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STILBENE PHYTOALEXINS FROM DISEASED MULBERRY[1]

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Two stilbene phytoalexins were isolated from fungus-infected xylem tissue of mulberry shoots and characterized as *trans*-2,3',4,5'-tetrahydroxystilbene (**1**, oxyresveratrol) and its 4'-(3-methyl-2-butenyl) derivative **2**.

In previous papers[2,3], we reported isolation and structures of four novel 2-phenylbenzofuran phytoalexins, moracin A, B, C, and D, from the **cortex** and **phloem** tissues of decorticated one-year-old mulberry shoots (*Morus alba* Linné) infected with *Fusarium solani* f. sp. *mori*. Two stilbene-type antifungal compounds (**1** and **2**) were isolated as major metabolites from acetone extracts of the fungus-infected **xylem** tissues of the mulberry shoots, guided by assay against *Cochliobolus miyabeanus*. We now wish to report the structure and antifungal activity of these compounds.

Fractionation of the acetone extracts by silica gel column chromatography (CH_2Cl_2-MeOH) and crystallization led to isolation of **1** and **2** in 0.018% and 0.0021% yield (from the dried tissues), respectively. These compounds (**1** and **2**) can be classified as phytoalexins, because those are not detected in the comparable extracts of healthy tissues.

The major compound (**1**), $C_{14}H_{12}O_4$, mp 199-200 °C; m/e 244 (M^+, base); λ_{max}^{EtOH} 218 nm (ε, 25000), 235 (sh, 17000), 290 (sh, 18300), 301 (19700), and 330 (27100), gave the tetramethyl ether (**1a**), $C_{18}H_{20}O_4$, (m/e 300.137), mp 83-84 °C, and the tetra-acetate (**1b**), mp 141-142 °C. The NMR spectrum (CD_3COCD_3) of **1a** showed signals for *trans* olefinic protons [δ 7.04 and 7.40 (each 1H, d, J = 16)], six aromatic protons [6.39, (1H, t, J = 2), 6.46-6.64, (2H, m), 6.70 (2H, d, J = 2), and 7.56 (1H, d, J = 9)], and four-methoxyl protons [3.79 (6H, s), 3.81 (3H, s), and 3.87 (3H, s)], suggesting the compound to be *trans*-2,3',4,5'-tetramethoxystilbene; namely, the tetramethyl ether of oxyresveratrol.[4,5] Permanaganate oxidation of **1a** giving 2,4- and 3,5-dimethoxybenzoic acids, confirmed the assignment of structure **1** to the major compound. Oxyresveratrol (**1**) was first isolated from rhizomes of *Veratrum grandiflorum*[4] and recognized later as an antifungal constituent of the durable wood of Osage orange.[5] Several *Morus* species have been reported to contain considerable amounts of oxyresveratrol in their heartwoods.[6] We also examined components of six-year-old healthy mulberry branches. It is to be noted that a large amount (1.7% of the dried tissues) of oxyresveratrol (**1**) was indeed isolated only from the heartwood (inside three ∿ four annual layers) but none of the compound detected from the sapwood (outside three ∿ two annual layers).

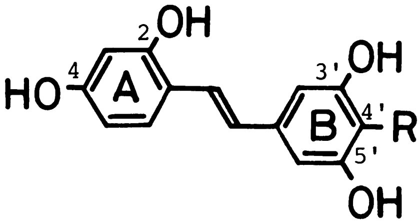
The second compound (2), $C_{19}H_{20}O_4$, mp 196-197 °C, m/e 312 (M^+, base) and 257 ($M^+-C_4H_7$, 91%), has a UV spectrum similar to that of 1; λ_{max}^{EtOH} 220 nm (ε, 28100), 240 (sh, 19700), 294 (sh, 21300), 303 (23100), and 330 (33100), and gave the tetramethyl ether 2a, $C_{23}H_{28}O_4$ (m/e 368.200), mp 90-91 °C. The NMR spectrum of 2a showed signals for four methoxyl groups [δ 3.83 (3H, s), 3.87 (6H, s), 3.89 (3H, s)] and for a <u>trans</u>-double bond [7.03 (1H, d, J = 17 and 7.39 (1H, d, J = 17)]. Signals at δ 7.58 (1H, d, J = 9) and 6.47-6.67 (2H, m), observed in the spectrum of 1a, indicated the presence of a 1,2,4-trisubstituted benzene (A ring) in 2a. The presence of a 3-methyl-2-butenyl (prenyl) group was demonstrated by signals at δ 1.63 (3H, s), 1.75 (3H, s), 3.31 (2H, br d, J = 7), and 5.16 (1H, br t, J = 7). A two-proton singlet at δ 6.80 due to aromatic protons is explainable by symmetrical disposition of two methoxyl and the prenyl group on another benzene ring (B ring) of 2a. The characteristic fragmentation peak at m/e 257 ($M^+-C_4H_7$, 91%) in the MS spectrum in 2 accounts for the prenyl group flanked by the two hydroxyl groups.[7] The structure of the second compound is therefore represented correctly by 2. It is of interest that these stilbene compounds possess structures closely related to those of 2-phenylbenzofuran phytoalexins.[2,3] Antifungal activity of oxyresveratrol (1) and 4'-prenyloxyresveratrol (2) against pathogenic and nonpathogenic fungi is given in Table 1.

Table 1 Antifungal activity of 1 and 2

Fungus	1 a)	2 a)
Fusarium roseum	18-35	28
F. lateritium f. sp. *mori*	140	56-112
F. solani f. sp. *mori*	285-560	>224
Diaporthe nomurai	70-140	112-224
Stigmina mori	140-280	28-56
Rosellinia necatrix	70-140	14-28
Cochliobolus miyabeanus b)	140	28-56

a) Minimum concentration (μg/ml) required for complete inhibition of fungal growth.

b) A non-pathogen against the mulberry.



1. R=H

2. R=$CH_2-CH=C\begin{matrix}CH_3\\CH_3\end{matrix}$

REFERENCES and NOTES

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