Two New Spirostanol Saponins from *Reineckia carnea*

by **Zhong-Quan Zhang**[a])[b]), **Jian-Chao Chen**[a])[b]), **Xian-Ming Zhang**[a]), **Zhong-Rong Li**[a]), and **Ming-Hua Qiu***[a])

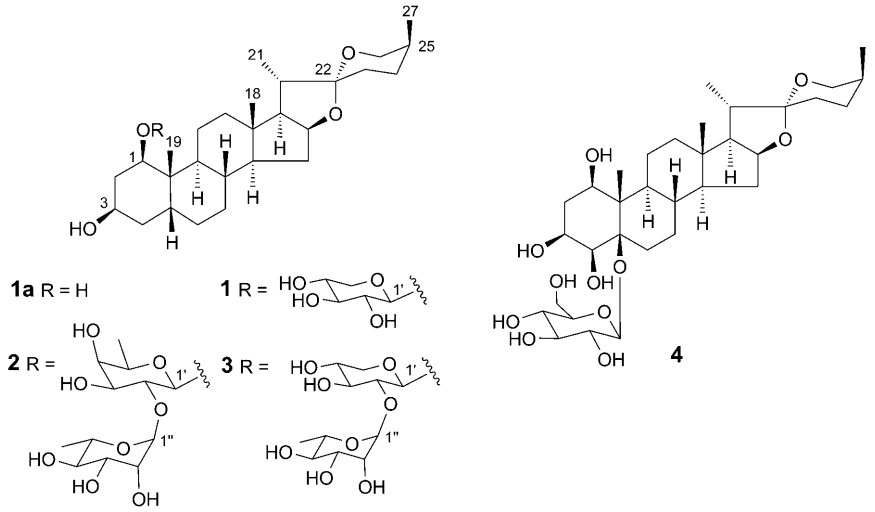
[a]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China (phone: +86-871-5223257; fax: +86-871-5223255; e-mail: mhchiu@mail.kib.ac.cn)

[b]) Graduate University of Chinese Academy of Sciences, Beijing 100049, P. R. China

Two new spirostanol saponins, (1*β*,3*β*,5*β*,25*S*)-spirostan-1,3-diol 1-(*β*-D-xylopyranoside) (**1**) and (1*β*,3*β*,5*β*,25*S*)-spirostan-1,3-diol 1-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-fucopyranoside] (**2**), along with two known compounds, (1*β*,3*β*,5*β*,25*S*)-spirostan-1,3-diol 1-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-xylopyranoside] (**3**) and (1*β*,3*β*,4*β*,5*β*,25*S*)-spirostan-1,3,4,5-tetrol 5-(*β*-D-glucopyranoside) (**4**) were isolated from the whole plant of *Reineckia carnea*. The structures of the new steroids were determined by detailed analysis of their 1D- and 2D-NMR spectra and chemical methods, and by comparison with spectral data of known compounds. Compounds **3** and **4** were isolated from the genus *Reineckia* for the first time.

Introduction. – Plants of the family Liliaceae are a rich source of steroids with diverse bioactivity [1][2]. As a perennial ever-green herb and the only species of the genus *Reineckia* (Liliaceae), *Reineckia carnea* (ANDR.) KUNTH is indigenous to China and Japan [3]. When cultivated as garden ornamental plant, it was thought to bring luck and fortune to people. *R. carnea* can produce a repairing effect on the soil that was polluted by copper and other heavy metals [4]. The whole plant is a highly valued traditional Chinese medicine as an antitussive, an antiarthritic, a hemostatic, and an antidote [5] used by the folk people of the Miao minority [6]. Recently, this plant was successfully developed into a medicine to cure cough and sore throats in China. Phytochemical investigation on *R. carnea* in the past 50 years has resulted in the isolation of some steroidal sapogenins and steroidal saponins [7–10]. The steroidal constituents from the underground parts of *R. carnea* exhibited inhibitory activity on cAMP phosphodiesterase, and a potent inhibitor, showing almost equal IC_{50} values compared to papaverine, was isolated [11]. Formerly, we reported two cholestane bisdesmosides from *R. carnea* [12]. On continuing the study of this plant, we have now isolated two new spirostanol saponins, namely (1*β*,3*β*,5*β*,25*S*)-spirostan-1,3-diol 1-(*β*-D-xylopyranoside) (**1**) and (1*β*,3*β*,5*β*,25*S*)-spirostan-1,3-diol 1-[*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-fucopyranoside] (**2**). This article deals with the isolation and structure elucidation of these new spirostanol saponins by detailed analysis of their 1D and 2D NMR spectra and acid hydrolysis.

Results and Discussion. – Compound **1** was obtained as an amorphous powder. The negative-ion HR-FAB-MS of **1** indicated a molecular formula $C_{32}H_{52}O_8$ which was derived from the quasi-molecular-ion peak at m/z 563.3588 ($[M-H]^-$) and confirmed



by the ^{13}C-NMR data. The IR spectrum showed absorption bands suggesting the presence of a (25*S*)-spirostan ($\tilde{\nu}_{max}$ 982, 923, 898, and 865 (923 > 898) cm^{-1}) [13][14]. Acid hydrolysis of **1** liberated D-xylose[1]), as identified by comparison with an authentic sample on TLC, and rhodeasapogenin (=(1*β*,3*β*,5*β*,25*S*)-spirostan-1,3-diol; **1a**) [15]. The ^{1}H-NMR and ^{13}C-NMR (DEPT) data (*Table*) of **1** and **1a** were consistent with the structure of (1*β*,3*β*,5*β*,25*S*)-spirostan-1,3-diol 1-(*β*-D-xylopyranoside) (**1**).

The ^{1}H-NMR spectrum of **1** showed the presence of two tertiary Me groups (*δ* 0.84 (*s*, Me(18)) and 0.88 (*s*, Me(19))), two secondary Me groups (*δ* 1.11 (*d*, *J* = 6.8, Me(21)) and 1.05 (*d*, *J* = 7.0, Me(27))), two oxygenated CH groups (*δ* 4.08 (br. *s*, H–C(1)) and 4.35 (br. *s*, H–C(3))), and a glycosyl moiety. The five characteristic signals at *δ* 102.4 (C(1′)), 75.2 (C(2′)), 78.9 (C(3′)), 71.3 (C(4′)), and 67.6 (C(5′)) in the ^{13}C-NMR (DEPT) spectrum of **1** (*Table*) suggested the presence of a xylose residue, while the *β*-configuration of the xylose was demonstrated by the large *J* value of the anomeric-proton signal at *δ* 4.87 (*d*, *J* = 7.6, H–C(1′)) in the ^{1}H-NMR spectrum. The remaining signals of **1** were nearly in line with those of rhodeasapogenin (**1a**) [15], except for the signals at *δ* 79.5 (C(1)), 29.9 (C(2)), and 66.6 (C(3)). The ^{13}C-NMR differences observed for **1** as a downfield shift of the C(1) signal at *δ* 79.5 (+6.1 ppm) and upfield shifts of the C(2) signal at *δ* 29.9 (−2.9 ppm) and the C(3) signal at *δ* 66.6 (−1.6 ppm) were explained by the glycosylation of OH–C(1) of **1a** [16][17]. In addition, from the heteronuclear multiple-bond coherence (HMBC) spectrum, long-range couplings were observed between H–C(1′) (*δ* 4.87 (*d*, *J* = 7.6)) and C(1) (*δ* 79.5), and between Me(19) (*δ* 0.88 (*s*)) and C(1) (*δ* 79.5) (*Fig.*), which confirmed that the glycosylation had occurred at C(1) of the aglycone. The negative-ion FAB-MS displayed characteristic fragment ions at *m*/*z* 563 ([*M* − H]$^-$), 431 ([*M* − 132 (xylose) − H]$^-$), and 413 ([*M* − 132 (xylose) − 18 (H_2O) − H]$^-$), indicating the presence of a xylose and OH group in **1**.

Compound **2** was obtained as white amorphous powder. The negative-ion HR-FAB-MS gave a quasi-molecular-ion peak at *m*/*z* 723.4336 ([*M* − H]$^-$) corresponding to the molecular formula $C_{39}H_{64}O_{12}$. The IR spectrum of **2** showed strong absorption bands, in agreement with a (25*S*)-spirostanol derivative, at 3400, 985, 945, 918, 898, and

[1]) The absolute configuration of the sugars was tentatively assigned from biogenetic considerations.

Table. *^{13}C-NMR Data* (100 MHz, C_5D_5N) *of* **1a** *and* **1**–**3**. δ in ppm.

Atom	**1a**	**1**	**2**	**3**
C(1)	73.4 (*d*)	79.5 (*d*)	75.1 (*d*)	74.3 (*d*)
C(2)	32.8 (*t*)	29.9 (*t*)	29.6 (*t*)	29.7 (*t*)
C(3)	68.2 (*d*)	66.6 (*d*)	70.0 (*d*)	70.0 (*d*)
C(4)	34.4 (*t*)	34.6 (*t*)	33.1 (*t*)	32.3 (*t*)
C(5)	31.2 (*d*)	30.3 (*d*)	30.4 (*d*)	30.4 (*d*)
C(6)	26.8 (*t*)	26.8 (*t*)	26.9 (*t*)	26.8 (*t*)
C(7)	26.7 (*t*)	27.1 (*t*)	26.8 (*t*)	27.1 (*t*)
C(8)	35.8 (*d*)	35.7 (*d*)	34.9 (*d*)	34.4 (*d*)
C(9)	42.1 (*d*)	41.8 (*d*)	42.6 (*d*)	42.5 (*d*)
C(10)	40.2 (*s*)	39.5 (*s*)	39.8 (*s*)	39.7 (*s*)
C(11)	21.1 (*t*)	22.9 (*t*)	22.9 (*t*)	22.9 (*t*)
C(12)	40.4 (*t*)	40.3 (*t*)	40.5 (*t*)	40.7 (*t*)
C(13)	40.7 (*s*)	40.8 (*s*)	40.7 (*s*)	40.6 (*s*)
C(14)	56.4 (*d*)	56.4 (*d*)	57.1 (*d*)	57.1 (*d*)
C(15)	32.2 (*t*)	32.1 (*t*)	32.2 (*t*)	32.2 (*t*)
C(16)	81.3 (*d*)	81.3 (*d*)	81.3 (*d*)	81.3 (*d*)
C(17)	63.1 (*d*)	63.0 (*d*)	63.1 (*d*)	62.9 (*d*)
C(18)	16.7 (*q*)	16.7 (*q*)	16.8 (*q*)	16.7 (*q*)
C(19)	19.3 (*q*)	19.7 (*q*)	19.0 (*q*)	19.1 (*q*)
C(20)	42.5 (*d*)	42.5 (*d*)	42.6 (*d*)	42.5 (*d*)
C(21)	14.9 (*q*)	14.9 (*q*)	14.9 (*q*)	14.9 (*q*)
C(22)	109.8 (*s*)	109.8 (*s*)	109.8 (*s*)	109.8 (*s*)
C(23)	26.4 (*t*)	26.4 (*t*)	26.4 (*t*)	26.4 (*t*)
C(24)	26.2 (*t*)	26.2 (*t*)	26.2 (*t*)	26.2 (*t*)
C(25)	27.6 (*d*)	27.6 (*d*)	27.6 (*d*)	27.6 (*d*)
C(26)	65.2 (*t*)	65.1 (*t*)	65.2 (*t*)	65.2 (*t*)
C(27)	16.3 (*q*)	16.3 (*q*)	16.3 (*q*)	16.4 (*q*)
C(1′)		102.4 (*d*)	99.8 (*d*)	99.7 (*d*)
C(2′)		75.2 (*d*)	77.1 (*d*)	79.2 (*d*)
C(3′)		78.9 (*d*)	74.4 (*d*)	77.3 (*d*)
C(4′)		71.3 (*d*)	74.2 (*d*)	71.5 (*d*)
C(5′)		67.6 (*t*)	71.4 (*d*)	66.9 (*t*)
C(6′)			17.2 (*q*)	
C(1″)			101.6 (*d*)	101.8 (*d*)
C(2″)			72.5 (*d*)	72.5 (*d*)
C(3″)			72.8 (*d*)	72.8 (*d*)
C(4″)			74.2 (*d*)	74.2 (*d*)
C(5″)			69.2 (*d*)	69.6 (*d*)
C(6″)			18.8 (*q*)	18.7 (*q*)

863 (918 > 898) cm^{-1}. The ^{13}C-NMR spectral features of **1** and **2** were nearly identical to each other, except for the sugar moieties, which suggested that **1** and **2** have the same aglycone. Acid hydrolysis of **2** produced rhodeasapogenin (**1a**), D-fucose[1]), and L-rhamnose[1]), as identified by TLC comparison with authentic samples. The ^{1}H- and ^{13}C-NMR (*Table*) data and their comparison with literature data established the structure of **2** as (1β,3β,5β,25*S*)-spirostan-1,3-diol 1-[α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside].

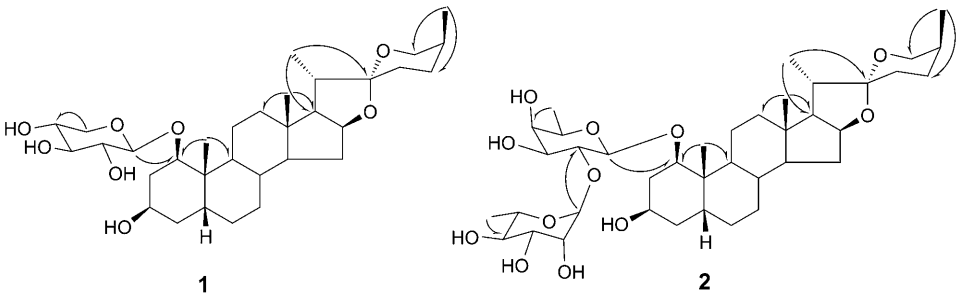


Figure. *Key HMBCs for compounds* **1** *and* **2**

Two anomeric protons (δ 5.43 (*s*, H–C(1′)) and 6.42 (*s*, H–C(1″))) in the ^{1}H-NMR spectrum of **2**, and two sets of ^{13}C-NMR signals (δ 99.8 (C(1′)), 77.1 (C(2′)), 74.4 (C(3′)), 74.2 (C(4′)), 71.4 (C(5′)), and 17.2 (C(6′)), and δ 101.6 (C(1″)), 72.5 (C(2″)), 72.8 (C(3″)), 74.2 (C(4″)), 69.2 (C(5″)), and 18.8 (C(6″))) corresponding to a D-fucopyranose and an L-rhamnopyranose moiety, resp., were readily recognized by comparison with literature data [10], which indicated that compound **2** may have the same sugar sequence at C(1) of the aglycone as the known compound convallamarogenin 1-[α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranoside] 3-(α-L-rhamnopyranoside) [10] (convallamarogenin = (1β,3β,5β)-spirost-25(27)-en-1,3-diol). The glycosylation-induced downfield shift of C(1) (+1.7 ppm) and C(3) (+1.8 ppm) and upfield shift of C(2) (−3.2 ppm) of **2** compared with those of rhodeasapogenin (**1a**) suggested that the sugar moiety was linked at C(1) of the aglycone [10]. The HMBC cross-peaks δ 6.42 (*s*, H–C(1″))/δ 77.1 (C(2′)), δ 5.43 (*s*, H–C(1′))/δ 75.1 (C(1)), and δ 0.88 (*s*, Me(19))/δ 75.1 (C(1)) were observed (*Fig.*), which confirmed the sugar sequence and the glycosylation position. The α-orientation of the rhamnose and β-orientation of the fucose were deduced from the *s* signal of the two anomeric protons at δ 5.43 (H–C(1′)) and 6.42 (H–C(1″)) [10]. Further, the fragment ions at *m*/*z* 723 ([*M* − H]$^-$), 577 ([*M* − 146 (rhamnose) − H]$^-$), and 431 ([*M* − 146 (rhamnose) − 146 (fucose) − H]$^-$) in the negative-ion FABMS of **2** were also observed.

Comparison of the physicochemical properties with the reported data allowed us to identify compounds **3** and **4** as (1β,3β,5β,25*S*)-spirostan-1,3-diol 1-[α-L-rhamnopyranosyl-(1 → 2)-β-D-xylopyranoside] (**3**) [18] and (1β,3β,4β,5β,25*S*)-spirostan-1,3,4,5-tetrol 5-(β-D-glucopyranoside) (**4**) [19]. Compounds **3** and **4** are isolated for the first time from the genus *Reineckia*.

The project was supported by the *Program of Promoting Development for Guizhou* (Qian-01-2005-01) *and Xibuzhiguang* of the Chinese Academy of Sciences (CAS) and the *Foundation of State Key Laboratory of Phytochemistry and Plant Resources in West China*, Kunming Institute of Botany, CAS.

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh and 10–40 μm; *Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, China), *Lichroprep RP-18* gel (40–63 μm; *Merck*, Darmstadt, Germany), and *Sephadex LH-20* (40–70 μm; *Amersham Pharmacia Biotech AB*, Uppsala, Sweden). TLC: precoated silica gel GF_{254} plates (*Qingdao Haiyang Chemical Co., Ltd*, Qingdao, China), detection by spraying with 10% H_2SO_4 soln. in EtOH followed by heating. Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR Spectra: *Bio-Rad FTS-135* spectrometer; KBr pellets; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometer; δ in ppm with Me_4Si as the internal standard, *J* in Hz, multiplicities of ^{13}C-NMR by DEPT. MS: *VG Autospec-3000* mass spectrometer, *m*NBA = 3-nitrobenzyl alcohol; in *m*/*z* (rel. %).

Plant Material. The whole plant of *R. carnea* was collected from Yulong Snow Mountain (Nov. 2004), Lijiang City, Yunnan Province, China, and a voucher specimen (identified by Prof. *Xi-wen Li* from the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences) was deposited with the State Key Laboratory of Phytochemistry of the Kunming Institute of Botany, Chinese Academy of Sciences (No. 200411011).

Extraction and Isolation. The air-dried, powdered whole plant of *R. carnea* (1.90 kg) was extracted with 90% MeOH/H_2O under reflux for 3 h each time (3 × 8 l). The extract was filtered, the filtrate concentrated *in vacuo* at 45°, and the resulting extract (557 g) suspended in H_2O (4 l) and then partitioned with AcOEt (3 × 3 l). The AcOEt extract (137 g) was subjected to CC (silica gel (800 g), $CHCl_3$/MeOH 1:0 → 2:1): *Fr. 1–4. Fr. 2* (24 g) was subjected to CC (silica gel (220 g), $CHCl_3$/MeOH 1:0 → 20:1; then repeated *RP-18*, 60–70% MeOH/H_2O): **1** (14 mg). *Fr. 3* (18 g) was subjected to CC (silica gel (200 g), $CHCl_3$/MeOH 12:1 → 5:1; then *Sephadex LH-20*, MeOH; then repeated *RP-18*, 30–50% MeOH/H_2O): **2** (16 mg), **3** (42 mg), and **4** (6 mg).

*Rhodeasapogenin 1-(β-*D*-Xylopyranoside)* (*=(1β,3β,5β,25*S*)-Spirostan-1,3-diol 1-(β-*D*-Xylopyranoside) = (1β,3β,5β,25*S*)-3-Hydroxyspirostan-1-yl β-*D*-Xylopyranoside*; **1**). White amorphous solid. $[\alpha]_D^{20} = +15.68$ ($c = 0.185$, MeOH). IR (KBr): 3400 (OH), 982, 923, 898, 865 (923 > 898). ^{1}H-NMR (400 MHz, (D_5)pyridine): 4.87 (*d*, *J* = 7.6, H–C(1′)); 4.55–4.57 (*m*, H–C(16)); 4.42–4.44 (*m*, H–C(2′)); 4.40 (*dd*, *J* = 4.8, 10.4, H_{eq}–C(5′)); 4.35 (br. *s*, H–C(3)); 4.18–4.20 (*m*, H–C(4′)); 4.16–4.18 (*m*, H–C(3′)); 4.08 (br. *s*, H–C(1)); 4.05 (*dd*, *J* = 4.8, 10.4, H_{eq}–C(26)); 3.75 (*t*, *J* = 10.4, H_{ax}–C(5′)); 3.33 (*d*, *J* = 10.7, H_{ax}–C(26)); 1.86–1.88 (*m*, H–C(17)); 1.11 (*d*, *J* = 6.8, Me(21)); 1.05 (*d*, *J* = 7.0, Me(27)); 0.88 (*s*, Me(19)); 0.84 (*s*, Me(18)). ^{13}C-NMR: *Table*. FAB-MS (neg.): 563 ([*M* – H]$^-$), 431 ([*M* – 132 (xylose) – H]$^-$), 413 ([*M* – 132 (xylose) – 18 (H_2O) – H]$^-$). HR-FAB-MS (neg.): 563.3588 ([*M* – H]$^-$, $C_{32}H_{51}O_8^-$; calc. 563.3583).

Acid Hydrolysis of **1**. A soln. of **1** (10 mg) in 1M HCl (dioxane/H_2O 1:1; 5 ml) was heated at 70° for 2 h. After evaporation of the solvent, the residue was extracted with $CHCl_3$ (3 ×). The $CHCl_3$ extract was subjected to CC (silica gel, $CHCl_3$/MeOH 100:1): **1a** (3.3 mg; identified by ^{1}H- and ^{13}C-NMR, see below).

D-Xylose was identified in the residue of the H_2O soln., after evaporation, by TLC comparison with an authentic sample. TLC (BuOH/Me_2CO/H_2O 4:5:1; detection: anisaldehyde/H_2SO_4); R_f 0.56 for D-xylose.

Rhodeasapogenin (*=(1β,3β,5β,25*S*)-Spirostan-1,3-diol*; **1a**). White amorphous solid. $[\alpha]_D^{27} = -84.25$ ($c = 0.102$, $CHCl_3$). ^{1}H-NMR (400 MHz, (D_5)pyridine): 4.40–4.42 (*m*, H–C(16)); 4.35 (*s*, H–C(3)); 4.02 (*s*, H–C(1)); 1.11 (*d*, *J* = 6.8, Me(21)); 1.05 (*d*, *J* = 7.0, Me(27)); 0.88 (*s*, Me(19)); 0.84 (*s*, Me(18)). ^{13}C-NMR: *Table*. FAB-MS (neg.): 431 ([*M* – H]$^-$, $C_{27}H_{43}O_4^-$).

*Rhodeasapogenin 1-[α-*L*-Rhamnopyranosyl-(1 → 2)-β-*D*-fucopyranoside]* (*=(1β,3β,5β,25*S*)-Spirostan-1,3-diol 1-[α-*L*-Rhamnopyranosyl-(1 → 2)-β-*D*-fucopyranoside] = (1β,3β,5β,25*S*)-3-Hydroxyspirostan-1-yl O-6-Deoxy-α-*L*-mannopyranosyl-(1 → 2)-6-deoxy-β-*D*-galactopyranoside*; **2**). White amorphous solid. $[\alpha]_D^{20} = +14.39$ ($c = 0.132$, MeOH). IR (KBr): 3400 (OH), 985, 945, 918, 898, 863 (918 > 898). ^{1}H-NMR (400 MHz, (D_5)pyridine): 6.42 (*s*, H–C(1″)); 5.43 (*s*, H–C(1′)); 4.87 (*s*, H–C(1)); 4.78–4.80 (*m*, H–C(2″)); 4.65–4.67 (*m*, H–C(3″)); 4.51–4.53 (*m*, H–C(16)); 4.38–4.40 (*m*, H–C(5″)); 4.34–4.36 (*m*, H–C(3)); 4.25–4.27 (*m*, H–C(4″)); 4.23–4.25 (*m*, H–C(3′)); 4.21–4.23 (*m*, H–C(2′)); 4.03–4.05 (*m*, H_{eq}–C(26)); 3.85–3.87 (*m*, H–C(4′)); 3.71 (*dd*, *J* = 4.3, 11.3, H–C(5′)); 3.21–4.23 (*m*, H_{ax}–C(26)); 1.65 (*d*, *J* = 6.1, Me(6″)); 1.49 (*d*, *J* = 6.2, Me(6′)); 1.11 (*d*, *J* = 6.8, Me(21)); 1.05 (*d*, *J* = 7.0, Me(27)); 0.88 (*s*, Me(19)); 0.83 (*s*, Me(18)). ^{13}C-NMR: *Table*. HR-FAB-MS (neg.): 723.4336 ([*M* – H]$^-$, $C_{39}H_{63}O_{12}^-$; calc. 723.4319). FAB-MS (neg.): 723 ([*M* – H]$^-$), 577 ([*M* – 146 (rhamnose) – H]$^-$), 431 ([*M* – 146 (rhamnose) – 146 (fucose) – H]$^-$).

Acid Hydrolysis of **2**. As described for the hydrolysis of **1**. Rhodeasapogenin (**1a**) was identified in the $CHCl_3$ extract by TLC comparison with the hydrolysis product of **1**, and D-fucose and L-rhamnose were detected in the H_2O soln. by TLC comparison with authentic samples. TLC: R_f 0.56 for D-fucose, and R_f 0.67 for L-rhamnose.

REFERENCES

[1] S. B. Mahato, A. N. Ganguly, N. P. Sahu, *Phytochemistry* **1982**, *21*, 959.
[2] S. B. Singh, R. S. Thakur, *J. Sci. Ind. Res.* **1983**, *42*, 319.
[3] Y. Tsukamoto, 'The Grand Dictionary of Horticulture', Shogakukan, Tokyo, 1988, Vol. 2, p. 36.
[4] P. Zhang, F.-Q. Lian, M.-Y. Zhu, M.-L. Xiong, *J. Jiangxi Agric. Univ.* **2004**, *26*, 941.
[5] 'Dictionary of Traditional Chinese Medicine', Ed. National Administration of Traditional Chinese Medicine, Shanghai Science and Technology Publishing House, 1999, Vol. 8, p. 7204 (in Chinese).
[6] 'Collection of the Drugs in Miao Minority', Ed. K.-M. Lu, Guizhou People's Publishing House, 1987, Vol. 1, p. 44 (in Chinese).
[7] K. Takeda, T. Okanishi, H. Minato, A. Shimaoka, *Tetrahedron* **1963**, *19*, 759.
[8] K. Sasaki, *Chem. Pharm. Bull.* **1961**, *9*, 684.
[9] K. Takeda, T. Okanishi, A. Shimaoka, *Yakugaku Zasshi* **1955**, *75*, 560.
[10] I. Kiyoshi, K. Tenji, K. Shiu, *Yakugaku Zasshi* **1987**, *107*, 140.
[11] T. Kanmoto, Y. Mimaki, Y. Sashida, T. Nikadido, K. Koike, T. Ohmoto, *Chem. Pharm. Bull.* **1994**, *42*, 926.
[12] Z.-Q. Zhang, J.-C. Chen, L. Zhou, M.-H. Qiu, *Helv. Chim Acta* **2007**, *90*, 616.
[13] C. R. Eddy, M. E. Wall, M. K. Scott, *Anal. Chem.* **1953**, *25*, 266.
[14] R. N. Jones, K. Katzenellenbogen, K. Dobriner, *J. Am. Chem. Soc.* **1953**, *75*, 158.
[15] K. Tori, S. Seo, Y. Terui, J. Nishikawa, F. Yashuda, *Tetrahedron Lett.* **1981**, *22*, 2405.
[16] Y. Takaishi, T. Ohashi, T. Tomimatsu, *Phytochemistry* **1989**, *28*, 945.
[17] D. de Castro Ferreira Gomes, L. Vilela Alegrio, *Phytochemistry* **1998**, *49*, 1365.
[18] K. Miyahara, K. Kudo, T. Kawasaki, *Chem. Pharm. Bull.* **1983**, *31*, 348.
[19] P. Shen, S. L. Wang, X. K. Liu, C. R. Yang, *Chem. Pharm. Bull.* **2003**, *51*, 305.

Received February 25, 2008