# Antitumor-Promoting Naphthoquinones from *Catalpa ovata*

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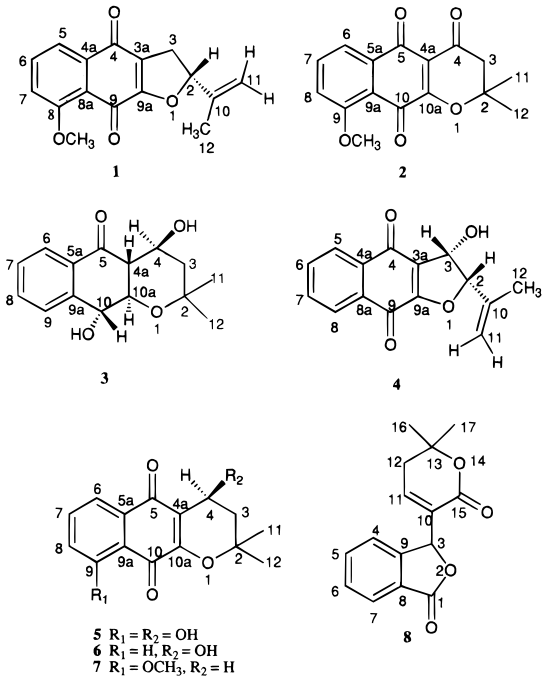
Bioassay-directed fractionation of an extract of the stem-bark of *Catalpa ovata* led to the isolation of three new naphthoquinones: 8-methoxydehydroiso-α-lapachone (**1**), 9-methoxy-4-oxo-α-lapachone (**2**), and (4*S*,4a*R*,10*R*,10a*R*)-4,10-dihydroxy-2,2-dimethyl-2,3,4,4α,10,10α-hexahydrobenzo[*g*]chromen-5-one (**3**), which is a 1,4-reductive form of **6**. The known compounds 3-hydroxydehydroiso-α-lapachone (**4**), 4,9-dihydroxy-α-lapachone (**5**), 4-hydroxy-α-lapachone (**6**), and 9-methoxy-α-lapachone (**7**), and catalpalactone (**8**) were also isolated. Their structures were elucidated by spectral methods. These compounds all exhibited significant inhibitory activity against 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced Epstein−Barr virus early antigen (EBV-EA) activation in Raji cells.

*Catalpa ovata* G. Don (Bignoniaceae), Japanese name "kisasage", is cultivated as an ornamental tree and is used as a crude drug in Japan and the central and south regions of China. In Japan, the fruits of the tree have been used for chronic nephritis and edema as a diuretic. In China, the root- and stem-bark have been used for the treatment of pyrexia, jaundice, and edema by nephritis, the fruits for chronic nephritis and albuminurea, and the leaves for burn. However, there is little published information on the chemical components of *C. ovata*.[1−3]

We previously isolated potent antitumor promoters from the bark of *Tabebuia avellanedae*, which is distributed in South America and belongs to the same family as *C. ovata*.[4] This prompted us to search for antitumor-promoting compounds from this plant. Bioassay-directed fractionation of the stem-bark extract of *C. ovata* led to the isolation of seven naphthoquinones **1**−**7**, including three new compounds and one phthalide derivative (**8**). In this paper, we describe structures of the new compounds (**1**−**3**) and in vitro antitumor-promoting activity of **1**−**8**.




## Results and Discussion

The air-dried stem-bark of *C. ovata* was extracted with MeOH by refluxing. The MeOH extract was partitioned between *n*-hexane and 90% aqueous MeOH. The residue from the aqueous MeOH layer was further partitioned between $CHCl_3$ and $H_2O$. The $CHCl_3$ layer, which showed antitumor-promoting activity, was purified by repeated Si gel column chromatography (CC) and preparative TLC to afford compounds **1**−**8**.

The HREIMS spectrum of **1** showed the molecular ion at *m*/*z* 270.0889, corresponding to the formula $C_{16}H_{14}O_4$. The IR and UV spectra of **1** suggested a naphthoquinone structure.[3] The $^1$H NMR spectrum showed aromatic proton signals (δ 7.25, 7.65, 7.74), an isopropenyl group (δ 1.78, 4.97, 5.11), a set of methylene protons (δ 2.99, 3.33), an oxymethine (δ 5.39), and an aromatic methoxyl group (δ 4.00). Thus, the structure of **1** was predicted to be a dehydroiso-α-lapachone analogue bearing a methoxyl group on the aromatic ring. The coupling constants and multiplicities of the aromatic protons suggested the methoxyl group to be at either C-5 or C-8. In the HMBC experiment, H-3 and H-5 showed correlations with C-4, and thus, the methoxyl group was determined to be attached to C-8. The absolute stereochemistry at C-2 was deduced to be *R* by analogy with (2*R*)-5-methoxydehydroiso-α-lapachone ($[\alpha]_D$ −17.4°).[5] Therefore, the absolute structure of **1** was assigned as (2*R*)-8-methoxydehydroiso-α-lapachone.

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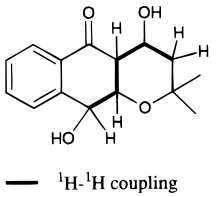


**Figure 1.** Planar structure of **3**.

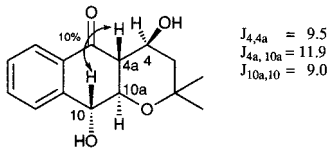


**Figure 2.** Difference NOE data and coupling constants of **3**.

The HREIMS spectrum of **2** showed the molecular ion at *m/z* 286.0835, corresponding to the formula $C_{16}H_{14}O_5$. Detailed analysis of the NMR data indicated that **2** is a naphthoquinone derivative analogous to the known compound (**7**)[3] bearing a 2,2-dimethylpyran ring system. The $^{13}$C NMR spectrum of **2** showed three carbonyl carbons, two of which were assigned to the naphthoquinone skeleton and the other assigned to either a 3-oxo or 4-oxo group at the pyran ring. The 4-oxo moiety was deduced from long-range connectivities between H-3 and C-11 and C-12 in the HMBC spectrum. From the coupling constants and multiplicities of the aromatic protons, the methoxyl group was assigned C-6. The HMBC spectrum showed a correlation between the proton signal at $\delta$ 7.81 (assignable to H-6) and the carbonyl signal appearing at lower field ($\delta$ 179.9). Considering the substitution pattern of the methoxyl group on the A ring in compounds **1** and **7** coexsisting in the same extract, the methoxyl group of **2** was more likely at C-9. Thus, **2** was determined to be 9-methoxy-4-oxo-α-lapachone.

The HREIMS spectrum of **3** showed the molecular ion at *m/z* 262.1210, corresponding to the formula $C_{15}H_{18}O_4$. The fact that **3** was colorless and did not show the UV spectrum characteristic of a naphthoquinone skeleton suggested that **3** is a dihydronaphthoquinone derivative lacking the conjugated ketone system. The $^{13}$C NMR data showed the presence of only one carbonyl carbon. The presence of geminal dimethyls in **3** and the comparison of the molecular formula with **6** ($C_{15}H_{14}O_4$) allowed us to deduce that the structure of **3** is the 1,4-reductive form of **6**. The planar structure of **3** was verified by the $^1$H,$^1$H−COSY spectrum as shown in Figure 1. The stereochemistry of **3** was elucidated from coupling constants and difference NOE data (Figure 2). The $J_{4,4a}$, $J_{4,10a}$, and $J_{10a,10}$ values were 9.5, 11.9, and 9.0 Hz respectively, suggesting that each pair of protons takes the *trans* diaxial form. These configurations were supported by difference NOE data in which irradiation of H-10 enhanced H-4a but not H-10a. Accordingly, the relative structure of **3** was determined as (4*S*,4a*R*,-10*R*,10a*R*)-4,10-dihydroxy-2,2-dimethyl-2,3,4,4a,10,10a-hexahydrobenzo[*g*]chromen-5-one.

3-Hydroxydehydroiso-α-lapachone (**4**), isolated previously from *R. sinica*,[6] was obtained in the present study as a mixture in which the 2*S*,3*S* stereoisomer predominates. This was determined from analysis of their respective CD spectra.[6]

**Table 1.** Relative Ratio of EBV-EA Activation with Respect to Positive Control in the Presence of *C. ovata* Bark Constituents

| | % to control (% viability)[a,b] at concentration (mol ratio/TPA) | | | |
|---|---|---|---|---|
| | 1000 | 500 | 100 | 10 |
| 1 | 0 (60) | 55.2 | 100 | 100 |
| 2 | 32.7 (70) | 63.8 | 88.1 | 100 |
| 3 | 0 (70) | 32.9 | 57.3 | 93.8 |
| 4 | 0 (30) | 0 (60) | 62.9 | 88.5 |
| 5 | 36.1 (60) | 88.4 | 100 | 100 |
| 6 | 11.2 (60) | 27.9 | 56.3 | 86.7 |
| 7 | 83.8 (70) | 92.0 | 100 | 100 |
| 8 | 0 (70) | 27.9 | 52.8 | 89.5 |
| β-carotene | 8.6 (70) | 34.2 | 82.1 | 100 |

[a] Values are EBV-EA activation (%) ± s.d. s (± 5.0%) in the presence of test compound relative to the positive control (100). The activation was caused by TPA (32pmol/mL). [b] Values in parentheses represent the viability % of Raji cells measured through Trypan Blue staining, followed by counting of the surviving cells 48 h after the concomitant treatment of the cells with TPA, *n*-butyrate, and test substances in a 0.25% phosphate buffer solution (pH 7.2).

Compounds **5**−**8** were identified by comparing their physical and spectral data with the literature values.[7,8]

Compounds **1**−**8** exhibited inhibitory activity against the 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced Epstein−Barr virus early antigen (EBV-EA) activation in a nonproducer Raji cell line[9−12] (Table 1). Compounds **3**, **4**, **6**, and **8** were found to be more effective than β-carotene, a vitamin A precursor that has been intensively studied in cancer prevention using animal models.[13] Structural comparison of **3**, **4**, and **6** with the other naphthoquinones indicated that the absence of substituents on the A ring and the presence of an alcoholic OH group on the C ring may be important structure−activity information. Furthermore, the present study showed that phthalide derivative **8** is also an antitumor promoter.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. UV spectra were recorded in MeOH using a Shimadzu UV−vis recording spectrophotometer UV-2200. IR spectra were recorded in $CHCl_3$ using a Shimadzu FTIR-8100A. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AC-300 spectrometer in $CDCl_3$ using TMS as the internal standard. Coupling constants (*J*) are given in Hz. For HMBC spectrum measurement, a JEOL LNM EX-400 FT-NMR spectrometer was used. Optical rotations were measured on a JASCO digital polarimeter DIP-181. CD spectra were recorded on a JASCO J-720 spectropolarimeter. CC was performed over silica gel 60 (70−230 and 230−400 mesh, Merck). Silica gel 60 $F_{254}$ (0.25 mm, Merck) and SIL G-50 (0.5 mm, Nagel) were used for analytical and preparative TLC, respectively.

**Plant Material.** The bark of *C. ovata* was collected at Kyoto University in October 1990. The voucher specimen have been deposited in the herbarium of the Department of Botany, Faculty of Science, Kyoto University.

**Extraction and Isolation.** The dried bark chips were extracted with MeOH using a reflux condenser. The MeOH extract was evaporated in vacuo and the

residue partitioned between *n*-hexane and 90% aqueous MeOH, followed by $CHCl_3$ and $H_2O$. The $CHCl_3$ layer was evaporated in vacuo, and a portion (66 g) of the total $CHCl_3$ extract (128 g) was subjected to Si gel flash chromatography eluting with $CHCl_3$ followed by stepwise addition of MeOH to yield 15 fractions. Fractions 4 and 6 exhibited the inhibitory activity against the TPA-induced EBV-EA activation in Raji cells. Fraction 4 (1.2 g) was chromatographed on Si gel with *n*-hexane/EtOAc and $CHCl_3$/MeOH gradient systems to yield 10 fractions. Fraction 4-2 (33 mg) was purified by preparative Si gel TLC developed with $CHCl_3/C_6H_6$/MeOH = 80:180:5 to afford compounds **4** (1.8 mg), **5** (5.4 mg), and **6** (2.8 mg). Fraction 4-4 (493 mg) was purified by preparative Si gel TLC using *n*-hexane/EtOAc = 60:40 and $CHCl_3$ as developing solvents to yield compounds **3** (2.0 mg) and **8** (250 mg). Fraction 4-5 (1 g) was chromatographed on Si gel eluting with *n*-hexane/EtOAc and $CHCl_3$/MeOH gradients followed by preparative Si gel TLC using $CHCl_3$ to yield compound **1** (3.2 mg). Fraction 6 (1.2 g) was chromatographed on Si gel with *n*-hexane/EtOAc and $CHCl_3$/MeOH gradients to yield seven fractions. Fraction 6-1 (42 mg) was purified by preparative Si gel TLC, developing with *n*-hexane/EtOAc = 40:60, to yield compound **2** (9.9 mg). Fraction 6-2 (137 mg) was purified by preparative Si gel TLC, developing with *n*-hexane/EtOAc = 50:50 to yield compound **7** (36.4 mg). Fraction 6-3 (61 mg) was purified by preparative Si gel TLC, developing with *n*-hexane/EtOAc = 50:50 and $C_6H_6$/EtOAc = 90:10 to yield compounds **1** (13.2 mg) and **8** (4.0 mg).

**(2*R*)-8-Methoxydehydroiso-α-lapachone (1):** yellow needles; mp 133−134 °C; $[\alpha]^{18}_D$ −26.2° (*c* 0.42, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 225 (4.18), 243 (sh) (4.13), 286 (3.99), 392 (3.63) nm; IR ($CHCl_3$) $\nu_{max}$ 3020, 2930, 1671, 1638, 1585, 1471, 1439, 1398, 1370, 1278, 1253, 1180, 1070, 968, 826 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.39 (1H, dd, $J$ = 8.3, 10.9, H-2), 2.99 (1H, dd, $J$ = 8.3, 16.8, H-3a), 3.33 (1H, dd, $J$ = 10.9, 16.8, H-3b), 7.74 (1H, dd, $J$ = 1.2, 7.6, H-5), 7.65 (1H, t, $J$ = 7.6, H-6), 7.25 (1H, dd, $J$ = 1.2, 7.6, H-7), 4.97 (1H, brs, H-11a), 5.11 (1H, brs, H-11b), 1.78 (3H, brs, H-12), 4.00 (3H, s, $OCH_3$); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 88.2 (C-2), 31.8 (C-3), 121.4 (C-3a), 181.8 (C-4), 135.6 (C-4a), 119.1 (C-5), 135.3 (C-6), 117.5 (C-7), 160.3 (C-8), 119.1 (C-8a), 176.7 (C-9), 160.9 (C-9a), 141.9 (C-10), 113.6 (C-11), 16.8 (C-12), 56.5 ($OCH_3$); EIMS *m*/*z* 270 $\{M\}^+$ (64.4), 255 (53.2), 242 (64.2), 227 (70.0), 199 (10.4), 173 (22.0), 163 (15.3), 149 (20.5), 134 (29.5), 104 (17.3), 76 (25.6), 57 (19.1); HREIMS *m*/*z* 270.0889 (calcd for $C_{16}H_{14}O_4$ 270.0892).

**9-Methoxy-4-oxo-α-lapachone (2):** yellow solid; mp 118 °C; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 217 (4.16), 240 (sh) (3.88), 282 (3.84), 394 (3.38) nm; IR ($CHCl_3$) $\nu_{max}$ 2928, 2369, 1723, 1709, 1688, 1682, 1584, 1572, 1397, 1300, 1283, 1238, 1179, 1021, 750 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 2.73 (2H, s, H-3), 7.81 (1H, dd, $J$ = 1.2, 8.0), 7.72 (1H, t, $J$ = 8.0), 7.26 (1H, dd, $J$ = 1.2, 8.0), 1.59 (3H, s, H-11), 1.59 (3H, s, H-12), 4.00 (3H, s, $OCH_3$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 84.3 (C-2), 48.5 (C-3), 189.7 (C-4), 112.0 (C-4a), 179.9 (C-5), 134.3 (C-5a), 119.5 (C-6), 136.5 (C-7), 116.9 (C-8), 159.8 (C-9), 119.3 (C-9a), 178.3 (C-10), 163.3 (C-10a), 26.1 (C-11), 26.1 (C-12), 56.5 ($OCH_3$); EIMS *m*/*z* 286 $\{M\}^+$ (100), 271 (13.0), 257 (2.2), 231 (59.2), 230 (43.6), 203 (27.8), 173 (51.9), 156 (6.9), 134 (12.2), 104 (12.7), 76 (14.6); HREIMS *m*/*z* 286.0835 (calcd for $C_{16}H_{14}O_5$ 286.0841).

**(4*S*,4a*R*,10*R*,10a*R*)-4,10-dihydroxy-2,2-dimethyl-2,3,4,4a,10,10a-hexahydrobenzo[*g*]chromen-5-one (3):** colorless solid; $[\alpha]^{17}_D$ +15.8° (*c* 0.3, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 248 (3.90), 290 (3.05) nm; IR ($CHCl_3$) $\nu_{max}$ 3400, 2984, 1734, 1719, 1671, 1603, 1561, 1543, 1459, 1368, 1300, 1258, 1136, 1128, 1071, 1022, 941 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.57 (1H, dd, $J$ = ∼9.0, 13.0, H-3a), 2.00 (1H, dd, $J$ = 4.7, 13.0, H-3b), 4.35 (1H, ddd, $J$ = 4.7, ∼9.0, 9.5, H-4), 2.30 (1H, dd, $J$ = 9.5, 11.9, H-4a), 8.02 (1H, dd, $J$ = 1.4, 7.8, H-6), 7.45 (1H, brt, $J$ = 7.8, H-7), 7.70 (1H, dt, $J$ = 1.4, 7.8, H-8), 7.85 (1H, brd, $J$ = 7.8, H-9), 4.94 (1H, d, $J$ = 9.0, H-10), 3.72 (1H, dd, $J$ = 9.0, 11.9, H-10a), 1.26 (3H, s, H-11), 1.34 (3H, s, H-12), 4.39 (1H, brs, 4-OH), 3.09 (1H, brs, 10-OH); $^{13}C$ NMR ($CDCl_3$) $\delta$ 74.1 (C-2), 43.3 (C-3), 65.4 (C-4), 55.4 (C-4a), 199.4 (C-5), 130.1 (C-5a), 126.6 (C-6), 128.1 (C-7), 135.2 (C-8), 126.4 (C-9), 142.8 (C-9a), 71.8 (C-10), 74.0 (C-10a), 23.2 (C-11), 31.3 (C-12); EIMS *m*/*z* 262 $\{M\}^+$ (7.7), 244 (100), 229 (10.8), 215 (5.49), 187 (11.6), 160 (14.59), 144 (20.8), 134 (97.6), 111 (56.0), 105 (31.4), 77 (14.2); HREIMS *m*/*z* 262.1210 (calcd for $C_{15}H_{18}O_4$ 262.1205).

**3-Hydroxydehydroiso-α-lapachone (4):** yellow needles; mp 164−165 °C; $[\alpha]^{19}_D$ +14.0° (*c* 0.3, $CHCl_3$); CD (MeOH) $\theta$ (nm) −8890 (207), +4005 (230), +4337 (248), −4617 (296), +1986 (362), +1063 (330); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 245 (4.10), 250 (4.14), 282 (3.93), 333 (3.30) nm; IR ($CHCl_3$) $\nu_{max}$ 3659, 3028, 2928, 1686, 1655, 1630, 1595, 1365, 1279, 953 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 5.13 (1H, d, $J$ = 4.2, H-2), 5.41 (1H, d, $J$ = 4.2, H-3), 8.10 (1H, dd, $J$ = 1.6, 7.3, H-5), 7.77 (1H, dt, $J$ = 1.6, 7.3, H-6), 7.72 (1H, dt, $J$ = 1.6, 7.3, H-7), 8.13 (1H, dd, $J$ = 1.6, 7.3, H-8), 5.02 (1H, brs, H-11a), 5.17 (1H, brs, H-11b), 1.80 (3H, brs, H-12), 3.65 (1H, brs, 3-OH); $^{13}C$ NMR ($CDCl_3$) $\delta$ 95.4 (C-2), 75.0 (C-3), 123.8 (C-3a), 182.6 (C-4), 133.0 (C-4a), 126.2 (C-5), 134.6 (C-6), 133.4 (C-7), 126.7 (C-8), 131.8 (C-8a), 178.1 (C-9), 160.8 (C-9a), 139.3 (C-10), 114.1 (C-11), 17.3 (C-12); EIMS *m*/*z* 256 $\{M\}^+$ (100), 238 (13.3), 227 (22.8), 215 (5.0), 199 (12.3), 173 (10.5), 149 (11.5), 105 (8.8), 102 (8.8), 76 (8.9); HREIMS *m*/*z* 256.0742 (calcd for $C_{15}H_{12}O_4$ 256.0736).

***In Vitro* EBV-EA Activation Experiments.** The inhibition of EBV-EA activation was assayed using the same method described previously.[4]

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