

STRUCTURES OF MULBERROFURANS F AND G, TWO NATURAL HYPOTENSIVE DIELS-ALDER
TYPE ADDUCTS FROM THE CULTIVATED MULBERRY TREE (*MORUS LHOUSER.*) KOIDZ.)¹

Toshio Fukai,^{a)} Yoshio Hano,^{a)} Kazuhiro Hirakura,^{a)} Taro Nomura,^{*,a)}
Jun Uzawa,^{b)} and Kazutaka Fukushima^{c)}

a) Faculty of Pharmaceutical Sciences, Toho University,
2-2-1, Miyama, Funabashi-shi, Chiba 274, Japan

b) The Institute of Physical and Chemical Research,
Wako-shi, Saitama 351, Japan

c) Research Institute of Chemobiodynamics, Chiba University,
1-8-1, Inohana-cho, Chiba-shi, Chiba 280, Japan

Abstract — From ethyl acetate extract of the root bark of cultivated mulberry tree (*Morus Lhou(ser.)* Koidz.), two 2-arylbenzofuran derivatives were isolated and named mulberrofurans F and G, whose structures were shown to be 1 and 2, respectively, on the basis of spectral and chemical evidences. Mulberrofurans F (1) and G (2) were derived from chalcemoracin (3) and mulberrofuran C (4), respectively, by the photocyclization in the acidic solution. Intravenous injection of mulberrofurans F (1), as well as G (2), caused a marked depressor effect in rabbit.

Previously we reported the structure determination of a series of natural Diels-Alder type adducts and isoprenylated flavonoids isolated from the root barks of the cultivated mulberry tree² and Chinese crude drug "Sang-Bai-pi" (Japanese name "Sohakuhi").³ In the course of our studies, two 2-arylbenzofuran derivatives, mulberrofurans F (1) and G (2) were isolated from the root bark of *Morus Lhou(ser.)* Koidz. (Japanese name "Rosō"). In this paper, the structure determinations of the compounds are described.

The ethyl acetate extract was fractionated sequentially by silica-gel column chromatography, resulting in the isolation of 1 (3×10^{-3} % yield from the root bark), and 2 (8.1×10^{-2} %). Single intravenous injection of 1 or 2 (both 0.1 mg/Kg) caused a marked depressor effect in rabbit by 26 mmHg and 16 mmHg, respectively.

Mulberrofuran F (1), amorphous powder, $[\alpha]_D^{23} +513^\circ$ ($c=0.024$, MeOH), FeCl₃ test (negative), gave the FD-MS which showed the molecular ion peak at m/z 630, and the ¹³C nmr spectrum which indicated the presence of thirty nine carbons [fifteen aliphatic carbons ($3 \times \text{CH}_3$ -, $2 \times \text{CH}_2$ -, $3 \times \text{CH}$ -, $2 \times \text{C}=\text{CH}$ -, $1 \times \text{CH}=\text{C}-\text{O}$ -, $1 \times \text{O}-\text{C}-\text{O}$ -) and twenty four aromatic carbons ($10 \times \text{CH}$, $6 \times \text{C}$, $8 \times \text{C}-\text{O}$)].

Treatment of 1 with dimethyl sulfate and potassium carbonate in acetone effected exhaustive methylation to give its pentamethyl ether (1a) as an amorphous powder. The molecular formula of 1a was determined to be C₄₄H₄₄O₈ by the high-resolution mass spectrum (m/z 700.3023), and hence 1 could be formulated as C₃₉H₃₄O₈. Work-up of 1 with acetic anhydride in pyridine gave the pentaacetate (1b) which showed a molecular ion peak at m/z 840 in its FD-MS. The compound (1) showed the following spectra: $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3380, 1615, 1610, 1600; $\nu_{\text{max}}^{\text{EtOH}}$ nm(log ϵ): 230 (infl. 4.51), 285(4.15), 296(sh 4.12), 306(infl. 4.26), 321(4.46), 335(4.39). The uv spectrum was similar to those of chalcemoracin (3)⁴ and mulberrofuran C (4)⁵ and suggested that 1 is one of the 4'-substituted 6,3',5'-trihydroxy-2-arylbenzofuran derivatives. This suggestion was

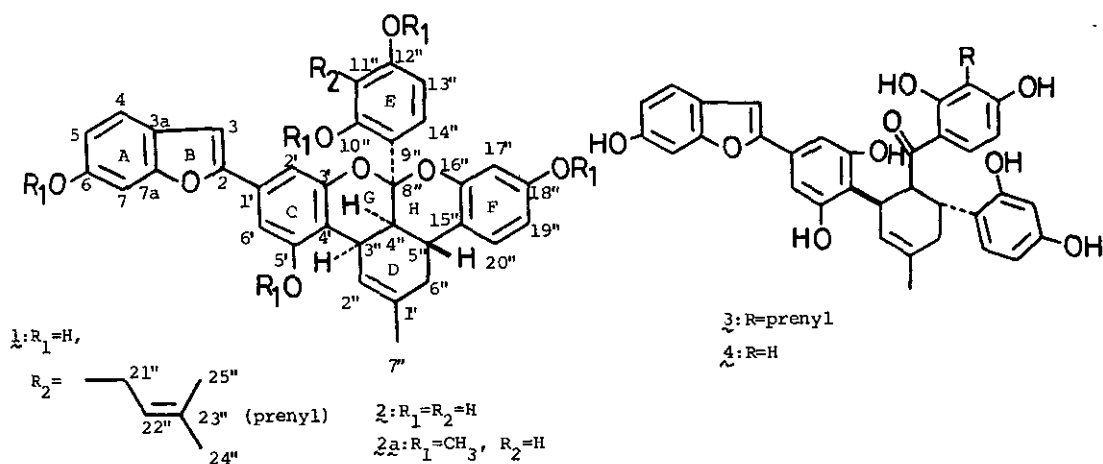


Fig. 1

Table 1 ^{13}C nmr chemical shifts (ppm) of $\underline{1}$ - $\underline{4}$ and $\underline{7}$

No of C	$\underline{1}$	$\underline{2}$	$\underline{7}$	$\underline{3}$	$\underline{4}$	No of C	$\underline{1}$	$\underline{2}$	$\underline{7}$	$\underline{3}$	$\underline{4}$
2	157.0*	157.7*	157.1	155.4*	155.3*	6"	35.7	36.2	121.3	33.9	34.5
3	101.9	102.2	103.2	102.9	102.7	7"	23.7	23.9	22.3	23.2	23.4
3a	122.6	122.5	123.0	121.2	121.3	8"	103.3	102.6	106.7	207.7	207.5
4	121.4	122.0	122.3	121.6	121.5	9"	117.2**	113.4	111.6	115.0	113.4
5	112.7	113.4	113.5	112.5	112.6	10"	154.0*	157.9*	160.2*	162.4*	164.6*
6	154.6*	155.0*	155.0*	153.8*	153.9*	11"	113.2	103.9	105.0	114.4	102.7
7	97.9	98.4	98.5	97.7	97.7	12"	155.9*	159.9*	158.2*	162.0*	164.6*
7a	155.9*	156.7*	157.4*	155.8*	157.1*	13"	106.8	107.3	111.6	106.2	106.4
1'	130.4	131.5	129.8	129.2	129.2	14"	127.3	130.3	131.6	131.2	128.5
2'	104.7	105.0	106.1	103.2	103.0	15"	116.4**	116.8**	115.7***	122.0	122.3
3'	157.0*	157.5*	160.8*	157.0*	157.1*	16"	152.7*	153.3*	152.7*	155.2*	155.3*
4'	116.6**	117.5**	122.5***	112.8	112.8	17"	103.4	104.0	106.5	102.6	101.5
5'	156.8*	156.7*	157.4*	157.0*	155.3*	18"	153.6*	154.5*	153.6*	155.2*	155.9*
6'	105.5	105.4	105.4	103.2	103.0	19"	109.8	109.9	107.4	107.2	108.1
1"	133.3	133.7	140.8	133.2	133.4	20"	125.5	127.9	122.3	131.2	133.4
2"	121.8	122.9	125.3	127.9	128.1	21"	22.2			21.0	
3"	37.0***	37.2***	130.1**	33.1	33.6	22"	124.4			122.0	
4"	28.5	28.5	116.0***	46.9	47.1	23"	131.4			130.4	
5"	35.3***	35.1***	132.7**	33.9	33.3	24"	25.8			25.4	
solvent	A	A	B	C	C	25"	17.8			17.6	

A: acetone- d_6 , B: CD_3OD , C: $dmsd-d_6$, ****: Assignments may be reversed.

Table 2 Chemical shifts (ppm) of $\underline{1}$ and $\underline{1b}$

	17"-H	19"-H	20"-H
$\underline{1}$	6.42	6.55	7.15
$\underline{1b}$	6.63	6.83	7.41
	-0.21	-0.28	-0.26

in acetone- d_6

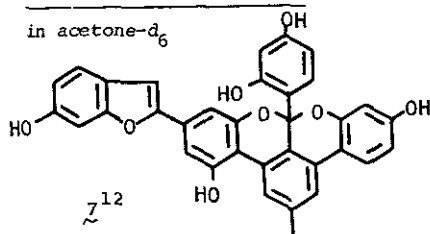


Table 3 Chemical shifts of $\underline{5}$ and $\underline{5a}$

	7-H	5-H	4-H
$\underline{5}$	6.99	6.78	7.40
$\underline{5a}$	7.28	6.99	7.55
	-0.29	-0.21	-0.15

in $CDCl_3$

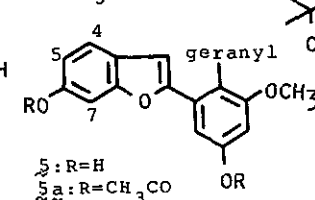


Table 4 Chemical shifts of $\underline{6}$ and $\underline{6a}$

	3'-H	5'-H	6'-H
$\underline{6}$	6.53	6.45	7.15
$\underline{6a}$	7.16	7.18	7.58
	-0.63	-0.73	-0.43

in acetone- d_6

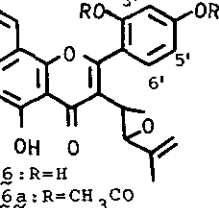


Fig. 2

supported through a comparative examination of the ^1H nmr spectrum of 1 (400 MHz, acetone- d_6) with those of 3, 4, 5 and other 2-arylbenzofuran derivatives.⁶ The chemical shifts and coupling constants (Hz) of the 2-arylbenzofuran moiety are shown as follows: δ 6.82 (1H, dd, $J=2.2$ and 8.5, C-5-H), 6.97 (1H, dd, $J=0.9$ and 2.2, C-7-H), 6.98 and 7.03 (each 1H, d, $J=1.5$, C-2'- and -6'-H), 7.05 (1H, d, $J=0.9$, C-3-H), 7.40 (1H, d, $J=8.5$, C-4-H). As the chemical shift values of the protons at the C-2' and 6' positions appeared nonequivalent, it is suggested that one of the hydroxyl groups in the C-ring formed the ether linkage.⁷ The ^{13}C nmr spectrum of 1 supported the substituted pattern on the C-ring. The chemical shifts of the carbon atoms of the 2-arylbenzofuran skeleton, except those of the carbon atoms at C-4' and 6', were similar to those of the relevant carbon atoms of 3 and 4 (Table 1). The presence of the following moieties and the substituted pattern on the F-ring of 1 were supported by comparing the ^1H nmr spectrum of 1 with those of prenylflavonoids² and by considering the following acetylation shifts in the ^1H nmr spectra. The signals of protons in a 2,4-dihydroxy-3-prenylphenyl moiety were observed at δ 1.57 and 1.73 (each 3H, s, C-23"-CH₃), 3.36 (2H, d, $J=6.8$, C-21"-Hx2), 5.23 (1H, m, C-22"-H), 6.38 (1H, d, $J=8.5$, C-13"-H), 7.17 (1H, d, $J=8.5$, C-14"-H) and those of aromatic protons in a 2,4-dioxygenated phenyl moiety were observed at δ 6.42 (1H, d, $J=2.5$, C-17"-H), 6.55 (1H, dd, $J=2.5$ and 8.5, C-19"-H), 7.15 (1H, d, $J=8.5$, C-20"-H). Comparison of the ^1H nmr spectra of 1 and 1b indicate that the acetylation of the hydroxyl group in the 2,4-dioxygenated phenyl moiety caused down field shifts (0.21-0.28 ppm) of the protons in the moiety (Table 2). Similar shifts were observed in the case of the A-ring protons of mulberrofuran A (5) and its acetate (5a) (Table 3).^{6a} On the other hand, the acetylation of the 2' and 4' hydroxyl groups of the compound (5)^{8a} caused larger down field shifts (0.43-0.73 ppm) of the protons in the B-ring (Table 4).⁸ These results suggest that 1 has a hydroxyl group in the 2,4-dioxygenated phenyl moiety and that the other oxygen atom formed the ether linkage.

The presence of trisubstituted methylcyclohexene ring was suggested by the examination of the ^1H nmr spectrum of 1a (400 MHz, acetone- d_6). The spectrum was analysed with the aid of sequential decoupling experiments, and the deduced structure is shown in Fig. 3 along with chemical shift values and the coupling constants of the protons of C_6H_9 moiety.

This methylcyclohexene ring is presumed to be substituted at the C-4' position from the biogenetic analogy of 3 and 4. This presumption was supported by the acetylation shift of the C-2" olefinic proton signal as follows: the C-2" proton signal of 1b shifted 0.47 ppm toward upper field from the corresponding proton of 1. In the ^{13}C nmr spectrum of 1, the singlet signal at 103.3 ppm suggested the presence of $-\text{O}-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-$ type moiety in the structure.⁹ All these results indicate that the structure of mulberrofuran F is possibly represented by 1 (except the stereochemistry at C-8" position).

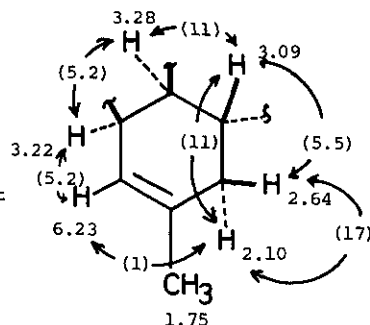


Fig. 3 $\underline{I_a}$ (): J (Hz)

In order to corroborate the structure, 1 was derived from 3 by the following reaction. A solution of 3 (40 mg) in ethanol containing 1.5% sulfuric acid was externally irradiated in a glass vessel with 100 W high-pressure mercury lamp for 9 h. The products were purified by preparative tlc to give 1 (4 mg). The ir and ¹H nmr spectra of the product along with the optical rotation value are in fair agreement with those of 1. The relative configuration of the E-ring with the methine protons on the methylcyclohexene ring was suggested by the coupling constants of the methylcyclohexene ring protons. The coupling constants of the ring protons were in good agreement with those expected for the relevant protons of the Dreiding model. From the above results, we propose the formula (1) for the structure of mulberrofuran F.

Mulberrofuran G (2), amorphous powder, $[\alpha]_D^{22} +546^\circ$ ($c=0.0326$, MeOH), FeCl_3 test (negative), gave the FD-MS which showed the molecular ion peak at m/z 562, and the ^{13}C nmr spectrum which indicated the presence of thirty four carbons (Table 1). Treatment of 2 with dimethyl sulfate gave pentamethyl ether (2a), mp 182-183°C (colorless needles), whose molecular formula was determined to be $\text{C}_{39}\text{H}_{36}\text{O}_8$ by the high-resolution mass spectrum (m/z 632.2372), and hence 2 could be formulated as $\text{C}_{34}\text{H}_{26}\text{O}_8$. The compound (2) showed the uv spectrum as follows: uv $\lambda_{\text{max}}^{\text{EtOH}}$ nm(log ϵ): 223(4.63), 285(4.29), 295(sh 4.24), 306(infl. 4.37), 321(4.57), 335(4.50). The uv spectrum and the mass spectrum of 2 were similar to those of 1, respectively. The mass spectrum of 2a showed the significant fragment ion at m/z 495 ($\text{M}^+ - \text{C}_8\text{H}_9\text{O}_2$)¹⁰ corresponding to the loss of a dimethoxyphenyl moiety. The mass spectrum of 1a showed the same fragment ion ($\text{M}^+ - \text{C}_{13}\text{H}_{17}\text{O}_2$)¹⁰. These results suggest that 2 is a deprenylmulberrofuran F. In the ^{13}C nmr spectrum of 2, all the carbon atoms except those of E ring were essentially of the same chemical shift values as compared with the relevant carbon atoms of 1 (Table 1). The ^1H nmr spectrum of 2 (400 MHz, acetone- d_6) was examined by comparison with that of 1 and showed the presence of the following moieties on the structure of 2: aromatic protons in a 2-arylbenzofuran moiety, δ 6.81 (1H, dd, $J=2$ and 8.6, C-5-H), 6.94 and 6.98 (each 1H, d, $J=1.5$, C-2' and -6'-H), 6.97 (1H, br d, $J=2$, C-7-H), 7.05 (1H, d, $J=0.7$, C-3-H), 7.41 (1H, d, $J=8.6$, C-4-H); aromatic protons in two 2,4-dioxygenated phenyl moieties, δ 6.23 (1H, dd, $J=2.4$ and 8.8, C-13"-H), 6.38 (1H, d, $J=2.5$, C-17"-H), 6.42 (1H, d, $J=2.4$, C-11"-H), 6.51 (1H, dd, $J=2.5$ and 8.3, C-19"-H), 7.14 (1H, d, $J=8.3$, C-20"-H), 7.24 (1H, d, $J=8.8$, C-14"-H). The protons in a methylcyclohexene ring moiety are shown in Fig. 4. The coupling constants of the protons of the methylcyclohexene ring of 2 were similar to those of the relevant protons of 1a. To confirm the structure of 2, the compound was derived from 4 as follows:

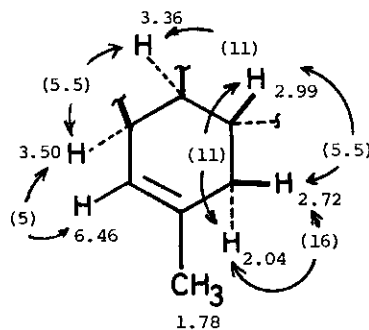


Fig. 4 2

a solution of 4 (30 mg) was treated as described in the case of 3 to give the product (19 mg). The ir and the ^1H nmr spectrum of the product along with the optical rotation value are in fair agreement with those of 2. From these results, the structure of mulberrofuran G is represented by the formula (2). Biogenetically, mulberrofurans F (1) and G (2) seems to be Diels-Alder type adducts derived from 3 and 4, respectively, by the intramolecular ketalization reaction of the carbonyl group with the two adjoining hydroxyl groups.

Recently, Rama Rao et al. reported the isolation of albanols A and B from *Morus alba* bark, and assigned the formula (2) and (7) for the compounds, respectively, on the basis of X ray study of albanol A pentamethyl ether.¹¹ Albanol A seems to be identical with mulberrofuran G by the comparison of the spectral and physical data of these compounds and their derivatives.^{12,13}

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12. Just after we contributed the abstract paper of the 26th Symposium on the Chemistry of Natural Products to the organizing committees (August 2nd, 1983), we found the report¹¹ of Rama Rao's group. In the abstract paper, we proposed the formula (1) and (2) for the structure of mulberrofurans F and G, respectively.
13. In addition to 1 and 2, the compound (3) was isolated from the same source. This compound seems to be albanol B¹¹ from the following spectral and physical data: mp 240°C (decomp.); $[\alpha]_D^{20}$ -15° (c=0.02, MeOH); FD-MS m/z 558(M⁺), 449; $uv \lambda_{max}^{EtOH}$ nm(log ϵ): 222(4.71), 275(sh 4.12), 285(4.18), 318(sh 4.34), 335(sh 4.54), 350(4.68), 365(4.65); ¹H nmr (100 MHz, CD₃OD), 2.46 (3H, s, C-1"-CH₃), 5.78 (1H, dd, J=2.5 and 9, C-19"-H), 6.06 (1H, d, J=9, C-20"-H), 6.18 (1H, d, J=2.5, C-17"-H), 6.40 (1H, C-2' or C-6'-H, combined the parts of double doublet signal), 6.45 (1H, dd, C-13"-H, combined with C-2' or C-6'-H signal), 6.65 (1H, dd, J=2 and 9, C-5-H), 6.82 (1H, d, J=2.5, C-11"-H), 6.85 (1H, br s, C-3-H), 6.92 (2H, br, C-7-H and C-2' or C-6'-H), 7.24 (1H, d, J=9, C-14"-H), 7.41 (1H, br s, C-2" or -6"-H), 7.50 (1H, d, J=9, C-4-H), 8.28 (1H, br s, C-2" or C-6"-H); ¹³C nmr chemical shifts are shown in Table 2, pentamethyl ether, mp 164-165°C (colorless prisms), C₃₉H₃₂O₈ (High-resolution mass spectrum, m/z 628.2074).

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