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COMPARISON OF SPOROPOLLENIN-LIKE ALGAL RESISTANT POLYMER FROM CELL WALL OF *BOTRYOCOCCUS, SCENEDESMUS* AND *LYCOPODIUM CLAVATUM* BY GC-PYROLYSIS

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Key Word Index—*Botryococcus*; *Scenedesmus*; *Lycopodium*; algae; algal resistant polymer; cell wall components; GC-pyrogramms; hydrocarbons, ketocarotenoids; polyethylene; sporopollenin; sporopollenin-like substances.

Abstract—Sporopollenin-like resistant, insoluble biopolymers from algal cell walls (ARP) of *Botryococcus braunii* and *Scenedesmus obliquus* (Chlorophyta) were compared by a pyrolysis-GC (Py-GC) method with synthetic polyethylene (PE) used as a model polyhydrocarbon and with a classical sporopollenin (SP) from *Lycopodium clavatum* (Lycopodiales). A series of *n*-alkanes and *n*-alkenes of carbon chain length of C_{13}–C_{31} were found as thermal degradation products of both kind of biopolymers ARP and PE. The results indicate essential differences of GC-pyrolysis pattern and so of chemical composition of SP-like algal polymer and SP of *L. clavatum* which can be used for distinguishing these biopolymers. The Py/GC or Py/GCMS can be used for identification of SP and SP-like insoluble biopolymers of various biological origins and for chemotaxonomical purposes.

INTRODUCTION

Recently it was shown that all algal strains (Chlorococcales, Chlorococcaceae) producing ketocarotenoids also form a sporopollenin-like biopolymer (ARP) which are known for their extraordinary resistance against chemical and biological agents. These polymers are deposited in the outer cell wall layer, i.e. in the so called trilaminar structure [1–5]. These cell wall (CW) preparations are coloured pinkish by carotenoids. An example is the CW of the alga *Scenedesmus obliquus*. In contrast the CW of natural strains not forming ARP, as well as mutants defective in ARP but derived from a wild strain forming this biopolymer, do not contain ketocarotenoids.

This fact seems to indicate that CW-carotenoids may be involved in the formation of ARP. There are at present no serious arguments supporting the hypothesis of Atkinson *et al.* [6] that carotenoids may be precursors or intermediates of sporopollenin (SP) or of sporopollenin-like algal biopolymer (ARP) [4, 5, 7].

Cell walls of *Botryococcus braunii* (Chlorococcales, Botryococcaceae) are another example containing a resistant biopolymer called PRB. Secondary carotenoids were not found in these CW or in cells from laboratory cultures of this alga, but were only found in cells grown under natural conditions. The PRB of this alga was identified as a polymer of hydrocarbon structure [8–10]. Recently, the existence of two types of PRB in CW of this alga were demonstrated.One was PRB A, an unbranched saturated hydrocarbon with chain length up to C_{31} cross-linked by ether and/or ester bounds. The other, PRB-B, was of terpenoic nature but not derived from carotenoids [9].

The taxonomic affinity of *Botryococcus* and *Scenedesmus* encouraged us to compare the resistant biopolymer of these algae using the GC-pyrolysis method. It was also interesting to study by this method a taxonomically distant species containing so called classical SP. These were sporae of *Lycopodium clavatum* (Lycopodiales). Additionally, polyethylene was used as a synthetic saturated high M_r polyhydrocarbon for comparative purposes as a defined model substance.

RESULTS AND DISCUSSION

The chromatographic patterns of pyrolysis products of ARP from cell walls of *Botryococcus braunii* and from maternal cell walls of the alga *Scenedesmus obliquus* were very similar (Fig. 1). Both pyrogramms were characterized by the presence of a series of peaks, each with one more carbon than the preceding peak. The pyrolysis-GC-MS data of ARP of *Scenedesmus obliquus* previously obtained showed that the main peaks are formed by *n*-hydrocarbons of the chain length C_{13}–C_{32} [11]. Some of the mentioned peaks, especially the peaks e–l, show a fine structure (doublets or triplets). A series of similar shaped maxima were observed in pyrogramms of polyethylene [12, and Burczyk, J. and Dworzański, J., unpublished results.] The last mentioned publication [12] showed that each triplet corresponds to a definite C number and that it comprises an α-olefin (main component), an *n*-alkane and an α,ω-di-olefine. It is difficult to explain structural factors of the biopolymer, which influence the intensity of the peaks of triplets or doublets.

In general there were no differences in the positioning of the main peaks formed by pyrolysis of the biopolymers of *Botryococcus* and *Scenedesmus*. However, the chromatographic pattern of pyrolysis products showed some quantitative differences. These were the especially high

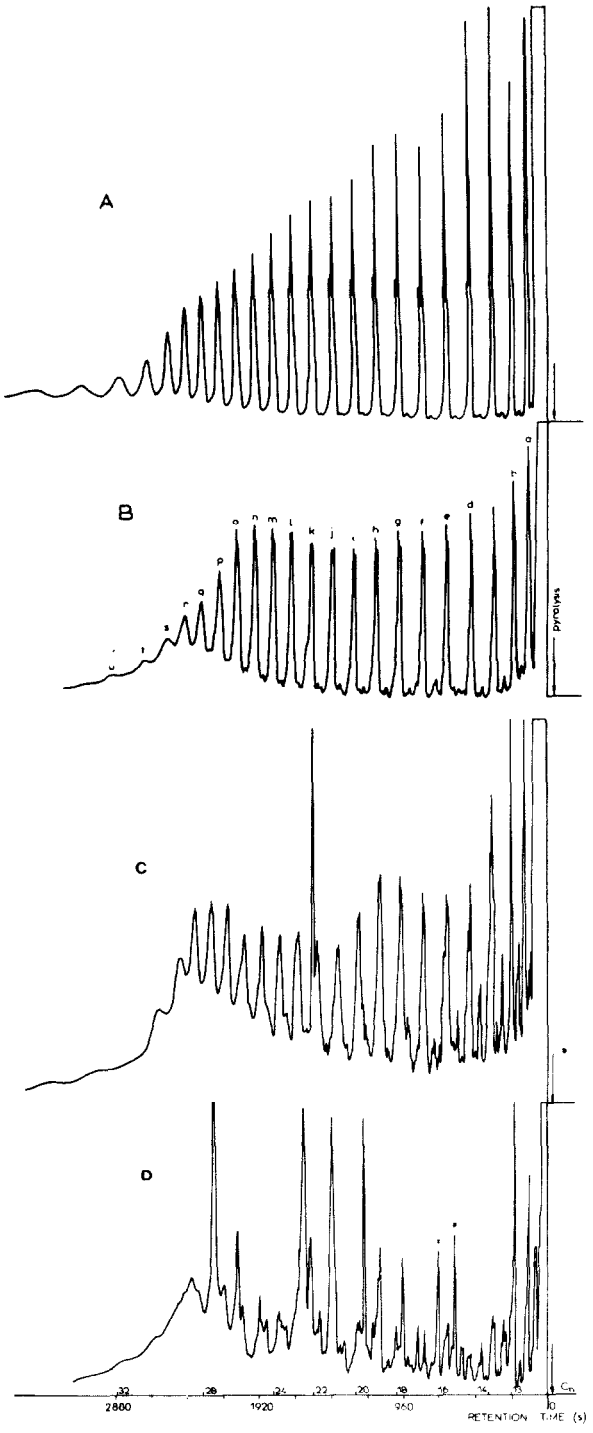

Fig. 1. GC-pyrogramm of: A, polyethylene, Hostalen (Hoechst, FRG); B, algal resistant polymer from maternal cell walls of *Scenedesmus obliquus*, strain 633 (ARP); C, resistant polymer of *Botryococcus braunii*; D, sporopollenin of *Lycopodium clavatum* spores.

peak of the region k-1 (of pyrogramm C). This seems to indicate a dominance of a corresponding fragment in the structure of the polyolefine of *Botryococcus*. However, at present the limited data does not allow the interpretation of the structural consequences of this fact.

Pyrogramms of ARP of both algae showed the presence of minor peaks located between the main peaks a-b, b-c, etc. Their intensity in the sample of *Botryococcus* PRB were higher than of ARP og *Scenedesmus*. At the present state of knowledge it is difficult to distinguish if these minor peaks originate from contamination of ARP caused by differences in preparation [8] or reflect other structural differences of both ARP.

The chemical similarity of the samples of ARP from *Botryococcus braunii* and *Scenedesmus obliquus* and their differences from SP of *L. clavatum* spores can be seen from Table 1 and Fig. 1.

The Py-GC patterns also showed the coincidence of R_t values of all the main splitting products (peaks) of synthetic PE and the investigated biopolymers. This indicates that the framework of the latter are built of very similar (or identical) long *n*-alkane or *n*-alkene units.

Some differences in the chromatographic patterns of both samples of ARP and PE concern the region of the peak k, especially the clear shoulder on the left side of peak k (Fig. 1B). Its positioning corresponds to that of peak k1 of previously analysed ARP samples of the same alga (see Fig 2B of ref. [11]). Another quantitative difference in the chromatographic pattern concerns the distribution of chain length of pyrolysis products of both samples. In the PE pyrogramm products representing shorter chain length prevail compared to the pattern for ARP.

The linear decrease of peak height with the increasing number of the chain length for synthetic PE is shown in Fig. 1. Under the same conditions of pyrolysis the ARP (Fig. 1B) gives the same main peaks but with some quantitative prevailance of longer fragments with chain length between C_{21} and C_{31}, which form a bimodal-like distribution. It is an open question if the preference for longer fragments in the ARP does not reflect definite aliphatic units, e.g. originating by splitting of long alcanoic-diesters or -hydroxydiesters taking part in the forming of ARP.

The similarity of the main pyrolysis products of ARP and PE does not necessarily mean their chemical identity before pyrolysis. In fact the essential differences of ARP and PE consist in the absence of oxygen in PE and presence of 16.8% oxygen in ARP of *Scenedesmus* and 8.8% in PRB of *Botryococcus*. A part of this oxygen probably exists as hydroxyls as indicated by the IR maxima described in the previous paper [4]. It is well known, that C–O (ester bonds) as well as C–C and N–C bonds are weak and that under pyrolysis conditions they cleave more easily than other bonds e.g. C–H, C=O and O–H. This permits the conclusion that the initial biopolymer may be a hydroxylated and/or esterified form of polyhydrocarbon chain.

The pyrogramm derived from *L. clavatum* SP differed markedly in the number of maxima (about 52 maxima in contrast to 21 peaks of ARP from the green algae belonging to the Chlorophyta. It does not show regularity of appearance of peaks (R_t values) observed in pyrogramms of ARP. This may indicate that the main framework of the biopolymer of *L. clavatum* probably do not show a hydrocarbon structure but another more highly unsaturated structure (calculated from about 50% of bromine) in bromosporopollenin [13]. However, to facilitate positioning of ARP derived pyrolysis products their R_t values were compared with those of standard *n*-alkanes (C_{13}–C_{28}) denoted on the abscissa. This was aimed at

Table 1. Elementary composition (%) of SP-like biopolymers of *Botryococcus braunii*, *Scenedesmus obliquus* and SP of *Lycopodium clavatum*

	C	H	O	N	P	Ash	Ref.
PRB	71.35	10.3	8.8	0.41	0.23	8	[8]
ARP (Sc)	69.93	9.53	16.88	1.50	n	n	
SP of *L. clavatum*	62.9	8	26.3	0.12	0.05	3	[8]

n: Not estimated.

eventually identifying the alkanes in a pyrogramm of SP of *L. clavatum*.

The positioning of peaks k and p corresponded to the retention times of xylene and phenol. These may be degradation products of lignin-like component of SP of *L. clavatum* [13–17]. However, this finding cannot serve as an equivocal argument for the presence of these compounds as structural elements of SP because some phenolic acids were confirmed also among the products of alkaline fusion of naturally occurring SP and synthetically derived SP from carotenoids.

The Py/GC alone, as well as in connection with other methods such as Py/GC/MS, of SP and SP-like resistant biopolymers derived from a wide range of biological subjects and natural sources seems to be a means of comparing this class of insoluble acetolysis resistant cell wall biopolymers. This may be useful for taxonomic purposes as well as for the elucidation of their chemical nature. Further studies are required to elucidate the chemical structure of sporopollenin and sporopollenin-like biopolymers from plant cell walls.

EXPERIMENTAL

Biological material and culture conditions. The culture conditions and medium for the alga *Scenedesmus obliquus*, strain 633 were the same as described in our previous papers [4, 5]. All cultivation data used for the alga *Botryococcus braunii* 807/1 have been described by Berkaloff *et al.* [8].

Isolation of algal resistant polymer (ARP). The ARP used for investigations were isolated from maternal cell walls accumulating in 30-day-old cultures of *Scenedesmus obliquus*, strain 633 of the author's Collection. The isolation procedures for CW of this alga were the same as previously described [3, 4].

All data concerning the isolation of an acetolysis resistant polymer from *Botryococcus braunii* have been described by Berkaloff *et al.* [8].

Pyrolysis-GC. A GCV gas chromatograph (Pye Unicam, Cambridge, Great Britain) equipped with Curie-point pyrolyser and a flame ionisation detector, was used for analysis. The detector was coupled to an integrator (Spetra-Physics, Santa Clara, Ca, U.S.A.) and a chart recorder (PM 8222, Phillips, Eindhoven, The Netherlands). The flame ionisation detector was supplied with 30 ml/min of hydrogen and 300 ml/min of air.

Samples of *ca* 200 μg of ARP and polyethylene (Hoechst, Hostalen) were pyrolysed at 770° for 8 sec under a flow of He carrier gas (25 ml/min) and the attenuation ranges were set at 10^{-11} A/mV for full scale deflection with a recorder chart speed of 30 cm/hr. Analyses were carried out on a 1.5 m × 2 mm i.d. glass column packed with Carbowax 20M chemically bonded to Chromosorb W (100–120 mesh, Supelco, Bellefonte, U.S.A.). The oven temperature was programmed from 70 to 230° at 4°/min and the final temperature being maintained until establishment of the base line.

A mixture of even-carbon *n*-paraffin (Applied Science Lab. U.S.A.) in Et_2O was injected into the GC under the same analytic conditions as standards for comparative purpose.

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