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KUWANON W, A NATURAL DIELS–ALDER TYPE ADDUCT FROM THE ROOT BARK OF *MORUS LHOU**

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Key Word Index—*Morus lhou*; Moraceae; mulberry tree; prenylated flavone; Diels–Alder type adduct; kuwanon W.

Abstract—A natural Diels–Alder type adduct, named kuwanon W, was isolated from ethyl acetate extracts of the root bark of cultivated mulberry tree, and its structure was determined on the basis of spectral and chemical evidence. Kuwanon W is regarded biogenetically as a Diels–Alder adduct of a chalcone derivative and dehydroprenyl-flavone.

INTRODUCTION

In a previous paper, we reported the structures of two natural Diels–Alder type adducts, kuwanons N and O, isolated from the root bark of *Morus lhou* Koidz. (Japanese name 'Rosō') [1]. Further extensive fractionation of ethyl acetate extracts of the root bark led to the isolation of a new Diels–Alder type adduct, named kuwanon W (**1**). We report herein the structural elucidation of this compound.

RESULTS AND DISCUSSION

Kuwanon W (**1**), amorphous powder, $[\alpha]_D^{17}$ −440°, $FeCl_3$ test (dark green), gave its heptamethyl ether (**1a**), amorphous powder, $FeCl_3$ test (negative), as the exhaustive methylation product. The field desorption (FD) mass spectrum of **1** and the electron impact (EI) mass spectrum of **1a** showed the $[M]^+$ peaks at m/z 758 and 856, respectively. The UV spectrum of **1** exhibited maxima at 210, 258 (infl.), 265, 278 (infl.) and 310 nm, which were similar to those of kuwanons C (**2**) [2], G (**3**) [3, 4] and H (**4**) [5]. These data led us to the assumption that kuwanon W (**1**) possesses a flavone skeletal structure like **2**, **3** and **4**. The EI mass spectrum of **1** and **1a** showed the significant fragments as the corresponding fragments of **4** and its octamethyl ether (**4a**), indicating that **1** must be a Diels–Alder type adduct such as **3** and **4** [4, 5]: **1**, m/z 420 (**5**), 377, 203 (**6**), 147 (**7**); **1a**, m/z 639 (**8**), 217. The ^1H NMR spectra (room temperature, acetone-d_6 or acetonitrile-d_3) showed a complex pattern with broad signals. This implies that **1** exists as an equilibrium mixture of conformational isomers in solution [6, 7]. In support of this, at higher temperature (75° in acetonitrile-d_3), the signals appeared more clearly. Analysis of the ^1H NMR spectra of **1** (room temperature, in acetone-d_6 or acetonitrile-d_3) by comparison with the spectrum of **4** [5] indicated that the chemical shifts and coupling constants of the protons of **1** were similar to those of the relevant protons of **4** except those of the E-ring protons, and also indicated the presence of a 2,2-dimethylpyran ring in the place of a γ,γ-dimethylallyl group on the E-ring of **4** (Table 1). The existence of two hydrogen-bonded hydroxyl groups in the structure of **1** was also supported by the ^1H NMR spectra. All these results indicate that the structure of kuwanon W is possibly represented by formula **1**.

Table 1. ^1H NMR spectral data of compounds 1 and 4 [270 (for 1) and 100 (for 4) MHz, $(CD_3)_2CO$]

	1	4
H-6	6.05 *s*	5.99 *s*
H-3′	6.68 *br s*	6.66 *d* (2)
H-5′	6.57 *m*	6.58 *dd* (2, 8)
H-6′	7.25–7.35 *m*	7.29 *d* (8)
H-9	[3.10 *br d* (7)]*	3.14 *br d* (7)
H-9		
H-10	5.00–5.20 *m*	4.90–5.50 *m*
Me-11	1.49 *s*	1.48 *s*
Me-11	1.62 *s*	1.62 *s*
H-14	[4.21 *br d* (10)]	4.30–4.85 *m*
H-15	5.21 *br s*	4.90–5.50 *m*
Me-16	1.55 *s*	1.57 *s*
H-18	[1.90–2.10 *m*]	1.80–2.20 *m*
H-18	[2.43 *dd* (10, 17)]	
H-19	~ 3.5 *m*	3.50–3.90 *m*
H-20	[4.50 *br t* (10)]	4.30–4.85 *m*
H-26	5.90 *br d* (8)	6.07 *d* (8)
H-27	7.25–7.35 *m*	7.29 *d* (8)
H-30	6.22 *br*	6.22 *d* (2)
H-32	6.05 *br*	6.00 *dd* (2, 8)
H-33	6.65–6.75 *m*	6.82 *d* (8)
H-34	6.45 *d* (10)	3.14 *br d* (7)
H-35	5.57 *d* (10)	4.90–5.50 *m*
Me-36	1.32 *s*	1.57 *s*
Me-36	1.32 *s*	1.67 *s*
OH-5	13.00 *s*, 13.01 *s*	13.16 *s*
OH-23	13.53 *s*, 13.41 *s*	13.58 *s*

Chemical shifts in ppm downfield from TMS. *J* (Hz) in parentheses.

*Data measured in CD_3CN are in square brackets.

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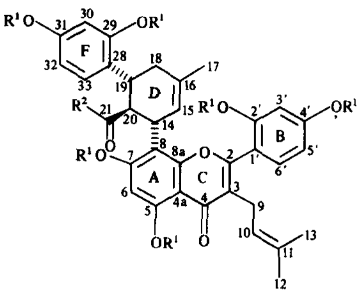
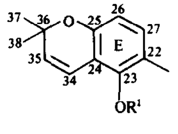
* Part 20 in the series "Constituents of the Cultivated Mulberry Tree" (Part 2 of "Constituents of the Root Bark of *Morus lhou*". For Part 1 see ref. [1]). For Part 19 see Hano, Y., Itoh, M., Koyama, N. and Nomura, T. (1984) *Heterocycles* **22**, 1791.



1(1') $R^1 = H$, $R^2 = A$
1a(1'a) $R^1 = Me$, $R^2 = A$
3 $R^1 = H$, $R^2 = C$
4 $R^1 = H$, $R^2 = B$
4a $R^1 = Me$, $R^2 = B$

A =



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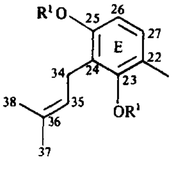

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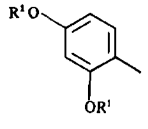

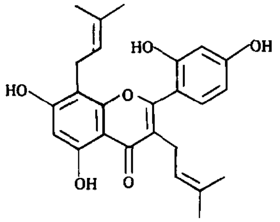

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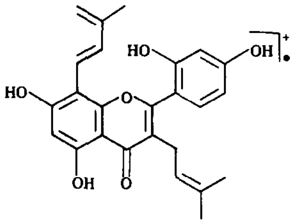

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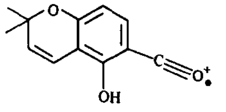

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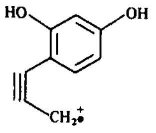

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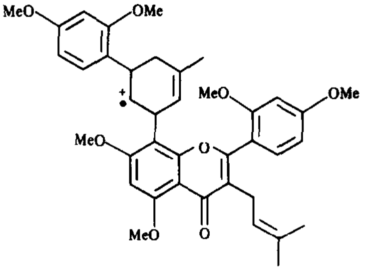

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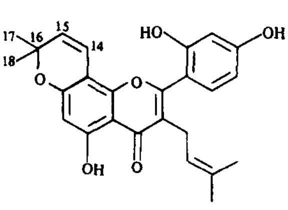

9

In order to corroborate the structure, 1 was derived from 4 by treating 4 with palladium chloride in 90% aqueous methanol solution [8, 9]. The IR and ^1H NMR spectra of the reaction product (1') were in fair agreement with those of 1. While the ^{13}C NMR spectrum of 1' showed a complex pattern at 35° in acetone-d_6, at higher temperature all the signals appeared more clearly so that the spectrum could be analysed by comparison of the spectra with those of 4 [5] and morusin (9) [2]. In the spectrum of 1', all the carbon atoms (except the signals of C-24, C-27 and C-34 to C-38) were essentially the same as those of the relevant carbon atoms of 4, and the chemical shift values of the carbon atoms (C-34 to C-38) were in good agreement with those of the relevant carbon atoms of 9 (Table 2). From the above results, kuwanon W is represented by formula 1.

Table 2. ^{13}C NMR spectral data of compounds **1'**, **4** and **9** (67.8 MHz, DMSO-d_6)

C	**1'***	**4**	C	**9**
2	158.5	159.3		
3	120.0	119.8		
4	181.9	181.8		
4a	103.3	103.9		
5	155.4	155.3		
6	98.0	97.3		
7	160.4	161.6		
8	106.8	106.9		
8a	159.7	160.3		
1'	111.9	111.6		
2'	156.1	156.4		
3'	103.3	102.7		
4'	158.3	160.9		
5'	107.0	106.9		
6'	130.8	129.5		
9	23.5	23.8		
10	122.1	121.9		
11	131.4	131.3		
12	25.2	25.5		
13	17.3	17.5		
14	37.7	[39.8]†		
15	123.6	123.4		
16	132.5	132.9		
17	22.4	22.6		
18	37.5	[39.2]		
19	37.7	[39.8]		
20	47.0	45.7		
21	208.7	208.4		
22	114.8	114.0		
23	161.0	162.1		
24	107.9	113.7		
25	161.4	161.6		
26	107.1	106.9		
27	128.1	131.3		
28	121.0	121 1		
29	156.0	155.9		
30	103.3	102.7		
31	155.4	155.9		
32	107.0	106.9		
33	130.8	131.3		
34	114.7	21.4	14	114.3
35	128.3	122.4	15	127.6
36	77.4	130.5	16	78.0
37	28.0	25.5	17	27.7
38	27.8	17.5	18	27.7

*Data measured at 120°.

†Data measured in CD$_3$OD are in square brackets.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were measured with TMS as internal reference. For TLC, Wakogel B-5FM was used, for prep. TLC, Wakogel B-5F, and for CC, Wakogel C-200.

Plant material. Root bark of the cultivated mulberry tree (Japanese name 'Rosō', a variety of *M. lhou*) was collected in the neighbourhood of Takasaki, Gunma prefecture, Japan, in December 1981.

Isolation of kuwanon W (**1**). The EtOAc extract (300 g) of the root bark of *M. lhou* (26 kg) [1] was chromatographed on silica gel (1.6 kg) using C_6H_6–MeOH as the eluant. The fraction eluted with C_6H_6 containing 5% MeOH was evapd to give 29 g of residue. This was rechromatographed on silica gel (200 g) using hexane–Me$_2$CO as the eluant. The fraction eluted with hexane containing 25% Me$_2$CO was evapd to give 12 g of residue From the residue (5 g), kuwanon W (**1**, 34 mg) was obtained using prep. TLC [solvent systems, C_6H_6–Me$_2$CO (3:1), CHCl$_3$–MeOH (10:1), Me$_2$CO–CHCl$_3$ (1:3)].

Kuwanon W (**1**). Kuwanon W (**1**) was obtained as an amorphous powder. Although only one spot was detected on TLC [C_6H_6–Me$_2$CO (3:1), CHCl$_3$–MeOH (10.1), Me$_2$CO–CHCl$_3$ (1:3)], **1** could not be isolated in a crystalline form. $[\alpha]_D^{17}$ $-440°$ (MeOH; *c* 0.03). FeCl$_3$ test: dark green → dark purple, Mg–HCl test: positive FDMS *m/z*: 758 $[M]^+$. EIMS 75 eV, *m/z* (rel. int): 420 (6), 377 (4), 203 (9), 147 (5). UV λ_{max}^{EtOH} nm (log ε): 210 (4 85), 258 (infl. 4.64), 265 (4 66), 278 (infl. 4.52), 310 (4.24), $\lambda_{max}^{EtOH+AlCl_3}$ 210 (4.85), 273 (4.70), 300 (sh 4.22), 340 (sh 4.08), 385 (3.90). IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1650, 1610, 1370.

Kuwanon W heptamethyl ether (**1a**) A mixture of kuwanon W (**1**, 3 mg), Me$_2$SO$_4$ (0.01 ml), dry K$_2$CO$_3$ (2 g), Me$_2$CO (15 ml) and crown ether (18-crown-6-ether, catalytic amount) was refluxed for 6 hr and treated as usual. The products were purified by prep. TLC [C_6H_6–Me$_2$CO (3:1)] to give an amorphous powder (**1a**, 0.7 mg). FeCl$_3$ test: negative. EIMS 75 eV, *m/z* (rel. int.) 856 $[M]^+$ (100), 639 (11), 476 (10), 217 (99), 151 (25).

Formation of kuwanon W(**1**)*from kuwanon H* (**4**). A mixture of **4** (49.8 mg) and PdCl$_2$ (1.3 mg) in 90% aq. MeOH soln (0.5 ml) was kept at room temp. for 15.5 hr. The products were purified by prep. TLC [CHCl$_3$–EtOAc (3:2)] to give an amorphous powder (**1'**, 12.9 mg) and **4** (1.9 mg). The IR spectrum of **1'** was in agreement with that of **1** The ^{1}H NMR spectrum of **1'** (400 MHz, 80°, in CD$_3$CN) was also in agreement with that of **1** (400 MHz, 75°, in CD$_3$CN). The compound (**1'**, 5 mg) gave the heptamethyl ether (**1'a**, 1 mg) by methylation, as described above The EIMS of **1'a** was in agreement with that of **1a**.

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