ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 11:5 (2017) 421-430

records of natural products

Cytotoxicity of Secondary Metabolites from *Dracaena viridiflora* Engl & Krause and their Semisynthetic Analogues

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(Received March 04, 2017; Revised May 04, 2017; Accepted May 12, 2017)

Abstract: The MeOH extract of *Dracaena viridiflora* was found to display significant cytotoxicity against some cancer cell lines. Systematic phytochemical investigation of this extract led to the isolation and structure elucidation of ten secondary metabolites including five spirostane (**1**-**5**) and one furostane (**6**) steroidal saponins. Furthermore, some acetylated spirostane analogues and three previously unreported derivatives with the 22,26-epoxycholesta-5,22-diene skeleton (**15**-**17**) were prepared from trillin (**1**), prosapogenin A of dioscin (**2**) and dioscin (**4**) by reaction with $ZnCl_2/Ac_2O$. Among the isolated and semisynthetic compounds, dioscin showed the most potent cytotoxicity against A549, Jurkat and Skov-3 cells with IC_{50} values of 0.42, 1.70 and 1.90 µg/mL, respectively. It was noteworthy that acetylation of the bioactive compounds led to semisynthetic derivatives which unfortunately did not present any activity. This is the first report on the phytochemical and pharmacological investigation of *Dracaena viridiflora.*

Keywords: *Dracaena viridiflora*; steroidal saponins; epoxycholesta-5,22-dienes; cytotoxicity.

1. Introduction

Dracaena viridiflora Engl & Krause is a member of the family Agavaceae which comprises more than 480 species distributed in tropical and subtropical regions of the world [1]. Although there are no scientific reports on the phytochemical and biological studies of this plant, its leaves are used in the West Region of Cameroon for the treatment of microbial infections, epilepsy and convulsions. In our continuous search of potentially bioactive steroidal glycosides from Cameroonian medicinal plants

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The article was published by ACG Publications
www.acgpubs.org/RNP © Published 06/13/2017 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.54.17.03.050

[2-5], we have investigated the MeOH extract from the leaves of *D. viridiflora*, leading to the isolation and structure elucidation of ten secondary metabolites including trillin **1**, prosapogenin A of dioscin **2**, prosapogenin B of dioscin **3**, dioscin **4**, gracillin **5**, methylprotodioscin **6**, cylicodiscoside **7**, stigmasterol **8**, stigmasterol 3-*O*-β-D-glucopyranoside **9**, and allantoin **10**. Since steroids have been a rich source of agents with potential pharmaceutical applications that have inspired the synthesis of new analogues with promising pharmacological activities [6], some isolated compounds were acetylated and three previously undescribed 22,26-epoxycholesta-5,22-diene derivatives **15-17** were prepared by rearrangement/acetylation in the presence of $ZnCl_2/Ac_2O$ (Figure 1). The structures of the isolated and semisynthetic compounds were determined mainly by extensive spectroscopic analysis (1D and 2D NMR) and HRESI-MS, as well as by comparison of their spectral data with those reported in the literature. Among the compounds biologically evaluated, dioscin (**4**) proved to be more active against the human lung carcinoma epithelial cells A549 than doxorubicin used as positive control (Table 2).

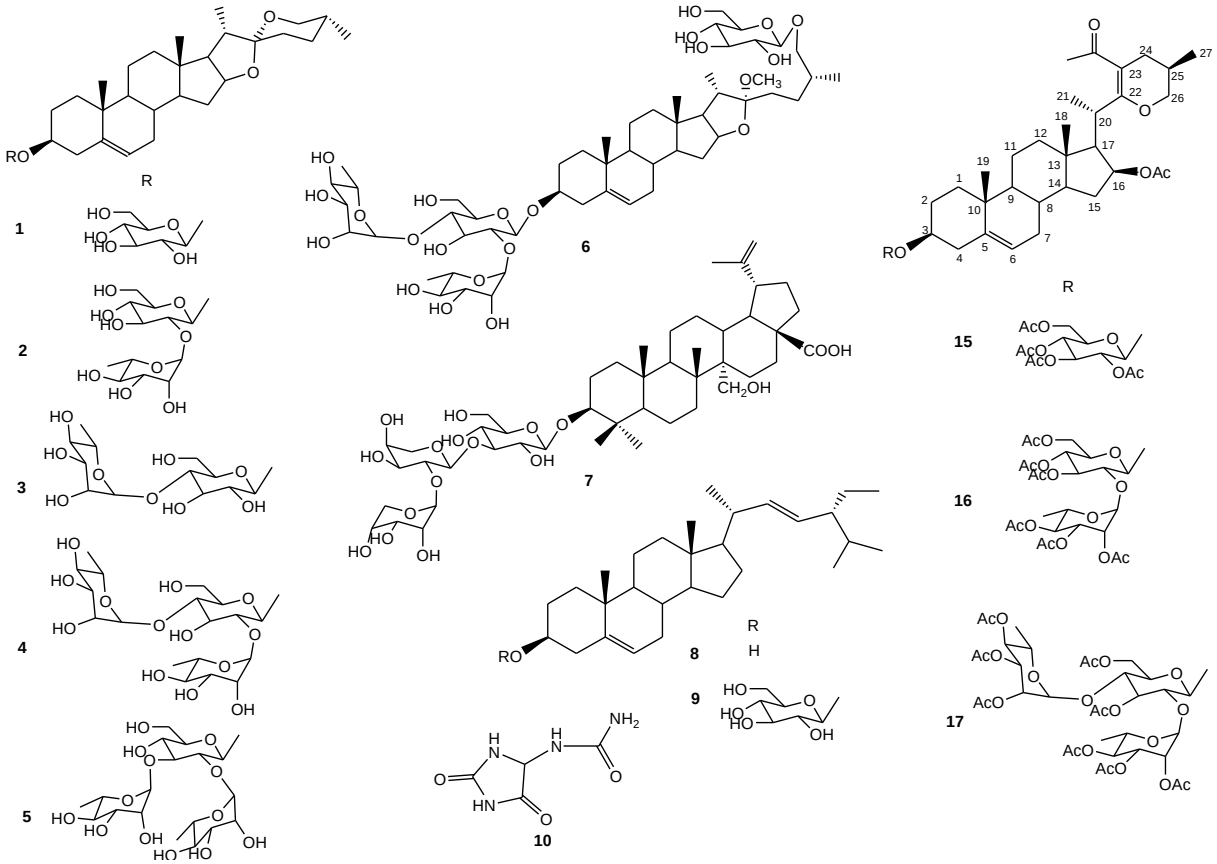


Figure 1. Structures of isolated compounds and some semisynthetic derivatives

2. Materials and Methods

2.1. General Experimental Procedures

Optical rotations were measured using the sodium D-line on a Perkin-Elmer 241 MC polarimeter at 25 ^{o}C. The IR absorption spectra were measured on a Bruker Tensor 27 FT-IR using a diamond ATR. Positive ion mode HRESI mass spectra were carried out on an Agilent 6320 Ion Trap Instrument. ^{1}H and ^{13}C NMR, COSY, HSQC, HMBC and NOESY spectra were recorded in deuterated MeOH, chloroform, pyridine and DMSO on a Bruker AVANCE III-600 MHz Spectrometer equipped

with a 5 mm cryogenic TCI-probe head using standard gradient-selected pulse sequences. Column chromatography was performed using sephadex LH-20 and silica gel (Fluka analytical silica gel 90 C-18 reversed phase for column chromatography and Macherey-Nagel Kieselgel 60 M 0.04–0.063 mm/230–400 mesh ASTM for column chromatography). The following solvent systems were used: MeOH for Sephadex column chromatography, MeOH-H_2O (1:1 and 6:4) for reversed phase column chromatography, Petroleum ether-Ethyl acetate 1:1 and EtOAc-MeOH-H_2O (80:20:10, 85:15:10, 90:10:5, 95:5:2 and 99:1:0) for normal phase column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (Merck) plates developed with EtOAc-MeOH-H_2O (80:20:10, 90:10:5 and 95:5:2). Substance bands were visualised after spraying with 10% H_2SO_4 followed by warming or directly on a 254 nm UV lamp for fluorescent spots.

2.2 Plant Material

The leaves of *D. viridiflora* were collected in Bangoua (West Region of Cameroon) in July 2012 and authenticated at the Cameroon National Herbarium, Yaoundé, where a voucher specimen is deposited (50698HNC).

2.3 Extraction and isolation

The dried and pulverized leaves of *D. viridiflora* (1.5 kg) were extracted with MeOH (2x8 L) for 24 hours. The filtrate obtained was concentrated under reduced pressure to yield a dark residue (170 g). A part of this extract (162 g) was submitted to silica gel (63–200 μm) column chromatography (CC), using a gradient of EtOAc in *n*-hexane, then with EtOAc-MeOH (with increasing amounts of MeOH) and five main fractions A-E were obtained. Fractions A (20.8 g) (eluted with hexane to hexane-EtOAc 7:3) and B (24.3 g) (with eluted hexane-EtOAc 6:4 to hexane-EtOAc 4:6) were shown to contain mainly oily compounds and chlorophylls, respectively. Fraction C (3.9 g) (eluted with hexane-EtOAc 3:7) was chromatographed using silica gel (32–63 μm) with petroleum ether-EtOAc (1:1) as eluent to afford stigmasterol **8** (150 mg). Fraction D (31.1 g) (EtOAc to EtOAc-MeOH 8:2) was purified by CC over silica gel (32–63 μm) using EtOAc-MeOH-H_2O (95:5:2) as eluent to yield four subfractions D_1-D_4. Subfraction D_1 (1.5 g) was chromatographed on a silica gel column eluted with AcOEt-MeOH (99:1) to afford trillin **1** (290 mg), allantoin **10** (45 mg) and stigmasterol 3-*O*-β-D-glycopyranoside **9** (250 mg). Subfraction D_2 (8.5 g) was repeatedly subjected to CC using EtOAc-MeOH-H_2O (95:5:2) as eluent to yield prosapogenin A of dioscin **2** (185 mg) and prosapogenin B of dioscin **3** (25 mg). Recrystallization of subfraction D_3 (3.5 g) gave a mixture (2 g) of dioscin and gracillin. Recristallization of subfraction D_4 (0.5 g) gave pure dioscin **4** (75 mg). Fraction E (EtOAc-MeOH 7:3 to EtOAc-MeOH 1:1) (26.5 g) was separated on a silica gel CC using EtOAc-MeOH-H_2O (8:2:1) to yield two subsfraction E_1 and E_2. Subfraction E1 (10.7 g) was a complex mixture and subfraction E_2 (1.2 g) consisted of a mixture of very polar compounds. E_2 was submitted to RP-18 CC eluted with MeOH-H_2O 1:1 to afford gracillin **5** (13 mg), cylicodiscoside **7** (75 mg) and methylprotodioscin **6** (180 mg).

2.4 Semisynthesis

2.4.1 Acetylation

Trillin (30 mg, 0.052 mmol) was dissolved in pyridine (1.5 mL), Ac_2O (1.5 mL) was added and the mixture was stirred at room temperature overnight. Excess pyridine and Ac_2O was removed by adding toluene and the mixture was concentrated to dryness under reduced pressure. The reaction

Table 1. ^{13}C and ^{1}H NMR data (150 and 600 MHz, resp.) of compounds **15** and **16** in $CDCl_3$.

	15		**16**	
Position	**δ_C**	**δ_H**	**δ_C**	**δ_H**
1	37.3	1.04 (m), 1.84 (m)	37.1	1.08 (m), 1.86 (m)
2	29.6	1.59 (m), 1.90 (m)	29.7	1.56 (m), 1.96 (m)
3	80.1	3.48 (m)	79.1	3.61 (m)
4	39.0	2.18 (m), 2.25 (m)	38.3	2.29 (m), 2.43 (m)
5	140.6	/	140.1	/
6	122.0	5.34 (brd, 5.1)	122.1	5.37 (brd, 5.1)
7	31.9	1.94 (o), 2.19 (o)	31.7	1.96 (o), 2.34 (o)
8	31.5	1.52 (o)	31.4	1.53 (o)
9	50.3	0.93 (m)	50.1	0.95 (o)
10	36.9	/	36.7	/
11	20.9	1.52 (o)	20.9	1.52 (o)
12	40.0	1.23 (m), 2.00 (m)	39.8	1.25 (m), 2.01 (m)
13	42.4	/	42.3	/
14	54.5	0.97 (m)	54.3	0.98 (m)
15	35.1	1.04 (m), 2.29 (m)	34.9	1.03 (m), 2.30 (m)
16	75.3	5.13 (m)	75.1	5.14 (td, 7.9, 4.5)
17	56.1	1.81 (o)	55.9	1.80 (o)
18	13.1	0.91 (s)	13.0	0.91 (s)
19	19.6	0.99 (s)	19.3	1.01 (s)
20	33.1	4.06 (m)	32.9	4.07(o)
21	19.6	1.18 (d, 6.9)	19.5	1.18 (d, 7.0)
22	171.5	/	171.4	/
23	107.1	/	106.9	/
24	31.8	2.23 (o), 2.44 (o)	31.7	2.24 (m), 2.43 (o)
25	26.7	1.89 (m)	26.6	1.90 (m)
26	71.7	3.45 (t, 10.2), 4.00 (ddd, 10.2, 2.3, 1.2)	71.6	3.45 (t, 10.1), 4.00 (m)
27	17.0	0.97 (d, 6.4)	16.9	0.97 (d, 6.4)
23-$COCH_3$	198.4, 30.0	2.20 (s)	198.3, 29.8	2.20 (s)
16-$OCOCH_3$	170.9, 21.4	1.85 (s)	170.7, 21.2	1.85 (s)
1'	99.8	4.59 (d, 8.0)	99.4	4.58 (d, 7.8)
2'	71.6	4.96 (dd, 8.0, 9.5)	75.3	3.72 (dd, 9.5, 7.8)
3'	73.0	5.20 (dd, 9.5, 9.8)	74.8	5.24 (o)
4'	68.6	5.08 (t, 9.8)	68.8	4.96 (t, 10.0)
5'	71.8	3.67 (m)	71.6	3.68 (ddd, 10.0, 4.9, 2.5)
6'	62.2	4.11 (dd, 12.2, 2.4), 4.25 (dd, 12.2, 4.8)	62.2	4.07 (o), 4.27 (dd, 12.2, 4.9)
2'-$OCOCH_3$	169.5, 20.9	2.05 (s)	/	/
3'-$OCOCH_3$	170.6, 20.8	2.00 (s)	170.3, 20.8	2.06 (s)
4'-$OCOCH_3$	169.6, 20.9	2.02 (s)	169.7, 20.9	1.99 (s)
6'-$OCOCH_3$	170.9, 21.0	2.08 (s)	170.7, 21.0	2.07 (s)
1"			97.3	4.96 (d, 2.0)
2"			70.2	4.99 (dd, 3.4, 1.8)
3"			68.6	5.23 (o)
4"			71.2	5.07 (t, 10.0)
5"			66.4	4.39 (m)
6"			17.3	1.19 (d, 6.3)
2"-$OCOCH_3$			169.9, 20.8	2.00 (s)
3"-$OCOCH_3$			170.0, 20.7	2.02 (s)
4"-$OCOCH_3$			170.1, 21.0	2.12 (s)

o: overlapped signals

mixture was purified by chromatography on a silica gel column eluted with petroleum ether-EtOAc (7:3) to give trillin tetraacetate (35 mg, 93%). Prosapogenin A of dioscin hexaacetate (38 mg, 94%)

was prepared from 30 mg of prosapogenin A of dioscin following the same procedure. Furthermore, a mixture of dioscin and gracillin (1.1 g) was acetylated to yield after purification dioscin octaacetate (400 mg), gracillin octaacetate (282 mg) and a mixture of both compounds (420 mg).

2.4.2 Reaction with $ZnCl_2/Ac_2O$

To a suspension of trillin tetraacetate (10 mg, 0.0134 mmol) in 2 mL of Ac_2O was added 2 mg (0.0147 mmol) of anhydrous $ZnCl_2$. The reaction mixture was stirred for 20 h at room temperature and quenched with ice. The organic phase was extracted with ethyl acetate, neutralized with a saturated $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to yield a powder which was chromatographed on a silica gel column eluted with petroleum ether-EtOAc (7:4) to give 8 mg of compound **15** (72%). Prosapogenin A of dioscin hexaacetate (20 mg, 0.020 mmol) and dioscin octaacetate (30 mg, 0.025 mmol) were submitted in the same reaction conditions to give 15 mg of compound (**16**) (69%) and 26 mg of compound **17** (81%), respectively.

*23-acetyl-16β-acetoxy-22,26-epoxycholesta-5,22-dien-3β-yl-O-(2,3,4,6-tetraacetyl)-O-β-D-glucopyranoside (**15**)*: White amorphous powder; $[\alpha]_D^{25}$ – 8.5 (*c* = 0.17, $CHCl_3$); IRν_{max} (ATR): 2939, 1757, 1664, 1568, 1222 cm^{-1}; ^{13}C and ^{1}H NMR data: see Table 1; HRESI-MS: *m/z* 829.4368 $[M+H]^+$ (Calcd for $C_{45}H_{65}O_{14}$: 829.4369).

*23-acetyl-16β-acetoxy-22,26-epoxycholesta-5,22-dien-3β-yl-O-[(2,3,4-triacetyl)-O-α-L-rhamnopyranosyl-(1→2)-O-(3,4,6-triacetyl)]-O-β-D-glucopyranoside **(16)***: White amorphous powder; $[\alpha]_D^{25}$ – 10.2 (*c* = 1.11, $CHCl_3$); IRν_{max} (ATR): 2939, 1749, 1667, 1575, 1224 cm^{-1}; ^{13}C and ^{1}H NMR data: see Table 1; HRESI-MS: *m/z* 1081.4969 $[M+Na]^+$ (Calcd for $C_{55}H_{78}O_{20}Na$: 1081.4979).

*23-acetyl-16β-acetoxy-22,26-epoxycholesta-5,22-dien-3β-yl-O-[(2,3,4-triacetyl)-O-α-L-rhamnopyranosyl-(1→2)-O-(2,3,4-triacetyl)-O-α-L-rhamnopyranosyl-(1→4)]-O-(3,6-diacetyl)-O-β-D-glucopyranoside (**17**)*: White amorphous powder; $[\alpha]_D^{25}$ – 24.3 (*c* = 1.00, $CHCl_3$). IRν_{max} (ATR): 2936, 1750, 1667, 1575, 1221 cm^{-1}; ^{13}C and ^{1}H NMR data: see Table 2; HRESI-MS: *m/z* 1289.5986 $[M+Na]^+$ (Calcd for $C_{65}H_{93}O_{26}Na$: 1289.5950).

2.5 Cytotoxic activity

2.5.1 Cell lines and culture conditions

The effect of compounds on cell growth was evaluated against human cancer cells including lung adenocarcinoma A549, human epithelial colorectal adenocarcinoma cells Caco-2, human ovarian carcinoma Skov-3 and human T-Cell leukemia cells JURKAT. SKOV-3 was cultured in IMDM (Iscove's Modified Dulbecco's Medium), Caco-2 and A549 were cultured on DMEM (Dulbecco's Modified Eagle Medium) high glucose with sodium pyruvate while JURKAT was maintained on RPMI medium containing β-mercaptoethanol 0.05mM. All media were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/fungizone (PSF) solution. The cell lines were cultured at 37°C in a humidified environment containing 5 % CO_2.

2.5.2 MTT assay

In vitro cytotoxicity test against above mentioned cell lines was performed by the [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] MTT assay. Briefly, cells were harvested in log phase using trypsin (0.05% trypsin, 0.02% EDTA, in PBS). The cell suspensions were diluted with appropriate growth medium to obtain the cell density of 10^4 cells/well. Aliquots of 100 µL of each suspension were seeded in 96 wells cell culture plates. The cells were incubated at 37°C in an

atmosphere of 5% CO_2 and 95% relative humidity in a CO_2 incubator. After 24 h of incubation, test materials (100 µL/well) at varying concentrations were added to the wells containing cells. Doxorubicin was used as positive control. Suitable controls with equivalent concentration of DMSO were also included. The plates were further incubated for 48h, then the medium in each well was aspirated and MTT solution (2 mg/mL in PBS) was diluted 1:10 with fresh medium and added to each well and the plates were incubated at 37°C for 4h. The medium was aspirated from the wells and DMSO was added to solubilize the formed formazan crystals. The absorbance was measured on FLUOstar OPTIMA microplate reader at 570 nm. The concentration causing 50% inhibition of cell growth (IC_{50}) was calculated from the concentration-inhibition response curve by regression analysis.

3. Results and Discussion

3.1. Structure elucidation

Phytochemical investigation of the MeOH extract from the leaves of *Dracaena viridiflora*, led to the isolation of trillin **1** [7], prosapogenin A of dioscin **2** [8], prosapogenin B of dioscin **3** [9], dioscin **4** [8], gracillin **5** [10], methylprotodioscin **6** [10], cylicodiscoside **7** [11]. Stigmasterol **8** [12], stigmasterol 3-*O*-glucopyranoside **9** [12], and allantoin **10** [13]. Furthermore, trillin tetraacetate **11** [14], prosapogenin A of dioscin hexaacetate **12** [15], dioscin octaacetate **13** [14], gracillin octaacetate **14** [14] were prepared and three previously undescribed 22,26-epoxycholesta-5,22-diene derivatives **15-17** were synthesized by rearrangement/acetylation of trillin tetraacetate, prosapogenin A of dioscin hexaacetate and dioscin octaacetate, respectively in the presence of $ZnCl_2/Ac_2O$.

Compound **15** was obtained as a white amorphous powder from petroleum ether-EtOAc (7:4). Its molecular formula $C_{45}H_{64}O_{14}$ was determined from the HRESI-MS which showed the pseudomolecular ion peak at *m/z* 829.4368 $[M+H]^+$. The presence of acetyl and conjugated carbonyl groups was deduced from the IR absorption bands at 1757 and 1664 cm^{-1}, respectively. The ^1H NMR spectrum of **15** showed signals for four steroid methyl groups at δ 1.18 (d, J = 6.9 Hz, H-21), 0.99 (s, H-19), 0.97 (d, J = 6.4 Hz, H-27) and 0.91 (s, H-18), an acetyl methyl protons singlet at δ 2.20 (s, Ac-23) as well as an olefinic proton at δ 5.34 (brd, J = 5.1 Hz, H-6). It also exhibited signals for two oxymethyne protons at δ 3.48 (m, H-3) and 5.13 (m, H-16), the methylene protons at δ 4.00 (ddd, J = 10.2, 2.3, 1.2, H-26a) and 3.45 (t, J = 10.2, H-26b). The above proton signals together with distinctive carbon signals at δ 198.4 (Ac-23), 171.5 (C-22), 140.6 (C-5), 122.0 (C-6), 107.1 (C-23), 80.1 (C-3), 75.3 (C-16) and 71.7 (C-26) indicated that the aglycone part was of the 22,26-epoxycholesta-5,22-diene type [6, 16]. The sugar part of the ^1H NMR spectrum showed the anomeric proton signal at δ 4.59 (d, J = 8.0 Hz, H-1′) which gave HSQC correlation with carbon at δ 99.8 (C-1') and some deshielded proton signals at δ 4.96 (dd, J = 8.0, 9.5 Hz, H-2′), 5.20 (dd, J = 9.5, 9.8 Hz, H-3′) and 5.08 (t, J = 9.8 HZ, H-4′) due to acetylation shifts [17]. In the ^1H NMR spectrum of compound **15**, the presence of acetoxy groups was indicated by methyl singlets at δ 2.08 (OAc-6′), 2.05 (OAc-2′), 2.02 (OAc-4′), 2.00 (OAc-3′), 2.08 (OAc-6′), 1.85 (OAc-16), whereas in the ^{13}C NMR spectrum, the signals of ester carbonyls at δ 170.9 (OAc-16 and OAc-6′), 170.6 (OAc-3′), 169.6 (OAc-4′), and 169.5 (OAc-2′) were observed. Intensive interpretation of the ^1H and ^{13}C NMR, ^1H-^1H COSY, HSQC and HMBC spectra (Table 1) and comparison with published data led to the elucidation of **15** as 23-acetyl-16β-acetoxy-22,26-epoxycholesta-5,22-dien-3β-yl-*O*-(2,3,4,6-tetraacetyl)-*O*-β-D-glucopyranoside.

Compounds **16** and **17** were also isolated as white amorphous powders. The ^1H and ^{13}C NMR data of their aglycone parts (Tables 1 and 2) were very similar to those of **15**, indicating that they derived from the same rearrangement. Their HRESI-MS spectra exhibited pseudomolecular ion peaks at *m/z* 1081.4969 and 1289.5986 $[M+Na]^+$ corresponding to the molecular formulas $C_{55}H_{78}O_{20}$ and $C_{65}H_{93}O_{26}$, respectively. Their structures were established as 23-acetyl-16β-acetoxy-22,26-epoxycholesta-5,22-dien-3β-yl-*O*-[(2,3,4-triacetyl)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-(3,4,6-triacetyl)]-*O*-β-D-glucopyranoside (**16**) and 23-acetyl-16β-acetoxy-22,26-epoxycholesta-5,22-dien-3β-yl-*O*-[(2,3,4-triacetyl)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-(2,3,4-triacetyl)-*O*-α-L-rhamnopyranosyl-

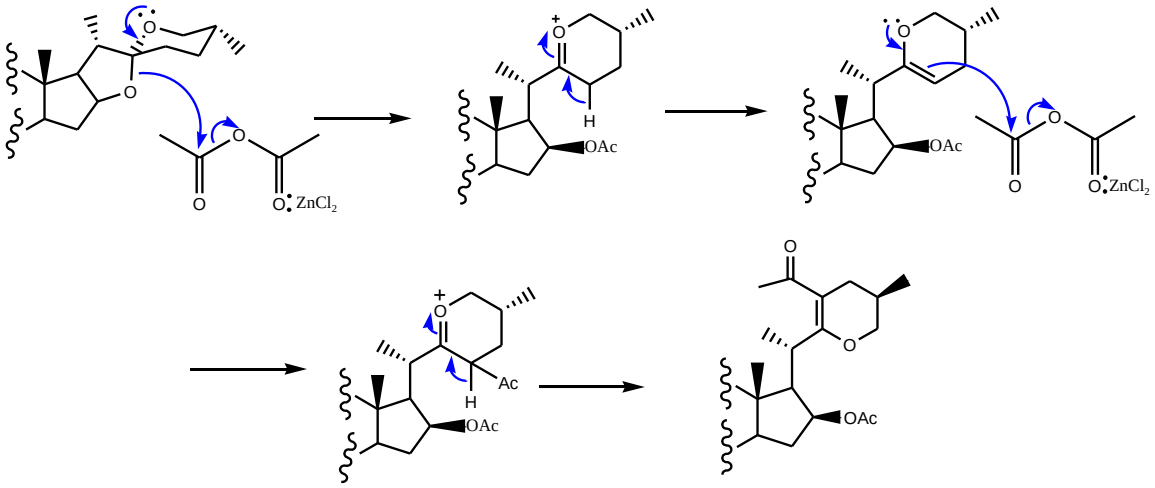
(1→4)]-*O*-(3,6-diacetyl)-*O*-β-D-glucopyranoside (**17**) from careful examination of the ^{1}H and ^{13}C NMR, HSQC, HMBC and ^{1}H–^{1}H COSY spectra. The plausible mechanism of the rearrangement/acetylation of spirostane steroids in the presence of $ZnCl_2/Ac_2O$ (Scheme 1) was proposed Sandoval-Ramírez *et al*. [18].

Table 2. ^{13}C and ^{1}H NMR data (150 and 600 MHz, resp.) of compound **17** in $CDCl_3$.

Position	δ_C	δ_H	Position	δ_C	δ_H
1	37.3	1.09 (m), 1.85 (m)	1'	99.7	4.56 (d, 7.8)
2	29.7	1.56 (m), 1.96 (m)	2'	76.4	3.57 (t, 7.8)
3	79.2	3.60 (o)	3'	75.5	5.28 (t, 9.4)
4	38.4	2.27 (m), 2.43 (m)	4'	77.8	3.73 (t, 9.5)
5	140.2	/	5'	72.4	3.60 (o)
6	122.2	5.36 (brd, 5.0)	6'	62.4	4.29 (dd, 12.3, 3.8), 4.44 (dd, 12.3, 1.5)
7	31.8	1.95 (o), 2.26 (o)	2'-$OCOCH_3$	/	/
8	31.5	1.52 (o)	3'-$OCOCH_3$	170.4, 21.1	2.14 (s)
9	50.2	0.96 (o)	4'-$OCOCH_3$	/	/
10	36.9	/	6'-$OCOCH_3$	170.9, 21.0	2.09 (s)
11	21.0	1.52 (o)	1"	97.4	4.90 (d, 1.7)
12	39.9	1.25 (o), 1.99 (o)	2"	70.4	5.00 (o)
13	42.4	/	3"	68.7	5.23 (dd, 10.0, 3.4)
14	54.5	0.98 (m)	4"	71.4	5.05 (o)
15	35.1	1.04 (m), 2.29 (m)	5"	66.6	4.37 (m)
16	75.3	5.14 (m)	6"	17.5	1.16 (d, 6.1)
17	56.1	1.80 (o)	2"-$OCOCH_3$	170.2, 21.0	2.01 (s)
18	13.1	0.90 (s)	3"-$OCOCH_3$	169.9, 20.9	1.98 (s)
19	19.4	1.00 (s)	4"-$OCOCH_3$	170.2, 21.1	2.13 (s)
20	33.1	4.07(m)	1"'	99.7	4.79 (d, 1.9)
21	19.6	1.17 (d, 7.0)	2"'	70.2	5.01 (o)
22	171.6	/	3"'	68.7	5.16 (dd, 10.0, 3.5)
23	107.1	/	4"'	70.7	5.00 (o)
24	31.9	2.24 (m), 2.42 (o)	5"'	68.1	3.84 (m)
25	26.7	1.90 (m)	6"'	17.3	1.15 (d, 6.2)
26	71.7	3.45 (t, 10.1), 4.00 (m)	2"'-$OCOCH_3$	170.2, 21.0	2.04 (s)
27	17.1	0.96 (d, 6.4)	3"'-$OCOCH_3$	170.3, 20.9	1.98 (s)
23-$COCH_3$	198.4, 29.8	2.19 (s)	4"'-$OCOCH_3$	170.2, 21.1	2.13 (s)
16-$OCOCH_3$	170.9, 21.5	1.84 (s)			

3.2 Cytotoxicity

The isolated compounds and the crude MeOH extract were screened for their cytotoxic effect against Jurkat, CaCo2, Skov-3, and A549 cancer cell lines using MTT assay. Their median inhibitory concentrations (IC_{50}) are listed in Table 3. The crude extract showed significant activity with IC_{50} values ranged between 11.76 µg/mL and 23.69 µg/mL. Out of the eleven compounds tested, three secondary metabolites (dioscin **4**, prosapogenin A of dioscin **2** and methylprotodioscin **8**) showed strong to moderate cytotoxic activity (IC_{50} values varying from 0.42 µg/mL and 16.13 µg/mL) while three others compounds (trillin **1**, trillin tetraacetate **11** and prosapogenin B of dioscin **3**) exhibited moderate to weak activity (with IC_{50} values ranged from 13.72 µg/mL and 93.46 µg/mL) against all the four cell lines used. Dioscin showed the most potent cytotoxicity against A549, Jurkat and Skov-3 with respective IC_{50} values of 0.42, 1.70 and 1.90 µg/mL.



Scheme 1. Plausible mechanism for the formation of compounds **15**-**17** [18].

Regarding the structure-activity relationships of the investigated compounds, some features that might influence the cytotoxic activity can be drawn when comparing their chemical structures. The most active compound, dioscin **4**, contains a 2,4-di-*O*-rhamnopyranosyl glucopyranoside unit linked to C-3. In comparison with prosapogenin A of dioscin **2** (containing a 2-*O*-rhamnopyranosyl glucopyranoside unit) and prosapogenin B of dioscin **3** (containing a 4-*O*-rhamnopyranosyl glucopyranoside unit), the 2,4-disubstitution pattern of the glucopyranosyl unit linked to C-3 turned to be essential for the cytotoxicity. Morever, the absence of a rhamnopyranosyl unit at C-4 of the glucopyranosyl unit linked to C-3 of the aglycone considerably reduces the activity. Besides, this activity is reduced when the spirostane skeleton turns to furostane with a glucopyranosyl unit at C-26 when comparing **4** and methylprotodioscin **6**. This observation is in agreement with several previous studies which reported that the biological activity of saponins is influenced both by the aglycone and the sugar moiety [19]. Except for trillin tetraacetate **11**, all the acetylated compounds were not active, even the new 22,26-epoxycholesta-5,22-diene derivatives.

4. Conclusion

Phytochemical investigation of the MeOH extract from the leaves of *D. viridiflora* led to the isolation and structure elucidation of ten secondary metabolites **1**-**10**. Furthermore, three previously unreported 22,26-epoxycholesta-5,22-diene derivatives were prepared by $ZnCl_2$ catalysed rearrangement/acetylation reaction. Although the semisynthetic derivatives showed not activity, the significant cytotoxicity exhibited by the methanol extract of this medicinal plant could be attributed to the presence of dioscin which proved to be more active against the human lung carcinoma epithelial cells A549 than doxorubicin used as positive control. To the best of our knowledge, this is the first report on the phytochemical and pharmacological investigation of *D. viridiflora* as well as the preparation of 22,26-epoxycholesta-5,22-diene derivatives from trillin, prosapogenin A of dioscin and dioscin. In addition, cylicodiscoside is reported here for the first time from a plant of the Agavaceae family.

Table 3. Cytotoxicity of extract, compounds and semisynthetic derivatives from *D. viridiflora* against human cancer cells lines.

Compounds	**IC_{50} (µg/mL)**			
	Jurkat	**CaCo2**	**Skov-3**	**A549**
MeOH Extract	11.76±0.88[a]	19.88±0.44[a]	16.08±1.13[a]	23.69±0.57[a]
1	22.36±1.40[c]	36.49±2.14[c]	64.78±1.91[c]	14.14±0.10[c]
2	2.06±0.12[b]	2.51±0.32[b]	5.69±0.88[e]	2.11±0.54[e]
3	21.74±1.80[c]	13.72±0.84[e]	62.33±1.42[c]	42.44±1.60[f]
4	1.70±0.38[b]	2.58±0.21[b]	1.90±0.86[b]	0.42±0.15[b]
6	4.82±0.33[e]	16.13±0.34[f]	7.07±0.39[f]	5.26±0.29[g]
7	>100	>100	>100	>100
11	20.32±1.18[c,d]	42.75±5.52[d]	93.46±3.59[d]	18.48±0.71[d]
12	>100	>100	>100	>100
13	>100	>100	>100	>100
14	>100	>100	>100	>100
17	>100	>100	>100	>100
Doxorubicin	0.61±0.04[f]	2.32±1.04[b]	0.84±0.08[g]	1.15±0.84[b]

Data represent the mean ± SD of three independent experiments; values with different letters are significantly different at $p < 0.05$, according to the Fisher's least significant difference (LSD) analysis. In bold are values of significant activity

Acknowledgments

This work was supported by the Rhineland-Palatinate Natural Products Research Center as well as by TWAS-DFG through the granting of a cooperation visit at the Institute of Organic Chemistry, Johannes Gutenberg University Mainz, Germany to R.B. Teponno. J.P. Dzoyem is grateful to the Philipps-Universität Marburg for a 3 months grant and to the German Academic Exchange Service (DAAD) for another 3 months research stay grant at Philipps-Universität Marburg. We thank Dr. Johannes C. Liermann as well as Dr. N. Hanold (deceased) of Johannes Gutenberg University for recording NMR and mass spectra.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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