# Terpenoid and flavonoid biosynthesis in *Astragalus membranaceus*

Jongwon Lee, Yeon Bok Kim, Md Romij Uddin, Jin Woong Cho*, Sang Un Park*

Department of Crop Science, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon, 305-764, Korea
Jin Woong Cho: jwcho@cnu.ac.kr (J.W. Cho) and supark@cnu.ac.kr (S.U. Park)

**Abstract:** Many natural products are high-value chemicals, since only small quantities can be isolated from their native biosynthetic organisms with the majority of them utilized as therapeutic agents for many diseases. The advent of genomics and metagenomics has allowed not only the discovery of a huge number of new promising metabolites, but also the elucidation of their biosynthetic pathways. As a result, the use of well-characterized hosts for their efficient, large scale production is now possible. *Astragalus membranaceus* is a herb that has traditionally been used in a wide variety of herbal blends and 'natural' remedies in Asiatic medicine, such as *Dang-gui buxue tang* (Astragalus paired with Angelicae Sinensis; also known as DBT). The genus Astragalus is a very large group of more than 2,000 species distributed worldwide, and is commonly known as milkvetch root. Astragalus contains the plant pigments: formononetin, astraisoflavan, astrapterocarpan, 2'-3'- dihydroxy-7, 4'- dimethooxyisoflavone, and isoliquiritigenin. Other major constituents include D-ß-asparagine,calycosin,cycloastragenol, astragalosides, choline, betaine, kumatakenin, sucrose, glucuronic acid, ß-sitosterol 1, and soyasaponin. The flavonoid content may also contribute to cardioprotection, as flavonoids tend to possess this mechanism, and the polysacccharide content can also aid this by being a potent anti-inflammatory agent and reducing cholesterol levels in a manner similar to psyllium husk (a fiber). It has recently been investigated more into due to its heart healthy (cardioprotective) effects, its anti-inflammatory effects, and the potential for Astragalus extract to enhance longevity and lifespan. Interestingly, the traditional chinese preparation method of DBT was shown to be a very effective way of extracting the main ingredients from the plant. A few of the research has been done in astragaloside biosynthesis in *A. membranaceus* and was not reported any one as a review. Therefore, we summrized the information related to astragalosides biosynthesis in this review paper. The biosynthesis of terpenoid and flavonoid in *Astragalus membranaceus* might play a key role for commercially used in the pharmaceuticals industry both in modern and traditional uses.
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## Introduction

*Astragalus membranaceus* Bge. var. *mongholicus* (Bge.) Hsiao is one of the most important traditional Chinese herbs. *A. membranaceus* is a flowering plant in the family Fabaceae and has a long history of use as a traditional medicine in China, Japan, Korea, and other Asian areas. The genus Astragalus is a very large group of more than 2,000 species distributed worldwide. In Europe, for example, there are 133 Astragalus species. In North America there are 368 Astragalus species, mostly confined to the western United States and Canada. The dry root of *A. membranaceus* Bge. or *A. membranaceus* var. Mongholicus (Bge.) Hsiao is grown mainly in northern China, Mongolia, and Siberia. Hwanggi has been used for centuries for general weakness, chronic illnesses, and to increase overall vitality (Wojciechowski *et al.,* 1999).

Hwanggi is an herbaceous perennial, growing to about 2 feet tall, with sprawling stems. The alternate leaves have 12–24 leaflets. Small, light yellow pea-like flowers arise from leaf axils. It is easily grown from seed, though the seed, like many legumes, has a hard coat.

Currently, Hwanggi has been researching for pharmacological study, immune-stimulating polysaccharides, and other active ingredients useful in treating immune deficiency conditions. Astragalus has illustrated a wide range of potential therapeutic applications in immunodeficiency syndromes, as an adjunct cancer therapy, and for its adaptogenic effect on the heart and kidneys. Astragalus root has been used to promote immune function and as a tonic to build stamina (Wagner et al.*,* 1997; Zheng, 2005).

### Terpenoid saponins

The terpenoids, also called isoprenoids are one of the largest groups of natural products found in nature with over 30,000 known examples and their number is growing steadily (Davis and Croteau 2000). Triterpenes are a large group of compounds arranged in a four or five ring configuration of 30 carbons with several oxygens attached and assembled from a C5 isoprene unit through the cytosolic mevalonate pathway (Lichtenthaler et al., 1997) to make a C30 compound and are steroidal in nature. Triterpenoid saponins are triterpenes which belong to the group of saponin compounds. Triterpenoid saponins are synthesized starting from the isoprenoid pathway throught farnesyl diphosphate (FPP) which is derived from the isoprenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), in a reaction catalyzed by farnesyl diphosphate synthase (FPS) (Ding et al., 2008). FPP is a precursor for squalene synthesis by squalene synthase (SS) in the first commited step toward sterol and triterpenoid biosynthesis (Abe et al., 1993; Huang et al., 2007). Squalene is oxidized by squalene epoxidase (SE) to 2,3-oxidosqualene, leading to the cyclization of triterpenoid skeletons such as oleanane, ursane, lupeol or dammarane (He et al., 2008).

*A. membranaceus* root possesses biologically active constituents such as saponins, isoflavonoids, polysaccharides, and astragalosides (Song *et al.*, 2004; Wu and Chen, 2004). Among them, astragalosides consist of a class of cycloartane triterpenoid type glycosides and play a role indicators of the root quality (Kwon *et al.*, 2013; Lai *et al.*, 2013; Pan *et al.*, 2008; Xu *et al.*, 2011). Astragalosides I, II, III, and IV are the major astragalosides in *A. membranaceus* root. Astragalosides have various therapeutic effects and are used clinically in the treatment of diabetes and cardiovascular disease (Liu *et al.*, 2001). Particularly, it was reported that Astragaloside IV had various beneficial effects on processes such as osteogenesis, angiogenesis, and metabolic syndrome, and to exert neuroprotective, antiflammatory, and cardioprotective functions (Kwon *et al.*, 2013; Lai *et al.*, 2013; Pan *et al.*, 2008; Xu *et al.*, 2011). Furthermore, it has affected as a natural product with both healing and antiscarring effects for wound treatment (Chen et al., 2012). Up to recently, many studies have been published about astragaloside of *A. membranaceus.* A few of the research has been done in astragaloside biosynthesis in *A. membranaceus* and was not reported any one as a review. Therefore, we summrized the information related to astragalosides biosynthesis in this review paper. Our results might provide a foundation to elucidate the mechanism of triterpenoid saponin, astragalosides, biosynthesis in *A. membranaceus*

**Flavonoid biosynthesis**

Flavonoids (from the Latin word flavus meaning yellow, their colour in nature) are one of the most abundant and a class of plant secondary metabolites with more than 6,000 compounds detected so far in higher plants (Ferrer et al., 2008). Flavonoids are also important nutritional compounds in foods and beverages, and a high or increased intake of flavonoids is related to a reduced risk of some kinds of cancer (Tang et al., 2009; Wilson et al., 2009). Some flavonoids are potent antioxidants and specific flavonoid compounds are beneficial in many physiological and pharmacological processes such as antiviral, antibacterial, estrogenic, and antiobesity (Wang et al., 2011). Lin et al. (2000) reported that eight flavonoids were identified as calycosin-7-O-beta-D-glucoside, calycosin-7-O-beta-D-glucoside-6"-O-malonate (2), ononin, (6aR,11aR)-3-hydroxy-9,10-dimethoxypterocarpan-3-O-beta-D-glucoside, calycosin, (3R)-7,2'-dihydroxy-3',4'-dimethoxy-isoflavan-7-O-beta-D-glucoside, formononetin-7-O-beta-D-glucoside-6"-O-malonate and formononetin by direct comparison with the isolated standards from Huangqi. In particular, calycosin and calycosin-7-O-β-D-glucoside (calycosin-7-glu) are 2 major isoflavones related to the bioactivity of the herb (Toda and Shiratakі, 1998; Wu et al., 2000). Calycosin has been shown to protect endothelial cells from hypoxia-induced barrier impairment (Fan et al., 2003). Calycosin-7- β-D-glucoside appears to be a potential natural anti-inflammatory and anti-osteoarthritic agent and might be used effectively to protect against proteoglycan degradation (Choi et al., 2005) and as a hyaluronidase inhibitory component (Lee et al., 2005). Nakamura et al. (1999) pointed out that these two compounds could be used as “marker compounds” for the chemical evaluation or product standardization of *A. membranaceus*.

The biosynthetic pathway of calycosin and calycosin-7-*O*-β-D-glucoside has been elucidated (Liu et al. 2003). Calycosin-7-*O*- β -D-glucoside is synthesized from L-phenylalanine through the isoflavonoid branch of the phenylpropanoid pathway.

Up to now, several enzymes were characterized in this pathway (Pan et al., 2007). The first enzyme of the phenylpropanoid pathway is a phenylalanine ammonia lyase (PAL) and PAL catalyzes the conversion of L-phenylalanine into *trans*-cinnamic acid. Following this, cinnamate-4-hydroxylase (C4H) is the first cytochrome P450-dependent monooxygenase of the phenylpropanoid pathway and it also catalyzes the hydroxylation of *trans*-cinnamic acid into *p*-coumaric acid (Russel, 1971). Next, a coenzyme-A (CoA) ligase (4CL) converts *p*-coumarate into its CoA ester. This product serves as a precursor for various phenylpropanoid biosynthetic derivatives, including lignins and flavonoids. Chalcone synthase (CHS) is a key enzyme of flavonoid biosynthesis. CHS catalyzes condensation of *p*-coumaroyl-CoA and three molecules of malonyl-CoA (Dixon et al. 1995). Isofavone synthase (IFS) is an important enzyme in isoflavone biosynthesis. Graham (2005) suggested that it may be regulated by wounding, glutathione, and UV irradiation. Isoflavone 3’-hydroxylase (I3’H) catalyzes hydroxylation reaction at the 3’-position of the B-ring. The formation of calycosin-7-*O*-β-D-glucoside from calycosin was catalyzed by UDP-glucose: calycosin 7-*O*-glucosyltransferase (UCGT).

**Conclusion**

The era of recombinant DNA technology for natural product biosynthesis emerged has remained the production platform of choice for many fine chemicals, including natural products. The rapid elucidation of biosynthetic pathways made possible through advanced genomic tools, has made natural product again the molecules of choice for drug development. Indeed, half of the drugs currently in clinical use are natural products and it is expected that the market size of biotech-derived small molecules will exceed 100 billion US$ in 2010 and 400 billion US$ in 2030. The above mention findings might provide a foundation to elucidate the mechanism of triterpenoid saponin, astragalosides, biosynthesis in *A. membranaceus*

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**Corresponding Author:**
Dr. Jin Woong Cho
Department of Crop Science, Chungnam National University, 99 Daehang-ro, Yuseong-Gu, Daejeon, 305-764, Korea
E-mail: jwcho@cnu.ac.kr

Dr. Sang Un Park
Department of Crop Science, Chungnam National University, 99 Daehang-ro, Yuseong-Gu, Daejeon, 305-764, Korea
E-mail: supark@cnu.ac.kr

**References**

1. Wojciechowski MF, Sanderson MJ, Hu JM. Evidence on the monophyly of *Astragalus* (Fabaceae) and its major subgroups based on nuclear ribosomal DNA ITS and chloroplast DNA *trn*L intron data. Syst Bot 1999; 24: 409-37.
2. Wagner H, Bauer R, Xiao PG, Chen JM, Michler G. Chinese drug monographs and analysis: *Radix Astragali* (Huangqi); Verlag Wald Germany, 1997 pp 1-17.
3. Zheng XY. Pharmacopoeia of the Peoples Republic of China, Vol. 1, Chinese edn. Beijing: Chemical Industry Press 2005: 212-213.
4. Davis EM, Croteau R. Cyclization enzymes in the biosynthesis of monoterpenes, sesquiterpenes, and diterpenes. In: Leeper FJ, Vederas JC (Eds.), Biosynthesis: Aromatic Polyketides, Isoprenoids, Alkaloids, vol. 209. Springer-Verlag, Heidelberg, 2000, pp. 53-95.
5. Lichtenthaler HK, Rohmer M, Schwender J. Two independent biochemical pathway for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants. Physiol Plant 19997;101:643–52.
6. Ding YX, Ou-Yang X, Shang CH, Ren A, Shi L, Li YX, Zhao MW. (2008) Molecular cloning, characterization, and differential expression of a farnesyl-diphosphate synthase gene from the basidiomycetous fungus *Ganoderma lucidum*. Biosci Biotechnol Biochem 2008; 72:1571-9.
7. Abe I, Rohmer M, Prestwich GD. Enzymatic cyclization of squalene and oxidosqualene to sterols and triterpenes. Chem Rev 1993; 93:2189–2206.
8. Huang Z, Jiang K, Pi Y, Hou R, Liao Z, Cao Y, Han X, Wang Q, Sun X, Tang K. Molecular cloning and characterization of the yew gene encoding squalene synthase from *Taxus cuspidata*. J Biochem Mol Biol 2007; 40:625-35.
9. He F, Zhu Y, He M, Zhang Y. Molecular cloning and characterization of the gene encoding squalene epoxidase in *Panax notoginseng*. DNA Seq 2008. 19:270-3.
10. Song ZH, Ji ZN, Lo CK, Dong TT, Zhao KJ, Li OT, Haines CJ, Kung SD, Tsim KW. Chemical and biological assessment of a traditional Chinese herbal decoction prepared from Radix Astragali and Radix Angelicae Sinensis: orthogonal array design to optimize the extraction of chemical constituents. Planta Medica 2004; 70: 1222-1227.
11. Wu F, Chen X. A review of pharmacological study on *Astragalus membranaceus* (Fisch.) Bge. *Zhong Yao Cai* 2004; 27: 232-234.
12. Kwon HJ, Hwang J, Lee SK, Park YD. Astragaloside content in the periderm, cortex, and xylem of *Astragalus membranaceus* root. Journal of Natural Medicines (In press) 2013.
13. Lai PK, Chan JY, Cheng L, Lau CP, Han SQ, Leung PC, Fung KP, Lau CB. Isolation of anti-inflammatory fractions and compounds from the root of *Astragalus membranaceus*. Phytotherapy Res 2013; 27:581-587.
14. Pan H, Wang Y, Zhang Y, Zhou T, Fang C, Nan P, Wang X, Li X, Wei Y. Phenylalanine ammonia lyase functions as a switch directly controlling the accumulation of calycosin and calycosin-7- O-b-D-glucoside in *Astragalus*

*membranaceus* var. *mongholicus* plants. Journal of Expt Bot 2008; 59: 3027-3037.

15. Xu RY, Nan P, Yang Y, Pan H, Zhou T, Chen J. Ultraviolet irradiation induces accumulation of isoflavonoids and transcription of genes of enzymes involved in the calycosin-7-O-β-d-glucoside pathway in *Astragalus membranaceus* Bge. var. *mongholicus* (Bge.) Hsiao. Physiologia Plantarum 2011;142: 265-273.
16. Liu ZQ, Li QZ, Qin GJ. Effect of *Astragalus* injection on platelet function and plasma endothelin in patients with early stage diabetic nephropathy. Chinese Journal of Integrative Medicine 2001; 21:274-76.
17. Chen X, Peng LH, Li N, Li QM, Li P, Fung, KP, Leung PC, Gao JQ. The healing and anti-scar effects of astragaloside IV on the wound repair *in vitro* and *in vivo*. J Ethnopharmacol 2012; 139:721-727.
18. Ferrer JL, Austin MB, Stewart CJ, Noel JP. Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. Plant Physiol Biochem 2008; 46: 356-70.
19. Tang NP, Zhou B, Wang B, Yu RB, Ma J. Flavonoids intake and risk of lung cancer: a meta-analysis. Jpn J Clin Oncol 2009; 39: 352-59.
20. Wilson RT, Wang J, Chinchilli V, Richie JP, Virtamo J, Moore LE, Albanes D. Fish, vitamin D, and flavonoids in relation to renal cell cancer among smokers. Am J Epidemiol 2009; 170: 717-29.
21. Wang Y, Chen S, Yu O. Metabolic engineering of flavonoids in plants and microorganisms. Appl Microbiol Biotechnol 2011; 91:949-956.
22. Toda S, Shirataki Y. Inhibitory effects of isoflavones in roots of Astragalus membranaceus Bunge (Astragali Radix) on lipid peroxidation by reactive oxygen species. Phytother Res 1998; 12:59-61.
23. Wu DZ, Fan Y, Hen ZF, Song CQ, Hu ZB. Effect of flavonoids isolated from Astragalus membranaceus on the changes of endothelial permeability induced by low osmolarity. Pharmacol Clin Chin Mater Med 2000; 16:16–18.
24. Fan Y, Wu DZ, Gong YQ, Zhou RY, Hu ZB. Effects of calycosin on the impairment of barrier function induced by hypoxia in human umbilical vein endothelial cells. Eur J Pharmacol 2003; 81: 33-40.
25. Choi S, Park SR, Heo TR. Inhibitory effect of astragali radix on matrix degradation in human articular cartilage. J Microbiol. Biotechnol 2005; 15: 1258–66.
26. Lee YM, Choi SI, Lee JW, Jung SM, Park SM, Heo TR. Isolation of hyaluronidase inhibitory component from the roots of Astraglus membranaceus Bunge Astragali radix. Food Sci Biotechnol 2005;14: 263–67.
27. Nakamura T, Hashimoto A, Nishi H, Kokusenya Y. Investigation on the marker substances of crude drugs in formulations. I. Marker substances for the identification of *Astragali Radix* in kampo and drinkable preparations. J Pharmaceut Soc Jap 1999; 119:391-400.
28. Liu CJ, Huhman D, Sumner LW, Dixon RA. Regiospecific hydroxylation of isoflavones by cytochrome P450 81E enzymes from *Medicago truncatula*. Plant J 2003;471–84.
29. Russel DW. The metabolism of aromatic compounds in higher plants. X. Properties of the cinnamic acid 4-hydroxylase of pea seedlings and some aspects of its metabolic and developmental control. J Biol Chem 1971; 246:3870-78.
30. Dixon RA, Paiva NL. Stress-induced phenylpropanoid metabolism. Plant Cell 1995; 7:1085–97.
31. Graham MY. The diphenylether herbicide lactofen induces cell death and expression of defense-related genes in soybean. Plant Physiol 2005; 139:1784–1794.

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