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STRUCTURE OF MULBERROFURAN M, A NOVEL 2-ARYLBENZOFURAN DERIVATIVE FROM THE CULTIVATED MULBERRY TREE (*MORUS ALBA* L.)

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Abstract —— A novel 2-arylbenzofuran derivative named mulberrofuran M (**1**) was isolated from an acetone extract of the reddish violet powder obtained from the surface of the root bark of cultivated mulberry tree (Ichinose, a cultivated variety of *Morus alba* L.). The structure was shown to be **1** on the basis of spectral evidence.

Previously we reported the structure determination of two 2-arylbenzofuran derivatives[1,2] and two stilbene derivatives[3] from the reddish violet powder obtained from the surface of the Morus root bark. In this paper, the structure determination is described of a novel 2-arylbenzofuran derivative, mulberrofuran M, isolated from the powder.

The acetone extract of the reddish violet powder (760 g) obtained from the surface of the Morus root bark (Ichinose, a cultivated variety of *Morus alba* L.[4]) was fractionated sequentially by the silica gel column chromatography and preparative thin layer chromatography, resulting in the isolation of **1** (2.3×10^{-3} % yield from the powder).

Mulberrofuran M (**1**), amorphous powder, $[\alpha]_D^{14}$ +15° (c=0.02, MeOH), $FeCl_3$ test (brown), gave the FD-MS which showed the molecular ion peak at *m/z* 590, and the ^{13}C nmr spectrum which indicated the presence of thirty-four carbon atoms [eight aliphatic carbons (1 x $-CH_3$, 1 x $-CH_2-$, 1 x $-CH\langle$, 1 x $\rangle C\langle^{/}_{O-}$, 1 x $-CH{=}C\langle^{/}_{O-}$, 1 x $\rangle C{=}C\langle^{/}_{O-}$), twenty-four aromatic carbons (11 x CH, 5 x C, 8 x C-O), and two carbonyl carbons] (Table 1). Treatment of **1** with acetic anhydride in pyridine gave a tetraacetate (**1a**) which showed a molecular ion peak at *m/z* 758 in its FD-MS. These results indicated the composition of mulberrofuran M to be $C_{34}H_{22}O_{10}$. The compound (**1**) showed the following spectra: ir ν_{max}^{KBr} cm^{-1}: 3350 (br), 1690 (sh), 1675, 1670

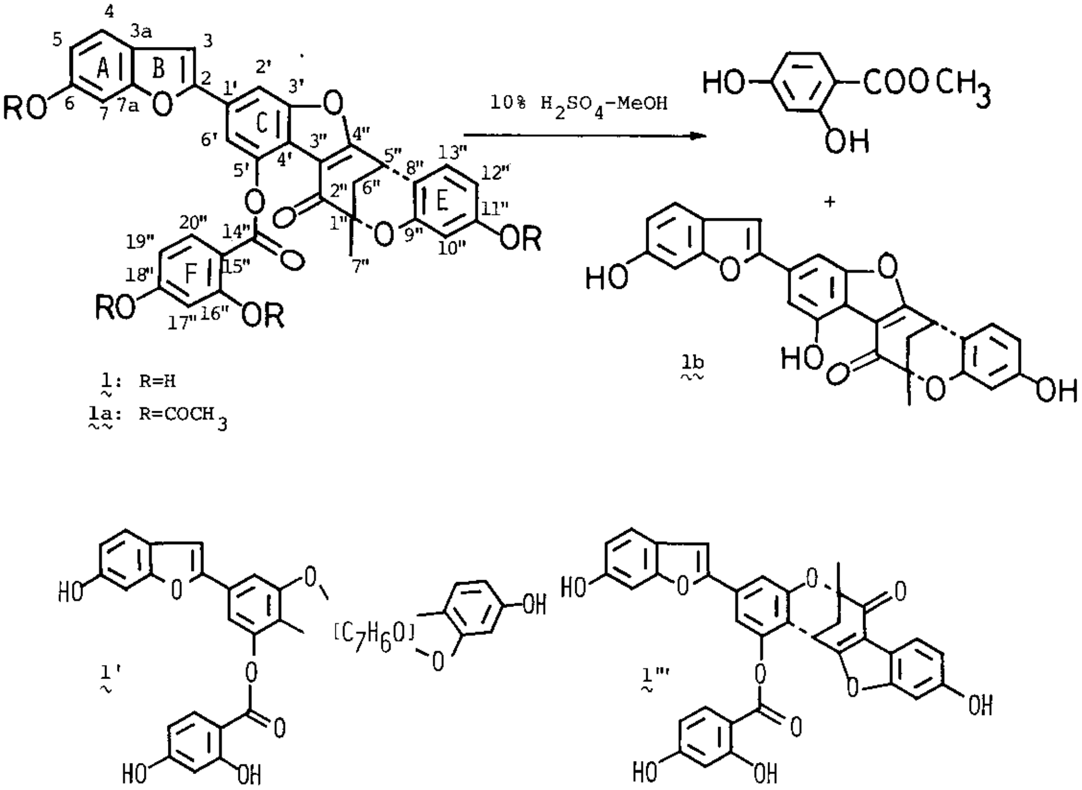

Fig. 1

Table 1 ^{13}C nmr chemical shifts of 1

C-2	159.1	C-8"	112.4
C-3	102.8	C-9"	156.6
C-3a	121.8	C-10"	104.1
C-4	121.8	C-11"	159.1
C-5	113.1	C-12"	115.3
C-6	154.3	C-13"	128.8
C-7	97.9	C-14"	169.1
C-7a	156.5	C-15"	105.4
C-1'	129.6	C-16"	164.4
C-2'	108.7	C-17"	103.4
C-3'	164.4	C-18"	164.9
C-4'	113.1	C-19"	108.7
C-5'	153.1	C-20"	133.3
C-6'	105.4		
C-1"	77.4		
C-2"	186.0		
C-3"	144.5		
C-4"	175.3		
C-5"	32.3		
C-6"	35.7		
C-7"	21.5		

(ppm) solvent: acetone-d_6

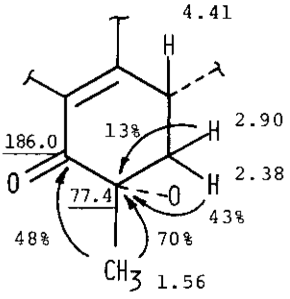

Fig. 2 Partial structure 1" and NOE results

(sh), 1650 (sh), 1620; uv λ_{max}^{EtOH} nm (log ε): 208 (4.71), 225 (infl. 4.42), 261 (4.30), 299 (infl. 4.26), 323 (sh 4.44), 335 (4.53), 350 (sh 4.45). The uv spectrum was similar to those of the 4'-substituted 6,3',5'-trioxygenated 2-arylbenzofuran derivatives obtained from the Morus root bark.[5] The ^1H nmr spectrum of **1** (400 MHz, acetone-d_6), analysed by a decoupling experiment and by comparison with the spectra of 2-arylbenzofuran derivatives, showed the signals of the following protons: 1) protons in a 2-arylbenzofuran moiety, δ 6.85 (1H, dd, J=2 and 8, C-5-H), 7.03 (1H, br d, J=2, C-7-H), 7.35 (1H, d, J=1, C-3-H), 7.46 (1H, d, J=8, C-4-H), 7.73 (1H, d, J=2, C-2'-H), 8.00 (1H, d, J=2, C-6'-H), 2) protons in two 2,4-dioxygenated phenyl moieties, δ 6.30 (1H, d, J=2, C-10"-H), 6.41 (1H, dd, J=2 and 8, C-12"-H), 7.21 (1H, d, J=8, C-13"-H); δ 6.47 (1H, d, J=2, C-17"-H), 6.60 (1H, dd, J=2 and 8, C-19"-H), 8.10 (1H, d, J=8, C-20"-H), 3) six aliphatic protons, δ 1.56 (3H, s, C-1"-CH_3), 2.38 (1H, dd, J=3 and 14, C-6"-H), 2.90 (1H, dd, J=3 and 14, C-6"-H), 4.41 (1H, t, J=3, C-5"-H), and 4) proton in hydrogen bonded hydroxyl group, δ 10.44 (1H, br s). The ^{13}C nmr spectrum was analysed by off-resonance decoupling technique and by comparison with the spectra of 2-arylbenzofuran derivatives[5] and 2,4-dihydroxybenzoyl esters, and showed the signals of following carbon atoms: 1) carbon atoms in a 2-arylbenzofuran moiety (C-2 ∼ C-6'), 2) carbon atoms in a 2,4-dioxygenated phenyl moiety (C-8" ∼ C-13"), 3) carbon atoms in a 2,4-dihydroxybenzoyl ester moiety (C-14" ∼ C-20"), and 4) seven aliphatic carbon atoms (C-1" ∼ C-7") (Table 1). The presence of 2,4-dihydroxybenzoyl ester moiety in the structure was comfirmed by the following result. When the compound (**1**) was hydrolyzed with 10% H_2SO_4 in methanol, β-resorcylic acid methyl ester and the compound (**1b**, M^+ 454) were obtained. The position of the ester linkage was suggested by comparing the ^1H nmr spectrum of **1** with that of **1a** (Table 2). In the ^1H nmr spectrum of **1a**, the C-ring protons showed little acetylation shift while the protons of the A, E, and F rings showed remarkable shifts. Comparison of the acetylation shift values of these protons suggested that the C ring has no hydroxyl

Table 2 Acetylation shifts of **1** and **1a**

	A-ring			C-ring		E-ring			F-ring		
	4-H	5-H	7-H	2'-H	6'-H	10"-H	12"-H	13"-H	17"-H	19"-H	20"-H
1	7.46	6.85	7.03	7.73	8.00	6.30	6.41	7.21	6.47	6.60	8.10
1a	7.64	7.04	7.40	7.70	8.06	6.59	6.67	7.42	7.14	7.32	8.44
Δ	−0.18	−0.19	−0.37	+0.03	−0.06	−0.29	−0.26	−0.21	−0.67	−0.72	−0.34

(ppm) measured in acetone-d_6

group, and that the E ring has one hydroxyl group while another oxygen atom of the E ring formed an ether linkage. The location of the ester linkage was also comfirmed by comparing the ^1H nmr spectrum of **1** with that of **1b**. In the ^1H nmr spectrum of **1b**, the C-2' and -6' proton signals shifted 0.51 and 0.45 ppm, respectively, toward upperfield from the corresponding protons of **1** (Table 3). From the above results, the partial structure (**1'**) was proposed. The remaining part of C_7H_6O moiety was indicated by the ^{13}C nmr spectrum to contain the following seven aliphatic carbon atoms: 1 x $-CH_3$, 1 x $-CH_2-$, 1 x $-CH\langle$, 1 x $\rangle C\langle_O$, 1 x $\rangle C{=}C\langle_O$, 1 x $\rangle C{=}O$ (Table 1). The presence of an α,β-unsaturated carbonyl group in the moiety was supported by the chemical shift value of the carbonyl carbon atom (δ 186.0 ppm). To clarify the nature of the moiety further data were obtained by the following long-range selective ^1H decoupling (LSPD) technique.[6] When the methyl proton signal at δ 1.56 (C-1"-CH_3) was weakly irradiated, the signal at δ 77.4 (C-1") varied in its shape and increased in area (ca. 70 %) as well as the signal at δ 186.0 (C-2") increased in its area (ca. 48 %). Irradiation of the signal at δ 2.38 (C-6"-H) increased the area (ca. 43 %) of the C-1" signal, and irradiation of the signal at δ 2.90 (C-6"-H) also increased the area (ca. 13 %) of the same carbon signal (Fig. 2). These spectral data, as well as the results of sequential decoupling experiment of the ^1H nmr spectrum, indicate the possible structure of the C_7H_6O moiety to be represented by **1"**. The location of the moiety on the C-ring was further supported by comparison of the ^{13}C nmr spectrum of **1** and those of the 4'-substituted 6,3',5'-trioxygenated 2-arylbenzofuran derivatives.[5] From the above results, the structure of mulberrofuran M is possibly represented by **1** or **1'''**.[7] The discrimination between **1** and **1'''** was carried out on the basis of comparison of the ^1H nmr spectra of **1** and **1b**. In the spectrum of **1b**, the C-1"-CH_3 signal shifted 0.19 ppm toward lower field from the corresponding protons of **1** (Table 3). From the above results, the structure **1** is considered to be more favorable than the structure **1'''**.

Table 3 Comparison of the ^1H nmr spectra of **1** and **1b**

	4-H	5-H	7-H	3-H	2'-H	6'-H	1"-CH_3	5"-H	6"-H	
1	7.46	6.85	7.03	7.35	7.73	8.00	1.56	4.41	2.38	2.90
1b	7.44	6.84	7.02	7.24	7.22	7.55	1.75	4.43	2.46	2.99
Δ	+0.02	+0.01	+0.01	+0.11	+0.51	+0.45	-0.19	-0.02	-0.08	-0.09

(ppm) in acetone-d_6

⟶ continue

Table 3

	10"-H	12"-H	13"-H
1	6.30	6.41	7.21
1b	6.34	6.44	7.21
Δ	-0.04	-0.03	±0.00

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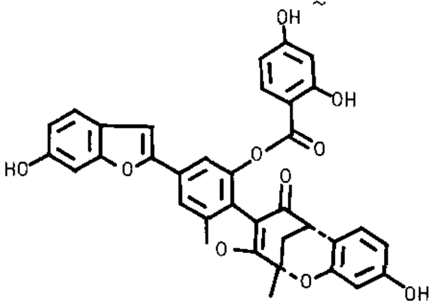
7. Considering hypothetic biogenetic route of mulberrofurans (e.g. mulberrofuran I, see ref. 1), another possible structure **A** may be represented. However, (1) as shown in Fig. 2, the NOE between 1"-CH_3 protons (δ 1.56) and carbonyl carbon (δ 186.0) was observed, and (2) in the ^1H nmr spectrum of **1b** obtained by hydrolysis of **1**, 1"-CH_3 protons showed remarkable downfield shift (-0.19 ppm). From these results, the structure **A** was excluded.



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