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Glycyrrhetinic acid derivatives as potent inhibitors of Na^+, K^+-ATPase. Synthesis and structure–activity relationships

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Summary — A series of ring A-modified GA derivatives (26 compounds) has been systematically synthesized and the structure–activity relationship investigated for inhibition of canine kidney Na^+, K^+-ATPase *in vitro*. The most potent inhibitory activity was found with a group of the 3-deoxygenated derivatives including **5**, **6**, **9**, **10** and **11**. The high inhibition may be closely related to the hydrophobic character of the A-ring of these compounds. This finding suggests that the ATP-binding site at the active center of the enzyme is located in a hydrophobic environment.

Na^+, K^+-ATPase inhibitor / ring A-modified glycyrrhetinic acid derivative / hydrophobic character / structure–activity relationship

Introduction

Glycyrrhizin (GL) and 18β-glycyrrhetinic acid (GA), a saponin and its aglycone from licorice root, are known to possess a variety of pharmacological properties including antiinflammatory, antiallergic, and antiulcer activities [1]. Recently, two of us (KI and TH) [2] found that GL and 18β-GA induce dose-dependent *in vitro* inhibition of canine kidney Na^+, K^+-ATPase. They also demonstrated that the enzyme inhibition is competitive with regard to ATP and that the inhibitory activity of 18β-GA (IC_{50} of 10^{-4} M) is 10 times more potent than that of GL (10^{-3} M).

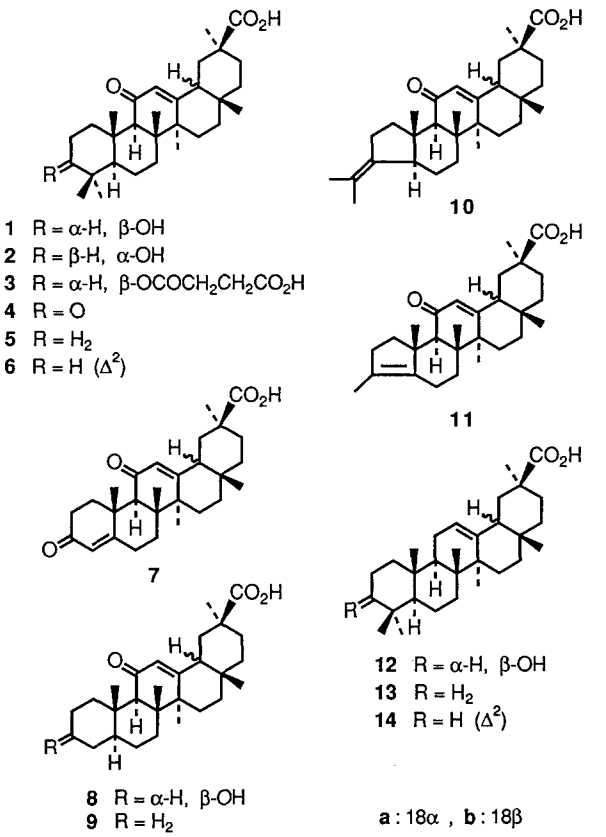
We have systematically prepared various GA derivatives of both 18α- and 18β-series, in which the ring A was primarily modified for structure–activity study. In the present paper, we report their syntheses and inhibitory effects on Na^+, K^+-ATPase activity.

Results and discussion

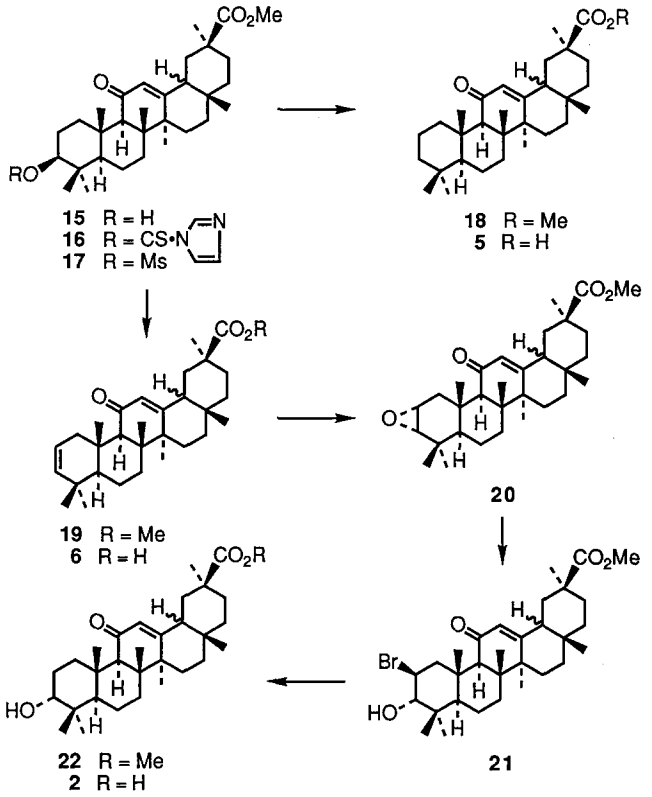
Chemistry

The GA derivatives tested in this study are shown in scheme 1. All were chemically synthesized starting from commercially available 18α- (**1a**) and 18β-GA (**1b**). Compounds **3**, **4**, **7**, **10**, **11** and **12** were prepared by literature procedures.

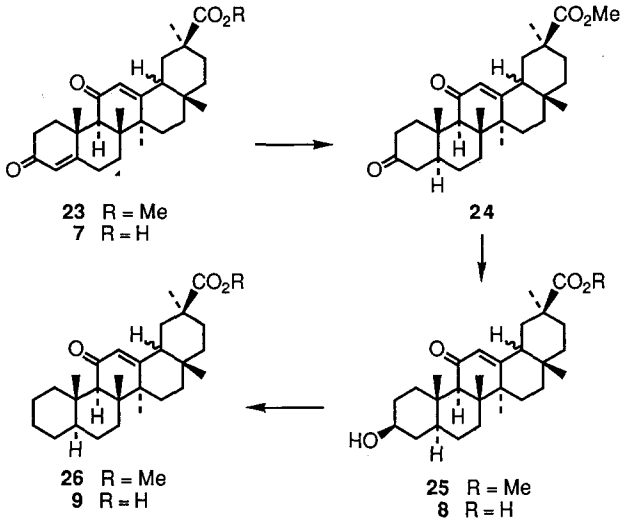
The corresponding methyl ester **15**, obtained from GA, was made to react with an axcess of *N,N'*-thiocarbonyldiimidazole at 100°C (scheme 2). When the



Scheme 1.



Scheme 2.



Scheme 3.

resultant 3-imidazolide **16** was treated with tributylstannane in the presence of azobisisobutyronitrile (AIBN) in refluxing toluene, facile deoxygenation occurred without rearrangement to give the 3-deoxy derivative **18** [3]. Subsequent ester cleavage was effected by refluxing with lithium chloride in 2,4,6-collidine to obtain the acid **5**. Alternatively, **15** was converted to the mesylate **17**. On heating in collidine at 180°C, **17** predominantly gave the 2-olefin **19** which was similarly demethylated to the acid **6**. In order to clarify the stereochemistry, 3-epi GA (**2**) was prepared in four steps from the above olefin **19**. Epoxidation of **19** with *m*-chloroperbenzoic acid gave the 2α,3α-oxide **20** as the major product. Oxide ring cleavage with hydrobromic acid led to the bromohydrin **21** with a 2β-bromo, 3α-hydroxy trans stereochemistry as expected. Reductive debromination of **21** with tributylstannane followed by demethylation gave the desired **2**.

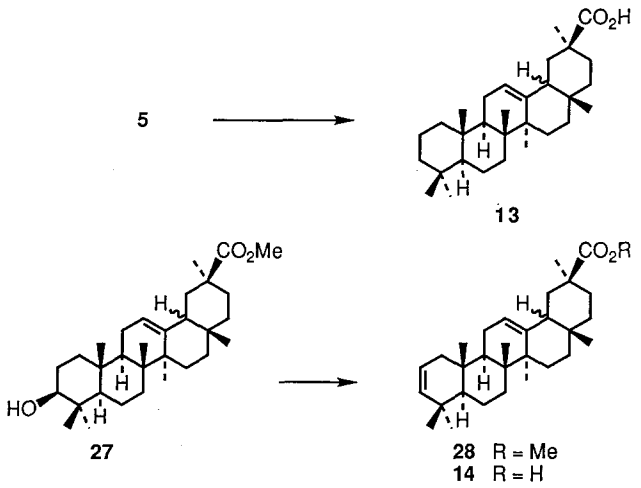
The 4,4-desmethyl analogs **8** and **9** were derived from the already known 3-keto-Δ^4-methyl ester **23** [1, 4, 5] which furnished on demethylation the acid **7**. Treatment of **23** with diisobutylaluminium hydride (DIBAL-H) in the presence of *t*-butylcopper and hexamethylphosphoramide (HMPA) stereoselectively induced conjugate reduction [6, 7] of only the Δ^4-3-keto moiety to predominantly give the 5α-ketone **24** (scheme 3). The A/B trans stereochemistry was clearly established by X-ray analysis [8]. Selective reduction of the 3-keto group with sodium borohydride and successive demethylation led to 4,4-desmethyl GA (**8**). When the intermediate ester **25** was subjected to the foregoing deoxygenation followed by demethylation, **9**, the 4,4-desmethyl analog of **5** was obtained.

The ring A-contracted compounds **10** and **11** were obtained likewise from the corresponding esters [1] which were previously prepared as intermediates in the synthesis of **23**.

In parallel with A-ring modification, reductive removal of the 11-oxo group was carried out for several compounds (scheme 4). The foregoing acid **5** was directly subjected to catalytic hydrogenation with platinium oxide in acetic acid [9–11] to obtain the 11-deoxo acid **13**. The known ester **27**, similarly obtained from **15**, was dehydrated *via* the mesylate as described above. The resulting diene **28** was finally demethylated to the acid **14**.

Bioassay

The inhibitory effects on Na^+, K^+-ATPase activity by synthetic GA derivatives and GA (**1**) and ouabain for comparison are listed in table I. The enzyme assay was carried out using a partially purified ouabain-sensitive Na^+, K^+-ATPase obtained from canine kidney according to the previously published procedure [2]. Many of the more active compounds were tested at several dose levels and their inhibitory activities were expressed as an average value of IC_{50}.



Scheme 4.

Table I. Inhibitory effects of glycyrrhetinic acid derivatives on Na^+, K^+-ATPase activity *in vitro*.

Compd	*IC_{50} (M)*	
	***a**: 18α*	***b**: 18β*
1	7.2×10^{-5}	6.7×10^{-5}
2	7.2×10^{-5}	4.2×10^{-5}
3	20[a]	6.1×10^{-4}
4	70[a]	8.7×10^{-5}
5	1.3×10^{-5}	8.1×10^{-6}
6	5.8×10^{-6}	8.0×10^{-6}
7	19[a]	2.7×10^{-4}
8	25[a]	8.4×10^{-4}
9	4.5×10^{-6}	9.5×10^{-6}
10	1.2×10^{-5}	8.7×10^{-6}
11	9.0×10^{-6}	8.8×10^{-6}
12	4.8×10^{-5}	1.1×10^{-4}
13	1.4×10^{-5}	4.2×10^{-5}
14	2.2×10^{-5}	5.5×10^{-5}
Ouabain	5.0×10^{-7}	

[a]Inhibition (%) at 2×10^{-4} M.

Structure–activity relationships

Table I shows that structural modification of the A-ring has a striking effect on inhibitory activity. In comparison with the parent GA (**1**), replacement of the 3β-OH by an α-OH or an oxo group showed essentially no variation for the inhibitory effect (**1** *vs* **2** or **4**). However, masking of the OH group by esterification, as in the case of carbenoxolone (**3b**), greatly reduced the enzyme inhibition (**1** *vs* **3**). In sharp contrast, elimination of the 3-oxygen functions led to more potent inhibition (**1** *vs* **5** or **6**). Removal of the 4,4-dimethyl groups significantly decreased the inhibition if an oxygen function was present at the 3-position (**1** *vs* **8**). In spite of the same modification, an equipotent inhibitory effect was retained in the 3-deoxy series (**5** *vs* **9**). Moreover, absence of oxygen functions at the 3-position appeared to be a predominant factor for the high inhibition over any skeletal alteration of the ring A (**8** *vs* **9**, **10**, or **11**). Other modifications, such as additional removal of the 11-oxo group exhibited no significant effect on the enzyme inhibition in a few cases (**1** *vs* **12**, **5** *vs* **13**). However, a substantial loss of activity was observed with the Δ^2-analog **14** (**6** *vs* **14**). Throughout this assay, no essential difference was found for the inhibitory effect between the 18α- and 18β-series.

Conclusion

A certain range of modifications of the ring A is undoubtedly responsible for the enhancement of the Na^+, K^+-ATPase inhibition. Among the 26 synthetic compounds tested, the most potent inhibitors were **5**, **6**, **9**, **10**, and **11** which have in common no oxygen function at the 3-position. Most of these compounds exhibited inhibitory activities (IC_{50} for 10^{-6} M) approximately 10 times as potent as the parent GA (**1**) (10^{-5} M). Thus, the more potent inhibition may be ascribed to the increased hydrophobicity in ring A of these derivatives. From this point of view, the ATP-binding site at the active center of Na^+, K^+-ATPase may be located in a hydrophobic environment.

Further study is under consideration on *in vivo* inhibition by our selected compounds as more potent inhibitors of Na^+, K^+-ATPase, which should be useful for therapeutic treatment of hypertension and cardiac malfunction.

Experimental protocols

Unless otherwise stated, melting points were determined on a Yanagimoto Micro Melting Point Apparatus and are uncorrected. ^1H NMR spectra, taken on a Varian VXR-200 200 MHz spectrometer, were run in $CDCl_3$ solution using Me_4Si as an internal standard. IR spectra were recorded in $CHCl_3$ solution on a Jasco IR-700 spectrometer. Ms spectra were obtained with a Hitachi M-68 spectrometer. Silica gel precoated plates (Merck, F-254, 20 x 20 x 0.05 cm) were used for preparative TLC. Silica gel 60 (Merck, particle size 0.063–0.2 mm) was used for ordinary column chromatography. Preparative HPLC was performed using some prepacked silica gel columns (Merck). Usual workup means washing extracts with water and then brine, drying over Na_2SO_4, filtration, and evaporation *in vacuo*.

18β- and 18α-GA (**1a** and **1b**) were purchased from Wako Pure Chemical Ind Ltd, and Sigma Chemical Co, respectively. Methylation with diazomethane afforded both methyl esters (**15a** and **15b**) which were also used as the starting materials in synthetic transformations.

3-O-(β-Carboxypropyl)-11-oxo-18ξ-olean-12-en-30-oic acid ***3***
This was prepared by the previously established procedure [12]. **3a**: mp 296–298°C ($CHCl_3$-acetone-*i*-propyl ether); ν_{max} (cm^{-1}) 3500–2500, 1711, 1654; δ (ppm) 0.73 (s, 3H, 28-H), 0.88 (bs, 6H, 23- & 24-H), 1.14 (s, 3H, 25-H), 1.22 (s, 3H, 26-H), 1.26 (s, 3H, 29-H), 1.34 (s, 3H, 27-H), 2.67 (m, 4H, ester CH_2), 4.55 (m, 1H, 3-H), 5.59 (s, 1H, 12-H); *m/e* 570 (M^+). **3b** (Carbenoxolone): mp 313–315°C ($CHCl_3$-MeOH-acetone-*i*-propyl ether); ν_{max} (cm^{-1}) 3500–2500, 1709, 1646; δ (ppm) 0.84 (s, 3H, 28-H), 0.88 (bs, 6H, 23- & 24-H), 1.13 (s, 3H, 25-H), 1.16 (s, 3H, 26-H), 1.22 (s, 3H, 29-H), 1.37 (s, 3H, 27-H), 2.67 (m, 4H, ester CH_2), 4.56 (m, 1H, 3-H), 5.70 (s, 1H, 12-H); *m/e* 570 (M^+).

3,11-Dioxo-18ξ-olean-12-en-30-oic acid ***4***
This was prepared by Jones oxidation of GA (**1**). **4a**: mp > 310°C (CH_2Cl_2-MeOH); ν_{max} (cm^{-1}) 3500–2500, 1694, 1652; δ (ppm) 0.75 (s, 3H, 28-H), 1.06 (s, 3H, 24-H), 1.10 (s, 3H, 23-H), 1.18 (s, 3H, 26-H), 1.27 (s, 3H, 25-H), 1.34 (s, 3H, 29-H), 1.36 (s, 3H, 27-H), 5.65 (bs, 1H, 12-H); *m/e* 468 (M^+). **4b**: mp 298–302°C (CH_2Cl_2-MeOH); ν_{max} (cm^{-1}) 3500–2500, 1694, 1647; δ (ppm) 0.85 (s, 3H, 28-H), 1.05 (s, 3H, 24-H), 1.11 (s, 3H, 23-H), 1.18 (s, 3H, 26-H), 1.23 (s, 3H, 29-H), 1.28 (s, 3H, 25-H), 1.38 (s, 3H, 27-H), 5.75 (s, 1H, 12-H); *m/e* 468 (M^+).

Methyl 11-oxo-18β-olean-12-en-30-oate ***18b***
A stirred mixture of **15b** (485 mg, 1 mmol) and 1,1'-thiocarbonyldiimidazole (446 mg, 2.5 mmol) in dry $ClCH_2CH_2Cl$ (5 ml) was heated at 100°C under N_2 for 2.5 h. The mixture was poured into cold N-HCl and extracted with CH_2Cl_2. The extract was washed with satd $NaHCO_3$ solution followed by usual workup. The crude imidazolide **16b** obtained (pure material, mp 242–243°C: ν_{max} (cm^{-1}) 1720, 1650, 1281) was dissolved in dry toluene (20 ml). To the resulting solution *n*-Bu_3SnH (0.54 ml, 582 mg, 2 mmol) was added together with AIBN (8.2 mg, 0.05 mmol). The mixture was heated at 125°C under N_2 for 1.5 h. The residue, obtained after removal of the solvent, was subjected to purification by preparative HPLC (Lobar Size B x 2, 20:1 $CHCl_3$-acetone). The material isolated was crystallized from CH_2Cl_2–ether to yield **18b** (318.7 mg, overall 68.0%), mp 222–223°C: ν_{max} (cm^{-1}) 1720, 1649; δ (ppm) 0.80 (s, 3H, 28-H), 0.84 (s, 3H, 24-H), 0.87 (s, 3H, 25-H), 1.13 (s, 3H, 23-H), 1.15 (s, 6H, 26-& 29-H), 1.38 (s, 3H, 27-H), 3.69 (s, 3H, 30-Me), 5.65 (bs, 1H, 12-H); *m/e* 468 (M^+).

Methyl 11-oxo-18α-olean-12-en-30-oate ***18a***
As above, **15a** (485 mg, 1 mmol) was deoxygenated to furnish **18a** (321.4 mg, overall 68.6%), mp 239–242°C (CH_2Cl_2-ether): ν_{max} (cm^{-1}) 1717, 1654; δ (ppm) 0.71 (s, 3H, 28-H), 0.84 (s, 3H, 24-H), 0.87 (s, 3H, 23-H), 1.14 (s, 3H, 25-H), 1.21 (s, 3H, 29-H), 1.22 (s, 3H, 26-H), 1.35 (s, 3H, 27-H), 3.69 (s, 3H, 30-OMe), 5.56 (bs, 1H, 12-H); *m/e* 468 (M^+).

11-Oxo-18β-olean-12-en-30-oic acid ***5b***
A stirred mixture of **18b** (328.1 mg, 0.7 mmol), LiI (328 mg, 2.45 mmol), and dry 2,4,6-collidine (9 ml) was refluxed under N_2 for 3 h. The mixture was poured into cold 2N-HCl and extracted with CH_2Cl_2. After usual workup, the product was crystallized from CH_2Cl_2 (MeOH)-ether to give **5b** (272.0 mg, 85.5%), mp 310–315°C: ν_{max} (cm^{-1}) 3500–2500, 1698, 1648; δ (ppm) 0.84 (s, 6H, 24- & 28-H), 0.87 (s, 3H, 25-H), 1.13 (s, 3H, 23-H), 1.15 (s, 3H, 26-H), 1.23 (s, 3H, 29-H), 1.38 (s, 3H, 27-H), 5.70 (bs, 1H, 12-H); *m/e* 454 (M^+). Anal $C_{30}H_{46}O_3$ (C, H).

11-Oxo-18α-olean-12-en-30-oic acid ***5a***
Similarly, **18a** (315 mg, 0.67 mmol) was demethylated with LiI (315 mg, 2.35 mmol) and dry collidine (9 ml) on refluxing for 3 h. The product was crystallized from CH_2Cl_2 (MeOH)-ether, giving **5a** (272.2 mg, 89.1%), mp 312–318°C: ν_{max} (cm^{-1}) 3500–2500, 1697, 1654; δ (ppm) 0.73 (s, 3H, 28-H), 0.84 (s, 3H, 24-H), 0.87 (s, 3H, 23-H), 1.14 (s, 3H, 25-H), 1.20 (s, 3H, 29-H), 1.26 (s, 3H, 26-H), 1.36 (s, 3H, 27-H), 5.60 (bs, 1H, 12-H); *m/e* 454 (M^+). Anal $C_{30}H_{46}O_3$ (C, H).

11-Oxo-18β-oleana-2,12-dien-30-oic acid ***6b***
Mesyl chloride (0.035 ml, 51.5 mg, 0.45 mmol) was added dropwise to a stirred solution of **15b** (145.4 mg, 0.3 mmol) in dry CH_2Cl_2 (3 ml) containing EtN_3 (0.125 ml, 91.1 mg, 0.9 mmol). The mixture was stirred at 2–3°C for 40 min, then poured into cold 2N-HCl and extracted with CH_2Cl_2. After usual workup, the crude mesylate (pure material, mp 175–177°C; ν_{max} (cm^{-1}) 1720, 1652, 1352, 1330, 1190) was dissolved in dry collidine (6 ml). The resultant mixture was heated at 180°C under N_2 for 2 h (at this stage, isolation of product gave the pure methyl ester **19b**, mp 224–228°C (acetone-pentane): *m/e* 466 (M^+), identical to the previously prepared material [12, 13]). To it, LiI (140.5 mg, 1.05 mmol) was added and the mixture was heated again at 180°C for 2.5 h. The solvent was removed and the residue was extracted with $CHCl_3$. The extract was washed with 2 N-H_2SO_4 followed by usual workup. The crude product was purified by preparative TLC (95:5 $CHCl_3$-MeOH), giving **6b** (117.4 mg, overall 86.5%), mp 290–296°C (CH_2Cl_2-ether). Recrystallization from $CHCl_3$-acetone afforded an analytical specimen, mp 300–304°C: ν_{max} (cm^{-1}) 3500–2500, 1700, 1646; δ (ppm) 0.87 (s, 3H, 28-H), 0.92 (s, 3H, 24-H), 0.98 (s, 3H, 25-H), 1.18 (s, 6H, 23- & 26-H), 1.24 (s, 3H, 29-H), 1.38 (s, 3H, 27-H), 5.40 (m, 2H, 2- & 3-H), 5.75 (s, 1H, 12-H); *m/e* 452 (M^+). Anal $C_{30}H_{44}O_3$ (C, H).

11-Oxo-18α-oleana-2,12-dien-30-oic acid ***6a***
In a similar manner, **15a** (145.4 mg, 0,3 mmol) was dehydrated *via* its mesylate (mp 189–192°C, ν_{max} (cm^{-1}) 1710, 1654, 1351, 1330, 1170) and followed by the foregoing ester cleavage. Preparative TLC of the crude product gave **6a** (121.7 mg, overall 89.6%), mp 292–298°C (CH_2Cl_2-ether). Recrystallization from $CHCl_3$-acetone afforded an authentic sample, mp 303–306°C; ν_{max} (cm^{-1}) 3500–2500, 1711, 1653; δ (ppm) 0.75 (s, 3H, 28-H), 0.92 (s, 3H, 24-H), 0.97 (s, 3H, 25-H), 1.16 (s, 3H, 29-H), 1.23 (s, 3H, 23-H), 1.27 (s, 3H, 26-H), 1.35 (s, 3H, 27-H), 5.39 (m, 2H, 2- & 3-H), 5.65 (bs, 1H, 12-H); *m/e* 452 (M^+). Anal $C_{30}H_{44}O_3$ (C, H). The extractive workup before ester cleavage furnished **19a**, mp 244–246°C (CH_2Cl_2-acetone-pentane): *m/e* 466 (M^+), identical to the previously prepared sample [13, 14].

Methyl 2α,3α-oxido-11-oxo-18β-olean-12-en-30-oate ***20b***
m-Chloroperbenzoic acid (*m*-CPBA, 164 mg, 0.95 mmol) was added portionwise to a stirred solution of **19b** (186.7 mg, 0.4 mmol) in dry CH_2Cl_2 (3 ml). The mixture was allowed to stand at room temperature overnight, then poured into cold satd $NaHCO_3$ solution, and extracted with CH_2Cl_2. After usual workup, the residue was crystallized from CH_2Cl_2-acetone gave **20b** (110.1 mg, 57.0%), mp 284–285°C: *m/e* 482 (M^+), identical to the previously prepared material [13, 14]. The mother-liquor residue was further purified by preparative TLC (10:1 benzene-EtOAc) which afforded an additional crop of **20b** (35.4 mg, 18.3%) together with the isomeric 2β,3β-oxide (18.7 mg, 9.7%), mp 240–245°C (CH_2Cl_2-acetone).

Methyl 2α,3α-oxido-11-oxo-18α-olean-12-en-30-oate ***20a***
As described above, **19a** (163.3 mg, 0.35 mmol) was oxidized with *m*-CPBA (120.8 mg, 0.7 mmol) in CH_2Cl_2 (4 ml) on standing at room temperature overnight. The crude product was purified by crystallization followed by preparative TLC, giving **20a** (128 mg, 75.8%), mp 264–267°C (CH_2Cl_2-acetone): *m/e* 482 (M^+) and the epimeric 2β,3β-oxide (10 mg, 5.9%), mp 271–275°C (CH_2Cl_2-acetone-hexane), identical to the previously synthesized material [13, 14].

3α-Hydroxy-11-oxo-18β-olean-12-en-30-oic acid ***2b***
To a stirred solution of **20b** (482.7 mg, 1 mmol) in dry dioxane (20 ml) dropwise 47% hydrobromic acid was added at 10–12°C. The mixture was stirred at the same temperature for 1 h, then poured into cold satd $NaHCO_3$ solution, and extracted with CH_2Cl_2. Usual workup yielded the crude bromohydrin **21b** (623 mg) which was dissolved in dry benzene (25 ml). To it, *n*-Bu_3SnH (0.4 ml, 436.6 mg, 1.5 mmol) and AIBN (8.2 mg, 0.05 mmol) were added. The mixture was refluxed under N_2 for 2.5 h and then concentrated *in vacuo*. The residue was eluted through a short column of silica gel with CH_2Cl_2. Removal of the solvent furnished the crude ester **22b** (606 mg) (pure material, mp 215–217°C (acetone-pentane): ν_{max} (cm^{-1}) 3614, 3444, 1719, 1648; *m/e* 484 (M^+)) which was demethylated as before using LiI (485 mg, 3.6 mmol) and collidine (13 ml) under refluxing for 3 h. Extraction with CH_2Cl_2 followed by usual workup gave a viscous syrup (630 mg) which was roughly chromatographed on silica gel (2 g). The fractions eluted with CH_2Cl_2-MeOH mixtures (30:1-10:1) were combined and evaporated to yield a crystalline residue. Recrystallization from CH_2Cl_2-MeOH afforded pure **2b** (total 355.1 mg, overall 75.4%), mp > 325°C: δ (ppm) 0.82 (s, 3H, 28-H), 0.86 (s, 3H, 24-H), 0.96 (s, 3H, 23-H), 1.13 (s, 3H, 25-H), 1.14 (s, 3H, 26-H), 1.19 (s, 3H, 29-H), 1.38 (s, 3H, 27-H), 3.42 (t, 1H, J = 2.5 Hz, 3-H), 5.65 (s, 1H, 4-H); *m/e* 470 (M+). Anal $C_{30}H_{46}H_4$ (C, H).

3α-Hydroxy-11-oxo-18α-olean-12-en-30-oic acid ***2a***
In a similar manner, **20a** (482.7 mg, 1 mmol) was converted to the crude bromohydrin **21a** (670 mg) which was further subjected to reductive debromination. After chromatography on silica gel the crude ester **22a** (590 mg) was isolated (pure material, mp 244–246°C (acetone-pentane): ν_{max} (cm^{-1}) 3614, 3476, 1717, 1653; *m/e* 484 (M^+)) which was finally demethylated as above. The crude product was purified by column chromatography followed by crystallization, affording pure **2a** (total 332 mg, overall 70.5%), mp > 325°C ($CHCl_3$-MecH): δ (ppm) 0.73 (s, 3H, 28-H), 0.87 (s, 3H, 24-H), 0.96 (s, 3H, 23-H), 1.15 (s, 3H, 25-H), 1.20 (s, 3H, 26-H), 1.24 (s, 3H, 29-H), 1.38 (s, 3H, 27-H), 3.43 (t, 1H, J = 2.5 Hz, 3-H), 5.56 (s, 1H, 12-H); *m/e* 470 (M^+). Anal $C_{30}H_{46}O_4$ (C, H).

4,4-Desmethyl-3,11-dioxo-18ξ-oleana-4,12-dien-30-oic acid 7
According to the method of the Searle group [1], the corresponding ester **23** was prepared in five to six steps from **15** in overall 20–25% yield. Finally, **23** was demethylated to **7**. **7a**: mp 318–323°C (CH_2Cl_2-acetone); ν_{max} (cm^{-1}) 3600–2500, 1695, 1657, 1614; δ (ppm) 0.77 (s, 3H, 28-H), 1.28 (s, 3H, 29-H), 1.33 (s, 6H, 26- & 27-H), 1.55 (s, 3H, 25-H), 5.72 (s, 1H, 12-H), 5.76 (s, 1H, 4-H); *m/e* 438 (M^+). **7b**: mp 308–313°C (CH_2Cl_2–acetone); ν_{max} (cm^{-1}) 3500–2500, 1698, 1657, 1617; δ (ppm) 0.89 (s, 3H, 28-H), 1.24 (s, 3H, 29-H), 1.33 (s, 3H, 26-H), 1.36 (s, 3H, 27-H), 1.43 (s, 3H, 25-H), 5.77 (s, 1H, 4-H), 5.83 (s, 1H, 12-H); *m/e* 438 (M^+).

Methyl 4,4-desmethyl-3,11-dioxo-18β-olean-12-en-30-oate ***24b***
To a stirred solution of CuI (190.5 mg, 1 mmol) in dry THF (5 ml) 1.54 M *t*-BuLi in pentane (0.8 ml, 1.2 mmol) was added dropwise at –50°C. After the mixture was stirred at the same temperature for 15 min, dry HMPA (3 ml) was added dropwise. The mixture was then cooled to –78°C and a solution of **23b** (452.6 mg, 1 mmol) in dry THF (3 mmol) was introduced. To the resultant mixture, 1 M diisobutylaluminium hydride (DIBAH) in THF (1.2 ml, 1.2 mmol) was added dropwise at –78°C. After stirring for 0.5 h, additional DIBAH in THF (1.2 ml, 1.2 mmol) was added. After 15 min, the temperature was allowed to rise to –40°C (2 h). The mixture was quenched with cold 3 N-HCl and extracted with CH_2Cl_2. The residue obtained by usual workup was treated with 8N-Jone reagent (1 mmol) in 1:1 CH_2Cl_2-acetone (25 ml) at room temperature for 2 h (for back-oxidation). After quenching with *i*-PrOH, the mixture was worked up as usual. The crude product was purified by preparative TLC (4:1 benzene-EtOAc with double development), giving **24b** (317 mg, 69.7%), mp 298–300°C (ether). This material proved to be a mixture of 5α- and 5β-epimers (68:32 by HPLC). Repeated crystallization from CH_2Cl_2-acetone and/or CH_2Cl_2-ether afforded pure **24b** (100% pure, 228 mg, 50.2%), mp 300–302°C; ν_{max} (cm^{-1}) 1708, 1651, 1616; δ (ppm) 0.83 (s, 3H, 28-H), 1.15 (s, 3H, 29-H), 1.19 (s, 3H, 26-H), 1.28 (s, 3H, 25-H), 1.38 (s, 3H, 27-H), 3.70 (s, 3H, 30-OMe), 5.73 (s, 1H, 12-H); *m/e* 454 (M^+). The stereostructure was ultimately established by X-ray analysis.

Methyl 4,4-desmethyl-3,11-dioxo-18α-olean-12-en-30-oate ***24a***
In a similar manner, **23a** (316.8 mg, 0.7 mmol) was subjected to conjugate reduction using CuI (133.3 mg, 0.7 mmol) in dry THF (2.5 ml), 1.54 M *t*-BuLi in pentane (0.55 ml, 0.84 mmol), dry HMPA (3 ml), dry THF (total 5.5 ml) and 1 M DIBAH in THF (total 3.5 ml, 3.5 mmol). After back-oxidation with 8N-Jone reagent, the mixture was worked up as usual. The crystalline residue was purified by preparative TLC (4:1 benzene-EtOAc with double development), giving **24a** (219.4 mg, 68.9 %) which proved to be a mixture of 5α- and 5β-epimers (71:29 by HPLC). Repeated crystallization from CH_2Cl_2-acetone gave the pure **24a** (94% pure, 149.9 mg, 47.1%), mp 305–307°C: ν_{max} (cm^{-1}) 1710, 1655, 1618; δ (ppm) 0.73 (s, 3H, 28-H), 1.19 (s, 3H, 29-H), 1.23 (s, 3H, 26-H), 1.34 (s, 3H, 25-H), 1.35 (s, 3H, 27-H), 3.70 (s, 3H, 30-OMe), 5.64 (bs, 1H, 12-H); *m/e* 454 (M^+). The stereostructure was finally established by X-ray analysis.

4,4-Desmethyl-3β-hydroxy-11-oxo-18β-olean-12-en-30-oic acid ***8b***
To a stirred solution of **24b** (90.9 mg, 0.2 mmol) in THF (3 ml) and MeOH (3 ml) $NaBH_4$ (7.6 mg, 0.2 mmol) was added in portions. Stirring was continued at room temperature for 40 min. The mixture was poured into cold NH_4Cl solution and extracted with $CHCl_3$. Usual workup gave a foamy product which was purified by preparative TLC (9:1 benzene-acetone with multiple development), furnishing the 3β-alcohol **25b** (76.9 mg, 84.2%), mp 253–255°C (CH_2Cl_2-acetone-dii-propyl ether): ν_{max} (cm^{-1}) 3602, 3452, 1720, 1649, 1615; *m/e* 456 (M^+). As described above, **25b** (68.5 mg, 0.15 mmol) was demethylated using LiI (70.3 mg, 0.53 mmol) in collidine (2 ml) at 180°C for 3.5 h. Preparative TLC (9:1 $CHCl_3$-MeOH) of the crude product gave **8b** (62.8 mg, 94.6%), mp 305–308°C ($CHCl_3$-acetone): ν_{max} (cm^{-1}) 3600, 3420, 1698, 1649; δ (ppm, 9:1 $CDCl_3$-CD_3OD) 0.83 (s, 3H, 28-H), 1.08 (s, 3H, 25-H), 1.14 (s, 3H, 26-H), 1.18 (s, 3H, 29-H), 1.39 (s, 3H, 27-H), 3.63 (s, 3H, 30-OMe), 5.67 (s, 1H, 12-H); *m/e* 442 (M^+). Anal $C_{28}H_{42}O_4$ (C, H).

4,4-Desmethyl-3β-hydroxy-11-oxo-18α-olean-12-en-30-oic acid ***8a***
Similarly, **24a** (90.9 mg, 0.2 mmol) was reduced with $NaBH_4$ (7.6 mg, 0.2 mmol) in 1:1 THF-MeOH (6 ml). The crude product was purified by preparative TLC (2:1 benzene-EtOAc) to yield the 3β-alcohol **25a** (71.3 mg, 78.1%), mp 240–242°C (CH_2Cl_2-ether): ν_{max} (cm^{-1}) 3602, 3456, 1717, 1655, 1618; *m/e* 456 (M^+). When **25a** (45.7 mg, 0.1 mmol) was heated at 180°C for 3.5 h with LiI (46.8 mg, 0.35 mmol) in collidine (1.5 ml), **8a** (41.0 mg, 92.6%) was obtained, mp 307–309°C ($CHCl_3$-acetone): δ (ppm, 9:1 $CDCl_3$-CD_3OD) 0.73 (s, 3H, 28-H), 1.14 (s, 3H, 25-H), 1.15 (s, 3H, 26-H), 1.24 (s, 3H, 29-H), 1.37 (s, 3H, 27-H), 3.61 (s, 3H, 30-OMe), 5.59 (bs, 1H, 12-H); *m/e* 442 (M^+). Anal $C_{28}H_{42}O_4$ (C, H).

4,4-Desmethyl-11-oxo-18β-olean-12-en-30-oic acid ***9b***
As described above, **25b** (88.7 mg, 0.194 mmol) was refluxed for 3 h with 1,1-thiocarbonyldiimidazole (71.3 mg, 0.4 mmol) in dry $ClCH_2CH_2Cl$ (2 ml). The product imidazolide (pure material, mp 228–229°C: ν_{max} (cm^{-1}) 1720, 1650) was deoxygenated by heating at 130°C for 3 h with *n*-Bu_3SnH (0.089 ml, 96.5 mg, 0.33 mmol) and AIBN (2.7 mg, 0.017 mmol) in dry toluene (3 ml). The crude product was purified by preparative TLC (9:1 cyclohexane-acetone with double development) to give the 3-deoxy ester **26b** (58.0 mg, 67.8%), mp 255–263°C (ether-pentane): ν_{max} (cm^{-1}) 1716, 1645, 1614; *m/e* 440 (M^+). Demethylation of **26b** (58 mg, 0.132 mmol) with LiI (61.7 mg, 0.46 mmol) in dry collidine (2 ml) at 180°C for 2 h followed by preparative TLC (9:1 $CHCl_3$-MeOH with double development) afforded **9b** (24.3 mg, 43.3%), mp 298–300°C ($CHCl_3$-acetone): ν_{max} (cm^{-1}) 3500–2500, 1697, 1648, 1617; δ (ppm) 0.84 (s, 3H, 28-H), 1.08 (s, 3H, 25-H), 1.14 (s, 3H, 26-H), 1.40 (s, 3H, 27-H), 1.23 (s, 3H, 29-H), 5.72 (s, 1H, 12-H); *m/e* 426 (M^+). Anal $C_{28}H_{42}O_3$ (C, H).

4,4-Desmethyl-11-oxo-18α-olean-12-en-30-oic acid ***9a***
In a similar manner, **25a** (59.4 mg, 0.13 mmol) was converted to the 3-O-thiocarbonylimidazolide by refluxing for 1.5 h with 1,1-thiocarbonyldiimidazole (57.9 mg, 0.325 mmol) in dry $ClCH_2CH_2Cl$ (2 ml). The crude imidazolide (73 mg) was gently refluxed for 2 h with *n*-Bu_2SnH (0.063 ml, 64.0 mg, 0.22 mmol) and AIBN (2.5 mg, 0.015 mmol) in dry toluene (3 ml). Preparative TLC (9:1 cyclohexane-acetone with double development) of the crude product furnished the 3-deoxy ester **26a** (39.7 mg, 69.3%), mp 245–252°C (ether-pentane): ν_{max} (cm^{-1}) 1717, 1653, 1618; *m/e* 440 (M^+). Refluxing of **26a** (39.7 mg, 0.09 mmol) with LiI (46.8 mg, 0.35 mmol) and dry collidine (3 ml) for 3.5 h gave the crude product which was purified by preparative TLC (2:1 benzene-acetone) to yield **9a** (13.1 mg, 34.1%), mp 296–302°C (CH_2Cl_2-acetone): δ (ppm) 0.74 (s, 3H, 28-H), 1.14 (s, 3H, 25-H), 1.15 (s, 3H, 26-H), 1.29 (s, 3H, 29-H), 1.38 (s, 3H, 27-H), 5.64 (bs, 1H, 12-H); *m/e* 426 (M^+). Anal $C_{28}H_{42}O_3$ (C, H).

3-Nor-4-isopropylidene-4,4-desmethyl-11-oxo-18ξ-olean-12-en-30-oic acid ***10*** *and 3-nor-4-desmethyl-11-oxo-18ξ-oleana-4,12-dien-30-oic acid* ***11***
Both corresponding esters [1], obtained as intermediates in the synthesis of **7**, were demethylated to **10** and **11**, respectively. **10a**: mp 199–205°C (ether-pentane); ν_{max} (cm^{-1}) 3500–2500, 1695, 1653, 1610; δ (ppm) 0.76 (s, 3H, 28-H), 0.91 (s, 3H, 25-H), 1.14 (s, 3H, 26-H), 1.27 (s, 3H, 29-H), 1.35 (s, 3H, 27-H), 1.61 (s, 3H, 23-H), 1.75 (s, 3H, 24-H), 5.71 (s, 1H, 12-H); *m/e* 452 (M^+). **10b**: mp 207–214°C (ether-pentane); ν_{max} (cm^{-1}) 3500–2500, 1697, 1648, 1610; δ (ppm) 0.86 (s, 3H, 28-H), 0.87 (s, 3H, 25-H), 1.14 (s, 3H, 26-H), 1.23 (s, 3H, 29-H), 1.37 (s, 3H, 27-H), 1.61 (s, 3H, 23-H), 1.75 (s, 3H, 24-H), 5.78 (s, 1H, 12-H); *m/e* 452 (M^+). **11a**: mp 263–270°C (acetone-pentane); ν_{max} (cm^{-1}) 3500–2500, 1697, 1652, 1610 (sh); δ (ppm) 0.77 (s, 3H, 28-H), 1.17 (s, 3H, 26-H), 1.22 (s, 3H, 25-H), 1.26 (s, 3H, 29-H), 1.28 (s, 3H, 27-H), 1.61 (s, 3H, 24-H), 5.75 (bs, 1H, 12-H); *m/e* 424 (M^+). **11b**: mp 253–257°C (acetone-pentane); ν_{max} (cm^{-1}) 3500–2500, 1697, 1647, 1610 (sh); δ (ppm) 0.88 (s, 3H, 28-H), 1.11 (s, 3H, 25-H), 1.23 (s, 6H, 26- & 29-H), 1.31 (s, 3H, 27-H), 1.61 (s, 3H, 24-H), 5.82 (s, 1H, 12-H); *m/e* 424 (M^+).

3β-Hydroxy-11-deoxo-18ξ-olean-12-en-30-oic acid ***12***
This was prepared by the already known procedure [9–11]. **12a**: mp 285–292°C ($CHCl_3$-MeOH-acetone); δ (ppm, 9:1 $CDCl_3$-CD_3OD) 0.67 (s, 3H, 28-H), 0.79 (s, 3H, 24-H), 0.97 (s, 3H, 23-H), 0.99 (s, 6H, 25- & 26-H), 1.16 (s, 3H, 27-H), 1.24 (s, 3H, 29-H), 5.20 (bt, 1H, 12-H); *m/e* 456 (M^+). **12b**: mp 320–325°C ($CHCl_3$-MeOH-acetone); ν_{max} (cm^{-1}) 3626, 3458, 1696; δ (ppm, 9:1 $CDCl_3$-CD_3OD) 0.79 (s, 6H, 24- & 28-H), 0.94 (s, 3H, 23-H), 0.97 (s, 3H, 26-H), 0.99 (s, 3H, 25-H), 1.15 (s, 3H, 27-H), 1.16 (s, 3H, 29-H), 5.28 (t, 1H, J = 3.5 Hz, 12-H); *m/e* 456 (M^+).

18β-Olean-12-en-30-oic acid ***13b***
A solution of **5b** (100 mg, 0.22 mmol) in HOAc (18 ml) was hydrogenated over PtO_2 (100 mg) under a pressure of 5.5 mg/cm^2 of H_2 at room temperature for 17 h. The catalyst was filtered off and the mixture was concentrated *in vacuo*. The residue was purified by preparative TLC (95:5 $CHCl_3$-MeOH with multiple development), giving **13b** (82.2 mg, 84.8%), mp 318–323°C ($CHCl_3$-MeOH-acetone): δ (ppm) 0.82 (s, 6H, 24- & 28-H), 0.87 (s, 3H, 23-H), 0.94 (s, 3H, 26-H), 0.97 (s, 3H, 25-H), 1.16 (s, 3H, 27-H), 1.21 (s, 3H, 29-H), 5.31 (t, 1H, J = 3.5 Hz, 12-H); *m/e* 440 (M^+). Anal $C_{30}H_{48}O_2$ (C, H).

18α-Olean-12-en-30-oic acid ***13a***
Similarly, **5a** (91 mg, 0.2 mmol) was hydrogenated with PtO_2 (91 mg) in HOAc (28 ml) to yield **13a** (57.3 mg, 65.0%), mp 290–294°C ($CHCl_3$-acetone): δ (ppm) 0.67 (s, 3H, 28-H), 0.82 (s, 3H, 24-H), 0.87 (s, 3H, 23-H), 0.96 (s, 3H, 26-H), 0.99 (s, 3H, 25-H), 1.16 (s, 3H, 27-H), 1.27 (s, 3H, 29-H), 5.20 (bs, 1H, 12-H); *m/e* 440 (M^+). Anal $C_{30}H_{48}O_2$ (C, H).

18β-Oleana-4,12-dien-30-oic acid ***14b***
As described in the preparation of **6**, known **27b** (94.1 mg, 0.2 mmol) was mesylated with MsCl (0.035 ml, 51.6 mg, 0.45 mmol) and EtN_3 (0.084 ml, 60.7 mg, 0.6 mmol) in CH_2Cl_2 (2 ml) on standing at 2–3°C for 1 h. The crude mesylate in dry collidine (5 ml) was heated at 180°C for 2 h. Then LiI (93.7 mg, 0.7 mmol) was added and heating was continued at 180°C for an additional 3 h. The crude product was purified by preparative TLC (95:5 $CHCl_3$-MeOH with triple development) afforded **14b** (55.7 mg, 63.5%), mp 285–290°C ($CHCl_3$-MeOH-acetone); ν_{max} (cm^{-1}) 3600–2500, 1720 (sh), 1692; δ (ppm) 0.83 (s, 3H, 28-H), 0.91 (s, 3H, 24-H), 0.97 (s, 6H, 23- & 26-H), 1.00 (s, 3H, 25-H), 1.16 (s, 3H, 27-H), 1.21 (s, 3H, 29-H), 5.34 (t, 1H, J = 3.5 Hz, 12-H), 5.41 (m, 2H, 2- & 3-H); *m/e* 438 (M^+). Anal $C_{30}H_{46}O_2$ (C, H).

18α-Oleana-4,12-dien-30-oic acid ***14a***
In a similar manner, **27a** (94.1 mg, 0.2 mmol) was converted to the corresponding mesylate which was demesylated followed by demethylation to give **14a** (67.9 mg, 77.4%), mp 265–270°C ($CHCl_3$-acetone-pentane): ν_{max} (cm^{-1}) 3600–2500, 1720 (sh), 1693; δ (ppm) 0.68 (s, 3H, 28-H), 0.90 (s, 3H, 24-H), 0.97 (s, 3H, 26-H), 0.99 (s, 3H, 23-H), 1.02 (s, 3H, 25-H), 1.16 (s, 3H, 27-H), 1.27 (s, 3H, 29-H), 5.22 (bt, 1H, 12-H), 5.41 (m, 2H, 2- & 3-H); *m/e* 438 (M^+). Anal $C_{30}H_{46}O_2$ (C, H).

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