Monatsh Chem (2010) 141:233–244
DOI 10.1007/s00706-009-0249-9

ORIGINAL PAPER

Preparation of new 18α-oleanane alcohols: synthesis, characterization, and cytotoxic activity

Miroslav Kvasnica · Iva Rudovska · Marian Hajduch · Jan Sarek

Received: 24 October 2008 / Accepted: 24 December 2009 / Published online: 4 February 2010

Abstract New oleanane alcohols and their acetates were prepared using classical reductive reagents ($LiAlH_4$, $NaBH_4$, and B_2H_6-DMS). In this research, we also studied the influence of these reagents on the stereoselectivity of reduction. All compounds prepared were fully characterized by ^1H and ^{13}C NMR spectra, IR spectra, MS, and elemental analysis. These products were tested for cytotoxic activity against T-lymphoblastic leukemia (CEM), human erythromyeloblastoid leukemia (K562), and human melanoma (SK-MEL1) cell lines. One of the compounds prepared exhibits significant cytotoxic activity against the mesenchymal type of cancer cell lines.

Keywords Reduction · Hydride · Ketone · Configuration · Cytotoxicity

M. Kvasnica
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 16610 Prague 6, Czech Republic

I. Rudovska
Department of Organic and Nuclear Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 12843 Praha 2, Czech Republic

M. Hajduch
Laboratory of Experimental Medicine, Departments of Pediatrics and Oncology, Faculty of Medicine, Palacky University in Olomouc, Puskinova 6, 77520 Olomouc, Czech Republic

J. Sarek (✉)
Department of Organic Chemistry, Faculty of Science, Palacky University in Olomouc, Trida Svobody 8, 77200 Olomouc, Czech Republic
e-mail: jan.sarek@gmail.com

Introduction

Oleanane derivatives, pentacyclic triterpenes occurring in plant species, exhibit different biological activities [1]. Within the worldwide research of new natural compounds in the field of possible therapeutics, a number of structural modifications and derivatizations of oleanane derivatives were studied to obtain compounds with significant hepatoprotective, anti-inflammatory, antiviral, anticancer, and many other activities [2–11]. From the prior results, it is known that several polar groups (e.g., hydroxyl, amine, or carboxyl) are necessary for higher biological activity. Many compounds with these groups were isolated from plants. There are several well-known oleanane acids, e.g., oleanolic acid (**1**), with anticancer (Fig. 1) [12], anti-inflammatory [13], and hepatoprotective [14] activity, glycyrrhetic acid (**2**) with cytotoxic [15], antiulcerogenic [16], and hepatoprotective [17] activity, β-boswellic acid (**3**) with cytotoxic [18] and anti-inflammatory [19] activity, and morolic acid (**4**) with anti-inflammatory activity [20].

In our team, we have prepared many lupane triterpenoids with high cytotoxic activity so far [21–23]; nowadays, we focus also on oleananes. Many polyhydroxylated oleanane derivatives with significant cytotoxic activity were the inspiration for the preparation of oleanane alcohols, for instance, preatroxigenin (**5**) [24], stachlic acid A (**6**) [25], arjulonic acid (**7**) [26], sericic acid (**8**) [27], and many others (Fig. 2). We report on the preparation of new oleanane alcohols for in vitro cytotoxic screening to discuss the structure–activity relationship. Complete structural analysis, especially NMR analysis, has been done to determine the structure and configuration of all the products.

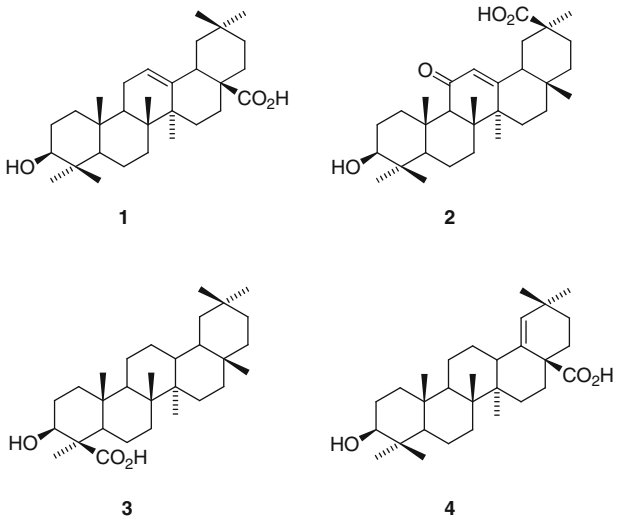


Fig. 1 The most widespread oleanane type acids

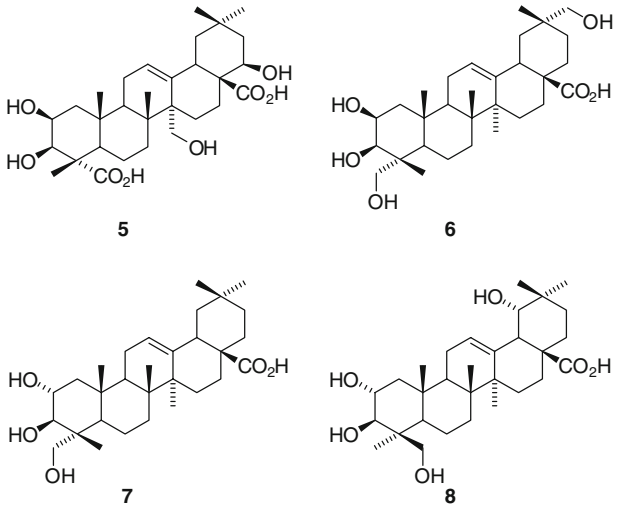


Fig. 2 Examples of polyhydroxylated oleanane derivatives

Results and discussion

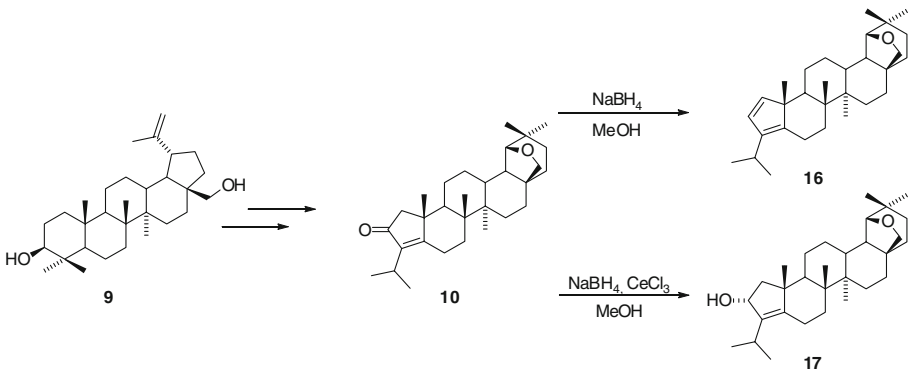
As a starting material for preparation of triterpenoids used for reductive reactions, we used betulin (**9**), which is easily accessible from birch bark (*Betula pendula*) by extraction with ethanol [21]. Using the known procedures [28] based on betulin (**9**), we synthesized several 18α-oleanane carbonyl compounds: unsaturated ketone **10**, unsaturated diketone **11**, *seco*-diketone **12**, unsaturated anhydride **13**, hydroxylactone **14**, and ester **15**.

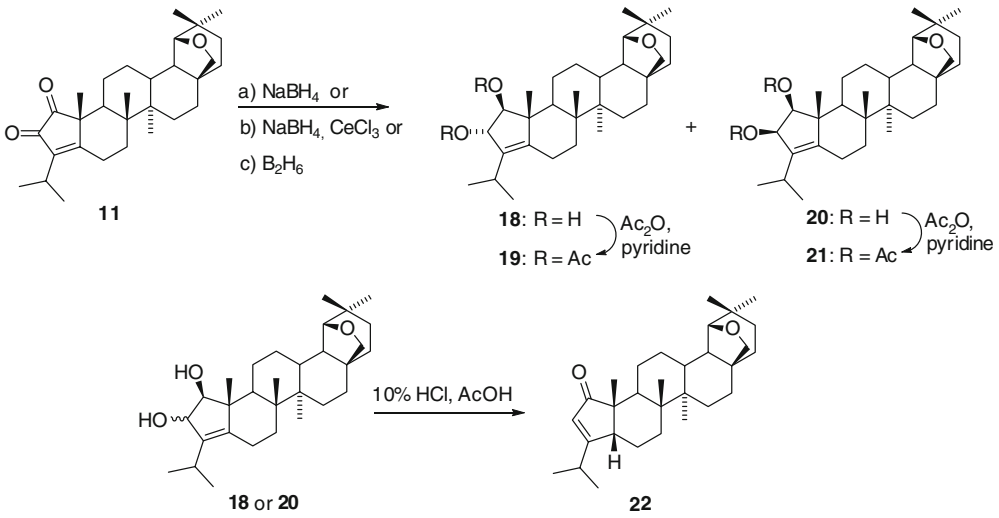
Reduction of unsaturated ketones is rarely connected with possible elimination of the allylic hydroxy groups prepared. Treatment of unsaturated ketone **10** with $NaBH_4$ also afforded the product of elimination, diene **16**. However, we could obtain the expected allylic alcohol **17** with an α configuration of the hydroxy group by addition of $CeCl_3$ to the borohydride reduction. Unfortunately, the allylic alcohol **17** was very unstable under strongly acidic conditions. Thus, a solution of citric acid instead of hydrochloric acid had to be used during the workup procedure. Even these conditions led to formation of a very small amount of diene **16** (Scheme 1).

Using the same conditions ($NaBH_4$ and $NaBH_4$ with $CeCl_3$) for reduction of the unsaturated diketone **11** led to two isomeric diols, **18** with 1β- and 2α-hydroxy configuration, and **20** with both configurations of β. No other combination of configurations was observed even when a diborane-dimethylsulfide complex (B_2H_6–DMS) was used as the reductive reagent; only the ratios of both isomers were different. This could be explained by a steric effect of the 25-methyl group, which prevents reagents from attacking the C-1 carbonyl group from the β position. Both diols were also sensitive to acidic conditions, as we observed during the workup procedure when a small amount of new compound could be observed. To determine the structure of this compound, both diols were treated with hydrochloric acid to afford the new unsaturated ketone **22** with 5β-H. Thus, the elimination of the allylic hydroxy group followed by migration of both double bonds is probably part of the mechanism for this reaction (Scheme 2).

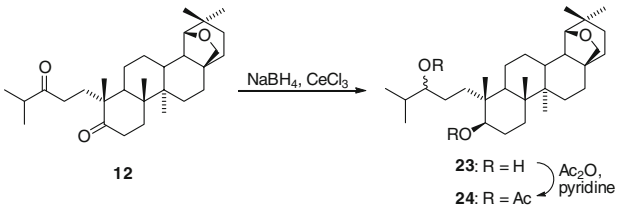
Reduction of *seco*-diketone **12** was a more problematic reaction. A very complicated mixture of compounds was obtained after reduction with $NaBH_4$ or B_2H_6-DMS. Due to this fact we had to use $NaBH_4$ with $CeCl_3$ again. Although

Scheme 1





Scheme 2



Scheme 3

these conditions did not lead to one compound, it was possible to isolate the major product, diol **23**, with a proven 5β-OH configuration. Determination of the configuration of 3-OH was not solved (Scheme 3).

In case of anhydride **13**, it was possible to use only $LiAlH_4$. However, direct reduction of anhydride **13** did not lead to any product under these conditions. Thus, at first we hydrolyzed anhydride **13** to dicarboxylic acid **25**. Separation and purification of acid **25** was impossible, as it formed anhydride **13** again very quickly. Esterification of acid **25** with diazomethane had to be done immediately after hydrolysis to avoid any formation of anhydride **13**. Reduction of the diester **26** with $LiAlH_4$ afforded the expectable diol **27** in good yield (Scheme 4).

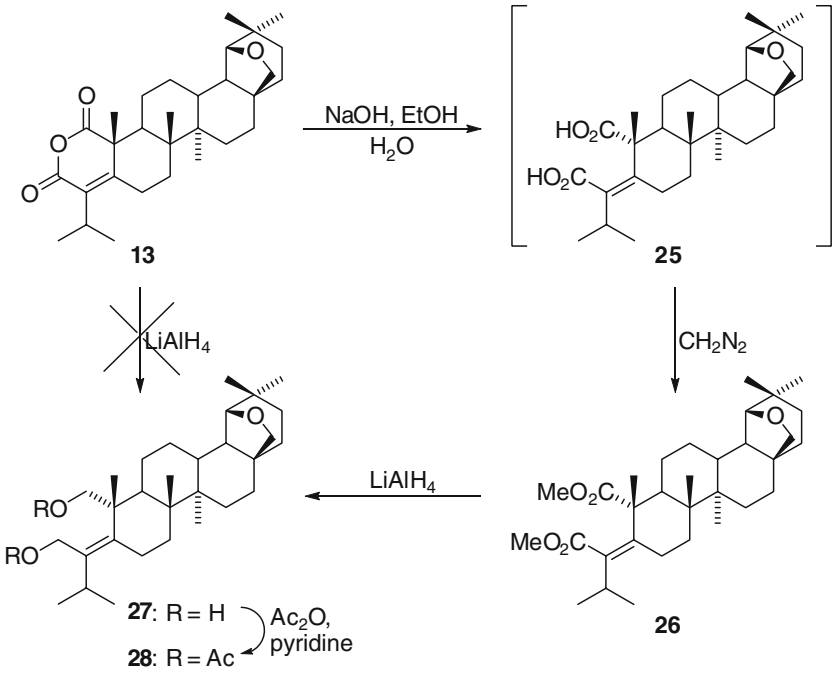
Reduction of hydroxylactone **14** and ester **15** could be done in several ways. At first we used $LiAlH_4$, which led to formation of diol **29** with 5β-OH configuration in case of both starting compounds, hydroxylactone **14** and ester **15**. Reduction of hydroxylactone **14** with $NaBH_4$ afforded only hydroxy acid **31** with 5β-OH. Unfortunately hydroxy acid **31** is not stable for a very long time, and formation of lactone **32** is observed in solvents after several hours. Reduction of ester **15** with the same reagent afforded two major products, hydroxy ester **33** with 5β-OH configuration and lactone **35** with 5β-H configuration. Using $NaBH_4$ with $CeCl_3$ for reduction of ester **15** afforded the same result as $NaBH_4$, and because of this fact, only the reaction procedure for $NaBH_4$ is included in "Experimental" (Scheme 5).

All hydroxy compounds were acetylated using a classical acetylation procedure (acetanhydride, pyridine), and we only did not obtain acetates in the case of alcohol **17** and hydroxy acid **31**. Using acetylation for alcohol **17** led only to the formation of diene **16**, and in case of hydroxy acid **31** we obtained only lactone **32**. All other acetates (**19**, **21**, **24**, **28**, **30**, and **34**) were obtained as expected.

NMR was the main method of analysis for determination of all structures. It was necessary to use not only 1D NMR analysis, but also 2D analysis, especially NOESY experiments, for determination of the configuration of new stereogenic centers. NOESY cross peaks for several compounds are shown in Table 1.

All synthesized hydroxy derivatives were tested for cytotoxic activity on the following tumor cell lines: T-lymphoblastic leukemia (CEM), human erythromyeloblastoid leukemia (K562), and human melanoma (SK-MEL1) cell lines. Normal cells of human fibroblast (BJ) were used as a control for toxicity. The lowest cytotoxic activity was found in the case of compounds **17**, **18**, **20**, **29**, and **33**, since activity of those substances increased only slightly when compared with inactive oxo derivatives. On the other hand, derivatives **23**, **27**, and **31** showed substantially better cytotoxic activity. Diol **23** was found to be the most active compound prepared in this work. This compound was significantly active against CEM (IC_{50} = 4.1 μmol dm^{-3}) and K562 (IC_{50} = 5.5 μmol dm^{-3}) cell lines. Diol **27** was significantly active mainly against the CEM cell line (IC_{50} = 9.3 μmol dm^{-3}), and hydroxy

Scheme 4



acid **31** was active mainly against the K562 cell line ($IC_{50} = 10.1$ μmol dm^{-3}). Unfortunately, no significant cytotoxic activity against the SK-MEL1 cell line was observed. No triterpenic derivative synthesized demonstrated a cytotoxic activity against normal human cells as in the case of human BJ fibroblast cells. This fact is very interesting and inspiring, too. IC_{50} values are summarized in Table 2.

Conclusion

Within the worldwide research on lupane and oleanane triterpenoids in the field of anti-tumor agents, a number of structural modifications have been studied. Our study demonstrates that reduction of oxo derivatives can result in hydroxy compounds with a higher cytotoxic activity against cell lines of mesenchymal histogenetic origin and simultaneously with no toxicity. Considering biological results, it is evident that especially A-*seco*-diols are the most active compounds. The alcohols prepared are also highly hydrophilic compounds (stated according to the solubility in polar solvents), which is very useful for MTT screening, where 10% DMSO in water is used, or for in vivo screenings, where using of water-based solutions is necessary.

Derivatization of the hydroxy groups with different substituents (e.g., esters, carbonates, ethers, etc.) also has potential for the synthesis of other modifications of the properties of these products. For this reason new triterpenic alcohols represent an interesting class of compounds for further studies and/or development.

Experimental

Melting points were determined using a Kofler block. Optical rotations were measured using $CHCl_3$ solutions (unless otherwise stated) on an Autopol III (Rudolph Research, Flanders, NJ) polarimeter, with an accuracy of ±2; they are given in 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded in chloroform (unless otherwise stated) on Nicolet AVATAR 370 FT IR; wave numbers are given in cm^{-1}. NMR spectra were recorded on a Varian UNITY INOVA 400 FT spectrometer (^{1}H NMR spectra at 399.95 MHz, ^{13}C NMR at 100.58 MHz) using $CDCl_3$ solutions (unless otherwise stated), with tetramethylsilane as the internal standard (in ^{13}C NMR, δ ($CDCl_3$) = 77.00 ppm). Chemical shift values (δ scale, ppm) and coupling constants (J, Hz) in the ^{1}H NMR spectra were obtained by first-order analysis. EI-MS spectra were recorded on an INCOS 50 (Finigan MAT) spectrometer at 70 eV and an ion source temperature of 150 °C. Relative abundances stated are given relative to the most abundant ion in the region of $m/z > 180$. TLC was carried out using silica gel 60 F_{254}; detection was by spraying with 10% aq. H_2SO_4 and heating to 150–200 °C. Column chromatography was performed using silica gel 60 (Merck 7734). The HPLC system used consisted of a high pressure pump (Gilson, model 361), an inject valve (Rheodyne), a preparative column

Scheme 5

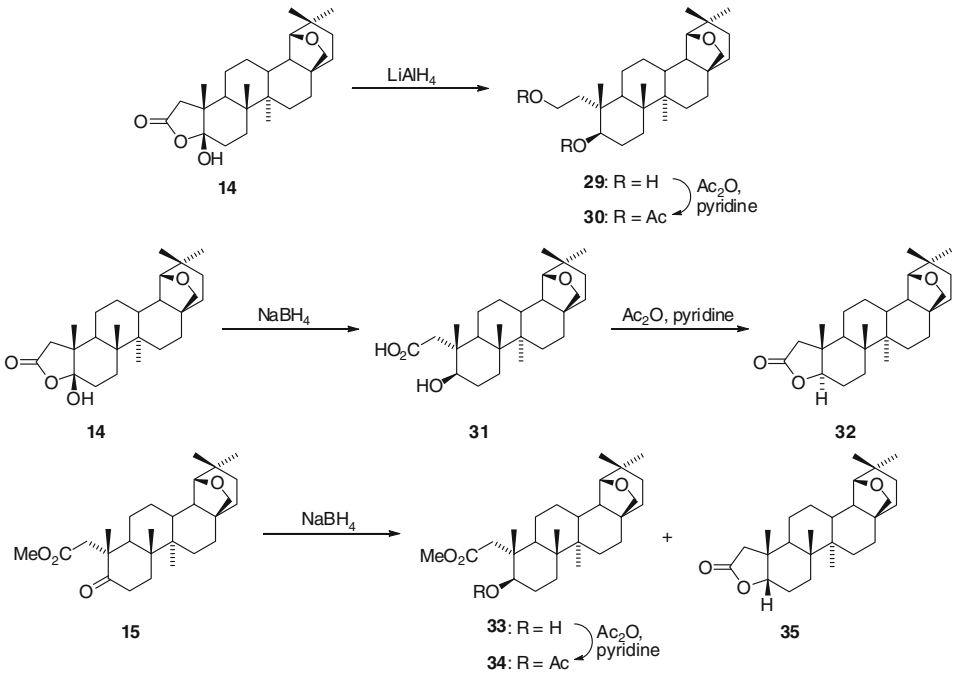


Table 1 Selected NOESY contacts for compounds **17**, **18**, **20**, **22**, **23**, **30**, **31**, **33**, and **35**

Compound	NOESY contacts
17	H-2β/H-25
18	H-1α/H-9α
	H-2β/H-25
20	H-1α/H-9α, H-2α
22	H-5β/H-25
23	H-5α/H-9α
30	H-5α/H-9α
31	H-5α/H-9α
33	H-5α/H-9α
35	H-5β/H-25

Table 2 Cytotoxic activity (IC_{50}) of compounds **16–24** and **26–35** against CEM, K562, and SK-MEL1 cells

Compound	IC_{50} (μmol/dm^3)[a]		
	CEM	K562	SK-MEL1
16	>250	>250	>250
17	53.2 (±5.7)	47.1 (±4.2)	121.2 (±14.2)
18	39.8 (±3.1)	49.0 (±5.0)	87.9 (±10.2)
19	>250	>250	>250
20	25.0 (±4.5)	19.4 (±2.9)	150.2 (±5.9)
21	>250	>250	>250
22	>250	>250	>250
23	4.1 (±1.1)	5.5 (±0.9)	73.4 (±8.1)
24	>250	>250	>250
26	>250	>250	>250
27	9.3 (±1.3)	14.9 (±1.7)	98.1 (±11.5)
28	>250	>250	>250
29	45.3 (±5.2)	51.9 (±7.0)	>250
30	>250	>250	>250
31	15.5 (±3.3)	10.1 (±2.0)	187.4 (±15.8)
32	>250	>250	>250
33	77.1 (±8.2)	61.0 (±7.8)	>250
34	>250	>250	>250
35	>250	>250	>250

[a] The lowest concentration that kills 50% of tumor cells. Value >250 μmol/dm^3 means that compound is not active

(25 × 250 mm) with filling Si gel (Biospher 7 μm), a differential refractometrical detector (Laboratorni pristroje, Praha, CZ) connected with a PC (software Chromulan), and an automatic fraction collector (Gilson, model 246). TLC was carried out on Kieselgel 60 F_{254} plates (Merck). Elemental analyses (C, H) were conducted using the Perkin-Elmer; their results were found to be in good agreement (±0.2%) with the calculated values.

General procedure for acetylation

To a solution of the appropriate hydroxy compound (0.1 mmol) in 3 cm^3 pyridine, 3 cm^3 acetic anhydride was added. The mixture was left at room temperature for 12 h. Then it was poured into 10% aqueous HCl, and the product was extracted twice with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$, and the solvent was evaporated. The crude product was purified by crystallization.

19β,28-Epoxy-4,5-seco-3,5-cyclo-18α-olean-1(2),3(5)-diene (**16**, $C_{30}H_{46}O$)

$NaBH_4$ (20 mg, 0.53 mmol) was added to 100 mg ketone **10** (0.23 mmol) dissolved in 2.5 cm^3 THF and 2 cm^3 MeOH. The mixture was stirred for 5 h. After that another portion of 20 mg $NABH_4$ (0.53 mmol) was added, and the mixture was stirred for 10 h. It was poured into 10% aqueous HCl, and the product was extracted twice with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$, and the solvents were evaporated. HPLC (EtOAc/hexane = 1/19, $R_f = 0.22$) and crystallization from MeOH-$CHCl_3$ afforded 65 mg (67%) **16**. M.p.: 155–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.35$ (d, $J = 5.3$ Hz, 1H, H-1), 6.26 (d, $J = 5.3$ Hz, 1H, H-2), 3.80 (dd, $J = 7.8$ Hz, $J = 1.1$ Hz, 1H, H-28*pro-S*), 3.54 (s, 1H, H-19α), 3.45 (d, $J = 7.8$ Hz, 1H, H-28*pro-R*), 2.75 (sept, $J = 6.9$ Hz, 1H, H-4), 2.51 (dt, $J = 13.1$ Hz, $J = 3.5$ Hz, 1H, H-6α), 2.12 (td, $J = 13.3$ Hz, $J = 4.0$ Hz, 1H, H-6β), 1.14 (s, 3H, CH_3), 1.09 (d, $J = 6.8$ Hz, 3H, CH_3), 1.06 (d, $J = 6.8$ Hz, 3H, CH_3), 0.97, 0.93, 0.79, 0.75 (all s, 3H, 4 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 146.29$ (C-1), 147.86 (C-5), 138.17 (C-3), 127.04 (C-2), 87.97 (C-19), 71.26 (C-28), 56.76 (C-10), 46.79 (C-18), 44.99 (C-9), 41.43 (C-8), 41.04 (C-17), 40.83 (C-14), 36.71 (C-16), 36.33 (C-7), 36.26 (C-20), 34.48 (C-13), 32.68 (C-21), 28.80 (C-30), 27.26 (C-22), 26.25 (C-12, C-15), 26.05 (C-11), 25.59 (C-4), 24.52 (C-29), 23.13 (C-23), 22.38 (C-24), 20.42 (C-6), 15.44 (C-25), 14.30 (C-26), 13.15 (C-27) ppm; IR (KBr): $\bar{\nu} = 1{,}606$, 1,453, 1,168, 1,031 cm^{-1}; MS (70 eV): $m/z = 422$ (M^+, 100), 407 (19), 379 (19), 351 (1), 329 (2), 287 (1), 269 (1), 245 (9), 215 (8), 203 (14), 189 (11); $[\alpha]_D^{20} = +49 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 0.29$, $CHCl_3$).

19β,28-Epoxy-4,5-seco-3,5-cyclo-18α-olean-3(5)-en-2α-ol (**17**, $C_{30}H_{48}O_2$)

$NaBH_4$ (40 mg, 1.05 mmol) and a 0.3 M solution of $CeCl_3$ in MeOH (2 cm^3) were added to 100 mg ketone **10** (0.23 mmol) dissolved in 2 cm^3 THF. The mixture was stirred for 10 h. It was poured into 5% aqueous solution of citric acid, and the product was extracted twice with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and the solvents were evaporated. HPLC (EtOAc/hexane = 3/17, $R_f = 0.25$) and lyophilization from benzene afforded 72 mg (72%) **17**. M.p.: 149–151 °C; ^{1}H NMR (400 MHz, C_6D_6): $\delta = 4.81$ (td, $J = 7.1$ Hz, $J = 3.2$, 1H, H-2β), 3.84 (dd, $J = 7.8$ Hz, $J = 1.7$ Hz, 1H, H-28*pro-S*), 3.67 (s, 1H, H-19α), 3.45 (d, $J = 7.9$ Hz, 1H, H-28*pro-R*), 2.71 (sept, $J = 7.0$ Hz, 1H, H-4), 2.32 (dt, $J = 14.1$ Hz, $J = 4.1$ Hz, 1H, H-6α), 2.16 (dd, $J = 12.0$ Hz, $J = 6.8$ Hz, 1H, H-1β), 1.96 (tt, $J = 13.7$ Hz, $J = 4.1$ Hz, 1H, H-6β), 1.28 (d, $J = 7.2$ Hz, 3H, CH_3), 1.23 (d, $J = 7.2$ Hz, 3H, CH_3), 1.15, 0.94, 0.91, 0.80, 0.76 (all s, 3H, 5 × CH_3) ppm; ^{13}C NMR (100 MHz, C_6D_6): $\delta = 145.28$ (C-5), 139.71 (C-3), 88.52 (C-19), 77.86 (C-2), 72.02 (C-28), 55.03 (C-1), 51.21 (C-9), 48.50 (C-10), 47.87 (C-18), 42.31 (C-17), 41.63 (C-14), 41.48 (C-8), 37.76 (C-16), 37.25 (C-20), 35.56 (C-13), 33.88 (C-21), 33.80 (C-7), 29.91 (C-30), 27.63 (C-15), 27.56 (C-4), 27.43 (C-12), 27.24 (C-22), 25.32 (C-29), 24.89 (C-23), 24.64 (C-11), 22.63 (C-25), 21.08 (C-6), 20.95 (C-24), 15.17 (C-26), 14.18 (C-27) ppm; IR (KBr): $\bar{\nu} = 3{,}450$, 1,448, 1,382, 1,034 cm^{-1}; MS (70 eV): $m/z = 440$ (M^+, 6), 422 (100), 407 (15), 379 (37), 245 (13), 203 (16), 187 (19); $[\alpha]_D^{20} = +79 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 0.16$, $CHCl_3$).

*Reduction of diketone **11***

Method A: 80 mg $NaBH_4$ (2.12 mmol) was added to 150 mg diketone **11** (0.33 mmol) dissolved in 4 cm^3 THF and 2 cm^3 MeOH. The mixture was stirred for 5 h. It was poured into 5% aqueous solution of citric acid, and the products were extracted twice with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and the solvents were evaporated. Separation of the products with HPLC (EtOAc/hexane = 3/7, $R_f = 0.18$ resp. $R_f = 0.21$) afforded two diols, **18** (65 mg, 43%) and **20** (73 mg, 48%).

Method B: 60 mg $NaBH_4$ (1.58 mmol) and a 0.3 M solution of $CeCl_3$ in MeOH (2 cm^3) were added to 100 mg diketone **11** (0.22 mmol) dissolved in 2 cm^3 THF. The mixture was stirred for 5 h. It was worked up according to the same procedure as described for method A. HPLC (EtOAc/hexane = 3/7) afforded diols **18** (72 mg, 71%) and **20** (18 mg, 18%).

Method C: B_2H_6–DMS (0.4 cm^3) was added to 200 mg diketone **11** (0.44 mmol) dissolved in 4 cm^3 THF. The mixture was stirred for 3 h. It was poured into water, and the products were extracted twice with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and the solvents were evaporated. HPLC (EtOAc/hexane = 3/7) afforded diols **18** (162 mg, 80%) and **20** (14 mg, 7%).

19β,28-Epoxy-4,5-seco-3,5-cyclo-18α-olean-3(5)-en-1β,2α-diol (**18**, $C_{30}H_{48}O_3$)

The title compound was obtained after lyophilization from benzene as white powder. M.p.: 137–139 °C; ^{1}H NMR (400 MHz, C_6D_6): $\delta = 4.52$ (dd, $J = 6.6$ Hz, $J = 3.5$ Hz, 1H, H-2β), 3.84 (dd, $J = 7.8$ Hz, $J = 1.5$ Hz, 1H, H-28*pro-S*), 3.70 (d, $J = 6.4$ Hz, 1H, H-1α), 3.68 (s, 1H, H-19α), 3.45 (d, $J = 7.9$ Hz, 1H, H-28*pro-R*), 2.71 (sept, $J = 7.0$ Hz, 1H, H-4), 2.30 (dt, $J = 14.5$ Hz, $J = 4.0$ Hz, 1H, H-6α), 1.94 (tt, $J = 13.6$ Hz, $J = 3.7$ Hz, 1H, H-6β), 1.82 (m, 1H, H-11α), 1.29 (d, $J = 7.2$ Hz, 3H, CH_3), 1.22 (d, $J = 7.0$ Hz, 3H, CH_3), 1.14, 1.04, 0.97, 0.84, 0.77 (all s, 3H, 5 × CH_3) ppm;

^{13}C NMR (100 MHz, C_6D_6): δ = 142.59 (C-5), 136.78 (C-3), 93.82 (C-1), 88.54 (C-19), 83.97 (C-2), 72.01 (C-28), 51.23 (C-9), 48.84 (C-10), 47.86 (C-18), 42.26 (C-17), 41.48 (C-14), 41.35 (C-8), 37.76 (C-16), 37.24 (C-20), 35.47 (C-13), 33.88 (C-21), 33.36 (C-7), 29.90 (C-30), 27.57 (C-15), 27.33 (C-4, C-12), 27.23 (C-22), 25.33 (C-29), 24.54 (C-23), 24.31 (C-11), 21.04 (C-6), 20.76 (C-24), 16.22 (C-25), 15.50 (C-26), 14.20 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu}$ = 3,607, 1,601, 1,449, 1,384, 1,032 cm^{-1}; MS (70 eV): *m/z* = 456 (M^+, 31), 438 (25), 423 (70), 413 (67), 395 (56), 245 (100), 215 (26), 203 (16), 192 (24); $[\alpha]_D^{20}$ = +77 × 10^{-1} deg cm^2 g^{-1} (*c* = 0.25, $CHCl_3$).

19β,28-Epoxy-4,5-seco-3,5-cyclo-18α-olean-3(5)-en-1β,2β-diol (**20**, $C_{30}H_{48}O_3$)

The title compound was obtained after lyophilization from benzene as white powder. M.p.: 149–151 °C; ^{1}H NMR (400 MHz, C_6D_6): δ = 4.18 (bt, *J* = 5.9 Hz, 1H, H-2α), 3.83 (dd, *J* = 7.8 Hz, *J* = 1.7 Hz, 1H, H-28*pro-S*), 3.66 (s, 1H, H-19α), 3.59 (dd, *J* = 9.0 Hz, *J* = 6.4 Hz, 1H, H-1α), 3.44 (d, *J* = 7.9 Hz, 1H, H-28*pro-R*), 2.83 (bd, *J* = 9.0 Hz, 1H, 1β-OH), 2.66 (sept, *J* = 6.8 Hz, 1H, H-4), 2.28 (ddd, *J* = 14.2 Hz, *J* = 4.2 Hz, *J* = 2.9 Hz, 1H, H-6α), 2.02 (td, *J* = 13.9 Hz, *J* = 4.4 Hz, 1H, H-6β), 1.98 (m, 1H, H-11α), 1.14 (s, 6H, 2 × CH_3), 1.11 (d, *J* = 6.8 Hz, 3H, CH_3), 0.99 (d, *J* = 7.0 Hz, 3H, CH_3), 0.98, 0.76, 0.74 (all s, 3H, 3 × CH_3) ppm; ^{13}C NMR (100 MHz, C_6D_6): δ = 148.14 (C-5), 137.63 (C-3), 88.52 (C-19), 83.39 (C-1), 74.87 (C-2), 71.98 (C-28), 51.74 (C-10), 51.49 (C-9), 47.85 (C-18), 42.23 (C-17), 41.48 (C-14), 41.32 (C-8), 37.75 (C-16), 37.22 (C-20), 35.53 (C-13), 33.87 (C-21), 33.57 (C-7), 29.88 (C-30), 27.73 (C-15), 27.38 (C-12), 27.33 (C-4), 27.23 (C-22), 25.28 (C-29), 24.94 (C-11), 23.74 (C-23), 22.68 (C-24), 21.21 (C-6), 19.47 (C-25), 15.47 (C-26), 14.17 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu}$ = 3,605, 1,602, 1,453, 1,385, 1,032 cm^{-1}; MS (70 eV): *m/z* = 456 (M^+, 42), 438 (37), 423 (51), 413 (39), 395 (36), 385 (5), 287 (11), 245 (100), 215 (29), 203 (24), 192 (27); $[\alpha]_D^{20}$ = +23 × 10^{-1} deg cm^2 g^{-1} (*c* = 0.30, $CHCl_3$).

19β,28-Epoxy-4,5-seco-3,5-cyclo-5β,18α-olean-2-en-1-on (**22**, $C_{30}H_{46}O_2$)

HCl (10%, 0.1 cm^3) was added to 50 mg diol **18**, resp. **20** (0.11 mmol) dissolved in 1 cm^3 AcOH. The mixture was stirred for 1 h. It was poured into saturated aqueous solution of $NaHCO_3$, and the product was extracted twice with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and the solvent was evaporated. HPLC (EtOAc/hexane = 1/9, R_f = 0.24) and crystallization from MeOH afforded 39 mg (81%) **22**. M.p.: 104–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.81 (s, 1H, H-2), 3.77 (dd, *J* = 7.8 Hz, *J* = 1.4 Hz, 1H, H-28*pro-S*), 3.55 (s, 1H, H-19α), 3.44 (d, *J* = 7.8 Hz, 1H, H-28*pro-R*), 2.62 (m, 1H, H-5β), 2.59 (sept, *J* = 6.9 Hz, 1H, H-4), 1.22 (d, *J* = 6.4 Hz, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.10 (d, *J* = 6.8 Hz, 3H, CH_3), 1.04, 0.93, 0.783, 0.778 (all s, 3H, 4 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 213.35 (C-1), 185.65 (C-3), 124.51 (C-2), 87.85 (C-19), 71.25 (C-28), 50.02 (C-10), 49.70 (C-9), 46.61 (C-18), 41.42 (C-17), 40.60 (C-8), 40.18 (C-14), 38.76 (C-5), 36.64 (C-16), 36.25 (C-20), 34.73 (C-13), 32.68 (C-21), 28.75 (C-30), 28.49 (C-4), 28.39 (C-7), 26.60 (C-15), 26.14 (C-22), 26.07 (C-12), 24.51 (C-29), 22.65 (C-11), 21.37 (C-23), 20.54 (C-24), 20.44 (C-6), 17.74 (C-25), 15.65 (C-26), 13.08 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu}$ = 1,690, 1,609, 1,451, 1,384, 1,263, 1,032, 909 cm^{-1}; MS (70 eV): *m/z* = 438 (M^+, 100), 423 (7), 408 (6), 395 (9), 367 (14), 257 (5), 217 (7), 203 (10), 189 (9); $[\alpha]_D^{20}$ = +9 × 10^{-1} deg cm^2 g^{-1} (*c* = 0.51, $CHCl_3$).

19β,28-Epoxy-4,5-seco-3,5-cyclo-18α-olean-3(5)-en-1β,2α-diol, diacetate (**19**, $C_{34}H_{52}O_5$)

According to the general procedure for acetylation, from 100 mg diol **18** (0.22 mmol) diacetate **19** was obtained as white powder (105 mg, 89%). M.p.: 174–175 °C (MeOH); ^{1}H NMR (400 MHz, C_6D_6): δ = 6.32 (dd, *J* = 5.8 Hz, *J* = 3.5 Hz, 1H, H-2α), 5.54 (d, *J* = 5.8 Hz, 1H, H-1α), 3.79 (bd, *J* = 7.8 Hz, 1H, H-28*pro-S*), 3.59 (s, 1H, H-19α), 3.42 (d, *J* = 7.8 Hz, 1H, H-28*pro-R*), 2.61 (sept, *J* = 7.0 Hz, 1H, H-4), 2.26 (td, *J* = 14.3 Hz, *J* = 2.5 Hz, 1H, H-6α), 1.89 (m, 1H), 1.90, 1.84 (both s, 3H, 2 × OAc), 1.76 (dd, *J* = 12.8 Hz, *J* = 3.2 Hz, 1H), 1.18 (d, *J* = 6.8 Hz, 3H, CH_3), 1.14, 1.10 (both s, 3H, 2 × CH_3), 1.02 (d, *J* = 7.0 Hz, 3H, CH_3), 0.88, 0.74, 0.60 (all s, 3H, 3 × CH_3) ppm; ^{13}C NMR (100 MHz, C_6D_6): δ = 171.26, 171.20 (2 × CH_3CO), 145.60 (C-5), 133.41 (C-3), 89.45 (C-1), 88.50 (C-19), 83.43 (C-2), 71.94 (C-28), 50.77 (C-9), 49.86 (C-10), 47.64 (C-18), 42.16 (C-17), 41.44 (C-14), 41.40 (C-8), 37.64 (C-16), 37.21 (C-20), 35.32 (C-13), 33.80 (C-21), 33.22 (C-7), 29.93 (C-30), 27.52 (C-15), 27.13 (C-22), 27.06 (C-12), 27.01 (C-4), 25.31 (C-29), 24.12 (C-23), 24.03 (C-11), 21.77, 21.70 (2 × CH_3CO), 21.05 (C-6), 20.43 (C-24), 17.69 (C-25), 15.46 (C-26), 13.93 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu}$ = 1,731, 1,603, 1,453, 1,374, 1,256, 1,032 cm^{-1}; MS (70 eV): *m/z* = 540 (M^+, not found), 480 (23), 438 (100), 420 (6), 395 (4), 245 (11), 215 (4), 201 (5), 189 (6); $[\alpha]_D^{20}$ = +23 × 10^{-1} deg cm^2 g^{-1} (*c* = 0.44, $CHCl_3$).

19β,28-Epoxy-4,5-seco-3,5-cyclo-18α-olean-3(5)-en-1β,2β-diol, diacetate (**21**, $C_{34}H_{52}O_5$)

According to the general procedure for acetylation, from 100 mg diol **20** (0.22 mmol) diacetate **21** was obtained as white powder (107 mg, 91%). M.p.: 168–170 °C (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.69 (d, *J* = 6.0 Hz, 1H, H-2α), 4.88 (d, *J* = 6.0 Hz, 1H, H-1α), 3.78 (bd, *J* = 7.8 Hz, 1H, H-28*pro-S*), 3.51 (s, 1H, H-19α), 3.45 (d, *J* = 7.8 Hz, 1H, H-28*pro-R*), 2.71 (sept, *J* = 6.9 Hz, 1H,

H-4), 2.42 (td, $J = 14.3$ Hz, $J = 3.4$ Hz, 1H, H-6α), 2.02–2.18 (m, 2H), 2.01, 2.00 (both s, 3H, 2 × OAc), 1.12, 1.08 (both s, 3H, 2 × CH_3), 1.01 (d, $J = 7.0$ Hz, 3H, CH_3), 0.95 (d, $J = 6.8$ Hz, 3H, CH_3), 0.92, 0.86, 0.78 (all s, 3H, 3 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 170.38, 170.25 (2 × CH_3CO), 148.64 (C-5), 132.98 (C-3), 88.02 (C-19), 80.96 (C-1), 74.15 (C-2), 71.25 (C-28), 49.95 (C-10), 49.89 (C-9), 46.70 (C-18), 41.42 (C-17), 40.65 (C-14), 40.45 (C-8), 36.68 (C-16), 36.25 (C-20), 34.41 (C-13), 32.65 (C-21), 32.23 (C-7), 28.79 (C-30), 26.68 (C-22), 26.15 (C-12, C-15), 25.80 (C-4), 24.50 (C-29), 23.73 (C-11), 22.03 (C-23), 21.58, 21.15 (2 × CH_3CO), 20.94 (C-24), 20.08 (C-6), 17.47 (C-25), 14.56 (C-26), 13.36 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu} =$ 1,735, 1,604, 1,454, 1,373, 1,255, 1,031 cm^{-1}; MS (70 eV): $m/z = 540$ (M^+, not found), 480 (27), 438 (100), 420 (19), 395 (5), 245 (13), 215 (4), 201 (10), 189 (6); $[\alpha]_D^{20} =$ $+12 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 0.75$, $CHCl_3$).

19β,28-Epoxy-4,5-seco-18α-oleanan-3ξ,5β-diol (**23**, $C_{30}H_{52}O_3$)

$NaBH_4$ (60 mg, 1.58 mmol) and a 0.3 M solution of $CeCl_3$ in MeOH (3 cm^3) were added to 100 mg diketone **12** (0.22 mmol) dissolved in 5 cm^3 THF. The mixture was stirred for 2 h at 5 °C. It was poured into 5% aqueous solution of citric acid, and the product was extracted twice with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and the solvents were evaporated. HPLC (EtOAc/hexane = 9/11, $R_f = 0.21$) and lyophilization from *t*-BuOH afforded 45 mg (45%) **23**. M.p.: 159–161 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.77$ (dd, $J = 7.8$ Hz, $J = 1.5$ Hz, 1H, H-28*pro-S*), 3.53 (s, 1H, H-19α), 3.45 (d, $J = 7.8$ Hz, 1H, H-28*pro-R*), 3.40 (m, 1H, H-5α), 3.32 (m, 1H, H-3ξ), 1.00 (s, 3H, CH_3), 0.94 (d, $J =$ 6.8 Hz, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.92 (d, $J = 6.8$ Hz, 3H, CH_3), 0.91, 0.84, 0.80 (all s, 3H, 3 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 87.93$ (C-19), 77.43 (C-3), 73.55 (C-5), 71.25 (C-28), 46.74 (C-18), 41.48 (C-17), 41.10 (C-8), 40.90 (C-10), 39.87 (C-14), 39.57 (C-9), 36.72 (C-16), 36.26 (C-20), 34.22 (C-13), 33.28 (C-1), 33.07 (C-4), 32.67 (C-21), 30.44 (C-7), 28.78 (C-30), 27.47 (C-6), 27.20 (C-2), 26.52 (C-15), 26.38 (C-12), 26.19 (C-22), 24.54 (C-29), 21.40 (C-11), 19.16 (C-24), 17.32 (C-23, C-25), 15.58 (C-26), 13.41 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu} =$ 3,613, 1,455, 1,386, 1,032 cm^{-1}; MS (70 eV): $m/z = 460$ (M^+, 6), 442 (8), 424 (13), 403 (100), 399 (22), 389 (29), 371 (3), 358 (41), 341 (22), 323 (23), 217 (17), 203 (45), 191 (40); $[\alpha]_D^{20} = +\ 54 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 0.37$, $CHCl_3$).

19β,28-Epoxy-4,5-seco-18α-oleanan-3ξ,5β-diol, diacetate (**24**, $C_{34}H_{56}O_5$)

According to the general procedure for acetylation, from 100 mg diol **23** (0.22 mmol) diacetate **24** was obtained as white powder (103 mg, 88%). M.p.: 61–63 °C (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.66$ (dd, $J = 10.4$ Hz, $J = 5.8$ Hz, 1H, H-5α), 4.60 (m, 1H, H-3ξ), 3.77 (dd, $J = 7.8$ Hz, $J = 1.5$ Hz, 1H, H-28*pro-S*), 3.53 (s, 1H, H-19α), 3.45 (d, $J = 7.9$ Hz, 1H, H-28*pro-R*), 2.04, 2.03 (both s, 3H, 2 × OAc), 1.02, 0.94, 0.92 (all s, 3H, 3 × CH_3), 0.89 (d, $J = 6.8$ Hz, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.87 (d, $J = 6.8$ Hz, 3H, CH_3), 0.81 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.01$, 170.72 (2 × CH_3CO), 87.93 (C-19), 79.05 (C-5), 75.70 (C-3), 71.24 (C-28), 46.71 (C-18), 41.50 (C-17), 41.15 (C-8), 39.74 (C-14), 39.66 (C-9), 36.70 (C-16), 36.28 (C-20), 34.21 (C-13), 33.52 (C-1), 32.69 (C-21), 31.22 (C-4), 30.12 (C-7), 28.79 (C-30), 26.50 (C-15), 26.24 (C-12), 26.14 (C-22), 24.57 (C-29), 24.48 (C-6), 23.84 (C-2), 21.24 (CH_3CO), 21.17 (C-11), 21.13 (CH_3CO), 18.69 (C-24), 18.38 (C-23), 17.63 (C-25), 15.58 (C-26), 13.43 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu} =$ 1,732, 1,455, 1,373, 1,251, 1,036 cm^{-1}; MS (70 eV): $m/z = 544$ (M^+, 18), 484 (21), 473 (100), 424 (93), 341 (52), 323 (22), 245 (19), 218 (34), 203 (42), 189 (40); $[\alpha]_D^{20} = +20 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 0.22$, $CHCl_3$).

Dimethyl 19β,28-epoxy-1,2:4,5-diseco-3,5-cyclo-18α-olean-3(5)-en-1,2-dioate (**26**, $C_{32}H_{50}O_5$)

KOH (150 mg, 2.68 mmol) dissolved in 10 cm^3 EtOH (96%) and 5 cm^3 water were added to 500 mg anhydride **13** (1.07 mmol) dissolved in 30 cm^3 toluene. The mixture was refluxed for 2 h. It was poured into 10% aqueous HCl, and the product was extracted twice with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and the solvents were evaporated. The crude product was dissolved in 10 cm^3 $CHCl_3$, and a solution of diazomethane in diethylether (5 cm^3) was added. After 30 min the solvent was evaporated. HPLC (EtOAc/hexane = 1/4, $R_f = 0.25$) and crystallization from MeOH afforded 373 mg (68%) **26**. M.p.: 190–192 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.77$ (d, $J = 7.8$ Hz, 1H, H-28*pro-S*), 3.61 (s, 6H, 2 × OCH_3), 3.52 (s, 1H, H-19α), 3.45 (d, $J = 7.9$ Hz, 1H, H-28*pro-R*), 2.90 (sept, $J = 7.0$ Hz, 1H, H-4), 2.57 (dt, $J = 15.4$ Hz, $J = 4.8$ Hz, 1H, H-6α), 2.39 (dd, $J = 12.8$ Hz, $J = 2.7$ Hz, 1H, H-9α), 2.22 (ddd, $J = 15.4$ Hz, $J = 12.1$ Hz, $J =$ 4.0 Hz, 1H, H-6*β*), 1.34 (s, 3H, CH_3), 1.09 (d, $J = 6.8$ Hz, 3H, CH_3), 1.06 (s, 3H, CH_3), 0.96 (d, $J = 7.2$ Hz, 3H, CH_3), 0.93 (s, 6H, 2 × CH_3), 0.78, (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 177.02$ (C-1), 169.82 (C-2), 140.10 (C-5), 133.37 (C-3), 87.83 (C-19), 71.27 (C-28), 53.86 (C-10), 51.68, 50.52 (2 × CH_3O), 46.60 (C-18), 44.22 (C-9), 41.50 (C-17), 41.27 (C-8), 39.27 (C-14), 36.66 (C-16), 36.25 (C-20), 34.64 (C-13), 32.65 (C-21), 31.55 (C-7), 29.08 (C-4), 28.76 (C-30) 26.41 (C-15), 26.31 (C-12), 26.14 (C-22), 24.51 (C-29), 23.73 (C-5), 22.95 (C-11), 21.72 (C-23), 20.42 (C-24), 19.48 (C-25), 15.81 (C-26), 13.13 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu} =$ 1,720, 1,619,

1,453, 1,238, 1,133, 1,032 cm^{-1}; MS (70 eV): $m/z = 514$ (M^+, 2), 499 (4), 482 (100), 454 (76), 439 (6), 245 (16), 215 (13), 201 (21), 189 (34); $[\alpha]_D^{20} = +15 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 0.33$, $CHCl_3$).

19β,28-Epoxy-1,2:4,5-diseco-3,5-cyclo-18α-olean-3(5)-en-1,2-diol (**27**, $C_{30}H_{50}O_3$)

$LiAlH_4$ (50 mg, 1.33 mmol) was added to 200 mg ketone **26** (0.39 mmol) dissolved in 15 cm^3 THF. The mixture was refluxed for 2 h. It was poured into 10% aqueous HCl, and the product was extracted twice with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and the solvents were evaporated. HPLC (EtOAc/hexane = 2/3, $R_f = 0.20$) and lyophilization from benzene afforded 115 mg (65%) **27**. M.p.: 222–225 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.37$ (d, $J = 12.1$ Hz, 1H, H-2a), 3.88 (d, $J = 12.2$ Hz, 1H, H-2b), 3.78 (d, $J = 7.8$ Hz, 1H, H-28*pro-S*), 3.54 (s, 1H, H-19α), 3.46 (d, $J = 7.8$ Hz, 1H, H-28*pro-R*), 3.43 (d, $J = 10.3$ Hz, 1H, H-1a), 3.32 (d, $J = 10.2$ Hz, 1H, H-1b), 3.01 (sept, $J = 6.9$ Hz, 1H, H-4), 2.49 (m, 1H, H-6a), 1.84–1.96 (m, 2H), 1.79 (dd, $J = 12.4$ Hz, $J = 3.7$ Hz, 1H, H-9α), 1.36 (s, 3H, CH_3), 1.07 (d, $J = 7.2$ Hz, 3H, CH_3), 0.96, 0.94 (both s, 3H, 2 × CH_3), 0.93 (d, $J = 7.0$ Hz, 3H, CH_3), 0.91, 0.81 (both s, 3H, 2 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 139.25$ (C-5), 138.68 (C-3), 88.05 (C-19), 71.05 (C-28), 67.70 (C-1), 55.95 (C-2), 46.93 (C-10), 46.69 (C-18), 42.06 (C-9), 41.49 (C-17), 40.94 (C-8), 39.97 (C-14), 36.44 (C-16), 36.11 (C-20), 34.81 (C-13), 33.98 (C-7), 32.51 (C-21), 31.50 (C-4), 28.53 (C-30), 26.99 (C-15), 26.07 (C-22), 25.72 (C-12), 25.20 (C-11), 24.79 (C-6), 24.35 (C-29), 21.64 (C-23), 20.57 (C-24), 19.86 (C-25), 17.69 (C-26), 13.43 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu} =$ 3,612, 1,580, 1,550, 1,461, 1,031 cm^{-1}; MS (70 eV): $m/z = 458$ (M^+, not found), 440 (22), 422 (11), 410 (53), 397 (50), 367 (49), 349 (44), 241 (100), 203 (26), 191 (77); $[\alpha]_D^{20} = +42 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 0.43$, $CHCl_3$).

19β,28-Epoxy-1,2:4,5-diseco-3,5-cyclo-18α-olean-3(5)-en-1,2-diol, diacetate (**28**, $C_{34}H_{54}O_5$)

According to the general procedure for acetylation, from 60 mg diol **27** (0.13 mmol) diacetate **28** was obtained as white powder (64 mg, 90%). M.p.: 180–183 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.81$ (d, $J = 12.2$ Hz, 1H, H-2a), 4.51 (d, $J = 12.4$ Hz, 1H, H-2b), 4.09 (d, $J = 10.7$ Hz, 1H, H-1a), 3.87 (d, $J = 10.9$ Hz, 1H, H-1b), 3.77 (d, $J = 7.6$ Hz, 1H, H-28*pro-S*), 3.53 (s, 1H, H-19α), 3.45 (d, $J = 7.8$ Hz, 1H, H-28*pro-R*), 2.85 (sept, $J = 6.8$ Hz, 1H, H-4), 2.48 (m, 1H, H-6a), 1.83–2.15 (m, 3H), 2.04, 2.03 (both s, 3H, 2 × OAc), 1.23 (s, 3H, CH_3), 0.98 (d, $J =$ 6.9 Hz, 3H, CH_3), 0.97, 0.94 (both s, 3H, 2 × CH_3), 0.94 (d, $J = 7.0$ Hz, 3H, CH_3), 0.92, 0.81 (both s, 3H, 2 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 171.18, 170.94 (2 × CH_3CO), 145.34 (C-5), 132.37 (C-3), 87.92 (C-19), 71.23 (C-28), 71.08 (C-1), 60.67 (C-2), 46.80 (C-18), 43.87 (C-10), 41.63 (C-8), 41.46 (C-9), 41.41 (C-17), 39.48 (C-14), 36.68 (C-16), 36.29 (C-20), 35.16 (C-13), 33.62 (C-7), 32.70 (C-21), 31.46 (C-4), 28.78 (C-30), 26.92 (C-15), 26.19 (C-22), 26.03 (C-12), 25.89 (C-11), 24.57 (C-29), 24.24 (C-6), 21.68, 21.27 (2 × CH_3CO), 20.95 (C-23), 20.81 (C-24), 20.33 (C-25), 20.27 (C-26), 13.15 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu} =$ 1,745, 1,457, 1,461, 1,383, 1,242, 1,037 cm^{-1}; MS (70 eV): $m/z = 542$ (M^+, 2), 482 (21), 471 (17), 422 (53), 409 (100), 244 (32), 203 (13), 189 (20); $[\alpha]_D^{20} =$ -74×10^{-1} deg cm^2 g^{-1} ($c = 0.16$, $CHCl_3$).

19β,28-Epoxy-4,5-seco-3,4,23,24-tetranor-18α-oleanan-2,5β-diol (**29**, $C_{26}H_{44}O_3$)

$LiAlH_4$ (20 mg, 0.53 mmol) was added to 100 mg lactone **14** (0.24 mmol) dissolved in 10 cm^3 THF. The mixture was refluxed for 3 h. It was poured into 10% aqueous HCl, and the product was extracted twice with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and the solvents were evaporated. HPLC (EtOAc/hexane = 1/4, $R_f = 0.19$) and crystallization from MeOH afforded 85 mg (88%) **29**. M.p.: 217–220 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.77$ (d, $J = 7.6$ Hz, 1H, H-28*pro-S*), 3.66 (t, $J = 5.5$ Hz, 2H, 2 × H-2), 3.55 (s, 1H, H-19α), 3.46 (d, $J = 7.8$ Hz, 1H, H-28*pro-R*), 3.37 (t, $J = 8.1$ Hz, 1H, H-5α), 1.80 (dt, $J = 15.0$ Hz, $J = 4.6$ Hz, 1H, H-1a), 1.02, 0.93, 0.90, 0.85, 0.80 (all s, 3H, 5 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 87.94$ (C-19), 76.56 (C-5), 71.16 (C-28), 58.22 (C-2), 46.55 (C-18), 43.66 (C-9), 43.48 (C-1), 41.37 (C-17), 41.19 (C-14), 40.89 (C-8), 40.13 (C-10), 36.52 (C-16), 36.13 (C-20), 34.18 (C-13), 32.54 (C-21), 30.37 (C-7), 28.62 (C-30), 26.67 (C-6), 26.44 (C-12), 26.33 (C-15), 26.07 (C-22), 24.39 (C-29), 21.86 (C-11), 15.65 (C-26), 13.37 (C-25), 13.06 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu} = 3{,}613$, 1,451, 1,385, 1,032, 1,008 cm^{-1}; MS (70 eV): $m/z = 404$ (M^+, 15), 386 (23), 368 (100), 356 (16), 333 (31), 297 (9), 217 (13), 203 (32), 191 (29); $[\alpha]_D^{20} = +23 \times 10^{-1}$ deg cm^2 g^{-1} ($c = 0.30$, $CHCl_3$).

19β,28-Epoxy-4,5-seco-3,4,23,24-tetranor-18α-oleanan-2,5β-diol, diacetate (**30**, $C_{30}H_{48}O_5$)

According to the general procedure for acetylation, from 30 mg diol **29** (0.07 mmol) diacetate **30** was obtained as white powder (28 mg, 77%). M.p.: 176–177 °C (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.67$ (dd, $J = 11.1$ Hz, $J = 4.7$ Hz, 1H, H-5α), 4.08 (t, 2H, $J = 7.3$ Hz, 2H, 2 × H-2), 3.77 (d, $J = 7.5$ Hz, 1H, H-28*pro-S*), 3.53 (s, 1H, H-19α), 3.45 (d, $J = 7.8$ Hz, 1H, H-28*pro-R*), 2.05, 2.02 (both s, 3H, 2 × OAc), 1.03 (s, 3H, CH_3), 0.94 (s, 6H, 2 × CH_3), 0.92, 0.80 (both s, 3H, 2 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.09$, 170.76 (2 × CH_3CO), 87.91 (C-19), 71.23 (C-28), 71.08 (C-5), 60.67

(C-2), 46.68 (C-18), 41.48 (C-17), 41.14 (C-9), 41.12 (C-14), 39.98 (C-8), 39.92 (C-10), 36.68 (C-1), 36.57 (C-16), 36.25 (C-20), 34.19 (C-13), 32.65 (C-21), 30.06 (C-7), 28.77 (C-30), 26.48 (C-15), 26.15 (C-12, C-22), 24.50 (C-29), 23.71 (C-6), 21.59 (C-11), 21.31, 21.05 (2 × CH_3CO), 17.69 (C-25), 15.65 (C-26), 13.36 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu}$ = 1,730, 1,371, 1,252, 1,030 cm^{-1}; MS (70 eV): *m/z* = 488 (M^+, 7), 473 (3), 428 (100), 385 (43), 356 (22), 332 (39), 297 (4), 217 (20), 203 (30), 191 (38); $[\alpha]_D^{20}$ = +25 × 10^{-1} deg cm^2 g^{-1} (*c* = 0.43, $CHCl_3$).

19β,28-Epoxy-5β-hydroxy-4,5-seco-3,4,23,24-tetranor-18α-oleanan-2-oic acid (**31**, $C_{26}H_{42}O_4$)

$NaBH_4$ (40 mg, 1.07 mmol) was added to 100 mg lactone **14** (0.24 mmol) dissolved in 5 cm^3 THF and 2 cm^3 MeOH. The mixture was stirred for 12 h. It was poured into 5% aqueous solution of citric acid, and the product was extracted twice with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and the solvents were evaporated. Column chromatography (toluene/Et_2O = 5/1, R_f = 0.13) and lyophilization from benzene afforded 86 mg (86%) **31**. M.p.: 140–142 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.78 (d, *J* = 7.6 Hz, 1H, H-28*pro-S*), 3.72 (m, 1H, H-5α), 3.55 (s, 1H, H-19α), 3.46 (d, *J* = 7.8 Hz, 1H, H-28*pro-R*), 2.61 (d, *J* = 13.7 Hz, 1H, H-1a), 2.29 (d, *J* = 13.7 Hz, 1H, H-1b), 1.14, 0.99, 0.96, 0.82, 0.76 (all s, 3H, 5 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.73 (C-2), 87.91 (C-19), 75.46 (C-5), 71.19 (C-28), 46.67 (C-18), 43.83 (C-1), 43.02 (C-10), 42.13 (C-9), 41.46 (C-17), 41.09 (C-14), 40.31 (C-8), 36.68 (C-16), 36.24 (C-20), 34.17 (C-13), 32.65 (C-21), 30.26 (C-7), 28.76 (C-30), 27.09 (C-6), 26.45 (C-15), 26.24 (C-22), 26.18 (C-12), 24.53 (C-29), 22.16 (C-11), 15.56 (C-25), 15.41 (C-26), 13.28 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu}$ = 3,608, 1,701, 1,453, 1,372, 1,239, 1,032 cm^{-1}; MS (70 eV): *m/z* = 418 (M^+, 8), 400 (62), 382 (85), 358 (19), 329 (100), 203 (28), 189 (38); $[\alpha]_D^{20}$ = +57 × 10^{-1} deg cm^2 g^{-1} (*c* = 0.23, $CHCl_3$).

19β,28-Epoxy-5β-hydroxy-4,5-seco-3,4,23,24-tetranor-18α-oleanan-2,5β-lactone (**32**, $C_{26}H_{40}O_3$)

Using the acetylation procedure from 50 mg acid **31** (0.12 mmol) lactone **32** was obtained as white powder (44 mg, 92%). M.p.: 224–226 °C (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.79 (dd, *J* = 12.7 Hz, *J* = 3.4 Hz, 1H, H-5α), 3.76 (dd, *J* = 7.9 Hz, *J* = 1.2 Hz, 1H, H-28*pro-S*), 3.52 (s, 1H, H-19α), 3.47 (d, *J* = 7.9 Hz, 1H, H-28*pro-R*), 2.29 (d, *J* = 15.3 Hz, 1H, H-1a), 2.15 (dd, *J* = 15.4 Hz, *J* = 1.2 Hz, 1H, H-1b), 1.06 (s, 3H, CH_3), 1.00 (d, *J* = 1.2 Hz, 3H, CH_3), 0.97, 0.94, 0.80 (all s, 3H, 3 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.29 (C-2), 88.82 (C-5), 87.92 (C-19), 71.12 (C-28), 47.81 (C-1), 46.71 (C-18), 45.15 (C-9), 44.77 (C-10), 41.45 (C-17), 41.12 (C-8), 41.02 (C-14), 36.69 (C-16), 36.22 (C-20), 33.86 (C-13), 32.57 (C-21), 31.21 (C-7), 28.73 (C-30), 26.27 (C-22), 26.09 (C-15), 25.67 (C-12), 24.46 (C-29), 23.60 (C-6), 21.09 (C-11), 16.59 (C-25), 15.11 (C-26), 13.41 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu}$ = 1,778, 1,453, 1,265, 1,192, 1,034 cm^{-1}; MS (70 eV): *m/z* = 400 (M^+, 53), 382 (69), 371 (11), 356 (6), 340 (8), 329 (100), 313 (18), 220 (5), 205 (21), 191 (25); $[\alpha]_D^{20}$ = +68 × 10^{-1} deg cm^2 g^{-1} (*c* = 0.55, $CHCl_3$).

Reduction of ketoester ***15***

$NaBH_4$ (60 mg, 1.61 mmol) was added to 150 mg ketoester **15** (0.35 mmol) dissolved in 10 cm^3 THF and 5 cm^3 MeOH. The mixture was stirred for 8 h. It was poured into 10% aqueous HCl, and the product was extracted twice with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and the solvents were evaporated. Separation of products with HPLC (EtOAc/hexane = 1/3, R_f = 0.22, resp. R_f = 0.27) afforded products **33** (97 mg, 64%) and **35** (29 mg, 21%).

Methyl 19β,28-epoxy-5β-hydroxy-4,5-seco-3,4,23,24-tetranor-18α-oleanan-2-oate (**33**, $C_{27}H_{44}O_4$)

The title compound was obtained after crystallization from MeOH as white powder. M.p.: 171–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.77 (dd, *J* = 7.8 Hz, *J* = 1.4 Hz, 1H, H-28*pro-S*), 3.68 (s, 3H, OCH_3), 3.67 (dd, *J* = 10.2 Hz, *J* = 5.8 Hz, 1H, H-5α), 3.54 (s, 1H, H-19α), 3.45 (d, *J* = 7.8 Hz, 1H, H-28*pro-R*), 2.58 (d, *J* = 13.6 Hz, 1H, H-1a), 2.26 (d, *J* = 13.6 Hz, 1H, H-1b), 1.98 (bs, 1H, 5β-OH), 1.00, 0.94, 0.91, 0.88, 0.80 (all s, 3H, 5 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.42 (C-2), 87.89 (C-19), 75.03 (C-5), 71.25 (C-28), 51.48 (CH_3O), 46.68 (C-18), 43.68 (C-1), 43.11 (C-10), 42.05 (C-9), 41.48 (C-17), 41.04 (C-14), 40.28 (C-8), 36.70 (C-16), 36.27 (C-21), 34.22 (C-13), 32.67 (C-21), 30.28 (C-7), 28.79 (C-30), 27.12 (C-6), 26.45 (C-15), 26.32 (C-12, C-22), 24.51 (C-29), 22.19 (C-11), 15.53 (C-25, C-26), 13.26 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu}$ = 3,615, 1,722, 1,452, 1,237, 1,032, 1,009 cm^{-1}; MS (70 eV): *m/z* = 432 (M^+, 4), 414 (3), 400 (4), 382 (100), 361 (5), 358 (39), 313 (9), 269 (10), 239 (8), 215 (19), 201 (20), 189 (15); $[\alpha]_D^{20}$ = +66 × 10^{-1} deg cm^2 g^{-1} (*c* = 0.58, $CHCl_3$).

19β,28-Epoxy-5α-hydroxy-4,5-seco-3,4,23,24-tetranor-18α-oleanan-2,5α-lactone (**35**, $C_{26}H_{40}O_3$)

The title compound was obtained after crystallization from MeOH as white powder. M.p.: 190–192 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.26 (t, *J* = 2.9 Hz, 1H, H-5α), 3.77 (dd, *J* = 7.8 Hz, *J* = 1.7 Hz, 1H, H-28*pro-S*), 3.54 (s, 1H, H-19α), 3.46 (d, *J* = 7.9 Hz, 1H, H-28*pro-R*), 2.54 (d, *J* = 16.9 Hz, 1H, H-1a), 2.21 (dd, *J* = 16.9 Hz, *J* = 0.9 Hz, 1H, H-1b), 2.02 (m, 1H), 1.84 (m, 1H), 1.14, 1.01, 0.94, 0.89, 0.80 (all s, 3H, 5 × CH_3) ppm; ^{13}C NMR

(100 MHz, $CDCl_3$): δ = 176.89 (C-2), 87.89 (C-19), 85.12 (C-5), 71.25 (C-28), 47.17 (C-1), 46.65 (C-18), 41.72 (C-10), 41.44 (C-17), 40.58 (C-14), 39.66 (C-9), 39.28 (C-8), 36.61 (C-16), 36.28 (C-20), 34.75 (C-13), 32.64 (C-21), 28.77 (C-30), 26.46 (C-15), 26.26 (C-12), 26.07 (C-22), 24.53 (C-29), 24.39 (C-7), 23.15 (C-11), 21.57 (C-6), 20.16 (C-25), 14.33 (C-26), 13.35 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu}$ = 1,761, 1,453, 1,269, 1,033, 908 cm^{-1}; MS (70 eV): *m/z* = 400 (M^+, 98), 382 (12), 370 (29), 329 (100), 215 (39), 201 (34), 191 (17); $[\alpha]_D^{20} = +15 \times 10^{-1}$ deg cm^2 g^{-1} (*c* = 0.21, $CHCl_3$).

Methyl 19β,28-epoxy-5β-acetoxy-4,5-seco-3,4,23,24-tetranor-18α-oleanan-2-oate (**34**, $C_{29}H_{46}O_5$)

According to the general procedure for acetylation, from 50 mg diol **33** (0.12 mmol) diacetate **34** was obtained as white powder (47 mg, 85%). M.p.: 127–129 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.80 (dd, *J* = 10.8 Hz, *J* = 4.5 Hz, 1H, H-5α), 3.77 (d, *J* = 7.8 Hz, 1H, H-28*pro-S*), 3.65 (s, 3H, OCH_3), 3.54 (s, 1H, H-19α), 3.45 (d, *J* = 7.8 Hz, 1H, H-28*pro-R*), 2.29 (s, 2H, 2 × H-1), 2.04 (s, 3H, OAc), 1.02, 0.99, 0.93, 0.91, 0.79 (all s, 3H, 5 × CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.00 (C-2), 170.47 (CH_3CO), 87.89 (C-19), 77.29 (C-5), 71.24 (C-28), 51.32 (CH_3O), 46.65 (C-18), 42.86 (C-1), 41.52 (C-9), 41.46 (C-17), 41.43 (C-10), 41.11 (C-14), 40.13 (C-8), 36.66 (C-16), 36.24 (C-20), 34.21 (C-13), 32.64 (C-21), 29.87 (C-7), 28.76 (C-30), 26.46 (C-22), 26.15 (C-12, C-15), 24.50 (C-29), 23.41 (C-6), 21.94 (C-11), 21.27 (CH_3CO), 17.16 (C-25), 15.54 (C-26), 13.31 (C-27) ppm; IR ($CHCl_3$): $\bar{\nu}$ = 1,736, 1,448, 1,360, 1,241, 1,032, 1,031 cm^{-1}; MS (70 eV): *m/z* = 474 (M^+, 6), 403 (19), 382 (100), 341 (10), 313 (10), 203 (11), 195 (13), 189 (9); $[\alpha]_D^{20} = +40 \times 10^{-1}$ deg cm^2 g^{-1} (*c* = 0.24, $CHCl_3$).

MTT cytotoxicity assay

Cell lines CEM, K562, and SK-MEL1 were purchased from the American Tissue Culture Collection (ATTC). Cell suspensions were prepared and diluted according to the particular cell type and the expected target cell density (2,500–30,000 cells/well based on cell growth characteristics). Cells were added by pipette (80 mm^3) into 96-well microtiter plates. Inoculates were allowed a pre-incubation period of 24 h at 37 °C and 5% CO_2 for stabilization. Fourfold dilutions, in 20 mm^3 aliquots, of the intended test concentration were added at time zero to the microtiter plate wells. All test compound (dissolved in 10 mm^3 of 10% DMSO) concentrations were examined in duplicate. Incubation of the cells with the test compounds lasted for 72 h at 37 °C, in a 5% CO_2 atmosphere at 100% humidity. At the end of the incubation period, aliquots (10 mm^3) of the MTT stock solution were pipetted into each well and incubated for a further 1–4 h. After this incubation period, the formazan produced was dissolved by the addition of 100 mm^3/well of 10% aq. SDS (pH = 5.5), followed by a further incubation at 37 °C overnight. The optical density (OD) was measured at 540 nm with a Labsystem iEMS Reader MF. Inhibition of tumor growth/survival (*IC*) was calculated using the following equation: *IC* = (ODdrug-exposed well/mean ODcontrol wells) × 100%. The IC_{50} value, the drug concentration lethal to 50% of the tumor cells, was calculated from appropriate dose-response curves.

Acknowledgments This study was supported by A/CZ0046/1/0022 of FM EEA/Norska, the Czech Science Foundation (203/05/P025 and 305/09/1216), and GAAS KAN200200651. Indirect costs were paid from the Czech Ministry of Education (MSM 6198959216). We are grateful to Stanislav Hilgard for measurement of IR spectra. We also thank Bohunka Sperlichova for measurement of optical rotation.

References

1. Dzubak P, Hajduch M, Vydra V, Hustova A, Kvasnica M, Biedermann D, Markova L, Urban M, Sarek J (2006) Nat Prod Rep 23:394
2. Sakai K, Fukuda Y, Matsunaga S, Tanaka R, Yamari T (2004) J Nat Prod 67:1088
3. Sun H-X, Ye Y-P, Pan Y-J (2004) J Ethnopharmacol 90:2
4. Akihisa T, Tabata K, Banno N, Tokuda H, Nishihara R, Nakamura Y, Kimura Y, Yasukawa K, Suzuki T (2006) Biol Pharm Bull 29:1976
5. Tu J, Sun H-X, Ye Y-P (2006) Chem Biodivers 3:69
6. Suh N, Wang Y, Honda T, Gribble GW, Dmitrovsky E, Hickey WF, Maue RA, Place AE, Porter DM, Spinella MJ, Williams CR, Wu G, Dannenberg AJ, Flanders KC, Letterio JJ, Mangelsdorf DJ, Nathan CF, Nguyen L, Porter WW, Ren RF, Roberts AB, Roche NS, Subbaramaiah K, Sporn MB (1999) Cancer Res 59:336
7. Fourie TG, Snyckers FO (1989) J Nat Prod 52:1129
8. Pinducciu G, Serra C, Cagetti MG, Cotti M, Deidda D, Pinza M, Pompei R (1995) Med Microbiol Lett 4:83
9. Adnyana IK, Tezuka Y, Banskota AH, Tran KQ, Kadota S (2000) Biol Pharm Bull 23:1328
10. Platanov VG, Zorina AD, Gordon MA, Chizhov NP, Balykina LV, Mikhailov YD, Ivanen DR, Kvi TK, Shavva AG (1995) Khim Farm Zh 29:42
11. Honda T, Janosik T, Honda Y, Han J, Liby KT, Williams CR, Couch RD, Anderson AC, Sporn MB, Gribble GW (2004) J Med Chem 47:4923
12. Kim YK, Yoon SK, Ryu SY (2000) Planta Med 66:485
13. Ringbom T, Segura L, Noreen Y, Perera P, Bohlin L (1998) J Nat Prod 61:1212
14. Liu Y, Kreppel H, Liu J, Choudhuri S, Klaassen CD (1993) J Pharmacol Exp Ther 266:400
15. Salvi M, Fiore C, Armanini D, Toninello A (2003) Biochem Pharmacol 66:2375
16. Farina C, Pinza M, Pifferi G (1998) Farmaco 53:22
17. Sundharsan PT, Mythili Y, Selvakumar E, Varalakshmi P (2006) Mol Cell Biochem 282:23
18. Streffer JR, Bitzer M, Schabet M, Dichgans J, Keller M (2001) Neurology 56:1219
19. Sailer ER, Subramanian LR, Rall B, Hoernlein RF, Ammon HPT, Safayhi H (1996) Br J Pharmacol 117:615
20. Giner-Larza EM, Manez S, Recio MC, Giner RM, Prieto JM, Cerda-Nicolas M, Rios JL (2001) Eur J Pharmacol 28:137

21. Sarek J, Klinot J, Dzubak P, Klinotova E, Noskova V, Krecek V, Korinkova G, Thomson JO, Janostakova A, Wang S, Parsons S, Fischer PM, Zhelev NZ, Hajduch M (2003) J Med Chem 46:5402
22. Kvasnica M, Sarek J, Klinotova E, Dzubak P, Hajduch M (2005) Bioorg Med Chem 13:3447
23. Sarek J, Kvasnica M, Urban M, Klinot J, Hajduch M (2005) Bioorg Med Chem Lett 15:4196
24. Lacaille-Dubois MA, Mitaine-Offer AC (2005) Phytochem Rev 4:2
25. Yang J-H, Wang Y-S, Huang R, Luo S-D, Zhang H-B, Li L (2006) Helv Chim Acta 89:2830
26. Efferth T, Kahl S, Paulus K, Adams M, Rauh R, Boechzelt H, Hao X, Kaina B, Bauer R (2008) Mol Cancer Ther 7:152
27. Iwu MM, Anyanwu BN (1982) Fitoterapia 53:25
28. Kvasnica M, Tislerova I, Sarek J, Sejbal J, Cisarova I (2005) Collect Czech Chem Commun 70:1447