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Original research article

Carotenoid glycoside isolated and identified from cyanobacterium *Cylindrospermopsis raciborskii*

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ABSTRACT

The freshwater cyanobacterium *Cylindrospermopsis raciborskii* was investigated for carotenoid composition. Besides β-carotene, echinenone and (9/9′*Z*)-echinenone a carotenoid glycoside was found to be the main component. This compound was isolated and subsequently acetylated for structural elucidation. The acetyl derivative was fully characterized by UV–vis, ECD, NMR and HRMS techniques. The detailed ^{1}H and ^{13}C NMR chemical shift assignment of the major carotenoid supported the unequivocal identification of (2′*S*)-2-hydroxymyxol 2′-α-L-fucoside.

1. Introduction

In natural product research many organisms and taxonomic groups are under investigation as a potent source for isolating bioactive metabolites and compounds of unique structures. One of the most promising groups is the ancient photoautotroph cyanobacteria possessing an interesting metabolite profile because of their long evolutionary history (Gademann and Portmann, 2008; Vasas et al., 2010).

Numerous cyanobacterial species are used as human or animal food and food supplements. Although the most popular edible genera are the *Spirulina*, *Nostoc* and *Aphanizomenon*, there are several species which are capable to multiply in natural conditions to pose huge masses and blooms. These species are good natural sources for investigating and exploring special metabolites such as alkaloids, peptides, terpenoids and other bioactive compounds. Cyanobacteria can also produce carotenoids. Besides the common β-carotene some special keto carotenoids (e.g. echinenone) and unique acyclic (e.g. oscillol, Hertzberg and Liaaen-Jensen, 1966) or monocyclic derivatives (e.g. myxol) can also be found in these species, as well as carotenoid glycosides (Takaichi and Mochimaru, 2007). The carbohydrate moieties, however, have not been identified in most of the cases, the characteristic carotenoid glycosides in cyanobacteria are frequently named as myxoxanthophyll and oscilloxanthin leaving the sugar part unclarified. (Takaichi et al., 2001). The biological role of these glycosides is still unknown, but the investigation of their exact structures can help to uncover their biosynthesis and possible functions in cyanobacteria. Herein we describe the isolation and structure identification of a myxol glycoside derivative in *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju (*C. raciborskii*), a nitrogen-fixing filamentous cyanobacterial species, which spread in the last decades and caused huge blooms worldwide.

C. raciborskii has become a well-studied species due to its unusual physiology, distribution and metabolism (Antunes et al., 2015). *C. raciborskii* was first observed in the island of Java, Indonesia in 1899–1900 and identified by Woloszynska and considered as a tropical species (Padisák, 1997). To this date, the presence of *C. raciborskii* has been reported in an increasing number of countries around the globe, both in the Northern and Southern hemispheres. The species multiply and represent a large biomass portion in rivers, shallow water bodies, lakes and reservoirs under optimal conditions (Moreira et al., 2011). Moreover, in several reported cases this species were identified as harmful organism due to the production of well-defined toxic compounds like cylindrospermopsin (CYN; Ohtani et al., 1992), paralytic shellfish poisoning (PSP) toxins (Lagos et al., 1999), and partly

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identified neurotoxic incidents (Vehovszky et al., 2013; Svirčev et al., 2016).

Some physiological studies elucidate partly the adaptation of *C. raciborskii* to a wider range of light intensities, and its influence in photosynthetic activity (O'Brien et al., 2009). Moreover, carotenoid and phycobilin concentrations, as well as photosynthetic activities, were significantly higher than those of other bloom forming cyanobacterial species (Wu et al., 2009).

A heavy and rather unusual *C. raciborskii* bloom was detected early November 2012 in the Eastern part of Hungary (Fancsika pond), resulting strong discoloration of the water. Instead of the well-known cyanobacterial pale green-bluish discoloration, the water turned to orange color which suggested uncommon pigments or uncommon pigment concentration in the water. (supplement Fig. S1.)

2. Material and methods

2.1. Reagents and standards

Analytical grade chemicals were used throughout the experiments. All solvents used in high-performance liquid chromatography (methanol, water, acetone and *tert*-butyl methyl ether) were of HPLC grade. All reagents and solvents were purchased from Scharlab Ma-gyarország Kft., Debrecen, Hungary. Organic solutions were dried over anhydrous Na_2SO_4 and concentrated in vacuum at 40 °C (bath temperature). Authentic reference samples (β-carotene, β-cryptoxanthin, echinenone) were taken from our in-house collection.

2.2. Cyanobacterial (Blue-green algae) material

A Hungarian *Cylindrospermopsis raciborskii* (*C. raciborskii*) (the cyanobacterial strains, were isolated from Fancsika pond, Debrecen and kept into BGSD-collection as *C. raciborskii* BGSD-2012) was investigated in our study for carotenoid composition. Environmental samples from Fancsika pond were collected from the water while blooming (deep orange colorization by the dens cyanobacterial filaments was observed) on the November 2, 2012 (supplement Fig. S1). The bloom-forming cyanobacterial cells were harvested by a 5 μm membrane filter and the species identified by their morphological characteristics (using an inverted microscope, LEICA DMIL). The bloom sample and also the laboratory isolate of the bloom forming cyanobacteria were filtered and lyophilized for further analysis. The *Cylindrospermopsis raciborskii* strain was isolated from the environmental sample and grown in liquid, nitrogen free medium of BG-10. The cultures were kept in glass flasks thermostatically maintained at 28 °C and illuminated with cool white fluorescent light (80 μmol·photons m^{-2} s^{-1}). Aeration and mixing was achieved by bubbling with sterile air. For collecting dry mass, samples were centrifuged (10,000*g*, 5 min, Beckman Avanti), and the pellets were lyophilized in dark (CHRIST-ALPHA 1-2 LDplus).

2.3. Phylogenetic analysis of C. raciborskii BGSD-2012

DNA isolation, PCR and sequencing of *C. raciborskii* strain Hungary (Fancsika) were conducted according to Ballot et al. (2016). The sequence was deposited in the European Nucleotide Archive (ENA) under the accession nr LT854187.

The phylogenetic analysis of the 16S rRNA sequence of *C. raciborskii* strain Hungary (Fancsika) was conducted using the Seqassem software package (version 07/2008) (Hepperle, 2017). The Align MS Windows-based manual sequence alignment editor (version 08/2016) (Hepperle, 2017) was used to obtain DNA sequence alignments, which were then corrected manually. Segments with highly variable and ambiguous regions and gaps making proper alignment impossible were excluded from further analyses. A 16S rRNA gene set containing1277 positions was used, and Gloeobacter violaceus PCC 7421 (AF132790) was employed as an outgroup in the 16S rRNA gene tree. The 16S rRNA sequences from 24 additional Nostocales sequences, derived from GenBank, were included in the 16S rRNA analyses.

A phylogenetic tree for the 16S rRNA gene was constructed using the maximum likelihood (ML) algorithm in Mega v. 7 (Kumar et al., 2016) (supplement Fig. S1.). The evolutionary substitution model T92 + G + I was found to be the best-fitting evolutionary model for the 16S rRNA gene and used for the calculation of the ML tree. ML analyses were performed with 1000 bootstrap replicates using Mega v.7 (Kumar et al., 2016).

2.4. Carotenoid extraction, isolation, derivatization

The freeze-dried cyanobacterium *C. raciborskii* (5.0 g) was sonicated in methanol-acetone 3:7 mixture (100 mL) for 5 min, and it was let to settle for an hour. After decantation the same extraction procedure was repeated for four times. Continuing the extraction, the remained freeze-dried material was mixed with methanol and kept for overnight. The extracts were combined and the solvent was evaporated.

The crude extract was subjected to open column chromatography (OCC) on a glass column (d: 60 mm) packed with calcium carbonate (Biogal, Debrecen, Hungary) using hexane as eluent, or was partitioned in aqueous methanol (15% H_2O) − hexane solvent system. The pigment present in the lower, polar phase was purified by OCC on modified silica gel (Kieselgel 60, particle size 0.063–0.200 mm, Merck, Darmstadt, Germany). (Nagy et al., 2009.)

Saponification of the pigments was executed in ether with 30% KOH/MeOH at room temperature in dark. (Deli et al., 1996.) Composition of the crude or saponified extracts, and that of the fractions obtained during chromatography was determined by HPLC.

Thin layer chromatography was performed on TLC Silica gel 60 F_{254} on Al sheets (Merck, Germany). Preparative layer chromatography was executed on PLC Silica gel 60 F_{254} 1 mm on glass plate (Merck, Germany).

Acetylation: 20 mg of the isolated carotenoid glycoside was acetylated in 1 mL of dry pyridine using 0.5 mL of acetic anhydride. The reaction was stirred for overnight, few drops of water were added and 5 min later the mixture was diluted by 50 mL diethyl ether and washed with 3 × 20 mL of brine. The organic phase was evaporated and purified on a calcium carbonate column using toluene as eluent. Crystallization from toluene:hexane resulted in 7 mg of the acetylated product, with a purity of 95% (HPLC).

Silylation: 5 mg of the isolated carotenoid glycoside was dissolved in 1 mL of dry dichloromethane and 3 mg of hexamethyldisilazane and 1 mg of ammonium thiocyanate were added (Jadhav et al., 2007). The reaction was stirred overnight, TLC in hexane:acetone 1:1 indicated the completion of the reaction. The mixture was diluted by 50 mL diethyl ether and washed with 20 mL of brine. The organic phase was evaporated and the crude compound was used as a sample for MS studies.

2.5. HPLC conditions

The HPLC analyses were performed with a DionexP680 quaternary analytical pump, a Dionex PDA 100 UV/vis detector (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with Chromeleon 6.8 software and a column temperature control module. Chromatograms were developed on a 250 × 4.6 mm stainless steel YMC C_{30}, 3 mm (YMC Europe GmbH, Dinslaken, Germany) endcapped column, with 1.00 mL/min flow rate, at 22 °C. Eluents were (A) MeOH/TBME/H_2O = 81/15/4 v/v%, (B) MeOH/TBME/H_2O = 6/90/4 v/v%. The gradient program was the following: 0–45 min from 100% A to 100% B (in linear steps). The chromatograms were registered at 450 nm wavelength.

HPLC–MS analyses were performed with an Agilent 6350 Accurate-Mass Q-TOF LC/MS, data acquisition was performed by Agilent MassHunter Qualitative Analysis B.04.00. For LC-(APCI)MS the positive ion mode was used, with TIC, scanning range 200–1500 *m/z*, corona voltage 2.6 kV, fragmentor voltage 150 V, skimmer 60 V, Oct 1RF Vpp

750 V. The flow rate of the dried nitrogen as nebulizer gas was 240 L/h and the vaporizer temperature was 400 °C.

2.6. Identification of carotenoids by HRMS, UV–vis and NMR spectroscopy

Carotenoids were identified on the basis of their UV–vis spectra (λ_{max}, spectral fine structure% III/II, and *cis* peak intensity% A_B/A_{II}), retention times in HPLC, and by spiking with authentic standards. Molecular masses were determined by LC–MS. For the identification of the carotenoid glycoside HRMS, ECD and NMR spectra were also applied.

HRMS analyses were performed on an LTQ FT Ultra (Thermo Fisher Scientific, Bremen, Germany) system. The ionization method was APCI operated in positive ion mode. The samples were dissolved in methanol. Data acquisition and analysis were accomplished with Xcalibur software version 2.0 (Thermo Fisher Scientific).

UV-VIS spectra were taken with a Jasco V-550 UV/VIS multiwavelength Spectrophotometer (Jasco Applied Sciences (UK) Ltd., Droxford, United Kingdom). Samples were dissolved in hexane or MeOH and the applied wavelength range was 250–600 nm. ECD spectra were recorded at room temperature with a J-810 spectropolarimeter (JASCO International Co. Ltd, Japan).

NMR spectra in $CDCl_3$ were recorded with a Bruker Avance III Ascend 500 spectrometer (500.12/125.4 MHz for $^1H/^{13}C$, respectively) at 25 °C. The ^{13}C and ^1H NMR assignments were made on the basis of 1D (^1H, ^{13}C APT) and 2D (COSY, HSQC, HMBC) experiments. All spectra were acquired using standard Bruker software. Chemical shifts were referenced to the residual solvent signals. The 1D and 2D data were processed using the programs MestReC 4.9.9.6. and ACD/NMR Processor 12.01.

3. Results and discussion

3.1. Isolation

After extraction of the freeze-dried cyanobacterium *C. raciborskii* (1.0 g) the extract was divided into two parts. One half was saponified with KOH/MeOH, and both the saponified material and the original extract were examined by HPLC. The chromatograms obtained for both samples were found to be similar, the saponified extract was choosen to establish the carotenoid composition: besides β-carotene (20.3%), echinenone (18.9%) and (9/9′*Z*)-echinenone (10.3%), an unknown carotenoid (40.4%) was found to be the main component. (Fig. 1.) The molar extinction coefficients of each carotenoid at 450 nm wavelength were assumed to be the same.

From the original extract, which also contained chlorophylls, the major carotenoid was separated in the most polar fraction by $CaCO_3$ column chromatography using hexane as eluent. Following workup and removal of the solvent the saponified extract was not completely soluble in toluene, the precipitate proved to be the same material as the major and unknown component gained by chromatography of the crude extract. The obtained major carotenoid was examined by NMR and MS, but no conclusive data were obtained, as this compound must have been partially decomposed during the measurements. However, the ^1H NMR spectrum indicated, that the unknown material contains a sugar (monosaccharide) moiety. For further structure elucidation studies the major carotenoid had to be derivatized. As the chemical modification requires higher amounts of starting material, large-scale extraction was necessary.

The crude extract obtained from 5.0 g freeze-dried *C. raciborskii* was partitioned in aqueous-methanol hexane solvent system. Because of the possible water solubility of the major carotenoid component, the lower methanolic hypophase was evaporated instead of the usual workup to avoid the contact with high amounts of water (Meyer et al., 1995).

The carotenoid composition of both phases were examined by HPLC. In the hypophase the unknown material enriched in high purity (78%), while the epiphase contained all the other components including chlorophylls (supplement Fig. S2.). The epiphase was saponified, which resulted in the disappearance of chlorophylls (supplement Fig. S3.). The remaining pigments were separated by OCC on $CaCO_3$ with a mixture of hexane/toluene (8:2) and gave five fractions containing carotenoids with different polarities. From these fractions β-cryptoxanthin (λ_{max}: 450, 478 in hexane, %III/II: 44, M_r: 552) (Fraction 2), echinenone (λ_{max}: 462 in hexane, M_r: 550) (Fraction 3), (9/9′*Z*)-echinenone (λ_{max}: 353, 456 in hexane, % A_B/A_{II}: 10, M_r: 550) (Fraction 4), β-carotene (λ_{max}: 450, 478 in hexane, %III/II: 38, M_r: 536) and it's (9*Z*)-isomer (λ_{max}: 341, 445, 473 in hexane, %III/II: 42, % A_B/A_{II}: 12 M_r: 536) (Fraction 5) were identified by their UV–vis spectra, mass spectra, HPLC retention times (Turcsi et al., 2016) and by co-chromatography with authentic samples.

The hypophase was subjected to chromatography on modified silica gel (Nagy et al., 2009.) using toluene: diethyl ether: methanol 100:25:20 as eluent. Three fractions were observed: a green fraction containing chlorophylls followed by an orange fraction of less polar

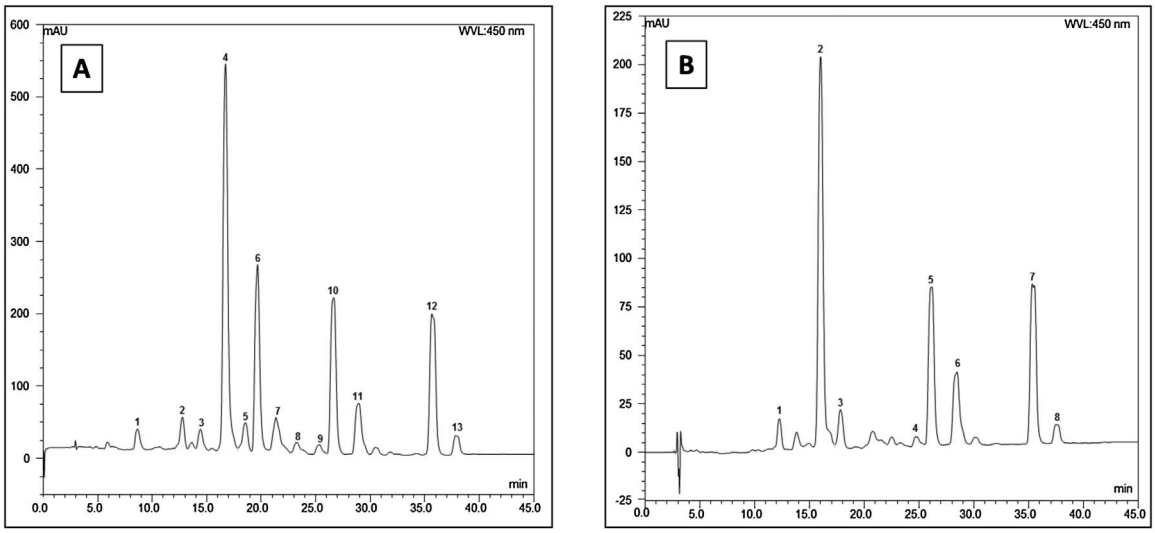


Fig. 1. HPLC chromatogram of the crude extract (A), and that of the saponified extract (B).
(A): Peak 4: major carotenoid, peak 6–7: chlorophylls, peak 9: β-cryptoxanthin, peak 10: echinenone, peak 11: (9/9′*Z*)-echinenone, peak 12: β-carotene, peak 13: (9*Z*)-β-carotene.
(B): Peak 2: major carotenoid (40.4%), peak 4: β-cryptoxanthin (1.1%), peak 5: echinenone (18.9%), peak 6: (9/9′*Z*)-echinenone (10.3%), peak 7: β-carotene (20.3%), peak 8: (9*Z*)-β-carotene (2.2%).

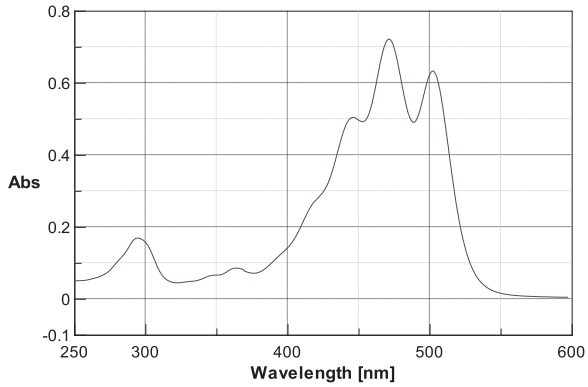


Fig. 2. UV–vis spectrum of (2*R*,3*R*,2′*S*)-2-hydroxymyxol 2′-α-L-fucoside pentaacetate (in MeOH) (λ_{max}: 295, 365, 447, 473, 504 nm).

carotenoids and a third polar red fraction containing the major carotenoid glycoside. This latter fraction was subsequently purified by preparative layer chromatography, it was dissolved in dichloromethane and methanol (9:1), and precipitated with hexane yielding 42 mg of the major carotenoid in 99.9% purity. (supplement Fig. S4.)

The obtained crystalline material was derivatized by acetylation and silylation. The acetyl and silyl derivatives could be obtained in pure forms (supplement Fig. S5.), and provided appropriate spectra.

3.2. Structure elucidation of the acetylated derivative

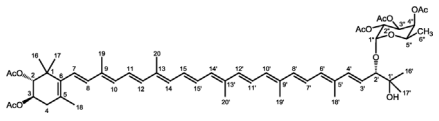
The UV–vis spectrum of the acetylated compound in methanol showed absorption maxima at 295, 365, 447, 473, and 504 nm wavelengths, the spectral fine structure of %III/II was 63. (Fig. 2.) It suggests that the major carotenoid contains 12 conjugated double bonds with one conjugated β-end group, and it is a derivative of β,ψ-carotene. (Takaichi and Shimada, 1992).

The ^{1}H NMR spectra of the sugar moiety and the end groups were identical with that of 2,3,2″,3″,4″-penta-*O*-acetyl-2-hydroxymyxol-2′-fucoside published by Iwai et al. (Iwai et al., 2008). Beside the ^{13}C APT and ^{1}H NMR spectra, on the basis of COSY, HSQC and HMBC spectra (supplement Fig. S6-S10.) all the ^{1}H and ^{13}C chemical shifts were assigned (Table 1.). The $^{3}J_{1'',2''}$ coupling constant (3.9 Hz) shows that the configuration of the glycosidic bond is α. The other ^{3}J H—H coupling constants of the sugar moiety confirmed the L-*galacto* configuration (Foss et al., 1986; Izumi, 1971).

In the β-end group, the H-2 and H-3 hydrogens are in axial positions ($^{3}J_{2,3}$ = 10.9 Hz), i.e. the two acetylated hydroxyl groups have a *trans* geometry. The *tertiary* hydroxyl group on C-1′ was not acetylated, as it is visible from the number of acetyl C═O signals in the ^{13}C spectrum, as well as the ^{1}H signal of the free OH at 2.48 ppm.

The ECD spectrum in MeOH showed a broad positive Cotton effect (CE) at 360 nm with a shoulder at 345 nm, a separated positive band with maxima at 287 and 299 nm, a broad negative ECD band at 256 nm and positive CE below 235 nm (supplement Fig. S11.). This ECD pattern was found congruent with that of phleixanthophyll pentaacetate having (2′*S*) absolute configuration and near mirror image of the ECD of plectaniaxanthin diester with (2′*R*) absolute configuration measured in EPA (Rønneberg et al., 1985). Moreover, the ECD pattern was quite different from that of (3*R*,2′*S*)-myxoxanthophyll tetraacetate, which had an additional chirality center at C-3 (Rønneberg et al., 1985). According to these correlations, it is the allylic C-2′ chirality center that determines primarily the ECD pattern, and thus the absolute configuration of C-2′ could be assigned as (*S*). However, the ECD data cannot be used for the configurational assignment of the C-2 and C-3 chirality centers. The ECD data showed significant solvent-dependence. In *n*-hexane, the 0.3 μM solution showed two intense ECD bands with opposite signs in the 400–700 nm region indicating aggregate formation and supramolecular origin of the ECD transitions. The ECD spectra

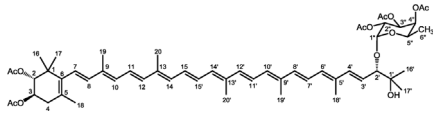
Table 1
^{1}H and ^{13}C NMR chemical shifts for (2*R*,3*R*,2′*S*)-2-hydroxymyxol 2′-α-L-fucoside pentaacetate (in $CDCl_3$, 500.12/125.4 MHz for ^{1}H/^{13}C).



	H	δ ^{1}H (*J*)		δ ^{13}C	C
β-end group	1		–	40.59 ppm	1
	2	d	5.04 ppm (10.9 Hz)	77.17 ppm	2
	3	ddd	5.13 ppm (6.8 Hz; 9.5 Hz; 10.5 Hz)	68.83 ppm	3
	4 eq	dd	2.62 ppm (6.5 Hz; 17.6 Hz)	37.51 ppm	4
	4 ax	dd	2.24 ppm (9.8 Hz; 17.4 Hz)		
	5		–	124.92 ppm	5
	6		–	136.92 ppm	6
	7	d	6.01 ppm (16.0 Hz)	124.41 ppm	7
	8	d	6.11 ppm (16.0 Hz)	139.65 ppm	8
	16	s	1.08 ppm	22.66 ppm	16
	17	s	1.00 ppm	25.63 ppm	17
	18	s	1.70 ppm	21.01 ppm	18
polyene chain	10	d	6.17 ppm (11.4 Hz)	131.95 ppm	10
	9, 13, 9′, 13′		–	136.63 ppm 136.57 ppm 135.82 ppm 135.27 ppm	9, 13, 9′, 13′
	12, 12′	m	6.36–6.41 ppm	139.02 ppm, 138.47 ppm	12, 12′
	10′ 14,14′	m	6.25–6.29 ppm	133.34 ppm 133.26 ppm 132.85 ppm 130.38 ppm 130.22 ppm	10′ 14,14′ 15,15′
	15,15′ 11, 11′	m	6.55–6.66 ppm		
				124.85 ppm, 124.10 ppm	11, 11′
	19	s	1.97 ppm	12.85 ppm 12.82 ppm 12.78 ppm 12.76 ppm	19, 19′ 20, 20′
	20	s	1.98 ppm		
	20′	s	1.98 ppm		
	19′	s	1.99 ppm		
ψ-end group	18′	s	1.92 ppm	12.93 ppm	18′
	17′	s	1.19 ppm	24.41 ppm	17′
	16′	s	1.16 ppm	25.71 ppm	16′
	8′	m	6.36–6.41 ppm	138.00 ppm	8′
	7′	m	6.55–6.66 ppm	125.00 ppm	7′
	6′	d	6.20 ppm (10.9 Hz)	133.49 ppm	6′
	5′		–	133.63 ppm	5′
	4′	d	6.34 ppm (15.6 Hz)	139.30 ppm	4′
	3′	dd	5.63 ppm (8.8 Hz; 15.6 Hz)	124.29 ppm	3′
	2′	d	3.75 ppm (8.8 Hz)	90.16 ppm	2′
	1′		–	72.99 ppm	1′
	OH	s	2.48 ppm	–	
sugar moiety	1"	d	5.16 ppm (3.9 Hz)	98.30 ppm	1"
	2"	dd	5.20 ppm (3.7 Hz; 10.8 Hz)	68.46 ppm	2"
	3"	dd	5.33 ppm (3.2 Hz; 10.7 Hz)	68.35 ppm	3"
	4"	d	5.30 ppm (2.8 Hz)	71.19 ppm	4"
	5"	m	4.14–4.21 ppm	64.93 ppm	5"
	6"	s	0.99 ppm	15.46 ppm	6"
	Ac-Me	s	2.15 ppm 2.09 ppm 2.08 ppm 2.03 ppm 1.98 ppm	21.08 ppm 20.95 ppm 20.72 ppm 20.63 ppm	Ac-Me
	Ac-CO		–	170.66 ppm 170.63 ppm 170.59 ppm	Ac-CO

(continued on next page)

Table 1 *(continued)*



H	δ ^{1}H (*J*)	δ ^{13}C	C
		170.23 ppm	
		170.07 ppm	

measured in acetonitrile or chloroform were also markedly different from that recorded in methanol (supplement Fig. S11.).

In natural myxol glycosides found in cyanobacteria, the C-3 configuration was determined to be *R* (in 4-ketomyxoxanthophylls it is *S*) because of the biosynthetic pathway (Takaichi and Mochimaru, 2007). Assuming the same absolute configuration in our compound, the diequatorial position of *O*-acetyl groups determines the absolute configuration of C-2 and C-3, which are *R*,*R,* respectively.

In the high-resolution full-MS spectrum of the acetylated compound the *m/z* at 957.53669 corresponds to $(M+H)^+$, *m/z* of 979.51776 is for $(M+Na)^+$, and the signal at *m/z* 667.43547 shows the loss of the triacetyl fucose moiety as an in-source fragment (lacking the OH function in position 2′). The silyl derivative gave *m/z* 1179.72050 $(M+H)^+$ and 799.53313 (M-trisilyl fucose)$^+$, which indicates that all the hydroxyl groups (the *tertiary* included) were silylated. (supplement Fig. S12-S13.)

4. Conclusions

Nostoxanthin, caloxanthin, and zeaxanthin were absent, whereas the major carotenoid in cyanobacterium *Cylindrospermopsis raciborskii* was identified as (2*R*,3*R,*2′*S*)-2-hydroxymyxol 2′-α-L-fucoside on the basis of UV/Vis, ECD, NMR and mass spectra of its acetylated derivative. Its semi-systematic name is (2*R*,3*R*,2′*S*)-2′-(α-L-fucopyranosyloxy)-3′,4′-didehydro-1′,2′-dihydro-β,ψ-carotene-2,3,1′-triol.

Although the same compound was previously found as one of the carotenoid component in the thermophilic cyanobacterial species *Thermosynechococcus elongatus* (Iwai et al., 2008), this glycoside is the major carotenoid in *C. raciborskii*. Our results suggest that the biomass of this cyanobacterial species would be an economically justified natural source for the production and purification of this unique compound for the industrial approaches.

In addition the presence of this hydrophilic antioxidant pigment suggests another explanation why this cyanobacterial species could spread and adapt efficiently and can built huge biomass in different aquatic habitats.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfca.2017.06.003.

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