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Synthesis of [2-^{13}C]-Oleanolic Acid and [2-^{13}C]-Myricerone

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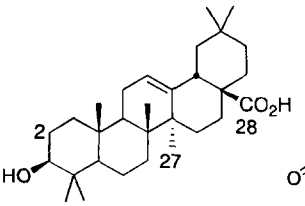
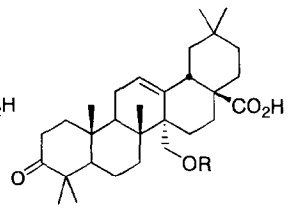
Abstract: Synthetic way for ^{13}C-labeled oleanolic acid **1** and myricerone **2** has been developed, starting from the parent **1** and **2**. The procedure involves ring opening and closure of the A rings of these oleanane triterpenes. ^{13}C was introduced into the 2-position by ^{13}C-MeLi as an isotope source. Chelation controlled addition of methyllithium to α-hydoxypentanone **11** is a common crucial step for labeling of **1** and **2**, and judicious choice of protecting groups is essential for **2**.

INTRODUCTION

Several oleanane triterpenes such as glycyrrhitic acid and myriceric acid A attract pharmaceutical interest as a traditional medicine and a drug candidate . Myriceric acid A is a potent endothelin A receptor antagonist that was isolated from a crude extract of twigs of the southern bayberry, *Myrica cerifera*.[1] It can be synthesized in a semi total synthesis from the most common and naturally abundant oleanane triterpene, oleanolic acid **1**, via key intermediate myricerone **2** by a multiple-step procedure.[2] Myriceric acid A and derivatives is expected to be useful for the treatment of various diseases including pulmonary hypertension, renal failure, congestive heart failure, and vasospasm.[3] Therefore, investigation of labeled compounds of **1** or **2** interested us in terms of elucidating their biosynthesis and their phamacokinetic properties. However, there had been no report on synthesis of the labeled compounds of these oleanane triterpenes or analogs, and development of the preparative procedure for labeling them was a synthetic challenge for us. We first started the synthetic study of ^{13}C-labeled **1** for several reasons. Compound **1** is a typical oleanane triterpene and shown to be converted to myriceric acid A by way of **2**. We envisioned that we could apply the procedure developed for ^{13}C-labeled **1** to the synthesis of **2**, and also to a synthesis of radioisotope(^{14}C)-labeled **1** and **2**, which will be useful for tracing the fate of these compounds in their biosynthetic pathway and their pharmacokinetic metabolic properties. ^{14}C-Labeled **1** was required for tracing of the biochemical conversion of **1** into **2**, which possibly enable the synthesis of **2** in less steps than the current procedure.[2]



oleanolic acid **1**



myricerone **2** (R = H)
myriceric acid A (R = caffeoyl)

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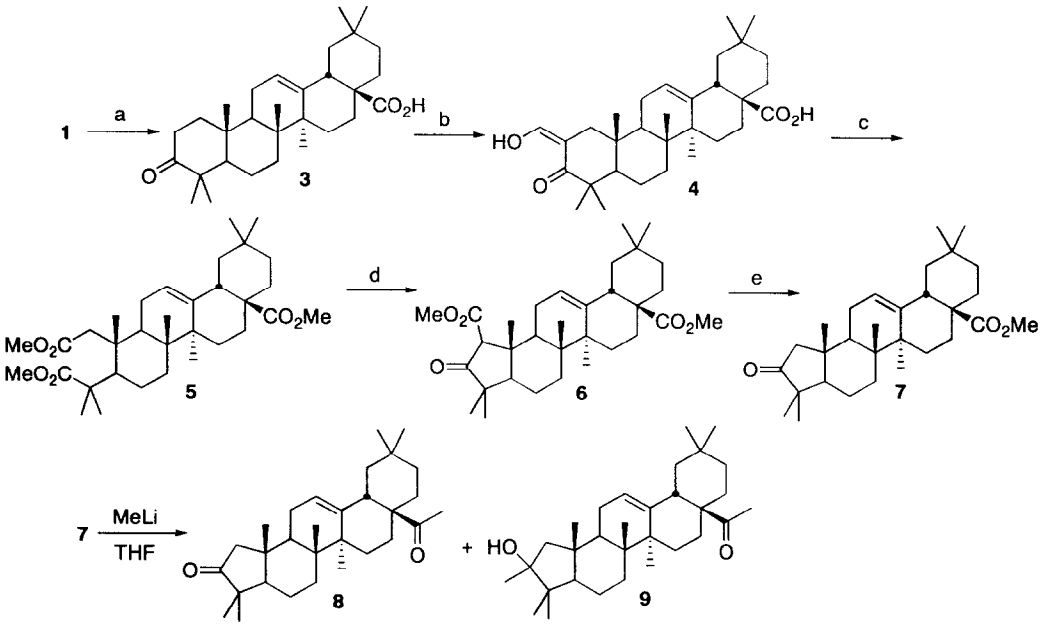
RESULTS

There have been established procedures for labeling steroids, and in most of them isotopic carbon was introduced into the 4-position.[4] However, we failed to label the 4-position of **1** by the known method,[5] and we next explored another strategy for introducing isotopic carbon on 2-position utilizing ^{13}C-MeLi as an isotope source,[6,7] and we found a successful one. Our procedure was shown in Scheme 1 and Scheme 2 using non-labeled MeLi as a model study. We prepared cyclopentanone **7** starting from **1** by a known process (Scheme 1). Compound **1** was oxidized to ketone **3** by Jones reagent, and **3** was condensed with ethyl formate to give 2-hydroxymethylene-oleanolic acid **4**.[8] The A ring of **4** was cleaved by alkaline hydrogen peroxide to give a tricarboxylic acid, which was esterified by diazomethane to yield trimethyl ester **5**.[9] Dieckmann condensation of **5** (*t*-BuOK/benzene) gave 5-membered keto ester **6**.[10,11] Saponification of the methoxycarbonyl group on the 1-position and subsequent decarboxylation of the transient carboxylic acid under a thermal condition gave cyclopentanone **7**. Methyl ester on the 28-position remained intact during the saponification because of extreme steric hindrance around this position. Having key precursor **7** for labeling, we tried to add the labeled one-carbon unit onto the 2-keto group by treating several methylmetal species under various conditions. However, starting ketone **7** was recovered unchanged in every attempt. Under forcing conditions such as treating **7** with a large excess of MeLi (10 eq.), 28-methyl ketone **8** was obtained as main product along with small amount of methyl adduct **9** derived from **8**. A facile enolate formation seemed to be the cause of sluggish addition of methylithium to the cyclopentanone moiety of **7**, and this was supported by an observation that more basic and bulkier butyllithium did not add to **7**.

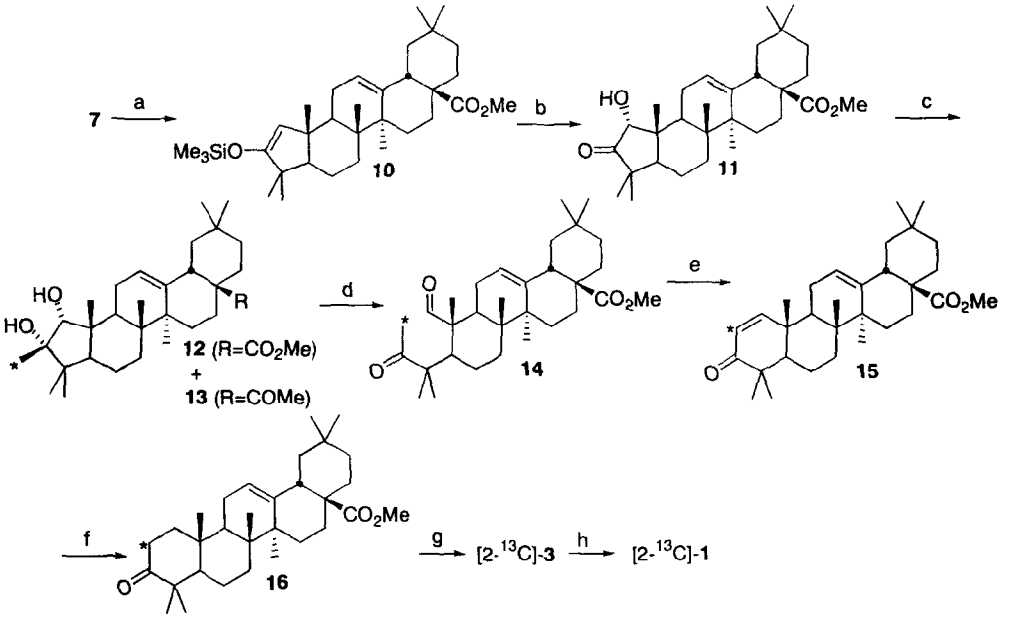


a) Jones reagent, CH_2Cl_2-acetone ; b) HCOOEt, 28% NaOEt, benzene ;
c) 1) 30% H_2O_2, 28% NaOEt, 2) CH_2N_2, MeOH 58% from1;
d) t-BuOK, benzene, 78% ; e) 50% aq. KOH, dioxane, 90%

Scheme 1. Synthesis of 5-membered ketone 7

In order to circumvent the sluggish addition of methyllithium, we then took advantage of the facile enolization of ketone **7** and converted the enolate to a derivative which would undergo addition of methyllithium (Scheme 2). The enolate generated by deprotonating **7** by BuLi was treated with Me_3SiCl to give silyl enol ether **10**, which was treated with *m*-CPBA in aqueous $KHCO_3$ buffer[12,13] to give hydroxy ketone **11**. In contrast to **7**, hydroxy ketone **11** underwent a preferential MeLi addition to 2-keto group to give syn-diol **12** with a small amount of methyl ketone **13** (6.5:1). Addition of MeLi to the cyclopentanone skeleton from the β-side can be explained by the chelating effect of the lithium alkoxide to the 2-ketone accelerating the addition of methyllithium from the unhidered β-side. Structures of **11** and **12** were determined by X-ray crystallography. Diol **12** was cleaved oxidatively by treatment with lead tetraacetate to give ketoaldehyde **14**, which was cyclized under a condition of aldol condensation and yielded 6-membered unsaturated ketone **15** regenerating the 3-ketooleanolic acid skeleton with 1,2-double bond. Final sequence of reactions to oleanolic acid **1** was a conventional one; the hydrogenation of 1,2-double bond, the methyl ester deprotection by LiI and the reduction of the 3-keto group by $NaBH_4$.

The whole sequence of the conversion was efficient in terms of total yield (5.9%) and labeling. We followed the whole procedure using ^{13}C-MeLi (20% enrichment).[14] Final oleanolic acid **1** prepared was shown to contain 20% ^{13}C on the 2-position by LSIMS and ^{13}C-NMR.

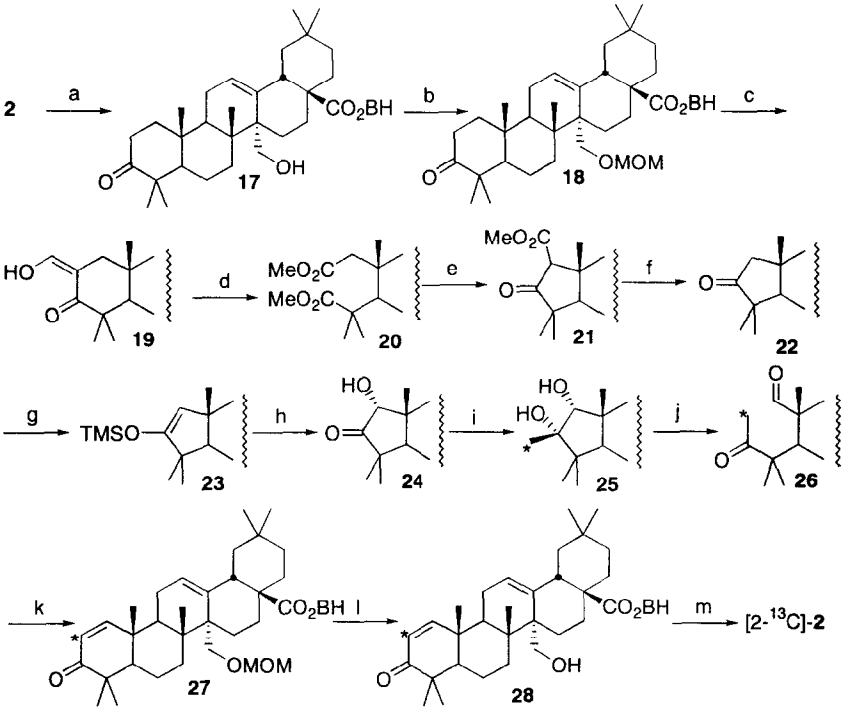


a) BuLi, TMSCl, THF ; b) *m*-CPBA, $KHCO_3$, hexane, 57% from **7** ;
c) MeLi, THF ; d) $Pb(OAc)_4$, $CHCl_3$-AcOH, 59% from **11** ;
e) 50% aq. KOH, dioxane, 78% ; f) H_2, 10% Pd-C, 99% ; g) LiI,
2, 6-lutidine, 80% ; h) $NaBH_4$, THF-MeOH, 70%

Scheme 2. Synthesis of [2-^{13}C]-**1**

^{13}C-Labeled myricerone **2** on the 2-position was synthesized in a similar way to that of oleanolic acid as shown in Scheme 3. In this case, choice of protecting group of the 27-hydroxy group and the 28-carboxyl group appeared to be crucial for successful conversion. After we surveyed several protective groups for both

functional groups, methoxymethyl (MOM) and diphenylmethyl (BH) were chosen as the protecting groups of the 27-hydroxy and 28-carboxy group, respectively. Protected myricerone **18** was prepared by a sequential treatment with diphenydiazomethane and methoxymethyl chloride. We applied the same sequence of reactions for **18** as that used for the preparation of ^{13}C-labeled oleanolic acid **1**. The conversions proceeded as well, and ^{13}C was introduced on the 2-potition by ^{13}C-methyllithium. Final deprotection and hydrogenation of **27** gave [2-^{13}C]-myricerone **2**.



a) Ph_2CN_2, CH_2Cl_2, 91%; b) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, 95%;
c) HCOOEt, 28% NaOEt, benzene ; d) 1) 30% H_2O_2, 28% NaOEt,
2) CH_2N_2, MeOH, 72% from **18** ; e) *t*-BuOK, benzene, 90% ;
f) 50% aq. KOH, dioxane, 64% ; g) BuLi, TMSCl, THF ; h) *m*-CPBA,
$KHCO_3$, hexane, 34% from**22** ; i) MeLi, THF, 41% ; j) $Pb(OAc)_4$,
$CHCl_3$-AcOH, 67% ; k) 50% aq. KOH, dioxane, 74% ; l) 2N-HCl,
THF-MeOH, 68% ; m) H_2, 10% Pd-C, 87%

Scheme 3. Synthesis of C-2 isotope labeled myricerone **2**

Synthetic scheme of ^{13}C-labeled oleanolic acid **1** and myricerone **2** that we have demonstrated has a possible application. Other isotopes such as ^{14}C would be introduced into **1** or **2**. Our scheme would also be applicable to other triterpenes having a similar structure.

EXPERIMENTAL

Reactions were carried out under a nitrogen atmosphere in anhydrous solvents (dried over molecular sievses type 4A). Organic extracts were dried over anhydrous $MgSO_4$. Solvent removal was accomplished

under aspirator pressure using a rotary evaporator. TLC was performed with Merck precoated TLC plates silica gel 60 F_{254}, and compound visualization was effected with 10% H_2SO_4 containing 5% ammonium molybdate and 0.2% ceric sulfate. Silica gel chromatography was done with Merck silica gel 60 (70-230 mesh). ^1H NMR and ^{13}C NMR were determined as $CDCl_3$ solution at 200 and 50.3 MHz, or 300 and 75.5 MHz, respectively. J values are given in hertz. ^{13}C NMR chemical shifts of enriched carbon-13's are indicated by asterisks. Elemental analysis result is for each unlabeled compound.

2-Hydroxymethylene-3-oxoolean-12-en-28-oic acid (4). 28% Sodium methoxide methanol solution (18 mL) and ethyl formate (170 mL) were added dropwise to a solution of **3** [2] (10.0 g, 22.0 mmol) in benzene (200 mL) at a room temperature. After being stirred for 24 h, the reaction mixture was cooled in ice bath. 1 N HCl (100 mL) and EtOAc (100 mL) were added to the mixture, and the layers were separated. The organic layer was washed with saturated NaCl, dried, and concentrated to give crude **4** (11.0 g), which was used in a following reaction without further purification. Analytically pure sample was obtained by silica gel chromatography of a small aliquot of the crude product: TLC (hexane/EtOAc (2/1)) *Rf* 0.55; mp 202–204 °C (hexane/EtOAc); IR (KBr) 3426, 1733, 1693, 1638, 1585 cm^{-1}; ^1H NMR δ 0.73–2.30 (20H, m), 0.82 (3H, s), 0.90 (3H, s), 0.91 (3H, s), 0.93 (3H, s), 1.09 (3H, s), 1.15 (3H, s), 1.18 (3H, s), 2.81–2.88 (1H, m), 5.32–5.34 (1H, m), 8.58 (1H, d, J = 2.8), 14.91 (1H, d, J = 2.8); ^{13}C NMR δ 14.4, 16.8, 19.4, 20.8, 22.9, 23.3, 23.5, 25.7, 27.6, 28.4, 30.7, 31.8, 32.4, 33.0, 33.8, 36.3, 39.1, 39.2, 40.0, 41.0, 41.7, 45.7, 45.8, 46.6, 52.0, 105.6, 122.4, 143.5, 183.9, 188.5, 190.5; $[\alpha]^{21.5}_D$ +106.1° (*c* 1.002 $CHCl_3$); HR-SIMS *m/z* 483.3473 $[M + H]^+$ (calcd for $C_{31}H_{47}O_4$, 482.3472). Anal. Calcd for $C_{31}H_{46}O_4$: C, 77.14; H, 9.61. Found: C, 77.05; H, 9.64.

Methyl 2,3-dimethoxy-2,3-dioxo-2,3-secoolean-12-en-28-oate (5). To a solution of **4** (11.8 g, 24.5 mmol) in MeOH (2.36 L) was added 28% sodium methoxide solution (236 mL) at a room temperature and then 30% aqueous hydrogen peroxide (275 mL) were added at 2–5 °C. The resulting mixture was stirred at the same temperature for 1 h. The mixture was concentrated to 300 mL and neutralized with 6 N HCl and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried, and concentrated. The residue was dissolved in MeOH (600 mL) and treated with diazomethane in Et_2O, and concentrated. MeOH was added and the resulting precipitate was collected by filtration to give **5** (7.4 g, 58% from **1**): TLC (hexane/EtOAc (5/1)) *Rf* 0.40; mp 163–165 °C (hexane); IR (KBr) 1741, 1723 cm^{-1}; ^1H NMR δ 0.73 (3H, s), 0.79–2.44 (19H, m), 0.89 (3H, s), 0.91 (3H, s), 0.98 (3H, s), 1.16 (3H, s), 1.22 (3H, s), 1.24 (3H, s), 2.59–2.68 (1H, m), 2.81–2.90 (1H, m), 3.60 (3H, s), 3.61 (3H, s), 3.62 (3H, s), 5.26–5.30 (1H, m); ^{13}C NMR δ 16.8, 18.9, 20.8, 23.1, 23.6, 23.70, 23.73, 25.5, 27.7, 27.8, 30.7, 31.8, 32.3, 33.1, 33.9, 39.2, 41.3, 41.4, 41.7, 42.3, 45.7, 46.1, 46.8, 48.7, 50.7, 51.4, 51.7, 122.3, 143.5, 171.8, 178.2, 179.8; $[\alpha]^{24}_D$ +47.5° (*c* 1.009 $CHCl_3$); HR-SIMS m/z 545.3843 $[M + H]^+$ (calcd for $C_{33}H_{53}O_6$, 545.3840). Anal. Calcd for $C_{33}H_{52}O_6$: C, 72.76; H, 9.62. Found: C,72.58; H, 9.63.

Methyl 1-methoxycarbonyl-2-oxo-A-norolean-12-en-28-oate (6). To a solution of **5** (6.1 g, 11.2 mmol) in benzene (240 mL) was added *t*-BuOK (1.0 M THF solution, 22 mL) at a room temperature, and the mixture was refluxed for 30 min. The mixture was neutralized with 1 N HCl, and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried, and concentrated. The residue was treated with MeOH and the precipitate was collected by filtration to give **6** (3.8 g). Addtional portion of **6** (0.7 g), (total 4.5 g,

78%) was obtained from the mother liquor by silica gel chromatography: TLC (hexane/EtOAc (5/1)) *Rf* 0.47. mp 108–110 °C; IR (KBr) 1760, 1730 cm^{-1}; ^1H NMR δ 0.78 (3H, s), 0.90(3H, s), 0.92 (3H, s), 1.03 (3H, s), 1.06 (3H, s), 1.12–2.05 (18H,m), 1.13 (3H, s), 1.20 (3H, s), 2.83–2.89 (1H, m), 3.04 (1H, s), 3.62 (3H, s), 3.72 (3H, s), 5.24–5.26 (1H, m); ^{13}C NMR δ 14.0, 17.2, 17.3, 21.0, 23.0, 23.6, 25.3, 26.2, 27.7, 28.3, 30.7, 32.3, 33.1, 33.8, 40.3, 41.4, 41.9, 45.4, 45.8, 46.3, 46.4, 46.7, 51.6, 51.8, 58.4, 68.8, 121.7, 144.1, 170.2, 178.2, 216.5; $[\alpha]^{25}_D$ +117.6° (*c* 0.902 $CHCl_3$); HR-SIMS *m/z* 513.3573 $[M + H]^+$ (calcd for $C_{32}H_{49}O_5$, 513.3577). Anal. Calcd for $C_{32}H_{48}O_5$: C, 74.96; H, 9.44. Found: C,74.70; H, 9.38.

Methyl 2-oxo-A-norolean-12-en-28-oate (7). To a solution of **6** (4.4 g, 8.58 mmol) in 1,4-dioxane (170 mL) was added 50% aqueous KOH solution (110 mL) and the mixture was refluxed for 8 h. The reaction mixture was cooled in ice bath and neutralized with 1 N HCl, and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried, and concentrated. The residue was purified by silica gel chromatography to give **7** (3.5 g, 90%): TLC (hexane/EtOAc (5/1)) *Rf* 0.54; mp 184–187 °C (MeOH); IR (KBr) 1741, 1723 cm^{-1}; ^1H NMR δ 0.79 (3H, s), 0.90 (3H, s), 0.92 (3H, s), 0.924 (3H, s), 0.98 (3H, s), 1.02 (3H, s), 1.07–2.20 (20H,m), 1.20 (3H, s), 2.84–2.90 (1H, m), 3.63 (3H, s), 5.29–5.30 (1H, m); ^{13}C NMR δ 17.4, 17.7, 18.6, 21.8, 23.6, 24.1, 25.8, 26.8, 28.2, 28.6, 31.2, 32.8, 33.1, 33.6, 34.4, 40.6, 41.2, 42.1, 42.5, 46.0, 46.3, 47.3, 52.1, 55.7, 59.6, 122.3, 145.0, 178.7, 225.0; $[\alpha]^{25}_D$ +164° (*c* 1.002 $CHCl_3$); HR-SIMS *m/z* 455.3515 $[M + H]^+$ (calcd for $C_{30}H_{47}O_3$ 455.3522). Anal. Calcd for $C_{30}H_{46}O_3$: C, 79.25; H, 10.20. Found: C,79.12; H, 10.16.

28-Methyl ketone (8), (9). To a solution of **7** (195 mg, 0.429 mmol) in THF (8.0 mL) was added MeLi in Et_2O solution (containing 20% ^{13}C-MeLi, diethyl ether solution, 4.12 mL of 1.04 M solution (4.29 mmol)) at –78 °C. The mixture was stirred for 1 h at –78 °C and then for 50 min at ice-cooled temperature. 1N HCl, H_2O and EtOAc were added to the reaction mixture and the layers were separated. The organic layer was washed with saturated NaCl, dried, and concentrated. The residue was purified by silica gel chromatography to give **8** (112 mg, 60%) and **9** (48 mg, 25%). **8** : TLC (hexane/EtOAc (5/1)) *Rf* 0.50; mp 231 °C (EtOAc); IR ($CHCl_3$) 1729, 1693 cm^{-1}; ^1H NMR δ 0.76 (3H, s), 0.92 (9H, s), 0.99 (3H, s), 1.02 (3H, s), 1.06–2.23 (20H, m), 1.21 (3H, s), 2.78–2.87 (1H, m), 5.32–5.35 (1H, m); ^{13}C NMR δ 16.9, 17.3, 18.1, 21.3, 22.6, 23.6, 25.1, 26.3, 27.5, 28.1, 30.7, 31.1, 32.5, 33.1, 33.8, 40.2, 40.6, 41.7, 42.1, 45.5, 45.8, 46.1, 51.9, 55.1, 59.1, 122.1, 144.7, 214.0, 224.3; $[\alpha]^{22}_D$ +165.8° (*c* 1.001 $CHCl_3$); LSIMS m/z 439 $[M + H]^+$. Anal. Calcd for $C_{30}H_{46}O_2$: C, 82.14; H, 10.57. Found: C,82.06; H, 10.55. **9** : TLC (hexane/EtOAc (5/1)) *Rf* 0.30; mp 231–235 °C; IR ($CHCl_3$) 3596, 1692 cm^{-1}; ^1H NMR δ 0.72 (3H, s), 0.87–2.18 (21H, m), 0.92 (3H, s), 0.93 (3H, s), 0.94 (6H, s), 1.12 (3H, s), 1.15 (3H, s), 1.24 (3H, s), 2.75–2.83 (1H, m), 5.30–5.33 (1H, m); ^{13}C NMR δ 15.8, 17.4, 19.0, 21.0, 22.6, 23.6, 25.2, 26.3, 27.6, 28.3, 29.4, 30.7, 31.1, 33.0, 33.1, 33.8, 40.3, 40.6, 41.7, 41.8, 42.0, 45.6, 46.1, 47.5, 51.9, 59.2, 62.9, 82.9, 122.8, 144.5, 214.2; $[\alpha]^{22}_D$ +69.5° (*c* 1.001 $CHCl_3$); LSIMS *m/z* 455 $[M + H]^+$ (calcd for $C_{31}H_{51}O_2$). Anal. Calcd for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C,81.62; H, 11.13.

Methyl 1α-hydroxy-2-oxo-A-norolean-1,12-dien-28-oate (11). To a solution of **7** (2.35 g, 5.17 mmol) in THF (240 mL) was added BuLi (1.6 M hexane solution, 9.69 mL, 6.06 mmol) dropwise at –78 °C, and the mixture was stirred for 30 min. Chlorotrimethylsilane (1.40 g, 12.9 mmol) was added, and the mixture was stirred for 30 min, and warmed to a room temperature. Saturated $NaHCO_3$ solution, EtOAc and H_2O were

added to the reaction mixture, and the layers were separated. The organic layer was washed with saturated NaCl, dried, and concentrated to give crude silyl enol ether **10**. The residue was dissolved in hexane and was added dropwise to the slurry of $KHCO_3$ (2.59 g, 25.9 mmol) and 3-chloroperoxybenzoic acid (1.23 g, 5.70 mmol) in hexane (97 mL) at an ice-cooled temperature. The mixture was stirred for 30 min at the same temperature, and then warmed to a room temperature. Saturated $Na_2S_2O_3$ solution, H_2O and EtOAc were added to the reaction mixture and the layers were separated. The organic layer was washed with saturated NaCl, dried, and concentrated. The residue was purified by silica gel chromatography to give **11** (1.39 g, 57%): TLC (hexane/EtOAc (5/1)) *Rf* 0.38; mp 245–248 °C (MeOH); IR (KBr) 3475, 1742, 1707 cm^{-1}; ^1H NMR δ 0.80 (3H, s), 0.81 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 0.97 (3H, s), 1.10–2.12 (18H,m), 1.12 (3H, s), 1.25 (3H, s), 2.42–2.48 (1H, m), 2.85-2.90 (1H, m) 3.40 (1H,d), 3.63 (3H, s), 5.29–5.31 (1H, m); ^{13}C NMR δ 15.5, 17.6, 21.8, 23.1, 23.6, 24.6, 26.1, 27.8 29.0, 30.7, 32.1, 32.3, 33.1, 33.8, 35.6, 39.6, 41.6, 42.2, 43.0, 44.2, 45.7, 46.8, 51.5, 52.6, 79.7, 121.8, 144.2, 178.3, 224.1; $[\alpha]^{23}_D$ +189.1° (*c* 1.007 $CHCl_3$); HR-SIMS *m/z* 471.3472 $[M + H]^+$ (calcd for $C_{30}H_{47}O_4$ 471.3472). Anal. Calcd for $C_{30}H_{46}O_4$: C, 76.55; H, 9.85. Found: C,76.17; H, 9.84.

Methyl 1α-hydroxy-2α-hydroxy-2β-[^{13}C]-methyl-A-norolean-12-en-28-oate (12). To a solution of **11** (125 mg, 0.266 mmol) in THF (6.5 mL) was added MeLi (containing 20% ^{13}C-MeLi, 1.05 M diethyl ether solution, 1.01 mL, 1.06 mmol) at an ice-cooled temperature.[14] The reaction mixture was stirred for 15 min at the same temperature. 1 N HCl, H_2O and EtOAc were added to the reaction mixture, and the layers were separated. The organic layer was washed with saturated NaCl, dried, and concentrated. The residue was purified by silica gel chromatography to give a mixture of **12** and 28-methyl ketone **13** (**12** : **13** = 6.5 : 1), from which analytically pure sample was separated by HPLC (column, Cosmosil 5C18 AR 20 mm x 250 mm; solvents, MeCN/ H_2O (9/1); flow rate, 6 ml/min; detection, 197 nm). **12**: TLC (hexane/EtOAc (3/1)) *Rf* 0.35; mp 240–246 °C (EtOAc); ^1H NMR δ 0.75 (3H, s), 0.89 (6H, s), 0.92 (3H, s), 0.93 (6H, s), 1.04–2.12 (17H, m), 1.19 (3H, s), 1.33 (3H, s), 2.28–2.34 (1H, m), 2.43 (1H, s) 2.82–2.88 (2H, m) 3.36 (1H,d), 3.62 (3H, s), 5.28–5.30 (1H, m); ^{13}C NMR δ 17.3, 17.4, 18.5, 22.0, 23.1, 23.6, 24.8, 26.2, 26.6, 27.0*, 27.9, 30.6, 32.3, 33.1, 33.8, 38.6, 40.0, 41.5, 42.1, 44.9, 45.1, 45.7, 46.7, 51.5, 56.4, 81.7, 86.1, 122.5, 143.7, 178.4; FABMS *m/z* 487 $[M + H]^+$ (calcd. for $C_{31}H_{51}O_4$). **13**: TLC (hexane/EtOAc (3/1)) *Rf* 0.35; IR ($CHCl_3$: 3612, 3464, 1692 cm^{-1}; ^1H NMR δ 0.73 (3H, s), 0.89 (3H, s), 0.91 (3H, s), 0.93 (3H, s), 0.94 (6H, s), 1.97–1.78 (14H, m), 1.90–2.16 (3H, m), 2.15 (3H, s), 2.29–2.35 (2H, m), 2.75–3.00 (2H, m), 3.36 (1H,s), 5.32–5.34 (1H, m); ^{13}C NMR δ 17.4, 17.7, 18.6, 22.1, 22.6, 23.6, 25.0, 25.2, 26.2, 26.5*, 27.2, 27.7, 30.7, 31.1, 32.3, 33.1, 33.8, 38.7, 40.1, 41.7, 42.3, 45.0, 45.2, 45.7, 46.0, 51.9, 56.5, 82.0, 86.4, 122.9, 144.1, 214.4; $[\alpha]^{22.5}_D$ +68.8° (*c* 0.551 $CHCl_3$); LSIMS *m/z* 471 $[M + H]^+$ (calcd for $C_{31}H_{51}O_3$). Anal. Calcd for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C,78.79; H, 10.71.

Methyl 1,3-dioxo-[2-^{13}C]-1,2-seco-olean-12-en-28-oate (14). To a solution of crude **12** (119 mg) in $CHCl_3$ (4.0 mL) was added acetic acid (2.0 mL) and lead tetraacetate (587 mg, 1.22 mmol) at a room temperature, and stirring was continued for 20 min. Saturated $Na_2S_2O_3$ solution, H_2O and $CHCl_3$ were added to the mixture, and the layers were separated. The organic layer was washed with saturated NaCl, dried, and concentrated. The residue was purified by silica gel chromatography to give **14** (76 mg, 59% from **11**): TLC (hexane/EtOAc (3/1)) *Rf* 0.51; mp 184–188 °C; IR (KBr) 1725, 1699 cm^{-1}; ^1H NMR δ 0.76 (3H, s), 0.90 (3H, s), 0.91 (3H, s),

0.98 (3H, s), 1.09–2.30 (18H, m) 1.14 (3H, s), 1.17 (3H, s), 1.20 (3H, s), 2.13 (3H, s) 2.41–2.45 (1H, m), 3.61 (3H, s), 5.21–5.24 (1H, m), 9.29 (1H, s); ^{13}C NMR δ 12.0, 16.6, 21.2, 22.1, 23.0, 23.5, 24.0, 24.8, 25.6, 26.0*, 27.6, 30.6, 31.7, 32.2, 33.0, 33.8, 36.7, 38.1, 41.4, 42.1, 45.6, 46.7, 46.9, 51.5, 52.0, 53.8, 121.4, 143.6, 178.0, 205.8, 214.1; $[\alpha]^{25}_D$ +42.1° (*c* 1.008 $CHCl_3$); FABMS m/z 485 $[M + H]^+$ (calcd. for $C_{31}H_{49}O_4$). Anal. Calcd for $C_{31}H_{48}O_4$: C, 76.82; H, 9.98. Found: C,76.61; H, 9.97.

Methyl 3-oxo-[2-^{13}C]-olean-1,12-dien-28-oate (15). To a solution of **14** (349 mg, 0.720 mmol) in 1,4-dioxane (40 mL) was added 10% KOH solution (20 mL) and the mixture was refluxed for 2.5 h. The reaction mixture was cooled in ice bath and neutralized with 6 N HCl and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried, and concentrated. The residue was purified by silica gel chromatography to give **15** (262 g, 78%): TLC (hexane/EtOAc (5/1)) *Rf* 0.53; mp 83–87 °C; IR (KBr) 1726, 1672 cm^{-1}; ^{1}H NMR δ 0.82 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 0.99–2.14 (18H, m), 1.09 (3H, s), 1.151 (6H, s), 1.158 (3H, s), 2.86–2.93 (1H, m), 3.63 (3H, s), 5.33–5.37 (1H, m), 5.80 (1H, d, *J* = 10.0), 7.03 (1H, d, *J* = 10.0); ^{13}C NMR: δ 17.3, 18.6, 18.8, 21.6, 22.9, 23.3, 23.6, 25.8, 27.6, 27.7, 30.6, 32.2, 32.4, 33.1, 33.8, 39.4, 40.0, 41.5, 41.7, 41.9, 44.5, 45.6, 46.7, 51.5, 53.4, 121.6, 125*.0, 144.2, 158.9, 178.1, 205.1; $[\alpha]^{24}_D$ +110.9° (*c* 1.002 $CHCl_3$); FABMS *m/z* 467 $[M + H]^+$ (calcd. for $C_{31}H_{47}O_3$). Anal. Calcd for $C_{31}H_{46}O_3$: C, 79.78; H, 9.94. Found: C,79.60; H, 9.90.

Methyl 3-oxo-[2-^{13}C]-olean-12-en-28-oate (16). To a solution of **15** (262 mg, 0.561 mmol) in THF (15 mL) was added 10% Pd-C (780 mg) and the mixture was stirred at a room temperature for 30 min under a hydrogen atmosphere. The reaction mixture was filtered through celite and concentrated to give **16** (261 g, 99%): TLC (hexane/EtOAc (5/1)) *Rf* 0.46; mp 189–190 °C; IR (KBr) 1725, 1703 cm^{-1}; ^{1}H NMR δ 0.77 (3H, s), 0.89 (3H, s), 0.92 (3H, s), 1.04 (6H, s), 1.07–2.04 (20H, m), 1.08 (3H, s), 1.14 (3H, s), 2.31–2.40 (1H, m), 2.49–2.60 (1H, m) 2.84–2.90 (1H, m), 3.63 (3H, s), 5.29–5.31 (1H, m); ^{13}C NMR δ 14.9, 16.7, 19.5, 21.4, 23.0, 23.4, 23.6, 25.8, 26.4, 27.6, 30.6, 32.1, 32.3, 33.1, 33.8, 34.1*, 36.7, 39.1, 39.2, 41.3, 41.7, 45.8, 46.7, 46.8, 47.4, 51.5, 55.3, 122.1, 143.8, 178.1, 217.5; $[\alpha]^{25}_D$ +92.2° (*c* 1.011 $CHCl_3$); HR-SIMS *m/z* 470.3713 $[M + H]^+$ (calcd. for $C_{30}C^*_1H_{49}O_3$ 470.3713). Anal. Calcd for $C_{31}H_{48}O_3$: C, 79.44; H, 10.32. Found: C,79.17; H, 10.26.

3-Oxo-[2-^{13}C]-olean-12-en-28-oic acid. To a solution of **16** (261 mg, 0.557 mmol) in 2,6-lutidine (26 mL) was added anhydrous LiI (3.59 g, 26.82 mmol) and the mixture was refluxed for 6 h. The mixture was cooled in ice bath, and neutralized with 1 N HCl, and then extracted with EtOAc. The organic layer was washed with saturated NaCl, dried, and concentrated. The residue was purified by silica gel chromatography to give [2-^{13}C]-**3** (202 g, 80%): TLC (hexane/EtOAc (2/1)) *Rf* 0.46; mp 169–172 °C; IR (KBr) 1725, 1703 cm^{-1}; ^{1}H NMR δ 0.81 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 1.03 (3H, s), 1.04 (3H, s), 1.08 (3H, s), 1.14 (3H, s), 1.20–1.98 (20H, m), 2.31–2.40 (1H, m), 2.49–2.60 (1H, m), 2.80–2.87 (1H, m), 5.29–5.31 (1H, m); ^{13}C NMR δ 14.9, 16.9, 19.5, 21.4, 22.8, 23.4, 23.5, 25.8, 26.4, 27.6, 30.6, 32.1, 32.4, 33.0, 33.8, 34.0*, 36.7, 39.0, 39.2, 40.9, 41.6, 45.8, 46.5, 46.8, 47.3, 55.2, 122.3, 143.6, 184.3, 217.4; $[\alpha]^{23}_D$ +95.9° (*c* 1.001 $CHCl_3$).

[2-^{13}C]-Oleanolic Acid. To a solution of [2-^{13}C]-**3** (187 mg, 0.411 mmol) in THF (4 mL) and MeOH (6 mL) was added $NaBH_4$ (77.8 mg, 2.06 mmol) at a room temperature, and the mixture was stirred for 30 min. The

mixture was cooled in ice bath, and neutralized with 1 N HCl, and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried, and concentrated. The residue was treated with EtOAc, and the resulting precipitate was collected by filtration to give [2-^{13}C]-**1** (113 mg). The mother liquor was concentrated, and purified by silica gel chromatography to give additional portion of [2-^{13}C]-**1** (11 mg), (total 124 mg, 70%): TLC (hexane/EtOAc (2/1)) *Rf* 0.34; ^{1}H NMR δ 0.72–2.00 (23H, m) 0.75 (3H, s), 0.77 (3H, s), 0.90 (3H, s), 0.91 (3H, s), 0.92 (3H, s), 0.98 (3H, s), 1.13 (3H, s), 2.80–2.88 (1H, m), 3.19–3.24 (1H, m), 5.27–5.29 (1H, m); ^{13}C NMR (CD_3OD): δ 15.4, 15.7, 17.0, 18.5, 23.2, 23.6, 23.7, 26.0, 26.9*, 27.8, 28.1, 30.8, 32.7, 32.9, 33.2, 34.0, 37.1, 38.7, 38.9, 39.4, 41.4, 41.9, 46.1, 46.6, 47.8, 55.5, 78.9, 122.5, 144.0, 181.3; HR-LSIMS *m/z* 480.3534 $[M + Na]^+$ (calcd. for C_{29} $C^*_1H_{48}O_3Na$, 480.3533).

Diphenylmethyl 27-hydroxy-3-oxoolean-12-en-28-oate (17). To a solution of **2** (10.1 g, 21.5 mmol) in CH_2Cl_2 (100 mL) was added diphenyldiazomethane (12.0 g, 61.8 mmol) at a room temperature, and the mixture was stirred for 1 h. H_2O (50 mL), AcOH (10 mL) and CH_2Cl_2 were added to the mixture, and the layers were separated. The organic layer was washed with saturated $NaHCO_3$ solution, dried, and concetrated. The residue was purified by silica gel chromatography to give **17** (12.4 g, 91%): TLC (hexane/EtOAc (2/1)) *Rf* 0.5; mp 120–125 °C; IR ($CHCl_3$) 1721, 1698 cm^{-1}; ^{1}H NMR δ 0.21 (3H, s), 0.86 (3H, s), 0.89 (3H, s), 0.96 (3H, s), 0.98 (3H, s), 1.03 (3H, s), 1.07–2.02 (10H, m) 2.32–2.46 (2H, m), 3.00–3.09 (1H, m), 3.20 (1H, d), 3.70 (1H, t), 5.79 (1H, m), 6.84 (1H, s), 7.25–7.31 (10H, m); ^{13}C NMR δ 15.3, 17.7, 19.4, 21.3, 22.7, 23.8, 23.9, 24.0, 26.5, 30.8, 31.9, 33.0, 33.4, 33.9, 36.6, 38.6, 39.5, 40.8, 44.9, 46.2, 47.2, 47.4, 47.5, 54.6, 62.9, 76.4, 127.1, 127.2, 127.7, 127.8, 128.3, 128.5, 129.3, 137.6, 140.3, 140.4, 175.9, 217.6; $[\alpha]^{23}_D$ +59.5° (*c* 1.010 $CHCl_3$); HR-SIMS *m/z* 637.4255 $[M + H]^+$ (calcd for $C_{43}H_{57}O_4$ 637.4254). Anal. Calcd for $C_{43}H_{56}O_4$: C, 81.09; H, 8.86. Found: C,80.98; H, 8.87.

Diphenylmethyl 27-methoxymethoxy-3-oxoolean-12-en-28-oate (18). To a solution of **17** (12.1 g, 19.0 mmol) in CH_2Cl_2 (36.0 mL) was added diisopropylethylamine (36.0 mL, 207 mmol) and MOM chloride (14.3 mL, 188 mmol) at a room temperature. The mixture was stirred for 1 h. Ice, 6 N HCl (30 ml) and CH_2Cl_2 were added to the mixture, and the layers were separated. The organic layer was washed with saturated $NaHCO_3$ solution, dried, and concentrated The residue was treated successively with EtOAc (8.3 mL) and hexane (41.7 mL). The resulting precipitate was collected by filtration to give **18** (10.2 g). The mother liquor was concentrated and purified by silica gel chromatography to give **18** (2.06 g), (total 12.26 g, 95%): TLC (hexane/EtOAc (4/1)) *Rf* 0.36; mp 183–184 °C; IR ($CHCl_3$) 1719, 1698 cm^{-1}; ^{1}H NMR δ 0.27 (3H, s), 0.88 (3H, s), 0.93 (3H, s), 0.95 (3H, s), 1.01 (3H, s), 1.04 (3H, s), 1.14–1.98 (20H, m), 2.23–2.60 (2H, m), 2.98–3.09 (1H, m), 3.35 (3H, s), 3.44 (1H, d, *J* = 11.2), 3.48 (1H, d, *J* = 11.2), 4.54 (1H, d, *J* = 6.7), 4.58 (1H, d, *J* = 6.7), 5.51 (1H, m), 6.85 (1H, s), 7.27-7.33 (10H, m); ^{13}C NMR δ 15.1, 17.7, 19.5, 21.4, 22.9, 23.3, 23.5, 23.6, 26.4, 30.6, 32.2, 32.9, 33.0, 33.7, 34.1, 36.7, 39.2, 39.8, 41.6, 45.0, 45.9, 46.6, 47.4, 47.5, 55.6, 71.1, 76.3, 96.9, 138.1, 140.5, 140.5, 176.2, 217.7; $[\alpha]^{23}_D$ +65.1° (*c* 1.000 $CHCl_3$); HR-SIMS *m/z* 703.4334 $[M + H]^+$ (calcd for $C_{45}H_{60}O_5Na$ 703.4335).

Diphenylmethyl 2-hydroxymethylene-27-methoxymethoxy-3-oxoolean-12-en-28-oate (19). 28% Sodium methoxide solution (13.3 mL) and ethyl formate (62 mL) was added dropwise to a solution of **18** (10.9 g, 16.0 mmol) in benzene (218 mL) at a room temperature. After being stirred for 2 h, the reaction mixture was

cooled in ice bath. 1 N HCl and EtOAc were added to the mixture, and the layers were separated. The organic layer was washed with saturated NaCl, dried, and concentrated to give crude **19** (12.0 g), which was used in the next reaction without further purification. Analytically pure sample was obtained by silica gel chromatography of a small aliquot of the crude product: TLC (hexane/EtOAc (4/1)) *Rf* 0.51; mp 98–102 °C; IR ($CHCl_3$) 1720, 1630, 1584 cm^{-1}; ^1H NMR δ 0.28 (3H, s), 0.79 (3H, s), 0.89 (3H, s), 0.96 (3H, s), 1.08 (3H, s), 1.15 (3H, s), 1.25–2.27 (20H, m), 2.99–3.06 (1H, m), 3.36 (3H, s), 3.45 (1H, d, *J* = 11.4), 3.65(1H, d, *J* = 11.4), 4.54 (1H, d, *J* = 6.6), 4.59 (1H, d, *J* = 6.6), 5.54–5.55 (1H, m), 6.85 (1H, s), 7.26–7.33 (10H, m), 8.55 (1H, d, *J* = 3.0), 14.90 (1H, d, *J* = 3.3); ^{13}C NMR δ 14.6, 17.5, 19.4, 20.8, 22.8, 23.3, 23.4, 23.5, 28.4, 30.6, 32.1, 32.7, 33.0, 33.7, 36.3, 39.3, 39.6, 40.0, 41.7, 45.0, 45.8, 46.2, 46.6, 52.3, 55.6, 71.0, 76.3, 96.8, 105.7, 126.4, 127.1, 127.2, 127.6, 127.7, 128.2, 128.4, 137.9, 140.4, 140.5, 176.2, 188.3, 190.6; $[\alpha]^{23}_D$ +72.3° (*c* 1.006 $CHCl_3$); LSIMS *m/z* 709 $[M + H]^+$ (calcd for $C_{46}H_{61}O_6$). Anal. Calcd for $C_{46}H_{60}O_6$ $0.4H_2O$: C, 77.15; H, 8.56. Found: C,77.18; H, 8.38.

Diphenylmethyl 2,3-dimethoxy-27-methoxymethoxy-2,3-dioxo-2,3-secoolean-12-en-28-oate (20). To a solution of **19** (3.0 g, 4.23 mmol) in MeOH (300 mL) was added 28% sodium methoxide solution (26.6 ml) and 30% aqueous hydrogen peroxide (22.4 ml) at a room temperature. The resulting mixture was stirred at the same temperature for 1 h, and treated with saturated $Na_2S_2O_3$ solution. The mixture was concentrated and neutralized with 6 N HCl, and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried, and concentrated. The residue was dissolved in MeOH (150 ml) and treated with diazomethane in Et_2O , and concentrated. The residue was purified by silica gel chromatography to give **20** (2.3 g, 72% from **18**): TLC (hexane/EtOAc (4/1)) *Rf* 0.41; mp 98–102 °C; IR ($CHCl_3$) 1719 cm^{-1}; ^1H NMR δ 0.23 (3H, s), 0.88 (3H, s), 0.93 (3H, s), 1.21 (3H, s), 1.93 (3H, s), 1.10–2.62 (24H, m), 1.15 (3H, s), 2.96–3.04 (1H, m), 3.39 (3H, s), 3.57 (1H, s), 3.58(1H, s), 4.57 (1H, d, *J* = 6.8), 4.67 (1H, d, *J* = 6.8), 5.46–5.49 (1H, m), 6.83 (1H, s), 7.26–7.33 (10H, m); ^{13}C NMR δ 17.6, 19.1, 20.7, 22.6, 23.3, 23.5, 23.7, 27.7, 30.6, 32.2, 32.7, 33.0, 33.8, 39.7, 40.1, 41.3, 41.4, 41.7, 45.1, 46.0, 46.2, 46.6, 48.5, 50.7, 51.6, 55.7, 70.7, 76.3, 97.1, 126.5, 127.2, 127.6, 127.7, 128.2, 128.4, 137.8, 140.5, 140.5, 171.4, 176.3, 179.8; $[\alpha]^{23}_D$ +29.9° (*c* 1.002 $CHCl_3$); HR-SIMS m/z 779.4494 $[M + Na]^+$ (calcd for $C_{47}H_{64}O_8Na$ 779.4495). Anal. Calcd for $C_{47}H_{64}O_8$: C, 74.57; H, 8.52. Found: C,74.49; H, 8.52.

Diphenylmethyl 1-methoxycarbonyl -27-methoxymethoxy-2-oxo-A-norolean-12-en-28-oate (21). To a solution of **20** (3.44 g, 4.54 mmol) in benzene (86 mL) was added *t*-BuOK (1.0 M THF solution, 22.5 mL) at a room temperature and the mixture was refluxed for 4.5 h. The reaction mixture was cooled in ice bath and neutralized with 1 N HCl and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried and concentrated. The residue was purified by silica gel chromatography to give **21** (2.96 g, 90%): TLC (toluene/EtOAc (4/1)) *Rf* 0.69; mp 90–94 °C; IR ($CHCl_3$) 1756, 1724 cm^{-1}; ^1H NMR δ 0.24 (3H, s), 0.89 (3H, s), 0.95 (3H, s), 1.00 (3H, s), 1.05–1.23 (18H, m), 1.01 (3H, s), 1.02 (3H, s), 2.96 (1H, s), 2.98–3.06 (1H, m), 3.19 (3H, s), 3.39 (3H, s), 3.47 (1H, d, *J* = 11.3), 3.91 (1H, d, *J* = 11.3), 3.71 (3H, s), 4.58 (1H, d, *J* = 6.8), 4.62 (1H, d, *J* = 6.8), 5.46–5.49 (1H, m), 6.85 (1H, s), 7.28–7.38 (10H, m); ^{13}C NMR δ 14.2, 17.2, 18.1, 20.9, 23.0, 23.1, 23.5, 25.3, 28.4, 30.6, 32.1, 32.8, 33.0, 33.7, 40.7, 41.5, 44.9, 45.3, 46.0, 46.2, 46.5, 46.8, 51.7, 55.7, 58.6, 68.8, 71.5, 76.4, 96.9, 125.9, 127.1, 127.2, 127.7, 128.3, 128.4, 138.5, 140.3, 140.5, 170.1, 176.2, 216.6;

$[\alpha]^{23}_D$ +84.4° (*c* 1.010 $CHCl_3$); FABMS m/z 747 $[M + Na]^+$ (calcd. for $C_{46}H_{60}O_7Na$ 747). Anal. Calcd for $C_{46}H_{60}O_7$ $0.2H_2O$: C, 75.83; H, 8.36. Found: C,75.78; H, 8.30.

Diphenylmethyl 27-methoxymethoxy-2-oxo-A-norolean-12-en-28-oate (22). To a solution of **21** (2.2 g, 3.03 mmol) in 1,4-dioxane (66 mL) was added 50% aqueous KOH solution (22 mL) and the mixture was refluxed for 7 h. The reaction mixture was cooled in ice bath and neutralized with 1 N HCl and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried and concentrated. The residue was purified by silica gel chromatography to give **22** (1.3 g, 64%): TLC (hexane/EtOAc (4/1)) *Rf* 0.36; mp 177–180 °C; IR ($CHCl_3$) 1727 cm^{-1}; ^1H NMR δ 0.26 (3H, s), 0.80 (3H, s), 0.89 (3H, s), 0.95 (6H, s), 0.97(3H, s), 1.20–2.16 (20H, m), 3.00–3.06 (1H, m) 3.36 (3H, s), 3.45 (1H, d, *J* = 11.4), 3.71 (1H, d, *J* = 11.4), 4.56 (1H, d, *J* = 6.6), 4.60 (1H, d, *J* = 6.6), 5.50–5.52 (1H, m), 6.85 (1H, s), 7.24–7.34 (10H, m); ^{13}C NMR δ 17.1, 18.0, 21.2, 23.0, 23.2, 23.5, 25.3, 28.1, 30.6, 32.2, 33.1, 33.2, 33.7, 40.5, 40.6, 41.9, 44.9, 45.4, 46.1, 46.3, 46.6, 55.2, 55.6, 59.3, 71.5, 76.3, 96.9, 126.0, 127.1, 127.3, 127.6, 127.7, 128.3, 128.4, 138.7, 140.4, 140.5, 176.2, 224.3; $[\alpha]^{23}_D$ +116.1° (*c* 0.950 $CHCl_3$); HR-SIMS m/z 689.4181 $[M + Na]^+$ (calcd for $C_{44}H_{58}O_5Na$ 689.4179). Anal. Calcd for $C_{44}H_{58}O_5$: C, 79.24; H, 8.77. Found: C,78.92; H, 8.75.

Diphenylmethyl 1α-hydroxy-27-methoxymethoxy-2-oxo-A-norolean-1,12-dien-28-oate (24). To a solution of **22** (400 mg, 0.60 mmol) in THF (32 mL) was added BuLi (1.69 M hexane solution, 816 mL, 1.38 mmol) dropwise at –78 °C and the mixture was stirred for 30 min. Chlorotrimethylsilane (130 mg, 1.20 mmol) was added and the mixture was stirred for 2 h. Saturated $NaHCO_3$ solution, EtOAc and H_2O were added to the reaction mixture and the layers were separated. The organic layer was washed with saturated NaCl, dried and concentrated to give crude **23**. The residue was dissolved in hexane and was added dropwise to the slurry of $KHCO_3$ (300 mg, 3.00 mmol) and 3-chloroperoxy benzoic acid (142 g, 0.658 mmol) in hexane(14 mL) at an ice-cooled temperature. The reaction mixture was stirred for 15 min at the same temperature. Saturated $Na_2S_2O_3$ solution, H_2O and EtOAc were added to the reaction mixture and the layers were separated. The organic layer was washed with saturated NaCl, dried and concentrated. The residue was purified by silica gel chromatography to give **24** (141 mg, 34%): TLC (hexane/EtOAc (3/1)) *Rf* 0.21; mp 103–107 °C; IR ($CHCl_3$) 3599, 1736 cm^{-1}; ^1H NMR δ 0.29 (3H, s), 0.68 (3H, s), 0.89 (3H, s), 0.94 (3H, s), 0.95(3H, s), 1.08 (3H, s), 1.20–2.02 (20H, m), 2.53–2.59 (1H, m) 3.00–3.06 (1H, m), 3.35 (1H, d), 3.38 (3H, s), 3.50 (1H, d, *J* = 11.4), 3.72 (1H, d, *J* = 11.4), 4.56 (1H, d, *J* = 6.6), 4.63 (1H, d, *J* = 6.6), 5.51–5.53 (1H, m), 6.85 (1H, s), 7.25–7.37 (10H, m); ^{13}C NMR δ 15.6, 17.5, 18.6, 21.7, 23.0, 23.2, 23.5, 24.7, 29.1, 30.6, 32.2, 32.8, 33.0, 33.8, 36.2, 40.2, 41.8, 43.1, 44.0, 44.9, 46.2, 46.7, 52.7, 55.5, 71.2, 76.4, 79.6, 96.9, 126.1, 127.2, 127.3, 127.6, 128.3, 128.4, 138.5, 140.5, 140.6, 176.3, 223.9; $[\alpha]^{23}_D$ +121.5° (*c* 0.904 $CHCl_3$); FABMS m/z 705 $[M + Na]^+$ (calcd. for $C_{44}H_{58}O_6Na$ 705). Anal. Calcd for $C_{44}H_{58}O_6$: C, 77.38; H, 8.56. Found: C,77.30; H, 8.58.

Diphenylmethyl 1α-hydroxy-2α-hydroxy-27-methoxymethoxy-2β-[^{13}C]-methyl-A-norolean-12-en-28-oate (25). To a solution of **24** (143 mg, 0.209 mmol) in THF (12 mL) was added MeLi (containing 20% ^{13}C-MeLi, 0.72M diethyl ether solution, 1.16 mL, 0.836 mmol) at an ice-cooled temperature. The reaction mixture was stirred for 30 min at the same temperature. 1 N HCl, H_2O and EtOAc were added to the reaction

mixture and the layers were separated. The organic layer was washed with saturated NaCl, dried and concentrated. The residue was purified by silica gel chromatography to give **25** (60 mg, 41%): TLC (hexane/EtOAc (2/1)) *Rf* 0.35; mp 207–210 °C; IR ($CHCl_3$) 3621, 3482, 1722 cm^{-1}; ^1H NMR δ 0.25 (3H, s), 0.82 (3H, s), 0.82 (3H, s), 0.85 (3H, s), 0.88(6H, s), 0.94 (3H, s), 1.06–2.02 (19H, m), 2.32–2.37 (1H, m), 2.59–2.70 (2H, m), 2.98–3.06 (1H, m), 3.31 (1H, m), 3.37 (1H, s), 3.50 (1H, d, J = 11.1), 3.65 (1H, d, J = 11.1), 4.55 (1H, d, J = 6.3), 4.61 (1H, d, J = 6.3), 5.51–5.53 (1H, m), 6.85 (1H, s), 7.26–7.34 (10H, m); ^{13}C NMR δ 17.5, 18.5, 18.5, 22.0, 23.1, 23.3, 23.6, 25.0, 26.7, 27.1*, 30.6, 32.2, 33.0, 33.1, 33.8, 39.4, 40.7, 41.7, 44.9, 45.2, 46.2, 46.6, 55.4, 56.6, 71.6, 76.3, 81.7, 86.2, 97.0, 126.5, 127.2, 127.3, 127.6, 127.7, 128.2, 128.4, 138.3, 140.6, 176.3; $[\alpha]^{23}_D$ +45.5° (*c* 1.000 $CHCl_3$); HR-SIMS m/z 722.4478 $[M + H]^+$ (calcd for $C_{44}C^*_1H_{62}O_6Na$ 722.4475).

Diphenylmethyl 27-methoxymethoxy-1,3-dioxo-[2-^{13}C]-1,2-seco-olean-12-en-28-oate (26). To a solution of crude **25** (60 mg, 85.84 mmol) in $CHCl_3$ (4.0 mL) was added acetic acid (2.0 mL) and lead tetraacetate (207 mg, 0.467 mmol) at a room temperature and stirred for 15 min. $NaHCO_3$ and saturated $Na_2S_2O_3$ solution and H_2O and $CHCl_3$ were added to the reaction mixture and the layers were separated. The organic layer was washed with saturated NaCl, dried and concentrated. The residue was purified by silica gel chromatography to give **26** (40 mg, 67%): TLC (hexane/EtOAc (2/1)) *Rf* 0.35; mp 83–86 °C; IR ($CHCl_3$) 1717 cm^{-1}; ^1H NMR δ 0.22 (3H, s), 0.89 (3H, s), 0.94 (6H, s), 1.05 (3H, s), 1.10 (3H, s), 2.10 (3H, s), 0.50–2.18 (18H, m), 2.97–3.06 (1H, m) 3.43 (3H, S), 3.48 (1H, d, J = 11.0), 3.70 (1H, d, J = 11.0), 4.56 (1H, d, J = 6.6); 4.63 (1H, d, J = 6.6), 5.43–5.45 (1H, m), 6.85 (1H, s), 7.26–7.34 (10H, m), 9.22 (1H, S); ^{13}C NMR δ 12.3, 17.6, 21.1, 21.9, 23.0, 23.2, 23.4, 24.0, 24.8, 25.8*, 26.7, 30.6, 32.1, 32.5, 33.0, 33.8, 37.1, 38.6, 41.9, 44.8, 46.0, 46.8, 46.9, 52.1, 53.9, 55.8, 70.7, 76.3, 96.9, 126.1, 127.0, 127.3, 127.7, 128.3, 128.4, 137.3, 140.3, 140.5, 176.1, 205.6, 213.8; $[\alpha]^{23}_D$ +35.9° (*c* 0.429 $CHCl_3$); HR-SIMS m/z 720.4319 $[M + H]^+$ (calcd for $C_{44}C^*_1H_{60}O_6Na$ 720.4318).

Diphenylmethyl 27-methoxymethoxy-3-oxo-[2-^{13}C]-olean-1,12-dien-28-oate (27). To a solution of **26** (40 mg, 57.39 mmol) in 1,4-dioxane (4 mL) was added 10% KOH solution (2 ml) and the mixture was refluxed for 2 h. The reaction mixture was cooled in ice bath and neutralized with 1 N HCl and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried and concentrated. The residue was purified by silica gel chromatography to give **27** (29 mg, 74%): TLC (hexane/EtOAc (2/1)) *Rf* 0.62; mp 84–87 °C; IR ($CHCl_3$): 1720, 1662 cm^{-1}; ^1H NMR δ 0.28 (3H, s), 0.89 (3H, s), 0.96 (3H, s), 1.04 (3H, s), 1.06(6H, s), 1.11 (3H, s), 1.13–2.09 (15H, m), 2.99–3.09 (1H, m) 3.31 (3H, s), 3.39 (1H, d, J = 10.8), 3.67 (1H, d, J = 10.8), 4.51 (1H, d, J = 6.0), 4.56 (1H, d, J = 6.0), 5.55–5.58 (1H, m), 5.75 (1H, d, J = 10.0), 6.99 (1H, d, J = 10.0), 6.86 (1H, s), 7.26–7.34 (10H, m); ^{13}C NMR δ 18.2, 18.8, 21.5, 23.0, 23.2, 23.4, 23.5, 26.8, 27.8, 30.6, 32.1, 33.0, 33.7, 39.4, 40.4, 41.7, 42.2, 44.5, 44.7, 46.0, 46.6, 53.7, 55.7, 71.0, 76.3, 96.8, 124.2, 124.8*, 125.9, 127.1, 127.3, 127.6, 127.7, 128.0, 128.4, 138.3, 140.4, 140.5, 159.2, 176.2, 205.2; $[\alpha]^{23}_D$ +83.5° (*c* 0.303 $CHCl_3$); HR-SIMS m/z 702.4209 $[M + H]^+$ (calcd for $C_{44}C^*_1H_{58}O_5Na$ 702.4212).

Diphenylmethyl 27-hydroxy-3-oxo-[2-^{13}C]-olean-1,12-dien-28-oate (28). To a solution of **27** (29 mg, 42.71 mmol) in THF (1 mL) and MeOH (1 mL) was added 2 N HCl (1 ml) at a room temperature and the mixture was refluxed for 3 h. H_2O and EtOAc were added to the reaction mixture and the layers were separated. The organic layer was washed with saturated NaCl, dried and concentrated. The residue was

purified by silica gel chromatography to give **28** (19 mg, 68%): TLC (hexane/EtOAc (2/1)) *Rf* 0.50; IR ($CHCl_3$) 3531, 1723, 1661 cm^{-1}; ^1H NMR δ 0.23 (3H, s), 0.82–2.20 (17H, m), 0.92 (3H, s), 0.99 (3H, s), 1.01 (3H, s), 1.05(6H, s), 1.11 (3H, s), 3.05–3.11 (1H, m), 3.23 (1H, d, *J* = 11.7), 3.71 (1H, t, *J* = 11.7), 5.75 (1H, d, *J* = 10.2), 5.83–5.85 (1H, m), 6.86 (1H, s), 6.93 (1H, d, *J* = 10.2), 7.27–7.35 (10H, m); ^{13}C NMR δ18.2, 18.7, 19.1, 21.5, 22.7, 23.7, 23.8, 24.0, 27.7, 30.8, 31.9, 32.2, 33.0, 33.5, 39.3, 40.2, 40.9, 42.2, 44.4, 44.9, 46.2, 47.7, 53.0, 63.0, 76.5, *125.1, 127.1, 127.2, 127.7, 127.8, 128.3, 128.5, 129.0, 138.0, 140.3, 140.4, 158.7, 175.8, 205.1; $[\alpha]^{22}_D$ +63.1° (*c* 0.955 $CHCl_3$); HR-SIMS m/z 636.4136 $[M + H]^+$ (calcd for $C_{42}C^*_1H_{55}O_4$ 636.4131).

[2-^{13}C]-Myricerone. To a solution of **28** (19 mg, 29.93 mmol) in THF (1 mL) and MeOH (1 mL) was added 10% Pd-C (48 mg) and the mixture was stirred at a room temperature for 10 min under hydrogen atmosphere. The reaction mixture was filtered through celite and concentrated to give [2-^{13}C]-**2** (12 mg, 87%): TLC (hexane/EtOAc (2/1)) *Rf* 0.28; ^1H NMR δ 0.76 (3H, s), 0.91 (3H, s), 0.96 (3H, s), 1.01 (6H, s), 1.08 (3H, s), 1.12–2.59 (24H, m), 2.89–2.98 (1H, m) 3.23 (1H, d), 3.78 (1H, d), 5.86 (1H, m); ^{13}C NMR ($CDCl_3$): δ 15.5, 18.3, 19.5, 21.3, 22.4, 23.8, 24.1, 24.4, 26.5, 30.8, 32.0, 32.2, 33.0, 33.4, *33.9, 36.8, 38.6, 39.7, 40.4, 44.8, 46.1, 47.2, 47.5, 47.6, 54.7, 63.0, 129.4, 137.7, 183.5, 217.4; HR-SIMS m/z 472.3505 $[M + H]^+$ (calcd for $C_{29}C^*_1H_{47}O_4$ 472.3505).

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