

Antifungal Steroid Saponins from *Dioscorea cayenensis*

M. Sautour¹, A.-C. Mitaine-Offer¹, T. Miyamoto², A. Dongmo³, M.-A. Lacaillle-Dubois¹

Abstract

From the rhizomes of *Dioscorea cayenensis* Lam.-Holl (Dioscoreaceae), the new 26-*O*- β -D-glucopyranosyl-22-methoxy-3 β ,26-dihydroxy-25(*R*)-furost-5-en-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside (**1**) was isolated together with the known dioscin (**2**) and diosgenin 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 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 μ g/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-H₂O (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 C₅₈O₂₆H₉₆. Acid hydrolysis of **1** yielded glucose, rhamnose (TLC) and an aglycone which was identified as the previously reported (3 β ,22 α ,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 ¹³C-

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Table 1 ¹H- and ¹³C-NMR data ^a of the aglycone part of **1** (in pyridine-*d*₅), δ in ppm, *J* in Hz

	δ_c	$\delta_H^{b,c}$
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.

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-3 β ,26-dihydroxy-25(*R*)-furost-5-en-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 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 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 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 μ g/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 μ g/mL.

Table 2 ^1H - 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 ^1H -NMR).

^b Overlapping ^1H -NMR signals are reported without designated multiplicities.

^c ^1H - and ^{13}C -NMR chemical shifts of substituted residues are italicized.

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 $\mu\text{g}/\text{mL}$. 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 ob-

Table 3 Antifungal activity of **1–3** and α -hederin against *Candida* species given as MIC ($\mu\text{g}/\text{mL}$)^a

Compounds	<i>Candida albicans</i>	<i>Candida glabrata</i>	<i>Candida tropicalis</i>
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 $\mu\text{g}/\text{mL}$ are considered not active.

^b Positive control.

served 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

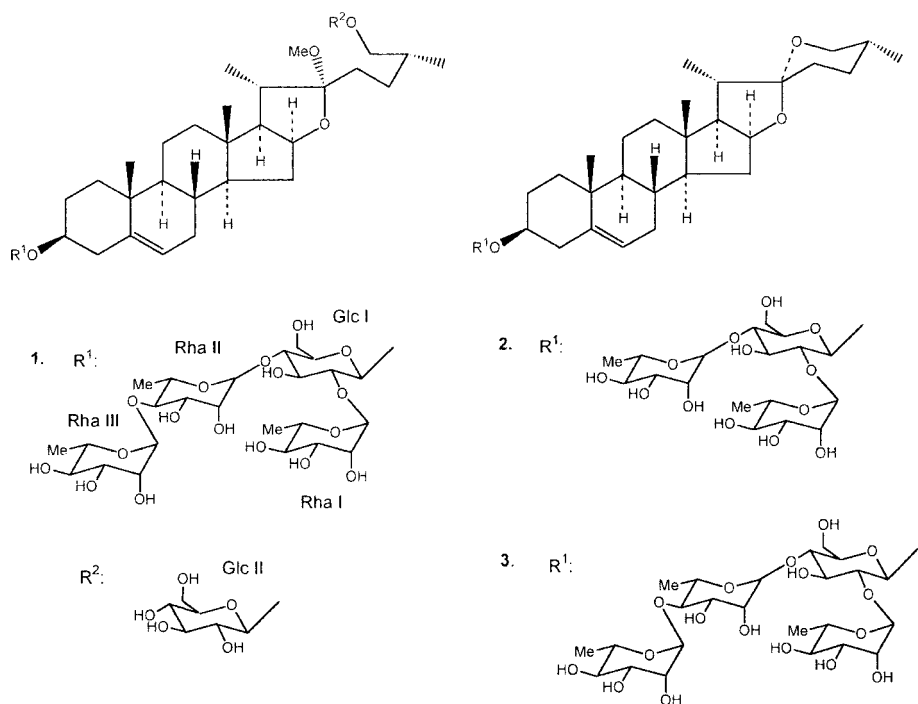
General experimental procedures: IR, FAB-MS, 2D-NMR and medium-pressure liquid chromatography (MPLC) instruments were as previously described [17]. Optical rotations were taken with a Perkin-Elmer 881 polarimeter. TLC and HPTLC: silica gel plates 60 F₂₅₄ (Merck), using solvent systems (a) for saponins CHCl_3 -MeOH-H₂O (13:7:2; lower phase), (b) for sapogenins CHCl_3 -MeOH (9:1), and (c) for sugars CHCl_3 -MeOH-H₂O (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-H₂O (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 C₁₈ reversed-phase (12×3 cm) using H₂O (100 mL), MeOH-H₂O 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-H₂O (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-3 β ,26-dihydroxy-25(R)-furost-5-en-3-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 4)-[α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside (1**):** White amorphous powder; [α]_D²⁰: -100° (MeOH, c 0.05); IR (KBr): ν_{max} = 3340 (OH), 2927 (CH), 1050 (C-O-C) cm^{-1} ; ^1H -NMR and ^{13}C -NMR, see Tables **1** and **2**; negative FAB-MS: m/z = 1207

Fig. 1 Chemical structures of 1–3.



[M–H]⁻, 1045 [M–H–162]⁻, 899 [M–H–162–146]⁻ (calcd. for C₅₈O₂₆H₉₆: 1208.62).

Dioscin (2): White amorphous powder; [α]_D²⁰: –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; [α]_D²⁰: –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 CF₃COOH (5 mL) was refluxed on a water bath for 3 h. After extraction with CH₂Cl₂ (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.

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