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Two New Cytotoxic C-21 Steroidal Glycosides from the Root of *Cynanchum auriculatum*

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Abstract—Guided by in vitro antineoplastic tests, two new cytotoxic C-21 steroidal glycosides, auriculosides A (**1**) and B (**2**), were isolated from the root of *Cynanchum auriculatum*. Their structures were determined on the basis of chemical evidence and extensive spectroscopic methods including one-dimensional and two-dimensional NMR. These compounds showed cytotoxic activities against PC_3, Hce-8693, Hela, and PAA cell lines.

Introduction

Species of genus Cynanchum (Asclepiadaceae) have been extensively studied for their bioactive C/D-*cis*-polyoxypregnane glycoside constituents.[1] The root of *Cynanchum auriculatum* Royle ex Wight (Asclepiadaceae) is a famous traditional tonic in the Oriental System of Medicine and has been used for nourishing the blood and prolonging life. Chen et al. reported the isolation of seven C-21 steroidal glycosides from this plant.[2] Pharmacological experiments indicated the crude glycoside from *C. auriculatum* had antineoplastic and immunopotentiating activities.[3,4] By screening using antineoplastic tests in vitro, chemical studies on the roots of this plant were undertaken and we have obtained two new cytotoxic steroidal glycosides named auriculosides A (**1**) and B (**2**) (Fig. 1).

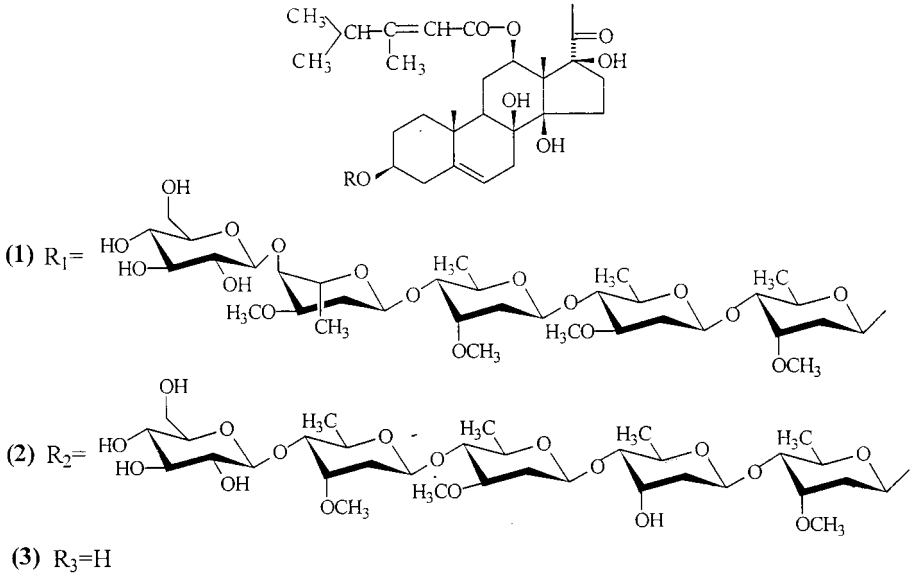

Figure 1. The chemical structures of **1** and **2**.

Keywords: antineoplastic tests; *Cynanchum auriculatum*; cytotoxic activities.
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Results and Discussion

The crude glycoside from *C. auriculatum* was chromatographed on silica gel by eluting with $CHCl_3$-MeOH (50:1, 30:1, 15:1, 5:1, V/V successively), and divided into five fractions. In the course of screening for cytotoxic activities using the MTT method, only fraction Fr2 exhibited more powerful cytotoxic activities than the crude glycoside. Further separation of the constituents of this fraction by HPLC led to the isolation of two new active glycosides, auriculosides A (**1**) and B (**2**). We have undertaken the structure elucidation of **1** and **2** as described below.

Auriculoside A (**1**) shows positive Lieberman–Buchard and Keller–Kiliani reactions suggesting the presence of a glycoside with 2-deoxy sugars. The negative HR-FAB-MS spectrum of compound **1** showed the molecular ion at *m/z* 1227.6495 in agreement with the molecular formula $C_{62}H_{100}O_{24}$ (calcd 1227.6526). Acidic hydrolysis of **1** afforded a mixture of sugars and the aglycone (**3**), which was identical with caudatin. ^1H and ^{13}C NMR assignments for **3** based on the analysis of NMR spectral data (proton, carbon, DEPT, ^1H–^1H COSY, HMQC, HMBC) were identical to those in the literature,[5] confirming that **3** is caudatin.

Inspection of the NMR spectral data of compound **1** (proton, carbon, DEPT, HMQC, HMBC) showed that it contained five anomeric carbons at δ 96.5 (Sa-C1), 101.9 (Sb-C1), 100.5 (Sc-C1, 97.4 (Sd-C1), 102.3 (Se-C1), corresponding to anomeric proton signals at δ 5.27 (1H, br d, J=11.3 Hz), 4.66 (1H, br d, J=9.7 Hz), 5.11 (1H, br d, J=9.7 Hz), 5.06 (1H, br d, J=2.6 Hz), 5.01 (1H, br d, J=7.7 Hz) respectively, which indicated that there were five sugar units in **1** with one α-linkage and four β-linkages. This is in good agreement with the FAB-MS spectral fragmentation pattern of **1**, which showed *m/z*: 1251 $[M+Na]^+$, 1071 $[M+Na-180]^+$, 761 $[M+1-180-144\times2]^+$, 617 $[M+1-180-144\times3]^+$, 473 $[M+1-180-144\times4]^+$, suggesting the presence of four hexose units. The assignments of the NMR data for the aglycone were recognized readily on the basis of NMR data (proton, carbon, DEPT, HMQC, HMBC), and glycosidation shifts of its carbon signals were observed at C-2 (−1.8 ppm), C-3 (+4.5 ppm), C-4 (−3.5 ppm), indicating that the sugar moiety is linked to the hydroxyl group at C-3 of **3**. Therefore, the remaining part of the NMR data of **1** attributed to the fragment of $C_{34}H_{60}O_{17}$ must belong to the pentasaccharide moiety of **1**. Although most of the signals at high field (δ 1.26–2.50, 3.39–3.60, 3.96–4.26) in the ^1H NMR spectrum, and middle-to-high field (δ 18.2–18.7, 37.1–37.2, 56.5–59.0, 78.5–79.2) in the ^{13}C NMR spectrum of **1** were severely overlapped, they could be readily recognized to be the resonances of the methyl, methine, methoxyl, and oxygenated methine groups respectively. The above five anomeric protons and two groups of well defined oxygenated methine protons (δ 3.36, 3.42) were used as starting points to assign the other proton and carbon atoms, the sugar linkage pattern, and the sugar sequence of **1** by examination of the long-range connectivity given in the HMBC[6] diagram and the heteronuclear correlation from the cross-sections in the HMQC[7] diagram. The heteronuclear correlation (HMQC) and the long-range correlation (HMBC) of the anomeric protons (Sa-H1: δ 5.27, Sb-H1: δ 4.66, Sc-H1: δ 5.11, Sd-H1: δ 5.06, Se-H1: δ 5.01) readily enabled the recognition of five fragments each composed of three carbons, one belonging to the aglycone or other sugar where the glycoside bonds were located, except for Sd-H1 which gave one more correlation with Sd-C5. Inspecting the HMQC and HMBC data of the protons at δ 3.51 (Sa-H4), 3.44 (Sb-H4), 3.36 (Sc-H4), and 3.42 (Sd-H4), allowed the addition of three more carbons and the corresponding connected protons to the above 3-carbon fragments except for the one containing C3 of the aglycone. The analysis of the ^1H–^{13}C long-range correlation of the protons at δ 4.07 (Sa-H3), 4.20 (Sb-H3), 3.99 (Sc-H3), 3.96 (Sd-H3) linked the above four 6-carbon fragments together, completing the NMR assignments for D-cymarose, D-oleandrose and L-cymarose, and the determination of the sugar sequence (D-glc→L-cym→D-cym→D-ole→D-cym→aglycone) and linkage (1→4). The internal D-cymarose was evident from the ^{13}C NMR assignments that were identical to those in other steroidal glycosides.[8] The ^{13}C NMR of the second sugar were similar to those for the internal D-cymarose except for the discrepancy between anomeric carbons caused by different chemical environments. However, the striking difference between Sa-H5 (δ 4.22) and Sb-H5 (δ 3.46) caused us to speculate that either the Sb-C3 methoxyl or Sb-H5, or both must be equatorial in order to reduce the γ-deshielding effect which caused Sa-H5 to be downfield relative to Sb-H5. Among those 2,6-dideoxy sugars, only D-oleandrose matches the above structural peculiarity. Additionally, Warashina et al. also reported the obvious difference between the chemical shifts of C5 in D-cymarose and D-oleandrose while the ^{13}C NMR data are relatively close.[9] Therefore, this sugar was deduced to be D-oleandrose on the basis of the above ^1H NMR evidence together with the ^{13}C NMR assignments in agreement with those in other compounds.[8,9] The central sugar could be readily discerned as D-cymarose because its NMR assignments were similar to those of the internal D-cymarose. The fourth sugar in the pentasaccharide chain, differs from D-cymarose and D-oleandrose, as was evident from the analysis of NMR data. The obvious upfield shifts were observed at Sd-C1-5 compared with Sa-C1-5, insinuating that an L-cymarose existed.

The NMR assignments for the terminal D-glucose were carried out on the basis of HMBC correlation (H6α→Se-C4, C-5, H6β→Se-C4; Se-H3→Se-C1, C2, C4), and were consistent with those in literature.[8]

The long-range correlation between Sa-H1 (δ 5.27) and C3 (δ 77.7), H3 (δ 3.85) and Sa-C1 (δ 96.5) further supported the above conclusion that the pentasaccharide was attached to the aglycone at the C3 hydroxyl group.

Therefore, compound **1** was concluded to be caudatin 3-*O*-β-D-glucopyranosyl-α-L-cymaropyranosyl-β-D-cymaropyranosyl-β-D-oleandropyranosyl-β-D-cymaropyranoside.

Auriculoside B (**2**), an amorphous powder, possessed the molecular formula $C_{61}H_{98}O_{24}$ on the basis of HR-FAB-MS. On acid hydrolysis, caudatin was obtained following TLC comparison with standard sample. The sugar moiety contained three methoxyl methyl signals at δ 3.45, 3.49, 3.53 (3H, each, s) and five anomeric proton signals at δ 5.45 (1H, br d, J=9.3 Hz), 5.21 (1H, br d, J=10.4 Hz),

4.64 (1H, br d, J=9.8 Hz), 5.14 (1H, br d, J=9.4 Hz), and 4.90 (1H, br d, J=7 Hz), corresponding to carbon signals at δ 96.4 (Sa-C1), 98.4 (Sb-C1), 102.0 (Sc-C1), 99.8 (Sd-C1), and 106.6 (Se-C1). This indicated that there were five sugar units in **2** all with β-linkages. Following the same methodology as described for **1**, the NMR assignments of the sugar moiety were obtained from HMQC and HMBC analysis using the well defined signals of the five anomeric protons above and three oxygenated methine protons at δ 3.37 (1H, br d, J=9.7 Hz), 3.46 (1H, br d, J=9.3 Hz), 3.65 (1H, br d, J=9.8 Hz) as starting points. The sugar sequence of **2** was also confirmed by HMBC NMR correlation δ 5.45 (1H, br d, J=9.3 Hz, H-1 of β-D-cymaropyranose) to δ 77.7 (C-3 of aglycone), and δ 5.21 (1H, br d, J=9.8 Hz, H-1 of β-D-digitoxopyranose) to δ 82.6 (C-4 of β-D-cymaropyranose), δ 4.64 (1H, br d, J=10.4 Hz, H-1 of β-D-oleandropyranose) to δ 83.2 (C-4 of β-D-digitoxopyranose), δ 5.14 (1H, br d, J=9.4 Hz, H-1 of β-D-cymaropyranose) to δ 83.2 (C-4 of β-D-oleandropyranose), δ 4.90 (1H, br d, J=7 Hz, H-1 of β-D-glucopyranose) to δ 83.4 (C-4 of β-D-cymaropyranose). This is in good agreement with the FAB-MS spectral fragmentation pattern of **2**, which showed *m/z*: 1237[M+Na]$^+$, 909 [M+1−162−144]$^+$, 765[M+1−162−144×2]$^+$, 473[M+1−162−144×3−130−18]$^+$. In comparison with caudatin, the glycosidation shifts of the aglycone moiety in **2** were observed at C-2 (−1.8 ppm), C-3 (+4.5 ppm), C-4 (−3.6 ppm), which indicated that the sugar moiety was linked to the C-3 hydroxyl group of the aglycone. Consequently, the structure of **2** was confirmed as caudatin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-cymaropyranoside.

By using MTT assay, auriculosides A and B were shown to have significant cytoxicity against four solid tumor cell lines—human cecum undifferentiated adenocarcinoma cell (Hce-8693), human prostatic carcinoma cell (PC_3), human cervical squamous carcinoma cell (Hela), human lung carcinoma cell (PAA).

Experimental

General methods

Melting points were determined on a Shimadzu LIBROR AEC-200 instrument. Optical rotations were measured in EtOH with a HORIBA SPEA-300 polarimeter. UV spectra were performed on a Shimadzu UV-220 spectrometer. IR spectra were obtained for a KBr pellet on a Perkin–Elmer 577 spectrometer. ^{1}H NMR, ^{13}C NMR, DEPT, HMQC and HMBC spectra were recorded at 500 MHz for ^{1}H, and 125 MHz for ^{13}C with a Bruker DRX 500 instrument, using tetramethylsilane as internal standard in C_5D_5N solution. FAB-MS was recorded on a VG AUTOSPEC 800 mass spectrometer with glycerol as matrix. Thin Layer Chromatography was performed on precoated Kieselgel 60 F_{254} plates and Rp-18 (Merk), and detection was achieved by spraying with 10% H_2SO_4 followed by heating. HPLC was carried out using a Shimadzu LC-6A instrument (ODS column 2.5×21 cm^2, RID-6A refractive index detector).

Plant material

Plant material used in this research was obtained from Sichuan province in China and identified as the root of *Cynanchum auriculatum* Royle ex Wight by Prof. Zhang Zhi-Guo (Institute of Materia Medica, Zhejiang Academy of Medical Sciences, Hangzhou).

Extraction and isolation

The root of *Cynanchum auriculatum* (10 kg) was extracted with MeOH three times under reflux for 2 h. The MeOH extract was evaporated in vacuo, yielding a black residue (880 g), which was extracted with $CHCl_3$ two times under reflux for half an hour. A dark brown residue (320 g) given by concentration of the $CHCl_3$ extract was poured into hexane. The hexane insoluble portion corresponded to a crude glycoside (305 g), which showed positive Lieberman–Burchard and Keller–Kiliani reactions. The crude glycoside (280 g) was subjected to column chromatography on silica gel eluting with $CHCl_3$-MeOH (50:1, 30:1, 15:1, 5:1, V/V) successively to give five fractions. Fraction 2 (8.0 g) was rechromatographed on silica gel and separated by HPLC with MeOH-H_2O (3:1) to afford auriculoside A (**1,** 497 mg), and B (**2**, 106 mg).

Auriculoside (**1**). An amorphous powder, mp 151–154°C, $[\alpha]_D^{28}$=−32.1 (c=0.04, EtOH). UV(EtOH) λ_{max}(log ϵ): 222.5 (4.05) nm. IR(KBr)ν_{max}: 3449, 2971, 2935, 1713, 1645, 1225, 1165 cm^{-1}. HR-FAB-MS: 1227.6495 [($C_{62}H_{100}O_{24}$−H)$^-$, calcd 1227.6526]. FAB-MS *m/z*: 1251 [M+Na]$^+$, 1071 [1251-H_2O-glc]$^+$, 761 [M+1-H_2O-glc-cym-cym]$^+$, 617 [761-ole]$^+$, 473 [617-cym]$^+$. ^{1}H NMR, ^{13}C NMR and HMBC data are shown in Table 1.

Acidic hydrolysis of **1**. A solution of **1** (78 mg) in 0.1N H_2SO_4-MeOH 5 ml was refluxed for 30 min, then neutralized with aq. saturated $Ba(OH)_2$. The precipitate was filtered off. The filtrate was concentrated and chromatographed on a column of silica gel (15 g) with $CHCl_3$–MeOH (200:1, 100:1, 60:1) successively to obtain caudatin {**3**, 14 mg, an amorphous powder, mp 157–160/191–196°C. $[\alpha]_D^{28}$=+20.3 (c=0.5, $CHCl_3$). UV(EtOH) λ_{max}(log ϵ): 221.0 (4.20) nm. IR(KBr)ν_{max}: 3446, 2971, 2935, 1713, 1680, 1646 cm^{-1}. HR-FAB-MS: 491.3009 [($C_{28}H_{42}O_7$+H)$^+$, calcd 491.3008]. FAB-MS *m/z*: 363 [M+1−$C_7H_{12}O_2$]$^+$, 111[$C_7H_{11}O$]$^+$} and a sugar mixture.

Auriculoside B (**2**). An amorphous powder, mp 141–146°C, $[\alpha]_D^{28}$=+4.4 (c=0.16, EtOH). UV(EtOH) λ_{max}(log ϵ): 222.5 (4.04) nm. IR(KBr)ν_{max}: 3447, 2971, 2935, 1712, 1646, 1225, 1164 cm^{-1}. HR-FAB-MS: 1213.6337 [($C_{61}H_{98}O_{24}$-H)$^-$, calcd 1213.6370]. FAB-MS *m/z*: 1237 [M+Na]$^+$, 909 [M+1-glc-cym]$^+$, 765 [909-glc-cym-ole]$^+$, 473 [M+1-sugar chain-H_2O]$^+$. ^{1}H NMR, ^{13}C NMR and HMBC data are shown in Table 2.

Acidic hydrolysis of **2.** A solution of **2** (32 mg) in 0.1 N H_2SO_4–MeOH 3 ml was refluxed for 30 min, then neutralized with aq. saturated $Ba(OH)_2$. The precipitate was filtered off. The filtrate was concentrated and chromatographed on a column of silica gel (10 g) with $CHCl_3$–MeOH (200:1, 100:1, 60:1) successively to obtain a sugar

mixture and caudatin (**3**, 10 mg), which was identified by TLC comparison with authentic sample ($CHCl_3$:MeOH=9:1, R_f=0.42; Me_2CO:Petrol=2:3, R_f=0.43; Me_2CO:Hexane=1:1, R_f=0.53).

Table 1. The ^1H, ^{13}C NMR and ^1H-^{13}C long-range correlation (HMBC) data of compound **1**

	Aglycone moieties		
	^{13}C NMR	^1H NMR	HMBC
1α	39.0 (t)	1.09–1.12, m	C-2, C-10
1β		1.77–1.83, m	
2α	29.9(t)	1.77–1.83, m	C-1, C-3
2β		2.00–2.14, m	
3	77.7(d)	3.85, m	C-2, C-5, Sa-C-1
4α	39.3(t)	1.78, m	C-3, C-5
4β		2.41, m	
5	139.4 (s)		
6	119.2 (d)	5.28, br s	C-8, C-10
7α	34.9 (t)	2.01	
7β		2.12, m	C-8, C-9
8	74.3 (s)	[5.04, br s (OH)]	C-8, C-9, C-14
9	44.6 (d)	1.72, br d (12.9)	C-8, C-10, C-11
10	37.1 (s)		
11α	25.1 (t)	2.14, m	C-9, C-10, C-12
11β		2.25, m	
12	72.6 (d)	5.03, m	C-11, C-13, C-17, C-1′
13	58.0 (s)		
14	89.5 (s)	[6.12, s (OH)]	
15	33.9 (t)	2.07, m	C-14, C-16, C-17, C-18
16α	33.0 (t)	2.02, m	C-13, C-14, C-15, C-17,
16β		3.25, m	
17	92.4 (s)	[6.44, s (OH)]	C-13, C-17, C-20
18	10.8 (q)	1.97, s	C-12, C-13, C-14, C-17
19	18.2 (q)	1.31, s	C-1, C-9, C-10
20	209.5 (s)		
21	27.6 (q)	2.50, d (0.8)	C-20
1′	166.0 (s)		
2′	114.2 (d)	5.85, s	C-1′, C-3′, C-4′, C-7′
3′	165.5 (s)		
4′	38.2 (d)	2.41, m	C-7′, C-5′, C-6′
5′	21.0 (q)	0.94, d (7.6)	C-3′, C-4′, C-6′, C-7′
6′	20.9 (q)	0.96, d (7.6)	C-3′, C-4′, C-5′, C-7′
7′	16.5 (q)	2.26, s	C-2′, C-3′, C-4′
		Sugar moieties	
D-cym.			
Sa-C1	96.5 (d)	5.27, br d (11.3)	C3, Sa-C2
Sa-C2	37.5 (t)	1.89, m; 2.32, m	Sa-C1, Sa-C3
Sa-C3	78.1 (d)	4.07, br s	Sa-C1, Sa-C5
Sa-C4	83.4 (d)	3.51, m	Sa-C3, Sa-C5, Sa-C6, Sb-C1
Sa-C5	69.1 (d)	4.22, m	Sa-C1, Sa-C6
Sa-C6	18.7 (q)	1.38, d (6.5)	Sa-C4, Sa-C5
Sa-3-OCH_3	58.9 (q)	3.60, d (0.9)	Sa-C3
D-ole.			
Sb-C1	101.9 (d)	4.66, br d (9.7)	Sa-C4, Sb-C2
Sb-C2	37.2 (t)	1.63, m; 2.25, m	Sb-C1, Sb-C3
Sb-C3	78.7 (d)	4.20, m	Sb-C1, Sb-C4, Sb-C5
Sb-C4	83.1 (d)	3.44, m	Sb-C3, Sb-C5, Sb-C6, Sc-C1
Sb-C5	71.9 (d)	3.46, dd (2.3, 9.1)	Sb-C1, Sb-C6
Sb-C6	18.7 (q)	1.38, d (6.5)	Sb-C4, Sb-C5
Sb-3-OCH_3	56.5 (q)	3.33, d (0.8)	Sb-C3
D-cym.			
Sc-C1	100.5 (d)	5.11, br d (9.7)	Sb-C4, Sc-C2
Sc-C2	37.5 (t)	1.78, m; 2.29, m	Sc-C1, Sc-C3
Sc-C3	77.8 (d)	3.99, m	Sc-C4, Sc-C4
Sc-C4	81.7 (d)	3.36, br d (8.95)	Sc-C3, Sc-C5, Sc-C6, Sd-C1
Sc-C5	69.0 (d)	4.16, m	Sc-C1, Sc-C6
Sc-C6	18.6 (q)	1.37, d (6.5)	Sc-C4, Sc-C5
Sc-3-OCH_3	59.0 (q)	3.55, d (0.9)	Sc-C3
L-cym.			
Sd-C1	97.4 (d)	5.06, br d (2.6)	Sc-C4, Sd-C2, Sd-C5
Sd-C2	34.9 (t)	1.78, m; 2.32, m	Sd-C1, Sd-C3
Sd-C3	73.7 (d)	3.96, m	Sd-C1, Sd-C4, Sd-C5
Sd-C4	79.1 (d)	3.42, dd (2.7, 9.1)	Sd-C3, Sd-C5, Sd-C6, Se-C1
Sd-C5	64.8 (d)	4.80, dq (6.5, 7.6)	Sd-C1, Sd-C3, Sd-C4, Sd-C6
Sd-C6	18.4 (q)	1.50, d (6.0)	Sd-C4, Sd-C5
Sd-3-OCH_3	57.1 (q)	3.49, d (0.8)	Sd-C3
D-glc.			
Se-C1	102.3 (d)	5.01, br d (7.7)	Sd-C4, Se-C-5
Se-C2	75.4 (d)	3.98, m	Se-C1, Se-C3
Se-C3	78.5 (d)	4.23, m	Se-C1, Se-C2, Se-C4
Se-C4	72.1 (d)	4.20, m	Se-C3, Se-C5, Se-C6
Se-C5	79.2 (d)	3.99, m	Se-C4, Se-C6
Se-C-6	63.0 (t)	4.37, dd (5.4, 11.6)	Se-C4, Se-C5
		4.56, br d (11.6)	Se-C4

Table 2. The ^1H, ^{13}C NMR and ^1H-^{13}C long-range correlation (HMBC) data for compound **2**[a]

	Sugar moieties		
	^{13}C NMR	^1H NMR	HMBC
D-cym.			
Sa-C1	96.4 (d)	5.45, br d (9.3)	C-3, Sa-C2
Sa-C2	37.4 (t)	1.78, m; 2.27, m	Sa-C1, Sa-C3
Sa-C3	78.2 (d)	3.97, m	Sa-C1, Sa-C5
Sa-C4	82.6 (d)	3.37, br d (9.7)	Sa-C3, Sa-C5, Sa-C6, Sb-C1
Sa-C5	68.6 (d)	4.23-4.26, m	Sa-C1, Sa-C6
Sa-C6	18.7 (q)	1.41, br d (4.9)	Sa-C4, Sa-C5
Sa-3-OCH_3	57.5 (q)	3.45, s	Sa-C3
D-digit.			
Sb-C1	98.4 (d)	5.21, br d (10.4)	Sa-C4, Sb-C2
Sb-C2	37.4 (t)	1.78, m; 2.27, m	Sb-C1, Sb-C3
Sb-C3	69.1 (d)	4.12-4.25, m	Sb-C4, Sb-C5
Sb-C4	83.2 (d)	3.50, m	Sb-C3, Sb-C5, Sb-C6, Sc-C1
Sb-C5	67.6 (d)	4.23-4.26, m	Sb-C1, Sb-C6
Sb-C6	18.7 (q)	1.37, br d (4.3)	Sb-C4, Sb-C5
D-ole.			
Sc-C1	102.0 (d)	4.64, br d (9.8)	Sb-C4, Sc-C2
Sc-C2	37.8 (t)	1.64, m; 2.24, m	Sc-C1, Sc-C3
Sc-C3	78.4 (d)	3.97, m	Sc-C1, Sc-C4, Sc-C5
Sc-C4	83.2 (d)	3.46, br d (9.3)	Sc-C3, Sc-C5, Sc-C6, Sd-C1
Sc-C5	71.8 (d)	3.45, d (6.3)	Sc-C1, Sc-C6
Sc-C6	18.7 (q)	1.37, d (4.3)	Sc-C4, Sc-C5
Sc-3-OCH_3	58.7 (q)	3.49, s	Sc-C3
D-cym.			
Sd-C1	99.8 (d)	5.14, br d (9.4)	Sc-C4, Sd-C2, Sd-C5
Sd-C2	37.4 (t)	1.78, m; 2.27, m	Sd-C1, Sd-C3
Sd-C3	77.7 (d)	3.97, m	Sd-C1, Sd-C4, Sd-C5
Sd-C4	83.4 (d)	3.65, br d (9.8)	Sd-C3, Sd-C5, Sd-C6, Se-C1
Sd-C5	69.7 (d)	4.15, m	Sd-C1, Sd-C6
Sd-C6	18.7 (q)	1.62, d (ca.6)	Sd-C4, Sd-C5
Sd-3-OCH_3	58.9 (q)	3.53, s	Sd-C3
D-glc.			
Se-C1	106.6 (d)	4.90, br d (ca.7)	Sd-C4, Se-C5
Se-C2	75.4 (d)	3.97, m	Se-C1, Se-C3
Se-C3	78.4 (d)	4.14, m	Se-C1, Se-C2, Se-C4
Se-C4	71.9 (d)	4.12, m	Se-C3, Se-C5
Se-C5	78.8 (d)	4.03, m	Se-C1, Se-C4, Se-C6
Se-C6	63.1 (t)	4.37, m	Se-C4, Se-C5
		4.56, br d (7.3)	Se-C4

[a] ^1H NMR, HMBC and ^{13}C NMR spectra were obtained at 500 and 125 MHz respectively, and recorded in C_5D_5N at room temperature. Multiplicity by DEPT experiments in parentheses; s, quaternary; d, methine; t, methylene and q, methyl carbons.

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