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Leopolic acid A, isolated from a terrestrial actinomycete, *Streptomyces* sp.

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ABSTRACT

Chemical analysis of a terrestrial-derived *Streptomyces* sp. isolated from the rhizosphere of the plant *Juniperus excels* collected from the Crimean Mountains (Ukraine) yielded a new acid, leopolic acid A (**1**). Leopolic acid A (**1**) was identified to possess a rare ureido dipeptide, Phe-CO-Val, attached to a 5-dihydro-3-hydroxy-pyrrole-2-one ring. A detailed spectroscopic and Marfey's analysis led to the structure elucidation of leopolic acid A (**1**).

During our recent investigations into the secondary metabolism of rare terrestrial-derived actinomycetes, we recovered a *Streptomyces* sp. (1–21) from a soil sample collected from Ukraine. HPLC-DAD-MS analysis of a small-scale liquid cultivation (100 mL) revealed the production of metabolite (**1**), *m*/*z* 529. HPLC fractionation of a larger scale liquid culture (6 L) permitted the recovery of **1**. An account of the spectroscopic analysis leading to the structural assignment is presented below.

HRESI(+)MS analysis of **1**[1] revealed a pseudomolecular ion ($[M+H]^+$) indicative of a molecular formula ($C_{29}H_{43}N_3O_6$) requiring 10 double bond equivalents. The ^{13}C NMR (DMSO-d_6) data (Table 1) revealed 4 ester/amide carbonyls (δ_C 157.8, 166.5, 172.5, and 174.2) and a further 8 sp^2 resonances (δ_C 126.6 to 141.7), accounting for 8 DBE and requiring 2 rings. Consideration of the 1D and 2D NMR data revealed a mono-substituted benzene ring [COSY correlations from H-5 to H-9 extended by HMBC correlations to the methylene C-3 (δ_C 37.9) which further extended to a deshielded methine C-2 (δ_C 54.3) and the amide NH (δ_H 6.35). HMBC correlations were observed from the amide NH (δ_H 6.35) and methine H-2 (δ_H 4.29) to the ester/amide carbonyl C-1 (δ_C 174.2)]. This led to the construction of the amino acid residue phenylalanine (C-1–C-10). Further examination of the ^1H and COSY NMR data documented an isolated spin system, indicative of two secondary methyls H_3-15 and H_3-16 ($\delta_{H's}$ 0.91 and 0.75) extending through the deshielded methine H-13 (δ_H 5.36) to the amide NH (δ_H 6.47). HMBC correlations were observed from the amide NH to the ester/amide carbonyl C-17 (δ_C 172.5). The generation of the fragment C-13–C-17 was reminiscent of the amino acid residue, valine. HMBC correlations from both the α protons H-2 (δ_H 4.29) and H-13 (δ_H 5.36) to the amide carbonyl C-11 (δ_C 157.8) linked the two amino acid residues together generating subunit A (Fig. 1). The remaining structure fragment of **1**, subunit B consisted of an isolated spin system, indicative of a primary methyl H_3-32 (δ_H 0.85) linked through a set of methylenes generating the aliphatic chain C-23–C-32.

A deshielded methylene H_2-22 (δ_H 4.00, 4.09) (δ_C 46.5) suggested its connectivity to a nitrogen, which in turn with the terminal methylene H_2-23 (δ_H 2.30) showed HMBC correlations to one another and to the quaternary carbons C-20 (δ_C 141.7) and C-21 (δ_C 129.2). The downfield chemical of C-20 suggested its attach-

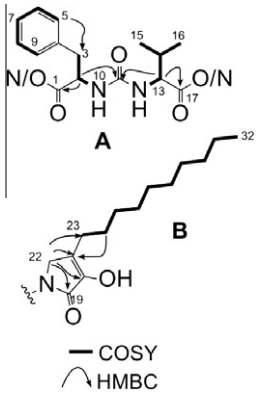

Figure 1. Key 2D NMR (500 MHz, DMSO-d_6) correlations for **1**.

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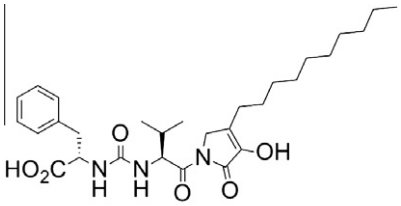
Table 1
NMR (500 MHz, DMSO-d_6) data for leopolic acid A (**1**)

Pos	δ_H, Mult (J in Hz)	δ_C	COSY	HMBC
1		174.2		
2	4.29, m	54.3	3a/b, 10	1, 3, 4, 11
3a	2.99, dd (13.7, 5.2)	37.9	2, 3b	1, 2, 4, 5, 9
3b	2.84, dd (13.7, 5.3)		2, 3a	1, 2, 4, 5, 9
4		137.9		
5	7.19, d (7.4)	129.6	6	3, 7, 9
6	7.28, dd (7.6, 7.4)	128.5	5, 7	4, 8
7	7.21, dd (7.6, 7.7)	126.6	6, 8	5, 9
8	7.28, dd (7.6, 7.4)	128.5	7, 9	4, 6
9	7.19, d (7.4)	129.6	8	3, 5, 7
10	6.35, d (8.0)		2	1, 2, 3, 11
11		157.8		
12	6.47, d (9.3)		13	11, 13, 14, 17
13	5.36, dd (9.3, 3.7)	56.9	12, 14	11, 14, 15, 16, 17
14	2.00, m	30.2	13, 15, 16	13, 15, 16, 17
15	0.91, d (6.8)	19.9	14	13, 14, 16
16	0.75, d (6.8)	16.6	14	13, 14, 15
17		172.5		
19		166.5		
20		141.7		
21		129.2		
22a	4.09, d (18.6)	46.5		17, 19, 20, 21, 23
22b	4.00, d (18.6)			17, 19, 20, 21, 23
23	2.30, m	25.2	24	19, 20, 21, 22, 24, 25
24	1.45, m	27.2	23, 25	21, 23, 25, 26
25	1.25, m[a]	29.2	24, 26	
26	1.25, m[a]	29.2	25, 27	
27	1.25, m[a]	29.2	26, 28	
28	1.25, m[a]	29.2	27, 29	
29	1.25, m[a]	29.2	28, 30	
30	1.25, m[a]	22.3	29, 31	31, 32
31	1.22, m[a]	31.7	30, 32	
32	0.85, t (6.4)	14.3	31	30, 31
20-OH	9.45, s			19, 20, 21

[a] Overlapping signals, ^{13}C shifts obtained from 2D HSQC and HMBC experiments.



1

Figure 2. Structure of leopolic acid A (**1**).

ment to a heteroatom, in this case hydroxyl functionality (δ_H 9.45). Also observed were correlations from H_2-22 to an amide carbonyl C-19 (δ_C 166.5). A large coupling (J = 18.6 Hz) for the methylene H_2-22 and to accommodate for the remaining DBE led to the construction of the 5-dihydro-3-hydroxy-pyrrole-2-one ring (C-19–C-22) (Fig. 1).

Finally a HMBC correlation from the methylene H_2-22 to C-17 of subunit A led to the formulation of the planar structure of **1** (Fig. 2). Marfey's analysis[2] of **1** (Fig. S5) confirmed the presence of L-Phe and L-Val residues, thereby assigning a 2*S*, 13*S* configuration (Fig. 2).

Ureido-peptides with varying number of amino acids have been previously isolated from actinomycetes. Noteworthy examples of ureido peptides include the microbial alkaline protease inhibitors, (MAPI)-α and β,[3] GE20372 factor A and B, as HIV-1 protease inhibitors,[4] and the pacidamycins,[5] exhibiting anti-*Pseudomonas aeruginosa* activity. Closest resemblance of the substituted pyrrole-2-one ring is found in the tetramic acid incorporating metabolites which include the nocamycins, isolated from a terrestrial *Nocardiopsis* sp.[6] Lydicamycin, isolated from *Streptomyces* showing selective Gram +ve antibacterial activity,[7] and TPU-0037-B, isolated from the marine-derived *Streptomyces* sp. exhibiting antimicrobial activity against MRSA.[8] The examples were not limited to microbes but spanned to cyanobacteria, isomalyngamide A isolated from *Lyngbya majuscule*.[9] Leopolic acid (**1**), has unprecedented structural features consisting of an aliphatic side chain attached to the novel pyrrole-2-one residue connected to the ureido dipeptide L-Phe-L-Val.

Leopolic acid A (**1**) was screened against a panel of Gram positive and negative bacterial strains, but to date did not show any signs of activity, neither did it exhibit any cytotoxic activity (Table S1). However, it did show weak antifungal and antibacterial activity against *Mucor hiemalis* and *Staphylococcus aureus* with an MIC of 32 and 16 μg/mL, respectively, (Table S1). In summary, the structural novelty of leopolic acid A (**1**) is intriguing and warrants an indepth analysis on the biosynthesis of this metabolite.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.09.046.

References and notes

1. Leopolic acid A (**1**): clear oil; $[\alpha]_D$ + 34 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.71), 260 (4.27); NMR (500 MHz, DMSO-d_6) see Table 1; HRESI(+)MS *m*/*z* 530.3198 (Calcd for $C_{29}H_{44}N_3O_6$, 530.32313).
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