Marine Steroids. Part III.[1] On the Structure of Marthasterone Glucoside, from the Starfish *Marthasterais glacialis*

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A ^{1}H n.m.r. method based on the chemical shift of the 10-methyl protons is used to locate the position of the glucose residue in marthasterone 6α-glucoside, which is characterised as its penta-acetate, 3β-acetoxy-23-oxo-5α-cholesta-9(11),24-dien-6α-yl tetra-*O*-acetyl-β-D-glucoside (20). The 3β- and 6α-tetra-*O*-acetylglucosides of several model 5α-steroids are used to establish chemical shift relationships.

ACIDIC hydrolysis of the crude saponin mixture obtained from the Atlantic starfish, *Marthasterias glacialis*,[2] gives marthasterone (1) and its 24,25-dihydro-derivative as the major aglycones.[3] During attempts to isolate a proposed[3] biogenetic precursor of marthasterone, milder conditions for the hydrolysis of the saponin mixture were investigated, and a polar compound was obtained. It was characterised as its high-melting penta-acetate, and shown to be a monoglucoside of marthasterone: it furnished marthasterone (1) and D-glucose upon complete hydrolysis. Evidence had previously been obtained that glucose was the last of the conjugating moieties to be released during hydrolysis of the *M. glacialis* saponins.[2] As the small amount of monoglucoside available discouraged the use of classical methods for determining which of the two hydroxy-groups of marthasterone was

[1] Part II, D. S. H. Smith and A. B. Turner, *J.C.S. Perkin I*, 1975, 1751.

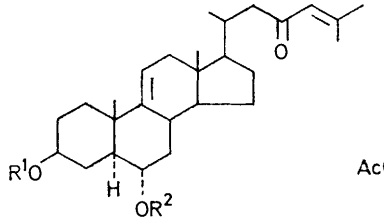
[2] A. M. Mackie and A. B. Turner, *Biochem. J.*, 1970, **117**, 543.

[3] D. S. H. Smith, A. B. Turner, and A. M. Mackie, *J.C.S. Perkin I*, 1973, 1745.

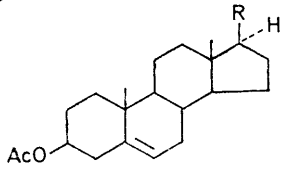
glycosidically linked, the ^1H n.m.r. method [4] involving the influence of nuclear substituents upon the chemical shift of the 10-methyl group was investigated for location of the sugar residue in a series of model conjugates. The effect of an acetoxy- or hydroxy-group at the 3β- or 6α-position on the resonance position of the angular methyl protons of 5α-steroids is well known,[4] but the corresponding effect of a fully acetylated glucose residue has not been studied.

A series of 3β-acetoxy-6α-alcohols (5)—(7), prepared by hydroboration of the Δ^5-steroids (2)—(4), were converted into the 6α-tetra-*O*-acetylglucosides (8)—(10) by conjugation with 1α-bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (11), with cadmium carbonate as catalyst.[5] Attempts at conjugation by the normal Koenigs–Knorr procedure with silver oxide gave inferior results. The 3β-glucosides (16)—(19) were prepared by the same method from the 3β-alcohols (12)—(15).

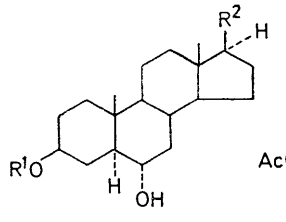
The presence of the acetylated glucose residue in the marthasterone conjugate and the model compounds was confirmed by mass spectral analyses. These showed the



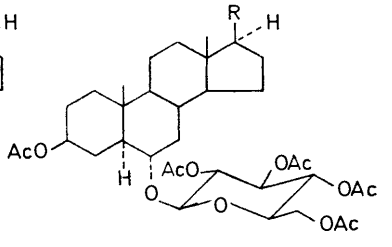
(1) $R^1 = R^2 = H$
(21) $R^1 = R^2 = Ac$
(22) $R^1 = Ac$, $R^2 = H$
(23) $R^1 = H$, $R^2 = Ac$



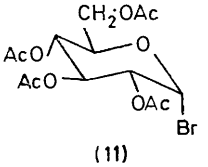
(2) R = OAc
(3) R = MeCH·[CH_2]$_3$·$CHMe_2$
(4) R = MeCH·[CHBr]$_2$·CHEt·$CHMe_2$



(5) R^1 = Ac, R^2 = OAc
(6) R^1 = Ac, R^2 = C_8H_{17}
(7) R^1 = Ac, R^2 = $C_{10}H_{18}Br_2$

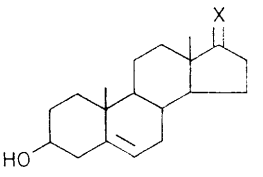


(8) R = OAc
(9) R = C_8H_{17}
(10) R = $C_{10}H_{18}Br_2$

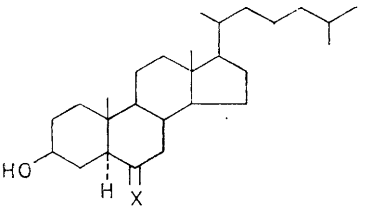


(11)

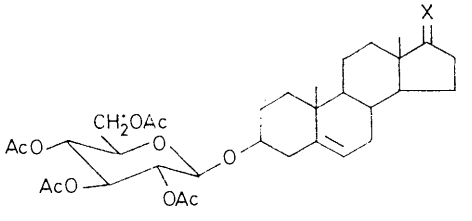
expected prominent ions at *m/e* 331.1028(8%, $C_{14}H_{19}O_9$), 169(27%), and 109(20%) resulting from breakdown of the sugar residue,[6] and although molecular ions were only rarely observed, fragments arising through loss of acetic acid or the steroid side chain were recorded. The side chain fragment at *m/e* 83, containing the 23-oxo-function and derived by McLafferty rearrangement followed by loss of a methyl group, was the base peak in the mass spectrum of the marthasterone conjugate. This fragment ion also features prominently in the spectrum of marthasterone diacetate.[3]



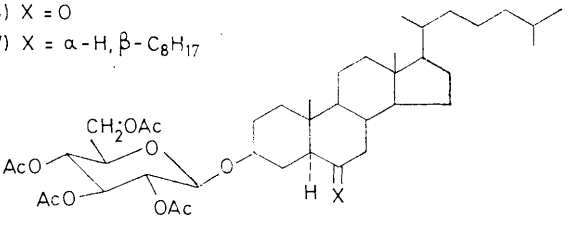
(12) X = O
(13) X = α-H,β-C_8H_{17}



(14) X = H_2
(15) X = O



(16) X = O
(17) X = α-H,β-C_8H_{17}



(18) X = H_2
(19) X = O

N.m.r. Data.—The n.m.r. spectrum of the marthasterone glucoside penta-acetate exhibited angular methyl signals at τ 9.37 and 9.03, those of side-chain olefinic methyl groups at 8.12 and 7.86, and signals for five acetoxy-groups at 8.00—7.93. The signals due to the methine protons of the sugar residue in the region τ 5.85—4.80 were very similar in pattern to those of the model glucosides, and the relatively large coupling constant of the anomeric proton (9 Hz; τ 5.46) established the β-configuration at the anomeric carbon atom.

The chemical shifts of angular methyl protons in the model conjugates are collected in Tables 1 and 2, together with the increments observed between the free alcohol and its glucoside ($\Delta\tau_1$), and between the acetate and the tetra-*O*-acetyl glucoside ($\Delta\tau_2$). The chemical shifts of the 10-methyl protons in the corresponding marthasterone derivatives are listed in Table 3, together with the shift differences between the same pairs of derivatives. The values for the marthasterone monoacetates

[4] R. F. Zurcher, *Helv. Chim. Acta*, 1961, **44**, 1380; 1963, **46**, 2054.
[5] R. B. Conrow and S. Bernstein, *J. Org. Chem.*, 1971, **36**, 863.
[6] H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Structure Elucidation of Natural Products by Mass Spectroscopy,' Vol. II, Holden–Day, San Francisco, 1964, pp. 203—213.

(22) and (23) are calculated from data on the diacetate (21) [3] by using the chemical shifts from Tables 1 and 2, together with literature data.[4] The values of $\Delta\tau$ for the diacetate (21) and the 3-monoacetate (22) are similar to those in Table 2, indicating conjugation of the glucose

TABLE 1

N.m.r. data of steroidal 3β-tetra-*O*-acetylglucosides

	τ		$\Delta\tau_1$(10-Me)		$\Delta\tau_2$(10-Me)	
Steroid	10-Me	13-Me	3β-OH	3β-TAG	3β-OAc	3β-TAG
(12)	8.96	9.11				
(12) acetate	8.95	9.11				
(16)	8.97	9.10	−0.01		−0.02	
(14)	9.20	9.35				
(14) acetate	9.18	9.36				
(18)	9.22	9.36	−0.02		−0.04	
(13)	9.00	9.32				
(13) acetate	8.98	9.32				
(17)	9.01	9.32	−0.01		−0.03	
(15)	9.25	9.34				
(15) acetate	9.23	9.34				
(19)	9.27	9.35	−0.02		−0.04	

TABLE 2

N.m.r. data of steroidal 6α-tetra-*O*-acetylglucosides

	τ		$\Delta\tau_1$(10-Me)		$\Delta\tau_2$(10-Me)	
Steroid	10-Me	13-Me	6α-OH	6α-TAG	6α-OAc	6α-TAG
(5)	9.16	9.22				
(5) acetate	9.09	9.22				
(8)	9.15	9.22	+0.01		−0.06	
(6)	9.17	9.35				
(6) acetate	9.11	9.34				
(9)	9.17	9.35	0.00		−0.06	
(7)	9.17	9.27				
(7) acetate	9.10	9.27				
(10)	9.16	9.26	+0.01		−0.06	

TABLE 3

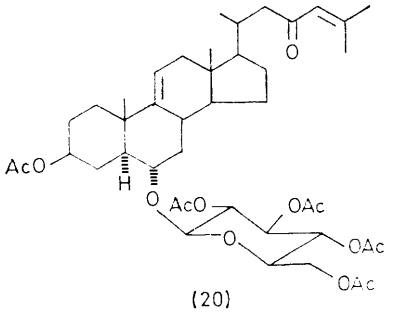
N.m.r. data on marthasterone derivatives

Steroid	τ(10-Me)	Δτ
(20)	9.03	
(21)	8.97	−0.06
(22)	9.04 *	+0.01
(23)	8.99 *	−0.04

* Calc. from (21).[4]

residue at the 6α-position. The value of $\Delta\tau$ for the 6-monoacetate (23) does not correlate with the 3β-glycoside derivatives of Table 1, being too large, also suggesting 6α-conjugation. Although the $\Delta\tau$ values are small the results are consistent for the two groups of conjugates studied, and we believe that the observed shift differences are sufficient to establish the position of conjugation in the present case.

It is clear that the chemical shift of the anomeric proton depends upon the position of glycosidation [τ 5.40 in the 3β-glucosides (16)—(19) and 5.51 in the 6α-glucosides (8)—(10)] and is little affected by the other substituents in the steroid nucleus. In the 6α-tri-*O*-acetyl deoxyglucoside (24), isolated by Sheikh and Djerassi [7] from the starfish, *Acanthaster planci*, the anomeric proton signal appears at τ 5.47, indicating that the 9,11-double bond is close enough to have a deshielding effect. The anomeric proton in marthasterone glucoside pentaacetate shows a similar resonance position (τ 5.46), providing further evidence for the 6α-structure (20) for the conjugate.



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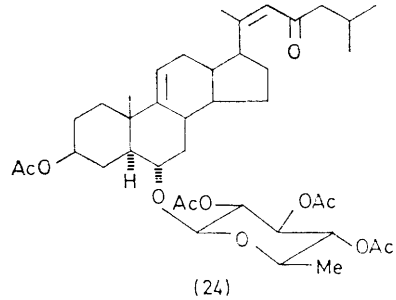

(24)

[7] Y. M. Sheikh and C. Djerassi, *Tetrahedron Letters*, 1973, 2927.
[8] S. M. Ali and A. B. Turner, *J.C.S. Perkin I*, 1974, 2225.

EXPERIMENTAL

N.m.r. spectra were recorded for solutions in deuteriochloroform on a Varian HA-100 spectrometer using tetramethylsilane as internal standard. Tabulated values for the chemical shifts of the angular methyl groups were checked by means of the frequency counter. For other general directions see refs. 3 and 8.

Hydrolysis of Saponins of M. glacialis.—A solution of the crude starfish saponins [1] (750 mg) in aqueous hydrochloric acid (2N; 30 ml) was heated for 45 min on a steam-bath. The brown precipitate (85 mg) was collected and dissolved in a mixture of pyridine (10 ml) and acetic anhydride (3 ml). After 48 h water (6 ml) was added and the solution was left for a further 12 h before it was evaporated *in vacuo* to give a brown gum (92 mg). T.l.c. of this material in benzene–ethyl acetate (4 : 1) gave marthasterone and dihydromarthasterone diacetates [1] (R_F 0.65) (38 mg) and the monoglucoside fraction (R_F 0.08) (21 mg). The latter was separated into the following two components by t.l.c. in benzene–ethyl acetate (20 : 1) after 5 developments: 3β-*acetoxy*-23-*oxo*-5α-*cholesta*-9(11),24-*dien*-6α-*yl tetra*-O-*acetyl*-β-D-*glucoside* (20) (8 mg), as needles, m.p. 250—253°, R_F 0.25 (Found: $M^+ - C_6H_{12}O$, 686.3303. $C_{37}H_{50}O_{12}$ requires 686.3301), $\lambda_{max.}$ (MeOH) 235 nm (ε 8 800), $\nu_{max.}$ 1 745, 1 690sh, 1 235, 1 040, and 760 cm^{-1}, τ 9.37 (s, 13-Me), 9.07 (d, *J* 6 Hz, 21-Me), 9.03 (s, 10-Me), 8.12 (s, 26-Me), 8.00—7.93 (m, 5 × Ac), 7.86 (s, 27-Me), 6.55 (m, 6β-H), 6.30 (m, 5′-H), 5.82 (m, 6′-H_2), 5.45 (d, *J* 8 Hz, 1′-H), 5.35—4.80 (m, 3α-, 2′-, 3′-, and 4′-H), 4.70 (m, olefinic 11-H), and 3.96 (m, olefinic 24-H), *m/e* 686 (0.3%), 438(3), 369(6), 331(8), 271(14), 169(27), 109(20), 98(18), 91(13), 83(100), and 55(35); and 3β-*acetoxy*-23-*oxo*-5α-*cholest*-9(11)-*en*-6α-*yl tetra*-O-*acetyl*-β-D-*glucoside* (4 mg) as needles, m.p. 248—252°, R_F 0.30 (Found: $M^+ - C_4H_6O_3$, 686.4031. $C_{39}H_{58}O_{10}$ requires 686.4007), $\nu_{max.}$ 1 750, 1 240, 1 040, and 760 cm^{-1}, τ 9.37 (s, 13-Me), 9.11—9.03 (m, 10-, 21-, 26-, and 27-Me), 8.00—7.92 (m, 5 × OAc), 6.65—6.25 (m, 5′- and 6β-H), 5.82 (m, 6′-H_2), and 5.6—4.7 (m, 1′-, 4′-, 3α-, and olefinic 11-H), *m/e* 729(0.1%),

686(0.1), 440(10), 381(26), 331(9), 281(9), 169(12), 109(11), 85(30), 44(100), and 43(32).

3β-*Acetoxy*-5α-*cholestan*-6α-*ol* (6).—Hydroboration–oxidation [9] of cholesteryl acetate gave the 6α-alcohol (18%), m.p. 127—128° (lit.,[9] 127—128°), τ 9.36 (s, 13-Me), 9.19 (s, 10-Me), 7.99 (s, OAc), and 6.50 (m, 6β-H).

Hydroboration of 3α-*Acetoxyandrost*-5-*en*-17-*one*.—Similar treatment of 3β-acetoxyandrost-5-en-17-one (2 g) gave 5α-androstane-3β,6α,17β-triol (0.16 g, 9%), m.p. 235—235.5° (from aqueous methanol) (Found: C, 69.9; H, 10.6%; M^+, 308.2350. $C_{19}H_{32}O_3,H_2O$ requires C, 69.9; H, 10.4%; M, 308.2351), $\nu_{max.}$ 3 300, 2 920, 1 450, and 1 050 cm^{-1}, τ[$(CD_3)_2$SO] 9.42 (s, 13-Me), 9.31 (s, 10-Me), 7.64br (m, 3α-, 6β-, and 17α-H); and 3β-*acetoxy*-5α-*androstane*-6α,17β-*diol* (0.43 g, 20%), m.p. 192—193° [Found: C, 71.8; H, 9.6%; (M − $C_2H_4O_2$), 290.2244. $C_{21}H_{34}O_3$ requires C, 72.0; H, 9.7%. $C_{19}H_{30}O_2$ requires *m/e* 290.2245], $\nu_{max.}$ 3 380, 2 930, 1 737, 1 710, and 1 265 cm^{-1}, τ[$(CD_3)_2$SO] 9.40 (s, 13-Me), 9.25 (s, 10-Me), 8.06 (s, OAc), 6.80br (m, 6β- and 17α-H), and 5.50br (m, 3α-H).

Hydroboration of 3β,17β-*Diacetoxyandrost*-5-*ene* (2).—Similar treatment of the diacetate (2) (2 g), with ether–hexane (2 : 1) as eluant in the final column chromatography, gave 3β,17β-*diacetoxy*-5α-*androstan*-6α-*ol* (5) (0.73 g, 36%), m.p. 163—164° [Found: C, 70.5; H, 9.5%; (M − H_2O)$^+$, 374.2456. $C_{23}H_{36}O_6$ requires C, 70.4; H, 9.2%. $C_{23}H_{34}O_4$ requires *m/e* 374.2456], $\nu_{max.}$ 3 550, 2 940, 1 745, 1 720, 1 260, and 1 050 cm^{-1}, τ[$(CD_3)_2$SO] 9.27 (s, 13-Me), 9.23 (s, 10-Me), 8.04 (s, 2 × OAc), 6.80 (m, 6β-H), and 5.48 (m, 3α- and 17β-H), τ($CDCl_3$) 9.22 (s, 13-Me), 9.16 (s, 10-Me), 7.98 (s, 2 × OAc), 6.60 (m, 6β-H), 5.40 (m, 3α- and 17β-H); 17β-*acetoxy*-5α-*androstane*-3β,6α-*diol* (0.30 g, 16%), m.p. 196—197.5° (from aqueous acetone) (Found: C, 68.7; H, 9.6%; M^+, 350.2453. $C_{21}H_{34}O_4$ requires C, 68.4; H, 9.8%; M, 350.2456), $\nu_{max.}$ 3 370, 2 930, 1 740, and 1 250 cm^{-1}, τ[$(CD_3)_2$SO] 9.27 (s, 10- and 13-Me), 8.02 (s, OAc), 6.80br (m, 3α- and 6β-H), 5.50 (m, 17α-H); and 5α-androstane-3β,6α,17β-triol (0.10 g, 6%), m.p. 235—235.5°, identical to material described above.

General Procedure for Conjugation of Steroid Alcohols.[5]—A mixture of the steroid (5 mmol) and cadmium carbonate (1.72 g, 10 mmol) in toluene (100 ml) was distilled until 25 ml of toluene had been removed. A solution of acetobromoglucose [10] (4.11 g, 10 mmol) in dry toluene (100 ml) was added dropwise while toluene was distilled off at the same rate. Distillation was continued for a further 30 min, during which the same quantity (*ca.* 50 ml) of dry toluene was added as was distilled off. The cooled mixture was filtered through Celite and evaporated *in vacuo*, and the residual oil was dissolved in acetone (30 ml) and poured into water (200 ml). The precipitate was collected on a pad of Celite, washed with water, and dissolved in dichloromethane. Evaporation of the dried (Na_2SO_4) solution gave the conjugate as a gum, which was crystallised from ethanol. The following conjugates were prepared by this method.

3β-*Acetoxy*-5α-*cholestan*-6α-*yl tetra*-O-*acetyl*-β-D-*glucopyranoside* (9) (53%), m.p. 220—225° [after separation by t.l.c. on silica gel with ethyl acetate–toluene (1 : 4)] (Found: C, 66.0; H, 8.3. $C_{43}H_{68}O_{12}$ requires C, 66.5; H, 8.8%), $\nu_{max.}$ 2 950, 1 755, 1 370, 1 230, and 1 040 cm^{-1}, τ 9.35 (s, 13-Me), 9.17 (s, 10-Me), 7.99—7.93 (m, 5 × OAc), 6.90—6.16 (m, 3α-, 6β-, and 5′-H), 5.82 (m, 6′-H_2), 5.51 (d, J 8 Hz, 1′-H), and 4.93 (m, 2′-, 3′-, and 4′-H), *m/e* 716 (M − $C_2H_4O_2$, 0.5%), 429(3), 428(4), 388(6), 370(15), 369(60), 331(5), 169(19), 109(16), 95(25), and 81(100).

3β-*Acetoxy*-22,23-*dibromo*-24-*ethyl*-5α-*cholestan*-6α-*yl tetra*-O-*acetyl*-β-D-*glycopyranoside* (10) (50%), m.p. 211—214° [after t.l.c. on silica gel with ethyl acetate–toluene (1 : 4)] (Found: M^+ − $3_{10}H_{21}Br_2$, 661.3223; M^+ − $C_{14}H_{18}O_9$, 632.2264. $C_{35}H_{49}O_{12}$ requires 661.3223; $C_{31}H_{52}{}^{79}Br^{81}BrO_3$ requires 632.2263), $\nu_{max.}$ 2 930, 1 760, 1 375, 1 235, and 1 040 cm^{-1}, τ 9.26 (s, 13-Me), 9.16 (s, 10-Me), 8.02—7.93 (m, 5 × OAc), 6.90—6.20 (m, 3α-, 6β-, and 5′-H), 5.84 (m, 6′-H_2), 5.51 (m, 22-, 23-, and 1′-H), and 4.94 (m, 2′-, 3′-, and 4′-H), *m/e* 661(0.2%), 632(0.2), 555(2), 331(10), 231(11), 149(21), 137(60), 110(80), 109(74), 77(100), and 95(100).

3β,17β-*Diacetoxy*-5α-*androstan*-6α-*yl tetra*-O-*acetyl*-β-D-*glucopyranoside* (8) (41%), m.p. 293—295° (Found: C, 61.9; H, 7.3. $C_{37}H_{54}O_{14}$ requires C, 61.6; H, 7.5%), $\nu_{max.}$ 2 940, 1 755, 1 730, 1 370, 1 227, and 1 040 cm^{-1}, τ 9.22 (s, 13-Me), 9.15 (s, 10-Me), 8.01—7.94 (m, 5 × OAc), 6.72 (m, 6β-H), 6.34 (m, 5′-H), 5.82 (m, 6′-H_2), 5.50 (d, J 8 Hz, 1′-H), 5.40 (m, 3α- and 17α-H), and 4.95 (m, 2′-, 3′-, and 4′-H), *m/e* 315(10%), 255(30), 169(7), 109(11), 107(17), 93(25), 81(100), 79(25).

17-*Oxoandrost*-5-*en*-3β-*yl tetra*-O-*acetyl*-β-D-*glucopyranoside* (16) (40%), m.p. 196—197° (lit.,[11] 96—97°), τ 9.10 (s, 13-Me), 8.97 (s, 10-Me), 8.00, 7.98, 7.95, and 7.93 (s, 4 × OAc), 7.74—6.14 (m, 3- and 5′-H), 5.82 (m, 6′-H_2), 5.40 (d, J 8 Hz, 1′-H), 4.94 (m, 2′-, 3′-, and 4′-H), 4.62 (m, 6-H), *m/e* 331(10%), 272(11), 721(75), 720(100), 169(75), and 109(50).

Cholest-5-en-3β-yl tetra-*O*-acetyl-β-D-glucopyranoside (17) (37%), m.p. 156—158° (lit.,[12] 156—158°), τ 9.32 (s, 13-Me), 9.01 (s, 10-Me), 8.00, 7.99, 7.96, and 7.92 (s, 4 × OAc), 6.70—6.20 (m, 3′- and 5′-H), 5.82 (m, 6′-H_2), 5.40 (d, J 8 Hz, 1′-H), 4.94 (m, 2′-, 3′-, and 4′-H), and 4.65 (m, olefinic 6-H), *m/e* 370(11%), 369(63), 368(100), 331(10), 169(71), 147(10), 109(25), and 95(11).

5α-Cholestan-3β-yl tetra-*O*-acetyl-β-D-glucopyranoside (18) (21%), m.p. 174—175° (lit.,[13] 174—175°), τ 9.36 (s, 13-Me), 9.22 (s, 10-Me), 8.00, 7.99, 7.97, and 7.94 (s, 4 × OAc), 6.60—6.20 (m, 3- and 5′-H), 5.83 (m, 6′-H_2), 5.41 (d, J 8 Hz, 1′-H), and 4.95 (m, 2′-, 3′-, and 4′-H), *m/e* 372(14%), 371(56), 257(10), 242(10), 217(11), 203(11), 200(14), 177(14), 169(50), 163(28), 157(32), 149(45), 135(35), 109(63), 95(100), 83(63), and 81(71).

6-*Oxo*-5α-*cholestan*-3β-*yl tetra*-O-*acetyl*-β-D-*glucopyranoside* (19) (14%), m.p. 183—184° (Found: C, 66.7; H, 8.7. $C_{41}H_{66}O_{11}$ requires C, 67.0; H, 9.0%), $\nu_{max.}$ 2 950, 1 753, 1 715, and 1 220 cm^{-1}, τ 9.35 (s, 13-Me), 9.27 (s, 10-Me), 8.00—7.93 (m, 4 × OAc), 6.60—6.24 (m, 3- and 5′-H), 5.83 (m, 6′-H_2), 5.40 (d, J 8 Hz, 1′-H), and 4.95 (m, 2′-, 3′-, and 4′-H), *m/e* 386(25%), 385(100), 384(17), 269(10), 368(10), 367(40), 331(5), 200(14), 169(32), 157(10), 145(10), 140(11), 109(14), 95(45), and 81(35).

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