

Structural comparison of algal microfossils with extant algaenans using ruthenium tetroxide degradation

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Algaenans are non-hydrolysable biopolymers present in the cell walls of specific freshwater and marine microalgae. As a consequence of their high resistance algaenans are selectively preserved upon sedimentation and burial in the sediments. Upon chemical analysis of algaenan isolated from some green microalgae it was shown that most algaenans share the same chemical features i.e. highly aliphatic and probably cross-linked by ether linkages (for a review de Leeuw and Largeau, 1993; Gelin *et al.*, 1997; Blokker *et al.*, 1998). Flash pyrolysis gas-chromatography mass spectrometry (py-GC/MS), fourier transform infrared spectroscopy (FTIR) and ^{13}C -nuclear magnetic resonance spectrometry (^{13}C -NMR) were used as tools to determine the chemical nature of these materials. However, these methods only provide information about the bulk chemical nature of the material but do not reveal much detail about the monomeric composition of the biopolymer. On the other hand, investigation of the algal lipids and information obtained from chemical degradations of the algaenans using chemical reagents like hydrogen iodide (HI) and ruthenium tetroxide (RuO_4) have provided a much more detailed insight in the composition of the algaenans. These techniques revealed that there are distinct differences between the monomers of the algaenans from different algal species. For example, the algaenan produced by *Tetraedron minimum* consists of unsaturated ω -hydroxy fatty acids of chain lengths C_{30} , C_{32} and C_{34} , whether the algaenan of *Scenedesmus communis* is composed of the same type of monomers with a chain length of C_{30} . *Pediastrum boryanum* uses C_{30} and C_{32} unsaturated and diunsaturated ω -hydroxy fatty acids to biosynthesize the resistant biopolymer in the cell walls. The monomers are intermolecularly linked via ester-linkages to form linear polyesters cross-linked by ether-linkages, providing an intricate three-dimensional network. *Botryococcus braunii* (races A, B and L) biosynthesizes an algaenan constructed of

linear polyaldehydes (Berthéas *et al.*, 1997) probably also containing ether cross-links (Gelin 1994). RuO_4 chemical degradation proved to be a useful tool in determining the chemical differences between the investigated algaenans by generating typical oxidation products that can be used to reconstruct the monomeric composition of the algaenans.

Although microscopy and chemical analysis using for example FTIR, ^{13}C -NMR and py-GC/MS showed that algaenans are probably an important constituent of Type I and II kerogens, it is not yet established whether these biopolymers accumulate in sediments without alterations. It is also unclear whether changes upon further burial in the sediments would affect the chemical properties of the algaenans. Therefore, a series of sediments containing high quantities of either *B. braunii*, *T. minimum* or *P. boryanum* microfossils, as established by microscopy, originating from different locations and ages, was investigated to determine the chemical composition of the resistant material present. Lacustrine sediments from the Messel oil shale in Germany, the Uddelermeer in the Netherlands, and from Funza and Fuquene in Colombia were analysed by FTIR and py-GC/MS. RuO_4 oxidation was used to provide information about the chemical composition of the microfossils and clearly revealed the presence of algaenans by generating the species specific oxidation products. Upon RuO_4 treatment of such ether cross-linked aliphatic biopolymers the vicinal ether-linked monomers are converted into two carboxylic acid containing fragments and single ether-linked into ketone group bearing fragments, thus releasing compounds from the biopolymer representative of the monomers used by the algae to biosynthesize these resistant materials (Fig. 1). Oxidation of for example *P. boryanum* algaenan will predominantly yield C_9 , C_{21} and C_{23} dicarboxylic acids originating from oxidation of vicinal ether-linked monomers (Fig. 1). These species specific oxidation products are

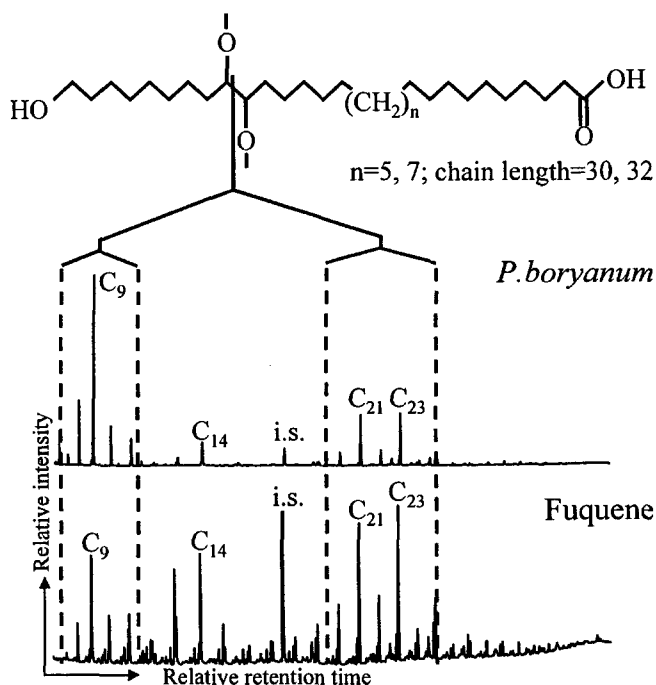


FIG. 1. A simplified model of *P. boryanum* algaenan and the gas chromatogram of the RuO_4 degradation product mixture of such an algaenan and of the Fuquene sediment. Both chromatograms are dominated by a series of linear dicarboxylic acids, generated upon RuO_4 degradation of *P. boryanum* algaenan.

also generated upon oxidation of the Fuquene sediment (Fig. 1). Although not all of the material is oxidized by the RuO_4 treatment, the distinct differences between the product mixtures of these sediments could be used to discriminate between the microfossils of *B. braunii*, *T. minimum* and *P. boryanum*.

Analysis of the RuO_4 product mixtures derived from the sediments and comparison with the RuO_4 degradation products from algaenans isolated from cultured algae showed that the algaenans present in these sediments are virtually identical to those from cultured algae and did not undergo severe alterations upon sedimentation and subsequent burial in the sediments. The differences between the sedimentary material and algaenans are probably mainly caused by the presence of other microfossils and because monomers that are only ester-linked to the polymer matrix in the case of *T. minimum* and *P. boryanum* algaenan are removed from the algaenan during diagenesis. These findings clearly illustrate that selective preservation of resistant algaenans can be

an important process in the preservation of sedimentary organic matter in lacustrine sediments.

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