Synthesis and Inhibitory Effect of Novel Glycyrrhetinic Acid Derivatives on IL-1β-Induced Prostaglandin E_2 Production in Normal Human Dermal Fibroblasts

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Olean-11,13(18)-dien-3β,30-diol dihemiphthalate (3), which was derived from glycyrrhetinic acid (GA), has been reported to produce a potent of anti-inflammatory effect in *in vivo* assays. Using 3 as a lead compound, we attempted to synthesize some modified compounds which varied in the following; i) the position of a carboxyl group in the phthalate moiety, ii) the number of carboxyls attached to the benzoyl group, iii) conversion of benzene ring to another ring system, iv) the linkage form between the benzene ring and oleanene skeleton at position 3 and/or 30. These were screened for their inhibitory activity against interleukin-1β (IL-1β)-induced prostaglandin E_2 (PGE_2) production in normal human dermal fibroblasts (NHDF). Although conversion of the *ortho*-carboxyl group of 3 into the *meta*-position or the *para*-position led to an increase in inhibitory activity, the elimination or increase of the carboxyl group resulted in loss of the inhibitory activity. Conversion of the ester bond to the amide bond at position 3 and/or 30 of 3 did not contribute to a significant increase in inhibitory activity. On the other hand, among the derivatives possessing an anthranilic acid moiety at position 30 of 3β-*O*-acetyl-olean-11,13(18)-dien-30-oic acid (20), 3β-hydroxy-30-nor-olean-11,13(18)-dien-20β-[*N*-(2-carboxyphenyl)]carboxamide (30) showed the most potent inhibitory activity (IC_{50} 1.0 μM) in this series.

Key words glycyrrhetinic acid derivative; anti-inflammatory; prostaglandin E_2; 3β-hydroxy-30-nor-olean-11,13(18)-dien-20β-[*N*-(2-carboxyphenyl)]carboxamide; olean-11,13(18)-dien-3β,30-diol dihemiphthalate; normal human dermal fibroblast

Glycyrrhizin (GL), a main saponin component of several *Glycyrrhiza* species (*G. uralensis*, *G. glabra*, and *G. inflata*) has been reported to possess various pharmacological activities such as anti-inflammation,[1] inhibition of prostaglandin E_2 (PGE_2) production in rat macrophages,[2] antiallergic,[3] antiviral[4,5] action, and interferon-γ-induction.[6] In Japan, a glycyrrhizin preparation, Stronger-Neo Minophagen C (SNMC), has been used extensively for more than 30 years to treat chronic hepatitis.

Glycyrrhetinic acid (GA), the aglycon of GL, is also known to have wide pharmacological effects such as anti-inflammation,[7,8] antitumor,[9] antihepatotoxic activity,[10] and inhibition of the growth of mouse melanoma.[11] In 1989, it was reported that GA strongly inhibits renal 11β-hydroxysteroid dehydrogenase (11β-HSD) in rat,[12] which has been regarded as a cause of pseudoaldosteronism occasionally induced by the administration of a GL preparation or Carbenoxolone.

We have synthesized various GA derivatives, such as deoxo-glycyrrhetol (**1**),[13] olean-11,13(18)-dien-3β,30-diol (**2**),[14] and their succinate or hemi-phthalate[14] forms (Fig. 1), in order to enhance the anti-inflammatory activity and suppress the pseudoaldosteronism due to the inhibition of mineralcorticoid metabolism. During the course of this work, **3** was reported to show various anti-inflammatory activities such as the inhibition of increasing vascular permeability,[15] 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear edema,[16] arachidonic acid-induced or capsaicin-induced mouse ear edema,[17,18] and carrageenin-induced rat paw edema.[19] It was suggested that one of the mechanisms by which **3** induces anti-inflammatory effects is the inhibition of cyclooxygenase or lipoxygenase activities at the inflammatory site.[20] Interestingly, it was found that **3** exhibited anti-acetic acid-induced writhing activity[15] and an antiulcer activity,[21] in addition to the anti-inflammation. In addition, we have reported that **3** did not inhibit the activity of 11β-HSD-2, which causes an adverse effect of GA.[22] These facts suggest that **3** is promising as a lead compound for a new type of anti-inflammatory agent, showing anti-inflammatory action as well as anti-ulcer action, different from conventional non-steroidal anti-inflammatory drugs (NSAIDs), which are mostly ulcerogenic.

Recently, confirmed that normal human dermal fibroblasts (NHDF) produce PGE_2 in response to IL-1β.[23] In fact, NHDF have been used to assess the effect of compounds such as Tranilast and Azelastine on PGE_2 production.[24,25] However, it is unclear whether **3** and its derivatives have an effect on COX-2 dependent PGE_2 production.

In this paper, we synthesized various new compounds derived from **3** and examined the effect of these compounds on IL-1β-induced PGE_2 production in NHDF.

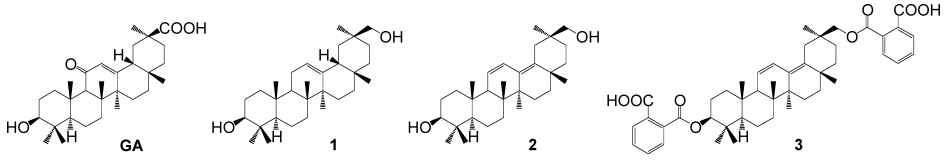

Fig. 1. Structures of Glycyrrhetinic Acid (GA) and Its Derivatives

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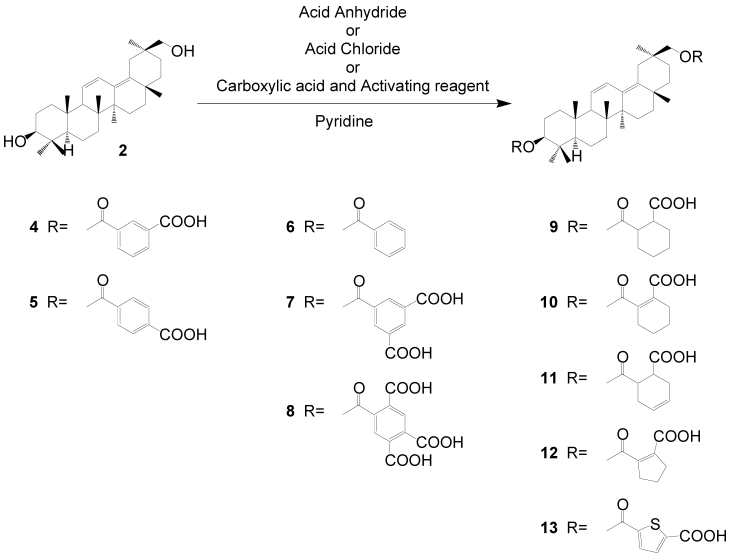

Chart 1

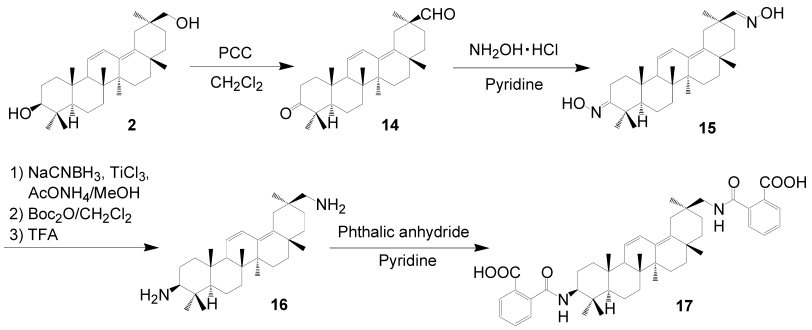

Chart 2

Chemistry

In regard to the modification of **3**, we focused on the following parts of **3**: i) the position of the carboxyl group in the phthalate moiety (**4**, **5**), ii) the number of carboxyls attached to the benzoyl group (**6**—**8**), iii) conversion of the benzene ring to other ring systems (**9**—**13**), and iv) the linkage form between the benzene ring and oleanene skeleton at position 3 and/or 30 (**17**, **23**—**31**).

As shown in Chart 1, **4** and **5** were obtained by the reaction of **2** with isophthaloyl dichloride or terephthaloyl dichloride in pyridine in 20% and 15% yield, respectively. Esterification of **2** with benzoyl chloride, 1,3,5-benzenetricarbonyl trichloride or pyromellitic dianhydride in pyridine afforded **6**, **7** and **8** in 82%, 16% and 35% yield, respectively.

Compounds (**9**—**12**), the benzoic acid of which were replaced by another ring system, were obtained in 10—45% yield by esterification of **2** with corresponding acid anhydride in pyridine. Compound (**13**) was prepared from **2** in 12% yield by activation of 2,5-thiophenedicarboxylic acid with 2-chloro-1-methylpyridinium iodide.[26,27]

Furthermore, in order to synthesize **17**, **23** and **24**, which were altered linkage forms between the oleanene skeleton and benzoic acid, **16**, **19** and **22** were prepared by the method of Sun *et al.*[28] (Charts 2—4). Namely, after deriving **14** (3,30-dioxo form, 50% yield) from **2** with pyridinium chlorochromate (PCC) in dichloromethane, it was reacted with hydroxylamine hydrochloride in pyridine to give dioxime (**15**) in 69% yield. Then, the reduction of **15** with sodium cyanoborohydride in the presence of titanium(III) chloride and ammonium acetate in methanol afforded a mixture of 3α,30- and 3β,30-diamino forms. Since it was difficult to purify the mixture using silica gel column chromatography, *t*-butoxycarbonylation was carried out by a general method, then the product was purified using silica gel column chromatography. Next, the fraction was subjected to de-*t*-butoxycarbonylation with trifluoroacetic acid (TFA) to give 3β,30-diamine (**16**) in 44% overall yield. Compound (**19**) was prepared by using a synthetic method of **16** from a 3-oxo form, which was obtained by selective acetylation of the C-30 hydroxy group in **2** with one equivalent of acetic anhydride, followed by oxidation with Jones reagent, as shown in Chart 3. Compound (**22**) was synthesized from **21**, which was prepared from **20**[29] and ethyl chlorocarbonate, followed by reduction with sodium borohydride[30] in THF (Chart 3). Condensation of **16**, **19** and **22** with phthalic anhydride in pyridine afforded **17**, **23** and **24** in 75%, 75% and 86% yield, respectively (Charts 2, 4). Furthermore, deacetylation of **23** and **24**, followed by their esterification with phthalic anhydride in pyridine, gave rise to **27** and **28**, respectively (Chart 4).

As shown in Chart 5, **29** was obtained in 46% yield by the condensation of C-30 acyl halide, which was derived from

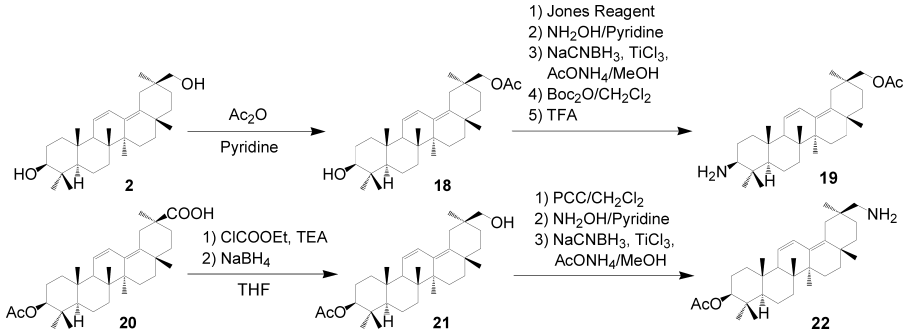

Chart 3

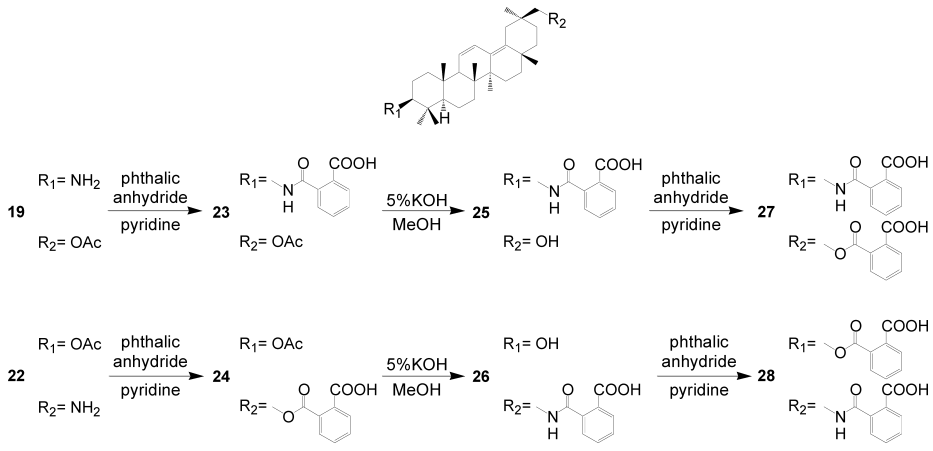

Chart 4

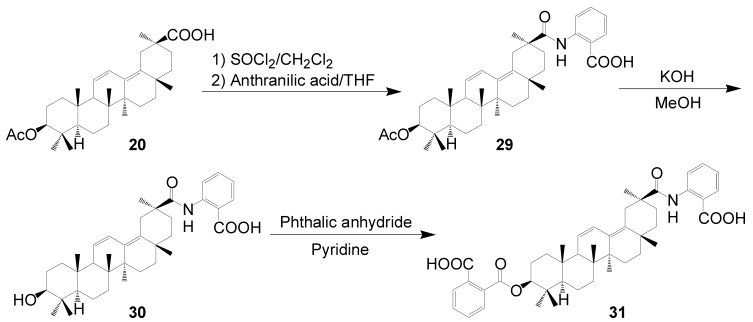

Chart 5

the halogenation of **20** with thionyl chloride in THF, and anthranilic acid.[31] Deacetylation of **29** gave **30** in 99% yield, and then **30** was esterified with phthalic anhydride in pyridine to give **31** in 67% yield.

Results and Discussion

All the newly synthesized compounds at a concentration of 1 μM were evaluated for their inhibitory activity on IL-1β-induced PGE_2 production in NHDF, and the results are summarized in Table 1. *N*-(2-Cyclohexyl-oxy-4-nitrophenyl)-methanesulfonamide (NS-398) (100 nM), a cyclooxygenase-2 (COX-2) inhibitor, completely inhibited PGE_2 production in response to IL-1β (data not shown), indicating that the IL-1β-induced PGE_2 is formed by the COX-2 dependent pathway.[23] The lead compound (**3**) showed weak activity (52±4% inhibition at 10 μM), whereas the *meta*- (**4**) or *para*-(**5**) carboxylic compounds led to an increase in the activity compared to that of **3**. However, the potency remained moderate (56±5% inhibition at 1 μM). Furthermore, elimination (**6**) or an increase (**7**, **8**) of the carboxyl group in the benzoic acid moiety resulted in loss of the inhibitory activity. These results clearly showed that the number or the position of the carboxyl group significantly affected the inhibitory activity.

Compounds (**9**—**13**), in which the benzene rings in the benzoyl group were replaced by another ring system, showed more potent activity than that of **3**. However, the potencies were only moderate in **10**—**12** (31—45% inhibition). Conversion of the ester bond at position 3 and/or 30 to the amide bond in **17**, **23**—**28** also failed to get the desired effects, while **28** showed higher potency (IC_{50} 2.0 μM). In comparison with **17** and **27**, **28** was potentiated in its biological activity by structural modification of the linkage between the

Table 1. Effect of Test Compounds on IL-1β-Induced PGE_2 Production in NHDF

Compound No.	1 μM Inhibition (%)	IC_{50} (μM)	Compound No.	1 μM Inhibition (%)	IC_{50} (μM)
3	52±4**[a)]	>10	**17**	18±7	
3	NA		**23**	NA	
4	56±5**	[b)]	**24**	16±7	
5	38±1*	[b)]	**25**	NA	
6	NA		**26**	9±10	
7	NA		**27**	16±1	
8	NA		**28**	39±10**	2.0
9	18±5		**29**	52±8**	1.4
10	45±12**	[b)]	**30**	54±8**	1.0
11	33±7*	[b)]	**31**	32±11	4.3
12	31±2**	[b)]			
13	16±15		NS-398		500 pM

a) The data which was measured in 10 μM.
b) The compound does not inhibit concentration-dependently. $*p<0.05$, $**p<0.01$ *vs.* control. NA: not activity, Values represent 0 or less than 0.

mother skeleton and the phthalic acid at positions 30 and/or 3.

Compounds (**29**—**31**), bearing a different amide linkage compared with **17** and **23**—**28**, exhibited the most potent activity among the newly synthesized compounds. Particularly, **30** showed the most potent activity in this series (IC_{50} 1.0 μM). In contrast with the relation of the activity of **26** and **28**, the inhibitory activity of **31** (IC_{50} 4.3 μM), in which phthalic acid was introduced into position 3 of **30**, was decreased compared with that of **30**.

In this series of the synthetic compounds, the introduction of anthranilic acid into position 30 is one of the significant factors for the expression of the activity. Thus, **30** may serve as a useful lead compound in the search for more powerful anti-inflammatory agents.

Experimental

General Experimental Procedures Melting points were taken on a Yanaco micro melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were measured using either a JEOL JNM-A500 (^{1}H: 500 MHz; ^{13}C: 125 MHz) or AL-400 (^{1}H: 400 MHz; ^{13}C: 100 MHz) NMR spectrometer, with chemical shifts represented in ppm and TMS used as an internal standard. MS data were obtained on a JEOL JMS-700 or JMS-GC-MATE.

3β,30-*O*-Bis(3-carboxybenzenecarbonyl)-olean-11,13(18)-dien (4) A solution of **2** (220 mg, 0.499 mmol) and isophthaloyl dichloride (1,3-benzenedicarbonl dichloride) (204 mg, 1.00 mmol) in pyridine (10 ml) was stirred for 4 h at room temperature. To the reaction mixture was added MeOH. The mixture was filtrated and then the filtrate was evaporated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to give **4** (60 mg, 20% in yield); mp >300 °C; ^{1}H-NMR (500 MHz, C_5D_5N) δ 8.73—7.57 (8H, m), 6.61 (1H, dd, $J_{9,11}$=2.5 Hz, $J_{11,12}$=10.4 Hz, H-11), 5.67 (1H, d, H-12), 5.02 (1H, dd, $J_{2\alpha,3}$=4.9 Hz, $J_{2\beta,3}$=11.6 Hz, H-3) 4.31, 4.28 (1H×2, each d, J_{gem}=11.0 Hz, H-30), 2.70, 2.09 (1H×2, each d, J_{gem}=14.7 Hz, H-19), 1.17, 1.10, 1.06, 1.01, 1.00, 0.94, 0.80 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (125 MHz, C_5D_5N) δ 168.6, 168.5, 166.0, 165.8 (C=O), 126.3 (C-12), 126.0 (C-11), 81.9 (C-3), 75.0 (C-30); FAB-MS *m/z*: 736.3967 (Calcd for $C_{46}H_{56}O_8$: 736.3975).

3β,30-*O*-Bis(4-carboxybenzenecarbonyl)-olean-11,13(18)-dien (5) A solution of **2** (440 mg, 0.998 mmol) and terephthaloyl dichloride (1,4-benzenedicarbonyl dichloride) (2.0 g, 9.9 mmol) in pyridine (5 ml) was heated for 10 min at 80 °C. To the reaction mixture was added 10% HCl, and the mixture was then filtered off. The filtrate was extracted with $CHCl_3$, and the organic layer was concentrated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to give **5** (113 mg, 15% in yield); mp >300 °C; ^{1}H-NMR (500 MHZ, C_5D_5N) δ 8.57—8.40 (8H, m), 6.38 (1H, dd, $J_{9,11}$=2.4 Hz, $J_{11,12}$=11.0 Hz, H-11), 5.56 (1H, d, H-12), 4.91 (1H, dd, $J_{2\alpha,3}$=4.3 Hz, $J_{2\beta,3}$=11.0 Hz, H-3), 4.24 (2H, s, H-30); ^{13}C-NMR (125 MHz, C_5D_5N) δ 168.4, 168.4, 166.0, 165.8 (C=O); FAB-MS *m/z*: 735.3905 (Calcd for $C_{46}H_{55}O_8$: 735.3897).

3β,30-*O*-Dibenzoyl-olean-11,13(18)-dien (6) To a solution of **2** (220 mg, 0.499 mmol) in pyridine (5 ml) was added benzoyl chloride (281 mg, 2.00 mmol). After stirring for 4 h at room temperature, MeOH was added to the reaction mixture. The precipitates were collected by filtration and washed with MeOH to give **6** as a white powder (265 mg, 82% in yield); mp 231—234 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.08—8.04 (4H, m), 7.58—7.26 (2H, m), 6.39 (1H, dd, $J_{9,11}$=3.1 Hz, $J_{11,12}$=11.0 Hz, H-11), 5.57 (1H, d, H-12), 4.77 (1H, dd, $J_{2\alpha,3}$=4.9 Hz, $J_{2\beta,3}$=11.8 Hz, H-3), 4.10 (2H, s, H-30), 1.11, 1.02, 1.00, 0.97, 0.95, 0.93, 0.74 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (125 MHz, C_5D_5N) δ 166.6, 166.3 (C=O), 125.9 (C-12), 125.5 (C-11), 81.5 (C-3), 74.7 (C-30); FAB-MS *m/z*: 648.4226 (Calcd for $C_{44}H_{56}O_4$: 648.4179).

3β,30-*O*-Bis(3,5-dicarboxybenzenecarbonyl)-olean-11,13(18)-dien (7) A solution of **2** (440 mg, 0.998 mmol) and 1,3,5-benzenetricarboxylic trichloride (800 mg, 3.01 mmol) in pyridine (20 ml) was heated for 20 min at 80 °C. To the reaction mixture was added water, then the mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 10% HCl, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. To the residue was added with MeOH, the mixture was filtrated, and then the filtrate was evaporated. The residue was purified on HPLC to give **7** (128 mg, 16% in yield); mp 266—267 °C; ^{1}H-NMR (400 MHZ, C_4D_8O) δ 8.88—8.84 (6H, m), 6.53 (1H, bd, $J_{11,12}$=10.5 Hz, H-11), 4.89 (1H, dd, $J_{2\alpha,3}$=5.6 Hz, $J_{2\beta,3}$=11.0 Hz, H-3), 4.21, 4.16 (2H, each d, J_{gem}=10.6 Hz, H-30), 1.19, 1.10, 1.08, 1.04, 0.99, 0.98, 0.82 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHZ, C_4D_8O) δ 165.3, 165.2, 164.2, 164.0 (C=O), 136.1, 134.6, 134.34, 134.27, 133.8, 132.1, 132.0, 131.8, 131.4 (C-13, C-18, Ar), 125.7, 125.5 (C-11, C-12), 81.8 (C-3), 74.7 (C-30); FAB-MS *m/z*: 823.3689 (Calcd for $C_{48}H_{55}O_{12}$: 823.3693).

3β,30-*O*-Bis(2,4,5-tricarboxybenzencarbonyl)-olean-11,13(18)-dien (8) A solution of **2** (206 mg, 0.468 mmol) and 1,2,4,5-benzentetracarboxylic anhydride (1.02 g, 4.68 mmol) in pyridine (10 ml) was heated for 3 h at 60 °C. To the reaction mixture was added 10% HCl (20 ml), and this was then stirred for 1 h. The precipitates were filtered off and washed with a small amount of MeOH to give **8** (151 mg, 35% in yield); mp 222—225 °C; ^{1}H-NMR (500 MHz, C_5D_5N) δ 8.92, 8.91, 8.76, 8.73 (1H×4, each s), 6.56 (1H, bd, $J_{9,11}$=8.5 Hz, H-11), 5.53 (1H, d, H-12), 5.09 (1H, dd, $J_{2\alpha,3}$=4.3 Hz, $J_{2\beta,3}$=11.6 Hz, H-3), 4.36 (2H, dd, J=11.0 Hz, H-30), 2.74, 2.10 (1H×2, each d, J_{gem}=14.0 Hz, H-19), 1.13, 1.07, 1.02, 1.00, 0.96, 0.83, 0.68 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (125 MHz, C_5D_5N) δ 169.8, 169.8, 169.8, 169.6, 169.4, 169.2, 168.2, 167.7 (C=O), 82.9 (C-3), 76.2 (C-30); FAB-MS *m/z*: 912.3536 (Calcd for $C_{50}H_{56}O_{16}$: 912.3568).

3β,30-*O*-Bis(2-carboxy-1-cyclohexsanecarbonyl)-olean-11,13(18)-dien (9) A solution of **2** (220 mg, 0.499 mmol) and cyclohexane dicarboxylic acid anhydride (385 mg, 2.50 mmol) in pyridine (10 ml) was heated for 3 d at 80 °C. The reaction mixture was poured into 10% HCl and extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated, then the residue was subjected to silica gel column chromatography and eluted with hexane–acetone (7 : 3) to give **9** (115 mg, 31% in yield); mp 218—219 °C; ^{1}H-NMR (400 MHz, C_4D_8O) δ 6.42 (1H, dd, $J_{9,11}$=2.4 Hz, $J_{11,12}$=10.2 Hz, H-11), 5.57 (1H, d, H-12), 4.52 (1H, dd, $J_{2\alpha,3}$=5.5 Hz, $J_{2\beta,3}$=11.0 Hz, H-3), 3.79 (2H, s, H-30),

2.82—2.76 (1H×4, m, cyclohexane–CH), 2.47, 1.86 (1H×2, each d, J_{gem}=14.5 Hz, H-19), 1.10, 0.99, 0.94, 0.88, 0.87, 0.81, 0.75 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, C_4D_8O) δ 174.6, 174.6, 173.3, 173.0 (C=O), 137.3, 135.1 (C-13, C-18), 126.4, 126.2 (C-11, C-12), 80.7 (C-3), 74.4 (C-30); FAB-MS *m/z*: 748.4921 (Calcd for $C_{46}H_{68}O_8$: 748.4914).

3β,30-*O*-Bis(2-carboxy-1-cyclohexenecarbonyl)-olean-11,13(18)-dien (10) To a solution of **2** (440 mg, 0.998 mmol) in pyridine (15 ml) was added 1-cyclohexene-1,2-dicarboxylic anhydride (1.50 g, 9.86 mmol). After stirring for 24 h at 120 °C, the mixture was poured into ice water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, 10% HCl and aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to give **10** (35 mg, 10% in yield); mp 141—142 °C; ^{1}H-NMR (400 MHz, CD_3OD) δ 6.41 (1H, dd, $J_{9,11}$=2.7 Hz, $J_{11,12}$=10.5 Hz, H-11), 5.59 (1H, d, H-12), 4.56 (1H, dd, $J_{2\alpha,3}$=6.6 Hz, $J_{2\beta,3}$=9.8 Hz, H-3), 3.88 (2H, s, H-30), 2.82—2.76 (1H×4, m, cyclohexane–CH), 2.47, 1.86 (1H×2, each d, J_{gem}=14.1 Hz, H-19), 1.10, 0.99, 0.96, 0.91, 0.87, 0.83, 0.75 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (400 MHz, CD_3OD) δ 171.9, 171.7, 170.3, 170.2 (C=O), 137.4, 135.7 (C-13, C-18), 137.4, 136.4, 136.0, 135.2 (cyclohexene-1,2), 83.2 (C-3), 76.2 (C-30); FAB-MS *m/z*: 744.4629 (Calcd for $C_{46}H_{64}O_8$: 744.4601).

3β,30-*O*-Bis(2-carboxy-4-cyclohexenecarbonyl)-olean-11,13(18)-dien (11) To a solution of **2** (220 mg, 0.499 mol) in pyridine (20 ml) was added *cis*-1,2,3,6-tetrahydrophthalic anhydride (760 mg, 5.00 mmol). The solution was stirred overnight at 120 °C, then the solvent was evaporated. The residue was dissolved with $CHCl_3$, washed with H_2O, and concentrated. The residue was purified by silica gel column chromatography using hexane–acetone (8 : 2) to give **11** (140 mg, 38% in yield); mp 205—206 °C; ^{1}H-NMR (400 MHz, CD_3OD) δ 6.40 (1H, d, $J_{11,12}$=10.5 Hz, H-11), 5.66, (4H, br s, cyclohexene–CH), 5.58 (1H, d, H-12), 4.51 (1H, dd, $J_{2\alpha,3}$=5.6 Hz, $J_{2\beta,3}$=11.0 Hz, H-3), 3.85 (2H, m, H-30), 1.10, 0.99, 0.95, 0.89, 0.87, 0.81, 0.74 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, CD_3OD) δ 176.8, 175.1, 174.7 (C=O), 137.4, 135.7 (C-13, C-18), 126.7, 126.34, 126.29, 126.10, 126.07 (C-11, C-12, cyclohexene–CH), 82.6 (C-3), 75.6 (C-30); FAB-MS *m/z*: 744.4565 (Calcd for $C_{46}H_{56}O_8$: 744.4601).

3β,30-*O*-Bis(2-carboxyl-1-cyclopentenecarbonyl)-olean-11,13(18)-dien (12) A solution of **2** (220 mg, 0.499 mmol) and 1-cyclopentene-1,2-dicarboxylic anhydride (600 mg, 4.34 mmol) in pyridine (10 ml) was heated for 4 h at 80 °C. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, 10% HCl and aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4, and concentrated. The residue was recrystallized from hexane–AcOEt (1 : 1) and filtered off. The crystals were washed with a small amount of MeOH to give **12** (157 mg, 45% in yield); mp 163—165 °C; ^{1}H-NMR (400 MHz, C_5D_5N) δ 6.60 (1H, dd, $J_{9,11}$=2.4 Hz, $J_{11,12}$=10.5 Hz, H-11), 5.54 (1H, d, H-12), 4.93 (1H, dd, $J_{2\alpha,3}$=4.6 Hz, $J_{2\beta,3}$=11.5 Hz, H-3), 4.21, 4.15 (2H, each d, J_{gem}=10.6 Hz, H-30), 2.90—2.78 (12H, m, cyclopenten–CH_2), 1.09, 1.09, 1.01, 1.00, 0.98, 0.84, 0.72 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, C_5D_5N) δ 168.9, 168.6, 165.8, 165.7 (C=O), 144.3, 143.0, 137.3, 136.4, 136.5, 134.5 (C-13, C-18, cyclopentene–CH), 125.9, 125.8 (C-11, C-12), 81.5 (C-3), 74.9 (C-30); FAB-MS *m/z*: 716.4282 (Calcd for $C_{44}H_{60}O_8$: 716.4288).

3β,30-*O*-Bis(5-carboxythiophenecarbonyl)-olean-11,13(18)-dien (13) A solution of **2** (220 mg, 0.499 mmol), 2,5-thiophenedicarboxylic acid (430 mg, 2.50 mmol), 2-chloro-1-methylpyridinium iodide (638 mg, 2.50 mmol) and tri-*n*-butylamine (463 mg, 2.50 mmol) in toluene (20 ml) was heated for 2 d at 120 °C. The reaction mixture was concentrated, then the residue was subjected to silica gel column chromatography and eluted with $CHCl_3$ to give **13** (43 mg, 12% in yield); mp >300 °C; ^{1}H-NMR (500 MHz, C_5D_5N) δ 7.95—7.90 (4H, m, thiophene–CH), 6.54 (1H, dd, H-11), 4.90 (1H, dd, H-3), 4.21 (2H, s, H-30); FAB-MS *m/z* 748.3145 (Calcd for $C_{42}H_{52}O_8S$: 748.3104).

3-Oxo-olean-11,13(18)-dien-30-al (14) To a solution of **2** (2.00 g, 4.55 mmol) in CH_2Cl_2 (21 ml) was added PCC (2.96 g, 13.7 mmol). After being stireed overnight at room temperature, the mixture was diluted with Et_2O and filtered through a Florisil column. The eluate was concentrated under a vacuum, then the residue was chromatographed on silica gel with hexane–AcOEt (9 : 1) to give **14** (1.0 g, 50% in yield); mp 206—208 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.51 (1H, s, H-30), 6.34 (1H, dd, $J_{9,11}$=2.9 Hz, $J_{11,12}$=10.5, H-11), 5.57 (1H, d, H-12); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 217.2 (C-3), 205.9 (C-30), 135.5, 135.4 (C-13, C-18), 126.0, 125.4 (C-11, C-12); EI-MS *m/z*: 436.3342 (Calcd for $C_{30}H_{44}O_2$: 436.3341).

Olean-11,13(18)-dien-3,30-dioxime (15) A solution of **14** (1.8 g, 4.1 mmol) and hydroxylamine hydrochloride (2.0 g, 29 mmol) in pyridine (10 ml) was heated for 3 h at 50 °C. After cooling to room temperature, the reaction mixture was diluted with $CHCl_3$ and washed with 10% HCl. The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel with hexane–AcOEt (5 : 1) to give **15** (1.3 g, 69% in yield); mp 272—274 °C; ^{1}H-NMR (400 MHz, C_5D_5N) δ 12.20, 11.92 (1H×2, s, OH), 7.75 (1H, s, H-30), 6.47 (1H, br d, $J_{11,12}$=10.2 Hz, H-11), 5.62 (1H, d, H-12), 3.40 (1H, m, H-2a), 2.75 (1H, d, J_{gem}=14.4 Hz, H-19), 2.49 (1H, m, H-2β), 2.24 (1H, d, H-19), 1.38, 1.15, 1.11, 1.10, 1.02, 1.02, 0.80 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, C_5D_5N) δ 164.0 (C-3), 158.3 (C-30), 136.2, 135.0 (C-13, C-18), 126.3, 125.8 (C-11, C-12); FAB-MS *m/z*: 467.3676 (Calcd for $C_{30}H_{47}O_2N_2$: 467.3638).

Olean-11,13(18)-dien-3β,30-diamine (16) Sodium cyanoborohydride (1.4 g, 22 mmol) was added to a methanol solution of **15** (1.0 g, 2.1 mmol) and ammonium acetate (1.8 g, 23 mmol). The solution was chilled in ice water, and 15% aqueous titanium trichloride (5.8 ml) was added dropwise over 20 min. The mixture was stirred at room temperature for 12 h and adjusted with 2 N sodium hydroxide to pH 10. The aqueous solution was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with distilled water and concentrated under reduced pressure to give a mixture of 3α,30- and 3β,30-diamine (880 mg). To the mixture in CH_2Cl_2 (15 ml) was added Boc_2O (517 mg, 2.4 mmol). The reaction mixture was stirred at room temperature for 2 h and then diluted with $CHCl_3$. The solution was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on silica gel with hexane–AcOEt (50 : 1→50 : 2→50 : 3→50 : 4) to give 3β,30-*N*,*N*′-di(*tert*-butoxycarbonyl)-11,13(18)-dien (254 mg, 50% in yield); ^{1}H-NMR ($CDCl_3$) δ 6.35 (1H, dd, $J_{9,11}$=3.1 Hz, $J_{11,12}$=10.4 Hz, H-11), 5.53 (1H, d, H-12), 4.64 (1H, t, $J_{30,N'H}$=6.1 Hz, N′H), 4.40 (1H, d, $J_{3,NH}$=10.4 Hz, NH), 3.30 (1H, ddd, $J_{2\alpha,3}$=9.9 Hz, $J_{2\beta,3}$=3.5 Hz, H-3), 2.98 (t, 2H, H-30), 1.45, 1.44 (9H×2, each s, *t*-Bu), 1.10, 0.96, 0.92, 0.86, 0.75, 0.73, 0.70 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR ($CDCl_3$) δ 156.3, 155.8 (C=O×2), 136.5, 134.2 (C-13, C-18) 125.7, 125.5 (C-11, C-12), 79.0, 78.9 (C-3, C-30). Following the addition of TFA (0.5 ml) to 3β,30-*N*,*N*′-di(*tert*-butoxycarbonyl)-11,13(18)-dien (484 mg), the mixture was stirred at room temperature for 15 min. The reaction mixture was adjusted with 1 N sodium hydroxide to pH 10. The aqueous solution was extracted with $CHCl_3$, and the organic layer was washed with distilled water and concentrated to give **16** (290 mg, 87%); mp 184—186 °C; ^{1}H-NMR (400 MHz, CD_3OD) δ 6.41 (1H, dd, $J_{9,11}$=2.9 Hz, $J_{11,12}$=10.5 Hz, H-11), 5.57 (1H, d, H-12), 3.31 (3H, m, H-3, H-30), 1.09, 0.98, 0.96, 0.90, 0.77, 0.75, 0.74 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, CD_3OD) δ 138.03, 135.3 (C-13, C-18), 126.6, 126.5 (C-11, C-12), 60.7, 56.9 (C-3, C-30); FAB-MS *m/z*: 439.4060 (Calcd for $C_{30}H_{51}N_2$: 439.4052).

3β,30-Bis[(2-carboxyphenyl)carboxamido]-olean-11,13(18)-dien (17) To a solution of **16** (290 mg, 0.661 mmol) in pyridine (10 ml) was added phthalic anhydride (900 mg, 6.08 mmol). After being stireed for 3 h at 80 °C, the reaction mixture was diluted with $CHCl_3$, then the $CHCl_3$ layer was washed with 10% HCl, dried over anhydrous Na_2SO_4, and concentrated. To the residue was added MeOH, then the precipitates were filtered off to give **17** (362 mg, 75% in yield); mp 212 °C; ^{1}H-NMR (400 MHz, C_5D_5N) δ 9.10 (1H, t, $J_{30,NH}$=6.1 Hz, N′H), 8.55 (1H, d, $J_{3,NH}$=9.2 Hz, NH), 8.18—7.36 (8H, m), 6.59 (1H, bd, $J_{11,12}$=10.4 Hz, H-11), 5.51 (1H, d, H-12), 4.42 (1H, ddd, $J_{2\alpha,3}$=4.3 Hz, $J_{2\beta,3}$=12.2 Hz, H-3), 3.76, 3.66 (1H×2, each d, J_{gem}=10.7 Hz, H-30), 1.39, 1.14, 1.13, 1.05, 1.02, 0.88, 0.75, (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, C_5D_5N) δ 170.7, 170.2, 169.8, 169.7 (C=O); FAB-MS *m/z*: 735.4416 (Calcd for $C_{46}H_{59}O_6N_2$: 735.4373).

30-*O*-Acetyl-olean-11,13(18)-dien-3β-ol (18) To a solution of **2** (2.0 g, 4.5 mmol) in pyridine (10 ml) was added acetic anhydride (0.86 ml) at 0 °C, then stirring was continued for 6 h. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, 10% HCl and aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on silica gel with hexane–AcOEt (20 : 1→10 : 1→5 : 1) to give **18** (1.2 g, 54% in yield); mp 185—186 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.34 (1H, dd, $J_{9,11}$=2.9 Hz, $J_{11,12}$=10.5 Hz, H-11), 5.55 (1H, d, H-12), 3.85, 3.81 (1H×2, each d, J_{gem}=10.7 Hz, H-30), 3.24 (1H, dd, $J_{2\alpha,3}$=5.1, $J_{2\beta,3}$=11.2 Hz, H-3), 2.08 (3H, s, Ac), 1.07, 0.99, 0.96, 0.89, 0.81, 0.78, 0.71, (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.2 (C=O), 136.0, 134.4 (C-13, C-18), 125.9, 125.4 (C-11, C-12), 79.0 (C-30), 74.4 (C-3); FAB-MS *m/z*: 482.3758 (Calcd for $C_{32}H_{50}O_3$: 482.3760).

30-*O*-Acetyl-olean-11,13(18)-dien-3β-amine (19) To a solution of **18** (105 mg, 0.218 mmol) in THF (5 ml) was added dropwise Jones reagent (5 drops), and then the mixture was stirred at room temperature for 15 min. The reaction mixture was filtered, then the filtrate was poured into ice water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on silica gel with hexane–AcOEt (20 : 1→10 : 1) to give 30-*O*-acetyl-olean-11,13(18)-dien-3-one (86 mg, 81% in yield); ^{1}H-NMR ($CDCl_3$) δ 6.38 (1H, dd, $J_{9,11}$=3.1 Hz, $J_{11,12}$=10.4 Hz, H-11), 5.54 (1H, d, H-12), 3.85, 3.82 (1H×2, each d, J_{gem}=10.4 Hz, H-30), 2.08 (3H, s, Ac), 1.10, 1.08, 1.04, 1.01, 0.98, 0.81, 0.75, (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR ($CDCl_3$) δ 217.4 (C-3), 171.3 (C=O), 136.7, 134.1 (C-13, C-18), 125.8, 125.3 (C-11, C-12), 74.3 (C-30). A solution of 30-*O*-acetyl-olean-11,13(18)-dien-3-one (229 mg, 0.476 mmol) and hydroxylamine hydrochloride (87 mg, 1.25 mmol) in pyridine (2 ml) was heated for 3 h at 50 °C. After being cooled to room temperature, the reaction mixture was diluted with $CHCl_3$ and washed with 10% HCl. The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel with hexane–AcOEt (5 : 1) to give 30-*O*-acetyl-olean-11,13(18)-dien-3-oxime (109 mg, 47% in yield); ^{1}H-NMR ($CDCl_3$) δ 6.36 (1H, d, $J_{11,12}$=10.4 Hz, H-11), 5.55 (1H, d, H-12), 3.85, 3.82 (1H×2, each d, J_{gem}=10.4 Hz, H-30), 3.11 (1H, m, H-2a), 2.40 (1H, d, J_{gem}=14.0 Hz, H-19), 2.29 (1H, m, H-2β), 2.09 (3H, s, Ac), 1.18, 1.07, 1.07, 1.00, 0.95, 0.81, 0.74, (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR ($CDCl_3$) δ 171.3 (C=O), 166.7 (C-3), 136.4, 134.3 (C-13, C-18), 125.7, 125.6 (C-11, C-12), 74.4 (C-30). Sodium cyanoborohydride (660 mg, 10.5 mmol) was added to a methanol solution of 30-*O*-acetyl-olean-11,13(18)-dien-3-oxime (475 mg, 0.462 mmol) and ammonium acetate (841 mg, 10.9 mmol). The solution was chilled in ice water, and 15% aqueous titanium trichloride (2.7 ml) was added dropwise over 20 min. The mixture was stirred at room temperature for 12 h and adjusted with 2 N sodium hydroxide to pH 10. The aqueous solution was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with distilled water and concentrated under reduced pressure to give the mixture 30-*O*-acetyl-olean-11,13(18)-dien-3-amine (373 mg). The mixture of 30-*O*-acetyl-olean-11,13(18)-dien-3-amine (690 mg, 1.43 mmol) was dissolved in CH_2Cl_2 (20 ml), then Boc_2O (500 mg, 2.29 mmol) was added to the mixture. The reaction mixture was stirred at room temperature for 12 h and then concentrated. The residue was chromatographed on silica gel with hexane–AcOEt (25 : 2) to give 30-*O*-acetyl-3β-*N*-(*tert*-butoxycarbonyl)-olean-11,13(18)-dien (500 mg, 59%).

A mixture of 30-*O*-acetyl-3β-*N*-(*tert*-butoxycarbonyl)-olean-11,13(18)-dien (500 mg) and TFA (1 ml) was stirred at room temperature for 15 min. The reaction mixture was adjusted with 1 N sodium hydroxide to pH 10. The aqueous solution was extracted with $CHCl_3$, then the $CHCl_3$ layer was washed with distilled water and concentrated to give **19** (373 mg, 92% in yield); mp 134—135 °C; ^{1}H-NMR (400 MHz, CD_3OD) δ 6.37 (1H, dd, $J_{9,11}$=2.9 Hz, $J_{11,12}$=10.5 Hz, H-11), 5.60 (1H, d, H-12), 3.83 (2H, s, H-30), 2.45 (1H, J_{gem}=12.0 Hz, H-19), 2.37 (1H, $J_{2\alpha,3}$=4.8 Hz, $J_{2\beta,3}$=11.3 Hz, H-3), 2.07 (3H, s, Ac), 1.10, 0.98, 0.96, 0.91, 0.82, 0.76, 0.74 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, CD_3OD) δ 172.9 (C=O), 137.2, 135.8 (C-13, C-18), 126.9, 126.4 (C-11, C-12), 75.4 (C-3); FAB-MS *m*/*z*: 482.3975 (Calcd for $C_{32}H_{52}O_2N$: 482.3975).

3β-*O*-Acetyl-olean-11,13(18)-dien-30-ol (21) To a solution of **20** (2.4 g, 4.8 mmol) in THF (20 ml) was added ethyl chlorocarbonate (2.3 ml) and triethylamine (3.3 ml) at −5 °C. The reaction mixture was stirred for 2 h at −5 °C and filtrated. To the filtrate was added sodium borohydride (1.0 g, 15.9 mmol) in water (3 ml), and the mixture was stirred for 30 min at −5 °C. The reaction mixture was evaporated, then the residue was dissolved in $CHCl_3$. The solution was washed with water, dried over anhydrous Na_2SO_4, and concentrated. To the residue was added MeOH, then the precipitates were filtrated off to give **21** (2.0 g, 85% in yield); mp 214—216 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.36 (1H, dd, $J_{9,11}$=2.8 Hz, $J_{11,12}$=10.6 Hz, H-11), 5.52 (1H, d, H-12), 4.52 (1H, dd, $J_{2\alpha,3}$=2.7 Hz, $J_{2\beta,3}$=5.4 Hz, H-3), 3.36 (2H, s, H-30), 2.37 (1H, d, J_{gem}=13.9 Hz, H-19), 2.05 (3H, s, Ac), 1.07, 0.96, 0.92, 0.87, 0.85, 0.78, 0.71 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8 (C=O) 136.6, 133.9 (C-13, C-18), 125.5, 125.3 (C-11, C-12), 80.8 (C-3), 74.1 (C-30); FAB-MS *m*/*z*: 482.3741 (Calcd for $C_{32}H_{50}O_3$: 482.3760).

3β-*O*-Acetyl-olean-11,13(18)-dien-30-amine (22) To a solution of **2** (1.3 g, 2.9 mmol) in CH_2Cl_2 (20 ml) was added PCC (1.6 g, 7.4 mmol). After being stirred for overnight at room temperature, the mixture was diluted with Et_2O and filtered through a Florisl column. The eluate was concentrated under a vacuum, then the residue was chromatographed on silica gel with hexane–AcOEt (9 : 1) to give 3-*O*-acetyl-olean-11,13(18)-dien-30-al (634 mg, 48% in yield); ^{1}H-NMR ($CDCl_3$) δ: 9.50 (1H, s), 6.30 (1H, dd, *J*=10.5, 2.9 Hz), 5.57 (1H, d, *J*=10.5 Hz), 4.52 (1H, dd, *J*=5.5, 2.8 Hz), 2.47 (1H, d, *J*=12.4 Hz), 2.05, 1.09, 0.98, 0.97, 0.92, 0.87, 0.86, 0.72 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29). A solution of 3-*O*-acetyl-olean-11,13(18)-dien-30-al (634 mg, 1.32 mmol) and hydroxylamine hydrochloride (320 mg, 4.60 mmol) in pyridine (5 ml) was heated for 3 h at 50 °C. After being cooled to room temperature, the reaction mixture was diluted with $CHCl_3$ and washed with 10% HCl. The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel with hexane–AcOEt (5 : 1) to give 3-*O*-acetyl-olean-11,13(18)-dien-30-oxime (648 mg, 99% in yield). Sodium cyanoborohydride (466 mg, 7.42 mmol) was added to a methanol solution of 3-*O*-acetyl-olean-11,13(18)-dien-30-oxime (680 mg, 1.97 mmol) and ammonium acetate (600 mg, 7.78 mmol). The solution was chilled in ice water, and 15% aqueous titanium trichloride (2 ml) was added dropwise over 20 min. The mixture was stirred at room temperature for 12 h and adjusted with 2 N sodium hydroxide to pH 10. The aqueous solution was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with distilled water and concentrated under reduced pressure to give **22** (542 mg, 82% in yield); mp 117—118 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.36 (1H, dd, $J_{9,11}$=2.6 Hz, $J_{11,12}$=10.6 Hz, H-11), 5.52 (1H, d, H-12), 4.52 (1H, dd, $J_{2\alpha,3}$=5.7 Hz, $J_{2\beta,3}$=10.6 Hz, H-3), 2.48 (2H, s, H-30), 2.33 (1H, d, J_{gem}=13.9 Hz, H-19), 2.05 (3H, s, Ac), 1.06, 0.96, 0.92, 0.87, 0.85, 0.75, 0.71 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8 (C=O), 137.0, 133.7 (C-13, C-18), 125.5, 125.3 (C-11, C-12), 80.8 (C-3); FAB-MS *m*/*z*: 482.4042 (Calcd for $C_{32}H_{52}O_2N$: 482.3998).

30-*O*-Acetyl-3β-(2-carboxyphenyl)carboxamido-olean-11,13(18)-dien (23) To a solution of compound **19** (236 mg, 0.490 mmol) in pyridine (10 ml) was added phthalic anhydride (900 mg, 6.08 mmol). After being stirred for 3 h at 80 °C, the reaction mixture was diluted with $CHCl_3$, and the $CHCl_3$ layer was washed with 10% HCl, dried over anhydrous Na_2SO_4, and concentrated. To the residue was added MeOH, then the precipitate was filtered off to give **23** (362 mg, 75% in yield); mp 239—239.5 °C; ^{1}H-NMR (500 MHz, C_5D_5N) δ 8.56 (1H, d, *J*=9.8 Hz, NH), 8.16 (1H, d, *J*=7.3 Hz), 7.75 (1H, d, *J*=7.3 Hz), 7.46 (1H, t, *J*=7.3 Hz), 7.37 (1H, t, *J*=7.3 Hz), 6.48 (1H, bd, $J_{11,12}$=10.4 Hz, H-11), 5.61 (1H, d, H-12), 4.01, 3.98 (1H×2, each d, J_{gem}=10.5 Hz, H-30), 2.52 (1H, d, J_{gem}=14.0 Hz, H-19), 2.12 (3H, s, Ac), 1.41, 1.09, 1.07, 1.03, 0.92, 0.90, 0.80 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (125 MHz, C_5D_5N) δ 170.9, 170.2, 169.7 (C=O), 141.1, 136.4, 134.9, 131.8, 131.4, 130.3, 128.9, 128.5, 125.9, 123.9 (Ar, C-11, C-12, C-13, C-18), 74.3 (C-30); FAB-MS *m*/*z*: 630.4207 (Calcd for $C_{40}H_{56}O_5N$: 630.4158).

3β-*O*-Acetyl-30-(2-carboxyphenyl)carboxamido-olean-11,13(18)-dien (24) 24 (201 mg, 86%) was obtained from **22** (180 mg) according to the above procedure; mp 236—237 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.88—7.71 (4H, m), 6.33 (1H, dd, $J_{9,11}$=2.4 Hz, $J_{11,12}$=10.4 Hz, H-11), 5.50 (1H, d, H-12), 4.51 (1H, dd, $J_{2\alpha,3}$=5.5 Hz, $J_{2\beta,3}$=10.4 Hz, H-3), 3.56 (2H, s, H-30), 2.05 (3H, s, Ac), 1.04, 0.94, 0.91, 0.86, 0.85, 0.83, 0.70 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); FAB-MS *m*/*z*: 630.4161 (Calcd for $C_{40}H_{56}NO_5$: 630.4158).

3β-(2-Carboxyphenyl)carboxamido-olean-11,13(18)-dien-30-ol (25) To a solution of **23** (204 mg, 0.324 mmol) in MeOH (5 ml) was added NaOMe (18 mg, 0.33 mmol). After being stirred overnight at room temperature, the reaction mixture was neutralized with Amberlite IR-120B, and the resin was removed by filtration. The filtrate was concentrated to give **25** (152 mg, 80% in yield); mp 214—216 °C; ^{1}H-NMR (500 MHz, C_5D_5N) δ 8.98 (1H, d, *J*=7.3 Hz, NH), 8.17 (1H, d, *J*=6.7 Hz), 7.89 (1H, d, *J*=7.3 Hz), 7.42 (1H, t, *J*=7.3 Hz), 7.37 (1H, t, *J*=6.7 Hz), 6.59 (1H, br d, $J_{11,12}$=9.2 Hz, H-11), 5.58 (1H, d, H-12), 4.40 (1H, m, H-3), 3.65 (2H, s, H-30), 2.71, 2.19 (1H×2, each d, J_{gem}=14.3 Hz, H-19), 1.35, 1.16, 1.10, 1.034, 1.026, 0.89, 0.80 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); FAB-MS *m*/*z*: 588.4066 (Calcd for $C_{38}H_{54}O_4N$: 588.4053).

30-(2-Carboxyphenyl)carboxamido-olean-11,13(18)-dien-3β-ol (26) **26** (159 mg, 75%) was obtained from **24** (171 mg), according to the above procedure. mp 178—179 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.99 (1H, d, *J*=7.8 Hz, NH), 7.53—7.46 (3H, m), 6.53 (1H, t, *J*=6.3 Hz), 6.33 (1H, dd, $J_{9,11}$=2.7 Hz, $J_{11,12}$=10.7 Hz), 5.53 (1H, d, H-12), 3.28—3.24 (2H, m, H-30), 2.41 (1H, d, J_{gem}=13.9 Hz, H-19), 1.06, 0.98, 0.96, 0.87, 0.83, 0.77, 0.70 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 169.1 (C=O), 137.1, 136.1, 134.4, 131.9, 131.3, 130.0, 127.6, 125.9, 125.3 (C-11, C-12, C-13, C-18, Ar), 79.0, 77.2 (C-3, C-30); FAB-MS *m*/*z*: 588.4048 (Calcd for $C_{38}H_{54}NO_4$: 588.4053).

3β-(2-Carboxyphenyl)carboxamido-30-*O*-phthaloyl-olean-11,13(18)-dien (27) To a solution of **25** (102 mg, 0.211 mmol) in pyridine (5 ml) was

added phthalic anhydride (120 mg, 0.810 mmol). After being stirred overnight at 80 °C, the reaction mixture was diluted with $CHCl_3$, and the $CHCl_3$ layer was washed with 10% HCl, dried over anhydrous Na_2SO_4, and concentrated. To the residue was added MeOH, then the precipitates were filtered off to give **27** (95 mg, 75% in yield); mp 185—187 °C; ^{1}H-NMR ($CDCl_3$) δ 7.90—7.44 (8H, m), 6.34 (1H, br d, $J_{11,12}$=10.4 Hz, H-11), 6.03 (1H, d, $J_{3,NH}$=7.9 Hz, NH), 5.51 (1H, d, H-12), 4.09 (2H, s, H-30), 4.00 (1H, dd, $J_{2\alpha,3}$=4.3 Hz, $J_{2\beta,3}$=12.2 Hz, H-3), 1.05, 1.03, 0.98, 0.87, 0.86, 0.81, 0.69, (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR ($CDCl_3$) δ 171.4, 170.2, 169.2, 168.3 (C=O); FAB-MS *m/z*: 734.4064 (Calcd for $C_{46}H_{56}NO_7$: 734.4057).

30-(2-Carboxyphenyl)carboxamido-3β-*O*-phthaloyl-olean-11,13(18)-dien (28) **28** (13 mg, 12%) was obtained from **26** (85 mg) according to the above procedure. mp 167—168 °C; ^{1}H-NMR (500 MHz, C_5D_5N) δ 9.07 (1H, t, *J*=6.4 Hz, NH), 8.16—7.36 (8H, m), 6.59 (1H, dd, $J_{9,11}$=2.4 Hz, $J_{11,12}$=11.0 Hz, H-11), 5.46 (1H, d, H-12), 5.08 (1H, dd, $J_{2\alpha,3}$=4.6 Hz, $J_{2\beta,3}$=11.9 Hz, H-3), 3.77—3.65 (2H, m, H-30), 1.13, 1.13, 1.12, 1.04, 0.97, 0.83, 0.73 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); FAB-MS *m/z*: 734.4049 (Calcd for $C_{46}H_{56}NO_7$: 734.4057).

3β-*O*-Acetyl-30-nor-olean-11,13(18)-dien-20β-[*N*-(2-carboxyphenyl)]-carboxamide (29) To a solution of **20** (2.5 g, 5.0 mmol) in CH_2Cl_2 (20 ml) was added $SOCl_2$ (3 ml). After being stireed for 3 h at 70 °C, the reaction mixture was concentrated. To the residue was added THF (25 ml), anthranilic acid (1.03 g, 7.51 mmol) and triethylamine (0.5 ml), then the mixture was stirred overnight at 70 °C. The reaction mixture was diluted with $CHCl_3$, and the $CHCl_3$ layer was washed with 5% HCl, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on silica gel with $CHCl_3$ to give **29** (1.4 g, 46% in yield); mp 252—254 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.21 (1H, s, NH), 8.82 (1H, d, *J*=8.3 Hz), 8.13 (1H, dd, *J*=7.9, 1.6 Hz), 7.60 (1H, dt, *J*=5.3, 2.6 Hz), 7.09 (1H, t, *J*=7.6 Hz), 6.43 (1H, dd, $J_{9,11}$=3.0 Hz, $J_{11,12}$=10.4 Hz, H-11), 5.57 (1H, d, H-12), 4.52 (1H, dd, *J*=5.4, 2.7 Hz, H-3), 2.84, 2.34 (1H×2, each d, J_{gem}=14.4 Hz, H-19), 2.06 (3H, s, Ac), 1.22, 1.14, 0.99, 0.90, 0.87, 0.85, 0.73 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H28, H-29); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.88 (C-30), 171.84, 170.94 (C=O), 142.42, 135.51, 135.32, 135.18, 131.62, 126.20, 125.23, 122.23, 120.38, 113.71 (C-13, C-18 C-11, C-12, Ar), 80.84 (C-3); FAB-MS *m/z*: 616.4002 (Calcd for $C_{39}H_{54}O_5N$: 616.4002).

3β-Hyroxy-30-nor-olean-11,13(18)-dien-20β-[*N*-(2-carboxyphenyl)]-carboxamide (30) To a solution of **29** (94 mg, 0.15 mmol) in MeOH (20 ml) was added KOH (92 mg). After being stireed overnight at 70 °C, the reaction mixture was neutralized with Amberlite IR-120B, then the precipitates were dissolved with $CHCl_3$, and the resin was removed from the solution. The solution was evaporated, then the residue was recrystallized from MeOH to give **30** (86 mg, 99% in yield); mp 286—288 °C; ^{1}H-NMR (400 MHz, C_5D_5N) δ 12.51 (1H, s, NH), 12.51 (1H, s), 9.34 (1H, d, *J*=8.5 Hz), 8.50 (1H, d, *J*=7.8 Hz), 7.60 (1H, t, *J*=6.7 Hz), 7.17 (1H, t, *J*=7.6 Hz), 6.65 (1H, dd, $J_{9,11}$=2.4 Hz, $J_{11,12}$=10.5 Hz, H-11), 5.67 (1H, d, H-12), 3.50 (1H, dd, $J_{2\alpha,3}$=5.7 Hz, $J_{2\beta,3}$=10.1 Hz, H-3), 3.17, 2.66 (1H×2, each d, J_{gem}=14.4 Hz, H-19), 1.43, 1.28, 1.19, 1.19, 0.97, 1.07, 0.81 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.6, 172.1 (C-30, C=O), 142.9, 135.9, 134.0, 132.0, 131.7 126.8, 125.6, 122.2, 120.2, 117.5 (C-11, C-12, C-13, C-18, Ar), 78.0 (C-3); FAB-MS *m/z*: 574.3893 (Calcd for $C_{37}H_{52}O_4N$: 574.3896).

3β-*O*-Phthaloyl-30-nor-olean-11,13(18)-dien-20β-[*N*-(2-carboxyphenyl)]carboxamide (31) To a solution of **30** (150 mg, 0.26 mmol) in pyridine (10 ml) was added phthalic anhydride (600 mg, 4.05 mmol). After being stirred overnight at 120 °C, the reaction mixture was diluted with $CHCl_3$, then the $CHCl_3$ layer was washed with 5% HCl (three times), dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed on silica gel with $CHCl_3$ to give **31** (125 mg, 67% in yield); mp 173—174 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.26 (1H, s, NH), 8.82 (1H, d, *J*=8.8 Hz), 8.15 (1H, d, *J*=7.8 Hz), 7.89—7.68 (2H, m), 7.59 (2H, m), 7.10 (1H, t, *J*=7.6 Hz), 6.41 (1H, d, $J_{11,12}$=10.7 Hz, H-11), 5.54 (1H, d, H-12), 4.77 (1H, dd, $J_{2\alpha,3}$=5.6 Hz, $J_{2\beta,3}$=11.2 Hz, H-3), 2.80, 2.39 (1H×2, each d, J_{gem}=14.4 Hz, H-19), 1.22, 1.13, 0.99, 0.94, 0.87, 0.84, 0.70 (3H×7, each s, H-23, H-24, H-25, H-26, H-27, H-28, H-29); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 178.1, 173.2, 173.1, 167.3 (C-30, C=O), 142.4, 135.7, 135.4, 135.3, 132.9, 131.9, 131.7, 131.0, 130.9, 129.4, 129.1, 126.2, 125.4, 122.5, 120.5, 114.1 (C-11, C-12, C-13, C-18, Ar), 83.1 (C-3); FAB-MS *m/z*: 722.4091 (Calcd for $C_{45}H_{56}O_7N$: 722.4057).

Bioassay. Reagents Pharmacological agents were purchased from the following firms: human recombinant IL-1β, BD Biosciences (Bedford, MA, U.S.A.); NS-398, Cayman Chemical Co. (Ann Arbor, MI, U.S.A.).

Normal Human Dermal Fibroblast Culture Normal human dermal fibroblasts (NHDF) from the foreskin were purchased from Kurabo (Osaka, Japan) and cultured in α-minimum essential medium (α-MEM) containing 10% fetal calf serum (FCS) and 60 mg/ml kanamycin.

Assay for Cytokines, PGE_2 Production NHDF (1—5×10^4 cells/0.25 ml/well) were seeded in 48 well plates (BD Biosciences) and cultured in α-MEM supplemented with 10% FCS. When cells grew to confluency, the medium was replaced with 0.25 ml α-MEM containing 0.5% FCS, and cells were kept for 48 h. These cells were then incubated with 1 ng/ml IL-1β in the presence or absence of various agents for 24 h. The culture supernatants were collected and kept at −80 °C until being for assay. PGE_2 (detection limit: Ca 12 pg/ml) in the culture supernatant was measured by an enzyme linked immunosorbent assay (ELISA) kit (Cayman Chemical Co.). The effect of test agents on the release of soluble mediators in response to IL-1β was expressed as percent inhibition in comparison with the control.

Statistical Analysis All results are expressed as the mean±S.E.M. The statistical significance of difference between the control and test groups was determined using the Tukey-Kramer multiple comparisons test after one-way analysis of variance (ANOVA). Appropriate groups were compared by Student's *t*-test. IC_{50} values were calculated with PRISM (Graphpad Software, Inc.).

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