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STRUCTURES OF KUWANONS Y AND Z, TWO NEW STILBENE DERIVATIVES FROM THE CULTIVATED MULBERRY TREE (MORUS ALBA L.)[1]

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Abstract —— Two novel stilbene derivatives named kuwanons Y and Z were isolated from the acetone extract of the reddish violet powder (lenticel) from the surface of the root bark of cultivated mulberry tree (Ichinose, a cultivated variety of Morus alba L.). The structures of kuwanons Y and Z were shown to be **1** and **2**, respectively, on the basis of spectral and chemical evidence. Kuwanon Y is regarded biogenetically as a Diels-Alder type adduct of a chalcone derivative and a dehydroprenylstilbene derivative, while kuwanon Z as a variation of a Diels-Alder type adduct.

Previously we reported several 2-arylbenzofuran and stilbene derivatives from the reddish violet powder (lenticel) obtained from the surface of the Morus root bark.[2-6] In this paper, we report the structure determination of two novel stilbene derivatives, kuwanons Y (**1**) and Z (**2**), isolated from the powder.

The acetone extract of the reddish violet powder was fractionated sequentially by silica gel column chromatography, and then by preparative tlc, resulting in the isolation of kuwanons Y (**1**) and Z (**2**).

Kuwanon Y (**1**), amorphous powder, $[\alpha]_D^{22}$ +172° (ethanol) gave the FD-MS showing a molecular ion peak at m/z 582, and ^{13}C nmr spectrum indicating the presence of thirty-four carbon atoms: nine aliphatic carbons, twenty-four aromatic carbons, and one carbonyl carbon (Table 1). These results indicate the composition of kuwanon Y to be $C_{34}H_{30}O_9$. The compound **1** gave a dark brown color in the methanolic ferric chloride test, and its ir spectrum showed absorption bands due to hydroxyl, conjugated carbonyl, and benzene ring moieties. The uv spectrum of

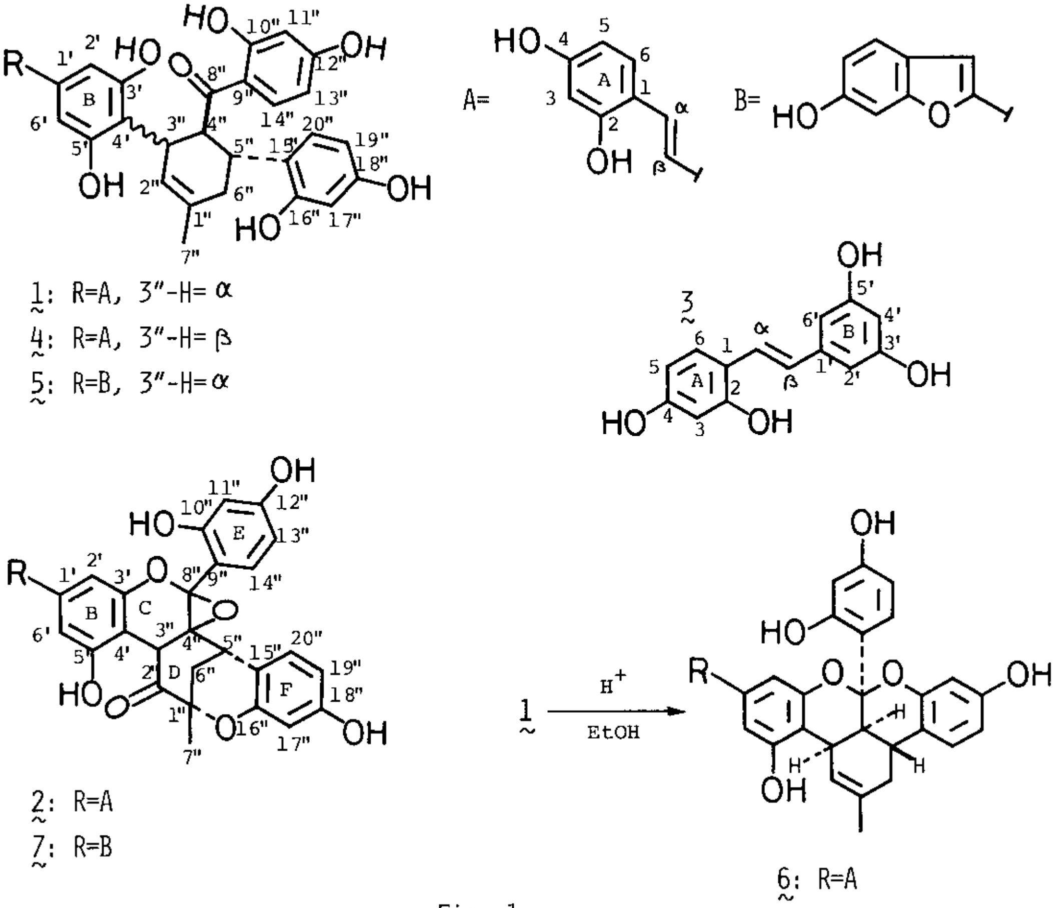


Fig. 1

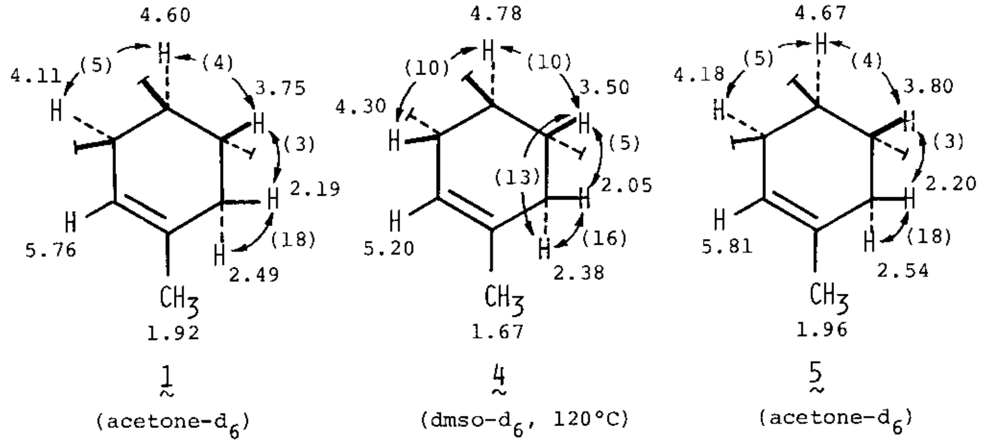


Fig. 2 ^{1}H nmr chemical shifts (ppm) and coupling constants (Hz) of methylcyclohexene rings of 1, 4, and 5.

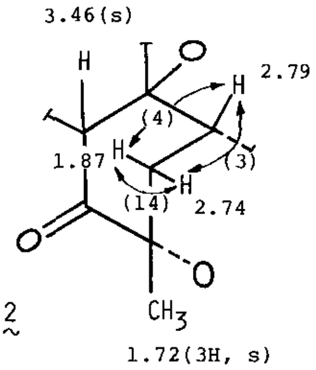
1 exhibited maxima at 218, 285, 300 (sh), and 326 nm, and showed a bathochromic shift in the presence of aluminum chloride. These uv characteristics were similar to those of oxyresveratrol (**3**)[7] and kuwanon X (**4**),[8] suggesting the presence of an oxyresveratrol moiety and a chelated carbonyl group. The ^{1}H nmr spectrum of kuwanon Y (**1**), analysed by comparison with the spectra of **4** and mulberrofuran C (**5**),[9] showed the signals of the following protons: 1) protons in a stilbene moiety, δ 6.36 (1H, dd, J=2 and 8, C-5-H), 6.44 (2H, s, C-2'-H and C-6'-H), 6.50 (1H, d, J=2, C-3-H), 6.77 (1H, d, J=16, C-β-H), 7.22 (1H, d, J=16, C-α-H), 7.34 (1H, d, J=8, C-6-H), 2) aromatic protons in a 2,4-dihydroxyphenyl moiety, δ 6.30 (1H, dd, J=2 and 8, C-19"-H), 6.40 (1H, d, J=2, C-17"-H), 6.98 (1H, d, J=8, C-20"-H), and 3) aromatic protons in a 2,4-dihydroxybenzoyl moiety, δ 6.24 (1H, d, J=2, C-11"-H), 6.35 (1H, dd, J=2 and 8, C-13"-H), 8.52 (1H, d, J=8, C-14"-H). The chemical shifts and coupling constants of protons of the methylcyclohexene ring are shown in Fig. 2. Comparative examination of the ^{1}H nmr spectra of **1**, **4**, and **5** revealed that the chemical shifts and coupling constants of protons of the methylcyclohexene ring of **1** resembled those of **5** better than those of **4** (Fig. 2). The ^{13}C nmr spectrum of **1** was analysed as shown in Table 1. Assignments of the carbon atoms in **1** were performed by comparison of the ^{13}C nmr spectra of **3**, **4**, and **5**. The chemical shift values of the carbon atoms of the A ring of **1** were similar to those of the relevant carbon atoms of **3**, while the carbon atoms of the B ring of **1** showed the chemical shift values different from the relevant carbon atoms of **3**. Moreover, the chemical shift values of the carbon atoms at C-2' and -6' positions were equivalent, and the chemical shift value of the carbon atom at C-4' was markedly shifted downfield as compared with that of the relevant carbon atom of **3** (Table 1). These results indicate that **1** is a 4'-substituted oxyresveratrol derivative. The chemical shift values of the carbon atoms of the 2,4-dihydroxyphenyl, 2,4-dihydroxybenzoyl, and methylcyclohexene ring moieties were similar to those of the relevant carbon atoms of **5** (Table 1). The above results suggest that kuwanon Y is a stereoisomer at the C-3" position on the methylcyclohexene ring of kuwanon X (**4**).

In order to corroborate the structure, kuwanon Y (**1**) was correlated to kuwanol A (**6**)[3] by means of the following reaction.[10] A solution of **1** in ethanol containing sulfuric acid was kept at room temperature. The products were purified by preparative tlc to give **6**. The ir and ^{1}H nmr spectra of the product are in fair agreement with those of kuwanol A (**6**).[3] From the above results, we propose the formula **1** for the structure of kuwanon Y.

Table 1 ^{13}C nmr chemical shifts (ppm) of **1**, **2**, **3**, **4**, **5**, and **7**

C No.	**1**	**3**	**4**	**5**	**2**	**7**
C-1	116.9	117.8	116.2		116.7	
C-2	157.1	156.8	156.0		158.1	
C-3	103.2	102.2	102.0		103.2	
C-4	158.3	158.6	158.1		158.3	
C-5	107.9	108.5	107.0		107.9	
C-6	124.1	124.6	123.1		123.6	
C-α	125.5	126.4	125.2		125.8	
C-β	127.6	128.5	127.1		127.7	
C-1'	138.5	142.0	137.2	130.9	140.5	132.1
C-2'	106.1	105.7	103.4	104.8	107.0	104.9
C-3'	156.8	159.0	155.6	156.5	160.2	160.2
C-4'	113.3	103.4	114.9	113.6	109.7	109.9
C-5'	156.8	159.0	155.6	156.5	154.3	154.6
C-6'	106.1	105.7	103.4	104.8	107.2	107.4
C-1"	133.3		131.2	134.1	75.3	75.2
C-2"	123.3		125.1	124.2	195.2	195.4
C-3"	36.0		39.0	33.2	49.0	49.0
C-4"	47.8		46.1	47.9	92.3	92.2
C-5"	33.1		37.7	33.2	37.9	37.9
C-6"	32.4		37.4	32.4	31.0	31.0
C-7"	23.7		22.7	23.8	22.4	22.4
C-8"	208.6		208.9	209.5	109.2	109.1
C-9"	114.3		115.2	116.4	114.4	114.3
C-10"	165.1		163.9	166.0	166.0	166.2
C-11"	103.1		105.0	102.0	98.2	97.9
C-12"	166.2		164.2	167.0	172.2	172.2
C-13"	108.2		107.6	108.8	102.7	102.8
C-14"	128.2		128.7	128.8	133.6	133.7
C-15"	121.6		121.1	122.5	111.6	112.8
C-16"	155.6		155.9	157.8	155.1	155.1
C-17"	103.1		105.0	103.6	99.6	98.2
C-18"	156.0		156.4	157.8	156.1	155.9
C-19"	107.0		106.4	107.5	111.3	111.8
C-20"	134.4		132.9	135.0	125.8	125.9
solv.	A	B	C	A	A	A

A: acetone-d_6 B: CD_3OD C: dmso-d_6(120 °C)



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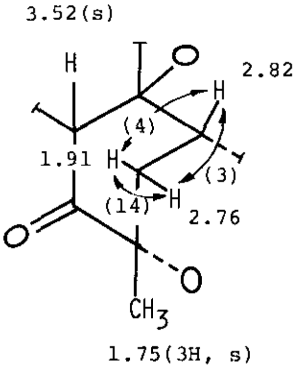

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Fig. 3 ^{1}H nmr chemical shifts (ppm) and coupling constants (Hz) of methylcyclohexanone rings of **2** and **7** (acetone-d_6).

Kuwanon Z (2), amorphous powder, $[\alpha]_D^{22}$ +40.5° (ethanol) gave the FD-MS showing a molecular ion peak at *m/z* 594, and ^{13}C nmr spectrum indicating the presence of thirty-four carbon atoms: nine aliphatic carbons, twenty-four aromatic carbons, and one carbonyl carbon (Table 1). These results indicated the composition of kuwanon Z to be $C_{34}H_{26}O_{10}$. The compound 2 was negative to the methanolic ferric chloride test, and its ir spectrum showed absorption bands due to hydroxyl, carbonyl, and benzene ring moieties. The uv spectrum of 2 exhibited maxima at 208, 283, 304, and 327 nm, and was similar to those of oxyresveratrol (3),[7] kuwanons X (4),[8] and Y (1). These results suggested that 2 is one of the 4'-substituted 2,4,3',5'-tetrahydroxystilbene derivatives. The ^{1}H nmr spectrum (acetone-d_6) of kuwanon Z (2), analysed by comparison with the spectra of oxyresveratrol derivatives[8] and mulberrofuran Q (7),[6] showed the signals of the following protons: 1) protons in a stilbene moiety, δ 6.24 (1H, br d, *J*=1, C-2'-H), 6.35 (1H, br d, *J*=1, C-6'-H), 6.36 (1H, dd, *J*=2 and 8, C-5-H), 6.57 (1H, br d, *J*=2, C-3-H), 6.83 (1H, d, *J*=16, C-β-H), 7.26 (1H, d, *J*=16, C-α-H), 7.43 (1H, d, *J*=8, C-6-H), and 2) aromatic protons in two 2,4-dioxygenated phenyl moieties, δ 6.34 (1H, dd, *J*=2 and 8, C-19"-H), 6.42 (1H, d, *J*=2, C-17"-H), 6.65 (1H, d, *J*=8, C-20"-H); δ 6.16 (1H, d, *J*=2, C-11"-H), 6.54 (1H, dd, *J*=2 and 8, C-13"-H), 7.36 (1H, d, *J*=8, C-14"-H). The chemical shifts and coupling constants of protons of the relevant methylcyclohexanone ring are shown in Fig. 3. The nonequivalency of the chemical shift values of the protons at C-2' and -6' positions suggested that one of the hydroxyl groups in the B ring formed ether linkage.[3,10] The chemical shift values and the coupling constants of the protons in the D, E, and F rings were similar to those of protons of 7[6,11] (Fig. 3). The ^{13}C nmr spectrum was analysed by comparison with the spectra of 3[3] and 7[6] (Table 1). In the spectrum of 2, the chemical shifts of the carbon atoms of the stilbene skeleton, except those of the carbon atoms at C-4' and -5' which were affected by additional substituent effect, were similar to those of the relevant carbon atoms of 3. On the other hand the chemical shifts 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 7 (Table 1). From these results, we propose the formula 2 for the structure of kuwanon Z.

Kuwanon Y (1) is regarded biogenetically as a Diels-Alder type adduct of a chalcone derivative and a dehydroprenylstilbene derivative, and an interesting intermediate to kuwanol A (6), while kuwanon Z (2) seems to be derived from the Diels-Alder type adducts, such as kuwanons X (4) and Y (1), through the hemiketal intermediate (8) and kuwanol B (9)[3] as shown in Fig. 4.[6]

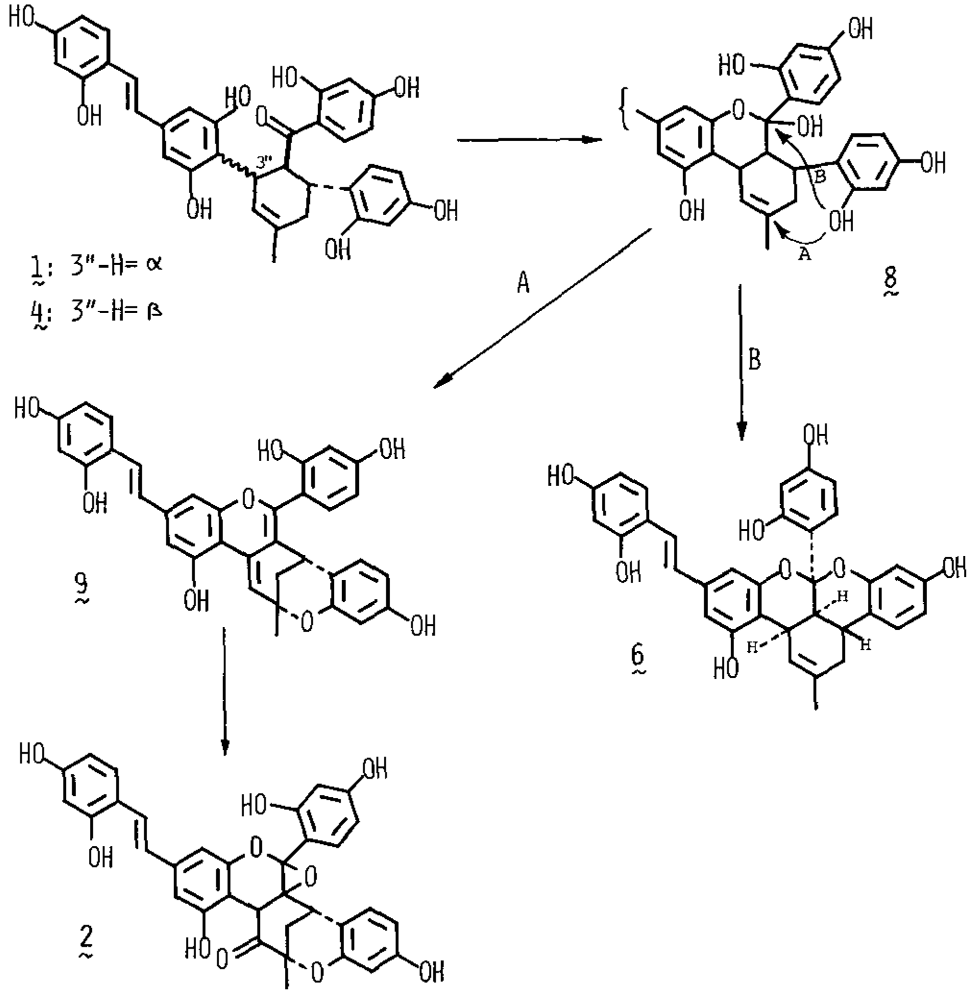

Fig. 4

EXPERIMENTAL

The general experimental procedures used are described in the previous paper.[10] The following instruments were used: uv spectrum; Hitachi 340 UV spectrometer, ir spectrum; Hitachi 260-30 IR spectrometer, optical rotation; JASCO DIP-4, ms; JEOL JMS01SG-2, ^{1}H and ^{13}C nmr spectra; JEOL GX-400 FT NMR spectrometer.

Isolation of Kuwanons Y (1) and Z (2)

The reddish violet powder (lenticel, 100 g) obtained from the surface of the root bark of cultivated mulberry tree (Ichinose, a cultivated variety of Morus alba L.) was extracted with acetone. Evaporation of the acetone solution to dryness yielded 8 g of residue. The residue (4 g) was chromatographed on silica gel (200 g) with chloroform-acetone as an eluent, each fraction being monitored by tlc. The fractions eluted with chloroform containing 20 % acetone were evaporated to give the residue (430 mg), which was fractionated by preparative tlc (solvent system: chloroform:methanol=6:1, benzene:ethyl acetate=3:5, benzene:acetone=1:1) to give kuwanons Y (1, 12 mg) and Z (2, 8 mg).

Kuwanon Y (1)

Compound 1 was obtained as an amorphous powder. $[\alpha]_D^{22}$ +172° (c=0.129, ethanol). FD-MS m/z: 582 (M^+). $FeCl_3$ test: positive (dark brown). ir ν_{max}^{KBr} cm^{-1}: 3370 (br), 1680 (sh), 1620, 1600 (sh). uv λ_{max}^{EtOH} nm (log ε): 218 (4.60), 285 (4.40), 300 (sh 4.34), 326 (4.44); $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 218 (4.60), 286 (4.39), 300 (4.37), 326 (4.43).

Kuwanon Z (2)

Compound 2 was obtained as an amorphous powder. $[\alpha]_D^{22}$ +40.5° (c=0.0543, ethanol). FD-MS m/z: 594 (M^+). $FeCl_3$ test: negative. ir ν_{max}^{KBr} cm^{-1}: 3350 (br), 1690, 1610. uv λ_{max}^{EtOH} nm (log ε): 208 (4.75), 283 (4.29), 304 (4.27), 327 (4.38).

Formation of Kuwanol A (6) from Kuwanon Y (1)

A mixture of 1 (10 mg), ethanol (5 ml), and 10 % sulfuric acid (1 ml) was kept at room temperature for 1.5 h. The reaction mixture was purified by preparative tlc (benzene:ethyl acetate=3:5, chloroform:methanol=8:1) to give an amorphous powder (6, 3 mg). The compound 6 thus obtained was identical with kuwanol A (6) on the basis of ir and ^1H nmr spectral comparisons.

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REFERENCES AND FOOTNOTES

1. Part XXXVIII on "Constituents of the Cultivated Mulberry Tree". Part XXXVII: Y. Hano, H. Kohno, S. Suzuki, and T. Nomura, Heterocycles, 1986, **24**, No 8, in press.
2. Y. Hano, T. Fukai, T. Nomura, J. Uzawa, and K. Fukushima, Chem. Pharm. Bull., 1984, **32**, 1260.

3. Y. Hano, M. Itoh, and T. Nomura, *Heterocycles*, 1985, **23**, 819.

4. Y. Hano and T. Nomura, *Heterocycles*, 1986, **24**, 1381.

5. Y. Hano, K. Hirakura, T. Someya, and T. Nomura, *Heterocycles*, 1986, **24**, 1251.

6. Y. Hano, H. Tsubura, and T. Nomura, *Heterocycles*, 1986, **24**, No 7, in press.

7. M. Takasugi, L. Munoz, T. Masamune, A. Shirata, and K. Takahashi, *Chem. Lett.*, 1978, 1241.

8. K. Hirakura, Y. Hano, T. Fukai, T. Nomura, J. Uzawa, and K. Fukushima, *Chem. Pharm. Bull.*, 1985, **33**, 1088.

9. T. Nomura, T. Fukai, J. Matsumoto, and T. Ohmori, *Planta Med.*, 1982, **46**, 28.

10. T. Fukai, Y. Hano, K. Hirakura, T. Nomura, J. Uzawa, and K. Fukushima, *Chem. Pharm. Bull.*, 1985, **33**, 3195.

11. The ^{1}H nmr spectrum of **7** showed the following proton signals of the E and F rings: δ 6.37 (1H, dd, J=2 and 9, C-19"-H), 6.38 (1H, d, J=2, C-17"-H), 6.69 (1H, d, J=9, C-20"-H); δ 6.17 (1H, d, J=2, C-11"-H), 6.56 (1H, dd, J=2 and 8, C-13"-H), 7.38 (1H, d, J=8, C-14"-H).

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