HETEROCYCLES, Vol. 19, No. 10, 1982

MULBERROFURAN D, A NEW ISOPRENOID 2-ARYLBENZOFURAN FROM THE ROOT BARKS OF THE MULBERRY TREE (MORUS AUSTRALIS POIR.)

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Abstract ——— From the benzene extract of the root barks of the mulberry tree (Morus australis Poir.), a novel isoprene-substituted 2-arylbenzofuran derivative, mulberrofuran D, was isolated whose structure was shown to be I on the basis of spectral and chemical data.

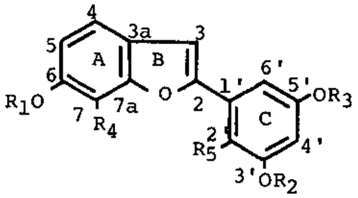
In the previous papers,[1,2] our group reported the isolation and structure determination of a series of isoprene-substituted flavonoids, as well as of isoprene-substituted 2-arylbenzofuran derivatives, obtained from the root barks of the Japanese cultivated mulberry tree. Recently, we also reported natural Diels-Alder adducts isolated from the same root barks[3] and the Chinese crude drug "Sāng-Bái-Pí" (Japanese name Sōhakuhi) imported from the People's Republic of China.[4] In the course of our studies on the constituents of the Morus root barks, we studied the phenolic constituents of the root barks of Morus australis Poir., and a new isoprene-substituted 2-arylbenzofuran derivative, mulberrofuran D (I), was isolated from the benzene extract. In this paper, we report the structure determination of this new compound.

The dried root barks (1.5 Kg) of Morus australis Poir. collected in San-Ti-Men, Taiwan, was extracted with n-hexane and then with benzene. The benzene extract was fractionated sequentially by the silica-gel column chromatography and by preparative thin layer chromatography. This procedure yielded a new benzofuran derivative, mulberrofuran D (I, 77 mg).

Mulberrofuran D (I) was obtained as colorless needles, mp 116-120°C, $C_{29}H_{34}O_4$,[5] m/z 446 (M^+), exhibiting both positive $FeCl_3$ test (brown) and Gibbs test. Mulberrofuran D (I) gave the absorption bands for hydroxyl and benzene ring in the ir spectrum (ν_{max}^{KBr} : 3360, 1630 cm^{-1}), and showed the absence of carbonyl function. The UV spectrum [λ_{max}^{EtOH} nm (log ε) : 250(sh 3.94), 275(sh 3.99), 310(4.27)] resembled those of mulberrofuran A (II)[2a] and other 6,3',5'-trioxygenated 2-arylbenzofuran derivatives[6] suggesting that I possesses a 6,3',5'-trioxygenated 2-arylbenzofuran skeleton.

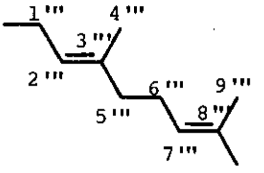
The presence of a geranyl (or neryl) and a prenyl group was supported by the ^1H nmr spectrum of I ($CDCl_3$, 270 MHz) as follows : δ 1.66, 1.76 or 1.78(each 3H,s, C-8'''-CH_3), 1.85(3H,s,C-3'''-CH_3), 2.08(4H,s,C-5'''-Hx2 and C-6'''-Hx2), 3.68(2H,d,J=7,C-1'''-Hx2), 5.05(1H,m,C-7'''-H), 5.42(1H,br t,J=7,C-2'''-H) ; 1.57, 1.76 or 1.78 (each 3H,s,C-3"-CH_3), 3.52(2H,d,J=7,C-1"-Hx2), 5.28(1H,br t,J=7,C-2"-H). Treatment of I with dimethyl sulfate and potassium carbonate in acetone (reflux, 20 min) effected exhaustive methylation to give its trimethyl ether (Ia) as an amorphous powder, m/z 488 (M^+), $FeCl_3$ test (negative).

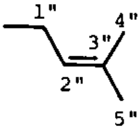
The ^1H nmr spectrum of I showed the signals corresponding to five hydrogens in the aromatic region. A sharp singlet at δ 6.76 was probably due to C-3-H, suggesting that C-7-position of the benzofuran ring was occupied by a substituent, since a small long-range coupling is observed between protons on C-3 and C-7 in C-7-unsubstituted 2-arylbenzofuran.[2a,7] The arrangement of substituents in the A-ring was suggested by an AB system, e.g. two doublets at δ6.84(1H,d,J=8,C-5-H) and at δ7.31(1H,d,J=8,C-4-H) indicating that the A-ring was substituted in the 4- and the 7- position or in the 6- and the 7- position. The chemical shifts of the two doublets were similar to those of 6-oxygenated benzofuran derivatives[8,9] and to those of 6-oxygenated 7-isoprene-substituted benzofuran derivative.[10] These results suggest that mulberrofuran D has a 6-oxygenated 7-isoprene-substituted benzofuran partial structure. The ^1H nmr spectrum (acetone-d_6, 100 MHz) of I showed the meta-coupled doublets (J=2 Hz) at δ6.49(C-4'-H) and at δ6.77(C-6'-H) which indicate that the C-ring is unsubstituted at the 4'- and the 6'- position or at the 3'- and the 5'- position. The chemical shifts and coupling patterns of these signals were similar to those of 3',5'-dioxygenated 2'-isoprene-substituted 2-arylbenzofuran derivative.[8] The biogenetic analogy to the other 2-arylbenzofuran derivatives isolated from Morus species led us to the assumption that the C-ring has the 3',5'-dioxygenated pattern.[2,6,8,10] In order to corroborate the structure of mulberrofuran D, the ^{13}C nmr spectrum was analysed as follows: δ in $CDCl_3$, 16.25(q,C-4'''),



II: $R_1=R_3=R_4=H$
$R_2=CH_3$
R_5=geranyl

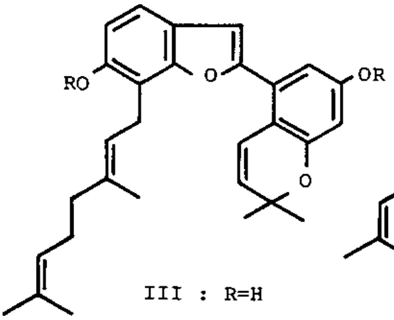
I : $R_1=R_2=R_3=H$

$R_4=$


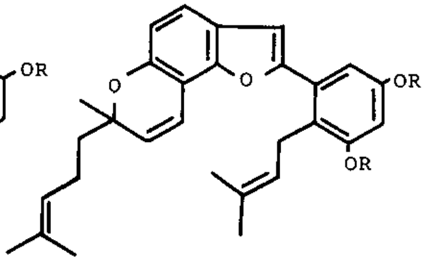
$R_5=$


Ia: $R_1=R_2=R_3=CH_3$
R_4=geranyl
R_5=prenyl

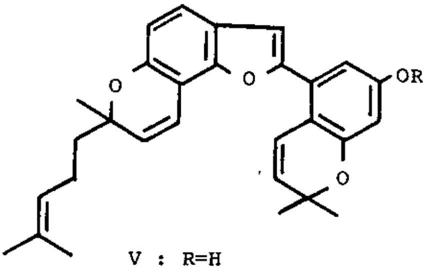
I': $R_1=R_2=R_3=H$
R_4=prenyl
R_5=geranyl



III : R=H
IIIa: $R=COCH_3$



IV : R=H
IVa: $R=COCH_3$



V : R=H
Va: $R=COCH_3$

17.68(q,C-4" or C-9'''), 17.94(q,C-4" or C-9'''), 23.07(t,C-1"), 25.65(q,C-5" and C-10'''), 26.42(t,C-1''' and C-6'''), 39.67(t,C-5'''), 103.86(d,C-3), 105.70(d,C-4'), 108.56(d,C-6'), 110.60(s,C-7), 112.83(d,C-5), 118.07(s,C-2'), 118.69(d,C-4), 121.08(d,C-2'''), 122.27(s,C-3a), 122.85(d,C-2"), 123.85(d,C-7'''), 132.09(s,C-8'''), 132.33(s,C-3"), 134.37(s,C-3'''), 139.03(s,C-1'), 152.20(s,C-7a), 154.02(s,C-3'), 154.52(s,C-6 and C-5'), 156.14(s,C-2). Assignment of the carbon atoms in I were performed by comparison of the ^{13}C nmr spectra of the model compounds, mulberrofuran A[2a] and the benzofuran derivatives[9c,11] and by off-resonance decoupling technique. The possibility of geranyl group was supported by comparing the chemical shifts of the signals of the C-4''' and the C-5''' of I with the shifts of the relevant carbon atoms of geraniol and nerol.[12] From these results, two possible structures, I and I', were assumed.

The discrimination between the structure (I) and (I') was supported by the following results. The mixture of I (16 mg) and DDQ (10 mg) in dry benzene was kept at room temperature for 2 h. The reaction mixture was purified by preparative TLC (solvent system, n-hexane : ethyl acetate = 3 : 1 silica gel) to give compound (III, 2.0 mg), (IV, 2.8 mg), and (V, 3.1 mg). The structures of III, IV, and V were supported by the following spectral data. Compound (III), amorphous powder, m/z 444 (M^+), ^1H nmr ($CDCl_3$, 270 MHz)[δ 1.46(6H,s,C-3"-CH_3x2), 1.58(3H,s, C-8'''-CH_3), 1.67(3H,s,C-8'''-CH_3), 1.87(3H,s,C-3'''-CH_3), 2.10(4H,br s,C-5'''-Hx2 and C-6'''-Hx2), 3.67(2H,d,J=8,C-1'''-Hx2), 5.07(1H,m,C-7'''-H), 5.41(1H,br t,J=8,C-2'''-H), 5.60(1H,d,J=10,C-2"-H), 6.36(1H,d,J=2,C-4'-H), 6.76(1H,s,C-3-H), 6.78(1H,d,J=2,C-6'-H), 6.80(1H,d,J=8,C-5-H), 6.83(1H,d,J=10,C-1"-H), 7.32(1H,d,J=8,C-4-H)]. Compound (IV), amorphous powder, m/z 444 (M^+), ^1H nmr ($CDCl_3$, 270 MHz)[δ 1.44(3H, s,C-3'''-CH_3), 1.58(6H,s,C-3"-CH_3 and C-8'''-CH_3), 1.66(3H,s,C-8'''-CH_3), 1.79(3H,s, C-3"-CH_3), 1.70-1.80(2H,m,C-5'''-Hx2), 2.10-2.20(2H,m,C-6'''-Hx2), 3.51(2H,d,J=6.5, C-1"-Hx2), 5.10(1H,m,C-7'''-H), 5.29(1H,br t,J=6.5,C-2"-H), 5.66(1H,d,J=10,C-2'''-H), 6.42(1H,d,J=2,C-4'-H), 6.68(1H,s,C-3-H), 6.74(1H,d,J=8,C-5-H), 6.78(1H,d,J=2,C-6'-H), 6.83(1H,d,J=10,C-1'''-H), 7.26(1H,d,J=8,C-4-H)]. Compound (V), amorphous powder, m/z 442 (M^+), ^1H nmr ($CDCl_3$, 270 MHz)[δ 1.44(3H,s,C-3'''-CH_3), 1.46(6H,s,C-3"-CH_3x2), 1.58(3H,s,C-8'''-CH_3), 1.66(3H,s,C-8'''-CH_3), 1.73-1.80(2H,m,C-5'''-Hx2), 2.05-2.12(2H,m,C-6'''-Hx2), 5.10(1H,m,C-7'''-H), 5.60(1H,d,J=10,C-2"-H or C-2'''-H), 5.67(1H,d,J=10,C-2"-H or C-2'''-H), 6.36(1H,d,J=2,C-4'-H), 6.73(1H,s,C-3-H), 6.74(1H,d,J=8,C-5-H), 6.78(1H,d,J=2,C-6'-H), 6.81(1H,d,J=10,C-1"-H or C-1'''-H), 6.86(1H, d,J=10,C-1"-H or C-1'''-H), 7.27(1H,d,J=8,C-4-H)].

Treatment of III, IV, and V with acetic anhydride in pyridine yielded the diacetates (IIIa and IVa) and the monoacetate (Va), respectively, and showed the following spectral data. Compound (IIIa), amorphous powder, m/z 528 (M^+), ^{1}H nmr ($CDCl_3$, 270 MHz) [δ 1.47(6H,s,C-3"-CH_3x2), 1.55(3H,s,C-8'''-CH_3), 1.62(3H,s,C-8'''-CH_3), 1.80(3H,s,C-3'''-CH_3), 2.10(4H,br s,C-5'''-Hx2 and C-6'''-Hx2), 2.30, 2.34(each 3H,s,$OCOCH_3$), 3.56(2H,d,J=7,C-1'''-Hx2), 5.05(1H,m,C-7'''-H), 5.30(1H,br t,J=7,C-2'''-H), 5.69(1H,d,J=10,C-2"-H), 6.62(1H,d,J=2,C-4'-H), 6.83(1H,d,J=10,C-1"-H), 6.83(1H,s,C-3-H), 6.95(1H,d,J=8,C-5-H), 6.98(1H,d,J=2,C-6'-H), 7.41(1H,d,J=8,C-4-H)]. Compound (IVa), amorphous powder, m/z 528 (M^+), ^{1}H nmr ($CDCl_3$, 270 MHz) [δ 1.44(3H,s,C-3'''-CH_3), 1.58(3H,s,C-3"-CH_3 or C-8'''-CH_3), 1.66(3H,s,C-3"-CH_3 or C-8'''-CH_3), 1.69(6H,s,C-3"-CH_3 and C-8'''-CH_3), 1.68-1.85(2H,m,C-5'''-Hx2), 2.05-2.22(2H,m,C-6'''-Hx2), 2.30, 2.31(each 3H,s,$OCOCH_3$), 3.47(2H,br d,J=5,C-1"-Hx2), 5.11(2H,m,C-2"-H and C-7'''-H), 5.66(1H,d,J=10,C-2'''-H), 6.74(1H,d.J=8,C-5-H), 6.75(1H,s,C-3-H), 6.82(1H,d,J=10,C-1'''-H), 6.91(1H,d,J=2,C-4'-H), 7.27(1H,d,J=8,C-4-H), 7.37(1H,d,J=2,C-6'-H)]. Compound (Va), amorphous powder, m/z 484 (M^+), ^{1}H nmr ($CDCl_3$, 270 MHz) [δ 1.44(3H,s,C-3'''-CH_3), 1.47(6H,s,C-3"-CH_3x2), 1.55, 1.58(each 3H,s,C-8'''-CH_3), 1.68-1.96(2H,m,C-5'''-Hx2), 2.02-2.30(2H,m,C-6'''-Hx2), 2.31(3H,s,$OCOCH_3$), 5.17(1H,m,C-7'''-H), 5.67(1H,d,J=10,C-2"-H or C-2'''-H), 5.70(1H,d,J=10,C-2"-H or C-2'''-H), 6.59(1H,d,J=2,C-4'-H), 6.74(1H,d,J=8,C-5-H), 6.75(1H,s,C-3-H), 6.85(1H,d,J=10,C-1"-H or C-1'''-H), 6.87(1H,d,J=10,C-1"-H or C-1'''-H), 7.02(1H,d,J=2,C-6'-H), 7.28(1H,d,J=8,C-4-H)].

The substituted position in the geranyl group was supported by comparison of the chemical shifts of the aromatic protons in the diacetate (IVa) and the monoacetate (Va) with those of IV and V, respectively. Although the signals of the C-4'- and C-6'- protons of IVa and Va appeared at lower applied magnetic fields than those of the protons of IV and V, respectively, the chemical shift values of the C-4- and the C-5- proton of IVa and Va were essentially the same as those of the relevant protons of IV and V, respectively. These results support the presence of geranyl group at the C-7 position. This conclusion was further supported by comparing the ^{1}H nmr spectra of III with those of IIIa as follows : the signals of all the aromatic protons of IIIa appeared at lower applied magnetic fields than those of the relevant protons of III. From the above results, we tentatively propose the formula (I) for a structure of mulberrofuran D. To the author's knowledge, mulberrofuran D is the first example of a geranylated and prenylated 2-arylbenzofuran derivative found in nature.[6,13]

ACKNOWLEDGEMENT We are grateful to Prof. S. Sakai, Faculty of Pharmaceutical Sciences, Chiba University, for mass spectrum measurement, and also grateful to Dr. M. Takase and to Dr. S. Terada, Research Laboratory of Zen-Yaku Kogyo Co., Ltd., for ^{1}H nmr spectrum (270 MHz) measurement.

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13 It is not clear that compound (I) is a stress compound (an abnormal secondary product) or a true natural product, because the yield of the compound is extremely low.

Received, 22nd June, 1982