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STRUCTURE OF MULBERROFURAN R, A NOVEL 2-ARYLBENZOFURAN DERIVATIVE FROM THE CULTIVATED MULBERRY TREE (MORUS LHOU KOIDZ.)[1]

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Abstract —— A novel 2-arylbenzofuran derivative, mulberrofuran R, was isolated from an ethyl acetate extract of the root bark of the cultivated mulberry tree (Rosō, a cultivated variety of Morus lhou Koidz.). Its structure was shown to be **1**, on the basis of spectral evidence. Mulberrofuran R is regarded biogenetically as a variation of a Diels-Alder type adduct of a chalcone derivative and a dehydroprenyl-2-arylbenzofuran derivative.

Previously we reported the structure determination of a series of phenolic compounds isolated from the root bark of the cultivated mulberry tree (Japanese name "Rosō", a cultivated variety of Morus lhou Koidz.).[2-9] Further extensive fractionation of ethyl acetate extract of the root bark led to the isolation of a new 2-arylbenzofuran derivative, mulberrofuran R. We herein report the characterization of this compound.

The dried root bark of the cultivated mulberry tree was extracted successively with n-hexane, benzene, and ethyl acetate. Mulberrofuran R (1) was isolated from the ethyl acetate extract as described in the Experimental section.

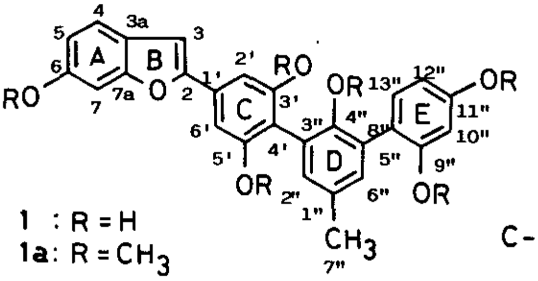
Mulberrofuran R (1), an amorphous powder, gave the EI-MS showing a molecular ion peak at m/z 456, and the ^{13}C nmr spectrum indicating the presence of twenty-seven carbon atoms [one methyl carbon, twenty-six olefinic or aromatic carbons (11xCH, 7xC, 8xC-O)] (Table 1). A work-up of **1** with dimethyl sulfate gave the hexamethyl ether (**1a**) which showed a molecular ion peak at m/z 540 in its EI-MS. These results indicated the composition of mulberrofuran R to be $C_{27}H_{20}O_7$. While mulberrofuran R was negative to the methanolic ferric chloride test, its ir spectrum showed absorption bands due to hydroxyl and benzene ring moieties. The uv spectrum of 1 exhibited maxima at 215, 285 (infl.), 326, and 335 (infl.), and showed no bathochromic shift in the presence of aluminum chloride. These uv characteristics were similar to those of 4'-substituted 6,3',5'-trioxygenated 2-arylbenzofuran derivatives.[4,6,7,10] The ^{1}H nmr spectrum of 1, analysed by a

Table 1. ^{13}C nmr data of **1** and **4**1) in acetone-d_6.

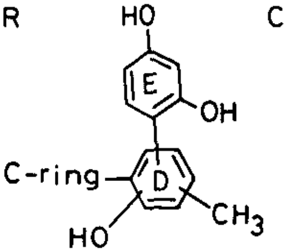
No. of C	1	4	No. of C	1	4	No. of C	1	4	
2	156.0^{*a}	156.5^{*a}	1'	131.4	130.5	5"	128.7^{*b}		
3	101.7	102.8	2',6'	104.2	103.9	6"	132.1^{*c}		
3a	122.1	122.0	3',5'	156.6	156.5	7"	20.6		
4	121.4	121.8	4'	121.6	115.9	8"	118.5^{*d}	(15")2)	122.5
5	112.7	113.1	1"	114.2^{*d}		9"	156.1^{*a}	(16")	157.1^{*a}
6	158.4^{*a}	158.2^{*a}	2"	132.0^{*c}		10"	104.1	(17")	104.6
7	98.0	98.4	3"	127.6^{*b}		11"	155.9^{*a}	(18")	157.1^{*a}
7a	155.0^{*a}	155.3^{*a}	4"	151.0^{*a}		12"	108.0	(19")	107.5
						13"	132.2	(20")	133.9

1): Data from K. Hirakura *et al.* (Ref. 4). 2): The numbers are those of the corresponding carbons of **4**. *a–d: Assignments may be interchanged in each column.

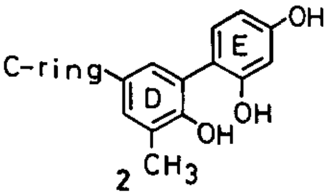
decoupling experiment and by comparison with the spectra of 2-arylbenzofuran derivatives,[4,6,7] showed the signals of the following protons: protons i) in a 2-arylbenzofuran moiety, δ 6.82 (1H, dd, *J* = 2 and 8 Hz), 6.99 (2H, s), 7.00 (1H, br d, *J* = 2), 7.06 (1H, d, *J* = 0.92), 7.42 (1H, d, *J* = 8), ii) in a 2,4-dioxygenated phenyl moiety (E-ring), δ 6.4580 (1H, d, *J* = 2), 6.4599 (1H, dd, *J* = 2 and 9), 7.13 (1H, d, *J* = 9), iii) in a 2,3,5-trisubstituted phenyl moiety (D-ring), δ 7.04 and 7.08 (each 1H, qd, *J* = 0.73 and 2), and iv) in a methyl group, δ 2.34 (3H, br s). A comparative examination of the ^{13}C nmr spectra of **1** and some 2-arylbenzofuran derivatives [mulberrofuran J (**4**)[4] and other similar compounds[6,7]] indicated the presence of a 4'-substituted 6,3',5'-trioxygenated 2-arylbenzofuran and a 2,4-dioxygenated phenyl moiety in the structure (Table 1). In the spectrum of **1**, the chemical shifts of the carbon atoms of the 2-arylbenzofuran moiety and the 2,4-dioxygenated phenyl moiety were similar to those of the relevant carbon atoms of **4**, except the carbon atoms at C-4' and 15" positions which were affected by additional substituents. From the above results, the partial structure (1') was suggested. The substitution pattern in the D-ring was supported by considering the following. Dhami *et al.* reported that the signal of the di*ortho*-substituted methoxyl carbon nucleus appears at δ *ca.* 60 ppm, while that of the mono*ortho*-substituted methoxyl nucleus at δ *ca.* 55 ppm.[11] In the ^{13}C nmr spectrum of hexamethylmulberrofuran R (**1a**, $CDCl_3$) the signals of methoxyl carbons appeared at δ 55.5, 55.8, 55.9, 56.2 (OCH_3 x 2), and 60.2 ppm, suggesting that one of the methoxyl groups is the di*ortho*-substituted methoxyl group and the group is located in the D-ring. From these results, three possible structures



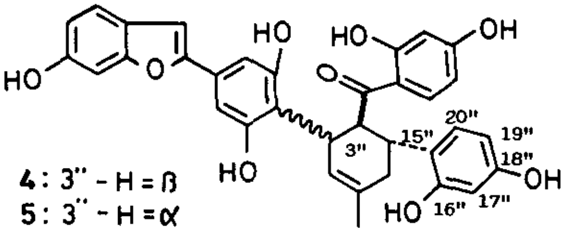
1 : R = H
1a: R = CH_3



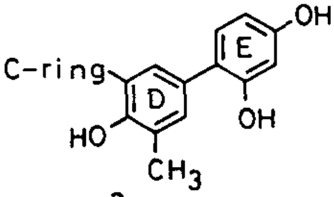
1'



2



4: 3" - H = β
5: 3" - H = α



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(**1**-**3**) can be suggested. Discrimination was carried out among the structures by the following decoupling experiment: irradiation of the methyl proton signal at δ 2.34 changes each of the signals at δ 7.04 (qd, J = 0.73 and 2) and 7.08 (qd, J = 0.73 and 2) into doublet signals (J = 2). Considering the benzylic coupling constants to be identical and the magnitude (J = 0.73 Hz) of the coupling constants, structures **2** and **3** could be excluded.[12]

From the above results, we propose that formula (**1**) is the structure of mulberrofuran R. Biogenetically, mulberrofuran R is regarded as a variation of Diels-Alder type adducts such as mulberrofurans J (**4**) and C (**5**).[13]

EXPERIMENTAL

Abbreviations: s = singlet, d = doublet, dd = double doublet, br = broad, sh = shoulder, infl. inflection. The general experimental procedures followed are described in our previous papers.[2-9] The following insturuments were used: uv spectra; Hitachi 340 UV spectrometer, ir spectra; Hitachi 260-30 IR spectrometer, ms; JEOL JMS 01SG-2, ^{1}H and ^{13}C nmr spectra; JEOL JNM-GX400 FT NMR spectrometer.

Isolation of Mulberrofuran R (1)

Ethyl acetate extract (300 g)[3,6] of the dried root bark of cultivated mulberry trees (26 Kg, Japanese name "Rosō", a cultivated variety of Morus lhou Koidz.), collected in the area around Takasaki, Gunma Prefecture, Japan, in December, 1981, was chromatographed on silica gel (1.6 Kg) with benzene-methanol as an eluent, each fraction being monitored by tlc. The fractions eluted with benzene containing 5% methanol were evaporated to leave a residue (10 g), which were rechromatographed on silica gel (250 g) with n-hexane-acetone as an eluent. The fractions eluted with n-hexane containing 30% acetone were evaporated to leave the residue (5 g). The residue (3 g) was fractionated by preparative tlc (solvent system, benzene:ethyl acetate=1:1, benzene: acetone=1:1, benzene:ethyl acetate=1:2) to give mulberrofuran R (**1**, 4 mg, 2 x 10^{-5}% yield from the root bark).

Mulberrofuran R (1)

Compound **1** was obtained as an amorphous powder. Although only one spot was detected on tlc (benzene:ethyl acetate=1:1, benzene:acetone=1:1, benzene:ethyl acetate=1:2), **1** could not be isolated in a crystalline form. $FeCl_3$ test: negative. EI-MS m/z: 456 (M^+), 242, 110, 107. uv λ_{max}^{EtOH} nm (log ε): 285 (infl. 4.01), 297 (infl. 4.10), 326 (4.83). ir ν_{max}^{KBr} cm^{-1}: 3380, 2920, 1620, 1595 (sh), 1440. ^1H nmr (δ in acetone-d_6, digital resolution: 0.00045 ppm): 2.34 (3H, br s, C-1"-CH_3), 6.4580 (1H, d, J = 2 Hz, C-10"-H), 6.4599 (1H, dd, J = 2 and 9 Hz, C-12"-H), 6.82 (1H, dd, J = 2 and 8 Hz, C-5-H), 6.99 (2H, s, C-2' and -6'-H), 7.00 (1H, br d, J = 2 Hz, C-7-H), 7.04 and 7.08 (each 1H, qd, J = 0.73 and 2 Hz, C-2" and -6"-H), 7.06 (1H, d, J = 0.92 Hz, C-3-H), 7.13 (1H, d, J = 9 Hz, C-13"-H), 7.42 (1H, d, J = 8 Hz, C-4-H). ^{13}C nmr spectra are described in the Table 1.

Mulberrofuran R Hexamethyl Ether (1a)

A mixture of mulberrofuran R (**1**, 1.2 mg), dimethyl sulfate (0.27 ml) and potassium carbonate (5 g) in acetone (30 ml) was refluxed for 1 h and treated as usual. The reaction product was purified by preparative tlc (n-hexane:ethyl ether=1:1) to give a hexamethyl ether (**1a**, 0.1 mg). EI-MS m/z: 540 (M^+), 526, 525, 270. ^1H nmr (δ in acetone-d_6+$CDCl_3$=1:1, 30 °C): 2.32 (3H, br s, C-1"-CH_3), 3.13, 3.79, 3.837, and 3.89 (each 3H, s, OCH_3), 3.842 (6H, s, OCH_3x2), 6.55 (1H, dd, J = 2 and 8 Hz, C-12"-H), 6.61 (1H, d, J = 2 Hz, C-10"-H), 6.89 (1H,dd, J = 2 and 8 Hz, C-5-H), 6.89 and 7.00 (each 1H, br s, C-2" and -6"-H), 7.15 (1H, br d, J = 2 Hz, C-7-H), 7.21 (2H, s, C-2' and -6'-H), 7.18 (1H, d, J = 8 Hz, C-13"-H), 7.22 (1H, br d, J = 1 Hz, C-3-H), 7.48 (1H, d, J = 9 Hz, C-4-H).

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