Structure of Three New Carotenoids with a 3-Methoxy-5-keto-5,6-seco-4,6-cyclo-β End Group from the Seeds of *Pittosporum tobira*

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Three new carotenoids with a 3-methoxy-5-keto-5,6-seco-4,6-cyclo-β end group (1—3) have been isolated from the seeds of *Pittosporum tobira*. Their structures were elucidated by detailed analyses of nuclear magnetic resonance and UV data.

Key words carotenoid; *Pittosporum tobira*; 3-methoxy-carotenoid; structure elucidation; NMR; ^{1}H–^{1}H nuclear Overhauser effect

In the course of studies on new carotenoids in natural products, we have reported the isolation and structure elucidation of the oyster carotenoids with unique end groups: crassostreaxanthins[1a,b]; the retrocarotenoid anhydroeschscholtzxanthin[1c]; the di-*Z*-carotenoids cucumariaxanthins[1d]; and the purple carotenoid rhodobacterioxanthin.[1e] In a previous paper, we reported the isolation and structure elucidation of the C_{69} carotenoids pittosporumxanthins A1 and A2 from the seeds of *Pittosporum tobira* of Pittosporaceae.[2] Recently, we have isolated three new carotenoids (**1**—**3**) with the new 3-methoxy-5-keto-5,6-seco-4,6-cyclo-β end group (3-methoxy-5-acetyl-4,18-dinor-β end group[3]) from the red seeds of *P. tobira* collected from the bank of the Kamogawa River, Kyoto, in December 1998. This paper reports the isolation and structural elucidation of the new carotenoids.

Results and Discussions

The seeds (20 kg) of *P. tobira* were extracted with methanol. The methanol extract was saponified with KOH–methanol and extracted with Et_2O–*n*-hexane. The extracted solution was washed with H_2O, dried, and evaporated under reduced pressure. The residual crude carotenoids were isolated by column chromatography on silica gel with increasing percentages of ether in *n*-hexane. The ether fractions purified by high-performance liquid chromatography (HPLC) on silica gel and octadecyl silica (ODS) furnished three carotenoids (**1**, 10 mg; **2**, 2 mg; **3**, 1 mg).

Carotenoids **1**—**3** were all obtained as a red amorphous powder. Acetylation of **1**—**3** with acetic anhydride in pyridine gave the mono-acetate of **1**—**3**, respectively. The molecular formulae of **1**—**3** were established to be $C_{41}H_{56}O_4$ using high-resolution electron-impact mass spectrometry (HR-EI-MS).

The ^{1}H-NMR spectra of **1**—**3** in $CDCl_3$ showed 11 singlet methyl signals, respectively. Among those, five methyl signals had common chemical shifts of δ 1.29, 1.42, 1.99, 2.31, and 3.35 in **1**—**3**. The ^{1}H-NMR data suggested that the carotenoids **1**—**3** have a common structural unit of a hitherto unknown type in the molecules. Thus the structural analysis of the chief product **1** was undertaken in detail.

Carotenoid **1** in Et_2O showed an absorption maximum at 457 nm, suggesting the existence of a conjugated decaene structural chromophore.[4] Both the ^{13}C- and the ^{1}H-NMR signals of **1** in $CDCl_3$ were assigned by distortionless enhancement by polarization transfer (DEPT), ^{13}C–^{1}H correlation spectroscopy (COSY), double-quantum filtered (DQF)-COSY, nuclear Overhauser effect (NOE), and ^{1}H-homodecoupling (including decoupling difference) experiments, and by comparison with those of related compounds.[5,6] Their assigned data are shown in Table 1. The ^{13}C-NMR and DEPT experiments with **1** in $CDCl_3$ established the presence of 41 carbons and 55 carbon-bonded protons (11 methyls, 3 methylenes, 16 methines). The ^{13}C–^{1}H COSY spectra of **1** established all the one-bond ^{13}C–^{1}H connectivities. The chemical shifts of carbons and protons of the half-partial structure (C1′ to C 20′) in **1** were identical to those of (3*S*,5*R*,8*R*)-auroxanthin.[5a]

The structure elucidation of the unassigned remaining half (C1 to C20 and a methoxyl group) in **1** was as follows. In the DQF-COSY spectra of **1**, the cross-peaks of ^{1}H spin-couplings were observed between following proton–proton pairs: H2–H3, H7–H8, H10–H11–H12, and H14–H15. The magnitude of their spin-coupling constants is shown in Table 1. In the low-power ^{1}H-homodecoupling difference experiments with **1**, irradiation of the methyl proton signals at δ 1.97 (H20) and 1.99 (H19) revealed the existence of long-range couplings between H20–H14 and between H19–H10. The NOE results in ^{1}H–^{1}H NOE difference experiments with **1** are summarized in Fig. 2.

As can be seen from Fig. 2, NOEs were observed between H20–H15, H11 and between H19–H11, H7 in the protons

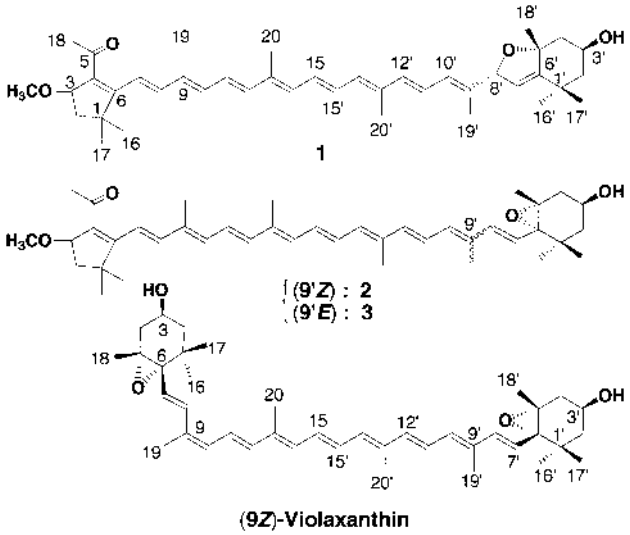

Fig. 1. Structures of **1**—**3**, and (9*Z*)-Violaxanthin

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Table 1. NMR Data of Carotenoids **1**—**3**, and (9*Z*)-Violaxanthin[6a] in $CDCl_3$

Carbon No.	**1**		**2**	**3**	(9*Z*)-Violaxanthin
	δ_C [a] mult.	δ_H [b] mult. (*J*, Hz)	δ_H [b] mult. (*J*, Hz)	δ_H [b] mult. (*J*, Hz)	δ_H mult. (*J*, Hz)
1	46.5 s	—	—	—	—
2	45.8 t	1.83 dd (13.5, 4)	1.84 dd (13.5, 4)	1.83 dd (13.5, 4)	1.26 dd (14.5, 10)
		1.97 dd (13.5, 7)	1.98 dd (13.5, 7)	1.97 dd (13.5, 7)	1.63 m
3	84.0 d	4.58 dd (7, 4)	4.58 dd (7, 4)	4.58 dd (7, 4)	3.92 m
4	136.8 s	—	—	—	1.64 dd (14.5, 9)
					2.41 ddd (14.5, 5.3, 1.5)
5	200.0 s	—	—	—	—
6	159.7 s	—	—	—	—
7	121.3 d	7.16 d (16)	7.17 d (16)	7.17 d (16)	5.93 d (15.5)
8	142.0 d	6.86 d (16)	6.86 d (16)	6.86 d (16)	6.84 d (15.5)
9	135.9 s	—	—	—	—
10	136.0 d	6.33 d (11)	6.33 d (11)	6.33 d (11)	6.08 d (11.5)
11	124.6 d	6.61 dd (15, 11)	6.63 dd (15, 11)	6.63 dd (15, 11)	6.76 dd (15, 11.5)
12	139.7 d	6.43 d (15)	6.43 d (15)	6.43 d (15)	6.29 d (15)
13	136.0 s	—	—	—	—
14	133.9 d	6.27 dm (10)	*ca.* 6.30 m	*ca.* 6.30 m	6.25 dm (12)
15	130.8 d	6.62 m	*ca.* 6.65 m	*ca.* 6.65 m	*ca.* 6.63 m
16	29.5 q	1.29 s	1.29 s	1.29 s	1.01 s
17	29.0 q	1.42 s	1.42 s	1.42 s	1.17 s
18	30.0 q	2.31 s	2.31 s	2.31 s	1.21 s
19	12.5 q	1.99 s	1.99 s	1.99 s	1.93 s
20	12.8 q	1.97 s	1.97 s	1.97 s	1.96 s
$O\underline{C}H_3$	56.3 q	3.35 s	3.35 s	3.35 s	—
1′	33.7 s	—	—	—	—
2′	46.7 t	1.51 dd (14, 3.5)	1.27 dd (14.5, 10)	1.24 dd (14.5,10)	1.24 dd (14.5, 10)
		1.77 dd (14, 4)	1.63 ddd (14.5, 3.5, 1.5)	1.63 ddd (14.5, 3.5, 1.5)	1.63 ddd (14.5, 3.5, 1.5)
3′	67.7 d	4,23 m	3.92 m	3.90 m	3.91 m
4′	47.4 t	1.99 dd (13.5, 4)	1.65 dd (14.5, 9)	1.63 dd (14.5,9)	1.63 dd (14.5, 9)
		2.13 dd (13.5, 4)	2.41 ddd (14.5, 5.5, 1.5)	2.38 ddd (14.5, 5, 1.5)	2.39 ddd (14.5, 5, 1.5)
5′	86.9 s	—	—	—	—
6′	154.1 s	—	—	—	—
7′	119.9 d	5.26 d (1)	5.94 d (15.5)	5.88 d (15.5)	5.88 d (15.5)
8′	87.7 d	5.17 d (1)	6.84 d (15.5)	6.29 d (15.5)	6.29 d (15.5)
9′	138.2 s	—	—	—	—
10′	127.2 d	6.20 d (11)	6.07 d (11.5)	6.19 d (11.5)	6.20 d (11.5)
11′	124.7 d	6.51 dd (15, 11)	6.76 dd (15, 11.5)	6.62 dd (15, 11.5)	6.60 dd (15, 11.5)
12′	137.5 d	6.32 d (15)	6.30 d (15)	6.38 d (15)	6.37 d (15)
13′	136.3 s	—	—	—	—
14′	132.2 d	6.23 dm (12)	6.25 dm (12)	6.25 dm (12)	6.26 dm (12)
15′	129.8 d	6.62 m	*ca.* 6.65 m	*ca.* 6.65 m	*ca.* 6.63 m
16′	31.4 q	1.17 s	1.01 s	0.98 s	0.98 s
17′	28.9 q	1.33 s	1.17 s	1.15 s	1.15 s
18′	29.0 q	1.62 s	1.22 s	1.19 s	1.19 s
19′	12.7 q	1.72 s	1.93 s	1.93 s	1.93 s
20′	12.9 q	1.95 s	1.97 s	1.97 s	1.97 s

^{1}H-(300 MHz) and ^{13}C-NMR (75 MHz) chemical shifts are reported downfield from internal tetramethylsilane (TMS) (=0.00). *a*) Assignments are based on DEPT and ^{13}C–^{1}H COSY, and on comparison with related carotenoids.[5] *b*) Assignments are based on DQF-COSY, ^{1}H–^{1}H NOE, ^{1}H-homodecoupling difference experiments, and comparison with those of the related carotenoids.[5,6]

from H7 to H15, H19, and H20. Based on the NMR experimental results mentioned above, the connections of C7 to C15, C19, and C20, and all-*E* geometries of Δ^7, Δ^9, Δ^{11}, and Δ^{13} were clarified. The connections of =C7–C6(=)–C1(C16,17)–C2–C3(O–)– in **1** were deduced on the basis of the observed NOEs between H7–H16, H17, and between H2–H17, and the COSY correlations between H2–H3. In addition, the carbon (C4) was connected to the C6 (ene structure) and acetyl group to satisfy the conjugated decaene structure in **1** estimated by the UV–visible data. The remaining connections of C3H (at δ_H 4.58)–C4 and C3–OCH_3 (at δ_H 3.35) in **1** were based on the NOEs between acetyl-methyl protons (δ_H 2.31) and H3, and between methoxy-methyl protons and H2, H3, respectively. Then the assigned structure of

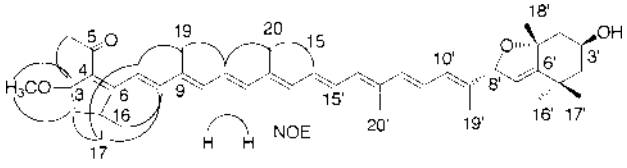

Fig. 2. Structure and NOE Data Summary of **1**

the end group and the geometry of $\Delta^{5,6}$ in **1** were straightforward for their ^{1}H- and ^{13}C-NMR data, and NOE data. Therefore the unique structure of the carotenoid **1** was assigned to be 3-methoxy-3′-hydroxy-5′,8′-epoxy-5′,8′-dihyodro-5,6-seco-4,6-cyclo-β,β-caroten-5-one as shown in Fig. 1.

Carotenoids **2** and **3** in Et_2O showed absorption maxima at

467 and 470 nm, respectively, suggesting the presence of a conjugated hendecaene structure in the molecule.[4] The ^{1}H-NMR data of **2** and **3** in $CDCl_3$ suggested the existence of (9*Z*)- and (all-*E*)-violaxanthin partial structures in addition to the partial structure found in **1**, respectively. Thus the NMR data of **2** and **3** were compared with those of (9*Z*)- and (all-*E*)-violaxanthin, respectively.[6a] The structure and ^{1}H-NMR data of (9*Z*)-violaxanthin are shown in Fig. 1 and Table 1, respectively. The ^{1}H data of H2,2′ to H20,20′ in (all-*E*)-violaxanthin are nearly the same as those of H2′ to H20′ in (9*Z*)-violaxanthin.[6] As shown in Table 1, the ^{1}H chemical shifts and their spin-couplings of H2′ to H20′ in **2** and **3** were almost identical with those of H2 to H20 and of H2′ to H20′ in (9*Z*)-violaxanthin, respectively. That is, the data indicated the inclusion of the (9*Z*)- and (all-*E*)-violaxanthin partial structure in the molecule of **2** and **3**. Thus the structures of **2** and **3** were determined to be (9′*Z*)- and (all-*E*)-3-methoxy-3′-hydroxy-5′,6′-epoxy-5′,6′-dihyodro-5,6-seco-4,6-cyclo-β, β-caroten-5-one, respectively, as shown in Fig. 1.

The elucidated structures of **1**, **2**, and **3** are unique 3-methoxy carotenoids that have not been reported previously in natural products to our knowledge. Therefore these are the first examples of carotenoids including the 3-methoxy-5-keto-5,6-seco-4,6-cyclo-β end group.

Experimental

General Experimental Procedures The UV–visible spectra were recorded on a Shimadzu UV-240 spectrophotometer in Et_2O. The EI-MS spectra were recorded using a JEOL JMS-SX 102AQQ mass spectrometer with ionization energy of 20 eV. The ^{13}C- (75.4 MHz) and ^{1}H- (300 MHz) NMR spectra were recorded on a Varian XL-300 NMR spectrometer in $CDCl_3$ with TMS as an internal standard. Two-dimensional (2D) experiments were acquired using the standard Varian pulse sequence programs and the software used to obtain 2D spectra was from Varian, version 6.1E or A. Circular dichroism (CD) spectra were recorded in Et_2O at room temperature with a JASCO J-500 spectropolarimeter. HPLC was performed on a Shimadzu LC-6AD instrument with a Shimadzu SPD-6AV spectrophotometer set at 450 nm. The columns used were a Shim-Pack PREP-SIL (Shimadzu, 20 mm×250 mm, 5 μm) and a Lichrospher 100 RP-18 (Cica Merck, 20×250 mm, 10 μm). Analytical thin-layer chromatography (TLC) was carried out on Silica gel 60 F_{254} plates (Merck) with the developing solvent of 30% acetone in *n*-hexane.

Plant Material The red colored seeds of *P. tobira* Aiton (Pittosporaceae) were collected in December 1998, on the banks of the Kamogawa River, Kyoto, Japan. Voucher specimens have been deposited at Kyoto Pharmaceutical University.

Extraction and Isolation of Carotenoids The seeds (20 kg) of *P. tobira* were washed with *n*-hexane and extracted with methanol. The methanol extract was transferred to Et_2O–*n*-hexane (1 : 1). The organic layer was washed with H_2O, dried, and evaporated under reduced pressure. The residue was saponified with 5% KOH–methanol for 12 h at 30 °C and extracted with Et_2O–*n*-hexane (1 : 1). The extracted solution was washed with H_2O, dried, and evaporated under reduced pressure. The residual crude carotenoids (1.0 g) were separated by column chromatography on silica gel with increasing percentages of ether in *n*-hexane. The ether fractions further separated by HPLC on silica gel with acetone–*n*-hexane (2 : 8) and purified by HPLC on ODS with $CHCl_3$–CH_3CN (2 : 8) furnished carotenoids **1** (10 mg), **2** (2 mg), and **3** (1 mg).

In addition to the new carotenoids **1**, **2**, and **3**, five known carotenoids were isolated and identified by UV–visible, EI-MS, ^{1}H-NMR, and CD spectral data[4—7]: antheraxanthin (10 mg); (9*Z*)-violaxanthin (30 mg); violaxanthin (10 mg); (3*S*,5*R*,8*R*)-auroxanthin (20 mg); neoxanthin (20 mg).

Carotenoid **1**: Red amorphous powder. TLC: *Rf* 0.45. HPLC: retention time, min: 13 (silica gel, acetone/*n*-hexane=2/8, 10 ml/min). UV–visible λ_{max} (Et_2O) nm: 457. ^{13}C- and ^{1}H-NMR ($CDCl_3$): Table 1. HR-EI-MS *m/z*,: 612.4178 (M^+) (Calcd for $C_{41}H_{56}O_4$: 612.4177).

Carotenoid **2**: Red amorphous powder. TLC: *Rf* 0.45. HPLC: retention time, min: 11 (silica gel, acetone/*n*-hexane=2/8, 10 ml/min). UV–visible λ_{max} (Et_2O) nm: 467. ^{1}H-NMR ($CDCl_3$): Table 1. HR-EI-MS *m/z*: 612.4164 (M^+) (Calcd for $C_{41}H_{56}O_4$: 612. 4177).

Carotenoid **3**: Red amorphous powder. TLC: *Rf* 0.45. HPLC: retention time, min: 12 (silica gel, acetone/*n*-hexane=2/8, 10 ml/min). UV–visible λ_{max} (Et_2O) nm: 470. ^{1}H-NMR ($CDCl_3$): Table 1. HR-EI-MS *m/z*,: 612.4170 (M^+) (Calcd for $C_{41}H_{56}O_4$: 612.4177).

Acetylation of 1—3 To the carotenoid **1** (*ca*. 50 μg, TLC: *Rf* 0.45) dissolved in dry piridine 1 ml was added acetic anhydride 0.5 ml. The reaction was carried out at room temperature, and monitored by TLC at regular intervals. After 1 h, a less polar product (monoacetate, UV–visible λ_{max} (Et_2O) 457 nm) with *Rf* 0.65 was formed by acetylation.

Acetylation of **2** and **3** was carried out in a similar manner to give less polar products (TLC: *Rf* 0.65 from **2** and *Rf* 0.65 from **3**), respectively.

As a comparative experiment, acetylation reaction of violaxanthin (TLC: *Rf* 0.3) was performed. The reaction was run in a similar manner to that above. Two less polar acetylation products (TLC: *Rf* 0.5, monoacetate and *Rf* 0.7, diacetate) were given.

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