A Sorbicillinoid Urea from an Intertidal *Paecilomyces marquandii*

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A ureido Diels−Alder adduct of sorbicillinol **3** has been isolated from an intertidal marine *Paecilomyces marquandii* strain, in a screening for new natural products. The structure was determined by spectroscopic methods, and the relative stereochemistry was confirmed by molecular modeling. The absolute stereochemistry was deduced by comparison of the CD curves with those of known members of the bisorbicillinol family. This is the first report of the isolation of a ureido sorbicillinol derivative.

Vertinoids are a family of fungal hexaketide-derived metabolites with more than 30 members, which may be monomeric or dimeric. The dimers may be Diels−Alder or Michael-type products, all derived from sorbicillinol (**1**), the key starting point of all these compounds. Bislongiquinolide (**2**),[1] bisorbicillinol,[2] and sorbiquinol[3] are interesting examples of this class of compound. The isolation of these compounds was previously not limited to a single strain and has been reported for many deuteromycetes such as *Trichoderma*[1], *Penicillium*,[4] and others.[4]

Prior to 2005, all the Diels−Alder products reported were formed from two vertinoid units, but in that year also dienophiles not related to **1** were proposed for the new metabolites, the rezishanones.[4] Also in 2005, the first Michael-adduct type sorbicillinol alkaloid was reported.[5] The biosynthetic origin of the nitrogen was proposed to be the amino acid alanine.

In the course of a program aiming at the isolation and structural elucidation of new antimicrobial fungal metabolites of diverse origin,[6−8] we have isolated a new sorbicillinoid compound with a urea group, together with known bioactive diketopiperazines from *Paecilomyces marquandii* (Massee) Hughes.

The *P. marquandii* (Massee) Hughes isolate BAFC 486 was obtained from a marine sediment and cultured in YPD medium in 100% artificial seawater. The EtOAc extract of the culture broth yielded an antibacterial fraction by chromatography on Sephadex LH-20, which yielded by HPLC the known diketopiperazines *cyclo*(L-prolyl-L-leucine), *cyclo*(L-phenylalanyl-L-valine), *cyclo*(L-phenylalanyl-L-leucine) and *cyclo*(L-leucyl-L-isoleucine)[9−11] and compound **3**. The diketopiperazines were elucidated by full NMR analysis, EIMS, and optical rotation. *cyclo*(L-Phenylalanyl-L-leucine) and *cyclo*(L-phenylalanyl-L-valine) were responsible for the weak antibacterial activity of the extract.

The molecular formula of compound **3** was shown to be $C_{17}H_{20}N_2O_5$ on the basis of its HRESIMS (*m*/*z* 331.1306 [M − H]$^-$). The ^{1}H NMR ($CDCl_3$−CD_3OD, 9:1) spectrum showed a sorboyl-like group (δ 7.33, 6.27, 6.24, 6.20, and 1.91), a three-hydrogen spin system at δ 4.76 (dd, J = 10.0 and 3.2 Hz), 4.00 (d, J = 10.0 Hz), and 3.28 (d, J = 3.2 Hz, H-4), and two methyl singlets, at δ 1.24 and 1.23. The ^{13}C NMR spectrum showed 17 carbon atoms corresponding to three methyl groups (δ 23.6, 18.4, and 8.9), three methine carbon atoms (δ 59.1, 50.2, and 46.4), four sp^2 carbon atoms at δ 142.9, 139.9, 130.5, and 117.4, and seven quaternary carbon atoms, two of which (δ 208.3 and 195.3) corresponded to carbonyl groups and one resembled a urea carbonyl at δ 163.1.

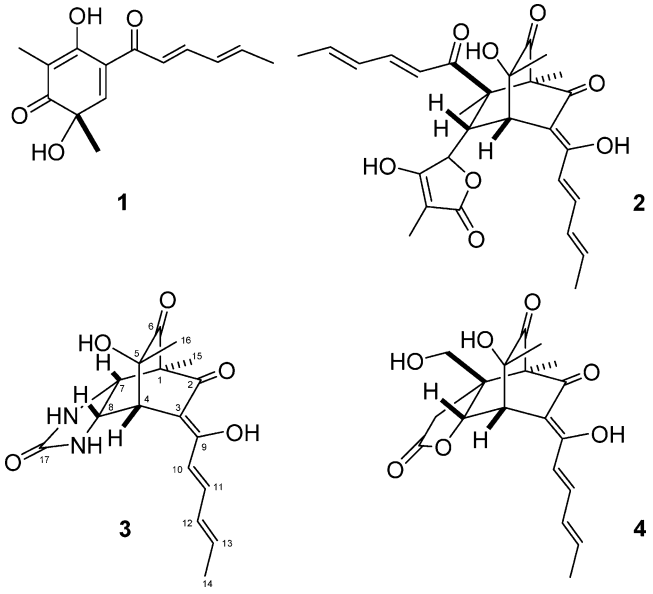

Figure 1. Structures of bislongiquinolide (**2**), rezishanone A (**4**), compound **3**, and its biosynthetic precursor sorbicillinol (**1**).

COSY and HSQC experiments allowed the confirmation of the sorboyl-like moiety and the three-hydrogen spin system mentioned above. Searches of the microbial natural products database Antibase[12] using these substructures gave bislongiquinolide (**2**)[1,13] as a close match, with ^{13}C NMR shifts of **3** being very similar to those of **2**. The HMBC spectrum allowed the connection of the above subunits and the carbonyl group at 163.1 ppm (Figure 2). These facts, combined with the evidence for the presence of two nitrogen atoms in the molecule, led us to conclude the planar structure of **3** as shown in Figure 1. NMR experiments were repeated using $(CD_3)_2SO$ as solvent to observe the exchangeable protons and their correlations. Two protons at δ 6.80 and 6.57 correlated to H-7 and H-8, respectively, in the COSY spectrum. This indicated that they were the NH protons attached to C-7 and C-8. Furthermore, the NH proton on C-7 correlated with C-8 and C17, the NH proton on C-8 correlated with C7, C8, and C17, and a proton at δ 6.09 (OH proton on C-5) correlated with C4 and C-5 in the HMBC spectrum. These additional data confirmed the above proposed structure for **3**.

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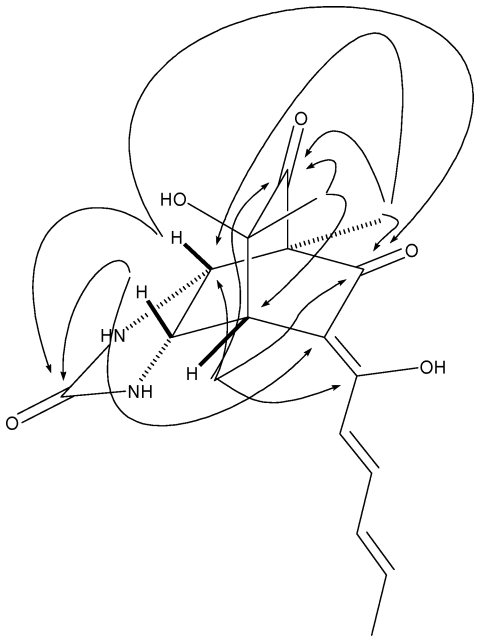

Figure 2. Key HMBC correlations (in $CDCl_3-CD_3OD$, 9:1).

The ESIMS/MS analysis of compound **3**, performed in the negative ion mode, also confirmed the proposed structure, as the main fragment corresponded to the retro-Diels−Alder fragmentation shown in Figure 3. This fragmentation was confirmed also in the EIMS spectrum, where an abundant fragment of *m*/*z* 84 was observed.

The relative stereochemistry at C-5 was based on the high-field chemical shift of the methyl group (δ 1.23) at this position, considering it is attached to an oxygen-bearing carbon atom. This observation can be attributed to the shielding effect of the enolized β-dicarbonyl system.[1] The ROESY data supported the structure; a strong correlation was observed between H-4 and H-10, and a weaker one between H-7 and H-8.

In order to confirm the *syn* configuration of H-7 and H-8, and to interpret the coupling constants between the protons H-4, H-7, and H-8, some semiempirical calculations were made using the Hyperchem Molecular Modeling Software program. Geometries of diasteroisomers with different relative configuration at C-7 and C-8 were calculated. The effects of the position of the urea protons on the coupling constants of **3** were also investigated, but appeared to have a minimal effect. Remarkably, all 16 isomers presented H7−C7−C8−H8 dihedral angles near 180° (*trans* isomers) or near 0° (*syn* isomers), predicting a large coupling constant between H-7 and H-8 in both cases, as observed in the natural compound **3**. In contrast, *trans* isomers presented H4−C4−C8−H8 dihedral angles near 90° (the expected coupling constant between H4 and H8 is close to 0 Hz), while *syn* isomers showed that angle around 60°. These calculated dihedral angles for *syn* configurations are in agreement with the observed J_{H4-H8} of 3.2 Hz in compound **3**. Although both *syn* configurations are plausible for **3**, considering the calculated dihedral angles, Diels−Alder reactions are *endo* additions, and as compound **3** clearly originated through this reaction, the configuration shown in Figure 1 was inferred. Biomimetic synthesis of bisorbicillinol[14] has confirmed this *endo* selectivity.

Compound **3** exhibited a CD curve with Cotton effects similar to all the other previously isolated Diels−Alder adducts of **1**[12,15] and particularly rezishanone A (**4**),[4] all possessing the same chiral centers. The absolute stereochemistry (1*R*, 4*R*, 5*S*, 7*R*, 8*S*) is proposed, taking into account that the same relative configuration was observed for the above compounds and **3**.

This is the first report of the isolation of a Diels−Alder product of **1** with a nitrogen-containing moiety and the second report of dienophiles not related to **1**.[4] However, none of the biosynthetic Diels−Alder precursors were detected in this work, nor any other related metabolites.

Although most organisms possess carbamoyltransferases, enzymes required for de novo biosynthesis of pyrimidines, there are few reports on the isolation of natural microbial products with a urea functionality. A steroidal derivative from the fungus *Chlorophyllum molybdites*,[16] biotin or biotin derivatives from *Streptomyces*[17] or *Phycomyces blakesleeanus*,[18] sugar derivatives from *Streptomyces* sp.,[19,20] and ureido-balhimycin from *Amycolatopsis* sp.[21] are examples of isolated ureido compounds.

Perhaps less surprising is the growing number and diversity of metabolites produced by marine fungal strains. Michael adducts of **1**[5,22] are examples of compounds isolated from these sources in the last year. Particularly, a unique spirochroman derivative[23] and a polyketide[24] were previously isolated from marine *Paecilomyces*.

Experimental Section

General Experimental Procedures. Optical rotation was recorded on a Perkin-Elmer polarimeter 343. The UV spectrum was taken on a Hewlett-Packard 8451 A diode array spectrophotometer. The CD spectrum was taken with a Jasco J-810. The FTIR spectrum was recorded on a Nicolet Magna-IR 550. NMR spectra were recorded on a Bruker AM-500 instrument at 500.13 MHz for ^1H (referenced to TMS,

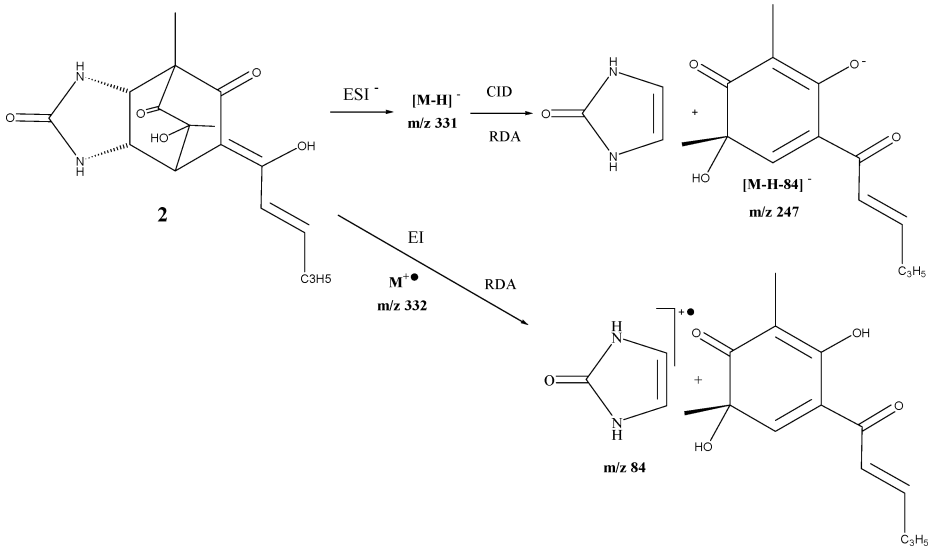

Figure 3. Main fragmentation observed by MS (RDA: retro-Diels−Alder).

$\delta = 0$) and at 125.13 MHz for ^{13}C NMR (referenced to the center line of $CDCl_3$, δ 77.0, or the center line of $(CD_3)_2SO$, δ 39.7). High-performance liquid chromatography used a variable-wavelength UV detector coupled with a refractive index detector (RefractoMonitor IV, Thermo Separation Products). EIMS was carried out on a Trio-2 VG Masslab mass spectrometer (Manchester, UK). HR-MS were recorded on a QTOF (Micromass, UK) in Qq-orthogonal time-of-flight configuration, operating at 7000 mass resolution, by R.H. and M.N.E. AM1 calculations were performed with Hyperchem 7.5 (Hyperchem Inc.).

Fungal Material and Fermentation. The fungus *Paecilomyces marquandii* (Massee) Hughes was isolated from an intertidal marine sediment sample collected at Miramar, Province of Buenos Aires, Argentina, and classified by two of the authors (R.M.A. and A.G.) according to the literature.[25] The strain was deposited in the BAFC Culture Collection (FCEN-UBA, CONICET) under the accession number BAFC 486.

The fermentation was carried out using YPD medium (0.5% Bacto yeast extract, 1% Bacto peptone, 1% dextrose in artificial seawater (Instant Ocean, 100%)). A 250 mL Erlenmeyer flask containing 75 mL of YPD medium was inoculated from agar slants of the strain *P. marquandii*. After a week at 25 °C, this medium was transferred to a 2 L Erlenmeyer flask containing 500 mL of YPD medium. These cultures were used to seed three fermentation flasks (19 L), each containing 5 L of YPD medium. Fermentation was carried out at 25 °C for 30 days under static conditions.

Extraction and Separation. The fermentation broth was filtered, and the filtrate was partitioned with EtOAc. The crude organic extract (1 g) was evaporated in vacuo and chromatographed on Sephadex LH-20 using MeOH as eluent. One of the fractions (474 mg) was subjected to preparative-scale HPLC (column: YMC C18, 5 μm, 22.5 × 2.5 cm; eluent: MeOH−H_2O (1:1), 5 mL/min; detection: UV 215 nm, RI), yielding the diketopiperazines *cyclo*(L-prolyl-L-leucine) (1.8 mg), *cyclo*(L-phenylalanyl-L-leucine) (1.0 mg), *cyclo*(L-phenylalanyl-L-valine) (2.2 mg), *cyclo*(L-leucyl-L-isoleucine) (2.7 mg), and compound **3** (3.1 mg).

Compound 3: yellow oil; $[\alpha]_D^{25}$ +50 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 362 (4.3) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$, $dm^3\ mol^{-1}\ cm^{-1}$) 351 (+7.18), 311 (−7.50) nm; FTIR (KBr) ν_{max} 3420 (NH), 3401 (NH), 3256 (OH), 2927 (CH), 2861 (CH), 1722 (CO), 1679 (CO), 1637 (CO), 1604 (CO), 1564, 1466, 1387 cm^{-1}; ^1H NMR ($CDCl_3$−CD_3OD 9:1, 500 MHz) δ 7.33 (dd, J = 14.8, 10.5 Hz, H-11), 6.27 (m, H-12), 6.24 (m, H-13), 6.20 (d, J = 14.8 Hz, H-10), 4.76 (dd, J = 10.0, 3.2 Hz, H-8), 4.00 (d, J = 10.0 Hz, H-7), 3.28 (d, J = 3.2 Hz, H-4), 1.91 (d, J = 6.4 Hz, H-14), 1.24 (s, H-15), 1.23 (s, H-16); ^1H NMR ($(CD_3)_2$-SO) 14.20 (br s, *H*O-9), 7.23 (dd, J = 14.8, 10.4 Hz, H-11), 6.80 (s, N*H*-C7), 6.57 (s, N*H*-C8), 6.44 (d, J = 14.8 Hz, H-10), 6.36 (m, H-12), 6.28 (m, H-13), 6.09 (s, *H*O-5), 4.50 (br m, H-8), 3.87 (br m, H-7), 3.23 (d, J = 2.8 Hz, H-4), 1.86 (d, J = 6.4 Hz, H-14), 1.07 (br s, H-16), 1.06 (br s, H-15); ^{13}C NMR ($CDCl_3$−CD_3OD, 9:1, 125 MHz) δ 208.3 (qC, C-6), 195.3 (qC, C-2), 169.8 (qC, C-9), 163.1 (qC, C-17), 142.9 (CH, C-11), 139.9 (CH, C-13), 130.5 (CH, C-12), 117.4 (CH, C-10), 106.2 (qC, C-3), 73.8 (qC, C-5), 64.8 (qC, C-1), 59.1 (CH, C-7), 50.2 (CH, C-8), 46.4 (CH, C-4), 23.6 (CH_3, C-15), 18.4 (CH_3, C-14), 8.9 (CH_3, C-16); HMBC ($(CD_3)_2SO$) H-4 (C-3, C-5, C-6, C-7, C-8), H-7 (C-2, C-15), H-10 (C-9, C-12), H-11 (C-13), H-13 (C-14), H-14 (C-12, C-13), H-15 (C-1, C-2, C-6, C-7), H-16 (C-4, C-5, C-6), *H*O-C5 (C-4, C-5), N*H*-C7 (C-8, C-17), N*H*-C8 (C-7, C-8, C-17); EIMS 70 eV, *m*/*z* 332 $[M]^{+\cdot}$ (9), 314 (1), 249 (19), 205 (11), 137 (13), 95 ($C_5H_7CO^+$, 100), 84 (53), 43 (79); HRESIMS *m*/*z* $[M - H]^-$ 331.1306 (calcd for $C_{17}H_{20}N_2O_5$, 331.1293; ESI MS/MS (331 u), *m*/*z* 331 $[M - H]^-$ (68), 247 $[M - H - 84]^-$ (100), 83 (6).

Antibiotic Activity. The antibiotic activity was determined by the agar diffusion method. *Bacillus subtilis* ATCC 6633 (BS) and *Escherichia coli* ATCC 25922 (EC) were used as test organisms. Gentamicin was used as the positive control compound. Inhibition halos: *cyclo*(L-Phe-L-Val), 9 mm (BS), 8 mm (EC); *cyclo*(L-Phe-L-Leu), 10 mm (BS), 8 mm (EC).

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