Antifungal Steroid Saponins from *Dioscorea cayenensis*

M. Sautour[1], A.-C. Mitaine-Offer[1], T. Miyamoto[2], A. Dongmo[3], M.-A. Lacaille-Dubois[1]

Abstract

From the rhizomes of *Dioscorea cayenensis* Lam.-Holl (Dioscoreaceae), the new 26-*O*-β-D-glucopyranosyl-22-methoxy-3β,26-dihydroxy-25(*R*)-furost-5-en-3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**1**) was isolated together with the known dioscin (**2**) and diosgenin 3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**3**). Their structures were established on the basis of spectral data. Compound **2** exhibited antifungal activity against the human pathogenic yeasts *Candida albicans*, *C. glabrata* and *C. tropicalis* (MICs of 12.5, 12.5 and 25 μg/mL, respectively) whereas **3** showed weak activity and **1** was inactive.

Dioscorea cayenensis Lam.-Holl belongs to the Dioscoreaceae family in which steroidal saponins are fairly widespread. Some of these are reported to exhibit antifungal and cytotoxic activities [1], [2], [3], [4]. *D. cayenensis*, an important economic tuber distributed in tropical West Africa [5], is used in African ethnomedicine as remedy for the treatment of burn and against fever [6], [7] but the chemical constituents have never been studied before. As part of our ongoing search for biologically active steroid saponins [8], [9], we report in this paper the isolation and characterization of a new furostanol glycoside together with two known spirostanol saponins. In addition, the antifungal activity of these compounds against three human pathogenic species of *Candida* is presented.

The *n*-BuOH-soluble fraction of the MeOH-H_2O (7 – 3) extract of the rhizome of *D. cayenensis* was subjected to repeated CC over silica gel to yield compounds **1 – 3**. Compound **1** showed in the FAB-MS (negative-ion mode) a quasi-molecular ion peak $[M - H]^-$ at *m/z* = 1207 consistent with the molecular formula $C_{58}O_{26}H_{96}$. Acid hydrolysis of **1** yielded glucose, rhamnose (TLC) and an aglycone which was identified as the previously reported (3β,22α,25*R*)-22-methoxyfurost-5-ene-3,26-diol, from the 2D NMR spectra of **1** (Tables **1** and **2**) [2], [10], [11]. The ^1H- and ^{13}C-NMR data of **1** (Tables **1** and **2**) obtained from its 2D NMR spectra were almost superimposable with those of methyl protodioscin [12] except for the presence of an additional terminal rhamnosyl moiety. The HMBC correlation between the Rha III H-1 (δ = 6.12) and Rha II C-4 (δ = 80.0) and the NOESY correlation between Rha II H-4 (δ = 4.31) and Rha III H-1 (δ = 6.12) indicated the attachment of this fourth sugar moiety at Rha II-4. On the basis of these results, **1** was deduced as 26-*O*-β-D-glucopyranosyl-22-methoxy-3β,26-dihydroxy-25(*R*)-furost-5-en-3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside, a new natural compound [13]. Compounds **2** and **3** were identified as dioscin and diosgenin 3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl(1→4)-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside, respectively (Fig. **1**) by comparison of the spectral data with literature values [1], [14].

The antifungal activity of saponins **1 – 3** (Table **3**) was evaluated at concentrations up to 200 μg/mL against strains of *Candida albicans*, *C. glabrata* and *C. tropicalis*. Concerning **2** (dioscin), its antifungal activity was previously reported against the fungus *Trichophyton mentagrophytes* [15]. We confirmed in this work its antifungal properties against *Candida* species with MIC values comparable to that of α-hederin, between 12.5 and 25 μg/mL.

Table **1** ^1H- and ^{13}C-NMR data [a] of the aglycone part of **1** (in pyridine-d_5), δ in ppm, *J* in Hz

	δ_C	δ_H [b,c]
1	37.2	0.93, 1.68
2	29.6	nd
3	77.9	3.94
4	38.6	2.74, 2.64
5	140.6	
6	121.6	5.31
7	32.0	1.84
8	31.4	1.92
9	50.0	0.82
10	36.8	
11	20.7	1.33, 1.38
12	39.5	1.03, 1.64
13	40.5	
14	56.3	0.96
15	31.8	1.36
16	81.1	4.40
17	63.8	1.70
18	16.0	0.75 s
19	19.1	0.98 s
20	40.2	2.16
21	16.0	1.12 d (6.9)
22	112.6	
23	37.2	0.93, 1.70
24	27.9	1.72
25	33.9	1.84
26	74.9	3.54
27	16.9	0.94 s
22-OMe	47.1	3.24 s

[a] Multiplicities were assigned from DEPT spectra.
[b] nd: not determined.
[c] Overlapping ^1H-NMR signals are reported without designated multiplicities.

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Table 2 ^{1}H- and ^{13}C-NMR data of the sugar moieties of **1** (in pyridine-d_5)[a,b,c], δ in ppm, J in Hz

	δ_C	δ_H
Sugars at C-3		
Glc I		
1	*100.0*	*4.89 d (7.5)*
2	*77.9*	*4.10*
3	77.1	4.10
4	*77.9*	*4.14*
5	76.5	3.58
6	60.9	3.98, 4.13
T-Rha I		
1	*101.9*	*6.20 s*
2	71.9	4.75
3	72.3	4.55
4	73.5	4.30
5	69.3	4.83
6	18.4	1.68 d (6.2)
Rha II		
1	*102.0*	*5.66 s*
2	72.3	4.48
3	72.6	4.43
4	*80.0*	*4.31*
5	68.2	4.72
6	18.0	1.48 d (6.0)
T-Rha III		
1	*102.8*	*6.12 s*
2	72.3	4.39
3	72.6	4.43
4	73.5	4.30
5	70.0	4.23
6	18.2	1.51 d (5.2)
Sugars at C-26		
Glc II		
1	*104.4*	*4.76 d (7.9)*
2	74.6	3.93
3	77.9	4.20
4	71.3	4.09
5	77.9	3.90
6	62.4	4.25, 4.46

[a] The assignments were based on the DEPT, HSQC, and HMBC experiments (150 MHz for ^{13}C-NMR, 600 MHz for ^{1}H-NMR).
[b] Overlapping ^{1}H-NMR signals are reported without designated multiplicities.
[c] ^{1}H- and ^{13}C-NMR chemical shifts of substituted residues are italicized.

Table 3 Antifungal activity of **1–3** and α-hederin against *Candida* species given as MIC (μg/mL)[a]

Compounds	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*
1	> 200	> 200	> 200
2	12.5	12.5	25
3	100	200	> 200
α-hederin	25	50	50
ketoconazole[b]	0.39	0.78	0.78

[a] Compounds with MIC values > 200 μg/mL are considered not active.
[b] Positive control.

Compound **3**, the analogue of **2** with a longer oligosaccharidic chain, possessed antifungal activity although showing lower inhibition capacity and a narrower spectrum of activity. It inhibited only *C. albicans* and *C. glabrata* with MICs between 100–200 µg/mL. We found here that an increasing sugar number decreases the antifungal properties. Finally, compound **1** having a furostan skeleton was devoid of activity against the tested fungi. Regarding the aglycone structure and by comparing the activities of **1** and **3**, having the same sugar sequence at C-3, we only observed antifungal activity with the spirostanol derivative whereas none was observed with the furostanol derivative. This confirms that the E and F rings of diosgenin play a key role in the antifungal properties [16].

Materials and Methods

General experimental procedures: IR, FAB-MS, 2D-NMR and medium-pressure liquid chromatography (MPLC) instruments were as previously described [17]. Optical rotations were taken with a Perkin-Elmer 881 polarimeter. TLC and HPTLC: silica gel plates 60 F_{254} (Merck), using solvent systems (a) for saponins $CHCl_3$-MeOH-H_2O (13 : 7 : 2; lower phase), (b) for sapogenins $CHCl_3$-MeOH (9 : 1), and (c) for sugars $CHCl_3$-MeOH-H_2O (8 : 5 : 1).

Plant material: The rhizomes of *Dioscorea cayenensis* Lam.-Holl were collected in October 2002 from Elounden (Yaoundé Province, Cameroon) and identified by the Dr. Nole Tsabang (Institut de recherches Médicales et d'études des Plantes Médicinales, IMPM). A voucher specimen (No. 14259 HNC) is deposited at the National Herbarium of Yaoundé, Cameroon.

Extraction and isolation: Dried powdered rhizomes (175 g) of *Dioscorea cayenensis* were refluxed with MeOH-H_2O (7 : 3, 6 L), concentrated, and 17.9 g were partitioned successively with hexane, CH_2Cl_2 and *n*-BuOH (each 3 × 200 mL) yielding the corresponding hexane (687 mg), CH_2Cl_2 (251 mg) and *n*-BuOH (1.2 g) fractions. The latter was submitted to vacuum liquid chromatography on C_{18} reversed-phase (12 × 3 cm) using H_2O (100 mL), MeOH-H_2O mixtures (1 : 4; 2 : 3; 3 : 2, each 100 mL) and finally MeOH (100 mL). The MeOH fraction (500 mg) containing the saponins was finally submitted to MPLC column chromatography on silica gel (15–40 µm), $CHCl_3$-MeOH-H_2O (13 : 7 : 2, lower phase), to give 9 fractions (F1 to F9). F7 (80 mL) was concentrated to give the pure compound **1** (11 mg). F3 (150 mg) was rechromatographed in the same conditions to give the pure compounds **2** (10 mg; 25 mL) and **3** (10 mg; 32 mL).

26-O-β-D-Glucopyranosyl-22-methoxy-3β,26-dihydroxy-25(R)*-furost-5-en-3-O-α-L-rhamnopyranosyl-*(1→4)*-α-L-rhamnopyranosyl-*(1→4)*-*[*α-L-rhamnopyranosyl-*(1→2)]*-β-D-glucopyranoside* (**1**): White amorphous powder; $[\alpha]_D^{20}$: –100° (MeOH, c 0.05); IR (KBr): ν_{max} = 3340 (OH), 2927 (CH), 1050 (C-O-C) cm^{-1}; ^{1}H-NMR and ^{13}C-NMR, see Tables **1** and **2**; negative FAB-MS: m/z = 1207

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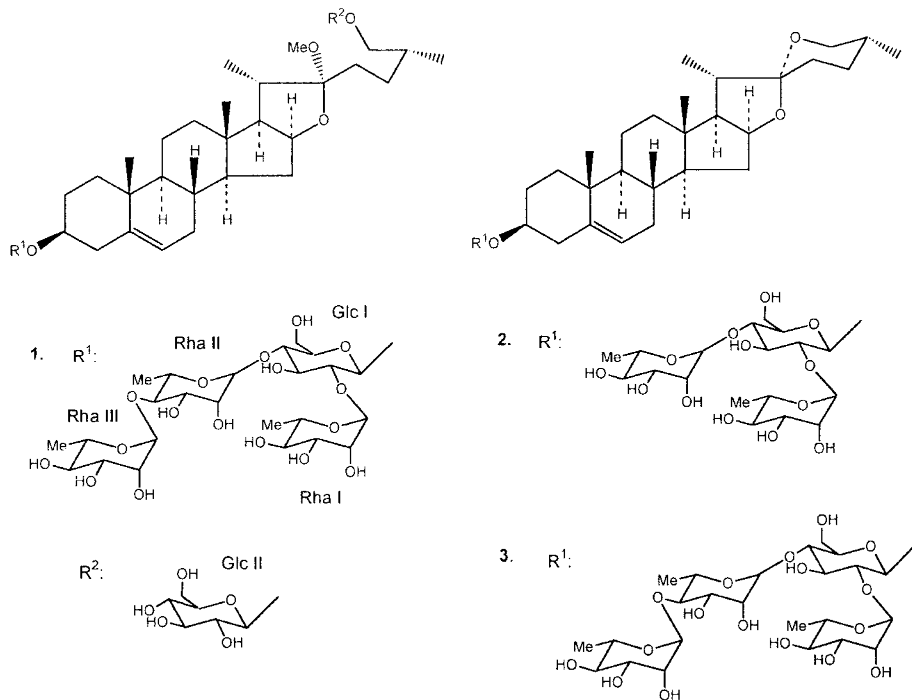

Fig. **1** Chemical structures of **1 – 3**.

[M – H]⁻, 1045 [M – H – 162]⁻, 899 [M – H – 162 – 146]⁻ (calcd. for $C_{58}O_{26}H_{96}$: 1208.62).

Dioscin (**2**): White amorphous powder; $[\alpha]_D^{20}$: –115° (MeOH, *c* 0.4). The spectral data were in full agreement with previously published data [1].

Diosgenin 3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl (1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**3**): White amorphous powder; $[\alpha]_D^{20}$: –113° (MeOH, *c* 0.57). The spectral data were in full agreement with previously published data [14].

Acid hydrolysis: A solution of compound **1** (3 mg) in 2 N aqueous CF_3COOH (5 mL) was refluxed on a water bath for 3 h. After extraction with CH_2Cl_2 (3 × 5 mL), the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral and then analyzed by silica gel TLC by comparison with standard sugars (solvent system c). The absolute configuration of sugar residues was determined by GC analysis as described in a previous paper [17].

Antifungal activity: Minimum inhibitory concentrations (MICs) were performed using the broth dilution test [18]. For these bioassays three human pathogenic yeasts were used: *Candida albicans* (IP 1180 – 79), *C. glabrata* and *C. tropicalis* (clinical isolates). The reference compounds ketoconazole (Sigma) and α-hederin (Extrasynthèse) [19] were used as positive controls.

Copies of the original spectra can be obtained from the author of correspondence.

References

1 Hu K, Dong A, Yao X, Kobayashi H, Iwasaki S. Antineoplasic agents; I. Three spirostanol glycosides from rhizomes of *Dioscorea collettii* var. *hypoglauca*. Planta Med 1996; 62: 573 – 5

2 Hu K, Dong A, Yao X, Kobayashi H, Iwasaki S. Antineoplasic agents; II. Four furostanol glycosides from rhizomes of *Dioscorea collettii* var. *hypoglauca*. Planta Med 1997; 63: 161 – 5

3 Dong M, Feng XZ, Wu LJ, Wang BX, Ikejima T. Two new steroidal saponins from the rhizomes of *Dioscorea panthaica* and their cytotoxic activity. Planta Med 2001; 67: 853 – 7

4 Liu HW, Hu K, Zhao QC, Cui CB, Kobayashi H, Yao XS. Bioactive saponins from *Dioscorea futschauensis*. Pharmazie 2002; 57: 570 – 2

5 Agbor-Egbe T, Treche S. Evaluation of the chemical composition of Cameroonian yam germplasm. J Food Comp Anal 1995; 8: 274 – 83

6 Brutus TC, Pierce-Noel AV. Les plantes et les légumes d'Haiti qui guérissent. Imprimerie de l'Etat, Port-au-Prince, Haiti: 1960

7 Altschul SVR. Drugs and Foods from Little Known Plants (Harvard Herbarium). Cambridge, MA: Harvard Univ. Press, 1973

8 Lin RC, Hanquet B, Lacaille-Dubois MA. Aferoside A, a steroidal saponin from *Costus afer*. Phytochemistry 1996; 3: 665 – 8

9 Lin RC, Lacaille-Dubois MA, Hanquet B, Correia M, Chauffert B. New diosgenin glycosides from *Costus afer*. J Nat Prod 1997; 60: 1165 – 9

10 Pereira Da Silva BP, Bernardo RR, Parente JP. A furostanol glycoside from rhizomes of *Costus spicatus*. Phytochemistry 1999; 51: 931 – 5

11 Agrawal PK, Jain DC, Pathak AK. NMR spectroscopy of steroidal sapogenins and steroidal saponins: an update. Mag Reson Chem 1995; 33: 923 – 53

12 Ju Y, Jia ZJ. Steroidal saponins from the rhizomes of *Smilax menispermoidea*. Phytochemistry 1992; 4: 1349 – 51

13 Buckingham JB. Dictionary of Natural Products on CD-ROM, Version 10 : 2. Chapman and Hall, CRC Press, London, UK: 2002

14 Matsuda H, Pongpiriyadacha Y, Morikawa T, Kishi A, Kataoka S, Yoshikawa M. Protective effects of steroid saponins from *Paris polyphylla* var. *yunnanensis* on ethanol – or indomethacin-induced gastric mucosal lesions in rats: structural requirement for activity and mode of action. Bioorg Med Chem Lett 2003; 13: 1101 – 6

15 Takechi M, Tanaka Y. Structure-activity relationships of the saponin α hederin. Phytochemistry 1990; 2: 451 – 2

16 Takechi M, Tanaka Y. Structure-activity relationships of synthetic diosgenyl monoglycosides. Phytochemistry 1991; 8: 2557 – 8

17 Haddad M, Miyamoto T, Laurens V, Lacaille-Dubois MA. Two new biologically active triterpenoidal saponins acylated with salicylic acid from *Albizia adianthifolia*. J Nat Prod 2003; 66: 372 – 7

18 Quiroga EN, Sampietro AR, Vattuone MA. Screening antifungal activities of selected medicinal plants. J Ethnopharmacol 2001; 74: 89 – 96

19 Favel A, Steinmetz MD, Regli P, Vidal-Ollivier E, Elias R, Balansard G. *In vitro* antifungal activity of triterpenoid saponins. Planta Med 1994; 60: 50 – 3

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