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STRUCTURE OF MULBERROFURAN C, A NATURAL HYPOTENSIVE DIELS-ALDER ADDUCT FROM ROOT BARKS OF THE CULTIVATED MULBERRY TREE (MORUS BOMBYCIS KOIDZUMI)

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A new 2-arylbenzofuran derivative with a fused dihydrochalcone partial moiety was isolated from root barks of the cultivated mulberry tree (Morus bombycis Koidzumi) and named mulberrofuran C. The structure was shown to be I on the basis of spectral data. Mulberrofuran C (I) is regarded biogenetically as a Diels-Alder adduct of a chalcone derivative and dehydromoracin C (II) or its equivalent. Intravenous injection of I (1 mg/Kg) produced a significant hypotension in rabbit.

In previous communications,[1,2] we reported that two new flavone derivatives having a hypotensive effect, kuwanon G (=moracenin B[3]=albanin F,[4] III) and H (moracenin A[5]=albanin G,[4] IV), were isolated from root barks of the cultivated mulberry tree (a variety of Morus alba L.), and the structures were shown to be III and IV, respectively. Further survey for hypotensive constituents has led to isolation of a novel hypotensive derivative of 2-arylbenzofuran, mulberrofuran C, from mulberry root barks (a variety of Morus bombycis Koidzumi). In this paper, we report the isolation and structure determination of mulberrofuran C.

The dried root bark of the cultivated mulberry tree (a variety of *Morus bombycis* Koidzumi) was extracted successively with hexane, benzene, and ethyl acetate. The ethyl acetate extract was fractionated sequentially by the polyamide column chromatography and by the preparative thin layer chromatography over silica gel to give chalcomoracin (V)[6,7] and a new 2-arylbenzofuran derivative, mulberrofuran C (I)[8] in 2.7 x 10^{-3}% and 2.5 x 10^{-3}% yield, respectively. The compound (I) showed a marked hypotensive effect (1 mg/Kg, i.v.) to rabbit.

Mulberrofuran C (I), amorphous powder,[9] $[\alpha]_D^{18}$ +153° (c=0.33 in methanol), gave the FD-MS spectrum which showed the molecular ion peak at m/e 580, and the ^{13}C nmr spectrum which indicated the presence of thirty-four carbons [nine aliphatic carbons (1xCH_3-, 1x-CH_2-, 3x>CH-, 1x>C=CH, 1x-CH=C-O-), twenty-four aromatic carbons (11xCH, 5xC, 8xC-O) and one carbonyl carbon]. These spectra suggested composition of mulberrofuran C to be $C_{34}H_{28}O_9$. Treatment of I with dimethyl sulfate and potassium carbonate in acetone (reflux, 9 h) effected exhaustive methylation to give its heptamethyl ether (Ia), amorphous powder,[9a] $[\alpha]_D^{14}$ +175° (c=0.12 in methanol), $FeCl_3$ test (negative). The molecular formula of Ia was determined to be $C_{41}H_{42}O_9$ by the high-resolution mass spectrum (m/e 678.2826, M^+), and hence mulberrofuran C (I) could be formulated as $C_{34}H_{28}O_9$. The compound (I) showed the following color reactions: Mg-HCl test (negative), $FeCl_3$ test (brown), and showed the following spectra: ir ν_{max}^{Nujol} cm^{-1}: 3350, 1625, 1620; uv λ_{max}^{MeOH} nm(log ε): 215(4.75), 281(4.43), 320(4.62), 333(4.56); $\lambda_{max}^{MeOH+AlCl_3}$: 220(4.80), 316(4.73), 360(sh 4.03). While the uv spectrum was similar to that of chalcomoracin (V),[6] comparison of the spectrum of I with that of moracin M (VI)[10]

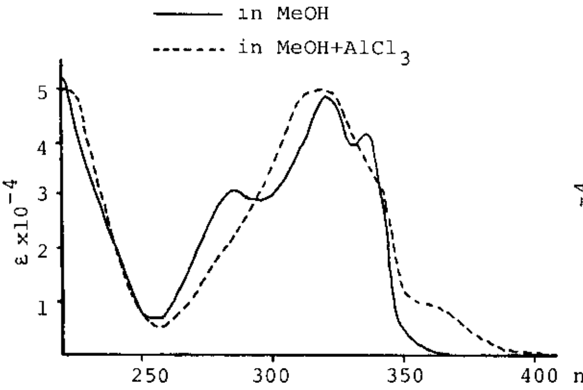

Fig. 1 The uv spectra of mulberrofuran C

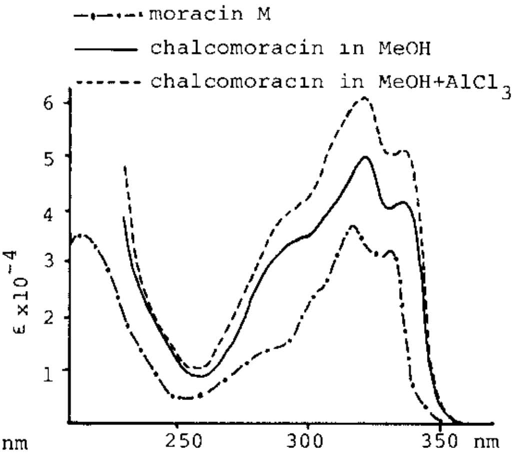

Fig. 2 The uv spectra

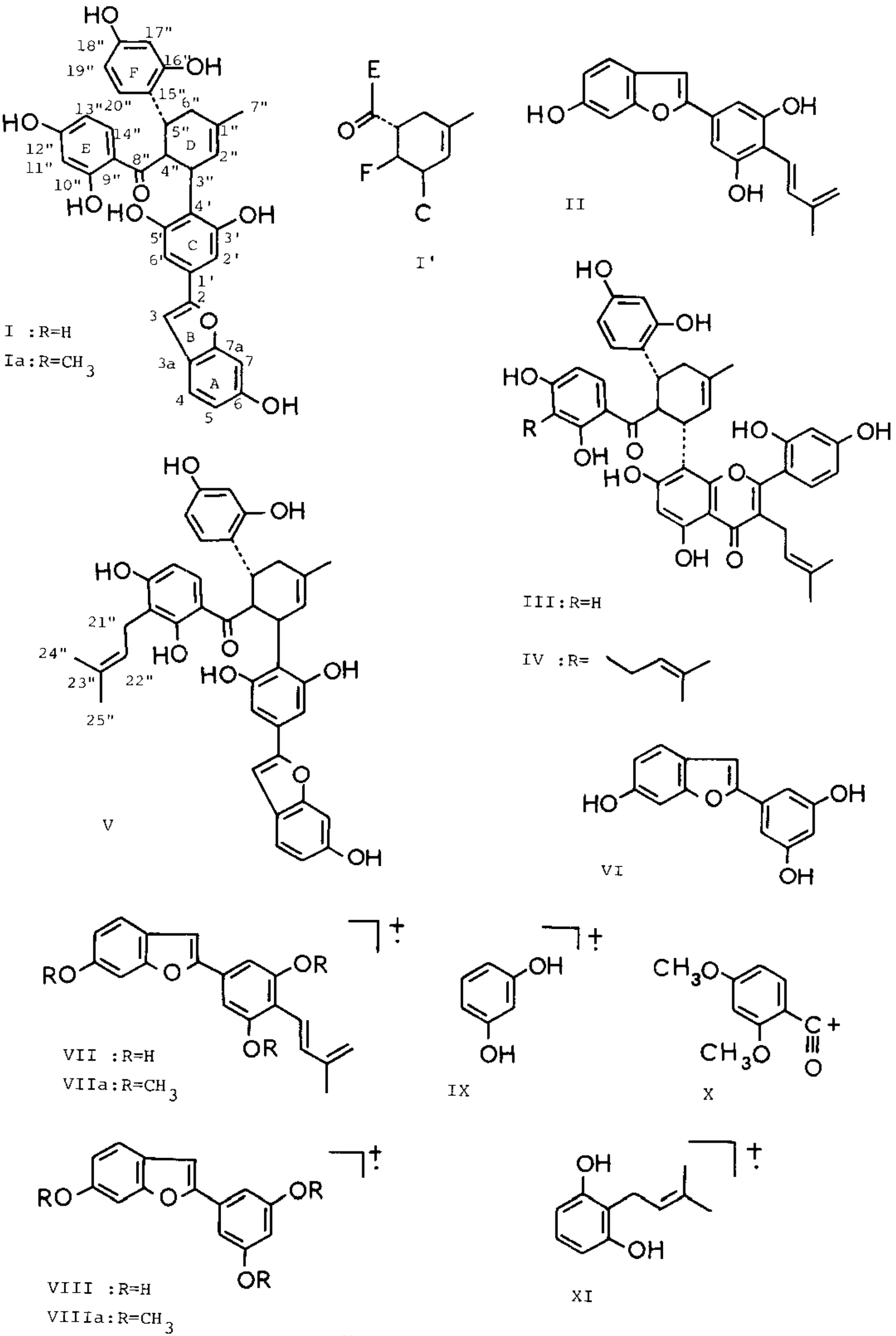
I :R=H
Ia:R=CH3
I'
II
III:R=H
IV :R=
V
VI
VII :R=H
VIIa:R=CH3
IX
X
VIII :R=H
VIIIa:R=CH3
XI
A
B
C
D
E
F
E
F
C

Chart 1

revealed the presence of an extra absorption at ~280 nm in the former (I) which would be ascribed to a conjugated carbonyl group[3] (Figs. 1,2). Moreover, the absorption at 280 nm induced a bathochromic shift in the presence of $AlCl_3$. Sherif et al. reported that when a prenyl group was located ortho to a chelated hydroxyl group no $AlCl_3$ induced shift was observed in the uv spectra.[11] These data, together with the fact that chalcomoracin (V) coexists with mulberrofuran C (I), led us to presume that I would probably be a deprenylchalcomoracin.

The mass spectra of I and Ia showed the following fragments: I, m/e 580(M^+),[12] 562,[12] 470,[12] 452,[12] 320, 308(VII), 242(VIII), 110(IX); Ia,[13] 678(M^+), 513 ($C_{31}H_{29}O_7$), 350(VIIa), 297($C_{18}H_{17}O_4$), 284(VIIIa), 165(X), 151($C_9H_{11}O_2$). On the other hand, the mass spectrum of chalcomoracin (V) showed the fragments at m/e 648(M^+),[12] 630,[12] 470,[12] 388, 308(VII), 242(VIII), 178(XI), 110(IX). These mass spectra also suggested that mulberrofuran C (I) would be a Diels-Alder adduct such as chalcomoracin (V),[6] regarded as a cycloaddition product with the chalcone and the 2-arylbenzofuran derivative. This was substantiated by detailed analysis of the ^1H nmr spectrum (200 MHz, CD_3COCD_3) using sequential decoupling and by comparison of the ^1H nmr spectrum of chalcomoracin (V).[6] The chemical shifts (δ) and coupling constants (Hz) of protons of the relevant cyclohexene ring are shown in Fig. 3, while the remaining protons are summarized as follows: protons in a 2-arylbenzofuran moiety, 6.96(2H,s,$C_{2'}$- and $C_{6'}$-H), 6.78-6.86(3H,m,C_3-,C_5-, and C_7-H), 7.41(1H,d,J=8.5,C_4-H); aromatic protons in a 2,4-dihydroxybenzoyl moiety, 6.27(1H,d,J=2.5,$C_{11''}$-H), 6.40(1H,dd,J=2.5 and 9,$C_{13''}$-H), 8.56(1H,d,J=9,$C_{14''}$-H), 12.61(1H,s,$C_{10''}$-OH); aromatic protons in a 2,4-dihydroxyphenyl moiety, 6.34(1H, dd,J=2.5 and 8.5,$C_{19''}$-H), 6.55(1H,d,J=2.5,$C_{17''}$-H), 7.04(1H,d,J=8.5,$C_{20''}$-H). All these results indicate that the structure of mulberrofuran C is possibly represented by I or I' (except the stereochemistry).

Detailed examination of the ^1H nmr spectrum of I with that of V,[6] revealed that the chemical shifts and coupling constants of protons of the relevant cyclohexene ring of I resembled closely those of V as shown in Figs. 3 and 4. This strongly suggested that mulberrofuran C (I) and chalcomoracin (V) have the same disposition concerning location of the dihydroxyphenyl and dihydroxybenzoyl moieties on the cyclohexene ring and also the same relative configuration. The ^{13}C nmr spectra of I and V were also measured, in which all the carbon atoms were assigned (Table 1) by comparison with the ^{13}C nmr spectra of model compounds, benzofuran derivatives,[8a,14,15] and kuwanon G[1] and H.[2] In the ^{13}C nmr spectrum

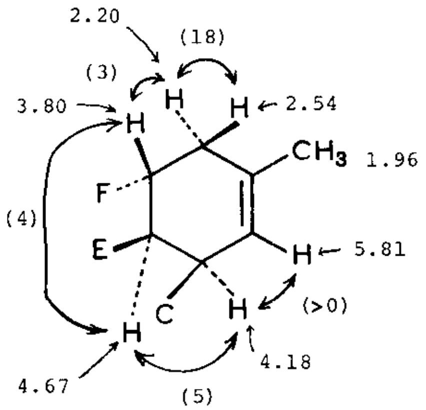

Fig. 3 (I)

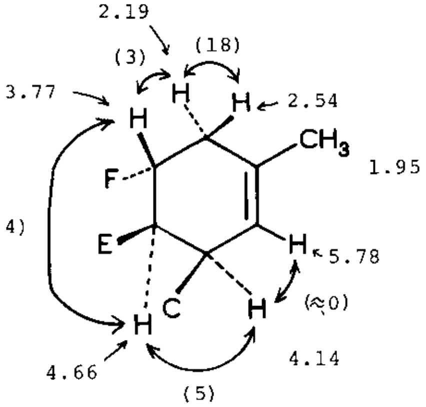

Fig. 4 (V)[6]

of I, the signal of the C-11" appeared at a higher applied magnetic field than that of the C-11" of V, whereas the chemical shift values of all the carbon atoms other than except the C-11" were essentially the same as to those of the relevant carbon atoms of V (Table 1).

Table 1 ^{13}C nmr chemical shifts in DMSO-d_6

	I		V		I		V		I		V
C-2	155.33*	s	155.40*	C-1"	133.37	s	133.21	C-15"	122.27	s	122.04
C-3	102.70	d	102.89	C-2"	128.09	d	127.90	C-16"	157.14*	s	155.21*
C-3a	121.27	s	121.19	C-3"	33.63	d	33.05	C-17"	101.47	d	102.62
C-4	121.54	d	121.56	C-4"	47.07	d	46.88	C-18"	155.90*	s	155.21*
C-5	112.60	d	112.53	C-5"	33.28	d	33.86	C-19"	106.40	d	107.17
C-6	153.94*	s	153.83*	C-6"	34.51	t	33.86	C-20"	133.37	d	131.17
C-7	97.69	d	97.65	C-7"	23.38	q	23.24	C-21"			21.03
C-7a	157.14*	s	155.83*	C-8"	207.45	s	207.68	C-22"			122.04
C-1'	129.24	s	129.17	C-9"	113.41	s	114.99	C-23"			130.36
C-2'	103.01	d	103.16	C-10"	164.61	s	162.42	C-24"			25.38
C-3'	155.33*	s	156.95*	C-11"	102.70	d	114.37	C-25"			17.56
C-4'	112.83	s	112.83	C-12"	164.61	s	162.03				
C-5'	155.33*	s	156.95*	C-13"	108.13	d	106.21				
C-6'	103.01	d	103.16	C-14"	128.47	d	131.17				

* Assignments may be reversed.

This result establishes that mulberrofuran C (I) is deprenylchalcomoracin.

Mulberrofuran C (I) is optically active and the second example of a natural product which is considered to be formed by a Diels-Alder type of enzymatic reaction process of a chalcone derivative and dehydromoracin C (II) or its equivalent.[6] The 2-arylbenzofuran derivatives were isolated as phytoalexins from diseased mulberry tree.[6,16] It is interesting that mulberrofuran C is a hypotensive constituent of Morus root bark.

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9a) Although only one spot was detected on tlc, this compound could not be isolated as crystalline form.

b) Elemental analysis gave a following result: Anal. Calcd. for $C_{34}H_{28}O_9 \cdot H_2O$: C, 68.22; H, 5.06. Found: C, 67.88; H, 5.02.

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