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Synthesis of 1-*O*-monoacyl or 12-*O*-monoacyl, 1-,12-*O*-diacyl-, and 11,12-dehydrated excisanin A 7,14-acetonides and their cytotoxic activity

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Abstract—1-*O*-Monoacyl, 12-*O*-monoacyl, 1-,12-*O*-diacyl, and 11,12-dehydrated excisanin A 7,14-acetonides were synthesized from excisanin A isolated from *Rabdosia excisa*. The structure and cytotoxic activity relationships (SAR) of the natural parent *ent*-kaurene diterpenes and these semisynthetic analogues were studied by using P388 murine leukemia cells.

1. Introduction

Plants of the genus *Rabdosia* (Labiatae) are rich in *ent*-kaurene diterpenoids, which are attracting much attention of late as they have various interesting biological activities such as cytotoxicity, antitumor, and antiinflammatory,[1] and a SAR study of cytotoxic and antitumor activity of natural *Rabdosia* diterpenoid is reported.[2] Recently, we reported isolation of some new *ent*-kaurene diterpenoids from *Rabdosia excisa* along with a large amount of known excisanin A (**1**), kamebanin (**2**), and kamebakaurin (**3**) (Fig. 1),[3] and an efficient transformation method of these 7,14-dihydroxy-*ent*-kaurenes (**1**–**3**) to *ent*-abietanes under Mitsunobu reaction conditions.[4] In our previous papers,[3,4] we assayed several natural *ent*-kaurenes and their analogues of compounds **1**–**3** for their cytotoxicity on P388 murine leukemia cells, which implies the importance of 12-*O*-acyl group in the cytotoxic activity (Fig. 2). In the present paper, we prepared more analogues of excisanin A 7,14-acetonides having 1-*O*-monoacyl, 12-*O*-monoacyl, 1-,12-*O*-diacyl, or 11,12-double bond groups and studied the effect of 1-*O*- and/or 12-*O*-acyl groups of excisanin A 7,14-acetonide on the cytotoxic activities.

Keywords: *ent*-Kaurene; Cytotoxic activity; P388 murine leukemia cells; Excisanin A; Semisynthesis; SAR.
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2. Chemistry

2.1. Direct acylation of 1- and 12-hydroxy groups (Table 1)

Acetylation of **6** under the reaction conditions shown in entry 2 in Table 1 with a mixture of pyridine and acetic anhydride at room temperature gave 1-,12-*O*-diacetate **8a** in 72% yield. When treated with acetic anhydride at 0 °C in the presence of triethylamine and 4-dimethylaminopyridine (DMAP), **6** gave a mixture of mono- (**8b**) (23%) and diacetylated (**8a**) (69%) compounds (entry 1 in Table 1). The structure of monoacetylated compound **8b** was determined to be 1-*O*-acetyl compound on the basis of ^{1}H and ^{13}C NMR spectra including 2D NMR spectrum: the HMBC correlation between the carbonyl carbon of the acetyl group and H-1 (Fig. 3) indicated that the acetylation took place at OH-1. Acylation of **6** with propionyl chloride at 0 °C for 1 h gave a mixture of 1-*O*-propionyl- (**10b**) and 1-,12-*O*-dipropionylexcisanin A 7,14-acetonide (**10a**) (entry 4 in Table 1), whereas the acylation at −78 °C for 1 h gave a 1-*O*-propionylexcisanin A 7,14-acetonide (**10b**) along with some starting material (**6**) (entry 5 in Table 1). Acylation with isobutyryl chloride having a bulkier alkyl group did not give 1-*O*-isobutyrylexcisanin A 7,14-acetonide (**11b**) but 12-*O*-isobutyrylexcisanin A 7,14-acetonide (**11c**) (entries 6, 7, and 8 in Table 1) along with

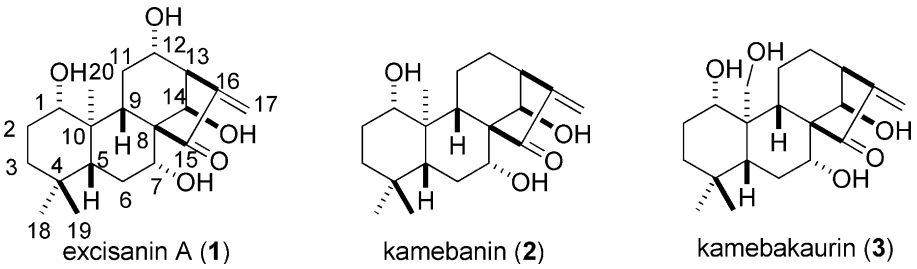


Figure 1. Structures of excisanin A (**1**), kamebanin (**2**), and kamebakaurin (**3**).

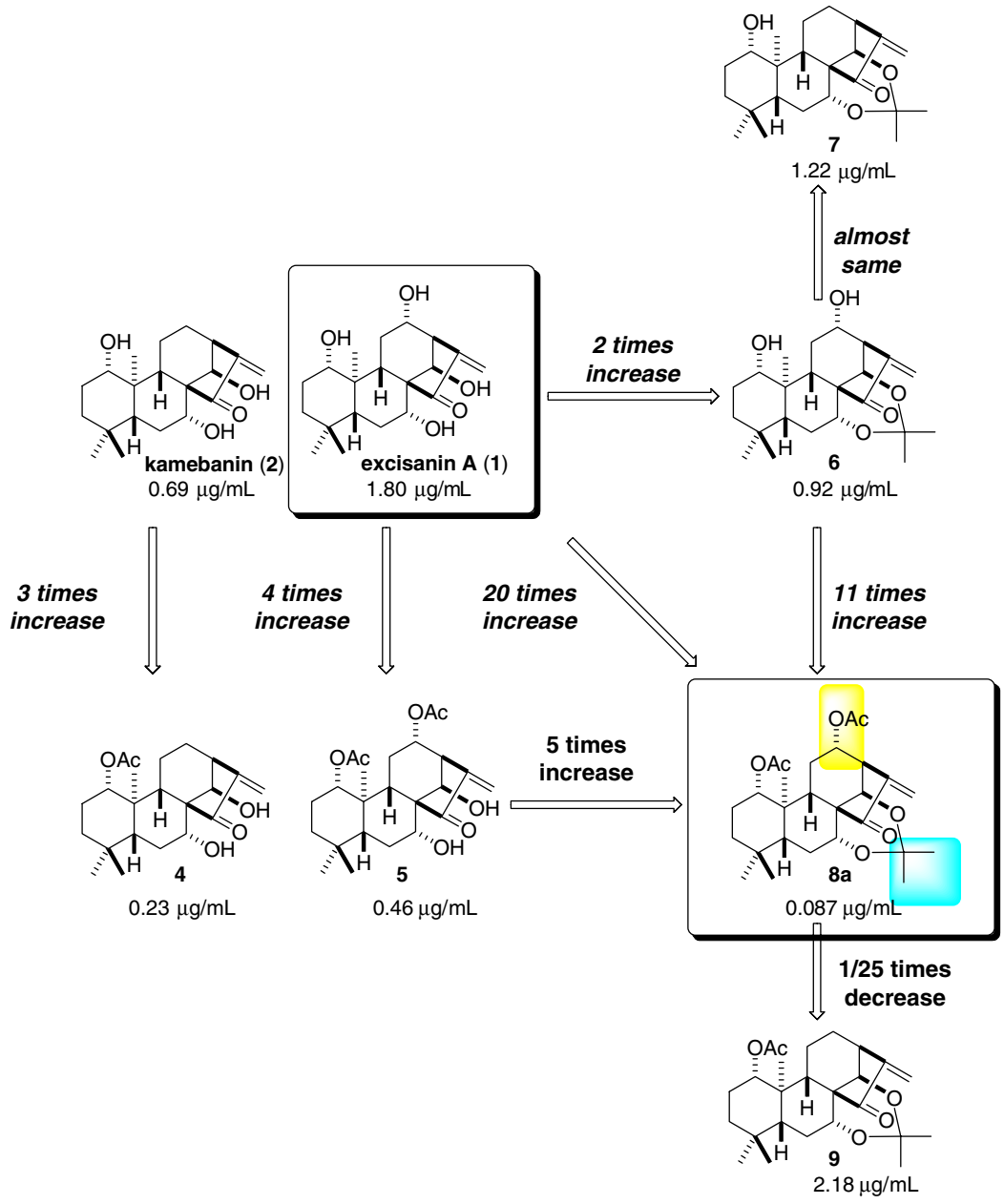
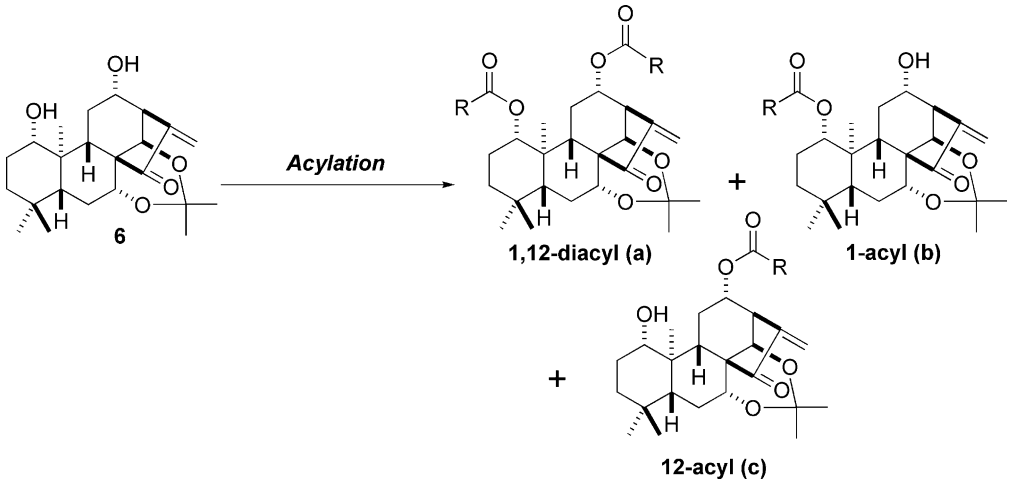


Figure 2. Preliminary cytotoxic SAR study for excisanin A (**1**) and kamebanin (**2**) analogues (**4**–**9**).

1-,12-*O*-diisobutyrylexcisanin A 7,14-dimethylacetonide (**11a**). The structure of 12-*O*-isobutyrylexcisanin A 7,14-acetonide (**11c**) was determined by the HMBC spectrum. When diacylation was carried out with isovaleryl chloride and triethylamine at 0 °C for 1 h and then at rt for 15 h, **12a** was obtained as a sole product (entry 9 in Table 1). On the other hand, when acylation of **6** with isovaleryl chloride was carried out

Table 1. Acylation of excisanin A 7,14-acetonide (6) under several reaction conditions



Entry	R	Acylating agents (equiv)	Base (equiv)	Reaction temperature (°C) and time (h)	Products (yields %)		
					diacyl (**a**)	1-acyl (**b**)	12-acyl (**c**)
1	Me[a]	Ac_2O (3.0)	Et_3N (5.0)/DMAP (cat.)	0 °C, 1 h	**8a** (69)	**8b** (23)	—
2	Me[a]	Ac_2O (19.0)	Pyridine (45.0)	rt 15 h	**8a** (72)	—	—
3	Me	AcCl	Et_3N (5.0)/DMAP (cat.)	0 °C, 1 h	—[c]	—[c]	—[c]
4	Et	EtCOCl (3.0)	Et_3N (5.0)/DMAP (cat.)	0 °C, 1 h	**10a** (33)	**10b** (25)	—
5	Et	EtCOCl (3.0)	Et_3N (5.0)/DMAP (cat.)	−78 °C, 1 h	—	**10b** (25)[b]	—
6	^{i}Pr	^{i}PrCOCl (3.8)	Et_3N (5.0)/DMAP (cat.)	0 °C, 1 h			**11c** (44)
7	^{i}Pr	^{i}PrCOCl (3.8)	Et_3N (5.0)/DMAP (cat.)	0 °C, 1 h then rt 15 h	**11a** (35)	—	**11c** (58)
8	^{i}Pr	^{i}PrCOCl (7.6)	Et_3N (10.0)/DMAP (cat.)	0 °C, 1 h then rt 15 h	**11a** (58)	—	**11c** (10)
9	^{i}Bu	^{i}BuCOCl (7.6)	Et_3N (10.0)/DMAP (cat.)	0 °C, 1 h then rt 15 h	**12a** (79)	—	—
10	^{i}Bu	^{i}BuCOCl (3.8)	Et_3N (5.0)/DMAP (cat.)	0 °C, 1 h	**12a** (2)	**12b** (5)	**12c** (49)
11	Ph	BzCl (8.0)	Et_3N (10.0)/DMAP (cat.)	0 °C, 1 h then rt 15 h	**13a** (30)	**13b** (9)	**13c** (59)
12	CF_3[a]	$(CF_3O)_2O$ (69.0)	Pyridine (238.0)	rt 15 h	**14a** (99)	—	—

[a] No solvents.
[b] The starting material **6** was recovered in 27% yield.
[c] Many products.

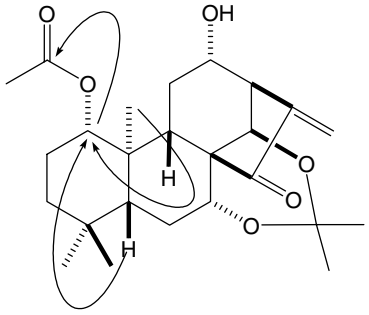
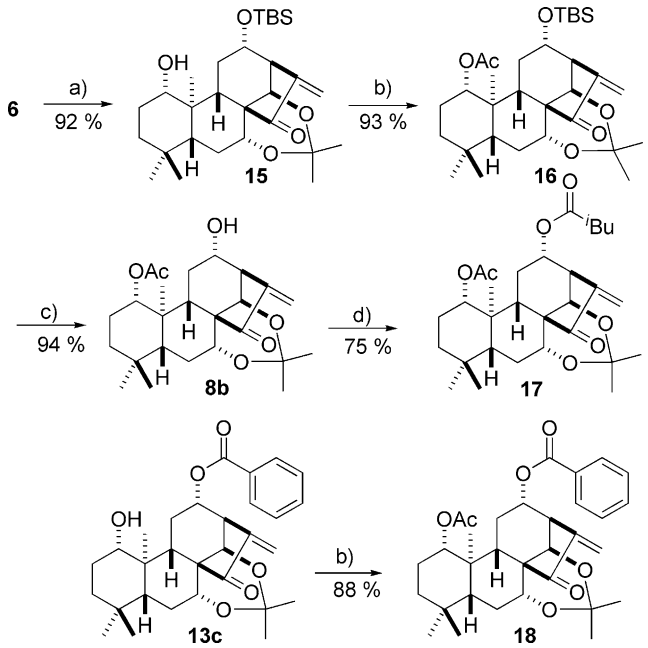


Figure 3. Selected HMBC spectrum of monoacyl compound **8b**.

in the presence of triethylamine at 0 °C for 1 h, 12-acylated compound **12c** was the major product (entry 10 in Table 1). Analogously, when **6** was treated with benzoyl chloride in the presence of triethylamine at 0 °C for 1 h and then at rt for 15 h, major product was **13c** (entry 11 in Table 1). Trifluoroacetylation of **6** produced ditrifluoroacetylated compound (**14a**), whose lipophilicity is different from those of the other diacylated analogues (entry 12 in Table 1).

2.2. Selective acylation

Selective acylation was performed first by selective introduction of ^{t}butyldimethylsilyl (TBS) group into the 12-hydroxy group of excisanin A 7,14-acetonides by treating **6** with 1.1 equiv of ^{t}butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), which was followed by acylation and deprotection. TBSOTf was used with **6** at 0 °C, silylation occurred at 12-hydroxy group to give a monosilyl compound **15** in 92% yield. The subsequent acetylation of 1-hydroxy group by the usual manner, followed by the deprotection of 12-*O*-TBS group, gave compound **8b** in 87% overall yield. The acylation of **8b** with isovaleryl chloride and triethylamine gave compound **17** in 75% (Scheme 1) yield. When 12-*O*-benzoylexcisanin A 7,14-acetonide (**13c**), prepared by direct acylation of **6** (entry 11 in Table 1), was treated with acetic anhydride and pyridine, 1-*O*-acetyl-12-*O*-benzoylexcisanin A 7,14-acetonide (**18**) was obtained in 88% yield (Scheme 1). On the other hand, the reaction of **6** with 3.0 equiv of TBSOTf gave disilylated compound **19** in 95% yield. Selective partial desilylation at *O*-12 of **19** with 1.0 M TBAF (tetrabutylammonium fluoride) in THF at room temperature gave 1-*O*-TBS-excisanin A



Scheme 1. Preparation of 1-*O*-acetyl-12-*O*-^{i}valeryl- (**17**) and benzoyl-excisanin A 7,14-acetonides (**18**). Reagents and conditions: (a) TBSOTf (1.2 equiv), 2,6-lutidine, CH_2Cl_2, 0 °C; (b) Ac_2O, pyridine, rt; (c) TBAF, THF, rt; (d) Et_3N, DMAP, ^{i}BuCOCl, CH_2Cl_2.

7,14-acetonide (**20**) in 58% yield. Subsequent acylation of **20** with acetic anhydride and pyridine gave **21**, which, by desilylation, gave 12-*O*-acetylexcisanin A 7,14-acetonide (**8c**). The structure of **8c** was established on the basis of ^{1}H and ^{13}C NMR and the HMBC correlations between the carbonyl carbon and H-12, as shown in Figure 4. The treatment of **8c** with isovaleryl chloride and Et_3N in the presence of DMAP gave 1-*O*-isovaleryl-12-*O*-acetyled compound (**22**) in 65% (Scheme 2). Treatment of **6** with the Mitsunobu reaction conditions gave 11,12-dehydrated excisanin A 7,14-acetonide (**23**) in a moderate yield. Acetylation of the alcohol (**23**) gave an acetate (**24**) in 85% yield (Scheme 3).

3. Biology

The presently prepared semisynthetic analogues of excisanin A (**6**, **8a–c**, **10a–b**, **11a**, **c**, **12a**, **c**, **13c**, **14a**, **15–22**, and **24**) and the parent natural excisanin A (**1**) and kamebanin (**2**) were assayed for their cytotoxic activities on P388 murine leukemia cells. The results are shown in Table 2.

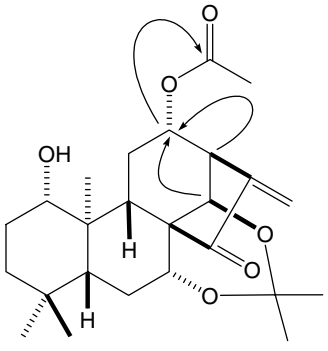


Figure 4. Selected HMBC spectrum of monoacyl compound **8c**.

As reported previously, the activity of **6** is 1.5 times that of excisanin A (**1**), and that of **8a** is still higher than that of **6**. The mono- and diacylation generally had a slight or moderate enhancing effect on the cytotoxic activity, excepting for **14a**, in which the two very bulky acyl groups somehow seem to make the compound less active. Among the series of diacyl excisanin A 7,14-acetonide (**8a**, **10a**, **11a**, **12a**, **14a**, **17**, **18**, and **22**), 1,12-*O*-diacetylexcisanin A 7,14-acetonide (**8a**) was the most active. Analogous results were obtained when the hydroxy groups were protected with TBS group. Namely, the activity of **19**, in which both 1-OH and 12-OH were protected with the bulky TBS group, was almost nil. Protection of either of the two hydroxyls with TBS, leading the other left free (**15** and **20**), or the protection of one of the two hydroxyls with TBS and the other with a smaller acyl group (**16** and **21**) gave a moderate enhancing effect on the activity. The cytotoxic activity of 1-*O*-acetyl-11,12-dehydrated excisanin A 7,14-acetonide (**24**) was much lower than those of diacyl or 12-monoacyl analogues. These facts suggest that 12-acyloxy groups are essential for the activity and that the steric factor provided by 1-*O*- and 12-*O*-protecting groups also seems to be important.

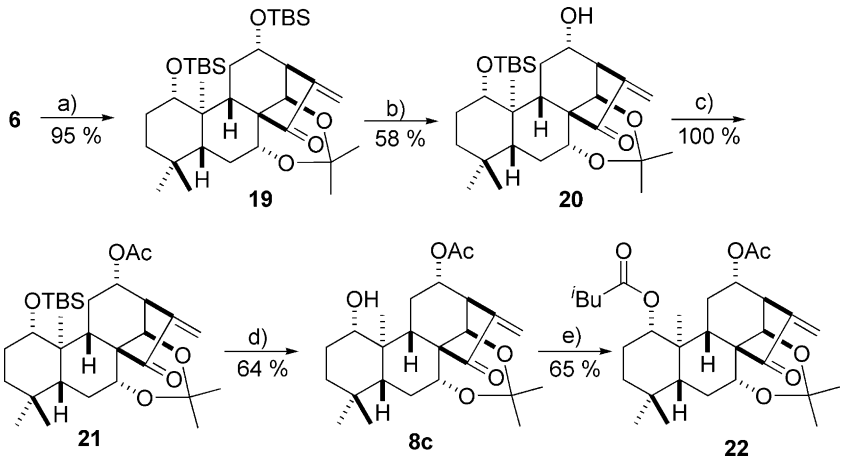
4. Conclusion

In this paper, a series of 1-*O*-monoacyl and 12-*O*-monoacyl, 1,12-*O*-diacyl, and 11,12-dehydrated excisanin A 7,14-acetonide analogues were synthesized from excisanin A isolated from *R. excisa*. Of those whose 1-OH and 12-OH were both protected, compounds **8a**, **16**, and **21** showed a significant cytotoxic activity on P388 murine leukemia cells, though **14a** and **19**, having two very bulky TBS or lipophilic trifluoromethyl groups introduced, had very low activity. This may imply that not only the hydrophilicity at 1-*O*- and/or 12-*O*- but also the steric effect may affect the activity.

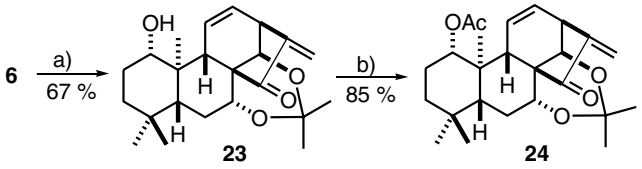
5. Experimental

5.1. General method

Melting points were determined on a Yanaco MP-3 apparatus and are recorded uncorrected. IR spectra were recorded on a JASCO FT/IR 620 spectrophotometer, optical rotation on a JASCO DIP-360 automatic digital polarimeter, and Mass spectra on a Micromass LCT (Manchester, UK) spectrometer. NMR spectra in $CDCl_3$ or pyridine–d_5 were recorded on a Brucker AM-400 and DRX-500 spectrometer at 300 K and the *J* values were given in Hz. The chemical shifts (δ) are reported in ppm relative to the residual $CHCl_3$ resonance at 7.26 ppm for ^{1}H NMR and to the resonance of $CDCl_3$ at 77.0 ppm for ^{13}C NMR. Preparative HPLC was carried out on a JASCO PU-986 equipped with a UV-970 UV detector (λ 220 nm) and an Inertsil



Scheme 2. Preparation of 12-*O*-acetyl- (**8c**) and 12-*O*-acetyl-1-*O*-^{i}valerylexcisanin A 7,14-acetonide (**22**). Reagents and conditions: (a) TBSOTf (1.2 equiv), 2,6-lutidine, CH_2Cl_2, 0 °C; (b) TBAF, THF, rt; (c) Ac_2O, pyridine, rt; (d) TBAF, THF, 0 °C; (e) Et_3N, DMAP, ^{i}BuCOCl, CH_2Cl_2.



Scheme 3. Preparation of 1-*O*-acetyl-11,12-dehydrated excisanin A 7,14-acetonide (**24**). Reagents and conditions: (a) benzoic acid, DEAD, PPh_3, THF, rt; (b) Ac_2O, pyridine, rt.

Table 2. Cytotoxic activity of natural and semisynthetic *ent*-kaurenes

Compound	IC_{50} (μg/mL)
1	0.97
2	0.68
6	0.63
8a	0.060
8b	0.21
8c	0.18
10a	0.26
10b	0.20
11a	0.090
11c	0.30
12a	0.25
12c	0.21
13c	0.38
14a	3.2
15	0.13
16	0.042
17	0.51
18	0.13
19	>100
20	0.090
21	0.046
22	0.19
24	1.7
Camptothecin[a]	0.0065

[a] Positive control.

PREP-ODS column (10 μm, 20 × 250 mm), by using a MeOH/H_2O or a MeCN/H_2O solvent system at a flow rate of 10 mL/min. Excisanin A (**1**) was obtained from aerial parts of the plant *R. excisa* (Labiatae), collected in Jing Yu county, Jinlin province of China, in August 2001, as reported previously.

5.2. Preparation of excisanin 7,14-acetonide (6)

p-Toluene sulfonic acid (cat. amount) was added to a mixture of excisanin A (1.04 g, 2.96 mmol), 2,2-dimethoxypropane (17.8 g, 171 mmol), and acetone (200 mL). The mixture was stirred at room temperature for 0.5 h. The solvent was evaporated in vacuo to give an oily residue, which was purified by silica gel column chromatography ($CHCl_3$/Me_2CO = 2:1) to give its acetonide (**6**) in quantitative yield. Colorless solid, mp 244–246 °C (MeOH–H_2O); $[\alpha]_D$ −74.0 (*c* 0.077, $CHCl_3$); ^{1}H NMR (500 MHz, 300 K, $CDCl_3$): δ 6.13 (1H, s), 5.38 (1H, s), 4.92 (1H, s), 4.22 (1H, dd, 9.0, 9.0), 4.11 (1H, br dd), 3.25 (1H, dd, 11.0, 4.1), 3.09 (1H, d, 2.7), 2.91 (1H, d, 16.8), 2.06–1.98 (3H, m), 1.78 (1H, ddd, 16.8, 9.6, 5.1), 1.68–1.50 (3H, m), 1.59 (3H, s), 1.41 (1H, ddd, 13.6, 3.3, 3.3), 1.29 (3H, s), 1.24 (3H, s), 1.31–1.18 (2H, m), 0.89 (3H, s), 0.86 (3H, s), 0.83–0.80 (1H, m); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ; 207.3, 145.0, 117.6, 97.3, 80.8, 72.5, 70.5, 66.6, 54.8, 54.2, 50.7, 50.2, 42.9, 39.3, 33.1, 32.7, 30.6, 30.0, 27.9, 27.3, 25.3, 21.2, 11.9; IR (film) 3262 (OH), 1731 (C=O), 1651 (C=C); HRMS (ESI): Calculated for $C_{23}H_{34}O_5Na$ (M^++Na): 413.2304. Found: 413.2283.

5.3. General procedure for acylation

5.3.1. Direct acylation (Table 1)

5.3.1.1. Excisanin A 7,14-acetonide (6). *With acid chloride.* Acyl chloride was added to a mixture of **6**, DMAP, Et_3N, and dry CH_2Cl_2 at a temperature specified in Table 1. After stirring for a time indicated in Table 1, the mixture was treated with H_2O and was extracted with AcOEt three times. The combined organic layer was washed with 5% HCl, satd aq $NaHCO_3$, and satd aq NaCl, successively, dried over $MgSO_4$, filtered, and evaporated in vacuo to give a residue, which was purified by MPLC to give the corresponding acylation product.

With acid anhydride. A mixture of **6**, acyl anhydride, and dry pyridine or dry Et_3N was stirred at the temperature and for the time given in Table 1. The reaction mixture was poured into ice-cooled water and the aqueous phase was extracted with AcOEt. The combined organic phase

was washed with 5% HCl, satd aq $NaHCO_3$, and satd aq NaCl, sequentially, dried over $MgSO_4$, filtered, and evaporated in vacuo to give oily residue, which was purified by MPLC to give an acylated compounds.

5.3.1.2. 1-,12-*O*-Diacetylexcisanin A 7,14-acetonide (8a). Colorless amorphous solid, mp 89–91 °C (MeOH–H_2O); $[\alpha]_D$ −34.1 (*c* 0.17, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.19 (1H, s), 5.48 (1H, s), 4.95 (1H, m), 4.81 (1H, d, 1.3), 4.42 (1H, dd, 10.6, 5.0), 4.22 (1H, dd, 11.3, 7.0), 3.12 (1H, d, 3.5), 2.24 (1H, d, 16.9), 2.16 (3H, s), 2.04–1.95 (2H, m), 1.97 (3H, s), 1.76 (1H, ddd, 17.0, 9.8, 5.6), 1.65 (1H, d, 9.4), 1.60 (3H, s), 1.62–1.56 (2H, m), 1.42 (1H, ddd, 17.4, 3.7, 3.7), 1.35 (1H, ddd, 13.2, 13.2, 4.8), 1.26 (3H, s), 1.23 (3H, s), 0.92 (3H, s), 0.89 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 205.5, 170.07, 170.05, 143.6, 119.3, 97.4, 82.7, 73.9, 70.3, 66.8, 54.1, 52.0, 50.5, 47.4, 41.8, 38.8, 33.0, 32.7, 30.4, 27.0, 25.3, 25.0, 24.2, 21.5, 21.4, 12.5; IR (film) 1737 (C=O), 1652 (C=C); HRMS (ESI): Calculated for $C_{27}H_{38}O_7Na$ (M^++Na): 497.2515. Found: 497.2501.

5.3.1.3. 1-*O*-Acetylexcisanin A 7,14-acetonide (8b). Colorless solid, mp 102–106 °C (MeOH–H_2O); $[\alpha]_D$ −49.4 (*c* 0.22, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.12 (1H, s), 5.38 (1H, s), 4.86 (1H, s), 4.44 (1H, dd, 9.8, 5.6), 4.19 (1H, dd, 9.5, 9.5), 4.06 (1H, m), 3.04 (1H, d, 2.7), 2.07 (1H, d, 16.2), 2.00 (3H, s), 2.01–1.97 (2H, m), 1.72 (1H, ddd, 16.2, 9.6, 5.5), 1.63 (1H, d, 8.8), 1.60–1.58 (2H, m), 1.56 (3H, s), 1.41 (1H, ddd, 13.7, 3.6, 3.6), 1.38–1.33 (1H, m), 1.34 (3H, s), 1.22 (3H, s), 0.91–0.88 (1H, m, overlapped), 0.91 (3H, s), 0.89 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 206.5, 170.1, 144.6, 118.0, 97.3, 83.3, 72.3, 70.3, 66.5, 54.7, 52.7, 51.0, 50.3, 41.8, 38.9, 33.0, 32.7, 30.2, 27.3, 26.9, 25.3, 25.1, 21.8, 21.5, 12.6; IR (film) 3464 (OH), 1784, 1737 (C=O), 1650 (C=C); HRMS (ESI): Calculated for $C_{25}H_{37}O_6$ (M^++H) : 433.2590. Found: 433.2592.

5.3.1.4. 1-,12-*O*-Dipropionylexcisanin A 7,14-acetonide (10a). Colorless amorphous solid, mp 64–68 °C (MeOH–H_2O); $[\alpha]_D$ −4.3 (*c* 1.53, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.19 (1H, s), 5.48 (1H, s), 4.98 (1H, dd, 4.0, 4.0), 4.80 (1H, s), 4.45 (1H, dd, 10.3, 5.2), 4.21 (1H, dd, 10.7, 7.6), 3.12 (1H, d, 2.7), 2.43 (2H, m), 2.24–2.19 (3H, m), 2.03–1.99 (2H, m), 1.76 (1H, ddd, 15.8, 9.8, 5.7), 1.66 (1H, d, 9.7), 1.63–1.53 (2H, m), 1.60 (3H, s), 1.43–1.32 (3H, m), 1.26 (3H, s), 1.23 (3H, s), 1.21 (3H, t, 7.6), 1.07 (3H, t, 7.5), 0.92 (3H, s), 0.89 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 205.5, 173.5, 173.3, 143.6, 119.3, 97.3, 82.5, 73.6, 70.4, 66.8, 54.1, 52.1, 50.5, 47.5, 41.9, 38.8, 33.0, 32.7, 30.5, 28.04, 28.00, 27.0, 25.3, 25.1, 24.3, 21.4, 12.6, 9.1, 8.9; IR (film) 1736 (C=O), 1652 (C=C); HRMS (ESI): Calculated for $C_{29}H_{43}O_7$ (M^++H): 503.3009. Found: 503.3023.

5.3.1.5. 1-*O*-Propionylexcisanin A 7,14-acetonide (10b). Colorless amorphous solid, mp 95–99 °C (MeOH–H_2O); $[\alpha]_D$ −20.8 (*c* 0.90, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.13 (1H, s), 5.38 (1H, s), 4.87 (1H, d, 1.1), 4.47 (1H, m), 4.21 (1H, dd, 2.4, 2.4), 4.05 (1H, br m), 3.04 (1H, d, 3.1), 2.33–2.21 (2H, m), 2.07 (1H, d, 16.0), 2.04–1.98 (3H, m), 1.72 (1H, ddd, 15.4, 9.6, 5.5), 1.64 (1H, d, 9.4), 1.61–1.55 (3H, m), 1.57 (3H, s), 1.43–1.34 (2H, m), 1.35 (3H, s), 1.23 (3H, s), 1.10 (3H, t, 12.6), 0.92 (3H, s), 0.89 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 206.4, 173.3, 144.6, 118.0, 97.3, 83.0, 72.3, 70.3, 66.5, 54.7, 52.7, 51.1, 50.3, 41.9, 38.9, 33.1, 32.7, 30.2, 28.4, 27.3, 27.0, 25.3, 25.2, 21.5, 12.6, 9.0; IR (film) 3480 (OH), 1733 (C=O), 1650 C=C); HRMS (ESI): Calculated for $C_{26}H_{39}O_6$ (M^++H): 447.2747. Found: 447.2788.

5.3.1.6. 1-,12-*O*-Diisobutyrylexcisanin A 7,14-acetonide (11a). Colorless solid, mp 53–56 °C (MeOH–H_2O); $[\alpha]_D$ −41 (*c* 0.30, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.19 (1H, s), 5.49 (1H, s), 5.00 (1H, m), 4.77 (1H, d, 0.7), 4.45 (1H, dd, 7.9, 7.7), 4.22 (1H, dd, 10.2, 8.1), 3.13 (1H, d, 3.3), 2.63 (1H, hep, 7.0), 2.41 (1H, hep, 7.0), 2.21 (1H, d, 16.9), 2.03–1.99 (2H, m), 1.78 (1H, ddd, 16.0, 9.9, 6.0), 1.67 (1H, d, 9.9), 1.60–1.55 (2H, m), 1.59 (3H, s), 1.41 (1H, ddd, 13.7, 3.7, 3.7), 1.38–1.30 (2H, m), 1.31 (3H, s), 1.24 (3H, d, 7.0), 1.231 (3H, s), 1.230 (3H, d, 7.0), 1.10 (3H, d, 7.0), 1.08 (3H, d, 7.0), 0.92 (3H, s), 0.89 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 205.6, 176.0, 175.8, 143.5, 119.4, 97.3, 82.5, 73.4, 70.4, 66.8, 54.2, 52.1, 50.4, 47.6, 42.1, 38.8, 34.4, 33.1, 32.7, 30.4, 27.0, 25.2, 25.0, 24.4, 21.4, 19.1, 19.0, 18.8, 18.3, 12.7; IR (film) 1734 (C=O), 1652 (C=C); HRMS (ESI): Calculated for $C_{31}H_{47}O_7$ (M^++H): 531.3322. Found: 531.3327.

5.3.1.7. 12-*O*-Isobutyrylexcisanin A 7,14-acetonide (11c). Colorless solid, mp 64–66 °C (MeOH–H_2O); $[\alpha]_D$ −46 (*c* 0.20, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.19 (1H, s), 5.50 (1H, s), 5.05 (1H, m), 4.79 (1H, d, 0.9), 4.23 (1H, dd, 9.0, 9.0), 3.24 (1H, m), 3.15 (1H, d, 3.3), 3.01 (1H, dd, 17.4, 0.5), 2.61 (1H, m), 2.02–1.99 (2H, m, overlapped), 1.85 (1H, ddd, 17.0, 9.7, 5.8), 1.65 (1H, m, overlapped), 1.64 (1H, d, 9.7, overlapped), 1.59 (3H, s), 1.50 (1H, m), 1.41 (1H, ddd, 13.9, 3.4, 3.4), 1.28 (1H, ddd, 14.0, 14.0, 3.9), 1.25 (1H, m), 1.24 (3H, s), 1.233 (3H, s), 1.232 (3H, d, 7.0), 1.21 (3H, d, 7.0), 0.90 (3H, s), 0.87 (3H, s), 0.81 (1H, m); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 206.5, 176.1, 144.0, 118.9, 97.3, 80.7, 73.6, 70.5, 67.0, 54.5, 53.2, 50.1, 47.7, 43.1, 39.2, 34.3, 33.1, 32.8, 30.4, 30.0, 27.2, 25.2, 24.9, 21.3, 19.1, 18.8, 11.7; IR (film) 3494 (OH), 1732 (C=O), 1652 (C=C); Calculated for $C_{27}H_{41}O_6$ (M^++H): 461.2930. Found: 461.2892.

5.3.1.8. 1-,12-*O*-Diisovaleroylexcisanin A 7,14-acetonide (12a). Colorless solid, mp 55–59 °C (MeOH–H_2O); $[\alpha]_D$ −34 (*c* 0.25, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.19 (1H, s), 5.49 (1H, s), 4.98 (1H, dd, 4.1, 4.1), 4.77 (1H, s), 4.44 (1H, dd, 10.2, 5.3), 4.21 (1H, dd, 10.6, 7.8), 3.12 (1H, d, 2.9), 2.31–2.21 (3H, m), 2.16 (1H, m), 2.11–1.98 (5H, m), 1.76 (1H, ddd, 16.0, 9.8, 5.8), 1.65 (1H, d, 9.8), 1.62–1.54 (4H, m), 1.43–1.32 (1H, m), 1.27 (3H, s), 1.22 (3H, s), 1.02 (6H, d, 6.6), 0.915 (6H, s), 0.908 (3H, d, 6.6), 0.902 (3H, d, 6.6), 0.89 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 205.6, 172.0 (overlapped), 143.6, 119.3, 97.3, 82.6, 73.4, 70.4, 66.8, 54.1, 52.1, 50.4, 47.5, 43.8, 43.7, 41.9,

38.8, 33.0, 32.7, 30.4, 27.0, 25.7, 25.5, 25.2, 25.1, 24.5, 22.5, 22.4, 22.31, 22.28, 21.4, 12.6; IR (film) 1736 (C=O), 1652 (C=C); Calculated for $C_{33}H_{51}O_7$ (M^++H): 559.3635. Found: 559.3640.

5.3.1.9. 12-*O*-Isovalerylexcisanin A 7,14-acetonide (12c). Colorless solid, mp 82–87 °C (MeOH–H_2O); $[\alpha]_D$ −44 (*c* 0.06, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.19 (1H, s), 5.51 (1H, s), 5.06 (1H, m), 4.79 (1H, d, 0.7), 4.22 (1H, dd, 9.6, 9.6), 3.24 (1H, m), 3.17 (1H, d, 3.3), 3.01 (1H, 17.3), 2.30–2.21 (2H, m), 2.16 (1H, hep, 6.8), 2.02–1.99 (2H, m), 1.85 (1H, ddd, 17.0, 9.7, 5.8), 1.63 (2H, m), 1.58 (3H, s), 1.50 (1H, ddd, 13.1, 7.7, 3.7), 1.41 (1H, ddd, 10.2, 3.4, 3.4), 1.29 (1H, dd, 13.7, 3.7), 1.24 (1H, m), 1.230 (3H, s), 1.227 (3H, s), 1.00 (3H, d, 6.6), 0.99 (3H, d, 6.6), 0.90 (3H, s), 0.87 (3H, s), 0.81 (1H, m); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 206.5, 172.1, 144.0, 118.9, 97.3, 80.7, 73.5, 70.5, 67.0, 54.5, 53.2, 50.1, 47.7, 43.8, 43.1, 39.2, 33.1, 32.8, 30.4, 30.0, 27.2, 25.7, 25.2, 25.0, 22.4 (overlapped), 21.3, 11.5 (q); IR (film) 3466 (OH), 1735 (C=O), 1651 (C=C); Calculated for $C_{28}H_{43}O_6$ (M^++H): 475.3060. Found: 475.3025.

5.3.1.10. 1-,12-*O*-Dibenzoylexcisanin A 7,14-acetonide (13a). Colorless solid, mp 273–276 °C ($CHCl_3$); $[\alpha]_D$ −8.3 (*c* 0.35, $CHCl_3$); ^1H NMR (400 MHz, 300 K, $CDCl_3$) δ 8.16 (2H, m), 7.82 (2H, dd, 8.1, 1.2), 7.68 (1H, m), 7.58 (2H, dd, 7.8, 7.8), 7.44 (1H, m), 7.15 (2H, 7.8, 7.8), 6.20 (1H, s), 5.51 (1H, s), 5.22 (1H, m), 4.97 (1H, d, 1.2), 4.72 (1H, dd, 10.9, 4.6), 4.29 (1H, dd, 10.9, 7.5), 3.29 (1H, d, 3.5), 2.41 (1H, d, 16.3), 2.18–2.07 (2H, m), 1.91–1.76 (2H, m), 1.73–1.68 (1H, m), 1.65 (3H, s), 1.60 (3H, s), 1.50–1.41 (2H, m), 1.31–1.25 (1H, m), 1.25 (3H, s), 1.03 (1H, m), 0.97 (3H, s), 0.95 (3H, s); ^{13}C NMR (100 MHz, 300 K, $CDCl_3$) δ 205.5, 165.4, 165.1, 143.5, 133.4, 133.0, 130.15, 130.12, 129.6, 129.4, 128.7, 128.3, 119.5, 97.3, 83.2, 74.1, 70.5, 66.9, 54.2, 52.5, 50.6, 47.6, 42.5, 38.8, 33.1, 32.7, 30.6, 27.1, 25.3, 25.0, 24.6, 21.4, 13.2; IR (film) 1716 (C=O), 1651 (C=C); Calculated for $C_{37}H_{43}O_7$ (M^++H): 599.3016. Found: 599.3009.

5.3.1.11. 12-*O*-Benzoylexcisanin A 7,14-acetonide (13c). Colorless solid, mp 74–77 °C (MeOH–H_2O); $[\alpha]_D$ −21 (*c* 0.08, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 8.08 (2H, d, 8.6), 7.60 (1H, dd, 7.4, 7.4), 7.49 (2H, dd, 7.9, 7.9), 6.24 (1H, s), 5.57 (1H, s), 5.33 (1H, m), 4.26 (1H, dd, 8.9, 8.9), 3.31 (1H, d, 3.2), 3.26 (1H, dd, 11.1, 4.5), 3.20 (1H, d, 17.4), 2.04 (1H, m), 2.03 (1H, d, 9.0), 1.97 (1H, ddd, 17.2, 9.8, 5.9), 1.70 (1H, d, 9.5), 1.64–1.54 (2H, m, overlapped), 1.61 (3H, s), 1.49 (1H, ddd, 13.0, 7.6, 3.7), 1.41 (1H, ddd, 13.6, 3.4, 3.4), 1.30 (1H, dd, 13.4, 3.9), 1.31 (3H, s), 1.26 (1H, m, overlapped), 1.24 (3H, s), 0.91 (3H, s), 0.88 (3H, s), 0.83 (1H, m); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 205.4, 164.7, 142.9, 132.1, 129.4, 128.6, 127.5, 118.2, 96.3, 79.8, 73.7, 69.6, 66.1, 53.6, 52.2, 49.1, 46.9, 42.2, 38.2, 32.1, 31.8, 29.5, 28.9, 26.2, 24.3, 24.0, 20.4, 10.8; IR (film) 3499 (OH), 1718 (C=O), 1651 (C=C); Calculated for $C_{30}H_{39}O_6$ (M^++H): 495.2747. Found: 495.2751.

5.3.1.12. 1-,12-*O*-Ditrifluoroacetylexcisanin A 7,14-acetonide (14a). Colorless solid (MeOH–H_2O), mp 62–64 °C; $[\alpha]_D$ −30.6 (*c* = 0.2, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.27 (1H, br s), 5.58 (1H, d, 0.6), 5.23 (1H, m), 4.78 (1H, d, 0.8), 4.61 (1H, dd, 7.0, 7.0), 4.23 (1H, dd, 11.9, 6.5), 3.26 (1H, d, 3.2), 2.11–1.99 (3H, m), 1.93 (1H, ddd, 17.3, 9.7, 5.7), 1.78–1.71 (3H, m), 1.59 (3H, s), 1.51 (1H, ddd, 13.9, 3.6, 3.6), 1.39–1.37 (1H, m), 1.33 (3H, s), 1.24 (3H, s), 0.95 (3H, s), 0.94 (1H, m), 0.93 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 204.4, 156.4, (J_{CF_3-C} 42 Hz), 156.3 (J_{CF_3-C} 43 Hz), 142.4, 120.6, 114.5 (J_{C-F} 284 Hz), 114.3 (J_{C-F} 284 Hz), 97.6, 88.0, 77.2, 70.4, 66.2, 53.9, 51.4, 50.5, 46.8, 42.3, 38.4, 33.0, 32.6, 30.3, 26.9, 25.2, 24.43, 24.35, 21.3, 12.0; IR (film) 1784, 1739 (C=O), 1653 (C=C); HRMS (ESI): Calculated for $C_{27}H_{33}O_7F_6$ (M^++H): 583.2130. Found: 583.2133.

5.3.2. Specific acylation (Schemes 1 and 2)

5.3.2.1. Preparation of 12-*O*-^{t}butyldimethylsilylexcisanin A 7,14-acetonide (15). TBSOTf (0.225 g, 0.85 mmol) was added to a solution of **6** (0.30 g, 0.76 mmol) and 2,6-lutidine (0.27 mL, 2.31 mmol) in dry CH_2Cl_2 (10 mL) at −10 °C under an argon atmosphere. After stirring at the same temperature for 15 min, the mixture was treated with AcOEt (15 mL) and satd aq NH_4Cl (10 mL), successively. The organic layer was separated and the aqueous phase was extracted with AcOEt (15 mL 2×) and the combined organic layer was washed with 5% HCl (20 mL 2×), satd aq $NaHCO_3$ (20 mL 2×), and satd aq NaCl (20 mL 2×). The organic phase was dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oily residue, which was purified by MPLC (hexanes/AcOEt = 6:1) to give the corresponding monosilylated compound (**15**) in 92% yield. Colorless amorphous solid, mp 175–177 °C. $[\alpha]_D$ −32.3 (*c* 0.74, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.10 (1H, s), 5.32 (1H, s), 4.88 (1H, s), 4.19 (1H, dd, 12.1, 6.5), 3.99 (1H, dd, 3.9, 3.9), 3.20 (1H, ddd, 11.2, 6.6, 4.8), 2.97 (1H, d, 3.7), 2.93 (1H, d, 16.4), 2.00 (1H, ddd, 12.6, 12.6, 12.6), 1.98 (1H, m), 1.69 (1H, m), 1.60 (1H, m, overlapped), 1.60 (1H, m, overlapped), 1.59 (3H, s), 1.53 (1H, m), 1.39 (1H, ddd, 13.6, 3.3, 3.3), 1.28 (3H, s), 1.28 (1H, m), 1.23 (3H, s), 1.05 (1H, dd, 6.7, 2.1), 0.93 (9H, s), 0.88 (3H, s), 0.85 (3H, s), 0.79 (1H, dd, 11.8, 2.8), 0.12 (3H, s), 0.09 (3H, s) ; ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 207.5, 145.1, 117.1, 97.0, 80.8, 73.2, 70.7, 66.8, 54.8, 54.6, 51.7, 50.2, 43.1, 39.4, 33.1, 32.7, 30.8, 30.0, 28.1, 27.3, 25.9, 25.1, 21.1, 18.2, 12.1, −5.0, −5.1; IR (film) 3478 (OH), 1732 (C=O), 1651 (C=C); HRMS (ESI): Calculated for $C_{29}H_{49}O_5Si$ (M^++H): 505.3349. Found: 505.3373.

5.3.2.2. Preparation of 1-,12-*O*-di^{t}butyldimethylsilylexcisanin A 7,14-acetonide (19). TBSOTf (0.60 g, 2.28 mmol) was added to **6** (0.30 g, 0.76 mmol) 2,6-lutidine (0.45 mL, 3.84 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C under an argon atmosphere. After the reaction mixture was stirred at the same temperature for 2 h, satd aq $NaHCO_3$ (30 mL) was added. The organic layer was separated. The aqueous phase was extracted with AcOEt (15 mL 3×) and the combined organic layer

was dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oily residue, which was purified by MPLC (hexanes/AcOEt = 9:1) to give the corresponding disilylated compound (**19**) in a 95% yield. Colorless amorphous solid, mp 67–69 °C; $[\alpha]_D$ −15.8 (*c* 0.58, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.12 (1H, s), 5.35 (1H, s), 4.86 (1H, s), 4.15 (1H, dd, 12.6, 5.5), 3.98 (1H, dd, 3.8, 3.8), 3.24 (1H, dd, 11.1, 3.8), 2.96 (1H, d, 3.4), 2.72 (1H, d, 16.1), 2.01 (1H, ddd, 12.3, 12.3, 12.3), 1.91 (1H, m), 1.66 (1H, m), 1.63 (1H, m), 1.58 (1H, d, 9.3), 1.55 (3H, s), 1.42 (1H, m), 1.36 (1H, ddd, 13.5, 3.3, 3.3), 1.22 (3H, s),, 1.22 (1H, m), 1.21 (3H, s), 0.93 (9H, s), 0.88 (3H, s), 0.86 (3H, s), 0.85 (9H, s), 0.75 (1H, d, 12.3), 0.14 (3H, s), 0.08 (3H, s), 0.02 (3H, s), −0.05 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 207.6, 145.2, 117.5, 97.0, 83.6, 73.1, 71.0, 66.8, 55.2, 53.5, 51.5, 50.3, 43.7, 39.5, 33.2, 33.0, 30.4, 29.6, 29.1, 27.2, 26.3, 25.8, 25.1, 21.7, 18.2, 18.0, 12.3, −3.5, −4.0, −4.6, −4.9; IR (film) 1737 (C=O), 1651 (C=C); HRMS (ESI): Calculated for $C_{35}H_{63}O_5Si_2$ (M^++H): 619.4214. Found: 619.4252.

5.3.2.3. Preparation of 1-*O*-acetyl-12-*O*-^{t}butyldimethylsilylexcisanin A 7,14-acetonide (16). A mixture of **15** (0.030 g, 0.059 mmol), Ac_2O (0.5 mL, 5.3 mmol), and dry pyridine (1.0 mL, 12.4 mmol) was stirred at rt for 2 days. The reaction mixture was poured into ice-cooled water (10 mL) and the aqueous phase was extracted with AcOEt (10 mL 3×). The combined organic phase was washed with 5% HCl (20 mL 2×), satd aq $NaHCO_3$ (20 mL 2×), and satd aq NaCl (20 mL 2×), dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oily residue, which was purified by MPLC (hexanes/AcOEt = 6:1) to give the corresponding acetylated compound (**16**) in 93% yield. Colorless amorphous solid ($CHCl_3$), mp 89–90 °C; $[\alpha]_D$ −37.5 (*c* 0.74, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.06 (1H, s), 5.29 (1H, s), 4.80 (1H, d, 1.0), 4.39 (1H, dd, 10.7, 4.9), 4.13 (1H, dd, 12.0, 6.3), 3.92 (1H, m), 2.91 (1H, d, 3.7), 2.03 (1H, dd, 16.0), 1.98 (1H, dd, 12.0, 12.0), 1.92 (3H, s, overlapped), 1.92 (1H, m, overlapped), 1.64 (1H, ddd, 15.7, 10.0, 5.2), 1.59 (1H, m), 1.58 (1H, m), 1.54 (1H, m, overlapped), 1.52 (3H, s, overlapped), 1.36 (1H, ddd, 13.7, 3.7, 3.7), 1.31 (3H, s), 1.29 (1H, ddd, 13.7, 13.7, 4.6), 1.18 (3H, s), 0.91 (9H, s), 0.88 (1H, m), 0.86 (3H, s), 0.83 (3H, s), 0.10 (3H, s), 0.07 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 206.6, 169.9, 144.7, 117.7, 97.0, 83.4, 72.9, 70.7, 66.5, 54.6, 53.3, 51.4, 50.5, 42.0, 39.0, 33.1, 32.7, 30.6, 27.8, 27.0, 25.8, 25.2, 25.0, 21.8, 21.4, 18.1, 13.0, −4.8, −5.0; IR (film) 1739 (C=O), 1651 (C=C); HRMS (ESI): Calculated for $C_{31}H_{51}O_6Si$ (M^++H): 547.3455. Found: 547.3458.

5.3.2.4. Preparation of 1-*O*-acetylexcisanin A 7,14-acetonide (8b). 1.0 M TBAF solution in THF (0.17 mL, 0.17 mmol) was added to a solution of **16** (0.030 g, 0.059 mmol) in dry THF (2.0 mL). After the reaction mixture was stirred at rt for 15 h, satd aq NH_4Cl (10 mL) was added. The aqueous phase was extracted with AcOEt (10 mL 3×). The combined organic layer was washed with satd aq NaCl (15 mL 3×), dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oily residue, which was purified by MPLC (hexanes/Me_2CO = 5:1) to give 1-*O*-acetylexcisanin A 7,14-acetonide (**8b**) in 94% yield, whose spectral data were identical with those of an acetylated compound obtained by the direct acylation of **6**.

5.3.2.5. Preparation of 1-*O*-acetyl-12-*O*-isovalerylexcisanin A 7,14-acetonide (17). Acylation of **8b** with isovaleryl chloride and Et_3N in CH_2Cl_2 gave **17** in 75% yield. Colorless solid (MeOH–H_2O), mp 56–61 °C; $[\alpha]_D$ −31 (*c* 0.83, $CHCl_3$); ^1H NMR (400 MHz, 300 K, $CDCl_3$) δ 6.19 (1H, s), 5.49 (1H, s), 4.98 (1H, m), 4.79 (1H, d, 1.1), 4.43 (1H, dd, 8.5, 7.1), 4.21 (1H, dd, 9.7, 7.0), 3.13 (1H, d, 3.4), 2.34–2.13 (4H, m), 2.04–1.98 (2H, m), 1.96 (3H, s), 1.78 (1H, m), 1.67–1.52 (2H, m), 1.59 (3H, s), 1.45–1.31 (1H, m), 1.26 (3H, s), 1.23 (3H, s), 1.021 (3H, d, 6.6), 1.018 (3H, d, 6.6), 0.91 (3H, s), 0.89 (3H, s); ^{13}C NMR (100 MHz, 300 K, $CDCl_3$) δ 205.6, 172.2, 170.0, 143.6, 119.4, 97.3, 82.9, 73.6, 70.4, 66.8, 54.1, 52.1, 50.4, 47.5, 43.8, 41.8, 38.8, 33.0, 32.7, 30.4, 27.0, 25.6, 25.2, 25.0, 24.3, 22.5, 22.4, 21.6, 21.4, 12.6; IR (film) 1738 (C=O), 1653 (C=C); HRMS (ESI): Calculated for $C_{30}H_{45}O_7$ (M^++H): 517.3158. Found: 517.3165.

5.3.2.6. Preparation of 1-*O*-acetyl-12-*O*-benzoylexcisanin A 7,14-acetonide (18). Acetylation of **13c** with Ac_2O and pyridine at rt by the general procedure gave **18** in 88% yield. Colorless solid (MeOH–H_2O), mp 87–91 °C; $[\alpha]_D$ −29 (*c* 0.46, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 8.09 (2H, d, 7.6), 7.62 (1H, dd, 7.6, 7.6), 7.52 (2H, dd, 7.6, 7.6), 6.24 (1H, s), 5.54 (1H, s), 5.22 (1H, m), 4.97 (1H, d, 1.1), 4.43 (1H, dd, 10.9, 4.6), 4.25 (1H, dd, 10.3, 8.4), 3.30 (1H, d, 3.1), 2.43 (1H, d, 17.2), 2.06–2.02 (2H, m), 1.94 (3H, s), 1.89 (1H, ddd, 17.2, 9.6, 5.8), 1.71 (1H, d, 9.6), 1.64 (3H, s), 1.61–1.47 (2H, m), 1.39 (1H, ddd, 13.5, 3.7, 3.7), 1.35 (1H, dd, 13.5, 4.2), 1.28 (3H, s), 1.25 (3H, s), 0.92 (1H, m, overlapped), 0.92 (3H, s), 0.87 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 205.5, 170.1, 165.7, 143.5, 133.3, 130.1, 129.6, 128.7, 119.5, 97.3, 82.7, 74.6, 70.4, 66.9, 54.1, 52.2, 50.5, 47.6, 42.0, 38.8, 33.0, 32.7, 30.5, 27.0, 25.3, 24.9, 24.2, 21.5, 21.4, 12.9; IR (film) 1737, 1717 (C=O), 1652 (C=C); HRMS (ESI): Calculated for $C_{32}H_{41}O_7$ (M^++H): 537.2852. Found: 537.2888.

5.3.2.7. Preparation of 1-*O*-^{t}butyldimethylsilylexcisanin A 7,14-acetonide (20). 1.0 M TBAF solution in THF (0.98 mL, 0.98 mmol) was added to a solution of **19** (0.203 g, 0.328 mmol) in dry THF (10 mL). After stirring at rt for 24 h, the reaction mixture was treated with satd aq NH_4Cl (10 mL). The aqueous phase was extracted with AcOEt (10 mL 3×). The combined organic layer was washed with satd aq NaCl (10 mL 3×), dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oily residue, which was purified by MPLC (hexanes/AcOEt = 2:1) to give 1-*O*-^{t}butyl-dimethylsilylexcisanin A 7,14-acetonide (**20**) in 58% yield. Colorless solid (MeOH–H_2O), mp 83–86 °C; $[\alpha]_D$ −30 (*c* 0.10, $CHCl_3$); ^1H NMR (400 MHz, 300 K, $CDCl_3$) δ 6.14 (1H, s), 5.39 (1H, s), 4.87 (1H, d, 1.3), 4.20 (1H, dd, 9.8, 8.1), 4.05 (1H, br m), 3.29 (1H, dd, 11.0, 4.2), 3.05 (1H, d, 2.4),

2.72 (1H, ddd, 16.3, 2.4, 2.4), 1.97 (1H, m), 1.94 (1H, dd, 8.5, 2.0), 1.76 (1H, ddd, 16.3, 9.3, 5.6), 1.70 (1H, m), 1.64–1.57 (3H, m), 1.55 (3H, s), 1.48 (1H, ddd, 13.6, 7.5, 3.5), 1.37 (1H, ddd, 13.6, 3.5, 3.5), 1.22 (3H, s), 1.21 (3H, s), 0.89 (3H, s), 0.87 (9H, s), 0.86 (3H, s), 0.80 (1H, m), 0.04 (3H, s), −0.03 (3H, s); ^{13}C NMR (100 MHz, 300 K, $CDCl_3$) δ 207.3, 145.2, 117.9, 97.5, 83.1, 72.6, 70.4, 66.8, 55.1, 53.0, 50.9, 49.9, 43.5, 39.4, 33.03, 32.99, 29.9, 29.2, 28.7, 27.3, 26.1, 25.5, 21.6, 18.2, 12.1, −3.1, −4.0; IR (film) 3404 (OH), 1734 (C=O), 1652 (C=C); HRMS (ESI): Calculated for $C_{29}H_{49}O_5Si$ (M^++H): 505.3349. Found: 505.3339.

5.3.2.8. Preparation of 12-*O*-acetyl-1-*O*-^{t}butyldimethylsilylexcisanin A 7,14-acetonide (21). A mixture of **20** (0.040 g, 0.08 mmol), Ac_2O (0.5 mL, 5.3 mmol), and dry pyridine (1.0 mL, 12.4 mmol) was stirred at rt for 15 h. The reaction mixture was poured into ice-cooled water (10 mL) and the aqueous phase was extracted with AcOEt (10 mL 3×). The combined organic phase was washed with 5% HCl (20 mL 2×), satd aq $NaHCO_3$ (20 mL 2×), and satd aq NaCl (20 mL 2×), successively, dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oily residue, which was purified by MPLC (hexanes/AcOEt = 10:1) to give an acetylated compound (**21**) in 100% yield. Colorless solid (MeOH–H_2O), mp 82–85 °C; $[\alpha]_D$ −23 (*c* 0.95, $CHCl_3$); ^{1}H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.19 (1H, s), 5.50 (1H, s), 5.05 (1H, m), 4.77 (1H, d, 0.7), 4.19 (1H, dd, 9.5, 8.8), 3.29 (1H, dd, 11.0, 4.2), 3.11 (1H, d, 2.8), 2.81 (1H, d, 16.8), 2.09 (3H, s), 1.97 (1H, dd, 11.0, 6.8), 1.96 (1H, d, 8.8), 1.77 (1H, ddd, 16.8, 9.5, 6.0), 1.64–1.56 (2H, m), 1.57 (3H, s), 1.48 (1H, ddd, 13.7, 7.5, 3.7), 1.37 (1H, ddd, 13.7, 3.4, 3.4), 1.26–1.19 (1H, m), 1.22 (3H, s), 1.17 (3H, s), 0.89 (3H, s), 0.87 (3H, s), 0.83 (9H, s), 0.79 (1H, m), 0.02 (3H, s), −0.04 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 206.5, 169.8, 144.1, 119.1, 97.4, 82.8, 73.4, 70.6, 67.0, 54.7, 52.5, 50.1, 47.7, 43.7, 39.3, 33.03, 32.97, 30.1, 29.3, 27.3, 26.0, 25.6, 25.5, 21.6, 21.3, 18.1, 11.8, 3.5, −3.8; IR (film) 1739 (C=O), 1654 (C=C); HRMS (ESI): Calculated for $C_{31}H_{51}O_6Si$ (M^++H): 547.3455. Found: 547.3466.

5.3.2.9. Preparation of 12-*O*-acetylexcisanin A 7,14-acetonide (excisanin B 7,14-acetonide) (8c). 1.0 M TBAF solution in THF (0.57 mL, 0.57 mmol) was added to a solution of **21** (0.31 g, 0.057 mmol) in dry THF (3 mL). After stirring at rt for 24 h, satd aq NH_4Cl (10 mL) was added to the reaction mixture. The aqueous phase was extracted with AcOEt (10 mL 3×). The combined organic layer was washed with satd aq NaCl (10 mL 3×), dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oily residue, which was purified by MPLC (hexanes/AcOEt = 2:1) to give a desilylated compound (**8c**) in 64% yield. Colorless solid (MeOH–H_2O), mp 92–95 °C; $[\alpha]_D$ −41 (*c* 0.29, $CHCl_3$); ^{1}H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.19 (1H, s), 5.50 (1H, s), 5.04 (1H, m), 4.80 (1H, s), 4.22 (1H, dd, 8.8, 8.8), 3.24 (1H, m), 3.15 (1H, br d), 3.01 (1H, d, 17.1), 2.12 (3H, s), 2.11 (1H, m, overlapped), 2.02–1.96 (2H, m), 1.84 (1H, ddd, 17.0, 9.6, 5.7), 1.67–1.56 (2H, m, overlapped), 1.60 (3H, s), 1.52–1.47 (1H, m), 1.41 (1H, ddd, 13.6, 3.4, 3.4), 1.28 (1H, ddd, 13.8, 13.8, 3.7), 1.23 (3H, s), 1.21 (3H, s), 0.90 (3H, s), 0.87 (3H, s), 0.82–0.80 (1H, m); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 206.4, 169.9, 144.0, 119.0, 97.4, 80.7, 73.8, 70.5, 67.0, 54.5, 53.1, 50.1, 47.7, 43.1, 39.2, 33.1, 32.8, 30.4, 29.9, 27.2, 25.3, 25.0, 21.5, 21.3, 11.4; IR (film) 3413 (OH), 1736 (C=O), 1649 (C=C); HRMS (ESI): Calculated for $C_{25}H_{37}O_6$ (M^++H): 433.2590. Found: 433.2563.

5.3.2.10. Preparation of 12-*O*-acetyl-1-*O*-isovalerylexcisanin A 7,14-acetonide (22). Acylation of **8c** with isovaleryl chloride gave **22** in 65% yield. Colorless solid (MeOH–H_2O), mp 65–69 °C; $[\alpha]_D$ −73 (*c* 0.17, $CHCl_3$); ^{1}H NMR (400 MHz, 300 K, $CDCl_3$) δ 6.19 (1H, s), 5.48 (1H, s), 4.96 (1H, m), 4.80 (1H, s), 4.44 (1H, dd, 10.8, 4.7), 4.22 (1H, m), 3.12 (1H, d, 3.0), 2.24 (1H, d,16.7), 2.15 (3H, s), 2.13–1.98 (5H, m), 1.74 (1H, ddd, 15.5, 9.7, 5.6), 1.60 (3H, s), 1.65 (1H, d, 10.2), 1.61–1.52 (3H, m), 1.44–1.31 (2H, m), 1.27 (3H, s), 1.23 (3H, s), 0.928 (3H, d, 6.8), 0.919 (3H, s), 0.918 (3H, d, 6.8), 0.89 (3H, s); ^{13}C NMR (100 MHz, 300 K, $CDCl_3$) δ 205.6, 172.0, 169.9, 143.6, 119.3, 97.3, 82.5, 73.7, 70.3, 66.8, 54.1, 52.1, 50.5, 47.5, 43.8, 41.8, 38.8, 33.0, 32.7, 30.4, 27.0, 25.5, 25.3, 25.0, 24.4, 22.33, 22.28, 21.4, 12.6; IR (film) 1737 (C=O), 1653 (C=C); HRMS (ESI): Calculated for $C_{30}H_{45}O_7$ (M^++H): 517.3165. Found: 517.3158.

5.3.2.11. Preparation of 11,12-dehydrated excisanin A 7,14-acetonide (23). Diethyl azodicarboxylate (0.021 g, 0.122 mmol) was added to a solution of **6** (0.022 g, 0.056 mmol), PPh_3 (0.044 g, 0.17 mmol), and benzoic acid (0.016 g, 0.131 mmol) in dry THF (2 mL) at 0 °C under an Ar atmosphere. The mixture was stirred at room temperature for 0.5 h. The solvent was evaporated in vacuo to give an oily residue, which was purified by MPLC (hexanes/AcOEt = 4:1) to give 11,12-dehydrated excisanin A 7,14-acetonide (**23**) in 67% yield. Colorless solid (MeOH–H_2O), mp 202–203 °C; $[\alpha]_D$ −258.5 (*c* 0.07, $CHCl_3$); ^{1}H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.30 (1H, dd, 9.9, 3.4), 5.93 (1H, ddd, 9.4, 7.0, 1.9), 5.82 (1H, s), 5.13 (1H, s), 4.65 (1H, d, 1.7), 4.28 (1H, dd, 10.1, 10.1), 3.35 (1H, br d), 3.26 (1H, d, 6.9), 2.06 (1H, br t), 2.01 (1H, d, 7.0), 1.99 (1H, dd, 5.0, 5.0), 1.62 (1H, m), 1.59 (3H, s), 1.54 (1H, m), 1.43 (1H, ddd, 13.6, 3.4, 3.4), 1.31 (1H, ddd, 13.6, 13.6, 3.8), 1.24 (3H, s), 1.21 (1H, br s), 1.04 (3H, s), 0.91 (3H, s), 0.89 (1H, m), 0.85 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 207.9, 147.9, 129.7, 129.3, 111.8, 97.2, 80.8, 70.9, 68.8, 57.0, 52.8, 49.7, 44.4, 43.9, 39.3, 33.1, 32.5, 30.8, 29.9, 27.3, 25.2, 21.5, 12.4; IR (film) 3443 (OH), 1731 (C=O), 1650 (C=C); HRMS (ESI): Calculated for $C_{23}H_{33}O_4$ (M^++H): 373.2379. Found: 373.2368.

5.3.2.12. Preparation of 1-*O*-acetyl-11,12-dehydrated excisanin A 7,14-acetonide (24). A mixture of **23** (0.029 g, 0.078 mmol), Ac_2O (0.5 mL, 5.3 mmol), and dry pyridine (1.0 mL, 12.4 mmol) was stirred at rt for 2 days. The reaction mixture was poured into ice-cooled water (10 mL) and the aqueous phase was extracted with $CHCl_3$ (10 mL 3×). The combined organic phase was washed with satd aq NaCl (10 mL 2×), dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oily

residue, which was purified by MPLC (hexanes/AcOEt = 5:1) to give an acetylated compound (**24**) in 85% yield. Colorless solid ($MeOH–H_2O$), mp 263–265 °C; $[\alpha]_D$ −240.1 (*c* 0.30, $CHCl_3$); ^1H NMR (500 MHz, 300 K, $CDCl_3$) δ 5.97 (1H, ddd, 9.5, 7.1, 2.1), 5.84 (1H, s), 5.53 (1H, dd, 9.9, 3.4), 5.15 (1H, s), 4.65 (1H, d, 1.9), 4.54 (1H, dd, 11.1, 4.4), 4.28 (1H, dd, 9.8, 8.9), 3.28 (1H, dd, 7.1, 0.9), 2.07 (1H, m), 2.02 (1H, m), 2.01 (3H, s), 2.00 (1H, m), 1.69 (1H, m), 1.60 (1H, m), 1.59 (3H, s), 1.43 (1H, m), 1.39 (1H, m), 1.24 (3H, s), 1.14 (3H, s), 0.99 (1H, m), 0.94 (3H, s), 0.89 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 207.1, 169.9, 147.5, 130.7, 127.6, 112.3, 97.2, 82.9, 70.8, 68.6, 56.0, 52.6, 50.1, 44.3, 42.7, 38.8, 33.0, 32.5, 30.7, 27.1, 25.3, 24.9, 21.7, 21.6, 13.5; IR (film) 1737 (C=O), 1650 (C=C); HRMS (ESI): Calculated for $C_{25}H_{35}O_5$ (M^++H): 415.2484. Found: 415.2492.

5.4. Assay for cytotoxic activity

The cytotoxic assay was performed by using the MTT assay method. The murine P388 leukemia cells were precultured in RPMI 1640 medium (Nissui Co. Ltd, Japan) supplemented with 5% heat-inactivated fetal bovine serum (FBS) and kanamycin (5.3 mL/L) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. A suspension of the cells (3×10^4 cells/mL, 100 μL) was added to each well (3×10^3 cells/well) of a 96-microwell plate (flat-bottomed, polystyrene-treated) and incubated for 24 h. Test compounds were dissolved in DMSO in various concentrations (100, 10, 1, and 0.1 μg/mL) and 10 μL of the test solutions or DMSO (control) was added to each well. The plate was incubated at 37 °C for 48 h. After termination of the cell culture by adding 5% MTT in PBS (20 μL) to each well, the plate was kept in the incubator for 4 h. To each well was added 100 μL of 10% SDS–0.01 N HCl and the plate was read on a microplate reader (MPR A4i, Toso) at 550 nm. A dose-response curve was plotted for each compound, and the concentrations giving 50% inhibition of the cell growth (IC_{50}) were recorded.

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