Pittosporumxanthins, Cycloaddition Products of Carotenoids with α-Tocopherol from Seeds of *Pittosporum tobira*

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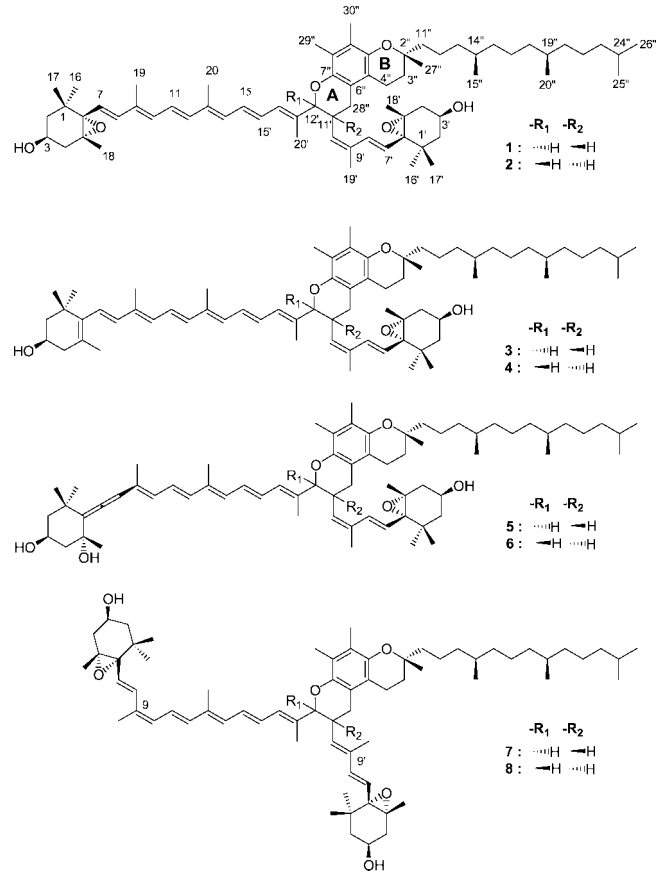
A series of carotenoid-α-tocopherol cycloaddition products, named pittosporumxanthins B1 (**3**), B2 (**4**), C1 (**5**), C2 (**6**), A3 (**7**), and A4 (**8**), were isolated from the seeds of *Pittosporum tobira*. The structures were determined to be cycloaddition products of 9′*Z*-antheraxanthin at the 11′ and 12′ positions with α-tocopherol (**3** and **4**), 9′*Z*-neoaxanthin with α-tocopherol (**5** and **6**), and 9*Z*-violaxanthin with α-tocopherol (**7** and **8**) on the basis of a detailed analyses of MS and NMR spectroscopic data. The configurations of the carotenoid end groups in the pittosporumxanthins were determined by a modified Mosher's method. Compounds **3**, **5**, and **7** were assigned (11′*R*,12′*S*) and **4**, **6**, and **8** (11′*S*,12′*R*) absolute configurations using CD measurements.

More than 700 naturally occurring carotenoids are known.[1] Some carotenoids exhibit biological effects, such as antioxidative, antitumor, anticarcinogenic, and immune-enhancing activities.[2] *Pittosporum tobira* Ait. (Pittosporaceae) is a small, slender, evergreen tree that grows on the southwestern Pacific coast of Japan. The seeds undergo a gradual change in color from green to red in late autumn to winter. The red color is due to carotenoids such as 9*Z*-violaxanthin, 9′*Z*-antheraxanthin, 9′*Z*-neoxanthin, and several unique seco-carotenoids named tobiraxanthins.[3,4] Two novel carotenoid−tocopherol complexes have been reporteed, named pittosporumxanthins A1 (**1**) and A2 (**2**),[5] which are diaistereomeric cycloaddition products of 9′*Z*-violaxanthin at the 11′ and 12′ positions with α-tocopherol having a O−C12′−C11′−C28″ linkage as shown. Further separation of the carotenoids from seeds of *P. tobira* afforded six additional carotenoid−tocopherol complexes named pittosporumxanthins B1 (**3**), B2 (**4**), C1 (**5**), C2 (**6**), A3 (**7**), and A4 (**8**). This paper reports the isolation and structural elucidation of **4**–**8**.

Results and Discussion

The red-colored seeds (20 kg) of *P. tobira* were extracted with MeOH, and the MeOH extract was saponified with 5% KOH−MeOH at room temperature. The unsaponifiable matter was subjected to column chromatography on silica gel and a series of HPLC separations on silica gel and ODS to afford **3** (3 mg), **4** (3 mg), **5** (5 mg), **6** (5 mg), **7** (3 mg), and **8** (3 mg).

Compounds **3**–**8** all were light yellow in color. The molecular formulas $C_{69}H_{104}O_5$ for **3** and **4** and $C_{69}H_{104}O_6$ for **5**−**8** were determined by high-resolution FABMS. The pittosporumxanthins all had very weak molecular ions (EIMS); however, fragment ions at *m*/*z* 584 and 430 for **3** and **4** and at *m*/*z* 600 and 430 for **5**−**8** were observed. These characteristic fragment ions were generated by apparent retro-Diels–Alder-like cleavage of the carotenoid and α-tocopherol moieties. Fragment ions at *m*/*z* 584 and 430 in **3** and **4** suggested that these compounds had antheraxanthin and α-tocopherol moieties. Collision-induced dissociation (CID) MS/MS experiments were performed in the EI mode in order to confirm



the partial structure of each compound. The CID MS/MS spectra of the fragment ion at *m*/*z* 584 in **3** and **4** showed the same product ions as that of antheraxanthin, as shown in Figure 1a,b. Furthermore, the CID MS/MS spectra of the fragment ion at *m*/*z* 430 in **3** and **4** also showed the same product ions as that of α-tocopherol (Figure 1c,d).[6] These results clearly indicated that the structures of **3** and **4** were consistent with addition products of antheraxanthin and α-tocopherol. Similarly, the presence of neoxanthin and α-tocopherol moieties in **5** and **6** and violaxanthin and α-tocopherol moieties in **7** and **8** was indicated by CID MS/MS.

The ^{1}H NMR and ^{13}C NMR spectra of **3**−**8** (in $CDCl_3$) are summarized in Tables 1 and 2, respectively. The data were assigned on the basis of the results of 2D NMR (COSY, ROESY, HSQC,

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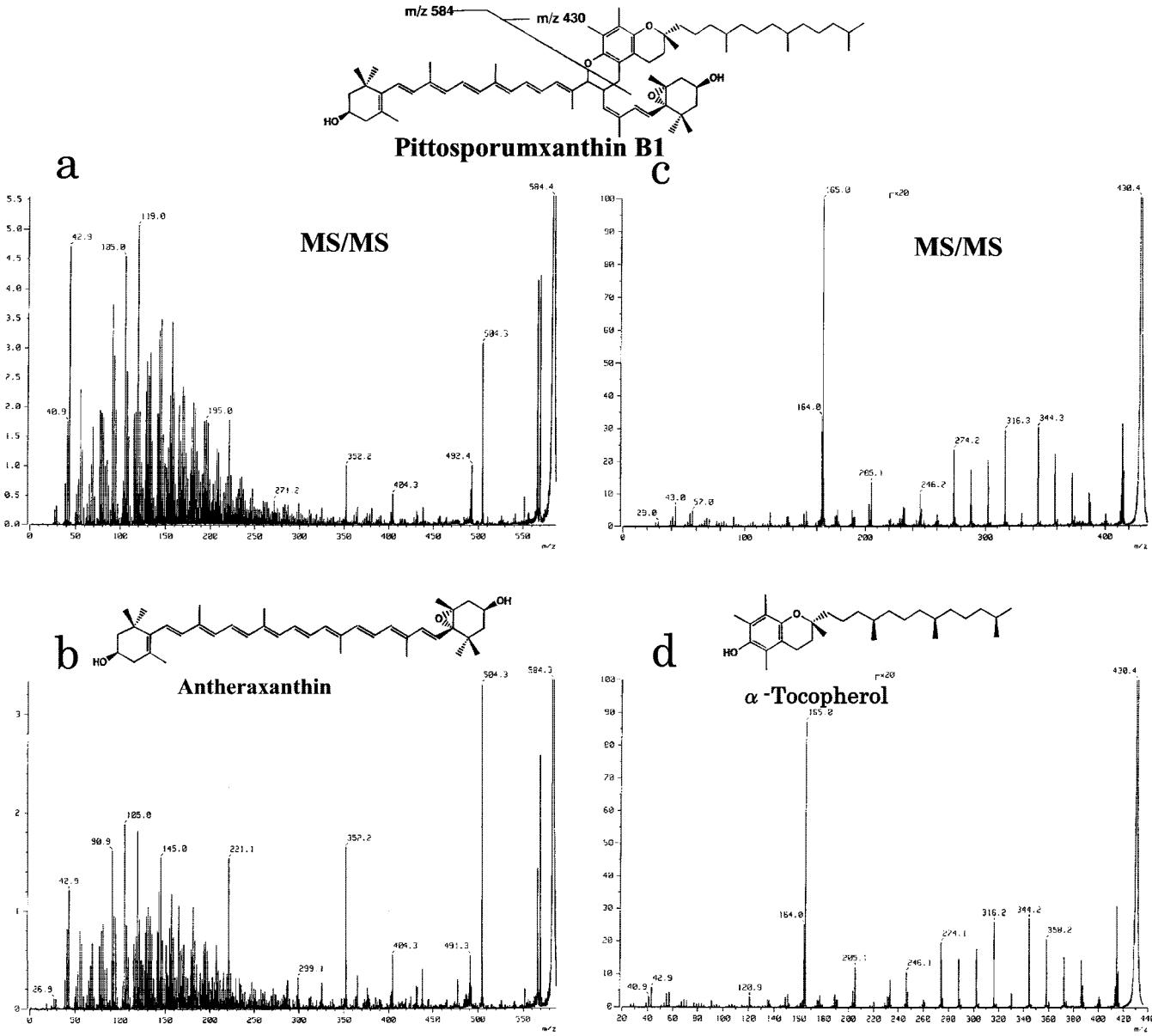

Figure 1. CID MS/MS of fragment ion at *m*/*z* 582 in **3** (a), fragment ion at *m*/*z* 430 in **3** (b), molecular ion of antheraxanthin (c), and molecular ion of α-tocopherol (d).

and HMBC) experiments and comparison with 9′*Z*-antheraxanthin,[7] 9′*Z*-neoxanthin,[7] 9*Z*-violaxanthin,[7] α-tocopherol,[8] and pittosporumxanthins A1 (**1**) and A2 (**2**).[5]

The ^{1}H and ^{13}C NMR signals of **3** and **4** were almost identical, except for the signals of H-28″, C-11″, and C-27″ as shown in Tables 1 and 2. The presence of 9′*Z*-antheraxanthin and α-tocopherol moieties in both compounds was also indicated by ^{1}H and ^{13}C NMR spectroscopy and confirmed in 2D NMR experiments. The ^{13}C NMR signals of C-11′ (δ 34.4), C-12′ (δ 84.7), and the characteristic five-spin coupling system H-10′, H-11′, H_2-28″, H-12 ($J_{H11'\text{-}H12'} = 9$ Hz, $J_{H11'\text{-}H28''} = 10.0$ and 5.5 Hz, and $J_{H10'\text{-}H11'} = 10$ Hz) revealed that the α-tocopherol moiety was substituted at the C-11′ and C-12′ positions of 9′*Z*-antheraxanthin with an O−C12′−C11′−C28″ linkage[5] as shown. Therefore, the planar structures of **3** and **4** were determined to be as indicated.

The planar structures of **5** and **6** were determined, in a similar manner, to be 11′, 12′ cycloaddition products of 9′*Z*-neoxanthin with α-tocopherol from the ^{1}H and ^{13}C NMR data, and the structures of **7** and **8** were determined to be 11′, 12′ cycloaddition products of 9*Z*-violaxanthin with α-tocopherol. The 9*Z* and 9′*E* geometry for **7** and **8** was determined from the ^{1}H chemical shift of olefinic protons[7] and ROESY correlations H-19 /H-7, H-19/H-10, H-10′/H-8′, and H-19′/H-7′ as shown in the Experimental Section.

The configuration of double bonds in the polyene chains of **3**–**8** were determined to be as shown from the ^{1}H chemical shifts, coupling constants, and ROESY correlations. ROESY correlations of H-11′/H-8′, H-11′/H-28″ (equatorial-like), H-11′/H-20′, H-12′/H-10′, H-12′/H-28″ (axial-like), and H-12′/H-14′ were observed for all pittosporumxanthins as shown in Figure 2a−d. The *trans* diaxial configuration of H-12′ and H-11′ was confirmed by the coupling constant of 9 Hz. These results indicated that the hexaene (or heptaene) chain at C-12′ and the diene chain at C-11′ were located at the equatorial position in pyran ring A, as shown in Figure 2e,f. For pittopsporumxanthin A2 (**2**),[5,9] the 1.1 ppm high-field shift of C-27″ in **4**, **6**, and **8** compared to that of **3**, **5**, and **7** indicated that the methyl group at C-27″ in **4**, **6**, and **8** was oriented more axially to the pyran ring B than that of **3**, **5**, and **7** (Figure 2e,f). Molecular modeling supported these conformations.[10]

The chirality of the carotenoid moiety in the pittosporumxanthins, (3*R*,3′*S*,5′*R*,6′*S*) for **3** and **4**, (3*S*,*R*,*R*′,*S*′,*R*′,*S*) for **5** and **6**, and (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*) for **7** and **8** was determined by a modified Mosher's method.[11,12] Although NMR and CD data could not

Table 1. ^{1}H NMR (300 MHz) Data for **3**, **4**, **5**, **6**, **7**, and **8** in $CDCl_3$[a]

position	**3**	**4**	*J* (Hz) **3** and **4**	**5**	**6**	*J* (Hz) **5** and **6**	**7**	**8**	*J* (Hz) **7** and **8**
2ax	1.48	1.48	dd (12, 12)	~1.34	~1.34	n.a.	1.26	1.26	dd (14, 13)
2eq	~1.77	1.77	n.a.	1.95	1.95	ddd (12, 3, 1.5)	1.64	1.64	ddd (14, 3.5, 1.5)
3	4.00	4.00	m	4.32	4.32	m	3.90	3.90	m
4ax	2.04	2.04	dd (17, 10)	1.41	1.41	dd (13, 12.5)	1.65	1.65	dd (14, 9)
4eq	2.39	2.39	ddd (17, 5, 1.5)	2.26	2.26	ddd (13, 4, 1.5)	2.40	2.40	ddd (14 5, 1.5)
7	6.11	6.11	d (16)				5.93	5.93	d (15.5)
8	6.13	6.13	d (16)	6.03	6.03	s	6.84	6.83	d (15.5)
10	6.15	6.15	d (11.5)	6.11	6.11	d (11.5)	6.06	6.06	d (11.5)
11	6.63	6.63	dd (15.5, 11.5)	6.57	6.57	dd (15.5, 11.5)	6.74	6.74	dd (15.5, 11.5)
12	6.35	6.35	d (15.5)	6.33	6.33	d (15.5)	6.26	6.27	d (15.5)
14	6.20	6.20	d (11)	6.20	6.20	d (11)	6.16	6.18	d (11)
15	6.56	6.56	dd (15, 11)	6.52	6.52	dd (15, 11)	6.50	6.53	dd (15, 11)
16	1.07	1.07	s	1.33	1.33	s	1.01	1.01	s
17	1.07	1.07	s	1.07	1.07	s	1.17	1.16	s
18	1.74	1.74	s	1.35	1.35	s	1.22	1.22	s
19	1.96	1.96	s	1.80	1.80	s	1.93	1.93	s
20	1.96	1.96	s	1.94	1.94	s	1.93	1.93	s
2'ax	~1.22	~1.22	n.a.	1.22	1.22	dd (14, 13)	~1.26	~1.26	dd (14, 13)
2'eq	1.61	1.61	ddd (14, 3.5 1.5)	1.61	1.61	ddd (14, 3.5 1.5)	~1.64	~1.64	n.a.
3′	3.88	3.88	m	3.88	3.88	m	3.90	3.90	m
4'ax	1.61	1.61	dd (14, 8.5)	1.61	1.61	dd (14, 8.5)	~1.64	~1.64	n.a.
4'eq	2.35	2.35	ddd (14, 5.5, 1.5)	2.35	2.35	ddd (14, 5.5, 1.5)	2.37	2.37	dd (14 5.5 1.5)
7′	5.89	5.89	d (15.5)	5.89	5.89	d (15.5)	5.73	5.72	d (15.5)
8′	6.58	6.58	d (15.5)	6.58	6.58	d (15.5)	6.17	6.16	d (15.5)
10′	5.12	5.12	d (10)	5.12	5.12	d (10)	5.24	5.24	d (10)
11′	3.13	3.13	dddd (10, 10, 9, 5.5)	3.13	3.13	dddd (10, 10, 9, 5.5)	2.95	2.94	dddd (10, 10, 9, 5.5)
12′	4.08	4.08	d (9)	4.08	4.08	d (9)	4.10	4.11	d (9)
14′	6.16	6.14	d (11)	6.16	6.14	d (11)	6.10	6.13	d (11)
15′	6.51	6.48	dd (15, 11)	6.51	6.48	dd (15, 11)	6.46	6.47	dd (15, 11)
16′	0.96	0.96	s	0.96	0.96	s	0.96	0.92	s
17′	1.12	1.13	s	1.12	1.13	s	1.12	1.09	s
18′	1.18	1.16	s	1.18	1.16	s	1.11	1.18	s
19′	1.81	1.81	d (1)	1.80	1.82	d (1)	1.75	1.74	s
20′	1.80	1.77	s	1.82	1.77	s	1.80	1.82	s
3″ax	~1.74	1.75	n.a.	1.74	1.75	m	1.75	1.75	m
3″eq	~1.74	1.75	n.a.	1.74	1.75	m	1.75	1.75	m
4″ax	2.43	2.46	dd (16.5, n.a.)	2.43	2.46	dd (16.5, n.a.)	2.47	2.45	dd (16.5, n.a.)
4″eq	2.51	2.53	dd (16.5, 10)	2.51	2.53	dd (16.5, 10)	2.57	2.54	dd (16.5, 10)
15″	0.85	0.85	d (6.5)	0.85	0.85	d (6.5)	0.85	0.85	d (6.5)
20″	0.84	0.84	d (6.5)	0.84	0.84	d (6.5)	0.84	0.84	d (6.5)
25″	0.87	0.87	d (6.5)	0.87	0.87	d (6.5)	0.87	0.87	d (6.5)
26″	0.87	0.87	d (6.5)	0.87	0.87	d (6.5)	0.87	0.87	d (6.5)
27″	1.25	1.21	s	1.25	1.21	s	1.25	1.21	s
28″ax	2.35	2.37	dd (16.5, 10)	2.35	2.37	dd (16.5, 10)	2.35	2.37	dd (16.5, 10)
28″eq	2.60	2.64	dd (16.5, 5.5)	2.60	2.64	dd (16.5, 5.5)	2.68	2.64	dd (16.5, 5.5)
29″	2.10	2.10	s	2.10	2.10	s	2.10	2.10	s
30″	2.09	2.09	s	2.09	2.09	s	2.09	2.09	s

[a] s: singlet d:doublet, m: multiplet, n.a. not assigned. Chemical shifts are indicated as δ ppm.

determine chirality of the α-tocopherol moiety, the (2″*R*,14″*R*,19″*R*) configuration of the α-tocopherol moiety for all of the pittosporumxanthins was tentatively postulated on the basis of biosynthetic considerations.[13] The chirality of the remaining asymmetric carbons (C-11′ and C-12′) was determined using CD data. Mirror image CD spectra were observed between pittosporumxanthins A1 (**1**) and A2 (**2**), between B1 (**3**) and B2 (**4**), between C1 (**5**) and C2 (**6**), and between A3 (**7**) and A4 (**8**), as shown in Figure 3. Pittosporumxanthins A1, B1, and C1 showed a positive Cotton effect in the hexaene (or heptaene) absorption band around 370 nm and a negative Cotton effect in the diene absorption band around 240 nm. On the other hand, pittosporumxanthins A2, B2, and C2 showed a negative Cotton effect in the hexaene (or heptaene) absorption band and a positive Cotton effect in the diene absorption band, as shown in Figure 3a−c. These CD spectra were assumed to originate from exciton coupling of $\pi\rightarrow\pi^*$ absorption of hexaene (or heptaene) and diene. From the exciton chirality rule of the CD spectrum,[14] it was deduced that the relation between hexane (or heptaene) and diene in compounds **1**, **3**, and **5** was positive chirality and that of compounds **2**, **4**, and **6** was negative chirality. Thus, the (11′*R*,12′*S*) configuration for **1**, **3**, and **5**, and the (11′*S*,12′*R*) configuration for **2**, **4**, and **6** were postulated. Compounds **7** and **8** showed positive and negative Cotton effects in the hexane absorption band, respectively (Figure 3d). However, these compounds showed more complex Cotton effects around 200–300 nm compared with those of **1** and **2** (Figure 3c) in the CD spectra. This might reflect 9*Z* geometry in the hexaene chain. Therefore, the (11′*R*,12′*S*) configuration for **7** and the (11′*S*,12′*R*) configuration for **8** were assigned from the sign of the first Cotton effect around 370 nm.

Pittosporumxanthins **1**−**8** are the first examples of carotenoid−tocopherol complexes to be found in nature.

Experimental Section

General Experimental Procedures. The UV–vis spectra were recorded with a Shimadzu UV-240 spectrophotometer in Et_2O. MS and MS/MS spectra were recorded using a JEOL JMS-HX/HX 110A mass spectrometer. The positive ions produced in the source were accelerated at 10 kV. In the FAB ionization, *m*-nitrobenzyl alcohol was used as a matrix. CID MS/MS were measured in the EI mode. The fragment ions at *m*/*z* 430 and 586 for **3** and **4** and at *m*/*z* 430 and 600 for **5**, **6**, **7**, and **8** in the EIMS were selected and collided with argon in a collision cell located in the third field-free region. Argon gas pressure was adjusted to attenuate the intensity of the precursor ion by 30%. The collision cell potential was 3 kV. The resulting product ions were acquired by accumulating several linked scans on MS2. NMR spectra were recorded with a Varian XL-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) or a Varian UNITY INOVA 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz)

Table 2. ^{13}C NMR (75 MHz) Data of **3**, **4**, **5**, **6**, **7**, and **8** in $CDCl_3$[a]

position	3	4	5	6	7	8
1	37.1	37.1	35.8	35.8	35.3	35.3
2	48.4	48.4	49.5	49.5	47.2	47.2
3	65.1	65.1	64.3	64.3	64.3	64.3
4	42.6	42.6	48.9	48.9	41.0	41.0
5	126.1	126.1	73.0	73.0	66.8	66.8
6	137.8	137.8	117.7	117.7	70.1	70.1
7	125.5	125.5	202.2	202.2	125.9	125.9
8	138.5	138.5	103.2	103.2	128.9	128.9
9	135.5	135.5	131.7	131.7	132.3	132.3
10	132.2	132.2	128.4	128.4	130.7	130.7
11	124.7	124.7	124.6	124.6	123.4	123.4
12	137.6	137.6	137.3	137.3	137.5	137.5
13	136.1	136.1	135.9	135.9	135.6	135.6
14	131.2	131.2	132.1	132.1	132.6	132.6
15	129.5	129.5	129.2	129.2	129.7	129.7
16	28.7	28.7	31.4	31.4	24.9	24.9
17	30.3	30.3	32.2	32.2	29.6	29.6
18	21.6	21.6	29.4	29.4	20.0	20.0
19	12.8	12.8	13.9	13.9	21.1	21.1
20	12.8	12.8	12.8	12.8	13.0	13.0
1′	35.2	35.2	35.2	35.2	35.2	35.2
2′	47.2	47.2	47.2	47.2	47.2	47.2
3′	64.3	64.3	64.3	64.3	64.3	64.3
4′	40.9	41.0	40.9	41.0	41.0	41.0
5′	66.5	66.8	66.5	66.8	67.0	67.0
6′	70.1	70.1	70.1	70.1	70.5	70.5
7′	126.1	126.0	126.1	126.0	123.7	123.8
8′	129.8	129.7	129.8	129.7	137.0	137.0
9′	132.0	132.0	132.0	132.0	133.6	133.6
10′	130.4	130.4	130.4	130.4	132.2	132.2
11′	34.4	34.4	34.4	34.4	35.8	35.8
12′	84.6	84.7	84.6	84.7	84.3	84.4
13′	137.5	137.4	137.5	137.4	137.1	137.1
14′	128.9	128.9	128.9	128.9	128.7	128.9
15′	129.5	129.5	129.5	129.5	129.7	129.7
16′	24.8	24.9	24.8	24.9	25.0	25.8
17′	29.6	29.6	29.6	29.6	29.6	29.6
18′	20.1	20.0	20.1	20.0	20.0	20.0
19′	20.8	20.8	20.8	20.8	13.0	13.0
20′	12.5	12.4	12.5	12.4	12.6	12.6
2″	74.5	74.5	74.5	74.5	74.6	74.5
3″	31.5	31.5	31.5	31.5	31.4	31.2
4″	19.7	19.7	19.7	19.7	19.7	19.8
5″	115.7	115.7	115.7	115.7	115.7	115.7
6″	144.9	145.0	144.9	145.0	144.9	145.0
7″	123.6	123.7	123.6	123.7	122.7	122.6
8″	122.8	122.9	122.8	122.9	123.0	122.9
9″	115.7	115.7	115.7	115.7	115.7	115.7
10″	145.5	145.5	145.5	145.5	145.5	145.5
11″	39.0	40.7	39.0	40.7	39.1	40.6
12″	21.0	21.0	21.0	21.0	21.0	21.0
13″	37.5	37.5	37.5	37.5	37.5	37.5
14″	32.7	32.7	32.7	32.7	32.7	32.7
15″	19.6	19.6	19.6	19.6	19.7	19.7
16″	37.5	37.5	37.5	37.5	37.5	37.5
17″	24.5	24.5	24.5	24.5	24.5	24.5
18″	37.5	37.5	37.5	37.5	37.5	37.5
19″	32.8	32.8	32.8	32.8	32.8	32.8
20″	19.8	19.8	19.8	19.8	19.8	19.8
21″	37.3	37.3	37.3	37.3	37.3	37.3
22″	24.8	24.8	24.8	24.8	24.8	24.8
23″	39.4	39.4	39.4	39.4	39.4	39.4
24″	28.0	28.0	28.0	28.0	28.0	28.0
25″	22.7	22.7	22.7	22.7	22.7	22.7
26″	22.6	22.6	22.6	22.6	22.6	22.6
27″	24.4	23.3	24.4	23.3	24.4	23.4
28″	30.2	30.1	30.2	30.1	29.7	29.8
29″	11.7	11.7	11.7	11.7	11.7	11.7
30″	11.8	11.8	11.8	11.8	11.8	11.8

[a] Chemical shifts are indicated as δ ppm.

spectrometer in $CDCl_3$ with TMS as an internal standard. CD spectra were recorded in Et_2O at room temperature with a Jasco J-500C spectropolarimeter. HPLC was performed on a Shimadzu LC-6AD with a Shimadzu SPD-6AV spectrophotometer set at 450 nm.

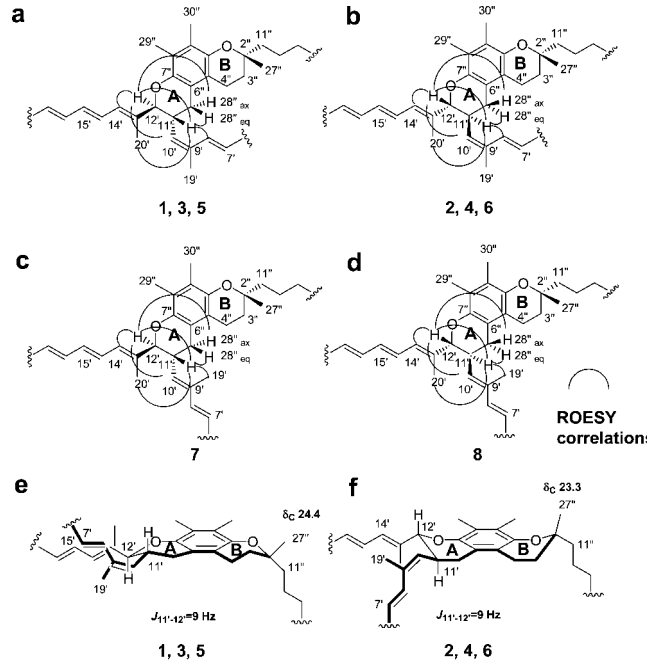

Figure 2. Partial structures and ROESY data of pittosporumxanthins (a, b, c, d) and relative stereochemistry of pyran rings A and B (e, f).

Plant Material. Seeds of *P. tobira* were collected in December 2000–2004 from plants growing on the bank of the Kamogawa River in Kyoto. Voucher specimens (RIPD-5) have been deposited at the Research Institute for Production Development.

Extraction and Isolation of Carotenoids. The seeds (20 kg) were washed with *n*-hexane to remove viscous matter on the surface and then extracted with MeOH. The MeOH extract was transferred to Et_2O−*n*-hexane (1:1) by the addition of water. The organic layer was washed with water and then evaporated under reduced pressure. The residual red-colored oil was saponified with 5% KOH−MeOH at room temperature for 12 h. Then unsaponifiable matter was extracted with Et_2O−*n*-hexane (1:1) and washed with water. After drying with anhydrous Na_2SO_4, the solution was evaporated and the residue was chromatographed on silica gel with *n*-hexane, Et_2O−*n*-hexane (1:1), Et_2O, Et_2O−Me_2CO (1:1), Me_2CO, and MeOH, successively. The fraction eluted with *n*-hexane contained β-carotene and wax. The fraction eluted with Et_2O−*n*-hexane (1:1) was subjected to HPLC on silica gel with Me_2CO−*n*-hexane (2:8) and followed by ODS with CH_2Cl_2−MeCN (2:8) to afford α-tocopherol. The fraction eluted with Et_2O was subjected to HPLC on silica gel with Me_2CO−*n*-hexane (2:8) followed by ODS with CH_2Cl_2−MeCN (2:8) to afford antheraxanthin (10 mg), 9′*Z*-antheraxanthin (15 mg), **3** (3 mg), **4** (3 mg), mutatoxanthin (5 mg), 9*Z*-violaxanthin (30 mg), violaxanthin (10 mg), luteoxanthin (20 mg), auroxanthin (20 mg), **1** (20 mg), **2** (20 mg), **7** (3 mg), and **8** (3 mg). The fraction eluted with Et_2O−Me_2CO (1:1) was subjected to HPLC on silica gel with Me_2CO−*n*-hexane (3:7) followed by ODS with CH_2Cl_2−MeCN (2:8) to afford 9′*Z*-neoxanthin (20 mg), 9′*Z*-neochrome (20 mg), **5** (5 mg), and **6** (5 mg).

Pittosporumxanthin B1 (3): yellow solid; UV–vis in Et_2O, λ_{max} nm (ϵ) 379 (120 000), 397 (115 000); CD λ, ($\Delta\epsilon$) in Et_2O, 209 (+23), 216 (0), 242 (−76), 267 (0), 280 (+25), 372 (+16), 430 (0); ^1H NMR (300 MHz $CDCl_3$), Table 1; ^{13}C NMR (75 MHz $CDCl_3$), Table 2; ROESY correlations H-16/H-3 and H-7, H-17/H-7, H-19/H-7 and H-11, H-20/H-11 and H-15, H-20′/H-15′, H-19′/H-7′ and H-10′; EIMS (probe) 70 eV, *m/z* (rel int) *m/z* 1012 $[M]^+$ (3), 584 (50), 520 (47), 430 (100), 428 (22), 165 (58); CID MS/MS of fragment ions at *m/z* 584 and 430, Figure 1; HRFABMS calc for $C_{69}H_{105}O_5$ ($M + H^+$) 1013.7962, found 1013.7974.

Pittosporumxanthin B2 (4): yellow solid; UV–vis in Et_2O, λ_{max} nm (ϵ) 379 (120 000), 397 (115 000); CD λ, ($\Delta\epsilon$) in Et_2O, 206 (−42), 217 (0), 238 (+93), 261 (0), 277 (−46), 375 (−15), 430 (0); EIMS (probe) 70 eV, *m/z* (rel int), 1012 $[M]^+$ (2.3), 760 (35), 744 (60), 584

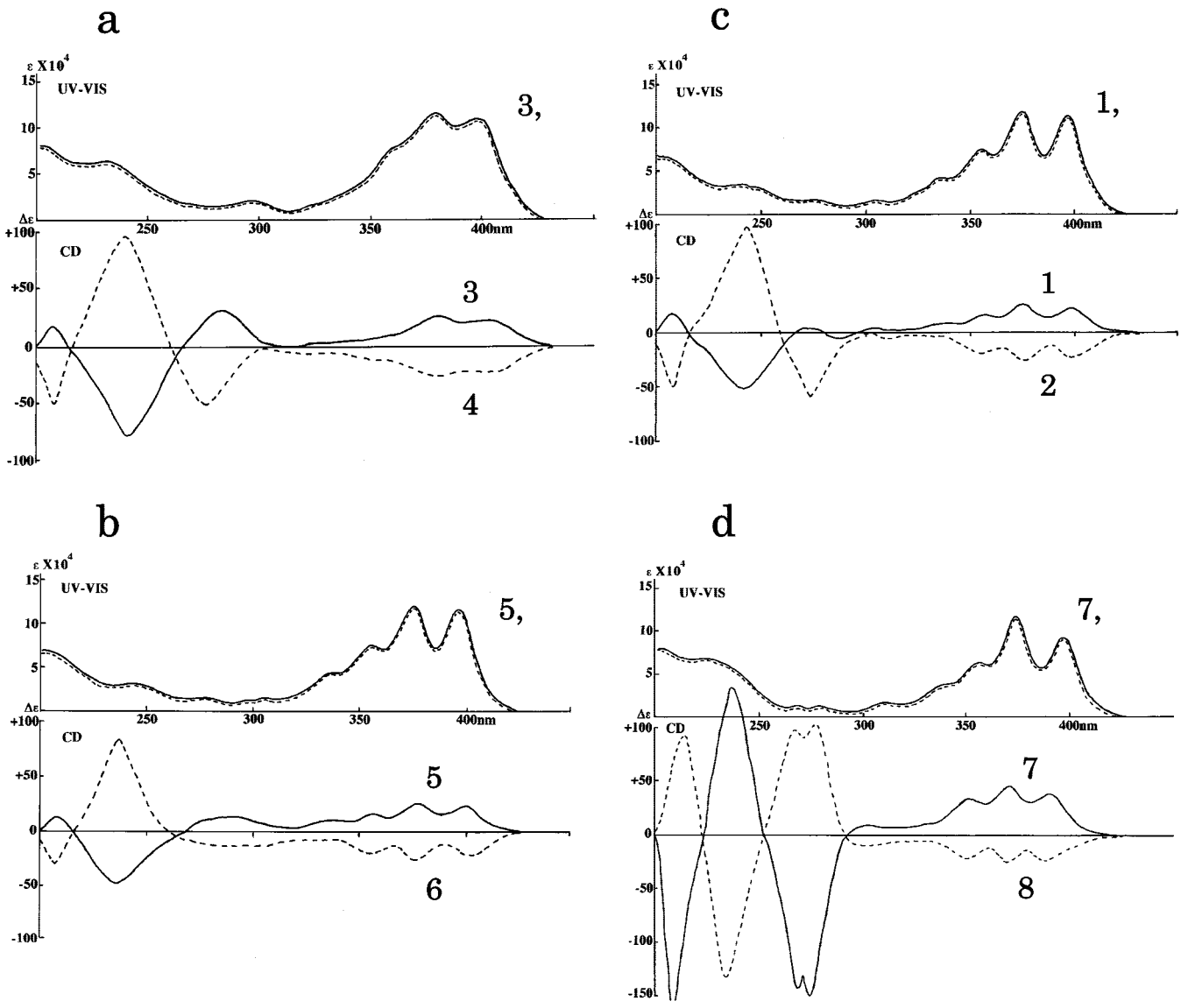

Figure 3. CD spectra of pittosporumxanthins.

(25), 520 (35), 430 (100), 428 (75), 165 (80); ^{1}H NMR (300 MHz $CDCl_3$), Table 1; ^{13}C NMR (75 MHz $CDCl_3$), Table 2; ROESY correlations H-16/H-3 and H-7, H-17/H-7, H-19/H-7 and H-11, H-20/H-11 and H-15, H-20′/H-15′, H-19′/H-7′ and H-10′; CID MS/MS of fragment ions at *m/z* 584 and 430 were the same as those of **3** (Figure 1); HRFABMS calc for $C_{69}H_{105}O_5$ (M + H$^+$) 1013.7962, found 1013.7917.

Pittosporumxanthin C1 (5): yellow solid; UV–vis in Et_2O, λ_{max} nm (ϵ) 356 (75 000), 374 (115 000), 397 (110 000); CD λ, ($\Delta\epsilon$) in Et_2O, 207 (+15), 214 (0), 235 (−50), 274 (0), 295 (+8), 325 (+4), 356 (+13), 376 (+20), 398 (+18), 410 (0); EIMS (probe) 70 eV, *m/z* (rel int) *m/z* 1028 [M]$^+$ (3), 600 (15), 520 (30), 430 (100), 165 (70); ^{1}H NMR (300 MHz $CDCl_3$), Table 1; ^{13}C NMR (75 MHz $CDCl_3$), Table 2; ROESY correlations H-16/H-3, H-19/H-8 and H-11, H-20/H-11 and H-15, H-20′/H-15′, H-19′/H-7′ and H-10′; CID MS/MS of fragment ion at *m/z* 600, *m/z* 582, 520, 433, 352, 287, 221, 167, 104, 91, 43; this spectrum was the same as that of neoxanthin; CID MS/MS of fragment ion at *m/z* 430 was the same as that of **3** (Figure 2c); HRFABMS calc for $C_{69}H_{105}O_6$ (M + H$^+$) 1029.7911, found 1029.7889.

Pittosporumxanthin C2 (6): yellow solid; UV–vis in Et_2O, λ_{max} nm (ϵ) 356 (75 000), 374 (115 000), 397 (110 000); CD λ, ($\Delta\epsilon$) in Et_2O, 206 (−33), 215 (0), 242 (+83), 272 (0), 290 (−12), 322 (−10), 356 (−23), 376 (−34), 394 (−30), 410 (0); EIMS (probe) 70 eV, *m/z* (rel int) *m/z* 1028 [M]$^+$ (5), 600 (13), 520 (28), 430 (100); 165 (75); ^{1}H NMR (300 MHz $CDCl_3$), Table 1; ^{13}C NMR (75 MHz $CDCl_3$), Table 2; ROESY correlations H-16/H-3, H-19/H-8 and H-11, H-20/H-11 and H-15, H-20′/H-15′, H-19′/H-7′ and H-10′; CID MS/MS of fragment ions at *m/z* 600 and 430 were the same as those of **5**; HRFABMS calc for $C_{69}H_{105}O_6$ (M + H$^+$) 1029.7911, found 1029.7828.

Pittosporumxanthin A3 (7): yellow solid; UV–vis in Et_2O, λ_{max} nm (ϵ) 354 (61 000), 372 (110 000), 394 (92 000); CD λ, ($\Delta\epsilon$) in Et_2O, 219 (−163), 227 (0), 236 (+138), 254 (0), 267 (−145), 278 (−145), 288 (0), 351 (+35), 370 (+45), 389 (+40), 410 (0); EIMS (probe) 70 eV, *m/z* (rel int) 1028 [M]$^+$ (3), 600 (18), 520 (100), 430 (90), 165 (40); ^{1}H NMR (300 MHz $CDCl_3$), Table 1; ^{13}C NMR (75 MHz $CDCl_3$), Table 2; ROESY correlations H-19/H-7 and H-10, H-20/H-11 and H-15, H-20′/H-15′, H-19′/H-7′, H-8′/H-10′; CID MS/MS of fragment ion at *m/z* 600, *m/z* 582, 520, 508, 454, 419, 352, 287, 221, 165, 119, 105, 91, 43; this spectrum was the same as that of violaxanthin; CID MS/MS of fragment ion at *m/z* 430 was the same as that of **3** (Figure 3c); HRFABMS calc for $C_{69}H_{105}O_6$ (M + H$^+$): 1029.7911, found 1029.7919.

Pittosporumxanthin A4 (8): yellow solid; UV–vis in Et_2O, λ_{max} nm (ϵ) 354 (61 000), 372 (110 000), 394 (92 000); CD λ, ($\Delta\epsilon$) in Et_2O, 215 (+92), 223 (0), 235 (−135), 254 (0), 267 (+98), 276 (+103), 285 (0), 351 (−12), 370 (−16), 289 (−14), 410 (0); EIMS (probe) 70 eV, *m/z* (rel int), 1028 [M]$^+$ (6), 600 (28), 520 (100), 430 (80), 165 (40); ^{1}H NMR (300 MHz $CDCl_3$), Table 1; ^{13}C NMR (75 MHz $CDCl_3$), Table 2; ROESY correlations H-19/H-7 and H-10, H-20/H-11 and H-15, H-20′/H-15′, H-19′/H-7′, H-8′/H-10′; CID MS/MS of fragment ions at *m/z* 600 and 430 were the same as those of **7**; HRFABMS calc for $C_{69}H_{105}O_6$ (M + H$^+$) 1029.7911, found 1029.7919.

Preparation of MTPA Esters of Pittosporumxanthins. Preparation of MTPA esters of pittosporumxanthins were according to the method described previously.[15] A solution of (+)-MTPA [α-methoxy-α-

(trifluoromethyl)phenylacetyl] chloride (20 mg) in anhydrous pyridine (2 mL) was added to a solution of pittosporumxanthin (0.5 mg) in pyridine (1 mL) at 0 °C. After 60 min, MTPA ester was extracted from the reaction mixture with *n*-hexane by addition of water, subjected to preparative TLC on silica gel G with acetone−hexane (3:7), and purified by HPLC on silica gel with acetone−hexane (3:7). The chirality of the carotenoid end group in the pittosporumxanthins was determined by $\Delta\delta$ values ($\Delta\delta = \delta S - \delta R$ in ppm) obtained from corresponding *S* and *R* MTPA esters in $CDCl_3$. The $\Delta\delta$ values were as follows: both **3** and **4**, H-16 (−0.03), H-17 (−0.01), H-18 (+0.03), H-7 (+0.02), H-8 (+0.03), H-16′ (−0.02), H-17′ (−0.03), H-18′ (+0.03), H-7′ (+0.04), H-8′ (+0.02); both **5** and **6**, H-16 (−0.03), H-17 (−0.01), H-4eq (+0.07), H-18 (+0.03), H-17′ (−0.03), H-16′ (−0.02), H-18′ (+0.03); both **7** and **8**, H-16 (−0.02), H-17 (−0.05), H-7 (−0.08), H-18 (+0.06), H-16′ (−0.02), H-17′ (−0.03), H-18′ (+0.03), H-8′ (+0.02).

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(6) In CID-MS/MS spectra, the characteristic fragment ions resulting from the loss of C_nH_{2n+2} at the alkyl terminal of the molecular ion of α-tocopherol and fragment ion at *m/z* 430 in the pittosporumxanthins produced in the source on MS1 were observed. These ions clearly revealed the structure of the isoprenoid chain of the α-tocopherol moiety in **1**–**8**.

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(9) The 1.1 ppm high-field shift at C-27″ (δ 24.4 for **1** and δ 23.3 for **2**) and 1.7 ppm low-field shift at C-11″ (δ 39.0 for **1** and δ 40.7 for **2** at C-11″) in **2** indicated that the methyl group at C-27″ in **2** is oriented more axially to the pyran ring B than **1** (see ref 5).

(10) Optimization of the conformations of pittosporumxanthins was carried out using molecular dynamics calculation software *NMR graf* Ver3.0 (Molecular Simulations, Inc., Waltham, MA). The calculation results showed that the hexaene (or heptaene) chain at C-11′ and diene chain at C-12′ were equatorial and that the dihedral angles between H-11′ and H-12′ were about 170° in **1**, **3**, **5**, and **7** and about 180° in **2**, **4**, **6**, and **8**. The conformation of the pyran ring B was shown to be a half-chair for **1**, **3**, **5**, and **7** and to be a half-boat for **2**, **4**, **6**, and **8**, and the methyl group C-27″ was oriented more axially in **2**, **4**, **6**, and **8** than in **1**, **3**, **5**, and **7**.

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(13) The configuration of naturally occurring α-tocopherol is (2*R*,4′*R*,8′*R*).

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