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Structure of pittosporumxanthins A1 and A2, novel C_{69} carotenoids from the seeds of *Pittosporum tobira*

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Abstract—Two novel C_{69} carotenoids containing an α-tocopherol moiety, named pittosporumxanthins A1 (**1**) and A2 (**2**), have been isolated from the seeds of *Pittosporum tobira*. Their structures were elucidated by NMR and CD spectral analysis and chemical means. © 2001 Elsevier Science Ltd. All rights reserved.

In the course of carotenoids research,[1] we isolated two novel C_{69} carotenoids, named pittosporumxanthins A1 (**1**) and A2 (**2**), from the seeds of *Pittosporum tobira*. This paper reports the isolation and the structural elucidation of the two carotenoids.

The red-colored seeds of *P. tobira* were collected from plants growing on the bank of the Kamogawa river of Kyoto in December. The seeds (5 kg) 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. The extracted solution was worked up in a similar manner as above. The residual crude carotenoids (300 mg) purified by column chromatography on silica gel with ether and by HPLC on silica gel furnished pittosporumxanthins A1 (**1**, 10 mg) and A2 (**2**, 10 mg).

Pittosporumxanthins A1 (**1**) and A2 (**2**) were both obtained as pale yellow amorphous powder. Acetylation of **1** and **2** with acetic anhydride in pyridine gave diacetate of **1** and **2**, respectively. The molecular formulae of **1** and of **2** were established as $C_{69}H_{105}O_6$ by HR FAB-MS.[2] The UV–vis spectra of **1** and **2** in Et_2O showed absorption maxima at 356, 375 and 396 nm, suggesting the existence of a conjugated hexaenes chromophore.[3] The CD spectra of **1** and **2** in Et_2O showed opposite Cotton effects with each other.[4] The ^{13}C NMR and DEPT experiments of **1** and **2** in $CDCl_3$ confirmed the presence of 69 carbons and 102 carbon-bonded protons (17 methyls, 16 methylenes and 19 methines).

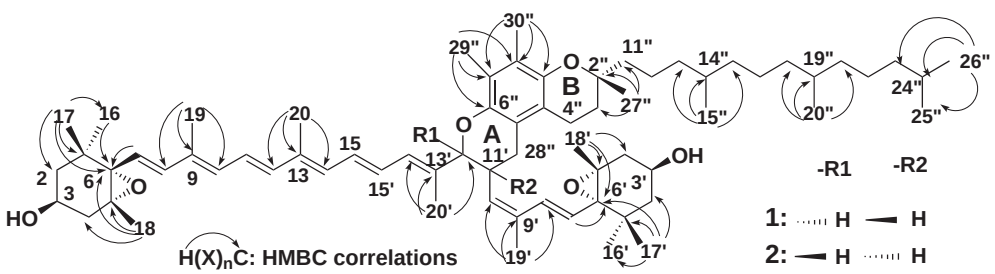

Figure 1. Structures and HMBC data summary of pittosporumxanthins A1 (**1**) and A2 (**2**).

Keywords: structure elucidation; pittosporumxanthins; *Pittosporum tobira* Aiton; ^1H and ^{13}C NMR; ^1H–^1H NOE.

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Table 1. ^{13}C (75.4 MHz) and ^{1}H (300 MHz) NMR data for **1** and **2** in $CDCl_3$

Carbon no.	**1**		**2**		Carbon no.	**1**		**2**	
	δ ^{13}C, mult.	δ ^{1}H	δ ^{13}C, mult.	δ ^{1}H		δ ^{13}C, mult.	δ ^{1}H	δ ^{13}C, mult.	δ ^{1}H
1	35.3 s	–	35.3 s	–	16′	24.8 q	0.954	24.9 q	0.953
2	47.2 t	1.25, 1.62	47.2 t	1.24, 1.63	17′	29.5 q	1.114	29.6 q	1.128
3	64.3 d	3.90	64.3 d	3.90	18′	20.1 q	1.182	20.0 q	1.155
4	41.0 t	1.62, 2.38	41.0 t	1.62, 2.38	19′	20.5 q	1.811	20.8 q	1.815
5	67.0 s	–	67.0 s	–	20′	12.5 q	1.803	12.4 q	1.769
6	70.3 s	–	70.3 s	–	2″	74.5 s	–	74.5 s	–
7	123.8 d	5.88	123.8 d	5.88	3″	31.4 t	1.74	31.2 t	1.75
8	137.3 d	6.29	137.3 d	6.29	4″	19.6 t	2.51, 2.43	19.6 t	2.53, 2.46
9	134.1 s	–	134.1 s		5″	115.7 s	–	115.7 s	–
10	132.2 d	6.19	132.2 d	6.19	6″(10″)	144.9 s	–	145.0 s	–
11	124.4 d	6.58	124.4 d	6.58	7″(8″)	123.6 s	–	123.7 s	–
12	138.2 d	6.35	138.2 d	6.35	8″(7″)	122.9 s	–	122.9 s	–
13	135.9 s	–	135.9 s	–	9″	115.7 s	–	115.7 s	–
14	132.5 d	6.21	132.5 d	6.19	10″(6″)	145.5 s	–	145.5 s	–
15	129.4 d	6.55	129.4 d	6.54	11″	39.0 t	Na	40.7 t	Na
16	24.9 q	0.978	24.9 q	0.975	12″	21.1 t	Na	21.0 t	Na
17	29.6 q	1.148	29.6 q	1.147	13″(21″)	37.5 t	Na	37.5 t	Na
18	20.0 q	1.187	20.0 q	1.186	14″	32.7 d	1.38	32.7 d	1.38
19	12.8 q	1.925	12.8 q	1.923	15″	19.6 q	0.85	19.7 q	0.86
20	13.0 q	1.946	13.0 q	1.943	16″	37.5 t	Na	37.5 t	Na
1′	35.2 s	–	35.2 s	–	17″	24.5 t	Na	24.5 t	Na
2′	47.1 t	1.23, 1.62	47.2 t	1.22, 1.62	18″	37.5 t	Na	37.5 t	Na
3′	64.2 d	3.88	64.3 d	3.88	l9″	32.8 d	1.35	32.6 d	1.35
4′	40.9 t	1.61, 2.35	41.0 t	1.61, 2.36	20″	19.8 q	0.84	19.8 q	0.86
5′	66.5 s	–	66.8 s	–	21″(13″)	37.3 t	Na	37.3 t	Na
6′	70.1 s	–	70.1 s	–	22″	24.8 t	~1.3	24.8 t	~1.3
7′	126.1 d	5.89	126.0 d	5.89	23″	39.4 t	1.15	39.4 t	1.15
8′	129.7 d	6.58	129.7 d	6.57	24″	28.0 d	1.52	28.0 d	1.52
9′	131.9 s	–	132.0 s	–	25″(26″)	22.7 q	0.866	22.7 q	0.866
10′	130.3 d	5.12	130.4 d	5.12	26″(25″)	22.6 q	0.866	22.6 q	0.866
11′	34.4 d	3.13	34.4 d	3.13	27″	24.4 q	1.246	23.3 q	1.210
12′	84.6 d	4.08	84.7 d	4.08	28″	30.2 t	2.35, 2.60	30.1 t	2.38, 2.64
13′	137.6 s	–	137.6 s	–	29″(30″)	11.7 q	2.100	11.7 q	2.098
14′	128.9 d	6.16	128.9 d	6.14	30″(29″)	11.8 q	2.093	11.8 q	2.089
15′	129.5 d	6.51	129.4 d	6.47					

J(H–H) of **1** (Hz): 2–2 = 14.0, 2–3 = 13.0, 3.5, 3–4 = 8.5, 5.0, 4–4 = 13.5, 7–8 = 15.5, 10–11 = 11.0, 11–12 = 15.0, 14–15 = 11.0, 2′–2′ = 14.0, 2′–3′ = 13.0, 3.5, 3′–4′ = 8.5, 4.5, 4′–4′ = 13.5, 7′–8′ = 15.5, 10′–11′ = 9.8, 10′–19′ = 1.0, 11′–12′ = 9.3, 11′–28″ = 10.0, 5.3, 14′–15′ = 10.5, 15–15′ = 14.5, 14″–15″ = 6.5, 19″–20″ = 6.5, 24″–25″(26″) = 6.8, 28″–28″ = 16.5. J(H–H) of **2** (Hz): 2–2 = 14.0, 2–3 = 13.0, 3.5, 3–4 = 8.5, 5.0, 4–4 = 13.5, 7–8 = 15.5, 10–11 = 11.2, 11–12 = 14.9, 14–15 = 10.7, 2′–2′ = 14.0, 2′–3′ = 13.0, 4.0, 3′–4′ = 8.5, 5.0, 4′–4′ = 13.5, 7′–8′ = 15.5, 10′–11′ = 9.8, 10′–19′ = 1.0, 11′–12′ = 9.3, 11′–28″ = 10.0, 5.2, 14′–15′ = 10.3, 15–15′ = 14.5, 14″–15″ = 6.5, 19″–20″ = 6.5, 24″–25″(26″) = 6.7, 28″–28″ = 16.5. ^{1}H and ^{13}C chemical shifts are reported downfield from internal TMS (=0.00). ^{13}C and ^{1}H NMR signals were assigned by DEPT, ^{13}C–^{1}H COSY, HMBC, LSPD, DQF-COSY, ROESY, and ^{1}H homodecoupling difference experiments and comparison with those of the related compounds (Ref. 6). Na: not assigned because of ^{1}H signals overlapping.

The chemical shifts of all carbons of **1** were almost identical to those of **2**, except two carbons (C11″ and C27″). These data suggested that the structures of **1** and **2** are the diastereoisomers.

Both the ^{13}C and the ^{1}H NMR signals of **1** and **2** in $CDCl_3$ were assigned by ^{13}C–^{1}H COSY, HMBC,[5a] LSPD, DQF-COSY, ROESY,[5b] and ^{1}H homodecoupling (including decoupling difference) experiments, and by comparison with those of the related compounds,[6] as shown in Table 1. The HMBC and the ROESY experimental results are summarized in Figs. 1 and 2. On the basis of these spectral data, the structures of **1** and **2** have been determined, as shown in Fig. 1.

The ^{13}C–^{1}H COSY spectra of **1** and **2** established all the one-bond ^{13}C–^{1}H connectivities. The characteristic four quaternary ^{13}C signals at δ 66.5, 67.0, 70.0, and 70.3, and the ^{1}H–^{1}H spin couplings of ^{1}H signals at δ 3.88 and 3.90 with the adjacent hydrogens in NMR spectra of **1** and **2** imply violaxanthin end-groups as a partial structure of carotenoid. Thus, the NMR data of **1** and **2** were compared with those of *trans*-violaxanthin.[6a] The ^{13}C and their attached ^{1}H chemical shifts and observed ^{1}H–^{1}H spin coupling constants of C1 to C20 and C1′ to C6′ in **1** and **2** were nearly identical to those of *trans*-violaxanthin. The additional experimental results of DQF-COSY and HMBC (Fig. 1), and the UV–vis data clarified the connection of C1 to C20 and

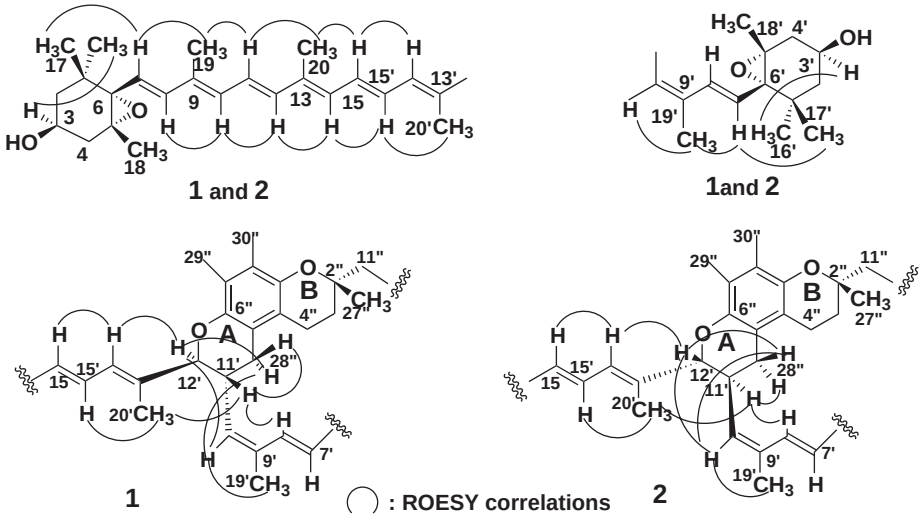

Figure 2. NOE data summary for pittosporumxanthins A1 (**1**) and A2 (**2**).

of C1′ to C20′, and generated 12′(–O–)-, 11′(–CH_2–)-substituted 11′,12′-dihydro-violaxanthin partial structure for both **1** and **2**. With regard to the remaining partial structures of **1** and **2**, their ^{1}H signals were unresolved owing to the signal overlapping, but their ^{13}C signals were resolved effectively. The chemical shifts of unassigned remaining ^{13}C signals in **1** and **2** were similar to those of α-tocopherol[6b] except that of one carbon (δ 30.2, C28″), suggesting the existence of an α-tocopherol partial structure. This assumption was also supported by the characteristic MS fragment ions at m/z 428 and 430 in EI-MS.[7]

The connection of the violaxanthin part with the α-tocophenol part was determined as follows. Based on the molecular formula, unassigned bonds of –OCH(12′)–CH(11′)CH_2– in the violaxanthin moiety and the existence of a methylene group (δ_C 30.2) instead of a methyl group in the α-tocopherol moiety for **1** and **2**, it was thought that the connection was constructed by a dihydropyran ring formation. The existence of the dihydropyran ring was also supported by the ^{1}H–^{1}H spin coupling constants of H11′ with the metylene hydrogens (J = 10.0 and 5.3 Hz) and with H12′ (J = 9.3 Hz). But the following connection was still unclear, i.e. whether C12′–O–C6″ = C5″–CH_2–C11′ or C12′–O–C6″ = C7″–CH_2–C11′. Thus, HMBC and low-power selective ^{13}C{^{1}H} NMR (LSPD) experiments for **1** and **2** were undertaken. In the LSPD of **1** and **2** when the methyl signals at δ 2.09 and 2.10 were irradiated (rB2/2π = 20 Hz) the four ^{13}C signals at δ 144.9 (C6″), 123.6 (C7″), 122.9 (C8″) and 145.5 (C10″) were effectively decoupled to give sharp signals, and when the proton signals at δ 2.55 (H4″ and H28″) were irradiated (rB2/2π ≅ 50 Hz) the two ^{13}C signals at δ 115.7 (C5″ and C9″) were effectively decoupled to result in sharp signals. Therefore, the whole chemical structures of **1** and **2** were determined as shown in Fig. 1.

The relative stereochemistries in **1** and **2** were established by ROESY experiments (Fig. 2). The *cis*-relationship between H10′ and H19′ in **1** and **2** was determined by the large NOE in ROESY and the long-range coupling in ^{1}H-homodecoupling (decoupling difference) between them. Also, *trans*-stereochemistry between H11′ and H12′ in **1** and **2** was elucidated by their spin coupling constant of 9.3 Hz and the fact that no NOE was detected between them (Table 1 and Fig. 2). The absolute configurations of 3(S) and 3′(S) in both **1** and **2** were determined on the basis of the modified Mosher method.[8] Those of 5(R), 6(S), 5′(R) and 6′(S) were also determined relative to the 3(S) and 3′(S). The chiralities of the α-tocopherol moiety in **1** and **2** were deduced to be 2″(R), 14″(R), 20″(R), because the absolute configurations of naturally occurring α-tocopherol are only known as 2(R), 14(R), 20(R). The chiralities of the remaining asymmetric carbons were estimated to be 11′(R), 12′(S) for **1** and 11′(S), 12′(R) for **2** on the basis of the opposite Cotton effects[9] at 374 {$\Delta\varepsilon$ +26 (**1**), −26 (**2**): π–π* of hexaenes} and 243 {$\Delta\varepsilon$ −52 (**1**), +99 (**2**): π–π* of dienes} nm in the CD spectra of **1** and **2**.

Finally, the ^{13}C chemical shift differences at C11″ ($\Delta\delta$ −1.7 ppm) and C27″ ($\Delta\delta$ 1.1 ppm) between **1** and **2** seem to be produced by a subtle difference between the conformations of the pyran ring B in **1** and **2**. That is, each conformation of the pyran ring A in **1** and **2**, which takes the mirror image of each other specifying their ^{1}H chemical shifts (H11′, H12′, and H28″) and ^{1}H–^{1}H spin-coupling constants ($J_{11'-12'}$ and $J_{11'-28''}$), influences those of the pyran ring B via steric (mutual) interaction between H28″ and H4″. On the basis of this assumption, a stereomodel examination showed that the methyl group on C2″(R) of **2** is oriented more axially to the pyran ring B plane than that of **1**. However, the present experimental results are not sufficient to relate the conformation of the pyran ring A in **1** and **2** to the orientation of the methyl group on C2″(R).

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2. HR FAB-MS: **1**, found m/z 1029.7885 $(MH)^+$, requires for $C_{69}H_{105}O_6$ 1029.7912; **2**, found m/z 1029.7927 $(MH)^+$, requires for $C_{69}H_{105}O_6$, 1029.7912.
3. UV–vis (Et_2O): Both **1** and **2**, λ (ε) 356 (79 300), 375 (123 000), 396 (118 000).
4. CD (Et_2O): **1**, λ ($\Delta\varepsilon$) 208 (+23), 214 (0), 243 (−52), 264 (0), 272 (+5), 277 (0), 283 (−5), 295 (0), 357 (+15), 364 (+13), 374 (+26), 386 (+13), 397 (+19), 426 (0); **2**, λ ($\Delta\varepsilon$), 208 (−51), 216 (0), 243 (+99), 260 (0), 273 (−59), 315 (−4), 357 (−20), 363 (−19), 374 (−26), 387 (−14), 395 (−20), 426 (0).
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7. EI-MS: **1**, m/z (%) 1028 (M^+, 0.2), 744 (3), 718 (4), 600 (18), 520 (100), 430 (88), 428 (10), 255 (20), 165 (40); **2**, m/z (%) 1028 (M^+, 0.7), 744 (5), 718 (5), 600 (28), 520 (100), 430 (78), 428 (16), 255 (20), 165 (40).
8. $\Delta\delta$ ($CDCl_3$, ppm): Both di-(*S* and *R*)-MTPA ester of **1** and **2**, H17 (−0.053), H16 (−0.015), H7 (+0.03), H18 (+0.063), H8 (+0.03), H17′ (−0.025), H16′ (−0.015), H7′ (+0.04), H18′ (+0.028), H8′ (+0.02). cf. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc*. **1973**, *95*, 512–519. Ohtani, I.; Kusumi, T.; Ishitsuka, M. O.; Kakisawa, H. *Tetrahedron Lett*. **1989**, *30*, 3147–3150.
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