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Steroidal Saponins from Fresh Stems of *Dracaena angustifolia*

Xu Min[†], Zhang Ying-Jun^{†,*}, Li Xing-Cong[‡], Melissa R. Jacob[‡], and Yang Chong-Ren^{†,‡}

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650204, P. R. China, National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA, Weihe Biotech Laboratory, 653100, Yuxi, Yunnan, P. R. China

[†]Kunming Institute of Botany.

[‡]University of Mississippi.

[‡]Weihe Biotech Laboratory.

Abstract

Six new steroidal saponins (**1-6**), angudracanosides A-F, were isolated from fresh stems of *Dracaena angustifolia* (Agavaceae), together with eight known compounds. The structures of compounds **1-6** were determined by detailed spectroscopic analyses and chemical methods. Antifungal testing of all compounds showed that **6** and **7** were active against *Cryptococcus neoformans* with IC₅₀s of 9.5 and 20.0 μg/mL, respectively.

The genus *Dracaena* (Agavaceae) contains more than 60 species that are distributed from the Old World tropic region to the Canary Islands. Some species contain C27 steroidal saponins¹, as evidenced in our previous phytochemical study of *D. cochinchinensis*.² The C27 steroidal saponins have demonstrated antitumor¹, anti-inflammatory³, and hypoglycemic⁴ activities as well as therapeutic potential for cardiovascular diseases.⁵ Some C27 steroidal saponins have antifungal activity, which varies with the aglycone structure and with the number and structure of monosaccharide units in their sugar chains.⁶

Dried roots and rhizomes of *D. angustifolia* Roxb (Agavaceae) collected in Vietnam have been studied, revealing the presence of several steroidal saponins with antiproliferative activity.⁷ Our interest in antifungal steroidal saponins prompted us to investigate fresh stems of this species that were collected in Southwest China. As a result, six new steroidal saponins (**1-6**), which were named angudracanosides A-F, and eight known compounds were isolated. The antifungal activity of these compounds against several human fungal pathogens was evaluated, and the results are presented in paper.

Results and Discussion

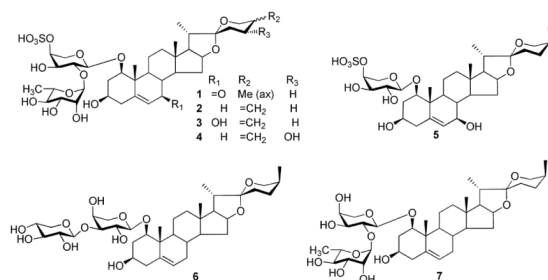
The fresh stems of *D. angustifolia* were extracted with methanol under reflux. A *n*-butanol-soluble portion of the methanol extract was chromatographed on the highly porous absorption resin Diaion HP-20, normal phase silica gel, Sephadex LH-20, and reversed-phase silica gel RP-18 to afford six new compounds (**1-6**). In addition, eight known compounds were identified as (25*S*)-spirost-5-en-1β,3β-diol-1-sulphate⁸, (25*R*)-

*Author to whom correspondence should be addressed. Tel.: +86-871-522-3235. Fax: +86-871-515-0124. zhangyj@mail.kib.ac.cn.

Supporting Information Available: The NMR spectra for angudracanoside A-F (**1-6**), structures of the known compounds (**8-14**) isolated from *Dracaena angustifolia*, important HMBC correlations for **1**, and selected ROESY correlations of **3**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

ruscogenin-1-*O*- α -L-rhamnopyranosyl-(1-2)-4-*O*-sulfo- β -D-arabinopyranoside⁹, 26-*O*- β -D-glucopyranosyl-furosta-5-ene-1 β ,3 β ,22 ζ ,26-tetrol-1-*O*- α -L-rhamnopyranosyl-(1-2)-*O*- β -D-xylopyranosyl-(1-3)- α -L-arabinopyranoside¹⁰, (2*S*)-ruscogenin-1-*O*- α -L-rhamnopyranosyl-(1-2)-*O*- β -D-xylopyranosyl-(1-3)- α -L-arabinopyranoside¹⁰, terreside^{11,12}, alliospiroside A (7)¹³, 3,4,5-trimethoxyphenyl-1-*O*- β -D-glucopyranoside¹⁴, and 3,4,5-trimethoxyphenyl-1-*O*- α -L-rhamnopyranosyl-(1-6)- β -D-gluco-pyranoside¹⁵, respectively, by comparison of the spectroscopic data with those reported in the literature. The new compounds were named angudracanoside A-F (**1-6**).

Angudracanoside A (**1**) was obtained as white amorphous powder. The negative ion HRFABMS and ¹³C NMR (DEPT) determined its molecular formula to be C₃₈H₅₈O₁₆S. The IR (KBr) spectrum of **1** displayed the absorptions characteristic for hydroxy groups (3441 cm⁻¹), a (2*S*)-spirostanol moiety [983, 918, 897, 838 cm⁻¹ (intensity 918>897)], and a S-O stretching band at 1227 cm⁻¹ indicative of a sulfate group.⁹ The ¹H NMR spectrum of **1** (Table 2) displayed two methyl singlets [δ 0.80 (s, Me-18) and 1.51 (s, Me-19)], three methyl doublets [δ 2.11 (t-like, *J* = 6.8 Hz, Me-21), 1.03 (d, *J* = 6.3 Hz, Me-27), and 1.75 (d, *J* = 6.0 Hz, Me-Rha)], and two anomeric proton signals [δ 4.64 (d, *J* = 7.5 Hz) and 6.29 (br s)]. In the ¹³C NMR and DEPT spectra (Table 1), 38 carbon signals were observed, including 27 resonances assigned to a C27 steroidal skeleton and 11 carbon resonances from two sugars. Compared to the literature values of similar compounds¹⁶, the carbon resonances at δ 202.0, 162.7, 128.1, 109.7, 101.8, and 100.3 could be assigned to a ketone functionality, two olefinic carbons, one spirostanol carbon of the aglycone, and two anomeric carbons, respectively. These NMR data indicated that compound **1** was a spirostanol saponin with two sugar moieties. Acid hydrolysis of **1** with 1 M HCl in dioxane afforded L-arabinose and L-rhamnose. The absolute configurations of both sugars were determined GC analysis of their corresponding trimethylsilylated L-cysteine adducts.¹⁷ The ¹H and ¹³C NMR data of the sugar moiety of **1** were identical to those of (2*S*)-ruscogenin-1-*O*- α -L-rhamnopyranosyl-(1-2)-4-*O*-sulfo- β -D-arabinopyranoside.⁹ The differences between **1** and the known compound were the molecular weight and the ¹³C NMR resonances of the B and F rings. Compound **1** was 14 mass units greater than (2*S*)-ruscogenin-1-*O*- α -L-rhamnopyranosyl-(1-2)-4-*O*-sulfo- β -D-arabinopyranoside. The obvious spectroscopic differences at C-6, C-7 and C-8 on the B ring (δ 128.1, 202.0 and 42.0 for **1**, respectively, versus δ 124.5, 33.5 and 33.0 for (2*S*)-ruscogenin-1-*O*- α -L-rhamnopyranosyl-(1-2)-4-*O*-sulfo- β -D-arabinopyranoside, respectively) suggested that the ketone functionality was located at C-7 in **1**. This was confirmed by the HMBC correlations of H-6, H-8, and H-9 with C-7 (δ 202.0). Other key HMBC correlations are shown in the Supporting Information. The IR absorption of **1** at 1655 cm⁻¹ was consistent with that of 7-ketodiosgenin from *Tamus edulis*.¹⁸ Finally, the typical F ring carbon resonances of C-23, C-24, C-25 and C-27 at δ 26.5, 26.3, 27.7 and 16.5, respectively, strongly supported a (2*S*)-spirostanol skeleton for **1**,^{9,16} in contrast to a (2*S*)-spirostanol skeleton for (2*S*)-ruscogenin-1-*O*- α -L-rhamnopyranosyl-(1-2)-4-*O*-sulfo- β -D-arabinopyranoside with distinctly different chemical shifts of those carbons at δ 32.2, 28.2, 30.8, and 17.4, respectively. Thus, the aglycone of **1** was determined to be as shown. The sequence of the sugar chain in **1** was determined to be the same as that of (2*S*)-ruscogenin-1-*O*- α -L-rhamnopyranosyl-(1-2)-4-*O*-sulfo- β -D-arabinopyranoside, by the HMBC and HSQC experiments. HMBC correlations from the anomeric signal at δ 6.29 (Rha H-1) to δ 76.0 (Ara C-2) and from δ 4.64 (Ara H-1) to δ 82.3 (Agly C-1) were observed. Moreover, the deshielded chemical shifts observed in the HSQC spectrum for the arabinosyl C-4 and H-4 at δ 76.1 and δ 5.32 indicated that the OH group at C-4 of the arabinosyl moiety in **1** was acylated, which was supported by the upfield shifts of the arabinosyl C-3 and C-5 at δ 75.2 and 66.2, respectively. This substituent was confirmed to be a SO₃H group by the FABMS (fragment ion peaks at *m/z* 80 and 97, respectively). Therefore, compound **1** was characterized as (2*S*)-spirost-5-en-7-keto-1 β ,3 β -diol-1-*O*- α -L-rhamnopyranosyl-(1-2)-(4-*O*-sulfo)- α -L-arabinopyranoside.



Angudracanoside B (**2**) was obtained as white amorphous powder. The negative ion HRFABMS and ^{13}C NMR (DEPT) analyses indicated an empirical molecular formula of $\text{C}_{38}\text{H}_{58}\text{O}_{15}\text{S}$, which is two hydrogen atoms fewer than that of (25*R*)-ruscogenin-1-*O*- α -*L*-rhamnopyranosyl-(1-2)-4-*O*-sulfo- β -*D*-arabinopyranoside. The overall ^1H and ^{13}C NMR data of **2** and (25*R*)-ruscogenin-1-*O*- α -*L*-rhamnopyranosyl-(1-2)-4-*O*-sulfo- β -*D*-arabinopyranoside were very similar. The structural difference between them was in the F ring, i.e., an *exo*-double bond between C-25 and C-27 in **2** replaced the C-27 methyl group in (25*R*)-ruscogenin-1-*O*- α -*L*-rhamnopyranosyl-(1-2)-4-*O*-sulfo- β -*D*-arabinopyranoside. The chemical shift values of the double bond carbons [δ 108.7 (CH₂) and 144.7 (C)] were consistent with those of analogs reported in the literature.¹⁶ HMBC correlations of H-27 (δ 4.78) with C-24 (δ 29.0) and C-26 (δ 65.2) confirmed the presence of this double bond. Thus, compound **2** was formulated as spirost-5,25(27)-en-1 β ,3 β -diol-1-*O*- α -*L*-rhamnopyranosyl-(1-2)-(4-*O*-sulfo)- α -*L*-arabinopyranoside.

The molecular formula of angudracanoside B (**3**) was determined as $\text{C}_{38}\text{H}_{58}\text{O}_{16}\text{S}$ from the negative ion HRFABMS and ^{13}C NMR (DEPT) spectra, one oxygen atom more than that of **2**. The NMR chemical shifts of **3** were closely related to those of **2**, except for signals due to the B ring. Compared to **2**, the olefinic proton and carbons in the B ring were shifted downfield to δ 6.03 and δ 129.0, 142.8 (versus δ 5.59 and δ 125.0, 139.6 in **2**). In addition, the methylene carbon (δ 1.52/1.68, δ 32.2) linked to the olefinic carbon (C-6) disappeared and was replaced by an oxygenated methine carbon (δ 4.00 and δ 65.0). These data indicated the presence of an OH group at C-7, which was supported by HMBC correlations of H-7 (δ 4.00) with C-5 (δ 142.8), C-6 (δ 129.0) and C-8 (δ 42.8). The orientation of the 7-OH group was determined by the ROESY experiment. As H-7 showed strong NOE correlations with H-14 (δ 1.68), H-15 α (δ 2.50), and H-6 (δ 6.03) it must be α -oriented; hence the 7-OH group must be β -oriented. Other key ROE correlations of **3** support the structure of compound **3** as being (25*S*)-spirost-5-en-1 β ,3 β ,7 β -triol-1-*O*- α -*L*-rhamnopyranosyl-(1-2)-(4-*O*-sulfo)- α -*L*-arabinopyranoside (see Supporting Information).

Angudracanoside D (**4**) had molecular formula $\text{C}_{38}\text{H}_{58}\text{O}_{16}\text{S}$, as deduced from the negative ion HRFABMS and ^{13}C NMR (DEPT) spectra, the same as that of **3**, and one more oxygen atom than that of **2**. The NMR chemical shifts of **4** were similar to those of **2** with exceptions of signals due to the F ring. The difference between **4** and **2** was an additional OH at C-24 (δ 67.0), as indicated by a downfield shift of the C-27 olefinic carbon ($\Delta\delta$, -2.2 ppm). HMBC correlations of H-27 (δ 5.64, 5.10) with C-24 (δ 67.0) and C-25 (δ 149.4) and H-23 (δ 1.98, 2.40) with C-24 (δ 67.0), C-25 (δ 149.4), and C-22 (δ 111.8) were also observed, which confirmed the OH at C-24. In addition, the 24-H proton [δ 5.00 (dd, J = 6.3, 10.5 Hz)] was coupled to 23-H by 6.3 and 10.5 Hz. The J values implied that the 24-H was axial in orientation. Thus, compound **4** was deduced to be spirost-5,25(27)-en-1 β ,3 β ,24 α -triol-1-*O*- α -*L*-rhamnopyranosyl-(1-2)-(4-*O*-sulfo)- α -*L*-arabinopyranoside.

Angudracanoside D (**5**) had molecular formula $C_{32}H_{50}O_{12}S$. In the ^{13}C NMR and DEPT spectra of **5** (Table 1), 32 carbon signals were observed including 27 resonances assigned to a steroidal skeleton and five resonances assigned to a sugar. Acid hydrolysis of **5** confirmed the presence of L -arabinose in the sugar residue. The sulphate group attached to C-4 of the arabinopyranosyl unit, which in turn was attached to the OH at C-1 of the aglycone, was confirmed by comparison of the 1H and ^{13}C NMR data with those of compounds **1-4** (Tables 1 and 2). The aglycone of **5** was identified as 7-hydroxy-(25*S*)-ruscogenin by comparison of its NMR data with those of compounds **3** and **1**, as it shared the same A/B/C/D/E rings as those of **3** and the same F-ring as that of **1**. 2D-NMR experiments including HMBC and ROESY spectra were also performed to facilitate the resonance assignments. Thus, the structure of compound **5** was determined to be (25*S*)-spirost-5-en-1 β ,3 β , 7 β -triol-1-*O*-(4-*O*-sulfo)- α -*L*-arabinopyranoside.

The molecular formula of angudracanoside E (**6**) was deduced as $C_{37}H_{58}O_{12}$ from its quasi-molecular ion peak at m/z 693.3853 (calcd. for $C_{37}H_{58}O_{12}$ 693.3850) in the HRFABMS and the ^{13}C NMR (DEPT) spectrum. Comparison of the 1H and ^{13}C NMR spectra of **6** with those of (25*S*)-ruscogenin-1-*O*- α -*L*-rhamnopyranosyl-(1-2)- β -*D*-xylopyranosyl-(1-3)- α -*L*-arabinopyranoside²⁰ showed that both compounds possess the same aglycone with a sugar moiety at C-1. The only difference was that compound **6** lacked a set of ^{13}C NMR resonances corresponding to a α -*L*-rhamnopyranosyl unit. Acid hydrolysis of **6** yielded L -arabinose and D -xylose as the sugar residues. HMBC correlation of the anomeric proton of xylose (δ 4.77) with C-3 of the arabinose (δ 84.0) confirmed the interglycosidic linkage. Therefore, compound **6** was determined to be (25*S*)-ruscogenin-1-*O*- β -*D*-xylopyranosyl-(1-3)- α -*L*-arabinopyranoside.

The isolated steroidal saponins were tested for their activity against the fungal pathogens *Candida albicans*, *C. glabrata*, *C. krusei*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*. Only compounds **6** and **7** exhibited antifungal activity selectively against *C. neoformans*, with minimum inhibitory concentrations (IC₅₀s) of 9.5 and 20.0 μ g/mL, respectively. Amphotericin B was used as a positive control and exhibited an IC₅₀ of 2.5 μ g/mL against *C. neoformans*. It was noted that both compounds possess (25*S*)-ruscogenin as their aglycone, and a different disaccharide sugar moiety at C-1. This study has further demonstrated that the antifungal activity of the C27 steroidal saponins is associated with individual aglycone and disaccharide sugar moieties at C-1.

It was interesting to note that the structures of steroidal saponins isolated in this study differed from those isolated from the same species that was collected in a different location.⁷ There was no single saponin that was isolated in both studies, although most saponins shared the basic 1,3-dihydroxylated steroidal skeleton with varying oxidation levels at different positions. The most distinguishing feature may be the presence of a sulphate group in the sugar moieties of some saponins isolated by us, while the saponins isolated by the Vietnam group had acetylated sugar moieties and a β -*D*-fucopyranosyl unit attached to the OH group at C-24 on the F-ring. It appears that geographic location, season of collection, and/or environmental conditions contribute to the chemical diversity of this plant species.

Experimental Section

General Experimental Procedures

Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter. IR (KBr) spectra were measured on a Bio-Rad FTS-135 spectrophotometer. NMR spectra were measured in pyridine- d_5 solution and recorded on a Bruker DRX-500 instrument (500 MHz for 1H NMR, and 125 MHz for ^{13}C NMR) at 25 °C, using TMS as an internal standard. Mass spectra were recorded on a VG Auto Spec-3000 mass spectrometer using glycerol as

matrix. Column chromatography (CC) was performed on Diaion HP20SS (Mitsubishi Chemical Co.), RP-18 (40-63 μm , Merck Chemical Co.), Sephadex LH-20 (Mitsubishi Chemical Co.), and Chromatorex ODS (100-200 mesh, Fuji Silysia Chemical Co. Ltd). Precoated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC. Detection was done by spraying the plates with 10% sulfuric acid, followed by heating. GC analysis was run on an Agilent Technologies HP5890 gas chromatograph equipped with a H_2 flame ionization detector. The column was a 30QC2/AC-5 quartz capillary column (30 m \times 0.32 mm) using the following conditions: column temperature: 180 $^\circ\text{C}$ /280 $^\circ\text{C}$; programmed increase, 3 $^\circ\text{C}/\text{min}$; carrier gas: N_2 (1 mL/min); injection and detector temperature: 250 $^\circ\text{C}$; injection volume: 4 μL , split ratio: 1/50. Silica gel (200-300 mesh and 10-40 μm) and reversed phase silica gel RP-8 (40-63 μm) were used for CC.

Plant Material

Fresh stems of *D. angustifolia* were collected in Xishuangbanna, Yunnan, China, during July 2005. The sample was identified by Prof. Chong-Ren Yang. A voucher specimen has been deposited at the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation

The chipped fresh stems of *D. angustifolia* (5.0 kg) were extracted with MeOH under reflux. The MeOH extract was condensed under reduced pressure. The viscous concentrate was partitioned between H_2O and *n*-BuOH. The *n*-BuOH fraction (27.938 g) was subjected to CC on highly porous absorption resin (Diaion HP-20) eluting with MeOH- H_2O (0:1-1:0) to afford five fractions (A-E). Fraction A (1.201 g) was chromatographed on MCI gel eluting with MeOH- H_2O (0:1-1:0), and then on silica gel with CHCl_3 -MeOH- H_2O (9:1:0.1-7:3:0.5), to give compounds **6** (22 mg), (25*S*)-spirost-5-en-1 β ,3 β -diol-1-sulphate (21 mg), (25*S*)-spirost-5-en-1 β ,3 β -diol-1-sulphate (29 mg), 3,4,5-trimethoxyphenyl-1-*O*- β - D -glucopyranoside (21 mg) and 3,4,5-trimethoxy-phenyl-1-*O*- α - L -rhamnopyranosyl-(1-6)- β - D -glucopyranoside (29 mg). Fraction B (2.113g) was subjected to RP-18 CC eluting with MeOH- H_2O (4:6-1:0) and silica gel CC eluting with CHCl_3 -MeOH- H_2O (9:1:0.1-7:3:0.5) to give **4** (4 mg) and **5** (20 mg). Fraction C (5.30 g) was subjected to ODS CC eluting with MeOH- H_2O (6:4-1:0), Sephadex LH-20 eluting with MeOH- H_2O (0:1-6:4), and silica gel CC with CHCl_3 -MeOH- H_2O (8:2:0.2-7:3:0.5) to give **1** (14 mg), **3** (274 mg), **2** (42 mg) and (25*S*)-ruscogenin-1-*O*- α - L -rhamnopyranosyl-(1-2)-*O*- β - D -xylopyranosyl-(1-3)- α - L -arabinopyranoside (14 mg). Fraction D (421 mg) was subjected to RP-18 CC eluting with MeOH- H_2O (4:6-1:0), Sephadex LH-20 eluting with MeOH- H_2O (0:1-7:3), and silica gel CC with CHCl_3 -MeOH- H_2O (8:2:0.2-7:3:0.5) to give 26-*O*- β - D -glucopyranosyl-furosta-5-ene-1 β ,3 β ,22 ζ ,26-tetrol-1-*O*- α - L -rhamnopyranosyl-(1-2)-*O*- β - D -xylopyranosyl-(1-3)- α - L -arabinopyranoside (29 mg) and terreside (95 mg); Fraction I (1.06 g) was subjected to RP-18 eluting with MeOH- H_2O (3:7-1:0) and silica gel CC with CHCl_3 -MeOH- H_2O (8:2:0.2-7:3:0.5) to give (25*S*)-ruscogenin-1-*O*- α - L -rhamnopyranosyl-(1-2)-*O*- β - D -xylopyranosyl-(1-3)- α - L -arabinopyranoside (4 mg) and alliospiroside A (**7**) (5 mg).

Angudracanoid A (1): white amorphous powder; $[\alpha]_{\text{D}}^{22} - 120.8$ (*c* 0.40, pyridine); IR (KBr) ν_{max} : 3441 (OH), 2907 (CH), 1655 (C=O), 1227 (S-O), 983, 918, 897, 838 (intensity 918>897, (25*S*)-spiroketal) cm^{-1} ; ^1H and ^{13}C NMR (pyridine-*d*₅): see Tables 1 and 2; FABMS (negative ion mode): m/z 801 $[\text{M} - 1]^-$. HRESIMS (negative ion mode): m/z 801.3379 $[\text{M} - \text{H}]^-$ (calcd. for $\text{C}_{38}\text{H}_{57}\text{O}_{16}$, 801.3367).

Angudracanoid B (2): white amorphous powder; $[\alpha]_{\text{D}}^{22} - 88.2$ (*c* 0.14, pyridine); IR (KBr) ν_{max} : 3439(OH), 2908 (CH), 1228 (S-O), 1092, 1050, 983, 917, 899, 837 cm^{-1} ; ^1H and ^{13}C

NMR (pyridine-*d*₅): see Tables 1 and 2; FABMS (negative ion mode): *m/z* 785 [M - H]⁻; HRESIMS (negative ion mode): *m/z* 785.3430 [M - H]⁻ (calcd. for C₃₈H₅₇O₁₅S, 785.3418).

Angudracanoside C (3): white amorphous solid, $[\alpha]_D^{22} - 108.0$ (*c* 0.11, pyridine). IR (KBr) ν_{\max} : 3425 (OH), 2935 (CH), 1229 (S-O), 1049, 983, 918, 900, 850 cm⁻¹; ¹H and ¹³C NMR (pyridine-*d*₅): see Tables 1 and 2; FABMS (negative ion mode): *m/z* 801 [M - H]⁻; HRESIMS (negative ion mode): *m/z* 801.3357 [M - H]⁻ (calcd. for C₃₈H₅₇O₁₆S, 801.3367).

Angudracanoside D (4): white amorphous solid; $[\alpha]_D^{22} - 71.9$ (*c* 0.28, pyridine); IR (KBr) ν_{\max} : 3439(OH), 2908 (CH), 1224 (S-O), 1092, 1050, 983, 917, 899, 837 cm⁻¹; ¹H and ¹³C NMR (pyridine-*d*₅): see Tables 1 and 2; FABMS (negative ion mode): *m/z* 801 [M - H]⁻; HRESIMS (negative ion mode): *m/z* 801.3382 [M - H]⁻ (calcd. for C₃₈H₅₇O₁₆S, 801.3367).

Angudracanoside E (5): white amorphous powder; $[\alpha]_D^{22} - 44.9$ (*c* 0.05, pyridine); IR (KBr) ν_{\max} : 3439 (OH), 2927 (CH), 1217 (S-O), 1053, 986, 918, 899, 849 [intensity 912>899, 25(*S*)-spiroketal] cm⁻¹; ¹H and ¹³C NMR (pyridine-*d*₅): see Tables 1 and 2; FABMS (negative ion mode): *m/z* 657 [M - H]⁻. HRESIMS (negative ion mode): *m/z* 657.2947 [M - H]⁻ (calcd. for C₃₂H₄₉O₁₂S, 657.2944).

Angudracanoside F (6): white amorphous powder, $[\alpha]_D^{22} - 85.1$ (*c* 0.09, pyridine). IR (KBr) ν_{\max} : 3425(OH), 2920 (CH), 986, 919, 895, 873(intensity 919>895, (25*S*)-spiroketal) cm⁻¹. ¹H and ¹³C NMR (pyridine-*d*₅): see Tables 1 and 2; FABMS (negative ion mode): *m/z* 694 [M - 1]⁻. HRESIMS (negative ion mode): *m/z* 693.3853 [M - H]⁻ (calcd. for C₃₇H₅₇O₁₂, 693.3850).

Acid Hydrolysis of 1-6: Compounds **1** (10 mg), **2-5** (5 mg), and **6** (8 mg) in 1M HCl-dioxane (1:1, v/v, 5 mL) were heated at 85° in a water bath for 6 h, respectively. The reaction mixtures were partitioned between CHCl₃ and H₂O four times. The aqueous layers were passed through Amberlite IRA-401 (OH⁻ form), and the eluate was concentrated to dryness to give a saccharide mixture. Solutions of the sugar residues of these compounds in 1.5 mL of pyridine were added to L-cysteine methyl ester hydrochloride (1.0 mg) and kept at 60 °C for 1h, respectively. Trimethylsilylimidazole (1.5 mL) was added to the reaction mixtures and they were kept at 60 °C for 30 min. The supernatants (4 μL) were analyzed by GC, respectively.²¹ The monosaccharides of compounds **1-4** were determined by GC analysis of the derivatives to be L-arabinose and L-rhamnose, of which the retention times were 14.250 min and 15.823 min, respectively. The monosaccharide of compound **5** was determined to be L-arabinose, of which the retention times were 14.449 min. The monosaccharides of compound **6** were determined to be L-arabinose and L-xylose, of which the retention times were 14.250 min and 14.474 min, respectively.

Antifungal and Antibacterial Bioassay—Susceptibility testing was performed using a modified version of the NCCLS methods^{22,23} using organisms obtained from the American Type Culture Collection (Manassas, VA) including *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906. The detailed procedures have been described in a previous paper.²⁴

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

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Table 1

 ^{13}C NMR Spectroscopic Data of Compounds **1-6** ($\text{C}_5\text{D}_5\text{N}$)^a

C	1	2	3	4	5	6
1	82.3	83.7	83.5	83.7	82.9	83.5
2	37.3	37.4	37.6	37.3	37.3	37.8
3	67.7	68.2	68.0	68.1	68.0	68.2
4	43.6	43.9	44.3	43.9	43.7	43.9
5	162.7	139.6	142.8	139.5	142.6	139.6
6	128.1	125.0	129.0	124.9	128.5	124.8
7	202.0	32.2	65.0	32.1	64.6	32.1
8	42.0	33.2	42.8	33.1	42.2	33.2
9	50.1	50.4	50.8	50.2	50.4	50.4
10	43.6	43.1	44.1	43.0	43.7	43.0
11	23.8	24.0	24.2	24.0	23.5	24.0
12	40.0	40.2	40.2	40.1	40.1	40.5
13	40.8	40.2	40.4	40.0	40.1	40.3
14	49.0	56.7	39.2	56.6	40.0	56.9
15	34.4	32.5	32.8	32.4	32.4	32.5
16	81.6	81.5	82.2	81.9	81.5	81.3
17	62.0	62.9	63.4	62.4	63.0	62.9
18	16.8	16.9	17.0	16.6	16.4	16.8
19	13.3	15.1	15.4	15.0	14.9	15.1
20	42.6	42.0	42.4	42.1	42.2	42.6
21	15.0	14.9	14.4	14.9	15.0	14.9
22	109.7	109.5	109.8	111.8	109.8	109.8
23	26.5	33.4	33.7	43.6	26.5	26.5
24	26.3	29.0	29.4	67.3	26.3	26.3
25	27.7	144.7	145.0	149.4	27.6	27.7
26	65.2	65.2	65.3	64.6	65.7	65.2
27	16.5	108.7	108.9	106.5	16.7	16.4
Att-1'	101.8	100.3	100.3	100.3	102.3	102.3

C	1	2	3	4	5	6
2'	76.0	75.9	76.3	76.2	72.9	71.7
3'	75.2	75.2	75.5	75.2	74.0	84.0
4'	76.1	76.4	76.3	76.2	76.4	69.3
5'	66.2	65.7	66.3	66.3	65.4	67.3
Rha-1''	100.3	101.3	101.3	101.7		
2''	72.4	72.6	72.9	72.6		
3''	72.4	72.5	72.8	72.6		
4''	74.3	74.7	74.7	74.5		
5''	69.6	69.5	69.4	69.8		
6''	19.0	19.0	19.0	19.0		
Xyl-1''						106.7
2''						75.5
3''						78.3
4''						71.2
5''						67.3

^a Assignments were based on DEPT, ¹H-¹H COSY, HMQC, and HMBC experiments.

Table 2

¹H NMR Signals of Compounds 1-6 (in C₅D₅N, δ in ppm and J in Hz)^a.

C	1	2	3	4	5	6
1	3.71 dd (11.7, 3.8)	3.76 dd (12.5, 3.0)	3.76 dd (11.7, 3.8)	3.78 dd (11.7, 2.6)	3.77 dd (11.0, 3.5)	3.87 dd (11.5, 3.5)
2	2.78 br d (11.7)	2.72 m	2.64 br d (11.7)	2.66 m	2.66 m	2.82 br d (11.5)
3	2.37 br d (11.7)	2.33 br d (12.5)	2.28 br d (11.7)	2.32 br d (11.7)	2.02 q-like (11.9)	2.16 br d (11.7)
4	3.86 m	3.77 m	3.84 m	3.85 m	3.82 m	3.85 m
5	2.21 br d (12.1)	2.74 br d (9.0)	2.51 dd (12.0, 4.8)	2.56 br d (12.1)	2.70 dd (11.5, 3.7)	2.60 dd (11.8, 4.4)
6	2.68 m	2.67 m	2.74 br d (12.0)	2.66 m	2.59 q-like (11.5)	2.69 br d (11.8)
7	5.94 s	5.59 br d (5.5)	6.03 br d (5.2)	5.55 br d (5.2)	6.02 br d (6.5)	5.57 br d (5.2)
8	-	1.68 m, 1.52 m	4.00 br s	1.83 m, 1.67 dd (11.9, 7.9)	4.02 br s	1.48 m, 1.88 dd (11.9, 4.7)
9	2.49 dd (4.5, 5.0)	1.75 m	2.18 m	1.49 m	2.09 m	1.72 m
10	1.88 m	1.46 m	2.06 m	1.47 m	2.06 m	1.46 m
11	1.68 m, 2.97 brd (10.6)	1.52 m, 2.87 br d (11.5)	1.74 m, 2.95 br d (10.6)	1.51 m, 2.86 br d (10.8)	1.50 m, 2.84 br d (11.5)	1.55 m, 2.88 br d (11.5)
12	1.52 m, 1.06 m	1.40 m, 1.21 ddd (11.5, 11.5, 3.6)	1.40 m, 1.28 ddd (10.6, 10.6, 4.0)	1.38 m, 1.20 ddd (11.8, 11.8, 3.6)	1.43 m, 1.17 m	1.69 m, 1.42 m
13	1.60 m	1.06 dd (7.0, 12.1)	1.68 dd (6.8, 12.1)	1.34 m	1.68 m	1.06 m
14	3.24 m, 1.65 m	1.46 m, 1.97 dd (8.0, 12.1)	2.50 m, 1.51 ddd (12.5, 6.8, 8.6)	1.98 m, 1.38 m	2.64 m, 1.54 m	1.46 m, 1.99 ddd (6.7, 12.0, 7.3)
15	4.50 q-like (8.6)	4.47 q-like (9.0)	4.59 q-like (8.6)	4.46 q-like (8.5)	4.54 q-like (7.0)	4.49 q-like (7.3)
16	1.67 m	1.67 m	1.64 m	1.66 m	1.79 dd (7.0, 6.5)	1.70 m
17	0.80 s	0.81 s	0.88 s	0.80 s	0.90 s	0.81 s
18	1.51 s	1.46 s	1.44 s	1.40 s	1.17 s	1.40 s
19	2.11 t-like (6.8)	2.19 t-like (7.0)	1.91 t-like (6.5)	2.05 t-like (12.0)	2.12 m	1.91 t-like (6.8)
20	1.06 d (7.0)	1.02 d (7.0)	1.00 d (7.0)	1.03 d (6.8)	1.07 d (7.0)	1.05 d (7.0)
21	1.85 m, 1.41 m	2.67 m, 2.21 m	1.68 m, 1.60 m	1.98 dd (10.5, 12.3), 2.40 dd (6.3, 12.3)	2.12 m, 1.87 m	1.99 m, 1.85 m
22	1.41 m, 1.31 m	2.21 m, 1.88 m	2.60 m, 2.18 m	5.00 dd (6.3, 10.5)	1.43 m, 1.24 m	1.49 m, 1.33 m
23	1.48 m	-	-	-	1.30 m	1.50 m
24	4.00 br d (11.5)	4.41 br d (12.0)	4.37 br d (12.0)	4.47 br d (11.5)	3.98 br d (10.5)	4.04 br d (11.5)
25	3.32 d (11.5)	3.98 d (12.0)	3.94 d (12.0)	4.24 dd (11.5, 3.3)	3.28 d (10.5)	3.66 d (11.5)
26	1.03 d (6.3)	4.78 brs	4.77 br s	5.64 br s, 5.20 br s	1.04 d (7.0)	1.03 d (6.3)
27	4.64 d (7.5)	4.63 d (7.5)	4.59 d, 7.5	4.72 d (7.5)	4.57 d (7.0)	4.77 d (7.5)

C	1	2	3	4	5	6
2'	4.52 dd (9.2, 7.5)	4.51 dd (9.0, 7.5)	4.63 dd (8.2, 7.5)	4.61 dd (9.3, 7.5)	4.32 dd (9.0, 7.0)	4.56 dd (8.2, 7.5)
3'	4.23 dd (9.2, 3.6)	4.24 dd (9.0, 3.5)	4.22 dd (8.2, 4.5)	4.21 dd (9.3, 3.5)	4.20 dd (9.0, 3.0)	4.19 dd (8.2, 3.5)
4'	5.32 m	5.29 m	5.27 m	5.27 m	5.48 br s	4.46 br d (3.1)
5'	4.65 dd (2.0, 12.0)	4.72 dd (12.0, 2.5)	4.63 m	4.64 dd (11.5, 2.1)	4.76 dd (12.0, 2.2)	4.29 br d (12.3)
	3.71 brd (12.0)	3.69 br d (12.0)	3.58 br d (12.5)	3.68 br d (11.5)	3.63 d (12.0)	3.71 dd (12.3, 3.1)
Rha-1''	6.29 brs	6.22 brs	6.27 br s	6.30 br s	-	5.30 d (7.5)
2''	4.65 m	4.61 m	4.71 br d (3.0)	4.67 br d (3.5)	-	4.01 dd (7.5, 8.2)
3''	4.61 m	4.64 dd (3.5, 9.1)	4.63 dd (3.0, 9.0)	4.63 dd (3.5, 10.1)	-	4.22 dd (3.5, 10.1)
4''	4.54 dd (8.4, 8.4)	4.31 dd (9.1, 9.1)	4.35 dd (9.0, 9.0)	4.21 m	-	4.15 m
5''	4.80 m	4.77 m	4.83 dq (9.0, 6.0)	4.80 dq (10.1, 6.0)	-	4.35 dd (11.0, 4.6)
6''	1.75 d (6.0)	1.71 d (6.0)	1.75 d (6.0)	1.72 d (6.4)	-	3.75 dd (11.0, 5.6)

^a Assignments were based on DEPT, HMQC, and HMBC experiments.