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Hualyzin, a Symmetrical Urea Derivative Isolated from *Penicillium herquei* Isolate GA4

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Penicillium herquei isolate GA4 was isolated from the infected *Conchocelis* of *Porphyra yezoensis*. A large-scale fermentation using yeast extract sucrose medium and repeated chromatography afforded a new symmetrical urea derivative, hualyzin (**1**). The structure was determined by detailed NMR spectroscopic investigations and MS fragmentation analysis.

Porphyra yezoensis is an edible red alga with some economic importance in China. A serious disease, the white-spot syndrome, causes the *Conchocelis* of *Porphyra yezoensis* to lose pigment and to decay. Thirty morphologically different fungal strains have been isolated from infected algae, and some exhibited strong antimicrobial activity. Among them, *Penicillium herquei* isolate GA4 was selected for further studies due to its high antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Streptomyces viridochromogenes*, *Mucor miehei*, and *Candida albicans*.

Along with a number of known compounds, isoherqueinone, herqueinone, norherqueinone, deoxyisoherqueinone, sclerodione, furan-3-carboxylic acid, indol-3-carboxylic acid, 3-indolylcarboxylic acid methyl ester, 5-hydroxymaltol, phenylacetic acid, *p*-hydroxyphenylacetic acid, *p*-hydroxyphenylacetic acid methyl ester, (*R*)-mevalonolactone, *cyclo*(prolylvalyl), and pentanoic acid, the extract afforded a new symmetrically disubstituted urea derivative, hualyzin (**1**). The urea substructure is quite common in microbial products and occurs in more than 500 compounds, the multitude of uracil, cytosin, and biotin derivatives not taken into account. If, however, some further heterocycles as well as the many ureido-peptides and ureido-sugars are subtracted, only a few compounds are left,[1] e.g., the formestins[2] and antiostatins.[3] Only one further compound, 1,3-diphenylethylurea, is symmetrically disubstituted.[4]

Hualyzin (**1**) was obtained as an oil with UV absorption at 254 nm and a gray color reaction on TLC with anisaldehyde/sulfuric acid. In the aromatic region, the ^{1}H NMR spectrum in CD_2Cl_2 displayed four protons as two *ortho*-coupled doublets at δ 8.36 and 7.83 and two singlets at δ 7.56 (NH) and 6.69. The aliphatic region showed one signal at δ 5.52 attributed to an oxymethine group, three methoxy groups (δ 3.95, 3.84, 3.83), and ethoxy signals (δ 4.07 q, 1.21 t). In addition, two methylene groups at δ 3.08 (dd) and 2.60 (d) and one methyl singlet at δ 1.98 were observed. HRESIMS showed a pseudomolecular peak at *m/z* 859 $[M + Na]^+$ and indicated the molecular formula $C_{43}H_{52}N_2O_{15}$. The ^{13}C NMR spectrum exhibited, however, only 22 carbon signals, assigned by APT as 10 quaternary sp^2 signals (including two carbonyls), three sp^2 methines, one sp^3 methine, three sp^3 methylenes, two methyls, and three methoxy signals, thus indicating a symmetrical compound with one carbon atom placed in the mirror plane. The H,H COSY spectrum confirmed the ethoxy fragment and the aromatic *ortho*-protons and indicated additionally a 2-hydroxypropane-1,3-diyl fragment.

According to the HMBC spectrum, the propanediyl fragment is sandwiched between an ethoxycarbonyl group and the aromatic system. Further correlations (Figure 1) confirmed a 4-(1,4,5-trimethoxynaphthalen-2-yl)butyric acid ethyl ester, which has an additional amide function at C-6, deduced from the coupling of the NH singlet at δ 7.56 with C-5 and C-7, as indicated in substructure I.

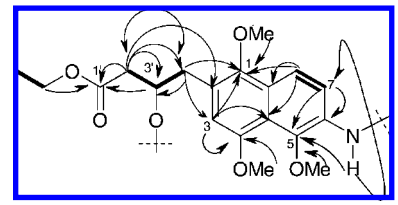

Figure 1. Selected HMBC (→, ↔) and COSY (—) correlations in substructure I of **1**.

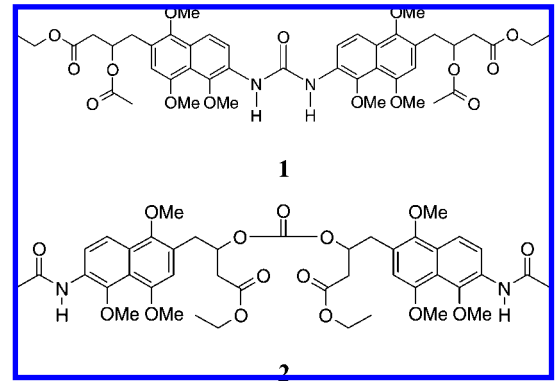

Figure 2. Alternative structures **1** and **2** of hualyzin.

According to the formula and the NMR data, two acetyl fragments and a carbonyl group (δ 153.0) were still unassigned. Thus two possible alternatives (Figure 2) had to be taken into account: a urea derivative **1**, with acetyl groups at both of the side chains, and a carbonate **2**.

The dimeric structure **1** was fully confirmed by the EI mass spectrum, which indicated four major peaks at *m/z* = 431, 405, 371, and 345. Fragments at *m/z* = 431 and 405 were explained by a cleavage at the urea moiety. A subsequent loss of acetic acid can occur only from *O*-acetates and gave the fragments *m/z* = 371 and 345 (Figure 3).

The intermediates **3** and **4** in the synthesis of daunomycinone are the only two reported naphthalene derivatives with structural relations to **1**.[5,6]

Penicillium sp. GA4 produced very low amounts of **1**, and efforts to reisolate it one year later were not successful. The biological activity of hualyzin (**1**) could not be tested due to the limited amount of material. We could not measure the optical rotation accurately.

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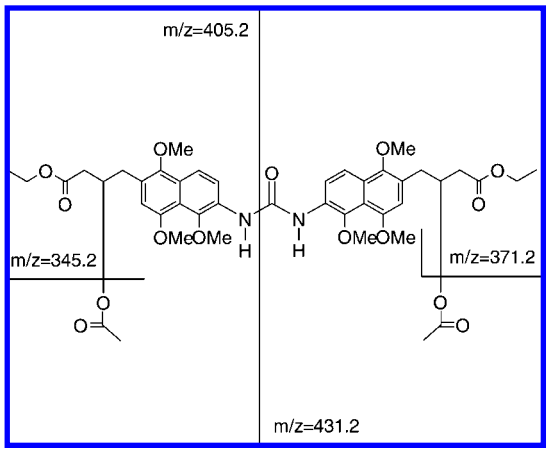
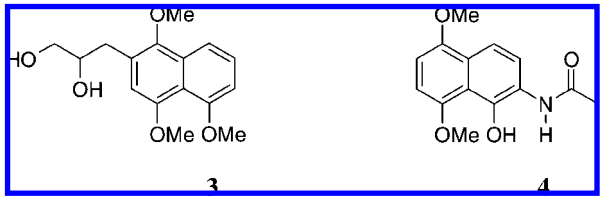


Figure 3. Selected EI mass fragments of hualyzin (**1**).



However, the CD spectrum indicated optical activity (positive Cotton effect at 225 nm), implying that the absolute configuration of the two stereogenic centers, although undefined at this stage, must be identical.

Experimental Section

General Experimental Procedures. UV/vis spectra were recorded on a Perkin-Elmer Lambda 15 UV/vis spectrometer. NMR spectra were measured on Bruker AMX 300 and Varian Inova 500 spectrometers. ESIMS was recorded on a Finnigan LCQ with Rheos 4000 quaternary pump (Flux Instruments). ESIHRMS were measured on a micromass LCT mass spectrometer coupled with a HP1100 HPLC with a diode array detector. Reserpine (MW = 608) and leucine-enkephalin (MW = 555) were used as standards in positive and negative mode. HRMS was recorded by ESIMS on an Apex IV 7 T Fourier-transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, MA). EIMS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV). Flash chromatography was carried out on silica gel (230−400 mesh). TLC was done on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). Size-exclusion chromatography was done on Sephadex LH-20 (Pharmacia). XAD-16 adsorber resin was obtained from Rohm and Haas, France.

Isolation, Fermentation, and Workup of *Penicillium herquei* Isolate GA4. Strain GA4 was isolated from the infected red alga *Porphyra yezoensis* obtained from a farm in Lianyungang, Jiangsu Province, China; its ITS1−5.8S−ITS2 rDNA sequence was deposited in the EMBL database (accession number EF536027). It was cultivated in 100 × 1 L Erlenmeyer flasks each containing 250 mL of YES medium (yeast extract 20 g, sucrose 150 g, $MgSO_4 \cdot 7H_2O$ 0.5 g in 1 L of artificial seawater). The culture broth was shaken on a linear shaker at 28 °C for 5 days, then mixed with ca. 1.5 kg of Celite and filtered under pressure. The water phase was passed through an XAD-16 (60 × 10 cm) column. The resin was washed with distilled H_2O (10 L) and eluted with MeOH (10 L). The water residue was extracted with EtOAc, while the mycelium was extracted with EtOAc (× 4) under ultrasonic irradiation. The combined organic phases were evaporated to yield 22 g of crude extract. Chromatography on silica gel (column 50 × 3 cm) using CH_2Cl_2/MeOH gave three fractions: A, B, and C. Compound **1** (1.1 mg) was obtained from fraction B by repeated chromatography on silica gel using a CH_2Cl_2/MeOH gradient. Isoherqueinone (4.5 mg), herqueinone (13 mg), norherqueinone (550 mg), deoxyisoherqueinone (8.3 mg), sclerodione (1.2 mg), furan-3-carboxylic acid (2.5 mg), indol-3-carboxylic acid (3.2 mg), 3-indolylcarboxylic acid methyl ester (1.2 mg), 5-hydroxymaltol (2.3 mg), phenylacetic acid (10 mg), *p*-hydroxyphenylacetic acid (1.4 mg), *p*-hydroxyphenylacetic acid methyl ester (1.4 mg), (*R*)-mevalonolactone (16 mg), *cyclo*(prolylvalyl) (10 mg), and pentanoic acid (3.2 mg) were obtained from other fractions.

Hualyzin (1): pale yellow solid, $R_f = 0.31$ (CH_2Cl_2/5% MeOH); IR (film) ν_{max} 3226, 2961, 2931, 2877, 1742, 1678, 1608, 1531, 1503, 1434, 1384, 1282, 1240, 1188, 1046, 1088, 918, 831, 755, 667 cm^{-1}; UV/vis (MeOH) λ_{max} (log ε) 350 (sh, 3.87), 313 (4.31), 270 (4.67), 222 (4.59) nm; ^1H NMR (CD_2Cl_2, 300 MHz) δ 8.36 (2H, d, J = 9.2 Hz, 2 × H-7), 7.83 (2H, d, J = 9.2 Hz, 2 × H-8), 7.56 (2H, s, 2 × NH), 6.69 (2H, s, 2 × H-3), 5.52 (2H, quint, 2 × H-3′), 4.07 (4H, q, J = 7.1 Hz, 2 × CH_2CH_3), 3.95 (6H, s, 2 × 4-OMe), 3.84 (6H, s, 2 × 5-OMe), 3.83 (6H, s, 2 × 1-OMe), 3.08 (4H, d, J = 6.9 Hz, 2 × H-4′), 2.60 (4H, d, J = 6.7 Hz, 2 × H-2′), 1.98 (6H, s, 2 × $COCH_3$), 1.21 (6H, t, J = 7.1 Hz, 2 × CH_2CH_3); ^{13}C NMR (CD_2Cl_2, 125 MHz) δ 170.6 (C_q-1′), 170.3 ($COCH_3$), 153.0 (*CO*), 151.7 (C_q-4), 148.5 (C_q-1), 144.2 (C_q-5), 130.1 (C_q-8a), 127.3 (C_q-6), 123.9 (C_q-2′), 120.8 (CH-7), 120.4 (C_q-4a), 119.0 (CH-8), 108.9 (CH-3), 70.8 (CH-3′), 62.4 (5-OCH_3), 62.2 (1-OCH_3), 60.8 (CH_2CH_3), 56.5 (4-OCH_3), 38.8 (CH_2-2′), 34.3 (CH_2-4′), 21.2 ($COCH_3$), 14.2 (CH_2CH_3); EIMS (70 eV) *m/z* 431 (90), 405 (100), 371 (100), 345 (32), 330 (24), 282 (25); (+)-ESIMS *m/z* 1696 $[2M + Na]^+$ (40), 859 $[M + Na]^+$ (100); (+)-HRESIMS *m/z* 837.3434550 $[M + H]^+$ (calcd for $C_{43}H_{53}N_2O_{15}$, 837.34458).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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