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Review Article

STABILITY OF FEVERFEW AND ITS ACTIVE PRINCIPLE PARTHENOLIDE: AN ELUSIVE ANTIMIGRAINE HERBAL MEDICINE

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ABSTRACT

Background

Feverfew is a traditional herbal remedy for the relief of arthritis, migraine, toothache, and menstrual difficulties. It is widely accepted that parthenolide, a sesquiterpene lactone, is its main active principle. However, the decrease of parthenolide in commercial preparations is a well-known process with no technical solution so far.

Aims

To review the evidence for the mechanism of the degradation of parthenolide and similar sesquiterpene lactones.

Methods

Systematic review.

Results and Conclusion

In conclusion, and without discarding any degradation of parthenolide into non-identifiable fragments, the fate of this compound in dry, powdered feverfew is to undergo a covalent binding to plant proteins resulting in a biologically inactive adduct - in accordance with the direct and indirect data found in the literature. This process seems to be virtually unstoppable, and temperature and light do not seem to be playing a significant role under normal storage conditions according to some authors. In the presence of a high level of humidity, parthenolide may undergo an acid-induced cyclisation giving rise to a guaianolide-type sesquiterpene lactone, a class of compound that is commonly found in Feverfew. Microbial degradations are not likely to play an important role if the formulation complies with Pharmacopoeial microbiological quality requirements. The experimental and clinical data in the literature do not report on any increase in the toxicity of stored feverfew.

Keywords

Sesquiterpene lactones, Chemical stability, Herbal medicines, Microbial degradation, Storage.

INTRODUCTION: FEVERFEW AND PARTHENOLIDE

Feverfew is a traditional herbal remedy for the relief of arthritis, migraine, toothache, and menstrual difficulties. These properties have solid pharmacological foundations (Wang & Li, 2015) and many feverfew-based medicines are regulated and sold in Europe under the Traditional Herbal Medicinal Products Directive. The plant is a perennial, strongly aromatic herb of the Compositae/Asteraceae family, and has been classified variously as *Tanacetum parthenium*, C*hrysanthemum parthenium*, *Leucanthemum parthenium*, or *Pyrethrum parthenium*, the former name being currently favoured. Of a range of sesquiterpene lactones of the germacrane (germacranolides) and guianane (guaianolides) groups characterized in the leaf material, the principal constituent and major active component is parthenolide (Figure 1). Parthenolide (CAS 20554-84-1) which can occur in up to 1%*w/w* in dried leaves, is a white crystalline solid with MW=248.3 and melting point of 112-115ºC. In comparison with other sesquiterpene lactones, parthenolide is remarkably soluble in water as well as in organic solvents (amphiphilic) and large quantities can be extracted into aqueous buffers (Heptinstall, 1998). The amount of parthenolide in feverfew depends on environmental conditions, feverfew chemovars, part of the plant and postharvest processing (Majdi et al.2013). Parthenolide content is higher in flowers, followed by leaves and stems but absent in roots (Majdi et al.2011). Other sesquiterpene lactones with an α-methylenebutyrolactone ring present in feverfew are canin, artecanin, tanaparthin-α- and -β peroxides, and seco-tanapartholide A and B (Figure 1) (Begley et al, 1989).

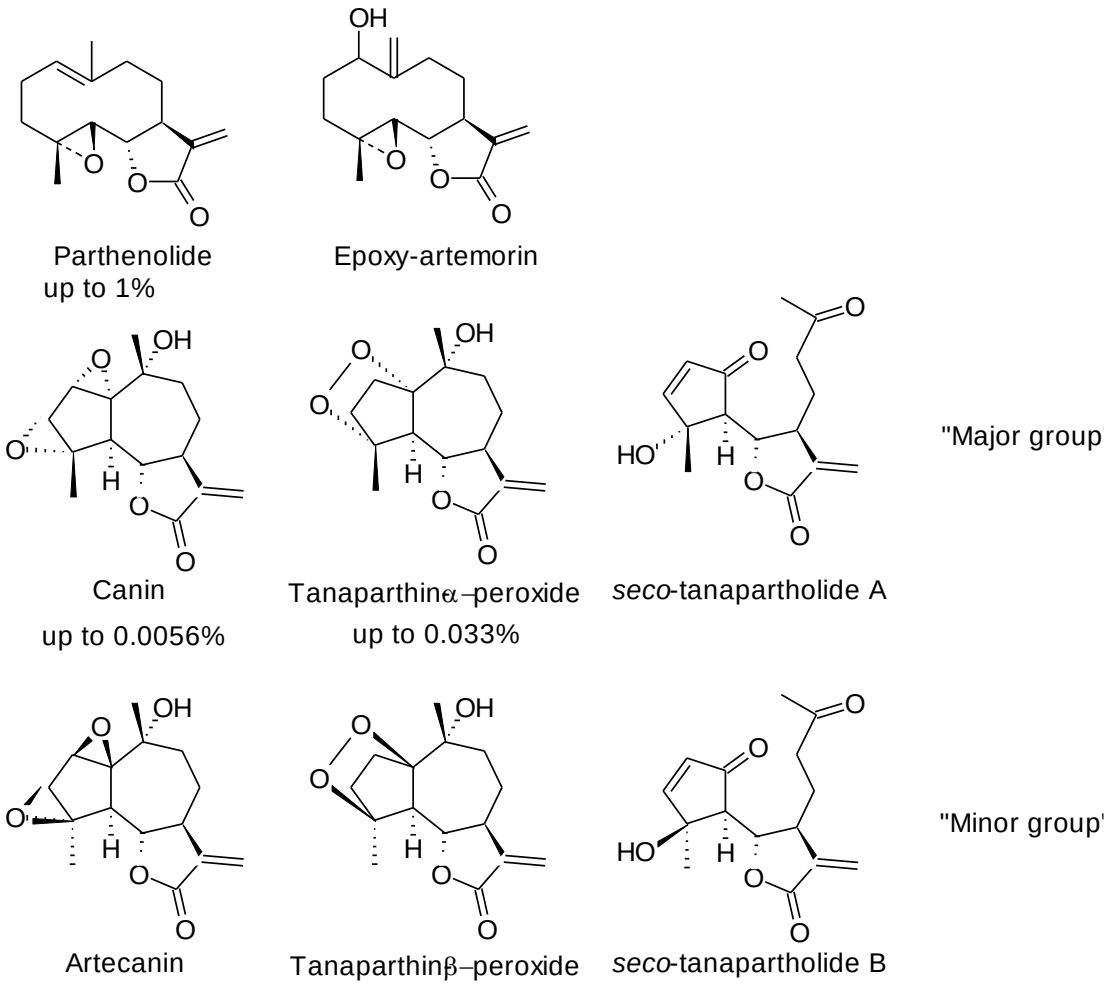

Figure 1. Main sesquiterpene lactones with α-methylenebutyrolactone group found in Feverfew.

THE EFFECT OF STORAGE OF FEVERFEW ON THE CONTENT OF PARTHENOLIDE.

The decrease of parthenolide in commercial preparations is a well-known process (Knight, 1995; Heptinstall and Awang, 1998) with no technical solution until now. The loss of parthenolide does not seem to be significantly affected by temperature or light exposure during storage (Fonseca et al, 2003; Tanko et al, 2003), so little can be done by controlling these parameters. Groenewegen et al (1992) reported fourteen years ago that there is little information on the stability of the sesquiterpene lactones during storage. The appearance of costunolide diepoxide when a chloroform solution of parthenolide was stored at room temperature after a few days, probably formed by aerial oxidation (Hoffman et al, 1977), and the suggestion that parthenolide can polymerize on prolonged storage were the only two references they could cite in their paper (Groenewegen et al, 1992). Since then, little work has been published and still today only indirect information is available. However, it is well known that the parthenolide content of dried leaf decreases on storage (Heptinstall et al, 1992).

Other herbal formulations containing sesquiterpene lactones face the same problems, the most important example coming from Arnica-containing formulations. Lactone-containing herbal remedies such as *Arnica spp.* preparations are frequently used in phytotherapy and sesquiterpene lactones are the main active compounds. When tinctures of *Arnica spp.* were stored at 4ºC, 25ºC and 30ºC, a decrease in the content of 11α, 13-dihydrohelenalin esters correlating with the temperature was observed. It was caused by addition of ethanol to the cyclopentenone moiety of these molecules (Schmidt et al, 2000). Bilia and co-workers (2006) studied the stability of lactones in different semisolid formulations under different conditions of storage and found great differences depending on the different excipients used. As a result, the cetomacrogol, polysorbate 60, and natrosol-based formulations were the least stable.

Several studies have been carried out to determine the effect of drying temperature and storage of feverfew on the concentration of parthenolide as its marker compound. Studies have examined the analysis of parthenolide using HPLC-UV after extraction from the feverfew leaves which had been dried, comminuted, and extracted. Drying the leaves at temperatures in the range of 30 to 60°C did not significantly affect the parthenolide concentration (Tanko et al, 2003). These authors also found that storage temperature in the range of -15 to 24°C did not significantly affect parthenolide concentration either. However, parthenolide concentration decreased as a function of time over 120 days of storage. In another study, Fonseca and co-workers (2003) reported that exposure to light (sunlight, or fluorescent light) in the drying process actually increases the content of parthenolide. Furthermore, light did not affect either dry feverfew powder or pure parthenolide during storage. Surprisingly, exposure of the stored material to heat (50-130ºC) during a short time (5 min) increases the extraction yield of parthenolide. In contrast these workers observed a drastic loss of parthenolide if the powder was dissolved in citrate buffers at various pH values (2.4 to 7.2).

Feverfew extract dissolved in different pH buffers to study the solution stability of parthenolide in feverfew. The degradation of parthenolide follows a first-order reaction model and is stable in the range of 5 to 7, becoming unstable when pH is less than 3 or more than 7. In solid feverfew extracts, moisture content and temperature are the most important parameters influencing the rate of parthenolide degradation which does not fit any obvious reaction model. Up to 40% loss in parthenolide were observed when extracts are stored at 50°C/31% RH and these losses go down to 18% to 32% after 6-month storage under 40°C/ 0% to 75% RH. When feverfew is mixed with excipients parthenolide levels remain constant at 5°C/31% RH for up to 6 months and exhibits good compatibility with common excipients under stress conditions up to 3-weeks (Jin et al, 2007).

Fonseca and co-workers (2007) studied the influence of pH, temperature and light on parthenolide content in powdered feverfew and parthenolide standard in citrate buffers at selected pH (2.4-7.2) were stored for 4 months. Parthenolide losses in solutions are greater at pH<5, and lower at pH=7.2. Parthenolide losses in dry samples were 30% percent after 320 days of storage. Short term studies (hours) show stability of the compound even at high temperatures though. Similar results were reported by Marete et al. (2011, 2013) in feverfew model beverages. Parthenolide hydrolysis increases with acidity and neutral infusions (pH 6.0) favour its stability but favour oxidation of phenolic substances. The best conditions are refrigerated storage -as parthenolide degradation with thermal treatment follows pseudo-first order kinetics- of mildly acidic infusions (pH 4.6) for both colour retention and parthenolide content with a shelf-life of approximately 4 months.

Finally, studies with “degraded” feverfew extracts found that they were pharmacologically active in an *in vivo* murine model of anti-serotonergic activity up to a 10% of loss of parthenolide (Mittra et al, 2000). The same authors did not report any toxic effect on rats treated with extracts degraded with up to a 33% loss of parthenolide.

REACTIVITY OF PARTHENOLIDE, GERMACRANOLIDES AND RELATED SESQUITERPENE LACTONES.

Generalities

It is a characteristic of sesquiterpenes to undergo *in vitro* acid-catalysed cyclizations. Cyclizations play a central role in the biosynthesis of guaianolides and eudesmanolides from germacranolides in the normal plant cell metabolism under enzymatic control (Cane, 1999), but they can also be reproduced *in vitro* in the presence of acids (Sethi et al, 1984; Castaneda- Acosta et al, 1993). In addition, germacranolides and related sesquiterpene lactones are frequently endowed with α, β unsaturated carbonyl groups making these molecules especially prone to react with nucleophiles following a Michael-type addition. The α, β unsaturated carbonyl groups is usually in the form of an α-methylene-γ-lactone group, also called an α-methylenebutyrolactone, and cyclopentenones. Other functional groups that are usually present in sesquiterpene lactones are endocyclic double bonds and epoxides, which can both react with electrophiles and/or induce rearrangements in the presence of UV radiation. Finally, high temperatures and strong irradiation may cause Cope rearrangements.

It is worth pointing out that reactivity does not necessarily infer chemical instability. The α-methylenebutyrolactone group – responsible for many of the biological activities of STLs -and possibly for their disappearance from the plant material over time- is often wrongly regarded as an unstable chemical feature. However, it is postulated that the exo double bond stabilizes the 5- membered ring of these sesquiterpenes. In fact, the biosynthesis of these compounds seems to proceed in such a manner as to favour the formation or retention of an exo double bond in a 5- membered ring and to avoid the formation or retention of an exo double bond in a 6-membered ring. With a few exceptions this generalization is supported by available data on the stability of cyclic esters, lactones, hemiacetals, and imides, and even in furanose and pyranose structures in the sugars and sugar acids. This generalization is in accord with available thermochemical data demonstrating that an atom of oxygen in a ring system can result in the same type of conformational effects as a CH_2 group (Brown et al, 1954). The lactone ring also has a strong influence on the thermal stability of germacrenes (de Kraker et al, 2000).

In vitro acid-catalysed cyclizations

Many sesquiterpene lactones, particularly germacranolides, and therefore parthenolide, undergo facile *in vitro* acid-catalysed cyclizations (Figure 2). Their own biosynthesis is based on a series of cyclizations, starting with farnesyl diphosphate, by electrophilic attack on to an appropriate double bond. Standard reactions of carbocations rationally explain most of the common structural skeletons encountered (Dewick, 2002). The formation of guaianolides or eudesmanolides probably occurs by cyclization of (+)-costunolide after C_4-C_5 epoxidation. or C_1-C_{10} epoxidation, respectively (Figure 2) (Piet et al, 1995; de Kraker et al, 2002).

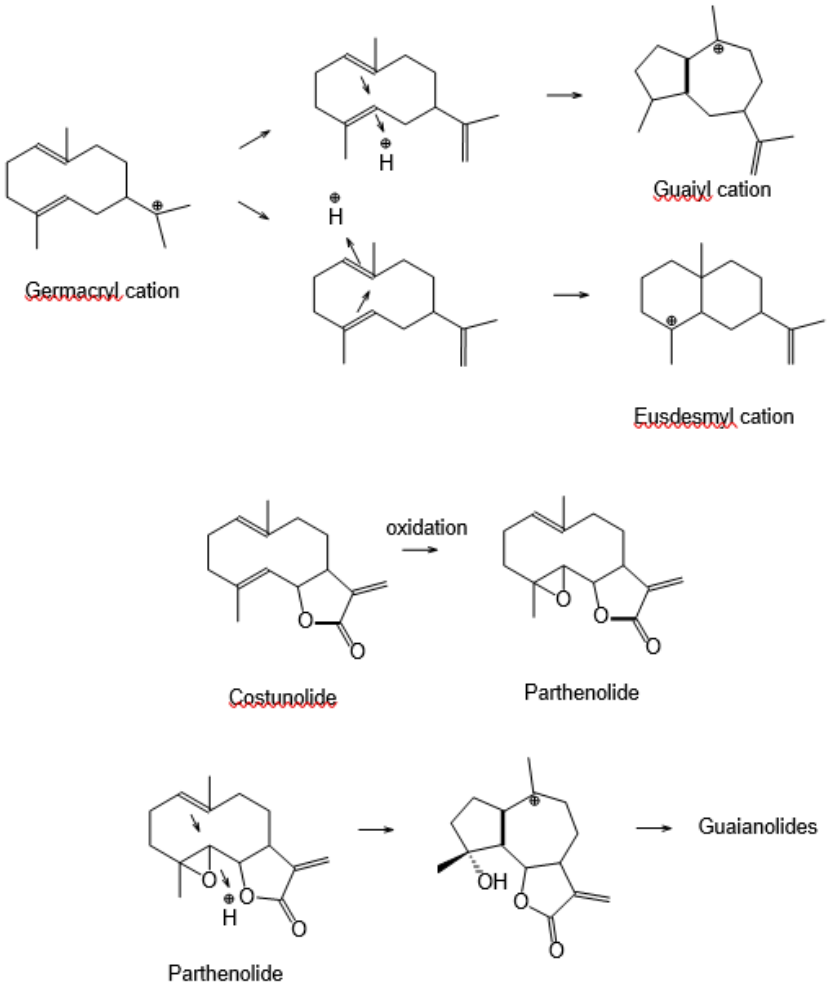

Figure 2. Mechanisms and intermediates of *in vivo* acid-catalysed cyclizations of germacranes and parthenolide.

Sethi and co-workers (1984) briefly reported the conversion of parthenolide into guaianolides after BF_3-catalyzed cyclization. A fuller description of these conversions was given by Castaneda-Acosta and co-workers (1993), by using the *in vitro* BF_3-mediated biomimetic approach in hexane. They explained the mechanisms as rearrangements involving carbocation intermediates giving rise to the major product micheliolide, and several minor products namely 10(14)-dehydro-5α-hydroxy-*trans*-guaianolide, 9,10-dehydro-5α-hydroxy-*trans*-guaianolide, the xanthanolide 2-desoxy-6-*epi*-parthemollin, 1,2-dehydro-4α-hydroxyguaianolide, 11,13-dehydrocompressanolide, and bicyclo[6.2.0]dec-10(14)-en-12,6-olide (Figure 3). All the identified products preserve the α-methylene-γ-lactone group.

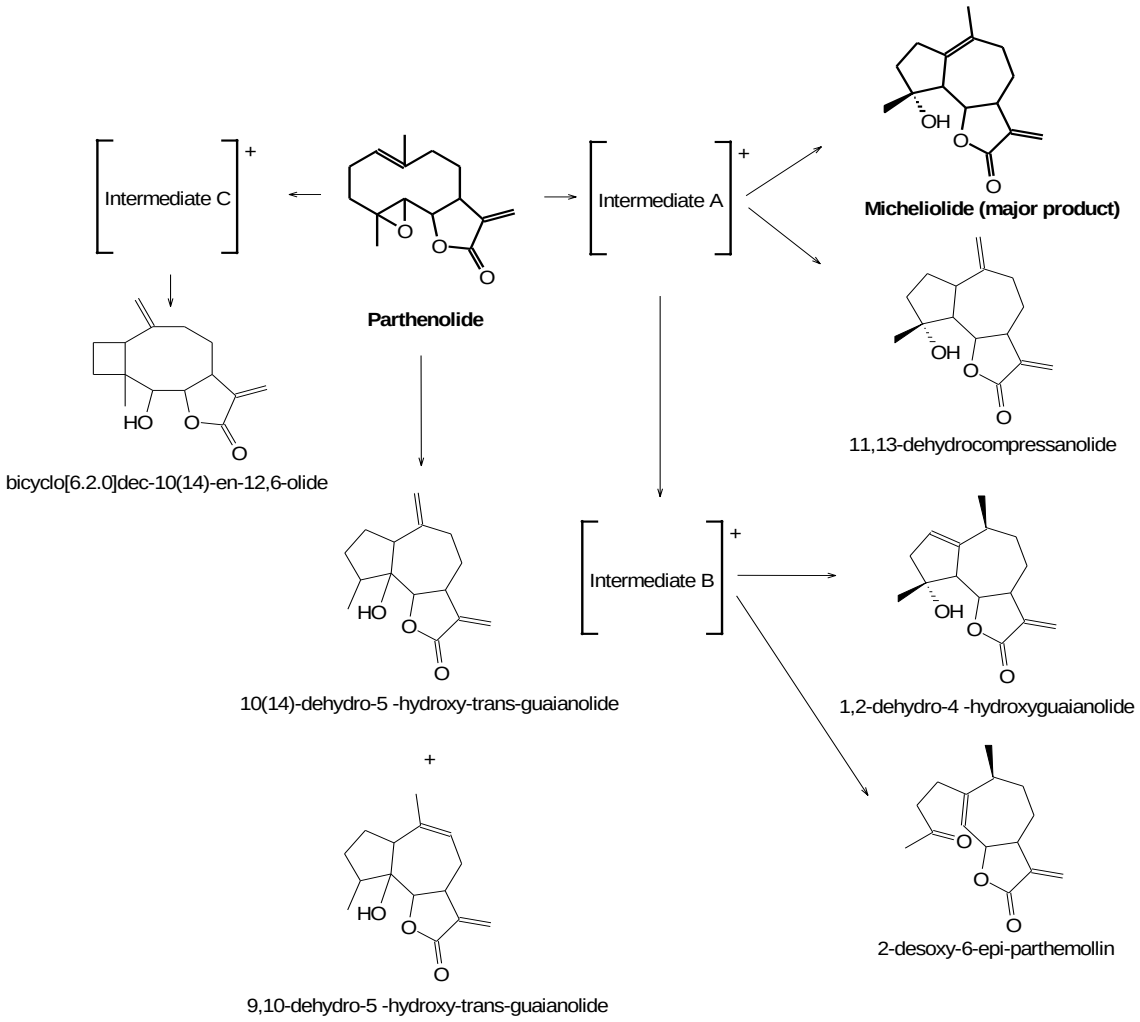

Figure 3. Products obtained after BF_3-initiated biomimetic cyclisations of parthenolide (As described by Castaneda-Acosta et al, 1993)

The cyclization of parthenolide in an aqueous acidic environment with R-OH acting as a nucleophile does not seem to be favoured but it may occur in the way depicted in Figure 4. If R=H, that is water is acting as the nucleophile, the resulting product is the guainolide partholide, that has been reported to be present in feverfew according to Johnson and co-workers (1982).

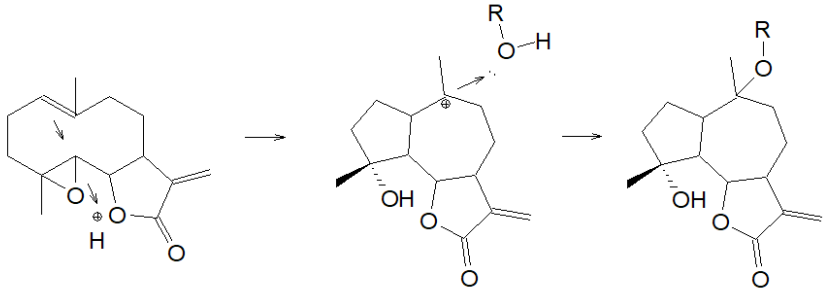

Figure 4. Cyclization of parthenolide in an aqueous acidic environment with R-OH acting as a nucleophile.

The possibility of a cyclization of parthenolide giving rise to a trans-decalin system, thus forming an eudesmanolide, (Figure 5) seems to be less likely as the carbocation cannot be stabilised on a tertiary carbon.

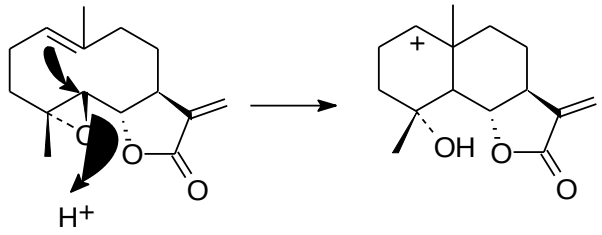

Figure 5. Cyclization of parthenolide giving rise to a trans-decalin system.

Nevertheless, such a type of transannular cyclization could be possible, and similar rearrangements have been reported to occur *in vitro with c*ostunolide, the analogue of parthenolide lacking the epoxide group. Costunolide can be smoothly converted into α- and β- cyclocostunolide by Amberlite cation exchange resin (Figure 6) (Roberts, 1972).

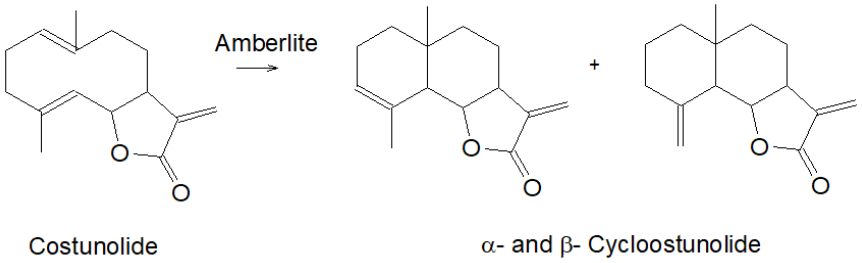

Figure 6. Amberlite-induced transannular cyclization of costunolide, the analogue of parthenolide lacking the epoxide group, into α- and β-cyclocostunolide.

Finally, the acid-catalysed cyclizations of tanaparthin peroxides give rise to the two cyclopentenone-containing lactones present in feverfew, *seco*-tanapartholides A & B. When tanaparthin-α-peroxide was treated with $BF_3 \cdot OEt_2$ ($CHCl_3$, 20ºC; 15 h), *seco*-tanapartholide A was the only product identified (Begley et al, 1989).

After cyclization, parthenolide gives rise mainly to an array of guanolides and xanthanolides. This kind of reaction might occur on the basis of the accelerated degradation of parthenolide when dissolved in citrate buffers at different pHs in the range of 2.4 to 7.2 as reported by Fonseca et al (2003). The resulting end-products are usually naturally occurring sesquiterpene lactones or compounds already present in feverfew like micheliolide, partholide, and *seco*- tanapartholides. In the case of micheliolide, it has been isolated from *Michelia champaca* where it occurs together with parthenolide (Sethi et al, 1984). This plant is reported to be endowed with leishmanicidal, antimicrobial, anti-inflammatory, and antipyretic activities (Takahashi et al, 2004; Khan et al, 2002).

Michael-type additions

The Michael addition is a facile reaction between nucleophiles and activated olefins and alkynes in which the nucleophile adds across a carbon–carbon multiple bonds. The Michael addition benefits from mild reaction conditions, high functional group tolerance, a large host of polymerizable monomers and functional precursors as well as high conversions and favorable reaction rates (Mather et al, 2006). The α,β-unsaturated carbonyl compounds can easily undergo these type of reactions, and α-methylenebutyrolactones fall into this class of compound.

Reaction of sesquiterpene lactones with amino acids and other thiol-containing molecules.

During the early '70s it was already evident to the scientific community that α- methylenebutyrolactones and cyclopentenone moieties exert their pharmacological and toxicological actions mainly by Michael-type additions with cysteine, glutathione, and a number of sulphydryl-bearing cell enzymes (Hall et al, 1979). This idea has been widely corroborated during the last decades and is nowadays accepted after findings that these compounds, including parthenolide, have the capacity to directly bind to the cysteine residue 179 in the activation loop of IKKβ (Kwok et al, 2001).

Dupuis and co-workers (1974) used alantolactone as a model compound to study how the α-methylenebutyrolactones undergoes Michael-type additions with different nucleophilic groups present in amino acids. As a result, the α-methylenebutyrolactone function reacted with the sulphydryl group of cysteine, with the imidazole group of histidine, and the ε-amino group of lysine, but not with the guanido group of arginine, the hydroxyl group of serine, or the thio ether function of methionine (Figures 7 and 8). It is important to point out that the sulphur atom of a thiol is rather more nucleophilic than the oxygen atom of an alcohol.

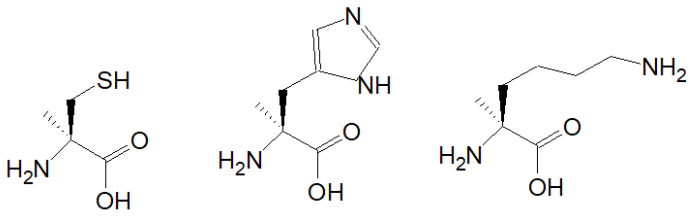

Figure 7. Amino acids with nucleophilic groups that react with α-methylenebutyrolactones (cysteine, histidine, and lysine).

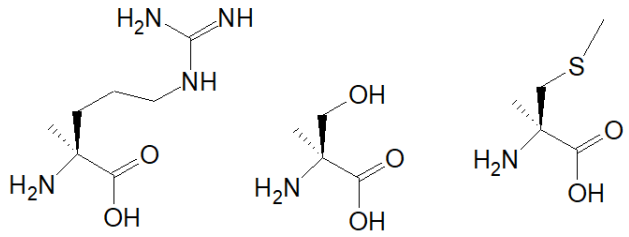

Figure 8. Amino acids with nucleophilic groups that do not react with α-methylenebutyrolactones (arginine, serine, and methionine).

The reaction of α-methylenebutyrolactone-containing sesquiterpene lactones with thiols has been well explored due to its biochemical and pharmacological implications. Sesquiterpene lactones with the cyclopentenone group react very quickly with glutathione (Gly-Cys-γ-Cys) which is present in high concentrations in all living cells, but this reaction is reversible. The α- methylenebutyrolactone is far less reactive with the tripeptide (Schmidt, 1999). In contrast, addition of free cysteine highly favours the exocyclic methylene group (Schmidt, 1997). This property has been exploited by Dolman and co-workers (1992) to set up a specific HPLC-based analyses of α-methylenebutyrolactone-containing sesquiterpene lactones by derivatization with 9-thiomethylanthracene. The thiol containing reagent reacts in a Michael type addition with the α-methylenebutyrolactone of the sesquiterpene lactone and increases the sensitivity, so that routinely nanogram quantities of the lactones can be detected by HPLC and monitoring at 369 nm.

Finally, many naturally occurring sesquiterpene lactones are found to be bound to other α, β unsaturated groups -for example dihydrohelenalin methacrylate- that seem to play no role as a structural element reacting in Michael-type reactions (Wagner et al, 2004)

Reaction of parthenolide with amino acids and other thiol-containing molecules.

Salan (1993) reported that the α-methylene-γ-butyrolactone of parthenolide is not reactive with $–NH_2$ and –OH groups of lysine and serine respectively, and therefore a reaction with such residues do not appear to interfere with the effects of feverfew. In contrast, according to Salan, the cysteine –SH group is very reactive to such a lactone function. In the case of parthenolide the epoxide group allows transannular cyclizations and the generation of a second alkylation site meaning that parthenolide can undergo two Michael-type additions. The reaction is initiated via protonation of the epoxide and may result in the attack of a sulphydryl -usually forming part of an amino acid, peptide or protein- onto the resultant carbocation. In addition, a second sulphydryl -or any other suitable nucleophilic group- can attack the α-methylenebutyrolactone. This chemical feature intensifies the reactivity of parthenolide as an alkylating agent, particularly towards thiols. (Figure 9) (Dewick, 2000).

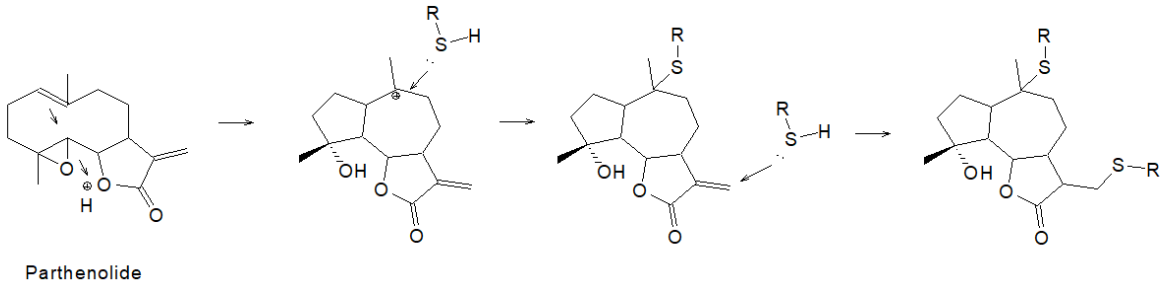

Figure 9. Reactivity of parthenolide with thiols.

The binding of sesquiterpene lactones to albumin, a protein with an important role in the bioavailability, toxicity and pharmacological behaviour of many xenobiotics, is an example of the complexity of the situation when different reactive residues are presented in a macromolecular structure. This protein contains a cysteine and a lysine residue that may react with α, β unsaturated carbonyls in lactones -*i.e*. α-methylenebutyrolactones and cyclopentenones. Franot and co-workers (1993) showed that α-methylene-γ-butyrolactones preferably react with lysine in human albumin, whereas they found that a reaction with cysteine only occurs under reducing conditions. The results of Wagner and co-workers (2004) showed that lactones were able to bind to albumin and other plasma proteins *in vitro* but the extent of the binding to cysteine residues does not explain this phenomenon, but rather the binding to other amino acids, like lysine, as well as non-covalent interactions. The results also showed that the extent of protein binding between individual sesquiterpene lactones was significantly different and in particular parthenolide binds to albumin to a greater extent than the helenalin derivatives do.

Reversibility of the formation of adducts between sesquiterpene lactones and sulphydryl-containing biomolecules.

As mentioned above, the adduct formed by sesquiterpene lactones with a cyclopentenone group reacting with glutathione (Gly-Cys-γ-Cys) is reversible (Schmidt, 1999). The addition of cysteine or other small –SH containing biomolecules to the α-methylenebutyrolactone group has also been investigated and shown to be reversible in several in vitro cell models. These adducts were oxidized by cytochrome P_{450} enzymes, thus releasing the intact lactone inside the cell (Heilmann et al, 2001).

The concept of parthenolide -and similar sesquiterpene lactones- binding to plant protein thiols -or other nucleophilic groups present in biomolecules- after the leaf cells have been disrupted by drying and powdering has been an intriguing hypothesis for many years. Salan (1993) provided the first proof of the presence of such adducts by reversing the Michael-type addition in “old” feverfew samples that did not contain anymore “free” parthenolide according to HPLC-UV analytical protocols. Sequential treatment of this plant material with an oxidant -to convert the putative sulphide into sulfone- and a weak base -to induce elimination- causes the regeneration of substantial amounts of parthenolide. Parthenolide was accompanied by the appearance of michenolide, due to the oxidation (epoxidation) of its endocyclic double bound by the excess of oxidising agent (oxone). Michenolide is however a naturally occurring compound in *Michelia compressa*. Mittra and co-workers (2000) claimed that they could reproduce these results in their laboratory.

Binding of γ- lactones to polysaccharides or polyhydroxypolymers.

Published data indicate that γ- and δ-lactones are able to bind to dispersed hydrated starch. Sorption of lactones is related to van der Waals interactions with polysaccharide molecules, which may lead to the formation of supramolecular structures: after the addition of organic substances into the starch gel, linear molecules of amylose form surrounding spirals and lactones are incorporated into the hydrophobic space between the spirals (Heinemann et al, 2001).

In some cases, these compounds cannot be isolated by extraction from the resulting complex. For example, Misharina and coworkers (2002) showed that a 25% concentration of sorbed γ-decalactone could not be desorbed by ether from gels of corn and potato starches. These authors also showed that the phenomenon of lactone sorption by polysaccharides increases with an increase in their concentration in the gel and lengthening of the alkyl substituent in the lactone molecule. They further proposed that this sorption occurs through a cooperative mechanism, which suggests the formation of supramolecular structures involving hydrated polysaccharide molecules and inclusion complexes with both amylose and lateral chains of amylopectin. The interaction between γ-heptalactone and γ-decalactone molecules with special polysaccharides with amino and sulphate groups (chitosan and carrageenan respectively) has also been studied by the same authors (Misharina et al, 2006).

Degradation of sesquiterpene lactones by the action of radiation and heat.

Ultraviolet (UV) radiation is reported to induce drastic rearrangements in germacranes. Two examples of this are the photoisomerization reported for Germacrene-D, that gives rise to α- and β-bourbonenes (Roberts, 1972), and how the irradiation of germacrene B alcohol, or its corresponding methyl ether, gives rise to three very different products (Roberts and Bryson, 1984) (Figure 10). UV radiation can also alter sesquiterpene lactones in a less extreme way. Bitter guaianolides present in *Chicorium intybus* L. (chicory) are modified by exposure to UV radiation (366nm) prior to extraction of inulin, a valuable food ingredient. The resulting lactones are not bitter as a result of the addition of a molecule of water to the double bound at C_1-C_{10}. The α-methylenebutyrolactone and cyclopentenone groups remained intact (Frey et al, 2002).

Germacrenes can undergo Cope rearrangements under high temperature conditions, irradiation, electronic impact (what is electronic impact?) or a combination of these physical factors. Germacranolides are apparently more resistant to Cope rearrangements because the lactone ring has an important influence on the thermal stability of germacrenes and makes the Cope rearrangement reversible. Cope rearrangements usually give rise to sesquiterpene lactones of the elemane type (Jain et al, 1970; 1971). Costunolide and dihydrocostunolide, that resemble parthenolide both chemically and biosynthetically, undergo Cope rearrangement at 200ºC to give dehydrosaussurealactone and saussurealactone respectively (de Kraker et al, 2000), which are naturally occurring compounds in *Saussurea* species (Roberts, 1972).

According to Cretnik and co-workers (2005) the thermal stability test indicated little degradation of parthenolide at 80°C: after 1 h the parthenolide content in acetonitrile decreased from 10 mg/10mL to 8.7 mg/10mL and after 5 h the content decreased to 8.4 mg/10 mL. This could be due to the strong influence of the lactone ring on the thermal stability of germacrenes (de Kraker et al, 2000) and a good example are some of the apparently unstable epoxidated sesquiterpene lactones found in feverfew such as the crystalline endoperoxide tanaparthin-α- peroxide, which is, in fact, a remarkably stable compound: it only undergoes a smooth, stereospecific, thermal rearrangement in rather extreme conditions (150ºC; C_6D_6; 1.5 h) to give canin, a major natural occurring guaianolide in feverfew (Begley et al, 1989).

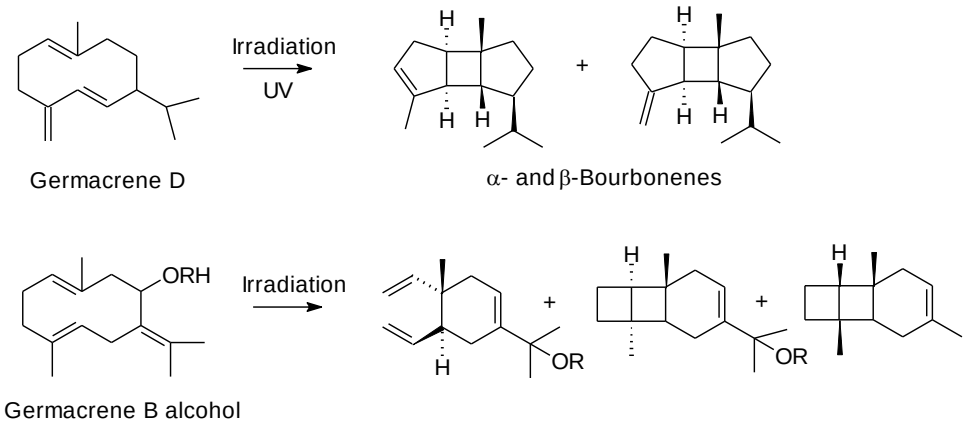

Figure 10. UV-induced rearrangements in germacrenes.

DEGRADATION OF SESQUITERPENE LACTONES BY MICROBIAL ACTION.

Sesquiterpene lactones can be degraded or transformed by microorganisms. The microbial transformation of parthenolide has been studied by Galal and co-workers (1999). Species of *Aspergillus*, *Candida*, *Cimninghamella*, *Gymnascella*, *Lindera*, *Penicillium*, *Rhizopus, Rhodotorula* and *Saccharomyces* were able to convert parthenolide into 11βH-dihydroparthenolide. The reduction of the C_{11}-C_{13} exocyclic double bond is comparable with the type of reactions catalysed by enoate reductases. This is a group of iron-sulphur flavoproteins that are involved in fatty-acid biosynthesis and can be found in many microorganisms (de Kraker et al, 2002) (Figure 11).

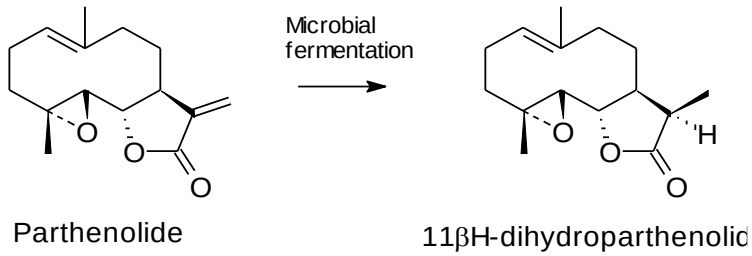

Figure 11. Main product of the microbial degradation of parthenolide.

Other identified minor metabolites were 9β-hydroxy-11βH-dihydroparthenolide, and 14- hydroxy-11βH-dihydroparthenolide that are products of allylic oxidation reactions, which are considered common microbial bioconversions of many unsaturated steroids and terpenoids (Fonken & Johnson, 1972). It was noticed that the microbial reaction proceeds with the retention of the *trans* configuration of the double bond.

The result of the microbial degradation of the α-methylene group is likely to reduce or eliminate the biological activity of sesquiterpene lactones (Ruengeler et al, 1999; Siedle et al, 2004). In addition, the increased polarity of the hydroxy compounds may improve their elimination in whole organisms. Detection of these metabolites by RP-HPLC-UV analyses of plant material may be difficult after a predictable decrease of their elution time and different UV spectral characteristics in comparison to parthenolide and other α-methylenebutyrolactones.

DISCUSSION

The data from the reviewed literature strongly support the idea of a complexation with –SH containing biomolecules, mostly plant proteins. Salan (1993) proved this theory by regenerating parthenolide from old material stored until it did not contain any trace of this compound. Many authors (Knight, 1995; Dewick,

2002, Mittra et al, 2000) also agree that this is the fate of parthenolide, and predictably the rest of sesquiterpene lactones, in dry, powdered feverfew.

The toxicological and pharmacological consequences of such a process have not been directly studied, but indirect data in the literature predict a complete loss of both activities. Michael-type additions imply the reaction of the α-methylene-γ-lactone group of the sesquiterpene lactone with a nucleophilic group giving rise to a covalent bound. This complexation removes the toxicological and pharmacological effects of sesquiterpene lactones in several experimental models (Dupuy et al, 1974; Schmidt et al, 1999).

Other physico-chemical possibilities of degradation can be acid-catalysed cyclizations, and heat or UV induced rearrangements. These reactions show a high degree of stability of the α- methylene-γ-lactone group. Parthenolide may undergo an acid-induced cyclization in aqueous solutions giving rise to a guaianolide-type sesquiterpene lactone - a type of compound that is common in feverfew (Begley et al, 1989). This type of sesquiterpene lactones has been described as a pharmacologically active form of parthenolide (Dewick, 2002). Moreover, it should be recognised that these “degradative processes” are initiated immediately after the harvest of feverfew, during the conditioning of the plant material (drying of leaves). Sorption of lactones with polyhydroxypolymers requires the direct contact of the compound with the polymers in a solution. This process is probably restricted to the formulation of pure parthenolide or parthenolide-rich extracts, which is not the case in formulations consisting of dry plant material mixed with such excipients. Moreover, the phenomenon is highly reversible.

The microbiological degradation of parthenolide usually consists of the selective reduction of the α-methylene group, and we can surmise that this will be accompanied by an important loss of its biological properties.

Finally, formulations of Feverfew used in clinical trials for 4-6 months did not show any toxicity and the reported adverse effects were mild, transient and similar to those reported by the placebo group (Murphy et al, 1998; Johnson et al, 1985; Palevitch et al, 1997) even though a certain degree of degradation would be expected during this time. There are reported differences in the toxicity of old feverfew material and fresh material in that dry encapsulated material is less allergenic than fresh feverfew (Schuller and Cupp, 2000; Mahady et al, 2001).

CONCLUSION

In conclusion, and without discounting a degradation of parthenolide into non-identifiable fragments, the fate of this compound in dry, powdered feverfew is to undergo a covalent binding to plant proteins resulting in a biologically inactive adduct - in accordance with the direct and indirect data found in the literature. This process seems to be virtually unstoppable, and temperature and light do not seem to be playing a significant role under normal storage conditions according to some authors. In the presence of a high level of humidity, parthenolide may undergo an acid-induced cyclisation giving rise to a guaianolide-type sesquiterpene lactone, a class of compound that is commonly found in Feverfew. Microbial degradations are not likely to play an important role if the formulation complies with Pharmacopoeial microbiological quality requirements. The experimental and clinical data in the literature do not report on any increase in the toxicity of stored feverfew. More work needs to be done to devise strategies to prevent loss of parthenolide in final medicinal products for their entire shelf-life (3 years at least) so this active component can be effectively used for the standardisation of feverfew extracts and products.

CONFLICT INTEREST

The author declares no personal or financial conflict of interest related to this work.

AUTHORS CONTRIBUTION

(JMP) Conceptualization, Methodology, Formal analysis, Investigation, Writing, Review & Editing.

REFERENCES

Begley, M.J., Hewlett, M.J., Knight, D.W. (1989) Revised structures for guaianolide α-methylenebutyrolactones from feverfew. Phytochemistry 28(3), 940-943.

Bilia, A.R., Bergonzi, M.C., Mazzi, G., Vincieri, F.F. (2006) Development and stability of semisolid preparations based on a supercritical CO_2 Arnica extract. Journal of Pharmaceutical and Biomedical Analysis 41, 449–454.

Brown, H.C., Brewster, J.H., Shechter, H. (1954) An interpretation of the chemical behaviour of five- and six-membered ring compounds. Journal of the American Chemical Society 76, 467-474.

Cane, D.E. (1999) Sesquiterpene biosynthesis: cyclization mechanisms. In Cane, D.E. (Ed) Comprehensive Natural Products Chemistry: Isoprenoids including caretenoids and steroids Vol 2, Elsevier, Oxford (UK), pp 155-200.

Castaneda-Acosta, J., Fischer, N.H., Vargas, D. (1993) Biomimetic transformations of parthenolide. Journal of Natural Products, 56(1), 90-98.

Cretnik, L., Skerget, M., Knez, Z. (2005) Separation of parthenolide from feverfew: performance of conventional and high-pressure extraction techniques. Separation and Purification Technology 41, 13–20.

de Kraker, J-W., Franssen, M.C.R., Joerinkm M., de Groot, A., Bouwmeester, H.J. (2002) Biosynthesis of costunolide, dihydrocostunolide, and leucodin. Demonstration of cytochrome P450-catalyzed formation of the lactone ring present in sesquiterpene lactones of chicory. Plant Physiology 129, 257–268.

Dewick, P.M. (2002) Medicinal Natural Products. John Wiley & Sons, London, pp 191-196.

Dolman, D.M., Knight, D.W., Salan, U., Toplis, D. (1992) A quantitative method for the estimation of parthenolide and other Sesquiterpene lactones containing α-methylenebutyrolactone functions present in feverfew. Phytochemical Analysis 3, 26- 31.

Dupuis, G., Mitchell, J.C., Towers, G.H.N. (1974) Reaction of alantolactone, an allergenic sesquiterpene lactone, with some amino acids. Resultant loss of immunologic reactivity. Canadian Journal of Biochemistry 52(7), 575-581.

Fonken, G.S., Johnson, R.A. (1972) Chemical oxidations with microorganisms. In: Belew, J.S. (ed) Oxidation in organic chemistry, vol 2. Marcel Dekker, New York, pp 185–212.

Fonseca, J.M., Rushing, J.W., Thomas, R.L., Riley, M.B., Rajapakse, N.C. (2003) Post-production conditions affect stability of parthenolide in feverfew. Horticultural Science 38, 1294.

Fonseca, J.M., Rushing, J.W., Thomas, R.L., Riley, M.B., and Rajapakse, N.C. (2007). Post-Production Stability of Parthenolide in Feverfew (Tanacetum parthenium). Journal of Herbs, Spices & Medicinal Plants 12, 139-152.

Franot, C., Benezra, C., Lepoittevin, J.P. (1993) Synthesis and interaction studies of ^{13}C labelled lactone derivatives with a model protein using ^{14}C NMR. Bioorganic and Medicinal Chemistry 1, 389-397.

Frey, U., Claude, J., Crelier, S., Juillerat, M.A. (2002) UV degradation of sesquiterpene lactones in chicory extracts: kinetics and identification of reaction products by HPLC- MS. Chimia 56, 292-293.

Galal, A.M., Ibrahim, A.S., Mossa, J.S., El-Feraly, F.S. (1999) Microbial transformation of parthenolide. Phytochemistry 51, 761-765.

Groenewegen, W.A., Knight, D.W., Heptinstall, S. (1992) Progress in the medicinal chemistry of parthenolide. In Ellis GP and Luscombe DK (Eds) Progress on Medicinal Chemistry Vol 29, Elsevier, Amsterdam, 217-238.

Hall, I.H., Lee, K.H., Starnes, C.O., Sumida, Y., Wu, R.Y., Waddell, T.G., Cochran, J.W., Gerhart, K.G. (1979) Anti-inflammatory activity of sesquiterpene lactones and related compounds. Journal of Pharmaceutical Sciences 68(5), 537-542

Heilmann, J., Wasescha, M.R., Schmidt, T.J. (2001) The influence of glutathione and cysteine levels on the cytotoxicity of helenanolide type sesquiterpene lactones against KB cells. Bioorganic & Medicinal Chemistry 9, 2189–2194.

Heinemann, C., Conde-Petit, B., Nuessli, J., Escher, F. (2001) Evidence of starch inclusion complexation with lactones. Journal of Agricultural and Food Chemistry 49(3), 1370– 1376.

Heptinstall, S., Awang, D.V., Dawson, B.A., Kindack, D., Knight, D.W., May, J. (1992) Parthenolide content and bioactivity of feverfew (Tanacetum parthenium (L.) Schultz- Bip.). Estimation of commercial and authenticated feverfew products. Journal of Pharmacy and Pharmacology 44(5), 391-395.

Heptinstall, S., Awang, D.V.C. (1998) Feverfew: a review of its history, its biological and medicinal properties, and the status of commercial preparations of the herb. In ACS Symposium Series Vol 691 (Phytomedicines of Europe), American Chemical Society, pp 158-175.

Hoffmann, J.J., Torrance, S.J., Wiedhopf, R.M., Cole. J,R. (1977) Cytotoxic agents from Michelia champaca and Talauma ovata: parthenolide and costunolide Journal of Pharmaceutical Sciences 66, 883-884.

Jain, T.C., Banks, C.M., McCloskey, J.E. (1970) Dehydrosaussurea lactone and reversibility in the germacranolide-Cope reaction. Tetrahedron Letters 11, 841–844.

Jain, T.C., McCloskey, J.E., Banks, C.M. (1971) Germacranolide-Cope reaction under electron-impact. Organic Mass Spectrometry 5, 751–755..

Johnson, E.S., Hylands, P.J., Hylands, D.M. (1982) Eur Pat EP 98401 (GB Pat, 1982, 82/14572).

Johnson, E.S., Kadam, N.P., Hylands, D.M., Hylands, P.J. (1985) Efficacy of feverfew as prophylactic treatment of migraine. British Medical Journal 291, 569-573.

Khan, M.R., Kihara, M., Omoloso, A.D. (2002) Antimicrobial activity of Michelia champaca. Fitoterapia 73(7-8), 744-748.

Knight DW. 1995. Feverfew: chemistry and biological activity. Natural Products Reports 12(3), 271-276.

Kwok, B.H.B., Koh, B., Ndubuisi, M.I., Elofsson, M., Crews, C.M. (2001) The anti- inflammatory natural product parthenolide from the medicinal herb Feverfew directly binds to and inhibits IκB kinase. Chemical Biology 8, 759–766.

Mahady, G.B., Fong, H.H.S., Farnsworth, N.R. (2001) Botanical dietary supplements: quality, safety and efficacy, Swets & Zeitlinger, Lisse (The Netherlands), pp 87-95.

Majdi, M., Liu, Q., Karimzadeh, G., Malboobi, M.A., Beekwilder, J., Cankar, K., Vos, R.D., Todorović, S., Simonović, A., and Bouwmeester, H. (2011). Biosynthesis and localization of parthenolide in glandular trichomes of feverfew (Tanacetum parthenium L. Schulz Bip.). Phytochemistry 72, 1739-1750.

Majdi, M., Charnikhova, T. & Bouwmeester, H. (2013) Genetical, developmental and spatial factors influencing parthenolide and its precursor costunolide in feverfew (*Tanacetum parthenium* L. Schulz Bip.). *Industrial Crops and Products*, 47, 270-276.

Marete, E. N., Jacquier, J.-C. & O'Riordan, D. (2013) Effect of processing temperature on the stability of parthenolide in acidified feverfew infusions. Food Research International, 50(2), 593-596.

Marete, E. N., Jacquier, J.-C. & O'Riordan, D. (2011) Feverfew as a source of bioactives for functional foods: Storage stability in model beverages. Journal of Functional Foods, 3(1), 38-43.

Mather, B.D., Viswanathan, K., Miller, K.M., Long, T.E. (2006) Michael addition reactions in macromolecular design for emerging technologies. Progress in Polymer Sciences 31, 487–531

Misharina, T.A., Terenina, M.B., Krikunova, N.I., Kalinchenko, M.A. (2006) Sorption of components from a mixture of odorants by polysaccharides of starch, chitosan, and carrageenan. Applied Biochemistry and Microbiology 42(1), 111–115.

Misharina, T.A., Terenina, M.B., Krikunova, N.I. (2002) Binding of Lactones by Polysaccharides of Corn and Potato Starches. Applied Biochemistry and Microbiology 38(6), 583–587.

Mittra, S., Datta, A., Singh, S.K., Singh, A. (2000) 5-Hydroxytriptamine-inhibiting property of Feverfew: role of parthenolide content. Acta Pharmacologica Sinica 21(12), 1116- 1114.

Murphy, J.J., Heptinstall, S., Mitchell, J.R. (1988) Randomised double-blind placebo- controlled trial of feverfew in migraine prevention. Lancet 2, 189-192.

Palevitch, D., Earon, G., Carasso, R. (1997) Feverfew (*Tanacetum parthenium*) as a prophylactic treatment for migraine: a double-blind placebo-controlled study. Phytotherapy Research 11(7), 508-511.

Piet, D.P., Schrijvers, R., Franssen, M.C.R., de Groot, Ae. (1995) Biotransformation of germacrene epoxides by Cichorium intybus L. Tetrahedron 51, 6303–6314.

Roberts, J.S., Bryson, I. (1984) Sesquiterpenoids. Natural Products Reports. 105-169.

Roberts, J.S. (1972) The sesquiterpenes. In Newman AA Ed. Chemistry of terpenes and terpenoids. Academic Press, London (UK) pp 88-146.

Ruengeler, P., Castro, V., Mora, G., Goren, N., Vichnewski, W., Pahl, H.L. 1999. Inhibition of transcription factor NF-kappaB by sesquiterpene lactones: a proposed molecular mechanism of action. Bioorganic and Medicinal Chemistry 7, 2343-2352.

Salan, U. (1993) Chemistry of Feverfew. Dissertation. Univ. of Nottingham, 135-168.

Schmidt, T.J. (1997) Helenanolide-type sesquiterpene lactones III. Rates and stereochemistry in the reaction of helenalin and related helenanolides with sulfhydryl containing biomolecules. Bioorganic and Medicinal Chemistry 5, 645-653.

Schmidt, T.J., Lyß, G., Pahl, H.L., Merfort, I. (1999) Helenanolide type sesquiterpene lactones. Part 5: the role of glutathione addition under physiological conditions. Bioorganic and Medicinal Chemistry 7, 2849-2855.

Schmidt, T.J., Matthiesen, U., Willuhn, G. (2000) On the stability of sesquiterpene lactones in the official Arnica Tincture of the German Pharmacopoeia. Planta Medica 66, 678- 681.

Schuller, B., Cupp, M.J. (2000) Feverfew. In Cupp MJ (Ed.) Forensic Science: Toxicology and Clinical Pharmacology of Herbal Products, Humana Press, Totowa (NJ, USA) pp 95-105.

Sethi, V.K., Thappa, R.K., Dhar, K.L., Atal, C.K. (1984) Constituents of Michelia champaca and Lewis acid catalyzed transformations of parthenolide into guaianolides. Planta Medica 50(4), 364.

Siedle, B., Garcia-Pineres, A.J., Murillo, R., Schulte-Monting, J., Castro, V., Rungeler, P., Klaas, C.A., Da Costa, F.B., Kisiel, W., Merfort, I. (2004) Quantitative structure-activity relationship of sesquiterpene lactones as inhibitors of the transcription factor NF-κB. Journal of Medicinal Chemistry 47, 6042-6054.

Takahashi, M., Fuchino, H., Satake, M., Agatsuma, Y., Sekita, S. (2004) In vitro screening of leishmanicidal activity in myanmar timber extracts. Biological and Pharmaceutical Bulletin 27(6), 921-925.

Tanko, H.M., Carrier, D.J., Sokhansanj, S., Crowe, T.G. (2003) Effects of drying temperature and storage on parthenolide concentration of feverfew (Tanacetum parthenium L.) leaves. Journal of Nutraceuticals, Functional & Medical Foods 4(1), 27-37.

Wagner, S., Kratz, F., Merfort, I. (2004) In vitro behaviour of sesquiterpene lactones and sesquiterpene lactone-containing plant preparations in human blood, plasma and human serum albumin solutions. Planta medica 70(3), 227-233.

Wang, M. & Li, Q. (2015) Parthenolide could become a promising and stable drug with anti-inflammatory effects. Natural Product Research, 29(12), 1092-1101.