Terpenoids for medicine

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Summary

The purpose of this thesis was to investigate aspects of terpenoid chemistry relevant to medicine. Specifically, to test vaporizing as a viable administration form for volatile terpenoids, to determine if the chemical profile of *Cannabis sativa* volatiles is useful for chemotaxonomy and reproducible, and to screen sesquiterpene lactones and phenolic diterpenes as drug leads. In **chapter 2** the gas chromatographic analysis of the vapor's produced by the volcano vaporizing device from *Lavandula angustifolia, Thymus vulgaris, Matricaria recutita, Salvia officinalis*, *Eucalyptus globulus,* and *Melissa officinalis* plant material is described. *Lavandula. angustifolia, T. vulgaris, M. recutita,* and *S. officinalis* vapor contained >0.1 mg/g of major essential oil components which increased in concentration with increased temperature. **Chapter 3** compared the vapor, smoke, and ethanol extracts with gas chromatography of three medicinal cannabis varieties produced by Bedrocan BV. The varieties Bedrocan, Bedrobinol, and Bediol produced cannabinoids and mono-terpenoids as major components of their smoke and vapor. Pyrolytic breakdown products were detected in cannabis smoke while none were observed in the vapor. No statistically significant differences in CB1 binding affinity *in-vitro* between pure Δ^9-THC, smoke, and vapor were observed at equivalent concentrations of Δ^9-THC.

In **chapter 4** a quantitative gas chromatography flame ionization detection method is described as well as its validation for analysis of cannabis monoterpenoids, sesquiterpenoids, and cannabinoids. The method was used to quantitatively analyze 11 cannabis varieties. In total 36 compounds were quantified in the 11 varieties and multi-variant data analysis was used to analyze the quantitative data. By using principal component analysis the cannabis varieties could be chemically distinguished from each other. Furthermore, a number of the cannabis varieties were grown in multiple batches to test the reproducibility of the volatile chemical profile. Cannabis varieties that were grown under the same environmental conditions and were of the same genetic stock had a reproducible chemical profiles. Minor deviations in growth period had minor effects on the quantitative levels of volatile compounds.

Chapters 5 and **6** focused on the isolation, structure elucidation, and bioactivity screening of sesquiterpene lactones from *Tanacetum parthenium* and *Inula britannica* respectively. Centrifugal partition chromatography was used as the main fractionation technique. Solvent systems composed of heptane: ethyl acetate: methanol: and water proved effective in separating sesquiterpene lactones from both plants. From *T. parthenium*, 11 sesquiterpenes were isolated and identified by NMR and high resolution mass spectrometry. These compounds were screened *in-vitro* on mouse primary cortical cultures for the ability to activate the Nrf2 pathway. Sesquiterpene lactones with the α-methylene-γ-lactone moiety were able to activate the Nrf2 pathway however they were also toxic towards the cultures. From *I. britannica*, 10 sesquiterpene lactones were isolated and identified by NMR, high resolution mass spectrometry, and infrared spectroscopy. Five of these compounds were never reported previously. Isolated compounds were screened for cytotoxic activity *in-vitro* on human cancer cell

lines, their derived multi-drug resistant cell lines, and normal human keratinocytes. Cytotoxic activity was generally mild, in the micromolar range, and the activity was similar between the different cell types.

In **chapter 7** the isolation of phenolic diterpenoids from *Salvia officinalis* is reported. In total 7 compounds were isolated with polyamide and centrifugal partition chromatography as the main fractionation methods. Carnosic acid, carnosol, epirosmanol, rosmanol, 12-methoxy-carnosic acid, sageone, and carnosaldehyde were identified by NMR and mass spectrometry. Isolated compounds were screened *in-vitro* on mouse primary cortical cultures for the ability to activate the Nrf2 pathway. Carnosic acid, carnosol, epirosmanol, and rosmanol were able to activate the Nrf2 pathway with minimal or no observed toxicity. 12-Methoxy-carnosic acid was inactive while sageone was inactive and toxic. Carnosaldehyde was active but toxic.

The conclusions and future perspectives of this thesis are discussed in detail in **chapter 8**. The main conclusions are that vaporizing is a promising administration form for volatile terpenoids. The volatile chemical profile of cannabis can be used for discriminating between varieties and that if grown under controlled conditions the chemical profile cannabis is reproducible. Sesquiterpene lactones display many biological activities however they often lack the specificity and are generally cytotoxic due to the reactive α-methylene-γ-lactone moiety. Finally, carnosic acid, carnosol, and rosmanol from *S. officinalis* represent interesting lead compounds for development as drugs against neurodegenerative disease.