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FOUR NEW NATURAL DIELS-ALDER TYPE ADDUCTS, MULBERROFURAN E, KUWANON Q, R, AND V FROM CALLUS CULTURE OF MORUS ALBA L.

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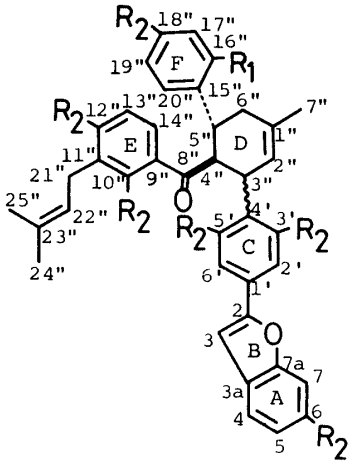
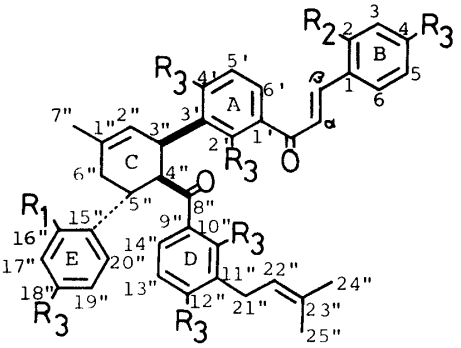
From callus tissues of Morus alba L. the four new Diels-Alder type adducts mulberrofuran E (3), kuwanon Q (4), R (5), and V (6) have been isolated. Their structures were established by chemical and spectroscopic means. Mulberrofuran E (3) was found to be an adduct of a chalcone and a dehydroprenyl-2-arylbenzofuran, whereas kuwanon Q (4), R (5), and V (6) were identified as adducts of two prenylchalcone derivatives.

KEYWORDS——Morus alba; tissue culture; mulberrofuran E; kuwanon Q; kuwanon R; kuwanon V; Diels-Alder type adduct; 2-arylbenzofuran; chalcone

Previously, we described the structure of a new Diels-Alder type adduct, kuwanon J (1), isolated along with chalcomoracin (2) from Morus alba L. callus tissues.[1] In the course of extended studies on M. alba callus constituents, we further isolated four new Diels-Alder type adducts named mulberrofuran E (3), kuwanon Q (4), R (5), and V (6) from the same cell line of the callus culture.[1] Preparative thin layer chromatography of the methanol extract of the callus tissues (233 g, fresh weight) on silica gel gave mulberrofuran E (3) (7.2 mg), kuwanon Q (4) (1.7 mg), R (5) (1.0 mg), and V (6) (0.3 mg) in addition to the known constituents.[1]

Mulberrofuran E (3),[2] an amorphous powder, $[\alpha]_D^{22}$ +302° (acetone), $FeCl_3$ test (brown), showed a molecular ion peak at m/z 632 in its FD-MS. The ^{13}C NMR spectrum of 3 indicated the presence of thirty nine carbons (Table 1). Treatment of 3 with dimethyl sulfate gave the hexamethyl ether (3a), $C_{45}H_{48}O_8$.[3] These results revealed the molecular formula of 3 to be $C_{39}H_{36}O_8$. The UV spectrum of 3, being similar to that of 2,[4] did not show any aluminum chloride-induced shift.[5] In the EI-MS of 3a, the fragment ions appeared at m/z 366 (8)[4] and 350 (9).[4,6] These results suggest that 3 is a Diels-Alder type adduct like 2.[4,6] This was supported through a comparative examination of the ^1H NMR spectrum of 3 with that of 2.[4] The chemical shifts and the coupling constants of the methylcyclohexene ring protons are shown in Fig. 2. In the ^{13}C NMR spectrum of 3, the chemical shifts of the carbon atoms, except those of the carbon atoms at C-4", 5", 6" and on the F ring, were similar to those of the relevant carbon atoms of 2[6] (Table 1). On the basis of these findings, together with the biogenetic analogy of the Diels-Alder type adducts obtained from the mulberry tree,[1,7] the structure of mulberrofuran E was inferred to be 3 or 3'. The location of the 2,4-dihydroxy-3-prenylbenzoyl and 4-hydroxyphenyl moieties, as well as the relative configuration of the substituents on the cyclohexene ring, were determined by comparing the signals of the methylcyclohexene ring protons of 3 with those





2 : $R_1 = R_2 = OH$, C-3"-H = α

3 : $R_1 = H$, $R_2 = OH$, C-3"-H = α

3' : $R_1 = H$, $R_2 = OH$, C-3"-H = β

3a : $R_1 = H$, $R_2 = OCH_3$, C-3"-H = α

1 : $R_1 = R_2 = R_3 = OH$

4 : $R_1 = H$, $R_2 = R_3 = OH$

4a : $R_1 = H$, $R_2 = R_3 = OCH_3$

5 : $R_2 = H$, $R_1 = R_3 = OH$

5a : $R_2 = H$, $R_1 = R_3 = OCH_3$

6 : $R_1 = R_2 = H$, $R_3 = OH$

Table 1. ^{13}C NMR Chemical Shifts in Acetone-d_6

Comp.	2	3		4	5	1
			C-1	115.3	127.4	115.1
C-2	156.4	155.6	C-2	159.9	131.7	160.0
C-3	101.9	101.8	C-3	103.6	116.7	103.6
C-3a	122.5	122.5	C-4	162.3	161.0	162.3
C-4	121.9	121.7	C-5	109.2	116.7	109.1
C-5	113.1	113.0	C-6	131.8	130.9	131.8
C-6	155.4	155.3	C-α	117.5	118.3	117.3
C-7	98.4	98.3	C-β	140.9	145.0	141.2
C-7a	156.4	155.6	C=O	193.3	192.9	193.4
C-1'	130.9	131.1	C-1'	114.2	113.9	114.0
C-2'	104.8	104.6	C-2'	166.0	165.8	165.7
C-3'	157.7	157.8	C-3'	116.2	117.3	116.2
C-4'	116.5	115.9	C-4'	163.6	163.7	163.4
C-5'	157.7	157.8	C-5'	109.2	110.2	110.1
C-6'	104.8	104.6	C-6'	130.8	131.7	130.6
C-1"	133.8	134.7	C-1"	135.2	134.8	134.7
C-2"	123.1	123.2	C-2"	123.2	123.2	123.2
C-3"	33.1	33.4	C-3"	33.3	32.5	32.4
C-4"	47.7	50.0	C-4"	50.0	47.4	47.4
C-5"	36.4	40.8	C-5"	40.4	36.4	36.4
C-6"	32.4	34.8	C-6"	36.1	32.4	32.4
C-7"	23.8	23.8	C-7"	23.8	23.8	23.8
C-8"	209.2	207.9	C-8"	207.6	209.5	209.5
C-9"	113.3	113.8	C-9"	114.1	113.3	113.3
C-10"	164.6	164.0	C-10"	164.2	164.6	164.5
C-11"	115.8	115.6	C-11"	115.5	115.8	115.8
C-12"	163.3	162.9	C-12"	162.7	163.7	163.4
C-13"	108.1	108.2	C-13"	108.1	108.1	108.2
C-14"	128.6	130.7	C-14"	131.8	128.6	128.6
C-15"	121.9	136.4	C-15"	136.7	121.7	121.7
C-16"	156.6	129.0	C-16"	129.2	156.4	156.5
C-17"	103.5	115.9	C-17"	116.0	103.6	103.6
C-18"	157.7	156.6	C-18"	156.6	157.9	157.9
C-19"	107.4	115.9	C-19"	116.0	107.5	107.4
C-20"	132.1	129.0	C-20"	129.2	132.9	132.1
C-21"	22.1	22.2	C-21"	22.2	22.2	22.1
C-22"	124.4	124.1	C-22"	123.2	123.4	123.5
C-23"	131.4	131.3	C-23"	131.4	131.4	131.4
C-24"	25.8	25.8	C-24"	25.8	25.8	25.8
C-25"	17.8	17.8	C-25"	17.8	17.8	17.8

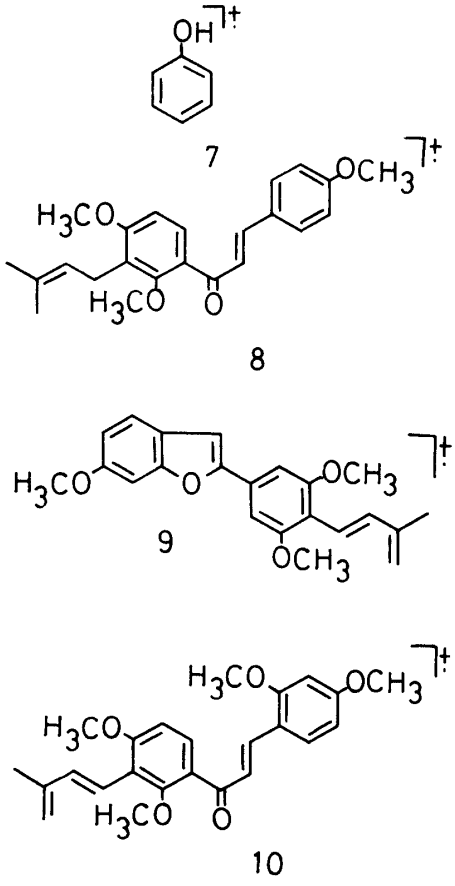


Fig. 1

of 2[4] and other Diels-Alder type adducts.[1,6,7] These results suggest that the structure of mulberrofuran E is represented by the formula (3).

Kuwanon Q (4),[8] an amorphous powder, $[\alpha]_D^{16}$ +161° (acetone), $FeCl_3$ test (brown), showed a molecular ion peak at m/z 662 in its FD-MS. The ^{13}C NMR spectrum of 4 revealed the presence of forty carbons (Table 1). Work-up of 4 with dimethyl sulfate gave the heptamethyl ether (4a), $C_{47}H_{52}O_9$.[3] The molecular formula of 4 was thus inferred to be $C_{40}H_{38}O_9$. The UV spectrum of 4, being similar to that of 1,[1] did not show any aluminum chloride-induced shift.[5] In the EI-MS of 4a, the fragment ions appeared at m/z 760 (M^+), 527, 394 (10),[9] 366 (8),[9] and 233. These results suggest that 4 is a Diels-Alder type adduct like 1.[1,9] The ^1H NMR spectrum of 4 was examined by comparison with that of 1.[1] The protons in a methylcyclohexene ring moiety are shown in Fig. 2. The chemical shifts and coupling constants of the protons of the methylcyclohexene ring of 4 were similar to those of the relevant protons of 3 (Fig. 2). Comparison of the ^{13}C NMR spectrum of 4 with those of 1 and 3 indicated that chemical shifts of the carbon atoms of the chalcone skeleton of 4 were similar to those of the relevant carbon atoms of 1, while those of the other carbon atoms of 4 were similar to those of the relevant carbon atoms of 3 (Table 1). From these results, the structure of kuwanon Q is represented by the formula (4).

Kuwanon R (5),[10] an amorphous powder, $[\alpha]_D^{17}$ +56° (acetone), $FeCl_3$ test (brown), showed a molecular ion peak at m/z 662 in its FD-MS. The ^{13}C NMR spectrum of 5 revealed the presence of forty carbons (Table 1). Work-up of 5 with dimethyl sulfate gave the heptamethyl ether (5a), $C_{47}H_{52}O_9$.[3] Comparing the UV spectrum of 5 with that of 4, a hypsochromic shift (about 20 nm) was observed in "band 1"[11] of 5. This result suggests that 5 is a 4-hydroxychalcone having no hydroxyl group at the C-2 position.[11] The chemical shifts and coupling constants of the protons of the methylcyclohexene ring were similar to those of the relevant protons of 1[1] (Fig. 2). Comparison of the ^{13}C NMR spectrum of 5 with that of 1 revealed that the chemical shifts of the carbon atoms, except those of the carbon atoms at the C-β and the B ring, are similar to those of the relevant carbon atoms of 1 (Table 1). These results suggest that the structure of kuwanon R is represented by the formula (5).

Kuwanon V (6),[12] an amorphous powder, $[\alpha]_D^{23}$ +145° (acetone), $FeCl_3$ test (brown), showed a molecular ion peak at m/z 646 in its FD-MS. The UV spectrum of 6, being similar to that of 5, did not show any aluminum chloride-induced shifts.[5] In the ^1H NMR spectrum of 6, the chemical shifts and coupling constants of the proton signals of the chalcone moiety were similar to those of the relevant proton signals of 5, while other proton signals were similar to those of the relevant proton signals of 4. From these results, the structure of kuwanon V is represented as 6.

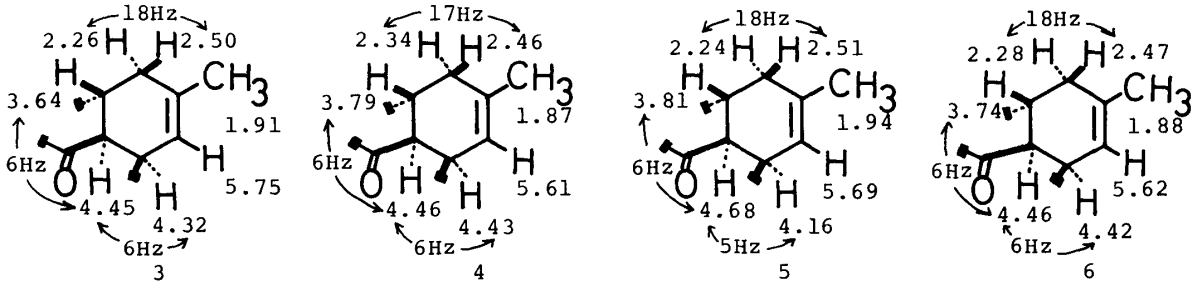


Fig. 2. ^1H NMR Chemical Shifts and Coupling Constants of Methylcyclohexene Ring Protons of mulberrofuran E (3), kuwanon Q (4), R (5) and V (6) in acetone-d_6.

It should be noted that all of the four possible stereochemically identical Diels-Alder type adducts consisting of two 4-hydroxy-prenylchalcone moieties, 1,[1] 4, 5 and 6, as well as two adducts, 2[1] and 3, which are composed of a dehydroprenyl-2-arylbenzofuran and a prenylchalcone, were isolated from the M. alba callus tissues.

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1) S. Ueda, T. Nomura, T. Fukai and J. Matsumoto, Chem. Pharm. Bull., 30, 3042 (1982), and references cited therein.

2) UV λ_{max}^{EtOH} nm(log ε); 220(4.64), 243(4.21,sh), 287(4.37,sh), 297(4.42,sh), 310 (4.51,sh), 321(4.59), 334(4.50). IR ν_{max}^{KBr} cm^{-1}: 3400, 1620. FD-MS m/z: 632(M^+), 454(M^+-178), 390(M^+-242). EI-MS m/z: 242,[6] 178,[6] 123, 110. 94(7). ^{1}H NMR (270 MHz, acetone-d_6) δ: protons in the 2-arylbenzofuran moiety, 6.77(1H,s,C-3 H), 6.77(1H,d,J=2,C-7 H), 6.78(1H,dd,J=2,8,C-5 H), 6.94(2H,s,C-2' and 6' H), 7.37(1H,d,J=8,C-4 H); protons in the 2,4-dihydroxy-3-prenylbenzoyl moiety, 1.57, 1.70(each 3H,s,C-23" CH_3), 3.24(2H,d,J=7,C-21" Hx2), 5.15(1H,br t,J=7,C-22" H), 6.54(1H,d,J=8,C-13" H), 7.89(1H,d,J=8,C-14" H), 12.92(1H,s,OH); protons in the 4-hydroxyphenyl moiety, 6.76(2H,d,J=8,C-17" and 19" H), 7.21(2H,d,J=8,C-16" and 20" H).

3) High resolution mass spectroscopy of the compound gave a satisfactory result.

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8) UV λ_{max}^{EtOH} nm(log ε): 222(4.37,sh), 266(3.94,sh), 300(4.06), 389(4.26). IR ν_{max}^{KBr} cm^{-1}: 3360, 1620(sh), 1610. FD-MS m/z: 662(M^+), 484(M^+-178), 324, 272. EI-MS m/z: 178,[1] 123,[1] 110,[1] 94(7). ^{1}H NMR(270 MHz, acetone-d_6) δ: protons in the chalcone moiety, 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), 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), 8.18(1H,d,J=15,C-β H); protons in the 2,4-dihydroxy-3-prenylbenzoyl moiety, 1.58, 1.68(each 3H,s,C-23" CH_3), 3.22(2H,d,J=7,C-21" Hx2), 5.14(1H,t,J=7,C-22" H), 6.51(1H,d,J=8.5,C-13" H), 7.89(1H,d,J=8.5,C-14" H); protons in the 4-hydroxy-phenyl moiety, 6.72(2H,d,J=8.5,C-17" and 19" H), 7.18(2H,d,J=8.5,C-16" and 20" H).

9) T. Nomura, T. Fukai, J. Matsumoto, A. Imashimizu, S. Terada and M. Hama, Planta medica, 46, 167 (1982).

10) UV λ_{max}^{EtOH} nm(log ε); 224(4.47,sh), 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), 340, 322, 256. EI-MS m/z: 322, 256, 178,[1] 123,[1] 110.[1] ^{1}H NMR(270 MHz, acetone-d_6) δ: protons in the chalcone moiety, 6.38(1H,d,J=9,C-5' H), 6.90(2H,d,J=9,C-3 and 5 H), 7.69(1H,d,J=15.5,C-α H), 7.70(2H,d,J=9,C-2 and 6 H), 7.78(1H,d,J=15.5,C-β H), 7.95(1H,d,J=9,C-6' H); protons in the 2,4-dihydroxy-3-prenylbenzoyl moiety, 1.59, 1.72(each 3H,s,C-23" CH_3), 3.27(2H,d,J=7,C-21" Hx2), 5.17(1H,t,J=7,C-22" H), 6.45(1H,d,J=9,C-13" H), 8.41(1H,d,J=9,C-14" H), 12.88(1H,s,OH); protons in the 2,4-dihydroxyphenyl moiety, 6.33(1H,dd,J=2.5,8.5,C-19" H), 6.55(1H,d,J=2.5, C-17" H), 6.98(1H,d,J=8.5,C-20" H); protons in the methylcyclohexene ring moiety are shown in Fig. 2.

11) T.J. Mabry, K.R. Markham and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, Berlin, 1970, Part II.

12) UV λ_{max}^{EtOH} nm(log ε); 226(3.85,sh), 296(3.46), 370(3.59). EI-MS m/z; 205, 178.[1] 123, 107, 94(7). ^{1}H NMR(270 MHz, acetone-d_6) δ: protons in the chalcone moiety, 6.36(1H,d,J=9,C-5' H), 6.91(2H,d,J=9,C-3 and 5 H), 7.69(1H,d,J=15,C-α H), 7.70 (2H,d,J=9,C-2 and 6 H), 7.78(1H,d,J=15,C-β H), 7.94(1H,d,J=9,C-6' H), 14.17(1H, s,OH); protons in the 2,4-dihydroxy-3-prenylbenzoyl moiety, 1.58, 1.68(each 3H, s,C-23" CH_3), 3.21(2H,d,J=7,C-21" Hx2), 5.14(1H,t,J=7,C-22" H), 6.49(1H,d,J=9, C-13" H), 7.89(1H,d,J=9,C-14" H), 12.83(1H,s,OH); protons in the 4-hydroxyphenyl moiety, 6.73(2H,d,J=8.5,C-17" and 19" H), 7.19(2H,d,J=8.5,C-16" and 20" H); protons in the methylcyclohexene ring moiety are shown in Fig. 2.

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