Terpenoids for medicine

Fischedick, J.

Chapter 8

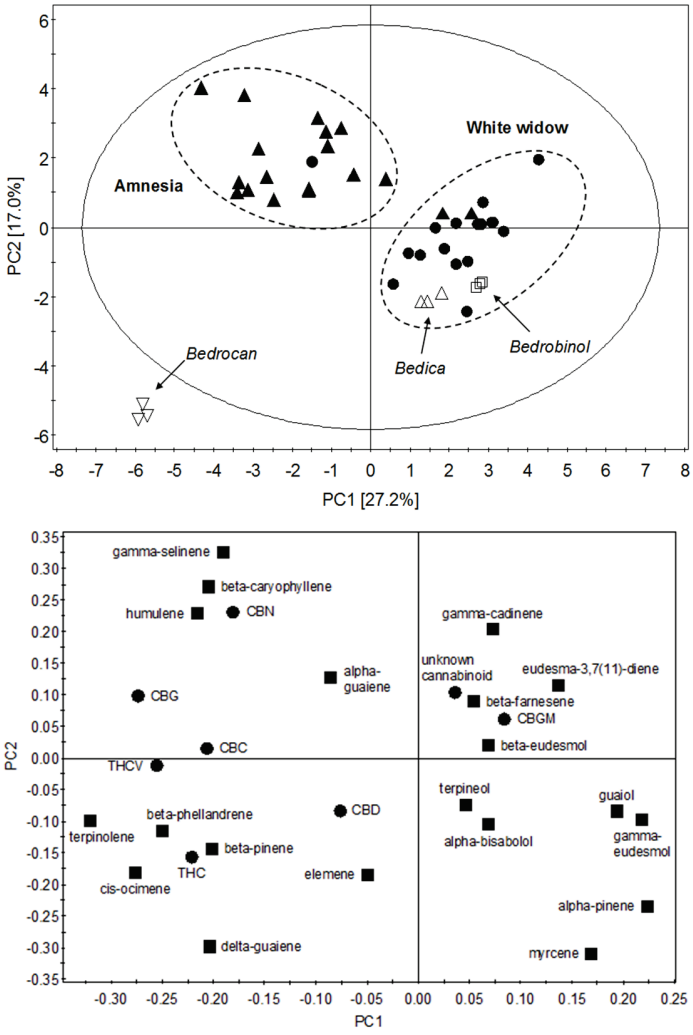
Conclusions and Future Perspectives

Throughout this thesis numerous aspects of terpenoid chemistry as it relates to medicine were explored. In chapter 2 *Lavandula angustifolia, Thymus vulgaris, Matricaria recutita, Salvia officinalis*, *Eucalyptus globulus,* and *Melissa officinalis* were tested in the Volcano vaporizer. These results confirmed that the vaporizer is capable of volatilizing the main essential oil components from *L. angustifolia, T. vulgaris, M. recutita,* and *S. officinalis* in a reproducible manner. Plants producing essential oils in secretory cavities such as *E. globulus* and those containing low amounts of essential oils such as the batches of *M. officinalis* used in the study are not useful to administer in the vaporizer. However, vaporizers such as the Volcano contain a wire-mesh which allows pure terpenoids or essential oils to be used in the device and heated in the same manner as with plant material. Therefore such devices allow many possibilities for designing clinical protocols. This could improve study design when assessing potential therapeutic effects of essential oil bearing plants.

A more detailed analysis of the vaporizer was performed in chapter 3 using *Cannabis sativa* (cannabis) as a model plant. The cannabis vapor produced by the Volcano did not contain pyrolytic degradation products found in cannabis smoke confirming that the vaporizing is a safer administration method. Furthermore the *in-vitro* CB1 binding of Δ^9-THC was not altered by impurities found in the vapor, smoke, or between the strains. Any additional potential biological effects of other components in cannabis besides Δ^9-THC would be difficult or impossible to study *in-vitro*. The question of whether or not herbal cannabis contains more active ingredients than Δ^9-THC is still an ongoing discussion (Russo, 2011). This question of multiple active ingredients, which is a common question when studying a herbal drug, can only truly be answered with properly designed clinical trials in humans using standardized and chemically defined plant sources.

In chapter 4, cannabis was used as a model plant to determine if the chemical profile could distinguish different varieties. A targeted metabolic profiling approach was developed and validated to chemically classify 11 cannabis varieties according to the terpenoid and cannabinoid content. Indeed the results demonstrated that cannabis varieties can be distinguished based on cannabinoid and terpenoid profiles. Interestingly cannabis varieties with similar levels of Δ^9-THC could be differentiated based on mono and sesquiterpenoid content. Cannabis varieties of the same genotype, grown in multiple batches under the same environmental conditions had reproducible chemical profiles. Although alterations in growth period (number of days vegetative or flowering) seemed to increase quantitative differences in a variety's profile, the differences were minor and qualitatively they were the same. These results would be expected if terpenoid and cannabinoid production in cannabis is genetically regulated and influenced by the environmental conditions.

Figure 1. PCA plot (top) and loading plot (bottom) from Hazekamp and Fischedick, 2012 reprinted with permission. Amnesia samples represented as black triangles, white widow samples as black circles.



The approach outlined in chapter 4 for analyzing cannabis was extended to evaluate two common cannabis varieties sold in Dutch coffeeshops, known as 'white widow' and 'amnesia'. In this study two 1 gram samples of each variety purchased in 2 separate visits to each coffeeshop (10 coffeeshops total) spread throughout the Netherlands were obtained. By analyzing quantitative data for the terpenoid and cannabinoids both varieties and 3 additional cannabis varieties produced by Bedrocan

BV were clearly distinguished from each other by PCA (Figure 1). These results again demonstrated that cannabis varieties containing similar Δ^9-THC levels could be distinguished by terpenoid profile (Hazekamp and Fischedick, 2012).

Overall the results from chapters 3 and 4 demonstrate that it is possible to standardize a plant producing volatile terpenoids and its administration form. Although cannabis was chosen as a model example due to its complex chemical profile and ongoing clinical research it is in theory possible to take such an approach with any plant or herbal product as long as certain criteria can be met. These criteria include:

1. Standardization of the plant source material or extract
2. Appropriate chemical characterization of the product
3. Standardization of the plant or extracts administration form

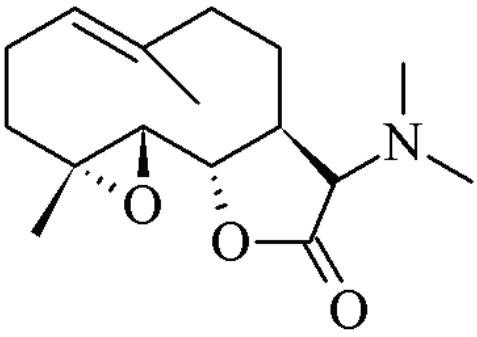
Standardization of plant material would be more difficult with plants that cannot be cultivated or propagated asexually. In these cases homogenization of the used plant material or production of standardized extracts would be an alternative. Obviously if an herbal drug is administered as a tea or eaten whole as opposed to inhaled, chemical characterization should utilize an appropriate analytical method such as NMR or HPLC to standardize the procedure. If cannabis is used as an example the following clinical protocol could be envisioned; as treatments two or more cannabis varieties with unique chemical profiles, the isolated essential oil of each variety, equivalent doses of Δ^9-THC as a positive control, and a negative control with no drugs. Administered to humans with a relevant illness one could then conclusively address the question over whether herbal cannabis is more or less therapeutically effective compared with pure Δ^9-THC for that particular illness.

The second half of this thesis (chapters 5-7) focused on sesquiterpene lactones and phenolic diterpenoids as lead compounds for drug development. In chapter 5, eleven sesquiterpene lactones from *Tanacetum parthenium* were screened for their ability to activate the Nrf2/ARE pathway in mouse primary cortical cultures. The structure activity relationship of the compounds confirms the importance of the α-methylene-γ-lactone moiety for activity. Although the data also suggests that other functional groups and the shape or flexibility of the molecule contribute to the activity as well. This observation is consistent with literature concerning the structure activity relationship of sesquiterpene lactones and cytotoxicity (Ghantous et al., 2010). The considerable toxicity of sesquiterpene lactones on primary cortical cultures *in-vitro* as well as available toxicity information in the literature demonstrates that further research is needed to assess the toxicity and selectivity of these compounds *in-vivo*.

In chapter 6, the structure of 5 new sesquiterpene lactones is described along with the structure of 5 known sesquiterpene lactones isolated from *Inula britannica*. Screening the compounds on cancer cell lines, multi-drug resistant cancer cell lines, and human keratinocytes demonstrated again that biological activity was mainly dependent on the presence of α-methylene-γ-lactone moiety. Although no strong trends in

selectivity against any of the cell lines were observed the sesquiterpene lactones were capable of inhibiting drug resistant cancer cell lines. One of the main complications of cancer treatment is the development of resistance of cancer cells to the chemotherapeutic agents. Drugs such as taxol can induce cancer cells to overexpress membrane transporter permeability-glycoprotein-1 which increases the cells ability to pump out the drug and or inhibit its uptake (Podolski-Renić et al., 2011). There is evidence that constitutive activation of NF-$_{\kappa}$B contributes to resistance of cancer cell lines against anti-cancer drugs (Sethi et al., 2012). Inhibition of NF-$_{\kappa}$B by parthenolide was demonstrated as a mechanism by which parthenolide was able to potentiate the ability of taxol to induce cell death in lung cancer both *in-vitro* and *in-vivo* (Zhang et al., 2009). Therefore the relationship between the mechanisms by which anti-cancer drugs induce resistance in cancer cells and the mechanisms by which sesquiterpene lactones may be able to prevent or reverse this process is a promising area for further research.

Figure 2. Dimethylamino-parthenolide.



It is important to realize that for over 40 years it has been known that the cytotoxic activity of sesquiterpene lactones is often dependent on the α-methylene-γ-lactone moiety (Kupchan et al., 1971). Furthermore, no sesquiterpene lactones with the α-methylene-γ-lactone moiety have been approved as drugs to date. This is likely due to their lack of selective biological activity and toxicity concerns. In fact as mentioned in chapter 1 sesquiterpene lactones that do not contain the α-methylene-γ-lactone, yet contain other functional groups such as ester side chains or an endoperoxide moiety, such as thapsigargin and artemisinin respectively, are actively being investigated as anticancer drugs. Parthenolide, which shows promising antitumor activity *in-vivo*, suffers from poor pharmacokinetic properties such as low water solubility and poor bioavailability. In order to overcome this problem analogues of parthenolide have been synthesized, such as dimethylamino-parthenolide which shows improved pharmacokinetic properties and maintains antitumor activity *in-vivo* (Figure 2) (Shanmugam et al., 2011).

For these reasons future research into sesquiterpene lactones should avoid compounds with the α-methylene-γ-lactone moiety. The same problems demonstrated in

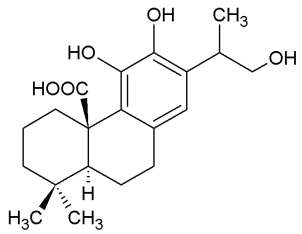
this thesis will continue to hinder drug development of these compounds. Bioactivity guided fractionation into plant families that often contain sesquiterpene lactones, such as the Asteraceae, may be biased towards sesquiterpene lactones due to the reactivity of the α-methylene-γ-lactone moiety. Researchers analyzing plant families rich in sesquiterpene lactones should aim early on in a bioactivity guided fractionation study to determine if they are present in a plant extract. Column material with active thiols or addition of biological thiols such as cysteine to the extract could potentially be used to remove or inactivate the α-methylene-γ-lactone moiety. This would make it easier for researchers to better assess if other components in the plant material have biological activity. Another area of future research is to chemically modify sesquiterpene lactones that have other interesting functional groups. For example tanaparthin-β-peroxide isolated in chapter 5 contains an endoperoxide moiety. Tanaparthin-β-peroxide was shown to potently activate the Nrf2/ARE pathway in mouse primary cortical cultures, but it was also toxic. Perhaps chemically reducing or modifying the α-methylene group would lead to a compound with more selective biological activity. Unfortunately the *T. parthenium* used in this study only produced low levels of tanaparthin- β-peroxide making such synthetic studies difficult. However, it has been reported that certain chemotypes of *T. parthenium* produce much larger amounts of tanaparthin peroxides (Begley et al., 1989).

In chapter 7, seven phenolic diterpenoids isolated from *S. officinalis* were screened for their ability to activate the Nrf2/ARE pathway in mouse primary cortical cultures. The catechol moiety was demonstrated as being necessary for activation of the pathway. Carnosaldehyde and sageone were toxic towards the cultures indicating structural features that are not desirable such as presence of an aldehyde at C-20 position. The presence of a functional group at the C-7 position also had an influence on activity, increasing hPAP activation in the case of carnosol and rosmanol when compared to carnosic acid. However further experiments are needed to determine which phenolic diterpenoids represent the most promising candidates for drug development. These experiments include determining if phenolic diterpenes are actually increasing cortical cell proliferation or if the MTS assay is giving false positives. Furthermore a direct demonstration of neuroprotection from the most promising compounds in our initial screening, carnosol and rosmanol, should be performed in both *in-vitro* and *in-vivo* experiments. At the time of this writing the follow up *in-vitro* experiments are being performed.

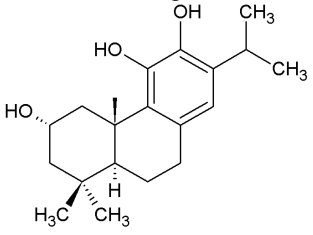
Finally many other *Salvia* species contain abietane diterpenoids with catechol moieties. Some examples include 16-hydroxycarnosic acid from *S. apiana* (Dentali and Hoffmann, 1990), euphraticol from *S. euphratica* (Ulubelen, 1989), and demethylcryptojaponol from *S. phlomoides* (Hueso-Rodríguez et al., 1983) (Figure 3). Another group of diterpenoids found in *Salvia* species that contain catechol moieties are the icetexanes, which include brussonol from *S. broussonetii* and przewalskin C from *S. przewalskii* (Figure 4) (Simmons and Sarpong, 2009). Future research should aim to investigate these and related compounds as potential neuroprotective agents as well. As discussed in previous chapters much of the research performed with *Salvia* species has

focused on the essential oil due to acetylcholinesterase inhibitory activity. Perhaps the phenolic diterpenoids in *Salvia* species may be essential for truly understanding the potential memory enhancing and neuroprotective effects of this valuable medicinal plant.

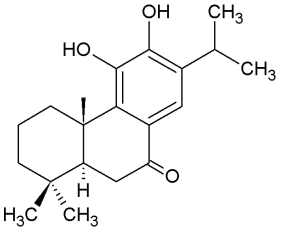
Figure 3. Abietane diterpenoids from *Salvia* species.



16-hydroxycarnosic acid

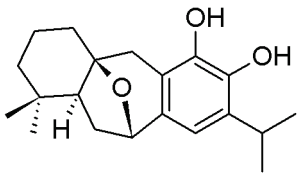


euphraticol

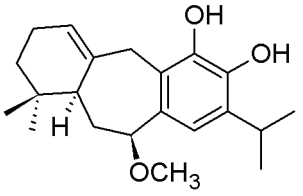


demethylcryptojaponol

Figure 4. Icetexanes from *Salvia* species.



brussonol



przewalskin C

References

Begley, M.J., Hewlett, M.J., Knight, D.W., 1989. Revised structures for guaianolide α-methylenebutyro-lactones from feverfew. Phytochemistry 28, 940–943.

Dentali, S.J., Hoffmann, J.J., 1990. 16-hydroxycarnosic acid, a diterpene from Salvia apiana. Phytochemistry 29, 993–994.

Ghantous, A., Gali-Muhtasib, H., Vuorela, H., Saliba, N.A., Darwiche, N., 2010. What made sesquiterpene lactones reach cancer clinical trials? Drug Discovery Today 15, 668–678.

Hazekamp, A., Fischedick, J.T., 2012. Cannabis - from cultivar to chemovar. Drug Testing and Analysis 4, 660–667.

Kupchan, S.M., Eakin, M.A., Thomas, A.M., 1971. Tumor inhibitors. 69. Structure-cytotoxicity relationships among the sesquiterpene lactones. Journal of Medicinal Chemistry 14, 1147–1152.

Podolski-Renić, A., Andelković, T., Banković, J., Tanić, N., Ruždijić, S., Pešić, M., 2011. The role of paclitaxel in the development and treatment of multidrug resistant cancer cell lines. Biomedicine & Pharmacotherapy 65, 345–353.

Hueso-Rodríguez, J.A., Jimeno, M.L., Rodríguez, B., Savona, G., Bruno, M., 1983. Abietane diterpenoids from the root of Salvia phlomoides. Phytochemistry 22, 2005–2009.

Russo, E.B., 2011. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. British Journal of Pharmacology 163, 1344–1364.

Sethi, G., Sung, B., Aggarwal, B.B., 2008. Nuclear factor-kappa B activation: from bench to bedside. Experimental Biology and Medicine 233, 21–31.

Shanmugam, R., Kusumanchi, P., Appaiah, H., Cheng, L., Crooks, P., Neelakantan, S., Peat, T., Klaunig, J., Matthews, W., Nakshatri, H., Sweeney, C.J., 2011. A water soluble parthenolide analogue suppresses in vivo tumor growth of two tobacco associated cancers, lung and bladder cancer, by targeting NF-κB and generating reactive oxygen species. International Journal of Cancer 128, 2481–2494.

Simmons, E.M., Sarpong, R., 2009. Structure, biosynthetic relationships and chemical synthesis of the icetexane diterpenoids. Natural Product Reports 26, 1195.

Ulubelen, A., 1989. Euphraticol and Euphracal, Two New Diterpenes from Salvia euphratica. Journal of Natural Products 52, 1313–1315.

Zhang, D., Qiu, L., Jin, X., Guo, Z., Guo, C., 2009. Nuclear factor-kappa B inhibition by parthenolide potentiates the efficacy of Taxol in non-small cell lung cancer in vitro and in vivo. Molecular Cancer Research 7, 1139–1149.