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Gel-permeation chromatography of solutions of D-glucose after irradiation by ultrasound and γ-rays

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ABSTRACT

Gel-permeation chromatography on the COO^- and COOH forms of Ultrahydrogel 120 of aqueous solutions of D-glucose irradiated with ultrasound and γ-rays, followed by g.l.c.–m.s. of the trimethylsilylated methoximated derivatives of the products, revealed derivatives of gluconic acid and hexulosonic acids after irradiation in the presence of air and 2-deoxy-*arabino*-hexonic and gluconic acid after irradiation under argon. A small proportion of products was formed with molecular weights larger than that of glucose and which contained an enolic structure.

INTRODUCTION

The products formed by irradiation of aqueous solutions of D-glucose with ultrasound are similar to those formed by exposure to ionising radiation[1–3]. Irradiation of aerated solutions leads to oxidation products, namely, gluconic acid, hexosuloses, and *gluco*-hexodialdose. In the absence of oxygen, in addition to these products, deoxyhexonic acids, deoxyhexosuloses, and deoxyhexodialdoses are produced. Since, during the sonolysis of water and in contrast to radiolysis, a small amount of oxygen is produced, the ratio of the deoxy compounds to the oxidation products is smaller in sonolysis. Degradation products with less than 6 carbon atoms are formed by irradiation of aerated solutions, but only minute proportions are present in deaerated solutions. An exception is malonaldehyde, which is a major product of sonolysis and radiolysis especially in alkaline solution. Malonaldehyde produces oligomers in aqueous solutions by 1,2- and 1,4-addition reactions even in the absence of irradiation[4]. The formation of polymers was observed[5–7] on exposure of aqueous solutions of D-glucose to ionising radiation in the absence of oxygen. The polymer was heterogeneous in molecular weight and chemical composition, and evidence was obtained that gluconolactone residues were incorporated. The purpose of the present work was to ascertain by gel-permeation chromatography whether polymers are produced by irradiation of aqueous solutions of glucose with ultrasound. For purposes of comparison, γ-irradiated solutions were also investigated.

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RESULTS

Aerated and argon-saturated aqueous solutions of D-glucose (0.05M) were irradiated with ultrasound and with γ-rays at 1.38 kGY/h, a dose rate that was shown by ferrous sulfate dosimetry to be equivalent to the ultrasound efficiency. The u.v. spectra of the irradiated solutions were similar and each contained 2 broad peaks: argon saturated, sonolysis λ_{max} 207 and 260 nm, radiolysis λ_{max} 200 and 260 nm; aerated, sonolysis λ_{max} 200 and 265 nm, radiolysis λ_{max} 199 and 265 nm.

Gel-permeation chromatography of the irradiated solutions was performed on Ultrahydrogel 120 (Waters), which is designed for the determination of the distribution of molecular weights of water-soluble polymers. Both the COO^- and COOH forms of the gel were used (obtained by prewashing with 0.01M sodium azide and 0.01M acetic acid, respectively). The results are shown in Fig. 1 and were obtained by using refractometer and u.v. (254 nm) detectors.

The eluates from the COO^- form of the column were separated into 3 fractions that involved peaks with retention times (*1*) smaller (14–22 min) than that of glucose, (*2*) similar to that (22–28 min) of glucose, and (*3*) larger (28–40 min) than that of glucose. The hydroxyl and carboxyl groups in the products were trimethylsilylated and the carbonyl groups were methoximated. G.l.c.–m.s. of the components in fractions 2 and 3

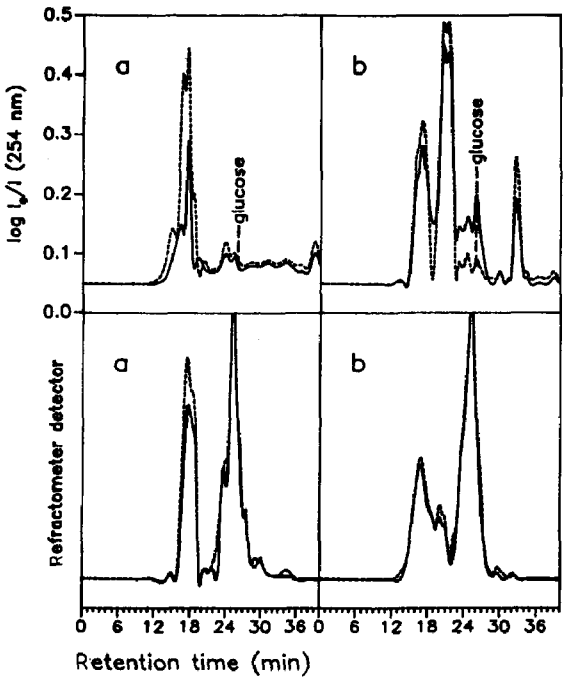


Fig. 1. Gel-permeation chromatograms of 0.05M D-glucose (0.2 mL injected) on Ultrahydrogel 120 (Waters) flushed first with 0.01M sodium azide and then eluted with water at 0.5 mL/min: (*a*) under argon; (*b*) aerated: ———, sonolysis for 96 h; ----, radiolysis for 70 h.

revealed derivatives of known products (D-glucono-1,4- and -1,5-lactones, hexosuloses, D-*gluco*-hexodialdose; additionally, in the absence of oxygen, deoxyhexonic acids, deoxyhexosuloses, and deoxyhexodialdoses, and, in the presence of oxygen, degradation products with <6 carbon atoms) of the sonolysis and radiolysis of glucose[1,2,3,8–11].

The u.v. spectra of fraction *1* contained 2 broad peaks: argon saturated, sonolysis λ_{max} 194 and 260 nm, radiolysis λ_{max} 197 and 263 nm; aerated, sonolysis λ_{max} 205 and 256 nm, radiolysis λ_{max} 207 and 260 nm. The same products were contained in fraction *1* of the sonolysis and the radiolysis, as shown by g.l.c.–m.s. of the trimethylsilylated methoximated derivatives, and they were as follows. Under argon: D-glucono-1,4- and -1,5-lactones, 2-deoxyhexonic acid, deoxyhexonolactones (since the lactone ring is not cleaved during derivatisation, the position of the deoxy group could not be determined by m.s.), D-arabinose, ?-deoxypentose, and 4-deoxypentose. Aeration: 4- and 5-hexulosonic acids, D-glucono-1,4- and 1,5-lactones, 5-pentulosonic acid, D-arabinose, and tetronic acid.

The gel-permeation chromatograms obtained on the COOH form of the column are shown in Fig. 2. The chromatograms of the products of sonolysis and radiolysis were similar and showed only differences in the peak intensities.

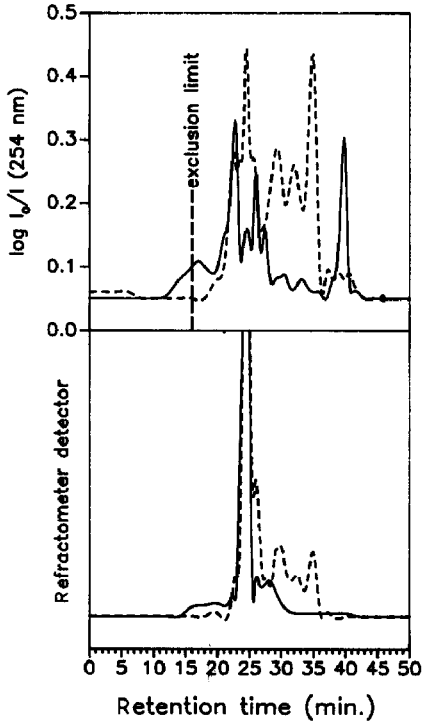


Fig. 2. Gel-permeation chromatograms of 0.05M D-glucose (0.2 mL injected), sonolysis for 96 h, on Ultrahydrogel 120 (Waters) flushed first with 0.01M acetic acid and then eluted with water at 0.5 mL/min: ———, under argon; ----, aerated.

The distinct peak with a retention time smaller than that of glucose, and present in Fig. 1, was absent from Fig. 2 and the refractometer detector showed only a small broad shoulder. The u.v. detector, for the samples irradiated under argon, gave a broad peak corresponding to molecular weights of 1000–5000, and a pronounced peak which could correspond to dimeric derivatives of glucose. In the chromatograms of the irradiated aerated solutions, only the latter peak was present and there were larger proportions of compounds with molecular weights similar to that of glucose which absorbed strongly at 254 nm.

The results with the COO^- form of the column demonstrated that non-ionic carbohydrates (mono- and di-saccharides, lactones) were separated according to their molecular weights, but acids appeared near to the exclusion limit of the column (corresponding to a retention time of 16.5 min under the conditions used). Thus, gluconolactone and glucose had the same retention time (24 min; *cf.* 18.0 min for gluconic acid). Vapour-phase osometry proved that gluconic acid was not present as an aggregate in aqueous solution; hence, the anomalous behaviour of the acids in gel-permeation chromatography must originate in the properties of the stationary phase.

The COOH form of the column did not show anomalous behaviour and the acids were separated according to their molecular weights, whereas the retention times of the non-ionic compounds and the exclusion limit of the column (tested with amylose) were not affected.

Irradiation of aerated and deaerated solutions for 96 h resulted in the consumption of $\sim 50\%$ of the glucose on sonolysis and $\sim 70\%$ on radiolysis. The fraction of glucose converted into gluconic acid for sonolysis and radiolysis in the presence of air was 0.3, and, under argon, 0.08 for radiolysis and 0.14 for sonolysis. Gluconic acid (fraction *1*) and gluconolactone (fraction *2*) were present in the ratio $\sim 2{:}1$. The concentrations of formaldehyde in the irradiated solutions of glucose were as follows: under argon, radiolysis 1.6×10^{-4}M and sonolysis 4×10^{-4}M; in the presence of air, radiolysis 6×10^{-4}M and sonolysis 6×10^{-4}M. No formaldehyde could be detected in fraction *1* (limit of detection, 5×10^{-6}M). In order to obtain more information on the composition of fraction *1*, electron impact (e.i.) and desorption chemical ionisation (d.c.i.) mass spectra of the underivatised samples were taken. The e.i.-mass spectra could not be interpreted. D.c.i.-mass spectrometry of fraction *1* obtained after sonolysis and radiolysis in the presence of air revealed three overlapping peaks. By comparison with an authentic sample, the main peak was shown to correspond to gluconic acid (m/z 197 for MH^+).

The second peak showed the fragmentation given in Scheme 1.

Based on m/z 177 for MH^+, this compound could have been derived from gluconolactone by elimination of 2 hydrogens. One possibility would be ascorbic acid, but its fragmentation is not consistent with this interpretation. The spectrum therefore should correspond to hexulosonolactones. The d.c.i.-mass spectrum of the third peak is given in Fig. 3. The ions with m/z 61, 91, 103, 133, and 163 were also formed from glucose (see Scheme 1). The ions m/z 181, 145, and 127, which are characteristic for glucose, were lacking and there were additional peaks at m/z 193, 177, 147, 117, and 75.

Fragmentation: $MH^+ = m/z$ 177
(% values are given in relation to the base peak = 100%)

m/z 177 (8%)

$$\xrightarrow[H_2O]{-18} 159\,(6\%) \xrightarrow[CO]{-28} 131\,(35\%) \xrightarrow[H_2O]{-18} 113\,(68\%)$$

$$\xrightarrow[CO]{-28} 149\,(11\%) \xrightarrow[H_2O]{-18\uparrow}$$

$$\xrightarrow[CO_2]{-44} 133\,(26\%) \xrightarrow[H_2O]{-18} 115\,(100\%) \xrightarrow[H_2O]{-18} 97\,(70\%)$$

Fragmentation of ascorbic acid

m/z 177 (8%)

$$\xrightarrow[CO]{-28} 149\,(22\%) \xrightarrow[H_2O]{-18} 131\,(52\%) \xrightarrow[H_2O]{-18} 113\,(100\%)$$

$$-30 \downarrow CH_2O$$

$$119\,(22\%)$$

Fragmentation of glucose
m/z 181 (MH^+, 10%)

$$\xrightarrow[H_2O]{-18} 163\,(82\%) \xrightarrow[H_2O]{-18} 145\,(60\%) \xrightarrow[H_2O]{-18} 127\,(22\%)$$

$$\downarrow \xrightarrow[CH_2O]{-30} 133\,(35\%) \xrightarrow[CH_2O]{-30} 103\,(55\%) \xrightarrow[CH_2O]{-30} 73\,(35\%)$$

$$\downarrow \xrightarrow[H_2O]{-18} 85\,(36\%)$$

$$\xrightarrow[CHOH=CHOH]{-60} 121\,(18\%) \xrightarrow[CH_2O]{-30} 91\,(100\%) \xrightarrow[CH_2O]{-30} 61\,(68\%)$$

Scheme 1. Fragmentations in d.c.i.-m.s. of underivatised sampes.

Therefore, fraction *1* contained no glucose. Possible structures corresponding to the observed spectrum are given in the Discussion.

D.c.i.-mass spectrometry of fraction *1*, obtained after sonolysis and radiolysis under argon, revealed only one broad peak with a complex mass spectrum and which probably contained several components, but structural assignments were not possible.

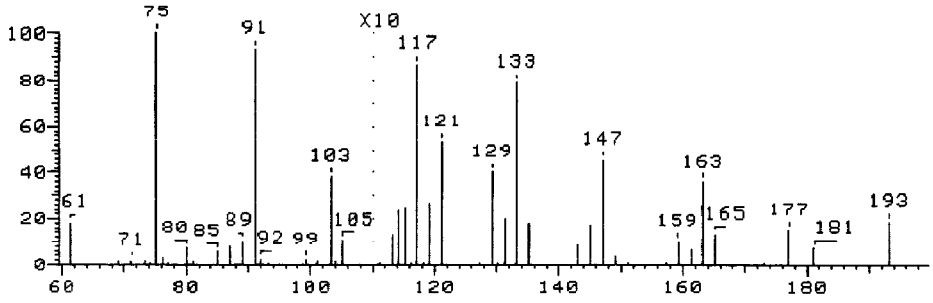


Fig. 3. D.c.i.-mass spectrum (isobutane) of one of the peaks of fraction 1.

The peaks of highest masses were at *m/z* 177 and 163, which correspond to MH^+ of hexulosonolactone and deoxyhexonolactone.

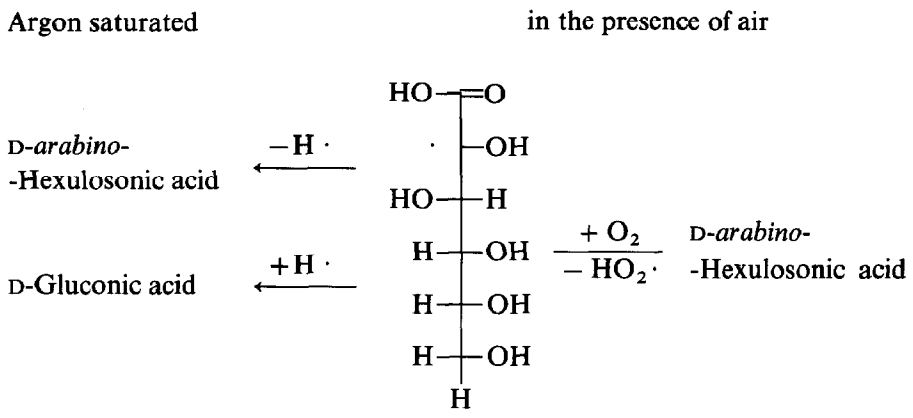
Dosimetry. — Both OH and H radicals are primary species in the sonolysis and radiolysis of water. The rate of production of these species is determined by the dose rate in γ-irradiation but, with ultrasound, additional parameters, such as geometrical arrangement, frequency, and concentration, and nature of dissolved gas, are important[12]. A γ-dose rate [1.38 kGy/h (0.138 Mrad/h)], which was equivalent to the ultrasound effect, was determined using the Fricke–ferrous sulfate dosimeter[13,14].

DISCUSSION

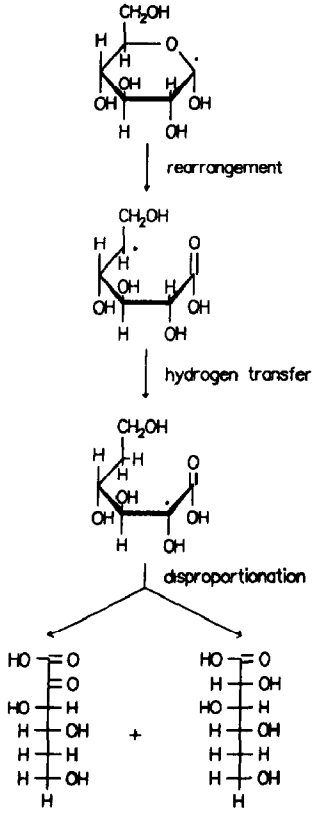
Gel-permeation chromatography on the COO^- form of Ultrahydrogel revealed products with retention times lower than that of glucose in all of the irradiated solutions. G.l.c.–mass spectrometry and d.c.i.-mass spectrometry showed that these products were mainly acids. After sonolysis and radiolysis in the presence of air, the main product was gluconic acid, and hexulosonic acids were present. Sonolysis and radiolysis under argon gave gluconic acid and deoxy acids. Arabinose was present in the irradiated aerated and deaerated solutions, and deoxypentoses were present in the latter solution. On the above column, non-ionic compounds were fractionated according to their molecular weights, whereas the acids appeared near the exclusion limit. Therefore, the pentose in fraction *1* must have been formed during concentration of the acidic eluates, and the precursor was probably D-*arabino*-2-hexulosonic acid. Pentoses have been isolated[15–18] as intermediates in the decomposition of D-*arabino*-2-hexulosonic acid and its enolic tautomer (ascorbic acid) into 2-furaldehyde. The D-*arabino*-2-hexulosonic acid probably is a secondary product formed from gluconic acid by abstraction of the labile H-2 (Scheme 2).

D-*arabino*-2-Hexulosonic acid was not present in fraction *1*, probably because it decomposed into arabinose. The 4- and 5-hexulosonic acids, which were detected in fraction *1* of the samples irradiated in the presence of air, must have a lower tendency to decarboxylation.

The precursor of the 4-deoxypentose, formed by irradiation in the absence of air,



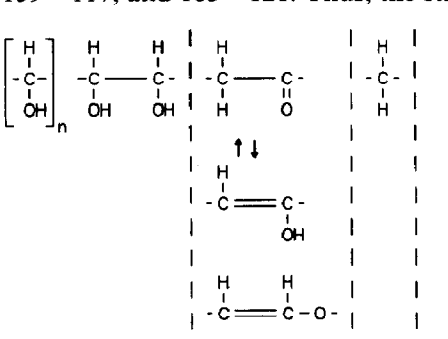
Scheme 2. Reaction mechanism for the formation of D-*arabino*-2-hexulosonic acid.



Scheme 3. Reaction mechanism for the formation of 5-deoxy-2-hexulosonic acid.

should be 5-deoxy-2-hexulosonic acid. The mechanism proposed for its formation is given in Scheme 3.

The results of g.l.c.–m.s. for the products with retention times smaller than that of glucose were supported by the d.c.i.-mass spectra. In the samples irradiated by ultrasound or ionising radiation in the presence of air, in addition to gluconic acid and hexulosonic acids, a compound was present with a molecular weight higher than that of glucose. The d.c.i.-mass spectrum (Fig. 3) is characterised by a series of ions with mass differences of 30 (*m/z* 61, 91, and 121; 103, 133, 163, and 193; 117, 147, and 177). This repeating unit could be either CH_2O or CH(OH). Formaldehyde was detected as a major product of sonolysis and radiolysis, but no formaldehyde was detected in fraction *1*. If formaldehyde is present as the monomer unit of a polymer, it should be released, at least partly, by depolymerisation. Another reason for rejecting the presence of CH_2O units is the occurrence of eliminations of H_2O in the mass spectrum: *m/z* 177→159, 147→129, 117→99, 121→103, and 103→85. Therefore, the repeating unit should be CH(OH). In addition, there are mass differences of 42: *m/z* 103→61, 117→75, 133→91, 159→117, and 163→121. Thus, the structural elements in Scheme 4 can be proposed.



n=0	61	103	117
n=1	91	133	147
n=2	121	163	177
n=3		193	

Scheme 4. Proposed structure for one of the components of fraction *1* based on the d.c.i.-mass spectrum given in Fig. 3.

The products in the irradiated solutions were fractionated also on the COOH form of Ultrahydrogel, on which ionic and non-ionic compounds are separated according to molecular weight. Oligomers or polymers should have retention times smaller than that of glucose. Only the u.v. detector revealed such peaks, one of which was observed in all samples and probably corresponded to dimeric derivatives of glucose. For each of the samples irradiated under argon, there was an additional broad peak that extended to the exclusion limit of the column (~5000 Da). Since the refractometer detector reflects the proportions of products present, it is concluded that only a small proportion of material with molecular weight higher than that of glucose was formed, which must have a high extinction coefficient at 254 nm indicative of enolic groups as structural units[19].

By ferrous sulfate dosimetry, a γ-dose rate of 1.38 kGy was found to be equivalent to the intensity of ultrasound used. On applying this dose rate to solutions of glucose,

the yield of products was found to be ~20% higher than for irradiation with ultrasound. Furthermore, h.p.l.c. showed that, on irradiation in presence of air, more degradation products and derivatives of glucose with high extinction coefficients at 254 nm were formed. This is consistent with published g.l.c.–m.s. data[1-3]. On sonolysis and radiolysis in the presence of air, the same proportion of glucose was converted into gluconic acid (irradiated for 96 h, 0.3), whereas, in the absence of air, a larger fraction (0.14) was converted on sonolysis than on radiolysis (0.08). This finding can be correlated with the results in the literature[3] that, for irradiation in the absence of air, the yield of deoxy compounds was smaller and the yield of oxidation products was larger on sonolysis than on radiolysis. This result reflects the fact that sonolysis of water in a closed system, in contrast to γ-irradiation, produces a small amount of molecular oxygen[20].

The results are only partly consistent with those of Barker *et al.*[5,6] who, on γ-irradiation of aqueous 1% D-glucose solution with a dose of 65 kGy in the absence of oxygen, obtained 7.6% of polymer. No polymer was found in aerated solutions.

In the present experiments, a total dose of 130 kGy was applied so that ~15% of polymer should be obtained. The refractometer detector indicated that, for sonolysis and radiolysis in the presence and absence of air, only a small proportion of material with molecular weight larger than that of glucose was obtained. That oxygen was absent was indicated by the fact that deoxy compounds were obtained. Barker *et al.*[5,6] assumed the polymer to be a polygluconic acid linked by C–C bonds at positions 6 and 2, and they suggested it to be formed by radical recombination. The formation of a polymer by such a process from a monomer that contained no polymerisable groups is improbable and, moreover, glucose units should be incorporated.

A possible explanation is that dialysis through cellophane is similar to gel-permeation chromatography with the COO^- form of Ultrahydrogel, whereby the diffusion of the free acids is prevented and the resulting polymer fraction would still contain gluconic acid. This would explain the fact that Barker *et al.*[5,6] found an elemental composition and i.r. spectra for the polymer that corresponded to gluconic acid. However, Snell[7] reported a high molecular weight for the polymer based on light-scattering measurements, although it was stated that the intrinsic viscosities were much lower than expected. The strong u.v. absorption at 254 nm of the small proportion of compounds with molecular weights larger than that of glucose, obtained in the present experiments, suggests that they contain enolic groups. The polymer may have originated from the products of oxidation or degradation of glucose by polyaddition reactions. Investigation of the problem is in progress.

EXPERIMENTAL

For ultrasound irradiation, an 800-kHz ultrasound generator (Physikalische Werkstätten PHYWE GmbH, Göttingen; transducer diameter, 2.5 cm; average intensity, 2W/cm^2) was applied. 0.05M D-glucose (10 mL, in bidistilled water) in a glass vial (2 cm i.d.) with a flat bottom and covered with an aluminium foil was placed on the ultrasound transducer. For irradiation in absence of air, argon was bubbled through the

solution for 30 min and the vials were sealed. The vial and the transducer were immersed in a water bath at 5°.

For γ-irradiation, the vials were placed at the appropriate distance from a 25 000 Curie ^{60}Co source to give a dose rate of 1.38 kGy/h at 20°. Dosimetry with the ferrous sulfate dosimeter was performed as described[13]. The mol. wt. of gluconic acid in aqueous solution was determined with a Knauer vapour-pressure osmometer.

Gel-permeation chromatography was performed on a column of Ultrahydrogel (Waters; cross-linked hydroxylated polymethacrylate with residual carboxyl group) with a pore size of 12 nm (efficiency: 14 000 plates/column, exclusion limit ~5000 Da) by elution with water. Aliquots of the eluate were concentrated, and the residues were trimethylsilylated and methoximated according to Lane and Sweeley[21].

A capillary column with cross-linked and surface-bonded silicone rubber phase (Durabond I) was used for g.l.c. For detection and identification, the gas chromatograph was coupled directly to a Finnigan MAT 212 mass spectrometer. Mass spectra were recorded on a cycle time of 3.5 s. Desorption c.i.-mass spectrometry[22] was performed using isobutane with heating at 5°/s.

Formaldehyde was determined by a colorometric method[23].

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