Supplementary Information

IB-01212, A NEW CYTOTOXIC CYCLODEPSIPEPTIDE ISOLATED FROM THE MARINE FUNGUS *Clonostachys pityrodes*

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EXPERIMENTAL PROCEDURES

General Experimental Procedures

NMR spectra were acquired on a 400 MHz for ^1H , 100 MHz for ^{13}C NMR spectrometer. Chemical shifts are reported in ppm referenced either to the CHCl_3 peak at 7.26 ppm for ^1H and 77.0 ppm for ^{13}C or to tetramethylsilyl chloride (TMS).

Mass spectra were recorded on a hybrid quadrupole time-of-flight (Q-TOF) mass spectrometer equipped with a gas nebulizer probe. The ESI drying and nebulizer was nitrogen, and the collision gas for MS/MS measurement was argon. The source temperature was 80° C, the ESI probe (needle) voltage was 3 kV and the sample cone voltage was 80 V. Samples were dissolved in water/methanol (v/v, 1:1) and experiments were performed in positive mode using a scan range from m/z 100 to 1500.

Marfey method

The stereochemistry of the amino acids in IB-01212 was determined by Marfey methods. Thus, by comparison of the retention times and co-elution, the three amino acids were confirmed to be L-Ser, L-*N*-MeLeu and L-*N*-MePhe (figure 4).

Figure 1. HPLC chromatograms of (a) amino-acid-DPAA standards, (b) DPAA derivatives of the hydrolyzed natural peptide (c) co-injection of the amino-acid-DPAA standards and DPAA derivatives of the hydrolyzed natural peptide. Samples were run on a Waters system equipped with a C₁₈ Symmetry column (4.6 x 150 mm, 5 μ m), using 0.045% aqueous TFA (Solvent A) and 0.036% TFA in ACN (Solvent B) in a linear gradient (20% to 40% B, 70 min) at a flow rate of 1 mL/min, and UV detection at 340 nm.

1-Menthol method

The 1-menthol method was used to determinate the absolute configuration of the N,N' -Me₂Leu-OH (figure 5). The stereochemistry of the N,N -Me₂Leu was clearly established to be L by comparison of the retention times and co-elution. HPLC-MS analysis revealed a peak corresponding to the 1-menthol derivative of N,N -Me₂-Leu (calculated m/z, 297.3 and found m/z, 298.4).

Figure 2. HPLC chromatograms of (a) co-elution of the 1-menthol derivatives of both N,N -Me₂Leu and the hydrolyzed natural peptide; (b) of the 1-menthol derivatives of both D- N,N -Me₂Leu and hydrolyzed natural peptide. Samples were run on a Waters system equipped with a Symmetry C₁₈ column (4.6 x 150 mm, 5 μ m), using 0.045% aqueous TFA (Solvent A) and 0.036% TFA in ACN (Solvent B) in a linear gradient (25% to 40% B, 70 min) at a flow rate of 1 mL/min, and UV detection at 260 nm.

Analysis by RP-HPLC

In order to confirm the proposed structure of IB-01212, the peptide was synthesized on solid phase. As observed in figure 6, the synthetic cyclodepsipeptide and an authentic sample of IB-01212 co-eluted by HPLC.

Figure 3. HPLC chromatograms of (a) IB-01212 natural; (b) synthetic cyclodepsipeptide obtained by solid phase synthesis;⁵ (c) co-elution of (a) and (b). Samples were run on a Waters system equipped with a Symmetry C₁₈ column (4.6 x 150 mm, 5 μ m), using 0.045% aqueous TFA (Solvent A) and 0.036% TFA in ACN (Solvent B) in a linear gradient (20% to 50% B, 40 min) at a flow rate of 1 mL/min, and UV detection at 220 nm.

Table 1. ^1H and ^{13}C NMR data of the Synthetic Cyclodepsipeptide in ACN- d_3

Position	^{13}C (δ)	^1H (δ)	Position	^{13}C (δ)	^1H (δ)
<i>N</i>-MePhe			Ser		
<i>N</i> -CH ₃	30.82	2.78 (S)	NH		8.28 (broad)
αCH	63.4	5.68 (broad)	αCH	49.7	5.02 (broad)
βCH_2	36.3	2.98 (m) 3.36(dd, 5.4, 14.7)	βCH_2	63.6	4.61 (broad) 4.36 (broad)
1-C	136.7		CO	166.3	
2,6-CH	129.1	7.27(m)	<i>N, N</i>-DiMeLeu		
3,5-CH	128.7	7.29(m)	<i>N</i> -CH ₃	40.5	2.88 (S)
4-CH	127.1	7.25(m)	<i>N'</i> -CH ₃	40.5	2.88 (S)
CO	170.7		αCH	65.6	4.02 (broad)
<i>N</i>-MeLeu			βCH_2	37.1	2.01 (m) 1.54 (m)
<i>N</i> -CH ₃	30.88	2.83 (S)	γCH	29.9	1.25 (m)
αCH	51.8	5.30 (broad)	δCH_3	21.7	0.93 (d,6.5)
βCH_2	38.1	1.10 (m) 0.97 (m)	δCH_3	23.4	0.90 (d,6.5)
γCH	24.8	0.94 (m)			
δCH_3	23.0	0.58 (d, 7.0)	CO	172.2	
δCH_3	23.2	0.62 (d, 7.1)			
CO	170.4				

NMR spectra were acquired on a 400 MHz at Barcelona Science Park. All the spectra were registered in ACN- d_3 , at 20°C and using TMS as reference.

Table 2. Fragments observed in the collision-induced dissociation (CID) of the peptide ion by nanoelectrospray ionization.

IB-01212 MW (found)[M+1] ⁺	Synthetic Peptide MW(found)[M+1] ⁺	MW (calculated)	Assigned structure
920.44	920.57	919.54
892.43	892.58	891.55
874.42	874.57	873.54
847.42	847.54	846.55
823.45	823.55	822.53
761.36	761.49	760.42
713.41	713.48	712.45

696.37	696.45	695.43
518.27	518.35	517.34
473.21	472.30	472.34
358.17	358.22	357.21
338.20	338.26	337.24
293.16	293.19	292.22
225.10	225.13	224.12
211.13	211.15	210.14
197.10	197.14	196.12
134.08	134.09	133.07
114.10	114.12	114.13