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22. BIOLOGICAL MARKER SIGNIFICANCE OF ORGANIC MATTER ORIGIN AND TRANSFORMATION IN SAPROPELS FROM THE PISANO PLATEAU, SITE 964[1]

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ABSTRACT

The organic matter in a series of 39 sapropel samples from Hole 964D drilled during Ocean Drilling Program Leg 160 in the Ionian Basin of the Eastern Mediterranean Sea has been characterized. Organic carbon contents exceed 20% in many sapropels of Pliocene to early Pleistocene age, but are conspicuously lower in those of the late Pleistocene and Holocene. The organic matter is predominantly of marine origin, with varying admixtures of terrigenous organic matter. The most abundant molecular algal markers are long-chain alkenones, alkandiols, and alkanolones (ketools) as well as a significant proportion of sterols. Terrigenous markers include long-chain alkanes, *n*-alcohols, and fatty acids. Although strong alteration of the organic matter by sulfate-reducing bacteria is inferred from the high total sulfur contents of the sapropels, there was little direct molecular evidence of a bacterial biomass contribution. High C_{org}:N ratios, exceeding values of 20, particularly in the most organic-carbon-rich sapropels, have to be interpreted as the result of partial degradation of the sinking organic matter with selective remineralization of nitrogen-bearing compounds.

INTRODUCTION

Organic-carbon-rich, dark-colored strata (sapropels) are common in post-Messinian sediments of the Mediterranean Sea. Bradley (1938) first proposed that periods of stagnation occurred in the Mediterranean during the Quaternary and caused the formation of sediment layers that contain high proportions of organic matter. This hypothesis was supported by data from sediment cores that were recovered by a Swedish deep-sea expedition to the Mediterranean in 1947/48 (Kullenberg, 1952). Their work initiated a multitude of investigations of the Mediterranean seafloor (e.g., Stanley, 1972; Ryan, Hsü, et al., 1973; Hsü, Montadert, et al., 1978; Kastens, Mascle, Auroux, et al., 1987) devoted to determining the occurrence of sapropels, the reasons for their formation, and the origin of the organic matter preserved in them.

Kidd et al. (1978) defined sapropels in the Mediterranean Sea as discrete, more than 1-cm-thick layers in open-marine (pelagic) deposits, with more than 2% organic carbon. A "sapropelic layer," according to the classification of the same authors, in sediments of the same type contains between 0.5% and 2% organic carbon. This strict distinction was not, however, used by many later investigators, and it was not adopted by the Leg 160 Shipboard Scientific Party, partly because it does not take into consideration the occurrence of sapropels altered by secondary processes ("burnt-out" sapropels; see Shipboard Scientific Party, 1996b), and phenomena related to sapropel formation according to a broad spectrum of characteristic element concentrations, but not represented by organic matter enrichment (see Wehausen and Brumsack, Chap. 17, this volume).

For a long time, sapropels were considered a special phenomenon of the Eastern Mediterranean Sea, but recent drilling campaigns have demonstrated that sedimentary layers with high contents of organic matter also occur in the western basins (Cita et al., 1973; Kidd et al., 1978). In many instances, the organic carbon contents were below 2%, however, and so these layers were not considered real sapropels. Ocean Drilling Program (ODP) Leg 107 revealed organic-carbon-rich sapropels also in the western Mediterranean Sea (Kastens, Mascle, Auroux, et al., 1987; Brosse and Herbin, 1990; Emeis et al., 1991).

Organic Geochemical Studies

The type of organic matter in Mediterranean sapropels is still a matter of debate. Some authors favor a predominantly terrigenous (Deroo et al., 1978; Sigl et al., 1978; Hahn-Weinheimer et al., 1978) or marine (e.g., Nesteroff, 1973; Cita and Grignani, 1982) origin of the organic matter. A dominance of amorphous organic matter and high concentrations of C_1 to C_{12} compounds were taken as evidence to support the latter view. Low C:N ratios of late Pleistocene sapropels indicate a marine origin (Calvert, 1979, cited by Thunell et al., 1984), while high C:N ratios in early Pleistocene sapropels are more consistent with a considerable proportion of terrigenous material (Sigl et al., 1978). C:N and $\delta^{13}C$ ratios of organic matter in the youngest sapropel layer (S_1) are in accordance with a marine organic matter origin (Sutherland et al., 1984).

Comprehensive organic geochemical investigations on a molecular level are too scarce so far to resolve the aforementioned discrepancies. According to the results of Comet (1984) on late Pliocene and early Pleistocene sapropels from DSDP holes, the organic matter is of a predominantly marine origin. The youngest sapropel layer (S_1; ≈9–7 ka) contains a mixture of marine and a significant proportion of terrigenous organic matter (Smith, 1984). High concentrations of dinosterol and long-chain alkenones in sapropels S_1 and S_7 were taken as evidence of an intense bloom of coccolithophorides and dinoflagellates at the time of sapropel formation and, thus, to support the high-productivity model for sapropels from the Eastern Mediterranean Sea (Smith et al., 1986; ten Haven, 1986; ten Haven et al., 1986, 1987).

This study provides preliminary information on the origin and preservation of the organic matter in the sapropels from ODP Site 964 on the Pisano Plateau (Fig. 1) by combining shipboard measurements with data on the composition of the extractable organic matter, particularly the polar low-molecular-weight lipid fractions, analyzed by gas chromatography (GC) and combined gas chromatography-

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mass spectrometry (GC-MS) as an initial step for paleoenvironmental assessment.

ANALYTICAL METHODS

We investigated a total of 39 sapropel core samples from Hole 964D (Table 1). After freeze-drying and grinding, the sediments were analyzed for total carbon (TC) and total sulfur (TS) contents by combustion in a LECO CS-444 instrument. Carbonate contents were determined after acidification as carbon dioxide using a UIC-Coulometrics CM 5012 device. Total organic carbon (TOC) content was calculated as the difference between total and inorganic carbon contents (Shipboard Scientific Party, 1996a).

Dry sediment (1 to 3 g) was solvent extracted (dichloromethane/1% methanol (v/v): 1 × 50 mL, 3 × 30 mL) in an ultrasonic bath. The decanted combined extracts were concentrated by rotary evaporation (2 mL) and dried by a stream of nitrogen at mild temperature (30°–35°C). Extract yields were determined gravimetrically.

Separation into fractions of different polarities was performed after addition of internal standards (squalane, anthracene-d_{10}, 5α-androstan-17-one, 5α-androstan-3β-ol and erucic acid [n-$C_{22:1}$ fatty acid]). Prior to column chromatographic separation, the *n*-hexane-insoluble fraction (asphaltenes) was precipitated. The *n*-hexane-soluble portion was separated by medium-pressure liquid chromatography (MPLC; Radke et al., 1980) into fractions of nonaromatic hydrocarbons, aromatic hydrocarbons and heterocompounds (NSO fraction). Elemental sulfur was removed with activated copper filings from the nonaromatic hydrocarbon fraction.

Subsequently, the ketones, esters, and *n*-alcohols ($>C_{20}$) were separated from the NSO-fraction by flash chromatography (Still et al., 1978) with a moderate overpressure of nitrogen. For this purpose, a 10-mm × 200-mm column was filled with 5 g silica gel 60 (40–63 µm, deactivated with 5% by weight of water) and washed with 50 mL dichloromethane. The unpolar fraction of ketones, esters, and *n*-alcohols ($>C_{20}$) was eluted with 60 mL dichloromethane. The other compounds were removed from the column with 100 mL of a mixture of dichloromethane and methanol (10% by volume). The polar portion of the NSO fraction was separated into an acid fraction and a steroid alcohol fraction using a column filled with KOH-impregnated silica gel (McCarthy and Duthie, 1962; modified according to Hinrichs et al., 1995). All fractions were concentrated as described for the whole extracts and weighed. For analysis of the molecular constituents, the heterocompound fractions were trimethylsilylated with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA).

Gas chromatography was performed on a Hewlett-Packard 5890 Series II instrument equipped with a temperature-programmed cold-injection system (Gerstel KAS 3) and a fused-silica capillary column (J&W DB-5, length = 30 m, inner diameter = 0.25 mm, film thickness = 0.25 µm). Helium was used as the carrier gas, and the temperature of the oven was programmed from 60°C (1 min isothermal) to 305°C (50 min isothermal) at a rate of 3°C/min.

GC/MS studies were performed with the same type of gas chromatograph (helium as carrier gas) and a temperature program from 60°C (1 min isothermal) to 300°C (50 min isothermal) at a rate of 3°C/min. The gas chromatograph was coupled to a Finnigan SSQ 710B mass spectrometer operated at 70 eV. Structural assignments of individual compounds are based on comparison of relative gas chromatographic retention times and mass spectra with those reported in the literature. Quantification was performed relative to the amount of internal standards with mass correction for trimethylsilylated compounds.

The determination of Kovats retention indices was carried out using two different gas chromatographic columns. The first column was fitted into a Hewlett Packard 5890 series II gas chromatograph coupled to a Hewlett Packard 5970 series mass selective detector. The sample was transferred on a 12 m (0.2 mm i.d.) fused silica column coated with HP1 by auto-splitless injection. The temperature was programmed from 40° to 300°C at 5°C/min with a hold time of 10 min at the final temperature. The carrier gas was helium. The second analysis was performed on a Finnigan Mat gas chromatograph coupled to a Finnigan Mat mass selective detector. Auto-splitless injection was made on a 30 m (0.25 mm i.d.) fused silica column coated with DB 5. The temperature program was the same as before. The carrier gas was helium at a linear velocity of 40 cm/s. C_{20}, C_{21}, and C_{22} *n*-alkane standards (Aldrich) were co-injected with the sample. In addition, two different isomers of a highly branched isoprenoid alkadiene were used as internal standards. $C_{25:2}$ (I) was from a diatom culture of *Haslea osfrearia,* $C_{25:2}$ (II) was from a Caspian Sea plankton catch. The Kovats retention indices were calculated according to the formula published by Braiswaithe and Smith (1996):

$$RI_C = 100\ Z + 100\ [(\log t'_{RC} - \log t_{RZ})/(\log t'_{R(Z+1)} - \log t'_{RZ})].$$

RESULTS AND DISCUSSION

Elemental Composition of Organic Matter

The results of elemental analysis for total organic carbon (TOC), carbonate, and total sulfur contents of the Hole 964D sapropels are

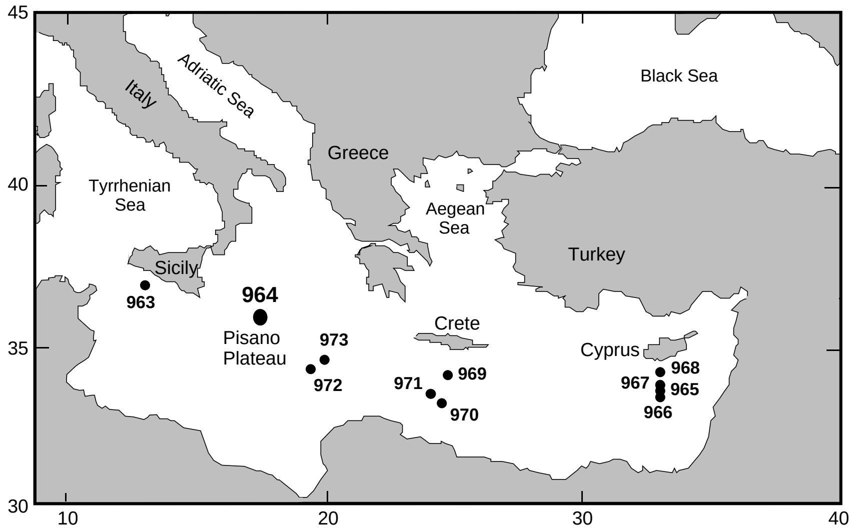

Figure 1. Drilling location of Site 964 in the Ionian Basin (Eastern Mediterranean Sea) and other ODP Leg 160 sites (after Shipboard Scientific Party, 1996a).

Table 1. Elemental data, alkenone ratios, and paleo–sea-surface temperatures for sapropels from Holes 964A (Scientific Shipboard Party, 1996b) and 964D.

Core, section, interval (cm)	Depth (mbsf)	Sapropel number	Organic carbon* (%)	$CaCO_3$** (%)	Sulfur** (%)	$U^{K'}_{37}$ /SST (–/°C)
160-964A-						
1H-1, 70–71	0.70	1	3.14	37.15	1.73	
2H-1, 94–95	7.74	3	5.78	27.41	2.18	
2H-1, 103–104	7.83	3	2.87	29.40	2.15	
2H-3, 122–123	11.02	4	3.50	37.98	2.99	
2H-3, 130–131	11.10	4	2.97	33.57	3.36	
2H-4, 69–70	11.99	5	7.30	12.66	3.93	
2H-4, 73–74	12.03	5	3.57	33.40	4.89	
2H-4, 133–134	12.63	6	3.15	31.49	2.29	
2H-4, 143–144	12.73	6	2.18	37.32	1.98	
3H-2, 108–109	18.88	8	2.43	36.99	2.73	
3H-4, 104–105	21.84	9	3.09	46.56	6.01	
3H-5, 92–93	23.22	10	2.59	48.40	2.36	
3H-6, 113–114	24.93	12	5.56	34.07	1.97	
4H-4, 38–39	30.68	16	4.95	43.98	3.24	
4H-4, 103–104	31.33	17	4.25	56.14	1.66	
4H-5, 56–57	32.36	18	16.38	2.33	3.94	
4H-6, 2–3	33.32	18	4.35	59.81	1.62	
4H-7, 16–17	34.96	19	2.69	43.48	2.29	
5H-1, 114–115	36.44	20	3.96	57.56	1.25	
5H-3, 29–30	38.59	21	13.22	1.67	2.34	
5H-3, 30–31	38.60	21	18.21	1.33	0.94	
5H-3, 31–32	38.61	21	14.22	3.42	4.67	
5H-3, 32–33	38.62	21	12.44	12.66	4.70	
5H-3, 33–34	38.63	21	17.19	1.83	5.80	
5H-3, 34–35	38.64	21	3.55	42.57	3.01	
5H-4, 102–103	40.82	22	13.11	3.83	4.19	
5H-5, 46–47	41.76	23	3.52	34.57	2.57	
5H-5, 100–101	42.30	24	20.10	2.25	8.70	
6H-1, 86–87	45.66	26	7.36	30.74	4.88	
6H-1, 87–88	45.67	26	1.98	58.48	2.54	
6H-3, 41–42	48.21	27	16.26	5.50	7.47	
6H-3, 44–45	48.24	27	2.25	50.90	4.58	
6H-4, 32–33	49.62	30	11.29	4.17	8.89	
6H-4, 38–39	49.68	30	7.11	37.40	8.00	
6H-5, 14–15	50.94	32	24.34	2.33	7.85	
6H-6, 83–84	53.13	35	9.28	39.40	6.10	
6H-7, 18–19	53.98	37	18.69	2.67	9.80	
7H-4, 107–108	59.87	39	8.84	29.65	5.48	
8H-1, 69–70	64.49	42	11.14	26.57	8.69	
8H-6, 22–23	71.52	48	4.79	59.14	4.70	
9H-3, 42–43	76.72	50	7.73	34.07	1.50	
9H-5, 35–36	79.65		13.51	14.49	0.77	
9H-5, 89–90	80.19	54	20.25	1.17	1.14	
9H-6, 14–15	80.94	55	14.42	3.00	18.24	
9H-6, 18–19	80.98	55	8.17	25.16	13.93	
9H-7, 4–5	82.34	57	3.85	12.41	2.76	
9H-CC, 25–27	83.11	58	24.62	1.17	9.66	
160-964D-						
1H-1, 72–74	0.72	1	2.80	39.0	1.75	0.55/16.8
1H-1, 74–76	0.74	1	2.85	38.5	1.79	0.62/18.7
2H-3, 67–69	7.77	2	2.79	40.0	2.20	0.46/14.4
2H-3, 69–71	7.79	3	2.99	37.5	4.68	
2H-3, 81–83	7.91	4	5.22	31.3	2.61	0.48/14.9
2H-3, 83–85	7.93	4	4.44	27.9	2.56	
2H-4, 23–25	8.83	5	5.07	34.4	4.54	
2H-4, 25–27	8.85	5	3.87	34.3	3.16	
2H-4, 84–86	9.44	6	2.66	34.6	2.01	
2H-4, 86–88	9.46	6	3.68	31.8	2.78	
2H-4, 92–94	9.52	6	2.44	35.9	2.37	0.54/16.4
2H-4, 94–96	9.54	6	2.97	35.1	2.50	
3H-2, 83–85	15.93	8	2.35	28.0	3.14	0.62/18.6
3H-4, 47–49	18.57	9	2.90	47.0	1.14	0.73/21.6
3H-5, 29–31	19.89	10	2.40	45.5	1.45	
3H-5, 31–33	19.91	10	2.40	47.0	2.62	
4H-5, 66–68	29.76	15	4.99	33.4	2.20	0.66/19.8
4H-5, 141–143	30.51	16	5.33	49.2	2.45	
4H-6, 92–94	31.52	17	17.5	2.10	4.01	
4H-7, 35–37	32.45	18	5.04	58.0	2.00	0.74/21.8
5H-4, 54–56	37.64	23	4.33	17.4	3.03	
5H-4, 56–58	37.66	23	4.17	28.0	3.44	
5H-4, 58–60	37.68	23	3.09	36.4	2.92	
5H-4, 60–62	37.70	23	2.93	37.8	2.71	
5H-4, 137–139	38.47	24	20.1	2.08	4.31	0.71/21.1
5H-4, 139–141	38.49	24	14.2	9.58	5.47	
6H-2, 70–72	44.30	27	7.75	38.8	3.10	
6H-3, 113–115	46.23	29	18.3	2.40	4.10	0.76/22.3
6H-5, 58–60	48.68	32	14.4	10.3	5.05	0.82/24.0
6H-7, 49–51	51.59	37	10.6	21.3	5.05	0.81/23.7
7H-1, 25–27	51.85	38	6.80	40.8	1.54	0.79/23.3
7H-6, 98–100	60.08	41	8.75	42.1	4.58	0.74/21.9
7H-CC, 01–03	61.13	43	19.5	5.08	8.87	
7H-CC, 03–05	61.15	43	13.4	21.2	5.84	0.85/24.9
9H-2, 149–150	73.59	48	4.01	38.1	1.95	
9H-3, 03–04	73.63	48	3.38	62.7	3.86	
10H-2, 01–03	81.61	54	15.0	3.92	5.57	0.83/24.4
10H-2, 62–64	82.22	55	20.0	2.75	10.2	
10H-3, 68–70	83.78	58	11.6	30.0	7.03	0.84/23.5

Notes: Sapropel numbers after Shipboard Scientific Party (1996b). * = calculated difference between total and carbonate carbon contents. ** = average of two measurements. $U^{K'}_{37}$ = alkenone index (see Prahl and Wakeham, 1987); SST = sea-surface temperature (calibration after Prahl and Wakeham, 1987).

compiled in Table 1. Together with the shipboard results on Hole 964A sapropels, the TOC data are also plotted vs. biostratigraphic age (Shipboard Scientific Party, 1996b) in Figure 2. The organic carbon contents in the sapropels vary, on a high level, by more than one order of magnitude. They range between about 2% and 5% for both investigated holes in the upper Pleistocene and show strong variations between 3% and 25% in the other time sections. The values are covariant for the upper sapropels (<1 Ma) in both holes. This correlation is not so pronounced in Figure 2 for the deeper part of the cores because sapropels selected from both cores do not match in all cases and because of some variation of organic carbon contents within a given sapropel particularly in the case of the extremely organic-carbon-rich sapropels (Shipboard Scientific Party, 1996b; Table 1). For several sapropels, however, there is a good match of organic carbon contents for samples from Holes 964A and 964D.

Carbonate contents are in the 30%–50% range in the upper part of the hole, but show large fluctuations in the section below about 30 mbsf (Table 1; Shipboard Scientific Party, 1996b). As an overall observation, carbonate contents are lowest in sapropels with very high TOC values. Carbonate and TOC contents are negatively correlated with each other (Hole 964A: R = 0.84, n = 48; Hole 964D: R = 0.83, n = 39). Carbonate dissolution in the organic-carbon-rich sapropels may be due to organic acids formed during diagenesis (e.g., by hydrolysis of esters), and this effect may have been stronger in the older, particularly organic-carbon-rich sapropels. This is consistent with the high proportions of free organic acids found in the polar extractable organic matter fractions of the sapropels (see “Molecular Investigations”).

Sulfur contents, as a general trend, are higher in the particularly organic-carbon-rich sapropels (Table 2; Shipboard Scientific Party, 1996b), but there is no clear relationship with TOC data (Hole 964A: R = 0.46, n = 48; Hole 964D: R = 0.77, n = 39). Most TOC:S ratios are lower in the sapropels than in present-day noneuxinic sediments, for which an average ratio of 2.8 was reported by Berner and Raiswell (1983), and thus indicate anoxic bottom-water conditions.

Shipboard analyses of sapropels by Rock-Eval pyrolysis (Shipboard Scientific Party, 1996b) formally revealed marine kerogen type II organic matter in many of the Hole 964A sapropels with hydrogen indices (HI) between 350 mg hydrocarbons (hc)/g TOC and slightly more than 500 mg hc/g TOC, whereas more than 50% of the sapropels revealed lower HI values and higher oxygen indices (OI) in the mixed kerogen type II/III range as is evident from Figure 3. This is not uncommon for black shales deposited in the deep ocean even at high levels of organic carbon (e.g., Herbin et al., 1986a, 1986b) and does not necessarily imply a strong contribution of terrigenous organic matter to the sediments. Actually, the terrigenous component in many cases was found to be low in many deep-sea sediments by organic petrography (e.g., between 2% and 14% along the continental margins of Peru and Oman; Lückge et al., 1996), and the explanation for the low hydrogen indices was microbial alteration of the organic

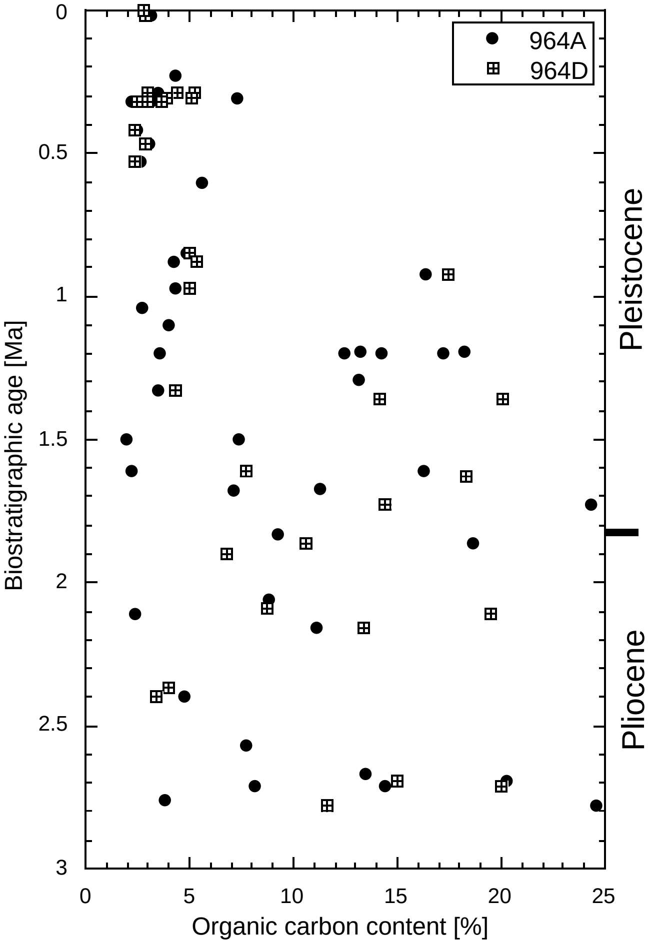

Figure 2. Organic carbon contents as a function of sediment age for sapropels from Holes 964A (shipboard data; Shipboard Scientific Party, 1996b) and 964D.

Table 2. Retention times and Kovats retention indices of unknown nonaromatic hydrocarbons and comparison with two highly branched isoprenoids.

Compound (see Fig. 5B)	Retention time (min)	Kovats retention index		Mass spectral type
		HP1	DB5	
a	53.04			II
b	53.25	2107	2096	II
c	53.87			(II)
d	54.02			I
e	54.40			I
f	54.55	2139	2116	I
g	54.73			I
h	55.10	2155	2151	II
i	55.74	2176	2161	II
HBI $C_{25:2}$ *(Haslea osfrarcia)*		2084	2063	
HBI $C_{25:2}$ *(Caspian Sea)*		2078	2059	

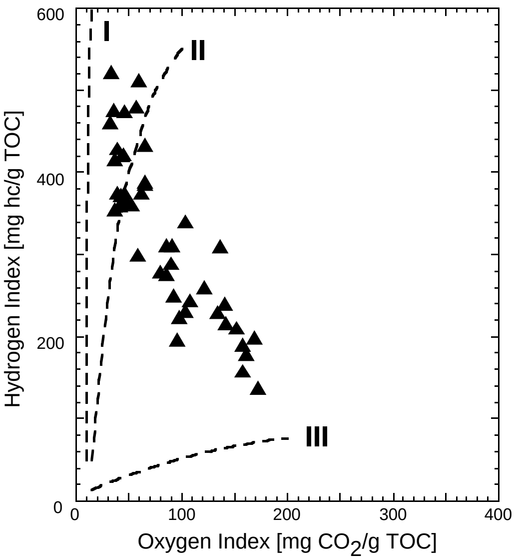

Figure 3. Van Krevelen–type diagram of hydrogen and oxygen index values for sapropels from Hole 964A (data taken from Shipboard Scientific Party, 1996b).

matter in the water column or in the upper sediment layers particularly by sulfate-reducing bacteria (Littke et al., 1991; Vetö et al., 1994). This type of alteration is largely dependent on the total organic carbon content as indicated by the TOC vs. HI diagram for Site 964 (Fig. 4A) and the other sites in the Eastern Mediterranean Sea occupied during Leg 160 (Fig. 4B). HI values increase with increasing organic-carbon content, indicating enhanced preservation of (labile) marine organic matter with increased organic-matter accumulation up to an organic carbon content of about 10%. Hydrogen indices of sapropels richer in organic carbon are “uniform” in the range of about 400–500 mg hc/gTOC.

Shipboard measurements of C_{org}:N ratios by themselves did not provide a definite indication as to the origin and extent of preservation of the organic matter in the sapropels. The C_{org}:N ratios for all sapropels exceeded a value of 10; the carbonate-rich sapropels had ratios between 10 and 15 and the carbonate-poor sapropels ratios between 15 and 22 (Shipboard Scientific Party, 1996b). As the high C_{org}:N ratios in many cases coincide with high hydrogen index values from Rock-Eval pyrolysis, early diagenetic alteration processes appear to have preferentially removed nitrogen-bearing compounds from the organic matter of marine biomass. This is independent of the presence of some terrigenous organic matter in all sapropels studied, but the C_{org}:N ratios certainly are not a measure of the terrigenous organic matter fraction in these sapropels. A recent study demonstrated the strong effect of molecular oxygen on the C_{org}:N ratios (Cowie et al., 1995) and suggested higher C_{org}:N ratios as the result of selective organic-matter degradation in anoxic sediments. For the formation of sapropels in the Eastern Mediterranean Sea, anoxic conditions are obvious by low TOC:S ratios, so in this case the high C_{org}:N must be interpreted as reflecting diagenetic alteration of organic matter.

Molecular Investigations

Extract yields, after removal of elemental sulfur, range between 15 mg/g TOC and 70 mg/g TOC. Despite some uncertainty due to the very low absolute amounts of extract (small sample size) and low accuracy of gravimetric determination in these cases, the extract yields are higher than in other deep-sea sediments with immature organic matter (e.g., Rullkötter et al., 1981) and may indicate degradation of biomacromolecules into soluble compounds by high bacterial activity and/or an exceptionally good preservation of labile low-molecular-weight compounds. The relative distributions of gross chromatographic fractions are dominated by asphaltenes and NSO compounds (each about 35% to 50% by weight). The main portion of the fractionated NSO compounds are the green- (in the younger sapropels) to brownish-colored acid fractions with a relative percentage of more than 50%. Nonaromatic and aromatic hydrocarbons in all sapropels represent only a minor amount of the total extract.

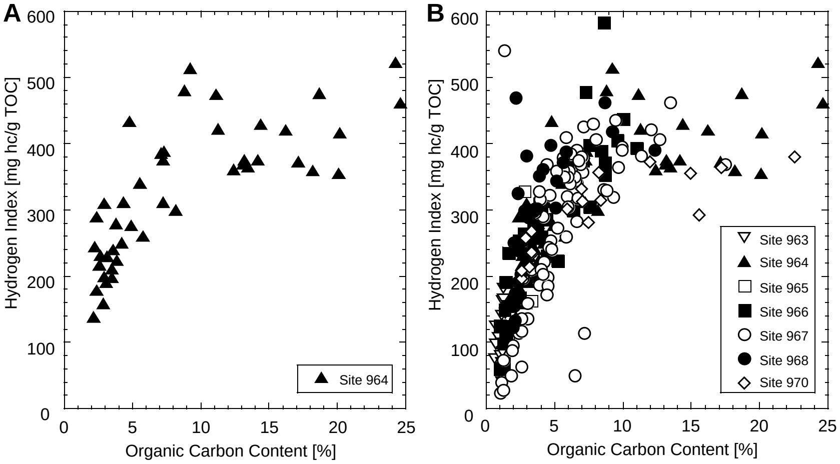

Figure 4. **A.** Hydrogen index values vs. organic carbon contents for sapropels from Hole 964A (data taken from Shipboard Scientific Party, 1996b). **B.** Hydrogen index values vs. organic carbon contents for sapropels from several holes in the Eastern Mediterranean Sea drilled during Leg 160 (data taken from Emeis, Robertson, Richter, et al., 1996).

Nonaromatic Hydrocarbons

Figure 5 shows gas chromatograms of the nonaromatic hydrocarbon fractions of Samples 160-964D-1H-1, 72–74 cm, and 10H-3, 68–70 cm, the shallowest and the deepest samples investigated, respectively. The *n*-alkane distribution patterns are very similar for all sapropel extracts studied and are typical of an origin of these compounds from cuticular waxes of higher land plants (Eglinton et al., 1962). The *n*-alkanes maximize at n-$C_{31}H_{64}$ in all samples, and Carbon Preference Index (CPI) values (Bray and Evans, 1961; corrected by Hunt, 1979) of *n*-alkanes of carbon numbers 25 to 33, as expected for a terrigenous source, consistently exceed a value of 2. At retention times between 90 min and 100 min, straight-chain C_{37} and C_{38} alkadienes (molecular weights 516 and 530, respectively; mass spectral base peak at *m/z* 96) elute from the GC column. They resemble the alkatrienes described by Volkman et al. (1980; and references therein) and, according to their carbon numbers, are most likely related to the long-chain alkenones (e.g., Volkman et al., 1980) that are present in the sapropels in high abundance. Phytane and pristane are absent or only detected in trace amounts (≈1 µg/g TOC), and thus cannot be used as redox indicators.

In samples buried more deeply than 35 m, branched and cyclic (steroid and triterpenoid) hydrocarbons increase in abundance relative to the *n*-alkanes due to a progress in diagenetic transformation of functionalized lipid compounds into nonaromatic (mostly olefinic) hydrocarbons. Among the pentacyclic triterpene hydrocarbons, oleanenes and ursenes together with the related des-A-triterpenoids, common in most of the samples, are further indicators of the terrigenous organic matter fraction in the sapropels but are outranged in abundance by sterenes of most likely marine origin. Even in the most deeply buried sapropel studied, diagenesis has not proceeded very far as is indicated by the exclusive presence of ster-2-enes and the absence of corresponding (thermodynamically more stable) ster-4- and -5-enes indicating a cool geothermal regime (Dastillung and Albrecht, 1977; Gagosian and Farrington, 1978; ten Haven et al., 1989). This is corroborated by the exclusive presence of hopanes with their unaltered biogenic 17β(H),21β(H)-22R sterical configuration in the triterpenoid series (Ensminger et al., 1977).

In the retention time range between 52 min and 58 min (Fig. 5B) the nonaromatic hydrocarbon fractions contain at least nine unknown compounds, partly in high concentrations, with a molecular weight of 348 u, corresponding to an elemental composition of $C_{25}H_{48}$ and indicating the presence of two degrees of unsaturation (double bond equivalents). The hydrocarbons exhibit two main types of mass spectra of which representative examples for two of the most abundant isomers are shown in Figure 6; the other isomers have mass spectra differing only slightly from those shown in relative intensity of major fragment ions. These compounds of unknown structure have retention times and Kovats indices that are slightly higher than those of C_{25} highly branched isoprenoids (HBIs) with two double bonds (Table 2) as reported from living organisms and sediments so far, and the unknown compounds also differ in their mass spectral fragmentation patterns from the C_{25} HBIs (Requejo and Quinn, 1983; Rowland et al., 1990; Belt et al., 1994). Any attempts to hydrogenate the unknown compounds, even under drastic conditions with an activated Adams catalyst, failed, so the novel compounds are likely to contain two rings instead of double bonds. Neither C_{20} nor C_{25} or C_{30} HBI pseudohomologs were detected in significant concentrations, indicating a low contribution of diatoms during times of sapropel formation consistent with the absence of diatom frustules in the sediments (Shipboard Scientific Party, 1996b). On the other hand, the carbon numbers of the novel bicyclic compounds suggest a relationship to isoprenoid biosynthesis and support speculations that the bicyclics may still be unknown biosynthetic products of diatoms or diagenetic transformation products of precursors from diatoms, whereas the absence of diatom frustules may be explained by post-depositional silicate dissolution. As reported by Bouloubassi et al. (Chap. 21, this volume) the C_{25} bicyclics dominate some of the nonaromatic hydrocarbon distributions of sapropels from sites farther to the east in the Mediterranean Sea. Structure elucidation and molecular isotope analysis are under way to solve the question of the origin of the novel bicyclic compounds.

n-*Alcohols*

n-Alcohols >C_{20} are the most abundant components in the least polar of the heterocompound fractions ("ketone fraction") (Fig. 7). *n*-Alcohols <C_{20} and phytol elute in the steroid alcohol fraction, and typically are present only in minor amounts (<1 µg/g TOC). In most samples, the distributions of long-chain *n*-alcohols have a marked preference of even-carbon-atom-number homologs, with a maximum at n-$C_{28}H_{57}OH$, which is a distribution typical of land-derived organic matter (Eglinton and Hamilton, 1963). The C_{20} to C_{30} *n*-alcohols with a strong even-over-odd carbon number predominance in the sapropels occur together with a similar range of fatty acids (C_{20}–C_{30}; see below) and a series of long-chain *n*-alkanes (C_{23}–C_{35}) with an odd-over-even carbon number predominance. All these compounds are related to a terrigenous organic matter supply (de Leeuw, 1986).

In four samples from the lower part of Hole 964D (Samples 160-964D-1H-1, 72–74 cm; 2H-3, 67–69 cm; 2H-3, 81–83 cm; and 2H-4, 92–94 cm), however, this pattern is overprinted by a series of straight-chain alcohols with an unusual odd-over-even carbon number predominance and a maximum at n-$C_{25}H_{51}OH$, which is of unknown origin and significance (Fig. 7A). The relatively high concentrations of monounsaturated *n*-alcohols of even-carbon-numbered homologs are also notable, because the $C_{30:1}$ compound in all samples

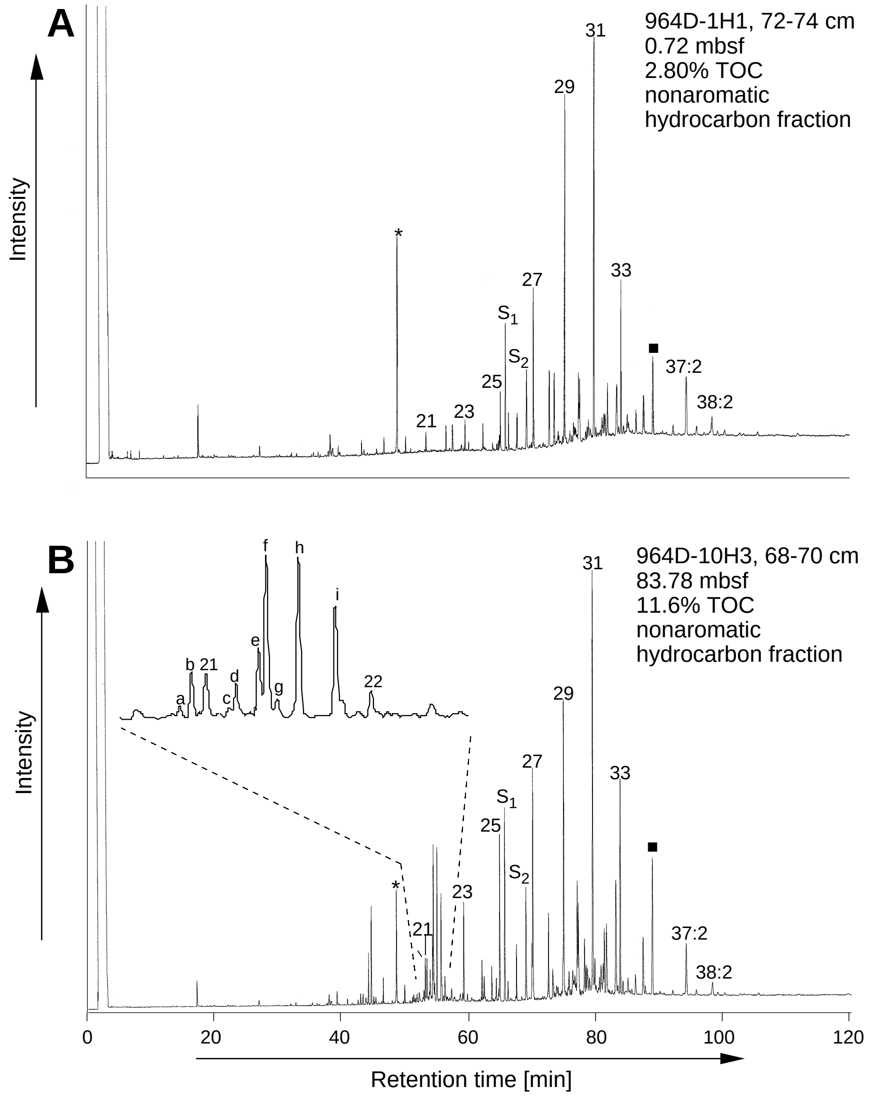

Figure 5. Gas chromatograms of the nonaromatic hydrocarbon fractions of the shallowest (**A**) and the deepest (**B**) sapropel sample from Hole 964D. The increase of branched and cyclic hydrocarbons relative to *n*-alkanes illustrates the difference in the extent of diagenetic transformation. Numbers refer to chain length of *n*-alkanes and alkadienes, respectively. S_1 = InjSTD (behenic acid methyl ester), S_2 = ISTD (squalane), square = tetraterpene, inset (expanded) = unknown bicyclic C_{25} compounds (molecular weight 348; cf. Table 2), * = contaminant.

exceeds the concentration of the related saturated analog (Fig. 7). This compound has been reported to occur in microalgae of the class *Eustigmatophyceae* (Volkman et al., 1992), which are also thought to biosynthesize the alkan-1,n-diols found in sediments (discussed separately).

Long-Chain Alkenones

Long-chain alkenones (C_{37}–C_{39}) are common in moderate to high abundances in the least polar heterocompound fractions (Fig. 7). These compounds are derived from marine prymnesiophyte species (Volkman et al., 1980). They are not restricted to sapropels younger than 268 ka, the age of the first occurrence of *Emiliania huxleyi* (Thierstein et al., 1977), or even younger than 70 ka, the beginning of the *E. huxleyi* acme zone (Gartner, 1977), respectively. They have apparently also been biosynthesized by their phylogenetic ancestors and related *Gephyrocapsaceae* species (Marlowe et al., 1990). The concentrations of total C_{37}–C_{39} alkenones vary between 125 µg/g TOC and 1876 µg/g TOC with no correlation to sediment sub-bottom depth or TOC content, but generally illustrate the strong marine influence on the organic matter in the sapropels.

Sterols

In Figure 8, the gas chromatogram shows the elution range of sterols for Sample 160-964D-5H-4, 137−139 cm. Compound identification is based on relative retention times and comparison with published mass spectra (e.g., Budzikiewicz, 1972; Brassell, 1980; McEvoy, 1983; cf. Table 3 and Appendix). The sterol distributions are complex, indicating a variety of primarily marine sources. The major compounds are the C_{27} to C_{29} sterols, although C_{30} sterols are present in significant concentrations; C_{26} sterols were only detected in trace amounts. The relative concentrations of C_{27} to C_{29} sterols are similar to each other, but with a slight predominance of C_{29} compounds, which varies among the samples (Fig. 9). This may be related to slight variations in the supply of terrigenous organic matter, even though most haptophyte algae also contain 24-ethylcholest-5-en-3β-ol, a sterol commonly assigned to a terrigenous higher plant source (Volkman, 1986). Similar sterol concentrations and distribution patterns with a predominance of C_{29} compounds were also found in modern sediments from the Peru upwelling region (Volkman et al., 1987). In that study it was concluded that an unreflected interpretation of steroid carbon number distribution using the Huang-Meinschein diagram in Figure 9 would lead to an overestimate of land-derived organic matter. Furthermore, a major portion of the sterols, and among them particularly those of marine sources, in the youngest sapropel is known to be bound as esters or sulfates due to the low progress in diagenesis (ten Haven, 1986). Thus, the distribution pattern in Figure 8 and the carbon number distributions in Figure 9 may not represent the total steroids present because bound components were not liberated prior to extraction in this study. But there is no correlation between the sterol carbon number distribution (or relative

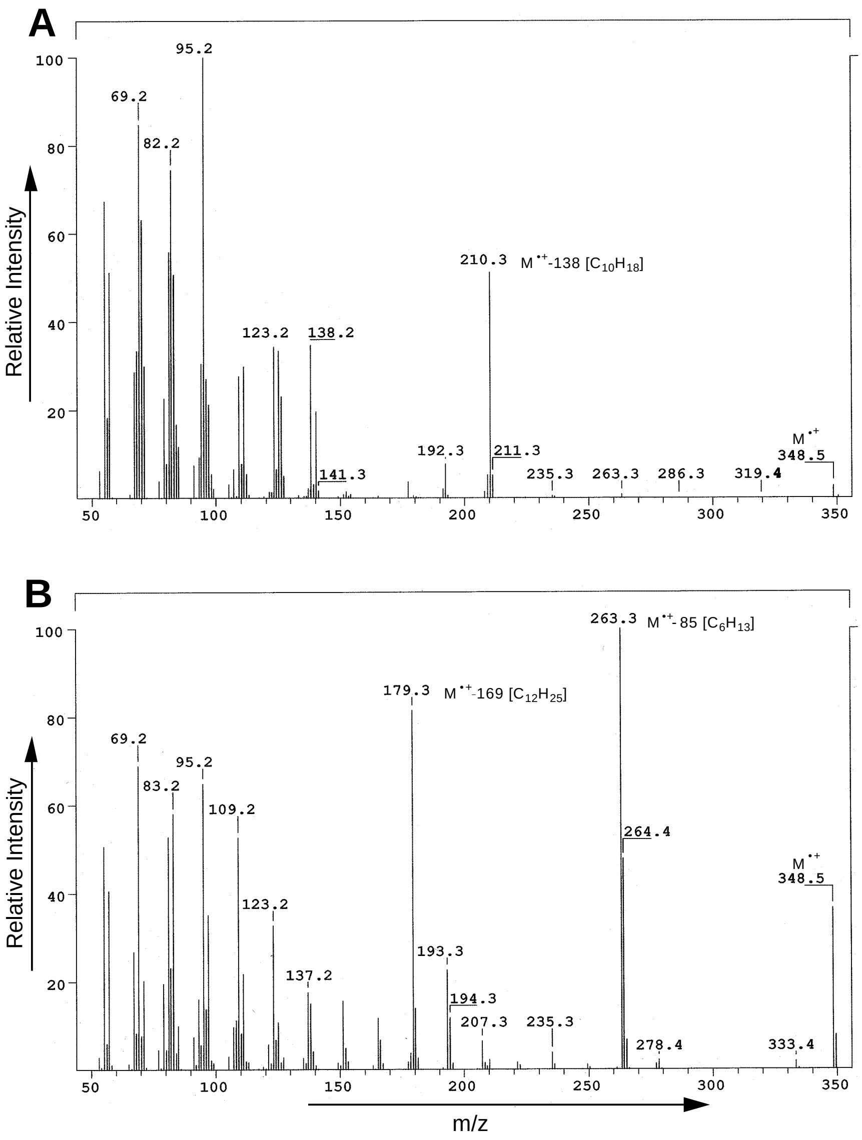

Figure 6. Representative mass spectra of unknown bicyclic C_{25} compounds in the hydrocarbon fraction of Sample 160-964D-10H-3, 68–70 cm (cf. Table 2).

proportion of C_{29} sterols; Fig. 9) with either organic carbon content or depth/age, that is, the type of sterols present does not depend on the preservation conditions but may rather reflect variations (mainly) of the phytoplanktonic community at a given time of sapropel deposition. On the other hand, the total free sterol concentrations correlate well (R = 0.87) with the total organic carbon contents (Fig. 10). This again may indicate enhanced preservation of labile organic matter in the particularly TOC-rich sapropels.

Long-Chain Alkan-1,n-diols and Alkan-1-ol-n-ones

C_{30}, C_{31}, and C_{32} alkan-1,15-diols and alkan-15-on-1-ols were initially reported to occur in immature Black Sea sediments (de Leeuw et al., 1981). Since then, these compounds and homologs with different chain lengths and positions of the midchain functionality have been found in various marine and lacustrine sediments (e.g., Morris and Brassell, 1988). *n*-Alkan-1,n-diols are now thought to be derived from eustigmatophyte algae (Volkman et al., 1992). Note that in our liquid chromatographic separation scheme, the alkandiols elute in the fatty acid fraction and the corresponding ketools in the sterol fraction, respectively.

In all investigated samples, the most abundant alkandiol (Fig. 8), and most abundant single compound in general with only one exception, is the C_{30}-1,15-diol (with coeluting isomers representing different positions of the midchain hydroxyl group). C_{28}- to C_{34}-diols with different positional isomers for the midchain functionality are also common and have a strong predominance of even-chain-length homologs. In Sample 160-964D-6H-3, 113–115 cm, a compound tentatively identified as C_{29}-1,14-diol is dominant and is accompanied by an admixture of the 1,12-isomer. The corresponding alkan-1-ol-n-ones are present in concentrations lower by a factor of 2 to 5 in all samples. Altogether, these compounds provide a strong marine molecular signal for the sapropels.

n-*Fatty Acids*

Saturated *n*-fatty acid distributions in the sapropels ranged in carbon number from 14 to 30 and contained a strong predominance of even-carbon-number homologs. The distributions were bimodal in all samples. One of the maxima occurs at C_{16} and the other one in the range of the terrigenous long-chain fatty acids (Kolattukudy, 1976). Most samples are dominated by the long-chain homologs, but a direct correlation between compound distribution pattern and depth or TOC content is not evident.

Mono- and diunsaturated fatty acids were detected in the range from C_{14} to C_{18} with a maximum at C_{18} and with a strong predominance of even-carbon-number homologs. The concentrations of the positional isomers of monounsaturated octadecenoic acids are only

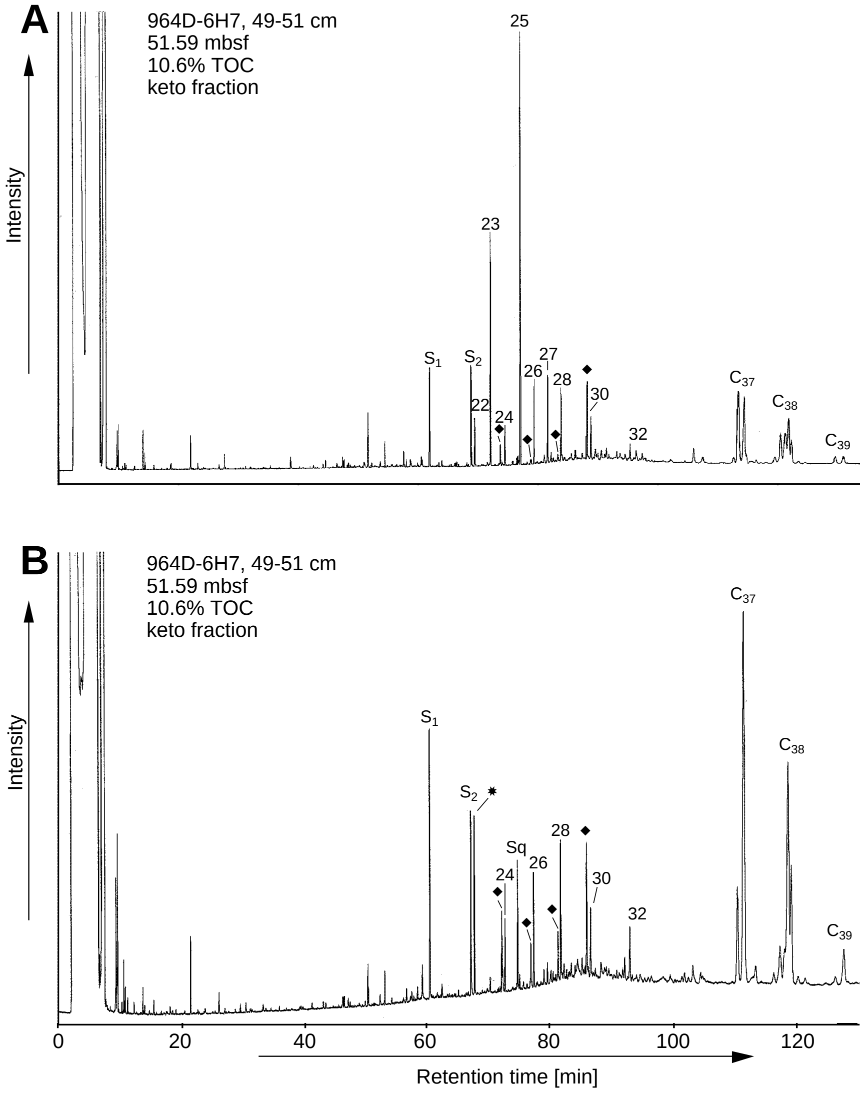

Figure 7. Gas chromatograms of two "ketone fractions" as discussed in the text. Note the differences in abundance of *n*-alcohols and long-chain alkenones. Chain lengths of *n*-alcohols are indicated by numbers, monounsaturated *n*-alcohols by diamonds. C_{37}, C_{38}, and C_{39} are long-chain alkenones. S_1 = ISTD (androstan-17-one), S_2 = InjSTD (behenic acid methyl ester), * = contaminant and C_{22} *n*-alcohol (trace).

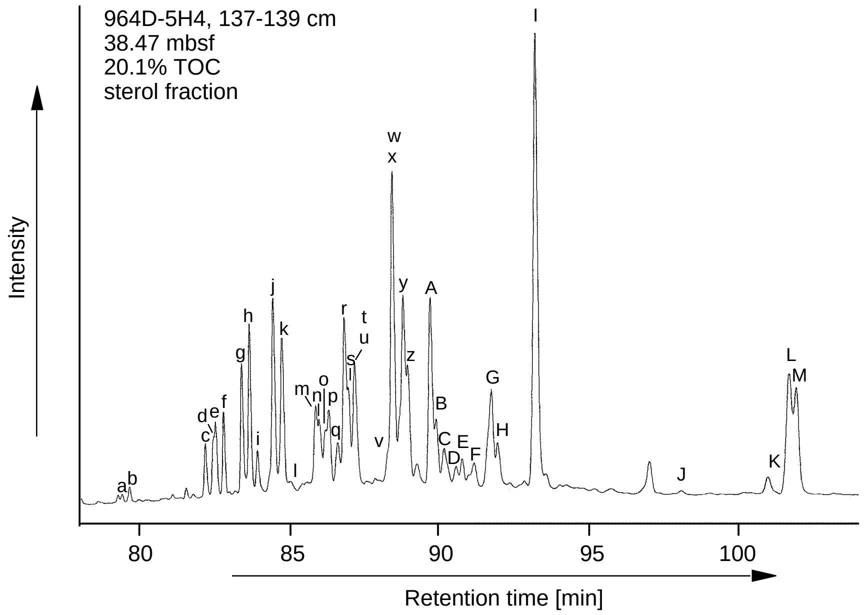

Figure 8. Gas chromatogram of the elution range of sterols extracted from Sample 160-964D-5H-4, 137–139 cm (see Table 3 for compound identification).

Table 3. Sterols and alkan-1-ol-n-ones in the sterol fractions (cf. Fig. 8).

Symbol	Compound	Structure (see Appendix)
a	24-*nor*-cholesta-5,22(E)-dien-3β-ol	b1
b	24-*nor* -5α-cholest-22(E)-en-3β-ol	a1
c	27-*nor*-24-methylcholesta-5,22(E)-dien-3β-ol	b4
d	27-*nor* -24-methyl-5α-cholest-22(E)-en-3β-ol	a4
e	cholesta-5,22(E)-dien-3β-ol	b2
f	5α-cholest-22(E)-en-3β-ol	a2
g	cholest-5-en-3β-ol	b3
h	5α-cholestan-3β-ol	a3
i	27-*nor*-24-methyl-5α-cholestan-3β-ol	a5
j	24-methylcholesta-5,22(E)-dien-3β-ol	b7
k	24-methyl-5α-cholest-22(E)-en-3β-ol	a7
l	5α-cholest-7-en-3β-ol	c3
m	C_{28}-steradienol	—
n	24-methylcholest-5-en-3β-ol	b6
o	24-methylcholesta-5,24(28)-dien-3β-ol	b8
p	24-methyl-5α-cholestan-3β-ol	a6
q	23,24-dimethylcholesta-5,22(E)-dien-3β-ol	b13
r	24-ethylcholesta-5,22(E)-dien-3β-ol	b10
s	23,24-dimethyl-5α-cholest-22(E)-en-3β-ol	a13
t	24-ethyl-5α-cholest-22(E)-en-3β-ol	a10
u	4α,24-dimethyl-5α-cholest-22(E)-en-3β-ol	d7
v	23,24-dimethylcholest-5-en-3β-ol	b12
w	24-ethylcholest-5-en-3β-ol	b9
x	+ 23,24-dimethyl-5α-cholestan-3β-ol (tr)	a12
y	24-ethyl-5α-cholestan-3β-ol	a9
z	+ 24-ethylcholesta-5,24(28)-dien-3β-ol	b11
A	4α,23,24-trimethyl-5α-cholest-22-en-3β-ol	d13
B	C_{30}-cholest-22-en-3β-ol	—
C	C_{30}-cholest-?en-3β-ol	—
D	4α,23,24-trimethyl-5α-cholest-7-en-3β-ol	e14
E	C_{30}-cholest-?-en-3β-ol	—
F	C_{30}-cholest-5-en-3β-ol	—
G	C_{30}-stanol	—
H	C_{30}-stanol	—
I	C_{30}-keto-1-ol	—
J	C_{31}-keto-1-ol	—
K	$C_{32:1}$-keto-1-ol	—
L	C_{32}-keto-1-ol	—
M	C_{32}-keto-1-ol (isomer of L)	—

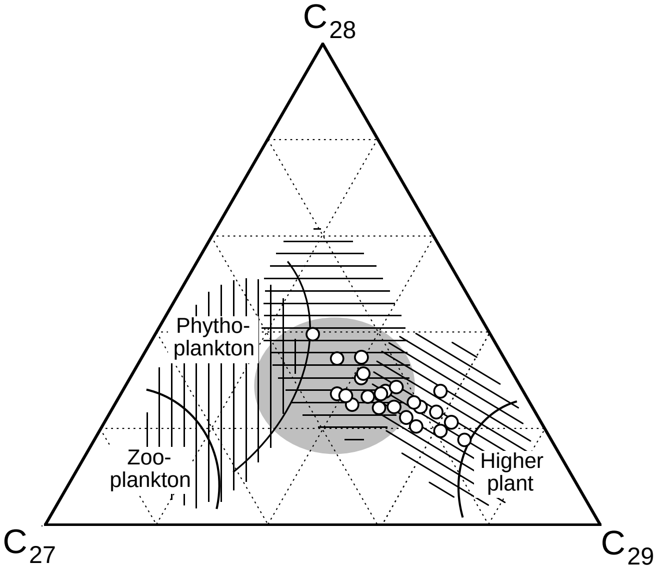

Figure 9. Triangular diagram of carbon number distributions of steroid alcohols in sapropels from Hole 964D analyzed in this study.

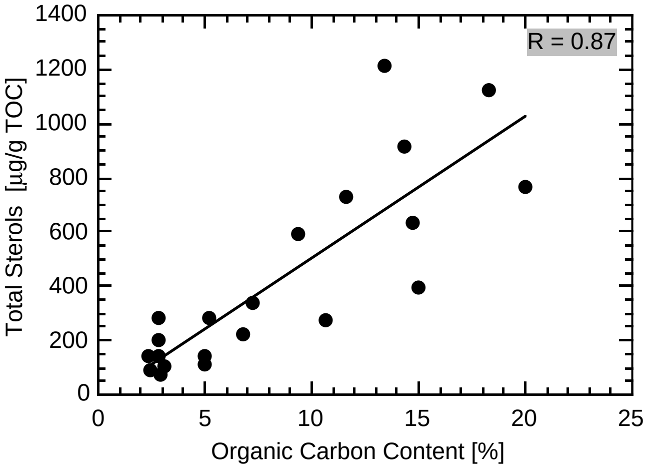

Figure 10. Linear regression of total sterol concentrations and organic carbon contents for sapropels from Hole 964D.

slightly lower than that of the saturated compound, while the monounsaturated hexadecenoic acids make up only 5%–10% of the concentration of the corresponding saturated fatty acid. Monounsaturated short-chain fatty acids are well known in many marine organisms. In microalgae the $C_{18:1}$ ω9-fatty acid (oleic acid) is in many cases more abundant than the saturated homolog, whereas this ratio is well balanced between the $C_{16:0}$ and the $C_{16:1}$ ω7-fatty acids (Cobelas and Lechado, 1989). The $C_{16:1}$ ω7 compound is a major fatty acid in diatoms (Smith et al., 1983; Volkman et al., 1989). This finding supports again the low contribution of siliceous organisms to the organic matter and contradicts speculations of a diatom origin of the novel C_{25} bicyclic hydrocarbons.

Alkenone-Based Sea-Surface Temperatures and Average Chain Lengths of n*-Alkanes as Climatic Indicators*

In Figure 11 the TOC values of 24 sapropels are plotted against the alkenone-derived paleo-sea-surface temperatures of the same samples. With one exception (160-964D-5H-4, 137–139 cm), the TOC contents of Pleistocene sapropels are in the range between 2% and 6% and thus distinctly lower than those of Pliocene sapropels (7%–20%). While the former were deposited under sea-surface temperature (SST) conditions with a relatively large fluctuation of 8°C between 14°C and 22°C, the Pliocene sapropels formed under more uniform and higher SSTs between 22°C and 25°C; the transition from the Pliocene to the Pleistocene reflects the global cooling at that time. Formation of (extremely) TOC-rich sapropels during times of elevated SSTs can be seen either as an effect of more sluggish circulation during the warmer Pliocene times or as a consequence of more stable stratification of the water column as a result of an enhanced freshwater inflow due to a higher humidity on the surrounding continents than during later times of sapropel formation in the Pleistocene. Although SSTs are not entirely uniform in a single sapropel (Table 1; Emeis et al., Chap. 26, this volume), the measurements appear to be largely representative of a given sapropel. This is supported by the fact that the $U^{K'}_{37}$ values (U^{K}_{37} = [37:3]/([37:3] + [37:2]) = 0.037 · SST – 0.07; Table 1; Prahl and Wakeham, 1987), determined using the chromatographically separated keto fractions for the Hole 964D samples and total extracts for the Hole 964A samples during the Leg 160 cruise (Shipboard Scientific Party, 1996b), respectively, are in agreement with each other.

The lowest SST was determined for the S_2 sapropel, which is known to have been deposited in a cool climate. In contrast to this, deposition of the youngest sapropel (S_1) started between 8.8 ka in shallow water depth and 8.2 ka in a water depth corresponding to that of the Site 964 location on the Pisano Plateau (Strohle and Krom, 1997), and thus the SST data correspond to the warmer present-day (Holocene) conditions. In fact, the SST data determined based on the alkenones in the S_1 sapropel (Table 1) match the present-day spring (April) SSTs in the Site 964 area (Anonymous, 1976). Likewise, the climate was warmer at the time of S_9 deposition (≈242 ka) than at the time of S_8 deposition (≈220 ka; e.g., Rossignol-Strick, 1985), and this is matched by a difference of 3°C in alkenone-based SST data in Table 1.

The carbon number distributions of terrestrial long-chain *n*-alkanes depend on the type of vegetation and, thus, on the terrestrial climatic conditions (Simoneit et al., 1977). A shift to higher chain-lengths can reflect (1) higher continental temperatures of the borderlands in general (Simoneit et al., 1977) or (2) an origin of the organic matter from vegetation zones with higher temperatures (Gagosian et al., 1981, 1987). For the Mediterranean Sea the origin of the land-derived organic matter may be sensitive to the strength of the wind systems, especially of the monsoonal southwesterlies, and the precipitation rate and area, respectively. As a proxy for the varying chain lengths of *n*-alkanes, Poynter (1989) has introduced the $ACL_{27–31}$ index and demonstrated a correlation between the pollen record and $ACL_{27–31}$ values for the West African coast (ODP Holes 658A and 658B) during the last 24 k.y., indicating varying origins of terrestrial material brought into the deep sea by eolian and fluviatile transport. In Poynter's (1989) study, the values range between 29.65 and 30.05.

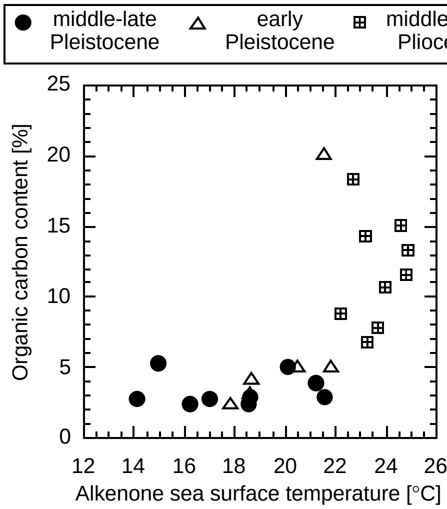

Figure 11. Organic carbon content vs. alkenone sea-surface temperatures for sapropels from Hole 964D. Biostratigraphic age (after Shipboard Scientific Party, 1996b) is indicated by different symbols.

The $ACL_{27–31}$ values of the investigated sapropels from the Pisano Plateau are lower (29.40–29.75) than for the West African coast and indicate lower growth temperatures for the higher land plants. The corresponding $ACL_{27–33}$ (Hinrichs, 1997) values correlate with other organic geochemical data of the Mediterranean sapropels in the following way (Fig. 12): (1) alkenone-derived SST values increase with increasing ACL values (R = 0.73) and (2) extremely TOC-rich sapropels were deposited at times when ACL values were high (R = 0.71). The relationship between ACL and continental floral assemblage is supported by the lowest measured value of the S_6 sapropel, deposited under a cold and dry land climate (e.g., Rossignol-Strick, 1985). These data suggest higher temperatures and more humid conditions during the deposition of middle–late Pliocene sapropels and can partly explain higher TOC contents as the result of elevated fresh-water inflow and thus, most probably, elevated nutrient supply and enhanced productivity.

CONCLUSIONS

Sapropels from ODP Site 964 at the Pisano Plateau (early Pliocene to Holocene) were investigated for bulk elemental composition and the distributions of solvent-extractable lipids. The bulk elemental data reflect the particular environmental conditions of sapropel formation in the Mediterranean Sea with enrichments of organic matter and sulfur otherwise only found in black shales of the Mesozoic oceans. TOC:S ratios indicate anoxic bottom water conditions.

The extractable lipids make up only a minor part of the total organic matter present, and their composition indicates a low level of diagenetic alteration. The molecular compositions of the sapropel extracts (with the exception of the fatty acids) are largely similar in all investigated samples, despite the large variation of TOC concentrations. They are dominated by marine-derived compounds (*n*-alkan-1,n-diols, *n*-alkan-1-ol-n-ones, sterols, and long-chain alkenones) with varying subordinate admixtures of terrigenous organic matter (long-chain *n*-alkanes, *n*-alkanols, and fatty acids) and less significant contributions from microbial sources. Lipid compositions are in general similar to those in sediments from modern upwelling areas (Farrimond et al., 1990; Poynter et al., 1989); a slightly enhanced proportion of land-derived organic matter may relate to a delicate balance between climatic changes, terrigenous supply, and sapropel formation. The correlation between SSTs and average chain length of *n*-alkanes and the relationship between TOC contents and average chain length of *n*-alkanes support land-climate-controlled conditions for sapropel formation.

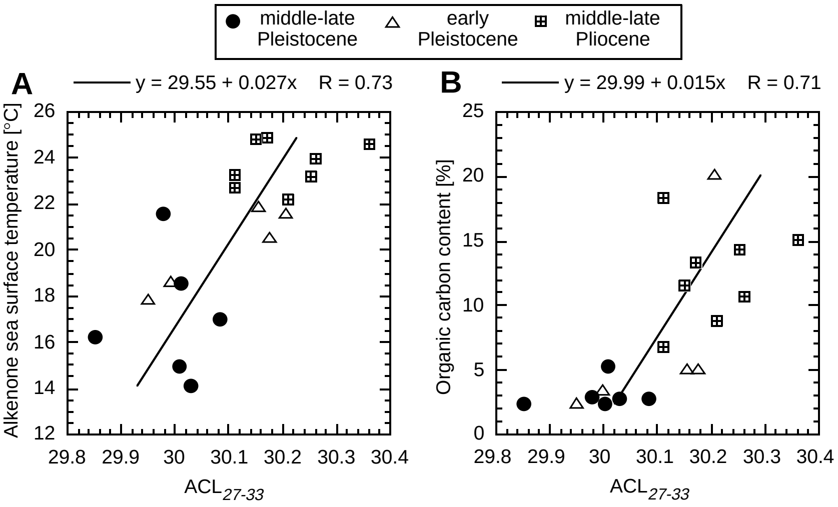

Figure 12. **A.** Alkenone sea-surface temperatures vs. average chain length (ACL) of *n*-alkanes for sapropels from Hole 964D. Biostratigraphic age (after Shipboard Scientific Party, 1996b) is indicated by different symbols. **B.** Organic carbon content vs. average chain length of *n*-alkanes for sapropels from Hole 964D. Biostratigraphic age (after Shipboard Scientific Party, 1996b) is indicated by different symbols.

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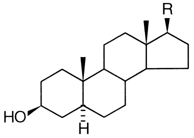
APPENDIX

Structures of sterols

Ring structures



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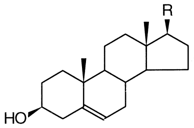

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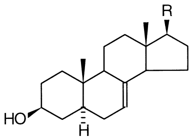

c / 5α-Cholest-7-en-
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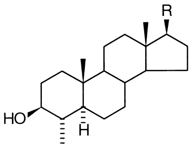
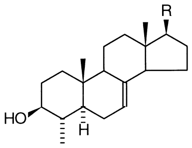


d / 4α-Methyl-5α-
cholestan-3β-ol series



e / 4α-Methyl-5α-
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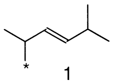
side-chain structures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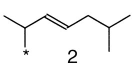
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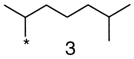
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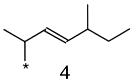
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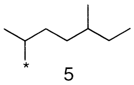
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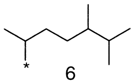
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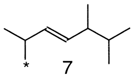
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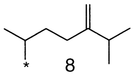
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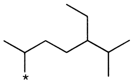
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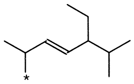
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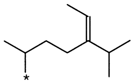
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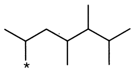
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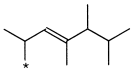
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