TWO NEW PHENOLIC COMPOUNDS, KUWANOLS C AND D, FROM THE ROOT BARK OF A MULBERRY TREE REDIFFERENTIATED FROM THE CALLUS TISSUES

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Abstract — A new isoprenoid-substituted flavanone, kuwanol C, and a new isoprenoid-substituted chalcone, kuwanol D, were isolated from the root bark of a mulberry tree redifferentiated from the callus tissues along with eight known phenolic compounds. On the basis of spectral evidence, structures of kuwanols C and D were shown to be 1 and 2, respectively.

We have already reported a series of isoprenoid-substituted phenolic compounds isolated from the root bark of mulberry trees. Some of those phenolic compounds showed interesting biological activities. While from the callus tissues induced from seedlings of Morus alba L., six natural Diels-Alder type adducts of prenylchalcone derivatives and dehydroprenylphenols have been isolated along with phytosterols. In continuation of these studies, we examined the constituents of the root bark of a mulberry tree redifferentiated from the callus tissues induced from the seedlings of M. alba. This paper describes the isolation of phenolic compounds and the characterization of two new phenolic compounds kuwanols C and D. The TLC and HPLC profiles of the methanol extract of the root bark of a mulberry tree redifferentiated from the callus tissues were similar to those of a cultivated mulberry tree described in the previous papers.

From the methanol extract of the root bark of a mulberry tree redifferentiated from the callus tissues, kuwanols C (1) and D (2) were isolated along with eight known phenolic compounds, morusin (3), kuwanons U (4), S (5), morachalcone A (6), 2,2',4,4'-tetrahydroxychalcone (7), moracins M (8), O (9), and P (10). Kuwanol C (1), an amorphous powder, \([\alpha]_D^{22} -10^\circ\), gave the EI-ms spectrum which
Table 1

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<th>C-9-H</th>
<th>C-10-H</th>
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<tr>
<td>1</td>
<td>6.63</td>
<td>5.58</td>
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<tr>
<td>1\sa</td>
<td>6.53</td>
<td>5.77</td>
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<td>(\Delta)</td>
<td>+0.10</td>
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\(\text{R=H}\)

\(\text{R=Ac}\)

**Fig. 1**

**Fig. 2**

\(\text{\(^1\text{H} Nmr\) chemical shift values for C-9-H (\(\delta\) in acetone-d\(_6\))}\)
showed the molecular ion peak at \( m/z \) 422. Treatment of \( \mathbf{1} \) with acetic anhydride in pyridine yielded the triacetate \( (\mathbf{1a}, \text{M}^+ 548) \). The compound \( \mathbf{1} \) exhibited positive ferric chloride reaction, magnesium hydrochloric acid test, and sodium borohydride test. The IR and UV spectra of \( \mathbf{1} \) disclosed the following data: IR \( \nu_{\text{KBr}}^{\text{max}} \) 3350 (br), 2900, 1640 (sh), 1620, 1600 (sh), 1570 (sh) cm\(^{-1}\); UV \( \lambda_{\text{EtOH}}^{\text{max}} \) 225, 268 (sh), 275, 295 (inf), 308 (sh) nm. The \( ^{1}H \) NMR spectrum of \( \mathbf{1} \) showed the characteristic proton signals at C-2 and C-3 positions of a flavanone skeleton \( [\delta 2.75 (1H, dd, J=3 \text{ and } 17 \text{ Hz}), 3.21 (1H, dd, J=13 \text{ and } 17 \text{ Hz})] \), and showed the hydrogen-bonded hydroxyl group \( [\delta 12.30 (1H, s)] \). These data suggest that kuwanol C \( (\mathbf{1}) \) possesses a flavanone skeleton. The NMR spectrum also revealed the presence of a 2-methyl-2-(4-methylpent-3-enyl)pyran ring \( [\delta 1.41, 1.56, 1.65 (each 3H, s), 2.04-2.07 (2H, m), 2.08-2.15 (2H, m), 5.11 (1H, m), 5.58 (1H, d, J=10 Hz)], 6.63 (1H, d, J=10 Hz)] \), ABC type aromatic protons \( [\delta 6.44 (1H, dd, J=2 \text{ and } 8 Hz), 6.48 (1H, d, J=2 Hz), 7.31 (1H, d, J=8 Hz)] \), and an aromatic proton \( [\delta 5.91 (1H, s)] \). Compound \( \mathbf{1} \) did not show aluminum chloride induced shift and it suggests that the ortho position of the hydrogen-bonded hydroxyl group is occupied by an isoprenoid substituent. The MS of \( \mathbf{1} \) showed the significant peak at \( m/z 203 (\mathbf{1}) \) arising from the A ring by a retro Diels-Alder fragmentation. The linear structure for kuwanol C was supported by the changes of chemical shifts in the pyran olefinic protons in \( \mathbf{1} \) compared with those in triacetate \( (\mathbf{1a}) \) (Table 1). These changes supported that the hydroxyl group is peri to C-9-H. From the above results, the structure of kuwanol C is represented by formula \( \mathbf{1} \) except the stereochemistry. Kuwanol D \( (\mathbf{2}) \), a yellow amorphous powder, gave the EI-MS spectrum which showed the molecular ion peak at \( m/z 408 \), and exhibited a positive ferric chloride reaction. The IR spectrum of \( \mathbf{2} \) disclosed absorption bands for hydroxyl and conjugated carbonyl groups, and aromatic ring as follows: IR \( \nu_{\text{KBr}}^{\text{max}} \) 3400 (br), 1660 (sh), 1640 (sh), 1610, 1590, 1560 (sh). The UV spectrum exhibited a resemblance to those of chalcone derivatives, showing absorption maxima at 260, 291 and 386 nm, which showed the red shifts in the presence of aluminum chloride. The \( ^{1}H \) NMR spectrum showed the characteristic signals of the protons at C-\( \alpha \) and C-\( \beta \) positions of the chalcone skeleton \( [\delta 7.76, 8.22 (each 1H, d, J=15 \text{ Hz})] \), and revealed the presence of ABC type aromatic protons \( [\delta 6.36 (1H, d, J=2 \text{ Hz}), 6.45 (1H, dd, J=2 \text{ and } 9 \text{ Hz}), 7.96 (1H, d, J=9 \text{ Hz})] \), two aromatic protons \( [\delta 6.56, 7.56 (each 1H, s)] \), a geranyl or neryl group \( [\delta 1.59, 1.64, 1.75 (each 3H, s), 2.00-2.10 (4H, m), 3.29 (2H, d, J=7 Hz), 5.14, 5.39 (each 1H, m)] \), and a hydrogen-bonded hydroxyl group \( [\delta 13.80 \ldots] \).
The 2',4'-dihydroxyphenyl structure for the B ring was supported by comparison of the chemical shift values of the ABC type protons of the model compounds (6, 7, isoliquiritigenin11 and broussochalcone A12). The 2,4-dihydroxy-type phenyl structure for the A ring was also supported by comparison of the chemical shift value of the C-8 proton of 2 with those of the relevant protons of the model compounds (Fig. 2). Discrimination between a geranyl and a neryl group was confirmed from the following results. Comparing the $^1$H nmr spectrum of 2 with those of geraniol and nerol, the shapes of the olefinic proton signals in the side chain of 2 were more similar to those of the relevant proton signals of geraniol than those of nerol (Fig. 3). From these results, we propose the formula 2 for the structure of kuwanol D.

**EXPERIMENTAL**

Abbreviations: s=singlet, d=doublet, dd=double doublet, m=multiplet, br=broad, sh=shoulder, infl=inflection. The general procedures followed and the instruments used are described in our previous papers.5

**Isolation of Kuwanols C (1), D (2) and Other Phenolic Compounds from the Root Bark of a Mulberry Tree Redifferentiated from the Callus Tissues**

The mulberry tree redifferentiated from the callus tissues5 induced from the seedlings of *M. alba* had been cultivated for eleven years in the herbal garden at Kyoto University. The dried root bark (60 g) of the mulberry tree was extracted with methanol at room temperature. Evaporation of the extract to dryness yielded 7 g of the residue. This residue was extracted with acetone. The acetone solution was concentrated to afford the residue (4 g), which was chromatographed on silica gel (120 g) with benzene-acetone as an eluent, each fraction being monitored by tlc. A part of the fraction eluted with benzene was evaporated to give a residue (630 mg), which was fractionated by preparative tlc (solvent system, n-hexane:acetone=2:1, n-hexane:ether=1:1, benzene:ethyl acetate=3:1, chloroform:ethyl acetate=5:1) and by HPLC analysis (solvent: n-hexane:ethyl acetate=4:1, flow rate: 3 ml/min,
column: Senshu Pak SSC-Silica 4251-N, detector: uv 280 nm) to give kuwanol C (1, 5 mg), morusin (2, 20 mg), kuwanons U (4, 5 mg), S (5, 2 mg). A part of the fractions eluted with benzene containing 10% acetone was evaporated to a residue (140 mg), which was fractionated by preparative tlc (solvent system, n-hexane:acetone=1:1, benzene:ethyl acetate=1:1, and 2:1, n-hexane:ethyl acetate=1:1) to give kuwanol D (2, 5 mg), morusin (3, 20 mg), 2,2',4,4'-tetrahydroxychalcone (7, 1 mg), moracins M (8, 2 mg), O (9, 2 mg), and P (10, 2 mg).

Kuwanol C (1)

Compound 1 was obtained as an amorphous powder. [α]D 22 -10° (c=0.31, ethanol), FeCl₃ test: violet, Mg-HCl test: violet, and NaBH₄ test: orange. Uv λ_{max} nm (log ε): 225 (3.69), 268 (sh 3.99), 275 (4.40), 295 (infl 3.59), 308 (sh 3.45). Uv λ_{max} nm (log ε): 224 (3.87), 275 (4.02), 284 (sh 3.93), 316 (infl 3.59).

EI-Ms: m/z 422 (M+), 262, 244, 226, 208, 190, 172, 154, 136, 118, 100, 82.

IR ν KBr cm⁻¹: 3320 (br), 2900, 1640 (sh), 1620, 1600 (sh), 1570 (sh).

'H Nmr (acetone-d₆): δ 1.41 (3H, s, C-11-CH₃), 1.56, 1.65 (each 3H, s, C-16-CH₃), 2.04-2.07 (2H, m, C-13-H x 2), 2.08-2.15 (2H, m, C-14-H x 2), 2.75 (1H, dd, J=3 and 17, C-3-H), 3.21 (1H, dd, J=13 and 17, C-3-H), 5.11 (1H, m, C-15-H), 5.58 (1H, d, J=10, C-10-H), 5.73 (1H, dd, J=3 and 13, C-2-H), 5.91 (1H, d, J=10, C-9-H), 6.30 (1H, s, C-8-H), 6.63 (1H, d, J=2 and 8, C-5'-H), 7.05 (1H, d, J=2, C-3'-H), 7.17 (1H, dd, J=2 and 8, C-6'-H), 7.76 (1H, d, J=8, C-5'-H).

Acetylation of 1

Kuwanol C (1, 5 mg) was acetylated with acetic anhydride (1.5 ml) and pyridine (0.5 ml) at room temperature overnight. The product was purified by preparative tlc (solvent system, benzene:ethyl acetate=1:1) to give the triacetate (1a, 1.5 mg).

The compound 1a was obtained as an amorphous powder, negative to FeCl₃ test. EI-Ms: m/z 548 (M+). 'H Nmr (acetone-d₆): δ 1.43 (3H, s, C-11-CH₃), 1.56, 1.64 (each 3H, s, C-16-CH₃), 2.05-2.15 (4H, m, C-13-H x 2 and C-14-H x 2), 2.28, 2.30, 2.35 (each 3H, s, COCH₃), 2.63 (1H, dd, J=3 and 17, C-3-H), 3.06 (1H, dd, J=13 and 17, C-3-H), 5.10 (1H, m, C-15-H), 5.74 (1H, dd, J=3 and 13, C-2-H), 5.77 (1H, d, J=10, C-10-H), 6.30 (1H, s, C-8-H), 6.53 (1H, d, J=10, C-9-H), 7.05 (1H, d, J=2, C-3'-H), 7.17 (1H, dd, J=2 and 8, C-6'-H), 7.76 (1H, d, J=8, C-5'-H).

Kuwanol D (2)

Compound 2 was obtained as a yellow amorphous powder. FeCl₃ test: brown. Uv λ_{max} nm (log ε): 260 (3.77), 291 (3.84), 386 (4.08). Uv λ_{max} nm (log ε): 280 (infl 3.76), 312 (3.84), 442 (4.16). IR ν KBr cm⁻¹: 3400 (br), 1660 (sh), 1640 (sh), 1610, 1590, 1560 (sh). 'H Nmr (acetone-d₆): δ 1.59 (3H, s, C-9-CH₃),
1.64, 1.75 (each 3H, s, C-14-CH, 3), 2.00-2.10 (4H, m, C-11-H x 2 and C-12-H x 2), 3.29 (2H, d, J=7, C-7-H), 5.14 (1H, m, C-13-H), 5.39 (1H, m, C-8-H), 6.36 (1H, d, J=2, C-3'-H), 6.45 (1H, dd, J=2 and 9, C-5'-H), 6.56 (1H, s, C-6-H), 7.76 (1H, d, J=15, C-α-H), 7.96 (1H, d, J=9, C-6'-H), 8.22 (1H, d, J=15, C-β-H), 13.80 (1H, s, C-2'-OH).

REFERENCES AND NOTES


6. Compound 7 seems to be the first example of isolation from natural sources. The data for this chalcone are as follows: yellow prisms, mp 140° (decomp.).

**EI-Ms:** m/z 254 (M⁺-H₂O), 226, 184, 137, 110. *Uv* *λ*ₘₐₓ (EtOH): 212 (4.35), 293 (3.89), 387 (4.15). *Uv* *λ*ₘₐₓ (EtOH+AlCl₃): 213 (4.46), 270 (3.89), 314 (3.93), 444 (4.24). *H Nmr* (acetone-d₆): 6.63 (1H, d, J=2, C-3'-H), 6.46 (2H, dd, J=2 and 8, C-5-H and C-5'-H), 6.52 (1H, d, J=2, C-3-H), 7.70 (1H, d, J=8, C-6-H), 7.80 (1H, d, J=15, C-α-H), 8.03 (1H, d, J=8, C-6'-H), 8.22 (1H, d, J=15, C-β-H), 13.78 (1H, s, C-2'-OH). Compound 7 was synthesized through the condensation of 2',4'-dimethoxymethylacetophenone with 2,4-dimethoxymethylbenzaldehyde followed by demethoxymethylation.

13. \( \mathcal{L} \) has two asymmetric centers at C-2 and C-11 positions. The stereochemistries of these positions are unidentified. However, as \( \mathcal{L} \) was the levorotatory like sanggenons \( \mathcal{L}^{15} \) and \( \mathcal{N}^{16} \), the configuration at C-2 position seems to be \( \mathcal{L} \).
14. The ABC type aromatic protons of \( \mathcal{L} \) have essentially the same chemical shift values as the B ring protons of \( \mathcal{J} \) and isoliquiritigenin \( \mathcal{J}^{2} \): 6.36 (C-3'-H), 6.45 (C-5'-H), 7.96 (C-6'-H), \( \mathcal{J} \): 6.36 (C-3'-H), 6.47 (C-5'-H), 8.03 (C-6'-H), isoliquiritigenin: 6.36 (C-3'-H), 6.47 (C-5'-H), 8.12 (C-6'-H), cf. \( \mathcal{J} \): 6.46 (C-5-H), 6.51 (C-3-H), 7.69 (C-6-H). This indicated that a geranyl group is located in the A ring. Another possible structure, 2,2',4,4'-tetrahydroxy-5'-geranylchalcone, was thus excluded.

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