DETERMINATION OF VALINE AND LEUCINE ISOMERS IN PEPTAIBOLS

GÁBOR ENDRE[1,2], DÓRA PAPP[1], TAMÁS MARIK[1], LÁSZLÓ KREDICS[1], MÓNIKA VARGA[1], CSABA VÁGVÖLGYI[1], ANDRÁS SZEKERES[1]

[1]University of Szeged, Faculty of Science and Informatics, Department of Microbiology, Közép fasor 52. Szeged H-6726
[2]Doctoral School in Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary
egabcy@gmail.com (G.E.), andras.j.szekeres@gmail.com (A.Sz.)

Peptaibols are peptid-like oligomers produced as extracellular secondary metabolites by *Trichoderma* species. Some peptaibols known as effective antibacterial and antifungal agents. They are amphiphilic, which allows them to form voltage-dependent ion channels in cell membranes causing leaks, which finally lead to the death of the cells. These compounds contain non-ribosomally synthetized, non-proteinogenic amino acids, like α-aminoisobutyric acid, ethyl-norvaline, isovaline, and hydroxyproline. Regarding these molecules, the N-terminus is acetylated, and the C-terminal is an amino-alcohol. Due to their diverse nature and bioactivities, the comprehensive elucidation of the structure of newly discovered peptaibols is important.
A lot of efforts have been made to analyse the amino acid sequence of peptaibols using mass spectrometry, however, the isobaric amino acids cannot be specified, thus the configuration of the compounds could not be determined. The hydrolysation of peptaibols followed by liquid chromatographic separation both problems could be solved-, e.g. isobaric amino acids could be separated, and due to chiral derivatisation D-, and L-isomers can be distinguished.
In this work, we focused on the achiral and chiral separation of the isobaric amino acids, valine, isovaline, leucine and isoleucine by HPLC-UV analysis. For these purposes, two HPLC methods were developed. For achiral separation the standard amino acid mixture was firstly derivatised with o-phtaldialdehyde (OPA) and then 9-fluorenylmethyloxycarbonyl chloride (Fmoc). For chiral derivatisation, Marfey's reagent was used to separate the D-, and L-amino acids. Finally, the developed methods were applied for hydrolysed pure peptaibol fractions.

Acknowledgements
This work was supported by the Hungarian Government and the European Union within the frames of the Széchenyi 2020 Programme through grant GINOP-2.3.2-15-2016-00012. AS was supported through the New National

Excellence Program of the Ministry of Human Capacities (ÚNKP-16-4). The infrastructural background was established with the support of GINOP-2.3.3-15-2016-00006 grant (Széchenyi 2020 Programme).