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Bioactive Constituents from the Rhizomes of *Sansevieria cylindrica*

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Abstract: Analysis of the MeOH extract of rhizomes of *Sansevieria cylindrica* using repeated silica gel and reversed-phase chromatographic separations led to the isolation of ten known phenolic compounds (**1-10**) belonging to five different classes. Their structures, including the absolute configuration, were identified by NMR and CD spectra analysis. The antiradical activity of isolated compounds was screened using the DPPH scavenging method. The cytotoxic effects were assayed against a Hela cell line. Compounds **2-10** have been isolated from a *Sansevieria* species for the first time and compounds **5**, **7-10** have been isolated from species of the Asparagaceae family for the first time. The structure of a homoisoflavanone isolated from *S. roxburghiana* has been revised.

Keywords: *Sansevieria cylindrical*; Asparagaceae; homoisoflavanone; cynnamoyl octopamine; tyramine derivatives; antiradical activity.

1. Introduction

The genus *Sansevieria* consists of about 70 species of flowering plants which are broadly distributed from Africa through Asia, including Myanmar and the islands of the Indian Ocean [1]. The genus *Sansevieria* was formely classified as belonging to the Dracaenaceae family. This family, however, has been included in the Asparagaceae by the APG III system of plant taxonomy for flowering plants (angiosperms), which is mostly molecular-based [2]. *S. cylindrica* Bojer ex Hook. is native to the subtropical African regions, and it is cultivated in Myanmar for ornamental purposes. It is also used as a traditional remedy, especially in remote rural areas of the country, where herbal preparations are still widely employed as medicines in the place of rarely available western drugs. The whole plant is employed for curing cuts, sprains and broken bones, whereas the roots are used to cure snakebites. *Sansevieri*a plants have been reported to be rich in steroidal sapogenins and saponins. Previous phytochemical investigations of leaves and aerial parts of *S. cylindrica* led to the isolation of steroidal saponins [3-5], one of which demonstrated inhibition of capillary permeability activity [3], whereas others exhibited moderate cytotoxicities *in vitro* [4,5]. In addition, (+)-trifasciatine B, with

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high radical scavenging activity activity [4], and dihydrochalcone (+)-trifasciatine C [5, 6], showing a moderate cytotoxicity against MCF7 cells, were isolated.

In our ongoing research on new bioactive constituents from medicinal plants collected in Myanmar [7], a new homoisoflavanone, together with four known congeners and dihydrochalcone (–)-trifasciatine C, were recently isolated from the methanolic extract of *S. cylindrica* rhizomes [8]. Upon completion of our investigation on this plant, in this paper we describe the isolation and the structure identification of ten additional compounds. They include two homoisoflavanones, (–)-trifasciatine A (**1**) [9] and (–)-(3*R*)-cambodianol (**2**) [10], one phenylpropane derivative, hydroxychavicol [3,4-dihydroxyallylbenzene (APC)] (**3**) [11], three alkaloids, *trans*-*N*-*p*-coumaroyl tyramine [synonyms, *N*-(*p*-hydroxyphenyl)ethyl *p*-hydroxycinnamide and paprazine] (**4**) [12], (–)-*trans*-*N*-*p*-coumaroyl octopamine (**5**) [13], and (–)-*trans*-*N*-feruloyl octopamine (**6**) [14]. Two furanoflavones, lanceolatin B (**7**) [15] and pongaglabol methyl ether (**8**) [16], one 3-methoxyflavone, de(s)methoxy kanugin (**9**) [17], and one pterocarpan, (–)-(6a*R*,11a*R*)-homopterocarpin (**10**) [18-20], were also isolated by repeated chromatographic separations on silica-gel and reversed-phase RP-18 columns. The structures of isolated compounds (Figure 1), including the absolute configuration, were identified by analysis of DART-HRMS, IR, NMR and CD spectra, and comparison with the literature. The antiradical properties of the isolated compounds were screened by a standard DPPH test. The cytotoxic effects of compounds **1**, **3**, **4**–**6** and **10** towards Hela cells were assayed in a standard MTT test.

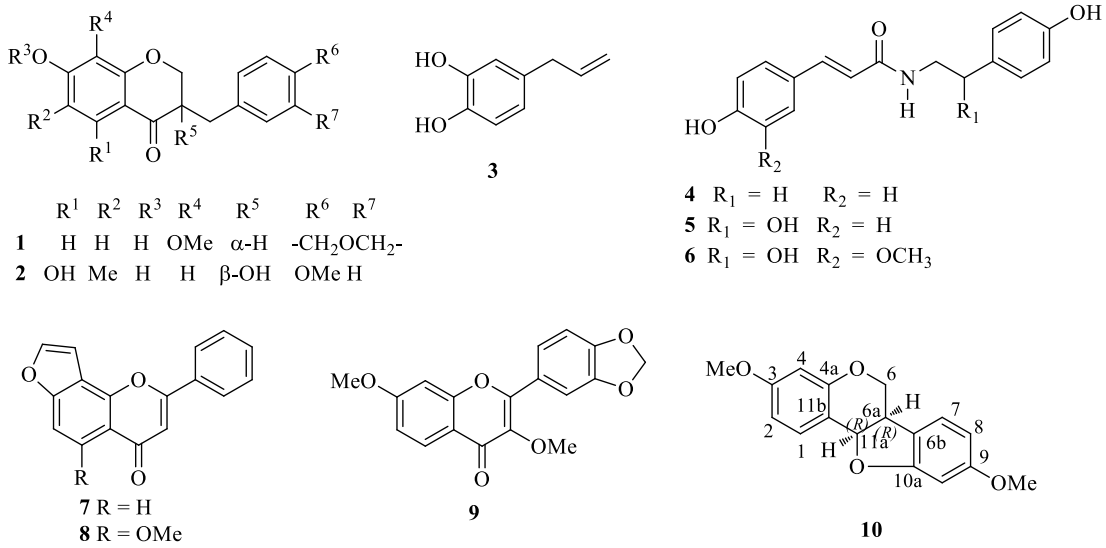

Figure 1. Chemical structures of compounds **1–10** isolated from *Sansevieria cylindrica*

2. Materials and Methods

2.1. General

UV spectra were recorded in MeOH on a JASCO V-560 UV–VIS spectrophotometer. IR spectra were recorded using KBr pellets on a JASCO FT/IR-410 spectrophotometer. Optical rotations were measured on a JASCO P-2200 polarimeter. CD spectra were recorded on a JASCO J-725 circular dichroism spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ or in methanol-d_4 on a Bruker Avance III HD600 spectrometer equipped with Prodigy liquid nitrogen cryoprobe. Chemical shifts for ^{1}H and ^{13}C NMR are given in ppm (δ) relative to TMS signal (δ_H 0.00) and solvent signal (δ_C 77.0 for $CDCl_3$ and 49.0 for methanol-d_4, respectively) as internal standards. Coupling constants (J) are in Hz. Positive mode DART-HRMS data were obtained by using an Exactive Plus instrument (Thermo Scientific). Column chromatographic separations were carried out on silica gel (BW-820MH, Fuji Silysia, Aichi, Japan). Analytical TLC was performed on precoated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany, Art. 5715). Spots were visualized under UV light at 254 nm and by spraying with anisaldehyde-sulfuric acid reagent, followed by heating at 200 °C for 2 min.

Semipreparative HPLC separations were performed on a chromatograph equipped with multiwavelength detector (JASCO) using a Develosil ODS UG-5 column (ϕ 20 × 250 mm, Nomura Chemical, Japan) and TSKgel ODS-120T (ϕ 7.8 × 300 mm, Tosoh, Japan).

2.2. Plant Material

Rhizomes of *Sansevieria cylindrica* Bojer ex Hook. (Asparagaceae) were collected in September 2016 at Thazi Township in Mandalay Region, Myanmar. The plant was identified by Professor Dr Soe Myint Aye, Department of Botany, University of Mandalay, Myanmar. A voucher specimen (N-11) has been deposited at the Department of Chemistry, University of Mandalay, Myanmar.

2.3. Extraction and Isolation

Air-dried rhizomes of *S. cylindrica* (1.0 Kg) were percolated with MeOH (4 L) for two weeks. The whole mixture was then filtered through filter paper and the filtrate was evaporated in vacuo at room temperature to provide a sticky residue (109 g), which was partitioned between EtOAc, H_2O and *n*-BuOH. The three layers were then evaporated in vacuo, separately, to provide residues A (9.1 g), B (24.9 g), and C (74.9 g), respectively. Subsequently, residue A was partitioned between *n*-hexane and MeCN at room temperature which, by evaporation in vacuo, afforded residues D (4.31 g) and E (4.36 g), respectively. Residue E was separated by flash column chromatography over octadecylsilanized silica gel (ODS). Elution with a gradient of MeCN in H_2O (from 70:30, v/v, to 100% MeCN) gave 30 main fractions (E1–E30). Fractions E4 (124 mg) and E12 (191 mg) were separated by semiprep. HPLC. Elution of the former with a gradient mixture of MeOH–H_2O (2:98, v/v) and MeOH, from 9:1 to 8:2 (v/v) yielded compounds **5** (5 mg) and **6** (2.9 mg). Elution of E12 (191 mg) using a gradient mixture of MeOH–H_2O (2:98, v/v) and MeOH, from 7:3 to 3:7 (v/v), afforded compounds **1** (3.8 mg) and **2** (5.0 mg). Flash column chromatography of fraction E13 (105 mg) over octadecylsilanized silica gel (ODS) using a gradient mixture of MeOH–H_2O (2:98, v/v) and MeOH, from 7:3 to 3:7 (v/v), gave compounds **8** (1.5 mg) and **9** (2.8 mg). Semiprep. HPLC separation of fraction E6 (379.9 mg), E8 (415.4 mg), E14 (253 mg), and E15 (67.3 mg) using a gradient mixture of MeOH–H_2O (2:98, v/v) and MeOH, yielded compounds **4** (2.2 mg), **3** (8.6 mg), **7** (1.6 mg), and **10** (10.8 mg), respectively. Although (-)-(6a*R*, 11a*R*)-homopterocarpin (**10**) is a known plant metabolite [18-20], we were unable to find a complete list of physical and spectroscopic data. Therefore, they are reported below for completeness.

2.3.1. (**–**)-*(6aR,11aR)-homopterocarpin* (**10**)

Colorless crystals from EtOH; mp. 86–88°C; $[\alpha]_D^{27}$ –205.71 (*c* 0.03, $CHCl_3$); CD λ_{max} (MeCN) nm ($\Delta\varepsilon$): 238 (–23.02), 288 (+9.95). UV λ_{max} (MeOH) nm (log ε): 205 (4.91), 229 (4.17), 286 (3.97). IR ν_{max} cm^{-1} 3396, 3070, 2937, 2835, 1619, 1496, 1444, 1346, 1276, 1199, 1159, 1031, 949, 834, 795, 631. DART-HRMS: *m*/*z* 285.1120 $[M+H]^+$ (calcd. for $C_{17}H_{17}O_4$ 285.1127). ^{1}H NMR ($CDCl_3$): δ 7.42 (1H, d, *J* = 8.5 Hz, H-1), 7.13 (1H, d, *J* = 8.8 Hz, H-7), 6.63 (1H, dd, *J* = 2.6, 8.5 Hz, H-2), 6.5 (1H, d, *J* = 2.6 Hz, H-4), 6.45 (1H, dd, *J* = 2.3, 8.8 Hz, H-8), 6.44 (1H, d, *J* = 2.3 Hz, H-10), 5.52 (1H, d, *J* = 6.8 Hz, H = 11a), 4.25 (1H, dd, *J* = 5.3, 10.9 Hz, C-6eq), δ 3.78 (3H, s, 3-OMe), δ 3.77 (3H, s, 9-OMe), 3.64 (1H, t, *J* = 10.9 Hz, C-6ax), 3.54 (1H, m, C-6a). ^{13}C NMR (chloroform-*d*): 161.3 (s, C-9), 161.1 (s, C-3), 160.7 (s, C-10a), 156.6 (s, C-4a), 131.8 (d, C-1), 124.7 (d, C-7), 119.1 (s, C-6b), 112.4 (s, C-11b), 109.2 (d, C-2), 106.4 (d, C-8), 101.6 (d, C-4), 96.9 (d, C-10), 78.6 (d, C-11a), 66.6 (t, C-6), 55.5 (q, C-3-O*Me*), 55.4 (q, C-9-O*Me*), 39.6 (d, C-6a).

2.4. Cytotoxicity Assay

Cytotoxic effects of isolated compounds against a HeLa (cervix adenocarcinoma) cell line were assayed by the MTT colorimetric method using a Cell Counting kit-8 that was based on the tetrazolium salt/formazan system [21]. HeLa cells (JCRB9004) were obtained from the Japanese Collection of Research Bioresources (JCRB) cell bank. Cells were cultured in minimum essential

media (MEM) supplemented with 10% fetal bovine serum. For the cytotoxicity assay, cells were seeded at a density of 5×10^3 cells/well in 0.2 mL of medium in 96-multiwell plates and adhered. Subsequently, samples were dissolved in saline containing 10% DMSO and sterilized by filtration. Subsequently, series of diluted samples (0.2 mL) were added to the cells. The plates were incubated under a humid atmosphere of 5% (*v*/*v*) CO_2 and 95% (*v*/*v*) at 37 °C for 48 h. Twenty microliters of cell counting kit-8 (based on the tetrazolium salt/formazan system) were added to each well, and the microplates were incubated for 1 h, after which cell densities were measured at 450 nm using a Bio-RAD Model 550 Microplate Reader. Cisplatin was used as the cytotoxic reference compound.

2.5. Radical Scavenging Activity Assay

Antiradical effects of isolated compounds were assayed by the DPPH scavenging activity test. A total of 500 µL of test solution at various concentrations (1–100 µM), 500 µL of 0.2 M acetate buffer pH 5.5, and 1000 µL of HPLC-grade EtOH were mixed in a test tube for water soluble compounds. For EtOH soluble compounds, 1000 µL of test solution at various concentrations (1-100 µM) and 1000 µL of 0.1 M acetate buffer pH 5.5 were mixed in a test tube. 500 µL of $5x10^{-4}$ M DPPH solution was added to the mixture which was then homogenized using a vortex in a dark place, shielded from UV light, and incubated for 30 min at room temperature. Subsequently, the UV absorbance (*A*) of the mixture at 517 nm was measured using a spectrophotometer. Vitamin C was used as the reference antiradical compound in the same concentration range as the tested compounds. A control solution was prepared in the same manner as the test mixture. The free-radical-scavenging activity [AA%] of each sample and the reference standard was determined according to the following formula: [AA%] = $100 - [(A_{sample} - A_{blank}) \times 100 / A_{control}]$, where A_{sample} is the absorbance of the sample with DPPH, A_{blank} is the absorbance of the sample without DPPH, and $A_{control}$ is the absorbance of $DPPH^{\cdot}$ in EtOH. The concentration (µM) of the sample reducing 50% DPPH (EC_{50}) was determined by plotting the percentage of inhibition against the sample concentration.

3. Results and Discussion

The residue E from the EtOAc subextract of the MeOH extract of *S. cylindrica* rhizomes was subjected to flash column chromatography over octadecylsilanized silica gel (ODS) followed by repeated semipreparative HPLC separations to afford ten known compounds (**1-10**). The physical and spectroscopic data of **1-10** nicely matched those reported in the literature. While browsing literature reports, we found that a compound isolated from *S. roxburghiana* [22] was claimed to have the same structure as (−)-cambodianol (**2**), isolated from *Dracaena cambodiana* [10]. However, upon carefully examining the ^{13}C NMR resonances determined for the homoisoflavanone from *S. roxburghiana* [22], we found that they were similar to the ^{13}C NMR signals of homoisoflavanone **11**, which was previously isolated from *Liriope muscari* [23] and *S. cylindrica* [8]. In contrast, the ^{13}C NMR data of the compound isolated from *S. roxburghiana* [22] were significantly different from those determined for cambodianol (**2**) [10], although the solvents were not the same.

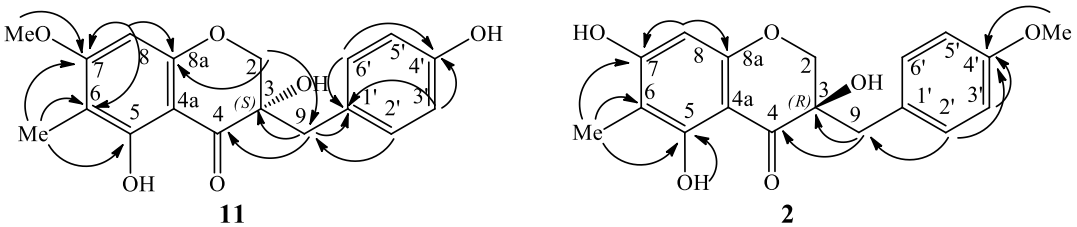

Figure 2. Representative HMBC correlations (H → C) for compound **11** and **2** isolated from *S. cylindrica*

Compounds **2** and **11** were re-isolated from *S. cylindrica* during our investigations on this plant and their ^{13}C NMR spectra were determined in the same solvent, $CDCl_3$ as the homoisoflavanone isolated from *S. roxburghiana* (Table 1). We reconfirmed that the structures of **2** and **11** are correct

and are isomeric. In fact, the two compounds differ for the position of the methoxy group, which is at C-7 in compound **11** [8, 23] and at C-4´ in cambodianol (**2**) [10]. Their location was confirmed by appropriate two bonds and three bonds correlations in the corresponding HMBC spectra (Figure 2) Moreover, comparison of the ^{13}C NMR spectra (Table 1) firmly indicated that the planar structure of the homoisoflavanone isolated from *S. roxburghiana* [22] must be revised from **2** to **11**.

Table 1. ^{13}C NMR signals (δ_C ; 150 MHz, $CDCl_3$) of compounds **11** and **2** isolated from *S. cylindrica* compared with literature

Carbon number	11[a,φ]	11[b,♣]	Homoisoflavanone[c,Φ]	2[d,§]
2	71.9	71.9	72.0	71.8
3	72.2	72.3	72.3	72.3
4	198.3	198.3	198.3	198.3
4a	100.1	100.2	100.2	100.3
5	160.1	160.1	160.2	161.5
6	106.5	106.5	106.6	104.5
7	166.5	166.5	166.5	163.2
8	90.9	91.0	91.0	95.0
8a	161.1	161.1	161.1	160.6
9	40.8	40.8	40.8	40.8
1´	126.4	126.2	126.2	126.2
2´/6´	131.8	131.8	131.8	131.6
3´/5´	115.2	115.3	115.3	113.8
4´	154.8	154.9	154.9	158.8
-OCH_3	55.9	56.0	56.0	55.2
-CH_3	6.9	6.9	6.9	6.7

[a][8] and this paper; [b]125 MHz, $CDCl_3$ [23]; [c]100 MHz, $CDCl_3$ [22]; [d]this paper, **2:** cambodianol
[φ]isolated from *S. cylindrical*; [♣] isolated from *Liriope muscari*; [Φ] isolated from *S. roxburghiana*; [§]isolated from *S. cylindrica*

The 3*S* configuration was attributed to compound (+)-**11** isolated from *Liriope muscari* [23] and *S. cylindrica* [8]. The 3*R* configuration has, instead, been proposed for the homoisoflavanone isolated from *S. roxburghiana* [22], whose stereostructure thus corresponds to that of *ent*-**11**. This finding is another example of enantiomeric congeners occurring in the same plant or in different species belonging to the same genus. Among homoisoflavanones arising from enantiodivergent biosynthetic pathways, other examples are trifasciatines B and C, whose enantiomers occur in *S. cylindrica* [4, 8] and in *S. trifasciata* [6, 9], respectively. A couple of biosynthetic routes have been proposed to explain the formation of enantiomeric sappanin-type homoisoflavonoids, in which antipodal compounds evolve from achiral intermediates by divergent enantioselective reactions [8, 9].

Homopterocapin (**10**), the three amides **4**–**6**, trifasciatine A, and hydroxychavicol (**3**) showed no cytotoxicity (IC_{50} > 80 μM) against Hela cells in a standard MTT test [21]. The other isolated compounds could not be tested due to their insolubility in saline solution containing 10% DMSO. In other experiments, significant cytotoxicities have been determined for cambodianol (**2**) [10], whereas hydroxychavicol (**3**) exhibited high antimicrobial, anti-inflammatory, and pancreatic lipase inhibitory effects [11]. On the other hand, lanceolatin B (**7**) showed high cancer chemopreventive potential [24], and anti-neuroinflammatory and analgesic properties [25]. In an antiradical screening test (DPPH) of compounds **1**-**10**, hydroxychavicol (APC) (**3**) exhibited the highest scavenging activity (EC_{50} = 2.33 μM). Actually, the high antioxidant activity of APC has been demonstrated by different tests, including the prevention of Fe(II)-induced lipid peroxidation (LPO) of liposomes and rat brain homogenates as well as *γ*–ray-induced damage of pBR322 plasmid DNA [26]. (–)-*trans*-*N*-Feruloyl octopamine (**6**) showed moderate radical scavenging activity (EC_{50} 61.8 μM), while the remaining isolated compounds displayed weak or no activity (EC_{50} value >100 μM); therefore, their antioxidant activity was no further investigated.

In conclusion, rhizomes of *S. cylindrica* are a rich source of bioactive phenolic compounds, among which rare homoisoflavonoids form a distinctive subclass. Asparagaceae and Fabaceae families and, to a minor extent, Liliaceae, have been associated with the largest number of isolated homoisoflavonoids [27] and our results confirm this chemotaxonomic finding. A thorough survey of the literature has revealed that compounds **2**–**10** have been isolated from a *Sansevieria* species for the first time, and that this is the first finding of compounds **5**, **7**–**10** in species belonging to the Asparagaceae family.

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Supporting Information

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