제 3 강연

식물성 사포닌의 생리활성

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Diverse Biological Properties of Plant Saponins

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The saponins are naturally occurring surface-active glycosides and mainly produced by many plant species, in both wild plants and cultivated crops. Saponins are generally classified into two groups, triterpenoid saponins and steroid saponins. Their physiological role in plants is considered a part of defense systems. The typical biological effects of saponins are mainly derived from the action on membranes.

Platycodin saponins(PS), the major and marker constituents of Platycodin Radix from *Platycodon grandiflorum* A.De(Campanulaceae) were investigated to examine their anti-obese, hypolipidemic and cholesterol-lowering properties through a series of *in vivo* and *in vitro* experiments. The underling mechanisms were futher investigated.

Initially, six of PS saponins were purified and the prosapogenins(PRS) were further prepared by base-hydrolysis of PS. 3-D-Glycosyl-platycodigenin(prs-GP), the major products of PRS, was purified, its methyl ester, prs-GPME was further prepared. These substances were subjected to the following experiments.

The obese-prone Sprague Dawley rats induced by high fat diet were administered with 35 and 70 mg/kg(p.o.) of PS. After 4 weeks of PS administration, the body weight reduction($13 \pm 4\%$ *vs.* HF control, $p<0.05$) showed high correlation to the food intake restriction(Pearson's linear coefficient $r=0.752$, $p<0.005$). The fecal TG excretion was dose dependently increased 2.1~3.2 folds, likely as a consequence of the lipase inhibition. The serum triglyceride(TG) and LDL-cholesterol concentrations were decreased without noticeable changes in HDL-cholesterol levels. The hepatic TG, cholesterol and the liver surface fat pads were decreased in ubiquity, but no abnormalities were noted in ALT and AST activities and in Masson' Trichrome Staining Test.

We attribute anti-obese effect of PS partly via the inhibition to pancreatic lipase. PD, one of major compounds of PS, inhibited the pancreatic lipase activity in a competitive manner, with the value of K_I being 0.18 ± 0.02 mM. The *in vitro* lipase inhibition attributed by each isolated compound and mixture of PS were virtually identical in potency and can be alternatively used as lipase inhibitors. The crucial moiety responsible for lipase inhibition is ascribed to PRS part in PS/PD.

During the study, a simple and trustworthy method to detect the inhibition kinetics for pancreatic lipase was

developed with a slight modification of known assay methods. The revised assay is sensitive, rapid and does not affect the accuracy to the kinetic properties, hence, is applicable not only to evaluate the kinetic properties of the pancreatic lipase, but also to high-throughput screening of pancreatic lipase activity.

These results suggest that PS could be a potential therapeutic alternative in the treatment of the obesity and hyperlipidemia.

A cholesterol-lowering effect of PD was investigated. An *in vivo* test with doses of 15, 30 or 50 mg/kg(p.o. male ICR mice) was performed for an eight week period. The body weights were decreased by 11.2±5%, 11.7± 5% and 23.4±7.9%, respectively, responding to the treatments of 15, 30 and 50 mg/kg PD. A decrease in daily food consumption was also noted in the initiating stage of animal test. TG and TC(total cholesterol) were decreased in serum and liver but increased in feces. To be noticeable, the cholesterol ester in liver tissue showed a significant decrease, which well matches the *in vitro* discoveries of hACAT activity inhibition, It evidences that PD can lower the formation of cholesterol ester in liver by inhibiting ACAT activity. No abnormalities in hepatic ALT and AST were noted.

In the *in vitro* study, PD's effects on atherogenic risk factors were investigated, with discoveries of an inhibition to the hACAT activity and a mild degree of antagonism to FXR binding. PD also showed high affinity to cholesterol, they formed insoluble 1:1 complex *in vitro.*

Taken together, PD has showed a promising cholesterol-lowering effect.

PRS, the derivatives of PS were found to reduce the hemolytic toxicity of PS and so is prsD, a major constituent of PRS. PRS still possessed anti-obese effect in SD rats. Many of remarkable changes in the biochemical and pharmacological properties were noted here.

Since we assume that the 28-sugar plays an important role in specific or nonspecific recognition of PS to membrane or enzymes, we tried to partly degrade the sugar composition by enzymatic degradation. In fact, the partly degraded sugar parts in 28-O-position brought about weakening cytotoxicity and sensory grades.

In conclusion, PS/PRS as well as PD/prsD, can represent an effective lead candidate for anti-obesity, anti-hyperlipidemia, cholesterol-lowering as well as atherogenesis-prevention without detectable hepatic toxicity.

Ⅰ. Introduction

Saponins are glycosides with a distinctive foaming characteristic. They are found in many plants, but get their name from the soapwort plant(*Saponaria*), the root of which was used historically as a soap(Latin *sapo* ---> soap). They consist of a polycyclic aglycone that is either a choline steroid or triterpenoid attached via C3 and an ether bond to a sugar side chain. The aglycone is referred to as the sapogenin and steroid saponins are called saraponins. The hydrolysis of saponins with acid or alkali yields sapongenins(=aglycone) and sugars. Generally, according to the structural features of sapogenin, saponins are classified into three main types(Table 1).

Most of sapongenins in nature bind sugars to form glycoside(some are bound ester bond) in a glycosic linkage. According to the number of sugar chains, saponins are classified into two groups, one is monodesmoside(desmos=chain, mono=one), like teasaponins and kaika saponin from *Pueraria thunbergiana.* The other is bidesmoside(bi=two), like platycodin saponins. Among a variety of saponins, triterpenoid glycosides are major types of naturally occurring saponins and more than 2,600 triterpene glycosides are isolated and

nominated by 1995(Ahmad, 2000).

Table 1. The types of the saponin according to the structure of sapongenin(aglycone)

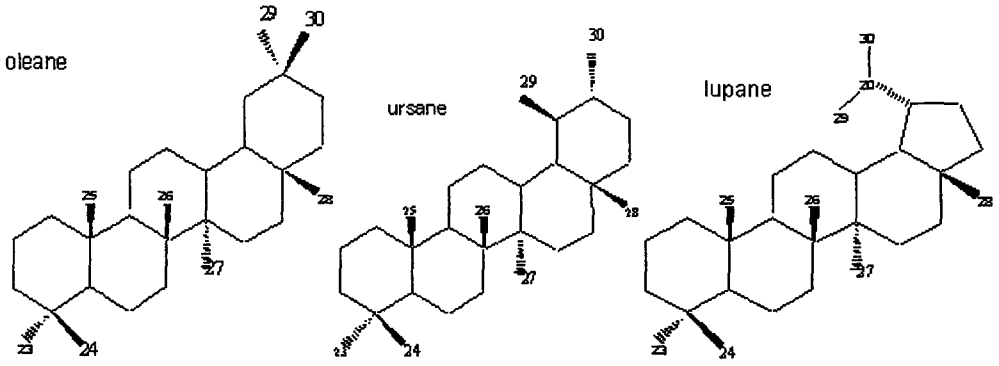
Triterpenoidal type	5-ring type	Oleanane, ursane, lupine, hopane, taraxerane
	4-ring type	Dammarane, lanostane, cholestane
Steroidal type	Furostane, spirostane	

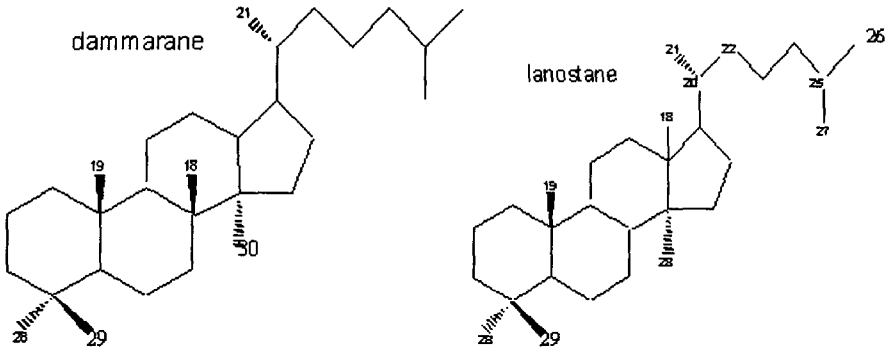
Ⅱ. Chemical Properties of Saponin Glycosides

Triterpene refers to a particular type of molecular structure that has a four- or five-ring and planar-base molecule containing 30 carbon atoms. It is synthesized from very simple compounds(acetate units) that are found in all plants, but is mainly synthesized in higher plants(flowering plants) by linking the acetate units "head to tail." The triterpenes have an acidic quality, an acrid-bitter taste, and their function in plants remains unknown. Glycoside refers to the attachment of various sugar molecules to the triterpene unit, which is known as the aglycone or genin when the sugar component is removed. When glycosides are consumed, the sugar molecule is usually cleaved off by enzymatic action either in the gut or in the blood stream.

The triterpenes are subdivided into about 20 groups, depending on their particular structures. The base structure that is found in the largest variety of medicinal plants is the oleanane triterpene. This type of compound may be represented by four of the most frequently occurring forms: oleanolic acid, ursolic acid, and alpha- and beta-amyrin(the latter three are sometimes put in the subdivision of ursane triterpenes). Ginseng is a highly valued herb in the Far East and has gained popularity in the West during the last decade. The major active components of ginseng are ginsenosides.

There is another type of saponins, called steroidal saponins, found in a variety of plants including many used as foodstuffs(Price, 1987), they are structurally even closer to cholesterol and steroid hormones, though their actions(when consumed) are not hormonal in nature.





Ⅲ. Biological Properties of Saponins

In additon to surfactant and soap-like properties, saponins also cause haemolysis(lysing red blood cells by increasing the permeability of the plasma membrane), and thus they are highly toxic when injected to blood stream. Table 2 illustrates typical biological properties of triterpenes in commonly used Chinese herbs.

However, saponins are relatively harmless when taken orally, and some of our valuable food materials, e.g. beans, lentils, soybeans, spinach, and oats contain significant amounts. Toxicity is minimized during ingestion by low absorption, and by hydrolysis. Due to its diversified biological activities, just as their structural diversity, it is hard to describet all of their chemical and pharmacological aspects here. We are hereby to set the focus

Table 2. Triterpene-containing Herbs Grouped by Therapeutic Actions

Action	Herbs	Comments
Hepato-protective; treats hepatitis	Forsythia, ligustrum, schizandra, imperata, bupleurum, melia, ginseng, swertia, licorice	Most of these herbs clear heat; in laboratory studies, they protect the liver from damage due to chemicals.
Resolves phlegm, relieves cough, treats bronchitis	Platycodon, centipeda, polygala, aster, schizandra, ginseng, licorice, bupleurum	These herbs are used in traditional formulas for lung diseases; most are warming in nature.
Treats urinary tract inflammation; diuretic	Alisma, hoelen, akebia (and substitute herb clematis), dianthus, dryopteris	These herbs are used in traditional formulas for urinary tract infections; most are cooling in nature.
Treats pain due to inflammation or injury	Melia, frankincense, platycodon, san-chi	These herbs are usually not combined together, but are used in a variety of formulas.
Sedative	Ginseng, jujube, hoelen, licorice, schizandra, ganoderma	These herbs are used in tonic sedative formulas.
Tonifies qi, raises qi, directs herbs upward	Ginseng, bupleurum, platycodon, cimicifuga, jujube, licorice	These herbs are frequently combined in formulas; bupleurum and cimicifuga "raise qi;" cimicifuga and platycodon direct herb actions to the upper body

on platycodin D which is attributed to the cholesterol and lipid metabolism. Saponins can enter into fatty materials and, in large enough quantity, break them up(just as soap can dissolve fat).

Ⅳ. Saponins on Hypocholesterolemia and Hypolipidemia

Over the past three decades, the prevalence of the obesity and non-insulin dependant diabetes, hyperlipidemia, hypertension and cardiovascular diseases has risen to reach epidemic proportions . Reduction in the concentration of serum lipids, especially cholesterol, is a major goal in several primary and secondary prevention initiatives. A variety of drugs and potent lead compounds have been developed and some have been commercially popularized. as a new trend of recent decades. Many types of natural products including some of saponins have been investigated and emerged as hopeful lead compounds or drug alternatives or diet-guided nutrition supplements in which saponins are added as active compounds/ingredients beneficial for hypocholesterolemia, hypolipidemia and hypoglycemia.

To control the lipid and cholesterol level in blood , the available strategies area inhibition of absorption, stimulation of catabolism or transfer to other metabolites, and increase elimination by the fecal excretion.

4-1 Absorption Inhibitors

Triglycerides:

Triglycerides, after uptake into the gut, will be digested by the pancreatic lipase into diglyceride, monoglycerides and free fatty acids in the end, the released free fatty acids and monoglycerides are absorbed into blood simply by diffusion. Lipase is believed to play a key role in digestion of triglycerides in small intestine.

Many diet foods and nutrition supplements exerts its functions by inhibiting the pancreatic lipase. One class of naturally occurring TG inhibitors is the saponins. In fact, saponins isolated from platycodin and tea were found to inhibit the pancreatic lipase activity *in vitro*(Han *et al.*, 2002).

Cholesterol :

Cholesterol absorption needs no enzymatic mediation, but bile acids are required to emulsify it to be easily absorbed in the intestinal lumen. Saponins are discovered to lower total plasma cholesterol of species including humans by inhibiting cholesterol and bile acid absorption. Saponin' s intervention to inhibit absorption has been proposed as follows:

First, saponins form insoluble complexes with cholesterol which prevents its absorption from the small intestine. Others cause an increase in the faecal excretion of bile acids, which is an indirect route for elimination of cholesterol.

Second, different saponins isolated from soybean and soapwort affect the absorption of the bile salt(sodium cholate) from perfused loops of small intestine, *in vivo*, reduced the rate of absorption of the bile salt. The formation of large mixed micelles by bile acid and saponin molecules in aqueous solution was observed(Sidhu

GS,1986). These aggregates can have molecular weights in excess of 10^6 daltons, consequently the bile acid molecules incorporated in them are not available for absorption. The saponins isolated from soy, *Saponaria officinalis* or *Quillaja saponaria* altered the size and shape of micelles(Oakenfull and Toppping, 1983).

Third, saponins form complexes with membrane cholesterol or extract cholesterol from membranes and this may explain the hemolytic activity of some saponins. Gypsophylla or saponaria saponins decrease permeability and active glucose transport in isolated everted jejunal sacs, with permeability changes resisting after saponin pretreatment(Johnson et al., 1986).

The experimental results indicate that the control of plasma cholesterol and nutrient absorption through dietary saponins could provide substantial health and nutritional benefits in humans. By the virtue of their purported intestinal action, saponins could be particularly well suited for combionation therapy with statins or other systemic hypolipidemic agents(Lee A, 1999). The examples of plant saponins that used as interstinal cholesterol absorption inhibitors and reduce plasma cholesterol concentrations in a variety of experimental animals, include steroid glycosides of digitonin, tomatine, sarsasaponin(which induce its precipatation *in vitro*, and inhibit cholesterol absorption without affecting bile acid absorption *in vivo*), and triterpenoid glycosides of alfalfa, sayya, *Quillaja saponaria*, gypsophila, ginseng, tea, platycodin(many of the triterpenoid saponins interfere with micelle size and structure and alter bile acid absorption in addition to inhibiting cholesterol absorption.)

Saponins as digestion/absorption inhibitors, however, exposed some unsolved drawbacks. First, theoretically, it is difficult to ascertain which mechanism is mediated for cholesterol absorption inhibition. Second, naturally occurring saponins are typically of low potency and the high does that are required to elicit hypocholesterolemia may induce secondary effects. Now, people take more trials by chemical modification of natural saponins to solve this problem(Harwood et al., 1993)

4-2 Cholesterol Esterification and Conversion to Bile Acid

ACAT(E.C.2.3.1.26) Catalyzes the formation of cholesteryl esters from cholesterol and long-chain fatty acyl-coenzyme A. Once cholesterol enters the intestinal enterocyte, it is esterified by acyl-CoA:cholesterol *O*-acyltransferase(ACAT), isoform 2(Oelkers et al., 1998), before its packaging into chylomicrons. It appears that this ACAT-mediated esterification prevents the back-diffusion of cholesterol rather than participating directly in the absorptive process. It has been suggested that ACAT plays a role in hepatic cholesterol homeostasis by preventing excessive accumulation of free cholesterol in intracellular membranes(Changiz et al., 2002). ACAT inhibitor is an attractive target for treatments of hypercholesterolemia and atherosclerosis. Several laboratories and pharmaceutical companies have developed ACAT inhibitors and are studying their cholesterol lowering properties. These compounds are thought to decrease plasma cholesterol levels by interfering with intestinal cholesterol absorption(Uchida et al., 1998; Umeda et al., 1998; Azuma et al., 1999). These agents are effective in reducing plasma cholesterol levels in animal models by 31 to 71%, depending on the particular model used. Success in reducing plasma cholesterol in human trials is lacking.

The conversion of cholesterol to bile acids in the liver is initiated by cholesterol 7-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis pathway. Transcription from the *CYP7A1*[1], which encodes cholesterol 7-hydroxylase, is regulated by hormones, dietary factors, and diurnal rhythm. The feedback repression

of *CYP7A1* transcription by bile acids is an important physiological mechanism for maintaining bile acid and cholesterol homeostasis(Kelli, S B, 2000)

Bile Acid Synthesis. Cholesterol derived from either de novo pools or dietary intake is metabolized to bile acids by a series of more than a dozen enzymes(Schwarz et al., 1998). Quantitatively, this metabolic pathway is the most important route for elimination of cholesterol carbon from the body.

Bile acids are synthesized by two biochemical pathways. The first, called the acidic or classical pathway, is contained within the liver and begins with the hydroxylation of cholesterol by cholesterol 7-hydroxylase(Cyp7a). 7-OH cholesterol is then subsequently hydroxylated at carbon 27, leading to the dihydroxy primary bile acid chenodeoxycholic acid. Alternatively, hydroxylation of 7-OH cholesterol at both carbon 12 and carbon 27, via a branchpoint in the biosynthetic pathway, leads to the trihydroxy bile acid, cholic acid. A different pathway begins with 27-hydroxylation of cholesterol, rather than 7-hydroxylation. The quantitative importance of this pathway has been only recently appreciated, and in most species studied to date accounts for as much as 50% of bile acid biosynthesis(Vlahcevic et al., 1997). The enzyme that mediates this reaction, sterol 27-hydroxylase, is expressed in numerous extra hepatic sites, such as spleen and endothelial cells. The product of this reaction, 27-hydroxy cholesterol, is taken into the liver and further hydroxylated by oxysterol 7-hydroxylase(Cyp7b). Additional biotransformation produces chenodeoxycholic acid.

From a pharmacologic perspective, most interest has focused on cholesterol 7-hydroxylase, the rate-limiting enzyme in the classic pathway. This enzyme is regulated by numerous hormones and physiologic conditions but, perhaps most importantly, is regulated by plasma cholesterol levels and bile acids. In the rat, ingestion of a cholesterol-enriched diet increases cholesterol 7-hydroxylase transcription, which leads to increased bile acid biosynthesis. Bile acids, recycled to the liver from the intestine via ASBT, are potent inhibitors of cholesterol 7-hydroxylase(Pandak et al., 1991). Increasing expression of cholesterol 7α-hydroxylase via adenoviral vectors(Spady et al., 1996, 1998) causes marked decreases in plasma cholesterol. Rabbits that over-express cholesterol 7-hydroxylase mRNA by 7-fold are resistant to increases in plasma cholesterol caused by a cholesterol-enriched diet(Poorman et al., 1993). These experiments suggest that pharmacologically increasing Cyp7a expression might lead to reductions in plasma cholesterol.

The Farnesoid X receptor (FXR) is a member of the nuclear hormone receptor superfamily that has been shown to play an important role in bile acid and cholesterol homeostasis.

4-3 Cholesrerol Biosynthesis

HMG CoA reductase inhibitors are currently used as cholesterol biosynthesis. Digoxin and hypothalamic digoxin(Maiquiz A Q, 2003) are well investigated saponins as the inhibitors of HMG CoA reductase , but since in principle they suppressed all post-mevalonate biosynthetic pathways, the selective inhibition of cholesterol biosynthetic pathways, is desirable pharmaceutical goals. The use of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors(statins) in clinical trials over the last 11 years supports this conclusion in a variety of populations, including patients with or without established cardiovascular disease and patients with severe or only moderate hypercholesterolemia(Najwa, 2000).

Squalene synthase(SS) and squalene epoxidase(SE)(EC 1.14.99.7) catalyze the conversion of squalene to (3s)2,3-oxidosqualene. the suppression to SS or SE is more specific than to HMG Co A Reductase in regulating cholesterol biosynthesis. soyasaponins are reported to inhibit the SS activity(Hayyasi H, 2003).

4-4 Saponins as Nutrient Components

Saponins are a hot new food ingredient and people are just starting to pay attention to it(Andrew, 2003). People found the saponin components from red wine is good for hypocholesterolemia and hypolipidemia. The saponins from orange juice, onion extract, soybean also showed good nutraceutical effects on hypocholesterolemia and hypolipidemia.

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