Three Steroids with Unique Structural Feature of 5β-Spirostan-1β,3β,17α-trihydroxyl from *Reineckia carnea*

Zhong-Quan ZHANG,[a,b] Jian-Chao CHEN,[a] Jian YAN,[a] and Ming-Hua QIU*,[a]

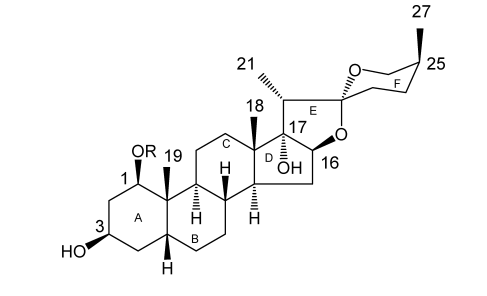
[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences; Kunming 650204, China: and* [b] *Research Institute of Resource Insects, Chinese Academy of Forestry; Kunming 650224, China.* Received July 7, 2010; accepted September 16, 2010; published online October 1, 2010

A new spirostanol sapogenin and two spirostanol saponins, tentatively named reineckiagenin A (1), reineckiagenoside A (2), and reineckiagenoside B (3), were isolated from the whole plant of *Reineckia carnea*. By detailed analysis of their 1D and 2D NMR spectra, chemical methods, and by comparison with spectra data of known compounds, the structures of the new steroids were determined to be 25(*S*)-5β-spirostan-1β,3β,17α-triol (1), 25(*S*)-5β-spirostan-1β,3β,17α-triol 1-*O*-β-D-xylopyranoside (2), 25(*S*)-5β-spirostan-1β,3β,17α-triol 1-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside (3). Compounds 1, 2, and 3 are the first naturally occurring steroids with unique structural feature of 5β-spirostan-1β,3β,17α-trihydroxyl.

Key words *Reineckia carnea*; steroid; reineckiagenin A; reineckiagenoside A; reineckiagenoside B

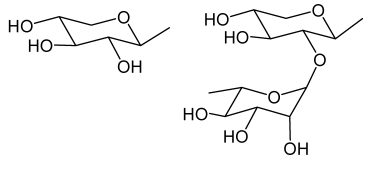
Reineckia carnea (ANDR.) KUNTH belongs to the family Liliaceae, it is the only species of the genus *Reineckia* and indigenous to China and Japan.[1)] As a perennial ever-green herb, it can grow well on soils polluted by heavy metals and can produce a repairing effect on the soil that was polluted by copper.[2)] The Chinese name "ji xiang cao" means it can bring luck and fortune to people, especially when it was cultivated as ornamental plant and flowers. The whole plant has been used as an antitussive, an antarthritic, a hemostatic, and an antitode in traditional Chinese medicine.[3)] It is one of the most important ingredients of many medical prescriptions of Miao Minority in China and has been successfully developed into a medicine to treat cough and sore throats.[4)] The genus *Reineckia* is taxonomically close to *Rohdea*, *Tupistra*, *Convallaria*, *Aspidistra*, *Liriope* and *Ophiopogon*, and their chemical constituents are characterised by steroidal saponins with polyhydroxylated skeletons.[5—9)] During the year 1955—1994, Japanese scholars reported some steroidal sapogenins and steroidal saponins from this plant.[10—13)] 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.[13)] Formerly, we reported three spirostanol steroids from *R. carnea*: 25(*S*)-5β-spirostan-1β,3β-diol (rhodeasapogenin, obtained through acid hydrolysis), 25(*S*)-5β-spirostan-1β,3β-diol 1-*O*-β-D-xylopyranoside, 25(*S*)-5β-spirostan-1β,3β-diol 1-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside,[14)] now, the 17α-OH derivatives of the three spirostanol steroids, corresponding to 25(*S*)-5β-spirostan-1β,3β,17α-triol (**1**), 25(*S*)-5β-spirostan-1β,3β,17α-triol 1-*O*-β-D-xylopyranoside (**2**), 25(*S*)-5β-spirostan-1β,3β,17α-triol 1-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside (**3**) were isolated from this plant. Compounds **1**, **2**, and **3** are the first naturally occurring steroids possessing unique structural feature of 5β-spirostan-1β,3β,17α-trihydroxyl. This paper describes the isolation and structural elucidation of these new compounds based on spectroscopic data and chemical transformation.

Compound **1** was isolated as a white amorphous solid with the molecular formula $C_{27}H_{44}O_5$ deduced from negative-ion high-resolution (HR)-FAB-MS *m/z* 447.3124 $[M-H]^-$, calculated: 447.3110 and also confirmed by ^{13}C-NMR evidence. The IR spectrum of **1** showed absorption bands ascribable to hydroxyl (3373 cm^{-1}) and spiroketal groups (977, 948, 918, 898, 822 cm^{-1}); the absorption at 918 cm^{-1} was of greater intensity than that at 898 cm^{-1} (25(*S*)-spiroketal).[15,16)] The ^{1}H-NMR spectrum of **1** showed signals for four typical steroid methyls; two appeared as singlets at δ 0.98 (H-18) and δ 1.34 (H-19) and the other two as doublets at δ 1.24 (*J*=7.1 Hz, H-21) and δ 1.03 (*J*=7.2 Hz, H-27). Two methine protons attached to the carbons bearing hydroxyl groups were also noted at δ 4.02 (1H, br s, H-1) and δ 4.36 (1H, br s, H-3). The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectrum of **1** showed a quaternary carbon signal at δ 110.6, which is the characteristic C-22 of a spirostanol skeleton.[17)] On comparison between the ^{13}C-NMR spectrum of **1** with that of rhodeasapogenin [25(*S*)-5β-spirostan-1β,3β-diol],[14,18)] the signal due to the C-17 carbon, which was observed at δ 63.1 (CH) in rhodeasapogenin, appeared at δ 89.6 as a quaternary carbon signal, accompanied by downfield shifts of the signals due to C-13 (+5.5 ppm), C-16 (+9.6 ppm), and C-20 (+2.7 ppm) and upfield shifts of signals due to C-12 (−10.4 ppm), C-14 (−3.5 ppm), and C-21 (−5.9 ppm); in addition, the molecu-



1 **2** **3**

R H



* To whom correspondence should be addressed. e-mail: mhchiu@mail.kib.ac.cn

lar formula of **1** ($C_{27}H_{44}O_5$) has one more oxygen atom than rhodeasapogenin ($C_{27}H_{44}O_4$). Thus, the introduction of a hydroxyl group onto C-17 was evident. The ^{13}C-NMR data of the C—F rings of **1** was closely related to those of pennogenin [25(*R*)-3β,17α-dihydroxy-spirost-5-ene],[19] and the characteristic ^{13}C signals that suggested the 17α-hydroxyl group as those for pennogenin on the *cis*-fused D/E ring system were: δ 46.2 (C-13), 90.0 (C-16), 89.6 (C-17), and 45.2 (C-20). The 17α-OH configuration was further supported by the rotating frame Overhauser effect spectroscopy (ROESY) correlations between 20-H (δ 2.24) and 18-CH_3 (δ 0.98) and between 16-H (δ 4.49) and 15α-H (δ 2.11) on a *cis*-fused D/E ring system (Fig. 1). The correlations of H-19 (δ 1.34) and H-5 (δ 2.24) in the ROESY spectrum of **1** supported the 5β-H configuration and the *cis*-fused A/B ring system; the broad singlet signal of H-1 (δ 4.02, br s) and H-3 (δ 4.36, br s) on the *cis*-fused A/B ring system were consistent with the 1β,3β-OH configuration.

Interpretation of the heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC), and correlation spectroscopy (COSY) spectrum allowed us to assign all the signals of compound **1** unambiguously (for the key HMBC correlations, see Fig. 2). Thus, the structure of **1** was elucidated to be 25(*S*)-5β-spirostan-1β,3β,17α-triol, namely, reineckiagenin A.

Compound **2** was obtained as a white amorphous powder, the negative-ion FAB-MS *m*/*z*: 579 [M−H]$^-$, 447 [M−132 (xylose)−H]$^-$. The molecular formula $C_{32}H_{52}O_9$ was determined by negative-ion HR-FAB-MS [*m*/*z* 579.3542 (M−H)$^-$, calculated: 579.3533] and ^{13}C-NMR. The IR spectrum of **2** showed characteristic absorptions due to hydroxyl (3400 cm^{-1}) and spiroketal groups (985, 916, 899, 863 cm^{-1}); the absorption at 916 cm^{-1} was of greater intensity than that at 899 cm^{-1} (25(*S*)-spiroketal). Acid hydrolysis of **2** with 1 M hydrochloric acid dioxane/water (1 : 1) gave **1** (identified by TLC comparison and further confirmed by ^{1}H- and ^{13}C-NMR spectrum) and D-xylose (determined by GC analysis of its trimethylsilylated L-cysteine adducts).[20] The presence of a β-xylopyranosyl moiety was demonstrated by the appearance of an anomeric proton signal at δ 4.83 (d, *J*=7.7 Hz) in the ^{1}H-NMR spectrum, and also by five characteristic ^{13}C signals at δ 102.3, 75.2, 78.9, 71.3, and 67.6.[21,22] The point of attachment of the sugar moiety in **2** was at 1β-OH rather than the more commonly observed 3β-OH group. This was supported by the ^{13}C-NMR spectrum of the aglycone **2** in which there was only a shift for C-1 downfield (+6.1 ppm, to 79.5 ppm), and by the HMBC correlations of H-1-xylose (δ 4.83) with C-1 (δ 79.5), this signal in turn coupled with H-19 (δ 1.35) (Fig. 3). Therefore, the structure of **2** was characterized as 25(*S*)-5β-spirostan-1β,3β,17α-triol 1-*O*-β-D-xylopyranoside, namely, reineckiagenoside A.

Compound **3** crystallized as needles from chloroform/methanol/water (7 : 3 : 0.1), negative-ion FAB-MS *m*/*z*: 725 [M−H]$^-$, 579 [M−147 (rhamnose)−H]$^-$, 447 [M−147 (rhamnose)−132 (xylose)−H]$^-$. Negative-ion HR-FAB-MS gave a quasi-molecular formula ion at *m*/*z* 725.4136 [M−H]$^-$, calculated: 725.4112 *m*/*z*, corresponding to an empirical molecular formula $C_{38}H_{62}O_{13}$. The IR spectrum showed absorption bands in agreement of 25(*S*)-spiroketal moiety (ν_{max} 980, 910, 894, 860 cm^{-1}, intensity 910>894). On acid hydrolysis with 1 M hydrochloric acid dioxane/water (1 : 1) at 90 °C, **3** liberated **1**, **2**, L-rhamnose, and D-xylose, **1** and **2** were identified by TLC comparison and further confirmed by ^{1}H- and ^{13}C-NMR spectrum, while L-rhamnose and D-xylose were determined by GC analysis of their trimethylsilylated L-cysteine adducts.

On comparison of the whole ^{13}C-NMR spectrum of **3** with that of **2**, a set of additional signals, corresponding to a terminal α-L-rhamnopyranosyl unit, appeared (δ 101.9, 72.2, 72.0, 74.5, 69.9, 18.8); the α-configuration of the L-rhamnose was readily confirmed by the ^{13}C shifts; remarkable differences in the ^{13}C shifts at C-3 and C-5 were recognized between α- and β-L-rhamnopyranosides.[22,23] The signal due to C-2 of the xylose was displaced downfield by 4 ppm and that due to C-1 of the xylose moved to upper field by 4.1 ppm, indicating that the C-2 position of the xylose unit was the glycosylated position to which the additional L-rhamnose was linked. The steroidal saponin with the same sugar sequence (α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl) has been isolated from *R. carnea* by Kiyoshi Iwagoe in 1987.[9]

The sequence of the sugars and linkage site to the aglycone of **3** were further supported by 2D NMR experiments. In the HMBC spectrum, correlations between H-1-rhamnose (δ 6.57) and C-2-xylose (δ 79.2), between H-1-xylose (δ 5.05, 1H, d, *J*=7.3 Hz) and C-1 (δ 76.0) which in turn coupled with H-19 (δ 1.34) were observed (Fig. 3).

On the basis of the above evidence, **3** was formulated as 25(*S*)-5β-spirostan-1β,3β,17α-triol 1-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside, namely, reineckiagenoside

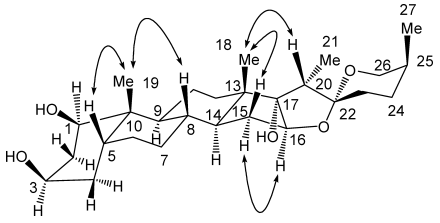
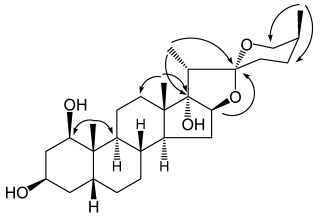
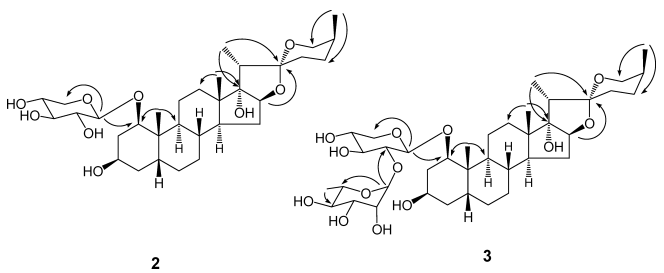


Fig. 1. Selected ROESY Correlations of **1**



1

Fig. 2. Key HMBC Correlations of **1**



2 **3**

Fig. 3. Key HMBC Correlations of **2** and **3**

B.

17α-OH steroids have been found from *Lilium pardarinum*[24] and *Paris axialis* Li.[25] *etc*. However, 17α-OH steroids was isolated for the first time from the genus *Reineckia*. Since 5β-spirostan with 1β,3β-dihydroxyl groups were found in very limited genera of plants such as *Rhodea*,[26] we estimated that 5β-spirostan with 1β,3β,17α-trihydroxyl groups would be very rare as natural products. Glycosylation of the two steroidal saponins occurred at 1β-OH rather than the more commonly observed 3β-OH group as those in the literature.[27] The three compounds are the first naturally occurring steroids with unique structural feature of 5β-spirostan-1β,3β,17α-trihydroxyl. The isolation of the new steroidal sapogenin may be of help in the chemotaxonomy of the family Liliceae.

Experimental

General Procedure Optical rotations were measured with a Horiba SEPA-300 polarimeter. IR spectral data were determined on a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were obtained on Bruker DRX-500 (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) instrument with tetramethylsilane (TMS) as the internal standard. The multiplicity of ^{13}C-NMR was determined as DEPT. MS data were obtained on a VG Autospec-3000 mass spectrometer. GC analysis was run on Agilent Technologies HP5890 gas chromatograph equipped with an H_2 flame ionization detector. The column was 30QC2/AC-5 quartz capillary column (30 m×0.32 mm) with the following conditions: column temperature: 180 °C/280 °C; programmed increase, 3 °C/min; carrier gas: N_2 (1 ml/min); injection and detector temperature: 250 °C; injection volume: 4 μl, split ratio: 1/50. Thin-layer chromatography (TLC) was performed on plates pre-coated with silica gel F_{254} (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). Silica gel (200—300 mesh and 10—40 μm), RP-18 (40—63 μm) and Sephadex LH-20 were used for column chromatography.

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 at the State Key Laboratory of Phytochemistry of Kunming Institute of Botany, Chinese Academy of Sciences (No. 200411011).

Extraction and Isolation The air-dried 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 filtrated, the filtrate concentrated *in vacuo* at 45 °C, 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 column chromatography over silica gel (800 g) eluting with $CHCl_3$/MeOH to afford four fractions. Fraction 2 (elution of $CHCl_3$/MeOH, 1 : 0—10 : 1, v/v, 24 g) was subjected to column chromatography over silica gel (220 g) eluting with $CHCl_3$/MeOH afford four fractions. Fraction 2.1 (elution of $CHCl_3$/MeOH, 1 : 0—80 : 1, 4 g) was rechromatographed on silica gel eluting with $CHCl_3$/MeOH (100 : 1) to obtain **1** (11 mg). Fraction 2.3 (elution of $CHCl_3$/MeOH, 20 : 1—10 : 1, 7 g) was chromatographed on silica gel eluting with $CHCl_3$/MeOH 15 : 1, then further purified by RP-18 (70% MeOH/H_2O) and sephadex LH-20 (MeOH) to afford **2** (18 mg). Fraction 3 (elution of

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data[a] of Compound **1** and the Aglycone Parts of Compounds **2** and **3**

Rhodeasapogenin[b]		**1**		**2**		**3**	
Position	C	C	H	C	H	C	H
1	73.4	73.4	4.02 (br s)	79.5	4.06 (br s)	76.0	4.39 (br s)
2ax	32.8	32.7	1.45—1.47 (m)	32.5	1.45—1.48 (m)	32.5	1.46—1.49 (m)
2eq			2.10—2.12 (m)		2.16—2.18 (m)		2.17—2.19 (m)
3	68.2	68.2	4.36 (br s)	66.5	4.27 (br s)	67.3	4.34 (br s)
4ax	34.4	34.3	1.68—1.70 (m)	34.4	1.62—1.64 (m)	34.0	1.60—1.62 (m)
4eq			1.92—1.94 (m)		1.94—1.96 (m)		1.93—1.95 (m)
5	31.2	31.5	2.23—2.25 (m)	31.6	2.21—2.24 (m)	31.8	2.48—2.50 (m)
6ax	26.8	26.7	1.40—1.44 (m)	26.5	1.42—1.44 (m)	26.4	1.46—1.49 (m)
6eq			1.82—1.84 (m)		1.90—1.94 (m)		1.90—1.94 (m)
7ax	26.7	26.6	1.20—1.24 (m)	26.4	1.33—1.36 (m)	26.2	1.33—1.36 (m)
7eq			1.68—1.70 (m)		1.60—1.62 (m)		1.60—1.62 (m)
8	35.8	36.3	1.68—1.71 (m)	36.2	1.67—1.69 (m)	36.1	1.67—1.69 (m)
9	42.1	41.9	1.27—1.29 (m)	41.6	1.26—1.29 (m)	41.8	1.29—1.30 (m)
10	40.2	40.4		39.4		39.4	
11	21.1	20.9	1.30—1.33 (m)	21.2	1.35—1.37 (m)	21.3	1.36—1.39 (m)
12ax	40.4	30.0	1.47—1.49 (m)	29.2	1.48—1.50 (m)	30.5	1.46—1.49 (m)
12eq			2.16—2.18 (m)		2.17—2.19 (m)		2.17—2.19 (m)
13	40.7	46.2		45.3		45.3	
14	56.4	52.9	2.08—2.10 (m)	52.8	2.21—2.24 (m)	52.8	2.15—2.17 (m)
15ax	32.2	31.5	2.10—1.12 (m)	30.0	2.13—2.15 (m)	31.6	2.18—2.20 (m)
15eq			1.42—1.44 (m)		1.44—1.46 (m)		1.46—1.48 (m)
16	81.3	90.9	4.49 (d, 7.1)	90.2	4.44 (d, 7.2)	90.2	4.43 (d, 7.1)
17	63.1	89.6		90.0		90.0	
18	16.7	17.8	0.98 (s)	17.5	0.96 (s)	17.5	0.95 (s)
19	19.3	19.4	1.34 (s)	19.8	1.35 (s)	19.5	1.34 (s)
20	42.5	45.2	2.23—2.25 (m)	45.4	2.21—2.23 (m)	45.4	2.20—2.22 (m)
21	14.9	9.0	1.24 (d, 7.1)	9.6	1.21 (d, 7.2)	9.6	1.20 (d, 7.1)
22	109.8	110.6		110.3		110.3	
23ax	26.4	26.7	1.58—1.60 (m)	26.6	1.58—1.60 (m)	26.6	1.60—1.62 (m)
23eq			1.92—1.94 (m)		1.92—1.94 (m)		1.92—1.94 (m)
24	26.2	26.6	1.89—1.90 (m)	25.7	1.90—1.92 (m)	25.7	1.90—1.92 (m)
24			2.19—2.20 (m)		2.18—2.20 (m)		2.19—2.20 (m)
25	27.6	27.4	2.17—2.19 (m)	27.4	2.17—2.19 (m)	27.4	2.18—2.20 (m)
26ax	65.2	63.9	3.16 (d, 11.1)	64.9	3.22 (d, 10.7)	64.9	3.23 (d, 10.8)
26eq			3.99 (dd, 2.2, 11.1)		4.03 (dd, 2.5, 10.7)		4.03 (dd, 2.1, 10.8)
27	16.3	16.9	1.03 (d, 7.2)	16.2	1.04 (d, 7.0)	16.2	1.04 (d, 7.0)

a) Measured in 500 Hz for ^{1}H, and 125 Hz for ^{13}C, in C_5D_5N; coupling constants (*J*) in Hz are given in parentheses. *b*) Data in ref. 14.

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data[a)] for Sugar Parts of Compounds **2** and **3**

2			**3**		
	C	H		C	H
xyl-1	102.3	4.83 (d, 7.7)	xyl-1	98.2	5.05 (d, 7.3)
2	75.2	4.00—4.02 (m)	2	79.2	4.18—20 (m)
3	78.9	4.17—4.19 (m)	3	76.9	4.25—27 (m)
4	71.3	4.22—4.24 (m)	4	71.6	4.12—4.14 (m)
5	67.6	3.77 (dd, 10.6, 10.7)	5	67.4	3.66 (dd, 10.1, 11.2)
		4.41—4.43 (m)			4.34 (dd, 5.2, 11.2)
			Rha-1	101.9	6.57 (s)
			2	72.2	4.77—4.79 (m)
			3	72.0	4.73—4.75 (m)
			4	74.5	4.21—4.23 (m)
			5	69.9	4.73 (m)
			6	18.8	1.73 (d, 6.1)

a) Measured in 500 Hz for ^{1}H, and 125 Hz for ^{13}C, in C_5D_5N; coupling constants (*J*) in Hz are given in parentheses.

$CHCl_3$/MeOH, 10 : 0—6 : 1, 18 g) was subjected to column chromatography over silica gel (200 g) eluting with $CHCl_3$/MeOH to afford four fractions. Fraction 3.3 (elution of $CHCl_3$/MeOH, 9 : 1—7 : 1, 4 g) was chromatographed over silica gel eluting with $CHCl_3$/MeOH (8 : 1) and further purified by RP-18 (60% MeOH/H_2O) and sephadex LH-20 (MeOH) to obtain **3** (280 mg).

Reineckiagenin A (**1**): $C_{27}H_{44}O_5$, white amorphous solid, $[\alpha]_D^{20}$ +10.4° (c=0.11, $CHCl_3$), IR ν_{max}^{KBr} (cm^{-1}): 3373, 2925, 1057, 977, 948, 918, 898, 822, intensity 918>898. Negative-ion FAB-MS *m/z*: 447 $[M-H]^-$. Negative-ion HR-FAB-MS *m/z* 447.3124 $[M-H]^-$, calculated: 447.3110. For the ^{1}H- and ^{13}C-NMR spectral data (see Table 1).

Reineckiagenoside A (**2**): $C_{32}H_{52}O_9$, white amorphous solid. $[\alpha]_D^{20}$ −3.7° (c=0.11, MeOH), IR ν_{max}^{KBr} (cm^{-1}): 3400, 2926, 1454, 1046, 985, 916, 899, 863, intensity 916>899. Negative-ion FAB-MS *m/z*: 579 $[M-H]^-$, 447 $[M-132-H]^-$. Negative-ion HR-FAB-MS *m/z:* 579.3542 $[M-H]^-$, calculated: 579.3533. For the ^{1}H- and ^{13}C-NMR spectral data (see Tables 1, 2).

Reineckiagenoside B (**3**): $C_{38}H_{62}O_{13}$, colorless needles, crystallized from chloroform/methanol/water (7 : 3 : 0.1), $[\alpha]_D^{20}$ −9.9° (c=0.14, MeOH), IR ν_{max}^{KBr} (cm^{-1}): 3400, 2930, 1450, 1040, 980, 910, 894, 860, intensity 910>894. Negative-ion FAB-MS *m/z*: 725 $[M-H]^-$, 579 $[M-147-H]^-$, 447 $[M-147-132-H]^-$. HR-FAB-MS *m/z* 725.4136 $[M-H]^-$, calculated: 725.4112. For the ^{1}H- and ^{13}C-NMR spectral data (see Tables 1, 2).

Acid Hydrolysis of 2 A solution of **2** (12 mg) in 1 M HCl (dioxane/water, 1 : 1, 6 ml) was heated at 90 °C for 2 h, the reaction mixture was diluted with 2 ml H_2O and extracted with $CHCl_3$ (5 ml×3), the $CHCl_3$ phase (8.6 mg) was subjected to CC over silica gel eluting with $CHCl_3$/MeOH (100 : 1 v/v) to give **1** (3.6 mg, identified by TLC comparison and further confirmed by ^{1}H- and ^{13}C-NMR spectrum). The aqueous layer was passed through an Amberlite IRA-401 (OH^- form) column and the eluate was concentrated to dryness to give D-xylose, which was detected by direct TLC analysis on a HPTLC silica gel 50000 F_{254} plate using *n*-BuOH–Me_2CO–H_2O (4 : 5 : 1, homogenous) as development and anisaldehyde-H_2SO_4 as detection, comparing with the authentic sample: xylose (*Rf* 0.58). The absolute configuration of monosaccharide was further determined by GC analysis of its derivatives to be D-xylose. A solution of the sugar residue (2.5 mg) in pyridine (2 ml) was added to L-cysteine methyl ester hydrochloride (3.3 mg) and kept at 60 °C for 1 h. Then trimethylsilylimidazole (0.5 ml) was slowly added to the reaction mixture and kept again at 60 °C for 30 min. The supernatant (4 μl) was analyzed by GC, and the retention time of D- and L-xylose were 13.35 and 14.01 min, respectively.

Acid Hydrolysis of 3 A solution of **3** (22 mg) in 1 M HCl (dioxane/water, 1 : 1, 12 ml) was heated at 90 °C for 1 h, the reaction mixture was diluted with 5 ml H_2O and extracted with AcOEt (10 ml×3), the AcOEt phase (18 mg) was subjected to CC over silica gel eluting with $CHCl_3$/MeOH (100 : 1—15 : 1) to give **1** and **2** [**1** (5.6 mg), **2** (6.8 mg), identified by TLC comparison and further confirmed by ^{1}H- and ^{13}C-NMR spectrum]. The aqueous layer was treated and analyzed as described for **2**, in which D-xylose and L-rhamnose was detected by HPTLC (Development solvent: *n*-BuOH/Me_2CO/H_2O, 4 : 5 : 1, D-xylose *Rf* 0.58, L-rhamnose *Rf* 0.67), and GC analysis (retention times for D- and L-xylose, and L-rhamnose were 13.35, 14.01, and 14.97 min, respectively).

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