Received Date: 18-Nov-2015

Accepted Date: 29-Feb-2016

Article Type: Full Paper

Cytotoxic and Apoptosis-inducing Activities of Taraxastane-type Triterpenoid Derivatives in Human Cancer Cell Lines

by **Motohiko Ukiya***[a]), **Chika Ohkubo**[a]), **Masahiro Kurita**[b]), **Makoto Fukatsu**[a]), **Takashi Suzuki**[b]), and **Toshihiro Akihisa**[a])[c])

[a]) College of Science and Technology, Nihon University, 1-8-14 Kanda Surugadai, Chiyoda-ku, Tokyo 101-8308, Japan (phone: +81-3-32590816; fax: +81-3-32937572; e-mail: ukiya.motohiko@nihon-u.ac.jp)

[b]) College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi-shi, Chiba 274-8555, Japan

[c]) Akihisa Medical Clinic, 1086-3 Kamo, Sanda-shi, Hyogo 669-1311, Japan

Twenty-eight taraxastane-type triterpenoid derivatives **4** – **31** were prepared from the naturally occurring triterpenoids faradiol (**1**) and heliantriol C (**3**). The cytotoxic activities of these compounds and arnidiol (**2**) were evaluated in leukemia (HL60), lung (A549), duodenal (AZ521), and breast (SK-BR-3) cancer cell lines. 21-Oxoarnidiol (**18**) and faradiol 3,16-di-*O*-L-alaninate (**31**) exhibited potent cytotoxicity, with 50% inhibitory concentrations of 0.5 – 2.7 μM. In particular, flow-cytometric analysis indicated that compound **31** induced typical apoptotic cell death in HL60 cells. These results suggested that taraxastane-type triterpenoid derivatives might provide useful antitumor agents with apoptosis-inducing activity.

Keywords: Taraxastane triterpenoid derivative, Cytotoxic activity, Apoptosis-inducing activity.

Introduction. - Natural products can provide important sources of new anticancer drugs [1]. In recent decades, many natural products have been isolated from natural resources and evaluated for their cytotoxic activities against human cancer cell lines. Among these, triterpenoids provide a unique group of agents with potential antitumor activities [2][3].

We have previously studied the bioactivities of triterpenoids derived from Compositae flowers and reported their anti-inflammatory [4 – 6], anti-tubercular [7], chemopreventive [6][8][9], and cytotoxic activities [6][8]. Among the compounds reported in these previous papers, the taraxastane-type triterpenoids, faradiol (**1**; taraxast-20-ene-3β,16β-diol), arnidiol

(**2**; taraxast-20(30)-ene-3β,16β-diol), and heliantriol C (**3**; taraxast-20-ene-3β,16β,22α-diol) exhibited potent antitumor promoting effects [10] and cytotoxic effects in a National Cancer Institute screen of 60 human cancer cell lines (NCI 60) [8].

The taraxastane skeleton is similar to the ursane skeleton, except that it shows ■inversed configurations?■ at C(18), C(19), and C(20). The ursane skeleton is known to be present in bioactive natural compounds because ursolic acid, an ursane-type triterpenoid, has been reported to show a broad range of bioactivities, including anticancer effects [11][12].

On the other hand, the ring *D*/*E* junction of taraxastane (the *trans*-form) differs from that of ursane (the *cis*-form), and taraxastane-type triterpenoids were thus expected to show unique bioactivities. We previously reported that taraxastane-type triterpenoids showed more potent cytotoxicity than ursane-type triterpenoids in the NCI 60 assay [8]. Although the structure–activity relationship (SAR) of synthetic taraxastane-type triterpenoid derivatives has been studied with respect to their anti-inflammatory effects [13], no systematic SAR studies of the anticancer activities of these compounds have been reported.

We therefore prepared 28 derivatives (compounds **4** – **31**) from the naturally occurring compounds **1** and **3**. Here, we describe the preparation of these taraxastane-type triterpenoid derivatives and evaluation of their cytotoxic activities against four human cancer cell lines.

Results and Discussion. - The present study focused on the *A*, *D*, and *E* rings of the taraxastane derivatives because these were considered to be important for the anti-inflammatory effects of these compounds [13]. The ring *E* structure is particularly characteristic of these taraxastane-type triterpenoids. Furthermore, acylation with an amino acid was reported to provide a useful means of improving the solubility of betulinic acid without reducing its cytotoxicity; this is a lupane-type triterpenoid that is a promising anticancer drug candidate [14]. On the basis of this information, we prepared taraxastane-type derivatives from **1** and **3** by *i*) modification of ring *E* (**4** – **28**) and *ii*) acylation of the OH groups at C(3) and C(16) in rings *A* and *D* (**29** – **31**).

Modification of Ring E. Starting from faradiol (**1**), twenty-five compounds (**4** – **28**) were synthesized as shown in *Schemes 1 – 5*. Firstly, the double bond in ring *E* of faradiol (**1**) was oxidized to prepare epoxide; this was expected to increase polarity and the ability to act as a H-bond acceptor, prior to further derivatization. Faradiol (**1**) was acetylated with Ac_2O to protect the OH groups, giving faradiol 3,16-di-*O*-acetate (**4**) in 89% yield. Epoxidation of the C(20)=C(21) bond of acetate **4** was achieved by treatment with *m*-chloroperbenzoic acid (*m*-CPBA) to give faradiol α-epoxide 3,16-di-*O*-acetate (**5**) in 78% yield. The *O*-acetate **5** was hydrolyzed by treatment with KOH in MeOH to give the corresponding alcohol, faradiol α-epoxide (**6**) in 89% yield (*Scheme 1*).

For preparing the ketone and epimeric drivatives of faradiol (**1**), compound **1** was oxidized with $K_2Cr_2O_7$ to afford three compounds that were oxidized at C(3) and/or C(16); **7** – **9** were obtained in 18%, 5%, and 65% yield, respectively. Two C=O groups at C(3) and C(16) of faradione (**9**) were reduced with $NaBH_4$ to give 16-epifaradiol (**10**) in 25% yield. In addition, faradione (**9**) was oxidized with *m*-CPBA to give the epoxide, faradione α-epoxide (**11**) in 48% yield (*Scheme 2*).

In order to introduce a conjugated enone structure as the *Michael* reaction acceptor in ring *E*, which was expected to increase cytotoxicity, compound **5** was treated with HCl in $CHCl_3$ to yield arnitriol A 3,16-di-*O*-acetate (**12**) and heliantriol F 3,16-di-*O*-acetate (**15**) in 41% and 28% yield, respectively. A previous study [15] showed that the HCl treatment of the epoxide at C(20) and C(21) of taraxastane-type triterpenoids produced a 20(30)-en-21-ol structure. However, we found that the treatment of epoxide **5** with HCl afforded both the expected compound (**12**) and an unexpected compound (**15**), which had a 20-en-30-ol structure. Compound **12** was acetylated or hydrolyzed to afford the corresponding peracetylated compound (**13**) and alcohol (**14**) in 80% and 77% yield, respectively. Compound **15** was acetylated or hydrolyzed to afford the corresponding peracetylated compound (**16**) and alcohol (**17**) in 80% and 85% yield, respectively. Selective oxidation of the OH group at C(21) of arnitriol A (**14**) with MnO_2 afforded 21-oxoarnidiol (**18**). Arnitriol 3,16-di-*O*-acetate (**12**) was oxidized with $K_2Cr_2O_7$ to afford 30-oxofaradiol 3,16-di-*O*-acetate

(**19**), which has an CHO group at C(20). A similar reaction yielding an α,β-unsaturated aldehyde from unsaturated alcohol was reported by *Gollnick* and *Schade* [16] for the preparation of 7,7-dimethylbicyclo[4.1.10]hept-3-ene-3-carboaldehyde. Compound **19** was hydrolyzed to give the corresponding alcohol **20** in 79% yield (*Scheme 3*).

In order to cleave the epoxy ring at C(20) and C(21) of compound **5**, thus increasing its polarity, the compound was treated with $HClO_4$, followed by acetylation (owing to partial hydrolysis of the products) to afford the unexpected ketones, **21** and **23** in 39% and 28% yield, respectively. Their structures were elucidated by spectral analyses, including HR-ESI-MS, IR, ^{1}H- and ^{13}C-NMR, DEPT, HMQC, HMBC, ^{1}H,^{1}H-COSY, and NOESY spectra. Briefly, compounds **21** and **23** were determined as $C_{34}H_{54}O_5$ using HR-ESI-MS (*m/z* 565.3874 and 565.3873 ($[M + Na]^+$), resp.) and ^{13}C-DEPT-NMR. The close similarity between their ^{1}H-NMR spectra and that of the parent compound **5**, with the exception of ring *E*, indicated that they had 3β,16β-diacetoxytaraxastane structures. HMBC analysis of **21** identified a correlation between the Me *singlet* at δ(H) 1.12 (Me(30)) and the ketone C-atom signal at δ(C) 211.2, indicating that this compound had a ketone group adjacent to C(20) bearing Me(30). Thus, the structure of **21** was determined to be 3β,16β-diacetoxytaraxastane-21-one. This conclusion was supported by comprehensive spectral analysis. The structure of **23** was also determined to be the epimer of **21** at C(20) by NOESY experiments. In the NOESY analysis of **21**, a correlation was observed between δ(H) 1.12 (Me(30)) and δ(H)

0.73 (Me(28)). However, no correlation between δ(H) 0.97 (Me(30)) and δ(H) 1.21 (MeC(28)) was observed in the NOESY analysis of **23**. These results indicated that the MeC(30) of **21** was β-oriented, while that of **23** was α-oriented. Interestingly, alkaline hydrolysis of **21** afforded a mixture of **22** and **24**. The production of **24** was thought to be due to a base-catalyzed enolization during hydrolysis (*Scheme 4*).

Heliantriol C (**3**), which was previously isolated from a chrysanthemum flower extract [4], was selected to probe the effect of the oxygen function at C(22). Compound **3** was acetylated to give heliantriol C 3,16,22-tri-*O*-acetate (**25**) in 75% yield. To prepare conjugated enone compound as a *Michael* reaction acceptor, oxidation of the OH group at the allylic position C(22) of compound **3** was achieved using MnO_2 to afford 22-oxofaradiol (**26**) in 30% yield. The C(20)=C(21) bond of compound **25** was oxidized by *m*-CPBA to afford heliantriol C α-epoxide 3,16,22-tri-*O*-acetate (**27**) in 66% yield, and compound **27** was hydrolyzed to the corresponding alcohol (**28**) in 51% yield (*Scheme 5*).

Acylation of the OH Groups at C(3) and C(16) in Rings A *and* D. In order to evaluate the influence of the acyl group, introduced at the OH groups at C(3) and C(16), on cytotoxicity, faradiol (**1**) was acylated with succinic anhydride and dimethylsuccinic anhydride to afford the corresponding compounds, **29** and **30**, in yields of 62% and 45%, respectively. In addition, acylation with *tert*-butoxycarbonyl (Boc)-protected L-alanine was

carried out, followed by deprotection of the Boc group using HCl to afford faradiol di-*O*-L-alaninate (**31**) in 38% yield (*Scheme 6*).

Cytotoxic Activity. The cytotoxic activities of compounds **1** – **31** against leukemia (HL60), lung (A549), duodenal (AZ521), and breast (SK-BR-3) cancer cell lines were determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-3*H*-tetrazolium bromide (MTT) assay. Cisplatin, which is one of the most effective and widely used chemotherapeutic drugs employed in the treatment of human cancers, was selected as a positive control. The growth inhibition in the presence of each compound was expressed as the 50% inhibitory concentration (IC_{50}) value, and these results are compiled in the *Table*.

Compounds with IC_{50} values that were lower than that of cisplatin were considered active. In particular, **18** and **31** showed potent cytotoxicities, with IC_{50} values of 0.5 – 2.7 μM against the cancer cell lines used in this study. On the other hand, **26** showed potent selective cytotoxicity against the A549 lung cancer cell line (IC_{50} 1.0 μM).

Comparison of compounds **2** and **18** indicated that the presence of a ketone group beside the C(20)=C(30) bond substantially enhanced cytotoxicity against all four cancer cell lines. This was the expected result for the conjugated enone structure. In addition, the presence of a ketone group neighboring the C(20)=C(21) bond considerably enhanced selective cytotoxicity against the A549 lung cancer cell line, as shown by compounds **1** and

26. The structural difference between **18** and **26** related to the orientation of the double bonds that interacted with their ketone groups. Compound **18** has an exocyclic double bond, whereas **26** has an endocyclic double bond. The selective cytotoxicity of **26** against A549 lung cancer cells may therefore be attributable to the position of the double bond. In addition, compounds **22** and **24**, which have the same structures as hydrogenated structure of compound **18** C(20)=C(30) bond, were much less cytotoxic than **18**, as expected. Furthermore, conjugated aldehyde **20** was more cytotoxic than the corresponding alcohol **17**. These results indicated that the conjugated enone structure was essential for cytotoxicity.

Faradiol (**1**), faradiol α-epoxide (**6**), arnitriol A (**14**), heliantriol F (**17**), 30-oxofaradiol (**20**), 3β,16β-dihydroxytaraxastan-21-one (**22**), and its 20-epimer (**24**) tended to show smaller IC_{50} values than their acetyl derivatives against one or more cancer cell lines. These compounds have taraxastane-type skeletons bearing OH groups at C(3) and C(6). In contrast, the cytotoxicities of heliantriol C (**3**) and heliantriol C α-epoxide (**28**), which have taraxastane-type skeletons bearing OH groups at C(3), C(16), and C(22), tended to show less cytotoxicity than their acetyl derivatives, **25** and **27**. These results suggested that the OH groups at C(3) and C(16) and the AcO group at C(22) played important roles, enhancing the cytotoxicity of these taraxastane-type triterpenoids against cancer cell lines.

The dihydroxy compounds, faradiol (**1**) and arnidiol (**2**), showed more potent cytotoxicities than their corresponding trihydroxy forms, heliantriol C (**3**) and arnitriol A (**14**). In addition, arnidiol (**2**) exhibited more potent cytotoxicity than faradiol (**1**). These results

were consistent with those of a previous study where the dihdyroxytaraxastane-type triterpenoids, faradiol (**1**) and arnidiol (**2**), showed marked cytotoxicities in the NCI 60 screen [8]. The number of OH groups and the position of the double bond were identified as important determinants of cytotoxicity against the human cancer cell lines used in the present study.

Although acetylation of faradiol (**1**) decreased cytotoxicity, faradiol 3,16-di-*O*-succinate (**29**), 22-dimethylsuccinate (**30**), and di-*O*-L-alaninate (**31**) showed marked cytotoxicities against human cancer cell lines. Since compounds **29**, **30**, and **31** have highly polar moieties at the ends of their acyl groups (COOH and NH_2 groups), acylation of taraxastane-type triterpenoids with a highly polar acyl group substantially enhanced their cytotoxicity against human cancer cell lines.

Apoptosis-Inducing Activity of Compounds **18** *and* **31**. Compounds **18** and **31** exhibited potent cytotoxic activities against HL60 (IC_{50} 0.3 and 0.5 μM), and these were evaluated for their effects on early apoptosis in this cell line. HL60 cells were incubated with these test compounds for 24 and 48 h, and then the cells were analyzed using flow cytometry with annexin V-propidium iodide (PI) double staining. Exposure of the membrane phospholipid, phosphatidylserine, to the external cellular environment is one of the earliest markers of apoptotic cell death [17]. Annexin V is a calcium-dependent phospholipid-binding protein with a high affinity for cell surface phosphatidylserine. PI does not enter whole cells with intact membranes and was therefore used to differentiate between early apoptotic

(annexin V-positive, PI-negative), late apoptotic (annexin V-positive, PI-positive), or necrotic (annexin V-negative, PI-positive) cell death. As shown in the *Figure*, the proportion of early apoptotic cells (lower right) was increased from 1.7% to 5.7% after treatment of HL60 with 5 μM **18** for 24 h and reached 13.6% at 48 h; the proportion of late apoptotic cells (upper right) was also increased from 1.0% to 5.4% after 24 h and reached 41.0% at 48 h. However, the proportion of necrotic cells (upper left) had also increased after 24 h (from 0.8% to 9.9%) and 48 h (18.0%). This observation of fewer early apoptotic cells, as compared to the number of necrotic cells, indicated that this α,β-unsaturated ketone (**18**) mainly induced HL60 cell death *via* necrotic pathways. In contrast, treatment of HL60 with 5 μM **31** increased the proportion of early apoptotic cells from 3.1% to 19.4% at 24 h and to 39.4% at 48 h; the proportion of late apoptotic cells was also increased after 24 h (from 0.9% to 19.7%) and 48 h (22.2%), while that of necrotic cells was 9.5% after 24 h and 6.5% after 48 h. This observation of more early apoptotic cells than necrotic cells indicated that compound **31**, a conjugate of a taraxastane-type triterpenoid and an amino acid, mainly induced HL60 cell death *via* the apoptotic pathway.

Conclusions. – In summary, 31 taraxastane-type triterpenoids, comprising 28 derivatives (**4** – **31**) prepared from faradiol (**1**) and heliantriol C (**3**), along with arnidiol (**2**), were evaluated for their cytotoxic activities against leukemia (HL60), lung (A549), duodenal (AZ521), and breast (SK-BR-3) cancer cell lines. 21-Oxoarnidiol (**18**) and faradiol di-*O*-L-

alaninate (**31**) exhibited potent cytotoxicity against the cancer cell lines used in this study and **31** was shown to induce apoptotic cell death in HL60 cells. These results suggested that the acylation of the OH groups at C(3) and C(16) of taraxastane-type triterpenoids with amino acids might provide a useful enhancement of their cytotoxic and pro-apoptotic activities. Further studies are underway in our laboratory to clarify the SAR of triterpenoid-amino acid conjugates.

We thank Mr. *Akira Takekawa* for his technical assistance and Prof. *Atsuyoshi Nishina* for his helpful advice. This work was partly supported by *JSPS KAKENHI* Grant Number 15K08004. We would like to thank *Editage* (www. editage.jp) for English language editing.

Experimental Part

General

Thin-layer chromatography (TLC): silica gel *60G* (*Merck & Co. Inc.*). Column chromatography (CC): silica gel *60* (SiO_2, 230 – 400 mesh; *Merck & Co. Inc.*, Darmstadt, Germany). Reversed-phase (RP) prep. HPLC: octadecyl silica column (*Pegasil ODSII* column, 25 cm × 10 cm i.d.; *Senshu Scientific Co., Ltd.*, Tokyo, Japan) at 25° with a mobile phase of MeOH (4 ml/min; HPLC system *I*) or with MeOH/H_2O 95:5 (4 ml/min; HPLC system *II*). Normal-phase HPLC: silica column (*Silica 4251-N* column, 25 cm × 10 mm i.d.; *Senshu Scientific Co., Ltd.*) at 25° with a mobile phase of hexane/AcOEt 6:4 (4 ml/min;

HPLC system *III*) or hexane/AcOEt 85:15 (2 ml/min; HPLC system *IV*). IR Spectra: *PerkinElmer Spectrum One* FT-IR spectrophotometer in KBr disks; $\tilde{\nu}$ in cm^{-1}. ^{1}H-NMR Spectra: *JEOL ECX-400* spectrometer (at 400 (^{1}H) and 100 MHz (^{13}C)) at r.t.; $CDCl_3$ or $CDCl_3$/CD_3OD soln.; δ in ppm rel. to Me_4Si as the internal standard, *J* in Hz. HR-ESI-MS: *Agilent 1100 LC/MSD TOF* (time-of-flight) system (ionization mode: positive or negative; cap. voltage: 3000 V; fragmentor voltage: 225 V). HR-APCI-MS: *Agilent 1100 LC/MSD TOF* system (ionization mode: positive; cap. voltage: 3000 V; fragmentor voltage: 40 V; corona current: 2.0 μA). Microplate reader: *Sunrise-Basic* (*Tecan Japan Co., Ltd.*, Kawasaki, Japan).

Chemicals and Materials. Compounds were purchased from the following sources: dimethylsuccinic anhydride, MTT, and DMSO from *Sigma-Aldrich Japan Co.* (Tokyo, Japan); 4-(dimethylamino)pyridine (DMAP), $NaBH_4$, succinic anhydride, and *m*-CPBA from *Tokyo Chemical Industry Co., Ltd.* (Tokyo, Japan); water-soluble carbodiimide (WSCD) from *Peptide Institute Inc.* (Osaka, Japan); *RPMI-1640* medium, fetal bovine serum (FBS), and penicillin-streptomycin from *Invitrogen Co.* (Auckland, NZ); rh annexin V/fluorescein isothiocyanate (FITC) kit (*Bender MedSystems*®) from *Cosmo Bio Co., Ltd.* (Tokyo, Japan); 1-hydroxybenzotriazole monohydrate (HOBt) from *Dojindo Molecular Technologies Inc.* (Kumamoto, Japan); and $K_2Cr_2O_7$, MnO_2, and cisplatin from *Wako Pure Chemical Industries Ltd.* (Osaka, Japan). All other chemicals and reagents were of anal. grade. Faradiol (**1**), arnidiol (**2**), and heliantriol C (**3**) were isolated from the compositae flower, as described previously [4].

Faradiol 3,16-Di-*O*-acetate (= **(3*β*,16*β*,18*α*,19*α*)-Urs-20-ene-3,16-diyl Diacetate**; **4**). To a soln. of **1** (700 mg, 1.6 mmol) in pyridine (12 ml), Ac_2O (12 ml, 128.2 mmol) was added and stirred at r.t. for 16 h. The mixture was then added to H_2O and extracted with Et_2O (3 × 100 ml). The Et_2O fraction was washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to yield **4** (686 mg, 82%) which was identified by spectral comparison with an authentic sample [4].

Faradiol *α*-Epoxide 3,16-Di-*O*-acetate (= **(3*β*,16*β*,18*α*,19*α*,20*β*,21*α*)-20,21-Epoxyursane-3,16-diyl Diacetate**; **5**). To a soln. of **4** (670 mg, 1.3 mmol) in CH_2Cl_2 (75 ml), $NaHCO_3$ (104 mg) and *m*-CPBA (219 mg, 1.3 mmol) were added and stirred at r.t. for 20 h. After this period, the mixture was washed with 1M aq. NaOH and H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to yield crude products (829 mg). The crude products were purified with Si-gel TLC (hexane/AcOEt 4:1) to give **5** (540 mg, 78%) which was identified by spectral comparison with an authentic sample [4].

Faradiol *α*-Epoxide (= **(3*β*,16*β*,18*α*,19*α*,20*β*,21*α*)-20,21-Epoxyursane-3,16-diol**; **6**). Compound **5** (20 mg, 0.04 mmol) was dissolved in 1M KOH/MeOH (5 ml) and allowed to stand at r.t. overnight. After this period, the mixture was poured into H_2O and extracted with Et_2O (2 × 30 ml), dried (Na_2SO_4), and evaporated under reduced pressure to yield **6** (15 mg, 89%) which was identified by spectral comparison with an authentic sample [4].

3β-Hydroxytaraxast-20-en-16-one (= **(3β,18α,19α)-3-Hydroxyurs-20-en-16-one**; **7**), **16β-Hydroxytaraxast-20-en-3-one** (= **(16β,18α,19α)-16-Hydroxyurs-20-en-3-one**; **8**), and **Faradione** (= **(18α,19α)-Urs-20-ene-3,16-dione**; **9**). A soln. of $K_2Cr_2O_7$ (234 mg, 0.8 mmol) and conc. H_2SO_4 (3.25 ml) in H_2O (16.3 ml) was added to **1** (500 mg, 1.1 mmol) dissolved in Et_2O (65 ml). The mixture was stirred at r.t. for 7 h, then the Et_2O fraction was washed with sat. $NaHCO_3$ aq. and H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to yield crude products (536.0 mg). These were purified with Si-gel TLC (hexane/AcOEt 4:1) to give **9** (322 mg, 65%) and a mixture of **7** and **8**, which was separated by HPLC (system *I*) to yield **7** (t_R 4.2 min; 89 mg, 18%) and **8** (t_R 2.7 min; 25 mg, 5%). These compounds were identified by spectral comparison with published data [18].

16-Epifaradiol (= **(3β,16α,18α,19α)-Urs-20-ene-3,16-diol**; **10**). To a soln. of **9** (84 mg, 0.2 mmol) in benzene (1.5 ml) and MeOH (3 ml), $NaBH_4$ (132 mg, 3.5 mmol) was added, and the mixture was stirred at r.t. for 5 h. After this period, 1M HCl (5 ml) was added to the mixture and stirred for a few min prior to extraction with Et_2O (2 × 20 ml). The Et_2O fraction was washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure to furnish **10** (21 mg, 25%), which was identified by spectral comparison with published data [18].

Faradione α-Epoxide (= **(18α,19α,20β,21α)-20,21-Epoxyursane-3,16-dione**; **11**). To a soln. of **9** (30 mg, 0.07 mmol) in CH_2Cl_2 (15 ml), $NaHCO_3$ (12 mg) and *m*-CPBA (24 mg, 0.14 mmol) were added and stirred at r.t. for 3 days. After this period, the mixture was washed with 1M aq. NaOH and H_2O, dried (Na_2SO_4), and evaporated under reduced pressure

to yield crude products (39.1 mg). These were purified with Si-gel TLC (hexane/AcOEt 4:1) to give **11** (15 mg, 48%).

Amorphous solid. IR (KBr): 752, 1118, 1384, 1459, 1701, 2952. ^{1}H-NMR (400 MHz): 0.83 (*s*, 3 H); 0.95 (*s*, 3 H); 1.03 (*s*, 3 H); 1.08 (*s*, 3 H); 1.09 (*s*, 3 H); 1.12 (*s*, 3 H); 1.18 (*d*, *J* = 6.4, 3 H); 1.36 (*s*, 3 H); 3.09 (*d*, *J* = 6.0, 1 H). HR-APCIMS (pos.): 455.3504 ([*M* + H]$^+$, $C_{30}H_{47}O_3^+$; calc. 455.3519).

Arnitriol A 3,16-Di-*O*-acetate (= **(3*β*,16*β*,18*α*,19*α*,21*α*)-21-Hydroxyurs-20(30)-ene-3,16-diyl Diacetate**; **12**) and **Heliantriol F 3,16-Di-*O*-acetate** (= **(3*β*,16*β*,18*α*,19*α*)-30-Hydroxyurs-20-ene-3,16-diyl Diacetate**; **15**). To a soln. of **5** (203 mg, 0.4 mmol) in $CHCl_3$ (120 ml), conc. HCl (0.2 ml) was added and stirred at r.t. for 1 day. After this period, the $CHCl_3$ fraction was washed with sat. aq. $NaHSO_4$, dried (Na_2SO_4), and evaporated under reduced pressure to yield a crude product (209 mg). This product was purified with Si-gel TLC (hexane/AcOEt 4:1) to furnish **12** (84 mg, 41%) and **15** (56 mg, 28%). Compound **15** was identified by spectral comparison with published values [19].

Data for **12**. Amorphous solid. IR (KBr): 754, 1026, 1247, 1371, 1735, 2945, 3436. ^{1}H-NMR (400 MHz): 0.83 (*s*, 6 H); 0.85 (*s*, 3 H); 0.87 (*s*, 3 H); 1.02 (*s*, 3 H); 1.07 (*s*, 3 H); 1.23 (*d J* = 7.1, 3 H); 2.01 (*s*, 3 H); 2.04 (*s*, 3 H); 4.38 (*dd*, *J* = 5.4, 9.0, 1 H); 4.48 (*dd*, *J* = 5.1, 10.7, 1 H); 4.72 (*dd*, *J* = 4.6, 11.7, 1 H); 4.92 (br. *s*, 1 H); 5.00 (br. *s*, 1 H). HR-ESI-MS (pos.): 565.3847 ([*M* + Na]$^+$, $C_{34}H_{54}NaO_5^+$; calc. 565.3868).

Arnitriol A 3,16,21-Tri-*O*-acetate (= **(3*β*,16*β*,18*α*,19*α*,21*α*)-Urs-20(30)-ene-3,16,21-triyl Triacetate**; **13**). To a soln. of **12** (15 mg, 0.03 mmol) in pyridine (2 ml), Ac_2O (2 ml, 21.2 mmol) was added and stirred at r.t. for 16 h. After this period, the mixture was added to H_2O and extracted with Et_2O (2 × 30 ml). The Et_2O fraction was washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to yield **13** (13 mg, 80%).

Amorphous solid. IR (KBr): 755, 979, 1023, 1247, 1370, 1735, 2944. ^{1}H-NMR (400 MHz): 0.84 (*s*, 3 H); 0.85 (*s*, 3 H); 0.87 (*s*, 3 H); 0.88 (*s*, 3 H); 1.02 (*s*, 3 H); 1.07 (*s*, 3 H); 1.16 (*d*, *J* = 6.9, 3 H); 2.00 (*s*, 3 H); 2.01 (*s*, 3 H); 2.04 (*s*, 3 H); 4.48 (*dd*, *J* = 4.8, 11.4, 1 H); 4.70 (*dd*, *J* = 4.5, 11.7, 1 H); 4.99 (br. *s*, 1 H); 5.10 (br. *s*, 1 H); 5.45 (*dd*, *J* = 4.8, 9.6, 1 H). HR-ESI-MS (pos.): 607.3966 ($[M + Na]^+$, $C_{36}H_{56}NaO_6^+$; calc. 607.3969).

Arnitriol A (= **(3*β*,16*β*,18*α*,19*α*,21*α*)-Urs-20(30)-ene-3,16,21-triol**; **14**). Compound **12** (26 mg, 0.05 mmol) was dissolved in 1M KOH/MeOH (5 ml) and allowed to stand at r.t. overnight. After this period, the mixture was poured into H_2O, extracted with Et_2O (2 × 30 ml), dried (Na_2SO_4), and evaporated under reduced pressure to yield **14** (17 mg, 77%). Amorphous solid. IR (KBr): 915, 986, 1031, 1136, 1389, 1418, 1446, 2937, 3399. ^{1}H-NMR ($CDCl_3$/CD_3OD 8:2, 400 MHz): 0.75 (*s*, 3 H); 0.76 (*s*, 3 H); 0.85 (*s*, 3 H); 0.96 (*s*, 3 H); 1.01 (*s*, 3 H); 1.03 (*s*, 3 H); 1.21 (*d*, *J* = 6.8, 3 H); 3.18 (*dd*, *J* = 7.0, 9.0, 1 H); 3.39 (*dd*, *J* = 4.1, 11.7, 1 H); 4.37 (*dd*, *J* = 5.4, 9.0, 1 H); 4.92 (br. *s*, 1 H); 5.00 (br. *s*, 1 H). HR-APCIMS (pos.): 441.3709 ($[M - H_2O + H]^+$, $C_{30}H_{49}O_2^+$; calc. 441.3732).

Heliantriol F 3,16,30-Tri-*O*-acetate (= **(3*β*,16*β*,18*α*,19*α*)-Urs-20-ene-3,16,30-triyl Triacetate**; **16**). To a soln. of **15** (7 mg, 0.01 mmol) in pyridine (2 ml), Ac_2O (2 ml, 21.2 mmol) was added and stirred at r.t. for 16 h. After this period, the mixture was added to H_2O and extracted with Et_2O (2 × 30 ml). The Et_2O fraction was washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to yield **16** (6 mg, 80%), which was identified by spectral comparison with published data [19].

Heliantriol F (= **(3*β*,16*β*,18*α*,19*α*)-Urs-20-ene-3,16,30-triol**; **17**). Compound **15** (7 mg, 0.01 mmol) was dissolved in 1M KOH/MeOH (5 ml) and allowed to stand at r.t. overnight. After this period, the mixture was poured into H_2O, extracted with Et_2O (2 × 30 ml), dried (Na_2SO_4), and evaporated under reduced pressure to yield **17** (5 mg, 85%), which was identified by spectral comparison with published data [13].

21-Oxoarnidiol (= **(3*β*,16*β*,18*α*,19*α*)-3,16-Dihydroxyurs-20(30)-en-21-one**; **18**). To a soln. of **14** (7 mg, 0.02 mmol) in dried $CHCl_3$ (3 ml), MnO_2 (98 mg, 1.1 mmol) was added and stirred at r.t. for 1 h. After this period, the mixture was filtered and evaporated under reduced pressure to yield a crude product (6 mg). This product was purified with Si-gel TLC (hexane/AcOEt 1:1) to furnish **18** (2 mg, 29%), which was identified by spectral comparison with published data [13].

30-Oxofaradiol 3,16-Di-*O*-acetate (= **(3*β*,16*β*,18*α*,19*α*)-30-Oxours-20-ene-3,16-diyl Diacetate**; **19**). To a soln. of **12** (40 mg, 0.07 mmol) in Et_2O (14 ml), $K_2Cr_2O_7$ (22 mg, 0.07 mmol) in 3M H_2SO_4 (1.5 ml) was added and stirred at r.t. for 7 h. After this period, the Et_2O fraction was washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to yield a crude product (35 mg). This product was purified by HPLC (system *III*) to furnish **19** (t_R 7.8 min; 14 mg, 35%), which was identified by spectral comparison with published data [18].

30-Oxofaradiol (= **(3*β*,16*β*,18*α*,19*α*)-3,16-Dihydroxyurs-20-en-30-al**; **20**). Compound **19** (9 mg, 0.02 mmol) was dissolved in 1M KOH/MeOH (5 ml) and allowed to stand at r.t. overnight. After this period, the mixture was poured into H_2O and extracted with Et_2O (2 × 30 ml), dried (Na_2SO_4), and evaporated under reduced pressure to yield **20** (6 mg, 79%), which was identified by spectral comparison with published data [13].

3*β*,16*β*-Diacetoxytaraxastan-21-one (= **(3*β*,16*β*,18*α*,19*α*,20*β*)-21-Oxoursane-3,16-diyl Diacetate**; **21**) and **3*β*,16*β*-Diacetoxy-20-epitaraxastan-21-one** (= **(3*β*,16*β*,18*α*,19*α*,20*α*)-21-Oxoursane-3,16-diyl Diacetate**; **23**). To a soln. of **5** (300 mg, 0.55 mmol) in THF (48 ml), 3M aq. $HClO_4$ (24 ml) was added and stirred at r.t. for 3 h. After this period, the mixture was poured into 4M aq. NaOH (24 ml) and extracted with $CHCl_3$ (2 × 30 ml). The $CHCl_3$ fraction was washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to yield a crude product. This product was partly hydrolyzed under acidic conditions and was therefore acetylated again to produce a mixture of **21** and **23** (290 mg).

This mixture was separated by HPLC (system *IV*) to furnish **21** (t_R 38.6 min; 118 mg, 39%) and **23** (t_R 27.0 min; 85 mg, 28%).

Data of **21**. Amorphous solid. IR (KBr): 755, 979, 1022, 1243, 1372, 1391, 1458, 1715, 1732, 2945. ^{1}H-NMR (400 MHz): 0.73 (*s*, 3 H); 0.84 (*s*, 3 H); 0.86 (*s*, 3 H); 0.88 (*s*, 3 H); 1.05 (*s*, 3 H); 1.10 (*s*, 3 H); 1.12 (*d*, *J* = 6.5, 3 H); 1.25 (*d*, *J* = 5.8, 3 H); 1.86 (*m*, 1 H); 2.02 (*s*, 3 H); 2.04 (*s*, 3 H); 2.15 (*d*, *J* = 12.4, 1 H); 2.22 (*d*, *J* = 12.4, 1 H); 4.48 (*dd*, *J* = 4.8, 11.3, 1 H); 4.85 (*dd*, *J* = 4.8, 11.3, 1 H). ^{13}C-NMR (100 MHz): 13.4 (C(28)); 14.3 (C(30)); 16.0 (C(26)); 16.1 (C(27)); 16.3 (C(25)); 16.5 (C(23)); 18.1 (C(6)); 21.2 (CO*Me*); 21.3 (CO*Me*); 21.4 (C(11)); 23.6 (C(2)); 26.3 (C(29)); 27.9 (C(24)); 28.2 (C(12)); 32.5 (C(15)); 34.3 (C(7)); 36.9 (C(10)); 37.8 (C(4)); 38.4 (C(1)); 39.1 (C(13)); 41.0 (C(19)); 41.3 (C(8)); 42.6 (C(14)); 43.4 (C(17)); 49.2 (C(18)); 49.4 (C(9)); 51.1 (C(22)); 52.4 (C(20)); 55.3 (C(5)); 77.0 (C(16)); 80.8 (C(3)); 170.6 (*C*OMe); 170.9 (*C*OMe); 211.2 (C(21)). HR-ESI-MS (pos.): 565.3874 ([*M* + Na]$^+$, $C_{34}H_{54}NaO_5^+$; calc. 565.3868).

Data of **23**. Amorphous solid. IR (KBr): 756, 979, 1022, 1242, 1370, 1453, 1734, 2943, 2974, 3436. ^{1}H-NMR (400 MHz): 0.75 (*d*, *J* = 6.8, 3 H); 0.85 (*s*, 3 H); 0.86 (*s*, 3 H); 0.89 (*s*, 3 H); 0.97 (*d*, *J* = 6.5, 3 H); 1.05 (*s*, 3 H); 1.08 (*s*, 3 H); 1.21 (*s*, 3 H); 2.00 (*s*, 3 H); 2.04 (*s*, 3 H); 4.48 (*dd*, *J* = 4.9, 11.7, 1 H); 4.70 (*dd*, *J* = 4.4, 11.3, 1 H). ^{13}C-NMR (100 MHz): 11.1 (C(30)); 16.0 (C(26)); 16.2 (C(27)); 16.3 (C(25)); 16.5 (C(23)); 16.7 (C(28)); 18.1 (C(6)); 19.7 (C(29)); 21.2 (CO*Me*); 21.3 (CO*Me*); 21.4 (C(11)); 23.7 (C(2)); 25.6 (C(12)); 27.9 (C(24)); 32.8 (C(15)); 34.0 (C(7)); 34.4 (C(19)); 37.0 (C(10)); 37.8 (C(4)); 38.5

(C(1)); 38.6 (C(13)); 39.2 (C(17)); 41.1 (C(8)); 42.6 (C(14)); 44.8 (C(20)); 48.5 (C(18)); 50.0 (C(9)); 51.7 (C(22)); 55.4 (C(5)); 79.4 (C(16)); 80.8 (C(3)); 170.4 (*C*OMe); 170.9 (*C*OMe); 213.6 (C(21)). HR-ESI-MS (pos.): 565.3873 ($[M + Na]^+$, $C_{34}H_{54}NaO_5^+$; calc. 565.3868).

3β,16β-Dihydroxytaraxastan-21-one (= **(3β,16β,18α,19α,20β)-3,16-Dihydroxyursan-21-one**; **22**) and **3β,16β-Dihydroxy-20-epitaraxastan-21-one** (= **(3β,16β,18α,19α,20α)-3,16-Dihydroxyursan-21-one**; **24**). Compound **21** (42 mg, 0.08 mmol) was dissolved in 1M KOH/MeOH (10 ml) and allowed to stand at r.t. overnight. After this period, the mixture was poured into H_2O and extracted with Et_2O (2 × 30 ml), dried (Na_2SO_4), and evaporated under reduced pressure to furnish a mixture of **22** and **24** (36 mg). This mixture was separated by HPLC (system *III*) to furnish **22** (t_R 11.7 min; 9 mg, 25%) and **24** (t_R 12.8 min; 12 mg, 34%).

Data of **22**. Amorphous solid. IR (KBr): 754, 1388, 1455, 1705, 2874, 2935, 3418. ^{1}H-NMR (400 MHz): 0.59 (*s*, 3 H); 0.73 (*s*, 3 H); 0.81 (*s*, 3 H); 0.93 (*s*, 3 H); 1.01 (*s*, 6 H); 1.08 (*d*, *J* = 6.8, 3 H); 1.21 (*d*, *J* = 5.5, 3 H); 1.83 (*quint.*, *J* = 6.9, 1 H); 2.08 (*d*, *J* = 11.9, 1 H); 2.52 (*d*, *J* = 11.9, 1 H); 3.16 (*dd*, *J* = 5.5, 10.5, 1 H); 3.49 (*dd*, *J* = 5.0, 11.5, 1 H). HR-ESI-MS (pos.): 459.3814 ($[M + H]^+$, $C_{30}H_{51}O_3^+$; calc. 459.3838).

Data of **24**. Amorphous solid. IR (KBr): 755, 1036, 1387, 1459, 1704, 2873, 2946, 3400. ^{1}H-NMR (400 MHz): 0.68 (*d*, *J* = 6.9, 3 H); 0.73 (*s*, 3 H); 0.82 (*s*, 3H); 0.92 (*d*, *J* = 7.3, 3 H); 0.93 (*s*, 3 H); 0.96 (*s*, 3 H); 1.01 (*s*, 3 H); 1.10 (*s*, 3 H); 1.87 (*d*, *J* = 19.2, 1 H); 2.59 (*d*, *J* = 19.2, 1 H); 3.02 (*quint.*, *J* = 6.9, 1 H); 3.15 (*dd*, *J* = 5.7, 10.8, 1 H); 3.36 (*dd*, *J* = 4.6, 11.5,

1 H). HR-ESI-MS (pos.): 459.3811 ([M + H]$^+$, $C_{30}H_{51}O_3^+$; calc. 459.3838).

Heliantriol C 3,16,22-Tri-*O*-acetate (= **(3β,16β,18α,19α,22α)-Urs-20-ene-3,16,22-triyl Triacetate**; **25**). To a soln. of **3** (230 mg, 0.5 mmol) in pyridine (10 ml), Ac_2O (10 ml, 105 mmol) was added and stirred at r.t. for 16 h. After this period, the mixture was added to H_2O and extracted with Et_2O (2 × 100 ml). The Et_2O fraction was washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to yield **25** (220 mg, 75%), which was identified by spectral comparison with published data [4].

22-Oxofaradiol (= **(3β,16β,18α,19α)-3,16-Dihydroxyurs-20-en-22-one**; **26**). To a soln. of **3** (10 mg, 0.02 mmol) in $CHCl_3$ (1 ml), MnO_2 (95 mg, 1.1 mmol) was added and stirred at r.t. for 12 h. After this period, the mixture was filtered and evaporated under reduced pressure to give a crude product (10 mg). This product was purified with Si-gel TLC (hexane/AcOEt 1:1) to give **26** (3 mg, 30%), which was identified by spectral comparison with published data [19].

Heliantriol C Epoxide 3,16,22-Tri-*O*-acetate (= **(3β,16β,18α,19α,20β,21α,22α)-20,21-Epoxyursane-3,16,22-triyl Triacetate**; **27**). To a soln. of **25** (31 mg, 0.05 mmol) in CH_2Cl_2 (10 ml), $NaHCO_3$ (5 mg) and *m*-CPBA (168 mg, 1.0 mmol) were added and stirred at r.t. for 19 h. After this period, the mixture was washed with 1M aq. NaOH and H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to yield crude products (76 mg). These crude products were purified with Si-gel TLC (hexane/AcOEt 4:1) to give **27** (21 mg, 66%).

Amorphous solid. IR (KBr): 978, 1023, 1240, 1375, 1449, 1739, 2947. ^{1}H-NMR (400

MHz): 0.84 (*s*, 3 H); 0.85 (*s*, 3 H); 0.87 (*s*, 3 H); 0.88 (*s*, 3 H); 1.01 (*s*, 3 H); 1.03 (*s*, 3 H); 1.13 (*d*, *J* = 6.0, 3 H); 1.33 (*s*, 3 H); 1.98 (*s*, 3 H); 2.05 (*s*, 3 H); 2.08 (*s*, 3 H); 3.29 (*d*, *J* = 6.0, 1 H); 4.48 (*dd*, *J* = 5.0, 11.0, 1 H); 4.67 (*d*, *J* = 6.0, 1 H); 5.05 (*dd*, *J* = 5.0, 11.9, 1 H). HR-ESI-MS (pos.): 623.3849 ([*M* + Na]$^+$, $C_{36}H_{56}NaO_7^+$; calc. 623.3918).

Heliantriol C Epoxide (= **(3*β*,16*β*,18*α*,19*α*,20*β*,21*α*,22*α*)-20,21-Epoxyursane-3,16,22-triol**; **28**). Compound **27** (10 mg, 0.02 mmol) was dissolved in 1M KOH/MeOH (5 ml) and allowed to stand at r.t. overnight. After this period, the mixture was poured into H_2O and extracted with Et_2O (2 × 30 ml), dried (Na_2SO_4), and evaporated under reduced pressure to give a crude product (7 mg). This product was purified with Si-gel TLC (hexane/AcOEt 1:1) to yield **28** (4 mg, 51%).

Amorphous solid. IR (KBr): 806, 1040, 1263, 1379, 1456, 1715, 2929, 3448. ^{1}H-NMR (400 MHz): 0.69 (*s*, 3 H); 0.76 (*s*, 3 H); 0.85 (*s*, 3 H); 0.94 (*s*, 3 H); 1.03 (*s*, 3 H); 1.11 (*s*, 3 H); 1.12 (*d*, *J* = 6.0, 3 H); 1.38 (*s*, 3 H); 3.20 (*dd*, *J* = 5.0, 11.4, 1 H); 3.36 (*d*, *J* = 6.2, 1 H); 3.99 (*dd*, *J* = 4.5, 11.4, 1 H); 4.00 (*d*, *J* = 6.2, 1 H). HR-ESI-MS (pos.): 497.3637 ([*M* + Na]$^+$, $C_{30}H_{50}NaO_4^+$; calc. 497.3601).

Faradiol 3,16-Di-*O*-succinate (= **4,4'-[(3*β*,16*β*,18*α*,19*α*)-Urs-20-ene-3,16-diylbis(oxy)]bis(4-oxobutanoic Acid)**; **29**). To a soln. of **1** (20 mg, 0.05 mmol) in pyridine (2 ml), succinic anhydride (21 mg, 0.2 mmol) and DMAP (10 mg, 0.08 mmol) were added and refluxed for 3 h. After this period, the mixture was poured into H_2O and extracted with Et_2O

(2 × 30 ml). The Et_2O fraction was washed with 1M aq. HCl and H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to yield a crude product (20 mg). This product was purified with Si-gel TLC (hexane/AcOEt 7:3) to furnish **29** (18 mg, 62%). Amorphous solid. IR (KBr): 757, 967, 1168, 1228, 1386, 1714, 1730, 2874, 2943. ^{1}H-NMR (400 MHz): 0.79 (*s*, 3 H); 0.83 (*s*, 3 H); 0.84 (*s*, 3 H); 0.87 (*s*, 3 H); 1.00 (*d*, *J* = 6.3, 3 H); 1.04 (*s*, 3 H); 1.05 (*s*, 3 H); 1.64 (br. *s*, 3 H); 4.51 (*dd*, *J* = 6.1, 10.5, 1 H); 4.76 (*dd*, *J* = 4.9, 11.7, 1 H); 5.24 (*d*, *J* = 6.8, 1 H). HR-ESI-MS (pos.): 665.4029 ($[M + Na]^+$, $C_{38}H_{58}NaO_8^+$; calc. 665.4029).

Faradiol 3,16-Di-*O*-dimethylsuccinate (= **4,4'-[(3*β*,16*β*,18*α*,19*α*)-Urs-20-ene-3,16-diylbis(oxy)]bis(2,2-dimethyl-4-oxobutanoic Acid)**; **30**). To a soln. of **1** (20 mg, 0.05 mmol) in pyridine (2 ml), dimethylsuccinic anhydride (50 mg, 0.4 mmol) and DMAP (10 mg, 0.08 mmol) were added and refluxed for 12 h. After this period, the mixture was poured into H_2O and extracted with Et_2O (2 × 30 ml). The Et_2O fraction was washed with 1M aq. HCl and H_2O, dried (Na_2SO_4), and evaporated under reduced pressure to yield a crude product (20 mg). This product was purified by HPLC (system *II*) to furnish **30** (t_R 8.0 min; 14.3 mg, 45%). Amorphous solid. IR (KBr): 757, 968, 991, 1136, 1202, 1311, 1365, 1388, 1448, 1475, 1705, 1730, 2875, 2945, 2974. ^{1}H-NMR (400 MHz): 0.77 (*s*, 3 H); 0.81 (*s*, 3 H); 0.84 (*s*, 3 H); 0.87 (*s*, 3 H); 1.00 (*d*, *J* = 6.0, 3 H); 1.04 (*s*, 3 H); 1.05 (*s*, 3 H); 1.28 (*s*, 3 H); 1.29 (*s*, 6 H); 1.30 (*s*, 3 H); 1.63 (br. *s*, 3 H); 4.51 (*dd*, *J* = 10.5, 5.5, 1 H); 4.75 (*dd*, *J* = 11.7, 4.3, 1 H); 5.25 (*d*, *J* = 6.4, 1 H). HR-ESI-MS (pos.): 721.4620 ($[M + Na]^+$, $C_{42}H_{66}NaO_8^+$; calc. 721.4650).

Faradiol 3,16-Di-*O*-alaninate (= **(3*β*,16*β*,18*α*,19*α*)-Urs-20-ene-3,16-diyl Bis(2-aminopropanoate)**; **31**). To a soln. of **1** (20 mg, 0.05 mmol), Boc-protected L-alanine (63 mg, 0.33 mmol), 1-hydroxybenzotriazole monohydrate (56 mg, 0.37 mmol) in DMF (420 µl), and WSCD (140 µl, 0.8 mmol) were added at –20°, and the mixture was allowed to stand for 48 h. at r.t. The mixture was diluted with H_2O and extracted with AcOEt (2 × 5 ml). The AcOEt fraction was washed with H_2O, 1M HCl, and sat. aq. $NaHCO_3$, dried (Na_2SO_4), and concentrated under reduced pressure to afford a Boc-protected **31**. Removal of the Boc group was achieved by treating with 3M HCl in 1,4-dioxane (200 µl) for 30 min at r.t. After deprotection, the mixture was suspended in Et_2O (800 µl) and filtered to remove unreacted Boc-protected **31**. The precipitate was added to $CHCl_3$ (300 µl) with Et_3N (20 µl), washed with H_2O, and evaporated under reduced pressure to give **31** (10 mg, 38%) without further purification. Amorphous solid. IR (KBr): 1052, 1455, 1628, 1733, 2347, 2943, 3448. ^{1}H-NMR (400 MHz): 0.82 (*s*, 3 H); 0.86 (*s*, 6 H); 0.89 (*s*, 3 H); 1.02 (*d*, *J* = 6.4, 3 H); 1.06 (*s*, 3 H); 1.08 (*s*, 3 H); 1.35 (*d*, *J* = 6.8, 1 H); 1.36 (*d*, *J* = 6.9, 1 H); 1.65 (br. *s*, 3 H); 3.53 (*m*, 2 H); 4.52 (*m*, 1 H); 4.77 (*m*, 1 H); 5.25 (*d*, *J* = 6.9, 1 H). HR-ESI-MS (pos.): 585.4623 ($[M + H]^+$, $C_{36}H_{61}N_2O_4^+$; calc. 585.4625).

Cytotoxicity Assay. Cytotoxicity assays were performed as described previously [20]. Briefly, 3×10^3 cells/well of HL60 (leukemia), A549 (lung), AZ521 (duodenum), and SK-BR-3 (breast) cell lines were treated with the indicated compounds for 48 h prior to adding MTT solution to each well. After incubation for 3 h, the generated blue formazan was

solubilized using 0.04M HCl in i-PrOH. The absorbances at 570 nm (top) and 630 nm (bottom) were measured by a microplate reader.

Annexin V-PI Double Staining. Apoptosis was detected in HL60 cells (1.5×10^5) exposed to test compound (final concentration: 5 μM) using an *rh Annexin V/FITC* kit [20]. Cells were washed with annexin binding buffer, stained for 10 min, and analyzed in a flow cytometer (*Cell Lab Quanta SC*; *Beckman Coulter K.K.*, Tokyo, Japan) using the FL1 and FL2 ranges for annexin V-FITC and PI, resp.

REFERENCES

[1] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2012**, *75*, 311.

[2] P. Dzubak, M. Hajduch, D. Vydra, A. Hustova, M. Kvasnica, D. Biedermann, L. Markova, M. Urban, J. Sarek, *Nat. Prod. Rep.* **2006**, *23*, 394.

[3] S. Safe, G. Chadalapaka, I. Jutooru, S. Chintharlapalli, S. Papineni, 'Pentacyclic Triterpenes as Promising Agents in Cancer', Ed. J. A. R. Salvador, Nova Science Publishers, New York, 2010, p. 277.

[4] M. Ukiya, T. Akihisa, K. Yasukawa, Y. Kasahara, Y. Kimura, K. Koike, T. Nikaido, M. Takido, *J. Agric. Food Chem.* **2001**, *49*, 3187.

[5] M. Ukiya, T. Akihisa, K. Yasukawa, K. Koike, A. Takahashi, T. Suzuki, Y. Kimura, *J. Nat. Prod.* **2007**, *70*, 813.

[6] M. Ukiya, T. Akihisa, K. Yasukawa, H. Tokuda, T. Suzuki, Y. Kimura, *J. Nat. Prod.*

2006, *69*, 1692.

[7] T. Akihisa, S. G. Franzblau, M. Ukiya, H. Okuda, F. Zhang, K. Yasukawa, T. Suzuki, Y. Kimura, *Biol. Pharm. Bull.* **2005**, *28*, 158.

[8] M. Ukiya, T. Akihisa, H. Tokuda, H. Suzuki, T. Mukainaka, E. Ichiishi, K. Yasukawa, Y. Kasahara, H. Nishino, *Cancer Lett.* **2002**, *177*, 7.

[9] M. Ukiya, T. Akihisa, H. Tokuda, K. Koike, J. Takayasu, H. Okuda, Y. Kimura, T. Nikaido, H. Nishino, *J. Agric. Food Chem.* **2003**, *51*, 2949.

[10] K. Yasukawa, T. Akihisa, Y. Kasahara, M. Ukiya, K. Kumaki, T. Tamura, S. Yamanouchi, M. Takido, *Phytomedicine* **1998**, *5*, 215.

[11] J. Liu, *J. Ethnopharmacol.* **2005**, *100*, 92.

[12] M. K. Shanmugam, A. H. Nguyen, A. P. Kumar, B. K. H. Tan, G. Sethi, *Cancer Lett.* **2012**, *320*, 158.

[13] H. Neukirch, M. D'Ambrosio, S. Sosa, G. Altinier, R. Della Loggia, A. Guerriero, *Chem. Biodiversity* **2005**, *2*, 657.

[14] M. Drag-Zalesinska, J. Kulbacka, J. Saczko, T. Wysocka, M. Zabel, P. Surowiak, M. Drag, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4814.

[15] Y.-H. Kuo, Y.-M. Chaiang, *Chem. Pharm. Bull.* **1999**, *47*, 498.

[16] K. Gollnick, G. Schade, *Tetrahedron* **1966**, *22*, 133.

[17] S. J. Martin, C. P. M. Reutelingsperger, A. J. McGahon, J. A. Rader, R. C. A. A. van Schie, D. M. LaFace, D. R. Green, *J. Exp. Med.* **1995**, *182*, 1545.

[18] J. S. Pyrek, *Rocz. Chem.* **1977**, *51*, 2331.

[19] J. S. Pyrek, *Pol. J. Chem.* **1979**, *53*, 1071.

[20] K. Tabata, K. Motani, N. Takayanagi, R. Nishimura, S. Asami, Y. Kimura, M. Ukiya, D. Hasegawa, T. Akihisa, T. Suzuki, *Biol. Pharm. Bull.* **2005**, *28*, 1404.

Received November 18, 2015

Accepted February 29, 2016

Table. Cytotoxic activities (IC_{50} ± S.D. [mM]) of taraxastane-type triterpenoid derivatives **1** – **31** against four human cancer cell lines[a]).

Compound	HL60 (leukemia)	A549 (lung)	AZ521 (duodenum)	SK-BR-3 (breast)
1	11.7 ± 8.7	> 100	52.4 ± 4.8	83.2 ± 4.9
2	10.1 ± 1.0	15.5 ± 2.2	13.8 ± 2.6	25.8 ± 0.2
3	> 100	> 100	> 100	> 100
4	> 100	> 100	> 100	> 100
5	39.6 ± 2.9	> 100	> 100	> 100
6	57.9 ± 6.6	> 100	7.8 ± 0.4	64.3 ± 5.8
7	81.9 ± 0.9	19.6 ± 3.6	75.0 ± 3.8	90.4 ± 2.9
8	31.9 ± 1.5	53.7 ± 12.5	59.9 ± 5.5	34.3 ± 4.4
9	95.4 ± 3.3	> 100	68.9 ± 5.4	> 100
10	35.8 ± 0.4	45.9 ± 0.1	38.7 ± 5.8	18.6 ± 0.9
11	> 100	> 100	> 100	> 100
12	> 100	12.7 ± 0.06	> 100	> 100
13	> 100	> 100	> 100	> 100
14	> 100	> 100	> 100	64.6 ± 0.6

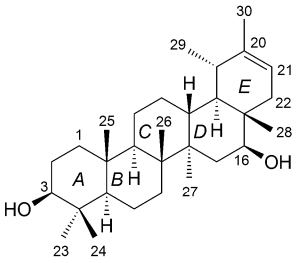
15	> 100	> 100	> 100	> 100
16	> 100	> 100	> 100	> 100
17	65.1 ± 7.9	42.2 ± 5.0	31.5 ± 3.1	> 100
18	0.3 ± 0.0	1.0 ± 0.1	0.7 ± 0.0	0.8 ± 0.1
19	> 100	32.0 ± 1.9	16.1 ± 2.7	29.0 ± 3.5
20	6.4 ± 0.7	14.6 ± 3.4	13.2 ± 1.0	> 100
21	> 100	> 100	45.0 ± 5.4	> 100
22	18.1 ± 8.1	> 100	> 100	38.4 ± 8.8
23	> 100	> 100	57.2 ± 4.4	> 100
24	83.1 ± 4.3	> 100	> 100	74.8 ± 1.8
25	15.9 ± 3.6	> 100	44.3 ± 4.1	> 100
26	63.9 ± 9.2	1.0 ± 0.2	46.3 ± 3.8	45.2 ± 8.3
27	> 100	56.7 ± 5.3	> 100	> 100
28	31.4 ± 7.9	> 100	> 100	> 100
29	35.4 ± 2.2	46.7 ± 4.1	63.2 ± 4.9	35.2 ± 3.1
30	2.1 ± 0.5	6.6 ± 1.3	79.0 ± 6.7	30.9 ± 0.1
31	0.5 ± 0.05	2.7 ± 0.1	1.4 ± 0.1	0.7 ± 0.3
Cisplatin[b])	4.2 ± 1.1	18.4 ± 1.9	9.5 ± 0.5	9.7 ± 0.6

[a]) Cells were treated with compounds ($1 \times 10^{-4} - 1 \times 10^{-6}$ M) for 48 h, and cell viability was analyzed by the MTT assay. IC_{50} Values based on triplicate five point.
[b]) Reference compound.

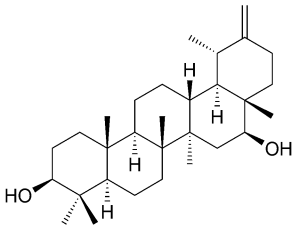
Caption:

Figure. Detection of early and late apoptosis. HL60 cells were cultured with 5 μM **18** or **31** for 24 or 48 h prior to annexin V-fluorescein isothiocyanate/propidium iodide (PI) double staining and flow cytometry analysis. The lower left quadrant includes live cells (annexin V^- /PI^-), the lower right quadrant shows early apoptotic cells (annexin V^+/PI^-), the upper right quadrant shows late apoptotic cells (annexin V^+/PI^+), and the upper left quadrant shows

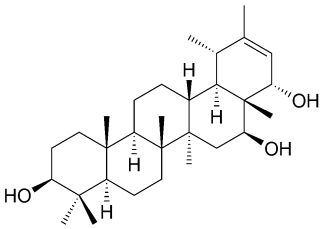
necrotic cells (annexin V^-/PI^+). Percentage values indicate the proportion of cells in the relevant quadrant. Each value is the mean of three experiments.



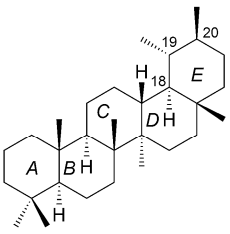
1



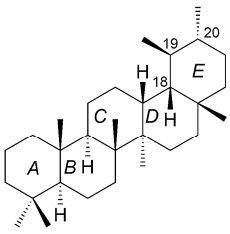
2



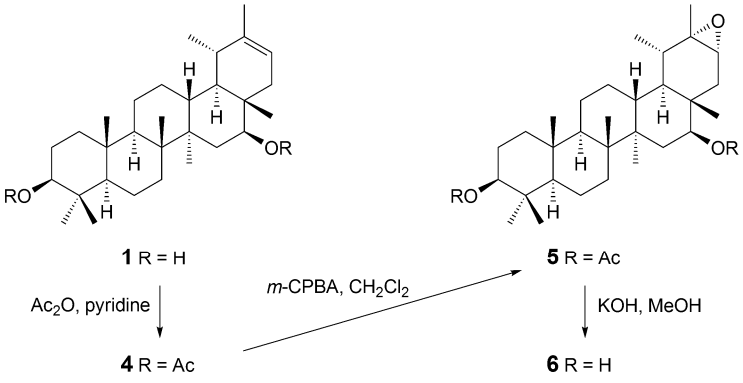
3

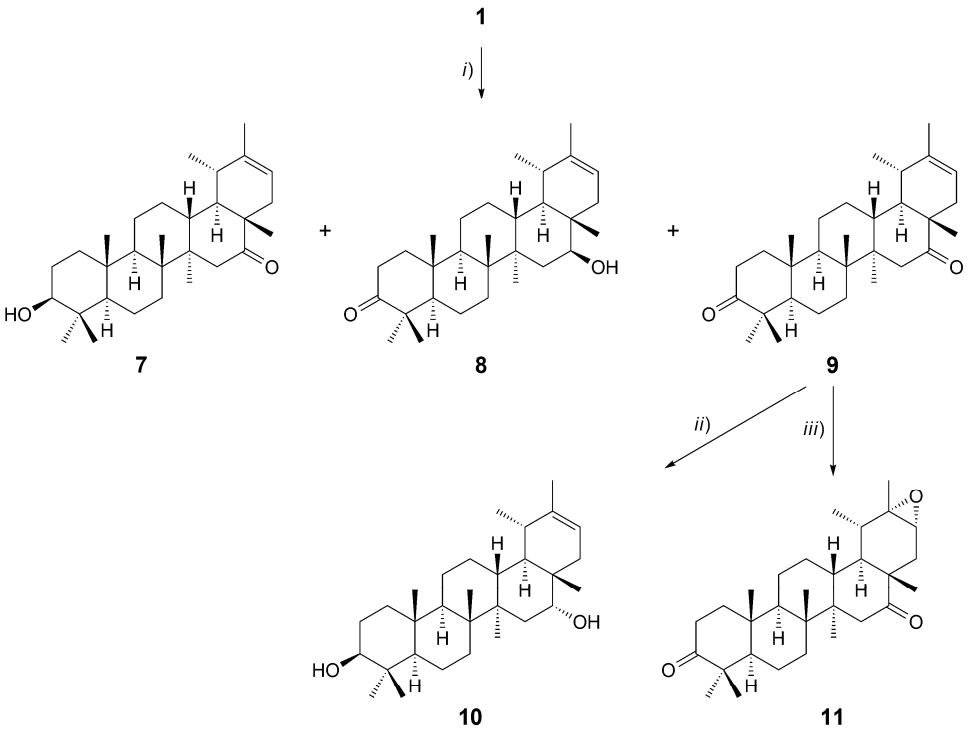


Taraxastane

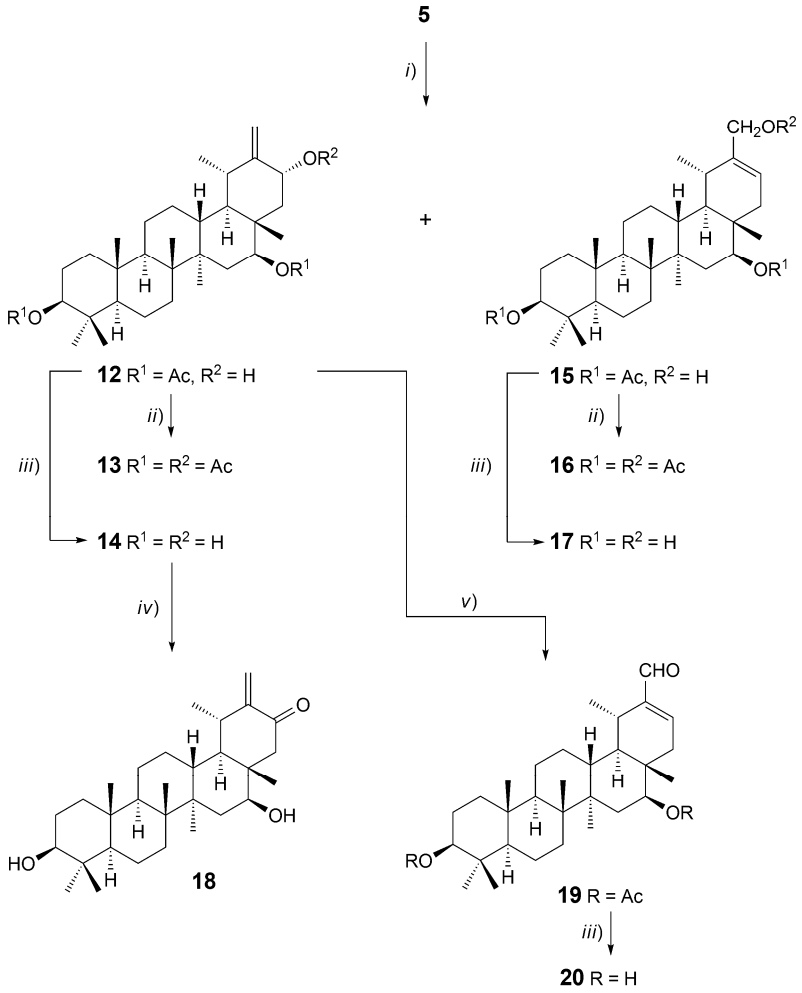


Ursane

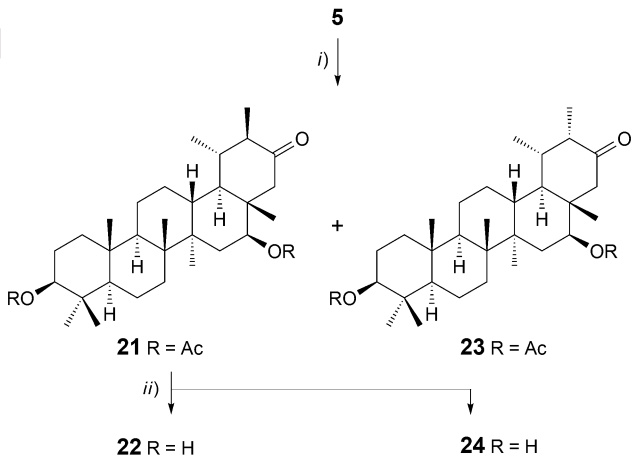




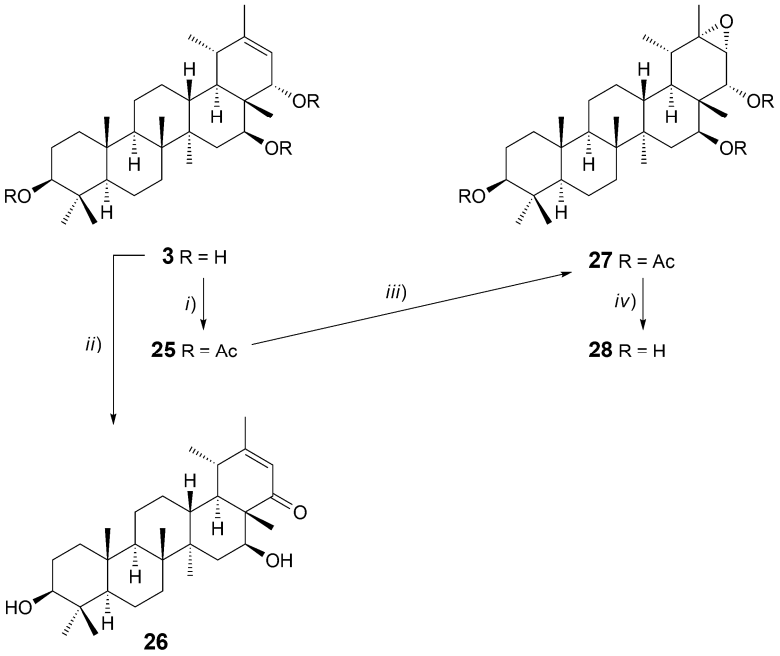
i) $K_2Cr_2O_7$, H_2SO_4, Et_2O. *ii*) $NaBH_4$, MeOH, C_6H_6. *iii*) *m*-CPBA, CH_2Cl_2



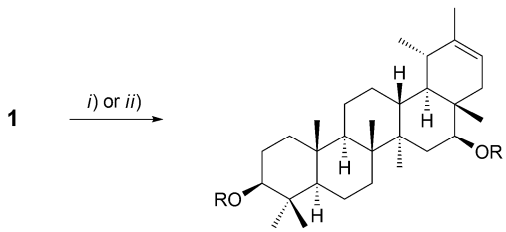
i) HCl, $CHCl_3$. *ii*) Ac_2O, pyridine. *iii*) KOH, MeOH. *iv*) MnO_2, $CHCl_3$. *v*) $K_2Cr_2O_7$, H_2SO_4, Et_2O.



i) 1. $HClO_4$, THF; 2. Ac_2O, pyridine. *ii*) KOH, MeOH.



i) Ac_2O, pyridine. *ii*) MnO_2, $CHCl_3$. *iii*) *m*-CPBA, $CHCl_3$. *iv*) KOH, MeOH.



29 R = $C(O)CH_2CH_2COOH$
30 R = $C(O)CH_2C(Me)_2COOH$
31 R = $C(O)CH(Me)NH_2$

i) Succinic anhydride (for **29**), dimethylsuccinic anhydride (for **30**), DMAP, pyridine.
ii) 1. Boc-L-Ala, HOBt, WSCD, DMF; 2. HCl, 1,4-dioxane (for **31**).