Natural Product Sciences
27(2) : 122-127 (2021)
https://doi.org/10.20307/nps.2021.27.2.122

Steroidal Saponins from *Dracaena humilis* (Dracaenaceae) and their Chemotaxonomic Significance

Cédric Mbiesset Mouzié[1], Beaudelaire Kemvoufo Ponou[1], Romuald Tematio Fouedjou[1], Rémy Bertrand Teponno[1,*], and Léon Azefack Tapondjou[1,*]

[1]*Department of Chemistry, Faculty of Science, University of Dschang, Box 67, Dschang, Cameroon*

Abstract – A new steroidal saponin, (23*S*,24*S*)-spirosta-5,25(27)-diene-1*β*,3*β*,23,24-tetrol 1-*O*-((2,3-diacetyl-α-L-rhamnopyranosyl)-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*α*-L-arabinopyranoside)-24-*O*-*β*-D-glucopyranoside (humilisoside) together with the known *β*-sitosterol 3-*O*-glucopyranoside, adenosine, dioscin, and methylprotodioscin were isolated from the leaves of *Dracaena humilis*. Their structures were elucidated by spectral techniques including mass spectrometry (ESIMS, HRESIMS, tandem MS-MS), 1D NMR (^{1}H, ^{13}C NMR), 2D NMR (HSQC, ^{1}H-^{1}H COSY, HMBC, NOESY), chemical method as well as by comparison with spectroscopic data reported in the literature. The chemotaxonomic significance of the isolation of these compounds is discussed. This is the first report on the phytochemical investigation of *D. humilis*.
Keywords – *Dracaena humilis*, Dracaenaceae, Steroidal saponins

Introduction

The Dracaenaceae family includes perennial plants with a more or less woody trunk of the genera *Dracaena* and *Sansevieria*, which occur in subtropical and tropical regions of the Old World.[1] Although Dracaenaceae are mainly ornamental plants, some of them are used in traditional medicine. It is the case of *D. humilis* whose leaves are used by the native people of the western highlands of Cameroon for the treatment of typhoid fever, rheumatism, and ulcers. A literature survey showed that previous phytochemical investigations on plants of the *Dracaena* genus led to the isolation of phenolic compounds[2,3] and steroidal saponins.[4-7] Steroidal saponins consist of a C_{27} skeleton, typically an oxidised cholesterol derivative, bearing varying numbers of sugar residues at different positions, reported to possess a large spectrum of biological activities including cytotoxic, anti-inflammatory, haemolytic, anti-fungal, and antimicrobial properties.[8] In our continuing search of bioactive compounds from medicinal plants growing in Cameroonian,[7,9-11] we have examined the chemical constituents of *D. humilis* and we herein report the isolation and structure elucidation of five secondary metabolites including a new steroidal saponin from the leaves of this plant.

*Author for correspondence
Rémy Bertrand Teponno, Department of Chemistry, Faculty of Science, University of Dschang, Box 67, Dschang, Cameroon.
Tel: +237-677312743; E-mail: remyteponno@gmail.com

Léon Azefack Tapondjou, Department of Chemistry, Faculty of Science, University of Dschang, Box 67, Dschang, Cameroon.
Tel: +237-675004826; E-mail: tapondjou2001@yahoo.fr

Experimental

General experimental procedures – Optical rotations were measured on Perkin Elmer 241 polarimeter at 25 ºC. Positive ion mode ESI mass spectra were carried out on an Agilent 6320 Ion Trap Instrument. HRESIMS was carried out with a Q-ToF ULTIMA-III quadrupole TOF mass spectrometer (Waters, Esch-Born, Germany). ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, and NOESY spectra were recorded in deuterated MeOH, Pyridine, and DMSO on a Bruker AVANCE III-600 MHz Spectrometer equipped with a 5 mm cryogenic probe head using standard gradient-selected pulse sequences. All chemical shifts (*δ*) are given in ppm units with reference to tetramethylsilane (TMS) as internal standard and the coupling constants (*J*) are in Hz. Column chromatography was performed using sephadex LH-20 (SIGMA life science Sephadex LH-20) and silica gel (Fluka analytical silica gel 90 C-18 reversed phase for column chromatography and MN 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, Hex-EtOAc, EtOAc, 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 were 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). TLC plates were visualised after spraying with 10% H_2SO_4 followed by warming or directly on a KW 245 nm UV lamp for fluorescent spots.

Plant material – The leaves of *D. humilis* were collected in Bafou village near the city of Dschang (West region of Cameroon) in September 2012. The plant was identified at the Cameroon National Herbarium, Yaoundé, where a voucher specimen (Ref: 2865/SRFK) was deposited.

Extraction and isolation – The dried and pulverized leaves (1.5 Kg) were extracted three times (each time for 24 hours) with MeOH. The filtrate obtained was concentrated under reduced pressure to yield a dark residue (149 g) which was fractionated on a silica gel column chromatography using a gradient of EtOAc in *n*-hexane, then a gradient of MeOH in EtOAc to give six main fractions (A-F). Fraction F (25.1 g) (eluted with EtOAc-MeOH 10% to 40%), the saponins rich one was submitted to silica gel column chromatography using EtOAc-MeOH-H_2O (85-15-10) as eluent, yielding three main subfractions (I-III). β-sitosterol 3-*O*-glucopyranoside (**4**) (105 mg) was obtained from subfraction I (2.5 g) by recrystallization in MeOH followed by simple filtration. Subfraction II (8.7 g) was repeatedly chromatographed on Sephadex LH20 column eluted with MeOH to yield adenosine (**5**) (100 mg) and a mixture containing mainly two compounds which was further purified on a silica gel column using EtOAc-MeOH-H_2O (90-10-5) as eluent to afford dioscin (**3**) (110 mg). Subfraction III 3.4 g was first chromatographed on Sephadex column eluted with MeOH, then on a silica gel column eluted with EtOAc-MeOH-H_2O (85-15-10) to yield methylprotodioscin (**2**) (10 mg) and a complex mixture. The above mixture was repeatedly purified on a RP-18 column chromatography eluted with MeOH-H_2O (1-1) to afford compound **1** (1.5 mg).

Humilisoside [(23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β,23,24-tetrol 1-*O*-((2,3-diacetyl-α-L-rhamnopyranosyl)-(1→2)-[β-*D*-xylopyranosyl-(1→3)]-α-L-arabinopyranoside)-24-*O*-β-*D*-glucopyranoside] (1) – White gum from methanol. $[\alpha]^{25}_D$ -34.4° (*c* = 0.13, CH_3OH); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1; ESIMS: *m/z* (%) = 1139 (14), 587 (60), 576 (87), 547 (85), 288 (100); HR-ESIMS: *m/z* = 1139.4893 $[M+Na]^+$ (Calcd. for $C_{52}H_{80}O_{25}Na^+$: 1139.4886).

Methylprotodioscin (2) – White powder. ^{1}H-NMR (CD_3OD, 600 MHz) δ_H: 5.36 (1H, m, H-6), 4.35 (1H, td, *J* = 8.1, 6.2 Hz, H-16), 3.71 (1H, o, 3.1 Hz, H-26a), 3.57 (1H, m, H-3), 3.35 (1H, o, H-26b), 3.11 (3H, s, *O*-Me), 2.42 (1H, dd, *J* = 4.7, 2.1 Hz, H-4a), 2.27 (1H, m, H-4b), 2.15 (1H, q, *J* = 6.9 Hz, H-20), 1.97 (m, H-7a), 1.94 (1H, dd, *J* = 6.1, 1.5 Hz, H-15b), 1.88 (1H, m, H-2a), 1.86 (1H, m, H-1a), 1.75 (1H, m, H-12a) 1.72 (1H, m, H-25), 1.70 (m, H-17), 1.63 (1H, m, H-8), 1.59 (1H, m, H-2b), 1.58 (1H, m, H-23b), 1.53 (1H, o, H-7b), 1.52 (1H, o, H-11), 1.43 (1H, m, H-24), 1.26 (1H, dd, *J* = 6.1, 1.6 Hz, H-15a), 1.16 (1H, d, *J* = 7 Hz, H-12b), 1.12 (1H, m, H-23a), 1.11 (1H, m, H-14), 1.05 (1H, m, H-1b), 1.02 (3H, s, H-19), 0.98 (3H, d, *J* = 7 Hz, H-21), 0.94 (1H, m, H-9), 0.92 (3H, d, *J* = 6.8 Hz, H-27), 0.81 (3H, s, H-18) for aglycone; 4.48 (1H, d, *J* = 7.9 Hz, H-1'), 3.38 (1H, o, H-2'), 3.29 (1H, o, H-3'), 3.49 (1H, o, H-4'), 3.56 (1H, o, H-5'), 3.77 (1H, o, H-6a'), 3.62 (1H, o, H-6b') for 3-*O*-glucose; 4.81 (1H, d, *J* = 1.8 Hz, H-1''), 3.80 (1H, dd, *J* = 3.3, 1.8 Hz, H-2'') 3.61 (1H, o, H-3''), 3.37 (1H, o, H-4''), 3.90 (1H, m, H-5''), 1.23 (3H, d, *J* = 6.3 Hz, H-6'') for 4'-*O*-rhamnose; 5.18 (1H, d, *J* = 1.7 Hz, H-1'''), 3.89 (1H, o, H-2'''), 3.63 (1H, o, H-3'''), 3.37 (1H, o, H-4'''), 4.10 (1H, m, H-5'''), 1.21 (3H, d, *J* = 6.3 Hz, H-6''') for 2'-*O*-rhamnose; 4.21 (1H, d, *J* = 7.8, 1.4 Hz, H-1''''), 3.16 (1H, brd, *J* = 1.3 Hz, H-2''''), 3.32 (1H, o, H-3''''), 3.25 (1H, o, H-4''''), 3.23 (1H, m, H-5''''), 3.83 (1H, dd, *J* = 11.9, 2.0 Hz, H-6a''''), 3.62 (1H, o, H-6b'''') for 26-*O*-glucose; ^{13}C-NMR (CD_3OD, 150 MHz) δ_C: 142.0 (C-5), 122.7 (C-6), 114.1 (C-22), 82.5 (C-16), 79.3 (C-3), 76.1 (C-26), 65.1 (C-17), 57.8 (C-14), 51.8 (C-9), 47.7 (*O*-Me), 42.0 (C-13), 41.3 (C-20), 40.9 (C-12), 39.6 (C-4), 38.7 (C-1), 38.1 (C-10), 35.1 (C-25), 33.3 (C-7), 32.9 (C-8), 31.5 (C-15), 30.8 (C-2), 29.1 (C-23), 28.1 (C-24), 22.0 (C-11), 20.0 (C-19), 17.4 (C-27), 16.9 (C-18), 16.3 (C-21) for aglycone; 100.5 (C-1'), 79.4 (C-2'), 76.7 (C-3'), 80.0 (C-4'), 78.2 (C-5'), 62.0 (C-6') for 3-*O*-glucose moiety; 103.0 (C-1''), 72.6 (C-2''), 72.3 (C-3''), 73.8 (C-4''), 70.7 (C-5''), 17.9 (C-6'') for 4'-*O*-rhamnose moiety; 102.4 (C-1'''), 72.2 (C-2'''), 72.4 (C-3'''), 74.0 (C-4'''), 69.9 (C-5'''), 18.1 (C-6''') for 2'-*O*-rhamnose moiety; 104.7 (C-1''''), 75.0 (C-2''''), 78.0 (C-3''''), 71.8 (C-4''''), 78.1 (C-5''''), 62.8 (C-6'''') for 26-*O*-glucose moiety.

Dioscin (3) – White powder. ^{1}H-NMR (CD_3OD, 600 MHz) δ_H: 5.38 (1H, m, H-6), 4.40 (1H, td, *J* = 8.0, 6.2 Hz, H-16), 3.60 (1H, dd, *J* = 5.9, 2.9 Hz, H-3), 3.44 (1H, ddd, *J* = 10.9, 4.5, 2.2 Hz, H-26a), 3.32 (1H, o, H-26b), 2.45 (1H, m, H-4a), 2.30 (1H, m, H-4b), 2.01 (1H, m, H-7a), 1.98 (1H, m, H-15a), 1.91 (1H, m, H-23), 1.90 (1H, m, H-20), 1.88 (1H, m, H-1a), 1.76 (1H, m, H-12b), 1.75 (1H, m, H-17), 1.66 (1H, m, H-8), 1.62 (1H, m, H-24a),

1.56 (1H, m, H-7b), 1.55 (1H, m, H-11), 1.42 (1H, dd, J = 13.3, 4.0 Hz, H-24b), 1.27 (1H, m, H-15b), 1.20 (1H, d, J = 4.6 Hz, H-12a), 1.15 (1H, m, H-14), 1.08 (1H, m, H-1b), 1.05 (3H, s, H-19), 0.97 (1H, o, H-9), 0.96 (3H, d, J = 7.0 Hz, H-21), 0.81 (3H, s, H-18), 0.79 (3H, d, J = 6.5 Hz, H-27) for the aglycone part; 4.50 (1H, d, J = 7.9 Hz, H-1'), 3.39 (1H, o, H-2'), 3.32 (1H, o, H-3'), 3.52 (1H, t, J = 9.2 Hz, H-4'), 3.58 (1H, o, H-5'), 3.79 (1H, dd, J = 12,1, 2,1 Hz, H-6'b), 3.64 (1H, m, H-6'a) for 3-*O*-glucose; 4.83 (1H, d, J = 1.8 Hz, H-1"), 3.83 (1H, dd, J = 3.4, 1.8 Hz, H-2"), 3.61 (1H, brd, J = 3.4, Hz, H-3"), 3.40 (1H, o, H-4"), 3.93 (1H, m, H-5"), 1.26 (3H, o, H-6") for 4'-*O*-rhamnose; 5.20 (1H, d, J = 1.7 Hz, H-1'"), 3.90 (1H, o, H-2'"), 3.66 (1H, brd, J = 2.7 Hz, H-3'"), 3.40 (1H, o, H-4'"), 4.13 (1H, m, H-5'"), 1.25 (3H, o, H-6'") for 2'-*O*-rhamnose; ^{13}C-NMR (CD_3OD, 150 MHz) δ_C: 141.9 (C-5), 122.7 (C-6), 110.7 (C-22), 82.3 (C-16), 79.3 (C-3), 67.9 (C-26), 63.8 (C-17), 57.9 (C-14), 51.8 (C-9), 43.0 (C-20), 41.5 (C-13), 41.0 (C-12), 39.6 (C-4), 38.6 (C-1), 38.1 (C-10), 33.3 (C-7), 32.9 (C-15), 32.8 (C-8), 32.5 (C-2), 31.5 (C-25), 30.9 (C-23), 29.9 (C-24), 22.1 (C-11), 19.9 (C-19), 17.6 (C-27), 16.8 (C-18), 15.0 (C-21) for the aglycone; 100.5 (C-1'), 79.4 (C-2'), 76.7 (C-3'), 79.9 (C-4'), 78.1 (C-5'), 61.9 (C-6') for 3-*O*-glucose; 103.0 (C-1"), 72.3 (C-2"), 72.2 (C-3"), 73.7 (C-4"), 70.7 (C-5"), 18.0 (C-6") for 4'-*O*-rhamnose; 102.3 (C-1'"), 72.1 (C-2'"), 72.5 (C-3'"), 73.9 (C-4'"), 69.9 (C-5'"), 17.9 (C-6'") 2'-*O*-rhamnose.

Adenosine (5) – White powder. ^{1}H-NMR (CD_3OD, 600 MHz) δ_H: 8.35 (1H, s, H-8), 8.21(1H, s, H-2) for the aglycone part; 5.99 (1H, d, J = 6.4 Hz, H-1'), 4.76 (1H, dd, J = 6.4, 5.1 Hz, H-2'), 4.35 (1H, dd, J = 5.1, 2.6 Hz, H-3'), 4.20 (1H, q, J = 2.6 Hz, H-4'), 3.91 (1H, dd, J = 12.5, 2.5 Hz, H-5'a), 2.77 (1H, dd, J = 12.5, 2.7 Hz, H-5'b) for the arabinofuranosyl moiety; ^{13}C-NMR (CD_3OD, 150 MHz) δ_C: 155,9 (C-6), 151,7 (C-2), 148,5 (C-4), 140,5 (C-8), 119,2 (C-5) for aglycone part; 89,5 (C-1'), 74,1 (C-2'), 71,3 (C-3'), 86,4 (C-4'), 62,1 (C-5') for the arabinofuranosyl moiety.

Acid hydrolysis of compound 1 – An amount of 0.5 mg of compound **1** was refluxed in 2N aqueous HCl (1 mL) at 90 °C for 2 h. The mixture was then extracted with CH_2Cl_2 (3 × 3 mL) and the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral and was then analysed by TLC using the solvent system EtOAc-MeOH-H_2O (8-2-1) as eluent. The Rf values of the products were identical to those of glucose, arabinose, xylose and rhamnose.

Results and Discussion

The MeOH extract from the leaves of *D. humilis* was repeatedly subjected to column chromatography yielding a new steroidal saponin **1** (Fig. 1) together with the known 26-*O*-β-*D*-glucopyranosyl-22-*O*-methyl-(25*R*)-furost-5-ene-3*β*,22,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (methyl-protodioscin),[12] diosgenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside

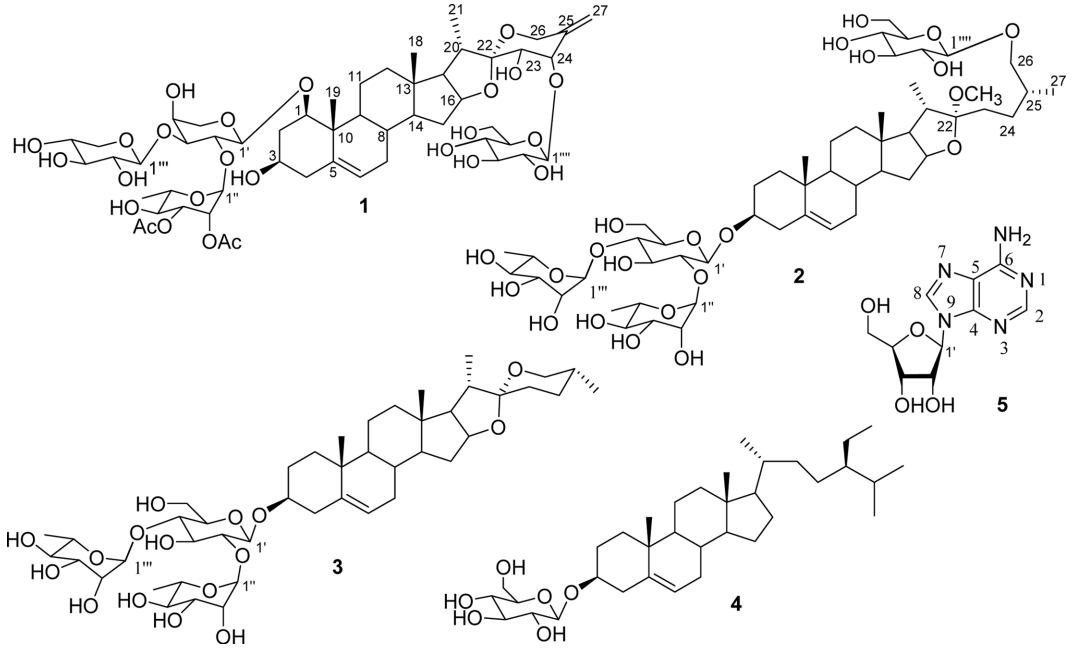

Fig. 1. Structures of compounds isolated from *D. humilis*.

(dioscin),[13] β-sitosterol 3-*O*-glucopyranoside,[14] and adenosine.[15]

Compound **1** was isolated as a white gum from methanol. Its molecular formula $C_{52}H_{80}O_{25}$ was deduced from the positive-ion mode HRESIMS which showed the pseudomolecular ion peak at *m/z* = 1139.4893 $[M+Na]^+$ (Calcd for $C_{52}H_{80}O_{25}Na^+$: 1139.4886). The ^1H NMR spectrum of **1** exhibited signals for two methyl singlets at $\delta = 0.86$ (Me-18) and 1.07 (Me-19), two methyl doublets at $\delta_H = 0.89$ ($J = 7.0$ Hz, Me-21) and 1.25 ($J = 6.1$ Hz, H-6"), an exomethylene group with protons at $\delta_H = 4.97$ and 5.09 (brs each, H-27) as well as an olefinic proton at $\delta_H = 5.53$ (brd, $J = 5.6$ Hz, H-6). The above proton signals together with the distinctive quaternary carbon signal at $\delta_C = 112.4$ (C-22) showing HMBC with methylene protons at $\delta_H = 3.69$ (1H, brd, $J = 12.0$ Hz, H-26a) 4.42 (1H, brd, $J = 12.0$ Hz, H-26b) and the olefinic carbon signals at $\delta_C = 144.6$ (C-25), 139.6 (C-5), 126.1 (C-6) and 114.4 (C-27) (Table 1) indicated that the aglycone was of the spirosta-5,25(27)-diene type.[5,9,16-18] Always in the aglycone region, the HMBC correlation between the two exomethylene protons at $\delta_H = 4.97$ (1H, brs, H-27a) and 5.09 (1H, brs, H-27b) and the carbon signal at $\delta_C = 83.6$ (C-24) as well as the ^1H-^1H COSY interaction between H-24 ($\delta = 4.33$ (d, $J = 4.2$ Hz)) and H-23 ($\delta = 3.48$ (d, $J = 4.2$ Hz)) suggested the presence of adjacent OH groups at C-23 and C-24. Furthermore, in the HMBC spectrum, the methyl proton at $\delta_H = 1.07$ (Me-19) showed long range correlation with carbon signals at $\delta_C = 43.5$ (C-10), 84.7 (C-1), 51.4 (C-9), and 139.6 (C-5). On the other hand, the olefinic proton H-6 ($\delta = 5.53$) exhibited long-range correlation with the methylene carbon at $\delta_C = 43.5$ (C-4). These findings indicated the presence of a double bond between C-5 and C-6, and that carbons C-1 and C-3 are oxygenated. The β orientation of the oxygen atom at

Table 1. ^{13}C NMR (150 MHz) and ^1H NMR (600 MHz) data of compound **1** in CD_3OD

Position	$\delta^{13}C$	δ^1H [m, *J* (Hz)]	Position	$\delta^{13}C$	δ^1H [m, *J* (Hz)]
1	84.7	3.36 (1H, dd, $J = 3.8, 11.6$ HZ)	Ara 1'	101.0	4.32 (1H, d, $J = 7.2$ Hz)
2	37.4	1.66 (1H, m); 2.14 (1H, m)	2'	74.4	3.81 (1H, o)
3	69.3	3.32 (1H, m)	3'	84.8	3.76 (1H, dd, $J = 3.3, 9.3$ Hz)
4	43.5	2.19 (1H, m)	4'	70.8	3.91 (1H, brm)
5	139.6	/	5'	67.1	3.49 (1H, o), 3.81 (1H, o)
6	126.1	5.53 (1H, brd, $J = 6.2$ Hz)	Rha 1"	99.0	5.44 (1H, brd, $J = 1.7$ Hz)
7	32.8	1.50 (1H, m)	2"	71.5	5.27 (1H, dd, $J = 1.7, 3.5$ Hz)
8	34.1	1.52 (1H, m)	3"	73.6	4.96 (1H, dd, $J = 3.4, 10.3$ Hz)
9	51.4	1.23 (1H, m)	4"	71.5	3.43 (1H, o)
10	43.5	/	5"	69.8	4.23 (1H, m)
11	24.9	2.50 (1H, m)	6"	18.7	1.25 (1H, d, $J = 6.1$ Hz)
12	41.5	1.20 (1H, m), 1.65 (1H, m)	$\underline{CH_3}CO$-2"	21.1	1.95 (3H, s)
13	41.7	/	$\underline{CH_3}CO$-3"	21.0	2.05 (3H, s)
14	58.1	1.13 (1H, m)	$CH_3\underline{C}O$-2"	172.5	/
15	33.1	1.40 (1H, m); 1.95 (1H, m)	$CH_3\underline{C}O$-3"	172.3	/
16	84.0	4.47 (1H, q, $J = 7.3$ Hz)	Xyl 1'"	106.5	4.31 (1H, d, $J = 7.5$ Hz)
17	62.6	1.67 (1H, m)	2'"	74.9	3.15 (1H, o)
18	17.3	0.86 (3H, s)	3'"	77.9	3.24 (1H, o)
19	15.4	1.07 (3H, s)	4'"	71.3	3.25 (1H, o)
20	38.3	2.54 (1H, m)	5'"	67.1	3.17 (1H, o); 3.81 (1H, o)
21	14.8	0.89 (1H, d, $J = 7.0$ Hz)	Glc 1'""	105.5	4.47 (1H, d, $J = 7.8$ Hz)
22	112.4	/	2'""	75.4	3.25 (1H, o)
23	70.9	3.48 (1H, d, $J = 4.2$ Hz)	3'""	78.0	3.33 (1H, o)
24	83.6	4.33 (1H, d, $J = 4.2$ Hz)	4'""	71.2	3.43 (1H, o)
25	144.6	/	5'""	78.0	3.15 (1H, o)
26	62.2	3.69 (1H, brd, $J = 12.0$ Hz); 4.42 (1H, brd, $J = 12.0$ Hz)	6'""	62.6	3.59 (1H, dd, $J = 5.3, 11.9$ Hz); 3.73 (1H, brd, $J = 11.9$ Hz)
27	114.4	4.97 (1H, brs); 5.09 (1H, brs)			

o: overlapped signal

C-1 and C-3 positions was revealed by the coupling constants of H-1 (dd, J = 3.8, 11.6) and was supported by the NOESY correlation between H-1 and H-3. The NOESY correlations depicted between the proton at δ_H 2.54 ppm (m, H-20) and the proton at 3.48 ppm (d, J = 4.2 Hz, H-23) as well as between H-23 and H-24 (δ_H 4.33, d, J = 4.2 Hz) supported the 23*S*, 24*S* configurations. Based on the above findings, the aglycone of **1** was identified as (23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β,23, 24-tetrol, and this was further confirm by comparison of ^{1}H and ^{13}C NMR spectroscopic data obtained from 2D NMR spectra of **1** (Table 1) with those reported in the literature.[9,16,18]

The analysis of the saccharide part of compound **1** revealed the presence of four sugar residues (Fig. 1) evidenced by anomeric proton signals at δ_H = 4.32 (1H, d, J = 7.2 Hz, H-1'), 5.44 (1H, brd, J = 1.7 Hz, H-1"), 4.31 (1H, d, J = 7.5 Hz, H-1'") and 4.47 (1H, d, J = 7.8 Hz, H-1"") giving HSQC correlations with carbons at δ_C = 101.0 (C-1'), 99.0 (C-1"), 106.5 (C-1'") and 105.5 (C-1""), respectively (Table 1). Complete assignment of the glycosidic proton and carbon signals was achieved by the analysis of ^{1}H-^{1}H COSY, HSQC, HMBC and NOESY spectra starting from the easily identifiable anomeric protons (Fig. 2). Evaluation of chemical shifts, spin couplings and comparison with published data allowed the identification of one arabinopyranosyl, one xylopyranosyl, one rhamnopyranosyl, and one glucopyranosyl units.[19] The coupling constants observed for the anomeric protons suggested that the anomeric configuration of xylose (J = 7.5 Hz) and glucose (J = 7.8 Hz) was β while that of arabinose (J = 7.2 Hz) and rhamnose (J = 1.7 Hz) was of α form. The location of two acetyl groups (characterized in the ^{13}C NMR spectrum by signals at δ_C = 172.5 and 172.3) on carbons C-2" and C-3" of the rhamnopyranosyl unit was deduced from the acylation schift of its H-2" (δ = 5.27) and H-3" (δ = 4.96) protons.[18,20] The sequence of the oligosaccharide chain was established by ^{13}C NMR chemical shift differences between individual sugar,[19] HMBC and NOESY experiments (Fig. 2). The cross-peak correlations observed in the NOESY spectrum between the protons at δ_H = 4.32 (H-1')) and the proton at δ = 3.36 (H-1) showed that the arabinopyranosyl unit was linked at C-1 of the aglycone. The fixation of the xylopyranosyl unit at C-3' position of arabinose was deduced from the HMBC correlation observed between H-1'" (δ = 4.31) and C-3' (δ = 84.7). Furthermore, the rhamnopyranosyl unit was located at C-2' as evidenced by the NOESY correlation depicted between H-1" (δ = 5.44) and H-2' (δ = 3.81). Finally, the linkage of the glucose at C-24 position was indicated by the long-range coupling (^3J) observed in the HMBC spectrum between the anomeric proton at δ_H = 4.47 (d, J = 7.8, H-1"") and the carbon at δ = 83.6 (C-24). Acid hydrolysis of **1** followed by co-TLC analysis of the hydrolysate with authentic samples of sugars confirmed the presence of glucose, rhamnose, arabinose, and xylose. The *D*-configuration for glucose and xylose, *L*-configuration for arabinose and rhamnose were assumed to be those of the most commonly encountered analogues in the plant kingdom.[21] The structure of **1** was also examined with the tandem mass spectrometry. The MS^2 of the ion at m/z = 1139 $[M+Na]^+$ gave fragments at m/z = 961 $[M+Na\text{-}162\text{-}H\text{-}CH_3]^+$, 731 $[M+Na\text{-}162\text{-}H\text{-}CH_3\text{-}2Ac\text{-}146]^+$ and 599 $[M+Na\text{-}162\text{-}H\text{-}CH_3\text{-}2Ac\text{-}146\text{-}132]^+$ corresponding to the loss of a hexose unit (glucose), a diacetyldeoxyhexose unit (rhamnose), and a pentose unit (xylose), respectively. Compound **1** was then elucidated as (23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β,23,24-tetrol 1-*O*-((2,3-diacetyl-α-L-

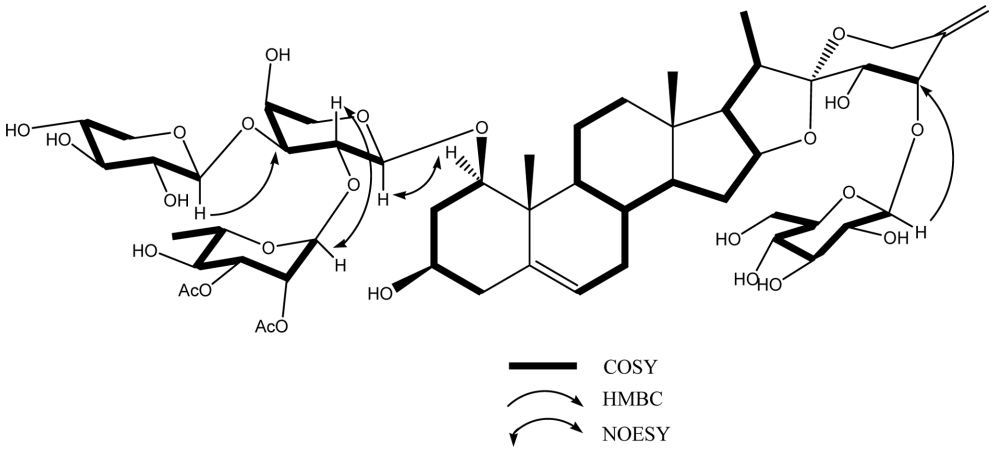

Fig. 2. Selected ^{1}H-^{1}H COSY, HMBC and NOESY correlations for compound **1**.

rhamnopyranosyl)-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside)-24-*O*-β-D-glucopyranoside, a new steroidal saponin to which we gave the trivial name humilisoside.

Although a large number of polyhydroxylated spirostanol saponins have been isolated from higher plants,[5,6,17,18,20,22-24] those very related to humilisoside were isolated only from *Sansevieria trifasciata*[9,16] and one of them has the same aglycone ((23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β, 23,24-tetrol) and the same sugar chain (1-*O*-(α-L-rhamnopyranosyl)-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside)-24-*O*-β-D-glucopyranoside) as humilisoside; the only difference being the presence of three *O*-acetyl groups on the rhamnose unit instead of two in humilisoside. These findings comforted the fact that the genera *Dracaena* and *Sansevieria* belong to the family Dracaenaceae as mentioned by Kim *et al*.[1] Furthemore, dioscin and methylprotodioscin were previously isolated from many *Dracaena* species, especially *Dracaena concinna*,[17] *Dracaena draco*,[22] *Dracaena viridiflora*[7] and should be considered as the chemotaxonomic markers for the genus *Dracaena*. To the best of our knowledge, this is the first report on the phytochemical study of *D. humilis*.

Compounds **2**, **3**, and **5** were tested for their antimicrobial activity against three yeasts (*Candida albicans* ATCC9028, *Candida glabrata*, and *Cryptococcus neoformans*) and three bacteria (*Staphylococcus aureus* ATCC1026, *Enterococcus faecalis* ATCC29212, and *Escherichia coli* ATCC25922) but no significant inhibition was observed (The MICs values were higher than 256 μg/mL). Nevetheless some of us have already measured the cytotoxicity of dioscin (**3**) and methylprotodioscin (**2**) isolated from *Dracaena viridiflora* Engl & Krause.[7] Dioscin was shown to exhibit a significant cytotoxicity against A549, Jurkat and Skov-3 cells with IC_{50} values of 0.42, 1.70 and 1.90 μg/mL, respectively.

Acknowledgements

This work was supported by TWAS through of a research grant (Nº 09-152 RG/CHE/AF/AC I-UNESCO FR:3240230339) to Prof. Teponno. We are grateful to Prof. Dr. Till Opatz of the Institute of Organic Chemistry Johannes Gutenberg University Mainz, Germany for allowing the measurement of NMR and mass spectra.

References

(1) Kim, J. H.; Kim, D. K.; Forest, F.; Fay, M. F.; Chase, M. W. *Ann. Bot*. **2010**, *106*, 775-790.
(2) Masaoud, M.; Ripperger, H.; Porzel, A.; Adam, G. *Phytochemistry* **1995**, *38*, 745-749.
(3) Luo, Y.; Wang, H.; Xu, X.; Mei, W.; Dai, H. *Molecules* **2010**, *15*, 8904-8914.
(4) Tapondjou, L. A.; Ponou, K. B.; Teponno, R. B.; Mbiantcha, M.; Djoukeng, J. D.; Nguelefack, T., B.; Watcho, P.; Gomez-Cadenas, A.; Park, H. J. *Arch. Pharm. Res*. **2008**, *31*, 653-658.
(5) Xu, M.; Zhang, Y. J.; Li, X. C.; Jacob, M. R.; Yang, C. R. *J. Nat. Prod*. **2010**, *73*, 1524-1528.
(6) Yokosuka, A.; Mimaki, Y.; Sashida, Y. *J. Nat. Prod*. **2010**, *62*, 1239-1243.
(7) Teponno, R. B.; Dzoyem, J. P.; Nono, R. N.; Kauhl, U.; Sandjo, L. P.; Tapondjou, L. A.; Bakowsky, U.; Opatz, T. *Rec. Nat. Prod.* **2017**, *11*, 421-430.
(8) Challinor, V. L.; Piacente, S.; De Voss, J. J. *Steroids* **2012**, *77*, 602-608.
(9) Teponno, R. B.; Tanaka, C.; Jie, B.; Tapondjou, L. A.; Miyamoto, T. *Chem. Pharm. Bull.* **2016**, *64*, 1347-1355.
(10) Ponou, B. K.; Teponno, R. B.; Tapondjou, A. L.; Lacaille-Dubois, M. A.; Quassinti, L.; Bramucci, M.; Barboni, L. *Fitoterapia* **2019**, *134*, 454-458.
(11) Kianfe, B. Y.; Kuhlborn, J.; Tchuenguem, R. T.; Tchegnitegni B. T.; Ponou, B. K.; Gross, J.; Teponno, R. B.; Dzoyem, J. P.; Opatz, T.; Tapondjou, L. A. *S. Afri. J. Bot.* **2020**, *133*, 161-166.
(12) Wang, G.; Chen, H.; Huang, M.; Wang, N.; Zhang, J.; Zhang, Y.; Bai, G.; Fong, W. F.; Yang, M.; Yao X. *Cancer Lett*. **2006**, *241*, 102-109.
(13) Hu, K.; Dong, A.; Yao, X.; Kobayashi, H.; Iwasaki, S. *Planta Med*. **1996**, *62*, 573-575.
(14) Gao, H.; Hou, B.; Kuroyanagi, M.; Wu, L. *Asian J. Trad. Med*. **2007**, *2*, 104-109.
(15) Kun, H. S.; Jae, C. D.; Sam, S. K. *Arch. Pharm. Res*. **1991**, *14*, 193-194.
(16) Mimaki, Y.; Inoue, T.; Kuroda, M.; Sashida, Y. *Phytochemistry* **1996**, *43*, 1325-1331.
(17) Mimaki, Y.; Kuroda, M.; Takaashi, Y.; Sashida, Y. *Phytochemistry* **1998**, *47*, 79-85.
(18) Kougan, G. B.; Miyamoto, T.; Tanaka, C.; Paululat, T.; Mirjolet, J. F.; Duchamp, O.; Sondengam, B. L.; Lacaille-Dubois, M. A. *J. Nat. Prod*. **2010**, *73,* 1266-1270.
(19) Agrawal, P. K. *Phytochemistry* **1992**, *31*, 3307-3330.
(20) Eskander, J.; Lavaud, C.; Harakat, D. *Phytochemistry* **2011**, *72*, 946-951.
(21) Mitaine-Offer, A. C.; Miyamoto, T.; Khan, I. A.; Delaude, C.; Lacaille-Dubois, M. A. *J. Nat. Prod*. **2002**, *65*, 553-557.
(22) Mimaki, Y.; Kuroda, M.; Ide, A.; Kameyama, A.; Yokosuko, A.; Sashida, Y. *Phytochemistry* **1999**, *50*, 805-813.
(23) Yang, Q. X.; Zhang, Y. J.; Li, H. Z.; Yang, C. R. *Steroids* **2005**, *70*, 732-737.
(24) Mimaki, Y.; Watanabe, K. *Helv. Chim. Acta* **2008**, *91*, 2097-2106.

Received March 13, 2021
Revised May 8, 2021
Accepted May 17, 2021