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A NEW PYRROLIDINE-2,4-DIONE DERIVATIVE, VERMELHOTIN, ISOLATED FROM UNIDENTIFIED FUNGUS IFM 52672

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Abstract - A new pyrrolidine-2,4-dione derivative, vermelhotin (**1**), was isolated along with the characteristic antifungal substance, dihydroepiheveadride (**2**), and its analog, deoxoepiheveadride (**3**), from unidentified fungus IFM 52672. The structure of **1** was elucidated by the spectroscopic and X-Ray crystallographic investigation. Velmelhotin (**1**) was the first example of 3-acylpyrrolidine-2,4-dione (tetramic acid) derivative having further pyrane ring.

INTRODUCTION

In the screening for new antifungal substances from fungal sources against pathogenic filamentous fungi, we recently reported the isolation and structure elucidation of dihydroepiheveadride (**2**),[1,2] as the characteristic antifungal agent against the filamentous fungi such as *Aspergillus fumigatus* FRESENIUS and *Trichophyton mentagrophytes* (ROBIN) BLANCHARD, and deoxoepiheveadride (**3**)[2] from unidentified fungus IFM 52672. Further purification of the $CHCl_3$-MeOH (1:1) extract of the rice cultivated by the above fungus brought us the isolation of a new pyrrolidine-2,4-dione (tetramic acid) derivative designated vermelhotin (**1**). The structure of **1** was mainly described in this paper.

RESULTS AND DISCUSSION

The molecular formula of vermelhotin (**1**), orange needles from CH_2Cl_2-MeOH, mp 212-214ºC (from CH_2Cl_2-MeOH), was confirmed as $C_{12}H_{10}NO_3$ from the analysis of high resolution FAB-MS. The IR spectrum of **1** showed the characteristic carbonyl bands of a ketone (1700 cm^{-1}) and an amide (3170, 1660

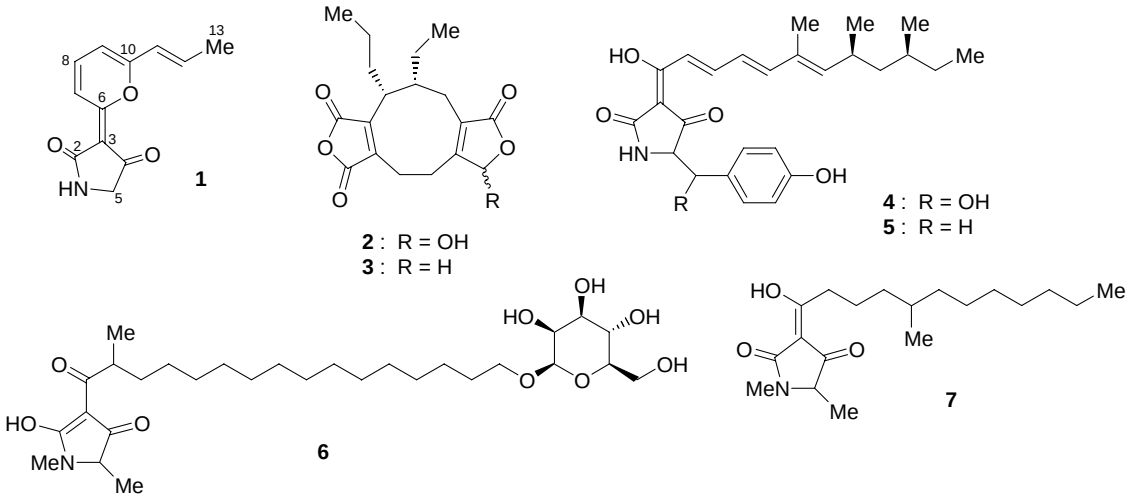

Figure 1. Structures of tetramic acid derivatives (**1**- **7**)

cm^{-1}), whereas the UV spectrum showed the absorption maxima at 456, 439, 332, 319, 276, 234, and 228 nm. The ^{1}H-NMR spectrum of **1** showed a methyl signal [δ 2.02 (dd, J=7.0, 1.5 Hz)], a sp^3 methylene signal [δ 3.79 (brs)], five olefinic proton signals [δ 6.16 (brd, J=15.3 Hz), 6.29 (brd, J=7.0 Hz), 7.40 (dq, J=15.3, 7.0 Hz), 7.42 (dd, J=9.2, 7.0 Hz), and 8.18 (brd, J=9.2 Hz)], and an amide proton [δ 5.76 (brs)], whereas the ^{13}C-NMR spectrum showed a methyl carbon (δ 19.0), a sp^3 methylene carbon (δ 50.4), five sp^2 methine carbons (δ 107.6, 116.0, 122.1, 138.6, 141.5), three sp^2 quarternary carbons (δ 98.1, 158.7, 165.5), and two carbonyl carbons (δ 172.4, 192.7). The detailed analysis of ^{1}H-^{1}H COSY and HMBC (Figure 2) spectra assumed the partial structure of vermelhotin (**1**). The structure of the tetramic acid moiety could not been confirmed, because the HMBC correlation from the methylene proton at δ 3.79 was observed only to the carbonyl carbons (amide and ketone).

In order to determine the exact structure of vermelhotin (**1**), the X-Ray crystallographic analysis was undertaken. Compound (**1**) was crystallized from CH_2Cl_2-MeOH as red needles, which is small but suitable for X-Ray analysis. Diffraction intensities were collected from a crystal of dimensions 0.20×0.03×0.02 mm on a Bruker SMART APEXII CCD area detector with monochromated Mo-*K*α radiation passed through a multilayer conforcal mirror. The crystal structure was solved by the direct methods using SHELXS-97[3] and the *R* (R_w) value reached to 0.0476 (0.0980). The crystal structure of **1** was established to be as shown in Figure 3. The bond lengths and angles are not significantly different from the expected ones. The molecular structure of vermelhotin was confirmed as shown in **1**.[4]

K. Schmidt *et al.* proposed that pyrrolidine-2,4-dione (tetramic acid) derivative such as militarinones B (**4**) and C (**5**), isolated from *Paecilomyces militaris*,[5] would be biosynthesized from amino acid (tyrosine or phenylalanine) and β-keto acid (unsaturated β-oxotetradecanoic acid). Epicoccamide (**6**), isolated from *Epicoccum purpurascens*,[6] and melophlin C (**7**), isolated from *Melophus sarassinorum*,[7] *etc.* were

also pyrrolidine-2,4-dione derivatives basically derived from alanine and β-keto acids (β-oxooctadecanoic acid or β-oxotetradecanoic acid, respectively), whereas vermelhotin (**1**) was derived from glycine and β-oxooctanoic acid followed by the further cyclization between 6-OH and C-10. Vermelhotin (**1**) is the fiest example of 3-acylpyrrolidine-2,4-dione (tetramic acid) derivative with further pyrane ring obtained from fungal sources.

No antifungal activity of vermelhotin (**1**) was observed against filamentous fungi *Aspergillus fumigatus* and *Aspergillus niger*, yeasts *Candida albicans* and *Cryptococcus neoformans*, and bacteria *Escherichia coli* and *Bacillus subtilis*.

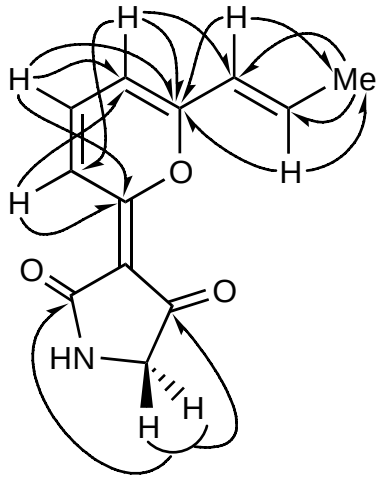

Figure 2. The HMBC correlations in vermelhotin (**1**)

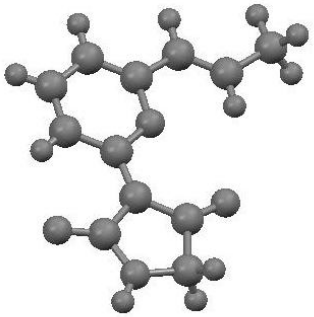

Figure 3. Crystal structure of vermelhotin (**1**)

EXPERIMENTAL

General. Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. EI-MS were taken with a JEOL JMS-HX110 spectrometer. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL Lambda-500 (1H, 500.00 MHz; 13C, 125.43 MHz) spectrometer, using tetramethylsilane as an internal standard. Column chromatography was performed using Kieselgel 60 (Art. 7734, Merck) and Wakogel C-200 (Art. 237-00071, Wako). Low-pressure liquid chromatography (LPLC) was performed with a Chemco Low-Prep 81-M-2 pump and glass column (200 × 10 mm) packed with Silica gel CQ-3 (30-50 μm, Wako). TLC was conducted on pre-coated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC were detected by UV light on 254 nm and/or by spraying with 5%-H_2SO_4 and then heating.

Isolation of vermelhotin (1) from unidentified fungus IFM 52672. The fungus IFM 52672 was cultured at 25°C for 28 d in 4 Roux flasks containing 125 g of moist rice in each flask. The cultivated rice was extracted with $CHCl_3$-MeOH (1:1) and the organic layer was evaporated *in vacuo*. The residue (18 g)

was extracted with hexane, benzene, and $CHCl_3$, in turn. The hexane and benzene soluble fraction was evaporated *in vacuo*. The residue was chromatographed on silica gel with hexane-benzene, benzene, and $CHCl_3$. The combined eluates were re-chromatographed on silica gel with the solvent system of benzene-$CHCl_3$-MeOH. The evaporated fraction, eluated after the fraction of dihydroepiheveadride (**2**) and deoxoepiheveadride (**3**) were obtained, was recrystallized from CH_2Cl_2-MeOH to give vermelhotin (**1**) (4 mg).

Vermelhotin (**1**): Red micro-needles, mp 212-214 °C (from CH_2Cl_2-MeOH). FAB-MS *m/z*: 218.0798 [(M $+$H)$^+$, 218.0817 for $C_{12}H_{11}NO_3$]. UV λ_{max}^{MeOH} nm (log ε): 228 (3.82), 234 (3.80), 276 (4.11), 319 (3.50), 332 (3.40), 439 (3.96), 456 (3.95). IR ν_{max}^{KBr} cm^{-1}: 3170 (NH), 3040, 1700 (CO), 1660 (CONH), 1610, 1550, 1495. ^{1}H-NMR δ in $CDCl_3$: 2.02 (3H, dd, J=7.0, 1.5 Hz, 13-H_3), 3.79 (2H, brs, 5-H), 5.76 (1H, brs, 1-NH), 6.16 (1H, brd, J=15.3 Hz, 11-H), 6.29 (1H, brd, J=7.0 Hz, 9-H), 7.40 (1H, dq, J=15.3, 7.0 Hz, 12-H), 7.42 (1H, dd, J=9.2, 7.0 Hz, 8-H), 8.18 (1H, brd, J=9.2 Hz, 7-H). ^{13}C-NMR δ in $CDCl_3$: 19.0 (C-13), 50.4 (C-5), 98.1 (C-3), 107.6 (C-9), 116.0 (C-7), 122.1 (C-11), 138.6 (C-12), 141.5 (C-8), 158.7 (C-10), 165.5 (C-6), 172.4 (C-2), 192.7 (C-4).

X-Ray structure analysis of vermelhotin (1). Vermelhotin (**1**) was grown slowly from CH_2Cl_2-MeOH to give red needles. Diffraction intensities were collected from a crystal of dimensions 0.20×0.03×0.02 mm on a Bruker SMART APEXII CCD area detector with Mo-*K*α radiation passed through a multilayer conforcal mirror. Of a total of 5284 reflections, 2149 independent reflections within a 2θ range of 27.54° were used in the solution and refinement of the structure. The data were corrected for Lorenz and polarization effects and also corrected for absorption effects by empirical method SADABS.[8]

Crystal Data $C_{12}H_{11}NO_3$, *M*= 217.22, monoclinic, space group $P2_1/n$, *a*= 4.8885 (9), *b*= 14.194 (3), *c*= 14.697 (3) Å, β= 93.096 (3)°, *V* = 1018.3 (3) Å^3, *Z*= 4, D_C= 1.417 g·cm^{-3}, *F*(000)= 456, μ(Mo*K*α) = 0.103 cm^{-1}, Mo-*K*α *X*-radiation (graphite monochromated), λ = 0.71073 Å, *T*= 90 K.

The structure was solved by direct method using SHELXS-97[9] and refined by the full-matrix least-squares method with SHELXL.[10] Although most of the hydrogen atoms were found from the difference Fourier synthesis, all of the hydrogen atoms used were calculated. In the final refinement, anisotropic thermal parameters were used for non-hydrogen atoms and the fractional and isotropic thermal parameters for hydrogen atoms were fixed. All calculations were performed using the APEX2.[11] The final *R* and R_w values were 0.0476 and 0.0980, respectively, for 2149 independent reflections.

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