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Chemical Modification of Glycyrrhetinic Acid in Relation to the Biological Activities[1)]

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18β-Olean-12-ene-3β,30-diol (deoxoglycyrrhetol) (**4a**) was prepared with a view to eliminating pseudoaldosteronism, a side-effect of glycyrrhetinic acid (**1b**), which is the sapogenin of Licorice saponin, glycyrrhizin (**1a**), while maintaining or enhancing the therapeutic activities.

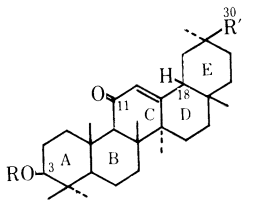
On reduction of the 11-keto and 30-carboxyl groups of **1b** with $NaAlH_2$–$(OCH_2CH_2OCH_3)_2$, olean-12-ene-3β,11β,30-triol (**9**) and olean-12-ene-3β,11α,30-triol (**10**) were obtained. On catalytic hydrogenation of **9** and **10** with Pd–C as a catalyst, **4a** was formed in an overall yield of 80%. On treatment with conc. HCl, **9** yielded 18β-olean-9(11),12-diene-3β,30-diol (**11a**), while **10** yielded olean-11,13(18)-diene-3β,30-diol (**12a**). Mono- and di-β-carboxy-propionyl and mono- and di-*o*-phthaloyl esters of **4a**, **11a** and **12a** were prepared to increase the hydrophilic character.

The competitive inhibition of 5β,Δ^4-reductase of corticosteroids in the liver which is caused by **1b** to induce pseudoaldosteronism was not observed in the case of **4a**. Compounds **4a**, **11a** and **12a** and their β-carboxypropionyl and *o*-phthaloyl esters were studied pharmacologically, and showed antiulcerogenic, antiallergic and antiinflammatory activities.

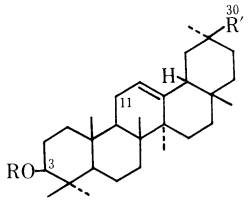
Keywords——glycyrrhizin; glycyrrhetinic acid; 18β-olean-12-ene-3β,30-diol; deoxoglycyrrhetol; 18β-olean-9(11),12-diene-3β,30-diol; 18β-olean-11,13(18)-diene-3β,30-diol

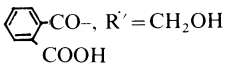
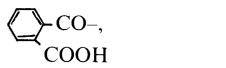
Glycyrrhizin (**1a**) and glycyrrhetinic acid (**1b**), a major saponin of licorice root (*Glycyrrhiza* spp. (Leguminosae)) and its aglycone, are known to be effective as antiallergic,[3)] antiinflammatory[4)] and antiulcer[5)] agents. Disodium 3-*O*-β-carboxypropionyl-glycyrrhetinate (carbenoxolone sodium)[6)] (**1c**) is used orally as a remedy for stomach ulcer, and a preparation of ammonium glycyrrhizinate combined with L-cysteine and glycine (strong neominophagen-C) (SNMC) is clinically applied by intravenous injection as an antiallergic agent. Recently SNMC was shown to be effective against chronic hepatitis in a clinical double blind trial.[7)] Antiviral activity of ammonium glycyrrhizinate *in vitro* was also reported.[8)]

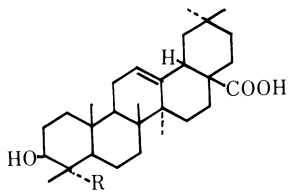
Thus glycyrrhizin and glycyrrhetinic acid are noteworthy drugs among phytotherapeutics, while the licorice root itself has long been used in traditional or folkloric medicine of both the East and the West. However, a side effect (induction of edema and hypertension) has been observed in patients given high doses of glycyrrhizin and glycyrrhetinic acid for prolonged periods. This side effect, a mineral corticoid-like action noted as pseudoaldosteronism, is due to the inhibition of the metabolic clearance of endogenous corticoid with



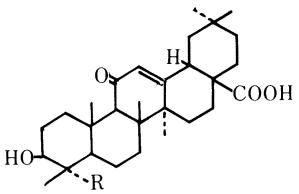
1a: R = GlcAβ(1→2)GlcA, R′ = COOH (glycyrrhizin)
1b: R = H, R′ = COOH (glycyrrhetinic acid)
1c: R = NaOOC(CH$_2$)$_2$–CO–, R′ = COOH
3: R = H, R′ = CH$_2$OH (glycyrrhetol)



2: R = H, R′ = COOH (deoxoglycyrrhetinic acid)[17]
4a: R = H, R′ = CH$_2$OH (deoxyoglycyrrhetol)
4b: R = HOOC(CH$_2$)$_2$–CO–, R′ = CH$_2$OH
4c: R = HOOC(CH$_2$)$_2$–CO–, R′ = CH$_2$OOC(CH$_2$)$_2$–COOH
4d: R =  R′ = CH$_2$OH
4e: R = 
R′ = CH$_2$–OOC  HOOC



5: R = CH$_2$OH (hederagenin)
7: R = CH$_3$ (oleanolic acid)



6: R = CH$_2$OH (11-oxohederagenin)
8: R = CH$_3$ (11-oxo-oleanolic acid)[18]

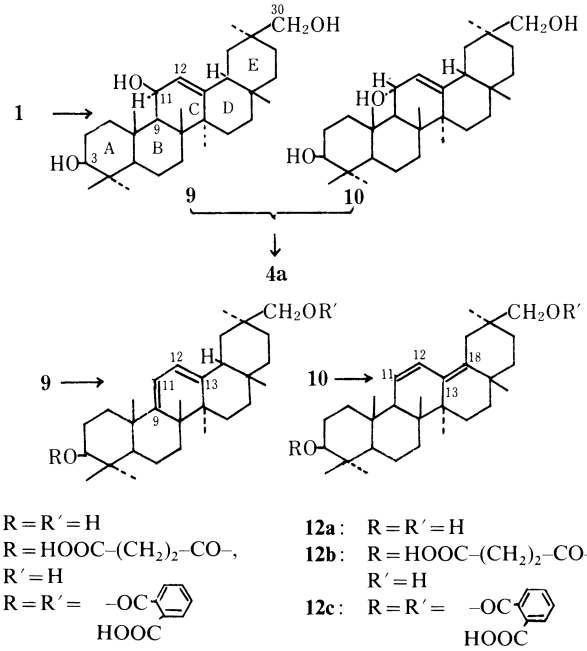
Chart 1

retention of Na^+ and water and excretion of K^+.

Kumagai *et al.*[9] and Atherden[10] found significant inhibition of the reduction of the Δ^4-3-keto system of cortical steroid by glycyrrhizin and glycyrrhetinic acid in rat liver, which contains soluble Δ^4-5β-reductase and microsomal Δ^4-5α-reductase (the former is the major enzyme in human liver). Atherden[10] showed that 11-deoxo-glycyrrhetinic acid (**2**) does not inhibit rat liver Δ^4-reductase and suggested that the presence of the 11-keto-$\Delta^{12(13)}$ system in ring C of glycyrrhetinic acid is essential for the inhibitory activity against the reductase. Baran *et al.*[11] prepared several derivatives of glycyrrhetinic acid to examine the reductase-inhibiting activities, and reached the same conclusion as above, while they observed loss of the antiulcer activity of the parent compound on modification of the structure. It seems plausible that the 11-oxo-$\Delta^{12(13)}$ system in ring C of glycyrrhetinic acid is competitive with the 3-oxo-$\Delta^{4(5)}$ system in ring A of cortical steroids at the active site of the reducing enzyme.

The present study has been designed to prepare modified compounds of glycyrrhetinic acid with a view to eliminating the pseudoaldosteronism while retaining or enhancing the therapeutic activities.

For the exclusion of pseudoaldosteronism, destruction of the 11-oxo-$\Delta^{12(13)}$ system in ring C of glycyrrhetinic acid was attempted, and for the enhancement of the therapeutic activities, a modification of the terminal carboxyl attached to ring E of the parent compound into hydroxymethyl was examined. The significance of the presence of a hydroxymethyl group attached to the terminal ring of oleanane-type triterpenoids for enhancing pharmacological activities was shown in the cases of saikosaponins a and d,[12] platycodins A and D,[13] *Aesculus hippocastanum* seed saponins, "aescin,"[14] and *Sapindus mukurossi* saponins and their aglycone, hederagenin.[15] It is remarkable to note that saikosaponin c is pharmacologically ineffective,[16] presumably because it possesses no hydroxymethyl group in ring A while the other part of the structure is almost the same as in its homologues a and d.



11a: R=R′=H
11b: R=HOOC–$(CH_2)_2$–CO–, R′=H
11c: R=R′= –OC(HOOC)

12a: R=R′=H
12b: R=HOOC–$(CH_2)_2$–CO–, R′=H
12c: R=R′= –OC(HOOC)

Chart 2

Chemistry

The target compound of the present study is 18β-olean-12-ene-3β-30-diol (18β-deoxoglycyrrhetol) (**4a**), which satisfies the above requirements. Some other oleanane derivatives (**2**, **3**, **5**, **6**, **7** and **8**) were also prepared as the reference compounds to compare their biological activities with those of deoxoglycyrrhetol (**4a**).

Almost all the above compounds except 11-oxohederagenin (**6**) are known in the literature, though the reported preparation procedures have been modified to some extent in the present study. Introduction of a keto group at the 11-position in ring C of oleanane-type triterpenes was performed by the following procedure: Acetylation of hederagenin (**5**) and oleanolic acid (**7**) at the hydroxyl group followed by oxidation with *tert*-butylchromate and deacetylation with alkali in dioxane afforded **6** and **8**, respectively.

Compound **4a** was synthesized by Ryabinin and Konovalova[19] starting from methyl glycyrrhetinate by catalytic reduction of the 11-keto group followed by reduction of the 30-carbomethoxyl to hydroxymethyl with $LiAlH_4$. Compound **4a** was also prepared by Canonica *et al.*[20] from naturally occurring olean-12-en-11-oxo-3β,30-diol (glycyrrhetol, a minor sapogenin of Licorice root) (**3**) by hydrogenation using PtO_2 as the catalyst.

During preliminary studies of the foregoing procedures, the following process was found to be the most efficient in giving a high yield of **4a**. Glycyrrhetinic acid (**1a**) was reduced with sodium bis(2-methoxyethoxy)aluminum hydride (vitride)[21] in tetrahydrofuran (THF) to afford olean-12-ene-3β,11β,30-triol (**9**), mp 252 °C $[\alpha]_D$ +89.6° (dioxane) and olean-12-ene-3β,11α-30-triol (**10**), mp 224 °C $[\alpha]_D$ +5.75° (dioxane), whose stereochemistry at the 11-position was established by the magnitude of the coupling constants of the proton nuclear magnetic resonance (^{1}H-NMR) signals due to 9α-H, 11ξ-H and 12-H. The 11-α-H of **9a** bisects the dihedral angle between 9α-H and 12-H (*ca.* 90°) to give a triplet ^{1}H-NMR signal (J=6 Hz) at δ 4.4. The double doublets (J_1=4 Hz and J_2=8 Hz) at δ 4.4 in the NMR spectrum of **10** agree well with the result given by Finucane and Thomson[24] for 3β-

acetoxyolean-12-en-11α-ol.

The major product possesses 11-β-hydroxyl (**9**) and the minor one 11α-hydroxyl (**10**), both of which are convertible to an equilibrium mixture on standing in chloroform at room temperature. The determination of the stereochemistry at C-11 and the assignment of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) chemical shifts of C-11 and C-12 of the triol (**9**) were performed by means of a deuteration experiment using $NaAlD_4$ as the reducing agent for methyl glycyrrhetinate (30-methyl ester of **1b**) a deuterated triol, $C_{30}H_{47}O_3D_3$, which gave the same *Rf* value on thin layer chromatography (TLC) as the major triol (**9**), was afforded as the major product. $LiAlD_4$ should attack the C-11 carbonyl of **1b** from the less-hindered α-direction to produce α-D, β-OH at this position. On acetylation, **9** and **10** gave the same triacetate, mp 96 °C, $[\alpha]_D$ +220°, having β-OAc at the 11-position. On acid treatment, **9** afforded only a homoannular diene (**11a**), while **10** yielded a heteroannular diene (**12a**).

On acid treatment the major deuterated triol having α-D, β-OH at C-11 was converted into a homoannular diene which retained the deuterium atom at C-11. The ^{13}C-NMR spectrum of this compound showed, in comparison with that of non-deuterated **11a**, a specific corruption of a doublet signal at δ 115.6, assignable to C-11. Thus the doublet signal at δ 121.5 was assigned to C-12, and those at δ 154.5(s) and 146.3(s) to C-9 and C-13 or *vice versa*.

On catalytic reduction of either **9** or **10** with Pd–C as the catalyst, **4a**, mp 250—251 °C, $[\alpha]_D$ +87.5° (dioxane), was produced in a high overall yield. The ^{1}H-NMR spectrum of **4a** showed that 12-H forms a dihedral angle of *ca.* 40° with 11-H_2, giving a triplet (1H) signal (J= 4 Hz) at δ 5.18.

Oxidation of **9** and **10** with manganese dioxide[23] in chloroform yielded a dienone compound, 11-oxo-18β-olean-12-ene-3β,30-diol(glycyrrhetol) (**3**), mp 282 °C, $[\alpha]_D$ +97.6° (dioxane). Compound **3** was also prepared from **4a** diacetate by the oxidation of its allylic methylene at C-11 with *tert*-butyl chromate[24] followed by deacetylation under the same conditions as used for the preparation of **6** and **8** from **5** and **7**, respectively. To obtain a water-soluble derivative of deoxoglycyrrhetol, the 3-*O*-β-carboxypropionate (**4b**) was prepared by the following procedure.

For the purpose of temporarily protecting the primary alcohol, **4a** was tritylated with trityl pyridinium fluoroborate[25] or with trityl chloride and 4-dimethylaminopyridine in dichloromethane.[26]

The 30-tritylated **4a** was reacted with succinic anhydride, 4-dimethylaminopyridine, triethylamine and pyridine in dichloromethane to afford the 3-*O*-β-carboxypropionate of the 30-trityl ether of deoxoglycyrrhetol, from which the trityl group was removed by heating with 80% acetic acid[27] or by the action of 10% Pd–C and 10% formic acid[28] to yield **4b** of deoxoglycyrrhetol. Compound **4b** was also prepared by partial hydrolysis of the 3β,30-di-β-carboxypropionate of **4a** (**4c**) with alkali. The 3β-*O*-hemiphthalate (**4d**) and 3β,30-di-*O*-hemiphthalate (**4e**) of **4a** were also prepared by the reaction of phthalic anhydride with **4a** in the presence of pyridine or triethylamine, and their sodium salts were employed for pharmacological experiments.

Biochemistry

To evaluate pseudoaldosteronism *in vitro*, the activities of **4a** and some related oleanane derivatives against the liver reductase of 3-keto-Δ^4-steroid were assayed using aldosterone as the substrate. The assay was carried out by the procedure developed by Kumagai *et al.*[29,30]

The experimental results were reported preliminarily.[1] Compound **4a** showed no inhibition of 5α or 5β-Δ^4-reductase (-7.1 ± 2.2% and 0 ± 1.1%, respectively), whereas glycyrrhetinic acid (**1b**) inhibited 9.2 ± 2.2% of the activity of 5α-reductase and 87.7 ± 2.2% of that of 5β-reductase. Thus the destruction of the αβ-unsaturated C=O system in ring C of

glycyrrhetinic acid appears to eliminate pseudoaldosteronism. The details of this experiment will be reported elsewhere.

Using our samples, Inoue *et al.*[31] reported that the disodium salt of **4e** showed a strong inhibition of 5- and 12-lipoxygenases. The disodium salt of the hemiphthalate of the homoannular diene (**11a**) and heteroannular diene (**12a**) compounds corresponding to **4e** also inhibited lipoxygenases. Since 5-lipoxygenase is involved in the biosynthesis of several leukotrienes,[32] the above triterpenoid compounds related to **4e** may be effective as antiinflammatory and antiallergic agents.

Pharmacology

The antiulcerogenic, antiallergic and antiinflammatory activities of deoxoglycyrrhetol (**4a**) and related compounds were reported preliminarily,[1] and further experiments have been performed using the hemisuccinates and hemiphthalates of this series of triterpenoid compounds. The results will be reported in detail elsewhere.

Experimental

Optical rotations were determined in dioxane with a Jasco J20 ORD/CD polarimeter. Ultraviolet (UV) spectra were measured with dioxane as a solvent. Melting points were taken on a Yanaco micro melting points apparatus and are uncorrected. NMR spectra were recorded on a JEOL FX-60 NMR spectrometer for all ^{13}C-NMR spectra using 10% solutions in 10 mm (o.d.) tubes at 60 MHz or on a JEOL PS 100 for all ^{1}H-NMR spectra in 20—30 mg/0.3 ml $CDCl_3$ or d_5-pyridine as a solvent, with Me_4Si as an internal standard. Chemical shifts are reported in parts per million upfield from this standard. Mass spectral analyses were performed with a JEOL G-300 instrument coupled with a JEOL data analysis system (model JMA 2000 disk system).

Liquid chromatography was performed using three different types of apparatus.

(A) The liquid chromatograph consisted of a Nihon Seimitsu NSP-800-12DX solvent pumping system with a Bellows Damper (NBD-III), Shodex SE-11 (RI-detector) and a Rheodyne model 7125 injection valve. Chromatograms were recorded on a Rikadenki model R 201 recorder. The experiments were performed in a column (30 cm × 8 mm i.d.) (Nihon Seimitsu, Tokyo) packed with Nucleosil 50-5 (5 μm) (Macherey, Nagel Co., Duren, G.F.R.) or Whatman Partisil 5 (5 μm) (Whatman Ltd., Springfield Mill, England) fitted with a guard column system, holder 140-200 and column SI-GU (Brownlee Labs. Inc., Santa Clara, U.S.A.).

(B) Du Pont model 830 liquid chromatograph. The experiments were performed on a column (50 cm × 8 mm i.d.) (Kyowa Seimitsu, Tokyo) packed with Lichrosorb SI-60 (5 μm) (E. Merck, Darmstadt, Dr. Theodor Schachardt Co.).

(C) Waters system 500A (Waters Associates, Inc., Milford, Mass, U.S.A.) Column; Prepak-500/silica.

The term "treated in the usual way" refers to the treatment of extracts, and indicates that they were washed with water till neutral, dried over Na_2SO_4, and evaporated to dryness.

High-performance liquid chromatography (HPLC) was applied for the separation and isolation of glycyrrhetinic acid derivatives. By using the Waters system 500A liquid chromatograph, olean-12-ene-3β,30-diol (**4a**) could be purified in large quantities within 10 min. Semipreparative HPLC was also efficient for the isolation of compounds for analysis.

18β-Glycyrrhetinic Acid (3β-Hydroxy-11-oxo-18β-olean-12-en-30-oic Acid) (1b)——Glycyrrhizin, a diglucuronide saponin of licorice root (*Glycyrrhiza* spp.), colorless needles, mp 335 °C, was hydrolyzed to prepare **1b**, which was used as a starting compound for chemical modification. ^{13}C-NMR (d_5-pyridine) δ ppm: 199.4 (s, C-11), 178.9 (s, C-30), 169 (s, C-13), 128.5 (d, C-12), 77.8 (d, C-3), 62.1 (d, C-9), 55.2 (d, C-5).

Methyl Ester: mp 235.5—237 °C. $[\alpha]_D$ (dioxane): +158.3°.

11-Deoxo-18β-glycyrrhetinic Acid (3β-Hydroxy-18β-olean-12-en-30-oic Acid) (2)——A solution of 4.0 g of **1b** dissolved in 170 ml of AcOH was added to prereduced Pt (prepared from 2.4 g of PtO_2) in 20 ml of AcOH, under an atmosphere of H_2. The mixture was stirred, and absorbed 420 ml of H_2 over 8 h at room temperature, when the reaction product separated out. Then 100 ml of AcOH was added, and the reaction mixture was heated on a boiling water bath and filtered. On evaporation of the solvent, crude **2** was obtained. Recrystallization from AcOH gave colorless needles, mp >330 °C, in 80% yield. ^{13}C-NMR (d_5-pyridine) δ ppm: 179.4 (s, C-30), 145.0 (s, C-13), 122.8 (d, C-12), 78.0 (d, C-3), 55.7 (d, C-5). MS *m/z* Calcd for $C_{30}H_{48}O_3$: M^+ 456.3603. Found: M^+ 456.3602.

18β-Olean-12-ene-3β,11α and β,30-triol——Glycyrrhetinic acid (**1b**) (0.94 g, 2 mM) dissolved in dry THF (20 ml) was added to a solution of $NaAlH_2(OCH_2CH_2OCH_3)_2$ (70% in toluene) (2.9 ml, 10 mM) in dry THF (20 ml) under vigorous stirring at 60 °C in an N_2 atmosphere. The reaction was continued for 1 h. After cooling, the reaction mixture was decomposed with 10% HCl to adjust the pH to 3—4. The filtrate was extracted with $CHCl_3$, and the

organic layer was washed with water 3 times then dried over anhydr. Na_2SO_4 for 6 h. The solvent was evaporated off to leave a residue, which was separated by HPLC (Waters system 500A flow rate 150 ml/min) with $CHCl_3$–EtOH–MeOH (94 : 5 : 1) as a solvent system to afford 18β-olean-12-ene-3β,11β,30-triol (yield 660 mg) at the retention time of 11 min, and olean-12-ene-3β,11α,30-triol (yield 165 mg) at the retention time of 14 min. The yield ratio of isomeric products at the 11-hydroxyl group was 4 : 1.

18β-Olean-12-ene-3β,11β,30-triol (**9**), colorless needles, mp 248—252 °C. ^{13}C-NMR (d_5-pyridine) δ ppm: 146.3 (s, C-13), 128.1 (d, C-12), 78.0 (d, C-3), 66.9 (d, C-11), 65.6 (t, C-30). ^{1}H-NMR ($CDCl_3$) δ ppm: 5.3 (d, 1H, J=5 Hz, C-12-H), 4.4 (t, 1H, J=6 Hz, C-11-H), 3.5 (s, 2H, C-30-H_2), 3.2 (t, 1H, J=8 Hz, C-3-H). MS m/z Calcd for $C_{30}H_{50}O_3$: M^+ 458.3760. Found: M^+ 458.3728.

18β-Olean-12-ene-3β,11α,30-triol (**10**), colorless needles, mp 222—224 °C. ^{1}H-NMR (d_5-pyridine) δ ppm: 5.8 (d, 1H, J=4 Hz, C-12-H), 4.4 (dd, 1H, J=4 Hz, J_2=8 Hz, C-11-H), 3.7 (dd, 2H, J_1=9 Hz, J_2=20 Hz, C-30-H_2), 3.5 (t, 1H, J=8 Hz, C-3-H). MS m/z Calcd for $C_{30}H_{50}O_3$: M^+ 458.3760. Found: M^+ 458.3706. Calcd for $C_{30}H_{48}O_2$: $M^+ - H_2O$ 440.3654. Found: M^+ 440.3736.

18β-Olean-12-ene-3β,11β,30-triol Triacetate——On acetylation, **9a** and **9b** gave the same triacetate, mp 96 °C $[\alpha]_D$ +220 . ^{13}C-NMR (d_5-pyridine) δ ppm: 170.6, 170.2, 169.9 (s, acetyl, C=O), 147.9 (s, C-13), 122.4 (d, C-12), 80.7 (d, C-3), 68.5 (d, C-11), 67.8 (t, C-30). ^{1}H-NMR ($CDCl_3$) δ ppm: 5.44 (t, 1H, J=6 Hz, C-11-H), 5.12 (d, 1H, J=6 Hz, C-12-H), 4.50 (t, 1H, J=9 Hz, C-5-H), 3.94 (br s, 2H, C-30-H_2), 2.00 (s, 9H, acetyl CH_3). MS m/z Calcd for $C_{36}H_{56}O_6$: M^+ 584.4077. Found: M^+ 584.4199.

18β-Olean-9(11),12-diene-3β,30-diol (11a)——A solution of 92 mg of **9** in $CHCl_3$ was acidified with 0.5 ml of conc. HCl at room temperature. After standing for 10 min the reaction mixture was evaporated at 60 °C to afford colorless needles, mp 230.5—232 °C. $[\alpha]_D$ +271° (dioxane). UV $\lambda_{max}^{dioxane}$ nm (log ε): 282 (3.79) (homoannular diene). Yield: 79 mg (90%). ^{13}C-NMR ($CDCl_3$) δ: 154.5 (s), 146.3 (s, C-9 or C-13 or *vice versa*), 121.1 (d, C-12), 115.7 (d, C-11). ^{1}H-NMR ($CDCl_3$) δ ppm: 5.60 (dd, 2H, J_1=4 Hz, J_2=8 Hz, C-11-H, C-12-H), 3.30 (t, 1H, J=7 Hz, C-3-H), 3.60 (dd, 2H, J_1=9 Hz, J_2=14 Hz, C-30-H_2). MS m/z Calcd for $C_{30}H_{48}O_2$: M^+ 440.3654. Found : M^+ 440.3663.

18β-Olean-11,13(18)-diene-3β,30-diol (12a)——A solution of 92 mg of **10** in $CHCl_3$ was acidified with 0.5 ml of conc. HCl at room temperature. After standing for 10 min, the reaction mixture was evaporated at 60 °C to afford colorless needles, mp 230—232 °C. $[\alpha]_D$ (dioxane) −61.5°. Yield: 79 mg (90%). UV $\lambda_{max}^{dioxane}$ nm (log ε): 254 (4.23), 260 (4.21) (heteroannular diene). ^{13}C-NMR (d_5-pyridine) δ ppm: 137.8 (s), 134.2 (s), 126.0 (d), 125.9 (d), 78.1 (d, C-3), 73.4 (t, C-30), 55.4, 54.6. ^{1}H-NMR ($CDCl_3$) δ ppm: 6.42 (dd, 1H, J_1=4 Hz, J_2=10 Hz, C-11-H), 5.60 (br d, 1H, J=10 Hz, C-12-H), 3.30 (m, 1H, C-3-H). MS m/z Calcd for $C_{30}H_{48}O_2$: M^+ 440.3654. Found: M^+ 440.3658 (bp).

18β-Olean-12-ene-3β,30-diol (Deoxoglycyrrhetol) (4a)——Olean-12-ene-3β,11α or β-30-triol (**10** or **9**) (920 mg, 2 mM) dissolved in EtOH (100 ml, pretreated with $NaHCO_3$) was hydrogenated over 10% Pd–C (3.7 g). After uptake of H_2 (20 mM), the reaction mixture was worked up in the usual way. The product was purified by HPLC (Du Pont solvent system, $CHCl_3$–MeOH (96 : 4)) to obtain **4a**, mp 250—251 °C. $[\alpha]_D$ +87.5° (dioxane). Yield: 500 mg. ^{13}C-NMR (d_5-pyridine) δ ppm: 145.0 (s, C-13), 122.5 (d, C-12), 78.2 (d, C-3), 66.0 (t, C-30). ^{1}H-NMR ($CDCl_3$) δ ppm: 5.18 (1H, t, J=9 Hz, C-12-H), 3.50 (2H, br s, C-30-H_2), 3.18 (1H, t, J=8 Hz, C-3-H). MS m/z Calcd for $C_{30}H_{50}O_2$: M^+ 442.3811. Found: M^+ 442.3842.

30-*O*-Tritylate——(1) A mixture of **4a** (88.5 mg, 0.2 mM), tritylchloride (279 mg, 1.0 mM), triethylamine (0.02 ml) and 4-dimethylaminopyridine (24.5 mg, 0.2 mM) in CH_2Cl_2 was stirred under reflux for 20 h. Chloroform (100 ml) and water (100 ml) were added to the reaction mixture, and the organic layer was separated. After removal of the solvent, the yellowish residue was purified by column chromatography to afford colorless needles, mp 131—133 °C. $[\alpha]_D$ (dioxane) + 103.2°. Yield: 123 mg (90%). ^{13}C-NMR (d_5-pyridine) δ ppm: 144.9 (s, C-13), 122.5 (d, C-12), 128.2, 127.3, 125.1 (d, phenyl C of trityl group), 86.4 (s, trityl C), 78.1 (d, C-3), 66.1 (t, C-30). ^{1}H-NMR ($CDCl_3$) δ ppm: 7.55—7.2 (m, trityl H), 4.9—5.2 (1H, m, C_{12}-H), 3.12 (1H, t, J=8 Hz, C_3-H), 2.85 (2H, br s, C-30-H_2).

(2) A solution of **4a** (3.3 g) in dry dimethylformamide was treated with tritylpyridinium fluoroborate (3.5 g), and the solution was stirred at 60 °C for 48 h. After cooling, the solution was diluted with water (400 ml) and extracted with chloroform 3 times (400 ml each). The combined chloroform solution was washed with water 3 times (500 ml each) and dried. The residue obtained on evaporation of the organic solvent was treated as above to give colorless needles, mp 131—133 °C (Yield: 67.7%), identical with the sample prepared by method (1).

3β-*O*-β-Carboxypropionyl-30-*O*-trityl 18β-Olean-12-ene-3β,30-diol (3-*O*-β-Carboxypropionyl-30-*O*-trityl-deoxoglycyrrhetol)——In a 200 ml round-bottomed flask, fitted with a magnetic stirrer and a condenser protected from moisture, 30 ml of dry pyridine and 30 ml of dry triethylamine were placed. Under vigorous stirring, 5 g of succinic anhydride was added to the above mixture and a solution of 30-*O*-trityl 18β-olean-12-ene-3β,30-diol (2.5 g) in 30 ml of triethylamine was subsequently added. The reaction mixture was maintained at 60 °C for 24 h in an oil bath, and then poured into 100 ml of ice-water and acidified with HCl. The solution was extracted with 150 ml of $CHCl_3$ 3 times, and the combined extracts were separated by HPLC to afford 3β-*O*-β-carboxypropionyl-30-*O*-trityl 18β-olean-12-ene, colorless needles, mp 126 °C, $[\alpha]_D$ +87.7°. ^{13}C-NMR (d_5-pyridine) δ ppm: 173.2 (s, succinyl C=O), 144.9 (s, C-13), 129.3, 128.1, 127.2 (phenyl C of trityl group), 86.3 (s, trityl C), 80.7 (d, C-3), 66.2 (t, C-30), 55.6 (d, C-5).

The conditions of HPLC were as follows: Whatman silica gel column, 8 mm i.d. × 30 cm; solvent: 1% MeOH/$CHCl_3$; flow rate: 3 ml/min; t_R: 9.0 min.

3β-*O*-β-Carboxypropionyl 18β-Olean-12-ene-3β,30-diol (3-*O*-β-Carboxypropionyldeoxoglycyrrhetol) (4b)——(1) The 30-*O*-tritylate of **4a** (1 g) was dissolved in MeOH (50 ml) containing 10% HCOOH. The mixture was added to a suspension of 10% Pd–C (2.5 g) in MeOH under an atmosphere of N_2. After a few hours, the catalyst was filtered off and washed successively with CH_3OH and $CHCl_3$, and the combined filtrate was evaporated. The product was purified by HPLC (Du Pont column, 50 cm × 8 mm, packed with Lichrosorb SI-60 (5 μm) using $CHCl_3$–MeOH–AcOH (95 : 5 : 0.05) as the solvent system (3 ml/min) retention time, 12 min) to obtain colorless needles, mp 172—174 °C. $[\alpha]_D$ (dioxan) +79.3°. Yield: 0.59 g.

(2) The 30-*O*-tritylate of **4a** (2 g) was dissolved in 80% CH_3COOH (200 ml) and heated at 110 °C for 1 h. The reaction mixture was extracted with $CHCl_3$. The extracts were separated over a silica gel column using $CHCl_3$–CH_3OH as the developing solvent to obtain colorless needles, mp 172—174 °C. Yield: 1.25 g.

Methyl Ester of **4b**: MS *m/z* Calcd for $C_{35}H_{56}O_5$: M^+ 556.4128. Found: M^+ 556.4116.

3β,30-*O*-Di-β-carboxypropionyl 18β-Olean-12-ene-3β,30-diol 3β,30-Di-*O*-β-carboxypropionyldeoxoglycyrrhetol) (4c)——Compound **4a** (6.5 g) was dissolved in dried pyridine (400 ml). Next, succinic anhydride (144.1 g) and triethylamine (67 ml) were successively added, and the mixture was heated under stirring in an oil bath at 90—100 °C for 7 h. After cooling, the reaction mixture was poured into ice and dil. HCl to obtain dark-colored precipitates which were separated and washed with water. The precipitates were extracted 3 times with $CHCl_3$ (each 700 ml), and the $CHCl_3$ layer was washed 3 times with water (each 500 ml). The $CHCl_3$ was distilled off from the dried extracts to obtain the crude product (3.7 g), which was recrystallized from CH_3OH to obtain pure **4c**, mp 226—228 °C.

Dimethyl Ester of **4c**: MS *m/z* Calcd for $C_{40}H_{62}O_8$: M^+ 670.4445. Found: M^+ 670.4482.

4b: Compound **4c** (6.4 g) was dissolved in boiling ethanol (225 ml). A solution of KOH (3.2 g) in water (70 ml) was added to an ethanolic solution of **4c**, and refluxed for 40 min on a boiling water bath.

Then 10% HCl was added to separate crystals, which were extracted 3 times with $CHCl_3$ (each 150 ml). The combined $CHCl_3$ layer was washed with water (each 200 ml) and dried over anhydr. Na_2SO_4. The solvent was distilled off to obtain a mixture of products (5 g) which was chromatographed over silica gel using $CHCl_3$–CH_3OH as a developing solvent to separate **4b** (2.9 g), mp 172—174 °C, and **4a**, needles (2 g), mp 250—251 °C. **4a** was submitted to re-esterification.

3β,30-*O*-Di-*o*-phthaloyl 18β-Olean-12-ene-3β,30-diol (Deoxoglycyrrhetol 3β,30-Dihemiphthalate) (4e)——Phthalic anhydride (110 g) and triethylamine (35 ml) were added to a solution of **4a** (13.2 g) in dried pyridine (200 ml). The mixture was heated at 90—100 °C in an oil bath for 7 h under stirring. After cooling, the reaction mixture was poured into an excess amount of dil. HCl mixed with ice. The precipitates formed were filtered off, washed with water, and then transferred to a 1 l beaker containing water (700 ml) and heated on a water bath under stirring. The precipitates were filtered off while hot and washed with hot water 3 times. The product was recrystallized from MeOH to obtain a colorless powder, mp 162.4—164 °C. $[\alpha]_D^{20}$ +65.8° (*c* = 0.41, THF). Yield: 20.5 g. ^{13}C-NMR (C_5D_5N) δ: 169.8, 169.6, 168.9, 168.3 (s, phthaloyl C=O), 144.4 (s, C-13), 122.7 (s, C-12), 82.4 (d, C-3), 69.4 (t, C-30). *Anal.* Calcd for $C_{46}H_{58}O_8 \cdot 2H_2O$: C, 71.29; H, 8.06. Found: C, 71.13; H, 8.01.

Dimethyl Ester: MS *m/z* Calcd for $C_{48}H_{62}O_8$: M^+ 767. Found: M^+ 767.

Di-Na Salt: **4e** (14.8 g) was dissolved in THF (300 ml), and $^1/_{10}$ N NaOH (400 ml) was added. The mixture was refluxed for 30 min on a boiling water bath, and then concentrated to form a paste. THF (400 ml) was added to the paste, and the mixture as refluxed again to obtain colorless crystals, mp 294—295 °C. Yield: 15.2 g.

3β,30-*O*-Di-*o*-diphthaloyl 18β-Olean-9(11),12-diene-3β,30-diol (11c)——Compound **11a** (26.4 g) was treated with phthalic anhydride and pyridine as above to afford the 3β,30-*O*-dihemiphthalate, mp 157—158 °C. $[\alpha]_D^{20}$ +62.9° (*c* = 0.39, pyridine). ^{13}C-NMR (C_5D_5N) δ: 168.7, 168.5, 168.0, 167.1 (s, phthaloyl C=O), 153.0 (s, C-9), 145.0 (s, C-13), 121.5 (d, C-12), 115.0 (d, C-11), 81.1 (d, C-3), 68.2 (t, C-30). UV λ_{max}^{EtOH} nm: 203.5, 226, 282. *Anal.* Calcd for $C_{46}H_{56}O_8 \cdot 2H_2O$: C, 71.48; H, 7.82. Found: C, 71.13; H, 7.69.

Dimethyl Ester: MS *m/z* Calcd for $C_{48}H_{60}O_8$: M^+ 765. Found: M^+ 765.

Di-Na Salt: mp 279.5—281 °C.

3β,30-*O*-Di-*o*-phthaloyl 18β-Olean-11,13(18)diene-3β,30-diol (12c)——Compound **12a** (13.2 g) was treated with phthalic anhydride and pyridine as above to afford the 3β,30-*O*-dihemiphthalate, mp 156 °C. $[\alpha]_D^{20}$ −22.4° (*c* = 0.42, pyridine). Yield: 21 g. ^{13}C-NMR (C_5D_5N) δ: 169.0, 168.9, 167.8, 167.3 (s, phthaloyl C=O), 135.4, 132.7 (s, C-13, C-18 or *vice versa*), 124.9, 124.8 (d, C-11, C-12 or *vice versa*), 81.2 (d, C-3), 74.8 (t, C-30). UV λ_{max}^{EtOH} nm: 204, 236, 242, 250, 260.

Dimethyl Ester: MS *m/z* Calcd for $C_{48}H_{60}O_8$: M^+ 765. Found: M^+ 765.

Di-Na Salt: mp 297—298 °C.

3β-*O*-β-Carboxypropionyl 18β-Olean-9(11),12-diene-3β,30-diol (11b)——Succinic anhydride (12 g) and triethylamine (5.5 ml) were added to a solution of **11a** (2.2 g) in dried pyridine (35 ml), and the mixture was refluxed for 7 h in an oil bath. After cooling, the reaction mixture was poured into ice water, and an excess of dil. HCl was added to obtain a dark-colored precipitate, which was extracted 3 time with $CHCl_3$ (50 ml each). Dark-colored crystals separated from the $CHCl_3$ solution on evaporation. On purification over a silica gel column using $CHCl_3$–MeOH (98 : 2) as the developing solvent, 3β,30-di-*O*-β-carboxypropionyl 18β-olean-9(11)12-diene-3β,30-diol, mp 224 °C (2.7 g), was isolated.

On partial hydrolysis of the 3β,30-di-*O*-β-carboxypropionate by refluxing for 2.5 h with 3.2% KOH (29 ml) in EtOH (100 ml), **11b** (1.4 g) was obtained from the acidified reaction mixture by extraction with $CHCl_3$ and chromatography of the extract on silica gel using $CHCl_3$–MeOH (99 : 1) as the developing solvent. mp 201—215 °C. $[\alpha]_D^{20}$ +254° (c=0.50, $CHCl_3$). UV λ_{max}^{EtOH} nm: 203, 208 (EtOH). ^{13}C-NMR (d_5-pyridine) δ ppm: 174.5, 172.3 (s, succinyl C=O), 154.2 (s, C-9), 147.1 (s, C-13), 121.3 (d, C-12), 116 (d, C-11), 80.7 (d, C-3), 65.7 (t, C-30).

Methyl Ester: MS m/z Calcd for $C_{35}H_{54}O_5$: M^+ 554.3957. Found: M^+ 554.3953.

3β-*O*-β-Carboxypropionyl 18β-Olean-11,13(18)-diene-3β,30-diol (12b)——**12a** (2.2 g) was treated as described above for **11a** to obtain **12b** (1.3 g). mp 224—226 °C. $[\alpha]_D^{20}$ −55° (c=1.00, $CHCl_3$). UV λ_{max}^{EtOH} nm: 243, 251, 260. ^{13}C-NMR ($CDCl_3$) δ ppm: 177.0, 171.8 (s, succinyl C=O), 136.8, 134.2 (s, C-13, C-18 or *vice versa*), 125.8, 125.5 (d, C-11, C-12 or *vice versa*), 81.6, (d, C-3), 74.1 (d, C-30).

Methyl Ester: MS m/z Calcd for $C_{35}H_{54}O_5$: M^+ 554.3957. Found: M^+ 554.3980.

3β,30-Di-*O*-β-carboxypropionyl 18β-Olean-11,13(18)diene-3β,30-diol: mp 183 °C.

3β,23-Dihydroxy-11-oxo-18β-olean-12-ene-28-oic Acid (11-Oxohederagenin) (6)——3,23-*O*-Diacetylhederagenin [prepared from hederagenin (**5**) (5.5 g) by treatment with Ac_2O and pyridine at room temperature for 12 h] was dissolved in CCl_4 (44.8 ml) and the solution was stirred with AcOH (11.2 ml) and Ac_2O (2.9 ml) at 55 °C for 45 min. *tert*-Butylchromate, CrO_2 (O-*tert*-Bu)$_2$ (CrO_3: 7.6 g), in CCl_4 (41.1 ml) and a mixture of AcOH (11.2 ml) and Ac_2O (2.9 ml) were added to the above solution, and the reaction was allowed to proceed overnight at 60—65 °C. After cooling of the mixture, 10% oxalic acid solution (207 ml) was added dropwise under stirring for 1 h. The reaction mixture was extracted three times with $CHCl_3$ (500 ml each).

The solution was evaporated *in vacuo* and the residue was purified by silica gel column chromatography using hexane–acetone (9 : 1) as the solvent. The product was deacetylated with 3% KOH in 50% dioxane (300 ml) at room temperature for 2 h. The reaction mixture was treated as usual and purified by chromatography using $CHCl_3$ as the solvent to obtain 11-oxo-hederagenin (**6**), colorless needles, mp 294 °C. Yield: 3.07 g (53.5% Calcd from hederagenin (**5**)). MS m/z Calcd for $C_{30}H_{46}O_5$: M^+ 486.3345. Found: M^+ 486.3334. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1688 (COOH), 1644 ($\alpha\beta$ unsat. 6 membered C=O). ^{13}C-NMR (d_5-pyridine) δ ppm: 199.9 (s, C-11), 179.5 (s, C-28), 169.5 (s, C-13), 128.0 (d, C-12), 72.7 (d, C-3), 67.2 (t, C-24), 62.3 (d, C-9). ^{1}H-NMR ($CDCl_3$) δ ppm: 5.88 (1H, s, C-12H), 4.13 (2H, br s, C-24H), 3.59 (1H, dd, C-18H), 2.53 (1H, s, C-9H).

11-Oxo-18β-olean-12-ene-3β,30-diol (Glycyrrhetol) (3)——AcOH (1.3 ml) and Ac_2O (0.34 ml) were added to a solution of **4a** diacetate (650 mg) in CCl_4 (5.2 ml), and the mixture was kept at 55—60 °C for 45 min under continuous stirring. A solution consisting of AcOH (1.3 ml), Ac_2O and *tert*-butyl chromate, CrO_2 (O-*tert*-Bu)$_2$ (CrO_3: 0.9 g), dissolved in CCl_4 (7.3 ml) was added to the above mixture, and the whole was kept at 60—65 °C for 20 h under stirring. After cooling of the reaction mixture to room temperature, 10% aq. oxalic acid solution (24 ml) was added dropwise. After stirring for additional 1 h, the reaction mixture was extracted with $CHCl_3$. After removal of the solvent, the residue was deacetylated with 5% KOH–50% aq. dioxane under stirring for 30 min at 80 °C. The reaction product was purified by HPLC on a Shodex silica gel column (8 mm i.d. × 30 cm) with hexane–acetone (75 : 25) as the solvent (flow rate 2.5 ml/min) to obtain colorless needles, mp 281—282 °C. ^{13}C-NMR (d_5-pyridine) δ ppm: 199.4 (s, C-11), 169.7 (s, C-13), 128.5 (d, C-12), 77.8 (d, C-3), 65.4 (t, C-30), 62.1 (d, C-9), 55.3 (d, C-5). ^{1}H-NMR ($CDCl_3$) δ ppm: 5.59 (1H, s, C-12H), 3.48 (2H, br s, C-30-H_2), 3.19 (1H, t, J=8 Hz, C-3-H), 2.75 (1H, dt, J=12, 4 Hz), 2.30 (1H, s, C-9-H). MS m/z Calcd for $C_{30}H_{48}O_3$: M^+ 456.3603. Found: M^+ 456.3624.

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References and Notes

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