New Diosgenin Glycosides from *Costus afer*

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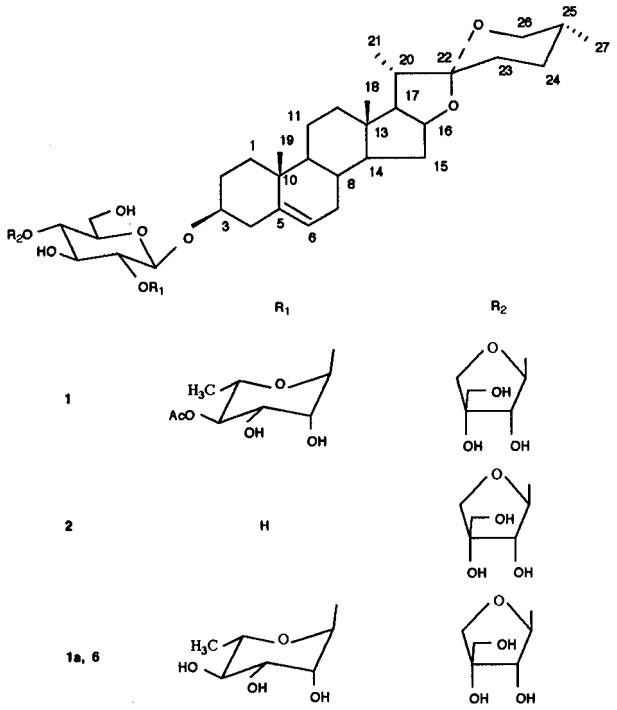
Two new steroidal saponins, aferosides B (**1**) and C (**2**), together with the known saponins, dioscin (**3**) and paryphyllin C (**4**), were isolated from the roots of *Costus afer*. The known flavonoid glycoside, kaempferol 3-*O*-α-L-rhamnopyranoside (**5**), was obtained from the aerial parts. The structures of the new compounds were elucidated principally by 2D NMR spectral methods. A structural revision of the sugar sequence was made for the previously reported saponin aferoside A (**6**) on the basis of detailed spectroscopic analysis. Saponins **1**−**4** and **6** did not show any ability to potentiate *in vitro* cisplatin cytotoxicity in a human colon cancer cell line.

Diosgenin, a steroidal sapogenin, is an important raw material used as a precursor for the synthesis of a number of steroidal drugs including corticosteroids, sex hormones, oral contraceptives, and anabolic agents.[1] For this reason there is an increasing interest of the constituents of *Costus* species which are rich sources of free and combined diosgenin.[2] *Costus afer* Ker-Gawl. (Zingiberaceae) (syn. *C. obliterans* Schum, *C. anomocalyx* Schum, *C. insularis* Chev.) is an African plant[3] which is used in ethnomedicine to treat cough, arthritis, and insufficiency of semen.[4] In our continuing search for biologically active saponins from plants,[5] we have reported the isolation and characterization of aferoside A, a new diosgenin glycoside, from a methanol extract of *C. afer* roots.[6] A detailed further examination of the same extract furnished four additional steroidal saponins (**1**−**4**), two of which are new, aferosides B (**1**) and C (**2**). From the methanol extract of the aerial parts, an additional compound was obtained and characterized as the known flavonoid kaempferol 3-*O*-α-L-rhamnopyranoside (**5**). This paper deals with the isolation and structural elucidation of **1** and **2** together with a revision in the sugar sequence of aferoside A (**6**). The influence of saponins **1**−**4** and **6** on the potentiation of the cytotoxicity of cisplatin in human colon cancer cells was also investigated.

The concentrated *n*-BuOH-soluble fraction of the methanol extract of the roots of *C. afer* was subjected to multiple chromatographic steps over silica gel (see ref 6) to yield aferosides B (**1**) and C (**2**), together with the known saponins dioscin (**3**)[7−8] and paryphyllin C (**4**).[7−8] Column chromatography of the methanol extract of the aerial parts over silica gel yielded the known flavonoid, kaempferol 3-*O*-α-L-rhamnopyranoside (**5**).[9] The structures of **1** and **2** were elucidated mainly by 500 MHz NMR analysis including 1D and 2D NMR (^{1}H−^{1}H DQFCOSY, HMQC, HMBC) spectroscopy.

Aferoside B (**1**) was obtained as a white, amorphous powder. The FAB mass spectrum (negative ion mode) of **1** exhibited a pseudomolecular ion peak at *m*/*z* 895 $[M - H]^-$ indicating a molecular weight of 896, compatible with the molecular formula $C_{46}H_{72}O_{17}$. Other fragments ions at *m*/*z* 853 $[(M - H) - 42]^-$, 721 $[(M - H) - 42 - 132]^-$, and 707 $[(M - H) - 42 - 146]^-$ indicated the respective elimination of one acetyl group, one terminal pentosyl, and one terminal desoxyhexosyl moiety.



Acid hydrolysis of **1** with 2 N TFA yielded rhamnose, apiose, and glucose (identified by co-TLC with authentic samples) and an aglycon which was identified as diosgenin [25(*R*)-spirost-5-en-3β-ol] on the basis of the DEPT, HMQC, and HMBC NMR spectra of **1**.[6]

Compound **1** was shown to contain three sugar residues from the HMQC spectrum. The anomeric protons at δ 6.22 (s), 5.93 (d, *J* = 3.5 Hz) and 4.97 (d, *J* = 8.1 Hz) giving correlations with carbon signals at δ 101.5, 111.3, and 99.7, respectively, were assigned to anomeric protons of α-rhamnopyranose, β-apiofuranose, and β-glucopyranose, respectively. Furthermore, from

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the cross peaks in the DQFCOSY spectrum and the correlations in the HMQC spectrum of **1** it was possible to assign the chemical shifts of a terminal β-D-apiofuranose (Api), a terminal α-L-rhamnopyranose (Rha), and a disubstituted β-D-glucopyranose (Glc), suggesting a branched oligosaccharide chain for **1**. Thus, in the ^{1}H−^{1}H COSY spectrum, from the anomeric proton Glc H-1 (δ 4.97, d, J = 8.1 Hz) it was possible to assign H-2 (δ 4.12, m), H-3 (δ 4.19, m), H-4 (δ 4.20, m), H-5 (δ 3.78, m), and H-6 (δ 4.22/4.32, m). H-2 and H-4 gave ^1J couplings in the HMQC spectrum of **1** with the carbons at δ 77.0 and 79.4, respectively, confirming that the glucose substituent was disubstituted at the C-2 and C-4 positions.

The point of attachment of this trisaccharide to the aglycon was then deduced from the HMBC spectrum of **1** which showed a cross peak between the Glc H-1 at δ 4.97 (d, J = 8.1 Hz) and C-3 of the aglycon at δ 77.8. Other correlations between Rha H-1 (δ 6.22, s) and Glc C-2 (δ 77.0) and between Api H-1 (δ 5.93, d, J = 3.5 Hz) and Glc C-4 (δ 79.4) indicated that the rhamnose was linked to the glucose by a 1 → 2 linkage and the apiose to the glucose by a 1 → 4 linkage. Long-range correlations between H-2 of Glc at δ 4.12 (m) and the anomeric carbon of Glc at δ 99.7 and the anomeric carbon of Rha at δ 101.5 confirmed the attachment of Rha at C-2 of Glc. Furthermore, correlations between H-4 of Glc at δ 4.20 and C-1 of Api at δ 111.3 proved the linkage of the Api sugar at C-4 of the Glc.

The IR and ^{13}C-NMR data, along with the HMQC and HMBC experiments, combined with the mass spectral results, and the presence in the ^{1}H-NMR spectrum of a 3H singlet at δ 2.10 (s) suggested that **1** was a monoacetylated saponin. A correlation in the HMQC spectrum at δ_C 76.3/δ_H 5.87 (t, J = 9.7 Hz) corresponded to the geminal proton of a secondary alcoholic function esterified by an acetic acid unit. Accordingly, from the cross peak in the COSY spectrum of **1**, it was possible to recognize from the anomeric proton of Rha (δ 6.22, s), H-2 (δ 4.74, m), H-3 (δ 4.65, dd, J = 9.5, 3.4 Hz), the deshielded H-4 (δ 5.87, t, J = 9.7 Hz), H-5 (δ 5.00, m), and H-6 (δ 1.5, d, J = 6.0 Hz), suggesting a 4-*O*-acetylrhamnosyl moiety. Furthermore, the HMBC experiment showed long-range couplings between the deshielded Rha-H-4 (δ 5.87) and the carbon of the acetoxyl group at δ 171.0, confirming that Rha was acetylated at the C-4 position. This conclusion was also confirmed by comparison between the ^{13}C-NMR spectral data of **1** and of **1a**, obtained after mild alkaline hydrolysis of **1**. All signals due to the sapogenin and the sugar moieties appeared at almost the same positions. With regard to the rhamnose carbon region, on going from **1a** to **1**, the signal for C-4 occurred downfield by +2.1 ppm, while the signals due to C-3 and C-5 were shielded by −2.6 and −2.3 ppm, respectively. Such changes in the chemical shifts can only be explained if the hydroxyl group at the 4-position of the rhamnose moiety is acylated.[10]

On the basis of the above results and the assumption that Glc and Api are members of the commonly found D series and rhamnose of the L series, the structure of **1** was determined as 3-*O*-{[4-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-[β-D-apiofuranosyl-(1→4)]-β-D-glucopyranosyl}-25(*R*)-spirost-5-en-3β-ol. According to several reports on the distribution of diosgenin glycosides in plants,[7,8] aferoside B (**1**) seems to be the first example of a diosgenin glycoside having an acetylated trisaccharide unit possessing an apiosyl moiety.

Table 1. ^{13}C-NMR Assignments for Compounds **1**, **1a**, **2**, and **6**[a]

position	DEPT	**1**	**1a**	**2**	**6**
1a/1b	CH_2	37.5	37.6	37.6	37.6
2a/2b	CH_2	30.2	30.2	30.4	30.3
3	CH	77.8	78.5	78.5	78.3
4a/4b	CH_2	39.0	39.1	39.4	39.1
5	C	140.8	140.4	141.9	140.4
6	CH	122.2	122.0	121.8	122.0
7a/7b	CH_2	32.5	32.5	32.6	32.5
8	CH	31.9	31.1	31.8	31.0
9	CH	50.5	50.4	50.5	50.5
10	C	37.3	37.9	37.4	37.8
11a/11b	CH_2	21.3	21.4	21.3	21.3
12a/12b	CH_2	40.0	40.1	40.1	40.1
13	C	40.6	40.2	40.8	40.2
14	CH	56.8	56.8	56.8	56.8
15a/15b	CH_2	32.4	32.4	32.5	32.4
16	CH	81.3	81.2	81.3	81.3
17	CH	63.0	62.9	63.0	63.0
18	CH_3	16.6	16.5	16.6	16.5
19	CH_3	19.6	19.5	19.6	19.6
20	CH	42.1	42.1	42.2	42.1
21	CH_3	15.2	15.4	15.2	15.2
22	C	109.1	109.3	110.2	109.1
23a/23b	CH_2	32.0	32.0	32.0	32.0
24a/24b	CH_2	29.4	29.4	29.4	29.4
25	CH	30.7	30.7	30.8	30.7
26a/26b	CH_2	67.1	66.9	67.1	67.0
27	CH_3	17.5	17.6	17.5	17.5
Glc[b]					
G1	CH	99.7	100.1	99.7	100.1
G2	CH	77.0	77.8	75.2	77.8
G3	CH	77.5	77.7	76.8	77.7
G4	CH	79.4	79.6	79.4	79.6
G5	CH	76.8	76.6	76.9	76.6
G6a/6b	CH_2	61.3	61.4	61.6	61.4
Api[b]					
A1	CH	111.3	111.3	111.1	111.3
A2	CH	77.5	77.5	77.6	77.5
A3	C	80.3	80.0	80.8	80.0
A4a/4b	CH_2	75.2	75.1	75.2	75.1
A5a/5b	CH_2	64.8	64.8	64.9	64.7
Rha[b]					
R1	CH	101.5	102.2		102.1
R2	CH	72.5	72.5		72.6
R3	CH	70.3	72.9		72.9
R4	CH	76.3	74.2		74.2
R5	CH	67.3	69.6		69.6
R6	CH_3	18.1	18.8		18.8
$COCH_3$	C	171.0			
$COCH_3$	CH_3	21.4			

[a] Spectra were measured in pyridine-d_5 at 125 MHz with reference to δ 135.5. Assignments were made on the basis of ^{1}H−^{1}H DQFCOSY, HMQC, HMBC, and DEPT experiments. [b] Rha = α-L-rhamnopyranosyl; Api = β-D-apiofuranosyl; Glc = β-D-glucopyranosyl.

Aferoside C (**2**) was obtained as an amorphous powder. The FABMS of **2** displayed a quasimolecular ion peak $[M - H]^-$ at m/z 707, in accordance with a molecular formula of $C_{38}H_{60}O_{12}$. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** and **2** indicated an identical aglycon moiety. The acid hydrolysis of **2** yielded apiose and glucose (TLC). In the HMQC spectrum, the anomeric protons at δ 6.03 (d, J = 3.4 Hz) and 4.98 (*d*, J = 8.0 Hz), giving correlations with carbon signals at δ 111.1 and 99.7, were assigned as the anomeric protons of β-apiofuranose and β-glucopyranose units, respectively. In the HMBC spectrum, cross peaks between H-1 (δ 4.98, *d*, J = 8.0 Hz) of Glc and C-3 at δ 78.5 of

Table 2. ^{1}H-NMR Assignments for Compounds **1**, **1a**, **2**, and **6**[a]

position	1	1a	2	6
1a/1b	1.03/1.70 (m)	0.95/1.73 (m)	0.95/1.68 (m)	0.97/1.72 (m)
2a/2b	1.30/2.10 (m)	1.85 (m)	1.27/2.08 (m)	1.83 (m)
3	3.91 (m)	3.90 (m)	3.90 (m)	3.89 (m)
4a/4b	2.61/2.78 (m)	2.75/2.78 (m)	2.41/2.71 (m)	2.73/2.76 (m)
5				
6	5.33 (m)	5.40 (m)	5.35 (m)	5.35 (m)
7a/7b	1.93 (m)	1.89 (m)	2.00/1.85 (m)	1.86 (m)
8	1.58 (m)	1.56 (m)	nd	1.55 (m)
9	0.91 (m)	nd	0.88 (m)	nd
10				
11a/11b	1.48/1.90 (m)	1.40 (m)	1.48/2.01 (m)	1.39 (m)
12a/12b	1.72/1.08 (m)	1.70/1.10 (m)	1.68/0.99 (m)	1.71/1.08 (m)
13				
14	1.10 (m)	nd	1.05 (m)	nd
15a/15b	1.50 (m)	1.48 (m)	nd	1.50 (m)
16	4.58 (m)	4.56 (m)	4.55 (m)	4.54 (m)
17	1.78 (m)	1.78 (m)	1.80 (m)	1.75 (m)
18	0.87 (s)	0.80 (s)	0.82 (s)	0.82 (s)
19	1.14 (s)	1.05 (s)	0.89 (s)	1.04 (s)
20	1.97 (m)	1.99 (m)	1.95 (m)	1.95 (m)
21	1.15 (d, 6.7)	1.14 (d, 7.0)	1.16 (d, 6.7)	1.12 (d, 6.9)
22				
23a/23b	1.67 (m)	1.60 (m)	nd	1.62 (m)
24a/24b	1.56 (m)	1.56/1.58 (m)	1.56 (m)	1.56/1.57 (m)
25	1.56 (m)	1.54 (m)	1.55 (m)	1.55 (m)
26a/26b	3.50/3.58 (m)	3.50/3.59 (m)	3.43/3.60 (m)	3.50/3.58 (m)
27	0.70 (d, 3.6)	0.69 (d, 3.7)	0.68 (d, 3.7)	0.70 (d, 3.7)
Glc[b]				
G1	4.97 (d, 8.1)	4.92 (d, 8.0)	4.98 (d, 8.0)	4.92 (d, 8.0)
G2	4.12 (m)	4.13 (m)	4.02 (m)	4.13 (m)
G3	4.19 (m)	4.18 (m)	4.28 (m)	4.18 (m)
G4	4.20 (m)	4.16 (m)	4.30 (m)	4.16 (m)
G5	3.78 (m)	3.73 (m)	3.82 (m)	3.73 (m)
G6a/6b	4.22/4.32 (m)	4.20/4.30 (m)	4.29/4.35 (m)	4.20/4.30 (m)
Api[b]				
A1	5.93 (d, 3.5)	5.89 (d, 3.0)	6.03 (d, 3.4)	5.88 (d, 3.0)
A2	4.78 (m)	4.75 (m)	4.80 (m)	4.73 (m)
A3				
A4a/4b	4.33/4.74 (d, 8.0)	4.30/4.75 (d, 8.0)	4.33/4.77 (d, 8.0)	4.32/4.72 (d, 8.0)
A5a/5b	4.15 (m)	4.12/4.15 (m)	4.16 (m)	4.12/4.16 (m)
Rha[b]				
R1	6.22 (s)	6.20 (s)		6.20 (s)
R2	4.74 (m)	4.80 (m)		4.78 (m)
R3	4.65 (dd, 9.5, 3.4)	4.58 (dd, 9.5, 3.5)		4.58 (dd, 9.5, 3.5)
R4	5.87 (t, 9.7)	4.34 (m)		4.33 (m)
R5	5.00 (m)	4.89 (m)		4.90 (m)
R6	1.50 (d, 6.0)	1.74 (d, 6.0)		1.73 (d, 6.0)
*C*OCH$_3$				
CO*C*H3	2.10 (s)			

[a] Spectra were measured at 500 MHz with reference to δ 7.56 in pyridine-d_5. Assignments were made on the basis of ^{1}H−^{1}H DQFCOSY, HMQC, HMBC, and DEPT experiments. Coupling constants (J in Hz) are given in parentheses. [b] Rha = α-L-rhamnopyranosyl; Api = β-D-apiofuranosyl; Glc = β-D-glucopyranosyl.

the aglycon indicated that the Glc moiety was attached at C-3 of the aglycon. In the ^{1}H−^{1}H COSY spectrum, from the anomeric proton of Glc (δ 4.98, d, J = 8.0 Hz), it was possible to assign H-2 (δ 4.02, m), H-3 (δ 4.28, m), H-4 (δ 4.30, m), H-5 (δ 3.82, m), and H-6 (δ 4.29/4.35, m) which gave ^1J couplings in the HMQC spectrum of **2** with carbons at δ 75.2, 76.8, 79.4, 76.9, and 61.6, respectively, suggesting that the Glc was substituted at the C-4 position. This was confirmed by the cross peak in the HMBC spectrum between H-4 of Glc at δ 4.30 (m) and C-1 of apiose at δ 111.1. On the basis of the above results and the assumption that Glc and Api are members of the commonly found D series, the structure of **2** was determined to be 3-*O*-{β-D-apiofuranosyl-(1→4)-β-D-glucopyranosyl}-25(*R*)-spirost-5-en-3β-ol. Aferosides B and C are new natural compounds.

After establishing the structures of **1** and **2**, we observed that the ^{1}H- and ^{13}C-NMR spectral data for the deacylated aferoside B (**1a**) were almost superimposable with those reported for aferoside A (**6**)[6] which was elucidated as 3-*O*-{[β-D-apiofuranosyl-(1→2)][α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-25(*R*)-spirost-5-en-3β-ol. This prompted us to revise the sugar sequence of aferoside A (**6**) by detailed 2D-NMR analysis. In the COSY spectrum of **6** from the anomeric proton Glc H-1 (δ 4.92, d, J = 8 Hz), it was possible to assign H-2 (δ 4.13 not 4.16), H-3 (δ 4.18 not 3.73), H-4 (δ 4.16 not 4.18), H-5 (δ 3.73 not 4.13), and H-6 (δ 4.20/4.30). The HMQC spectrum of **6** showed that the Glc H-2 (δ 4.13) and Glc H-4 (δ 4.16) resonances were correlated to the Glc C-2 and Glc C-4 resonances at δ 77.8 (not 79.6) and 79.6 (not 77.8), respectively. All ^{13}C-NMR assignments are given in Table 1. The HMBC spectrum of **6** showed long-range ^3J couplings between Rha H-1 (δ 6.20, s) and Glc C-2 (δ 77.8), between Api H-1 (δ 5.88, d, J = 3.0 Hz) and Glc C-4 (δ 79.6), indicating that Rha was linked to Glc by a 1→2 linkage (not 1→4) and Api to Glc by a 1 → 4 linkage (not 1 →

2). These attachment positions of the sugars were confirmed by ^3J couplings in the HMBC spectrum between Glc H-2 (δ 4.13), Rha C-1 (δ 102.1), and Glc C-1 (δ 100.1) and between Glc H-4 (δ 4.16) and Api C-1 (δ 111.3). After detailed analysis of these sugars NMR spectral assignments, the structure of aferoside A previously designated as 3-*O*-{[β-D-apiofuranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-25-(*R*)-spirost-5-en-3β-ol has been revised as 3-*O*-{[α-L-rhamnopyranosyl-(1→2)]-[β-D-apiofuranosyl-(1→4)]-β-D-glucopyranosyl}-25(*R*)-spirost-5-en-3β-ol (**6**) and was identical with the structure of deacetyl aferoside B (**1a**). Thus aferoside B (**1**) was considered to be the acetyl aferoside A, the acetyl moiety being located at the 4-position of rhamnosyl moiety.

The known compounds **3**−**5** were identified by comparing their MS, ^{1}H-NMR, and ^{13}C-NMR data obtained from 2D NMR experiments with published data as dioscin,[11] paryphyllin C,[12] and kaempferol 3-*O*-α-L-rhamnopyranoside,[9] respectively.

Since cisplatin and digitonin (a steroid saponin) have been shown to interact synergistically to increase tumor cell (ovarian carcinoma 2008 cell line) lethality *in vitro*,[13] we have tested the effect of aferosides A−C, dioscin, and paryphyllin C on the cytotoxicity of cisplatin in the human cancer colon HT-29 cell line.[14] However, none of these saponins showed any effect on the potentiation of the cytotoxicity of cisplatin.

Experimental Section

General Experimental Procedures. The NMR spectra were obtained with a Bruker DRX 500 spectrometer (500 MHz for ^{1}H and 2D ^{1}H−^{1}H COSY spectra and 125 MHz for ^{13}C spectra). The carbon type (methyl, methylene, methine, and quaternary) was determined by DEPT experiments. Atom connectivities were determined using HMQC, HMBC, and DQFCOSY spectra. All chemical shifts are given in ppm, and the samples were solubilized in pyridine-d_5. FABMS was recorded on a JEOL DX 300 spectrometer with a JMA-3500 system. Optical rotations were taken with a Perkin-Elmer 241 polarimeter. The MPLC separations were performed on a system equipped with a Gilson pump M 303, a 25SC head pump, a M 802 manometric module, a Büchi column (460 × 25 mm), a Büchi precolumn (110 × 15 mm), with the stationary phase silica gel 60 (40−63 μm, Merck). Column and flash chromatography were carried out on silica gel 60 (63−200 μm, Merck) and silica gel 60 (40−63 μm, Merck), respectively.

Plant Material. The whole plant (roots and aerial parts) of *C. afer* was collected in February 1989 near Dhabli (Ivory Coast). A voucher specimen (no. 89/29) is deposited in the Herbarium of the Laboratory of Pharmacognosy, University of Burgundy, Dijon, France.

Extraction and Isolation. Compounds **1** (20 mg), **2** (10 mg), **3** (5 mg), and **4** (6 mg) were isolated from the concentrated *n*-BuOH-soluble fraction (9.4 g) of the MeOH extract (17.4 g) of the roots of *C. afer* (700 g) by multiple chromatographic steps over silica gel as described in a preceding paper.[6] The fractionation was monitored by TLC on silica gel (CH_2Cl_2−MeOH−H_2O, 13:7:2, lower layer).

The dried powdered aerial parts (600 g) of *C. afer* were extracted in a Soxhlet apparatus with petroleum ether (2 L), EtOAc (2 L) and then MeOH (3 L). The MeOH extract (20 g) was fractionated on a Sephadex LH-20 column eluted with MeOH and monitored by TLC on Si gel (toluene−EtOAc−HCOOH, 5:4:4). A fraction containing the main flavonoid was purified by successive column chromatography on silica gel using a discontinuous gradient of CH_2Cl_2−MeOH (100:0 to 100:3) yielding **5** (10 mg), which was eluted with CH_2Cl_2−MeOH (60:40).

Acid Hydrolysis of 1 and 2. A 2 mg quantity of each sample was refluxed in MeOH and 2 N aqueous CF_3COOH (2 mL) for 3 h. After this period, the reaction mixture was diluted with H_2O (5 mL) and then extracted with CH_2Cl_2 (3 × 5 mL). The residue of the CH_2Cl_2 extracts was detected by TLC on silica gel (toluene−acetone, 4:1) with an authentic sample of diosgenin. The H_2O layer was evaporated under reduced pressure by adding MeOH to remove the acid, and the residue was compared with standard sugars by silica gel TLC (CH_2Cl_2−MeOH−H_2O, 8:5:1) detected by (diphenylamino)-phosphoric acid, which showed the sugars to be rhamnose, glucose, and apiose for compound **1** and glucose and apiose for compound **2**.

Mild Alkaline Hydrolysis of 1. Compound **1** (15 mg) was hydrolyzed with KOH (1%) at room temperature. After 30 min the mixture was neutralized with dilute HCl and extracted with *n*-BuOH, yielding the deacylated saponin **1a** (7 mg).

Aferoside B (1): white amorphous powder; $[\alpha]^{26}_D$ −90.0° (*c* 0.35, MeOH); IR (KBr) ν_{max} 3400 (OH), 2940 (CH), 1720 (C=O, ester), 1360, 1230, 1040, 970 cm^{-1}; ^{1}H- and ^{13}C-NMR data (pyridine-d_5), see Tables 1 and 2; negative-ion FABMS using thioglycerol as matrix *m*/*z* 895 [M − H]$^-$, 853 [(M − H) − 42]$^-$, 721 [(M − H) − 42 − 132]$^-$, 707 [(M − H) − 42 − 146]$^-$.

Deacylated aferoside B (1a): white amorphous powder; $[\alpha]^{20}_D$ −108.0° (*c* 0.16, MeOH); IR (KBr) ν_{max} 3400 (OH), 2940 (CH), 1450, 1360, 1230, 1040, 970 cm^{-1}; ^{1}H- and ^{13}C-NMR spectral data (pyridine-d_5), see Tables 1 and 2; negative-ion FABMS using thioglycerol as matrix *m*/*z* 853 [M − H]$^-$, 721 [(M − H) − 132]$^-$, 707 [(M − H) − 146]$^-$.

Aferoside C (2): white amorphous powder; $[\alpha]^{26}_D$ −72.9° (*c* 1.58, MeOH); IR (KBr) ν_{max} 3400 (OH), 2940 (CH), 1450, 1370, 1230, 1040, 970 cm^{-1}; ^{1}H- and ^{13}C-NMR spectral data (pyridine-d_5), see Tables 1 and 2; negative-ion FABMS using thioglycerol as matrix *m*/*z* 707 [M − H]$^-$.

Dioscin (3). Spectroscopic properties were identical to those previously described.[10]

Paryphyllin C (4). ^{1}H- and ^{13}C-NMR data were in good agreement with literature data.[11]

Kaempferol 3-*O*-α-L-rhamnopyranoside (5). ^{1}H- and ^{13}C-NMR data were in good agreement with literature data.[9]

Aferoside A (6). For revised ^{1}H- and ^{13}C-NMR assignments, see Tables 1 and 2.

Bioassay. The test of potentiation of cisplatin cytotoxicity *in vitro* was performed using human colon cancer cells, according to ref 14.

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