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EIGHT MINOR PHYTOALEXINS FROM DISEASED PAPER MULBERRY1)

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The fungus-inoculated Broussonetia papyrifera shoot cortical tissues produced eight minor antifungal compounds: two flavans named broussinol and demethylbroussin, four 1,3-diphenylpropanes named broussonin C - F, and two chalcones. Isolation and structure elucidation of these compounds are described.

In a series of studies on phytoalexins of the family Moraceae, we have reported isolation and structure determination of many 2-phenylbenzofuran-type and stilbene-type phytoaleixns from diseased mulberry (Morus alba L.)2) and two phytoalexins, broussonin A (1) and B (2), with 1,3-diphenylpropane skeleton from diseased shoot cortical tissues of paper mulberry (Broussonetia papyrifera Vent.).3) Continuing studies on phytoalexins of the inoculated tissues of paper mulberry led to isolation of eight additional antifungal compounds (3 - 10), of which 3 was identified as broussonin C, obtained previously from the wounded xylem tissues of paper mulberry.4) Compounds 4, 5, 6, 7, and 8 were designated as broussinol, demethylbroussin, and broussonin D, E, and F, respectively. These eight compounds were not detected in the uninoculated shoot cortical tissues and active against Bipolaris leersiae at concentration of 10^{-4}M. We report herein the isolation and structure elucidation of these phytoalexins.

Acetone extracts of the shoot cortical tissues of paper mulberry, which had been inoculated with Fusarium solani f. sp. mori, were fractionated by chromatography over silica gel with dichloromethane and dichloromethane-methanol (97:3) as eluents. The major phytoalexins (1 and 2) had been isolated from dichloromethane eluates.3) Fractionation of less-polar fractions of the dichloromethane eluates afforded 7 and 8 in 0.0032 and 0.033% yields (from the dried tissues), while careful separation of the dichloromethane-methanol eluates by chromatography over silica gel and/or Sephadex LH-20 columns afforded 4, 5, 3, and 6, and two chalcones (9 and 10) in 0.014, 0.0014, 0.038, 0.012, 0.0032, and 0.0014% yields, respectively.

Broussinol (4), mp 127-128 °C, $[\alpha]_D$ -21.8° (EtOH), had molecular formula $C_{20}H_{22}O_4$ [m/z 326.1517 (M^+)] and gave its triacetate (4a), amorphous, m/z 452 (M^+). The UV spectrum (EtOH) of 4 [288 nm (ε 4500, sh), 282 (5900), and 224 (18700)] resembled that of broussin4) (11), suggesting the presence of two isolated benzenoid chromophores with the same oxygenation pattern as that in 11, which was substantiated by the ^{1}H NMR spectrum. The spectrum (CD_3COCD_3) indicated that 4 contained three hydroxyl groups with two phenolic ones (δ 4.09, 8.41, and 8.53), one prenyl group [δ 1.69 (3H, s), 1.69 (3H, d, J = 1.0 Hz), 3.31 (2H, br d, J = 6.8), and 5.33 (br t, J = 6.8)], and a pair of three aromatic protons [δ 6.29 (1H, d, J = 2.4, 8-H), 6.38 (1H, dd, J = 8.3 and 2.4, 6-H), and 6.88 (1H, d, J = 8.3, 5-H); 6.82 (1H, d, J = 8.3, 5'-H), 7.06 (1H, dd, J = 8.3 and 2.4, 6'-H), and 7.14 (1H, d, J = 2.4, 2'-H)]. The four remaining protons were assigned by decoupling studies to those of a moiety Ar-CH_2-CH(OH)-CH(O-)-Ar' [δ 4.64 (1H, d, J = 7.3 Hz, 2β-H), 4.03 (1H, ddd, J = 8.8, 7.3, and 5.4, 3α-H), 2.72 (1H, dd, J = 15.6 and 8.8, 4β-H), and 2.91 (1H, dd, J = 15.6 and 5.4, 4α-H)]. The ^{13}C NMR spectrum (CD_3COCD_3) was consistent with this moiety [δ 82.9 (d, 2-C), 68.2 (d, 3-C), and 33.6 (t, 4-C)]. The mass spectrum exhibited two peaks due to retro-Diels-Alder fragmentation at m/z 204 ($C_{13}H_{16}O_2$) and 123 ($C_7H_7O_2$) together with two peaks at m/z 175 ($C_{12}H_{15}O$) and 148 ($C_9H_8O_2$), which was consistent with a catechin structure with one hydroxyl group in the A ring and one prenyl and one hydroxyl groups in the B ring, respectively. These facts, coupled with a negative Gibbs test and similarity in chemical shift of the prenyl carbon atoms between 4 and 3 [δ 29.0 (t) and 29.1 (t)], indicate that the compound is represented best by formula 4. The absolute configuration of C-2 and C-3 was assigned R and S by comparison of the CD spectrum of 4 [[θ] (MeOH), 260 nm (-760), 270 (-2920), 280 (-5840), 285 (-5330), 290 (-4570), and 295 (-630)] with that of natural catechins.[5] Broussinol (4) is the first phytoalexin possessing a catechin structure.

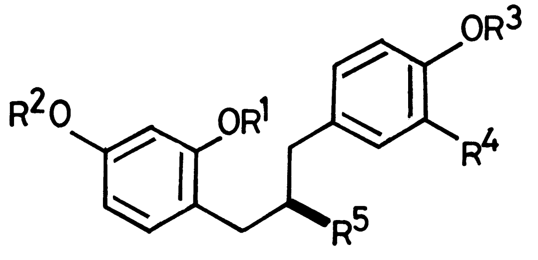
Demethylbroussin (5), mp 194-195 °C, $[\alpha]_D$ -30.5° (MeOH), had molecular formula $C_{15}H_{14}O_3$ [m/z 242.0946 (M^+)] and exhibited the following spectra: UV (MeOH), 290 nm (ε 3680, sh), 281 (5980), and 224 (23500); MS, m/z 242, 123, and 120 (base). The ^{1}H NMR spectrum was closely similar to that of 11[4] except the absence of protons due to the methoxyl group in 11, suggesting 5 to be a demethyl derivative of 11. In fact, the spectra (MS, IR, and ^{1}H NMR) of its dimethyl ether (5a) were identical with those of (±)-7,4'-dimethoxyflavan, derived from (±)-broussin.[4] The absolute configuration of C-2 in 5 was assigned S on the basis of the CD spectrum similar to that of 11. Coxon et al. recently reported isolation of 7,4'-dihydroxyflavan as a phytoalexin of daffodil bulbs (Amaryllidaceae) without any comments on its optical activity.[6]

Broussonin D (6), mp 73-74 °C, $[\alpha]_D$ -0.8° (MeOH), had molecular formula $C_{16}H_{18}O_4$ [m/z 275.1217 (M^+)] and gave its triacetate (6a), oil, m/z 400 (M^+). The UV spectrum (EtOH) of 6 [284 nm (ε 4530, sh), 278 (5130), and 225 (18000)] was very similar to that of 1.[3] The ^{1}H[7] and ^{13}C NMR spectra [δ (CD_3COCD_3) 38.7 (t), 43.2 (t), and 75.0 (d)] revealed the presence of two benzene rings with two hydroxyl and one methoxyl groups as well as a moiety Ar-CH_2-CH(OH)-CH_2-Ar'. The mass spectrum showed three peaks at m/z 137 (base, $C_8H_9O_2$), 167 ($C_9H_{11}O_3$), and 107 (C_7H_7O), which were associated with cleavage at two benzylic positions, indicating that one hydroxyl and one methoxyl groups are located in one of the two benzene rings, and another hydroxyl group in another ring. These results, combined with a positive Gibbs test, indicated that broussonin D is represented by formula 6. The absolute configuration of C-2 was assigned R by comparison of its ORD spectrum [[Φ] (MeOH), 340 nm (-55°), 320 (-110°), and 300 (-220°)] with the reported ones of natural 1,3-diphenylpropan-2-ols.[8)]

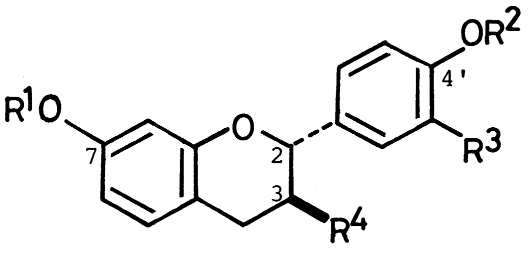
Broussonin E (7), mp 72-74 °C, $[\alpha]_D$ 0°, had molecular formula $C_{17}H_{20}O_4$ [m/z 288.1367 (M^+)] and the following UV spectrum (EtOH): 285 nm (ε 4000, sh), 280 (4500), and 220 (11500, sh). The ^1H NMR spectrum[7)] revealed the presence of two 1,2,4-trisubstituted benzene rings with two hydroxyl and two methoxyl groups as well as a moiety $Ar-CH_2-CH_2-CH_2-Ar'$. The mass spectrum exhibited two characteristic peaks at m/z 137 (base, $C_8H_9O_2$) and 151 ($C_9H_{11}O_2$), indicating that one hydroxyl and one methoxyl groups are located in each of the two rings. Relative disposition of these groups was deduced from comparison of 7 with 1,3-(2,3'-dihydroxy-4,4'-dimethoxydiphenyl)propane, which was prepared by two-step reduction (i, H_2/Pd-C in Et_2O and ii, Zn-Hg in HCl aq) of 2',3-dihydroxy-4,4'-dimethoxychalcone. Hence the compound is represented by formula 7.

Broussonin F (8), oil, $[\alpha]_D$ 0°, had the same molecular formula $C_{17}H_{20}O_4$ [m/z 288.1361 (M^+)] as 7 and displayed almost the same UV and mass spectra as 7. The ^1H NMR spectrum[7)] also indicated the presence of two hydroxyl and two methoxyl groups, suggesting that 8 would be an isomer concerning these groups. The whole structure was determined to be 8 by direct comparison with an authentic sample prepared by two-step reduction (i, H_2/Pd in EtOH, and ii, $LiAlH_4$-$AlCl_3$) of 4,4'-dibenzyloxy-2',3-dimethoxychalcone. A compound assigned the same structure has been reported to be isolated from trunk wood of *Iryanthera coriacea* (Myristicaceae) along with its related 1,3-diphenylpropanes.[9)]

One (9) of the two chalcones, mp 191-192 °C, had molecular formula $C_{15}H_{12}O_4$ [m/z 256.0769 (M^+)]. This compound was presumed to be a chalcone on the basis of the ^1H NMR spectrum[7)] and was indeed identified as 2',4,4'-trihydroxychalcone, isoliquiritigenin with spasmolytic activity[10)] by direct comparison with an authentic sample.[11)]



1 $R^1=R^3=R^4=R^5=H$, $R^2=CH_3$
2 $R^2=R^3=R^4=R^5=H$, $R^1=CH_3$
3 $R^1=R^2=R^3=R^5=H$, $R^4=CH_2CH=C(CH_3)_2$
6 $R^1=R^3=R^4=H$, $R^2=CH_3$, $R^5=OH$
7 $R^1=R^5=H$, $R^2=R^3=CH_3$, $R^4=OH$
8 $R^2=R^3=R^5=H$, $R^1=CH_3$, $R^4=OCH_3$



4 $R^1=R^2=H$, $R^3=CH_2CH=C(CH_3)_2$, $R^4=OH$
5 $R^1=R^2=R^3=R^4=H$
5a $R^1=R^2=CH_3$, $R^3=R^4=H$
11 $R^1=R^3=R^4=H$, $R^2=CH_3$

Another chalcone (**10**), mp 214-215 °C, had molecular formula $C_{16}H_{14}O_4$ [m/z 270.0873 (M^+)] and exhibited the following spectra: UV (EtOH), 350 nm (ε 25600) and 235 (12900); ^{1}H NMR (CD_3COCD_3), δ 3.92 (3H, s), 6.57 (1H, dd, J = 9 and 2.5 Hz), 6.61 (1H, br s), 6.93 (2H, d, J = 8.5), 7.55 (2H, s), 7.59 (2H, d, J = 8.5), 7.61 (1H, d, J = 9), 9.12 and 9.33 (each 1H, s). The compound was identified as 4,4'-dihydroxy-2'-methoxychalcone by direct comparison with an authentic sample.12) While this chalcone (**10**) has not been isolated yet from natural sources as a normal constituent, it was obtained from excreted bile of a rat after administration of 2',4,4'-trimethoxychalcone (Vesidryl, a choleretic drug) by Chabannes *et at.*12) It is to be noted that Carlson and Dolphin have recently isolated this chalcone as a stress metabolite from *Pisum sativum* treated with copper(II) chloride.13)

References

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7) **6**: δ (CD_3COCD_3) 2.64 - 2.86 (4H, m), 3.72 (3H, s), 4.11 (1H, m), 4.95 (1H, d, J = 4 Hz, OH), 6.34 (1H, dd, J = 8.4 and 2.6), 6.39 (1H, d, J = 2.6), 6.75 (1H, d, J = 8.4), 6.89 (1H, d, J = 8.0), and 7.06 (1H, d, J = 8.4). **7**: δ 1.84 (2H, m), 2.54 and 2.58 (each 2H, t, J = 7.5 Hz), 3.70 and 3.79 (each 3H, s), 6.35 (1H, dd, J = 8 and 2.5), 6.44 (1H, d, J = 2.5), 6.62 (1H, dd, J = 2 and 8), 6.71 (1H, d, J = 2), 6.82 and 6.98 (each 1H, d, J = 8), 7.49 and 8.37 (each 1H, br s). **8**: δ 1.80 (2H, m), 2.54 (4H, t, J = 7.5 Hz), 3.76 and 3.81 (each 3H, s), 6.35 (1H, dd, J = 8 and 2), 6.45 (1H, d, J = 2), 6.63 (1H, dd, J = 8 and 1.5), 6.74 (1H, d, J = 8), 6.79 (1H, d, J = 1.5), 6.91 (1H, d, J = 8), 7.38 and 8.30 (each 1H, s). **9**: δ 6.37 (1H, d, J = 2 Hz), 6.45 (1H, dd, J = 9 and 2), 8.09 (1H, d, J = 9), 6.90 (2H, d, J = 8), 7.71 (2H, d, J = 8), 7.76 (2H, s), 9.2 (2H, br), and 13.56 (1H, s).

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