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MULBERROFURAN A, A NEW ISOPRENOID 2-ARYLBENZOFURAN FROM THE ROOT BARK OF THE CULTIVATED MULBERRY TREE (MORUS ALBA L.)

Taro Nomura* and Toshio Fukai
Faculty of Pharmaceutical Science, Toho University,
2-2-1, Miyama, Funabashi-shi, Chiba 274, Japan

Jun Uno and Tadashi Arai
Reserch Institute for Chemobiodynamics, Chiba University,
1-8-1, Inohana-cho, Chiba-shi, Chiba 280, Japan

From the benzene extract of the root bark of the cultivated mulberry tree (a variety of Morus alba L.), a novel isoprene-substituted 2-arylbenzofuran derivative, mulberrofuran A, was isolated whose structure was shown to be I on the basis of spectral data. The antimicrobial activities of I are reported.

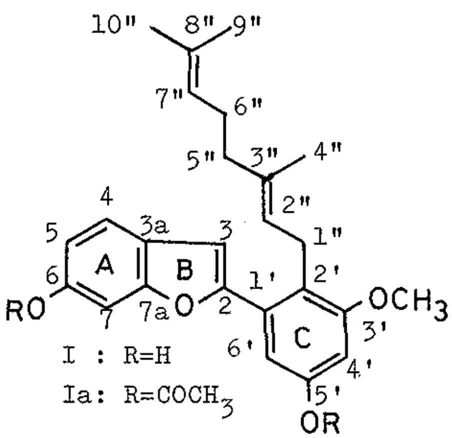
The structures were reported[1] of a series of prenylflavonoids isolated from the root bark of Morus alba L., a plant of Moraceae family. In the course of our studies on the constituents of the root bark, a new isoprene substituted 2-arylbenzofuran derivative, mulberrofuran A (I), was isolated from the benzene extract. In this paper, we report the isolation, structure determination and antimicrobial activities of I.

The dried root bark (5.0 Kg) of the cultivated mulberry tree (a variety of Morus alba L.), collected in Gunma Prefecture, was finely cut and extracted with n-hexane and then with benzene. The benzene extract was dissolved in methanol (500 ml), and allowed to stand until semi-solid mass was separated which was removed by filtration. After evaporation, the residue (54 g) was dissolved in benzene (200 ml), and the solution was allowed to stand for a day at room temperature. Concentration of the benzene solution gave a brown residue (34 g), which was dissolved in ether. The ether solution was extracted successively with 5 % aqueous sodium bicarbonate, 5 % aqueous sodium carbonate, and 5 % aqueous sodium hydroxide solution. The 5 % aqueous sodium hydroxide solution was acidified with dilute hydrochloric acid and extracted with ether. From the ether solution, a mixture of phenolic material (20 g) was obtained, and was chromatographed on silica gel using benzene-methanol (99.7:0.3) as an eluent to give a mixture of fluorescent materials (2.7 g) containing I and other unidentified compounds. From this mixture, I (26 mg) was isolated by preparative TLC (ether:chloroform=1:4, silica gel, and ethyl acetate:benzene=1:1, silica gel[2]).

Mulberrofuran A (I) was obtained as colorless plates, mp 100-103°, M^+ 392.1958 (Calcd. for $C_{25}H_{28}O_4$:392.1986), $C_{25}H_{28}O_4$, exhibiting negative ferric chloride reaction and Gibbs reaction. Mulberrofuran A (I) gave the absorption bands for hydroxyl and benzene ring in the ir spectrum (ν_{max}^{Nujol}: 3400, 1625, 1595 cm^{-1}) and showed the absence of carbonyl function. The uv spectra [λ_{max}^{EtOH} nm(log ε): 216(4.51), 280(sh 4.08), 311(4.37); $\lambda_{max}^{EtOH+NaOH}$:

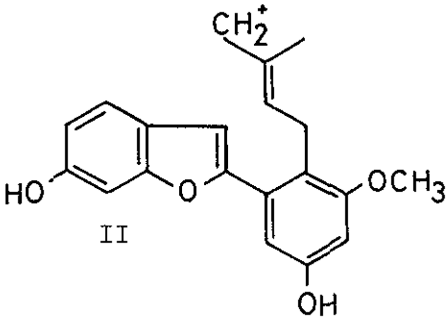
300(sh 4.08), 328(4.37)] resembled those of 2-arylbenzofuran derivatives[3] suggesting that I possesses a 2-arylbenzofuran skeleton. The mass spectrum of I showed the fragments[4] at m/e 323($C_{20}H_{19}O_4$, II)[5], 269($C_{16}H_{13}O_4$, III)[5], 123(C_9H_{15})[5], 69(C_5H_9)[5b]. The significant peak at m/e 269 indicates the loss of m/e 123 (due to side chain) from the molecular ion[1h], suggesting the presence of a geranyl (or neryl) group in the formula (I). The presence of a geranyl (or neryl) group and a methoxyl group was supported by the ^1H nmr spectrum of I in $CDCl_3$: 1.58, 1.64(each 3H, s, $C_{8''}$-CH_3), 1.70 (3H, s, $C_{3''}$-CH_3), 2.01(4H, br s, $C_{5''}$-H×2 and $C_{6''}$-H×2), 3.48(2H, br d, J=6.4Hz, $C_{1''}$-H×2), 3.81(3H, s, OCH_3), 4.95-5.30(4H, m, C_6-OH, $C_{5'}$-OH, $C_{2''}$-H, and $C_{7''}$-H, on addition of D_2O, this signal was altered to 2H, m). On treatment of I with acetic anhydride in pyridine at room temperature for 7 min, a diacetate (Ia) was obtained as a viscous oil which exhibits the following spectroscopic properties, ir[ν_{max}^{Nujol} 1770, 1625(sh), 1615(sh), 1585 cm^{-1}], ^1H nmr (δ in $CDCl_3$)[1.59, 1.65, 1.71(each 3H, s, $C_{3''}$-CH_3 and $C_{8''}$-CH_3), 2.03(4H, br s, $C_{5''}$-H×2 and $C_{6''}$-H×2), 2.32, 2.34(each 3H, s, OAc), 3.53(2H, br d, J=7Hz, $C_{1''}$-H×2), 5.15(2H, m, $C_{2''}$ and $C_{7''}$-H), 6.68(1H, d, J=2.5Hz, $C_{4'}$-H), 6.86(1H, br s, C_3-H), 6.99(1H, dd, J=2 and 8.5Hz, C_5-H), 7.10(1H, d, J=2.5Hz, $C_{6'}$-H), 7.28(1H, C_7-H, overlapping with the solvent)[6], 7.55(1H, d, J=8.5Hz, C_4-H)], ms [m/e 476(M^+), 434, 365, 353, 323, 312, 311, 281, 269, 123, 69]. These results indicate that I possesses two hydroxyl groups on aromatic rings.

The ^1H nmr spectrum of I showed the signals corresponding to six hydrogens in the aromatic region. The broad singlet at δ 6.76 is probably due to C_3-H[3b] and the arrangement of substituents in the

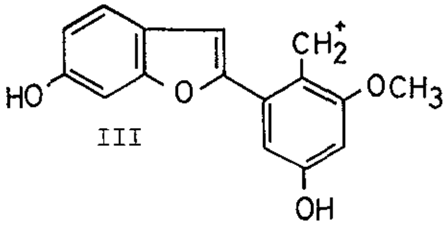


I : R=H

Ia: R=$COCH_3$



II



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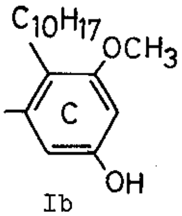
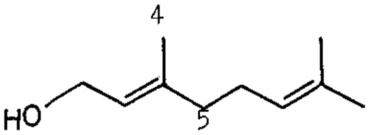
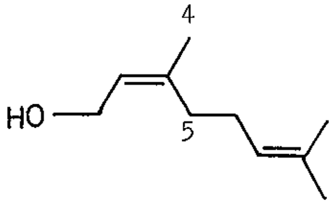


Ib



IV



V

A ring was assumed by the ABX type signals as follows: A double doublet signal at δ 6.78(1H, J=2.1 and 8.4Hz, C_5-H), doublet at δ 6.99(1H, br d, J=2.1Hz, C_7-H), and doublet at δ 7.40(1H, d, J=8.5Hz, C_4-H) indicated that A ring of I was substituted in the 5- or 6-position. The chemical shifts and coupling patterns of these signals were similar to those of 6-oxygenated-benzofuran derivatives.[3c,d,7] The broad doublet signal at δ 6.99 showed that the small long-range coupling were operative between protons at C_3 and C_7 of benzofurans.[8] In the ^{1}H nmr spectrum of Ia, the signals of the protons at C_5 and

C_7 underwent a downfield shift relative to I of *ca*. 0.2-0.3 ppm. This shift is ascribed to loss of the shielding effect of a phenolic hydroxyl group *ortho* to the C_5 and C_7 protons. These results support that I has a hydroxyl group attached at C_6. The ^1H nmr spectrum of I showed the *meta*-coupled doublet (J=2.5Hz) at δ 6.48($C_{4'}$-H) and δ 6.82($C_{6'}$-H) which indicates that the C-ring is unsubstituted at 2' and 4', at 3' and 5' or 4' and 6' positions. The biogenetic analogy to the other 2-arylbenzofuran derivatives isolated from Morus species[3c,f] led us to the assumption that C ring has the 3',5'-dioxygenated pattern. If geranyl (or neryl) side chain is adjacent to a methoxyl group, additional rearrangements occur with loss of C_8H_{15} (111 mass unit).[9] Mulberrofuran A (I) shows the fragment ion at m/e 281(M^+ - C_8H_{15}, 95 %)[4] indicating that a geranyl (or neryl) group is adjacent to a methoxyl group. The presence of a geranyl (or neryl) group at 2' position was supported by the nuclear Overhauser effect (NOE) in I: The irradiation of the methylene signal (δ 3.48, $C_{1''}$-H×2) increased the area (+ 12.9 %) of the olefinic proton (δ 6.76, C_3-H), but the NOE was not observed between the methylene signal and the aromatic protons in the C ring. The NOE was also observed (+ 17.8 %) between the methoxyl signal (δ 3.81) and the proton (δ 6.48, $C_{4'}$-H), but the NOE was not observed between the proton at δ 6.82 ($C_{6'}$-H).[10] From these results, the partial structure (Ib) is possible for ring C. In order to corroborate the structure of I, the ^{13}C nmr spectrum was analysed as follows: δ in $CDCl_3$, 16.3(q, $C_{4''}$), 17.7(q, $C_{9''}$), 25.6(q, $C_{10''}$), 25.8(t, $C_{1''}$), 26.7(t, $C_{6''}$), 39.7(t, $C_{5''}$), 55.8(q, OCH_3), 98.2(d, $C_{4'}$),[11a] 99.5(d, $C_{6'}$),[11a] 105.2(d, C_7), 107.0(d, C_3), 111.9(d, C_5), 121.2(d, $C_{2''}$), 121.4(s, C_{3a}), 122.9(s, $C_{2'}$), 123.8(d, C_4), 124.4(d, $C_{7''}$), 131.2(s, $C_{8''}$), 131.7(s, $C_{1'}$), 135.0(s, $C_{3''}$), 153.5(s, C_6),[11b] 154.3(s, C_2),[11b] 154.4(s, $C_{5'}$),[11b] 155.4(s, C_{7a}),[11b] 159.3(s, $C_{3'}$). Assignments of the carbon atoms in I were performed by off-resonance decoupling technique, and by comparison of the ^{13}C nmr spectra of the model compounds, the benzofuran derivatives[7,12a] and geranylated compounds.[12b] The possibility of geranyl group is supported by the ^{13}C nmr spectrum of I as follows: The signals of C_4 and C_5 of geraniol (IV) appear at δ 15.7 and δ 38.9, respectively, while the signals of C_4 and C_5 of nerol (V) are at δ 22.8 and δ 31.5, respectively.[13] The signals of $C_{4''}$ and $C_{5''}$ of I appeared at δ 16.3 and 39.7, respectively. From the consideration of above results, we tentatively propose the formula (I) for a structure of mulberrofuran A. To the author's knowledge, I is the first example of a geranylated 2-arylbenzofuran derivative found in nature.

Recently 2-arylbenzofuran derivatives were isolated as phytoalexin from diseased mulberry tree (Morus alba L.), and their antifungal activities were reported.[3f] Therefore, the antimicrobial spectrum of I was determined by the agal streak method. Mulberrofuran A (I) was effective against Gram-positive bacteria but inactive against Gram-negative bacteria, and showed weak activity against fungi at a level insufficient for further interest. The minimum inhibitory concentrations (MIC) against a variety of microorganisms are shown in Table.

Table Antimicrobial spectra of mulberrofuran A

Test organism	MIC* (mcg/ml)
Staphylococcus aureus 209p	6.25
Streptococcus faecalis	3.12
Basillus sultilis PCI 219	3.12
Mycobacterium sp. 607	1.56
Escherichia coli F_1	> 100.0
Pseudomonas aeruginosa	> 100.0
Trichophyton mentagrophytes*1	25.0
Fusarium roseum*2	100.0
Gibberella saubinetti*2	100.0
Helminthosporium sesamum*1	100.0

* Agar streak method.
Medium: Heart infusion agar 37°C, 24 or 48 hr for bacteria.
*1 Sabouraud agar 27°C, 48 or 72 hr.
*2 Potato dextrose agar 27°C, 48 or 78 hr.

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