—Reviews—

A Drug over the Millennia: Pharmacognosy, Chemistry, and Pharmacology of Licorice

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Licorice, the root of *Glycyrrhiza* spp. (Fabaceae), has been used since ancient Egyptian, Greek, and Roman times in the West and since the Former Han era (the 2nd—3rd century B.C.) in ancient China in the East. In traditional Chinese medicine, licorice is one of the most frequently used drugs. In Japan, the oldest specimen of licorice introduced from China in the middle of the 8th century still exists in Shosoin, the Imperial Storehouse, in Nara. Extracts of licorice were recommended as a remedy for gastric ulcer by Revers of the Netherlands in 1946, which was soon withdrawn owing to its side effects. Carbenoxolon sodium, glycyrrhetinic acid (GA) hemisuccinate Na, was prepared from licorice to treat peptic ulcer in the UK. In Japan for the past 60 years, a glycyrrhizin (GL) preparation under the name of Stronger Neo-Minophagen C (SNMC) has been used clinically as an antiallergic and antihepatitis agent. GL and GA sometimes induce edema, hypertension, and hypokalemia in patients treated with higher doses and long-term administration. The mechanism of this side effect, pseudoaldosteronism, has been explained as due to the 11-hydroxy-steroid dehydrogenase inhibitory activity of GL and GA. The excess of endogenous cortisol produced combines with the renal mineral corticoid receptor, which promotes an aldosterone-like action. GL and GA reduce alanine transaminase (ALT) and aspartate transaminase (AST) values in the serum. This hepatoprotective effect has recently been explained as the inhibitory effects of GL and GA on immune-mediated cytotoxicity against hepatocytes and on nuclear factor (NF)-κB, which activates genes encoding inflammatory cytokines in the liver. To exclude the side effects and enhance the therapeutic activities, chemical modification of GL and GA has been performed. Deoxoglycyrrhetol (DG), homo- and heteroannular diene homologs of dihemiphthalates, showed a remarkable improvement in antiinflammatory, antiallergic, and antiulcer activities in animal experiments. Immunomodulating effects of GL, GA, and DG derivatives, which induce interferon-γ and some other cytokines, have been demonstrated in relation with their antiviral activities. Antiinflammatory, antitumorigenic, and antimalarial effects of licorice flavonoids have also been investigated.

Key words——licorice; glycyrrhizin; hepatoprotective effect; isoliquiritigenin; licochalcone A; deoxoglycyrrhetol

History of Licorice

Licorice (liquorice), the root of the wild leguminous plant *Glycyrrhiza* spp., has been used since ancient Egyptian times as a drug for catarrh of the respiratory organs. It was described in the Codex Hammurabi (2100 B.C.) and in the Ebers Papyrus (1552 B.C.). Licorice also appeared in the "De Historia Plantarum" and "De Causis Plantarum" of Theophrast (371—286 B.C.) in ancient Greece and in the "De Materia Medica" of Dioscurides (40—90 A.D.) in Rome. Under the title of Glukoriza (sweet root), Dioscurides wrote that the expressed sap of its root was used for diseases of the stomach, liver, and kidney. Chewing the root relieved thirst and applying the root powder healed wounds.

In China, licorice first appeared among the descriptions of 250 kinds of drugs in the medical document "Recipes for Fifty-two Maladies (五十二病方)" found in the tomb of Ma-Wang (馬王堆) built in 186 B.C. in Chang-sha (長沙). In the *Shen-Nung-Pen-Cao-Ching* (神農本草経) written by an unknown author in the first century and revised by Tao-Hung-Ching (陶弘景, 452—536), licorice was listed in the superior class of drugs. One hundred twenty drugs classified in the superior class are nontoxic and effective in prolonging life. The same number of drugs classified in the general class are toxic or nontoxic and effective in preventing the progress of disease. The 125 drugs classified in the inferior class are more or less toxic but effective in curing disease. Licorice was described in the *Shen-Nung* herb book as an agent to strengthen muscle and bone, smooth the skin, and act as an antidote. In the first century, during the later Han Dynasty in China, Chang-Zhong-Jing (張仲景, 142—220) wrote the medical book *Shang-Han-Za-*

Dedicated to the memory of Dr. Kyosuke Tsuda

Bing-Lun (傷寒雑病論). The original book was lost, and later Wan-Su-He (王叔和, 210—285) recompiled it into two volumes, the *Shang-Han-Lun* (傷寒論) and *Jin-Gui-Yao-Lue* (金匱要略). In the *Shang-Han-Lun*, 113 prescriptions were cited, 80% of which contain licorice as a significant component. Therefore licorice has been said to harmonize the effects of other drug components and used the most frequently in Chinese medical prescriptions. A Japanese physician, Yoshimasu-Todo (1702—1773), analyzed the actions of drugs composing prescriptions cited in the *Shang-Han-Lun* based on his clinical experience and described the results in his work *Yaku-cho* (藥徵), in which licorice was reported generally to relieve acute symptoms such as spasm, cramp, abdominalgia, pharyngalgia, and arthralgia.

The oldest substantial evidence of the introduction of traditional Chinese medicine into Japan is some of medicaments stored in Shosoin. Thirty-eight of 60 medicaments, originally dedicated to the Great Buddha of Todaiji Temple in Nara by the Empress Dowager Komyo in memory of the late Emperor Shomu, remain at present. Medicaments in Shosoin, which are of plant, animal, and mineral origins, were brought from China, Korea, and other Asian countries by way of China during the Tang Dynasty. In the original list of medicaments at the time of dedication, 214 kg of licorice was recorded. It was consumed rapidly, and only 10 kg remained after 100 years (Fig. 1).

The medicaments stored in Shosoin have been scientifically investigated since 1948 after 1200 years of storage. The characteristic sweet taste of licorice remains even in the long-stored material.

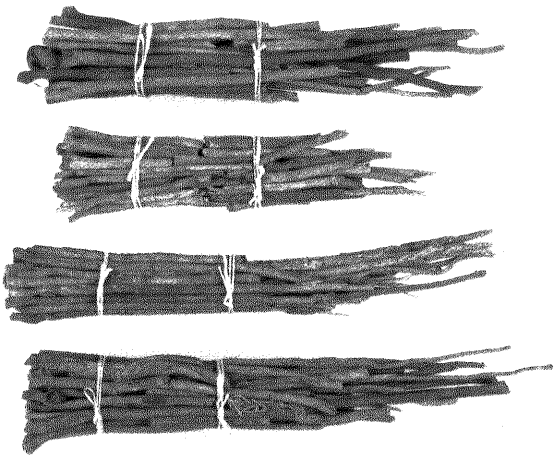


Fig. 1. Licorice Stored in Shosoin, Imperial Repository in Nara, Since 756 A.D. Glycyrrhizin Still Exists with Sweet Taste in the Root

Photo from "Shosoin Medicaments," edited by Office of Shosoin Treasure House, Imperial Household Agency.

Botanical Background

The original licorice plants, *Glycyrrhiza* spp., are widely distributed over the dry region of the Eurasian continent. They occur in Mongolia, northeastern and northwestern China, Xinjiang province in China, Afghanistan, Pakistan, other central Asian countries, Iran, Iraq, Turkey, and even in the southern part of Europe, in Italy and Spain. Licorice consists of pale yellow and sweet roots and stolons of various species of *Glycyrrhiza* (Fabaceae [Leguminosae]). *Glycyrrhiza* plants yielding the medical and sweetening agents are listed in Table 1.

The following species of *Glycyrrhiza* are rarely used for medical and commercial purposes: *G. echinata* L. (=*G. macedoniaca* BOISS & ORPH.), and *G. pallidiflora* MAXIM.

Some local production of *Glycyrrhiza* plants has been reported, such as *G. yunnanensis* P.C. LI (Malay licorice) in southwestern China and *G. squamulosa* Franch in the Central Asian region.

A large amount of licorice and its extracts are on the world drug market as medicinal materials and sweetening agents. In Japan, 2000—9000 tons of

Table 1. *Glycyrrhiza* Plants Most Commonly Used as Medicinal and Sweetening Agents

Glycyrrhiza plant	Region found
G. uralensis FISCHER	Northeastern China, Far East Russia
G. glabra L. var. *typica* REG. & HERD	Spain, Italy
G. glabra L. var. *violacea* BOISS	Turkey, Iran
G. glabra L. var. *glandulifera* WALDST & KIT	China, Russia, Central Asia
G. inflata BATALIN	Xinjiang (China)
G. eurycarpa P.C. LI (*G. korshinskyi* GRIGORJ) (=*G. uralensis*×*G. inflata*)	Xinjiang (China), Russia, Central Asia
G. aspera PALLAS	Xinjiang (China)

licorice have annually been imported in the past several years, since there is no domestic production.

Chemical Pinciples of Licorice

Triterpenoid Saponins and Sapogenins: The yield of the principal sweet-tasting saponin, 18β-GL, is on average 4—5% of the dried root. The total content of flavonoid in the root is 1—2%. GL is D-glucurono (β1→2) D-glucuronide of GA. Ruzicka and coworkers[1,2] established the structure of GA as 18β-olean-11-oxo-12-ene-3β-ol-30-oic acid.

The stereochemistry of GL was proposed using the classic molecular rotation method to be an α-linked glucurono (β1→2) glucuronide moiety attached at the 3β-hydroxyl of GA.[3] This was revised later to a β-linkage of the sugar moiety by Khalilov *et al.*[4] and Shibata[5] using the ^{1}H and ^{13}C NMR method (Fig. 2).

Several minor satellite oleanane-type saponins were isolated from *G. uralensis* and *G. inflata*, and their chemical structures were fully elucidated.[6-8] As their aglycones, the structures of deoxo-GA, GA 22-lactone, 24-hydroxy-GA, liquiritic acid (30α-COOH), 24-hydroxy-deoxo-GA and uralenic acid (18α-GA) were determined. Some other homologous triterpenes were isolated from the roots of *G. glabra*, *G. uralensis*, *G. yunnanensis*, *G. inflata*, and other species of *Glycyrrhiza* as the aglycones of saponins. The total number of these types of compounds so far determined is about 50.

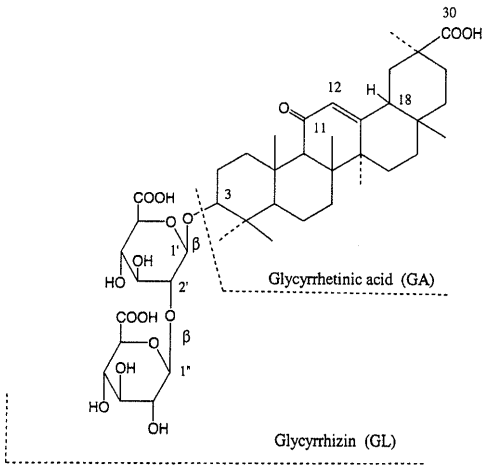
Phenolic Compounds: About 300 kinds of phenolic compounds have so far been isolated from various species of *Glycyrrhiza*, about half of which are new and characteristic of licorice. About 70 phenolics are from *G. glabra* root, about 60 from *G. uralensis* root, about 60 from *G. inflata* root, about 40 from *G. aspera* root, about 40 from *G. eurycarpa* root, and about 30 from *G. pallidiflora* root. All these phenolic compounds were documented in the review article "Phenolic constituents of licorice" published by T. Nomura and T. Fukai.[9]

These phenolic compounds are structurally classified into chalcone, dibenzoylmethane, flavanone, isoflavanone, flavone, flavonol, isoflavone, isoflavane, isoflav-3-ene, 5-arylcoumarin, pterocarpan, coumestan, 2-arylbenzofuran, dihydrostilbene, and dihydrophenanthrene. Among these phenolic compounds, isoliquiritin and its aglycone, isoliquiritigenin (chalcones), liquiritin and its aglycone, liquiritigenin (flavanones), and ononin and formononetin (isoflavones) are widely distributed in several species of licorice.

Licochalcone A, B, C, and D, which are reversely constructed chalcones, were originally found in the root of *G. inflata* (Xinjiang licorice). Later, some of them were isolated from the roots of *G. glabra* and *G. uralensis*, collected in Xinjiang province, but not from those of other localities. Total yields of phenolic compounds in licorice are about 1—2% of its dried root. The content of licochalcone A in the root of *G. inflata* is very high (*ca.* 0.8%).

Biological Activities of GL and GA

A number of studies on the biological activities and pharmacological effects of licorice have been reported, mainly focusing on the major saponin GL and its sapogenin GA. In 1946, Revers[10] reported the application of licorice extracts for peptic ulcer. Its



^{13}C-NMR Spectrum of Glycyrrhizin

Glycyrrhetinic acid moiery		Glucuronic acid moiery	
C-3	88.1 ppm	C-1'	103.3 ppm
C-11	198.8	C-2'	82.5
C-12	127.2	C-3'	75.0
C-13	169.5	C-4'	71.1
C-18	47.9	C-5'	75.7
C-30	177.5	C-6'	170.0
		C-1"	104.6
		C-2"	74.5
		C-3"	75.5
		C-4"	71.4
		C-5"	76.1
		C-6"	169.8

^{1}H-NMR signal of anomeric protons

4.31 ppm (^{1}H d, J = 6.9 Hz)

4.47 ppm (^{1}H d, J = 7.3 Hz)

Fig. 2. Structures of Glycyrrhetinic Acid (GA) and Glycyrrhizin (GL)

clinical use, however, was soon interrupted by the incidence of a side effect inducing edema, hypertension, and hypokalemia. GL and GA induce a mineral corticoid-like action, pseudoaldosteronism, which retains Na^+ and water and excludes K^+. Pseudoaldosteronism appears during long-term and high-dose intravenous administration of GL, and more markedly during oral administration of GA. In Europe, GA hemisuccinate sodium was later used under the trade name "Carbenoxolone sodium" for peptic ulcer by oral administration. In Japan, a GL preparation combined with L-cysteine and glycine has been used for more than 60 years under the trade name of "Stronger Neo-Minophagen C" (SNMC). At first, it was used as an antidote and antiallergic agent. For 30 years, it has been used as a drug for chronic hepatitis by intravenous administration, remarkably reducing serum AST (GOT) and ALT (GPT) levels in patients. "Glycyron" (GL + L-methionine, glycine) has been applied orally as a supplement to SNMC. In 1977, Suzuki *et al.*[11] performed a double-blind clinical trial of SNMC for the treatment of chronic hepatitis and found a significant decrease in plasma transaminase activity in the group treated with SNMC. Histologic improvement in the liver of SNMC-treated patients was also observed.

Several groups have studied the mechanism of the side effects of GL and GA, so-called pseudoaldosteronism. First, in 1950, Molhuysen *et al.*[12] found a deoxycorticosterone-like action in licorice extracts. In 1951, Groen *et al.*[13] noted that licorice extract or GL is effective for minimal Addison's disease, but ineffective for severe cases and that GL is ineffective in adrenalectomized rats.[14] Tamura, *et al.*[15] reported that GL and GA in rat liver preparation significantly inhibited $\Delta^4 5\beta$-reductase, which is involved in the metabolism of cortisol, aldosterone, and testosterone. Accordingly, it is considered that the inhibition of metabolic transformation of cortical steroids by GL and GA induces pseudoaldosteronism. Recently, it has become clear that GA suppresses the activity of 11-hydroxysteroid dehydrogenase by retaining excess cortisol.[16,17] Cortisol is bound to a receptor of mineral corticoid in the kidney, expressing the mineral corticoid action. 3-Deoxo-GA, 3-keto-GA, 3-epi-GA, and 11-deoxo-GA also inhibit 11-hydroxy-steroid dehydrogenase activity in rat liver microsomes.[18] 18α-Isomers of those compounds were 1/10 less inhibitory of 11β-hydroxysteroid dehydrogenase activity, whereas 18α-isomers were potent in inhibiting 3α-hydroxysteroid dehydrogenase which is related to antiinflammatory activity. 18α-GA showed stronger inhibitory activity than 18β-GA against 3α-hydroxysteroid dehydrogenase, suggesting a stronger antiinflammatory effect of 18α-GA than of 18β-GA (Fig. 3).

11β-Hydroxysteroid dehydrogenase is a naturally

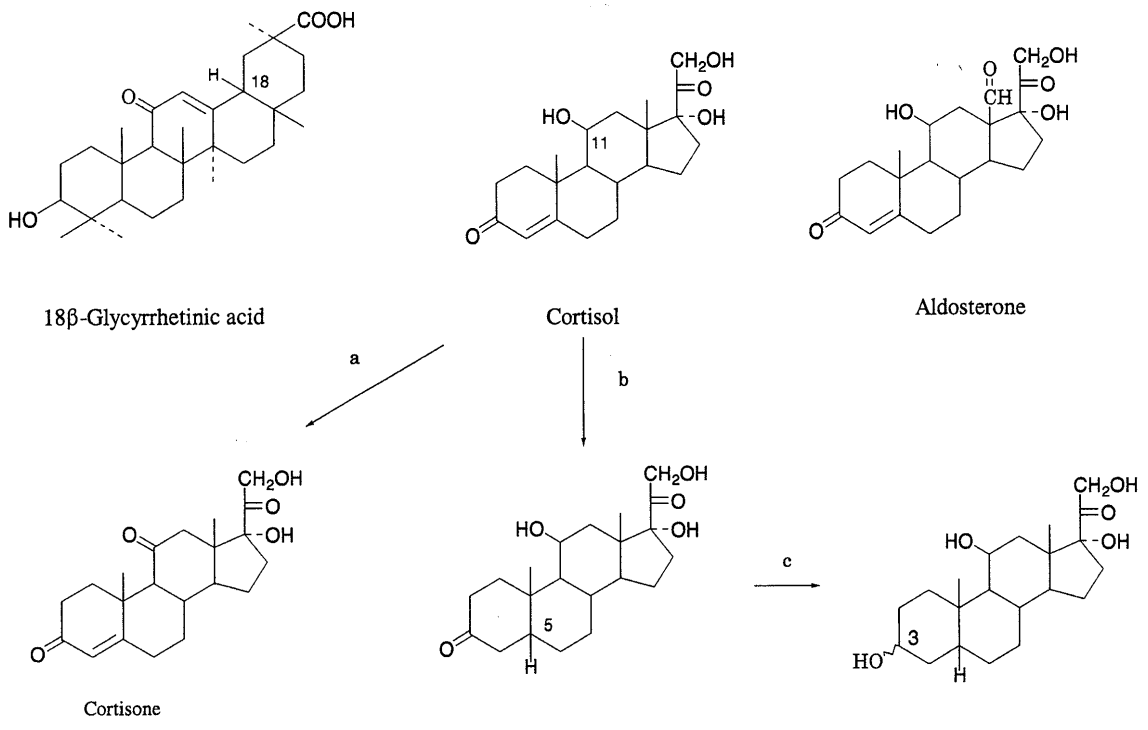


Fig. 3. Pseudoaldosteronism of GA and the Inhibition of Metabolic Enzymes of Endogenous Cortisol

occurring enzyme in the skin. GA locally inhibits this enzyme and significantly potentiates the antiinflammatory activity of natural cortisol in the lung tissue.[19]

Hepatoprotective Effect of GL and GA

For the past few decades in Japan, the GL preparation SNMC has been clinically used in patients with chronic hepatitis by intravenous administration. When administrated intravenously, GL is rapidly eliminated from sera and transformed to GA-monoglucuronide by hepatic β-glucuronidase. When administered orally, GL is readily hydrolyzed into GA by human intestinal bacteria and absorbed, with pharmacological effects. It was clinically observed that intravenously administrated SNMC significantly decreased elevated AST and ALT values in patients with hepatitis. This effect was also demonstrated in isolated rat hepatocytes incubated with anti-liver cell membrane antibody and complement. In this experiment, release of AST from the hepatocytes was observed, and GL and GA remarkably suppressed the release. The hepatoprotective effect of GA was much stronger than that of GL. GL and GA also reduced morphologic damage to liver tissue in a hepatitis model.[20] Several groups have recently investigated the mechanism of the hepatoprotective effects of GL and GA.

Chronic viral hepatitis involves immune-mediated cytotoxicity by cytotoxic T lymphocytes (CTLs) and tumor necrosis factor-α (TNF-α). Using an antigen-specific murine CD4$^+$ T hydrodoma line, Yoshikawa *et al.*[21] showed that GL inhibits immune-mediated cytotoxicity against hepatocytes. This function decreases elevated plasma levels of AST and ALT, which are released by the apoptosis of hepatocytes induced by liver injury. It has been known that hepatocytes are sensitive to Fas, a type II membrane protein. Fas ligand and Fas antigen are expressed in the liver of patients infected with chronic hepatitis C virus (HCV).[22,23] GL inhibited anti-Fas antibody-induced elevation of ALT in mice. Thus the decrease in ALT in the chronic HCV patients treated with SNMC may be due to the inhibition of Fas-mediated hepatic injury.[24]

An unusual activation of gene expression inducing pathogenic proteins results in inflammatory disease. A transcription activator, NFκB, induces genes encoding pathogenic proteins such as proinflammatory cytokines. NFκB ordinarily exists in cytosol, forming an inactive complex, IκB-NFκB. NFκB released from the inactive complex after stimulating factors such as CTL, TNF-α, or interleukin(IL)-1, translocates into the nucleus and activates transcription of genes.[25,26] Wang *et al.*[27] reported that GL inhibits the NFκB activity in the murine liver injury induced by CCl_4-ethanol. This mechanism may explain the hepatoprotective effect of GL preparation in human hepatitis.

Antiviral Effects of GL

Pompei *et al.*[28] reported that GL inhibits the growth and cytopathic effect of *Vaccinia* virus, herpes simplex virus type I (HSV-I), Newcastle disease, vesicular stomatitis viruses, and polio virus type I. Baba and Shigeta[29] studied the antiviral activity of GL against *Varicella zoster* virus (VZV) in cell culture. Ito *et al.*[30] reported that GL inhibits the replication of human immunodeficiency virus type I (HIV-I) *in vitro*, the etiologic agent of acquired immune deficiency syndrome (AIDS). Hirabayashi and our collaborators[31] studied *in vitro* anti-HIV-I and anti-HSV-I activities of GL and some chemically modified derivatives of GL. Among them, an 11-deoxo-heteroannular diene analogue showed the most potent antiviral activity against HIV-I in MT-4 and Molt-4 cells at a concentration of 0.16 mM (GL: 0.62 mM) (Fig. 4).

In 1987, Gotoh *et al.*[32] conducted a long-term study of SNMC (5 mg GL/kg) by drip infusion to AIDS patients with high CD4/CD8 ratios before treatment. In this clinical study, the count of CD4 lymphocytes and CD4/CD8 ratio in asymptomatic carriers (AC) or patients with AIDS-related complex (ARC), were elevated. A significant clinical improvement was obtained in almost half of the treated patients.

The doses of SNMC were 200 ml (400 mg GL)/d by i.v. infusion to AC daily for 1—3 weeks, 400 ml/d to ARC patients every 2 d for 4—8 weeks, and 800 ml/d to AIDS patients twice a week for 9—11 weeks. In addition, 6—9 tablets/d of Glycyron (25 mg GL/tab) were given to all patients in the study for 12 weeks.

Ikegami *et al.*[33] conducted a study of long-term oral administration of GL tablets (Glycyron) at Na-

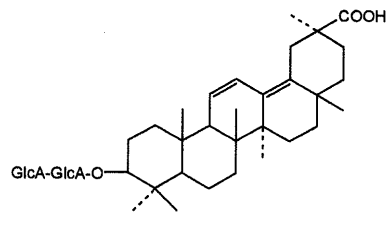


Fig. 4. 11-Deoxoheteroannular Diene Homolog of GL

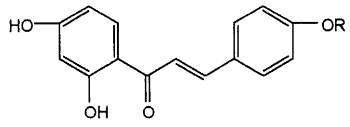
tional Osaka Hospital from 1984 to the present. Nine tablets (225 mg GL/d) administrated to AC have prevented the progress of disease in AC with high CD4/CD8 ratios. On the other hand, Abe *et al.*[34] found that GL and GA have an interferon γ-inducing activity and natural killer cell (NK)-enhancing effect in both animal experiments and human clinical trials. Consequently, a dual biological action was shown for GL and GA. Oral administration of GL tablets (225 mg GL/d) results in only 2 μg/ml GA in the serum. Thus the anti-HIV action of orally administrated GL must be a biological reaction modifying (BRM) action, not a direct cytotoxic antiviral action. Recently, Ito[35] found that GL administrated to mice enhanced the production of IL-12 to 7-fold that in controls.

Biological Activities of Phenolic Compounds

In recent chemical investigations, numerous phenolic compounds have been isolated from various *Glycyrrhiza* spp. root. In every species of licorice, 40—70 kinds of flavonoid and other phenolic compounds occur, with some species specificity in their chemical structures. The total contents of phenolics are circa 1—2% of the weight of the dried root. Isoliquiritin and its aglycone, isoliquiritigenin (2',4',4-trihydroxychalcone), commonly occur in various species of licorice, and their pharmacological activities have been reported. In earlier investigations, the presence of isoliquiritigenin explained the antispasmodic action of licorice. Yamamoto *et al.*[36] reported that isoliquiritigenin is effective in preventing tumorigenesis. In experiments in mice, topical application of isoliquiritigenin prevented papilloma induced by dimethyl-benz[*a*]anthracene (DMBA) initiation and tetradecanoylphorbol 13-acetate (TPA) promotion. Baba *et al.*[37] found that oral administration of isoliquiritigenin, which was also found in scallion, the tuber of *Allium bakeri*, suppressed the colon cancer formation induced by azoxymethane (AOM) in mice (Fig. 5).

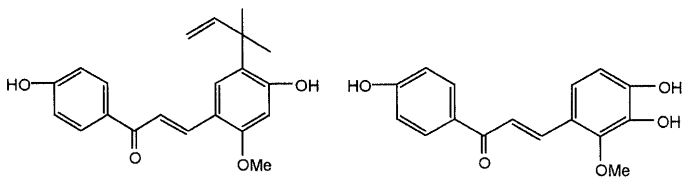
In 1975, Saito and Shibata[38] isolated structurally unique chalcones called licochalcone A and B from Xinjiang licorice (*Glycyrrhiza inflata*) root. These chalcones possess a reversely constructed structure in contrast to ordinary naturally occurring chalcones. Biosynthetically, the A-ring of licochalcone A and B is derived from shikimate and the B-ring from acetate-malonate, while they are reversed in ordinary chalcones (Fig. 6).[39] In 1991, Shibata *et al.*[40] reported on the antiinflammatory and antitumor promoting activities of licochalcone A. Topical application of licochalcone A (0.5 mg per ear) to the mouse ear significantly suppressed inflammatory edema induced by arachidonic acid (2 mg per ear) or TPA (2 μg per ear). The two-stage carcinogenesis model in mice (DMBA initiation and TPA promotion) was used, and licochalcone A remarkably inhibited the papilloma formation on the backs of mice. According to Hatano *et al.*,[41] licochalcone A and B and some other flavonoid compounds of licorice are effective in inhibiting the cytopathic activity of HIV. Based on the antitumor activity of licochalcone A, several homologous chalcone derivatives were synthesized, and their topical antitumorigenic activities were tested in mice.[42] Among them, 3'-methyl-3-hydroxy- and 4'-methyl-3-hydroxy- chalcones (3'Me-3C and 4'Me-3C) showed potent antitumorigenic activity when topically applied to skin papilloma and orally administrated *in vivo* in mice with AOM-induced colon cancer.[37]

As the mechanism of antitumorigenesis of these licorice chalcones and their synthetic homologs, competitive binding to the estrogen type II binding site was discussed.[43] Usually, antitumor and antiinflammatory activity function in parallel in most biologically active compounds, but antitumorigenically ac-



R=Glc Isoliquiritin R=H Isoliquiritigenin

Fig. 5. Chalcones Generally Occurring in Licorice



Licochalcone A Licochalcone B

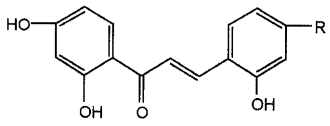
Fig. 6. Reversely Constructed-Chalcones in Xinjiang Licorice

tive 3'Me-3C and 4'Me-3C do not inhibit arachidonic acid- and TPA-induced inflammation. It is noteworthy, however, that all these compounds are mutually potent inhibitors of ornithine decarboxylase (ODC), preventing the formation of polyamines.

In response to the general demand for new antimalarial agents, Chen *et al.*[44] reported that licochalcone A inhibits the *in vitro* growth of both chloroquine-susceptible and -resistant *Plasmodium falciparum* strains at all stages of growth. The *in vivo* activity of licochalcone A was tested in mice infected with *P. yoelli* by intraperitoneal or oral administration for 3—6 days. Similar experiments were performed against *Leishmania major* and *L. donovam*, demonstrating *in vitro* the inhibition of their growth. An *in vivo* study was also carried out in mice and hamsters infected with *Leishmania* parasites.[45] Intraperitoneal or oral administration of licochalcone A resulted in the reduction of parasite load in the liver and spleen of infected mice by more than 96%. An analogous synthetic chalcone, 2,4-dimethoxy-4'-butoxy-chalcone, showed potent antimalarial activities.[46]

Human aldose reductase plays an important role in the diabetic complications such as cataract, keratopathy, and retinopathy. Diabetic complications result from the osmotic pressure difference that occurs in- and outside the cells due to the accumulation of sorbitol in them. Sorbitol is formed by the action of aldose reductase from excess glucose in the blood of diabetic patients. Screening for aldose, reductase inhibitors in natural products has been carried out and flavonoids appear to be one of the most promising groups of compounds.[47,48]

Aida *et al.*[49] reported that isoliquiritigenin of licorice remarkably inhibited rat lens aldose reductase. Using recombinant human aldose reductase, Iwata *et al.*[50] investigated the inhibitory activities of some natural chalcones, finding potent inhibitory effects in isoliquiritigenin (IC_{50}, 7.0×10^{-7}), echinatin, and licochalcone A from licorice. They also investigated the activities of synthetic chalcones and found more potent activities in 2',4',2-trihydroxy- and 2',4',2,4-tetrahydroxy chalcone, with IC_{50} values of 7.4×10^{-9} M and 1.6×10^{-7} M, respectively (Fig. 7).



R=H 2',4',2-Trihydroxychalcone
R=OH 2',4',2,4-Tetrahydroxychalcone

Fig. 7. Synthetic Chalcone Inhibiting Human Aldose Reductase

Biological Activities of Deoxoglycyrrhetol and Its Homologs

Natural resources of *Glycyrrhiza* spp. in central and southwestern Asia are sufficient for a plentiful, inexpensive supply of licorice to the drug market (¥112—200/kg, US$1—1.5/kg). The yield of its major principle, GL, is high (4—5% of the weight of the dried root), and it can readily be employed for medicines and for starting materials for new medicinal derivatives.

Lichochalcone A, for which the biological activities have recently been revealed, is obtained in a good yield of 1—0.8% from Xinjiang licorice (*G. inflata*) root and is used as a medicament. A great store of knowledge of the biological activities of GL, GA, and their derivatives, as well as those of licorice chalcones, has accumulated. Licorice and its principles are therapeutically multifunctional: antiallergic; antiinflammatory; antiulcer; antihepatitis; antiviral; antitumorigenic; antimicrobial; antimalarial; aldose reductase inhibiting; immunomodulating, *etc.* Total synthesis of GL and GA is difficult, if not impossible, owing to their complex stereochemical structures closely related to their biological activities. As the next step in the medical application of licorice, chemical modification of GL and GA should be pursued to reduce their side effects and to enhance their therapeutic value. A plentiful supply of GL and GA makes it possible for them to serve as the starting materials for developing that program, if necessary on a commercial scale.

The following have already been studied to some extent.

1. **Stereochemical conversion of 18β-H into 18α-H in GL and GA, and structural transformation into heteroannular and homoannular diene homologs of GL and GA**
   The conversion and transformation may potentiate antiinflammatory hepatoprotective effects and other biological activities. It is noteworthy that 18α-GL has been tested clinically in China against hepatitis, showing stronger AST- and ALT-reducing effects than 18β-GL (1994).
2. **Deoxoglycyrrhetol and its homologs**
   A partial structural modification was attempted to reduce pseudoaldosteronism. Baran *et al.*[51] failed in an attempt at therapeutic enhancement of GA owing to the destruction of the carbonyl system in the C-ring, which might be responsible

for pseudoaldosteronism. Based on the same idea for decreasing pseudoaldosteronism, we prepared deoxoglycyrrhetol (DG) (18β-olean-12-ene-3β-30-diol) from GA simultaneously by reducing 11-carbonyl and 30-carboxyl using vitride —sodium aluminum bis-ethoxy-methoxy-hydride = $NaAlH_2(OCH_2OCH_2OCH_3)_2$—in tetrahydrofuran (THF), followed by catalytic hydrogenation using Pd/C as the catalyst. As intermediate by-products, homo- and heteroannular diene derivatives were obtained. Hemisuccinate and hemiphthalate derivatives of these products were subjected to pharmacological experiments (Fig. 8).[52,53]

Anti-ulcer activity—Oral administration (12 mg/kg) of dihemiphthalates of DG and its homo- and heteroannular diene derivatives intensively inhibited the incidence of water-immersion stress-induced gastric ulcer in rats by 76%. They are not

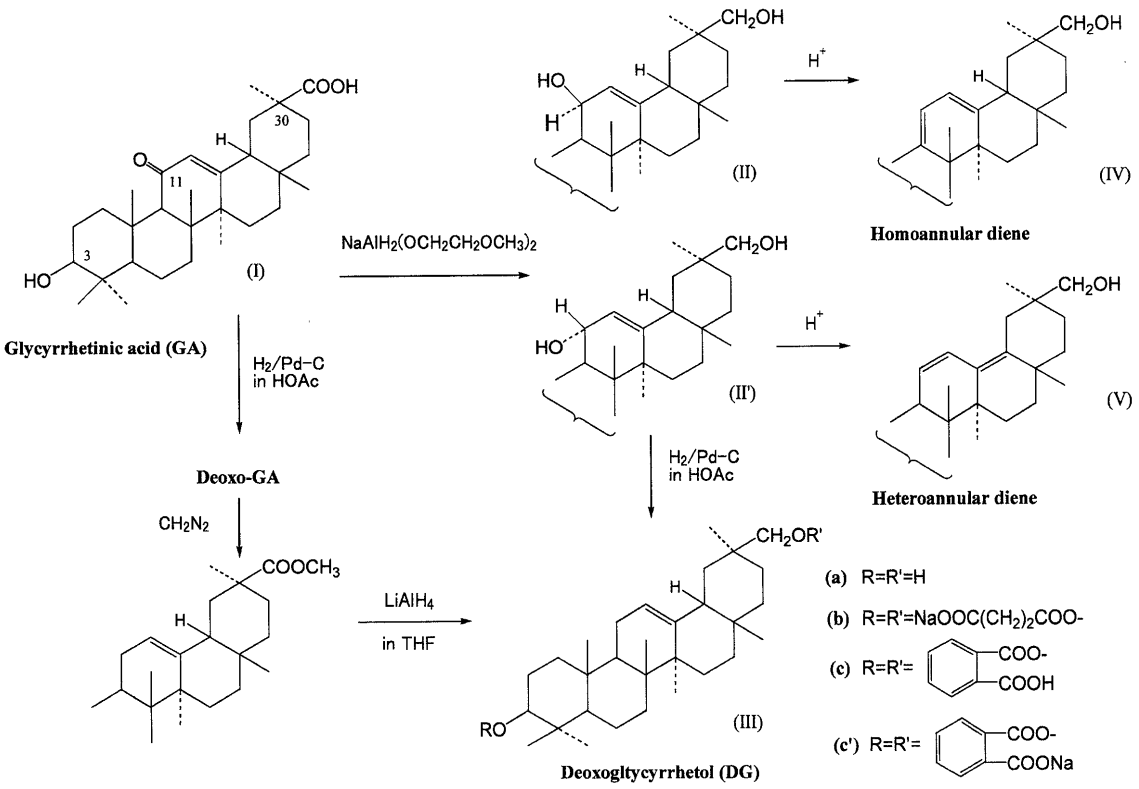


Fig. 8. Chemical Conversion of GA into Deoxoglycyrrhetol (DG) and Homo- and Hetero-Annular Diene Homologs

Table 2. Effect of 18β-Deoxoglycyrrhetol (DG) and Related Compounds on Stress-Induced Gastric Erosion in Rats under Restraint-Water Immersion at 25°C for 6 h

	Dose (mg/kg, *p.o.*)	No. of rats	Inhibition (%)
Glycyrrhetinic acid hemisuccinate Na (Ib′)	200	9	7
	500	9	67($p<0.05$)
DG dihemisuccinate Na (IIIb′)	200	10	48($p<0.05$)
	500	9	66($p<0.001$)
DG dihemiphthalate Na (IIIc′)	12	8	76($p<0.05$)
	25	8	**98**($p<0.001$)
Homoannular diene dihemiphthalate Na (IVc′)	12	10	63($p<0.05$)
	25	10	**80**($p<0.01$)
Heteroannular diene dihemiphthalate Na (Vc′)	12	10	80($p<0.01$)
	25	10	**86**($p<0.01$)
Phthalic acid Na	12	8	14

Values of inhibition are expressed as percent of the control compounds were given 30 min before water immersion.

effective in inhibiting gastric juice secretion, but protect the stomach wall membrane (Table 2).[54] Both topical and oral administration of DG and its homo- and heteroannular diene derivatives inhibited arachidonic acid-induced mouse ear edema,[55] which was mediated by leukotrienes (LTs) and prostaglandin E_2 (PGE_2) (Tables 3 and 4). The same three compounds are also effective in inhibiting TPA-induced mouse ear edema. It is presumed that PGE_2 is a mediator of TPA-induced edema (Tables 5 and 6).[56] Thus these compounds are dual inhibitors of lipoxygenase and cyclooxygenase in the arachidonic acid cascade (Table 7).[57,58]

Most antiinflammatory agents irritate the stomach, sometimes causing gastric injury. It is noteworthy that both DG and its homologs are antiinflammatory and antiulcer agents. DG and its homo- and heteroannular diene derivative dihemiphthalates are analgesic, showing inhibitory effects against acetic acid-induced writhing in mice[59] (ED_{50}: 14, 31, and 22 mg/kg *p.o.*, respectively). GA is less active (ED_{50}: 200 mg/kg *p.o.*). DG and its homologous derivatives significantly inhibited PGE_2 production in the peritoneal fluid of mice treated with acetic acid (Table 8). GA showed only minor activity in mice against paw swelling induced by vasoactive agents such as carrageenan (Table 9), histamine, bradykinin and

Table 3. Inhibition of Glycyrrhetinic Acid (GA) Derivatives Applied Topically on Arachidonic Acid (AA)-Induced Mouse Ear Edema

Compound	Dose (mg/ear)	Inhibition (%)
Glycyrrhetinic acid (GA) (I)	1	11
GA hemiphthalate (Ic)	1	0
Deoxoglycyrrhetol (DG) dihemiphthalate (IIIc)	1	**30**($p<0.01$)
Homoannular diene dihemiphthalate (IVc)	1	**25**($p<0.01$)
Heteroannular diene dihemiphthalate (Vc)	1	**36**($p<0.01$)
Aspirin (PG inhibitor)	1	0
AA 861 (LT inhibitor)	1	42($p<0.001$)
NDGA (dual inhibitor)	1	43($p<0.001$)

Test compounds were applied 30 min before AA application. NDGA: nordihydroguaiaretic acid. AA 861: 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone.

Table 4. Inhibition of Glycyrrhetinic Acid (GA) Derivatives Administered Orally on Arachidonic Acid (AA)-Induced Mouse Ear Edema

Compound	Dose (mg/kg)	Inhibition (%)
Glycyrrhetinic acid (GA) (I)	200	2
Deoxoglycyrrhetol (DG) (III)	200	0
DG dihemiphthalate Na (IIIc′)	12.5	0
	25	**20**($p<0.05$)
	50	**41**($p<0.001$)
	100	**52**($p<0.001$)
Homoannular diene dihemiphthalate Na (IVc′)	12.5	6
	25	**20**($p<0.05$)
	50	**26**($p<0.05$)
	100	**45**($p<0.001$)
Heteroannular diene dihemiphthalate Na (Vc′)	12.5	0
	25	**20**($p<0.05$)
	50	**29**($p<0.05$)
	100	**54**($p<0.001$)
Aspirin (PG inhibitor)	200	18

Test compounds were orally administered 30 min before AA application. ($n=8$)

Table 5. Inhibition of Glycyrrhetinic Acid (GA) Derivatives Applied Topically on TPA-Induced Mouse Ear Edema

Compound	Dose (mg/ear)	Inhibition (%) pre-treat	Inhibition (%) post-treat
Glycyrrhetinic acid (GA) (I)	1	81***	24**
GA hemiphthalate (Ic)	1	88***	23***
Deoxoglycyrrhetol (DG) dihemiphthalate (IIIc)	1	**93*****	40***
Homoannular diene dihemiphthalate (IVc)	1	**91*****	41***
Heteroannular diene dihemiphthalate (Vc)	1	**96*****	48***
Aspirin (PG inhibitor)	1	8	16**
Indomethacin (PG inhibitor)	1	32**	31**
AA 861 (LT inhibitor)	1	38***	24**
NDGA (dual inhibitor)	1	19**	30**
Dexamethasone (steroid)	0.1	—	83**

Compounds were applied 30 min before and after TPA treatment. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$. ($n=8$)

Table 6. Inhibition of Glycyrrhetinic Acid (GA) Derivatives Administered Orally on TPA-Induced Mouse Ear Edema

Compound	Dose (mg/kg)	Inhibition (%)
Glycyrrhetinic acid (I)	200	8
Deoxoglycyrrhetol (DG) (III)	200	0
DG dihemiphthalate Na (IIIc′)	25	10
	50	**28**($p<0.05$)
	100	**54**($p<0.01$)
	150	**96**($p<0.001$)
Homoannular diene dihemiphthalate Na (IVc′)	25	1
	50	28($p<0.01$)
	100	**43**($p<0.01$)
	150	**78**($p<0.001$)
Heteroannular diene dihemiphthalate Na (Vc′)	25	18
	50	**29**($p<0.05$)
	100	**71**($p<0.001$)
	150	**93**($p<0.001$)

Test compounds were orally administered 30 min before TPA application. ($n=8$)

platelet activating factor, whereas DG and its homologous compound dihemiphthalates remarkably inhibited the edema formation induced by the above agents (Table 10). This is a distinct difference in the activities of DG and its homologs from the parent compound GA.[60]

Tachykinin NK_1-receptor antagonists, histamine, and/or serotonin antagonists were shown to inhibit capsaicin-induced mouse ear edema in previous studies, but arachidonate metabolite antagonists did not. Oral administration of DG dihemiphthalate and its homologous compounds inhibited mouse ear edema induced by capsaicin, substance P (SP), and compound 48/80, whereas GA and DG showed no inhibitory effects (Tables 11 and 12). Dihemiphthalates of DG and its homo- and heteroannular diene compounds at high dose can suppress vasodilation and plasma extravasation induced by SP in capsaicin-induced edema.[61] Thus these compounds chemically mod-

Table 7. Inhibition of Lipoxygenase and Cyclooxygenase Activities by Glycyrrhetinic Acid (GA) and Its Modified Compounds

Compounds	Conc. (M)	Lypoxygenase		Cyclooxygenase
		5-Lip	12-Lip	
Glycyrrhizin	10^{-4}	14	16	5
GA (I)	10^{-4}	32	43	26
	10^{-5}	19	8	
GA hemiphthalate Na (Ic′)	10^{-4}	45	68	23
	10^{-5}	11	40	
Deoxoglycyrrhetol (DG) (III)	10^{-4}	55	64	25
DG dihemisuccinate Na (IIIb′)	10^{-4}	55	75	47
	10^{-5}	10	37	
DG dihemiphthalate Na (IIIc′)	10^{-4}	**97**	**100**	**60**
	10^{-5}	62	69	21
Homoannular diene dihemiphthalate Na (IVc′)	10^{-4}	**91**	**96**	**48**
	10^{-5}	34	36	17
Heteroannular diene dihemiphthalate Na (Vc′)	10^{-4}	**94**	**85**	**71**
	10^{-5}	28	36	25

Values of inhibition are expressed as percentage of the control. Similar results were obtained in three separate experiments.

Table 8. Inhibition of Acetic Acid-Induced Writhing and PGE_2 Production by Deoxoglycyrrhetol (DG) Dihemiphthalate and Its Derivatives in Mice

Compound	Dose (mg/kg)	No. of writhings /30 min	PGE_2 content (pg/ml)
Control		30 ±4	200.8±35.3
DG dihemiphthalate Na (IIIc′)	25	9 ±3***	91.6±11.8*
Homoannular diene dihemiphthalate Na (IVc′)	25	8 ±1***	79.0± 7.3*
Aspirin (PG inhibitor)	100	9.3±3***	6.4± 0.8***

Test compounds were orally administered 45 min before intraperitoneal injection of 0.7% acetic acid. PGE_2 was extracted peritoneal fluid 20 min after irritant treatment. Values are expressed as mean±s.e. of 7—8 animals. * $p<0.05$ and *** $p<0.001$.

ified from GA may be effective for skin diseases including neurogenic inflammatory response.

3. Immunomodulating activities of GL, GA, and their homologous triterpenoid compounds

Immunomodulating effects of several triterpenoid compounds *in vitro* and *in vivo* have been reported.[62] Otsuki and Ishida[63] studied interferon (IFN)-inducing activity by intraperitoneal administration of SNMC (330 mg/kg for DDI mice) in the presence of concanavalin A, resulting in the maximum value (800 IU/ml) of IFN-γ in NK cells after 21 h. Intraperitoneal (50 mg/kg) or intravenous (20 mg/kg) administration of 18β-DG induced IFN-γ (maximum 300 IU/ml) in serum 24 h after injection. As previously mentioned, Ikegami *et al.*[33] reported that oral administration of GL tablets prevented disease progress in HIV-infected individuals and maintained a high CD4/CD8 ratio in them. This may be due to the immunomodulating effect of GL rather than due to its direct antiviral action.

Table 9. Inhibition of Glycyrrhetinic Acid (GA) Derivatives on Carrageenan-Induced Rat Paw Edema

Compound	Dose (mg/kg)	Inhibition (%)
Glycyrrhetinic acid (GA) (I)	200	15($p<0.05$)
GA hemiphthalate Na (Ic)	200	23
Deoxoglycyrrhetol (DG) (III)	200	3
DG dihemiphthalate Na (IIIc′)	25	**28**($p<0.01$)
	50	**43**($p<0.001$)
	100	**55**($p<0.001$)
	200	**73**($p<0.001$)
Homoannular diene dihemiphthalate Na (IVc′)	25	**29**($p<0.05$)
	50	**41**($p<0.001$)
	100	**52**($p<0.001$)
	200	**66**($p<0.001$)
Heteroannular diene dihemiphthalate Na (Vc′)	25	**24**($p<0.01$)
	50	**37**($p<0.001$)
	100	**42**($p<0.001$)
	200	**62**($p<0.001$)
Indomethacin (PG inhibitor)	5	60($p<0.001$)
Cyproheptadine	10	0

Test compounds were orally administered 30 min before injection of 1% carrageenan suspension. Paw edema was examined 3 h after irritant injection. ($n=6$)

As preclinical experimental results on the pharmacological and immunomodulating effects of GA and its natural and synthetic homologous compounds have accumulated, therapeutic applications might be achieved in the next stage. Licorice has been used over the millennia since ancient times in both the East and West, and it is still providing several biologically effective ingredients and their chemical derivatives. Further investigations will contribute to human healthcare in the new century.

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Table 10. Inhibition of Glycyrrhetinic Acid (GA) Derivatives on Mouse Paw Edema Induced by Vasoactive Agents

Compound	Dose (mg/kg)	Inhibition (%)			
		Histamine	5-HT	Bradykinin	PAF-acether
GA (I)	200	0	11	8	0
GA hemisuccinate Na (Ib′)	200	20	0	16	22
Deoxoglycyrrhetol dihemiphthalate Na (IIIc′)	25	38*	—	0	18
	50	52	22	45***	31
	100	67***	28	53**	56**
Homoannular diene dihemiphthalate Na (IVc′)	25	27	—	19	20
	50	19	17	33	46**
	100	47**	19	49**	48***
Heteroannular diene dihemiphthalate Na (Vc′)	25	28	—	12	23
	50	28	0	30	29*
	100	61***	6	64***	45***
Aspirin	200	0	8	13	0
Cyproheptadine	20	83	78***	43**	43**
Pyrilamine	20	47**	—	10	0

Test compounds were orally administered 30 min before injection of each irritant. Swelling was measured 15 min after irritant treatment. Statistical significance from the control at * $p<0.05$, ** $p<0.01$ and *** $p<0.001$. ($n=7$)

Table 11. Inhibition of Glycyrrhetinic Acid (GA) Derivatives on Capsaicin-Induced Mouse Ear Edema

Compound	Dose (mg/kg)	Route	Inhibition (%)
Glycyrrhetinic acid (GA) (I)	200	*p.o.*	−15
GA hemiphthalate Na (Ic′)	200	*p.o.*	29
Deoxoglycyrrhetol (DG) (III)	200	*p.o.*	−11
DG dihemiphthalate Na (IIIc′)	25	*p.o.*	**25**($p<0.05$)
	50		**53**($p<0.001$)
	100		**69**($p<0.001$)
	200		**76**($p<0.001$)
Homoannular diene dihemiphthalate Na (IVc′)	25	*p.o.*	**37**($p<0.05$)
	50		**63**($p<0.001$)
	100		**67**($p<0.001$)
	200		**79**($p<0.001$)
Heteroannular diene dihemiphthalate Na (Vc′)	25	*p.o.*	**30**($p<0.05$)
	50		**53**($p<0.001$)
	100		**68**($p<0.001$)
	200		**74**($p<0.001$)
Indomethacin (PG inhibitor)	10	*p.o.*	−20
AA 861 (LT inhibitor)	1	t.a.	−10
NDGA (dual inhibitor)	1	t.a.	−1
Dexamethasone (steroid)	0.1	t.a.	76($p<0.001$)
Chlorpheniramine (histamine H_1 antagonist)	4	i.v.	29($p<0.01$)
RP 67580 (NK_1 antagonist)	0.5	i.v.	83($p<0.001$)

Oral and topical (t.a.) administration of test compounds were performed 30 min (but dexamethasone was given 3 h) before capsaicin application (250 μg/ear). Chlorpheniramine and RP 67580 were administered intravenously 15 min before capsaicin treatment. Ear edema was examined 30 min after capsaicin application. ($n=6$—7)

Table 12. Inhibition of Glycyrrhetinic Acid (GA) Derivatives on Substance P-Induced Mouse Ear Edema

Compound	Dose (mg/kg)	Route	Inhibition (%)
Glycyrrhetinic acid (I)	200	*p.o.*	0
Deoxoglycyrrhetol dihemiphthalate Na (IIIc′)	25	*p.o.*	4
	50		**42**($p<0.01$)
	100		**58**($p<0.001$)
Homoannular diene dihemiphthalate Na (IVc′)	25	*p.o.*	8
	50		**51**($p<0.001$)
	100		**67**($p<0.001$)
Heteroannular diene dihemiphthalate Na (Vc′)	25	*p.o.*	14
	50		**50**($p<0.001$)
	100		**67**($p<0.001$)
Chloropheniramine (histamine H_1 antagonist)	4	i.v.	30($p<0.05$)
RP 67580 (NK_1 antagonist)	0.5	i.v.	66($p<0.001$)

Test compounds were orally administered 30 min (except for chlorpheniramine and PR 67580 were intravenously given 15 min) before intradermal injection of substance P (100 pmol/site). Ear edema was examined 30 min after substance P treatment. ($n=6$)

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