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Chemical Modification of Glycyrrhetinic Acid in Relation to the Biological Activities

Several modified derivatives of glycyrrhetinic acid were prepared for obtaining compounds which are devoid of aldosterone-like properties and retain or enhance the therapeutic activities of the mother compound. Among them, olean-12-en-3β,30-diol showed antiallergic and antiulcerous activities without inhibition of Δ^4-5α- and Δ^4-5β-reductases of 3-keto-Δ^4-steroids. This compound was prepared in a good yield from glycyrrhetinic acid by reduction with sodium bis(2-methoxyethoxy)aluminum hydride followed by catalytic hydrogenation of the intermediate with Pd-C.

Keywords——glycyrrhetinic acid; pseudoaldosteronism; chemical modification; olean-12-en-3β,30-diol; antiallergic activity; antiulcerous activity; enzymatic reduction of 3-keto-Δ^4-steroids

Pharmacological activities of glycyrrhizin, a saponin of licorice root, and its aglycone, glycyrrhetinic acid, have been studied extensively, and their antiinflammatory,[1] antiulcerous[2] and antiallergic effects[3] have been reported. Sodium salt of 3-O-hemisuccinate of glycyrrhetinic acid[4] is orally administered as a remedy of stomach ulcer,[1] and a preparation of ammonium salt of glycyrrhizin combined with glycine and cysteine[5] is clinically used by intravenous injection as an antiallergic drug. The same drug has recently been proved to be effective by the clinical double blind trial in chronic hepatitis and also in some cases of liver cirrhosis.[6] However, administration of glycyrrhizin and glycyrrhetinic acid preparations in higher dosage for a long period has resulted in a side effect which is noted as pseudoaldosteronism inducing edema and hypertension in patients. This mineral corticoid-like action of glycyrrhizin and glycyrrhetinic acid producing Na ion retention and K ion excretion was earlier observed by Molhuysen *et al.*[7] and this becomes manifest only by the existence of endogenous or exogenous mineral corticoids. Kumagai *et al.*,[8] and Atherden[9] as well, found an inhibitory activity of glycyrrhizin and glycyrrhetinic acid on reductive metabolism of corticoids in the liver which results in delaying their clearance, and subsequently demonstrated, using a rat liver homogenate preparation, that glycyrrhetinic acid strongly inhibits Δ^4-5β-reductase of 3-keto-Δ^4-steroids.[8b] Since Atherden[9] demonstrated that 11-deoxo-glycyrrhetinic acid inhibits the rat liver reductase to a small extent, it would be suggested that 11-oxo-$\Delta^{12(13)}$-system in the C-ring of glycyrrhetinic acid is essential as an active site. Baran *et al.*[10] prepared a series of modified compounds derived from glycyrrhetinc acid with testing biological activities, and reached the same conclusion.

On the basis of the above findings and in considering that the 11-oxo-$\Delta^{12(13)}$-system in the C-ring glycyrrhetinic acid is competitive with the 3-oxo-$\Delta^{4(5)}$-system in the A-ring of some steroid hormones at the active site of the reducing enzyme, several modified compounds of

1) R.S.H. Finney and A.L. Tárnoky, *J. Pharm. Pharmacol.*, **12**, 49 (1960).
2) M.H. Khan and F.M. Sullivan, "Symposium on Carbenoxolone Sodium," ed. by J. Robson and F. Sullivan, Butterworths Scientific Publications, London, 1968, p. 5.
3) A. Kumagai, *Minophagen Med. J.*, **12**, 14 (1967).
4) Carbenoxolone.
5) Strong Neominophagen C.
6) H. Suzuki, *Proc. Symp. Wakan-yaku*, **12**, 114 (1979) (in Japanese).
7) J.A. Molhuysen, J. Gerbrandy, L.A. de Vries, J.C. de Jong, L.B. Lenstra, K.P. Turner, and J.C. Borst, *Lancet*, **2**, 381 (1950).
8) *a*) A. Kumagai, S. Yano, M. Otomo, and K. Takeuchi, *Endocrinol. Jpn.l* **4**, 17 (1957); *b*) Y. Tamura, T. Nishikawa, K. Yamada, M. Yamamoto, and A. Kumagai, *Arzneim.-Forsch.*, **29**, 647 (1979).
9) L.M. Atherden, *Biochem. J.*, **69**, 75 (1958).
10) J.S. Baran, D. Langford, C.-D. Liang, and B.S. Pitzel, *J. Med. Chem.*, **17**, 184 (1973).

glycyrrhetinic acid have been prepared for eliminating the pseudoaldosteronism but retaining or enhancing the therapeutical activities of glycyrrhetinic acid. Among those compounds, an 11-deoxo-30-hydroxyl derivative of glycyrrhetinic acid, olean-12-en-3β,30-diol (III), has been shown to be most promising in animal and enzymatic experiments.

III was prepared by Ryabinin and Konovalova[11] starting from methyl glycyrrhetinate (I') by the catalytic reduction of 11-keto group followed by the action of $LiAlH_4$, and by Canonica *et al.*[12] from naturally occurring olean-12-en-11-oxo-3β,30-diol (=glycyrrhetol isolated from licorice root) (V) by the catalytic hydrogenation with platinum dioxide as the catalyst. In the present study the foregoing processes were traced, while the following procedures showed a good result on preparing III: Glycyrrhetinic acid (I) was reduced with sodium bis-(2-methoxyethoxy)aluminum hydride, $NaAlH_2(OCH_2OCH_3)_2$, in tetrahydrofuran to yield olean-12-en-3β,11ξ,30-triol (II), which was catalytically hydrogenated with Pd–C as the catalyst to afford III, $C_{30}H_{50}O_2$ (MS: M^+ *m/e* Calcd 442.73; Found 442.38), mp 251°, in a yield of 80% (Chart 1).

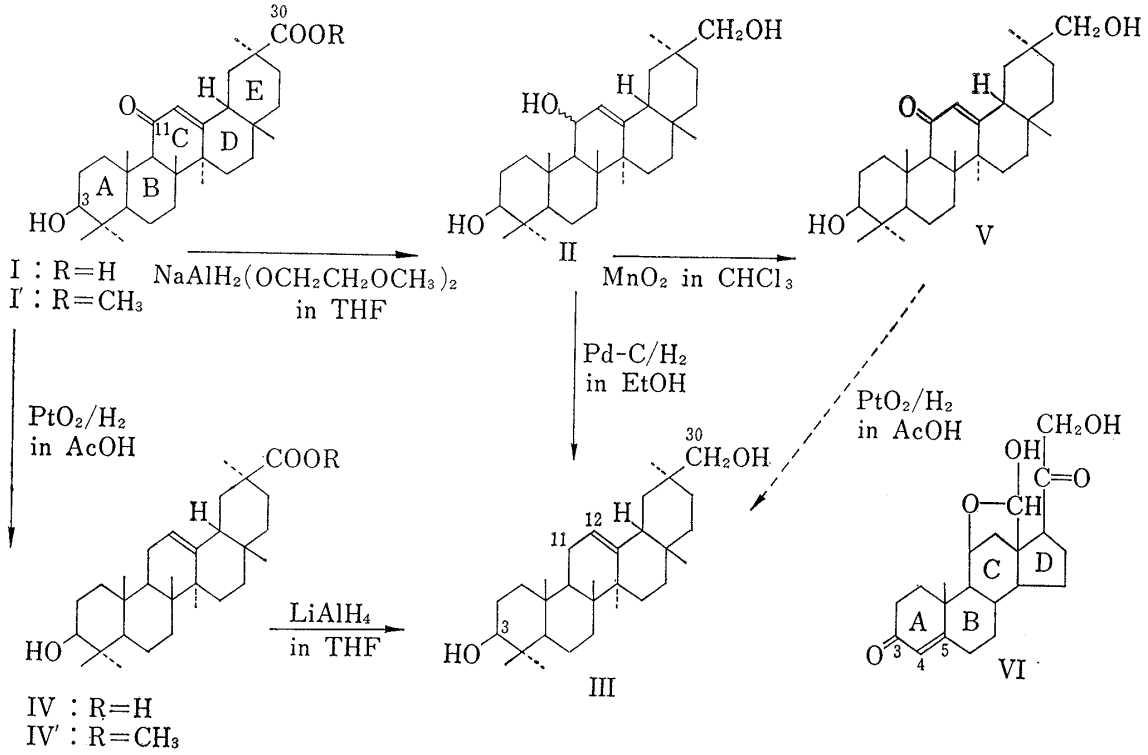

Chart 1

The activities of glycyrrhetinic acid derivatives in inhibiting the Δ^4-5α- and Δ^4-5β-reductases of 3-keto-Δ^4-steroids prepared from the rat liver were measured by the method previously reported by Tamura *et al.*[8b] The results given in Table I reveal that III is completely inactive in inhibiting both Δ^4-5α- and Δ^4-5β-reductases.

The toxicities (oral and *i.p.* LD_{50}) of I and III were determined by the Litchfield–Wilcoxon method. Effects of these samples on acetic acid induced writhing syndrome, carrageenin induced edema, stress induced gastric erosions, and aspirin induced gastric lesions were examined. As to antiallergic activities, PCA (passive cutaneous anaphylaxis) test and Arthus

11) A.A. Ryabinin and N.E. Konovalova, *Zh. Obshch. Khim.*, **32**, 644 (1962) [*C.A.*, **58**, 1500 *a* (1963)].
12) L. Canonica, B. Danieli, P. Manitto, G. Russo, and E. Bombadelli, *Gazz. Chim. Ital.*, **97**, 1347 (1967).

Table I. *In Vitro* Effects of Glycyrrhetinic Acid and Its Derivatives on Δ^4-5α and Δ^4-5β Reduction of Aldosterone in Rat Liver[a]

Compounds	Inhibition (%)	
	5α-Reductase ($p<$)[b]	5β-Reductase($p<$)
Control	0.0±2.5[c]	0.0±3.4
I	9.2±2.2 (NS)[d]	87.7±2.2 (0.001)
III	−7.1±2.2 (NS)	0.0±1.1 (NS)
IV	−0.5±6.6 (NS)	20.5±4.1 (0.01)
V	26.5±5.6 (0.001)	88.4±4.7 (0.001)

a) Aldosterone was used as a substrate. Molar ratio of aldosterone (VI) and glycyrrhetinic acid (I) or its derivatives (III, IV, and V) was equal. See ref. 6*b* as regards the preparation of 5α- and 5β-reductases and the procedure of measurements of enzyme inhibition.
b) Statistically significant level as compared with control.
c) Mean±S.E.
d) Not significant.

Table II. Pharmacological Properties of Glycyrrhetinic Acid and Its Derivatives

Test	Animal, Route	I	III	Glycyrrhizin	Positive control
LD_{50}	Mouse, *p.o.*	560(518—605) mg/kg[a]	>5 g/kg		
	Mouse, *i.p.*	455(433—478) mg/kg	>4 g/kg		
Writhing induced by acetic acid	Mouse, *p.o.*	400 mg/kg[b] 47%[g]	100 mg/kg 38%[g]	800 mg/kg −2%	ASP[c] 200 mg/kg *p.o.* 98%[g]
Edema induced by carrageenin	Rat, *p.o.*	200 mg/kg 22%	200 mg/kg 28%	400 mg/kg 5%	ASP 200 mg/kg *p.o.* 80%[g]
Stress ulcer induced by restraint and water immersion	Mouse, *p.o.*	200 mg/kg 34%	200 mg/kg 55%[f]		ATR[d] 10 mg/kg *s.c.* 95%[g]
	Rat, *p.o.*	300 mg/kg 19%	300 mg/kg 50%[f]		ATR 10 mg/kg *s.c.* 98%[g]
Gastric lesion induced by ASP	Rat, *i.d.*	320 mg/kg 6%	320 mg/kg 56%		
PCA Test	Rat, *p.o.*	100 mg/kg 2%	100 mg/kg 22%	200 mg/kg −52%	
	Rat, *i.p.*	200 mg/kg 53%[f]	100 mg/kg 58%[f]	200 mg/kg 52%[f]	Pred.[e] 5 mg/kg *i.p.* 52%[g]
Arthus phenomenon test	Guinea Pig, *i.p.*	200 mg/kg 47%	200 mg/kg 81%[f]	200 mg/kg 100%[f]	

a) 95% Fiducial limit.
b) Figures indicate drug dose and inhibition percentage.
c) ASP : aspirin.
d) ATR : atropine sulfate.
e) Pred. : prednisolone.
f) Significantly different from control, $p<0.05$.
g) $p<0.01$.

phenomenon test were also performed using the same series of compounds. The results are summarized in Table II.

III showed antiallergic activities in a similar fashion to glycyrrhizin and I. On the other hand, III produced a prominent prevention of experimental gastric lesions without any inhibitory activity on Δ^4-5α- and Δ^4-5β-reductases of 3-keto-Δ^4-steroids, suggesting that it might be possible to prepare promising derivatives of glycyrrhetinic acid devoid of pseudoaldosteronism.

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Labdane and Bisnorlabdane Type Diterpenes from *Alpinia speciosa* K. SCHUM.

Two new diterpenes were isolated from the rhizomes of *Alpinia speciosa* K. SCHUM. (Zingiberaceae) and their structures were established by the spectral evidences as I and II. The latter has an unusual bisnorlabdane carbon skeleton. It is the first example that diterpenes were obtained from Alpinia genus.

Keywords——Zingiberaceae; *Alpinia speciosa* K. SCHUM.; diterpene; bisnorditerpene; labdane; labda-8(17),12-diene-15,16-dial; 15,16-bisnorlabda-8(17),11-dien-13-one; ^{13}C-NMR

The seeds of *Alpinia speciosa* have been used as an aromatic stomachic in Japan, but none of their active constituents has so far been characterized.[1] In the course of our extensive studies on the Zingiberaceous plant having pharmacological activities against excised ileum of guinea pigs,[2] we isolated two new diterpenes from this plant. Only a few instances have been recorded of the isolation of diterpenes from Zingiberaceous plant.[3]

The fresh rhizomes of the plant were extracted with methanol, and the aqueous methanolic extracts were shaken with petroleum ether. Chromatographic purification of the petroleum ether soluble fraction furnished compound (I) and (II).

Compound (I) obtained as an unstable oil, $C_{20}H_{30}O_2$(M$^+$: 302.226, Calcd: 302.225), $[\alpha]_D^{25}$ $-15°$ (c=0.04, EtOH), showed the UV absorption maxima at 235 and 292 (infl.) nm (ε=8900 and 340, EtOH) and IR bands at 1729 and 1680 cm^{-1} (liquid film). The ^{1}H-NMR spectrum

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