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SESQUITERPENE *O*-NAPHTHOQUINONES FROM THE ROOT BARK OF *ULMUS DAVIDIANA*

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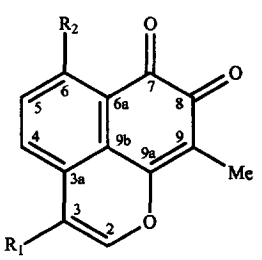
Key Word Index—*Ulmus davidiana*; Ulmaceae; root bark; sesquiterpene *o*-naphthoquinones; davidianones A–C; antioxidative activity.

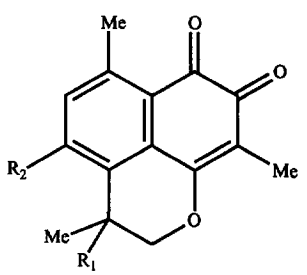
Abstract—Three new sesquiterpene *ortho*-naphthoquinones, davidianones A, B and C, together with four known compounds, mansonones E, F, H and I, were isolated from the root bark of *Ulmus davidiana*. On the basis of spectral data including pulse field gradient two-dimensional NMR spectroscopy, the structures of new compounds were established as 3-hydroxymethyl-6,9-dimethylnaphtho(1,8-*b*,*c*)pyran-7,8-dione, 6-methoxycarbonyl-3,9-dimethylnaphtho(1,8-*b*,*c*)pyran-7,8-dione, 6-dimethoxymethyl-3,9-dimethylnaphtho(1,8-*b*,*c*)pyran-7,8-dione, respectively. Their antioxidative activities were evaluated by a thiobarbituric acid method using rat liver microsomes, with mansonone F showing the greatest activity. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Ulmus davidiana Planch is a deciduous tree which is widely distributed in Korea. The stem and root bark of this species have been used in oriental traditional medicine for the treatment of oedema, mastitis, gastric cancer and inflammation [1, 2]. As a part of our search for new biologically active substances from traditional medicines [3, 4], we isolated seven sesquiterpene ortho-naphthoquinones from the 80% aqueous methanolic extract of the root bark of *U. davidiana*. The isolation and structural elucidation of three new compounds, davidianones A (**1**), B (**2**) and C (**3**), as well as four known compounds, mansonones E (**5**), F (**4**), H (**6**) and I (**7**), are described, along with their antioxidative activities, in the present paper.



	R_1	R_2
1	CH_2OH	Me
2	Me	COOMe
3	Me	$CH(OMe)_2$
4	Me	Me



	R_1	R_2
5	H	H
6	H	OH
7	OH	H

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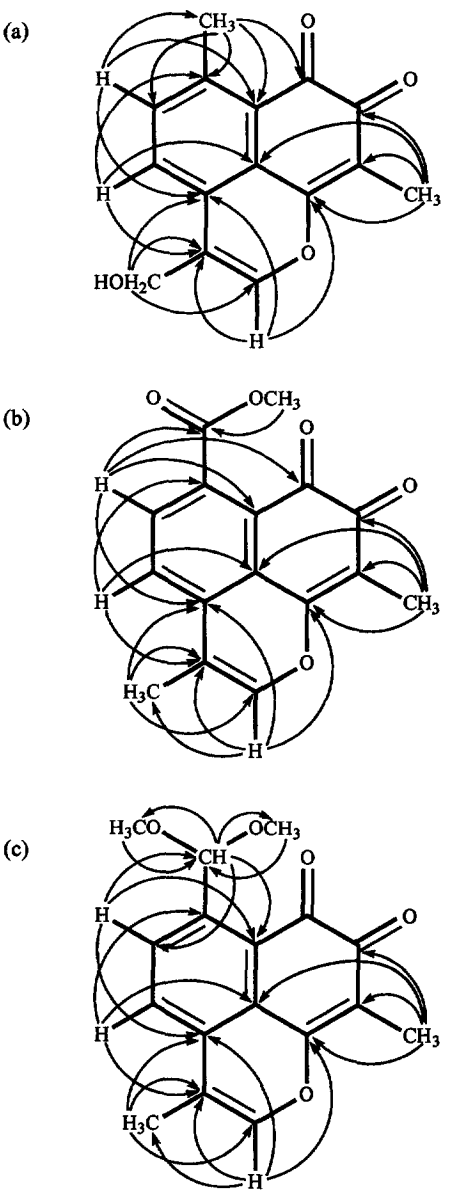

Fig. 1. ^{1}H, ^{13}C long-range correlations in PFG-HMBC spectra of compounds **1** (a), **2** (b) and **3** (c).

RESULTS AND DISCUSSION

The 80% aqueous methanolic extract of the root bark of *U. davidiana* was successively partitioned with *n*-hexane and $CHCl_3$. Column chromatography of the *n*-hexane and $CHCl_3$ extracts on silica gel and Sephadex LH-20 successively, followed by preparative TLC on silica gel and HPLC on a C-18 column yielded seven sesquiterpene *ortho*-naphthoquinones. From the $CHCl_3$ extract, three new compounds, davidianones A (**1**), B (**2**) and C (**3**), were obtained together with the known mansonones F (**4**), H (**6**) and I (**7**). Mansonone E(**5**) was purified from the *n*-hexane layer. Mansonones, a group of sesquiterpene *ortho*-naphthoquinones, were originally isolated from the West African tree *Mansonia altissima* Chev [5–9] and several other plant species [10–14]; as well as in the sapwood of *U. americana* and other elm species in response to infection by *Ceratocystis ulmi*, or other stresses [15–18]. However, mansonones H and I have never been reported from Ulmaceae.

The physico-chemical properties of davidianones A–C (**1–3**) are very similar to those of manosone F (**4**). They show IR absorptions at 1680–1690 (C=O), 1620–1640 (olefinic C=C), and 1570–1610 cm^{-1} (aromatic C=C). UV and visible spectra of these violet-coloured compounds in methanol are very similar to those of mansonone F, whose maximum absorptions are at 235, 255(sh), 335, and 550 nm [12]. These facts suggest that they have the same 1-oxa-phenalenequinone-7,8 chromophoric group. The quinonoid nature of these compounds was indicated by disappearance of the colour from an alcoholic solution of the compounds on the addition of sodium dithionite and their carbonyl absorptions in their IR spectra at 1680–1690 cm^{-1}. The EI mass spectra were diagnostic, showing the relatively intense $[M+2]^{+}$ ion peaks that are characteristic of *ortho*-naphthoquinones [19] such as the mansonones (**4–7**), but not displayed by *para*-naphthoquinones [19].

The molecular formula of davidianone A (**1**) was determined to be $C_{15}H_{12}O_4$ from the molecular ion at *m/z* 256.0751 $[M]^{+}$ in the high-resolution EI-mass spectrum. The IR spectrum of compound **1** exhibited the characteristic absorption of hydroxyl groups (3380 cm^{-1}). The fragment ion at *m/z* 211 $[M-CO-OH]^{+}$ in the EI-mass spectrum confirmed the presence of an hydroxyl group. From these observations, compound **1**, which contains one more oxygen atom than compound **4**, was presumed to be a hydroxylated mansonone F. The ^{1}H NMR spectrum was very similar to that of compound **4**; a signal for aromatic methyl protons was strongly deshielded (δ 2.70) due to the methyl being peri to a carbonyl of the quinone, one isolated methyl on the quinone ring appeared as a sharp singlet at δ 2.00, and two *ortho*-coupled aromatic protons appeared at δ 7.70 and 7.73, almost identical chemical shifts to those of compound **4**. However, the ^{1}H NMR spectral data of compound **1** differed from those of compound **4**, mainly in the chemical shifts and couplings of hydroxymethyl protons and olefinic methine proton attached to C-3 and C-2, respectively (Table 1). In place of the signal at δ 2.11 for the 3-methyl group of compound **4**, davidianone A showed a doublet ($J = 4.5$ Hz) at δ 4.65 for the C-3 methylene protons coupled with the hydroxyl group. The olefinic methine proton (H-2) signal appeared as a singlet with increment of chemical shift to δ 7.28, which in compound **4** is a doublet at δ 7.08. These facts strongly supported the presence of a hydroxymethyl group instead of a methyl group, as found on the heterocyclic ring of compound **4**. The complete structure of compound **1** was confirmed by the ^{1}H and ^{13}C NMR assignments coupled with pulse field gradient (PFG)-HMQC and HMBC experiments. In the ^{13}C NMR

Table 1. ^{1}H NMR spectral data of compounds **1–7***

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**
2	7.28 (1H, *s*)	7.09 (1H, *d*, 1.5)	7.09 (1H, *d*, 1.2)	7.08 (1H, *d*, 1.2)	4.23 (1H, *dd*, 10.7, 5.1)	4.28 (1H, *dd*, 10.3, 3.5)	4.24 (1H, *d*, 10.8)
					4.41 (1H, *dd*, 10.7, 3.9)	4.40 (1H, *br d*, 10.3)	4.30 (1H, *d*, 10.8)
3					3.10 (1H, *m*)	3.21 (1H, *m*)	
4	7.73 (1H, *d*, 8.4)	7.58 (1H, *d*, 8.3)	7.61 (1H, *d*, 8.3)	7.47 (1H, *d*, 8.3)	7.35 (1H, *d*, 7.8)		7.82 (1H, *d*, 7.8)
5	7.70 (1H, *d*, 8.4)	7.52 (1H, *d*, 8.3)	7.98 (1H, *d*, 8.3)	7.41 (1H, *d*, 8.3)	7.25 (1H, *d*, 7.8)	6.33 (1H, *s*)	7.44 (1H, *d*, 7.8)
3-Me		2.11 (3H, *d*, 1.5)	2.12 (3H, *d*, 6.8)	2.11 (3H, *d*, 1.2)	1.37 (3H, *d*, 6.8)	1.24 (3H, *d*, 7.3)	1.56 (3H, *s*)
6-Me	2.78 (3H, *s*)			2.70 (3H, *s*)	2.63 (3H, *s*)	2.48 (3H, *s*)	2.63 (3H, *s*)
9-Me	2.00 (3H, *s*)	1.97 (3H, *s*)	1.97 (3H, *s*)	1.97 (3H, *s*)	1.94 (3H, *s*)	1.85 (3H, *s*)	1.92 (3H, *s*)
CH_2OH	4.65 (2H, *d*, 4.5)						
COOMe		3.96 (3H, *s*)					
$CH(OMe)_2$			6.15 (1H, *s*)				
			3.53 (6H, *s*)				

*Recorded in $CDCl_3$ (**1–5**) and CD_3OD (**6**, **7**), chemical shift values are reported as δ from TMS at 300 MHz for **1** and 600 MHz for **2–7**; number of protons, signal multiplicity and coupling constants (Hz) are shown in parentheses.

spectrum, 15 carbons were observed with two carbonyl carbons of quinone resonating at δ 178.7 and 182.5. The presence of a hydroxymethyl group was indicated by the characteristic ^{13}C NMR chemical shift of δ 58.3 (Table 2). In the HMBC spectrum, the hydroxymethyl protons showed three correlations within the heterocyclic ring (Fig. 1) due to the ^3J couplings with C-2 (δ 143.2) and C-3a (δ 129.1), and the ^2J coupling with C-3 (δ 117.5). The olefinic methine proton at δ 7.28 (H-2) also showed correlations with C-3, C-3a and C-9a. Although the correlation from H-2 to hydroxymethyl carbon was not observed, the above observations were enough to lead to the conclusion that the hydroxymethyl group is attached to C-3. Other carbon signals of compound **1** were assigned as shown in Table 2 by the HMBC correlations in Fig. 1. Therefore, the structure of compound **1** was determined as 3-hydroxymethyl-6,9-dimethylnaphtho(1,8-*b*,*c*)pyran-7,8-dione.

Davidianone B (**2**) showed similar patterns to those of compound **4** in its UV-vis, IR and EI-mass spectra (see Experimental), suggesting that it also has an oxaphenalenequinone skeleton. It showed the $[M]^+$ at *m/z* 284.0686 in the high-resolution EI-mass spectrum, which corresponds to the empirical formula $C_{16}H_{12}O_5$. In addition to the IR absorptions possessed in common between compounds **1** and **4**, it showed an absorption at 1737 cm^{-1} corresponding to an ester functionality. In the EI-mass spectrum, the presence of methoxyl group was indicated by the ion peak at *m/z* 253, considered to correspond to a $[M-MeO]^+$ fragment ion peak. The ^1H NMR spectrum showed a very close resemblance to that of compound **4**, but differed in that the strongly deshielded aromatic methyl group (δ 2.70) *peri* to a carbonyl in compound **4** was not observed. Instead of this methyl group, a singlet corresponding to one carbomethoxyl group was observed at δ 3.96 in compound **2**.

In the ^{13}C NMR spectral data, in addition, to two quinone carbonyls at δ 178.0 and 179.0, one ester carbonyl at δ 168.6 together with one methoxyl carbon at δ 53.4 was observed. The empirical formula ^1H and ^{13}C NMR spectral data suggested that the aromatic methyl group *peri* to a quinone carbonyl of compound **4** was replaced by a carbomethoxyl group in compound **2**. This was confirmed by the HMBC correlations of the carbonyl carbon at δ 168.6 with an aromatic proton at δ 7.52 (H-5) and the methoxyl protons at δ 3.96 as shown in Fig. 1(b). In this HMBC spectrum, the ^4J long-range correlation of H-5 (δ 7.52) with a carbonyl carbon of quinone at δ 179.0 (C-7) could be observed by changing the pulse interval from 60 msec to 120 msec. Thus, the structure of compound **2** was concluded to be 6-methoxycarbonyl-3,9-dimethylnaphtho(1,8-*b*,*c*)pyran-7,8-dione.

Davidianone C (**3**), with the formula $C_{17}H_{16}O_5$ (*m/z* 300.0994 $[M]^+$ in high-resolution EI-mass spectra) also showed some similarities with davidianones A and B. Except for common UV-visible and IR absorptions of davidianones mentioned earlier, no other functionality was observed in compound **3**. The ^1H NMR spectrum was very similar to that of compound **2**. Like compound **2**, it differed from compound **4** only in the functionality attached to C-6. Instead of the 6-methyl group of compound **4**, a singlet methine and a methoxyl signal were observed at δ 6.15 and 3.53, respectively.

Table 2. ^{13}C NMR spectral data of compounds **1–7***

C	1	2	3	4‡	5‡	6	7
2	143.1	141.9	141.1	140.4	71.4	73.8	76.0
3	117.5	111.7	112.2	112.1	31.3	27.5	67.1
3a	129.1	132.1	131.3	129.5	136.9	118.9	140.9
4	130.4	128.9	129.1	128.4	132.6	156.0	136.7
5	137.6	130.3	130.7	136.4	134.9	121.8	131.7
6	148.0	137.0	144.6	146.6	142.8	148.3	144.9
6a	126.5	125.3	126.2	126.3	127.3	129.2	127.9
7	182.5	179.0	181.7	182.0	182.2	183.2	182.9
8	178.7	178.0	179.0	178.0	180.2	180.2	181.2
9	113.8	114.3	113.5	113.4	116.8	114.8	117.4
9a	163.7	161.0	161.8	161.7	162.4	165.6	164.8
9b	124.1	124.2	124.2	124.0	126.8	129.4	127.1
3-Me		12.8	12.9	12.6	17.5	17.4	26.0
6-Me	23.4			23.2	22.5	23.8	22.8
9-Me	7.7	7.6	7.7	7.7	7.8	7.9	7.8
CH_2OH	58.3						
$\underline{C}OOMe$		168.6					
$COO\underline{Me}$		53.4					
$\underline{C}H(OMe)_2$			101.5				
$CH(O\underline{Me})_2$			56.5†				

*Recorded in $CDCl_3$ (**1** – **5**) and CD_3OD (**6**–**7**), chemical shift values are reported as δ from TMS at 75.5 MHz for **1** and 150.8 MHz for **2**–**7**.

†Overlapped.

‡Assignments differed from those in the literature [12]. All carbons were assigned with the aid of PFG-HMQC and PFG-HMBC.

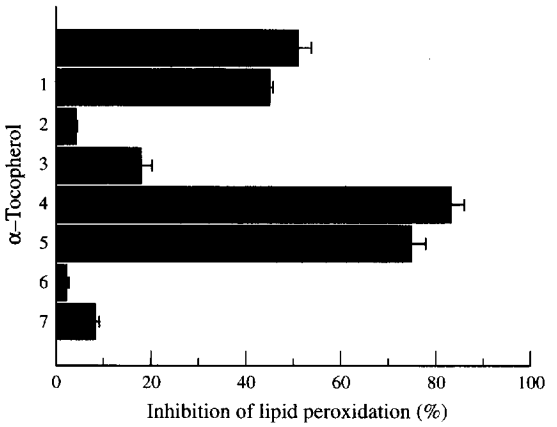

Fig. 2. Antioxidative activity of compounds **1**–**7**. Each sample was added at a final concentration of 0.1 μg ml^{-1} based on the total volume of reaction system. The values are mean±s.e. of three independent test tubes.

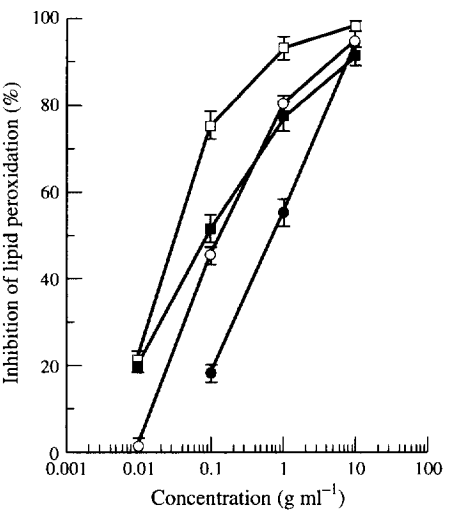

Fig. 3. Concentration effects of antioxidative activity of compounds 1 (○), 3 (●), 4 (□) and α-tocopherol (■). The values are mean±s.e. of three independent test tubes.

The methoxyl signal at δ 3.53 represented two overlapping methoxyl groups by its integration of six protons. In ^{13}C NMR spectrum, the carbon signals of these methine and methoxyl groups appeared at δ 101.5 and 56.5, respectively. The chemical shift of this methine carbon indicated that it is geminal with the methoxyl groups of the acetal. In the HMBC spectral data (Fig. 1), the methine proton at δ 6.15 showed long-range correlations with these methoxyl carbons (δ 56.5), C-5 (δ 130.7) and C-6a (δ 126.2). Other ^{1}H and ^{13}C NMR signals which were almost the same as those of compounds **2** and **4**, were assigned as shown in Table 2. These spectral data established the structure of compound **3** as 6-dimethoxymethyl-3,9-dimethylnaphtho(1,8-*b*,*c*)pyran-7,8-dione. It was considered that it might be an artefact formed during the methanol extraction from the corresponding aldehyde which was not detected in this study.

The ^{1}H and ^{13}C NMR assignments of the four known compounds, mansonones E, F, H and I, were performed with the aid of HMQC and HMBC spectral data, as shown in Tables 1 and 2. This is the first report to present the ^{13}C NMR spectral data for compound **7**. When compared with the literature [12], however, the carbon assignments of C-3a, C-6a, and C-9b of compounds **4** and **5** of the literature, which had been made with the aid of DEPT experiments, were not compatible with our data obtained in the same solvent. Our results correct the carbon chemical shift assignments for C-3a, C-6a, and C-9b of compounds **4** and **5** made with the aid of DEPT experiments [12].

The antioxidative activities of compounds **1**–**7** were evaluated by a thiobarbituric acid method [20] using rat liver microsomes. Figure 2 shows that compounds **1** and **3**–**5** are active, and the most active compound is **4**. Concentration effects of compounds **1**, **3** and **4** on lipid peroxidation of microsomes are shown in Fig. 3. The IC_{50} values of compounds **1**–**5** were 0.12, 6.90, 0.80, 0.03 and 0.04 μg ml^{-1}, respectively. When compared with α-tocopherol (IC_{50} 0.10 μg ml^{-1}), compounds **4** and **5** exhibited greater antioxidative activities in this system.

EXPERIMENTAL

General. Mps: uncorr. UV: MeOH. IR: KBr discs. NMR: 300 and 600 MHz for ^{1}H, and 75.5 and 150.8 MHz for ^{13}C in $CDCl_3$ or CD_3OD with TMS as int. standard. Chemical shifts are given in δ from TMS. The 2D-NMR spectroscopy experiments were carried out on a JEOL α-600 NMR spectrometer operating at 600.05/150.8 MHz with $^nJ_{CH}$ = 8.3 Hz for HMBC and $^1J_{CH}$ = 145 Hz for HMQC, using a pulse field gradient of GRAD 1 = 20% (28.8 G cm^{-1}) and GRAD 2 = 10% (14.4 G cm^{-1}). EI-MS (probe): 70 eV. HREI-MS: JEOL JMS-HX 10A mass spectrometer. Analytical TLC: silica gel (Merck, Kiesel gel 60F_{254}, 0.25 mm). Prep. TLC plates (Merck, Kiesel gel 60F_{254}, 0.5 mm) were used without activation. Prep. HPLC was performed on a C-18 Maxsil column (22.5 × 250 mm, 5 μm, Phenomenex) using H_2O–MeOH systems as a mobile phase, and by monitoring with a photodiode-array detector (190–650 nm).

Plant material. The root bark of *U. davidiana* was collected from Mt Wonhyo, Kyungnam Province, Korea in May 1994, and identified by Prof. Jong-Hee Park, College of Pharmacy, Pusan National University, Korea. Fresh root bark was dried in a dark, well-ventilated place. The voucher specimen is deposited in the Herbarium of this college.

Extraction and isolation of compounds. The dried root bark (4 kg) was milled and extracted with 80% aq. MeOH at room temp. for 3 days. The MeOH extract was filtered and concd under red. pres. The residue (185 g) was subjected to successive extraction with

n-hexane and $CHCl_3$. The $CHCl_3$ layer was concd under vacuum, and the residue (6.4 g) was applied to CC on a silica gel column eluted with $CHCl_3$ and $CHCl_3$–MeOH mixture with increasing proportion of MeOH. The frs were collected and combined by monitoring with analytical TLC to yield three frs (1–3) in order of increasing polarity. Fr. 1 was rechromatographed on a Si gel column eluting with $CHCl_3$–MeOH (100:1) and on Sephadex LH-20 with $CHCl_3$–MeOH (1:1) followed by C-18 (YMC-gel ODS-A, 60–230/70 μm) CC eluted with 40% and then 60% aq. MeOH. The 60% aq. MeOH-eluted fr. was subjected to silica gel prep. TLC and prep. HPLC (70% aq. MeOH) to yield **2** (1.9 mg), **3** (12.6 mg) and **4** (96.3 mg). Through the Sephadex LH-20 CC (MeOH) and Si gel prep. TLC, followed by prep. HPLC (60% aq. MeOH), **7** (14.4 mg) was purified from fr. 2, and **1** (12.2 mg) and **6** (10.3 mg) were obtained from fr. 3. From the *n*-hexane layer, compound **5** (183.0 mg) was purified by the combination of the above methods. Purity of compounds was confirmed by HPLC on a Cosmosil C-18 column (4.6 × 150 mm, 5 μm) with 65% aqueous MeOH at 1.0 ml min^{-1}.

Davidianone A (**1**). Violet coloured needles, mp 248°C, UV-vis λ_{max} nm (log ε): 232 (4.48), 255 (sh), 336 (3.58), 548 (3.61). IR λ_{max}^{KBr} cm^{-1}: 3380, 1680, 1625, 1595, 1565, 1380, 1330, 1245, 1190, 1160. HREI-MS *m/z* 256.0751 ($C_{15}H_{12}O_4$ requires 256.0736). EI-MS *m/z* (rel. int.): 258 $[M+2]^+$ (24), 256 $[M]^+$ (42), 228 $[M-CO]^+$ (100), 211 $[M-CO-OH]^+$ (16). ^1H and ^{13}C NMR: Tables 1 and 2.

Davidianone B (**2**). Violet coloured needles, mp 203°C, UV-vis λ_{max} nm (log ε): 232 (4.07), 255 (sh), 344 (3.18), 570 (3.06). IR λ_{max}^{KBr} cm^{-1}: 2955, 2850, 1737, 1695, 1625, 1605, 1575, 1380, 1365, 1290, 1230, 1190, 1145. HREI-MS *m/z* 284.0686 ($C_{16}H_{12}O_5$ requires 284.0685). EI-MS *m/z* (rel. int.): 286 $[M+2]^+$ (14) 284 $[M]^+$ (17), 256 $[M-CO]^+$ (100), 253 $[M-MeO]^+$ (42), 241 $[M-CO-Me]^+$ (11), 226 $[M-CO-2Me]^+$ (26), 213 $[M-2CO-Me]^+$ (29), 197 $[M-2CO-MeO]^+$ (49). ^1H and ^{13}C NMR: Tables 1 and 2.

Davidianone C (**3**). Violet coloured needles, mp 187°, UV-vis λ_{max} nm (log ε): 234 (4.16), 255 (sh), 336 (3.54), 570 (3.45). IR λ_{max}^{KBr} cm^{-1}: 2925, 2850, 1735, 1690, 1635, 1605, 1585, 1320, 1260, 1190, 1155, 1125, 1095, 1045. HREI-MS *m/z* 300.0994 ($C_{17}H_{16}O_5$ requires 300.0997). EI-MS *m/z* (rel. int.): 300 $[M]^+$ (43), 285 $[M-Me]^+$ (47), 272 $[M-CO]^+$ (8), 241 $[M-CO-MeO]^+$ (100). ^1H and ^{13}C NMR: Tables 1 and 2.

The known compounds **4–7** were characterized by comparison of their physico-chemical properties and spectral data with those of literature data [7, 12].

Antioxidative activity. Antioxidative activities were evaluated by the inhibitory activities of compounds against lipid peroxidation in rat liver microsomes according to the method of ref. [20] with minor modifications. Reaction was initiated by the addition of 100 μM $FeSO_4 \cdot 7H_2O$ into a mixture of ascorbic acid (0.2 mM) and microsomal suspension (0.5 μg protein ml^{-1}). Lipid peroxidation was assessed by measuring the thiobarbituric acid reactive products at 532 nm.

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