Chapter 9
Lipid Components of the Coprolites

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INTRODUCTION

This chapter concerns lipids that were found in several coprolites from the Ciniza Lake Beds. For comparisons, lipids also were extracted from a sample of the enclosing lake shales and from a sample of undescribed cuticle coal of the same approximate age. They were extracted by standard lipid-extraction procedures and separated by thin-layer chromatography into various lipid groups. These fractions were analyzed by gas chromatography, and, when feasible, the individual lipid structures were verified by GC-mass spectrometry.

Although during the process of digestion and/or fossilization, many changes may have occurred in the quantity and nature of the lipid components, it is suggested that the lipids generally reflect the type of material ingested by animals living in and adjacent to Lake Ciniza and hence, to a certain extent, reflect the plant and animal life of the immediate area of the lake. Also they give us some indication of the nature of the lipids that existed during Late Triassic time.

PREVIOUS INVESTIGATION

During the past several years, interest in the nature of organic substances found in rock formations and fossils has increased. Some of the reasons that investigation of organic substances in rock formations have been made include a desire to explain the origin of oil (Hills and others 1970), to provide evidence that life existed at specific geological time periods (Schopf 1970, Spoczynska 1971), and/or to compare the chemical compounds of fossil plants and animals with present-day organisms (Hoering 1967, Schopf 1970, Wyckoff 1972). Breger (1963) summarized the literature on organic substances in rocks in his book on organic geochemistry. The literature relating to evolutionary events of organic substances in nonvascular plants has been summarized by Schopf (1970).

Organic Substances in Rocks

Most of the analyses of organic substances in rocks have been very general in nature, although specific compounds such as carbohydrates, amino acids, and lipids have been reported (Breger 1963). More than 100 specimens have been analyzed for organic material. These analyses represent diverse samples from all over the world collected from very different environments. The types of organic substances detected will be discussed briefly, and only the lipid compounds will be discussed in detail. The types of lipid compounds detected in rock formations include straight-chain hydrocarbons, aromatic hydrocarbons, porphyrins, and fatty acids (Breger 1963, Bergmann 1963).

Abelson (1967) reported that the most recent sediments contain almost no unsaturated fatty acids, and about 0.1 percent saturated fatty acids, predominately palmitic acid (C_{16}). The fatty acids in living blue-green algae communities were high in oleic acid ($C_{18:1}$). Apparently unsaturated fatty acids disappear during the sedimentation process. In examining the older sediments, Abelson (1967) found that there was a relative increase in fatty acids in ancient sediments and in crude oil. Normally the fatty acids in living organisms have a predominance of even-numbered carbon chains (Abelson 1967). In the Green River shale of Eocene age, C_{14}, C_{16}, and C_{18} saturated fatty acids were the most common (Abelson and Parker 1962). In the more ancient sediments Cooper and Bray (1963) suggested that the odd-chain fatty acids occurred by decarboxylation of the even-numbered fatty acids followed by oxidation of the intermediate alkyl radical to yield the odd-numbered fatty acids. Another possible explanation is that the even-numbered fatty acids were utilized as a food source by microorganisms, leaving the odd-chain fatty acids to accumulate. Another possible source of odd-chain fatty acids is from partially degraded kerogen (Abelson 1967).

Kerogen is a complex, insoluble, organic substance that accounts for about 95 percent of the world's organic matter in rock formations. Abelson (1967) predicted that kerogen was the most probable source of hydrocarbons in petroleum. Some of the pigments, such as porphyrengrins, which are found in rot extracts are probably related to chlorophyll or to hemoglobin (Blumer and Rudrum 1970). During the pressures that develop in rock formation, these compounds are probably converted to kerogen.

Waxes from numerous soils and peat mosses contain a variety of even- and odd-numbered hydrocarbons (Breger 1963, Abelson 1967). Although relatively small amounts of hydrocarbon are present in rock formations, some of the hydrocarbons could have come from kerogen (Abelson 1967). In crude oils the ratio of odd- to even-chain length in hydrocarbons is about equal (Breger 1963). In contrast, alkanes (hydrocarbons) in living organisms have a predominance of odd-chained alkanes (Weete and others 1970). It has been proposed that the heat process of rock formation produces a random distribution of even and odd hydrocarbons (Abelson 1967). In the older rocks, which have experienced slightly elevated temperatures, the even- and odd-numbered hydrocarbons are present in about equal amounts (Hoering 1967). Normal alkanes of high molecular weight have been detected in shales and are considered to be of biological origin (Degens 1965). Since microorganisms normally do not metabolize isoprenoids, the carotenoids represent a potential residual type of hydrocarbon in rock formations (Calvin 1969). Isoprenoid hydrocarbons such as phytol and porphyrins have been detected in shales (Calvin 1969). Compounds like sterols or those resembling sterols and carotenoids are often found in coals, water sediments, and soils (Degens 1965). Some of these compounds resemble the typical cholesterol or phytosterol found in animal and plant tissues, respectively.

Biological Compounds in Fossils

Schopf (1970) reviewed the literature on the fossils of Precambrian microorganisms and showed that our knowledge of them is very limited. Chemical fossils, such as proteins, unsaturated fatty acids, and many normal alkanes with odd-numbered carbons, have been detected in Tertiary sediments (Vibal and Roshoff 1971, Uspenski 1969, Hoering 1967). They are less frequent in the Paleozoic deposits and may be completely absent from sediments of greater geological age (Albrecht and Ourisson 1971). One of the problems in analyzing the ancient geological strata is their low organic content (Degens 1965; Swain and Rogers 1966; Swain and others 1967a, 1967b; Leo and Barghoorn 1970).

Leo and Barghoorn (1970) detected phenolic aldehydes in fossil wood. The cell wall residue of fossil wood from Senftenberg, Germany, was analyzed by Morey and Morey (1971). Fossil cell wall fragments from a 7000-year-old Black Sea sediment were analyzed by Degens and others (1970).

Galactose and glucose were the predominant sugars in some Paleozoic plant fossils analyzed by Swain and others (1967, 1976). In the mid-Devonian Onondaga beds of Pennsylvania the average carbohydrate content (mainly mannose, glucose, and xylose) of the limestones is about 50 parts per million (Swain and Rogers 1966). The lowest content of carbohydrates was found in the Jurassic reef limestones (Palacas and others 1960, Swain and others 1967). Swain and others (1967, 1970) and Rogers (1965) concluded that no relationship existed between the carbohydrate content and the geological age of the fossil specimens.

The types of organic compounds found in fossil plants has been discussed by Knoche and others (1968).

The D and L forms of amino acids in fossil shells were determined by Hare and Abelson (1967). Jope (1969) separated the proteins of fossil shells by gel electrophoresis and determined their amino acid composition. The protein and amino acids in fossil shells have been determined by several investigators (Akiyama and Wyckoff 1970, Akiyama and others 1971, Matter and others 1969, Gregoire and Vossfouc 1970, Bricteux and others 1968, and Doberenz and others 1969).

Lipids such as fatty acids have been detected in fossil bones (Everts and others 1968) and fossil teeth (Das and Harris 1970). Douglas and Powell (1969) developed a method for separating fatty acids of fossil lipids by thin-layer chromatography methods. Blumer and Rudrum (1970) detected fossil porphyrins with a molecular weight of about 1100. A fossil polyethylene was found by Heidberg and Krejcigr (1969).

The type of lipids obtained from fossils of certain age indicate that lipids were synthesized in the geologic past by pathways present in biological systems today (Van Hoeven and others 1969, Abelson 1967). This would suggest that at least 2 m.y. ago life had already evolved to a very complex state and basic biochemical pathways were already well selected.

Organic Substances in Coprolites

The composition of a number of coprolites has been analyzed (Hantzschel and others 1968) for carbonites, calcium phosphate, iron, magnesium, oxides, silica, clays, and general organic matter.

Scott (1977) analyzed coprolites from the Carboniferous of Britain and concluded that they contained plant materials. They contained lycopod megaspore fragments, microspores, and indeterminate plant debris. While he did not know which Palaeozoic animal produced the coprolites, he concluded that plant materials in the coprolites were direct evidence that animals were using plants of the Carboniferous period for their food. Other workers have reported the presence of plant material in coprolites. Harris (1957) reported the presence of Caytonia pollen and leaf cuticle of both *Sagenopteris* and *Gingko* in small coprolites from the Jurassic of Yorkshire. Bryant and Williams-Dean (1975) described subfossil human coprolites that contained plant material and pollen.

Since coprolites represent a type of concentrated form of fossil organic material (0.9 to 7.2%) (Hantzschel and others 1968), it is of interest to determine if the chemical components are similar to present-day plants and animals. Edwards (1973) analyzed four coprolites from the Oligocene deposits of the White River Group of Nebraska and Wyoming and compared them with two specimens of Recent feces (bison and margay cat). He used X-ray fluorescence to analyze the specimens and concluded that there were many similarities. In spite of the fact that his animals were zoo dwellers and not wild, Edwards (1973b) concluded that coprolites from carnivores were dominantly fossil bone fragments as indicated by X-ray fluorescence analyses.

The instruments available for lipid separation and characterization make lipid components a feasible group of organic compounds to analyze. In a recent literature review on coprolites by Hantzschel and others (1968), the authors reported no extensive lipid analysis of coprolites. The only report was in 1933 when Fikentscher (1933) extracted coprolites with ether and reported the presence of porphyrin on the basis of spectroanalysis. The logic in analyzing the coprolites was that the organic material is a concentration of the plant and animal material eaten by the organisms in their time period and would be reflective of the plant and animal life of that geological period. It is recognized, of course, that, during the process of digestion and fossilization, many changes could occur in the quantity and nature of the lipid components.

MATERIALS ANALYZED

Sample Number	Description	Amount Analyzed (grams)
W1-J	Small coprolites	21.0
W3-K	Small coprolites	13.6
W4-L	Small coprolites	30.2
W7-M	Gray shale in which coprolites were imbedded	116.5
W8-N	A large coprolite	51.8
W9-O	Small coprolite taken from the surface of the lake bed	12.6
W10-T	Specimen of cuticle coal from Petrified Forest National Park, Arizona (USGS fossil plant locality 10090)	200.0

EXTRACTION PROCEDURE

Although the coprolites were separate specimens, they were similar in general appearance. The plant fossils from the cuticle coal were similar to the plant fossils found in the lake beds (Ash 1973). Each coprolite, the brown shale, and the cuticle coal were powdered separately between aluminum foil with metal blocks. Care was taken to not use plastic bags because of the possibility that some plasticizers (phthalates)

would appear as contaminants. After the samples were powdered, they were ground separately to a fine powder in an acid-washed mortar and pestle. The powder from each sample was weighed, and then lipid materials were extracted as described below using spectrograde solvents in order to avoid contamination.

The powdered samples were extracted with 50 ml of heptane with the exception of samples W7-M, W8-N and W10-T. Because of the large size of these samples, 150 ml of heptane were utilized. After one hour at 50°C the heptane extract was separated from the fossilized powder, and 50 ml of benzene and chloroform (3:1 v/v) were added to the sample powder residues. In the case of samples W7-M, W8-N and W10-T, 150 ml of the benzene and chloroform (3:1 v/v) solvent were used. After an hour at 50°C, the benzene and chloroform solvent was separated from the coprolite residue. Finally 50 ml of methanol (150 ml of methanol in the cases of W7-M, W8-N and W10-T) were added to the residue and incubated for one more hour at 50°C. All the extracts from the coprolites were combined, and the mixture of extracts was taken to dryness at 50°C under a stream of purified nitrogen. The extract was then redissolved in heptane, benzene, and chloroform (1:1:1 v/v) and the concentrated extract applied to a thin-layer silica gel G plate. The samples on the thin-layer plates were separated in a solvent system of benzene:chloroform(9:1). After separation, the thin-layer silica gel G plates were placed in an iodine chamber to detect the lipid bands. After the bands were marked (fig. 1), the iodine was evaporated from the TLC plates in a hood. Identical bands were scraped from several silica gel plates and combined in order to obtain a sufficient quantity of sample for analysis. An example of the type of lipid separation that was obtained with the silica gel G plates (thin-layer chromatography) is shown in figure 1. The lipids were extracted from the silica gel G powder as follows: the sample from band 1 silica powder was eluted with heptane followed by water-saturated ether. Bands 2 and 3 were eluted separately with chloroform and water-saturated ether. Band 4 was eluted from the silica powder with methanol and water-saturated ether.

The concentrated extracts from the silica gel powders from the individual bands were coded by adding the number of the band onto the specimen number. For example, W1-J-1 is the first band from the W1-J specimen, and W1-J-3 is the third band from this specimen. The fractions from bands 1 and 2 of each specimen were injected directly into the Packard Gas Chromatography (Model 7300) and separated into compounds using a SE-30 packed column (either 2.1 mm ID × 2 m stainless steel or 2 mm ID × 2 m glass column). The temperature program was from 50° to 300°C at 2° or 4°C per minute. The nitrogen flow rate was 35 ml/min.

In the case of bands 3 and 4, the fraction for each of the samples was methylated by using boron trifluoride and methanol (Morrison and Smith 1964). After gas chromatographic analysis of the fractions, the samples were characterized using a Varian MAT 111 gas chromatograph-mass spectrometer. A 2 mm ID × 2 m SE-30 glass column was used and programmed from 50° to 300°C at 2° or 4°C per minute. The helium flow rate was 15ml/min. The scan conditions of the mass spectrometer was 100M/sec., ion source, 200°C, and 80 ev ionization energy.

The unknown spectra were compared with known mass spectra. In addition the NIH mass spectro computer analysis system was utilized. The major fragments and intensities of the individual mass spectra were submitted to a computer in Bethesda, Maryland, by means of teletype via the telephone. The computer scanned approximately 15,000 compounds and reported the best fit of the unknown data with spectra of the compounds in the computer library.

RESULTS

The results from the thin-layer chromatography separation as indicated by the iodine vapors suggested that lipid components were present in all the samples analyzed (fig. 1). Band 1 was normally the darkest, followed in intensity by band 4. The dried extracts of the total lipid samples contained a considerable amount of yellow crystalline material, and in other cases white powdery material was observed in the bottom of the vials.

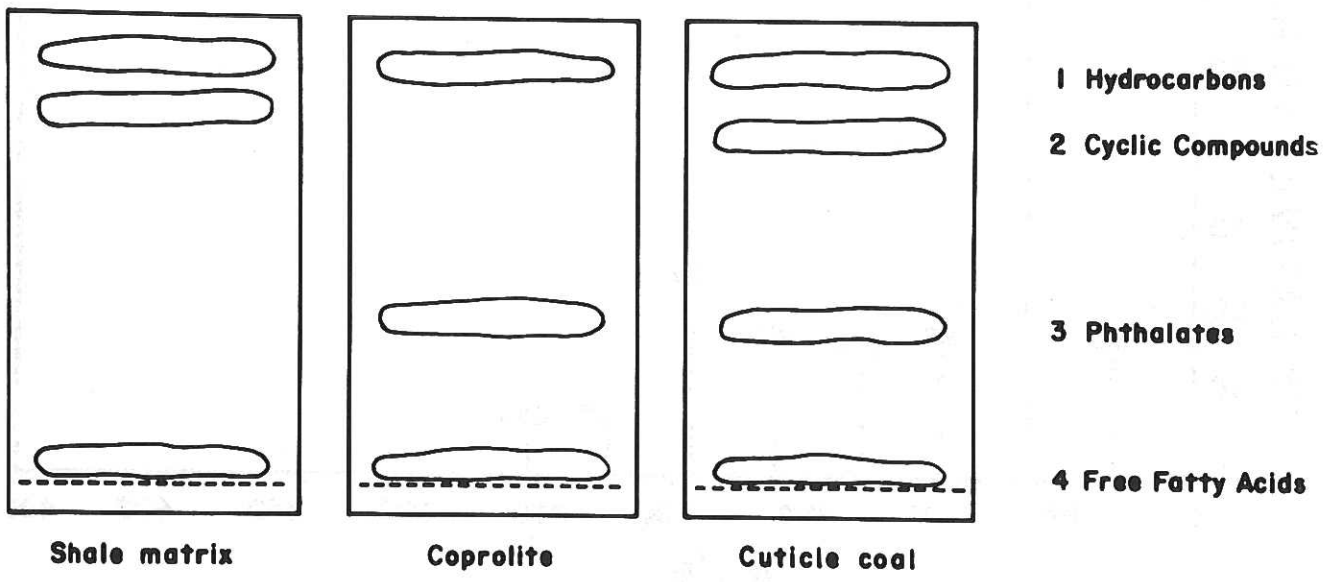


FIGURE 1.—Diagrams of thin-layer silica gel G plates of the lipid extracts of coprolites, cuticle coal, and shale. Solvent system was benzene and chloroform (9:1).

Sample W8-N-1 contained a large number of the yellow crystals. While all the fractions contained crystals, the coprolite extracts contained a higher concentration of them. Later analysis of this yellow crystalline material by direct probe mass spectrometry indicated that it was sulfur (S_8). Mass spectra of S_8 are shown in figure 2. While it is possible that the sulfur was bound with an organic compound, the fragmentation pattern of the spectra obtained indicated that sulfur was the predominant substance present.

Analysis of Band 1

Hydrocarbon compounds were detected in band 1 after TLC of the lipid extracts from the coprolites, the shale matrix, and the cuticle coal. The gas chromatographic pattern obtained with the band 1 fraction of the lipid extract of the shale matrix (W7-M-1) is shown in figure 3. The GC-mass spectra analysis of this fraction (W7-M-1) is summarized in table 1. The numbers on the peaks in the gas chromatogram (fig. 3) correlate with the peak numbers in table 1. A branched-chain hydrocarbon series and a straight-chain hydrocarbon series were detected in the shale (figure 4 and table 1). The mass spectra for peak 33 is characteristic of terpanes. The relative percent of straight-chain alkanes is higher than the branched-chain alkanes (table 1). The distribution of even and odd-chain alkanes was nearly equal in the shale (W7-M-1).

The band 1 extract of the coprolites (W1-J, W3-K, W4-L, W7-N, and W9-O) was analyzed by GC-mass spectrometry. The concentration of the individual compounds varied in the different coprolite samples, but the mass spectra of the compound from individual GC peaks were the same. The amount of lipid in the band 1 from the coprolites was low as compared to the shale. This is probably a reflection of the smaller sample size of the coprolites. The mass spectra of straight-chain hydrocarbons are characterized by having a very weak parent ion. Still, the hydrocarbons have a characteristic small-ion fragmentation pattern. The retention time of the hydrocarbons on the gas chromatograph correlates with the numbers of carbon atoms in the chain. In those cases where the parent ion was weak, by comparing the gas chromatogram of known hydrocarbons and the lower mass fragmentation pattern, it was possible to estimate the chain length of the hydrocarbons present in the coprolite extracts. The compounds detected in the coprolite extract are listed in table 2 for samples W4-L-1 and W9-0-1. A straight-chain alkane series from C_{20} to C_{29} is present (table 2). There is no predominance of odd-chain compounds over even-chain compounds. The pattern is not, however, a simple gaussian distribution as illustrated by the large peak (rel. %) for C_{22}. Peak 6 (table 2) was very large, and the mass fragmentation pattern was characterized by multiples of 32 mass units. The lack of small mass fragments characteristic of short-chain car-

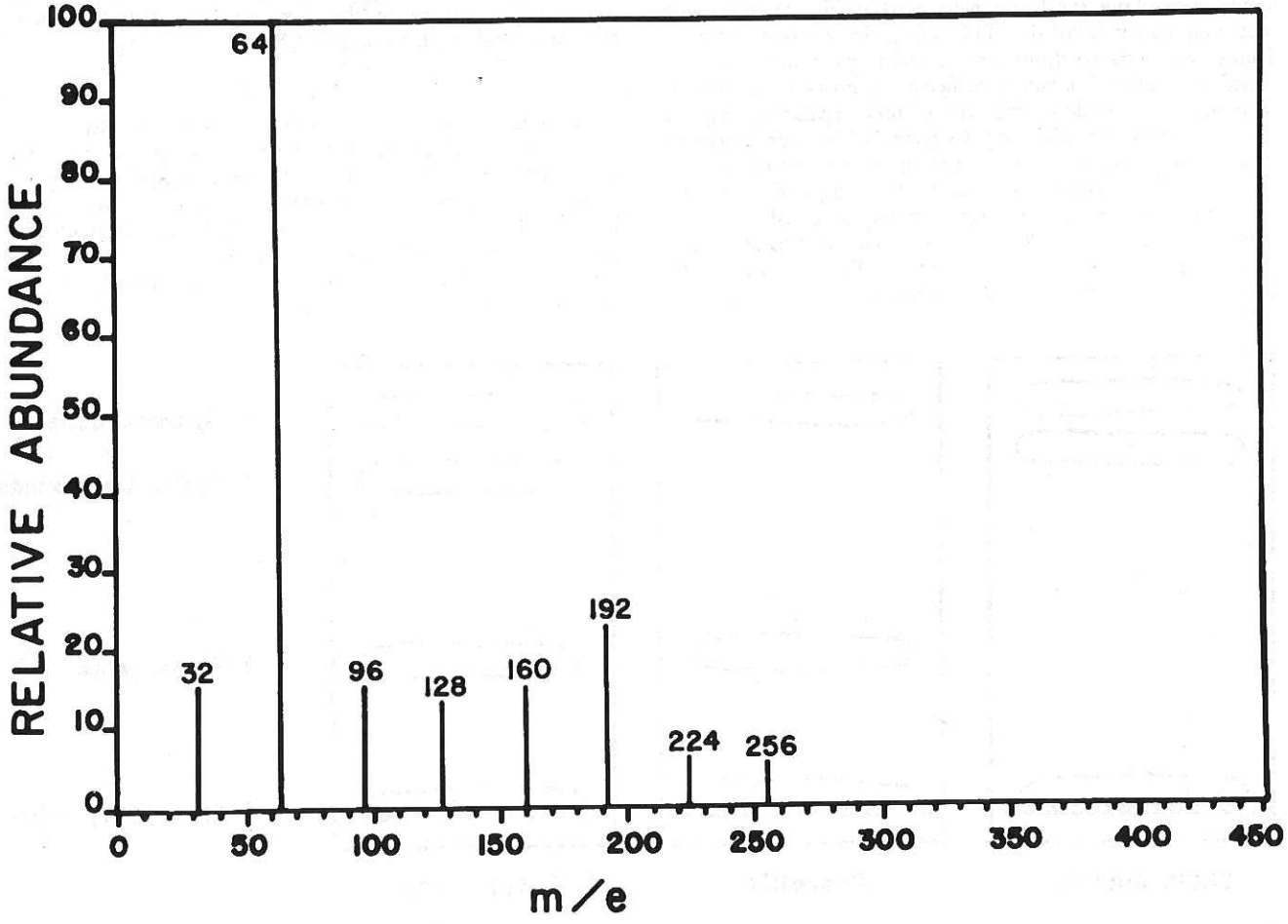


FIGURE 2.—Mass spectra of sulfur (S_8) and yellow crystalline material from lipid extract of coprolites.

bon compounds and the limited number of combination of 32 mass compounds resulted in the conclusion that the compound was a sulfur polymer (S_8). Comparison of the unknown spectra with the known spectra of sulfur (S_8) verified the identification (fig. 2). The compound could be connected to some small carbon compound, but there was an absence of small carbon mass fragments.

The compounds present in band 1 of the lipid extract from the cuticle coal are shown in table 3. The alkane series was from C_{16} to C_{29}, with the pattern being similar to band 1 of the coprolite extract. The sulfur peak was also the predominant peak, being 41.6 percent of the fraction.

Analysis of bands 2 and 3

In present-day plant lipid extracts, the bands 2 and 3 normally contain aromatic compounds, cyclic compounds, and natural esters of fatty acids. The shale band 2, as shown in table 4, contained several branched-chain hydrocarbons from C_{13} to about C_{17}, a number of higher-chain-length hydrocarbons, terpanes, and some heterocyclic compounds. The dominant peaks were the branched-chain compounds and cyclic compounds. Some sulfur was detected but at a low level (2 percent). Band 2 was not present in detectable amounts in the lipid extract of the coprolites. A small amount of material was detected in the lipid extract of the plant tissue matrix (table 5). The compounds in the plant tissue matrix extract in band 2 were difficult to identify but appeared to be phenolic compounds and some phthalates (fig. 5). The hydrocarbon lower mass fragmentation pattern was apparent in several spectra.

Table 6 lists the compounds detected in the band 3 from lipid extracts from coprolites (fig. 1). A series of phthalates

TABLE 1

COMPOUNDS PRESENT IN BAND 1 OF LIPID EXTRACT OF SHALE MATRIX (W7-M-1) ANALYZED BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Peak	Compound	Rel.%	Identification Method	Major Ions (Intensity)
1	$C_{12:0}$ Hyd	0.3	GC-MS	43(100), 57(84), 71(76), 85(33), 99(10), 113(11), 127(7), 141(6), 170*(10)
2	$C_{13:0}$ Hyd 4,8-Dimethylundecane	4.1	MS	57(100), 85(29), 99(12), 113(71), 127(4), 141(4), 155(3), 169(3), 184*(1)
3	$C_{13:0}$ Hyd	7.0	GC-MS	43(91), 57(100), 71(57), 85(29), 99(22), 113(41), 127(5), 141(3), 155(2), 134*(4)
4	$C_{14:0}$ Hyd Branched 3-Methyltridecane	8.4	MS	57(100), 99(10), 113(14), 127(6), 141(3), 155(4), 183(3), 169(8), 198*(2)
5	$C_{14:0}$ Hyd	9.2	GC-MS	43(83), 57(100), 71(60), 85(37), 99(8), 113(5), 127(3), 141(3), 155(2), 169(1), 198*(2)
6	$C_{15:0}$ Hyd Branched 3,8 Dimethylpentodecane	4.8	MS	57(100), 99(16), 113(15), 127(6), 141(8), 155(3), 169(2), 183(5), 197(3), 212*(2)
7	$C_{15:0}$ Hyd	6.4	MS	57(100), 85(40), 99(13), 113(7), 127(7), 141(4), 155(4), 169(2), 183(11), 212*(3)
8	C_{16} Branched 4 methyl pentadecane	1.6	GC-MS	Hyd Pattern
9	$C_{16:0}$ Hyd	3.7	GC-MS	57(100), 71(61), 85(31), 99(11), 113(7), 127(6), 141(5), 155(3), 169(4), 183(3), 197(3), 226*(3)
10	C_{18} Branched 3,5,7,8 tetramethyl tetradecane	2.2	GC-MS	57(100), 71(58), 85(34), 99(23), 113(18), 127(10), 141(6), 155(3), 169(8), 183(6), 254(1)
11	$C_{17:0}$ Hyd	8.9	GC-MS	43(70), 57(100), 71(90), 85(31), 99(12), 141(4), 155(4), 169(3), 183(6), 197(2), 211(1)
12	$C_{17:1}$ H_{34} alkene C_{17} Branched	1.5	GC-MS	Hyd Pattern
13	$C_{18:0}$ Hyd alkane	5.7	GC-MS	57(100), 85(45), 99(14), 113(11), 127(6), 141(6), 155(4), 169(2), 183(2), 197(3), 211(31), 225(1), 254*(3)
14	C_{19} Branched	2.1	GC	
15	$C_{19:0}$ Hyd	3.9	GC-MS	57(100), 85(37), 99(2), 113(12), 127(7), 141(6), 155(4), 169(4), 183(3), 197(3), 211(2), 225(2), 268*(2)
16	C_{20} Branched	0.2	GC	
17	$C_{20:0}$ Hyd	3.5	GC-MS	57(100), 85(41), 99(16), 113(9), 141(5), 155(4), 169(2), 183(3), 197(2), 211(2), 225(1), 239(2), 253(2), 282*(2)
18	C_{21} Branched	0.7	GC	
19	$C_{21:0}$ Hyd	3.2	GC-MS	57(100), 71(64), 85(40), 99(17), 113(13), 127(7), 141(5), 155(4), 169(3), 183(3), 197(3), 211(3), 225(2), 239(2), 296*(2)
20	C_{22} Branched	0.9	GC	
21	$C_{22:0}$ Hyd	3.1	GC-MS	Hyd Pattern
22	C_{23} Branched	1.0	GC	
23	$C_{23:0}$ Hyd	3.3	GC-MS	Hyd Pattern
24	C_{24} Branched		GC	
25	$C_{24:0}$ Hyd	2.2	GC-MS	Hyd Pattern
26	C_{25} Branched	0.3	GC	
27	$C_{25:0}$ Hyd	2.0	GC-MS	Hyd Pattern
28	C_{26} Branched	0.5	GC	
29	$C_{26:0}$ Hyd	1.4	GC-MS	Hyd Pattern
30	$C_{27:0}$ Hyd		GC	
31	Terpane	0.9	GC	
32	Terpane	0.9	GC	
33	Terpane	2.0	MS	43(95), 57(100), 71(99), 85(63), 95(48), 109(44), 121(29), 137(25), 163(17), 169(13), 172(26), 191(47)
34	Terpane	0.8	GC	
35	Terpane	0.1	GC	
36	Terpane	0.06	GC	

*Molecular ion (parent ion)

were detected beginning with dimethyl phthalate and continuing to dioctyl phthalate. One or two oxygen-containing compounds were also present. Since dioctyl phthalate is a common contaminant from diffusion oil, it was a major concern to determine whether the dioctyl phthalates were from the sample (coprolites) or from the diffusion pump. Since the samples, shale, cuticle coal, and coprolites were all analyzed under similar conditions and on the same machine, one would expect phthalates to be present in all samples. Also one would expect a predominant dioctyl phthalate as the contaminant. Since dioctyl phthalate gives a strong 149 and 167 mass fragment, it can easily be detected in other spectra and the background spectra. The indications were that the phthalates came from the coprolite sample.

The band 3 from the cuticle coal also had phthalates present in the fraction (table 7). The phthalates were the predominant compounds present. A methyl ester of a fatty acid, C_{18}, was detected and is probably a natural ester although the fraction was artificially methylated.

In present-day plant lipid extracts, the band 4 normally contains free fatty acids. The band 4 is near the origin of the thin-layer plates (fig. 1) and was present in the two matrixes and the coprolites. The major fatty acids were C_{18} (4 percent), C_{17} (2.6 percent), C_{16} (3.7 percent), C_{15} (0.8 percent), and C_{14} (1.6 percent) (table 8). It was of interest to note that while the even- and odd-chain fatty acids were present, the even-chain length fatty acids were predominant (64 percent of the fatty acids in the band 4 fraction were even-chained). Some phthalate compounds and some hydrocarbon lower mass fragment patterns were detected.

Analysis of band 4

The band 4 of the lipid extract from the cuticle coal (W10-T-4) contained a dominant series of long-chain fatty

TABLE 2
COMPOUNDS PRESENT IN BAND 1 OF LIPID EXTRACT OF COPROLITES (SAMPLES W4-L-1 AND W9-0-1) ANALYZED BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Peak	Compound	Rel.%	Identification Method	Major Ions (Intensity)
1	Sulfur S_8	6.9	MS	64(100), 96(15), 128(13), 160(14), 192(23), 224(5), 256*(4)
2	Dimethyl phthalate	2.8	GC-MS	194(12), 51(53), 77(66), 94(32), 135(30), 163(100), 207(16)
3	Hydrocarbon type	2.8	MS	Hyd Pattern
4	Unknown	2.1		
5	S_8	1.4	MS	64(100), 96(12), 128(16), 160(14), 192(19), 224(7), 256*(16)
6	Sulfur S_8	52.7	GC-MS	64(100), 96(12), 128(16), 160(14), 192(19), 224(7), 256*(16)
7	$C_{20:0}$ alkane	1.3	GC	
8		1.9		
9	$C_{21:0}$ alkane	1.6	GC	
10	C_{22} alkane	3.8	GC	
11	$C_{23:0}$ alkane	2.6	GC	
12	$C_{24:0}$ alkane	2.1	GC	
13	$C_{25:0}$ alkane	5.5	GC	
14	$C_{26:0}$ alkane	3.0	GC	
15	$C_{27:0}$ alkane	4.1	GC	
16	$C_{28:0}$ alkane	3.0	GC	
17	$C_{29:0}$ alkane	2.3	GC	

*Molecular ion (Parent ion)

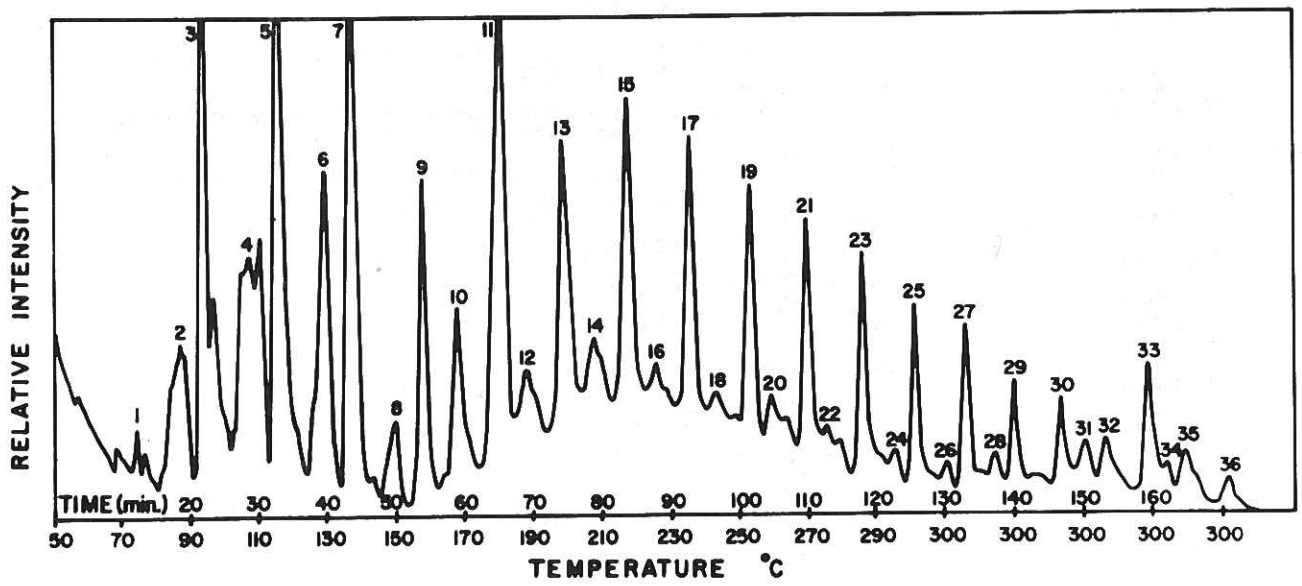


FIGURE 3.—Gas chromatographic pattern (ion monitor detector) of band 1 obtained from TLC of a lipid extract from the Ciniza Lake Beds.

acids as shown in table 9 (fig. 6). The fatty acids ranged from C_{13} to C_{34}. In addition, several were branched.

DISCUSSION

One of the goals of the investigation was to determine whether the lipid components in the coprolites could be determined. It was speculated that the lipid analysis might give some indication of the type of lipid components that existed in that time period. Results indicate that the coprolites contain lipid components of a biological nature. Identification of individual lipid compounds was limited by the size of the individual coprolites which placed a restriction on the quantity of lipid that could be obtained for analysis. Results indicate that lipid compounds in the different coprolites were very similar in types of compounds but varied in the amounts present.

The results of this study suggest that, if a number of coprolites from Lake Ciniza were combined, the results would probably be quite similar to those obtained for an individual coprolite.

The shale in which the coprolites were imbedded contained compounds that differ from the coprolites in the case of the first three TLC bands, bands 1, 2 and 3, analyzed. These results suggest that the coprolites contain unique compounds and are not merely a reflection of the shale in which they were imbedded. At the same time, the shale contained fatty acids very similar to the coprolite fatty acids. This is difficult to explain, but one of the following may have occurred: endogenous organisms (such as bacteria) may be present in the shale, or the shale may contain decayed biological material, or the shale may contain a high amount of powdered coprolite material from which some of the lipid material has been leached out but with a residue of fatty acids remaining. Other shale from the lake deposit has been analyzed, and the lipid content was very low.

The fatty acids series in the cuticle coal suggests that the fatty acids at the time of fossilization were of higher chain lengths than are commonly found in plants and animals today. Some yeasts (Welch and Burlingame 1973) have been reported to have long-chain fatty acids up to C_{34}. It also suggests that in the process of passing through the intestine of the animal, either the plant or the animal tissues lost some of the higher chain fatty acids, or at least the concentration was decreased as reflected by a lower chain length in the fatty acids in the coprolites.

Results indicate the following: (1) the shale in which the fossils were present normally contained little organic material although it was a large amount for shale (see chap. 2). Four different shale analyses were made. Three of them gave indication of very low levels of organic material. The fourth was richer in organic material, and the results indicate the presence of a hydrocarbon mixture as well as lipid components such as fatty acids. The coprolites were similar to

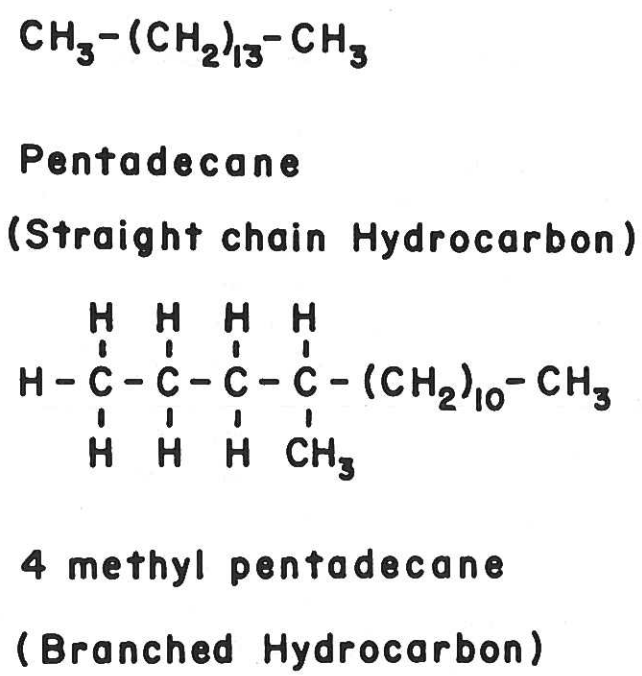


FIGURE 4.–Straight-chained and branched-chained hydrocarbons.

TABLE 3
COMPOUNDS PRESENT IN BAND 1 IN THE LIPID EXTRACT OF THE CUTICLE COAL MATRIX, ANALYZED BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Peak	Compound	Rel.%	Identification Method	Major Ions (Intensity)
1	Sulfur S_8	2.7	GC-MS	64(100), 96(15), 128(19), 160(16), 192(28), 224(5), 256(6)
2	C_{16} hydrocarbon alk.	1.5	GC-MS	Hyd Pattern
3	Hydrocarbon type	2.7	MS	Hyd Pattern
4	Pyridine or quinoline	5.7	MS	57(35), 83(30), 128(18), 150(24), 152(26), 178(100)
5	Hydrocarbon C_{17}	4.5	GC-MS	43(100), 55(85), 69(54), 95(52), 131(35), 137(36), 204(43)
6	C_{18} alkane	17.6	GC-MS	43(76), 57(100), 71(71), 85(34), 95(28), 111(17), 123(15), 127(20), 166(11), 192(12)
7	Sulfur S_8	41.6	GC-MS	64(100), 96(15), 128(14), 160(7), 192(10), 224(4), 256(7)
8	C_{20} hyd	2.4	GC	
9	C_{21} hyd	1.7	GC	
10	C_{22} hyd	2.8	GC	
11	C_{23} hyd	2.6	GC	
12	C_{24} hyd	2.6	GC	
13	C_{25} hyd	3.5	GC	
14	C_{26} hyd	1.8	GC	
15	C_{27} hyd	2.4	GC	
16	C_{28} hyd	2.7	GC	
17	C_{29} hyd	1.1	GC	

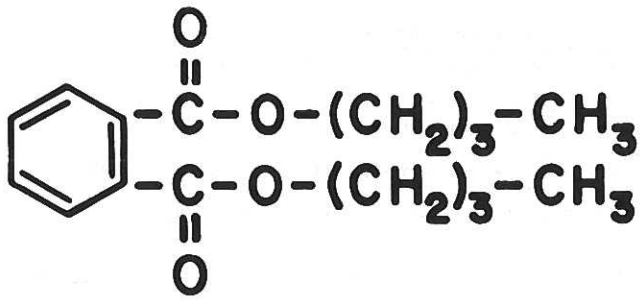
each other, suggesting that from a lipid view, the types of plants or animals eaten were similar in lipid composition, or, at least, the lipid composition was similar after digestion had occurred in the animal. In terms of materials detected, the hydrocarbons present appeared to fall within a general range similar to many of the waxes present in modern-day plants, although the length of the carbon chain in the fossilized material is generally smaller in molecular weight than the present-day hydrocarbons. (2) Many unknown lipid components were found. It was difficult to identify them because of the lack of a strong parent ion and comparable known standards. The third fraction containing the free fatty acids indicates that the fatty acids were similar to those found in lipids from plants and animals (Kolattukudy 1970) today with even-chain fatty acids being predominant (C_{16} and C_{18}). The lipid analyses tend to support the concept that the cuticle coal was plant material. Further detailed analysis of the individual components may give indications about types of metabolic pathways that functioned in early plants or types of compounds not utilized by the digestive systems of the animals eating the plants or animals. A major limitation should be recognized: The coprolites represent the remains of the digested food of the animal. Whether lipid analysis can be useful for characterization of individual coprolites was not tested by this investigation. The basic concept is that coprolites do represent a type of concentrated food material, and

TABLE 4
COMPOUNDS PRESENT IN BAND 2 FROM LIPID EXTRACT OF THE SHALE MATRIX (W7-M-2) ANALYZED BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Peak	Compound	Rel.%	Identification Method	Major Ions (Intensity)
1	Alkane, branched hydrocarbon	0.4	GC-MS	44(100), 57(99), 71(71), 85(12), 99(19), 113(18), 155(14)
2	$C_{13:0}$ hyd 4,8 dimethyl lyndecane	1.3	GC-MS	57(100), 85(40), 99(10), 113(5), 127(4), 141(5), 155(1), 184(3)
3	C_{14} branched alkane hydrocarbon	0.2	GC-MS	43(60), 57(100), 71(58), 85(31), 99(10), 113(9), 127(7), 183(4)
4	$C_{14:0}$ hyd branched 3 methyl tridecane	6.9	GC-MS	57(100), 85(37), 113(6), 127(3), 141(5), 155(4), 169(7), 198*(2)
5	$C_{14:0}$ hyd nor-tetradecane	8.4	GC-MS	43(100), 57(97), 71(48), 85(31), 99(7), 113(4), 127(2), 141(2), 155(1), 169(1), 198*(1)
6	–		–	
7	$C_{15:0}$ hyd branched at 3 position	5.8	GC-MS	57(100), 85(41), 99(13), 113(10), 127(7), 141(6), 155(4), 169(4), 183(4), 197(2), 212*(1)
8	$C_{15:0}$ hyd pentadecane	6.9	GC-MS	57(100), 99(10), 113(6), 127(4), 141(3), 155(4), 169(2), 183(2), 212*(2)
9	Alkane	2.7	MS	57(100), 97(9), 111(3), 123(9), 133(2), 155(2), 169(2), 207(2), 222(1)
10	Alkane branched C_{16}	0.6	GC	
11	Alkane branched C_{16} cyclo hexyl ether	1.3	GC-MS	57(100), 71(61), 85(33), 97(20), 109(12), 111(12), 113(10), 149(9), 182(9)
12	$C_{16:0}$ hyd	4.3	GC-MS	57(100), 113(5), 127(4), 141(3), 155(3), 169(3), 183(2), 226*(2)
13	Alkane $C_{17:0}$ branched	0.3	GC-MS	43(100), 69(73), 83(43), 97(37), 119(22), 165(19), 183(30), 198(13)
14	$C_{17:0}$ branched at 7 carbon	3.7	GC-MS	57(100), 85(24), 99(13), 113(6), 127(4), 141(4), 155(5), 169(3), 183(3), 197(2), 240*(1)
15	Cyclic hydrocarbon	4.1	MS	57(100), 85(26), 97(14), 109(8), 155(4), 169(4), 178(9)
16	Cyclic hydrocarbon	4.1	MS	57(100), 85(25), 99(10), 113(10), 127(6), 141(4), 155(4), 183(6), 210(2)
17	Oxygen heterocyclics	0.9		Hyd pattern
18	Oxygen heterocyclics	10.1	MS	57(100), 85(33), 97(20), 134(6), 141(6), 150(5), 155(5), 183(4), 192(5)
19	Aromatic nitrogen or terpene	4.5	MS	55(100), 71(49), 83(42), 96(42), 109(28), 119(19), 123(17), 133(13), 192(28)
20	S_8 sulfur	2.9	MS	
21	S_8 sulfur	0.9	MS	64(100), 96(13), 128(15), 160(17), 192(11), 224(7), 256(11)
22	S_8 sulfur	2.5	MS	
23	2, 6-di-T-Butyl-4-methyl phenol (Ionol)	1.6	MS	57(100), 71(51), 85(37), 97(23), 123(12), 165(7), 220(9)
24	Hydrocarbon alkane	1.3	MS	Hyd pattern
25	2, 6-di-T-Butyl-4 ethyl phenol	2.6	MS	57(100), 71(57), 85(34), 95(25), 109(16), 119(13), 155(6), 203(6), 219(7), 234(10), 246(8)
26	di-isobutyl fummarate dibutyl malate	0.6	MS	57(100), 69(81), 81(63), 95(48), 109(46), 125(24), 228(56)
27	Hydrocarbon	1.5	MS	Hyd Pattern
28	Hydrocarbon	0.1	MS	57(100), 71(84), 85(39), 95(32), 123(13), 149(13), 169(9), 242(9)
29	Hydrocarbon	0.4	MS	Hyd Pattern
30	Hydrocarbon	0.4	MS	57(100), 71(52), 81(48), 95(45), 109(29), 123(21), 256(18)
31	Hydrocarbon	0.9	MS	Hyd Pattern
32	Terpane	2.8	MS	55(100), 69(63), 81(73), 95(83), 109(39), 123(43), 149(29), 191(42), 357(5), 372(7)
33	C_{27} H_{48} Terpane	1.8		
34	C_{29} H_{50} Terpane	4.5	MS	81(100), 95(89), 109(71), 123(58), 137(29), 149(20), 163(21), 177(34), 191(56), 383(8), 398(9)
35	Unknown	2.0		
36	Unknown	2.4		
37	Unknown	2.0		
38	Unknown	1.4		

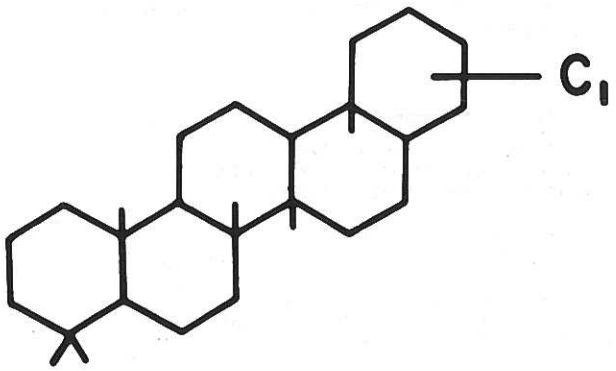
*Molecular Ion (Parent Ion)

(Phenolic type)



Dibutyl Phthalate

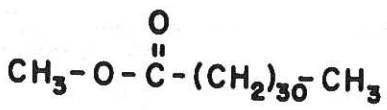
(Phthalate type)



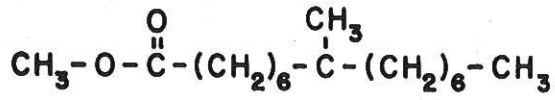
Terpane

FIGURE 5.–Aromatic compounds found in lipid extracts.

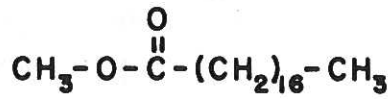
therefore the possibility of an analytical analysis is somewhat increased. To obtain sufficient plant cuticle on an individual basis is a tedious and difficult task, whereas a coprolite properly analyzed might also reflect the types of plant tissue present. The cuticle coal is similar to plants indigenous to this lake area and is probably similar to plants eaten by the animals around the lake. Similarity between analyses of cuticle coal and coprolites would imply that the lipid components were not changed by the digestive process, except for the long-chain fatty acids. In terms of taxonomic value in distinguishing one coprolite from another or in establishing that they are truly coprolites, a much greater number and variety of coprolite samples would have to be analyzed to determine whether lipid components from coprolites from a certain area are characteristic. Logically, one would anticipate that plants ingested by an animal would be reflected in the coprolite, and the lipid components might be a general biological marker of the whole area. Present-day taxonomic analysis of lipid components suggests that in warmer environments plants have longer-chain hydrocarbons in their wax cuticle in order to survive the higher temperatures (Herbin and Robins 1969, Kolattukudy 1970). If this suggestion is true, hydrocarbons of the particular plants in the coprolites analyzed in this investigation suggest that the environment was warm but not extremely hot. The prediction of the environment temperature on the basis of hydrocarbon chain length is only a speculative concept but could be useful if a more complete analysis of various time periods were made. The even-chain fatty acid pattern was higher than the odd-chain fatty acid pattern, but the ratio was closer to types of fatty acids found in older sediments (Abelson and Parker 1962). The ratio of even- and odd-chain hydrocarbons in the shale matrix also indicates an older sediment (Abelson 1967).



Long chain fatty acid methyl ester



Branched chain fatty acid methyl ester



Methyl Sterate

(Methyl ester of a fatty acid)

FIGURE 6.–Branched- and straight-chain methyl esters of fatty acids.

ACKNOWLEDGMENTS

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TABLE 5

COMPOUNDS PRESENT IN BAND 2 FROM LIPID EXTRACT OF THE CUTICLE COAL MATRIX (W10-T-2) ANALYZED BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Peak	Compound	Rel.%	Identification Method	Major Ions (Intensity)
1	Unknown	2.5	MS	44(41), 75(35), 123(20), 125(23), 153(86), 181(100), 209(9), 224(10)
2	Unknown	7.5		
3	Unknown	2.7	MS	43(100), 57(61), 69(65), 71(55), 78(48), 166(82), 167(79), 195(48), 196(43)
4	Di-isobutyl phthalate	2.7	MS	41(69), 57(49), 149(100), 167(13), 205(11), 223(9)
5	Hydrocarbon	8.4		Hyd Pattern
6	Phenolic compound	6.3	MS	41(100), 55(73), 69(54), 81(67), 95(56), 139(89), 151(53), 168(76), 179(43), 196(59)
7	Dibutyl phthalate	15.6	MS	41(40), 57(37), 77(16), 93(15), 104(11), 121(9), 149(100), 169(8)
8	Unknown	4.4		
9	Unknown	3.5		
10	Phenolic compound	1.7	MS	41(100), 57(98), 69(42), 71(65), 83(59), 87(55), 95(64), 119(49), 192(48), 220(55)
11		3.6		
12	Alkyl benzene phthalate	2.8	MS	65(45), 71(41), 91(100), 104(32), 105(24), 109(23), 123(26), 206(18), 207(20)
13	Hydrocarbon	6.2	MS	57(38), 65(79), 77(100), 95(45), 170(36), 215(39), 233(28), 326(39)
14	Nitrogen compound	3.0	MS	44(100), 55(91), 71(39), 83(43), 112(27), 129(81), 147(19)
15	Hydrocarbon	6.5	MS	Hyd Pattern
16	Dioctyl phthalate	11.0	MS	57(68), 71(39), 83(28), 97(25), 109(12), 149(100), 167(34), 279(11)
17	Hydrocarbon	6.7	MS	Hyd pattern
18	Hydrocarbon	3.0	MS	Hyd pattern
19	Hydrocarbon	2.3	MS	Hyd pattern

*Molecular ion (Parent ion)

TABLE 6

COMPOUNDS PRESENT IN BAND 3 IN LIPID EXTRACT FROM COPROLITES (W1-J-3, W3-K-3, W4-L-3, W7-N-3, W9-0-3) ANALYZED BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Peak	Compound	Rel.%	Identification Method	Major Ions (Intensity)
1	Dimethyl phthalate	1.6	MS	77(51), 105(11), 133(18), 135(14), 163(100), 194(15)
2	A dialkyl phthalate	2.7	MS	65(41), 75(22), 93(13), 105(13), 121(11), 149(100), 177(24)
3		1.8	MS	79(57), 115(50), 128(57), 143(83), 171(100), 184(70)
4		1.4		
5	Br_2 compound	0.4	MS	41(100), 44(59), 71(45), 253(54), 332(29)
6	Di-isobutyl phthalate	4.0	MS	41(40), 57(37), 77(16), 78(17), 93(15), 104(11), 121(9), 149(100), 167(18)
7		6.4		
8	Di-isobutyl phthalate	9.9	MS	65(6), 77(11), 93(6), 121(5), 149(100), 205(4), 223(3)
9	Phthalate unknown ester	1.1	MS	41(100), 63(65), 65(65), 69(27), 149(25), 179(46), 223(26)
10	Aromatic thiazole	4.3	MS	43(78), 57(40), 71(45), 73(79), 83(46), 135(100), 197(40), 207(40)
11		2.8		
12		2.9		
13	2,5 diten,amg.quinore	1.8	MS	43(100), 57(66), 71(42), 83(40), 123(30), 144(60), 217(45), 218(44), 219(38), 247(42)
14		3.3	MS	65(45), 69(21), 71(41), 91(100), 104(32), 109(23), 123(26), 206(18), 207(20)
15	Silicone	3.9	MS	57(100), 71(67), 85(56), 99(17), 113(13), 127(11), 141(11), 169(14), 326(16)
16		6.1	MS	55(100), 57(68), 65(70), 77(53), 95(47), 169(33), 324(44), 325(51)
17	Hydrocarbon	5.6		
18	Dioctyl phthalate	9.8	MS	57(68), 71(39), 83(28), 97(25), 149(100), 167(34)
19	Hydrocarbon	5.3		
20		4.1		
21	Hydrocarbon	5.4		
22		9.6	MS	55(100), 69(94), 81(55), 95(61), 109(37), 135(28), 368(17), 370(18)
23		2.1		
24		1.6		
25	Mixture	1.9	MS	55(100), 69(46), 79(33), 81(61), 93(34), 95(34), 159(36), 174(40), 187(40)

*Molecular ion (Parent ion)

TABLE 7

COMPOUNDS PRESENT IN BAND 3 IN LIPID EXTRACT OF CUTICLE COAL EXTRACT (W10-T-3) ANALYZED BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Peak	Compound	Rel.%	Identification Method	Major Ions (Intensity)
1	Phthylic acid Anhydride	3.2	MS	44(17), 50(52), 148(12), 76(85), 104(100)
2	Alkyl benzene	5.2	MS	44(57), 65(43), 91(100), 120(12), 137(7), 155(22), 184(15), 198(14)
3	Alkyl benzene	4.1	MS	44(31), 65(37), 91(100), 104(12), 155(53), 184(38), 198(13)
4	Dibutyl phthalate	7.3	MS	76(17), 19(9), 104(18), 121(6), 149(100), 205(5), 223(4)
5	Phthalate	1.3	MS	41(98), 43(83), 55(58), 57(60), 69(33), 71(29), 85(100), 95(24), 97(29), 115(21), 149(34)
6	Hydrocarbon	1.4	MS	Hyd Pattern
7	Phthalate	0.9	MS	41(96), 43(65), 55(80), 57(84), 73(100), 104(47), 155(51), 147(80)
8	Phthalate	2.1	MS	41(86), 46(71), 55(62), 52(51), 69(46), 85(100), 47(36), 115(28), 149(28)
9	$C_{18}O$ FAME	40	GC-MS	94(100), 87(77), 143(11), 199(6), 255(8), 267(5), 269(5), 298*(4)
10	Phthalate	1.2	MS	41(100), 43(98), 55(81), 69(48), 85(81), 97(37), 149(80), 207(20)
11	Phthalate	65.6	MS	41(31), 57(27), 76(24), 77(26), 104(25), 133(13), 149(100), 207(9), 263(14)
12	Hydrocarbon	1.2	MS	41(100), 45(90), 55(67), 57(86), 71(62), 85(56), 95(28), 97(25), 105(36), 149(49)
13	A dioctyl phthalate	2.0	MS	43(100), 57(77), 71(47), 81(28), 113(14), 149(87), 167(27)
14	Phthalate	0.7	MS	41(95), 43(69), 55(78), 57(100), 69(36), 71(39), 85(30), 99(21), 109(21), 117(26), 149(71)

*Molecular ion (Parent ion)

TABLE 8

COMPOUNDS PRESENT IN BAND 4 IN LIPID EXTRACT OF COPROLITES (W1-J-7, W3-K-4, W4-L-4, W7-N-4, W9-0-4) AND SHALE MATRIX (W10-M-4) ANALYZED BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Peak	Compound	Rel.%	Identification Method	Major Ions (Intensity)
J,0-I			MS	73(100), 191(18), 207(50), 267(26), 281(24), 355(22)
J-II			MS	76(100), 105(91), 191(20), 207(44), 267(15), 281(14), 355(12)
J,0-III			MS	73(100), 147(21), 207(21), 241(47), 429(14)
1		3.4	MS	50(39), 77(87), 92(18), 105(8), 133(18), 135(11), 163(100), 194(13)
2	A Dialkyl phthalate	4.5	MS	65(14), 76(16), 77(13), 93(16), 104(13), 121(13), 149(100), 163(6), 177(21)
3	$C_{14:0}$ FAME	1.6	GC-MS	74(100), 87(57), 143(16), 199(9), 211(6), 213(4), 242*(5)
4		0.4	MS	43(88), 55(39), 74(81), 105(100), 121(48), 135(48), 149(40)
5	$C_{15:0}$ FAME	0.8	GC-MS	74(100), 87(55), 143(15), 213(18), 221(16), 223(6), 256*(6)
6	Disobutyl phthalate	0.8	MS	57(51), 65(11), 93(8), 121(8), 149(100), 205(5), 223(11)
7		0.8		
8	$C_{16:0}$ FAME	3.7	GC-MS	74(100), 87(49), 143(12), 199(5), 241(3), 270(3)
9	Dibutyl phthalate	3.7	MS	65(10), 76(12), 93(8), 104(14), 121(6), 123(4), 149(100), 205(6), 278(1)
10		0.9	MS	41(100), 51(94), 71(34), 79(42), 87(32), 107(33), 115(36), 134(53), 143(34), 149(27), 167(29), 199(21), 227(27), 234(26)
11	$C_{17:0}$ FAME	2.6	GC-MS	74(100), 87(63), 143(20), 199(18), 241(13), 284(13)
12		1.2	MS	57(44), 91(26), 117(100), 129(19), 133(21), 149(21), 163(19), 215(15), 232(26), 233(28), 238(16)
13		4.1	MS	55(100), 57(32), 69(53), 74(39), 83(42), 96(30), 111(18), 123(18), 207(8), 213(9), 247(10), 264(9)
14	$C_{18:0}$ FAME	4.1	GC-MS	74(100), 87(81), 143(18), 199(5), 255(5), 267(4), 269(2), 298(5)
15		11.4	MS	41(100), 71(47), 85(32), 97(35), 109(25), 125(23), 149(27), 154(26), 240(30)
16		9.6	MS	57(100), 71(57), 85(46), 99(18), 113(10), 127(11), 141(17), 197(16), 211(71), 233(4), 325(7), 326(5), 327(5)
17L-3				
M-3		4.2		
N-3				
O-3				
K-3			MS	57(100), 71(26), 86(37), 101(21), 125(32), 153(13), 155(10), 199(12), 221(5), 225(6), 227(6)
J-3	Butyl phthalyl		MS	57(17), 77(23), 104(14), 121(5), 133(15), 149(100), 207(11), 263(25)
18		4.2	MS	57(100), 71(42), 85(43), 99(19), 113(10), 127(23), 143(9), 153(14), 155(12), 199(9), 225(6), 227(9), 256(6), 290(7), 300(6)
19		0.8	MS	57(100), 69(62), 71(46), 81(62), 91(35), 95(39), 129(31), 141(29), 197(52), 239(73), 243(28), 285(64), 300(19)
21J-3			MS	57(100), 71(58), 85(31), 104(23), 117(14), 149(45), 207(12), 239(7), 263(14), 341(10), 355(6)
K,L,M,N,O-3		4.1	MS	57(100), 71(56), 85(51), 97(24), 111(21), 113(18), 127(12), 155(12), 169(9), 183(13), 243(9), 261(9), 281(8), 368(13)
22		4.1	MS	44(67), 57(100), 71(71), 83(49), 97(43), 125(33), 207(31)
23		6.7	MS	57(100), 71(84), 85(37), 97(20), 99(21), 113(12), 127(10), 141(11), 169(5), 183(10), 197(6), 211(4), 225(4), 239(5), 253(4), 281(4), 295(4)
24		5.0		
25		4.2		
26		3.0		
27		2.0		
28		0.9		
29		0.7		

*Molecular Ion (Parent ion)

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TABLE 9

COMPOUNDS PRESENT IN BAND 4 FROM LIPID EXTRACT FROM CUTICLE COAL MATRIX (W10-T-4) AS ANALYZED BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Peak	Compound	Rel.%	Identification Method	Major Ions (Intensity)
1		1.3	MS	
2		1.1	MS	73(100), 79(45), 267(30), 355(14)
3				73(93), 78(69), 79(100), 147(43), 241(39)
4		0.3	MS	44(100), 79(60), 137(16), 153(21), 286(15)
5		0.3	MS	41(59), 43(71), 73(77), 79(100), 149(63)
6		0.2	MS	79(100), 127(56), 155(59), 186(34)
7	$C_{13:0}$ FAME	1.3	GC-MS	74(100), 87(71), 143(8), 197(6), 199(4), 228*(4)
8	C_{14} branched at 2 carbons	0.9	MS	74(100), 87(58), 101(30)
9	$C_{14:0}$ FAME	5.8	GC-MS	74(100), 87(59), 141(21), 199(9), 211(7), 213(3), 242*(4)
10		0.3	MS	43(79), 44(72), 55(58), 67(80), 91(58), 95(38), 109(57), 163(26), 211(26)
11	C_{15} branched Sat. FAME	2.3	MS	41(100), 74(64), 87(65)
12	$C_{15:0}$ FAME	3.1	GC-MS	74(100), 87(58), 143(13), 199(3), 213(4), 225(3), 229(3), 256*(4)
13	Di-isobutyl phthalate	3.1	GC-MS	41(53), 57(48), 104(21), 149(100), 223(12)
14	C_{16} branched at C_8 Sat. FAME	5.0	GC-MS	41(100), 74(86), 87(71), 143(19)
15	$C_{16:0}$ FAME	8.4	GC-MS	74(100), 87(64), 143(15), 199(4), 127(8), 239(4), 241(2), 270*(5)
16	C_{17} branched at C_8	1.9	MS	41(100), 74(68), 87(62), 143(19)
17	$C_{17:0}$ FAME	9.4	GC-MS	74(100), 87(76), 143(15), 199(6), 241(9), 284*(8)
18	C_{18} branched Sat. FAME	2.2	MS	55(100), 74(70), 87(56)
19	$C_{18:0}$ FAME	10.3	GC-MS	74(100), 87(64), 143(16), 199(8), 255(5), 267(4), 269(4), 298*(7)
20	$C_{19:0}$ FAME	10.3	GC-MS	74(100), 87(87), 143(12), 199(5), 281(6), 312*(10)
21	C_{20} branched at 2 carbons	2.0		74(100), 87(80), 101(20)
22	$C_{20:0}$ FAME	10.5	GC-MS	74(100), 87(70), 143(20), 199(4), 297(5), 326*(4)
23	$C_{21:0}$ FAME	3.7	GC-MS	74(100), 87(65), 143(13), 199(7), 240*(6)
24	$C_{22:0}$ FAME	3.0	GC-MS	74(100), 87(93), 143(18), 199(7), 354*(4)
25	$C_{23:0}$ FAME	2.9	GC-MS	74(100), 87(85), 143(21)
26	$C_{24:0}$ FAME	2.3	GC-MS	41(100), 74(93), 87(74), 143(21), 199(8), 239(5), 351(5), 353(5)
27	$C_{25:0}$ FAME	1.5	GC-MS	74(100), 87(47), 143(26)
28	$C_{26:0}$ FAME	1.3	GC-MS	74(100), 87(47), 143(18)
29	$C_{27:0}$ FAME	1.4	GC-MS	74(100), 87(52), 143(16)
30	$C_{27:0}$ FAME	1.0	GC-MS	74(100), 87(90), 143(15)
31	$C_{29:0}$ FAME	0.8	GC-MS	74(100), 87(78), 143(21)
32	$C_{30:0}$ FAME	0.6	GC-MS	41(100), 74(82), 87(68), 143(28)
33	$C_{31:0}$ FAME	0.5	GC-MS	43(100), 74(61), 87(59), 143(24)
34	$C_{32:0}$ FAME	0.4	GC-MS	
35	$C_{33:0}$ FAME		GC	
36	$C_{34:0}$ FAME		GC	

*Molecular Ion (parent ion)

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