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Four New Steroidal Saponins from the Roots of *Dracaena cambodiana* with NO Production Inhibition Activity in LPS Activated RAW 264.7 Cells

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Seven steroidal saponins including three new 16,23-cyclocholestanes (**1**-**3**) and one new pregane (**4**) were isolated from the roots of *Dracaena cambodiana* Pierre ex Gagnep. Their chemical structures were elucidated to be (23*R*,25*R*)-26-*O*-β-D-glucopyranosyl-16,23-cyclocholesta-5,17(20)-dien-22-one-3β,16α,26-triol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside (**1**), (23*R*,25*R*)-26-*O*-β-D-glucopyranosyl-16,23-cyclocholesta-5,17,20(22)-trien-3β,22,26-triol-3-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside (**2**), (23*R*,25*R*)-16,23-cyclocholesta-5,16,20(22)-trien-3β,22,26-triol-3-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside (**3**), 3β-[(*O*-α-L-rhamnopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-gluco-pyranosyl)oxy]-pregna-5,17(20)-diene-16-one-20-carboxylic acid 4''''-*O*-β-D-glucopyranosylisopentyl ester (**4**), cambodianoside A (**5**), diosbulbiside C (**6**), and diosbulbiside D (**7**), by IR, HR-ESI-MS, 1D and 2D NMR spectra. Compounds **1** and **4**–**7** inhibited nitric oxide (NO) production in lipopolysaccharide activated RAW 264.7 cells with IC_{50} values ranging from 19.03 ± 1.84 to 67.92 ± 3.81 μM, whereas compounds **2** and **3** were inactive with IC_{50} values over 100 μM.

Introduction

The plant *Dracaena cambodiana* Pierre ex Gagnep. (family Asparagaceae), known as a famous traditional medicine in Vietnam for the treatment of leucorrhea, wounds, diarrhea fractures, and piles, as well as intestinal and stomach ulcers for a long time.[1,2] It has been reported that steroidal saponins are main constituents of the trunks and roots of *D. cambodiana*, which show anti-inflammatory, antibacterial, antifungal, antioxidant, and cytotoxic activities.[1–6] In our screening anti-inflammatory components from the plants, the methanol extract of the roots of *D. cambodiana* showed significantly nitric oxide production inhibition with the inhibition percentage of 81.2% at 100 μg/mL, and therefore was selected for further study. In the previous paper, we have reported the steroidal saponins from the trunks of *D. cambodiana* with their NO inhibition activity.[7] The present paper reports four new and three known steroidal saponins isolated from the roots of *D. cambodiana* and their NO inhibition activity *in vitro*.

Results and Discussion

The bio-guided fractionation of the methanol extract of *D. cambodiana* roots led to the isolation of seven steroidal saponins (Figure 1). Compound **1** was yielded as a colorless amorphous powder. The IR spectrum indicated the presence of hydroxy (3407 cm^{-1}), carbonyl (1701 cm^{-1}), olefinic (1453 cm^{-1}), and ether (1041 cm^{-1}) functionalities. The molecular formula of **1** was $C_{51}H_{80}O_{22}$, as determined by HR-ESI-MS (found *m/z* 1067.5053 $[M+Na]^+$, calcd for $[C_{51}H_{80}O_{22}Na]^+$: 1067.5033), indicating 12 degrees of unsaturation. The ^{1}H-NMR spectrum of **1** supported three singlet methyl groups (δ_H 1.12, 1.34, 1.77, each 3H, s), one doublet methyl group (δ_H 1.00, d, J = 6.6 Hz), one olefinic proton (δ_H 5.42), one methine carbinol (δ_H 3.66, m), one oxygenated methylene group (δ_H 3.45 and 3.77), four anomeric protons at δ_H 4.55 (d, J = 7.8 Hz), 4.28 (d, J = 7.8 Hz), 4.92 (d, J = 1.2 Hz), and 5.00 (d, J = 1.2 Hz), and two rhamnose methyl groups at δ_H 1.28 and 1.29 (each 3H, d, J = 7.0 Hz). These suggested a steroidal aglycone bearing two glucose and two rhamnose moieties.[3,8–10] The ^{13}C NMR and HSQC spectra of **1** revealed the presence of 51 carbon signals, including 27 of the steroidal aglycone and 24 of four hexose sugar moieties.[3,9, 10] All the NMR data of **1** were assigned with the aid of HSQC, COSY, and HMBC spectra (Table 1, Figure 2). The ketone group

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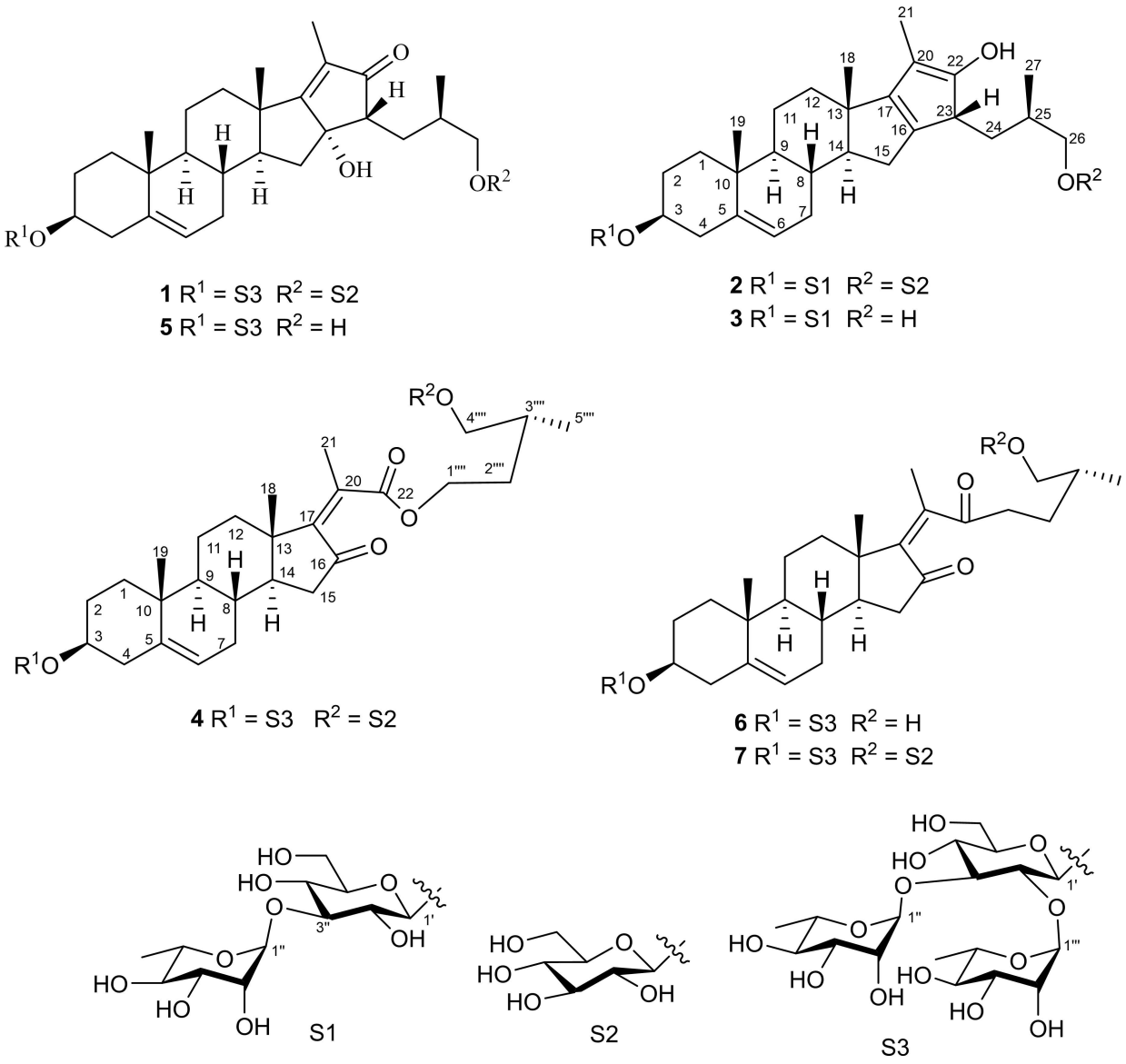

Figure 1. Chemical structures of compounds **1**–**7**.

at δ_C 214.7 (C-22), an oxygenated quaternary carbon at δ_C 84.1 (C-16), and the double bond at δ_C 183.4 (C-17) and 129.9 (C-20) were identified.[3,9] The NMR data of **1** were remarkably similar to those of known compound **5** (cambodianoside A)[3] except for the additional signals of one glucose moiety at C-26. These suggestions were further confirmed by HMBC correlations from H_3-18 (δ_H 1.34) to C-12 (δ_C 36.7)/C-13 (δ_C 45.1)/C-14 (δ_C 55.1)/C-17 (δ_C 183.4), from H_3-21 (δ_H 1.77) to C-17/C-20 (δ_C 129.9)/C-22 (δ_C 214.7), and from H-23 (δ_H 2.33) to C-22 (δ_C 214.7)/C-16 (δ_C 84.1)/C-24 (δ_C 29.4)/C-25 (δ_C 32.3). The 16,23-cyclo structure was clearly evident by HMBC correlations from H-23 to C-16/C-22/C-24/C-25, and from H-24 to C-16. The 16,23-cyclo steroidal compound, cambodianoside A, has been previously isolated from *D. Cambodiana*.[3] The large $^3J_{3,4}$ value (10.8 Hz) suggested the *axial*/α orientation for H-3. The down field shift of C-26 (δ_C 77.0) suggested that one sugar linked to C-26, similar to that of trillikamtoside N,[9] which was further indicated by HMBC correlations from H_2-26 (δ_H 3.45 and 3.77) to C-1′′′′ (δ_C 104.8) and from H-1′′′′ (δ_H 4.28) to C-26. The sequence of sugar chain attached to C-3 in **1** was identical with that of compound **5**[3] by comparison of their NMR data, the HMBC correlations (Figure 2), together with the results of acid hydrolysis. Acid hydrolysis of **1** yielded D-glucose and L-rhamnose, which were identified by comparison with authentic samples via thin-layer chromatography (TLC), and from the positive sign of the optical rotations.[11,12] The large *J* values of the anomeric protons at δ_H 4.55 and 4.28 ($J = 7.8$ Hz) suggested β-form and the small *J* values of the anomeric protons at δ_H 4.92 and 5.00 ($J = 1.2$ Hz) suggested α-form of the glycosidic linkages. The 25*R*-configuration of **1** was suggested from the small $\delta_{Ha\text{-}6}$–$\delta_{Hb\text{-}6}$ value ($\Delta = 0.32$), less than 0.48.[11,13,14] The carbon chemical shifts from C-15 to C-17 and from C-20 to C-24 of **1** (δ_C 38.6, 84.1, 183.4, 129.9, 8.4, 214.7, 57.9, and 29.4, respectively) were similar to those of trillikamtoside N[9] (δ_C 38.5, 83.0, 182.2, 128.2, 8.6, 212.4, 57.6,

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1–3**.

No.	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.5	1.12–1.13 (m)/1.94*	38.2	1.12 (td, 10.8, 3.6)/1.93*	38.3	1.15 (td, 10.8, 3.6)/1.93*
2	30.7	1.63–1.64 (m)/1.93*	30.8	1.63–1.64 (m)/1.97*	30.9	1.64–1.65 (m)/1.97*
3	79.0	3.66–3.67 (m)	79.9	3.64–3.65 (m)	79.9	3.64–3.65 (m)
4	39.3	2.50 (brd, 10.8) 2.32 (dd, 10.8, 10.8)	39.5	2.48 (dd, 11.4, 2.4) 2.28 (dd, 11.4, 10.8)	39.5	2.47 (dd, 11.4, 2.4) 2.28 (dd, 11.4, 10.8)
5	142.0	–	141.1	–	141.2	–
6	122.4	5.44 (t-like, 2.4)	122.8	5.43 (t-like, 1.8)	122.8	5.43 (t, 3.0)
7	32.5	1.62–1.63 (m)/2.04–2.05 (m)	33.3	1.75–1.76 (m)/2.35–2.36 (m)	33.3	1.74–1.75 (m)/2.35–2.36 (m)
8	33.8	1.81-1.82 (m)	30.7	1.82-1.83 (m)	30.7	1.83-1.84 (m)
9	51.7	1.05-1.06 (m)	50.5	1.18 (td, 10.8, 4.2)	50.5	1.19 (td, 10.8, 4.2)
10	38.1	–	38.1	–	38.1	–
11	21.5	1.74*	20.8	1.52–1.53 (m)/1.58–1.59 (m)	20.8	1.52–1.53 (m)/1.58–1.59 (m)
12	36.7	1.62-1.63 (m)/2.27–2.28 (m)	37.7	1.48–1.49 (m)/2.03–2.04 (m)	37.7	1.48–1.49 (m)/2.03-2.04 (m)
13	45.1	–	41.7	–	41.7	–
14	55.1	1.22-1.23 (m)	48.4	2.38-2.39 (m)	48.5	2.35-2.36 (m)
15	38.6	1.67*/2.16 (dd, 12.6, 6.6)	34.0	1.82*/1.94*	34.2	1.82*/1.93*
16	84.1	–	172.9	–	172.9	–
17	183.4	–	135.4	–	135.3	–
18	15.8	1.43 (s)	20.0	1.43 (s)	20.0	1.43 (s)
19	19.9	1.12 (s)	19.7	1.07 (s)	19.7	1.07 (s)
20	129.9	–	107.3	–	107.3	–
21	8.4	1.77 (s)	13.2	2.15 (s)	13.1	2.14 (s)
22	214.7	–	159.9	–	159.9	–
23	57.9	2.33 (dd, 9.6, 4.2)	49.9	2.34*	49.8	2.34*
24	29.4	1.52–1.53 (m)/1.82–1.83 (m)	28.0	1.03*	27.8	1.01*
25	32.3	2.05–2.06 (m)	34.4	1.76–1.77 (m)	36.7	1.58–1.59 (m)
26	77.0	3.45*/3.77 (dd, 10.2, 6.6)	75.6	3.37*/3.74 (dd, 9.6, 6.0)	68.1	3.35-3.39*
27	17.0	1.00 (d, 6.0)	16.9	0.95 (d, 6.0)	16.7	0.92 (d, 6.6)
3-*O*-Glc			3-*O*-Glc		3-*O*-Glc	
1′	100.3	4.55 (d, 7.8)	102.4	4.42 (d, 7.8)	102.4	4.42 (d, 7.8)
2′	79.4	3.44 (dd, 9.0, 7.8)	75.5	3.27 (dd, 9.0, 7.8)	75.6	3.27 (dd, 9.0, 7.8)
3′	88.3	3.60 (t, 9.0)	84.5	3.53 (t, 9.0)	84.5	3.52 (t, 9.0)
4′	70.6	3.40*	70.2	3.36*	70.2	3.37*
5′	77.5	3.30–3.31 (m)	77.8	3.32–3.33 (m)	77.8	3.30–3.31 (m)
6′	62.6	3.69 (dd, 12.0, 5.4) 3.87 (dd, 12.0, 1.8)	62.7	3.68* 3.88*	62.7	3.68* 3.88*
3′- *O*-Rha			3′- *O*-Rha		3′- *O*-Rha	
1″	103.8	4.92 (d, 1.2)	102.7	5.18 (d, 1.2)	102.7	5.18 (d, 1.2)
2″	72.3	3.90 (dd, 3.0, 1.2)	72.4	3.96 (dd, 3.0, 1.2)	72.4	3.96 (dd, 3.0, 1.2)
3″	72.5	3.65 (dd, 9.0, 3.0)	72.2	3.72 (dd, 9.0, 3.0)	72.2	3.73 (dd, 9.0, 3.0)
4″	73.6	3.44 (t, 9.0)	74.0	3.43 (t, 9.0)	74.0	3.41 (t, 9.0)
5″	70.9	3.95–3.97 (m)	70.1	4.00–4.01 (m)	70.1	4.00–4.01 (m)
6″	17.8	1.29 (d, 6.6)	18.0	1.28 (d, 6.6)	17.9	1.27 (d, 6.6)
2′- *O*-Rha			26-*O*-Glc			
1‴	102.7	5.00 (d, 1.2)	104.6	4.24 (d, 7.8)		
2‴	72.2	3.86 (dd, 3.0, 1.2)	75.1	3.19 (dd, 9.0, 7.8)		
3‴	72.4	3.68 (dd, 9.0, 3.0)	78.1	3.36*		
4‴	73.8	3.44 (t, 9.0)	71.7	3.29*		

Table 1. continued

No.	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
5‴	70.1	4.13-4.14 (m)	77.9	3.26–3.27 (m)		
6‴	18.1	1.28 (d, 7.0)	62.7	3.68*/3.88*		
26- *O*-Glc						
1⁗	104.8	4.28 (d, 7.8)				
2⁗	75.2	3.20 (dd, 9.0, 7.8)				
3⁗	78.1	3.36*				
4⁗	71.7	3.28*				
5⁗	77.9	3.27–3.28 (m)				
6⁗	62.8	3.69*/3.87*				

[*] Overlapped signals

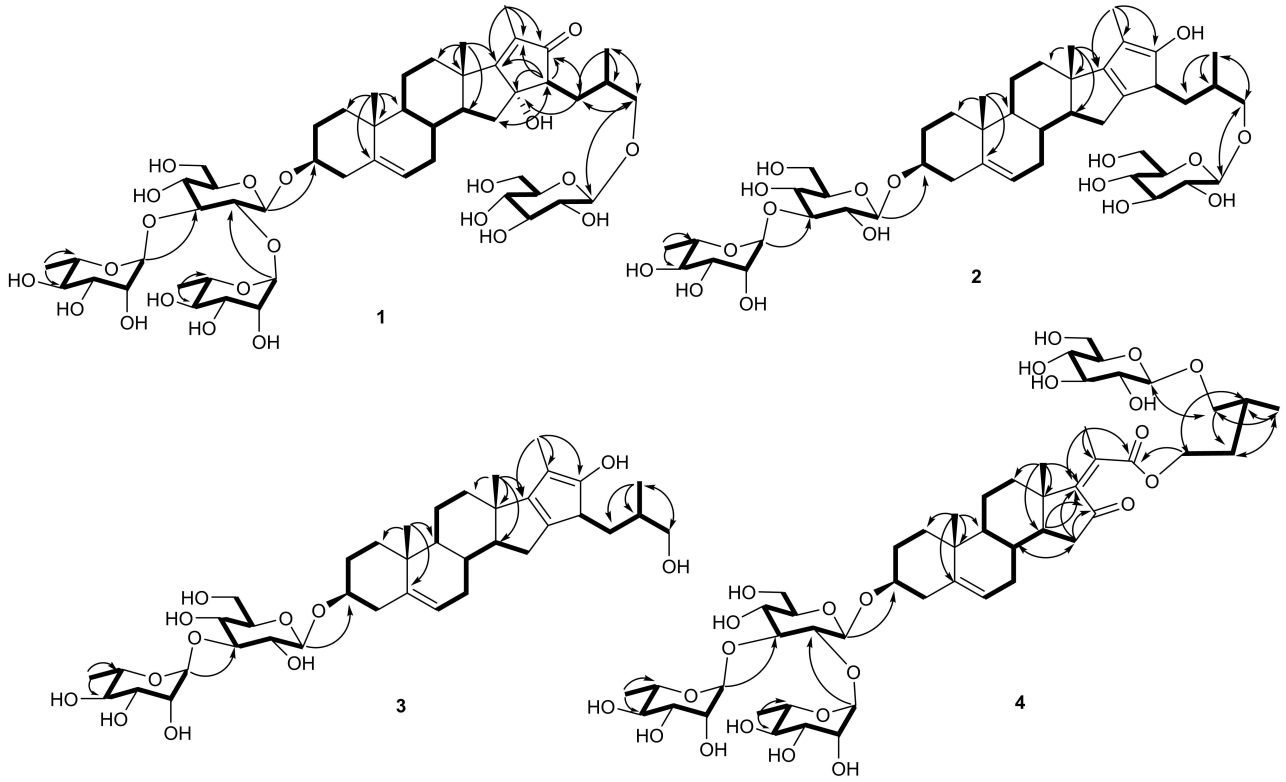

Figure 2. The key HMBC and COSY correlations of compounds **1–4**.

and 29.3, respectively) and ypsiyunnoside A[10] (δ_C 38.5, 83.0, 182.1, 128.3, 8.6, 212.3, 57.6, and 29.3, respectively) suggesting that these compounds have the same relative structure and substituted groups at the ring E. This was further indicated by NOESY spectrum. The NOESY cross peaks of H_3-18/H-8, H-8/H_β-15 (δ_H 1.67), and H_β-15/H-23 suggested β-oriented for H-23.[10] In addition, the NOESY correlation between H_β-15 and H-23 also suggested α-oriented for 16-OH group, similar to that in cambodianoside A,[3] trillikamtoside N,[9] and ypsiyunnoside A[10] (Figure 3). Thus, compound **1** was determined to be (23*R*,25*R*)-26-*O*-β-D-glucopyranosyl-16,23-cyclocholesta-5,17-diene-22-ketone-3β,16α,26-triol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside, a new compound named as cambodianoside G (Figures S1-S12).[3]

Compound **2** was yielded as a colorless amorphous powder. The IR spectrum of **2** suggested the presence of hydroxy (3371 cm^{-1}), double bond (1639, 1594, 1508 cm^{-1}), and C-*O*-C groups (1071 cm^{-1}). The molecular formula of **2** was determined to be $C_{45}H_{70}O_{17}$ by HR-ESI-MS ion peak at *m/z* 905.4518, $[M+Na]^+$ (calcd for $[C_{45}H_{70}O_{17}Na]^+$: 905.4505). The ^{1}H-NMR spectrum of **2** showed three methyl singlet signals at δ_H 1.07, 1.43, and 2.15 (each 3H), a double methyl signal at δ_H 0.95 (3H, J= 6.0 Hz), and one olefinic methine proton at δ_H 5.43, suggesting a Δ^5-steroidal aglycone.[3,9] In addition, three anomeric protons

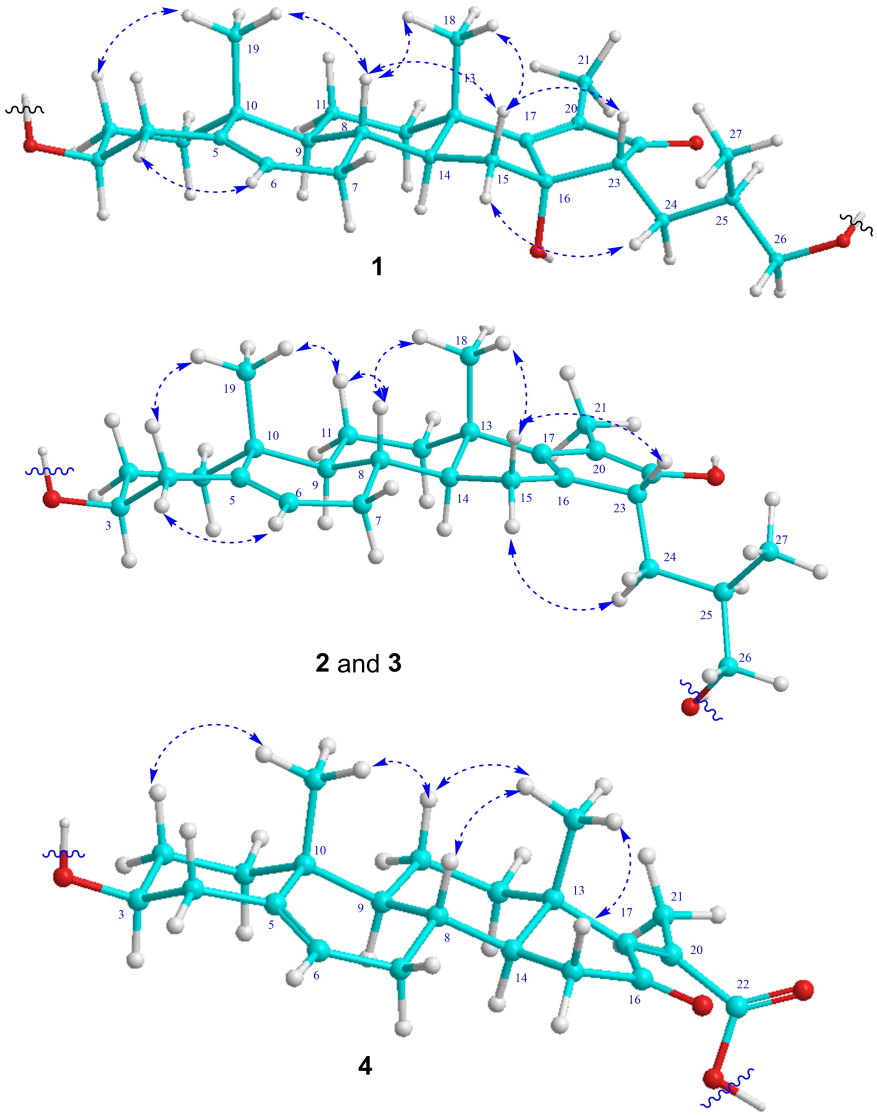

Figure 3. The important NOESY correlations of the aglycones of compounds **1–4**.

at δ_H 4.24 (d, J=7.8 Hz), 4.42 (d, J=7.8 Hz), and 5.18 (d, J= 1.2 Hz) suggested two glucose and one rhamnose moieties.[2–6] The ^{13}C NMR and HSQC spectra of **2** supported 45 carbons including 27 of the steroidal aglycone and 18 of three hexose sugars. Of these, three double bonds at δ_C 141.1 (C-5), 122.8 (C-6), 172.9 (C-16), 135.4 (C-17), 107.3 (C-20), 159.9 (C-22), four methyls at δ_C 19.7 (C-19), 20.0 (C-18), 13.2 (C-21), and 16.9 (C-27), one methine carbinol group at δ_C 79.9 (C-3), and one oxygenated methylene carbon at δ_C 75.6 were assigned for the aglycone.[3,8,9] All the NMR data were assigned with the aid of HSQC, COSY, and HMBC spectra (Table 1, Figure 2). In the HMBC spectra, H-19 (δ_H 1.07) correlated with C-1 (δ_C 38.2), C-5 (δ_C 141.1), C-9 (δ_C 50.5), and C-10 (δ_C 38.1) indicating C-5/C-6 double bond. H_3-18 (δ_H 1.43) showed strong ^3J HMBC correlations with C-12 (δ_C 37.7)/ C-13 (δ_C 41.7)/C-14 (δ_C 48.4)/C-17 (δ_C 135.4) and weak ^4J HMBC correlation with C-16 (δ_C 172.9), while H_3-21 (δ_H 2.15) showed strong ^3J HMBC correlations with C-17 (δ_C 135.4)/C-20 (δ_C 107.3)/C-22 (δ_C 159.9), and weak ^4J HMBC correlation with C-16 (δ_C 172.9) indicating C-16/C-17 and C-20/C-22 double bonds. The down field shift of C-22 (δ_C 159.9) suggested one hydroxy group attached to C-22, which was further confirmed by the above HR-ESI-MS results.[3] Moreover, HMBC correlations from H-1′ glc (4.42) to C-3 (δ_C 79.9), from H-1′′ rha (5.18) to C-3′ (δ_C 84.5), and from H-1′′′ (4.24) to C-26 (δ_C 75.6) were observed indicating the rhamnose sugar linked to C-3 of the glucose, which attached to C-3, and the other glucose linked to C-26. Two H_2-26 protons appeared at δ_H 3.37 and 3.74 (Δ=0.37) suggesting the (25*R*)-configuration.[11,13,14] The large $^3J_{3,4}$ value (10.8 Hz) indicated from H_{ax}-4 signal (δ_H 2.28, dd, J= 11.4, 10.8 Hz) suggested H-3 was *axial*/α orientation. The large $^3J_{1',2'}$ and $^3J_{1''',2'''}$ values (7.8 Hz) of anomeric protons at δ_H 4.42 (H-1′) and δ_H 4.24 (H-1′′′) suggested the β-form, and smaller $^3J_{1'',2''}$ value (1.2 Hz) of the other anomeric proton at δ_H 5.18 (H-2′′) indicated α-form of the glycosidic linkages. Acid hydrolysis of **2** yielded D-glucose and L-rhamnose, which were identified by comparison with authentic samples via thin-layer chroma-

tography (TLC), and from the positive sign of the optical rotations.[11,12] The NOESY cross peaks of H_3-18/H_β-15 (δ_H 1.82) and H_β-15/H-23 (δ_H 2.34) suggested β-oriented for H-23 (Figure 3), similar to that of **1**. Thus, compound **2** was elucidated to be (23*R*,25*R*)-26-*O*-β-D-glucopyranosyl-16,23-cyclocholesta-5,16,20(22)-trien-3β,22,26-triol-3-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside, a new compound named as cambodianoside H (Figures S13-S24).

Compound **3** had a molecular formula of $C_{39}H_{60}O_{12}$, as determined from its HR-ESI-MS (found *m/z* 743.3966 $[M+Na]^+$, calcd for $[C_{39}H_{60}O_{12}Na]^+$: 743.3977) indicating 11 degrees of unsaturation. The IR spectrum of **3** suggested the presence of hydroxy (3317 cm^{-1}), double bond (1639, 1594, 1508 cm^{-1}), and ether (1036 cm^{-1}) functionalities. The ^1H, ^{13}C NMR, and HSQC spectra of **3** were closely resembling those of **2** (Table 1) except for the loss of glucose moiety signals at C-26, and carbon signals for C-26 was shifted upfield (from δ_C 75.6 in **2** to δ_C 68.1 in **3**). The NMR assignments of **3** were firstly revealed by directly comparison with the corresponding data of **2** and further indicated by HSQC, HMBC, and COSY spectra (Table 1, Figure 2). The HMBC correlations from H-1″ to C-3′ and from H-1′ to C-3 confirmed 3-*O*-rhamnopyranosyl-(1→3)-glucopyranoside moiety. In addition, H-18 correlated with C-13/C-14/C-15/C-17, H-21 correlated with C-17/C-20/C-22, H_3-27 correlated with C-24/C-25/C-26, and H_2-26 correlated with C-24/C-25/C-27 in the HMBC spectrum confirming two double bonds at C-16/C-17 and C-20/C-22 and the hydroxy group linked to C-26. The large *J* value (10.8 Hz) between H-3 and H_{ax}-4 suggested an *axial* orientation for H-3. The similarities between NMR chemical shifts and *J* values of sugar moieties of **2** and **3** suggested the same sugar moiety linked to C-3. Acid hydrolysis of **3** yielded D-glucose and L-rhamnose, which were identified by comparison with authentic samples via thin-layer chromatography (TLC), and from the positive sign of the optical rotations.[11,12] The NOESY cross peaks of H_3-18/H_β-15 (δ_H 1.82) and H_β-15/H-23 (δ_H 2.34) suggested β-oriented for H-23 (Figure 3), similar to that of **1** and **2**. Thus, compound **3** was determined to be (23*R*,25*R*)-16,23-cyclocholesta-5,16,20(22)-trien-3β,22,26-triol-3-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside, a new compound named as cambodianoside I (Figures S25-S36).

The molecular formula of compound **4** was determined as $C_{39}H_{60}O_{12}$ by its HR-ESI-MS analysis (found *m/z* 1083.4975 $[M+Na]^+$, calcd for $[C_{39}H_{60}O_{12}Na]^+$: 1083.4983), indicating 12 degrees of unsaturation. The IR spectrum of **4** revealed the presence of hydroxy (3418 cm^{-1}), carbonyl (1713 cm^{-1}), double bond (1630 cm^{-1}), and C-*O*-C groups (1041 cm^{-1}). The NMR spectra of **4** were similar to those of **1–3** suggesting a steroidal saponins (Table 2). In the ^1H-NMR spectrum of **4**, three quaternary methyl

Table 2. ^1H-NMR (δ_H, multi, *J* in Hz) and ^{13}C NMR spectral data of **4** in CD_3OD.

C	δ_C	δ_H	C	δ_C	δ_H	C	δ_C	δ_H
1	38.2	1.16–1.17 (m)/1.94*	3-*O*-Glc			Isopentyl moiety		
2	30.7	1.64–1.65 (m)/1.95*	1′	100.3	4.55 (d, 7.8)	1‴′	64.8	4.28–4.29 (m)
3	79.0	3.67–3.68 (m)	2′	79.4	3.44 (dd, 9.0, 7.8)	2‴′	33.2	1.54–1.55 (m)/1.91–1.92 (m)
4	39.3	2.51 (dd, 10.8, 2.4) 2.34 (dd, 10.8, 10.8)	3′	88.4	3.60 (t, 9.0)	3‴′	31.8	1.92–1.93 (m)
5	142.1	–	4′	70.6	3.40*	4‴′	75.6	3.43*/3.78 (dd, 9.6, 6.6)
6	122.3	5.43 (t-like, 2.4)	5′	77.6	3.30-3.31 (m)	5‴′	17.3	1.01 (d, 6.0)
7	32.6	1.68*/2.02-2.03 (m)	6′	62.6	3.68 (dd, 12.0, 5.4) 3.87 (dd, 12.0, 1.8)			
8	32.0	1.76–1.77 (m)	3′- *O*-Rha			4‴′-*O*-Glc		
9	51.2	1.18–1.19 (m)	1″	103.8	4.92 (d, 1.8)	1‴′′	104.6	4.26 (d, 7.8)
10	38.0	–	2″	72.3	3.90 (dd, 3.0, 1.8)	2‴′′	75.2	3.20 (dd, 9.0, 7.8)
11	21.9	1.78-1.79*	3″	72.4	3.65 (dd, 9.0, 3.0)	3‴′′	78.1	3.37*
12	37.2	1.77*/2.38–2.39 (m)	4″	73.6	3.44 (t, 9.0)	4‴′′	71.7	3.30*
13	44.8	–	5″	70.9	3.96–3.97 (m)	5‴′′	77.9	3.28–3.29 (m)
14	51.6	1.60–1.61 (m)	6″	17.8	1.29 (d, 6.6)	6‴′′	62.8	3.68*/3.87*
15	38.7	2.11 (dd, 16.0, 14.0) 2.24 (dd, 16.0, 7.2)	2′- *O*-Rha					
16	206.5	–	1‴	102.7	5.00 (d, 1.8)			
17	145.5	–	2‴	72.2	3.86 (dd, 3.0, 1.8)			
18	16.9	1.14 (s)	3‴	72.5	3.68 (dd, 9.0, 3.0)			
19	19.8	1.11 (s)	4‴	73.8	3.44 (t, 9.0)			
20	136.0	–	5‴	70.9	3.96–3.97 (m)			
21	16.3	2.15 (s)	6‴	17.8	1.29 (d, 6.6)			
22	173.6	–						

[*] Overlapped signals

groups [δ_H 1.11 (H_3-19), 1.14 (H_3-18), 2.15 (H_3-21)], one secondary methyl group [δ_H 1.01, d, J=6.6 Hz (H_3-27)], one olefinic proton (δ_H 5,43, H-5), and four anomeric protons [δ_H 4.55 (d, J=7.8 Hz), 4.26 (d, J=7.8 Hz), 4.92 (d, J=1.8 Hz), and 5.00 (d, J=1.8 Hz)] were identified. Two carbonyl carbons (δ_C 206.5 and 173.6), two double bonds [δ_C 142.1 (C), 122.3 (CH), 145.5 (C), and 136.0 (C)) were also identified by ^{13}C NMR and HSQC spectra. The sugar moiety, 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside attached to C-3 as determined by comparing NMR data of **4** with the corresponding data of **1** (Tables 1 and 2) and by the observed HMBC correlations as shown in Figure 2. The NMR data of aglycone of **4** were similar to those of pregna-5,17(20)-diene-20-carboxylic acid methyl ester-3-ol-16-one-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (trillikamtoside M)[9], which were further confirmed by HSQC, COSY, and HMBC spectra (Figure 2). In addition, the 4-hydroxyisopentyl alcohol chain was elucidated from ^{1}H and ^{13}C NMR signals, δ_C/δ_H: 64.8/4.28 (CH_2), 33.2/1.54 and 1.91 (CH_2), 31.8/1.92 (CH), 75.6/3.43 and 3.78 (CH_2), and 17.3/1.01 (3H, d, J=6.6 Hz). These were assigned by COSY, HSQC, and HMBC spectra (Figure 2). This side chain linked to C-22 by an ester linkage as evident by HMBC correlation from H_2-1′′′′ (δ_H 4.28) to C-22 (δ_C 173.6). The remaining glucose sugar linked to C-4′′′′ of the side chain as determined by HMBC correlations from H_2-4′′′′ to C-1′′′′′ (δ_C 104.6) and from H-1′′′′′ (δ_H 4.26) to C-4′′′′ (δ_C 75.6). The small difference between the chemical shifts of two H_2-4′′′′ (δ_H 3.43 and 3.78, Δ=0.35) provided evidence for 3′′′′-*R* configuration.[11,13,14] Acid hydrolysis of **4** yielded D-glucose and L-rhamnose, which were identified by comparison with authentic samples via thin-layer chromatography (TLC), and from the positive sign of the optical rotations.[11,12] Thus, compound **4** was determined to be 3β-[(*O*-α-L-rhamnopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl)oxy]-pregna-5,17(20)-diene-16-one-20-carboxylic acid 4′′′′-*O*-β-D-glucopyranosylisopentyl ester, a new compound named as cambodianoside J (Figures S37-S48).

The known compounds were identified to be cambodianoside A (**5**),[3] diosbulbiside C (**6**),[15] and diosbulbiside D (**7**),[15] by comparison of their NMR data with those reported in the literature (Table S2, Figures S49-S60).

Compounds **1–7** were screened for their NO production inhibition in LPS stimulated RAW 264.7 cells. Compounds **1–7** did not show significant cytotoxic activity at a concentration of 100 μM in the MTT assay (Table S1). Therefore, levels of NO production in the cell medium were measured in the presence of **1–7** at serially diluted concentrations. As shown in Table 3, compounds **1** and **5** showed significantly NO inhibition with IC_{50} values of 19.03±1.84 and 25.52±2.45 μM, respectively, compounds **4**, **6**, and **7** showed moderate inhibition with IC_{50} values of 41.02±2.14, 67.92±3.81, and 62.12±2.56 μM, respectively. Whereas, compounds **2** and **3** were inactive with IC_{50} values over 100 μM, compared to that of the positive control compounds, dexamethasone, which showed IC_{50} values of 13.66±1.08 μM. These results suggested that the presence 22-OH group may have reduced the NO inhibition activity of these compounds.

Table 3. NO inhibition effects in LPS-activated RAW 264.7 cells of compounds

Compounds	NO inhibition (IC_{50}, μM)
1	19.03±1.84
2	>100
3	>100
4	41.02±2.14
5	25.52±2.45
6	67.92±3.81
7	62.12±2.56
Dexamethasone*	13.66±1.08

[*] positive control compound

Conclusions

Seven steroidal saponins including four new compounds named as cambodianoside G (**1**), cambodianoside H (**2**), cambodianoside I (**3**), cambodianoside J (**4**) were isolated from the roots of *Dracaena cambodiana*. Their chemical structures were elucidated by IR, HR-ESI-MS, 1D and 2D NMR spectra. Except compounds **2** and **3** were inactive with IC_{50} values over 100 μM, the remaining compounds exhibited NO production inhibition activity in lipopolysaccharide activated RAW 264.7 cells with IC_{50} values ranging from 19.03±1.84 to 67.92±3.81 μM.

Experimental Section

General

The optical rotations were measured on a Jasco P2000 polarimeter. The infrared spectra (IR) were recorded on a Spectrum Two FT-IR spectrometer. The high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was acquired on an Agilent 6530 Accurate Mass Q-TOF LC/MS. The NMR spectra were recorded on a Bruker 600 MHz spectrometer. Semi-preparative high-performance liquid chromatography (HPLC) were run on an Agilent 1260 system including binary pump, autosampler, DAD detector, and semi-preparative HPLC column YMC J'sphere ODS–H80 (4 μm, 20×250 mm). Isocratic mobile phase with the flow rate of 2.5 mL/min was used in Semi-prep-HPLC. The compound was monitored at wavelengths of 205, 230, 254, and 280 nm. Flash column chromatography was performed using silica gel, reversed phase C-18, and diaion HP-20 resins as stationary phase. Thin layer chromatography was carried out on pre-coated silica gel 60 F_{254} and RP-18 F_{254S} plates. The spots were detected by spraying with aqueous solution of H_2SO_4 5% followed by heating with a heat gun.

Plant material

The roots of *Dracaena cambodiana* Pierre ex Gagnep. were collected at Quang Tri province, Vietnam in March 2023, and taxonomically identified by Dr. Le Tuan Anh at the Vietnam National Museum of Nature, VAST and Dr Nguyen The Cuong at the Institute of Ecology and Biological Resource, VAST. A voucher

specimen (NCCT-170) was kept at Institute of Marine Biochemistry, VAST.

Extraction and isolation

The dried roots of *D. cambodiana* were powdered (4.1 kg) and ultrasonically extracted with methanol (three times, each 10 L, 1 h) to obtain the crude extract (180 g). This was suspended into 2.5 L of distilled water, and then successively extracted with ethyl acetate to give the EtOAc extract (DCRE, 27.0 g) and water layer (DCRW). These extracts were further screened for their NO inhibition activity and the DCRW fraction significantly showed effects with the inhibition percentage of 84.5% at 100 μg/mL. Therefore, the DCRW fraction was isolated on a Diaion column (HP-20) eluting with MeOH/H_2O (1/3, 1/1, 3/1, and 1/0, each 3 L) to give 4 fractions, DCRW1– DCRW4, respectively. Fraction DCRW2 (27.3 g) was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (20/1, 10/1, 5/1, 1/1; each 1.3 L) to give 4 four fractions, DCRW2 A-DCRW2D. Fraction DCRW2 C (4.3 g) was chromatographed on an YMC RP18 column eluting with MeOH/H_2O (3/1) to get four fractions, DCRW2 C1-DCRW2 C4. Fraction DCRW2 C2 (256 mg) was purified on a semi-pre-HPLC using ACN 26% in water to get compounds **1** (17.2 mg, t_R = 43.68 min) and **7** (21.4 mg, t_R = 58.34 min). Fraction DCRW2 C3 (168 mg) was purified on a semi-pre-HPLC using ACN 27% in water to get compound **4** (14.5 mg, t_R = 56.57 min). Fraction DCRW3 (18.25 g) was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (30/1, 15/1, 7/1, 1/1; each 1.2 L) to give 4 four fractions, DCRW3 A-DCRW3D. Fraction DCRW3B was isolated on an YMC RP18 column eluting with MeOH/H_2O (4/1) to get three fractions, DCRW3B1-DCRW3B3. Fraction DCRW3B2 (198 mg) was purified on a semi-pre-HPLC using ACN 24% in water to get compounds **2** (15.2 mg, t_R = 55.80 min) and **3** (26.0 mg, t_R = 59.31 min). Fraction DCRW3B3 (158 mg) was purified on a semi-pre-HPLC using ACN 29% in water to get compounds **5** (10.0 mg, t_R = 51.01 min) and **6** (13.6 mg, t_R = 61.93 min).

Cambodianoside G (1)

White amorphous powder; $[\alpha]_D^{25} = -97.6$ (*c* 0.08, MeOH); UV (MeOH) λ_{max} (nm): 242. IR (KBr) ν_{max} (cm^{-1}): 3407, 2933, 1701, 1660, 1453, 1380, 1041. ECD (MeOH) $\theta_{(\lambda\ nm)}$ mdeg: $-67.2_{(242)}$. HR-ESI-MS *m/z* 1067.5053 $[M+Na]^+$, calcd for $[C_{51}H_{80}O_{22}Na]^+$: 1067.5033, Δ = 1.9 ppm). ^{1}H-NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are shown in the Table 1.

Cambodianoside H (2)

White amorphous powder; $[\alpha]_D^{25}$=-64.5 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (nm): 225. IR (KBr) ν_{max} (cm^{-1}): 3371, 2903, 1639, 1594, 1508, 1071. ECD (MeOH) $\theta_{(\lambda\ nm)}$ mdeg: $+5.0_{(226)}$, $-1.8_{(250)}$. HR-ESI-MS *m/z* 905.4518 $[M+Na]^+$, calcd for $[C_{45}H_{70}O_{17}Na]^+$: 905.4505, Δ = + 1.4 ppm). ^{1}H-NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are shown in the Table 1.

Cambodianoside I (3)

White amorphous powder; $[\alpha]_D^{25} = -68.7$ (*c* 0.09, MeOH); UV (MeOH) λ_{max} (nm): 226. IR (KBr) ν_{max} (cm^{-1}): 3317, 2935, 1639, 1594, 1508, 1036. ECD (MeOH) $\theta_{(\lambda\ nm)}$ mdeg: $+4.7_{(227)}$, $-0.2_{(256)}$. HR-ESI-MS *m/z* 743.3966 $[M+Na]^+$, calcd for $[C_{39}H_{60}O_{12}Na]^+$: 743.3977, Δ=-1.5 ppm). ^{1}H-NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are shown in the Table 1.

Cambodianoside J (4)

White amorphous powder; $[\alpha]_D^{25} = -103.6$ (*c* 0.08, MeOH); UV (MeOH) λ_{max} (nm): 250. IR (KBr) ν_{max} (cm^{-1}): 3418, 2932, 1713, 1630, 1379, 1266, 1041. ECD (MeOH) $\theta_{(\lambda\ nm)}$ mdeg: $+4.3_{(213)}$, $+8.3_{(252)}$, $-8.1_{(348)}$. HR-ESI-MS *m/z* 1083.4975 $[M+Na]^+$, calcd for $[C_{39}H_{60}O_{12}Na]^+$: 1083.4983, Δ=-0.7 ppm). ^{1}H-NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are shown in the Table 2.

Acid hydrolysis of compounds 1–4

Acid hydrolysis of compounds **1–4** were the same as described in previous work[11,12] referred to Supplementary information.

Nitric oxide assay

The NO assay protocol is the same as described in previous papers[16,17] referred to Supplementary information.

Supplementary Material

Additional references cited within the Supporting Information.[11,12, 16, 17]

Author Contributions

Kiem PV, Tai BH, Nhiem NX, Yen PH, Thu VK designed experiments, elucidated chemical structures and wrote the paper. Hoang NH, Dung DT, Huong PTT, Dung NV, Ha NT, Trang DT extracted and isolated compounds and prepared sample for bioassay.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: *Dracaena cambodiana* · Asparagaceae · cambodianoside G–J · nitric oxide inhibition activity · steroidal saponin

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