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Carbon isotope variability in monosaccharides and lipids of aquatic algae and terrestrial plants

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ABSTRACT: The stable carbon-isotope compositions of individual monosaccharides and lipids, as well as the bulk stable carbon-isotope composition of total cell material from different aquatic and terrestrial plants were determined. With the exception of a *Phaeocystis* sp. bloom sample, monosaccharides were generally enriched in ^{13}C by 0 to 9‰ compared to the total cell material and significantly enriched (1 to 16‰) in ^{13}C compared to lipids (fatty acids, phytol, sterols and alkenones) within single organisms. The depletion of ^{13}C in *n*-alkyl lipids relative to monosaccharides was larger than the depletion of ^{13}C in isoprenoid lipids relative to monosaccharides. In addition, an isotopic enrichment was observed in the ^{13}C content of C_5 monosaccharides compared to glucose in some of the organisms studied, indicating isotopic heterogeneity within carbohydrates. The magnitude of the differences between monosaccharides, total cell material and lipids was far greater than previously reported. Thus, selective assimilation of ingested carbohydrates can lead to isotopic enrichments of heterotrophic biomass and zooplankton and subsequent trophic levels. In addition, since dissolved organic carbon (DOC) and particulate organic carbon (POC) has a significant carbohydrate fraction, the $\delta^{13}C_{POC}$ and $\delta^{13}C_{DOC}$ signal will be significantly influenced by the relative amounts and the $\delta^{13}C$ values of the carbohydrates present. This has significant implications for the isotopic integrity of the organic matter fractions during carbon cycling in food chains of aquatic ecosystems.

KEY WORDS: Carbohydrates · ^{13}C · Stable carbon isotope · Food web · Lipids · Aquatic · Terrestrial

INTRODUCTION

Carbohydrates are the most abundant biochemicals on earth (Aspinall 1983). For instance, carbohydrates comprise about 75 wt% of vascular plant tissues (Sjöström 1981) and 20 to 40 wt% of plankton (Parsons et al. 1984). The carbohydrates comprise the bulk of the biomass in marine and terrestrial environments together with proteins and lignins (Tissot & Welte 1978, Parsons et al. 1984). In addition, carbohydrates are a significant component of oceanic dissolved organic matter (DOM; Mopper et al. 1980, Benner et al. 1992, Pakulski & Benner 1994, McCarthy et al. 1996, Borch & Kichman 1997, Skoog & Benner 1997, Burdige et al. 2000). DOM represents one of the largest dynamic reservoirs of reduced carbon on earth (~10^{18} g carbon, i.e. the same magnitude as all living vegetation on the earth's continents), and is larger than the atmospheric CO_2 pool (Hedges 1992). Because carbohydrates, together with proteins and lignins, represent >40% of the degraded carbon, they are important nutrients for the benthic and pelagic communities (Cowie et al. 1992). Even at low concentrations, excreted carbohydrates provide a substantial food source for heterotrophic bacteria and zooplankton (Wright & Hobbie 1966, Gocke 1975, Williams & Yentsch 1976), as indicated by their rapid turnover rates.

In the assessment of food web structures in freshwater and marine ecosystems, the potential of stable carbon-isotope ($\delta^{13}C$) ratios as natural tracers in food chains is increasingly acknowledged (e.g. Michener & Schell 1994) and a number of studies utilizing stable

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carbon isotopes have been published (e.g. Yoshii et al. 1999, Van der Zanden et al. 2000). These studies show that the $\delta^{13}C$ of the heterotrophic biomass is primary influenced by the $\delta^{13}C$ of the autotrophic food source. Consequently, because carbohydrates are an important constituent of autotrophic carbon and selective assimilation can take place (e.g. Volkman et al. 1980, Grice et al. 1998), the carbon-isotope composition of carbohydrates will significantly influence the carbon-isotope composition of the heterotrophs and zooplankton. Thus, information on the carbon-isotope composition of carbohydrates is of primary importance for reconstructing food web relations based on isotopic compositions. In addition, since carbohydrates are an important component of DOM their carbon-isotope composition will significantly influence the bulk DOM carbon-isotope composition.

Until now, using bulk measurements, carbohydrates were generally thought to be slightly enriched in ^{13}C. The magnitude of this enrichment was found to be up to 3 to 4‰ relative to total cell material and 4 to 10‰ relative to lipids (e.g. Deines 1980). Wong et al. (1975) reported an enrichment in ^{13}C of bacterial carbohydrates of up to 6‰ relative to the total cell material and up to 16‰ in ^{13}C relative to the lipids.

Very little is known about the $\delta^{13}C$ values of carbohydrates at the molecular level. In contrast, much attention has been paid to another class of biochemicals, the lipids. This is mainly because of the easy accessibility of these compounds by gas chromatography techniques due to their apolar nature. Differences between $\delta^{13}C$ values of individual lipids within single organisms were found to be large (Schouten et al. 1998). However, the magnitude of the biosynthetic enrichment of $\delta^{13}C$ in monosaccharides relative to total cell material and lipids is unclear and detailed molecular isotopic analyses of individual monosaccharides are lacking. Recently, we developed a new method (Van Dongen et al. 2001) to reliably measure the $\delta^{13}C$ values of individual monosaccharides. In this study we determined the stable carbon-isotope composition of individual monosaccharides in a range of marine and freshwater algae and terrestrial higher plants. These were compared with the ^{13}C content of the total cell material and individual lipids to infer the implications for carbon isotopic studies of food webs and carbon cycling in aquatic systems.

MATERIALS AND METHODS

Cell material. The aquatic and terrestrial plants that were analyzed in this study and their culture conditions or sampling sites are listed in Table 1. The freshwater species *Tetraedron minimum*, *Scenedesmus communis* and *Pediastrum boryanum* were cultured under conditions described by Blokker et al. (1998a,b). Two different cultures of *T. minimum* were cultured, 1 continuous culture and 1 batch culture. The continuous culture was kept at a constant pH of 7.0, while the pH of the batch culture was between 5 and 6 at the start and between 8 and 9 at the end. Cultures of the marine species *Isochrysis galbana* and *Rhodomonas* sp. were grown in Erlenmeyer flasks (3 l) with a continuous air supply. Light was kept on a 16:8h light: dark regime and dilution rates ranged between 0.13 and 0.22 d^{-1}. The algae were grown on *F*/2 medium (Guillard 1975). The alga *Phaeocystis* sp. was collected with plankton nets (50 µm mesh) during spring blooms in the western Dutch Wadden Sea and the southern North Sea in 1991 and 1992 and stored frozen (–20°C) until use. Field specimens of *Sphagnum cuspidatum* and the roots of *Erica tetralix* were obtained from the peat bog reserve 'Bargerveen' (Zwartemeer, SE Drenthe, The Netherlands: Baas et al. 2000). Immediately after collection, the plants were washed repeatedly with water and any non-indigenous material was removed. The samples were stored frozen until use.

Table 1. Aquatic and terrestrial plants analyzed in this study with their culture or field condition

Organism	Class	Culture type	Sampling site	T (°C)	CO_2 source
Freshwater					
Tetraedron minimum	Chlorophyceae	Batch/Continuous	–	19	Air + 2% CO_2
Scenedesmus communis	Chlorophyceae	Batch	–	19	Air + 2% CO_2
Pediastrum boryanum	Chlorophyceae	Batch	–	19	Air + 2% CO_2
Marine					
Isochrysis galbana	Haptophyceae	Continuous	–	15	Air
Rhodomonas sp.	Cryptophyceae	Continuous	–	15	Air
Phaeocystis sp.	Haptophyceae	–	North Sea bloom	–	Air
Terrestrial					
Sphagnum cuspidatum	Bryophyta	–	Bargerveen peat bog	–	Air
Erica tetralix	Ericaceae	–	Bargerveen peat bog	–	Air

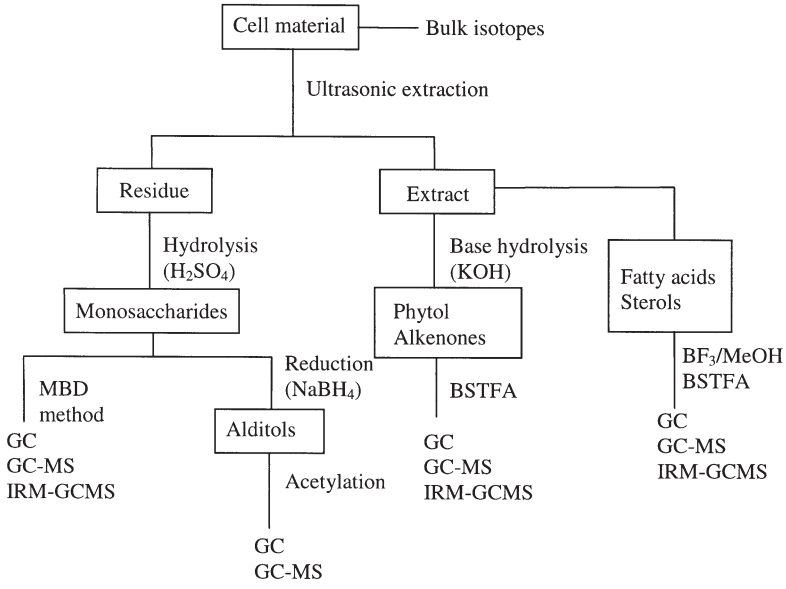

Fig. 1. Extraction and isolation scheme of plant cell material

Monosaccharide analysis. Cell material of the plants was collected, freeze-dried and extracted as described by Schouten et al. (1998). The extraction, separation and identification of the monosaccharides as well as the lipids are schematically depicted in Fig. 1. Briefly, cell material was ultrasonically extracted with methanol (3×), methanol/dichloromethane (1:1, 3×) and hexane/dichloromethane (hex/DCM, 1:1, v/v mixture; 3×). The extracts were analyzed for lipids as described in the following subsection. Approximately 7 to 15 mg of dried residues left after extraction were stirred with 4 ml 12 M H_2SO_4 at room temperature for 2 h. The solution was diluted to 1 M and the polysaccharides were hydrolyzed for 4.5 h at 85°C. The acidic solution was neutralized with $BaCO_3$. The precipitate was removed by centrifugation, the residue washed twice with bidistilled water and the combined water layers were freeze-dried. The hydrolyzed material was analyzed for monosaccharides using 2 different methods. To determine the relative amounts of the different monosaccharides, they were analyzed using the alditol acetate method as described by Klok et al. (1982, 1984). The methylboronic derivatization method (MBD method; described in subsection 'Instrumental analyses') was not used to determine the relative amounts of all the monosaccharides since the derivatization of a number of the monosaccharides is incomplete (Van Dongen et al. 2001). The alditol acetate method involves the reduction of monosaccharides with $NaBH_4$ to alditols and acetylation of the alcohol groups using acetic anhydride and pyridine with the use of myo-inositol as internal standard. Because the monosaccharides are measured as their alditol acetates, the origin of some of the alditol acetates is uncertain. For instance, glucitol could originate from glucose as well as fructose (Macko et al. 1998). In these cases the results of the MBD method, which preserves the original isomeric structure, in combination with the alditol acetate method, were used to infer the origin of the different alditol acetates. The MBD method, a derivatization method to make monosaccharides GC-amenable, was used to determine the $\delta^{13}C$ values of the different monosaccharides as described by Van Dongen et al. (2001). Typically, 0.5 ml of a solution of 10 mg methylboronic acid in 1 mL pyridine was added to the hydrolyzed material and the solution was heated at 60°C for 30 min. Subsequently, 15 µl N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added and the solution was stirred at 60°C for 5 min. To remove any solid material, the solution was filtered through a pipette filled with $MgSO_4$ with ethyl acetate as an eluent. The solution was concentrated using evaporation under nitrogen as necessary. The monosaccharide fractions were analyzed by gas chromatography (GC), gas chromatography-mass spectro-metry (GC/MS), and isotope-ratio monitoring GC/MS (IRM-GC/MS) as described in 'Instumental analysis'.

Lipids. The separation and identification of lipids are schematically depicted in Fig. 1. Saponification, methylation and silylation were performed as described by Schouten et al. (1998). In some instances, phytol and sterols were present in relatively low amounts compared to fatty acids, and $\delta^{13}C$ values could not be determined accurately. In these cases, the total ex-tracts were filtered over an Al_2O_3-column using ethyl acetate as an eluent to remove the fatty acids. The total extracts of *Erica Tetralix* and *Sphagnum cuspidatum* were eluted over a SiO_2-column using hex/DCM (9:1, v/v mixture) as eluent to collect the *n*-alkanes and DCM/methanol (1:1, v/v mixture) to collect the fatty acids and sterols and phytol. The long-chain alkenones present in *Isochrysis galbana* were measured in the saponified fraction to avoid co-elution with the long-chain alkenoates present. The lipid fractions were analyzed by GC, GC/MS and IRM-GC/MS as described in the following section.

Instrumental analyses. Gas chromatography (GC) was performed with a Hewlett Packard 6890 instrument and gas chromatography-mass spectrometry (GC-MS) using a Hewlett-Packard 5890 gas chromatograph interfaced with a VG Autospec Ultima mass

spectrometer. Lipid analysis was carried out as described by Schouten et al. (1998). For analysis of alditol acetates, a fused silica capillary column (l = 25 m; i.d. = 0.32 mm) coated with CP Sil-88 (film thickness 0.25 µm) was used. Samples were dissolved in ethyl acetate and injected at 70°C. The temperature was raised to 150°C at 20°C min^{-1}, 3°C min^{-1} to 240°C and held for 10 min. Since the number of added carbon atoms during the derivatization reaction is different for the different monosaccharides the relative amounts were corrected for added carbon. In order to measure The δ^{13} values of the monosaccharides, the MBD method was used for the derivatization as described by Van Dongen et al. (2001). The derivatized compounds were separated on a fused silica capillary column (l = 30 m; i.d. = 0.32 mm) coated with CP Sil-19 (film thickness 0.12 µm). Samples were injected at 70°C and the temperature was raised to 80°C (20°C min^{-1}) and held for 10 min. Subsequently, the GC was heated at 4°C min^{-1} to 180°C followed by 10°C min^{-1} to 280°C and held for 1 min.

The alditol acetates and methyl boronic acid derivatives were identified on the basis of known relative retention times established by the analysis of standard mixtures and on the basis of mass spectra (Reinhold et al. 1974, Klok et al. 1982, 1984, Van Dongen et al. 2001).

Isotope-ratio-monitoring gas chromatography-mass spectrometry (IRM-GC-MS) was performed using a Finnigan DELTA-C. The same columns, column conditions and temperature program was used as in the case of GC analysis. The δ^{13}C values reported were obtained by at least 2 analyses and the results were averaged to obtain a mean value. The MBD method implies the introduction of extra carbon atoms from the derivatization agent: 2 in the case of arabinose and xylose and 5 in the case of glucose (Fig 2). To correct for the alteration in the stable carbon-isotope composition due to the introduction of these additional carbon atoms, the δ^{13}C of the derivatization agents (methylboronic acid and BSTFA) were determined (Van Dongen et al. 2001). The corrections for the introduced carbon due to the methylation or silylation of the fatty acids, phytol and sterols were performed as described by Schouten et al. (1998). The stable carbon-isotope compositions are reported in delta notation as determined against the Vienna PDB ^{13}C standard.

The stable carbon-isotope composition of the bulk cell material ($\delta^{13}C_{bulk}$) was determined by automatic online combustion (Carlo Erba CN analyser 1502 series) followed by conventional isotope ratio-mass spectrometry (Fisons optima: Fry et al. 1992).

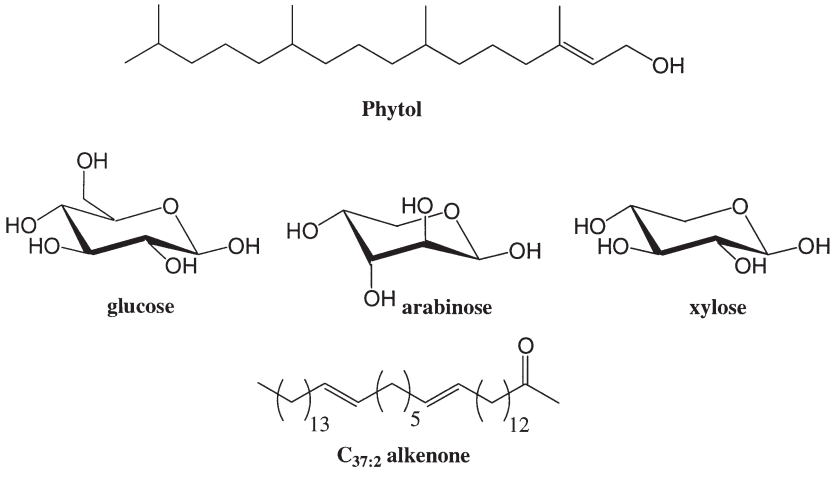

Fig. 2. Structures of the compounds analyzed

RESULTS AND DISCUSSION

^{13}C content of monosaccharides

The monosaccharide fraction of the freshwater algae and the marine alga *Rhodomonas* sp. mainly consisted of glucose (65 to 90%) and other C_6-monosaccharides (mannose, rhamnose and galactose: Fig. 3). In *Isochrysis galbana* the major component was also glucose (30%), but it was relatively less abundant. *Phaeocystis* sp. had as major monosaccharide arabinose (46%), and the terrestrial plants *Sphagnum cuspidatum* and *Erica tetralix* xylose (24 and 47%). In addition to these monosaccharides, considerable amounts of other C_5 (mainly ribose), C_6 (rhamnose, mannose and galactose) and C_7-monosaccharides were found.

Using the MBD method, the δ^{13}C values of the different monosaccharides (where possible) were determined (Table 2). For the cultured algae (except *Isochrysis galbana*) only the δ^{13}C of glucose could be reliably determined. The contribution of other monosaccharides was too small and consequently could not been determined or, in the case of mannose, the derivatization by the MBD method was incomplete (Van Dongen et al. 2001) and the δ^{13}C values obtained were deemed not reliable. For *Isochrysis galbana*, *Phaeocystis* sp. and both terrestrial plants, the δ^{13}C of glucose and the C_5-monosaccharides arabinose and/or xylose could be determined (Table 2). Sometimes small amounts (<6%) of other monosaccharides were co-eluting with the C_5-monosaccharides, i.e. fucose co-eluted

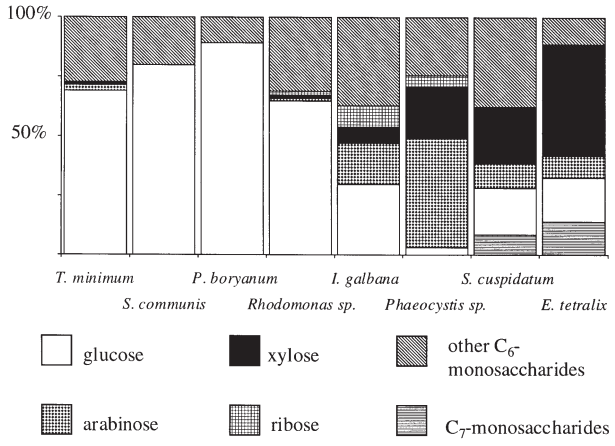

Fig. 3. Percentage of monosaccharides relative to total amount of alditols in different aquatic and terrestrial organisms. Other C_6-monosaccharides included rhamnose, mannose, galactose and fucose. The structures of the C_7-monosaccharides are unknown. Full specific names as in Table 1

with arabinose and ribose co-eluted with xylose. In those instances an average $\delta^{13}C$ value for co-eluting peaks was determined.

Except for *Phaeocystis* sp., all $\delta^{13}C$ values of the monosaccharides were enriched in ^{13}C compared with $\delta^{13}C_{bulk}$ (values of bulk cell material; Table 2, Fig. 4), i.e. 2 to 9‰ for glucose, 5 to 7‰ for xylose and 6 to 8‰ for arabinose. In contrast, the monosaccharides $\delta^{13}C$ were depleted up to 4‰ in ^{13}C relative to the $\delta^{13}C_{bulk}$ value, for *Phaeocystis* sp. Compared with previous reports (e.g. Wong et al. 1975, Deines 1980) the magnitude of these differences is greater than has been reported on the basis of bulk determinations of monosaccharides. These differences are also far more variable than previously reported. This indicates a large heterogeneity in the relative isotopic compositions of monosaccharides in photoautotrophic organisms. The large enrichment (up to 8‰) in ^{13}C of the individual monosaccharides relative to $\delta^{13}C_{bulk}$ in the case of the *Sphagnum* species (*S. cuspidatum*) is surprising, since the ^{13}C content of monosaccharides versus bulk material found by Macko et al. (1990) for a *Sphagnum* species were significantly different (depleted by up to 1‰). The reason for this could be that different *Sphagnum* species have different isotopic contents. Another difference between the 2 studies is the fact that Macko et al. used the alditol acetate method for isotopic analysis; this is prone to larger errors in $\delta^{13}C$ determination (Van Dongen et al. 2001).

To explain the large ^{13}C-enrichment of monosaccharides we have to take into account that other biochemicals, such as lipids, are generally depleted in ^{13}C compared with monosaccharides (see below). The reason why the $\delta^{13}C_{bulk}$ of the *Phaeocystis* sp. was enriched compared with the $\delta^{13}C$ values of the different monosaccharides could be the fact that this was a bloom sample. It is possible that isotopic fractionation within the cell during bloom conditions may be different than during non-bloom conditions. In addition, it is possible

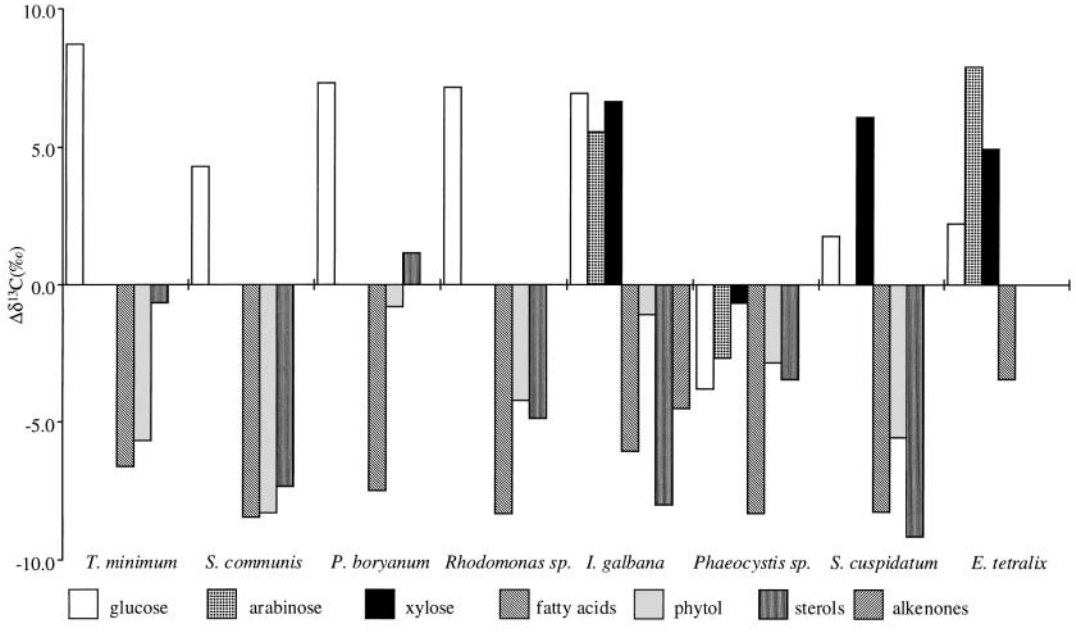

Fig. 4. $\delta^{13}C$ values of monosaccharides and lipids relative to $\delta^{13}C_{bulk}$ in different aquatic and terrestrial organisms. The $\delta^{13}C$ values of the fatty acids and sterols used are the average values of the C_{14}-C_{18} fatty acids and C_{27}-C_{29} sterols, respectively. Full specific names as in Table 1

Table 2. $\delta^{13}C_{bulk}$ and $\delta^{13}C$ values (‰) of monosaccharides, fatty acids, phytol, sterols and alkenones in different aquatic and terrestrial organisms (with analytical error, which includes error in measurement as well as error through derivatization). Full specific names as in Table 1

Component	*T. minimum*	*S. communis*	*P. boryanum*	*Rhodomonas* sp.	*I. galbana*	*Phaeocystis* sp.	*S. cuspidatum*	*E. tetralix*
Bulk material	−47.9 ± 0.1	−39.8 ± 0.1	−40.1 ± 0.1	−12.1 ± 0.2	−13.8 ± 0.2	−17.5 ± 0.3	−25.6 ± 0.1	−25.9 ± 0.2
Monosaccharides								
Arabinose					−8.2 ± 0.6[a]	−20.2 ± 0.6[a]		−18.0 ± 1.0[a]
Xylose					−7.1 ± 0.9	−18.2 ± 0.9[b]	−19.5 ± 0.7	−21.0 ± 0.9
Glucose	−39.2 ± 0.8	−35.5 ± 0.4	−32.8 ± 0.4	−4.9 ± 0.4	−6.8 ± 0.3	−21.3 ± 0.9	−23.8 ± 0.7	−23.7 ± 0.8
Fatty acids[c]								
C_{14}				−21.4 ± 0.4	−19.1 ± 0.1	−24.5 ± 0.5		
C_{16}	−53.9 ± 0.1	−50.2 ± 0.6	−47.3 ± 0.3	−20.4 ± 0.5	−23.0 ± 0.3	−24.0 ± 0.1	−35.0 ± 0.2	−28.8 ± 0.6
C_{18}	−54.6 ± 0.4	−47.0 ± 0.4	−47.7 ± 0.5	−20.2 ± 0.2	−19.0 ± 0.1	−27.8 ± 0.3	−33.1 ± 0.5	−29.3 ± 0.3
Phytol	−53.6 ± 0.7	−48.3 ± 0.2	−40.9 ± 0.7	−16.3 ± 0.2	−14.8 ± 0.3	−20.3 ± 0.7	−31.2 ± 0.5	
Sterols[c]								
C_{27}						−22.2 ± 0.5[d]		
C_{28}	−48.7 ± 0.4[e]	−47.0 ± 0.6[e]	+38.4 ± 0.7[e]	−16.9 ± 0.2[f]	−21.8 ± 0.1[g]	−19.7 ± 0.5[f]	−35.1 ± 0.2[h]	
C_{29}	−48.5 ± 0.4[h]	−47.3 ± 0.4[i]	+39.5 ± 0.5[i]				−34.4 ± 0.4[j]	
Alkenones[c]								
$C_{37:n}$[k]					−18.6 ± 0.1			
$C_{38:n}$[k]					−17.9 ± 0.4			

[a]Small contribution of fucose
[b]Small contribution of ribose
[c]Subscripts indicate number of carbon atoms
[d]Cholest-5-enol
[e]24-methylcholest-7-enol and 24-methylcholesta-7,22-dienol
[f]24-ethylcholesta-5,22-dienol
[g]24-methylcholest-5,22-dienol
[h]24-methylcholest-5-enol and 24-methylcholesta-5,22-dienol
[i]24-ethylcholest-7-enol
[j]24-ethylcholest-5-enol and 24-ethylcholesta-5,22-dienol
[k]Average of n = 2 to n = 4

that other undetermined ^{13}C-enriched carbon material, present in the bloom but not derived from *Phaeocystis* sp., was influencing the $\delta^{13}C_{bulk}$. Alternatively, there may be other, as yet undetermined, ^{13}C-enriched (compared with the $\delta^{13}C_{bulk}$) components present in *Phaeocystis* sp. itself.

Comparison of the $\delta^{13}C$ values of the C_5-monosaccharides arabinose and xylose with those of glucose within the same organism revealed significant differences. Xylose was up to 4‰ enriched in ^{13}C and arabinose up to 6‰ enriched in ^{13}C compared with glucose, with the exception of *Isochrysis galbana*. To the best of our knowledge this is the first time that such large differences between C_5 and C_6-monosaccharides have been reported. It is possible that a fractionation effect occurs during the decarboxylation of glucose to form C_5-monosaccharides. However, such a kinetic isotope effect is likely to result in a depletion in ^{13}C of the product formed (C_5-monosaccharide) relatively to the reactant available (C_6-monosaccharide) at the reaction site (Hayes 1993, 2001). It is also possible that there are significant differences between the carbon-isotope compositions of the different carbon atoms in glucose, as found by Ivlev et al. (1987) and Rossmann et al. (1991), which then lead to an isotopic difference between glucose and the C_5-monosaccharides. In order to differentiate between these 2 possible explanations more detailed information about the biosynthesis of C_5-monosaccharides and the ^{13}C fractionation of the enzymes involved needs to be obtained.

^{13}C content of lipids

The most dominant lipids present in the total extracts of all the algae except *Isochrysis galbana* were C_{14}-C_{18} saturated and unsaturated fatty acids, phytol, and C_{28}-C_{29} sterols, with a distribution similar to those reported by Wood (1988). Phytol (Fig. 2) and sterols were also found in *I. galbana*, but the most abundant compounds were C_{37} and C_{38} alkenones (Fig. 2; Marlowe et al. 1994). Only traces of fatty acids were found. The dominant compounds in the total extract of *Sphagnum cuspidatum* and the roots of *Erica tetralix* were C_{16}-C_{18}

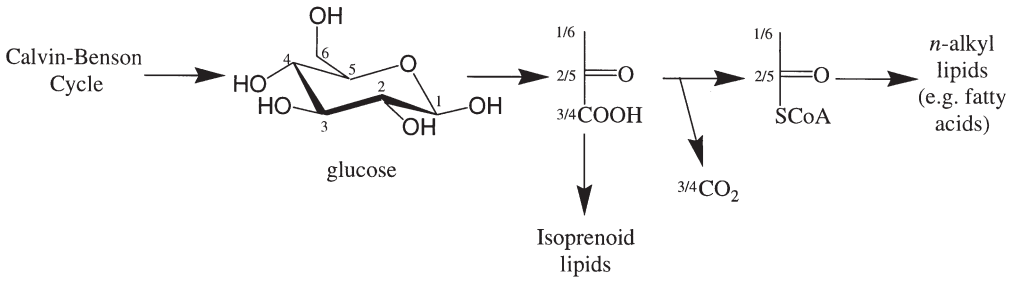

Fig. 5. Biosynthesis of fatty acids and isoprenoids starting from glucose. Carbon atoms are numbered according to their original position in the glucose carbon skeleton

fatty acids and C_{28}-C_{29} sterols. Minor amounts of C_{21}-C_{23} *n*-alkanes, triterpenoids and wax esters were found, with a distribution similar to those reported for *S. cuspidatum* by Baas et al. (2000). Significant amounts of phytol were found in *S. cuspidatum*. Phytol is the main alkyl side chain of chlorophyll and no chlorophyll is present in roots, and hence it was not present in the roots of *E. tetralix*.

When possible, the $\delta^{13}C$ of the lipids were determined, for comparison with the $\delta^{13}C$ of the different monosaccharides (Table 2). As the unsaturated and saturated C_{18} fatty acids were often co-eluting, an average $\delta^{13}C$ value of the total cluster of unsaturated and saturated C_{18} fatty acids was determined. For the same reasons, the average $\delta^{13}C$ value of the C_{37} and C_{38} alkenones were determined in *Isochrysis galbana*. The fatty acids were considerably depleted in ^{13}C compared with glucose (Table 2, Fig. 4), i.e. the C_{14}-C_{18} fatty acids were 12 to 16‰ depleted for freshwater and marine algal cultures, 3 to 6‰ for *Phaeocystis* sp., and 5 to 13‰ for terrestrial plants. Phytol and sterols were generally less depleted in ^{13}C compared with glucose (Table 2) than the fatty acids, i.e. phytol was 8 to 14‰ depleted for the freshwater and marine algal cultures and about 7‰ depleted in ^{13}C for *Sphagnum cuspidatum*. In contrast, the $\delta^{13}C$ value of phytol from *Phaeocystis* sp. was similar to that of glucose. The sterols were 6 to 15‰ depleted compared with glucose in the freshwater and marine algal cultures and about 11‰ depleted in ^{13}C in *S. cuspidatum*. In contrast, the C_{27} sterol as well as the C_{28} sterol measured in *Phaeocystis* sp. were similar in ^{13}C compared with glucose. The alkenones in *I. galbana* were 11 to 12‰ depleted in ^{13}C compared to glucose. The isotopic differences between the fatty acids, phytol, the sterols and the alkenones in the algal cultures are comparable to those reported previously by Schouten et al. (1998).

An enrichment in ^{13}C of monosaccharides compared to lipids has been generally assumed (Hayes 1993, 2001). However, the magnitude of the difference between monosaccharides and lipids determined here is far greater than has been anticipated. The reason for the depletion of lipids versus monosaccharides is assumed to be a consequence of kinetic isotope effects during enzymatic reactions. Glucose is formed in the Calvin-Benson cycle during photosynthesis. Subsequently, the glucose is cleaved to 2 pyruvate molecules, which are converted to acetyl coenzyme A through decarboxylation (Fig. 5). *n*-alkyl lipids are synthesized starting from acetyl coenzyme A and isoprenoid lipids are synthesized from pyruvate (Hayes 1993, 2001, Rohmer et al. 1993, Schwender et al. 1996, Lichtenthaler et al. 1997, Disch et al. 1998, and present Fig 5.). The decarboxylation from pyruvate to acetyl coenzyme A is associated with a kinetic isotopic fractionation effect (e.g. Monson & Hayes 1980, 1982a,b, Melzer & Schmidt 1987), leading to a depletion in ^{13}C in the carboxyl atom of acetyl coenzyme A. Thus, *n*-alkyl lipids are usually depleted in ^{13}C relative to the isoprenoid lipids constructed from pyruvate (Schouten et al. 1998). The different pathways may explain the differences in ^{13}C depletion between individual lipids and monosaccharides.

An alternative explanation for the ^{13}C depletion of lipids relative to monosaccharides may be the heterogeneous distribution of ^{13}C in glucose. During the decarboxylation of pyruvate to acetyl coenzyme A the original glucose carbon atoms 3 or 4 are removed. According to Rossmann et al. (1991), these carbon positions are relatively enriched in ^{13}C (about 2 to 6‰). This would mean that the acetyl coenzyme A unit formed is relatively depleted in ^{13}C compared with the pyruvate unit and consequently compared to glucose. Because straight-chain lipids are mainly constructed from acetyl coenzyme A, they are depleted in ^{13}C compared with glucose. However, as pointed out by Hayes (1993, 2001), this relative enrichment in ^{13}C of carbon atoms within glucose is too small to explain the observed large depletion in $\delta^{13}C$ values of lipids. In addition, the difference between monosaccharides and lipids are even larger (up to 16‰ in $\delta^{13}C$), and thus an even larger enrichment in ^{13}C within glucose is required. The observed differences between C_5-monosaccharides and glucose indicate that the loss of a sin-

gle carbon atom can lead to a significant difference in $\delta^{13}C$ values (up to 6‰). This means that the potential difference in ^{13}C between the carbon atoms in glucose could be significantly larger than reported by Rossman et al. (1991). This makes the alternative explanation for the depletion of lipids, the heterogeneous distrubution of ^{13}C in glucose, more likely, although little is known about the pathways by which these C_5-monosaccharides are formed.

Influence of culture conditions on isotopic heterogeneity of biochemicals

The large range in differences in ^{13}C content between the monosaccharides, lipids and the bulk material for the different cultured algae could either be due to species-specific fractionation differences or to the culture conditions (e.g. Riebesell et al. 2000). In batch cultures, algae experience different growth conditions at the end of the log growth phase than at the beginning. This can result in changes in the culture medium, such as increasing pH. In water there is a balance between the concentration of CO_2, HCO_3^- and CO_3^{2-}. If the pH increases, this equilibrium is shifted to CO_3^{2-}, causing a relatively lower dissolved CO_2 concentration (Hayes 1993, 2001). This means that less CO_2 is available and relatively more enriched CO_2 is incorporated, which will result in less ^{13}C-depletion. Thus, these conditions probably result in less ^{13}C-depletion at the end of the end of the log growth phase. In comparison, in the continuous culture there are constant limiting nutrient conditions, which will result in different, but consistent, isotopic fraction patterns. This will probably influence the differences in isotopic composition between the compounds in a single organism. Therefore, we cultured the same algae (*Tetraedron minimum*) under different conditions, i.e. under continuous culture and under batch culture conditions, and compared the two (Table 3). Glucose was approximately 7‰ enriched compared with $\delta^{13}C_{bulk}$ and generally 10‰ enriched compared with the fatty acids in the continuous culture. Under batch culture conditions, glucose was approximately 9‰ enriched compared with $\delta^{13}C_{bulk}$ and generally 15‰ enriched enriched compared with the fatty acids.

Table 3. *Tetraedron minimum.* $\delta^{13}C_{bulk}$ and the $\delta^{13}C$ values (‰) of glucose and fatty acids (weighted mean average of C_{14}-C_{18} fatty acids, with analytical error, which includes error in measurement as well as error through derivatization) under different culture conditions

Component	Batch	Continuous
Bulk material	−47.9 ± 0.1	−34.7 ± 0.1
Glucose	−39.2 ± 0.8	−27.6 ± 0.8
Fatty acids	−53.6 ± 0.3	−37.6 ± 0.3

The differences in isotopic content between the monosaccharides, lipids and the bulk material for the different cultures of *Tetraedron minimum* (batch versus continuous: Table 3) seem to support the assumption that culture conditions can influence the differences in ^{13}C content between monosaccharides, lipids and bulk material. Both types of cultures showed the same isotopic differences between fatty acids, monosaccharides and bulk. However, it seems that the different culture conditions influenced the isotopic composition of the fatty acids more than glucose compared with bulk-material values.

Differing growth conditions may also explain the difference between the results for cultured algae and those for the natural bloom sample.

Conclusions

From our results it is clear that large isotopic differences can exist in ^{13}C content between monosaccharides, lipids and bulk material in photoautotrophic organisms. These organisms comprise the base of the aquatic food-web chain and, depending on the (relative) assimilation of their different biochemicals components, they determine the isotopic composition of heterotrophic bacteria and zooplankton. Zooplankton can alter their bulk isotopic composition through selective assimilation of ingested compounds. Recent studies showed that components like carbohydrates are relatively easily digested and selectively assimilated (e.g. Cowie & Hedges 1996). Thus, not the $\delta^{13}C$ values of the algae but the $\delta^{13}C$ values of the carbohydrates (and amino acids) produced by these algae will mainly determine the $\delta^{13}C$ values of the zooplankton.

Suspended particulate organic carbon (POC) and DOM originate from various sources—primary production, resuspended sediment and terrestrial detritus discharged by rivers. The $\delta^{13}C_{DOM}$ and $\delta^{13}C_{POC}$ signal is often used as a biomarker indicating the origin of the organic matter (e.g. Salomons & Mook 1981, Fry & Sherr 1984, Laane et al. 1990, Mook & Tan 1991). Organic matter produced by marine phytoplankton is assumed to have an average $\delta^{13}C$ value of –21‰, while terrestrial C-3 plant-derived organic matter has a content of –27‰. DOM and POC consist, to a significant degree, of carbohydrates and, as shown in this study, monosaccharides are significantly enriched in ^{13}C compared with the isotopic composition of the total cell material. This would mean that if the amount of carbohydrates present in the DOM and POC differ significantly, the $\delta^{13}C_{DOM}$ and $\delta^{13}C_{POC}$ signals will also differ

significantly. This will make it more difficult to distinguish between the different sources of the organic matter based solely on $\delta^{13}C_{DOM}$ and $\delta^{13}C_{POC}$ signals, and alternative methods need to be used, e.g. ^{14}C analysis (Megens et al. 1998).

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