Major Carotenoid Isolated from *Paracoccus schoinia* NBRC 100637^T Is Adonixanthin Diglucoside

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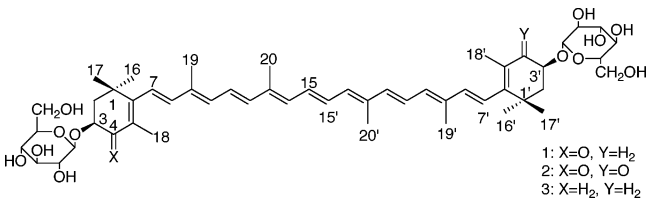
The structure of a novel major carotenoid glycoside (nearly 80% of total carotenoids) from a newly isolated bacterium, *Paracoccus schoinia* NBRC 100637^T, was determined to be adonixanthin diglucoside using spectral data. By contrast, carotenoid diglycosides are rare and are usually minor carotenoids in nature. The minor carotenoids in this bacterium included astaxanthin diglucoside, zeaxanthin diglucoside, canthaxanthin, echinenone, zeaxanthin, β-cryptoxanthin, and β-carotene.

The genus *Paracoccus* consists of Gram-negative cocci or short rods that show substantial metabolic versatility. Phylogenetically, the genus belongs to the α-3 subclass of the Proteobacteria. Nearly 20 species have been described to date.[1] Some species produce carotenoids: astaxanthin 3-glucoside and adonixanthin 3-glucoside from *Paracoccus* sp. N81106 (formerly *Agrobacterium aurantiacum*),[2] astaxanthin and canthaxanthin from *Paracoccus marcusii*,[3] astaxanthin from *Paracoccus* sp. MBIC 03024 (formerly *Alcaligenes* PC-1),[4] zeaxanthin from *Paracoccus zeaxanthinifaciens* R-1534 (formerly *Flavobacterium* sp. R-1534),[5] and astaxanthin (with insufficient data) from *Paracoccus carotinifaciens*[6] and *Paracoccus haeundaensis*.[7] On the other hand, eight species, including the type species of the genus *Paracoccus*, *Paracoccus denitrificans*, produce no carotenoids.[3]

A newly isolated bacterium from coastal seawater in Tokyo Bay is a marine Gram-negative bacterium that is red-colored, nonmotile, spherical to short rod-shaped, and aerobic. The results of a taxonomic study and a phylogenetic analysis based on 16S rRNA gene sequence comparisons classified the newly isolated strain as a representative of a novel species within the genus *Paracoccus*, and the name *Paracoccus schoinia* has been proposed. The present paper reports the isolation and structural elucidation of a novel carotenoid diglycoside, adonixanthin diglucoside, as the major carotenoid from *P. schoinia*.

The HPLC elution profiles of the organic-solvent-soluble pigments extracted from *P. schoinia* showed one major peak (nearly 80% of total carotenoids) and some minor peaks of carotenoids (Figure 1).



The major peak, carotenoid **1**, appeared to be polar, as determined by Si gel TLC and C18-HPLC. These chromatographic properties are characteristics of carotenoid glycosides.[8] The UV−vis absorption spectrum of **1** in MeOH showed a broad absorption maximum at around 470 nm and small maxima at 263 and 300 nm, indicating the presence of a conjugated carbonyl group, which was comparable to that of echinenone, but not canthaxanthin.[8] The molecular formula of **1** was determined to be $C_{52}H_{74}O_{13}$ by HRFABMS. Trimethylsilylation of **1** provided an octatrimethylsilyl derivative as determined by FDMS (*m*/*z* 1482 $[M]^+$), indicating the presence of eight hydroxyl groups in **1**. The ^{1}H and ^{13}C NMR data of **1** in CD_3OD (Table 1) indicated the presence of adonixanthin as the carotenoid moiety and two hexose moieties. The ^{1}H signals of the hexose moieties were overlapped except for two anomeric protons at δ_H 4.52 (d, J = 7.5 Hz, H-G1) and 4.40 (d, J = 6.5 Hz, H-G1′). Therefore, we compared the ^{13}C NMR data[9] and found the two hexoses were β-D-glucopyranose. On the basis of the HMBC correlations and the coupling constants of the anomeric protons (Table 1), the glucose moieties were attached to the hydroxyl groups at C-3 and C-3′ of the adonixanthin moiety by β-glycosidic linkages. The all-*trans* geometry of the polyene chain was determined by ROESY correlations. The CD spectrum of **1** in MeOH was comparable to those of (3*S*,3′*R*)-adonixanthin[10] and (3*S*,3′*R*)-adonixanthin 3-glucoside.[2] Thus, the structure of the carotenoid **1** was identified to be adonixanthin diglucoside (also known as

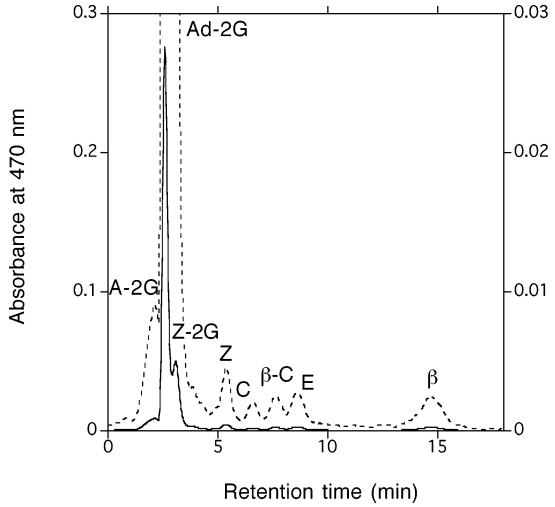


Figure 1. HPLC elution profiles of pigments eluted with MeOH. A-2G: astaxanthin diglucoside, Ad-2G: adonixanthin diglucoside, Z-2G: zeaxanthin diglucoside, Z: zeaxanthin, C: canthaxanthin, β-C: β-cryptoxanthin, E: echinenone, β: β-carotene.

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Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Spectroscopic Data for Adonixanthin Diglucoside (**1**) in CD_3OD

position	δ_C, mult.	δ_H (*J* in Hz)	HMBC (H to C)	position	δ_C, mult.	δ_H (*J* in Hz)	HMBC (H to C)
1	38.6,qC[a]			1′	38.1, qC[a]		
2	46.3, CH_2	1.94 dd (14, 14)	C-3, 4	2′	47.5, CH_2	1.54 dd (11, 11)	C-3′, 4′
		2.24 dd (14, 5)	C-3, 4			1.94 overlapped	C-3′, 4′
3	78.3, CH	4.62 dd (14, 5)	C-G1	3′	73.7, CH	4.12 m	C-G1′
4	201.4, qC[a]			4′	40.1, CH_2	2.12 dd (18, 11)	
						2.49 dd (18, 6)	
5	129.5, qC[a]			5′	127.8, qC[a]		
6	164.1, qC[a]			6′	139.8, qC[a]		
7	124.5, CH	6.32 d (16)		7′	126.9, CH	6.13 d (16)	
8	143.9, CH	6.50 d (16)		8′	140.0, CH	6.16 d (16)	
9	n.a.[b]			9′	n.a.		
10	136.2, CH	6.32 d (11)		10′	132.6, CH	6.17 d (11.5)	
11	125.1, CH	6.72 overlapped		11′	125.4, CH	6.72 overlapped	
12	136.7, CH	6.50 d (15.5)		12′	138.2, CH	6.40 d (15.5)	
13	n.a.			13′	n.a.		
14	133.9, CH	6.32 d (11)		14′	135.1, CH	6.32 d (11)	
15	132.2, CH	6.72 overlapped		15′	132.2, CH	6.72 overlapped	
16	26.6, CH_3	1.354 s	C-1, 2, 6	16′	29.1, CH_3	1.082 s	C-1′, 2′, 6′
17	31.2, CH_3	1.223 s	C-1, 2, 6	17′	30.8, CH_3	1.065 s	C-1′, 2′, 6′
18	24.2, CH_3	1.891 s	C-4, 5, 6	18′	21.8, CH_3	1.741 s	C-4′, 5′, 6′
19	12.7, CH_3	2.012 s	C-8, 10	19′	12.7, CH_3	1.980 s	C-8′,10′
20	12.7, CH_3	1.986 s	C-12, 14	20′	12.7, CH_3	1.986 s	C-12′, 14′
G1	106.1, CH	4.52 d (7.5)	C-3	G1′	103.1, CH	4.40 d (6.5)	C-3′
G2	72.4, CH	3.66 dd (10, 7.5)		G2′	77.2, CH	3.50 dd (10, 6.5)	
G3	74.4, CH	3.52 overlapped		G3′	74.7, CH	3.53 overlapped	
G4	69.7, CH	3.83 m		G4′	69.7, CH	3.83 m	
G5	76.3, CH	3.52 overlapped	C-G4, G6	G5′	76.3, CH	3.52 overlapped	C-G4′, G6′
G6	62.6, CH_2	3.74 m	C-G4	G6′	62.6, CH_2	3.74 m	C-G4′
		3.74 m	C-G4			3.74 m	C-G4′

[a] Chemical shifts were estimated by HMBC spectrum. [b] n.a. : not assigined.

4-ketozeaxanthin diglucoside). The IUPAC-IUB semisystematic name is (3*S*,3′*R*)-3,3′-di(β-D-glucopyranosyloxy)-β,β-caroten-4-one.

The composition of adonixanthin diglucoside (**1**) including *cis*-forms was 76% (mol % of total carotenoids). The minor compounds were determined to be astaxanthin diglucoside (**2**) (1%) and zeaxanthin diglucoside (**3**) (15%) on the basis of the UV−vis absorption spectra and the shorter retention times on C18-HPLC (Figure 1). The composition of these carotenoid diglucosides was more than 90% of the total. Canthaxanthin (1%), echinenone (1%), zeaxanthin (2%), β-cryptoxanthin (1%), and β-carotene (3%) were also determined on the basis of the comparable absorption spectra and the corresponding retention times on C18-HPLC (Figure 1) with those of cyanobacteria *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120.[8] The composition of these carotenoids was less than 10% of the total.

In the present study, a major carotenoid in *P. schoinia* was identified to be adonixanthin diglucoside (**1**) (nearly 80%). This is a novel carotenoid diglucoside[11] and is precedented only by small amounts of adonixanthin 3-glucoside being found from *Paracoccus* sp. N81106.[2] Some minor carotenoid diglucosides and carotenoids were also found. In the biosynthesis of the carotenoids, astaxanthin diglucoside (**2**) is the final product. Adonixanthin lacks one keto group from astaxanthin, and adonixanthin diglucoside (**1**) was accumulated as a major component. The minor carotenoids found were logical intermediates in the biosynthesis of astaxanthin diglucoside (**2**) from β-carotene.

Carotenoid diglycosides, such as zeaxanthin diglucoside (**3**) from *Pantoea ananatis* (formerly *Erwinia uredovora*)[12] and dihydroxylycopene diglucoside from two species of *Halorhodospira*,[13] are very rare in nature.[11] In fact, these carotenoid glycosides comprise less than 25% of the total in these species, while the total content in *P. schoinia* is more than 90%. In the genus *Paracoccus*, *P. schoinia* was the first species found to have the carotenoid diglucoside adonixanthin diglucoside (**1**) as the major carotenoid. *Paracoccus* sp. N81106 produces the carotenoid monoglucosides astaxanthin 3-glucoside and adonixanthin 3-glucoside,[2] while other species produce only free astaxanthin or zeaxanthin and not carotenoid glycosides.[3] The differences within *Paracoccus* might be the presence or absence of glucosyltransferase and the activity and/or substrate specificity of the enzyme.

Experimental Section

General Experimental Procedures. The UV−vis absorption spectra in the HPLC eluent of MeOH described below were measured with Otsuka Electronics MCPD-3600 photodiode array spectrophotometer.[8] The CD spectrum in MeOH was measured with a JASCO J-500 spectropolarimeter. The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra in CD_3OD were measured with a Varian UNITY INOVA 500 spectrometer. The residual solvent signals (δ_H 3.30 and δ_C 49.0 ppm) were used as references. COSY, ROESY, TOCSY, gHSQC ($^{1}J_{CH}$ = 142 Hz), and gHMBC ($^{n}J_{CH}$ optimized for 8 Hz) spectra were acquired using the standard Varian pulse programs with the software Varian version 6.1A. Quaternary carbon signals in ^{13}C NMR of **1** were not completely observed due to the small amount of sample remaining for NMR measurement (less than 1 mg), and then the chemical shifts of these quaternary carbons were estimated from the HMBC spectrum (Table 1). The positive ion HRFABMS spectrum was recorded using a JEOL JMS-HX/HX 110A mass spectrometer with *m*-nitrobenzyl alcohol as a matrix.

Isolation and Cultivation of Bacterium. *Paracoccus schoinia* NBRC 100637^T (=CIP 108500^T) was isolated as a red-colored colony grown on a Marine Agar 2216 (Difco) plate at 20 °C that had been inoculated with a diluted sample of seawater from Tokyo Bay. This isolate was proposed to be a new species on the basis of the almost complete 16S rRNA gene sequence (DDBJ accession number AB185957), which was only 96% similar to the phylogenetically closest type strain, *Paracoccus aminophilus* (NBRC 16710^T).[14] It was cultured in Marine Broth 2215 (Difco), and the cells were harvested by centrifugation.

Extraction and Isolation of Carotenoids. Carotenoids were extracted with MeOH several times from the wet cell pellets (ca. 20 g) using an ultrasonicator for several seconds. The extract was partitioned with $CHCl_3$ and H_2O, and then the $CHCl_3$ layer was evaporated. The HPLC system was equipped with a μBondapak C18 column (Waters, USA) eluted with MeOH.[8] A major carotenoid was purified by preparative C18-TLC (Whatman) developed with MeOH, and preparative Si gel TLC (Merck) developed with CH_2Cl_2/EtOAc/acetone/MeOH (2:4:2:5), followed by C18-HPLC eluted with MeOH/H_2O (9:1). The

yield of the major carotenoid **1** was ca. 1 mg. All procedures were performed under dim light.

Adonixanthin diglucoside (1): UV−vis (MeOH) λ_{max} 263, 300, 470 nm; CD (MeOH) λ ($\Delta\epsilon$) 235 (−15), 260 (+17), 300 (−21); ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (125 MHz), see Table 1; HRFABMS *m*/*z* 929.5020 $[M + Na]^+$ (calcd for $C_{52}H_{74}O_{13}Na$, 929.5028).

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References and Notes

(1) van Spanning, R. J. M.; Stouthamer, A. H.; Baker, S. C.; van Verseveld, H. W. In *Bergey's Manual of Systematic Bacteriology, Second Edition, Volume 2, Part C*; Brenner, D. J., Krieg, N. R., Staley, J. T., Eds.; Springer: New York, 2005; pp 197−204.
(2) Yokoyama, A.; Adachi, K.; Shizuri, Y. *J. Nat. Prod.* **1995**, *58*, 1929−1933.
(3) Harker, M.; Hirschberg, J.; Oren, A. *Int. J. Syst. Bacteriol.* **1998**, *48*, 543−548.
(4) Misawa, N.; Kajiwara, S.; Kondo, K.; Yokoyama, A.; Satomi, Y.; Saito, T.; Miki, W.; Ohtani, T. *Biochem. Biophys. Res. Commun.* **1995**, *209*, 867−876.
(5) Pasamontes, L.; Hug, D.; Tessier, M.; Hohmann, H.-P.; Schierle, J.; van Loon, A. P. G. M. *Gene* **1997**, *185*, 35−41.
(6) Berry, A.; Janssens, D.; Hümbelin, M.; Jore, J. P. M.; Hoste, B.; Cleenwerck, I.; Vancanneyt, M.; Bretzel, W.; Mayer, A. F.; Lopez-Ulibarri, R.; Shanmugam, B.; Swings, J.; Pasamontes, L. *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 231−238.
(7) Lee, J. H.; Kim, Y. S.; Choi, T.-J.; Lee, W. J.; Kim, Y. T. *Int. J. Syst. Evol. Microbiol.* **2004**, *54*, 1699−1702.
(8) Takaichi, S.; Mochimaru, M.; Maoka, T.; Katoh, H. *Plant Cell Physiol.* **2005**, *46*, 497−504.
(9) Burgess, M. L.; Barrow, K. D.; Gao, C.; Heard, G. M.; Glenn, D. *J. Nat. Prod.* **1999**, *62*, 859−863.
(10) Matsuno, T.; Maoka, T.; Ikuno, Y. *Comp. Biochem. Physiol.* **1986**, *83B*, 335−337.
(11) Britton, G., Liaaen-Jensen, S., Pfander, H. *Carotenoids Handbook*; Birkhäuser Verlag: Basel, 2005.
(12) Misawa, N.; Nakagawa, M.; Kobayashi, K.; Yamano, S.; Izawa, Y.; Nakamura, K.; Harashima, K. *J. Bacteriol.* **1990**, *172*, 6704−6712.
(13) Takaichi, S.; Maoka, T.; Hanada, S.; Imhoff, J. F. *Arch. Microbiol.* **2001**, *175*, 161−167.
(14) Khan, S. T.; Takaichi, S.; Harayama, S. Unpublished.

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