

## New C<sub>27</sub> Steroidal Bisdesmosides from the Fresh Stems of *Dracaena cambodiana*

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Two new steroidal bisdesmosides, cambodracanosides A and B (**1** and **2**, resp.), were isolated from the fresh stems of *Dracaena cambodiana*, together with seven known glycosides. The structures of the new saponins were elucidated on the basis of detailed spectroscopic analyses, including 1D- and 2D-NMR techniques, and acidic hydrolysis.

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**Introduction.** – The genus *Dracaena* (Agavaceae) contains about 60 species and is distributed from the Old World tropic region to the Canary Islands. The resins from several species (e.g., *D. draco*, *D. cinnabari*, and/or *D. loureiri*) were used as a source of Dragon's Blood, a traditional medicine from ancient time, for the treatment of wounds, leucorrhoea, fractures, diarrhea, and piles, as well as for intestinal and stomach ulcers [1]. In China, the red resin of *D. cochinchinensis* S. C. CHEN, called 'Long-Xue-Jie' (Chinese dragon's blood) has been used as a substitute from 1970s [1]. Several steroidal glycosides from the fruits and stems of *D. cochinchinensis* and numerous phenolic compounds from its red resins have been reported [2–9].

*D. cambodiana* PIERRE EX GAGNEP, one of the related species of *D. cochinchinensis*, is distributed in Indo-China Peninsula and extended to the south part of Yunnan Province of China. Even though *D. cambodiana* was treated as a synonym of *D. cochinchinensis* and combined as one species by some taxonomists [10], it shows distinct difference in morphological characters and ecological habit from *D. cochinchinensis*. In order to compare these two species from the view of chemical composition, a detailed phytochemical study on the fresh stems of *D. cambodiana* was carried out. This led to the isolation of two new steroidal bisdesmosides **1** and **2**, together with seven known glycosides, **3–9**. This article describes the isolation and structure elucidation of the new compounds.

**Results and Discussion.** – The MeOH extract of the fresh stems of *D. cambodiana* was suspended in H<sub>2</sub>O and partitioned sequentially with petroleum ether and BuOH. The BuOH-soluble portions were subjected to a macroporous polymer polystyrene (*Diaion HP20SS*) column and then chromatographed over *MCI-gel CHP20P*, *Chromatorex ODS*, *RP-8* and silica gel columns, to afford nine compounds (**1–9**, see *Fig. 1*). Thereof, compounds **3–9** were the known glycosides namonin D (**3**) [11], 26-

*O*- $\beta$ -D-glucopyranosylfurosta-5,20(22),25(27)-triene-1 $\beta$ ,3 $\beta$ ,26-triol-1-*O*- $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\alpha$ -L-arabinopyranoside (**4**) [12], namonin C (**5**) [11], cantalasaponin-1 (**6**) [13], 26-*O*- $\beta$ -D-glucopyranosyl-22-*O*-methylfurosta-5,25(27)-diene-1 $\beta$ ,3 $\beta$ ,22 $\zeta$ ,26-tetrahydroxy-1-*O*- $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\alpha$ -L-arabinopyranoside (**7**) [14], syringin (**8**) [15], and (3,4,5-trimethoxyphenyl)-1-*O*- $\beta$ -D-apiofuranosyl-(1  $\rightarrow$  6)- $\beta$ -D-glucopyranoside (**9**) [16], respectively, which were revealed by comparison of the spectroscopic data with those reported in the literature. Compounds **1** and **2** were identified as new steroidal bisdesmosides and named as cambodracanosides A and B.

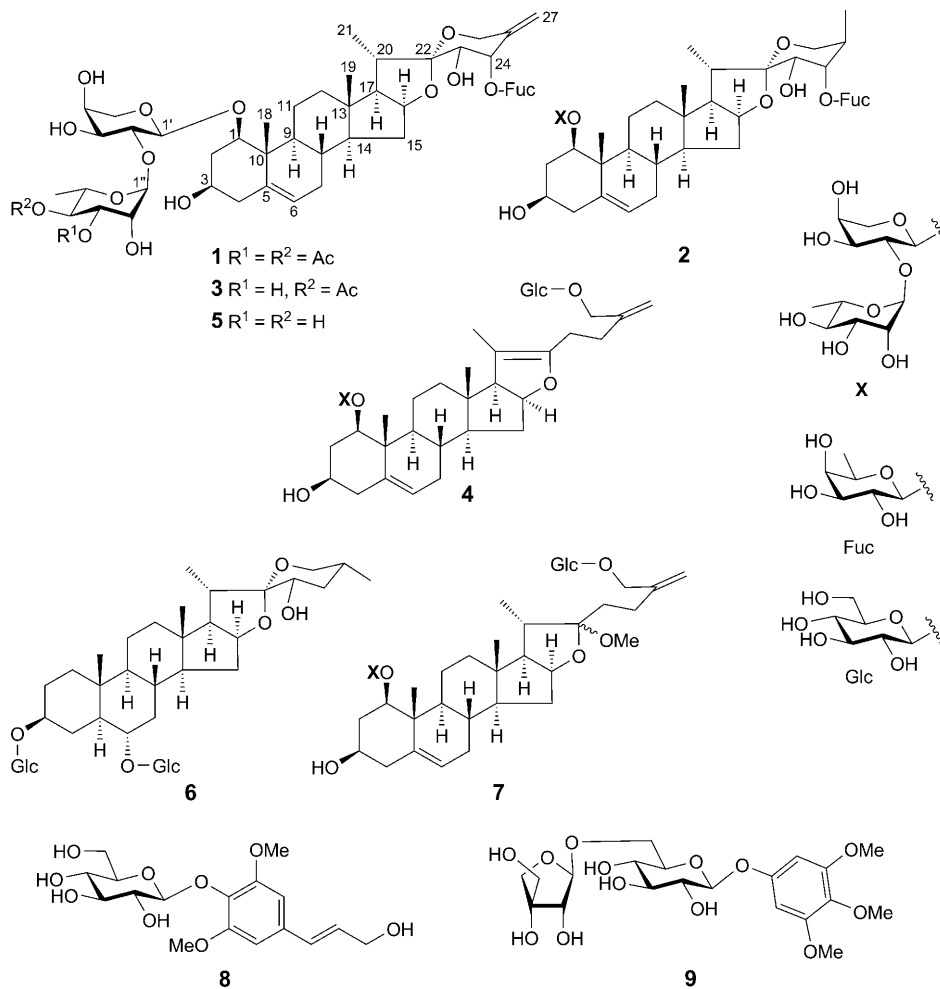


Fig. 1. Structures of compounds 1–9

Cambodracanoside A (**1**), obtained as a white amorphous powder, has the molecular formula C<sub>48</sub>H<sub>72</sub>O<sub>20</sub>, as deduced from the HR-FAB-MS (negative-ion mode; *m/z* 967.4555 ([*M* – H]<sup>–</sup>)) and <sup>13</sup>C-NMR (DEPT) spectra. On the basis of 1D- and 2D-NMR spectral data and acidic hydrolysis, **1** was established to be (1 $\beta$ ,3 $\beta$ ,23 $S$ ,24 $S$ )-1-[[2-

*O*-(3,4-di-*O*-acetyl-6-deoxy- $\alpha$ -L-mannopyranosyl)- $\alpha$ -L-arabinopyranosyl]oxy}-3,23-dihydroxyspirosta-5,25(27)-dien-24-yl 6-deoxy- $\beta$ -D-galactopyranoside.

The IR spectrum of **1** indicated the presence of AcO groups (1739 cm<sup>-1</sup>). The <sup>1</sup>H-NMR spectrum of **1** displayed three Me signals of a typical steroidal skeleton at  $\delta$ (H) 0.96, 1.36 (*s*, each 3 H) and 1.05 (*d*, *J* = 6.8, 3 H), as well as signals of three anomeric H-atoms at  $\delta$ (H) 4.65 (*d*, *J* = 7.7, 1 H), 5.16 (*d*, *J* = 7.9, 1 H), and 6.27 (*br. s*, 1 H), of an olefinic H-atom at  $\delta$ (H) 5.62 (*br. d*, *J* = 4.7, 1 H), and two signals for an O-bearing CH<sub>2</sub> group at  $\delta$ (H) 5.22 and 5.09 (*br. s*, each 1 H). The <sup>13</sup>C-NMR and DEPT spectrum (Table) showed a quaternary C-atom signal at  $\delta$ (C) 111.8, which is characteristic for C(22) of a spirostanol skeleton [17][18], and four olefinic C-atom signals at  $\delta$ (C) 139.5 (C), 124.5 (CH), 143.5 (C), and 113.8 (CH<sub>2</sub>), respectively. This indicated that **1** is a  $\Delta^{5,25(27)}$ -spirostanol triglycoside derivative. The NMR spectral features of **1** were very similar to those of namonin C (**5**) [11], except for the appearance of two additional AcO units in **1**. The obvious differences at C(3), C(4) and C(5) in the rhamnosyl unit ( $\delta$ (C) 69.6, 73.7, and 66.5 for **1**;  $\delta$ (C) 72.6, 74.3, and 69.5 for **5**, resp.) suggested that the two AcO groups were attached at the rhamnosyl C(3) and C(4) positions. Acid hydrolysis of **1** with 1M HCl in dioxane/H<sub>2</sub>O 1:1 yielded L-arabinose, L-rhamnose, and D-fucose as sugar residues, which were determined by GC analysis of their corresponding trimethylsilylated L-cysteine adducts [19]. In the HMBC spectrum of **1**, correlations of  $\delta$ (H) 4.65 (Ara H–C(1')) with  $\delta$ (C) 83.6 (C(1)),

Table. <sup>13</sup>C-NMR Spectral Data of Compounds **1** and **2** (at 125 MHz in (D<sub>5</sub>)pyridine;  $\delta$  in ppm)

	<b>1</b>	<b>2</b>		<b>1</b>	<b>2</b>
1	83.6	83.7	25	143.5	35.4
2	37.4	37.3	26	61.5	61.7
3	68.0	68.3	27	113.8	13.2
4	43.9	43.9	MeCO	170.7	
5	139.5	139.8	MeCO	170.8	
6	124.5	124.7	MeCO	20.9	
7	31.9	31.8	MeCO	20.8	
8	32.4	33.1	Ara-1'	100.2	100.5
9	50.3	50.5	Ara-2'	74.6	75.2
10	42.8	43.0	Ara-3'	75.9	76.0
11	23.9	24.0	Ara-4'	69.6	70.1
12	40.3	40.5	Ara-5'	67.6	67.3
13	40.7	40.6	Rha-1''	100.8	101.7
14	56.6	56.8	Rha-2''	69.6	72.7
15	32.9	32.5	Rha-3''	73.7	72.6
16	82.9	83.2	Rha-4''	72.0	74.8
17	61.4	61.7	Rha-5''	66.5	69.7
18	16.8	16.9	Rha-6''	18.1	19.0
19	14.8	15.0	Fuc-1'''	106.3	106.0
20	37.6	37.5	Fuc-2'''	73.4	73.4
21	14.8	15.1	Fuc-3'''	75.4	75.4
22	111.8	111.8	Fuc-4'''	73.1	72.9
23	70.4	70.1	Fuc-5'''	71.6	71.8
24	82.2	81.6	Fuc-6'''	17.3	17.3

$\delta(\text{H})$  6.27 (Rha H–C(1'')) with  $\delta(\text{C})$  74.6 (Ara C(2')), and  $\delta(\text{H})$  5.16 (Fuc H–C(1''')) with  $\delta(\text{C})$  82.2 (C(24)) revealed the sugar sequence and linkage site to the aglycone of **1** (Fig. 2). In addition, HMBCs of both rhamnosyl H–C(3'') and H–C(4'') with the two AcO CO groups ( $\delta(\text{C})$  170.8, 170.7) confirmed that both OH groups of the rhamnosyl moiety C(3'') and C(4'') were esterified with acetic acid. Moreover, the ROESY correlations between Me(18) and H–C(20), and H–C(17) and H–C(16) evidenced the *cis* D/E ring junction and the (20 $\alpha$ ) and (22 $\alpha$ ) configurations. The small coupling constants of H–C(23) ( $\delta(\text{H})$  3.75 (*d*,  $J = 3.8, 1 \text{ H}$ )) and H–C(24) ( $\delta(\text{H})$  4.79 (*d*,  $J = 3.8, 1 \text{ H}$ )), as well as the ROESY correlations (Fig. 3) between H–C(23)/H–C(24) and Me(21), revealed the (23*S*) and (24*S*) configuration, as well as a usual configuration at C(22). Therefore, the structure of cambodracanoside A was elucidated as shown in formula **1**.

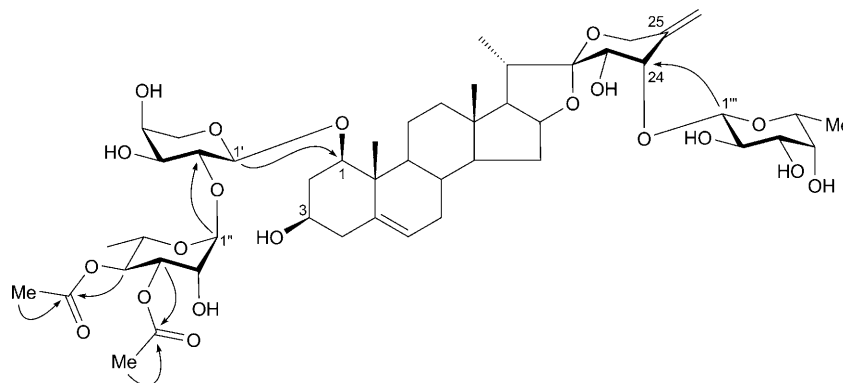


Fig. 2. Important HMBCs of **1**

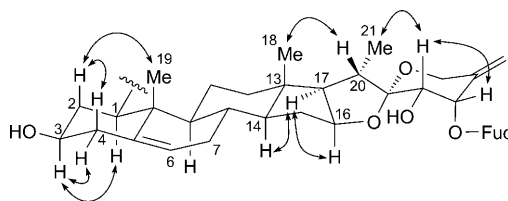


Fig. 3. Important ROESY correlations of **1**

Cambodracanoside B (**2**) has the molecular formula  $\text{C}_{44}\text{H}_{70}\text{O}_{18}$ , as deduced from the HR-FAB-MS (negative-ion mode,  $m/z$  885.4479 ( $[M - \text{H}]^-$ )). By comparison of the NMR data with those of compound **5** [12] and further 2D-NMR spectral data analysis, the structure of compound **2** was determined as (1 $\beta$ ,3 $\beta$ ,23*S*,25*R*)-1-[[2-*O*-(6-deoxy- $\alpha$ -L-mannopyranosyl)- $\alpha$ -L-arabinopyranosyl]oxy]-3,23-dihydroxyspirost-5-en-24-yl 6-deoxy- $\beta$ -D-galactopyranoside.

The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra of **2** showed typical Me signals at  $\delta(\text{H})$  0.98 (*s*, 3 H), 1.06 (*d*,  $J = 6.8, 3 \text{ H}$ ), 1.07 (*d*,  $J = 6.1, 3 \text{ H}$ ), and 1.42 (*s*, 3 H), three anomeric H-atoms ( $\delta(\text{H})$  4.97 (*d*,  $J = 6.6, 1 \text{ H}$ ), 5.12 (*d*,  $J = 7.7, 1 \text{ H}$ ), and 6.20 (*br. s*, 1 H)), two olefinic C-

atom signals at  $\delta(\text{C})$  139.8 and 124.7, and a quaternary C-atom signal at  $\delta(\text{C})$  111.8 (C(22)), suggesting that **2** is a  $\Delta^5$ -spirostanol triglycoside. The NMR data of **2**, including the sugar residues, were closely related to those of **5**, except for signals arising from ring *F* of the aglycone. Instead of signals for a C=C bond at  $\delta(\text{C})$  144 and 114 in **5** [11], a Me group ( $\delta(\text{H})$  1.06 (*d*,  $J = 6.8$ , 3 H),  $\delta(\text{C})$  13.2) and a CH group ( $\delta(\text{C})$  35.4) appeared in **2**. These observations suggested that **2** had a Me(27) group attached at C(25) of ring *F*, instead of the exocyclic C=C bond between C(25) and C(27) in **5**. This was confirmed by the HMBC of the Me(27) signal at  $\delta(\text{H})$  1.06 with C(25) ( $\delta(\text{C})$  35.4). The absolute configuration at C(25) was deduced as (*R*) based on the  $^{13}\text{C}$ -NMR of ring *F* and ROESY correlations [20]. Other HMBCs confirmed the structure of cambodracanoside B (**2**) as shown in Fig. 1.

Previous research showed that the fresh stems of *D. cochinchinensis* contained a lot of steroids with only one sugar linkage position at C(3) of plenty types of aglycones, including C<sub>21</sub> pregnane, C<sub>22</sub> pregnane, furostane, and spirostane derivatives, whereas the saponin composition in *D. cambodiana* was simple and only comprised of furosta and spirosta. In addition, various glycosylations at C(1), C(3), C(6), and C(24) positions were observed in *D. cambodiana*. From the phytochemical evidence, it is difficult to link these two species. Therefore, more evidences from protein and DNA levels are necessary to evaluate the relation between these two plants.

### Experimental Part

*General.* Column chromatography (CC): silica gel (SiO<sub>2</sub>; 200–300 mesh; Qingdao Marine Chemical Factory), Diaion HP20SS (Mitsubishi Chemical Industry, Ltd.), MCI-gel CHP20P (75–150  $\mu\text{m}$ ; Mitsubishi Chemical Industry, Ltd.), or Chromatorex ODS (100–200 mesh; Fuji Silysia Chemical Co. Ltd.). TLC: on silica-gel G pre-coated plates (Qingdao Haiyan Chemical Co.) with CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 7:3:0.5; spots were detected by spraying with 10% of H<sub>2</sub>SO<sub>4</sub>, followed by heating. GC Analysis: Agilent Technologies HP5890 gas chromatograph, equipped with a H<sub>2</sub> flame ionization detector; 30QC2/AC-5 quartz cap. column (30 m  $\times$  0.32 mm); conditions: column temp.: 180°/280°; programmed increase, 3°/min; carrier gas: N<sub>2</sub> (1 ml/min); injection and detector temp.: 250°; injection volume: 4  $\mu\text{l}$ , split ratio: 1:50. Optical rotations: SEPA-3000 automatic digital polarimeter. IR Spectra: Bio-Rad FTS-135 spectrometer; in cm<sup>-1</sup>. 1D- and 2D-NMR spectra: Bruker DRX-500 MHz instrument with TMS as internal standard. MS Spectra: VG Autospect 3000 spectrometer.

*Plant Material.* The fresh stems of *D. cambodiana* were collected at Xishuangbanna, Yunnan, P. R. China, and identified by C.-R. Y. A voucher specimen was deposited with the Herbarium of Kunming Institute of Botany (KIB), Chinese Academy of Sciences (CAS).

*Extraction and Isolation.* The fresh stems of *D. cambodiana* (14.8 kg) were extracted with MeOH (3  $\times$  10 l). After solvent evaporation, the residue (169 g) was suspended in H<sub>2</sub>O and extracted sequentially with petroleum ether (PE) and BuOH. The BuOH fraction (5.1 g) was put directly on a Diaion HP20SS column, eluting with a gradient of H<sub>2</sub>O/MeOH (1:0 to 0:1), to give three fractions (Frs. 1–3). Fr. 1 (1.7 g) was subjected to repeated CC over SiO<sub>2</sub> (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 9:1:0.1 to 7:3:0.5) and MCI-gel CHP20P (40 to 100% MeOH) to yield **8** (27 mg) and **9** (12 mg). Fr. 2 (2.0 g) was chromatographed over Chromatorex ODS (40 to 100% MeOH) and RP-8 (70% MeOH) columns to give **1** (8 mg), **2** (9 mg), **3** (16 mg), **4** (19 mg), **5** (123 mg), **6** (5 mg), and **7** (28 mg).

*Acid Hydrolysis of 1 and 2.* Compounds **1** and **2** (5 mg each) in 1M HCl/dioxane (1:1, v/v, 4 ml) were heated at 86° on a water bath for 6 h. The mixtures were partitioned between CHCl<sub>3</sub> and H<sub>2</sub>O four times. The aq. layer was passed through Amberlite IRA-401 (OH<sup>-</sup> form), and the eluate was concentrated to dryness to give a saccharide mixture. Fucose, rhamnose, and arabinose were identified as being present in the mixture by direct TLC analysis compared with authentic samples: R<sub>f</sub> 0.60 (fucose); R<sub>f</sub> 0.43

(arabinose);  $R_f$  0.69 (rhamnose) (iPrOH/MeOH/H<sub>2</sub>O 25:1:2). The soln. of the sugar residue of compounds **1** and **2** in 1.5 ml pyridine was added to L-cysteine methyl ester hydrochloride (1.0 mg) and kept at 60° for 1 h. Then, 1-(trimethylsilyl)-1H-imidazole (1.5 ml) was added to the mixture and kept again at 60° for 30 min. 4  $\mu$ l of the supernatant were analyzed by GC, and the retention times of L-rhamnose, L-arabinose, and D-fucose were 15.967, 14.113, and 15.672 min, resp.

**Cambodianoside A** (= (1 $\beta$ ,3 $\beta$ ,23S,24S)-1-[[2-O-(3,4-Di-O-acetyl-6-deoxy- $\alpha$ -L-mannopyranosyl)- $\alpha$ -L-arabinopyranosyl]oxy]-3,23-dihydroxyspirosta-5,25(27)-dien-24-yl 6-Deoxy- $\beta$ -D-galactopyranoside; **1**). White amorphous powder.  $[\alpha]_D^{25} = -21.74$  ( $c = 0.46$ , pyridine). IR (KBr): 3431 (OH), 2930 (CH), 1739, 1631, 1050, 999. <sup>1</sup>H-NMR ((D<sub>5</sub>)pyridine, 500 MHz): 0.96 (s, Me(18)); 1.05 (d,  $J = 6.8$ , Me(21)); 1.08–1.10 (m, H–C(14)); 1.25–1.26 (m, H<sub>a</sub>–C(12)); 1.36 (s, Me(19)); 1.39 (d,  $J = 5.6$ , Me(6<sub>Rha</sub>)); 1.39–1.40 (m, H<sub>a</sub>–C(15)); 1.40–1.42 (m, H–C(8)); 1.44–1.46 (m, H–C(9)); 1.45–1.47 (m, H<sub>a</sub>–C(7)); 1.49 (d,  $J = 6.3$ , Me(6<sub>Fuc</sub>)); 1.54–1.55 (m, H<sub>b</sub>–C(11)); 1.69 (dd,  $J = 8.1, 6.2$ , H–C(17)); 1.78–1.79 (m, H<sub>b</sub>–C(7)); 1.79–1.80 (m, H<sub>b</sub>–C(15)); 1.97 (s, AcO); 2.12 (s, AcO); 2.36–2.38 (m, H–C(20)); 2.62–2.65 (m, H<sub>b</sub>–C(4)); 2.70–2.74 (m, H<sub>a</sub>–C(4)); 2.70–2.71 (m, H<sub>a</sub>–C(2)); 2.80 (br. d,  $J = 8.8$ , H<sub>a</sub>–C(11)); 2.83–2.85 (m, H<sub>b</sub>–C(2)); 3.65 (d,  $J = 11.9$ , H<sub>a</sub>–C(5'<sub>Ara</sub>)); 3.75 (dd,  $J = 11.9, 3.8$ , H–C(1)); 3.75 (d,  $J = 3.8$ , H–C(23)); 3.78–3.79 (m, H–C(3)); 3.97 (br. d,  $J = 9.3$ , H<sub>a</sub>–C(26)); 4.24 (dd,  $J = 11.9, 5.2$ , H<sub>b</sub>–C(5'<sub>Ara</sub>)); 4.49–4.51 (m, H–C(2'<sub>Ara</sub>)); 4.56 (q-like,  $J = 6.1$ , H–C(16)); 4.65 (d,  $J = 7.7$ , H–C(1'<sub>Ara</sub>)); 4.79 (d,  $J = 3.8$ , H–C(24)); 4.87 (d,  $J = 9.3$ , H<sub>b</sub>–C(26)); 5.09 (br. s, H<sub>a</sub>–C(27)); 5.16 (d,  $J = 7.9$ , H–C(1''<sub>Fuc</sub>)); 5.22 (br. s, H<sub>b</sub>–C(27)); 5.62 (br. d,  $J = 4.7$ , H–C(6)); 5.66 (dd,  $J = 4.4, 12.6$ , H–C(4''<sub>Rha</sub>)); 5.92 (dd,  $J = 12.3, 12.6$ , H–C(3''<sub>Rha</sub>)); 6.27 (br. s, H–C(1''<sub>Rha</sub>)). <sup>13</sup>C-NMR (125 MHz, (D<sub>5</sub>)pyridine): Table. FAB-MS (neg.): 968 ( $M^-$ ). HR-FAB-MS (neg.): 967.4555 ( $[M - H]^-$ , C<sub>48</sub>H<sub>71</sub>O<sub>20</sub>; calc. 967.4539).

**Cambodianoside B** (= (1 $\beta$ ,3 $\beta$ ,23S,25R)-1-[[2-O-(6-Deoxy- $\alpha$ -L-mannopyranosyl)- $\alpha$ -L-arabinopyranosyl]oxy]-3,23-dihydroxyspirost-5-en-24-yl 6-Deoxy- $\beta$ -D-galactopyranoside; **2**). White amorphous powder.  $[\alpha]_D^{25} = -35.1$  ( $c = 1.52$ , pyridine). IR (KBr): 3423 (OH), 2926 (CH), 1632, 1059, 973, 914, 897. <sup>1</sup>H-NMR (500 MHz, (D<sub>5</sub>)pyridine): 0.98 (s, Me(18)); 1.06 (d,  $J = 6.8$ , Me(27)); 1.07 (d,  $J = 6.1$ , Me(21)); 1.22–1.24 (m, H<sub>a</sub>–C(12)); 1.42 (s, Me(19)); 1.42–1.43 (m, H–C(8), H<sub>a</sub>–C(15)); 1.44–1.46 (m, H<sub>a</sub>–C(7)); 1.49–1.51 (m, H<sub>a</sub>–C(12)); 1.50 (d,  $J = 6.3$ , Me(6<sub>Fuc</sub>)); 1.52–1.53 (m, H<sub>b</sub>–C(12)); 1.70–1.72 (m, H–C(17)); 1.71 (d,  $J = 6.1$ , Me(6<sub>Rha</sub>)); 1.78–1.80 (m, H<sub>b</sub>–C(7), H<sub>b</sub>–C(15)); 2.46–2.49 (m, H–C(25)); 2.59 (dd,  $J = 12.0, 5.7$ , H<sub>b</sub>–C(4)); 2.59–2.61 (m, H<sub>a</sub>–C(2)); 2.76 (dd,  $J = 12.0, 5.7$ , H<sub>a</sub>–C(4)); 2.90–2.92 (m, H<sub>b</sub>–C(11)); 2.94–2.96 (m, H<sub>b</sub>–C(2)); 3.75 (d,  $J = 3.2$ , H–C(23)); 3.82 (dd,  $J = 11.7, 3.9$ , H–C(1)); 3.85–3.86 (m, H–C(3)); 3.94–3.95 (m, H–C(16)); 4.43 (br. d,  $J = 11.7$ , H<sub>a</sub>–C(26)); 4.73 (d,  $J = 3.2$ , H–C(24)); 4.86 (dd,  $J = 11.7, 2.8$ , H<sub>b</sub>–C(26)); 4.97 (d,  $J = 6.6$ , H–C(1'<sub>Ara</sub>)); 5.12 (d,  $J = 7.7$ , H–C(1''<sub>Fuc</sub>)); 5.56 (d,  $J = 5.3$ , H–C(6)); 6.20 (br. s, H–C(1''<sub>Rha</sub>)). <sup>13</sup>C-NMR (125 MHz, (D<sub>5</sub>)pyridine): Table. FAB-MS (neg.): 885 ( $[M - H]^-$ ), 753 ( $[M - Rha]^-$ ). HR-FAB-MS (neg.): 885.4479 ( $[M - H]^-$ , C<sub>44</sub>H<sub>69</sub>O<sub>18</sub>; calc. 885.4484).

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