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CHALCOMORACIN, A NATURAL DIELS-ALDER ADDUCT FROM DISEASED MULBERRY[1)]

Mitsuo TAKASUGI,* Shigemitsu NAGAO,* Tadashi MASAMUNE,*
Akira SHIRATA,** and Kokichi TAKAHASHI**
*Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060
**The Sericultural Experiment Station, Yatabe-cho, Tsukuba-gun 305

The structure elucidation of a new phytoalexin, isolated from diseased mulberry leaves and designated as chalcomoracin, is described. The compound is regarded biogenetically as a Diels-Alder adduct of a chalcone, isolated newly from diseased mulberry shoots, and a substituted 2-phenylbenzofuran.

In previous papers we reported the structures of eight 2-phenylbenzofuran phytoalexins,[2)] moracins A ∿ H, and two stilbene phytoalexins[3)] isolated from cortical and xylem tissues of mulberry shoots (Morus alba Linné) infected with Fusarium solani f. sp. mori, respectively. Inoculation of the mulberry leaves with the same fungus induced formation of a different type of an antifungal compound, designated as chalcomoracin (1), as a major phytoalexin of the leaves. The compound completely inhibited germination of Fusarium roseum and Bipolaris leersiae at concentrations of 10^{-4} ∿ 10^{-5} M. We now wish to report the isolation and structure elucidation of chalcomoracin (1).

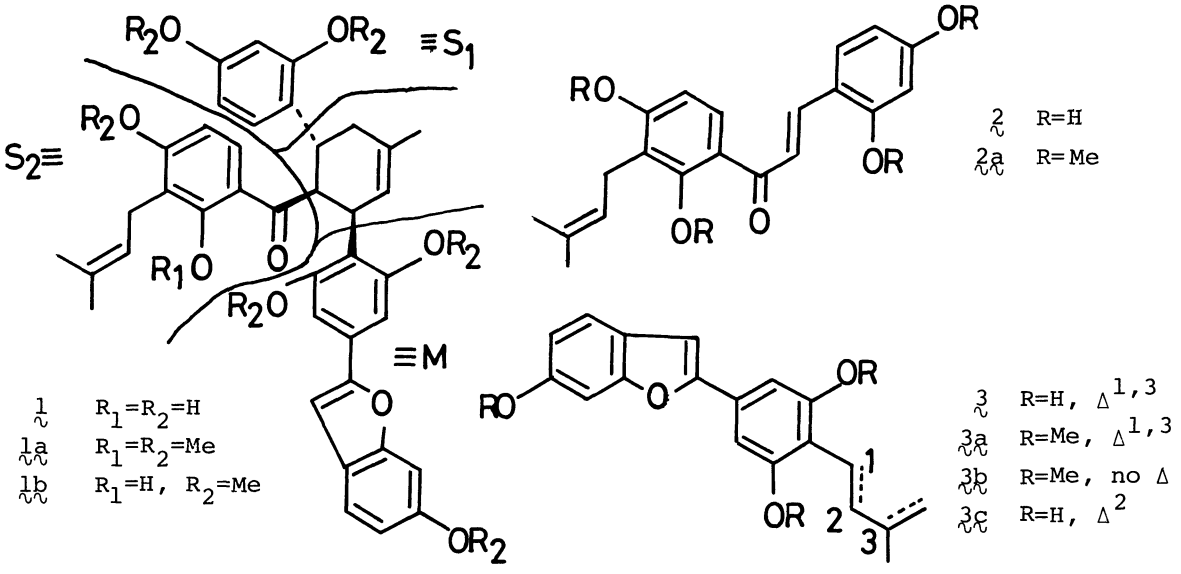
Acetone extracts of the infected mulberry leaves were fractionated successively by column chromatography over silica gel (CH_2Cl_2-CH_3OH) and then with Sephadex LH-20 (CH_3OH) to give 1 in a 0.013% yield from the dried leaves, which was not detected in the corresponding extracts of healthy leaves.

Chalcomoracin (1), mp 183 °C (dec) and $[\alpha]_D$ +194° (acetone), exhibited the following spectra: m/e 648 (M^+, FD-MS); λ_{max} (C_2H_5OH) 218 nm (ε 58600), 294 (sh, 33900), 329 (50500), and 334 (41300); ν_{max} (KBr) 1620 cm^{-1}. Methylation [$(CH_3)_2SO_4$ and K_2CO_3 in acetone, reflux, 3 h] of 1 afforded its heptamethyl and hexamethyl ethers (1a and 1b) in 43 and 44% yields, respectively; 1a, mp 154-156 °C and $[\alpha]_D$ +412° ($CHCl_3$); m/e 746 (18%), 542 (1), 396 (6), 350 (100), and 233 (69); 1b, mp 95-97 °C and $[\alpha]_D$ +429° ($CHCl_3$); m/e 732 (30%), 528 (1), 382 (3), 350 (100), 219 (38), and 163 (32). The molecular formula of the former (1a) was determined to be $C_{46}H_{50}O_9$ by the high-resolution mass spectrum (m/e 746.3510, M^+) and elementary analyses, and hence that of chalcomoracin (1) could be formulated as $C_{39}H_{36}O_9$.

Pyrolysis (280 °C, 0.2 Torr, 20 min) of the heptamethyl ether (1a) gave two fragmentation products (2a and 3a), corresponding to two fragments at m/e 396 ($C_{24}H_{28}O_5$) and 350 ($C_{22}H_{22}O_4$) of the mass spectrum, in 59 and 61% yields, respectively, with the unchanged starting material (1a, 35%). The latter (3a) was unstable and hydrogenated (Pd-$CaCO_3$) immediately to form its tetrahydro

derivative (3b), $C_{22}H_{26}O_4$, mp 119-120 °C, quantitatively, which was identified as trimethyldihydromoracin C by direct comparison with an authentic sample prepared (methylation and hydrogenation) from moracin C[4] (3c), a known mulberry phytoalexin. The former (2a), oil, was presumed to be trans-2,2',4,4'-tetramethoxy-3'-prenylchalcone on the basis of the spectral data (will be described in a full paper). The structure was confirmed by direct comparison with an authentic sample prepared by condensation of 2,4-dimethoxy-3-prenylacetophenone (obtained by methylation of the corresponding acetophenone[5]) with 2,4-dimethoxybenzaldehyde. Interestingly, the demethylated compound (2), mp 138 °C (dec), of 2a was also isolated from the cortical and xylem tissues of mulberry shoots infected with the fungus and designated as morachalcone A: 2, m/e 340 (M^+, 1%), 324 (97, $C_{20}H_{20}O_4$ by the high-resolution technique), 268 (72), and 267 (82); λ_{max} (C_2H_5OH) 250 nm (sh, 6500), 317 (7400), and 388 (24600); ν_{max} (KBr) 1622 and 1603 cm^{-1}; δ (CD_3COCD_3) 1.63 and 1.75 (each 3H, s), 3.18 (2H, br d, J = 7 Hz), 5.30 (1H, br t, J = 7), 6.46 (1H, dd, J = 8 and 2), 6.52 (1H, d, J = 2), 6.51 (1H, d, J = 9), 7.68 (1H, d, J = 8), 7.78 (1H, d, J = 15), 7.89 (1H, d, J = 9), 8.23 (1H, d, J = 15), 8.90, 9.21, 9.26, and 14.03 (each 1H, s, D_2O exchangeable).

The facile formation of the two compounds (2a and 3a) by pyrolysis of the heptamethyl ether (1a) strongly suggested that chalcomoracin (1) would probably be represented by either one (1 or 1') of Diels-Alder adducts of the respective demethylated compounds (2 and 3). This was substantiated by detailed analysis of the ^{1}H-NMR spectrum (270 MHz, CD_3COCD_3) using sequential decoupling. The chemical shifts (δ) and coupling constants (Hz) of protons of the relevant cyclohexene ring are shown in Fig. 1, while the remaining protons are summarized as follows: a prenyl group, 1.58 and 1.71 (each 3H, s), 3.28 (2H, br d, J = 7), and 5.17 (1H, br t, J = 7); a 2-phenylbenzofuran moiety, 6.74 (1H, dd, J = 8 and 2), 6.75 (2H, s), 6.89 (1H, s), 6.89 (1H, d, J = 2), and 7.32 (1H, d, J = 8); aromatic protons in two substituents (S_1 and S_2) in formulas 1 or 1', 6.31 (1H, dd, J = 8 and 2), 6.44, 6.49, 6.98, and 8.40 (each 1H, d, J = 9, 2, 8, and 9, respectively), 8.0 ∿ 9.0 (6H, br, six phenolic OH) and 12.84 (1H, s, chelated OH).

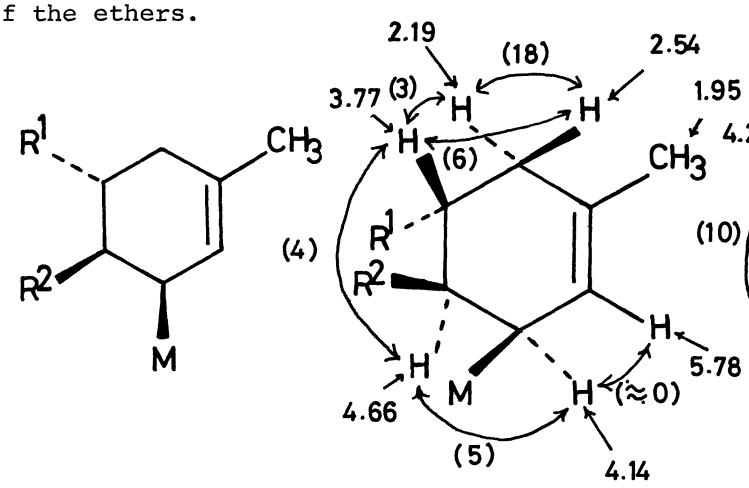


1 $R_1=R_2=H$
1a $R_1=R_2=Me$
1b $R_1=H$, $R_2=Me$

2 R=H
2a R=Me

3 R=H, $\Delta^{1,3}$
3a R=Me, $\Delta^{1,3}$
3b R=Me, no Δ
3c R=H, Δ^2

The coupling constants between adjacent protons on the cyclohexene ring (Fig. 1) indicated that the cyclohexene ring would probably take a half-chair conformation with the three substituents, R^1, R^2, and M axial, axial, and quasi-equatorial, respectively. The trans-configuration of the two substituents R^1 and R^2 was deduced more clearly from the signal pattern (Fig. 2) obtained by analysis of the ^{1}H-NMR spectrum of the methyl ether (1a). The summarized results indicated that, contrary to the axial conformation of R^1 and R^2 in 1, the substituents in 1a are both oriented equatorial on the half-chair cyclohexene ring. Naturally the ethers (1a and 1b) take the same (relative) configuration as that of the phenol (1), since the latter was recovered unchanged under the basic conditions used for the preparation of the ethers.



1 $R^1=S_1$, $R^2=S_2$
1' $R^1=S_2$, $R^2=S_1$

Fig. 1 (1)

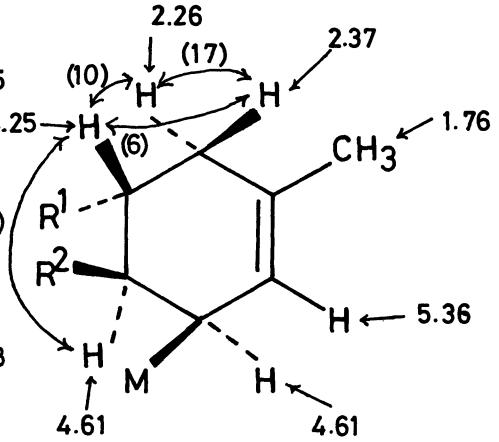


Fig. 2 (1a)

Hydride reduction ($LiAlH_4$) of the hexamethyl ether (1b) afforded a mixture of two alcohols (4a and 4b), which were isolated by preparative TLC in 40 and 44% yields, respectively. Fortunately, the former (4a), amorphous and $[\alpha]_D$ +191°, m/e 734 (M^+, 0.2%), 716 (24), and 511 (100), displayed well-separated signals due to aliphatic protons in the ^{1}H-NMR spectrum and hence spin-decoupling studies effected elucidation of the whole signal pattern due to the cyclohexene ring protons and two newly-formed protons, the result being shown in Fig. 3. Irradiation of the hydroxy proton (H_F) (J = 3, D_2O exchangeable) identified the chemical shift (δ 5.03) of a new methine proton (H_E), and subsequent irradiation of the latter (H_E) revealed the signal (δ 2.76) due to the relevant proton (H_D). The proton (H_D) remained unchanged on irradiation of either one of the geminal protons (H_A and H_B), confirming that the proton (H_D) is not located adjacent to the two geminal protons and the formula 1' should be eliminated. In accordance with the assigned formula (1), high-resolution mass spectra of minor fragmentation peaks of the methyl ethers (1a and 1b) at m/e 542 and 528 indicated that these fragments are formulated as $C_{33}H_{34}O_7$ and $C_{32}O_{32}O_7$, which were corresponding to formulas 5a and 5b. Chalcomoracin is therefore represented correctly by formula 1 (except the absolute configuration).

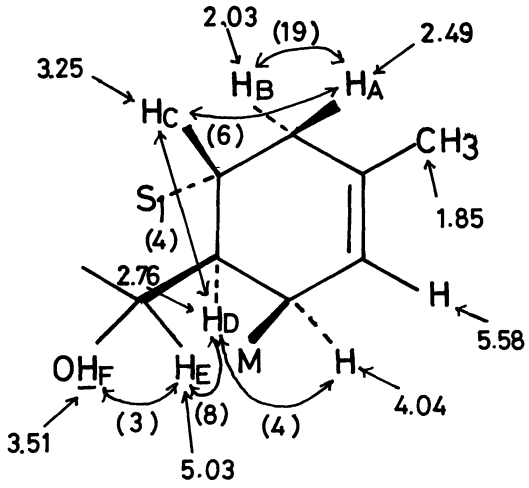
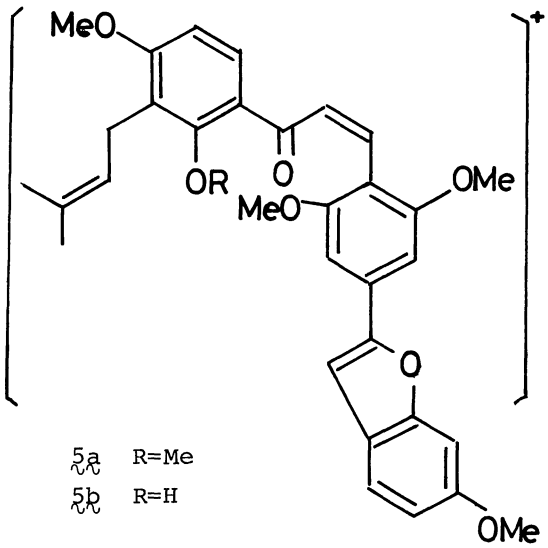


Fig. 3 (4a)



5a R=Me
5b R=H

Chalcomoracin (1) is optically active and considered to be formed by a Diels-Alder type of enzymatic reaction process of morachalcone A (2) and dehydromoracin C (3) or its equivalents. The co-occurrence of morachalcone A (2), moracin D[4] equivalent to dehydromoracin C, and chalcomoracin (1) as minor phytoalexins in the infected cortical tissues of mulberry shoots supports this biogenetic hypothesis. Although compounds such as thamnosin,[6a] alflabene,[6b] and heliocides[6c] were assumed to be formed by Diels-Alder reactions in vivo, the natural products showed no optical activity in contrast with chalcomoracin.

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References and Notes

1) Part 7 in the series "Studies on Phytoalexins of the Moraceae;" Part 6, M. Takasugi, Y. Kumagai, S. Nagao, T. Masamune, A. Shirata, and K. Takahashi, Chem. Lett. (the preceding paper).
2) M. Takasugi, S. Nagao, T. Masamune, A. Shirata, and K. Takahashi, Tetrahedron Lett., 1979, 4675 and refs cited therein.
3) M. Takasugi, L. Muñoz, T. Masamune, A. Shirata, and K. Takahashi, Chem. Lett. 1978, 1241.
4) M. Takasugi, S. Nagao, S. Ueno, T. Masamune, A. Shirata, and K. Takahashi, Chem. Lett., 1978, 1239.
5) A. C. Jain, P. Lal, and T. R. Seshadri, Indian J. Chem., 7, 1072 (1969).
6) a) J. P. Kutney, T. Inaba, and D. L. Dreyer, J. Am. Chem. Soc., 90, 813 (1968). b) I. Mori, Y. Nakachi, K. Ueda, D. Uemura, and Y. Hirata, Tetrahedron Lett., 1978, 2297. c) A. A. Bell, R. D. Stipanovic, D. H. O'Brien, and P. A. Fryxell, Phytochemistry, 17, 1297 (1978).

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