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Discovery of new triterpene glycosides from *Dendrobium officinale* with their α-glucosidase and α-amylase inhibitory activity†

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In this study, seven new pentacyclic triterpene glycosides, named dendrocinaosides A–G (**1–7**), and six known ones (**8–13**) were isolated from the whole plants of *Dendrobium officinale*. Their structures were determined by analyses of HR-ESI-MS, 1D and 2D NMR spectra. Compounds **1–4**, **8**, and **9** potentially inhibited α-glucosidase and α-amylase activities with the IC_{50} values ranging from 31.3 ± 2.2 to 42.4 ± 2.5 μM for anti α-glucosidase and from 36.5 ± 1.8 to 56.4 ± 2.0 μM for anti α-amylase activities, respectively, which were lower than that of the positive control, acarbose, showing IC_{50} values of 47.1 ± 1.4 μM for anti α-glucosidase and 145.7 ± 2.2 μM for anti α-amylase.

Introduction

The plant *Dendrobium officinale* Kimura & Migo (Orchidaceae family, Vietnamese name Lan Thạch Hộc) is an epiphytic herb that grows on large tree branches or on moist cliffs. The flat stem has longitudinal grooves divided into many segments, the stem is narrow, the tip is thicker, and the colour is light yellow. Short leaves with sheaths. The flowers are pink or white and pink, growing in short clusters between fallen leaves. The fruit is long and diamond shaped. This plant is distributed in the countries with tropical and subtropical climates such as China, Myanmar, Laos, and Vietnam. This plant has been used in traditional Chinese medicine for thousands of years used to protect the liver, treat cancer, gastritis, enhance immunity, stimulate digestion, reduce blood sugar and blood fat.[1,2] In Vietnam, *D. officinale* has been found in the mountains and forests of Vinh Phu, Quang Tri, Quang Nam, Da Nang, Gia Lai, Lam Dong provinces. The previous studies reported terpenoids,[3] bibenzyl derivatives,[1–7] polysaccharides,[8–15] are the main components of this plants. Some of which exhibited anti-tumor,[7,14,15] anti-inflammatory activities[8–13] and alleviates type 2 diabetes mellitus.[9] Therefore, the whole plants of *D. officinale* were selected for further study. This paper reported the isolation, structural identification of thirteen triterpene glycosides (**1–13**), including seven undescribed compounds (**1–7**) and their anti α-glucosidase and α-amylase activities *in vitro*.

Experimental

General experimental procedures

The optical rotations were measured on a Jasco P2000 polarimeter. IR spectra were recorded on a PerkinElmer SpectrumTwo FTIR spectrometer. The NMR spectra were measured on a Bruker Avance 600 MHz FT-NMR spectrometer. The HR-ESI-MS were measured on a SCIEX X500 QTOF or Agilent 6530 QTOF system. The semi-preparative HPLC was performed on an Agilent 1260 infinity II system including binary pump, autosampler, DAD detector, and YMC J'sphere ODS-H80 (20 × 250 mm, 4 μm) HPLC column. Mobile phases were an isocratic system of methanol/water or acetonitrile/water at flow rate of 3 mL min^{-1}. Thin layer chromatography was performed on precoated plates with silica gel or reversed phase C18 (RP18). Column chromatography was performed using silica gel (40–63 μm) or RP18 (150 μm) as adsorbents.

Plant material

Plant samples, *Dendrobium officinale* Kimura & Migo, were collected in Tam Dao, Vinh Phuc province, Vietnam during April 2022 and identified by Dr Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. Voucher specimen (NCCT-P160) was kept at the Institute of Marine Biochemistry, VAST.

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† Electronic supplementary information (ESI) available: HR-ESI-MS, 1D, and 2D-NMR spectra of new compounds of new compounds would be found. See DOI: https://doi.org/10.1039/d4ra01483a

Extraction and isolation

Dried powdered *D. officinale* whole plants (8.1 kg) was ultrasonically extracted with MeOH at 40 °C for three times (each, 20 L, 2 h) to obtain MeOH residue (300 g). This was suspended with 5.0 L of water and then successively partitioned with *n*-hexane, EtOAc to give *n*-hexane (DO1, 100 g), EtOAc (DO2, 28 g), and water-soluble extracts (DO3, 160 g). These fractions were screened for their anti α-glucosidase and α-amylase activities *in vitro* at the concentration of 200 μg mL^{-1}. Fraction DO3 showed significant anti α-glucosidase (71.2%) and anti α-amylase (68.0%) activity. Therefore, this fraction was selected for further study. The DO3 fraction was run on a Diaion (HP-20) column using MeOH/H_2O (1/4, 1/1, 3/4, and 1/0, each 3 L) as eluent to give four fractions, DO3A (45.6 g), DO3B (32 g), DO3C (27.0 g), and DO3D (18.5 g), respectively. Fraction DO3B (32 g) was isolated on an RP18 column eluting with MeOH/H_2O (3/1) to get five fractions, DO3B1–DO3B5. Fraction DO3B2 (5.7 g) was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (3/1) to get three fractions, DO3B2A-DO3B2C. Fraction DO3B2B (212 mg) was isolated on HPLC using CH_3CN 35% in water to get two compounds **10** (22.5 mg, t_R = 35.19 min) and **11** (20.3 mg, t_R = 32.17 min). Fraction DO3B2C (183 mg) was purified on the HPLC using CH_3CN 25% to get compound **12** (15.5 mg, t_R = 59.31 min). Fraction DO3B4 (6.1 g) was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (4/1) to get four fractions, DO3B4A–DO3B4D. Fraction DO3B4B (248 mg) was purified on the HPLC using CH_3CN 35% to get two compounds, **8** (51.0 mg, t_R = 34.00 min) and **9** (23.4 mg, t_R = 43.46 min). Fraction DO3B4C (176 mg) was purified on the HPLC using CH_3CN 31% to get two compounds, **5** (16.5 mg, t_R = 37.70 min) and **6** (15.2 mg, t_R = 25.53 min). Fraction DO3C (27.1 g) isolated on an RP18 column eluting with MeOH/H_2O (4/1) to get four fractions, DO3C1–DO3C4. Fraction DO3C2 (2.7 g) was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (5/1) to get six fractions, DO3C2A–DO3C2F. Fraction DO3C2B (178 mg) was purified on the HPLC using CH_3OH 70% in H_2O to get compound **1** (15.8 mg, t_R = 45.56 min). Fraction DO3C2C (157 mg) was purified on the HPLC using CH_3OH 70% in H_2O to get compound **2** (7.0 mg, t_R = 35.10 min). Fraction DO3C3 (1.9 g) was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (6/1) to get three fractions, DO3C3A–DO3C3C. Fraction DOC3B (217 mg) was chromatographed on the HPLC using CH_3CN 25% to get two compounds, **3** (18.2 mg, t_R = 23.81 min) and **4** (17.0 mg, t_R = 40.29 min). Fraction DO3D (18.5 g) isolated on an RP18 column eluting with MeOH/H_2O (5/1) to get four fractions, DO3D1–DO3D4. Fraction DO3D2 (2.9 g) was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (7/1) and then purified on the HPLC using CH_3CN 25% to get compound **7** (19.0 mg, t_R = 43.37 min). Fraction DO3D3 (1.8 g) was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (8/1) and then purified on the HPLC using CH_3OH 75% in H_2O to get compound **13** (20.1 mg, t_R = 38.5 min).

Dendrocinaoside A (1). Colourless powder; mp 216–218 °C. $[\alpha]_D^{25}$: −31.6 (*c* 0.1, MeOH); UV λ_{max}: 220, 245, 331 nm. IR (KBr) ν cm^{-1}: 3404, 2939, 1755, 1649, 1464, 1388, 1175, 1064; HR-ESI-MS *m*/*z*: 1227.5586 $[M + Na]^+$, (calcd for $[C_{61}H_{88}O_{24}Na]^+$, 1227.5558, Δ = +2.3 ppm); *m*/*z*: 1205.5728 $[M + H]^+$, (calcd for $[C_{61}H_{89}O_{24}]^+$, 1205.5739, Δ = −0.9 ppm). ^{1}H- and ^{13}C-NMR data were shown in the Table 1.

Dendrocinaoside B (2). Colorless powder; mp 207–208 °C. $[\alpha]_D^{25}$: −43.5 (*c* 0.1, MeOH); UV λ_{max}: 220, 245, 335 nm. IR (KBr) ν cm^{-1}: 3422, 2942, 1732, 1639, 1460, 1390, 1228, 1063; HR-ESI-MS *m*/*z*: 1223.5854 $[M + H]^+$, (calcd for $[C_{61}H_{91}O_{25}]^+$, 1223.5844, Δ = +0.8 ppm); ^{1}H- and ^{13}C-NMR data were shown in the Table 1.

Dendrocinaoside C (3). Colorless powder; mp 212–214 °C. $[\alpha]_D^{25}$: −28.0 (*c* 0.1, MeOH); UV λ_{max}: 230, 315 nm. IR (KBr) ν cm^{-1}: 3414, 2939, 1730, 1642, 1548, 1467, 1391, 1376, 1254, 1170, 1074; HR-ESI-MS *m*/*z*: 1221.6071 $[M + H]^+$, (calcd for $[C_{62}H_{93}O_{24}]^+$, 1221.6051, Δ = +1.6 ppm); ^{1}H- and ^{13}C-NMR data were shown in the Table 1.

Dendrocinaoside D (4). Colorless powder; mp 221–223 °C. $[\alpha]_D^{25}$: −33.8 (*c* 0.1, MeOH); UV λ_{max}: 219, 245, 300, 330 nm. IR (KBr) ν cm^{-1}: 3444, 2939, 1731, 1751, 1698, 1634, 1456, 1259, 1170, 1075; HR-ESI-MS *m*/*z* 1235.5839 $[M–H]^-$, (calcd for $[C_{62}H_{91}O_{25}]^-$, 1235.5855, Δ = −1.3 ppm), *m*/*z* 1271.5568 $[M + {}^{35}Cl]^-$, (calcd for $[C_{62}H_{92}O_{25}{}^{35}Cl]^-$, 1271.5521, Δ = −4.2 ppm), *m*/*z* 1273.5563 $[M + {}^{37}Cl]^-$, (calcd for $[C_{62}H_{92}O_{25}{}^{37}Cl]^-$, 1273.5592, Δ = −2.3 ppm); ^{1}H- and ^{13}C-NMR data were shown in the Table 1.

Dendrocinaoside E (5). Colorless powder; mp 198–199 °C. $[\alpha]_D^{25}$: −45.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (nm): terminal absorption at 190–240 nm; IR (KBr) ν cm^{-1}: 3441, 2943, 1715, 1645, 1069; HR-ESI-MS *m*/*z* 1075.5679 $[M + H]^+$, (calcd for $[C_{53}H_{87}O_{22}]^+$, 1075.5684, Δ = −0.5 ppm), *m*/*z* 1097.5476 $[M + Na]^+$, (calcd for $[C_{53}H_{86}O_{22}Na]^+$, 1097.5503, Δ = −2.5 ppm); ^{1}H- and ^{13}C-NMR data were shown in the Table 2.

Dendrocinaoside F (6). Colorless powder; mp 192–194 °C. $[\alpha]_D^{25}$: −31.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (nm): terminal absorption at 190–240 nm; IR (KBr) ν cm^{-1}:3412, 2937, 1737, 1645, 1457, 1389, 1066; HR-ESI-MS *m*/*z* 1061.5553 $[M + H]^+$, (calcd for $[C_{52}H_{85}O_{22}]^+$, 1061.5527, Δ = +2.5 ppm), *m*/*z* 1083.5373 $[M + Na]^+$, (calcd for $[C_{52}H_{84}O_{22}Na]^+$, 1083.5347, Δ = +2.4 ppm); ^{1}H- and ^{13}C-NMR data were shown in the Table 2.

Dendrocinaoside G (7). Colorless powder; mp 194–196 °C. $[\alpha]_D^{25}$: −46.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (nm): terminal absorption at 190–250 nm; IR (KBr) ν cm^{-1}: 3415, 2942, 1715, 1612, 1456, 1418, 1078; HR-ESI-MS *m*/*z* 929.5128 $[M + H]^+$, (calcd for $[C_{47}H_{77}O_{18}]^+$, 929.5105, Δ = +2.5 ppm), *m*/*z* 951.4913 $[M + Na]^+$, (calcd for $[C_{47}H_{76}O_{18}Na]^+$, 951.4913, Δ = −1.7 ppm); ^{1}H- and ^{13}C-NMR data were shown in the Table 2.

Acid hydrolysis of compounds 1–7

Acid hydrolysis of compounds **1–7** were the same as described in previous reports[16,17] and referred to ESI.†

α-Glucosidase and α-amylase inhibitory assay

The α-glucosidase and α-amylase inhibitory assay protocols are the same as described in previous reports[18,19] and referred to ESI.†

Table 1 ^{1}H-NMR and ^{13}C-NMR spectral data for **1–4**

	1		**2**		**3**		**4**	
No.	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)
1	39.8	0.97 (m)/1.63 (m)	39.7	0.97 (m)/1.63 (m)	39.7	0.97 (m)/1.62 (m)	39.7	0.96 (m)/1.62 (m)
2	27.0	1.70(m)/1.86 (m)	27.0	1.70(m)/1.85 (m)	26.9	1.70(m)/185 (m)	27.0	1.70(m)/1.85 (m)
3	90.8	3.12 (dd, 12.0, 4.2)	90.8	3.13 (dd, 12.0, 4.2)	90.8	3.12 (dd, 12.0, 4.8)	90.8	3.12 (dd, 12.0, 4.2)
4	40.8	—	40.7	—	40.7	—	40.7	—
5	56.9	0.72 (br d, 11.4)	57.5	0.76 (br d, 11.4)	57.0	0.75 (br d, 11.4)	57.0	0.75 (br d, 12.0)
6	19.3	1.37 (m)/(1.49)	19.3	1.37 (m)/1.50 (m)	19.3	1.37 (m)/1.50 (m)	19.3	1.35 (m)/1.48 (m)
7	33.9	1.29 (m)/1.48 (m)	33.9	1.30 (m)/1.48 (m)	33.9	1.30 (m)/1.48 (m)	33.9	1.29 (m)/1.46 (m)
8	40.1	—	40.1	—	40.1	—	40.1	—
9	49.0	1.58 (m)	49.0	1.58 (m)	49.0	1.60 (m)	49.0	1.58 (m)
10	37.9	—	37.9	—	37.9	—	37.9	—
11	24.6	1.90–1.92 (m)	24.6	1.90–1.92 (m)	24.7	1.90–1.92 (m)	24.6	1.90–1.92 (m)
12	124.3	5.34 (t, 3.0)	124.2	5.31 (t, 3.0)	124.2	5.28 (t, 3.6)	123.9	5.28 (t, 3.6)
13	144.2	—	144.0	—	144.8	—	144.8	—
14	42.9	—	42.9	—	42.8	—	42.9	—
15	28.9	1.13 (m)/1.80 (m)	28.9	1.10 (m)/1.78 (m)	29.0	1.10 (m)/1.78 (m)	28.9	1.08 (m)/1.77 (m)
16	24.2	1.85 (m)/2.17[a]	24.1	1.77(m)/2.12 (td, 13.2, 3.6)	24.0	1.77(m)/2.07 (*t*d, 13.2, 2.4)	24.0	1.72 (m)/2.05 (*t*d, 13.2, 3.0)
17	48.5	—	47.9	—	48.4	—	48.3	—
18	48.4	2.75 (dd, 13.8, 4.8)	44.7	2.81 (dd, 13.8, 4.2)	41.4	2.90 (dd, 13.2, 4.2)	41.8	2.90 (dd, 13.2, 3.6)
19	42.6	2.10 (dd, 13.8, 4.6) 2.57 (t, 13.8)	47.6	1.47[a] 2.00 (t, 13.8)	41.4	1.11[a] 1.83 (t, 13.8)	41.4	1.12[a] 1.83 (t, 13.4)
20	149.6	—	71.3	—	36.8	—	36.8	—
21	30.9	2.15[a] 2.24 (td, 13.2, 3.6)	35.6	1.51 (m)/1.65 (m)	29.5	1.18 (m)/1.52 (m)	29.3	1.18 (m)/1.50 (m)
22	38.5	1.55 (m)/1.91 (m)	39.8	1.67 (m)/1.75 (m)	32.6	1.65 (m)/1.74 (m)	32.6	1.65 (m)/1.74 (m)
23	28.5	0.95 (s)	28.5	0.93 (s)	28.5	0.93 (s)	28.5	0.93 (s)
24	16.9	0.73 (s)	16.9	0.73 (s)	16.9	0.72 (s)	16.9	0.72 (s)
25	16.1	0.95 (s)	16.0	0.95 (s)	16.0	0.93 (s)	16.0	0.94 (s)
26	17.8	0.80 (s)	18.0	0.80 (s)	17.8	0.79 (s)	17.8	0.79 (s)
27	26.3	1.18 (s)	26.2	1.18 (s)	26.3	1.17 (s)	26.3	1.17 (s)
28	177.3	—	177.6	—	178.0	—	178.0	—
29	—	—	—	—	74.2	3.21 (s)	74.3	3.21 (s)
30	107.5	4.64 (br s) 4.65 (br s)	25.0	1.25 (s)	19.7	0.95 (s)	19.7	0.95 (s)
3-*O*-Arabinose			3-*O*-Arabinose		3-*O*-Arabinose		3-*O*-Arabinose	
1′	105.2	4.49 (d, 7.8)	105.2	4.49 (d, 7.2)	105.2	5.49 (d, 7.2)	105.2	5.48 (d, 7.8)
2′	74.2	5.15 (dd, 9.0, 7.8)	74.2	5.15 (dd, 9.0, 7.2)	74.2	5.15 (dd, 9.0, 7.2)	74.2	5.15 (dd, 9.0, 7.2)
3′	72.8	3.76 (dd, 9.0, 3.0)	72.8	3.76 (br d, 9.0)	72.8	3.76 (br d, 9.0)	72.8	3.76 (br d, 9.0)
4′	70.2	3.88 (br s)	70.2	3.88 (br s)	70.2	3.88 (br s)	70.2	3.87 (br s)
5′	67.1	3.62 (d, 12.0) 3.92 (dd, 12.0, 2.4)	67.1	3.61 (br s, 12.0) 3.92 (br d, 12.0)	67.1	3.61 (br s, 12.0) 3.92 (br d, 12.0)	67.1	3.61 (br s, 11.4) 3.92 (br d, 11.4)
28-*O*-Glucose			28-*O*-Glucose		28-*O*-Glucose		28-*O*-Glucose	
1″	95.9	5.32 (d, 7.8)	95.8	5.34 (d, 7.8)	95.8	5.35 (d, 7.8)	95.8	5.35 (d, 7.8)
2″	73.8	3.33[a]	73.8	3.33[a]	73.8	3.34[a]	73.9	3.34[a]
3″	78.2	3.40[a]	78.2	3.40[a]	78.2	3.40[a]	78.2	3.40[a]
4″	71.0	3.40[a]	71.0	3.40[a]	71.0	3.40[a]	71.0	3.42[a]
5″	78.1	3.52 (m)	78.1	3.52 (m)	78.2	3.52 (m)	78.1	3.52 (m)
6″	69.4	3.78[a] 4.08 (d, 12.0, 1.8)	69.5	3.80[a] 4.09 (d, 12.0, 1.8)	69.4	3.82[a] 4.09 (d, 12.0, 1.8)	69.4	3.82[a] 4.09 (br d, 12.0)
6″-*O*-Glucose			6″-*O*-Glucose		6″-*O*-Glucose		6″-*O*-Glucose	
1‴	104.2	4.41 (d, 7.8)	104.3	4.41 (d, 7.8)	104.2	4.42 (d, 7.8)	104.2	4.43 (d, 7.8)
2‴	75.3	3.24 (dd, 9.0, 7.8)	75.3	3.24[a]	75.3	3.24 (dd, 9.0, 7.8)	75.3	3.25 (dd, 9.0, 7.8)
3‴	76.7	3.48 (*t*, 9.0)	76.7	3.48 (*t*, 9.0)	76.7	3.48 (*t*, 9.0)	76.7	3.48 (*t*, 9.0)

Table 1 (*Contd.*)

6″-*O*-Glucose			6″-*O*-Glucose		6″-*O*-Glucose		6″-*O*-Glucose	
4‴	79.6	3.56 (*t*, 9.0)	79.7	3.56[a]	79.7	3.55 (*t*, 9.0	79.6	3.55 (*t*, 9.0
5‴	76.8	3.30 (m)	76.9	3.31 (m)	76.9	3.31 (m)	76.9	3.31 (m)
6‴	61.9	3.67 (dd, 11.4, 4.2) 3.84 (dd, 11.4, 1.8)	61.9	3.67[a] 3.82[a]	61.9	3.67[a] 3.82[a]	61.9	3.67 (dd, 11.4, 5.4) 3.83[a]
4‴-*O*-Rhamnose			4‴-*O*-Rhamnose		4‴-*O*-Rhamnose		4‴-*O*-Rhamnose	
1⁗	102.9	4.86 (d, 1.2)	103.0	4.86 (d, 1.2)	103.0	4.85 (d, 1.2)	103.0	4.86 (d, 1.2)
2⁗	72.4	3.86 (dd, 3.0, 1.2)	72.4	3.85[a]	72.4	3.85[a]	72.4	3.85[a]
3⁗	72.2	3.66 (dd, 9.0, 3.0)	72.2	3.65[a]	72.2	3.65[a]	72.2	3.64[a]
4⁗	73.8	3.43 (t, 9.0)	73.8	3.43[a]	73.8	3.4[a]	73.8	3.43[a]
5⁗	70.7	3.98 (m)	70.7	3.98[a]	70.7	3.97[a]	70.7	3.98[a]
6⁗	17.8	1.29 (d, 6.6)	17.8	1.29 (d, 6.6)	17.8	1.28 (d, 6.6)	17.8	1.29 (d, 6.6)
Caffeoyl			Caffeoyl		Coumaroyl		Caffeoyl	
1⁗′	127.8	—	127.8	—	127.0	—	127.8	—
2⁗′	115.1	7.05 (d, 1.8)	115.0	7.05 (d, 1.8)	131.1	7.46 (d, 8.0)	115.1	7.05 (d, 1.8)
3⁗′	146.9	—	146.9	—	117.0	6.83 (d, 8.0)	146.8	—
4⁗′	149.4	—	149.6	—	161.7	—	149.6	—
5⁗′	116.7	6.80 (d, 7.8)	116.6	6.80 (d, 8.0)	117.0	6.83 (d, 8.0)	116.6	6.80 (d, 8.0)
6⁗′	122.9	6.95 (dd, 7.8, 1.8)	122.9	6.95 (dd, 8.0, 1.8)	131.1	7.46 (d, 8.0)	122.9	6.95 (dd, 8.0, 1.8)
7⁗′	147.1	7.58 (d, 15.6)	147.1	7.58 (d, 16.2)	146.8	7.65 (d, 15.6)	147.1	7.58 (d, 16.2)
8⁗′	115.4	6.29 (d, 15.6)	115.5	6.29 (d, 16.2)	115.3	6.35 (d, 15.6)	115.5	6.29 (d, 16.2)
9⁗′	168.6	—	168.6	—	168.6	—	168.6	-

[a] Overlapped signal.

Results and discussion

The whole part methanol extract of *D. officinale* was separated into *n*-hexane, EtOAc, and water-soluble fractions. The water portion was isolated on a Diaion HP20 column chromatography, then on silica gel or ODS reversed phase, and further purified by semipreparative HPLC to afford thirteen pentacyclic triterpene glycosides (**1–13**).

The known saponins were identified to be yemuoside YM14 (**8**),[20] yemuoside YM11 (**9**),[20] 3-*O*-α-L-arabinopyranosyl-oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**10**),[21] 3-*O*-α-L-arabinopyranosyl-29-hydroxyoleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**11**),[22] 3-*O*-α-L-arabinopyranosyl-serratagenic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**12**),[23] 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**13**)[24] by comparisons of their physical and NMR data with those reported in the literature. New saponins **1–7** differed from above known ones by either containing addional coumaroyl, caffeoyl, and sugar moiety or loss of methyl group at C-20 in the oleanane-type backbone.

Compound **1** (Fig. 1) had a molecular formula, $C_{61}H_{88}O_{24}$, as determined by the sodium adductive ion $[M + Na]^+$ at *m*/*z* 1227.5586 (calcd for $[C_{61}H_{88}O_{24}Na]^+$, 1227.5558) and proton adductive ion $[M + H]^+$ at *m*/*z* 1205.5728 (calcd for $[C_{61}H_{89}O_{24}]^+$, 1205.5739) in the HR-ESI-MS. The IR spectrum of **1** suggested the presence of hydroxy (3404 cm^{-1}), carbonyl (1755 cm^{-1}), aromatic ring and olefinic double bond (1649 cm^{-1}), and C–O–C (1064 cm^{-1}) functional groups. The ^{1}H-, ^{13}C-NMR, and HSQC spectra of **1** showed signals corresponding to five quaternary methyl groups [δ_C/δ_H: 28.5 (C-23)/0.95 (H_3-23), 16.9 (C-24)/0.73 (H_3-24), 16.1 (C-25)/0.95 (H_3-25), 17.8 (C-26)/0.80 (H_3-26), 26.3 (C-27)/1.18 (H_3-27)], two double bonds [δ_C 124.3 (CH, C-12)/δ_H 5.34 (*t*, $J = 3.0$ Hz) and δ_C 144.2 (C, C-13)] and [δ_C 149.6 (C, C-20) and δ_C 107.5 (CH_2, C-30)/δ_H 4.64 and 4.65 (each 1H, br s)], one *trans*-caffeoyl group [δ_H 7.05 (d, 1.8 Hz), 6.80 (d, 7.8 Hz), 6.95 (dd, 7.8, 1.8 Hz), 7.58 (d, 15.6 Hz), and 6.29 (d, 15.6 Hz)] and δ_C 168.6 (C=O), four sugar moieties [δ_C 105.2/δ_H 4.49 (d, 7.8 Hz, H-1′), δ_C 95.9/δ_H 5.32 (d, 7.8 Hz, H-1″), δ_C 104.2/δ_H 4.41 (d, 7.8 Hz, H-1‴), δ_C 102.9/δ_H 4.86 (d, 1.2 Hz, H-1⁗)], and a carboxylate group [δ_C 177.3, C-28]. The NMR data of **1** were similar to the corresponding data of compound **8** (yemuoside YM14)[20] except the additional signals due to the caffeoyl group (Table 1). The aglycone of **1** was indicated to be 29-noroleana-12,20(30)-dien-28-oic acid and the sugar moieties, 28-*O*-α-rhamnopyranosyl-(1→4)-β-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester and 3-*O*-α-arabinopyranosyl, were indicated by HSQC, COSY, and HMBC correlations as shown in Fig. 2, in comparison with the corresponding NMR data of compound **8** (yemuoside YM14).[20] The caffeoyl moiety linked to C-2′ of the arabinose sugar as confirmed by ^{1}H–^{1}H COSY cross peak of H-1′ (δ_H 4.49)/H-2′ (δ_H 5.15) and HMBC correlation from H-2′ (δ_H 5.15) to C-9⁗′ (δ_C 168.6). The HMBC correlations from H_3-27 (δ_H 1.18) to C-13 (δ_C

Table 2 ^{1}H-NMR and ^{13}C-NMR spectral data for **5–7**

	5		**6**		**7**	
No.	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)
1	39.8	0.99 (m)/1.63 (m)	39.8	0.98 (m)/1.64 (m)	39.78	0.98 (m)/1.64 (m)
2	27.0	1.72 (m)/1.87 (m)	27.0	1.72 (m)/1.86 (m)	27.0	1.70 (m)/1.87 (m)
3	90.7	3.15 (dd, 11.4, 4.2)	90.7	3.16 (dd, 11.4, 4.2)	90.7	3.15 (dd, 11.4, 4.2)
4	40.7	—	40.8	—	40.8	—
5	57.1	0.80 (br d, 11.4)	57.1	0.80 (br d, 11.4)	57.1	0.80 (br d, 11.4)
6	19.4	1.43 (m)/1.56 (m)	19.4	1.43 (m)/1.56 (m)	19.4	1.42 (m)/1.55 (m)
7	33.9	1.33 (m)/1.52 (m)	33.9	1.33 (m)/1.52 (m)	34.0	1.33 (m)/1.50 (m)
8	40.1	—	40.2	—	40.2	—
9	49.0	1.60 (m)	49.0	1.60 (m)	49.0	1.59 (m)
10	37.9	—	38.0	—	37.9	—
11	24.6	1.92–1.94 (m)	24.6	1.90–1.92 (m)	24.6	1.90–1.93 (m)
12	124.0	5.31 (t, 3.6)	123.8	5.34 (t, 3.0)	123.9	5.29 (t, 3.0)
13	144.6	—	144.6	—	144.8	—
14	42.9	—	42.9	—	42.9	—
15	28.9	1.10 (m)/1.78 (m)	28.9	1.10 (m)/1.80 (m)	28.9	1.10 (m)/1.80 (m)
16	24.3	1.76 (m)/2.10 (*t*d, 13.2, 3.0)	23.8	1.74 (m)/2.04 (td, 13.2, 3.0)	24.1	1.73 (m)/2.07 (*t*d, 13.8, 2.4)
17	49.0	—	47.7	—	48.4	—
18	41.9	2.85 (dd, 13.2, 4.2)	41.5	3.09 (dd, 13.8, 4.2)	41.8	2.91 (dd, 13.8, 4.2)
19	42.5	1.40[a]/1.62[a]	46.1	1.47[a] 1.84 (t, 13.8)	41.4	1.11[a] 1.83 (t, 13.8)
20	36.1	—	69.8	—	36.8	—
21	29.9	1.31 (m)/1.52 (m)	34.1	1.50 (m)/1.56 (m)	29.3	1.19 (m)/1.50 (m)
22	32.8	1.58 (m)/1.72 (m)	32.5	1.57 (m)/1.94 (m)	32.5	1.66 (m)/1.74 (m)
23	28.6	1.06 (s)	28.6	1.06 (s)	28.6	1.06 (s)
24	17.0	0.87 (s)	17.0	0.88 (s)	17.0	0.87 (s)
25	16.1	0.98 (s)	16.2	0.98 (s)	16.1	0.98 (s)
26	17.9	0.82 (s)	18.0	0.84 (s)	17.9	0.83 (s)
27	26.3	1.19 (s)	26.3	1.17 (s)	26.3	1.19 (s)
28	178.1	—	178.0	—	178.0	—
29	28.0	0.92 (s)	31.5	1.20 (s)	74.3	3.21 (s)
30	66.3	3.45[a]/3.54[a]	—	—	19.6	0.95 (s)
3-*O*-Arabinose			3-*O*-Arabinose		3-*O*-Arabinose	
1′	107.1	4.30 (d, 6.6)	107.1	4.30 (d, 6.6)	107.1	4.30 (d, 6.6)
2′	72.8	3.59 (dd, 9.0, 6.6)	72.8	3.59 (dd, 9.0, 6.6)	72.8	3.58 (dd, 9.0, 6.6)
3′	74.3	3.52[a]	74.3	3.52[a]	74.3	3.53[a]
4′	69.5	3.82 (br s)	69.5	3.82 (br s)	69.5	3.82 (br s)
5′	66.3	3.53[a] 3.84[a]	66.3	3.53[a] 3.84[a]	66.3	3.53[a] 85 (dd, 9.6, 2.4)
28-*O*-Glucose			28-*O*-Glucose		28-*O*-Glucose	
1″	95.8	5.35 (d, 7.8)	96.1	5.32 (d, 7.8)	95.8	5.38 (d, 7.8)
2″	73.9	3.35[a]	73.8	3.36[a]	73.8	3.35[a]
3″	78.2	3.40[a]	78.2	3.43[a]	78.2	3.43[a]
4″	71.1	3.42[a]	71.1	3.43[a]	71.0	3.44[a]
5″	78.0	3.52 (m)	78.0	3.52 (m)	77.8	3.54 (m)
6″	69.6	3.78 (dd, 12.0, 5.4) 4.10 (dd, 12.0, 1.8)	69.8	3.78 (dd, 12.0, 5.4) 4.10 (dd, 12.0, 1.2)	69.6	3.78 (dd, 11.4, 5.4) 4.10 (dd, 11.4, 1.8)
6″-*O*-Glucose			6″-*O*-Glucose		6″-*O*-Glucose	
1‴	104.4	4.41 (d, 7.8)	104.6	4.39 (d, 7.8)	104.7	4.37 (d, 7.8)
2‴	75.3	3.25 (dd, 9.0, 7.8)	75.3	3.25 (dd, 9.0, 7.8)	75.2	3.22 (dd, 9.0, 7.8)
3‴	76.7	3.48 (t, 9.0)	76.8	3.48 (t, 9.0)	78.0	3.37 (t, 9.0)
4‴	79.6	3.54[a]	79.8	3.54[a]	71.6	3.31[a]
5‴	76.9	3.31 (m)	76.9	3.33 (m)	78.0	3.27 (m)
6‴	62.0	3.68 (dd, 11.4, 5.4) 3.82[a]	62.0	3.68 (dd, 11.4, 5.4) 3.82[a]	62.0	3.69 (dd, 12.0, 6.6) 3.87 (dd, 12.0, 1.8)

Table 2 (*Contd.*)

4‴-*O*-Rhamnose			4‴-*O*-Rhamnose	
1⁗	102.9	4.87[a]	102.9	4.86 (d, 1.2)
2⁗	72.4	3.86[a]	72.4	3.85 (dd, 3.0, 1.2)
3⁗	72.3	3.66[a]	72.3	3.65 (dd, 9.0, 3.0)
4⁗	73.8	3.42[a]	73.8	3.42[a]
5⁗	70.7	3.98 (m)	70.7	3.99 (m)
6⁗	17.9	1.29 (d, 6.6)	17.9	1.29 (d, 6.0)

[a] Overlapped signal.

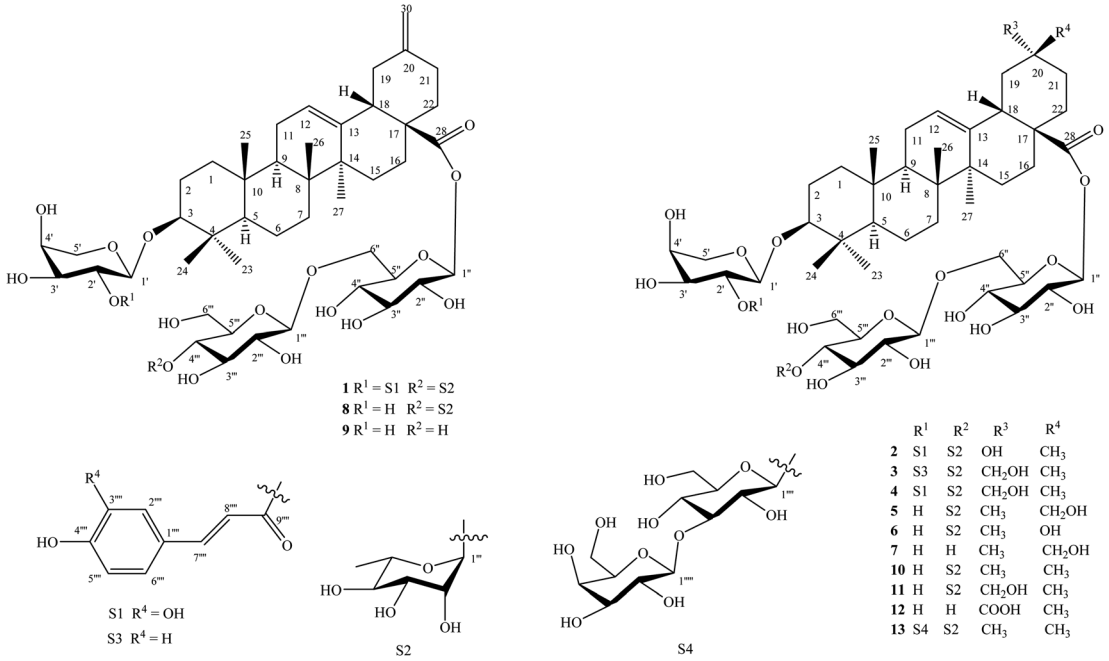

Fig. 1 Structures of compounds **1–13** isolated from the stems of *D. officinale*.

144.2) and from H_2-30 (δ_H 4.64 and 4.65) to C-19 (δ_C 42.6)/C-20 (δ_C 149.6)/C-21 (δ_C 30.9) indicated the location of double bonds at C-12/C-13 and C-20/C-30. The small *J* value (1.2 Hz) of an rhamnose anomeric proton at δ_H 4.86 suggested α-form of this glycosidic linkage, and the large *J* values (7.8 Hz) of the glucose anomeric protons at δ_H 5.32 and 4.41 suggested β-form of these glycosidic linkages, while the large *J* values (7.8 Hz) of the arabinose anomeric proton at δ_H 4.49 indicated α-form of this glycosidic linkage.[20] The *trans*-configuration of the caffeoyl moiety was evidenced by large *J* value of the olefinic protons (15.6 Hz). In addition, proton H-3 (δ_H 3.12) showed NOESY correlation with H-5 (δ_H 0.77) suggesting alpha/axial orientation of H-3 (Fig. 3). Acid hydrolysis of **1** yielded D-glucose, L-arabinose, and L-rhamnose which were identified by comparison with authentic samples (D-glucose, L-arabinose, and L-rhamnose, Sigma-Aldrich) *via* thin-layer chromatography, and from the positive sign of the optical rotations. Consequently, the structure of **1** was established to be 3-*O*-{(2-*trans*-caffeoyl)-α-L-arabinopyranosyl}-29-noroleana-12,20(30)-dien-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester and named as dendrocinaoside A.

Compound **2** had a molecular formula, $C_{61}H_{90}O_{25}$, as determined by the proton adductive ion at *m*/*z* 1223.5854, (calcd for $[C_{61}H_{91}O_{25}]^+$, 1223.5844), indicating 17 degrees of unsaturation. The IR pectrum of **2** suggested the presence of hydroxy (3422 cm^{-1}), carbonyl (1732 cm^{-1}), aromatic ring and olefinic double bond (1639 cm^{-1}), and C–O–C (1063 cm^{-1}) functional groups. The NMR data of **2** were very similar to those of **1** except for the absence of C-20/C-30 double bond signals and the additional signals of one quaternary methyl group [δ_C 25.0 (C-30)/δ_H 1.25 (3H, s, H_3-30) and one oxygenated quaternary carbon at δ_C 71.3 (C-20). The HMBC correlations from H-30 to C-19 (δ_C 47.6, CH_2)/C-20 (δ_C 71.3, C)/C-21 (δ_C 35.5, CH_2) were observed. These data suggested that the $\Delta^{20(30)}$ double bond was replaced by one hydroxy group at C-20 of the 29-noroleanolic acid aglycone. In addition, the caffeoyl, the sugar moieties 28-*O*-α-rhamnopyranosyl-(1→4)-β-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester and 3-*O*-α-arabinopyranosyl were identified by

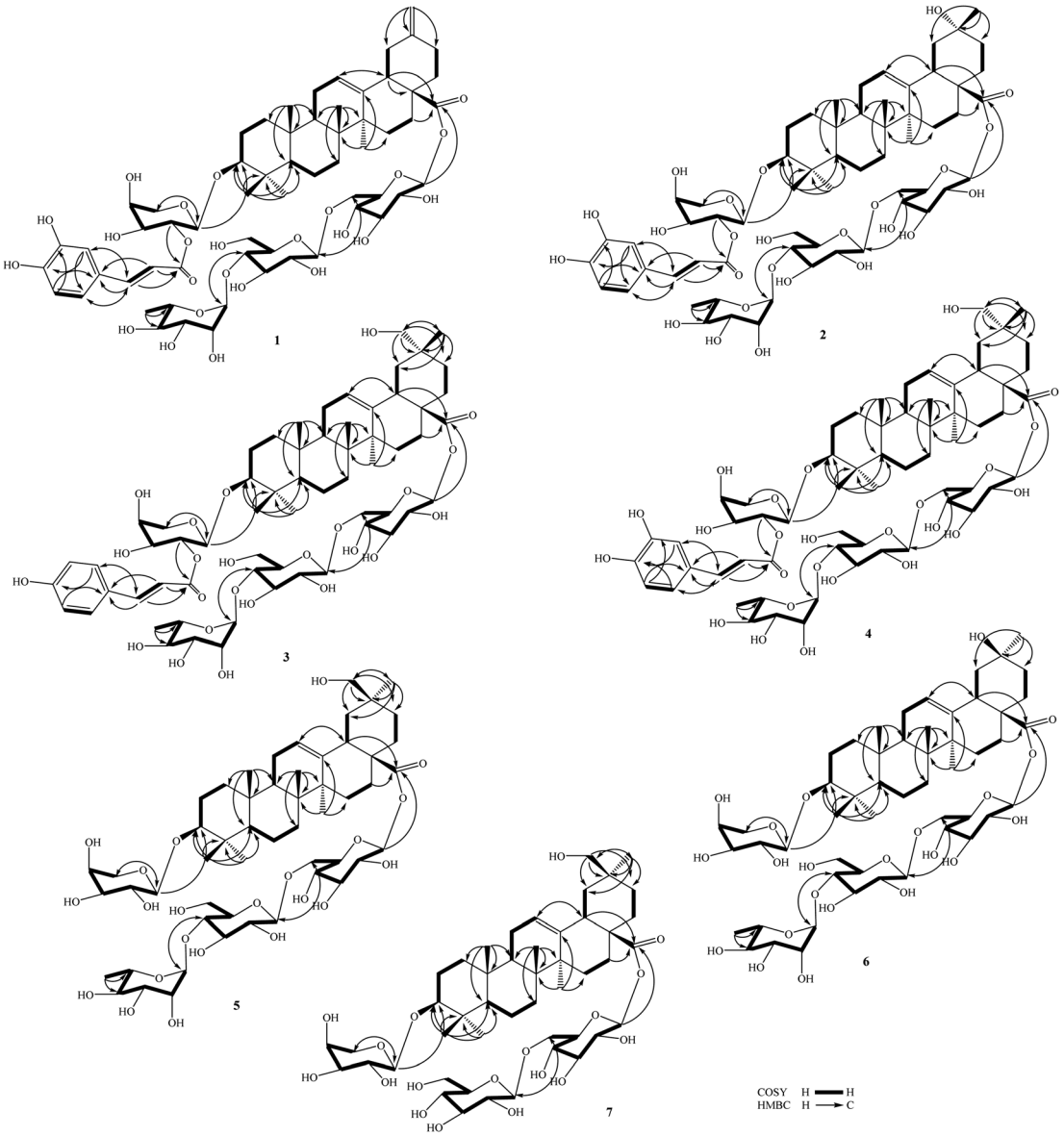

Fig. 2 Key HMBC and COSY correlations of compounds **1–7**.

comparison the NMR data with those of **1** (Table 1) and further confirmed by HSQC, COSY, and HMBC correlations (Fig. 2). The HMBC correlations from H-2′ to C-9′′′′′ (C═O), from H-1′ ara to C-3, from H-1′′′′ rha to C-4′′′, from H-1′′′ to C-6′′, and from H-1′′ to C-28 were observed. The large $^{3}J_{2,3}$ value (12.0 Hz) of H-3 together with the NOE cross peak of H-3/H-5 indicated alpha/axial orientation of H-3. In addition, The large $^{3}J_{18,19}$ value (13.8 Hz) and H_3-30 (δ_H 1.25) showed NOE cross peak with H-18 (δ_H 2.81) confirmed beta/axial orientations of H-18 and the methyl group at C-20 (or 20α-hydroxy group) (Fig. 3). The low *J* value for rhamnose H-1′′′′ proton (1.2 Hz) indicated an α-form, and high *J* values for the two glucose anomeric protons H-1′′ and H-1′′′ (7.8 Hz) suggested β-forms for the glycosidic linkages. In addition, the high *J* value for arabinose H-1′ proton (7.2 Hz) suggested α-form of this glycosidic linkage. Acid hydrolysis of **2** yielded D-glucose, L-arabinose, and L-rhamnose which were identified by comparison with authentic samples (D-glucose, L-arabinose, and L-rhamnose, Sigma-Aldrich) *via* thin-layer chromatography, and from the positive sign of the optical rotations. Thus, compound **2** was determined to be 3-*O*-[(2-*trans*-caffeoyl)-α-L-arabinopyranosyl]-20α-hydroxy-29-noroleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester and named as dendrocinaoside B.

The molecular formula of **3** was determined to be $C_{62}H_{92}O_{24}$ from the pseudo molecular ion peak $[M + H]^+$ at *m*/*z* 1221.6071 (calcd for $[C_{62}H_{93}O_{24}]^+$, 1221.6051), indicating 17 degrees of unsaturation. The IR spectrum of **3** suggested the presence of hydroxy, carboxylate, aromatic ring, double bond, and C–O–C functionalities. The ^{13}C NMR of **3** displayed 62 carbons, comprising 30 from an oleanolic acid aglycone, 9 from a coumaroyl moiety, and 23 from four sugar moieties.[16] The NMR data for **3** closely resembled those of compound **2** (Table 1), with

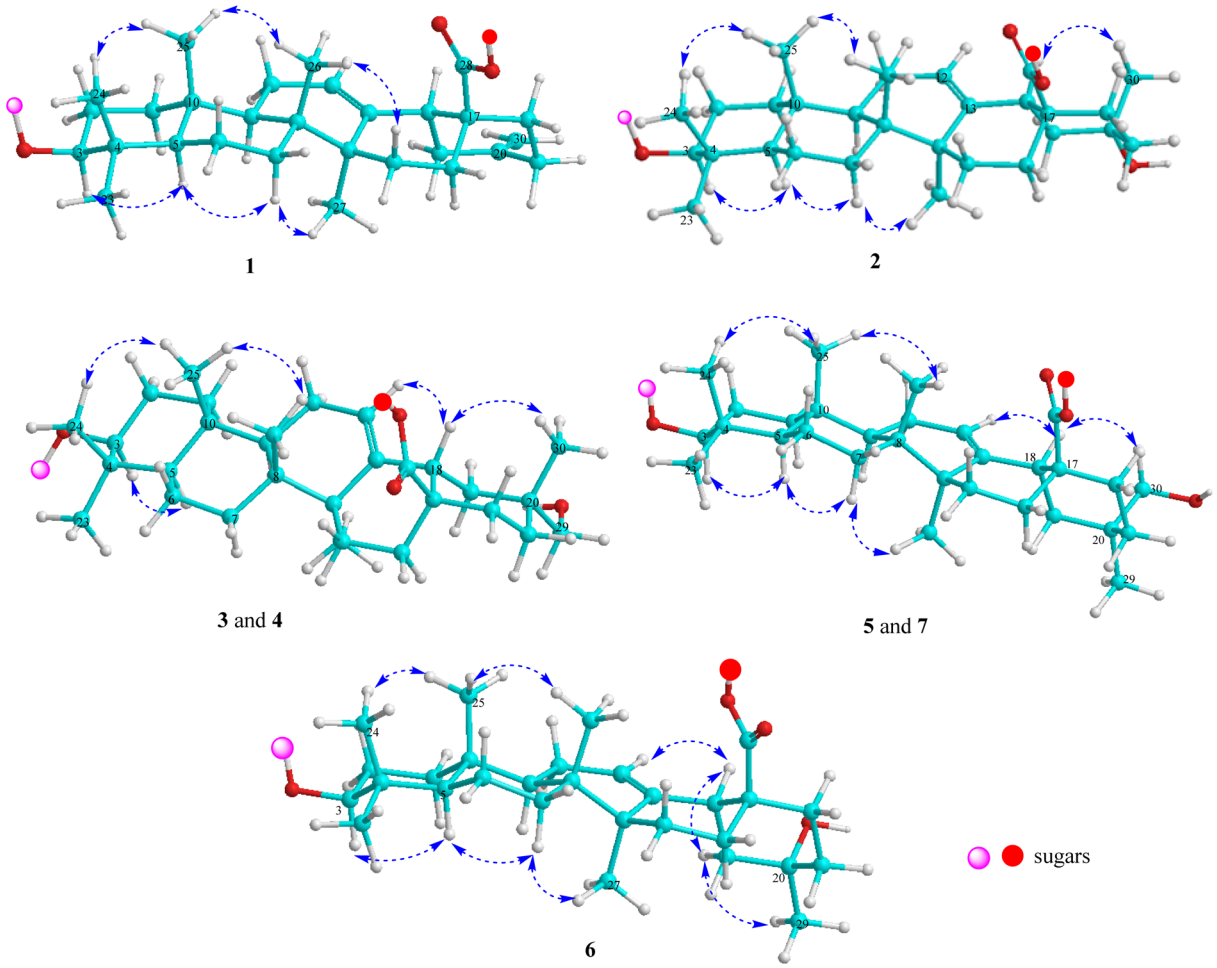

Fig. 3 Important NOESY correlations of compounds **1**–**7**.

the exception of additional signals for one oxygenated methylene group at C-20 [δ_C 74.2/δ_H 3.21 (2H, s)] and a caffeoyl group was replaced by a coumaroyl group (Table 1). The sugar moieties, 3-*O*-α-L-arabinopyranosyl and 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester were identified. The observed HMBC correlations from H-7′′′′′ and H-8′′′′′ to C-9′′′′′, from H-2′ to C-9′′′′′, and from H-1′ to C-3 indicated the coumaroyl group linked to C-2′ of the arabinose sugar, and this sugar linked to C-3 of the aglycone by an ether linkage. Protons H-1′′′′ (δ_H 4.85), H-1′′′ (δ_H 4.42), and H-1′′ (δ_H 5.35) showed HMBC correlations with C-4′′′ (δ_C 79.6), C-6′′ (δ_C 69.4), and C-28 (δ_C 178.0), respectively, confirming rhamnopyranosyl-(1→4)-glucopyranosyl-(1→6)-glucopyranosyl moiety, which linked to C-28 by an ester linkage. The hydroxy group was at C-29 as determined by HMBC correlations from H-30 (δ_H 0.95) to C-19 (δ_C 41.4)/C-20 (δ_C 36.8)/C-21 (δ_C 29.5)/C-29 (δ_C 74.2), from H-29 (δ_H 3.21) to C-19/C-20/C-21/C-30 (δ_C 19.7), and from NOESY cross peak between H-18 (δ_H 2.90) and H-30 (δ_H 0.95) (Fig. 2 and 3). All the $^3J_{1,2}$ values of the anomeric protons were remarkably like those of **1** and **2** (Table 1) suggesting the same form of glycosidic linkages. Acid hydrolysis of **3** yielded D-glucose, L-arabinose, and L-rhamnose, which were identified by comparison with authentic samples (D-glucose, L-arabinose, and L-rhamnose, Sigma-Aldrich) *via* thin-layer chromatography, and from the positive sign of the optical rotations. Thus, compound **3** was determined to be 3-*O*-[(2-*trans*-coumaroyl)-α-L-arabinopyranosyl]-29-hydroxyoleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester and named as dendrocinaoside C.

The molecular formula of **4** was determined to be $C_{62}H_{92}O_{25}$ from the pseudo molecular ion peak $[M–H]^-$ at *m*/*z* 1235.5839 (calcd for $[C_{62}H_{91}O_{25}]^-$, 1235.5855), indicating 17 degrees of unsaturation. The IR spectrum of **4** suggested the presence of hydroxy, carboxylate, aromatic ring, double bond, and C–O–C functionalities. The ^{1}H, ^{13}C NMR, HSQC, and HMBC spectra of **4** were almost similar to those of **3** except for the coumaroyl signals were replaced by caffeoyl signals with the presence of additional signals for an aromatic ABX coupled proton system [δ_H 7.05 (d, 1.8 Hz), 6.80 (d, 8.0 Hz), and 6.95 (dd, 8.0, 1.8 Hz)] (Table 1). The hydroxy group was at C-29 as determined by HMBC correlations from H_3-30 to C-19/C-20/C-21/C-29 and from H_2-29 to C-19/C-20/C-21/C-30 along with NOESY cross peak from H-18 to H_3-30. H-3 proton was alpha/axial orientation as evident by the high $^3J_{2,3}$ value (12.0 Hz) along with NOESY cross peak between H-3 and H-5. The *trans*-configuration of the coumaroyl group was indicated by high *J* value (16.2 Hz) of the double

bond. All the $^3J_{1,2}$ values of the anomeric protons were terribly like those of **1–3** (Table 1) suggesting the same form of glycosidic linkages. Acid hydrolysis of **4** yielded D-glucose, L-arabinose, and L-rhamnose, which were identified by comparison with authentic samples (D-glucose, L-arabinose, and L-rhamnose, Sigma-Aldrich) *via* thin-layer chromatography, and from the positive sign of the optical rotations. Thus, compound **4** was determined to be 3-*O*-[(2-*trans*-caffeoyl)-α-L-arabinopyranosyl]-29-hydroxyoleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester and named as dendrocinaoside D.

Compound **5** (Fig. 1) has a molecular formula, $C_{53}H_{86}O_{22}$, as determined by its HR-ESI-MS (found *m*/*z* 1075.5679 $[M + H]^+$), (calcd for $[C_{53}H_{87}O_{22}]^+$, 1075.5684, $\Delta = -0.5$ ppm), found *m*/*z* 1097.5476 $[M + Na]^+$, (calcd for $[C_{53}H_{86}O_{22}Na]^+$, 1097.5503, $\Delta = -2.5$ ppm), indicating 11 degrees of unsaturation. The IR spectrum of **5** supported the presence of hydroxy (3441 cm^{-1}), carbonyl (1715 cm^{-1}), aromatic ring (1645 cm^{-1}), and C–O–C (1069 cm^{-1}) functional groups. The NMR spectra of **5** were like those of **4** except for the absence of the caffeoyl group signals. The oxygenated methylene group was located at C-20 identified by HMBC correlation from its protons (δ_H 3.45) and the neighbour methyl protons (δ_H 0.92) to C-19/C-20/C-21. However, the carbon chemical shifts for the oxygenated methylene group (δ_C 66.3) and methyl group (δ_C 28.0) were quite different from those of **4** (δ_C 74.3 and 19.7, respectively) suggesting 30-hydroxy group in **5**. This was further confirmed by NOESY cross peak between H-18 (δ_H 2.85) and H-30 (δ_H 3.45). Proton H-3 was axial orientation as indicated by NOESY cross peak between H-3 and H-5, along with the high $^3J_{2,3}$ value (11.4 Hz) of H-3. In the HMBC spectrum, H-1′ correlated to C-3, H-1″ correlated to C-28, H-1‴ correlated to C-6″, and H-1⁗ correlated to C-4‴ confirming 3-*O*-arabinopyranosyl and 28-*O*-rhamnopyranosyl-(1→4)-glucopyranosyl-(1→6)-glucopyranosyl moieties. All the $^3J_{1,2}$ values of the anomeric protons were similar to those of **1–4** (Table 1) suggesting the same form of glycosidic linkages. Acid hydrolysis of **5** yielded D-glucose, L-arabinose, and L-rhamnose, which were identified by comparison with authentic samples (D-glucose, L-arabinose, and L-rhamnose, Sigma-Aldrich) *via* thin-layer chromatography, and from the positive sign of the optical rotations. Thus, compound **5** was determined to be 3-*O*-α-L-arabinopyranosyl-30-hydroxyoleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester and named as dendrocinaoside E.

The molecular formula of **6** was $C_{52}H_{84}O_{22}$ determined from its HR-ESI-MS (found *m*/*z* 1061.5553 $[M + H]^+$, (calcd for $[C_{52}H_{85}O_{22}]^+$, 1061.5527, $\Delta = +2.5$ ppm), found *m*/*z* 1083.5373 $[M + Na]^+$, (calcd for $[C_{52}H_{84}O_{22}Na]^+$, 1083.5347, $\Delta = +2.4$ ppm)), indicating 11 degrees of unsaturation. The IR spectrum of **6** suggested the presence of hydroxy (3412 cm^{-1}), carbonyl (1737 cm^{-1}), aromatic ring (1645 cm^{-1}), and C–O–C (1066 cm^{-1}) functional groups. The NMR spectra of **6** were closely related to those of compound **2** except for the absence of the caffeoyl group signals (Tables 1 and 2). The arabinose (δ_C 107.1, 72.8, 74.3, 69.5, and 66.3), a rhamnose (δ_C 102.9, 72.4, 72.2, 73.8, 70.7, and 17.9), and two glucose sugars were identified. These sugar moieties and their glycosidic linkages were suggested to be like those of compounds **1–5** determined by HSQC, COSY, HMBC spectra (Fig. 2), along with $^3J_{1,2}$ values of the anomeric protons and acid hydrolysis results. The hydroxy group was at C-20 confirmed by HMBC correlation from H-29 to C-19/C-20/C-21. The carbon chemical shift of the methyl group at C-20 (δ_C 31.5) in **6** was vast different from that in **2** (δ_C 25.0). This evidence suggested 20α-hydroxy in **6** instead of 20β-hydroxy in **2**. This was further confirmed by NOESY cross peaks between $H_{alpha/axial}$-19 (δ_H 1.84, dd, $J = 13.8$, 13.8 Hz) and H_3-29 (δ_H 1.20). Thus, compound **6** was determined to be 3-*O*-α-L-arabinopyranosyl]-20β-hydroxy-29-noroleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester and named as dendrocinaoside F.

Compound **7** had a molecular formula of $C_{47}H_{76}O_{18}$, as determined by its HR-ESI-MS (found *m*/*z* 929.5128 $[M + H]^+$), (calcd for $[C_{47}H_{77}O_{18}]^+$, 929.5105, $\Delta = +2.5$ ppm), found *m*/*z* 951.4913 $[M + Na]^+$, (calcd for $[C_{47}H_{76}O_{18}Na]^+$, 951.4924, $\Delta = -1.7$ ppm)), indicating 10 degrees of unsaturation. The IR spectrum suggested the presence of hydroxy, carboxylate, double bond, and ether functional groups. The NMR data of **7** were closely related to those of compound **5** except for the absence of rhamnose moieties. For the aglycone of **7**, one Δ^{12}-double bond [δ_C 123.9/δ_H 5.29 (*t*, 3.0 Hz) and δ_C 144.8], the oxygenated methylene group [δ_C 74.3/δ_H 3.21 (2H, s)], one methine carbinol group (δ_C 90.7/δ_H 3.15 (dd, 11.4, 4.2 Hz), and six quaternary methyl groups were identified (Table 2). In addition, three anomeric protons at δ_H 4.30, 5.38, and 4.37 showed HSQC corrections with carbons at δ_C 107.1, 95.8, and 104.7, respectively, suggesting one arabinose and two glucose sugars. In the HMBC spectrum, H-1′ correlated with C-3, H-1‴ correlated with C-6″, and H-1″ correlated with C-28 confirming the arabinose linked to C-3, one glucose linked to C-6 of the remaining glucose, which linked to C-28 by an ester linkage. The high $^3J_{1,2}$ values of the anomeric protons at δ_H 4.30 ($J = 6.6$ Hz), 5.38 ($J = 7.8$ Hz), and 4.37 ($J = 7.8$ Hz) suggesting α-form of the arabinosyl linkage and β-form of the glucosyl linkages. H-3 proton was axial orientation as supported by the high $^3J_{2,3}$ value (11.4 Hz) of H-3 at δ_H 3.15, and by NOESY cross peak from H-3 to H-5. In addition, H-18 showed NOESY cross peak with H_3-30 (δ_H 0.95) indicating this methyl group was beta/axial orientation (or 29-hydroxy group). Acid hydrolysis of **7** yielded D-glucose and L-arabinose, which were identified by comparison with authentic samples (D-glucose and L-arabinose, Sigma-Aldrich) *via* thin-layer chromatography, and from the positive sign of the optical rotations. Thus, compound **7** was determined to be 3-*O*-α-L-arabinopyranosyl-29-hydroxyoleanolic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester and named as dendrocinaoside G.

In the digestive system, α-amylase and α-glucosidase are two important enzymes that hydrolyse starch yielding oligosaccharide and finally glucose. Inhibition of α-amylase and/or α-glucosidase slow down the hydrolysis processes which are helpful to reduce levels of glucose in the blood. The α-amylase and/or α-glucosidase inhibitors therefore could be lead compounds for new drug developments in the treatment of type 2 diabetes. Therefore, compounds **1–13** were screened for both anti α-glucosidase and α-amylase activities at the concentration of 200 μM (Table S1†). Acarbose, an antidiabetic drug, was used as

Table 3 α-Glucosidase and α-amylase inhibitory effects of 1–13

Compounds	Inhibition (IC_{50}, μM)	
	α-Glucosidase	α-Amylase
1	31.3 ± 2.2	52.1 ± 2.3
2	37.7 ± 1.7	45.3 ± 1.9
3	33.1 ± 1.5	56.4 ± 2.0
4	33.8 ± 1.6	36.5 ± 1.8
5	105.1 ± 2.8	>200
6	99.6 ± 3.4	135.2 ± 3.3
7	148.1 ± 2.5	>200
8	40.5 ± 1.9	44.5 ± 2.3
9	42.4 ± 2.5	39.8 ± 2.5
10	107.7 ± 2.9	>200
11	136.5 ± 3.7	148.6 ± 4.2
12	>200	127.4 ± 3.1
13	>200	166.5 ± 3.7
Acarbose[a]	47.1 ± 1.4	145.7 ± 2.2

[a] Acarbose was used as a positive control.

a positive control in both tests. Compounds **1–11** showed anti α-glucosidase activity and compounds **1–4**, **6**, **8**, **9**, **11–13** showed anti α-amylase activity with inhibitory percentages over 50%. Therefore, further evaluation of α-glucosidase and α-amylase inhibition of these compounds were assayed to determine their IC_{50} values. The results (Table 3) indicated that compounds **1–4**, **8**, and **9** potentially inhibited both anti α-glucosidase (IC_{50} values: 31.3 ± 2.2 to 42.4 ± 2.5 μM) and anti α-amylase (IC_{50} values: 36.5 ± 1.8 to 56.4 ± 2.0 μM) activities, which were lower than that of the positive control, acarbose, showing IC_{50} values of 47.1 ± 1.4 μM (anti α-glucosidase) and 145.7 ± 2.2 μM (anti α-amylase). These results suggested that 29-noroleana-12,20(30)-dien-28-oic acid framework and the presence of the caffeoyl or coumaroyl moieties may play significant role in anti α-glucosidase and α-amylase activities of the isolated saponins, and further study on anti-diabetes of this plant should be continued.

Conclusions

The bio-guided fractionation study on the whole plants of *Dendrobium officinale* led to the isolation of thirteen saponins, including seven new compounds namely dendrocinaosides A–G. Their chemical structures were determined by spectroscopic methods, including NMR spectroscopy and HRESIMS analysis. Compounds **1–4**, **8**, and **9** potentially inhibited α-glucosidase (IC_{50} values from 31.3 ± 2.2 to 42.4 ± 2.5 μM) and α-amylase (IC_{50} values from 36.5 ± 1.8 to 56.4 ± 2.0 μM), which were lower than that of the positive control, acarbose.

Author contributions

PH Yen, PV Kiem, BH Tai, contributed to research idea and writing; DTT Hang, LDT Lam, DT Dung, DT Trang, DTH Yen, NH Hoang, PTT Huong, NV Dung, NA Bang, ND Duy contributed to isolation; PH Yen, PV Kiem, BH Tai contributed to structure elucidation and bioassay.

Conflicts of interest

There are no conflicts to declare.

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