Phytochemistry, Vol. 27, No. 2, pp. 551–557, 1988.
Printed in Great Britain.

0031–9422/88 $3.00 + 0.00

RESISTANT BIOPOLYMER IN THE OUTER WALLS OF *BOTRYOCOCCUS BRAUNII, B* RACE

A. KADOURI, S. DERENNE, C. LARGEAU, E. CASADEVALL and C. BERKALOFF*

Laboratoire de Chimie Bioorganique et Organique Physique, UA CNRS 456, E.N.S.C.P., 11 Rue Pierre et Marie Curie, 75231 Paris Cedex 05, France; *Laboratoire de Botanique-Cytophysiologie Végétale, UA CNRS 311, E.N.S., 24 Rue Lhomond, 75231 Paris Cedex 05, France

(*Revised received* 15 *May* 1987)

Key Word Index—*Botryococcus braunii, B* race; Chlorophyceae; resistant biopolymer of outer wall; electron microscopy; FT IR; solid state ^{13}C NMR; pyrolysis; comparison with sporopollenins; comparison with the resistant biopolymer of *A* race.

Abstract—A biopolymer highly resistant to non-oxidative treatments (PRB *B*) occurs in the outer walls of the *B* race of *Botryococcus braunii*. PRB *B* accounts for *ca* 10% of the total algal biomass and fulfils the usual requirements to be regarded as a sporopollenin; however, the structural information obtained indicate it does not derive from carotenoids. A complete lack of relationship was also noted between PRB *B* and the isoprenoid hydrocarbons produced in large amount by the *B* race. The PRB *B* structure is based upon saturated, normal, hydrocarbon chains up to C_{31}; these chains are probably linked by ether bridges. PRB *B* also comprises ester and hydroxyl groups protected from external attacks by the polymeric network. PRB *B* and PRB *A* (the resistant biopolymer isolated from the outer walls of the *A* race of *B. braunii*, which produces non-isoprenoid hydrocarbons) are not identical. Nevertheless they show the same major structural features and belong to the same group of resistant biopolymers (probably derived from the polymerisation of very long chain fatty acid derivatives). Analogies between PRB *A* and PRB *B* would have resulted in the formation, via fossilization of *A* and *B* races, of kerogens with a similar structure and a high oil potential.

INTRODUCTION

Several species of microalgae possess an outer wall unusually resistant to non-oxidative chemical treatments [1–17]; it is generally assumed that such walls contain a sporopollenin-like material. Sporopollenins are highly resistant biopolymers, building up the outer walls, exines, of spores and pollens [18–22] and derive from the oxidative polymerization of carotenoids and/or carotenoid esters [2, 23–32]. The supposed relationship between resistant algal outer wall constituents and sporopollenins is usually based upon the following common features: resistance to drastic treatments including acetolysis, UV fluorescence, similarities in the IR spectra of the acetolysis-resistant residues. Furthermore, in the case of several Chlorococcales, a close connection was noted in various strains, including mutants, between the ability to produce secondary carotenoids and the presence of a resistant outer wall [3, 33, 34].

A high content of resistant material, termed PRB *A*, was observed [13] in the outer walls of the *A* race of the hydrocarbon-rich green alga *Botryococcus braunii* (The *A* race exclusively produces non-isoprenoid hydrocarbons with odd carbon number from C_{23} to C_{33}; see below for a discussion on the different races of *B. braunii*). PRB *A* fulfilled all the usual requirements, listed above, to be considered as a sporopollenin. The chemical features, however, of PRB *A* indicated that its structure is based upon long, unbranched, saturated hydrocarbon chains [13, 35, 36]. Accordingly PRB *A* does not derive from carotenoids and should not be considered as a sporopollenin. These findings were ascertained by feeding experiments that indicated that PRB *A* may originate from the polymerization of unsaturated hydrocarbons or of very long chain unsaturated fatty acid derivatives [35]. PRB *A* thus provided what seemed to be the first example of a new class of resistant biopolymers distinct from sporopollenins. It also appeared that the criteria generally used for identifying the resistant materials of algal walls as 'sporopollenins' were not sufficient by themselves. (More recently, resistant constituents different from sporopollenins, and possibly related to PRB *A*, were identified in the outer walls of lichen phycobionts [16] and in higher plant cuticles [37].)

The first studies on *B. braunii* lipids [38–44] revealed that some samples (collected in Nature and from laboratory cultures) contained only odd carbon numbered, C_{23}–C_{31}, dienic and trienic, unbranched, non-isoprenoid hydrocarbons. In sharp contrast other samples, collected in Nature, exclusively contained isoprenoid C_{30}–C_{37} hydrocarbons, termed botryococcenes. Such differences were initially assumed to be related to physiological state: green actively growing cells would produce non-isoprenoid hydrocarbons, whereas brown resting ones would only contain botryococcenes [40]. However, on the basis of this assumption, the complete lack of non-isoprenoid hydrocarbons in the botrycoccenes-containing samples seemed surprising. *B. braunii* being unable, under various culture conditions, to efficiently degrade its non-isoprenoid hydrocarbons [45]. In addition, all attempts at obtaining botryococcene formation, by starting with laboratory cultures producing non-isoprenoid hydrocarbons, were unsuccessful, even after prolonged resting stages [46]. On the other hand green apparently actively dividing colonies, exclusively containing botryococcenes, were observed in Nature [47]. Finally, it was demon-

strated that laboratory cultures of several strains, derived from botryococcene-containing samples, formed only the latter hydrocarbons whatever their physiological stage, including exponential growth [48]. These findings indicated the existence of two distinct races of *B. braunii*, differing in the nature of the hydrocarbons they produce in large amounts: non-isoprenoid hydrocarbons in the *A* race, botryococcenes in the *B*. These two races show a similar general morphology; however the ultrastructure of the outer walls, where hydrocarbons are formed and stored [49], is sometimes substantially different [48–50].

In the present work the occurrence of a resistant wall constituent was tested in the *B* race of *B. braunii*. Its chemical structure was examined using FT IR, solid state ^{13}C NMR and pyrolysis (spectroscopic study of pyrolysis residues and identification of pyrolysate constituents). Our purpose was to determine whether the major differences, in hydrocarbon chemical structure and biosynthetic origin in *A* and *B* races, are paralleled by differences in resistant wall constituents.

RESULTS AND DISCUSSION

Occurrence, localization and abundance of resistant biopolymer

Direct acetolysis of the algal biomass has been commonly used as a simple way of testing for the presence of a resistant material in numerous species [1, 3, 6–12, 14, 15, 17]. This method affords a large amount of residue from *B. braunii*, *B* race. However, it recently appeared that acetolysis has two important drawbacks, (i) it may produce artefacts, via polymerization of carotenoids and other constituents into resistant clumps [16] and (ii) it results in the partial acetylation of the isolated material hampering discussion and comparison of IR spectra [13]. Therefore we employed the successive treatments, already used for isolating PRB *A* from the *A* race [13], which completely eliminates all the usual algal constituents except the resistant biopolymers.

These treatments were carried out both on algae collected in Nature (Lake Darwin, Australia; lakes Paquemar and La Manzo, French West Indies) and from laboratory cultures. The occurrence of a resistant constituent, termed PRB *B*, was demonstrated in all these samples of *B. braunii B* race. (Weight losses, as % of the initial biomass, due to the different chemical treatments (extraction with organic solvents, saponification, action of concentrated H_3PO_4) were respectively *ca* 45%, 30% and 15%.) Ultrastructural observations by transmission electron microscopy (Fig. 1) showed that PRB *B* is located in the outer walls. The latter retain their initial shape following the drastic chemical attacks required for isolating the resistant material, while the other colony elements are entirely eliminated. PRB *B* is always a major constituent of the biomass (9–12% of the initial algal dry wt). Similar amounts were previously obtained for PRB *A* with *B. braunii*, *A* race [13], but they are markedly higher than the levels of resistant material reported for other algae: 0.6% in *Chlorella fusca* [3] and 0.3% in *Prototheca wickerhamii* [51]. These differences are consistent with the occurrence, in the latter species, of only a single very thin outer wall around each cell.

Silica is a major constituent of some algal walls and is sometimes associated with a resistant organic material [3, 52]. The complete degradation of PRB *B* by a strong

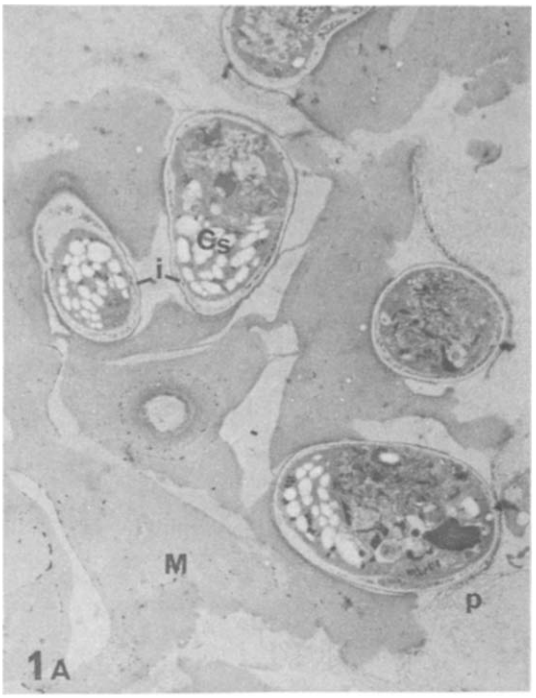

Fig. 1A. A part of an untreated colony of *Botryococcus braunii*, *B* race. Each cell is enclosed in a thin polysaccharidic inner wall (i). The thick outer walls are aggregated, forming the general matrix (M) of the colony. The basal part of each cell is occupied by a chloroplast crowded with starch (C_S). The apical part of the cells bears polysaccharidic fibrils (p). G: × 2100.

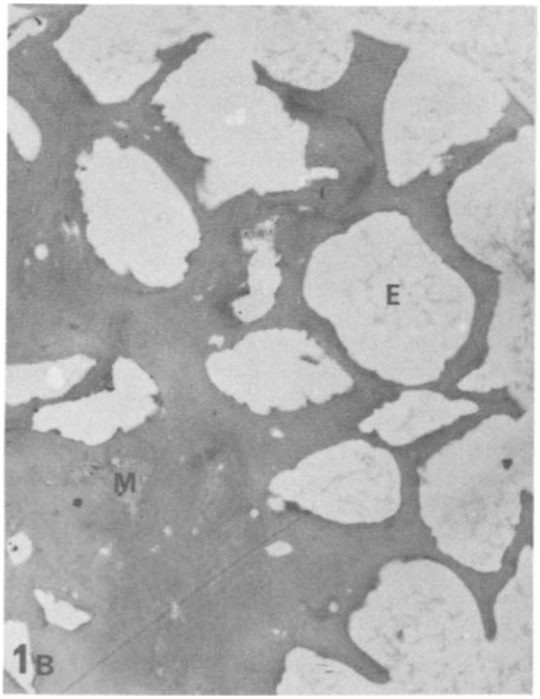

Fig. 1B. After the chemical treatments required for isolating PRB; all colony constituents, but the outer walls, have disappeared. The original organization of the matrix (M) is retained and the original positions of the cells, now emptied, are still well recognizable (E). G: × 4800.

oxidizing agent, chromic acid, shows it is chiefly composed of a resistant organic biopolymer. The absence or very low level of silica in PRB *B* is also confirmed, see below, by IR spectroscopy and elemental analysis.

UV fluorescence of PRB *B* was observed both after treatment with primuline (secondary fluorescence) and from untreated samples (auto-fluorescence). Following excitation at 300–400 nm, PRB *B* fluoresces in the range 450–600 nm.

According to the above features, resistance to non-oxidative treatments and UV fluorescence; PRB *B* may be related either to sporopollenins or to PRB *A*. The chemical structure of PRB *B* was therefore examined with different methods suitable for such an insoluble and chemically resistant material.

Chemical structure of PRB B

PRB *B* contains a low level of ash so that a significant oxygen determination can be carried out [29]. The oxygen content is markedly lower than that observed in classical sporopollenins (Table 1) while it is similar to that of PRB *A*. In fact the resistant biopolymers isolated from the two races of *B. braunii* show parallel elemental compositions, with low oxygen and high hydrogen contributions. A low level of nitrogen and phosphorus is retained in PRB *B*, as well as in PRB *A*, even after prolonged H_3PO_4 treatments. This may be the result of PRB deposition on surfaces promoting polymerization and composed, as in the case of exines [53–62], of lipoproteins, lipopolysaccharides and mucopolysaccharides. These minor constituents would be protected from external agents by accumulations of the resistant material.

FT IR and solid state ^{13}C NMR observations are in agreement with the lack of relationships between PRB *B* and sporopollenins, whereas close spectroscopic features are noted relative to PRB *A*. When compared with sporopollenins the FT IR spectrum of PRB *B* is characterized by a lower content of hydroxyl groups, a lower content of CH_3 relative to CH_2 and the presence of long $(CH_2)_n$ chains. In addition FT IR indicates the presence in PRB *B* of other oxygenated functions (ether, carbonyl and/or carboxyl) and of *cis* unsaturations. On the other hand, the spectra of PRB *A* and PRB *B* are almost identical (see [13] for PRB *A* FT IR spectrum and for a complete discussion of the attributions of the different bands).

High resolution ^{13}C NMR (dipolar decoupling, cross polarization and magic angle spinning) confirms the above findings. The PRB *B* spectrum appears again to be closely related to that previously described for PRB *A* [13, 35]. Low levels of unsaturation and of oxygenated functions are detected. The spectrum is dominated by a broad band at 10–50 ppm corresponding to saturated carbons not linked to heterosubstituents. This band shows a sharp maximum at 29 ppm (long methylenie chains) and two shoulders centered at 15 ppm and 45 ppm. However these shoulders attributed to Me groups and to tertiary or quaternary carbons, respectively, seem slightly more important in PRB *B* compared to PRB *A* (Fig. 2).

All the above spectroscopic observations, added to elemental composition, point to a close relationship between the structures of PRB *B* and PRB *A*. However, these methods only afford gross information on the nature and relative abundance of the different functions, so a more precise comparison was required. This was obtained by analysis of the complex mixture obtained on pyrolysis of PRB *B*.

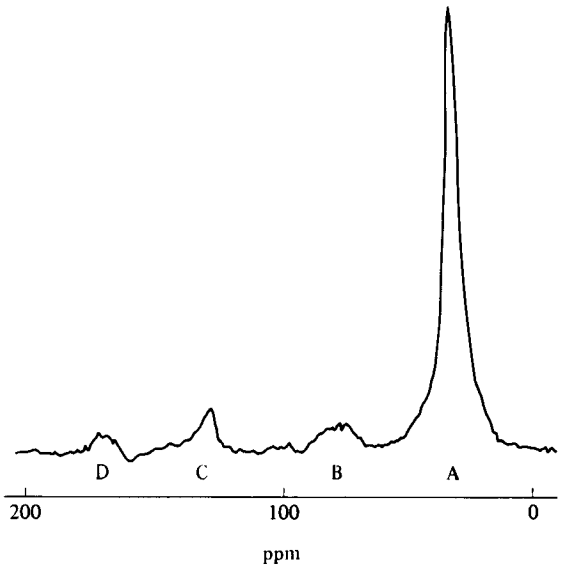

Fig. 2. Solid state ^{13}C NMR spectrum of PRB *B*. A: saturated carbons not linked to heterosubstituents; B: C–O of alcohols and ethers; C: olefinic carbons; D: C=O of esters.

Table 1. Elemental composition (%) of PRB *B*, comparison with PRB *A* and sporopollenins*

	C	H	O	N	P	Ash	Formula†
PRB *B*‡	75.1	11.3	8.6	0.4	0.5	4	$C_{90}H_{162}O_8$
PRB *A*	71.35	10.3	8.8	0.52	0.38	8	$C_{90}H_{156}O_8$ [13]
Lycopodium clavatum spores	62.9	8	26.3	§	§	3	$C_{90}H_{138}O_{28}$ [13]
Lilium henryii pollen	60	7.9	32	§	§	§	$C_{90}H_{142}O_{36}$ [2]

*Only classical sporopollenins, which formation via carotenoid derivative polymerization was supported by structural and/or biosynthetic studies, were considered here for comparison.

†In order to make easier comparison with previous results, the formulae are arbitrarily based on a C_{90} unit (19) and the low levels of N and P detected are not taken into account.

‡Average values, the resistant biopolymers isolated from the different samples (collected and laboratory grown) of *B. braunii*, *B* race, showed close elemental compositions.

§Not determined.

To derive significant structural information from pyrolysis experiments it was necessary to achieve an efficient fragmentation of the biopolymer while minimizing secondary reactions of the products formed. This was obtained by heating the sample at 400° under a helium flow. The material was first heated at 300° to eliminate products possibly adsorbed on PRB *B* and not removed by the chemical treatments. The subsequent heating at 400° resulted in extensive fragmentation that yielded a weight loss corresponding to *ca* 85% of the initial organic material. The medium and low volatility products so generated accounted for *ca* 45% by weight of the starting biopolymer. (Similar quantitative observations were obtained [36] by 400° pyrolysis of PRB *A*.) No attempt was made to analyse the high volatility products, also formed in large amounts, since they provide little significant structural information.

FT IR of the pyrolysis residue shows, as expected from the large weight loss achieved at 400°, very important variations relative to unheated PRB *B*, viz., a complete elimination of carboxyl and/or carbonyl functions (the residual oxygen is located in ether and OH groups) and a major decrease in both Me and CH_2 groups (the 720 cm^{-1} absorption due to $(CH_2)_n$ chains disappears), an important aromatization. These observations are consistent with a large decrease in the H/C atomic ratio of the residue (0.46 vs 1.74 in PRB *B*) and with the nature of the trapped products.

The crude pyrolysate shows a very complex composition and was first separated into three fractions by column chromatography. These were composed, as shown by IR and ^{1}H NMR, of hydrocarbons, ketones and polar compounds (chiefly fatty acids). The three fractions account for 64, 22 and 14%, respectively, of the pyrolysate. A similar distribution was noted in the case of PRB *A* [36].

The GC trace of the total hydrocarbon fraction was still complex, with a background of relatively minor peaks dominated by a regular series of doublets. A further fractionation was therefore carried out using TLC on $AgNO_3$–silica gel and afforded three subfractions whose constituents were identified (Table 2). The highest R_f subfraction (I) is exclusively composed of saturated hydrocarbons. It is dominated (Table 2) by a regular series (series 1) of C_{13}–C_{31} *n*-alkanes with a regular decrease in intensity from C_{18} and no odd/even predominance. Series 2 shows a maxima at C_{18} and corresponds to a cyclohexane substituted by a C_8–C_{23} *n*-alkyl chain (base peak *m/z* 83 or 82). A minor series (series 3), was also detected by selective ion detection (SID) at *m/z* 97 and probably corresponds to a cyclohexane substituted both by a methyl and a C_7–C_{12} *n*-alkyl chain; however the relative position of these two substituents was not determined.

The IR and ^{1}H NMR spectra of the intermediate subfraction (II) indicate the presence of aromatic structures and of *trans*-unsaturations. The predominant series (series 4) was identified as C_{13}–C_{28} *trans n*-alkenes and shows a maximum at C_{17} with no odd/even predominance. SID revealed two minor series, attributed to other *n*-alkenes with a different position of *trans*-unsaturation. Series 5 and 6 correspond to a cyclohexane and a methyl cyclohexane substituted by a C_8–C_{15} *n*-alkenyl chain, respectively. Three series of alkylbenzenes (series 7–9) were also detected. The first one (base peak *m/z* 91 or 92) corresponds to monosubstitution by a normal, C_8–C_{12} alkyl chain. The others (base peaks *m/z* 105 and 106, respectively) are attributed to an aromatic ring substituted both by one methyl and a C_7–C_{17} chain [63].

The lowest R_f subfraction (III) is mainly composed of olefins with a terminal double bond. It is dominated by a regular series (series 10) of C_{13}–C_{27} *n*-1-alkenes with a smooth decrease in intensity from C_{19} and no odd/even predominance. Several minor series attributed to other *n*-alkenes were also noted, resulting from positional isomerism of the double bond. Taking into account the R_f of (III) this unsaturation is probably *cis*. Series 11 corresponds to

Table 2. Nature and relative abundance of the hydrocarbon homologous series identified in PRB *B* pyrolysate*

Subfraction	Series	Structure	Carbon number range	CPI†	Relative abundance‡
I	1	*n*-Alkanes	C_{13}–C_{31}	0.98	1
	2	*n*-Alkylcyclohexanes	C_{14}–C_{29}		0.48
	3	Methyl-*n*-alkylcyclohexanes	C_{14}–C_{19}		0.11
II	4	*n*-Alkenes (*trans*)	C_{13}–C_{28}	1.01	0.17
	5	*n*-Alkenyl (*trans*) cyclohexanes	C_{14}–C_{21}		0.025
	6	Methyl-*n*-alkenyl (*trans*) cyclohexanes	C_{15}–C_{22}		0.02
	7	*n*-Alkylbenzenes	C_{14}–C_{18}		0.03
	8	Methyl-*n*-alkylbenzenes	C_{14}–C_{24}		0.03
	9	Methyl-*n*-alkylbenzenes	C_{14}–C_{24}		0.04
III	10	*n*-1-Alkenes	C_{13}–C_{27}	1.04	0.81
	11	*n*-1-Alkenylcyclohexanes	C_{16}–C_{22}		0.23
	12	Methyl-*n*-1-alkenylcyclohexanes	C_{16}–C_{19}		0.2

*Identifications were carried out using IR, ^{1}H NMR, GC and GC-MS. In some cases the analyses were also performed after inclusion in molecular sieves and/or hydrogenation. See [36] for a complete discussion of the mass spectra of series 2, 10 and 11. Some additional minor series were identified (corresponding to position isomerism of the double bond in *cis* and *trans*-*n*-alkenes) but their carbon number range and relative abundance could not be accurately determined.

†Carbon preference Index (CPI), allowing to detect a possible odd/even predominance in the *n*-series, was calculated according to [80]. In the series containing a substituted cyclohexane or benzene it is noted that the alkyl or alkenyl chains also show no odd/even predominance.

‡The relative abundance of each homologous series was calculated, within the corresponding carbon range, with respect to the dominant series 1.

a cyclohexane substituted by a C_{10}–C_{16} *n*-alk-1-enyl chain. Finally SID also disclosed a series (series 12) where the ring is substituted, in addition, by a methyl group (*m/z* 97).

The nature, distribution and relative abundance of the major hydrocarbons generated by pyrolysis provide further information on the structure of PRB *B*. Taking into account the functions identified in the biopolymer and in its pyrolysis residue, by FT IR and ^{13}C NMR, such hydrocarbons may derive from the rupture of either esters or of C–C bonds. However, different distributions would be obtained in these two cases, ester pyrolysis would mainly afford *n*-alkanes with an odd predominance (decarboxylation of the fatty acid moiety) and *n*-1-alkenes with an even dominance (alcohol dehydration) [64–70]. However, such distributions are not observed in series 1 and 10 which largely dominate the total hydrocarbon of the PRB *B* pyrolysate. Moreover, as shown below in the study of the polar products, no decarboxylation of the fatty acids released by PRB *B* takes place under the pyrolytic conditions employed. Cracking of C–C bonds generates radicals which would undergo chain reactions. From saturated hydrocarbon chains, including cross-linked structures, the resulting hydrocarbon series (alkanes and monounsaturated alkenes with mainly a terminal double bond) would be characterized by (i) a smooth decrease in intensity with carbon number, (ii) a maximal length $\leqslant$ to that of the initial chains, (iii) the lack of odd/even predominance [71–76]. Normal alkanes and alkenes are also released by C–C cracking from polycondensed cyclohexyl structures. However, in this case, products with specific carbon numbers $(6+4n)$ dominate the different series [77].

In the present work the lack of odd/even dominance was noted for the different series of *n*-alkanes and *n*-alkenes generated by PRB *B* pyrolysis. But their distributions (maxima located in the range C_{17}–C_{19}, followed by a smooth decrease) seemed to be different to that expected for C–C cracking. However, the maxima merely reflect the loss of shorter hydrocarbons during the different steps of the fractionation. Indeed, direct analyses of PRB *B* pyrolysis products, by PY/GC, clearly show a regular decrease in intensity of the major alkane and alkene series with carbon number. So it appears that most of the hydrocarbons released by PRB *B* derive from cracking of C–C bonds in long hydrocarbon chains. These chains, probably cross-linked by ether bridges, are saturated (as indicated by a lack of dienic hydrocarbons in the pyrolysate) and normal, with carbon number up to C_{31}. Some cyclohexanes and aromatic rings are also detected in the hydrocarbon fraction; however, they are always associated with long, saturated, or monounsaturated unbranched alkyl chains. Moreover some of the aromatic structures may have been formed by olefin cyclization and aromatization during the heat treatment. So it appears that the bulk of the carbon atoms of PRB *B* are located in long $(CH_2)_n$ chains and that the structure of the biopolymer is most likely based on the cross linking of such chains.

The carbonyl fraction of the pyrolysate shows a very complex chromatogram. Furthermore, no separation in subfractions containing a limited number of homologous series was obtained by CC or TLC. Nevertheless, the presence of C_{13}–C_{25} normal, saturated or monoenic ketones, was detected. In addition some of these series were shown, on the basis of MacLafferty rearrangement in their mass spectra, to correspond to methyl ketones. The formation of aliphatic ketones was observed in the pyrolysis of different materials, including kerogens [70] and PRB *A* [36] (the same types of ketones occur in the pyrolysates of PRB *A* and PRB *B*), but their origin and relationship to the initial structure are not clearly established. Nevertheless, the formation of such ketones is consistent with a very large contribution of long $(CH_2)_n$ units in the structure of the biopolymer.

The polar fraction is chiefly composed of fatty acids whose distribution (Table 3) is characterized by the exclusive occurrence of even fatty acids and the lack of very long chain ($\geqslant C_{20}$). Mono and polyunsaturated fatty acids with *cis* double bonds make a substantial contribution and the fraction is dominated by oleic acid. Previous observations on model compounds [78], added to the differences discussed above between the spectra of unheated PRB *B* and pyrolysis residue, indicate that such acids originate from the cleavage of ester bonds occurring in PRB *B*. *cis*-Unsaturations were detected in PRB *B*, by spectroscopic methods. It appears that they are located in fatty acyl chains simply bound to the polymeric network and not in bridging structures.

PRB *B* contains ester functions able to survive the drastic saponification and acidic treatments required for its isolation. This is probably related to steric protection of esters by the 3-dimensional network of the biopolymer. A similar protection takes place for the hydroxyl groups which do not undergo dehydration during the prolonged H_3PO_4 treatment.

All the structural information obtained account for the very high resistance of PRB *B* to chemical degradation. In fact this biopolymer is based upon long, saturated, unbranched hydrocarbon chains, probably cross-linked by ether bridges. PRB *B* contains few functions which are protected from external attacks by the polymeric network.

Comparison of the pyrolysis products from PRB A *and PRB* B

The resistant biopolymers isolated from the two races of *B. braunii* show several similar spectroscopic features.

Table 3. Nature and relative abundance (%) of the fatty acids of PRB *B* pyrolysate; comparison with PRB *A* [36]

	Fatty acids							
	12:0	14:0	16:0	16:1*	18:0	18:1†	18:2‡	18:3§
PRB *B*	2	2	10	2	6	51	8	19
PRB *A*	4	7	25	6	8	38	4	8

*Palmitoleic acid. †Oleic acid. ‡Linoleic acid. §Linolenic acid.

This relationship was confirmed by identification of the major constituents of the pyrolysates. The same types of ketones and the same fatty acids are released from the two biopolymers. Some differences in relative abundances of fatty acids are noted (Table 3), although oleic acid is always predominant. Regarding hydrocarbons (Table 2), alkylbenzenes (series 7–9) and methylcyclohexyl derivatives (series 3, 6, 12) are detected from PRB *B* but not from PRB *A*. These series comprise *ca* 14% of the total hydrocarbons released from PRB *B*. Their formation reveals structural differences and is consistent with the variation detected by ^{13}C NMR, viz., more important shoulders corresponding to methyl and to tertiary or quaternary carbons in PRB *B* spectrum. However, the major hydrocarbon series released on pyrolysis of the two biopolymers are the same and, furthermore, they show identical distribution and carbon number ranges. Taken together these observations indicate that PRB *A* and PRB *B*, while not identical, probably belong to the same family of resistant biopolymers. This is in agreement with the results of a study [79], involving feeding experiments and lipid analysis in the two races, suggesting that both PRB *A* and PRB *B* are produced via polymerization of very long chain fatty acid derivatives. These feeding experiments also pointed to the lack of relationships between PRB *B* and the terpenic constituents of the alga (including carotenoids and botryococcenes). The present observations on the structure of PRB *B*, especially the dominance of unbranched saturated hydrocarbon chains, confirms that it derives neither from carotenoids (and so cannot be considered as a sporopollenin) nor from botryococcenes.

The presence of resistant biopolymers with fairly close structures, in the two races of *B. braunii*, may have important implications for the formation of some algal kerogens exhibiting a high oil potential. A comparative study [35, 36] of PRB *A*, isolated from laboratory grown *B. braunii*, and of an immature Torbanite (kerogen chiefly composed of the fossil remains of *Botryococcus* colonies) indicated important similarities in chemical structure. Thus, it appeared that Torbanites were generated via the selective preservation of the resistant biopolymer, present in the sedimentated algal biomass, while the other constituents including hydrocarbons were almost entirely degraded. Accordingly due to the analogy between PRB *A* and PRB *B*, the fossilization of the *A* and *B* races of *B. braunii* should result, in spite of major differences in some of the initial biomass constituents, to kerogens with quite similar structures.

EXPERIMENTAL

The description of the samples collected from Lakes Darwin (Australia), Paquemar and La Manzo (French West Indies), the isolation of unialgal strains of *B* race from Paquemar and La Manzo samples, the growth conditions of laboratory cultures, were as previously reported [46, 48, 49].

Ultrastructural observations, isolation of PRB *B* by drastic chemical treatments, direct acetolysis of the algal biomass, oxidative degradation of PRB *B*, UV fluorescence, spectroscopic studies (FT IR and CP-MAS ^{13}C NMR), pyrolysis under a He flow, fractionation and identification of pyrolysis products, were carried out as previously described [13, 35, 36, 49, 50].

Acknowledgements—This work was supported by the CNRS as a part of the 'Programme Interdisciplinaire de Recherche sur les Sciences pour l'Energie et les Matières Premières' and by Société Nationale Elf-Aquitaine (Production). We are grateful to Dr Hillen (Aeronautical Research Laboratories, Melbourne) and to Dr Coute (Museum National d'Histoire Naturelle, Paris) for providing *B. braunii* samples. GC-MS analyses were carried out by Dr Sellier, Mrs Mauroy and Cazaussus (Laboratoire de Spectrométrie de Masse, ENSCP). We thank Mr B. Rousseau (electron microscopy) for helpful technical assistance.

REFERENCES

1. Swift, E. and Rensen, C. C. (1970) *J. Phycol.* **6**, 79.
2. Shaw, G. (1971) in *Sporopollenin* (Brooks, J., Grant, P., Muir, M. D., Shaw, G. and van Gijzel, P., eds) p. 305. Academic Press, London.
3. Atkinson, A. W., Cunning, B. E. S. and John, P. C. L. (1972) *Planta* **107**, 1.
4. Syrett, P. J. and Thomas, E. M. (1973) *New Phytol.* **72**, 1307.
5. Hegevald, Von, E. and Schnepf, E. (1974) *Arch. Hydrobiol.* **46**, 151.
6. Staehelin, L. A. and Pickett-Heaps, J. D. (1975) *J. Phycol.* **11**, 163 and 186.
7. Marchant, H. J. (1977) *J. Phycol.* **13**, 102.
8. Good, B. H. and Chapman, R. L. (1978) *Am. J. Botany* **65**, 27.
9. König, J. and Peveling, E. (1980) *Pflanzenphysiol.* **98**, 459.
10. Ashraf, M. and Godward, M. B. E. (1980) *Ann. Botany* **46**, 485.
11. Morrill, L. C. and Loeblich, A. R. (1981) *J. Phycol.* **17**, 315.
12. Honegger, R. and Brunner, U. (1981) *Can. J. Botany* **59**, 2713.
13. Berkaloff, C., Casadevall, E., Largeau, C., Metzger, P., Peracca, S. and Virlet, J. (1983) *Phytochemistry* **22**, 389.
14. Devries (1983) *Acta Bot. Nederl.* **32**, 25.
15. König, J. and Peveling, E. (1984) *Lichenologist* **16**, 129.
16. Brunner, U. and Honegger R. (1985) *Can J. Botany* **63**, 2221.
17. Aken, M. E. and Pienaar, R. N. (1985) *S. Afr. J. Botany* **51**, 408.
18. Zetsche, F. and Kalin, O. (1931) *Helv. Chim. Acta* **14**, 58.
19. Zetsche, F., Kalt, P., Leichti, J. and Ziegler, E. (1937) *J. Prakt. Chem.* **148**, 267.
20. Heslop-Harrison, J. (1968) *Science* **161**, 230.
21. Brooks, J. (1971) in *Sporopollenin* (Brooks, J., Grant, P., Muir, M. D., Shaw, G. and Van Gijzel, P. eds) p. 351. Academic Press, London.
22. Heslop-Harrisson, J. (1974) *Phil. Trans. R. Soc. London Ser. B* **190**, 275.
23. Brooks, J. and Shaw, G. (1968) *Nature (London)* **219**, 532.
24. Brooks, J. and Shaw, G. (1968) *Grana Palynol.* **8**, 227.
25. Fawcett, P., Green, D., Hollyhead, R. and Shaw, G. (1970) *Grana* **10**, 246.
26. Fawcett, P., Green, D., Hollyhead, R. and Shaw, G. (1970) *Nature (London)* **227**, 195.
27. Shaw, G. (1970) in *Phytochemical Phylogeny* (Harbone, J. B., ed.) Academic Press, London.
28. Gooday, G. W., Fawcett, P., Green, D. and Shaw, G. (1973) *J. Gen. Microbiol.* **74**, 233.
29. Gooday, G. W., Green, D., Fawcett, P. and Shaw, G. (1974) *Arch. Microbiol.* **101**, 145.
30. Achari, R. G., Shaw, G. and Hollyhead, R. (1973) *Chem. Geol.* **12**, 229.
31. Libert, P. (1974) Thesis, Université de Bordeaux.
32. Brooks, J. and Shaw, G. (1978) *Grana* **17**, 91.
33. Burczyk, J., Szkawran, H., Zontek, I. and Czygan, F. H. (1981) *Planta* **151**, 247.

34. Burczyk, J. (1987) *Phytochemistry* **26**, 113 and 121.
35. Largeau, C., Casadevall, E., Kadouri, A. and Metzger, P. (1984) *Org. Geochem.* **6**, 327.
36. Largeau, C., Derenne, S., Casadevall, E., Kadouri, A. and Sellier, N. (1986) *Org. Geochem.* **10**, 1023.
37. Nip, M., Tegelaar, E. W., De Leeuw, J. W., Schenck, P. A. and Holloway, P. J. (1986) *Org. Geochem.* **10**, 769.
38. Swain, F. M. and Gilby, J. M. (1964) *Publ. Stn Zool. Napoli* **33**, 361.
39. Maxwell, J. R., Douglas, A. G., Eglinton, G. and McCormick, A. (1968) *Phytochemistry* **7**, 2157.
40. Brown, A. C., Knights, B. A. and Conway, E. (1969) *Phytochemistry* **8**, 543.
41. Gelpi, E., Oro, J., Schneider, H. J. and Bennett, E. O. (1968) *Science* **161**, 700.
42. Gelpi, E., Schneider, H. J., Mann, J. and Oro, J. (1970) *Phytochemistry* **9**, 603.
43. Knights, B. A., Brown, A. C., Conway, E. and Middleditch, B. S. (1970) *Phytochemistry* **9**, 1317.
44. Cox, R. E., Burlingame, A. L., Wilson, D. M., Eglinton, G. and Maxwell, J. R. (1973) *J. Chem. Soc. Chem. Commun.* 284.
45. Largeau, C., Casadevall, E. and Berkaloff, C. (1980) *Phytochemistry* **19**, 1081.
46. Casadevall, E., Dif, D., Largeau, C., Gudin, C., Chaumont, D. and Desanti, O. (1985) *Biotechnol. Bioeng.* **27**, 286.
47. Wake, L. V. and Hillen, L. W. (1981) *Aust. J. Mar. Freshwater Res.*, **32**, 353.
48. Metzger, P., Berkaloff, C., Casadevall, E. and Coute, A. (1985) *Phytochemistry* **24**, 2305.
49. Largeau, C., Casadevall, E., Berkaloff, C. and Dhamelincourt, P. (1980) *Phytochemistry* **19**, 1043.
50. Berkaloff, C., Rousseau, B., Coute, A., Casadevall, E., Metzger, P. and Chirac, C. (1984) *J. Phycol.* **20**, 377.
51. Puel, F., Largeau, C. and Giraud, G. (1987) *J. Phycol.* (in press).
52. Millington, W. F. and Gawlik, S. R. (1967) *Nature* (*London*) **216**, 68.
53. Rowley, J. R. and Southworth, D. (1967) *Nature* (*London*) **213**, 703.
54. Heslop-Harrison, J. (1971) in *Sporopollenin* (Brooks, J., Grant, P., Muir, M. D., Shaw, G. and van Gijzel, P., eds) p. 1. Academic Press, London.
55. Willemse, M. T. M. (1971) in *Sporopollenin* (Brooks, J., Grant, P., Muir, M. D., Shaw, G. and van Gijzel, P., eds) p. 68. Academic Press, London.
56. Van Campo, M. and Lugardon, B. (1973) *Pollen Spores* **15**, 171.
57. Dickinson, H. G. and Heslop-Harrison, J. (1968) *Nature* (*London*) **220**, 926.
58. Dickinson, H. G. (1972) *Planta* **107**, 205.
59. Sengupta, S. and Rowley, J. R. (1974) *Grana* **14**, 143.
60. Dickinson, H. G. (1976) *Pollen Spores* **18**, 321.
61. Rowley, J. R. and Prijanto, B. (1977) *Geophytol.* **7**, 1.
62. Steer, M. W. (1977) *J. Cell. Sci.* **25**, 125.
63. Philp, R. P. (1985) in *Fossil Fuel Biomarkers. Applications and Spectra.* Elsevier, Amsterdam.
64. Brooks, J. D. and Smith, J. W. (1969) *Geochim. Cosmochim. Acta* **33**, 1183.
65. Douglas, A. G., Eglinton, G. and Henderson, W. (1970) in *Advances in Organic Geochemistry* (Hobson, G. D. and Speers, G. C., eds) p. 369. Pergamon Press, Oxford.
66. Connan, J. (1974) in *Advances in Organic Geochemistry* 1973 (Tissot, B. and Bienner, F., eds) p. 73. Technip, Paris.
67. Douglas, A. G., Coates, R. C., Bowler, B. F. and Hall, K. (1977) in *Advances in Organic Geochemistry* 1975 (Campos, R. and Goni, J., eds) p. 357. Enadimsa, Madrid.
68. Harrison, W. E. (1978) *Chem. Geol.* **21**, 315.
69. Larter, S. R. and Douglas, A. G. (1980) *Geochim. Cosmochim. Acta* **44**, 2087.
70. Van De Meent, D., Brown, S. C., Philp, R. P. and Simoneit, B. R. T. (1980) *Geochim. Cosmochim. Acta* **44**, 999.
71. Henderson, W., Eglinton, G., Simmonds, P. and Lovelock, J. E. (1968) *Nature* (*London*) **219**, 1012.
72. Kiran, E. and Gilham, J. K. (1976) *J. Appl. Polym. Sci.* **20**, 2045.
73. Raley, J. H. (1980) *Fuel* **39**, 419.
74. Allan, J., Bjoroy, M. and Douglas, A. G. (1980) in *Advances in Organic Geochemistry* 1979 (Douglas, A. G. and Maxwell, J. R., eds) p. 599. Pergamon Press, Oxford.
75. Evans, R. J. and Felbeck Jr., G. T. (1983) *Org. Geochem.* **4**, 135.
76. Saxby, J. D., Bennett, A. J. R., Corcoran, J. F., Lambert, D. E. and Riley, K. W. (1986) *Org. Geochem.* **9**, 69.
77. Bandurski, E. (1982) *Energy Sources* **6**, 47.
78. Ravey, M. (1983) *J. Polym. Sci.* **21**, 1.
79. Laureillard, J., Largeau, C., Waeghemaeker, F. and Casadevall, E. (1986) *J. Nat. Prod.* **49**, 794.
80. Bray, E. E. and Evans, E. D. (1961) *Geochim. Cosmochim. Acta* **22**, 2.