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 $FeCl_3$-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]

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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-$FeCl_3$.

Kuwanon A (I), $C_{25}H_{24}O_6$ (M^+ 420), amorphous powder,[4] ultraviolet (UV) λ_{max}^{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 $(CD_3)_2CO$, 1.40 (6H, s, C_{14}-$CH_3\times2$), 1.45 (3H, s, C_{11}-CH_3), 1.58 (3H, s, C_{11}-CH_3), 3.11 (2H, d, J=8 Hz, C_9-H×2), 5.13 (1H, m, C_{10}-H), 5.69 (1H, d, J=10 Hz, C_{13}-H), 6.26 (1H, d, J=2 Hz, C_6-H), 6.32 (1H, d, J=2 Hz, C_8-H), 6.68 (1H, d, J=9 Hz, $C_{5'}$-H), 6.75 (1H, d, J=10 Hz, C_{12}-H), 7.08(1H, d, J=9 Hz, C_6'-H); Mass Spectrum *m/e*: 420 (M^+), 405 (M^+-CH_3), 365 (M^+-C_4H_7), 153[5] (VII). Treatment of I with ethereal CH_2N_2 yielded a dimethyl ether (Ia),[6] $C_{27}H_{28}O_6$, mp 147°, which showed a violet color with methanolic-$FeCl_3$. I forms a diacetate (Ib),[6] $C_{29}H_{28}O_8$, mp 167—169°, on treatment with Ac_2O in pyridine at room temperature.

Kuwanon B (II)[7] $C_{25}H_{24}O_6$, mp 250—254°, UV λ_{max}^{MeOH} nm (log ε), 234 (4.49), 260 (4.38), 280 (sh 4.10), 330 (sh 4.00); PMR, δ in $(CD_3)_2CO$, 1.42 (3H, s, C_{11}-CH_3), 1.47 (6H, s,C_{14}-$CH_3\times2$), 1.58 (3H, s, C_{11}-CH_3), 3.12 (2H, d, J=6 Hz, C_9-H×2), 5.11 (1H, t, J=6 Hz, C_{10}-H), 5.72 (1H, d, J=10 Hz, C_{13}-H), 6.26 (1H, d, J=2 Hz, C_6-H), 6.31 (1H, d, J=2 Hz, C_8-H), 6.46 (1H, d, J=9 Hz, $C_{5'}$-H), 6.77 (1H, d, J=10 Hz, C_{12}-H), 7.08 (1H, d, J=9 Hz, C_6'-H); Mass Spectrum *m/e*: 420 (M^+), 405 (M^+—CH_3), 365 (M^+—C_4H_7), 153. On treatment with ethereal CH_2N_2, II forms a dimethyl ether (IIa), $C_{27}H_{28}O_6$ (M^+ 448), mp 115—118°, which showed a violet color with methanolic-$FeCl_3$. Treatment of II with Ac_2O in pyridine at room temperature yielded a diacetate (IIb), $C_{29}H_{28}O_8$ (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 C_{12}-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 C_{12}- and C_{13}-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 obtserved by many investigators for a number of similar compound, in which the hydroxyl group is *peri* to C_{12}-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 $CHCl_3$ 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: $C_{25}H_{24}O_8$ (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, C_9-H), 3.67 (1H, dd, J=2 and 16 Hz, C_9-H), 4.83 (1H, dd, J=2 and 9 Hz, C_{10}-H); Mass Spectrum *m/e*: 452 (M^+), 436 (M^+—O), 421 (M^+—O—CH_3), 377[9] (M^+—$C_3H_7O_2$), 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 $C_{2'}$ 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 C[7] (III), $C_{25}H_{26}O_6$, mp 148—150°, UV λ_{max}^{MeOH} nm (log ε): 210 (4.63), 264.5 (4.49), 315 (4.06); Mass Spectrum *m/e*: 422 (M^+), 407 (M^+—CH_3), 379 (M^+—C_3H_7), 367 (M^+—C_4H_7),

4) Although only one spot was detected on TLC, this compound (I) could not be isolated as crystalline form.
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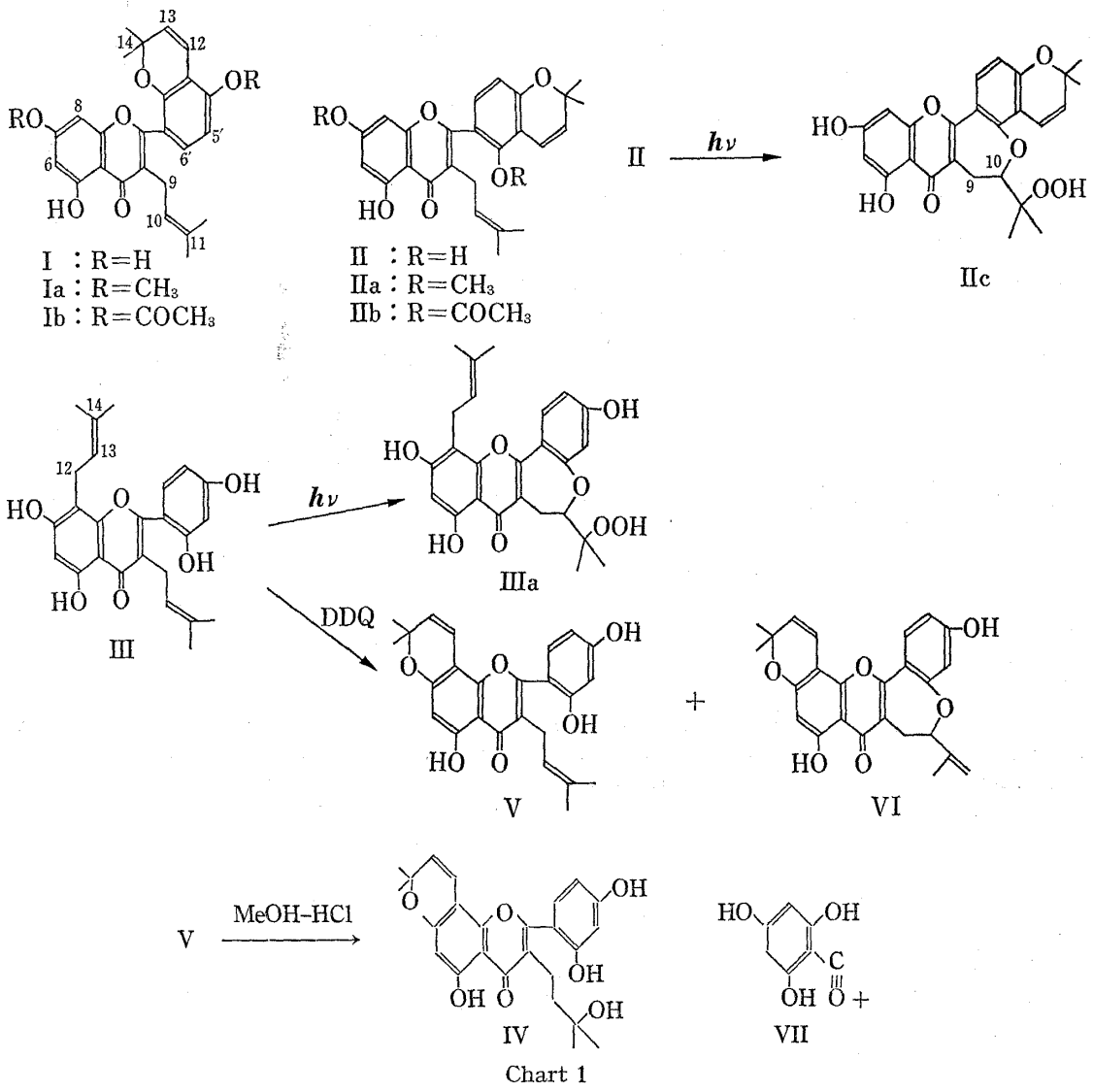

Chart 1

Table I. Chemical Shift (ppm) for C_{12}-H and C_{13}-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 $(CD_3)_2CO$

323 ($M^+-C_3H_7-C_4H_8$)[10]; PMR, δ in $(CD_3)_2CO$, 1.43 (3H, s, C_{11}-CH_3), 1.57 (9H, s, C_{11}-CH_3 and C_{14}-$CH_3\times2$), 3.12 (2H, d, J=8 Hz, C_9-H×2), 3.35 (2H, d, J=8 Hz, C_{12}-H×2), 5.20 (2H, m, C_{10} and C_{13}-H), 6.31 (1H, s, C_6-H), 6.43 (1H, dd, J=2 and 8 Hz, $C_{5'}$-H), 6.52 (1H, d, J=2 Hz, $C_{3'}$-H), 7.20 (1H, d, J=8 Hz, $C_{6'}$-H). These spectral data suggest that the structure of kuwanon

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C closely resembles that of mulberrin.[1] 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: $C_{25}H_{26}O_8$ (M^+ 454), mp 162—163°, PMR, δ in pyridine-d_5, 2.93 (1H, dd, J=10 and 18 Hz, C_9–H), 3.77—4.10 (3H, m, C_9–H and C_{12}–H×2), 4.78 (1H, dd, J=2 and 10 Hz, C_{10}–H), 5.62 (1H, m, C_{13}–H); Mass Spectrum *m/e*: 454 (M^+), 438 (M^+—O), 395 (M^+—O–C_3H_7), 379[9] (M^+—$C_3H_7O_2$), negative to the Gibbs test. Treatment of III with 2,3-dichloro-5,6-dicyanobenzoquinone[11] yielded morusin[3] (V) and compound[3] (VI). From the consideration of these data, we propose the formula (III) as a structure of kuwanon C.

Oxydihydromorusin (IV), $C_{25}H_{26}O_7$,[7] mp 215—216° UV λ_{max}^{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 ν_{max}^{Nujol} cm^{-1}: 3380; PMR, δ in pyridine-d_5, 1.39 (12H, s, CH_3×4), 2.00—2.30 (2H, m, C_{10}–H×2), 2.98—3.28 (2H, m, C_9–H×2); Mass Spectrum *m/e*: 438 (M^+), 423 (M^+—CH_3), 420 (M^+—H_2O), 405 (M^+—CH_3–H_2O), 379 (M^+—C_3H_7O), 365 (M^+—C_4H_9O), 203[3] (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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