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The absolute stereostructures of three rare D:B-friedobaccharane skeleton triterpenes from the leaves of *Salacia chinensis*

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ABSTRACT

Three new rare D:B-friedobaccharane skeleton triterpenes named foliasalacins D_1 (**1**), D_2 (**2**), and D_3 (**3**) together with the 20 known ones (**4**–**23**) were isolated from the leaves of *Salacia chinensis* Linn., collected in Thailand. The absolute stereostructures of the D:B-friedobaccharane-type compounds (**1**–**3**) were characterized on the basis of chemical and physiochemical evidences.

1. Introduction

In the course of our characterization studies on bioactive constituents from *Salacia* species,[1–13] we have reported the isolation and absolute stereostructural elucidation of 13 megastigmane glycosides, 7 phenolic glycosides, and 8 triterpenes from the leaves of *Salacia chinensis* Linn. (Hippocrateaceae) together with the 20 known constituents.[14–17] As a continuing study on this herbal medicine, three new triterpenes possessing the rare D:B-friedobaccharane skeleton, named foliasalacins D_1 (**1**), D_2 (**2**), and D_3 (**3**) were isolated together with the 20 known ones (**4**–**23**). This paper deals with the absolute stereostructural elucidation of these three new constituents (**1**–**3**).

2. Results and discussion

The dried leaves of *S. chinensis* Linn. collected in Thailand were finely cut and extracted with methanol (MeOH) under reflux to provide a methanolic extract (13.0%). The MeOH extract was partitioned into an EtOAc–H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (4.1%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (2.4%) and an H_2O-soluble fraction (6.6%). The EtOAc-soluble fraction was subjected to normal- and reversed-phase silica gel column chromatographies, and finally to HPLC to give three new triterpenes, foliasalacins D_1 (**1**, 0.00042%), D_2 (**2**, 0.00025%), and D_3 (**3**, 0.00024%) (Chart 1), together with the 20 known ones, 3β-hydroxy-20-oxo-30-norlupane (**4**, 0.0028%),[18] 29-norlupan-3,20-dione (**5**, 0.00004%),[19] lup-20(29)-en-3β,15α-diol (**6**, 0.00053%),[20] betulin (**7**, 0.0013%),[21] lup-20(29)-en-3-on-28-ol (**8**, 0.00007%),[22] betulinic acid (**9**, 0.00087%),[21] lup-20(29)-ene-3β,30-diol (**10**, 0.00028%),[23] 30-hydroxy lup-20(29)-en-3-one (**11**, 0.014%),[21] 3β,20-dihydroxylupane (**12**, 0.00009%),[24] friedelin (**13**, 0.042%),[21] 4-epifriedelin (**14**, 0.0011%),[25] friedelan-3-one-29-ol (**15**, 0.00009%),[21] octandronol (**16**, 0.00009%),[26] 12β-hydroxy D:A-friedooleanan-3-one (**17**, 0.00004%),[26] oleanoic acid (**18**, 0.00033%),[21] erythrodiol (**19**, 0.00037%),[21] ursolic acid (**20**, 0.00034%),[27] uvaol (**21**, 0.00037%),[21] 19a(*H*)-taraxastane-3β,20α-diol (**22**, 0.00007%),[28] and isoursenol (**23**, 0.00005%)[29] (Chart 2).

Foliasalacin D_1 (**1**), $[\alpha]_D^{23}$ +24.6 ($CHCl_3$), was isolated as a white powder. Absorption for hydroxyl (3420 cm^{-1}), double bond (1640 cm^{-1}), and ether function (1078 cm^{-1}) was revealed in the IR spectrum. The ^1H ($CDCl_3$) and ^{13}C NMR (Table 1) spectra[30] of **1** indicated the presence of 8 methyl groups [δ 0.87, 0.91, 0.98, 1.04, 1.08, 1.14, 1.32, 1.32 (3H each, all s, H_3-28, 25, 26, 23, 27, 24, 29, 30)], a methine bearing an oxygen function [δ 3.47 (1H, t-like, *J*=2.5 Hz, H-3)], a disubstituted double bond [δ 5.58 (1H, d, *J*=15.8 Hz, H-21), 5.64 (1H, dd-like, *J*=6.9, 15.8 Hz, H-20)], and a tri-substituted double bond [δ 5.62 (1H, m, H-6)], together with 9 methylenes, 2 methine, and 6 quaternary carbons. As shown in Figure 1, the ^1H–^1H COSY experiment on **1** indicated the presence of partial structure drawn in bold lines, and in the HMBC experiment, long-range correlations were observed between the following protons and carbons: H-6 and C-8, 10; H_2-18 and C-12, 14, 16, 19; H_2-19 and C-16,

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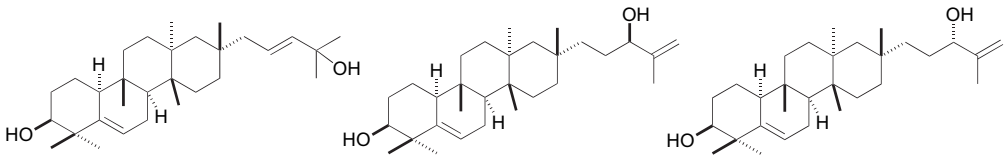
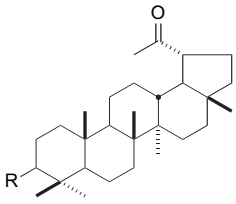


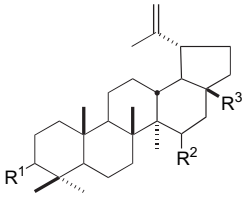
foliasalacin D_1 (**1**) foliasalacin D_2 (**2**) foliasalacin D_3 (**3**)

Chart 1.

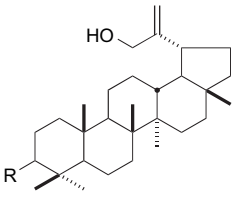
18, 21; H-20 and C-17, 22; H-21 and C-19, 29, 30; H_3-23 and C-3–5, 24; H_3-24 and C-3–5, 23; H_3-25 and C-8–11; H_3-26 and C-8, 13–15; H_3-27 and C-12–14, 18; H_3-28 and C-16–19; H_3-29 and C-21, 22, 30; and H_3-30 and C-21, 22, 29. The spectra of **1** were similar to those of baruol,[31] which was determined as the uncommon natural triterpenoid with D:B-friedobaccharane skeleton. EIMS of **1** exhibited molecular ion peak at *m*/*z* 442 and HREIMS analysis revealed the molecular formula to be $C_{30}H_{50}O_2$. In addition, the EIMS of **1** showed the characteristic fragments at *m*/*z* 152 for $C_{10}H_{16}O$ (i) and 290 for $C_{20}H_{34}O$ (ii) as shown in Figure 2, which were derived from the retro Diels–Alder cleavage, regarded as suggestive of a Δ^5-compound. Consequently, **1** was characterized as D:B-friedo-baccharan-5,20-dien-3β,22-diol. Next, the NOE correlations between the following proton pairs (H-3 and H_3-23, H_3-24, H-2α, H-2β; H-8 and H-10, H_3-27; H-10 and H_3-23; H-18β and H_3-28; H-18α and H_2-19; and H_2-19 and H_3-27) were observed in the NOESY experiment on



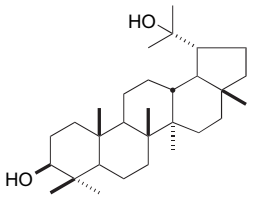
R	
4:	β-OH
5:	=O



	R^1	R^2	R^3
6:	β-OH	α-OH	CH_3
betulin (**7**):	β-OH	H	CH_2OH
8:	=O	H	CH_2OH
betulinic acid (**9**):	β-OH	CH_3	COOH



R	
10:	β-OH
11:	=O



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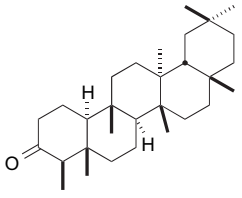
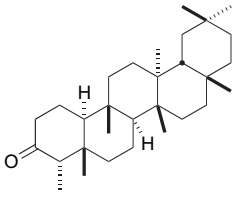
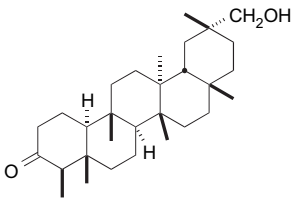


friedelin (**13**)



4-epifriedelin (**14**)



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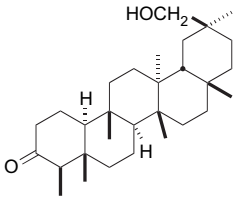
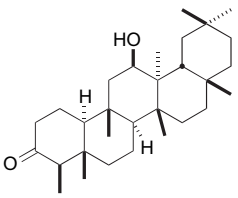


octandronol (**16**)



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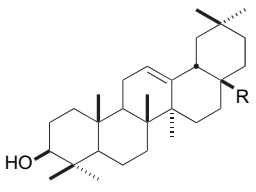
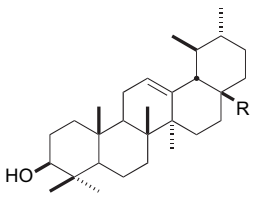
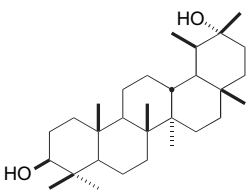


R	
oleanoic acid (**18**):	COOH
erythrodiol (**19**):	CH_2OH



R	
ursolic acid (**20**):	COOH
uvaol (**21**):	CH_2OH



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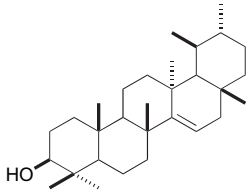
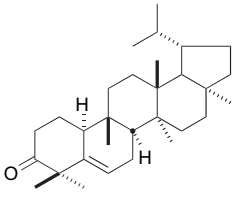


Isoursenol (**23**)



3-dehydrohancokinol

Chart 2.

Table 1
^{13}C NMR data for **1**–**3** and related compound (**1a**)

Position	1	1a	2	3	Position	1	1a	2	3
1	18.1	21.4	18.1	18.1	16	33.7	33.7	34.6	34.7
2	27.7	38.1	27.7	27.8	17	32.3	32.3	31.6	31.6
3	76.3	215.4	76.3	76.3	18	45.3	45.3	44.6	44.4
4	40.8	50.0	40.8	40.8	19	45.5	45.4	38.4	38.6
5	142.0	142.7	142.0	142.0	20	124.2	124.2	29.6	29.7
6	122.0	121.3	122.0	122.0	21	140.7	140.7	76.9	77.0
7	23.7	23.7	23.7	23.7	22	70.8	70.8	147.4	147.4
8	44.6	44.2	44.6	44.6	23	29.0	28.4	29.0	29.0
9	35.5	35.7	35.5	35.5	24	25.5	24.6	25.5	25.5
10	50.0	51.0	50.0	50.0	25	17.5	17.1	17.5	17.5
11	34.2	33.6	34.2	34.2	26	15.2	14.9	15.2	15.2
12	32.7	32.6	32.7	32.8	27	20.6	20.7	20.2	20.2
13	36.6	36.6	36.5	36.5	28	32.9	32.8	32.9	34.0
14	37.8	37.9	37.9	37.9	29	29.9	29.9	111.4	111.3
15	29.2	29.1	29.2	29.2	30	30.0	30.0	17.3	17.3

Measured in $CDCl_3$ at 125 MHz.

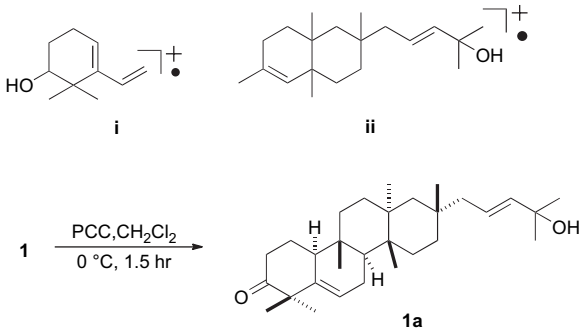


Figure 2.

1. The evidence mentioned above indicated that the steric configurations of the H-8, H-10, Me-4 (H_3-23), Me-13 (H_3-27), and C(19) to be α-axial, while those of Me-9 (H_3-25) and Me-14 (H_3-26) to be β-axial. Furthermore, the steric configuration of the H-3 was determined to be α-equatorial, which was also supported since the proton and carbon signals at A and B rings (H-1–H-10 and H-23–25) of **1** in the ^{1}H and ^{13}C NMR spectra were superimposable on those of baruol[31] with the steric configuration of Hα(equatorial)-3, Hα(axial)-10, and Meβ(axial)-9 (H_3-25). Finally, the oxidation of **1** with pyridinium chlorochromate (PCC) was carried out to give **1a** as the product (Fig. 2). The CD spectrum (MeOH) of **1a** showed Cotton effect at 298 nm ($\Delta\varepsilon$ −0.82) in MeOH and 300 nm ($\Delta\varepsilon$ −0.92) in dioxane, carbonyl n→π*, which was very similar to that of 3-dehydrohancokinol[32] (Chart 2) [295 nm ([θ] −4100) in dioxane] reported by Lou et al. On the basis of the structural similarities of A- and B-ring, the absolute stereostructure at the 10-position in **1a** was elucidated to be in *S* orientation. Consequently, **1** was determined to be (3*S*,8*S*,9*R*,10*S*,13*S*,14*R*,17*S*)-D:B-friedobacchar-5,20-dien-3β,22-diol.

Foliasalacin D_2 (**2**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{29}$ +17.1 in $CHCl_3$). The IR spectrum of **2** showed absorption bands at 3422, 1636, and 1096 cm^{-1} ascribable to hydroxyl, double bond, and ether functions. The ^{1}H ($CDCl_3$) and ^{13}C NMR (Table 1) spectra[30] showed signals assignable to 7 methyls [δ 0.86, 0.89, 0.96, 1.02, 1.05, 1.13, 1.70 (3H each, all s, H_3-28, 25, 26, 23, 27, 24, 30)], 2 methines bearing an oxygen function [δ 3.46 (1H, s-like, H-3), 3.99 (1H, t-like, *J*=6.2 Hz, H-21)], a terminal double bond [δ 4.83, 4.91 (1H each, both br s, H_2-29)], and a tri-substituted double bond [δ 5.59 (1H, m, H-6)], together with 10 methylenes, 2 methines, and 5 quaternary carbons. The ^{1}H–^{1}H COSY experiment on **2** indicated the presence of partial structure drawn in bold lines, and in the HMBC experiment, long-range correlations were observed between the following proton and carbon pairs: H-6 and C-8, 10; H_2-18 and C-14, 16, 19; H_2-19 and C-16, 18; H-21 and C-19, 29, 30; H_3-23 and C-3–5, 24; H_3-24 and C-3–5, 23; H_3-25 and C-8–11; H_3-26 and C-8, 13–15; H_3-27 and C-12–14, 18; H_3-28 and C-16–19; H_3-29 and C-21, 22, 30; and H_3-30 and C-21, 22, 29. Furthermore, the molecular formula $C_{30}H_{50}O_2$ of **2** was determined based on the results of the molecular ion peak at *m*/*z* 442 and HREIMS measurement, and the fragment ion peaks were identical to those of **1**, which were observed at *m*/*z* 152 for $C_{10}H_{16}O$ and 290 for $C_{20}H_{34}O$. In the NOESY experiment, the NOE correlations were observed similar to those of **1** as shown in Figure 1, which suggested that the stereochemistry of **2** was the same as that of **1** except for the side chain. Finally, the (*R*)- and (*S*)-MTPA esters (**2a** and **2b**) were derived from **2** upon reaction with (*R*)- and (*S*)-MTPA in the presence of EDC·HCl and 4-DMAP. The protons on the 29- and 30-position of the (*S*)-MTPA ester (**2b**) resonated at higher field than those of the (*R*)-MTPA ester (**2a**) [Δδ: negative], while the protons on the 8-, 11-, 12-, 15-, 16-, 18–20-, and 26–28-position of **2b** were observed at lower fields compared to those of **2a** [Δδ: positive] (Fig. 3). Consequently, the absolute stereostructure of the 21-position in **2** was elucidated to be in *R* orientation, and the stereostructure of **2** was determined to be (3*S*,8*S*,9*R*,10*S*,13*S*,14*R*,17*S*,21*R*)-D:B-friedobacchar-5,22-dien-3β,21-diol.

Foliasalacin D_3 (**3**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{29}$ +20.8 in $CHCl_3$). Its molecular formula $C_{30}H_{50}O_2$ was determined based on the results of the molecular ion peaks at *m*/*z* 442 and HREIMS measurement. The ^{1}H ($CDCl_3$) and ^{13}C NMR (Table 1) spectra[30] indicated that the stereostructures of A–D rings and the planar structure of the side chain part were the same as those of **2**. Finally, the (*R*)- and (*S*)-MTPA esters (**3a** and **3b**) were

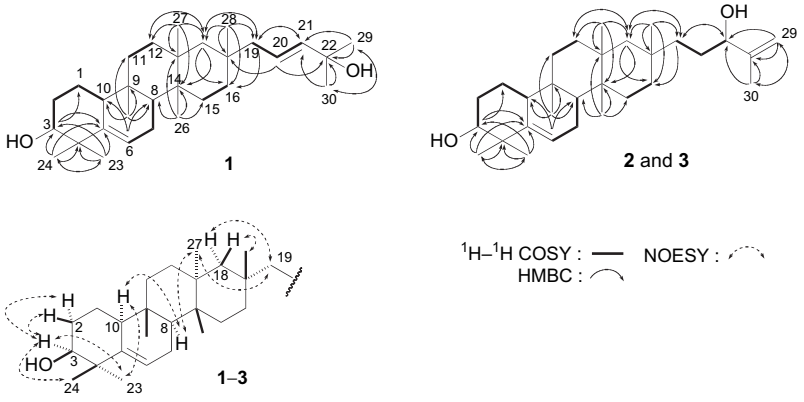


Figure 1. ^{1}H–^{1}H COSY, HMBC, and NOE correlations of **1**–**3**.

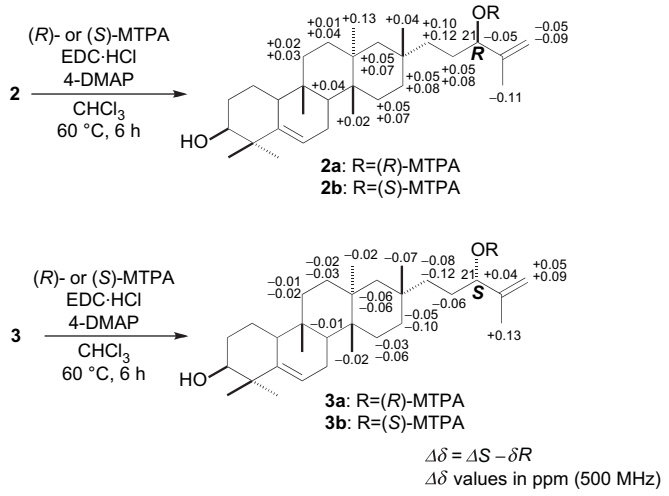


Figure 3. Application of the modified Mosher's method to **2** and **3**.

derived from **3** upon reaction with (*R*)- and (*S*)-MTPA in the presence of EDC·HCl and 4-DMAP. The protons on the 29- and 30-position of the (*S*)-MTPA ester (**3b**) resonated at lower field than those of the (*R*)-MTPA ester (**3a**) [$\Delta\delta$: positive], while the protons on the 8-, 11-, 12-, 15-, 16-, 18–20-, and 26–28-position of **3b** were observed at higher fields compared to those of **3a** [$\Delta\delta$: negative] (Fig. 3). Thus, **3** was elucidated to be the 21-isomer of **2** and the absolute stereochemistry was characterized as shown.

3. Experimental

3.1. General

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); CD spectra, JASCO J-720WI spectrometer; UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H NMR spectra, JEOL JNM-LA500 (500 MHz) spectrometer; ^{13}C NMR spectra, JEOL JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; EIMS and HREIMS, JEOL JMS-GCMATE mass spectrometer; FABMS and HRFABMS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV–vis detectors. HPLC column, Cosmosil 5C_{18}-MS-II (Nacalai Tesque Inc., 250×4.6 mm i.d. and 250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); Diaion HP-20 column chromatography (Nippon Rensui); TLC, pre-coated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

3.2. Plant material

The dried leaves of *S. chinensis* Linn. were collected at Nakhon Si Thammarat province, Thailand in 2006 and identified by Dr. Pongpiriyadacha Y. (Rajamangala University of Technology Srivijaya). A voucher of the plant is on file in our laboratory (2006. Thai-06).

3.3. Extraction and isolation

The dried leaves of *S. chinensis* Linn. (5.8 kg) were finely cut and extracted three times with methanol (MeOH) under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (756 g, 13.0%). The MeOH extract (712 g) was partitioned into an EtOAc–H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (222 g, 4.1%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (130 g, 2.4%) and an H_2O-soluble fraction (361 g, 6.6%). The EtOAc fraction (200 g) was subjected to ordinary-phase silica gel column chromatography [3.8 kg, hexane–EtOAc (40:1 to 10:1 to 5:1 to 1:1, v/v)–$CHCl_3$–MeOH–H_2O (10:3:1, v/v/v, lower layer)–MeOH] to give 16 fractions [Fr. 1 (0.7 g), Fr. 2 (1.3 g), Fr. 3 (28.3 g), Fr. 4 (0.9 g), Fr. 5 (9.0 g), Fr. 6 (14.9 g), Fr. 7 (3.2 g), Fr. 8 (10.7 g), Fr. 9 (9.1 g), Fr. 10 (6.2 g), Fr. 11 (4.2 g), Fr. 12 (13.8 g), Fr. 13 (4.1 g), Fr. 14 (43.2 g), Fr. 15 (16.9 g), and Fr. 16 (26.3 g)]. Fraction 3 (28.3 g) was subjected to ordinary-phase silica gel column chromatography [1.0 kg, hexane–hexane–EtOAc (200:1 to 150:1 to 100:1 to 50:1 to 20:1 to 10:1, v/v)–EtOAc] to afford 16 fractions [Fr. 3-1 (10 mg), Fr. 3-2 (5170 mg), Fr. 3-3 (7570 mg), Fr. 3-4 (790 mg), Fr. 3-5 (1260 mg), Fr. 3-6 (3390 mg), Fr. 3-7 (1020 mg), Fr. 3-8 (3480 mg), Fr. 3-9 (250 mg), Fr. 3-10 (260 mg), Fr. 3-11 (300 mg), Fr. 3-12 (110 mg), Fr. 3-13 (350 mg), Fr. 3-14 (300 mg), Fr. 3-15 (420 mg), and Fr. 3-16 (1800 mg)]. Fr. 3-6 (3390 mg) was further recrystallized with $CHCl_3$–MeOH (1:1, v/v) to give friedelin (**13**, 1860.0 mg, 0.042%). Fraction 4 (0.9 g) was isolated with ordinary-phase silica gel column chromatography [40 g, hexane–hexane–EtOAc (200:1 to 150:1 to 75:1 to 50:1 to 10:1, v/v)–EtOAc] to furnish 13 fractions [Fr. 4-1 (19 mg), Fr. 4-2 (24 mg), Fr. 4-3 (84 mg), Fr. 4-4 (94 mg), Fr. 4-5 (34 mg), Fr. 4-6 (45 mg), Fr. 4-7 (100 mg), Fr. 4-8 (61 mg), Fr. 4-9 (78 mg), Fr. 4-10 (64 mg), Fr. 4-11 (126 mg), Fr. 4-12 (101 mg), and Fr. 4-13 (121 mg)]. Fr. 4-7 (100 mg) was recrystallized with $CHCl_3$–MeOH (1:1, v/v) to give 4-epifriedelin (**14**, 48.8 mg, 0.0011%). Fraction 10 (6.2 g) was subjected to Sephadex LH-20 column chromatography [200 g, MeOH–$CHCl_3$ (1:1, v/v)] to give two fractions [Fr. 10–1 (2400 mg) and Fr. 10–2 (3600 mg)]. Fr. 10-2 (3600 mg) was subjected to reversed-phase silica gel column chromatography [120 g, CH_3CN–H_2O (75:25 to 85:15 to 90:10 to 100:5, v/v)↑–CH_3CN–$CHCl_3$] to give nine fractions [Fr. 10-2-1 (150 mg), Fr. 10-2-2 (164 mg), Fr. 10-2-3 (192 mg), Fr. 10-2-4 (817 mg), Fr. 10-2-5 (204 mg), Fr. 10-2-6 (70 mg), Fr. 10-2-7 (49 mg), Fr. 10-2-8 (159 mg), and Fr. 10-2-9 (583 mg)]. Fr. 10-2-2 (164 mg) was further purified by HPLC [MeOH–H_2O (92:8, v/v)] to furnish betulinic acid (**9**, 28.0 mg, 0.00062%), Fr. 10-2-2-4 (12.8 mg), and Fr. 10-2-2-7 (5.5 mg). Fr. 10-2-2-4 (12.8 mg) was further purified by HPLC [CH_3CN–H_2O (75:25, v/v)] to give lup-20(29)-en-3-on-28-ol (**8**, 3.2 mg, 0.00007%). Fr. 10-2-2-7 (5.5 mg) was purified by HPLC [MeOH–H_2O (88:12, v/v)] to afford 29-norlupan-3,20-dione (**5**, 1.8 mg, 0.00004%). Fr. 10-2-3 (192 mg) was separated with HPLC [MeOH–H_2O (92:8, v/v)] and finally HPLC [MeOH–H_2O (88:12, v/v)] to furnish foliasalacin D_2 (**2**, 2.3 mg, 0.00005%), lup-20(29)-en-3β,15α-diol (**6**, 23.8 mg, 0.00053%), and 30-hydroxylup-20(29)-en-3-one (**11**, 23.5 mg, 0.00052%). Fr. 10-2-4 (817 mg) was subjected to HPLC [MeOH–H_2O (92:8, v/v)] and HPLC [MeOH–H_2O (88:12, v/v)] to give foliasalacins D_1 (**1**, 13.1 mg, 0.00029%), D_2 (**2**, 8.8 mg, 0.00020%), and D_3 (**3**, 10.7 mg, 0.00024%) together with 3β-hydroxy-20-oxo-30-norlupane (**4**, 55.2 mg, 0.0013%), 12β-hydroxy D:A-friedooleanan-3-one (**17**, 1.8 mg, 0.00004%), and 30-hydroxy-lup-20(29)-en-3-one (**11**, 588.8 mg, 0.013%). Fr. 10-2-5 (204 mg) was purified with HPLC [MeOH–H_2O (92:8, v/v)] and finally HPLC [MeOH–H_2O (88:12, v/v)] to afford 3β-hydroxy-20-oxo-30-norlupane (**4**, 16.9 mg, 0.00038%) and octandronol (**16**, 4.2 mg, 0.00009%). Fraction 11 (4.2 g) was subjected to reversed-phase silica gel column chromatography [150 g, MeOH–H_2O (70:30 to 80:20 to 90:10, v/v)–MeOH–$CHCl_3$] to afford five fractions [Fr. 11-1

(45 mg), Fr. 11-2 (54 mg), Fr. 11-3 (40 mg), Fr. 11-4 (799 mg), and Fr. 11-5 (1960 mg)]. Fr. 11-4 (799 mg) was separated by HPLC [MeOH–H_2O (92:8, v/v)] to give 16 fractions [Fr. 11-4-1 (5.0 mg), Fr. 11-4-2 (9.1 mg), Fr. 11-4-3 (7.1 mg), Fr. 11-4-4 (55.9 mg), Fr. 11-4-5 (18.5 mg), Fr. 11-4-6 (107.7 mg), Fr. 11-4-7 (17.7 mg), Fr. 11-4-8 (29.2 mg), Fr. 11-4-9 (34.5 mg), Fr. 11-4-10 (54.7 mg), Fr. 11-4-11 (64.5 mg), Fr. 11-4-12 (78.6 mg), Fr. 11-4-13 (32.2 mg), Fr. 11-4-14 (15.7 mg), Fr. 11-4-15 (18.1 mg), and Fr. 11-4-16 (16.2 mg)]. Frs. 11-4-4 and 11-4-10 were identified as betulin (**7**, 55.9 mg, 0.0012%) and 3β-hydroxy-20-oxo-30-norlupane (**4**, 54.7 mg, 0.0012%). Fr. 11-4-5 (18.5 mg) was purified by HPLC [MeOH–H_2O (88:12, v/v)] to give betulin (**7**, 1.8 mg, 0.00004%) together with betulinic acid (**9**, 11.9 mg, 0.00026%). Fr. 11-4-6 (107.7 mg) was isolated with HPLC [CH_3CN–MeOH–H_2O (65:16:19, v/v/v)] to furnish oleanoic acid (**18**, 14.8 mg, 0.00033%), ursolic acid (**20**, 15.1 mg, 0.00034%), erythrodiol (**19**, 16.5 mg, 0.00037%), uvaol (**21**, 16.2 mg, 0.00037%), and foliasalacin D_1 (**1**, 6.0 mg, 0.00014%). Fr. 11-4-7 (17.7 mg) was separated by HPLC [MeOH–H_2O (88:12, v/v)] to afford isoursenol (**23**, 2.2 mg, 0.00005%). Fr. 11-4-11 (64.5 mg) was isolated with HPLC [CH_3CN–MeOH–H_2O (65:16:19, v/v/v)] and finally HPLC [MeOH–H_2O (88:12, v/v)] to give lup-20(29)-ene-3β,30-diol (**10**, 6.4 mg, 0.00014%). Fr. 11-4-12 (78.6 mg) was subjected to HPLC [CH_3CN–MeOH–H_2O (65:16:19, v/v)] to furnish lup-20(29)-ene-3β,30-diol (**10**, 6.4 mg, 0.00014%) and 30-hydroxylup-20(29)-en-3-one (**11**, 38.2 mg, 0.00085%). Fr. 11-4-14 (15.7 mg) was isolated with HPLC [MeOH–H_2O (88:12, v/v)] to give 19a(*H*)-taraxastane-3β,20α-diol (**22**, 3.0 mg, 0.00007%). Fr. 11-4-15 (18.1 mg) was further subjected to HPLC [MeOH–H_2O (88:12, v/v)] to afford friedelan-3-one-29-ol (**15**, 3.8 mg, 0.00009%) and 3β,20-dihydroxy lupane (**12**, 4.0 mg, 0.00009%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, ^1H NMR, ^{13}C NMR, and MS) with those of reported values.

3.3.1. *Foliasalacin D_1 (**1**)*

A white powder, $[\alpha]_D^{23}$ +24.6 (*c* 0.60, $CHCl_3$). High-resolution EIMS calcd for $C_{30}H_{50}O_2$ $(M)^+$: 442.3811, found: 442.3814. IR (KBr): 3420, 2932, 2870, 1640, 1456, 1385, 1215, 1096, 972, 756 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.87, 0.91, 0.98, 1.04, 1.08, 1.14, 1.32, 1.32 (3H each, all s, H_3-28, 25, 26, 23, 27, 24, 29, 30), 1.07 (1H, d, *J*=13.7 Hz, H-18α), 1.24 (1H, d, *J*=13.7 Hz, H-18β), 1.43 (1H, dd, *J*=4.1, 11.6 Hz, H-8), 1.96, 2.36 (1H each, both dd, *J*=6.9, 13.7 Hz, H_2-19), 2.08 (1H, m, H-10), 3.47 (1H, t-like, *J*=2.5 Hz, H-3), 5.58 (1H, d, *J*=15.8 Hz, H-21), 5.62 (1H, m, H-6), 5.64 (1H, dd-like, *J*=6.9, 15.8 Hz, H-20). ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C: given in Table 1. EIMS: *m*/*z* 442 (M^+, 2), 424 (10), 406 (63), 343 (27), 325 (100), 290 (8), 191 (67), 152 (18).

3.3.2. *Foliasalacin D_2 (**2**)*

A white powder, $[\alpha]_D^{29}$ +17.1 (*c* 0.43, $CHCl_3$). High-resolution EIMS calcd for $C_{30}H_{50}O_2$ $(M)^+$: 442.3811, found: 442.3806. IR (KBr): 3422, 3069, 3015, 2948, 1636, 1096, 897, 758 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.86, 0.89, 0.96, 1.02, 1.05, 1.13, 1.70 (3H each, all s, H_3-28, 25, 26, 23, 27, 24, 30), 1.05, 1.72 (1H each, both m, H_2-19), 1.07 (1H, d, *J*=14.5 Hz, H-18α), 1.24 (1H, d, *J*=14.5 Hz, H-18β), 1.43 (1H, dd, *J*=4.8, 12.4 Hz, H-8), 2.06 (1H, m, H-10), 3.46 (1H, s-like, H-3), 3.99 (1H, t-like, *J*=6.2 Hz, H-21), 4.83, 4.91 (1H each, both br s, H_2-29), 5.59 (1H, m, H-6). ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C: given in Table 1. EIMS: *m*/*z* 442 (M^+, 6), 424 (23), 406 (89), 391 (33), 343 (10), 325 (58), 290 (71), 275 (23), 257 (37), 203 (33), 152 (40), 134 (100).

3.3.3. *Foliasalacin D_3 (**3**)*

A white powder, $[\alpha]_D^{29}$ +20.8 (*c* 0.53, $CHCl_3$). High-resolution EIMS calcd for $C_{30}H_{50}O_2$ $(M)^+$: 442.3811, found: 442.3803. IR (KBr): 3422, 3069, 3015, 2948, 1636, 1094, 897, 758 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88, 0.91, 0.98, 1.04, 1.05, 1.11, 1.71 (3H each, all s, H_3-28, 25, 26, 23, 27, 24, 30), 1.06 (1H, d, *J*=14.5 Hz, H-18α), 1.18, 1.59 (1H each, both m, H_2-19), 1.25 (1H, d, *J*=14.5 Hz, H-18β), 1.43 (1H, dd, *J*=4.8, 12.4 Hz, H-8), 2.08 (1H, m, H-10), 3.46 (1H, s-like, H-3), 3.99 (1H, t-like, *J*=6.4 Hz, H-21), 4.83, 4.91 (1H each, both br s, H_2-29), 5.61 (1H, m, H-6). ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C: given in Table 1. EIMS: *m*/*z* 442 $[M]^+$ (10), 424 (22), 406 (7), 391 (2), 343 (10), 325 (18), 290 (53), 275 (22), 257 (31), 203 (100), 152 (36), 134 (72).

3.3.4. *PCC oxidation of **1***

A solution of **1** (1.9 mg) in dry CH_2Cl_2 was treated with pyridinium chlorochromate (PCC, 5.0 mg) and the whole mixture was stirred at 0 °C for 1.5 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The EtOAc extract was washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$, and filtrated. Removal of the solvent under reduced pressure gave a crude product, which was purified by silica gel column chromatography [500 mg, hexane–EtOAc (10:1 to 5:1, v/v)] to furnish **1a** (0.6 mg, 32%).

*Compound **1a***. A white powder, $[\alpha]_D^{19}$ +36.6 (*c* 0.02, $CHCl_3$). CD [MeOH, nm, ($\Delta\varepsilon$)]: 298 (−0.82), CD [dioxane, nm, ($\Delta\varepsilon$)]: 300 (−0.92). High-resolution EIMS calcd for $C_{30}H_{50}O_2$ $(M)^+$: 440.3654, found: 440.3651. IR (KBr): 3456, 2928, 1713, 1466, 1377, 1228, 1176, 972, 756 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.87, 0.88, 0.95, 1.10, 1.23, 1.24, 1.33, 1.33 (3H each, all s, H_3-25, 28, 26, 27, 24, 23, 29, 30), 2.41–2.50 (2H, m, H_2-2), 5.58 (1H, d, *J*=15.6 Hz, H-21), 5.67 (1H, m, H-6), 5.64 (1H, dd, *J*=6.8, 15.6 Hz, H-20). ^{13}C NMR data (125 MHz, $CDCl_3$) δ_C: given in Table 1. EIMS: *m*/*z* 440 (M^+, 1), 422 (29), 406 (63), 341 (99), 340 (53), 290 (2), 191 (67), 150 (3), 189 (49), 177 (77), 82 (100).

3.3.5. *Preparation of the (R)-MTPA ester (**2a**) and (S)-MTPA ester (**2b**) from **2***

A solution of **2** (3.6 mg) in dehydrated $CHCl_3$ (1.0 mL) was treated with (*R*)-2-methoxy-2-trifluoromethylphenylacetic acid [(*R*)-MTPA, (31.5 mg)] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 24.9 mg) and 4-dimethyl-aminopyridine (4-DMAP, 10.5 mg), and the mixture was stirred under reflux at 60 °C for 6 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The EtOAc extract was washed successively with 5% HCl, $NaHCO_3$–saturated H_2O, and brine, and dried over Na_2SO_4, and filtered. Removal of the solvent from the filtrate under reduced pressure afforded the residue that was subjected to normal-phase silica gel CC [500 mg, hexane–EtOAc (20:1 to 10:1 to 5:1)] to give **2a** (2.0 mg, 37%). Using a similar procedure, (*S*)-MTPA ester (**2b**, 1.8 mg, 39%) was obtained from **2** (3.1 mg) with (*S*)-MTPA (30.2 mg), EDC·HCl (26.9 mg), and 4-DMAP (8.8 mg).

*Compound **2a***. Colorless oil. ^1H NMR (500 MHz, $CDCl_3$) δ 0.81, 0.82, 0.90, 0.94, 1.07, 1.15, 1.73 (3H each, all s, H_3-28, 27, 25, 26, 23, 24, 29), 1.51, 1.60 (1H each, both m, H_2-1), 1.67, 1.86 (1H each, both m, H_2-2), 1.87 (2H, m, H_2-7), 1.36 (1H, dd, *J*=4.6, 11.6 Hz, H-8), 2.08 (1H, m, H-10), 1.47, 1.59 (1H each, both m, H_2-11), 0.87, 1.54 (1H each, both m, H_2-12), 1.13, 1.27 (1H each, both m, H_2-15), 1.19, 1.54 (1H each, both m, H_2-16), 0.97, 1.19 (1H each, both d, *J*=14.1 Hz, H_2-18), 1.00, 1.56 (1H each, both m, H_2-19), 1.50, 1.72 (1H each, both m, H_2-20), 3.49 (1H, br s, H-3), 3.55 (3H, s, –COOCH_3), 4.97, 5.05 (1H each, both br s, H_2-30), 5.37 (1H, dd, *J*=5.2, 7.7 Hz, H-21), 5.64 (1H, m, H-6), 7.37–7.52 (5H, m, Ph–H).

*Compound **2b***. Colorless oil. ^1H NMR (500 MHz, $CDCl_3$) δ 0.85, 0.90, 0.95, 0.96, 1.07, 1.15, 1.62 (3H each, all s, H_3-28, 25, 27, 26, 23, 24, 29), 1.51, 1.60 (1H each, both m, H_2-1), 1.67, 1.86 (1H each, both m, H_2-2), 1.87 (2H, m, H_2-7), 1.40 (1H, dd, *J*=4.6, 11.6 Hz, H-8), 2.08 (1H, m, H-10), 1.49, 1.62 (1H each, both m, H_2-11), 0.91, 1.55 (1H each, both m, H_2-12), 1.20, 1.32 (1H each, both m, H_2-15), 1.27, 1.59

(1H each, both m, H_2-16), 1.04, 1.24 (1H each, both d, J=14.0 Hz, H_2-18), 1.10, 1.67 (1H each, both m, H_2-19), 1.58, 1.77 (1H each, both m, H_2-20), 3.49 (1H, br s, H-3), 3.54 (3H, s, –COOCH_3), 4.92, 4.96 (1H each, both br s, H_2-30), 5.32 (1H, dd, J=6.4, 6.4 Hz, H-21), 5.64 (1H, m, H-6), 7.37–7.52 (5H, m, Ph–H).

*3.3.6. Preparation of the (R)-MTPA ester (**3a**) and (S)-MTPA ester (**3b**) from **3***

A solution of **3** (2.1 mg) in dehydrated $CHCl_3$ (1.0 mL) was reacted with (*R*)-MTPA (20.0 mg) in the presence of EDC·HCl (20.0 mg) and 4-DMAP (8.0 mg), and the mixture was stirred under reflux at 60 °C for 6 h. Workup of the reaction mixture as described for **2** gave **3a** (1.2 mg, 38%). Using a similar procedure, (*S*)-MTPA ester (**3b**, 1.3 mg, 42%) was obtained from **3** (2.1 mg) with (*S*)-MTPA (33.2 mg), EDC·HCl (27.1 mg), and 4-DMAP (11.0 mg).

*Compound **3a***. Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85, 0.90, 0.97, 1.00, 1.05, 1.14, 1.59 (3H each, all s, H_3-28, 25, 26, 27, 23, 24, 29), 1.50, 1.59 (1H each, both m, H_2-1), 1.67, 1.85 (1H each, both m, H_2-2), 1.88 (2H, m, H_2-7), 1.42 (1H, dd, J=4.6, 11.9 Hz, H-8), 2.08 (1H, m, H-10), 1.50, 1.60 (1H each, both m, H_2-11), 0.92, 1.58 (1H each, both m, H_2-12), 1.14, 1.34 (1H each, both m, H_2-15), 1.30, 1.60 (1H each, both m, H_2-16), 1.05, 1.28 (1H each, both m, H_2-18), 1.18, 1.70 (1H each, both m, H_2-19), 1.62 (2H, m, H_2-20), 3.49 (1H, br s, H-3), 3.56 (3H, s, –COOCH_3), 4.91, 4.95 (1H each, both br s, H_2-30), 5.30 (1H, dd, J=6.4, 6.4 Hz, H-21), 5.62 (1H, m, H-6), 7.39–7.52 (5H, m, Ph–H).

*Compound **3b***. Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.78, 0.90, 0.95, 0.98, 1.05, 1.14, 1.72 (3H each, all s, H_3-28, 25, 26, 27, 23, 24, 29), 1.50, 1.59 (1H each, both m, H_2-1), 1.67, 1.85 (1H each, both m, H_2-2), 1.88 (2H, m, H_2-7), 1.41 (1H, dd, J=4.3, 12.3 Hz, H-8), 2.08 (1H, m, H-10), 1.48, 1.59 (1H each, both m, H_2-11), 0.89, 1.56 (1H each, both m, H_2-12), 1.08, 1.31 (1H each, both m, H_2-15), 1.20, 1.55 (1H each, both m, H_2-16), 0.99, 1.22 (1H each, both m, H_2-18), 1.06, 1.62 (1H each, both m, H_2-19), 1.56 (2H, m, H_2-20), 3.49 (1H, br s, H-3), 3.54 (3H, s, –COOCH_3), 4.96, 5.04 (1H each, both br s, H_2-30), 5.34 (1H, dd, J=6.9, 6.9 Hz, H-21), 5.62 (1H, m, H-6), 7.39–7.52 (5H, m, Ph–H).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.05.054.

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