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Constituents of *Morus alba* L. Cell Cultures. (1). Structures of Four New Natural Diels–Alder Type Adducts, Kuwanons J, Q, R, and V[1,2]

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From callus tissues of *Morus alba* L., four new yellow pigments, kuwanons J (**1**), Q (**2**), R (**3**), and V (**4**), have been isolated. Chemical and spectroscopic evidence revealed that these compounds are stereochemically identical Diels–Alder type adducts of two 4-hydroxyprenylchalcone moieties bearing all the four possible patterns of hydroxylation at the 2 and/or 16″ positions.

Keywords——callus ; *Morus alba*; mulberry; Moraceae; kuwanon J; kuwanon Q; kuwanon R; kuwanon V; Diels–Alder type adduct; chalcone

A series of natural Diels–Alder type adducts as well as a series of isoprenylated flavonoids has been isolated by us from the root bark of cultivated mulberry tree and from the Chinese crude drug "Sang-Bai-Pi" (Japanese name "Sōhakuhi") imported from the People's Republic of China, and their structures have been elucidated.[3] Some of the Diels–Alder type adducts, such as kuwanon G, sanggenon C, and mulberrofuran F, showed a significant hypotensive effect,[3] and some of the Diels–Alder type adducts as well as the prenylflavonoids showed strong activity against beef heart adenosine 3′,5′-cyclic monophosphate phosphodiesterase.[4] Moreover, a series of mulberry phytoalexins such as chalcomoracin and other 2-arylbenzofuran derivatives was reported by Takasugi *et al.*,[5] and a glycoprotein, moran A, which has a hypoglycemic effect on normal and alloxan-induced hyperglycemic mice, was reported by Hikino *et al.*[6] A few reports on mulberry tissue culture have been published during the past decade or so.[7] Apart from the isolation of β-sitosterol[7c] and detection of yellow substances which have not been characterized at all,[7a,c] these studies were mainly concerned with the response of the callus growth to the culture conditions.

In order to examine the production and biosynthesis of secondary metabolites in plant cell cultures, and to identify the constituents of the *Morus* root bark, we attempted to obtain pigment-producing callus cultures of *Morus alba* L. This paper describes the structure determination of four new Diels–Alder type adducts of two prenylchalcone derivatives, kuwanons J (**1**), Q, (**2**), R (**3**), and V (**4**), obtained from *Morus alba* L. cell cultures.

Callus tissues induced from the seedlings were subcultured under specified conditions[8] and subjected to selection over nine years, giving rise to cell strains having a high pigment productivity. The methanol extract of the callus tissues was dissolved in acetone. The acetone extract was fractionated by preparative thin layer chromatography (preparative TLC) on silica gel to give kuwanons J (**1**), Q (**2**), R (**3**), and V (**4**).

Kuwanon J (**1**), a yellow amorphous powder, $[\alpha]_D^{17}+85°$. The field desorption mass spectrum (FD-MS) showed the molecular ion peak at m/z 678. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** revealed the presence of forty carbons: fourteen aliphatic

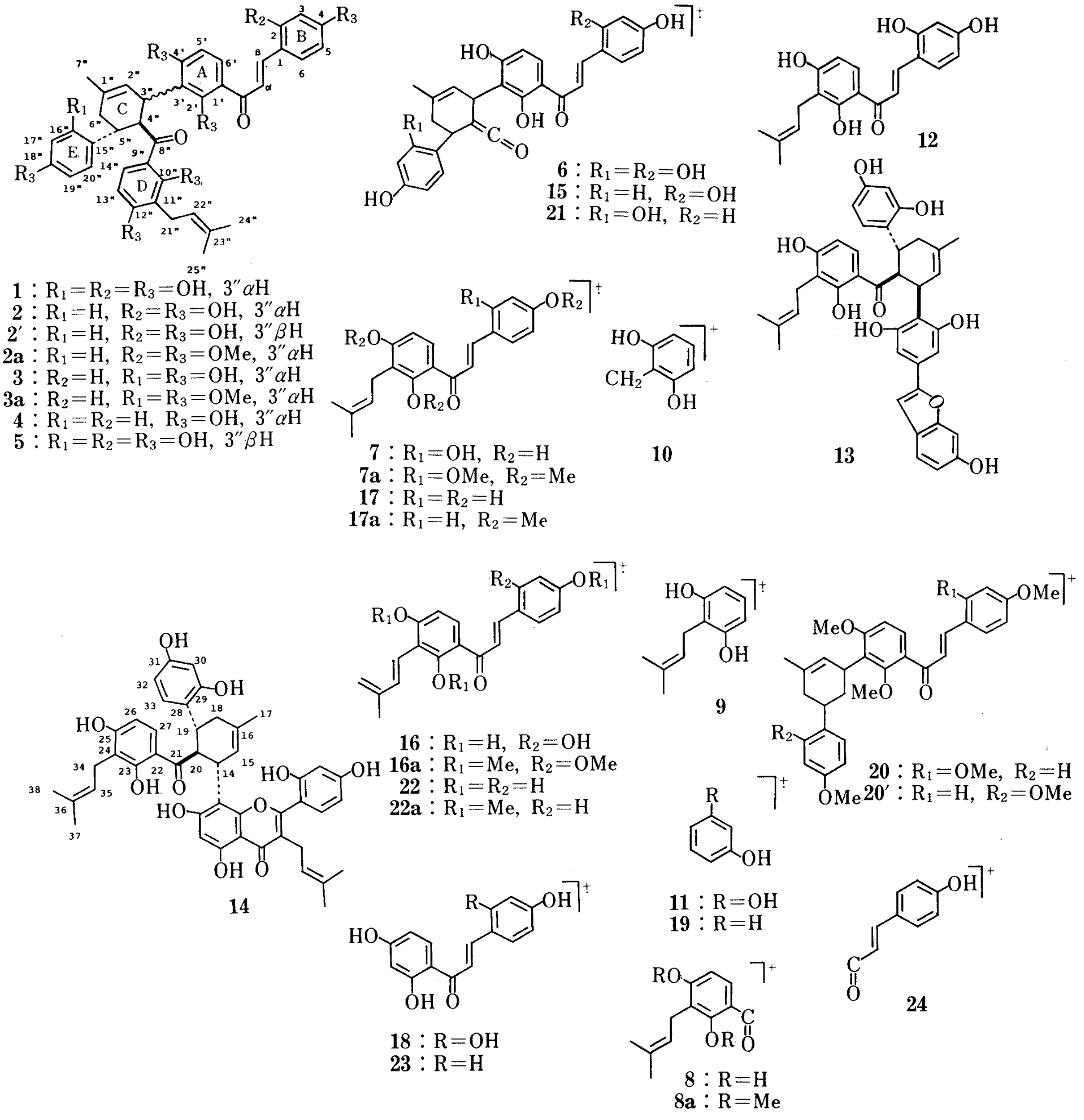

Fig. 1

carbons ($CH_3\text{-}\times 3$, $\text{-}CH_2\text{-}\times 2$, $CH\times 3$, $>C=C<^{H}\times 2$, $^{H}>C=C<_{H}\times 1$), twenty-four aromatic carbons ($CH\times 10$, $C\times 6$, $C\text{–}O\times 8$), and two carbonyl carbons (Table I). These spectra suggested the composition of kuwanon J to be $C_{40}H_{38}O_{10}$. The compound **1** gave a brown color in the ferric chloride test, and was negative to the magnesium hydrochloric acid and sodium borohydride test.[9] The infrared (IR) spectrum of **1** disclosed absorption bands due to hydroxyl, conjugated carbonyl, and benzene ring moieties as follows: $\nu_{\max}^{\text{KBr}}$ 3350, 1640 (sh), 1630 (sh), 1625 (sh), 1615 (sh), and 1605 cm^{-1}. The ultraviolet (UV) spectrum of **1**, exhibiting maxima at 264, 298, and 390 nm, was substantially similar to that of kuwanon I (**5**)[10] and to those of chalcone derivatives.[11] Moreover, the UV spectrum of **1** showed no red shift upon addition of aluminum chloride. It was reported that no aluminum chloride-induced shift is observed in UV spectra when a prenyl group is located *ortho* to a chelated hydroxyl group.[12] The proton magnetic resonance (^{1}H-NMR) spectrum of **1** showed signals assignable to two

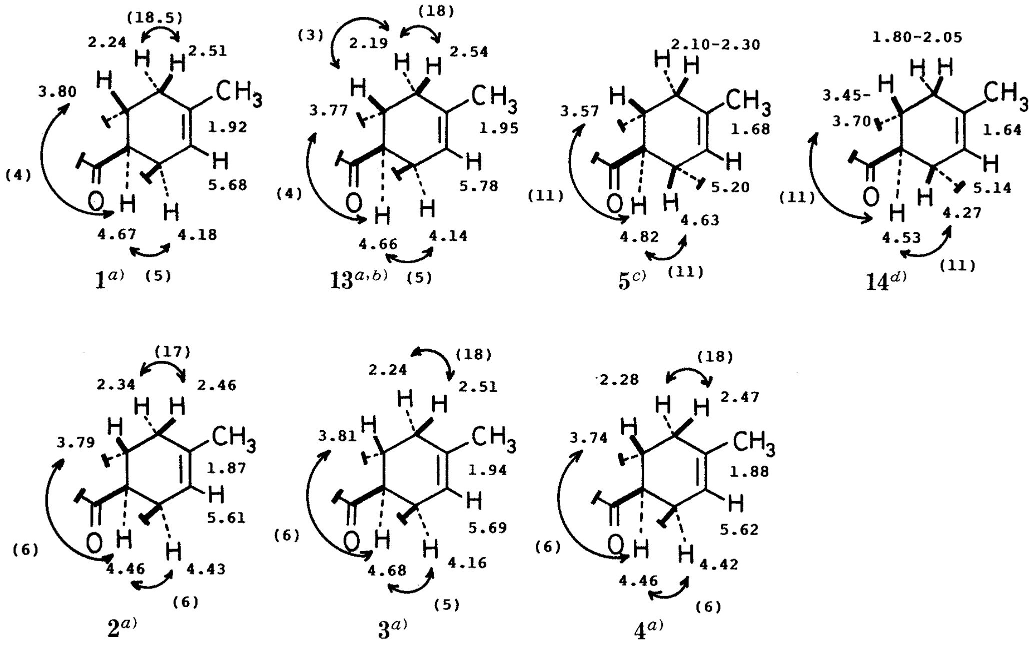

Fig. 2. ^{1}H-NMR Chemical Shifts (ppm) and Coupling Constants (Hz) of Cyclohexene Ring Protons of Kuwanons J (**1**), Q (**2**), R (**3**), and V (**4**) and Related Compounds **5**, **13**, and **14**

a) In $(CD_3)_2CO$.
b) Data from reference 5*b*.
c) In DMSO-d_6 at 120 °C, data from reference 10.
d) In DMSO-d_6 at 100 °C, data from reference 10.

hydrogen-bonded hydroxyl groups at δ 12.86 (1H, s) and 14.28 (1H, s). These data led us to presume that kuwanon J (**1**) possesses a chalcone partial structure, and that each of the *ortho* positions of the two hydrogen-bonded hydroxyl groups is substituted by an isoprene unit. In the FD-MS, fragment ions of **1** appeared at *m*/*z* 661[10] and 500 (**6**),[13] while in the electron-impact mass spectrum (EI-MS), they appeared at *m*/*z* 340 (**7**),[10] 205 (**8**),[13] 178 (**9**), 123 (**10**), and 110 (**11**).[10,13] These data also suggested that kuwanon J (**1**) is Diels–Alder type adduct resembling kuwanon I (**5**), which is regarded as a cycloaddition product of a chalcone and a dehydroprenylchalcone derivative, and that the isoprene unit is located at the dihydroxybenzoyl group. The ^{1}H-NMR spectrum of **1** showed signals of a 3,3-dimethylallyl group at δ 1.58 and 1.70 (each 3H, br s), 3.27 (2H, br d, $J=7$ Hz), and 5.17 (1H, br t, $J=7$ Hz) and the following signals of protons excluding those on the cyclohexene ring: double doublets, δ 6.31 (1H, $J=2.5$ and 8.5 Hz) and 6.43 (1H, $J=2.5$ and 9 Hz); *ortho*-coupled doublets, δ 6.37 (1H, $J=9$ Hz), 6.45 (1H, $J=9$ Hz), 6.98 (1H, $J=8.5$ Hz), 7.63 (1H, $J=9$ Hz), 7.84 (1H, $J=9$ Hz), and 8.36 (1H, $J=9$ Hz); *meta*-coupled doublets, δ 6.49 ($J=2.5$ Hz) and 6.52 (1H, $J=2.5$ Hz); doublets of vinyl protons on a *trans* double bond, δ 7.71 (1H, $J=15.5$ Hz) and 8.14 (1H, $J=15.5$ Hz). These spectral data suggest that **1** comprises two 1,2,4-trisubstituted and two 1,2,3,4-tetrasubstituted benzene rings with a *trans* double bond structure. Comparisons of the ^{13}C-NMR spectrum of **1** with those of **5** and morachalcone A (**12**)[5b] revealed that the chemical shifts of the carbon atoms, except those of the cyclohexene ring, are similar to the chemical shifts of the corresponding carbon atoms of **5** and **12** (Table I). These facts suggest that kuwanon J (**1**) is an isomer of **5** regarding the location of the substituents on the methylcyclohexene ring or the relative configuration of the substituents. Considering the biogenetic analogy of the Diels–Alder type adducts obtained from the *Morus* root bark,[3] the locations of the 2,4-dihydroxyphenyl and 2,4-dihydroxy-3-γ,γ-dimethylallylbenzoyl moieties seem to be at the C-5″ and -4″ positions, respectively. This assumption and the relative

Table I. ^{13}C-NMR Data for **1**, **2**, **3**, and Related Compounds

Carbon No.	**1**	**1**	**2**	**3**	**5**[b]	**12**	**13**	Carbon No.	**14**[c]
C-1	115.1	113.2[e]	115.3[d]	127.4	113.5	114.4			
C-2	160.0	159.0	159.9	131.7	159.0	159.2			
C-3	103.6	102.5	103.6	116.7	103.0	102.4			
C-4	162.3	161.4	162.3[e]	161.0	161.4[e]	161.5			
C-5	109.1	107.9[d]	109.2	116.7	108.1[d]	108.0[d]			
C-6	131.8	130.0	130.8	131.7	129.9	130.4			
C-α	117.3	116.2	117.5	118.3	116.2	115.6			
C-β	141.2	139.4	140.9	145.0	139.8	139.7			
C=O	193.4	191.5	193.3	192.9	192.0	191.9			
C-1′	114.0[d]	112.2	114.2[d]	113.9[e]	112.8	112.7			
C-2′	165.7	164.8	166.0	165.8	164.5	163.4			
C-3′	116.2	115.5	116.2	117.3	116.2	113.3			
C-4′	163.4	163.0	163.6	163.7	162.2	161.9			
C-5′	110.1	107.5[d]	109.2	110.2	107.6[d]	107.2[d]			
C-6′	130.6	130.0	129.2	130.9	128.6	129.6			
C-1″	134.7	132.2	135.2	134.8	131.8		133.8	C-16	132.9
C-2″	123.2[e]	121.7	123.2	123.2[d]	124.3		123.1	C-15	123.4
C-3″	32.4	33.0	33.3	32.5	(39.4 br)[f]		33.1	C-14	(39.8)[f]
C-4″	47.4	46.6	50.0	47.4	44.8		47.7	C-20	45.7
C-5″	36.4	35.8	40.4	36.4	(39.4 br)[f]		36.4	C-19	(39.8)[f]
C-6″	32.4	33.0	36.1	32.4	(38.2 br)[f]		32.4	C-18	(39.2)[f]
C-7″	23.8	23.3	23.8	23.8	22.5		23.8	C-17	22.6
C-8″	209.5	207.5	207.6	209.5	208.7		209.2	C-21	208.4
C-9″	113.3[d]	114.2	114.1[d]	113.3[e]	114.1		113.3	C-22	208.4
C-10″	164.5	162.0	164.2	164.6	161.8[e]		164.6	C-23	162.1
C-11″	115.8	113.6[e]	115.5[d]	115.8	114.4		115.8	C-24	113.7
C-12″	163.4	161.3	162.7[e]	163.7	161.4[e]		163.3	C-25	161.6
C-13″	108.2	105.9	108.1	108.1	106.2		108.1	C-26	106.9
C-14″	128.6	130.2	129.2	128.6	129.9		128.6	C-27	131.3
C-15″	121.7	122.4	136.7	121.7	120.7		121.9	C-28	121.2
C-16″	156.5	155.3	131.8	156.4	156.0		156.6	C-29	155.9
C-17″	103.6	102.3	116.0	103.6	103.0		103.5	C-30	102.7
C-18″	157.9	155.8	156.6	157.9	155.7		157.7	C-31	155.9
C-19″	107.4	106.5	116.0	107.5	106.2		107.4	C-32	106.9
C-20″	132.1	132.2	131.8	132.9	129.5		132.1	C-33	131.3
C-21″	22.1	21.1	22.2	22.2	21.1		22.2	C-34	21.4
C-22″	123.5[e]	122.4	123.2	123.4[d]	122.5		124.4	C-35	122.4
C-23″	131.4	130.2	131.4	131.4	129.9		131.4	C-36	130.5
C-24″	25.8	25.3	25.8	25.8	25.2		25.8	C-37	25.5
C-25″	17.8	17.5	17.9	17.8	17.5		17.8	C-38	17.5
Solvent[a]	A	B	A	A	B[g]	A	A		B

a) A, $(CD_3)_2CO$; B, DMSO-d_6. *b*) Data from reference 10. *c*) Data from reference 3*b*. *d*—*e*) Assignments may be interchanged in each column. *f*) In CD_3OD at 35 °C. *g*) At 90 °C.

configuration of the substituents on the methylcyclohexene ring were supported by comparing the ^{13}C- and ^{1}H-NMR spectra of **1** with those of **5**, chalcomoracin (**13**),[5b] and kuwanon H (**14**).[13] Kuwanon J (**1**) resembles **13** more than **5** and **14** in terms of the chemical shifts of the carbon atoms of the methylcyclohexene ring (Table I) and also in the chemical shifts and coupling constants of the protons of the methylcyclohexene ring (Fig. 2). On the basis of these findings, we propose formula **1** for the structure of kuwanon J.

Kuwanon Q (**2**), a yellow amorphous powder, $[\alpha]_D^{16}+160°$, gave a brown color in the ferric chloride test. The FD-MS showed the molecular ion peak at m/z 662. The ^{13}C-NMR spectrum of **2** revealed the presence of the forty carbons: fourteen aliphatic carbons, twenty-four aromatic carbons (CH × 11, C × 6, C–O × 7), and two carbonyl carbons (Table I). Work-up of **2** with dimethyl sulfate gave the heptamethyl ether (**2a**), $C_{47}H_{52}O_9$, which showed a negative ferric chloride test. The molecular formula of **2** was thus inferred to be $C_{40}H_{38}O_9$.

The UV spectrum of **2**, exhibiting maxima at 222 (sh), 266 (sh), 300, and 389 nm, was similar to that of **1** and showed no red shift upon addition of aluminum chloride. In the FD-MS of **2**, fragment ions appeared at *m/z* 662 (M^+), 484 ($M^+ - 178$, **15**),[13] 338 (**16**),[10,13] 324 (**17**),[10] 272 (**18**),[13] while in the EI-MS, they appeared at *m/z* 178 (**9**), 123 (**10**), 110 (**11**),[10,13] 94 (**19**). In the EI-MS of **2a**, the fragment ions appeared at *m/z* 760 (M^+), 527 (**20**),[10,13] 394 (**16a**),[10,13] 366 (**17a**),[10] 233 (**8a**).[10,13] These results suggest that **2** is a Diels–Alder type adduct like **1**. As described in Experimental, the ^{1}H-NMR spectrum of **2** examined by comparison with that of **1** revealed the presence of a 3,3-dimethylallyl group, a 1,2,4-trisubstituted benzene ring, two 1,2,3,4-tetrasubstituted benzene rings, a 1,4-disubstituted benzene ring, and a *trans* double bond. The chemical shifts and the coupling constants of the protons on the methylcyclohexene ring are shown in Fig. 2. In the ^{13}C-NMR spectrum of **2**, the chemical shifts of the carbon atoms, except those of the carbon atoms at the C-4″, -5″, and -6″ positions and on the E-ring, were similar to those of the corresponding carbon atoms of **1** (Table I). On the basis of these findings, together with the biogenetic analogy of the Diels–Alder type adducts,[3] the structure of kuwanon Q was inferred to be **2** or **2′**.

Discrimination between these structures (**2** and **2′**) was done by comparing the signals of the methylcyclohexene ring protons of **2** with those of **1** and other Diels–Alder type adducts.[3] Kuwanon Q (**2**) resembled **1** and **13**[5b] more than **5**[10] and **14**[13] in terms of the chemical shifts and coupling constants of the corresponding protons of the methylcyclohexene ring (Fig. 2). From these results, the structure of kuwanon Q was concluded to be represented by the formula (**2**).

Kuwanon R (**3**), a yellow amorphous powder, $[\alpha]_D^{17} + 56°$, gave a brown color in the ferric chloride test. The FD-MS showed the molecular ion peak at *m/z* 662. The ^{13}C-NMR spectrum of **3** revealed the presence of the forty carbons: fourteen aliphatic carbons, twenty-four aromatic carbons, and two carbonyl carbons (Table I). Work-up of **3** with dimethyl sulfate gave the heptamethyl ether (**3a**), $C_{47}H_{52}O_9$, which showed a negative ferric chloride test. The molecular formula of **3** was thus inferred to be $C_{40}H_{38}O_9$.

The UV spectrum of **3**, exhibiting maxima at 224 (sh), 299, and 370 nm, showed no red shift upon addition of aluminum chloride. Comparing the UV spectrum of **3** with that of **2**, a hypsochromic shift (about 20 nm) was observed in "band I"[11] of **3**. This result suggests that **3** is a 4-hydroxychalcone having no hydroxyl group at the C-2 position.[11] In the FD-MS of **3**, fragment ions appeared at *m/z* 662 (M^+), 484 ($M^+ - 178$, **21**)[10,13] 340 (**7**)[10] 322 (**22**),[10,13] 256 (**23**),[13] 205 (**8**),[10,13] 203,[10] while in the EI-MS of **3a** they appeared at *m/z* 760 (M^+), 527 (**20′**),[10,13] 396 (**7a**),[10] 364 (**22a**),[10,13] 233 (**8a**).[10] These results suggest that **3** is a Diels–Alder type adduct. As described in Experimental, the ^{1}H-NMR spectrum of **3** showed the presence of a 3,3-dimethylallyl group, a 1,2,4-trisubstituted benzene ring, a 1,4-disubstituted benzene ring, two 1,2,3,4-tetrasubstituted benzene rings, and a *trans* double bond. The chemical shifts and coupling constants of the protons on the methylcyclohexene ring were similar to those of the corresponding protons of **1**, **2**, and **13**[5b] (Fig. 2). Comparison of the ^{13}C-NMR spectrum of **3** with that of **1** revealed that the chemical shifts of the carbon atoms, except those of the carbon atoms at C-β and the B-ring, are similar to those of the corresponding carbon atoms of **1** (Table I). These results suggest that the structure of kuwanon R is represented by the formula (**3**).

Kuwanon V (**4**), a yellow amorphous powder, $[\alpha]_D^{23} + 145°$, gave a brown color in the ferric chloride test. The FD-MS showed the molecular ion peak at *m/z* 646. The UV spectrum of **4**, exhibiting maxima at 226 (sh), 296, and 370 nm, was similar to that of **3** and did not show any aluminum chloride-induced shifts. In the EI-MS of **4**, fragment ions appeared at *m/z* 205 (**8**)[10] 149, 147 (**24**), 123 (**10**), and 107. As described in Experimental, the ^{1}H-NMR spectrum of **4** showed the presence of a 3,3-dimethylallyl group, two 1,2,3,4-tetrasubstituted benzene rings, two 1,4-disubstituted benzene rings, and a *trans* double bond. The chemical shifts and coupling constants of the protons of the methylcyclohexene ring are shown in Fig. 2. Comparing the ^{1}H-NMR spectrum of **4** with those of **2** and **3**, the chemical shifts and coupling constants of the proton signals of the chalcone moiety were similar to those of the corresponding proton signals of **3**, while other proton signals were similar to those of the corresponding proton signals of **2**. From these results, the structure of kuwanon V was concluded to be represented as the formula (**4**).

Kuwanons Q and R were also obtained from *M. alba* cell suspension cultures grown in the same medium but without agar.

It is noteworthy that kuwanons J (**1**), Q (**2**), R (**3**), and V (**4**), the stereochemically identical Diels–Alder type adducts of two 4-hydroxyprenylchalcone moieties bearing all the four possible patterns of hydroxylation at the 2 and/or 16″ positions, were isolated from *M. alba* callus tissues.

Experimental

^{1}H- and ^{13}C-NMR spectra were measured with tetramethylsilane (TMS) as an internal reference. Chemical shifts are expressed in ppm downfield from TMS and coupling constants (J) in Hz. Abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, sh = shoulder, infl. = inflection. The following instruments were used: UV spectra, Hitachi 340 UV spectrometer; IR spectra, Hitachi 260-30 IR spectrometer; ^{1}H-NMR spectra, JEOL FX-270 spectrometer; ^{13}C-NMR spectra, JEOL FX-270 and Hitachi R-900 FT NMR spectrometers; MS, JEOL JMS 01SG-2 and Hitachi RMU mass spectrometers; optical rotations, JASCO DIP-4 digital polarimeter. For TLC and preparative TLC, Wakogel B-5FM and B-5F were used, and for column chromatography, Wakogel C-200.

Isolation of Kuwanons J (1), Q (2), R (3), and V (4)——The callus tissues (233 g, fresh weight) were extracted three times with MeOH. The MeOH extract was concentrated under reduced pressure to afford a residue (13.57 g), which was extracted twice with $(CH_3)_2CO$. The $(CH_3)_2CO$ extract was concentrated under reduced pressure to afford the residue (995.6 mg), which was fractionated by preparative TLC (solvent system, benzene : AcOEt = 2 : 3) to give crude kuwanon J (**1**, 21.0 mg), a crude mixture of kuwanons Q (**2**) and R (**3**) (24.0 mg), and crude kuwanon V (**4**, 5.8 mg). The crude **1** (21.0 mg) was purified by preparative TLC (benzene : $(CH_3)_2CO$ = 6 : 1) to give **1** (6.0 mg). The crude mixture of **2** and **3** (24.0 mg) was fractionated by preparative TLC ($(CH_3)_2CO$: *n*-hexane = 1 : 1) to give crude **2** (2.0 mg) and **3** (2.8 mg). The crude **2** (2.0 mg) was further purified by preparative TLC ($CHCl_3$: MeOH = 6 : 1, *n*-hexane : AcOEt = 1 : 2) to give **2** (1.0 mg). The crude **3** (2.8 mg) was purified by preparative TLC ($CHCl_3$: MeOH = 6 : 1, *n*-hexane : AcOEt = 1 : 2) to give **3** (1.7 mg). The crude **4** (5.8 mg) was purified by preparative TLC ($(CH_3)_2CO$: *n*-hexane = 1 : 1, $CHCl_3$: MeOH = 10 : 1, $CHCl_3$: AcOEt = 2 : 1) to give **4** (0.3 mg).

Kuwanon J (1)——Compound **1** was obtained as an amorphous yellow powder, $[\alpha]_D^{17}$ +85° (c = 0.040, MeOH). $FeCl_3$ test (brown), Mg–HCl test (negative), $NaBH_4$ test (negative). *Anal.* Calcd for $C_{40}H_{38}O_{10} \cdot 3/2H_2O$: C, 68.07; H, 5.86. Found: C, 68.29; H, 5.84. UV λ_{max}^{EtOH} nm (log ε): 223 (sh 4.56), 264 (sh 4.03), 298 (4.30), 390 (4.47); $\lambda_{max}^{EtOH+AlCl_3}$: 223 (sh 4.58), 266 (sh 4.04), 301 (4.30), 392 (4.44), 460 (infl. 3.65). IR ν_{max}^{KBr} cm^{-1}: 3350, 1640 (sh), 1630 (sh), 1625 (sh), 1615 (sh), 1605. FD-MS *m/z*: 678 (M^+), 661, 500 ($M^+ - 178$, **6**), EI-MS (30 eV, In-beam) *m/z* (relative intensity, %): 340 (4, **7**), 205 (7, **8**), 178 (98, **9**), 123 (base peak, **10**), 110 (85, **11**). ^{1}H-NMR ($(CD_3)_2CO$, 270 MHz) δ: 1.58, 1.70 (each 3H, br s, $C_{23''}$-CH_3), 1.92 (3H, br s, $C_{1''}$-CH_3), 2.24, 2.51 (each 1H, br d, J = 18.5, $C_{6''}$-H), 3.27 (2H, br d, J = 7, $C_{21''}$-H × 2), 3.80 (1H, m, $C_{5''}$-H), 4.18 (1H, m, $C_{3''}$-H), 4.67 (1H, m, $C_{4''}$-H), 5.17 (1H, br t, J = 7, $C_{22''}$-H), 5.68 (1H, br s, $C_{2''}$-H), 6.31 (1H, dd, J = 2.5, 8.5, $C_{19''}$-H), 6.37 (1H, d, J = 9, $C_{5'}$-H), 6.43 (1H, dd, J = 2.5, 9, C_5-H), 6.45 (1H, d, J = 9, $C_{13''}$-H), 6.49 (1H, d, J = 2.5, C_3-H), 6.52 (1H, d, J = 2.5, $C_{17''}$-H), 6.98 (1H, d, J = 8.5, $C_{20''}$-H), 7.63 (1H, d, J = 9, C_6-H), 7.71 (1H, d, J = 15.5, C_α-H), 7.84 (1H, d, J = 9, $C_{6'}$-H), 8.14 (1H, d, J = 15.5, C_β-H), 8.36 (1H, d, J = 9, $C_{14''}$-H), 12.86 (1H, s, $C_{10''}$-OH), 14.28 (1H, s, $C_{2'}$-OH).[14] The ^{13}C-NMR ($(CD_3)_2CO$, 67.5 MHz): = Table I.

Kuwanon Q (2)——Compound **2** was obtained as an amorphous yellow powder, $[\alpha]_D^{16}$ +161° (c = 0.081, $(CH_3)_2CO$). $FeCl_3$ test (brown). UV λ_{max}^{EtOH} nm (log ε): 222 (sh 4.37), 266 (sh 3.94), 300 (4.06), 389 (4.26); $\lambda_{max}^{EtOH+AlCl_3}$: 222 (sh 4.38), 266 (sh 3.94), 300 (4.06), 389 (4.24). IR ν_{max}^{KBr} cm^{-1}: 3360. 1620 (sh), 1610. FD-MS *m/z*: 662 (M^+), 484 ($M^+ - 178$, **15**), 338 (**16**), 324 (**17**), 272 (**18**). EI-MS (75 eV) *m/z* (relative intensity, %): 178 (11, **9**), 123 (35, **10**), 110 (35, **11**), 94 (11, **19**), 44 (base peak). ^{1}H-NMR ($(CD_3)_2CO$, 270 MHz) δ: 1.58, 1.68 (each 3H, br s, $C_{23''}$-CH_3), 1.87

(3H, br s, $C_{1''}$-CH_3), 2.34, 2.46 (each 1H, br d, $J=17$, $C_{6''}$-H), 3.22 (2H, br d, $J=7$, $C_{21''}$-H × 2), 3.79 (1H, m, $C_{5''}$-H), 4.43 (1H, m, $C_{3''}$-H), 4.46 (1H, m, $C_{4''}$-H), 5.14 (1H, br t, $J=7$, $C_{22''}$-H), 5.61 (1H, br s, $C_{2''}$-H), 6.37 (1H, d, $J=8.5$, $C_{5'}$-H), 6.43 (1H, dd, $J=2$, 8.5, C_5-H), 6.50 (1H, d, $J=2$, C_3-H), 6.51 (1H, d, $J=8.5$, $C_{13''}$-H), 6.72 (2H, d, $J=8.5$, $C_{17''}$- and $C_{19''}$-H), 7.18 (2H, d, $J=8.5$, $C_{16''}$- and $C_{20''}$-H), 7.65 (1H, d, $J=8.5$, C_6-H), 7.73 (1H, d, $J=15$, C_α-H), 7.86 (1H, d, $J=8.5$, $C_{6'}$-H), 7.89 (1H, d, $J=8.5$, $C_{14''}$-H), 8.18 (1H, d, $J=15$, C_β-H), 12.86 (1H, s, $C_{10''}$-H), 14.37 (1H, s, $C_{2'}$-OH). The ^{13}C-NMR (($(CD_3)_2CO$, 67.5 MHz): Table I.

Kuwanon Q Heptamethyl Ether (2a)——A mixture of **2** (5.3 mg), $(CH_3)_2SO_4$ (0.2 ml) and K_2CO_3 (5 g), in $(CH_3)_2CO$ (30 ml) was refluxed for 6 h, and treated as usual. The product was purified by preparative TLC (benzene : MeOH = 40 : 1) to give kuwanon Q heptamethyl ether (**2a**, 2.4 mg). The compound was obtained as an amorphous powder, $FeCl_3$ test (negative). High-resolution MS: Calcd for $C_{47}H_{52}O_9$ (M^+): *m/z* 760.3607. Found: *m/z* 760.3559. Calcd for $C_{33}H_{35}O_6$ (**20**): *m/z* 527.2431. Found: *m/z* 527.2429. Calcd for $C_{24}H_{26}O_5$ (**16a**): *m/z* 394.1779. Found: *m/z* 394.1805. Calcd for $C_{23}H_{26}O_4$ (**17a**): *m/z* 366.1829. Found: *m/z* 366.1850. Calcd for $C_{14}H_{17}O_3$ (**8a**): *m/z* 233.1176. Found: *m/z* 233.1169.

Kuwanon R (3)——Compound **3** was obtained as an amorphous yellow powder, $[\alpha]_D^{17}+56°$ ($c=0.165$, $(CH_3)_2CO$). $FeCl_3$ test (brown). UV λ_{max}^{EtOH} nm (log ε): 224 (sh 4.47), 299 (4.22), 370 (4.35); $\lambda_{max}^{EtOH+AlCl_3}$: 224 (sh 4.98), 299 (4.22), 370 (4.35). IR ν_{max}^{KBr} cm^{-1}: 3350, 1625 (sh), 1615 (sh), 1605. FD-MS *m/z*: 662 (M^+), 484 (M^+-178, **21**), 340 (**7**), 322 (**22**), 256 (**23**), 205 (**8**), 203. EI-MS (75 eV) *m/z* (relative intensity, %): 322 (15), 256 (7), 178 (7, **9**), 123 (13, **10**), 110 (3, **11**). ^{1}H-NMR (($(CD_3)_2CO$, 270 MHz) δ: 1.59, 1.72 (each 3H, br s, $C_{23''}$-CH_3), 1.94 (3H, br s, $C_{1''}$-CH_3), 2.24, 2.51 (each 1H, br d, $J=18$, $C_{6''}$-H), 3.27 (2H, br d, $J=7$, $C_{21''}$-H × 2), 3.81 (1H, m, $C_{5''}$-H), 4.16 (1H, m, $C_{3''}$-H), 4.68 (1H, m, $C_{4''}$-H), 5.17 (1H, br t, $J=7$, $C_{22''}$-H), 5.69 (1H, br s, $C_{2''}$-H), 6.33 (1H, dd, $J=2.5$, 8.5, $C_{19''}$-H), 6.38 (1H, d, $J=9$, $C_{5'}$-H), 6.45 (1H, d, $J=9$, $C_{13''}$-H), 6.55 (1H, d, $J=2.5$, $C_{17''}$-H), 6.90 (2H, d, $J=9$, C_3- and C_5-H), 6.98 (1H, d, $J=8.5$, $C_{20''}$-H), 7.69 (1H, d, $J=15.5$, C_α-H), 7.70 (2H, d, $J=9$, C_2- and C_6-H), 7.78 (1H, d, $J=15.5$, C_β-H), 7.95 (1H, d, $J=9$, $C_{6'}$-H), 8.41 (1H, d, $J=9$, $C_{14''}$-H), 12.88 (1H, s, $C_{10''}$-OH), 14.28 (1H, s, $C_{2'}$-OH). The ^{13}C-NMR (($(CD_3)_2CO$, 22.6 Hz): Table I.

Kuwanon R Heptamethyl Ether (3a)——A mixture of **3** (5.6 mg), $(CH_3)_2SO_4$ (0.2 ml) and K_2CO_3 (5 g) in $(CH_3)_2CO$ (30 ml) was refluxed for 6 h, and treated as usual. The product was purified by preparative TLC (benzene : MeOH = 40 : 1) to give kuwanon R heptamethyl ether (**3a**, 3.9 mg). The compound was obtained as an amorphous powder, $FeCl_3$ test (negative). High-resolution MS, Calcd for $C_{47}H_{52}O_9$ (M^+): *m/z* 760.3608. Found: *m/z* 760.3593. Calcd for $C_{33}H_{35}O_6$ (**20′**): *m/z* 527.2431. Found: *m/z* 527.2430. Calcd for $C_{24}H_{28}O_5$ (**7a**): *m/z* 396.1935. Found: *m/z* 396.1940. Calcd for $C_{23}H_{24}O_4$ (**22a**): *m/z* 364.1673. Found: *m/z* 364.1667. Calcd for $C_{14}H_{17}O_3$ (**8a**): *m/z* 233.1176. Found: *m/z* 233.1150.

Kuwanon V (4)——Compound **4** was obtained as an amorphous yellow powder, $[\alpha]_D^{23}+145°$ ($c=0.11$, $(CH_3)_2CO$). $FeCl_3$ test (brown). UV λ_{max}^{EtOH} nm (log ε): 226 (sh 4.70), 296 (4.37), 370 (4.56); $\lambda_{max}^{EtOH+AlCl_3}$: 226 (sh 3.86), 296 (3.47), 370 (3.59). IR ν_{max}^{KBr} cm^{-1}: 3390, 1650 (sh), 1640 (sh), 1600. FD-MS *m/z*: 646 (M^+). EI-MS (75 eV) *m/z* (relative intensity, %): 205 (20, **8**), 149 (27), 127 (17, **24**), 123 (30, **10**), 107 (20). ^{1}H-NMR (($(CD_3)_2CO$, 270 MHz) δ: 1.58, 1.68 (each 3H, br s, $C_{23''}$-CH_3), 1.88 (3H, br s, $C_{1''}$-CH_3), 2.28 (1H, dd, $J=5.5$, 18, $C_{6''}$-H), 2.47 (1H, br d, $J=18$, $C_{6''}$-H), 3.21 (2H, br d, $J=7$, $C_{21''}$-H × 2), 3.74 (1H, m, $C_{5''}$-H), 4.42 (1H, m, $C_{3''}$-H), 4.46 (1H, m, $C_{4''}$-H), 5.14 (1H, br t, $J=7$, $C_{22''}$-H), 5.62 (1H, br s, $C_{2''}$-H), 6.36 (1H, d, $J=9$, $C_{5'}$-H), 6.49 (1H, d, $J=9$, $C_{13''}$-H), 6.73 (2H, d, $J=8.5$, $C_{17''}$- and $C_{19''}$-H), 6.91 (2H, d, $J=9$, C_3- and C_5-H), 7.19 (2H, d, $J=8.5$, $C_{16''}$- and $C_{20''}$-H), 7.69 (1H, d, $J=15$, C_α-H), 7.70 (2H, d, $J=9$, C_2- and C_6-H), 7.78 (1H, d, $J=15$, C_β-H), 7.89 (1H, d, $J=9$, $C_{14''}$-H), 7.94 (1H, d, $J=9$, $C_{6'}$-H), 12.83 (1H, s, $C_{10''}$-OH), 14.17 (1H, s, $C_{2'}$-OH).

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References and Notes

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