

Anthranoyl-substituted Norditerpene Alkaloids from *Aconitum vulparia* Rchb. and Their Cytotoxic Activities

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Extensive chromatographic purification of the alkaloid fraction of *Aconitum vulparia* Rchb. led to the isolation of a new norditerpene alkaloid, vulparine (**1**), besides the known compounds septentriodine (**2**), finetiadine (**3**), anthranoyllycoctonine (**4**), *N*-methyl-*N*-deethyllycoctonine (**5**) and delectinine (**6**). The structure of the new compound was determined by means of HRMS, 1D and 2D NMR spectroscopy. Detailed NMR studies, including ¹H-¹H COSY, NOESY, HSQC and HMBC experiments, resulted in complete and unambiguous ¹H chemical shift assignments for **2** and **6**, and revision of some ¹³C NMR data. Compounds **1**–**4** were evaluated for their cytotoxic activities, and **1**, **3** and **4** were found to exhibit marginal cell growth inhibitory activity against breast adenocarcinoma (MCF-7) and cervix adenocarcinoma (HeLa) cells.

Key words: *Aconitum vulparia*, Norditerpene Alkaloid, Cytotoxicity

Introduction

Aconitum, *Delphinium* and *Consolida* species are known to produce various hydroxy-, alkoxy- and ester-substituted diterpene and norditerpene alkaloids. Many C₁₉ lycoctonine-type alkaloids exhibit high toxicity and a broad spectrum of pharmacological activities, including antinociceptive, anti-inflammatory, local anaesthetic, antioxidant and tyrosinase inhibitory activities [1–3]. Moreover, norditerpene alkaloids with an *N*-substituted anthranilate ester side-chain, *e.g.* methyllycaconitine (MLA), display extremely great affinity and high selectivity for the $\alpha 7$ nicotinic acetylcholine receptor (nAChR) subtype, and are therefore prime lead compounds for the design and synthesis of new drugs for the therapy of Alzheimer's disease, epilepsy and schizophrenia [4, 5]. In recent years it was demonstrated that norditerpene alkaloids can act on biological targets other than neuroreceptors: they exhibit cytotoxic effect against insect Sf9 and different human tumour cell lines with strong molecular selectivity [6–8]. In a previous paper, we reported the isolation of a new norditerpene alkaloid, acovulparine,

from *A. vulparia*, together with lycoctonine and delcosine [9]. On completion of our studies on this plant, we now describe the isolation and structure elucidation of a further new norditerpene alkaloid, vulparine (**1**), and five known compounds: septentriodine (**2**), finetiadine (**3**), anthranoyllycoctonine (**4**), *N*-methyl-*N*-deethyllycoctonine (**5**) and delectinine (**6**). The complete and unambiguous ¹H and ¹³C NMR signal assignments of compounds **2** and **6**, and the results of the cytotoxic assay of vulparine (**1**), septentriodine (**2**), finetiadine (**3**), anthranoyllycoctonine (**4**) on breast adenocarcinoma (MCF-7) and cervix adenocarcinoma (HeLa) cells are also reported in this paper.

Results and Discussion

Compound **1** was isolated as an amorphous solid. The molecular formula C₃₆H₅₀N₂O₁₁ was derived from the HR mass spectrum ($[M+H]^+$ $m/z = 687.3437$, $[M+H]^+$ calcd. 687.3493) combined with the NMR data. The ¹H NMR spectrum of **1** indicated the presence of aromatic protons ($\delta_H = 7.11$ t, 7.55 t, 7.96 d, 8.70 d), which were attributed to an anthranoyl group,

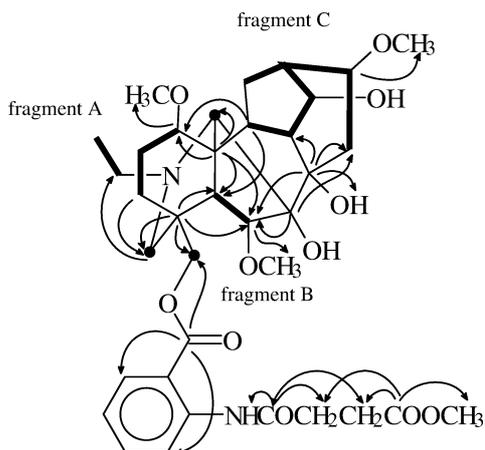


Fig. 1. Selected ^1H - ^1H COSY (—) and HMBC (C \rightarrow H) correlations for **1**.

bond correlations were observed between the quaternary carbon at $\delta_{\text{C}} = 48.4$ (C-11) and the protons at $\delta_{\text{H}} = 3.02$ (H-1), 1.80 (H-5), 3.89 (H-6) and 3.28 (C-17), and between the carbon at $\delta_{\text{C}} = 37.9$ (C-4) and the protons at $\delta_{\text{H}} = 1.80$ (H-5), 3.89 (H-6), 4.20 (H-18a), 4.15 (H-18b) and 2.78 (H-19a), indicating that fragment A and the quaternary carbons C-4 and C-11 form a six-membered ring. The correlations of the carbon at $\delta_{\text{C}} = 89.3$ (C-7) with the protons at $\delta_{\text{H}} = 3.89$ (H-6) and 2.50 (H-15 β), of the carbon at $\delta_{\text{C}} = 76.3$ (C-8) with the protons at $\delta_{\text{H}} = 3.89$ (H-6), 3.13 (H-9) and 2.50 (H-15 β) and of the carbon at $\delta_{\text{C}} = 46.1$ (C-10) with the protons at $\delta_{\text{H}} = 3.02$ (H-1) and 1.80 (H-5) proved the linkage of structural parts C and B with the six-membered ring, as depicted in Fig. 1. The HMBC spectrum also provided information on the locations of the four methoxy groups, one anthranoyl group, and one hydroxy group. The long-range correlations of the carbons of the norditerpene core at $\delta_{\text{C}} = 76.3$ (C-8), 81.8 (C-16), 84.8 (C-1) and 90.6 (C-6) with the protons at $\delta_{\text{H}} = 3.53$ (1H), 3.36, 3.26 and 3.39 (each 3H) revealed the positions of one hydroxy group at C-8 and three methoxy groups at C-16, C-1 and C-6. The correlation of the carbon at $\delta_{\text{C}} = 52.6$ (C-19) with the proton at $\delta_{\text{H}} = 2.97$ (H-20a) confirmed the position of the *N*-ethyl group. The position of the anthranoyl group at C-18 was determined *via* the correlations of the carbonyl carbon at $\delta_{\text{C}} = 168.1$ (C-7') with the protons at $\delta_{\text{H}} = 8.70$ (H-3'), 7.96 (H-6'), 4.20 (H-18a) and 4.15 (H-18b). In view of the molecular formula of **1** and the ^{13}C chemical shifts of C-7 ($\delta_{\text{C}} = 89.3$) and C-14 ($\delta_{\text{C}} = 75.3$), the presence of further two hydroxy groups was concluded, situated at C-7 and C-14. Moreover, the

presence of a $-\text{CO}-\text{CH}_2-\text{CH}_2-\text{COOCH}_3$ side-chain, attached to the amino group of the anthranoyl moiety, was demonstrated by several correlations of carbonyl carbons [$\delta_{\text{C}} = 170.2$ (C-1'') and 172.0 (C-4'')] with methylene protons [$\delta_{\text{H}} = 2.76$ (4H, H-2'', H-3'')], a methoxy group ($\delta_{\text{H}} = 3.70$) and the NH group ($\delta_{\text{H}} = 11.07$) (Table 1).

The relative stereochemistry of **1** was studied by means of a NOESY experiment and evaluation of the coupling constants. As starting point, the stereochemistry of H-5 was considered to be β , as characteristic for norditerpene alkaloids [10]. The coupling constant ($J = 0$ Hz) between H-5 and H-6 demonstrated the α position of H-6 [9]. The Overhauser effect between H-5 and H-18 was indicative of the β position of H-18. Consequently, H-19 must be in the α position, and the *N*-containing bridge between C-19 and C-17 is below the plane of the six-membered ring. The NOESY correlation between H-17 and H-16 pointed to an α -oriented H-16. The stereochemistry of C-1 was determined from the NOESY cross-peaks between H-19 and H-2 α and between H-2 β and H-1. The β orientation of H-1 was corroborated by comparison of its coupling constant ($J = 10.3, 7.1$ Hz) with those published for this type of norditerpene alkaloids [11, 12]. Further important NOEs were detected between H-9 and H-14, between H-9 and 8-OH, and between H-13 and H-14, which confirmed the same stereostructure of compound **1** as that of the co-occurring lycocotinine, with β -oriented H-9, H-13, H-14 and 8-OH [9]. Additionally, some NOESY correlations were suitable for the steric differentiation of methylene protons (C-2, C-3 and C-15). Such correlations were observed between H-19a and H-2 α ; H-2 β and H-3 β ; H-16 and H-15 α ; and H-17 and H-15 α . All of the above evidence confirmed the structure **1** for this compound, named vulparine.

Compound **2** was identified as septentriodine (= cashmiradelphine) by comparison of its physical and spectral data with those reported in the literature [13, 14]. This compound was previously isolated from *Aconitum septentrionale* [13] and from *A. barbatum* var. *puberulum* [15] under the name septentriodine, and from *Delphinium cashmirianum* under the name cashmiradelphine. In these earlier publications, the structure was presented with a β -oriented 1-OMe group. In a later publication by the Pelletier group, this substituent was placed in the α position [16]. Our NOESY experiment provided unequivocal evidence for a 1 α -OMe group, since Overhauser effects were

Atom	¹ H	¹³ C	HMBC (C→H)	COSY (H No.)
1	3.02 dd (7.1, 10.3)	84.8	1-OMe	2 α , 2 β
2 α	2.15 m	25.4	–	1, 2 β , 3 α , 3 β
2 β	2.05 m	–	–	1, 2 α , 3 β
3 α	1.82 m	32.3	18a, 18b, 19b	2 α , 3 β
3 β	1.50 m	–	–	2 α , 2 β , 3 α
4	–	37.9	5, 6, 18a, 18b, 19a	–
5	1.80 s	50.7	17, 18a, 19a	6, 17*
6	3.89 s	90.6	5, 17, 6-OMe	5
7	–	89.3	6, 15 α	–
8	–	76.3	6, 8-OH, 9, 15 β	–
9	3.13 brs	45.2	–	10, 14
10	1.84 m	46.1	1, 5, 9, 12	9
11	–	48.4	1, 5, 6, 17	–
12	1.87 m (2H)	27.6	9	13
13	2.43 m	36.5	–	12, 14
14	4.00 brs	75.3	–	9, 13, 14-OH
15 α	2.50 m	33.2	8-OH	15 β
15 β	1.82 m	–	–	15 α
16	3.48 m	81.8	15 β , 16-OMe	13, 15 α
17	3.28 s	65.0	1, 5, 19a	5*
18a	4.20 d (11.0)	69.8	5, 19b	–
18b	4.15 d (11.0)	–	–	–
19a	2.78 m	52.6	5, 17, 18a, 18b, 20a	19b
19b	2.53 d (8.9)	–	–	19a
20a	2.97 dq (14.0, 7.1)	51.1	21	20b, 21
20b	2.85 dq (14.0, 7.1)	–	–	20a, 21
21	1.07 t (7.1)	14.1	20a	20a, 20b
1'	–	114.7	3', 5', NH	–
2'	–	141.7	4', 6'	–
3'	8.70 'd' (8.3)	120.7	5'	4'
4'	7.55 't' (7.6)	134.9	6'	3', 5', 6'
5'	7.11 't' (8.0)	122.6	3'	4', 6'
6'	7.96 'd' (8.0)	130.3	4'	4', 5'
7'	–	168.1	3', 6', 18a, 18b	–
1''	–	170.2	2'', 3'', NH	–
2''	2.76 m (2H)	29.0	3''	–
3''	2.76 m (2H)	32.8	2''	–
4''	–	172.0	2'', 3'', 4''-OMe	–
1-OMe	3.26 s	55.9	1	–
6-OMe	3.39 s	58.4	6	–
16-OMe	3.36 s	56.6	–	–
4''-OMe	3.70 s	51.8	–	–
8-OH	3.53 s	–	–	–
14-OH	4.33 brs	–	–	14
NH	11.07 brs	–	–	–

Table 1. NMR spectral data of vulparine (**1**) [500 MHz (¹H), 125 MHz (¹³C), CDCl₃, δ (ppm) ($J = \text{Hz}$)].

* ⁴J_{H,H} correlations.

detected between H-1 and H-5, and between H-1 and H-10. A detailed NMR study, including ¹H-¹H COSY, HSQC and HMBC measurements, allowed (previously unpublished) ¹H chemical shift assignments for all the protons of **2** (Table 2). On the basis of the HMBC experiment, the ¹³C NMR chemical shifts of **2** were reinvestigated, and some signal assignments were corrected. The long-range correlations of the carbon at $\delta_{\text{C}} = 50.4$ (C-5) with H-18, and of the carbon at $\delta_{\text{C}} = 43.1$ (C-9) with H-13, H-14 and H-15a, dictated amendment of the previous assignments of C-5 and

C-9. Similarly, the HMBC cross-peaks of the carbon at $\delta_{\text{C}} = 45.9$ (C-10) with H-9 and H-13, and of the carbon at $\delta_{\text{C}} = 37.9$ (C-13) with H-9, H-14 and H-15a, showed that the published assignments of C-10 and C-13 must be reversed.

Compound **3** was found to be identical in all of its characteristics, including the ¹H and ¹³C NMR spectral data, with finetiadine isolated earlier from *A. finetianum* [17]. Alkaloids **1–3** are structurally closely related to the highly potent nAChR ligand MLA, and appear worthy of pharmacological studies. Norditer-

Table 2. ¹H NMR spectral data of compounds **2** and **6** [500 MHz, CDCl₃, δ (ppm), (J = Hz)].

H	2	6
1	3.03 m	2.96 m
2a	2.18 m (α)	2.12 m
2b	2.06 m (β)	2.03 m
3a	1.85 m (α)	1.66 m
3b	1.55 m (β)	1.47 m
5	1.74 s	1.77 s
6	3.91 s	3.83 brs
9	3.08 dd (6.8, 5.0)	3.13 m
10	1.97 m	1.82 m
12a	2.41 d (11.3) (α)	1.83 m (2H)
12b	1.85 m (β)	
13	2.34 dd (7.1, 4.6)	2.42 m
14	3.61 t (4.6)	3.99 brs
15a	2.61 dd (15.3, 8.9) (α)	2.49 dd (15.3, 7.8)
15b	1.69 dd (15.3, 7.0) (β)	1.81 m
16	3.22 t (7.6)	3.46 m
17	2.98 s	3.23 s
18a	4.20 d (11.6)	3.62 d (10.3)
18b	4.17 d (11.6)	3.35 d (10.3)
19a	2.87 d (11.7)	2.61 d
19b	2.80 m	2.32 d
20a	3.03 m	2.94 m
20b	2.87 m	2.82 m
21	1.07 t (7.1)	1.05 t (7.0)
3'	8.69 d (8.5)	–
4'	7.55 dt (8.0, 1.5)	–
5'	7.10 dt (7.6, 0.7)	–
6'	7.96 dd (8.0, 1.5)	–
2'', 3''	2.75m (4H)	–
1-OMe	3.26 s	3.26 s
6-OMe	3.37 s	3.44 s
14-OMe	3.40 s	–
16-OMe	3.34 s	3.36 s
4''-OMe	3.70 s	–
8-OH	3.91 brs	3.61 s
14-OH	–	4.31 brs
NH	11.06 brs	–

pene alkaloids substituted with 2-succinimidobenzoate groups (*e. g.* MLA) often occur in *Aconitum* and *Delphinium* species, but compounds in which the succinimido part is opened (such as **1–3**) are rare [10].

The ¹³C chemical shifts and physical data for **4** confirmed that this compound is identical with anthranoyllycoctonine (= inuline), previously detected in many species of the genera *Aconitum*, *Delphinium*, *Consolida* and *Inula* [10].

The NMR spectral data on compound **5** were very similar to those of lycoctonine. The only difference was the absence of the signals of the *N*-ethyl group (δ_H = 2.93 m, 2.79 dq, 1.04 t; δ_C = 51.1, 14.1 in lycoctonine) [9] and the appearance of an *N*-methyl signal (δ_H = 2.62 s; δ_C = 44.4). The good agreement of the other ¹H and ¹³C NMR data for **5** and lycoc-

Table 3. Cytotoxic activity of compounds **1–4**.

Compound	Concentration (μg/mL)	Inhibition (%) ± SEM	
		MCF-7	HeLa
Vulparine (1)	10	< 15	< 15
	30	< 15	< 15
Septentriodine (2)	10	< 15	< 15
	30	34.50 ± 3.72	< 15
Finetiadine (3)	10	19.28 ± 4.24	19.91 ± 1.76
	30	39.48 ± 3.42	25.59 ± 1.87
Anthranoyllycoctonine (4)	10	29.82 ± 5.69	< 15
	30	20.70 ± 3.02	17.33 ± 1.60
Cisplatin	3	53.01 ± 2.33	42.55 ± 2.92
	30	86.90 ± 1.24	99.93 ± 0.26

tonine proved the structure *N*-methyl-*N*-deethyllycoctonine for compound **5**. Previously, only the mass spectrum of *N*-methyl-*N*-deethyllycoctonine had been reported [18].

Compound **6** was a trimethoxy-tetrahydroxy-substituted lycoctonine-type alkaloid, which, with the aid of 2D NMR measurements, was identified as delectinine. The previously reported spectral data [10] on delectinine (**6**) were supplemented with complete ¹H NMR assignments (Table 2), and with the correction of some of the ¹³C NMR chemical shifts. Diagnostic long-range correlations between C-5/H-17, C-9/H-10 and C-9/H-14, and ¹H-¹H COSY correlations between H-10/H-9, H-13/H-12, H-13/H-14 and H-13/H-16, were indicative of the need to correct the ¹³C NMR chemical shifts of C-5 and C-9, and C-10 and C-13.

The isolated norditerpene alkaloids **1–4** were tested for their cytotoxic effect on MCF-7 and HeLa cells using MTT assay. The cell growth-inhibitory potencies of compounds **1–4** are shown in Table 3. Finetiadine (**3**) exhibited the highest cytotoxicity (39.48 ± 3.42 % and 25.59 ± 1.87 % inhibition on MCF-7 and HeLa cells, respectively, at 30 μg/mL). Septentriodine (**2**) and anthranoyllycoctonine (**4**) displayed marginal tumour cell inhibitory activity, while vulparine (**1**) was found to be inactive. In all cases MCF-7 cells were more sensitive than HeLa cells. The present results demonstrate that some anthranoyl-substituted norditerpene alkaloids possess weak cytotoxic potencies against human tumour cells depending on their substitutions pattern.

Experimental Section

General procedure

Optical rotations were determined in CHCl₃ with a Perkin-Elmer 341 polarimeter. UV spectra were recorded on

a Shimadzu UV-2101 PC spectrometer. NMR spectra were recorded in CDCl₃ on a Bruker Avance DRX 500 spectrometer, at 500 MHz (¹H) and 125 MHz (¹³C), with TMS as internal standard. 2D data were acquired and processed with standard Bruker software. HRMS measurement was performed on a VG ZAB SEQ hybrid mass spectrometer equipped with a Cs SIMS ion source. The resolution was 10000. The sample was dissolved in methanol and this solution was measured with glycerol as matrix. The glycerol cluster peak at *m/z* = 645 was used in peak matching experiments. Centrifugal TLC was performed with a Chromatotron (Model 8924, Harrison Research).

Plant material

Aconitum vulparia Rchb. was collected in the region of Nagyvázsony, Hungary, in September 2002, and was identified by András Mészáros, Balaton Uplands National Park, Veszprém, Hungary. A voucher specimen (No. 617) has been deposited in the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

Extraction and isolation

Dried whole plants of *A. vulparia* (250 g) were crushed in a blender and then percolated with MeOH (12 L). After evaporation, the crude extract was dissolved in 4% aq. H₂SO₄ (250 mL) and extracted exhaustively with CHCl₃, which yielded organic layer I and aqueous layer II. After adjustment of the pH to 12, aqueous layer II was extracted with CHCl₃, affording organic layer III. This fraction (III) was evaporated under reduced pressure, and fractionated by VLC (VLC I) on Al₂O₃, using a gradient system of *n*-hexane/EtOAc/MeOH (50:50:1, 50:50:1.5, 50:50:2, 50:50:3, 50:50:4, 50:50:5, 50:50:10). Fractions obtained with *n*-hexane/EtOAc/MeOH 50:50:3, 50:50:4 and 50:50:5 were further fractionated by VLC on Al₂O₃, using a gradient system of *n*-hexane/CHCl₃ (7:3, 6:4, 1:1, 4:6, 3:7, 2:8, 1:9). Compound 2 was obtained in pure form from the fractions eluted with *n*-hexane/CHCl₃ 6:4. The fractions eluted with *n*-hexane/CHCl₃ 1:1 were further purified by gel chromatography, using MeOH/CHCl₃ 1:1 as solvent, yielding compound 1. Organic layer I was extracted with 2% aqueous H₂SO₄, which afforded aqueous layer IV. Aqueous layer IV was then rendered alkaline with 5% aqueous NaOH solution, and extracted with CHCl₃ (organic layer V). Phase V was evaporated *in vacuo*, and separated by VLC (VLC II), using a gradient system of *n*-hexane/EtOAc/MeOH (Al₂O₃; 50:50:0.5, 50:50:1, 50:50:1.5, 50:50:2, 50:50:3, 50:50:4, 50:50:5, 50:50:10, 1:1:1). The fractions obtained by eluting with *n*-hexane/EtOAc/MeOH 50:50:0.5 were further purified by gel chromatography (solvent: MeOH/CHCl₃ 1:1) and preparative TLC (SiO₂,

petroleum ether/diethyl ether/MeOH 50:50:7), affording compound 4. The fractions eluted from VLC II with *n*-hexane/EtOAc/MeOH 50:50:2 and 50:50:3 were further purified by centrifugal TLC (SiO₂, gradient elution with cyclohexane/EtOAc/EtOH 1:0:0, 9:1:0, 8:2:1, 6:3:1, 0:1:1, 0:0:1). The fractions derived from centrifugal TLC with *n*-hexane/EtOAc/MeOH 8:2:1 were subjected to preparative TLC (SiO₂, toluene/acetone/EtOH/NH₃ 70:40:12:3), yielding compound 3. The fractions eluted from VLC II with *n*-hexane/EtOAc/MeOH 50:50:3 and 50:50:4 were further fractionated by column chromatography on SiO₂ with mixtures of cyclohexane/CHCl₃/MeOH. The diterpene alkaloid-containing fractions from this chromatography were subjected to RP-VLC (RP-SiO₂, MeOH/H₂O 1:1, 55:45, 60:40, 65:35, 70:30, 80:20, 90:10). The final purification was performed by preparative TLC (SiO₂, toluene/acetone/EtOH/NH₃ 70:50:35:6), affording compound 5. The fractions eluted from VLC II with *n*-hexane/EtOAc/MeOH 50:50:10 were subjected to preparative TLC (SiO₂, toluene/acetone/EtOH/NH₃ 70:50:35:6) and gel chromatography (CHCl₃/MeOH 1:1), yielding compound 6.

Vulparine (1): amorphous solid; $[\alpha]_D^{27} = +47$ (*c* = 0.1, CHCl₃). – UV (MeOH): λ_{\max} (log ϵ) = 223 (3.04), 227 sh (3.01), 252 (2.75), 260 sh (2.65), 311 (2.32) nm. – ¹H and ¹³C NMR data, see Table 1. – HRMS: *m/z* (%) = 687.3437 [M+H]⁺ (calcd. 687.3493 for C₃₆H₅₁N₂O₁₁).

Septentriodine (2): amorphous solid; $[\alpha]_D^{27} = +37$ (*c* = 0.05, CHCl₃). – UV (MeOH): λ_{\max} (log ϵ) = 222 (2.99), 227 sh (2.97), 252 (2.71), 260 sh (2.61), 310 (2.28) nm. – ¹H NMR data, see Table 2. – ¹³C NMR (CDCl₃): δ = 83.8, 83.6 (C-1, C-14), 25.8 (C-2), 32.2 (C-3), 37.4 (C-4), 50.4 (C-5), 90.8 (C-6), 88.3 (C-7), 77.5 (C-8), 43.1 (C-9), 45.9 (C-10), 48.9 (C-11), 28.5 (C-12), 37.9 (C-13), 33.6 (C-15), 82.4 (C-16), 64.2 (C-17), 69.7 (C-18), 52.4 (C-19), 50.7 (C-20), 13.8 (C-21), 114.5 (C-1'), 141.5 (C-2'), 120.5 (C-3'), 134.6 (C-4'), 122.4 (C-5'), 130.1 (C-6'), 167.8 (C-7'), 170.0 (C-1''), 28.8 (C-2''), 32.5 (C-3''), 172.7 (C-4''), 55.5 (1-OMe), 57.9 (6-OMe), 57.6 (14-OMe), 56.0 (16-OMe), 51.6 (4''-OMe).

Finetiadine (3): amorphous solid; $[\alpha]_D^{27} = +27$ (*c* = 0.05, CHCl₃). – UV (MeOH): λ_{\max} (log ϵ) = 222 (3.06), 228 sh (3.02), 253 (2.77), 260 sh (2.68), 311 (2.36) nm. – ¹H and ¹³C NMR data identical with those reported by Wu *et al.* [17].

Anthranoyllycoctonine (4): amorphous solid; $[\alpha]_D^{27} = +80$ (*c* = 0.025, CHCl₃). – UV (MeOH): λ_{\max} (log ϵ) = 220 (3.08), 248 (2.57), 340 (2.39) nm. – ¹H and ¹³C NMR data identical with those published by Hardick *et al.* [5].

***N*-Deethyl-*N*-methyllycoctonine (5):** amorphous solid; ¹H NMR (CDCl₃): δ = 2.93 (1H, dd, *J* = 10.0, 7.3 Hz, H-1), 3.85 (1H, s, H-6), 3.07 (1H, t, *J* = 5.7 Hz, H-9), 2.81 (1H,

d, $J = 1.6$, H-17), 2.62 (1H, d, $J = 9.1$ Hz, H-18a), 3.45, 3.42, 3.34, 3.27 ($4 \times 3H$, $4 \times s$, $4 \times OCH_3$), 2.62 (3H, s, $N-CH_3$). – ^{13}C NMR ($CDCl_3$): $\delta = 84.7$ (C-1), 26.1 (C-2), 29.7 (C-3), 49.5 (C-5), 90.5 (C-6), 77.9 (C-8), 43.3 (C-9), 46.2 (C-10), 28.8 (C-12), 38.1 (C-13), 84.0 (C-14), 33.7 (C-15), 82.6 (C-16), 66.1 (C-17), 55.5 (C-18), 67.9 (C-19), 56.2, 58.0, 57.8, 56.3 ($4 \times OCH_3$), 44.4 ($N-CH_3$). – ^{13}C NMR signals of C-4, C-7 and C-11 were not observed.

Delectinine (6): amorphous solid; $[\alpha]_D^{27} = +31$ ($c = 0.05$, $CHCl_3$). – UV (MeOH): λ_{max} ($\log \epsilon$) = 203 (2.95), 218 sh (2.78), 280 (2.06) nm. – 1H NMR data, see Table 2. – ^{13}C NMR ($CDCl_3$): $\delta = 85.2$ (C-1), 25.4 (C-2), 31.7 (C-3), 38.9 (C-4), 49.6 (C-5), 90.2 (C-6), 89.1 (C-7), 76.3 (C-8), 45.1 (C-9), 46.1 (C-10), 48.2 (C-11), 27.5 (C-12), 36.4 (C-13), 75.3 (C-14), 33.1 (C-15), 81.8 (C-16), 65.4 (C-17), 67.8 (C-18), 52.7 (C-19), 51.2 (C-20), 14.3 (C-21), 56.0 (1-OMe), 58.1 (6-OMe), 56.5 (16-OMe).

Bioassay

Cytotoxic effects were measured *in vitro* on HeLa (cervix adenocarcinoma) and on breast adenocarcinoma (MCF-7) cell lines, using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay. The cyto-

toxicity tests were carried out in 96-well microtitre plates, using 5000 cells per well in all cases, which were allowed to adhere overnight before the drugs were introduced. The original medium was then removed, 200 μ L culture medium containing the compounds of interest was added and the cells were incubated for 72 h. The tested compounds were dissolved in DMSO. The final concentration of DMSO never exceeded 0.3 %, and therefore had no essential effect on the cell growth. Next the living cells were assayed: aliquots (20 μ L at 5 mg/mL) of the MTT stock solution were pipetted into each well and reduced by viable cells to an insoluble formazan product during a further 4 h. After this contact period, the medium was removed and the formazan crystals were dissolved in 100 μ L DMSO by gentle shaking for 60 min. Finally, the absorbance was measured at 545 nm with a microplate reader [19]. In this way the cell growth or drug toxicity was determined. All *in vitro* experiments were carried out on two microplates with at least 5 parallel wells.

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