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CHOLESTANE- AND PREGNANE-TYPE GLYCOSIDES FROM THE ROOTS OF *TRIBULUS CISTOIDES**†

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Key Word Index—*Tribulus cistoides*; Zygophyllaceae; roots; cardioactive saponin-3; cholestane-type glycosides; pregnane-type glycoside; D-(+)-pinitol; sucrose.

Abstract—From the methanolic extract of the roots of *Tribulus cistoides* the cardioactive saponin-3, which is known to occur in the leaves, was isolated along with tribulosin, a pregnane-type glycoside and eight new cholestane glycosides. D-(+)-Pinitol and sucrose were major constituents. The structures were established by spectroscopic studies of the isolated compounds, their acetylated derivatives and their hydrolysation products. Chemical conversions revealed the configurations at C-22 and C-25, respectively.

INTRODUCTION

Tribulus cistoides L. is widely distributed in Middle America and the Southern U.S.A. [1]. The plant is used in folk medicine against various diseases [2, 3]. We previously reported on the isolation of various new spirostanol- and furostanol-type glycosides from the aerial parts of *T. cistoides*, among them tribulosin (**1**) and the cardioactive compounds cistocardin (**2**) and saponin-3 (**3**) [1].

This work describes the phytochemical analysis of the roots.

RESULTS AND DISCUSSION

The methanolic root extract was diluted with water and then extracted with chloroform. Chromatographic separation of this chloroform fraction (= M/$CHCl_3$) by column chromatography and then HPLC yielded tribulosin (**1**) [4]and the cardioactive spirostanol glycoside saponin-3 (**3**) [1], which is known to occur in the aerial parts of *T. cistoides*, and also the new cholestane-type glycosides **4**–**9** (Table 1).

From the aqueous fraction, in addition,we isolated the bisdesmodic cholestenol glycosides **10** and **11** and the new pregnane-type glycoside **12** along with D-(+)-pinitol (**13**) [5] and sucrose; the last two compounds represent the major constituents of the methanolic root extract and of the extractable aerial parts of the plant.

The structures were established mainly by spectroscopic studies of the isolated compounds, their acetyl derivatives and their hydrolysis products.

Analysis of the ^{13}C NMR spectra (Table 2) of the isolated genuine glycosides **4**–**12** revealed the number of participating hexose and desoxyhexose moieties; the configurations of the sugars were established by ^1H NMR studies of the peracetyl derivatives, which also provided information on the sequence of the sugar units in **4**–**9**.

The mass spectra run by direct chemical ionization with NH_3 not only revealed the size of the molecules and their aglycones, but the occurrence of characteristic fragment ions also indicated whether an isolated glycoside had a monodesmodic or bisdesmodic structure.

The positions of the individual sugars in the bisdesmodic compounds **10** and **11** were established by ^1H NMR studies of the acetylated degradation products obtained by partial hydrolysis. The structure of the trisaccharidic sugar moiety in **12** resulted from long-range heteronuclear NMR (by HMBC [6]) and from NOE studies of the genuine glycoside.

The structures of the aglycones were also mainly determined by ^1H and ^{13}C NMR studies. Provided that the amounts of isolated glycosides allowed, the corresponding aglycones **14**–**17** or the spontaneous cyclization product **18** (from **6**, see below) were prepared by acid hydrolysis and then used for the structure work. Comparison of the NMR spectra with the literature data corroborated partial structures of the aglycones for either C-1 to C-13 with a correspondingly substituted ring system A–B–C or of ring D including the atoms of the side chain at C-17 [7, 8, 9]. The positions of the hydroxy groups at C-16 and C-22 in **14** (obtained by hydrolysis of

*Dedicated to Prof. Dr B. Unterhalt, Institut für Pharmazeutische Chemie, Universität Münster, FRG, on the occasion of his 60th birthday.

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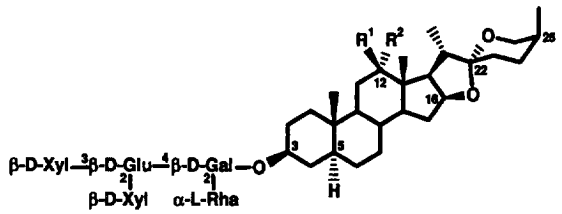
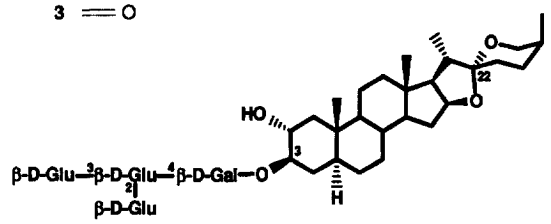
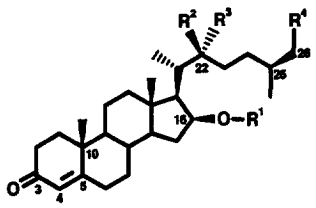


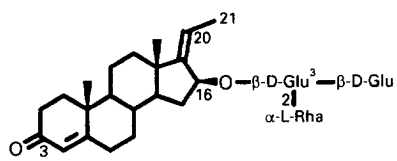
	R^1	R^2
1	H	H
3	=O	



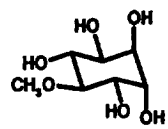
Cistocardin (**2**)



	R^1	R^2	R^3	R^4
4	α-L-Rha-(1 → 2)-β-D-Gal	OH	H	H
5	β-D-Glu-(1 → 3)-β-D-Gal	OH	H	H
6	β-D-Glu-(1 → 3)-β-D-Gal		=O	OH
7	β-D-Glu-(1 → 3)-β-D-Gal	OH	H	OH
8	α-L-Rha-(1 → 2)-β-D-Gal	OH	H	OH
9 = 4,5α-dihydro-**8**				
14	H	OH	H	H
15	H	OH	H	OH



Glycoside-9 (**12**)



D-(+)-Pinitol (**13**)

4) were corroborated by oxidation and subsequent NMR studies on the produced cholest-4-ene-3,16,22-trione [10]. The configuration at C-16 was revealed from the ^{1}H-coupling constants observed for H-16 in the ^{1}H NMR. To establish the configuration of **14** at C-22, the cyclic carbonic diester **19** was prepared by reaction of **14** with phosgene and the stereochemistry of the 1,3-dioxepan-2-one ring system generated was studied by ^{1}H NMR (Fig. 1).

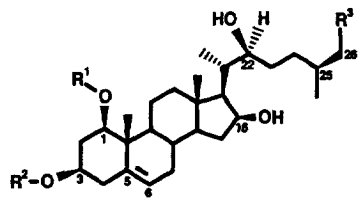
The spectroscopic data of **15** (obtained by hydrolysis of **7** and **8**) revealed complete structural agreement with **14** but with an additional hydroxy group at C-26 (Table 2). The configuration at the chiral C-25 was determined by the following reaction sequence, which converted the aglycone of **8** into (25*S*)-neospirost-4-en-3-one (**18**) [11]: glycoside-5 (**8**) was selectively silylated at the 26-hydroxy group using *t*-butyl-dimethylsilyl chloride [12, 13] and subsequently oxidized with pyridinium chlorochromate [14]. Final hydrolysis removed the silyl group and the sugar and resulted in the formation of the spiroketal **18**. These results also corroborated the structure of the glycoside-3 (**6**).

Table 1. Compounds isolated from the root extract of *Tribulus cistoides*

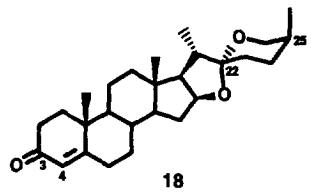
Compound class	Compound	Fraction*	Content [%]†	Refs
Steroid glycosides				
(a) Spirostanol-type	Tribulosin (**1**)	$M/CHCl_3$	0.15	[1, 4]
	Saponin-3 (**3**)	$M/CHCl_3$	0.08	[1]
(b) Cholestane-type	Glycoside-1 (**4**)	$M/CHCl_3$	0.2	
	Glycoside-2 (**5**)	$M/CHCl_3$	0.05	
	Glycoside-3 (**6**)	$M/CHCl_3$	0.15	
	Glycoside-4 (**7**)	$M/CHCl_3$	0.25	
	Glycoside-5 (**8**)	$M/CHCl_3$	0.4	
	Glycoside-6 (**9**)	$M/CHCl_3$	0.05	
	Glycoside-7 (**10**)	M/H_2O	0.05	
	Glycoside-8 (**11**)	M/H_2O	0.05	
(c) Pregnane-type	Glycoside-9 (**12**)	M/H_2O	0.05	
Cyclitols	D-(+)-Pinitol (**13**)	M/H_2O	8	[5]
Sugars	Sucrose	M/H_2O	30	

*$M/CHCl_3$: mainly from chloroform-soluble fraction of the aqueous MeOH extract; M/H_2O: residue after extraction with $CHCl_3$.

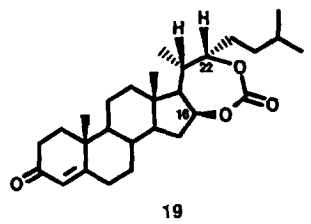
†Dry wt of original MeOH-extract = 100%.



	R^1	R^2	R^3
10	α-L-Rha	β-D-Gal	H
11	α-L-Rha	β-D-Gal	OH
16	H	H	H
17	H	H	OH
20a	H	β-D-Gal	H
20b	α-L-Rha	H	H
21	α-L-Rha	H	OH



18



19

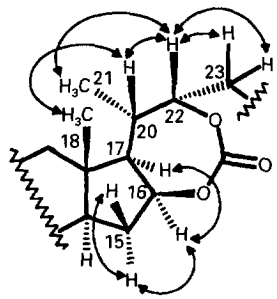


Fig. 1. Major NOEs observed in **19**.

The structure of **9** was easily established from its NMR data. Careful hydrolysis of **10** (or **11**) followed by chromatographic separation yielded the aglycone **16** (or **17**) along with the monodesmodic glycosides **20** (or **21**), which contained only one sugar moiety. Again, NMR studies established the same 1β, 3β, 16β-trihydroxycholest-5-ene ring system for **10** and **11** with a monohydroxylated side-chain at C-17 in **10**, like that found in **4** and **5**, whereas **11** had a dihydroxylated side-chain like that found in **7** or **8**.

Further column chromatography of the partially hydrolysed **10** yielded **20a** and **20b**. Compound **20a** contained only the galactose moiety and, correspondingly, on acetylation gave a heptaacetyl derivative, whose ^1H NMR spectrum, when compared with that of acetylated **10**, exhibited a strong downfield shift for H-1 ($\Delta\delta_{H-1} = 1.4$ ppm) but an almost unchanged signal for H-3 indicating that the galactose was linked to C-3.

Surprisingly, the partial hydrolysis product of **11** was not a mixture: investigation of the product after acetylation revealed a heptaacetyl derivative with rhamnose at C-1 and the H-3 significantly shifted by the ester formation.

Table 2. ^{13}C NMR data of the isolated glycosides **4**–**12** and the aglycones **14**–**18** (δ [ppm] in CD_3OD)

C	4	5	6	7	8	9	10	11	12	14	15	16	17	18
1	36.6	36.7	36.7[a]	36.7	36.7	39.7	83.1	83.2	36.7	36.7	36.7	79.1	79.1	36.8
2	34.0[a]	34.0[a]	33.9[b]	34.0[a]	34.0[a]	38.9	35.6	35.6	33.9[a]	34.0[a]	33.9[a]	42.4[a]	42.3[a]	33.9[a]
3	202.4	202.4	202.3	202.4	202.4	214.9	82.5	82.5	202.4	202.3	202.3	69.1	69.0	202.2
4	124.1	124.1	124.1	124.1	124.1	45.5	43.2	43.2	124.1	124.1	124.1	44.0[a]	44.0[a]	124.2
5	175.5	175.4	175.2	175.4	175.5	48.2	139.2	139.2	175.2	175.1	175.2	139.9	140.1	174.9
6	34.7[a]	34.7[a]	34.7[b]	34.7[a]	34.7[a]	30.1	126.4	126.4	34.7[a]	34.7[a]	34.7[a]	125.8	125.8	34.7[a]
7	33.3	33.3	33.3	33.3	33.3	32.9	32.2	32.2	33.1	33.3	33.3	32.6[b]	32.3[b]	33.5
8	36.7[b]	36.6	36.4	36.4[b]	36.6	36.5	34.5	34.5	36.4	36.4[b]	36.6[b]	32.8[b]	32.8[b]	36.5
9	55.5[c]	55.4[b]	55.3	55.4	55.5[b]	56.0	51.9	51.9	55.7	55.1[c]	55.1[c]	52.2	52.1	55.3
10	40.0	40.0	40.0	40.0	40.0	36.8	43.0	43.0	40.1	40.0	40.0	43.0	43.0	40.1
11	21.9	21.9	21.9	21.9	21.9	22.3	25.6	25.7	21.9	21.9	21.9	24.7	24.7	22.0
12	40.6	41.0	41.0	41.0	40.7	40.9	41.6	41.6	37.3	41.3	41.3	41.9	41.8	40.8
13	43.5	43.3	42.9	43.3	43.5	43.7	43.4	43.4	44.0	43.5	43.5	43.3	43.2	41.6
14	55.6[c]	55.5[b]	54.8	55.4	55.6[b]	55.3	56.5	56.5	52.2	55.3[c]	55.4[c]	56.0	55.9	56.9
15	33.7	37.1[c]	36.6[a]	36.9	33.5	33.5	37.7	37.6	36.4	37.2	37.2	37.6	37.6	32.6
16	81.0	83.0	82.1	83.2	81.1	81.2	72.6[a]	72.6[a]	80.4	72.5	72.6	72.6	72.5	82.2
17	59.2	58.7	58.0	58.8	59.2	59.3	58.8	58.9	152.8	58.9	58.6	58.8	58.7	63.6
18	14.2	13.6	13.9	13.5	14.2	14.3	14.7	14.7	19.9	14.4	14.4	14.4	14.3	16.8
19	17.7	17.7	17.7[c]	17.6[c]	17.7	11.7	12.1	12.1	17.7	17.7	17.7	13.6[c]	13.6[c]	17.7
20	36.9[b]	36.1	44.7	36.6[b]	36.9	36.9[a]	36.3	36.4	119.1	36.6[b]	36.5[b]	36.5	36.6	43.5
21	12.1	12.1	17.2[c]	12.1	12.1	12.1	13.9	13.9	14.7	13.6	13.6	13.7[c]	13.7[c]	14.7
22	74.8	74.3	217.6	74.3	74.4	74.5	76.3	76.4	—	76.0	75.9	76.1	75.8	111.1
23	37.9[d]	37.1[c]	39.4	31.3[d]	38.3	38.4	34.1	33.5	—	32.6	32.1	34.0	34.0	26.8[b]
24	38.1[d]	37.2[c]	27.9	33.6[d]	32.0	32.0	37.1	31.4	—	37.2	31.5	37.2	31.4	27.0[b]
25	29.2	29.7	36.4	37.2	36.9	37.0[a]	29.7	37.2	—	29.3	36.9	29.4	36.9	28.5
26	23.2	23.2	67.8	68.2	68.6	68.6	23.2	68.2	—	23.0	68.5	23.0	68.5	66.2
27	23.7	23.2	17.6[c]	17.7[c]	17.4	17.4	23.2	17.5	..	23.1	17.1	23.1	17.3	16.4
1′	104.8	105.7	105.6[d]	105.8	104.7	104.7	98.0	98.0	103.6					
2′	75.4	71.9	71.9	71.9	75.6	75.7	72.9[a]	72.9[a]	77.4					
3′	76.6	85.4	85.3	85.4	76.5	76.5	72.9[a]	72.9[a]	89.1					
4′	71.3	69.5	69.4	69.5	71.3	71.3	73.6	73.6	70.3					
5′	76.8	76.0	76.1	76.1	76.8	76.8	70.1	70.2	77.4					
6′	62.2	62.3	62.3	62.3	62.2	62.2	18.1	18.1	62.6[b]					
1″	101.7	106.7	105.7[d]	106.7	101.7	101.7	107.3	107.3	102.2					
2″	71.7[e]	75.4	75.4	75.4	71.9[c]	71.9[b]	70.8	70.9	72.0[c]					
3″	72.2[e]	77.6[d]	77.7[e]	77.7[e]	72.2[c]	72.2[b]	74.3	74.4	72.2[c]					
4″	74.1	71.2	71.2	71.2	73.9	74.0	69.1	69.1	74.0					
5″	69.8	77.9[d]	77.9[e]	77.9[e]	69.8	69.8	75.3	75.3	69.6					
6″	18.3	62.3	62.3	62.3	18.3	18.3	62.6	62.3	18.7					
1‴									104.5					
2‴									75.2					
3‴									78.2[d]					
4‴									71.5					
5‴									78.3[d]					
6‴									62.8[b]					

[a–e]Assignments may be interchanged.

NMR studies established the pregna-4,17(20)-dien-3-one structure for the aglycone of **12** [15] with the stereochemistry β at C-16 and *Z* for the 17(20)-double bond corroborated by NOE measurements.

The absolute configurations of the cholest-4-en-3-one moieties are based on their Cotton effects: comparison of the CD curves with that of diosgenone [16] (prepared by PCC-oxidation of diosgenin [17]) exhibited excellent agreement and, thereby, indicated identical (10*R*)-configurations for **4**–**8**, **12**, **14**, **15** and **18**. Similarly, **9** showed the CD in good agreement with that of neotigogenone [prepared by oxidation of neotigogenin, the aglycone of tribulosin (**1**)].

The absolute configurations of the sugars were determined in the course of our studies of the constituents from the aerial parts of *T. cistoides* [1]. We assume the same configurations for the sugar moieties of the constituents found in the roots.

As far as the isolated amounts of compounds permitted, the glycosides were tested for cardioactivity using papillary muscle [18]: only saponin-3, which is also a component of the leaf extract of *T. cistoides*, was shown to be active. In the plate diffusion test against *Bacillus subtilis* all isolated compounds were inactive, whereas the bisdesmodic cholestane-type glycoside **10** exhibited significant antifungal activity against *Saprolegnia asterophora*.

In contrast to the leaf extract, which contained only spirostanol-type glycosides [1] in addition to pinitol and sucrose, glycosides derived from cholestane prevail in the root extract; a pregnane-type glycoside has also been isolated.

Except for **1** and **3**, the glycosides isolated from *T. cistoides* represent hitherto unknown natural products. However, the aglycones **14** and **16**, connected to other sugars, were recently described from *Allium schubertii* [19] and **16** has been isolated from other *Allium* species [20]. Pregnane-type glycosides with a $\Delta^{17(20)}$ double bond have not previously been reported as plant constituents and an aglycone of this type has been reported only once from the Indian *Commiphora mukul* (Burseraceae) [15]. Therefore, compound **12** represents the first pregn-17(20)-ene-type glycoside isolated from plants.

EXPERIMENTAL

Plant material. See [1].

General. TLC was performed on ready-made plates (nanoplates® SIL-20/UV_{254}, Macherey-Nagel) using S-1 = $CHCl_3$–MeOH–H_2O (75:25:2); S-2 = petrol–EtOH (97:3); S-3 = $CHCl_3$–MeOH (97:3). Detection: UV and anisaldehyde reagent [21]. Unless stated otherwise, IR were run in KBr; $[\alpha]_D$, UV and CD measurements in MeOH. ^1H NMR at 400 MHz and ^{13}C NMR at 100 MHz in CD_3OD with TMS as int. standard. MS were recorded by EIMS (70 eV) using a direct inlet system or by DCIMS or CIMS with NH_3 as reactand gas. FABMS (positive ions) in glycerol (xenon, 8 kV). Si gel 60 (Macherey-Nagel) and Sephadex® LH 20 (Pharmacia) were used for CC, Si gel 60 for MPLC (at *ca* 3 bar); HPLC was done on Nucleosil ® RP-18 (Macherey-Nagel) or LiChrosorb® RP-18 (Merck) with the solvent systems S-4 = MeOH–H_2O (7:3), S-5 = MeOH–H_2O (65:35) or S-6 = MeOH–H_2O (6:4).

Acetylation. See [1].

Oxidation with PCC (= *pyridinium chlorochromate*). See [14].

Hydrolysis. The glycoside (5–35 mg) was dissolved in a mixture of 5 ml MeOH and 4 ml 2 N H_2SO_4, and refluxed at 80° for 5 hr. Subsequent neutralization with 2 N NaOH, evapn, solution in H_2O and extraction with $CHCl_3$ yielded the crude product, which was purified by CC on silica gel with $CHCl_3$–MeOH mixtures to give the pure aglycone.

Extraction and chromatography. Roots (450 g) extracted with MeOH at room temp. to obtain the MeOH extract (72 g), from which 42 g were redissolved in 350 ml MeOH and diluted with 700 ml H_2O. Repeated extraction with 2000 ml $CHCl_3$ and subsequent evapn yielded the $CHCl_3$ extract (= M/$CHCl_3$; 4.5 g) and the H_2O-soluble residue (= M/H_2O; 37.5 g). CC of M/$CHCl_3$ (2 g) on silica gel using $CHCl_3$–MeOH–H_2O (75:25:2) gave 9 frs of crude substances. Frs Nos 3–9 were first subjected to CC on LH 20 (with MeOH) and then HPLC on RP-18 with MeOH–H_2O mixtures to yield compounds **1** and **3**–**9**. The M/H_2O extract was sepd by MPLC (2 × 18 g) on silica gel with $CHCl_3$–MeOH–H_2O (75:25:2) into 7 frs. Frs Nos 1–6 by HPLC on RP-18 with MeOH–H_2O mixtures yielded compounds **1** and **3**–**13** along with sucrose.

Tribulosin (**1**) [1, 4]. Amorphous (12 mg). TLC: R_f 0.10 (S-1); anisaldehyde: yellow-green. HPLC: R_t 50 min (S-4). All physicochemical properties identical with literature data [1, 4].

Saponin-3 (**3**) [1]. Amorphous (7.5 mg). TLC: R_f 0.10 (S-1); anisaldehyde: yellow-green. HPLC: R_t 11 min (S-4). Substance identical with an authentic sample [1].

Glycoside-1 (= 16(S)-[α-L-*rhamnopyranosyl*-(1 → 2)-β-D-*galactopyranosyloxy*]-22(S)-*hydroxycholest*-4-*en*-3-*one*, **4**). Crystals from $CHCl_3$:MeOH (2:1) (48 mg), mp 182–185°. TLC: R_f 0.39 (*S*-1); anisaldehyde, brown-green. HPLC: R_t 11 min (*S*-4). $[\alpha]_D^{20}$ − 2° (MeOH; *c* 0.9). IR ν_{max} cm^{-1}: 3401, 2946, 1657. UV λ_{max} nm (log ε): 241 (4.22). CD λ_{max} nm (Δε): 250 (+ 0.55), 313 (− 1.16). ^1H NMR: δ0.80–1.03 (13H, *m*), 1.18–1.30 (7H, *m*) including 1.21 (3H, *d*, *J* = 6 Hz, Me-6″) and 1.23 (3H, *s*, Me-19), 1.40–1.74 (11H, *m*), 1.86–1.93 (1H, *m*), 1.97–2.14 (4H, *m*), 2.19–2.34 (4H, *m*), 2.42–2.54 (2H, *m*), 3.37 (1H, *dd*, $J_1 = J_2 = 10$ Hz, H-4″), 3.44 (1H, *ddd*, $J_1 \sim J_2 \sim 6$, $J_3 = 1$ Hz, H-5′), 3.53–3.60 (2H, *m*), 3.70–3.79 (5H, *m*), 3.91 (1H, *dd*, $J_1 = 3.5$, $J_2 = 1.5$ Hz, H-2″), 3.98 (1H, *dq*, $J_1 = 10$, $J_2 = 6$ Hz, H-5″), 4.21 (1H, *d*, *J* = 7.5 Hz, H-1′) overlapped by 4.21–4.27 (1H, *m*), 5.41 (1H, *d*, *J* = 1.5 Hz, H-1″), 5.70 (1H, *s*, H-4). ^{13}C NMR: Table 2. DCIMS *m/z* (rel. int.): 725 $[M + H]^+$ (25), 580 (10), 579 $[M - 146 + H]^+$ (24), 434 (10), 418 (32), 417 $[M - 308 + H]^+$ (100), 416 $[M - 308]^+$ (16), 400 (21), 399 (88), 371 (18), 301 (31), 299 (39).

Heptaacetylglycoside-1. Compound **4** (5 mg) after acetylation and purification (HPLC, MeOH–H_2O,

88:12) gave 4 mg heptaacetylglycoside-1 (oil). TLC: R_f 0.31 (S-2); anisaldehyde, brown-green. $[\alpha]_D^{20}$ + 5° ($CHCl_3$; *c* 0.4). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2954, 1747, 1663. ^1H NMR (C_6D_6): δ0.47–0.78 (6H, *m*) including 0.74 (3H, *s*, Me-18), 0.95–2.09 (52H, *m*) including 0.96 (3H, *s*, Me-19), 1.13 and 1.15 (3H each, *d*, J = 7 Hz, Me-26 and Me-27), 1.35 (3H, *d*, J = 7 Hz, Me-21), 1.42 (3H, *d*, J = 6 Hz, Me-6″), 1.59, 1.60, 1.69, 1.70, 1.79, 1.87 and 2.05 (3H each, *s*, Ac), 2.11–2.35 (4H, *m*), 2.55–2.64 (1H, *m*), 3.32 (1H, *ddd*, $J_1 \sim J_2 \sim 7$, J_3 = 1.5 Hz, H-5′), 4.14 (2H, *d*, J = 7 Hz, H-6′$_{A/B}$), 4.42 (1H, *dd*, J_1 = 10, J_2 = 8 Hz, H-2′), 4.61 (1H, *dq*, J_1 = 10, J_2 = 6 Hz, H-5″), 4.72 (1H, *ddd*, $J_1 \sim J_2 \sim 8$, J_3 = 4 Hz, H-16), 4.81 (1H, *d*, J = 8 Hz, H-1′), 5.32 (1H, *dd*, J_1 = 10, J_2 = 3 Hz, H-3′), 5.49 (1H, *dd*, J_1 = 3, J_2 = 1.5 Hz, H-4′), 5.52–5.56 (1H, *m*, H-22) overlapped by 5.55 (1H, *dd*, J_1 = 3.5, J_2 = 1.5 Hz, H-2″), 5.59 (1H, *dd*, $J_1 \sim J_2 \sim$ 10 Hz, H-4″), 5.64 (1H, *d*, J = 1.5 Hz, H-1″), 5.73 (1H, *dd*, J_1 = 10, J_2 = 3.5 Hz, H-3″), 5.85 (1H, *s*, H-4).

16(S),22(S)-*Dihydroxycholest*-4-*en*-3-*one* (**14**) *by hydrolysis of glycoside*-1 (**4**). Hydrolysis of **4** (19 mg; 5 hr) and subsequent purification by CC using $CHCl_3$–MeOH (99:1) yielded the aglycone **14** (7 mg).

16(S),22(S)-*Dihydroxycholest*-4-*en*-3-*one* (**14**) [19]. Amorphous (7 mg). TLC: R_f 0.42 (S-3); anisaldehyde, brown-green. $[\alpha]_D^{20}$ + 49° ($CHCl_3$; *c* 0.3) (lit. [19] $[\alpha]_D$ + 36° ($CHCl_3$)). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3446, 2956, 1662, 1614. UV λ_{max} nm (log ε): 241 (4.25). CD λ_{max} nm ($\Delta\varepsilon$): 255 (+ 0.12), 313 (− 1.21). ^1H NMR: δ0.85–1.10 (15H, *m*) including 0.90 and 0.91 (3H each, *d*, J = 6.5 Hz, Me-26 and Me-27), 0.94 (3H, *d*, J = 6.5 Hz, Me-21), 0.95 (3H, *s*, Me-18), 0.98–1.10 (1H, *m*, H-7α), 1.13–1.29 (6H, *m*) including 1.24 (3H, *s*, Me-19) and 1.19–1.27 (1H, *m*, H–15β), 1.37 (1H, *dd*, J_1 = 11.5, J_2 = 7.5 Hz, H-17) overlapped by 1.39–1.60 (6H, *m*), 1.64–1.73 (2H, *m*, H-1α and H-8), 1.86–1.93 (1H, *m*, H-7β), 1.99–2.13 (3H, *m*, H-12β, H-1β and H-20), 2.20–2.34 (3H, *m*, H-15α, H-2α and H-6α), 2.42–2.55 (2H, *m*, H-2β and H-6β), 3.68–3.73 (1H, *m*, H-22), 4.35 (1H, *ddd*, $J_1 \sim J_2 \sim$ 7.5, J_3 = 4.5 Hz, H-16), 5.71 (1H, *br s*, H-4); ($CDCl_3$; 360 MHz): δ0.85–1.74 (30H, *m*) including 0.91 and 0.92 (3H each, *d*, J = 6.5 Hz, Me-26 and Me-27), 0.96 (3H, *s*, Me-18) overlapped by 0.98 (3H, *d*, J = 7 Hz, Me-21) and 1.20 (3H, *s*, Me-19), 1.83–1.90 (1H, *m*, H-7β), 1.96–2.05 (2H, *m*, H-12β, H-1β), 2.20–2.48 (6H, *m*, H-15β, H-20, H-2α, H-6α, H-2β, H-6β), 3.63 (1H, *ddd*, J_1 = 9.5, $J_2 \sim J_3 \sim$ 2.5 Hz, H-22), 4.35 (1H, *ddd*, J_1 = 8, J_2 = 7, J_3 = 4.5 Hz, H-16), 5.73 (1H, *br s*, H-4). ^{13}C NMR (63 MHz): Table 2. EIMS *m/z* (rel. int.): 416 $[M]^+$ (0.5), 398 $[M - 18]^+$ (4), 317 (10), 299 (26), 298 (100), 283 (43), 269 (33), 175 (26), 55 (42). DCIMS (isobutane): 417 $[M + H]^+$ (100).

Cholest-4-*ene*-3,16,22-*trione* [10] *by oxidation of* **14**. Compound **14** (2.5 mg) oxidized with pyridinium chlorochromate [14]; from reaction mixture amorphous cholest-4-ene-3,16,22-trione (1.8 mg) isolated by CC on silica gel using cyclohexane–EtOAc (7:3). TLC: R_f 0.67 (*S*-3); anisaldehyde, red. $[\alpha]_D^{20}$ − 91° ($CHCl_3$; *c* 0.1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2957, 1736, 1712, 1665, 1616. UV λ_{max} nm (log ε): 240 (4.07). ^1H NMR: δ0.87 (3H, *s*, Me-18), 0.92 (6H, *d*, J = 7 Hz, Me-26 and Me-27), 1.05 (3H, *d*, J = 7 Hz, Me-21), 1.09–1.22 (2H, *m*, including H-7α), 1.27 (3H, *s*, Me-19), 1.39–1.52 (2H, *m*, H-24$_{A/B}$), 1.53–1.88 (9H, *m*) including 1.82 (1H, *dd*, J_1 = 18, J_2 = 13 Hz, H-15β), 2.04–2.14 (2H, *m*, including H-1β), 2.21 (1H, *dd*, J_1 = 18, J_2 = 7.5 Hz, H-15α), 2.27–2.36 (2H, *m*, H-2α and H-6α), 2.44–2.56 (2H, *m*, H-2β and H-6β) overlapped by 2.57 (1H, *d*, J = 10.5 Hz, H-17), 2.59–2.75 (3H, *m*, H-20 and H-23$_{A/B}$), 5.73 (1H, *br s*, H-4). EIMS *m/z* (rel. int.): 412 $[M]^+$ (6), 397 (6), 357 (22), 356 (83), 342 (9), 341 (38), 314 (29), 313 (50), 299 (21), 126 (32), 99 (60), 81 (66), 69 (49), 55 (31), 43 (100).

Preparation of **19** *from* **14**. To a soln of 1.5 mg **14** in 2 ml $CHCl_3$–toluene (1:3) 250 μl of a phosgene soln (1.93 M in toluene) was added dropwise. After 2 hr the solvent was evapd and residue purified by CC on silica gel with $CHCl_3$–MeOH (99:1) to obtain 1 mg of **19**.

(16*S*,22*S*)-*Cholest*-4-*en*-3-*one*-16,22-*diyl carbonate* (**19**). Amorphous. TLC: R_f 0.88 (S-3); anisaldehyde, brown-green. $[\alpha]_D^{20}$ − 16° ($CHCl_3$; *c* 0.1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2929, 1740, 1663. UV λ_{max} nm (log ε): 240 (3.98). ^1H NMR ($CDCl_3$; 360 MHz): δ0.83–1.75 (30H, *m*) including 0.90 and 0.91 (3H each, *d*, J = 6 Hz, Me-26 and Me-27), 0.92 (3H, *s*, Me-18), 1.01 (3H, *d*, J = 7 Hz, Me-21), 1.21 (3H, *s*, Me-19), 1.41 (1H, *dd*, J_1 = 11, J_2 = 7 Hz, H-17), 1.50–1.58 (2H, *m*, H-23$_{A/B}$), 1.53–1.63 (1H, *m*, H-15β) and 1.65–1.74 (1H, *m*, H-1α), 1.83–1.90 (1H, *m*, H-7β), 1.93 (1H, *ddd*, J_1 = 13, $J_2 \sim J_3 \sim$ 3 Hz, H-12β), 2.02 (1H, *ddd*, J_1 = 13, J_2 = 5, J_3 = 3 Hz, H-1β), 2.22–2.49 (6H, *m*, H-15α, H-20, H-2α, H-6α, H-2β, H-6β), 4.19 (1H, *m*, H-22), 4.86 (1H, *ddd*, J_1 = 8, J_2 = 7, J_3 = 4.5 Hz, H-16), 5.74 (1H, *br s*, H-4). CIMS (isobutane) *m/z* (rel. int.): 444 (32), 443 $[M + H]^+$ (100), 399 $[M - 44 + H]^+$ (12), 381 $[M - 62 + H]^+$ (70), 269 (30).

Glycoside-2 (= 16(S)-[β-D-*glucopyranosyl*-(1 → 3)-β-D-*galactopyranosyloxy*]-22(S)-*hydroxycholest*-4-*en*-3-*one*, **5**). Crystals from $CHCl_3$–MeOH (2:1) (12 mg), mp 173–175°. TLC: R_f 0.36 (S-1); anisaldehyde, brown-green. HPLC: R_t 20 min (S-4). $[\alpha]_D^{20}$ + 16° (MeOH; *c* 0.6). IR ν_{max} cm^{-1}: 3436, 2951, 1663. UV λ_{max} nm (log ε): 241 (4.13). CD λ_{max} nm ($\Delta\varepsilon$): 220 (+ 0.90), 315 (− 0.39). ^1H NMR (250 MHz): δ0.85–1.76 (30H, *m*) including 0.91 (6H, *d*, J = 6.5 Hz, Me-26 and Me-27), 0.92 (3H, *d*, J = 7 Hz, Me-21), 0.97 (3H, *s*, Me-18) and 1.30 (3H, *s*, Me-19), 1.86–1.97 (1H, *m*), 2.01–2.35 (6H, *m*), 2.40–2.57 (2H, *m*), 3.25–3.43 (4H, *m*), 3.50 (1H, *ddd*, $J_1 \sim J_2 \sim$ 6, J_3 = 1 Hz, H-5′), 3.58 (1H, *dd*, J_1 = 10, J_2 = 3 Hz, H-3′), 3.64–3.78 (5H, *m*), 3.65 (1H, *dd*, J_1 = 12, J_2 = 2 Hz, H-6′$_A$), 4.10 (1H, *dd*, J_1 = 3, J_2 = 1 Hz, H-4′), 4.18–4.27 (2H, *m*) including 4.21 (1H, *d*, J = 8 Hz, H-1′ or H-1″), 4.55 (1H, *d*, J = 8 Hz, H-1″ or H-1′), 5.70 (1H, *br s*, H-4). ^{13}C NMR (63 MHz): Table 2. DCIMS *m/z* (rel. int.): 758 $[M + NH_4]^+$ (3), 741 $[M + H]^+$ (17), 579 $[M - 162 + H]^+$ (6), 418 (11), 417 $[M - 324 + H]^+$ (22), 416 $[M - 324]^+$ (8), 399 (27), 397 (10), 298 (50), 180 (100).

Octaacetylglycoside-2. Compound **5** (2.4 mg) was acetylated and purified by HPLC (MeOH–H_2O, 82:18) to give 1.4 mg of oily octaacetylglycoside-2. TLC: R_f 0.26 (S-2); anisaldehyde, brown-green. $[\alpha]_D^{20}$ + 10° ($CHCl_3$; *c* 0.1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2938, 1756, 1662. ^1H NMR

(360 MHz; C_6D_6): δ0.50–2.33 (63H, *m*) including 0.72 (3H, *s*, Me-18), 0.91 (3H, *s*, Me-19), 1.01 and 1.02 (3H each, *d*, J = 6.5 Hz, Me-26 and Me-27), 1.13 (3H, *d*, J = 7 Hz, Me-21), 1.66, 1.67, 1.75, 1.82, 1.83, 1.85, 1.96 and 2.14 (3H each, *s*, Ac), 3.28 (1H, *ddd*, J_1 = 10, J_2 = 3.5, J_3 = 2.5 Hz, H-5″), 3.50 (1H, *br dd*, $J_1 \sim J_2 \sim$ 6.5 Hz, H-5′), 3.70 (1H, *dd*, J_1 = 10, J_2 = 3.5 Hz, H-3′), 4.05 (1H, *dd*, J_1 = 12.5, J_2 = 3.5 Hz, H-6′$_A$), 4.25 (1H, *dd*, J_1 = 11, J_2 = 6.5 Hz, H-6′$_A$), 4.30 (1H, *dd*, J_1 = 11, J_2 = 6.5 Hz, H-6′$_B$), 4.40 (1H, *dd*, J_1 = 12.5, J_2 = 2.5 Hz, H-6″$_B$), 4.51 (1H, *d*, J = 8 Hz, H-1′) overlapped by 4.54 (1H, *ddd*, $J_1 \sim J_2 \sim$ 8, J_3 = 4.5 Hz, H-16), 4.62 (1H, *d*, J = 8 Hz, H-1″), 5.15 (1H, *dd*, J_1 = 10, J_2 = 8 Hz, H-2″), 5.23 (1H, *dd*, $J_1 \sim J_2 \sim$ 10 Hz, H-4″), 5.39 (1H, *dd*, $J_1 \sim J_2 \sim$ 10 Hz, H-3″) overlapped by 5.38–5.43 (1H, *m*, H-22), 5.45 (1H, *br d*, J = 3.5 Hz, H-4′), 5.60 (1H, *dd*, J_1 = 10, J_2 = 8 Hz, H-2′), 5.84 (1H, *br s*, H-4).

Glycoside-3 (= (25S)-16(S)-[β-D-*glucopyranosyl*-(1 → 3)-β-D-*galactopyranosyloxy*]-26-*hydroxycholest*-4-*ene*-3,22-*dione*, **6**). Crystals from $CHCl_3$–MeOH (2:1) (37 mg), mp 178–182°. TLC: R_f 0.31 (*S*-1); anisaldehyde, yellow-green. HPLC: R_t 14 min (S-4). $[\alpha]_D^{20}$ + 18° (MeOH; *c* 0.4). IR ν_{max} cm^{-1}: 3401, 2935, 1702, 1657. UV λ_{max} nm (log ε): 241 (4.24). CD λ_{max} nm ($\Delta\varepsilon$): 255 (+ 1.06), 2.95 (− 1.45). ^{1}H NMR: δ0.89–1.09 (9H, *m*, H-7α, H-9, H-14) including 0.92 (3H, *d*, J = 7 Hz, Me-27) and 0.95 (3H, *s*, Me-18), 1.13 (3H, *d*, J = 7 Hz, Me-21), 1.18–1.35 (5H, *m*, H-12α, H-24$_A$) including 1.23 (3H, *s*, Me-19), 1.45–1.74 (7H, *m*, H-11α, H-11β, H-8, H-25, H-15β, H-24$_B$ and H-1α), 1.81 (1H, *dd*, J_1 = 11, J_2 = 8 Hz, H-17), 1.86–1.94 (1H, *m*, H-7β), 1.99 (1H, *br d*, J = 12.5 Hz, H-12β), 2.07 (1H, *ddd*, J_1 = 14, J_2 = 5, J_3 = 3 Hz, H-1β), 2.20 (1H, *ddd*, J_1 = 14, $J_2 \sim J_3 \sim$ 7 Hz, H-15α), 2.24–2.34 (2H, *m*, H-6α, H-2α), 2.42–2.53 (2H, *m*, H-6β, H-2β), 2.68–2.86 (2H, *m*, H-23$_{A/B}$), 3.08 (1H, *dq*, J_1 = 11, J_2 = 7 Hz, H-20), 3.25–3.38 (4H, *m*, H-2″, H-3″, H-4″, H-5″), 3.40 (2H, *d*, J = 6 Hz, H-26$_{A/B}$), 3.45 (1H, *br dd*, $J_1 \sim J_2 \sim$ 6 Hz, H-5′), 3.52 (1H, *dd*, J_1 = 10, J_2 = 3 Hz, H-3′), 3.60 (1H, *dd*, J_1 = 10, J_2 = 8 Hz, H-2′), 3.65–3.75 (3H, *m*, H-6′$_{A/B}$, H-6″$_A$), 3.83 (1H, *dd*, J_1 = 12, J_2 = 4 Hz, H-6″$_B$), 4.00 (1H, *d*, J = 8 Hz, H-1′), 4.07 (1H, *br d*, J = 3 Hz, H-4′), 4.10 (1H, *ddd*, $J_1 \sim J_2 \sim$ 8, $J_3 \sim$ 5 Hz, H-16), 4.51 (1H, *d*, J = 8 Hz, H-1″), 5.70 (1H, *br s*, H-4). ^{13}C NMR: Table 2. DCIMS *m/z* (rel. int.): 772 [M + NH_4]$^+$ (1), 754 [M]$^+$ (1), 737 [M − 18 + H]$^+$ (2), 593 [M − 162 + H]$^+$ (4), 575 [M − 180 + H]$^+$ (10), 431 [M − 324 + H]$^+$ (7), 413 [M − 342 + H]$^+$ (69), 180 (100).

Octaacetylglycoside-3. Acetylation of **6** (9 mg) and purification by HPLC (LiChrosorb® RP2, MeOH–H_2O, 75:25) yielded 2 mg of octaacetyl-**6** (oil). TLC: R_f 0.19 (S-2); anisaldehyde, yellow-green. $[\alpha]_D^{20}$ + 42° ($CHCl_3$; *c* 0.1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2943, 1756, 1663. ^{1}H NMR (C_6D_6): δ0.40–1.15 (19H, *m*) including 0.72 and 0.81 (3H each, *s*, Me-18 and Me-19), 0.96 (3H, *d*, J = 7 Hz, Me-27) and 1.06 (3H, *d*, J = 7 Hz, Me-21), 1.23–2.37 (38H, *m*) including 1.66, 1.67, 1.78, 1.81, 1.82, 1.84, 1.96 and 2.12 (3H each, *s*, Ac), 2.46–2.65 (2H, *m*, H-23$_{A/B}$), 2.91 (1H, *dq*, J_1 = 11, J_2 = 7 Hz, H-20), 3.46 (1H, *br dd*, $J_1 \sim J_2 \sim$ 7 Hz, H-5′), 3.56 (1H, *ddd*, J_1 = 10, J_2 = 4, J_3 = 3 Hz, H-5″), 3.83 (1H, *dd*, J_1 = 10, J_2 = 3 Hz, H-3′), 3.90 (1H, *dd*, J_1 = 11, J_2 = 7 Hz, H-26$_A$), 4.06 (1H, *dd*, J_1 = 12, J_2 = 4 Hz, H-6″$_A$), 4.13 (1H, *dd*, J_1 = 11, J_2 = 5 Hz, H-26$_B$), 4.18–4.26 (3H, *m*, H-1′, H-6′$_A$, H-16), 4.34 (1H, *dd*, J_1 = 12, J_2 = 7 Hz, H-6′$_B$), 4.49 (1H, *dd*, J_1 = 12, J_2 = 3 Hz, H-6″$_B$), 4.73 (1H, *d*, J = 8 Hz, H-1″), 5.20 (1H, *dd*, J_1 = 10, J_2 = 8 Hz, H-2″), 5.28 (1H, *dd*, $J_1 \sim J_2 \sim$ 10 Hz, H-4″), 5.48 (1H, *dd*, $J_1 \sim J_2 \sim$ 10 Hz, H-3″), 5.52 (1H, *br d*, J = 3 Hz, H-4′) overlapped by 5.54 (1H, *dd*, J_1 = 10, J_2 = 8 Hz, H-2′), 5.85 (1H, *br s*, H-4).

(25S)-*Neospirost*-4-*en*-3-*one* (**18**) *by hydrolysis of* **6**. Hydrolysis of **6** (17 mg; 5 hr) and purification of the reaction mixture ($CHCl_3$–MeOH, 199:1) gave 4 mg of amorphous (25*S*)-neospirost-4-en-3-one (**18**) [11]. TLC: R_f 0.71 (S-3); anisaldehyde, yellow-green. $[\alpha]_D^{20}$ − 23° ($CHCl_3$; *c* 0.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940, 1662, 1616, 985, 919, 900, 850. UV λ_{max} nm (log ε): 240 (4.20). CD λ_{max} nm ($\Delta\varepsilon$): 260 (+ 0.28), 315 (− 1.44). ^{1}H NMR: δ0.86 (3H, *s*, Me-18), 0.93–1.12 (8H, *m*, H-9, H-7α) including 0.99 (3H, *d*, J = 7 Hz, Me-21) and 1.08 (3H, *d*, J = 7 Hz, Me-27), 1.13–1.26 (5H, *m*, H-14, H-11α) including 1.24 (3H, *s*, Me-19), 1.28–1.39 (2H, *m*, H-15β, H-23$_{eq}$), 1.40–1.46 (1H, *m*, H-24$_{eq}$), 1.47–1.62 (2H, *m*, H-11β and H-12), 1.64–1.96 (8H, *m*, H-25, H-1α, H-17, H-12$_{\alpha/\beta}$, H-8, H-20, H-7β and H-23$_{ax}$), 1.98–2.10 (3H, *m*, H-15α, H-24$_{ax}$, H-1β), 2.24–2.35 (2H, *m*, H-2α, H-6α), 2.42–2.55 (2H, *m*, H-2β, H-6β), 3.25 (1H, *br d*, $J \sim$ 11 Hz, H-26$_{ax}$) overlapped by solvent peak, 3.93 (1H, *dd*, J_1 = 11, J_2 = 3 Hz, H-26$_{eq}$), 4.40 (1H, *ddd*, J_1 = 9, J_2 = 8, J_3 = 6.5 Hz, H-16), 5.71 (1H, *br s*, H-4). ^{13}C NMR (63 MHz): Table 2. EIMS *m/z* (rel. int.): 412 [M]$^+$ (10), 353 (5), 343 (22), 340 (16), 299 (24), 298 (61), 283 (9), 269 (24), 140 (12), 139 (100), 69 (37), 55 (26).

Glycoside-4 (= (25S)-16(S)-[β-D-*glucopyranosyl*-(1 → 3)-β-D-*galactopyranosyloxy*]-22(S),26-*dihydroxycholest*-4-*en*-3-*one*, **7**). Crystals from $CHCl_3$–MeOH (2:1) (65 mg), mp 175–177°. TLC: R_f 0.22 (S-1); anisaldehyde: brown-green. HPLC: R_t 14 min (S-5). $[\alpha]_D^{20}$ + 15° (MeOH; *c* 0.3). IR ν_{max} cm^{-1}: 3411, 2931, 1656. UV λ_{max} nm (log ε): 241 (4.22). CD λ_{max} nm ($\Delta\varepsilon$): 255 (+ 0.21), 315 (− 1.00). ^{1}H NMR: δ0.88–1.08 (12H, *m*) including 0.92 (6H, *d*, J = 7 Hz, Me-21 and Me-27), 0.96 (3H, *s*, Me-18), 1.13–1.74 (15H, *m*) including 1.23 (3H, *s*, Me-19), 1.87–1.95 (1H, *m*), 2.01–2.17 (3H, *m*), 2.20–2.34 (3H, *m*), 2.42–2.54 (2H, *m*), 3.26–3.46 (5H, *m*), 3.50 (1H, *br dd*, $J_1 \sim J_2 \sim$ 6 Hz, H-5′), 3.57 (1H, *dd*, J_1 = 10, J_2 = 3 Hz, H-3′), 3.63–3.81 (6H, *m*), 3.84 (1H, *dd*, J_1 = 12, J_2 = 2 Hz, H-6″), 4.09 (1H, *br d*, J = 3 Hz, H-4′), 4.21 (1H, *d*, J = 8 Hz, H-1′ or H-1″) overlapped by 4.21–4.26 (1H, *m*), 4.53 (1H, *d*, J = 8 Hz, H-1″ or H-1′), 5.70 (1H, *br s*, H-4). ^{13}C NMR: Table 2. DCIMS *m/z* (rel. int.): 757 [M + H]$^+$ (6), 595 [M − 162 + H]$^+$ (9), 433 [M − 324 + H]$^+$ (18), 415 [M − 342 + H]$^+$ (21), 301 (22), 180 (100).

Nonaacetylglycoside-4. Compound **7** (9 mg) was acetylated and purified by HPLC (LiChrosorb® RP-2, MeOH–H_2O, 75:25) to give nonaacetyl-**7** (amorphous; 2.5 mg). TLC: R_f 0.20 (*S*-2); anisaldehyde, brown-green. $[\alpha]_D^{20}$ + 43° ($CHCl_3$; *c* 0.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 1756, 1666. ^{1}H NMR (C_6D_6): δ0.50–2.33 (63H, *m*) including

0.72 and 0.89 (3H each, *s*, Me-18 and Me-19), 0.97 (3H, *d*, $J = 7$ Hz, Me-27), 1.11 (3H, *d*, $J = 7$ Hz, Me-21), 2 × 1.67, 1.77, 2 × 1.82, 2 × 1.84, 1.98 and 2.16 (3H each, *s*, Ac), 3.42–3.57 (2H, *m*, H-5′ and H-5″), 3.85 (1H, *dd*, $J_1 = 10$, $J_2 = 3$ Hz, H-3′), 3.92 (1H, *dd*, $J_1 = 11$, $J_2 = 7$ Hz, H-26$_A$), 4.09 (1H, *dd*, $J_1 = 12.5$, $J_2 = 3.5$ Hz, H-6′$_A$) overlapped by 4.13 (1H, *dd*, $J_1 = 11$, $J_2 = 5.5$ Hz, H-26$_B$), 4.24–4.33 (2H, *m*, H-6′$_{A/B}$), 4.47 (1H, *dd*, $J_1 = 12.5$, $J_2 = 3$ Hz, H-6″$_B$), 4.50–4.56 (1H, *m*, H-16), 4.59 (1H, *d*, $J = 8$ Hz, H-1′), 4.72 (1H, *d*, $J = 8$ Hz, H-1″), 5.20 (1H, *dd*, $J_1 = 10$, $J_2 = 8$ Hz, H-2″), 5.27 (1H, *dd*, $J_1 \sim J_2 \sim 10$ Hz, H-4″), 5.36 (1H, *br d*, $J = 10$ Hz, H-22), 5.47–5.53 (2H, *m*, H-3″ and H-4′), 5.63 (1H, *dd*, $J_1 = 10$, $J_2 = 8$ Hz, H-2′), 5.85 (1H, *br s*, H-4).

(25S)-16(S),22(S),26-*Trihydroxycholest*-4-*en*-3-*one* (**15**) *by hydrolysis of* **7**. Hydrolysis of **7** (25 mg, 8 hr) and subsequent CC on silica gel gave 9 mg **15**. TLC: R_f 0.24 (*S*-3); anisaldehyde, brown-green. $[\alpha]_D^{20} + 36°$ ($CHCl_3$; *c* 0.8). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3417, 2951, 1662, 1615. UV λ_{max} nm (log ε): 241 (4.16). CD λ_{max} nm ($\Delta\varepsilon$): 260 (+ 0.13), 3.10 (− 0.26). ^{1}H and ^{13}C NMR, MS identical with the aglycone from glycoside-5 (see below).

Glycoside-5 (= (25S)-16(S)-[α-L-*rhamnopyranosyl*-(1 → 2)-β-D-*galactopyranosyloxy*]-22(S),26-*dihydroxycholest*-4-*en*-3-*one*, **8**). Crystals from $CHCl_3$–MeOH (2:1) (108 mg), mp 189–191°. TLC: R_f 0.20 (*S*-1); anisaldehyde, brown-green. HPLC: R_t 8 min (S-5). $[\alpha]_D^{20} - 3°$ (MeOH; *c* 1.3). IR ν_{max} cm^{-1}: 3401, 2938, 1657. UV λ_{max} nm (log ε): 241 (4.25). CD λ_{max} nm ($\Delta\varepsilon$): 252 (+ 0.39), 313 (− 1.13). ^{1}H NMR: δ0.85–1.07 (12H, *m*, H-9, H-14, H-7α) including 0.94 (3H, *d*, $J = 7$ Hz, Me-21), 0.95 (3H, *d*, $J = 7$ Hz, Me-27), 0.96 (3H, *s*, Me-18), 1.13–1.25 (7H, *m*, H-12α) including 1.21 (3H, *d*, $J = 6.5$ Hz, Me-6″), 1.23 (3H, *s*, Me-19), 1.29–1.40 (1H, *m*, H-24$_A$), 1.41–1.73 (10H, *m*, H-15β, H-24$_B$, H-17, H-8, H-23$_{A/B}$, H-11$_{z/\beta}$, H-25, H-1α), 1.86–1.93 (1H, *m*, H-7β), 2.00–2.10 (2H, *m*, H-12β, H-1β), 2.17–2.33 (4H, *m*, H-20, H-15α, H-2α, H-6α), 2.42–2.54 (2H, *m*, H-6β, H-2β), 3.37 (1H, *dd*, $J_1 = 11$, $J_2 = 5.5$ Hz, H-26$_A$) overlapped by 3.38 (1H, *dd*, $J_1 \sim J_2 \sim 10$ Hz, H-4″), 3.43–3.47 (2H, *m*, H-26$_B$ and H-5′), 3.55–3.59 (1H, *m*, H-22) overlapped by 3.60 (1H, *dd*, $J_1 = 10$, $J_2 = 3$ Hz, H-3′), 3.69–3.77 (4H, *m*, H-6′$_{A/B}$, H-2′, H-3″), 3.78 (1H, *br d*, $J = 3$ Hz, H-4′), 3.91 (1H, *dd*, $J_1 = 3.5$, $J_2 = 1.5$ Hz, H-2″), 3.99 (1H, *dq*, $J_1 = 10$, $J_2 = 6.5$ Hz, H-5″), 4.22 (1H, *d*, $J = 8$ Hz, H-1′) overlapped by 4.20–4.25 (1H, *m*, H-16), 5.40 (1H, *d*, $J = 1.5$ Hz, H-1″), 5.70 (1H, *br s*, H-4). ^{13}C NMR: Table 2. DCIMS *m/z* (rel. int.): 741 $[M + H]^+$ (3), 595 $[M - 146 + H]^+$ (11), 434 (10), 433 $[M - 308 + H]^+$ (36), 413 (20), 164 (27), 130 (63), 113 (100).

Octaacetylglycoside-5. Acetylation of **8** (5 mg) gave 6 mg of amorphous octaacetyl-**8**. TLC: R_f 0.25 (*S*-2); anisaldehyde, brown-green. $[\alpha]_D^{20} + 3°$ ($CHCl_3$; *c* 0.5). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2947, 1747, 1663. ^{1}H NMR (C_6D_6): δ0.53–0.76 (6H, *m*) including 0.74 (3H, *s*, Me-18), 0.90–2.34 (56H, *m*) including 0.97 (3H, *s*, Me-19), 1.13 (3H, *d*, $J = 7$ Hz, Me-27), 1.35 (3H, *d*, $J = 7$ Hz, Me-21), 1.42 (3H, *d*, $J = 6$ Hz, Me-6″), 1.60, 1.61, 1.69, 1.75, 1.80, 1.86, 1.89 and 2.06 (3H each, *s*, Ac), 2.58 (1H, *dq*, $J_1 = 12$, $J_2 = 7$ Hz, H-20), 3.66 (1H, *br dd*, $J_1 \sim J_2 \sim 7$ Hz, H-5′), 4.12 (2H, *d*, $J = 6.5$ Hz, H-26$_{A/B}$), 4.20 (2H, *d*, $J = 7$ Hz, H-6′$_{A/B}$), 4.46 (1H, *dd*, $J_1 = 10$, $J_2 = 8$ Hz, H-2′), 4.62 (1H, *dq*, $J_1 = 10$, $J_2 = 6$ Hz, H-5″), 4.73 (1H, *ddd*, $J_1 \sim J_2 \sim 7.5$, $J_3 \sim 4$ Hz, H-16), 4.88 (1H, *d*, $J = 8$ Hz, H-1′), 5.43 (1H, *dd*, $J_1 = 10$, $J_2 = 3$ Hz, H-3′), 5.50 (1H, *br d*, $J = 10$ Hz, H-22), 5.55 (1H, *dd*, $J_1 = 3$, $J_2 = 1.5$ Hz, H-2″), 5.57–5.62 (2H, *m*, H-4′ and H-4″), 5.67 (1H, *br s*, H-1″) overlapped by 5.69 (1H, *dd*, $J_1 = 10$, $J_2 = 3$ Hz, H-3″), 5.85 (1H, *br s*, H-4).

(25S)-16(S),22(S), 26-*Trihydroxycholest*-4-*en*-3-*one* (**15**) *by hydrolysis of* **8**. Compound **8** (35 mg) was hydrolysed (3 hr). Purification by CC with $CHCl_3$:MeOH (95:5) gave 7 mg amorphous **15**. TLC: R_f 0.24 (*S*-3); anisaldehyde, brown-green. $[\alpha]_D^{20} + 30°$ ($CHCl_3$; *c* 0.5). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3423, 2951, 1663, 1614. UV λ_{max} nm (log ε): 241 (4.09). CD λ_{max} nm ($\Delta\varepsilon$): 257 (+ 0.09), 310 (− 0.35). ^{1}H NMR: δ0.87–1.10 (12H, *m*, H-14, H-7α) including 0.92 (3H, *d*, $J = 6.5$ Hz, Me-27), 0.95 (3H, *d*, $J = 7$ Hz, Me-21) and 0.96 (3H, *s*, Me-18), 1.12–1.75 (15H, *m*, H-24$_A$, H-15β, H-17, H-23$_{A/B}$, H-1α, H-8) including 1.24 (3H, *m*, Me-19), 1.86–1.94 (1H, *m*, H-7β), 2.00–2.14 (3H, *m*, H-24$_B$, H-1β, H-20), 2.20–2.34 (3H, *m*, H-15α, H-2α, H-6α), 2.42–2.55 (2H, *m*, H-2β, H-6β), 3.34 (1H, *dd*, $J_1 = 10.5$, $J_2 = 6.5$ Hz, H-26$_A$), 3.43 (1H, *dd*, $J_1 = 10.5$, $J_2 = 5.5$ Hz, H-26$_B$), 3.72 (1H, *br d*, $J = 8$ Hz, H-22), 4.35 (1H, *ddd*, $J_1 \sim J_2 \sim 7.5$, $J_3 \sim 4.5$ Hz, H-16), 5.71 (1H, *br s*, H-4). ^{13}C NMR (22.5 MHz): Table 2. EIMS *m/z* (rel. int.): 432 $[M]^+$ (9), 414 (11), 382 (8), 367 (22), 317 (38), 316 (88), 299 (25), 298 (58), 283 (42), 271 (31), 269 (68), 175 (41), 99 (100), 81 (69), 69 (51), 55 (99), 43 (81).

(25S)-*Neospirost*-4-*en*-3-*one* (**18**) *from* **8**. Compound **8** (30 mg) was selectively silylated at C-26 by treatment with *t*-butyldimethylsilylchloride [12, 13] yielding 40 mg of the corresponding silyl ether. Subsequent oxidation with PCC [14] and work-up via a short CC (silica gel; $CHCl_3$–MeOH, 9:1) gave 15 mg of the crude 22-oxo-26-silyloxy product, which was refluxed in 2 N H_2SO_4–MeOH (1:1) (80°, 7 hr). Neutralization and purification by CC (silica gel; $CHCl_3$–MeOH, 99:1) gave 1 mg of (25S)-neospirost-4-en-3-one (**18**) [11].

Glycoside-6 (= 5S,25S)-16(S)-[α-L-*rhamnopyranosyl*-(1 → 2)-β-D-galactopyranosyloxy]-22(S),26-*dihydroxycholestan*-3-*one*, **9**). Crystals from $CHCl_3$–MeOH (2:1) (16 mg), mp 151–154°. TLC: R_f 0.20 (*S*-1); anisaldehyde, brown-green. HPLC: R_t 11 min (*S*-5). $[\alpha]_D^{20} - 8°$ (MeOH; *c* 0.4). IR ν_{max} cm^{-1}: 3401, 2929, 1703. UV λ_{max} nm (log ε): 205 (3.65). CD λ_{max} nm ($\Delta\varepsilon$): 285 (+ 0.72), 320 (− 0.06). ^{1}H NMR: δ0.72–1.80 (34H, *m*) including 0.92 (3H, *s*, Me-18), 0.93 and 0.95 (3H each, *d*, $J = 7$ Hz, Me-21 and Me-27), 1.06 (3H, *s*, Me-19), 1.21 (3H, *d*, $J = 6$ Hz, Me-6″), 1.96–2.07 (3H, *m*), 2.16–2.30 (3H, *m*), 2.36 (1H, *dd*, $J_1 = J_2 = 14$ Hz, H-4β), 2.48 (1H, *ddd*, $J_1 = J_2 = 14$, $J_3 = 6$ Hz, H-2β), 3.33–3.40 (2H, *m*), 3.42–3.48 (2H, *m*), 3.54–3.62 (2H, *m*), 3.69–3.79 (5H, *m*), 3.91 (1H, *dd*, $J_1 = 4$, $J_2 = 1.5$ Hz, H-2″), 3.98 (1H, *dq*, $J_1 = 10$, $J_2 = 6$ Hz, H-5″), 4.19–4.25 (2H, *m*) including 4.21 (1H, *d*, $J = 8$ Hz, H-1′), 5.41 (1H, *d*, $J = 1.5$ Hz, H-1″). ^{13}C NMR: Table 2. DCIMS *m/z* (rel. int.): 743 $[M + H]^+$ (3), 597 $[M - 146 + H]^+$ (10), 435

$[M - 308 + H]^+$ (25), 417 (12), 388 (17), 298 (18), 281 (16), 180 (16), 164 (30), 130 (50), 113 (100).

Octaacetylglycoside-6. Compound **9** (2.5 mg) was acetylated to give 2.5 mg of the octaacetyl derivative (oil). TLC: R_f 0.28 (*S*-2); anisaldehyde, brown-green. $[\alpha]_D^{20} \pm 0°$ ($CHCl_3$; *c* 0.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2935, 1747. ^1H NMR (360 MHz; C_6D_6): δ0.60–2.34 (65H, *m*) including 0.62 and 0.99 (3H each, *s*, Me-18 and Me-19), 1.13 (3H, *d*, J = 7 Hz, Me-27), 1.36 (3H, *d*, J = 7 Hz, Me-21), 1.40 (3H, *d*, J = 6 Hz, Me-6″), 1.60, 1.61, 1.69, 1.74, 1.80, 1.86, 1.89 and 2.05 (3H each, *s*, Ac), 2.55–2.65 (1H, *m*, H-20), 3.67 (1H, *br dd*, $J_1 \sim J_2 \sim$ 6 Hz, H-5′), 4.12 (2H, *d*, J = 6 Hz, H-6′$_{A/B}$) overlapped by 4.15 (1H, *dd*, J_1 = 11, J_2 = 6.5 Hz, H-26$_A$), 4.23 (1H, *dd*, J_1 = 11, J_2 = 7 Hz, H-26$_B$), 4.46 (1H, *dd*, J_1 = 10, J_2 = 8 Hz, H-2′), 4.59–4.67 (1H, *m*, H-5″), 4.71–4.78 (1H, *m*, H-16), 4.88 (1H, *d*, J = 8 Hz, H-1′), 5.43 (1H, *dd*, J_1 = 10, J_2 = 3 Hz, H-3′), 5.48–5.53 (1H, *br d*, J = 10 Hz, H-22) overlapped by 5.54 (1H, *dd*, J_1 = 3.5, J_2 = 1.5 Hz, H-2″), 5.55–5.62 (2H, *m*, H-4′ and H-4″), 5.67 (1H, *d*, J = 1.5 Hz, H-1″) overlapped by 5.69 (1H, *dd*, J_1 = 10, J_2 = 3.5 Hz, H-3″).

Glycoside-7 (= 1(R*)-(α-L-*rhamnopyranosyloxy*)-3(R*)-(β-D-*galactopyranosyloxy*)*cholest*-5-*ene*-16(S*),22(S*)-*diol*, **10**). Crystals from $CHCl_3$–MeOH (1:1) (18 mg), mp 212°. TLC: R_f 0.30 (*S*-1); anisaldehyde, light-blue. HPLC: R_t 9 min (*S*-6). $[\alpha]_D^{20}$ − 20° (MeOH; *c* 0.4). IR ν_{max} cm^{-1}: 3400, 2933. UV λ_{max} nm: end absorption. ^1H NMR: δ0.87–0.97 (15H, *m*, Me-26, Me-27, Me-21 and Me-18), 1.06 (3H, *s*, Me-19), 1.10–1.62 (16H, *m*) including 1.23 (3H, *d*, J = 6 Hz, Me-6′), 1.93–2.26 (6H, *m*), 3.35–3.76 (12H, *m*), 3.82 (1H, *br d*, J = 3 Hz, H-4″), 4.13 (1H, *d*, J = 7.5 Hz, H-1″), 4.17 (1H, *ddd*, $J_1 \sim J_2 \sim$ 8, J_3 = 5 Hz, H-16), 4.59 (1H, *br s*, H-1′), 5.59 (1H, *br d*, J = 6 Hz, H-6). ^{13}C NMR: Table 2. DCIMS *m/z* (rel. int.): 743 $[M + H]^+$ (5), 598 (6), 597 $[M - 146 + H]^+$ (5), 581 $[M - 162 + H]^+$ (10), 577 (14), 545 (10), 434 $[M - 308]^+$ (9), 417 (10), 399 (17), 180 (34), 130 (55), 113 (100).

Nonaacetylglycoside-7. Compound **10** (2.5 mg) after acetylation gave 3 mg of nonaacetylglycoside-7 (amorphous). TLC: R_f 0.31 (*S*-2); anisaldehyde, light-blue. $[\alpha]_D^{20}$ − 16° ($CHCl_3$; *c* 0.3). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2957, 1747. ^1H NMR (C_6D_6): δ0.85–1.88 (56H, *m*) including 0.98, 0.99, 1.00, 1.01 (overlapping signals, 12H, Me-18, Me-19, Me-26, Me-27), 1.12 (3H, *d*, J = 7 Hz, Me-21), 1.32 (3H, *d*, J = 6 Hz, Me-6′), 1.58, 1.66, 1.68, 1.70, 1.72, 1.74, 1.77 and 1.78 (3H each, *s*, Ac), 1.92–2.06 (4H, *m*) including 2.05 (3H, *s*, Ac), 2.10–2.42 (7H, *m*), 3.24 (1H, *ddd*, $J_1 \sim J_2 \sim$ 7.5, J_3 = 1 Hz, H-5″), 3.51 (1H, *dd*, J_1 = 11.5, J_2 = 4 Hz, H-1), 4.09–4.19 (3H, *m*, H-6″$_{A/B}$, H-5′), 4.61 (1H, *d*, J = 8 Hz, H-1″) overlapped by 4.60–4.66 (1H, *m*, H, 16), 4.70–4.77 (1H, *m*, H-3), 5.08 (1H, *d*, J = 1.5 Hz, H-1′), 5.20 (1H, *dd*, J_1 = 10, J_2 = 3 Hz, H-3″), 5.40–5.45 (3H, *m*, H-4″, H-22, H-6), 5.59 (1H, *dd*, $J_1 \sim J_2 \sim$ 10 Hz, H-4′) overlapped by 5.61 (1H, *dd*, J_1 = 3, J_2 = 1.5 Hz, H-2′) overlapped by 5.64 (1H, *dd*, J_1 = 10, J_2 = 8 Hz, H-2″), 5.77 (1H, *dd*, J_1 = 10, J_2 = 3 Hz, H-3′).

Hydrolysis of glycoside-7 (**10**). Hydrolysis of **10** (5 mg; 6 hr) and subsequent chromatography by CC using $CHCl_3$–MeOH (9:1) yielded the aglycone **16** (2 mg) along with two further compounds, which were acetylated and identified as the heptaacetyl derivative of **20a** (1 mg) and the hexaacetyl derivative of **20b** (1.5 mg) (see below).

Cholest-5-*ene*-1 (R*), 3(R*),16(S*),22(S*)-*tetraol* (**16**) [19, 20]. Amorphous (2 mg). TLC: R_f 0.40 (*S*-3); anisaldehyde, brown blue. $[\alpha]_D^{20}$ − 16° ($CHCl_3$; *c* 0.2) (ref. [19] $[\alpha]_D$ − 60° ($CHCl_3$)). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 2955. ^1H NMR: δ0.90 and 0.91 (3H each, *d*, J = 6.5 Hz, Me-26 and Me-27) overlapped by 0.91 (3H, *s*, Me-18), 0.94 (3H, *d*, J = 7 Hz, Me-21), 1.03 (3H, *s*, Me-19), 1.09–1.23 (4H, *m*), 1.27–1.65 (10H, *m*), 1.90–2.00 (3H, *m*), 2.07 (1H, *ddq*, J_1 = 11.5, J_2 = 1.5, J_3 = 7 Hz, H-20), 2.15–2.27 (4H, *m*), 3.25–3.32 (*m*, H-1, overlapped by solvent signal), 3.40 (1H, *dddd*, J_1 = 12, $J_2 \sim J_3 \sim$ 8, J_4 = 5 Hz, H-3), 3.67–3.72 (1H, *m*, H-22), 4.33 (1H, *ddd*, J_1 = 8, J_2 = 7.5, J_3 = 5 Hz, H-16), 5.54 (1H, *br d*, J = 5 Hz, H-6). ^{13}C NMR (90 MHz): Table 2. CIMS *m/z* (rel. int.): 435 $[M + H]^+$ (15), 418 (26), 417 (100), 399 (43), 381 (11).

Heptaacetyl derivative of **20a** (= 3(R*)-(2′,3′,4′,6′-tetra-O-*acetyl*-β-D-*galactopyranosyloxy*)-1(R*),16(S*),22(S*)-*triacetoxycholest*-5-*ene*). Oil (1 mg). TLC: R_f 0.42 (*S*-2); anisaldehyde, brown-blue. $[\alpha]_D^{20}$ + 16° ($CHCl_3$; *c* 0.1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2928, 1747. ^1H NMR (C_6D_6): δ0.80–2.50 (58H, *m*) including 0.94 (3H, *s*, Me-18 or Me-19), 1.01 and 1.02 (3H each, *d*, J = 6.5 Hz, Me-26 and Me-27), 1.05 (3H, *s*, Me-19 or Me-18), 1.09 (3H, *d*, J = 7 Hz, Me-21), 1.52, 1.56, 2 × 1.68, 1.74, 1.85, 2.05 (3H each, *s*, Ac), 3.21 (1H, *ddd*, $J_1 \sim J_2 \sim$ 7 Hz, J_3 = 1 Hz, H-5′), 4.10 (1H, *dd*, J_1 = 11, J_2 = 7 Hz, H-6′$_A$), 4.15 (1H, *dd*, J_1 = 11, J_2 = 7 Hz, H-6′$_B$), 4.57–4.63 (1H, *m*, H-16) overlapped by 4.60 (1H, *d*, J = 8 Hz, H-1′), 4.77–4.85 (1H, *m*, H-3), 4.90 (1H, *dd*, J_1 = 12, J_2 = 5 Hz, H-1), 5.19 (1H, *dd*, J_1 = 10.5, J_2 = 3 Hz, H-3′), 5.39–5.44 (3H, *m*, H-4′, H-22, H-6), 5.64 (1H, *dd*, J_1 = 10.5, J_2 = 8 Hz, H-2′).

Hexaacetyl derivative of **20b** (= 1(R*) − (2′,3′,4′-*tri*-O-*acetyl*-α-L-*rhamnopyranosyloxy*)-3(R*),16(S*),22(S*)-*triacetoxycholest*-5-*ene*). Oil (1.5 mg). TLC: R_f 0.40 (*S*-2); anisaldehyde, blue. $[\alpha]_D^{20}$ − 10° ($CHCl_3$; *c* 0.1) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 1748. ^1H NMR (C_6D_6): δ0.78–1.95 (51H, *m*) including 0.87 (3H, *s*, Me-18), 0.89 and 0.90 (3H each, *d*, J = 6.5 Hz, Me-26 and Me-27), 1.04 (3H, *s*, Me-19), 1.11 (3H, *d*, J = 6 Hz, Me-21), 1.33 (3H, *d*, J = 6 Hz, Me-6′), 1.67, 1.70, 1.72, 1.74, 1.79 and 1.80 (3H each, *s*, Ac), 2.16–2.45 (7H, *m*), 3.50 (1H, *dd*, J_1 = 11.5, J_2 = 4 Hz, H-1), 4.09–4.16 (1H, *m*, H-5′), 4.46 (1H, *ddd*, $J_1 \sim J_2 \sim$ 8, $J_3 \sim$ 5 Hz, H-16), 4.70–4.78 (1H, *m*, H-3), 5.09 (1H, *d*, J = 1.5 Hz, H-1′), 5.40 (1H, *br d*, J = 6 Hz, H-6), 5.53–5.63 (3H, *m*, H-22, *H*-2′, H-4′), 5.78 (1H, *dd*, J_1 = 10, J_2 = 3 Hz, H-3′).

Glycoside-8 (= (25S*)-1(R*)-(α-L-*rhamnopyranosyloxy*)-3(R*)-(β-D-*galactopyranosyloxy*)*cholest*-5-*ene*-16(S*),22(S*),26-*triol*, **11**). Crystals from $CHCl_3$–MeOH (1:1) (20 mg), mp 245°. TLC: R_f 0.14 (S-1); anisaldehyde, light-blue. HPLC: R_t 6 min (S-6). $[\alpha]_D^{20}$ − 26° (MeOH; *c* 1.0). IR ν_{max} cm^{-1}: 3400, 2937. UV λ_{max} nm: end absorption. ^1H NMR (360 MHz): δ0.91 and 0.92 (3H each, *d*, J = 7 Hz, Me-21 and Me-26) overlapped by 0.93 (3H, *s*, Me-18), 1.06 (3H, *s*, Me-19), 1.18–1.63 (17H, *m*) including 1.23 (3H, *d*, J = 6 Hz, Me-6′), 1.92–2.01 (2H, *m*),

2.08–2.30 (6H, *m*), 3.32–3.51 (8H, *m*), 3.52–3.76 (6H, *m*), 3.83 (1H, *dd*, $J_1 = 3$, $J_2 = 1$ Hz, H-4″), 4.13 (1H, *d*, $J = 8$ Hz, H-1″), 4.18 (1H, *ddd*, $J_1 \sim J_2 \sim 7.5$, $J_3 = 4.5$ Hz, H-16), 4.89 (1H, *d*, $J = 1.5$ Hz, H-1′), 5.59 (1H, *br d*, $J = 6$ Hz, H-6). ^{13}C NMR: Table 2. DCIMS *m/z* (rel. int.): 759 $[M + H]^+$ (7), 725 (7), 613 $[M - 146 + H]^+$ (11), 597 $[M - 162 + H]^+$ (22), 579 (7), 451 $[M - 308 + H]^+$ (7), 433 (6), 196 (11), 180 (20), 164 (22), 146 (50), 113 (100).

Decaacetylglycoside-8. Acetylation of **11** (3 mg) and purification by CC (silica gel; petrol–EtOH, 9:1) gave 3 mg of oily decaacetylglycoside-8. TLC: R_f 0.24 (*S*-2); anisaldehyde, light-blue. $[\alpha]_D^{20}$ −15° ($CHCl_3$; *c* 0.3). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2955, 1747. ^{1}H NMR (C_6D_6): δ0.80–1.87 (57H, *m*) including 0.93 (3H, *d*, $J = 7$ Hz, Me-27), 0.97 and 0.99 (3H each, *s*, Me-18 and Me-19), 1.09 (3H, *d*, $J = 7$ Hz, Me-21), 1.32 (3H, *d*, $J = 6$ Hz, Me-6′), 1.60, 1.66, 1.68, 1.69, 1.72, 1.74, 1.76, 1.78, 1.80 (3H each, *s*, Ac), 2.00–2.09 (4H, *m*) including 2.07 (3H, *s*, Ac), 2.17–2.43 (6H, *m*), 3.36 (1H, *br dd*, $J_1 \sim J_2 \sim 7$ Hz, H-5″), 3.51 (1H, *dd*, $J_1 = 11.5$, $J_2 = 4$ Hz, H-1), 4.00 and 4.03 (2H each, *dd*, $J_1 = 10.5$, $J_2 = 6$ Hz, H-26$_A$ and H-26$_B$), 4.08–4.21 (3H, *m*, H-6″$_A$, H-5′ and H-6″$_B$), 4.57–4.64 (1H, *m*, H-16) overlapped by 4.63 (1H, *d*, $J = 8$ Hz, H-1″), 4.74 (1H, *dddd*, $J_1 \sim J_2 \sim 11$, $J_3 \sim J_4 \sim 5.5$ Hz, H-3), 5.08 (1H, *d*, $J = 1.5$ Hz, H-1′), 5.23 (1H, *dd*, $J_1 = 10.5$, $J_2 = 3$ Hz, H-3″), 5.38 (1H, *br dd*, $J_1 = 10$, $J_2 = 4$ Hz, H-22) overlapped by 5.42 (1H, *br d*, $J = 6$ Hz, H-6), 5.44 (1H, *br d*, $J = 3$ Hz, H-4″), 5.59 (1H, *dd*, $J_1 \sim J_2 \sim 10$ Hz, H-4′) overlapped by 5.60 (1H, *dd*, $J_1 = 3.5$, $J_2 = 1.5$ Hz, H-2′) overlapped by 5.64 (1H, *dd*, $J_1 = 10.5$, $J_2 = 8$ Hz, H-2″), 5.77 (1H, *dd*, $J_1 = 10$, $J_2 = 3.5$ Hz, H-3′).

Hydrolysis of glycoside-8 (**11**). Compound **11** was hydrolysed (6 hr) and subsequently chromatographed by CC on silica gel ($CHCl_3$–MeOH, 9:1) to yield the aglycone **17** (2 mg) and a crude substance, which was purified after acetylation and identified as the heptaacetyl derivative of **21** (1.5 mg).

(25S*)-*Cholest*-5-*ene*-1(R*),3(R*),16(S*),22(S*),26-*pentaol* (**17**). Amorphous (2 mg). TLC: R_f 0.20 (*S*-3); anisaldehyde, blue. $[\alpha]_D^{20}$ −41° ($CHCl_3$; *c* 0.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 2937. ^{1}H NMR: δ0.92 (3H, *d*, $J = 7$ Hz, Me-21 or Me-27) overlapped by 0.92 (3H, *s*, Me-18), 0.95 (3H, *d*, $J = 7$ Hz, Me-27 or Me-21), 1.02 (3H, *s*, Me-19), 1.06–1.65 (14H, *m*), 1.90–2.00 (3H, *m*), 2.08 (1H, *ddq*, $J_1 = 10$, $J_2 = 1.5$, $J_3 = 7$ Hz, H-20), 2.15–2.28 (4H, *m*), 3.27–3.46 (4H, *m*, H-1, H-26$_A$, H-3, H-26$_B$ overlapped by solvent signal), 3.71 (1H, *br d*, $J = 8$ Hz, H-22), 4.33 (1H, *ddd*, $J_1 = J_2 = 8$, $J_3 = 5$ Hz, H-16), 5.54 (1H, *br d*, $J = 5$ Hz, H-6). ^{13}C NMR (90 MHz): Table 2. CIMS *m/z* (rel. int.): 451 $[M + H]^+$ (100), 433 (76), 415 (16), 397 (3), 299 (11).

Heptaacetyl derivative of **21** (= (25S*)-1(R*)-(2′,3′4′-*tri*-O-*acetyl*-α-L-*rhamnopyranosyloxy*)-3(R*),16(S*),22(S*),26-*tetraacetoxycholest*-5-*ene*). Oil (1.5 mg). TLC: R_f 0.33 (*S*-2); anisaldehyde, blue. $[\alpha]_D^{20}$ −2° ($CHCl_3$; *c* 0.1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2951, 1734. ^{1}H NMR (C_6D_6; 360 MHz): δ0.68–0.80 (4H, *m*) including 0.78 (3H, *d*, $J = 7$ Hz, Me-27), 0.88 (3H, *s*, Me-18), 0.97–1.14 (9H, *m*) including 1.04 (3H, *s*, Me-19) and 1.08 (3H, *d*, $J = 7$ Hz, Me-21), 1.18–1.47 (10H, *m*) including 1.33 (3H, *d*, $J = 6$ Hz, Me-6′), 1.55–1.85 (25H, *m*) including 1.68, 1.70, 1.71, 1.73, 1.75, 1.79, 1.81 (3H each, *s*, Ac), 2.12–2.22 (2H, *m*), 2.24–2.44 (5H, *m*), 3.49 (1H, *dd*, $J_1 = 11.5$, $J_2 = 4$ Hz, H-1), 3.84 and 3.87 (1H each, *dd*, $J_1 = 12$, $J_2 = 6$ Hz, H-26$_A$ and H-26$_B$), 4.12 (1H, *dq*, $J_1 = 10$, $J_2 = 6$ Hz, H-5′), 4.69–4.78 (1H, *m*, H-3), 5.09 (1H, *d*, $J = 1.5$ Hz, H-1′), 5.12–5.17 (1H, *m*, H-16), 5.40 (1H, *br d*, $J = 6$ Hz, H-6), 5.54–5.61 (3H, *m*, H-4′, H-2′, H-22), 5.77 (1H, *dd*, $J_1 = 10$, $J_2 = 3$ Hz, H-3′).

Glycoside-9 (= 16(S)-[α-L-*rhamnopyranosyl*-(1 → 2)-[β-D-*glucopyranosyl*-(1 → 3)]-β-D-*glucopyranosyloxy*]*pregna*-4,17(20)*Z*-*dien*-3-*one*, **12**). Crystals from $CHCl_3$–MeOH (2:1) (15 mg), mp 172–175°. TLC: R_f 0.13 (*S*-1); anisaldehyde, violet. HPLC: R_t 28 min (*S*-6). $[\alpha]_D^{20}$ −6° (MeOH; *c* 0.1). IR ν_{max} cm^{-1}: 3400, 2932, 1656. UV λ_{max} nm (log ε): 240 (4.21). CD λ_{max} nm (Δε): 215 (+3.45), 315 (−0.78). ^{1}H NMR: δ0.81 (1H, *ddd*, $J_1 = 14$, $J_2 = 11$, $J_3 = 7$ Hz, H-14), 0.88–1.30 (12H, *m*, H-9, H-7α, H-12α) including 0.99 (3H, *s*, Me-18), 1.14 (3H, *d*, $J = 6$ Hz, Me-6″) and 1.25 (3H, *s*, Me-19), 1.48–1.75 (8H, *m*, H-11β, H-15β, H-11α, H-1α, H-8) including 1.71 (3H, *d*, $J = 7$ Hz, Me-21), 1.80–1.86 (1H, *m*, H-12β), 1.92–1.99 (1H, *m*, H-7β), 2.05–2.12 (1H, *m*, H-1β), 2.24–2.39 (3H, *m*, H-2α, H-6α, H-15α), 2.43–2.55 (2H, *m*, H-2β, H-6β), 3.25 (1H, *dd*, $J_1 = 10$, $J_2 = 8$ Hz, H-2‴) overlapped by 3.25–3.39 (5H, *m*, H-5′, H-5‴, H-4″, H-3‴, H-4‴), 3.45 (1H, *dd*, $J_1 \sim J_2 \sim 9$ Hz, H-4′), 3.57 (1H, *dd*, $J_1 = 9$, $J_2 = 8$ Hz, H-2′), 3.60–3.66 (2H, *m*, H-6″$_A$, H-3″), 3.71 (1H, *dd*, $J_1 = 11.5$, $J_2 = 5$ Hz, H-6′$_A$) overlapped by 3.72 (1H, *dd*, $J_1 \sim J_2 \sim 9$ Hz, H-3′), 3.88 (1H, *dd*, $J_1 = 11.5$, $J_2 = 2$ Hz, H-6′$_B$) overlapped by 3.90 (1H, *dd*, $J_1 = 11.5$, $J_2 = 2$ Hz, H-6″$_B$), 3.93 (1H, *dd*, $J_1 = 3$, $J_2 = 2$ Hz, H-2″), 4.01 (1H, *dq*, $J_1 = 10$, $J_2 = 6$ Hz, H-5″), 4.48 (1H, *d*, $J = 8$ Hz, H-1‴), 4.50 (1H, *d*, $J = 8$ Hz, H-1′), 4.56 (1H, *br dd*, $J_1 \sim J_2 \sim 7$ Hz, H-16), 5.31 (1H, *dq*, $J_1 = 2$, $J_2 = 7$ Hz, H-20), 5.38 (1H, *d*, $J = 2$ Hz, H-1″), 5.70 (1H, *br s*, H-4). ^{13}C NMR: Table 2. DCIMS *m/z* (rel. int.): 656 $[M - 146 + NH_4]^+$ (1), 639 $[M-146 + H]^+$ (1), 623 $[M - 162 + H]^+$ (1), 316 (21), 315 $[M - 470 + H]^+$ (21), 300 (20), 299 (100); FABMS *m/z* (rel. int.): 807 $[M + Na]^+$ (17), 785 $[M + H]^+$ (8), 317 (100), 315 (90).

Nonaacetylglycoside-9. Acetylation of **12** (1.5 mg) gave 2.6 mg of nonaacetylglycoside-9. Amorphous. TLC: R_f 0.17 (S-2); anisaldehyde, violet, $[\alpha]_D^{20}$ −10° ($CHCl_3$; *c* 0.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2939, 1747, 1666. ^{1}H NMR (C_6D_6): δ0.58–2.35 (56H, *m*) including 0.75 and 0.96 (3H each, *s*, Me-18 and Me-19), 1.34 (3H, *d*, $J = 6$ Hz, Me-6″), 1.64, 1.68, 1.69, 1.71, 1.78, 1.80, 1.86, 1.89 (3H, *s*, Ac), 2.00 (3H, *d*, $J = 7$ Hz, Me-21) and 2.09 (3H, *s*, Ac), 3.48–3.54 (1H, *m*, H-5′), 3.62 (1H, *ddd*, $J_1 = 10$, $J_2 = 4$, $J_3 = 2$ Hz, H-5‴), 3.90 (1H, *dd*, $J_1 = 13$, $J_2 = 2$ Hz, H-6‴$_A$) overlapped by 3.91 (1H, *dd*, $J_1 = 9.5$, $J_2 = 8$ Hz, H-2′), 4.11 (1H, *dd*, $J_1 \sim J_2 \sim 9.5$ Hz, H-3′), 4.21 (1H, *dd*, $J_1 = 12.5$, $J_2 = 2$ Hz, H-6′$_A$), 4.28 (1H, *d*, $J = 8$ Hz, H-1′), 4.35 (1H, *br dd*, $J_1 \sim J_2 \sim 7$ Hz, H-16), 4.41 (1H, *dd*, $J_1 = 12.5$, $J_2 = 5$ Hz, H-6′$_B$), 4.48 (1H, *dd*, $J_1 = 13$, $J_2 = 4$ Hz, H-6‴$_B$), 4.58 (1H, *dq*, $J_1 = 10$, $J_2 = 6$ Hz, H-5″), 4.95 (1H, *d*, $J = 8$ Hz, H-1‴), 5.08 (1H, *dd*, $J_1 \sim J_2 \sim 9.5$ Hz, H-4′),

5.24 (1H, *dd*, $J_1 = 9.5$, $J_2 = 8$ Hz, H-2‴), 5.35 (1H, *dd*, $J_1 = 10$, $J_2 = 9.5$ Hz, H-4‴) overlapped by 5.39 (1H, *dq*, $J_1 = 1$, $J_2 = 7$ Hz, H-20), 5.57 (1H, *dd*, $J_1 = J_2 = 10$ Hz, H-4″), 5.64 (1H, *br s*, H-1″) overlapped by 5.66 (1H, *dd*, $J_1 \sim J_2 \sim 9.5$ Hz, H-3‴), 5.73 (1H, *dd*, $J_1 = 10$, $J_2 = 4$ Hz, H-3″), 5.83 (1H, *br s*, H-4) overlapped by 5.85 (1H, *dd*, $J_1 = 4$, $J_2 = 1.5$ Hz, H-2″).

D-(+)-*Pinitol* (**13**). Crystals from MeOH (70 mg), mp 179° (ref. [5] mp 185–186°). TLC: R_f 0.08 (*S*-1); anisaldehyde, light blue. $[\alpha]_D^{20} + 60°$ (H_2O; *c* 6.5) (ref. [22] $[\alpha]_D^{20} + 64.6°$ (H_2O; *c* 1.0)). Substance identical with an authentic sample [1].

Sucrose. Crystals from MeOH (100 mg), mp 172°. TLC: R_f 0.05 (*S*-1); anisaldehyde, brown-blue. $[\alpha]_D^{20} + 65°$ (H_2O; *c* 9.0) (ref. [23] $[\alpha]_D^{22} + 66.5°$ (H_2O)). Substance identical with an authentic sample.

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