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An experimental study of the low-temperature sulfurization of carbohydrates

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Abstract

Sulfurization of carbohydrates has been suggested as an important mechanism for the preservation of organic matter. To study this process, different monosaccharides were sulfurized under laboratory conditions at relatively low temperature (50 °C). The products formed after cleavage of polysulfide linkages were analyzed using gas chromatography (GC) and gas chromatography/mass spectrometry after appropriate derivatization. Selected products were isolated by preparative GC and their structures were identified by nuclear magnetic resonance spectroscopy. During these experiments all monosaccharides were completely converted into organic sulfur compounds (OSCs) and monosaccharides with the carbonyl function replaced by sulfur formed a substantial part of the GC-amenable OSCs. The structures of other OSCs formed indicated that cleavage of C–C bonds and racemization also took place during these experiments. The yield of recoverable OSCs after cleavage of polysulfide linkages was relatively low (<5% of the starting monosaccharide), indicating that most of the sulfurization products were still non GC-amenable and thus, for example, linked through monosulfide linkages. Flash pyrolysates of the sulfurized carbohydrate material contained in all cases relatively high amounts of short-chain alkylated (C_0–C_5) thiophenes, comparable to those obtained from S-rich kerogen. The structure of the monosaccharide used in the experiments had no effect on the alkylthiophene distribution. These results provide experimental evidence that sulfurization of monosaccharides at relatively low temperatures can result in the formation of OSCs, most likely starting with sulfurization of the carbonyl functionality. Preservation of carbohydrates through sulfurization may thus be an important pathway of preservation of organic matter in anoxic depositional environments.

1. Introduction

Organic sulfur compounds (OSCs) have been identified in many sediments and oils (for a review see Sinninghe Damsté and de Leeuw, 1990) and the characterization of these compounds is still a major topic of geochemical interest because they provide clues to the timing and preservation effects of sulfurization of organic matter (OM) and, ultimately, petroleum quality (Stock et al., 1989; Orr and Sinninghe Damsté, 1990; Sinninghe Damsté et al., 1999). A large part of the OSCs is in a macromolecular form as shown by the presence of large amounts of organically bound sulfur in, for instance, kerogens of immature sediments (Orr, 1986; Sinninghe Damsté and de Leeuw, 1990). OSCs are formed during

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early diagenesis through the incorporation of inorganic sulfur species into functionalized lipids (Valisolalao et al., 1984; Brassell et al., 1986; Sinninghe Damsté et al., 1989; Ten Haven et al., 1990; Kohnen et al., 1990; Sinninghe Damsté and de Leeuw, 1990; Wakeham et al., 1995; Werne et al., 2000; Kok et al., 2000a). Recent studies indicate that, besides lipids, carbohydrates may also be preserved through the incorporation of sulfur species (van Kaam-Peters et al., 1998; Sinninghe Damsté et al., 1998b; Kok et al., 2000b) although carbohydrates are generally thought to be remineralized during early diagenesis in the water column and in the sediment, and not preserved in substantial amounts (Arnosti et al., 1994; Arnosti and Repeta, 1994; Arnosti, 1995).

Laboratory simulations have shown that sulfurization of algal matter results in the loss of labile carbohydrate material through the formation of a macromolecular structure composed of (poly)sulfide cross-linked carbohydrate skeletons (Kok et al., 2000b). Flash pyrolysis of these macromolecular structures yields short-chain alkylated thiophenes which are often important pyrolysis products in S-rich, immature petroleum source rocks (Sinninghe Damsté et al., 1989, 1998a; Eglinton et al., 1990b) such as, for example, the Kimmeridge Clay Formation (KCF; van Kaam-Peters et al., 1998; Sinninghe Damsté et al., 1998b). The enriched $\delta^{13}C$ values of the short-chain alkylated thiophenes relative to the *n*-alkanes/*n*-alkenes in the KCF kerogen pyrolysates (van Kaam-Peters et al., 1998) provides further circumstantial evidence for a carbohydrate origin for the alkylthiophenes as monosaccharides can be enriched up to 16‰ in ^{13}C compared to lipids in the same organisms (Sinninghe Damsté et al., 2001; van Dongen et al., 2002). However, further evidence for the sulfurization of carbohydrates is required since only flash pyrolysis was performed on the material formed (Kok et al., 2000b). This analytical method provides no insight into the reaction steps that result in the formation of S-rich macromolecules. In addition, the alkylated thiophenes in pyrolysates represent only a relatively small part (6%) of the total amount of organic sulfur as observed in the KCF (Eglinton et al., 1990a), indicating that the remaining part of the organic sulfur may still have a different origin.

In order to provide a more detailed insight into the carbohydrate sulfurization process, a number of different monosaccharides were sulfurized in laboratory experiments under an inert atmosphere and at relatively low temperature (50 °C). The material formed was analyzed to provide information about the mechanisms involved in the formation of the sulfurized material and the effect of the original structure of the monosaccharide on this sulfurization process. The results provide direct evidence that sulfurization of monosaccharides is possible and that the carbonyl functionality plays an important role in this process.

2. Experimental

2.1. Materials

The carbohydrates used were the C_4 monosaccharide erythrose (**I**), the C_5 monosaccharides arabinose (**II**), lyxose (**III**) and ribose (**IV**), and the C_6 monosaccharides galactose (**V**), glucose (**VI**), mannose (**VII**), fructose (**VIII**) and fucose (**IX**) obtained from either Aldrich, Acros, Baker or Merck.

2.2. Sulfurization of monosaccharides

Sulfurization of the different monosaccharides was carried out according to the procedure described by Kok et al. (2000b; Fig. 1). The monosaccharides (ca. 200 mg) were dissolved in 6 ml of doubly distilled water, stirred and heated at 50 °C under a nitrogen atmosphere for 4 weeks after addition of 560 mg NaHS and 16 mg of elemental sulfur. After the reaction, activated Cu curls were added to remove elemental sulfur and stirred for 24 h at room temperature. Subsequently, the solution was filtered and freeze-dried. The residual materials were used for further analysis as indicated in Fig. 1. A

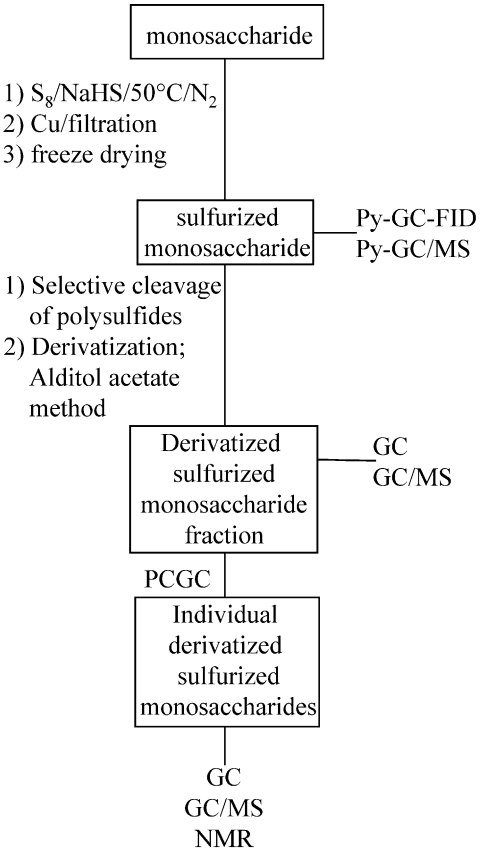
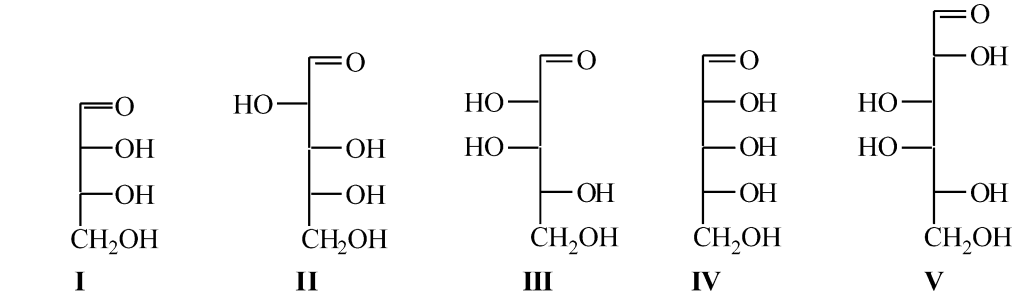
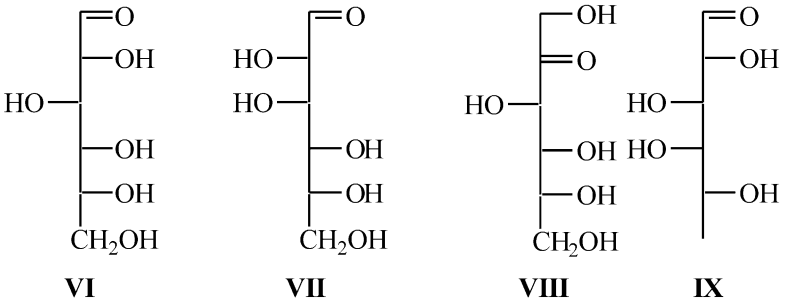
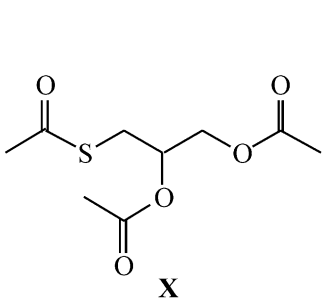
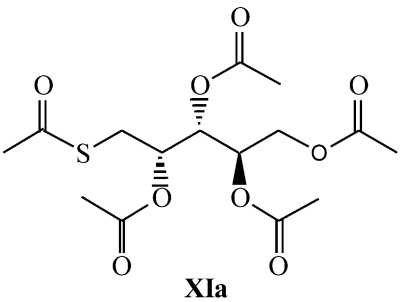


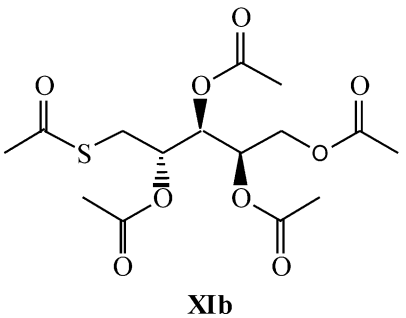
Fig. 1. Analytical flow diagram.

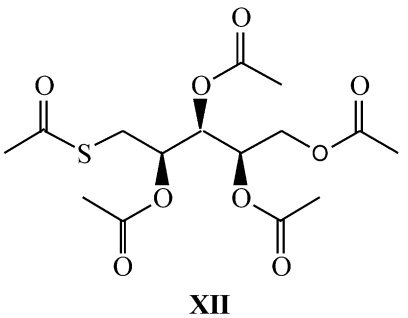












similar procedure was used for a control experiment. In this case, 200 mg of lyxose (**III**) was treated under the same reaction conditions, including treatment with Cu curls, without the addition of NaHS and elemental sulfur.

Reaction products were analyzed by Curie-point pyrolysis-gas chromatography/flame ionisation detection (Py-GC/FID) and Curie-point pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). In addition, the products of all sulfurized C_5 aldoses, the sulfurized C_6 aldoses, except galactose (**V**), and the control experiment were used for further analyses by selective cleavage of polysulfides and derivatization using the alditol acetate method, as described below.

2.3. Selective cleavage of polysulfide bonds and derivatization using the alditol acetate method

In a typical methyllithium (MeLi)/methyl iodide (MeI) experiment (modified after Kohnen et al., 1991) the sulfurized monosaccharides were suspended in 4 ml

of MeLi (1.4 M solution in diethyl ether) at room temperature and stirred. After 5 min 200 μl of MeI was added and the solution was stirred for 1 h until the sulfurized material was totally dissolved. Subsequently, drops of doubly distilled water were carefully added. The layers were separated and the water layer was washed three times with hexane. The sulfurized monosaccharides present in the water layer were derivatized using the alditol acetate method as described by Moers et al. (1989). In the case of glucose (**VI**) the organic layer was also checked for the presence of OSCs but none were detected. *Myo*-inositol was added as an internal standard and the monosaccharides were reduced by $NaBH_4$ to give the corresponding alditols. After neutralisation with $BaCO_3$ all the alcohol groups were acetylated through a reaction with acetic anhydride and pyridine. The collected fractions were dissolved in a small amount of ethyl acetate and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). In the case of the control experiment the reaction products were analysed using the same procedure but without the treatment with MeLi/MeI (i.e. using only the alditol acetate method).

2.4. Preparative capillary gas chromatography (PCGC)

In the case of sulfurized lyxose (**III**) preparative capillary gas chromatography (PCGC) was performed to isolate the most abundant OSCs formed. PCGC was performed on a HP 6890 gas chromatograph equipped with a Gerstel temperature programmable injector, a 30 m × 0.32 mm i.d. CP-SIL 19CB capillary column (d_f = 1 μm) and a Gerstel preparative fraction collection system cooled with a cryostatic bath at 20 °C. Details of the trapping procedure are given by Eglinton et al. (1996). Samples were dissolved in ethyl acetate and injected at 70 °C. The oven temperature was rapidly raised to 130 °C (20 °C/min) and further programmed at 3 °C/min to 300 °C at which it was held isothermally for 20 min; 209 injections were performed to trap sufficient material.

The trapped fractions were dissolved in a small amount of ethyl acetate and analyzed by GC and GC/MS, or dissolved in $CDCl_3$ and analyzed by nuclear magnetic resonance spectroscopy (NMR).

2.5. GC and GC/MS

Gas chromatography (GC) was performed using a Hewlett Packard 5890 instrument with an on-column injector. A 30 m × 0.32 mm i.d. CP-SIL 19CB capillary column (d_f = 0.25 μm) was used with helium as carrier gas. The effluent was monitored by both a flame ionisation detector (FID) and a sulfur-selective flame photometric detector (FPD), applying a stream-splitter with a split ratio of FID:FPD = ca. 1:2. Samples were dissolved in ethyl acetate and injected at 70 °C. The temperature programme was rapidly raised to 130 °C (20 °C/min) and further programmed at 4 °C/min to 300 °C at which it was held isothermally for 10 min.

Gas chromatography/mass spectrometry (GC/MS) was performed using a Hewlett-Packard 5890 gas chromatograph interfaced to a VG Autospec Ultima mass spectrometer or a HP6890 interfaced to a mass selective detector. The same columns, column conditions and temperature program were used as in the case of GC.

2.6. Curie-point pyrolysis-gas chromatography/flame ionisation detection (Py-GC/FID) and Curie-point pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS)

Py-GC/FID was conducted on a Hewlett-Packard 5890 gas chromatograph using a FOM-5LX unit for pyrolysis. The powdered samples were pressed on a flattened ferromagnetic wire with a Curie-point temperature of 610 °C. The wire was inserted into a glass liner, subsequently introduced into the pyrolysis unit and inductively heated for 9 s. The desorbed fragments were flushed into the capillary column using helium as a carrier gas. The gas chromatograph, equipped with a cryogenic unit, was programmed from 0 °C (5 min) to 300 °C (5 min) at a rate of 3 °C/min. Separation was achieved using a fused silica capillary column (25 m × 0.32 mm) coated with CP-Sil 5CB (film thickness 0.4 μm). The temperature of the flame ionisation detector (FID) was 320 °C.

Py-GC/MS was conducted on a VG Autospec Ultima mass spectrometer connected to a Hewlett Packard 5890 series II gas chromatograph equipped with a FOM-4LX pyrolysis unit. Pyrolysis and chromatographic conditions were identical to those during Py-GC-FID. Compounds were ionised at 70 eV and mass analyzed over a range of m/z 50–800 and a cycle time of 1.8 s (resolution 1000). The alkylated thiophenes were quantified by integration of their peaks in the partial summed mass chromatograms of m/z 84 + 97 + 98 + 111 + 112 + 125 + 126 + 139 + 140 + 153 + 154. The different isomers were identified on the basis of relative retention time and on the basis of mass spectral data in comparison with literature data (Sinninghe Damsté et al., 1988, 1998a, 1999; Kok et al., 2000b).

2.7. NMR

All samples were dissolved in $CDCl_3$. ^1H and ^{13}C NMR experiments were performed on a Bruker ARX400 (5 mm ^1H–^{13}C dual probe; 25 °C) and a Bruker AV-750 (5 mm BBI-ZGRAD probe; 25 °C) spectrometer. Besides normal ^1H and ^{13}C NMR experiments COSY (correlated spectroscopy), long-range COSY, NOESY (nuclear overhauser effect spectroscopy),

TOCSY (total correlation spectroscopy), HMQC (heteronuclear multiple quantum correlation) and HMBC (heteronuclear multiple bond correlation) experiments were also performed. Proton and carbon shifts were referenced to internal $CDCl_3$ (7.24/77.0 ppm). In the two-dimensional experiments the number of complex points and sweep widths were 2 K points/5.5 ppm. Quadrature detection in the indirect dimension was achieved with the time-proportional-incrementation method. The data were processed with a NMRSuite software package. After apodization with a 90 shifted sinebell, zero filling to 512 points were applied for the indirect dimensions. For the direct dimensions zero filling to 2 K real points, Lorentz transformations were used.

3. Results

3.1. Sulfurization

Different monosaccharides (**I–IX**) were sulfurized in an aqueous solution under an inert atmosphere for 4 weeks at 50 °C. Removal of elemental sulfur and freeze-drying yielded in all cases a dark brown material. Carbohydrate analysis of the reaction products using the alditol acetate method did not reveal any monosaccharide or any other GC-amenable product, suggesting the formation of high-molecular-weight (HMW) organic material. The control experiment yielded a light yellow material which, upon carbohydrate analysis, only yielded the original monosaccharide (recovery ca. 86%). This indicates that, although other reactions could have taken place, no sulfurization occurred and the largest part of the original monosaccharide was still intact.

3.2. Methyllithium/methyl iodide treatment

To analyze the reaction mixture from the sulfurized C_5 aldoses (**II–IV**), the sulfurized C_6 aldoses (**VI** and **VII**) and the control experiment, selective cleavage of polysulfide bonds by MeLi/MeI (Kohnen et al., 1991) was applied. In this way, carbon skeletons bound through polysulfide linkages into HMW material would be released as methyl thioethers (Eliel et al., 1976; Kohnen et al., 1991; Schouten et al., 1993, 1994). In order to make these products, which still are likely to contain multiple hydroxyl groups, GC-amenable, a derivatization is performed using the alditol acetate method (Fig. 1; Klok et al., 1981; Moers et al., 1989). Besides acetylation of the hydroxyl groups, methyl thioether groups formed upon methyllithium and methyl iodide treatment are then transformed into thioacetates (Angyal and Le Fur, 1980). In all experiments OSCs were released as revealed by the FPD chromatogram of the product mixture (Fig. 2B and D). GC and GC/MS analysis revealed that similar OSCs are present in all the reaction mixtures, although their distributions are different. If the polysulfide cleavage was not applied prior to the alditol acetate method (which includes $NaBH_4$ treatment) no OSCs were detected, indicating that the OSCs released were part of the non GC-amenable material, presumably comprised of sulfur cross-linked monosaccharide skeletons. The yield of GC-amenable OSCs after polysulfide cleavage was $<5\%$ of the original amount of the monosaccharide. Analysis of the control experiment (including the polysulfide cleavage with MeLi/MeI) only yielded the alditol form of the original monosaccharide. This indicates that the MeLi/MeI treatment had no effect other than the cleavage of polysulfide bonds.

To identify their structures, the three most abundant OSCs (**X**, **XI** and **XII**; Fig. 2) formed after MeLi/MeI treatment and derivatization of sulfurized lyxose (**III**) were isolated using preparative capillary gas chromatography (PCGC). This resulted in three colourless fractions (ca. 1 mg each) containing $>95\%$ (as determined by GC analysis) of the isolated OSC.

High-field ^1H and ^{13}C NMR analysis of component **XI**, the most abundant GC-amenable OSC (Fig. 2A), led to the complete assignment of proton and carbon chemical shifts and showed that the isolated component **XI** was actually a mixture of two, coeluting components (**XIa** and **XIb**; Table 1). Carbon multiplicities were established by APT spectra in combination with an inverse ^1H–^{13}C correlation experiment (HMQC), which revealed how the signals in the ^1H spectrum and the ^{13}C spectrum are correlated (Table 1) and the integral of the amount of protons in the ^1H spectrum. It was revealed that a total of ten quaternary carbonyl carbon atoms, six O–CH units, two S–CH_2 units, two O–CH_2 units and ten methyl groups were present. The proton spectrum showed ten methyl groups (all singlets) in the 2.0–2.3 ppm range, four CH_2 units (all double doublets) in the 2.9–3.3 and 4.0–4.3 ppm range and six CH units (two double doublets and four multiplets) in the 5.1–5.4 ppm range. A ^1H–^1H COSY experiment (Table 2) showed that the two protons of the CH_2 unit with signals at 2.88 and 3.20 ppm, besides the strong geminal coupling, also showed a coupling with the proton of the CH unit at 5.25 ppm. Besides this coupling, this proton at 5.25 ppm was also coupled to the proton of a CH unit at 5.38 ppm, which was also coupled to the proton of a CH unit at 5.11 ppm. This last proton was coupled to a CH_2 unit with signals at 4.12 and 4.25 (also coupled to each other) and this formed the end of a carbon skeleton comprised of five carbon atoms. The remaining two CH_2 and three CH units were coupled in a similar way (Table 2). Long-range COSY, TOCSY and HMBC experiments confirmed the interactions observed by the COSY experiments. One of the terminal CH_2 units and

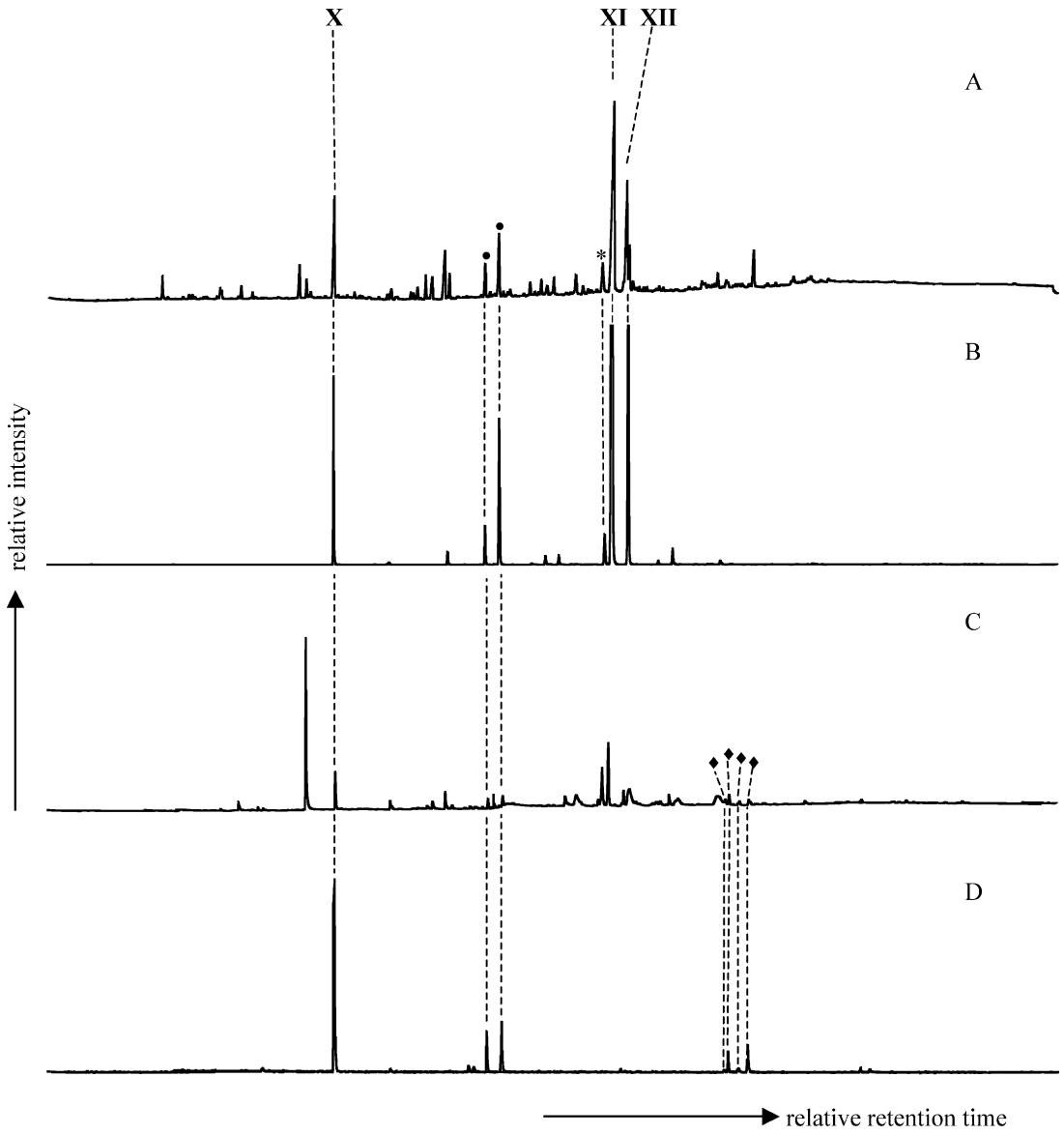
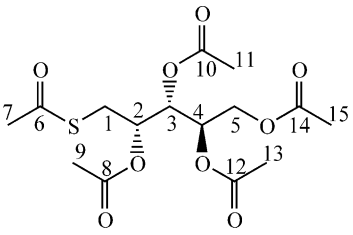


Fig. 2. FID (A and C) and FBD (B and D) chromatograms of products released after polysulfide cleavage and derivatization of sulfurized (A and B) lyxose (**III**) and (C and D) mannose (**VII**). **X**, **XI** and **XII** represent the components isolated by PCGC, filled circles represent a 2,3,4-tri-*O*-acetyl-*S*-acetyl-1-thio-tetrose, the asterisk represent 2,3,4,5-tetra-*O*-acetyl-*S*-acetyl-1-thio-pentose, and filled diamonds represent 2,3,4,5,6-penta-*O*-acetyl-*S*-acetyl-1-thio-hexoses.

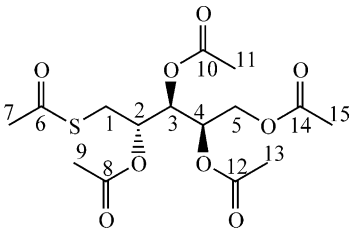
the three central CH units were in both skeletons bound by oxygen, as indicated by their chemical shifts. Since the FPD signal already revealed that sulfur must be present, the remaining CH_2 unit in both skeletons must be bound by sulfur, which was confirmed by the chemical shift in the ^{1}H-spectrum. The chemical shifts in the ^{13}C spectrum showed that these oxygen and sulfur atoms were acetyl-bound (Table 1; Jarosz et al., 2001; Kamo et al., 2001), indicating that all the oxygen and sulfur atoms connected to the central C_5 carbon skeleton were acetylated. No correlation was observed in all the experiments between any of the atoms from the two different structures, indicating that these were indeed two separate structures not connected to each other. The integrals of proton signals in the ^{1}H spectrum indicated the mixture was comprised of 60% **XIb** and 40% **XIa**.

Comparison of the proton coupling constants of both OSCs with those reported for structurally closely-related alditol acetates (Angyal and Le Fur, 1980; Osawa et al., 1991; Jarosz et al., 2001; Kamo et al., 2001) resulted in the complete assignment of the stereochemistry of the carbon atoms of both components. The stereochemistry

Table 1
^{1}H and ^{13}C data of 2,3,4,5-tetra-*O*-acetyl-*S*-acetyl-1-thio-arabinose (**XIa**) and 2,3,4,5-tetra-*O*-acetyl-*S*-acetyl-1-thio-lyxose (**XIb**)



XIa



XIb

Component	C-number	H-shift (ppm)	C-shift (ppm)[a]			
			CH_3	CH_2	CH	C_q
XIa	1	2.88 (1H, dd, J = 14.0 and 8.1 Hz), 3.20 (1H, dd, J = 14.0 and 5.5 Hz)		29.35		
	2	5.25 (1H, m, J = 8.2, 5.7 and 2.7 Hz)			68.84	
	3	5.38 (1H, dd, J = 8.7 and 2.7 Hz)			69.55	
	4	5.11 (1H, m, J = 8.7, 5.0 and 2.7 Hz)			68.36	
	5	4.12 (1H, dd, J = 12.5 and 5.0 Hz), 4.25 (1H, dd, J = 12.5 and 2.7 Hz)		61.81		
	6	–				194.24[b]
	7	2.332 (3H, s)	30.42			
	8	–				169.90
	9	2.063 (3H, s)	20.65[c]			
	10	–				169.85
	11	2.152 (3H, s)	20.67[c]			
	12	–				169.96
	13	2.056 (3H, s)	20.70[c]			
	14	–				170.61
	15	2.065 (3H, s)	20.72[c]			
XIb	1	2.99 (1H, dd, J = 14.6 and 6.6 Hz), 3.30 (1H, dd, J = 14.6 and 3.3 Hz)		29.42		
	2	5.14 (1H, m, J = 7.8, 6.5 and 3.3 Hz)			68.77	
	3	5.34 (1H, dd, J = 7.8 and 3.1 Hz)			70.06	
	4	5.37 (1H, m, J = 6.9, 4.9 and 3.1 Hz)			68.27	
	5	3.97 (1H, dd, J = 11.8 and 7.0 Hz), 4.28 (1H, dd, J = 11.8 and 4.9 Hz)		62.00		
	6	–				194.47[b]
	7	2.333 (3H, s)	30.42			
	8	–				169.73
	9	2.022 (3H, s)	20.65[c]			
	10	–				169.81
	11	2.154 (3H, s)	20.67[c]			
	12	–				170.09
	13	2.082 (3H, s)	20.70[c]			
	14	–				170.44
	15	2.044 (3H, s)	20.82[c]			

[a] Multiplicity of signals determined by APT.
[b] Assignments may be interchanged with the isomer.
[c] Assignment may be interchanged.

of **XIa** was 2*R*, 3*R* and 4*R*, thereby identifying this component as 2,3,4,5-tetra-*O*-acetyl-*S*-acetyl-1-thio-D-arabinose, and that of **XIb** was 2*R*, 3*S* and 4*R*, and thus component **XIb** is 2,3,4,5-tetra-*O*-acetyl-*S*-acetyl-1-thio-D-lyxose.

These structural assignments were supported by data obtained by GC/MS. Mass spectrometry revealed a molecular ion at *m/z* 378 (Fig. 3), which confirms the molecular weight ($C_{15}H_{22}O_9S$). The inferred electron ionisation fragmentation pattern of these components is

Table 2
Selected COSY cross peaks observed for 2,3,4,5-tetra-*O*-acetyl-*S*-acetyl-1-thio-D-arabinose (**XIa**) and 2,3,4,5-tetra-*O*-acetyl-*S*-acetyl-1-thio-D-lyxose (**XIb**)

Component	Proton	Shift (ppm)	COSY cross peaks observed
XIa	1	2.88	1′, 2
	1′	3.20	1, 2
	2	5.25	1, 1′, 3
	3	5.38	2, 4
	4	5.11	3, 5, 5′
	5	4.12	4, 5′
	5′	4.25	4, 5
XIb	1b	2.99	1′, 2
	1b′	3.30	1, 2
	2b	5.14	1, 1′, 3
	3b	5.34	2, 4
	4b	5.37	3, 5, 5′
	5b	3.97	4, 5′
	5b′	4.28	4, 5

shown in Fig. 3. Most of the primary fragments can be explained by either an elimination of an acetoxyl radical or acetic acid from the molecular ion or by cleavage of the alditol chain. These primary fragments undergo further fragmentation by subsequent elimination(s) comparable to those of normal acetylated alditols (e.g. Kamerling and Vliegenthart, 1974). The fragmentation pattern for **XIa** is not drawn but is identical to that of **XIb**.

The identification of the two other isolated OSCs, **X** and **XII**, was performed using GC/MS and ^{1}H NMR. The mass spectrum of **XII** also revealed a molecular ion at *m*/*z* 378 and a fragmentation pattern similar to that of the mixture of **XIa** and **XIb** (Fig. 3). ^{1}H NMR of **XII** also showed a great structural similarity with the OSCs **XIa** and **XIb**. Three methyl groups (all singlets) in the 2.0–2.4 ppm range, two CH_2 units (all double doublets) in the 2.9–3.3 and 4.0–4.4 ppm range and three CH units (a triplet and two multiplets) in the 5.2–5.4 ppm range were observed (Table 3). A ^{1}H–^{1}H COSY experiment (Table 3) showed similar couplings to those observed for **XIa** and **XIb**. This confirmed that **XII** indeed was another sulfurized and acetylated C_5-monosaccharide. Comparison of the coupling constants with those observed for closely related acetylated alditols (Angyal and Le Fur, 1980; Osawa et al., 1991; Jarosz et al., 2001; Kamo et al., 2001) resulted in the stereochemical assignment, i.e. 2*S*, 3*S* and 4*R*, indicating that **XII** was 2,3,4,5-tetra-*O*-acetyl-*S*-acetyl-1-thio-D-xylose. The mass spectrum of another OSC, eluting just before **XI** and **XII** (marked with an asterisk in Fig. 2) was virtually identical to those of **XIa**, **XIb** and **XII**, indicating that this OSC is likely another acetylated C_5 monosaccharide with the original carbonyl function replaced with sulfur.

The ^{1}H NMR spectrum of OSC **X** showed three CH_3 groups (all singlets) in the 2.1–2.4 ppm range, two CH_2 units (all double doublets) in the 3.1–3.3 and 4.1–4.3 ppm range and one CH unit (a multiplet) at 5.2 ppm (Table 4). A ^{1}H–^{1}H COSY experiment (Table 4)

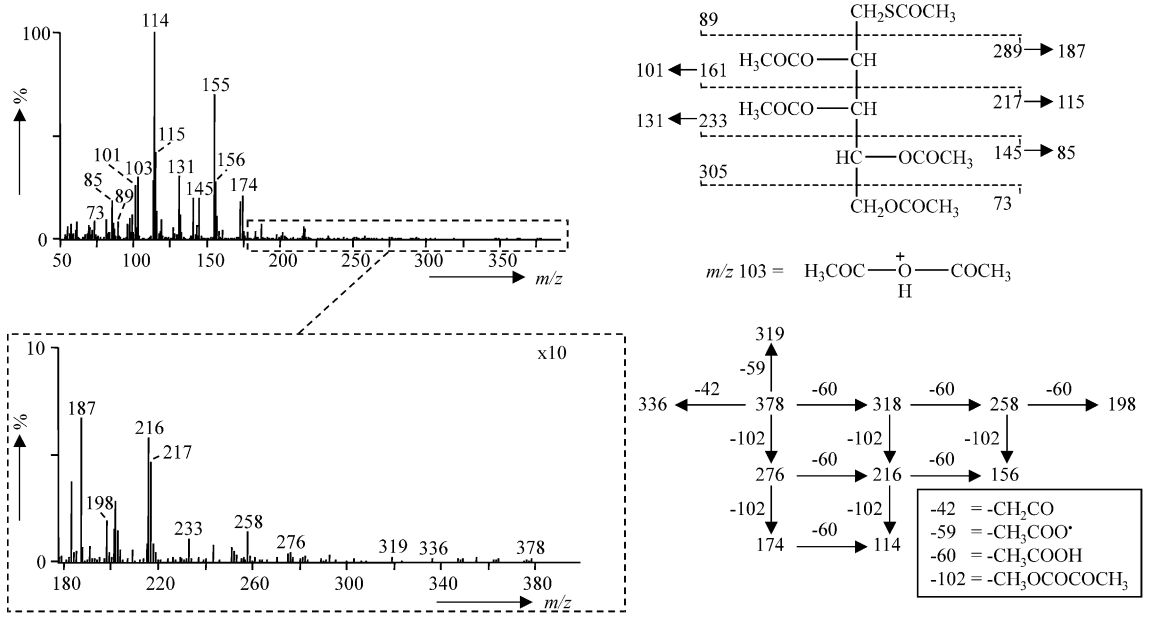
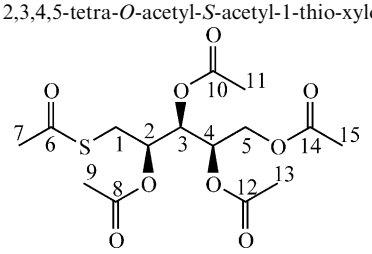


Fig. 3. Mass spectrum of the mixture of 2,3,4,5-tetra-*O*-acetyl-*S*-acetyl-1-thio-D-arabinose (**XIa**) and 2,3,4,5-tetra-*O*-acetyl-*S*-acetyl-1-thio-D-lyxose (**XIb**) and inferred fragmentation pathway.

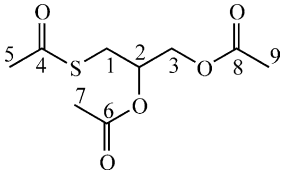
Table 3
^{1}H data of 2,3,4,5-tetra-*O*-acetyl-*S*-acetyl-1-thio-xylose (**XII**)



C-number	H-shift (ppm)	COSY cross peaks observed
1	2.92 (1H, dd, J = 7.8 and 14.1 Hz),	1′, 2
	3.30 (1H, dd, J = 4.6 and 14.2 Hz)	1, 2
2	5.21 (1H, m, J = 7.8, 5.0 and 4.3 Hz)	1, 1′, 3
3	5.37 (1H, t, J = 5.2 Hz)	2, 4
4	5.32 (1H, m, J = 5.6, 5.7 and 4.2 Hz)	3, 5, 5′
5	4.02 (1H, dd, J = 6.1 and 12.0 Hz),	4, 5′
	4.36 (1H, dd, J = 4.1 and 12.0 Hz)	4, 5
7	2.36	
9	2.15[a]	
11	2.14[a]	
13	2.11[a]	
15	2.09[a]	

[a] Assignment may be interchanged.

Table 4
^{1}H data of component 2,3-di-*O*-acetyl-*S*-acetyl-1-thio-glyceraldehyde (**X**)



C-number	H-shift (ppm)	COSY cross peaks observed
1	3.09 (1H, dd, J = 6.6 and 14.2 Hz),	1′, 2
	3.28 (1H, dd, J = 5.6 and 14.2 Hz)	1, 2
2	5.17 (1H, m)	1, 1′, 3, 3′
3	4.15 (1H, dd, J = 5.9 and 12.0 Hz),	2, 3′
	4.29 (1H, dd, J = 3.9 and 12.0 Hz)	2, 3
5	2.38	
7	2.11[a]	
9	2.10[a]	

[a] Assignment may be interchanged.

revealed that the two protons of the CH_2 unit with signals at 2.09 and 3.28 ppm showed, besides a strong geminal coupling, a coupling to the proton of the CH unit at 5.17 ppm and to the protons of the other CH_2 unit with signals at 4.15 and 4.29. These latter signals also showed a strong geminal coupling. This indicated that the basis of structure **X** is a C_3 carbon skeleton. The proton chemical shifts indicate that one of the CH_2 units is bound by sulfur and the remaining CH_2 and CH unit by oxygen. Comparison of the chemical shift with those observed for OSCs **XIa**, **XIb** and **XII** indicated that these oxygen and sulfur groups were acetylated. The mass spectrum of **X** (Fig. 4) is in agreement with this

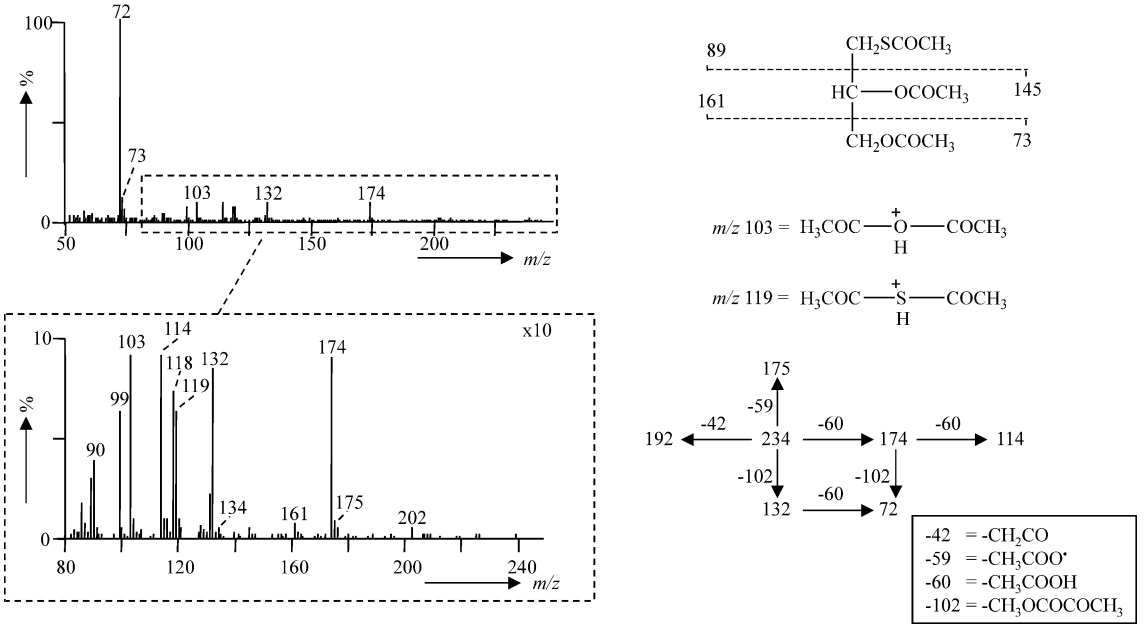


Fig. 4. Mass spectrum of 2,3-di-*O*-acetyl-*S*-acetyl-1-thio-glyceraldehyde (**X**) and inferred fragmentation pathway.

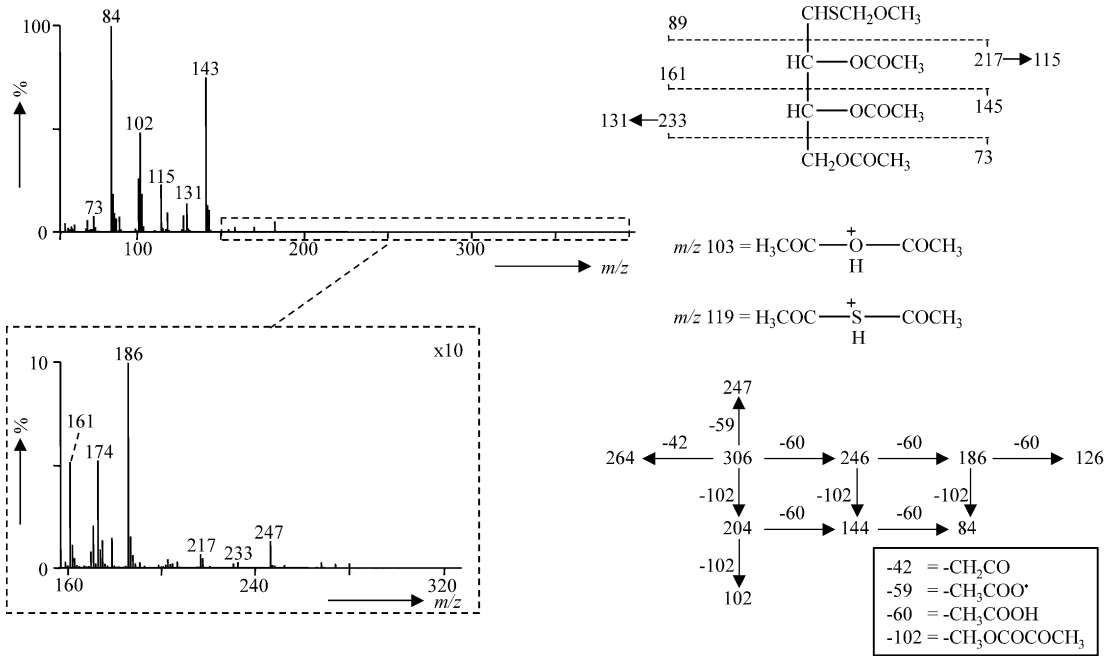


Fig. 5. Mass spectrum of a 2,3,4-tri-*O*-acetyl-*S*-acetyl-1-thio-tetrose and the inferred fragmentation pathway.

structural assignment, although no molecular ion was observed. However, the observed fragmentation pattern is consistent with those of **XIa** and **XIb** (Fig. 3) and normal acetylated alditols (e.g. Kamerling and Vliegenthart, 1974). These findings indicated that **X** is an acetylated C_3 monosaccharide unit in which the oxygen at position 1 is replaced by sulfur, i.e. 2,3-di-*O*-acetyl-*S*-acetyl-1-thio-glyceraldehyde.

Using the structural information obtained from the isolated OSCs, the other OSCs present in the reaction mixture after MeLi/MeI treatment and derivatization using the alditol acetate method of the sulfurized C_5 and C_6 monosaccharides (Fig. 2) could be tentatively identified. The mass spectra of the OSCs eluting between derivatives **X** and **XI** (marked with a filled circle in Fig. 2) and those with a longer retention time than derivative **XII** (only present in the reaction mixtures of C_6 monosaccharides and marked with a filled diamond in Fig. 2) reveal a fragmentation pattern (Figs. 5 and 6), which resembles those of **X**, **XI** and **XII**, and of normal alditols (e.g. Kamerling and Vliegenthart, 1974), although no molecular ion peak could be observed. This suggests that these components are most likely acetylated C_4 and C_6 monosaccharides, in which the oxygen at position 1 is replaced by sulfur. This means that the components containing sulfur and eluting between derivatives **X** and **XI** (marked with a filled circle in Fig. 2) are likely 2,3,4-tri-*O*-acetyl-*S*-acetyl-1-thio-tetroses and those with a higher retention time than derivative **XII** (marked with a filled diamond in Fig. 2) are likely 2,3,4,5,6-penta-*O*-acetyl-*S*-acetyl-1-thio-hexoses.

3.3. Flash pyrolysis

To obtain additional information on the material formed upon sulfurization (OSCs identified so far represent $<5\%$ of the original monosaccharides) flash pyrolysis was performed on the reaction products of all the sulfurized monosaccharides (Fig. 7). For this purpose, product mixtures of monosaccharides with a different chain length, compounds **I**–**VII**, as well as the same chain length but a different structure, components **VIII** (a ketose) and **IX** (a 6-deoxy C_6 monosaccharide) were chosen.

Flash pyrolysates of the reaction mixtures were generally dominated by series of C_0–C_4 alkylated thiophenes and furans (Table 5 and Fig. 8). Using the peak areas of all the C_0–C_3 alkylated thiophenes the weighted average number of carbon atoms of these alkylated thiophenes was calculated (Table 6). This average number varied from 5.0 for those formed from sulfurized erythrose (**I**) to 5.7 for those formed from sulfurized fucose (**IX**). The percentage of C_2 alkylated thiophenes with a linear carbon skeleton, 2-ethylthiophene and 2,5-dimethylthiophene, versus the other thiophene isomers, ranged between 25 and 37% (Table 6).

4. Discussion

Our results indicate that monosaccharides can be sulfurized through a reaction with reduced inorganic sulfur species at relatively low-temperatures. The presence of the thioacetate moiety only at position C-1 (Fig. 9) in all

the OSCs identified (after the derivatization of the products formed upon polysulfide cleavage) showed that the carbonyl function reacts preferentially with inorganic sulfur species and that the hydroxyl groups of the monosaccharides are relatively inert. The preferential attack at C-1 also indicates that the polysulfides reacted with the open-chain form (containing the carbonyl moiety) of the monosaccharide (Fig. 9), which is only present in minor amounts in aqueous solutions. It has been previously demonstrated that under similar reaction conditions aldehyde and keto groups react much faster than alcohols with polysulfide ions (Fig. 9;

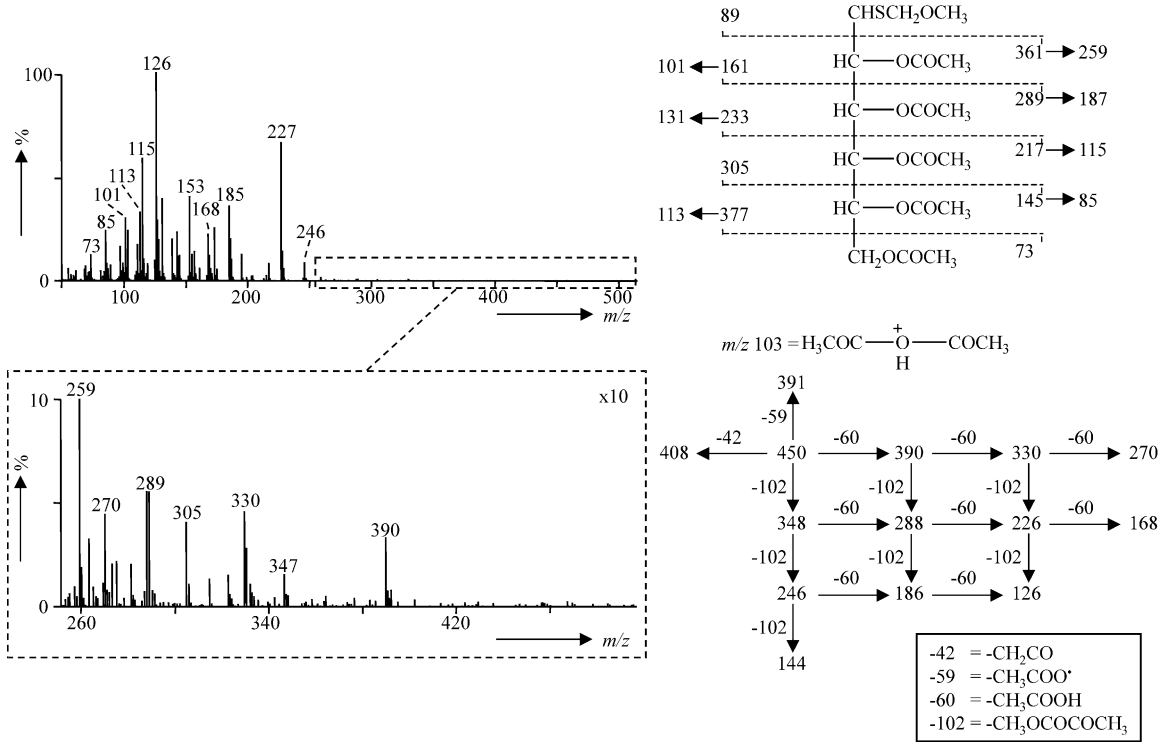


Fig. 6. Mass spectrum of a 2,3,4,5,6-penta-*O*-acetyl-*S*-acetyl-1-thio-hexose and inferred fragmentation pathway.

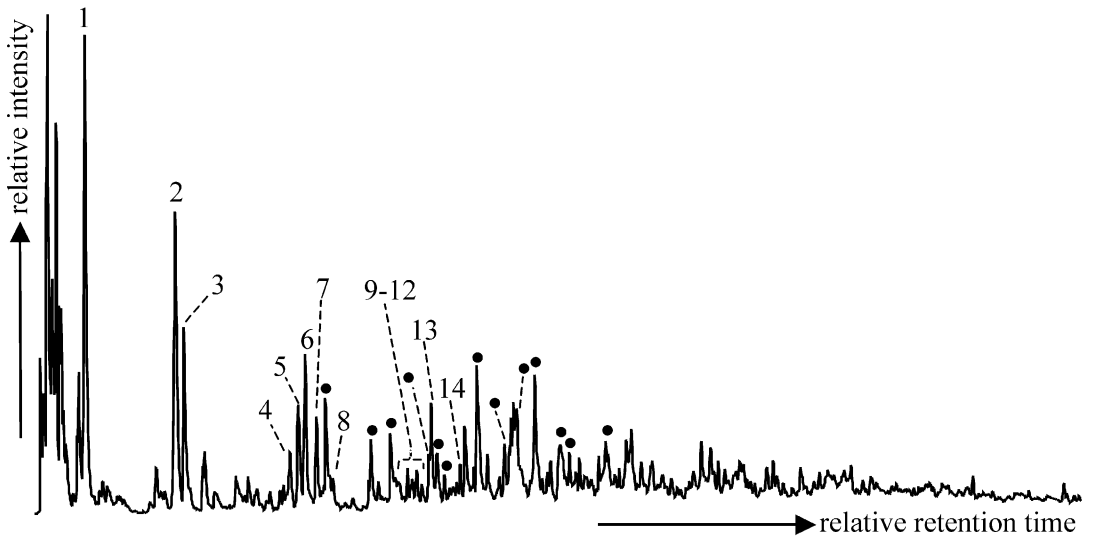


Fig. 7. Total ion chromatogram (TIC) of the pyrolysate of sulfurized lyxose (**III**). Compound assignments are listed in Table 5. Filled circles represent series of alkylated furans.

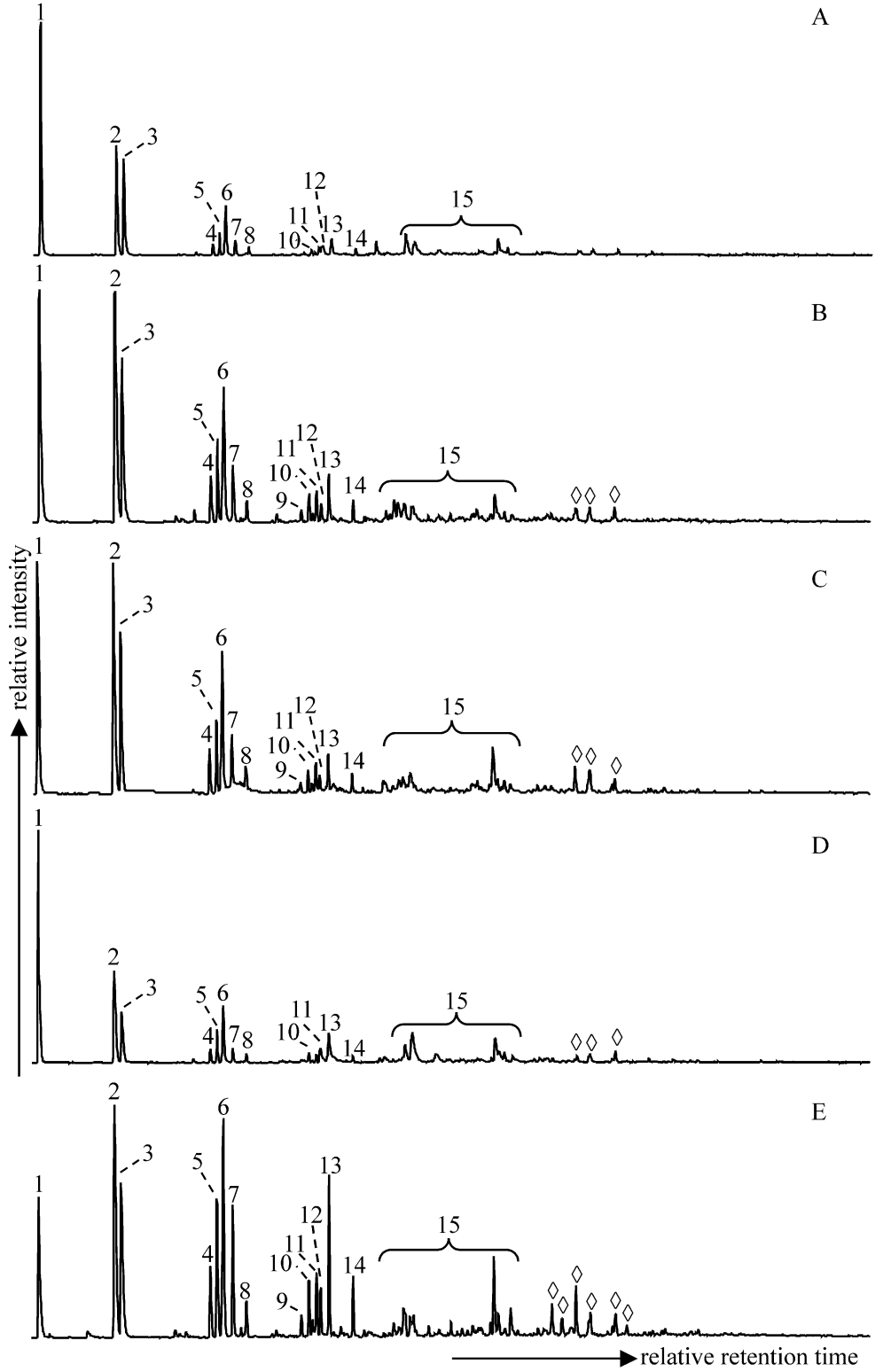


Fig. 8. Distribution of alkylthiophenes in the pyrolysates of sulfurized (A) erythrose (**I**), (B) lyxose (**III**), (C) glucose (**VI**), (D) fructose (**VIII**) and (E) fucose (**IX**), as revealed by the partial accurate mass chromatograms of m/z 84 + 97 + 98 + 111 + 112 + 125 + 126 + 139 + 140 + 153 + 154. Compound assignments are listed in Table 5. Open diamond represent series of C_5 + alkylated thiophenes.

Table 5
Thiophenes identified in the flash pyrolysates of sulfurized monosaccharides[a]

1	Thiophene
2	2-Methylthiophene
3	3-Methylthiophene
4	2-Ethylthiophene
5	2,5-Dimethylthiophene
6	2,4-Dimethylthiophene
7	2,3-Dimethylthiophene
8	3,4-Dimethylthiophene
9	2-Propylthiophene
10	2-Ethyl-5-methylthiophene
11	2-Ethyl-4-methylthiophene
12	Ethylmethylthiophene
13	2,3,5-Trimethylthiophene
14	2,3,4-Trimethylthiophene
15	$C_8H_{12}S$[b]

[a] Numbers refer to Figs. 7 and 8.
[b] Mixture of 2-methyl-5-propylthiophene, 2,5-diethylthiophene, 2-butylthiophene, 2-ethyl-3,5-dimethylthiophene, ethyldimethyl-thiophene and/or 5-ethyl-2,3-dimethylthiophene.

Table 6
Average number of carbon atoms of the C_0–C_3 alkylated thiophenes and the percentage of the 2-ethylthiophene and 2,5-dimethylthiophene of all C_2 alkylated thiophenes for the pyrolysates of the sulfurized monosaccharides

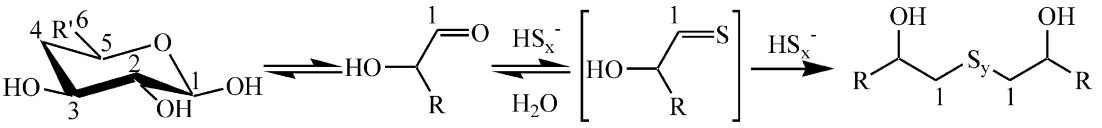
Monosaccharide	Average number of carbon atoms	2-ET and 2,5-DMT (%)[a]
Erythrose (**I**)	4.99	25
Arabinose (**II**)	5.23	35
Lyxose (**III**)	5.25	32
Ribose (**IV**)	5.20	33
Galactose (**V**)	5.26	37
Glucose (**VI**)	5.24	29
Mannose (**VII**)	5.31	34
Fructose (**VIII**)	5.19	31
Fucose (**IX**)	5.70	33

[a] ET = ethylthiophene, DMT = dimethylthiophene.

Schouten et al., 1993, 1994; Schneckenburger et al., 1998), which is in good agreement with our data. Since the monosaccharides only contain one aldehyde group, it is logical that sulfurization resulted in the formation of dimers. If these dimers consisted of two monomers linked by a polysulfide bond cleavage of this bond, would indeed result in the main OSCs observed (Fig. 2).

Besides sulfurization, a second process must take place since our results showed clearly that stereoisomers of the main OSCs were formed (Fig. 2). This indicates that isomerisation of chiral centres (three in the case of a C_5 monosaccharide and four in the case of a C_6 monosaccharide) also takes place. For example, in the case of the OSCs formed from lyxose (**III**), besides the sulfurized form with the original configuration (derivative **XIb**), isomers with an inversed stereochemistry at C-2 (derivative **XII**) and C-3 (derivative **XIa**) were formed (Fig. 2). This isomerization may be due to the alkaline circumstances, caused by the chemical reagents added (NaHS and elemental sulfur) and not by heating since in the control experiment no racemization was observed. El khadem et al. (1987) showed that during alkaline treatment of monosaccharides various isomers are formed. The amounts of both derivatives (**XIa** and **XII**) are approximately equal, indicating that isomerisation at C-2 and C-3 occurs at approximately the same rate.

In addition to stereoisomers of sulfurized monosaccharides with the original carbon chain length, OSCs with a shorter chain length were also found (Fig. 2). This indicates that a third process, cleavage of C–C bonds, also occurred during the simulation experiments. Again, as in the case of the isomerisation, this bond cleavage reaction is not caused by the heating to 50 °C since C–C bond cleavage was not observed in the control experiment. Thus, these cleavages must again be the result of a reaction with the reagents (NaHS and elemental sulfur) Harsch et al. (1984) showed that under alkaline conditions sugars may be cleaved by retroaldol reactions. Such a process might explain the formation of monosaccharides with shorter chain length than the original chain length. Fragmentation and recombination by a retroaldol reaction followed by an aldol reaction reaction might also partly explain the isomerization observed. These reaction products would subsequently be subjected to sulfurization.



R= $C_3H_7O_2$, $C_4H_9O_3$, ...

R'=H, CH_2OH,.....

X in HS_X^-=1,2,.....

Y in S_y=2,3,.....

Fig. 9. Inferred reaction pathway for the formation of a polysulfide dimer resulting from sulfurization.

Only a small part (<5%) of the monosaccharide was retrieved as GC-amenable OSCs after the derivatization of the products formed upon polysulfide cleavage. Since no monosaccharide could be retrieved after the reaction, this indicates that the original monosaccharide was converted into non GC-amenable material. However, polysulfide bond cleavage only succeeded in partly breaking this material down, suggesting that monosulfide linkages were also present. To obtain additional information on the HMW material, flash pyrolysis was performed. Flash pyrolysis of the reaction products revealed substantial amounts of short-chain alkylated (C_0–C_5) thiophenes with a distribution comparable to alkylthiophenes in pyrolysates of sulfurised carbohydrates (Kok et al., 2000b; Sinninghe Damsté et al., 1998b) and S-rich kerogen (van Kaam-Peters et al., 1998a; Sinninghe Damsté et al., 1998b). It has been shown that these alkylthiophenes are thermodynamically-stable end products of sulfur-rich HMW material formed by thermal rearrangement reactions during pyrolysis (Krein and Aizenshtat, 1994; Sinninghe Damsté et al., 1998a). This implies that a substantial part of the product mixture is part of HMW structures since LMW (including dimeric) structures would have evaporated prior to formation of these alkylated thiophenes. In addition, the large amount of alkylated thiophenes relative to furans in the pyrolysates shows that a much larger part than the <5% liberated upon polysulfide cleavage must be connected through sulfur. However, the mode of bonding cannot be solely through polysulfide bridges since these would have been broken upon polysulfidic cleavage. This indicates that a large amount of the cross-linkages was either through a monosulfidic-linkage or a non-sulfidic linkage. Kok et al. (1995) showed that monosulfidic linkages can indeed be formed through sulfurization of double bond functionalities and that if sediment is used instead of a phase transfer catalyst (PTC) during the simulation experiments, these are the predominant linkages formed. Since in the present sulfurization experiments no PTC was used, it is likely that in our experiments mostly monosulfide linkages were formed. However, even if we infer the involvement of cross-linking of monosaccharides through monosulfide linkages, sulfurization of functional groups other than the aldehyde has to be invoked in order to account for the formation of HMW material. Replacement of the polysulfide group in the end product shown in Fig. 9 by a monosulfide moiety would not explain the formation of alkylthiophenes upon flash pyrolysis. Therefore, we must infer that the hydroxy groups (albeit at a much slower rate than the aldehyde functionalities) are also prone to sulfurization and that this results in the formation of sulfur-rich HMW products from the monosaccharides.

Alternatively, it is also possible that processes other than sulfurization result in the formation of HMW material. A control experiment without the addition of the reagents (NaHS and elemental sulfur) indicated some (14%) loss of monosaccharide perhaps resulting from such "non-sulfur" polymerisation reactions. It is possible that if the reagents were present the amount of non-sulfur-containing linkages would be even higher, due to the higher pH. However, the relatively high amount of alkylthiophenes in the pyrolysates probably indicates that "sulfur" polymerisation reactions predominate.

The flash pyrolysis experiments also showed that, in all cases besides alkylthiophenes with linear carbon skeletons (i.e. 2,5-dimethylthiophene), alkylthiophenes with non-linear carbon skeletons (i.e. 2,3-dimethylthiophene) were formed in relatively high amounts (Table 6). Since the original monosaccharides had linear carbon skeletons, this indicates that during pyrolysis substantial C–C bond formation as well as cleavage occurred. This can in our view only be explained if the material formed upon sulfurization is cross linked by a substantial number of (poly)sulfide bonds, more than would be possible through sulfurization of only the carbonyl functionalities, hinting at sulfurisation of hydroxy groups.

Besides the large amounts of alkylthiophenes with linear carbon skeletons, substantial amounts of alkylthiophenes with non-linear carbon skeletons were observed in S-rich kerogen pyrolysates (Eglinton et al., 1990b; Sinninghe Damsté and de Leeuw, 1992; van Kaam-Peters et al., 1998; Sinninghe Damsté et al., 1998b). Our results indicate that these alkylthiophenes with non-linear carbon skeletons can originate from sulfurized monosaccharides. In addition, the distribution of the alkylthiophenes in S-rich kerogen pyrolysates was comparable in all cases. Our results show that the structure of the original monosaccharide had no direct influence on the distribution of the alkylated thiophenes formed (Fig. 8, Table 6), maybe due to the fact that during formation of the macromolecular structures other processes, like for instance breaking of C–C bonds, played an important role. Thus, the macromolecular structures formed upon sulfurization were comparable in composition, which explains why in all pyrolysates of S-rich kerogens comparable distributions of alkylthiophenes are observed.

5. Conclusions

Sulfurization of different monosaccharides under laboratory conditions resulted in the complete conversion into sulfur-containing macromolecular structures. The products formed after cleavage of polysulfide linkages of this material showed that sulfurization of the carbonyl functionality plays an important role in this respect. Other OSCs indicated that in addition to sulfurization, cleavage of C–C bonds and isomerisation also took place. The yield of the recoverable OSCs after

cleavage of polysulfide linkages was relatively low (< 5%), indicating that only a small part of the HMW material is bound solely through polysulfidic linkages. This indicates that most of the HMW material was still linked otherwise, among others through monosulfide linkages. Flash pyrolysis revealed in all cases, short-chain alkylated (C_0–C_5) thiophenes, with linear as well as non-linear carbon skeletons, indicating that during pyrolysis C–C bond formation as well as C–C cleavage had occurred. The structure of the monosaccharide used had no direct influence on the alkylated thiophenes formed. These results provide direct experimental evidence that sulfurization of monosaccharides at a relatively low temperature can result in the formation of sulfurized material and confirms the idea that preservation of carbohydrates through sulfurization may be an important pathway of preservation of organic matter in anoxic depositional environments, like for instance in the case of the KCF.

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