

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

Ling Ding,^{†,‡} Serge Fotso,[§] Fuchao Li,[†] Song Qin,[†] and Hartmut Laatsch^{*,§}

Institute of Oceanology, Chinese Academy of Sciences, Nanhai Road 7, 266071 Qingdao, People's Republic of China, Graduate School of the Chinese Academy of Sciences, Yuquan Road 19A, 100039 Beijing, People's Republic of China, and Department of Organic and Biomolecular Chemistry, University of Göttingen, Tammannstrasse 2, D-37077 Göttingen, Germany

Received December 29, 2007

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, isotherqueinone, herqueinone, norherqueinone, deoxyisotherqueinone, 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 formestins² 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 ¹H NMR spectrum in CD₂Cl₂ 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 C₄₃H₅₂N₂O₁₅. The ¹³C NMR spectrum exhibited, however, only 22 carbon signals, assigned by APT as 10 quaternary sp² signals (including two carbonyls), three sp² methines, one sp³ methine, three sp³ 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

* To whom correspondence should be addressed. Tel: +49 551 393211. Fax: +49 551 399660. E-mail: hlaatsch@gwdg.de.

[†] Institute of Oceanology, Chinese Academy of Sciences.

[‡] Graduate School of the Chinese Academy of Sciences.

[§] University of Göttingen.

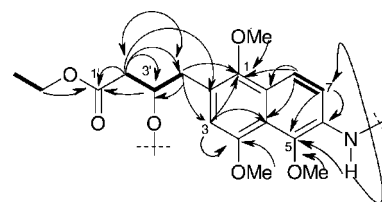


Figure 1. Selected HMBC (→, ↔) and COSY (---) correlations in substructure I of **1**.

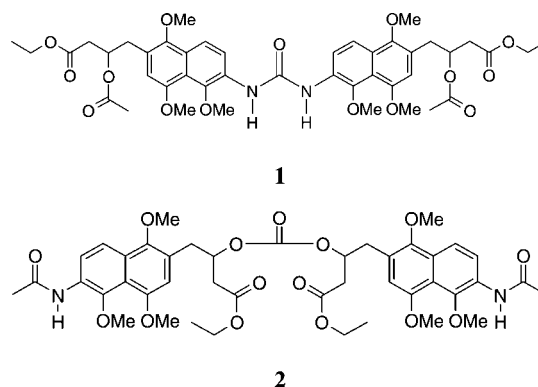


Figure 2. Alternative structures **1** and **2** of hualyzin.

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.

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.

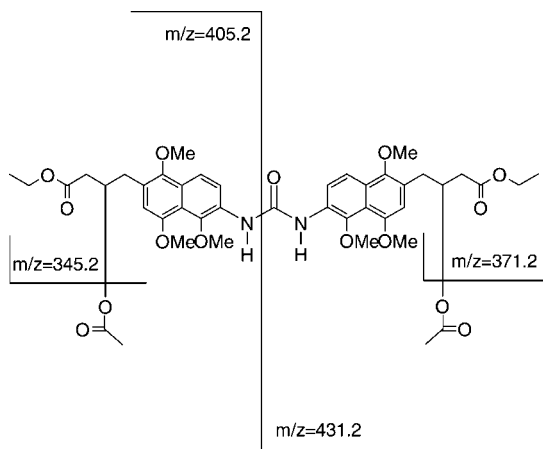
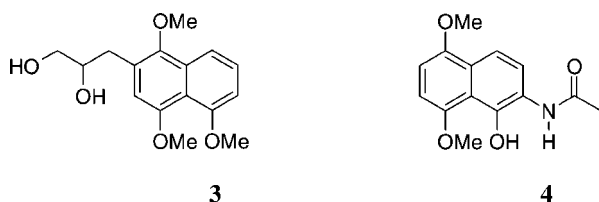


Figure 3. Selected EI mass fragments of hualyzin (1).



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/UV₂₅₄ (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, MgSO₄·7H₂O 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 H₂O (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 CH₂Cl₂/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 CH₂Cl₂/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(propylvalyl) (10 mg), and pentanoic acid (3.2 mg) were obtained from other fractions.

Hualyzin (1): pale yellow solid, *R_f* = 0.31 (CH₂Cl₂/5% 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 (CD₂Cl₂, 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 × CH₂CH₃), 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 × COCH₃), 1.21 (6H, t, *J* = 7.1 Hz, 2 × CH₂CH₃); ¹³C NMR (CD₂Cl₂, 125 MHz) δ 170.6 (C_q-1'), 170.3 (COCH₃), 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-OCH₃), 62.2 (1-OCH₃), 60.8 (CH₂CH₃), 56.5 (4-OCH₃), 38.8 (CH₂-2'), 34.3 (CH₂-4'), 21.2 (COCH₃), 14.2 (CH₂CH₃); 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 C₄₃H₅₃N₂O₁₅, 837.34458).

Acknowledgment. We thank R. Machinek for the NMR spectra, Dr. H. Frauendorf for the mass spectra, and F. Lissy and A. Kohl for technical assistance. This work was supported by the Key Innovative Project of the Chinese Academy of Science (KZCX2-YW-216, KZCX2-YW-209, 2007-1), the National High Technology Research and Development Program of China (2007AA09Z412), and the CAS/SAFEA International Partnership Program for Creative Research Teams (Research and Applications of Marine Functional Genomics).

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

References and Notes

- Laatsch, H. *AntiBase 2007, A Data Base for Rapid Dereplication and Structure Determination of Microbial Natural Products*. Wiley-VCH: Weinheim, Germany, 2007 (and annual updates); see: <http://www-wuser.gwdg.de/ucoc/laatsch/AntiBase.htm>.
- Takeuchi, T.; Aoyanagi, T.; Hamada, M.; Finuma, H.; Ogawa, K. *Jpn. Kokai Tokkyo Koho JP 01 050895*, 27.02.1989.
- Mo, C. J.; Shin-Ya, K.; Furihata, K.; Furihata, K.; Shimazu, A.; Hayakawa, Y.; Seto, H. *J. Antibiot.* **1990**, *43*, 1337.
- Iwai, Y.; Hirano, A.; Awaya, J.; Matsuo, S.; Omura, S. *J. Antibiot.* **1978**, *31*, 375–376.
- Delgado, A.; Garcia, J. M.; Mauleon, D.; Minguillon, C.; Subirats, J. R.; Feliz, M.; Lopez, F.; Velasco, D. *Can. J. Chem.* **1988**, *66*, 517–527.
- Parker, K. A.; Kallmerten, J. *J. Am. Chem. Soc.* **1980**, *102*, 5881–5886.

NP700751B