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KUWANON D, NEW ISOPRENOID FLAVANONE FROM THE ROOT BARK OF THE CULTIVATED MULBERRY TREE (MORUS ALBA L.)

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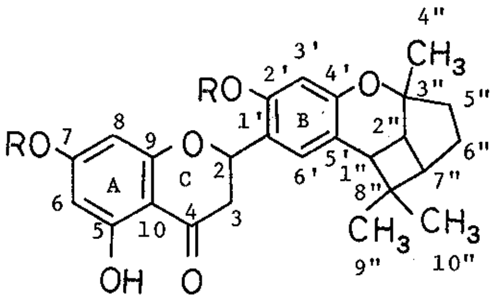
From the benzene extract of the root bark of the cultivated mulberry tree (a variety of Morus alba L.), a novel isoprene-substituted flavanone, kuwanon D, was isolated whose structure was shown to be I on the basis of spectral data.

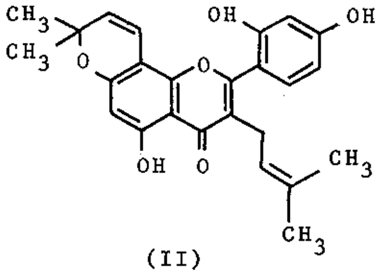
The structures of a series of prenylflavones isolated from the root bark of Morus alba L., a plant of Moraceae family, were reported.[1-5] In the course of our studies on the constituents of the root bark, a novel isoprene-substituted flavanone, kuwanon D (I), has been isolated from the benzene extract. In this paper, we report the isolation and structure determination of I.

The dried root bark (2.0 Kg) of the cultivated mulberry tree (a variety of Morus alba L.), collected in Gunma Prefecture, was finely cut and extracted with n-hexane and then with benzene. The benzene extract was dissolved in the minimal amount of methanol, and allowed to stand until semi-solid mass separated. The methanolic mother liquor was evaporated, and the residue was dissolved in ether. The ether solution was extracted successively with 5 % aqueous sodium bicarbonate, 5 % aqueous sodium carbonate, and 5 % aqueous sodium hydroxide solution. The 5 % aqueous sodium hydroxide solution was acidified with dilute hydrochloric acid and extracted with ether. From the ether solution, a mixture of phenolic material was obtained, and was chromatographed on silica gel using benzene-methanol as an eluent to give a mixture of phenolic materials containing I, morusin (II),[3] kuwanon A,[4] kuwanon B,[4] and other unidentified phenolic compounds. This mixture was extracted with 5 % aqueous sodium carbonate solution, and the aqueous layer was acidified with dilute hydrochloric acid, and extracted with ether. From the ether extract, I (10 mg) was obtained by using preparative TLC (ether : chloroform = 1 : 4, silica gel).

Kuwanon D (I), $C_{25}H_{26}O_6$,[6] mp 230-232°(colorless prisms from ether-methanol), uv [λ_{max}^{EtOH} nm(log ε): 213(4.59), 227(sh 4.07), 290(4.30), 320(sh 3.86); $\lambda_{max}^{EtOH+AlCl_3}$ 213(4.58), 220(sh 4.53), 308(4.39), 373(3.67)], $[\alpha]_{589}^{20}$ 0°(c=0.15 in acetonitrile, from ORD measurement), which gave an intense reddish violet color with methanolic ferric chloride and was positive to magnesium-hydrochloric acid, sodium borohydride test,[7] but negative to zinc-hydrochloric acid test. I gave the absorption bands for hydroxyl, conjugated carbonyl, and benzene ring in the ir spectrum (ν_{max}^{Nujol}: 3250, 1645, 1600 cm^{-1}). Treatment of I with acetic anhydride in pyridine at room temperature for five min yielded a diacetate (Ia), $C_{29}H_{30}O_8$ (M^+ 506), mp 162-166°(colorless prisms from n-hexane - ether), uv [λ_{max}^{EtOH} nm(log ε): 210.5(4.93), 278(4.28), 335(3.75); $\lambda_{max}^{EtOH+AlCl_3}$: 211(4.98), 289(4.29), 385(3.91)], which gave a reddish violet color with methanolic ferric chloride test. These findings show that I is a flavanone derivative which has at least



(I) : R = H
(Ia): R = $COCH_3$



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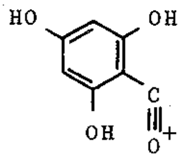

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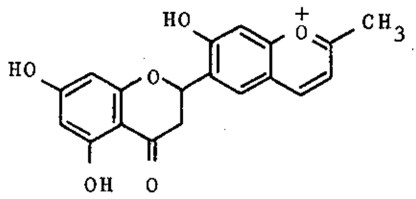

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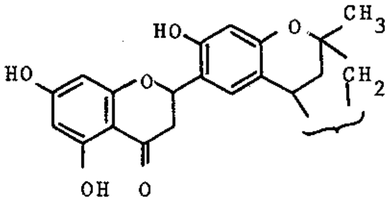

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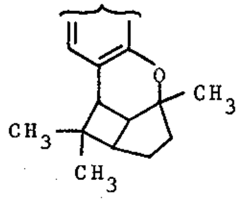
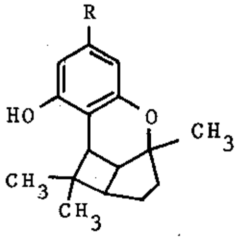
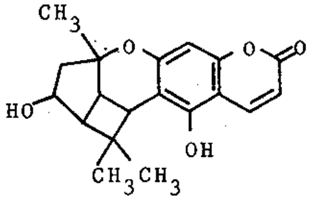


(VI)

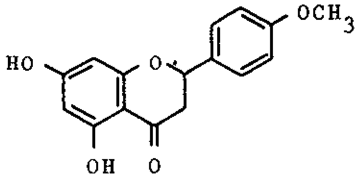


(VII)

R = n - C_5H_{11}



(VIII)



(IX)

three phenolic hydroxyl groups and one of them is hydrogen bonded. This was substantiated by the ^{1}H nmr spectrum[8] of I as follows : δ 3.08(1H, dd, J = 3 and 17 Hz, C_3 - H, cis), 3.47(1H, dd, J = 13 and 17 Hz, C_3 - H, trans), 6.23(1H, dd, J = 3 and 13 Hz, C_2 - H), 12.99 (1H, s, C_5 - OH, disappeared on addition of D_2O). The ^{1}H nmr spectrum of I showed the absence of signals for any olefinic protons, and I was recovered unchanged on the catalytic hydrogenation procedure using Adams catalyst. This fact shows the lack of ethylenic bond in I.

The meta-coupled doublet (J = 2 Hz) at δ 6.41(C_6 - H) and δ 6.49 (C_8 - H) indicated that the A ring was unsubstituted at 6 - and 8 - position.[9] The arrangement of substituents in the B ring was assumed by the ^{1}H nmr spectrum of I as follows: two singlet signals at δ 6.87 ($C_{3'}$ - H), and δ 7.39($C_{6'}$ - H) supported that the B ring of I was substituted in the 2' -, the 4' -, and the 5' - positions.
The biogenetic analogy to other prenylflavones which have been isolated from Morus alba L.,[1-5] suggests that B ring has the 2' -, 4' - dioxygenated pattern. Comparing the ^{1}H nmr spectrum of I with that[10] of Ia, the signal of the proton (C_2 - H) of Ia shifted 0.38 ppm toward upper field from the corresponding proton of I. This finding exhibits that I is a flavanone derivative having a hydroxyl group attached to the 2' - position.

The mass spectrum of I gave the flagments at m/e 407(M^+ - CH_3), 404(M^+ - H_2O), and 153(III).[11] Moreover, the base peak, m/e 339 ($C_{19}H_{15}O_6$),[12] appeared most significantly, which was corresponding to the oxonium ions (IV) expected for flavanone derivatives containing a pyran ring fused to the aromatic nucleus.[13-16] The combined evidence thus pointed strongly to the partial structure (V) plus a C_5 unit.

As there was no other double bond in the molecule, it was apparent that I has a hexacyclic structure and the formula (I) was suggested for kuwanon D.

The "cyclol" (VI) unit[17] in the formula (I) was supported by the ^{1}H nmr spectrum of I as follows: δ 0.78(3H, s, $C_{8''}$ - CH_3), 1.27(3H, s, $C_{3''}$ - CH_3 or $C_{8''}$ - CH_3), 1.33(3H, s, $C_{3''}$ - CH_3 or $C_{8''}$ - CH_3), 1.40 - 2.10(4H, m, $C_{5''}$ - H x 2 and $C_{6''}$ - H x 2), 2.28(1H, m, $C_{7''}$ - H), 2.52(1H, q, J = 7.5 and 9.6 Hz, $C_{2''}$ - H), 3.02(1H, d, J = 9.6 Hz, $C_{1''}$ - H, overlapping with the signal at 3.08(C_3 - H, cis). Irradiation on the proton doublet at δ 3.02 changes the proton quartet at δ 2.52 into a doublet (J = 7.5 Hz), and the irradiation on the proton multiplet at δ 2.28 changes the proton quartet at δ 2.52 into a doublet (J = 9.6 Hz). The chemical shifts and the splitting patterns of these signals are very similar to those of the corresponding protons of compounds, such as cannabicyclol (VII)[14,17,18] and hydroxyeriobrucinol (VIII),[16] which have the "cyclol" (VI) unit in the formula. Moreover, a δ value 0.78 has precedent in other methyl cyclobutanes (e.g. the C - 9 of trans pinane, δ 0.82 in CCl_4)[19] and one methyl in I may, from the molecular model, also be receiving a positive aryl shielding contribution. A similar phenomenon has been reported by Crombie and Ponsford in the case of VII.[14b]

In order to corroborate the structure of I, the ^{13}C nmr spectrum[20] was analysed as follows : δ in pyridine-d_5, 19.4, 25.7, 27.2(each q, $C_{4''}$, $C_{9''}$, and $C_{10''}$), 34.6(t, $C_{5''}$ or $C_{6''}$), 38.5, 38.9, 39.4, 39.8 ($C_{2''}$, $C_{7''}$, $C_{8''}$, and $C_{5''}$ or $C_{6''}$), 42.9(t, C_3), 46.8(d, $C_{1''}$), 75.3(d, C_2), 84.1(s, $C_{3''}$), 96.2(d, C_8), 97.2(d, C_6), 103.1(s, C_{10}), 105.5(d, $C_{3'}$), 115.7(s, $C_{5'}$), 119.4(s, $C_{1'}$), 128.9(d, $C_{6'}$), 155.2(s, $C_{2'}$), 155.4(s, $C_{4'}$), 164.8(s, C_9), 165.4(s, C_5), 168.6(s, C_7), 197.3(s, C_4). Assignments of the carbon atoms in I were performed by the off-resonance decoupling technique, and by the use of the model compound, isosakuranetin (IX),[21] taking into account the substituent effects of the alkyl group and of the hydroxyl group in B ring.[5,22]

From the considerations of above results, we tentatively propose the formula (I) for a structure of kuwanon D.[23]

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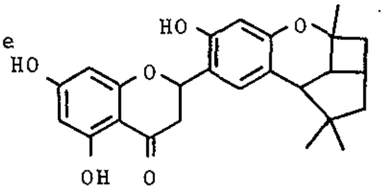
23 The formula (I') for a structure of kuwanon D can not be excluded completely, but the signal (δ 0.78) of one of the methyl groups at $C_{8''}$ was in good agreement with that (δ 0.78 in $CDCl_3$) of cannabicyclol (VII).[14b] It is probable that the structure (I) is more reasonable than I'.



(I')

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