Four New Ursane-Type Triterpenes, Olibanumols K, L, M, and N, from Traditional Egyptian Medicine Olibanum, the Gum-Resin of *Boswellia carterii*

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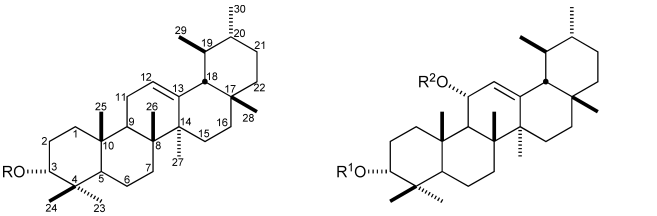
Four new ursane-type triterpenes, olibanumols K (1), L (2), M (3), and N (4), were isolated from traditional Egyptian medicine olibanum, the exuded gum-resin from *Boswellia carterii* Birdw. Their structures were elucidated on the basis of chemical and physicochemical evidence.

Key words olibanumol; olibanum; *Boswellia carterii*; triterpene; Burseraceae

During the course of our studies on bioactive constituents from Egyptian and Yemeni natural medicines,[1—12] we found that 80% aqueous acetone extract of exuded gum-resin of *Boswellia carterii*, so called olibanum (frankincense), showed anti-inflammatory effect by monitoring the inhibitory activity of nitric oxide (NO) production induced by lipopolysaccharide (LPS) in mouse peritoneal macrophages.[10] From the extract, three monoterpenes, olibanumols A—C, a diterpene, olibanumol D, and six triterpenes, olibanumols E—J, were isolated together with five monoterpenes and two triterpenes, epilupeol (**5**) and isofouquierol (**21**).[10,13] Among them, several constituents including olibanumols A, D, E, and H exhibited the inhibitory effect on the NO production.[10] As a continuing study on this natural medicine, we further isolated four new ursane-type triterpenes, olibanumols K (**1**), L (**2**), M (**3**), and N (**4**), together with 19 known triterpenes. This paper deals with the isolation and structure elucidation of these newly isolated triterpenes (**1**—**4**).[14]



Olibanumol K (**1**): R = HCO
3-Epi-α-amyrin (**1a**): R = H

Olibanumol L (**2**): R^1 = Ac, R^2 = H
Olibanumol M (**3**): R^1 = H, R^2 = CH_3
Olibanumol N (**4**): R^1 = Ac, R^2 = CH_3

Chart 1

The gum-resin from *B. carterii* collected in Yemen and purchased at Cairo of Egypt was extracted with 80% aqueous acetone at room temperature to give an aqueous acetone extract (78.9% from the natural medicine), as was reported.[10] By the intensive chromatographies on the aqueous acetone extract, **1** (0.0013%), **2** (0.043%), **3** (0.0043%), and **4** (0.0035%) have been newly isolated together with seven lupane-type triterpenes, epilupeol acetate[15] (**6**, 2.87%), lup-20(30)-ene-3α,29-diol[16] (**7**, 0.0050%), glochidiol[17] (**8**, 0.0017%), lupeol[18] (**9**, 0.72%), lup-20(29)-ene-2α,3β-diol[19] (**10**, 0.0026%), 3β-acetoxylup-20(29)-en-11β-ol[20] (**11**, 0.043 %), and lupenone[21,22] (**12**, 0.33%), five ursane-type triterpenes, urs-9(11),12-dien-3β-ol[23,24] (**13**, 0.0012%), neoilexonol[25] (**14**, 0.039%), neoilexonol acetate[26,27] (**15**, 0.27%), urs-12-ene-3β,11α-diol[28] (**16**, 0.0041%), and urs-12-ene-3α,11α-diol[28] (**17**, 0.015%), and five dammarane-type and two nordammarane-type triterpenes, dammarenediol II[29,30] (**18**, 0.089%), dammarenediol II acetate[30] (**19**, 0.45%), 3-*O*-acetyl-3β,20*S*,24-trihydroxydammar-25-ene[31] (**20**, 0.0022%), isofouquierol acetate[31,32] (**22**, 0.066%), ocotillol acetate[33] (**23**, 0.0044%), 3β-hydroxymansumbin-13(17)-en-16-one[34] (**24**, 0.0065%), and mansumbinol[35] (**25**, 0.0071%).

Structure of Olibanumol K (1) Olibanumol K (**1**) was obtained as a white powder with positive optical rotation ($[\alpha]_D^{27}$+16.2 in MeOH). The IR spectrum of **1** showed absorption bands at 1725 cm^{-1} ascribable to an ester carbonyl function. In the positive-ion fast atom bombardment (FAB)-MS of **1**, a quasimolecular ion peak was observed at *m*/*z* 477 $(M+Na)^+$, and high-resolution positive-ion FAB-MS analysis revealed the molecular formula of **1** to be $C_{31}H_{50}O_2$. The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[36] showed signals assignable to eight methyls [δ 0.80, 0.89, 0.92, 0.92, 1.11, 1.13 (3H each, all s, 28, 23, 24, 25, 27, 26-H_3), 0.90 (3H, d, J=7.4 Hz, 29-H_3), 0.92 (3H, d, J=7.0 Hz, 30-H_3)], a methine bearing an oxygen function [δ 4.77 (1H, br s, 3-H)], an olefin [δ 5.13 (1H, dd-like, 12-H)], and a formyl group [δ 8.13 (1H, s, HCO–)] together with nine methylenes, five methines, and six quaternary carbons. The ursane-type triterpene structure of **1** was constructed on the basis of the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) and heteronuclear multiple bond correlation (HMBC) experiments as shown in Fig. 1. Thus, ^{1}H–^{1}H COSY experiment indicated the presence of partial structures in bold lines. In the HMBC experiment on **1**, long-range correlations were observed between the following protons and carbons: 3-H and the formyl ester carbonyl carbon (δ_C 160.7); 23-H_3 and 3—5, 24-C; 24-H_3 and 3—5, 23-C; 25-H_3 and 1, 5, 9, 10-C; 26-H_3 and 7—9, 14-C; 27-H_3 and 8, 13—15-C; 28-H_3 and 16—18, 22-C; the formyl proton and 3-C. Finally, alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide (KOH)–50% aqueous 1,4-dioxane (1 : 1, v/v) liberated 3-epi-α-amyrin[37] (**1a**), so that the stereostructure of olibanumol K was determined to be 3-epi-α-amyrin formate (**1**).

Structures of Olibanumols L (2), M (3), and N (4) Olibanumol L (**2**) was also obtained as a white powder with positive optical rotation ($[\alpha]_D^{28}$+14.5 in MeOH) and showed absorption bands at 3432 and 1739 cm^{-1} due to hydroxyl and

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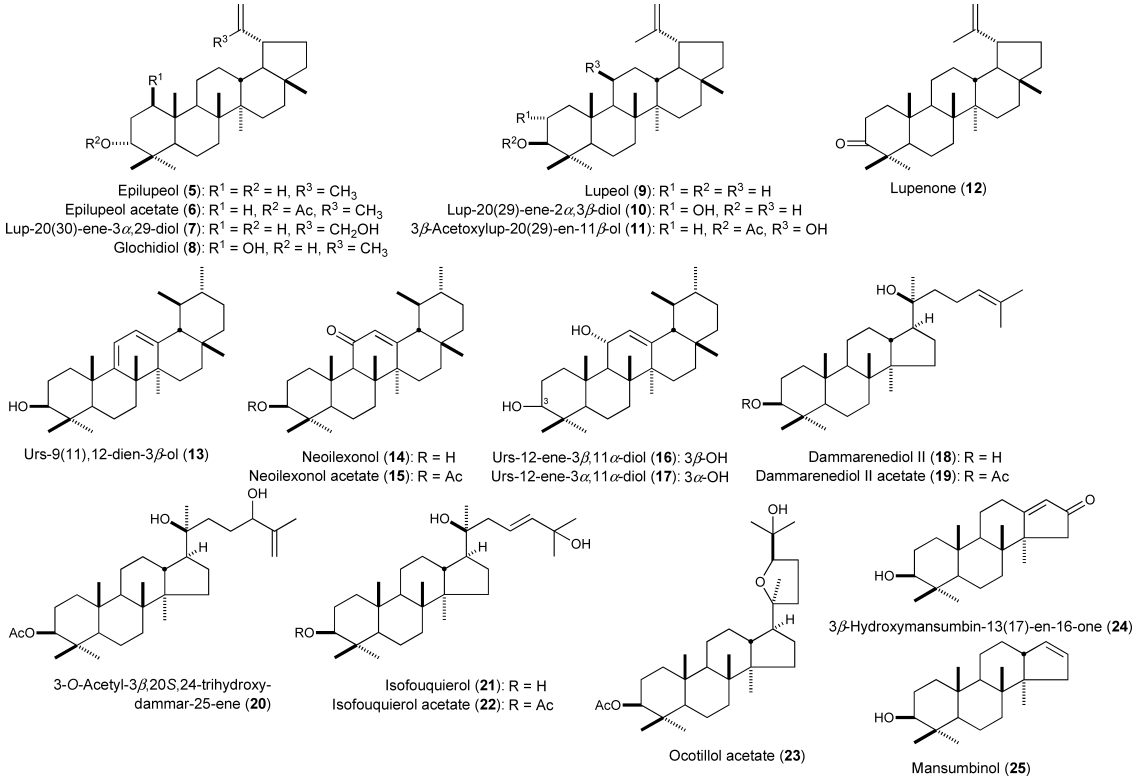

Chart 2

Table 1. ^{13}C-NMR Data for Olibanumols K (**1**), L (**2**), M (**3**), and N (**4**)

Position	1	2	3	4
1	33.8	35.7	33.6	34.9
2	23.0	22.8	25.5	22.9
3	78.5	78.0	75.9	78.1
4	36.5	36.5	37.5	36.5
5	50.0	49.9	48.8	49.8
6	18.2	18.1	18.3	18.1
7	32.8	33.3	33.6	33.1
8	40.2	43.4	43.5	43.0
9	47.5	55.5	51.8	52.6
10	36.9	38.0	38.1	38.0
11	23.3	68.1	76.6	76.7
12	124.3	128.4	124.0	123.5
13	139.5	142.7	143.4	143.3
14	42.2	42.1	42.1	42.1
15	28.2	27.9	27.9	27.9
16	26.7	26.7	26.7	26.8
17	33.8	33.5	33.4	33.5
18	59.1	58.0	58.5	58.4
19	39.7	39.4	39.5	39.5
20	39.7	39.3	39.3	39.3
21	31.3	31.1	31.1	31.1
22	41.6	41.2	41.4	41.4
23	28.8	28.6	28.6	28.1
24	22.0	22.0	22.4	22.0
25	15.6	16.6	16.8	16.9
26	17.0	18.1	18.2	18.2
27	23.5	23.2	22.6	22.6
28	28.1	28.7	28.7	28.7
29	17.6	17.5	17.4	17.3
30	21.5	21.3	21.3	21.4
H<u>C</u>O–	160.7			
CH$_3$<u>C</u>O–		170.5		170.6
<u>C</u>H$_3$CO–		21.4		21.3
<u>C</u>H$_3$O–			54.2	54.7

Measured in $CDCl_3$.

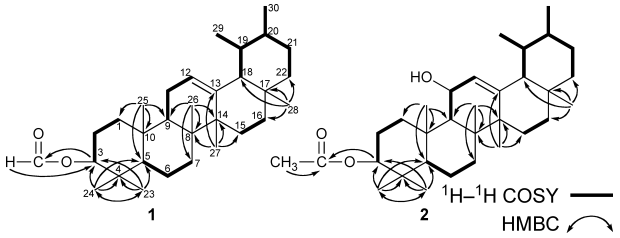

Fig. 1. ^{1}H–^{1}H COSY and HMBC Experiments of **1** and **2**

ester carbonyl functions in the IR spectrum. The molecular formula $C_{32}H_{52}O_3$ was determined from the positive-ion FAB-MS [*m/z* 507 $(M+Na)^+$] and by high-resolution MS measurement. The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **2** indicated the presence of eight methyls [δ 0.81, 0.86, 0.93, 1.19 (3H each, all s, 28, 24, 23, 27-H$_3$), 1.11 (6H, s, 25, 26-H$_3$), 0.86, 0.88 (3H each, both d, J=7.6 Hz, 29, 30-H$_3$)], an acetyl [δ 2.03 (3H, s, CH_3CO–)], two methines bearing an oxygen function [δ 4.27 (1H, dd-like, 11-H), 4.61 (1H, br s, 3-H)], and an olefin [δ 5.18 (1H, br s, 12-H)] together with eight methylenes, five methines, and seven quaternary carbons. As shown in Fig. 1, the ^{1}H–^{1}H COSY experiment on **2** indicated the presence of partial structures written in bold lines. In the HMBC experiment on **2**, long-range correlations were observed between the following protons and carbons: 3-H and the acetyl carbonyl carbon (δ_C 170.5); 23-H$_3$ and 3—5, 24-C; 24-H$_3$ and 3—5, 23-C; 25-H$_3$ and 1, 5, 9, 10-C; 26-H$_3$ and 7—9, 14-C; 27-H$_3$ and 8, 13—15-C; 28-H$_3$ and 16—18, 22-C. Thus, the connectivities of quaternary carbons (4, 8, 10, 13, 14, 17-C) and position of the acetyl group in **2** were clarified and its urs-12-ene-3,11-diol structure was elucidated. Finally, deacetylation of **2** with 0.14%

sodium methoxide (NaOMe)–MeOH yielded urs-12-ene-3α,11α-diol (**17**). Consequently, the stereostructure of olibanumol L was determined to be 3-*O*-acetyl-urs-12-ene-3α,11α-diol (**2**).

Olibanumol M (**3**), a white powder with positive optical rotation ($[\alpha]_D^{27}$+21.0 in MeOH), showed absorption bands at 3475 and 1735 cm^{-1} due to hydroxyl and olefin functions in the IR spectrum. The positive-ion FAB-MS spectrum of **3** showed a quasimolecular ion peak at *m/z* 479 $(M+Na)^+$, and the molecular formula was determined to be $C_{31}H_{52}O_2$ by high-resolution MS measurement. The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **3** indicated the presence of an urs-12-ene-3,11-diol part [δ 0.80, 0.87, 0.97, 1.08, 1.11, 1.16 (3H each, all s, 28, 24, 23, 25, 26, 27-H_3), 0.86, 0.88 (3H each, both d, J=7.6 Hz, 29, 30-H_3), 3.39 (1H, br s, 3-H), 3.85 (1H, dd, J=3.9, 9.2 Hz, 11-H), 5.33 (1H, d-like, 12-H)] together with a methoxyl group [δ 3.26 (3H, s, CH_3O–)]. On the other hand, olibanumol N (**4**), $C_{33}H_{54}O_3$, was also obtained as a white powder with positive optical rotation ($[\alpha]_D^{22}$+1.1 in MeOH). The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **4** were similar to those of **2**, except for the signals due to the 11-methoxyl group [δ 3.28 (3H, s, CH_3O–)]. Methylation of **2** with methyl iodide (CH_3I) and potassium carbonate (K_2CO_3) yielded **4**, whereas acetylation of **3** with Ac_2O/pyridine furnished **4**. On the basis of this evidence, the stereostructures of olibanumols M and N were determined to be 11α-methyurs-12-ene-3α,11α-diol (**3**) and its acetate (**4**).

Experimental

The following instruments were used to obtain spectral and physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H-NMR spectra, JEOL JNM-LA500 (500 MHz) and EX-270 (270 MHz) spectrometers; ^{13}C-NMR spectra, JEOL JNM-LA500 (125 MHz) and EX-270 (68 MHz) spectrometers with tetramethylsilane as an internal standard; FAB-MS and high resolution FAB-MS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV–VIS detectors; HPLC column, YMC-Pack ODS-A and YMC-Pack SIL (YMC Co., Ltd., Kyoto, Japan) (250 mm×4.6 mm i.d.) and (250 mm×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography (CC), silica gel 60N (Kanto Chemical Co., Ltd., 63—210 mesh, spherical, neutral); reversed-phase silica gel CC, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); normal-phase TLC, pre-coated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm); reversed-phase TLC, pre-coated TLC plates with silica gel RP-18 F_{254S} (Merck, 0.25 mm); reversed-phase HPTLC, pre-coated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm), detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Plant Material This item was described in a previous report.[10]

Extraction and Isolation The gum-resin from *B. carterii* (2.7 kg) was extracted three times with 80% aqueous acetone at room temperature for 24 h. Evaporation of the solvent under reduced pressure provided an aqueous acetone extract (2131 g, 78.9%). The aqueous acetone extract (360.0 g) was subjected to normal-phase silica gel CC [3.0 kg, *n*-hexane–EtOAc (30 : 1→20 : 1→10 : 1→1 : 1, v/v)→MeOH] to give 11 fractions [Fr. 1 (6.3 g), Fr. 2 (11.2 g), Fr. 3 (30.5 g), Fr. 4 (181.4 g), Fr. 5 (12.7 g), Fr. 6 (9.3 g), Fr. 7 (18.2 g), Fr. 8 (14.6 g), Fr. 9 (7.1 g), Fr. 10 (5.9 g), and Fr. 11 (15.6 g)], as reported previously.[10] The fraction 2 (11.2 g) was subjected to normal-phase silica gel CC [345 g, *n*-hexane→*n*-hexane–EtOAc (100 : 1→80 : 1→30 : 1→20 : 1→10 : 1, v/v)→EtOAc] and HPLC [YMC-Pack SIL, UV detector (254 nm), *n*-hexane–EtOAc (300 : 1, v/v)] to give olibanumol K (**1**, 5.8 mg, 0.0013%) together with olibanumol F[13] (37.8 mg, 0.0083%). The fraction 3 (3.0 g) was subjected to normal-phase silica gel CC [150 g, *n*-hexane–EtOAc (30 : 1, v/v)→MeOH] to give epilupeol acetate (**6**, 1288.9 mg, 2.87%) and lupenone (**12**, 148.2 mg, 0.33%). The fraction 5 (12.7 g) subjected by reversed-phase silica gel CC [381 g, MeOH–H_2O (70 : 30→80 : 20→90 : 10, v/v)→MeOH] and HPLC [YMC-Pack ODS-A, RI detector, MeOH–H_2O (90 : 10, v/v)] to furnish lupeol (**9**, 3289.2 mg, 0.72%) together with olibanumol D[13] (22.8 mg, 0.0050%). The fraction 6 (3.0 g) was subjected by reversed-phase silica gel CC [150 g, MeOH–H_2O (70 : 30→80 : 20→90 : 10, v/v)→MeOH] and HPLC [YMC-Pack ODS-A, RI detector, MeOH–H_2O (90 : 10 or 95 : 5, v/v)] to give neoilexonol acetate (**15**, 338.2 mg, 0.23%) and dammarenediol II acetate (**19**, 661.8 mg, 0.45%). The fraction 7 (9.0 g) was subjected to normal-phase silica gel CC [270 g, *n*-hexane–EtOAc (10 : 1→5 : 1→1 : 1, v/v)→MeOH] to give six fractions [Fr. 7-1 (95.0 mg), Fr. 7-2 (369.7 mg), Fr. 7-3 (2.00 g), Fr. 7-4 (694.3 mg), Fr. 7-5 (3.50 g), and Fr. 7-6 (2.10 g)], as reported previously.[13] The fraction 7-3 (2.0 g) was purified by HPLC [YMC-Pack ODS-A, RI detector, MeOH–H_2O (95 : 5, v/v)] to furnish 3β-acetoxylup-20(29)-en-11β-ol (**11**, 97.0 mg, 0.043%) and mansumbinol (**25**, 16.0 mg, 0.0071%). The fraction 7-4 (694.3 mg) was purified by HPLC [YMC-Pack ODS-A, RI detector, MeOH–H_2O (95 : 5, v/v)] to furnish olibanumol L (**2**, 97.0 mg, 0.043%) together with olibanumol G[13] (11.3 mg, 0.0050%). The fraction 7-5 (3.5 g) was subjected by reversed-phase silica gel CC [175 g, MeOH–H_2O (70 : 30→80 : 20→90 : 10, v/v)→MeOH] and HPLC [YMC-Pack ODS-A, RI detector, MeOH–H_2O (90 : 10 or 95 : 5, v/v)] to give olibanumol N (**4**, 7.9 mg, 0.0035%), glochidiol (**8**, 3.8 mg, 0.0017%), neoilexonol (**14**, 88.0 mg, 0.039%), and **15** (97.0 mg, 0.043%). The fraction 7-6 (2.1 g) was purified by HPLC [YMC-Pack ODS-A, RI detector, MeOH–1% aqueous AcOH (95 : 5, v/v)] to give dammarenodiol II (**18**, 200.8 mg, 0.089%) and ocotillol acetate (**23**, 9.9 mg, 0.0044%). The fraction 9 (7.1 g) subjected to reversed-phase silica gel CC [200 g, MeOH–H_2O (70 : 30→90 : 10, v/v)→MeOH] and HPLC [YMC-Pack ODS-A, RI detector, MeOH–H_2O (95 : 5, v/v)] to give lup-20(30)-ene-3α,29-diol (**7**, 22.8 mg, 0.0050%), lup-20(29)-ene-2α,3β-diol (**10**, 11.8 mg, 0.0026%), urs-9(11), 12-dien-3β-ol (**13**, 5.5 mg, 0.0012%), urs-12-ene-3β,11α-diol (**16**, 18.7 mg, 0.0041%), urs-12-ene-3α,11α-diol (**17**, 68.3 mg, 0.015%), and 3-*O*-acetyl-3β,20*S*,24,trihydroxydammar-25-ene (**20**, 10.0 mg, 0.0022%) together with olibanumol E[13] (13.2 mg, 0.0029%). The fraction 10 (5.9 g) was subjected by reversed-phase silica gel CC [180 g, MeOH–H_2O (70 : 30→80 : 20→90 : 10, v/v)→MeOH] and HPLC [MeOH–H_2O (95 : 5, v/v)] to furnish olibanumol M (**3**, 19.7 mg, 0.0043%), isofouquierol acetate (**22**, 301.9 mg, 0.066%), and 3β-hydroxymansumbin-13(17)-en-16-one (**24**, 29.7 mg, 0.0065%).

Olibanumol K (**1**): A white powder, $[\alpha]_D^{27}$ +16.2 (c=0.30, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{31}H_{50}O_2Na$ $(M+Na)^+$ 477.3709; Found 477.3703. IR (KBr, cm^{-1}): 2940, 1725, 1457, 1379. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.80, 0.89, 0.92, 0.92, 1.11, 1.13 (3H each, all s, 28, 23, 24, 25, 27, 26-H_3), 0.90 (3H, d, J=7.4 Hz, 29-H_3), 0.92 (3H, d, J=7.0 Hz, 30-H_3), 4.77 (3H, br s, 3-H), 5.13 (1H, dd-like, 12-H), 8.13 (1H, s, HCO–). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. Positive-ion FAB-MS *m/z*: 477 $(M+Na)^+$.

Olibanumol L (**2**): A white powder, $[\alpha]_D^{28}$ +14.5 (c=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{32}H_{52}O_3Na$ $(M+Na)^+$ 507.3805; Found 507.3814. IR (KBr, cm^{-1}): 3432, 2962, 1739, 1456, 1370. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.81, 0.86, 0.93, 1.19 (3H each, all s, 28, 24, 23, 27-H_3), 1.11 (6H, s, 25, 26-H_3), 0.86, 0.88 (3H each, both d, J=7.6 Hz, 29, 30-H_3), 2.03 (3H, s, CH_3CO–), 4.27 (1H, dd-like, 11-H), 4.61 (1H, br s, 3-H), 5.18 (1H, br s, 12-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. Positive-ion FAB-MS *m/z*: 507 $(M+Na)^+$.

Olibanumol M (**3**): A white powder, $[\alpha]_D^{27}$ +21.0 (c=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{31}H_{52}O_2Na$ $(M+Na)^+$ 479.3814; Found 479.3805. IR (KBr, cm^{-1}): 3475, 2926, 1735, 1456, 1389. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.80, 0.87, 0.97, 1.08, 1.11, 1.16 (3H each, all s, 28, 24, 23, 25, 26, 27-H_3), 0.86, 0.88 (3H each, both d, J=7.6 Hz, 29, 30-H_3), 3.26 (3H, s, CH_3O–), 3.39 (1H, br s, 3-H), 3.85 (1H, dd, J=3.9, 9.2 Hz, 11-H), 5.33 (1H, d-like, 12-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. Positive-ion FAB-MS *m/z*: 479 $(M+Na)^+$.

Olibanumol N (**4**): A white powder, $[\alpha]_D^{22}$ +1.1 (c=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{33}H_{54}O_3Na$ $(M+Na)^+$ 521.3814; Found 521.3805. IR (KBr, cm^{-1}): 2926, 1732, 1458, 1374. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.81, 0.86, 0.93, 1.04, 1.08, 1.19 (3H each, all s, 28, 24, 23, 25, 26, 27-H_3), 0.86, 0.88 (3H each, both d, J=7.6 Hz, 29, 30-H_3), 2.03 (3H, s, CH_3CO–), 3.28 (3H, s, CH_3O–), 3.77 (1H, dd-like, 11-H), 4.61 (1H, br s, 3-H), 5.39 (1H, d-like, 12-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. Positive-ion FAB-MS *m/z*: 521 $(M+Na)^+$.

Deformylation of 1 A solution of **1** (3.0 mg) in 10% aqueous potassium hydroxide (KOH)–50% aqueous 1,4-dioxane (1 : 1, v/v, 2.0 ml) was stirred at 37 °C for 1 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form), and the resins were removed by filtration. After removal of the

solvent under reduced pressure, the residue was separated by normal-phase silica gel CC [1.0 g, *n*-hexane–EtOAc (3 : 1, v/v)] to give 3-epi-α-amyrin (**1a**, 0.8 mg), which was identified by comparison of its physical and spectral data ($[\alpha]_D$, ^{1}H-NMR, and MS) with reported values.[37]

Deacetylation of 2 A solution of **2** (10.0 mg) in 0.14% sodium methoxide (NaOMe)–MeOH (2.0 ml) was stirred at room temperature for 3 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form), and the resins were removed by filtration. After removal of the solvent under reduced pressure, the residue was separated by normal-phase silica gel CC [1.0 g, *n*-hexane–EtOAc (3 : 1, v/v)] to give urs-12-ene-3α,11α-diol (**17**, 7.5 mg), which was identified by comparison of its physical and spectral data ($[\alpha]_D$, ^{1}H-NMR, and MS) with reported values.[28]

Methylation of 2 To a solution of **2** (10.0 mg) in dry dimethylformamide (DMF, 2.0 ml) was added methyl iodide (CH_3I, 15 μl) and potassium carbonate (K_2CO_3, 66.4 mg), and the mixture was stirred at 80 °C for 3 h. The reaction mixture was poured into brine and extracted with EtOAc. The extract was successively washed with saturated aqueous $NaHCO_3$ and brine, and filtrated. After removal of the solvent under reduced pressure, the residue was purified by normal-phase silica gel CC [1.0 g, *n*-hexane–EtOAc (3 : 1, v/v)] to give **4** (7.0 mg).

Acetylation of 3 To a solution of **3** (5.0 mg) in dry pyridine (2.0 ml) was added acetic anhydride (1.0 ml), and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, and filtrated. After removal of the solvent under reduced pressure, the residue was purified by normal-phase silica gel CC [1.0 g, *n*-hexane–EtOAc (2 : 1, v/v)] to give **4** (3.0 mg).

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