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Anti-tumor-promoter activity of modified glycyrrhetinic acid derivatives. Synthesis and structure–activity relationships

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Summary — A series of modified derivatives of 18α- and 18β-glycyrrhetinic acid was systematically synthesized and their structure–activity relationships investigated for inhibition of the promoter activity of 12-*O*-tetradecanoylphorbol-13-acetate *in vitro*. Increased hydrophobicity in the A-ring moiety and the presence of an 11-oxo function were shown to be important for the high inhibition. Replacement of the carboxy to the hydroxymethyl group at the 20-position also enhanced the inhibitory activity.

anti-tumor-promoter activity / modified glycyrrhetinic acid derivatives / structure–activity relationships / hydrophobicity

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 anti-inflammatory, anti-allergic, and anti-ulcer activities [1]. Very recently, we systematically prepared ring A-modified GA derivatives of both 18α- and 18β-series and tested them for inhibition of canine kidney Na^+ ,K^+-ATPase (Terasawa *et al*, submitted for publication). Higher inhibitory activity was found with several of the 3-deoxygenated derivatives. Nishino *et al* [3] recently showed that some oleanane-type triterpenes including GA inhibit the tumor-promoting action induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) *in vitro* and *in vivo*. In addition, they found that replacement of the inherent carboxy by a hydroxymethyl group markedly enhanced the inhibitory activity.

In the present paper, we describe the *in vitro* anti-tumor promoter activities of synthetic modified GA derivatives and their structure–activity relationships.

Results and discussion

Chemistry

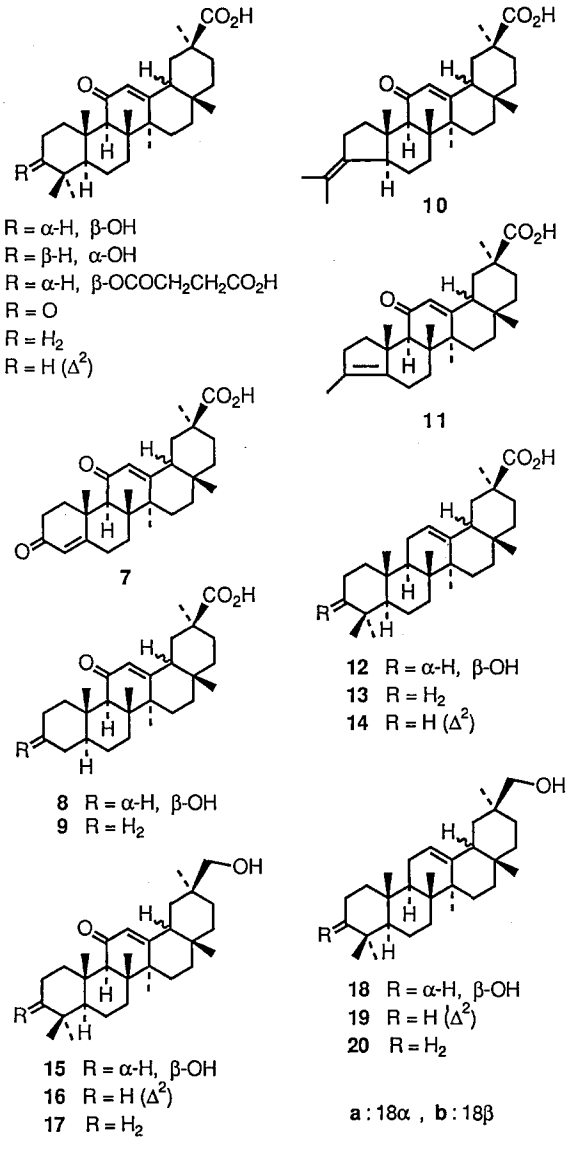
The modified GA derivatives examined in this study are shown in scheme 1. Compounds **2–14** were previously derived by chemical synthesis from commercially available 18α- (**1a**) and 18β-GA (**1b**), respectively. In order to further clarify the effect of the 11-oxo and 20-carboxy groups for structure–activity study, additional compounds **15–20** were prepared. Compounds **15**, **16** and **17** were obtained by hydride reduction ($Na[(MeOCH_2CH_2O)_2AlH_2]$ or $LiAlH_4$) of the corresponding acids or esters followed by oxidation with manganese dioxide. Compounds **18**, **19** and **20** were also prepared by similar hydride reduction of the corresponding acids or esters.

Bioassay

The anti-tumor-promoter activity was evaluated by the inhibitory effect on TPA-induced stimulation of ^{32}Pi incorporation into phospholipids of HeLa cells. The assay was carried out as previously described [2–4], where HeLa cells were incubated with one of the test compounds (25 μg/ml) and after 1 h, ^{32}Pi (20 μCi/culture) was added with or without TPA (50 nM). Incubation was continued for 4 h and then the radioactivity incorporated into the phospholipid fraction was measured. Data are expressed as the percentage of inhibition of TPA-enhanced ^{32}Pi incorporation, which is the mean of duplicate experiments.

Structure–activity relationships

Table I shows that the structural modification in the A-ring of 18α- and 18β-GA exerts a slight but significant effect on the inhibitory activity. As compared with the parent GA (1), most of the compounds with



Scheme 1.

no 3-oxygen function induced a relatively higher inhibition which was retained even in the A-ring contracted derivatives. Thus, increasing the hydrophobicity in ring A led to maximum inhibitory activity with **6** and **11**. This tendency agreed with the findings from the *in vitro* Na^+, K^+-ATPase inhibition. However, there was one exception – **5** did not show the expected higher inhibitory potency. Substantial enhancement of the inhibitory effect was also observed for removal of the 4,4-dimethyl groups. Table II explains the effect of the 11-oxo and 20-carboxy groups on the anti-tumor-promoter activity.

Table I. Inhibitory effects of ring A-modified GA derivatives on the enhanced ^{32}Pi incorporation into phospholipids of HeLa cells induced by TPA.

Compound	*Inhibition (%)*	
	***a**: 18α*	***b**: 18β*
1	46.6	30.7
2	0.0	2.5
3	35.0	38.0
4	26.6	17.2
5	39.4	38.9
6	58.8	67.7
7	49.6	47.1
8	63.7	42.0
9	51.9	48.5
10	57.1	63.9
11	56.9	53.6

Elimination of the 11-oxo function led to substantial loss of the inhibition. Replacement of the inherent carboxy by a hydroxymethyl group at the 20-position significantly increased the inhibitory activity, although a minor discrepancy was observed with the modified derivatives of **5**. Throughout this assay, no essential difference was found for the inhibitory effects between the 18α- and 18β-series.

Conclusions

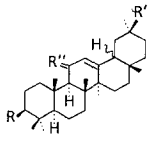
Our structure–activity study showed the predominant factors for the high anti-tumor-promoter activity in GA series compounds to be the hydrophobic character of the A-ring moiety, the presence of an 11-oxo function, and the introduction of a 20-hydroxymethyl group. The most potent inhibition was found with **16**.

Further studies will be conducted on the *in vivo* inhibition of the tumor–promoting action of selected compounds.

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. Usual workup means washing extracts with water and then brine, drying over Na_2SO_4, filtration, and evaporation *in vacuo*. 18β- and 18α-GA (**1b** and **1a**) were purchased from Wako Pure Chemical Ind Ltd and Sigma Chemical Co, respectively.

Table II. Inhibitory effects of oleanane-type triterpenoids (25 μg/ml) derived from GA on the enhanced ^{32}Pi incorporation into phospholipids of HeLa cells induced by TPA (50 nM).



R	*R'*	*R''*		*Inhibition (%)* *a: 18α*	*b: 18β*
OH	CO_2H	H_2	**(12)**	11.3	7.2
OH	CH_2OH	O	**(15)**	51.2	61.3
OH	CH_2OH	H_2	**(18)**	47.9	8.8
H	CO_2H	H_2	**(13)**	22.5	14.3
H	CH_2OH	O	**(17)**	25.5	36.8
H	CH_2OH	H_2	**(20)**	32.3	28.2
H (Δ^2)	CO_2H	H_2	**(14)**	52.5	55.0
H (Δ^2)	CH_2OH	O	**(16)**	70.1	81.3
H (Δ^2)	CH_2OH	H_2	**(19)**	63.8	79.3

11-Oxo-18β-olean-12-ene-3β,30-diol (glycyrrhetol) ***15b***
$LiAlH_4$ (85.4 mg, 2.25 mmol) was added in portions to a stirred solution of **1b** (141.2 mg, 0.3 mmol) in dry THF (6 ml). The mixture was stirred at 60°C under nitrogen for 1 h, then cooled, and quenched with 2 N–HCl. After dilution with cold water, the mixture was extracted with $CHCl_3$. The extract was washed with 2 N–HCl and worked up as usual. The crude product was dissolved in $CHCl_3$ (15 ml) containing THF (1 ml) and activated MnO_2 (2 g) charged. The mixture was vigorously shaken for 25 h and then filtered. The filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC (4:1 $CHCl_3$–acetone), giving **15b** (99.7 mg, 72.8%), mp 244–252°C. Recrystallization from acetone–pentane afforded an analytical sample, mp 274–277°C: ν_{max} (cm^{-1}) 3614, 3448, 1647, 1614; δ (ppm) 0.81 (s, 3H, 28-H), 0.86 (s, 3H, 24-H), 0.93 (s, 3H, 29-H), 1.01 (s, 3H, 23-H), 1.13 (bs, 6H, 25- and 26-H), 1.38 (s, 3H, 27-H), 3.23 (t, 1H, J = 8 Hz, 3-H), 3.48, 3.56 (ABq, 2H, J = 11.5 Hz, 30-H_2), 5.59 (s, 1H, 12-H); *m/e* 456 (M^+), identical to the material obtained previously [5].

11-Oxo-18α-olean-12-ene-3β,30-diol ***15a***
In a similar manner, **1a** (188.3 mg, 0.4 mmol) was subjected to $LiAlH_4$ reduction followed by MnO_2 oxidation to give **15a** (111.8 mg, 61.2%), mp 271–276°C (acetone–ether): ν_{max} (cm^{-1}) 3614, 3444, 1651, 1618; δ (ppm) 0.68 (s, 3H, 28-H), 0.81 (s, 3H, 24-H), 0.93 (s, 3H, 29-H), 1.00 (s, 3H, 23-H), 1.14 (s, 3H, 25-H), 1.20 (s, 3H, 26-H), 1.35 (s, 3H, 27-H), 3.22 (q, 1H, J = 11.6 Hz, 3-H), 3.33 (s, 2H, 30-H_2), 5.55 (bs, 1H, 12-H); *m/e* 456 (M^+). Anal $C_{30}H_{48}O_3$ Calcd: C, 78.89; H, 10.59. Found: C, 78.61; H, 10.47.

11-Oxo-18β-oleana-2,12-dien-30-ol ***16b***
A stirred suspension of **6b** (60.2 mg, 0.133 mmol) and $LiAlH_4$ (25.2 mg, 0.665 mmol) in dry THF (2 ml) was heated at 60°C under nitrogen for 1.5 h. The mixture was poured into cold 2 N–H_2SO_4 and extracted with $CHCl_3$. After usual workup, the residue was dissolved in $CHCl_3$ (6 ml) and activated MnO_2 (1.2 g) was charged. The suspension was vigorously stirred. After 22 h, additional fresh MnO_2 (0.7 g) and $CHCl_3$ (6 ml) were introduced. Stirring was continued for a further 65 h. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC (4:1 $CHCl_3$–acetone) to afford **16b** (30.2 mg, 51.8%), mp 175–182°C (ether–pentane): ν_{max} (cm^{-1}) 3618, 3434, 1646, 1613; δ (ppm) 0.87 (s, 3H, 28-H), 0.92 (bs, 6H, 24- and 29-H), 0.97 (bs, 6H, 23- and 25-H), 1.16 (bs, 6H, 26- and 27-H), 3.47, 3.56 (ABq, 2H, J = 10.5 Hz, 30-H_2), 5.40 (m, 2H, 2- and 3-H), 5.61 (s, 1H, 12-H); *m/e* 438 (M^+). Anal $C_{30}H_{46}O_2$ Calcd: C, 82.13; H, 10.57. Found: C, 81.84; H, 10.38.

11-Oxo-18α-oleana-2,12-dien-30-ol ***16a***
As described above, **6a** (54.3 mg, 0.12 mmol) was converted to **16a** (27.5 mg, 52.3%), mp 138–143°C (pentane): ν_{max} (cm^{-1}) 3620, 3428, 1650, 1619; δ (ppm) 0.69 (s, 3H, 28-H), 0.92 (s, 3H, 24-H), 0.93 (s, 3H, 29-H), 0.97 (s, 3H, 25-H), 1.16 (s, 3H, 23-H), 1.23 (s, 3H, 26-H), 1.34 (s, 3H, 27-H), 3.34 (s, 2H, 30-H_2), 5.40 (m, 2H, 2- and 3-H), 5.58 (bs, 1H, 12-H); *m/e* 438 (M^+). Anal $C_{30}H_{46}O_2$ Calcd: C, 82.13; H, 10.57. Found: C, 81.75; H, 10.46.

11-Oxo-18ξ-olean-12-en-30-ol ***17***
Red-Al [$(MeOCH_2CH_2O)_2AlH_2$, 3.4 M] solution in toluene (0.28 ml, 1 mmol) was added dropwise to a stirred solution of the Me ester of **5** (93.7 mg, 0.2 mmol) in dry THF (2 ml). The mixture was heated at 60°C for 1 h, then cooled, poured into cold 2 N–HCl, and extracted with $CHCl_3$. The extract was washed with cold 2 N–HCl and satd $NaHCO_3$ followed by usual workup. The crude product was dissolved in $CHCl_3$ (10 ml) and activated MnO_2 (2 g) was charged. The resulting suspension was vigorously stirred at room temperature. After 24 h, the mixture was filtered. Fresh MnO_2 (2 g) was introduced to the filtrate. The suspension was shaken again for 24 h. The inorganic precipitate was filtered off. Concentration *in vacuo* left a syrupy residue which was purified by prepara-

tive TLC (9:1 $CHCl_3$–acetone), giving the pure material of **17**. **17a**: 34.1 mg (38.7%), mp 275–277°C (MeOH); ν_{max} (cm^{-1}) 3676, 3616, 3446, 1653, 1622; δ (ppm) 0.67 (s, 3H, 28-H), 0.84 (s, 3H, 24-H), 0.87 (s, 3H, 25-H), 0.92 (s, 3H, 29-H), 1.14 (s, 3H, 23-H), 1.20 (s, 3H, 26-H), 1.35 (s, 3H, 27-H), 3.33 (s, 2H, 30-H_2), 5.53 (bs, 1H, 12-H); *m/e* 440 (M^+). Anal $C_{30}H_{48}O_2$ Calcd: C, 81.76; H, 10.98. Found: C, 81.47; H, 10.79. **17b**: 25.3 mg (28.7%), mp 200–203°C (ether–pentane); ν_{max} (cm^{-1}) 3676, 3620, 3440, 1647, 1618 (sh); δ (ppm) 0.84 (s, 3H, 28-H), 0.86 (s, 3H, 24-H), 0.88 (s, 3H, 25-H), 0.93 (s, 3H, 29-H), 1.13 (s, 3H, 23-H), 1.15 (s, 3H, 26-H), 1.39 (s, 3H, 27-H), 3.47, 3.56 (ABq, 2H, J = 11 Hz, 30-H_2), 5.58 (s, 1H, 12-H); *m/e* 440(M^+). Anal $C_{30}H_{48}O_2$ Calcd: C, 81.76; H, 10.98. Found: C, 81.53; H, 10.82.

18α-Olean-12-ene-3β,30-diol ***18a***
The Me ester of **12a** (47.1 mg, 0.1 mmol) was similarly reduced with $LiAlH_4$ (9.5 mg, 0.25 mmol) in dry THF (2 ml) on heating at 60°C for 45 min. The crude product was purified by preparative TLC (9:1 $CHCl_3$–acetone), affording **18a** (27.1 mg, 61.2%), mp 282–284°C (CH_2Cl_2–acetone–pentane): δ (ppm) 0.63 (s, 3H, 28-H), 0.79 (s, 3H, 24-H), 0.93 (s, 3H, 29-H), 0.96 (s, 3H, 26-H), 0.99 (s, 3H, 23-H), 1.00 (s, 3H, 25-H), 1.15 (s, 3H, 27-H), 3.22 (m, 1H, 3-H), 3.32 (s, 1H, 30-H_2), 5.16 (bs, 1H, 12-H); *m/e* 442 (M^+). Anal $C_{30}H_{50}O_2$ Calcd: C, 81.39; H, 11.38. Found: C, 81.17; H, 11.22.

18β-Olean-12-ene-3β,30-diol(11-deoxoglycyrrhetol) ***18b***
In the same manner, the Me ester of **12b** (188.3 mg, 0.4 mmol) was converted to **18b** (156.0 mg, 88.1%), mp 246–250°C ($CHCl_3$–acetone–hexane): δ (ppm) 0.79 (s, 3H, 28-H), 0.83 (s, 3H, 24-H), 0.90 (s, 3H, 29-H), 0.94 (s, 3H, 26-H), 0.96 (s, 3H, 23-H), 1.00 (s, 3H, 25-H), 1.15 (s, 3H, 27-H), 3.23 (q, 1H, J = 10.5, 5.5 Hz, 3-H), 3.49, 3.56 (ABq, 2H, J = 11 Hz, 30-H_2), 5.18 (t, 1H, J = 4 Hz, 12-H); *m/e* 442 (M^+), identical to the material obtained previously [5].

18ξ-Oleana-2,12-dien-30-ol ***19***
As described above, the Me ester of **14** (45.3 mg, 0.1 mmol) was reduced with $LiAlH_4$ (9.5 mg, 0.25 mmol) in dry THF (1.5 ml) at 60°C for 1 h. Usual workup left a syrupy residue which was purified by preparative TLC (9:1 benzene–EtOAc) to give the pure material of **19**. **19a**: 34.8 mg (81.9%), mp 193–198°C (ether–pentane): δ (ppm) 0.64 (s, 3H, 28-H), 0.90 (s, 3H, 29-H), 0.93 (s, 3H, 24-H), 0.97 (s, 3H, 26-H), 0.99 (s, 3H, 23-H), 1.01 (s, 3H, 25-H), 1.16 (s, 3H, 27-H), 3.33 (s, 2H, 30-H_2), 5.20 (m, 1H, 12-H), 5.41 (m, 2H, 2- and 3H); *m/e* 424 (M^+). Anal $C_{30}H_{48}O$ Calcd: C, 84.84; H, 11.39. Found: C, 84.71; H, 11.33. **19b**: 38.0 mg (89.5%) mp 164–167°C (ether–pentane): δ (ppm) 0.84 (s, 3H, 28-H), 0.90 (bs, 6H, 24- and 29-H), 0.97 (bs, 6H, 23- and 26-H), 1.00 (s, 3H, 25-H), 1.16 (s, 3H, 27-H), 3.49, 3.56 (ABq, 2H, J = 10.5 Hz, 30-H_2), 5.23 (t, 1H, J = 3.5 Hz, 12-H), 5.41 (m, 2H, 2- and 3-H); *m/e* 424 (M^+). Anal $C_{30}H_{48}O$ Calcd: C, 84.84; H, 11.39. Found: C, 84.55; H, 11.23.

18ξ-Olean-12-en-30-ol ***20***
Similarly, **13** (44.0 mg, 0.1 mmol) in dry THF (2 ml) was reduced with $LiAlH_4$ (19 mg, 0.5 mmol). The crystalline residue obtained by usual workup was recrystallized from CH_2Cl_2–MeOH to give an analytical sample. **20a**: 35.4 mg (83.0%), mp 200–203°C; δ (ppm) 0.63 (s, 3H, 28-H), 0.82 (s, 3H, 24-H), 0.87 (s, 3H, 25-H), 0.93 (s, 3H, 29-H), 0.96 (s, 3H, 26-H), 0.99 (s, 3H, 23-H), 1.16 (s, 3H, 27-H), 3.33 (s, 2H, 30-H_2), 5.17 (bs, 1H, 12-H); *m/e* 426 (M^+). Anal $C_{30}H_{50}O$ Calcd: C, 84.44; H, 11.81. Found: C, 84.15; H, 11.64. **20b**: 34.4 mg (80.6%), mp 177–179°C; δ (ppm) 0.82 (s, 3H, 28-H), 0.83 (s, 3H, 24-H), 0.87 (s, 3H, 25-H), 0.90 (s, 3H, 29-H), 0.93 (s, 3H, 26-H), 0.96 (s, 3H, 23-H), 1.16 (s, 3H, 27-H), 3.48, 3.56 (ABq, 2H, J = 11 Hz, 30-H_2), 5.20 (t, 1H, J = 3.5 Hz, 12-H); *m/e* 426 (M^+). Anal $C_{30}H_{50}O$ Calcd: C, 84.44; H, 11.81. Found: C, 84.21; H, 11.70.

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