COMPOSITION AND ORIGIN OF COMPLEX ORGANIC MATTER IN RECENT MARINE SEDIMENTS

Significance of bacterial biomarkers

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2012 5020

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PROEFSCHRIFT ter verkrijging van de graad
van doctor in de technische wetenschappen
aan de Technische Hogeschool Delft,
op gezag van de Rector Magnificus,
prof.ir. B.P.Th. Veltman,
in het openbaar te verdedigen
ten overstaan van
het College van Dekanen
op donderdag 24 mei 1984 te 14.00 uur
door

JACOB KLOK

doctorandus in de chemie
geboren te Brielle

DELFT/1984

Dit proefschrift is goedgekeurd door de promotor
prof.drs. P.A. Schenck

Dr. H.C. Cox en dr. J.W. de Leeuw hebben in hoge mate bijgedragen aan de begeleiding bij het tot standkomen van het proefschrift en zijn als zodanig aangewezen door het College van Dekanen.

Aan mijn ouders
Aan Monique
Voor Jaap, Erik en Jan

CONTENTS

CHAPTER 1

INTRODUCTION

The occurrence of organic matter on earth is not limited to the conspicuous forms like biomass and the explorable fossil resources coal, petroleum and natural gas. In fact these forms constitute only a very small part of the total organic carbon present throughout the earth's crust. The major part is present in sedimentary rocks in a disseminated and insoluble form which is generally known as kerogen. It has been estimated that about 90% of all the organic carbon on earth is present as kerogen (Hunt, 1979).

The word kerogen derived from the Greek "*keros*" (= wax) was proposed to describe the organic content of oil shale which produces, by destillation, oil with a waxy (paraffinic) consistency (Steuart, 1912). However the term kerogen covered and still covers a variety of concepts which historically originate from the exploitation of oil shales and which are closely linked to research dealing with the origin of petroleum, especially with theories on its organic origin. In the definition stated by Durand (1980) and used here, kerogen is defined as the fraction of sedimentary organic matter which is insoluble in the usual organic solvents. In practise the term kerogen designates a polycondensed or polymerized state of organic matter not belonging to the living realm.

In the current concept of kerogen formation (Hunt, 1979; Tissot and Welte, 1979; Durand, 1980) biopolymers are at the very start of the diagenesis of organic matter. After the death of organisms, during sedimentation and afterwards the activity of burrowing organisms and microbes, governed by environmental conditions, results in the partial decomposition of biogenic polymers. During this process biopolymers are partly mineralized and partly resynthesized in (microbial) cell material, thus ultimately leaving an organic residue unassimilable by microorganisms under these conditions. This residue is now incorporated into a new, humic polycondensate precursing kerogen (Tissot and Welte, 1979). In a spin-off from the biogeochemical cycle part of the microbially

released biomonomers are thought to condense and to be incorporated in the polycondensate as well (Hunt, 1979). Apart from the insoluble fraction (kerogen) sedimentary organic matter comprises a minor amount of free hydrocarbons and related compounds at the end of the diagenetic period. These hydrocarbons originate from compounds synthesized by living organisms and are incorporated in the sediment with no or minor changes and which can be considered as geochemical fossils reflecting the environment of deposition.

Because of its quantitative importance and because it is considered to constitute the source material for oil and natural gas it is worthwhile to understand the composition and origin of kerogen. Due to its insolubility kerogen is less amenable to structural investigation from an analytical point of view. Moreover when studying its origin the absence of a morphological relationship with biological structures is a great disadvantage. However, the cooccurrence of geochemical fossils in the soluble fraction of sedimentary organic matter could yield some information about the origin. In the case of coal studies the morphology of the natural precursor is partly retained upon coalification, which simplifies both the chemical characterization and the interpretation of the processes that led to the present condition of this type of organic matter.

In order to make kerogen accessible to structural elucidation chemical degradation techniques must be employed. Degradation processes should be as specific as possible in order to obtain smaller identifiable compounds which still retain a structural relationship with the kerogen. Various types of chemical degradation and functional group analyses have been carried out, many of which have been reviewed by Vitorović (1980). Furthermore pyrolysis methods have shown to be valuable tools in structural characterization of kerogens (e.g. Larter and Douglas, 1980; Solli *et al.*, 1980; van de Meent *et al.*, 1980). However the above methods are of limited value from a quantitative point of view. Moreover it is often impossible to relate the structure of the products released to that of the original complex organic matrix.

Since in the current concept the genesis of kerogen is considered to be the result of a sequence of biochemical and geochemical transformations of organic matter primarily derived from biomass, one could consider the organic matter present in a recent sediment to represent an intermediate stage between biomass and kerogen. For this reason studies of the organic matter from recent sediments could yield valuable information about the starting structures of future kerogen.

When the organic matter in a recent marine sediment is regarded as an accumulation of biopolymers in various stages of decomposition the structural characterization is directed in the first place by analytical methods aiming at

the release and quantitation of biopolymers or building blocks of biopolymers. In the second place organic compounds not recognizable as such, bust most probable derivatives thereof, must be investigated.

Various attempts have been undertaken to quantify the contribution of the major compound classes in the marine environment viz. carbohydrates, proteins, lipids and nucleic acids and of "humic substances" to sedimentary organic carbon. Carbohydrates determined in marine and lake sediments (e.g. Swain and Rogers, 1966; Swain *et al.*, 1967; Swain *et al.*, 1970; Swain, 1971; Modzeleski *et al.*, 1971; Fleischer, 1972; Handa and Mizuno, 1973; Degens and Mopper, 1976; Mopper, 1977; Hatcher *et al.*, 1977; Böhm *et al.*, 1980; Ferguson and Ibe, 1981; Uzaki and Ishiwatari, 1983) have been shown to represent from several ppm in ancient sedimentary rocks to about 45% of the total organic carbon in a recent mangrove lake sediment. Similarly proteins (e.g. Brown *et al.*, 1972; Casagrande, 1974; Whelan, 1975; Morris, 1975; Hatcher *et al.*, 1977; Degens and Mopper, 1976; Henrichs and Farrington, 1979) have been estimated to comprise up to 33% of the total sedimentary organic carbon. Lipids constitute in general less than 10% of the organic carbon (Boon, 1978; Hunt, 1979 and references cited therein). The contribution of nucleotides is generally an order of magnitude less than those of carbohydrates, proteins or lipids (van der Velden, 1976 and references cited therein). Organic matter releasable from sediment samples by extraction with base and structurally less defined (often denoted as humic substances) constitutes up to 40% of the total sedimentary organic carbon (Debyser *et al.*, 1977; Pelet and Debyser, 1977; Hunt, 1979; Jocteur-Monrozier and Jeanson, 1981).

Because of the poor specificity of the common monosaccharides and amino acids the above authors interpret their data with some caution when the origin of the bulk constituents like carbohydrates and proteins is concerned. However during carbohydrate component analyses in the distribution of monosaccharides in general the characteristics of structural carbohydrates of the contributing organisms (algae) are recognized. In the case of amino acid analyses some minor amino acids with a marker value for the presence of bacteria indicate a bacterial contribution to the sedimentary organic matter in addition to that of the primary algal producer. Among the lipids releasable from marine sediments in general both the contributions of the primary algal producer and of bacteria are distinguishable.

In the present study samples from recent marine sediments are investigated in order to elucidate the composition and origin of the organic matter. An attempt is made to set up a complete inventory of recognizable, well defined organic compounds viz. proteins, carbohydrates and lipids. This is partly

realized by non-degradative extraction of biopolymers using water or water based extractants. Biopolymers not releasable in this way are depolymerized to yield the biomonomers e.g. monosaccharides, amino acids and lipid components. By means of the application of acid hydrolysis or base saponification it is possible to break down the greater part of the covalent linkages between monomer building blocks of biomacromolecules viz. ester-, glycosidic- and amide bonds. The nature and strength of the chemical treatment and the choice of the extractant offer the possibility to distinguish between the various classes of monomeric building blocks released.

The various classes thus obtained were analysed qualitatively and quantitatively. For that purpose it was necessary to adapt a number of analytical methods in order to make them applicable to the analysis of sediment samples.

In Chapter 2 the investigation of organic matter present in a recent marine diatomaceous ooze sample from the Namibian Shelf (S.W. Africa) is presented. The sediment was analysed for carbohydrates, proteins and lipids releasable upon extraction with water and acid of increasing concentration or with organic solvents using analytical procedures which are introduced hereafter. The organic components released but not identified by these procedures and the ultimate insoluble organic matter after extraction were analysed further by pyrolysis - mass spectrometry and pyrolysis - gas chromatography - mass spectrometry in order to make a complete inventory of the organic matter.

A method was developed for the analysis of carbohydrates in samples from recent marine sediments. Upon acid hydrolysis complex mixtures of monosaccharides were obtained, which were analysed as their alditol acetates by gas chromatography (Chapter 3). Gas chromatographic - mass spectrometric analysis revealed the presence of a large number of partially methylated and deoxy alditol acetates. The identification of these components required the synthesis of appropriate standards. In Chapter 4 the analysis of synthetic mixtures of partially methylated alditol acetates is described. The results of the ultimate identification of mixtures of alditol acetates obtained from sediment hydrolyzates are summarized in Chapter 5. In this comparative study the geochemical significance of partially methylated and deoxy monosaccharides is discussed.

The alditol acetate method is also applied to the analysis of carbohydrates in a recent stromatolytic deposit in order to study the diagenesis of carbohydrates upon burial. The carbohydrate composition and the contribution of carbohydrate carbon relative to the total organic carbon are discussed in terms of the potential to become part of the organic matter that survives geological periods of time (Chapter 6).

In Chapter 7 a special and partly new method is presented for the analysis of total lipids in sediments. Lipid components obtained after extraction with water or organic solvents and released after acid hydrolysis and/or saponification with base were analysed without any preseparation by gas chromatography and gas chromatography - mass spectrometry after appropriate derivatization. In this way free, esterified and amide bound lipids were distinguished. Two classes of lipids were detected in significant amounts. On the one hand the abundance of β-hydroxy fatty acids, amide bound to the organic matrix, point to a bacterial contribution to the organic matter present in the sediment. On the other hand the loliolide - type lactones point to an algal contribution to the sedimentary organic matter as well. Moreover these lactones are thought to be indicators for early diagenetic transformations of carotenoids from an algal source (Chapter 8).

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CHAPTER 2

QUALITATIVE AND QUANTITATIVE CHARACTERIZATION OF THE TOTAL ORGANIC MATTER IN A RECENT MARINE SEDIMENT II.*

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ABSTRACT

The total organic matter of a recent marine diatomaceous ooze is studied by analysis of its water and acid extracts and residues. The extraction data relative to the total organic carbon reveal major contributions of carbohydrates (22%), "volatiles" (12%) and proteins (11%) and a smaller contribution of lipids (4%) and volatile fatty acids (1%). The residual organic carbon (50%) mainly shows aliphatic characteristics. Among the compounds identified in the extracts some with an algal and others with a bacterial origin are distinguishable.

*In: *Advances in Organic Geochemistry 1983* (P.A. Schenck *et al.*), in press.

INTRODUCTION

Organic matter in both recent and ancient sediments insoluble in organic solvents, has been subject of many structural investigations because of its quantitative importance when compared to the soluble fraction. The study of the organic matter in recent sediments, being at the start of a sequence diagenetic processes, may offer the possibility to obtain information on the starting structures of the insoluble organic matter in ancient sediments, the so-called kerogen.

In connection with a former study (Klok *et al.*, 1983) an attempt is made here to characterize the total organic matter in a recent marine sediment. For this purpose we have investigated a Namibian Shelf diatomaceous ooze sample. This sediment is characterized by unique input parameters and the absence of terrestrial material (Boon, 1978). Because of the relatively young age and the shallow depth of the sediment it may be expected that at least part of the biopolymers in the remains of contributing organisms are still present. By means of a sequence of extractions with water and acid of increasing concentration, the intention was to release the maximum amount of the organic carbon present, with minimal chemical degradation. The organic carbon thus released is further analysed for the constituent monomeric building blocks of biopolymers e.g. amino acids, monosaccharides, volatile fatty acids and lipid components. The residual, insoluble organic matter is further investigated applying pyrolysis techniques.

In this way some insight is obtained, not only into the amount and mode of occurrence of the released moieties, but also into the primary diagenetic changes occurring in the sediment and/or in the predepositional stage of the sedimentary organic matter.

EXPERIMENTAL

Ooze samples from the Namibian Shelf were collected on a cruise during December 1968 - January 1969 (Eisma, 1969). The KD6 core ($22^{o}30'S$ $14^{o}05.7'E$), sampled underneath a water column of 106 m, was sectioned and stored at $-20^{o}C$ until use. The 40-75 cm section used for this study was homogenized and lyophilized prior to extraction as outlined in Fig. 1.

A 50.0 gram aliquot of the dry sample (R1) was treated ultrasonically with 400 ml cold water during 3×5 minutes and the mixture was subsequently allowed to heat under reflux for 69 hours. After cooling the suspension was centrifuged for 10 minutes (2700 g) and the supernatant (and several washings) were filtered over a G4 filter. The residue (R2) was lyophilized. An aliquot of the combined

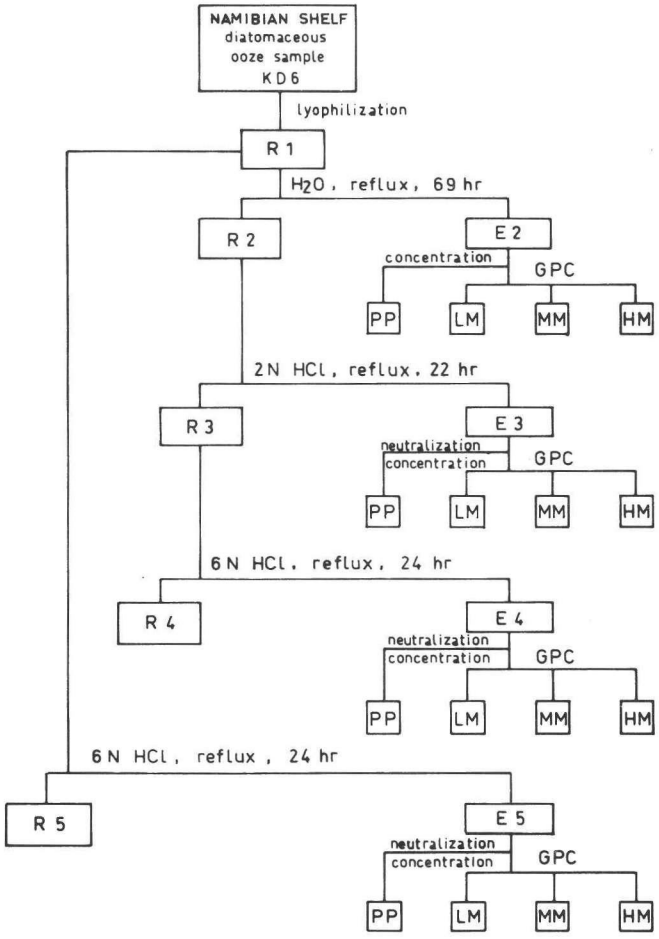

Fig. 1. Extraction scheme. For description of the fractions (PP, LM, etc.) see Experimental.

extracts and washings (E2) was dried in an oven (at 90 - 100°C) and subsequently *in vacuo* over KOH for a dry weight determination.

The lyophilized residue after water extraction (R2, 25.0 gram) was suspended in 400 ml 2N HCl and heated under reflux for 22 hours. Similarly, 10.0 gram of the dry starting material (R1) and residue R3 were each suspended in 100 ml 6N HCl and heated under reflux for 24 hours. About 50 mg of cellulose powder (MN 300 HR, Macherey, Nagel & Co.) was hydrolysed in a similar way. The various resulting hydrolyzates were treated as described above.

Aliquots of the extracts were dried for dry weight and chloride (Volhard titration) determinations. The pH of other aliquots was raised to 3 with sodium hydroxide before total lipid extraction.

The remaining portions of the extracts were stored at -20°C until use.

Gel permeation chromatography (GPC)

Aliquots of the extracts were lyophilized (if necessary after neutralization with ammonia to pH 7) and stored *in vacuo* over KOH. The dry extract was suspended in water. The insoluble fraction (defined precipitate = PP) was removed by centrifugation, washed with water, dried *in vacuo* over KOH and weighed. The soluble part of the extract including the precipitate washings was chromatographed over a Bio-gel P2 column (Biorad, 200-400 mesh, 17×2.4 cm) with water as eluent (flow rate: 1 ml/min). The refractive index of the eluate was recorded using a Waters-R401 RI-detector. Three fractions were collected representing high molecular weight material (HM, mol.w. > 2000 D), medium molecular weight material (MM, ∿400 < mol.w. < ∿2000 D) and low molecular weight material (LM, mol.w. < ∿400 D). Each fraction was analysed for organic carbon and its dry weight was determined.

Carbon measurements

Total carbon in residues and precipitates was determined using an automatic Perkin Elmer 240 CHN-analyser. Carbonate carbon was determined titrimetrically. Carbonate carbon and total carbon present in aqueous solutions were determined using a wet combustion apparatus operated at 150°C and 900°C respectively. The amount of organic carbon is calculated by subtraction.

Amino acids

Amino acids in lyophilized residues and in extracts were released upon hydrolysis with 6N HCl during 24 hours at 105°C under nitrogen in sealed ampoules. Nor-leucine was added before hydrolysis as an internal standard. The amino acids present in the hydrolyzates were analysed using a Kontron Liquimat III equipped with a Durrum Resin DC-4A and Picobuffer system 2 as eluent. Quantitation was performed after ninhydrin derivatization and detection at 570 nm.

Carbohydrates

Neutral monosaccharides were determined in the starting material (R1), residue R2 and extract E2. About 500 mg of each lyophilized residue and a lyophilized aliquot of about 50 ml of extract E2 were hydrolysed in 15 ml 0.5N H_2SO_4 during 18 hours at 100°C in sealed ampoules under nitrogen. Derivatization into the corresponding alditol acetates was performed as described earlier (Klok *et al.*, in press). Myo-inositol was added as an internal standard. Identification was based on GC retention data and on comparison of the mass spectra obtained by capillary GC-MS with those published by Jansson *et al.* (1976) and with mass spectra of synthesized reference compounds (Klok *et al.*, 1982).

Volatile fatty acids

Volatile fatty acids released from the starting material (R1) after saponification with 8N NaOH were analysed as their benzyl esters as will be described elsewhere (Klok *et al.*, in preparation).

Total lipids

The procedure for the determination of total lipids in residues is summarized briefly below. Details on the applied procedure are given elsewhere (de Leeuw *et al.*, in press; Klok *et al.*, submitted). The lipids obtained after saponification were esterified with diazomethane and silylated with Trisil-Z (Pierce). The resulting derivatives were subjected to column chromatography on Lipidex-5000 (Packard) for removal of very polar compounds. The eluent was concentrated and analysed by capillary GC and GC-MS.

Total lipids in extracts were extracted with dichloromethane and derivatized and analysed in a similar way.

Gas chromatography of the lipid fractions was performed using a Carlo Erba 4160 gas chromatograph equipped with an FID and an on-column injection system (Grob and Grob, 1978). A glass capillary column coated with SE52 (20 m, I.D. 0.32 mm) was used with helium as the carrier gas. Samples were injected at 125°C and the temperature was programmed at a rate of 4°C perminute to 310°C.

Gas chromatography-mass spectrometry was carried out on a Varian 3700 gas chromatograph connected to a Varian Mat 44 quadrupole mass spectrometer. Electron impact mass spectra were obtained at 80 eV.

Pyrolysis-mass spectrometry and pyrolysis-gas chromatography-mass spectrometry (Py-MS and Py-GC-MS)

The automated Curie-point Py-MS system used has been described elsewhere (Windig *et al.*, 1980). The instrument was operated using the following experimental conditions: sample size, 5-25 µg (in 0.1M phosphate buffer, pH 7); wire equilibrium temperature, 610°C; temperature rise time, 0.1 s; total heating time, 0.9 s; MS inlet temperature, 150°C; scan rate 0.1 s/scan (m/z 15-180); total scanning time, 10 s. Each sample was analysed in quadruplicate.

Py-GC-MS was carried out using a pyrolysis reactor as described by Meuzelaar *et al.* (1975), modified for use at high temperatures (van de Meent *et al.* 1980a). Gas chromatographic separation was performed using the following conditions: glass capillary column (CPsil5, 25 m, 0.3 mm I.D.); carrier gas, helium; temperature program, 0°C (5 min) - 3°C/min - 300°C (20 min); wire equilibrium temperature, 610°C; pyrolysis time, 10 s; mass spectrometer, Varian Mat 44 operated at 80 eV; cycle time, 1 s; m/z 25-500 up to scan 250 and m/z 50-500 after scan 250;m/z 28,32,40,44 were omitted in the reconstructed ion current (RIC).

RESULTS

Table 1 summarizes the data on dry weight and organic carbon of residues, total water and acid extracts and GPC fractions of extracts as mentioned in Fig. 1. The dry weight determinations are based on 50.0 g (≡ 100%) lyophilized starting material. Similarly the organic carbon measurements are based on 2320 mg (≡ 100%) organic carbon present in this amount of starting material.

Table 1. Results of mass balance and organic carbon balance determinations relative to R1[1]

	Mass balance (50.0 g ≡ 100.0%)					
sample	$\%W_{tot}$	$\%W_{PP}$	$\%W_{LM}$	$\%W_{MM}$	$\%W_{HM}$	$\%W_{rec}$[2]
R1	100.0	-	-	-	-	-
E2	17.3[3]	1.0	15.0	0.2	0.1	16.3
R2	81.8	-	-	-	-	-
E3	16.6[4]	4.8	10.2[4]	0.9	0.3	16.2
R3	66.4	-	-	-	-	-
E4	1.6	0.35	1.06	0.17	0.06	1.6
R4	66.2	-	-	-	-	-
E5	33.2[4]	3.4	28.2[4]	2.0	0.2	33.8
R5	67.2	-	-	-	-	-
	Organic C balance (2320 mg in R1 ≡ 100%)					
	$\%C_{tot}$	$\%C_{PP}$	$\%C_{LM}$	$\%C_{MM}$	$\%C_{HM}$	$\%C_{rec}$[2]
R1	100.0	-	-	-	-	-
E2	4.7	0.5	1.8	0.8	1.6	4.7
R2	91.8	-	-	-	-	-
E3	24.1	6.9	6.5	5.8	2.4	21.6
R3	60.8	-	-	-	-	-
E4	4.5	0.9	0.9	1.1	0.2	3.1
R4	54.4	-	-	-	-	-
E5	32.5	6.6	10.3	10.8	0.8	28.5
R5	60.0	-	-	-	-	-

[1] R1 : organic carbon, 4.64% ; carbonate carbon, 1.12%
[2] $\%W_{rec} = \%W_{PP} + \%W_{LM} + \%W_{MM} + \%W_{HM}$ (similarly $\%C_{rec}$)
[3] indigenous chloride content , 7.6% (based on dry weight R1)
[4] percentages adjusted for contribution of chloride due to HCl treatment

The dry weight- and organic carbon balances are seen in Fig. 2.

The molar distribution and the contribution to the organic carbon of the individual amino acids are summarized in Table 2. Fig. 3 represents these data obtained after 6N HCl treatment of the starting material (E5). Fig. 4 shows the gas chromatogram of the neutral carbohydrates present in the water extract (E2), analysed as alditol acetates. Identifications of major peaks are given in the figure caption. Table 3 summarizes the quantitative results of the analysis of neutral monosaccharides in the starting material (R1), the residue after water extraction (R2) and in the water extract (E2). FID respons factors of the individual alditol acetate derivatives have been assumed equal on a

weight basis, compared to myo-inositol hexaacetate (internal standard).

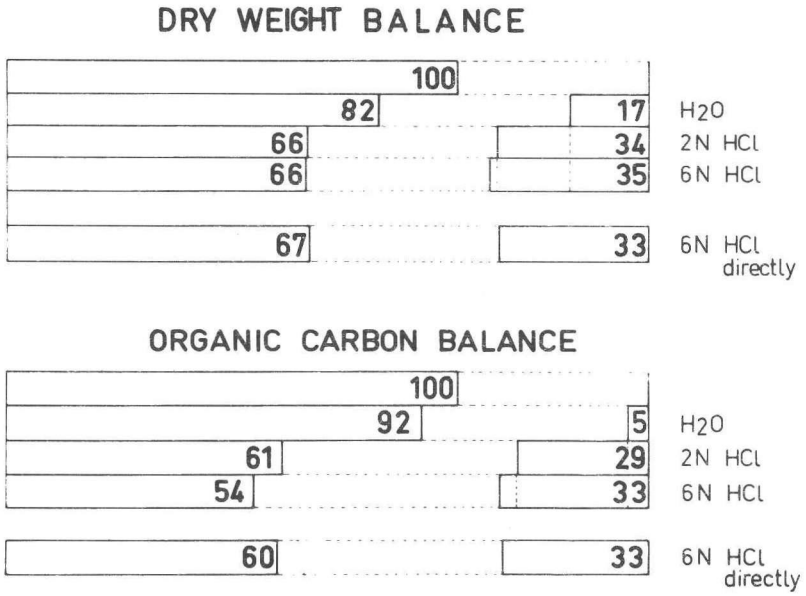

Fig. 2. Dry weight and organic carbon balances. All percentages refer to the total amount of dry starting material (50.0 g R1 ≡ 100%) and total amount of organic carbon (2320 mg ≡ 100%) present therein (Table 1).

Among the individual chromatograms of the derivatized total lipids extracted both from water- and acid extracts and from the residues mentioned in Fig. 1, three types could be distinguished based on the distribution of the observed compounds. From each type a typical example is shown in Fig. 5 : total lipids present in the extracts (E3 shown), in the starting material and non-acid treated residues (R1 shown) and in the acid treated residues (R5 shown) respectively. Quantitation of the lipid components observed in the chromatograms is based on peak area integration. For convenience the FID responses of the individual components are assumed equal on a weight basis. Absolute calibration of the GC apparatus was achieved with palmitic acid methyl ester. The total amount of extractable lipid carbon was calculated to be 4.2% of the total organic carbon present in the starting material (R1). Short chain fatty acids (C_1-C_4) analysed as their benzyl esters comprise 0.7% of the total organic carbon (Klok *et al.*, in preparation).

The Py-MS data obtained from residues and fractions of extracts are given in Table 4. Some typical pyrolysis mass spectra are shown in Fig. 6. The reconstructed total ion current (RIC) of the Py-GC-MS analysis of residue R5 is shown in Fig. 7. Identifications are listed in Table 5 (scan numbers correspond to the numbers in Fig. 7).

DISCUSSION

Bulk parameters

The various treatments of a lyophilized sample of the Namibian Shelf sediment, as depicted in Fig. 1, resulted in the solubilization of one third of the starting material (Table 1, Fig. 2). About 15% of the dry starting material is present as salts (mainly chlorides) and is recovered in the low molecular weight fraction of the water extract (E2, LM). During the successive extractions about 46% of the organic carbon (including volatile compounds) originally present in the starting material (R1) was released (Table 1, Fig.2). Similarly the direct acid treatment with 6N HCl resulted in a release of about 40% (including volatile compounds). The discrepancy between the amount of organic carbon retained in the residues R4 and R5 might be ascribed to the occurrence of condensation reactions, which are more severe during the direct acid treatment.

About 12% and 8% of the organic carbon was not recovered after the successive extractions and after the direct acid treatment respectively. These losses are explained by the fact that very volatile compounds, such as short chain aliphatics and organo sulphur compounds, are not recovered during the various procedures. In a separate study (Klok *et al.*, in preparation) it is shown that the free and bound volatile acids (C_1-C_4) present in this sediment sample, account for 0.7% of the organic carbon present in the starting material. The amount of organic carbon not recovered will be further denoted as "volatile".

Quantitative and qualitative data on individual component classes

Amino acids

From Table 2 it is clear that a substantial part of the organic carbon that solubilizes during the successive extractions and similarly during the direct acid hydrolysis consists of proteinaceous carbon. In both ways about 11% of the total amount of organic carbon present in the starting material was identified as amino acid carbon.

Fig. 3 illustrates both the contributions of the individual amino acids to the organic carbon and the relative composition of amino acid units analysed in the direct 6N HCl hydrolyzate of the starting material (extract E5). The contribution to the organic carbon of the amino acids leucine and phenylalanine exceeds 1%, which makes them the most abundant building blocks that could be released from the organic matter present in this sample.

All amino acids encountered in this study, except ornithine, are regarded as proteinaceous amino acids. Ornithine is thought to originate from ornithine

Table 2. Amino acids quantitated in the extracts after hydrolysis in 6N HCl

amino acid	molar distribution (mole %) E2	E3	E4	E5	% amino acid C of organic C E2	E3	E4	E5
cys	1.7	0.6	0.6	0.5	0.001	0.03	0.01	0.03
asp	12.6	12.4	5.6	10.2	0.009	0.85	0.13	0.93
thr	5.2	6.0	4.8	5.8	0.004	0.41	0.11	0.53
ser	6.5	5.9	4.7	5.8	0.004	0.31	0.08	0.40
glu	9.0	8.4	5.9	7.3	0.008	0.72	0.17	0.84
pro	4.2	4.6	3.9	4.1	0.004	0.40	0.11	0.46
gly	19.7	16.8	10.2	13.6	0.007	0.58	0.12	0.62
ala	11.2	10.7	10.3	10.2	0.006	0.55	0.08	0.70
val	5.6	5.8	8.7	6.5	0.005	0.50	0.25	0.75
met	0.5	0.6	1.3	1.6	0.000	0.06	0.04	0.18
ile	5.9	6.5	8.9	7.1	0.006	0.67	0.30	0.98
leu	7.2	7.5	13.6	9.0	0.008	0.77	0.46	1.24
tyr	2.5	1.6	3.1	2.8	0.004	0.25	0.16	0.59
phe	2.8	4.3	9.2	5.3	0.005	0.66	0.17	1.09
orn	0.5	0.7	0.8	0.8	0.001	0.06	0.02	0.10
lys	1.9	3.3	4.9	4.9	0.002	0.34	0.17	0.67
his	0.7	1.2	1.2	1.4	0.001	0.13	0.04	0.20
arg	1.1	3.1	2.4	3.1	0.001	0.32	0.08	0.42
total (%)	98.8	100.0	100.1	100.0	0.08	7.6	2.9	10.7
total µmole	35	3331	1090	4417				

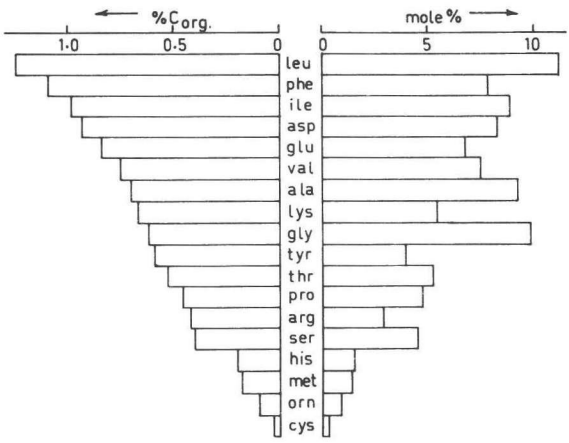

Fig. 3. Amino acids analysed in extract E5 expressed as percentage of the total organic carbon present in the starting material and of the molar distribution of the individual components (Table 2).

lipids occurring in bacteria (Lechevalier, 1977). These lipids are also characterized by the presence of amide linked β-hydroxy fatty acids. The significant presence of the β-hydroxy fatty acids among the lipids analysed in the residues after acid treatment (see below) may indicate that ornithine-containing lipids are the source of the observed ornithine. The extent in which the high concentration of other amino acids (e.g. glycine and alanine) might be interpreted as contributions from a non-proteinaceous source is not clear.

Carbohydrates

Fig. 4 shows the gas chromatogram of the alditol acetates obtained from the water extract (E2) after hydrolysis and derivatization. Apart from the well known, major components a large number of less common, minor components are observed.

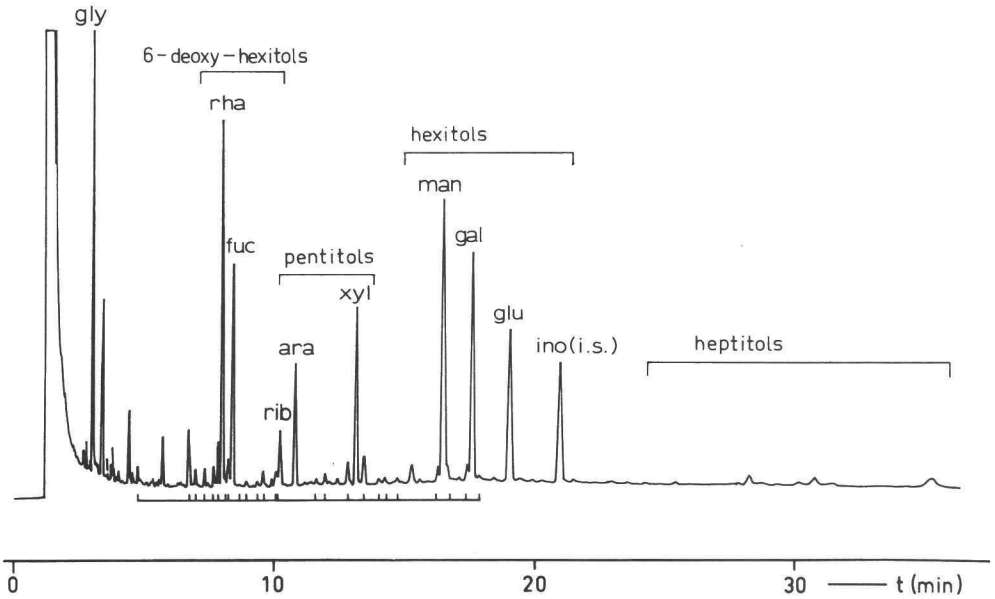

Fig. 4. Gas chromatogram of the alditol acetates obtained from extract E2. Identifications: gly = glycerol, rha = rhamnitol, fuc = fucitol, rib = ribitol, ara = arabitol, xyl = xylitol, man = mannitol, gal = galactitol, glu = glucitol and ino = myo-inositol (internal standard). The connected vertical lines in the figure indicate the observed partially methylated alditols.

Table 3. Relative distribution of neutral monosaccharides in starting material (R1), residue R2 and extract E2 released upon hydrolysis with 0.5N H_2SO_4.

monosaccharide[1]	R1	R2	E2
rhamnose	12.7	12.5	12.8
fucose	7.3	7.4	8.1
ribose	3.9	4.6	2.4
arabinose	5.1	5.9	5.0
xylose	9.0	8.2	7.8
mannose	14.1	13.0	17.2
galactose	16.6	15.9	14.9
glucose	13.0	12.0	10.6
others	18.3	20.5	21.3
total monosaccharides (mg)	236	176	24
% monosaccharide C of organic C	4.1	3.1	0.4

[1] analysed as alditol acetates (conditions see Experimental)

Since the results of the quantitative determination of monosaccharide

building blocks in complex carbohydrates are influenced by both the efficiency of the hydrolysis and the derivatization procedure (Dutton, 1973; Mopper,1977; Albersheim *et al.*, 1967; Torello *et al.*, 1980) the amount of carbohydrate carbon determined (Table 3), comprising more than 4% of the total organic carbon present in the starting material (R1), is interpreted as a minimum value.

Table 3 also reveals the similarity in the relative composition of the hydrolyzable monosaccharides obtained from the starting material (R1), the residue after water extraction (R2) and from the water extract (E2). Obviously a representative part (about 10%, Table 3) of the hydrolyzable carbohydrates was solubilized by the water extraction procedure. The hydrolyzate of the high molecular weight fraction of this water extract (E2, HM) obtained by GPC revealed the presence of neutral monosaccharides with a distribution pattern similar to that obtained from a hydrolyzate of the total extract (E2). The carbohydrate nature of the high molecular weight fraction was also confirmed by Py-MS data as will be discussed below.

The major monosaccharides encountered during this study are omnipresent constituents of natural carbohydrates, while the less common, predominantly partially methylated- and deoxy monosaccharides and heptoses (Klok *et al.*, in press) are mainly reported to occur in carbohydrates associated with bacterial cell walls, such as lipopolysaccharides (Weckesser *et al.*, 1979). These lipopolysaccharides are also characterized by the presence of amide linked β-hydroxy fatty acids (Weckesser *et al.*, 1979). The abundance of β-hydroxy fatty acids among the lipids extractable from the residues after acid treatment, as will be discussed below, supports a common origin of β-hydroxy fatty acids and the minor monosaccharides from lipopolysaccharides. We attempted, therefore, to isolate from the sediment sample lipopolysaccharides possibly present (Klok *et al.*, submitted). A high molecular weight substance was indeed obtained, which upon hydrolysis produced monosaccharides and also fatty- and β-hydroxy fatty acids typical of lipopolysaccharides. Taking this into account the observed methylated monosaccharides probably are good indicators for the presence of structures associated with bacterial cell walls in the sediment sample.

Lipids

The gas chromatograms of the total lipid mixtures obtained from both the water- and acid extracts and the residues mentioned in Fig. 1, can be devided into three types based upon the distribution of the observed compounds. Fig. 5 shows the gas chromatograms of typical representatives of each type: the extracts E2 - E5 (E3 shown), the starting material (R1) and residue R2 (R1 shown) and the residues after acid treatment R3 - R5 (R5 shown). The contribution of the extractable lipid carbon to the total organic carbon present in

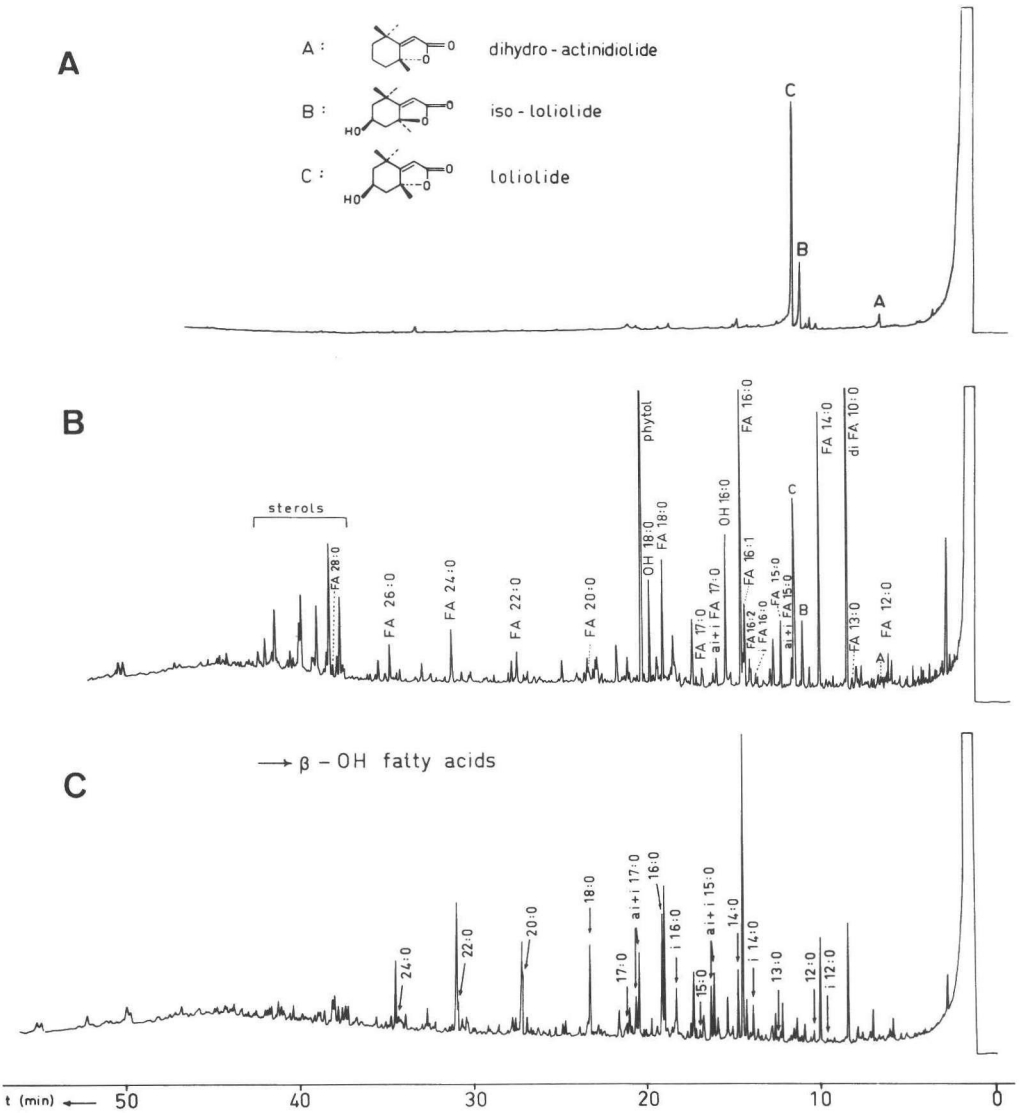

Fig. 5. Total lipid derivatives obtained from A) extract E3, B) starting material and C) the ultimate residue R5. For conditions: see Experimental.

the starting material is estimated to be 4%.

A brief review of the lipid compounds encountered in the various chromatograms is given below. Detailed data on these lipids will be given elsewhere (Klok *et al.*, submitted).

The middle trace shown in Fig. 5 shows the distribution pattern of lipid classes and individual lipids extracted from the starting material. As in other recent marine sediments acids, alcohols (especially phytol) and sterols are the major lipid classes obtained. The lower chromatogram of lipids obtained from the residue after direct 6 N HCl hydrolysis is characterized by the

abundance of fatty acids and β-hydroxy fatty acids (the latter marked with arrows). The β-hydroxy fatty acids are released from the sample, without solubilization, during the acid treatment and can be explained by the presence of amide linked hydroxy fatty acids. They are known to occur as such in bacterial lipopolysaccharides and ornithine containing lipids as already mentioned before. Their abundance points to a considerable bacterial contribution to the extractable lipid carbon present in this sample.

The upper trace is dominated by the presence of three compounds with a distinct structural relationship (molecular structures depicted in the chromatogram). Component A was identified as dihydroactinidiolide. The other and more abundant compounds were identified as isololiolide and loliolide (compounds B and C respectively). The natural occurrence of these terpenoid compounds is mainly reported in plant material (Marx and Sondheimer, 1966; Pailer and Haschke-Hofmeister, 1969; Holub *et al.*, 1975; Kodama *et al.*, 1982) but also as a sex pheromone of the red fox *Vulpes vulpes* (Albone, 1975), in molluscs (Petitt *et al.*, 1980) and brown algae (Ravi *et al.*, 1982). These compounds are also reported to be generated during photo-oxidation of carotenoids (Isoe *et al.*, 1969; Isoe *et al.*, 1971; Isoe *et al.*, 1972). Since fucoxanthin is the major carotenoid to be expected in the Namibian Shelf upwelling area, because it is the predominant carotenoid in *Bacillariophyceae* and *Phaeophyceae* (Johansen *et al.*, 1974; Goodwin, 1980; Repeta and Gagosian, 1983) it is suggested that the loliolides are products of the photo-oxidation of this carotenoid (Klok *et al.*, in preparation).

Pyrolysis characteristics of the unidentified organic carbon

Using the analytical procedures described above only a part (∿20%) of the total organic carbon present in the starting material (R1) could be identified as amino acid, monosaccharide, volatile fatty acid or lipid carbon. We therefore applied pyrolysis mass-spectrometry (Py-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) to investigate the structures of the as yet unidentified organic constituents present in residues and extracts. For this purpose the residues (R), the precipitates (PP), the medium (MM) and the high molecular (HM) weight fractions were analysed by Py-MS (Table 4, Fig. 6). The starting material (R1) and residue R5 were also studied by Py-GC-MS (Fig. 7, Table 5).

Py-MS data of the residues as summarized in Table 4 and seen in Fig. 6 reveal the abundance of hydrochloric acid (m/z 36, 38) as a result of indigenous sea salt and the hydrochloric acid used. Similarly, specific ions of sulphur compounds are relatively abundant (m/z: 34, 48, 64, 66, 76). Elementary sulphur (m/z 64, 96, 128, 160) present in the starting material (R1) was partly

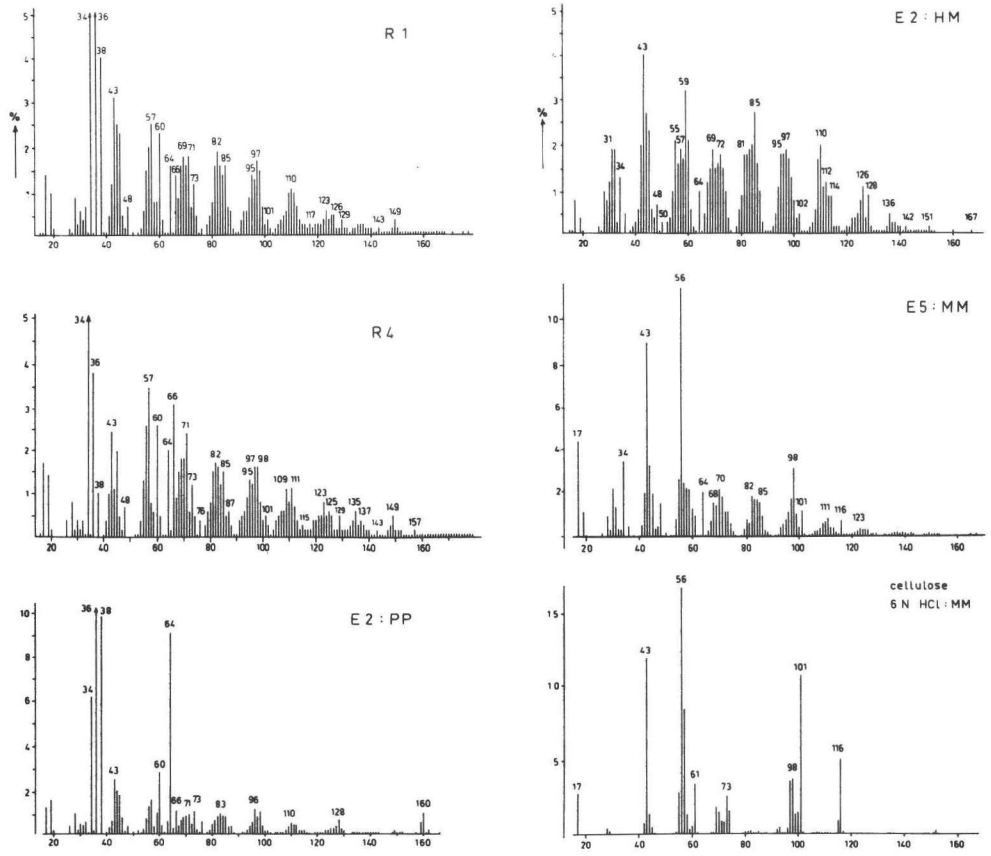

Fig. 6. Pyrolysis mass spectra of some of the residues and fractions of extracts obtained after extraction of a lyophilized Namibian Shelf sediment sample and of the MM fraction of cellulose treated with 6N HCl. For conditions: see Experimental.

Table 4. Py-MS data obtained from residues and fractions of extracts as mentioned in Fig. 1. For conditions: see Experimental.

	Residues					Precipitates				MM fractions				HM fractions				cellulose
characteristics[1]	R1	R2	R3	R4	R5	E2	E3	E4	E5	E2	E3	E4	E5	E2	E3	E4	E5	fractions
chlorides (HCl)	+++	+++	++	+	++	+++	+++	++	++	+		+						
sulphur compounds	++	++	++	++	++	+++	+	+	++	+	+	++	+	+	+	+	++	
proteins	+	+																
carbohydrates	+	+								++				+++				
amino carbohydr.														++				
"levulinic acid"							+	+	++		++	++	++		++	++	++	+++
alkanes	++	++	++	++	++	+	+	+	+	+			+	+				
alkenes	+	+	+	+	+					+			+	+				
fatty acids	+	+	+	+	+	+	+	+	+						+	+	+	

[1] characteristic m/z values : HCl : 36,38 ; sulphur compounds : 34,48,64,66,76 ; proteins : 67,69,83, 92,94,108,117,131 ; carbohydrates : 43,68,82,96,98,110,112,114,126,128 ; amino carbohydrates : 59, 109,125,137,151 ; "levulinic acid" : 43,56,98,101,116 ; alkanes : 57,71,85,99,... ; alkenes : 55,69, 83,97,... ; fatty acids : 60,73,87,101,115,129,... (after Meuzelaar *et al.*, 1982)

+ arbitrary units (+ = present , ++ = abundant , +++ = predominant)

solubilized during refluxing with water and recovered in the precipitate of the water extract (E2: PP , Fig. 6). Water extraction and acid-mediated depolymerization of carbohydrate and proteinaceous structures resulted in the release of these component classes from the sample. The absence of specific carbohydrate and protein derived pyrolysis products (van de Meent *et al.*, 1982; Meuzelaar *et al.*, 1982) in the pyrolysis mass spectra of the ultimate residues (Fig. 6, Table 4) clearly indicates this process. Similarly comparison of the Py-GC-MS analyses of starting material (R1) and residue (R5) reveals the disappearance of specific carbohydrate and protein derived pyrolysis products (van de Meent *et al.*,1983) after acid treatment. About 10% of the hydrolyzable carbohydrates present in the starting material were solubilized during water extraction (Table 3). The high molecular weight nature of the released carbohydrates is demonstrated by the predominance of m/z values indicative for carbohydrates (Meuzelaar *et al.*, 1982) observed in the pyrolysis mass spectrum of the high molecular weight fraction of the water extract (E2: HM, Fig. 6). Moreover, this spectrum indicates the presence of amino sugar constituents (Table 4), which are not included in the carbohydrate component analysis discussed before.

The pyrolysis data from the residues demonstrate that the aliphatic nature of the organic matter present therein is consolidated after each extraction step (Fig. 6, Table 4), resulting in a pronounced aliphatic profile in the Py-GC-MS trace of residue R5 (Fig. 7, Table 5). Moreover, the isoprenoid nature of a number of the observed aliphatics is shown.

Apart from the observed aliphatics a number of aromatic and heterocyclic compounds were generated upon pyrolysis (Fig. 7 , Table 5). The alkyl-substituted thiophenes may indicate the presence of organo-sulphur compounds in the sample. Similarly the alkylsubstituted pyrroles indicate the presence of organic nitrogen. The latter compounds have been reported to be generated pyrolytically from tetrapyrroles (Whitten *et al.*, 1966). However, flash pyrolysis studies in our laboratory on pure porphyrins (unpublished data) and chlorophylls (van de Meent *et al.*, 1980b) do not confirm these results.

The mainly aliphatic (partly isoprenoid) nature of the insoluble organic matter present in this Namibian Shelf sediment sample and the observed aromatic and heterocyclic compounds during pyrolysis studies, indicate that the organic matter present in the ultimate residue is to some extent similar to kerogens obtained from ancient sediments when analysed with these pyrolysis techniques (van de Meent *et al.*, 1980a). Whether this similarity is indigenous

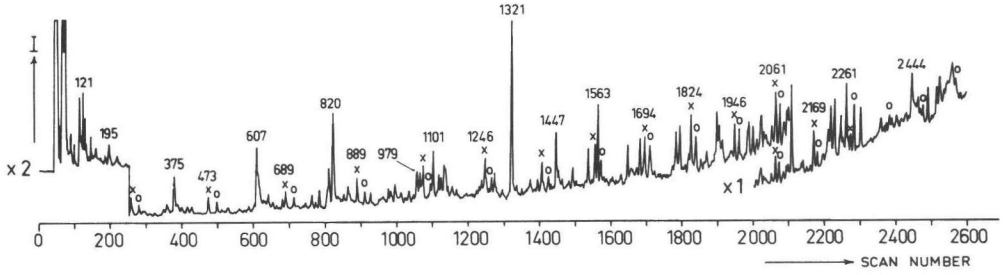

Fig. 7. Reconstructed total ion current of the Py-GC-MS trace of residue R5. Identifications are given in Table 5. Peaks marked "x" and "o" are n-alk-1-enes and n-alkanes respectively. For conditions: see Experimental.

Table 5. Identifications of pyrolysis products observed during (Cu)Py-GC-MS analysis of residue R5 as shown in Fig. 7.

scan	RRT[1]	identification	scan	RRT[1]	identification	scan	RRT[1]	identification
250	-	short chain aliphatics up to C_5 , H_2S, methanethiol	1010	954	$C_{10}H_{20}$ + C_3-thiophene	1782	1457	unknown alkene
			1013	956	C_3-benzene	1793	1466	isopr. $C_{16}H_{34}$
257	-	n-hex-1-ene	1028	964	C_3-benzene	1808	1478	methylketone
279	600	n-hexane	1034	967	C_3-thiophene	1818	1485	C_2-naphthalene
290	605	C_6H_{10}	1046	974	β-pyronene[2]	1824	1490	n-pentadec-1-ene
295	607	C_6H_{12}	1055	979	C_3-benzene	1837	1500	n-pentadecane
323	620	methylcyclopentane	1064	984	terp. $C_{10}H_{16}$	1897	1550	C_8-benzene[2]
376	644	benzene	1074	989	n-dec-1-ene	1903	1555	phthalate
387	649	thiophene	1094	1000	n-decane	1912	1562	C_9-thiophene[2]
391	651	cyclohexane	1098	1002	C_4-thiophene[2]	1946	1590	n-hexadec-1-ene
399	655	C_6H_8	1101	1004	C_3-benzene	1957	1600	n-hexadecane
413	661	C_6H_{10}	1113	1011	C_4-benzene[2]	1982	1621	6-phenylundecane
426	667	cyclohexene	1117	1013	$C_{10}H_{22}$[2]	1986	1625	5-phenylundecane
473	689	n-hept-1-ene	1122	1016	terp. $C_{10}H_{18}$	1997	1635	4-phenylundecane
498	700	n-heptane	1125	1018	C_3-pyrrole	2021	1656	3-phenylundecane
607	751	toluene	1132	1022	C_3-pyrrole	2050	1681	methylketone
613	753	methylthiophene	1133	1023	indene	2061	1691	n-heptadec-1-ene
628	760	C_7H_{12}	1155	1036	C_4-thiophene[2]	2062	1692	2-phenylundecane
632	762	methylthiophene	1167	1043	C_4-thiophene[2] + C_4-benzene	2071	1700	n-heptadecane
641	767	C_8H_{18}				2084	1712	pristane
654	773	C_8H_{16}	1173	1046	C_4-thiophene[2]	2092	1719	6-phenyldodecane
664	776	C_8H_{16}	1222	1075	C_4-benzene	2097	1724	5-phenyldodecane
684	785	C_8H_{16}	1237	1084	C_4-pyrrole	2106	1732	pristene
689	789	n-oct-1-ene	1246	1089	n-undec-1-ene	2111	1737	4-phenyldodecane
713	800	n-octane	1265	1100	n-undecane	2133	1757	3-phenyldodecane
754	816	C_8H_{14}[2]	1273	1105	C_4-pyrrole	2138	1761	$C_{19}H_{36}$[2]
763	825	terp. C_9H_{14}	1321	1135	C_4-benzene	2144	1767	1-phenylundecane
773	830	C_9H_{20}	1336	1145	C_5-thiophene[2]	2160	1782	methylketone
784	836	terp. C_9H_{14}	1374	1169	C_5-pyrrole	2169	1790	n-octadec-1-ene
805	846	ethylbenzene	1406	1189	n-dodec-1-ene	2174	1794	2-phenyldodecane
809	848	C_2-thiophene	1423	1200	n-dodecane	2180	1800	n-octadecane
810	849	C_9H_{18}	1426	1202	C_5-benzene	2211	1830	methylketone
819	854	C_2-thiophene	1447	1216	branched $C_{13}H_{28}$	2217	1836	phytene
820	854	m-/p-xylene	1452	1220	branched $C_{13}H_{26}$	2228	1847	phytene
835	862	C_2-thiophene	1481	1239	C_6-thiophene[2]	2253	1871	1-phenyldodecane
851	870	C_2-thiophene	1493	1247	C_2-indene	2261	1879	phytadiene
863	876	o-xylene	1494	1248	C_6-thiophene[2]	2265	1883	methylketone
868	879	C_9H_{16}	1536	1276	isopr. $C_{14}H_{30}$	2272	1889	n-nonadec-1-ene
889	889	n-non-1-ene	1555	1289	n-tridec-1-ene	2283	1900	n-nonadecane + phytadiene
911	900	n-nonane	1563	1295	isopr. $C_{14}H_{28}$			
926	908	C_9H_{18}	1571	1300	n-tridecane	2301	1918	phthalate
976	936	C_9H_{20}[2]	1628	1342	C_7-thiophene[2]	2358	1976	ethylketone
982	939	C_3-thiophene	1647	1355	unknown alkene	2382	2000	n-eicosane
983	939	n-propylbenzene	1680	1380	isopr. $C_{15}H_{32}$	2475	2100	n-heneicosane
995	946	C_3-thiophene	1694	1390	n-tetradec-1-ene	2485	2111	C_{16}-thiophene[3]
998	948	C_3-benzene	1708	1400	n-tetradecane + C_7-benzene[2]	2513	2142	C_{16}-thiophene[3]
1000	949	C_3-benzene				2565	2200	n-docosane

[1] RRT = relative retention time calculated by linear interpolation of the scan numbers corresponding with n-alkanes
[2] tentative identification
[3] tentative identification, mass spectrum described by Van Graas (1982)

or promoted by the artificial leaching and the acid treatment of the sample is not clear. The similar distribution patterns of alkenes/alkanes and also of alkylsubstituted thiophenes in both starting material (R1) and in the ultimate residue, indicate that this is not the case for the precursors of these pyrolysis products. However, among the aromatic compounds marked differences are observed, indicating an increase of the contribution of aromatic structures relative to the alkene/alkane fraction in the pyrolysis gas chromatogram after acid treatment, probably indicative for the occurrence of chemical transformations due to the acid treatment. The observed C_{11}- and C_{12}-alkylbenzenes (Fig. 7, Table 5) are thought to originate from contamination (Ishiwatari *et al.*, 1983).

The pyrolysis mass spectra of all fractions of the acid extracts are characterized by the abundance of fragments with m/z : 43,56,98,101 and 116 (Fig. 6, Table 4). These m/z values correspond with those observed in the 80 eV mass spectrum of 4-oxo-pentanoic acid (levulinic acid). Levulinic acid is derived from monosaccharides by an acid catalyzed reaction in aqueous solution upon heating (Feather and Harris, 1973). Moreover Anderson and Russell (1976) observed significant amounts of levulinic acid in the hydrolyzate (6N HCl) of the fulvic acid fraction (partly consisting of polysaccharides) of Humus Podzol Bh. These findings support the suggestion that the m/z values mentioned above correspond to pyrolysis products originating from a carbohydrate precursor. In order to verify this hypothesis, cellulose was hydrolyzed with 6N HCl and the various molecular weight fractions of the hydrolyzate were analysed by Py-MS (Table 4). One of the typical pyrolysis mass spectra obtained is shown in Fig. 6. The similarities between the spectra of the various fractions of acid treated cellulose and sediment samples point to a carbohydrate origin for a major part of the released and unidentified organic carbon. Carbohydrate component analysis already revealed that about 4% of the organic carbon present in the sediment sample could be identified as carbohydrate carbon (Table 3). When the amount of unidentified released organic carbon is ascribed to carbohydrates the total amount of carbohydrate carbon, relative to the total organic carbon, exceeds 20%. This consideration necessarily implies that as a result of the mild acid hydrolysis, preceeding the carbohydrate component analysis, only a limited part of the carbohydrate carbon has become identifiable. This may be caused by the fact that a great part of the sedimentary carbohydrates have been (slightly) transformed making them unrecognizable in component analysis.

CONCLUSION

As a result of the extraction procedure outlined in Fig. 1 a large part of the organic carbon present in a Namibian Shelf diatomaceous ooze sample is released. Part of this releasable organic carbon is not recovered and is therefore ascribed to volatile organic compounds. The organic carbon recovered from the extracts and ultimate residues was identified partly as amino acid , neutral monosaccharide, volatile fatty acid or lipid carbon (Fig. 8).

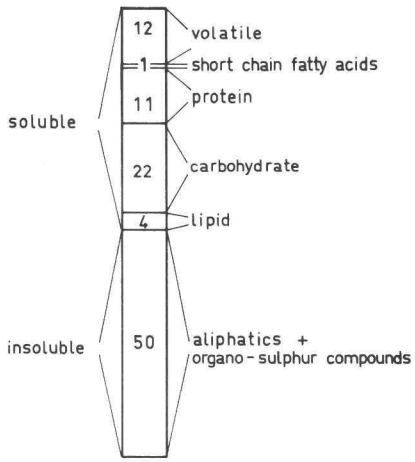

Fig. 8. Relative composition of the total organic carbon present in the Namibian Shelf diatomaceous ooze sample (KD6).

Some new lipid compounds were encountered in the water and acid extracts. Among the released compounds which were identified, a clear contribution of both bacterial and algal origin is distinguishable. The remaining organic carbon present in the extracts (Fig. 1) is mainly attributed to slightly transformed carbohydrates, which were unrecognizable in carbohydrate component analysis.

The residual organic carbon, about 50% of the organic carbon originally present, mainly shows aliphatic characteristics. Aromatic hydrocarbons (partly induced by the acid treatment) and alkylated thiophenes and pyrroles are also observed upon pyrolysis. The aliphatic (partly isoprenoid) nature of the residual organic matter indicates that the ultimate organic matter is to some extent similar to kerogens of ancient sediments.

ACKNOWLEDGEMENT

The pyrolysis mass spectrometry, associated computer assisted data analysis and its interpretation (project nr. 1983-B3 and -B4) were carried out by the research group on flash pyrolysis of macromolecular systems at the FOM-Institute for Atomic and Molecular Physics in Amsterdam.
We thank Dr. J.J. Boon (FOM-Institute) for his cooperation and for many helpful discussions.

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CHAPTER 3

CAPILLARY GAS CHROMATOGRAPHIC SEPARATION OF MONOSACCHARIDES AS THEIR ALDITOL ACETATES*

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ABSTRACT

During organic geochemical research dealing with the occurrence and composition of polysaccharides in recent marine sediments, a gas-liquid chromatographic (GLC) separation is required by which mixtures of monosaccharide derivatives can be baseline separated.

To avoid complex mixtures of anomeric monosaccharides, the hydrolytically released monosaccharides can be reduced to the corresponding alditols. The alditol mixture is subsequently derivatized into the alditol acetates. The GLC separation of alditol acetates on columns packed with OV-275[1] and ECNSS-M[2-5] has been reported. In the latter case the column is usually operated under conditions close to the maximum operating temperature, which limits column life[6]. Moreover, a baseline separation of some of the alditols used in these studies is not achieved. Holzer *et al.*[7] applied a glass capillary column coated with a chiral phase. Their results show that the separation of rhamnitol/fucitol and

*Published in *Journal of Chromatography*, 207 (1981) 273-275.

ribitol/arabitol is not complete. This report describes the baseline separation of ten alditol acetates using a glass capillary column coated with OV-275.

EXPERIMENTAL

The ten alditols used as standards in this study are listed in Table I. They are commercially available from various companies. The standard mixture of the alditol acetates was prepared by acetylation of a mixture of the alditols, containing equal amounts (by weight) of the individual alditols. The acetylation was performed in a sealed vial with pyridine-acetic anhydride (1:1) at 100°C during 2 h. After evaporation of the acetylation reagent the alditol acetate mixture was dissolved in dichloromethane.

The natural mixture of monosaccharides was obtained from a diatomaceous ooze sample from the Namibian Shelf (S.W. Africa, 22°51.5' S, 14°14.5' E)[8]. The sample was lyophilized and hydrolysed with 1 *M* H_2SO_4 during 3 h at 100°C. The hydrolysate was neutralized with $BaCO_3$ and reduced with $NaBH_4$. Subsequent acetylation was performed as described above.

GLC was carried out on a Varian 3700 gas chromatograph equipped with a glass capillary column (25 m × 0.25 mm I.D.) coated with OV-275 (Chrompack, Middelburg, The Netherlands). The temperature was programmed from 190 to 215°C at 1°C/min. Further GLC conditions: injector, 250°C; flame ionization detector, 250°C; carrier gas, helium at a flow-rate *ca.* 1.5 ml/min; helium pressure, 18 p.s.i.; splitter, 30 ml/min; attenuator, $1 \cdot 10^{-11}$ mA.

Identification of the acetates was based on the retention times of the individual alditol acetates.

RESULTS AND DISCUSSION

Fig. 1 shows the gas chromatogram of the standard alditol acetate mixture. The peak numbers correspond to the alditols listed in Table I. All components are baseline separated, thus allowing a complete qualitative and quantitative

Table I. The alditols used as standards in this study

Alditol	No.	Alditol	No.
Erythritol	1	Xylitol	6
Rhamnitol	2	Mannitol	7
Fucitol	3	Galactitol	8
Ribitol	4	Sorbitol	9
Arabitol	5	*m*-Inositol	10

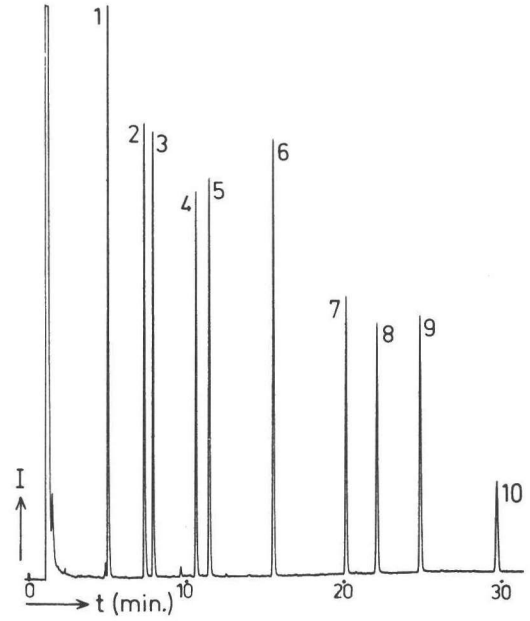

Fig. 1. Gas chromatogram of a standard mixture of ten alditol acetates. The peak numbers correspond to the alditols listed in Table I.

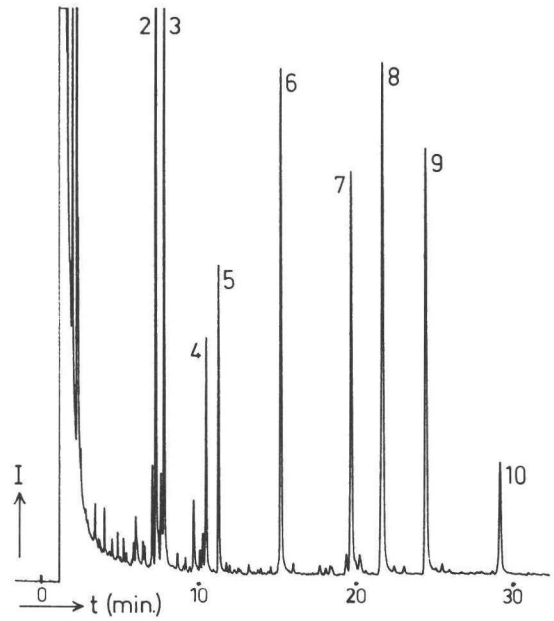

Fig. 2. Gas chromatogram of the alditol acetates obtained from a recent sediment. m-Inositol (10) was added as an internal standard. The peak numbers correspond to the alditols listed in Table I.

analysis of monosaccharides as their alditol acetates.

Fig. 2 shows the gas chromatogram of the mixture obtained from the diatomaceous ooze sediment after hydrolysis, reduction and derivatization. *m*-Inositol was added as an internal standard. The relative retention times of the main peaks correspond exactly to those of the alditol acetates in Fig. 1. Ultimate identification of both major and minor peaks has to be achieved by GLC-mass spectrometry.

The abundance of rhamnose and fucose in the ooze sample is not unexpected since these monosaccharides are major building blocks of algal polysaccharides[9-12].

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CHAPTER 4

ANALYSIS OF SYNTHETIC MIXTURES OF PARTIALLY METHYLATED ALDITOL ACETATES BY CAPILLARY GAS CHROMATOGRAPHY, GAS CHROMATOGRAPHY-ELECTRON IMPACT MASS SPECTROMETRY AND GAS CHROMATOGRAPHY-CHEMICAL IONIZATION MASS SPECTROMETRY*

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SUMMARY

The identification of naturally methylated neutral monosaccharides in acid hydrolysates as their alditol acetates requires appropriate standards. The availability of such standards also facilitates the analysis of complex mixtures of partially methylated alditol acetates (PMAAs) which appear upon methylation analysis of polysaccharides.

For this purpose the alditols of eight common monosaccharides have been partially methylated using the Haworth methylation. The resulting mixtures of partially methylated alditols have been acetylated and analysed by capillary gas chromatography, gas chromatography-electron impact mass spectrometry and gas chromatography-chemical ionization mass spectrometry.

Identification of the obtained PMAAs is further elaborated by reduction of the aldoses with sodium borodeuteride and the use of partially methylated aldoses or disaccharides.

*Published in *Journal of Chromatography*, 253 (1982) 55-64.

INTRODUCTION

Until recently very few naturally occurring methylated sugars had been reported. Over the past few years they have been more frequently encountered as building blocks of, for example, polysaccharides of bacteria[1,2], cyanobacteria[3] and coccoliths[4] and of lipopolysaccharides of bacteria[5] and cyanobacteria[6,7].

In the geochemical literature partially methylated monosaccharides have only been reported to occur in soils[8] and in peat samples[9]. During the course of our geochemical research on the occurrence and composition of carbohydrates in recent sediments we demonstrated the presence of various methylated monosaccharides in acid hydrolysates[10]. The monosaccharides obtained were analysed as their alditol acetates by capillary gas-liquid chromatography[11].

In order to identify the observed methylated sugars we systematically analysed the mono-, di-, tri- and tetra-O-methyl ethers of the common pentitols, hexitols and 6-deoxyhexitols. For this purpose we synthesized mixtures of partially methylated alditol acetates (PMAAs) starting from each alditol. These standard mixtures were analysed by capillary gas chromatography (GC), capillary gas chromatography-electron-impact mass spectrometry (GC-EI-MS) and capillary gas chromatography-chemical-ionization mass spectrometry (GC-CI-MS). This paper presents the identification of methylated monosaccharides in complex mixtures and gives details of the procedure used.

EXPERIMENTAL

Some of the alditols used in this study were commercially available {ribitol, L-arabitol, xylitol, D-mannitol, D-galactitol and D-glucitol (=sorbitol)}. The others (L-rhamnitol and L-fucitol) were prepared by reduction of the corresponding aldoses.

Reduction of the aldoses

The reduction of the aldoses was carried out by addition of sodium borohydride or sodium borodeuteride to an ammoniacal solution of the mono- or disaccharide. After at least 3 h the excess of borohydride was decomposed by addition of glacial acetic acid. In order to remove the boric acid methanol was added and the mixture was evaporated to dryness. The residue was suspended in dry methanol and again evaporated to dryness. This procedure was repeated twice.

Synthesis of PMAA mixtures (Haworth methylation)

The alditol, reduced monosaccharide or reduced disaccharide (50-100 mg) was dissolved in 0.5 ml 4 *M* sodium hydroxide and freshly distilled dimethyl sulphate (100 µl) was added. After 1 h at 70°C the reaction mixture was cooled, acidified with glacial acetic acid and evaporated to dryness *in vacuo*. The dry residue was dissolved in 1 ml acetic anhydride and 100 mg sodium acetate were added. The mixture was heated in a closed vial for 2 h at 100°C and subsequently evaporated *in vacuo*. The dry residue was suspended in 2 ml dichloromethane washed several times with water and dried over anhydrous sodium sulphate. Aliquots of 1 µl of the resulting dichloromethane solution were injected into the gas chromatograph.

Methylation of the aldoses was carried out by the method of Hirst and Percival[12]. The aldose (100 mg) was dissolved in 300 µl water, then 140 µl dimethyl sulphate and 450 µl 8.8 *M* sodium hydroxide were added in small portions at 0°C. A second portion of 140 µl dimethyl sulphate was added and the temperature was raised to 35°C. Subsequently 450 µl 8.8 *M* sodium hydroxide was added and the mixture was stirred for 3 h. The resulting methyl glycosides were hydrolysed by adding sulphuric acid to an end concentration of 0.5 *M*. The mixture was kept in a closed vial at 100°C for at least 10 h. The pH was raised to 9 by adding concentrated ammonia solution and the resulting mixture was reduced and acetylated as described above.

Gas-liquid chromatography

Gas-liquid chromatography of the alditol acetates (AAs) and PMAAs on a glass capillary coated with OV-275 (25 m × 0.25 mm I.D., Chrompack, Middelburg, The Netherlands) was carried out as described earlier[11]. The temperature was programmed from 165 to 215°C at 2°C/min and finally kept isothermal at 215°C.

As an example of the separation which can be obtained on this stationary phase, Fig. 1 shows the separation of a standard mixture of 24 AAs, of which 22 are clearly separated. The relative retention times of the components shown in Fig. 1 are listed in Table I.

Mass spectrometry

GC-MS was carried out on a Varian 3700 gas chromatograph connected to a Varian/Mat-44 mass spectrometer. In the EI mode the mass spectrometer was operated at 70 eV and a source temperature of 200°C. For CI isobutane was used as reagent gas at 200 eV, keeping the source temperature at 150°C and the pressure of the ionization chamber at 0.5 Torr.

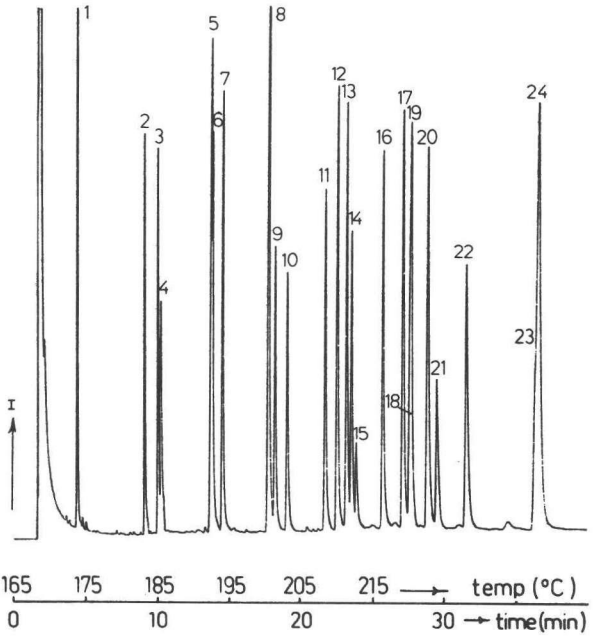


Fig. 1. Gas chromatogram of a standard mixture of 24 alditol acetates. Identifications are given in Table I.

Table I. Relative retention times of standard alditol acetates shown in Fig. 1 The retention time of xylitol pentaacetate is taken as standard.

Peak number	Identity	Rel. retention time
1	Glycerol	184
2	2,3,4,6-Tetra-O-methylglucitol	392
3	Erythritol	431
4	Digitoxitol	440
5	2-Deoxyribitol	589
6	Rhamnitol	596
7	Fucitol	625
8	6-Deoxyglucitol	764
9	Ribitol	784
10	Arabitol	820
11	1,4-Anhydromannitol	936
12	1,5-Anhydromannitol	971
13	Xylitol	1000
14	2-Deoxyglucitol	1016
15	2-Deoxygalactitol	1033
16	Allitol	1110
17	Mannitol	1170
18	3-O-Methylglucitol	1188
19	Altritol	1192
20	Galactitol	1249
21	4-O-Methylglucitol	1277
22	Glucitol	1366
23	Iditol	1584
24	myo-Inositol	1589

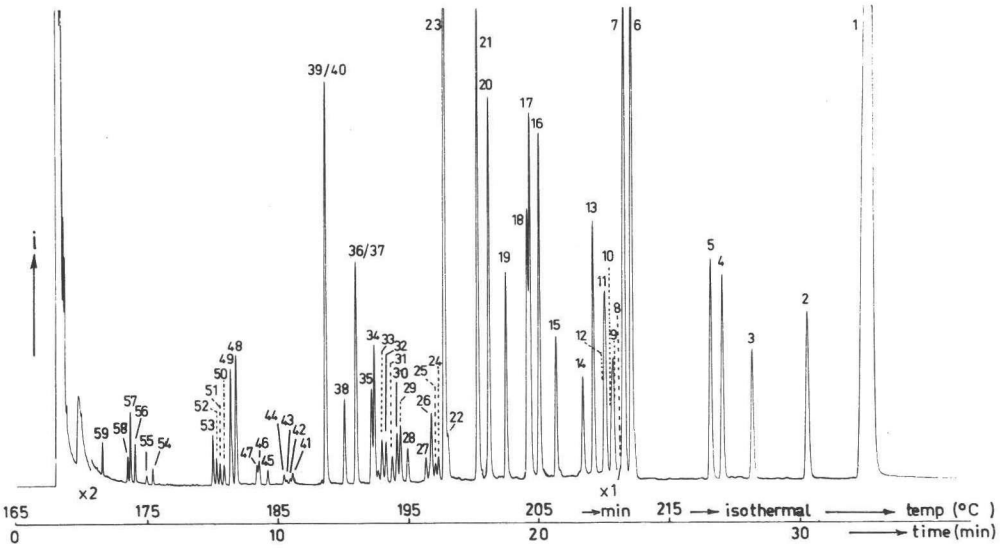


Fig. 2. Gas chromatogram of the glucitol-derived PMAA mixture. Identifications are given in Table III.

Table III. Identification of the glucitol-derived PMAAs shown in fig. 2

Peak number	Position(s) of the O-methyl groups	Peak number	Position(s) of the O-methyl groups
1	none	25	2,3,5
2	4	26	1,4,5
3	3	27	2,3,4
4	5	28	1,3,5
5	2	29	1,2,5
6	6	30	2,5,6
7	1	31	3,5,6
8	3,5	32	3,4,6
9	3,4	33	1,3,4
10	2,3	34	1,4,6
11	2,5	35	2,4,6
12	4,5	36	1,2,3
13	1,4	37	4,5,6
14	2,4	38	1,3,6
15	3,6	39	1,2,6
16	4,6	40	1,5,6
17	2,6	41-45	Tetra
18	1,5	46	2,3,4,6
19	1,3	47	1,3,4,5
20	5,6	48-51	Tetra
21	1,2	52	1,3,4,6
22	2,3,6	53	Tetra
23	1,6	54-58	Penta
24	3,4,5	59	Hexa†

†Tentative identification.

RESULTS AND DISCUSSION

Partial methylation of alditols results in mixtures of methyl ethers. Depending on the symmetry of the starting alditol different numbers of derivatives are expected theoretically. For each alditol the number of positional isomers within each group of O-methyl ethers (mono-, di-, tri-O-methyl ethers, etc.) are given in Table II. Since enantiomers are not separated on the non-chiral OV-275 stationary phase they have not been considered.

Table II. Number of positional isomers of methylated alditols

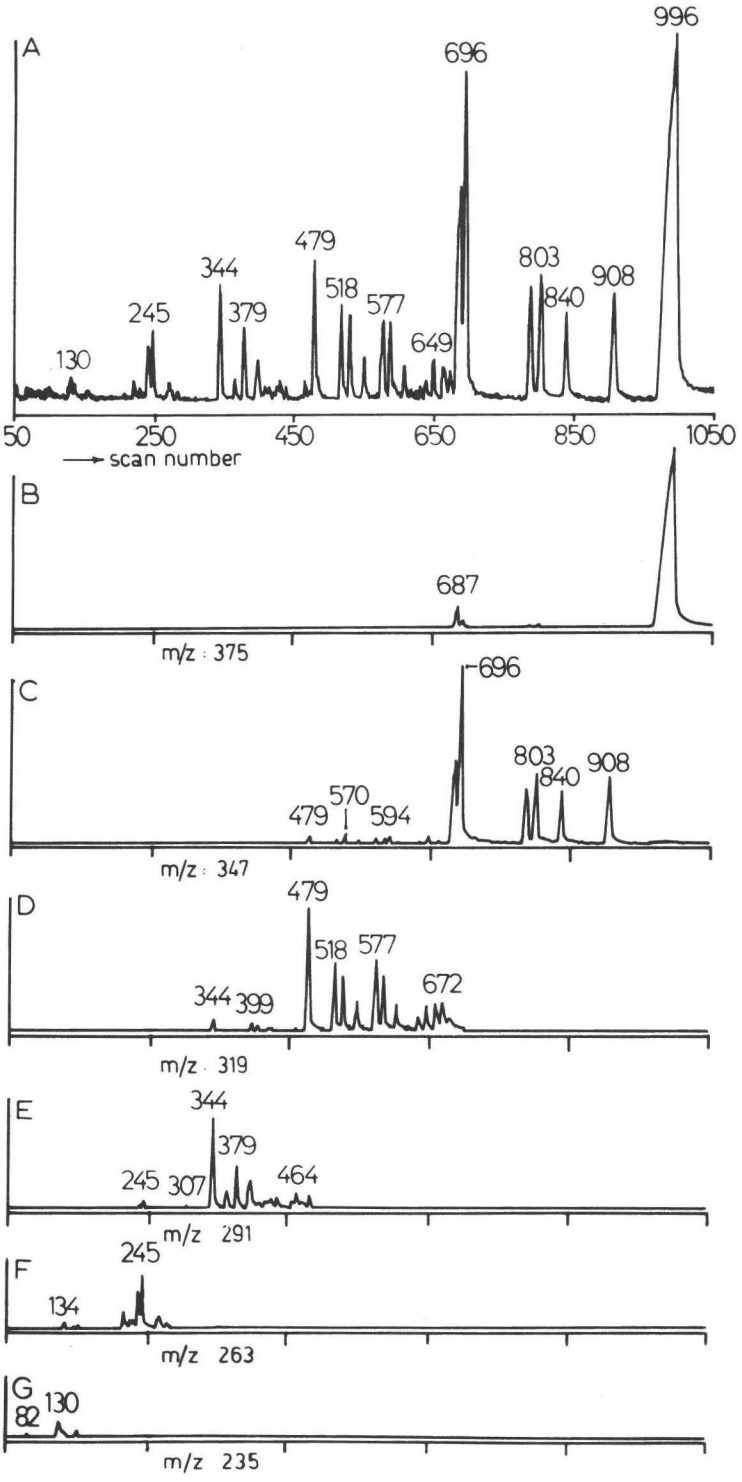
Alditol	Number of methyl substituents						
	0	1	2	3	4	5	6
Rhamnitol	1	5	10	10	5	1	-
Fucitol	1	5	10	10	5	1	-
Ribitol	1	3	6	6	3	1	-
Arabitol	1	5	10	10	5	1	-
Xylitol	1	3	6	6	3	1	-
Mannitol	1	3	9	10	9	3	1
Galactitol	1	3	9	10	9	3	1
Glucitol	1	6	15	20	15	6	1

From Table II it is clear that glucitol yields the most complex mixture of PMAAs. As an example of the application of this procedure, the identification of the PMAAs in this mixture will be elaborated in more detail. Fig. 2 shows the gas chromatogram of this mixture. Peak numbers correspond with those in Table III.

Identification of the individual components is based on the GC-EI-MS and GC-CI-MS (isobutane) data. The simplicity of the CI-fragmentation patterns, in which the masses $M + 1 - 60$, $M + 1 - 32$ and $M + 1$ dominate[13,14], offers the possibility of discriminating between the mono-, di-, tri-, tetra- and penta-O-methyl derivatives by mass chromatography. Fig. 3 shows the total ion current and the appropriate mass chromatograms of the D-glucitol PMAA mixture.

In Table IV the *m/z* values of the expected fragments are compiled for the

Fig. 3. Total ion current of the gas chromatogram of the glucitol-derived PMAAs (A) and mass chromatograms of M + 1 - 60 ion of hexitol hexaacetate and M + 1 - 32 ion of mono-O-methylhexitol pentaacetates (B); M + 1 - 60 ion of mono-O-methylhexitol pentaacetates and M + 1 - 32 ion of di-O-methylhexitol tetraacetates (C); M + 1 - 60 ion of di-O-methylhexitol tetraacetates and M + 1 - 32 ion of tri-O-methylhexitol triacetates (D); M + 1 - 60 ion of tri-O-methylhexitol triacetates and M + 1 - 32 ion of tetra-O-methylhexitol diacetates (E); M + 1 - 60 ion of tetra-O-methylhexitol diacetates and M + 1 - 32 ion of penta-O-methylhexitol acetates (F); and M + 1 - 60 ion of penta-O-methylhexitol acetates and M + 1 - 32 ion of hexa-O-methylhexitol (G).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D
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518
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399
672
m/z : 319
E
344
379
245
307
464
m/z 291
F
245
134
m/z 263
G
82
130
m/z 235

Table IV. m/z Values of the various ions resulting from chemical ionization of PMAAs derived from pentitols, hexitols and deoxyhexitols

Parent	Fragment	Number of methyl substituents						
		0	1	2	3	4	5	6
Deoxyhexitol	*M* + 1	377	349	321	293	265	237	
	M + 1 - 32	-	317	289	261	233	205	
	M + 1 - 60	317	289	261	233	205	-	
Pentitol	*M* + 1	363	335	307	279	251	223	
	M + 1 - 32	-	303	275	247	219	191	
	M + 1 - 60	303	275	247	219	191	-	
Hexitol	*M* + 1	435	407	379	351	323	295	267
	M + 1 - 32	-	375	347	319	291	263	235
	M + 1 - 60	375	347	319	291	263	235	-

PMAAs derived from pentitols, hexitols and deoxy-hexitols. The *M* + 1 - 60 fragment is the ion with the highest intensity in the CI mass spectra of most PMAAs[13,14]. Discrimination by mass chromatography of *e.g.*, mono-O-methylhexitol pentaacetates only on account of their *M* + 1 - 60 ion is prevented by the *M* + 1 - 32 ion of the di-O-methylhexitol tetraacetates interfering (both *m/z* 347, Table III). Nevertheless mass chromatography of the *M* + 1 - 60 and *M* + 1 - 32 ions clearly distinguishes between groups of derivatives with different degrees of methyl substitution (mono-, di-, tri-O-methyl derivatives, etc.) as shown in Fig. 3.

The identity of a number of the components in the D-glucitol PMAA mixture can be established directly by comparison of the EI mass spectra with data published by Jansson *et al.*[15]. However in many cases pairs of components are encountered in the chromatogram which give very similar mass spectra. In this way the mono-O-methylglucitol pentaacetates consist of three pairs of derivatives (1- and 6-, 2- and 5-, and 3- and 4-O-methylglucitol pentaacetates). Final identification of these components is achieved by comparison of the gas chromatograms of the D-glucitol- and D-glucose-derived PMAAs and/or by comparison of the EI mass spectra of the D-glucitol- and D-glucitol (1-^{2}H)-derived PMAAs.

D-Glucose, because of its pyranose structure, yields PMAAs in which the 1- and 5-O-methyl derivatives are absent. Thus comparison of the gas chromatograms of D-glucose- and D-glucitol-derived PMAAs allows one to distinguish between the 1- and 6-, and 2- and 5-, but not between 3- and 4-O-methylglucitol pentaacetates. The latter problem can be solved by interpretation of the EI mass spectra of the D-glucitol (1-^{2}H) PMAAs. On the basis of the known EI

Table V. Relative retention times of partially methylated alditol acetates on an OV-275 glass capillary column.
(The retention time of Xylitol pentaacetate was taken as standard. Temperature programme: 165°C then 2°C/min to 215°C (isothermal). The observed deviation was in the order of 1%. Derivatives marked "-" were not clearly distinguishable among the PMAAs.)

Position of O-methyl group	Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glu
none	596	625	784	820	1000	1170	1249	1366
1	370	386	518	534	666	865	918	983
2	511	541	640	685	777	1035	1098	1123
3	589	623	623	714	768	1166	1247	1188
4	554	611	640	707	777	1166	1247	1277
5	445	505	518	550	666	1035	1098	1142
6						865	918	995
1,2	274	278	357	379	443	671	715	743
1,3	296	300	335	368	430	746	798	789
1,4	337	347	397	425	492	857	875	931
1,5	276	304	333	339	411	758	799	824
1,6						586	631	689
2,3	398	459	424	493	545	883	989	961
2,4	390	425	408	498	492†	922	994	914
2,5	-	-	397	433	492	893	974	951
2,6						758	799	829
3,4	383	475	424	509	545	951	1049	963
3,5	-	-	335	384	430	922	994	980
3,6						857	875	871
4,5	-	-	357	401	443	883	989	949
4,6						746	798	844
5,6						671	715	761
1,2,3	-	-	195	232	256	498	579	543
1,2,4	-	-	210	252	254	565	602	-
1,2,5	-	-	216	232	266	560	629	616
1,2,6						446	469	494
1,3,4	-	-	218	250	277	590	661	585
1,3,5	-	-	182	194	231	577	612	625
1,3,6						493	501	524
1,4,5	-	-	216	227	266	623	678	667
1,4,6						493	501	573
1,5,6						446	469	494
2,3,4	234	297	216	305	314	657	776	654
2,3,5	-	279	218	255	277	690	756	673
2,3,6						623	678	694
2,4,5	-	-	210	258	254	690	756	-
2,4,6						577	612	568
2,5,6						560	629	610
3,4,5	-	-	195	239	256	657	776	679
3,4,6						590	661	593
3,5,6						565	602	602
4,5,6						498	579	543
1,3,4,5						387	447	388
1,3,4,6						315	361	317
2,3,4,6						387	447	392
2,3,5,6						396	432	-

†The 2,4-di-O-methylxylitol derivative probably co-elutes with the 1,4- and 2,5-di-O-methylxylitol derivatives.

fragmentation[16], predictions can be made which fragment will carry the deuterium label. In addition this method confirms the identifications based on the D-glucose derived PMAAs.

Within the group of di-O-methylglucitol derivatives, all the components can be identified as described above, except for the 1,2-, 5,6- and 1,6-di-O-methylglucitol tetraacetates for which no spectra are available in the literature. The presence in this group of two components having identical EI spectra implies that this pair represents the 1,2- and 5,6-di-O-methylglucitol derivatives. Consequently the third component must be 1,6-di-O-methylglucitol tetraacetate. The EI fragmentation patterns of the D-glucitol (1-^{2}H)-derived PMAAs allow distinction between 1,2- and 5,6-di-O-methylglucitol tetraacetate and confirm the identity of 1,6-di-O-methylglucitol tetraacetate.

Components with three or more O-methyl groups per molecule can be identified similarly. In some cases additional information was obtained from a study of the PMAAs derived from sodium-borohydride-reduced maltose.

Although many PMAAs having a high degree of methyl substitution were synthesized from the alditols used in this study, some are not considered, partly because their identification was too tentative and partly because they are less relevant both for our purposes and for methylation studies on polysaccharides.

The PMAAs derived from all the other alditols are characterized as described for the glucitol PMAAs. Table V is a compilation of the relative retention times of all the identified PMAAs.

CONCLUSIONS

The synthesis of PMAAs outlined above affords mixtures of all the theoretically possible derivatives of each alditol which can be well separated using a glass capillary column coated with OV-275.

With the help of these standard mixtures the preliminary identification of the methylated monosaccharides encountered in acid hydrolysates of recent marine sediments has been possible[10].

In addition the data obtained after analysis of these synthesized mixtures will be of importance for structural analyses of polysaccharides, because it is possible to synthesize all the possible PMAAs that might appear upon methylation analysis. Thus it overcomes the problem of the non-availability of individual reference compounds for GC and GC-MS studies.

ACKNOWLEDGEMENTS

The authors are grateful to Mrs. A.H. Knol-Kalkman for performing the mass-spectrometric analyses. Dr. P.J.W. Schuyl is thanked for many helpful discussions and for critically reading the manuscript.

Copies of the chromatograms of the various PMAA mixtures and of the EI mass spectra of the components identified are available from the authors on request.

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CHAPTER 5

CARBOHYDRATES IN RECENT MARINE SEDIMENTS I. ORIGIN AND SIGNIFICANCE OF DEOXY- AND O-METHYL-MONOSACCHARIDES *

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ABSTRACT

A qualitative and partly quantitative survey of the carbohydrates encountered in acid hydrolyzates of some recent marine sediments reveals the presence of a large variety of known and hitherto unknown monosaccharides. Apart from the well known major monosaccharides a great number of minor components (notably deoxy- and O-methyl monosaccharides) are encountered. These minor components are considered to originate from bacteria. Since significantly larger amounts of major monosaccharides are encountered in carbohydrates associated with bacterial cell walls the greater part of the carbohydrate carbon in these sediments is ascribed to these structures. Superimposed on the bacterial contribution the characteristics of the carbohydrates originating from the primary producers are recognizable. The results indicate that bacterial biopolymers formed by *de novo* synthesis in the sediment should be considered as a potential source for the insoluble organic matter in these sediments.

*In: *Organic Geochemistry* (in press)

INTRODUCTION

Geochemical studies concerning the presence of carbohydrates in marine and freshwater sediments are relatively scarce, partly due to the lack of adequate analytical techniques. A limited number of reports is known dealing with the analysis of carbohydrate compounds in ancient sediments ranging from Precambrian to Cenozoic (Swain and Rogers, 1966; Swain *et al.*, 1967; Swain *et al.*, 1970), recent sediments (Handa, 1969; Handa and Tominaga, 1969; Handa and Yanagi, 1969; Modzeleski *et al.*, 1971; Swain, 1971; Handa and Mizuno, 1973; Cranwell, 1976; Hatcher *et al.*, 1977; Mopper, 1977; Boon *et al.*, 1983; Klok *et al.*, 1983) and contemporary marine deposits (Boon *et al.*,1983; de Leeuw *et al.*, 1983). The minor interest in carbohydrates in marine sediments, compared to that of carbohydrates in soils, may also be a consequence of the current concept of kerogen formation (Tissot and Welte, 1978; Hunt, 1979; Durand, 1980). In this view microbes alter and partly mineralize the original biopolymers resulting in monomeric units which are chemically polymerized in new polycondensed structures which are precursor of kerogen.

It should be emphasized that bacteria biosynthesize new polymers from enzymatically released fragments, which in turn might contribute to the insoluble organic matter. In this respect it is worthwhile mentioning that constituents of archaebacterial cell membranes have been shown to be present in some kerogens (Michaelis *et al.*, 1979). The decrease in the amount of hydrolyzable carbohydrates with depth in sediment cores and the observed decrease of the O/C_{org} ratio in sedimentary organic matter with age does not necessarily imply the simultaneous loss of carbon originally present as carbohydrate.

The larger part of the organic carbon in the biosphere is present as carbohydrates, which can be devided into structural and storage components. As a consequence of their function the storage carbohydrates are highly susceptible to (auto-)biodegradation and keep participating in the biocycle of organic carbon. The carbohydrates which are structural elements, give body, stability and protection to the cell or to the organism as a whole (e.g. chitin, pectin, cellulose), and are therefore considered to be more stable. In many cases these carbohydrates occur linked to e.g. proteins (in peptidoglycans), lipids (in glyco-lipids and lipopolysaccharides), phenolic substances (lignin) and even minerals (silicates, phosphates and carbonates). As a result they show greater resistance towards biological decay, which enhances their potential to become part of the insoluble organic matter in the sediment.

The fate of carbohydrates, abundant in organisms and relatively poorly present as such in marine sediments seems to be largely determined in the very first stages of diagenesis. For this reason we decided to investigate the car-

bohydrates in recent marine sediments and we chose samples from different depositional environments for a comparative study. The samples from Solar Lake and Sabkha Gavish represent *in situ* bacterial deposits of different composition and the Namibian Shelf, Black Sea Unit 1 and 2 samples represent sediments underneath a water column with algae as the primary producers. The latter three are comparable in so far that they are deposited underneath a water column and are all anaerobic. They differ, however, as far as their primary input of organisms is concerned (diatoms, coccolithophores and dinoflagellates respectively). To some extent the two Solar Lake samples offer possibilities to study diagenetic effects on the carbohydrates synthesized in the top mat and a further contribution of carbohydrates generated by microbial populations at greater depths.

The development of a sensitive analytical technique offers the possibility of identifying both major and minor components in the complex mixture of monosaccharides released upon hydrolysis of sediment samples (Klok *et al.*, 1983). The monomers are analysed as their alditol acetates by capillary gas chromatography-mass spectrometry (Klok *et al.*, 1981; Klok *et al.*, 1982).

SAMPLE DESCRIPTION

Sabkha Gavish

The Sabkha Gavish depression is located about 15 km north of Sharm e Sheikh (Ophira) near Nabq at 400 m from the coast of the Sinai along the Gulf of Aqaba. This environment is characterized by extreme conditions. Due to the constant inflow of sea water and the high evaporation rate, hypersaline conditions have developed leading to precipitation of gypsum at the surface. Occasional rainfall induces mud loaded sheet floods which may reach the depression and cover the ecosystem. Despite the extreme conditions, life proliferates under the gypsum crust. As a consequence of the variable conditions some niches are dominated by algae, protozoa, insects, others by prokaryotes e.g. cyanobacteria, purple sulphur phototrophs and archaebacteria (mainly halophiles), which have adapted themselves to these conditions.

The sample used for the carbohydrate component analysis was collected in November 1980 in the zone permanently covered with water. The microbial mats previously described by Krumbein *et al.* (1979) were covered by a yellow brown mud layer of about 0.5 cm thickness deposited during the sheet floods in winter 1979 - 1980. The sedimentary material used for the analysis had a slimy viscous nature and represented the decomposed remains of the top of the microbial mat before the first sheet flood (de Leeuw *et al.*, in press).

Solar Lake

Solar Lake is a small (140 × 50 m) lake situated 18 km south of Eilat on the coast of the Sinai along the Gulf of Aqaba. Samples were collected in November 1980 (Boon *et al.*, 1983).

In this hypersaline, mesothermal lake a continuous sequence of laminated cyanobacterial mats is present with a total thickness of about one meter (Krumbein *et al.*, 1977). The accumulation of these sediments has continued since about 2400 years b.p. (Krumbein and Cohen, 1974). Stable hydrographic conditions throughout the depositional history are the main reason that the present day environmental conditions prevailing in the photosynthetic communities at the benthic surface resemble those when microbial mat accumulation began (Cohen *et al.*, 1977).

From microscopic observations it became clear that the top few millimeters of the Solar Lake core (SL6) consisted of a lawn of green *Microcoleus* in twisted bundles of 5 - 10 filaments, surrounded by a very thin external sheath. Occasionally other cyanobacteria are observed (*Chloroflexis*, *Aphanotheca*). The 625 - 658 mm sample of the SL6 core (about 2400 years old) consisted of rock fragments, carbonate and colourless sheath remains, which were much less tightly packed than in the upper layers (Boon *et al.*, 1983).

Namibian Shelf

The Namibian Shelf ooze samples were collected on a cruise (December 1968 - January 1969; Eisma, 1969). The sample investigated comprised the 40 - 75 cm section of core KD6 ($22^{\circ}30'S$ $14^{\circ}05.7'E$) sampled underneath a water column of 106 m in the Walvis Bay area on the continental shelf. The Namibian Shelf area is characterized by the occurrence of upwelling cold and nutrient-rich water from the Atlantic (Benguela current) which mixes with warm and nutrient-poor surface water. These conditions induce the development of dense planktonic populations. Depletion of certain nutrients causes massive death resulting in the sedimentation of algal remains. The high primary production leads to a rapid accumulation of organic matter on the anoxic bottom. The anaerobic conditions preclude the evolution of benthic organisms (Boon, 1978).

Microscope studies confirm an almost exclusively diatomaceous input, since mainly frustules of the vegetative stages of *Actinodiscus*, *Chaetoceros*, *Coscinodiscus* and *Navicula* species and cysts of *Chaetoceros* species are observed.

Black Sea

The Black Sea samples were collected from the western abyssal plain underneath a water column of about 2000 m during a cruise in May, 1975. Algal remains of diatoms, dinoflagellates and coccolithophores in the top two meters

of the abyssal Black Sea sediments are tracers for the phytoplankton succession during the gradual salinity change of the water column over the last 10,000 years. Specific diatoms are good indicators for the freshwater conditions which existed prior to this period, but their amounts in the sediments are low (Maynard, 1974). Very large amounts of dinoflagellate cysts and acritarchs occur in the Unit 2 sediment (core 137, sapropel layer, 18 - 59 cm depth below the top sediment, $30^{o}31.5'E$ $42^{o}43.5'N$) which was deposited from 7000 - 3000 years b.p. at the anoxic bottom. Dominant are dinoflagellate cysts from the motile stages of *Gonyaulax polyedra* and *spinifera* and from *Peridinium trochoideum* (Boon, 1978).

Coccoliths from *Emiliania huxleyi* are apparent from 3000 years b.p. and constitute the Unit 1 coccolith ooze (core 42/0, top 23 cm of the sediment $29^{o}42.5'E$ $43^{o}0'N$). The predominance of this coccolith and the appearance of marine diatoms in the Unit 1 sample indicate that from 3000 years b.p. marine conditions have developed in the surface water of the Black Sea (Degens and Ross, 1974).

All samples described above were stored at $-20^{o}C$ until use.

EXPERIMENTAL

Organic carbon

Total carbon was determined using an automatic Perkin Elmer 240 CHN-analyser. Carbonate carbon was determined titrimetrically or by acid digestion with HCl combined with gravimetric analysis of the CO_2 evolved following the method of Pieters (1948). The amount of organic carbon was calculated by subtraction.

Sample treatment

An amount of about 0.5 gram of the lyophilized sediment sample or 30 mg of purified coccoliths (kindly donated by Dr. E.W. de Jong, De Jong *et al.*, 1976) is treated with 0.25M H_2SO_4 for removal of carbonates. After centrifugation of the acid suspension the supernatant is neutralized with $BaCO_3$. The precipitate is removed by centrifugation, washed with water and the resulting solution is concentrated and added to the corresponding residue. Myo-inositol is added as internal standard. The combined fractions are hydrolysed in 0.25M H_2SO_4 in sealed ampoules under nitrogen for 18 hours at $100^{o}C$. After neutralization with $BaCO_3$, removal and repeated washing of the precipitates, the pH of the resulting solution is raised to 8 - 9 by adding 10% (v/v) tri-ethylamine in water for hydrolysis of lactones. After about 30 minutes an excess of solid $NaBH_4$ is added to reduce the released monosaccharides to the corresponding

alditols. The residual $NaBH_4$ after 2 hours at room temperature is decomposed by addition of glacial acetic acid. After effervescence has ceased the solution is evaporated to dryness under reduced pressure. Boric acid is removed by repeated addition of methanol and evaporation to dryness. The samples are desiccated *in vacuo* over KOH.

Acetylation is performed in closed vials in pyridine/acetic anhydride (1:1) for two hours at 100°C. The acetylation reagent is evaporated under reduced pressure and the resulting sample is desiccated overnight over P_2O_5 and KOH *in vacuo*. Subsequently water (4 ml) is added and the solution is extracted three times with an equal amount of dichloromethane. The combined dichloromethane extracts are dried on anhydrous Na_2SO_4 and concentrated.

Gas liquid chromatography and gas liquid chromatography-mass spectrometry

Gas liquid chromatography of the alditol acetates on a glass capillary column coated with OV-275 (25 m × 0.25 mm I.D., Chrompack, Middelburg, The Netherlands) is carried out as described earlier (Klok *et al.*, 1981). The temperature is programmed from 165 to 215°C at 2°C/min and finally kept isothermal at 215°C.

Quantitation of the major components (rhamnitol, fucitol, ribitol, arabitol, xylitol, mannitol, galactitol and glucitol peracetates) is achieved by peak area integration. Response factors for the alditol acetates have been assumed equal on a weight basis compared to myo-inositol hexaacetate (internal standard).

To obtain an optimum quantitation of the greater part of the mono-O-methyl alditol acetates a Carlo Erba Fractovap 4160 gas chromatograph fitted with a flame ionization detector and a non-vaporizing septumless on-column injector of the Grob type (Grob and Grob, 1978) is equipped with a glass capillary column coated with CPsil88 (25 m × 0.32 mm I.D., Chrompack, Middelburg, The Netherlands). Helium is used as carrier gas. Samples in ethyl acetate are injected at 100°C, the oven temperature is then rapidly raised to 200°C, further programmed at 2°C/min to 230°C, and finally kept isothermal at this temperature. Peak areas of the mono-O-methyl alditol acetates are measured as height times width at half height. Since the enantiomeric pair 2-/4-O-methyl xylitol tetraacetate coelutes with 3-O-methyl xylitol tetraacetate the separate determination of peak areas is impossible for these components on CPsil88 under the conditions used. The ratio in which these derivatives are present in the various hydrolyzates is calculated from the corresponding peak areas in the chemical ionization mass spectrometry mass chromatograms at m/z 275 (Klok *et al.*, 1982) on OV-275 as stationary phase.

Gas chromatography-mass spectrometry is carried out as described earlier

(Klok *et al.*, 1982).

It should be emphasized that only neutral monosaccharide derivatives are detected by the analytical procedures presented here.

Synthesis of reference compounds

The partially methylated alditol acetates of 8 alditols were synthesized as described earlier (Klok *et al.*, 1982).

5-Deoxy-xylitol tetraacetate is prepared by reduction of 5-deoxy-D-xylo-furanose (Serva, Feinbiochemica, Heidelberg) with $NaBH_4$ and acetylation as described above.

Partially formylated alditol acetates (PFAA) are synthesized by performing the acetylation in 3% (v/v) formic acid in pyridine/acetic acid anhydride (1:1) as described above.

RESULTS

Since the various glycosidic bonds behave differently during acid hydrolysis and since the various monosaccharides are degraded by acid at different rates (Dutton, 1973; Mopper, 1977) the hydrolysis conditions for polysaccharides are inevitably a compromise between incomplete hydrolysis of the more resistant glycosidic bonds and partial decomposition of the less stable monosaccharides. Moreover the results of the quantitative determination of the released monosaccharides are influenced by the reduction and acetylation procedures (Albersheim *et al.*, 1967; Torello *et al.*, 1980). In order to obtain comparable results care was taken to ensure maximum reproducibility during treatment of all samples.

The observed alditol derivatives are supposed to be mainly derived from the corresponding aldoses (or ketoses) although the occurrence of alditols as such in nature is known (Hough and Richardson, 1967a; Percival and McDowell, 1967a; Modzeleski *et al.*, 1971).

Fig. 1 shows a gas chromatogram of the neutral monosaccharides obtained from the Solar Lake (0 - 3 mm) sample analysed as their alditol acetates. Peak numbers correspond to the numbers mentioned in Table 1. Identification is based on retention data, EI mass spectra (Jansson *et al.*, 1976) and CI mass spectra (McNeil and Albersheim, 1977; Laine, 1981; Klok *et al.*, 1982) of reference compounds.

The results of the quantitative determination of the major components in the various hydrolyzates are summarized in Table 2. The percentage of carbohydrate carbon relative to the total organic carbon is given in the last column of Table 2. Fig. 2 shows the graphical representation of these data.

Table 1. Identification of the alditol acetates indicated in Fig. 1

peak number	identification	RRT†	peak number	identification	RRT†
1	glycerol	180	35	2-/4-OMe-xylitol¶	784
2	2,3,4-tri-OMe-rhamnitol§	234	36	ribitol	784
3	2,3,4-tri-OMe-fucitol§	297	37	2,6-di-OMe-galactitol§	799
4	5-deoxy-pentitol§	319	38	arabitol	820
5	2,3-di-OMe-6-deoxy-hexitol§	354	39	2,6-di-OMe-glucitol§	829
6	3,4-di-OMe-rhamnitol	383	40	3,6-di-OMe-mannitol§	857
7	2,4-di-OMe-rhamnitol	390	41	6-OMe-mannitol	865
8	2,3-di-OMe-rhamnitol§	398	42	3,6-di-OMe-glucitol§	871
9	2,4-di-OMe-fucitol	425	43	3,6-di-OMe-galactitol§	875
10	erythritol	431	44	2,4-di-OMe-glucitol§	914
11	2,3-di-OMe-fucitol	459	45	6-OMe-galactitol	918
12	3,4-di-OMe-fucitol§	475	46	3,4-di-OMe-mannitol§	951
13	2,3-di-OMe-arabitol§	493	47	2,3-di-OMe-galactitol§	989
14	2,4-di-OMe-arabitol	498	48	2,4-di-OMe-galactitol§	994
15	2-OMe-rhamnitol	511	49	6-OMe-glucitol	995
16	threitol	513	50	xylitol	1000
17	3,6-dideoxy-hexitol§	539	51	2-OMe-mannitol	1035
18	2-OMe-fucitol	541	52	3,4-di-OMe-mannitol§	1049
19	4-OMe-rhamnitol	554	53	2-OMe-galactitol	1098
20	3-OMe-rhamnitol	589	54	allitol	1110
21	3,6-dideoxy-hexitol	589	55	2-OMe-glucitol	1123
22	rhamnitol	596	56	3-/4-OMe-mannitol	1166
23	4-OMe-fucitol	611	57	mannitol	1170
24	3-OMe-fucitol	623	58	3-OMe-glucitol	1188
25	3-OMe-ribitol	623	59	altritol	1192
26	fucitol	625	60	3-/4-OMe-galactitol¶	1247
27	2-/4-OMe-ribitol¶	640	61	galactitol	1249
28	2-OMe-arabitol	685	62	4-OMe-glucitol	1277
29	4-OMe-arabitol	707	63	glucitol	1366
30	3-OMe-arabitol	714	64	myo-inositol (int. stand.)	1589
31	6-deoxy-hexitol	744	65	heptitol	1700
32	2,6-di-OMe-mannitol§	758	66	2-/6-OMe-heptitol¶§	1760
33	6-deoxy-glucitol	764			
34	3-OMe-xylitol	768	F	mono-O-formyl-hexitol	

† RRT = relative retention time. The retention time of xylitol pentaacetate is taken as standard (=1000)
¶ enantiomeric alditol acetates are not separated on OV-275
§ tentative identification

Minor components are determined qualitatively and the results are summarized in Table 3. In cases where the presence of certain compounds is not indicated their identity could not be established from both relative retention time and mass spectrometric data or their concentration was below the limit of detection. The last two columns of Table 3 comprise the reported occurrence in eukaryotes and prokaryotes of the minor components encountered in this study.

Based upon the assumption of a similar specific FID response for the various components the minor components are estimated to make up about 5 - 15% of the total monosaccharides mentioned in Table 2.

The relative distribution patterns of the greater part of the mono-O-methyl alditols are given in Fig. 3.

The mono-formyl hexitol peracetates observed in the gas chromatograms (marked "F" in Fig. 1) are due to the presence of formic acid in the original sediment samples (Klok *et al.*, in preparation). The formation of these derivatives was confirmed by control experiments. Partially formylated alditol acetates (PFAA) show a larger retention time than the corresponding alditol acetates on the polar stationary phases used in this study. The retention time increases with the degree of formylation of the PFAA. The formyl derivatives as shown in Fig. 1 are mainly glucitol derivatives since glucose is the predominant monosaccharide in this particular hydrolyzate (Table 2).

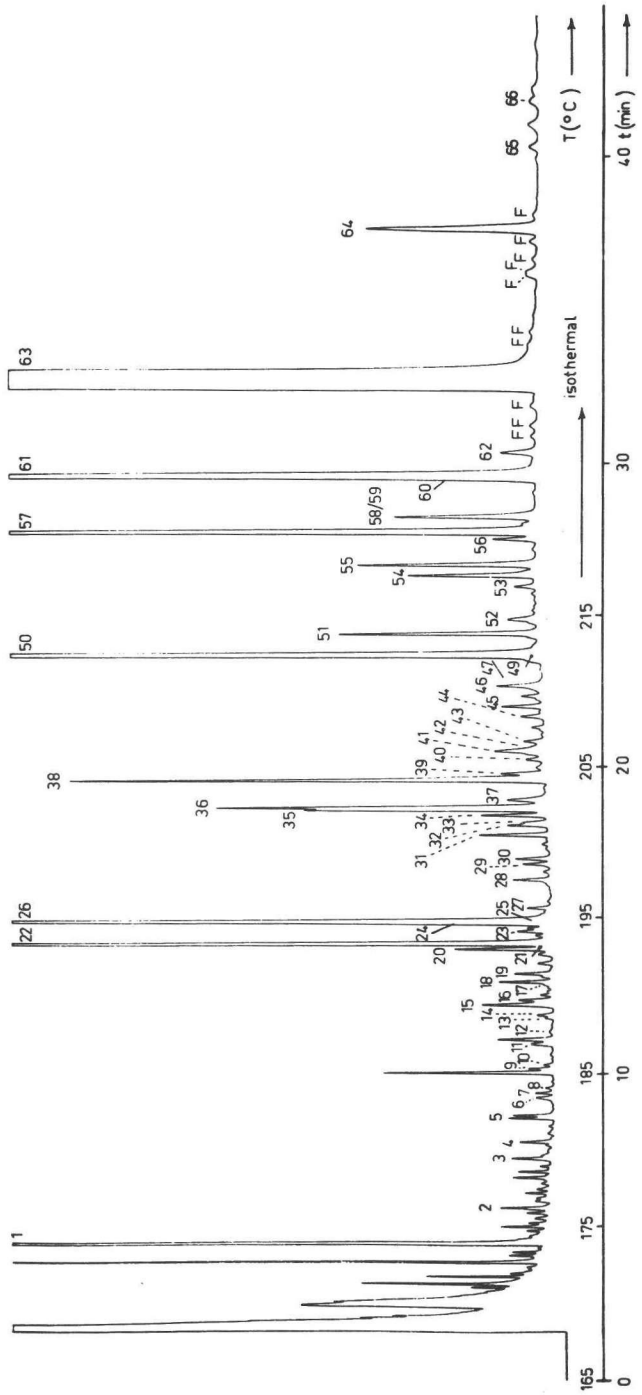

Fig. 1. Gas chromatogram of the alditol acetates obtained from the hydrolyzate of the Solar Lake (0 - 3 mm) sample. Peak numbers correspond to the numbers mentioned in Table 1.
(for conditions : see Experimental)

Table 2. The distribution of neutral monosaccharides in recent marine sediments after hydrolysis in 0.5N H_2SO_4

Sample	% C_{org} †	% Rha	% Fuc	% Rib	% Ara	% Xyl	% Man	% Gal	% Glu	Σ§ mg/g	% carbohydrate carbon of C_{org}
Sabkha Gavish	6.6	5.6	9.9	1.9	6.2	14.9	13.0	18.6	29.8	16.1	9.8
Solar Lake (0-3mm)	19.5	3.8	4.6	4.6	3.8	10.6	3.6	7.8	61.3	104.4	21.4
Solar Lake (628-658mm)	4.5	8.0	8.7	2.9	7.0	24.1	11.2	19.1	19.0	7.2	6.4
Namibian Shelf (KD6)	4.6	15.0	10.7	5.4	6.8	14.3	14.1	19.4	14.2	2.4	2.1
Black Sea (Unit 1)	6.3	8.9	7.0	9.4	6.9	14.0	14.2	18.9	20.5	5.3	3.4
Black Sea (Unit 2)	16.4	13.7	10.5	5.4	7.4	10.7	12.8	18.0	21.4	21.1	5.1

† calculated on dry weight basis
§ mg neutral monosaccharides per gram dry sediment

DISCUSSION

From Fig. 1 (Table 1) and Table 3 it is clear that the hydrolyzates of all samples investigated exhibit a great variety of neutral monosaccharides. Apart from the well known major compounds a multitude of O-methylated and/or deoxy monosaccharides could be identified in lower concentrations. Some of these compounds have as yet not been reported to occur in nature. Table 3 and Figs. 2 and 3 show that each sample is characterized by a specific distribution.

Comparison of Sabkha Gavish and Solar Lake 0-3mm samples

For a better understanding of the distribution of monosaccharides we first want to focus on the Sabkha Gavish and Solar Lake (0-3 mm) samples. These two samples represent a living community and the first stages of decomposition of an exclusively (cyano-)bacterial deposit. Compounds encountered in these samples therefore originate from bacteria.

The large carbohydrate contribution to the organic carbon in these particular samples (Table 2) -mainly caused by the high glucose content- is considered to be indicative for the presence of storage polysaccharides. Cyanobacteria are known to accumulate cyanophycean starch under favourable conditions (Lehmann and Wöber, 1976; Smith, 1982).

Among the monosaccharides encountered in the hydrolyzates (Table 3) a small number of the minor components have been reported to occur in eukaryotes (Table 3). A much larger number of these components have been reported to occur in cyanobacteria and other gram-negative bacteria. In these organisms they occur as lipopolysaccharide (LPS) constituents and also in other less well-defined carbohydrates associated with cell walls (Table 3). The occurrence of 3,6-dideoxy hexoses has been reported exclusively in LPS (Wilkinson, 1977; Weckesser *et al.*, 1979; Mikheyskaya *et al.*, 1981; Elkin *et al.*, 1982). Heptoses and O-methyl monosaccharides are also characteristic LPS constituents (references in Table 3). The cooccurrence of these classes of monosaccharides in these

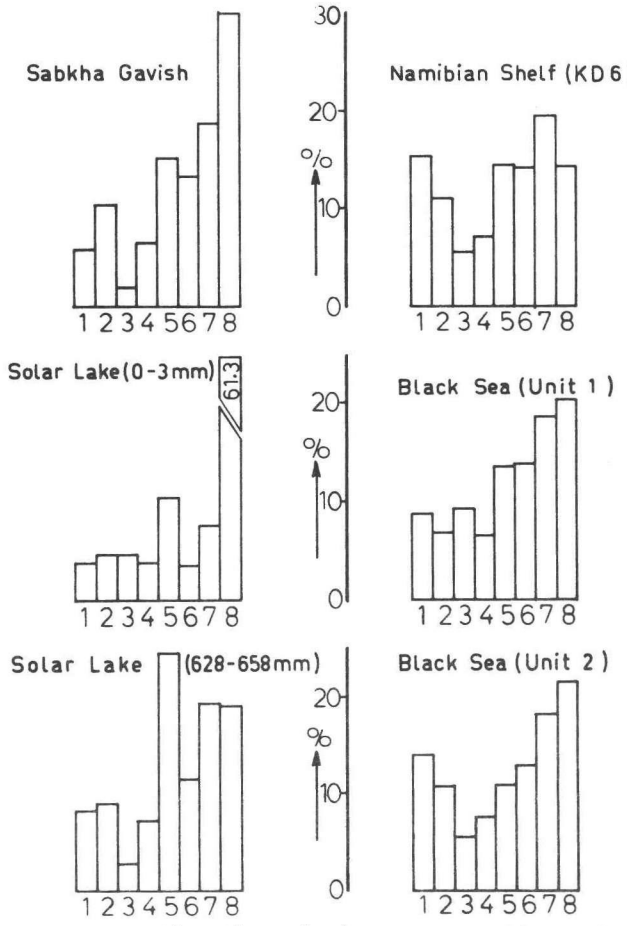

Fig. 2. Diagrams representing the relative concentrations of eight monosaccharides encountered in the hydrolyzates of recent marine sediments as presented in Table 2. (Identifications: 1 = rhamnose, 2 = fucose, 3 = ribose, 4 = arabinose, 5 = xylose, 6 = mannose, 7 = galactose, 8 = glucose)

samples points to an origin from bacterial LPS. The distribution of these components in LPS is strain specific and is therefore frequently used in the chemotyping of bacteria (Weckesser *et al.*, 1979; Schmidt *et al.*, 1980a; Schmidt *et al.*, 1980b). For this reason the observed monosaccharide patterns (Fig. 3) are considered as fingerprints of the bacterial communities in the Sabkha Gavish and Solar Lake (0-3 mm) samples. The differences in the patterns are due to the differences in the microbial populations in each sample: mainly filamentous cyanobacteria in the Solar Lake top mat *vs.* coccoid cyanobacteria, purple sulphur phototrophs and archaebacteria in the Sabkha Gavish deposit.

The natural occurrence of the greater part of the O-methylated monosaccharides has not (yet) been described in literature. This may be caused by the fact that the bacterial communities in sediments consist of hitherto unknown

component class	component name	I[†]	II	III	IV	V	VI	VII	VIII	IX
tri-OMe-6-deoxy-hexitol	2,3,4-rha	234				(+)	+			1
	2,3,4-fuc	297				(+)	+			
tri-OMe-pentitol	2,3,4-ara	305				+	(+)			2
	2,3,4-xyl	314				+	(+)			
	2,3-?	(354)	+			+	+			
	3,4-rha	383	+	+		+	+	+		3
	2,4-rha	390	+	+	+	(+)	+	+		3
di-OMe-6-deoxy-hexitol	2,3-rha	398	+	(+)		+	+	+	4	
	2,4-fuc	425	+	+	+	+	+	+		3
	2,3-fuc	459	+	+	+	(+)	+	+	3	5
	3,4-fuc	475	+		+	+	+	+		
	2,3-ara	493	+		+	+	+	+		
di-OMe-pentitol	2,4-ara	498	+	+	(+)			+		
	3,4-ara	509			(+)		+	(+)		
	2,3-xyl	545				(+)	+	(+)		
	2,6-man	758	(+)					(+)		
	2,6-gal	799	+			(+)	+	(+)		
	2,6-glu	829	+		+	(+)	+			
	4,6-glu	844	(+)				+			
	3,6-man	859			+			+		
	3,6-glu	871			(+)	(+)	+	(+)		
di-OMe-hexitol	3,6-gal	875						+		
	2,3-man	883	+							
	2,4-glu	914	+			(+)	+	+		
	2,4-man	922			(+)					
	3,4-man	951				(+)				
	3,4-glu	963				+				
	2,3-gal	989	(+)			(+)	+	(+)		6
	2,4-gal	994	(+)		(+)	(+)	+	(+)		
	3,4-gal	1049	+		+	+	+	+		
	2-rha	511	+	+	+	+	+	+		3,7,8,9,10
	2-fuc	541	+	+	+	+	+	+	3,11	3,5,12
mono-OMe-6-deoxy-hexitol	4-rha	554	+	+	+	+	+	+		
	3-rha	589	+	+	+	+	+	+	3	6,9,10,12,13,14,15
	4-fuc	611	+	+	+	+	+	+		3
	3-fuc	623	+	+	+	+	+	+	3	9
	3-rib	623	(+)	(+)		(+)	(+)			6,9
	2-/4-rib[¶]	640	+	+		+	+	+		3
	2-ara	685	+	+	+	+	+	+		
mono-OMe-pentitol	4-ara	707	+	+	+	+	+	+		
	3-ara	714	+	+	+	+	+	+		16[§]
	3-xyl	768	+	+	+	+	+	+	4	10,12
	2-/4-xyl[¶]	784	+	+	+	+	+	+	3,11	5,10,12
	6-man	865	+	+	+	+	+	+	4	10,17
	6-gal	918	+	+	+	+	+	+	3,18	9
	6-glu	995	+	+	+	+	+	+		3,19,20
	2-man	1035	+	+	+	+	+	+		5,21
mono-OMe-hexitol	2-gal	1098	+	+	+	+	+	+		6
	2-glu	1123	+	+	+	+	+	+		
	3-/4-man	1166	+	+	+	+	+	+		2,3,6,10,12,17,19,20
	3-glu	1188	+	+	+	+	+	+	22	9,23
	3-/4-gal[¶]	1247	+	+	+	+	+	+	3,18	24
	4-glu	1277	+	+	+	+	+	+		
mono-OMe-heptitol	3-hept	(1665)			+					
	(2-/6-hept)[¶,φ]	(1760)			+					5
	5-deoxy-pentitol	313				(+)	(+)			
	5-deoxy-pentitol	319	+	(+)		(+)	(+)			
	5-deoxy-pentitol	340	+		+	+	+	+		
	5-deoxy-xylitol	450				(+)	(+)	+		
	rhamnitol (rha)	596	+	+	+	+	+	+		
deoxy-alditol	fucitol (fuc)	625	+	+	+	+	+	+		
	6-deoxy-hexitol	(744)	+	+	+	+	+	+		
	6-deoxy-glucitol	764	+	+	+	+	+	+		
	3,6-dideoxy-hexitol	(498)	+		+	+	+	+		
	3,6-dideoxy-hexitol	(516)						(+)		
	3,6-dideoxy-hexitol	(539)	(+)				+			
	3,6-dideoxy-hexitol	(589)	(+)			+	+	(+)		
	glycerol	180	+	+	+	+	+	+		
	erythritol	431	+	+	+	+	+	+		
	threitol	513	+	+	+	+	+	+		
	ribitol (rib)	784	+	+	+	+	+	+		
	arabitol (ara)	820	+	+	+	+	+	+		
	xylitol (xyl)	1000	+	+	+	+	+	+		
alditol	allitol	1110	+	(+)	+	+	+	+		
	mannitol (man)	1170	+	+	+	+	+	+		
	altritol	1192	+	+	+	+	+	+		
	galactitol (gal)	1249	+	+	+	+	+	+		
	glucitol (glu)	1366	+	+	+	+	+	+		
	heptitol	(1700)	+			+	(+)	(+)		
	gluco-heptitol	1880	+		(+)	(+)	+	+		
	heptitol	(2120)			(+)					

Table 3. (opposite page) Neutral monosaccharides identified in the hydrolyzates of recent marine sediments analysed as their alditol acetates on an OV-275 glass capillary column. (for conditions : see Experimental)

I relative retention time (RRT)
II Sabkha Gavish
III Solar Lake (0-3 mm)
IV Solar Lake (628-658 mm)
V Black Sea (Unit 1)
VI Black Sea (Unit 2)
VII Namibian Shelf (KD6)
VIII occurrence reported in eukaryotes (references are summarized below)
IX occurrence reported in prokaryotes (references are summarized below)
1: Lechevalier, 1977; 2: Schmidt *et al.*, 1980a; 3: Hough and Richardson, 1967b; 4: Fichtinger-Schepman *et al.*, 1979; 5: Schmidt *et al.*, 1980b; 6: Tharanathan *et al.*, 1978; 7: Liptak, 1982b; 8: Cheshire, 1977; 9: Kennedy, 1980; 10: Weckesser *et al.*, 1979; 11: Aspinall, 1980; 12: Wilkinson, 1977; 13: Weckesser *et al.*, 1974; 14: Jackson *et al.*, 1982; 15: Villé and Gastambide-Odier, 1970; 16: Liptak, 1982a; 17: Schrader *et al.*, 1982; 18: Percival and McDowell, 1967b; 19: Ballou, 1981; 20: Tonn and Gander, 1979; 21: Kanamura *et al.*, 1982; 22: Barbier, 1981; 23: Saadat and Ballou, 1983; 24: Lechevalier and Gerber, 1970
(RRT) from this particular component no standard was available for identification purposes
\+ identification of the particular component is based on RRT, EI- & CI-mass spectrum
(+) identification of the particular component is based on RRT, EI- or CI-mass spectrum
† the retention time of xylitol pentaacetate is taken as standard (=1000)
¶ enantiomeric alditol acetates are not separated on OV-275
§ occurrence reported as 3-OMe-L-lyxose
Φ tentative identification

or scarsely studied species. For this reason it is not (yet) possible to link the observed components to individual bacterial species.

Comparison of Solar Lake (0-3 mm) and Solar Lake (628-658 mm) samples

Since the Solar Lake sequences have been deposited under almost identical conditions for at least 2400 years (Krumbein and Cohen, 1974) the 628-658 mm sample represents a layer of the deposit which has undergone microbial influences during this period. Hence, the differences observed in the monosaccharide patterns (Table 3, Figs. 2 and 3) are thought to originate from a specific contribution of microbial populations present in the sediment column and/or selective biodegradation of the original carbohydrates.

The high glucose contribution in the 0-3 mm sample rapidly decreases with depth (Boon *et al.*, 1983) obviously as a result of biodegradation of storage carbohydrates. The relatively large amount of ribose in the top 3 mm might indicate the high metabolic activity in the top mats, since this pentose is a component of many metabolically important molecules (RNA, ATP, NAD(P)H). An alternative contribution of ribitol moieties from bacterial teichoic acids (Rogers *et al.*, 1980) cannot, however, be precluded. The Solar Lake (0-3 mm) sample and likewise the 628-658 mm sample and also samples at intermediate depth (Boon *et al.*, 1983; Klok *et al.*, submitted), are characterized by a relatively important contribution of xylose. Microscope observations show that the 628-658 mm layer consists almost entirely of empty sheaths of *Microcoleus*, while these sheaths occur abundantly in the other Solar Lake samples. Xylose is therefore suggested to be a major carbohydrate building block of this resistant sheath material.

No increase of the relative contribution of any of the minor monosaccharide components is observed. Therefore it cannot be concluded whether the dominant

components present in the 628-658 mm layer (E , H and P , Fig. 3) represent LPS constituents originating from microbial populations living in the sediment column or that they represent more stable components of the LPS of the primary community, which forms the present day top layer of the deposit.

A more detailed investigation of the hydrolyzable carbohydrates in the Solar Lake sequences will be discussed elsewhere (Klok *et al.*, accepted).

Comparison of Namibian Shelf and Black Sea samples

The Namibian Shelf, Black Sea Unit 1 and Unit 2 sediments represent marine deposits underneath a water column in which diatoms, coccolithophores and dinoflagellates were the major primary producers respectively. Although there are large differences in the origin of the primary input of organic matter, they all have similarities in their sedimentation history. The remains of algae and their predators living in a brackish or marine water column migrate to an anoxic bottom. In the (pre-)depositional period bacteria start to decompose the organic matter leaving behind the more resistant structures. The decomposed organic matter is utilized by bacteria to build up their biomass and to cover their energy requirements. Thus one might wonder to what extent the originally eukaryotic biomass is transformed into prokaryotic biomass during the process of sedimentation and afterwards. The carbohydrate data obtained for these sediments are discussed in this context. The sugar patterns of both major and minor components (Table 3, Figs 2 and 3) show similarities. The minor components encountered in the hydrolyzates (notably the O-methyl monosaccharides, the 3,6-dideoxy hexoses and the heptoses) indicate an origin from bacterial cell wall material as discussed above. The similarities in the patterns are not unexpected since the similar sedimentation history presumably involves the development of related bacterial populations. Nevertheless, distinct differences are observed. When focussing on the patterns of minor components (Table 3, Fig. 3) the Black Sea Unit 1 sediment is characterized by the abundance of 3-O-methyl xylose (component K in Fig 3). This particular monosaccharide is reported to be a constituent of the polysaccharide which forms a structural part of the coccoliths of *Emiliania huxleyi* (Fichtinger-Schepman *et al.*, 1979). Moreover, our investigation of a hydrolyzate of coccoliths obtained from a culture of this organism confirmed the abundance of 3-O-methyl xylose. Because these coccoliths are the main constituents observed microscopically in the Unit 1 ooze, part of the 3-O-methyl xylose is suggested to originate from the eukaryotic coccolithophore.

The other mono-methyl monosaccharide constituent of the coccolith polysaccharide, 6-O-methyl mannose (component N in Fig. 3), Fichtinger-Schepman *et al.*, 1979) is also detected in the Unit 1 ooze. Its relative contribution compared

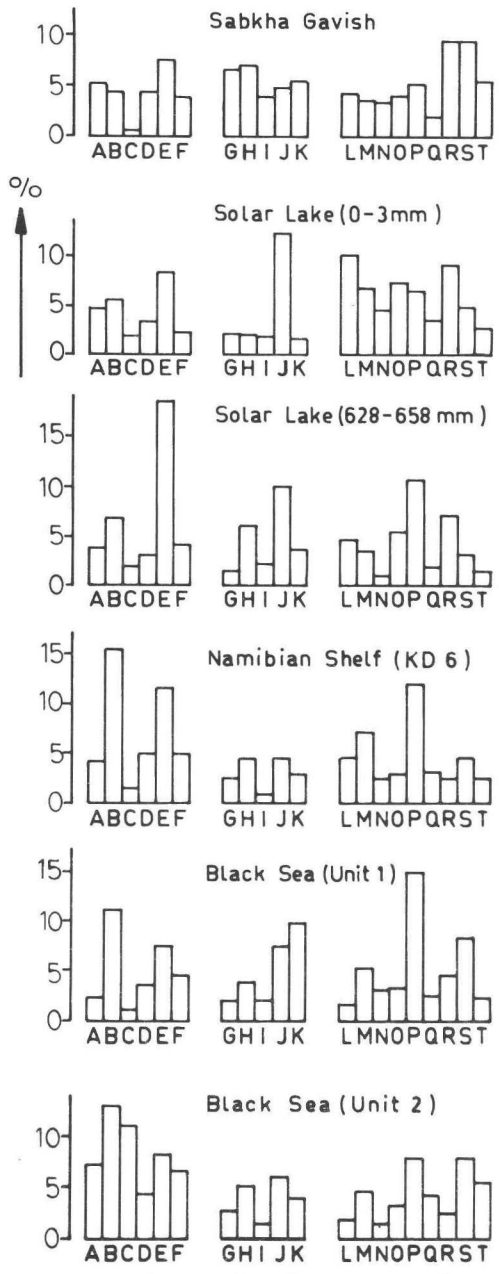

Fig. 3. Diagrams representing the relative concentration of mono-O-methyl monosaccharides as percentage of the total peak area of the corresponding mono-O-methyl alditol peracetates subdivided into classes: 6-deoxy hexoses (A: 2-OMe-Rha, B: 3-OMe-Rha, C: 4-OMe-Rha, D: 2-OMe-Fuc, E: 3-OMe-Fuc and F: 4-OMe-Fuc), pentoses (G: 2-OMe-Ara, H: 3-OMe-Ara, I: 4-OMe-Ara, J: 2-/4-OMe-Xyl and K: 3-OMe-Xyl) and hexoses (L: 2-OMe-Man, M: 3-/4-OMe-Man, N: 6-OMe-Man, O: 2-OMe-Gal, P: 3-/4-OMe-Gal, Q: 6-OMe-Gal, R: 2-OMe-Glu, S: 4-OMe-Glu and T: 6-OMe-Glu).

to the Namibian Shelf and Unit 2 mono-O-methyl monosaccharides (Fig. 3) is less pronounced than that of 3-O-methyl xylose.

Similarly one might speculate about the origin of "sediment specific" O-methyl monosaccharides observed in the Namibian Shelf and Unit 2 samples (e.g. components E, L and C, F, T respectively in Fig.3). They may be the result of differences in bacterial populations that developed during sedimentation and afterwards. On the other hand they may be suggested to originate partly from diatoms and dinoflagellates respectively, superimposed on a bacterial contribution. Therefore the presence or absence of the components in diatoms and dinoflagellates remains to be established by carbohydrate analyses of relevant organisms.

The occurrence of the minor monosaccharides in the hydrolyzates and the supposed relation with the presence of bacterial cell wall constituents in the samples agrees with the assumption that there is also a contribution of major (and less specific) monosaccharides from this source. This contribution might be as much as an order of magnitude larger, since the minor monosaccharides occur as minor components in carbohydrates associated with bacterial cellwalls.

Because of similar reasons as described above for the minor components the composition of the fractions of major monosaccharides show similarities. The striking differences in the major monosaccharide patterns (Fig. 2) may be correllated with the differences in the primary producers of the organic matter as a distinct eukaryotic contribution superimposed on the bacterial contribution. The Namibian Shelf and Black Sea Unit 2 sediments show a relatively large contribution of rhamnose and fucose. This is in accordance with the findings of Degens and Mopper (1976) who also found a similar contribution of these 6-deoxy hexoses in comparable samples from the Namibian Shelf and the Black Sea among twelve sediment samples investigated. Part of the rhamnose and fucose can be considered as a contribution from algae since these monosaccharides are known to be important building blocks in algal polysaccharides (Parsons *et al.*, 1961; Handa, 1969; Modzeleski *et al.*, 1971; Handa and Mizuno, 1973; Hecky *et al.*, 1973; Myklestad, 1974; Smestad *et al.*, 1974 and 1975; Mori *et al.*, 1982). Similarly an abundance of ribose in the Black Sea Unit 1 sample, also in accordance with the findings of Degens and Mopper (1976), is observed. This observation points to a coccolithophoric origin since this pentose is a constituent of the coccolith polysaccharide mentioned above (Fichtinger-Schepman *et al.*,1979). In this case it is unlikely that ribose is a marker for high metabolic activity in the Black Sea Unit 1 ooze, as is suggested for the Solar Lake (0 - 3 mm) sample.

When the Namibian Shelf sediment sample is refluxed with water and the water extract is hydrolysed, the same classes of monosaccharides are encountered in similar proportions compared to those obtained after hydrolysis of the total sediment sample (Klok *et al.*, 1984). The prolonged exposure of the algal remains to water leaching during sedimentation and deposition, apart from the degradative action of microbes at the same time, makes these easily extractable carbohydrates unlikely to originate from algae. An *in situ* biogenesis of polysaccharides by bacteria is suggested to be a more appropriate explanation for the presence of these polymers.

In these sediment samples the amount of carbohydrate carbon calculated from the amount of identifiable monosaccharide derivatives observed in the gas chromatograms comprises about 2-5% of the total organic carbon (Table 2). Apart from the incomplete release of monosaccharides these calculations represent minimum values since losses of certain components during hydrolysis and derivatization steps must also be considered. Nevertheless, a significant part of the organic matter is characterized as carbohydrates. A great variety of minor monosaccharides has mainly been reported to occur in carbohydrates associated with bacterial cell walls (compare Table 3, columns VIII and IX). Only some specific O-methyl monosaccharides are found in phytoplankton, as mentioned

before. We therefor want to suggest that the presence of minor monosaccharides in the hydrolyzates points to a mainly bacterial origin. Consequently the greater part of the major monosaccharides present will also originate from these carbohydrates. Future experiments will have to reveal the simultaneous presence of a: other specific compounds from bacterial cell wall polymers (e.g. β-hydroxy fatty acids, aminosugars, 2-keto-3-deoxy-D-manno-octonate) and b: the intact polymers themselves (e.g. LPS) in order to confirm the supposed relationship.

When the carbohydrate part of the organic matter mainly originates from bacteria it is reasonable to suggest that in these environments an important part of the sedimentary organic matter is of bacterial origin. The present findings contribute to our knowledge about the origin and diagenesis of the organic matter in recent anoxic marine sediments. In our view biopolymers of the primary producers are degraded to a large extent by the action of microbes, which in turn resynthesize from them their own biomass. A succession of bacterial populations, depending on the ecological conditions at the various depositional stages, partly causes a bioconversion of the primary eukaryotic organic matter. The conversion proceeds until conditions deteriorate and continuation of life is precluded.

ACKNOWLEDGEMENTS

The authors thank Dr. E.W. de Jong (Leiden State University, The Netherlands) for the gift of coccoliths of *Emiliania huxleyi*. The Koninklijke/Shell Exploratie en Produktie Laboratorium (Shell Research B.V.) Rijswijk, The Netherlands is kindly acknowledged for supplying the Black Sea samples. Mrs. E.H. Nieberg-van Velzen is thanked for her skilful GC assistance. Mrs. A.H. Knol-Kalkman is acknowledged for performing the mass-spectrometric analyses.

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CHAPTER 6

CARBOHYDRATES IN RECENT MARINE SEDIMENTS II. OCCURRENCE AND FATE OF CARBOHYDRATES IN A RECENT STROMATOLITIC DEPOSIT : SOLAR LAKE , SINAI. *

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ABSTRACT

In a study on the diagenesis of carbohydrates in a recent stromatolitic deposit (Solar Lake , Sinai) monosaccharides are quantitated after mild acid hydrolysis and gas chromatographic analysis as alditol acetates. From the depth profiles of the individual monosaccharides relative to the total organic carbon three categories of carbohydrates can be distinguished. The behaviour of the various categories upon burial is discussed in terms of their potential to become a part of the organic matter that survives geological periods. The distribution patterns of especially the mono-O-methyl monosaccharides at various depths are characteristic for the bacterial communities present in the corresponding parts of the sediment column.

*In: *Organic Geochemistry* (in press)

INTRODUCTION

In connection with the study on the origin of carbohydrates in recent marine sediments (Klok *et al.*, 1984) the present investigation aims the elucidation of the fate of carbohydrates upon burial. For this purpose we selected the recent stromatolitic deposit in Solar Lake, Sinai. Due to the absence of an influx of organic matter from the water column an exclusively cyanobacterial deposit has developed, which represents a suitable model system.

In this hypersaline, mesothermal Solar Lake a continuous sequence of laminated cyanobacterial mats is present with a total thickness of about one meter (Krumbein *et al.*, 1977). The accumulation of these sediments started about 2400 years b.p. and still continues (Krumbein and Cohen, 1974). Stable hydrographic conditions throughout the depositional history is the main reason that the present day environmental conditions prevailing in the photosynthetic cyanobacterial communities at the benthic surface closely resemble those when microbial mat accumulation began (Cohen *et al.*, 1977). These parameters make the deposit a well defined system and offer the possibility of evaluating the carbohydrate stratigraphy of these sediments in terms of processes of degradation, transformation, *de novo* synthesis and preservation, because the living generation in the top mat is underlaid by the debris of similar previous generations in various stages of decomposition. In the lower layers active carbonate precipitation interferes with biological transformation processes and presumably leads to an efficient preservation of organic matter (Krumbein *et al.*, 1977).

Apart from the determination of some bulk parameters (dry weight, organic carbon and carbonate carbon) we made a quantitative survey of the monosaccharides which could be identified after acid hydrolysis of samples from various depths. As a result of the specific behaviour of the various monomers in the sediment core three categories of carbohydrates could be distinguished, the geochemical fate of which will be discussed in more detail.

EXPERIMENTAL

Site description

Samples were taken from the shallow benthic cyanobacterial mats present in Solar Lake, a small lake (140 × 50 m) situated 18 km south of Eilat on the coast of the Sinai desert along the Gulf of Aqaba in November 1980 (Boon *et al.* 1983). Underneath a water column of about 70 cm the dark green top mat, mainly representing a lawn of *Microcoleus* cells in twisted bundles of 5-10 filaments surrounded by a thin external sheath, was observed. The core for this study

(code: SL6) was 67 centimeter in length and showed a continuous sequence of laminations. Three major sections could be distinguished: the top section (∿ 1 cm) predominantly oxic, the intermediate sulfide containing section (∿ 1 - 20 cm) and the bottom non-reducing section (20 - 67 cm). Microscope observations of the samples taken at various depths revealed the abundance of empty sheath remains of *Microcoleus* of which the 628 - 658 millimeter layer (about 2400 years in age) is constituted almost entirely.

A more extensive description of the site and of the sampling is given by Boon *et al.* (1983).

Organic carbon and carbonate carbon

Total carbon was determined using an automatic Perkin Elmer 240 CHN-analyser. Carbonate carbon was determined by acid digestion with HCl and gravimetric analysis of the CO_2 evolved following the method of Pieters (1948). The amount of organic carbon was calculated by subtraction.

Carbohydrate component analysis

0.2 - 0.5 gram of the lyophilized sediment sample is treated with 0.25M H_2SO_4 to remove carbonates. The acid suspension is centrifuged. The supernatant is removed and neutralized with $BaCO_3$; the resulting precipitate is removed by centrifugation, washed with water and the resulting solution is concentrated and added to the corresponding residue of the decarbonatization procedure. Myo-inositol is added as an internal standard. The combined fractions are hydrolysed with 0.25M H_2SO_4 in sealed glass ampoules for 18 hours at 100°C. After neutralization with $BaCO_3$, removal and repeated washing of the precipitates the pH of the resulting solution is raised to 8 - 9 by adding a 10% (v/v) tri-ethylamine solution in water for hydrolysis of lactones. After about 30 minutes an excess of solid $NaBH_4$ is added to reduce the released monosaccharides into the corresponding alditols. At room temperature after 2 hours the residual $NaBH_4$ is decomposed by addition of glacial acetic acid and the solution is evaporated to dryness under reduced pressure. Boric acid is removed by repeated addition of methanol and evaporation to dryness under nitrogen. The samples are desiccated *in vacuo* over KOH.

Acetylation is performed in closed vials with pyridine/acetic acid anhydride (1:1) for two hours at 100°C. The acetylation reagent is evaporated under reduced pressure and the resulting sample is desiccated overnight over P_2O_5 and KOH *in vacuo*. Subsequently, 4 ml water is added and the suspension is extracted three times with an equal amount of dichloromethane. The combined dichloromethane extracts are dried on anhydrous Na_2SO_4 and concentrated.

Gas liquid chromatography of the alditol acetates on a glass capillary

column coated with OV-275 (25 m × 0.25 mm I.D., Chrompack, Middelburg, The Netherlands) is carried out as described earlier (Klok *et al.*, 1981). The temperature is programmed from 165 to 215°C at 2°C/min and finally kept isothermal at 215°C. Quantitation of the components is achieved by peak area integration. Response factors for the alditol acetates have been assumed equal on a weight basis compared to myo-inositol hexaacetate (internal standard).

To enable an optimum quantitative survey of the greater part of the mono-O-methyl alditol acetates, a Carlo Erba Fractovap 4160 gas chromatograph fitted with a flame ionization detector and a non-vaporizing septumless on-column injector of the Grob-type (Grob and Grob, 1978) is equipped with a glass capillary column coated with CPsil 88 (25 m × 0.32 mm I.D., Chrompack, Middelburg, The Netherlands). Helium is used as carrier gas. Samples in ethyl acetate are injected at 100°C, the oven temperature is then rapidly raised to 200°C, further programmed at 2°C/min to 230°C and finally kept isothermal at this temperature. Individual peak areas of the mono-O-methyl alditol acetates are measured as height times width at half height.

Gas chromatography - mass spectrometry is carried out as described earlier (Klok *et al.*, 1982).

It should be emphasized that, by the analytical procedures presented here only the derivatives of neutral monosaccharides are detected.

RESULTS

In Table 1 the results of the dry weight, total carbon and organic carbon determinations are summarized. The depth profile of the organic carbon obtained from these data is vizualized in Fig. 1. This figure additionally shows the schematic sectioning of the SL6 core.

Table 1. Results of dry weight, total carbon, organic carbon determinations and of the individual monosaccharide contribution relative to the organic carbon in the various Solar Lake samples.

depth (mm)	% dry weight	% C_{tot}	% C_{org}	% Rha	% Fuc	% Rib	% Ara	% Xyl	% Man	% Gal	% Glu	% Others	% $C_{carbohydr.}$
0-0.5	29.8	26.6	26.6	11.2	10.5	6.0	3.6	17.7	12.3	20.7	218.1	27.3	34.1
0.5-3	25.8	15.8	15.2	9.5	10.7	5.0	3.9	20.2	11.0	18.4	59.4	17.4	15.4
3-10	21.3	10.3	9.5	5.1	7.8	13.0	4.2	17.7	11.8	20.2	32.8	13.5	12.5
10-20	29.0	8.0	4.9	7.2	10.7	5.7	5.7	22.0	24.5	22.8	35.1	19.6	15.2
115-145	24.4	6.5	4.7	7.5	12.1	3.4	6.8	22.9	13.6	21.3	29.8	13.6	12.9
265-295	28.2	7.3	5.9	4.5	7.1	2.0	4.7	20.3	8.1	14.2	18.3	8.1	8.8
385-415	27.7	8.1	4.7	4.7	6.5	2.6	4.3	17.9	8.5	11.1	11.9	11.9	7.8
628-658	30.8	8.3	4.5	5.8	5.8	1.8	4.4	15.1	7.1	12.4	12.4	10.7	7.5

Since the various glycosidic bonds behave differently during acid hydrolysis and since the various monosaccharides are degraded by acid at different rates (Dutton, 1973; Mopper,1977) and the results of the quantitative deter-

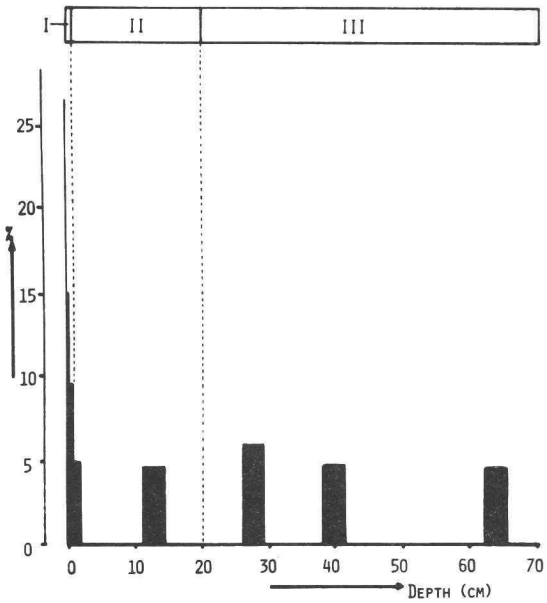

Figure 1. Depth profile of the organic carbon as percentage of the dry weight. (I : top oxic section, II : sulfide containing section and III : bottom , non-reducing section)

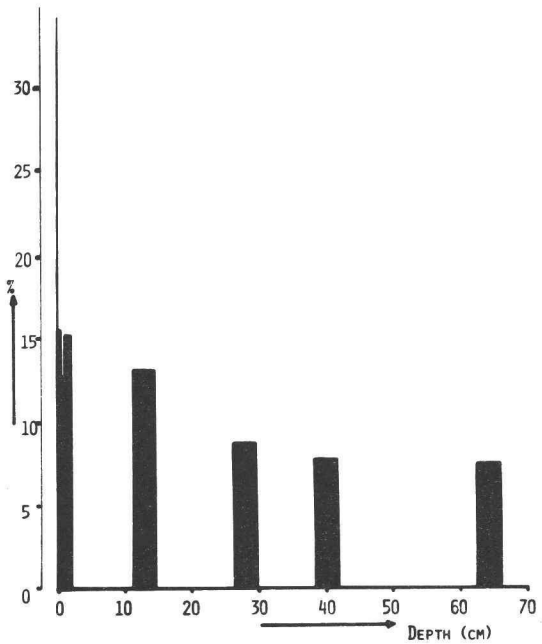

Figure 3. Depth profile of hydrolyzable carbohydrate carbon relative to the total carbon.

mination of the released monosaccharides are influenced by the reduction and acetylation procedures (Albersheim *et al.*, 1967; Torello *et al.*, 1980) care was taken to ensure maximum reproducibility during treatment of all samples to obtain comparable results. The observed alditol derivatives are supposed to be mainly derived from the corresponding aldoses (or ketoses) although the occurrence of alditols as such in nature is known (Hough and Richardson,1967a). Fig. 2 shows a gas chromatogram of the neutral monosaccharides from the 628 - 658 mm sample analysed as their alditol acetates. Peak numbers correspond to the numbers mentioned in Table 2. Identification is based on retention data, EI mass spectra (Jansson *et al.*, 1976) and CI mass spectra (McNeil and Albersheim, 1977; Laine, 1981; Klok *et al.*, 1982) of reference compounds.

Table 2. Identifications of the alditol acetates indicated in Fig. 2.

peak number	identification	RRT†	peak number	identification	RRT†
1	glycerol	180	33	4,6-di-OMe-glucitol	844
2	5-deoxy-pentitol	340	34	3,6-di-OMe-mannitol	857
3	3,4-di-OMe-rhamnitol§	383	35	6-OMe-mannitol	865
4	2,4-di-OMe-rhamnitol	390	36	3,6-di-OMe-glucitol§	871
5	2,4-di-OMe-fucitol	425	37	2,4-di-OMe-glucitol§	914
6	erythritol	431	38	6-OMe-galactitol	918
7	2,3-di-OMe-fucitol	459	39	2,4-di-OMe-mannitol§	922
8	3,4-di-OMe-fucitol	475	40	3,4-di-OMe-glucitol	963
9	2,3-di-OMe-arabitol	493	41	2,3-di-OMe-galactitol§	989
10	2,4-di-OMe-arabitol§	498	42	2,4-di-OMe-galactitol§	994
11	3,6-dideoxy-hexitol	498	43	6-OMe-glucitol	995
12	3,4-di-OMe-arabitol§	509	44	xylitol	1000
13	2-OMe-rhamnitol	511	45	2-OMe-mannitol	1035
14	threitol	513	46	3,4-di-OMe-mannitol	1049
15	2-OMe-fucitol	541	47	2-OMe-galactitol	1098
16	4-OMe-rhamnitol	554	48	allitol	1110
17	3-OMe-rhamnitol	589	49	2-OMe-glucitol	1123
18	rhamnitol	596	50	3,6-anhydro-hexitol§	1148
19	4-OMe-fucitol	611	51	3-/4-OMe-mannitol	1166
20	3-OMe-fucitol	623	52	mannitol	1170
21	fucitol	625	53	3-OMe-glucitol	1188
22	2-OMe-arabitol	685	54	altritol	1192
23	4-OMe-arabitol	707	55	3-/4-OMe-galactitol¶	1247
24	3-OMe-arabitol	714	56	galactitol	1249
25	6-deoxy-hexitol	744	57	4-OMe-glucitol	1277
26	6-deoxy-glucitol	764	58	glucitol	1366
27	3-OMe-xylitol	768	59	myo-inositol (int. stand.)	1589
28	2-/4-OMe-xylitol¶	784	60	3-OMe-heptitol	1665
29	ribitol	784	61	heptitol§	1700
30	2,6-di-OMe-galactitol§	799	62	2-/6-OMe-heptitol¶	1760
31	arabitol	820	63	glucoheptitol§	∿1880
32	2,6-di-OMe-glucitol	829	64	heptitol	∿2120

† RRT = relative retention time. The retention time of xylitol pentaacetate is taken as standard (=1000)
§ tentative identification
¶ enantiomeric alditol acetates are not separated on OV-275

Table 1 also summarizes the results of the quantitative determination of the observed components. The depth profile of the amount of hydrolyzable carbohydrate carbon relative to the total organic carbon as presented in Table 1 is given in Fig. 3. Fig. 4 shows a graphical representation of the *pro rata* contribution to the organic carbon of the individual components as presented in Table 1.

The patterns of the relative distribution of the greater part of the mono-O-methyl alditols observed in the gas chromatograms are given in Fig. 5. The peracetates of the enantiomeric pair 2-/4-O-methyl-ribitol, of 3-O-methyl-

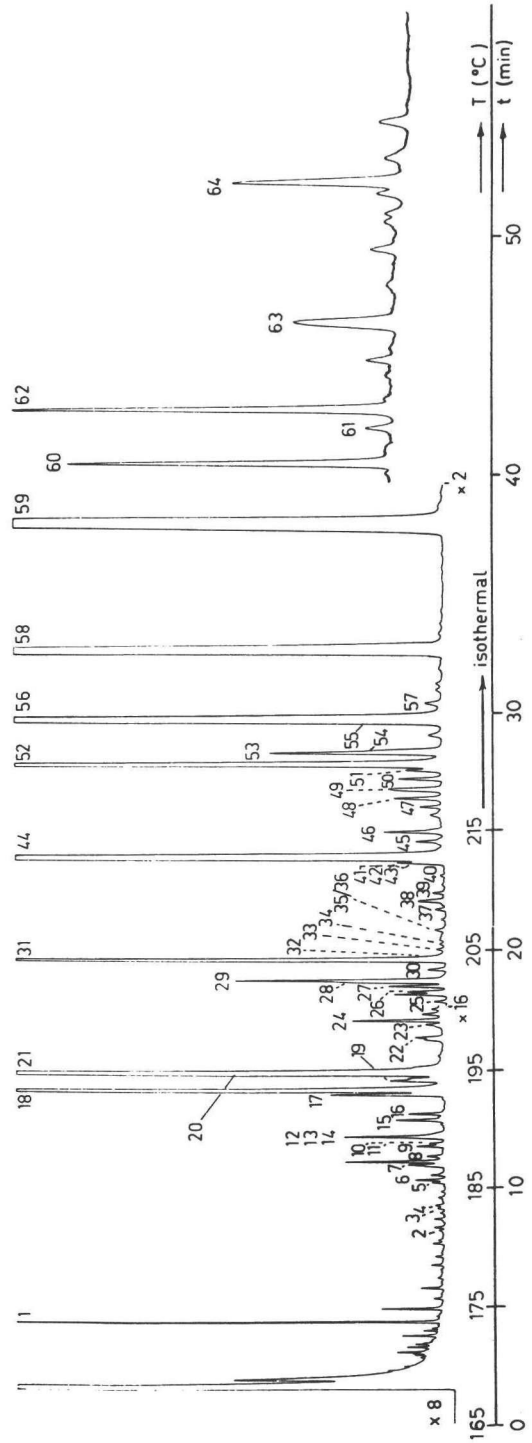

Fig. 2. Gas chromatogram of the alditol acetates obtained from the hydrolyzate of the Solar Lake 628-658 mm sample. Peak numbers correspond to the numbers listed in Table 2. (For conditions : see Experimental)

ribitol and of 3-O-methyl-glucitol coelute with other components when CPsil 88 was used as stationary phase and consequently they were omitted from this survey. Since the separate determination of the peak areas of the coeluting enantiomeric pair 2-/4-O-methyl-xylitol and 3-O-methyl-xylitol peracetates (components J and K) was not possible on this stationary phase they are regarded as a single component in Fig. 5.

DISCUSSION

The Solar Lake stromatolitic environment is characterized by the presence of a benthic cyanobacterial population, which is overgrown by a similar new one in an annual cycle. As a result a laminated deposit has developed in a continuous process for 2400 years. The top mat represents the living primary photosynthetic producer. The underlying layers represent the successive stages of bacterial decomposition of the original organic matter. Each sample is characterized by the presence of residual organic matter originating from the primary producers on the one hand and organic matter (also in various stages of decomposition) which was generated by bacteria that live and have lived in the particular layer on the other hand.

The mode and degree of decomposition of the organic constituents is thought to be determined mainly by their biodegradability. However, at a certain stage the biodegradation might be inhibited because of lithification (Boon and de Leeuw, 1983). By this lithification process structural components of the organisms, notably carbohydrates, might be preserved (Golubic, 1983).

Bulk parameters

The total amount of organic carbon as a function of depth in the core shows a strong decrease from about 27% to 5% in the top 10 millimeters, which coincides with the top oxic part of the deposit (Table 1, Fig. 1). Underneath these 10 millimeters the changes in the percentages of organic carbon in the anoxic (sulfide containing) zone and the lower non-reducing part, starting immediately below it, are minimal. The initially strong decrease in the percentage of organic carbon relative to the total sedimentary material is caused partly by biodegradation under oxic conditions of organic matter generated in the living top mat. The decrease of the relative amount of organic carbon coincides with an increase of fixed carbonate as a result of lithification, which starts immediately below the living top (0.5 millimeter) mat. Therefore not only bicarbonate present in the pore water but also the carbonate produced during bacterial mineralization of organic matter must be considered as a carbonate source. The lithification process causes a considerable increase of the

inorganic sedimentary material and as a result this process is largely responsible for the observed decrease in the relative amount of organic carbon especially in the top layers of the deposit.

Individual monosaccharides

Monosaccharides present in the hydrolyzates are derivatized and analysed by GLC. Fig. 2 shows a typical example of a gas chromatogram of the alditol acetates. This chromatogram demonstrates the complexity of the distribution of common (major) and less common (minor) monosaccharides released upon hydrolysis (Table 2).

Fig. 3 shows the visual representation of the hydrolyzable carbohydrate carbon relative to the total organic carbon as a function of depth (Table 1). The observed profile shows that the relative composition of the organic matter changes with depth. The decrease of the relative contribution to the organic carbon of hydrolyzable carbohydrates can be interpreted by suggesting that carbohydrates are (bio-)degraded or (bio-)transformed more selectively than other organic component classes. On the other hand it cannot be precluded that tightly bound or (microbially) altered carbohydrates escape from our analyses. The slightly, temporary increase of the relative amount of carbohydrates in the sulfide containing zone can be explained by opposite arguments : more selective (bio-)degradation of non-carbohydrate organic components and/or bacterial biogenesis of carbohydrate-rich organic matter, which in turn is subjected to degradation at greater depth. The relative contribution to the organic carbon of the individual monosaccharides will be discussed in this context.

When the variations in the relative contribution to the organic carbon of the various monosaccharides (Table 1, Fig. 4) as a function of depth are studied, the monosaccharides can be devided into four classes.

Class A represents glucose. Glucose shows initially a very strong decrease until about 10 millimeter depth and stabilizes at greater depth. The strong decrease is thought to be mainly the result of the disappearance of storage carbohydrates (cyanophycean starch) synthesized in the top layer of the deposit. Cyanobacteria are known to accumulate polyglucose under certain conditions (Lehmann and Wöber, 1976; Smith, 1982).

Class B monosaccharides rhamnose and "others". Rhamnose and the less common (minor) monosaccharides making up the group of "others" show a similar behaviour as glucose although the initial decrease is much less drastic (Table 1, Fig. 4). The greater part of the minor monosaccharides are O-methylated monosaccharides (Fig. 2). These compounds are thought to originate mainly from gram negative (cyano-)bacterial cell wall associated carbohydrates

(Klok *et al.*, 1984). Rhamnose and glucose are also well known building blocks of these carbohydrates (Hough and Richardson, 1967b; Schmidt *et al.*, 1980a; Weckesser *et al.*, 1979).

Hence the similarity in the initial decrease of rhamnose and "others" (and partly of glucose as well) in the top 10 millimeter may be interpreted in terms of a selective degradation of these cell wall associated carbohydrates when compared with other carbohydrates present in the living top mat (except cyanophycean starch). The small and temporary increase of the contribution of class B monosaccharides in the sulfide containing zone may be the result of the genesis of structural carbohydrates by sulphate reducing bacteria, since in this part of the core a completely new population of bacteria develops, which continue to biodegrade the organic matter still present.

Class C monosaccharides (fucose, arabinose, xylose, mannose and galactose) show a slightly increasing or meandering profile (Fig. 4) in the top 10 millimeter of the deposit. Their contribution maximizes in the sulfide containing zone. Underneath this section the contribution of the class C monosaccharides stabilizes or slightly decreases (Fig. 4). This behaviour can be explained by suggesting that these monosaccharides mainly are part of carbohydrate structures originating from the primary (cyanobacterial) producers, with a much higher resistance towards degradation than storage carbohydrates. These more resistant carbohydrates are probably part of the sheath material produced by the original cyanobacteria (Boon *et al.*, 1983). The environmental change (from oxic to anoxic conditions) which coincides with the development of a new population of (sulphate reducing) bacteria with a molecular composition probably enriched in (these)carbohydrates might account for the temporary increase of the contribution of class C monosaccharides.

Class D comprises only ribose, the contribution of which to the organic carbon slightly decreases with depth (Table 1, Fig. 4). Only in 3 - 10 millimeter sample a twofold relative contribution is observed compared to the two samples just above indicating a very specific source of ribose in this particular sample. Ribose is a constituent of many metabolically important molecules like RNA, nucleosides and NAD(P)H and is suggested therefore to be a marker for the biological activity in the samples. It cannot be precluded however that ribitol, a constituent of bacterial teichoic acids, also contributes to the amount of ribose determined (Rogers *et al.*, 1980).

Minor components

The greater part of the less common monosaccharides grouped as "others" in Table 1 consists of mono-O-methyl monosaccharides. These components present in this environment must originate mainly from carbohydrates associated with bac-

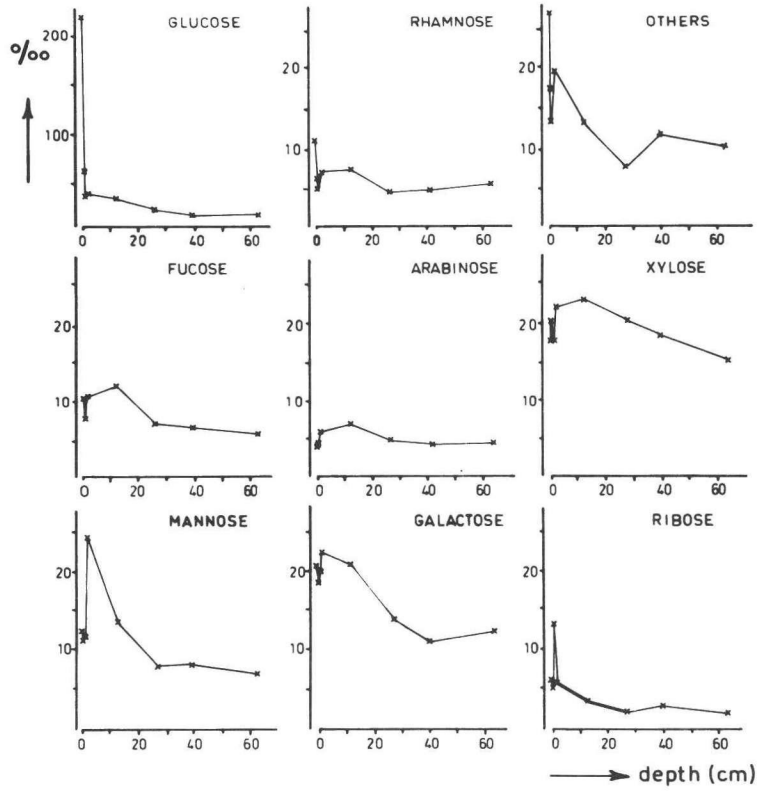

Fig. 4. Depth profiles of the concentration of the various monosaccharides relative to the total organic carbon.

terial cell walls (Klok *et al.*, 1984). Since these carbohydrates have a strain specific monosaccharide composition, the diagram of the 0 - 0.5 millimeter sample (Fig. 5), representing the living top mat, may be interpreted as a fingerprint of the (cyano-)bacterial population (Klok *et al.*, 1984). The relation of the individual minor components with the organisms microscopically observed is not clear however, since the natural occurrence of the greater part of the identified monosaccharides has not (yet) been described in the literature. A second problem is the fact that the bacterial communities present in these sediment samples consist of scarcely studied species. The diagram corresponding with the samples taken from other parts of the core (Fig. 5) represent the cumulative contribution of bacterial populations that live and have lived in the particular sample.

The 0.5 - 3 millimeter sample represents the first decompositional stages of the cyanobacterial mats. When the diagram corresponding with the 0 - 0.5 millimeter and 0.5 - 3 millimeter samples are compared (Fig. 5) a number of differences are observed. The drastic decrease in the relative contribution of component T (6-OMe-glucose) is most striking. This component therefore must be

very specific for the organisms living in the top 0.5 millimeter of the mats. A poly-6-O-methyl glucose has been described to occur in gram positive mycobacteria in which it probably serves as lipid carrier for the unusually large and mycobacteria-specific fatty acids in the cell (Ballou, 1981). The abundance of component T in the top 0.5 millimeter might therefore be related to the presence of carbohydrates with similar functions in related organisms. Moreover the cyanophycean starch may also be suggested as a possible origin, since the decrease in the relative amount of this component T coincides with the decrease in the amount of glucose storage polysaccharides.

The other mono-O-methyl monosaccharides also show considerable changes in their relative contributions. These changes have to be ascribed directly to the decomposition of the top mat material. The strong decrease in the relative contribution of the group "others" to the organic carbon (Table 2) coincides with these changes. Obviously the selective decomposition of O-methyl monosaccharide containing carbohydrates starts immediately below the top 0.5 millimeter. They are partly replaced by similar carbohydrates of the newly developed bacterial population, which are characterized by a different fingerprint of O-methyl monosaccharides.

The differences between the mono-O-methyl monosaccharide patterns of the 0.5 - 3 millimeter sample and the next stages of decomposition (3 - 10 millimeter sample) as shown in Fig. 5 are small compared to the differences between those corresponding with the upper two samples. The similarity between the patterns indicates that the bacterial community responsible for the degradation process does not change significantly. The selective degradation of the carbohydrates associated with the cell walls obviously has continued, since the relative contribution of the group "others" further decreases (Table 1 , Fig. 4).

The 10 - 20 millimeter sample represents the beginning of the sulfide containing zone. The increase in the relative contribution of the minor monosaccharides (Table 1) and also the increase in the total amount of hydrolyzable carbohydrates relative to the organic carbon (Table 1, Fig. 3) indicate a probable increase of the amount of cell wall associated carbohydrates from the newly developed population of sulphate reducing bacteria. As a result the 10 - 20 millimeter mono-O-methyl monosaccharide pattern (Fig. 5) shows large differences when compared with all the patterns obtained for the samples taken in the upper part of the core. It is reasonable to suggest that the components that show a relative increase compared to the 3 - 10 millimeter sample (components A, B, D, E, H, J/K, P and R) mainly originate from bacteria that live at this depth, since the contribution of the minor monosaccharides ("others") increased significantly (Table 1, Fig. 3). The diagram of the 115 - 145 millimeter

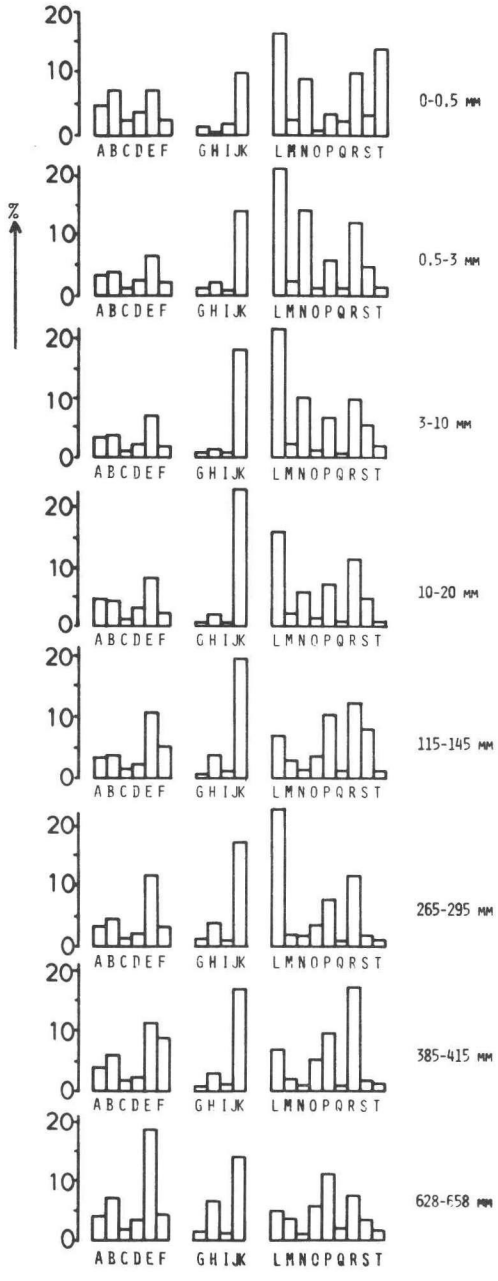

Fig. 5. Diagrams representing the relative concentration of mono-O-methyl monosaccharides as percentage of the total peak area of the corresponding mono-O-methyl alditol peracetates subdivided in groups : 6-deoxy-hexoses (A: 2-OMe-Rha, B: 3-OMe-Rha, C: 4-OMe-Rha, D: 2-OMe-Fuc, E: 3-OMe-Fuc and F: 4-OMe-Fuc), pentoses: (G: 2-OMe-Ara, H: 3-OMe-Ara, I: 4-OMe-Ara, J: 2-/4-OMe-Xyl and K: 3-OMe-Xyl) and hexoses (L: 2-OMe-Man, M: 3-/4-OMe-Man, N: 6-OMe-Man, O: 2-OMe-Gal, P: 3-/4-OMe-Gal, Q: 6-OMe-Gal, R: 2-OMe-Glu, S: 4-OMe-Glu and T: 6-OMe-Glu).

sample again differs from the preceeding sample (10 - 20 millimeter), from which it is separated by an anoxic part of about 10 centimeter length. This sample represents (cyano-)bacterial remains that have also undergone the influences of degradation of a sequence of sulphate reducing bacteria.

The three samples from the lower part of the deposit are taken from the non-reducing section. The conditions in this part of the core might coincide with the development of new bacterial communities. For the interpretation of the observed mono-O-methyl monosaccharide patterns (Fig. 5) similar arguments may be used as described above. Although the percentage of organic carbon in these samples as well as the amount of hydrolyzable carbohydrate carbon hardly shows any change with depth (Fig. 1 and 3) there still exists a significant variation in the distribution patterns of mono-O-methyl monosaccharides (Fig. 5).

Since the group of less common monosaccharides comprises a great number of potential markers for carbohydrate structures associated with bacterial cell walls e.g. lipopolysaccharides (LPS), the variations observed in the mono-O-

methyl monosaccharide patterns throughout the deposit (Fig. 5) suggest that these carbohydrates are subjected to a rapid turnover at all depths. Saddler and Wardlaw (1980) studied the distribution of autochthonous LPS in recent marine sediments and observed a rapid decrease with depth. They also observed that the carbohydrate part of allochthonous LPS incubated in the sediment was readily biodegraded. This is in accordance with our findings and it confirms our suggestion that bacteria in the sediment column are active in transforming and/or mineralizing the carbohydrates associated with cell walls originating from bacteria of previous populations. Because in general only a minor part of the LPS consists of O-methylated and other specific monosaccharides and the major part is formed by other neutral, but less strain specific, monosaccharide building blocks, the latter must be subjected to a rapid turnover also.

CONCLUSIONS

From the data discussed above it is clear that in the Solar Lake deposit a number of carbohydrate categories may be distinguished.

Category I carbohydrates comprise the storage carbohydrates, which are generated in relatively large amounts in the top mat and which are readily degraded.

Category II carbohydrates comprise the remaining (non-storage), mainly structural carbohydrates biosynthesized in the top mat. Among this category carbohydrates associated with bacterial cell walls are included, e.g. lipopolysaccharides which appear to be susceptible to a rapid turnover. The more resistant part of the category II carbohydrates originates from other structural carbohydrates as present e.g. in the sheath of *Microcoleus*. This resistance might be enhanced by preservation due to lithification, henceforth inhibiting further microbial decomposition.

Category III carbohydrates are the carbohydrates formed by the bacteria that are living on the remains of the cyanobacterial mats. This category comprises apart from the carbohydrates characterized by a rapid turnover (e.g. lipopolysaccharides) also more stable structural carbohydrates. The latter could also be prevented from biodegradation by the preservative lithification process.

The present data show that carbohydrates in the stromatolitic Solar Lake deposits represent a significant part of the organic matter, also after a period of 2400 years. Carbohydrates with a high potential to become part of the organic matter that survives geological periods originate from the resistant structures among the categories II and III. Since high amounts of specific carbohydrate pyrolysis products are generated from the samples under

investigation (Boon *et al.*, 1983) and also from kerogens derived from fossil stromatolites (Zumberge and Nagy, 1979; Nagy, 1976; Nagy *et al.*, 1977; Sklarew and Nagy, 1979) these resistant structures may be suggested to be precursors for the carbohydrates thought to be present in ancient stromatolites.

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CHAPTER 7

THE MODE OF OCCURRENCE OF LIPIDS IN A NAMIBIAN SHELF DIATOMACEOUS OOZE WITH EMPHASIS ON THE β-HYDROXY FATTY ACIDS*

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ABSTRACT

Lipids present in a recent marine sediment sample are extracted, derivatized and analysed by GC and GCMS, without any preseparation. This method offers the possibility for the simultaneous determination of various classes of lipids. By means of selective treatments prior to the total lipid extraction, we are able to speculate about the mode of occurrence in the sediment and about the origin of individual lipids released. The significant contribution and the structural features of the β-hydroxy fatty acids among the lipids released after acid treatment point to the presence of bacterial cell wall material in the sediment sample. This suggestion is supported by a separate isolation of bacterial cell wall lipopolysaccharide.

*Submitted for publication to *Organic Geochemistry*

INTRODUCTION

As a part of an investigation on the composition and origin of the total organic matter in a recent marine sediment we intend the release and identification of a maximum amount of the organic carbon. For this purpose a Namibian Shelf diatomaceous ooze sample is extracted subsequently with water and acid of increasing concentration. The organic compounds thus released are further analysed for the presence of component classes e.g. amino acids, monosaccharides, volatile fatty acids and lipids (Klok *et al.*, in press A).

Lipids present in residues and extracts are analysed separately according to the slightly modified "total lipid" procedure, previously described by de Leeuw *et al.* (in press). The application of this particular procedure offers the possibility to study simultaneously various classes of lipids without the intervenience of TLC or other types of preseparations. In this way one can study the relative quantities in which not only the various individual lipids, but also the various classes of lipids occur in a sample. By studying the total lipids present in both water- and acid extracts and in residues before and after extraction, we are able to speculate about the mode of occurrence of individual lipids in the sedimentary material and consequently about their origin.

In this study a considerable amount of β-hydroxy fatty acids is encountered among the total lipids releasable from residues after acid treatment, indicating an amide type linkage. Since amide bound β-hydroxy fatty acids are strong indicators for bacterial cell wall material and because of their cooccurrence with other specific components associated with these walls e.g. O-methylated-, deoxy monosaccharides and heptoses (Klok *et al.*, in press B), we suggest the presence of bacterial cell wall material in the sediment sample. This suggestion is supported by a separate isolation of a high molecular weight substance, which upon hydrolysis reveals to consist of β-hydroxy- and other fatty acids and monosaccharides specific for bacterial cell wall lipopolysaccharides.

EXPERIMENTAL

Sediment samples from the Namibian Shelf were collected on a cruise during December 1968 - January 1969 (Eisma, 1969). The KD6 core ($22^{o}30'S$ $14^{o}05.7'E$), sampled underneath a water column of 106 m, was sectioned and stored at $-20^{o}C$ until use. The 40-75 cm section used for this study was homogenized and lyophilized prior to extraction as outlined in Fig. 1. A brief summary of the extraction procedure is given below; details will be published elsewhere (Klok

et al., in press A). The dry starting material was refluxed with water during 69 hours. After cooling the suspension was centrifuged during 10 minutes at 2700 g and the supernatant and several washings were filtered over a G4 filter. The residue (R2) was lyophilized and refluxed with 2N HCl during 22 hours. After separation of the extract and several washings with water, the residue was lyophilized. Similarly residue R3 and also the starting material (R1) were refluxed with 6N HCl during 24 hours and treated as described above.

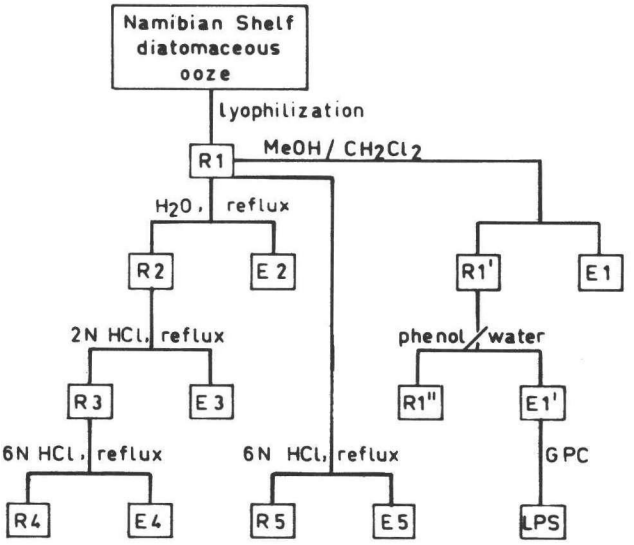

Fig. 1. Extraction scheme (R = residue, E = extract, LPS = lipopolysaccharide)

Total lipids in residues

The procedure for the determination of total lipids is schematically presented in Fig. 2. About 200 mg of the lyophilized starting material (R1) and residues R2 - R5 each were saponified in 4 ml 1N KOH in 96% methanol under reflux during 1 hour. After centrifugation (5 minutes, 3600 g) the supernatant was transferred into a separatory funnel. The residue was washed with 4 ml of 2N HCl in water/methanol (1:1), water/methanol (1:1), dry methanol (two times) and with dichloromethane (three times) subsequently. The combined extract and washings (pH ∿3) were phase separated by addition of about 5 ml of a saturated solution of NaCl in water. The dichloromethane layer was separated and the water layer was washed twice with dichloromethane. The combined dichloromethane extracts containing the total lipids were washed with water, dried on anhydrous Na_2SO_4 and evaporated to dryness under nitrogen. Free carboxyl groups were esterified with diazomethane in diethyl ether. Free hydroxyl groups were silylated using Trisil-Z (Pierce). After derivatization the lipid sample was diluted with cyclohexane/pyridine/HMDS (98:1:1) and chromatographed with this

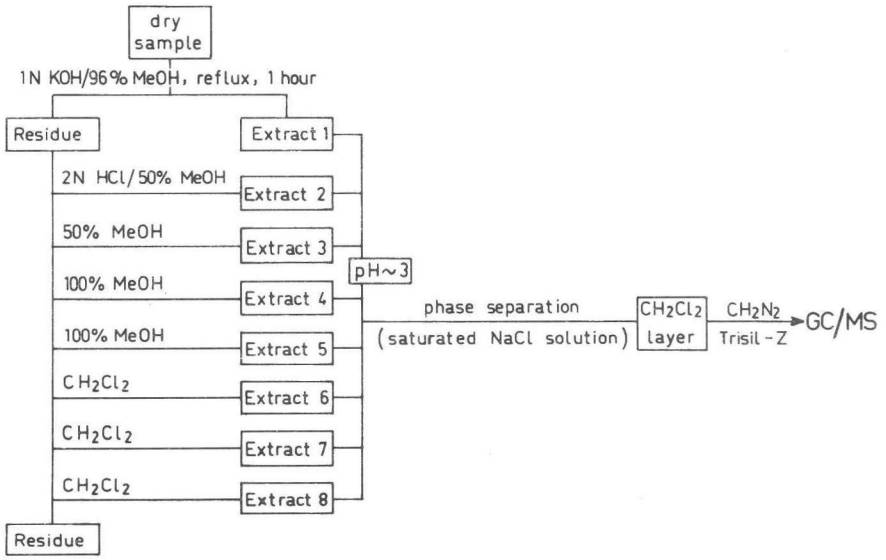

Fig. 2. Schematic representation of the procedure for determination of total lipids.

eluent over Lipidex 5000 (Packard) in order to remove very polar compounds. The resulting solution was concentrated and analysed by capillary gas chromatography and capillary gas chromatography-mass spectrometry.

Free lipids present in the starting material (R1) were extracted with methanol and dichloromethane as described by Bligh and Dyer (1959). Aliquots of the extract thus obtained were evaporated to dryness under nitrogen and derivatized a: as such , b: after saponification with 1N KOH and c: after hydrolysis with 4N HCl in a sealed glass ampoule at 100°C during 6 hours and subsequent saponification with 1N KOH.

Total lipids in the residue after phenol/water extraction (R'') were analysed after steam distillation and lyophilization of this residue prior to hydrolysis with 4N HCl and saponification with 1N KOH as described above.

Total lipids in extracts

An aliquot of the water extract (E2) was lyophilized, saponified with 1N KOH in methanol and derivatized as described above. After the pH of the acid extracts E3 - E5 was adjusted to ∿3 using NaOH, the lipids were extracted with dichloromethane (two times). The dichloromethane extract was evaporated to dryness under nitrogen and saponified as described above.

Gas chromatography and gas chromatography - mass spectrometry

Gas chromatography was carried out on a Carlo Erba 4160 instrument equipped with a flame ionization detector, an on-column injection system

(Grob and Grob, 1978) and a glass capillary column coated with SE52 (20 m, I.D. 0.32 mm). Helium was used as the carrier gas. Samples in dichloromethane or ethyl acetate were injected at 125 °C and the temperature was programmed at 4 °C per minute to 310 °C.

Gas chromatography-mass spectrometry was carried out using a Varian 3700 gas chromatograph connected to a Varian Mat 44 quadrupole mass spectrometer operated at 80 eV.

Isolation of lipopolysaccharide (LPS)

The experimental conditions for the isolation and component analysis of sedimentary LPS were previously tested using lyophilized *Escherichia coli* MRE 600 cells according to the procedure described by Westphal and Jann (1965) with some modifications.

5 g of the dry residue R1' (Fig.1) or 0.7 g dry *E. coli* cells were suspended in 100 ml (30 ml in the case of *E. coli*) 45% phenol/water (v/v) at 68 - 70 °C. The mixture was stirred vigorously during 25 minutes at this temperature, cooled on ice until the temperature was below 10 °C and centrifuged at 2700 g for 30 minutes to obtain optimal phase separation. After separation of the water phase the remaining residue and phenol layer were washed with water by stirring at 68 - 70 °C as described above. The combined water phases were concentrated at reduced pressure and chromatographed on Bio-gel P10 (100-200 mesh 34 cm, I.D. 1.2 cm) using 0.05M NH_4Ac buffer (pH 7-8) as eluent. The refractive index of the eluate was recorded using a Waters-R401 RI-detector. The high molecular weight fraction (mol. w. > 10.000 D) was collected. After the addition of ∿30 µg RNaseI (Boehringer), activated in 0.9% NaCl for 10 minutes at 80 °C, the RNA possibly present was fragmented during incubation at 37 °C for 1 hour. The resulting solution was concentrated and chromatographed on the Bio-gel P10 column as described above. The high molecular weight fraction was collected and evaporated to dryness under reduced pressure.

RESULTS

Lipids present in the residues and extracts (Fig. 1) are analysed by the total lipid procedure schematically presented in Fig. 2. Based on similarities in the distribution of individual lipids and lipid derivatives the chromatograms of total lipids are categorized into three distinct types. Fig. 3 shows the gas chromatograms of typical representatives of each type : starting material (R1) and residue R2 (R1 shown, Fig. 3a), residues after acid treatment R3, R4 and R5 (R5 shown, Fig. 3b) and the extracts E2, E3, E4 and E5 (E3 shown, Fig. 3c). The distribution of lipids and lipid derivatives obtained

after 4N HCl hydrolysis of residue R1'' is very similar to those obtained from the other residues after acid treatment (Fig. 3b). Identifications of the peaks indicated in Fig. 3 are given in Table 1.

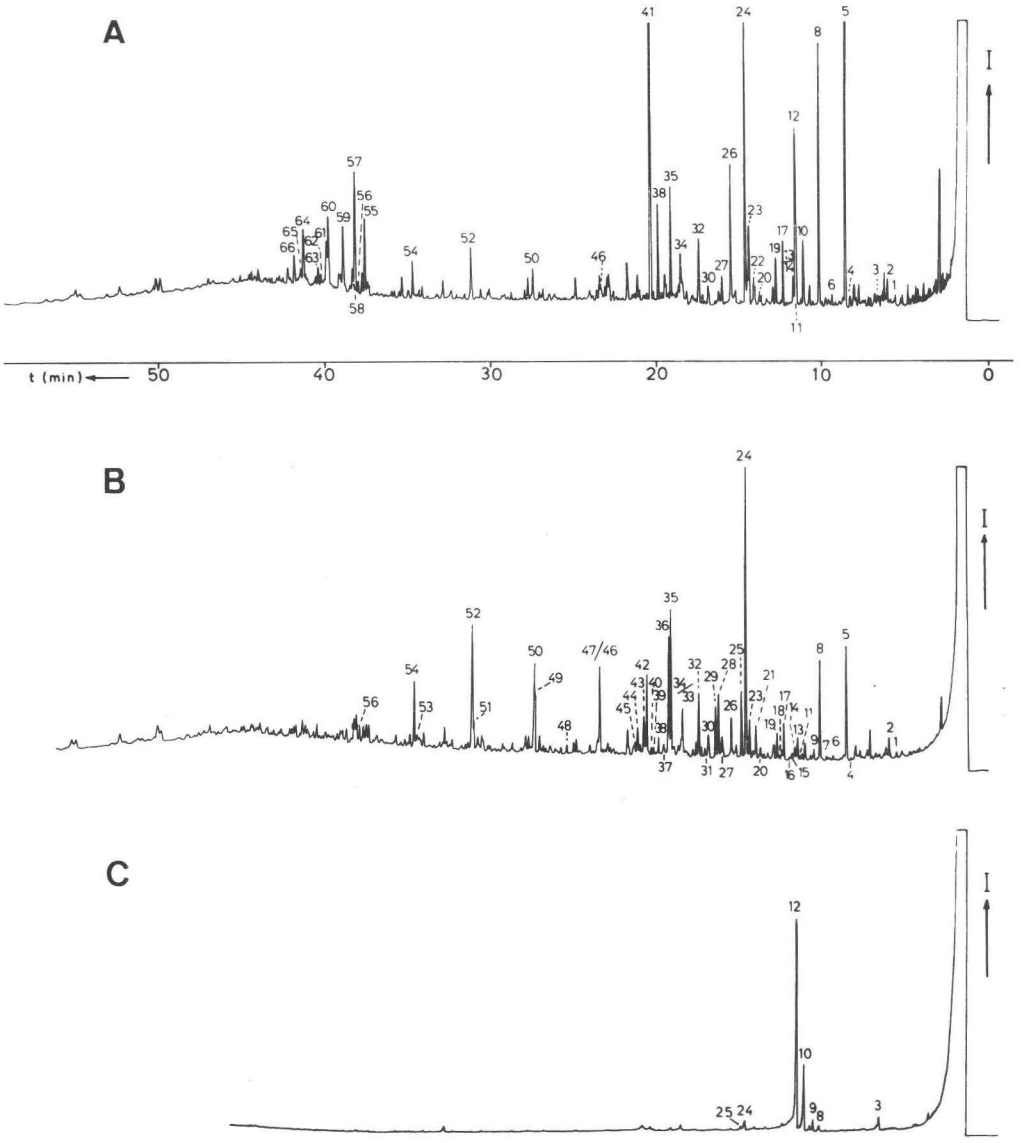

Fig. 3. Gas chromatograms of total lipids obtained from : a starting material R1, b residue R5 and c extract E3. Peak numbers correspond to the identifications given in Table 1.

Prior to the isolation of lipopolysaccharides from the sediment sample the experimental conditions are tested using *Escherichia coli* MRE 600 cells.

Table 1. Identifications of the components indicated in Figs. 3 and 4

nr.	identification[1]	nr.	identification[1]
1	i-C12:0-FA	34	phytanic acid[2]
2	n-C12:0-FA	35	n-C18:0-FA
3	dihydroactinidiolide	36	n-C16:0-βOH-FA
4	n-C13:0-FA	37	C16-thiophene[3]
5	n-C10:0-diFA	38	n-C18:0-OH
6	i-C14:0-FA	39	i-C19:0-FA
7	i-C12:0-βOH-FA	40	ai-C19:0-FA
8	n-C14:0-FA	41	phytol
9	n-C12:0-βOH-FA	42	i-C17:0-βOH-FA
10	iso-loliolide	43	ai-C17:0-βOH-FA
11	n-C14:0-OH	44	n-C19:0-FA
12	loliolide	45	n-C17:0-βOH-FA
13	i-C15:0-FA	46	n-C20:0-FA
14	ai-C15:0-FA	47	n-C18:0-βOH-FA
15	i-C13:0-βOH-FA	48	n-C21:0-FA
16	ai-C13:0-βOH-FA	49	n-C20:0-βOH-FA
17	n-C15:0-FA	50	n-C22:0-FA
18	n-C13:0-βOH-FA	51	n-C22:0-βOH-FA
19	hexahydrofarnesylacetone	52	n-C24:0-FA
20	i-C16:0-FA	53	n-C24:0-βOH-FA
21	i-C14:0-βOH-FA	54	n-C26:0-FA
22	n-C16:2-FA	55	cholesta-5,22-dien-3β-ol
23	n-C16:1-FA	56	n-C28:0-FA
24	n-C16:0-FA	57	cholest-5-en-3β-ol
25	n-C14:0-βOH-FA	58	5-α(H)-cholestan-3β-ol
26	n-C16:0-OH	59	24-methylcholesta-5,22-dien-3β-ol
27	i + ai-C17:0-FA	60	24-methylcholesta-5,24(28)-dien-3β-ol
28	i-C15:0-βOH-FA	61	24-methylcholest-5-en-3β-ol
29	ai-C15:0-βOH-FA	62	23,24-dimethylcholesta-5,22-dien-3β-ol
30	n-C17:0-FA	63	24-ethylcholesta-5,22-dien-3β-ol
31	n-C15:0-βOH-FA	64	24-ethylcholest-5-en-3β-ol
32	unknown	65	24-ethyl-5α(H)-cholestan-3β-ol
33	i-C16:0-βOH-FA	66	4,23,24-trimethylcholest-22-en-3β-ol

1 acid functions are esterified with methanol ; hydroxyl functions are etherified with trimethylsilyl groups
2 tentative identification
3 tentative identification ; mass spectrum described by van Graas (1982)

A high molecular weight fraction from these cells is obtained, which upon hydrolysis reveals to consist of monosaccharides, fatty acids and β-hydroxy fatty acids as previously described by Wilkinson (1977). Since no ribose is detected among the neutral monosaccharides released, the RNase digestion is supposed to be effective. The high molecular weight fraction obtained the sediment sample after GPC of the water phase of the phenol/water extract is hydrolysed with 0.5N sulphuric acid. The monosaccharides thus released are analysed as their alditol acetates (Klok *et al.*, in press B). Rhamnose, arabinose, xylose, mannose, galactose, glucose and one heptose are encountered. The lipid fraction of the high molecular weight fraction obtained after hydrolysis in 4N HCl and subsequent saponification in 1N KOH and derivatization is shown in Fig. 4. Identifications of major peaks indicated in this

chromatogram are given in Table 1. Peaks A (n-C14:1-FAMe), B (n-C14:0-βOH(free) FAMe) and C (n-C14:0-βOH(acetyl)-FAMe) are the result of partial loss of water or the silyl group or esterification with acetic acid of the abundant n-C14:0-βOH-FA respectively. Minor fatty acids, not indicated in Fig. 4 but also identified, are the fatty acids n-C14:0 and n-C17:0 and the βOH-fatty acids n-C12:0, n-C13:0, n + i + ai-C15:0, n + i-C16:0, i + ai-C17:0 and n + i-C18:0.

The results of the total lipid analyses of residues and extracts mentioned in Fig. 1 are briefly summarized in Table 2.

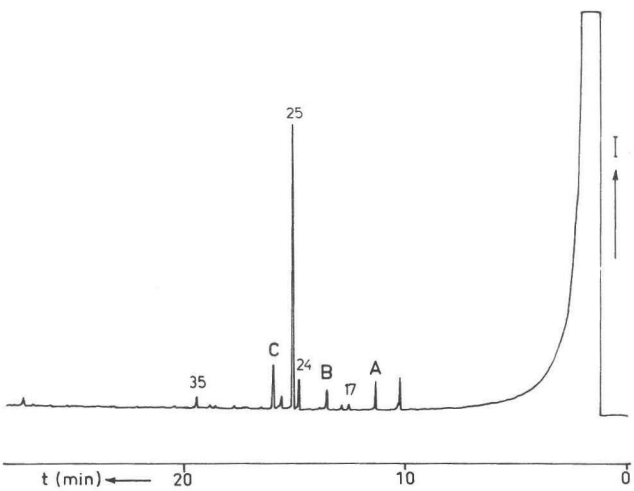

Fig. 4. Gas chromatogram of the lipids obtained from the high molecular weight fraction isolated from the sediment by extraction with phenol/water. Peak numbers correspond to the identifications given in Table 1 ; A: n-C14:1-FAMe, B: n-C14:0-βOH(free)-FAMe and C: n-C14:0-βOH(acetyl)-FAMe.

DISCUSSION

As in most other recent marine sediment samples the major classes of lipids observed in the total lipid chromatogram obtained from the Namibian Shelf diatomaceous ooze comprise acids, alcohols and sterols. Additionally several loliolide type lactones are identified (Klok *et al.*, submitted). The lipid classes observed among the total lipids obtained from the starting material (R1) and the residue R2 (Fig. 3a) are also observed in the total lipid traces corresponding to the residues after acid treatment (R3 - R5, Fig. 3b). However, phytol and the loliolides, major components among the total lipids derived from the residues before acid treatment, have disappeared. β-Hydroxy fatty acids, not detected in the gas chromatograms of the total lipids from the residues before acid treatment, contribute significantly to the amount of lipid material extractable from the residues after acid treatment (Fig. 3b).

Table 2. Overall results of the total lipid analyses of residues and extracts mentioned in Fig. 1.

sample	treatments[1]	FA[2]	βOH-FA[3]	n-alcohols	phytol	sterols	loliolides
R1	(-),1N KOH	++	tr	+	+++	++	++
R2	(H_2O),1N KOH	++	tr	+	+++	++	++
R3	(H_2O,2N HCl),1N KOH	+++	++	+	-	++	+
R4	(H_2O,2N HCl,6N HCl),1N KOH	+++	++	+	-	+	-
R5	(6N HCl),1N KOH	+++	++	+	-	+	-
R1''	(MeOH/CH_2Cl_2,phenol/water),4N HCl,1N KOH	+++	++	+	-	-	-
E1	(-),-	+	-	-	-	+	+++
E1	(-),1N KOH	++	-	-	+++	+	++
E1	(-),4N HCl,1N KOH	++	++	-	++[4]	+	+++
E2	(H_2O),1N KOH	+	-	+	-	-	+++
E2	(H_2O),4N HCl,1N KOH	+	-	+	-	-	+++
E3	(2N HCl),1N KOH	+	tr	tr	-	-	+++
E4	(6N HCl),1N KOH	+	tr	-	-	-	+++
E5	(6N HCl),1N KOH	+	tr	-	-	-	+++
LPS	(phenol/water,GPC),1N HCl	+++	+	-	-	-	-
LPS	(phenol/water,GPC),4N HCl,1N KOH	+	+++	-	-	-	-

[1] treatments in parentheses are indicated in Fig. 1
[2] FA = fatty acids
[3] βOH-FA = β-hydroxy fatty acids
[4] encountered as phytadienes

\+ arbitrary units (+ = present, ++ = abundant, +++ = predominant)
\- not detected
tr trace amount detected

Dihydroactinidiolide, isololiolide and loliolide, stepwise released by extraction with water and acid, are recovered from the extracts. Phytol however is not recovered as such after acid treatment, neither from the acid extracts, nor from the corresponding residues (Table 2). Phytol is therefore thought to be dehydrated to form phytadienes and phytadiene polymers (de Leeuw 1974; Larter *et al.*, 1983) no longer amenable for component analysis.

The results of the total lipid analyses summarized in Table 2 reveal that dihydroactinidiolide and the loliolides are the main free lipids present in the sediment sample, since they are observed as principle components after direct extraction of the starting material (R1) with MeOH/CH_2Cl_2 followed by derivatization. Fatty acids and sterols are also encountered among the free lipids.

Ester linked lipids, released from the residues by saponification with methanolic KOH, mainly consist of phytol and other alcohols, fatty acids and sterols.

Only traces of β-hydroxy fatty acids are encountered among the total lipids obtained from the non-acid treated samples. Acid treated samples, on the contrary show a relative abundance of β-hydroxy fatty acids among the total lipids (Table 2). Previous reports dealing with the analysis of β-hydroxy fatty acids in recent sediments mention heating under nitrogen, harsher (Kawamura and Ishiwatari, 1982) or prolonged saponification with base (Cardoso and Eglinton, 1983) or acid hydrolysis (Cranwell, 1981; Parker *et al.*, 1982) preceeding the lipid extraction for a better recovery of the sedimentary β-hydroxy fatty acids. This behaviour can be explained by suggesting an amide type linkage of these acids, because the amide bond is more slowly hydrolysed

with base. Amide linked β-hydroxy fatty acids are known to occur in various lipid containing structures associated with bacterial cell walls e.g. lipopolysaccharides and ornithine containing lipids (Lechevalier, 1977; Weckesser *et al.*, 1979). The coocurrence of β-hydroxy fatty acids and other specific building blocks of these bacterial cell wall structures in this sediment sample has been noticed before. In a previous study we demonstrated the presence of O-methylated- and deoxy monosaccharides and heptoses specific for lipopolysaccharides and ornithine and O-methylated monosaccharides specific for ornithine containing lipids (Klok *et al.*, in press A). Moreover the relatively high amount of iso- and anteiso β-hydroxy fatty acids point to a contribution to the sedimentary organic matter of bacterial origin (Boon *et al.*, 1977).

An attempt was made to isolate lipopolysaccharide (LPS), a well known cell wall constituent of gram negative bacteria, from the sediment sample as such. For this purpose residue R1' (Fig. 1) is extracted with phenol / water according to the method described by Westphal and Jann (1965) for the isolation of LPS from bacteria. After phase separation the water layer is concentrated and chromatographed over a Bio-gel column for a molecular weight separation. A high molecular weight fraction is obtained. After hydrolysis of this fraction with 0.5N H_2SO_4 several monosaccharides are obtained among which a heptose. Upon hydrolysis with 4N HCl the total lipid fraction contains a variety of β-hydroxy fatty acids and fatty acids (β-hydroxy myristic acid predominating , Fig. 4). Saddler and Wardlaw (1980) reported a similar predominance among the fatty acids present in a phenol/water extractable sedimentary lipopolysaccharide. The presence of specific elements of bacterial LPS (heptoses and amide linked β-hydroxy fatty acids) strongly suggest that the high molecular weight fraction comprises bacterial LPS.

In this way we indirectly demonstrated the occurrence of bacterial cell wall structures in the sediment sample. The significant presence of β-hydroxy fatty acids among the lipids releasable from the acid treated sediment samples (∿30% of the total peak area in the chromatogram shown in Fig. 3b) points to a considerable contribution of bacterial origin to the organic matter present therein.

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CHAPTER 8

LOLIOLIDES AND DIHYDROACTINIDIOLIDE IN A RECENT MARINE SEDIMENT PROBABLY INDICATE A MAJOR TRANSFORMATION PATHWAY OF CAROTENOIDS*

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ABSTRACT

In the course of our investigation dealing with the composition of the total organic matter in a recent marine sediment[1] we encountered dihydroactinidiolide, loliolide and iso-loliolide among the free lipid components present. Based on literature data and because of the structural relationship of these compounds with carotenoids we suggest that they are the photo-oxidation products of carotenoids originating from marine algae.

*To be submitted

EXPERIMENTAL

Sediment samples were collected during a cruise in December 1968 - January 1969[2]. Core KD6, sampled underneath a water column of 106 meter (22°30'S 14° 0.57'E) on the Namibian Shelf was sectioned and stored at -20°C until use. The 40 - 75 centimeter section of the core was lyophilized and refluxed subsequently with water, 2N HCl and 6N HCl. The starting material, the intermediate and final residues and also the water and acid extracts were analysed for total lipids.

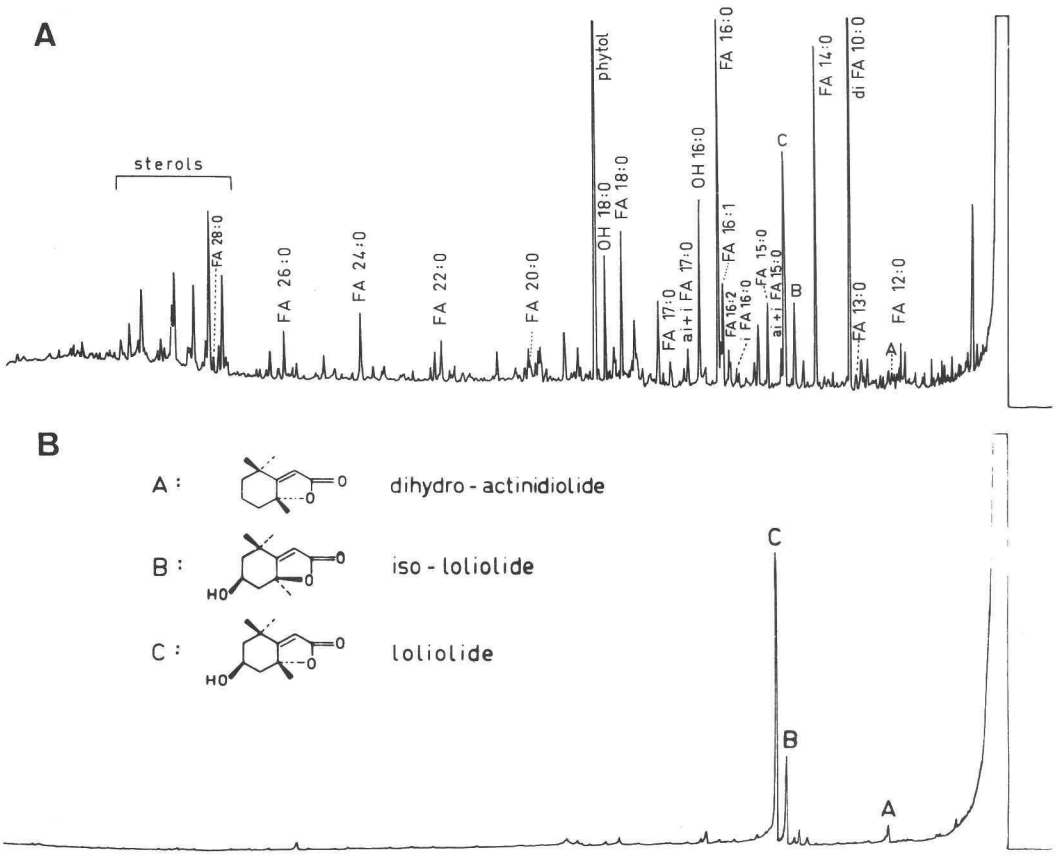

Fig. 1. Capillary gas chromatogram of total lipids obtained from a) the Namibian Shelf sediment and b) the 2N HCl extract. A Carlo Erba Fractovap 4160 gas chromatograph equipped with an on-column injection system and a 20 m × 0.32 mm glass capillary column coated with SE-52 was used. Samples in dichloromethane were injected at 125°C and the temperature was programmed with 4°C per minute to 310°C.

Total lipids were released from the lyophilized residues after saponification with 1N methanolic KOH under reflux. After 1 hour the mixture was centrifuged and the extract was transferred into a separatory funnel. The residue

was washed subsequently with 2N HCl/50% MeOH, 50% MeOH, 100% MeOH (two times) and CH_2Cl_2 (three times). After adjustment of the pH to ∿3 the combined extract and washings were phase separated by the addition of a saturated solution of NaCl in water. The CH_2Cl_2 layer containing the total lipids was dried over anhydrous Na_2SO_4. The total lipids thus obtained were derivatized with diazomethane to esterify free carboxyl groups and subsequently with Trisil-Z (Pierce) to silylate free hydroxyl groups. The resulting mixture was chromatographed over Lipidex-5000 (Packard) to remove very polar compounds. The eluate was concentrated and analysed with capillary GC and GC-MS.

Free lipids present in the sediment sample were extracted with MeOH and CH_2Cl_2 and derivatized in a similar way. The lipids present in water- and acid extracts were extracted with CH_2Cl_2, in the case of acid extracts after the pH had been adjusted at ∿3 and saponified and derivatized as described above.

The identification of dihydroactinidiolide and loliolide are based on capillary gas chromatographic and mass spectrometric data. The GC retention times of component *A* and *C* (Fig. 1) are identical to those of the authentic dihydroactinidiolide and loliolide respectively upon coinjection on SE-52. The EI mass spectra of components *A* and *C* and those of the standard dihydroactinidiolide and loliolide are shown to be identical (Fig. 2). Since no standard of iso-loliolide was available the identification is based on the mass spectrometric fragmentation of the silylderivative, which is similar to the fragmentation pattern of silyl loliolide (Fig. 2).

RESULTS AND DISCUSSION

The natural occurrence of dihydroactinidiolide and loliolide is mainly reported in plant material [3-6]. They have become especially known as flavour compounds in tea[7] and tobacco[8,9]. Some authors suggest that these terpenoid flavour compounds are at least partly generated from carotenoid precursors during harvesting and/or curing treatments. These compounds are also reported to occur in the animal kingdom. Loliolide is isolated from the marine mollusc *Dolabella ecaudata*[10], whereas dihydroactinidiolide is known as the sexpheromone of the red fox *Vulpes vulpes*[11].

Since dihydroactinidiolide and the loliolides could also be isolated from the sediment sample by a simple extraction with MeOH and CH_2Cl_2 without any base or acid, we beleave that they are not artificial degradation products of carotenoids generated during the extraction and/or derivatization procedures. The significant presence of these free components in the Namibian Shelf diatomaceous ooze sample (up to ∿0.2% of the total organic matter) suggests an

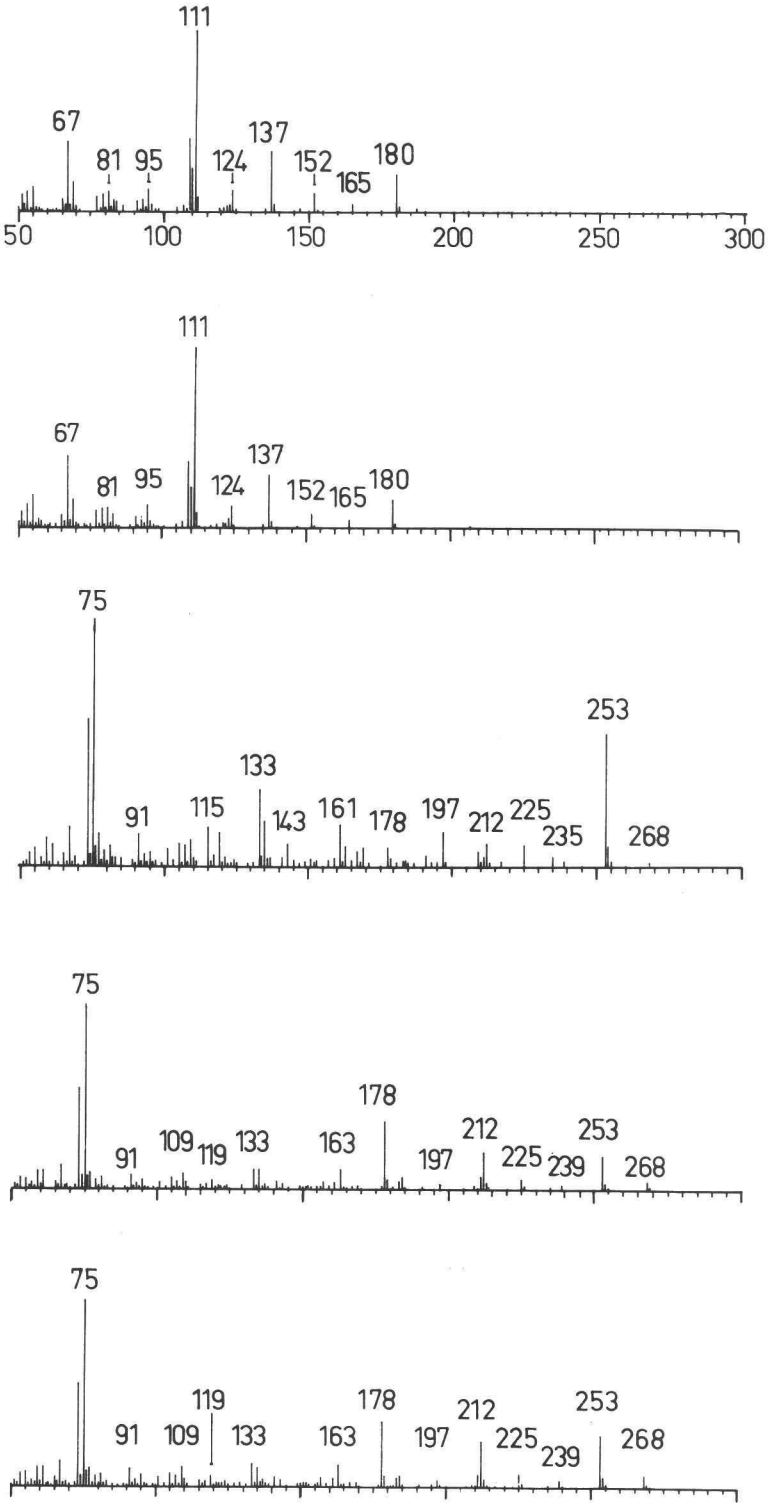
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Fig. 2. (Opposite page) Electron impact mass spectra of a) authentic dihydroactinidiolide, b) component A, c) component B (isololiolide-TMSi), d) authentic loliolide-TMSi and e) component C. Mass spectra were recorded using a Varian 3700 gas chromatograph equipped with a 25 m × 0.2 mm glass capillary column coated with CPsil 5 connected with a Varian Mat 44 mass spectrometer operated at 80 eV.

origin from diatoms and/or dinoflagellates.

Dihydroactinidiolide and loliolides are generally observed as major products of the photo- or chemical oxidation of carotenoids depending on the functionality at C-3[12-16] (Fig. 3). The absence of an oxygen function

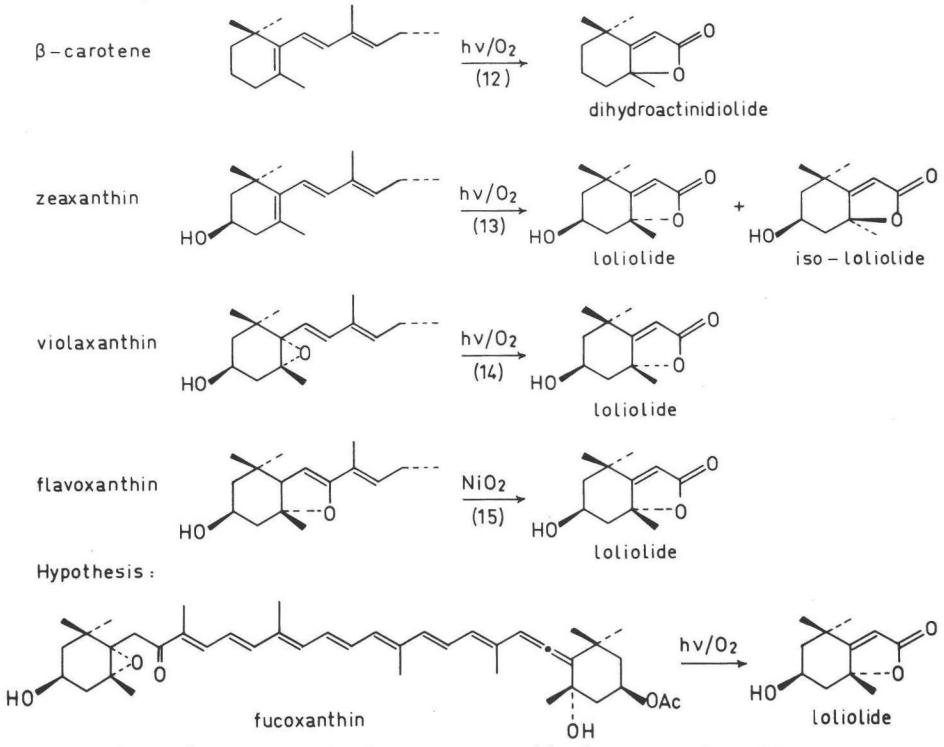

Fig. 3. Schematic representation of the oxidative transformation of carotenoids and the proposed photo-oxidative degradation of fucoxanthin in the marine environment. The numbers underneath the arrows correspond with the numbers in the reference list.

(hydroxyl group or epoxide) at C-5 is not prohibitive for the occurrence of this reaction. Because of the predominance of fucoxanthin among the carotenoids of diatoms and dinoflagellates[17], the principle primary producers in this environment of deposition[18], we suggest a direct formation of the loliolides from fucoxanthin in the oxic zone of the water column. Dihydroactinidiolide may have been formed in an analogous way from carotenoids not functionalized at C-3 (Fig. 3).

ACKNOWLEDGEMENTS

We thank Dr. F. Kienzle (Hoffman-La Roche & Co., Basel, Switzerland) and Dr. O. Takazawa (T. Hasegawa Co. Ltd., Kawasaki, Japan) for supplying us with the authentic samples of loliolide and dihydroactinidiolide.

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SUMMARY AND CONCLUSIONS

This thesis comprises the results of an organic geochemical investigation on the composition and origin of the total organic matter present in recent marine sediments. This organic matter can be considered as in an intermediate stage between biomass and kerogen. The structural elucidation therefore might give valuable information about the "starting structures" of the future kerogen. The release of biopolymers or building blocks of biopolymers from this recent organic matter, which is regarded as an accumulation of biopolymers in various stages of (bio-)chemical degradation, and their quantitation are the main objectives of this investigation. To obtain, in a specific case, a complete inventory of the sedimentary organic matter a diatomaceous ooze from the Namibian Shelf (S.W. Africa) was extracted with water and subsequently with acid of increasing concentration and/or with organic solvents (Chapter 2).

Some analytical techniques for the quantitative determination of neutral monosaccharides were adapted to make them applicable to the analysis of sediment samples.

The analytical data obtained reveal major contributions of carbohydrates, proteins, lipids and volatile compounds to the total organic matter. The components released from the sediment sample point to an algal as well as a bacterial contribution to the sedimentary organic matter. On the one hand the algal contribution is demonstrated by the abundance of building blocks of algal polysaccharides among the neutral monosaccharides quantitated in a sediment hydrolyzate. Moreover some sterols and the loliolide type lactones observed point to an algal source as well. On the other hand a distinct bacterial imprint is demonstrated by the cooccurrence of specific monosaccharides and lipid building blocks from biopolymers associated with bacterial cell walls e.g. lipopolysaccharides.

A major part of the organic matter released by water or acid treatment is of high molecular weight. Pyrolysis experiments have indicated that this material consists of carbohydrates or carbohydrate derivatives.

The residual organic matter insoluble in water, acid and/or in organic solvents was further studied using analytical pyrolysis techniques and showed mainly aliphatic characteristics. Aromatic hydrocarbons (probably partly induced by the chemical treatments), alkylated thiophenes and pyrroles were also observed. The pyrograms of the organic matter in the ultimate residue reveal an aliphatic, partly isoprenoid nature, to some extent comparable to that of pyrograms obtained for kerogens from ancient sediments.

Carbohydrates in recent marine sediment samples were released upon acid hydrolysis. The resulting monosaccharides were derivatized into the corres-

ponding alditol acetates (Chapter 3). Gas chromatographic analysis of the resulting mixtures revealed the presence of the alditol acetates of well known monosaccharides but also of a large number of minor components. For the gas chromatographic-mass spectrometric identification of these components appropriate standards were synthesized (Chapter 4). Ultimate identification showed that partially methylated mono- and/or deoxy monosaccharides were present among the neutral monosaccharides released from various recent marine sediment samples. From a comparative study it was demonstrated that these components might mainly be attributable to bacteria, since their natural occurrence is mainly reported in carbohydrate structures associated with cell walls of gram-negative bacteria (Chapter 5).

The alditol acetate procedure was also applied to a study on the diagenesis of carbohydrates in a recent stromatolitic deposit (Chapter 6). It was demonstrated that carbohydrates represent the major part of the organic matter in the top few millimeters of the sediment and that they still represent a considerable part of the organic matter at a depth of 65 centimeter ($\sim$2400 years b.p.). In this study various types of carbohydrates are distinguishable, each showing a different behaviour upon burial. The structural carbohydrates of the primary cyanobacterial producers are suggested to be preserved, probably as a result of early lithification. This enhances their potential to become a part of the insoluble organic matter precursing kerogen.

In Chapter 7 a method for the analysis of integral lipid extracts from sediment samples is applied. By means of different chemical treatments prior to the lipid extraction the type of linkage of individual lipids or classes of lipids in the sedimentary organic matter from a Namibian Shelf sediment sample was determined. Of particular interest is the substantial contribution of amide bound β-hydroxy fatty acids. The mode of occurrence of these acids and their structural features and distribution strongly indicate the presence of bacterial cell wall structures in the sediment. This suggestion is supported by the cooccurrence in this sediment sample of monosaccharides specific for carbohydrates associated with bacterial cell walls. An attempt was made to isolate lipopolysaccharides as such from the sediment sample. A fraction was obtained, which upon hydrolysis appeared to consist of lipids and monosaccharides specific for the suggested bacterial lipopolysaccharides.

During the analysis of lipid extracts a significant amount of loliolide type lactones were encountered among the free lipids. In Chapter 8 the identification of these components is described. Since these lactones are mainly reported as products of (photo-)chemical transformation of carotenoids, their occurrence in the sediment sample is interpreted as a result of a possible (photo-)chemical transformation of carotenoids in the marine environment.

In view of the results obtained the current concept of kerogen formation as described in Chapter 1 needs some modification. First it should be noted that microbial degradation of organic substrates is generally accompanied by an efficient use of the monomers released. Part of the fragments released is used for the microbial energy requirements, another part is used for biosynthesis. Therefore it is less likely that microbially released monomers should randomly polymerize to a polycondensate. Secondly the biomass of bacteria active in transforming the sedimentary organic matter also represents a certain amount of organic carbon, which, especially when ecological conditions deteriorate, might give a significant contribution to the insoluble organic matter. The presence of distinct bacterial cell wall constituents among the components released and the separate isolation of a bacterial lipopolysaccharide support this suggestion.

The part of the organic matter in the Namibian Shelf diatomaceous ooze ultimately insoluble in water, acid and/or organic solvents shows, when studied with pyrolysis techniques, mainly aliphatic characteristics comparable to some extent to those obtained for kerogens from ancient sediments. These features, which hold for as much as 50% of the total organic matter in this recent marine sediment, indicate that the fate of the greater part of the sedimentary organic matter has already been determined in a very early stage of diagenesis.

SAMENVATTING EN CONCLUSIES

Dit proefschrift beschrijft de resultaten van een organisch geochemisch onderzoek naar de samenstelling en herkomst van het totale organische materiaal aanwezig in recente mariene sedimenten. Aangezien dit organisch materiaal kan worden beschouwd als zijnde in een stadium tussen biomassa en kerogeen, kan de opheldering van de structuur ervan waardevolle informatie opleveren over de "begin-structuur" van het toekomstige kerogeen.

Uit dit recente organische materiaal, beschouwd als een opeenhoping van biopolymeren in verschillende stadia van (bio)chemische afbraak,zijn in dit onderzoek biopolymeren of bouwstenen daarvan vrijgemaakt en kwantitatief bepaald. Teneinde in een bepaald geval tot een volledige inventarisatie van de sedimentaire organische koolstof te komen, werd een sedimentmonster -een diatomeeën-slik afkomstig van het continentale plat voor de kust van Namibië (Z.W. Afrika)- geëxtraheerd met water, vervolgens met zuur van toenemende concentratie en/of met organische oplosmiddelen (hoofdstuk 2).

Het bleek noodzakelijk enige bestaande analysetechnieken voor de kwantificering van neutrale monosacchariden aan te passen, zodat ze toepasbaar werden voor de analyse van sedimentmonsters.

De analyseresultaten geven aan dat koolhydraten, eiwitten, lipiden en vluchtige organische verbindingen belangrijke bestanddelen zijn van het in het monster aanwezige organische materiaal. De organische verbindingen die kunnen worden vrijgemaakt en geïdentificeerd, wijzen op een bijdrage van zowel algen als bacteriën. De algenbijdrage wordt duidelijk uit de relatief hoge concentratie van monosaccharide-bouwstenen voorkomend in algen polysacchariden en uit de aanwezigheid van sterolen en van loliolide-achtige lactonen. Anderzijds blijkt een duidelijke bacteriële bijdrage uit de gelijktijdige aanwezigheid van specifieke monosaccharide- en lipide-bouwstenen van biopolymeren, die met celwanden van bacteriën zijn geassocieerd (b.v. lipopolysacchariden).

Het belangrijkste gedeelte van het in water en zuur opgeloste organisch materiaal blijkt hoogmoleculair te zijn en op grond van pyrolyse-experimenten te bestaan uit koolhydraten, dan wel koolhydraat derivaten.

Het organisch materiaal dat niet oplosbaar blijkt in water, zuur en/of in organische oplosmiddelen is eveneens bestudeerd met analytische pyrolyse technieken. Daarbij is een hoofdzakelijk alifatisch karakter aan het licht gekomen. Bovendien zijn aromatische koolwaterstoffen (die gedeeltelijk kunnen zijn ontstaan als een gevolg van de chemische behandelingen), alkyl-thiophenen en alkyl-pyrrolen aangetoond. De pyrogrammen van het organisch materiaal in het uiteindelijke residu vertonen een aliphatisch, gedeeltelijk isoprenoid, karakter enigszins vergelijkbaar met dat aanwezig in pyrogrammen van kerogenen uit

oude mariene sedimenten.

Koolhydraten aanwezig in recente mariene sedimentmonsters zijn vrijgemaakt d.m.v. zure hydrolyse. De daarbij ontstane monosacchariden zijn vervolgens gereduceerd en gederivatiseerd tot de overeenkomstige alditol acetaten (hoofdstuk 3). Gaschromatografische analyse van de verkregen mengsels toont de aanwezigheid aan van alditol acetaten van de bekende neutrale monosacchariden, maar ook van een groot aantal, uit kwantitatief oogpunt minder belangrijke, andere componenten. Om deze laatste te kunnen identificeren m.b.v. gaschromatografie-massaspectrometrie zijn een aantal standaarden gesynthetiseerd (hoofdstuk 4). De uiteindelijke identificatie heeft aangetoond, dat onder de neutrale monosacchariden in de hydrolysaten van verschillende recente mariene sedimenten partieel gemethyleerde mono- en/of deoxy monosacchariden voorkomen. In een vergelijkende studie wordt aannemelijk gemaakt, dat deze verbindingen hoofdzakelijk zijn toe te schrijven aan bacteriën, aangezien het voorkomen ervan in de natuur voornamelijk gerapporteerd is in de koolhydraat-structuren, die zijn geassocieerd met celwanden van gram-negatieve bacteriën (hoofdstuk 5).

De alditol acetaat methode is eveneens toegepast bij een studie van de diagenese van koolhydraten in een stromatolitische afzetting (hoofdstuk 6). Er is aangetoond dat koolhydraten de grootste bijdrage leveren aan het totaal aanwezige organisch materiaal in de bovenste millimeters van deze afzetting en dat ze nog steeds een significante bijdrage leveren aan het organisch materiaal op ca. 65 centimeter diepte (ca. 2400 jaar oud). Uit deze studie blijkt dat verschillende typen koolhydraten kunnen worden onderscheiden, die ieder een verschillend gedrag vertonen naarmate ze dieper in de sedimentkolom terecht komen. Verondersteld wordt dat de structurele koolhydraten van de primaire cyano-bacteriën worden gepreserveerd, vermoedelijk als gevolg van lithificatie in een zeer vroeg stadium. De kans wordt daardoor vergroot dat zij deel gaan uitmaken van het organisch materiaal dat uiteindelijk tot kerogeen leidt.

In hoofdstuk 7 wordt een methode toegepast voor de analyse van integrale lipide extracten van sedimentmonsters. D.m.v. verschillende chemische behandelingen voorafgaande aan de extractie kan de aard van de binding van individuele lipiden of klassen lipiden in het sedimentaire organisch materiaal van een sedimentmonster van het Namibische continentale plat worden bepaald. Van bijzonder belang is de substantiële bijdrage van amide-gebonden β-hydroxy vetzuren. De wijze van voorkomen van deze verbindingen in het sediment, alsmede hun structuur en verdelingspatroon, vormen een sterke aanwijzing voor de aanwezigheid van bacteriële celwand structuren in het sediment. Deze interpretatie wordt nog gesteund door het gelijktijdig voorkomen van monosacchariden, die specifiek zijn voor koolhydraten geassocieerd met deze celwanden. Er is een poging ondernomen om lipopolysacchariden als zodanig uit het sediment te iso-

leren. Daarbij is een fraktie verkregen, die na hydrolyse bleek te zijn samengesteld uit lipiden en monosacchariden, die specifiek zijn voor bacteriële lipopolysacchariden.

Tijdens de analyse van de lipide extracten is een significante hoeveelheid loliolide-achtige lactonen aangetroffen onder de lipiden die vrij in het sediment aanwezig zijn. In hoofdstuk 8 wordt de identificatie van deze verbindingen besproken. Aangezien deze lactonen voornamelijk zijn beschreven als de (foto-) chemische omzettingsprodukten van carotenoiden, wordt hun voorkomen in het sedimentmonster geïnterpreteerd als het resultaat van een mogelijke (foto-)chemische omzetting van carotenoiden in het mariene milieu.

In het licht van de verkregen resultaten is het o.i. noodzakelijk het gangbare concept van de kerogeenvorming, zoals dat in hoofdstuk 1 is geschetst, enigszins te herzien. In de eerste plaats dient men te bedenken dat microbiële afbraak van organische structuren in het algemeen gepaard gaat met een effectief gebruik van de vrijgemaakte monomeren. Een gedeelte van de vrijgemaakte fragmenten wordt gebruikt voor de energiebehoefte van het micro-organisme ; een ander gedeelte wordt gebruikt voor biosynthese. Daarom is het minder waarschijnlijk dat de langs microbiële weg vrijgemaakte monomeren zullen bijdragen tot de vorming van een polycondensaat. In de tweede plaats vertegenwoordigt de biomassa van bacteriën, die bezig zijn het in het sediment aanwezige organisch materiaal om te zetten, een zekere hoeveelheid organisch materiaal, die, zeker wanneer de ecologische condities extreem worden, een belangrijke bijdrage zou kunnen leveren aan het onoplosbare organische materiaal. De aanwezigheid van duidelijk als bouwstenen van bacteriële celwanden te kenmerken componenten in de sedimentextracten en een afzonderlijke isolatie van bacterieel lipopolysaccharide ondersteunen deze gedachtengang.

Het gedeelte van het organisch materiaal van het sedimentmonster van het Namibische continentale plat dat uiteindelijk onoplosbaar blijkt in water, zuur en organische oplosmiddelen, vertoont bij studie met pyrolysetechnieken voornamelijk een alifatisch karakter en levert pyrolyseprodukten tot op zekere hoogte vergelijkbaar met die van kerogenen uit oude sedimenten. Deze eigenschap, die karakteristiek is voor ongeveer 50% van het organische materiaal in dit recente mariene sediment, geeft een aanwijzing dat het "lot" van het grootste deel van het sedimentaire organisch materiaal al is bepaald in een zeer vroeg stadium van de diagenese.

CURRICULUM VITAE

Jacob Klok werd op 10 september 1954 te Brielle geboren. Na het behalen van het diploma h.b.s.-B aan de toenmalige RHBS in zijn geboorteplaats begon hij in 1972 zijn studie aan de Rijksuniversiteit Leiden. In juni 1975 slaagde hij voor het kandidaatsexamen scheikunde, waarna hij voor zijn doktoraalfase als hoofdvak biochemie en als bijvakken respectievelijk moleculaire genetica en organische fotochemie koos. Zijn afstudeeronderzoek (Translatie van een plantevirus RNA in een cel-vrij *E. coli* systeem) verrichtte hij bij Prof. Dr. L. Bosch onder leiding van Drs. A. Castel en Dr. B. Kraal. Na het behalen van het doktoraalexamen scheikunde (september 1978) trad hij op 1 februari 1979 in dienst van de Technische Hogeschool Delft voor het verrichten van een promotieonderzoek naar de struktuur en de herkomst van complex organisch materiaal in recente mariene sedimenten, waarvan dit proefschrift de verslaggeving omvat. Sinds 1 december 1983 is hij als research chemist werkzaam bij DOW-Chemical (Nederland) B.V. te Terneuzen.

NAWOORD

Gaarne wil ik op deze plaats iedereen memoreren, die op enigerlei wijze aan de totstandkoming van dit proefschrift heeft meegewerkt. In de eerste plaats zijn dat mijn ouders, die mij altijd hebben gesteund en die mij mede in staat hebben gesteld mijn voortgezette opleiding te volgen en af te ronden.
Ik denk met veel plezier terug aan de periode, welke ik bij de onderzoeksgroep Organische Geochemie van de vakgroep Algemene Scheikunde aan de Technische Hogeschool Delft heb doorgebracht. Aan allen die hetzij direct, hetzij indirect het onderzoek hebben ondersteund ben ik veel dank verschuldigd.
De studenten die ik in deze periode in de vorm van praktica heb mogen begeleiden wens ik toe, dat het ook voor hen een vruchtbare tijd is geweest.
De leden van de vakgroep Organische Scheikunde van de Technische Hogeschool Delft ben ik dankbaar voor de hulp bij het verrichten van de vele massaspektrometrische analyses. Voorts spreek ik mijn dank uit aan de leden van de afdeling Biomoleculaire Fysica van het FOM-instituut voor Atoom- en Molecuulfysica te Amsterdam voor het verrichten van pyrolyse massaspektrometrische analyses.
In het bijzonder wil ik ook een woord van dank uitspreken aan het adres van mijn huidige werkgever DOW Chemical (Nederland) B.V., die mij zoveel ruimte heeft gegeven voor de afronding van het manuscript.
Tenslotte, maar zeker niet in de minste plaats wil ik mijn echtgenote danken voor haar voortdurende steun. Door haar en de jongens heeft het allemaal een dimensie meer gekregen.

STELLINGEN

1. Het ontstaan van 2-cyclopentenon en 2,3-dimethyl maleinezuuranhydride bij pyrolyse van polymaleinezuur en het ontstaan van deze verbindingen in relatief kleine hoeveelheden bij pyrolyse van organisch materiaal uit bodems rechtvaardigt niet de conclusie, dat een deel van dat organisch materiaal bestaat uit polycarbonzuren.

   Bracewell J.M., Robertson G.W. and Williams B.L. *J. Anal. Appl. Pyrol.*, 2 (1980) 53-62

   Bracewell J.M., Robertson G.W. and Welch D.I. *J. Anal. Appl. Pyrol.*, 2 (1980) 239-248

2. Bij de verklaring van de aanwezigheid van 4-methylsterolen in sedimentmonsters sluiten Smith *et al.* ten onrechte een mogelijke herkomst van dinoflagellaten uit.

   Smith D.J., Eglinton G., Morris R.J. and Poutanen E.L. *Oceanol. Acta*, 5 (1982) 365-378

   Smith D.J., Eglinton G., Morris R.J. and Poutanen E.L. *Oceanol. Acta*, 6 (1983) 211-219

3. In het algemeen is het niet raadzaam om een krachtveld te "re-parameteriseren" op grond van een kleine hoeveelheid experimentele gegevens.

   Jaime C. and Osawa E. *Tetrahedron*, 39 (1983) 2769-2778

   Van der Graaf B., Baas J.M.A. and Widya H.A. *Recl. Trav. Chim. Pays-Bas*, 100 (1981) 59-61

4. De recentelijk door Gehrke *et al.* afgeleide 5' conformatie van het alfalfa mozaiek virus RNA-4 levert een door de auteurs niet onderkende, doch aantrekkelijke verklaringsmogelijkheid op voor de aanwezigheid van een tweede overlappende startplaats voor de synthese van manteleiwit zoals vastgesteld door Castel *et al.*

   Gehrke L., Auron P.E., Quigly G.J., Rich A. and Sonenberg N. *Biochemistry*, 22 (1983) 5157-5164

   Castel A., Kraal B., Kerklaan P.R.M., Klok J. and Bosch L. *Proc. Natl. Acad. Sci. USA*, 74 (1977) 5509-5513

   Castel A., Kraal B., Konienczny A. and Bosch L. *Eur. J. Biochem.*, 101 (1979) 123-133

5. 2-Methyl-2-cyclopentenon en een trimethyl-2-cyclopentenon, componenten die voorkomen in pyrolysaten van zoet-water huminezuur, kunnen zeer wel een andere herkomst hebben dan het pyrolyseprodukt 2-cyclopentenon.

   Wilson M.A., Philp R.P., Gillam A.H., Gilbert T.D. and Tate K.R. *Geochim. Cosmochim. Acta*, 47 (1983) 497-502

6. Bij de scheiding van eiwitten op aluminiumoxide m.b.v. een fosfaatgradiënt moet, behalve met de twee door Laurent *et al.* genoemde mechanismen, tenminste met nog een derde mechanisme rekening gehouden worden.

   Laurent C.J.C.M., Billiet H.A.H., de Galan L., Buytenhuys F.A. and van der Maeden F.P.B. In: Thesis C.J.C.M. Laurent, Delft University of Technology, Chapter 5 (1983)

7. In verband met analytische toepassingen van Fourier Transform massa spectrometers verdient het aanbeveling in commerciële folders de gevoeligheid op te nemen.

8. De naam Westvoorne voor de bij de gemeentelijke herindeling van Voorne-Putten en Rozenburg samengevoegde gemeenten Rockanje en Oostvoorne is om historisch geografische redenen onjuist.

   De informatie op ten Staet ende Gesteltenisse der Stede van den Brielle en de landen van Voorne by commissarissen van 's Conincs wegen, genomen in Augusti 1565 pp. 7, 15 and 22

   van Alkemade and van der Schelling (1729) *Caartboek van Voorne* Chapter VI, page 6

   Werken van de Nederlandse Letterkunde (1876) pp. 276-312 (Leiden)

   J. Kluit *De landen van Oost-, West- en Zuidvoorne, beschouwt in haar Opkomst, Aanwas, Tegenwoordige Staat en Regering*, Part II, Chapter 1 , 2

   W. Plokker *De Geschied- en Aardrijkskundige beschrijving van het eiland Voorne en Putten* , page 1

   J.Klok *Voorne en Putten* , Thesis , Utrecht , The Netherlands (1939)

   Handelingen van de Tweede Kamer , 8 september 1977, page 481

   Staatsblad 470 , Wet van 17 maart 1979

   Van Westvoorne tot St. Adolfsland. Historische verkenningen op Goeree-Overflakkee (1979) De Motte , Ouddorp

9. Het verdient aanbeveling dat de Stichting voor Leerplanontwikkeling een onderzoek instelt naar de wijze, waarop de vaardigheid van een deel der hedendaagse jeugd in het hanteren van de verfspuitbus kan worden benut bij het schrijfonderricht op de basisschool.

   n.a.v. cartoon van S. Verwey, *De Volkskrant* , 11 september 1980

Stellingen bij het proefschrift:

"COMPOSITION AND ORIGIN OF COMPLEX ORGANIC MATTER IN RECENT MARINE SEDIMENTS. Significance of bacterial biomarkers"

24 mei 1984

J. Klok

Delft University Press