V. PHARMACOLOGY
11. THE BIOLOGICAL/PHARMACOLOGICAL ACTIVITY OF THE *SALVIA* GENUS

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INTRODUCTION

Sage (*Salvia* species) has been used as a herb with beneficial healing properties for millennia. The name itself comes from the Latin word for health (*salvare* or heal). Ancient authors called it *elelisphakon*. This term most likely refers to several species, such as *Salvia fruticosa* Mill., *Salvia officinalis L. and Salvia pomifera* L. (Rivera *et al.,* 1994). A tenth century Salerno School called it *Salvia salviatrix,* whereas the Spanish call it *ierba buena* or "good herb". Both terms admire feats attributed to sage. A proverb assures us, that a man who has sage in his garden needs no doctor. Sage became very popular also in China in the eighteenth century where the merchants would exchange two crates of best tea for a crate of sage (Toussaint-Samat, 1996).

Until the discovery of antibiotics, sage was a frequent component of herbal tea mixtures, recommended in patients with tuberculosis to prevent sudation. The essential oil of sage is still employed in flavouring condiments, cured meats, liqueurs and bitters. Besides the usage as a flavouring and antioxidant agent, sage (*S. officinalis* L.) leaves exhibit a range of biological activities, i.e. antibacterial, micostatic, virustatic, astringent and antihidrotic (Anonymus, 1994). Sage was found to be an active ingredient in combined plant preparations for treatment of acute and chronic bronchitis. Animal studies show hypotensive activity and central nervous system (CNS) depressant action of sage extracts (Newall *et al., 1996*). Because of antimicrobial effects (Dobrynin *et al.,* 1976; Cherevatyi *et al.,* 1980; Farag *et al.,* 1986) and tannin-based astringent activities of sage this is used as an active ingredient of dental-care preparations. It reduces growth of plaques, inhibits gingival inflammation, and has beneficial effects on caries prophylaxis (Willershausen *et al.,* 1991).

The traditional Chinese herbal drug Dan-Shen (Tan-shen, *S. miltiorrkiza* Bge.) is described to have sedative, antimicrobial, antispasmodic, anti-inflammatory and antioxidant properties. Tan-shen is mentioned in Chinese Pharmacopoeia as a drug that treats problems associated with heart and circulatory system (oral preparations: decoction or tablet with *Panax notoginseng*) insomnia (dry, oral preparation: decoction with *Polygala tenuifolia* and *Zizyphus spinosa*) and as a drug used in the

treatment of acute arthritic pain in patients with rheumatism (Xiao, 1989). Also, use of the decoction of tan-shen together with other herbs such as *Angelica sinensis* and *Curcuma zedoaria,* is recommended in the treatment of amenorrhea, dysmenorrhea and other menstrual disorders. When studying the effects of *S. miltiorrhiza* on endocrine function of ovary-uterus in immature rats, increased level of estradiol (E2) in plasma, weight of uterus and ovarian PGF2 alpha content were observed by Li *et al.* (1992). *S. Miltiorrhiza* stimulated ovulation in immature mice, inhibited function of corpus luteum in pseudopregnant rats and decreased concentration of progesterone in plasma.

S. haematodes Wall., known as red sage, was found to posses significant CNS depressant (anticonvulsant) properties (Akbar *et al.,* 1985). Further pharmacological screening revealed a broad variety of pharmacological effects. When tested in animal models, the ethanolic extract of red sage showed anti-inflammatory and analgesic effects, hypothermic response in non-pyretic rats and enhancement of the wound healing process (Akbar, 1989). The ethanolic extract of *S. haematodes* had significant inotropic and chronotropic effects on isolated rabbit hearts. It also had a parasympathomimetic effect on isolated rabbit duodenum. Unfortunately, active substances responsible for these effects have been so far unknown, although different constituents are probably involved.

S. desoleana Atzei & Picci, an indigenous Sardinian species, is used in folk medicine to treat menstrual and digestive disorders and diseases of the central nervous system. Peana and Satta (1992) reported that the essential oil from the leaves of *S. desoleana* had a dose dependent central nervous-depressant effect in mice. In further pharmacological screening, the essential oil was tested also for its choleretic effects in rats and was found to significantly increase bile flux at 1 h after administration of essential oil at 250 mg/kg *i.p.* or its alcoholic (linalool and alpha-terpineol) fraction at 62 mg/kg i.p. (Peana *et al.,* 1994). Intraperitoneal administration of essential oil produced stronger choleretic effects than subcutaneous administration. The amount of dry bile residue of essential oil treated rats was higher than that of control values at 1 and at 2 h after treatment. Linalool and alpha-terpineol fractions of the essential oil fractions showed the strongest choleretic activity.

A wide variety of species (900 known species) of the *Salvia* genus shows also much variety in bioactivity. There are, however, many differences in pharmacol ogical effects amongst these species. Aerial parts of these plants usually contain flavonoids and triterpenoids as well as essential oils with volatile compounds such as monoterpenoids. Diterpenoids are the main compounds in roots. Some of these compounds have been isolated and their structures elucidated, however, many compounds are still scientifically challenging. Many more studies on structure-activity relationship within the *Salvia* species are needed in order to explain mechanisms of biological activity.

ANTIMICROBIAL AND ANTIVIRAL ACTIVITIES

Extensive literature on the antimicrobial potency of the *Salvia* genus reveals a broad variability with regard to microorganisms sensitivity as well as to the efficiency

(measured as minimal inhibitory concentration, MIC) of tested compounds, when different species are considered. Most frequently, essential oils with volatile monoterpenoids as their major constituents are reported to be antibacterially active in those *Salvia* species that are rich in essential oil (*S. officinalis* L., *S. lavandulifolia* Vahl., *S. triloba* L.=*S. fruticosa* Mill.).

Less evidence exists on the antifungal potency of these essential oils. Generally, Gram-negative bacteria are not sensitive or are less sensitive for sage essential oil when compared with the sensitivity of Gram-positive bacteria. This is in agreement with observations of Maruzella and Henry (1958) and of Yousef and Tawil (1980), while some authors report that there is no relationship between susceptibility of tested bacteria to essential oils and their Gram reaction (Deans and Ritchie, 1987, Shapiro *et al*., 1994).

When compared with some other species from *Labiatae* family (especially *Thymus* spp. and *Origanum* spp.), essential oils of *Salvia* species show relatively low antibacterial and/or antifungal activity (Thompson *et al.,* 1986).

Sage oil turned out to exhibit inhibitory effects on many of oral bacteria, such as obligate anaerobes *(Fusobacterium nucleatum, Peptostreptococcus anaerobius, Porphyromonas gingivalis, Treponema denticola, Treponema vincentii)* and capnophilic microaerophiles (*Actinobacillus actinomycetemcomitans, Capnocytophaga* spp., *Eikenella corrodens*) at concentrations between 0.06 % (w/v) and 0.2 % (w/v). When compared to obligate anaerobes, the facultative anaerobe group of oral bacteria *(Actinomyces viscosus, Strepococcus sanguis, Streptococcus sobrinus)* was generally less sensitive to administered sage oil. Sage oil inhibited the growth of the facultative group at concentrations between 0.3% (w/v) and 0.6 % (w/v) (Shapiro *et al.,* 1994).

The Egyptian sage essential oil, composed mostly of thujone (41.5%) and of limonene (14.7%), shows antibacterial activity against Gram-positive *Sarcina* spp. (MIC=2.0 mg/ml), *Staphylococcus aureus* (MIC =1.0 mg/ml), *Bacillus subtilis* (MIC=0.75 mg/ml) and against yeast *Saccharomyces cerevisiae* (MIC 2.0 mg/1) (Farag *et al.,* 1989a). According to Kustrak and Pepeljnjak (1989), the antimicrobial activity (against *Bacillus subtilis*) of sage oil depended on composition, i.e. contents of 1, 8-cineole, p-cymene, *a*- and *ß*-thujone and camphor as well as on the relationship between 1, 8-cineole, p-cymene and ketonic compounds. The antimicrobial activity of Dalmatian sage oil, was attributed to its thujone contents (Jalsenjak *et al*., 1987). Antibacterial activity was not reduced even when essential oil was microencapsulated into gelatin-acacia capsules (although a certain time lag in achieving full activity was observed), microencapsulation, however, inhibited antifungal activity of sage oil.

According to Deans and Ritchie (1987), who tested 50 essential oils against 25 genera of bacteria, sage (*S. officinalis* L.) essential oil (undiluted) was moderately effective against the growth of *Bacillus subtilis, Brevibacterium linens, Micrococcus luteus, Seratia marcescens* bacteria. When tested against eight bacteria *(Bacillus subtilis, Escherichia coli, Hafnia alvei, Micrococcus luteus, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus faecalis)* and five fungi (*Aspergillus niger, Aspergillus terreus,* two strains of *Candida albicans,*

Fusarium spp.) commercial sage essential oil (probably issued from a mixture of *S. triloba* L. and of *S. lavandulifolia* Vahl.) had almost no effect (Biondi *et al.,* 1993). Similarly, the essential oil from *S. triloba* showed no fungistatic activity against soil-borne pathogens *(Fusarium oxysporum, Macrophomina phaseolina)* or against foliar plant pathogens *(Botrytis cinerea, Exserohilum turcicum)* (Shimoni *et al.,* 1993). Ground sage (2%), as a component of Malt Extract Agar (MEA) medium, showed no fungistatic activity against food-contaminating fungi *(Trichoderma harzianum, Alternaria alternata, Fusarium oxysporum, Mucor circinelloides f. griseo-cyanus, Rhizopus stolonifer, Cladosporium cladosporioides, Fusarium culmorum, Aspergillus versi-color, Penicillium citrinum)* (Schmitz *et al.,* 1993).

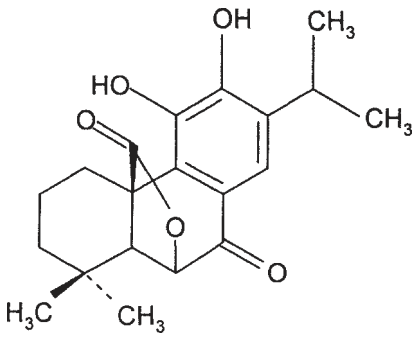
Contrary to this, by measuring the antifungal property of sage essential oil against *Alternaria alternata* and against *Aspergillus parasiticus,* a strong fungistatic effect was observed (Crisan and Hodisan, 1975; Farag *et al.,* 1989b). Volatile oils showed much stronger fungistatic properties than tested extracts (Crisan and Hodisan, 1975).

Concentration of 2.0 mg/ml sage oil reduced *Aspergillus parasiticus* mould growth by 87.6% and inhibited total aflatoxin (B and G groups) production by more than 96% (Farag *et al.,* 1989b). Like antibacterial activity, the mould growth inhibitory effect of sage oil was mainly due to thujone as a major component in essential oil. According to Farag *et al.* (1989a, 1989b), a relationship between the chemical structures of the most prevalent compounds in essential oils and antimicrobial activity was observed. The antimicrobial activity of sage essential oil was lower than that of essential oils (thyme oil, clove oil) containing thymol or other phenolic-OH structure compounds (eugenol). A well known inductive effect of polar functional groups (e.g. hydroxyl or isopropyl) on aromatic nucleus seems of great importance in explaining the correlation between structure and antimicrobial activity of the essential oil compound.

The essential oil of *S. plebeia* is also reported to have fungitoxic potential, inhibiting the growth of storage fungus *Aspergillus flavus* by 54% (at a concentration of 5000 ppm) (Mishra and Dubey, 1990).

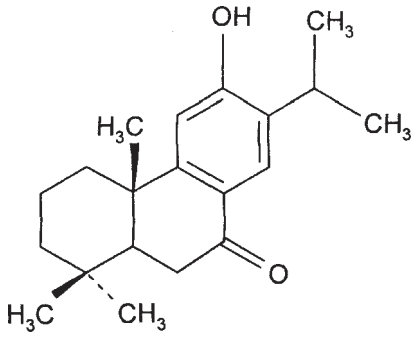
Much emphasis has also been placed on the investigation of compounds (i.e. diterpenoids, flavonoids) in extracts of different *Salvia* species, which show significant inhibitory activity against bacteria (G-negative and/or G-positive) and fungi.

Significant antibacterial (towards gram-negative *Klebsiella pneumoniae* at a concentration 400 μg/ml and against gram-positive *Bacillus subtilis* at 300 μg/ml and *Staphylocoecus aureus* (200 μg/ml) and antifungal (towards *Candida albicans* at concentration of 200 μg/ml) compounds (carnosic acid, 16-hydroxycarnosic acid and their derivatives) were found in the diterpene acid fraction of extract of *S. apiana,* whereas its essential oil (composed primarily of 1, 8-cineole and camphor) as well as a mixture of oleanolic and ursolic acid were inactive even at 1000 μg/ml against tested organisms (Dentali and Hoffmann, 1992). Antibacterial activity (against *Staphylococcus aureus*) of carnosic acid (referred to as salvin) has been reported already by Dobrynin *et al.* (1976) and Pavlenko *et al.* (1989). The dry methanolic extract of *S. officinalis,* dissolved in DMSO (50 mg/ml DMSO) inhibited the growth of Gram-positive *Staphylococcus aureus* at a concentration 100 μg/ml, while no

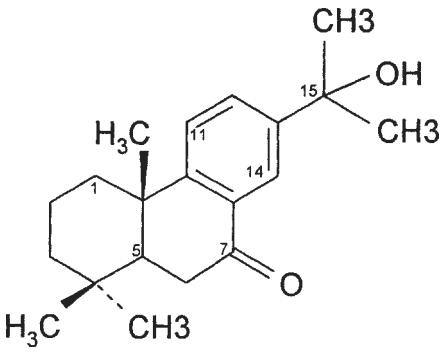


Structure 1

antibacterial activity against Gram-negative bacteria *E. coli* or *Pseudomonas aeruginosa* strains was observed (Baricevic *et al.,* 1996). An abietane diterpene galdosol *(structure 1)* with antibacterial properties against *Bacillus subtilis, Micrococcus luteus and Staphylococcus aureus* was isolated from the aerial parts of *S. canariensis* L., a plant endemic to Canary Islands (Gonzalez *et al.,* 1989a; Gonzalez *et al.,* 1989b; Darias *et al.,* 1990). This shrub has been used in folk medicine as an antispasmodic, febrifuge and hypoglycemiant. Abietane diterpenes, sugiol *(structure 2)* and 15-hydroxy-7-oxo-abiet-8,11,13-triene *(structure 3),* were isolated from S. *albocaerulea* Lindl., a species, which is native to the south-eastern intertropical region of Mexico. These two diterpenes are responsible for antimicrobial activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus,* MIC 50 μg/ml) and for a moderate activity against *Candida albicans* (at 100 μg/ml), but are inactive against Gram-negative bacteria (Pereda-Miranda *et al.,* 1992). Another abietane diterpene, forskalinone *(structure 4)* isolated from roots of *S. forskahlei* L., showed slight antimicrobial activity against *Enterococcus faecalis* (168 μg/ml) (Ulubelen *et al.,* 1996). Free catechol grouping (or



Structure 2.



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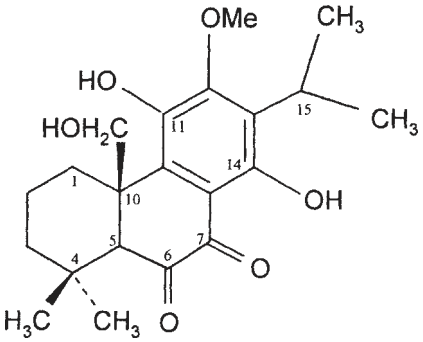

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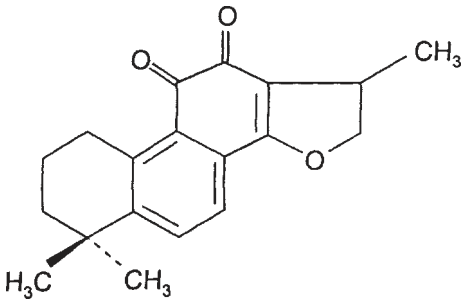
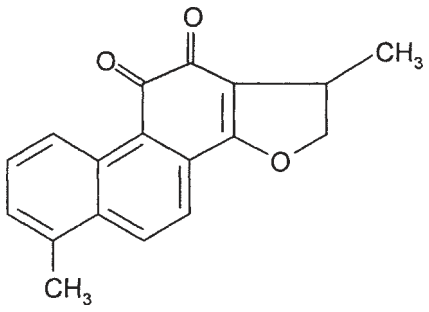


Structure 5

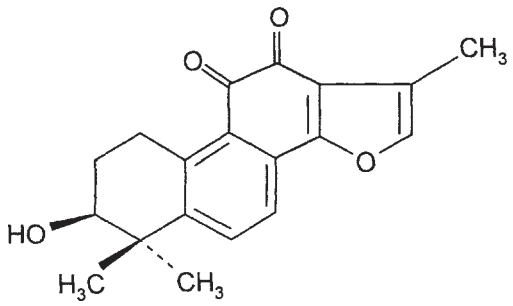
its oxidised quinone structure) is responsible for the antimicrobial activity of *Salvia* abietane diterpenes against Gram-positive bacteria (Moujir *et al.,* 1993).

A series of phenanthrene quinone derivatives has been identified in S. *miltiorrhiza* (Kakisawa *et al.,* 1968; Kakisawa *et al.,* 1969; Shibata *et al.,* 1982; Xiao and Fu,

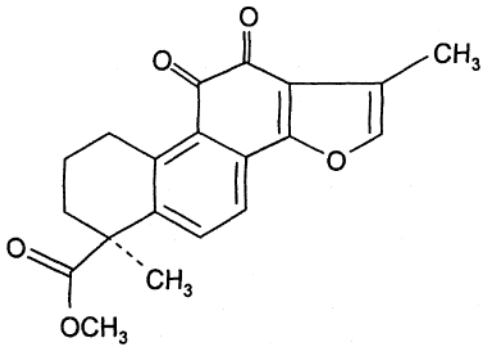
1987). The medicinal mixture known as tanshinone, as well as components of mixture such as cryptotanshinone *(structure 5)*, dihydrotanshinone I *(structure 6)*, hydroxytanshinone II-A *(structure 7)*, methyltanshionate *(structure 8)* and tanshinone II-B *(structure 9)*, were shown to have bacteriostatic activity, especially on



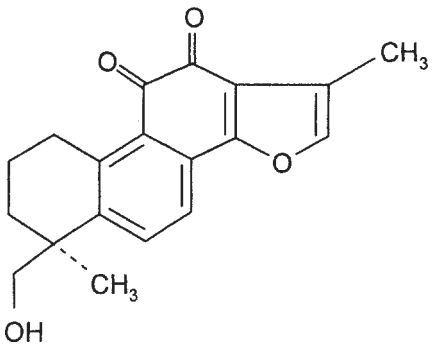
Structure 6



Structure 7

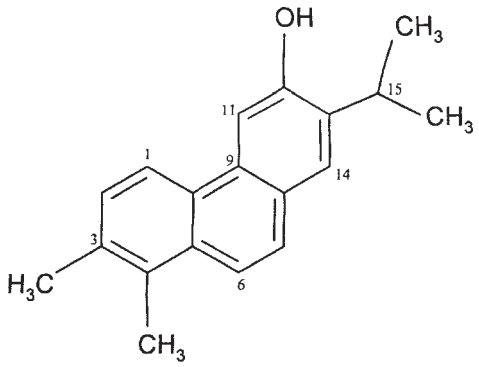


Structure 8

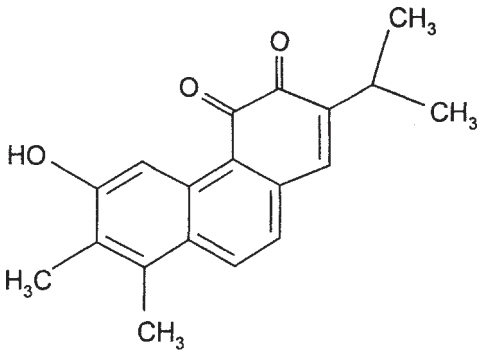


Structure 9

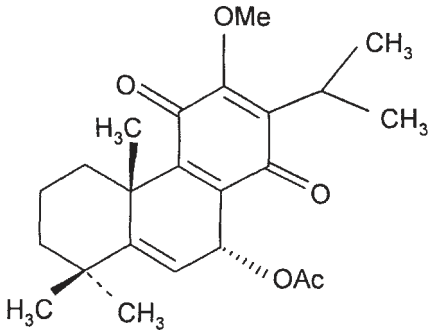
Staphylococcus aureus strains cultured *in vitro*. Tanshinones didn't show any antimicrobial activity against gram-negative bacteria *(E. coli, Pseudomonas aeruginosa, Citrobacter freundii, Serratia marcescens)* and yeasts *(Candida albicans, C. krusei, C. mycoderma, C. tropicalis, C. utilis, Saccharomyces sake)* at a concentration of 100 μg/ml (Honda *et al.*, 1988). While having a similar inhibitory effect against gram-positive bacteria (MIC 0.195–50 μg/ml), dihydrotanshinone I and cryptotanshinone differ fundamentally in their activity against dermatophytes. Dihydrotanshinone I (but not cryptotanshinone) was proved to be a potent antidermatophytic substance. This compound inhibited the mycelial growth of six dermatophytes (*Trichophyton rubrum, T. mentagrophytes, T. tonsulans* var. *sulfureum, Mycrosporum gypseum, Sabourandites canis, Epidermophyton floccosum*) at a concentration as low as 1.56 to 6.25 μg/ml, an activity being comparable to that of griseofulvin (Honda *et al.*, 1988). Tanshinone demonstrated inhibitory activity against *Mycobacterium tuberculosis* H 37 Rv and two related dermatophytes. Tablets and ointment of tanshinone provided satisfactory clinical results in 455 cases of infection mainly with *Staphylococcus aureus* (Gao *et al.*, 1979; Xiao and Fu, 1987). A significant antituberculous activity, tested on *Mycobacterium tuberculosis* H 37 Rv, was showed



Structure 10



Structure 11

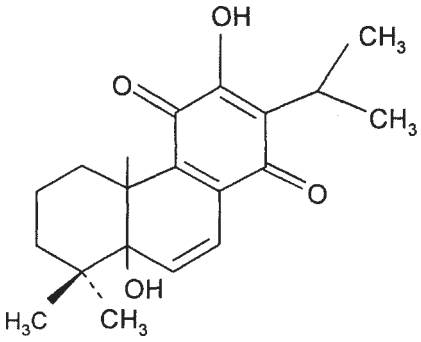


Structure 12

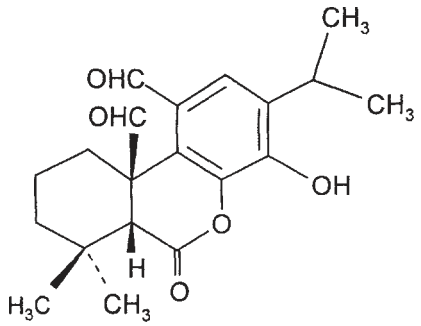
also by norditer-penoids and diterpenoids from *S. multicaulis,* the most potent substances being 12-demethylmulticaulin *(structure 10),* 12-demethylmultiorthoquinone *(structure 11)* and 12-methyl-5-dehydroacetylhorminone *(structure 12)* (Ulubelen *et al.,* 1997). Among anitibacterial active hypargenins, i.e. abietane diterpenoids isolated from the root extract of *S. hypargeia,* hypargenin F *(structure 13)* showed antituberculous activity (Ulubelen *et al.,* 1988).

Also some flavonoids proved to be active against Gram positive and/or Gram negative bacteria. Cirsimaritin, a flavonoid isolated from the leaves of *S. palaestina* Bentham, showed a high activity against standard strains of *Staphylococcus aureus* (MIC=31.25 μg/ml; minimum bactericidal concentration, MBC=125 μg/ml), *Staphylococcus epidermidis* (MIC=62.5 μg/ml ; MBC=125 μg/ml), *E. coli* (MIC=45 μg/ml ; MBC=90 g/ml), *Pseudomonas aeruginosa* (MIC=31.25 μg/ml; MBC=125 μg/ml), *Proteus vulgaris* (MIC=31.25 μg/ml ; MBC=125 μg/ml) and *Klebsiella pneumoniae* (MIC=45 μg/ml ; MBC=90 μg/mJ) (Miski *et al.,* 1983).

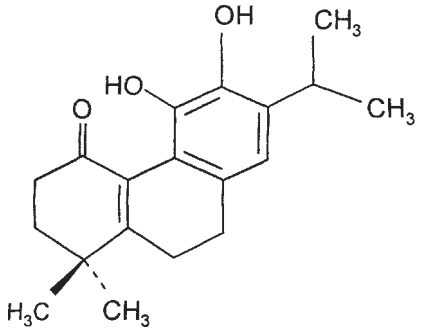
A potent antiviral activity of crude extracts of sage *(S. officinalis)* is displayed by two abietane diterpenoids, which were isolated from sage aerial parts and their structure elucidated. Safficinolide *(structure 14)* was active against VSV (vesicular stomatitis virus), while sageone *(structure 15)* showed virus inactivation activity



Structure 13



Structure 14



Structure 15

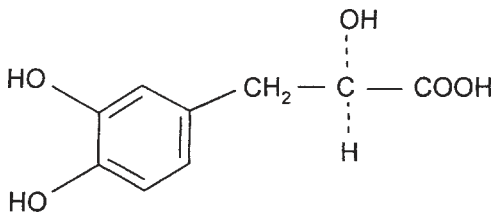
against HSV (herpes simplex virus type 1) (Tada *et al.*, 1994). Also, Sivropoulou *et al.* (1997) report on the high virucidal activity of essential oil of *S. triloba* against HSV. According to Bulgarian researchers, water and alcoholic extracts of sage were active against influenza, herpes simplex and vaccinia viruses (Manolova *et al.*, 1995). This preparation was officially approved for clinical use in Bulgaria.

CARDIOVASCULAR AND RENAL ACTIVITIES

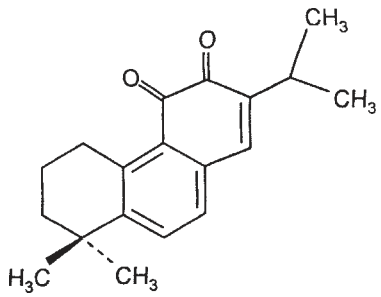
In China, herbal remedies made from *S. miltiorrhiza* roots are used by modern medicine to treat diseases such as cardio-cerebral ischemia, thrombosis, in the treatment of neurasthenic insomnia and in the prevention of myocardial infarction because they are capable of reducing aggregation of blood platelets, mobilising blood circulation, and of removing stasis (Chen, 1984; Chang and But, 1986; Lee *et al.,* 1987; Liu *et al.,* 1992). Danshensu, *ß* (3, 4-dihydroxyphenyl)-lactic acid *(structure 16),* obtained from the water-soluble fraction of *S. miltiorrhiza,* is reported to dilate coronary artery and significantly antagonise constricting responses elicited by morphine and propranolol (Dong and Jiang, 1982; Xiao and Fu, 1987).

The roots of *S. miltiorrhiza* were proved to inhibit cellular cholesterol biosynthesis (Sun and Cai, 1989) and to have vasodilatory, hypotensive, and anticoagulant properties. They are beneficial to patients with chronic renal failure (Chung *et al.,* 1986; Yokozawa *et al.,* 1990). However, use of the decoction of *S. Miltiorrhizae* in hypertension is questionable because it induces both vasodilatation and vaso-constriction what depends on the dosage and the target vessel (Lei and Chiou, 1986a; Lei and Chiou, 1986b). *S. Miltiorrhiza* dilated coronary vessels both at lower (3 mg/ml) or higher (10 mg/ml) concentrations, however, it contracted renal, femoral and mesenteric arteries at higher concentration (10.0 mg/ml) only.

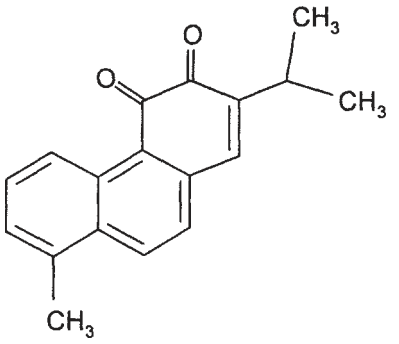
Some sources identify phenolic compounds as the main source of a wide range of pharmacological properties. Among stasis-eliminating compounds abietane diterpenoids (miltirone—*structure 17,* Ro09–0680—*structure 18* and salvinone—



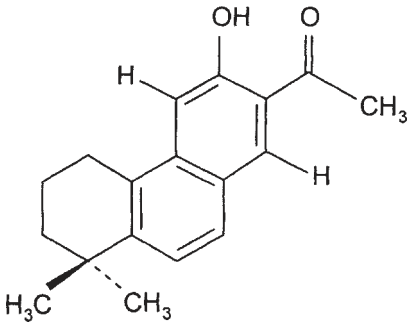
Structure 16



Structure 17



Structure 18

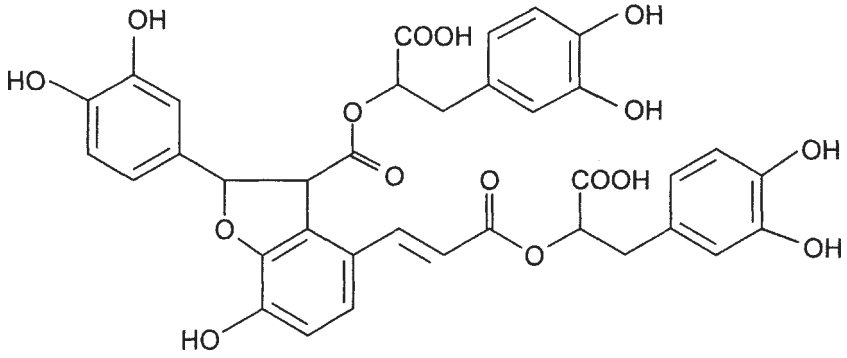


Structure 19

structure 19) showed a dose-dependent *in vivo* inhibiting properties in platelet aggregation in rabbits induced by collagen (Wang *et al.,* 1989). Yu and Xu (1994) report on the significant *in vitro* and *in vivo* inhibitory effect of acetylsalvianolic acid A (ASAA) on rat and rabbit platelet aggregation, induced by ADP, collagen and arachidonic acid. While inhibiting platelet aggregation, ASAA was found also to have a suppressive effect on collagen-induced 5-HT release. Zou *et al.* (1993) report on the antithrombotic effect of rosmarinic acid, which might be associated with its inhibition of platelet aggregation and promotion of fibrinolytic activity.

From the water soluble fraction of *S. miltiorrhiza,* an active principle, danshensuan B *(structure 20)*, a compound refered as lithospermic acid B by Tanaka *et al.* (1989), was isolated. This compound promotes fibrinolysis and increases coronary blood flow (Xiao and Fu, 1987). A potent inhibitory effect of compound IH764–3, isolated from *S. miltiorrhiza,* on fibroblast proliferation and on their ability to synthesise collagen, was reported by Liu *et al.* (1992).

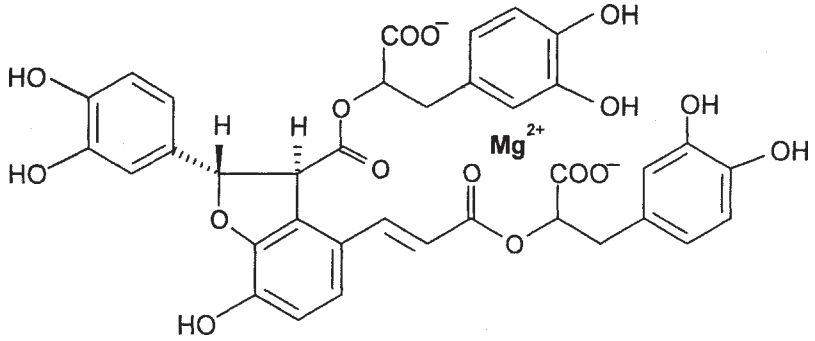
S. Miltiorrhiza aqueous extract (decoction) was mentioned as a useful anti-anginal agent as it dilates coronary vessels (Lei and Chiou, 1986a; Lei and Chiou, 1986b). In isolated whole-heart preparations *S. Miltiorrhiza* it significantly



Structure 20

increased coronary blood flow for 15 min and had positive inotropic action for 3 min after pulse injection. The results of *in vivo* and *in vitro* studies (rat and rabbit blood vessels, four types of dog vasculature) indicate, that vasodilatation of coronary arteries and vasodilatation of renal, mesenteric and femoral arteries at low concentrations (3 mg/ml) after administration of extracts of *Salviae Miltiorrhizae* might be ascribed to increased utilisation of extracellular calcium ions (activity of the drug plant on isolated blood vessels of rabbits was enhanced by 2 mM Ca^{2+}). According to Li *et al.* (1990), the vasodepressor effect, which might account for positive inotropic and for negative chronotropic effects (the latter through modulation of cholinergic activity) of *Salviae Miltiorrhizae* extracts, was probably angiotensin- and/or bradykinin-related.

When Chung *et al.* (1987) studied the effects of *Salviae miltiorrhizae* extract on renal function in normal rats, they established that this extract, after intraperitoneal single dose (10 mg/100 g body weight), markedly increased urine volume, and urinary urea, creatinine, sodium, potassium and inorganic phosphate excretion. No potassium retention was observed and no changes in the renin-angiotensin system or in aldosterone level were observed, what implies that natriuretic effect of extract was not mediated via reduced aldosterone secretion. Acute administration of *Salviae miltiorrhizae* extract also significantly increased glomerular filtration rate in renal plasma flow and increased renal blood flow, which might influence and increase in urinary urea and creatinine (Chung *et al.,* 1987). Aqueous extract of *Salviae miltiorrhizae radix,* when chronically administered to uremic rats with mild or moderate (but not severe) uremic state, was reported to decrease urea nitrogen, creatinine, methylguanidine and guanidinosuccinic acid levels and to increase serum guanidinoacetic acid concentration as well as to increase renal tissue blood flow while decreasing renal vascular resistance and blood pressure (Chung *et al.,* 1986; Yokozawa *et al.,* 1987). These observations indicate, that neurological and humoral factors responsible for lowering blood pressure may mediate an increase in renal blood flow. Diminished renal vascular resistance and attendant increase in renal blood flow by *Salviae miltiorrhizae radix* extract may contribute to the increase of glomerular filtration rate and the increase in urinary



Structure 21

excretion of uremic toxins such as urea and creatinine. In the eighties, magnesium lithospermate B *(structure 21),* a tetramer of caffeic acid, was isolated from *Salviae miltiorrhizae radix* and was proved to be responsible for most of the above mentioned effects that facilitate renal function in normal rats or in rats with mild or moderate renal failure. Magnesium lithospermate B was found to cause significant decrease of urea nitrogen, creatinine, inorganic phosphate, methyl guanidine and guanidinosuccinic acid levels in blood of adenine diet-induced uremic rats and showed remarkable improving effect on uremie symptoms of these rats (Tanaka *et al.,* 1989; Yokozawa *et al.,* 1989b; Yokozawa *et al.,* 1990a).

After intraperitoneal administration of magnesium lithospermate B (10 mg/kg) to rats with adenine-induced renal failure, the levels of glomerular filtration rate, renal plasma flow and renal blood flow increased, while renal vascular resistance decreased (Yokozawa *et al.,* 1989a; Yokozawa *et al.,* 1989b; Yokozawa *et al.,* 1990a). Also, urinary excretions of prostaglandin E_2 (PGE_2) and 6-keto-prostaglandin F_1 (6-keto-PGF1), which have vasodilating effects on mesangial cells of glomeruli and on small vessel system of kidney, increased in magnesium lithospermate B treated rats, while there were no significant changes in excretion of vaso-constrictive prostanoid thromboxane B_2 (TXB_2). Significant increase in PGE_2 and 6-keto-PGF_1 were achieved also in normal rats, after intraperitoneal administration of magnesium lithospermate B (at 10 mg/kg body weight), while its effect became weaker as renal failure progressed due to the prolonged administration of adenine (24 days). Urinary sodium excretion decreased gradually with the progression of renal failure, whereas administration of magnesium lithospermate B significantly increased urinary sodium as well as potassium excretion together with a decrease in mean blood pressure, increase in renal tissue blood flow and increase in cerebral blood flow (Yokozawa *et al.,* 1992; Yokozawa *et al.,* 1993). Increase in renal tissue blood flow was associated with a significant increase in excretion of urinary urea, creatinine and inorganic phosphate. These results suggest that magnesium lithospermate B reduces renovascular hypertension by improving urinary electrolyte excretion and haemodynamics. These effects might be due to magnesium lithospermate B-influenced increase in formation of

prostaglandin E_2 in renal tissue (which contribute to improvement of renal blood flow and to reduction in renal vascular resistance) and have a protective effect against renal failure providing renal tissues are still functioning.

Kidneys play an important role in the pathogenesis of hypertension as a consequence of a primary defect in renal haemodynamics that influences retention of fluid and electrolytes. It was found that magnesium lithospermate B does not affect renin-angiotensine-aldosterone system (Yokozawa *et al.,* 1990 b), but induces dilation of blood vessels, increase in renal blood flow and improvement of renal func tion by enhancing production and secretion of PGE_2 in kidney through activation of the kallikrein system (Yokozawa *et al.,* 1994). The kinin-kallikrein system, together with the prostaglandin system, is involved in the mechanism of blood pressure regulation and regional blood flow as well as the metabolism of water and electrolytes. Oral administration of magnesium lithospermate B (10 mg/kg) in spontaneously hypertensive rats resulted in significant decrease of systolic, mean and diastolic blood pressures, effects having depended on duration of administration period. Also, the low urinary kallikrein level in spontaneously hypertensive rats increased with a parallel increase in excretion of PGE_2, sodium and potassium. These findings suggest that magnesium lithospermate B, which proved to be a safety compound when administered orally (LD50 > 3000 mg/kg), reduces hypertension by improving renal circulatory state, at least partly through activation of the kinin-kallikrein-prostaglandin system in kidney.

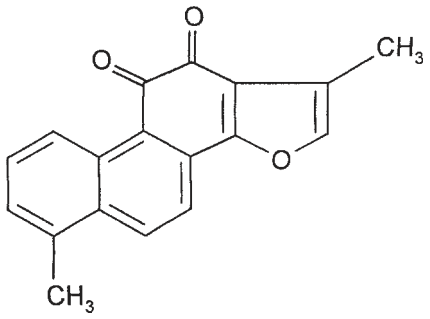
Guoji *et al.* (1994) report that the polysaccharide fraction isolated from *Salviae miltiorrhizae radix,* which contains a large amount of uronic acids, reduces the symptoms of aminonucleoside (puromycin, PA)-induced experimental nephrosis in rats. A decreased urinary protein excretion, increased serum albumin and less severe lesions of the epithelial cells were observed in rats treated with PA (60 mg/kg, *i.v.*) after both oral (40 mg/kg) or intramuscular (2.5 mg/kg) injection of polysaccharide active fraction (AF). According to Guoji *et al.* (1994), this improvement was probably influenced by polyaninonic nature of AF due to many carboxyl groups of galacturonic acids, that might stabilise and restore glomerular basement mebrane in PA-induced nephrosis.

The hexane extract from *S. miltiorrhiza* root was shown to have strong antioxidant properties, similar to those of dihydrotanshinone I, isolated from non-polar extracts of Danshen (Gordon and Weng, 1992). Sodium tanshinone II-A sulfonate (STS) is a water-soluble derivative of tanshinone II A and displays marked cardiovascular activity. After STS-treatment of patients with cardiovascular diseases and cerebral thromoboembolism the symptoms like anginal pain and feeling of chest tightness were reduced and ischemic alterations in the electrocardiogram (EGG) showed a more normal course (Chen *et al.,* 1979; Xiao and Fu, 1987). Lithospermic acid B, when infused at 5.5 mol/kg into post ischemic rabbit heart, reduced the myocardial damage found in saline control by 62 10% (Fung *et al.,* 1993). STS was found to significantly reduce the myocardial infarct in a rabbit 1-hr ischemia and 3-hr reperfusion model (Wu *et al.,* 1993). STS did not inhibit oxygen uptake by xantine oxidase (XO) which is a key enzyme in free radical generation. Also, STS significantly prolonged the survival of cultured human saphe nousvein endothelial cells but not human ventricular

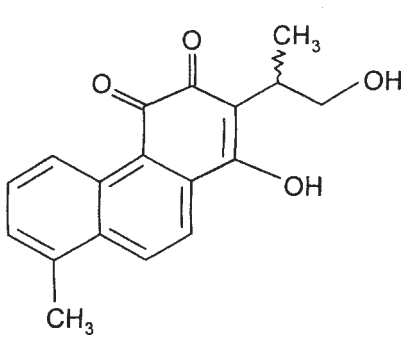
myocytes *in vitro* when these cells were separately exposed to XO-generated oxyradicals. STS was found to be a cardioprotective substance, which may beneficially influence vascular endothelium, a key site of oxidant generation and heart attack.

Bai and Wang (1994) clinically studied the haemodynamic effects of *Salvia miltiorrhiza* and compared it to nitroglycerin, and established that both drugs had similar vaso-dilating effects. Both drugs reduced the filling pressure of the left ventricle and increased the cardiac output, although the effect of *Salvia miltiorrhiza* was markedly superior and was more persistent than that of nitroglycerin.

Based on preclinical studies, the aqueous extract of *S. miltiorrhiza* was found to significantly reduce the mortality rate and to have a protective role in chemically (isoproterenol or $BaCl_2$) induced acute myocardial ischemia and arrhythmia (Cheng *et al.,* 1990), and in cardial ischemia induced by ligation of the coronary artery (Cheng *et al.,* 1992). Tanshinones are reported to protect myocardium against disturbances in cardiac function and metabolism induced by oxygen deficiency (Yagi *et al.,* 1989; Takeo *et al.,* 1990; Yagi *et al.,* 1991; Yagi *et al.,* 1994). When isolated rat hearts were subjected to hypoxic perfusion (20 min) in the presence of either 37.5 nM tanshinone I *(structure 22),* 29.5 nM cryptotanshinone *(structure 5),* or 37.5 nM tanshinone VI *(structure 23),* and tanshinone VI derivatives (42 nM), the decreased



Structure 22



Structure 23

cardiac contractile force was significantly recovered after subsequent 45 min heart reoxygenation, while little recovery of cardiac contractile force was observed upon reoxygenation in control rats. Tanshinones prevented the hypoxia/reoxygenation-induced increase in tissue sodium and calcium and decrease in tissue potassium and magnesium. Also, resting tension (a marker for cardiac contractile failure after oxygen deficiency) at 45 min reoxygenation in hearts pre-treated with these compounds was significantly lower than that without treatment. Concomitantly, tanshinones dimin ished the release of creatine kinase (CK) and ATP metabolites such as adenosine, inosine and hypoxanthine from hypoxic/reoxygenated hearts (Yagi *et al.,* 1994). Release of CK from myocardium is considered to be an indicator of cardiac cell necro sis or of an increase in cell membrane permeability, while release of ATP metabolites in hypoxia/reoxygenation or ischemia/reperfusion hearts serves as an indicator of loss of purine nucleosides from myocardium. These results suggest that enhanced recovery of contractile force of rat heart upon reoxygenation by tanhinones may be at least partly due to restoration of heart tissue ionic concentrations, prevention of cardiac cell necro sis, preservation of cell membrane integrity and due to the improvement of restoration of myocardial high-energy phosphates in myocardium, which in turn may enhance restoration of ATP when oxygen is replenished.

Free radicals play an important role in pathogenesis of acute viral myocarditis (AVM). A clinical study of 60 children with AVM showed that *S. miltiorrhiza* as an effective antioxidant significantly decreased the levels of plasma lipid peroxide (LPO) and that of erythrocyte membrane microviscosity (EMMV) (Meng *et al.,* 1992). In most patients treated with *S. miltiorrhiza,* LDH, GOT and EGG recovered after one course. These results announce the myocardium- protective effects of the drug in acute viral myocarditis. Another clinical study on infantile acute toxic myocarditis also showed an efficiency of *Salvia miltiorrhiza* treatment and its advan tages over the western medicine control group, that can be seen in a significantly shorter period of hospitalisation and EGG normalisation when compared to the control group (Wang, 1993).

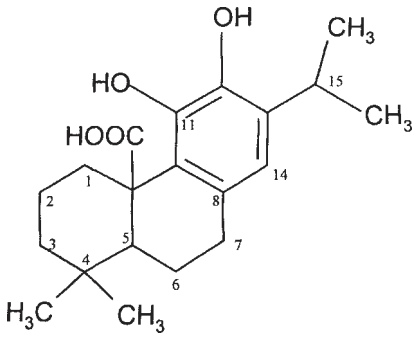
As stated above, cardiovascular activity is almost exclusively attributed to *Salvia miltiorrhiza* and its preparations. However, animal studies show that also *Salvia officinalis* posses the potential in lowering the blood pressure in animal studies (Newall *et al.,* 1996; Todorov *et al.,* 1984). When applied intravenously or duo denally, the aqueous-alcoholic extract of *S. officinalis* induced a moderate but prolonged lowering of blood pressure in cats.

BIO-ANTIOXIDATIVE, ANTIINFLAMMATORY AND TUMORIGENESIS-PREVENTING ACTIVITIES

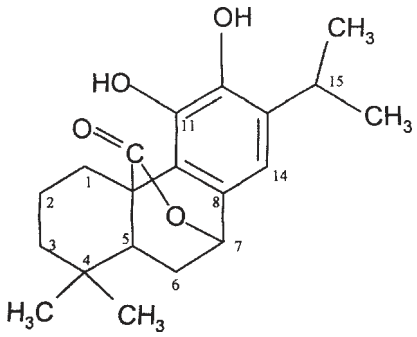
Within the last decade, the importance of free radicals in the aetiology of disease has been increasingly recognised and has led to development of new approaches in biochemical evaluation of events associated with mutagenesis, tumorigenesis and/or cancer promotion. Biomembranes (microsomes, plasma membrane…) are rich in polyunsaturated fatty acids, which are very sensitive to the peroxidative damage

induced by free radicals. Free radicals are generated by metabolic pathways within the body or can also be caused by transformation of specific xenobiotic molecules and by ecological pollutants. Dietary supplies of natural antioxidants act as protective agents against such free radicals and can, in sufficient amounts, act as efficient scavengers of free radicals before any tissue damage occurs (Deighton *et al.,* 1993).

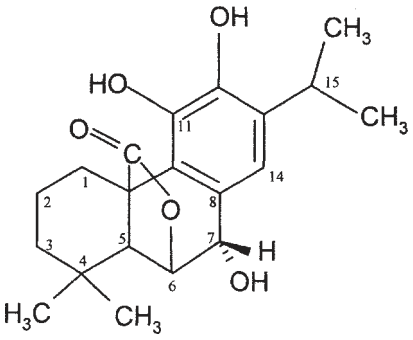
Leaves of sage (*S. officinalis* L.) are well known for their phenolic structure-based antioxidative potency (Chipault *et al.,* 1956; Farag *et al.,* 1989; Lamaison *et al.,* 1990; Schwarz and Ternes, 1992; Cuvelier *et al.,* 1994). Commercially available extracts of sage are mainly utilised by the food processing industry, but may be applicable in human health. Main sage phenolic diterpenes, which show high antioxidative activity are carnosic acid *(structure 24),* which is known for its instability, and its degradation derivatives carnosol *(structure 25),* rosmanol *(structure 26),* its isomer epirosmanol *(structure 27),* 7-methyl-epirosmanol (Cuvelier *et al.,* 1994; Schwarz *et al.,* 1992; Schwarz and Ternes, 1992) as well as rosmanol 9-ethyl ether (Markovic *et al.,* 1996). Rosmarinic acid *(structure 28)* also



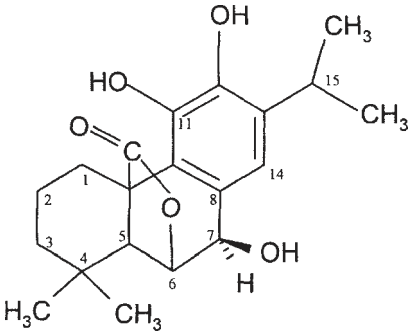
Structure 24



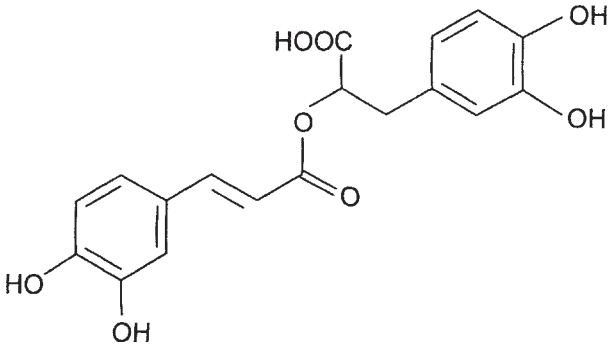
Structure 25



Structure 26

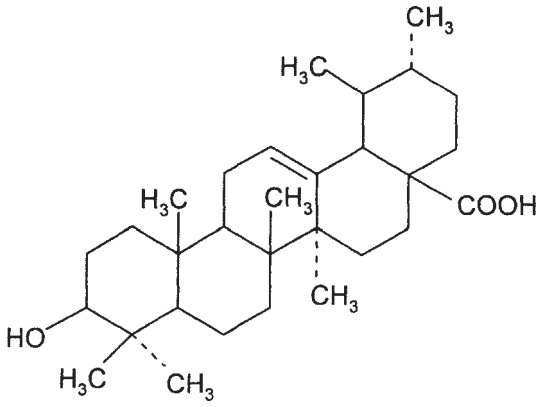


Structure 27



Structure 28

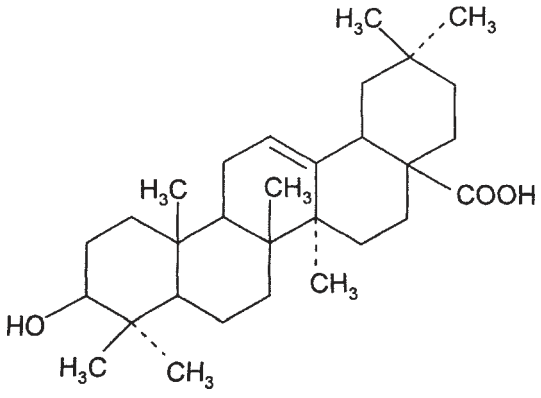
accounts for the antioxidant activity of sage. When measuring the radical scavenger effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, the antioxidative effect of rosmarinic acid (EC_{50}=2.7 μg/ml) was comparable to that of ascorbic acid (Lamaison *et al.,* 1991). A variety of plant phenolic compounds were investigated for



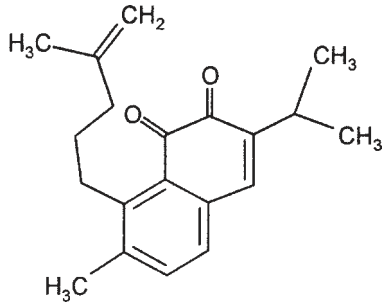
Structure 29

their antioxidative potential using human aortic endothelial cells (HAEC) to mediate oxidation of low-density lipoprotein (LDL) (Pearson *et al.,* 1997). All anti-oxidants produced a dose-dependent inhibition of LDL oxidation. Most potent antioxidants in HAEC system were carnosic acid, carnosol and rosmarinic acid.

Some strong natural antioxidants like carnosol were proved to exhibit anti-inflammatory and inhibitory effects with regard to tumor-initiation activities in mice test systems (Huang *et al.,* 1994). Also some sage compounds (ursolic and/or oleanolic acid) that show no antioxidant activity (Wu *et al.,* 1982) may turn promising in future research of inflammation and of cancer prevention. A squalene derived triterpenoid ursolic acid *(structure 29)* and its isomer oleanolic acid *(structure 30)* (up to 4% in sage leaves, dry weight basis) (Brieskorn and Kapadia, 1980), act anti-inflammatory and inhibit tumorigenesis in mouse skin (Tokuda *et al.,* 1986; Huang *et al.,* 1994; Ho *et al.,* 1994). Recent data on the anti-inflammatory activity of sage (*S. officinalis* L.) extracts when applied topically (ID_{50}= 2040 μg/cm^2) and evaluated as oedema inhibition after Croton oil—induced dermatitis in mouse ear, confirm/ suggest ursolic acid to be the main active ingredient, responsible for sage anti-inflammatory effect (Baricevic *et al.,*



Structure 30



Structure 31

2000). The data on the pharmacological effects of these metabolites promise new therapeutic possibilities of sage extracts.

The importance and therapeutic potential of naturally occurring o-naphthoquinones, compounds that might be closely involved in inflammatory process inhibition, has been stressed. It has been reported (Hernández-Pérez *et al.,* 1995), that naphthoquinone derivatives of *S. aethiopis* have a similar pharmacological profile as NSAI (Non-Steroidal-Anti-Inflammatory) substances with regard to reducing oedema induced by carrageenan and contractions induced by phenyl-p-quinone. An o-naphthoquinone diterpenoid, aethiopinone *(structure 31)*, isolated from *S. aethiopis* L. roots, showed strong anti-inflammatory and antinociceptive effects in rodents' model systems and increased bleeding time in mice with similar potency as some of NSAI drugs (Hernández-Pérez *et al.,* 1995). In anti-inflammatory studies, measured by inhibition of Carrageenan paw oedema in mouse, aethiopinone at 100 mg/kg *p.o.* inhibited oedema formation similarly to NSAI drugs (Aspirin, Ibuprofen, Piroxicam) used as reference at 50 mg/kg *p.o.* Also in the TPA-induced ear inflammation model, aethiopinone significantly reduced ear oedema induced by phorbolic ester, when administered topically (but not orally) at 1.0 mg/ear. It is therefore as effective as some NSAI drugs, but less effective than steroidal reference drugs used at a dose as low as 0.1 mg/ear. Aethiopinone exerted its analgesic effect especially against thermal painful stimuli measured by tail immersion test (significant increase in reaction time of mice at 100 mg/kg p.o.), indicating the presence of a central analgesic action, although a moderate peripheral analgesia, was also conformed by the phenylquinone writhing test, when 100 mg/kg of aethiopinone was administered orally. Even though the structure of aethiopinone is similar to that of tanshinones, which had been reported as antipyretic agents, no such properties were found when aethiopinone was assayed against yeast-induced hyperthermia in rats.

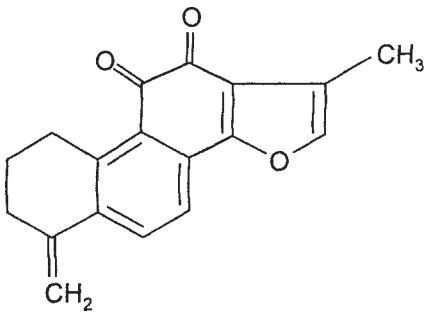
Ursolic acid showed significant cytotoxicity in lymphatic leukemia cells P-388 (ED_{50}=3.15 μg/ml) and L-1210 (ED_{50}=4.00 μg/ml) as well as human lung carcinoma cell A-549 (ED_{50}=4.00 μg/ml) (Lee et *al.,* 1987; Fang and Mc Laughlin, 1989). Both carnosol and ursolic acid are referred to as being strong inhibitors of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ornithine decarboxylase activity and of TPA-induced tumor promotion in mouse skin. The tumorigenesis-prevention potential of ursolic acid was comparable to that of retinoic acid (RA)—a known

inhibitor of tumor promotion (Tokuda *et al.,* 1986; Huang *et al.,* 1994). Both ursolic acid- and oleanolic acid- treatment (41 nmol of each), when applied continuously before each TPA-treatment (4.1 nmol), delayed the formation of papillomas in mouse skin, significantly reduced the rate of papilloma-bearing mice and reduced the number of papillomas per mouse, when compared with the control group (only TPA treatment). Ursolic acid acted more effectively in a single application before initial TPA-treatment when compared to the effect of RA and/or oleanolic acid. So, the mechanism of the inhibitory action of ursolic acid (inhibition of the first critical cellular event in tumor promotion step caused by TPA) may differ slightly from those of RA and/or oleanolic acid, which block a critical second stage process in tumor promotion by TPA (induction of ornithine decarboxylase and polyamine levels).

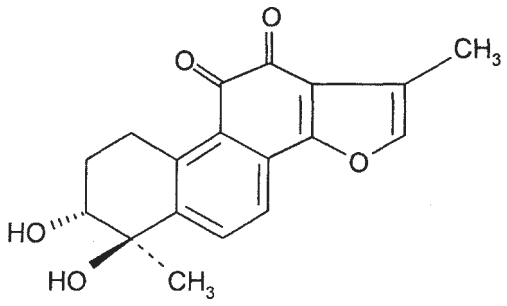
A possible tumorigenesis preventing effect can be predicted for abietane diterpene galdosol (structure 1), isolated from *S. canariensis* L., which showed significant cytostatic activity (ID_{50}=0.50 μg/ml) when inhibition of development of single-layer culture of HeLA 229 cells was measured in *in vitro* experiment (Darias *et al.,* 1990).

One of the most dangerous environmental sources of cytogenetic damage is ionizing radiation, which acts either directly or by secondary reactions and induces ionization in tissues. Interaction of ionizing radiation with water and other protoplasmatic constituents in oxidative metabolism causes formation of harmful oxygen radicals. DNA lesions, caused by reactive oxygen species in mammalian cells are the initial event which may lead to possible mutagenesis and/or carcinogenesis and form the basis of spontaneous cancer incidence (Hanawalt, 1998; Lutz, 1998). Free radi cals play an important role in preventing deleterious alterations in cellular DNA and genotoxic effects caused by ionizing radiation in mammalian tissues. Many drugs and chemicals (for example sulfhydryl compounds) are known to increase the survival rate in animals. Based on animal models studies, *S. miltiorrhiza* and its extracts were shown to have a potential to prevent X-radiation-induced pulmonary injuries (Du *et al.,* 1990) and high dosage gamma-irradiation-induced platelet aggregation lesions (Wang *et al.,* 1991).

The antiproliferative activity of tanshinones against five human tumor cells, i.e. A-549 (lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF-498 (central nerve



Structure 32



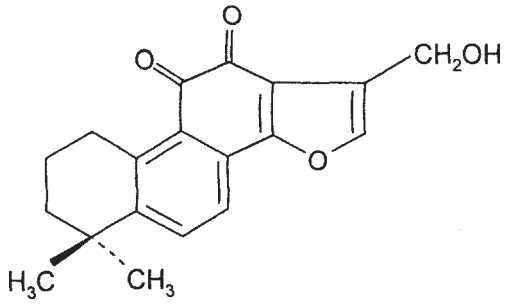
Structure 33

system) and HCT-15 (colon), was evaluated by sulfrhodamine-B method (Ryu *et al.*, 1997). 18 isolated tanshinones exhibited significant but presumably nonspecific cytotoxicity against all tested tumor cells, which might be attributed to common naphtoquinone skeleton rather than to substituents attached to it. Methylenetanshi quinone *(structure 32)* and tanshindiol C *(structure 33)* exhibited most powerful cytotoxic effects against tested tumor cells, with IC_{50} ranging from 0.4 μg/ml in A-549 cells to 2.2 μg/ml in SK-MEL-2 cells and IC_{50} from 0.3 μg/ml in SK-MEL-2 cells to 0.9 μg/ml in SK-OV-3 cancer cell lines respectively.

From *S. przewalskii* Maxim, var. *mandarinorum* Stib., a strong bacteriostatic compound, przewaquinone A *(structure 34)* was isolated. Przewaquinone A was reported (Xiao and Fu, 1987) to possess potential for inhibiting Lewis lung carcinoma and melanoma B-16.

ANTIMUTAGENIC ACTIVITY

Studies of bio-antimutagenesis, with emphasis on natural antimutagens from sage began in the late 80-ies when water extracts of *S. officinalis* were tested for their ability to suppress mutagenicity towards *Salmonella typhimurium* TA 98 of Trp-P-2,



Structure 34

a carcinogen which occurs in some foods. Sage water extracts suppressed the mutagenicity of Trp-P-2 by 90% (Natake *et al.,* 1989). To assess the antimutagenic potential of sage (*S. officinalis* L.) extracts, our laboratory used a *E. coli* test system involving the repair proficient strain WP2 *(trp)* and the excision repair deficient strain WP2uvrA. Methanolic extracts applied at nontoxic doses reduced the number of UV induced revenants in both strains, but the reduction was significantly more efficient in the repair proficient strain. The extracts were further tested for the abillity to reduce spontaneous mutation rate in mismatch repair defficient mutator strains *E. coli* IB101, *E. coli* IB 102 and *E. coli* IB 103 and non of the tested extracts reduced spontaneous mutation rate. (Baricevic *et al.,* 1996; Filipic and Baricevic, 1997).

These results are in agreement with previous data on suppression of UV-induced mutation frequency in E. *coli* repair proficient strains with ethanolic extracts of cultivated sage, that were not active in repair deficient strains (Vukovic-Gacic *et al.,* 1993). The potential antimutagenic effect of extracts of cultivated and wild *Salvia officinalis* was investigated also on new *E. coli* K12 reversion assay system for identifying antimutagens. Among three extracts tested, only extract 1, with the highest content of monoterpenoid camphor, showed bio-antimutagenic effect. Extract 1 or camphor alone suppressed UV induced mutagenesis when tested with the *E. coli* repair proficient strain, while no effect was observed when tested with the mismatch repair deficient strains (Simic *et al.,* 1997). The results obtained by comparing model bio-antimutagens with sage extracts on the same *E. coli* K12 assay system indicate, that bio-antimutagenic agents from cultivated sage enhance error-free recombina tional DNA repair by intervening in a formation of RecA-DNA complex and channelling it into recombination reaction (Simic *et al.,* 1997; Simic *et al.,* 1998). Results of the study, where several plant extracts were tested for their effect on UV-induced beta-galactosidase activity (SOS gene expression) proved no antimutagenic potential of sage extract. Moreover, the level of UV-induced enzyme was even higher after addition of sage extract (Vukovic-Gacic *et al.,* 1993; Simic *et al.,* 1997).

Recently we showed that n-hexane and chloroform sage extracts inhibited UV induced SOS response in *Salmonella typhimurium* TA1535/pSK1002 (Filipic and Baricevic, 1998). Contrary to our results Simic *et al.* (1997) observed no effect of ethanol sage extracts on UV induced SOS response. Possible reason for these differencies might be, that different active principles were isolated due to the different extraction procedures used by the two groups. Another reason might be due to the fact that different test systems having different target genes for detection of SOS response were used. In our laboratory we used *Salmonella typhimurium* TA1535/pSK1002 that has the *umuC-lacZ* fused gene while Simic *et al.* (1997) used *E. coli* IB100 that has the *sfiA-lacZ* fused gene.

Based on these results, bio-antimutagens from sage extracts could be used as preventive agents in intervention strategies against cancer but further investigation on active principles of the extracts is needed.

Mutagenic and/or antimutagenic effects are reported also in *S. miltiorrhiza* Bge. 4 tanshinones (dihydrotanshinone I, cryptotanshinone, tanshinone I, and tanshinone IIA), isolated from ether extract of *S. miltiorrhiza,* were recognised to be modulators

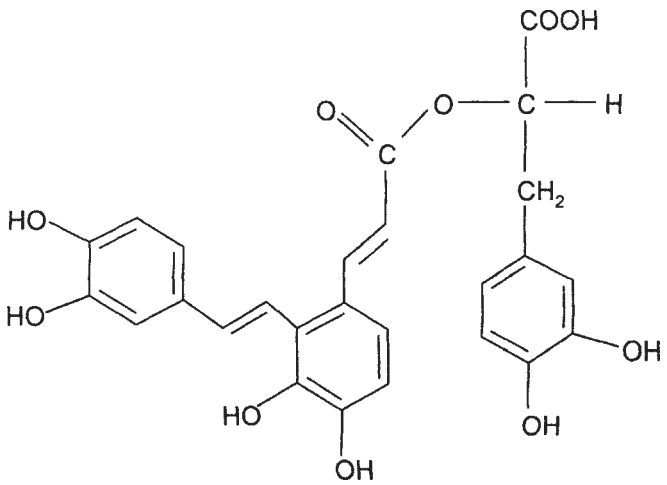
of Trp-P-1 and BP (benzopyrene) mutagenic activities in *Salmonella typhimurium* TA98. They enhanced both mutagens at low concentrations by 8 to 24-fold at 20 g/plate), but suppressed mutagens at high concentrations. Dihydrotanshinone I suppressed Trp-P-1 activity completely at 100 μg/plate (Sato *et al.,* 1992).

PEPTIC-ANTIULCER ACTIVITY

Salvianolic acid A (Sal A) *(structure 35)* was found to be a strong inhibitor of gastric H^+, K^+-ATPase and to be effective in inhibition of acid secretion and in inhibition of stress-induced gastric lesions (Murakami *et al.,* 1990). Hydroxyl groups were identified as an important moiety of Sal A in competitive interaction with ATP, thereby reducing the phosphorylation of gastric enzyme, responsible for acid secretion. Sal A was about 10 times stronger as H^+, K^+-ATPase inhibitor but less effective in antisecretory and antiulcer activities than the well known anti-ulcer agent omeprazole. This was probably due to metabolic changes at hydroxyl groups.

ANTISPASMODIC ACTIVITY

Also, antispasmodic action *in vitro* has been reported for sage (*S. officinalis* L. and *S. triloba* L.) extracts, which inhibited smooth-muscle contractions induced by acetylcholine, histamine, serotonin and barium chloride by 60 to 80%. Contrary to this, a same experiment with vervain sage (*S. verbenacea* L.) extracts showed, that this species increased spasmogenic effect of applied spasmogens on isolated smooth muscle segments of guinea-pig ileum (Todorov *et al.,* 1984). Although some of sage



Structure 35

essential oil components like pinene (Taddei *et al.,* 1988) or borneol (in higher doses) (Cabo *et al.,* 1986) show spasmogenic activity *per se,* dose-dependent antispasmodic activities of sage essential oil *in vitro* (guinea pig ileum) (Taddei *et al.,* 1988) and *in vivo* (Giachetti *et al.,* 1986) have been reported. Camphor and borneol from the essential oil of *S. lavandulifolia* Vahl., were tested for spasmolytic activity on isolated rat duodenal tissue and showed significant inhibitory activity against at least one of the chemical spasmogenic agents ($BaCl_2$ and acetylcholine) (Cabo *et al.,* 1986).

According to Giachetti *et al.* (1988) intravenous injection of *S. officinalis* essential oil emulsions resulted in a partial (10–25 mg/kg) or total (50 mg/kg) unblockage of contracted guinea pig Oddi's sphincter, induced by intravenous morphine hydrochloride (1 mg/kg *i.v.*).

HYPOGLYCAEMIC ACTIVITY

Based on ethnopharmacological data and pharmacological studies, *S. officinalis* L. (Essway *et al.,* 1995), *S. lavandulifolia* Vahl. (Jimenez *et al.,* 1985; Zarzuelo *et al.,* 1990), *S. triloba* L.(Yaniv *et al.,* 1987; Perfumi *et al,* 1991) and *S. aegyptyaca* (Shabana *et al.,* 1990) possess strong hypoglycaemic properties. In *S. officinalis* essential oil (1950 mg/kg, *i.p.*) was tested and proved to be hypoglicaemically active in normal or in alloxan-induced diabetic rats. Results with laboratory rats treated with *S. lavandulifolia* Vahl. aqueous extract indicate, that hypoglycaemic action may be a result of several synchronous mechanisms (Zarzuelo *et al.,* 1990). These include potentiation of insulin release induced by glucose, increased peripheral uptake of glucose, decreased intestinal absorption of glucose. In case of chronic treatment, hyperplasia of pancreatic islet beta cells was suggested to act as physiological background for the hypoglycaemic activity of the *S. lavandulifolia* aqueous extract.

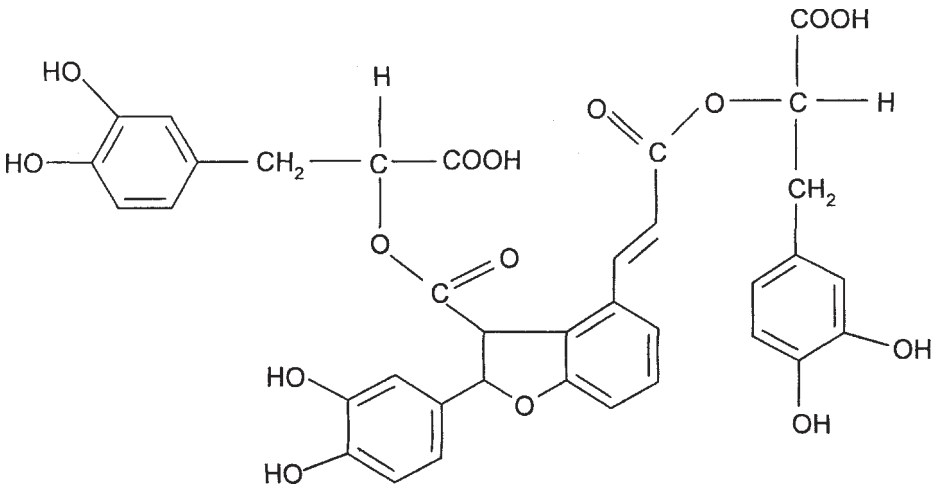
Water extracts of leaves of *S. triloba,* used in folk medicine of the eastern Mediterranean regions as a hypoglycaemic agent, were assayed on normoglycaemic rabbits and in rabbits made hyperglycaemic by alloxan administration (Perfumi *et al.,* 1991). Oral dose of 0.250 g/kg body weight caused a statistically significant reduction in blood glucose levels in alloxan-hyperglycaemic rabbits, but not in normoglycaemic animals. Contrary to this, hypoglycaemic effect was induced by single oral dose of water extract in both normoglycaemic and alloxanhyper glycaemic rabbits orally loaded with glucose. However, in these animals the *S. triloba* extract did not modify plasma insulin levels. The hypoglycaemic effect of the drug was not demonstrated in rabbits which received glucose load intravenously. These data suggest that the *S. triloba* treatment produces hypoglycaemia mainly by reducing the intestinal absorption of glucose.

HEPATOPROTECTIVE EFFECTS

The damage of cell biomembranes integrity (due to lipid peroxidation of their unsaturated fatty acids) caused by free radicals is considered as a pathway in some experimental liver injuries and clinical liver diseases. Various factors have been reported to injure liver, and especially free radicals derived from oxygen and other

chemicals are thought to be strong noxious agents. Therefore, it can be assumed that drugs with antioxidative properties might be effective in protecting the liver against oxidative stress-induced injuries. Many data on the beneficial effects of natural compounds in experimental liver injuries support this hypothesis.

For example, a strong radical scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and inhibitory activity against free radical generation as well as cytoprotective effect against t-BHP in cultured liver cell serve as examples of anti oxidative potential of methanolic extract of *S. miltiorrhiza* Bge. roots (Kang *et al.,* 1997). Also aqueous extract of *S. miltiorrhiza* roots was reported to have scavenging oxygen free radical activity and to act protectively against liver injury caused by chemicals such as carbon tetrachloride (CCl_4) (Yang *et al.,* 1990; Yu *et al.,* 1992). Liu *et al.* (1992) studied effects of seven phenolic compounds isolated from aqueous extract of *S. miltiorrhiza* on peroxidative damage to liver microsomes, hepatocytes and erythrocytes of rats. Among tested phenolic compounds, the action of salvianolic acid (Sal A) against peroxidative damage/MDA production of rat liver microsomes and hepatocytes (induced by iron/cysteine and Vitamin C/NADPH) and against hemolysis of rat erythrocytes (induced by H_2O_2) was the most potent. The site of protective action of Sal A against peroxidative damage is thought to be at the initiation stage of lipid peroxidation of polyunsaturated fatty acids of bio membranes. The potency of Sal A biomembranes-protecting activity can be explained by multiple phenolic hydroxyl groups. Also, the antioxidant activity of some other compounds like Vitamin E and butylated hydroxyl toluene is closely related to the existence of a phenolic hydroxyl group. Another water soluble polyphenolic antioxidant, salvianolic acid B (Sal B) *(structure 36),* which was also isolated from the roots of *S. miltiorrhiza,* was likewise found to scavenge DPPH. Sal



Structure 36

B prevented endothelial damage by its antioxidant potential, increased the content of vitamin E in LDL, inhibited LDL oxidative modification in hyperchol esterolemic animals, and had plasma cholesterol-lowering effect (Wu *et al.,* 1998).

Further studies show lithospermate B to be an active constituent of the water extract of *S. miltiorrhiza* which inhibits experimental liver injuries, induced either chemically (CCl_4) or immunologically (D-galactosamine/lipopolysaccharide, D-GalN/LPS, an active principle of endotoxin) (Hase *et al.,* 1997). Lithospermate B, which contains two carboxylic groups and seven phenolic hydroxy groups, was identified as a mixture of magnesium and calcium (3:1) salts of lithospermic acid B. Lithospermate B (0.1–100 μg/ml) showed a concentration-dependent protective effect on CCl_4 induced cultured hepatocytes necrosis (measured as aspartate aminotransferase (AST) concentrations in *in vitro* medium 60 min after CCl_4 challenge). The effect of lithospermate B (at 5–10 μg/ml) was more potent than that of glycyrrhizin (at 10 μg/ml), a clinically used drug for liver diseases in Japan. Results of *in vivo* experiments indicate, that lithospermate B significantly protect both CCl_4-induced liver injury in rats (at 50 and 200 mg/kg), measured as serum alanin aminotransferase (ALT), AST and lactic dehydrogenase (LDH) enzyme levels and D-GalN/LPS induced liver injury in mice (at 500 mg/kg *p.o.* or 50 mg/kg *s.c.*), measured as blood AST levels. The effects of lithospermate B in liver damage control in both animal studies were as strong as that of glycyrrhizin.

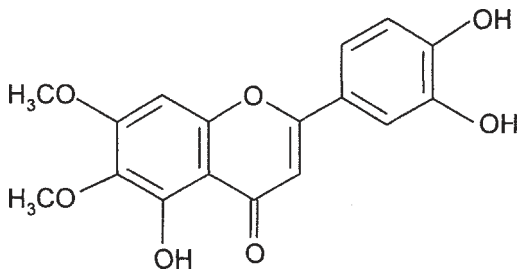
Both oleanolic acid and ursolic acid have antihyperlipidemic properties and were shown to be effective in protecting against chemically induced liver injury in laboratory animals (Liu, 1995). Multiple and toxicant-dependent mechanisms are believed to be involved in hepatoprotective effects of oleanolic acid, which protects many (e.g. CCl_4, acetaminophen, cadmium, bromo-benzene-furosemide, colchicine, D-galactosamine…) but not all of the hepatotoxicants. In comparison, ursolic acid is even more potent than oleanolic acid in decreasing the chemically induced liver injury. Suppression of hepatic cytochrome P-450 enzymes (inhibition of toxicant activation), enhancement of body defence systems, preventing liver lesions from progressing to fibrosis and stimulating liver regeneration are some of the important mechanisms of hepatoprotection.

The crude extract of *S. miltiorrhiza* dried roots and one of its main abietanoid diterpenes, tanshinone IIA, enhanced adenylate cyclase (AC) activity in purified rat liver plasma membranes in a progressive, time-dependent manner of stimulatory response (Bombardelli *et al.,* 1992).

Experimental evidence, i.e. demonstration of liver protection in test animals, has been presented for aerial parts of *S. plebeia,* a species traditionally used in folk medicine for treatment of hepatitis in Taiwan (Lin and Kan, 1990).

CENTRAL NERVOUS SYSTEM ACTIVITY

In cat model experiments, the extract of *S. miltiorrhiza* Bge. inhibited discharges of visceral pain in posterior nucleus of thalamus. It was suggested that this analgesic effect was exerted through the central nervous system (Liu *et al.,* 1990). The Central Nervous System (CNS) inhibitory effects of Danshen extracts could be attributed to



Structure 37

the interaction of active compounds with benzodiazepine (BDZ) sites of GABA receptors (Liao *et al.,* 1995). Several groups of researchers have reported the presence of benzodiazepine receptor ligands in extracts of plants, used in traditional medicine as anticonvulsants and tranquilizers (Nielsen *et al.,* 1988; Medina *et al.,* 1990; Wolfmann *et al.,* 1994; Viola *et al.,* 1994; Viola *et al.,* 1995). Active principles were found to be flavonoid derivatives with low micromolar affinities for brain BDZ receptor and in some cases, they were characterised as partial agonists, exhibiting selective anxiolytic, but not sedative properties (Wofman *et al.,* 1994; Viola *et al.,* 1995). Contrary to this, cirsiol (*structure 37*), a flavonoid isolated from aerial parts of *S. guaranitica* St. Hil., possesses cut-sedative and hypnotic properties, but shows no myorelaxant or anticonvulsant activities (Viola *et al.,* 1997). Cirsiol was shown to have a different pharmacological profile than some other naturally-occurring flavonoids (chrysin or apigenin), that specifically recognise central BDZ receptor, due to a differential interaction with a subpopulation of BDZ binding sites. Cirsiol was found to be a potent low affinity competitive ligand for type I benzodiazepine receptor in rat cerebral cortex. This interaction expresses sedative and hypnotic effects, but does not induce anxiolysis and muscle relaxation.

In some *in vitro* studies, diterpenes like carnosic acid and carnosol, which bind to the chloride channel of the GABA/benzodiazepine receptor complex in brain tissue, were considered as active inhibitory agents in the central nervous system (Rutherford *et al.,* 1992).

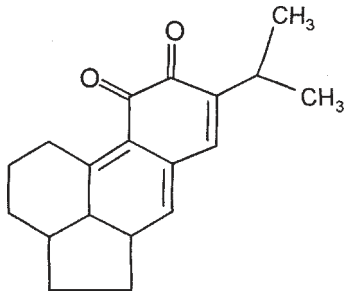
As shown in step-down and step-through tests, Sal A could at 3 and 10 mg/kg *(i.v.)* improve the impaired memory function induced by cerebral ischemia-reperfusion in mice (Du and Zhang, 1997). In the Sal A-treated group, a lower number of errors was observed and latency was longer than that of the control group. When administered intravenously at the same dosages Sal A was also found to reduce malondialdehyde contents in cortex, hippocampus and corpus striatum of cerebral ischemia-reperfusion rats *in vivo*. Sal A (10–100 nM) was shown to inhibit lipid-peroxidation of brain and to scavenge free hydroxyl radicals. These results suggest, that the protective effect in brain injury and ameliorating effect on learning and memory may be related to anti-oxidant activity of Sal A.Rosmarinic acid, lithospermic acid and its methyl ester derivatives are polyphenolic acids isolated from EtOAc fraction of methanolic extract of *S. miltiorrhiza,* and were proved to

have strong inhibitory effects on adenylate cyclase (AC) in both rat brain and in rat erythrocytes (Kohda *et al.,* 1989). Lee *et al.* (1991) observed that abietane-derived diterpene quinones (tanshinones) from *S. miltiorrhiza* strongly inhibited binding of ^{3}Hflunitrazepam, radiolabeled benzo diazepine, to the central BDZ receptor in bovine cerebral cortical membranes. Benzodiazepines are synthetic psychotropic drugs, clinically used as sedatives/ hypnotics, muscle relaxants, anxiolytic and anticonvulsant drugs in the treatment of sleep disturbances, muscle spasms, anxiety, and convulsive disorders (Rall, 1990).

Among tanshinones isolated, miltirone displayed the highest potency in central BDZ receptor binding assay (IC_{50}=0.3 μM). It behaved as a partial agonist in central BDZ receptor binding and behavioural tests. Miltirone *(structure 17),* when administered orally (10–60 mg/kg) to mice, showed tranquilizing activity. In contrast to diazepam (a full BDZ receptor agonist) miltirone produced no muscle relaxant effect. Chronic treatment of mice with miltirone (10 mg/kg, *p.o.*) twice daily in the period of 17 days did not cause sedation and did not induce drug dependence and withdrawal reactions. On the basis of these results, Chang *et al.* (1991) synthesised 22 related compounds in order to identify the key structural elements involved in interaction of miltirone with the central benzodiazepine receptor. It was found that quinone structure plays an important role in receptor interaction. Ring A and iso propyl group on ring C which can be replaced with a methyl group with minimal reduction in affinity, are essential moieties of miltirone for its interaction with the central BDZ receptor. When rings A and B were synthetically linked with an ethylene bridge, the analogue 89 *(structure 38)* was obtained, which showed higher potency in inhibiting of binding of ^{3}Hflunitrazepam to the central benzodiazepine receptor (IC_{50}=0.05 μM). Miltirone and its synthetic analogue 89 can be thus considered as potential non-sedative and non-addictive anxiolytic drugs.

PEST-TOXIC AND REPELLENT ACTIVITY

Aromatic plants and their essential oils are considered as the most effective new group of ecological products in insect and spider mite pest control. Many experiments have been carried out which show insecticidal/acaricidal and/or



Structure 38

repellent potential of the *Salvia* genus plants. Essential oils and their monoterpenoids are the most prevalent active constituents. These show either fumigant (*S. triloba* L.) or topical toxicity (*S. cardiophylla* Benth., *S. triloba* L.) as well as antifeedant or repellent (*S. officinalis* L., *S. sclarea* L., *S. triloba* L.) effects when concentration is high enough (Polyakov *et al.,* 1977; Mansour *et al.,* 1986; Hirschfeld and Klingauf, 1988; Shaaya *et al.,* 1991; Konstantopoulou *et al.,* 1992; Schmeda-Hirschmann and Rojas de Arias, 1992; Lee *et al.,* 1997).

TOXICITY

Sage and its commercial preparations, when either inhaled or ingested, were found to provoke convulsions that originate in the central nervous system. This effect has been known for more than a century (Cadeac and Meunier 1881; Grimaud-Gaspari, 1979). Several cases of human poisoning accompanied by tonico-clonic convulsions as the major symptom were observed (Millet *et al.,* 1981). Based on the experimental study of sage neurotoxicity in rats, the subconvulsive limit dose of sage essential oil was 0.3 g/kg. Convulsions started at 0.50 g/kg and became lethal with 1.25 g/kg (Millet *et al.,* 1979). Daily repeated injection of subclinical doses of sage oil had cumulative toxic effects that resulted in electrocortical clonic seizures (Millet *et al.,* 1981). Furthermore, sage essential oil has a potency to reduce epileptogenic threshold and to facilitate kindling what was showed in rats experimental model of epilepsy (Dury *et al.,* 1986). The toxicity of sage oil is apparently caused by ketone terpenoids—thujone and camphor content, so the oil should not be ingested (Millet *et al.,* 1979, Millet *et al.,* 1981, De Vincenzi and Mancini, 1997).

When *Salvia* genus essential oil content was observed only a few species, i.e. *S. officinalis* L., *S. lavandulifolia* Vahl., *S. triloba* L. and *S. sclarea* L. had permanently high levels of it. Other species contained essential oil only in traces. Also, its content might have been beneath a detectable limit (Mate *et al.,* 1993; De Vincenzi and Maialetti, 1992; Hoppe, 1975). Although there exists a great variability in the composition of major constituents of essential oils, which depends on the origin of plants, it can be generally assumed that thujone and camphor are the prevalent components in *S. officinalis* essential oil. In *S. lavandulifolia* 1, 8-cineole, camphor, *ß*-pinene and sabinyl acetate prevail. In *S. triloba* 1, 8-cineole is the most prevalent with minor camphor and thujone content and in *S. sclarea* essential oil linalool, linalyl acetate, terpineol, geranyl acetate and sclareol were identified as major constituents (Hoppe, 1975; Lawrence, 1983; Putievsky and Ravis, 1985; Tucker and Maciarello, 1990; Sivropoulou *et al.,* 1997; Souleles and Argyriadou, 1997; Lawrence, 1998). Both thujone and camphor are known to be highly toxic if they are used in prolonged treatment. Especially camphor, even when ingested in small amounts or when its administration is associated with other factors (for example febrile seizures) can cause serious or fatal consequences in small children (Calvelli *et al.,* 1987; Galland *et al.,* 1992; Liebelt and Shannon, 1993; Theis and Koren, 1995). Given the toxicity of sage essential oil, also the crude drug or its extracts should be used carefully. Its interactions with other drugs can also be dangerous. Sage may interfere with existing hypoglycaemic and anticovulsant therapies and may

potentiate sedative effects of other drugs (Newall *et al.,* 1996). Yu *et al.* (1997) report on the interaction of the extract of *S. miltiorrhiza* with warfarin, an anticoagulant drug used in the prevention of thromboembolic diseases. This interaction can provoke danshen-induced overcoagulation with severe abnormalities of clothing in patients with rheumatic heart disease.

Due to a high proportion of *a-* and *ß-* thujones in essential oil, which are known to possess abortifacient property, sage is contraindicated in pregnancy. Also, haemorrhoids and acute inflammation processes represent contraindication for use of sage or its preparations (Anonymus, 1994). Acute LD_{50} values for sage oil are documented as 2.6 g/kg (orally, rat) and 5g/kg (intradermal, rabbit) (Newall *et al.,* 1996). Because of its moderate skin irritating effects, sage oil is not recommended in aromatherapy (Newall *et al.,* 1996).

S. lavandulifolia Vahl. was also reported to be abortifaciently active, what is due to the relatively high content in sabinyl acetate in some of the chemotypes (Pages *et al.,* 1992; Fournier *et al.,* 1993). Recently an abortifacient property of *S. triloba* was observed, although compounds responsible for this effect have not as yet been discovered. Ingestion of S. *triloba* aqueous (800 mg/kg) or ethanolic extracts (400 mg/kg) in a duration of a longer period (30 consecutive days) reduced the number of implantations or viable foetuses and increased the number of resorbtions in pregnant rats (Elbetieha *et al.,* 1998). Crude drugs and their preparations should therefore be used with care during pregnancy.

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