

role as an intermediate in the esterification reaction leading to the formation of III: the enzyme system for the formation of III is assumed to be involved by two enzymes, one a ligase catalyzing the formation of the CoA thioester of II and the other a transferase catalyzing the formation of III from the CoA thioester.

The conversion of *trans*-cinnamic acid (IV) to (–)-(trans-cinnamoyl) lupinine (V) by enzyme preparations obtained from the 5–6 day's growth of *Lupinus* seedlings was negligible although the cinnamoyl-CoA ligase activity (about 3/10 that shown toward II), measured by the FeCl₃-hydroxylamine method by Gross, *et al.*⁷⁾ and by Rhodes, *et al.*,⁸⁾ was clearly observed.

The question whether or not the (–)-(trans-cinnamoyl)lupinine (V), which is tentatively found in the young parts of *Lupinus* plant, is alternatively an intermediate in the biosyntheses of III and (–)-(trans-4'-hydroxy-3'-methoxycinnamoyl)lupinine (VI), and an attempt to study a physiological role of these lupin alkaloids in the intact plants are currently under investigation (Fig. 1).

III and VI are partially present as the rhamnosides (VII and VIII) in varying concentration at different times in young seedlings and particularly in the growing aerial parts of *Lupinus* plant.⁹⁾

Faculty of Pharmaceutical Sciences,
University of Chiba
1-33 Yayoi-cho, Chiba, 280, Japan

Hoshi College of Pharmacy
2-4-41 Ebara, Shinagawa-ku,
Tokyo, 141, Japan

ISAMU MURAKOSHI
MAKOTO OGAWA
KAZUO TORIIZUKA
JOJU HAGINIWA
SHIGERU OHMIYA
HIROTAKE OTOMASU

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Kuwanon A, B, C and Oxydihydromorusin, Four New Flavones from the Root Bark of the Cultivated Mulberry Tree (*Morus alba* L.)

The structures of four new flavone derivatives, kuwanon A, B, C and oxydihydromorusin, which were isolated from the root bark of the cultivated mulberry tree (a variety of *Morus alba* L.), were shown to be I, II, III and IV, respectively.

Keywords—kuwanon A; kuwanon B; kuwanon C; oxydihydromorusin; *Morus alba* L.; morusin; flavone; photooxidation

The structures of a series of prenyl flavones isolated from the root bark of *Morus alba* L., a plant of Moraceae family, have been reported.^{1–3)} In the course of our studies on the con-

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stituents of the root bark, four new flavone derivatives, kuwanon A (I), B (II), C (III) and oxydihydromorusin (IV) have been isolated from the benzene extract of the root bark of the cultivated mulberry tree (a variety of *Morus alba* L.). In this communication, we report the structure determination of the four new flavone derivatives, I, II, III and IV, which gave characteristic color reactions for flavones and were positive with methanolic-FeCl₃.

Kuwanon A (I), C₂₅H₂₄O₆ (M⁺ 420), amorphous powder,⁴⁾ ultraviolet (UV) $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 208 (4.49), 260.5 (4.26), 283 (sh 4.11), 325 (sh 3.93); proton magnetic resonance (PMR), δ in (CD₃)₂CO, 1.40 (6H, s, C₁₄-CH₃ × 2), 1.45 (3H, s, C₁₁-CH₃), 1.58 (3H, s, C₁₁-CH₃), 3.11 (2H, d, $J=8$ Hz, C₉-H × 2), 5.13 (1H, m, C₁₀-H), 5.69 (1H, d, $J=10$ Hz, C₁₃-H), 6.26 (1H, d, $J=2$ Hz, C₆-H), 6.32 (1H, d, $J=2$ Hz, C₈-H), 6.68 (1H, d, $J=9$ Hz, C₅-H), 6.75 (1H, d, $J=10$ Hz, C₁₂-H), 7.08 (1H, d, $J=9$ Hz, C₆'-H); Mass Spectrum m/e : 420 (M⁺), 405 (M⁺-CH₃), 365 (M⁺-C₄H₇), 153⁵⁾ (VII). Treatment of I with ethereal CH₂N₂ yielded a dimethyl ether (Ia),⁶⁾ C₂₇H₂₈O₆, mp 147°, which showed a violet color with methanolic-FeCl₃. I forms a diacetate (Ib),⁶⁾ C₂₉H₂₈O₈, mp 167–169°, on treatment with Ac₂O in pyridine at room temperature.

Kuwanon B (II)⁷⁾ C₂₅H₂₄O₆, mp 250–254°, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ), 234 (4.49), 260 (4.38), 280 (sh 4.10), 330 (sh 4.00); PMR, δ in (CD₃)₂CO, 1.42 (3H, s, C₁₁-CH₃), 1.47 (6H, s, C₁₄-CH₃ × 2), 1.58 (3H, s, C₁₁-CH₃), 3.12 (2H, d, $J=6$ Hz, C₉-H × 2), 5.11 (1H, t, $J=6$ Hz, C₁₀-H), 5.72 (1H, d, $J=10$ Hz, C₁₃-H), 6.26 (1H, d, $J=2$ Hz, C₆-H), 6.31 (1H, d, $J=2$ Hz, C₈-H), 6.46 (1H, d, $J=9$ Hz, C₅-H), 6.77 (1H, d, $J=10$ Hz, C₁₂-H), 7.08 (1H, d, $J=9$ Hz, C₆'-H); Mass Spectrum m/e : 420 (M⁺), 405 (M⁺-CH₃), 365 (M⁺-C₄H₇), 153. On treatment with ethereal CH₂N₂, II forms a dimethyl ether (IIa), C₂₇H₂₈O₆ (M⁺ 448), mp 115–118°, which showed a violet color with methanolic-FeCl₃. Treatment of II with Ac₂O in pyridine at room temperature yielded a diacetate (IIb), C₂₉H₂₈O₈ (M⁺ 504), mp 125–127°. From these data, both kuwanon A (I) and B (II) can be regarded as flavone derivatives having the 2,2-dimethylchromene in the B ring and γ,γ -dimethylallyl group attached to the 3-position of chromone ring. The relation of the C₁₂-H in chromene ring to the hydroxyl group in ring B is proposed to be *peri* both in I and II on consideration of changes in chemical shift for C₁₂- and C₁₃-H when I and II are acetylated (Table I). The changes for I and II are of the same sign and of the same order of magnitude as those observed by many investigators for a number of similar compound, in which the hydroxyl group is *peri* to C₁₂-H.⁸⁾ These results indicate that both kuwanon A and B are represented as I or II. Final proof for the structures of kuwanon A and B was obtained by the photooxidation.⁹⁾ When a solution of II in CHCl₃ was irradiated with a high pressure mercury lamp (40w) for 5 hr, kuwanon B hydroperoxide (IIc) was obtained in 60% yield. IIc shows the following data: C₂₅H₂₄O₈ (M⁺ 452), mp 203°, PMR (δ in pyridine-*d*₅) showing the AMX pattern, such as 3.00 (1H, dd, $J=9$ and 16 Hz, C₉-H), 3.67 (1H, dd, $J=2$ and 16 Hz, C₉-H), 4.83 (1H, dd, $J=2$ and 9 Hz, C₁₀-H); Mass Spectrum m/e : 452 (M⁺), 436 (M⁺-O), 421 (M⁺-O-CH₃), 377⁹⁾ (M⁺-C₃H₇O₂), 153. On the other hand, this photooxidation did not occur in kuwanon A (I). In the light of the result of photooxidation of morusin (V),⁹⁾ it is concluded that kuwanon B (II) has the hydroxyl group at C₂' and the isolated double bond in prenyl group attached to the 3-position of the chromone ring. From these considerations, we propose the formula (I) for kuwanon A and the formula (II) for kuwanon B.

Kuwanon C⁷⁾ (III), C₂₅H₂₆O₆, mp 148–150°, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 210 (4.63), 264.5 (4.49), 315 (4.06); Mass Spectrum m/e : 422 (M⁺), 407 (M⁺-CH₃), 379 (M⁺-C₃H₇), 367 (M⁺-C₄H₇),

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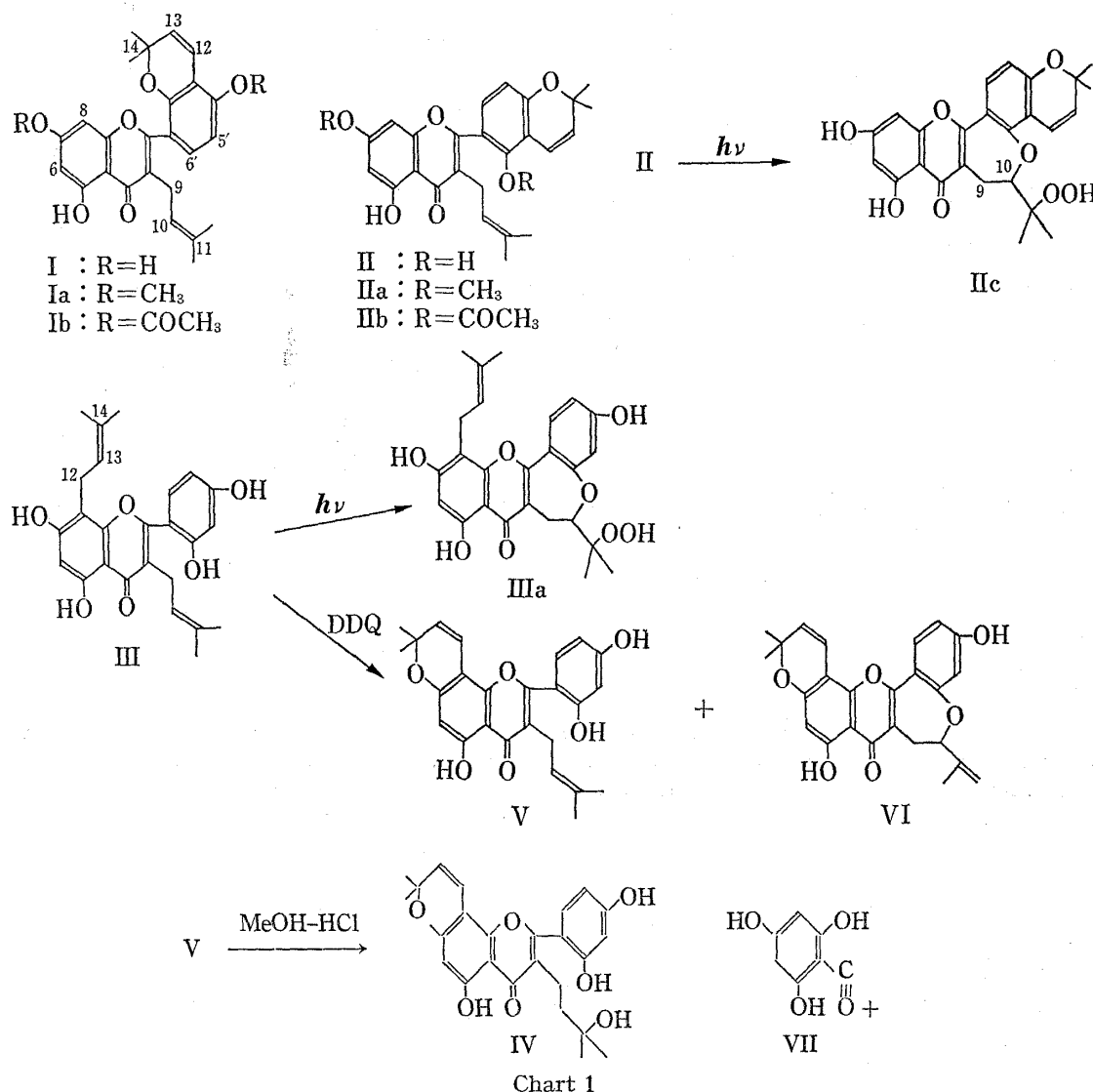


TABLE I. Chemical Shift (ppm) for C₁₂-H and C₁₃-H in I, Ib, II, and IIb^{a)}

Compound	C ₁₂ -H	C ₁₃ -H
I	6.75	5.69
Ib	6.53	5.88
Δ	+0.22	-0.19
II	6.77	5.72
IIb	6.46	5.90
Δ	+0.31	-0.18

^{a)} measured in (CD₃)₂CO

323 (M⁺-C₃H₇-C₄H₈)¹⁰⁾; PMR, δ in (CD₃)₂CO, 1.43 (3H, s, C₁₁-CH₃), 1.57 (9H, s, C₁₁-CH₃ and C₁₄-CH₃×2), 3.12 (2H, d, J =8 Hz, C₉-H×2), 3.35 (2H, d, J =8 Hz, C₁₂-H×2), 5.20 (2H, m, C₁₀ and C₁₃-H), 6.31 (1H, s, C₆-H), 6.43 (1H, dd, J =2 and 8 Hz, C₅'-H), 6.52 (1H, d, J =2 Hz, C₃'-H), 7.20 (1H, d, J =8 Hz, C₆'-H). These spectral data suggest that the structure of kuwanon

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C closely resembles that of mulberrin.¹¹ Unequivocal evidences for the structure (III) of kuwanon C were obtained as follows. When a solution of III in CHCl_3 was irradiated with high pressure mercury lamp for 10 hr, kuwanon C hydroperoxide (IIIa) was obtained in 30% yield in analogy with morusin (V)⁹ and kuwanon B (II). IIIa shows the following data: $\text{C}_{25}\text{H}_{26}\text{O}_8$ (M^+ 454), mp 162–163°, PMR, δ in pyridine- d_5 , 2.93 (1H, dd, $J=10$ and 18 Hz, $\text{C}_9\text{-H}$), 3.77–4.10 (3H, m, $\text{C}_9\text{-H}$ and $\text{C}_{12}\text{-H} \times 2$), 4.78 (1H, dd, $J=2$ and 10 Hz, $\text{C}_{10}\text{-H}$), 5.62 (1H, m, $\text{C}_{13}\text{-H}$); Mass Spectrum m/e : 454 (M^+), 438 ($\text{M}^+ - \text{O}$), 395 ($\text{M}^+ - \text{O} - \text{C}_3\text{H}_7$), 379⁹ ($\text{M}^+ - \text{C}_3\text{H}_7\text{O}_2$), negative to the Gibbs test. Treatment of III with 2,3-dichloro-5,6-dicyanobenzoquinone¹¹ yielded morusin³ (V) and compound³ (VI). From the consideration of these data, we propose the formula (III) as a structure of kuwanon C.

Oxydihydromorusin (IV), $\text{C}_{25}\text{H}_{26}\text{O}_7$,⁷ mp 215–216° UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 206 (4.38) 225 (4.26), 242 (4.26), 269.5 (4.44), 300 (sh 3.82), 350 (sh 3.64); IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3380; PMR, δ in pyridine- d_5 , 1.39 (12H, s, $\text{CH}_3 \times 4$), 2.00–2.30 (2H, m, $\text{C}_{10}\text{-H} \times 2$), 2.98–3.28 (2H, m, $\text{C}_9\text{-H} \times 2$); Mass Spectrum m/e : 438 (M^+), 423 ($\text{M}^+ - \text{CH}_3$), 420 ($\text{M}^+ - \text{H}_2\text{O}$), 405 ($\text{M}^+ - \text{CH}_3 - \text{H}_2\text{O}$), 379 ($\text{M}^+ - \text{C}_3\text{H}_7\text{O}$), 365 ($\text{M}^+ - \text{C}_4\text{H}_9\text{O}$), 203³ (formed from the ion at 423 by a reverse Diels-Alder reaction). From these data, the structure of oxydihydromorusin was deduced as formula (IV). Final proof for the structure (IV) assigned to oxydihydromorusin was obtained by comparing the mixed melting point with those of compound (IV) obtained by the action of 4% methanolic hydrogen chloride on morusin (V). From these results, oxydihydromorusin is represented by the formula IV.

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Faculty of Pharmaceutical Science,
Toho University
542, Miyama-cho, Funabashi-shi
Chiba, 274, Japan

TARO NOMURA
TOSHIO FUKAI
MASA KATAYANAGI

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