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Novel Phenolics from Phytopathogenic Fungus Helicobasidium mompa

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Novel phenolics HM-1(**3**), HM-2(**5**), HM-3(**6**), and HM-4(**7**) were obtained from phytopathogenic fungus Helicobasidium mompa which causes violet root rot against mulberry and several kinds of fruit trees. Compounds **3** and **7** could be precursors of helicobasidin and deoxyhelicobasidin, which have already been isolated from the same origin, while **5** and **6** were unusual types of C_{15}-compound.

Recent improvements in agricultural techniques have caused new plant diseases. Violet root rot is one of these. Avoiding contagion from the pathogenic fungus Helicobasidium mompa Tanaka is very difficult since it spreads under the ground. Recently, one of the authors found actinomycetes, which suppress the growth of H. mompa.[1] Helicobasidin (**1**), deoxyhelicobasidin(**2**), and mompain were isolated from the title fungus and their structures were determined by Natori in the 1960's.[2,3] However, no other metabolites have been reported since. Thus, in the course of our study on the molecular mechanism of violet root rot, we had to rescrutinize the compounds produced by this fungus.

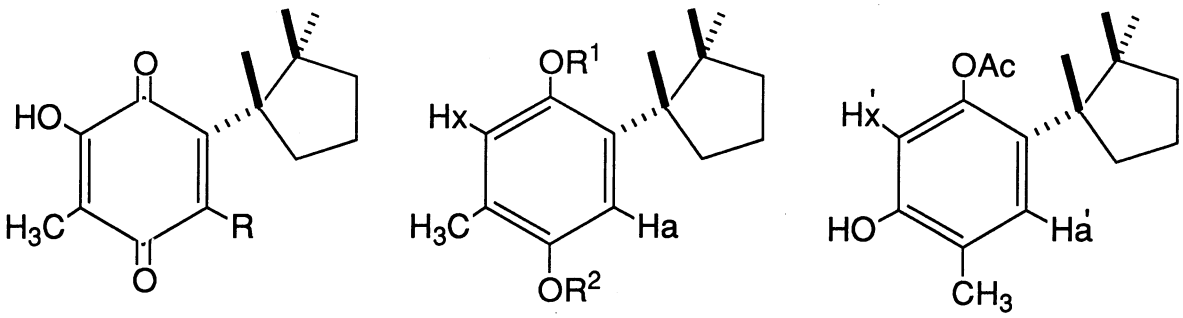
H. mompa, isolated by Takahashi from infected mulberry roots, was incubated in PD-medium at 25 °C for 2 months. The methanol extracts of the grown mycelium were subjected to a combination of column chromatographies on Sephadex LH-20 (benzene: MeOH=3:1) and silica gel(hexane:AcOEt=30:1→3:1), to afford three homogeneous components designated HM-1—3 along with **1** and **2**.

HM-1(**3**), colorless needles of mp 139.5-140.0 °C, $[\alpha]_D$ -55.0°($CHCl_3$), showed

a molecular ion peak at m/z 248 in the EI-MS and proton signals as listed in Table 1 in the ^{1}H-NMR spectrum. These signals were similar to those of **1** and **2**. Although the coupling constant of aromatic protons Ha and Hx showed that they were situated para to each other, the locations of the other substituents were still ambiguous. To clarify the orientation of each substituent, the NOESY spectrum of the acetate of HM-1(**4**) was measured. In this spectrum we observed NOEs between the Ha signal and the methyl signals attributed to a methoxyl group and quarternary methyl groups on the cyclopentane ring; we also observed the long-range couplings between the methyl group attached to the aromatic ring and both Ha and Hx, which were also detected in the ^{1}H-^{1}H COSY spectrum. Furthermore, the NOEs were observed between the acetoxy methyl and methyl groups on the side chain. As a result, the structure of **3** was determined as shown in Fig.1.[4)]

HM-2(**5**), a colorless oil of $[\alpha]_D$ -37.0°($CHCl_3$), showed a molecular ion peak at m/z 276 in the EI-MS. The ^{1}H-NMR data was shown in Table 1. Based on the facts that signals due to both aromatic protons were singlet and that the one at δ 6.89 (Ha') exhibited the NOEs between the methyl protons on the cyclopentane ring in the NOESY spectrum, the side chain and another aromatic proton(Hx') should be located on the o- and p-positions to Ha, respectively. The acetoxy methyl signal also had NOE with methyl groups on the cyclopentane ring. Therefore, the acetoxy group should orientate on the o-position of the side chain. However, the locations of the hydroxy and phenylic methyl groups were not confirmed with the NOESY spectrum, since the phenylic methyl signal correlated with both of the aromatic protons in both the NOESY and ^{1}H-^{1}H COSY spectrum. To clarify the orientation of the substituents, the ^{1}H-NMR spectrum of **5** was compared with that of **4** in detail. As a result, we found that each signal at δ 6.70 and δ 2.16 in **4** shifted to δ 6.53 and δ 2.07 in **5**, respectively, and the aromatic signals having NOEs with the side chain gave almost the same chemical shift values. Thus, the structure of HM-2 was elucidated as **5** in Fig.1.

HM-3(**6**), colorless needles, mp 158.5-160.0 °C, $[\alpha]_D$ -8.1°($CHCl_3$), showed a molecular ion peak at m/z 276 in the EI-MS. Proton signals were observed as listed in Table 2 in the ^{1}H-NMR spectrum. The coupling pattern exhibited each aromatic proton(H_α,H_β) to be located on the neighbouring carbons, and only one NOE was observed between the phenylic methyl group and one of the aromatic protons at δ 6.69 in the NOESY spectrum. The structure of HM-3 was thus determined as **6**.



1 : R=OH
2 : R=H

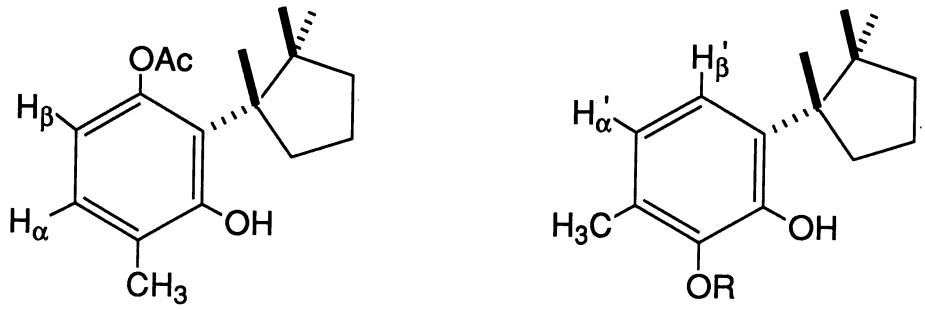
3 : R^1=H, R^2=CH_3
4 : R^1=Ac, R^2=CH_3

5

Fig. 1. Structures of compound **1-5**.

Table 1. ^{1}H-NMR Spectra of Compound **3-5**

	Ha(Ha')	Hx(Hx')	Ar-CH_3	-OMe	-OAc	CH_3 x3
3	6.80	6.48	2.14	3.77	-	1.40, 1.18, 0.78
4	6.88	6.70	2.16	3.80	2.27	1.28, 1.15, 0.76
5	6.89	6.53	2.07	-	2.29	1.39, 1.16, 0.76



6

7: R=H
8: R=Me

Fig. 2. Structures of compound **6-8**.

Table 2. ^{1}H-NMR Spectra of Compound **6-8**

	H_α(H_α')	H_β(H_β')	Ar-CH_3	-OMe	-OAc	-OH	CH_3 x3
6	6.69 (d, J=8 Hz)	7.08 (d, J=8 Hz)	2.11	-	2.37	5.15	1.40, 1.15, 0.74
7	6.59 (d, J=8 Hz)	6.78 (d, J=8 Hz)	2.22	-	-	5.57, 4.95 (bs) (bs)	1.41, 1.18, 0.76
8	6.58 (d, J=8 Hz)	6.94 (d, J=8 Hz)	2.28	3.80	-	6.09	1.40, 1.15, 0.73

This structure was also supported by the fact that NOEs were not observed between the acetyl group and the phenylic methyl group, or between the H_β and the side chain in the NOESY spectrum.

When the fungus was incubated for a shorter time(1 month), HM-3 was not produced, though HM-1 and 2 were generated. However, the existence of another compound, HM-4(**7**), was recognized on TLC. HM-4(**7**), colorless needles, mp 72.0-73.0 °C, $[\alpha]_D$ -57.4°($CHCl_3$), showed a molecular ion peak at m/z 234 in the EI-MS and proton signals(Table 2) in the ^{1}H-NMR spectrum. The coupling pattern of the aromatic protons was of the AB type. Methylation of HM-4 with diazomethane at 0 °C afforded a monomethyl ether(**8**), and its ^{1}H-NMR spectrum exhibited signals as shown in Table 2. In the NOESY spectrum of **8**, the methoxy signal showed the NOEs with the phenylic methyl signal and the hydroxy proton signal, which also showed the NOE with proton signals at the side chain. Thus the structure of HM-4 could be depicted as **7**.

Consequently, we obtained four phenolics from H. mompa. Since phenolics can be generally transformed to quinones by air as well as by enzymes, HM-1(**3**) and HM-4(**7**) could be precursors of **1** or **2**. HM-2(**5**) and HM-3(**6**), having the methyl group at different positions on the aromatic ring, are regarded as unusual compounds from a biogenetic view point. All of the newly obtained phenolics are expected to be anti-oxidants because their structures are similar to those of tocopherol and the reduced form of ubiquinone. They are also expected to be antibiotics because of our observation that the H. mompa colonies formed a growth-inhibiting area against certain bacteria.

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References

1) Unpublished. Takahashi will soon report in detail.

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4) As for this compound, the occurance was suggested by Nozoe et al. in the course of studies on the biosynthesis of **1**. S.Nozoe, M.Morisaki, and H.Matsumoto, J. Chem. Soc., Chem. Commun., 1970, 926.

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