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STRUCTURES OF MORACINS E, F, G, AND H, NEW PHYTOALEXINS FROM DISEASED MULBERRY[1]

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Abstract: The structures and antifungal activity of four new mulberry phytoalexins, designated as moracins E, F, G, and H, are described.

In previous papers[2,3] we reported the structures of four novel 2-phenylbenzofuran phytoalexins, moracins A, B, C, and D (1 ∿ 4), which were isolated from acetone extracts of cortex and phloem tissues of decorticated mulberry shoots (Morus alba Linné) infected with Fusarium solani f. sp. mori. Further fractionation of the extracts on silica gel, polyamide, and/or Sephadex LH-20 columns led to isolation of four new antifungal compounds, designated as moracins E, F, G, and H (5 ∿ 8), in 0.02, 0.0002, 0.001, and 0.009% yields from the dried tissues, respectively, which were not detected in the corresponding extracts of healthy tissues. We report herein the structure elucidation of these compounds, based on comparison with the spectral data of the known moracins, and their antifungal activity.

Moracin E (5), $C_{19}H_{16}O_4$,[4] mp 184-185 °C, m/e 308 (M^+) and 293 (M - CH_3, base), gave its diacetate (5a), mp 87-88 °C, and its dihydro derivative (5b), mp 216-217 °C, on acetylation and hydrogenation (Pd-$CaCO_3$), respectively. The UV spectrum[4] of 5b [λ_{max} 326 nm (sh) (ε 28000), 314 (36000), and 217 (35400)] resembled that of moracin C[3] (3), suggesting the presence of a 2-substituted 6-hydroxybenzofuran moiety in 5, which was supported by the ^{1}H-NMR spectrum:[4] 5, δ 7.48 (1H, d, J = 8, 4-H), 6.86 (1H, do d, J = 8 and 2, 5-H), 7.04 (1H, br d, J = 2, 7-H), 6.88 (1H, d, J = 0.8, 3-H),[2,5] and 8.64 (2H, br s, 6- and 7'-OH). The ^{1}H-NMR spectra of 5, 5a and 5b also exhibited signals ascribed to a chromene moiety with two meta-oriented aromatic hydrogen atoms and a hydroxyl group. Disposition of these on the chromene-benzene ring was deduced from long-range couplings[3,6] between the chromene proton at C-4' and one of the relevant aromatic protons at C-8': 5, δ 6.85 (1H, do d, J = 10 and 0.8,[3,6] 4'-H), 5.68 (1H, d, J = 10, 3'-H), 1.44 (6H, s, CH_3 at C-2'), 6.39 (1H, do d, J = 2.4 and 0.8,[3,6] 8'-H), 6.83 (1H, d, J = 2.4, 6'-H), and 8.64 (2H, br s, 7'- and 6-OH); 5a, δ 6.68 (1H, do d, J = 2 and 0.5, 8'-H), 7.09 (1H, d, J = 2, 6'-H), and

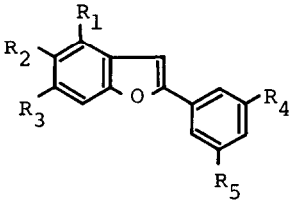
2.28 and 2.29 (total 6H, each s, 7'- and 6-OCOC$\underline{H}_3$); 5b, δ 2.89 and 1.82 (each 2H, t, J = 7, 4'- and 3'-$\underline{H}$), 6.32 and 6.94 (each 1H, d, J = 2.4, 8'- and 6'-$\underline{H}$), and 8.41 (2H, br s, 7'- and 6-O$\underline{H}$). Combination of these two moieties led to assignment of structure 5 to moracin E. Naturally, no absorptions due to carbonyl functions were observed in the IR spectrum.[4]

Moracin F (6), $C_{16}H_{14}O_5$, mp 188-189 °C, m/e 286 (M^+, base), displayed an UV spectrum [λ_{max} 334 nm (ε 26000), 321 (30700), 291 (13900), 282 (12100), and 217 (32500)] closely similar to that of moracin B[2] (2) and gave its dimethyl ether (6a), mp 101-103 °C, which was identified as moracin B dimethyl ether[2] in every respect. Moracin F was then assigned formula 6 on the basis of the whole ^{1}H-NMR spectrum [δ 3.83 and 3.88 (each 3H, s, OC$\underline{H}_3$ at C-5 and C-6), 6.36 (1H, t, J = 2, 4'-$\underline{H}$), 6.85 (2H, d, J = 2, 2'- and 6'-$\underline{H}$), 7.01 (1H, d, J = 0.8, 3-$\underline{H}$), 7.09 (1H, s, 4-$\underline{H}$), 7.16 (1H, br s, 7-$\underline{H}$), and 8.37 (2H, s, 3'- and 5'-O$\underline{H}$)] and specially, the signal patterns due to the two protons at C-2' and C-6' (equivalent) and the two methoxy protons (non-equivalent).

Moracin G (7), $C_{19}H_{16}O_4$, mp 198-199 °C, m/e 308 (M^+) and 293 (M - CH_3, base) showed almost the same type of UV spectrum as moracin C[3] (3), suggesting the existence of a 2-phenylbenzofuran skeleton. The ^{1}H-NMR spectrum, compared with that of moracin F (6), indicated that the phenyl group in question was substituted by two hydroxyl groups at C-3' and C-5': 7, δ 6.43 (1H, t, J = 2, 4'-$\underline{H}$), 6.94 (2H, d, J = 2, 2'- and 6'-$\underline{H}$), and 8.57 (2H, br s, 3'- and 5'-O$\underline{H}$). The spectrum also revealed that only three protons were located on the benzofuran ring and that the ring was substituted at C-7, as demonstrated by appearance of the C-3 proton as a sharp singlet:[5] δ 7.11 (1H, s, 3-H), 7.38 and 6.95 (each 1H, d, J = 8, 4- and 5-$\underline{H}$). The remaining eight protons were then attributed to those of a dihydrooxepin moiety, considering the chemical shifts and multiplicities: δ 4.47 (2H, br s, 2"-$\underline{H}$), 1.58 (3H, s, C$\underline{H}_3$ at C-3"), 5.74 (1H, m, 4"-$\underline{H}$), and 3.74 (2H, m, 5"-$\underline{H}$).[7] All these spectral data, coupled with the ^{13}C-NMR spectrum[4] [δ 19.9 (q), 22.4 (t), 73.1 (t), 101.5 (d), 102.4 (d, intense), 102.8 (d), 116.5 (d), 118.0 (s), 118.2 (d), 119.2 (d), 124.3 (s), 131.0 (s), 134.5 (s), 151.1 (s), 155.1 (s), 155.2 (s), and 158.4 (s, intense)], indicate that moracin G is represented favorably by formula 7.

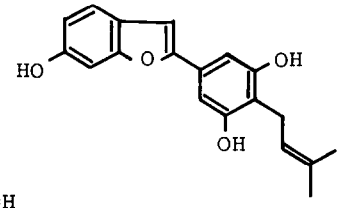
Moracin H (8), $C_{20}H_{18}O_5$, mp 191-192 °C, m/e 338 (M^+) and 323 (M - CH_3, base), resembled moracin A[2] (1) rather than moracin B[2] (2) in the UV spectrum: λ_{max} 329 nm (ε 22000), 315 (33400), 306 (31000), and 217 (33000). The compound (8) exhibited essentially the same ^{1}H-NMR spectrum as moracin G (7) except signals due to methoxy protons [δ 3.92 (3H, s)], indicative of the presence of a 3',5'-dihydroxyphenyl group [δ 6.41 (1H, t, J = 2, 4'-$\underline{H}$), 6.93 (2H, d, J = 2, 2'- and 6'-$\underline{H}$), and 8.51 (2H, br s, 3'- and 5'-O$\underline{H}$)] and a dihydrooxepin moiety [δ 4.48 (2H, s, 2"-$\underline{H}$), 1.60 (3H, s, C$\underline{H}_3$ at C-3"), 5.75 (1H, m, 4"-$\underline{H}$), and 3.67 (2H, m, 5"-$\underline{H}$)]. The remaining two protons were observed as sharp singlets[5] at

δ 7.10 and 6.51, which were ascribed to protons at C-3 and C-5 (or C-4) on the benzofuran ring, respectively. Disposition of the methoxyl group at C-4 rather than at C-5 was based on the UV spectrum, the chemical shift (δ 6.51) of one of two protons on the benzofuran ring, and the ^{13}C-NMR spectrum [δ 20.2 (q), 22.3 (t), 55.6 (q), 73.1 (t), 99.0 (d), 99.2 (d), 102.6 (d, intense), 103.0 (d), 110.5 (s), 114.5 (s), 120.4 (d), 131.5 (s), 134.6 (s), 150.9 (s), 152.2 (s), 154.1 (s), 156.4 (s), and 158.8 (s, intense)].[8] Acetylation of 8 afforded its diacetate (8a), mp 140-141 °C, in which four protons at C-3, C-2', C-6', and C-4' were shifted to lower field, whereas other protons remained unchanged in the ^{1}H-NMR spectrum: δ 7.32 (1H, s, 3-H), 7.54 (2H, d, J = 2, 2'- and 6'-H), 6.94 (1H, t, J = 2, 4'-H), and 2.31 (6H, s, 3'- and 5'-OCOCH$_3$). All these data indicated that moracin H is represented reasonably by formula 8.



1 R_1=R_3=OCH_3, R_4=R_5=OH, R_2=H

2 R_3=R_5=OCH_3, R_2=R_4=OH, R_1=H



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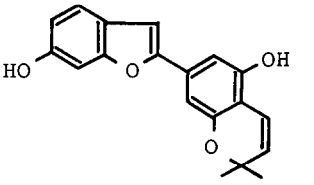

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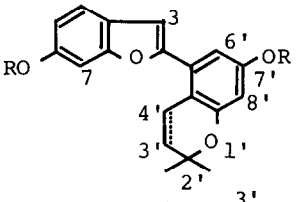
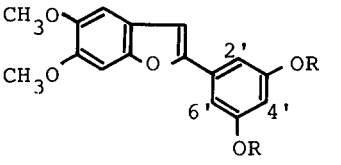


5 R=H, with $\Delta^{3'}$

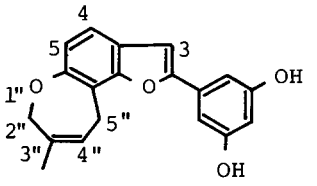
5a R=$COCH_3$, whith $\Delta^{3'}$

5b R=H, with no $\Delta^{3'}$

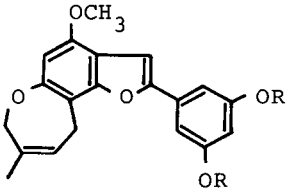


6 R=H

6a R=CH_3



7



8 R=H

8a R=$COCH_3$

All moracins so far isolated from cortex and phloem tissues of diseased mulberry shoots involve a 2-phenylbenzofuran unit as the fundamental skeleton and are related structurally and biogenetically to stilbene-type phytoalexins[1] isolated from the corresponding xylem tissues. It is to be noted that moracins

G and H are the first phytoalexins with a dihydrooxepin moiety fused with a benzene ring, and only several natural compounds possessing such an unique structure have recently been reported.[7] Antifungal activity of moracins E ∿ H against pathogenic and non-pathogenic fungi is described in Table 1.

Table 1. Antifungal activity of moracins E ∿ H (5 ∿ 8).[a]

Fungus	5	6	7	8
Fusarium roseum	15	∿50	15	15
F. lateritium f. sp. mori	∿50	∿200	∿500	∿100
F. solani f. sp. mori	∿200	∿1000	∿500	∿1000
Diaporthe nomurai	∿15	∿1000	∿50	∿50
Stigmina mori	∿200	∿50	∿50	∿50
Sclerotinia sclerotiorum	∿50	∿50	∿500	∿200
Cochlioborus miyabeanus[b]	∿100	∿100	∿50	∿50
Rosellinia necatrix	15	∿50	15	15

a) Minimum concentration (μg/ml) required for complete inhibition of fungal growth. b) A non-pathogenic fungus.

References and Notes

1) Part 4 of "Studies on Phytoalexins of the Moraceae;" Part 3, M. Takasugi, L Muñoz, T. Masamune, A. Shirata, and K. Takahashi, Chem. Lett., 1241 (1978).

2) M. Takasugi, S. Nagao, T. Masamune, A. Shirata, and K. Takahashi, Tetrahedron Lett., 797 (1978).

3) M. Takasugi, S. Nagao, S. Ueno, T. Masamune, A. Shirata, and K. Takahashi, Chem. Lett., 1239 (1978).

4) All the molecular formulas were determined by high resolution mass spectra and/or elementary analyses. The UV, IR, ^{1}H- and ^{13}C-NMR spectra were measured in ethanol, potassium bromide, acetone-d_6, and dimethyl sulfoxide-d_6, respectively.

5) J. A. Elvidge and R. G. Foster, J. Chem. Soc., 590 (1963).

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8) Examination of the ^{13}C-NMR spectra of all moracins indicated that, if the methoxyl group in question were located at C-5, a singlet peak would have to be observed at δ 140-145. Cf., T. Okuyama and T. Fueno, Bull. Chem. Soc. Jpn., 47, 1263 (1974); G. L. Nelson, G. C. Levy, and J. D. Cargioli, J. Am. Chem. Soc., 94, 3089 (1972).

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