New Steroidal Saponins from the Leaves of *Yucca elephantipes*

by **Ying Zhang**[a])[b]), **Chong-Ren Yang**[a])[c]), and **Ying-Jun Zhang***[a])

[a]) State Key Laboratory of Phytochemistry and Plant Resources of West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P. R. China (phone/fax: +86-871-5223235; e-mail: zhangyj@mail.kib.ac.cn)

[b]) College of Chinese Medicine, Jilin Agricultural Science and Technology College, Jilin 132101, P. R. China

[c]) Weihe Biotech Research and Development Center, Yuxi 653101, P. R. China

Two new spirostanol saponins, namely elephanosides G and H (**1** and **2**, resp.) were isolated from the leaves of *Yucca elephantipes* (Agavaceae), together with the two known furostanol saponins **3** and **4** and the six known flavonoid *O*- and *C*-glycosides **5**–**10**. The new structures were elucidated as (3*β*,25*S*)-spirost-5-en-3-yl *O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**1**) and (3*β*,5*β*,25*R*)-3-[(2-*O*-*β*-D-glucopyranosyl-*β*-D-galactopyranosyl)oxy]spirostan-12-one (**2**) on the basis of detailed spectroscopic analysis and acidic hydrolysis.

Introduction. – *Yucca elephantipes* REGEL. (Agavaceae) is an evergreen ornamental plant native to Mexico and introduced to China later on. It is the tallest in the genus *Yucca*, reaching up to 30 feet in height with glossy bluish green leaves that can grow up to 4 feet long with a narrow width of about 3 inches. The leaf extract of *Yucca elephantipes* was reported to have antiviral activity on TMV (Tobacco mosaic virus) [1]. Our previous chemical study on the stems of *Yucca elephantipes* led to the isolation of ten steroidal saponins with *cis*-fused *A*/*B* rings. Among them, two smilagenin glycosides exhibited moderate antifungal activity against *Candida albicans* and *Cryptococcus neoformans* with $IC_{50} = 5.0–15$ μg/ml [2]. As a continuation of our work on the title plant, two new spirostanol steroidal saponins namely elephanosides G and H (**1** and **2**, resp.) were identified from the leaves, together with the two known furostanol steroidal saponins **3** and **4** and the six known flavonoid *O*- and *C*-glycosides **5**–**10**. The present article describes the isolation and structure elucidation of the two new saponins by means of to detailed spectroscopic analysis and acidic hydrolysis.

Results and Discussion. – The BuOH soluble fraction obtained after liquid–liquid partition of the MeOH extract of the leaves of *Yucca elephantipes* was subjected to various column chromatographies (SiO_2, *MCI* gel *CHP20P*, *Sephadex LH-20*, *Diaion HP-20*, and reversed phase silica gel (*RP-8*)) to afford the ten compounds **1**–**10** (*Fig. 1*). Among them, **3**–**10** proved to be known compounds. They were identified as two furostanol saponins, *i.e.*, (3*β*,22*ξ*,25*R*)-22-hydroxyfurost-5-ene-3,26-diyl bis(*β*-D-glucopyranoside) (**3**) [3], (3*β*,22*ξ*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-hydroxyfurost-5-en-3-yl 4-*O*-*β*-D-glucopyranosyl-*β*-D-galactopyranoside (=(25*R*)-epimer of Po-8; **4**) [4], and six flavonoid glycosides, *i.e.*, kaempferol 3-{*O*-*β*-D-glucopyranosyl-(1→

2)-*O*-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-galactopyranoside} (**5**) [5], quercetin 3-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) (= rutin; **6**) [5], 4′-*O*-methylquercetin 3-(6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) (**7**) [6], quercetin 3-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-galactopyranoside} (**8**) [5], 6,8-bis(β-D-glucopyranosyl)apigenin (**9**) [7], and 2″-*O*-(α-L-rhamopyranosyl)vitexin (= 8-(2-*O*-α-L-rhamnopyranoyl-β-D-glucopyranosyl)apigenin; **10**) [8], respectively, on the basis of spectroscopic and physico-chemical comparison with authentic samples and literature data (kaempferol = 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one; quercetin = 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxyphenyl-4*H*-1-benzopyran-4-one; apigenin = 5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one; α-L-rhamose = 6-deoxy-α-L-mannose). Compounds **3**–**10** were found for the first time in *Y. elephantipes*.

The new saponins **1** and **2** showed a positive reaction (green color) with the anisaldehyde reagent but a negative reaction with the *Ehrlich* reagent, indicating the presence of a spirostanol skeleton [9].

Compound **1**, a white amorphous powder, had a molecular formula $C_{45}H_{72}O_{18}$, as deduced by the negative-ion-mode HR-ESI-MS (*m*/*z* 899.4652 ($[M-H]^-$, $C_{45}H_{71}O_{18}$)) and ^{13}C-NMR (DEPT) spectra. The ^{1}H- and ^{13}C-NMR spectra (*Table*) of saponin **1** showed the presence of a typical steroidal skeleton with four Me groups at δ(H) 0.80 (*s*, Me(18)), 0.87 (*s*, Me(19)), 1.06 (*d*, *J* = 7.0 Hz, Me(27)), and 1.13 (*d*, *J* = 6.9 Hz, Me(21)), and a characteristic C(22) resonance at δ(C) 109.7 [4]. In addition, the appearance of one olefinic H-atom at δ(H) 5.30 (*d*, *J* = 4.5 Hz) and two olefinic C-atoms at δ(C) 141.0 (C) and 121.7 (CH) indicated the existence of a C=C bond between C(5) and C(6) [10]. The (25*S*) configuration in the aglycone was deduced by the signals of the *F* ring at δ(C) 26.4 (C(23)), 26.2 (C(24)), 27.5 (C(25)), 65.1 (C(26)), and 16.3 (C(27)) [11]. The aforementioned data were identical to those of yamogenin, revealing the aglycone of **1** to be (3β,25*S*)-spirost-5-en-3-ol (= yamogenin) [11]. In the negative-ion-mode FAB-MS of **1**, the characteristic fragment-ion peaks at *m*/*z* 737 ($[M-162-H]^-$), 575 ($[M-162-162-H]^-$), and 413 ($[M-162-162-162-H]^-$) revealed the existence of three hexosyl units, which was coincident with the ^{1}H-NMR (three anomeric H-atoms at δ(H) 4.90 (*d*, *J* = 7.5 Hz), 5.14 (*d*, *J* = 7.8 Hz), and 5.22 (*d*, *J* = 7.4 Hz)) and ^{13}C-NMR data (*Table*); the large *J* values indicated the β-configuration at these anomeric centers. Acidic hydrolysis of **1** afforded D-galactose and D-glucose, in a ratio of 1:2, as sugar residues, which were determined by GC analysis of their trimethylsilylated L-cysteine derivatives [12]. The full assignment of each sugar moiety was achieved by the HMQC-TOCSY experiment. Taking into account the known effects of *O*-glycosylation and the result of acid hydrolysis, the presence of a terminal β-D-glucopyranosyl unit (δ(C) 107.0, 76.8, 79.0, 70.3. 78.1, and 61.6), a 3-*O*-substituted β-D-glucopyranosyl unit (δ(C) 105.3, 75.1, 86.2, 71.8, 78.5, and 63.2), and a 4-*O*-substituted β-D-galactopyranosyl unit (δ(C) 102.6, 73.3, 75.6, 81.1, 77.7, and 60.4) was deduced. In the HMBC spectrum (*Fig. 2*), the anomeric H-atoms at δ(H) 5.22 (terminal glucosyl), 5.14 (3-*O*-substituted glucosyl), and 4.90 (4-*O*-substituted galactosyl) were correlated with the C-atoms at δ(C) 86.2 (C(3) of the 3-*O*-substituted glucosyl), 81.1 (C(4) of the 4-*O*-substituted galactosyl), and 78.2 (C(3) of the aglycone), respectively, leading to the triglycoside structure *O*-β-D-glucopyranosyl-

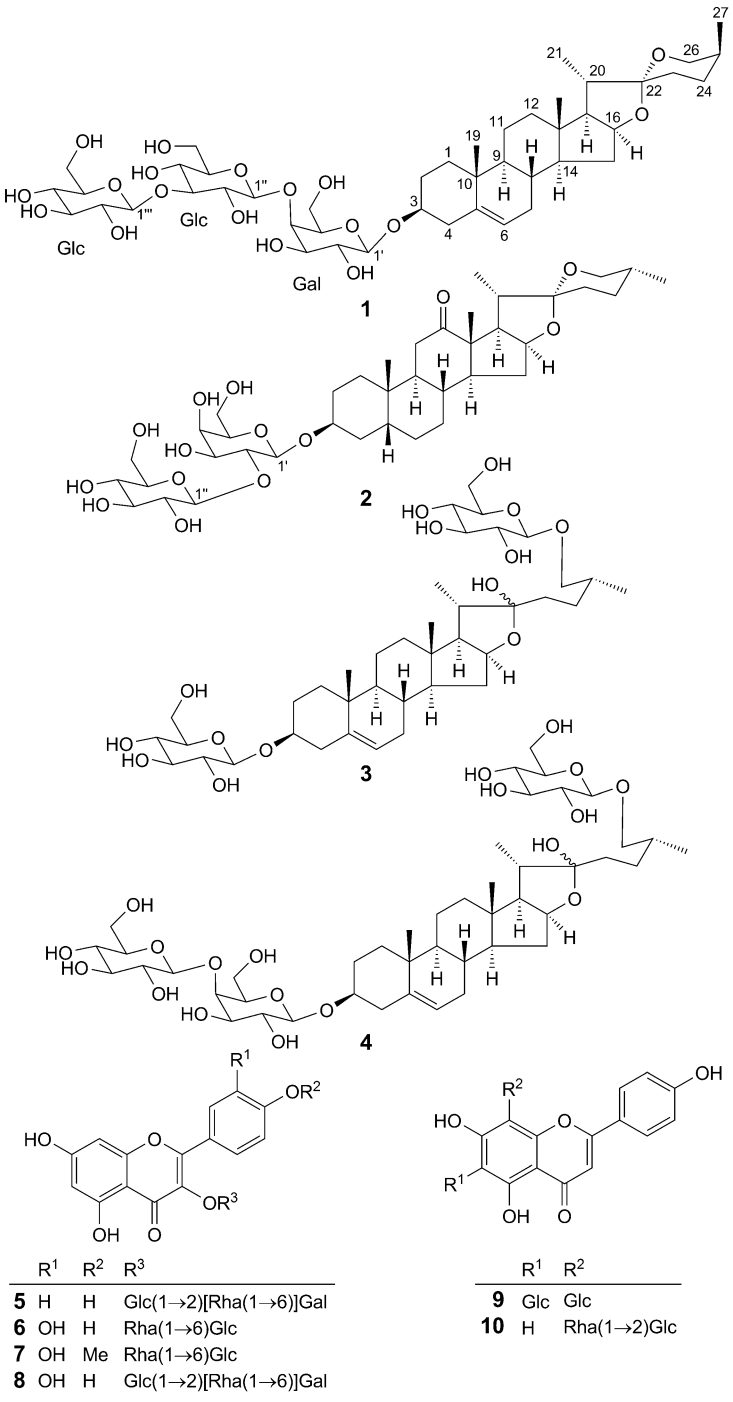

Fig. 1. *Compounds* **1**–**10**, *isolated from the leaves of* Yucca elephantipes

Table. *^{13}C-NMR Data* ((D$_5$)pyridine) *of Compounds* **1** *and* **2.** δ in ppm, *J* in Hz.

C-Atom	**1**	**2**	C-Atom	**1**	**2**
C(1)	37.5	30.7	Gal C(1′)	102.6	102.5
C(2)	30.2	26.8	C(2′)	73.3	81.9
C(3)	78.2	75.3	C(3′)	75.6	75.2
C(4)	39.2	30.5	C(4′)	81.1	69.9
C(5)	141.0	36.5	C(5′)	77.7	77.0
C(6)	121.7	26.4	C(6′)	60.4	62.8
C(7)	32.3	26.5	Glc C(1″)	105.3	106.2
C(8)	31.6	34.7	C(2″)	75.1	76.7
C(9)	50.2	41.9	C(3″)	86.2	78.1
C(10)	37.0	35.7	C(4″)	71.8	71.7
C(11)	21.1	37.8	C(5″)	78.5	78.5
C(12)	39.8	213.1	C(6″)	63.2	62.2
C(13)	40.4	55.6	Glc C(1‴)	107.0	
C(14)	56.6	56.0	C(2‴)	76.8	
C(15)	32.2	31.8	C(3‴)	79.0	
C(16)	81.2	79.8	C(4‴)	70.3	
C(17)	62.7	54.3	C(5‴)	78.1	
C(18)	16.4	16.1	C(6‴)	61.6	
C(19)	19.4	23.2			
C(20)	42.4	42.6			
C(21)	14.9	13.9			
C(22)	109.7	109.5			
C(23)	26.4	31.4			
C(24)	26.2	28.9			
C(25)	27.5	30.6			
C(26)	65.1	67.0			
C(27)	16.3	17.3			

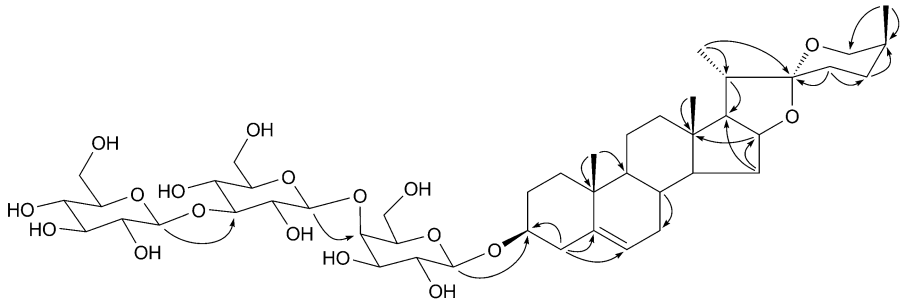

Fig. 2. *Selected HMBCs of* **1**

(1 → 3)-*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-galactopyranoside and its linkage to OH-C(3) group of the aglycone yamogenin. Accordingly, the structure of **1** was characterized as (3*β*,25*S*)-spirost-5-en-3-yl *O*-*β*-D-glucopyranosyl-(1 → 3)-*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-galactopyranoside, and named elephanoside G.

Compound **2** was obtained as a white amorphous powder. Its molecular formula $C_{39}H_{62}O_{14}$ was assigned by the negative-ion-mode HR-ESI-MS (*m/z* 753.4059 ($[M-H]^-$, $C_{39}H_{61}O_{14}^-$) and ^{13}C-NMR (DEPT) spectra. The ^{1}H- and ^{13}C-NMR spectra (*Table*) of **2** showed the presence of four Me signals at δ(H) 0.67 (*d*, $J=5.0$ Hz, Me(27)), 0.96 (*s*, Me(19)), 1.06 (*s*, Me(18)), and 1.31 (*d*, $J=6.8$ Hz, Me(21)), and a characteristic C(22) resonance at δ(C) 109.5, corresponding to a typical steroidal skeleton [4]. The C-atom signal of Me(19) at δ(C) 23.2, the downfield shifted CH_2(11) group at δ(C) 37.8, and a CO group at δ(C) 213.1, revealed the existence of a C(12)=O group. Moreover, a set of C-signals arising from rings *A* and *B* at δ(C) 30.7 (C(1)), 26.8 (C(2)), 30.5 (C(4)), 36.5 (C(5)), 26.4 (C(6)), and 26.5 (C(7)), and from ring *F* at δ(C) 31.4 (C(23)), 28.9 (C(24)), 30.6 (C(25)), 67.0 (C(26)), and 17.3 (C(27)) were observed in the ^{13}C-NMR and DEPT spectra, indicating the *cis* fusion of rings *A* and *B* (5β-H type) and the (25*R*) configuration of the aglycone skeleton [11]. Thus, the aglycone of **2** was deduced as gloriogenin (=(3β,5β,25*R*)-3-hydroxyspirostan-12-one) [13] by further comparison of their ^{13}C-NMR data. The negative-ion-mode FAB-MS displayed a quasi-molecular-ion peak at *m/z* 753 ($[M-H]^-$) and a fragment-ion peak at *m/z* 591 ($[M-162-H]^-$), indicating the existence of a terminal hexosyl residue in **2**. In the ^{1}H-NMR spectrum, two anomeric H-atom signals at δ(H) 4.88 (*d*, $J=7.6$ Hz) and 5.29 (*d*, $J=7.6$ Hz) suggested the existence of two sugar units with β-configurations. The HMQC-TOCSY experiment achieved the full assignment of the two hexosyl moieties, which were identical with the sugar moiety of Ys-II (=smilagenin 3-(2-*O*-β-D-glucopyranosyl-β-D-galactopyranoside) isolated from *Yucca gloriosa* [14]. In the HMBC spectrum of **2**, the correlations of δ(H) 5.29 (Glc H–C(1)) with δ(C) 81.9 (Gal C(2)), and of δ(H) 4.88 (Gal H–C(1)) with δ(C) 75.3 (C(3)) confirmed the linkage of the sugar units in **2**, *i.e.*, the inner galactosyl unit was attached to C(3) of the aglycone, and the terminal glucosyl unit to C(2) of the inner galactosyl unit. Therefore, the structure of **2** was determined as (3β,5β,25*R*)-3-[(2-*O*-β-D-glucopyranosyl-β-D-galactopyranosyl)oxy]spirostan-12-one and named elephanoside H.

Previous phytochemical studies on the genus were mainly focused on steroidal saponins. No flavonoid glycosides were reported. Our investigations revealed differences in the secondary metabolites profile of the stems and leaves. While saponins identified in the stems had a saturated *B* ring and (25*R*) configuration [2], the three saponins **1**, **3**, and **4** isolated from the leaves possess a C(5)–C(6) bond, and one thereof, *i.e.*, **1**, has (25*S*) configuration.

The authors are grateful to the staff of the Analytical Group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for measurements of all spectra. This work was supported by the *973 Program of Ministry of Science & Technology of China* (2011CB915503) and the *12th Five Year National Science & Technology Supporting Program* (2012BAI29B06).

Experimental Part

General. GC: *Agilent Technologies-HP5890* gas chromatograph; for the determination of the absolute configurations of monosaccharides (see below) [12]. Column chromatography (CC): *Diaion HP-20* (*Mitsubishi*, Japan), silica gel (SiO_2; 200–300 mesh, *Qingdao Makall*, P. R. China), *Sephadex LH-20* (*Pharmacia*, Sweden), *MCI* gel *CHP-20P* (*Mitsubishi*, Japan) and reversed phase silica gel *RP-8*

(40–63 μm; *Merck*, Germany). Optical rotations: *SEPA-3000* Automatic digital polarimeter. NMR Spectra: *Bruker-AM-400* and *-DRX-500* instrument; in (D_5)pyridine and (D_6)DMSO with $SiMe_4$ as internal standard; δ in ppm, J in Hz. FAB-MS (negative-ion mode) and HR-ESI-MS (negative-ion mode): *VG-AutoSpec-3000* and *API-Qstar Pulsar* LC/TOF spectrometers, resp; in m/z.

Plant Material. The dried leaves of *Yucca elephantipes* were collected from the East Garden of the Kunming Botanical Garden, Chinese Academy of Sciences, Yunnan, P. R. China, on August, 2005. A voucher specimen (KUN No. 0831640) has been deposited with the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The dried leaves (3.8 kg) of *Yucca elephantipes* were extracted three times with MeOH (each 5 l) under reflux for 2 h each time. After evaporation of the solvent, the residue was suspended in H_2O (5 l), and then partitioned with petroleum ether (each 1 l, 5 times), $CHCl_3$ (each 1 l, 5 times), and BuOH (each 1 l, 5 times), successively. The BuOH fraction (150 g) was subjected to CC (SiO_2 (3700 g), $CHCl_3$/MeOH/H_2O 7:3:0.5): *Fractions 1–6*. *Fr. 1* (20 g) was subjected to CC (*MCI* gel *CHP-20P* H_2O/MeOH 1:0 → 0:1, then SiO_2, $CHCl_3$/MeOH/H_2O 9:1:0.1 → 7:3:0.5, and finally *RP-8*, H_2O/MeOH 4:6 → 0:1): **1** (10 mg), **2** (6 mg), and **3** (8 mg). *Fr. 2* (27 g) was applied to CC (*MCI* gel *CHP-20P*, H_2O/MeOH 1:0 → 0:1, then *RP-8*, H_2O/MeOH, 6:4 → 0:1, then *Sephadex LH-20*, H_2O/MeOH 1:0 → 0:1, and finally SiO_2, $CHCl_3$/MeOH/H_2O 7:3:0.5): **4** (31 mg) and **7** (170 mg). *Fr. 4* (11 g) was subjected to CC (*Diaion HP-20*, H_2O/MeOH 1:0 → 0:1, then *MCI* gel *CHP-20P*, MeOH/H_2O 2:8 → 1:0, and finally SiO_2, $CHCl_3$/MeOH/H_2O 6:4:1): **6** (34 mg) and **10** (90 mg). *Fr. 5* (20 g) was applied to CC(*MCI*-gel *CHP-20P*, MeOH/H_2O 0:1 → 1:0, then *RP-8*, MeOH/H_2O 2:8 → 1:0), and finally SiO_2, $CHCl_3$/MeOH/H_2O 6:4:1): **5** (170 mg). *Fr. 6* (14 g) was subjected to CC(*MCI* gel *CHP-20P*, MeOH/H_2O 0:1 → 1:0 and then SiO_2, $CHCl_3$/MeOH/H_2O 6:4:1): **5** (450 mg), **8** (80 mg), and **9** (60 mg).

Elephanoside G (=*(3β,25*S)*-Spirost-5-en-3-yl* O-*β*-D-*Glucopyranosyl-(1 → 3)*-O-*β*-D-*glucopyranosyl-(1 → 4)-β*-D-*galactopyranoside*; **1**). White amorphous powder. $[\alpha]_D^{26} = -35.9$ ($c = 0.12$, MeOH). ^{1}H-NMR (C_5D_5N, 500 MHz): 0.80 (*s*, Me(18)); 0.83–0.86 (*m*, H–C(9)); 0.87 (*s*, Me(19)); 0.94–0.97 (*m*, H_a–C(1)); 0.99–1.03 (*m*, H_a–C(12)); 1.03–1.06 (*m*, H–C(14)); 1.06 (*d*, $J = 7.0$, Me(27)); 1.13 (*d*, $J = 6.9$, Me(21)); 1.29–1.31 (*m*, H_a–C(7)); 1.32–1.34 (*m*, H_a–C(24)); 1.36–1.38 (*m*, H_a–C(15)); 1.39–1.41 (*m*, H_a–C(23)); 1.42–1.45 (*m*, H–C(11)); 1.49–1.53 (*m*, H–C(8)); 1.56–1.59 (*m*, H–C(25)); 1.64–1.67 (*m*, H_b–C(12)); 1.69–1.71 (*m*, H_b–C(1)); 1.72–1.74 (*m*, H_a–C(2)); 1.77 (*dd*, $J = 6.5, 8.5$, H–C(17)); 1.84–1.86 (*m*, H_b–C(7)); 1.87–1.89 (*m*, H_b–C(23)); 1.91–1.93 (*m*, H–C(20)); 1.95–2.01 (*m*, H_b–C(15)); 2.07–2.10 (*m*, H_b–C(2)); 2.12–2.15 (*m*, H_b–C(24)); 2.44 (*t*, $J = 11.5$, H_a–C(4)); 2.66 (*dd*, $J = 2.5, 11.5$, H_b–C(4)); 3.35 (br. *d*, $J = 11.0$, H_a–C(26)); 3.89–3.92 (*m*, H–C(3)); 3.93–3.95 (*m*, Glc H–C(5‴)); 4.03 (br. *d*, $J = 11.0$, H_b–C(26)); 4.05–4.07 (*m*, Glc H–C(2‴)); 4.08–4.10 (*m*, Gal H–C(5′)); 4.11–4.13 (*m*, Gal H–C(3′)); 4.14 (*t*, $J = 8.0$, Glc H–C(3″)); 4.20–4.23 (*m*, Glc H–C(4‴)); 4.45–4.48 (*m*, Gal H–C(2′)); 4.49–4.52 (*m*, H–C(16)); 4.56 (br. *s*, Gal H–C(4′)); 4.90 (*d*, $J = 7.5$, Gal H–C(1′)); 5.14 (*d*, $J = 7.8$, Glc H–C(1″)); 5.22 (*d*, $J = 7.4$, Glc H–C(1‴)); 5.30 (*d*, $J = 4.5$, H–C(6)). ^{13}C-NMR (C_5D_5N, 125 MHz): *Table*. FAB-MS (neg.): 899 ($[M-H]^-$), 737 ($[M-162-H]^-$), 575 ($[M-162-162-H]^-$), 413 ($[M-162-162-162-H]^-$). HR-ESI-MS (neg.): 899.4652 ($[M-H]^-$, $C_{45}H_{71}O_{18}^-$; calc. 899.4640).

Elephanoside H (=*(3β,5β,25*R)*-3-[(2-*O-*β*-D-*Glucopyranosyl-β*-D-*galactopyranosyl)oxy]spirostan-12-one*; **2**). White amorphous powder. $[\alpha]_D^{23} = -21.3$ ($c = 0.46$, MeOH). ^{1}H-NMR (C_5D_5N, 400 MHz): 0.67 (*d*, $J = 5.0$, Me(27)); 0.88–0.92 (*m*, H_a–C(7)); 0.96 (*s*, Me(19)); 1.06 (*s*, Me(18)); 1.16–1.19 (*m*, H_b–C(7)); 1.31 (*d*, $J = 6.8$, Me(21)); 1.40–1.43 (*m*, H–C(14)); 1.64–1.67 (*m*, H–C(25)); 1.68–1.69 (*m*, H–C(9)); 1.82–1.84 (*m*, H–C(8)); 1.90–1.95 (*m*, H–C(20)); 2.17 (*dd*, $J = 4.8, 13.9$, H_a–C(11)); 2.19–2.25 (*m*, H–C(5)); 2.34 (*t*, $J = 13.9$, H_b–C(11)); 2.81 (*dd*, $J = 7.1, 8.1$, H–C(17)); 3.49 (br. *t*, $J = 10.2$, H_a–C(26)); 3.58 (br. *d*, $J = 10.2$, H_b–C(26)); 3.82–3.86 (*m*, Glc H–C(5″)); 4.01–4.04 (*m*, Gal H–C(5′)); 4.21 (*t*, $J = 8.6$, Glc H–C(3″)); 4.23–4.25 (*m*, H–C(3)); 4.09 (*t*, $J = 8.6$, Glc H–C(2″)); 4.25 (*dd*, $J = 2.6, 9.0$, Gal H–C(3′)); 4.33 (*t*, $J = 8.6$, Glc H–C(4″)); 4.52–4.54 (*m*, H–C(16)); 4.56 (*d*, $J = 2.6$, Gal H–C(4′)); 4.67 (*t*, $J = 9.0$, Gal H–C(2′)); 4.88 (*d*, $J = 7.6$, Gal H–C(1′)); 5.29 (*d*, $J = 7.6$, Glc H–C(1″)). ^{13}C-NMR (C_5D_5N, 100 MHz): *Table*. FAB-MS (neg.): 753 ($[M-H]^-$), 591 ($[M-162-H]^-$). HR-ESI-MS (neg.): 753.4059 ($[M-H]^-$, $C_{39}H_{61}O_{14}^-$; calc. 753.4061).

Acid Hydrolysis of **1** *and the Determination of the Absolute Configuration of the Sugar Units.* Saponin **1** (4 mg) was dissolved in 2M HCl/dioxane 1:1 (2 ml) and hydrolyzed at 95° for 6 h. The mixture was

diluted with H_2O and extracted with $CHCl_3$. The aq. layer was passed through an *Amberlite IRA-401* (OH^- form) column to afford the monosaccharide mixture. Glucose (R_f 0.27) and galactose (R_f 0.29) were detected by comparison with authentic samples through HP-TLC (BuOH/^{i}PrOH/H_2O 10:5:4, detection by anisaldehyde (H_2SO_4). The abs. configurations of monosaccharides were determined as D-glucose and D-galactose by GC analysis after derivatization [12], as previously reported [2][15]. The retention times of corresponding trimethylsilylated L-cysteine derivatives of and D- and L-glucose, and D- and L-galactose were 18.12 and 18.70, and 18.98 and 19.56 min, resp.

REFERENCES

[1] Y. H. Wang, Ph. D. Thesis, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China, 2006.
[2] Y. Zhang, Y. J. Zhang, M. R. Jacob, X. C. Li, C. R. Yang, *Phytochemistry* **2008**, *69*, 264.
[3] B. P. Silva, R. R. Bemardo, J. P. Patente, *Fitoterapia* **1998**, *69*, 528.
[4] X. C. Li, C. R. Yang, M. Ichikawa, H. Matsuura, R. Kasai, K. Yamasaki, *Phytochemistry* **1992**, *31*, 3559.
[5] S. M. Sang, X. F. Cheng, N. Q. Zhu, R. E. Stark, V. Badmaev, G. Ghai, R. T. Rosen, C. T. Ho, *J. Agric. Food Chem.* **2001**, *49*, 4478.
[6] C. V. R. Sastry, C. Rukmini, L. R. Row, *Indian J. Chem.* **1967**, *5*, 613.
[7] D. Q. Yu, J. S. Yang, 'The Handbook of Analytical Chemistry', No. 7, 2nd edn., 1999.
[8] L. Z. Xu, Z. Chen, N. J. Sun, *Acta Bot. Sin.* **1993**, *35*, 138.
[9] S. Kiyosawa, M. Hutoh, *Chem. Pharm. Bull.* **1968**, *16*, 1162.
[10] O. Espejo, C. J. Llavot, H. Jung, F. Giral, *Phytochemistry* **1982**, *21*, 413.
[11] P. K. Agrawal, D. C. Jain, R. K. Gupta , R. S. Thakur, *Phytochemistry* **1985**, *24*, 2479.
[12] S. Hara, H. Okabe, K. Mihashi, *Chem. Pharm. Bull.* **1987**, *35*, 501.
[13] K. Nakano, Y. Midzuta, Y. Hara, K. Murakami, Y. Takaishi, T. Tomimatsu, *Phytochemistry* **1991**, *30*, 633.
[14] K. Nakano, T. Yamasaki, Y. Imamura, K. Murakami, Y. Takaishi, T. Tomimatsu, *Phytochemistry* **1989**, *28*, 1215.
[15] Y. Zhang, H. Z. Li, Y. J. Zhang, M. R. Jacob, S. I. Khan, X. C. Li, C. R. Yang, *Steroids* **2006**, *71*, 712.

Received October 19, 2012