Arch. Pharm. Res. 8(4), 237~242 (1985)

Studies on Triterpenoid Corticomimetics (VI)

Anti-inflammatory Activities of 11-Keto-derivatives of Pomolic Acid, β-Boswellic Acid and Presenegenin

Byung Hoon Han, Yong Nam Han, Eun Tae Park*,
Kyung Mi Kim* and Tae Hee Kim*

Natural Products Research Institute, Seoul National University, Seoul 110, Korea
**College of Pharmacy, Sookmyung Women's University, Seoul 151, Korea*

(Received September 16, 1985)

Abstract □ 11-Keto-derivatives of pomolic acid, β-boswellic acid and presenegenin were compared with those of oleanolic acid, hederagenin and glycyrrhetinic acid in respects of inhibitions on corticoid-5β-reductase and anti-inflammatory activities. Hydrophilicity of ring A and hydrophobicity of rings C/D enhanced the inhibition on the enzyme. However, the former induced edema and the latter caused to exhibit anti-inflammatory activity.

Keywords □ 11, 19-diketo-18, 19-secoursolic acid, 11-keto-β-boswellic acid, 11-ketopresenegenin, inhibition on corticoid-5β-reductase, corticoid-5β-reductase, anti-inflammatory agents.

Many kinds of oleanene and ursene triterpenoids contain alkenyl linkages at carbon atom 12, but not 11-keto group. Exceptionally, glycyrrhetinic acid (GA) from licorice involves 11-keto-Δ^{12}-system. It has been stated that GA strongly inhibits Δ^4-reductase of 3-keto-Δ^4-steroids, and 11-keto of GA is required in order to be a good inhibitor.[1,2] Pharmacological activities of GA 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.

Baran and his co-workers had 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 keto group and replacement of 20-carboxylic acid with carbinol.[5]

However, the effects of alteration of carboxyl group and presences of another functional groups on the enzyme have not studied. We have prepared some artificially derived 11-keto-triterpenoids. It has been demonstrated that 11-keto-oleanolic acid and 11-ketohederagenin strongly inhibited the enzyme more than GA,[6,7] and the former exhibited more potent anti-inflammatory activity than hydrocortisone.[7]

In the present study, 11-keto-derivatives of pomolic acid, β-boswellic acid and presenegenin were compared with those of oleanolic acid, hederagenin and GA in respects of their inhibitory activities on corticoid-5β-reductase and their anti-inflammatory activities. And structure-activity relationship is discussed.

EXPERIMENTAL METHODS

Materials

11-Keto-derivatives of pomolic acid[9], β-bos-

wellic acid[8], presenegenin[10], oleanolic acid[6,7] and hederagenin[6] were prepared as previously reported. NADPH and carrageenin (λ type) were purhased from Sigma Co.

Corticoid-5β-reductase Activity

The enzyme activity was assayed by observing the decrease in optical density at 240nm which occurred when the Δ^4-3-ketone of hydrocortisone (substrate) was reduced, as previously described[6]. The measurements were made on methylene chloride extracts after the reaction mixtures were alkalized with 5% KOH in order to eliminate the effects of artificial inhibitors.

Five hundred μl of enzyme (10,000g supernatant of rat liver homogenate) was mixed with 0.1ml of 2mM hydrocortisone, 0.1ml of 2mM inhibitor, 0.2ml of 0.1M phosphate buffer (pH 7.0) and 0.1ml of 6mM NADPH. The reaction mixtures were incubated at 37°C for 30 min, and then 0.1 ml of 5% KOH and 5 ml of dichloromethane were added. After vigorous agitation, optical density of dichloromethane layer was determined at 240nm on a UV/visible spectrophotometer (Gilford system 2,600).

Carrageenin Edema Test

Anti-inflammatory activity utilizing rat hind paw carrageenin edema test was ascertained as previously described.[6] Parallel experiments were conducted with hydrocortsione or phenylbutazone as reference. The inhibition percent of edema induced by each agent was calculated for each animal group with respect to its vehicle-treated control group.

RESULTS

Inhibitory Effects of 11-Keto-triterpenoids on Corticoid-5β-reductase

The structures of 11-keto-triterpenoids tested for inhibition on corticoid-5β-reductase are shown in Chart 1.

To determine the Ki values of the 11-keto compounds, the maximum concentration of the substrate hydrocortisone in reaction mixtures was made to 0.4mM, it was serially diluted to 2, 3, 4 and 5 times, and the concentration of

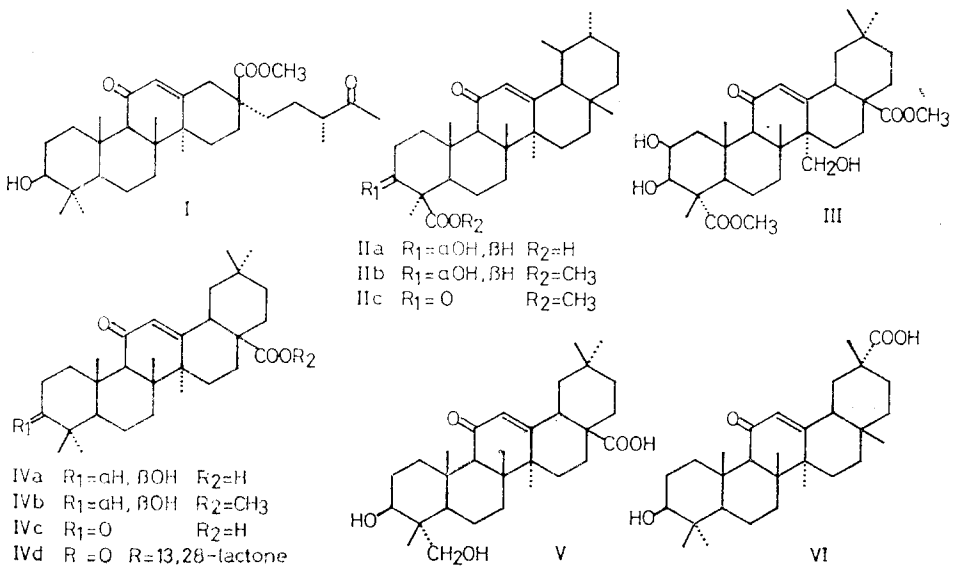

Chart 1

inhibitor was given to 0.2mM. 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. (Table I) All the 11-keto-triterpenoids competitively inhibited the enzyme, as shown in Fig. 1.

Table I. 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

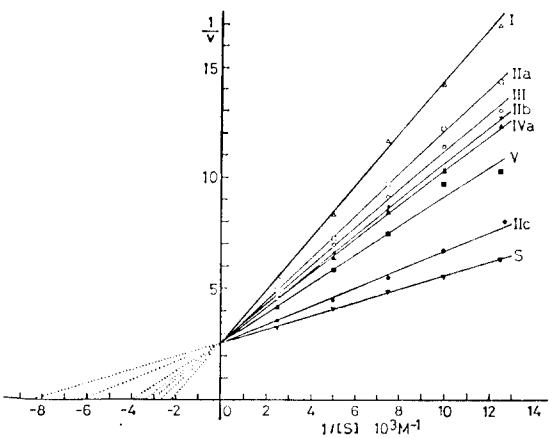

Fig. 1. Competitive inhibitions of some artificially derived 11-keto-triterpenoids on corticoid-5 β-reductase. The maximum concentration of the substrate (S), hydrocortisone was made to 0.4 mM, and it was serially diluted to 2, 3, 4 and 5 times. The concentrations of inhibitors were given to 0.2mM.

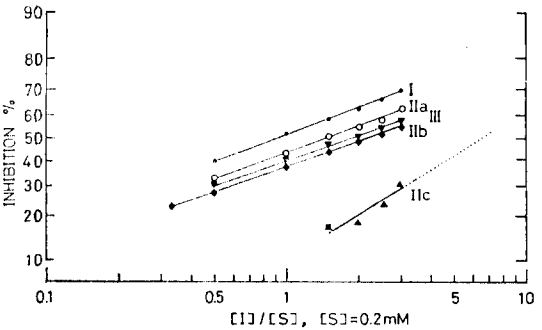

Fig. 2. Determination of 50% inhibition concentration (IC_{50}) of some 11-keto-triterperoids on corticoid-5β-reductase. The substrate concentration was given to 0.2mM, and the inhibitor concentration was changed.

For determination of 50% inhibition concentration (IC_{50}), the substrate concentration was given to 0.2mM, and the inhibitor concentrations were varied. Linearization of inhibition percent was achieved on a probit plot or a logit plot. (Fig. 2) Values for IC_{50} are summarized in Table I.

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).

The Anti-inflammatory Activities of Compounds I, IIa, IIb and III

The anti-inflammatory activities of 11, 19-diketo-18, 19-secoursolic acid methyl ester (I), 11-keto-β-boswellic acid (IIa), its methyl ester (IIb) and 11-ketopresenegenin dimethyl ester (III) were investigated, utilizing carrageenin-induced emeda test in rat hind paw, and the results were compared with that of hydrocortisone or phenylbutazone as reference. (Table II to IV)

Compounds were given twice at 6 hr and 0.5 hr before carrageenin injection, and edema volumes were determined every one hr after the last injection of compounds.

Administration of I, 100 and 33mg.kg, s.c., and hydrocortisone, 50mg/kg, s.c., showed the significant suppression of the edema volumes by 45.6, 36.8 and 66.7% on average 4 hrs after the last injection of the compounds, respectively. (Table II) The results indicate that I exhibits an anti-inflammatory acitiviy less potent than hydrocortisone.

When compounds IIa and IIb, each 100 and 33 mg/kg, were subcutaneously given, they

Table II. Anti-inflammatory effect of 11, 19-diketo-18, 19-secoursolic acid methyl ester

Compound	Dose (mg/kg, s.c.)	No. of Animals	Edema Increased Percent (Inhibition Percent)				
			1hr	2hr	3hr	4hr	5hr
Control	1% CMC	6	25.3±6.6	37.5±5.0	60.8±6.9	57.0±6.5	45.5±4.2
Hydrocortisone	50	7	4.0±2.2	8.8±2.6	19.3±1.9 (68.3)*	18.7±4.7 (68.3)*	1.7±0.5 (66.7)*
11, 19-Diketo-18, 19-seco ursolic acid methyl ester(I)	100	7	9.9±2.1	32.1±3.5	37.1±4.4 (39.0)**	31.0±3.9 (45.6)**	6.2±1.8
〃	33	7	11.1±2.6	21.1±4.8	35.3±3.7 (41.9)**	35.6±4.5 (36.8)***	29.3±5.7

*$p<0.001$, **$p<0.01$, ***$p<0.05$ (as Student:s t-test)

Table III. Anti-inflammatory effects of 11-keto-β-boswellic acid and its methyl ester

Compound	Dose (mg/kg, p.o)	No. of Animals	Edema Increased Percent (Inhibition Percent)				
			1hr	2hr	3hr	4hr	5hr
Control	1% CMC	5	23.8±7.9	68.8±18.7	90.7±21.0	101.0±18.0	71.3±13.4
Phenylbutazone	100	4	19.3±2.4	15.5± 3.1	43.5*±7.9 (52.0)	57.3*±9.3 (43.3)	39.4±12.6
11-Keto-β-boswellic acid (IIa)	100	5	40.3±4.6	84.4±10.1	102.7±12.5 (−13.2)	98.9±13.0 (2.1)	81.3± 9.7
〃	33	5	26.7±4.2	84.3± 6.4	96.8± 8.4 (−6.7)	101.4± 8.6 (0)	91.5± 8.8
11-Keto-β-boswellic acid methylester (IIb)	100	4	30.4±12.7	77.9±13.6	100.8±10.2 (−11.1)	84.6±10.6 (16.2)	74.2± 7.7
〃	33	5	24.3± 3.3	57.7± 7.2	70.2± 4.1 (22.6)	72.3± 4.8 (28.4)	52.4± 5.7

*$p<0.05$ (as Student's t-test)

Table IV. Anti-inflammatory effect of 11-ketopresenegenin dimethyl ester

Compound	Dose (mg/kg, s.c)	No. of Animals	Edema Increased Percent (Inhibition Percent)				
			1hr	2hr	3hr	4hr	5hr
Control	1% CMC	6	17.1±12.0	24.3±11.8	39.4±28.4	48.2±25.9	36.3±27.3
Hydrocortisone	33	6	0	2.7± 2.0	14.7±11.3 (62.7)	14.8±11.4 (69.3)	12.4± 7.1
11-Keto presenegenin dimethyl ester (III)	100	6	13.3±13.1	33.8±23.2	47.1±2.31 (−19.5)	57.9±18.8 (−20.1)	48.1±17.9
〃	33	6	8.1± 7.4	34.9±20.1	51.4±26.9 (−30.5)	49.9±27.3 (−3.5)	37.5±27.3

barely suppressed edema. (data not shown) On autopsy of animals tested, it was found that major samples administered s.c. were remained in injection sites without absorbing. Oral administration of them revealed no potency, rather tending to increase edema volumes. (Table III) Compound III at dose of 100 and 33mg/kg, s.c. increased edema volumes more than vehicle-treated control group. (Table IV)

DISCUSSION

By introducing keto groups on carbon atom 11 of pentacyclic triterpenoids such as oleanene and ursene, we have synthesized some artificially derived 11-keto-triterpenoids,[6,7] and have investigated their inhibitory activities on corticoid-5 β-reductase and their anti-inflammatory activities. It has been demonstrated that 3, 11-diketo-oleanolic acid (IVc), 11-keto-oleanolic acid (IVa) and 11-keto-hederagenin (V) inhibited more strongly than glycyrrhetinic acid (VI), [6,7] and that IVc exhibiting the most potent inhibitory activity among them showed more stronger anti-inflammatory activity than hydrocortisone.[7]

In the present studies, 11-keto derivatives of pomolic acid (I)[9], β-boswellic acid (IIa, IIb, IIc)[8] and presenegenin (III)[10] were compared with the above-mentioned compounds for investigating the effects of the position changes of carboxyl groups and the presences of another functional groups on the enzyme and 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 I) 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 shows 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 enzyme. 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 E ring. The hydrophobicity of rings C/D decreases in the order of I, IVb and IVa. The decrease of hydrophobicity on ring C/D tends to lower the activity.

It has been assumed that the more stronger inhibitors on the enzyme may be the better anti-inflammatory agents. By a rough estimation, the relative anti-inflammatory activities of the artificially derived 11-keto-triterpenoids are compared with that of hydrocortisone as summarized in Table V.

All the 11-ketooleanolic acid derivatives exhi-

Table V. 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[7]
3, 11-Dioxo-oleanolic acid (IVc)	1.5[7]
11-Oxo-oleanolic acid (IVa)	1.0[7]
3, 11-Dioxo-oleanolic acid lactone (IVd)	1.0[7]
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

bited strong anti-inflammatory activities, and among them 11-ketooleanolic acid methyl ester (IVb) was the most potent. The order of their anti-inflammatory activities well coincides with that of their inhibitory actions on corticoid-5 β-reductase.

However, 11, 19-diketo-18, 19-secoursolic acid methyl ester 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-ketopresenegenin dimethyl ester (III) rather increased edema: hydrophilicity of their A-rings enhance the inhibitory activities on corticoid-5 β-reductase *in vitro*, but caused to edema *in vivo*. These might result in affecting aldosterone catabolism.

Final conclusion must be waited until 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 also confirm that α,β-unsaturated ketone on C_{11}-position, and C_3-hydroxy function play critical roles for corticomimetic activities.

ACKNOWLEDGEMENTS

This work was supported by a research grant from the Korean Science and Engineering Foundation.

LITERATURE CITED

1) Tamura, Y., Nishikawa, T., Yamada, K., Yamamoto, M. and Kumagai, A.: Effects of glycyrrhetinic acid and its derivatives on Δ^5-5α-and 5β-reductase in rat liver. *Arzneim.-Forsch/Drug Res.* **29**, Nr. 4, 647 (1979),

2) Atherden, L.M.: *J. Biol. Chem.* **69**, 75 (1958).

3) Baran, J.S., Langford, D.D., Liang. C.D. and Pitzele, B.S.: Synthesis and biological activities of substituted glycyrrhetic acids. *J. Med. Chem.* **17**, 184 (1974).

4) Pitele, B.S.: Synthesis of 2-oxygenated glycyrrhetic acid derivatives. *J. Med. Chem.* **17**, 191 (1974).

5) Shibata, S.: Medicinal chemistry of triterpenoid saponins and sapogenins. *Proceedings of 4th Asian symposium on medicinal plants and spices*, Bangkok, Thailand, 59 (1980).

6) Han, B.H., Lee, H.J. and Han, D.S.: Studies on triterpenoid corticomimetics. (I). Inhibition of corticoid-5β-reductase by 11-oxo-oleanolic acid and 11-oxo-hederagenin. *Yakhak Hoeji* **26**, 1 (1982).

7) Han, B.H., Kim-Cho, M.S., Han, Y.N. and Park, M.H.: Studies on triterpenoid corticomimetics. (II). Synthesis of 11-oxo-oleanolic acid derivatives and their anti-inflammatory activities. *Korean Biochem. J.* **17**, 91 (1984).

8) Han, B.H., Kim, K.M., Park, J.H., Park, M.H., Kim, T.H. and Han, Y.N.: Studies on triterpenoid corticomimetics. (III). Determination of acetyl-11-keto-β-boswellic acid and 11-keto-β-boswellic acid in olibanum. *Arch. Pharm. Res.* 8(4), 213 (1985).

9) Han, Y.N., Han, B.H., Park, E.T. and Kim, T.H.: Studies on triterpenoid corticomimetics. (IV). E-Ring opening of pomolic acid by retrograde aldol condensation. *Arch. Pharm. Res.* 8(4), 221 (1985).

10) Han, B.H. and Han, Y.N.: Studies on triterpenoid corticomimetics. (V). Oxidation of presenegenin with chromium trioxide-acetic acid to yield 11-keto and 12-keto derivatives. *Arch. Pharm. Res.* 8(4), 229 (1985).