STRUCTURES OF KUWANOLS A AND B, TWO NOVEL STILBENE DERIVATIVES FROM THE CULTIVATED MULBERRY TREE (MORUS BOMBYCIS KOIDZ.)

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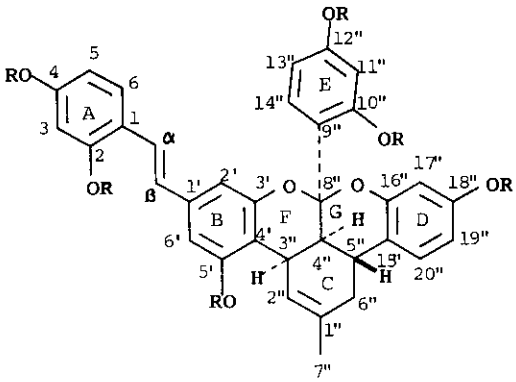
Abstract ——— From an acetone extract of the reddish violet powder obtained from the surface of the root bark of cultivated mulberry tree (Ohshimaso, a variety of Morus bombycis Koidz.), two novel stilbene derivatives were isolated and named kuwanols A and B, whose structures were shown to be **1** and **2**, respectively, on the basis of spectral evidence. These compounds are regarded biogenetically as variations of a Diels-Alder type adduct of a chalcone derivative and a dehydroprenylstilbene derivative.

Previously we reported the structure determination of a novel 2-arylbenzofuran derivative named mulberrofuran I (**3**) from the reddish violet powder obtained from the surface of the Morus root bark (Ohshimaso, a variety of Morus bombycis Koidz.[2]).[1] In the course of extended studies of the reddish violet powder, we isolated two stilbene derivatives, kuwanols A (**1**) and B (**2**) from the powder. In this paper, the structure determination of the compounds are described.

The acetone extract of the powder (260 g) was dissolved in ether, and the ether extract was fractionated by the preparative thin-layer chromatography on silica gel to give kuwanols A (**1**, 13 mg) and B (**2**, 5 mg).

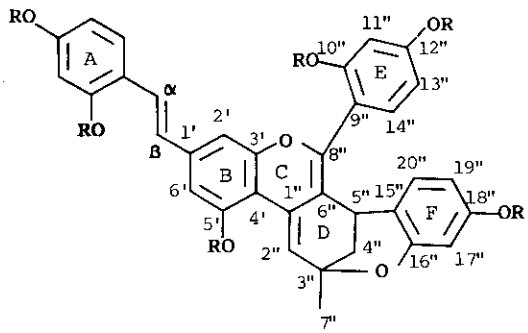
Kuwanol A (**1**), amorphous powder, $[\alpha]_D^{24}$ +557° (c=0.111, MeOH), $FeCl_3$ test (negative), gave the FD-MS which showed the molecular ion peak at m/z 564, and the ^{13}C nmr spectrum which indicated the presence of thirty-four carbon atoms[ten aliphatic carbons (1 x CH_3-, 1 x -CH_2-, 3 x >CH-, 1 x -CH=CH-, 1 x >C=CH-, 1 x -O-$\overset{|}{\underset{|}{C}}$-O-) and twenty-four aromatic carbons (11 x CH, 5 x C, 8 x C-O)]. Treatment of **1** with dimethyl sulfate and potassium carbonate in acetone gave a hexamethyl ether (**1a**) as an amorphous powder. The molecular formula of **1a** was determined to be $C_{40}H_{40}O_8$



1: R=H

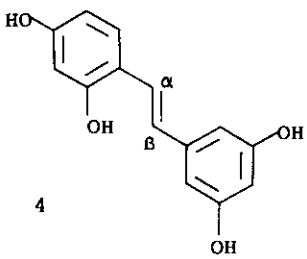
1a: R=CH_3

1b: R=$COCH_3$



2: R=H

2a: R=CH_3



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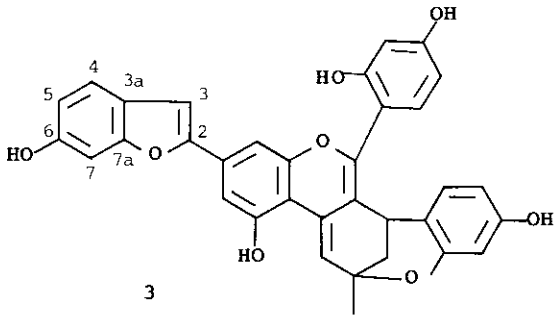

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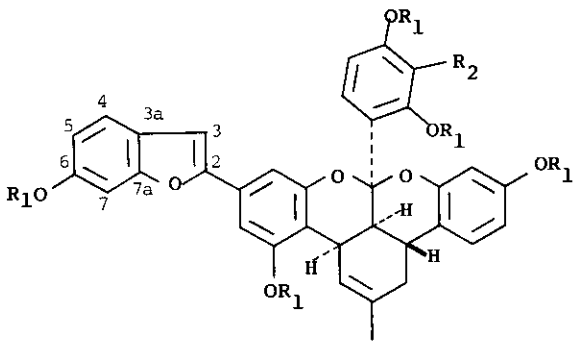
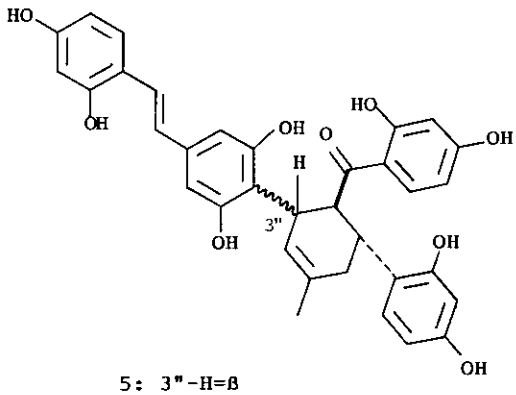


6: R_1=R_2=H

7: R_1=$COCH_3$, R_2= (prenyl)



5: 3"-H=β

8: 3"-H=α

Fig. 1

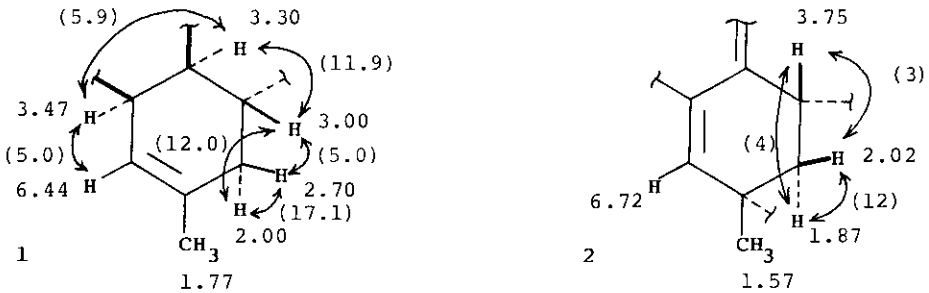


Fig. 2 J:(Hz)

Table 1 ^{13}C nmr chemical shifts (ppm) of **1** – **4** and **6**

No of C	**1**	**4**		**6**	**2**		**3**
C-1	117.1	117.8	(C-2)	156.7*	117.0	(C-2)	155.0*
C-2	156.9*	156.8	(C-3)	102.2	156.2*	(C-3)	102.3
C-3	103.2	102.2	(C-3a)	122.5	103.7	(C-3a)	121.3
C-4	158.4*	158.6	(C-4)	122.0	157.2*	(C-4)	121.2
C-5	108.0	108.5	(C-5)	113.4	108.2**	(C-5)	112.5
C-6	124.0	124.6	(C-6)	155.0*	124.8	(C-6)	154.0*
C-α	125.1	126.4	(C-7)	98.4	125.6	(C-7)	97.4
C-β	127.8	128.5	(C-7a)	157.7*	128.5	(C-7a)	156.9*
C-1'	138.8	142.0		131.5	140.1		130.5
C-2'	106.4	105.7		105.0	108.1**		106.0
C-3'	156.9*	159.0		157.5*	159.5*		156.6*
C-4'	116.7**	103.4		117.5**	119.1		119.0
C-5'	157.2*	159.0		156.7*	157.5*		156.0*
C-6'	106.7	105.7		105.4	106.7		103.7
C-1"	132.7			133.7	127.9		126.6
C-2"	122.7			122.9	120.2		119.9
C-3"	37.2***			37.2***	73.2		72.3
C-4"	28.4			28.5	34.1		33.1
C-5"	35.0***			35.1***	33.8		32.8
C-6"	36.1			36.2	114.2		116.9
C-7"	23.8			23.9	28.5		28.5
C-8"	102.1			102.6	142.7		142.3
C-9"	111.4			113.4	111.5		112.9
C-10"	157.0*			157.9*	157.8*		156.7*
C-11"	103.5			103.9	103.8***		102.9
C-12"	159.3*			159.9*	160.6*		159.0*
C-13"	106.7			107.3	108.7**		107.2
C-14"	129.7			130.3	133.3		132.2
C-15"	116.6**			116.8**	118.0		118.1
C-16"	153.4*			153.3*	152.8*		153.2
C-17"	104.2			104.0	103.9***		102.9
C-18"	152.5*			154.5*	154.8*		155.9*
C-19"	109.3			109.9	108.6**		107.2
C-20"	127.2			127.9	130.3		129.4
solv.	A	B		A	A		A

solvent: A; acetone-d_6 B; CD_3OD

* — ***: Assignments may be interchangeable in each column.

from the high-resolution mass spectrum (m/z 648.2696), and hence **1** could be formulated as $C_{34}H_{28}O_8$. Work-up of **1** with acetic anhydride in pyridine gave a hexaacetate (**1b**) which showed a molecular ion peak at m/z 816 in its EI-MS. The compound (**1**) showed the following spectra: ir ν_{max}^{KBr} cm^{-1} : 3400, 1610, 1570, 1510; uv λ_{max}^{EtOH} nm (log ε): 224 (sh 4.59), 285 (4.29), 304 (4.26), 330 (4.33), 342 (sh 4.30). The uv spectrum was similar to those of oxyresveratrol (**4**)[3] and kuwanon X (**5**),[4] and suggested that **1** is one of the 4'-substituted 2,4,3',5'-tetrahydroxystilbene derivatives.

This suggestion was supported through a comparative examination of the ^1H nmr spectrum of **1** (400 MHz, acetone-d_6) with that of **4**.[5] The chemical shifts and coupling constants (Hz) of the stilbene moiety are shown as follows: δ 6.38 (1H, dd, J=2.4 and 8.4, C-5-H), 6.43 (1H, d, J=2.4, C-3-H), 6.62 (1H, d, J=1.5, C-6'-H), 6.64 (1H, d, J=1.5, C-2'-H), 6.88 (1H, d, J=16.5, C-α-H), 7.32 (1H, d, J=16.5, C-ß-H), 7.39 (1H, d, J=8.4, C-6-H). The chemical shift values of the protons at C-2' and 6' positions appeared nonequivalent, suggesting that one of the hydroxyl groups in the B ring formed the ether linkage.[6,7] The presence of two 2,4-dioxygenated phenyl moieties and a methylcyclohexene ring moiety were supported by comparing the ^1H nmr spectrum of **1** with that of mulberrofuran G (**6**).[7] The signals of protons in two 2,4-dioxygenated phenyl moieties (D and E rings) were observed as follows: E ring protons δ 6.23 (1H, dd, J=2.4 and 8.6, C-13"-H), 6.40 (1H, d, J=2.4, C-11"-H), 7.23 (1H, d, J=8.6, C-14"-H); D ring protons δ 6.35 (1H, d, J=2.4, C-17"-H), 6.49 (1H, dd, J=2.4 and 8.4, C-19"-H), 7.13 (1H, d, J=8.4, C-20"-H). The protons in a methylcyclohexene ring moiety are shown in Fig. 2. The chemical shifts and coupling constants of the protons of these moieties of **1** were similar to those of the relevant protons of **6**. The location of the methylcyclohexene ring was supported by the acetylation shift of the C-2" olefinic proton signal as follows: the C-2" proton signal of **1b** shifted 0.44 ppm toward upper field from the corresponding proton of **1**. The similar shift (+0.47 ppm) was observed in the case of mulberrofuran F pentaacetate (**7**).[7]

In the ^{13}C nmr spectrum of **1**, the chemical shifts of the carbon atoms of the stilbene skeleton, except those of the carbon atoms at C-1', 3', and 4' which were affected by additional substituent effects, were similar to those of the relevant carbon atoms of **4**,[8] while those of the carbon atoms of the C, D, and E rings as well as the carbon atom at C-8", were essentially the same as those of the relevant carbon atoms of **6**[7] (Table 1). All these results indicate that the structure of

kuwanol A is represented by formula **1**.

Kuwanol B (**2**), a reddish violet amorphous powder, $[\alpha]_D^{17}$ +103.5° (c=0.17, MeOH) gave the FD-MS which showed the molecular ion peak at m/z 562, and the ^{13}C nmr spectrum indicating the presence of thirty-four carbons (Table 1). Treatment of **2** with dimethyl sulfate in acetone gave a hexamethyl ether (**2a**), of which mass spectrum showed the fragment ions as follows: m/z 646.2562 (M^+, $C_{40}H_{38}O_8$, 21.2 %), 644.2406 ($C_{40}H_{36}O_8$, base peak), 507.1763 ($C_{32}H_{28}O_6$, 98 %). These results indicated the molecular formula of **2** to be $C_{34}H_{26}O_8$. The compound (**2**) showed the following spectra: ir ν_{max}^{KBr} cm^{-1}: 3300 (br), 1675, 1620, 1600; uv λ_{max}^{EtOH} nm (log ε):286 (4.08), 310 (infl. 4.12), 326 (sh 4.23), 336 (4.24). The shape of the uv spectrum was similar to that of **4**,[3] and suggested that **2** is a 2,4,3',5'-tetraoxygenated stilbene derivative. Acidic solution of **2** showed a red color, which faded when neutralized. The uv spectrum in the acidic solution showed a bathochromic shift as follows: uv $\lambda_{max}^{EtOH+HCl}$ nm (log ε): 286 (4.00), 310 (infl. 4.04), 326 (4.14), 336 (4.14), 470 (sh 3.35), 555(3.69). The same phenomenon was observed in the case of mulberrofuran I (**3**).[1]

The presence of the following moieties on the structure of **2** was supported by a detailed analysis of the ^1H nmr spectrum (400 MHz, acetone-d_6) and by comparing the spectrum of **2** with those of **4**[5] and **3**[1] as follows: protons in a 4'-substituted 2,4,3',5'-tetraoxygenated stilbene moiety, δ 6.37 (1H, dd, J=2.4 and 8.3, C-5-H), 6.43 (1H, d, J=2.4, C-3-H), 6.63 (1H, d, J=2.0, C-2'-H), 6.78 (1H, d, J=2.0, C-6'-H), 6.87 (1H, d, J=16.1, C-α-H), 7.36 (1H, d, J=16.1, C-β-H), 7.39 (1H, d, J=8.3, C-6-H); protons in two 2,4-dioxygenated phenyl moieties (E and F rings),δ6.15 (1H, dd, J=2.4 and 8.3, C-19"-H), 6.19 (1H, d, J=2.4, C-17"-H), 6.53 (1H, d, J=8.3, C-20"-H), 6.56 (1H, dd, J=2.4 and 8.3, C-13"-H), 6.59 (1H, d, J=2.4, C-11"-H), 7.26 (1H, d, J=8.3, C-14"-H). The protons in a methylcyclohexene ring moiety are shown in Fig. 2. The nonequivalent of the chemical shift values of the protons at C-2' and 6' suggested that one of the hydroxyl groups in the B ring formed the ether linkage.[6,7] The chemical shift values and the coupling constants of the protons in the D, E, and F rings were similar to those of relevant protons of **3**.[1] In the ^{13}C nmr spectrum of **2**, the chemical shifts of the carbon atoms of the stilbene skeleton, except those of the carbon atoms at C-1', 2', 4', and 5' which were affected by additional substituent effects, were similar to those of the relevant carbon atoms of **4**, while those of the carbon atoms of the D, E, and F rings as well as the carbon atom at C-8" were essentially the same as those of the relevant carbon atoms

of **3** (Table 1). From these results, we propose formula **2** for the structure of kuwanol B.

Biogenetically kuwanol A seems to be a Diels-Alder type adduct derived from the compound (**8**), which had not been isolated from the Morus root bark, by the intramolecular ketalization reaction of the carbonyl group with the two adjoining hydroxyl groups.[7] On the other hand, kuwanol B seems to be a derivative induced from the Diels-Alder type adduct, such as kuwanon X (**5**)[4] or its stereoisomer (**8**) through the hemiketal intermediate.[1] Considering the biogenetic route of 2-arylbenzofuran derivatives involving oxidative cyclization process of hydroxystilbenes,[9] kuwanols A (**1**) and B (**2**) seem to be interesting intermediates to examine the biogenetic route of mulberrofuran G (**6**) and I (**3**).

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5. ^{1}H nmr (90 MHz, acetone-d_6) **δ** : 6.20 (1H, t, J=2, C-4'-H), 6.35 (1H, dd, J=2 and 8, C-5-H), 6.41 (1H, d, J=2, C-3-H), 6.49 (2H, d, J=2, C-2' and 6'-H), 6.86 (1H, d, J=16, C-**α**-H), 7.29 (1H, d, J=16, C-ß-H), 7.36 (1H, d, J=8, C-6-H).

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