Triterpenoids from the Floral Spikes of *Betula platyphylla* var. *japonica* and Their Reversing Activity against Multidrug-Resistant Cancer Cells

Yoshiki Kashiwada,*,[†] Michiko Sekiya,[‡] Kimihisa Yamazaki,[‡] Yasumasa Ikeshiro,[‡] Toshihiro Fujioka,[§] Takashi Yamagishi,[⊥] Shuji Kitagawa,[‖] and Yoshihisa Takaishi[†]

Graduate School of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima 770-8505, Japan, Faculty of Pharmaceutical Sciences, Niigata University of Pharmacy and Applied Life Sciences, Higashijima 265-1, Niigata 956-8603, Japan, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814-0180, Japan, Kitami Institute of Technology, Kitami, Hokkaido 090, Japan, and Kobe Pharmaceutical University, Motoyamakita-machi 4-19-1, Higashinada-ku, Kobe 658-8558, Japan

Four new triterpenes, together with 16 known triterpenes, were isolated from the floral spikes of *Betula platyphylla* var. *japonica* in a search for compounds capable of reversing multidrug resistance in cancer cells. The structures of the new triterpenes were elucidated as 3,4-*seco*-olean-4(23),13(18)-dien-3-oic acid (**1**), 3,4-*seco*-urs-4(23),20(30)-dien-3-oic acid (**2**), 3-*O*-methylmalonylepiocotillol II (**6**), and 3-*O*-methylmalonylcabraleahydroxylactone (**16**) by spectroscopic examination. The cytotoxicity of the isolated triterpenes against human cancer cell lines as well as multidrug-resistant cancer cell lines was evaluated. Most of the isolated triterpenes showed very weak cellular toxicities. Although no discernible differences were found in the cytotoxicities for the tested compounds against sensitive and resistant cell lines, the cytotoxicities for several triterpenes against multidrug-resistant cancer cell lines (KB-C2 or K562/Adr) were enhanced in the presence of nontoxic concentrations of colchicine or doxorubicin. Compound **10** reversed the cytotoxicity of colchicine against KB-C2 cells at 8.1 μM and showed comparable potency to 5 μM verapamil.

One of the major problems of cancer chemotherapy is intrinsic or acquired drug resistance, and searching to overcome drug resistance has been a major effort in clinical oncology.[1] A primary mechanism of multiple-drug resistance (MDR) is due to the overexpression of P-glycoprotein (P-gp) in the plasma membrane of resistant cells, which mediates the efflux of MDR drugs, reducing intracellular accumulation of anticancer drugs.[2] A variety of compounds have been found that modify, modulate, or reverse the MDR phenotype, including verapamil, nifedipine, trifluoperazine, progesterone, quinidine, cyclosporin, and some tetracyclines. In addition to these compounds, synthetic derivatives, including SDZPSC833, SDZ 280-446, XR9051, GF120918, and ardeemin, were also found to be effective in reducing MDR.[3] Among others, verapamil, trifluoperazine, cyclosporin A, and nifedipine have been subjected to clinical trials.[4,5] The use of cancer chemotherapeutic agents in combination with these MDR-reversing agents, however, has frequently shown a lack of desired effectiveness and may cause unacceptable side effects or toxicity at the concentration needed to reverse MDR.[4−6] Thus, there still remains a need to develop new classes of MDR-reversing agents with less toxicity.

Betula platyphylla Sukatchev var. *japonica* (Miq.) Hara is widely distributed in Japan, mainland China, Korea, and eastern Russia, and its bark has been used in Chinese traditional medicine for the treatment of pneumonia, choloplania, nephritis, and chronic bronchitis.[7] Betulin, together with several related triterpenes, is a well-known constituent of the outer bark of *Betula* spp., while the isolation of diarylheptanoids and arylbutanoids from their inner bark and dammarane-type triterpenes from the leaves, the root bark, and the pollen has been reported.[8−17]

During a continuing search for plant-derived MDR-reversing agents from natural sources, a MeOH extract of the floral spikes of *B. platyphylla* var. *japonica* was found to potentiate the activities of anticancer drugs in multidrug-resistant KB-C2 cells at nontoxic

concentrations. Thus, the MeOH extract showed a weak toxicity against KB-C2 cells with an IC_{50} value of 50.9 μg/mL in the presence of a nontoxic concentration (2.5 μM) of colchicine, although it was nontoxic against KB and KB-C2 cells (>100 μg/mL).

Bioassay-guided fractionation and repeated chromatography of this extract has led to the isolation of two new secotriterpenoids (**1** and **2**) and methylmalonyl esters of a trisnortriterpenoid (**16**) and a triterpenoid (**6**), together with 16 known triterpenoids (**3−5, 7−15, 17−20**). We report herein the structure elucidation of these new triterpenoids and the MDR-reversing activity for all of the triterpenoids isolated from the floral spikes of *B. platyphylla* var. *japonica*.

Results and Discussion

The floral spikes of *B. platyphylla* var. *japonica* were extracted with MeOH at room temperature. The MeOH extract was partitioned successively between EtOAc, BuOH, and water. The EtOAc-soluble portion was further partitioned between hexane and 90% MeOH. Among these fractions, the hexane-soluble fraction was not cytotoxic against KB-C2 cells, having an IC_{50} value of >100 μg/mL. However, it showed cytotoxicity against KB-C2 cells with an IC_{50} value of 31.0 μg/mL in the presence of a noncytotoxic concentration of colchicine (2.5 μM), suggesting the presence of an MDR-reversing compound in this fraction. Repeated silica gel chromatography and semipreparative HPLC has led to the isolation of two new secotriterpenoids (**1** and **2**) and two new methylmalonyl esters of a trisnortriterpenoid (**16**) and a triterpenoid (**6**), together with 14 known triterpenoids (**3, 4, 7, 8, 10−15, 17−20**). Two known triterpenoids (**5** and **9**) were also isolated from the 90% MeOH-soluble fraction. The known triterpenoids, 3-epiocotillol II (**3**),[10] 3-epiocotillol II acetate (**4**),[10] 3-*O*-malonylepiocotillol II (**5**),[18] ocotillone (**7**),[19] 12β-acetoxy-20(*S*),24(*R*)-epoxy-3α,25-dihydroxydammarane (**8**),[20] papyriferic acid (**9**),[21] methyl papyriferate (**10**),[22] 3α,12β-diacetoxy-20(*S*),24(*R*)-epoxy-25-hydroxydammarane (**11**),[23] cabraleone (**12**),[19] methyl shoreate (**13**),[24] cabralelactone (**14**),[24] cabraleahydroxylactone (**15**),[24] betulonic acid (**17**),[25] hydroxyhopanone (**18**),[15] 20(*S*),24(*S*)-dihydroxydammara-26-en-3-one (**19**),[26] and hydroxydammarenone II (**20**),[27] were identified as shown by

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* To whom correspondence should be addressed. Tel/Fax: +81-88-633-7276. E-mail: kasiwada@ph.tokushima-u.ac.jp.
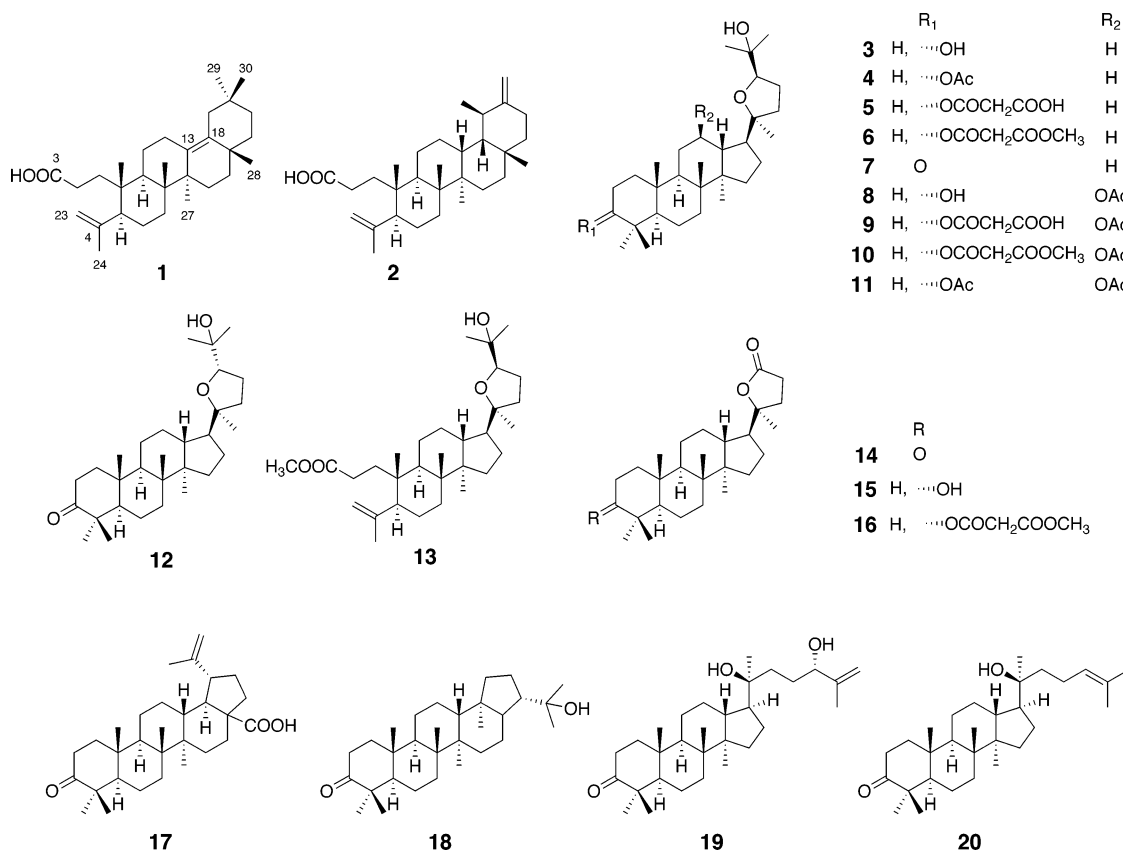† University of Tokushima.
‡ Niigata University of Pharmacy and Applied Sciences.
§ Fukuoka University.
⊥ Kitami Institute of Technology.
‖ Kobe Pharmaceutical University.

Chart 1

comparison of their physical and spectroscopic data with those reported in the literature.

Compound **1** displayed an M$^+$ ion peak in the positive FABMS at *m/z* 440, and its molecular formula of $C_{30}H_{48}O_2$ was determined by positive HRFABMS. The ^1H NMR spectrum of **1** revealed the presence of six tertiary methyls (δ 0.75, 0.88, 0.90, 0.97, 1.04, 1.20), a vinyl methyl (δ 1.80), and an exomethylene [δ 4.87, 4.96 (each br s)]. The presence of a carboxylic acid (δ_C 176.5) and a tetrasubstituted double bond (δ_C 133.4 and 134.9) was shown by the ^{13}C NMR resonances. The occurrence of a 3,4-*seco*-type triterpene skeleton was deduced from the following HMBC correlations: δ_H 4.87 and 4.96 (C=CH_2) and 1.80 (C=C−CH_3) with δ_C 50.2 (CH); δ_H 0.88 (CH_3-C) with δ_C 35.2 (CH$_2$), 39.8 (C), 40.9 (CH), and 50.2 (CH); δ_H 1.91 and 1.96 (CH_2) and 2.56 and 2.68 (CH_2) with δ_C 176.5 (COOH). Further detailed $^1H-^1H$ COSY, HSQC, and HMBC spectroscopic analysis indicated that **1** is a 3,4-*seco*-4(24)-oleanene-type triterpenoid (Table 1). The carbon resonance at δ_C 134.9 showed HMBC correlations with H-12, H-19, and Me-27, with other HMBC correlations between H-12, H-16, H-19, and Me-28 and δ_C 133.4 also being observed. Thus, the location of the tetrasubstituted double bond was concluded to be at C-13 and C-18. The NOE correlations from Me-26 and Me-25 to Me-28 suggested the stereochemistry for **1** is the same as that of oleanolic acid. On the basis of the evidence described above, the structure of **1** was assigned as 3,4-*seco*-olean-4(23),13(18)-dien-3-oic acid (**1**).

Compound **2** exhibited an [M − H]$^-$ ion peak at *m/z* 439 in the negative FABMS, and its molecular formula ($C_{30}H_{48}O_2$), confirmed by negative HRFABMS, was identical with that of **1**. The ^1H NMR spectrum exhibited the presence of four tertiary methyls (δ 0.88, 0.90, 0.95, and 1.02), a secondary methyl [δ 1.04 (d, *J* = 6.5 Hz)], a vinyl methyl (δ 1.80), and two exomethylenes [δ 4.72, 4.77, 4.88, and 4.96 (each br s)]. The occurrence of a carboxylic acid group was also revealed by the carbon resonance at δ_C 176.5. Detailed interpretation of the $^1H-^1H$ COSY, HSQC, and HMBC data

indicated that **2** is a 3,4-*sec*o-ursane-type triterpenoid (Table 1). The location of the additional exomethylene group at C-20 was deduced from the HMBC correlations between H$_2$-30 and C-19, C-20, and C-21. The observation of NOE enhancements from H-13 and Me-26 to Me-28 as well as those from Me-27 and H-19 to Me-29 suggested the configurations at H-13, H-18, and Me-29 to be β. From this evidence, the structure of **2** was elucidated as 3,4-*sec*o-urs-4(23),20(30)-dien-3-oic acid (**2**).

Compound **6**, a white, amorphous powder, $[\alpha]_D$ +8.8 (CHCl$_3$), gave ^1H and ^{13}C NMR spectra that resembled those of **5** except for the observation of a methoxyl [δ_H 3.77 (3H, s); δ_C 52.3 (q)] signal. The positive FABMS of **6** showed a quasi-molecular ion peak at *m/z* 583 [M + Na]$^+$, which was 14 mass units more than **5**. These data suggested compound **6** to be a methyl ester of **5**. Treatment of **5** with diazomethane gave a monomethylate, which was identical with **6**.

Compound **16**, a white, amorphous powder, $[\alpha]_D$ +1.2 (CHCl$_3$), gave a quasi-molecular ion peak at *m/z* 539 [M + Na]$^+$ in the positive FABMS, and its molecular formula was confirmed as $C_{31}H_{48}O_6$ by HRFABMS. The ^1H and ^{13}C NMR spectra of **16** showed the presence of a γ-lactone ring and six tertiary methyls, including a methyl adjacent to the lactone ring, for which the features were similar to those of cabraleahydroxylactone (**15**).[24] The observation of additional signals for a methylene [δ_H 3.42 (2H, s); δ_C 41.8 (t)], two ester carbonyls [δ_C 165.9 and 167.2 (s)], and a methoxyl [δ_H 3.77 (3H,s); δ_C 52.3 (q)], together with the downfield-shifted H-3 [δ_H 4.68 (1H, t, *J* = 4 Hz); δ_C 80.1 (d)], as compared with those of **15**, indicated the presence of a methyl malonyl group at the C-3 hydroxyl group of **16**. The structural confirmation was obtained by the preparation of **16** from **15**. Thus, **15** was reacted with malonyl dichloride in the presence of pyridine and then treated with diazomethane to furnish a compound that was identical with **16**. Therefore, the structure of **16** was confirmed as 3-*O*-methyl-malonylcabraleahydroxylactone (**16**).

Table 1. NMR Spectroscopic Data (400 MHz, pyridine-d_5) for Compounds **1** and **2**

		1			2	
position	δ_C	δ_H (*J* in Hz)	HMBC	δ_C	δ_H (*J* in Hz)	HMBC
1	35.2	1.91, 1.96, m	3	35.2	1.95, m	3
2	29.5	2.56, 2.68, m	3	29.4	2.49, 2.65, m	3
3	176.5			176.5		
4	148.0			148.1		
5	50.2	2.15, br d (10.5)	4, 10, 24, 25	50.2	2.11, br d (10)	4, 10, 24, 25
6	25.0	1.35, 1.42, m		24.9	1.35, 1.74, m	
7	33.5	1.31, 1.36, m		32.8	1.28, m	
8	40.9			40.7		6
9	40.9	1.83, m		39.5	1.52, m	25
10	39.8			39.5		
11	22.6	1.53, 1.81, m		22.2	1.23, 1.59, m	
12	25.3	1.80, 2,60, m	13, 18	26.3	1.10, 1.60, m	
13	134.9			40.8	1.66, m	
14	45.3			42.6		
15	26.9	1.72, m		26.9	0.92, m	
16	36.8	1.28, 1.44, m	17, 18	38.4	1.09, 1.21, m	
17	34.8			34.7		
18	133.4			48.7	0.95, m	
19	38.9	1.68, d (13.5) 2.28, d (13.5)	13, 18	39.5	2.11, m	
20	33.5			154.8		
21	39.7	1.34, m		25.9	2.19, 2.47, m	
22	35.6	1.11, 1.16, m		39.0	1.33, 1.39, m	18
23	113.8	4.87, 4.96, br s	4, 5, 24	113.7	4.88, 4.96, br s	4, 5, 24
24	24.0	1.80, s	4, 5, 23	23.6	1.80, s	4, 5, 23
25	20.7	0.88, s	1, 5, 9, 10	20.6	0.88, s	1, 5, 9, 10
26	18.2	0.90, s	8, 9, 7, 14	16.1	1.02, s	8, 9, 7, 14
27	21.5	1.20, s	8, 13, 14, 15	14.8	0.95, s	8, 13, 14, 15
28	23.7	1.04, s	16, 17, 18, 22	19.8	0.90, s	16, 17, 18, 22
29	32.4	0.97, s	19, 20, 21, 30	25.5	1.04, d (6.5)	18, 20, 21, 30
30	24.3	0.75, s	19, 20, 21, 29	107.5	4.72, 4.77, br s	19, 20, 21

Compounds **6** and **16** have not been previously reported, though they might be artifacts as a result of using MeOH during the process of extraction and separation.

The cytotoxic activities of compounds **1**−**20** were evaluated against a variety of human cancer cell lines, namely, the KB (human epidermoid carcinoma of the nasopharynx), K562 (leukemia), MCF7 (breast carcinoma), and COLO205 (colon carcinoma) cell lines, as well as two mutidrug-resistant human cancer cell lines, including KB-C2 (cochicine-resistant KB) and K562/Adr (doxorubicin-resistant K562). Among these compounds, **17** showed significant cytotoxic activities against the KB and KB-C2 cell lines with IC$_{50}$ values of 3.8 and 2.3 μM, respectively. In contrast, most of the compounds showed little or no cytotoxicity, and no discernible differences were found in the cytotoxic activities for the test compounds against the sensitive and resistant cell lines. However, some of them showed enhanced cytotoxicity against KB-C2 cells in the presence of 2.5 μM colchicine. At this concentration level, colchicine had no effect on the growth of KB-C2 cells. Thus, while compounds **7**, **12**, **14**, **15**, and **20** were not cytotoxic against KB-C2 cells (IC$_{50}$ values >100 μM), they showed moderate cytotoxic activity, with IC$_{50}$ values ranging from 20.6 to 64.2 μM in the presence of 2.5 μM colchicine. The enhanced cytotoxicity against KB-C2 cells in the presence of 2.5 μM colchicine as compared with those in the absence of colchicine was observed in **1**, **3**, **4**, **6**−**8**, **10**−**17**, **19**, and **20**, suggesting that these triterpenes might show some MDR-reversing effects. In contrast, similar cellular toxicity enhancements against K562/Adr cells in the presence of doxorubicin (1 μM) were observed only for compounds **10** and **16**. In order to elucidate further the MDR-reversing effects of these triterpenes, compounds **1**, **4**, **6**−**8**, **10**−**16**, **19**, and **20** were selected to determine the recovery of the cytotoxicity of colchicine against KB-C2 cells. As shown in Table 3, compound **10** demonstrated the most potent recovery effect. Thus, 5 μg/mL (8.1 μM) of compound **10** showed a comparable MDR-reversing effect to that of 5 μM verapamil. Compounds **14** and **15** also showed some reversing effect at 5 μg/mL (12.0 μM), whereas the other compounds showed weaker effects. Compounds **5** and **9**, both of

which contain a 3-*O*-malonyl ester group, did not enhance the cellular toxicity of colchicine against KB-C2 cells. In contrast, a potent MDR-reversing effect was observed in the methyl ester of **9** (compound **10**), while the methyl ester of **5** (compound **6**) showed a weak MDR-reversing effect. These results suggest that modification of these compounds might yield more potent derivatives, and thus these substances appear to be potential leads as MDR-reversing agents.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ^{1}H and ^{13}C NMR spectra were measured on a JEOL JNM-A-400 spectrometer with TMS as an internal standard. Mass spectra were determined on a JEOL HX-110 spectrometer. Preparative TLC was performed on precoated silica gel 60 F254 plates (1 mm, Merck).

Plant Material. The spikes of male and female flowers of *Betula platyphylla* Sukatchev var. *japonica* (Miq.) Hara were collected in April 1996 in Hokkaido, Japan. Identification was carried out by one of the authors (T.Y.). A voucher specimen (NUPULS-BPJ960401) was deposited at the herbarium of the Faculty of Pharmaceutical Sciences, Niigata University of Pharmacy and Applied Life Sciences.

Extraction and Isolation. The dried spikes of *B. platyphylla* var. *japonica* (1.06 kg) were extracted three times with MeOH at room temperature. The MeOH extract was concentrated under reduced pressure to give a residue (295 g), which was partitioned with EtOAc and H$_2$O. The EtOAc layer, after removal of the solvent by evaporation, was further partitioned with hexane and 90% aqueous MeOH to give a hexane-soluble fraction (58 g) and a 90% MeOH-soluble fraction (120 g). The aqueous layer was subsequently extracted with *n*-BuOH, yielding a *n*-BuOH-soluble fraction (35 g) and a water-soluble fraction (80 g).

The hexane-soluble fraction was subjected to chromatography over silica gel with benzene containing increasing amounts of EtOAc to give eight fractions: 1 (5.0 g), 2 (14.5 g), 3 (5.0 g), 4 (6.8 g), 5 (2.1 g), 6 (6.5 g), 7 (9.5 g), and 8 (12.0 g). Compound **18** (390 mg) was obtained from fraction 3 by crystallization (from EtOAc). The mother liquor of fraction 3 was combined with fraction 2 and was chromato-

Table 2. Cytotoxicity ($IC_{50}{}^{a}$ in μM) of Compounds **1**−**20** against Human Cancer Cell Lines and Multidrug-Resistant Human Cancer Cell Lines[b] with or without Colchicine (for KB-C2) or Doxorubicin (for K562/Adr) *in Vitro*

	KB	KB-C2	KB-C2 (+colchicine 2.5 μM)	K562	K562/Adr	K562/Adr (+doxorubicin 1 μM)	MCF7	COLO205
1	27.5 ± 1.75	58.0 ± 2.00	36.3 ± 3.81	28.0 ± 1.07	25.9 ± 0.57	24.8 ± 0.20	37.4 ± 2.13	38.1 ± 2.04
2	>100	>100	>100	>100	>100	>100	>100	>100
3	43.4 ± 0.48	55.3 ± 1.13	28.6 ± 0.76	48.5 ± 1.63	73.9 ± 2.67	45.2 ± 2.58	57.9 ± 1.91	67.5 ± 3.02
4	44.8 ± 2.55	89.6 ± 1.37	43.6 ± 2.72	41.6 ± 2.63	50.5 ± 0.93	68.3 ± 1.19	47.5 ± 1.95	54.2 ± 0.84
5	60.8 ± 0.79	69.6 ± 1.10	65.5 ± 1.70	57.5 ± 1.61	47.2 ± 0.80	42.5 ± 1.37	64.1 ± 0.99	55.6 ± 0.60
6	64.2 ± 3.07	87.0 ± 0.89	13.6 ± 1.73	35.4 ± 2.64	31.1 ± 1.39	41.1 ± 4.37	63.6 ± 1.57	57.2 ± 1.71
7	>100	>100	64.2 ± 3.25	>100	>100	>100	>100	>100
8	36.5 ± 1.18	25.7 ± 0.56	3.5 ± 0.69	27.5 ± 2.72	15.4 ± 1.10	15.3 ± 1.39	34.2 ± 1.48	36.7 ± 4.07
9	47.8 ± 0.84	66.0 ± 1.77	60.1 ± 1.97	47.6 ± 2.55	42.5 ± 1.12	34.5 ± 3.95	71.7 ± 1.21	52.2 ± 1.12
10	33.8 ± 1.08	31.1 ± 1.24	3.7 ± 1.60	32.6 ± 1.52	36.8 ± 1.49	10.6 ± 2.63	48.9 ± 3.77	49.6 ± 2.36
11	35.1 ± 1.05	62.2 ± 0.61	3.0 ± 0.98	26.2 ± 3.82	33.2 ± 2.60	23.9 ± 1.91	78.4 ± 4.69	58.0 ± 1.98
12	>100	>100	62.4 ± 9.24	>100	>100	>100	>100	>100
13	66.6 ± 3.74	93.5 ± 0.96	20.1 ± 2.17	45.3 ± 2.82	50.9 ± 0.59	47.1 ± 2.31	59.7 ± 2.07	67.7 ± 2.09
14	63.5 ± 0.82	>100	28.5 ± 2.73	>100	>100	>100	97.9 ± 9.07	>100
15	>100	>100	20.6 ± 1.82	>100	>100	>100	>100	>100
16	58.0 ± 4.99	30.4 ± 2.32	3.3 ± 0.77	47.2 ± 5.92	56.1 ± 1.28	26.5 ± 5.07	>100	>100
17	3.8 ± 0.53	2.3 ± 0.26	0.64 ± 0.22	16.1 ± 1.54	73.5 ± 1.65	48.4 ± 5.67	49.0 ± 3.30	36.8 ± 2.68
18	>100	>100	>100	>100	>100	>100	>100	77.1 ± 9.74
19	70.2 ± 1.42	78.5 ± 2.05	29.8 ± 0.85	40.2 ± 0.92	35.9 ± 3.71	50.4 ± 4.75	73.1 ± 2.90	58.8 ± 3.18
20	>100	>100	38.2 ± 0.97	>100	>100	>100	>100	>100

[a] Data are mean ± SE from three or four experiments. [b] Cell lines: KB (human epidermoid carcinoma of the nasopharynx), KB-C2 (multidrug-resistant KB), K562 (leukemia), K562/Adr (multidrug-resistant K562), MCF7 (breast carcinoma), and COLO205 (colon carcinoma).

Table 3. MDR-Reversing Effects of Triterpenes on the Cytotoxicity of Colchicine ($IC_{50}{}^{a}$ in μM) against KB-C2 Cells in the Presence of the Indicated Concentrations of Triterpenes

			IC$_{50}$ value of colchicine			
compound	0	+0.5 μg/mL	+1 μg/mL	+2.5 μg/mL	+5 μg/mL	+10 μg/mL
1			20.6 ± 0.86		19.0 ± 1.60	
4					9.0 ± 0.10	7.0 ± 0.34
6			11.1 ± 0.31	7.3 ± 0.26		
7					8.3 ± 0.19	7.0 ± 0.29
8		8.9 ± 0.23	6.1 ± 0.08			
10			11.2 ± 0.24		0.40 ± 0.02	
11		11.2 ± 0.16	9.0 ± 0.23			
12					7.2 ± 0.49	7.1 ± 0.41
13				10.2 ± 0.39	7.1 ± 0.41	
14				5.7 ± 0.13	3.0 ± 0.15	
15				5.6 ± 0.13	3.0 ± 0.15	
16			15.3 ± 0.47		7.4 ± 0.25	
19			10.2 ± 0.13	8.1 ± 0.09		
20					7.7 ± 0.04	5.9 ± 0.22
colchicine	18.2 ± 0.49					
verapamil (positive control)						
1 μM		4.1 ± 0.21				
2 μM		2.5 ± 0.08				
5 μM		0.42 ± 0.04				

[a] Data are mean ± SE from three or four experiments.

graphed over silica gel [*n*-hexane−EtOAc (10:1 → 1:2)] to yield five fractions (2-1−2-5). Silica gel chromatography of fraction 2-2 with CHCl$_3$−EtOAc (1:0 → 1:1) followed by crystallization (from EtOAc) gave a mixture of **1** and **2**. The mixture was subsequently separated by semipreparative scale HPLC with YMC-Pack R&D ODS [MeOH−2% AcOH (98:2)] to furnish pure samples: **1** (50 mg) and **2** (28 mg). Fraction 2-3 was purified by silica gel chromatography with hexane−acetone (20:1) to afford **19** (62 mg). Compound **17** crystallized from fraction 2-4 (from MeOH) to give a pure sample (510 mg), and the mother liquor was separated by silica gel chromatography [benzene−acetone (50:1 → 10:1)] to furnish three further fractions (2-4-1−2-4-3). Fraction 2-4-1 was subsequently separated into two fractions by silica gel chromatography [toluene−acetone (30:1 → 10:1)], of which the former was purified by preparative TLC [benzene−EtOAc (5:1)] to furnish compound **13** (65 mg), while the latter was separated by HPLC on YMC-Pack ODS (97% MeOH) to yield **12** (16 mg). Preparative HPLC of fraction 2-4-3 by YMC-Pack ODS (97% MeOH) gave **4** (24 mg). Compound **7** (1.55 g) was obtained from fraction 4 by crystallization (from EtOAc), and the mother liquor was subjected to silica gel chromatography [benzene−EtOAc (20:1 → 5:1)] to afford five fractions (4-1−4-5). Fractions 4-2 and 4-4 were separately purified by chromatography on silica gel [benzene−acetone (20:1 → 15:1)] to give **6** (320 mg) and **16** (45 mg), respectively. Fraction 4-3 consisted mainly of compound **14**, and crystallization from MeOH furnished a

pure sample (94 mg). Compound **3** (2.15 g) crystallized from the EtOAc solution of fraction 7, and its mother liquor was chromatographed over silica gel [hexane−acetone (7:1 → 1:1)] to yield five fractions (6-1−6-5). Crystallization of fraction 6-2 gave **15** (128 mg). Fractions 6-3 and 6-5 were separately purified by chromatography over silica gel [benzene−acetone (15:1 → 5:1)] to yield **11** (40 mg) and **10** (145 mg), respectively. Fraction 7 was separated by silica gel chromatography [benzene−acetone (12:1 → 1:1)] to give five fractions (7-1−7-5). Compound **20** (40 mg) crystallized from the EtOAc solution of fraction 7-3, and the mother liquor was repeatedly chromatographed over silica gel [hexane−acetone (3:1 → 1:1) and benzene−acetone (10:1 → 5:1)] to give **8** (225 mg). The 90% MeOH-soluble fraction was separated by chromatography over silica gel [CHCl$_3$−MeOH (40:1 → 4:1)] to give eight fractions (9−17). Fraction 13 was rechromatographed over ODS to give six further fractions (13-1−13-6). Fractions 13-2 and 13-4 were separately chromatographed over silica gel [CHCl$_3$−MeOH (20:1 → 10:1)] to furnish **5** (320 mg) and **9** (463 mg), respectively.

3,4-*seco*-Olean-4(23),13(18)-dien-3-oic acid (1): colorless needles; mp 202−204 °C; $[\alpha]_D{}^{16}$ −36.6 (*c* 1.23, pyridine); ^{1}H NMR (pyridine-*d*$_5$, 400 MHz), see Table 1; ^{13}C NMR (pyridine-*d*$_5$, 100 MHz), see Table 1; HRFABMS (positive) *m/z* 440.3648 [M]$^+$ (calcd for C$_{30}$H$_{48}$O$_2$, 440.3654).

3,4-*seco*-Urs-4(23),20(30)-dien-3-oic acid (2): colorless needles; mp 268−270 °C; $[\alpha]_D{}^{16}$ +87.0 (*c* 0.54, pyridine); ^{1}H NMR (pyridine-*d*$_5$,

400 MHz), see Table 1; ^{13}C NMR (pyridine-d_5, 100 MHz), see Table 1; HRFABMS (negative) m/z 439.3575 $[M - H]^-$ (calcd for $C_{30}H_{47}O_2$, 439.3577).

3-*O*-Methylmalonylepiocotillol II (6): white, amorphous powder; $[\alpha]_D^{24}$ +8.8 (*c* 0.91, CHCl$_3$); ^{1}H NMR (pyridine-d_5, 400 MHz) δ 0.85 (3H, s, CH$_3$-28), 0.86 (3H, s, CH$_3$-18), 0.89 (3H, s, CH$_3$-29), 0.91 (3H, s, CH$_3$-30), 0.96 (3H, s, CH$_3$-19), 1.12 (3H, s, CH$_3$-21), 1.13 (3H, s, CH$_3$-27), 1.21 (3H, s, CH$_3$-26), 3.42 (2H, s, malonyl −CH$_2$−), 3.75 (1H, t, *J* = 8 Hz, H-24), 3.77 (3H, s, OCH$_3$), 4.68 (1H, br s, H-3); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 15.5 (C-19), 16.0 (C-18), 16.5 (C-30), 18.0 (C-6), 21.4 (C-11), 21.6 (C-29), 22.8 (C-2), 24.3 (C-21), 25.7 (C-12), 26.1 (C-23), 27.3 (C-16), 27.5 (C-27), 27.8 (C-26), 27.9 (C-28), 31.4 (C-15), 34.1 (C-1), 34.6 (C-7), 35.7 (C-22), 36.8 (C-10), 37.1 (C-4), 40.6 (C-8), 41.8 (malonyl −CH$_2$-), 42.9 (C-13), 49.5 (C-17), 50.1 (C-14), 50.6 (C-9), 50.8 (C-5), 52.3 (COO−CH$_3$), 70.9 (C-25), 80.1 (C-3), 83.3 (C-20), 86.4 (C-24), 166.0 (malonyl −CO−), 167.2 (malonyl −CO−); HRFABMS (positive) m/z 583.3971 $[M + Na]^+$ (calcd for $C_{34}H_{56}O_6Na$, 583.3975).

3-*O*-Methylmalonylcabraleahydroxylactone (16): white, amorphous powder; $[\alpha]_D^{24}$ +1.2 (*c* 0.85, CHCl$_3$); ^{1}H NMR (pyridine-d_5, 400 MHz) δ 0.86 (3H, s, CH$_3$-19), 0.86 (3H, s, CH$_3$-28), 0.89 (3H, s, CH$_3$-29), 0.92 (3H, s, CH$_3$-30), 0.96 (3H, s, CH$_3$-18), 1.36 (3H, s, CH$_3$-21), 2.00 (1H, dt, *J* = 5, 12 Hz, H-23a), 2.12 (1H, dt, *J* = 9.7, 12 Hz, H-23b), 2.54 (1H, ddd, *J* = 4.5, 10, 18.5 Hz, H-22a), 2.66 (1H, ddd, *J* = 9, 10, 18.5 Hz, H-22b), 3.42 (2H, s, malonyl −CH$_2$−), 3.77 (3H, s, OCH$_3$), 4.68 (1H, t, *J* = 4 Hz, H-3); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 15.5 (C-18), 16.0 (C-19), 16.3 (C-30), 18.0 (C-6), 21.2 (C-11), 21.6 (C-29), 22.7 (C-2), 25.0 (C-21), 25.4 (C-16), 27.9 (C-28), 29.2 (C-23), 31.1 (C-15), 31.2 (C-22), 34.1 (C-1), 35.0 (C-7), 36.8 (C-4), 37.1 (C-10), 40.5 (C-8), 41.8 (malonyl −CH$_2$−), 43.1 (C-13), 49.3 (C-17), 50.2 (C-14), 50.4 (C-9), 50.7 (C-5), 52.3 (COO−CH$_3$), 80.1 (C-3), 90.1 (C-20), 165.9 (malonyl −CO−), 167.2 (malonyl −CO−), 176.7 (C-24); HRFABMS (negative) m/z 539.3348 $[M + Na]^+$ (calcd for $C_{31}H_{48}O_6Na$, 539.3348).

Cell Lines and Cell Culture. 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 2 μg/mL colchicine. MCF7, COLO205, and K562 cells were cultured in RPMI1640 supplemented with 10% FBS. K562/Adr cells were cultured in RPMI1640 medium containing 10% FBS and 0.5 μM doxorubicin. All cells were incubated at 37 °C in a humidified atmosphere with 5% CO$_2$−95% air.

Cytotoxicity Assays. Cells in exponential growth were trypsinized, dispersed in a single cell suspension, and dispensed in 100 μL volumes into 96-well plates. For each assay, 1×10^4 cells/well for K562 and K562/Adr, 5×10^3 cells/well for KB and KB-C2, or 5×10^3 cells for MCF7 and COLO205 were inoculated in 100 μL of medium containing 10% FBS and incubated for 24 h. Test samples were dissolved in small amounts of DMSO and diluted in the appropriate culture medium (final concentration of DMSO < 0.5%). After removal of preincubated culture medium, 100 μL of medium containing various concentrations of each test compound was added, and the mixture was further incubated for 48 h. Cell proliferaion was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[28] IC$_{50}$ values are defined as the concentration of each test sample that reduced absorbance to 50% of vehicle-treated controls.

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