Pergamon

PII: S0031-9422(96)00340-8

Phytochemistry, Vol. 43, No. 3, pp. 665–668, 1996

0031-9422/96 $15.00 + 0.00

AFEROSIDE A, A STEROIDAL SAPONIN FROM *COSTUS AFER*

RUI-CHAO LIN, BERNARD HANQUET* and MARIE-ALETH LACAILLE-DUBOIS†

Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université de Bourgogne, 7, Bd Jeanne d'Arc, 21033 Dijon Cedex, France; *Laboratoire de Synthèse et d'Electrosynthèse Organométalliques, CNRS URA 1685, Université de Bourgogne, 6, Bd Gabriel, Dijon, France

(*Received* 12 *February* 1996)

Key Word Index—*Costus afer*; Zingiberaceae; steroidal saponin; diosgenin.

Abstract—A new steroidal saponin, aferoside A, has been isolated from the roots of *Costus afer*. Its structure was established as 3-*O*-{[β-D-apiofuranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-25(*R*)-spirost-5-en-3β-ol by chemical transformations and various spectroscopic methods, mainly 2D NMR techniques (COSY, HMQC and HMBC). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Costus afer Ker-Gawl (Syn.: *C. obliterans* Schum, *C. anomocalyx* Schum, *C. insularis* Chev.), commonly called ginger lily, is an indigenous plant to west Africa [1] that is used in African ethnomedicine to treat coughs, arthritis and insufficiency of semen, respectively [2]. The rhizome is claimed to contain an alkaloid responsible for a papaverine-like smooth muscle relaxant effect and antispasmodic, diuretic and CNS depressant activities in laboratory animals. The saponin fraction pocesses abortifacient activity and produces proliferative changes in the uterus and vagina of sprayed rats [2]. Clinical trials of *C. afer* extracts showed that patients suffering from rhumatoid arthritis were relieved of their symptoms. The same extracts showed a good anti-inflammatory effect in several *in vivo* test systems [2]. There are few reports on the chemical constituents of *C. afer* except that it contains lanosterol, tigogenin, diosgenin, stigmasterol, sitosterol and costugenin [3]. No chemical studies have been carried out on the steroid glycosides of this plant. As part of our ongoing investigation for biologically active saponins [4–8], we report here the isolation and structural elucidation of a new saponin, aferoside A (**1**). Its structure was established mainly by 500 MHz NMR analysis including 1D and 2D NMR (COSY, HMQC and HMBC) spectroscopy.

RESULTS AND DISCUSSION

The roots of *C. afer* were extracted successively with cyclohexane, dichloromethane and methanol. After concentration under reduced pressure, the methanol extract was partitioned between water and *n*-butanol. The butanolic extract showed the presence of five spots in the R_f range 0.35–0.65 on TLC [solvent system (a)], which were detected with Liebermann–Burchard reagent. A combination of column chromatography, MPLC and flash chromatography on normal phase silica gel resulted in the isolation of five saponins of which compound **1** was the major component. Compound **1** was obtained as a white, amorphous compound. The FAB-mass spectrum (glycerol matrix, negative ion mode) showed a quasi-molecular ion peak $[M-H]^-$ at *m/z* 853, indicating a molecular weight of 854 compatible with the molecular formula $C_{44}H_{70}O_{16}$. Other significant peaks visible at *m/z* 707 $[M-H-146]^-$ and 721 $[M-H-132]^-$ correspond to the losses of a terminal desoxyhexose and a terminal pentose, respectively.

Acid hydrolysis of **1** with 2 N trifluoroacetic acid yielded glucose, apiose, rhamnose (TLC) and an aglycone identified as diosgenin (TLC and ^1H and ^{13}C NMR) [9]. The structure of diosgenin was also confirmed by analysis of the HMQC and HMBC spectra of **1**. Most of the ^{13}C NMR signals of the steroid was assigned through two- and three-bond ^1H–^{13}C couplings of the four angular methyls (Table 1).

The ^1H NMR spectrum displayed signals for two tertiary methyl groups at δ 0.82 (*s*) and 1.04 (*s*), two secondary methyl groups (δ 0.70, *d*, $J = 3.7$ Hz; 1.12, *d*, $J = 6.9$ Hz) and one olefinic proton (δ 5.35, *m*, H-6) that are characteristic of a steroidal sapogenin. A secondary methyl proton signal at δ 1.73 (*d*, $J = 6$ Hz) was due to the rhamnosyl unit. Three anomeric protons in the range δ 4.9–6.5 proved the presence of three sugars in **1**. In full agreement with the results from ^1H NMR spectroscopy, the ^{13}C NMR spectrum showed three anomeric carbon signals (δ 111.3, 102.1 and 100.1), one olefinic carbon signal (δ 122.0) and a

---

†Author to whom correspondence should be addressed.

Table 1. NMR spectral data for the aglycone of **1*** [δ in ppm, pyridine-d_5, 125 MHz for δ_C, 500 MHz for δ_H, coupling constants (J in Hz) are given in parentheses]

		H–C one bond		H–C long range
Position	DEPT	δ_C	δ_H	δ_C
1a/1b	CH$_2$	37.6	0.97/1.72	
2	CH$_2$	30.3	1.83	C-4
3	CH	78.3	3.89	
4a/4b	CH$_2$	39.1	2.73/2.76	C-2, C-10, C-3, C-6, C-5
5	C	140.4	—	
6	CH	122.0	5.35	C-7, C-4, C-10
7a/7b	CH$_2$	32.5	1.86	
8	CH	31.0	1.55	
9	CH	50.5	3.58	
10	C	37.8	—	
11a/11b	CH$_2$	21.3	1.39	
12a/12b	CH$_2$	40.1	1.71/1.08	
13	C	40.2	—	
14	CH	56.8	n.d.	
15a/15b	CH$_2$	32.4	1.50	C-14, C-16
16	CH	81.3	4.54	
17	CH	63.0	1.75	C-18, C-21
18	Me	16.5	0.82 *s*	C-12, C-13, C-14, C-17
19	Me	19.6	1.04 *s*	C-1, C-10, C-9, C-5
20	CH	42.1	1.95	C-21, C-23, C-13, C-16, C-17
21	Me	15.2	1.12 *d* (6.9)	C-20, C-17, C-22
22	C	109.1	—	
23a/23b	CH$_2$	32.0	1.62	
24a/24b	CH$_2$	29.4	1.56/1.57	
25	CH	30.7	1.55	
26a/26b	CH$_2$	67.0	3.50/3.58	C-24, C-25, C-22
27	Me	17.5	0.70 *d* (3.7)	C-24, C-25, C-26

*The assignments were made by DEPT, HMQC and HMBC NMR experiments.
n.d. = not determined.

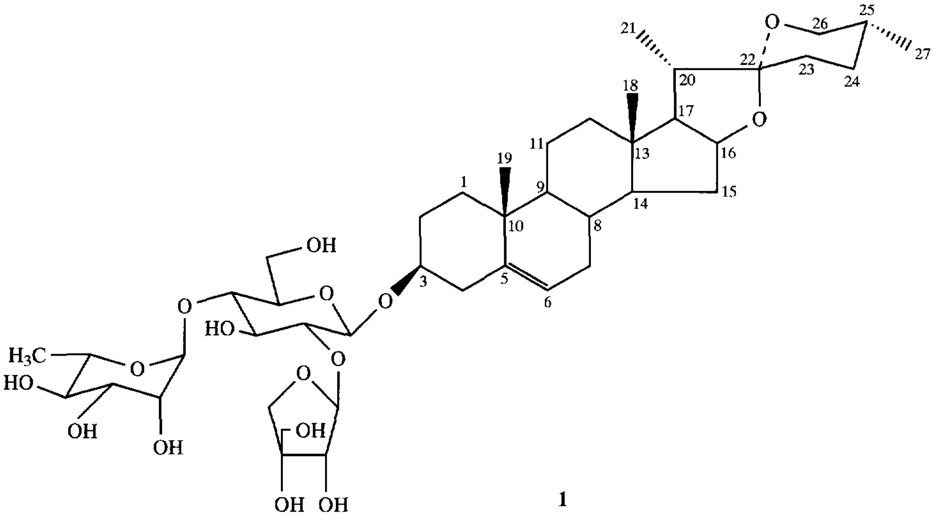
carbon signal at δ 109.1 due to a spiro-atom C-22 of the steroidal sapogenin.

The above data suggested that **1** must be a monodesmosidic saponin in which three sugars units should be bound by a glycosidic linkage to the diosgenin at C-3. The full structural elucidation of **1** was performed by 2D NMR techniques [^{1}H-^{1}H COSY and HMQC (correlation via one bond coupling)] [10] and HMBC (correlation via two- and three-bond coupling) [11] experiments. We can confirm that **1** contains three sugar residues as shown by the HMQC spectrum. The anomeric protons at δ 6.2 (*s*), 5.88 (*d*, $J = 2.7$ Hz) and 4.92 (*d*, $J = 8$ Hz), giving correlations with carbon signals at δ 102.1, 111.3 and 100.1, were assigned to anomeric protons of α-rhamnopyranose, β-apiofuranose and β-glucopyranose, respectively. Furthermore, from the cross peaks in the COSY spectrum and the correlation in the HMQC spectrum of **1** it was easy to assign the chemical shifts of a terminal β-D-apiofuranose, a terminal α-L-rhamnopyranose and a disubstituted β-D-glucopyranose, suggesting a branched oligosaccharide chain for **1**. Namely, from the anomeric proton glucose-H-1 (δ 4.92) it was possible to assign H-2 (δ 4.16), H-3 (δ 3.73) and H-4 (δ 4.18). H-2 and H-4 gave ^1J couplings in the HMQC spectrum of **1** with carbons at δ 79.6 and 77.6, respectively, confirming that glucose was substituted at the C-2 and C-4 positions.

The site of attachment of the sugar residues to the aglycone and the sugar sequence were ascertained by long range heteronuclear correlation spectroscopy (HMBC). A cross peak in the HMBC spectrum of **1** between the glucose-H-1 (δ 4.92) and C-3 (δ 78.3) of the aglycone confirmed the attachment of glucose at C-3. The HMBC spectrum showed long range couplings (^3J) between apiose-H-1 (δ 5.88) and glucose-C-2 (δ 79.6) and between rhamnose-H-1 (δ 6.20) and glucose-C-4 (δ 77.6), indicating that the apiose was linked to the glucose by a $1 \rightarrow 2$ linkage and the rhamnose to the glucose by a $1 \rightarrow 4$ linkage. These results were confirmed by comparison with glycosylation-induced shifts reported in the literature [12].

Based on the above results and the assumption that glucose and apiose are members of commonly found D series and rhamnose of the L series, the structure of **1** was determined as 3-*O*-{[β-D-apiofuranosyl-($1 \rightarrow 2$)]-[α-L-rhamnopyranosyl-($1 \rightarrow 4$)]-β-D-glucopyranosyl}-25(*R*)-spirost-5-en-3β-ol.

Several publications have reported the distribution of diosgenin glycosides in plants [9, 13, 14]. To our knowledge, **1** seems to be the first example of a



1

diosgenin glycoside having an apiosyl unit. The other saponins (B–E) are also derivatives of diosgenin. Their chemical and pharmacological investigations are in progress in order to evaluate the therapeutic profile of the *C. afer* saponins.

EXPERIMENTAL

Instruments and general methods. NMR spectra were obtained with a Bruker DRX 500 spectrometer (500 MHz for ^{1}H and 2D ^{1}H–^{1}H COSY and 125 MHz for ^{13}C). The carbon type (Me, CH_2, methine) was determined by DEPT experiments. The ^{1}H-detected one-bond [10] and multiple-bond ^{13}C multiple-quantum coherence spectra [11] (HMQC and HMBC), respectively, were measured at 500 MHz with an DRX 500 spectrometer, which was equipped to allow reverse detection. The magnitude of the delay for optimizing one-bond correlations in the HMQC spectrum and suppressing them in the HMBC spectrum was 3.45 msec, and the evolution delay for long range couplings in the latter was set to 60 msec. All 1D and 2D spectra were recorded using standard software, and data manipulation of the 2D spectra was performed on a silicon Graphics Indigo Computer. All chemical shifts (δ) are given in ppm and the samples were solubilized in pyridine-d_5. FABMS: negative ion mode, thioglycerol matrix; Jeol DX 300 with JMA-3500 system. The target was bombarded with 6 keV Xe atoms. Optical rotations were taken with a Perkin-Elmer 241 polarimeter. Isolations were carried out using a MPLC system [pump Gilson M 303, head pump 25SC, manometric module M 802, Büchi column (460 × 25 mm), Büchi precolumn (110 × 15 mm), stationary phase: silica gel 60 (40–63 μm, Merck]. Silica gel 60 (63–200 μm, Merck) and silica gel 60 (40–63 μm, Merck) were used for CC and flash chromatography, respectively.

TLC employed precoated silica gel plates $60F_{254}$ (Merck). The following solvent systems were used: for saponins, (a) CH_2Cl_2–MeOH–H_2O (13:6:2, lower layer), (b) CH_2Cl_2–MeOH–*n*-PrOH–H_2O (100:20:6:3) and (c) CH_2Cl_2–MeOH–*n*-PrOH–H_2O (70:20:6:3); for sapogenins, (d) toluene–Me_2CO (4:1); for monosaccharides, (e) CH_2Cl_2–MeOH–H_2O (8:5:1). Spray reagents were Liebermann–Burchard (for saponins) and diphenylaminophosphoric acid reagents (for sugars).

Plant material. The roots of *C. afer* were 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 of saponins. Dried powdered roots (700 g) were submitted to successive extractions in a Soxhlet by cyclohexane (3 l, 24 hr), CH_2Cl_2 (3 l, 24 hr) and MeOH (4 l, 72 hr). After evapn under red. pres. of the solvent, 5 g cyclohexane extract, 1.8 g CH_2Cl_2 extract and 17.4 g MeOH extract were obtained. The MeOH extract was dissolved in 400 ml H_2O and extracted 2× with H_2O-satd *n*-BuOH. On evapn of the solvent, 9.4 g residue was obtained and fractionated on a silica gel column eluted with a discontinuous gradient of CH_2Cl_2–MeOH (100:0 to 1:1). The saponin fr. (4 g) was submitted to MPLC on a silica gel column eluted with a discontinous gradient of CH_2Cl_2–MeOH (49:1 to 3:7). Frs 33–38 were purified by successive flash chromatography on silica gel eluted with CH_2Cl_2–MeOH-*n*-PrOH–H_2O (70:20:6:3) [15] to yield 5 saponins: aferoside A, (20 mg), B (10 mg), C (10 mg), D (6 mg) and E (5 mg). Final purification of the compounds was carried out on a Sephadex LH 20 column eluted with MeOH.

Aferoside A (**1**). Amorphous powder, $[\alpha]_D^{20}$ −108° (MeOH; *c* 0.16). TLC R_f 0.40 [system (a)]. Green spots by spraying with Liebermann–Burchard reagent; negative ion FABMS *m/z*: 853 $[M-H]^-$, 721 $[(M-H)-132]^-$, 707 $[(M-H)-146]^-$. ^{1}H NMR (pyridine-d_5, 500 MHz), ^{13}C NMR (pyridine-d_5, 125 MHz), direct ^{1}H–^{13}C correlations (HMQC), long range ^{1}H–^{13}C

Table 2. ^{13}C NMR and ^{1}H NMR spectral data for sugar moities of **1*** [pyridine-d_5, δ in ppm, 125 MHz for δ_C, 500 MHz for δ_H, coupling constants (J in Hz) are given in parentheses]

			H–C one bond		H–C long range
	Position	DEPT	δ_C	δ_H	δ_C
R	1	CH	*102.1*	6.20 *s*	R-5, R-2, R-3, G-4
	2	CH	72.6	4.78	
	3	CH	72.9	4.58	
	4	CH	74.2	4.33	R-5, R-3
	5	CH	69.6	4.90	
	6	Me	18.8	1.73 *d* (6.0)	R-5, R-4
A	1	CH	*111.3*	5.88 *d* (2.7)	A-4, G-2
	2	CH	77.5	4.73	
	3	C	80.0	—	
	4a/4b	CH_2	75.1	4.32/4.72	A-5, A-3/A-5, A-1
	5a/5b	CH_2	64.7	4.12/4.16	A-4, A-2, A-3, A-1
G	1	CH	*100.1*	4.92 *d* (8)	Agly-C3
	2	CH	*79.6*	4.16	
	3	CH	76.6	3.73	
	4	CH	*77.6*	4.18	
	5	CH	77.8	4.13	
	6a/6b	CH_2	61.4	4.20/4.30	

R = α-L-rhamnopyranosyl; A = β-D-apiofuranosyl; G = β-D-glucopyranosyl.

*The assignments were made on the basis of ^{1}H–^{1}H COSY, HMQC, HMBC and DEPT experiments (^{13}C chemical shifts of substituted residues are italicized).

correlations (HMBC) used for defining the aglycone and sugars (Tables 1 and 2).

Acid hydrolysis of aferoside A (**1**). A soln of **1** (12 mg) in H_2O (10 ml) and 2 N aq. TFA (20 ml) was refluxed on a water bath for 3 hr. After this period, the reaction mixt. was diluted with H_2O (15 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined CH_2Cl_2 extracts were washed with H_2O and dried with Na_2SO_4; evapn of the solvent gave diosgenin (5 mg), which was identical by TLC [solvent system (d)], MS, ^{1}H and ^{13}C NMR with lit. data [9] and with an authentic sample of diosgenin. After repeated evapns of the solvent from the aq. layer by adding MeOH to remove the acid, the sugars were analysed by TLC [solvent system (e)].

Acknowledgement—The authors thank Prof. H. Shoyama (Department of Pharmacognosy, Fukuoka, University of Kyushu, Japan) for the negative-ion FAB MS spectra.

REFERENCES

1. Oliver-Bever, B. (1986) *Medicinal Plants in Tropical West Africa*, 375 pp. Cambridge University Press, Cambridge, U.K.
2. Iwu, M. M. and Anyanwu, B. N. (1982) *J. Ethnopharmacol.* **6**, 263.
3. Iwu, M. M. (1982) *Planta Med.* **44**, 413.
4. Lacaille-Dubois, M. A., Hanquet, B., Rustaiyan, A. and Wagner, H. (1993) *Phytochemistry* **34**, 489.
5. Brandao, M. G. L., Lacaille-Dubois, M. A., Teixera, M. A. and Wagner, H. (1993) *Phytochemistry* **34**, 1123.
6. Guinea, M. C., Parellada, J., Lacaille-Dubois, M. A. and Wagner, H. (1994) *Planta Med.* **60**, 163.
7. Dirsch, V., Lacaille-Dubois, M. A. and Wagner, H. (1994) *Nat. Prod. Letters* **4**, 29.
8. Lacaille-Dubois, M. A., Hanquet, B., Cui, Z.-H., Lou, Z.-C. and Wagner, H. (1995) *Phytochemistry* **40**, 509.
9. Agrawal, P. K., Jain, R. K., Gupta, R. K. and Thakur, R. S. (1985) *Phytochemistry* **24**, 2479.
10. Summers, M. F., Marzilli, L. G. and Bax, A. (1986) *J. Am. Chem. Soc.* **108**, 4285.
11. Bax, A. and Summers, M. F. (1986) *J. Am. Chem. Soc.* **108**, 2093.
12. Agrawal, P. K. (1992) *Phytochemistry* **31**, 3307.
13. Voigt, G. and Hiller, K. (1987) *Sci. Pharm.* **55**, 201.
14. Hostettmann, K. and Marston, A. (1985) *Saponins*, 548 pp. Cambridge University Press, Cambridge, U.K.
15. Ma, J. C. N. and Lau, F. W. (1985) *Phytochemistry* **24**, 1561.