

KUWANON E, A NEW FLAVANONE DERIVATIVE FROM THE ROOT BARK
OF THE CULTIVATED MULBERRY TREE (MORUS ALBA L.)

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A new flavanone derivative named kuwanon E was isolated from the root bark of the cultivated mulberry tree (a variety of Morus alba L.). The structure was presumed to be 5,7,2',4'-tetrahydroxy-5'-geranylflavanone (I) by spectral data.

The structures were reported¹⁻⁶ of a series of prenylflavonoids isolated from the root bark of Morus alba L., a plant of Moraceae family. In the course of our studies on the constituents of Morus alba L., a new flavanone derivative, kuwanon E (I), was isolated from the methanol extract. In this paper, we report the isolation and structure determination of I.

The dried root bark (5.2 Kg) of the cultivated mulberry tree (a variety of Morus alba L.), collected in Gunma Prefecture, was finely cut and extracted successively with n-hexane, benzene, and methanol. After the methanol extract was evaporated, the residue (800 g) was dissolved in ethyl acetate (1:1), and the solution was

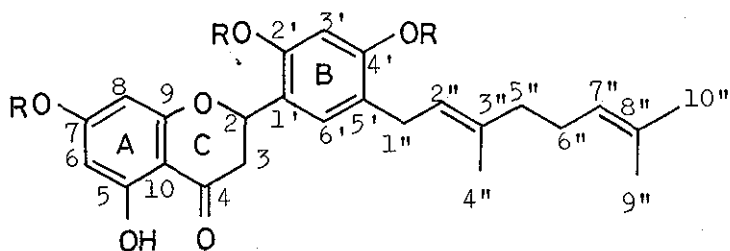
allowed to stand for 6 days at room temperature. Concentration of the ethyl acetate solution gave a brown residue (250 g), which was extracted repeatedly with ether on heating. Evaporation of the solvent afforded the residue (170 g), which was positive to methanolic ferric chloride test. This residue (100 g) was chromatographed on silica gel using benzene-methanol (99.5 : 0.5) as an eluent to give a mixture of phenolic materials (6 g) which contained I and other unidentified phenolic compounds. From this mixture, I (40 mg) was isolated by preparative TLC (ether : chloroform = 1 : 2, silica gel).

Kuwanon E (I), $C_{25}H_{28}O_6$, mp 132-136° (colorless prisms from ether-n-hexane), uv [λ_{max}^{EtOH} nm(log ϵ) : 212(4.56), 289(4.26), 320(sh 3.73); $\lambda_{max}^{EtOH+AlCl_3}$: 211.5(4.55), 220(sh 4.19), 308(4.33), 374(3.57); $\lambda_{max}^{EtOH+NaOAc}$: 214(4.66), 295(sh 3.97), 327(4.49); $\lambda_{max}^{EtOH+NaOMe}$: 325.5(4.16), 497(4.17)]. $[\alpha]_{589}^{20}$ 0° (c = 1.5 in methanol, from ORD measurement), gave an intense reddish violet color with methanolic ferric chloride and was positive both magnesium-hydrochloric acid test and to sodium borohydride test⁸ and gave a pale violet color with zinc-hydrochloric acid test. Kuwanon E gave the absorption bands for hydroxyl, conjugated carbonyl, and benzene ring in the ir spectrum (ν_{max}^{Nujol} : 3360, 1650, 1595 cm^{-1}). Treatment of I with acetic anhydride in pyridine at room temperature for five min yielded a triacetate (Ia), $C_{31}H_{34}O_9$ (M^+ 550), mp 87-90° (colorless prisms from ether-n-hexane), uv [λ_{max}^{EtOH} nm(log ϵ) : 212(4.70), 277.5(4.16), 334(3.69); $\lambda_{max}^{EtOH+AlCl_3}$: 212(4.72), 301(4.19), 390(3.75); $\lambda_{max}^{EtOH+NaOMe}$: 325(4.22), 495(4.27)], which gave a reddish violet color with methanolic ferric chloride test. These findings show that

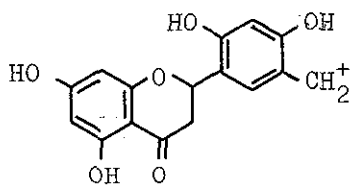
I is a flavanone derivative which has at least four hydroxyl groups, and that one hydroxyl group is hydrogen bonded. This assumption was substantiated by the ^1H nmr spectrum (acetone- d_6) of I in which the signals were observed at δ 2.68(1H, dd, $J = 3$ and 18 Hz, $\text{C}_3 - \text{H}$, cis), 3.16(1H, dd, $J = 13$ and 18 Hz, $\text{C}_3 - \text{H}$, trans), 5.72(1H, dd, $J = 3$ and 13 Hz, $\text{C}_2 - \text{H}$), 8.28, 8.39, 9.63(each 1H, br s, OH, disappeared on addition of D_2O), 12.83(1H, s, $\text{C}_5 - \text{OH}$, disappeared on addition of D_2O).

The ^1H nmr spectrum⁹ of I in pyridine- d_5 showed the meta-coupled doublet ($J = 2$ Hz) at δ 6.38($\text{C}_6 - \text{H}$) and δ 6.47($\text{C}_8 - \text{H}$), which indicated that the A ring was unsubstituted at 6- and 8-positions.¹⁰ The arrangement of substituents in the B ring was assumed by the ^1H nmr spectrum of I in acetone- d_6 : two singlet signals at δ 6.52 ($\text{C}_3, - \text{H}$) and δ 7.23($\text{C}_6, - \text{H}$) supported that the B ring of I was substituted in 2',-4',- and 5'-positions. The biogenetic analogy to the other prenylflavonoids isolated from Morus alba L.¹⁻⁶ suggests that B ring has the 2',4'-dioxxygenated pattern.

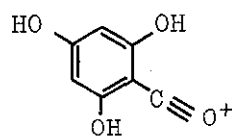
The mass spectrum of I showed the fragments¹¹ at m/e 406($\text{C}_{25}\text{H}_{26}\text{O}_5$), 301($\text{C}_{16}\text{H}_{13}\text{O}_6$, II),^{12,13} 153($\text{C}_7\text{H}_5\text{O}_4$, III),¹⁴ 149($\text{C}_9\text{H}_9\text{O}_2$, IV),¹²⁻¹⁴ 123(C_9H_{15}),^{12,13} 69(C_5H_9 , base peak).¹³ The significant peak at m/e 301 indicates the loss of m/e 123 (due to side chain) from the molecular ion. The characteristic peak at m/e 149 suggests the presence of two hydroxyl groups and a geranyl (or neryl) group on the ring B. The geranyl (or neryl) group in formula (I) was supported by the ^1H nmr spectrum of I in acetone- d_6 ^{12,15,16}: δ 1.58, 1.63(each 3H, s, $\text{C}_8'' - \text{CH}_3$), 1.72(3H, s, $\text{C}_3'' - \text{CH}_3$), 2.05(4H, br s, $\text{C}_5'' - \text{H} \times 2$ and $\text{C}_6'' - \text{H} \times 2$, overlapping with the solvent), 3.23(2H, br d, $J = 8$ Hz,



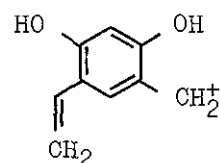
(I) : R = H, (Ia) : R = COCH₃



(II)



(III)



(IV)

C_{1''} - H × 2), 5.13(1H, br t, J = 8 Hz, C_{7''} - H), 5.37(1H, br t, J = 8 Hz, C_{2''} - H). The possibility of the geranyl group is supported by the nuclear Overhauser effect (NOE) in I : The irradiation of the higher methylene signal (δ 2.05) increased the area (+ 25.0 ± 1.0 %) of the proton (δ 5.37, C_{2''} - H) attached to the double bond, while the irradiation of the lowest methyl signal (δ 1.72, C_{3''} - CH₃) showed no effect. The NOE was also observed (+ 20.5 ± 1.0 %) between the methylene signal (δ 2.05) and the olefinic proton (δ 5.13, C_{7''} - H).

In order to corroborate the structure of I, the ¹³C nmr spectrum¹⁷ was analysed as follows : δ in CD₃OD, 16.2(C_{4''}), 17.8(C_{9''}), 25.8(C_{1''}), 27.5(C_{10''}), 28.3(C_{6''}), 40.5(C_{5''}), 42.6(C₃), 76.0(C₂), 96.4(C₈), 97.0(C₆), 103.3(C₁₀),^{18a} 103.7(C₃),^{18a} 117.3(C₅),^{18b} 120.8(C₁),^{18b} 123.9(C_{2''}), 125.3(C_{7''}), 128.8(C₆), 132.1(C_{8''}), 136.8(C_{3''}), 154.1(C₂),

156.6(C₄), 165.0(C₉), 167.6(C₅), 167.8(C₇), 198.5(C₄). The carbon shift assignment is based on the use of naringenin (5,7,4'-trihydroxyflavanone)¹⁹ as a model and additive substituent parameters.²⁰

From the considerations of above results, we tentatively propose the formula (I) for the structure of kuwanon E.

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