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Structures of tobiraxanthins A1, A2, A3, B, C and D, new carotenoids from the seeds of *Pittosporum tobira*

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Abstract—Six new carotenoids, named tobiraxanthins A1–A3, B, C and D, have been isolated from the seeds of *Pittosporum tobira*. Their structures were elucidated by NMR and MS spectral analyses. The chemical relation of the tobiraxanthins to the previously reported carotenoids having a 4-acetyl-3-methoxycyclopentene ring was also studied.

In the course of our carotenoids studies,[1,2] we investigated the carotenoids of the red-colored seeds of *Pittosporum tobira* and have already reported the isolation and structure elucidation of unique carotenoids having a 4-acetyl-3-methoxycyclopentene group represented by the structure **1**.[2] However, from the viewpoint of the carotenoid structure and possible biosynthesis pathway, it was questionable whether the 3-methoxy ones are naturally occurring or not.[3] Hence, further studies on the carotenoid constituents of the seeds, by use of extraction separation without saponification,[4] resulted in the isolation of six new carotenoids, named tobiraxanthins A1–A3, B, C and D, most of which were converted into the corresponding carotenoids having the 4-acetyl-3-methoxycyclopentene moiety. This report deals with the isolation and structure elucidation of the six carotenoids, and the chemical and structural analogy between these and the previously reported 4-acetyl-3-methoxycyclopentene ones.[2]

The red-colored seeds of *P. tobira* were collected from plants growing on the bank of the Kamogawa River in Kyoto in December. The seeds (30 kg) were washed with *n*-hexane to remove the viscous matter on the surface and extracted with methanol. The methanol extract was transferred to Et_2O–*n*-hexane (1:1) by addition of water. The organic layer was washed with H_2O, dried and evaporated under reduced pressure. The residual red-colored viscous oil was chromatographed on silica gel with *n*-hexane/Et_2O/acetone and divided into seven fractions. The crude carotenoids from fraction-4 (*n*-hexane/Et_2O, 2:8), purified by HPLC on silica gel and on ODS (CH_2Cl_2/CH_3CN, 2:8) furnished tobiraxanthins A1 (**2**, 8 mg), A2 (**3**, 6 mg) and A3 (**4**, 6 mg). Similarly, tobiraxanthin B (**5**, 3 mg) from fraction-5 (Et_2O), and tobiraxanthins C (**6**, 4 mg) and D (**7**, 1 mg) from fraction-6 (acetone) were obtained.

Tobiraxanthins A1–A3 (**2**, **3** and **4**) were obtained as a red-colored amorphous powder. The UV–vis spectrum of **2** in *n*-hexane showed absorption maxima at 440, 468 and 500 nm, suggesting the existence of a capsorubin-type chromophor.[5] The IR spectrum of **2** in $CHCl_3$ showed three carbonyl absorptions at 1793, 1718, and 1654 cm^{-1}. The molecular formula of **2** was determined as $C_{68}H_{108}O_8$ by HR FABMS.[6] The ^{13}C NMR spectrum of **2** showed 34 carbon signals. On the basis of the ^{13}C NMR and the HR FABMS data, the structure of **2** is symmetrical in the molecule. The ^1H and ^{13}C NMR spectra of **2** in $CDCl_3$ showed the characteristic signals of a saturated fatty acid ester: methyl (δ_C 14.2, δ_H 0.88 t), methylene (δ_C 34.1 ~ 22.7, δ_H ~1.25) and carbonyl (δ_C 173.8) groups. The oxymethine (δ_C 67.4, δ_H 5.24 m) groups, of which the signals are resonated at lower field than that of general hydroxyl methines suggest the existence of an *n*-saturated fatty acid ester moiety. The assigned NMR data of **2** in $CDCl_3$ and the numbering system are shown with those of the other tobiraxanthins in Table 1 and Fig. 1, respectively. In FABMS of **2**, the characteristic fragment ions were observed at *m*/*z* 825 $[M-(C_{14}H_{27}O_2)]^+$ and 598 $[M-2(C_{14}H_{27}O_2)]^+$ in addition to the molecular ion. Thus, the structure of **2** was determined as the dimyristyl esters of a carotenoid.

Keywords: structure elucidation; carotenoid; tobiraxanthin; *Pittosporum tobira*; NMR; ^1H–^1H NOE; FABMS.

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Table 1. ^{13}C (75 MHz)[b] and ^{1}H (300 MHz)[a] NMR data of tobiraxanthins A1 (**2**), B (**5**), C (**6**) and D (**7**) in $CDCl_3$

Carbon no.	**2**		Carbon no.	**5**		**6**		**7**	
	^{13}C, δ mult	^{1}H, δ (mult, J, Hz)		^{13}C, δ mult	^{1}H, δ (mult, J, Hz)	^{13}C, δ mult	^{1}H, δ (mult, J, Hz)	^{13}C, δ mult	^{1}H, δ (mult, J, Hz)
1, 1′	45.2s	–	1′	35.3s	–	35.8s	–	39.7s	–
2, 2′	43.9t	1.80 (dd, 14.5, 3) 2.12 (dd, 14.5, 9)	2′	47.2t	1.27 (dd, 14.5, 10) 1.65 (ddd, 14.5, 3.5, 1.5)	49.8t	Na ∼1.95 (m)	46.8t	1.54 (m) 1.77 (m)
3, 3′	67.4d	5.24 (m)	3′	64.3d	3.92 (m)	64.3d	4.32 (m)	67.4d	4.27 (m)
4, 4′	49.0t	2.71 (dd, 15.5, 5.5) 2.59 (dd, 15.5, 7.5)	4′	41.0t	1.65 (dd, 14.5, 9) 2.41 (ddd, 14.5, 5, 1.5)	49.0d	∼1.41 (m) 2.26 (ddd, 13.5, 4, 2)	45.2t	1.61 (m) 2.12 (m)
5, 5′	205.6s	–	5′	67.1s	–	73.0s	–	76.6s	–
6, 6′	202.9s	–	6′	70.5s	–	117.7s	–	79.3s	–
7, 7′	119.2d	6.50 (d, 15)	7′	126.2d	5.95 (d, 15.5)	202.8s	–	129.8d	5.87 (d, 15.5)
8, 8′	146.9d	7.37 (d, 15)	8′	129.3d	6.84 (d, 15.5)	103.2d	6.03 (s)	133.1d	6.42 (d, 15.5)
9, 9′	134.0s	–	9′	132.4s	–	132.3s	–	132.6s	–
10, 10′	140.4d	6.55 (d, 11.5)	10′	130.7d	6.08 (d, 11.5)	128.3d	6.11 (d, 11.5)	131.9d	6.22 (d, 11.5)
11, 11′	124.1d	6.65 (dd, 15, 11.5)	11′	124.1d	6.79 (dd, 15.5, 11.5)	125.3d	∼6.57 (m)	125.2d	∼6.61 (m)
12, 12′	141.7d	6.52 (d, 15)	12′	137.6d	6.30 (d, 15.5)	137.1d	6.35 (d, 15.5)	137.8d	6.38 (d, 15.5)
13, 13′	136.9s	–	13′	135.9s	–	137.4s	–	134.8s	–
14, 14′	135.0d	6.35 (d, 10)	14′	133.6d	6.25 (d, 10)	135.8d	6.25 (d, 10)	135.9d	6.25 (d, 10)
15, 15′	130.8d	6.69 (m)	15′	129.7d	∼6.67 (m)	129.7d	∼6.67 (m)	129.6d	∼6.67 (m)
16, 16′	24.6q	1.19 (s)	16′	25.0q	1.01 (s)	29.3q	1.33 (s)	28.6q	0.82 (s)
17, 17′	26.2q	1.20 (s)	17′	29.6q	1.17 (s)	32.1q	1.07 (s)	29.2q	1.25 (s)
18, 18′	29.6q	2.14 (s)	18′	12.0q	1.22 (s)	31.4q	1.35 (s)	27.8q	1.10 (s)
19, 19′	12.8q	1.98 (s)	19′	21.1q	1.94 (s)	14.1q	1.81 (s)	13.2q	1.93 (s)
20, 20′	12.9q	1.98 (s)	20′	13.1q	1.98 (s)	14.0q	1.94 (s)	14.1q	1.98 (s)

The ^{13}C and ^{1}H data of C1 to C20 in **5**, **6** and **7** are the same as those of C1(1′) to C20(20′) in **2**.

The ^{13}C and ^{1}H data of fatty acid part in **2**, **5**, **6** and **7** are as follows: δ_C, 173.8 s, 22.7 d, 23.7 d, 29.2 d, 29.4 d, 29.6 d, 29.7 d, 30.5 d, 31.9 d, 34.1 d, 14.2 q, and δ_H, 2.09 t (7.5), ∼1.25 m, 0.88 t (6.5).

^{1}H and ^{13}C chemical shifts are reported downfield from internal TMS (=0.00).

[a] Assignments are based on ^{1}H–^{1}H NOE, INDOR and ^{1}H–{^{1}H} (including decoupling difference) experiments.

[b] Assignments are based on DEPT, ^{13}C–^{1}H COSY, LSPD experiments and comparison with those of the related compounds. (cf. Refs. 7 and 9). Na: not assigned because of ^{1}H signals overlapping.

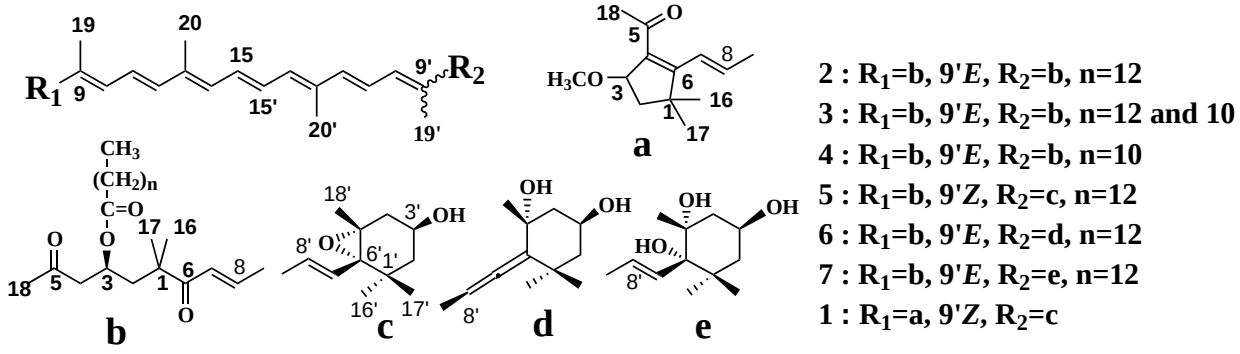

Figure 1. Structures of tobiraxanthins A1(**2**), A2 (**3**), A3 (**4**), B (**5**), C (**6**) and D (**7**), and 3-methoxy carotenoid 2 (**1**).

The structure of the carotenoid moiety in **2** was determined by ^{1}H homodecoupling (^{1}H–{^{1}H}), ^{13}C–^{1}H COSY, LSPD and ^{1}H–^{1}H NOE experiments. The results are summarized in Fig. 2. The ^{13}C–^{1}H COSY spectrum of **2** established all the one-bond ^{13}C–^{1}H connectivities. In the polyene part of **2**, the ^{1}H and ^{13}C signals of C8 to C8′ including C19(19′) and C20(20′) were similar to those of capsorubin.[7] The conjugated

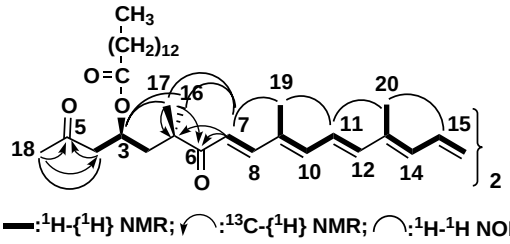

Figure 2. NMR data summary for **2**.

polyene partial structure was also supported by ^{1}H–{^{1}H} NMR and ^{1}H–^{1}H NOE difference experiments (Fig. 2).

As for the remaining unassigned structure in **2**, the connections of H2(2′) to H4(4′) were characterized by simple ^{1}H–{^{1}H} NMR experiments. As regards the connections of the quaternary carbons of C1(1′), C5(5′) and C6(6′), long-range ^{1}H–^{13}C spin-couplings were observed between H18(18′), H4(4′) and C5(5′), and between H16, 17 (16′,17′), H7(7′) and C1(1′), C6(6′) in the LSPD experiments of **2**.[8] Thus, the ^{13}C signals of C1(1′), C5(5′) and C6(6′) were assigned and the connections of C7(7′) to C1(1′) and C18(18′) in **2** were clarified (Fig. 2). These results were also supported by the NOEs between H18(18′) and H2(2′) and between H16,17(16′,17′) and H7(7′), H3(3′). Therefore, the total structure of **2** was determined to be 3,3′-dihydroxy-5,6,5′,6′-diseco-β,β-carotene-5,6,5′,6′-tetraone dimyristate, as shown in Fig. 1. The molecular formulae of **3** and **4** were determined as $C_{66}H_{104}O_8$ and $C_{64}H_{100}O_8$, respectively, by HR FABMS.[6] Also, the characteristic fragment ions of **3** and **4** in FABMS were observed at m/z 825 $[M-(C_{12}H_{23}O_2)]^+$, 797 $[M-(C_{14}H_{27}O_2)]^+$ and 598 $[M-(C_{14}H_{27}O_2)-(C_{12}H_{23}O_2)]^+$ and at m/z 797 $[M-(C_{12}H_{23}O_2)]^+$ and 598 $[M-2(C_{12}H_{23}O_2)]^+$ in addition to their molecular ions, respectively. The other spectral data of **3** and **4** were almost identical with those of **2**. Therefore, the structures of **3** and **4** were determined to be 3-myristyloxy-3′-lauryloxy- and 3,3′-dilauryloxy-analogs of **2**, respectively (Fig. 1).

Tobiraxanthins B (**5**), C (**6**) and D (**7**) were obtained as a red-colored amorphous powder. The molecular formulae of **5**, **6** and **7** were determined as $C_{54}H_{82}O_6$, $C_{54}H_{82}O_6$ and $C_{54}H_{84}O_7$, respectively, by HR FABMS.[6] The ^{1}H and ^{13}C NMR signals of C1 to C20 and of the fatty acid ester moiety in **5**, **6** and **7** were almost identical with those of **2**. (Table 1) Also, the ^{1}H and ^{13}C signals of the remaining unassigned (C1′ to C20′) in **5**, **6** and **7** were almost identical with those of 9*Z*-violaxanthin,[9a] neoxanthin,[9b] and neoflor,[9b,c] respectively. Thus, the structures of **5**, **6** and **7** were determined as shown in Fig. 1.

The 3(3′)*S* chirality for the tobiraxanthins was tentatively postulated on the basis of the following: violaxanthin diester taking 3*S*, 3′*S* chirality was isolated from the same sources.[10] It can be assumed that the tobiraxanthins were derived by biogenetic selective oxidative cleavage of C5(5′)–C6(6′) bond(s)[11] in the ester of violaxanthin, neoxanthin, or latoxanthin,[9b] and held 3(3′)*S* chirality as well as that of the precursor. The CD spectra of **2**,[12] **3** and **4** in Et_2O showed nearly the same Cotton effects. These tobiraxanthins are the first examples of seco-carotenoids including 3(3′)-acyloxy group(s) in the molecule.

Chemical relation of tobiraxanthins (5, 6 and 7) to the previous 3-methoxy carotenoids[2]

The 3-methoxy carotenoids were not isolated by the present procedure, that is, by extraction separation without saponification.[2] Thus, the chemical changes in toberaxanthins with a base were examined. The confirmed reaction scheme of **5** with a base is summarized in Fig. 3. The treatment of **5** with 0.1% KOH in THF resulted in the elimination of myristilic acid to give compound **8** in high yield (>80%). In the degree of the HC–O bond cleavage the elimination reaction somewhat resembles the formation of the characteristic fragment ion which is generated by elimination of the myristyloxy group and is observed at m/z 599 [M–

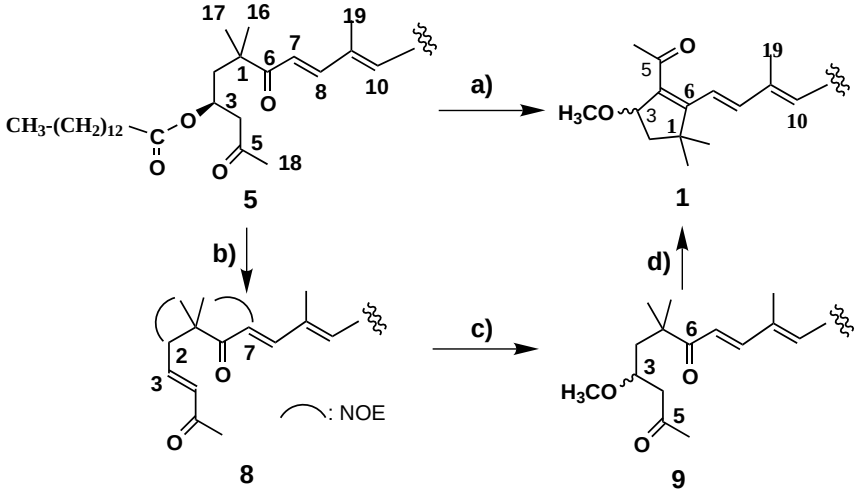

Figure 3. *Reagents and conditions*: (a) 5% KOH in MeOH, rt, 3 h; (b) 0.1% KOH in THF, rt, 1 h; (c) 0.3% KOH in MeOH, rt, 0.5 h; (d) 5% KOH in MeOH, rt, 1 h.

$C_{14}H_{27}O_2]^+$ in the FABMS of **5**. Next, the treatment of **8** with 0.3% KOH in MeOH resulted in addition of MeOH to give compound **9**. The treatment of **9** with 5% KOH in MeOH underwent intra-molecular aldol condensation to give the 3-methoxy carotenoid 2(**1**),[2] while the treatment of **5** with 5% KOH in MeOH resulted in the formation of **1**. These results showed that the treatment of **5** with 5% KOH in MeOH underwent the successive reactions of elimination, addition and aldol condensation to yield **1**.

The structures of **8** and **9** were determined by the MS and ^{1}H NMR data analyses.[13] Also, the treatment of **6** and **7** with 5% KOH in MeOH resulted in the formation of their structurally corresponding 3-methoxy carotenoids.

Thus, it has been proved that the previously reported unique carotenoids having the 4-acetyl-3-methoxycyclopentene moiety were the artifacts resulting from the stepwise reactions of the structurally corresponding tobiraxanthins with the base of the saponification process.

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6. HR FABMS: **2** m/z 1052.8071 (M^+), calcd for $C_{68}H_{108}O_8$ 1052.8040; **3** m/z 1024.7760 (M^+), calcd for $C_{66}H_{104}O_8$ 1024.7727; **4** m/z 996.7516 (M^+), calcd for $C_{64}H_{100}O_8$ 996.7414; **5** m/z 826.6155 (M^+), calcd for $C_{54}H_{82}O_6$ 826.6109; **6** m/z 826.6116 (M^+), calcd for $C_{54}H_{82}O_6$ 826.6109; **7** m/z 844.6221 (M^+), calcd for $C_{54}H_{84}O_7$ 844.6213.
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8. In the LSPD of **2**, when each proton signal at δ 2.14 [H18(18′)] and 2.6 [H4(4′)] irradiated ($\Upsilon B2/2\pi = 40$ Hz) the signal at δ 205.6 [C5(5′)] was effectively decoupled to give a sharp signal, and when each proton signal at δ 1.20 [H16,17 (16′,17′)] and 6.50 [H7(7′)] irradiated ($\Upsilon B2/2\pi =$ ca. 30 Hz) the signals at δ 45.1 [C1 (1′)] and 202.9 [C6(6′)] were effectively decoupled to result in sharp signals.
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10. In the present isolation, the following additional known carotenoids were isolated and identified: violaxanthin diester, violaxanthin monoester, neoxanthin 3′-ester, and 6-*epi*-latoxanthin 3′-ester.
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12. CD (Et_2O): $\lambda(\Delta\varepsilon)$ 220 (+9), 235 (0), 248 (–2.5), 255 (0), 294 (+18.6), 313 (0), 368 (–5.8).
13. **8** HR FABMS: m/z 598.4031 (M^+), calcd for $C_{40}H_{54}O_4$ 598.4020; ^{1}H NMR ($CDCl_3$) δ 2.23 (3H, s, H18), 6.71 (H, d, $J=16.0$, H4), 6.50 (H, d-like, $J=16.0$, H3) 2.58 (2H, m, H2), 1.22/1.23 (3H/3H, s, H16/17), 6.50 (H, d, $J=15.5$, H7), 7.43 (H, d, $J=15.5$, H8); NOE: obsd between H16/H17 and H2, H7; UV–vis (Et_2O): 457 nm.
9 HR FABMS: m/z 630.4293 (M^+), calcd for $C_{41}H_{58}O_5$ 630.4281; ^{1}H NMR ($CDCl_3$) δ 2.16 (3H, s, H18), 2.47 (H, dd, $J=16.0$, 6.5, H4), 2.68 (H, dd, $J=16.0$, 5.5, H4), 4.22 (H, m, H3) 3.17 (3H, s, Me-O), 1.86 (H, dd, $J=14.5$, 3.5, H2), 2.1 (H, m, H2) 1.21/1.18 (3H/3H, s, H16/17), 6.52 (H, d, $J=15.5$, H7) 7.30 (H, d, $J=15.5$, H8); UV–vis (Et_2O): 457 nm.