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Cyclic lipoundecapeptide lokisin from *Pseudomonas* sp. strain DSS41

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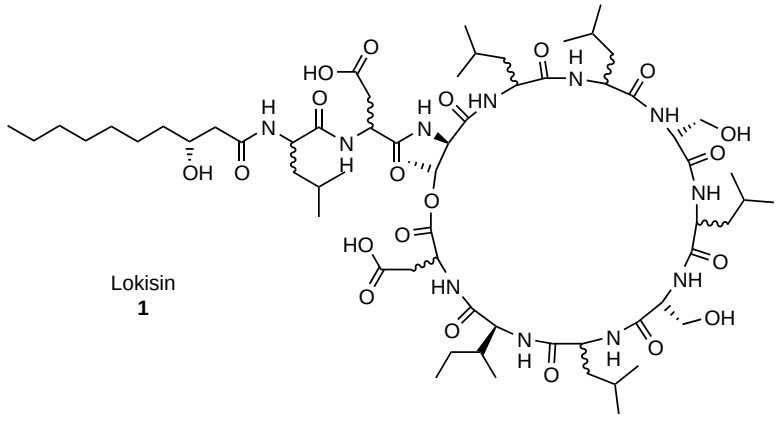
Abstract—Lokisin was isolated from *Pseudomonas* sp. strain DSS41 as part of a study of prospective anti-fungal bio-control agents. Based on NMR and MS studies, lokisin was tentatively identified as pholipeptin. However, detailed analysis of the amino acid constituents by chiral gas chromatography revealed a different D-/L-leucine ratio of 3:2 and the *allo*-isomer of threonine. Lokisin represents a new structural variation in the cyclic lipoundecapeptide class. © 2002 Elsevier Science Ltd. All rights reserved.

The use of an organism to antagonize and suppress other organisms in a specific ecosystem is called biological control. Our present study aims at the recovery of anti-fungal fluorescent *Pseudomonas* spp. from sugar beet rhizosphere, evaluation of their utility as bio-control agents and characterization of their bioactive metabolites. The expression of a diverse array of secondary metabolites is characteristic for fluorescent *Pseudomonas* spp. classified as plant disease bio-control phenotypes.[1] We have previously isolated the cyclic lipopeptides viscosinamide and tensin from soil-derived *Pseudomonas* spp. and described their activity against fungal root pathogens *Pythium ultimum* and *Rhizoctonia solani*.[2,3] X-Ray crystallographic structures were established for the cyclic lipoundecapeptides tensin and amphisin.[4,5] The latter was isolated as part of further investigations of anti-fungal fluorescent *Pseudomonas* spp. The present paper reports the structure of the analogous cyclic lipoundecapeptide lokisin **1**. These structures provide initial data for studies of anti-fungal structure–activity relationships as well as information regarding the nature of the implicated non-ribosomal peptide synthetases (NRPS).

In our collection of soil-derived *Pseudomonas* spp., the strain DSS41 was selected as a potential bio-control agent based on the antagonistic characteristics and HPLC analysis of the metabolite profile.[6] DSS41 was



Lokisin
1

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cultured on 50 Petri dishes of Potato Dextrose Agar (Difco 0013-17-6) at 25°C for 4 days. Extraction with EtOAc yielded 414 mg of a crude extract. After initial sample preparation steps by C18 VLC and Si60 MPLC, preparative C18 HPLC afforded 20.1 mg pure lokisin **1** as a white powder. We were not able to obtain suitable crystals for X-ray crystallography.

The $[M+H]^+$ ion in the ESI-QTOF-MS at m/z 1354.809 was appropriate for the molecular formula $C_{64}H_{111}N_{11}O_{20}$ ($[M+H]^+$ calcd 1354.809). ^1H, ^{13}C, COSY, HMQC, HMBC, and ROESY spectra of **1** in DMSO-d_6 were acquired on a Varian 400 MHz FT-NMR spectrometer. Initial analysis of these data led to the erroneous conclusion that **1** was identical to the phospholipase C inhibitor pholipeptin (Fig. 1).[7]

The NMR spectra of lokisin **1** and pholipeptin are virtually identical. The major part of the observations and arguments used throughout the structural elucidation of pholipeptin was directly reproduced for lokisin **1** and led to the establishment of the same primary sequence.[7]

The lactone is formed by C-terminal/threonine–hydroxyl linkage rather than the C-terminal side chain linkage assumed for pholipeptin. The Asp(12) γ-CO to Thr(4) β-H HMBC correlation argument, used in the elucidation of pholipeptin, did not seem valid. The particular correlation was also observed in the NMR spectrum of amphisin and, as this interpretation would contradict the established crystal structure, the signal was reassigned as Asp(12) α-CO to Thr(4) β-H.

Furthermore, during the structural elucidation of amphisin from the related *Pseudomonas* sp. strain DSS73, it was necessary to confirm the stereochemistry indicated in the X-ray crystallographic structure. The peptide was hydrolyzed with 6 M HCl and the constituents, derivatized as pentafluoropropionyl amide isopropyl esters, were subsequently analyzed by chiral gas chromatography (GC). Lokisin **1** was used for comparison and to test the procedure for chiral GC. The only difference between lokisin **1** and amphisin in the chiral GC-chromatogram was the presence of an additional D-Ser and lack of D-Glu. Further co-injections of prepared standards and the establishment of the X-ray crystallographic structure for amphisin, confirmed that the amino acid composition of **1** is 3×D-Leu, 2×L-Leu, L-Ile, D-Asp, L-Asp, 2×D-Ser, and *allo*-Thr. These stereochemical characteristics do not correspond with the data from pholipeptin and the structure of lokisin **1** must be different. It was not possible to obtain an original sample of pholipeptin for comparison.

Until the exact total configuration of **1** can be confirmed by total synthesis, we propose that the present structure **1** should be adopted. A hypothesis regarding the possible configuration may be derived from comparison with closely related compounds (Fig. 1). The structures of amphisin and tensin were determined by X-ray crystallography and differ only by the Asp(12)/Glu(12) amino acids. Also, lokisin **1** differs only from amphisin by the Ser(9)/Gln(9) substitution. Given their common origin by NRPS, the initial assumption would be that their overall stereo-configurations are similar. The structure of lokisin **1** may be of interest to researchers wishing to synthesize pholipeptin analogs as phospholipase C inhibitors for signal transduction studies.

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Figure 1. Comparison of structures: lokisin **1**, amphisin **2**, tensin **3**,† and pholipeptin **4**. #, ¤ marks interchangeable stereochemistry assignments. 3HDA is 3-hydroxydecanoic acid.

† Fig. 1 in the original article depicts the correct stereochemistry of the 3-hydroxydecanoic acid as (*R*)—it was erroneously stated as (*S*) in the text.

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