N-Acetyl-γ-hydroxyvaline Lactone, an Unusual Amino Acid Derivative from a Marine Streptomycete

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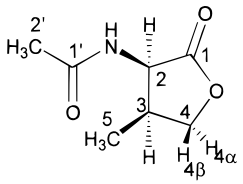
We report the isolation and structure elucidation of *N*-acetyl-γ-hydroxyvaline lactone (**1**) from a streptomycete obtained from marine sediments collected in the north coastline of São Paulo State, Brazil.

Signal-transduction mediated by low molecular weight γ-butyrolactone derivatives has been described as a key factor for the control of the secondary metabolism and other biochemical processes of several microorganisms belonging to genera *Streptomyces*, *Vibrio*, *Erwinia*, *Pseudomonas*, *Serratia*, and *Agrobacteria*.[1] The regulation of secondary metabolism by structurally related γ-butyrolactone derivatives includes the start of secondary metabolism production in wild-type strains of *Streptomycetes* and the enhancement of secondary metabolism production in strains that produce these chemical mediators.[1] Another chemically mediated signaling between bacteria has been recently described as the key factor for the recognition of the bacterial population density.[2] These chemical mediators, called bacteria quorum-sensing signals, have been identified as being proteins, as well as acyl-homoserine lactone (acyl-HSL) derivatives, which are present only in Gram-negative bacteria. It has been demonstrated that different acyl-HSL derivatives are the key signaling factors for the control of bacterial aggregation implied, for example, in tissue infection by *Pseudomonas aeruginosa*, in the antibiotic production by the plant-associated bacterium *P. aureofasciens*, and in the light emission by marine bacteria belonging to the genus *Vibrio*.[1,2] Therefore, these γ-butyrolactone derivatives are of prime importance to better understand the biochemical process involved in the production of economically important natural products, as well as quorum-sensing signals, which are implied in human infection by pathogen bacteria.

Marine microorganisms represent one of the less explored sources of biologically active natural products.[3−13] During a chemical screening of crude extracts obtained from a streptomycete isolated from marine sediments, we have obtained a major compound isolated by Si gel chromatography. Herein we report the isolation and structure elucidation of a new amino acid derivative, *N*-acetyl-γ-hydroxyvaline lactone (acyl-HVL, **1**), isolated from the culture medium of a marine streptomycete.

N-Acetyl-γ-hydroxyvaline lactone (**1**) was isolated as a glassy solid, $[\alpha]_D$ + 20.1 (*c* 0.01, MeOH). HRFABMS analysis of **1** indicated a formula $C_7H_{11}NO_3$ (measured for M + 1: 158.08120, Δmu 3.28 ppm), with three unsaturation degrees. The IR spectrum of **1** showed the presence of



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secondary amide (ν_{N-H}: 3257, 3200 cm^{-1}) and lactone ($\nu_{C=O}$: 1779, 1172, 1137, 1079, 1056 cm^{-1}) functional groups. The ^1H, ^{13}C (BBD and DEPT), and HSQC NMR spectra indicated the presence of two methyl groups, at δ 0.98 (doublet, 7.2 Hz; $\delta^{13}C$ 12.8) and at 2.08 (singlet; $\delta^{13}C$ 22.7); the latter was assigned to a methyl group in position α to a carbonyl. Additionally, we observed signals of a diastereotopic methylene at δ 4.45 (dd, 5.1 and 9.2) and 4.11 (d, 9.2) (δ ^{13}C 72.8); two methine groups at δ 3.00 (ddq; $\delta^{13}C$ 34.0) and 4.77 (dd, 6.4 and 6.3 Hz; $\delta^{13}C$ 53.5); and two quaternary carbons (δ 170.7 and 175.3). The presence of a *N*-acetyl moiety was inferred because we detected an active hydrogen signal at δ 6.64, which was coupled in the HMBC spectrum with the δ 170.7 carbonyl quaternary carbon, and the latter was connected with the methyl signal at δ 2.08. The double doublet methine proton signal at δ 4.77 suggested an α-amino acid derivative moiety. The $^1H-^1H$ COSY spectrum showed a spin system with sequential couplings between the N−H proton at δ 6.64 and the methine signal at δ 4.77; the latter was coupled with the ddq methine at δ 3.00, which in turn has been shown to be adjacent to the methyl group at δ 0.98 and to the diastereotopic methylene signals at δ 4.45 and 4.11. Based on the $^1H-^1H$ COSY spectrum analysis, the structure of a cyclized amino acid derivative was envisaged. Our hypothesis was corroborated by analysis of the long-range correlations observed in the HMBC (Figure 1) and HSQC−TOCSY spectra.

The relative stereochemistry of **1** was established by molecular modeling analysis after calculation of the minimal conformational energy for each of the two possible diastereomers. Only the stereochemistry of **1** showed agreement with the coupling constants observed in the ^1H NMR spectrum and the dihedral angles measured after the calculation of the minimal conformation energy for each of the two possible stereoisomers. The key coupling constant analyzed was that measured between H4β and H3, which is 0 Hz. Therefore, the dihedral angle H4β−C4−C3−H3 must be very close to 90°. The calculated value for the

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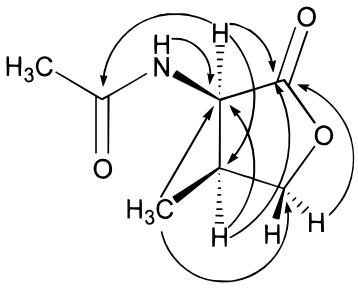

Figure 1.

H4β−C4−C3−H3 dihedral angle of **1** was 90.4°, with an excellent agreement with the experimental value of the coupling constant between H4β and H3. The molecular modeling analysis was confirmed by differential NOE experiments. Irradiation of the H-2 methine proton signal at δ 4.77 increased the intensity of the H-3 methine proton signal at δ 3.00 and of the H-4α proton signal at δ 4.45, indicating these three hydrogens were at the same side of the lactone ring. Additionally, irradiation of the doublet methyl signal at δ 0.98 increased the intensity of the H-4β doublet signal at δ 4.11, confirming the relative stereochemistry. We have, therefore, established the complete stereostructure of acyl-HVL, excluding the absolute stereochemistry.

Biogenetically it seems obvious that **1** is derived from the oxidation of valine, followed by lactonization and *N*-acetylation. The amino acid γ-hydroxyvaline is present in aureobasidin D, a cyclic peptide isolated from the terrestrial bacterium *Aureobasidium pullulans*.[14] The close similarity between the structure of acyl-HSL derivatives and γ acyl-HVL (**1**) suggests that **1** may be a chemical mediator of a related biochemical process in the marine streptomycete isolated from marine sediments collected in Brazilian coastline. Clearly, further studies are required to establish the biochemical role of acyl-HVL in the marine streptomycete that we have isolated, and we are endeavoring to address this issue.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a FTIR Bomem MB102 infrared spectrometer. NMR spectra were run either on a Bruker AC-4.7 T spectrometer, operating at 200.1 MHz for ^{1}H and 50.3 MHz for ^{13}C NMR spectra, or on a Bruker ARX 9.4 T instrument, operating at 400.35 MHz for ^{1}H and 100.10 MHz for ^{13}C channels. All the NMR spectra were obtained at 28 °C using tetramethylsilane as internal reference. HRFABMS were obtained on hybrid Kratos concept IIHQ equipment. Solvents employed for extraction and column chromatography were glass-distilled prior to use. TLC analysis was performed with Aldrich precoated TLC sheets of silica gel on polyester with 254-nm fluorescent indicator eluting with two eluents: hexanes−ethyl acetate, 1:1, and CH_2Cl_2−MeOH, 9:1. Plates were developed by observing at 254 nm and subsequently by spraying with phosphomolybdic acid reagent in ethanol and further heating at 120°.

Microorganism Collection, Isolation, and Growth. The bacterium, designated SS99BA-2, was obtained from samples of sediments collected with a Kojak apparatus, at depths between 12 and 15 m in the São Sebastião channel (north coastline of São Paulo State, Brazil). Samples of sediments were immediately processed at the Marine Biology Station of the Universidade de São Paulo; aliquots of sediments were inoculated in Petri dishes containing different culture media. Marine streptomycetes were selected using standard growing conditions:[15] soluble starch 1 g/100 mL; casein 0.1 g/100 mL, agar 1.5 g/100 mL, pH 7.0−7.5. Spread plates were incubated at 25 °C for two weeks, then single colonies were harvested and restreaked for purity. Each species of streptomycete was grown in 1 L of marine broth 2216 (Difco) with 1% soluble starch, at 25 °C for 5 days at 200 rpm. Culture media were processed as follows: ethyl acetate was added to the culture medium and left overnight under magnetic stirring. After filtration through a Whatman #1 paper, ethyl acetate was separated from the aqueous phase by liquid−liquid partition. The organic phase was evaporated, yielding crude extracts, which were analyzed by ^{1}H NMR spectroscopy and by TLC. Based on the chemical and spectroscopic analyses, the crude extract of strain SS99BA-2 was selected for isolation and identification of chemical constituents.

Isolation of *N*-Acetyl-γ-hydroxyvaline Lactone (1). The crude extract of strain SS99BA-2 (0.63 g) was subjected to liquid chromatography on a Si gel open column with a gradient of EtOAc in CH_2Cl_2, then a gradient of MeOH in CH_2Cl_2. Crude acyl-HVL (**1**) was obtained in the fractions eluted with 7:3 CH_2Cl_2−EtOAc. A second Si gel chromatography of crude **1** with a gradient of EtOAc in CH_2Cl_2 yielded 14 mg of pure acyl-HVL lactone (**1**).

***N*-Acetyl-γ-hydroxyvaline Lactone (1):** glassy solid; IR (film) 3257, 3200, 3065, 2973, 2920, 1779, 1660, 1553, 1451, 1391, 1374, 1326, 1294, 1218, 1172, 1137, 1079, 1056, 989, 976, 921, 872, 716, 656, 613, 584, 531, 514, 394 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.98 (d, 7.2; H_3C-5); 2.08 (s; H_3C-2′); 3.00 (ddq, 5.1, 6.4, 9.3; H-3); 4.11 (d, 9.3; H-4β); 4.44 (dd, 5.1 and 9.2; H-4α); 4.77 (dd, 6.4 and 6.4; H-2); 6.64 (br s; N−H); ^{13}C NMR ($CDCl_3$, 100 MHz): 12.8 (H_3C-5); 22.7 (H_3C-2′); 34.0 (CH-3); 53.5 (CH-2); 72.7 (CH_2-4); 170.7 (C-1′); 175.3 (C-1); HRFABMS *m*/*z* 158.08120 [M + 1] (calcd). For $C_7H_{12}NO_3$, 158.08172); FABMS *m*/*z* (rel int) 158 (49), 135 (17), 123 (29), 109 (55), 95 (80), 83 (100), 71 (90).

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Supporting Information Available: LSIMS scan of compound **1**, acyl-HVL, $C_7H_{11}NO_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

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