ABSTRACT (EN)

This work aims at preparation, analysis and isolation of intermediates of biosynthetic pathways of 4-alkyl-L-proline derivatives for their structural elucidation. Compounds with incorporated 4-alkyl-L-proline derivatives include clinically used lincosamide antibiotic, lincomycin A, antitumor pyrrolobenzodiazepines and bacterial hormone hormaomycin. Detailed knowledge of biosynthetic pathways of these biological active substances can be used to prepare new, more efficient derivatives.

The first part of this work focuses on yellow-coloured dicarboxylic intermediates **1** and **2** of the biosynthetic pathway of 4-propyl-L-proline – the precursor of lincomycin A. In the presence of the methylation agent, *S*-adenosyl-L-methionine, and LmbW C--methyltransferase, **1** was partially converted into intermediate **2**. Using ultra-high performance liquid chromatography, both intermediates were identified from absorption and mass spectrometry spectra. A semi-preparative chromatographic method for isolation of both intermediates was developed. Surprisingly, a significantly lower stability of **2** compared to intermediate **1** was observed in an *in vitro* enzymatic reaction mixture.

The second part of the work focuses on 4-ethylidene-L-proline – the precursor of tomaymycin belonging to pyrrolobenzodiazepines. After optimization of the solid-phase extraction method, 4-ethylidene-L-proline was identified in the culture medium of *Streptomyces purpureus* using ultra-high performance liquid chromatography with mass spectrometry detection. For the development of the method for 4-ethylidene-L-proline isolation, structurally similar 4-alkyl-L-proline derivatives were used. For separation of the analytes in the semi-preparative high performance liquid chromatography mode, ammonium formate and ammonium acetate of different ionic strengths and pH as the aqueous part of the mobile phase were tested. The optimized isocratic method was used to isolate 0.7 mg of 4-ethylidene-L-proline from culture broth for subsequent determination of *E*/*Z* configuration at the exocyclic double bond by nuclear magnetic resonance.