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Studies on Triterpenoid Corticomimetics

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Abstracts—It was our working hypothesis that introduction of 11-keto groups to 12-oleanene/ursene series of triterpenoids should endow them with corticoid-like activities, since pharmacological actions of glycyrrhetinic acid (GA) are known to be caused by inhibition on corticoid-Δ^4-reductase. 11-Keto-triterpenoids derived artificially in these studies, such as 11, 19-diketo-18, 19-secoursolic acid methyl ester(I), 11-keto-β-boswellic acid derivatives (IIa-IIc), 11-keto-presenegenin dimethyl ester (III), 11-keto-oleanolic acid derivatives (IVa-IVd) and 11-keto-hederagenin (V) possess the fundamental functions of α, β-unsaturated ketone on C-11 and hydroxyl group on C-3, as like GA (VI). Additionally, they involve the carboxyl groups on rings A (II, III), D (I, III, IV, V) and E (VI), and the hydroxyl groups on rings A (III, V) and C (III). All the compounds competitively inhibited corticoid-5β-reductase, and the highest inhibitory potency appeared in I. All of them except 3, 11-diketo-β-boswellic acid methyl ester (IIc) were more effective about five times to twice than GA. On carrageenin-induced edema test, compounds I and IVa-IVd showed anti-inflammatory activities, but III enhanced rather edema. Structure-activity relations were found in the aspects of hydrophilicity of ring A and hydrophobicity of rings C/D. The more they were hydrophilic in ring A and hydrophobic in rings C/D, the more they inhibited the enzyme. And the more they were hydrophobic in rings C/D, the more they exhibited antiiflammatory activities. However, the increased hydrophilicity in ring A resulted in increasing edema, probably due to a nonspecific inhibition on aldosterone-5β-reductase.

Keywords—Corticoid-like activities · pomolic acid · oleanolic acid · β-boswellic acid · hederagenin · presenegenin · 11-keto-triterpenoids · corticoid-5β-reductase · enzyme inhibition · antiinflammatory activity · E-ring opening of triterpenoid

Triterpenoids are widely distributed in plants. They have relatively complex pentacyclic and tetracyclic structures, most being either alcohols, aldehydes or carboxylic acids. Many kinds of oleanene/ursene triterpenoids possess alkenyl linkages at carbon atom 12, but not 11-keto groups. Exceptionally, glycyrrhetinic acid (GA) from licorice involves 11-keto-Δ^{12}-system. It has been stated that GA strongly inhibits Δ^4-reductase for 3-keto-Δ^4-steroids, and that the 11-keto-Δ^{12}-system of GA is essential in order to be a good inhibitor on the enzyme.[1,2] Pharmacological properties of GA such as anti-inflammatory, anti-ulcer and anti-allergic actions have been assumed to be caused by the inhibition of reductive catabolism of corticoids in liver which might result in delaying the clearance of corticoids.[1]

Baran and his co-workers has prepared a series of modified derivatives of GA, such as

3-and/or 2-oxygenated and 11-deoxo compounds, in the purpose of separating the property of sodium ion retention from other potentially useful medicinal properties.[3,4] Recently, Shibata has done a similar approach to the subject and prepared some derivatives of GA by elimination of its 11-keto group and replacement of its 20-carboxylic acid with carbinol.[5] However, the 11-deoxo derivatives of GA were turned to lose the main actions of GA. Kumagai and his coworkers demonstrated that GA inhibited Δ^4-5β-reductase of corticoids more effectively than Δ^4-5α-reductase, and that 11-keto group of GA was not only essential for the inhibition on the enzymes but also 3β-hydroxyl group of ring A was important.[1]

However, the effects of alteration of carboxy group and appearances of another functional groups in other carbon positions on corticoid-5β-reductase have not studied. Introduction of 11-keto groups to 12-oleanene/ursene series of triterpenoids could endow them with corticoid-like activities, because the artificially derived 11-keto-triterpenoids become to contain the fundamental functions of α,β-unsaturated ketone in their C-rings, which will be competitive with 3-keto-Δ^4-system of corticoids at active site of corticoid-5β-reductase. And we also assumed that the artificial 11-keto-triterpenoids may exhibit anti-inflammatory activities.

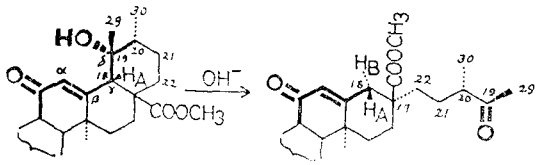
We chose oleanolic acid, hederagenin, β-boswellic acid, pomolic acid and presenegenin, which involved one or two carboxyl groups at different carbon position from carbon atom 20 of GA. And some of them possess additional hydroxyl group near to C-3 hydroxyl group or C-12 alkenyl group. In this paper, we describe the synthesis of artificially-derived 11-keto-triterpenoids, their inhibitory activities on corticoid-5β-reductase, their anti-inflammatory activities, and finally a structure-activity relationship.

The structures of 11-keto-triterpenoids synthesized for these studies are shown in Chart 1.

1. Synthesis of 11-Keto-triterpenoids

We obtained the 11-keto-triterpenoids such as 11, 19-diketo-18, 19-diketo-18, 19-secoursolic acid methyl ester (I) (from pomolic acid), 11-keto-β-boswellic acid (IIa), 11-ketopresenegenin dimethyl ester (III), 11-keto-hederagenin (V) through serial reactions of acetylation, methylation, CrO_3/HAc oxidation and then deacetylation of each triterpenoid. 3, 11-Diketo-triterpenoids such as 3, 11-diketo-β-boswellic acid methyl ester (IIc) and 3, 11-diketo-oleanolic acid (IVc) were also prepared by CrO_3/pyridine oxidation of their 11-keto-derivatives.[6-11]

Pomolic acid was obtained by HIO_4 oxidation of Ziyu-glycosides isolated from Sanguisorbae Radix. Mild alkaline hydrolysis of 11-keto-acetylpomolic acid methyl ester synthesized first induced E-ring opening to yield a 18, 19-seco-compound, and further hydrolysis of it advanced deacetylation to give I. The E-ring opening was thought to be due to retrograde aldol reaction in a system with both functional groups of α,β-unsaturated ketone and δ-hydroxyl group, of which r-δ bond was cleaved. To the best of our knowledge, this E-ring opening is the first finding in the triterpenoid chemistry.[9]



It is well known that boswellic acid (BA) mainly occurs as the acetate in olibanum from *Boswellia carterii*, and β-BA (ursene type) is predominant and is accompanied by small amounts of α-BA (oleanene type). Recently, Indian workers reported that acetyl-11-keto-β-BA and 11-keto-β-BA were isolated from *B. serrata*, but they did not mention the co-ocurrence of the α-compounds. Thus, we also isolated four triterpenoids from olibanum. By their spectral data

and GC/MS analysis, it was found that acetyl-BA and BA were turned to be the mixture of α and β with the ratio of 1 to 2 and 1 to 4, respectively, and that acetyl-11-keto-BA and 11-keto-BA were naturally occurred as all β-forms in olibanum. And it was also found that, among acetyl-11-keto-BA or 11-keto-BA which was synthetically derived from BA and acetyl-BA, only β-form was each crystallized from the mixtures of α and β. Therefore, we prepared three kinds of 11-keto-β-derivatives, i.e., 11-keto-β-boswellic acid (IIa), its methyl ester (IIb) and 3, 11-diketo-β-boswellic acid methyl ester (IIc).[8]

Presenegenin was obtained by HIO_4 oxidation of tenuifolin which was a prosapogenin of Polygalae radix saponins. Oxidation of presenegenin dimethyl ester triacetate with chromium trioxide in acetic acid gave two major compounds, 11-ketone and 12-ketone derivatives. On mild alkaline hydrolysis, the former affored 11-keto-persenegenin dimethyl ester (III), whereas the latter did 12-keto-presenegenin dimethyl ester-12, 27-hemiketal.[10]

11-Keto-oleanolic acid (IVa) and its methyl ester (IVb), and 11-keto-hederagenin were known in literatures, although their synthetical processes have been modified in some extent.[6] Oleanolic acid and hederagenin were isolated from *Eugenia caryophilata* and *Kalopanax pictum* var. *typicum*, respectively. 3-Keto-oleanolic acid was obtained by CrO_3/pyridine oxidation, and from it, 3, 11-diketo-oleanolic acid (IVc) was made by CrO_3/HAc oxidation. The lactone (IVd) was obtained as a by-product.[7]

2. Inhibitory Activities of 11-Keto-triterpenoids on Corticoid-5β-reductase

The enzyme activity was assayed by observing the decrease in absorbance at 240 nm which occurred when the Δ^4-3-ketone of hydrocortisone as a substrate was reduced, using NADPH as a coenzyme. The measurements were made on methylene chloride extracts after the enzyme reactions were alkalized with 5% KOH. The alkaline treatment was carried out in order to eliminate the effects of the artificial inhibitors from reaction mixtures, because they showed some great interferences due to their absorption at 240 nm when the extraction with dichloro methane.[6]

Table I. Inhibitory effects of 11-oxo-derivatives

Inhibitors([I]=[S]=0.2 mM)	Inhibition rate(%)
11-Oxo-oleanolic acid (IVa)	54
11-Oxo-hederagenin (V)	43
Glycyrrhetinic acid (VI)	23
Oleanolic acid	0
Oleanolic acid acetate	0

The effects of 11-keto-oleanolic acid (IVa) and 11-keto-hederagenin (V) on corticoid-5β-reductase were compared with that of GA (VI), as shown in Table I. The inhibitory activities of IVa and V were more stronger than that of GA. Oleanolic acid and its acetate showed no inhibition on the enzyme.[6]

To determine Ki values of the 11-keto compounds, the maximum concentration of the substrate hydrocortisone in reaction mixtures was

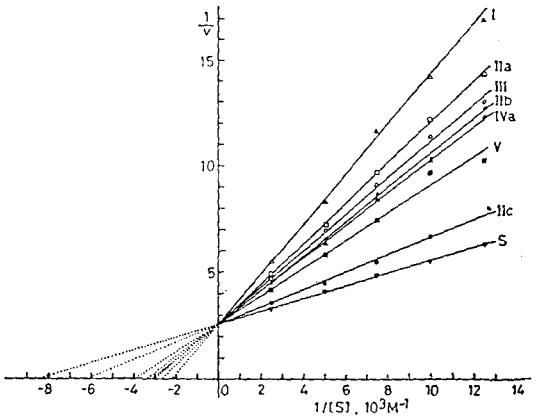


Fig. 1. Competitive inhibitions of some artificially derived 11-keto-triterpenoids on corticoid-5β-reductase. The maximum concentration of the substrate (S), hydrocortisone was made to 0.4 mM, and it was serially diluted to 2, 3, 4 and 5 times. The concentrations of inhibitors were given to 0.2 mM.

Table II. Corticomimetic activities of 11-keto-triterpenoids

Compounds	Ki (10^{-4}M)	IC_{50} (mM)
11, 19-Diketo-18, 19-secoursolic acid methyl ester(I)	0.69	0.18
11-Keto-β-boswellic acid(IIa)	0.95	0.29
11-Keto-presenegenin dimethyl ester(III)	1.14	0.37
11-Keto-oleanolic acid methyl ester(IVb)	1.18	0.42
11-Keto-β-boswellic acid methyl ester(IIb)	1.19	0.42
3, 11-Diketo-oleanolic acid(IVc)	1.24	0.30
11-Keto-oleanolic acid(IVa)	1.36	0.32
11-Keto-hederagenin(V)	1.78	0.52
Glycyrrhetinic acid(VI)	3.32	0.97
3, 11-Diketo-β-boswellic acid methyl ester(IIc)	5.88	1.30

made to 0.4 mM, it was serially diluted to 2, 3, 4 and 5 times, and the concentration of inhibitor was given to 0.2 mM. After performance of enzyme reactions, the results were plotted by Lineweaver-Burk equation (Fig. 1), and Ki values determined from the curves are summarized in the order to their inhibitory activities[6,7,11] in Table II. All the 11-keto-triterpenoids competitively inhibited the enzyme, as shown in Fig. 1.

For determination of 50% inhibition concentration (IC_{50}), the substrate concentration was given to 0.2 mM, and the inhibitors' concentrations were varied. Linearization of inhibition percent was achieved on a probit plot or a logit plot[11]. Values for IC_{50} are summarized in TableII.

The highest inhibitory potency appears in 11, 19-diketo-18, 19-secoursolic acid methyl ester(I), which is 4-ring membered. All the compounds except 3, 11-diketo-β-boswellic acid methyl ester (IIc) are more effective about five times to twice than GA (VI).

3. Anti-inflammatory Activities of 11-Keto-triterpenoids

The anti-inflammatory activities of the artificially derived 11-keto-triterpenoids were investigated, utilizing carrageenin-induced edema test in rat hind paw. Compounds were subcutaneously or orally given twice at doses of 10, 30 and 100 mg/kg body weight, 6 and 0.5 hours before carrageenin injection. Edema volumes were determined every one hour from after carrageenin injection. Parallelled experiments were conducted with hydrocortisone as a reference, at a dose of 30 mg/kg body weight. Data on average three hours after the treatment of carrageenin were compared with that of hydrocortisone, and relative activities of 11-keto-derivatives for hydrocortisone are summarized in Table III.[7,11]

Table III. Relative anti-inflammatory activities of some artificial 11-keto-triterpenoids

Compounds	Relative Activities
Hydrocortisone	1.0
11-Oxo-oleanolic acid methyl ester(IVb)	2.5
3, 11-Dioxo-oleanolic acid(IVc)	1.5
11-Oxo-oleanolic acid(IVa)	1.0
3, 11-Dioxo-oleanolic acid lactone(IVd)	1.0
11, 19-Diketo-18, 19-secoursolic acid methyl ester(I)	0.3
11-Oxo-boswellic acid(IIa)	0
11-Oxo-boswellic acid methyl ester(IIb)	0
11-Oxo-presenegenin dimethyl ester(III)	—*

* Rather increase edema volume

All the 11-keto-oleanolic acid derivatives exhibited strong anti-inflammatory activities, and among them 11-keto-oleanolic acid methyl ester (IVb) was the most potent. However, 11, 19-diketo-18, 19-secoursolic acid methyl ester (I) which showed the most potent inhibitory activity on the enzyme, exhibited less potent anti-inflammatory activity than hydrocortisone. Moreover, all the 11-keto-β-boswellic acid derivatives revealed no potency, and 11-keto-presenegenin dimethyl ester(III) rather increased edema.

4. Structure-activity Relationships

By introducing keto groups on carbon atom 11 of pentacyclic triterpenoids such as oleanene/ur-

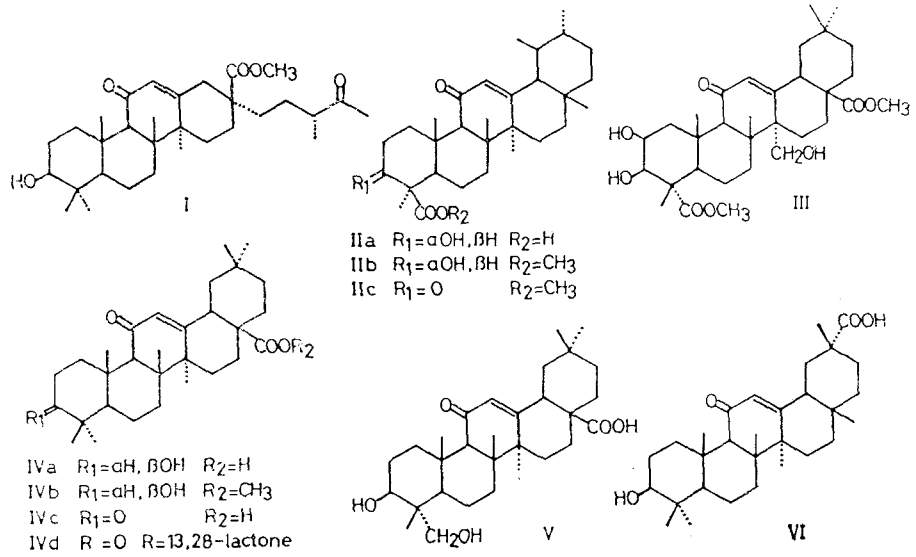


Chart 1

sene, we have synthesized the artificially derived 11-keto-triterpenoids, and have investigated their inhibitory activities on corticoid-5β-reductase and their anti-inflammatory activities. As shown in Chart 1, all the compounds fundamentally possess the 11-keto-Δ^{12}-systems and oxygen functions on C_3. Additionally, they contain the carboxyl groups on rings A (II, III), D (I, III, IV, V) and E (VI), and the hydroxyl functions on rings A (III, V) and C (III).

Inhibitory actions of the 11-keto-triterpenoids on corticoid-5β-reductase are arranged in the order of Ki values (Table II). Structure-activity relations are found in the aspects of hydrophilicity of ring A and hydrophobicity of rings C/D. In case of 11-keto-β-boswellic acid derivatives, the hydrophilicity of ring A decreases in the order of IIa, IIb and IIc, and the decrease incurs low activity. This tendency is also found in III, V and VI. That is, the increase of the hydrophilicity of ring A enhances the inhibitory activity on the enyme. Exceptionally, compound I exhibits the strongest inhibitory activity. This may be due to more exposure of 11-keto-Δ^{12}-system on the molecule of I by the opening of its ring E.

On the other hand, the decrease of hydrophobicity on rings C/D tends to lower the activity. The hydrophobicity of rings C/D decreases in the order of I, IVb and IVa. That is, the increase of hydrophilicity tends to lower the activity.

It is possible to postulate that the more stronger inhibitors on corticoid-5β-reductase may be the better anti-inflammatory agents. By a rough estimation, the relative anti-inflammatory activities of the 11-keto-compounds are compared with that of hydrocortisone as summarized in Table III. All the 11-keto-oleanolic acid derivatives exhibited strong anti-inflammatory activities. The order of their activities well coincides with that of their inhibitory actions on the enzyme.

However, 11, 19-diketo-18, 19-secoursolic acid methyl ester (I) which showed the most potent inhibition on the enzyme, exhibited less potent anti-inflammatory activity than hydrocortisone. Moreover, all the 11-keto-β-boswellic acid derivatives(II) revealed no potency, and 11-ketopresenegenin dimethyl ester (III) rather increased edema; hydrophilicity of their A-rings enhance the inhibitory activities on corticoid-5β-reductase *in vitro*, but it caused to edema *in vivo*. This might result in affecting aldosterone catabolism.

Final conclusion for the triterpenoid corticomimetics studies must be waited until the effects

of the artificially derived 11-keto-triterpenoids on the metabolism of aldosterone and others Δ^4-3-keto steroids will be tested. But we could confirm that both of α, β-unsaturated ketone on C_{11} and hydroxyl group on C_3 play critical roles for their corticoid-like activities.

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