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HYPOTENSIVE CONSTITUENT, KUWANON H, A NEW FLAVONE DERIVATIVE FROM THE ROOT BARK OF THE CULTIVATED MULBERRY TREE (MORUS ALBA L.)

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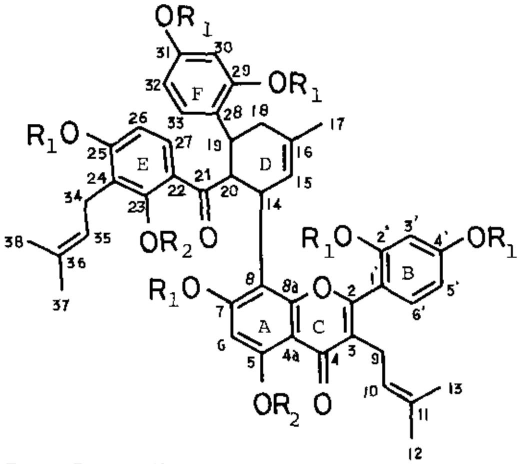
A new flavone derivative, containing condensed dihydrochalcone partial structure named kuwanon H, was isolated from the root bark of the cultivated mulberry tree (a variety of Morus alba L.). The structure was shown to be I on the basis of chemical and spectral data. The compound (I) to rabbit (1 mg/Kg, i.v.) produced a significant hypotension.

In the previous communication,[1] we reported that a new flavone derivative, containing a hypotensive effect named kuwanon G (II), was isolated from the root bark of the cultivated mulberry tree (a variety of Morus alba L.), and the structure was shown to be II. In this paper, we report the isolation and structure determination of a new flavone derivative, kuwanon H (I), isolated from the ethyl acetate extract, and clarified as having hypotensive action in rabbit.[2]

The dried root bark of the cultivated mulberry tree was extracted succesively with n-hexane, benzene, and ethyl acetate. The ethyl acetate extract was fractionated sequentially by the polyamide column chromatography, and by combined HPLC systems (Waters prep PAK-500/C_{18} followed by Waters prep PAK-500/silica gel) resulting in the isolation of a new prenylated flavone derivative, kuwanon H (I) in 0.13 % yield. The compound (I) to rabbit (lmg/Kg, i.v.) showed a marked hypotensive effect.[2]

Kuwanon H (I), amorphous powder,[3] m.p. 186-190°(decomp.), $[\alpha]_D^{22}$ = -536°

(c=0.166 in methanol), had a molecular formula of $C_{45}H_{44}O_{11}$(Anal. Calcd. for $C_{45}H_{44}O_{11}\cdot H_2O$: C, 69.40; H, 5.95. Found: C, 69.62; H, 5.85. ms m/e M^+ 760), and showed the following color reactions: Mg-HCl test (red); Zn-HCl test (orange); $FeCl_3$ test (dark green ⟶ dark purple), and showed the following spectra: ir ν_{max}^{Nujol} cm^{-1}: 3300, 1665(sh), 1655, 1625; uv λ_{max}^{MeOH} nm(log ε): 224(4.57), 263(4.45), 285(4.27), 325(sh 4.12); $\lambda_{max}^{MeOH+AlCl_3}$: 224(4.59), 273.5(4.54), 325 (4.08), 385(3.85); $\lambda_{max}^{MeOH+NaOMe}$: 223(4.63), 274.5(4.50), 343(4.48), 380(sh 4.05). The uv spectra were similar to those of kuwanon C (III)[4] suggesting that I possesses a kuwanon C partial structure. The treatment of I with dimethyl sulfate in acetone gave the following methyl ethers as an amorphous powder: hexamethyl ether (Ia), $C_{51}H_{56}O_{11}$ (M^+ 844), $FeCl_3$ (green), Gibbs test (positive), pmr ($CDCl_3$, δ 12.78 and 12.88, each 1H,s,2xOH, disappeared on addition of D_2O); octamethyl ether (Ib), $C_{53}H_{60}O_{11}$ (M^+ 872), $FeCl_3$ test (negative). These findings indicate that I has eight hydroxyl groups and two of them are hydrogen bonded. The mass spectrum of I showed the fragments[5] at m/e 582($C_{34}H_{30}O_9$, IV), 555($C_{33}H_{31}O_8$, V), 420($C_{25}H_{24}O_6$, VI), 377($C_{22}H_{17}O_6$), 354($C_{20}H_{18}O_6$, VII), 205($C_{12}H_{13}O_3$, VIII), 147 ($C_9H_7O_2$,IX), 110($C_6H_6O_2$,XI). On the other hand, the mass spectrum of kuwanon G (II)[1] showed the fragments at m/e 582(IV), 555(V), 420(VI), 377, 354(VII), 147(IX), 137($C_7H_5O_3$, X), 110(XI), but the fragment at m/e 205(VIII) could not be detected. The hexamethyl ether (Ia, 170 mg) was pyrolysed at 330°. The reaction products were purified by preparative tlc to give 2'-hydroxy-3'-γ,γ-dimethylallyl-2,4,4'-trimethoxychalcone (XII, 10.5 mg), $C_{23}H_{26}O_5$(M^+ 382), $FeCl_3$ test (olive green), uv λ_{max}^{MeOH} nm: 203, 250(sh), 320(sh), 381, pmr, δ in $CDCl_3$, 1.66, 1.78(each 3H,s,$C_{3''}$-CH_3), 3.37(2H,br d,J=8 Hz,$C_{1''}$-Hx2), 3.84(3H,s,OCH_3), 3.91(6H,s,OCH_3x2), 5.22(br t, J=8,$C_{2''}$-H), 6.45(d,J=9,$C_{5'}$-H), 6.46-6.57(2H,m,C_3- and C_5-H), 7.53(d,J=8,C_6-H), 7.58(d,J=16,C_α-H), 7.77(d,J=9,$C_{6'}$-H), 8.09(d,J=16,C_β-H), 13.79(s,$C_{2'}$-OH). The ms of XII showed the fragments at m/e 382(M^+, $C_{23}H_{26}O_5$), 367(M^+ - CH_3), 351 (M^+ - OCH_3), 339(M^+ - C_3H_7), 327(M^+ - C_4H_7), 219, 218(XIII),[6] 163(XIV).[6] These findings indicate that I has a condensed structure of dihydrochalcone derivative and kuwanon C, and that the isoprene unit is located at the C-24 position. The cmr spectrum was analysed as shown in Table 1. Assignments of the carbon atoms in I were performed by off-resonance decoupling technique as well as by comparison of the cmr spectra of the model compounds, such as, kuwanon G (II), kuwanon C (III),[7] and 2',4'-dihydroxyacetophenone (XV).[8] The chemical shift values of the carbon atoms of I were similar to those of II except the signals of carbon atoms which

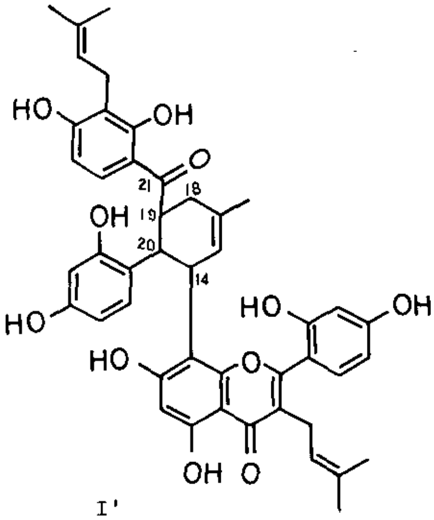


I : $R_1=R_2=H$

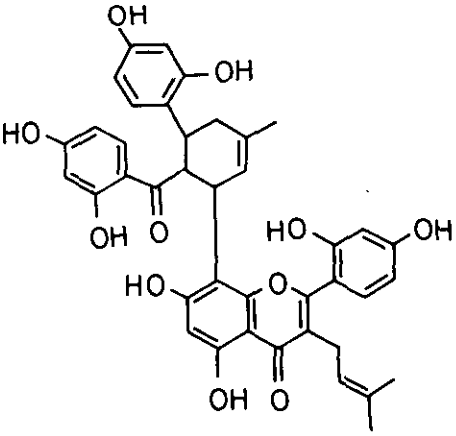
Ia : $R_1=CH_3$, $R_2=H$

Ib : $R_1=R_2=CH_3$

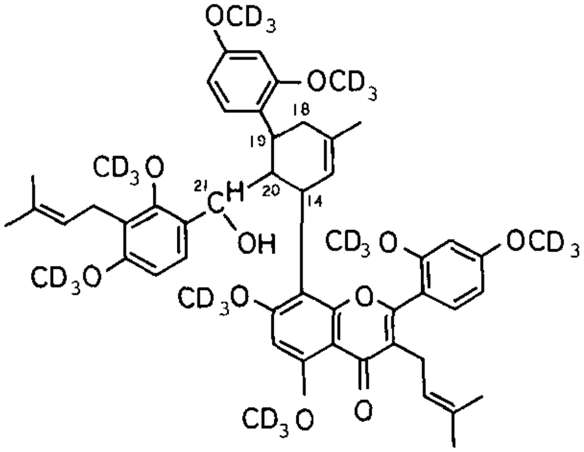
Ic : $R_1=R_2=CD_3$



I'



II



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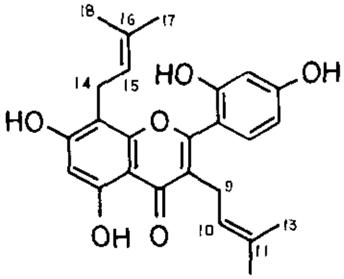

III

Chart 1

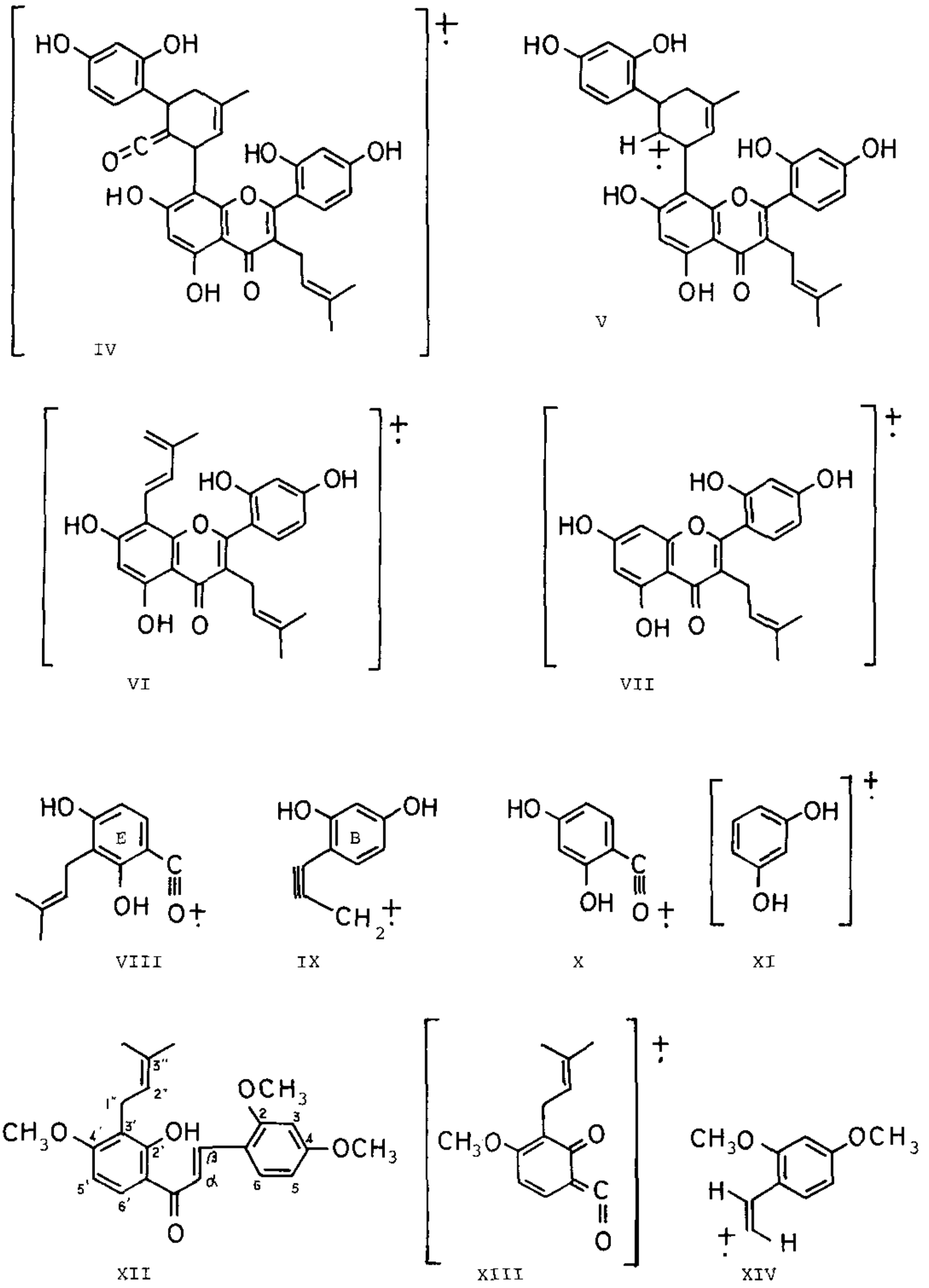
IV
V
VI
VII
VIII
E
IX
B
X
XI
XII
XIII
XIV
HO
OH
O
OH O
$O=C$
H
CH_2
OCH_3
CH_3O
1″
2″
3″
2′
3′
4′
5′
6′
2
3
4
5
6
α
β

Chart 2

Table 1. Cmr Spectra of Kuwanon H(I), G(II), C(III), and XV

carbon	kuwanon H(I)	kuwanon G(II)	kuwanon C(III)	XV	carbon	I
2	159.33(s)	159.18(s)	158.92(s)		34	21.37(t)
3	119.80(s)	119.69(s)	119.41(s)		35	122.39(d)
4	181.83(s)	181.72(s)	181.84(s)		36	130.52(s)
4a	103.93(s)	103.74(s)	103.45(s)		37	25.46(q)
5	155.29(s)	155.21(s)	155.01(s)		38	17.49(q)
6	97.73(d)	97.46(d)	97.93(d)			
7	161.61(s)	161.34(s)	161.72(s)			
8	106.86(s)	106.75(s)	105.48(s)			
8a	160.34(s)	160.26(s)	160.30(s)			
9	23.76(t)	23.53(t)	23.53(t)			
10	121.93(d)	121.81(d)	121.70(d)			
11	131.25(s)	131.17(s)	131.17(s)			
12	25.46(q)	25.42(q)	25.38(q)			
13	17.49(q)	17.33(q)	17.29(q)			
14	23.19(d)^{*1}	22.92(d)^{*1}	21.06(t)			
15	123.43(d)	123.23(d)	122.07(d)			
16	132.91(s)	132.83(s)	130.73(s)			
17	22.57(q)	22.45(q)	25.38(q)			
18	39.20(t)^{*2}	38.28(t)^{*3}	17.29(q)			
19	39.77(d)^{*1,2}	38.28(d)^{*1,3}				
20	45.72(d)	45.84(d)				
21	208.41(s)	208.11(s)		$(\underline{\mathrm{C}}=\mathrm{O})$ 205.11		
22	113.99(s)	114.03(s)		(C-1') 114.34		
23	162.07(s)	164.22(s)		(C-2') 165.00		
24	113.68(s)	102.58(d)		(C-3') 103.67		
25	161.61(s)	164.22(s)		(C-4') 165.61		
26	106.86(d)	107.21(d)		(C-5') 109.67		
27	122.92(d)	130.83(d)		(C-6') 135.21		
28	121.08(s)	120.73(s)				
29	155.90(s)	155.79(s)				
30	102.70(d)	101.97(d)				
31	155.90(s)	155.79(s)				
32	106.86(d)	106.75(d)				
33	131.25(d)	132.36(d)				
1'	111.56(s)	111.37(s)	111.32(s)			
2'	156.44(s)	156.33(s)	156.46(s)			
3'	102.70(d)	102.58(d)	102.69(d)			
4'	160.87(s)	160.83(s)	161.18(s)			
5'	106.86(d)	106.75(d)	106.73(d)			
6'	129.51(d)	131.17(d)	131.17(d)			

δ : ppm in dmso-d_6. *1 : Assignments may be reversed. *2 : measured in CD_3OD.
*3 : measured in pyridine-d_5.

Table 2. Pmr Spectra of Kuwanon H(I), G(II), and C(III)

	kuwanon H(I)	kuwanon G(II)	kuwanon C(III)
6-H	5.99(s)	5.98(s)	6.31(s)
3'-H	6.66(d,J=2 Hz)	6.67(d,J=2)	6.52(d,J=2)
5'-H	6.58(dd,J=2 and 8)	6.55(dd,J=2 and 8)	6.43(dd,J=2 and 8)
6'-H	7.29(d,J=8)	7.29 or 7.41(d,J=8)	7.20(d,J=8)
9-Hx2	3.14(br d,J=7)	3.17(br d,J=7)	3.12(br d,J=8)
10-H	4.90-5.50(m)	4.95-5.40(m)	5.02-5.32(m)
11-CH$_3$	1.48(s) and 1.62(s)	1.48(s) and 1.62(s)	1.43(s) and 1.57(s)
14-H	4.30-4.85(m)	4.30-4.70(m)	3.35(br d,J=8)
15-H	4.90-5.50(m)	4.95-5.40(m)	5.02-5.32(m)
16-CH$_3$	1.57(br s)	1.52(br s)	1.57(s,6H)
18-Hx2	1.80-2.20(m)	1.80-2.20(m)	
19-H	3.50-3.90(m)	3.30-3.90(m)	
20-H	4.30-4.85(m)	4.30-4.70(m)	
24-H		6.03(d,J=2)	
26-H	6.07(d,J=8)	5.93(dd,J=2 and 8)	
27-H	7.29(d,J=8)	7.29 or 7.41 (d,J=8)	
30-H	6.22(d,J=2)	6.21(d,J=2)	
32-H	6.00(dd,J=2 and 8)	6.08(dd,J=2 and 8)	
33-H	6.82(d,J=8)	6.78(d,J=8)	
34-Hx2	3.14(br d,J=7)		
35-H	4.90-5.50(m)		
36-CH$_3$	1.57(br s) and 1.67(s)		
OH	7.88(2H) and 8.70-9.70(4H)	7.60-9.63(6H)	
5-OH	13.16 or 13.58	13.19 or 13.23	13.13
23-OH	13.16 or 13.58	13.19 or 13.23	

(ppm) in acetone-d_6

were affected by the additional substituent effect. This result suggests that both I and II have the same skeletal structure. The pmr spectrum of I was analysed as shown in Table 2, and the assighnments of the signals in I were performed by comparison of the pmr spectra of II[1] and III.[4] The presence of two γ,γ-dimethylallyl groups was supported by the pmr spectrum. The location of these groups were suggested by the following results. In the cmr spectrum of I, the chemical shift value of C-24 appeared at a lower applied magnetic field than those of the C-24 of II and of the C-3' of XV. These results suggest that a γ,γ-dimethylallyl group is located at the C-24 position. The chemical shift values of the C-3 and C-9 of I were in good agreement with those of the C-3 and C-9 of II and III, whereas those of the C-8 and C-14 of I and II were shifted to a lower applied magnetic field than those of the C-8 and C-14 of III(Table 1). This results supports the presence of γ,γ-dimethylallyl group at C-3 position, as shown in II and III. The C-6 substituted prenylflavone structure for I was excluded for the following reason: Chari, et al reported that C-8 substituted prenylflavone is discriminated from C-6 substituted prenylflavones by comparison of the chemical shift values of the C-6 and C-8 signals.[9] On the other hand, the chemical shift values of the C-6 and C-8 signals of I (Table 1) were in good agreement with those of the C-8 substituted prenylflavones.[7,9] By comparing the pmr spectra of I and III, the signal of the C_6-H of I appeared at a higher applied magnetic field than that of III (Table 2). The proton may be receiving a positive aryl shielding contribution. This result suggests that the dihydrochalcone partial structure was not condensed with the isoprene unit at C-3 position, but with the isoprene unit substituted at C-8 position.

The arrangements of substituents in the D ring were assumed by following pmr data. The pmr spectrum of I showed the signals at δ 1.80-2.20(2H,m,C_{18}-Hx2), 3.50-3.90(1H,m,C_{19}-H), 4.30-4.85(2H,m,C_{14}- and C_{20}-H),[10] 4.90-5.50(3H,m,C_{10}-,C_{15}-, and C_{35}-H). The chemical shift values and coupling patterns were similar to those of II (Table 2), and the irradiation on the signals at δ 1.80-2.20 changed the multiplet at δ 3.50-3.90 to a broad doublet (J=10 Hz). From the above results, the structure of kuwanon H is possibly represented by I or I'.

The discrimination between I and I' was carried out on the basis of the pmr spectra of octadeuteromethyl ether (Ic) and the alcohol (Id) obtained by sodium borohydride reduction of Id. The compounds (Ic and Id) showed the following data: Ic, amorphous powder; ms m/e 896(M^+); pmr, δ in $CDCl_3$, 1.30-2.20(17H,CH_3x5 and

C_{18}-Hx2), 3.50-4.08(1H,m,C_{19}-H), 4.16-4.67(2H,m,C_{14}- and C_{20}-H),[10] 4.83-5.50(3H,m, C_{10}-,C_{15}-, and C_{35}-H). Id, amorphous powder; ms m/e 880(M^+ - H_2O); pmr, δ in $CDCl_3$, 1.35-2.00(17H,CH_3x5 and C_{18}-Hx2), 2.80-3.60(6H,m,C_9-Hx2,C_{34}-Hx2,C_{19}-, and C_{20}-H), 3.70-4.10(1H,C_{21}-OH, disappeared on addition of D_2O), 4.30-4.80(2H,m,C_{14}- and C_{21}-H), 4.90-5.35(3H,m,C_{10}-,C_{15}-, and C_{35}-H); ir ν_{max}^{Nujol} cm^{-1}: 3250(br), cmr, δ in $CDCl_3$, 177.40(C-4), 75.70(C-21). The signal of C_{20}-H of Id shifted to a higher applied magnetic field than that of Ic. If the structure of kuwanon H could be represented by I', three proton signals (C_{14}-,C_{20}-, and C_{21}-H) would appear at 4.0-5.0 ppm. From the above results, the structure I is considered to be more favorable than the structure I'.[11]

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10 The C_{20}-H signal appeared at lower aplied magnetic field. From the molecular model, the proton may be receiving a deshielding effect of aromatic anisotropy.

11 At the 100 th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 2, 1980, our group proposed orally the formula I and I' for a structure of kuwanon H and suggested that the structure I is more favorable than the structure I'.

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