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KUWANON M, A NEW DIELS-ALDER ADDUCT FROM THE ROOT BARKS OF THE CULTIVATED MULBERRY TREE (MORUS LHOU(SER.) KOIDZ.)

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Abstract ——— Kuwanon M (I), a new Diels-Alder adduct of two prenylflavone derivatives, has been isolated from the cultivated mulberry tree (Morus Lhou(ser.) Koidz.). The structure was shown to be I on the basis of spectral data. The compound (I) produced a hypotensive effect to spontaneously hypertensive rat (2 mg/Kg, i.v.).

In the previous papers[1,2], we reported the structure determination of a series of natural Diels-Alder adducts and isoprenylated flavonoids, isolated from the root barks of cultivated mulberry tree and the Chinese crude drug "Sāng-Bái-Pí" (Japanese name "Sōhakuhi") imported from the People's Republic of China. Some of the Diels-Alder adducts showed a significant hypotensive effect. In the course of our studies on the constituents of the Morus root barks, we examined the phenolic constituents of the root barks of Morus Lhou(ser.) Koidz. (Japanese name "Rosō"). The ethyl acetate extract of the root barks was fractionated by repeated silica gel column chromatography (benzene-MeOH, $CHCl_3$-AcOEt) and repeated preparative thin layer chromatography on silica gel ($CHCl_3$:AcOEt=1:1, $CHCl_3$:MeOH=15:1). Consequently, kuwanon M (I), a new Diels-Alder adduct type flavone, was isolated in 9.2×10^{-4} % yield, and was found to have hypotensive action in spontaneously hypertensive rat (2 mg/Kg, i.v.). We report herein the structure determination of the compound, based on comparison with the spectral data of the known compounds.

Kuwanon M (I), mp 252-254 °C and $[\alpha]_D^{25}$ -2.0° (MeOH), showed the molecular ion peak at m/z 840 in its FD-MS spectrum. The ^{13}C nmr spectrum (dmso-d_6) of I revealed the presence of the following fifty carbons: twenty four aliphatic carbons (CH_3- x7, $-CH_2-$ x3, $>CH-$ x3, $>C<$ x1, $>C{=}C<^{H}$ x3, $>C{=}C<$ x2), twenty four aromatic carbons (CH x8, C x8, C-O x8), and two carbonyl carbons. These results suggest the composition of kuwanon M (I) to be $C_{50}H_{48}O_{12}$[3]. This substance (I) gave a dark green color with methanolic iron(III) chloride, and was positive to the magnesium

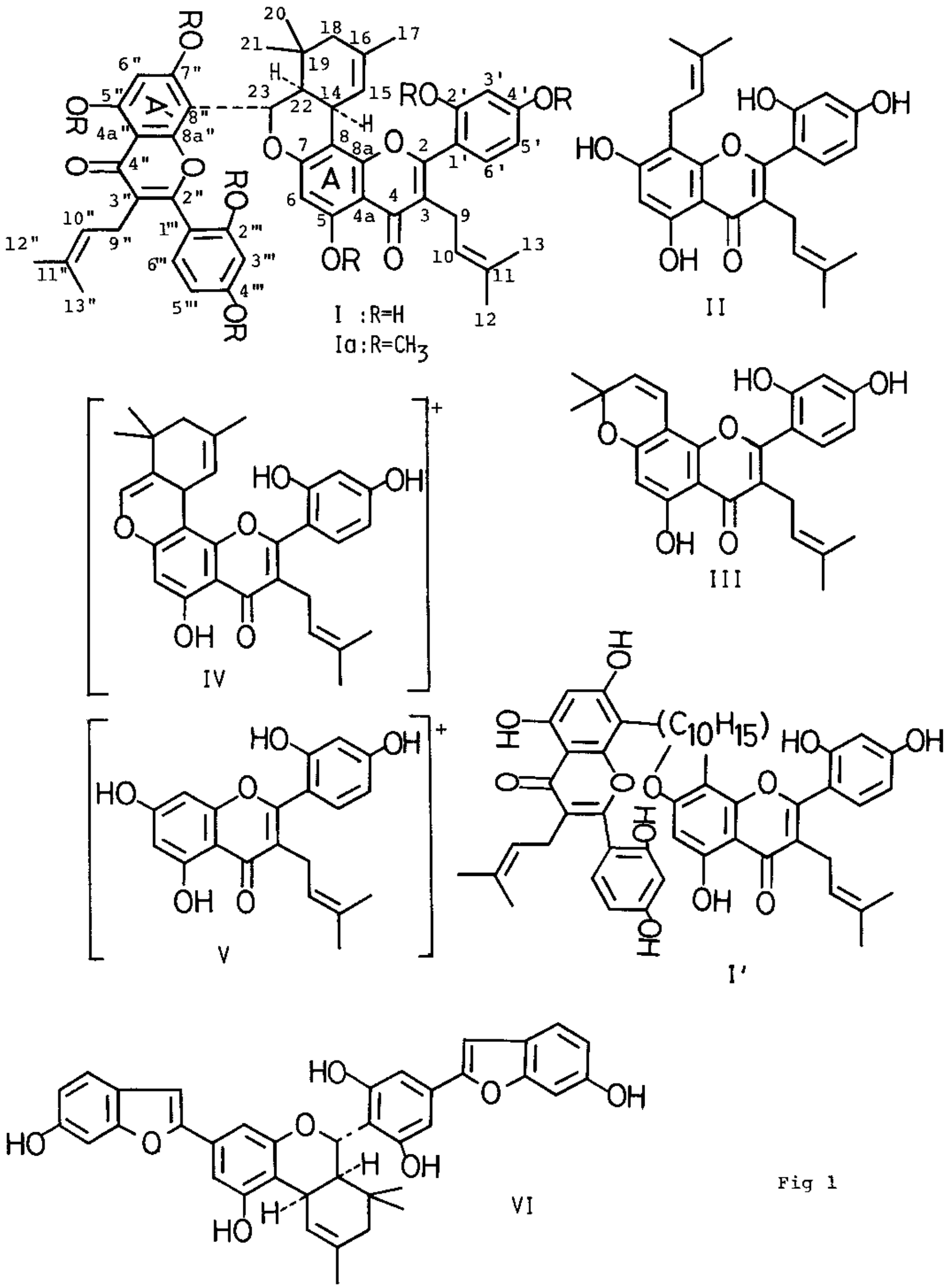


Fig 1

Table 1 ^{13}C nmr chemical shifts (ppm)

	I	III		I	II
C-2	159.0	158.6	C-2"	160.1	158.9
C-3	120.2	120.0	C-3"	119.8	119.4
C-4	181.8	181.8	C-4"	181.8	181.8
C-4a	104.3	100.5	C-4a"	103.7	103.4
C-5	154.5	151.9	C-5"	156.8	155.0
C-6	98.5	98.9	C-6"	97.7	97.9
C-7	161.7	162.0	C-7"	161.7	161.7
C-8	105.8	104.4	C-8"	107.1	105.5
C-8a	160.4	160.7	C-8a"	160.5	160.3
C-1'	110.6	110.9	C-1"'	111.1	111.3
C-2'	156.8	156.7	C-2"'	156.4	156.5
C-3'	102.8	103.0	C-3"'	102.6	102.7
C-4'	161.2	161.1	C-4"'	160.5	161.2
C-5'	106.8	107.0	C-5"'	106.6	106.7
C-6'	131.2	131.3	C-6"'	130.8	131.2
C-9	23.7	23.7	C-9"	23.7	23.5
C-10	121.5	121.6	C-10"	121.5	121.7
C-11	131.2	131.3	C-11"	131.1	131.2
C-12	25.4	25.4	C-12"	25.4	25.4
C-13	17.3	17.3	C-13"	17.3	17.3

solvent; dmso-d_6

Table 2 ^{1}H nmr spectra

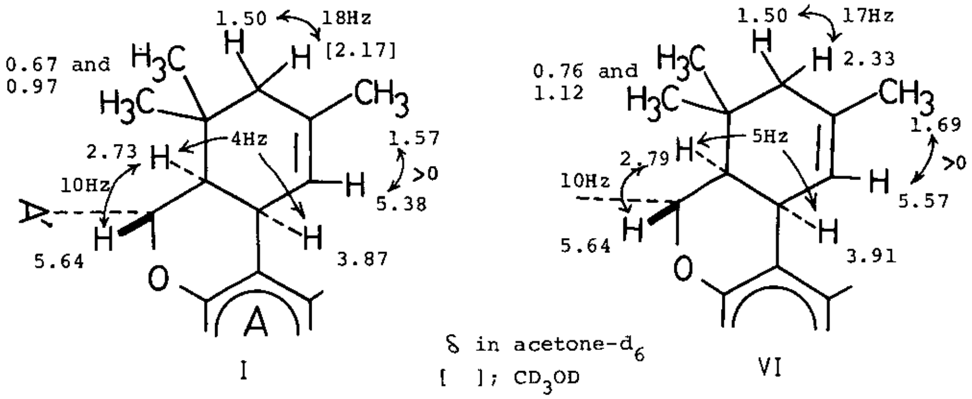
	I	III	II
6-H	6.00(1H,s)	6.21(1H,s)	
3'-H	6.43(1H,d,J=2)	6.45(1H,d,J=2)	
5'-H	6.37(1H,dd,J=2,8)	6.36(1H,dd,J=2,8)	
6'-H	7.05(1H,d,J=8)	7.14(1H,d,J=8)	
9-Hx2	3.09(2H,br d,J=7)	3.02(2H,br d,J=7)	
10-H	5.11(1H,br t,J=7)	5.03(1H,br t,J=7)	
11-CH_3	1.42,1.57(each 3H,s)	1.42,1.57(each 3H,s)	
6"-H	6.38(1H,s)		6.31(1H,s)
3"' -H	6.56(1H,d,J=2)		6.52(1H,d,J=2)
5"' -H	6.51(1H,dd,J=2,8)		6.43(1H,dd,J=2,8)
6"' -H	7.28(1H,d,J=8)		7.20(1H,d,J=8)
9"-Hx2	3.18(2H,m)		3.12(2H,br d,J=8)
10"-H	5.15(1H,br t,J=7)		5.20(1H,m)
11"-CH_3	1.44,1.57(each 3H,s)		1.43,1.57(each 3H,s)

solvent; acetone-d_6

hydrochloric acid test. The compound (I) showed the following spectra: UV λ_{max}^{EtOH} nm(log ε): 208(4.99), 265(4.84), 310(sh 4.39), 326(4.40); $\lambda_{max}^{EtOH+AlCl_3}$ nm(log ε): 210(5.02), 275(4.87), 320(sh 4.34), 332(4.36), 380(4.27). IR ν_{max}^{Nujol} cm^{-1}: 3480(br), 3220(br), 1655, 1620(sh), 1615. The UV spectra were similar to those of kuwanon C (II)[1a] suggesting that I possesses a partial structure with kuwanon C type of chromophore. On the basis of molecular formula and the UV spectra of I, kuwanon M seems to be a dimer of kuwanon C type of prenylflavones. The treatment of I with dimethyl sulfate in acetone gave the heptamethyl ether (Ia) as an amorphous powder, which was negative to the methanolic iron(III) chloride test and showed the following spectra: UV λ_{max}^{EtOH} nm(log ε): 205(5.16), 222(sh 4.93), 260(4.89), 296 (4.53), 316(4.54); $\lambda_{max}^{EtOH+AlCl_3}$ nm(log ε): 206(5.16), 222(sh 4.95), 260(4.90), 296 (4.53), 316(4.56). IR ν_{max}^{Nujol} cm^{-1}: 1644, 1637(sh), 1616(sh), 1610(sh), 1605, 1583 (sh). FD-MS spectrum: m/z 939(M^++1), 938(M^+). ^1H nmr, δ in $CDCl_3$ at 50°C, 0.55, 0.93(each 3H, s, $C_{19}-CH_3$), 1.30(1H, m, $C_{18}-H$), 1.38, 1.42, 1.58, 1.59(each 3H, s, $C_{11}-CH_3$ and $C_{11''}-CH_3$), 1.51(3H, s, $C_{16}-CH_3$), 1.80-2.00(1H, m, $C_{18}-H$), 2.55(1H, m, $C_{22}-H$), 3.00(2H, m, C_9-Hx2), 3.06(2H, m, $C_{9''}-H$x2), 3.60, 3.78, 3.79, 3.81, 3.86, 3.92, 4.00(each 3H, s, OCH_3), 5.10-5.25(2H, m, $C_{10}-H$ and $C_{10''}-H$), 5.25(1H, br s, $C_{15}-H$), 5.64(1H, d, J=10, $C_{23}-H$), 6.11(1H, br s, C_6-H), 6.30-6.42(3H, m, $C_{3'}-$, $C_{5'}-$ and $C_{6''}-H$), 6.53(1H, d, J=2, $C_{3'''}-H$), 6.55(1H, dd, J=2 and 8, $C_{5'''}-H$), 7.00-7.10(1H, m, $C_{6'}-H$), 7.29(1H, d, J=8, $C_{6'''}-H$)[4].

In the ^{13}C nmr spectrum of I, all the carbon atoms were assigned by the off-resonance decoupling technique as well as by comparison of the ^{13}C nmr spectra of the model compounds, kuwanon C (II)[1a], morusin (III)[1a]. The chemical shift values of the carbon atoms of the two prenylflavone skeletons were similar to those of the relevant carbon atoms of II and III except that the signals of carbon atoms at C-5, -8, -4a, -5", and -8" were affected by additional substituent effect (Table 1)[5]. This result indicated the presence of two partial structures with kuwanon C (II) and morusin (III) type of chromophores. This assumption was further supported by detailed analysis of the ^1H nmr spectrum (270 MHz, acetone-d_6) of I using sequential decoupling and by comparison of the spectra with those of II[1a] and III[1a] (Table 2)[6]. From the above results, the partial structure (I') was proposed. The EI-MS spectrum of I showed the following fragmentation species: m/z 486 (IV), 471 (IV-CH_3), 354 (V), 311 (V-C_3H_7, base peak)[7]. This fragmentation was interpreted reasonably by the partial structure (I').

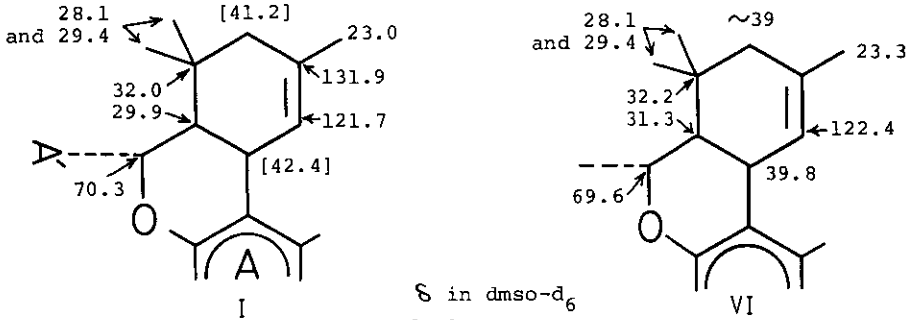
The remaining part ($C_{10}H_{15}$) of the C_{10} side chain consisted of the following ten aliphatic carbons: $-CH_3$ x3, $-CH_2-$, $>CH-$ x2, $>C<$, $>C{<}^{O-}_{H}$, $^{H}{>}C{=}C{<}$. In order to clarify the complete structure of the C_{10} side chain, the ^1H nmr spectrum of I was analysed with the aid of sequential decoupling experiments, and the deduced structure of the C_{10} side chain is shown in Fig. 2. This partial structure was supported by the comparison of the ^1H nmr spectrum and the ^{13}C nmr spectrum of I with those of dimoracin (VI)[8] as shown in Fig. 2 and 3. The EI-MS spectrum of Ia showed the following fragmentation species[8]: m/z 477(VII), 476(VIII), 462(IX). This fragmentation was interpreted reasonably by assuming that the reaction was initiated by retro-Diels-Alder reaction followed by cleavage of the benzyl-carbon and oxygen



δ in acetone-d_6

[ ]; CD_3OD

Fig 2



δ in dmso-d_6

[ ]; CD_3OD

Fig 3

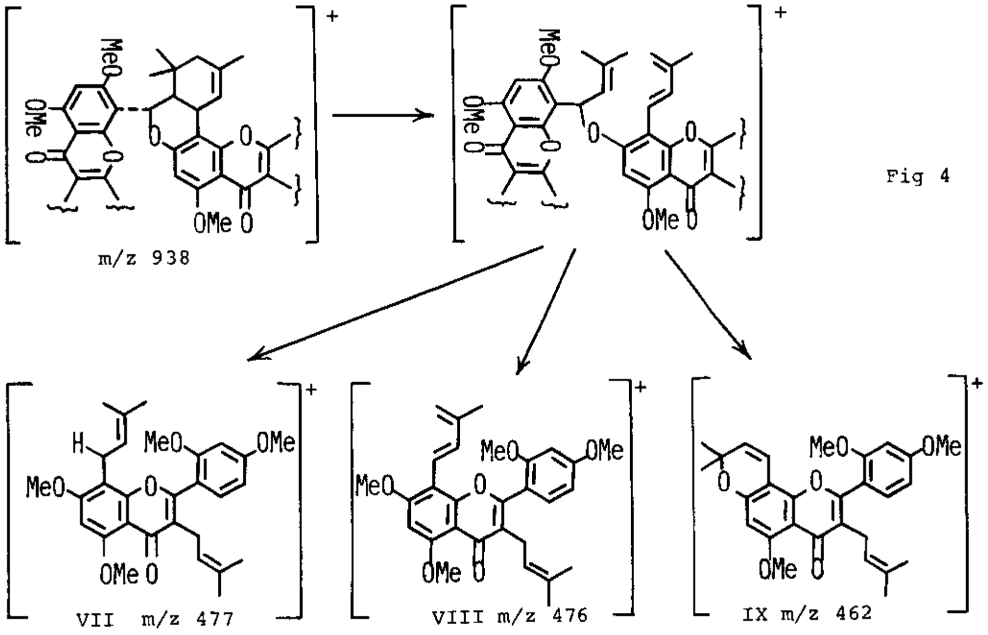


Fig 4

bond as shown in Fig. 4[8].

From the above results, we propose the formula I with the indicated (relative) configuration for kuwanon M. Kuwanon M (I) is optically active and is considered to be formed by a Diels-Alder type of enzymatic reaction process of kuwanon C (II)[1a], coexisting in the same root barks[9], and its dehydro derivative and subsequent cyclodehydrogenation[10].

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3 Elemental analysis gave a following result: Anal. Calcd. for $C_{50}H_{48}O_{12}\cdot H_2O$: C, 69.93; H, 5.82. Found: C, 69.56; H, 5.75.

4 The signal of C_{14}-H could not be detected. The signal may be overlapping with those of the methoxyl groups.

5 J.B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, 1972,p 196.

6 The signal of the C_6-H of I appeared at a higher applied magnetic field than that of III. The proton may be receiving a positive aryl shielding contribution. The similar phenomena were observed in Diels-Alder adducts obtained from the mulberry root barks[1a,e,f].

7 The formulae of the fragment ions were supported by the high-resolution mass spectrometry.

8 M. Takasugi, S. Nagao, and T. Masamune, Chem. Letters, 1982, 1217.

9 Meanwhile, the co-occurrence of I and II in Morus Lhou(ser.) Koidz. was noticed. T. Nomura unpublished data.

10 After completion of our work, Dr. Mitsuo Takasugi, Hokkaido University, imformed

us orally that his group assigned the same formula to the compound obtained from mulberry (October 20, 1982) and that the result had been described in the master's thesis presented by S. Ishikawa to Hokkaido University. Kuwanon M was indistinguishable(only ^{1}H nmr) from the compound obtained by Takasugi's group.

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