Bidentatoside I, a New Triterpene Saponin from *Achyranthes bidentata*

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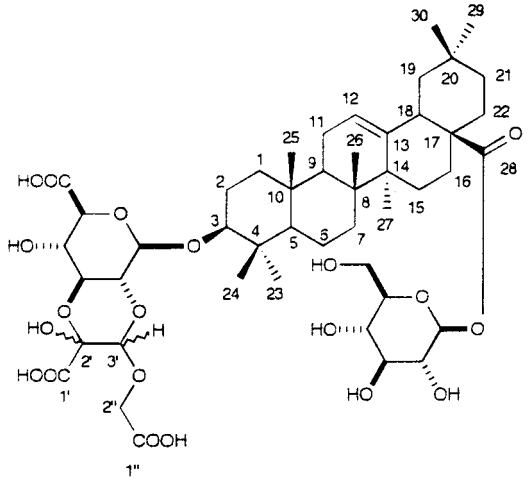
Bidentatoside I (**1**) is a new triterpene saponin bearing an unusual dioxopropionic acid unit, isolated from the roots of *Achyranthes bidentata*. Structural elucidation was performed mainly by chemical and homo- and heteronuclear 2D NMR techniques. This compound did not show any potentiation of the in vitro cytotoxicity of cisplatin in the HT 29 human colon cancer cell line.

Achyranthes bidentata Blume (Amaranthaceae), an indigenous plant of tropical areas of Asia and Africa, is used in traditional medicine as a tonic,[1] diuretic, and antifertility and immunostimulatory agent. It has also been used to treat blisters of the mouth and gastroenteritis.[2] A decoction of the roots was shown to increase the blood flow in rat hind limbs and to cause a vasodilatory effect. It also exhibited an analgesic action in mice and a reversible hypotensive effect in rabbits.[3] Three saponins were previously isolated and characterized as known oleanolic acid glycosides.[4] We describe in this note the isolation and structural elucidation of a new triterpene saponin, bidentatoside I (**1**). The influence of **1** on the potentiation of the cytotoxicity of cisplatin in human cancer colon cells was also investigated.



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A concentrated *n*-BuOH-soluble fraction of the MeOH extract of the roots of *A. bidentata* was purified by precipitation with diethyl ether and subjected to multiple chromatographic steps over Sephadex LH-20 and Si gel to yield bidentatoside I (**1**). The structure of **1** was elucidated mainly by 600 MHz NMR analysis, including 1D and 2D NMR (^{1}H−^{1}H DQF-COSY, HSQC, HMBC, TOCSY) spectroscopy.

Bidentatoside I (**1**) was obtained as a white, amorphous powder. Its FABMS (positive-ion mode) showed a $[M + K]^+$ ion at *m/z* 993 and a $[M + Na]^+$ ion at *m/z* 977, indicating a molecular weight of 954, compatible with a molecular formula of $C_{47}H_{70}O_{20}$. Its ESIMS (negative-ion mode) showed a quasimolecular ion peak $[M - H]^-$ at *m/z* 953 and a $[M + K - 2H]^-$ ion at *m/z* 991, which confirmed the proposed molecular weight. Another fragment ion peak at *m/z* 791 $[(M - H) - 162]^-$ indicated the elimination of one terminal hexosyl, and a peak at *m/z* 455 was attributed to the aglycon moiety.

On acid hydrolysis, **1** gave a product that cochromatographed on TLC with authentic samples of oleanolic acid, glucose, and glucuronic acid, consistent with the structural assignments made.

The ^{1}H NMR and ^{13}C NMR data of **1**, obtained from HSQC and HMBC spectra (Table 1), showed that the signals of the aglycon were in good agreement with the literature data of oleanolic acid.[5] Compound **1** was shown to contain two sugar residues from the HSQC spectrum: the anomeric protons at δ 4.51 (d, J = 7.5 Hz) and δ 5.40 (d, J = 7.5 Hz) gave correlations with carbon signals at δ 106.3 and 95.5, respectively. The sugar moieties were assigned mainly from ^{1}H−^{1}H DQF-COSY, TOCSY, HSQC, and HMBC experiments. Evaluation of spin−spin couplings and chemical shifts allowed the identification of one β-glucuronopyranose (GlcA) unit and one β-glucopyranose (Glc) unit. The common D-configurations for these two molecules were assumed to be those of the most commonly encountered analogues in the plant kingdom.

The HMBC experiment showed a correlation between signals at δ_H(GlcA-1) 4.51 and δ_C(Agly-C-3) 91.6 and another between δ_H(Glc-1) 5.40 and δ_C(Agly-C-28) 178.7. These data proved that **1** is a 3,28-bidesmosidic saponin having glucuronic acid linked to the C-3 position of the oleanolic acid by a glycosidic linkage, while glucose was bound to the C-28 of the aglycon through a glycosidic ester linkage. The HSQC experiment, which correlated all the proton resonances with those of each corresponding carbon, allowed the assignments of GlcA-2, GlcA-3, and GlcA-4 at δ 72.2, 73.1, and 71.0, respectively. After subtraction of the

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Table 1. ^{13}C NMR and ^{1}H NMR Data of the Aglycon of Compound **1** (δ ppm, CD_3OD as solvent)[a]

position	mult.[b]	δ ^{13}C	δ ^{1}H
1	CH_2	39.3	nd[c]
2	CH_2	26.6	nd
3	CH	91.6	3.22
4	C	39.5	
5	CH	56.6	nd
6	CH_2	19.1	nd
7	CH_2	33.1	nd
8	C	40.0	
9	CH	48.2	nd
10	C	37.1	
11	CH_2	24.3	nd
12	CH	123.4	5.28 (br s)
13	C	144.5	
14	C	42.4	
15	CH_2	28.1	nd
16	CH_2	24.2	nd
17	C	47.0	
18	CH	42.5	2.84
19	CH_2	46.5	nd
20	C	31.0	
21	CH_2	34.0	nd
22	CH_2	34.2	nd
23	CH_3	27.8	1.07 (s)
24	CH_3	16.4	0.86 (s)
25	CH_3	15.6	0.96 (s)
26	CH_3	17.3	0.79 (s)
27	CH_3	25.9	1.17 (s)
28	C	178.7	
29	CH_3	33.2	0.92 (s)
30	CH_3	24.1	0.94 (s)

[a] Measured at 600 MHz for ^{1}H and 150 MHz for ^{13}C with reference to CD_3OD at δ 49.0. Assignments were made on the basis of $^{1}H-^{1}H$ DQF-COSY, HSQC, and HMBC experiments. [b] Multiplicities were assigned from HSQC and ^{13}C NMR spectra. [c] nd: not determined.

^{1}H and ^{13}C NMR signals of oleanolic acid, GlcA, and Glc from the total HSQC and ^{13}C NMR spectrum of **1**, the signals of a $C_5H_6O_6$ portion (*m*/*z* 162) remained. The relatively small upfield shifts at δ_C(GlcA-2) 72.2 and at δ_C(GlcA-3) 73.1 suggested an unusual substitution pattern by comparison with related glycosidation shifts. In the HSQC spectrum the deshielded proton at δ_H 4.87 (s) gave a correlation with a carbon at δ_C 96.8 typical of an acetal methine such as in the achyrantosides[6−8] and betavulgarosides.[9−11] Furthermore, this proton gave long-range correlations in the HMBC spectrum with the carbon signal at δ_C(GlcA-2) 72.2, a carboxylic acid at δ_C 174.5, and a methylene at δ_C 67.3. The protons of this methylene observed in the HSQC spectrum at δ_H 4.20 and 3.94 (each H, d, J = 14.0 Hz) gave correlations in the HMBC spectrum with the carboxylic carbon signal at δ_C 177.1, suggesting a glycolyl residue. By comparing with the published spectroscopic data of the achyrantosides[6−8] and betavulgarosides,[9−11] the above data are characteristic of a rare bridging system between GlcA-2 and GlcA-3 through a bisacetal with dioxopropionic acid having a 3′-*O*-glycolyl substituent.

On the basis of the above results, bidentatoside I (**1**) is a derivative of oleanolic acid 28-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucuronopyranoside having a substituent resulting from bridging between GlcA-2 and GlcA-3, through a bisacetal unit with dioxopropionic acid, possessing a 3′-*O*-glycolyl substituent. The stereochemistry at the C-2′ and C-3′ positions of **1** has not been determined with certainty. According to a literature search and from previous reviews on triterpene saponins,[12−14] analogous bridged substituents were found only in the achyrantosides and betavulgarosides at the C-3 and C-4 positions of the GlcA unit, but this unique type of bridging at C-2 and C-3 of the GlcA is encountered in saponins for the first time in the present investigation, underscoring the structural novelty of bidentatoside I.

Because cisplatin and digitonin (a steroid saponin) have been shown to interact synergistically to increase tumor cell (ovarian carcinoma 2008 cell line) lethality in vitro,[15] we have evaluated **1** for potentiation of the cisplatin cytotoxicity in the human cancer colon HT-29 cell line.[16] However, no significant effect could be found in this bioassay with this compound.

Experimental Section

General Experimental Procedures. The optical rotation was taken with a Perkin-Elmer 241 polarimeter. The IR spectrum was measured with a Perkin-Elmer 881 spectrophotometer. A Bruker DRX-600 spectrometer operating at 599.19 MHz for ^{1}H and 150.858 for ^{13}C using the UXNMR software package was used for NMR measurements in CD_3OD. The $^{1}H-^{1}H$ DQF-COSY and inverse-detected $^{1}H-^{13}C$ HSQC and HMBC experiments were run by employing conventional pulse sequences. The 1D TOCSY NMR data were acquired using waveform generator-based GAUSS-shaped pulses, with a mixing time ranging from 100 to 120 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5 ms trim pulse. FABMS was conducted in the positive-ion mode (thioglycerol matrix) on a Micromass ZAB 2-SEQ instrument, and ESIMS in the negative-ion mode on a Micromass Quattro LS instrument. TLC and HPTLC 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); for monosaccharides (c) $CHCl_3-MeOH-H_2O$ (8:5:1). The spray reagent was 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, 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 column chromatography on Si gel eluted with $CHCl_3-MeOH-H_2O$ (8:5:1), yielding 12 fractions. Fraction 7 (500 mg) was further purified by successive MPLC on a Si gel column eluted with the same solvent system to give **1** (15 mg).

Bidentatoside I (**1**): obtained as a colorless amorphous powder; $[\alpha]^{25}_D$ +44.0° (*c* 0.05, MeOH); IR (KBr) ν_{max} 3419 (OH), 2926 (CH), 1736 (CO ester), 1707 (CO carboxylic acid), 1630, 1440, 1090 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Tables 1 and 2; long-range correlations in the HMBC spectrum used for defining the aglycon of **1**, δ 0.79 (Me-26) → C-7 (33.1), C-8 (40.0), C-14 (42.4), C-9 (48.2); δ 0.86 (Me-24) → C-23 (27.8), C-4 (39.5), C-5 (56.6), C-3 (91.6); δ 0.92 (Me-29) → C-30 (24.1), C-20 (31.0), C-21 (34.0), C-19 (46.5); δ 0.94 (Me-30) → C-20 (31.0), C-29 (33.2), C-21 (34.0); δ 0.96 (Me-25) → C-10 (37.1), C-1 (39.3), C-9 (48.2), C-5 (56.6); δ 1.07 (Me-23) → C-24 (16.4), C-4 (39.5), C-5 (56.6), C-3 (91.6); δ 1.17 (Me-27) → C-15 (28.1), C-8 (40.0), C-14 (42.4), C-13

Table 2. ^{13}C NMR and ^{1}H NMR Data of Functional Groups at C-3 and C-28 of the Aglycon from Compound **1** (δ ppm, CD_3OD as solvent)[a]

position	δ ^{13}C	δ ^{1}H
3-*O*-		
GlcA 1	106.3	4.51 (d, 7.5)
2	72.2	3.49 (dd, 7.5, 2.0)
3	73.1	4.12 (dd, 8.5, 2.0)
4	71.0	4.03
5	78.0	3.45
6	175.9	
1′	174.5	
2′	100.1	
3′	96.8	4.87 (s)
1″	177.1	–
2″	67.3	4.20 (d, 14.0)
		3.94 (d, 14.0)
28-*O*-		
Glc 1	95.5	5.40 (d, 7.5)
2	73.2	3.41
3	77.4	3.50
4	70.6	3.41
5	78.0	3.45
6	61.4	3.86 (dd, 12.5, 2.5)
		3.72 (dd, 12.5, 4.5)

[a] Measured at 600 MHz for ^{1}H and 150 MHz for ^{13}C with reference to CD_3OD at δ 49.0 ppm. Assignments were made on the basis of $^{1}H-^{1}H$ DQF-COSY, TOCSY, HSQC, and HMBC experiments. β-D-glucuronopyranose (GlcA), β-D-glucopyranose (Glc).

(144.5); positive FABMS *m*/*z* 993 $[M + K]^+$, 977 $[M + Na]^+$; negative ESIMS *m*/*z* 991 $[M + K - 2H]^-$, 953 $[M - H]^-$, 791 $[(M - H) - 162]^-$; TLC R_f 0.3 (system a); pink-violet spot developed on spraying with aqueous H_2SO_4 (50%).

Acid Hydrolysis of 1. A solution of compound **1** (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; glucose and glucuronic acid were identified with R_f 0.4 and 0.2, respectively (solvent system c). The chloroform layer was evaporated to dryness and then analyzed by Si gel TLC by comparison with standard aglycons; oleanolic acid, R_f 0.7 (solvent system b), was identified as the aglycon.

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.[16]

References and Notes

(1) Tang, W.; Eisenbrand, G. *Chinese Drugs of Plant Origin*; Springer-Verlag: Berlin, 1992; pp 13−18.
(2) Bishit, G.; Sandhu, H.; Verma, S. *Fitoterapia* **1993**, *64*, 85.
(3) Sun, S. P.; Li, X. H.; Sun S. G. *Henan Trad. Chin. Med.* **1985**, 39−40.
(4) Marouf, A.; Desbene, S.; Khanh, T. C.; Wagner, H.; Correia, M.; Chauffert, B.; Lacaille-Dubois, M.-A. *Pharm. Biol.* **2000**, in press.
(5) Massiot, G.; Dijoux, M.-G.; Lavaud, C.; Le Men-Olivier, L.; Connolly, J. D.; Sheeley, D. M. *Phytochemistry* **1994**, *37*, 1667−1670.
(6) Ida, Y.; Satoh, Y.; Katoh, M.; Katsumata, M.; Nagasao, M.; Yamaguchi, K.; Kamei, H.; Shoji, J. *Tetrahedron Lett.* **1994**, *35*, 6887−6890.
(7) Ida, Y.; Satoh, Y.; Katsumata, M.; Nagasao, M.; Shoji, J. *Chem. Pharm. Bull.* **1995**, *43*, 896−898.
(8) Ida, Y.; Satoh, Y.; Katsumata, M.; Nagasao, M.; Hirai, Y.; Kajimoto, T.; Katada, N.; Yasuda, M.; Yamamoto, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2555−2558.
(9) Yoshikawa, M.; Murakami, T.; Kadoya, M.; Matsuda, H.; Muraoka, O.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1996**, *44*, 1212−1217.
(10) Yoshikawa, M.; Murakami, T.; Kadoya, M.; Yamahara, J.; Matsuda, H. *Chem. Pharm. Bull.* **1998**, *46*, 1758−1763.
(11) Murakami, T.; Matsuda, H.; Inadzuki, M.; Hirano, K.; Yoshikawa, M. *Chem. Pharm. Bull.* **1999**, *47*, 1717−1724.
(12) Hostettmann, K.; Marston, A. In *Saponins*; Chemistry and Pharmacology of Natural Products Series; Phillipson, J. D., Ayres, D. C., Baxter, H., Eds.; Cambridge University Press: Cambridge, UK, 1995.
(13) Mahato, S. B.; Garai, S. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, Ch., Eds.; Springer-Verlag: Vienna, 1998; Vol. 74, pp 1−196.
(14) Lacaille-Dubois, M.-A.; Wagner, H. In *Studies in Natural Products Chemistry. Bioactive Natural Products (Part B)*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 2000; Vol. 21, pp 633−687.
(15) Jenuken, A. P.; Shalinsky, D. R.; Hom, D. K.; Albright, K. D.; Health, D.; Howel, S. B. *Biochem. Pharmacol.* **1993**, *45*, 2079−2085.
(16) Assem, M.; Bonvalot, S.; Beltramo, J.-L.; Garrido, C.; Dimanche-Boitrel, M.-T.; Genne, P.; Rebibou, J.-M.; Caillot, D.; Chauffert, B. *Br. J. Cancer* **1994**, *70*, 631−635.

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