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Straightforward partial synthesis of four diastereomeric 2,3-dihydroxy-olean-12-en-28-oic acids from oleanolic acid

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ABSTRACT

The four diastereomeric 2,3-dihydroxy-olean-12-en-28-oic acids (maslinic, augustic, bredemolic and 3-*epi*-maslinic acid) were easily accessed from one single starting material, oleanolic acid. The procedures allow the medium-to-large scale preparation of these valuable starting materials. Except for maslinic acid, the triterpenoic acids showed only a low cytotoxicity towards several human tumor cell lines.

1. Introduction

Secondary plant metabolites are an inexhaustible reservoir for isolation, modification, and finally their biological testing. Among other secondary plant metabolites, more than 20.000 triterpenes have been isolated so far. Pentacyclic triterpenes are among the most abundant, many of these compounds are bioactive, and they show antitumor, antiviral, anti-inflammatory as well as antidiabetic activities. Only a few compounds (e.g., corosolic acid (**1**, Fig. 1) as a dietary supplement for the treatment of diabetes mellitus) are already on the market, while several of them are regarded as suitable candidates for an extended biological screening including initial preclinical and clinical trials. This seems especially be true for derivatives of betulinic acid (**2**) and of maslinic acid (**3**). Recently, we were able to show that a maslinic acid derivative ('EM2')[1] shows high cytotoxicity for several human tumor cell lines while being significantly less toxic for non-malignant human fibroblasts. In the course of a more detailed look at analogs of this compound, we became interested to access the four diastereomeric 2,3-dihydroxy-olean-12-en-28-oic acids, i.e., maslinic acid (**3**), augustic acid (**4**), bredemolic acid (**5**) and 3-*epi*-maslinic acid (**6**) in significant amount, and we outlined syntheses of maslinic acid (**3**) from oleanolic acid (**7**) with augustic acid (**4**) being a side product of this preparation. In this contribution we extend our strategy to the partial synthesis of all four diastereomers from one single precursor, oleanolic acid.

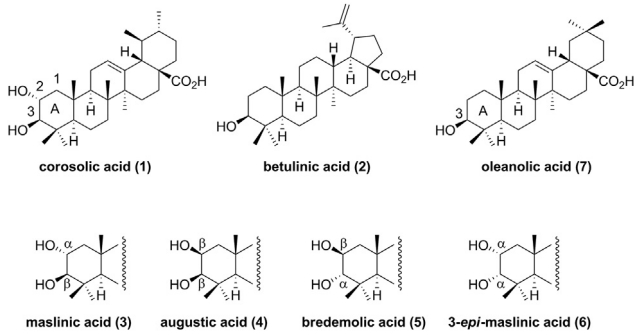

Fig. 1. Structure of corosolic acid (**1**), betulinic acid (**2**), maslinic acid (**3**), augustic acid (4), bredemolic acid (**5**), 3-*epi*-maslinic acid (**6**) and oleanolic acid (**7**).

2. Results and discussion

Maslinic acid (**3**) (showing a 2α, 3β-configuration in ring A, Fig. 1) can easily be obtained in large amounts either by extractive work-up of solid wastes of the olive oil pressing or from green or black olives[1–4] being easily bought in almost unlimited quantity and—as an alternative—by a short partial synthesis[5] from

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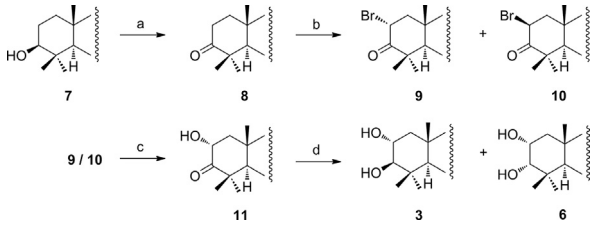
reasonably priced oleanolic acid (**7**). This approach allowed the partial synthesis of maslinic acid by a convenient and chromatography-free four-step synthesis in an over-all yield of 41% from oleanolic acid. Accessing the C2/C3 epimers of maslinic acid, however, seemed more challenging.

Augustic acid (**4**), being epimeric to maslinic acid with respect to position C-2 has previously been isolated in small amounts from the leaves[6] of *Perilla frutescense* (L.), from *Byrsonima crassifolia*,[7] from the roots of *Ambroma augusta*[8] and from *Rosmarinus officinals* (L.).[9] During our expedient partial synthesis of **3** [5], **4** has been obtained as a by-product. Augustic acid, however, seems of special interest to pharmaceutical chemists, since this compound induces apoptosis[10] in murine melanoma B16-F10 cells, and it is an inhibitor of the tyrosine phosphatase 1B GST fusion protein as well as of the glycogen phosphorylase (rabbit).[11] In addition, it is vasodilative in rats,[12] antiproliferative (Raji-cells)[13] and anti-inflammatory (ICR mouse).[13]

Bredemolic acid (**5**) was first described by Tschesche et al.[14] as early as 1960, and has been isolated[14] from *Bredemeyera floribunda* in small amounts; a lengthy partial synthesis has been described by the same group[15] in 1963 and more recently by Cheng et al.[16] Bredemolic acid also is an inhibitor[16] of the muscle glycogen phosphorylase in rabbit. Nothing is known, however, of its cytotoxicity for human tumor cells.

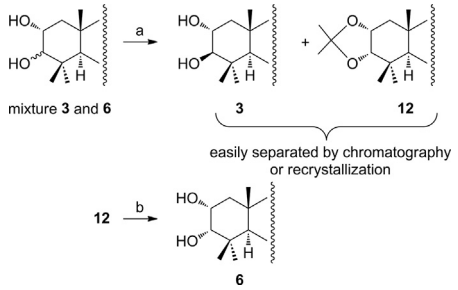
Finally, 3-*epi*-maslinic acid (**6**) has previously been isolated—among other triterpenoids—from *Potentilla chinensis*,[17] *Centella asiatica*,[18] *Prunella vulgaris*[19,20] and Salvia officinalis.[21] A lengthy partial synthesis[11] of this inhibitor of the muscle glycogen phosphorylase has been described.

The partial synthesis (Scheme 1) of maslinic acid (**3**) started from oleanolic acid (**7**) that was oxidized with silica gel supported Jones reagent[22] to yield 3-oxo **8**,[23] whose bromination with pyridiniumtribromide in acetic acid[24,25] gave a 7:3 mixture of bromides **9**/**10** in quantitative yield. These epimeric bromides can be obtained in analytical pure form by chromatography—but their separation is not necessary for the synthesis of **3**. Thus, this mixture of bromides was treated with sodium hydroxide in DMF under inert gas to yield (2α) configurated **11** whose reduction with $NaBH_4$ gave a mixture of maslinic acid (**3**) and 3-*epi*-maslinic acid (**6**).



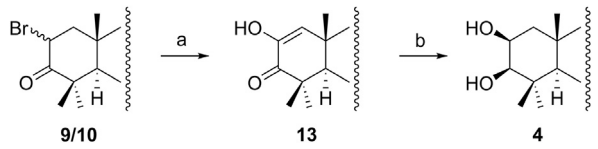
Scheme 1. a) SiO_2, Jones reagent, acetone, 30 min, 0 °C, 98%; b) pyridiniumtribromide, AcOH, 2 h, 25 °C, quant.; c) NaOH, DMF, inert gas, 0 °C, quant.; d) $NaBH_4$, MeOH, THF, 0 °C, 1 h, 48% (of **3**) and 45% (of **6**).

Mixtures of these two triterpenoic acids can be separated quite easily (Scheme 2). Thus, treatment of mixtures of **3** and **6** with acetone in the presence of sulfuric acid at 0 °C for 30 min led to the formation of the (2α,3α) acetonide **12**.[26] This acetonide was cleaved by dilute acids, by treatment with an ion exchange resin or even by prolonged contact with silica gel.



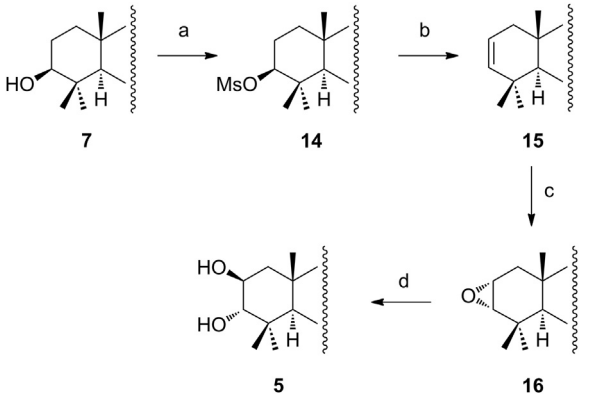
Scheme 2. Separation of maslinic acid (**3**)/3-*epi*-maslinic acid (**6**) mixtures by acetalization and de-acetalization [(a) acetone, H_2SO_4, 0 °C, 30 min; b) IR 120H$^+$, MeOH, 25 °C, 95%].

The bromides **9**/**10** also served as a suitable starting material for the partial synthesis (Scheme 3) of augustic acid (**4**). Thus, treatment of **9**/**10** with sodium hydroxide in DMF at 60 °C[27] under access of air gave a 95% yield of **13** whose treatment with sodium borohydride gave augustic acid (**4**) in 76% isolated yield.



Scheme 3. Partial synthesis of augustic acid (**4**): a) NaOH, DMF, 0 °C, 20 min then 60 °C under access of air, 1 h, 95%; b) $NaBH_4$, MeOH, 25 °C, 76%.

Our partial synthesis (Scheme 4) of bredemolic acid (**5**) started from, oleanolic acid (**7**) whose mesylation afforded a mesylate **14** in almost quantitative yield. Treatment of **14** with lithium carbonate in dry DMF furnished alkene **15**[28] whose epoxidation[29] with *m*-CPBA yielded compound **16**. Ring opening of this epoxide in the presence of aqueous perchloric acid yielded the target compound, bredemolic acid (**5**).



Scheme 4. Partial Synthesis of bredemolic acid (**5**): a) MsCl, pyridine, 80 °C, 1 h, 99%; b) Li_2CO_3, DMF, reflux, 1 h, 85%; c) *m*CPBA, DCM, 25 °C, 2 h, 85%; d) $HClO_4$, THF, H_2O, 25 °C, 30 min, 71%.

Thus, all four diastereomeric 2,3-dihydroxylated triterpenoic acids **3**–**6** were readily available in good yields and significant amounts from a single starting material, oleanolic acid (**7**). These compounds can be identified using their physico-chemical properties (mp, mmp, ^{1}H and ^{13}C NMR, $[\alpha]_D$) while their identification using TLC by comparing their R_f values with authentic samples remains still a problem. Applying 'usual' eluents and mixtures thereof (e.g., hexanes, chloroform, ethyl acetate, THF, DCM, toluene, benzene, methanol, isopropanol) allows a distinction between **3** and **5**. Unambiguous identification of **3** (R_f=0.29), **5** (R_f=0.31) and **6** (R_f=0.37)/**4** (R_f=0.38) was found for a four component system consisting of toluene/ethyl acetate/heptane/formic acid (80:20:30:4).

No problem was the identification of the compounds using ^{1}H NMR spectroscopy.[30] Identification of these triterpenoic acids as well as a proof for the absolute configuration at centers C-2/C-3 can be obtained from their respective ^{1}H NMR data (Table 1).

Thus, the signal for H-3_{ax} is detected (as in **3** and **4**) at lower frequencies than H-2_{eq} (as in **5** and **6**), and the same applies for H-2. The coupling constant $^3J_{H\text{-}2,H\text{-}3}$ is large for **3** and **5** (reflecting an axial/axial or an equatorial/equatorial configuration) while being small for **4** and **6**.

Table 1
Selected NMR spectroscopic data for triterpenoic acids **3**–**6** (500 MHz (for ^{1}H) or 125 MHz (for ^{13}C), pyridine-d_5, 25 °C)

	Maslinic acid (**3**)	Augustic acid (**4**)	Bredemolic acid (**5**)	3-*epi*-Maslinic acid (**6**)
δ H-2	4.11_{ax}	4.41_{eq}	4.36_{eq}	4.21_{ax}
δ H-3	3.41_{ax}	3.44_{ax}	4.00_{eq}	3.77_{eq}
J (H-2, H-3)	9.4	4.0	7.5	2.6
J (H-2, H-1_{ax})	11.2	2.8	6.6	11.6
J (H-2, H-1_{eq})	4.4	4.0	6.6	4.5
δ C-2	69.0	71.8	71.1	66.5
δ C-3	84.3	78.7	78.7	79.7

Performing photometric SRB assays[31] for compounds **3**–**6** allowed exploring their cytotoxic activities. The EC_{50} values from these tests employing several different human cancer cell lines and mouse fibroblasts NIH 3T3 are compiled in Table 2.

Table 2
Cytotoxicity of selected compounds (EC_{50} values in μM from SRB assays after 96 h of treatment; the values are averaged from three independent experiments performed each in triplicate; confidence interval CI=95%; cut-off 30 μM). Human cancer cell lines: 518A2 (melanoma), FaDu (hypopharyngeal carcinoma), HT29 (colorectal adenocarcinoma), MCF7 (breast adenocarcinoma), A549 (lung adenocarcinoma); NIH 3T3: nonmalignant mouse fibroblasts

EC_{50}	518A2	FaDu	HT29	MCF7	A549	NIH 3T3
3	13.7±0.9	24.9±1.1	28.8±0.5	>30	23.4±0.5	21.1±0.2
5	25.7±0.7	29.1±1.0	>30	25.1±2.1	26.4±1.0	>30
6	29.6±1.9	>30	>30	>30	>30	>30
10	27.9±1.4	>30	>30	27.1±0.9	>30	>30

The effect of **3** onto cancer cell lines has been investigated by several groups. Thus, Reyes et al.[32–35] demonstrated that **3** is able inducing apoptotic cell death in colon cancer cell lines HT29 and Caco-2. It was cytotoxic for the breast cancer cell line MCF7 [12]. But compound **3** also exhibits some selectivity between non-malignant and cancer cells.[36] For example, its cytotoxicity for malignant 518A2 melanoma cells was twice as for non-malignant mouse fibroblasts. The highest cytotoxicity was established for **3** and the human melanoma cell line 518A2 while being significantly less cytotoxic for other human tumor cell lines and for non-malignant mouse fibroblasts.

3. Conclusion

All four diastereomeric 2,3-dihydroxy-olean-12-en-28-oic acids **3**–**6** were semi-synthesized in short sequence from commercially available oleanolic acid (**7**). These procedures allow the medium-to-large scale production in a very straightforward way. For most steps no purification or simple re-crystallization affords sufficiently pure material. The cytotoxicity of the triterpenoic acids was evaluated in photometric SRB assays. These experiments showed acid **3** slightly higher cytotoxic than acids **4**–**6** for 518A2 human melanoma cells.

4. Experimental section

4.1. General—Chemistry

Melting points are uncorrected (*Leica* hot stage microscope), NMR spectra were recorded using the Varian spectrometers Gemini 2000 or Unity 500 (δ given in ppm, J in Hz, internal Me_4Si; typical experiments: H–H-COSY, HMBC, HMQC, NOESY, DQF-COSY, INADEQUATE), MS spectra were taken on a Finnigan MAT LCQ 7000 (electrospray, voltage 4.1 kV, sheath gas nitrogen) instrument. The optical rotation was measured on a Perkin–Elmer polarimeter at 20 °C; TLC was performed on silica gel (Merck 5554); elemental analyses were performed on a Vario EL (CHNS). The solvents were dried according to usual procedures. The purity of the compounds was determined by HPLC and found to be >98%. Oleanolic acid was obtained from different commercial suppliers in bulk quantities.

4.2. General—biological screening

The SRB assay was performed as previously described.[31,37]

4.3. Synthesis of maslinic acid (3)

*4.3.1. 3-Oxoolean-12-en-28-oic acid (**8**).* A suspension of oleanolic acid (**7**, 19.72 g, 43.18 mmol) and silica gel (150 mL) in acetone (1000 mL) was refluxed for 30 min. The mixture was cooled to 0 °C and Jones reagent was added [freshly prepared from CrO_3 (5.18 g, 51.82 mmol), water (16.8 mL) and concentrated H_2SO_4 (5 mL)]. After stirring for 30 min at 0 °C, MeOH (4 mL) was added, and stirring was continued for 1 h before evaporating to dryness. The resulting solid was subjected to a Soxhlet extraction with diethyl ether for 6 h; the solvent was removed and **8** (19.31 g, 98%) was obtained as a slightly yellowish solid; an analytical sample showed mp: 225–227 °C (lit.: 226–229 °C).[23]

*4.3.2. (2α)-Bromo-3-oxoolean-12-en-28-oic acid (**9**) and (2β)-bromo-3-oxoolean-12-en-28-oic acid (**10**).* To a solution of **8** (19.25 g, 42.34 mmol) in acetic acid (500 mL) pyridiniumtribromide (90%, 15.35 g, 43.18 mmol) was added at 25 °C in several portions within 1 h. After stirring for 2 h, the mixture was cooled (0 °C) and diluted with ice-cold water (1.5 L). The precipitate was filtered off and thoroughly washed with water. After drying ($CaCl_2$) a mixture of compounds **9** and **10** (22.57 g, 100%) was obtained as an off white solid. The mixture was used for the next steps. Separation of **9** from **10** was accomplished by chromatography (silica gel, hexane/ethyl acetate 8:1), and gave analytical samples.

Data for (**9**): mp 155–158 °C; R_f=0.15 (silica gel, hexane/ethyl acetate, 8:2); $[\alpha]_D$=+43.88° (c=0.35, $CHCl_3$); IR (KBr): ν=3422*v*, 2949*s*, 2866*s*, 1724*s*, 1697*s*, 1461*s*, 1387*s*, 1365*m*, 1305*w*, 1270*m*, 1187*m*, 1163*m*, 1065*m*, 1012*m*, 763*s*, 727*m*, 646*s*, 603*s* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.28 (dd, J=3.6, 3.6 Hz, 1H, H-12), 5.06 (dd, J=13.4, 6.1 Hz, 1H, H-2), 2.82 (dd, J=13.8, 4.3 Hz, 1H, H-18), 2.56 (dd, J=13.0, 6.1 Hz, 1H, H-1a), 2.14–1.88 (*m*, 4H, H-1b+H-16a+H-11a+H-11b), 1.88–1.54 (*m*, 8H, H-5+H-9+H-19a+H-7a+H-7b+H-15a+H-16a+H-16b), 1.54–1.40 (*m*, 3H, H-6a+H-6b+H-22a), 1.39–1.01 (*m*, 6H, H-2+H-19b+H-22b+H-21a+H-21b+H-15b), 1.21 (*s*, 3H, H-25), 1.20 (*s*, 3H, H-27), 1.12 (*s*, 3H, H-23), 1.09 (*s*, 3H, H-24), 0.93 (*s*, 3H, H-29), 0.90 (*s*, 3H, H-30), 0.80 (*s*, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=206.9 (C-3), 184.3 (C-28), 144.0 (C-13), 122.0 (C-12), 56.7 (C-2), 52.4 (C-1), 52.3 (C-5), 49.5 (C-4), 47.1 (C-9), 46.7 (C-17), 45.9 (C-19), 41.9 (C-14), 41.1 (C-18), 40.0 (C-8), 39.5 (C-10), 33.9 (C-21), 33.2 (C-30), 32.5 (C-7), 32,3 (C-22), 30.8 (C-20), 27.8 (C-15), 26.5 (C-27), 26.0 (C-23), 23.7 (C-29), 23.6 (C-11), 22.8 (C-16), 22.1 (C-24), 19.4 (C-6), 17.3 (C-26), 15.4 (C-25) ppm; MS (ESI): m/z (%)=531.3 ($[C_{30}H_{45}^{79}BrO_3-H]^-$, 48), 533.3 ($[C_{30}H_{45}^{81}BrO_3-H]^-$, 52), 1063.1 ($[2C_{30}H_{45}{}^{79}BrO_3-H]^-$, 92), 1065.1 ($[C_{30}H_{45}{}^{79}BrO_3+C_{30}H_{45}{}^{81}BrO_3-H]^-$, 100); Anal. Calcd for $C_{30}H_{45}BrO_3$ (533.58): C 67.53, H 8.50; found: C 67.31, H 8.74.

Data for (**10**): mp 148–151 °C; R_f=0.22 (silica gel, hexane/ethyl acetate 8:2); $[\alpha]_D$=+120.03° (c=0.25, $CHCl_3$); IR (KBr): ν=3425*m*, 2949*s*, 1731*s*, 1697*s*, 1636*w*, 1459*s*, 1388*s*, 1365*m*, 1305*m*, 1268*m*, 1208*m*, 1162*m*, 1062*m*, 774*s*, 709*m*, 646*m*, 606*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.30 (dd, J=3.4, 3.4 Hz, 1H, H-12), 5.08 (dd, J=11.0, 9.5 Hz, 1H, H-2), 2.84 (dd, J=13.9, 3.9 Hz, 1H, H-18), 2.50 (dd,

J=13.6, 11.3 Hz, 1H, H-1a), 2.10–1.91 (*m*, 3H, H-11a+H-16a+H-1b), 1.92–1.44 (*m*, 10H, H-16b+H-5+H-9+H-22a+H-22b+H-15a+H-19a+H-11b+H-7a+H-6a), 1.44–1.30 (*m*, 3H, H-6b+H-7b+H-21a), 1.30–1.02 (*m*, 3H, H-21b+H-19b+H-15b), 1.17 (*s*, 3H, H-27), 1.14 (*s*, 3H, H-23), 1.13 (*s*, 3H, H-29), 0.93 (*s*, 3H, H-24), 0.91 (*s*, 3H, H-30), 0.88 (*s*, 3H, H-25), 0.76 (*s*, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=209.2 (C-3), 183.1 (C-28), 143.6 (C-13), 122.4 (C-12), 53.5 (C-1), 52.7 (C-5), 51.2 (C-2), 47.7 (C-4), 46.9 (C-9), 46.8 (C-17), 45.9 (C-19), 42.1 (C-14), 41.4 (C-18), 39.6 (C-8), 39.3 (C-10), 34.0 (C-21), 33.2 (C-30), 32.5 (C-22), 31,7 (C-7), 30.8 (C-20), 29.6 (C-23), 27.8 (C-15), 25.9 (C-27), 23.7 (C-29), 23.6 (C-11), 23.1 (C-16), 20.4 (C-24), 20.1 (C-6), 18.3 (C-25), 16.6 (C-26) ppm; MS (ESI): *m*/*z* (%)=531.3 ([$C_{30}H_{45}{}^{79}BrO_3-H]^-$, 100), 533.3 ([$C_{30}H_{45}{}^{81}BrO_3-H]^-$, 92), 1063.1 ([$2C_{30}H_{45}{}^{79}BrO_3-H]^-$, 54), 1065.1 ([$C_{30}H_{45}{}^{79}BrO_3+C_{30}H_{45}{}^{81}BrO_3-H]^-$, 60); Anal. Calcd for $C_{30}H_{45}BrO_3$ (533.58): C 67.53, H 8.50; found: C 67.39, H 8.72.

*4.3.3. (2α)-Hydroxy-3-oxoolean-12-en-28-oic acid (**11**).* To a solution of **9**/**10** (11.28 g, 21.14 mmol) in DMF (130 mL) at 0 °C under inert gas 2M NaOH (22.20 mL, 44.39 mmol) was added, and 20 min later, the resulting suspension was re-dissolved by adding MeOH (250 mL), then acidified with 2 M HCl (25 mL) and finally treated with water (1.5 L) at 0 °C. The resulting solid was collected and thoroughly washed with water, dried ($CaCl_2$) and **11** (9.95 g, 100%) was obtained as an off white solid; mp 143–146 °C; R_f=0.41 (silica gel, hexane/ethyl acetate, 6:4); $[\alpha]_D$=+64.23° (*c*=0.35, $CHCl_3$); IR (KBr): *ν*=3446*v*, 2944*s*, 1700*s*, 1636*m*, 1559*w*, 1540*w*, 1507*w*, 1458*m*, 1388*s*, 1268*m*, 1162*m*, 1101*m*, 1056*m*, 1029*s*, 994*m*, 646*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.28 (dd, *J*=3.6, 3.6 Hz, 1H, H-12), 4.54 (dd, *J*=12.6, 6.6 Hz, 1H, H-2), 2.83 (dd, *J*=13.7, 4.3 Hz, 1H, H-18), 2.40 (dd, *J*=12.6, 6.6 Hz, 1H, H-1a), 2.03–1.89 (*m*, 2H, H-11a+H-11b), 1.82–1.65 (*m*, 2H, H-22a+H-15a), 1.66–1.40 (*m*, 9H, H-9+H-19a+H-7a+H-7b+H-22b+H-16a+H-16b+H-6a+H-6b), 1.40–1.04 (*m*, 6H, H-1b+H-5+H-19b+H-21a+H-21b+H-15b), 1.26 (*s*, 3H, H-25), 1.16 (*s*, 3H, H-23), 1.10 (*s*, 3H, H-24), 0.93 (*s*, 3H, H-29), 0.93 (*s*, 3H, H-30), 0.81 (*s*, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=216.7 (C-3), 183.9 (C-28), 143.9 (C-13), 122.3 (C-12), 69.3 (C-2), 57.8 (C-5), 49.5 (C-1), 47.9 (C-4), 47.5 (C-9), 46.6 (C-17), 45.9 (C-19), 41.8 (C-14), 41.1 (C-18), 39.6 (C-8), 37.9 (C-10), 33.9 (C-21), 33.2 (C-30), 32.6 (C-7), 32,5 (C-22), 30.8 (C-20), 27.8 (C-15), 26.1 (C-27), 24.9 (C-23), 23.8 (C-29), 23.7 (C-11), 23.0 (C-16), 21.7 (C-24), 19.3 (C-6), 17.4 (C-26), 16.2 (C-25) ppm; MS (ESI): *m*/*z* (%)=469.5 ([M−H]$^-$, 100), 939.2 ([2M−H]$^-$, 66), 961.7 ([2M−2H+Na]$^-$, 28); Anal. Calcd for $C_{30}H_{46}O_4$ (470.68): C 76.55, H 9.85; found: 76.32, H 9.98.

*4.3.4. (2α,3β)-Dihydroxyolean-12-en-28-oic acid (maslinic acid) (**3**).* To a solution of **11** (9.95 g, 21.14 mmol) in THF (120 mL) and MeOH (20 mL) $NaBH_4$ (0.40 g, 10.57 mmol) was added at 0 °C. The mixture was stirred for 1 h. An aqueous solution of HCl (2 m, 20 mL) and water (1.5 L) were added, the precipitate was filtered off and dried ($CaCl_2$). This mixture (consisting of **5** and **6**) was dissolved in acetone (200 mL) at 60 °C. It was cooled to 0 °C, H_2SO_4 (concentrated, 4 mL) in acetone (80 mL) was added, and the mixture was stirred for 30 min. After adding water (1.5 L), the precipitate was filtered off, dried ($CaCl_2$) and **3** (4.84 g, 48%) was obtained as white solid [recrystallized once from EtOAc (150 mL)]; mp 265–267 °C (lit.:[11] 266–269 °C); R_f=0.22 (hexane/ethyl acetate, 6:4); $[\alpha]_D$=+55.52° (*c*=0.5, $CHCl_3$).; [(lit.:[38] $[\alpha]_D$=34°, *c*=0.2, MeOH; IR (KBr): *ν*=3424*m*, 2944*s*, 1697*m*, 1462*m*, 1385*m*, 1050*m* cm^{-1}; ^{1}H NMR (800 MHz, pyridine-d_5): δ=5.46 (dd, 1H, $^3J_{H,H}$=3.7, 3.4, H-12), 4.07 (ddd, 1H, $^3J_{H,H}$=11.4, 9.4, 4.4 Hz, H-2), 3.37 (d, 1H, $^3J_{H,H}$=9.4 Hz, H-3), 3.28 (dd, 1H, $^3J_{H,H}$=13.6, 4.5 Hz, H-18), 2.23 (dd, 1H, $^2J_{H,H}$=−12.5 Hz, $^3J_{H,H}$=4.4 Hz, H-1b), 2.16 (ddd, $^2J_{H,H}$=13.6 Hz, $^3J_{H,H}$=14.3, 4.4 Hz, 1H, H-15b), 2.09 (ddd, 1H, $^2J_{H,H}$=13.2 Hz, $^3J_{H,H}$=14.3, 4.0 Hz, H-16a), 2.02 (ddd, 1H, $^2J_{H,H}$=13.9 Hz, $^3J_{H,H}$=4.1, 2.6 Hz, H-22b), 2.00 (ddd, 1H, $^2J_{H,H}$=18.5 Hz, $^3J_{H,H}$=10.6, 3.7 Hz, H-11b), 1.96 (ddd, 1H, $^2J_{H,H}$=18.5 Hz, $^3J_{H,H}$=7.5, 3.3 Hz, H-11a), 1.94 (ddd, 1H, $^2J_{H,H}$=13.2 Hz, $^3J_{H,H}$=4.4, 2.5 Hz, H-16b), 1.80 (ddd, 1H, $^2J_{H,H}$=13.9 Hz, $^3J_{H,H}$=14.5, 4.3 Hz, H-22a), 1.79 (dd, 1H, $^3J_{H,H}$=10.6 Hz, 7.5 Hz, H-9), 1.77 (dd, 1H, $^2J_{H,H}$=14.0 Hz, $^3J_{H,H}$=13.6 Hz, H-19a), 1.56 (dddd, 1H, $^2J_{H,H}$=13.1 Hz, $^3J_{H,H}$=4.4, 2.9, 1.6, H-6a), 1.51 (ddd, 1H, $^2J_{H,H}$=13.0 Hz, $^3J_{H,H}$=11.8, 4.4 Hz, H-7a), 1.43 (ddd, 1H, $^2J_{H,H}$=13.2 Hz, $^3J_{H,H}$=4.3, 2.6 Hz, H-21a), 1.38 (dddd, 1H, $^2J_{H,H}$=13.1 Hz, $^3J_{H,H}$=13.1, 11.8, 4.9, H-6b), 1.31 (ddd, 1H, $^2J_{H,H}$=13.0 Hz, $^3J_{H,H}$=4.9 Hz, 2.9, H-7b), 1.27 (dd, 1H, $^2J_{H,H}$=12.5 Hz, $^3J_{H,H}$=11.4 Hz, H-1a), 1.26 (dd, 1H, $^2J_{H,H}$=14.0 Hz, $^3J_{H,H}$=4.5 Hz, H-19b), 1.25 (*s*, 3H, C*H*$_3$ (24)), 1.24 (*s*, 3H, C*H*$_3$ (27)), 1.19 (ddd, 1H, $^2J_{H,H}$=13.2 Hz, $^3J_{H,H}$=14.5, 4.1 Hz, H-21b), 1.17 (ddd, 1H, $^2J_{H,H}$=13.6 Hz, $^3J_{H,H}$=4.0, 2.5 Hz, H-15a), 1.05 (*s*, 3H, C*H*$_3$ (23)), 1.02 (dd, 1H, $^3J_{H,H}$=13.1, 1.6 Hz, H-5), 1.00 (*s*, 3H, C*H*$_3$ (25)), 0.98 (*s*, 3H, C*H*$_3$ (29)), 0.97 (*s*, 3H, C*H*$_3$ (26)), 0.92 (*s*, 3H, C*H*$_3$ (30)) ppm; ^{13}C NMR (125 MHz, pyridine-d_5): δ=180.2 (C-28, $^1J_{C,C}$=54.2 Hz), 144.9 (C-13, $^1J_{C,C}$=41.3 Hz), 122.5 (C-12, *C*H=C, $^1J_{C,H}$=154 Hz, $^1J_{C,C}$=41.3 Hz), 83.9 (C-3, $^1J_{C,H}$=142 Hz, $^1J_{C,C}$=39.5, 36.9 Hz), 68.6 (C-2, $^1J_{C,H}$=141 Hz, $^1J_{C,C}$=39.5, 37.2 Hz), 56.0 (C-5, $^1J_{C,H}$=125 Hz, $^1J_{C,C}$=35.2, 34.2, 32.9 Hz), 48.2 (C-9, $^1J_{C,H}$=120 Hz, $^1J_{C,C}$=35.0, 33.8, 32.9 Hz), 47.8 (C-1, $^1J_{C,H}$=128 Hz, $^1J_{C,C}$=37.2, 31.7 Hz), 46.7 (C-17, C_q, $^1J_{C,C}$=54.2, 34.9, 34.5, 31.2 Hz), 46.5 (C-19, $^1J_{C,H}$=127 Hz, $^1J_{C,C}$=33.1, 32.3 Hz), 42.3 (C-14, C_q, $^1J_{C,C}$=41.3, 35.6, 35.0, 33.6 Hz), 42.0 (C-18, $^1J_{C,H}$=130 Hz, $^1J_{C,C}$=39.4, 34.9, 32.3 Hz), 39.9 (C-4, C_q, $^1J_{C,C}$=36.9, 36.8, 36.6, 34.2), 39.9 (C-8, C_q, $^1J_{C,C}$=35.5, 35.2, 33.6, 32.9 Hz), 38.6 (C-10, C_q, $^1J_{C,C}$=35.8, 33.8, 33.7, 32.9 Hz), 34.3 (C-21, $^1J_{C,H}$=127 Hz, $^1J_{C,C}$=34.3, 34.1 Hz), 33.3 (C-7, $^1J_{C,H}$=128 Hz, $^1J_{C,C}$=35.2, 33.1 Hz), 33.3 (C-30, $^1J_{C,H}$=122 Hz, $^1J_{C,C}$=36.4 Hz), 33.2 (C-22, $^1J_{C,H}$=128 Hz, $^1J_{C,C}$=34.1, 31.2 Hz), 31.0 (C-20, C_q, $^1J_{C,C}$=36.4, 35.9, 34.3, 33.1 Hz), 29.4 (C-24, $^1J_{C,H}$=125 Hz, $^1J_{C,C}$=36.6 Hz), 28.3 (C-15, $^1J_{C,H}$=128 Hz, $^1J_{C,C}$=35.0, 33.5 Hz), 26.2 (C-27, $^1J_{C,H}$=126 Hz, $^1J_{C,C}$=35.6 Hz), 24.0 (C-11, $^1J_{C,H}$=129 Hz, $^1J_{C,C}$=41.3, 35.0 Hz), 23.8 (C-16, $^1J_{C,H}$=124 Hz, $^1J_{C,C}$=34.5, 33.5 Hz), 23.8 (C-29, $^1J_{C,H}$=126 Hz, $^1J_{C,C}$=35.9 Hz), 18.9 (C-6, $^1J_{C,H}$=126 Hz, $^1J_{C,C}$=35.2, 33.1 Hz), 17.7 (C-23), $^1J_{C,H}$=125 Hz, $^1J_{C,C}$=36.8 Hz), 17.5 (C-25, $^1J_{C,H}$=125 Hz, $^1J_{C,C}$=35.5 Hz), 16.9 (C-26, $^1J_{C,H}$=125 Hz, $^1J_{C,C}$=35.8 Hz) ppm; MS (ESI, MeOH, $C_{30}H_{48}O_4$): *m*/*z*=471.6 ([M−H]$^-$, 63%), 517.2 ([M+HCO$_2$]$^-$, 72%), 943.3 ([2M−H]$^-$, 100%); Anal. Calcd for $C_{30}H_{48}O_4$: C 76.23, H 10.24; found: C 76.11, H 10.39.

4.4. Synthesis of 3-*epi*-maslinic acid (6)

*4.4.1. (2α,3α)-Dihydroxyolean-12-en-28-oic acid [3-epi-maslinic acid (**6**)] and (2α,3α)-(isopropylidenedioxy)-olean-12-en-28-oic acid (**12**).* Crude mixtures of **3** and **6** (obtained as described above, 10 g) were dissolved in acetone (200 mL) at 60 °C. The solution was filtered, and at 0 °C a freshly prepared solution of conc. H_2SO_4 (4 mL, 98%) in acetone (80 mL) was slowly added. The mixture was stirred for 30 min, water (1.5 L) was added, and the precipitate was filtered off, dried ($CaCl_2$) and recrystallized from ethanol to yield pure **3**. The filtrate was evaporated; flash chromatography (silica gel, hexane–ethyl acetate, 7:3) furnished crude **12**. An analytical sample was obtained by re-crystallization from acetone. A solution of **12** (5.13 g, 10 mmol) in methanol (100 mL) was stirred with ion exchange resin (IR 120H$^+$) for 2 h. The resin was filtered off, the solvents were evaporated, and **6** (4.49 g, 95%) was obtained as a colorless solid (being pure enough for the next transformations). An analytical sample was obtained by re-crystallization from ethanol.

Data for **6**: mp 289–293 °C (lit.:[11] 295–297 °C); R_f=0.40 (silica gel, hexane/ethyl acetate, 1:1); $[\alpha]_D$=55.34° (*c*=0.11, pyridine); IR (KBr): *ν*=2946*vs*, 2876*s*, 1698*s*, 1462*m*, 1388*m*, 1366*m*, 1266*m*, 1234*m*, 1184*m*, 1162*m*, 1034*m*, 994*m* cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5): δ=5.48 (dd, 1H, *J*=3.4, 3.4 Hz, H-12), 4.31 (ddd, 1H, *J*=11.6, 4.1, 3.4 Hz, H-2), 3.77 (d, 1H, *J*=2.6 Hz, H-3), 3.30 (dd, 1H, *J*=14.0, 4.2 Hz, H-18), 2.23–1.89 (*m*, 8H, H-15a+H-

11a+H11b+H16a+H16b+H-22a+H-1a+H-9a), 1.86–1.63 (*m*, 4H, H-22b+H-1b+H-19a+H-5), 1.60–1.15 (*m*, 8H, H-7a+H-7b+H-6a+H-6b+H-21a+H-21b+H-19b+H-15b), 1.28 (*s*, 3H, C*H*$_3$ (24)), 1.19 (*s*, 3H, C*H*$_3$ (27)), 1.04 (*s*, 3H, C*H*$_3$ (26)), 1.00 (*s*, 3H, C*H*$_3$ (29)), 0.98 (*s*, 3H, C*H*$_3$ (25)), 0.93 (*s*, 3H, C*H*$_3$ (30)), 0.91 (*s*, 3H, C*H*$_3$ (23)) ppm; ^{13}C NMR (125 MHz, pyridine-d_5): δ=180.5 (C-28), 145.2 (C-13), 122.9 (C-12), 79.7 (C-3), 66.5 (C-2), 49.2 (C-5), 48.4 (C-9), 47.0 (C-17), 46.8 (C-19), 43.2 (C-1), 42.6 (C-14), 42.4 (C-18), 40.4 (C-8), 39.2 (C-4), 39.1 (C-10), 34.6 (C-21), 33.6 (C-30), 33.6 (C-22), 33.6 (C-7), 31.3 (C-20), 29.9 (C-24), 28.7 (C-15), 26.5 (C-27), 24.3 (C-11), 24.1 (C-29), 24.1 (C-16), 22.7 (C-23), 18.9 (C-6), 17.9 (C-26), 17.0 (C-25) ppm; MS (ESI): *m*/*z* (%)=471.4 ([M−H]$^-$, 100), 943.2 ([2M−H]$^-$, 68), 965.7 ([2M−2H+Na]$^-$, 18); Anal. Calcd for $C_{30}H_{48}O_4$: C 76.23, H 10.24; found: C 76.01, H 10.29.

Data for **12**: colorless solid; mp 158–161 °C; R_f=0.63 (silica gel, hexane/ethyl acetate, 7:3); $[\alpha]_D$=+83.74° (*c* 0.29, $CHCl_3$); IR (ATR): ν=2949*s*, 2871*w*, 1695*m*, 1642*m*, 1366*m*, 1217*m*, 1166*w*, 1127*m*, 1048*vs*, 938*m*, 861*m*, 759*m*, 497*s* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.29 (dd, *J*=3.6, 3.6 Hz, 1H, H-12), 4.17 (ddd, *J*=10.6, 6.1, 4.5 Hz, 1H, H-2), 3.69 (d, *J*=4.3 Hz, 1H, H-3), 2.83 (dd, *J*=13.6, 4.3 Hz, 1H, H-18), 1.98 (ddd, *J*=13.5, 13.5 4.0 Hz, 1H, H-16a), 1.93–1.89 (*m*, 2H, H-11a, H11-b), 1.83–1.66 (*m*, 3H, H-1a+H-22a+H-15a), 1.66–1.54 (*m*, 4H, H-19a+H-22b+H-16b+H-9), 1.53–1.45 (*m*, 2H, H-6a+H-7a), 1.47 (*s*, 3H, C*H*$_3$ (32)), 1.38–1.27 (*m*, 3H, H-21a+H-7b+H-6b), 1.32 (*s*, 3H, C*H*$_3$ (33)), 1.24–1.06 (*m*, 4H, H-21b+H-5+H-19b+H-15b), 1.15 (*s*, 3H, C*H*$_3$ (27)), 1.07 (*s*, 3H, C*H*$_3$ (24)), 1.04–0.97 (*m*, 1H, H-1b), 0.92 (*s*, 3H, C*H*$_3$ (29)), 0.90 (*s*, 3H, C*H*$_3$ (30)), 0.89 (*s*, 3H, C*H*$_3$ (23)), 0.88 (*s*, 3H, C*H*$_3$ (25)), 0.73 (*s*, 3H, C*H*$_3$ (26)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=183.5 (C-28), 143.6 (C-13), 122.8 (C-12), 107.2 (C-31), 83.0 (C-3), 72.0 (C-2), 49.8 (C-5), 47.2 (C-9), 46.7 (C-17), 46.1 (C-19), 42.4 (C-1), 41.9 (C-14), 41.2 (C-18), 39.5 (C-8), 38.3 (C-10), 35.6 (C-4), 34.0 (C-21), 33.2 (C-30), 32.6 (C-22), 32.4 (C-7), 30.8 (C-20), 29.0 (C-32), 28.5 (C-24), 27.8 (C-15), 26.7 (C-33), 26.1 (C-27), 24.0 (C-23), 23.7 (C-29), 23.4 (C-11), 23.1 (C-16), 18.5 (C-6), 17.2 (C-26), 15.6 (C-25) ppm; MS (ESI): *m*/*z* (%)=511.5 ([M−H]$^-$, 59), 1023.3 ([2M−H]$^-$, 100), 1045.6 ([2M-2H+Na]$^-$, 38); Anal. Calcd for $C_{33}H_{52}O_4$: C 77.30, H 10.22; found: C 77.11, H 10.34.

4.5. Synthesis of augustic acid (4)

*4.5.1. 2-Hydroxy-3-oxoolean-1,12-dien-28-oic acid (**13**).* To a solution of **9**/**10** (11.28 g, 21.14 mmol) in DMF (130 mL) 2 M NaOH (22.20 mL, 44.39 mmol) was added at 0 °C, and the mixture was stirred for 20 min. The suspension was heated to 60 °C and 2M NaOH (22.30 mL, 44.39 mmol) was added under access of air. Stirring was continued for 1 h, the mixture was cooled to 0 °C, diluted with methanol MeOH (250 mL), acidified with 2M HCl (50 mL), and finally water (1.5 L) was added. The precipitate was collected and thoroughly washed with water, dried ($CaCl_2$), and **13** (9.43 g, 95%) was obtained as an off white solid; mp 138–141 °C; R_f=0.50 (silica gel, hexane/ethyl acetate 6:4); $[\alpha]_D$=+100.97° (*c*=0.31, $CHCl_3$); UV–vis ($CHCl_3$): λ_{max} (log ε)=272 nm (4.31); IR (KBr): ν=3441*s*, 2947*s*, 1713*s*, 1697*s*, 1652*m*, 1463*s*, 1429*m*, 1396*s*, 1385*s*, 1234*m*, 1149*m*, 1123*s*, 1056*s*, 803*m*, 458*s* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.34 (*s*, 1H, H-1), 5.94 (*s*, 1H, OH-2), 5.34 (dd, *J*=3.6, 3.6 Hz, 1H, H-12), 2.85 (dd, *J*=13.7, 4.3 Hz, 1H, H-18), 2.20–1.92 (*m*, 3H, H-16a+H-11a+H-11b), 1.91–1.85 (*m*, 1H, H-9), 1.84–1.67 (*m*, 2H, H-15a+H-22a), 1.67–1.43 (*m*, 6H, H-6a+H-16b+H-22b+H-7a+H-19a+H-5), 1.43–1.06 (*m*, 6H, H-6b+H-15b+H-7b+H-21a+H-21b+H-19b), 1.22 (*s*, 3H, H-26), 1.22 (*s*, 3H, H-23), 1.13 (*s*, 3H, H-27), 1.10 (*s*, 3H, H-24), 0.94 (*s*, 3H, H-29), 0.91 (*s*, 3H, H-30), 0.83 (*s*, 3H, H-25) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=201.2 (C-3), 183.5 (C-28), 144.0 (C-2), 143.9 (C-13), 128.3 (C-1), 122.2 (C-12), 54.0 (C-5), 46.7 (C-4), 45.8 (C-19), 44.0 (C-17), 43.2 (C-9), 42.1 (C-14), 41.3 (C-18), 40.1 (C-8), 38.6 (C-10), 34.0 (C-21), 33.2 (C-30), 32.6 (C-7), 32,5 (C-22), 30.8 (C-20), 27.8 (C-15), 27.4 (C-23), 26.0 (C-27), 23.7 (C-29), 23.6 (C-11), 23.0 (C-16), 21.9 (C-24), 19.3 (C-26), 18.8 (C-6), 17.7 (C-25) ppm; MS (ESI): *m*/*z* (%)=467.3 ([M−H]$^-$, 54), 935.3 ([2M−H]$^-$, 28), 958.6 ([2M−2H+Na]$^-$, 24); Anal. Calcd for $C_{30}H_{44}O_4$ (468.67): C 76.88, H 9.46; found: C 76.61, H 9.83.

*4.5.2. (2β,3β)-Dihydroxyolean-12-en-28-oic acid (augustic acid) (**4**).* To a solution of **13** (9.43 g, 20.12 mmol) in THF (120 mL) and MeOH (20 mL) $NaBH_4$ (0.76 g, 20.12 mmol) was added at room temperature. The mixture was stirred for 1 h, an aqueous solution of HCl (2M, 20 mL) and water (1.5 L) were added. The precipitate was filtered off, dried ($CaCl_2$), and **4** (7.22 g, 76%) was obtained as a colorless solid [recrystallized from EtOAc (500 mL)]; mp 310–314 °C (decomp.; (lit.:[11] 308–310 °C)); R_f=0.49 (silica gel, hexane/ethyl acetate, 1:1); $[\alpha]_D$=+88.05° (*c*=0.31, THF) (lit.:[16] $[\alpha]_D$=+93.5° (*c*=0.17, pyridine); IR (KBr): ν=2946*vs*, 1704*vs*, 1464*m*, 1388*m*, 1378*m*, 1362*m*, 1322*m*, 1304*m*, 1264*s*, 1188*s*, 1162*m*, 1150*m*, 1060*m*, 1024*s*, 988*m*, 932*m* cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5): δ=5.47 (dd, *J*=3.6, 3.6 Hz, 1H, H-12), 4.12–4.40 (*m*, 1H, H-2), 3.44 (d, *J*=4.0 Hz, 1H, H-3), 3.33 (dd, *J*=13.8, 4.5 Hz, 1H, H-18), 2.32 (dd, *J*=14.1, 2.8 Hz, 1H, H-1a), 2.26–1.95 (*m*, 6H, H-15a+H-16a+H-16b+H-22a+H-11a+H-11b), 1.88–1.80 (*m*, 2H, H-22b+H-19a), 1.73–1.43 (*m*, 5H, H-9+H-6a+H-6b+H-7a+H-21a), 1.53 (*s*, 3H, H-25), 1.42–1.18 (*m*, 5H, H-7b+H-19b+H-1b+H-21b+H-15b), 1.37 (*s*, 3H, H-23), 1.32 (*s*, 3H, H-27), 1.28 (*s*, 3H, H-24), 1.09 (*s*, 3H, H-26), 1.05–1.00 (*m*, 1H, H-5), 1.02 (*s*, 3H, H-29), 0.96 (*s*, 3H, H-30) ppm; ^{13}C NMR (125 MHz, pyridine-d_5): δ=180.5 (C-28), 145.2 (C-13), 123.1 (C-12), 78.7 (C-3), 71.8 (C-2), 56.4 (C-5), 48.9 (C-9), 47.1 (C-17), 46.9 (C-19), 45.3 (C-1), 42.7 (C-14), 42.4 (C-18), 40.3 (C-8), 39.2 (C-4), 37.8 (C-10), 34.6 (C-21), 33.7 (C-7), 33.7 (C-30), 33.6 (C-22), 31.4 (C-20), 30.6 (C-24), 28.6 (C-15), 26.6 (C-27), 24.4 (C-11), 24.2 (C-29), 24.1 (C-16), 19.0 (C-6), 18.5 (C-23), 17.9 (C-26), 17.0 (C-25) ppm; MS (ESI): *m*/*z* (%)=471.3 ([M−H]$^-$, 100), 943.3 ([2M−H]$^-$, 30); Anal. Calcd for $C_{30}H_{48}O_4$: C 76.23, H 10.24; found: C 76.15, H 10.31.

4.6. Synthesis bredemolic acid

*4.6.1. (3β)-Methylsulfonyloxy-olean-12-en-28-oic acid (**14**).* To a solution of oleanolic acid (**7**, 5.00 g, 10.95 mmol) in pyridine (40 mL), methanesulfonyl chloride (2.51 g, 21.92 mmol) was added, and the mixture was stirred for 1 h at 80 °C. The mixture was poured into ice cold aq HCl (1 M, 600 mL) and extracted with DCM (500 mL). The organic layer was washed with water (3×500 mL) and brine (1×500 mL), dried ($MgSO_4$), filtrated, and the filtrate was concentrated under reduced pressure to yield **14** (5.78 g, 99%) being sufficiently pure for the next synthetic step. An analytical sample was obtained by flash chromatography (florisil, hexane/ethyl acetate, 8:2) as a colorless solid; mp 135 °C; R_f=0.16 (silica gel, hexane/ethyl acetate, 8:2); $[\alpha]_D$=+59.56° (*c*=1.02, $CHCl_3$); IR (KBr): ν=2948*vs*, 2880*s*, 1728*m*, 1698*s*, 1466*m*, 1356*s*, 1332*s*, 1268*m*, 1176*vs*, 1110*m*, 978*m*, 934*s*, 910*vs*, 876*s*, 836*m*, 532*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.26 (dd, *J*=3.7, 3.7 Hz, 1H, H-12), 4.35 (dd, *J*=10.9, 5.8 Hz, 1H, H-3), 3.01 (*s*, 3H, C*H*$_3$ (31)), 2.82 (dd, *J*=13.9, 4.6 Hz, 1H, H-18), 2.02–1.81 (*m*, 5H, H-16a+H-11a+H-11b+H-2a+H-2b), 1.81–1.52 (*m*, 8H, H-22a+H-22b+H-15a+H-1a+H-19a+H-16b+H-6a+H-9), 1.48–1.11 (*m*, 6H, H-7a+H-7b+H-6b+H-21a+H-21b+H-19b), 1.12 (*s*, 3H, C*H*$_3$ (27)), 1.10–1.01 (*m*, 2H, H-15b+H-1b), 1.02 (*s*, 3H, C*H*$_3$ (24)), 0.94 (*s*, 3H, C*H*$_3$ (25)), 0.92 (*s*, 3H, C*H*$_3$ (29)), 0.90 (*s*, 3H, C*H*$_3$ (30)), 0.87–0.82 (*m*, 1H, H-5), 0.85 (*s*, 3H, C*H*$_3$ (23)), 0.74 (*s*, 3H, C*H*$_3$ (26)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=184.3 (C-28), 143.8 (C-13), 122.5 (C-12), 90.6 (C-3), 55.6 (C-5), 47.9 (C-9), 46.7 (C-17), 46.0 (C-19), 41.7 (C-14), 41.1 (C-18), 39.4 (C-8), 39.0 (C-31), 38.7 (C-4), 38.3 (C-1), 37.0 (C-10), 33.9 (C-21), 33.2 (C-30), 32.6 (C-22), 32.6 (C-7), 30.8 (C-20), 28.4 (C-24), 27.8 (C-15), 26.0 (C-27), 25.3 (C-2), 23.7 (C-29), 23.5 (C-11), 23.0 (C-16), 18.5 (C-6), 17.3 (C-26), 16.5 (C-23), 15.5 (C-25) ppm; MS (ESI): *m*/*z* (%)=533.2 ([M−H]$^-$, 100), 1067.3

([2M−H]$^-$, 16), 1089.3 ([2M-2H+Na]$^-$, 12); Anal. Calcd for $C_{31}H_{50}SO_5$: C 69.62, H 9.42, S 6.00; found: C 69.57, H 9.51, S 5.87.

*4.6.2. Olean-2,12-dien-28-oic acid (**15**).* A suspension of compound **14** (5.78 g, 10.81 mmol) and Li_2CO_3 (4.79 g, 64.86 mmol) in dry DMF (50 mL) was heated under reflux for 1 h. The mixture was poured into ice cold aq HCl (0.2 M, 500 mL) and extracted with Et_2O (500 mL). The organic layer was washed with water (4×500 mL) and brine (1×500 mL); dried ($MgSO_4$), filtrated and concentrated under reduced pressure. The residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 8:2) to yield compound **15** (4.03 g, 85%) as a colorless solid; mp 144–146 °C; R_f=0.53 (silica gel, hexane/ethyl acetate, 8:2); $[\alpha]_D$=+107.58° (*c*=0.90, $CHCl_3$), lit.:[28] $[\alpha]_D$=+74° (*c*=1, $CHCl_3$); IR (KBr): *ν*=2950*vs*, 2886*s*, 1696*vs*, 1462*m*, 1384*m*, 1364*m*, 1304*m*, 1270*m*, 1208*w*, 1162*w*, 1032*w*, 1018*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): *δ*=5.44–5.28 (*m*, 3H, H-2+H-3+H-12), 2.84 (dd, *J*=13.6, 4.2 Hz, 1H, H-18), 2.04–1.68 (*m*, 6H, H-16a+H-11a+H-11b+H-1a+H-22a+H-15a), 1.68–1.54 (*m*, 5H, H-19a+H-16b+H-9+H-1b+H-22b), 1.54–1.08 (*m*, 9H, H-6a+H-6b+H-7a+H-7b+H-21a+H-21b+H-19b+H-5+H-15b), 1.15 (*s*, 3H, C*H*$_3$ (27)), 0.96 (*s*, 3H, C*H*$_3$ (24)), 0.95 (*s*, 3H, C*H*$_3$ (25)), 0.94 (*s*, 3H, C*H*$_3$ (29)), 0.91 (*s*, 3H, C*H*$_3$ (30)), 0.88 (*s*, 3H, C*H*$_3$ (23)), 0.80 (*s*, 3H, C*H*$_3$ (26)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): *δ*=184.5 (C-28), 143.6 (C-13), 138.1 (C-3), 123.0 (C-12), 121.6 (C-2), 52.2 (C-5), 46.8 (C-17), 46.3 (C-9), 46.0 (C-19), 41.9 (C-14), 41.2 (C-18), 40.9 (C-1), 39.6 (C-8), 36.4 (C-4), 34.6 (C-10), 34.0 (C-21), 33.2 (C-30), 32.6 (C-22), 32.2 (C-7), 32.0 (C-24), 30.8 (C-20), 27.8 (C-15), 26.0 (C-27), 23.7 (C-29), 23.4 (C-11), 23.1 (C-16), 22.9 (C-23), 19.6 (C-6), 17.3 (C-26), 15.7 (C-25) ppm; MS (ESI): *m*/*z* (%)=437.5 ([M−H]$^-$, 100), 875.4 ([2M−H]$^-$, 20), 898.6 ([2M-2H+Na]$^-$, 38); Anal. Calcd for $C_{30}H_{46}O_2$: C 82.14, H 10.57; found C 82.02, H 10.65.

*4.6.3. (2α,3α)-Epoxy-olean-12-en-28-oic acid (**16**).* To a solution of compound **15** (570 mg, 1.30 mmol) in dry DCM (30 mL) *m*-CPBA (250 mg, 1.45 mmol) was added, and the mixture was stirred at room temperature for 2 h. Further DCM (50 mL) was added, and the mixture was washed with saturated aq $NaHCO_3$ solution (2×50 mL), water (2×50 mL) and brine (1×50 mL), dried ($MgSO_4$), filtrated, and the filtrate was concentrated in vacuo. The residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 8:2) to yield compound **16** (502 mg, 85%) as a colorless solid; mp 176–177 °C; R_f=0.44 (silica gel, hexane/ethyl acetate, 8:2); $[\alpha]_D$=+74.33° (*c*=0.87, $CHCl_3$); IR (KBr): *ν*=2942*vs*, 2866*s*, 1698*vs*, 1462*s*, 1386*s*, 1364*s*, 1304*s*, 1280*s*, 1238*s*, 1162*s*, 1122*m*, 1064*m*, 1012*m*, 942*m*, 828*s* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): *δ*=5.28 (dd, *J*=3.6, 3.6 Hz, 1H, H-12), 3.20–3.17 (*m*, 1H, H-2), 2.84–2.79 (*m*, 1H, H-18), 2.79 (d, *J*=3.8 Hz, 1H, H-3), 2.00–1.47 (*m*, 10H, H-16a+H-16b+H-1a+H-11a+H-11b+H-22a+H-22b+H-15a+H-19a+H-9), 1.47–1.37 (*m*, 2H, H-6a+H-7a), 1.37–1.24 (*m*, 4H, H-21a+ H-1b+H-6b+H-7b), 1.23–1.05 (*m*, 3H, H-21b+H-19b+H-15b), 1.09 (*s*, 6H, C*H*$_3$ (27)+C*H*$_3$ (24)), 0.99 (*s*, 3H, C*H*$_3$ (23)), 0.96–0.91 (*m*, 1H, H-5), 0.92 (*s*, 3H, C*H*$_3$ (29)), 0.91 (*s*, 3H, C*H*$_3$ (25)), 0.89 (*s*, 3H, C*H*$_3$ (30)), 0.71 (*s*, 3H, C*H*$_3$ (26)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): *δ*=184.3 (C-28), 143.6 (C-13), 122.6 (C-12), 61.8 (C-3), 52.8 (C-2), 47.0 (C-5), 46.8 (C-17), 46.2 (C-9), 45.9 (C-19), 41.9 (C-14), 41.2 (C-18), 40.1 (C-1), 39.3 (C-8), 36.2 (C-4), 34.0 (C-21), 33.2 (C-30), 32.6 (C-22), 32.6 (C-10), 32.1 (C-7), 30.8 (C-20), 28.2 (C-24), 27.8 (C-15), 25.8 (C-27), 23.7 (C-29), 23.3 (C-11), 23.0 (C-16), 22.3 (C-23), 18.9 (C-6), 17.9 (C-25), 16.7 (C-26) ppm; MS (ESI): *m*/*z* (%)=453.5 ([M−H]$^-$, 100), 907.5 ([2M−H]$^-$, 74), 929.7 ([M-2H+Na]$^-$, 88); Anal. Calcd for $C_{30}H_{46}O_3$: C 79.25, H 10.20; found: C 79.13, H 10.32.

*4.6.4. (2β,3α)-Dihydroxy-olean-12-en-28-oic acid, bredemolic acid (**5**).* Compound **16** (500 mg, 1.10 mmol) was dissolved in THF (20 mL) and water (4 mL). Aqueous perchloric acid (4 mL, 65%) was added, and the mixture was stirred at room temperature for 30 min. Et_2O (200 mL) was added, and the mixture was washed with saturated aq $NaHCO_3$ solution (2×100 mL), water (2×100 mL) and brine (1×100 mL), dried ($MgSO_4$), filtrated, and the filtrate was evaporated under reduced pressure. The residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 1:1) to yield compound **6** (370 mg, 71%) as a colorless solid; mp 234–237 °C (lit.:[14] 288–292 °C); R_f=0.31 (silica gel, hexane/ethyl acetate, 1:1); $[\alpha]_D$=+91.13° (*c*=0.29, pyridine) (lit.:[14] +93.5° (*c*=0.17, pyridine); IR (KBr): *ν*=2946*vs*, 2868*s*, 1696*s*, 1462*m*, 1386*m*, 1366*m*, 1304*m*, 1268*m*, 1240*m*, 1210*m*, 1182*m*, 1164*w*, 1112*w*, 1064*m*, 1010*m* cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5): *δ*=5.53 (dd, *J*=3.4, 3.4 Hz, 1H, H-12), 4.36 (ddd, *J*=7.2, 6.6, 6.6 Hz, 1H, H-2), 4.00 (d, *J*=7.5 Hz, 1H, H-3), 3.32 (dd, *J*=13.6, 4.2 Hz, 1H, H-18), 2.22–1.99 (*m*, 6H, H-15a+H-16a+H-1a+H-11a+H-11b+H-22a), 1.99–1.75 (*m*, 5H, H-16b+H-1b+H-9+H-22b+H-19a), 1.61–1.39 (*m*, 5H, H-7a)+H-5+H-6a+H-6b+H-21a), 1.38–1.15 (*m*, 4H, H-7b+H-19b+H-15b+H-21b), 1.32 (*s*, 3H, C*H*$_3$ (25)), 1.31 (*s*, 3H, C*H*$_3$ (23)), 1.29 (*s*, 3H, C*H*$_3$ (24)), 1.26 (*s*, 3H, C*H*$_3$ (27)), 1.08 (*s*, 3H, C*H*$_3$ (26)), 1.01 (*s*, 3H, C*H*$_3$ (29)), 0.94 (*s*, 3H, C*H*$_3$ (30)) ppm; ^{13}C NMR (125 MHz, pyridine-d_5): *δ*=180.6 (C-28), 145.2 (C-13), 123.1 (C-12), 78.7 (C-3), 71.1 (C-2), 51.2 (C-5), 49.0 (C-9), 47.1 (C-17), 46.8 (C-19), 46.1 (C-1), 42.8 (C-14), 42.5 (C-18), 40.4 (C-8), 38.3 (C-4), 38.1 (C-10), 34.6 (C-21), 33.6 (C-30), 33.6 (C-22), 33.4 (C-7), 31.3 (C-20), 28.6 (C-15), 27.5 (C-24), 26.5 (C-27), 24.3 (C-11), 24.1 (C-29), 24.1 (C-16), 23.8 (C-23), 20.1 (C-6), 20.0 (C-25), 17.7 (C-26) ppm. MS (ESI): *m*/*z* (%)=471.4 ([M−H]$^-$, 100); Anal. Calcd for $C_{30}H_{48}O_4$: C 76.23, H 10.24; found: C 76.17, H 10.38.

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Supplementary data

Supplementary data related to this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2015.09.037

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