# A New Steroidal Saponin from *Dioscorea cayenensis*

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**The new 26-*O*-$\beta$-D-glucopyranosyl-3$\beta$,26-dihydroxy-20,22-seco-25(*R*)-furost-5-en-20,22-dione-3-*O*-$\alpha$-L-rhamnopyranosyl-(1→4)-$\alpha$-L-rhamnopyranosyl-(1→4)-[$\alpha$-L-rhamnopyranosyl-(1→2)]-$\beta$-D-glucopyranoside (1), along with the known methyl protodioscin (2), asperoside (3) and prosapogenin A of dioscin (4) were isolated from the rhizomes of *Dioscorea cayenensis* LAM.-HOLL (Dioscoreaceae). Their structures were established mainly on the basis of 600 MHz 2D-NMR spectral data. 4 exhibited antifungal activity against the human pathogenic yeasts *Candida albicans*, *C. glabrata* and *C. tropicalis* (MICs of 20.8, 6.25, 25 $\mu$g/ml, respectively), whereas saponins 1—3 were inactive.**

**Key words** *Dioscorea cayenensis*; Dioscoreaceae; steroidal saponin; antifungal activity; 2D-NMR

Our previous phytochemical studies on the methanolic extract of the rhizome of *Dioscorea cayenensis* LAM.-HOLL led to the isolation of a new furostanol saponin.[1)] Further detailed investigation of the same extract has resulted in the isolation of a new furostanol glycoside (**1**) together with three known spirostanol saponins **2**—**4**. Their structures were elucidated mainly by 1D and 2D NMR experiments (COSY, TOCSY, NOESY, HSQC and HMBC), and by HR-ESI-MS and FAB-MS. 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**—**4**. Compound **1**, an amorphous powder, showed IR absorptions at 3371 (OH), 2929 (CH), 1736 (CO), 1680 and 1035 $cm^{-1}$. The high-resolution ESI mass spectrometry (HR-ESI-MS) (positive-ion mode) of **1** exhibited a pseudomolecular ion peak at *m*/*z* 1231.5697 $[M+Na]^+$ (Calcd 1231.5724), consistent with a molecular formula of $C_{57}H_{92}O_{27}Na$. Its negative-ion FAB-MS showed a quasimolecular ion peak at *m*/*z* 1207 $[M-H]^-$, indicating a molecular weight of 1208. Acid hydrolysis of **1** yielded glucose, rhamnose (TLC) and an aglycone which was identified as a furostanol-type steroid by comparison of the NMR data of **1** (Table 1) with those of known spirostane-type steroids.[1—3)] The comparison of NMR data of **1** with literature data allowed the identification of the aglycone as the previously reported (3$\beta$,25*R*)-20,22-seco-25-furost-5-en-20,22-dione-3,26-diol (aglycone of dioscoreside D),[2,3)] and the five sugar residues as two $\beta$-glucopyranosyl (Glc) and three $\alpha$-rhamnopyranosyl (Rha) moieties. The absolute configurations of glucose and rhamnose were determined to be D and L, respectively, by GC analysis of chiral derivatives of the sugars in the acid hydrolysate (see experimental section). The $^1$H- and $^{13}$C-NMR data of **1** (Tables 1, 2) obtained from its 2D NMR spectra were almost superimposable with those of dioscoreside D,[3)] except for the presence of an additional terminal $\alpha$-L-rhamnopyranosyl moiety. The sequence of the four sugars at the C-3 position was indicated by long-range coupling ($^3J$) in the HMBC spectrum between Rha H-1 ($\delta$=6.10) and Glc I C-2 ($\delta$=78.0), Rha II H-1 ($\delta$=5.58) and Glc I C-4 ($\delta$=77.9) and Rha III H-1 ($\delta$=6.03) and Rha II C-4 ($\delta$=79.8). The linkage of Rha III at the 4-position of Rha II was confirmed by the NOESY correlation observed between the anomeric proton of Rha III at $\delta$=6.03 (s) and the H-4 of Rha II at $\delta$=4.26. The linkage of the fifth remaining sugar at the C-26 position was indicated by long-range coupling ($^3J$) in the HMBC spectrum between Glc II H-1 ($\delta$=4.75) and C-26 ($\delta$=75.6) of the aglycone. On

Table 1. $^1$H- and $^{13}$C-NMR Data[a)] of the Aglycone Part of **1**, $\delta$ in ppm (*J* in Hz)

| | $\delta_C$ | DEPT[b)] | $\delta_H$[c,d)] |
|---|---|---|---|
| 1 | 37.0 | $CH_2$ | 0.90, 1.66 |
| 2 | 31.2 | $CH_2$ | nd |
| 3 | 77.9 | CH | 3.84 |
| 4 | 38.3 | $CH_2$ | 2.60, 2.70 |
| 5 | 140.0 | C | |
| 6 | 121.5 | CH | 5.30 br s |
| 7 | 31.6 | $CH_2$ | 1.80 |
| 8 | 31.2 | CH | 1.94 |
| 9 | 49.8 | CH | 0.80 |
| 10 | 36.4 | C | |
| 11 | 20.6 | $CH_2$ | 1.30, 1.34 |
| 12 | 39.2 | $CH_2$ | 1.02, 1.64 |
| 13 | 40.4 | C | |
| 14 | 56.2 | CH | 0.95 |
| 15 | 31.9 | $CH_2$ | 1.40 |
| 16 | 72.3 | CH | 5.04 |
| 17 | 66.7 | CH | nd |
| 18 | 15.9 | $CH_3$ | 0.74 s |
| 19 | 19.0 | $CH_3$ | 0.95 s |
| 20 | 196.3 | C | |
| 21 | 31.2 | $CH_3$ | 1.98 s |
| 22 | 170.0 | C | |
| 23 | 33.9 | $CH_2$ | 2.25, nd |
| 24 | 24.7 | $CH_2$ | 1.48, nd |
| 25 | 31.6 | CH | 1.80 m |
| 26 | 75.6 | $CH_2$ | 3.96, 4.08 m |
| 27 | 16.7 | $CH_3$ | 0.94 d (6.43) |

*a*) Measured at 600 MHz for $^1$H and 150 MHz for $^{13}$C with reference to pyridine-$d_5$. *b*) Multiplicities were assigned from DEPT spectra. *c*) nd: not determined. *d*) Overlapping $^1$H-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] $\delta$ in ppm ($J$ in Hz)

| | $\delta_C$ | $\delta_H$ |
|---|---|---|
| Sugars at C-3 | | |
| Glc I | | |
| 1 | 99.7 | 4.84 d (7.4) |
| 2 | 78.0 | 4.16 |
| 3 | 77.1 | 4.04 |
| 4 | 77.9 | 4.06 |
| 5 | 77.8 | 3.86 |
| 6 | 60.8 | 3.98, 4.12 |
| T-Rha I | | |
| 1 | 101.6 | 6.10 br s |
| 2 | 71.6 | 4.68 |
| 3 | 71.6 | 4.56 |
| 4 | 73.5 | 4.20 |
| 5 | 69.2 | 4.78 |
| 6 | 18.3 | 1.65 d (6.2) |
| Rha II | | |
| 1 | 101.7 | 5.58 s |
| 2 | 72.0 | 4.35 |
| 3 | 71.0 | 4.02 |
| 4 | 79.8 | 4.26 |
| 5 | 68.1 | 4.64 |
| 6 | 18.0 | 1.46 d (6.4) |
| T-Rha III | | |
| 1 | 102.6 | 6.03 s |
| 2 | 72.0 | 4.38 |
| 3 | 71.9 | 4.44 |
| 4 | 73.5 | 4.26 |
| 5 | 69.9 | 4.19 |
| 6 | 18.3 | 1.70 d (6.0) |
| Sugars at C-26 | | |
| Glc II | | |
| 1 | 104.1 | 4.75 d (7.8) |
| 2 | 74.4 | 3.90 |
| 3 | 77.6 | 4.18 |
| 4 | 71.0 | 4.00 |
| 5 | 77.9 | 3.90 |
| 6 | 62.1 | 4.18, 4.40 |

*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.

Table 3. Antifungal Activity of **1**—**4** and Ketoconazole against *Candida* Species Given as MIC ($\mu$g/ml)[a]

| Compounds | *Candida albicans* | *Candida glabrata* | *Candida tropicalis* |
|---|---|---|---|
| **1**—**3** | >200 | >200 | >200 |
| **4** | 20.80 | 25 | 6.25 |
| Ketoconazole[b] | 1.56 | 0.78 | 0.78 |

*a*) Compounds with MIC values >200 $\mu$g/ml are considered not active. *b*) Positive control.

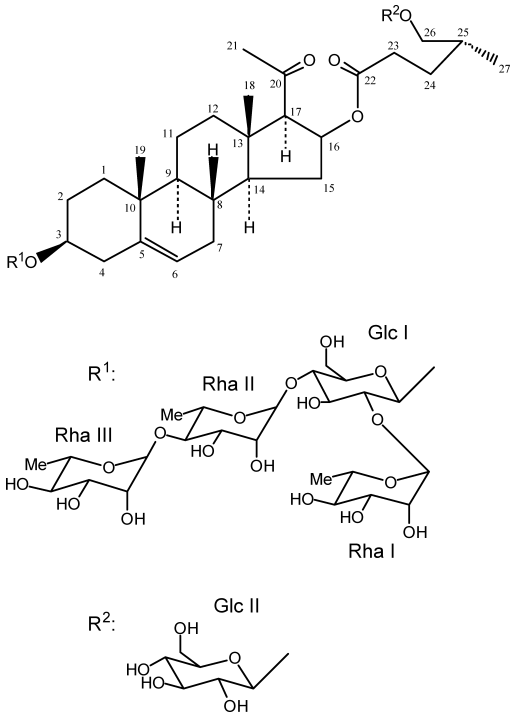


Fig. 1. Chemical Structure of **1**

the basis of the above results, compound **1** was deduced to be 26-*O*-$\beta$-D-glucopyranosyl-3$\beta$,26-dihydroxy-20,22-seco-25(*R*)-furost-5-en-20,22-dione-3-*O*-$\alpha$-L-rhamnopyranosyl-(1→4)-$\alpha$-L-rhamnopyranosyl-(1→4)-[$\alpha$-L-rhamnopyranosyl-(1→2)]-$\beta$-D-glucopyranoside (Fig. 1), a new natural compound.[4]

Compounds **2**—**4** were identified by interpretation of their spectral data, mainly FAB-MS and 2D-NMR (COSY, TOCSY, NOESY, HSQC and HMBC), as well by comparison with literature data as methyl protodioscin (**2**),[5] asperoside (**3**),[6] and prosapogenin A of dioscin (**4**),[7] respectively.

The antifungal activity of saponins **1**—**4** (Table 3) was evaluated at concentrations of less than 200 $\mu$g/ml against strains of *Candida albicans*, *C. glabrata* and *C. tropicalis*. Compound **1** presented MIC values above 200 $\mu$g/ml, and was considered inactive against the yeasts tested. Compounds **2**—**3**, having a furostan skeleton, were also devoid of activity against the tested fungi. Compound **4**, having a spirostan skeleton, presented antifungal activity against *Candida* species with MIC values between 6.25 and 25 $\mu$g/ml. Regarding the aglycone structure, we confirmed the presence of antifungal activity only with the spirostanol derivative, whereas none was observed with the furostanol derivatives.[1] This confirms that the E and F rings of diosgenin play a key role in the antifungal properties.[8]

## Experimental

**General Experimental Methods** IR, FAB-MS, 2D-NMR (COSY, TOCSY, NOESY, HSQC and HMBC), medium-pressure liquid chromatography (MPLC) instruments and GC analysis were as previously described.[9] HR-ESI-MS was carried out on a Q-TOF 1-micromass spectrometer. Optical rotations were taken with a Perkin-Elmer 881 polarimeter. TLC and HPTLC were performed on silica gel plates 60 $F_{254}$ (Merck), using the following 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 (Yaounde Province, Cameroon), and identified by the Dr. Nole Tsabang (Institut de Recherches Médicales et d'Etudes des Plantes Médicinales, IMPM). A voucher specimen (No. 14259 HNC) is deposited at the National Herbarium of Yaoundé, Cameroon.

**Extraction and Separation** Dried powdered rhizomes (800 g) of *D. cayenensis* were refluxed with MeOH–$H_2O$ (7 : 3, 6 l), and evaporated to dryness yielding 17.9 g of MeOH–$H_2O$ extract. This was partitioned successively with hexane, $CH_2Cl_2$ and *n*-BuOH (each 3×200 ml) yielding after evaporation of the solvents the corresponding hexane (2 g), $CH_2Cl_2$ (1.3 g) and *n*-BuOH (7 g) fractions. 5 g of the *n*-BuOH residue was dissolved in MeOH and purified by precipitation with diethyl ether (3×300 ml), yielding a crude saponin mixture (3.45 g). This latter was submitted to the vacuum liquid chromatography on $C_{18}$ reversed-phase (12×3 cm) using $H_2O$ (100 ml), MeOH–$H_2O$ mixtures (5 : 5; 4 : 1, each 100 ml) and finally MeOH

(100 ml). The fraction eluted with MeOH–$H_2O$ (4 : 1) was submitted to MPLC column chromatography (Si gel (15—40 $\mu$m), $CHCl_3$–MeOH–$H_2O$ (13 : 7 : 2, lower phase)), yielding 7 fractions, 1—7. Fraction 2 was concentrated to dryness to give the pure compound **1** (8 mg). Fractions 4 and 7 were rechromatographed under the same conditions to give the pure compounds **2** (10 mg) and **3** (7 mg), respectively. The fraction eluted with 100% MeOH was submitted to an additional MPLC in the same conditions to give 6 fractions, 1—6. Fraction 2 was rechromatographed by using MPLC in the same conditions to give the pure compound **4** (7 mg).

26-*O*-$\beta$-D-Glucopyranosyl-3$\beta$,26-dihydroxy-20,22-seco-25(*R*)-furost-5-en-20,22-dione-3-*O*-$\alpha$-L-rhamnopyranosyl-(1→4)-$\alpha$-L-rhamnopyranosyl-(1→4)-[$\alpha$-L-rhamnopyranosyl-(1→2)]-$\beta$-D-glucopyranoside (**1**): white amorphous powder, HR-ESI-MS (positive ion-mode) *m/z*: 1231.5697 $[M+Na]^+$, (Calcd for $C_{57}H_{92}O_{27}Na$: 1231.5724). FAB-MS (negative ion mode) *m/z*: 1207 $[M-H]^-$. $[\alpha]_D^{20}$ +80° (*c*=0.025, MeOH). IR $\nu_{max}$ ($CHCl_3$) $cm^{-1}$: 3371 (OH), 2929 (CH), 1736 (CO), 1680 and 1035. $^1$H- and $^{13}$C-NMR: see Tables 1 and 2.

**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 glucose was identified by TLC with a standard using $CHCl_3$–MeOH–$H_2O$ (8 : 5 : 1). Furthermore, a silylated derivated of the sugar was prepared according to the procedure previously described.[9] L-cysteine methyl ester hydrochloride (0.06 mol/l) and HMDS–TMCS (hexamethyldisilazane–trimethylchlorosilane, 3 : 1) were added to the aqueous residue. After centrifugation of the precipitate, the supernatant was concentrated and partitioned between *n*-hexane and $H_2O$, and the hexane layer was analyzed by GC. D-Glucose and L-rhamnose were detected.

**Antifungal Activity** Minimum inhibitory concentrations (MICs) were performed using the broth dilution test.[10] For these bioassays, three human pathogenic yeasts were used: *Candida albicans* (IP 1180-79), *C. glabrata* and *C. tropicalis* (clinical isolates). The reference compound ketoconazole (Sigma)[11] was used as a positive control.

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