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PHYTOCHEMISTRY

Phytochemistry 69 (2008) 1234–1241

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Geranylated flavanones from the secretion on the surface of the immature fruits of *Paulownia tomentosa*

Teigo Asai[a], Noriyuki Hara[a], Sawa Kobayashi[b], Shiro Kohshima[b], Yoshinori Fujimoto[a,*]

[a] *Department of Chemistry and Materials Science, Graduate School of Science and Engineering,*
Tokyo Institute of Technology, Meguro, Tokyo 152-8551, Japan
[b] *Department of Biological Sciences, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Meguro, Tokyo 152-8551, Japan*

Received 22 June 2007; received in revised form 12 October 2007

Abstract

Chemical investigation of the methanol extract of the viscous secretion on the surface of immature fruits of *Paulownia tomentosa* furnished nine geranylated flavanones, 6-geranyl-5,7-dihydroxy-3′,4′-dimethoxyflavanone (**1**), 6-geranyl-3′,5,7-trihydroxy-4′-methoxyflavanone (**2**), 6-geranyl-4′,5,7-trihydroxy-3′,5′-dimethoxyflavanone (**3**), 6-geranyl-4′,5,5′,7-tetrahydroxy-3′-methoxyflavanone (**4**), 6-geranyl-3,3′,5,7-tetrahydroxy-4′-methoxyflavanone (**5**), 4′,5,5′,7-tetrahydroxy-6-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]-3′-methoxyflavanone (**6**), 3,3′,4′,5,7-pentahydroxy-6-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]flavanone (**7**), 3,3′,4′,5,7-pentahydroxy-6-[7-hydroxy-3,7-dimethyl-2(*E*)-octenyl]flavanone (**8**), and 3,4′,5,5′,7-pentahydroxy-3′-methoxy-6-(3-methyl-2-butenyl)flavanone (**9**), along with six known geranylated flavanones. Among these, compounds **4**, **6**–**9** and the known 6-geranyl-3′,4′,5,7-tetraahydroxyflavanone (diplacone), 6-geranyl-3,3′,4′,5,7-pentahydroxyflavanone (diplacol) and 3′,4′,5,7-pentahydroxy-6-[7-hydroxy-3,7-dimethyl-2(*E*)-octenyl]flavanone showed potent radical scavenging effects towards DPPH radicals.

Keywords: *Paulownia tomentosa*; Scrophulariaceae; Flavanone; Flavanol; DPPH radical; Anti-oxidant activity

1. Introduction

Paulownia tomentosa Steud. (Scrophulariaceae) is an ornamental tree widely distributed throughout China, Korea and Japan. Its stem bark has been used in Chinese herbal medicine as a component of remedies for infectious diseases such as gonorrhea and erysipelas (Kang et al., 1999). Earlier studies of *P. tomentosa* led to the isolation of iridoids (Plouvier, 1971; Adriani et al., 1981; Damtoft and Jensen, 1993) and lignans (Ina et al., 1987; Takahashi and Nakagawa, 1966). Recently, diplacone, a geranylated flavanone, has been reported from the flower (Du et al., 2004).

Plants often have unique small structures on their surface. However, in most cases, functions of these structures have remained unclear. Systematic chemical analysis of the constituents contained in the small structures would be helpful in understanding their possible ecological functions. The surface of immature fruits of *P. tomentosa* is coated with viscous materials, at least part of which are likely to be secreted from the glandular trichomes, a fine structure located on the surface of immature fruits. This paper describes the isolation and structure elucidation of nine new flavanones **1**–**9** from this secretion, together with six known flavanones **10**–**15**. Anti-oxidant activities of the isolated flavanones were tested using the diphenylpicrylhydrazyl (DPPH) assay.

2. Results and discussion

The viscous oily material on the surface of fresh immature fruits of *P. tomentosa* was dissolved in MeOH by

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[*] Corresponding author. Tel./fax: +81 357342241.
E-mail address: fujimoto.y.aa@m.titech.ac.jp (Y. Fujimoto).

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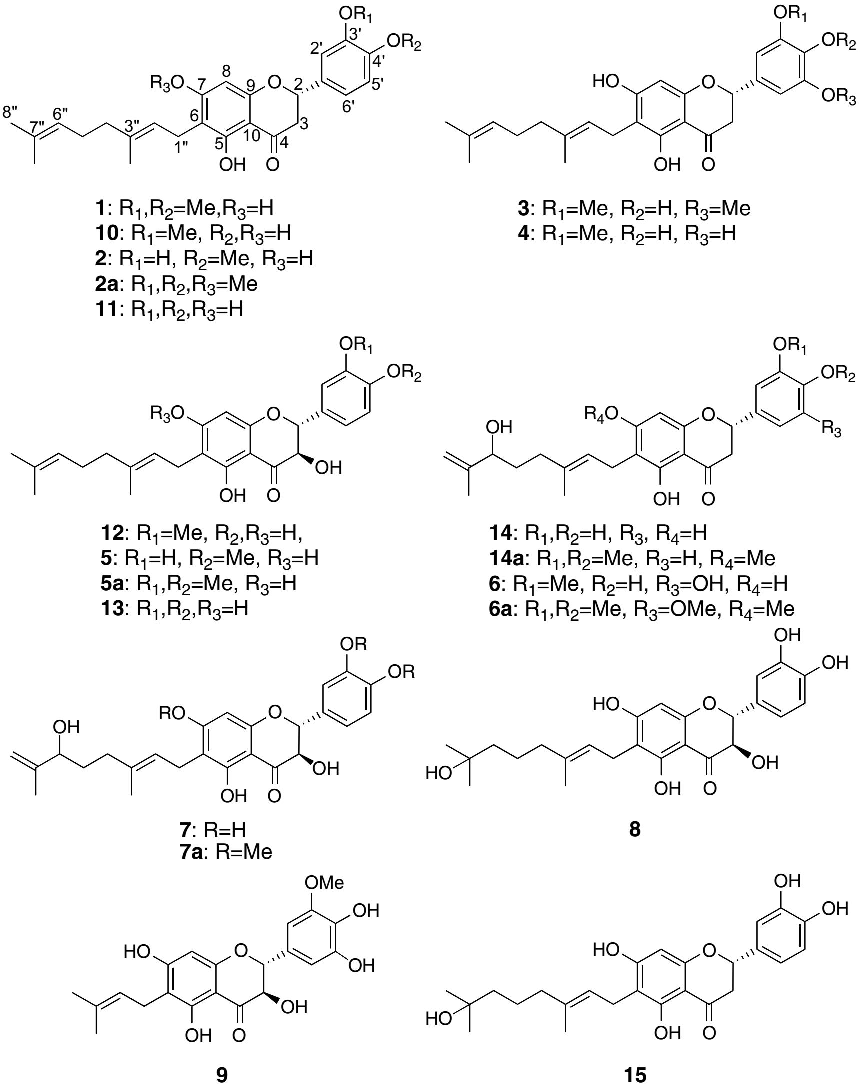
doi:10.1016/j.phytochem.2007.11.011

briefly dipping the fruits in the solvent. The concentrated MeOH extract was separated on silica gel, Sephadex LH-20 and reversed-phase HPLC to give the new flavanones **1**–**9** (Fig. 1), together with the known flavanones **10**–**15**. These compounds were all found to bear a C_{10} substituent (geranyl or its metabolite) at the C-6 position, except for compound **9** which has a prenyl group at C-6.

Compound **1** was assigned the molecular formula $C_{27}H_{32}O_6$ on the basis of HREIMS data. The ^1H NMR spectrum of compound **1** showed three protons at δ 2.80 (*dd*, $J = 17.3$, 2.9 Hz), 3.10 (*dd*, $J = 17.3$, 13.1 Hz) and 5.34 (*dd*, $J = 13.1$, 2.9 Hz), typically assignable to H_2-3 and H-2 of a flavanone skeleton. In addition, signals for three protons at δ 6.99 (*d*, $J = 1.9$ Hz), 6.96 (*dd*, 8.2, 1.9 Hz), 6.89 (*d*, $J = 8.2$ Hz) assignable to the aromatic protons of a 3,4-disubstituted B-ring, resonances for protons of a geranyl group, a downfield proton at δ 12.41 assignable to C-5-OH chelated to C-4 carbonyl, and an additional aromatic proton at δ 6.01 (*s*) were observed (Table 1). Furthermore, two methoxy signals appeared at δ 3.92 and 3.90. These spectroscopic data suggested that compound **1** was a 5-hydroxyflavanone substituted with a geranyl group, two methoxy groups and an additional hydroxy group. The substitution pattern was unambiguously established by HMBC correlations (Fig. 2) which gave evidence for the 3′,4′-dimethoxy B-ring and the 6-geranyl-5,7-dihydroxy A-ring structures. This substitution pattern was also consistent with the EIMS data, which exhibited ions at *m*/*z* 288 and 164 from a retro-Diels-Alder fragmentation. Hence, the structure of **1** was determined to be 6-geranyl-5,7-dihydroxy-3′,4′-dimethoxyflavanone. Complete assignments of ^1H and ^{13}C signals of **1**, obtained by 2D NMR studies (H-H COSY, HMQC, HMBC) are listed in Tables 1 and 2. The absolute stereochemistry at C-2 of **1** was elucidated as *S* on the basis of the CD data (a positive absorption at 328 nm and a negative absorption at 288 nm) (Gaffield, 1970). The C-2 configurations of the other flavavones **2**, **3**, **4** and **6** were studied in the same manner and established to be *S*.



1: R_1,R_2=Me,R_3=H
10: R_1=Me, R_2,R_3=H
2: R_1=H, R_2=Me, R_3=H
2a: R_1,R_2,R_3=Me
11: R_1,R_2,R_3=H

3: R_1=Me, R_2=H, R_3=Me
4: R_1=Me, R_2=H, R_3=H

12: R_1=Me, R_2,R_3=H,
5: R_1=H, R_2=Me, R_3=H
5a: R_1,R_2=Me, R_3=H
13: R_1,R_2,R_3=H

14: R_1,R_2=H, R_3, R_4=H
14a: R_1,R_2=Me, R_3=H, R_4=Me
6: R_1=Me, R_2=H, R_3=OH, R_4=H
6a: R_1,R_2=Me, R_3=OMe, R_4=Me

7: R=H
7a: R=Me

8

9

15

Fig. 1. Chemical structures of flavonoids **1**–**15**.

Table 1
^{1}H NMR data (500 MHz) of compounds **1**–**9**[a]

No.	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[c]	**7**[c]	**8**[c]	**9**[c]
2	5.34 (*dd*, 13.1, 2.9)	5.30 (*dd*, 13.2, 2.9)	5.29 (*dd*, 13.0, 2.9)	5.25 (*dd*, 13.0, 3.0)	4.95 (*d*, 12.0)	5.23 (*dd*, 13.0, 2.9)	4.91 (*d*, 12.0)	4.89 (*d*, 12.0)	4.89 (*d*, 12.0)
3	3.10 (*dd*, 17.3, 13.1) 2.80 (*dd*, 17.3, 2.9)	3.05 (*dd*, 17.4, 13.0) 2.76 (*dd*, 17.4, 3.2)	3.08 (*dd*, 17.2, 13.0) 2.78 (*dd*, 17.2, 2.9)	3.04 (*dd*, 17.4, 13.0) 2.76 (*dd*, 17.4, 2.9)	4.52 (*brd*, 12.0)	3.04 (*dd*, 17.4, 13.0) 2.73 (*dd*, 17.3, 2.9)	4.50 (*d*, 12.0)	4.48 (*d*, 12.0)	4.50 (*d*, 12.0)
8	6.01 (*s*)	5.99 (*s*)	6.01 (*s*)	6.00 (*s*)	5.97 (*s*)	5.97 (*s*)	5.96 (*s*)	5.95 (*s*)	5.97 (*s*)
2′	6.99 (*d*, 1.9)	7.13 (*d*, 1.9)	6.68 (*brs*)	6.56 (*d*, 1.7)	7.13 (*d*, 1.9)	6.54 (*brs*)	7.00 (*d*, 1.7)	6.99 (*d*, 1.7)	6.64 (*d*, 1.7)
5′	6.89 (*d*, 8.2)	6.88 (*d*, 8.1)			6.89 (*d*, 8.2)		6.89 (*d*, 8.0)	6.87 (*d*, 8.2)	
6′	6.96 (*dd*, 8.2, 1.9)	7.02 (*dd*, 8.1, 1.9)	6.68 (*brs*)	6.67 (*d*, 1.7)	7.01 (*dd*, 8.2, 1.9)	6.66 (*brs*)	6.92 (*dd*, 8.0, 1.7)	6.91 (*dd*, 8.2, 1.7)	6.74 (*d*, 1.7)
1″	3.37 (*d*, 7.1)	3.37 (*d*, 7.1)	3.36 (*d*, 7.1)	3.36 (*d*, 7.4)	3.37 (*d*, 7.1)	3.28 (*d*, 7.1)	3.29 (*d*, 6.8)	3.28 (*d*, 7.1)	3.29 (*d*, 6.8)
2″	5.26 (*brt*, 7.2)	5.26 (*brt*, 7.2)	5.26 (*brt*, 7.2)	5.25 (*brt*, 7.3)	5.26 (*brt*, 7.2)	5.29 (*brt*, 7.1)	5.27 (*brt*, 6.8)	5.20 (*brt*, 7.1)	5.23 (*brt*, 7.0)
3″-Me	1.81 (*s*)	1.80 (*s*)	1.80 (*s*)	1.80 (*s*)	1.81 (*s*)	1.79 (*s*)	1.79 (*s*)	1.76 (*s*)	1.70 (*s*)
4″	2.07 (*m*)	2.06 (*m*)	2.06 (*m*)	2.07 (*m*)	2.07 (*m*)	2.05 (*brt*, 7.3)	2.04 (*m*)	1.98 (*m*)	1.79 (*s*)
5″	2.09 (*m*)	2.08 (*m*)	2.09 (*m*)	2.08 (*m*)	2.09 (*m*)	1.65 (*m*)	1.65 (*m*)	1.41 (*m*)	
6″	5.05 (*brt*, 6.7)	5.05 (*brt*, 6.7)	5.05 (*brt*, 6.7)	5.05 (*brt*, 6.8)	5.06 (*brt*, 6.7)	4.01 (*t*, 6.1)	4.01 (*t*, 6.1)	1.62 (*m*)	
7″-Me	1.59 (*brs*)	1.59 (*s*)	1.59 (*s*)	1.59 (*s*)	1.59 (*s*)	1.70 (*s*)	1.70 (*s*)	1.18 (*s*)	
8″	1.67 (*brs*)	1.67 (*s*)	1.67 (*s*)	1.67 (*s*)	1.67 (*s*)	4.88, 4.80 (*brs*)	4.88, 4.81 (*brs*)	1.18 (*s*)	
OMe	3.92 (*s*) 3.90 (*s*)	3.91 (*s*)	3.91 (*s*×2)	3.89 (*s*)	3.91 (*s*)	3.89 (*s*)			3.91 (*s*)
OH	12.41 (*s*) 6.24 (*s*)	12.38 (*s*) 6.29 (*s*) 5.71 (*s*)	12.38 (*s*) 6.47 (*s*) 5.63 (*s*)	12.37 (*s*) 6.48 (*s*) 5.61 (*s*)	11.56 (*s*) 6.45 (*s*) 5.73 (*s*)	12.24 (*s*)	11.56 (*s*)	11.55 (*s*)	11.55 (*s*)

[a] Multiplicity and coupling constant (*J* in Hz) assigned in parentheses.
[b] Recorded in $CDCl_3$.
[c] Recorded in $CDCl_3$–CD_3OD (9:1).

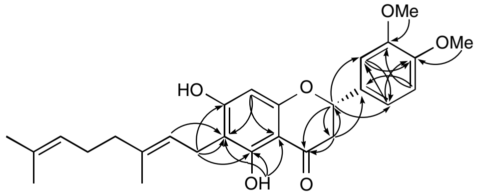


Fig. 2. Pertinent HMBC correlations from H to C of flavanone **1**.

Compound **2** could not be isolated in pure form, but was obtained as a 1:4 mixture of **2** and **10**. An attempted separation of **2** from **10** by PTLC and reversed-phase HPLC was not successful. EIMS data suggested that compounds **2** and **10** have the identical molecular formula, $C_{26}H_{30}O_6$. The major constituent **10** was identified as 6-geranyl-4′,5,7-trihydroxy-3′-methoxyflavanone (3′-*O*-methyldiplacone) (Wollenweber et al., 1989; Phillips et al., 1996; Phommart et al., 2005) by comparison of the ^{1}H and ^{13}C NMR spectroscopic data with reported values. The minor constituent **2** was an isomer of **10** with regard to the substitution pattern on the B-ring, since the ^{1}H signals of the 3′,4′-substituted B-ring protons [δ 7.13 (*d*, $J = 1.9$ Hz), 7.02 (*dd*, 8.1, 1.9 Hz), 6.88 (*d*, $J = 8.1$ Hz)] were different from those [δ 6.97 (*d*, $J = 1.9$ Hz), 6.90 (*dd*, 8.2, 1.9 Hz), 6.93 (*d*, $J = 8.2$ Hz)] of **10**. A methoxy signal of **2** at δ 3.91 showed an NOE correlation with the *ortho*-coupled aromatic proton at 6.88 (H-5′), indicating 3′-hydroxy-4′-methoxy B-ring structure for **2**. The ^{1}H and ^{13}C NMR spectroscopic data of the A-ring signals for **2** and **10** were essentially identical, thus indicating the same 6-geranyl-5,7-dihydroxy A-ring structure for **2**. This was confirmed by HMBC correlations recorded for the mixture **2**/**10**. The structure of **2** was, therefore, determined to be 6-geranyl-3′,5,7-trihydroxy-4′-methoxyflavanone. Diazomethane treatment of the mixture **2**/**10** yielded 6-geranyl-5-hydroxy-3′,4′,7-trimethoxyflavanone as a single methylated product, which further supported the structures of **2** and **10**.

Compound **3** possesses the molecular formula $C_{27}H_{32}O_7$. Analysis of the ^{1}H NMR spectroscopic data of **3** established that the A-ring structure is the same as for compounds **1** and **2**, and the B-ring [δ 6.68 (2H, *s*)] is symmetrically substituted with a hydroxy and two methoxy [δ 3.91 (3H×2)] groups. In NOE experiments, the methoxy signal showed a correlation with the 4′-OH resonance (δ 5.63), thus assigning 4′-hydroxy-3′,5′-dimethoxy B-ring structure. The structure of **3** was, therefore, determined to be 6-geranyl-4′,5,7-trihydroxy-3′,5′-dimethoxyflavanone.

Compound **4** was assigned the molecular formula $C_{26}H_{30}O_7$. The ^{1}H NMR spectroscopic data, together with the molecular formula, suggested that the B-ring is substituted with two hydroxy and one methoxy (δ 3.89) groups. The two *meta*-coupled aromatic protons at δ 6.56 (*d*, $J = 1.7$ Hz) and 6.67 (*d*, $J = 1.7$ Hz), assignable to those

Table 2
^{13}C NMR spectroscopic data of compounds **1**–**9**

C	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[b]	**7**[b]	**8**[b]	**9**[b]
2	79.02	78.67	79.37	79.09	82.95	79.16	83.37	83.02	83.53
3	43.39	43.02	43.59	43.39	72.30	43.27	72.30	71.99	72.22
4	196.03	196.18	195.94	196.07	194.75	195.76	195.85	195.48	195.61
5	161.21	161.17	161.21	161.20	161.04	160.87	160.87	160.49	160.63
6	106.83	107.31	107.07	107.08	107.89	107.11	108.89	108.79	108.38
7	164.07	163.99	164.05	164.06	164.60	164.26	165.14	164.84	164.98
8	95.68	95.46	95.63	95.64	95.83	94.74	95.22	94.97	95.10
9	161.04	160.94	160.92	160.95	160.65	160.41	160.69	160.35	160.58
10	102.89	102.72	102.80	102.83	100.40	102.42	100.22	99.93	100.11
1′	130.96	131.50	129.58	130.30	129.18	130.42	128.29	127.98	127.54
2′	109.40	112.65	103.16	107.04	113.62	108.37	114.35	114.08	109.02
3′	149.27	145.72	147.18	147.18	145.62	147.56	144.53	144.17	147.48
4′	149.67	146.90	135.14	132.75	147.33	135.27	145.58	145.21	133.98
5′	111.14	110.65	147.18	144.10	110.55	143.10	115.12	114.86	144.47
6′	118.79	118.10	103.16	101.29	119.79	102.54	120.04	119.77	102.82
OMe	55.98	55.92	56.37	56.24	55.88	56.18			56.13
OMe	55.96		56.37						
1″	21.07	20.97	21.04	21.04	20.94	20.88	21.00	20.64	20.95
2″	121.26	121.32	121.27	121.28	121.17	122.93	122.85	122.03	121.96
3″	139.61	138.44	139.25	139.25	138.46	135.27	135.53	135.25	132.29
3″-Me	16.20	16.09	16.16	16.17	16.09	15.88	16.02	15.65	17.64
4″	39.70	39.63	39.68	39.68	39.64	35.78	35.93	39.53	25.64
5″	26.31	26.31	26.33	26.36	26.36	32.70	32.81	22.10	
6″	123.65	123.70	123.72	123.80	124.15	75.71	75.71	42.75	
7″	132.13	131.80	132.01	132.04	131.78	147.15	147.28	71.04	
7″-Me	25.67	25.59	25.62	25.65	25.58	17.49	17.64	28.56	
8″	17.70	17.61	17.66	17.68	17.61	110.86	111.00	28.56	

[a] Recorded in $CDCl_3$.

[b] Recorded in $CDCl_3$–CD_3OD (9:1).

of the B-ring, showed HMBC correlations with C-2 of the flavanone. Hence, compound **4** was determined to be 6-geranyl-4′,5,5′,7-tetrahydroxy-3′-methoxyflavanone.

Compound **5** was obtained as a 2:3 mixture of **5** and **12**. The two compounds had the identical molecular formula $C_{26}H_{30}O_7$. In the ^{1}H NMR spectrum, the major compound **12** showed two protons at δ 4.98 (*d*, $J = 12.0$ Hz) and 4.55 (*d*, $J = 12.0$ Hz) typical of H-2 and H-3 of a 3-hydroxyflavanone. The minor compound **5** exhibited the corresponding ^{1}H signals at δ 4.95 (*d*, $J = 12.0$ Hz) and 4.52 (*d*, $J = 12.0$ Hz). Compound **12** was identified as 6-geranyl-3,4′,5,7-tetrahydroxy-3′-methoxyflavanone (diplacol 3′-*O*-methyl ether) by comparison of the NMR data with reported values (Phillips et al., 1996). The ^{1}H NMR data for the B-ring of **5** [δ 7.13 (*d*, $J = 1.9$ Hz), 7.01 (*dd*, 8.2, 1.9 Hz), 6.89 (*d*, $J = 8.2$ Hz)] were identical to those of **2**, thus indicating a 3′-hydroxy-4′-methoxy B-ring structure for **5**. The HMBC spectrum confirmed the B-ring and 6-geranyl-5,7-dihydroxy A-ring structures. The *trans* relationship of H-2 and H-3 was obvious from the large coupling constant ($J = 12.0$ Hz) between the two hydrogens. Diazomethane treatment of the mixture **5**/**12** yielded 6-geranyl-3,5-dihydroxy-3′,4′,7-trimethoxyflavanone as a single product. Thus, the structures of **5**/**12** were determined as described above. The absolute stereochemistry of **5** was elucidated as 2*R*,3*R* based on the CD data of **5**/**12** (a positive absorption at 328 nm and a negative absorption at 292 nm). All 3-hydroxyflavanones **5**, **7**, **8** and **9** isolated in the present study were found to have the 2*R*,3*R* absolute stereochemistry.

Compound **6** was obtained as a 1:4 mixture of **6** and **14**. An attempted separation of the two components on PTLC and reversed-phase HPLC was not successful. It appeared that the molecular weights of the two constituents are 470 and 440 on the basis of EIMS data. The major constituent **14** was identified as 3′,4′,5,7-tetrahydroxy-6-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]flavanone (tanariflavanone) by comparison of its ^{1}H and ^{13}C NMR spectroscopic data with those reported (Phommart et al., 2005). Compound **6** was therefore assigned the molecular formula, $C_{26}H_{30}O_8$. Analysis of the ^{1}H NMR data of the mixture indicated that compound **6** has the same 5,7-dihydroxyflavanone skeleton substituted with 6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl group [δ 5.29 (1H, *brt*, 7.1 Hz), 4.88 (1H, *brs*), 4.80 (1H, *brs*), 4.01 (1H, *t*, $J = 6.1$ Hz), 3.28 (2H, *d*, $J = 7.1$ Hz), 2.05 (2H, *brt*, $J = 7.3$ Hz), 1.65 (2H, *m*), 1.79 (3H, *s*), 1.70 (3H, *s*)] at C-6, as found in compound **14**. The B-ring structure of **6**, different from that of **14**, was readily assigned as 4′,5′-dihydroxy-3′-methoxybenzene ring, since the chemical shifts of the *meta*-coupled two protons [δ 6.66 (*brs*) and 6.54 (*brs*)] and a methoxy group [δ 3.89 (*s*)] were closely similar to those found in compound **4**. Diazomethane treatment of the mixture **6** and **14** yielded 5-hydroxy-6-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]-3′, 4′,7-trimethoxyflavanone (**14a**) and 5-hydroxy-6-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]-3′,4′,5′,7-tetramethoxyflavanone (**6a**), which were separable from each other. Hence, the structure of compound **6** was unambiguously established to be (2*R*,3*R*)-4′,5,5′,7-tetrahydroxy-3′-methoxy-6-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]flavanone. In order to obtain information of the configuration at C-6″ of **6**, the methyl ether **6a** was converted to the 6″-*O*-(*S*)-MTPA ester derivative. The ester showed pairs of ^{1}H signals, for example, 9″-Ha at δ 4.99 and 4.94, and 9″-Hb at δ 4.90 and 4.89, in a 1:1 intensity. This finding established that compound **6** is a 1:1 epimeric mixture at C-6″. Compound **14** was similarly found to be a 1:1 epimeric mixture at C-6″.

Compound **7** was assigned the molecular formula $C_{25}H_{28}O_8$ on the basis of HREIMS data. The ^{1}H NMR spectrum exhibited signals for H-2, H-3 and H-8 of a C-6 substituted 3,5,7-trihydroxyflavanone and signals due to a 6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl group. In addition, the spectrum showed signals for H-2′, H-5′ and H-6′ [δ 7.00 (1H, *d*, $J = 1.7$ Hz), 6.92 (1H, *dd*, $J = 8.0$, 1.7 Hz) and 6.89 (1H, *d*, $J = 8.0$ Hz)] of a 3′,4′-dihydroxy B-ring. The ^{13}C NMR spectroscopic data of **7** were in good agreement with these structures. The structure of **7** was therefore determined to be (2*R*,3*R*)-3,3′,4′,5,7-pentahydroxy-6-[6-hydroxy-3,7-dimethyl-2(*E*),7-octadienyl]flavanone. Compound **7** was found to be a 1:1 epimeric mixture at C-6″ from the ^{1}H NMR data of the 3-*O*,6″-*O*-di-(*S*)-MTPA ester prepared from the methyl ether **7a** which was obtained by diazomethane treatment of **7**.

Compound **8** was assigned the molecular formula $C_{25}H_{30}O_8$ on the basis of HREIMS data. The ^{1}H and ^{13}C NMR spectroscopic data of **8** resembled those of **7** except for the signals of the terminal portion of the C_{10} side chain at C-6. The ^{1}H NMR signals of **8** resonating at δ 1.18 (two tertiary methyls), δ 5.20 (*brt*, $J = 7.1$ Hz), δ 1.98 (2H, *m*), 1.62 (2H, *m*) and 1.41 (2H, *m*) and the ^{13}C signal of a quaternary carbon at δ 71.04 (C-7″) established the C_{10} moiety to be 7-hydroxy-3,7-dimethyl-2(*E*)-octenyl group. Thus, the structure of compound **8** was determined to be 3,3′,4′,5,7-pentahydroxy-6-[7-hydroxy-3,7-dimethyl-2(*E*)-octenyl]flavanone.

Compound **9** was assigned the molecular formula $C_{21}H_{22}O_8$ on the basis of HREIMS data. The ^{1}H NMR spectrum showed signals of 3-methyl-2-butenyl (prenyl) group [δ 3.29 (2H, *d*, $J = 6.8$ Hz), 5.23 (1H, *t*, $J = 7.0$ Hz), 1.70 (3H, *s*)] and 1.79 (3H, *s*) and H-2′ [δ 6.74 (*d*, $J = 1.7$ Hz)] and H-6′ [δ 6.64 (*d*, $J = 1.7$ Hz)] for 4′,5′-dihydroxy-3-methoxy B-ring, in addition to the signals of H-2 [δ 4.89 (*d*, $J = 12.0$ Hz)], H-3 [δ 4.50 (*d*, $J = 12.0$ Hz)] and H-8 [δ 5.97 (*s*)]. Thus, the structure of **9** was determined to be 3,4′,5,5′,7-pentahydroxy-3′-methoxy-6-(3-methyl-2- butenyl)flavanone. The ^{13}C NMR spectroscopic data of **9** corroborated the assigned structure.

Table 3
Anti-oxidant activity of compounds **1**–**15**

Compound	SC$_{50}$(μg/ml)
1	>50
2/10	>50
3	>50
4	4.5
5/12	34
6/14	2.0
7	2.9
8	1.8
9	1.8
11	3.2
13	1.9
15	1.9
α-Tocopherol	4.7
(+)-Taxifolin	1.6

The structures of known compounds **10** (Wollenweber et al., 1989; Phillips et al., 1996; Phommart et al., 2005), **12** and **14** have been described above, and the additional known compounds **11**, **13** and**15** were identified as 6-geranyl-3′,4′,5,7-tetrahydroxyflavanone (diplacone, nymphaerol A) (Phillips et al., 1996; Phommart et al., 2005; Kumazawa et al., 2004), 6-geranyl-3,3′,4′,5,7-pentahydroxyflavanone (diplacol) (Phillips et al., 1996; Lee et al., 2001) and 3′,4′,5,7-pentahydroxy-6-[7-hydroxy-3,7-dimethyl-2(*E*)-octenyl]flavanone (Kumazawa et al., 2005), respectively, by comparison of their spectroscopic data with those reported.

Anti-oxidant activity of compounds **1**–**15** towards DPPH radicals was examined and the results are summarized in Table 3. Compounds **4**, **6/14**, **7**, **8**, **9**, **11**, **13** and **15** were found to be more active than α-tocopherol. In particular, compounds **6/14**, **8**, **9**, **13** and **15** showed significant antiradical activities, comparable to well-known highly active flavonoids such as luteorin 5-*O*-glucoside, quercetin and taxifolin (3,3′,4,5,7-pentahydroxyflavanone) (Seyoum et al., 2006). It should be noted that the active flavonoids in the present study all have an *ortho*-dihydroxybenzene structure in the B-ring. It can be considered that the substitution of a geranyl group or its oxidized congeners at C-6 of flavanols does not significantly alter the anti-oxidant activity as evidenced by comparison of the activity of compound **13** and taxifolin.

3. Concluding remarks

In this study, we demonstrated that the viscous materials on the surface of the immature fruits of *P. tomentosa* contain a number of flavonoids substituted with either a geranyl group or its derivative at C-6. Some of these compounds showed notable anti-oxidant activity. It is worth noting that the extract is readily available from the fruits and the flavonoid mixture is a major component of the extract. The finding therefore suggests that the fruits of *P. tomentosa* provide a source of natural antioxidants. The flavonoid constituents were found to exist exclusively on the fruit surface rather than the internal parts of the fruits. We presume these flavonoids are biosynthesized in the microstructure glandular trichomes on the fruit surface (Gang et al., 2002). After the submission of our manuscript, a related work on the flavonoid constituents of the *P. tomentosa* fruits collected in Czech has been published (Smejkal et al., 2007). Interestingly, the flavonoid constituents reported in this report are different from those isolated in the present study, except compound **12**, although they mostly have a C_{10} substituent at C-6. It is suggested that these flavonoids may serve as antifeedents and as protection against excessive UV radiation (Smejkal et al., 2007).

4. Experimental

4.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$ or $CDCl_3/CD_3OD$ (9:1) solution. Tetramethylsilane signal was used as an internal standard for ^{1}H spectra, and $CDCl_3$ ($\delta = 77.00$) signal was used as a reference for ^{13}C spectra. EIMS (70 eV) and negative FABMS were obtained on a JEOL JMS-700 spectrometer. UV spectra were recorded on a UV-1600PC spectrometer. Optical rotations were measured on a JASCO DIP-360 polarimeter. CD spectra were measured with a JASCO J-500C polarimeter at 25 °C in MeOH solution. HPLC analysis was carried out on a Shimadzu LC-6A apparatus equipped with a UV detector using a reversed-phase column (Inertsil ODS-3, 15 cm×4.6 mm i.d.) under isocratic solvent condition.

4.2. Plant material

Fruits of the *P. tomentosa* Steud. (Scrophulariaceae) were collected in September 2005 in the campus of Tokyo Institute of Technology, and the plant source was confirmed by S. Kohshima, an author of this paper. A voucher specimen (CMS17-01) is deposited in the Department of Chemistry and Materials Science, Tokyo Institute of Technology.

4.3. Extraction and isolation

Fresh fruits of *P. tomentosa* (320 g) were dipped into MeOH (300 ml) to extract surface components, and the MeOH solution was concentrated to dryness (5 g). The extract was subjected to silica gel CC eluting with a gradient of $CHCl_3$-MeOH (20:1–10:1) to give five fractions (Frs.1-5). Fr. 1 was further separated by silica gel flash CC eluting with hexane–EtOAc (20:1–8:1) to give **1** (20 mg) and **2/10** (120 mg). Fr. 2 was purified by Sephadex LH-20 CC eluting with EtOAc to give **3** (120 mg). Fr. 3 was purified by silica gel flash CC eluting with hexane–EtOAc (10:1–5:1) to give **4** (250 mg). Fr. 4 was separated by silica gel CC eluting with $CHCl_3$–MeOH (20:1–10:1) to give **5/12** (60 mg) and **11** (72 mg). Fr. 5 was subjected to silica gel CC eluting with a gradient of hexane–EtOAc–MeOH (2:1:0.1–1:1:0.2) and then Sephadex LH-20 CC eluting with $CHCl_3$–MeOH (3:2) to give a purified Fr. 5. This was finally applied to reversed-phase HPLC (flow rate: 1.0 ml/min, solvent: 25% aqueous MeOH, UV detection at 282 nm) to furnish **9** (2 mg), **8** (6 mg), **7** (2 mg), **15** (6 mg), **6/14** (4 mg) and **13** (14 mg) in the order of elution.

4.4. 6-Geranyl-5,7-dihydroxy-3′,4′-dimethoxyflavanone (1)

Yellow colored oil; $[\alpha]_D^{25}$ −6.6 (*c*, 1.0, $CHCl_3$); UV λ_{max}^{MeOH} ($\log\varepsilon$): 204 (4.88), 232 (4.50), 292 (4.34) 342 (shoulder); CD (MeOH) $\Delta\varepsilon_{328}$ +4.49, $\Delta\varepsilon_{311}$ 0, $\Delta\varepsilon_{288}$ −18.8; EIMS: *m/z* 452 $[M]^+$ (81), 409 (5), 383 (20), 367 (21), 353 (22), 329 (72), 316 (10), 288 (6), 245 (5), 219 (100), 203 (30), 191 (8), 177 (10), 165 (38), 164 (32), 151 (26); HREIMS: *m/z* 452.2208 $[M]^+$ (calcd. for $C_{27}H_{32}O_6$, 452.2199).

4.5. A mixture of 6-geranyl-4′,5,7-trihydroxy-3′-methoxyflavanone (2) and (10)

Obtained as a 1:4 mixture of **2** and **10**; yellow colored oil; UV λ_{max}^{MeOH} 202, 232, 292, 342 (shoulder); CD (MeOH) $\Delta\varepsilon_{328}$ +2.22, $\Delta\varepsilon_{312}$ 0, $\Delta\varepsilon_{289}$ −8.4; EIMS: *m/z* 438 $[M]^+$ (60), 395 (5), 381 (4), 369 (12), 353 (15), 315 (62), 302 (7), 288 (4), 245 (6), 231 (6), 219 (100), 203 (12), 177 (12), 165 (41), 150 (13), 135 (10); HREIMS: *m/z* 438.2055 $[M]^+$ (calcd. for $C_{26}H_{30}O_6$, 438.2042).

A mixture of compound **2/10** (3.0 mg) and ethereal CH_2N_2 (3 ml) was stirred overnight at room temperature. The reaction mixture was concentrated and purified by PTLC to give 6-geranyl-5-hydroxy-3′,4′,7-trimethoxyflavanone (**2a**) as a yellow powder (1.6 mg, 50%), EIMS: *m/z* 466 $[M]^+$ (75), 451 (4), 423 (5), 397 (13), 396 (11), 383 (12), 368 (10), 355 (11), 343 (70), 330 (13), 302 (5), 293 (3), 287 (3), 249 (87), 245 (8), 233 (100), 219 (17), 191 (15), 179 (37), 164 (16), 151 (20), 149 (17); HREIMS: *m/z* 466.2317 $[M]^+$ (calcd. for $C_{28}H_{34}O_6$, 466.2355); ^{1}H NMR ($CDCl_3$) δ 12.01 (1H, *s*, 5-OH), 7.00 (1H *d*, $J = 1.7$ Hz, H-2′), 6.99 (1H, *dd*, $J = 8.1$, 1.7 Hz, H-6′), 6.90 (1H, *d*, $J = 8.4$ Hz, H-5′), 6.09 (1H, *s*, H-8), 5.35 (1H, *dd*, $J = 13.2$, 3.0 Hz, H-2), 5.20 (1H, *brt*, $J = 7.0$ Hz, H-2″), 5.08 (1H, *brt*, $J = 6.8$ Hz, H-6″), 3.92 (3H, *s*, 3′-OMe), 3.91 (3H, *s*, 4′-OMe), 3.83 (3H, *s*, 7-OMe), 3.28 (2H, *d*, $J = 7.1$, H-1″), 3.11 (1H, *dd*, $J = 16.8$, 13.2 Hz, H-3α), 2.78 (1H, *dd*, $J = 17.1$, 3.2 Hz, H-3β), 2.02 (2H, *m*, H-5″), 1.98 (2H, *m*, H-4″), 1.76 (3H, *s*, Me-3″), 1.65 (3H, *s*, H_3-8″), 1.58 (3H, *s*, Me-7″).

4.6. 6-Geranyl-4′,5,7-trihydroxy-3′,5′- dimethoxyflavanone (3)

Yellow colored oil; $[\alpha]_D^{25}$ −21.2 (*c*, 2.3, $CHCl_3$); UV λ_{max}^{MeOH} ($\log\varepsilon$): 209 (4.93), 232 (4.59), 292 (4.46) 342 (shoulder); CD (MeOH) $\Delta\varepsilon_{328}$ +4.32, $\Delta\varepsilon_{315}$ 0, $\Delta\varepsilon_{290}$ −14.3; EIMS: *m*/*z* 468 [M]$^+$ (55), 399 (12), 383 (20), 360 (21), 345 (52), 315 (22), 219 (100), 203 (50), 180 (78), 165 (73), 151 (19), 137 (16); HREIMS: *m*/*z* 468.2169 [M]$^+$ (calcd. for $C_{27}H_{32}O_7$, 468.2148).

*4.7. 6-Geranyl-4′,5,5′,7-tetrahydroxy-3′- methoxyflavanone (**4**)*

Yellow oil; $[\alpha]_D^{25}$ −17.6 (*c*, 1.6, $CHCl_3$); UV λ_{max}^{MeOH} (log ε): 206 (4.94), 233 (4.52), 294 (4.41) 342 (shoulder); CD (MeOH) $\Delta\varepsilon_{328}$ +3.88, $\Delta\varepsilon_{313}$ 0, $\Delta\varepsilon_{289}$ −12.2; EIMS: *m*/*z* 454 [M]$^+$ (56), 438 (5), 385 (18), 369 (20), 345 (18), 331 (70), 315 (40), 219 (100), 203 (24), 177 (16), 165 (83); HREIMS: *m*/*z* 454.1947 (calcd. for $C_{26}H_{30}O_7$, 454.1992).

*4.8. A mixture of 6-geranyl-3,3′,5,7-tetrahydroxy-4′-methoxyflavane (**5**) and (**12**)*

Obtained as a 2:3 mixture of **5** and **12**; yellow colored oil; UV λ_{max}^{MeOH} 206, 232, 292, 342 (shoulder); CD (MeOH) $\Delta\varepsilon_{328}$ +6.31, $\Delta\varepsilon_{315}$ 0, $\Delta\varepsilon_{292}$ −22.2; EIMS: *m*/*z* 454 [M]$^+$ (60), 436 (8), 385 (10), 331 (58), 301 (24), 289 (52), 219 (53), 203 (15), 177 (24), 165 (100), 137 (72); HREIMS: *m*/*z* 454.1945 [M]$^+$ (calcd. for $C_{26}H_{30}O_7$, 454.1992).

Compound **5**/**12** (3.0 mg) and ethereal CH_2N_2 (3 ml) was stirred overnight at room temperature. The reaction mixture was concentrate and purified by PTLC to give 6-geranyl-3,5-dihydroxy-3′,4′,7-trimethoxyflavanone (**5a**) as a yellow powder (1.6 mg, 50%); EIMS: *m*/*z* 482 [M]$^+$ (78), 464 (10), 453 (5), 439 (4), 413 (10), 397 (12), 395 (13), 368 (19), 359 (62), 341 (16), 329 (20), 317 (12), 303 (67), 276 (10), 256 (8), 233 (55), 219 (10), 191 (16), 179 (100), 165 (20), 161 (80); HREIMS: *m*/*z* 482.2263 [M]$^+$ (calcd. for $C_{28}H_{34}O_7$, 482.2305); ^{1}H NMR ($CDCl_3$) δ 11.23 (1H, *s*, 5-OH), 7.11 (1H, *dd*, *J* = 8.4, 1.8 Hz, H-6′), 7.07 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.95 (1H, *d*, *J* = 8.4 Hz, H-5′), 6.10 (1H, *s*, H-8), 5.18 (1H, *brt*, *J* = 7.0 Hz, H-2″), 5.08 (1H, *brt*, *J* = 6.8 Hz, H-6″), 5.00 (1H, *d*, *J* = 11.7 Hz, H-2), 4.57 (1H, *dd*, *J* = 11.7, 1.5 Hz, H-3α), 3.94 (3H, *s*, 3′-OMe), 3.91 (3H, *s*, 4′-OMe), 3.84 (3H, *s*, 7-OMe), 3.28 (2H, *d*, *J* = 6.9 Hz, H-1″), 2.04 (2H, *m*, H-5″), 1.98 (2H, *m*, H-4″), 1.77 (3H, *s*, Me-3″), 1.65 (3H, *s*, H_3-8″), 1.58 (3H, *s*, Me-7″).

*4.9. A mixture of 4′,5,5′,7-tetrahydroxy-3′-methoxy-6-[6-hydroxy-3,7-dimethyl-2(E),7-octadienyl]flavanone (**6**) and (**14**)*

Obtained as a 1:4 mixture of **6** and **14**; yellow colored oil; UV λ_{max}^{MeOH} 205, 232, 292, 342 (shoulder); CD (MeOH) $\Delta\varepsilon_{327}$ +3.49, $\Delta\varepsilon_{316}$ 0, $\Delta\varepsilon_{291}$ −13.5; EIMS: *m*/*z* 470 [M]$^+$ (7), 452 (13), 384 (10), 369 (10), 331 (31), 287 (8), 231 (8), 219 (31), 203 (30), 179 (14), 165 (100); HREIMS: *m*/*z* 470.1938 [M]$^+$ (calcd. for $C_{26}H_{30}O_7$, 470.1941).

A mixture of compound **6**/**14** (1.0 mg) and ethereal CH_2N_2 (3 ml) was stirred overnight at room temperature. The reaction mixture was concentrated, separated by PTLC, and finally purified by reversed-phase HPLC (solvent, 20% aqueous MeOH) to yield **6a** (0.15 mg, 12.5%) and **14a** (0.6 mg, 50%). **6a**: EIMS: *m*/*z* 512 [M]$^+$ (40), 497 (8) 426 (66), 413 (20), 387 (10), 373 (90), 368 (60), 360 (30), 343 (30), 329 (10), 313 (9), 285 (11), 255 (22), 236 (40), 219 (33), 194 (44), 179 (100), 165 (10), 151 (26); HREIMS: *m*/*z* [M]$^+$512.2365 (calcd. for $C_{29}H_{34}O_8$, 512.2410); ^{1}H NMR ($CDCl_3$) δ 12.04 (1H, *s*, 5-OH), 6.67 (2H, *s*, H-2′ and 6′), 6.10 (1H, *s*, H-8), 5.33 (1H, *dd*, *J* = 13.3, 2.9 Hz, H-2), 5.23 (1H, *brt*, *J* = 7.3 Hz, H-2″), 4.89 (1H, *brs*, Ha-8″), 4.81 (1H, *brs*, Hb-8″), 4.01 (1H, m, H-6″), 3.89 (6H, *s*, 3′ and 5′-OMe), 3.87 (3H, *s*, 4′-OMe), 3.84 (3H, *s*, 7-OMe), 3.27 (2H, *d*, *J* = 6.8 Hz, H-1″), 3.09 (1H, *dd*, *J* = 17.1, 13.3 Hz, Ha-3), 2.79 (1H, *dd*, *J* = 17.1, 2.9 Hz, Hb-3), 2.02 (2H, *m*, H-4″), 1.79 (3H, *s*, Me-3″), 1.71 (3H, *s*, Me-7″), 1.58 (2H, *m*, H-5″). **14a**: ^{1}H NMR ($CDCl_3$) δ 12.04 (1H, *s*, 5-OH), 6.99 (1H, *d*, *J* = 2.4 Hz, H-2′), 6.98 (1H, *d*, *J* = 8.2, 2.4 Hz, H-6′), 6.90 (1H, *d*, *J* = 8.2 Hz, H-5′), 6.09 (1H, *s*, H-8), 5.34 (1H, *dd*, *J* = 13.3, 2.9 Hz, H-2), 5.23 (1H, *brt*, *J* = 7.3 Hz, H-2″), 4.89 (1H, *brs*, Ha-8″), 4.81 (1H, *brs*, Hb-8″), 4.02 (1H, *m*, H-6″), 3.93 (3H, *s*, 3′-OMe), 3.91 (3H, *s*, 4′-OMe), 3.83 (3H, *s*, 7-OMe), 3.27 (2H, *d* *J* = 6.8 Hz, H-1″), 3.11 (1H, *dd*, *J* = 17.1, 13.2 Hz, Ha-3), 2.79 (1H, *dd*, *J* = 17.1 Hz, 2.9 Hz, Hb-3), 2.02 (2H, *m*, H-4″), 1.79 (3H, *s*, Me-3″), 1.71 (3H, *s*, Me-7″), 1.58 (2H, *m*, H-5″).

(*R*)-MTPA-Cl (1 μl) was added to a solution **6a** (0.3 mg) in pyridine (30 μl) and the mixture was allowed to stand for 1 h at room temperature. The mixture was concentrated after addition of MeOH (30 μl), and purified by PTLC to give the 6″-*O*-(*S*)-MTPA ester; ^{1}H NMR ($CDCl_3$) δ 12.07 (1H, *s*, 5-OH), 6.09 (0.5H, *s*, H-8), 6.08 (0.5H, *s*, H-8), 4.99 (0.5H, *brs*, H-8″), 4.94 (0.5H, *brs*, H-8″), 4.90 (0.5H, *brs*, H-8″), 4.89 (0.5H, *brs*, H-8″), 3.84 (1.5H, *s*, 7-OMe), 3.83 (1.5H, *s*, 7-OMe), 3.54 (1.5H, *brs*, MTPA-OMe), 3.52 (1.5H, *brs*, MTPA-OMe), 3.27 (0.5H, *d*, *J* = 7.1 Hz, H-1″), 3.25 (0.5H, *d*, *J* = 7.1 Hz, H-1″), 1.76 (1.5H, *s*, Me-7″), 1.71 (1.5H, *s*, Me-7″).

*4.10. 3,3′,4′,5,7-Pentahydroxy-6-[6-hydroxy-3,7-dimethyl-2(E),7-octadienyl]flavanone (**7**)*

Yellow colored oil; $[\alpha]_D^{25}$ +30.1° (*c*, 0.20, MeOH); UV λ_{max}^{MeOH} (log ε): 203 (4.84), 232 (4.42), 292 (4.31) 342 (shoulder); CD (MeOH) $\Delta\varepsilon_{332}$ +3.50, $\Delta\varepsilon_{317}$ 0, $\Delta\varepsilon_{293}$ −11.2; EIMS: *m*/*z* 456 [M]$^+$ (6), 438 (14), 370 (12), 355 (14), 331 (14), 317 (34), 301 (42), 299 (14), 287 (14), 219 (27), 203 (20), 177 (18), 165 (100); HREIMS: *m*/*z* 456.1775 [M]$^+$ (calcd. for $C_{26}H_{30}O_7$, 456.1784).

Compound **7** (1.0 mg) and ethereal CH_2N_2 (3 ml) was stirred overnight at room temperature. The reaction mixture was concentrated and purified by PTLC to give **7a** (0.6 mg, 50%); EIMS: *m*/*z* 498 [M]$^+$ (20), 480 (8) 412 (10), 383 (5), 368 (30), 359 (40), 343 (16), 329 (33), 319 (22), 301 (8), 233 (20), 219 (8), 179 (100), 165 (14), 151 (28); HREIMS: *m*/*z* [M]$^+$ 498.2285 (calcd. for $C_{28}H_{34}O_8$, 498.2254); ^{1}H NMR ($CDCl_3$) δ 11.23 (1H, *s*, 5-OH), 7.11

(1H, *dd*, $J = 8.2$, 2.4 Hz, H-6′), 7.07 (1H, *d*, $J = 2.4$ Hz, H-2′), 6.94 (1H, *d*, $J = 8.2$ Hz, H-5′), 6.10 (1H, *s*, H-8), 5.22 (1H, *brt*, $J = 7.3$ Hz, H-2″), 5.02 (1H, *d*, $J = 12.0$ Hz, H-2), 4.58 (1H, brd, $J = 12.0$ Hz, H-3), 4.89 (1H, *brs*, Ha-8″), 4.81 (1H, *brs*, Hb-8″), 4.03 (1H, *m*, H-6″), 3.94 (3H, *s*, 3′-OMe), 3.91 (3H, *s*, 4′-OMe), 3.84 (3H, *s*, 7-OMe), 3.28 (2H, *d*, $J = 6.8$ Hz, H-1″), 2.02 (2H, *m*, H-4″), 1.79 (3H, *s*, Me-3″), 1.71 (3H, *s*, Me-7″), 1.58 (2H, *m*, H-5″).

Conversion of **7a** (0.3 mg) to the 3-*O*,6″-*O*-di-(*S*)-MTPA ester was carried out as described for **6a**. **7a**: ^{1}H NMR ($CDCl_3$) δ 11.41 (0.5H, *s*, 5-OH), 11.40 (0.5H, *s*, 5-OH), 6.12 (0.5H, *s*p, H-8), 6.11 (0.5H, *s*, H-8), 5.00 (0.5H, *brs*, H-8″), 4.94 (0.5H, *brs*, H-8″), 4.90 (0.5H, *brs*, H-8″), 4.89 (0.5H, *brs*, H-8″), 3.84 (1.5H, *s*, 7-OMe), 3.83 (1.5H, *s*, 7-OMe), 3.55 (1.5H, *brs*, MTPA-OMe), 3.52 (1.5H, *brs*, MTPA-OMe), 3.28 (0.5H, *d*, $J = 7.1$ Hz, H-1″), 3.26 (0.5H, *d*, $J = 7.1$ Hz, H-1″), 1.75 (1.5H, *s*, Me-7″), 1.71 (1.5H, *s*, Me-7″).

*4.11. 3′,4′,5,7-Tetrahydroxy-6-[7-hydroxy-3,7-dimethyl-2(E)-octenyl]flavanone (**8**)*

Yellow colored oil; $[\alpha]_D^{25}$ +18.3 (*c*, 0.60, MeOH); UV λ_{max}^{MeOH} ($\log\varepsilon$): 203 (4.80), 232 (4.42), 292 (4.30) 342 (shoulder); CD (MeOH) $\Delta\varepsilon_{332}$ +3.25, $\Delta\varepsilon_{317}$ 0, $\Delta\varepsilon_{293}$ −12.9; HRFABMS(−): *m/z* 457.1891 $[M-H]^-$ (calcd. for $C_{25}H_{29}O_8$, 457.1862); EIMS: *m/z* 458 $[M]^+$ (12), 440 (42), 422 (8), 355 (10), 317 (68), 299 (18), 289 (30), 233 (10), 219 (24), 203 (12), 177 (12), 165 (100), 150 (24), 123 (42).

*4.12. 6-(3-Methyl-2-butenyl)-4′,5,5′,7-tetrahydroxyflavanone (**9**)*

Yellow colored oil; $[\alpha]_D^{25}$ +19.1 (*c*, 0.24, MeOH); UV λ_{max}^{MeOH} ($\log\varepsilon$): 204 (4.73), 232 (4.34), 295 (4.22) 342 (shoulder); CD (MeOH) $\Delta\varepsilon_{328}$ +3.39, $\Delta\varepsilon_{316}$ 0, $\Delta\varepsilon_{292}$ −11.4; EIMS: *m/z* 402 $[M]^+$ (60), 384 (5), 373 (18), 369 (7), 346 (12), 317 (40), 221 (42), 219 (14), 203 (10), 180 (74), 165 (100), 153 (28), 139 (8), 123 (8); HREIMS: *m/z* 402.1278 $[M]^+$ (calcd. for $C_{21}H_{22}O_8$, 402.1315).

4.13. Anti-oxidant activity

The DPPH radical scavenging activity was evaluated as reported previously (Jayasinghe et al., 2006). The concentrations in μg/ml (mean values of triplicated experiments) required to scavenge DPPH free radicals by 50% (SC_{50}) were determined. The antioxidants α-tocopherol and (+)-taxifolin were used as reference compounds.

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