Sesquiterpene Lactones from the Roots of *Ferula varia* and Their Cytotoxic Activity

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The ethyl acetate-soluble fraction from a MeOH extract of the roots of *Ferula varia* gave six new sesquiterpene lactones (**1**–**6**) and five known sesquiterpenes (**7**–**11**). Their structures were established on the basis of spectroscopic evidence. The cytotoxic activities of **1**–**11** were evaluated against selected human cancer cell lines. Compound **4** showed significant selective cytotoxicity against multidrug-resistant cancer cells (KB-C2). The cytotoxicities of compounds **1**, **3**, **5**, **8**, and **11** against KB-C2 cells were enhanced in the presence of nontoxic concentrations of colchicine.

The exclusively Old World genus *Ferula* (Umbelliferae) has about 130 species distributed from the Mediterranean region to Central Asia. Several species of this genus have been used in folk medicine as tranquilizers and for the treatment of digestive disorders, rheumatism, headache, arthritis, dizziness, and toothache.[1] Compounds commonly found in this genus include sesquiterpenes (especially daucanes, humulanes, himachalanes, and guaianes),[2–9] sesquiterpene coumarins,[10,11] and dimeric coumarins.[12] The roots of *Ferula varia* (Shrenk) Trautv. (Umbelliferae) are used traditionally in Uzbekistan to treat fever and intestinal parasites and as a mouth rinse.[13] Investigation of the root constituents of *F. varia,* as part of our ongoing studies of Uzbekistan folk medicinal plants, has resulted in the isolation of six new (**1**–**6**) and five known (**7**–**11**) sesquiterpene lactones. We describe herein the isolation and characterization of these compounds. Cytotoxic activity of **1**–**11** against selected human cancer cell lines, including two multidrug-resistant human cancer cell lines, is also described.

Results and Discussion

Dried roots of *F. varia* were extracted with MeOH. The MeOH extract was separated by repeated column chromatography to give six new (**1**–**6**) and five known sesquiterpene lactones (**7**−**11**). The known sesquiterpenes were identified as dehydrooopodin (**7**),[14] oopodin (**8**),[15] spathulenol (**9**),[16] ferupennin L (**10**),[17] and 8α-angeloyloxy-10β-hydroxyslov-3-en-6,12-olide (**11**)[18] by comparison of their physical and spectroscopic data with those reported in the literature.

Compound **1** was a colorless oil. It showed absorbance for OH (3433 cm^{-1}) and carbonyl (1763, 1718 cm^{-1}) groups in the IR spectrum. The HRESIMS of **1** showed the $[M + Na]^+$ ion peak at *m/z* 369.1678, which indicated a molecular formula of $C_{20}H_{26}O_5$. The ^1H NMR spectrum of **1** had signals indicating two vinyl methyl groups [δ_H 1.98 (3H, brd, J = 7.2 Hz, Me-4′) and 1.85 (3H, s, Me-5′)], two tertiary methyl groups [δ_H 1.49 and 0.88 (each 3H, s, Me-13 and 14)], two methylenes [δ_H 2.25, 1.15 (each 1H, m, H_2-9), and 1.92 (2H, m, H_2-8)], two methines [δ_H 2.39 (1H, m, H-5) and 2.91 (1H, m, H-7)], three olefinic protons [δ_H 6.32 (1H, d, J = 9.7 Hz, H-3), 6.06 (1H, q, J = 7.2 Hz, H-3′), and 5.84 (1H, dd, J = 5.6, 9.7 Hz, H-2)], an exomethylene [δ_H 5.48 and 5.24 (each 1H, s, H_2-15)], and two oxygenated methines [δ_H 4.86 (1H, d, J = 5.6 Hz, H-1), and 4.81 (1H, dd, J = 8.1, 9.8 Hz, H-6)] (Table 1). The ^{13}C NMR spectrum of **1** showed 20 carbon signals, including six olefin, four methyl, two methylene, two methine, two carbonyl, a quaternary carbon, and three oxygenated carbons (Table 2). The $^1H-^1H$ COSY spectrum of **1** confirmed the connectivity of C-1 to C-3, and C-5 to C-9. The HMBC spectrum of **1** showed correlations of Me-14 to C-1, C-5, C-9, and C-10; H_2-15 to C-3, C-4, and C-5; H-6 to C-11 and C-12; and Me-13 to C-7 and C-11, which supported a eudesmane skeleton in **1**. An angeloyl group was elucidated from the $^1H-^1H$ COSY correlations between H-3′ and Me-4′, the HMBC correlations of Me-5′ to C-1′, C-2′, and C-3′, and the NOESY correlation of H-3′ to Me-5′. The correlation of H-1 to C-1′ in the HMBC spectrum confirmed that the angeloyl group was at C-1. The relative configuration of **1** was deduced from the NOESY spectrum of **1**. The coupling pattern and J-values of H-6 (dd, $J_{5,6}$ = 9.8 Hz, $J_{6,7}$ = 8.1 Hz), together with the NOE correlation of H-5 to H-8α, indicated that ring B adopts a boat conformation, similar to that of oopodin (**8**).[14] Furthermore, the β-orientation of Me-13 was confirmed by the NOE correlation of H-7 to Me-13. Thus, the structure of **1** was concluded to be as illustrated.

Compound **2** was obtained as a colorless oil with molecular formula $C_{25}H_{32}O_7$ (HRESIMS). The ^1H and ^{13}C NMR spectra of **2** resembled those of **1**; differences were found in the appearance of signals due to an oxygenated methylene (δ_H 4.43, 4.24; δ_C 64.6), an additional angeloyl group, and the absence of a tertiary methyl signal. HMBC correlations of H_2-13 (δ_H 4.43) to C-7, C-11, and C-12 confirmed that C-13 had changed from a methyl to an oxygenated methylene group. The angeloyl groups were located at C-1 and C-13 from the HMBC correlations of H-1 to C-1′ and H_2-13 to C-1″. Accordingly, the structure of compound **2** was elucidated as shown.

The molecular formula of **3** was determined to be $C_{25}H_{32}O_8$ (HRESIMS). The IR spectrum of **3** revealed absorption bands for OH (3428 cm^{-1}) and carbonyl (1776, 1712 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **3** were similar to those of **2** except for the appearance of oxygenated methines [δ_H 3.67 (2H, m); δ_C 52.5, 58.1] instead of the olefinic bond, which indicated that the olefinic bond in **2** was replaced by an epoxide (δ_C 52.5, C-2 and δ_C 58.4, C-3) in **3**. The relative configurations of H-1, H-6, H-7, and H_2-13 were concluded to be the same as those of **2** from the NOE correlations of Me-14 to H-1, H-6, and H-7, and H-7 to H_2-13, as well as the coupling pattern and J-value of H-6 (t, J = 9.9 Hz). The conformation of ring B was considered to be a boat form, as seen

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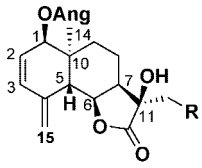

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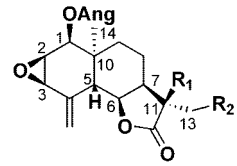

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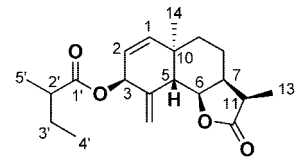

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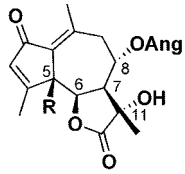

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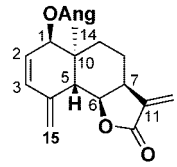

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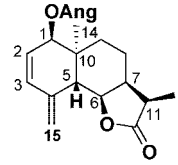

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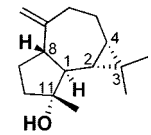

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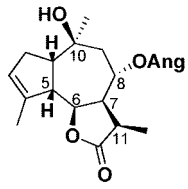

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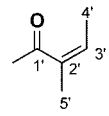

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in compounds **1** and **2**. The NOE correlation of Me-14 to H-2 indicated that H-2 and H-3 are located on the same side of the molecule. Thus, the structure of **3** was determined to be as shown.

Compound **4** showed carbonyl absorbance (1783, 1714 cm^{-1}) in the IR spectrum. The molecular formula of **4** was assigned as $C_{25}H_{32}O_7$ (HRESIMS). The ^1H and ^{13}C NMR spectra of **4** were similar to those of **3**, except for a tertiary methyl signal (δ_H 1.54, δ_C 20.7) in place of the oxygenated methylene signals. The $^1H-^1H$ COSY and HMBC spectrum of **4** supported a eudesmane skeleton and two angeloyl groups in the structure. One of the angeloyl groups was placed at C-1 on the basis of the HMBC correlation of H-1 to C-1′. The other angeloyl group was concluded to be at C-11 from the downfield shift of C-11 as compared with that of **2**. Thus, structure **4** was assigned as illustrated.

The molecular formula of compound **5** was established to be $C_{20}H_{28}O_4$ by HRESIMS. The IR spectrum showed carbonyl absorption bands (1766, 1733 cm^{-1}). The ^1H NMR spectrum of **5** showed signals due to a *cis*-oriented olefinic group [δ_H 5.78 (1H, d, J = 9.4 Hz, H-1) and 5.59 (1H, dd, J = 4.4, 9.4 Hz, H-2)], an exomethylene [δ_H 5.50 and 5.24 (each 1H, s, H_2-15)], two oxygenated methines [δ_H 5.63 (1H, d, J = 4.4 Hz, H-3) and 4.83 (1H, dd, J = 6.0, 9.5 Hz, H-6)], and a tertiary methyl group [δ_H 0.89 (3H, s, Me-14)]. It also showed a secondary methyl group [δ_H 1.27 (3H, d, J = 6.0 Hz, Me-13)] instead of the tertiary methyl group due to Me-13 seen in **1**, along with signals ascribable to a 2-methylbutanoyl group [δ_H 2.34 (1H, sext, J = 7.0 Hz, H-2′), 1.60 and 1.46 (each 1H, m, H-3′), 1.11 (3H, d, J = 7.0 Hz, Me-5′), and 0.89 (3H, t, J = 7.2 Hz, Me-4′)]. The exomethylene signals due to H_2-15 showed HMBC correlations with an oxygenated methine carbon (δ_C 69.9), whereas the tertiary methyl signal (δ_H 0.89) due to Me-14 gave an HMBC correlation with an olefinic carbon (δ_C 143.9). Therefore, the double bond was present at C-1 and C-2 and the oxygenated methine group was at C-3. The 2-methylbutanoyl group was concluded to be at C-3 from the downfield shift of H-3 in the ^1H NMR and the HMBC correlations of H-3 to C-1. The NOE enhancement of Me-14 on irradiation of H-6 suggested that that they are on the same side of the molecule. The coupling constant values of H-6 ($J_{5,6}$ = 9.5 Hz; $J_{6,7}$ = 6.0 Hz) were also similar to those observed in the foregoing compounds. On the other hand, the coupling constant of H-3 (d, J = 4.4 Hz), together with the NOESY correlation from H-3 to H_2-15 (δ_H 5.50 and 5.24), confirmed the β-orientation of H-3. The orientation of Me-13 was concluded to be α from the NOESY correlation between Me-13 and H-5. Consequently, the structure of **5** was assigned as shown.

Compound **6** ($C_{20}H_{24}O_6$ by HREIMS) had OH (3382 cm^{-1}) and carbonyl (1781, 1689 cm^{-1}) absorptions in the IR spectrum. The ^1H NMR spectrum of **6** showed signals for five methyls, a methylene, two methines, two olefinic protons, and two oxygenated methines. The ^{13}C NMR spectrum of **6** had 20 signals, including an olefin, five methyls, a methylene, two methines, and three oxygenated carbons. On the basis of these findings, **6** was assumed to be a guaianolide-type sesquiterpene with an angeloyl group. Except for the absence of an acetoxyl group at C-5, the ^{13}C NMR data for **6** were similar to those of ferupennin L (**10**).[17] Therefore, compound **6** was elucidated to be as shown.

It should be noted that dehydrooopodin (**7**) was considered to possess 6,7-*cis* orientation, like that for the other sesquiterpene lactones isolated from *F. varia* based on the spectroscopic analysis, although the reported structure of it has 6,7-*trans* orientaion.[14]

The sesquiterpenes isolated from *F. varia* were evaluated for their cytotoxic activity against human cancer cell lines, including KB (human epidermoid carcinoma of the nasopharynx), K562 (leukemia), MCF7 (breast carcinoma), and COLO205 (colon carcinoma), as well as the multidrug-resistant human cancer cell lines KB-C2 (colchicine-resistant KB) and K562/Adr (doxorubicin-resistant K562). Compounds **7** and **8** showed moderate cytotoxicities against most tested cell lines, with IC_{50} values ranging from 24.7 to 56.9 μg/mL. Compound **4** demonstrated significant selective cytotoxicity against KB-C2 cells with an IC_{50} value of 15.7 μg/mL, although doxorubicin, tested as a positive control, was nontoxic against the resistant cell lines (>100 μg/mL). Cytotoxicity of **4** against KB-C2 was 4.6 times more potent than that (IC_{50} 72.8 μg/mL) against sensitive cells (KB). Compound **4** was also slightly more cytotoxic against K562/Adr cells than against K562 cells. Compound 8 also showed moderate cytotoxcity against these resistant cell lines (IC_{50} 24.7 and 25.4 μg/mL). Compounds **1**, **3**, **5**, and **11** were nontoxic against all the tested cell lines (IC_{50} values were >100 μg/mL), whereas they showed moderate cytotoxicity against KB-C2 cells with IC_{50} values ranging from 25.4 to 67.8 μg/mL in the presence of 2.5 μM colchicine, whose concentration had no effect on the growth of KB-C2 cells. This data suggested that these compounds have some MDR-reversing effect. Since MDR

Table 1. ^{1}H NMR Data for Compounds **1–7** [400 MHz, δ, ppm (multi., J in Hz)] in $CDCl_3$

position	1	2	3	4	5	6	7
1	4.86 (d, 5.6)	4.86 (d, 5.6)	4.68 (d, 3.7)	4.65 (d, 4.0)	5.78 (d, 9.4)		4.83 (d, 5.6)
2	5.84 (dd, 5.6, 9.7)	5.85 (dd, 5.6, 9.7)	3.67 (m)	3.66 (m)	5.59 (dd, 4.4, 9.4)		5.79 (dd, 5.6, 9.7)
3	6.32 (d, 9.7)	6.31 (d, 9.7)	3.67 (m)	3.66 (m)	5.63 (d, 4.4)	6.18 (m)	6.29 (m)
5	2.91 (d, 9.8)	2.85 (d, 9.8)	2.71 (d, 9.9)	2.55 (d, 11.2)	2.50 (d, 9.5)	4.00 (d, 10.8)	2.35 (d, 9.6)
6	4.81 (dd, 8.1, 9.8)	4.86 (t-like)	4.83 (t, 9.9)	4.99 (dd, 9.8, 11.2)	4.83 (dd, 6.0, 9.5)	4.40 (dd, 7.9, 10.8)	4.88 (dd, 8.0, 9.6)
7	2.39 (m)	2.67 (m)	2.62 (m)	3.09 (m)	2.83 (m)	2.67 (m)	3.30 (m)
8	1.92 (2H, m)	1.96 (2H, m)	1.76 (2H, m)	1.81, 1.74 (m)	1.76, 1.64 (m)	5.74 (ddd, 2.0, 6.6, 8.9)	2.02 (m)
9	2.25, 1.15 (m)	2.24, 1.16 (m)	2.28, 1.06 (m)	1.90, 1.08 (m)	1.72, 1.56 (m)	3.05, 2.67 (m)	1.80 (m), 1.16 (dt, 3.8, 13.6)
11					2.83 (m)		
13	1.49 (s)	4.43, 4.24 (each d, 11.4)	4.41, 4.20 (each d, 11.4)	1.54 (s)	1.27 (d, 6.0)	1.60 (s)	6.29 (m), 5.53 (d, 3.3)
14	0.88 (s)	0.88 (s)	0.86 (s)	0.84 (s)	0.89 (s)	2.32 (s)	0.87 (brs)
15	5.48, 5.24 (s)	5.46, 5.23 (s)	5.59, 5.44 (s)	5.59, 5.52 (each d, 1.9)	5.50, 5.24 (s)	2.28 (s)	5.47 (s), 5.22 (brs)
2′					2.34 (sext, 7.0)		
3′	6.06 (q, 7.2)	6.16 (q, 7.2)	6.14 (m)	6.15 (m)	1.60, 1.46 (m)	6.18 (m)	6.05 (q, 7.3)
4′	1.98 (brd, 7.2)	2.00 (d, 7.2)	2.02 (d, 7.3)	2.04 (d, 7.3)	0.89 (t, 7.2)	2.00 (brd, 7.3)	1.92 (d, 7.2)
5′	1.85 (s)	1.90 (s)	1.92 (s)	1.94 (s)	1.11 (d, 7.0)	1.90 (brs)	1.80 (s)
3″		6.06 (q, 7.2)	6.14 (m)	6.15 (m)			
4″		1.98 (d, 7.2)	1.98 (d, 7.2)	1.99 (d, 7.3)			
5″		1.85 (s)	1.89 (s)	1.88 (s)			

Table 2. ^{13}C NMR Data for Compounds **1–7** (100 MHz, δ, ppm) in $CDCl_3$

position	1	2	3	4	5	6	7
1	73.4	73.4	73.6	74.8	143.9	130.5	73.1
2	123.1	123.1	52.5	52.5	121.3	195.5	122.7
3	134.5	134.4	58.1	58.4	69.9	135.6	134.3
4	141.9	141.7	140.9	139.8	140.7	171.0	140.6
5	42.6	43.0	40.1	38.2	45.3	49.3	43.5
6	79.4	79.4	77.5	75.0	76.2	80.4	78.0
7	42.6	38.6	37.7	40.6	35.9	51.7	39.4
8	17.7	17.5	17.4	19.1	19.2	67.0	19.3
9	29.7	30.0	29.7	30.9	34.1	41.5	27.3
10	37.0	36.8	38.5	36.6	36.4	146.4	36.8
11	74.6	75.5	75.3	79.0	37.9	73.0	137.2
12	178.1	175.8	175.5	174.8	179.5	176.1	169.9
13	24.0	64.6	64.6	20.7	11.9	24.2	119.6
14	17.7	18.0	18.9	20.8	21.8	20.9	17.0
15	117.1	117.2	118.1	118.2	115.8	20.1	117.2
1′	167.3	167.3	167.3	167.1	176.2	167.0	167.0
2′	127.8	126.8	127.4	127.1	40.9	127.0	127.5
3′	138.7	140.1	139.4	139.4	27.0	140.4	138.4
4′	15.8	15.9	15.9	15.8	11.4	16.0	15.7
5′	20.6	20.4	20.6	20.6	16.4	20.5	20.5
1″		167.1	167.1	166.6			
2″		127.7	126.8	126.8			
3″		138.7	140.1	140.5			
4″		15.8	15.9	16.0			
5″		20.6	20.5	20.3			

inhibitors are known to inhibit the efflux of anticancer drugs by interfering with P-glycoprotein (P-gp) function, compounds **3**, **4**, and **11** were also examined for their effects on P-gp function in KB-C2 cells. First, we examined the effects on rhodamine 123 accumulation in P-gp-overexpressing KB-C2 cells. Verapamil, a modulator of P-gp function, showed an increase of rhodamine 123 accumulation by 32% at 2 μM and by 80% at 5 μM. Compounds **3**, **4**, and **11** increased rhodamine 123 accumulation to 169, 134, and 114%, respectively, at 50 μM. Next, we examined the effects on rhodamine 123 effluxes from KB-C2 cells. Thus, after rhodamine 123 was accumulated in the KB-C2 cells, the cells were further incubated with new medium in the absence of rhodamine 123. The effluxes of rhodamine 123 were evaluated by measuring the remaining rhodamine 123 in the KB-C2 cells at 60 min. These compounds, however, showed no effect on the efflux of rhodamine 123 from KB-C2 cells at 60 min, suggesting that these sesquiterpenes changed the toxicity of colchicine against KB-C2 cells by other than interfering with P-gp function.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO Fourier transform infrared spectrometer (FT-IR 420). NMR spectra were measured on a Bruker AVANCE-400 instrument (^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz) using TMS as internal standard. MS was obtained on a JEOL SX-102A instrument and a Waters LCT Premier. Column chromatography: silica gel 60 (Merck, 63–210 μm) and Toyopearl HW-40 (TOSHO); HPLC: GPC (Shodex H-2001, 2002, $CHCl_3$), silica gel (YMC-pack SIL-06 Sh-043-5-06, 250 Shodex H-2001 20 mm).

Plant Material. Roots of *Ferula varia* were collected in Kyzylkum, Uzbekistan, in April 2001. Herbarium specimens (ESM-01ky-10) are deposited in the herbarium of the Graduate School of Pharmaceutical Sciences, Kyoto University.

Extraction and Isolation. The dried roots of *F. varia* (1.4 kg) were crushed and extracted with MeOH for 4 h at 60 °C three times. The MeOH extracts were concentrated *in vacuo* to give a gum (227 g), which was partitioned between EtOAc and H_2O, and then BuOH and H_2O. The EtOAc layer was concentrated to a residue (112 g), which was fractionated by column chromatography (CC) over silica gel using solvents of increasing polarity [3 kg, 10 × 65 cm, *n*-hexane−EtOAc (5:1) to EtOAc−MeOH (7:3)] to give fractions 1–11. Fraction 4 (14 g) was subjected to silica gel CC [481 g, 7 × 25 cm, hexane−$CHCl_3$ (1:1) to $CHCl_3$−MeOH (1:1)] to

Table 3. Cytotoxicity (IC_{50}[a] in μg/mL) of Compounds **3**, **4**, **8**, and **11** against Multidrug-Resistant Human Cancer Cell Lines[b] with or without Colchicine (for KB-C2) *in Vitro*

	KB	KB-C2	KB-C2 (+2.5 μM col.)	K562	K562/Adr
3	91.4 ± 5.3	98.0 ± 6.4	48.3 ± 5.4	>100	>100
4	72.8 ± 3.6	15.7 ± 0.86	26.5 ± 2.0	>100	52.1 ± 4.0
7	46.1 ± 2.2	56.9 ± 2.2	55.8 ± 2.0	36.8 ± 2.3	>100
8	34.7 ± 0.77	24.7 ± 0.39	13.8 ± 0.79	35.1 ± 0.97	25.4 ± 0.36
11	>100	>100	25.4 ± 2.2	>100	>100
doxorubicin	0.22 ± 0.01	>100		0.45 ± 0.01	15.27 ± 0.43

[a] Data are mean ± SE from three experiments. [b] Cell lines: KB (human epidermoid carcinoma of the nasopharynx), KB-C2 (multidrug-resistant KB), K562 (leukemia), K562/Adr (multidrug-resistant K562). col. = colchicine.

yield 12 fractions (4.1–4.12). Toyopearl CC [3 × 20 cm, $CHCl_3$−MeOH (2:1)] and HPLC [silica gel, hexane−EtOAc (2:1)] of fraction 4.6 (455 mg) yielded dehydrooopodin (**7**, 185 mg). Toyopearl [7 × 25 cm, $CHCl_3$−MeOH (2:1)] and silica gel [393 g, 5 × 40 cm, $CHCl_3$−MeOH (99:1 to 7:3), and 106 g, 3 × 30 cm, hexane−EtOAc (3:1 to 1:2)] CC of fraction 4.7 afforded six fractions (4.7.1–4.7.6) and oopodin (**8**, 52 mg). Spathulenol (**9**, 9 mg) was isolated from fraction 4.7.2 by Toyopearl CC [3 × 30 cm, $CHCl_3$−MeOH (2:1)]. Fraction 4.9 was separated by Toyopearl CC [7 × 25 cm, $CHCl_3$−MeOH (2:1)] to give seven fractions (4.9.1–4.9.7). Fraction 4.9.5 was repeatedly chromatographed over silica gel [112 g, 3 × 30 cm, $CHCl_3$−MeOH (100:0 to 7:3)], Toyopearl [3 × 20 cm, $CHCl_3$−MeOH (2:1)], silica gel [55 g, 3 × 15 cm, hexane−EtOAc (1:1 to 1:3)], HPLC [silica gel, $CHCl_3$−MeOH (99:1)], GPC ($CHCl_3$), and HPLC [silica gel, hexane−EtOAc (3:1)] to give **2** (11 mg) and **4** (26 mg). Compound **5** (4 mg) was obtained from fraction 4.9.6 by repeated CC on silica gel [294 g, 5 × 30 cm, hexane−EtOAc (1:1 to 1:3), and 35 g, 3 × 10 cm, $CHCl_3$−MeOH (99.5:0.5 to 8:2)], Toyopearl [3 × 20 cm, $CHCl_3$−MeOH (2:1)], HPLC [silica gel, $CHCl_3$−MeOH (99.5:0.5), and hexane−EtOAc (2:1)], and GPC ($CHCl_3$). Fraction 4.9.7 afforded five fractions (4.9.7.1–4.9.7.5) by CC on silica gel [111 g, 3 × 30 cm, hexane−EtOAc (3:1 to 1:2)]. Fraction 4.9.7.1 was purified by HPLC [silica gel, $CHCl_3$−MeOH (99.5:0.5)] and GPC ($CHCl_3$) to give **1** (2 mg). Fraction 4.9.7.2 was subjected to HPLC [silica gel, $CHCl_3$−MeOH (99.5: 0.5)] and then purified by PTLC [$CHCl_3$−MeOH (99:1)] to give 8α-angeloyloxy-10β-hydroxyslov-3-en-6,12-olide (**11**, 5 mg) and **3** (11 mg). Chromatography of fraction 7 on Toyopearl [7 × 25 cm, $CHCl_3$−MeOH (2:1)] and silica gel [478 g, 7 × 25 cm, $CHCl_3$−MeOH (100:0 to 8:2), and 59 g, 3 × 15 cm, hexane−EtOAc (2:1 to 1:3)] afforded five fractions (7.1–7.5). Preparative HPLC [silica gel, hexane−EtOAc (2:1)] of fraction 7.2 yielded **6** (9 mg). Fraction 7.4 was purified by HPLC [silica gel, hexane−EtOAc (1:1)] to give ferupennin L (**10**, 16 mg). The configurations of **1**–**11** as illustrated are relative rather than absolute.

Compound 1: colorless oil; $[\alpha]_D$ −235.7 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3433, 1763, 1718 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 369.1678 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_5Na$, 369.1780).

Compound 2: colorless oil; $[\alpha]_D$ −234.2 (*c* 1.8, $CHCl_3$); IR (KBr) ν_{max} 3424, 1776, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 467.2022 $[M + Na]^+$ (calcd for $C_{25}H_{32}O_7Na$, 467.2046).

Compound 3: pale yellowish oil; $[\alpha]_D$ −122.9 (*c* 1.1, MeOH); IR (KBr) ν_{max} 3428, 1776, 1712 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 483.1989 $[M + Na]^+$ (calcd for $C_{25}H_{32}O_8Na$, 483.1995).

Compound 4: colorless oil; $[\alpha]_D$ −184.4 (*c* 2.5, $CHCl_3$); IR (KBr) ν_{max} 1783, 1714 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 467.2026 $[M + Na]^+$ (calcd for $C_{25}H_{32}O_7Na$, 467.2046).

Compound 5: colorless oil; $[\alpha]_D$ −34.8 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max}1766, 1733 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 355.1873 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_4Na$, 355.1988).

Compound 6: colorless oil; $[\alpha]_D$ +39.1 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3382, 1781, 1689 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 360.1556 $[M]^+$ (calcd for $C_{20}H_{24}O_6$, 360.1573).

Cell Lines and Cell Culture. Cell culture was performed as described previously.[19] KB (human epidermoid carcinoma of the nasopharynx), MCF7 (breast carcinoma), COLO205 (colon carcinoma), K562 (leukemia), and K562/Adr (multidrug-resistant human erythromyelogenous leukemia) cells were obtained from the Cell Resource Center for Biomedical Research (Tohoku University). Multidrug-resistant human epidermoid carcinoma KB-C2 cells were kindly provided by Prof. Shin-ichi Akiyama (Kagoshima University, Japan). KB cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS). KB-C2 cells were maintained in DMEM medium in the presence of 10% FBS and 5 μM colchicine. MCF7, COLO205, and K562 cells were cultured in RPMI1640 supplemented with 10% FBS. K562/Adr (doxorubicin-resistant K562 cell line) cells were cultured in RPMI1640 medium containing 10% FBS and 0.5 μM doxoruibicin. All cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2−95% air.

Cytotoxicity. Cytotoxicity assays were performed as described previously.[19]

Cellular Accumulation of P-gp Substrates. Cellular accumulation of P-gp substrates was measured as described previously.[20,21]

Efflux of Rhodamine 123. Following previous reports,[20,21] the efflux of rhodamine 123 was also measured using cells incubated with 20 μM rhodamine 123 and samples for 2 h as described above. Cells were then washed twice with DMEM without serum to remove the fluorescence probe from the medium and were incubated again with the medium in the presence or absence of test compounds. After various incubation times, the cells were washed twice with an excess volume of ice-cold PBS and lysed with 0.1% Triton X-100 (in PBS), and the fluorescence intensity was measured as described above. The amount of rhodamine 123 retained in the cells was recorded.

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