Communications to the Editor

[Chem. Pharm. Bull. 31(8)2936—2939(1983)]

STRUCTURE OF KUWANON P, A NEW DIELS-ALDER TYPE ADDUCT FROM THE ROOT BARK OF THE CULTIVATED MULBERRY TREE (MORUS LHOU (SER.) KOIDZ.)

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From the ethyl acetate extract of the root bark of cultivated mulberry tree (Morus Lhou (ser.) Koidz.), a new stilbene derivative with a fused dihydrochalcone partial moiety was isolated and named kuwanon P. The structure was shown to be 1 on the basis of spectral data. Kuwanon P (1) is regarded biogenetically as a Diels-Alder type adduct of a chalcone derivative and a dehydroprenylstilbene derivative.

KEYWORDS —— Morus Lhou (ser.); Moraceae; mulberry tree; kuwanon P; Diels-Alder adduct; oxyresveratrol; stilbene; chalcone; ^{1}H NMR spectra; ^{13}C NMR spectra

In the previous papers, we reported the structure determination of a series of natural Diels-Alder type adducts and isoprenylated flavonoids isolated from the root bark of the cultivated mulberry tree[1] and Chinese crude drug "Sāng-Bái-Pí" (Japanese name "Sōhakuhi").[2] In the course of our studies, a new Diels-Alder adduct, named kuwanon P (1), was isolated from the root bark of Morus Lhou (ser.) Koidz. (Japanese name "Rosō"). In this paper, the structure determination of the compound is described. The ethyl acetate extract was fractionated sequentially by polyamide column chromatography, and then preparative thin layer chromatography, resulting in the isolation of kuwanon P (1) in 5×10^{-3}% yield from the root bark.

Kuwanon P (1), amorphous powder, $[\alpha]_D^{17}$ -509° (MeOH), which showed a molecular ion peak at m/z 582 in its FD-MS. The ^{13}C NMR spectrum of 1 revealed the presence of the following thirty-four carbons: nine aliphatic carbons (CH_3- x1, $-CH_2$- x1, >CH- x3, $^{H}>C=C<_{H}$ x1, $>C=C<^{H}$ x1), twenty-four aromatic carbons (CH x11, C x5, C-O- x8) and one carbonyl carbon (Table 1). Treatment of 1 with dimethyl sulfate in acetone gave the octamethyl ether (1a) as an amorphous powder which was negative to the methanolic ferric chloride test, and showed a molecular ion peak at m/z 694 ($C_{42}H_{46}O_9$)[3] in the EI-MS. From these data the composition of kuwanon P was considered to be $C_{34}H_{30}O_9$. The compound (1a) showed the following spectra: UV λ_{max}^{EtOH} nm (log ε): 218(sh 4.55), 283(sh 4.23), 297(infl. 4.28), 304(4.29), 330 (4.28); $\lambda_{max}^{EtOH+AlCl_3}$ nm(log ε): 220(sh 4.55), 283(sh 4.23), 297(infl. 4.29), 304 (4.30), 330(4.29). IR ν_{max}^{Nujol} cm^{-1}: 1670, 1600(sh), 1585(sh).

Kuwanon P (1), giving a reddish violet color with methanolic ferric chloride, was negative to the magnesium-hydrochloric acid test. The compound (1) showed the following spectra: UV λ_{max}^{EtOH} nm(log ε): 218(4.42), 284(4.19), 310(sh 4.12), 331 (4.16); $\lambda_{max}^{EtOH+AlCl_3}$ nm(log ε): 221(4.43), 299(sh 4.25), 305(4.23), 331(sh 4.15). IR ν_{max}^{Nujol} cm^{-1}: 3300, 1630(sh), 1618(sh), 1600, 1590. ^{1}H NMR(100 MHz, acetone-d_6): δ 1.78(3H, s), 2.25(1H, m), 2.50-2.85(1H, m), 3.60-3.80(1H, m), 4.21(1H,br d, J=10),

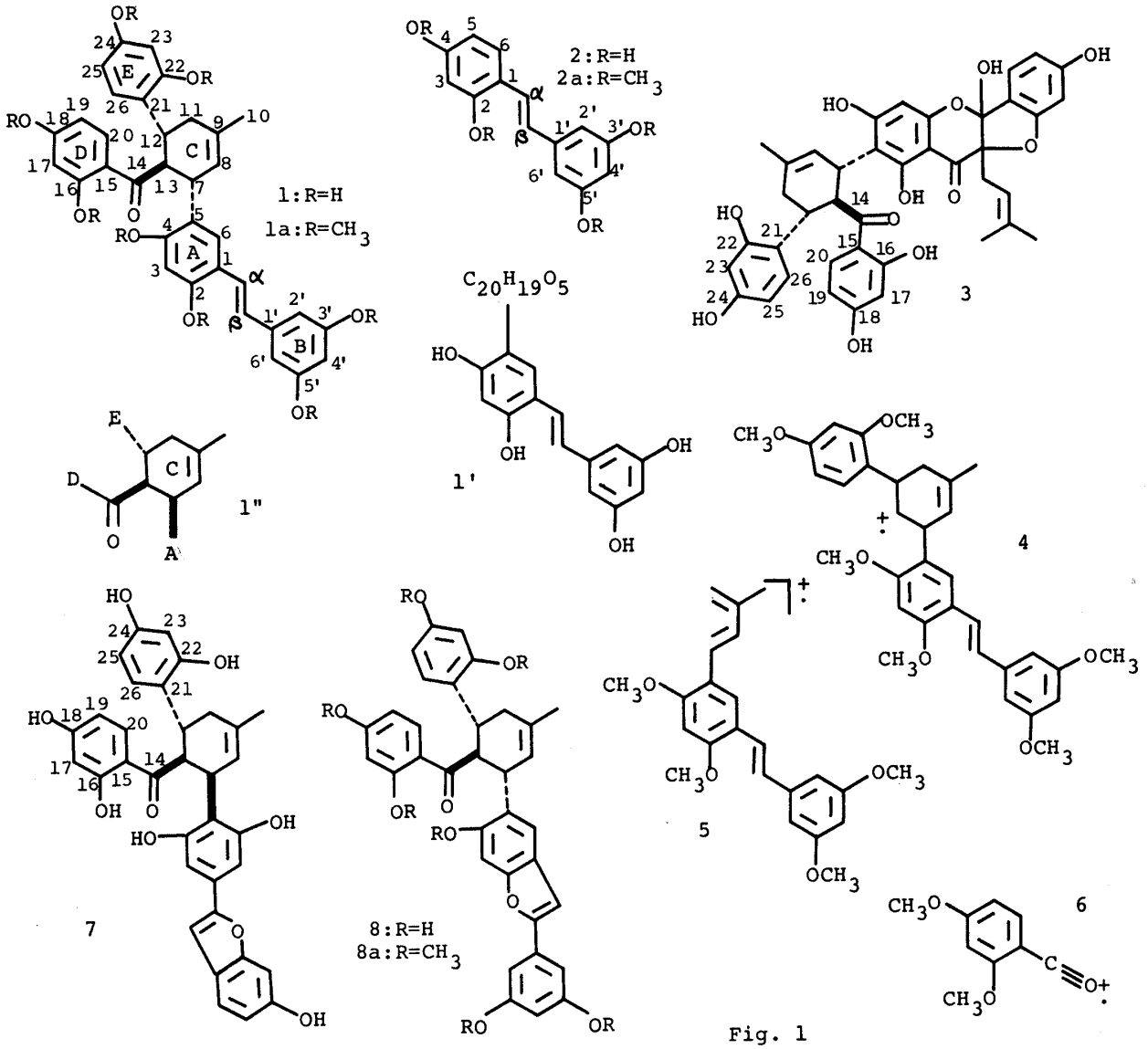


Fig. 1

Table 1. ^{13}C NMR Chemical Shifts

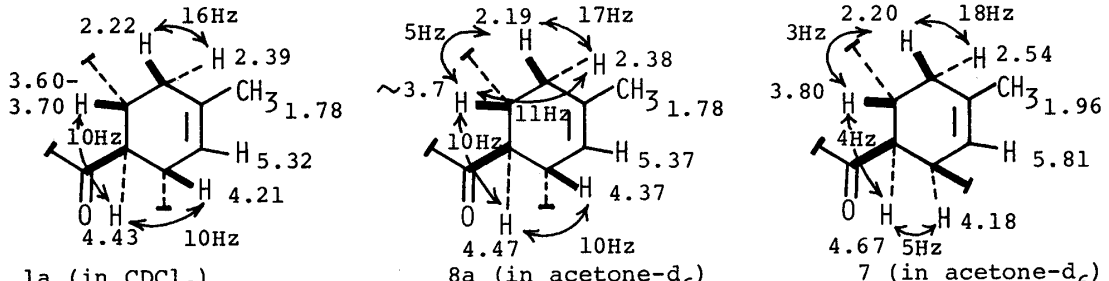
Comp.	1*	2**		1*	3**	7***
C-1	117.3	117.8	C-10	23.4		
C-2	154.9	156.8	C-11	37.7		
C-3	102.2	102.2	C-12	42.3		
C-4	157.4	158.6	C-13	50.1		
C-5	124.7	108.5	C-14	209.7	210.7 br	207.5
C-6	122.7	124.6	C-15	115.8	116.0	113.4
C-α	126.2	126.4	C-16	164.8	165.6	164.6
C-β	130.5	128.5	C-17	103.5	103.8	102.7
C-1'	141.7	142.0	C-18	166.0	165.6	164.6
C-2'	105.4	105.7	C-19	107.7	107.5	108.1
C-3'	159.4	159.0	C-20	127.9	130.9 br	128.5
C-4'	103.7	103.4	C-21	121.6	121.4	122.3
C-5'	159.4	159.0	C-22	156.1	156.8	155.3
C-6'	105.4	105.7	C-23	102.8	103.2	101.5
C-7	42.3		C-24	156.4	156.8	155.9
C-8	126.2		C-25	107.4	108.4	106.4
C-9	134.0		C-26	134.3	134.3 br	133.4

solvent *; acetone-d_6 **; CD_3OD ***; DMSO-d_6

4.56(1H, br t, J=10), 5.41(1H, br s), 6.03(1H, d, J=2), 6.14(1H, dd, J=2 and 9), 6.17(1H, dd, J=2 and 9), 6.20-6.30(3H, m), 6.56(2H, d, J=2), 6.86(1H, d, J=16), 6.97(1H, d, J=9), 7.29(1H, d, J=16), 7.45(1H, s), 7.82(1H, d, J=9), 13.38(1H, s). The UV spectrum of 1 was similar to that of oxyresveratrol (2).[4] Comparison of the spectrum of 1 with that of 2 disclosed an extra absorption in the former at ~285 nm which must be ascribed to a conjugated carbonyl group.[2c,5] In the spectrum of 1 the absorption at ~285 nm showed a bathochromic shift in the presence of aluminum chloride.[6] In the ^{13}C NMR spectrum of 1, all the carbon atoms were assigned by the off-resonance decoupling technique as well as by comparison of the ^{13}C NMR spectra of the model compounds, oxyresveratrol (2), sanggenon D (3),[2d] and other Diels-Alder adducts[1,2] obtained from Morus species. The chemical shift values of the carbon atoms of the stilbene skeleton were similar to those of the relevant carbon atoms of 2 except that the signals of carbon atoms at C-4, -5, and -6 were affected by additional substituent effect (Table 1). These results indicated the presence of a partial structure with the oxyresveratrol type of chromophore. This assumption was further supported by examination of the ^{1}H NMR spectra of 1a (270 MHz, $CDCl_3$) by comparison with the spectrum of oxyresveratrol tetramethyl ether (2a)[4] as follows: δ 6.23(1H, s, C-3 H), 6.37(1H, t, J=2, C-4' H), 6.70(2H, d, J=2, C-2' and C-6' H), 6.94(1H, d, J=16, C-α H), 7.35(1H, d, J=16, C-β H), 7.42 (1H, s, C-6 H). From these results, the partial structure (1') was suggested.

The EI-MS of 1a showed the following species: m/z 529 (4), 366 (5),[7] 165 (6).[7] This result suggests that kuwanon P (1) may be a Diels-Alder adduct regarded as a cycloaddition product with a chalcone and the dehydroprenyloxyresveratrol. The presence of the following moieties on the structure of 1a was supported by a detailed analysis of the ^{1}H NMR spectrum of 1a by comparing it with those of the natural Diels-Alder adducts[1,2] as follows: aromatic protons in a 2,4-dihydroxyphenyl moiety, δ 6.16(1H, dd, J=2 and 8.5, C-25 H), 6.26(1H, d, J=2, C-23 H), 7.06 (1H, d, J=8.5, C-26 H); aromatic protons in a 2,4-dihydroxybenzoyl moiety, δ 5.97 (1H, d, J=2, C-17 H), 6.01(1H, dd, J=2 and 9, C-19 H), 7.09(1H, d, J=9, C-20 H); protons in a methylcyclohexene ring moiety were shown in Fig. 2.[8] The presence of 2,4-dihydroxyphenyl and 2,4-dihydroxybenzoyl moieties was also supported by a comparison of the ^{13}C NMR spectrum of 1 with those of sanggenon D (3)[2d] and mulberrofuran C (7)[1d] as shown in Table 1. On the basis of these results and the biogenetic analogy of the Diels-Alder adducts obtained from Morus species,[1,2] the structure of kuwanon P seems to be represented by 1 or 1".

The location of the 2,4-dihydroxyphenyl and 2,4-dihydroxybenzoyl moieties and the relative configuration of the substituents on the cyclohexene ring of 1 were



1a (in $CDCl_3$) 8a (in acetone-d_6) 7 (in acetone-d_6)

Fig.2. ^{1}H NMR Chemical Shifts and Coupling Constants of Cyclohexene Ring Protons of Kuwanon P Octamethyl Ether (1a), Albafuran C Heptamethyl Ether (8a) and Mulberrofuran C (7)

determined by comparing the ^{1}H NMR spectrum of 1a with those of albafuran C heptamethyl ether (8a)[9] and mulberrofuran C (7)[1d] (Fig. 2). Substance 1a resembled 8a in the chemical shifts and coupling constants of the relevant protons of the methylcyclohexene ring except that the signal of the proton at C-7 was affected by the substituent effect. On the basis of these findings, we propose formula 1 for the structure of kuwanon P. Kuwanon P (1) is optically active and is the first example of a natural product which is considered to be formed by a Diels-Alder type of enzymatic process of a chalcone derivative and a dehydroprenylstilbene derivative.

Recently, Takasugi et al.[9] reported albafuran C (8) obtained from the mulberry shoot, and that the compound (8) was regarded as a Diels-Alder adduct of a chalcone and a dehydromoracin N with a 2-arylbenzofuran skeleton. Considering the biogenetic route of 2-arylbenzofuran derivatives involving oxidative cyclization process of hydroxystilbenes,[10] kuwanon P (1) seems to be an interesting intermediate to examine the biogenetic route of albafuran C (8).

ACKNOWLEDGEMENT We are grateful to Dr. T. Endo, Tsumura Laboratory, for kind donation of the ^{13}C NMR spectrum of oxyresveratrol, and also grateful to Dr. I. Yokoe, Faculty of Pharmaceutical Sciences, Josai University, for High-resolution MS measurement, and also grateful to Dr. K. Fukushima, Research Institute for Chemobiodynamics, Chiba University, for FD-MS measurement, and also grateful to Dr. S. Terada, Research Laboratory of Zen-Yaku Kogyo Co., Ltd., for ^{1}H NMR measurement.

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(Received June 7, 1983)