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# Cholestane steroid glycosides from the root of *Dioscorea villosa* (wild yam)

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## Abstract

Phytochemical investigation of the MeOH extract of *Dioscorea villosa* root resulted in the isolation of two new bidesmosidic cholestane steroid glycosides, dioscoreavillosides A and B (**1** and **2**). In addition, the extract yielded 12 previously known furostane and spirostane steroid glycosides (**3-14**), along with diosgenin (**15**). Compounds **3-7**, **9**, **14**, and **15** were isolated for the first time from *D. villosa*. The structures of the isolated compounds were determined using spectroscopic and chemical methods including 1D and 2D NMR. The antimicrobial action of most of these compounds was tested against five fungal and five bacterial strains.

### Keywords

*Dioscorea villosa*; Dioscoreaceae; Wild yam; Cholestane steroid; Dioscoreavilloside A; Dioscoreavilloside B

## 1. Introduction

Steroid glycosides are mostly found in Dioscoreaceae, Agavaceae, Liliaceae, and Smilacaceae. Based on the aglycone moiety, steroid saponins can be classified into cholestane, spirostane, furostane, stigmastane, ergostane, and pregnane types. *Dioscorea* plants are mostly known for their spirostane and furostane steroid glycosides,[1] but a few cholestane, ergostane, and pregnane glycosides have also been reported from this plant.[2-4] The presence of clerodane diterpenes,[5] quinones,[6] cyanidins,[7] phenolics,[8] and nitrogen containing compounds[9] in *Dioscorea* have also been demonstrated. *Dioscorea*, a genus of over 600 species of flowering plants, is found in tropical and sub-tropical regions. The



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tuberous rhizomes of many *Dioscorea* species, known as “Yams”, contain starch and are therefore cultivated for food. *Dioscorea villosa* L., also known as wild yam, colicroot, rheumatism root or devil's bones, is widely distributed in North America, particularly in the central and southern regions of the United States. It contains diosgenin and diosgenin-based glycosides. Diosgenin, a phytoestrogen, is a spirostane steroid and is used as a source in synthesis of a sex hormone called progesterone. To date, six spirostane and four furostane steroid saponins,[1,10,11] along with two flavanol glycosides,[11] have been reported from *D. villosa*. In the present study, a detailed phytochemical investigation of the root of *D. villosa* was conducted to explore new chemical constituents. Fifteen steroidal compounds (Fig. 1), including two previously undiscovered metabolites, were isolated from its root. The new compounds were characterized as cholestane type steroid glycosides, dioscoreavillosides A and B (**1** and **2**). As only seven cholestane steroid glycosides have been reported from three *Dioscorea* species (*D. septemloba, D. bulbifera, and D. spongiosa*) so far,[2,4,12,13] our finding of this class of glycosides from *D. villosa* has chemotaxonomic importance. Structure elucidation of the isolated compounds was accomplished using spectroscopic and chemical data analyses including 1D and 2D NMR. Previous studies show that among C-27 steroid glycosides, spirostanes possess better antifungal activity, whereas glycosides of furostanes and cholestanes have minor to no activity.[14-16] Compounds **1-5** and **7-13** were tested for *in vitro* antimicrobial activities against five fungal strains (*Candida albicans, C. glabrata, C. krusei, Cryptococcus neoformans*, and *Aspergillus fumigatus*) (Table 2) and five bacterial strains (*Staphylococcus aureus*, MRSA, *Escherichia coli, Pseudomonas aeruginosa*, and *Mycobacterium intracellulare*).

## 2. Results and discussion

From the MeOH extract of *D. villosa* root, 15 compounds were isolated using a combination of different types of chromatography over Sephadex LH-20, silica gel, and RP-18 silica gel. The isolated compounds include mostly spirostane, furostane, and cholestane type steroid glycosides. Two new compounds, **1** and **2**, are glycosides of cholestane-type steroids. The molecular formula of compound **1** was deduced to be $C_{39}H_{66}O_{14}$ from a sodiated ion $[M + Na]^+$ at *m/z* 781.4353 in the HRESI-MS (TOF) (calcd for $C_{39}H_{66}O_{14}Na$, 781.4350). The $^{13}C$ NMR spectrum exhibited 39 resonances, of which 27 were attributed to a steroid skeleton and 12 to two sugar units. A DEPT NMR experiment was used to differentiate 39 $^{13}C$ NMR resonances as five methyl, 10 methylene, 21 methine, and three quaternary carbons. The IR spectrum showed absorption due to hydroxy groups at 3376 $cm^{-1}$. The resonances for two tertiary methyls [$\delta_H/\delta_C$ 1.32/11.0 ($CH_3$-18) and 0.86/19.4 ($CH_3$-19)], three secondary methyls [$\delta_H/\delta_C$ 1.63 (d, $J$ = 7.2 Hz)/13.8 ($CH_3$-21) and 0.85 (d, $J$ = 6.6 Hz)/23.0 ($CH_3$-26, 27)], a double bond [$\delta_H/\delta_C$ 5.25/122.0 (CH-6) and $\delta_C$ 140.9 (C-5)], four oxygenated methines [$\delta_H/\delta_C$ 3.88/78.2 (CH-3), 3.68/78.2 (CH-12), 4.46/84.8 (CH-16), and 4.37/76.1 (CH-22)] along with two β-glucopyranose moieties [$\delta_H$ 4.99 (d, $J$ = 7.7 Hz, H-1′) and 4.82 (d, $J$ = 7.8 Hz, H-1″) and $\delta_C$ 102.5 (C-1′), 107.2 (C-1″), 75.3, 75.5 (C-2′, C-2″), 78.4, 78.5, 78.9 (C-5′ and C-5″, C-3″, C-3′), 71.7 (C-4′, C-4″), and 62.8, 62.9 (C-6′, C-6″)] were observed in the $^1H$ and $^{13}C$ NMR spectra (Table 1). The $^1H$ and $^{13}C$ NMR spectroscopic data assignment in **1** (Table 1) was based on gHSQC, gHMBC (Fig. 2), and $^1H$-$^1H$ COSY spectroscopic data analyses. The C-5—C-6 double bond and oxygen

atoms at C-3, C-12, C-16, and C-22 were supported by the HMBC correlations of H-4 with C-3, C-5, C-6; H-6 with C-8, C-10; H-19 with C-5; H-18 with C-12; H-15, H-17, H-20 with C-16; H-16 with C-13; and H-17, H-21, H-23 with C-22 (Fig. 2). The HMBC correlation of anomeric proton H-1″ ($\delta_H$ 4.82) with C-16 ($\delta_C$ 84.8) revealed the location of sugar unit at C-16. Though the HMBC correlation, observed between anomeric proton H-1′ ($\delta_H$ 4.99) and C-3 ($\delta_C$ 78.2), is not clear due to close chemical schifts of C-3′ and C-5′ inside the sugar unit, but the downfield shift of C-3 from usual chemical shift (71 ppm, if OH is attached) to (78 ppm) strongly supported the sugar unit at C-3. The NMR spectroscopic data of **1** were similar to those of dioseptemloside I,[13] except for the fact that the resonances for an additional β-glucopyranose unit were present in **1**. The absolute configuration of **1** was found to be similar to that of dioseptemloside I based on their similar NMR data.[13] Furthermore, the β-orientation of OH-12 and OH-16 was also supported from the NOESY correlations of H-12 with biogenetically α-oriented H-9, H-14, and H-17 and of H-16 with H-14 and H-17. The *S*-configuration at C-22 was inferred by comparing its NMR data with those of the *S* and *R* stereoisomers.[13,17] The sugars were identified as glucose by co-TLC (EtOAc-$CHCl_3$-MeOH-$H_2O$, 6:4:4:1) of standard sugars from Sigma-Aldrich with the sugar mixture obtained via acid hydrolysis of **1**. Both glucose units were found to be β based on the characteristic coupling constant values of their anomeric protons ($J$ = 7.7 Hz (H-1′) and $J$ = 7.8 Hz (H-1″)) in the $^1$H NMR spectrum. The absolute configuration of glucose was determined to be D (see experimental). Accordingly, the structure of dioscoreavilloside A (**1**) was established as (22*S*)-3β,16β-di-(*O*-β-D-glucopyranosyl)-12β,22-dihydroxycholest-5-ene.

The HRESI-MS (TOF) of **2** yielded a sodiated molecular ion $[M + Na]^+$ at *m/z* 779.4190, which in conjunction with the $^{13}$C NMR data, indicated a molecular formula of $C_{39}H_{64}O_{14}$. The IR spectrum of **2** indicated hydroxyl and carbonyl functions due to absorptions at 3386 and 1699 $cm^{-1}$, respectively. The $^1$H and $^{13}$C NMR data assignment (Table 1) in the usual manner indicated that compound **2** was also comprised of the cholestane-type steroid with two sugar units. The NMR data of **2** were found to be close to those of **1** except that the resonances for C-12 oxygenated methine ($\delta_H/\delta_C$ 3.68/78.2) and C-22(*S*) were replaced by those of an oxo group ($\delta_C$ 214.6) and C-22(*R*), respectively in **2**. The oxo function was also supported by the IR spectrum (1699 $cm^{-1}$) and the mass difference of two units between **1** and **2**. The C-12 position of the oxo group was evident from the HMBC correlations of H-11 and H-18 with C-12 ($\delta_C$ 214.6) (Fig. 2). The oxo instead of β-hydroxy group at C-12 resulted in significant downfield carbon chemical shifts for C-9, C-11, C-13, C-14, and C-18 and upfield shift for C-17 (Table 1). Challinor et al.,[17] revised the absolute configurations of bethosides B and C at C-22 by preparing 22-*R* and 22-*S* analogues. Based on their $^{13}$C NMR resonances ($\delta_C$ 73.4 for 22-*R* and $\delta_C$ 75.5 for 22-*S*), the configuration at C-22 was assigned as *R* in **2**.[17] The sugars and their positions in **2** were found to be similar to those of **1**. The $^1$H and $^{13}$C NMR spectroscopic data assignment in **2** (Table 1) was based on gHSQC, gHMBC (Fig. 2), and $^1$H-$^1$H COSY spectroscopic data analyses. Thus, the structure of dioscoreavilloside B (**2**) was elucidated as (22*R*)-3β,16β-di-(*O*-β-D-glucopyranosyl)-22-hydroxycholest-5-en-12-one.

The name, huangjiangsu A, for compound **5** has been assigned to two different compounds in literature. It was first reported in *D. zingiberensis* by Bai and Sun in 2006 without NMR data.[18] In 2009, Wang et al., had reported it as (25*R*)-26-*O*-{β-D-glucopyranosyl}-furost-5,20(22)-dienyl-3-*O*-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopynosyl-(1→2)]-β-D-galactopyranoside with a galactose unit instead of glucose.[19] In the current study, the structure of **5** was elucidated with glucose unit as reported by Bai and Sun[18] instead of galactose, despite the similarity of its NMR spectroscopic data with those reported by Wang et al.[19] The sugars were identified as glucose and rhamnose by co-TLC (EtOAc-$CHCl_3$-MeOH-$H_2O$, 6:4:4:1) of standard sugars from Sigma-Aldrich with the sugar mixture obtained via acid hydrolysis of **5**. The absolute configurations of glucose and rhamnose were determined to be D and L, respectively (see experimental).

25(*R*)-Dracaenoside G (**14**) was isolated and identified for the first as a single isomer with a 25*R* configuration at C-25 similar to that found in compounds **10**-**13**. Previously, this compound was reported in mixture form as 25(*R*,*S*)-dracaenoside G.[20]

Other known compounds were identified as 26-*O*-β-D-glucopyranosyl-3β,26-diol-25(*R*)-furost-5,20(22)-dien-3-*O*-α-L-rhamnopyranosyl(1→2)-*O*-β-D-glucopyranoside (**3**), [21] pseudoprotodioscin (**4**),[22] protobioside (**6**),[23,24] methyl protobioside (**7**),[24,25] protodioscin (**8**),[26] protodeltonin (**9**),[27] progenin III (**10**),[10] dioscin (**11**),[10] deltonin (**12**),[10] zingiberensis saponin I (**13**),[10] and diosgenin (**15**).

In accordance to the previous report, spirostane glycosides **11** and **12** showed antifungal activity against *C. albicans* and *C. glabrata* (Table 2).[14] Antifungal activities of **10** and **13** against *C. glabrata* and **12** and **13** against *C. neoformans* were also observed. The antifungal activity results of three types of C-27 steroid glycosides (Table 2), in compliance to the previous reports,[14-16] showed that spirostane glycosides have better activity than furostane and cholestane types. It is also concluded that the antifungal activity in a respective class of steroid glycosides depends on the types and sequence of the sugars. None of the compounds showed antibacterial activity up to the test concentration of 20 μg/mL.

## 3. Experimental

### 3.1. General methods

Specific rotations were measured at ambient temperature using a Rudolph Research Analytical Autopol IV automatic polarimeter. IR spectra were acquired on a Bruker Tensor 27 spectrophotometer. NMR spectra were recorded on a Varian Unity Inova 600 MHz NMR spectrometer, whereas HRESI-MS (TOF) data were obtained on an Agilent Series 1100 SL mass spectrometer. TLC was carried out on aluminum-backed plates pre-coated with silica gel $F_{254}$ (20 × 20 cm, 200 μm, 60 Å, Merck). Visualization was accomplished by spraying with 5% vanillin (Sigma) solution in conc. $H_2SO_4$-95% EtOH (5:95) followed by heating. Chromatography was performed using silica gel (40 μm for flash chromatography, 60 Å, J. T. Baker), reversed-phase RP-C18 silica (Polarbond, JT Baker), and Sephadex LH-20 (Sigma). HPLC was carried out on a Waters Alliance 2695, equipped with a 996 photodiode array detector (Waters Corp., Milford, MA), with Waters Empower-2 software. A Luna C-18 column (150 × 4.6 mm, 5 μm particle size, Phenomenex Inc., Torrance, CA) was

protected with a 2 cm LC-18 guard column (Phenomenex Inc.). The solvents (Fisher brand) used for HPLC and other chromatographic procedures were of HPLC and certified grades, respectively. All organisms were obtained from the American Type Culture Collection (Manassas, VA). Drug controls [Ciprofloxacin (99.3%) for bacteria and Amphotericin B (92%) for fungi] were obtained from ICN Biomedicals, Aurora, OH.

### 3.2. Plant material

Root powder of *D. villosa* was purchased from Starwest Botanicals, USA in April, 2011. Authenticity of the plant material was confirmed by comparison of the TLC of its MeOH extract with that of a limited quantity of an authentic plant material grown at the Medicinal Botanical Garden (MBG) at the University of Mississippi. The plant material obtained from the MBG was identified by Dr. Aruna Weerasooriya of the University of Mississippi. A voucher specimen (No. 9800) and a sample specimen of the purchased material (No. 9412) were deposited at the National Center for Natural Products Research, University of Mississippi.

### 3.3. Extraction and isolation

Root powder (0.9 kg) was extracted with MeOH (3.0 L × 4 × 24 h) at room temperature. Following the removal of the solvent, a gummy residue (75 g) was obtained. An aliquot (55 g) was subjected to size exclusion chromatography using Sephadex LH-20 (17 × 10 cm) and eluted with MeOH to furnish 2 aliquots, A (7.0 L, 45.2 g) and B (5.0 L, 9.4 g). Part A (30 g) was separated by normal phase column chromatography (NPCC) using silica gel (30 × 10 cm) into 12 fractions (A1-A12) with lower layer of $CHCl_3$-MeOH-$H_2O$ (13:7:2) [A1 (0.5 L, 782 mg), A2 (1.0 L, 730 mg), A3 (0.5 L, 304 mg), A4 (0.5 L, 767 mg), A5 (0.5 L, 490 mg), A6 (0.5 L, 1.8 g), A7 (1.0 L, 657 mg), A8 (1.0 L, 2.8 g), A9 (1.0 L, 1.9 g), A10 (1.5 L, 4.4 g), A11 (1.5 L, 4.5 g), and A12 (1.5 L, 5.9 g)]. Fractions A3 and A4 were individually subjected to NPCC [silica gel (87 × 1.3 cm), EtOAc-$CHCl_3$-MeOH-$H_2O$ (10:6:4:1), 1.0 L] to yield compounds **10** (375 mg) and **11** (126 mg). Fraction A5 yielded seven subfractions (A5a-A5g) by NPCC [silica gel (87 × 1.3 cm), EtOAc-$CHCl_3$-MeOH-$H_2O$ (10:6:4:1), 1.5 L]. Compounds **1** (34 mg) and **2** (38 mg) were purified from subfraction A5g (112 mg) by reversed phase chromatography (RPC) [RP-18 silica gel (45 × 1.3 cm), acetone-$H_2O$ (2:3), 0.5 L], and **14** (3 mg) was obtained from subfraction A5e (26 mg) by RPC [RP-18 silica gel (45 × 1.3 cm), MeOH-$H_2O$ (9:1), 0.3 L]. Compounds **12** (785 mg) and **13** (90 mg) were obtained from fractions A6 and A8, respectively, as MeOH insoluble materials. Compounds **3** (32 mg) and **7** (325 mg) were purified from the MeOH soluble part of fraction A8 by NPCC [silica gel (87 × 2.5 cm), $CHCl_3$-MeOH-$H_2O$ (32:8:1), 4.5 L] and RPC [RP-18 silica gel (37 × 2.5 cm), acetone-$H_2O$ (1:1), 0.5 L]. Fraction A9 was resolved by NPCC [silica gel (37 × 2.5 cm), lower layer of $CHCl_3$-MeOH-$H_2O$ (13:7:2), 2.7 L] into three subfractions (A9a-A9c). Compound **6** (22 mg) was obtained from subfraction A9a (113 mg) by RPC [RP-18 silica gel (37 × 1.3 cm), acetone-$H_2O$ (1:1), 0.5 L]. Compounds **4** (45 mg) and **8** (212 mg) were purified from subfraction A9c (665 mg) by RPC [RP-18 silica gel (45 × 2.5 cm), acetone-$H_2O$ (2:3), 0.7 L]. Compounds **5** (646 mg) and **9** (1.3 g) were purified from fraction A11 by RPC [RP-18 silica gel (50 × 3.7 cm), acetone-$H_2O$ (2:3), 2.5 L]. Part B (5 g) was fractionated into seven fractions (B1-B7) by CC over silica gel (45 × 7.5 cm) and eluted with $CHCl_3$-MeOH gradients [B1 (1:0, 2.0 L, 117 mg), B2 (1:0, 2.0 L, 22 mg), B3

(1:0, 2.0 L, 48 mg), B4 (19:1, 4.0 L, 1.1 g), B5 (9:1), 3.0 L, 618 mg), B6 (4:1), 4.0 L, 594 mg), and B7 (0:1), 2.0 L, 1.7 g)]. Fraction B4 was subjected to RPC [RP-18 silica gel (50 × 1.7 cm), MeOH-$H_2O$ (9:1, 0.5 L), (1:0, 1.0 L) and acetone (0.5 L)] to give 11 subfractions (B4a-B4k). Compound **15** (11 mg) was found as a pure substance in subfraction B4e.

### 3.4. Identification of compounds

**3.4.1. Dioscoreavilloside A (1)—**Colorless solid; $[\alpha]^{22}_D$ – 34.0 (*c* 0.1, MeOH); IR $\nu_{max}$ (NaCl): 3376 (OH), 2943, 1649, 1367, 1075, 1024 $cm^{-1}$; $^1H$ NMR (600 MHz, $C_5D_5N$) and $^{13}C$ NMR (150 MHz, $C_5D_5N$) spectroscopic data: see Table 1; HRESI-MS: *m/z* 781.4353 $[M + Na]^+$ (calcd for $C_{39}H_{66}O_{14}Na$, 781.4350).

**3.4.2. Dioscoreavilloside B (2)—**Colorless solid; $[\alpha]^{22}_D$ – 14.0 (*c* 0.1, MeOH); IR $\nu_{max}$ (NaCl): 3386 (OH), 2939, 1699 (C=O), 1456, 1370, 1073, 1029 $cm^{-1}$; $^1H$ NMR (600 MHz, $C_5D_5N$) and $^{13}C$ NMR (150 MHz, $C_5D_5N$) spectroscopic data: see Table 1; HRESI-MS: *m/z* 779.4190 $[M + Na]^+$ (calcd for $C_{39}H_{64}O_{14}Na$, 779.4194).

**3.4.3. Huangjiangsu A (5)—**$^{13}C$ NMR ($C_5D_5N$, 150 MHz) spectroscopic data: $\delta_C$ (ppm) 37.8 (C-1), 30.4 (C-2), 78.7 (C-3), 39.2 (C-4), 141.0 (C-5), 122.1 (C-6), 32.7 (C-7), 31.7 (C-8), 50.6 (C-9), 37.4 (C-10), 21.5 (C-11), 39.9 (C-12), 43.7 (C-13), 55.2 (C-14), 34.7 (C-15), 84.8 (C-16), 64.8 (C-17), 14.4 (C-18), 19.7 (C-19), 103.9 (C-20), 12.1 (C-21), 152.6 (C-22), 24.0 (C-23), 31.7 (C-24), 33.8 (C-25), 75.2 (C-26), 17.6 (C-27), 100.2 (C-1′), 78.5 (C-2′), 77.6 (C-3′), 82.2 (C-4′), 76.4 (C-5′), 62.1 (C-6′), 102.1 (C-1″), 72.7 (C-2″), 73.0 (C-3″), 74.3 (C-4″), 69.8 (C-5″), 18.9 (C-6″), 105.4 (C-1‴), 75.2 (C-2‴), 78.0 (C-3‴), 71.5 (C-4‴), 78.4 (C-5‴), 62.3 (C-6‴), 105.1 (C-1⁗), 75.5 (C-2⁗), 78.8 (C-3⁗), 72.0 (C-4⁗), 78.9 (C-5⁗), 63.1(C-6⁗); $^1H$ NMR ($C_5D_5N$, 600 MHz) spectroscopic data: $\delta_H$ (ppm) 1.73, 0.98 (H-1a/b), 2.11 (H-2a/b), 3.86 (m, H-3), 2.75, 2.71 (H-4a/b), 5.31 (br. s H-6), 1.95, 1.40 (H-7a/b), 1.48 (H-8), 0.89 (H-9), 1.48 (H-11a/b), 1.74, 1.16 (H-12a/b), 0.90 (H-14), 2.11, 1.50 (H-15a/b), 4.80 (m, H-16), 2.45 (d, *J* = 10.8 Hz, H-17), 0.72 (s, H-18), 1.02 (s, H-19), 1.64 (s, H-21), 2.22 (H-23a/b), 1.80 (H-24a/b), 1.96 (H-25), 4.03, 3.61 (H-26a/b), 1.01 (d, *J* = 6.6 Hz, H-27), 4.95 (d, *J* = 72 Hz, H-1′), 4.44-3.85 (H-2′/3′/3‴/5‴/3⁗/5⁗), 4.23 (H-4′), 3.86 (H-5′), 4.58-4.29 (H-6a′/6b′/6a‴/6b‴/6a⁗/6b⁗), 6.24 (s, H-1″), 4.75 (s, H-2″), 4.58 (H-3″), 4.35 (H-4″), 4.94 (H-5″), 1.76 (d, *J* = 6.0 Hz, H-6″), 5.13 (d, *J* = 7.2 Hz, H-1‴), 4.06, 4.02 (H-2‴/2⁗), 4.26 (H-4‴/4⁗), 4.84 (d, *J* = 7.2 Hz, H-1⁗).

### 3.5. Sugar analyses

Compound **1** (3 mg) was dissolved in 1 mL of 2 N HCl in dioxane-$H_2O$ (1:1) and heated at 95 °C for 3 h. The mixture was diluted with $H_2O$ (1 mL) on cooling, then neutralized with $NH_4OH$ and extracted with EtOAc (2 × 2 mL). The residue obtained after drying the aqueous layer was dissolved in pyridine (1 mL) and 0.1 M cysteine methyl ester hydrochloride in pyridine (1 mL) was added. The reaction mixture was heated at 60 °C for 1h. An equal volume of phenyl isothiocyanate in pyridine (10 mg/mL) was added and heated at 60 °C for 1h. The mixture was filtered and analyzed by reversed-phase HPLC. Acetonitrile (ACN) with 0.1% HOAc (A) and $H_2O$ with 0.1% HOAc (B) were used as the mobile phase at a flow rate of 1 mL/min with the following gradient: 10 % A for 20 min and

55 % A for 25 min. The chromatographic peaks were detected at 250 nm. The process was repeated for compounds **2** and **5**. The standard sugar (Sigma-Aldrich) derivatives were prepared identically and analyzed by HPLC under similar conditions. A pair of isomers (major & minor) was detected in each case. D-Glucose in compounds **1** and **2** and D-glucose and L-rhamnose in compound **5** were identified by comparing the retention times of their derivatives with those of authentic sugar samples [D-glucose: 12.6 min (minor)/15.3 min (major), L-glucose: 13.1 min (minor)/15.2 min (major), and L-rhamnose: 14.4 min (minor)/ 17.4 min (major)].

### 3.6. Assays for *in vitro* antimicrobial activity

Assays were performed as described earlier.[14]

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Highlights

► Phytochemical investigation of the MeOH extract of *Dioscorea villosa* root. ► Fifteen compounds including two new bidesmosidic cholestane steroid glycosides. ► Structures elucidation by spectroscopic and chemical methods. ► Antimicrobial action of the isolated compounds.

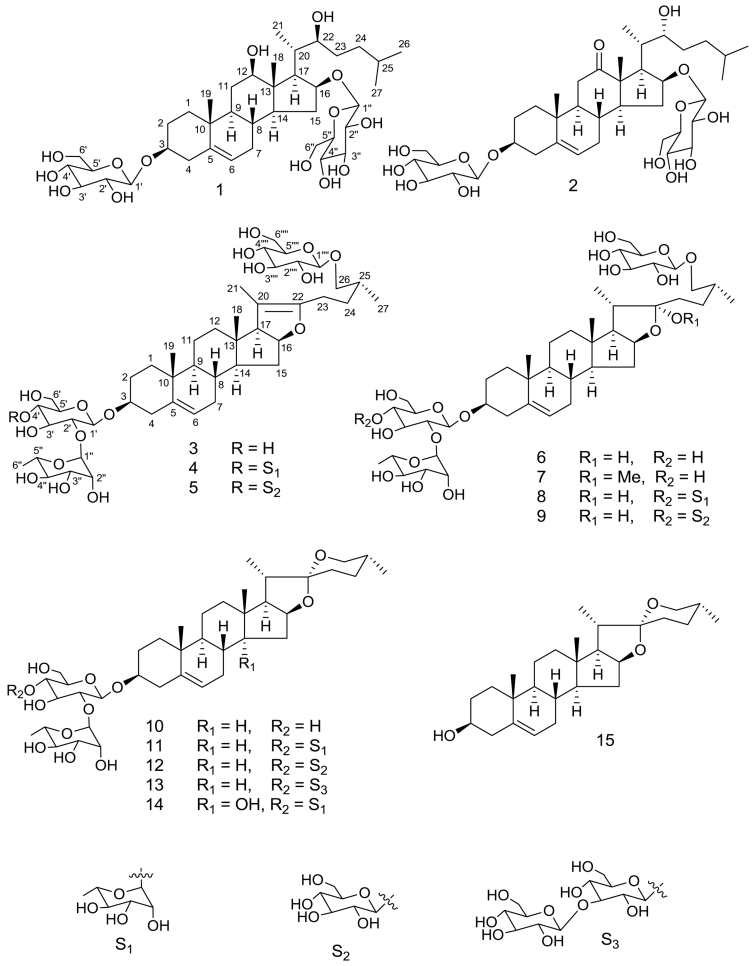


**Fig. 1.**
The structures of compounds **1**–**15**.

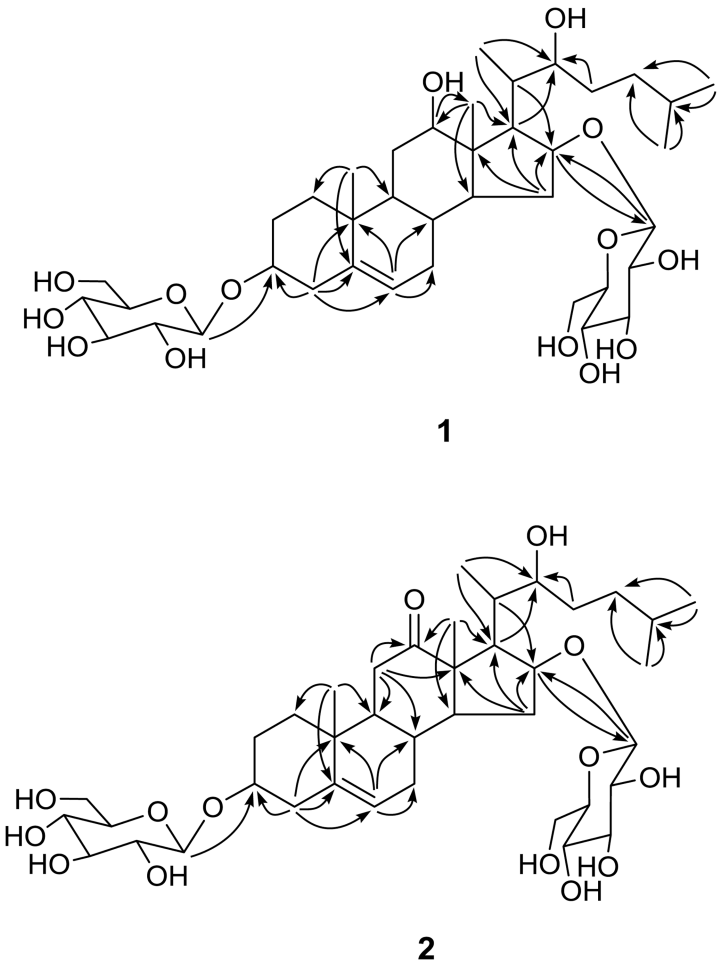


**Fig. 2.**
Key HMBC correlations of **1** and **2**.

## Table 1

$^{1}H$ and $^{13}C$ NMR spectroscopic data in $C_5D_5N$ for compounds **1** and **2**.

| Position | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H{}^a$ (HMQC) | $\delta_C$ | $\delta_H{}^a$ (HMQC) |
| 1 | 37.5 | 1.76, 0.91 m | 37.2 | 1.45 m, 0.80 td (13.0, 4.3) |
| 2 | 30.2 | 2.03 m, 1.73 | 30.1 | 2.02 m, 1.63 |
| 3 | 78.2 | 3.90 m | 78.0 | 3.88 m |
| 4 | 39.3 | 2.68 dd (13.0, 3.0), 2.40 t (13.0) | 39.1 | 2.66 dd (12.8, 5.4), 2.37 |
| 5 | 140.9 | | 140.8 | |
| 6 | 122.0 | 5.25 br. d (4.7) | 121.9 | 5.19 br. d (4.0) |
| 7 | 31.8 | 1.74, 1.39 | 31.7 | 1.67, 1.26 |
| 8 | 30.6 | 1.28 m | 32.1 | 1.54 m |
| 9 | 49.9 | 0.99 td (12.2, 6.1) | 54.7 | 1.26 td (12.0, 4.8) |
| 10 | 37.0 | | 38.0 | |
| 11 | 31.0 | 1.85, 1.73 | 38.3 | 2.65 t (12.2), 2.16 dd (12.2, 5.4) |
| 12 | 78.2 | 3.68 dt (9.3, 4.5) | 214.6 | |
| 13 | 48.9 | | 57.4 | |
| 14 | 53.9 | 0.79 td (12.2, 6.0) | 57.3 | 1.11 td (12.0, 7.7) |
| 15 | 36.0 | 2.36 dt (13.7, 7.2), 1.92 td (13.7, 4.0) | 37.2 | 2.37, 1.91 td (13.0, 3.6) |
| 16 | 84.8 | 4.46 br. td 7.5, 4.0) | 81.8 | 4.48 br. td (6.5, 3.4) |
| 17 | 63.0 | 1.80 dd (12.9, 11.1) | 49.4 | 2.97 dd (11.0, 7.7) |
| 18 | 11.0 | 1.32 s | 13.5 | 1.26 s |
| 19 | 19.4 | 0.86 s | 19.1 | 0.92 s |
| 20 | 38.1 | 2.81 m | 35.4 | 2.45 m |
| 21 | 13.8 | 1.63 d (7.2) | 13.2 | 1.20 d (6.9) |
| 22 | 76.1 | 4.37 | 73.3 | 4.29 |
| 23 | 35.2 | 1.83, 1.72 | 34.0 | 1.82 |
| 24 | 36.5 | 1.67, 1.47 | 36.9 | 1.91, 1.61 |
| 25 | 28.6 | 1.54 m | 29.0 | 1.57 m |
| 26 | 23.0 | 0.85 d (6.6) | 23.2 | 0.88 d (6.1) |
| 27 | 23.0 | 0.85 d (6.6) | 23.3 | 0.88 d (6.1) |
| 3-Glc | | | | |
| 1′ | 102.5 | 4.99 d (7.7) | 102.6 | 4.96 d (7.2) |
| 2′ | 75.3[b] | 4.01 t like (8.9) | 75.3[b] | 3.99 t like (9.0) |
| 3′ | 78.9[c] | 4.18 t (9.0) | 78.7[c] | 4.13 t (9.0) |
| 4′ | 71.7[d] | 4.19 t (9.0) | 71.7[d] | 4.18 t (9.0) |
| 5′ | 78.4 | 3.90 | 78.2[e] | 3.83 |
| 6′ | 62.8[e] | 4.36, 4.52 | 62.9 | 4.34, 4.48 |
| 16-Glc | | | | |
| 1″ | 107.2 | 4.82 d (7.8) | 107.0 | 4.71 d (7.5) |

| Position | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$[a] (HMQC) | $\delta_C$ | $\delta_H$[a] (HMQC) |
| 2″ | 75.5[b] | 4.01 t like (8.9) | 75.5[b] | 3.99 t like (9.0.) |
| 3″ | 78.5[c] | 4.27 t (8.9) | 78.6[c] | 4.27 t (9.0) |
| 4″ | 71.7[d] | 4.19 t (8.9) | 71.8[d] | 4.22 t (9.0) |
| 5″ | 78.4 | 3.88 | 78.4[e] | 3.90 |
| 6″ | 62.9[e] | 4.36, 4.52 | 62.9 | 4.34, 4.50 |

[a] multiplicity is not clear for some resonances due to overlapping, chemical shifts are in ppm, J in parenthesis are in hertz.

[b,c,d,e] Interchangeable within the column.

**Table 2**

**Antifungal activity of compounds 1-5 and 7-13**

| Compound[a] | *C. albicans* $IC_{50}$ (μg/mL) | *C. glabrata* $IC_{50}$ (μg/mL) | *C. krusei* $IC_{50}$ (μg/mL) | *A. fumigatus* $IC_{50}$ (μg/mL) | *C. neoformans* $IC_{50}$ (μg/mL) |
|---|---|---|---|---|---|
| 1 | NA | NA | NA | NA | NA |
| 2 | NA | NA | NA | NA | NA |
| 3 | NA | NA | NA | NA | NA |
| 4 | NA | NA | NA | NA | NA |
| 5 | NA | NA | NA | NA | NA |
| 7 | NA | NA | NA | NA | NA |
| 8 | NA | NA | NA | NA | NA |
| 9 | NA | NA | NA | NA | NA |
| 10 | NA | 15.99 | NA | NA | NA |
| 11 | 3.97 | 3.26 | NA | NA | NA |
| 12 | 15.24 | 7.35 | NA | NA | 13.17 |
| 13 | NA | 3.09 | NA | NA | 9.70 |
| Amphotericin B | 0.14 | 0.20 | 0.73 | 1.18 | 0.35 |

[a] purity of compounds is 92% to 99%

NA = Not active up to the highest concentration of 20 μg/mL.