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Bachelor Thesis: Michal Kamenický

Title: Mass spectrometric analysis of tricarboxylic acid cycle metabolites

Tutor Evaluation: Petr Šimek

Michal Kamenický started his study and laboratory work at the Laboratory of Analytical Biochemistry of the Institute of Entomology two years ago. His laboratory training proceeded rather in time blocks than continuously, because the students are heavily tasked in two lines of the Austrian and Czech study program. Nevertheless, his approach was always enthusiastic during the elaboration of the bachelor study.

In order to accomplish the work, the student had to fulfill three main tasks:

- (i) to perform library search and study the role of the TCA cycle in the cell metabolism;
- (ii) to get basic laboratory training and acquire manual skills in sample preparation techniques and operation of sophisticated GC-MS and LC-MS mass spectrometric equipment;
- (iii) to learn how to critically interpret and visualize the measured data, particularly mass spectra.

Michal Kamenický adapted successfully to these high-level demands going beyond the Bc. study, prepared smoothly samples for his experiments and performed by himself the reported measurements on the mass spectrometers. Our regular communication and numerous discussions about various biochemical and analytical topics led me to the opinion that Michal is a skilled, active student always eager new knowledge and evidently capable to perform research tasks.

Metabolites of the TCA cycle represent key intermediates in the cell metabolome and their analysis is an indispensable part of current metabolomic analytical strategies. The TCA metabolites represent mainly organic acids and their analysis by GC is therefore most feasible method. In his pilot work, Michal examined two sample preparation strategies, i.e. silylation and alkyl chloroformates, proving the latter as the more efficient. We and other noticed earlier that the TCA components do not provide a single product upon derivatization with the alkyl



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chloroformate – alcohol – pyridine reagent complex. And it was the task of Michal to measure carefully the derivatization reaction products of eight metabolites and aim to elucidate their chemical structures by means of homologous and deuterium-labeled reagents and organic acids standards. Although it was not possible to accomplish the work within the timeframe of this Bc. study, his experimental work is a significant step forward to accomplish the task and to understand reaction routes associated with the powerful alkyl chloroformate esterification reactions proceeding immediately in aqueous media. The work thus represent an important contribution to the development an efficient analytical strategy to the cell metabolome and will be an important piece of a future publication prepared within the research project which financially supported this work.

In conclusion, I would like to appreciate the personal commitment and capability of Michal to solve the demanding university study program, his enthusiastic approach and questions and comments that stimulated very positively research of my group. Therefore, I can easily recommend the thesis for defence before the Bc. University Examination Committee.

České Budějovice, September 15, 2012.

RNDr. Petr ŠIMEK, CSc.