TWO NEW DIELS-ALDER TYPE ADDUCTS, MULBERROFURAN T AND KUWANOL E, FROM CALLUS TISSUES OF MORUS ALBA L.[1,2]

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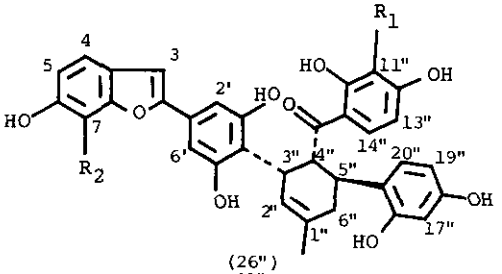
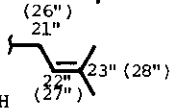
Abstract —— Two new Diels-Alder type adducts, mulberrofuran T and kuwanol E, were isolated from callus tissues of Morus alba L. On the basis of spectral evidence, the structures of mulberrofuran T and kuwanol E were shown to be **1** and **2**, respectively.

Previously we reported a series of isoprenoid-substituted phenolic compounds isolated from the root bark of mulberry tree and related plants.[3] Some of these phenolic compounds showed interesting biological activities.[3] On the other hand, cell cultures of high pigment productivity have been obtained through the selection of callus tissues induced from the seedlings or the leaves of Morus alba L.[4,5] From those callus tissues and cell suspension cultures six natural Diels-Alder type adducts of prenylchalcone derivatives and dehydroprenylphenols have been isolated, along with phytosterols.[4,6,7] In continuation of these studies, we isolated two new natural Diels-Alder type adducts, mulberrofuran T and kuwanol E, from the callus tissues. This paper describes the characterization of the compounds.

The methanol extract of callus tissues[4,5] induced from the leaves of Morus alba L. under specified conditions[8] was fractionated sequentially by silica gel column chromatography, preparative tlc, and then hplc analysis, resulting in the isolation of mulberrofuran T (**1**) and kuwanol E (**2**).

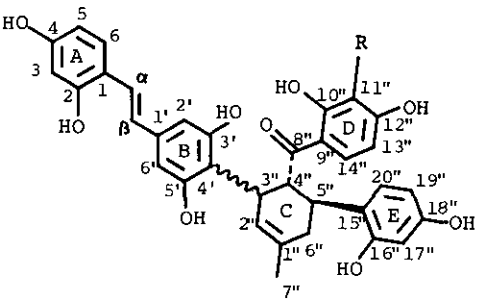
Mulberrofuran T (**1**), a white crystalline powder, mp 168-169 °C (decomp.), $[\alpha]_D^{22}$ +139° (ethanol), gave the FAB-ms spectrum showing a molecular ion at m/z 716. The compound **1** gave a brown color with methanolic ferric chloride, and its ir spectrum showed absorption bands due to hydroxyl, conjugated carbonyl, and benzene ring moieties. The uv spectrum of **1** exhibited maxima at 204, 218, 290 (infl), 296 (infl), 310 (infl), 320, and 333 (sh) nm, and was similar to those of mulberrofuran

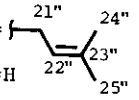


1 : $R_1=R_2=$ 

3 : $R_1=R_2=H$

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



2 : 3"-(S), R= 

5 : 3"-(R), R=H

6 : 3"-(S), R=H

Table 1 ^{1}H Nmr spectral data of **1** and **4** (δ in acetone-d_6)

	1	**4**
3-H	6.90 (s)	6.93 (br s)
4-H	7.18 (d, J=8)	7.36 (d, J=8)
5-H	6.80 (d, J=8)	6.78 (dd, J=2 and 8)
7-H	——	6.93 (br d, J=2)
2'-H 6'-H	6.81 (2H, s)	6.78 (2H, s)
1"-CH_3	1.94 (3H, br s)	1.94 (3H, br s)
2"-H	5.79 (br s)	5.78 (br s)
3"-H	4.13 (br)	4.13 (br)
4"-H	4.65 (dd, J=4 and 5)	4.65 (dd, J=4 and 5)
5"-H	3.76 (m)	3.77 (m)
6"-H	2.19 (br d, J=17)	2.20 (br d, J=17)
	2.50 (br d, J=17)	2.51 (br d, J=17)
13"-H	6.45 (d, J=9)	6.44 (d, J=9)
14"-H	8.45 (d, J=9)	8.44 (d, J=9)
17"-H	6.51 (d, J=2)	6.51 (d, J=2)
19"-H	6.32 (dd, J=2 and 8)	6.32 (dd, J=2 and 8)
20"-H	7.00 (d, J=8)	7.00 (d, J=8)
21"-H	3.27 (2H, br d, J=7)	3.27 (2H, br d, J=7)
22"-H	5.16 (m)	5.17 (m)
23"-CH_3	1.57 (3H, br s)	1.58 (3H, br s)
	1.72 (3H, br s)	1.72 (3H, br s)
26"-H	3.59 (2H, br d, J=7)	
27"-H	5.40 (m)	
28"-CH_3	1.64 (3H, br s)	
	1.85 (3H, br s)	

Table 2 ^{1}H Nmr spectral data of **2** and **6** (δ in acetone-d_6)

	2	**6**
3-H	6.40 (d, J=2)	6.40 (d, J=2)
5-H	6.35 (dd, J=2 and 8)	6.36 (dd, J=2 and 8)
6-H	7.34 (d, J=8)	7.34 (d, J=8)
α-H	7.21 (d, J=17)	7.22 (d, J=16)
β-H	6.76 (d, J=17)	6.77 (d, J=16)
2'-H 6'-H	6.43 (2H, s)	6.44 (2H, s)
1"-CH_3	1.92 (3H, br s)	1.92 (3H, br s)
2"-H	5.77 (br s)	5.76 (br s)
3"-H	4.08 (br)	4.11 (br)
4"-H	4.61 (dd, J=4 and 5)	4.60 (dd, J=4 and 5)
5"-H	3.74 (m)	3.75 (m)
6"-H	2.17 (br d, J=18) 2.50 (br d, J=18)	2.19 (br d, J=18) 2.49 (br d, J=18)
11"-H	----	6.24 (d, J=2)
13"-H	6.43 (d, J=9)	6.35 (dd, J=2 and 8)
14"-H	8.42 (d, J=9)	8.52 (d, J=8)
17"-H	6.50 (d, J=2)	6.50 (d, J=2)
19"-H	6.31 (dd, J=2 and 8)	6.30 (dd, J=2 and 8)
20"-H	6.99 (d, J=8)	6.98 (d, J=8)
21"-H	3.27 (2H, br d, J=7)	
22"-H	5.17 (m)	
23"-CH_3	1.59 (3H, br s) 1.72 (3H, br s)	

Table 3 ^{13}C Nmr spectral data of **2**, **4**, **5**, and **6**

	2*	**6***	**5****	**4***		**2***	**6***	**5****	**4***
C-1	117.5	116.9	116.2		C-8"	209.7	208.6	208.9	209.8
C-2	157.4	156.0	156.0		C-9"	113.7	113.3	115.2	113.6
C-3	103.6	103.2	102.0		C-10"	164.7	166.2	164.2	164.7
C-4	159.0	158.3	158.1		C-11"	115.9	103.1	105.0	116.0
C-5	108.5	107.9	107.0		C-12"	163.1	165.1	163.9	163.2
C-6	124.8	124.1	123.1		C-13"	108.0	108.2	107.6	108.0
C-α	126.1	125.5	125.2		C-14"	132.2	134.4	132.9	132.1
C-β	128.2	127.6	127.1		C-15"	122.1	121.6	121.1	122.7
C-1'	139.2	138.5	137.2		C-16"	156.8	155.6	155.9	156.4
C-2'	106.6	106.1	103.4		C-17"	103.6	103.1	105.0	103.6
C-3'	157.5	156.8	155.6		C-18"	157.9	158.3	156.4	157.9
C-4'	115.1	114.3	114.9		C-19"	107.6	107.0	106.4	107.6
C-5'	157.5	156.8	155.6		C-20"	128.8	128.2	128.7	128.8
C-6'	106.6	106.1	103.4		C-21"	22.2			22.2
C-1"	133.7	133.3	131.2	134.0	C-22"	123.2			123.2
C-2"	123.9	123.3	125.1	124.4	C-23"	131.5			131.5
C-3"	33.2	33.1	39.0	33.3	C-24"	17.9			17.9
C-4"	47.9	47.8	46.1	47.9	C-25"	25.8			25.8
C-5"	36.4	36.0	37.7	36.5					
C-6"	32.4	32.4	37.4	32.4					
C-7"	23.8	23.7	22.7	23.8					

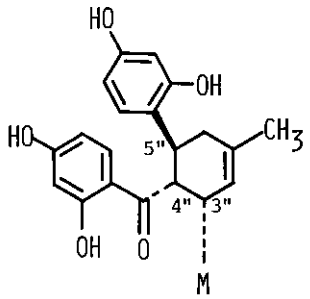
Solvent: *; acetone-d_6, **; DMSO-d_6 at 120 °C

C (**3**)[9] and chalcomoracin (**4**)[10]. The uv spectrum of **1** in the presence of aluminum chloride showed no bathochromic shift.[11] Taking into account of the molecular ion of the FAB-ms spectrum and the uv spectrum, it was assumed that mulberrofuran T is a monoprenylated derivative of **4**. This assumption was substantiated by comparing the ^{1}H nmr spectrum of **1** with that of **4** (Table 1). In the ^{1}H nmr spectrum of **1**, chemical shifts and coupling constants of all the proton signals, except those of the benzofuran ring and the additional prenyl (3,3-dimethylallyl) group, resembled those of the relevant proton signals of **4**. Furthermore, neither a proton signal at the C-7 position of **1** nor a long-range coupling between the protons at the C-3 and the C-7 positions could be observed. From these results, it was confirmed that an additional prenyl group is located at the C-7 position, and the formula **1** is represented for the structure of mulberrofuran T.

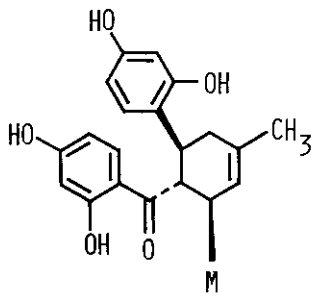
Kuwanol E (**2**), a white amorphous powder, $[\alpha]_D^{20}$ +171° (ethanol), gave the FAB-ms spectrum showing a protonated molecular ion at m/z 651. Compound **2** gave a brown color with methanolic ferric chloride, and its ir spectrum showed absorption bands due to hydroxyl, conjugated carbonyl, and benzene ring moieties. The uv spectrum of **2** exhibited maxima at 204, 215 (sh), 292, 300 (sh), 330 nm, and was similar to those of kuwanon X (**5**)[12] and Y (**6**).[13] Comparison of ^{1}H nmr spectrum of **2** with that of **6** revealed that chemical shifts and coupling constants of all the proton signals of **2**, except those of the 2,4-dihydroxy-3-prenylbenzoyl moiety, resembled those of the relevant proton signals of **6** (Table 2). These results suggest that kuwanol E is 11"-prenylkuwanon Y. This suggestion was substantiated by comparing the ^{13}C nmr spectrum of **2** with those of **4**, **5**, and **6** (Table 3). The chemical shift values of all the carbon atoms of **2**, except those of the carbon atoms at the C-10", 11", and 12" positions and those of the carbon atoms of the prenyl group, were in good agreement with those of the relevant carbon atoms of **6**. Furthermore, the chemical shift values of all the carbon atoms of the C, D, and E rings of **2**, along with those of the carbon atoms of the prenyl group, were in good agreement with those of the relevant carbon atoms of **4**. The chemical shift values of the carbon atoms of the methylcyclohexene ring of **2** were similar to those of the relevant carbon atoms of **6** rather than to those of **5**. From these results, the structure of kuwanol E is represented by the formula **2**.

In previous papers,[14,15] we reported that absolute configurations of the chiral centers on the methylcyclohexene ring in the cis-trans adducts (**7**, in relative configuration of the three substituents on the methylcyclohexene ring) were

established to be 3"S, 4"R, 5"S, while in the all-trans adducts (**8**) to be 3"R, 4"R, 5"S. Furthermore, the cis-trans adducts exhibited positive optical rotation values while the all-trans adducts exhibited negative ones. In the cases of mulberrofuran T (**1**) and kuwanol E (**2**), the relative configurations of the three substituents on the methylcyclohexene rings are the cis-trans type, and the optical rotation values of **1** and **2** are positive. Therefore, the absolute configurations of **1** and **2** were determined to be 3"S, 4"R, 5"S. On the other hand, for the biosynthesis of chalcomoracin (**4**), we reported that the 2-arylbenzofuran skeleton of **4** is formed by a cyclization of the cinnamoylpolyketide precursor, followed by decarboxylation.[5] It is noteworthy that kuwanol E (**2**) and chalcomoracin (**4**) coexist in the Morus cell cultures. This fact suggests the biogenetic relationship between the stilbene derivatives and the 2-arylbenzofuran derivatives occurring in the mulberry tree.[3]



7 (cis-trans): 3"S, 4"R, 5"S



8 (all-trans): 3"R, 4"R, 5"S

EXPERIMENTAL

Abbreviations: s=singlet, d=doublet, dd=double doublet, m=multiplet, br=broad, sh=shoulder, infl=inflection. The general procedures followed those described in the previous paper.[7] The following instruments were used: Yazawa micro-melting points apparatus (hot-stage type), Shimadzu UV-265 spectrophotometer, Hitachi 260-30 IR spectrophotometer, JEOL JMS-DX 303 mass spectrometer, JEOL JNM GX-400 FTNMR spectrometer and SSC (Senshu Scientific Co.)-High Pressure Liquid Chromatography System with ERC-7211 as a uv detector.

Isolation of Mulberrofuran T (**1**) and Kuwanol E (**2**)

Lyophilized M. alba callus tissues[4,6,7] (12.0 g) were extracted with methanol (120 ml) for five days at room temperature. Evaporation of the extract to dryness yielded 4.6 g of the residue. The residue was extracted with acetone (40 ml). The acetone solution was concentrated to afford a residue (0.8 g), which was chromatographed on silica gel (60 g) with chloroform and an increasing content of acetone

as eluents, each fraction being monitored by tlc. The fraction eluted with chloroform containing 25% acetone was evaporated to give a residue (52 mg), which was fractionated sequentially by preparative tlc (silica gel, solvent system, n-hexane:acetone=1:1) and by preparative hplc (solvent, ether only, column, SSC Silica 4251-N) to give mulberrofuran T (**1**, 5 mg). The fraction eluted with chloroform containing 50% acetone was evaporated to give a residue (40 mg), which was fractionated sequentially by preparative tlc (chloroform:methanol=6:1) and by preparative hplc (ether only) to give kuwanol E (**2**, 5 mg).

Mulberrofuran T (**1**)

Compound **1** was recrystallized from n-hexane-ether to give a white crystalline powder, mp 168-169 °C (decomp.). $[\alpha]_D^{22}$ +139° (c=0.098, ethanol). $FeCl_3$ test: brown. FAB-ms: m/z 716 (M^+). Uv λ_{max}^{EtOH} nm (log ε): 204 (4.91), 218 (4.78), 290 (infl 4.50), 296 (infl 4.56), 310 (infl 4.63), 320 (4.70), 333 (sh 4.59). Uv $\lambda_{max}^{EtOH+AlCl_3}$: no shift. Ir ν_{max}^{KBr} cm^{-1}: 3400 (br), 3300 (sh), 1620, 1610 (sh), 1600 (sh), 1580, 1515, 1500, 1450.

Kuwanol E (**2**)

Compound **2** was obtained as an amorphous powder, $[\alpha]_D^{20}$ +171° (c=0.018, ethanol), $FeCl_3$ test: brown. FAB-ms: m/z 651 $(M+H)^+$. Uv λ_{max}^{EtOH} nm (log ε): 204 (4.80), 215 (sh 4.65), 292 (4.37), 300 (sh 4.36), 330 (4.36). Uv $\lambda_{max}^{EtOH+AlCl_3}$: no shift. Ir ν_{max}^{KBr} cm^{-1}: 3350 (br), 1610, 1600 (sh), 1570 (sh), 1510, 1500, 1440.

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