# Studies on the Preparation of Bioactive Lignans by Oxidative Coupling Reaction. VI.[1] Oxidation of Methyl (*E*)-3-(4,5-Dihydroxy-2-methoxyphenyl)-2-butenoate and Lipid Peroxidation-Inhibitory Effects of the Produced Caffeoquinone

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**Oxidation of hydroxy-α-methylcinnamate 5 derived from 4-methylesculetin afforded the α-methylcaffeoquinone derivative 7 without formation of the oxidative coupling product. The reaction of the α-methylferulate derivative 13 afforded a complex mixture of products. Thus, the hydroxy-α-methylcinnamates seem not to be suitable substrates for oxidative coupling.**

**Compound 7 was tested for inhibitory effect on lipid peroxidation. It showed more potent activity than idebenone in rat brain homogenate,and was much more potent than (±)-α-tocopherol in rat liver microsomes.**

**Key words** α-methylcaffeoquinone; oxidative coupling; lipid peroxidation inhibitory effect; 4-methylesculetin; 3′-methylcinnamate

In parts II—V of this series of papers,[1-4] we dealt with the oxidative coupling reaction of hydroxycinnamate derivatives obtained from coumarins, and the products were examined for the ability to inhibit lipid peroxidation. Various types of products were obtained depending upon the substitution pattern of the benzene ring and the position of the free hydroxy group, and some of them showed prominent anti-peroxidative activity. Since 4-methylcoumarins are easily accessible through the Pechmann reaction of phenols with acetoacetate,[5] we were interested in the oxidative coupling reaction of hydroxy-α-methylcinnamate derivatives obtained from 4-methylcoumarins. The presence of the α-methyl group in the hydroxycinnamate substrate might influence the mode of the coupling reaction, leading to the formation of novel products. First, we examined the oxidation of the hydroxy-α-methylcinnamate **5** obtained from 4-methylesculetin (**1**) and then that of α-methyl ferulate **13** for comparison.

## Results and Discussion

**Preparation and Oxidative Coupling Reactions of Methyl (*E*)-3-(4,5-Dihydroxy-2-methoxyphenyl)-2-butenoate (5) and Ethyl (*E*)-3-(4-Hydroxy-3-methoxyphenyl)-2-butenoate (13)** When the bismethoxymethoxy (MOM) derivative **2**, derived from 4-methylesculetin (**1**), was treated with sodium methoxide in MeOH to open the coumarin ring, the reaction proceeded slowly. After refluxing of the reaction mixture for 10 d, a monodemethoxymethylated compound **3** was obtained in 37% yield and none of the desired ring-opened product was detected. The remaining MOM group was located at the C-6 position, based on the observation of a correlation between the signals of the hydroxyl proton ($\delta$ 7.13) and the aromatic carbons at the 6, 7 and 8 positions ($\delta$ 142.0, 150.5, 103.6) in the correlation spectroscopy *via* long-range coupling (COLOC) NMR technique. The unusual cleavage of the 7-MOM group by the methoxide anion, which is a relatively poor nucleophile, is noteworthy, and may be ascribed to stabilization of the transition state through electron delocalization involving the lactone carbonyl group, as shown in Chart 2.

Subsequently the bis-MOM compound **2** was subjected to opening of the coumarin ring with concomitant etherification by using dimethyl sulfate and aqueous NaOH solution, which afforded the desired product **4** in 62% yield. Removal of the MOM groups and esterification of the carboxyl group in **4** were achieved simultaneously with MeOH and a catalytic amount of HCl to give the oxidation substrate **5** in 31% yield. The observation of nuclear Overhauser effect (NOE, 4, 8%) between the H-$\beta$ signal ($\delta$ 5.90) and the H-6 signal ($\delta$ 6.68) indicated that the trisubstituted double bond in **5** has *E*-configuration.

Compound **5** was treated with silver oxide in benzene–acetone at room temperature to afford a single product **7** in 75% yield. The molecular formula of **7** was determined to be $C_{12}H_{12}O_5$ by elemental analysis and MS measurement [*m/z*: 236 ($M^+$)], indicating that **7** was a simple oxidation product. This suggested that **7** would be an *ortho*-quinone formed by oxidation of the *ortho*-dihydroxy groups in **5**. The appearance of two signals at $\delta$ 177.7 and 180.1 in the $^{13}$C-NMR spectrum was consistent with this supposition. When the oxidation of **5** was conducted with potassium hexacyanoferrate(III) and 1% aqueous $Na_2CO_3$ solution in $CHCl_3$ at room temperature, the same *ortho*-quinone **7** was obtained in lower yield (21%). Some data have been reported for caffeoquinone derivatives,[6-8] but not for the α-methylcaffeoquinone derivative **7**. We found that one-electron oxidation of the hydroxycinnamate derivative **5** with the α-methyl group in the side chain failed to result even in $\beta$–$\beta'$ coupling (desired product, **6**), but instead led to the formation of the *ortho*-quinone **7**.

Next we examined the oxidative coupling reaction of the α-methyl ferulate analog **13** in order to see whether the coupling reaction could occur for the 4-methyl substrate without the easily oxidizable *ortho* dihydroxy groups. Acetovanillone (**10**) was converted to the MOM

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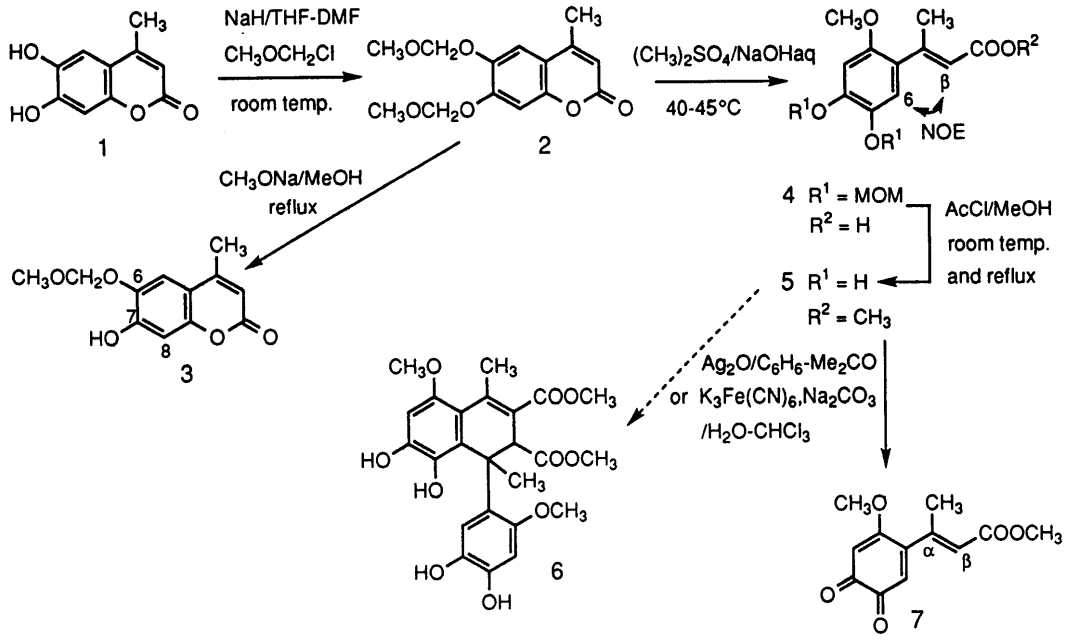


Chart 1

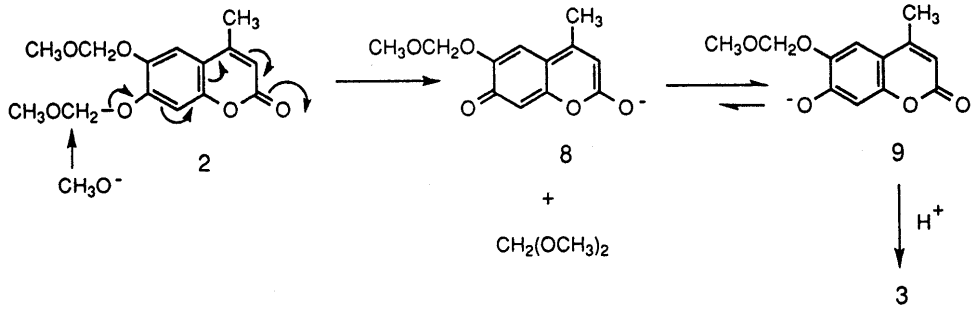


Chart 2

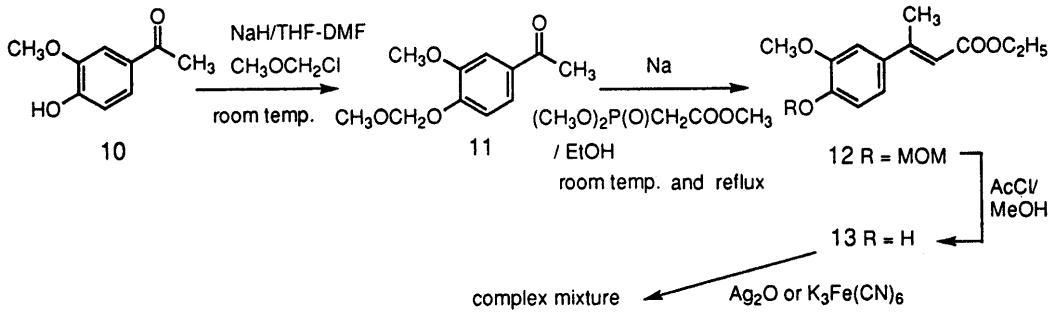


Chart 3

derivative **11**, which was treated with trimethyl phosphonoacetate to give α-methyl MOM cinnamate **12** in 97% yield, and deprotection gave the desired compound **13** in 76% yield. The oxidative coupling reaction has been investigated using silver oxide, potassium hexacyanoferrate(III) and iron(III) chloride.[1-4,9] However, the reaction of **13** with silver oxide in acetone–benzene or potassium hexacyanoferrate(III)–$Na_2CO_3$ in $CHCl_3$ at room temperature led to the formation of a complex mixture of inseparable products. Treatment of **13** with iron(III) chloride in aqueous acetone for 3 d at room temperature resulted in recovery of the starting material. Thus, β–β′ coupling was not the major course, at least in the reaction of the hydroxy-α-methylcinnamate, which might indicate instability of the β-radical derived from it due to the *peri* type strain between the methyl group and the *ortho* hydrogen atom. Thus, hydroxy-α-cinnamate derivatives are not suitable substrates for oxidative coupling.

**Inhibitory Effect on Lipid Peroxidation** We examined the effect of compound **7** on lipid peroxidation in rat brain homogenate and rat liver microsomes, according to the method described in a previous paper.[9] The results are summarized in Table 1.

Interestingly, in rat brain homogenate, the *o*-benzoquinone compound **7** was more potent than the *p*-quinone, idebenone, which is a nootropic drug. Furthermore, when compound **7** was tested in rat liver microsomes, it was found to be much more potent than that (±)-α-

Table 1. Inhibitory Effect of the Caffeoquinone on Lipid Peroxidation in Rat Brain Homogenate[a] and Rat Liver Microsomes[b]

| Compound | Inhibition (%)[c] Brain homogenate | | | $IC_{50}$ ($10^{-6}$ M)[d] Liver microsomes |
|---|---|---|---|---|
| | $10^{-4}$ M | $10^{-5}$ M | $10^{-6}$ M | |
| **7** | 99 | 97 | 34 | 3.24 (3.11—3.40) |
| Idebenone | 93 | 27 | — | — |
| (±)-α-Tocopherol | — | — | — | 976 (880—1149) |

*a*) Control values of MDA production were 250—300 nmol/g wet tissue. *b*) Control values of MDA production were 20—28 nmol/g protein. *c*) The inhibition (%) values are the averages of three or four experiments. *d*) $IC_{50}$ values and their 95% confidence limits were calculated by probit analysis by using 4 determinations at 3—4 different concentrations (geometric ratio = 1.4) for each compound.

tocopherol.

## Experimental

Details of the analytical procedures used and the evaluation of inhibitory effects on lipid peroxidation have been given in Parts I and III of this series of papers.[3,9]

**Bis(methoxymethyl)-4-methylesculetin (2)** A solution of 4-methylesculetin (**1**) (100.0 g, 0.52 mol) in dry tetrahydrofuran (THF)–*N*,*N*-dimethylformamide (DMF) (900 ml, 5:3) was added dropwise to a suspension of sodium hydride (60%, in oil) (45.8 g, 1.14 mol) in dry THF–DMF (1.3 l, 5:1) at 0 °C under nitrogen atmosphere. The mixture was stirred at room temperature for 3 h, and then chloromethyl methyl ether (83 ml, 1.09 mol) was added dropwise at 0 °C. Stirring was continued at room temperature for 17 h, then the mixture was worked up as usual to give, after recrystallization from MeOH, **2** (116.1 g, 80%) as yellow scales, mp 96—98 °C. IR (KBr): 1715 (C=O) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 2.40 (3H, d, $J$=1 Hz, 4-$CH_3$), 3.52, 3.55 (each 3H, s, $CH_2OCH_3$ × 2), 5.27, 5.31 (each 2H, s, $CH_2OCH_3$ × 2), 6.18 (1H, d, q, $J$=1 Hz, 3-H), 7.16, 7.32 (each 1H, s, ArH × 2). *Anal.* Calcd for $C_{14}H_{16}O_6$: C, 59.99; H, 5.77. Found: C, 60.11; H, 5.77.

**6-Methoxymethyl-4-methylesculetin (3)** To a solution of **2** (60.0 g, 0.21 mol) in dry MeOH (600 ml) was added a sodium methoxide solution (28% in MeOH) (330 ml, 1.7 mol) and the mixture was refluxed for 10 d. The reaction mixture was concentrated and poured into ice-water, and extracted with ether. The aqueous portion was acidified with 6 M HCl and extracted with AcOEt. The extract was purified by chromatography on a silica gel column ($CH_2Cl_2$–EtOH, 98:2) and recrystallization from benzene, giving **3** (26.2 g, 39%) as colorless scales, mp 102—103 °C. IR (KBr): 3287 (OH), 1702 (C=O) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 2.38 (3H, d, $J$=1 Hz, 4-$CH_3$), 3.56 (3H, s, $CH_2OCH_3$), 5.26 (2H, s, $CH_2OCH_3$), 6.14 (1H, q, $J$=1 Hz, 3-H), 6.92 (1H, s, 8-H), 7.13 (1H, s, OH), 7.27 (1H, s, 5-H). $^{13}$C-NMR ($CDCl_3$) δ: 18.8 (4-$CH_3$), 56.5 ($CH_2OCH_3$), 96.4 ($CH_2OCH_3$), 103.6 (C8), 110.3 (C5), 111.9 (C3), 112.7 (C4a), 142.0 (C6), 150.2 (C8a), 150.5 (C7), 152.8 (C4), 161.7 (C2). *Anal.* Calcd for $C_{12}H_{12}O_5$: C, 61.01; H, 5.13. Found: C, 61.05; H, 5.09. MS *m/z*: 236 ($M^+$).

**(*E*)-3-[4,5-Bis(methoxymethoxy)-2-methoxyphenyl]-2-butenoic Acid (4)** Compound **2** (44.5 g, 0.16 mol) was dissolved in 29% NaOH solution (600 ml) by heating and dimethyl sulfate (309 ml, 3.2 mol) was added dropwise to this solution at 40—45 °C during 8 h. The mixture was stirred at room temperature for 17 h. After the excess of dimethyl sulfate was decomposed by the addition of 5% ammonia solution, the mixture was acidified with 6 M HCl-ice and extracted with AcOEt. The residue obtained by evaporation of the solvent was recrystallized from MeOH, giving **4** (31.0 g, 62%) as colorless prisms, mp 119—120 °C. IR (KBr): 1699 (C=O) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 2.14 (3H, d, $J$=1 Hz, $ArCH_3C$=), 3.47, 3.53, 3.73 (each 3H, s, $ArOCH_3$ and $CH_2OCH_3$ × 2), 5.10, 5,24 (each 2H, s, $CH_2OCH_3$ × 2), 5.90 (1H, q, $J$=1 Hz, =CHCOO), 6.77, 6.86 (each 1H, s, ArH × 2). *Anal.* Calcd for $C_{15}H_{20}O_7$: C, 57.68; H, 6.47. Found: C, 58.08; H, 6.49.

**Methyl (*E*)-3-(4,5-Dihydroxy-2-methoxyphenyl)-2-butenoate (5)** Acetyl chloride (3 ml) was added to a solution of **4** (30.0 g, 0.10 mol) in dry MeOH (900 ml), and the reaction mixture was stirred at room temperature for 16 h, then refluxed for 1 h. The solution was neutralized with aqueous saturated $NaHCO_3$, and concentrated. Ice-water was added to the residue, and then the mixture was extracted with AcOEt. The extract was purified by chromatography on a silica gel column (*n*-hexane–AcOEt, 5:1) and recrystallization from benzene, giving **5** (7.0 g, 31%) as pale yellow scales, mp 112—113 °C. IR (KBr): 3518, 3338 (OH), 1699 (C=O) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 2.45 (3H, d, $J$=1 Hz, $ArCH_3C$=), 3.73, 3.74 (each 3H, s, $ArOCH_3$ and $COOCH_3$), 5.72, 5.88 (each 1H, s, OH × 2), 5.90 (1H, q, $J$=1 Hz, =CHCOO), 6.53 (1H, s, 3-H), 6.68 (1H, s, 6-H). *Anal.* Calcd for $C_{12}H_{14}O_5$: C, 60.49; H, 5.93. Found: C, 60.48; H, 5.87.

**Oxidation of 5 with Silver Oxide** Compound **5** (1.0 g, 4.2 mmol) was dissolved in benzene–acetone (60 ml, 2:1) and stirred with silver oxide (0.74 g, 3.2 mmol) at room temperature for 21 h under nitrogen atmosphere. The suspension was filtered, the filtrate was evaporated and the residue was recrystallized from ether, giving methyl (*E*)-3-(6-methoxy-3,4-dioxo-1,5-cyclohexadien-1-yl)-2-butenoate (**7**) (0.75 g, 75%) as red-yellow needles, mp 115—116 °C. IR (KBr): 1720, 1658 (C=O) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 2.37 (3H, d, $J$=1 Hz, $ArCH_3C$=), 3.77 (3H, s, $COOCH_3$), 3.91 (3H, s, $ArOCH_3$), 5.84 (1H, s, 6-H), 5.94 (1H, q, $J$=1 Hz, =CHCOO), 6.23 (1H, s, 3-H). $^{13}$C-NMR ($CDCl_3$) δ: 18.8 ($ArCH_3C$=), 51.3 ($COOCH_3$), 57.1 ($ArOCH_3$), 103.3 (C6), 120.4 (=CHCOO), 128.5 (C3), 150.8 (C1), 152.6 ($ArCH_3C$=), 165.6 ($COOCH_3$), 167.3 (C2), 177.7 (C4), 180.1 (C5). *Anal.* Calcd for $C_{12}H_{12}O_5$: C, 61.01; H, 5.13. Found: C, 60.93; H, 4.94. MS *m/z*: 236 ($M^+$).

**Oxidation of 5 with Potassium Hexacyanoferrate(III)–Sodium Carbonate** A solution of potassium hexacyanoferrate(III) (2.8 g, 8.5 mmol) and anhydrous $Na_2CO_3$ (1.3 g, 12 mmol) in water (140 ml) was added dropwise to a solution of **5** (2.0 g, 8.4 mmol) in $CHCl_3$ (800 ml) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 1 h at 0 °C and for another 2 h at room temperature, then the organic layer was separated and the water layer was extracted with $CH_2Cl_2$. The combined organic layers were washed with saturated aqueous NaCl, dried over $MgSO_4$, and evaporated to leave a residue, which was purified by chromatography on a silica gel column ($CH_2Cl_2$–EtOH, 99:1) and recrystallization from ether, giving **7** (0.4 g, 21%).

**Methoxymethyl Acetovanillone (11)** A solution of acetovanillone (**10**) (50.0 g, 0.30 mol) in dry THF–DMF (300 ml, 5:3) was added dropwise to a suspension of sodium hydride (60%, in oil) (13.2 g, 0.33 mol) in dry THF–DMF (500 ml, 5:1) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 3 h at room temperature, then chloromethyl methyl ether (24 ml, 0.32 mol) was added dropwise at 0 °C. Stirring was continued for another 2 h at room temperature, then the mixture was concentrated. Ice-water was added, and the product was extracted with AcOEt. Recrystallization from EtOH gave **11** (41.4 g, 66%) as colorless needles, mp 50—52 °C. IR (KBr): 1672 (C=O) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 2.57 (3H, s, $COCH_3$), 3.52 (3H, s, $CH_2OCH_3$), 3.94 (3H, s, $OCH_3$), 5.31 (2H, s, $CH_2OCH_3$), 7.16—7.20 (1H, m, ArH), 7.52—7.55 (2H, m, ArH × 2).

**Ethyl (*E*)-3-(3-Methoxy-4-methoxymethoxyphenyl)-2-butenoate (12)** Trimethyl phosphonoacetate (25 ml, 0.15 mol) was added dropwise to a solution of Na (3.8 g, 0.17 mol) in dry EtOH (300 ml) at 0 °C. The mixture was stirred for 10 min at 0 °C, then a solution of **11** (31.5 g, 0.15 mol) in dry EtOH (100 ml) was added. The whole was stirred for 16 h at room temperature, refluxed for 23 h, poured into 6 M HCl-ice water, and extracted with AcOEt. The residue obtained by usual work-up was purified by chromatography on a silica gel column (*n*-hexane–AcOEt, 5:2), giving **12** (40.6 g, 97%) as colorless oil. IR (KBr): 1710 (C=O) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 1.32 (3H, t, $J$=7 Hz, $CH_2CH_3$), 2.57 (3H, m, $ArCH_3C$=), 3.52 (3H, s, $CH_2OCH_3$), 3.92 (3H, s, $ArOCH_3$), 4.22 (2H, q, $J$=7 Hz, $CH_2CH_3$), 5.26 (2H, s, $CH_2OCH_3$), 6.11 (1H, m, =CHCOO), 7.02—7.16 (3H, m, ArH × 3).

**Ethyl (*E*)-3-(4-Hydroxy-3-methoxyphenyl)-2-butenoate (13)** Acetyl chloride (3 ml) was added to a solution of **12** (20.0 g, 71.3 mol) in dry MeOH (400 ml), and the reaction mixture was stirred at room temperature for 16 h. Work-up as before gave an oily product, which was distilled under reduced pressure, giving **13** (12.8 g, 76%) as colorless oil, bp 162 °C (3 mmHg). $^1$H-NMR ($CDCl_3$) δ: 1.32 (3H, t, $J$=7 Hz, $CH_2CH_3$), 2.56 (3H, m, $ArCH_3C$=), 3.92 (3H, s, $ArOCH_3$), 4.21 (2H, q, $J$=7 Hz, $CH_2CH_3$), 5.85 (1H, s, OH), 6.10 (1H, m, =CHCOO), 6.89—7.26 (3H, m, ArH × 3).

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**References and Notes**

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