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STRUCTURE OF MORACIN A AND B, NEW PHYTOALEXINS FROM DISEASED MULBERRY

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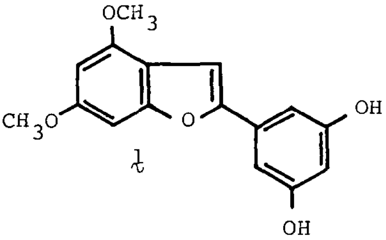
Phytoalexins are antimicrobial compounds produced by plants in response to microbial infection and their production is believed to be an important disease resistance mechanism in higher plants.[1] We report herein the isolation and structure elucidation of two metabolites, designated as moracin A and B, from diseased mulberry (*Morus alba* Linné), which, as far as we know, are the first phytoalexins found in the family *Moraceae*.

Acetone extracts of cortex and phloem tissues of mulberry shoots, infected with *Fusarium solani* f. sp. *mori*, were fractionated sequentially by column and preparative thin-layer chromatography over silica gel, resulting in the isolation of two antifungal metabolites (Table 1), moracin A and B (**1** and **2**) in 0.012 and 0.032% yields, respectively. These compounds could not be detected in comparable extracts of the corresponding uninoculated tissues.

Moracin A (**1**), mp 83-85°C, had a molecular formula of $C_{16}H_{14}O_5$,[2] formed its diacetate (**1a**), mp 126-127°C, and showed the following spectra:[2] UV (C_2H_5OH), λ_{max} 326 nm (ε 20,900), 313 (29,400), 304 (24,800), and 217 (26,700); PMR (CD_3COCD_3), δ 3.88 and 3.96 (each 3H, s, $2OC\underline{H}_3$), 8.62 (2H, br s, $2O\underline{H}$), 6.44 (1H, t, J = 2 Hz), 6.45 (1H, d, J = 2), 6.82 (1H, do d, J = 2 and 0.8), 6.94 (2H, d, J = 2), and 7.13 (1H, d, J = 0.8[3]); CMR (CD_3SOCD_3), δ 55.7 (q), 55.8 (q), 88.5 (d), 94.5 (d), 98.9 (d), 102.3 (d, intense[4]), 102.7 (d), 112.3 (s), 131.4 (s), 152.9 (s), 153.1 (s), 155.6 (s), 158.6 (s, intense), and 158.9 (s). The spectra suggested that **1** would possess a 2-phenylbenzofuran skeleton.[3] Ozonolysis of **1a** in ethyl acetate at -70°C afforded aldehyde ester (**3**), mp 152-156°C, in 23% yield; MS, m/e 402 (M^+), 221, and 181; PMR ($CDCl_3$), δ 10.21, which on saponification (5% KOH in aq CH_3OH, room temp, 2.5 h) gave 2-hydroxy-4,6-dimethoxybenzaldehyde and 3,5-dihydroxybenzoic acid in 71 and 95% yields, respectively. The result indicates that moracin A is represented correctly by structure **1**.

Moracin B (**2**), mp 184-185°C, had the same molecular formula $C_{16}H_{14}O_5$ as **1** and exhibited the following spectra: UV (C_2H_5OH), λ_{max} 337 nm (ε 24,900), 325 (27,300), 294 (13,900), 285 (12,400), and 218 (29,300); PMR (CD_3COCD_3), δ 3.83

and 3.94 (each 3H, s, $2OCH_3$), 7.33 and 8.50 (each 1H, s, 2OH), 6.43 (1H, t, J = 2 Hz), 6.97 (2H, m), 7.04 (1H, s), 7.08 (1H, d, J = 0.8[3]), and 7.21 (1H, br s); CMR (CD_3SOCD_3), δ 55.1 (q), 56.1 (q), 95.7 (d), 100.7 (d), 101.4 (d), 102.1 (d), 103.8 (d), 105.4 (d), 121.1 (s), 132.0 (s), 143.9 (s), 147.1 (s), 148.5 (s) 154.1 (s), 158.9 (s), and 160.9 (s). Compound **2** formed the following derivatives: diacetate (**2a**), mp 82-84°C; dimethyl ether (**2b**), mp 104-105°C; diethyl ether (**2c**) [with $(C_2H_5)_2SO_4$ and K_2CO_3 in acetone], mp 88-89°C. Ozonolysis of **2c** afforded aldehyde ester (**4**), mp 128-131°C, in 15% yield; MS, m/e 374 (M^+), 196, and 179; PMR ($CDCl_3$), δ 10.08, which was hydrolyzed with base to give phenol and acid in 73 and 65% yields, respectively. These compounds were identified as 3-ethoxy-6-hydroxy-4-methoxybenzaldehyde[5] and 3-ethoxy-5-methoxybenzoic acid,[6] respectively, by direct comparison with authentic samples. Hence moracin B must be represented by structure **2**.



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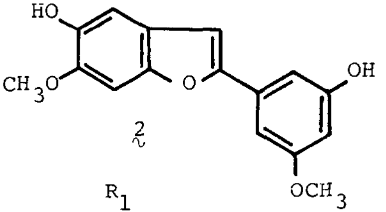

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Table 1 Antifungal activity of **1** and **2**

Fungus	**1**[a]	**2**[a]
Fusarium roseum	6-12	12-25
F. lateritium f. sp. mori	25-49	49
Diaporthe nomurai	6-12	12
Stigmina mori	6-12	12-25
Cochliobolus miyabeanus	12-25	49

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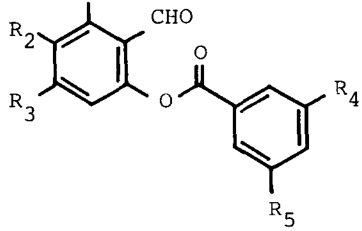
a) Minimal concn (μg/ml) causing 100% inhibition of spore germination.



3 $R_1=R_3=OCH_3$, $R_4=R_5=OCOCH_3$, $R_2=H$

4 $R_3=R_5=OCH_3$, $R_2=R_4=OCH_2CH_3$, $R_1=H$

References and Footnotes

1) J. Friend and D. R. Threlfall, Ed., "Biochemical Aspects of Plant-Parasite Relationships," Academic Press, London (1976), Chap. 11.

2) All the compounds gave elementary analyses and MS, UV, IR, and PMR spectra in good accord with the assigned structures. The abbreviations "s, d, t, q, m, br, and do" in the NMR spectra denote "singlet, doublet, triplet, quartet, multiplet, broad, and double," respectively.

3) Small long-range couplings have been observed between protons at C_3 and C_7 of benzofurans; cf., J. A. Elvidge and R. G. Foster, J. Chem. Soc., **1963**, 590.

4) Intense signals indicate the presence of two equivalent carbon atoms.

5) F. S. H. Head and A. Robertson, J. Chem. Soc., **1932**, 2434.

6) A. von Wacek, Ber., **63**, 2984 (1930).