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Bioactive Fabaceous Saponins and Structure-Activity Relationship

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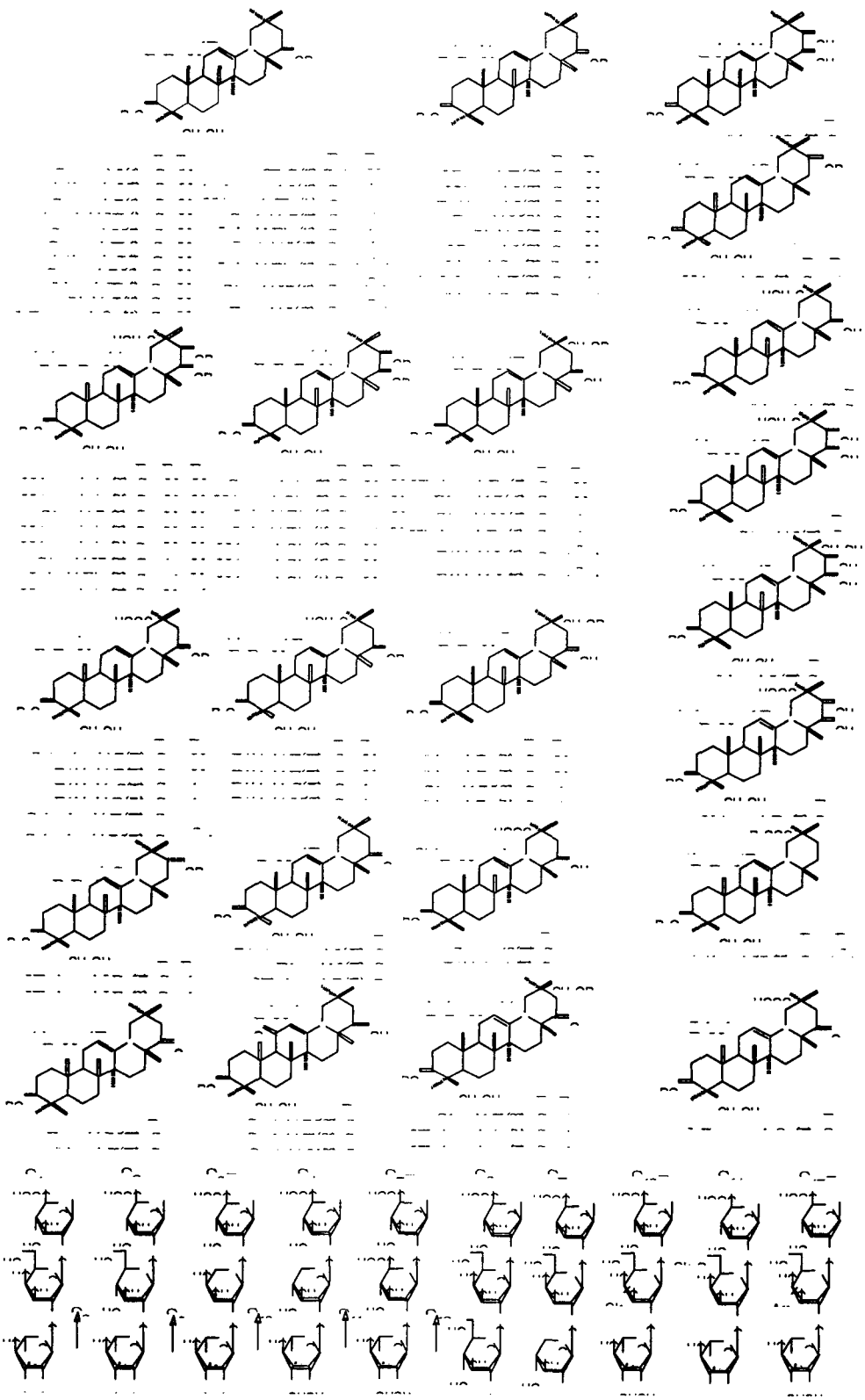
Saponins are glycosidic compounds present in many edible and inedible plants. Structurally, they are composed of a lipid-soluble aglycone consisting of either a sterol or, more commonly, a triterpenoid and water-soluble sugar residues differing in type and amount of sugars [1]. Because of their amphiphilic nature, they are highly surface-active. Their biological activity is closely related to the chemical structures that determine the polarity, hydrophobicity and acidity of compounds [1]. A recent review by Safayhi and Sailer shows that pentacyclic triterpenes, like as oleanane skeleton, might be a rich natural source of lead compounds for anti-inflammatory drug development [2]. Some oleanane-type triterpene saponins are known to exhibit anti-hepatic (hepatoprotective) activity. Among them, glycyrrhizin and saikosaponins [1] are the most well-known. Oleanene-glucuronide (OG) would be defined as an olean-12-ene type triterpene with a C-17 methyl group and a glucuronic acid moiety linked at the C-3 of the triterpene. Soyasaponin I (**1**) is a representative OG, and glycyrrhizin also belongs to the OG species. It has been revealed that OGs are widely distributed in the fabaceous (Faboideae in Leguminosae) plants and show several biological activities. For example, anti-hepatitis, anti-hypercholesteremia, anti-urolithiasis, anti-inflammatory, and anti-nephritic activities were confirmed in experimental *in vivo* models. And, antiviral, anti-complementary, and calcium-dependent potassium channel-opening activitieswere examined using *in vitro* models [1]. In view of the fact that leguminous plants are widely distributed and used as foodstuff and folk medicines, we have focused on these plants and have been trying to develop natural medicines after proving the effectiveness of these crude drugs and to find the lead compounds among these natural sources.

Herein, we describe the structures of OGs and some of their biological activities, probing into the structure-activity relationships.

1. Isolation and Structures of OGs

As the results of our continuing study on the chemical constituents of leguminous plants, we have so far obtained over 191 OGs from 40 plants. Among them, 73 OGs are new glycosides, and 21 are those of novel sapogenols. Many OGs have been isolated from the leguminous plants, and therefore, they seemed to be ubiquitous ingredients in the family. However, their distribution is localized to Faboideae in Leguminosae, *i.e.*, Fabaceae in the present botanical taxonomy.

These saponins have two common features: one, a methyl group at C-17 of the sapogenol; the other, glucuronic acid linked at C-3. Further, most of them possess some oxgen functional groups in the E-ring.

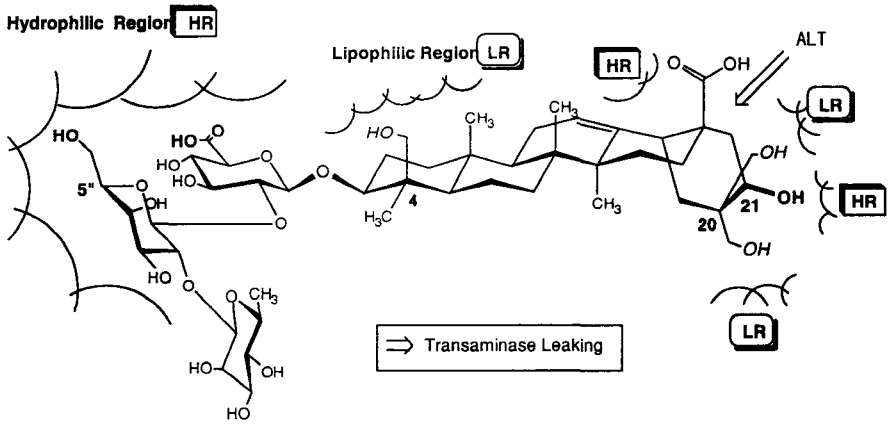


2. Hepatoprotective Effects and Structure-Activity Relationships [3]

In the course of our study on hepatoprotective drugs, we devised the conditions for an *in vitro* assay method using immunologically induced liver injury on primary cultured rat hepatocytes and confirmed hepatoprotective actions of more than 40 OGs and the related compounds. Structure-activity relationships for the sapogenol moiety suggested that the β-hydroxy group at C-21 would enhance hepatoprotective activity; on the contrary, the hydroxy group at C-23, 24, 29 and 30 could reduce the activity. The free carboxylic acid group at C-17 would mediate cytotoxicity toward liver cells.

The structure-hepatoprotective relationships of the sugar moiety suggested that glucuronic acid linked at C-3 of the sapogenol is a crucial unit in exhibiting hepatoprotective activity. In the case of a disaccharide chain bound at C-3, an hydroxymethyl group at C-5" seems to enhance the activity. The terminal sugar unit seems not to give influence on the activity.

The mechanism of our immunological liver injury is regarded as being caused by complement-mediated cell damage. OGs could recognize the hepatocyte membrane, that is, the lipophilic region (LR) around at C-4, C-20; hydrophilic region (HR) around at C-21, C-5". Therefore, OGs seem to prevent the complement system from injuring the hepatocyte membrane. In view of its strong affinity toward hepatocyte membranes, free carboxylic acid at C-17 might induce transaminase (ALT) leaking, *i.e.*, hepatotoxicity.



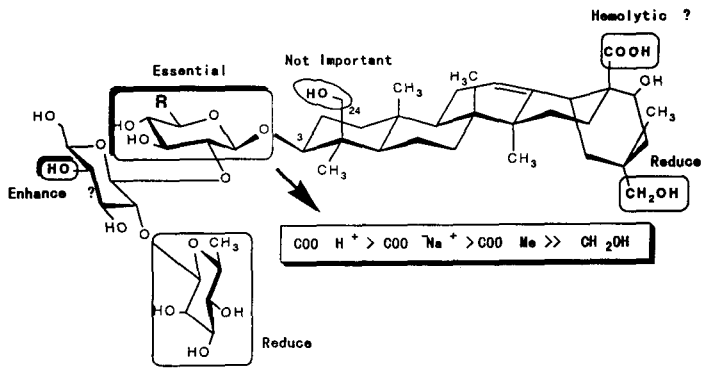
3. Anticomplementary Activity and Structure-Activity Relationships [5]

As described in the former section, the mechanism of our immunological liver injury is regarded as being caused by complement-mediated cell damage. The complement system is a humoral effector of inflammation which is activated by a cascade mechanism through the classical and/or alternative pathway [3]. Activation of the system is normally beneficial for the host. However, excessive activation may evoke pathological reaction in a variety of immunological and degenerative diseases and hyperacute rejection in transplantation. Therefore, the modulation of complement activity should be useful in the therapy of inflammatory diseases.

Since OGs were known to have not only anticomplementary but also antinephritic activities, we tested some OGs toward the classical pathway. Monoglucuronides and diglycosides were most potent then followed by triglycosides, whereas the aglycones exhibited increase in hemolysis. These results indicate that the glucuronic acid moiety is important for exhibition of the anticomplementary activity. The anticomplementary activity of the OGs

having a free glucuronic acid was more potent than that of its sodium salt or methylester. Furthermore, reduction of the glucuronic acid moiety decreased significantly their activity. The free acid form of the glucuronic acid moiety seemed to contribute to the potency. The hydroxy group at C-24 did not affect the anticomplementary activity except for the methylester forms.

Some inflammations, including hepatitis and nephritis, are caused by excessive immunoreaction. Therefore, it might be possible that OGs in the edible fabaceous plants play an important role for suppression of a kind of inflammation.



Acknowledgement

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