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Cytotoxic diterpenoid alkaloid from *Aconitum japonicum* subsp. *subcuneatum*

Hiroshi Yamashita¹, Mai Miyao¹, Kazuki Hiramori¹, Daisuke Kobayashi¹, Yuji Suzuki¹, Megumi Mizukami¹, Masuo Goto², Kuo-Hsiung Lee^{2,3}, Koji Wada¹

¹Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University of Science, 4-1, Maeda 7-jo 15-choume, Teine-ku, Sapporo 006-8585, Japan

²Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599 7568, USA

³Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung 40402, Taiwan

Abstract

We explored new cytotoxic C₁₉-diterpenoid alkaloid, lipojesaconitine (**1**), from rhizoma of *Aconitum japonicum* THUNB. subsp. *subcuneatum* (NAKAI) KADOTA. Two additional non-cytotoxic new C₁₉-diterpenoid alkaloids, 10-hydroxychasmanine (**2**) and 3-hydroxykaracoline (**3**), together with eight known C₁₉- and C₂₀-diterpenoid alkaloids (**4–11**) were also isolated. Their structures were elucidated by extensive spectroscopic methods including NMR (1D and 2D), IR, and MS (HRMS). Six known diterpenoid alkaloids, foresticine (**5**), neoline (**6**), aconicarchamine A (**7**), 9-hydroxynominine (**8**), kobusine (**9**), and torokanine (**10**), were isolated for the first time from *A. japonicum* subsp. *subcuneatum*. Alkaloid **1** showed cytotoxicity with IC₅₀ values ranging from 6.0 to 7.3 μM against four human tumor cell lines, except a multidrug-resistant subline, suggesting that **1** was likely exported by P-glycoprotein.

Keywords

Lipojesaconitine; 10-Hydroxychasmanine; 3-Hydroxykaracoline; *Aconitum japonicum* THUNB. subsp. *subcuneatum* (NAKAI) KADOTA; Cytotoxicity

Introduction

The rhizoma of *Aconitum* plants, Kampo medicine (traditional Japanese medicine) known as “bushi”, are used in Japan for the treatment of hypometabolism, dysuria, cardiac weakness, chills, neuralgia, gout, and certain rheumatic diseases [1, 2]. Diterpenoid alkaloids are major pharmaceutically active constituents of *Aconitum* rhizoma [2]. In prior phytochemical investigations of *A. japonicum* THUNB. subsp. *subcuneatum* (NAKAI) KADOTA (Ranunculaceae), the structures of isolated C₁₉-diterpenoid alkaloids were elucidated [3–9]. As previously

kowada@hus.ac.jp.

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reported [10–13], several diterpenoid alkaloids from the genera *Aconitum* and *Delphinium* and their derivatives revealed mild cytotoxic activity against human tumor cell lines, including A172 (glioblastoma), A549 (lung carcinoma), DU145 (prostate), KB (identical to cervical carcinoma HeLa-derivative AV-3), and KB-VIN (vincristine-resistant KB subline overexpressing P-glycoprotein). In our course of studies on synthetic derivatives from C₁₉-diterpenoid alkaloid delpheline, and C₂₀-diterpenoid alkaloids kobusine and pseudokobusine, we found several derivatives showing significant cytotoxic activity. The cytotoxic properties of *Aconitum* diterpenoid alkaloids and extracts have been reviewed [14].

In a previous paper [15], the isolation and elucidation of 19 C₁₉-diterpenoid alkaloids and two C₂₀-diterpenoid alkaloids from the rhizoma of *A. japonicum* subsp. *subcuneatum* were reported. Continued investigations on the constituents of this plant have now resulted in the isolation of three new C₁₉-diterpenoid alkaloids, lipojesaconitine (**1**), 10-hydroxychasmanine (**2**), and 3-hydroxykaracoline (**3**), together with three known C₁₉-diterpenoid alkaloids, lipoaconitine (**4**) [16], foresticine (**5**) [17], neoline (**6**) [18], and five known C₂₀-diterpenoid alkaloids, aconicarchamine A (**7**) [19], 9-hydroxynominine (**8**) [20], kobusine (**9**) [21], torokanine (**10**) [22], and dehydrolucicoline (**11**) [23]. Six of the known alkaloids (**5**–**10**) were isolated for the first time from this plant. Two of the new C₁₉-diterpenoid alkaloids (**1**, **3**) and five of the known diterpenoid alkaloids (**4**, **6**–**8**, **10**) were evaluated for cytotoxic activity against five human tumor cell lines (A549, MDA-MB-231, MCF-7, KB, and KB-VIN). To our knowledge, alkaloid **1** is the most potent aconitine-type C₁₉-diterpenoid alkaloid with an aconitine cytoskeleton.

Results and discussion

Alkaloid **1** was obtained as a colorless amorphous solid, $[\alpha]_D^{25} + 13.4^\circ$. The IR spectrum indicated the presence of hydroxy (3490 cm⁻¹) and carbonyl (1712 cm⁻¹) groups. The ¹H-NMR spectrum of **1** (Table 1) revealed the presence of an *N*-ethyl unit (δ_H 1.10, 3H, t, *J* = 7.2 Hz; 2.39 and 2.74, each 1H, m), five methoxy resonances (δ_H 3.26, 1'-OMe; 3.16, 6'-OMe; 3.76, 16'-OMe; 3.30, 18'-OMe; 3.85, aromatic-OMe; each 3H, s), and four aromatic protons at δ_H 6.92 and 7.97 (each 2H, d, *J* = 8.6 Hz, H-Ar). The ¹H-NMR spectrum of **1** was very similar to that of lipoaconitine (**4**) [16], included a fatty acid (steroyl, oleoyl, linoleoyl, linolenoyl, and palmitoyl acid) ester moiety at the C-8 position, except for the absence of a resonance for a benzoyl group and the presence of a resonance for an anisoyl group. The ¹³C-NMR spectrum of **1** (Table 1, Fig. 1) indicated two ester carbonyl carbons (δ_C 175.2 and 165.8), four aromatic carbons (δ_C 113.9, 122.3, 131.8, and 163.6), nine oxygenated carbons (δ_C 71.6, 74.1, 76.8, 78.7, 79.0, 82.4, 83.5, 90.1 and 91.7), and five methoxy carbons (δ_C 55.4, 55.9, 58.2, 59.1, and 61.3). The presence of an IR absorption at 1712 cm⁻¹, the molecular formula C₅₁H₈₁NO₁₂ (steroyl), C₅₁H₇₉NO₁₂ (oleoyl), C₅₁H₇₇NO₁₂ (linoleoyl), C₅₁H₇₅NO₁₂ (linolenoyl), C₄₉H₇₇NO₁₂ (palmitoyl), as deduced from the apparent molecular ion ([M+H]⁺) at *m/z* 900.5855 (C₅₁H₈₂NO₁₂, calcd. 900.5857), 898.5662 (C₅₁H₈₀NO₁₂, calcd. 898.5681), 896.5531 (C₅₁H₇₈NO₁₂, calcd. 896.5524), 894.5358 (C₅₁H₇₆NO₁₂, calcd. 894.5368), 872.5516 (C₄₉H₇₈NO₁₂, calcd. 872.5524), calculated from the HR-FABMS, and ¹³C-NMR signals at δ_C 175.2 (carbonyl) suggested

that, like **4**, compound **1** contained a fatty acid ester moiety at the C-8 position. In the HMBC spectrum of **1** (Fig. 2), correlations were observed between signals at δ_{H} 2.11 (1H, m, H-10), 2.84 (1H, s, H-7), 2.89 (1H, m, H-9 β), 3.08 (1H, s, H-17), 4.03 (1H, d, J = 6.8 Hz, H-6), 4.43 (1H, d, J = 5.5 Hz, H-15 β), and 4.82 (1H, d, J = 5.0 Hz, H-14 β) with an angular carbon at δ_{C} 91.7 (s, C-8). Correlations were also observed between signals at δ_{H} 3.16 (3H, s, OCH₃) with δ_{C} 83.5 (C-6), δ_{H} 3.26 (3H, s, OCH₃) with δ_{C} 82.4 (C-1), δ_{H} 3.30 (3H, s, OCH₃) with δ_{C} 76.8 (C-18), δ_{H} 3.76 (3H, s, OCH₃) with δ_{C} 90.1 (C-16), and δ_{H} 4.82 (1H, d, J = 5.0 Hz, H-14 β) with δ_{C} 165.8 (C=O), 94.7 (C-8), and 74.1 (C-13). Key two- and three-bond heteronuclear connectivities and the combined spectroscopic data verified the structure of **1** as lipojesaconitine. Lipojesaconitine (**1**) is the third reported natural C₁₉-diterpenoid alkaloid possessing a fatty acid ester moiety at C-8 with oxygenation at C-3, C-13, and C-15.

Alkaloid **2** was obtained as a white amorphous solid, $[\alpha]_{\text{D}}^{24} + 12.2^\circ$. The EIMS ions observed at m/z 467 (M⁺), 452 ([M-CH₃]⁺) and 436 ([M-OCH₃]⁺) as well as HR-EIMS ion at m/z 452.2645 ([M-CH₃]⁺) corresponded to the formula C₂₄H₃₈NO₇ (calcd. 452.2648). The IR spectrum of **2** indicated the presence of a hydroxy (3414 cm⁻¹) group. The ¹H-NMR of **2** (Table 1) revealed the presence of an *N*-ethyl unit (δ_{H} 1.08, 3H, t, J = 7.5 Hz; 2.45 and 2.53, each 1H, m), four methoxy groups at δ_{H} 3.25, 3.310, 3.314, and 3.34 (each 3H, s), and five oxygenated protons at δ_{H} 3.29 and 3.76 (each 1H, d, J = 8.2 Hz, H₂-18), 3.38 (1H, d, J = 9.6 Hz, H-16 α), 3.65 (1H, m, H-1 β), 4.15 (1H, d, J = 6.9 Hz, H-6 β), and 4.70 (1H, t, J = 5.5 Hz, H-14 β). The ¹H-NMR of **2** was very similar to that of chasmanine [24]. The ¹³C-NMR spectrum of **2** (Table 1) indicated an *N*-ethyl unit (δ_{C} 13.7 and 49.3), seven oxygenated carbons (δ_{C} 71.2, 74.2, 78.9, 80.7, 81.5, 81.7, and 82.4), and four methoxy carbons (δ_{C} 55.9, 56.4, 57.6, and 59.2). The ¹³C-NMR spectrum of **2** was very similar to that of chasmanine [24], except for the absence of a resonance at δ_{C} 38.4 (d) and the presence of a resonance at δ_{C} 81.7 (s). A comparison of the ¹³C-NMR spectrum of **2** and chasmanine showed that the C-10 resonance in **2** was shifted downfield due to an α effect, the C-9, C-11, and C-12 resonances in **2** were shifted downfield due to a β effect, and the C-1, C-5, C-8, C-13, and C-14 resonances in **2** were shifted upfield due to an γ effect [2, 24], as expected with the presence of a hydroxyl group at C-10 in **2**. In the HMBC spectra of **2** (Fig. 2), correlation of a proton at δ_{H} 2.45 (1H, t, J = 8.3 Hz, H-13), 3.65 (1H, m, H-1 β), and 4.70 (1H, t, J = 5.5 Hz, H-14 β) with a carbon at δ_{C} 81.7 (C-10) confirmed the presence of the hydroxyl group at C-10. Correlations were also observed between signals at δ_{H} 3.25 (3H, s, OCH₃) with δ_{C} 78.9 (C-1) as well as δ_{H} 3.310 (3H, s, OCH₃) with δ_{C} 80.7 (C-18), δ_{H} 3.314 (3H, s, OCH₃) with δ_{C} 82.4 (C-6), and δ_{H} 3.34 (3H, s, OCH₃) with δ_{C} 81.5 (C-16). Key two- and three-bond heteronuclear connectivities also substantiated the structure of **2**. The spectroscopic data were typical of a C₁₉-diterpenoid alkaloid and consistent with the structure of 10-hydroxychasmanine for **2**. 10-Hydroxychasmanine (**2**) is the fourth reported aconitine-type C₁₉-diterpenoid alkaloid possessing a 10-hydroxy group with a 18-methoxy moiety and non-oxygenation at C-3, C-13, and C-15.

The EIMS and HR-EIMS of alkaloid **3**, a white amorphous solid, $[\alpha]_{\text{D}}^{26} + 5.3^\circ$, contained ions at m/z 393 (M⁺) and 376 ([M-OH]⁺) and **3** contained a molecular ion at m/z 394 ([M+H]⁺)

corresponding to the molecular formula $C_{22}H_{36}NO_5$ (found m/z 394.2581, calcd. 394.2593). The IR spectrum contained an absorption for a hydroxy (3400 cm^{-1}) group. The $^1\text{H-NMR}$ spectrum of **3** (Table 1) revealed the presence of an *N*-ethyl unit (δ_{H} 1.09, 3H, t, $J = 7.5\text{ Hz}$; 2.42 and 2.52, each 1H, m), an angular methyl group (δ_{H} 0.91, 3H, s), a methoxy group (δ_{H} 3.34, 3H, s) and four oxygenated methines at δ_{H} 3.40 (1H, m, H-16 α), 3.76 (1H, m, H-3 β), 3.78 (1H, m, H-1 β), and 4.20 (1H, t, $J = 5.5\text{ Hz}$, H-14 β), and the $^{13}\text{C-NMR}$ spectrum (Table 1) indicated the presence of *N*-ethyl carbons (δ_{C} 13.5 and 48.8), an angular methyl carbon (δ_{C} 21.3), and five oxygenated carbons (δ_{C} 72.0, 73.5, 74.8, 75.7, and 82.1). The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra of **3** were very similar to those of karacoline [25, 26], except for the absence of a resonance at δ_{C} 31.5 (t) and the presence of a resonance at δ_{C} 74.8 (d). When the $^{13}\text{C-NMR}$ spectra of **3** and karacoline [26] were compared, the C-3 resonance in **3** was shifted downfield due to an α effect, the C-2 and -4 resonances in **3** were shifted downfield due to a β effect, and the C-1, -5, -18, and -19 resonances in **3** were shifted upfield due to an γ effect [2, 24], as expected due to the presence of a hydroxyl group at C-3 in **3**. In HMBc experiments (Fig. 2), signals at δ_{H} 0.91 (H₃-18), 2.01 and 2.09 (H₂-2), 2.10 and 2.35 (H₂-19), and 3.78 (H-1) was correlated with signal at δ_{C} 74.8 (C-3). From these correlations, the signal at δ_{C} 74.8 was assigned to C-3. Additional key two- and three-bond heteronuclear connectivities were elucidated from the spectroscopic data, and compound **3** was fully identified as 3-hydroxykaracoline. 3-Hydroxykaracoline (**3**) is the fourth reported aconitine-type C₁₉-diterpenoid alkaloid possessing a 3-hydroxy group with an 18-methyl moiety.

The known diterpenoid alkaloids were assigned as three known C₁₉-diterpenoid alkaloids, lipoaconitine (**4**) [16], foresticine (**5**) [17], neolinine (**6**) [18], and five known C₂₀-diterpenoid alkaloids, aconicarchamine A (**7**) [19], 9-hydroxynominine (**8**) [20], kobusine (**9**) [21], torokonine (**10**) [22], and dehydroluciculine (**11**) [23] by comparison of the spectroscopic data with those reported. Six of the known alkaloids (**5**–**10**) were isolated for the first time from this plant.

Two new C₁₉-diterpenoid alkaloids (**1**, **3**) and five known diterpenoid alkaloids (**4**, **6**–**8**, **10**) were evaluated for cytotoxic activity against five human tumor cell lines [A549, MDA-MB-231 (estrogen and progesterone receptors-negative and HER2-negative triple-negative breast cancer), MCF-7 (estrogen receptor-positive, HER2-negative breast cancer), KB, and multidrug-resistant KB subline KB-VIN] (Table 2). The new C₁₉-diterpenoid alkaloid, lipojesaconitine (**1**), showed significant cytotoxicity against the four tested cell lines with IC₅₀ values of 6.0–7.3 μM , but weak cytotoxicity against KB-VIN (IC₅₀ = 18.6 μM). These observations suggested that **1** may be partially pumped out of KB-VIN cells by P-gp. The known diterpenoid alkaloid (**4**) was weakly cytotoxic (IC₅₀ = 13.7–20.3 μM) against all five human tumor cell lines. The new (**3**) and four known (**6**–**8**, **10**) alkaloids were non-toxic at 40 μM against all five cell lines. In our prior study, the two known diterpenoid alkaloids **9** and **11** were also non-cytotoxic (IC₅₀ > 20 μM) against A549, DU145, KB, and KB-VIN human tumor cell lines [13]. When the cytotoxic activities of 17 aconitine-type C₁₉-diterpenoid alkaloids (**1**, **4**, lipomesaconitine [15], aconitine [13], jesaconitine [13], mesaconitine [13], deoxy-aconitine [13], deoxyjesaconitine [13], hypaconitine [13], aljesaconitine A [13], hokbusine A [13], 3,15-diacetyljesaconitine [13], 3-acetylmesaconitine [13], secojesaconitine [13], 14-anisoyllasianine [15], 14-anisoyl-*N*-

deethylaconine [15], and *N*-deethylaljesaconitine A [15]) were compared, compound **1** (lipojesaconitine) showed significant cytotoxicity against four tested cell lines (A549, MDA-MB-231, MCF-7, and KB) and lipomesaconitine exhibited mild cytotoxicity against the KB cell line ($IC_{50} = 9.9 \mu M$) [15]. These results suggest that the fatty acid ester at C-8 and the anisoyl group at C-14 found in **1** may be important to the cytotoxic activity of aconitine-type C_{19} -diterpenoid alkaloids.

Conclusion

In this report, we describe the isolation of three new aconitine-type C_{19} -diterpenoid alkaloids (**1–3**) from the rhizoma of *A. japonicum* subsp. *subcuneatum*. The structures of **1–3** were elucidated from 1D and 2D NMR spectroscopic data. Lipojesaconitine (**1**) is the third natural C_{19} -diterpenoid alkaloid possessing a fatty acid ester moiety at C-8 with oxygenation at C-3, C-13, and C-15. 10-Hydroxychasmanine (**2**) is the fourth reported aconitine-type C_{19} -diterpenoid alkaloid possessing a 10-hydroxy group with a 18-methoxy moiety and non-oxygenation at C-3, C-13, and C-15. 3-Hydroxykaracoline (**3**) is the fourth reported aconitine-type C_{19} -diterpenoid alkaloid possessing a 3-hydroxy group with a 18-methyl moiety. In addition, we isolated three known C_{19} -diterpenoid alkaloids, lipoaconitine (**4**), foresticine (**5**), neoline (**6**), and five known C_{20} -diterpenoid alkaloids, aconicarchamine A (**7**), 9-hydroxynominine (**8**), kobusine (**9**), torokonine (**10**), and dehydroluciculine (**11**). Six of the known alkaloids (**5–10**) were isolated for the first time from this plant. Lipojesaconitine (**1**) showed significant cytotoxicity against the four tested cell lines with IC_{50} values of 6.0–7.3 μM , but weak cytotoxicity against KB-VIN ($IC_{50} = 18.6 \mu M$). These observations suggested that **1** may be partially pumped out of KB-VIN cells by P-gp. Lipoaconitine (**4**) was weakly cytotoxic ($IC_{50} = 13.7\sim 20.3 \mu M$) against all five human tumor cell lines.

Experimental section

General experimental procedures

Optical rotations were measured on a Jasco Model P-2300 polarimeter (Jasco, Tokyo, Japan). IR spectra were recorded using a PerkinElmer Model Spectrum 100 spectrometer (PerkinElmer Japan, Yokohama, Japan). NMR spectra were recorded in $CDCl_3$ on a JEOL Model ECZ400 or ECA600 spectrometer (JEOL, Tokyo, Japan) with TMS as an internal standard. MS and HRMS were recorded on a JEOL Model JMS-700 mass spectrometer (JEOL, Tokyo, Japan).

Isolation procedure

The rhizoma of *A. japonicum* THUNB. subsp. *subcuneatum* (NAKAI) KADOTA were collected from Zenibako, Otaru City, Hokkaido, Japan in August 2009. The plant was identified by Dr. Koji Wada of the Hokkaido University of Science, where a voucher specimen has been deposited. Twenty-one alkaloids were extracted and isolated previously from the rhizoma of *A. japonicum* THUNB. subsp. *subcuneatum* (NAKAI) KADOTA [15]. The remaining crude alkaloidal material was chromatographed on silica gel with Et_2O and/or $CHCl_3$ saturated with 28% aq. NH_3 -MeOH mixture of increasing polarity. Purification of fraction E by

repeated column chromatography gave three new C₁₉-diterpenoid alkaloids, lipojesaconitine (**1**, 17 mg), 10-hydroxychasmanine (**2**, 2 mg), and 3-hydroxykaracolone (**3**, 7 mg), together with three known C₁₉-diterpenoid alkaloids, lipoaconitine (**4**, 4 mg) [16], foresticine (**5**, 2 mg) [17], neolinine (**6**, 34 mg) [18], and five known C₂₀-diterpenoid alkaloids, aconicarchamine A (**7**, 17 mg) [19], 9-hydroxynominine (**8**, 23 mg) [20], kobusine (**9**, 5 mg) [21], torokonine (**10**, 33 mg) [22], and dehydroluciculine (**11**, 30 mg) [23].

Lipojesaconitine (**1**) colorless amorphous solid; $[\alpha]_D^{25} + 13.4^\circ$ (c 1.23, CHCl₃); IR (ATR) $\nu_{\max}$ cm⁻¹: max cm : 3490, 2923, 2853, 1712, 1606, 1257, 1169, 1097; ¹H-NMR (CDCl₃, 600 MHz) and ¹³C-NMR (CDCl₃, 150 MHz) spectroscopic data, see Table 1; FABMS m/z 900 (stearoyl, [M+H]⁺), 898 (oleoyl, [M+H]⁺), 896 (linoleoyl, [M+H]⁺), 894 (linolenoyl, [M+H]⁺), 872 (palmitoyl, [M+H]⁺); HR-FABMS m/z 900.5855 [M+H]⁺ (calcd. for C₅₁H₈₂NO₁₂, 900.5857), 898.5662 [M+H]⁺ (calcd. for C₅₁H₈₀NO₁₂, 898.5681), 896.5531 [M+H]⁺ (calcd. for C₅₁H₇₈NO₁₂, 896.5524), 894.5358 [M+H]⁺ (calcd. for C₅₁H₇₆NO₁₂, 894.5368), 872.5516 [M+H]⁺ (calcd. for C₄₉H₇₈NO₁₂, 872.5524).

10-Hydroxychasmanine (**2**) white amorphous solid; $[\alpha]_D^{25} + 12.2^\circ$ (c 0.16, CHCl₃); IR (ATR) $\nu_{\max}$ cm⁻¹: 3414, 2925, 2820, 1087; ¹H-NMR (CDCl₃, 600 MHz) and ¹³C-NMR (CDCl₃, 150 MHz) spectroscopic data, see Table 1; EIMS m/z 467 [M]⁺, 452 [M-CH₃]⁺, 437 (base peak), 436 [M-OCH₃]⁺; HR-EIMS m/z 452.2645 [M-CH₃]⁺ (calcd. For C₂₄H₃₈NO₇, 452.2648).

Cell culture and cytotoxicity assay

All cell lines were obtained from American Type Culture Collection (ATCC, VA, USA) or UNC Lineberger Comprehensive Cancer Center (NC, USA), except vincristine-resistant KB subline KB-VIN, which was a gift from Professor Y.-C. Cheng (Yale University, CT, USA). Cytotoxicity was determined by the sulforhodamine B (SRB) assay, as described previously [27]. Cells were cultured with test compounds for 72 h. The IC₅₀ was calculated from at least three independent experiments performed in duplicate.

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Fig. 1.
Chemical structures of compounds **1–11**

Fig. 2.

Key ¹H-¹H COSY and HMBC correlations of compounds **1–3**

Table 1

¹³C- and ¹H-NMR spectroscopic data for compounds **1–3** in CDCl₃ (δ in ppm; *J* in Hz)

Position	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	82.4	3.13 dd (7.2, 5.9)	78.9	3.65 m	72.0	3.78 m
2	33.5	2.37 m, 1.98 m	25.8	2.34 m, 1.97 m	41.0	2.09 m, 2.01 m
3	71.6	3.78 m	35.0	1.65 m, 1.52 m	74.8	3.76 m
4	43.1	–	39.3	–	39.0	–
5	46.5	2.11 d (6.8)	46.3	2.21 d (6.8)	44.0	1.94 d (7.6)
6	83.5	4.03 d (6.8)	82.4	4.15 d (6.8)	25.6	4.06 d (6.2)
7	44.7	2.84 s	52.2	2.05 s	44.6	2.10 m
8	91.7	–	71.2	–	73.5	–
9	44.3	2.89 m	58.0	2.05 s	46.8	2.30 t (5.5)
10	41.0	2.11 m	81.7	–	44.6	1.84 m
11	50.1	–	55.6	–	48.9	–
12	35.7	2.69 m, 2.11 m	38.5	2.58 d (16.5), 1.71 dd (16.5, 8.3)	27.6	1.95 m, 1.81 m
13	74.1	–	38.3	2.45 d (8.3)	38.8	2.36 m
14	78.7	4.82 d (5.0)	74.2	4.70 t (5.5)	75.7	4.20 t (5.5)
15	79.0	4.43 d (5.5)	40.1	2.45 d (18.6), 2.09 d (18.6)	40.4	2.42 m, 2.09 m
16	90.1	3.33 d (5.5)	81.5	3.38 d (9.6)	82.1	3.40 m
17	61.0	3.08 s	63.4	2.98 s	62.4	2.99 s
18	76.8	3.61 d (9.1), 3.46 d (9.1)	80.7	3.76 d (8.9), 3.29 d (8.9)	21.3	0.91 s
19	47.0	2.87 d (11.4), 2.35 d (11.4)	53.7	2.65 d (9.6), 2.51 d (9.6)	57.2	2.35 d (11.7), 2.10 d (11.7)
N-CH ₂	48.9	2.74 m, 2.39 m	49.3	2.53 m, 2.45 m	48.8	2.52 m, 2.42 m
CH ₃	13.3	1.10 t (7.2)	13.7	1.08 t (7.5)	13.5	1.09 t (7.5)
1'	55.9	3.26 s	55.9	3.25 s	–	–
6'	58.2	3.16 s	57.6	3.314 s	–	–
16'	61.3	3.76 s	56.4	3.34 s	56.4	3.34 s
18'	59.1	3.30 s	59.2	3.310 s	–	–
8' C=O	175.2	–	–	–	–	–
14' C=O	165.8	–	–	–	–	–

Position	1		2		3	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1''	122.3	-	-	-	-	-
2'' 6''	131.8	7.97 d (8.6)	-	-	-	-
3'' 5''	113.9	6.92 d (8.6)	-	-	-	-
4''	163.6	-	-	-	-	-
OCH ₃	55.4	3.85 s	-	-	-	-

The assignments were based on ¹H-¹H COSY, HMQC and HMBC experiments

Table 2Cytotoxicity of diterpenoid alkaloids **1**, **3**, **4**, **6-8**, **10**, and lipomesaconitine

Alkaloids	IC ₅₀ (μM) ^a					
	A549	MDA-MB-231	MCF-7	KB	KB-VIN	
Lipojesaconitine (1)	7.3 ± 0.3	6.0 ± 0.2	6.7 ± 0.2	6.0 ± 0.2	18.6 ± 0.9	
3-Hydroxykaracoline (3)	> 40	> 40	> 40	> 40	> 40	
Lipoaconitine (4)	17.4 ± 1.1	15.5 ± 0.5	16.0 ± 0.3	13.7 ± 1.3	20.3 ± 1.1	
Neoline (6)	> 40	> 40	> 40	> 40	> 40	
Aconitine (7)	> 40	> 40	> 40	> 40	> 40	
9-Hydroxynonimine (8)	> 40	> 40	> 40	> 40	> 40	
Torokone (10)	> 40	> 40	> 40	> 40	> 40	
Lipomesaconitine [15]	17.2 ± 2.3	20.0 ± 0.2	19.0 ± 1.0	9.9 ± 3.3	21.5 ± 0.9	
Paclitaxel (nM) ^b	6.3 ± 0.3	7.8 ± 0.1	12.4 ± 0.1	6.8 ± 0.8	2237.6 ± 271.4	

^aValues are mean ± standard deviation^bPaclitaxel was used as an experimental control