Two New Cholestane Bisdesmosides from *Reineckia carnea*

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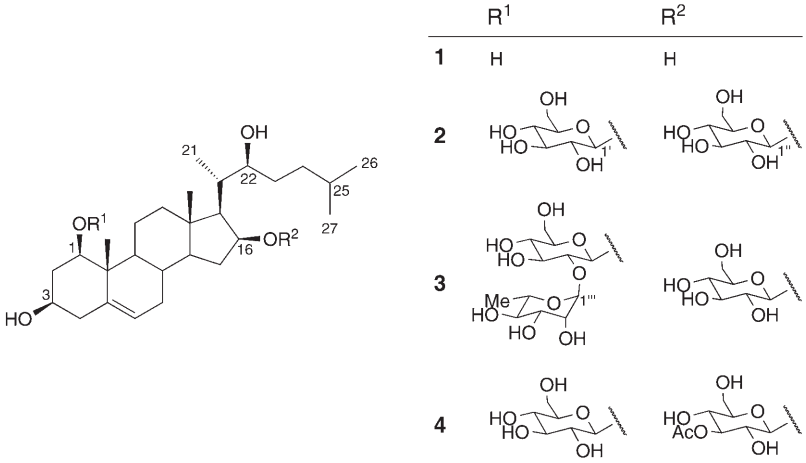
Three cholestane bisdesmosides, together with the corresponding aglycone, were isolated from the whole plant of *Reineckia carnea*. By detailed analysis of the 1D- and 2D-NMR spectra, chemical methods, and comparison with spectral data of known compounds, the structures were determined to be (1*β*,3*β*,16*β*,22*S*)-cholest-5-ene-1,3,16,22-tetrol (**1**), (1*β*,3*β*,16*β*,22*S*)-cholest-5-ene-1,3,16,22-tetrol 1,16-di(*β*-D-glucopyranoside) (**2**), (1*β*,3*β*,16*β*,22*S*)-cholest-5-ene-1,3,16,22-tetrol 1-[*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside] 16-(*β*-D-glucopyranoside) (**3**), (1*β*,3*β*,16*β*,22*S*)-cholest-5-ene-1,3,16,22-tetrol 1-(*β*-D-glucopyranoside) 16-(3-*O*-acetyl-*β*-D-glucopyranoside) (**4**). Compounds **3** and **4** appeared to be new compounds, while compound **1** was isolated for the first from a natural source. Compound **2** was isolated from the genus *Reineckia* for the first time.

1. Introduction. – *Reineckia carnea* KUNTH, the only species of the genus *Reineckia* (Liliaceae), is indigenous to China and Japan [1]. The Chinese name 'ji xiang cao' means it can bring luck and fortune to people. As a perennial ever-green herb, it is the pollution-resistant garden plant of choice for it can produce a repairing effect on the soil that was polluted by copper [2]. The whole plant has been used as an antitussive, an antarthritic, a hemostatic, and an antidote in traditional Chinese medicine [3]. As a major ingredient of many medical prescriptions, *R. carnea* is one of the most important traditional Chinese medicines of the Miao minority [4]. It was known that the genus *Reineckia* is close to *Rohdea*, *Tupistra*, *Convallaria*, *Aspidistra*, *Liriope*, and *Ophiopogon*, and all of them are rich in steroidal saponins with polyhydroxylated skeletons [5–9]. From the 1950s to the 1990s, the investigation on *R. carnea* by a few research groups in Japan have led to the isolation of some steroidal sapogenins and steroidal saponins from the aerial parts of the plant [10], as well as sapogenins from the saponified MeOH extract of the whole plant [11][12]. However, only one cholestane bisdesmoside, (1*β*,3*β*,16*β*,22*S*)-cholest-5-ene-1,3,16,22-tetrol 1-(*α*-L-rhamnopyranoside) 16-(*β*-D-glucopyranoside), was isolated from the genus [13]. The cholestane bisdesmosides from *Nolina recurvata* exhibited inhibitory activity on cAMP phosphodiesterase [14]. Despite of the former studies on *R. carnea*, some of the ethnomedical applications need more support for the medicinal effects of the chemical constituents. As a part of our study on the major medicinal plants of Miao ethnomedicine in China, we have now phytochemically investigated the whole plant *R. carnea*, collected from Tongzi county, Guizhou Province. This paper refers to the structural elucidation of the

new cholestane bisdesmosides based on spectroscopic data and chemical transformation.

2. Results and Discussion. – Compound **1** of the molecular formula $C_{27}H_{46}O_4$ (negative-ion FAB-MS: *m/z* 587 $[M+154\ (m\text{NBA})-H]^-$, 433 $[M-H]^-$, 417 $[M-18\ (H_2O)+H]^-$) was obtained as an amorphous, optically active solid. Analysis of the ^{1}H- and ^{13}C-NMR spectrum of **1** indicated that the structure of **1** was identical to the aglycone of the cholestane bisdesmoside schubertoside D [15][16]. Although this aglycone has been already obtained as a partial hydrolysate from schubertoside D, this is the first example of the isolation of **1** from a plant source.



The ^{1}H-NMR spectra of **1** in (D_5)pyridine showed signals for five steroid Me groups: three appeared as *d* at δ 1.18 ($J=7.0$ Hz, Me(21)), 0.87 ($J=6.5$ Hz, Me(26)), and 0.87 ($J=6.5$ Hz, Me(27)) and the other two as *s* at δ 1.17 (Me(18)) and 1.24 (Me(19)).

On comparison of the NMR data (*Tables 1* and *2*) and physicochemical properties with similar known compounds, the structure of **2** was determined to be (1β,3β,16β,22*S*)-cholest-5-ene-1,3,16,22-tetrol 1,16-di-(β-D-glucopyranoside). Compound **2** has been isolated from *Nolina recurvata* [14]; however, this is the first time that **2** is obtained from the genus *Reineckia*.

Compound **3**, an amorphous, optically active, solid, had the molecular formula $C_{45}H_{76}O_{18}$, as determined by negative-ion HR-FAB-MS (*m/z* 903.4938 ($[M-H]^-$)) and DEPT-NMR. A broad IR absorption near 3400 cm^{-1} was attributable to OH groups. Acid hydrolysis of **3** with 0.2M HCl in dioxane/H_2O (1:1) resulted in the production of **1**, **2**, D-glucose, and L-rhamnose. The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*), the HMBC correlations (*Fig.*), and further spectroscopic data established the structure of **3** to be (1β,3β,16β,22*S*)-cholest-5-ene-1,3,16,22-tetrol 1-[*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside] 16-(β-D-glucopyranoside).

The presence of three sugar units in **3** was readily recognized from three anomeric proton signals at δ 4.88 (*d*, $J=7.6$ Hz), 4.73 (*d*, $J=7.6$ Hz), and 6.31 (br. *s*), correponding to three anomeric C-atom signals at δ 99.9, 107.1, and 101.6. Comparison of the ^{13}C-NMR spectrum of **3** with that of **2** (*Tables 1* and *2*),

Table 1. *^{1}H- and ^{13}C- NMR Data* ((D_5)pyridine) *of the Aglycone Parts of Compounds* **2**–**4**[a])

	2		**3**		**4**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
H–C(1)	82.9 (*d*)	3.91–3.93 (*m*)	83.7 (*d*)	3.80–3.82 (*m*)	83.0 (*d*)	3.92–3.94 (*m*)
H_{ax}–C(2)	37.6 (*t*)	2.02–2.04 (*m*)	37.7 (*t*)	2.05–2.07 (*m*)	37.6 (*t*)	2.02–2.04 (*m*)
H_{eq}–C(2)		2.55–2.57 (*m*)		2.58–2.60 (*m*)		2.60–2.62 (*m*)
H–C(3)	68.1 (*d*)	3.78–3.82 (*m*)	68.1 (*d*)	3.81–3.83 (*m*)	68.0 (*d*)	3.72–3.74 (*m*)
H_{ax}–C(4)	43.8 (*t*)	2.53–2.55 (*m*)	43.9 (*t*)	2.51–2.53 (*m*)	43.7 (*t*)	2.53–2.55 (*m*)
H_{eq}–C(4)		2.62–2.64 (*m*)		2.65–2.66 (*m*)		2.61–2.63 (*m*)
C(5)	139.5 (*s*)		139.5 (*s*)		139.6 (*s*)	
H–C(6)	124.8 (*d*)	5.47 (*d*, *J* = 4.3)	124.9 (*d*)	5.46 (*d*, *J* = 4.9)	124.8 (*d*)	5.51 (*d*, *J* = 4.7)
H_{ax}–C(7)	31.9 (*t*)	1.39–1.42 (*m*)	31.9 (*t*)	1.42–1.44 (*m*)	31.8 (*t*)	1.41–1.43 (*m*)
H_{eq}–C(7)		1.75–1.79 (*m*)		1.78–1.79 (*m*)		1.78–1.80 (*m*)
H–C(8)	33.2 (*d*)	1.30–1.32 (*m*)	33.3 (*d*)	1.32–1.34 (*m*)	33.2 (*d*)	1.34–1.36 (*m*)
H–C(9)	50.3 (*d*)	1.36–1.39 (*m*)	50.5 (*d*)	1.50–1.52 (*m*)	50.3 (*d*)	1.33–1.36 (*m*)
C(10)	42.9 (*s*)		42.8 (*s*)		42.8 (*s*)	
H_{ax}–C(11)	23.9 (*t*)	1.55–1.57 (*m*)	24.1 (*t*)	1.63–1.65 (*m*)	23.8 (*t*)	1.50–1.52 (*m*)
H_{eq}–C(11)		2.65–2.68 (*m*)		2.76–2.78 (*m*)		2.72–2.74 (*m*)
H_{ax}–C(12)	40.6 (*t*)	1.30–1.32 (*m*)	40.8 (*t*)	1.35–1.38 (*m*)	40.5 (*t*)	1.33–1.36 (*m*)
H_{eq}–C(12)		1.96–1.98 (*m*)		1.98–2.00 (*m*)		2.00–2.02 (*m*)
C(13)	42.2 (*s*)		42.2 (*s*)		42.2 (*s*)	
H–C(14)	55.2 (*d*)	0.88–0.90 (*m*)	55.3 (*d*)	0.92–0.94 (*m*)	55.2 (*d*)	0.88–0.90 (*m*)
H_a–C(15)	37.3 (*t*)	1.74–1.76 (*m*)	37.3 (*t*)	1.73–1.75 (*m*)	37.0 (*t*)	1.75–1.77 (*m*)
H_b–C(15)		2.23–2.25 (*m*)		2.26–2.28 (*m*)		2.25–2.27 (*m*)
H–C(16)	82.8 (*d*)	4.47–4.50 (*m*)	82.8 (*d*)	4.52–4.54 (*m*)	82.9 (*d*)	4.46–4.48 (*m*)
H–C(17)	58.2 (*d*)	1.95–1.97 (*m*)	58.2 (*d*)	1.96–1.98 (*m*)	58.1 (*d*)	1.90–1.92 (*m*)
Me(18)	13.9 (*q*)	1.07 (*s*)	13.9 (*q*)	1.07 (*s*)	13.8 (*q*)	1.00 (*s*)
Me(19)	14.8 (*q*)	1.22 (*s*)	15.1 (*q*)	1.42 (*s*)	14.8 (*q*)	1.22 (*s*)
H–C(20)	36.0 (*d*)	2.62–2.64 (*m*)	36.1 (*d*)	2.64–2.65 (*m*)	35.9 (*d*)	2.43–2.45 (*m*)
Me(21)	12.6 (*q*)	1.14 (*d*, *J* = 6.8)	12.6 (*q*)	1.16 (*d*, *J* = 6.8)	12.6 (*q*)	1.12 (*d*, *J* = 6.8)
H–C(22)	73.3 (*d*)	3.96–3.98 (*m*)	73.2 (*d*)	3.96–3.98 (*m*)	73.2 (*d*)	4.19–4.21 (*m*)
H_a–C(23)	33.9 (*t*)	1.52–1.56 (*m*)	33.9 (*t*)	1.62–1.63 (*m*)	33.8 (*t*)	1.55–1.57 (*m*)
H_b–C(23)		1.78–1.80 (*m*)		1.77–1.79 (*m*)		1.77–1.79 (*m*)
H_a–C(24)	36.8 (*t*)	1.53–1.55 (*m*)	36.8 (*t*)	1.63–1.65 (*m*)	36.6 (*t*)	1.55–1.58 (*m*)
H_b–C(24)		1.75–1.77 (*m*)		1.83–1.85 (*m*)		1.78–1.79 (*m*)
H–C(25)	28.9 (*d*)	1.58–1.60 (*m*)	28.9 (*d*)	1.62–1.64 (*m*)	28.9 (*d*)	1.58–1.60 (*m*)
Me(26)	23.1 (*q*)	0.90 (*d*, *J* = 6.5)	23.1 (*q*)	0.90 (*d*, *J* = 6.5)	22.9 (*q*)	0.89 (*d*, *J* = 6.5)
Me(27)	23.2 (*q*)	0.90 (*d*, *J* = 6.5)	23.2 (*q*)	0.90 (*d*, *J* = 6.5)	23.0 (*q*)	0.89 (*d*, *J* = 6.5)

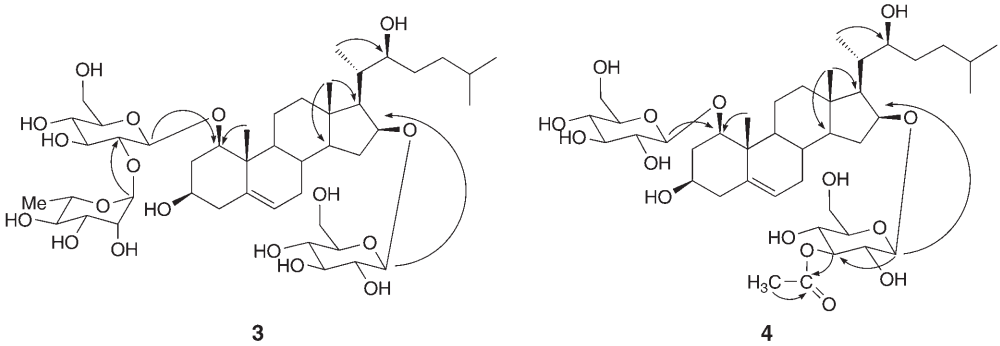
[a]) Measured in 500 Hz for ^{1}H, and 125 Hz for ^{13}C; δ in ppm, *J* in Hz.

showed that **3** displayed one more set of signals assignable to a terminal α-L-rhamnopyranosyl unit. This was supported by the subsequential losses of the sugar units in the positive-ion FAB-MS spectrum: (*m*/*z* 905 $[M+H]^+$, 869 $[M-36\ (2\,H_2O)+H]^+$, 723 $[M-146\ (Rha)-36\ (2\,H_2O)+H]^+$, 561 $[M-162\ (Glc)-146\ (Rha)-36\ (2\,H_2O)+H]^+$, 399 $[M-162\ (Glc)-162\ (Glc)-146\ (Rha)-36\ (2\,H_2O)+H]^+$. *O*-Glycosylation-induced chemical shifts due to the glucose moiety attached to C(1) of the aglycone were significant, while the remaining signals were in good agreement with those of **2**. On comparison with those of **2**, the signal of C(2′) of **3** was shifted downfield to δ 79.9 (+4.4 ppm), accompanied by the upfield shifts of C(1′) (−1.4 ppm) and C(3′) (−1.8 ppm) (*Table 2*), indicating that the additional L-rhamnose was linked at the C(2′) position of the glucose unit. Furthermore, the HMBC

Table 2. *^1H- and ^{13}C- NMR Data* ((D_5)pyridine) *for the Sugar Units of Compounds* **2**–**4**[a])

	2		**3**		**4**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
1-*O*-β-D-Glucopyranosyl:						
H–C(1′)	101.3 (*d*)	4.96 (*d*, *J* = 7.6)	99.9 (*d*)	4.88 (*d*, *J* = 7.6)	101.3 (*d*)	4.87 (*d*, *J* = 7.6)
H–C(2′)	75.5 (*d*)	3.91–3.94 (*m*)	79.9 (*d*)	4.21–4.24 (*m*)	75.4 (*d*)	3.86–3.89 (*m*)
H–C(3′)	78.7 (*d*)	4.12–4.14 (*m*)	76.9 (*d*)	4.20–4.22 (*m*)	78.7 (*d*)	4.09–4.11 (*m*)
H–C(4′)	72.4 (*d*)	4.00–4.01 (*m*)	72.6 (*d*)	4.15–4.18 (*m*)	72.5 (*d*)	4.00–4.01 (*m*)
H–C(5′)	78.2 (*d*)	3.81–3.83 (*m*)	77.9 (*d*)	3.81–3.83 (*m*)	78.0 (*d*)	3.74–3.76 (*m*)
CH_2(6′)	63.6 (*t*)	4.22–4.23 (*m*), 4.25–4.27 (*m*)	63.7 (*t*)	4.15–4.20 (*m*), 4.25–4.31 (*m*)	63.6 (*t*)	4.21–4.23 (*m*), 4.43–4.45 (*m*)
16-*O*-β-D-Glucopyranosyl:						
H–C(1″)	107.1 (*d*)	4.72 (*d*, *J* = 7.6)	107.1 (*d*)	4.73 (*d*, *J* = 7.6)	106.6 (*d*)	4.69 (*d*, *J* = 7.7)
H–C(2″)	75.7 (*d*)	3.93–3.96 (*m*)	75.7 (*d*)	3.95–3.98 (*m*)	73.5 (*d*)	3.92–3.94 (*m*)
H–C(3″)	78.8 (*d*)	4.17–4.19 (*m*)	78.8 (*d*)	4.18–4.20 (*m*)	79.9 (*d*)	5.62–5.64 (*m*)
H–C(4″)	71.8 (*d*)	4.15–4.18 (*m*)	71.8 (*d*)	4.12–4.16 (*m*)	69.5 (*d*)	4.15–4.17 (*m*)
H–C(5″)	78.2 (*d*)	4.03–4.05 (*m*)	78.2 (*d*)	4.13–4.15 (*m*)	78.1 (*d*)	3.80–3.82 (*m*)
CH_2(6″)	63.0 (*t*)	4.30–4.32 (*m*), 4.37–4.39 (*m*)	62.9 (*t*)	4.30–4.32 (*m*), 4.46–4.48 (*m*)	62.4 (*t*)	4.30–4.32 (*m*), 4.35–4.37 (*m*)
*Me*CO					21.2 (*q*)	1.90 (*s*)
Me*C*O					170.8 (*s*)	
2′-*O*-α-L-Rhamnopyranosyl:						
H–C(1‴)			101.6 (*d*)	6.31 (br. *s*)		
H–C(2‴)			72.7 (*d*)	4.60–4.62 (*m*)		
H–C(3‴)			72.8 (*d*)	4.59–4.61 (*m*)		
H–C(4‴)			74.3 (*d*)	4.44–4.46 (*m*)		
H–C(5‴)			69.5 (*d*)	5.06–5.09 (*m*)		
Me(6‴)			19.2 (*q*)	1.78 (*d*, *J* = 6.0)		

[a]) Measured in 500 Hz for ^1H, and 125 Hz for ^{13}C; δ in ppm, *J* in Hz.



3 **4**

Figure. *Key HMBC correlations of compounds* **3** *and* **4**

correlations of δ 6.31 (*s*, H–C(1‴)) with δ 79.9 (C(2′)), of δ 4.88 (*d*, *J* = 7.6 Hz, H–C(1′)) with δ 83.7 (C(1)) and 79.9 (C(2′)), and of δ 4.73 (*d*, *J* = 7.6 Hz, H–C(1″)) with δ 82.8 (C(16)) were observed (*Fig.*).

By interpretation of the HSQC and HMBC spectra, the complete assignment of the ^{1}H- and ^{13}C-NMR data of **3** was performed unambiguously. The ^{1}H-NMR signal for Me(6‴) of the rhamnose unit was shifted to a relatively lower field (δ 1.78) compared with that of the usual terminal rhamnose unit of oligosaccharides (*ca.* δ 1.70) [17], while the signal of Me(19) of the aglycone part was shifted prominently downfield, by 0.3 ppm, as compared with those of **1** and **2**. The downfield shifts of these Me signals seemed due to the interaction between Me(19) and the Me(6‴). Molecular-mechanics and molecular-dynamics calculation studies revealed that the steric proximity of the Me groups resulted in the reduction of electron density around the protons of the Me(19) and Me(6‴), which caused downfield shifts of their δ(H) [14].

Compound **4** was obtained as an amorphous optically active solid. The negative-ion HR-FAB-MS showed an $[M-H]^-$ ion peak at *m*/*z* 799.4457, in accordance with the empirical molecular formula $C_{41}H_{68}O_{15}$, which was supported by the ^{13}C-NMR spectrum and DEPT data. Treatment of **4** with 3% NaOMe in MeOH afforded the deacetyl derivative **2**. On the basis of the ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*), the HMBC correlations (*Fig.*), and further spectroscopic data, **4** was formulated as (1β,3β,16β,22*S*)-cholest-5-ene-1,3,16,22-tetrol 1-(*β*-D-glucopyranoside) 16-(3-*O*-acetyl-*β*-D-glucopyranoside).

A *s* signal at δ 1.90, which was correlated to the δ 21.2 resonance in the HSQC spectrum and to δ 170.8 in the HMBC spectrum, indicated the presence of an acetyl group in **4**. The ^{13}C-NMR spectrum of **4** was in accordance with that of **2**, except for the two signals of the acetyl group. This was supported by fragment-ion peaks at *m*/*z* 799 $[M-H]^-$, 757 $[M-42\ (MeCO)-H]^-$, 595 $[M-162\ (Glc)-42\ (MeCO)-H]^-$, and 433 $[M-162\ (Glc)-162\ (Glc)-42\ (MeCO)-H]^-$ in the negative-ion FAB-MS of **4**. When the ^{1}H- and ^{13}C-NMR spectra of **4** were compared with those of **2**, the downfield shifts due to *O*-acylation could be recognized at the H–C(3″) (+1.44 ppm) and C(3″) (+1.1 ppm) signals of the glucose moiety of **4**. This suggested that the acetyl group was involved in an ester linkage at C(3″) of the glucose unit. In the HMBC spectrum of **4**, correlations between δ 4.87 (*d*, *J* = 7.6 Hz, H–C(1′)) and δ 83.0 (C(1)), between δ 4.69 (*d*, *J* = 7.7 Hz, H–C(1″)) and δ 82.9 (C(16)), between δ 5.63 (*m*, H–C(3″)) and δ 170.8 (MeCO), and between δ 4.69 (*d*, *J* = 7.7 Hz, H–C(1″)) and δ 79.9 (C(3″)) further supported the conclusion (*Fig.*).

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Experimental Part

General. TLC: plates precoated with silica gel F_{254} (*Qingdao Haiyang Chemical Co.*, *Ltd*, Qingdao, China) and *RP-18* plates (*Merck*). Column chromatography (CC): silica gel (200–300 mesh and 10–40 μm), *RP-18* (40–63 μm; *Merck*), and *Sephadex LH-20* (*Pharmacia*). Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr pellets, $\tilde{\nu}$ in cm^{-1}; 1D-NMR Spectra: *Bruker AM*-400-MHz (^{1}H) and -100-MHz (^{13}C) spectrometer, δ in ppm with $SiMe_4$ as the internal standard, *J* in Hz; multiplicities of ^{13}C-NMR by DEPT. 2D-NMR Spectra: *Bruker DRX*-500-MHz spectrometer. MS: *VG Autospec-3000* spectrometer, *m*NBA = 3-nitrobenzyl alcohol; in *m*/*z* (rel %).

Plant Material. The whole plant of *R. carneae* was collected in December 2004 from Tongzi County, Guizhou Province, China, and a voucher specimen (identified by Prof. *Du Jiang* from the Traditional Chinese Medical College of Guizhou) was deposited at the State Key Laboratory of Phytochemistry of the Kunming Institute of Botany, CAS (No. 200412013).

Extraction and Isolation. The air-dried whole plant of *Reineckia carneae* (2.0 kg) was extracted with 90% MeOH/H_2O under reflux to give a crude extract which was suspended in H_2O and then partitioned with AcOEt and BuOH. The AcOEt extract (140 g) was subjected to CC (silica gel (800 g), $CHCl_3$/MeOH 1:0 → 2:1): *Fractions 1–4*. *Fr. 2* (28 g) was subjected to CC (silica gel (220 g), $CHCl_3$/MeOH 1:0 → 20:1) and then to repeated CC (*Sephadex LH-20*): **1** (26 mg; $[\alpha]_D^{27} = -60$ ($c = 0.10$, $CHCl_3$)). *Fr. 4* (28 g) was subjected to CC (silica gel (280 g), $CHCl_3$/MeOH 12:1 → 3:1) and repeated CC (*RP-18*), 30–70% H_2O/MeOH): **2** (220 mg), **3** (220 mg), and **4** (25 mg).

*(1β,3β,16β,22S)-Cholest-5-ene-1,3,16,22-tetrol 1-[O-α-*L*-rhamnopyranosyl-(1 → 2)-β-*D*-glucopyranoside] 16-(β-*D*-Glucopyranoside)* (= *(1β,3β,16β,22S)-16-(β-*D*-Glucopyranasyloxy)-3,22-dihydroxycholest-5-en-1-yl 2-*O*-(6-Deoxy-α-*L*-mannopyranosyl)-β-*D*-glucopyranoside*; **3**; $C_{45}H_{76}O_{18}$). Amorphous solid. $[\alpha]_D^{20} = -4.0$ ($c = 0.12$, MeOH). IR: 3400 (OH), 2940 (CH), 1455, 1380, 1125, 1050, 985, 823, 810, 695. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (neg.): 903 (95, $[M-H]^-$), 760 (23), 570 (25), 463 (100). FAB-MS (pos.): 905 (3, $[M+H]^+$), 869 (2, $[M-36\ (2\ H_2O)+H]^+$), 723 (5, $[M-146\ (Rha)-36\ (2\ H_2O)+H]^+$), 561 (4, $[M-162\ (Glc)-146\ (Rha)-36\ (2\ H_2O)+H]^+$), 399 (30, $[M-162\ (Glc)-162\ (Glc)-146\ (Rha)-36\ (2\ H_2O)+H]^+$), 381 (40, $[M-18\ (H_2O)-162\ (Glc)-162\ (Glc)-146\ (Rha)-36\ (2\ H_2O)+H]^+$). HR-FAB-MS (neg.): 903.4938 ($[M-H]^-$; calc. 903.4953).

Partial Hydrolysis of **3**. A soln. of **3** (12 mg) in 0.2M HCl in dioxane/H_2O 1:1 (5 ml) was refluxed at 90° for 30 min under Ar. After concentration of the soln., the residue was extracted with AcOEt (3×). Compounds **1** and **2** were detected in the AcOEt extract, and D-glucose and L-rhamnose in the H_2O phase after evaporation and TLC comparison of the residue with authentic samples (BuOH/Me_2CO/H_2O 4:5:1): R_f (D-glucose) 0.36, R_f (L-rhamnose) 0.67.

*(1β,3β,16β,22S)-Cholest-5-ene-1,3,16,22-tetrol 1-(β-*D*-Glucopyranoside) 16-(3-*O*-Acetyl-β-*D*-glucopyranoside)* (= *(1β,3β,16β,22S)-16-[3-*O*-Acetyl-β-*D*-glucopyranosyl)oxy]-3,22-dihydroxycholest-5-en-1-yl β-*D*-Glucopyranoside*; **4**; $C_{41}H_{68}O_{15}$). Amorphous solid. $[\alpha]_D^{20} = -1.0$ ($c = 0.15$, MeOH). IR: 3400 (OH), 2945 (CH), 1720 (C=O), 1460, 1380, 1360, 1250, 1060, 980, 825. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (neg.): 954 (100, $[M+154\ (m\text{NBA})]^-$), 799 (35, $[M-H]^-$), 757 (14, $[M-42\ (MeCO)-H]^-$), 595 (5, $[M-162\ (Glc)-42\ (MeCO)-H]^-$), 433 (4, $[M-162\ (Glc)-162\ (Glc)-42\ (MeCO)-H]^-$). HR-FAB-MS (neg.): 799.4457 ($[M-H]^-$; calc. 799.4479).

Alkaline Methanolysis of **4**. Compound **4** (12 mg) was treated with 3% NaOMe in MeOH (3 ml) at r.t. for 1 h. After concentration of the soln., the residue was extracted with MeOH (3×). The MeOH extract was fractionated by CC (silica gel, CH_3Cl/MeOH 4:1): **2** (6.3 mg).

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