

Synthesis and Antiinflammatory Effects of a New Tricyclic Diterpene and Its Analogues as Potent COX-2 Inhibitors

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Abstract

The cyclooxygenase enzymes catalyze the oxidative conversion of arachidonic acid into prostaglandin H₂ which mediates both beneficial and pathological effects. The COX-1 is constitutively expressed in most tissues and in blood platelets whereas the expression of COX-2 isoform is induced in response to inflammatory stimuli such as cytokines. Thus the identification of a novel COX-2 selective inhibitor should offer excellent antiinflammatory activity with minimal side effects such as gastrointestinal toxicity.

Recently, a group of structurally unique and biologically active pimarane diterpenoids has been isolated from indigenous Korean medicinal plants. These new diterpenoids turned out to be potential analgesic and antiinflammatory agent due to their potent inhibitory activities of prostaglandin synthesis. We have also found that the inhibition of PGE₂ synthesis is attributed to the potent COX inhibition by pimarane diterpenoid in arachidonic acid cascade. In conjunction with development of new analgesic and nonsteroidal antiinflammatory agent, a series of works on these diterpenoids have been extensively carried out in our laboratories. These efforts involve the structure-activity relationship of pimaradienoic acid, molecular modelings and COX inhibitory activities as well as antiinflammatory effects of its structural analogues. In addition, the total syntheses of the new natural pimarane

diterpenoids, their stereoisomers and other structural variants were intensively investigated.

1. Mechanistic and molecular modeling studies of acanthoic acid as a novel bioactive component of *Acanthopanax koreanum*

As we anticipated, *in vitro* purified enzyme assay of the natural acanthoic acid revealed the COX-2 inhibitory activities. Moreover, we disclosed that the COX-2 inhibitory activity of acanthoic acid is possibly attributed to the interaction of the carboxylic group of acanthoic acid with arginine120 and tyrosine355 of the COX-2 active site. The docking study of acanthoic acid of the COX-2 active sites shows the hydrogen bonding of carboxyl group with phenolic hydrogen of tyrosine355 and guanidine of arginine120. Other hydrophobic part of acanthoic acid seems to be well fit to the hydrophobic channel of the active site..

2. Structure-activity relationship of natural acanthoic(pimaradienoic) acid.

For the studies on structure-activity relationship of pimaradienoic (acanthoic) acid, sixty structural analogues or key skeletons of pimaradienoic acid have been synthesized and their inhibitory activities of PGE₂ synthesis have been examined. Consequently, the congener of pimaradienoic acid which is responsible for the potent antiinflammatory effect has been elucidated. Moreover, the structure-activity relationship envisioned the desired structure for the enhanced COX-2 inhibitory activities and antiinflammatory effect. The details for SAR and structural variation of pimaradienoic acid will be discussed.

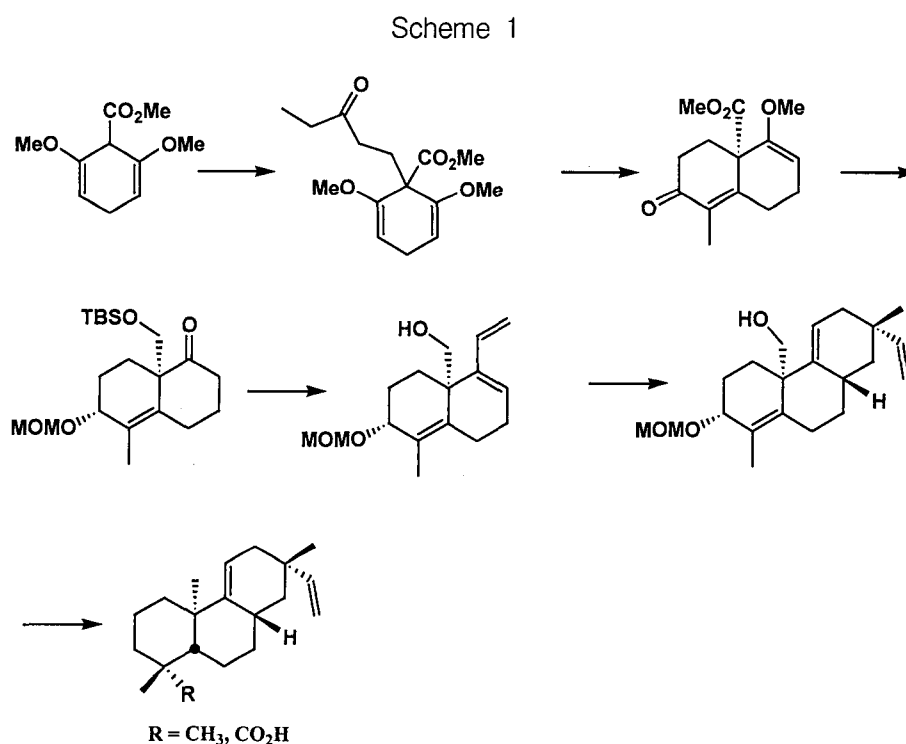
3. Studies on the COX inhibitory activities and antiinflammatory effects of pimaradienoic acid and its analogues.

The structural analogues of pimaradienoic acid designed on the basis of the structure-activity relationship have been tested for COX-2 inhibitory activities as well as antiinflammatory effects. Several analogues have shown to be highly potent COX-2 inhibitor. Their COX-2 inhibitory activities are comparable to that of NS-398. Two of eleven analogues selected on the

basis of COX inhibitory activities have shown excellent antiinflammatory effect in animal model.

4. Stereoselective synthesis of the natural pimaradienoic acid.

We have developed a new stereoselective synthetic route to the natural pimaradienoic acid for the supplement of parent compound which would be derivatized diversely. Scheme 1 summarize our synthesis. The synthesis was commenced by preparation of A,B-ring moiety which unusually possesses angular hydroxymethyl group. Methyl ester derived from 2,6-dimethoxybenzoic acid was alkylated for hydroxypentanyl side chain as a cyclization precursor. Robinson annelation of the resulting 1,5-diketone afforded the bicyclooctenone system.



After the diene moiety was introduced to the B-ring system, stereoselective

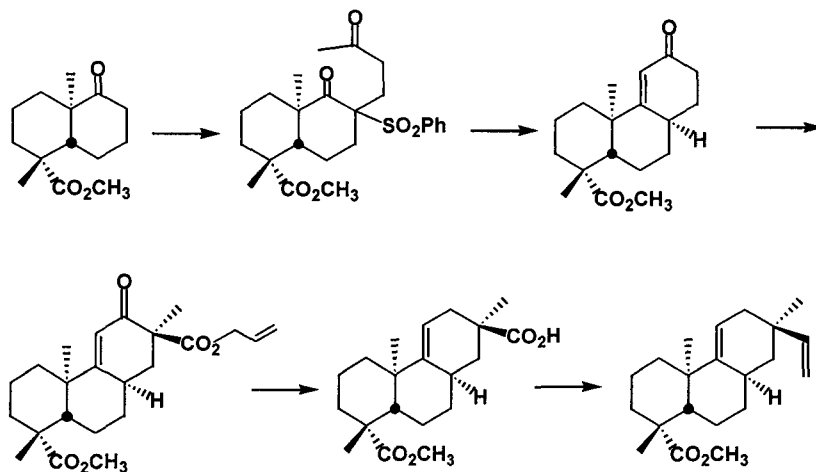
introduction of C-ring moiety was successfully achieved by intramolecular Diels-Alder reaction which provides two chiral centers and one olefin with correct stereo and regiochemistry through single reaction step. The vinyl functionality of the quaternary carbon center was obtained by standard Wittig reaction to give the second key intermediate which is utilized for the synthesis of natural product and its derivatives. The synthetic intermediates including A,B-ring and B,C-ring moieties and derivatives of the natural pimaradienoic acid have been tested for the inhibitory activities of PGE₂ synthesis.

5. Synthesis of methyl 8,13-epi-pimara-9(11),15-dien-19-oate as a stereoisomer of natural pimaradienoic (acanthoic) acid

Since we reported isolation and bioactivities of natural pimaradienoic acid, we have been interested in synthesis of its stereoisomer for the detailed structure-activity relationship.

The total synthesis of C-8 and C-13 epimer is outlined in Scheme 2. Introduction of C-ring to the A,B-ring moiety was achieved by combination of Michael-Aldol reaction. Intramolecular aldol condensation of the 1,4-diketone introduces C-ring moiety as a cyclic enone system. Consecutive allyloxycarbonylation and methylation of the cyclic enone affords quaternary carbon center in C-ring which has an opposite configuration of natural product. For the completion of the synthesis, the carbonyl group of enone system was removed by standard Barton procedure then the allyl ester was deprotected to give the free acid. Finally, conversion of acid to aldehyde followed by Wittig olefination provided methyl 8,13-epi-pimara-9(11),15-dien-19-oate as a stereoisomer of the natural diterpenoid.

Scheme 2



REFERENCES

1. Kim, Y. H., Chung, B. S. and Sankawa, U. *J. Nat. Prod.* **1998**, *51*, 1080.
2. Suh, Y. G., Jun, R. O., Jung, J. K. and Ryu, J. S. *Synth. Commun.* **1997**, *27*, 587.
3. Suh, Y. G., Kim, H. S., Kumar, P. R., Choi, N. S. and Jung, J. K. *Tetrahedron*, **1998**, *54*, 15161