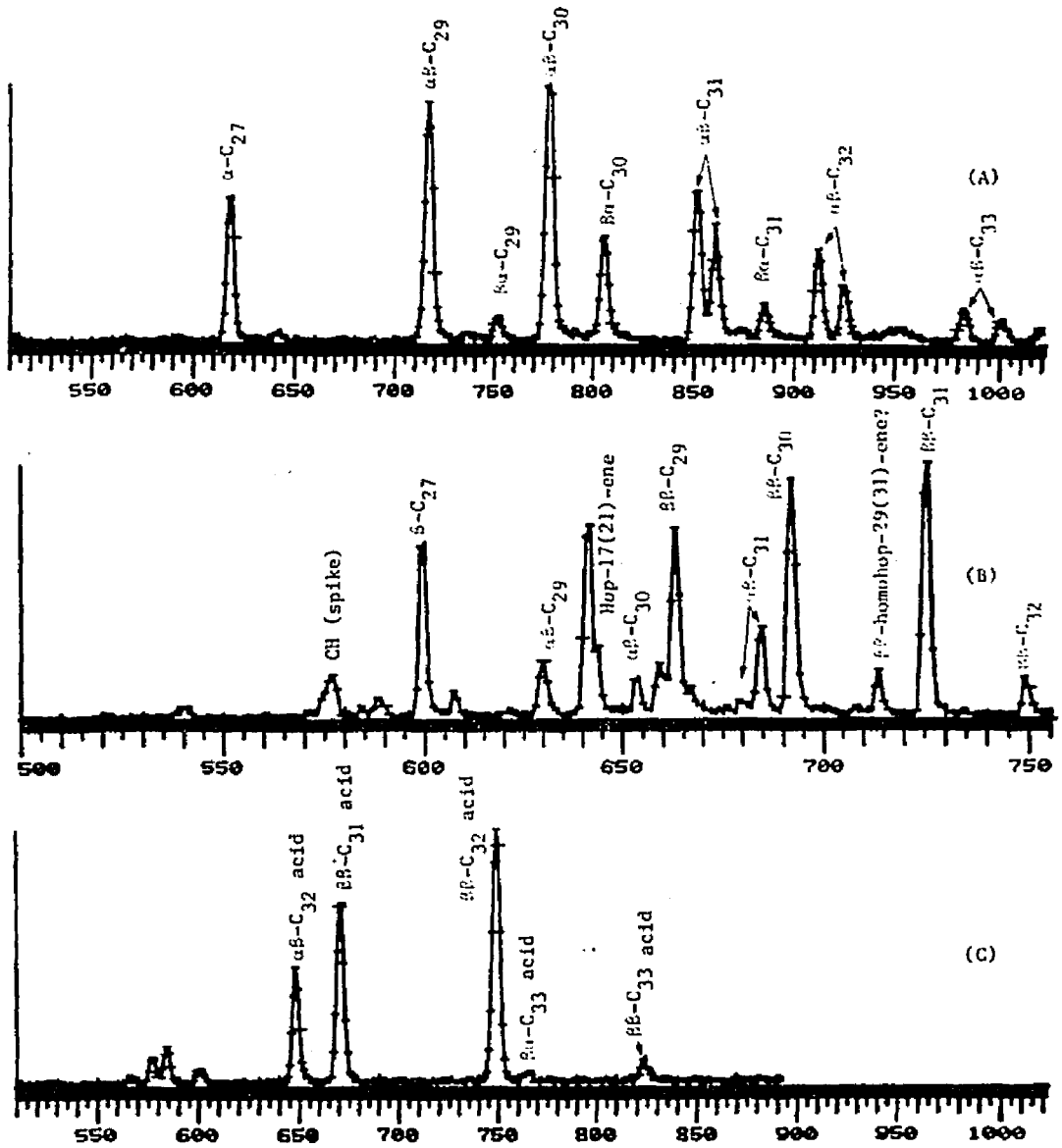

α-C27
αβ-C29
αβ-C30
βα-C30
αβ-C31
βα-C31
ββ-C32
ββ-C33
(A)
550
600
650
700
750
800
850
900
950
1000
CH (spike)
δ-C27
αβ-C29
Hop-17(21)-ene
αβ-C30
ββ-C29
ββ-C31
ββ-C30
ββ-homohop-29(31)-ene?
ββ-C31
ββ-C32
(B)
500
550
600
650
700
750
αβ-C32 acid
ββ-C31 acid
ββ-C32 acid
βα-C33 acid
ββ-C33 acid
(C)
550
600
650
700
750
800
850
900
950
1000

Figure A4. Mass spectra of unsaturated hopanoid hydrocarbons.

A. Hop-22(29)-ene (Diploptene).
B. Hop-17(21)-ene.
C. ββ-Homohop-29(31)-ene ??

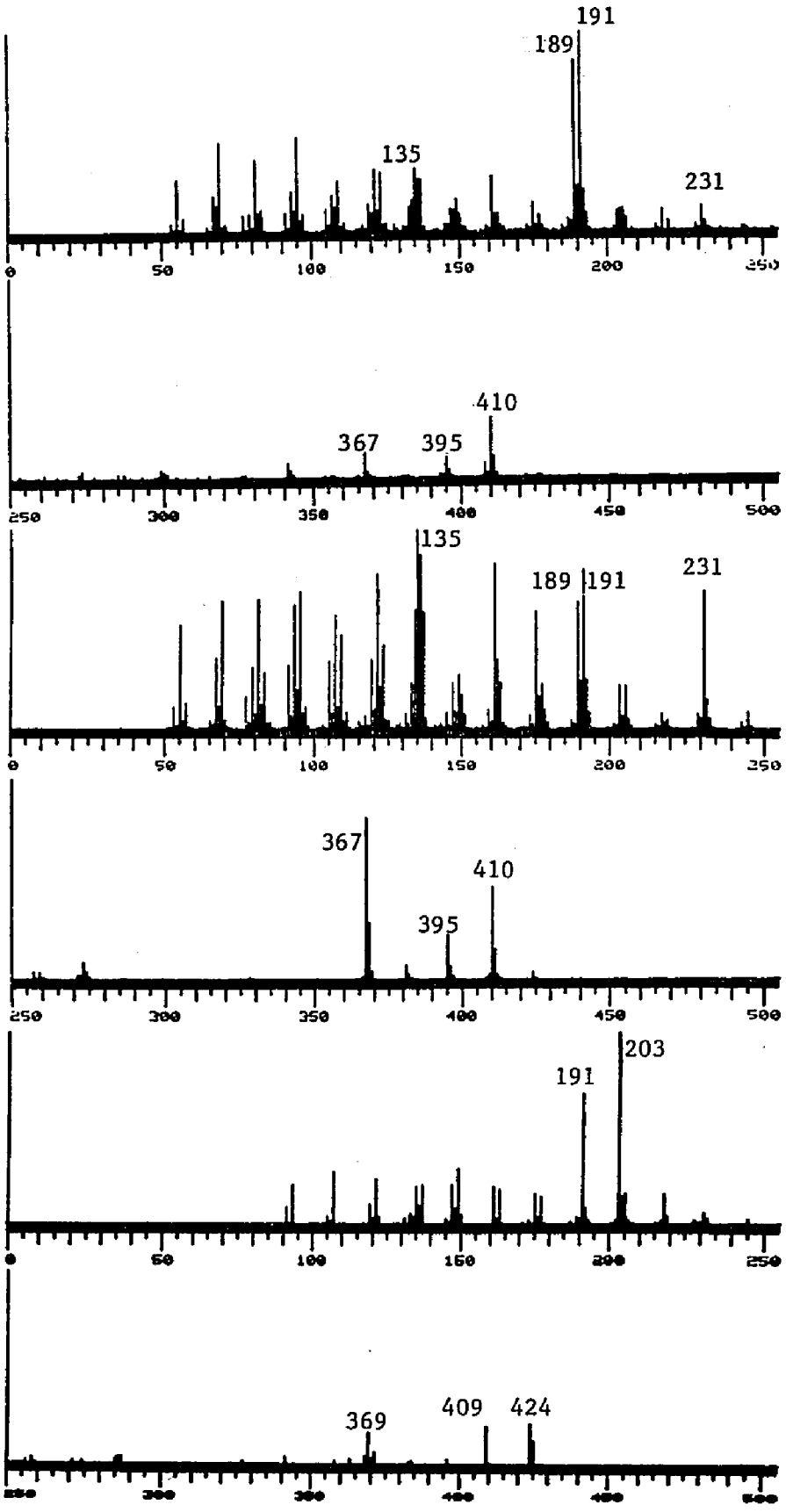

191
189
135
231
0
50
100
150
200
250
410
367
395
250
300
350
400
450
500
(A)
135
189
191
231
0
50
100
150
200
250
367
410
395
250
300
350
400
450
500
(B)
203
191
0
50
100
150
200
250
369
409
424
250
300
350
400
450
500
(C)

Figure A5. Mass spectra of 17β,21β-hopanes.

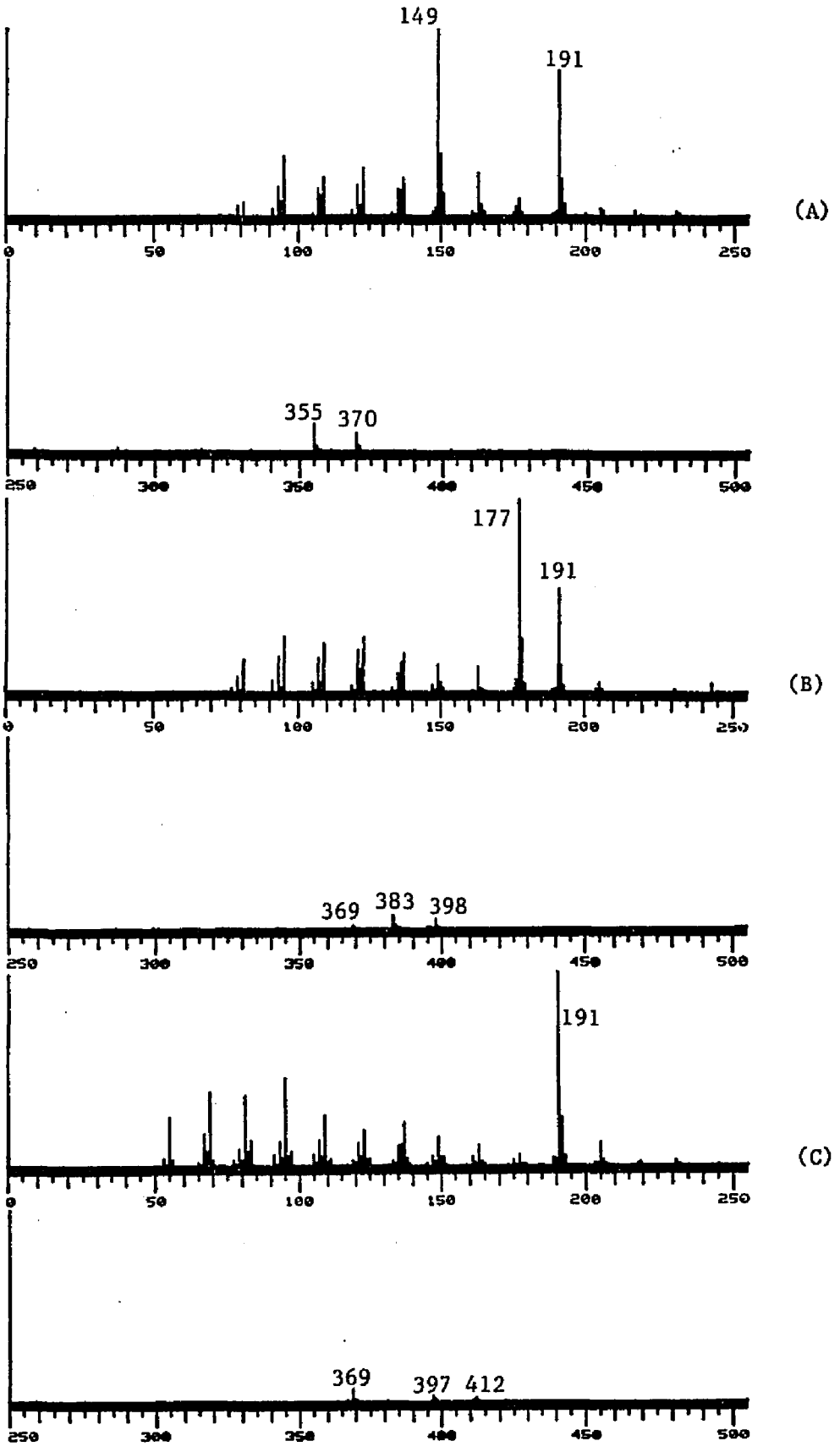
A. β-C_{27} hopane.

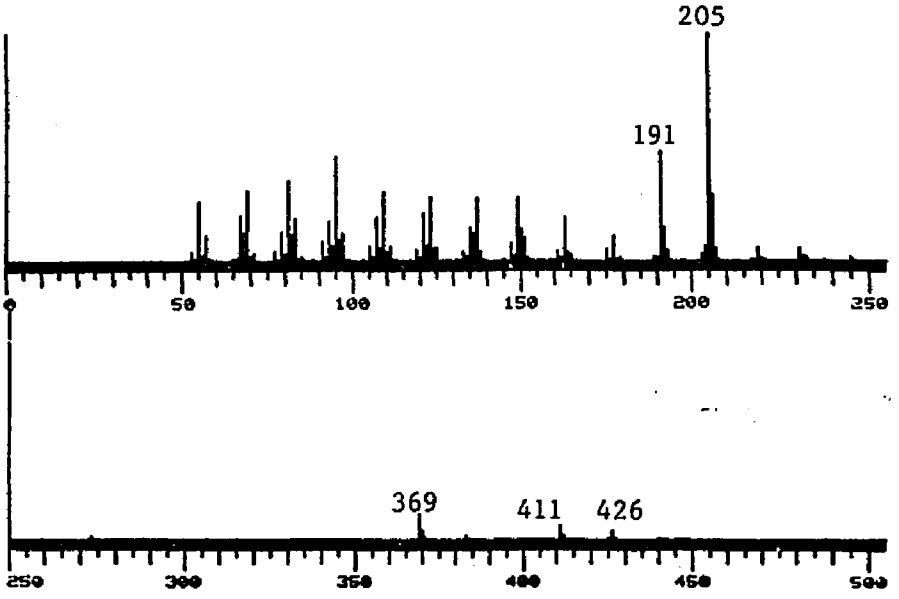
B. ββ-C_{29} hopane.

C. ββ-C_{30} hopane.

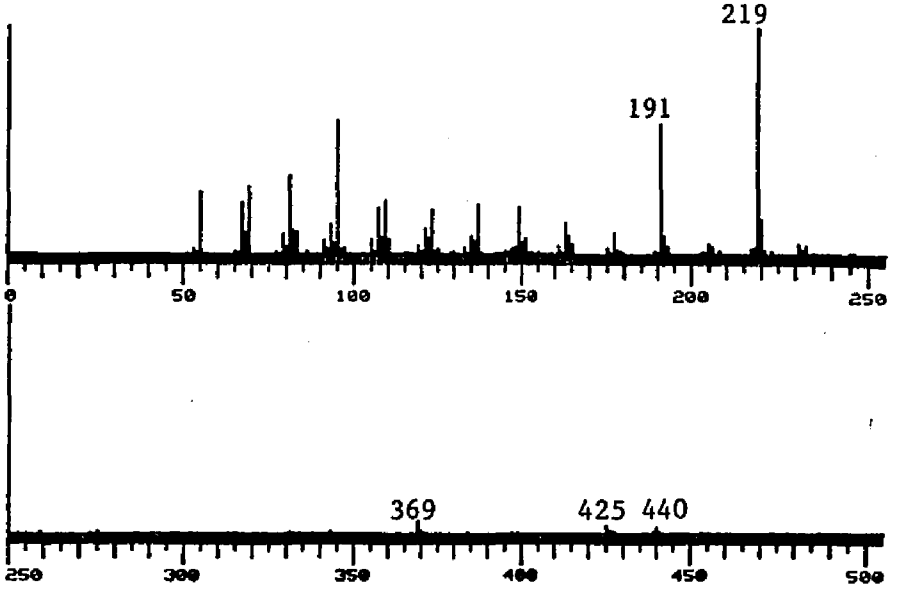
D. ββ-C_{31} hopane.

E. ββ-C_{32} hopane.


149
191
(A)
0
50
100
150
200
250
355
370
250
300
350
400
450
500
177
191
(B)
0
50
100
150
200
250
369
383
398
250
300
350
400
450
500
191
(C)
0
50
100
150
200
250
369
397
412
250
300
350
400
450
500


205
191
0
50
100
150
200
250
369
411
426
250
300
350
400
450
500

(D)


219
191
0
50
100
150
200
250
369
425
440
250
300
350
400
450
500

(E)

Figure A6. Mass spectra of 17β,21α-hopanes.

A. βα-C_{29} hopane.

B. βα-C_{30} hopane.

C. βα-C_{31} hopane.

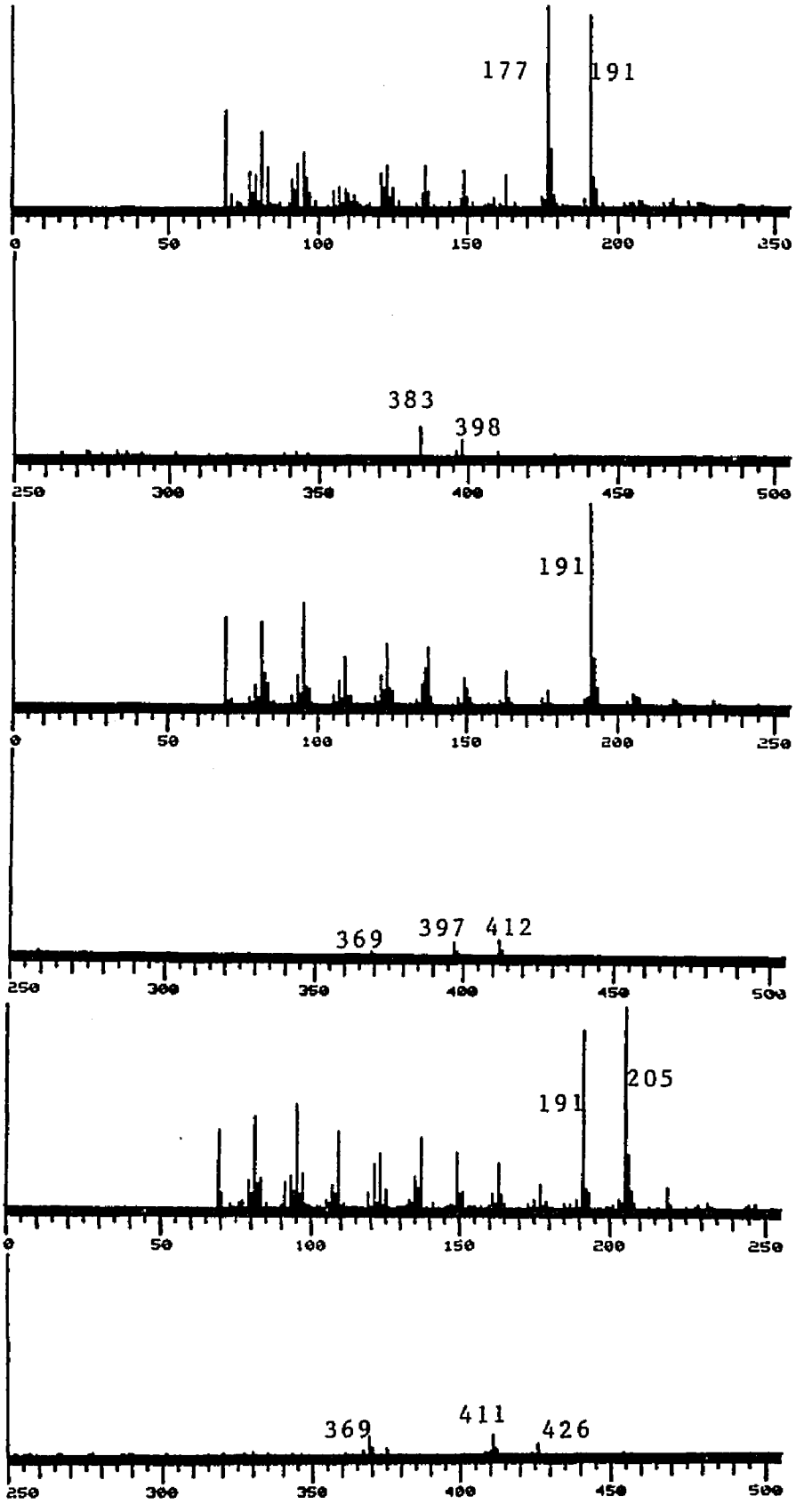

177
191
(A)
0
50
100
150
200
250
383
398
250
300
350
400
450
500
191
(B)
0
50
100
150
200
250
369
397
412
250
300
350
400
450
500
191
205
(C)
0
50
100
150
200
250
369
411
426
250
300
350
400
450
500

Figure A7. Mass spectra of 17α,21β-hopanes.

A. α-C_{27} hopane.

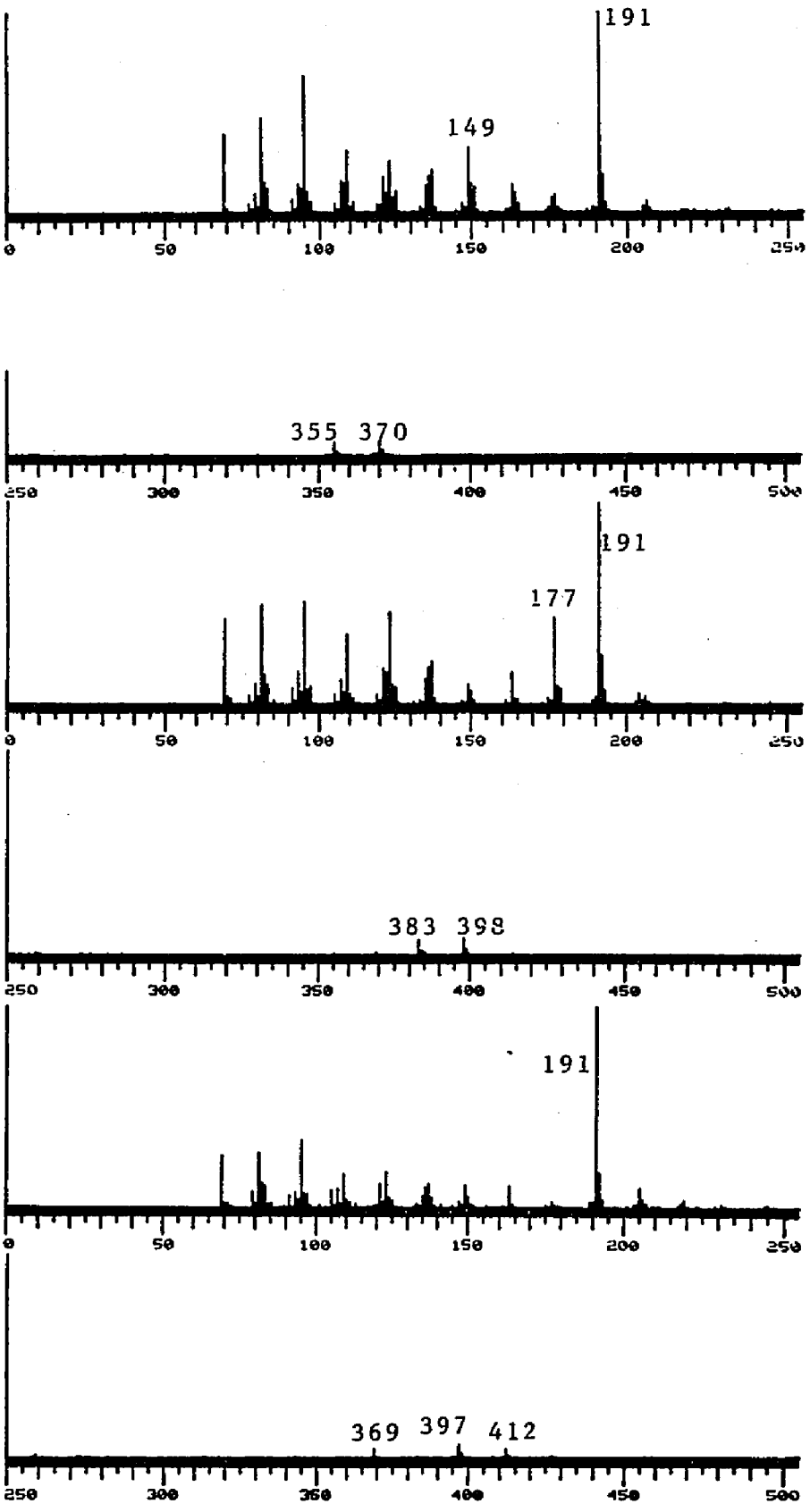
B. αβ-C_{29} hopane.

C. αβ-C_{30} hopane.

D. αβ-C_{31} hopane.

E. αβ-C_{32} hopane.

F. αβ-C_{33} hopane.


191
149
(A)
0
50
100
150
200
250
355 370
250
300
350
400
450
500
191
177
(B)
0
50
100
150
200
250
383 398
250
300
350
400
450
500
191
(C)
0
50
100
150
200
250
369 397 412
250
300
350
400
450
500

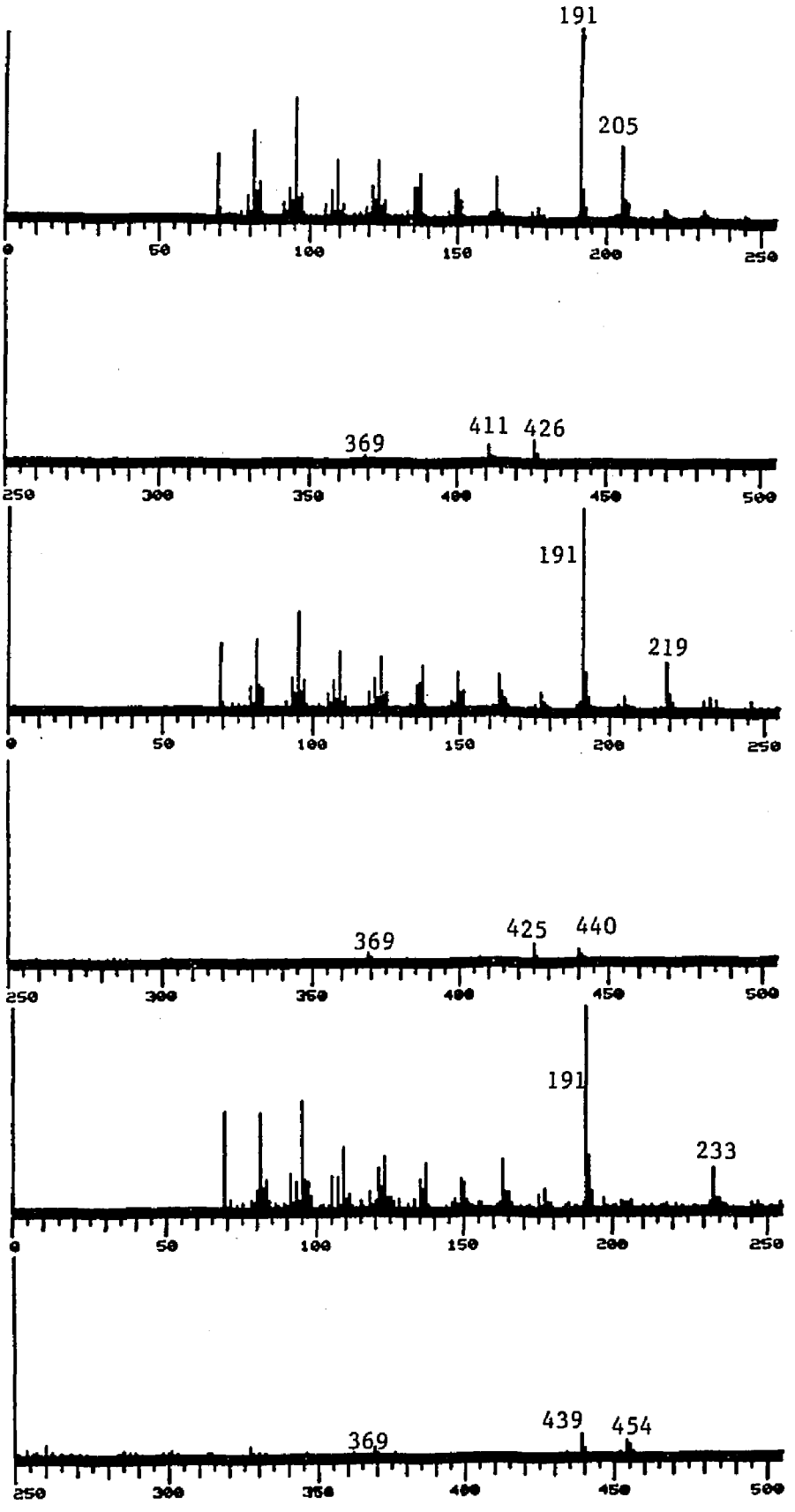

191
205
0
50
100
150
200
250
369
411
426
250
300
350
400
450
500
(D)
191
219
0
50
100
150
200
250
369
425
440
250
300
350
400
450
500
(E)
191
233
0
50
100
150
200
250
369
439
454
250
300
350
400
450
500
(F)

Figure A8. Mass spectra of hopanoid acid methyl esters.

A. $\beta\beta$-C_{30} hopanoid acid.

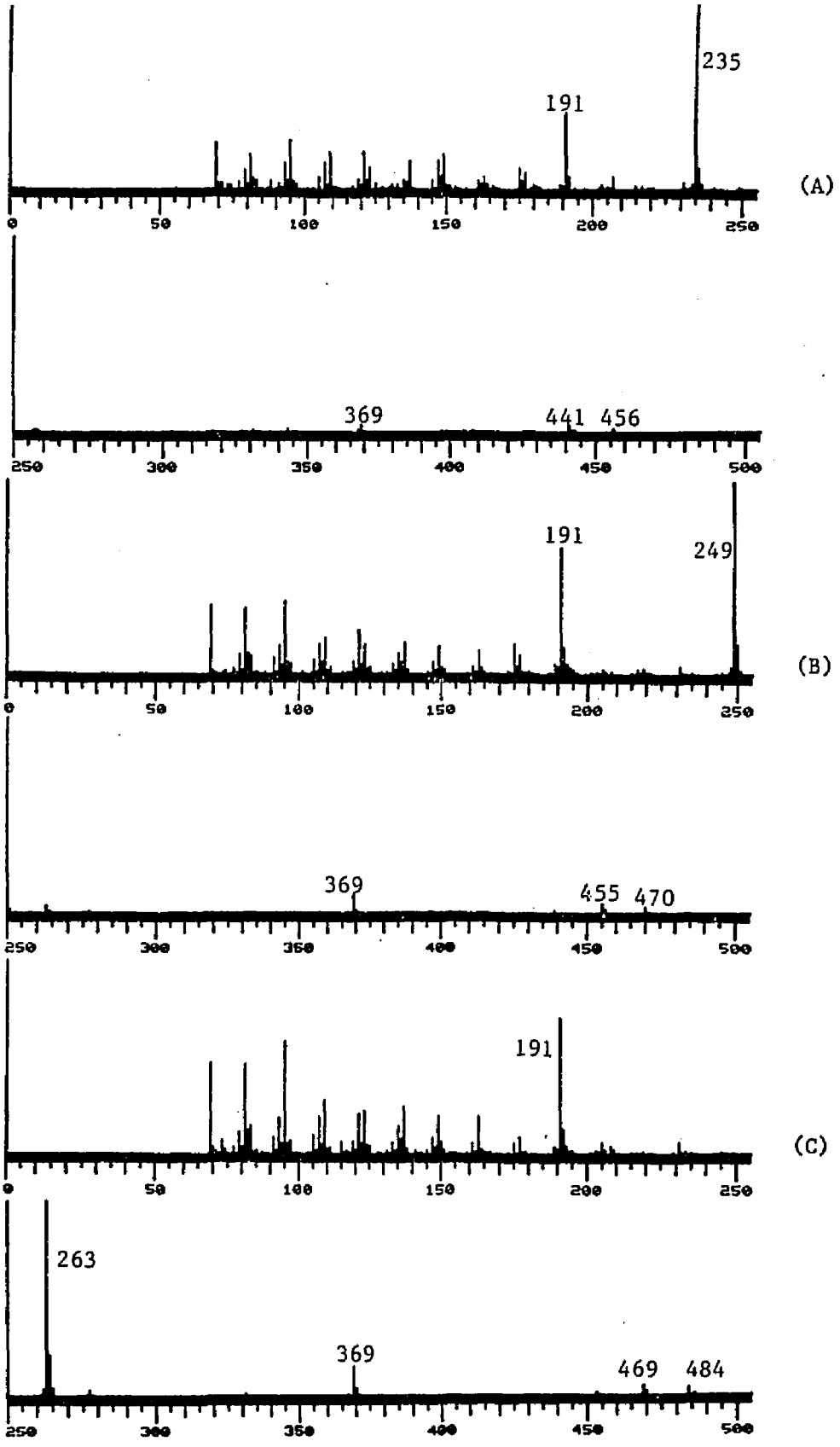
B. $\beta\beta$-C_{31} hopanoid acid.

C. $\beta\beta$-C_{32} hopanoid acid.

D. $\beta\beta$-C_{33} hopanoid acid.

E. $\beta\alpha$-C_{31} hopanoid acid.

F. $\alpha\beta$-C_{32} hopanoid acid.


235
191
(A)
0
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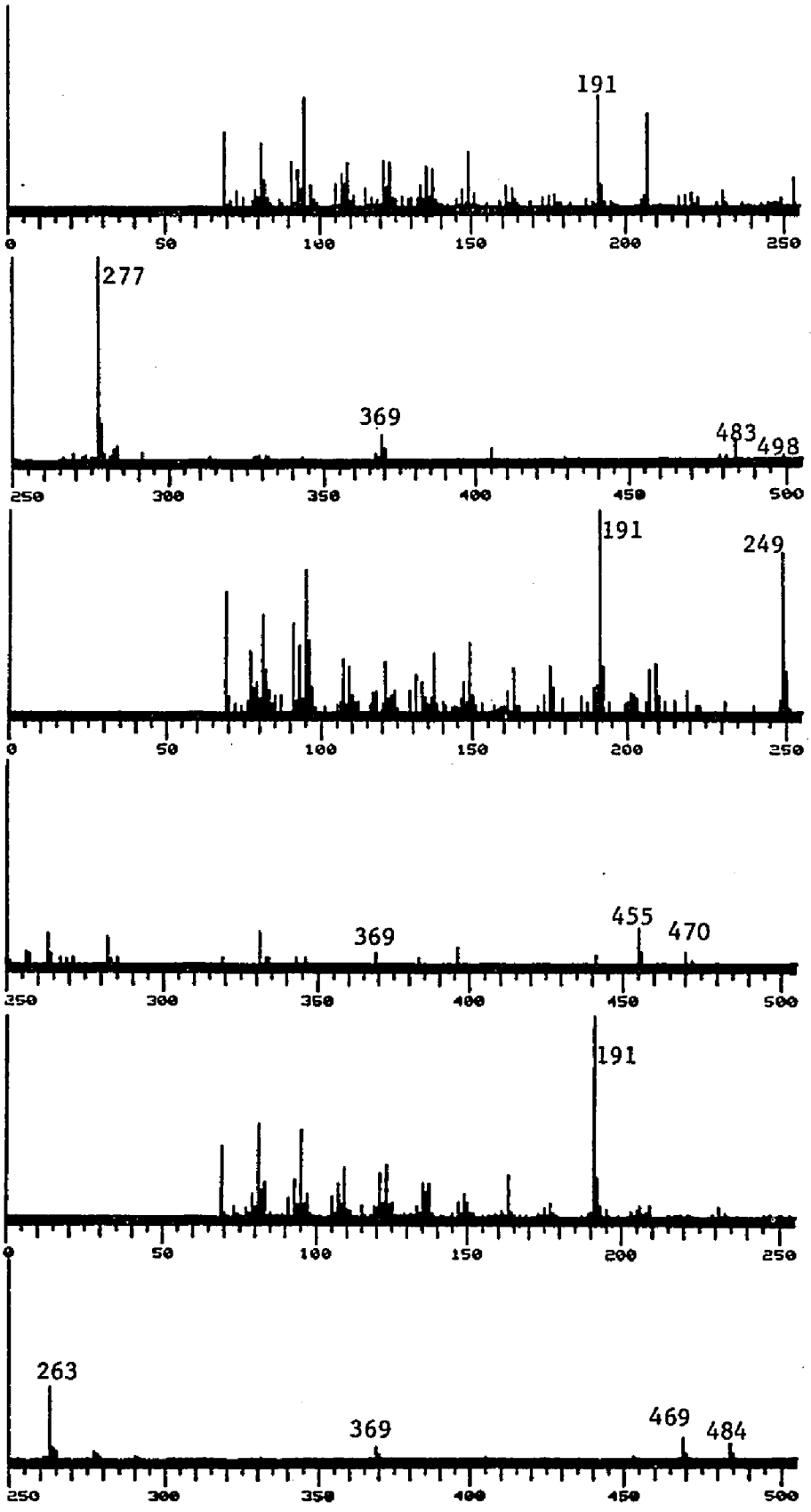
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