Four New Cycloartane (= 9,19-Cyclolanostane) Saponins from the Aerial Parts of *Thalictrum fortunei*

by **Hui Sun**[a]), **Xian-Tao Zhang**[a]), **Lei Wang**[a]), **Xiao-Qi Zhang**[b]), **Ying Wang**[b]), **Si-Bao Chen**[c]), **Pei-Gen Xiao**[c]), and **Wen-Cai Ye***[a])[b])

[a]) Department of Phytochemistry, China Pharmaceutical University, Nanjing 210009, P. R. China (phone: +86-25-8532-2132; fax: +86-25-8530-1528; e-mail: chywc@yahoo.com.cn)

[b]) Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou 510632, P. R. China

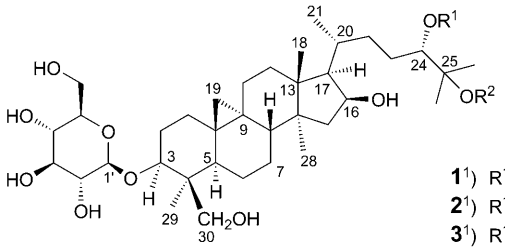
[c]) Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100094, P. R. China

The four new cycloartane (= 9,19-cyclolanostane) glycosides **1**–**4** were isolated from the aerial parts of *Thalictrum fortunei* (Ranunculaceae). The structures of these new glycosides were elucidated as (3*β*,16*β*,24*S*)-cycloartane-3,16,24,25,30-pentol 3,25-di-*β*-D-glucopyranoside (**1**), (3*β*,16*β*,24*S*)-24-(acetyloxy)cycloartane-3,16,25,30-tetrol 3,25-di-*β*-D-glucopyranoside (**2**), (3*β*,16*β*,24*S*)-24-(acetyloxy)-3-(*β*-D-glucopyranosyloxy)cycloartane-16,25,30-triol 25-[*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside] (**3**), and (3*β*,16*β*,24*S*)-24-(acetyloxy)-3-(*β*-D-glucopyranosyloxy)cycloartane-16,25,30-triol 25-[*β*-D-glucopyranosyl-(1 → 4)-*β*-D-glucopyranoside] (**4**). The structure elucidations were accomplished by 1D- and 2D-NMR methods, HR-ESI-MS, and hydrolysis.

Introduction. – The genus *Thalictrum* (Ranunculaceae) contains more than 200 species growing in northern temperate regions, and about 70 of them are distributed in China. Many plants belonging to the genus *Thalictrum* have been used as anticancer, antibacterial, and anti-inflammatory agents in traditional Chinese medicine. Previous phytochemical investigations revealed that the main constituents of the genus *Thalictrum* are alkaloids, together with some flavonoids and triterpene saponins [1]. The plant *Thalictrum fortunei* S. MOORE was used as an anti-inflammatory agent in Chinese folk medicine. However, only a small amount of alkaloids and flavonoids had been reported in this plant [2][3].

We had isolated some triterpene glycosides from several ranunculaceous plants [4–8]. Recently, several new cycloartane glycosides were obtained from *T. fortunei* [9]. Further investigation of the plant had led to the purification of several minor saponin constituents. In the present paper, we describe the isolation and structure elucidation of the four new cycloartane glycosides **1**–**4**. Their structures were determined with the aid of 1D- and 2D-NMR techniques, HR-ESI-MS, and hydrolysis.

Results and Discussion. – The dried aerial parts of *T. fortunei* were extracted with 95% EtOH. The extract was concentrated and the residue suspended in H_2O and then successively extracted with petroleum ether, AcOEt, and BuOH. The BuOH-soluble fraction was separated by column chromatography (silica gel, *Sephadex LH-20*, and *RP-18*). Then, prep. reversed-phase HPLC was applied to afford cycloartane glycosides **1**–**4**.



1[1]) R^1 = H, R^2 = β-D-Glc
2[1]) R^1 = Ac, R^2 = β-D-Glc
3[1]) R^1 = Ac, R^2 = β-D-Glc-(1→6)-β-D-Glc
4[1]) R^1 = Ac, R^2 = β-D-Glc-(1→4)-β-D-Glc

Compound **1** was obtained as an amorphous powder. Positive results from both *Liebermann–Burchard* and *Molish* reactions indicated that **1** was a saponin. The HR-ESI-MS showed a quasimolecular ion at *m/z* 815.4796 ($[M-H]^-$), consistent with a molecular formula $C_{42}H_{72}O_{15}$. Acid hydrolysis of **1** afforded D-glucose, which was identified by gas-chromatographic analysis (see *Exper. Part*). Based on the ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*), DEPT, ^{1}H,^{1}H-COSY, HSQC, and HMBC data, the

Table 1. *^{13}C-NMR Data* (125 MHz, C_5D_5N) *of Compounds* **1**–**4**[1]). δ in ppm. Glc = β-D-Glucopyranosyl.

	1	**2**	**3**	**4**		**1**	**2**	**3**	**4**
C(1)	32.0	31.5	32.2	32.2	C(27)	22.6	21.8	22.4	22.4
C(2)	30.1	29.4	30.1	30.1	C(28)	20.4	19.8	20.5	20.5
C(3)	89.4	88.8	89.4	89.4	C(29)	21.2	20.5	21.2	21.3
C(4)	45.1	44.5	45.1	45.1	C(30)	63.4	62.7	63.4	63.4
C(5)	47.9	47.3	47.9	47.9	AcO		170.6, 20.6	171.2, 21.3	171.2, 21.3
C(6)	22.0	21.4	22.0	22.0	Glc′:				
C(7)	26.9	26.2	26.9	27.1	C(1′)	106.0	105.7	106.2	106.3
C(8)	48.7	48.1	48.9	48.5	C(2′)	75.7	75.0	75.5	75.7
C(9)	21.3	20.5	21.3	21.1	C(3′)	78.8	78.2	78.8	78.9
C(10)	25.7	25.0	25.8	25.7	C(4′)	71.9	71.2	71.8	71.9
C(11)	26.4	25.8	26.4	26.4	C(5′)	78.4	77.9	78.5	78.5
C(12)	33.3	32.7	33.3	32.8	C(6′)	62.8	62.4	62.9	63.0
C(13)	45.7	45.1	45.8	45.6	Glc″:				
C(14)	46.9	46.3	46.9	47.0	C(1″)	97.7	98.4	99.0	99.0
C(15)	48.9	48.9	49.6	49.6	C(2″)	75.6	74.7	75.3	75.3
C(16)	71.7	70.8	71.4	71.9	C(3″)	78.8	78.2	78.7	78.7
C(17)	57.6	56.8	57.5	56.9	C(4″)	71.9	71.0	71.7	82.8
C(18)	19.6	19.0	19.7	19.6	C(5″)	78.6	77.5	77.5	78.0
C(19)	30.0	29.5	30.4	30.1	C(6″)	63.0	62.4	70.3	63.0
C(20)	29.5	30.2	30.9	30.5	Glc‴:				
C(21)	18.3	17.6	18.3	18.0	C(1‴)			105.5	107.0
C(22)	33.9	33.0	33.7	34.0	C(2‴)			75.3	75.6
C(23)	28.5	26.9	27.6	26.6	C(3‴)			78.5	78.7
C(24)	75.0	78.9	79.6	80.5	C(4‴)			71.9	71.9
C(25)	80.5	78.2	78.8	79.1	C(5‴)			78.2	78.0
C(26)	22.9	23.7	24.4	24.9	C(6‴)			62.9	63.0

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

Table 2. *^{1}H-NMR Data* (500 MHz, C_5D_5N) *of the Saccharide Moieties of* **1**–**4**. δ in ppm, J in Hz. Glc = β-D-Glucopyranosyl.

		1	**2**	**3**	**4**
Glc′:	H–C(1′)	5.00 (*d*, *J* = 7.8)	5.04 (*d*, *J* = 7.7)	4.94 (*d*, *J* = 7.7)	5.04 (*d*, *J* = 7.7)
	H–C(2′)	3.94 (*dd*, *J* = 7.8, 8.9)	3.98 (*dd*, *J* = 7.7, 8.9)	3.89 (*dd*, *J* = 7.7, 8.9)	3.90 (*dd*, *J* = 7.7, 9.1)
	H–C(3′)	4.12[a])	4.21[a])	4.12[a])	4.10[a])
	H–C(4′)	4.11[a])	4.19[a])	4.14[a])	4.13[a])
	H–C(5′)	3.80[a])	3.96[a])	3.80[a])	3.83[a])
	H_a–C(6′)	4.55 (*dd*, *J* = 11.8, 2.4)	4.55 (*dd*, *J* = 11.8, 2.5)	4.54 (*dd*, *J* = 11.8, 2.5)	4.56 (br *d*, *J* = 11.6)
	H_b–C(6′)	4.41[a])	4.42[a])	4.40[a])	4.42[a])
Glc″:	H–C(1″)	5.05 (*d*, *J* = 7.8)	5.08 (*d*, *J* = 7.7)	5.03 (*d*, *J* = 7.7)	5.07 (*d*, *J* = 7.6)
	H–C(2″)	3.90 (*dd*, *J* = 7.8, 9.0)	3.92[a])	3.88 (*dd*, *J* = 7.7, 8.8)	3.90[a])
	H–C(3″)	4.17[a])	4.18[a])	4.14[a])	4.16[a])
	H–C(4″)	4.16[a])	4.16[a])	4.10[a])	4.25[a])
	H–C(5″)	3.92[a])	3.85[a])	4.07[a])	3.83[a])
	H_a–C(6″)	4.51 (*dd*, *J* = 11.8, 2.4)	4.53 (*dd*, *J* = 11.8, 2.5)	4.78 (br *d*, *J* = 11.5)	4.66 (br *d*, *J* = 11.5)
	H_b–C(6″)	4.40[a])	4.45[a])	4.30[a])	4.38[a])
Glc‴:	H–C(1‴)			5.11 (*d*, *J* = 7.7)	4.63 (*d*, *J* = 7.8)
	H–C(2‴)			3.99 (*dd*, *J* = 7.7, 8.8)	3.93[a])
	H–C(3‴)			3.96[a])	4.10[a])
	H–C(4‴)			4.01[a])	4.16[a])
	H–C(5‴)			3.83[a])	3.83[a])
	H_a–C(6‴)			4.52 (*dd*, *J* = 11.8, 2.5)	4.51(*dd*, *J* = 11.7, 2.3)
	H_b–C(6‴)			4.47[a])	4.41[a])

[a]) Overlapped signals.

structure of **1** was established as (3β,16β,24*S*)-cycloartane-3,16,24,25,30-pentol 3,25-di-β-D-glucopyranoside[1]).

The ^{1}H-NMR spectrum of **1** showed two signals at δ(H) 0.22 (*d*, *J* = 3.7 Hz) and 0.47 (*d*, *J* = 3.7 Hz), indicating the presence of a cyclopropane ring. Furthermore, the signals for five tertiary Me groups at δ(H) 0.86, 1.32, 1.38, 1.44, and 1.53, as well as one secondary Me group at δ(H) 1.02 (*d*, *J* = 6.5 Hz) were observed. The ^{13}C-NMR data of **1** displayed 42 signals including 6 Me, 13 CH_2, 17 CH, and 6 quaternary C-atoms. The ^{13}C-NMR also suggested the presence of a cyclopropane CH_2 at δ(C) 30.0, a CH_2 bearing an O-atom at δ(C) 63.4 (C(30)), three CH groups bearing an O-atom at δ(C) 71.7 (C(16)), 75.0 (C(24)), and 89.4 (C(3)), and a quaternary C-atom bearing an O-atom at δ(C) 80.5 (C(25)). The ^{13}C-NMR data of **1** showed the presence of two anomeric C-atoms at δ(C) 106.0 and 97.7, which correlated with the H-atoms at δ(H) 5.00 (*d*, *J* = 7.8 Hz) and 5.05 (*d*, *J* = 7.8 Hz), respectively, in the HSQC spectrum, indicating the presence of two β-D-glucopyranosyl units. Comparison of the ^{1}H- and ^{13}C-NMR data of compound **1** with those of cyclofoetigenin B (= (3β,16β,24*S*)-cycloartane-3,16,24,25,30-pentol = (3β,4β,16β,24*S*)-9,19-cyclolanostane-3,16,24,25,28-pentol) indicated that the aglycone of **1** was cyclofoetigenin B [10][11]. Glycosylation at C(3) and C(25) was indicated by the ^{1}H,^{13}C-long-range (HMBC) correlations between δ(H) 5.00 (H–C(1′)) and δ(C) 89.4 (C(3)), as well as between δ(H) 5.05 (H–C(1″)) and δ(C) 80.5 (C(25)).

Compound **2** was obtained as an amorphous powder. The molecular formula of **2** was determined to be $C_{44}H_{74}O_{16}$ by HR-ESI-MS (*m*/*z* 857.4850 ($[M-H]^-$)). Acid hydrolysis of **2** afforded D-glucose, which was identified by gas-chromatographic analysis (see *Exper. Part*). The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) of **2** were

similar to those of **1**, except for the appearance of the signals for an additional Ac group. Based on the results of alkaline hydrolysis of **2** (see *Exper. Part*), as well as 1D- and 2D-NMR analyses, **2** could be identified as 24-*O*-acetylated derivative of **1**. Thus, the structure of **2** was that of (3*β*,16*β*,24*S*)-24-(acetyloxy)cycloartane-3,16,25,30-tetrol 3,25-di-*β*-D-glucopyranoside[1]).

The ^{1}H-NMR spectrum of **2** showed two cyclopropane signals at δ(H) 0.22 (*d*, *J* = 3.7 Hz) and 0.47 (*d*, *J* = 3.7 Hz), six tertiary Me groups at δ(H) 0.87, 1.34, 1.44, 1.47, 1.58, and 2.09, a secondary Me group at δ(H) 1.02 (*d*, *J* = 6.6 Hz), as well as two anomeric H-atoms at δ(H) 5.08 (*d*, *J* = 7.7 Hz) and 5.04 (*d*, *J* = 7.7 Hz). The ^{1}H-NMR data of **2** were similar to those of **1**, except for an additional Ac group at δ(H) 2.09 (*s*). Correspondingly, in the ^{13}C-NMR spectrum of **2**, the signals of this additional Ac group (δ(C) 170.6 and 20.6) were also observed. Comparison of the ^{13}C-NMR data of **2** with those of **1** indicated that the additional acetyl group was located at O–C(24), which was supported by the correlation between H–C(24) (δ(H) 5.47 (*dd*, *J* = 10.0, 1.7 Hz)) and C=O (δ(C) 170.6) of the Ac group in the HMBC plot.

Compound **3** had a molecular formula $C_{50}H_{84}O_{21}$ according to the quasimolecular ion at *m*/*z* 1019.5389 ([*M* – H]$^-$) in the HR-ESI-MS. D-Glucose was identified by GC analysis after acid hydrolysis (see *Exper. Part*). Comparison of the NMR data of **2** with those of **3** indicated that the latter contained an additional glucopyranosyl residue. Further analysis of ^{13}C-NMR (*Table 1*), HSQC, and HMBC data of **3** revealed that the additional glucopyranosyl residue was attached to O–C(6″) of the glucose moiety at C(25). These findings led to the assignment of **3** as (3*β*,16*β*,24*S*)-24-(acetyloxy)-3-(*β*-D-glucopyranosyloxy)cycloartane-16,25,30-triol 25-[*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside][1]).

The ^{1}H-NMR spectrum of **3** showed two cyclopropane signals at δ(H) 0.19 (*d*, *J* = 3.7 Hz) and 0.40 (*d*, *J* = 3.7 Hz), six tertiary Me groups at δ(H) 0.76, 1.29, 1.40, 1.43, 1.49, and 2.04, a secondary Me group at δ(H) 0.98 (*d*, *J* = 6.6 Hz), as well as three anomeric H-atoms at δ(H) 5.11 (*d*, *J* = 7.7 Hz), 5.03 (*d*, *J* = 7.7 Hz), and 4.94 (*d*, *J* = 7.7 Hz). The ^{13}C-NMR data of **3** were similar to those of **2**, except for the appearance of an additional glucose unit. The linkage positions and sequence of the sugar moieties could be assigned by the HMBC experiment. Hence, in the HMBC spectrum, the correlations δ(H) 4.94 (H–C(1′))/δ(C) 89.4 (C(3)), δ(H) 5.03 (H–C(1″))/δ(C) 78.8 (C(25)), as well as δ(H) 5.11 (H–C(1‴))/δ(C) 70.3 (C(6″)) were observed.

Compound **4** was isolated as an amorphous powder. The HR-ESI-MS displayed an [*M* – H]$^-$ ion at *m*/*z* 1019.5398, consistent with the molecular formula $C_{50}H_{84}O_{21}$. Acid hydrolysis of **4** also afforded D-glucose, which was identified by gas-chromatographic analysis (see *Exper. Part*). Analysis of the ^{1}H- and ^{13}C-NMR data (*Table 1*) of **4** and a comparison with those of **3** indicated that compound **4** was very similar to **3**, except for the interglycosidic linkage of the sugar chain at C(25). Based on the 1D- and 2D-NMR experiments, the structure of **4** was identified as (3*β*,16*β*,24*S*)-24-(acetyloxy)-3-(*β*-D-glucopyranosyloxy)cycloartane-16,25,30-triol 25-[*β*-D-glucopyranosyl-(1 → 4)-*β*-D-glucopyranoside][1]).

The ^{1}H-NMR spectrum of **4** showed two cyclopropane H-atom signals at δ(H) 0.22 (*d*, *J* = 3.7 Hz) and 0.47 (*d*, *J* = 3.7 Hz), six tertiary Me groups at δ(H) 0.76, 1.10, 1.48, 1.51, 1.52, and 2.09, a secondary Me group at δ(H) 0.88 (*d*, *J* = 6.3 Hz), as well as three anomeric H-atoms at δ(H) 5.07 (*d*, *J* = 7.6 Hz), 5.04 (*d*, *J* = 7.7 Hz), and 4.63 (*d*, *J* = 7.8 Hz). The ^{13}C-NMR data of **4** indicated the presence of the same aglycone as in **2** and **3**. The linkage positions and sequence of the sugar moieties could be deduced by an

HMBC experiment. Thus, in the HMBC spectrum, the correlations H–C(1′)/C(3), H–C(1″)/C(25), as well as H–C(1‴)/C(4″) were observed.

Cycloartane glycosides are commonly found in *Thalictrum* species, such as *T. minus*, *T. foetidum*, *T. squarrosum*, and *T. thunbergii*, *etc.* [12]. Our present report has made the cycloartane compounds more diverse in the genus *Thalictrum*.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical Plant*, Qingdao, P. R. China), *Sephadex LH-20* (*Pharmacia*), and *RP-C18* (10–40 μm; *Merck*). Prep. HPLC: *Agilent-1100* apparatus; *Zorbax-XDB-18* column (10 mm i.d. × 15 cm; 5 μm); *Varian-ProStar-355* RI detector; flow rate = 3 ml/min. TLC: precoated silica gel GF_{254} plates (*Qingdao Marine Chemical Plant*, Qingdao, P. R. China), precoated *RP-18* F_{254}-*S* plates (*Merck*). GC: *HP-1-TCD* instrument (*Hewlett-Packard*); *HP-Chiral* column (30 m × 0.25 mm × 1.0 μm; 20% permethylated β-cyclodextrin). M.p.: *XT-4* micro-melting-point apparatus; uncorrected. Optical rotation: *Perkin-Elmer 241* polarimeter. IR Spectra (KBr): *Nicolet-Impact-410* FT-IR spectrometer; in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR Spectra: *Bruker-AV-500* spectrometer; δ in ppm rel. to Me_4Si, *J* in Hz. HR-ESI-MS: *Applied-Biosystems-Mariner-5140* spectrometer; in *m*/*z*.

Plant Material. The aerial parts of *T. fortunei* were collected in Wuhu city, Anhui province, P. R. China, in April 2004, and authenticated by Prof. *Min-Jian Qin* (China Pharmaceutical University). A voucher specimen (No. 040192) was deposited with the herbarium of the China Pharmaceutical University, Nanjing, P. R. China.

Extraction and Isolation. The dried aerial parts of *T. fortunei* (4.8 kg) were extracted with 95% EtOH (3 × 20 l; each 2 h) under reflux. The concentrated EtOH extract was suspended in H_2O (3 l) and then successively extracted with petroleum ether (60–90°; 3 × 3 l), AcOEt (3 × 3 l), and BuOH (3 × 3 l). The BuOH soln. was concentrated to give a residue (207 g), which was separated by CC (SiO_2, $CHCl_3$ → $CHCl_3$/MeOH 1:1): *Fractions A – F. Fr. C* was purified by CC (*Sephadex LH-20*, MeOH): *Fr. C.1 – C.3. Fr. C.2* was subjected to reversed-phase CC (ODS, MeOH/H_2O 60:40 → 70:30), followed by HPLC (MeCN/H_2O 30:70): **1** (51 mg; t_R 37 min), **2** (153 mg; t_R 41 min), **3** (82 mg; t_R 26 min), and **4** (32 mg; t_R 28 min), resp.

*(3β,4β,16β,24*S*)-25-(β-*D*-Glucopyranosyloxy)-16,24,28-trihydroxy-9,19-cyclolanostan-3-yl β-*D*-Glucopyranoside* (**1**): Amorphous powder. M.p. 259–261° (MeOH). $[\alpha]_D^{25} = +21.0$ ($c = 0.18$, MeOH). IR (KBr): 3421, 2935, 2871, 1633, 1076, 1037. ^1H-NMR (C_5D_5N; aglycone): 0.22 (*d*, *J* = 3.7, 1 H–C(19)); 0.47 (*d*, *J* = 3.7, 1 H–C(19)); 0.86, 1.32, 1.38, 1.44, 1.53 (5 *s*, Me(30), Me(18), Me(26), Me(27), Me(29)); 1.02 (*d*, *J* = 6.5, Me(21)); 3.72 (*dd*, *J* = 11.9, 4.3, H–C(3)); 3.75 (*d*, *J* = 10.9, 1 H–C(28)); 4.46 (*d*, *J* = 10.9, 1 H–C(28)); 4.62–4.64 (*m*, H–C(16)); 4.02 (*dd*, *J* = 8.8, 2.2, H–C(24)); sugar resonances in *Table 2*. ^{13}C-NMR (C_5D_5N): *Table 1*. HR-ESI-MS (neg.): 815.4796 ($[M-H]^-$, $C_{42}H_{71}O_{15}^-$; calc. 815.4793).

*(3β,4β,16β,24*S*)-24-(Acetyloxy)-25-(β-*D*-glucopyranosyloxy)-16,28-dihydroxy-9,19-cyclolanostan-3-yl β-*D*-Glucopyranoside* (**2**): Amorphous powder. M.p. 268–270° (MeOH). $[\alpha]_D^{25} = +17.1$ ($c = 0.09$, MeOH). IR (KBr): 3421, 2937, 2871, 1724, 1629, 1076, 1037. ^1H-NMR (C_5D_5N; aglycone): 0.22 (*d*, *J* = 3.7, 1 H–C(19)); 0.47 (*d*, *J* = 3.7, 1 H–C(19)); 0.87, 1.34, 1.44, 1.47, 1.58 (5 *s*, Me(30), Me(18), Me(27), Me(26), Me(29)); 1.02 (*d*, *J* = 6.6, Me(21)); 2.09 (*s*, Ac); 3.77 (*dd*, *J* = 11.9, 4.3, H–C(3)); 3.82 (*d*, *J* = 10.5, 1 H–C(28)); 4.50 (*d*, *J* = 10.5, 1 H–C(28)); 4.59–4.61 (*m*, H–C(16)); 5.47 (*dd*, *J* = 10.0, 1.7, H–C(24)); sugar resonances in *Table 2*. ^{13}C-NMR (C_5D_5N): *Table 1*. HR-ESI-MS (neg.): 857.4850 ($[M-H]^-$, $C_{44}H_{73}O_{16}^-$; calc. 857.4899).

*(3β,4β,16β,24*S*)-24-(Acetyloxy)-3-(β-*D*-glucopyranosyloxy)-16,28-dihydroxy-9,19-cyclolanostan-25-yl 6-*O*-β-*D*-Glucopyranosyl-β-*D*-glucopyranoside* (**3**): Amorphous powder. M.p. 242–244° (MeOH).

$[\alpha]_D^{25} = +8.29$ ($c = 0.07$, MeOH). IR (KBr): 3421, 2935, 3873, 1720, 1635, 1074, 1040. ^{1}H-NMR (C_5D_5N; aglycone): 0.19 (*d*, *J* = 3.7, 1 H–C(19)); 0.40 (*d*, *J* = 3.7, 1 H–C(19)); 0.76, 1.29, 1.40, 1.43, 1.49 (5*s*, Me(30), Me(18), Me(27), Me(26), Me(29)); 0.98 (*d*, *J* = 6.6, Me(21)); 2.04 (*s*, Ac); 3.76 (*dd*, *J* = 11.9, 4.3, H–C(3)); 3.76 (*d*, *J* = 10.6, 1 H–C(28)); 4.49 (*d*, *J* = 10.6, 1 H–C(28)); 4.55–4.57 (*m*, H–C(16)); 5.40 (*dd*, *J* = 9.9, 1.8, H–C(24)); sugar resonances in *Table 2*. ^{13}C-NMR (C_5D_5N): *Table 1*. HR-ESI-MS (neg.): 1019.5389 ($[M-H]^-$, $C_{50}H_{83}O_{21}^-$; calc. 1019.5427).

*(3β,4β,16β,24*S*)-24-(Acetyloxy)-3-(β-*D*-glucopyranosyloxy)-16,28-dihydroxy-9,19-cyclolanostan-25-yl 4-O-β-*D*-Glucopyranosyl-β-*D*-glucopyranoside* (**4**): Amorphous powder. M.p. 249–251° (MeOH). $[\alpha]_D^{25} = +24.4$ ($c = 0.37$, MeOH). IR (KBr): 3417, 2937, 2873, 1721, 1635, 1074, 1035. ^{1}H-NMR (C_5D_5N; aglycone): 0.22 (*d*, *J* = 3.7, 1 H–C(19)); 0.47 (*d*, *J* = 3.7, 1 H–C(19)); 0.76, 1.10, 1.48, 1.51, 1.52 (5*s*, Me(30), Me(18), Me(27), Me(26), Me(29)); 0.88 (*d*, *J* = 6.3, Me(21)); 2.09 (*s*, Ac); 3.70 (*dd*, *J* = 11.9, 4.3, H–C(3)); 3.77 (*d*, *J* = 10.6, 1 H–C(28)); 4.43 (*d*, *J* = 10.6, 1 H–C(28)); 4.55–4.57 (*m*, H–C(16)); 5.47 (*dd*, *J* = 10.0, 1.7, H–C(24)); sugar resonances in *Table 2*. ^{13}C-NMR (C_5D_5N): *Table 1*. HR-ESI-MS (neg.): 1019.5398 ($[M-H]^-$, $C_{50}H_{83}O_{21}^-$; calc. 1019.5427).

Alkaline Hydrolysis of **2**. Compound **2** (30 mg) and 5N NH_4OH in 50% EtOH (10 ml) was refluxed at 80° for 24 h. The mixture was neutralized with 2N HCl and extracted with BuOH. The BuOH soln. was concentrated to give a residue, which was subjected to CC (*ODS*, 70% MeOH/H_2O) to yield a deacetylation product. The product was identical to compound **1** by NMR and direct comparison with an authentic sample.

Acid Hydrolysis and Identification of Sugars in **1**–**4**. The soln. of each of compounds **1**–**4** (10 mg) in 30 ml of 1M HCl (MeOH/H_2O 1:1) was heated under reflux for 3 h. After removal of the solvent, the residue was partitioned between $CHCl_3$ and H_2O. The aq. layer was neutralized with *Dowex* (HCO_3^-) and then filtered. The filtrate was concentrated to 2 ml and then treated with $NaBH_4$ (20 mg) at r.t. for 3 h. Excessive $NaBH_4$ was removed with 30% AcOH. After evaporation at 60° and washing with 0.1% HCl/MeOH, the mixture was heated for drying at 105° for 15 min, followed by the addition of anh. pyridine (0.5 ml) and Ac_2O (0.5 ml). The mixture was incubated in a water bath at 100° for 1 h and then partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated for GC analysis (front inlet 250°, column temp. 80° → 230°, 5°/min). All monosaccharides of each of compounds **1**–**4** were identified as D-glucose (t_R (D-glucose from **3**) 33.052 min, t_R (reference D-glucose) 33.073 min, t_R (reference L-glucose) 34.463 min).

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