Development of Anti-arthritic Therapeutics from Saponins

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Although steroidal- or non-steroidal anti-inflammatory drugs are usually selected for treatment of arthritic disease, patients with this disease very often depends on the oriental medicinal drugs or folkloric medicinal herbs mainly to avoid the adverse effect of the chemical therapeutics due to long-term administration.

We have isolated many saponins from Kalopanacis Cortex, Rubi Fructus, Akebiae Caulis, the herb of *Pleurospermum kamtschaticum*, and the leaves of *Acanthopanax chiisanensis* by chromatographic methods and investigated the pharmacological action mainly on antinociceptive and antiiflammatory activity using animals to develop anti-arthritic therapeutics from saponin-containing herbal drugs. The isolated compounds were shown in Fig. 1-5.

The extracts of those plant materials, their fractions, the isolated saponins and sapogenins obtained by hydrolysis of the saponins were subjected to antinociceptive activity tests by the writhing-, hot plate- and tail-flicks methods using mice and to anti-inflammatory activity test by carrageenan or Freund's complete adjuvant reagent (FCA) using rats. (1,2) Many saponins tested displayed significant antinociceptive and antiifnammatory activities possibly due to the active moiety, sapogenins.

Kalopanaxsaponins isolated from the stem bark of *Kalopanax pictus* were hydrolyzed by human intestinal bacteria to yield many prosapogenins and finally to hederagenin. This compound, which was fully hydrolyzed from kalopanaxsaponins by the bacteria, (3) exhibited prominent antinociceptive and anti-inflammatory activities indicating that kalopanaxsaponins are pro-drugs for hederagenin. Preteatment of rats with hederagenin inhibited the edema caused by carrageenan or FCA reagent together with ROS generating enzyme activities of a hepatic drug metabolizing system. (1,2)

Five saponins isolated from Akebiae Radix were identified as the glycosides of oleanolic acid or hederagenin. (4) Hederagenin showed more significant effects as assessed by antinociceptive and anti-inflammatory assays in mice and rats. The antinociceptive effects were shown in Table 2. Although kalopanaxsaponin A of the five saponins produced inhibition of apoptosis and NO-, PGE_2- and TNF-α formations in the cell via the inhibition of genetic expression, (5) the sapogenin,

hederagenin exhibited more pronounced antinociceptive and anti-inflammatory effects than the saponins, kalopanaxsaponin A, when orally treated. (6) These results suggest that true active compounds of the saponins should be the sapogenins.

We have isolated the triterpenoid glycosides with the sapogenin 19α-hydroxyursane-type triterpenes from the unripe fruits of *Rubus coreanus* (7) and the roots of *Rosa rugosa*, (8) which are both Rosaceae plants and showed the bioactivity similar to kalopanaxsaponins. The triterpenoids, euscaphic acid, tormentic acid, and 23-hydroxytormentic acid and their glycosides were isolated from those Rosaceae plants and these were subjected to in vivo antinociceptive and anti-inflammatory tests. The 19α-hydroxyursane-tye triterpenoids isolated exhibited more potent activities than the 28-O-glucosides of the isolates. (7,8) Since the sapogenins produced potent or weak bioactivities, it was assumed that the crude saponin would be available for the commercial or clinical uses rather than the isolated saponins. Niga-ichigoside F_1 and 23-hydroxytormentic acid reduced gastric lesion caused by hydrochloric acid-ethanol, suggesting that the saponins from Rosacease plants may substitute cyclooxygenase-2 inhibitors with adverse effects such as gastric disease. Saponins may be available for anti-inflammatory therapeutics that do not cause gastric lesion by their long-term administration.

We also isolated a large amount of a saponin, chiisanoside, from the leaves of Acanthopanax chiisanensis and then prepared a sapogenin, chiisanogenin. The latter compound showed a more pronounced antinociceptive/anti-inflammatory effect than the former. (9) In the carrageenan-induced edema test on saponins and sapogenins, the bioactive substances inhibited xanthine oxidase and cytochrome P450 activities and increased superoxide dismutase, catalase and glutahione peroxidase activities. The compounds also reduced serum hydroxyl radical concentration. These results suggest that triterpenoids produce anti-inflammatory response via the reduction of reactive oxygen species (ROS). Rheumatoid arthritis systemically exerts the increase of ROS level in serum and causes the damage of arthritic tissue, suggesting that the inhibition of lipid peroxidation is the mechnism of the therapy of this disease.

In addition, the saponins, buddlejasaponin IV and buddlejasaponin IVa, from *Pleurospermum kamtschaticum* and their sapogenin, saikogenin A and H, were used for the in vitro anti-inflammatory activity tests in LPS-activated macrophage 264.7 cells. Buddlejasaponin IV more potentially inhibited NO, PGE_2 and TNF-α production in the cell than other compounds. (10) Saikogenin A and -H produced a very low viability in the effective concentration. In addition, acid hydrolysate of the crude saponin, which may be composed of genuine or artifact sapogenins, exhibited a potent inhibition in NO production with relatively higher cellular viability, indicating that the hydrolysate may be an available potent NO inhibitory substance. These results also suggest that the saponin extract from *P. kamtschaticum* displays the bioactivity in more complicated fashion due to many substances possibly producible in the biosystem.

By our continuous efforts on the bioactivity of saponins, it was found that a variety of natural saponins showed antinociceptive and anti-inflammatory effects regardless of the activity potency. It was also revealed that the saponins should be transformed to substantially active sapogenins. Therefore, crude saponins or their hydrolysates could be available for functional foods, nutraceuticals or therapeutics for treatment of rheumatoid arthritis disease. These efforts may create many available saponin mixtures using saponin- containing crude drugs. Clinical tests could lead the increase of the availability of these saponins for the industrialization.

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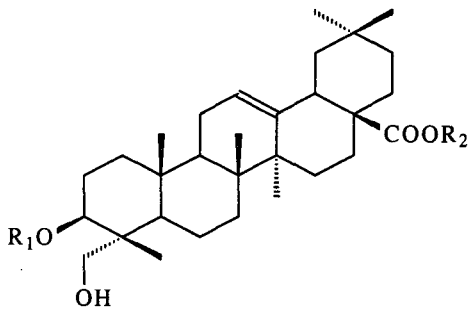
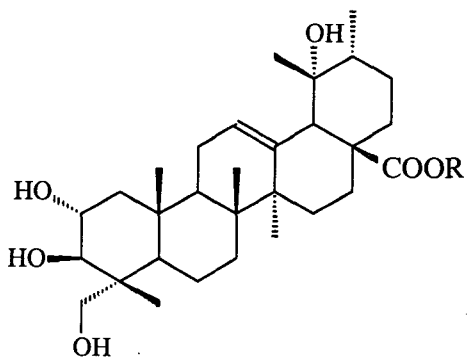


1: R_1=α-L-rha(*p*) (1→2)-α-L-ara(*p*); R_2=α-L-rha(*p*) (1→2)-β-D-glc (1→6)-β-D-glc(*p*)
2: R_1=β-D-xyl(*p*) (1→3)-α-L-rha(*p*) (1→2)-α-L-ara(*p*); R_2=α-L-rha(*p*) (1→2)-β-D-glc(*p*) (1→6)-β-D-glc(*p*)
3: R_1=β-D-glc(*p*) (1→4)-β-D-xyl(*p*) (1→3)-α-L-rha(*p*) (1→2)-α-L-ara(*p*); R_2=α-L-rha(*p*) (1→2)-β-D-glc(*p*) (1→6)-β-D-glc(*p*)
4: R_1=α-L-rha(*p*); R_2=H
5: R_1=α-L-rha(*p*) (1→2)-α-L-ara(*p*); R_2=H
6: R_1=β-D-xyl(*p*) (1→3)-α-L-rha(*p*) (1→2)-α-L-ara(*p*); R_2=H
7: R_1=β-D-glc(*p*) (1→4)-β-D-xyl(*p*) (1→3)-α-L-rha(*p*) (1→2)-α-L-ara(*p*); R_2=H

Fig. 1. Structures of sapnins isolated from the stem bark of *K. pictus*



1: Niga-ichigoside F_1 (R=Glc)
1a: 23-Hydroxytormentic acid (R=H)
Fig. 2. The structure of compounds **1** and **1a** isolated from *R. coreanus*.

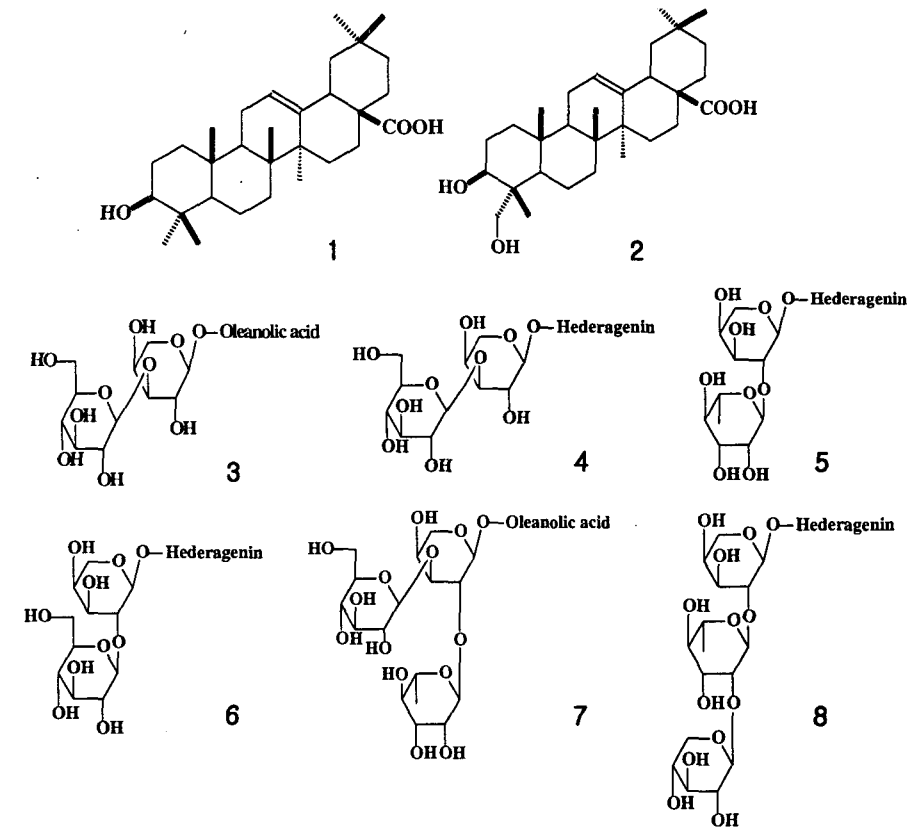


Fig. 3. Structures of triterpenes (**1–2**) and triterpenoid saponins (**3-7**) isolated from *A. quinata* and a reference compound (**8**)

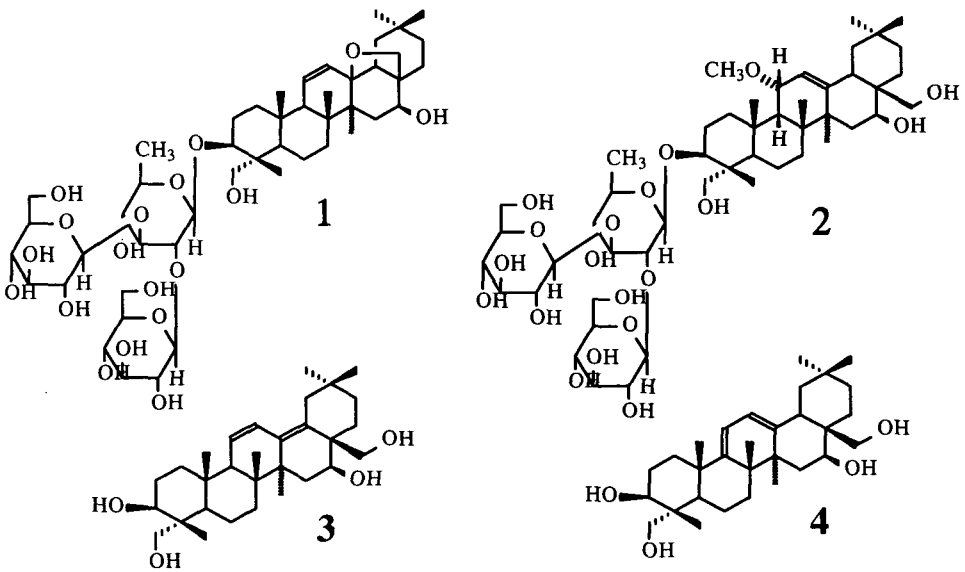


Fig. 4. Structure of compounds **1-4** obtained from *P. kamtschaticum*