Two Triterpene Saponins from *Achyranthes bidentata*

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Bidentatoside II (1) and chikusetsusaponin V methyl ester (2) are two further triterpene saponins isolated from the roots of *Achyranthes bidentata*. Chemical and homo and heteronuclear two-dimensional (2D) NMR techniques have led to the structural elucidation of 1 which is a new *seco*-glycoside of oleanolic acid and the full ^{1}H- and ^{13}C-NMR assignments of 2. These compounds did not show any potentiation of the *in vitro* cytotoxicity of cisplatin in the HT 29 human colon cancer cell line.

Key words *Achyranthes bidentata*; Amaranthaceae; triterpene-saponin

In the search for new saponins possessing the ability to potentiate *in vitro* the cisplatin cytotoxicity in human cancer colon cells, we have previously isolated three known oleanolic acid glycosides[1] and one new triterpene saponin, bidentatoside I,[2] from *Achyranthes bidentata* BLUME (Amaranthaceae). Furthermore, as part of our ongoing studies, we report here the isolation, structure elucidation and influence on the potentiation of cisplatin cytotoxicity in human colon cancer cells of one new further triterpene saponin, bidentatoside II (**1**) and chikusetsusaponin V methyl ester (**2**).

A concentrated *n*-BuOH-soluble fraction of the MeOH extract of the roots of *Achyranthes bidentata* was purified by precipitation with diethyl ether and subjected to multiple chromatographic steps over Sephadex LH-20 and Si gel to yield bidentatoside II (**1**) and chikusetsusaponin V methyl ester (**2**). The structures of these two compounds were elucidated mainly by 600 MHz NMR analysis, including one- and two-dimensional (1D, 2D) NMR [^{1}H–^{1}H double quantum filtered-correlation spectroscopy (DQF-COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), ^{1}H-detected heteronuclear multiple bond connectivity (HMBC)] spectroscopy.

Bidentatoside II (**1**) was obtained as a very polar compound which displayed in the electrospray ionization (ESI)-MS spectrum (negative-ion mode) a quasimolecular ion peak $[M-H]^-$ at *m/z* 749, a fragment ion peak at *m/z* 731 $[M-H_2O]^-$ and two molecular adduct ions $[M+Na-H]^-$ at *m/z* 772 and $[M+2Na-3H]^-$ at *m/z* 793 compatible with the molecular formula of $C_{40}H_{62}O_{13}$. These data suggested the presence of two carboxylic acid functions in compound **1**.[3] Other fragment ion peaks at *m/z* 587 $[(M-H)-162]^-$ and *m/z* 631 $[(M+2Na-3H)-162]^-$ indicated the loss of a terminal hexose. Furthermore, a negative fragment ion at *m/z* 455 $[(M-H)-162-132]^-$ corresponding to the aglycon (Agly), revealed the presence of a $C_4H_5O_5$ supplementary residue.

On acid hydrolysis, **1** gave oleanolic acid and glucose, which were compared by TLC with authentic samples.

DQF-COSY and HMQC experiments of **1** allowed the identification of the Agly as oleanolic acid and the sugar moiety as one β-D-glucopyranose (Glc) (Tables 1, 2). A correlation observed in the HMBC spectrum between δ_H (Glc-1) 5.25 and δ_C (Agly-C-28) 175.6 proved a glycosidic ester linkage to the C-28 of the Agly. The substitution at the C-3 position of the oleanolic acid by the $C_4H_5O_5$ residue is confirmed by the deshielded carbon at δ_C 88.5. This type of fragment containing two carboxylic acids has already been encountered in saponins of *Pisonia umbellifera*[3] and *Chenopodium album.*[4] It was described as a *seco*-glycopyranosyl moiety possessing two characteristic groups such as a methine represented by a pseudo-anomeric carbon and a methylene. Analogous signals corresponding to this structure were detected in compound **1**: In the HMBC experiment, a carbonyl signal of one carboxylic acid at δ_C 173.0 gave a correlation with a proton at δ_H 3.60 (d, J=14.0 Hz) of a methylene which correlated in the HMQC spectrum with a carbon at δ_C 69.5. The singlet at δ_H 5.17 of a methine at δ_C 100.7, corresponding to a pseudo-anomeric carbon, didn't show any long-range correlation with the second carbonyl which was assigned at δ_C 171.0 from the ^{13}C-NMR spectrum. This observation was previously reported for the saponins of *Pisonia umbellifera.*[3]

Based on the above results, bidentatoside II (**1**) is characterized as 3-*O*-β-[2′-(2″-*O*-glycolyl)-glyoxylyl]-oleanolic

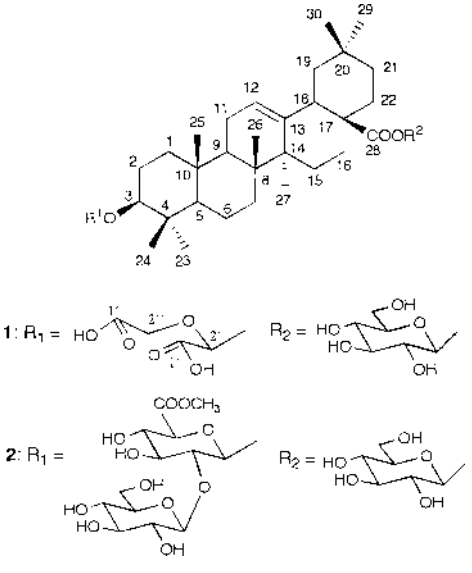

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Table 1. ^{13}C- and ^{1}H-NMR Data[a] of the Agly of Compounds **1** and **2**

	Mult.[b]	**1**		**2**	
		C	H[c]	C	H[c]
1	CH_2	38.1	1.50, nd	38.0	1.09, 1.35
2	CH_2	25.4	nd	26.5	nd
3	CH	88.5	3.00	88.3	3.00
4	C	38.5	—	38.8	—
5	CH	55.0	0.70	54.9	0.72
6	CH_2	17.6	nd	17.5	nd
7	CH_2	32.0	nd	32.1	nd
8	C	38.9	—	39.0	—
9	CH	46.9	1.49	47.0	1.47
10	C	36.1	—	36.1	—
11	CH_2	22.3	nd	22.0	nd
12	CH	122.0	5.17 (br s)	121.5	5.16 (br s)
13	C	143.4	—	143.3	—
14	C	41.0	—	41.1	—
15	CH_2	27.3	nd	27.0	nd
16	CH_2	24.1	1.50, nd	24.3	nd
17	C	45.8	—	45.8	—
18	CH	40.5	2.75	40.6	2.75
19	CH_2	45.1	1.05, 1.63	45.4	1.08, 1.65
20	C	30.2	—	30.2	—
21	CH_2	33.0	nd	33.1	nd
22	CH_2	31.5	nd	31.5	nd
23	CH_3	27.8	0.99 (s)	27.3	0.98 (s)
24	CH_3	16.5	0.76 (s)	16.0	0.75 (s)
25	CH_3	15.1	0.90 (s)	15.0	0.86 (s)
26	CH_3	17.1	0.70 (s)	16.5	0.69 (s)
27	CH_3	25.9	1.09 (s)	25.3	1.08 (s)
28	C	175.6	—	175.0	—
29	CH_3	32.6	0.88 (s)	32.6	0.89 (s)
30	CH_3	23.5	0.88 (s)	23.2	0.88 (s)

a) Measured at 600 MHz for ^{1}H and 150 MHz for ^{13}C with reference to dimethylsulfoxide-d_6 (DMSO-d_6) at δ 39.5 ppm. *b*) Multiplicities were assigned from distorsionless enhancement by polarization transfert (DEPT) spectrum. *c*) Coupling constants (*J*) in Hz are given in parentheses. nd: not determined.

Table 2. ^{13}C- and ^{1}H-NMR Data[a] of Functional Groups at C-3 and C-28 of the Agly from Compounds **1** and **2**

	1			**2**	
	C	H[b]		C	H[b]
3-*O*-			3-*O*-		
1′	171.0	—	GlcA-1	103.5	4.45 (d, 7.3)
2′	100.7	5.17 (s)	2	80.5	3.41
1″	173.0	—	3	75.6	3.41
2″	69.5	3.60 (d, 14.0)	4	71.3	3.37
		nd	5	74.7	3.80 (d, 9.0)
28-*O*-			6	169.3	
Glc 1	94.0	5.25 (d, 7.5)	OCH_3	51.7	3.70 (s)
2	72.3	3.12	Glc-1	103.5	4.48 (d, 7.7)
3	76.5	3.25	2	75.0	3.00
4	69.5	3.15	3	77.6	3.15
5	77.6	3.15	4	69.4	3.05
6	60.5	3.72, 3.40	5	76.7	3.04
			6	60.5	3.42, nd
			28-*O*-		
			Glc 1	93.9	5.25 (d, 7.5)
			2	72.2	3.10
			3	76.0	3.22
			4	68.8	3.12
			5	76.5	3.07
			6	60.8	3.38, nd

a) Measured at 600 MHz for ^{1}H and 150 MHz for ^{13}C with reference to DMSO-d_6 at δ 39.5 ppm. β-D-glucuronopyranose (GlcA), β-D-glucopyranose (Glc). *b*) Coupling constants (*J*) in Hz are given in parentheses. nd: not determined.

acid-28-*O*-β-D-glucopyranoside. The novelty of this molecule in comparison with the literature data about the saponins described as *seco*-glycosides, is the linkage of the unusual *seco*-glycopyranosyl moiety with the C-3 of the Agly instead of the C-3 position of a glucuronic acid.[3,4] The nature of the asymmetric center on the *seco*-glycopyranosyl remains to be determined.

Chikusetsusaponin V methyl ester (**2**) was obtained as a white, amorphous powder. Its ESI-MS (positive-ion mode) showed a ion peak $[M+Na]^+$ at *m/z* 993 compatible with a molecular formula of $C_{49}H_{78}O_{19}$ and a fragment ion peak at *m/z* 831 $[(M+Na)-162]^+$ which indicated the elimination of one terminal hexosyl. Its FAB-MS (positive-ion mode) showed a quasimolecular ion peak $[M+H]^+$ at *m/z* 971 which confirmed the proposed molecular weight.

On acid hydrolysis, **2** gave products which co-chromatographed on TLC with authentic samples of oleanolic acid and glucose consistent with the structural assignments made.

The ^{1}H- and ^{13}C-NMR data of **2**, obtained from HMQC and HMBC spectra (Tables 1, 2), showed that most of the signals were in agreement with literature data of chikusetsusaponin V.[1] A long-range correlation in the HMBC experiment between a carbon at δ_C 169.3 and a proton at δ_H 3.70 which correlated in the HMQC with a carbon at δ_C 51.7, suggested a methyl ester group. The HMBC experiment showed equally a correlation between signals at δ_C 169.3 and δ_H (GlcA-5) 3.80 (d, J=9.0 Hz). The signal of the corresponding carbon of the latter at δ_C (GlcA-5) 74.7 revealed the methyl ester form of the glucuronic acid. This compound was prepared by methylation of chikusetsusaponin V with diazomethane[5,6] and also isolated from a natural source[7,8] but the NMR data were not yet described. So we proposed the assignments of the carbons and protons of this molecule in Tables 1 and 2.

As we systematically did with the triterpene saponins isolated from a natural source, we have tested **1** and **2** for potentiation of the cisplatin cytotoxicity in the human cancer colon HT-29 cell line.[9] However, no significant effect could be found in this bioassay with these two further compounds.

Experimental

General Methods Optical rotations were taken with a Perkin-Elmer 241 polarimeter. The IR spectrum was measured with a Perkin-Elmer 881 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker DRX-600 spectrometer with standard pulse sequences, operating at 600 and 150 MHz, respectively. The chemical shifts (δ) were referenced to the solvent peaks (DMSO-d_6). FAB-MS was conducted in the positive-ion mode (thioglycerol with 1% trifluoroacetic acid (TFA) matrix) on a Micromass ZAB 2-SEQ instrument, ESI-MS in the positive- and negative-ion mode on a Micromass Quattro LS instrument. TLC and HPTLC (high performance thin-layer chromatography) employed precoated Si gel 60F_{254} plates (Merck). The following TLC solvent systems were used: for saponins (a) $CHCl_3$–MeOH–AcOH–H_2O (15 : 8 : 3 : 2); for sapogenins (b) CH_2Cl_2–MeOH (19 : 1) and (c) toluene–Me_2CO (4 : 1); for monosaccharides (d) $CHCl_3$–MeOH–H_2O (8 : 5 : 1). The spray reagent for saponins and sapogenins was Komarowsky reagent, a mixture (5 : 1) of *p*-hydroxybenzaldehyde (2% in MeOH) and ethanolic H_2SO_4 (50%) and for the sugars, aqueous H_2SO_4 (50%). Isolations were carried out using a medium-pressure liquid chromatography (MPLC) system [Gilson pump M 303, head pump 25 SC, manometric module M 802], with a Rheodyne 7125 injector, a Büchi column (230×15 mm), a Büchi precolumn (110×15), and Si gel 60 (15—40 μm, Merck). For column chromatography (CC), Si gel 60 (63—200 μm, Merck) was used.

Plant Material The roots of *A. bidentata* were collected in June 1993, in the northeast region of Hanoi, and the plant was identified by Dr. T. C. Khanh, College of Pharmacy, University of Hanoi, Hanoi, Vietnam. A voucher specimen (No. 5004) has been deposited in the Herbarium of the Laboratory of Pharmacognosy, Faculty of Pharmacy, Dijon, France.

Extraction and Isolation The dried powdered roots (428 g) were submitted to successive Soxhlet extractions with hexane, CH_2Cl_2, and MeOH. The MeOH extract was concentrated to dryness and the residue was diluted with H_2O (300 ml) and partitioned between *n*-BuOH (300 ml×3) and water. The residue from the *n*-BuOH layer was solubilized in a small amount of MeOH and precipitated with diethyl ether (300 ml×3) yielding 11.5 g of crude saponins. One portion of this mixture (5.5 g) was separated by CC on Si gel eluted with $CHCl_3$–MeOH–H_2O (8 : 5 : 1) yielding twelve fractions. Fraction 1 (124 mg) and fraction 7 (500 mg) were further purified by successive MPLC on a Si gel column eluted with the same solvent system to give **2** (8 mg) and **1** (10 mg), respectively.

Bidentatoside II (**1**): Obtained as a white amorphous powder; $[\alpha]_D^{25}$ +6° (*c*=0.1, MeOH). IR (KBr) cm^{-1}: 3410 (OH), 2926 (CH), 1741 (CO ester), 1718 (CO carboxylic acid), 1619, 1422, 1077. ^{1}H- and ^{13}C-NMR (DMSO-d_6): see Tables 1 and 2. Negative ESI-MS *m*/*z*: 749 $[M-H]^-$, 731 $[M-H_2O]^-$, 772 $[M+Na-H]^-$, 793 $[M+2Na-3H]^-$, 587 $[(M-H)-162]^-$, 631 $[(M+2Na-3H)-162]^-$, 455 $[(M-H)-162-132]^-$. TLC *Rf* 0.2 (system a); pink-violet spot developed on spraying with Komarowsky reagent.

Chikusetsusaponin V Methyl Ester (**2**): Obtained as a white amorphous powder; $[\alpha]_D^{25}$ +6° (*c*=0.07, MeOH). IR (KBr) cm^{-1}: 3409 (OH), 2924 (CH), 1736 (CO ester), 1653, 1458, 1072. ^{1}H- and ^{13}C-NMR (DMSO-d_6): see Tables 1 and 2. Positive ESI-MS *m*/*z*: 993 $[M+Na]^+$, 831 $[(M+Na)-162]^+$. Positive FAB-MS *m*/*z*: 971 $[M+H]^+$. TLC *Rf* 0.7 (system a); pink-violet spot developed on spraying with Komarowsky reagent.

Acid Hydrolysis The same method was employed for the compounds **1** and **2**. A solution of saponin (3 mg) in 2 N aqueous CF_3COOH (5 ml) was heated on a water bath for 3 h. After extraction with $CHCl_3$, the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral and then analyzed by Si gel TLC by comparison with standard sugars; for the two compounds, glucose was identified with *Rf* 0.4 (solvent system d). The chloroform layer was evaporated to dryness and then analyzed by Si gel TLC by comparison with standard Agly; oleanolic acid, *Rf* 0.7 (solvent system b) and 0.5 (solvent system c), was identified as the Agly of **1** and **2**.

Bioassays The potentiation of the *in vitro* cisplatin cytotoxicity in human colon cancer cell line was evaluated according to the method of Assem *et al.*[9]

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