A New Carotenoid Glycosyl Ester Isolated from a Marine Microorganism, *Fusarium* Strain T-1

Hideyuki Sakaki,[§] Hirokazu Kaneno,[†] Yasuji Sumiya,[†] Miyuki Tsushima,[‡] Wataru Miki,[⊥] Noriaki Kishimoto,[†] Tokio Fujita,[†] Sadayoshi Matsumoto,[†] Sadao Komemushi,[†] and Akiyoshi Sawabe*[,†]

Department of Agricultural Chemistry, Faculty of Agriculture, Kinki University, 3327-204, Nakamachi, Nara 631-8505, Japan, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8412, Japan, Kobe Creative Center, Senju Pharmaceutical Ltd, 1-5-4, Murotani, Nishiku, Kobe 651-2241, Japan, and Institute for Fundamental Research, Suntory Ltd, 1-1-1, Wakayama-dai, Shimamoto, Mishima, Osaka 618-0024, Japan

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A new carotenoid glycosyl ester, neurosporaxanthin *β*-D-glucopyranoside (**2**), together with neurosporaxanthin (**1**), *β*-carotene, *γ*-carotene, and torulene were isolated from cultured cells of a marine microorganism, strain T-1, which was identified as *Fusarium* sp. Their structures were determined by chemical and spectral data.

The surface of the sea in tropical and subtropical regions is a severe environment for the growth of organisms because active oxygen and free radicals are generated by intense irradiation with strong sunlight. Recently, it has been reported that the marine microorganism *Agrobacterium aurantiacum* produced astaxanthin,[1] a pigment that has one of the strongest $^1O_2$ quenching activities.[2] As a result, we have focused our natural product efforts on fungal-type marine microorganisms, especially those that produce carotenoids with antioxidative activity. Here, we report on the isolation and structural determination of a new carotenoid from a marine microorganism.

Strain T-1, which produces an orange pigment, was isolated from the surface of seawater at Tanegashima, Kagoshima Prefecture in Japan, in August 1999 and identified as a *Fusarium* sp. The microorganism was cultured in 10 L scale at 25 °C for 7 days on a reciprocal shaker using a modified marine broth medium.[3] The cultured cells were concentrated by centrifugation and then extracted with acetone. The extracted solution was fractionated between ethyl acetate and an aqueous NaCl solution. The organic layer was concentrated below 40 °C and fractionated by Si gel CC with *n*-hexane−Me_2CO (8:2) and $CHCl_3$−MeOH (1:1) as eluents to yield crude **1** and **2**. Each crude fraction was further purified by a carotenoid C30 column to afford **1** (4.8 mg) and **2** (1.2 mg).

Compound **1** showed a molecular ion at *m*/*z* 498.3503, compatible with the formula $C_{35}H_{46}O_2$, and exhibited visible absorption maxima at 474.6 nm (Et_2O), suggesting the presence of an undecaene chromophore. The characteristic ^{13}C NMR signals at *δ* 171.9 indicated the presence of a carboxyl group (Table 2).[4] The partial structure of the *β*-end group and the polyene chain in **1** were characterized by ^1H NMR (Table 1) and ^{13}C NMR data.[4] From the spectral data described above, the structure of **1** was deduced to be 4′-apo-*β*-carotene-4′-oic acid (=neurosporaxanthin) (Figure 1).

Compound **2** showed absorption maxima at 474.6 nm (Et_2O), indicating the presence of the same chromophore as that found in **1**. By HRFABMS, the molecular formula of **2** was established as $C_{41}H_{56}O_7$, compatible with a **1**-hexoside. From extensive ^1H NMR studies, **2** appeared

Table 1. ^1H NMR Data (*δ* mult, *J* in Hz) of Neurosporaxanthin (**1**), Neurosporaxanthin *β*-D-Glucopyranoside (**2**), and Neurosporaxanthin *β*-D-Glucopyranoside Tetraacetate (**3**)

position	**1**[a]	**2**[b]	**3**[a]
H-2	1.47 (m)	1.47 (m)	1.47 (m)
H-3	1.62 (m)	1.62 (m)	1.61 (m)
H-4	2.02 (m)	2.02 (m)	2.02 (m)
H-6′	7.39 (d, 11)	7.43 (d, 11)	7.35 (d, 11)
H-7	6.19 (d, 16)	6.20 (d, 16)	6.19 (d, 16)
H-7′	6.51 (dd, 15, 11)	6.52 (dd, 15, 11)	6.48 (dd, 15, 11)
H-8	6.13 (d, 16)	6.13 (d, 16)	6.13 (d, 16)
H-8′	6.66 (d, 15)	6.68 (d, 15)	6.68 (d, 15)
H-10	6.16 (d, 11)	6.16 (d, 11)	6.16 (d, 11)
H-10′	6.37 (d, 11)	6.37 (d, 11)	6.40 (d, 11)
H-11	6.67 (dd, 15, 11)	6.68 (dd, 15, 11)	6.68 (dd, 15, 11)
H-11′	6.63 (dd, 15, 11)	6.64 (dd, 15, 11)	6.63 (dd, 15, 11)
H-12	6.36 (d, 15)	6.36 (d, 15)	6.36 (d, 15)
H-12′	6.47 (d, 15)	6.48 (d, 15)	6.48 (d, 15)
H-14	6.26 (d, 11)	6.27 (d, 11)	6.26 (d, 11)
H-14′	6.32 (d, 11)	6.34 (d, 11)	6.34 (d, 11)
H-15	6.67 (m)	6.67 (m)	6.69 (m)
H-15′	6.65 (m)	6.65 (m)	6.67 (m)
H-16	1.03 (s)	1.04 (s)	1.03 (s)
H-17	1.03 (s)	1.04 (s)	1.03 (s)
H-18	1.72 (s)	1.72 (s)	1.72 (s)
H-18′	2.00 (s)	2.02 (s)	2.02 (s)
H-19	1.98 (s)	1.99 (s)	1.98 (s)
H-19′	2.00 (s)	2.00 (s)	1.99 (s)
H-20	1.99 (s)	1.99 (s)	1.99 (s)
H-20′	1.98 (s)	1.99 (s)	1.98 (s)
H-1′		5.58 (d, 8)	5.78 (d, 8)
H-2″		3.48 (m)	5.26 (dd, 9.5, 8)
H-3″		3.60 (m)	5.17 (t, 9.5)
H-4″		3.51 (m)	5.30 (t, 9.5)
H-5″		3.41 (m)	3.89 (ddd, 9.5, 4.5, 2.5)
H-6″$_A$		3.77 (dd, 12, 5.5)	4.12(dd, 12, 2.5)
H-6″$_B$		3.88 (dd, 12, 2)	4.32 (dd, 12, 4.5)
acetyl methyl			2.01 (s)
acetyl methyl			2.03 (s)
acetyl methyl			2.04 (s)
acetyl methyl			2.09 (s)

[a] Data recorded in $CDCl_3$ at 500 MHz. [b] Data recorded in $CDCl_3$−CD_3OD (4:1) at 300 MHz.

* To whom correspondence should be addressed. Tel: 81-742-43-1511. Fax: 81-742-43-1445. E-mail: sawabe@nara.kindai.ac.jp.
† Kinki University.
‡ Kyoto Pharmaceutical University.
§ Kobe Creative Center, Senju Pharmaceutical Ltd.
⊥ Institute for Fundamental Research.

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Table 2. ^{13}C NMR Data for Neurosporaxanthin (**1**)[a]

carbon	δ (mult.)	carbon	δ (mult.)
1	34.3 (s)	1′	
2	39.7 (t)	2′	
3	19.3 (t)	3′	
4	33.1 (t)	4′	171.9 (s)
5	129.4 (s)	5′	124.6 (s)
6	137.9 (s)	6′	140.3 (d)
7	126.9 (d)	7′	122.8 (d)
8	137.7 (d)	8′	145.1 (d)
9	136.4 (s)	9′	135.0 (s)
10	130.8 (d)	10′	136.6 (d)
11	125.5 (d)	11′	124.5 (d)
12	137.1 (d)	12′	141.0 (d)
13	137.3 (s)	13′	136.1 (s)
14	132.3 (d)	14′	134.4 (d)
15	131.2 (d)	15′	129.7 (d)
16	29.0 (q)	16′	
17	29.0 (q)	17′	
18	21.8 (q)	18′	12.6 (q)
19	12.8 (q)	19′	12.8 (q)
20	12.9 (q)[b]	20′	12.8 (q)[b]

[a] Data recorded in $CDCl_3$ at 125 MHz. [b] These assignments may be interchangeable.

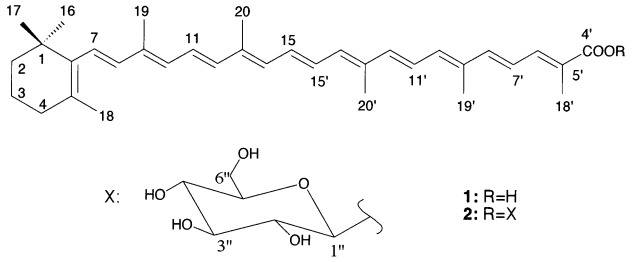

Figure 1. Structures of **1** and **2**

to consist of both carotenoid and sugar moieties (Table 1).[4] Except for the ^1H chemical shift of the sugar moieties, the ^1H NMR spectral data of the aglycone of **2** closely resembled those of **1**. After acetylation of **2**, the sugar moiety was identified as β-glucopyranose by assignment of ^1H NMR data, including decoupling experiments (Table 1). From the chemical shift of an anomeric proton [δ_H ($CDCl_3$) 5.87, 1H, d, J = 8 Hz, H-1″], C-1″ of the glucose was found to be linked to C-4′ of the carotenoid molecule by a β-glycosidic linkage. Sugar analysis of tetraacetyl derivative **3** gave D-glucose. Thus, **2** was determined to be 4′-apo-β-caroten-4′-oic acid β-D-glucopyranoside (=neurosporaxanthin β-D-glucopyranoside) (Figure 1).

More than 650 carotenoids have been isolated from natural sources.[5] Among these isolated compounds, compound **2** is a new carotenoid glycosyl ester. Thus, compound **2** is the first naturally occurring neurosporaxanthin glycoside.

Experimental Section

General Experimental Procedures. Optical rotation was measured with a JASCO DIP-1000. Visible spectra were recorded on a Shimadzu UV-265FS spectrophotometer. Concentrations were calculated using $E^{1\%}_{1cm}$ = 2500 at λ_{max} (in Et_2O). EIMS were recorded on a JEOL JMS-GCmate mass spectrometer with a direct inlet system with an ionization energy of 70 eV. FABMS were measured with a JEOL JMS-SX 102 mass spectrometer. The ^1H NMR spectra were measured with Varian XL-300 (300 MHz) and JEOL GSX-500 (500 MHz) instruments in $CDCl_3$ or $CDCl_3$−CD_3OD (4:1) with TMS as internal standard. HPLC was performed on a Shimadzu LC-10ATvp, C-R8A instrument supplied with a Shimadzu SPD-10Avp spectrometer set at 470 nm. The column used was a carotenoid C30 (YMC, 250 × 20 mm i.d.) with solvent A (MeOH−MTBE−H_2O, 81:15:4) for 60 min or a linear gradient from 100% solvent A to 100% solvent B (MeOH−MTBE−H_2O, 8:90:4) for 60 min.

Material. *Fusarium* sp. strain T-1 was isolated from the surface of seawater collected at Tanegashima Island of Kagoshima Prefecture in Japan in August 1999. The microorganism was cultured in 10 L scale (50 mL of medium/500 mL flask × 20) at 25 °C for 7 days on a reciprocal shaker at 120 strokes/min (5 cm span), using a modified marine broth medium [1000 mL of distilled H_2O, 37.4 g of BACTO Marine Broth 2216, 20 g of glucose].

Extraction and Isolation. The cultured cells were concentrated by centrifugation, then extracted with acetone. The extracted solution was fractionated between ethyl acetate and an aqueous NaCl solution. The organic layer was dried over Na_2SO_4, then concentrated to dryness. The residue was subjected to column chromatography on Si gel using a mixture of *n*-hexane−Me_2CO and $CHCl_3$−MeOH as the eluent. Compound **1** (4.8 mg, 45% of the total carotenoid) was eluted with Me_2CO−*n*-hexane (2:8) from a Si gel column and was further purified by reversed-phase HPLC (carotenoid C30, YMC, 250 × 20 mm i.d.) with a linear gradient from 100% solvent A (MeOH−MTBE−H_2O, 81:15:4) to 100% solvent B (MeOH−MTBE−H_2O, 8:90:4) for 60 min. Compound **2** (1.2 mg, 11%) was eluted with $CHCl_3$−MeOH (1:1) from a Si gel column and further purified by reversed-phase HPLC with solvent A.

The following additional carotenoids were identified from *Fusarium* sp.: β-carotene (5% of the total carotenoid), γ-carotene (32%), and torulene (3%).

Acetylation of 2. A mixture of **2** (1.2 mg), pyridine (2 mL), and acetic anhydride (1 mL) was kept at 28−30 °C for 3 days. Water was added to the reaction mixture, which was then extracted with EtOAc after 30 min. The EtOAc extract was concentrated, and the residue was chromatographed on Si gel with $CHCl_3$−EtOAc (9:1) as an eluent to give the acetylated product (1.5 mg, **3**).

Sugar Analysis. To a solution of **3** in MeOH−THF (1:1, 9 mL) was added 1 M MeONa (1 mL). After being stirred for 1 h at room temperature, the reaction mixture was treated with Dowex 50WX8-100 and filtered. The filtrate was concentrated in vacuo. A mixture of the residue and 0.5 M HCl (2 mL) was heated at 96 °C for 1 h and concentrated in vacuo. EtOAc and water were added to the residue. The aqueous layer was evaporated in vacuo to give a sugar, D-glucose ($[\alpha]_D^{22}$ +45.6° (*c* 0.07, H_2O)).

Neurosporaxanthin (=4′-apo-β-caroten-4′-oic acid) (1): Vis (Et_2O) λ_{max} 474.6 nm; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS (70 eV) *m*/*z* 498 $[M]^+$ (100), 483 (3), 454 (3), 419 (4), 406 (29), 392 (25), 340 (8); HREIMS *m*/*z* 498.3503 (calcd for $C_{35}H_{46}O_2$, 498.3498).

Neurosporaxanthin β-D-glucopyranoside (=4′-apo-β-caroten-4′-oic acid β-D-glucopyranoside) (2): Vis (Et_2O) λ_{max} 474.6 nm; ^1H NMR data, see Table 1; FABMS *m*/*z* 660 $[M]^+$ (5), 683 $[M + Na]^+$ (5), HRFABMS *m*/*z* 660.4022 (calcd for $C_{41}H_{56}O_7$, 660.4026).

Neurosporaxanthin β-D-glucopyranoside tetraacetate (3): Vis (Et_2O) λ_{max} 474.6 nm; ^1H NMR data, see Table 1; EIMS (70 eV) *m*/*z* 828 $[M]^+$ (7).

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References and Notes

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