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journal homepage: www.elsevier.com/locate/orggeochem Occurrence of C₃₅–C₄₅ polyprenols in filamentous and unicellular cyanobacteriaThorsten Bauersachs^{a,*}, Stefan Schouten^a, Justine Compaoré^b, Lucas J. Stal^b, Jaap S. Sinninghe Damsté^a^a NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry, P.O. Box 59, 1790 AB Den Burg, The Netherlands^b NIOO-KNAW Netherlands Institute of Ecology, Department of Marine Microbiology, P.O. Box 140, 4400 AC Yerseke, The Netherlands

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ABSTRACT

Polyprenols, regular (head-to-tail) isoprenoid alcohols with 7–9 prenyl units, were tentatively identified in several cultivated cyanobacteria. Heptaprenol (C₃₅), octaprenol (C₄₀) and a suite of nonaprenols (C₄₅) were present in unicellular and filamentous non-heterocystous cyanobacteria, while they were absent in heterocystous species. Structurally related components, e.g. long-chain ($\geq$ C₃₅) acyclic regular isoprenoid hydrocarbons, have been reported from petroleum and cyanobacterial polyprenols may be potential precursors.

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1. Introduction

Polyprenols are isoprenoid alcohols with 6–12 prenyl units that are common constituents of bacterial cell membranes (Řezanka and Votruba, 2001). They occur in di-*trans* or poly-*cis* configuration, with the latter containing one or a small number of saturated prenyl units (Hemming, 1985). The most common bacterial poly-prenol is bactoprenol, a C₅₅ isoprenoid alcohol, which was originally identified in three species of *Lactobacillus* (Thorne and Kodicek, 1962) and has subsequently been reported from *Staphylococcus aureus* (Sandermann and Strominger, 1971). A suite of C₄₅–C₆₀ polyprenols has also been described from *Bacillus acidocaldarius* (De Rosa et al., 1973), while polyprenols ranging from C₅₀ to C₆₀ have been found in *Streptococcus mutans* (Szabo et al., 1978). More recently, Wolucka and de Hoffmann (1998) reported the presence of C₃₅–polyprenyl-phospho-mannose in *Mycobacterium smegmatis*.

Polyprenols are thus present in several bacteria, where they act as lipid carriers in the biosynthesis of cell surface polymers (Řezanka and Votruba, 2001). However, they have not been described from cyanobacteria. In this study, we report the tentative identification of C₃₅–C₄₅ polyprenols in unicellular and filamentous cyanobacteria. In addition, we discuss the role of cyanobacterial polyprenols as potential precursors of long chain acyclic isoprenoid hydrocarbons in petroleum.

2. Materials and methods

Cyanobacterial cultures were obtained from the culture collection Yerseke (CCY) at the Netherlands Institute of Ecology

(NIOO-KNAW). All strains were grown as axenic batch cultures in 250 mL Erlenmeyer flasks containing 100 ml culture medium. Detailed information on the culture conditions are given in Table 1. Cyanobacterial cultures were grown for six weeks, harvested and washed with 5 ml double distilled water. The cells were subsequently freeze-dried and stored at –20 °C until analysis.

Freeze-dried cells (30–50 mg) were ultrasonically extracted (3x) with dichloromethane (DCM)/MeOH (2:1, v/v) to give the total lipid extract (TLE). Apolar and polar fractions of the TLE were obtained using Al₂O₃ column chromatography and eluted with hexane/DCM (9:1 v/v) and MeOH/DCM (1:1 v/v), respectively. Poly-prenols were detected in the polar fraction after conversion to trimethylsilyl (TMS) ether derivatives using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine (1:1; 20 min at 60 °C).

In order to characterize the structures of the components, aliquots of the polar hydrocarbon fraction were hydrogenated by bubbling H₂ into 2 ml ethyl acetate containing acetic acid and using Adams catalyst (PtO₂). The resulting saturated poly-prenols were converted to alkyl iodides by refluxing with 56 wt.% HI (in H₂O) for 1 h and further reduced to isoprenoid hydrocarbons by refluxing with LiAlH₄ in 1,4-dioxane for 1 h (Schouten et al., 1998).

Gas chromatography-mass spectrometry (GC-MS) was performed using a Thermofinnigan Trace GC coupled to a Thermofinnigan Trace DSQ operated at 70 eV with a range of *m/z* 50–800 and cycle time of 1.0 s. The gas chromatograph was equipped with a fused silica column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.12 mm). The carrier gas was He. The samples were injected on-column at 70 °C and the oven was programmed to 130 °C at 20 °C/min and then at 4 °C/min to 320 °C (held 20 min).

3. Results and discussion

The polar fraction of the filamentous cyanobacterium *Leptolyngbya* CCY0004 contained high abundances of phytadienes, phytol,

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Table 1
Cyanobacterial strains and culture conditions [LPP: morphotypes of *Lyngbya–Plectonema–Phormidium*; BG11°, freshwater medium without NaNO₃ (Rippka et al., 1979); ASN3, artificial seawater medium with NaNO₃ (Rippka et al., 1979); AB, mixture of 1/2 ASN3 + 1/2 BG11°; BA, mixture of 1/3 ASN3 + 2/3 BG11°; BA°, mixture of 1/3 ASN3 + 2/3 BG11° without NaNO₃].

Organism	Strain	Growth medium	Nitrogen source	Salinity (PSU)	Light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Temp. (°C)
<i>Anabaena</i> sp.	CCY9402	BG11°	N ₂	0	30	14
<i>Nodularia</i> sp.	CCY9416	BA°	N ₂	0	30	14
<i>Leptolyngbya</i> sp.	CCY0004	ASN3	NO ₃	33	5	14
LPP sp.	CCY0022	ASN3	NO ₃	33	5	14
<i>Oscillatoria</i> sp.	CCY0001	ASN3	NO ₃	33	5	14
<i>Oscillatoria</i> sp.	CCY0028	AB	NO ₃	16	5	14
<i>Synechococcus</i> sp.	CCY9510	BA	NO ₃	11	15	18
<i>Synechococcus</i> sp.	CCY9506	BA	NO ₃	11	15	18

α -tocopherol, and C₁₆ and C₁₈ fatty acids as well as C₂₆ and C₂₈ *n*-alcohols (Fig. 1). In addition, a homologous series of unknown components (I–V) eluted in the high molecular weight range of the chromatogram. The mass spectrum of the trimethylsilyl (TMS) derivative of the most abundant of these (III) exhibited a molecular ion at *m/z* 706 (Fig. 2A). The ion at *m/z* 616 (*M*⁺–90) was in agreement with the presence of one TMS ether group. The molecular weight of the alcohol was thus deduced to be 634, corresponding to C₄₅H₇₈O or a C₄₅ alkanol with seven double bonds or rings. The base peak at *m/z* 69 represented the ω -terminal prenyl residue, which retained charge upon fragmentation (Wellburn et al., 1967). The ion at *m/z* 135 likely derived from the α terminal and adjacent prenyl residue upon loss of the trimethylsilyl ether group. A series of ions at *m/z* 135, 203, 271 and 339 corresponded to sequential losses of prenyl units (68 Da). Hydrogenation of the polar fraction and silylation resulted in a dominant component with a molecular weight of 720, which represented the hydrogenated derivative of III (Fig. 2B). The increase in molecular weight by 14 Da confirmed the presence of seven double bonds within III and the ion at *m/z* 103 indicated the presence of a terminal TMS ether group. In order to identify the carbon skeleton, HI/LiAlH₄ treatment was performed on the hydrogenated polar fraction, resulting in the cleavage of the TMS ether group and yielding the fully saturated hydrocarbon derivative of III (Fig. 2C). Its mass spectrum was in agreement with that of a regular acyclic isoprenoid hydrocarbon, i.e. 2,6,10,14,18,22,26,30,34-nonamethyltetracontane. Consequently, III was identified as a C_{45:7} nonaprenol (Fig. 3). Other components of the polar fraction (e.g. I and II) exhibited mass spectra similar to the one obtained for III (Fig. 2D–E) though with molecular ions at *m/z* 572 and *m/z* 640, suggesting that they were C₃₅ and C₄₀ alkanols. Hydrogenation and subsequent conversion to their saturated hydrocarbon derivatives indicated that they were

head-to-tail linked isoprenoid alcohols with 4 and 5 double bonds, respectively. Therefore, we tentatively assigned I as C_{35:4} heptaprenol and II as C_{40:5} octaprenol (Fig. 3). In addition, two isoprenoid alcohols with molecular ions at *m/z* 704 (IV) and *m/z* 702 (V) were observed, which eluted shortly after the C_{45:7} nonaprenol (III). These components showed a fragmentation pattern similar to that of III, suggesting they were also unsaturated nonaprenols. However, the lower molecular weight by 2 and 4 Da compared to III indicated that they contained one and two additional double bonds, respectively. Therefore, they were tentatively assigned as C_{45:8} (IV) and C_{45:9} (V) nonaprenols.

For the eight cyanobacterial strains analyzed, polyprenols were abundant in the polar fraction of all nitrate-utilizing species (Table 2). In contrast, they were not detected in N₂-fixing heterocystotous species, i.e. strains of the genera *Anabaena* and *Nodularia*. In general, the distribution of polyprenols was variable, ranging from simple distributions of C_{40:5} (II) and C_{45:8} (IV) in species of *Synechococcus* to the most complex distribution found in *Leptolyngbya* CCY0004, which included a total of six different polyprenols.

Although polyprenols are ubiquitously distributed in animals and plants, they have not been described from cyanobacteria. Reports on the polyprenol content in bacteria indicate that they occur as higher molecular weight homologues, i.e. $\geq$ C₅₅ (Řezanka and Votruba, 2001). In contrast, polyprenols found in cyanobacteria are of shorter chain length, i.e. C₃₅, C₄₀, and C₄₅. However, it has to be noted that the analytical technique used in this study, GC/MS, precluded the detection of polyprenols with molecular weights higher than C₄₅ and their presence in cyanobacteria cannot be excluded.

Short chain regular acyclic isoprenoid hydrocarbons (<C₂₀) are usually the most prevalent class of hydrocarbons in crude oils and rock extracts after normal alkanes (Moldovan and Seifert,

Fig. 1. Gas chromatogram of polar fraction of solvent extract of *Leptolyngbya* CCY0004. Insert shows the distribution of C_{45:7} (III), C_{45:8} (IV) and C_{45:9} (V) nonaprenols. Roman numerals refer to polyprenols in Fig. 3.

Fig. 2. Mass spectra of (A) $C_{45:7}$ nonaprenol (III), (B) $C_{45:0}$ nonaprenol, (C) C_{45} regular isoprenoid hydrocarbon, (D) $C_{35:4}$ heptaprenol (I) and (E) $C_{40:5}$ octaprenol (II).

1979). Despite their low abundance, regular series of acyclic isoprenoid hydrocarbons extending towards the C_{45} homologues have also been found in a number of crude oils (Albaigés et al., 1978;

Albaigés, 1980). The structural similarity of these long chain acyclic isoprenoid hydrocarbons and the cyanobacterial polyisoprenoids identified in this study suggests a generic relationship between both

Fig. 3. Generalized structures of C_{35:4} heptaprenol (I), C_{40:5} octaprenol (II) as well as C_{45:7} (III), C_{45:8} (IV) and C_{45:9} nonaprenols (V). Note that the position of the double bonds has not been established.

Table 2
Relative abundance of polyprenols (% of total polyprenol content) in axenic cultures of cyanobacteria obtained from the culture collection Yerseke (CCY). The genus LPP consists of a group of cyanobacteria belonging to morphotypes of *Lyngbya*–*Plectonema*–*Phormidium*.

Organism	Strain	C _{35:4} (I)	C _{40:5} (II)	C _{45:7} (III)	C _{45:8} (IV)	C _{45:9} (V)
<i>Anabaena</i> sp.	CCY9402	–	–	–	–	–
<i>Nodularia</i> sp.	CCY9416	–	–	–	–	–
<i>Leptolyngbya</i> sp.	CCY0004	17	19	48	13	3
LPP sp.	CCY0022	–	6	65	16	13
<i>Oscillatoria</i> sp.	CCY0001	–	5	–	49	46
<i>Oscillatoria</i> sp.	CCY0028	–	10	–	75	15
<i>Synechococcus</i> sp.	CCY9501	–	15	–	85	–
<i>Synechococcus</i> sp.	CCY9506	–	11	–	89	–

compound classes and it is likely that acyclic isoprenoid hydrocarbons can be formed from polyprenols by maturation. We, therefore, suggest that cyanobacterial polyprenols may be diagenetic precursor components for regular acyclic isoprenoid hydrocarbons in petroleum.

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