REVIEW

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Pharmacological activities and mechanisms of natural phenylpropanoid glycosides

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The pharmacological activities and mechanisms of action of natural phenylpropanoid glycosides extracted from a variety of plants are summarized in this review, such as antitumor, antivirus, anti-inflammation, antibacteria, antiartherosclerosis, anti-platelet-aggregation, antihypertension, antifatigue, analgesia, hepatoprotection, immunosuppression, protection of sex and learning behavior, protection of neurodegeneration, reverse transformation of tumor cells, inhibition of telomerase and shortening telomere length in tumor cells, effects on enzymes and cytokines, antioxidation, free radical scavenging and fast repair of oxidative damaged DNA. Molecular modeling is discussed as well as structure-activity relationships.

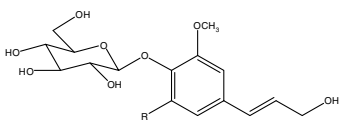
1. Introduction

Phenylpropanoid glycosides (PPGs) are present in many plants, for example *Rehmannia glutinosa*, *Lippia trisphylla* and *Forsythia*, which are distributed in China, Peru, Japan and elsewhere. Many of these plants have long been used in traditional medicine to treat a variety of illness. In recent years, many different PPGs have been isolated and identified, and their bioactivities have also been studied. Their structures are listed in the Table.

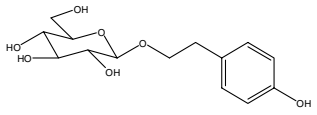
2. Pharmacological activities

2.1. Immunosuppressive activity

Rehmannia glutinosa is one of the most important crude drugs of Chinese traditional medicine. The dried root of *Rehmannia* is used as a tonic, antianemic and antipyretic agent by the local inhabitants in China and Japan. Jinoside A and B extracted from *Rehmannia* are the immunosuppressive principles. They were first found to exhibit activity on immune responses and can suppress the induction of hemolytic plaque-forming cells in mice. Their immunosuppressive action may be mainly due to the phenylethyl alcohol moiety in PPG [1].



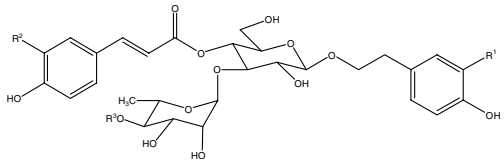
Coniferin R=H
Syringin R=OCH_3



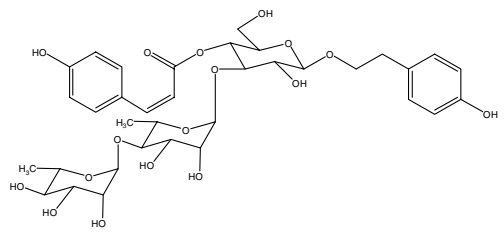
Salidroside

2.2. Antibacterial and antiviral activities

Four caffeoyl glycosides are extracted from the callus tissue of Rehmannia glytinosa: acteoside (the synonym of verbascoside), forsythiaside, 3,4-dihydroxy-β-phenethyl-*O*-β-D-glucopyranosyl-(1→3)-*O*-α--rhamopyranosyl-(1→6)-4-*O*-caffeoyl-β-D-glucopyranoside (compound **1**) and 3,4-dihydroxy-β-phenethyl-*O*-β-D-glucopyranosyl-(1→3)-4-*O*-caffeoyl-β-D-glucopyranoside (compound **2**). A significant antibiotic activity of compound **2** has been observed against *Pseudomonas capacia* and *P. maltophilia* at con-

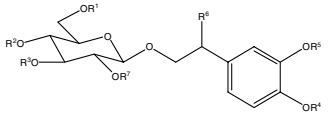


Ligupurpuroside A	R^1=OH,	R^2=OH,	R^3=H
trans-Ligupurpuroside B	R^1=H,	R^2=H,	R^3=H
Osmanthuside B	R^1=H,	R^2=H,	R^3=H



cis-Ligupurpuroside B

Table: Chemical structures of some PPGs discussed in this review



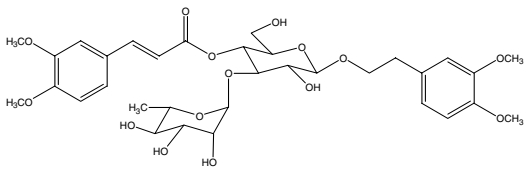
	R^1	R^2	R^3	R^4	R^5	R^6	R^7
Acteoside(verbascoside)	H	caf	α-L-rha	H	H	H	H
2′-Acetylacteoside	H	caf	α-L-rha	H	H	H	Ac
Arenarioside	β-D-xyl	caf	α-L-rha	H	H	H	H
Ballotetroside	β-D-api	caf	α-L-arab(1 → 2) α-L-rha				
Brandioside	α-L-rha	caf	α-L-rha	H	H	H	H
Calceolarioside A	H	caf	H	H	H	H	H
Calceolarioside B	caf	H	H	H	H	H	H
Campneoside I	H	caf	α-L-rha	H	H	OCH_3	H
Cistanoside C	H	caf	α-L-rha	H	CH_3	H	H
Cistanoside D	H	fer	α-L-rha	H	CH_3	H	H
Compound 1	H	caf	β-D-glu	H	H	H	H
Compound 2	α-L-rha	caf	β-D-glu	H	H	H	H
Darendoside B	H	H	α-L-rha	CH_3	H	H	H
Deacyllavandulifolioside dimethyl ether	H	H	α-L-arab(1 → 2) α-L-rha	CH_3	CH_3	H	H
Deacylacteoside dimethyl ether	H	H	α-L-rha	CH_3	CH_3	H	H
Deacylforsythoside-B dimethyl ether	β-D-api	H	α-L-rha	CH_3	CH_3	H	H
Echinacoside	β-D-glu	caf	α-L-rha	H	H	H	H
Ferruginoside B	β-D-glu	H	H	H	H	H	H
Forsythiaside	α-L-rha	caf	H	H	H	H	H
Forsythoside B	β-D-api	caf	α-L-rha	H	H	H	H
Incanoside	caf	H	β-D-glu(1 → 2) α-L-rha	H	H	H	H
Incanoside C	H	fer	β-D-glu(1 → 2) α-L-rha	H	H	H	H
Incanoside D	H	fer	β-D-glu(1 → 2) α-L-rha	Me	H	H	H
Incanoside E	H	fer	β-D-glu(1 → 2) α-L-rha	H	Me	H	H
Isomartynoside	fer	H	α-L-rha	CH_3	H	H	H
Isoaceteoside (Isoverbascoside)	caf	H	α-L-rha	H	H	H	H
Jionoside A	β-D-gal	fer	α-L-rha	H	H	H	H
Jionoside B	β-D-gal	fer	α-L-rha	CH	H	H	H
Leucosceptoside A	H	fer	α-L-rha	H	H	H	H
Leucosceptoside B	β-D-api	fer	α-L-rha	CH_3	H	H	H
Luecosceptoside	H	fer	α-L-rha	H	H	H	H
Lugrandoside	β-D-glu	caf	H	H	H	H	H
Martynoside	H	fer	α-L-rha	CH_3	H	H	H
Pedicularioside A	α-L-rha	caf	β-D-api	H	H	H	H
Pedicularioside M	β-D-api	fer	α-L-rha	H	H	H	H
Pedicularioside N	β-D-api	fer	α-L-rha	CH_3	H	H	H
Phlinoside A	H	caf	β-D-glu(1 → 2) α-L-RHA	H	H	H	H
Plantainoside D	caf	H	β-D-GLU	H	H	H	H
Poliumoside	α-L-rha	caf	α-L-RHA	H	H	H	H
Samioside	H	caf	β-D-api(1 → 4) α-L-rha	H	H	H	H
Suspensaside	α-L-rha	caf	H	H	H	OH	H
Teucrioside	H	caf	α-L-lyx-(1 → 2) α-L-rha	H	H	H	H
Tubuloside B	caf	H	α-L-rha	H	H	H	Ac

caf = caffeoyl, fer = feruloyl, rha = rhamnosyl, glu = glucosyl, api = apiosyl, lyx = lyxose, isofer = isoferuloyl

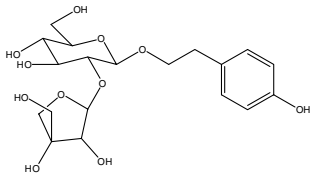
centrations of 0.2–0.5 mg/disk. Forsythiaside and desrhamnosyl acteoside also show antibiotic activity somewhat weaker than that of compound 2 [2].

Scrophularia scorodonia is distributed in Southwestern Spain and Northwest Africa. Acteoside isolated from it has been found to have antiviral activity against vesicular stomatitis virus. The percentage of cellular viability at the non-toxic limit concentration of the active compound was 53.6% at 500 μg/ml [3].

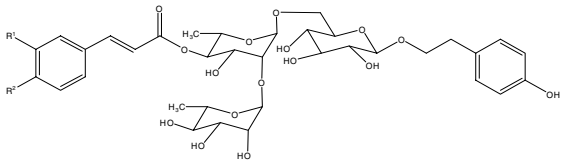
Echinacea angustifolia and *E. purpurea* (L.) belonging to the Compositae that have long been used by the native Indians of North America to cure wounds and snake bites. Nowadays, *Echinacea* is used as a immunomodulating agent, especially in Germany. Two major components, chicoric acid and echinacoside, have been isolated from *E. pallida*. In yield reduction of the vesicular stomatitis virus system in mouse L-929 cells, dose dependent antiviral activity has been observed with both components,



Permethyl acteoside



Hattashoside	R = syringyl
Pholmisethanoside	R = vanilloyl
Darendoside A	R = H



4-cinnamoylmussatioside	R^1= H	R^2 = H
4-dimethylcaffeoylmussatioside	R^1 = OMe	R^2 = OMe
4-*p*-methoxycinamoylmussatioside	R^1 = H	R^2 = OMe

and was accompanied by marked inhibitory effects on cell growth and DNA metabolism [4].
Campneoside I extracted from *Paulownia tomentosa* stem also shows antibacterial activity against *Streptococcus faecium* MD8b, *Staphylococcus aureus* (SG 511, 285 and 503) and *Staphylococcus pyogenes* (A308 and A77) etc. The methoxy group of PPG has been postulated to be the essential element for the antibacterial activity [5].

2.3. Analgesic and anti-inflammatory activities

In ancient times, many fruits and plants were used as anti-inflammatories, such as the fruits of *Forsythia* species, cedron and so on. The fruits of *Forsythia* species have been used for treatment of allergic and inflammatory diseases for many years in China and Japan.
Cedron (*Lippia triphylla*) has long been used in Peru as a calmative and carminative and for the treatment of stomach ache etc. Acteoside isolated from this plant shows analgesic activity. The compound exhibited analgesia on acetic acid-induced writhing and on tail pressure pain in mice with oral administration of 300 and 100 mg/kg, respectively. It also causes weak sedation as demonstrated by its effect on the prolongation of pentobarbital-induced anesthesia and on the depression of locomotion enhanced by methamphetamine. An intravenous injection of acteoside reduced the effective dose to 2 mg/kg as determined by the writhing method. Investigation of the active structure of acteoside showed that modification of the sugar moiety varies the potency. In addition, deletion or migration of the rhamnose, an additional glucose or galactose subtituent, lake of caffeoyl and phenethyl moieties will decrease their analgesic activity in different level [6].
Three PPGs isolated from *Marrubium vulgare* are able to inhibit the activity of cyclooxygenase 2 (Cox 2), which catalyses prostaglandin biosynthesis and is strongly associated with inflammation [7].
A preliminary screening of successive petroleum ether, chloroform and methanol extracts of the aerial parts of *Verbena officinalis* Linn. (Verbenaceae) for anti-inflammatory activity has been carried out using a carrageenan paw oedema model. All three extracts were found to exhibit anti-inflammatory activity with the chloroform extract being the most active. Acteoside was found as a constituent of methanol extract [8].

2.4. Activity on tumor cells

2.4.1. Antitumor activity

Acteoside can be extracted from many plants, such as *Phlomis armeniaca*, *Lippia dulcin* etc and exhibits many forms of biological activity such as protective action on KCN-induced anoxia, immunosuppressive activity, antihypertensive activity and anti-tumor activity [9]. Acteoside expresses cytotoxic activity against some cancer cells such as S-180, P-388/D1, DRLh-84 and HL-60, but not against primary-cultured rat hepatocytes. That is to say, acteoside is selectively sensitive to tumor cells rather than normal cells. In addition, the o-dihydroxy aromatic system is necessary for the cytotoxic activity of PPGs. Acteoside can induce cell death in promyelocytic leukemia HL-60 cells with an IC_{50} value of 26.7 μM, induce the internucleosomal breakdown of chromatin DNA characteristic of apoptosis, and decrease the DNA content of the G_0/G_1, S and G_2/M phases with incubation. There is a possibility that acteoside elicits a pro-oxidant action, resulting in the induction of apoptosis. Acteoside shows a biphasic effect on cancer cells, that is cytostatic and/or cytotoxic activity, depending on cell type, indicating that not all cancer cells are always sensitive to acteoside [10].
Acteoside, leucosceptoside A, martynoside, forsythoside B, phlinoside B, phlinoside C and teuerioside isolated from methanol extracts of *Phlomis armeniaca* and *Scutellaria salviifolia* have been found to show activity against several kinds of cancer cells. However, they did not affect the growth and viability of primary-cultured rat hepatocytes. It is very important that the PPGs tested are selectively sensitive to cancer cells. Study of the structure activity relationship indicated that ortho-dihydroxy aromatic systems are necessary for the cytotoxic and cytostatic activities auf PPGs [11].
Castillleja linariaefolia Benth, known as Indian paintbrush, is found in mountainous areas of Northern Arizona and Southern Utah, USA. While some 50 species of the large Scrophulariaceae family have been used in primitive cancer treatment, acteoside and isoacteoside isolated from this plant showed activities against murine P-388 lymphocytic leukemia cells with ED_{50} of 2.6 and 10 μg/ml, respectively [12].

2.4.2. Reverse transformation of tumor cells

Our results showed that redifferentiation of HL-60 cells can be induced by isoacteoside at 20–25 μM; HL-60 cells treated by isoacteoside at 20 μM were delayed in G_1 phase at 12 hours and G_2/M phase at 72 hours [13]. After being treated with 20 μM acteoside, we found that the growth curve and mitotic index of human gastric adenocarcinoma MGc80–3 cells decreased remarkably, cell doubling time being delayed from 48 h to 68.5 h, while the cellular growth inhibitory rate was 53.2%, and the cell electrophoresis velocity decreased to 28.4%. There was a 75% decrease of the tumorgenicity for treated cells compared with untreated cells inoculated subcutaneously in BALB/C nude mice. Scanning electron microscopy revealed that the microvilli on the surface of treated cells

MGc80-3 were obviously reduced [14]. Electron microscopy revealed that the nuclear-cytoplasmic ratio was decreased in treated cells, the nucleolus lessened, and heterochromatin reduced. The microvilli at cell surface were obviously reduced [15]. It is confirmed that the natural antioxidant isoacteoside can reverse the malignant phenotypic characteristics of MGc80–3 cells, and can certainly induce redifferentiation of gastric carcinoma cells.

To study the structure-activity relationship between PPGs and their anti-tumor activity, we tested the antitumor activities of six PPGs, isoacteoside (**1**), acteoside (**2**), echinacoside (**3**), pedicularioside A (**4**), pedicularioside N (**5**), and permethyl acteoside (**6**), on three different tumor cell lines, human hepatoma cells SMMC-7721, human pulmonary adenocarcinoma cells L_{342} and human gastric adenocarcinoma cells MGc80-3 [16, 17]. The activities of the PPGs with four phenolic hydroxyl groups, compounds **1**, **2**, **3** and **4**, are stronger than that of compound **5** with two phenolic hydroxyl groups. The 50% inhibition concentrations are approximately 91.5–105.4 μg/ml for the first four PPGs, and 256.7–289.4 μg/ml for compound **5**. All the phenolic hydroxyl groups of compound **6** are methoxylated, so compound **6** has no phenolic hydroxyl group remaining and is no longer able to inhibit the growth of tumor cells.

2.5. Inhibition of telomerase activity and shorting telomere length of tumor cells

Screening of natural products for anti-tumor activity as telomerase inhibitors is a new development in the field of tumor therapy. Using telomerase PCR ELISA, telomere DNA hybridization and flow cytometry analysis, we examined the effects of acteoside, a phenylpropanoid glucoside extracted from *Pedicularis striata* Pall, on telomerase activity, telomere length and cell cycle of human gastric carcinoma cells MKN45 *in vitro* [18]. After being treated with a 50% inhibition concentration of acteoside (17.8 μg/ml), telomerase activity in the cells was significantly inhibited although not in the cellular supernatant, the average telomere length became remarkably short, and a sub-G0/G1 peak and G2/M arrest were also displayed when compared to the control cells. These results suggest that acteoside-mediated cell differentiation and apoptosis may be affected by telomere-telomerase-cell cycle dependent modulation. Thus, the antitumor mechanism of acteoside is again demonstrated once more by its inhibiting effect on telomerase activity in tumor cells, and the telomerase assay may provide a valuable screening method for antitumor activity of natural products.

2.6. Hepatoprotective activity

Cistanche deserticola (Orobanchaceae) is a parasitic plant growing mainly in the north and northwest of China. The stems are used as a traditional Chinese tonic drug for kidney deficiency characterized by impotence, pain in the loins and knees, female sterility, and constipation due to dryness of the bowel in the senile. PPGs are the major constituents of this plant and are believed to be responsible for the various actions of the drug. Four PPGs have been isolated from the stems of this plant, acteoside, 2′-acetylacteoside, isoacteoside and tubuloside B. These compounds significantly suppress NADPH/CCl_4-induced lipid peroxidation in rat liver microsomes, prevent CCl_4-induced hepatocyte lipid peroxidation and AST (aspartate aminotranferase D-galactosamine) release from the cells, exhibit hepatoprotective activity against D-galactosamine intoxication, and show anti-hepatotoxic activity against CCl_4 *in vivo*. The anti-CCl_4-toxicity activity of these compounds is believed to be partly based on their free radical scavenging activity and anti-lipid peroxidative effect [19].

Acteoside, an antioxidative PPG widely distributed in medicinal plants, effectively inhibits TNF-α-mediated hepatic apoptosis, upstream of the liver injury process, and the subsequent necrosis and lethality in D-galactosamine-induced fulminant hepatitis in mice. Although multiple pathways such as detoxification of D-galactosamine and inactivation of caspase-3 may be implicated in the effect of acteoside on apoptosis, its antioxidant activity (i.e. interception of reactive oxygen intermediate signal transduction) seems to be the major contributor. Results suggest that a reactive oxygen intermediate may be functionally involved in the hepatic apoptosis induced by D-galactosamine in mice, and that some antioxidants such as acteoside, can suppress hepatic apoptosis and subsequent liver failure [20].

2.7. Antihypertensive effect

Acteoside isolated from the violet flowers of *Syringa vulgaris* shows a definite antihypertensive effect, decreasing blood pressure $39.40 \pm 2.38\%$ within 2–3 min at a high dose of 10 mg/kg ip, while heart rate was also decreased. On the whole, acteoside gives a dose dependent decrease in systolic, diastolic and mean arterial blood pressure in pentothal anaesthetized rats. In terms of structure-activity relationships, the effects of this drug on blood pressure depend on the occurrence of an OH group in the aromatic ring [9].

The PPG fraction (100 μg/ml) isolated from *Phlomis pungens* var. *pungens* is able to protect rat aorta against free radical-induced impairment of endothelium-dependent relaxation in response to acetylcholine, while the iridoid glycoside fraction (150 μg/ml) is ineffective. The protective activity of PPGs may be related to their free radical scavenging property [21].

2.8. Effect on muscle contraction

In recent years we have also studied the biological activities of PPGs extracted mainly from *Pedicularis* species. *Pedicularis* is a herb used in Chinese folk medicine, called 'native ginseng' by the local residents in Northwest China, and is generally used as a cardiac-tonic to treat collapse, exhaustion, spontaneous sweating, seminal emission, and senility, to invigorate the circulation of blood, to aid digestion, to recover vitality and to relieve uneasiness of body and mind. Many PPGs may be isolated from it, such as acteoside, isoacteoside, martynoside, pedicularioside A, M and N etc.

Our previous study [22] showed that skeletal muscles suffer from a considerable amount of oxygen consumption during normal activity which may initiate free radical-induced damage. Continuous intensive contraction of muscle increases oxygen consumption and the corresponding production of oxygen radicals. Free radicals play a possible role in various pathological disorders of skeletal muscle. Intensive contraction of muscle is known to heighten free radical signals in skeletal muscle, to enhance free radical induced lipid peroxidation indexed by expired alkanes in breath and by thiobarbituric-acid reactive-substances (TBARS) in skeletal muscle, and to induce muscle membrane damage indexed by efflux of intracellular lactate dehydrogenase (LDH) from muscle *in vivo* and *in vitro*. Nowadays, there is substantial evidence showing that continuous intensive contraction of skeletal muscle causes

formation of reactive oxygen species (ROS). ROS scavengers are able to counteract fatigue induced in mice during swimming stress. Two PPGs, acteoside and martynoside isolated from *Pedicularis*, reduced electrically induced muscle fatigue in a concentration-dependent manner [23]. The number of phenol hydroxyl groups in PPGs is important for their antifatigue activities. The inhibitiory actions of acteoside on free radical formation and lipid peroxidation in rat skeletal muscle have been reported [24]. Our experiments suggest that ROS induced and promoted muscle fatigue, while classical antioxidants counteracted muscle fatigue. Therefore, it would be expected that the stronger the ROS scavenging and antioxidizing activities of these PPGs, the stronger the protection against muscle fatigue.

Acteoside isolated from the dried leaves of *Stachytarpheta cayennensis* showed an inhibitory effect on histamine and bradykinin induced contractions of guinea-pig ileum, *in vivo* anti-inflammatory activity and antinociceptive activity, while its anti-inflammatory properties seemed to be partly due to the inhibition of bradykinin and histamine [25].

2.9. Effects on sex and learning behavior

The effects of the constituents of *Cistanchis herba* on sex and learning behaviors in IV-CS strain adult male mice were studied using the chronic hanging stress method. The phenylpropanoid glycoside fraction (20 mg/kg, p.o.) prepared from *Cistanchis herba* showed marked protective effects on both sex and learning behaviors in the hanging stress loaded mice. The constituents, acteoside (10 mg/kg, p.o.), cistanoside A (10 mg/kg, p.o.) and cistanoside C (10 mg/kg, p.o.) also showed very similar effects. Echinacoside (10 mg/kg, p.o.) showed a protective effect against decreases of sex behavior, but had little effect on learning behavior [26].

2.10. Antiaggregation effect on platelets

The bark of "chamairo", an undetermined *Mussatia* (Bignoniaceae), a liana widely known in Bolivia and Peru, is appreciated for its euphoric and medicinal effects when chewed alone or mixed with "coca". Three PPGs (4-dimethylcaffeoylmussatioside, 4-cinnamoylmussatioside and 4-p-methoxycinnamoylmussatioside) isolated from this plant were found to possess an inhibitory action on ADP-induced rat platelet aggregation [27].

2.11. Antihemolysis

We found that five PPGs (isoacteoside, acteoside, echinacoside, pedicularioside A and cistanoside D) from *Pedicularis* can protect against oxidative hemolysis of red blood cells *in vitro*. The inhibitory activity is related to the number of phenolic hydroxy groups [28]. Four PPGs, acteoside, 2′-acetylacteoside, poliumoside and brandioside isolated from *Brandisia hancei* were shown to have inhibitory effect on free radical-induced hemolysis of red blood cells [29].

2.13. Antineurodegeneration

Four PPGs, (2-*O*-acetyl-3-*O*-(*E*)-*p*-methoxycinnamoyl-alpha-L-rhamnopyranoside, 2-O-acetyl-3,4-di-O-(E)-p-methoxycinnamoyl-alpha-L-rhamnopyranoside, 2-*O*-acetyl-3-*O*-(*Z*)-*p*-methoxycinnamoyl-alpha-L-rhamnopyranoside and 4-*O*-(*E*)-*p*-methoxycinnamoyl-alpha-L-rhamnopyranoside) isolated from *Scrophularia hueriana*, are able to attenuate glutamate-induced neurotoxicity in a dose-dependent manner when added to primary cultures of rat cortical cells. These results demonstrated that the above four PPGs might exert significant protective effects against glutamate-induced neurodegeneration in primary cultures of cortical neurons [30].

3. Mechanisms

PPGs show many kinds of bioactivity, but how do they function? Many reports have indicated possible mechanisms of action of PPGs.

3.1. Effects on enzymes and cytokines

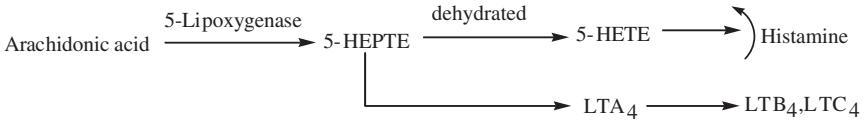
A number of enzymes, cytokines and other intracellular molecules are very important modulators of many physiological and pathological processes. PPGs are found to show the ability to change the activity of some enzymes and the level of some cytokines. Therefore, PPGs participate indirectly in the modulation of some pathologic processes.

The twigs and leaves of *Brandisa hancei* Hool. F. (Scrophulariaceae) have long been used as a folk remedy for the treatment of diseases such as hepatitis, edema, rheumatoid arthritis and osteomyelitis. Isoacteoside isolated from *Brandisa hancei* (Scrophulariaceae) inhibited xanthine oxidase (a key enzyme associated with the incidence of hyperuricemia-related disorders) with an IC_{50} value of 45.48 μM. Isoacteoside was found to be the first PPG that substantially decreased the formation of uric acid by competitively inhibiting xanthine oxidase. The structure-activity relationship suggested that the caffeoylation of the 6′-hydroxyl group of the phenylethanoids was essential for their enzyme inhibitory action [31].

Leukotrienes are involved in immunoregulation and in a variety of diseases. In the presence of 5-lipoxygenase, free arachidonic acid is always metabolized as shown in the Scheme.

Therefore, specific 5-lipoxygenase inhibitors should be useful not only in the therapy of the allergic disease asthma and inflammation, but also in studies on the biosynthesis and function of leukotriene. The genus *Phillyrea*, is widely represented in the Mediterranean and Iberian flora, and consists of a group of plants, many of which are used in traditional medicine. For example,

Scheme



Phillyrea latifolia L. is used as an astringent and diuretic and for the treatment of ulcers and mouth inflammations by the inhabitants in Spain, Mediterranean, Europe and North Africa. Three PPGs (salidroside, syringin and coneferin) have been isolated from this plant. These compounds are able to exert inhibitory actions on the enzymes of the arachidonate cascade in calcium-stimulated mouse peritoneal macrophages and human platelets [32]. Salidroside exerts a preferential effect on the cyclooxygenase pathway, inhibiting the release of prostaglandin E_2 and to a lesser extent reducing thromboxane B_2 levels.

Four caffeoyl glycosides: forsythiaside, suspensaside, β-hydroxyacteoside and acteoside have been isolated from the fruits of *F. suspensa* and *F. viridisscima*. These compounds have been found to be the inhibitors of 5-lipoxygenase [33]. They can inhibit selectively the formation of 5-HETE and LB_4 in human peripheral polymorphonuclear leukocytes and the formation of 5-HETE in rat peritoneal cells. Their concentrations for IC_{50} (50% inhibition) for the formation of 5-HETE in human peripheral polymorphonuclear leukocytes are 1.92, 49.0, 4.85 and 40.0 μM. The IC_{50} values for the formation of LB_4 in human peripheral polymorphonuclear leukocytes are 1.01, 8.85, 2.93 and 8.50 μM, and for the formation of 5-HETE in rat peritoneal cells are 2.50, 7.97, 5.27 and 19.3 μM, respectively. From analysis of the structure- activity relationship, two adjacent phenolic hydroxyl groups have been found to be essential for potent inhibition of the formation of 5-HETE and LTB_4.

Cytokines are a class of polypeptide, which are secreted from many kinds of cell, and can regulate cell growth, differentiation and immunity, and participate in the occurrence of inflammation, as well as in wound healing. Acteoside has been found to induce interleukin (IL-1 and IL-6) and tumor necrosis factor α (TNF-α) in macrophage-like cell line J774.A1 at 1–100 ng/ml. In addition acteoside stimulated IL-60 production in bovine glomerular endothelial cell line GEN-T. These stimulatory activities were not abolished by treatment with polymixin B, which inactivates lipopolysaccharide (LPS), indicating that the action was not a contamination of LPS [34].

Protein kinase C (PKC), a Ca^{2+}/phospholipid-dependent protein kinase, plays a crucial role in signal transduction, cellular proliferation and differentiation. Acteoside isolated from *Lantana camara* is an inhibitor of PKC from the rat brain. Acteoside interacts with the catalytic domain of PKC and is a non-competitive inhibitor with respect to the phosphate acceptor. This effect is further evidenced by the fact that acteoside inhibits native PKC and its catalytic fragment identically and does not affect [^{3}H]-phorboc-12, 13-dibutyrate biding to PKC. The antitumour activity of acteoside against L-1210 cells measured *in vitro* might be due at least in part to inhibition of PKC [35]. In adddition, many PPGs isolated from *Digitalis purpurea* and *Penstemon linarioides* showed inhibitory activity against PKCa [36].

3.2. Antioxidative and free radical scavenging activities

An increasing amount of experimental epidemiological evidence implicates the involvement of free radicals, especially reactive oxygen species, in the pathogenesis of various diseases, such as cancer, Alzheimer disease, diabetes, arthris, hepatitis, atheroscleorosis, stroke, cataract, autoimmunity, AIDs and even aging processes. Therefore, free radical scavengers and antioxidants may open the door to a new class of drugs for the possible treatment or prevention of certain free radical induced diseases. PPGs, as a class of natural polyphenols, have also been widely studied for their antioxidative and free radical scavenging activities.

The whole plant of *Caryopteris incana* (Thunb.) has long been used in China as a folk medicine to relieve cold, cough and rheumatic pain. Incanoside has been isolated from the whole plant together with four known PPGs, acteoside, isoacteoside, phlinoside A and 6-*O*-caffeoyl-β-*O*-glucose. These compounds exhibit potent radical scavenging activity against DPPH, ·OH and $O_2^{\bullet -}$ radicals. And the number of phenolic hydroxyl groups may play an important role in their scavenging activity [37, 38]. PPGs isolated from the genus *Phillyrea* were also found to have antioxidant activity [32]. In addition, the four caffeoylglycosides isolated from the fruits of *E. suspense* and *E. viridisscima* have been found to be effective free radical scavengers. They can scavenge 80–95% of DPPH radicals at levels of 10^{-5}–10^{-4} M [33].

Incanoside C, incanoside D and incanoside E isolated from the whole plant of *Caryopteris incana* exhibit radical scavenging activity against DPPH radicals and inhibitory activity against the oxidation of linoleic acid [37, 38]. Samioside isolated from the aerial parts of *Phlomis samia*, also demonstrates scavenging properties towards the DPPH radical and antimicrobial activity against Gram-positive and -negative bacteria [39].

Quanbo et al. found that nine PPGs (acteoside, isoacteoside, 2′-acetylacteoside, tubuloside B, echinacoside, tubuloside A, syringalide A 3′-alpha-rhamnopyranoside, cistanoside A and cistanoside F) extracted from the stem of Cistanche desertic showed strong free radical scavenging activity. These nine compounds all showed stronger free radical scavenging activities than α–tocopherol on the DPPH radical and the xanthine/xanthine oxidase generated superoxide anion radical ($O_2^{\bullet -}$) [40]. In further studies, they also found that each of them exhibited significant inhibition on both ascorbic acid/Fe^{2+} and ADP/NADPH/Fe^{3+} induced lipid peroxidation in rat liver microsomes, being more potent than α–tocopherol or caffeic acid [19]. The structure-activity analysis showed that the antioxidative effect was found to be potentiated by an increasing in the number of phenolic hydroxyl groups in the molecule.

By comparing the radical scavenging activities of 19 PPGs (calceolarioside A, acteoside, isoacteoside, lugrandoside, echinacoside, forsythoside B, alyssonoside, leucosceptoside A, isomartynoside, martynoside, leucosceptoside B, hattushoside, phlomisethanoside, ferruginoside B, darendoside B, deacylacteoside dimethyl ether, deacylforsythoside B dimethyl ether, deacyllavandulifolioside dimethyl ether and darendoside A), the structure-activity relationship of PPGs were demonstrated as follows: the antioxidant activity is mainly related to the number of aromatic methoxy and hydroxy groups and the structure of the acyl moiety (C_6-C_1 or C_6-C_3) [41].

The hydroxyl radical is the presently known substance which is the simplest in structure, and the strongest carcinogen; it can attack DNA bases, the sugar moiety of nucleotides, fatty acids and other biological targets. Using a pulse radiolysis technique, we examined the reaction between the hydroxyl radical and seven PPGs: echinacoside, acteoside, leucosceptoside A, martynoside, and pedicularoside A, M and N. All seven PPGs react with hydroxyl radical at high rate constants in the range of $0.97–1.91 \times 10^{10}\ M^{-1} \cdot s^{-1}$, suggesting that they are effective hydroxyl radical scavengers. The results also

demonstrate that the numbers of phenolic hydroxyl groups and O-dihydroxy groups are essential for their scavenging activities [42, 43].

In another experiment, the action of six PPGs, leucosceptoside A, martynoside, acteoside and pedicularioside A, M and N on superoxide anion and hydroxyl radicals was studied by the spin trapping method. We found that the scavenging activities of the PPGs on superoxide and hydroxyl radicals are affected by their structures and concentrations: (i) the number of phenolic hydroxyl groups in PPGs may play an important role in their scavenging activity; (ii) the O-dihydroxyl group is also important [44].

In addition, we also studied the activities of six PPGs (acteoside (**1**), isoacteoside (**2**), echinacoside (**3**), pedicularioside A (**4**), cistanoside D (**5**) and permethyl acteoside (**6**)) as chain-breaking antioxidants for the autoxidation of linoleic acid in cetyl trimethylammonium bromide (CTAB) micelles at 37 °C. The results showed that PPGs can inhibit linoleic acid peroxidation in micelles, and the number of phenolic hydroxyl groups is critical for their inhibitory activity [45]. The antioxidant activities of PPGs measured in mouse liver microsomes were similar to those in the chemical system. Compounds **1**, **2**, **3** and **4**, all with four phenolic groups, inhibit microsomal lipid peroxidation concentration-dependently and efficiently. Their inhibitory effects were stronger than that of compound **5** with two phenolic groups. Compound 1, with a 50% inhibition concentration of 5.57 μM, was the strongest antioxidant, while **6**, possessing no Ph-OH group did not inhibit microsomal lipid peroxidation even when the concentration was as high as 65.0 μM [17]. Five PPGs (compounds **1**, **2**, **3**, **4** and **5**) can also protect against oxidative hemolysis [16]. When mouse erythrocytes were incubated in a 10% suspension in 0.9% NaCl aqueous solution at 37 °C, they were relatively stable and little hemolysis (<10%) was observed within 5 h. When AAPH (2,2′-azobisC2-amidinopropandihydro-chloride), the water-soluble initiator of lipid peroxidation, was added to the aqueous suspensions of erythrocytes, hemolysis increased in a concentration dependent manner. The inhibitory activity was related to the number of phenolic hydroxy groups.

As mentioned above, we found that the activities of PPGs for inhibiting lipid peroxidation and scavenging oxygen radicals depend mainly on the number of phenolic hydroxyl groups. This activity-structure relationship has subsequently been demonstrated [19].

Iron-dependent lipid peroxidation is thought to play a central role in pathologically relevant oxy-radical-induced tissue damage *in vitro*. Thus, agents that inhibit iron-dependent lipid peroxidation may represent a rational approach to the management of oxy-radical-related diseases [15]. Our study showed that under physiological condition PPG-Fe^{2+} chelates are sufficiently stable, and thus PPGs are able to inhibit Fe^{2+}-dependent lipid peroxidation *in vivo* through chelating Fe^{2+} and show therapeutic potential by the same mechanism *in vitro*. So, the inhibitory effects on lipid peroxidation of PPGs with phenolic hydroxyl groups depend on their chelating properties [46].

Oxidized low-density lipoproteins (OX-LDL) might be involved in the pathogenesis of atherosclerosis, and it has been reported that polyphenols inhibit LDL peroxidation and atherogenesis. In the Yunnan province of China, the species *Ligustrum purpurascens* is used to brew a bitter tea. It has been claimed to act as a stimulant to the central nervous system, a diuretic, a treatment for sore throat, an aid to weight loss, and to relief hypertension. Acteoside, isoacteoside and ligupurpuroside A purified from this plant protect against LDL peroxidation, whereas cis-ligupurpuroside B, trans-ligupurpuroside B and osmanthuside B exhibit no protection to human LDL from Cu^{2+}-mediated oxidation. Acteoside, isoacteoside and ligupurpuroside A are also effective in preventing peroxyl free radical-induced oxidation of alpha-tocopherol in human LDL. The antioxidative activity of these PPGs relates to their number of hydroxyl groups, that is, their electron- or hydrogen-donating potency. In other words, their antioxidative activities were most likely attributable to their proton-donating capacities [47]. Another report also indicates that PPGs (acteoside, forsythoside B, arenarioside and balloletroside) from the European plant *Ballota nigra* L, of which flowered aerial parts and polar extracts are commonly used in European medicine for their neurosedative activity, are strong inhibitors of Cu^{2+}-induced LDL oxidation, independent of any capacity to act as Cu^{2+} chelators [48].

Acteoside and cistanoside F (not a PPG) have been studied with regard to lipid peroxidation in fresh rat liver mitochondria, in freeze-thawed mitochondria and in mitochondrial lipid liposomes induced by Fe^{2+}/ADP. Oxygen consumption, formation of thiobarbituric acid reactive substances (TBARs), glutathione concentration and radical scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) were determined simultaneously during lipid peroxidation. Results showed that: (a) acteoside and cistanoside F cause very similar concentration-dependent inhibition of lipid peroxidation in all three systems, and were much more effective than TX-1847 (a synthetic acteoside analogue); (b) the efficacy of PPG in inhibition of TBARs formation during lipid peroxidation in either freshly isolated or freeze-thawed mitochondria are ranked as follows: acteoside = cistanoside F > TX-1847; (c) as far as effect on GSH concentration is concerned, when acteoside is added, the GSH concentration is maintained at 69% of the starting value, while with cistanoside F or TX-1847 the GSH concentration is kept at or near the same level as at the beginning of the incubation; (d) the free radical scavenging abilities of the three compounds is ranked as follows: TX-1847 > acteoside > cistanoside F, acting as free radical chain-breaking antioxidants; (e) the catechol group and sugar moieties and/or the conformational structure of the compounds seem to be the chief contributor to their inhibitory effects on lipid peroxidation, while the free radical scavenging activity depends on the number of phenol groups contained in the PPG (an exception is cistanoside F, which possesses only one catechol group, but is effective as acteoside which has two catechol groups) [49].

3.3. Fast repair of PPGs on oxidative damaged DNA

Environmental agents, such as ionizing radiation, UV light and a variety of chemical agents, cause damage to DNA bases. Even in normal metabolism, products of oxidative DNA damage, such as thymine glycol (Tg), 8-hydroxydeoxy guanosine (8-OH-dG) and hydroxy methydeoxyuridine, can be detected in the urine of mammalian animals and in cultured cells *in vitro*. The excretion of damaged DNA bases is positively correlated with metabolic rate and negatively correlated with life span [50]. DNA damaged by oxygen radicals has been implicated as a causative event in a wide range of diseases, including hereditary syndromes, autoimmune syndromes, atherosclerosis, strokes, cancers, normal aging and age-related degenerative disease. Previous experimental results indicated that hydroxylated 2′-deoxyguanosine and hydroxy-

lated 2′-deoxyadenosine formed by ·OH, are the most abundant base lesion and are believed to be important lesions leading to mutation, cancer and aging [51]. Fortunately, a series of enzymes, such as DNA polymerase, DNA ligase, DNA glycosylases and apurinic (AP) endonucleases are involved in the repair of oxidative DNA damage, although not all DNA damage can be repaired, especially in the case of oxidative stress and aging. This means that DNA damage always exists. So it may be very significant to look for ways in which either oxygen radicals are scavenged prior to DNA damage or damaged DNA is repaired to supplement the cell's inadequate repair capacity. Recently, we have found evidence to prove that natural antioxidants occuring in plants can play a protective role in DNA damage.

Thymine is easily attacked by hydrated electrons (e_{aq}) to produce the thymine radical anion. By using pulse radiolysis technique, we found that the thymine radical anion could be repaired within microseconds through one electron transfer between the radical anion and PPG. Electrophilic phenyl-substituted unsaturated carboxylic groups contained in the PPG's structure are able to capture electrons from thymine radical anions before they undergo reversible protonation [52]. The reaction rate constants of electron transfer from thymine radical anion to PPGs are mainly within the range $1.16–2.75 \times 10^9\,M^{-1} \cdot s^{-1}$ [53]. The repair effects of PPGs (acteoside and pedicularioside A) on the thymine-hydroxyl adduct has also been studied using the pulse radiolysis technique. Rapid electron transfer from PPGs to T-OH^- was observed, while phenoxyl radicals of PPGs were also generated via an one-electron-transfer reaction. This result shows that PPGs also exhibit repair activities on oxidizing T-OH^-. The reaction rate constants of electron transfer from the above 2 PPGs were 1.27×10^{9} and $1.29 \times 10^9\,M^{-1} \cdot s^{-1}$, respectively [54, 55].

On pulse irradiation of nitrogen saturated deoxynucleotide aqueous solution containing one of the PPGs (acteoside, cistanoside C and pedicularioside A), the transient absorption spectra of the radical cations of nucleotide (dAMP, dGMP and dCMP) decay with the formation of those of the radical cation of PPGs within several tens of microseconds after electron pulse irradiation. The results indicate that deoxy-nucleotide radical cations could be repaired by PPGs. The rate constants of the repair reactions have been determined to be $0.48–1.1 \times 10^9$, $0.64–1.80 \times 10^9$ and $2.12–4.410^9\,M^{-1} \cdot s^{-1}$ for dAMP, dGMP and dCMP radical cations, respectively. The rate constants of the repair reaction depend on the number of phenolic hydroxyl groups contained in the PPGs, reducing activity of PPGs and its concentration in the repaired system [51, 56, 57].

As far as the hydroxyl adducts of dGMP or dAMP are concerned, the same conclusion can be drawn. Our results show that dGMP or dAMP hydroxyl adducts can also be repaired by PPGs (pedicularioside A and cistanoside C). The rate constants of the repair reactions are deduced to be $0.641–1.28 \times 10^9$ and $0.2–0.491 \times 10^9\,M^{-1} \cdot s^{-1}$ for dGMP-OH and dAMP-OH, respectively [56]. Two PPGs, acteoside and angoroside C, and two iridoid glycosides, harpagoside and harpagide, were isolated from *Scrophularia ningpoensis*. At 0.1 mM, both PPGs were able to repair the oxidized OH adducts dAMP and dGMP significantly, while both iridoid glycosides had no such effect [58]. TMP radical anions can also be repaired by acteoside, cistanoside C and pedicularioside A. The rated constants of the repair reactions have been deduced to be $1.64–2.75 \times 10^9\,M^{-1} \cdot s^{-1}$ [53].

The repair effect on polyA-$OH^{\bullet}$, polyG-$OH^{\bullet}$ and poly$C^{\bullet+}$ was similar to that of bases or nucleotides. After pulse irradiation, there is a new transient absorption spectrum assigned to PPG-$PhO^{\bullet}$ generated via electron transfer from PPG to polynucleotide cations. That is to say, poly $C^{\bullet+}$, poly A-$OH^{\bullet}$ and polyG-$OH^{\bullet}$ can also be repaired by PPG. DsDNA and ssDNA can also be repaired by PPG via electron transfer to them of PPGs (acteoside and pedicularioside A).

In a word, we found that PPGs are able to fast repair DNA damage in all four structural levels: base → nucleoside → nucleotide → polynuleotide and even DNA fragments, such as ssDNA and dsDNA.

In DNA there are mechanisms of electron and positive hole transfer by which the initially generated and randomly distributed electron gain and loss centers are funneled into the T and G 'traps', respectively. Therefore, it is reasonable to say that by repairing radical cations of dAMP, dGMP and dCMP, PPGs can intercept the 'charge hole' to dGMP in the direction of T(C) → A → G, indirectly repair thymine radical cations produced in cellular DNA by irradiation or other oxidizing intermediates, and protect DNA from strand breaks and other stable lesions [59].

We know that damaged DNA can be repaired by enzymatic systems, but enzymatic repair of DNA damage is carried out in a time scale of hours. Another disadvantage of enzymatic repair is that the enzymes themselves are sensitive to ROS and would be damaged by ROS thus losing their repair ability. However, in our study, the fast repair reaction by PPG was initiated and finished in a time scale of microseconds, so that the fast repair may occur before replication or transcription of damaged DNA in the cell cycle, and may avoid the damaged DNA being transferred to cells of the next generation. As a result, fast repair is able to prevent transient products from reaction with other biological macromolecules, and to protect against secondary damage that would result in further loss of cellular physiological function. Despite the above fast repair being conducted in a chemical system, it is suggested that non-enzymatic fast repair exists in cells due to a series of endogenous reductants such as ascorbate, thiols etc., which can react with both dGMP-$OH^{\bullet}$ and $dGMP^{+\bullet}$ at a high rate implying a potentially effective repair process. Based on this suggestion, it is proposed that non-enzymatic fast repair is restricted to the damage induced by reactive oxygen species, and that the enzymatic repair system works for repair of steady state lesions of DNA. These two repair systems complement each other. Therefore a deeper understanding of this new mechanism will undoubtedly help researchers explore new medcines for prevention and/or intervention [59].

3.4. Molecular modeling of PPG interact with DNA

According to our experiments using pulse radiolysis and transient absorption spectral techniques as well as molecular modeling studies, we propose that the electron transfer between DNA-base radical and PPGs occurs on the exocyclic nitrogen N2 of guanine, so that the damaged DNA can be fast repaired. The activation energy Ea, corresponding to the key step of interaction with the guanine radical is very weak (<10 kal/mol). The distance O−N is found to be about 2.8 Å in transition state complexes. The electron clouds of the two atoms (O and N) overlap each other, and, thus, the electron of the guanine radical is able to transfer to PPG. A docking study performed with junction minimization of nucleic acid (JUMNA) software

on the DNA decamer ATGACGTCAT or an telemetric DNA fragment $(TTAGGG)_3$ for PPG-DNA complexes shows that several complexes are feasible, and it seems that the hydroxyl of the non-conjugated arm of PPGs is clearly less active than the conjugated arm, most often due to the presence of the methyl group in the PPG producing a steric hindrance. The value of the complexation energy is a fine balance of several terms. For all complexes with an adequate O−N distance, the interaction energy is found to be between −19 and −45 kal/mol. The PPG molecules can be docked into the minor groove of DNA and form complexes with the geometry suitable for an electron transfer between the guanine radical and the PPG. Such complexes can be found without major distortions of DNA structure and are further stabilized by interaction with the PPG's rhamnosyl sidegroup [60–62]. The mechanism that we propose on the theoretical ground is in agreement with our experimental results.

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