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THE CO-OCCURRENCE OF FLAVAN AND 1,3-DIPHENYLPROPANE DERIVATIVES IN WOUNDED PAPER MULBERRY[1)]

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Two antifungal compounds, designated as broussin and broussonin C , were isolated from wounded xylem tissues of paper mulberry shoots and identified as flavan and 1,3-diphenylpropane derivatives, respectively.

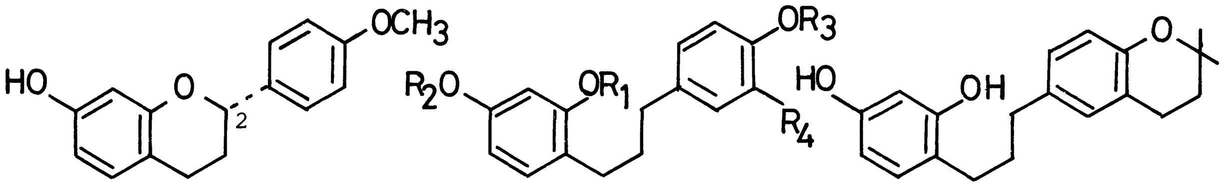
In a previous paper,[2)] we reported the structures of two phytoalexins, broussonins A (**1**) and B (**2**), produced by diseased cortical tissues of paper mulberry shoots (Broussonetia papyrifera Vent.). Continuing studies on phytoalexins of the paper mulberry led to isolation of two antifungal compounds designated as broussin (**3**) and broussonin C (**4**), from wounded xylem tissues of the shoots. We report herein the isolation and structure elucidation of the compounds.

Acetone extracts (5.4 g) of wounded (not inoculated)[3)] xylem tissues of paper mulberry shoots (2.1 kg), which had been chopped and kept at room temperature for 9 days (10 °C for 3 d and 20 °C for 6 d), were partitioned between hexane and methanol. The methanol extracts (4.0 g) were then separated by chromatography over silica gel to give broussin (**3**, 26 mg) and broussonin C (**4**, 370 mg). These compounds were not detected in the acetone extracts of the healthy[3)] xylem tissues and active against Bipolaris leersiae at concentrations of $10^{-4} \sim 10^{-5}$ M, and hence were qualified as phytoalexins.

Broussin (**3**), $C_{16}H_{16}O_3$ (m/e 256.1099, M^+), had mp 120-122 °C and $[\alpha]_D$ -17.4° ($CHCl_3$), and exhibited the following spectra: $[\theta]_{max}$ (CH_3OH) 275 nm (-3800), 280 (-3670), and 289 (sh, -1880); m/e 256 (65%), 134 (100), and 121 (28); λ_{max} (C_2H_5OH) 224 nm (ε 23700), 282 (5400), and 289 (sh, 3700); ν_{max} ($CHCl_3$) 3580, 1615, and 1595 cm^{-1}. The ^1H-NMR spectrum of **3** indicated the presence of seven aromatic protons, one-hydroxy and one-methoxy protons, and five protons [δ ($CDCl_3$) 4.98 (1H, dd, J = 8 and 4), 2.12 and 2.77 (each 2H, m)] due to a moiety $ArCH(\overset{|}{-O})CH_2CH_2Ar'$ (by spin-decoupling studies). All these spectral data indicated that the compound was assigned reasonably a flavan structure **3**, in which the methoxyl group was presumed to be located in the B-ring from the MS base peak (m/e 134) due to a cation formed by retro-Diels-Alder fragmentation. In fact, natural broussin revealed the UV, IR, and NMR spectra identical with those of a known, synthetic racemic sample[4)] formulated as **3**. The relevant absolute configuration (S at C-2) of **3** was deduced from comparison of the CD spectrum with those of natural flavans.[5)] It is noteworthy that it possesses a flavan skeleton unique as a phytoalexin.[6)]

Broussonin C (4), $C_{20}H_{24}O_3$ (m/e 312.1732), oil, displayed the UV spectrum [λ_{max}(C_2H_5OH) 225 nm (sh, ε 15500), 281 (5600), and 287 (sh, 4500)] similar to those[2)] of 1 and 2. The ^{1}H-NMR spectrum indicated the presence of three hydroxyl groups, one prenyl group [δ (CD_3COCD_3) 1.70 (6H, s), 3.30 (2H, d, J = 7 Hz), and 5.34 (1H, t, J = 7), six aromatic protons, and six protons [1.85 (2H, m), 2.54 and 2.55 (each 2H, t, J = 7)] due to three methylene groups flanked by two benzene rings. The MS spectrum showed three prominent peaks at m/e 312 (98%, M^+), 175 (100), and 123 (97), indicative of a 1,3-diphenylpropane structure, in which one benzene ring involves two hydroxyl groups and the other one hydroxyl and one prenyl groups. Broussonin C, when treated with formic acid (room temp, 4 h), gave a cyclization product (5), $C_{20}H_{24}O_3$ (m/e 312.1712), oil, δ ($CDCl_3$) 1.31 (6H, s), 1.77 and 2.77 (each 2H, t, J = 7), proving that the prenyl group is located ortho to the hydroxyl group. Compound 4 formed its trimethyl ether (4a), oil, which on oxidation ($KMnO_4$ in aqueous acetone) followed by methylation, gave two esters, which were identified as methyl 2,4-dimethoxybenzoate and dimethyl 4-methoxyisophthalate, respectively, by direct comparison with authentic specimens. These results lead to assignment of formula 4 to broussonin C.[7)]

The co-occurrence of the optically active flavan (3) and the 1,3-diphenylpropane (4) in the same tissues suggests close biosynthetic relationship between the two types of compounds and indicates that 1,3-diphenylpropanes can be regarded as flavonoids from the biosynthetic viewpoint.



1: $R_1=R_3=R_4=H$, $R_2=CH_3$
3 2: $R_2=R_3=R_4=H$, $R_1=CH_3$ 5
4: $R_1=R_2=R_3=H$, $R_4=CH_2CH=C(CH_3)_2$
4a: $R_1=R_2=R_3=CH_3$, $R_4=CH_2CH=C(CH_3)_2$

References and Notes

1) Part 6 in the series "Studies on Phytoalexins of the Moraceae." For Part 5, see ref 2.
2) M. Takasugi, M. Anetai, T. Masamune, A. Shirata, and K. Takahashi, Chem. Lett., 1980, 339.
3) A. Shirata and K. Takahashi, Ann. Phytopath. Soc. Jpn., 45, 156 (1979).
4) K. Freudenberg, J. H. Stocker, and J. Porter, Chem. Ber., 90, 957 (1957).
5) G. Cardillo, L. Merlini, G. Nasini, and P. Salvadori, J. Chem. Soc. (C), 1971, 3967.
6) After completion of our work three flavan phytoalexins from daffodil bulbs (Amaryllidaceae) were reported; D. T. Coxon, T. M. O'Neill, J. W. Mansfield, and A. E. A. Porter, Phytochemistry, 19, 889 (1980).
7) Other 1,3-diphenylpropanes have been isolated only from the family Myristicaceae, together with flavans and dihydrochalcone; R. B. Filho, M. S. da Silva, and O. R. Gottlieb, Phytochemistry, 19, 1195 (1980) and refs cited therein.

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