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Synthesis of cardenolide glycosides and putative biosynthetic precursors of cardenolide glycosides

Melitta Luta, Andreas Hensel, and Wolfgang Kreis

Friedrich-Alexander-Universität Erlangen-Nürnberg, Institut für Botanik und Pharmazeutische Biologie, Erlangen, Germany

A rapid and efficient procedure for glycosylation of steroids was established using a modified Koenigs–Knorr procedure. Peracetylated β-glycosides were synthesized by reaction of cardenolides, various pregnanes and 23-nor-5,20(22)E-choldienic acid at room temperature with the peracetylated 1-bromo derivatives of D-glucose, D-galactose, D-fucose and cellobiose. Subsequent deprotection was performed by alkaline hydrolysis with sodium methoxide. Structures of the respective glycosides were established by NMR techniques. The complete protocol was shown to be non-destructive at all stages to the sugar moiety and the steroidal nucleus. The γ-unsaturated lactone ring of the cardenolides was shown to remain intact and no formation of C-14 unsaturated compounds was observed. (*Steroids* **63**:44–49, 1998) © *1998 by Elsevier Science Inc.*

Keywords: cardenolides; glycosylation; norcholenic acid; pregnanes; NMR

Introduction

Despite considerable progress in carbohydrate chemistry in the past few years, the stereoselective formation of O-glycosidic bonds between carbohydrates and steroids is usually a time consuming process with relatively low yields of the newly formed glycosides.[1,2] This is due to the low reactivity of the secondary alcohol functions in the steroid moiety and the necessity to activate the glycosyl donors. Methods described for glycosidation of steroids are mostly based on the classical Koenigs–Knorr synthesis with α-1,2-*cis* halogenated carbohydrates as reactants.[1] These methods are not generally applicable but have to be adapted to the specific requirements of the substrates.[3] The catalysts and solvents employed, which influence stereoselectivity, and the non-destructive removal of the blocking groups must be optimized to obtain acceptable yields. In contrast to these multi-stage procedures the few cases of direct synthesis of O-glycosides using unprotected carbohydrates,[4] highly reactive glycosyl donors[5] and enzymatic glycosylation[6] are very effective, but are restricted to special applications.

The aim of the present study was to establish a method for O-glycosylation of steroids, capable of condensing any mono- or oligosaccharide in acceptable yields stereoselectively to the hydroxyl function at C-3 of a given steroid. Such a method should be applicable widely for rapid synthesis of new pharmacological active compounds such as new cardenolide glycosides or putative biosynthetic precursors of steroid pathways such as pregnane glycosides or glycosides of norcholenic acids. The glycosidation method described here is a versatile variation of the Koenigs–Knorr methodology which was applied to a variety of steroids, using as carbohydrate components several hexoses, the sensitive 6-deoxyhexose fucose and cellobiose as a model substance for an oligosaccharide.

Experimental

General

Chemicals used were purchased from Merck (Darmstadt, Germany) in analytical grade quality. Acetobromoglucose, acetobromogalactose and 21-acetoxy pregnenolone were purchased from Sigma (Steinheim, Germany). Disilver maleinate was prepared as described previously.[7] 23-Nor-5,20(22)E-chol-dienic acid-3β-ol was synthesized according to Maier et al.[8] ^{1}H and ^{13}C NMR spectra were measured in deuterochloroform and deuterated methanol on a Bruker AM 360 spectrometer at 360 and 90 MHz, respectively. Chemical shifts are expressed in δ ppm downfield relative to tetramethylsilane as internal standard. Coupling constants are given in Hertz. Thin layer chromatography was performed on precoated plates Silica G60 F_{254} (Merck, Darmstadt, Germany). Mobile phase I for the separation of peracetylated sugars: Ethyl acetate/CH_2Cl_2 (70:30 v/v); mobile phase II for the separation of peracetylated and deprotected steroid glycosides: CH_2Cl_2/MeOH/H_2O (80:18:2 v/v/v). Spots were visualized by spraying of the developed plates with a mixture of anisaldehyde (0.5 mL), acetic acid (10 mL), methanol (85 mL) and sulfuric acid (5 mL) with subsequent heating to 110°C for 5 min. Cardenolide

Address reprint requests to A. Hensel, Friedrich-Alexander-Universität Erlangen-Nürnberg, Institut für Botanik und Pharmazeutische Biologie, Staudtstr. 5, 91058 Erlangen, Germany.
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glycosides were analyzed by HPLC.[9] Melting points (m.p.) were determined with a Büchi 535 system and are uncorrected. Column chromatography was performed on Sephadex LH 20 (Pharmacia, Upsala, Sweden) with methanol as the mobile phase (1 mL/min); column dimensions: 80 × 2 cm. Elemental analysis was performed with a Carlo Erba Elemental System. For additional purity testing a minimum purity of not less than 95% was specified which was determined by TLC or HPLC. All compounds described here conform to this specification.

General procedure for the peracetylation of carbohydrates

The dried carbohydrate (5 mmol) was dissolved in absolute pyridine (10 mL) and treated at 0°C with acetic anhydride (5 mL). The reaction mixture was stirred for 2 h at 0°C and then kept at 4°C for 24 h. The excess of acetic anhydride was destroyed under ice cooling by dropwise addition of water (1 mL). The solution was extracted three times with CH_2Cl_2; the organic phases were combined and subsequently washed with diluted H_2SO_4, Na_2CO_3 and water. Multiple evaporation of the residue with ethanol afforded pure products. 1,2,3,4-Tetra-O-acetyl-β-D-fucopyranose from D-fucose (0.82) was obtained as a colorless syrup (1.58 g, 94%); TLC: R_f(l) = 0.42. 1,2,2′,3,3′,4′,6,6′-Octa-0-acetyl-cellobiose from D-cellobiose (1.71 g); the crude product was crystallized from CH_2Cl_2 with pentane affording white crystals (2.6 g, 77%); m.p. 226–228°C (lit.[10] 228°C), TLC: R_f(l) = 0.57.

General procedure for the activation of peracetylated carbohydrates to the α-D halogenoses

The peracetylated carbohydrates (5 mmol) were dissolved in dry CH_2Cl_2 (15 mL) and treated with HBr 33% in glacial acetic acid (5 mL). The reaction mixture was stirred for 30 min at room temperature. The solution was extracted three times with CH_2Cl_2; the organic phases were combined and subsequently washed with diluted H_2SO_4, Na_2CO_3 and water. The solvent was evaporated and the residue dried at reduced pressure.

2,3,4-tri-O-acetyl-α-D-fucopyranosyl bromide from 1,2,3,4-Tetra-O-acetyl-β-D-fucopyranose (1.65 g) was obtained as a slightly yellow syrup (1.74 g, 99%); TLC: R_f(l) = 0.33; ^{1}H-NMR ($CDCl_3$): δ ppm 1.22 (d, $J_{5,6}$ = 6.5 Hz, 3H H-6), 2.01, 2.11, 2.17 (3 × s, 3 × 3 H, 3 × CH_3-CO), 4.41 (q, $J_{5,6}$ = 6.5 Hz, 1H, H-5), 5.03 (q, $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 10.4 Hz, 1H, H-2), 5.2–5.4 (m, 2H, H-3,4), 6.70 (d, $J_{1,2}$ = 3.9 Hz, 1H, H-1). Data conform to the lit.[6] 2,2′,3,3′,4′,6,6′-Hepta-O-acetyl-cellobiosyl bromide from 1,2,2′,3,3′,4′,6,6′-Octa-O-acetyl-cellobiose (2.26 g). The crude product was dissolved in acetone and crystallized from pentane at 4°C (2.0 g, 86%); m.p 175–184°C (lit.[10] 180°C); TLC: R_f(l) = 0.45; ^{1}H-NMR ($CDCl_3$): δ ppm: 1.99–2.15 (7 × s, 7 × 3H, 7 × CH_3-CO), 3.67–3.87 (m, 2H, H-3′, H-4′), 3.84 (m, 1H, H-2′), 4.05 (m, 1H, H-6_B′), 4.17 (m, 1H, H-6_B), 4.38 (m, 1H, H-$6'_A$), 4.52 (m, 1H, H-5′), 4.55 (d, *J* = 8 Hz, H-1′), 4.77 (m, 1H, H-6_A), 4.95 (m, 1H, H-2), 5.10 (m, 2H, H-3, H-4), 6.53 (d, *J* = 8Hz, H-1).

General procedure for glycosylation of steroid alcohols

1 mmol of steroid and 2.75 mmol of disilver maleinate were dissolved in a mixture of 12 mL of dry Et_2O/CH_2Cl_2 (3:1 v/v). 2.5 mmol of peracetylated halogenose were added. The reaction mixture was stirred for 72 h at room temperature. The mixture was filtered and the clear solution evaporated to dryness. For deacetylation the residue obtained was dissolved in 10 mL of methanol and treated with 50 μmol of sodium methoxide at 60°C for 8 h. After neutralization and evaporation the residue was dissolved in 2 mL of methanol and purified by column chromatography on Sephadex LH20.

3β-O-(β-D-glucopyranosyl)-digitoxigenin (2) from 375 mg of digitoxigenin (1). Afforded 247 mg (46%) product; m.p.: 226–228°C (lit.[11] 228–230°C); TLC: R_f (II) = 0.31; HPLC purity: 100% (t_R 12.2 min); ^{1}H-NMR(CD_3OD) δ ppm: 0.88 (s, 3H, H-18), 0.96 (s, 3H, H-19), 2.83 (m, 1H, H-17), 3.18 (m, 1H, H-2′), 3.24 (m, 1H, H-5′), 3.30 (m, 2H, H-3′, H-4′), 3.65 (m, 1H, H-$6'_A$), 3.83 (dd, 1H, H-$6'_B$), 4.08 (br.s, 1H, H-3), 4.31 (d, $J_{1',2'}$ = 6.5 Hz, 1H, H-1′), 4.85 (dd, *J* = 18.1 Hz, 1H, H-21), 5.89 (t, *J* = 3 Hz, 1H, H-22); ^{13}C-NMR(CD_3OD) δ ppm: 16.40 (C-18), 24.06 (C-19), 62.84 (C-6′), 71.76 (C-4′), 75.21 (C-2′), 75.35 (C-3), 77.85 (C-5′), 78.25 (C-3′), 102.69 (C-1′), 117.78 (C-22), 177.25 (C-23), 178.46 (C-20); data conform to lit.[11]

3β-O-(β-D-galactopyranosyl)-digitoxigenin (3) from 375 mg of digitoxigenin (1). Afforded 262 mg (49%) product; m.p. 228°C (lit.[11] 211°C); TLC: R_f(II) = 0.29; HPLC purity: 99% (t_R 10.3 min); ^{1}H-NMR(CD_3OD) δ ppm: 0.88 (s, 3H, H-18), 0.96 (s, 3H, H-19), 2.83 (m, 1H, H-17), 3.28 (m, 1H, H-2′), 3.52 (dd, 1H, H-6_A′), 3.80 (dd, 1H, H-6_B′), 4.07 (s, 1H, H-3), 4.27 (d, $J_{1',2'}$ = 7 Hz, 1H, H-1′), 4.87 (dd, *J* = 18.1 Hz, 1H, H-21), 5.89 (t, 1H, H-22); ^{13}C-NMR(CD_3OD) δ ppm: 16.44 (C-18), 24.09 (C-19), 62.43 (C-6′), 70.30 (C-4′), 72.70 (C-2′), 75.14 (C-3′), 75.36 (C-3), 76.48 (C-5′), 103.41 (C = 1′), 117.80 (C-22), 177.25 (C-23), 178.48 (C-20); data conform to lit.[11]

3β-O-(β-D-fucopyranosyl)-digitoxigenin (4) from 375 mg of digitoxigenin (1). Afforded 223 mg (43%) product; m.p. 195°C (lit.[6]: 195°C); TLC: R_f(II) = 0.49; HPLC purity: 95% (t_R 27.4 min); ^{1}H-NMR(CD_3OD) δ ppm: 0.88 (s, 3H, H-18), 0.97 (s, 3H, H-19), 1.32 (d, $J_{5,6}$ = 6.5 Hz, 1H, H-6′), 2.87 (m, 1H, H-17), 3.56–3.63 (m, 3H, H-2′,3′,5′), 3.74 (m, 1H, H-4′), 4.03 (br.s, 1H, H-3), 4.23 (d, $J_{1',2'}$ = 7.6 Hz, 1H, H-1′), 4.88 (dd, *J* = 18.1 Hz, 2H, H-21), 5.87 (s, 1H, H-22); data conform to lit.[6]; ^{13}C-NMR(CD_3OD) δ ppm: 16.41 (C-18), 17.14 (C-6′), 24.08 (C-19), 71.81 (C-5′), 72.37 (C-4′), 73,04 (C = 2′), 83.80 (C-3′), 75.38 (C-3), 102.4 (C-1′), 117.80 (C-22), 177.28 (C-23), 178.50 (C-20).

3β-O-(β-D-cellobiosyl)-digitoxigenin (5) from 375 mg of digitoxigenin (1). Afforded 271 mg (39%) product; m.p. 212°C; TLC: R_f(II) = 0.12; HPLC purity: 100%, (t_R 9.7 min); ^{1}H-NMR(CD_3OD) δ ppm: 0.88 (s, 3H, H-18), 0.95 (s, 3H, H-19), 2.83 (m, 1H, H-17), 4.06 (br.s, 1H, H-3), 4.31 (d, $J_{1'',2''}$ = 8 Hz, 1H, H-1″), 4.44 (d, $J_{1',2'}$ = 8 Hz, 1H, H-1′), 4.88 (dd, *J* = 18.1 Hz, 1H, H-21), 5.89 (s, 1H, H-22); ^{13}C-NMR(CD_3OD) δ ppm: 16.42 (C-18), 24.08 (C-19), 72.02 (C-4″), 75.29 (C-3), 83.35 (C-4′), 103.20 (C-1″), 103.60 (C-1′), 117.80 (C-22), 177.23 (C-23), 178.48 (C-20).

3β-O-(β-D-fucopyranosyl)-pregn-5-en-3β-ol-20-one (11) from 316 mg of pregn-5-en-3β-ol-20-one (10). Afforded 152 mg (33%) product; m.p. 173–176°C; TLC: R_f(II) = 0.65; ^{1}H-NMR(CD_3OD) δ ppm: 0.63 (s, 3H, H-18), 1.00 (s, 3H, H-19), 1.35 (d, $J_{5',6'}$ = 6.5 Hz, 3H, H-6′), 2.12 (s, 3H, H-21), 2.53 (t, $J_{16,17}$ = 9 Hz, 1H, H-17), 3.53–3.66 (m, 3H, H-2′, H-3′, H-5′), 3.72 (br.s, 1H, H-4′), 4.15 (m, 1H, H-3), 4.32 (d, $J_{1',2'}$ = 7.6 Hz, 1H, H-1′), 5.36 (dd, $J_{6,7}$ = 6 Hz, 1H, H-6); ^{13}C-NMR (CD_3OD) δ ppm: 13.55 (C-18), 16.77 (C-6′), 19.82 (C-19), 71.79 (C-5′), 72.59 (C-4′), 73.16 (C-2′), 75.33 (C-3′), 75.91 (C-3), 103.36 (C-1′), 122.14 (C-6), 142.44 (C-5), 215.41 (C-20). Analysis calculated for $C_{27}H_{42}O_6$ (462.64): C 70.10, H 9.15. Found: C, 70.15; H, 9.27.

Table 1 Chemical Shifts (ppm) in ^{1}H- and ^{13}C-NMR Spectra of β-D-fucose, Unglycosylated **6** and Fucosylated **7** 5β-pregnan-3β-ol-20-one

	^{1}H-NMR			^{13}C-NMR		
Atom	β-D-Fucose	**6**	**7**	β-D-Fucose	**6**	**7**
3	—	3.50	3.93	—	71.0	79.25
17	—	2.57	2.57	—	63.7	64.82
18	—	0.52	0.52	—	13.4	13.80
19	—	0.89	0.88	—	12.3	12.71
20	—	—	—	—	209.2	212.40
21	—	2.03	2.03	—	—	—
1′	5.3	—	4.16	97.10	—	103.27
2′	3.36	—	3.45	72.71	—	73.12
3′	3.52	—	3.45	73.92	—	75.26
4′	3.62	—	3.57	72.42	—	72.43
5′	3.69	—	3.57	71.70	—	71.69
6′	1.15	—	1.17	16.59	—	16.83

3β-O-(β-D-fucopyranosyl)-pregn-5-en-20-one-3β,21-diol (14) from 374 mg of pregn-5-en-20-one-3β,21-diol-21 acetat (13). Afforded 154 mg (32%) product. m.p. 163–165°C; TLC: R_f(II) = 0.55; ^{1}H-NMR(CD_3OD) δ ppm: 0.73 (s, 3H, H-18), 1.03 (s, 3H, H-19), 1.27 (d, $J_{5',6'}$ = 6.5 Hz, 3H, H-6′), 2.53 (t, 1H, H-17), 3.53–3.66 (m, 3H, H-2′, H-3′, H-5′), 3.72 (br.s, 1H, H-4′), 4.15 (m, 1H, H-3), 4.30 (t, 1H, H-21), 4.40 (d, $J_{1',2'}$ = 8 Hz, 1H, H-1′), 5.37 (dd, 1H, H-6); ^{13}C-NMR(CD_3OD) δ ppm: 13.60 (C-18), 16.84 (C-6′), 19.85 (C-19), 57.54 (C-17), 71.79 (C-5′), 72.35 (C-4′), 73.04 (C-2′), 73.78 (C-3′), 75.25 (C-3), 102.95 (C-1′), 122.50 (C-6), 142.11 (C-5), 212.36(C-20). Analysis calculated for $C_{27}H_{42}O_7$ (478.63): C,67.76; H, 8.85. Found: C, 67.73; H, 8.91.

3β-O-(β-D-fucopyranosyl)-5α-pregnan-3β-ol-20-one (9) from 318 mg of 5α-pregnan-3β-ol-20-one (8). Afforded 178 mg (39%) product; m.p. 185–187°C; TLC: R_f(II) = 0.61; ^{1}H-NMR δ ppm: 0.60 (s, 3H, H-18), 0.84 (s, 3H, H-19), 1.26 (d, $J_{5',6'}$ = 6.5 Hz, 3H, H-6′), 2.10 (s, 1H, H-21), 2.62 (t, 1H, H-17), 3.49 (m, 3H, H-2′,3′,5′), 3.61 (m, 1H, H-4′), 4.00 (br. s, 1H, H-3), 4.31 (d, $J_{1',2'}$ = 7.6 Hz, 1H, H-1′); ^{13}C-NMR(CD_3OD) δ ppm: 12.71 (C-19), 13.80 (C-18), 16.85 (C-6′), 45.37 (C-5), 64.82 (C-17), 71.77 (C-5′), 72.35 (C-4′), 73.06 (C-2′), 75.19 (C-3′), 79.25 (C-3), 102.78 (C-1′), 212.40 (C-20). Analysis calculated for $C_{27}H_{44}O_6$ (464.64): C, 69.79; H, 9.55. Found: C, 69.12; H, 9.55.

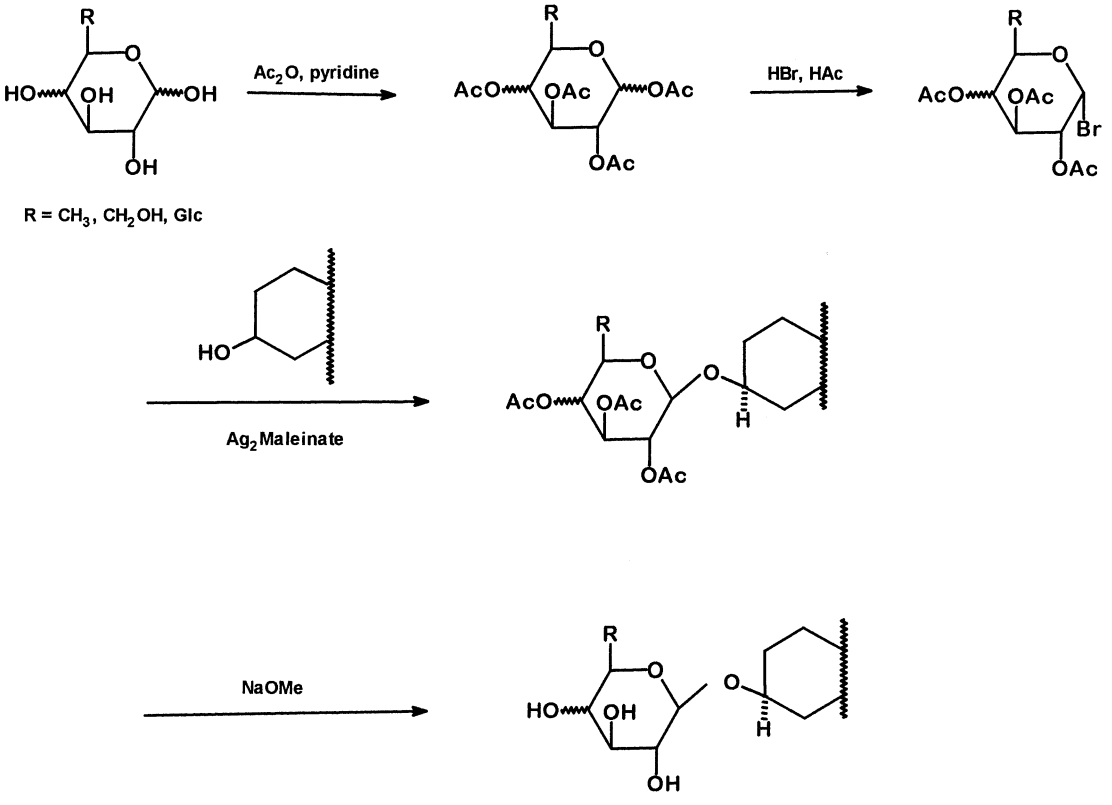

Figure 1 General reaction for β-glycosylation of steroids.

3β-O-(β-D-fucopyranosyl)-5β-pregnan-3β-ol-20-one (7) from 318 mg of 5β-pregnan-3β-ol-20-one (6). Afforded 173 mg (38%) product; m.p. 193°C; TLC: R_f(II) = 0.63; ^{1}H-NMR (CD_3OD) δ ppm: 0.52 (s, 3H, H-18), 0.88 (s, 3H, H-19), 1.25 (d, $J_{5',6'}$ = 6.5 Hz, 3H, H-6′), 2.03 (s, 1H, H-21), 2.57 (t, 1H, H-17), 3.45 (m, 3H, H-2′,3′,5′), 3.59 (dd, 1H, H-4′), 3.93 (br. s, 1H, H-3), 4,16 (d, $J_{1',2'}$ = 7.6 Hz, 1H, H-1′); ^{13}C-NMR (CD_3OD) δ ppm: 13.78 (C-18), 16.83 (C-6′), 23.81 (C-19), 64.89 (C-17), 71.69 (C-5′), 72.43 (C-4′), 73.12 (C-2′), 75.26 (C-3′), 75.73 (C-3), 103.27 (C-1′), 212.45 (C-20). Analysis calculated for $C_{27}H_{44}O_6$ (464.64): C, 69.79; H, 9.55. Found: C, 69.36; H, 9.61.

3β-O-(β-D-fucopyranosyl)-23-Nor-5,20(22)E-choladienic acid (17) from 387 mg 23-nor-5,20(22)E-choladienic acid ethyl ester[8] (16). Afforded 153 mg (30%) product; m.p. 160°C start of decomposition; TLC: R_f(II) = 0.63; ^{1}H-NMR (CD_3OD) δ ppm: 0.63 (s, 3H, H-18), 1.02 (s, 3H, H-19), 1.37 (d, $J_{5',6'}$ = 6.5 Hz, 3H, H-6′), 2.16 (s, 1H, H-21), 2.37 (t, 1H, H-17), 3.67 (m, 3H, H-2′,3′,5′), 3.84 (br.s, 1H, H-4′), 4.02 (br.s, 1H, H-3), 4.32 (d, $J_{1',2'}$ = 7.6 Hz, 1H, H-1′), 5.44 (dd, 1H, H-6), 5.71 (s, 1H, H-22), 8.54 (s, 1H, -COOH); ^{13}C-NMR (CD_3OD) δ ppm: 14.29 (C-18), 16.77 (C-61′), 122.48 (C-6), 127.97 (C-22), 142.16 (C-5), 161.14 (C-20), 179.65 (C-23). Analysis calcu-

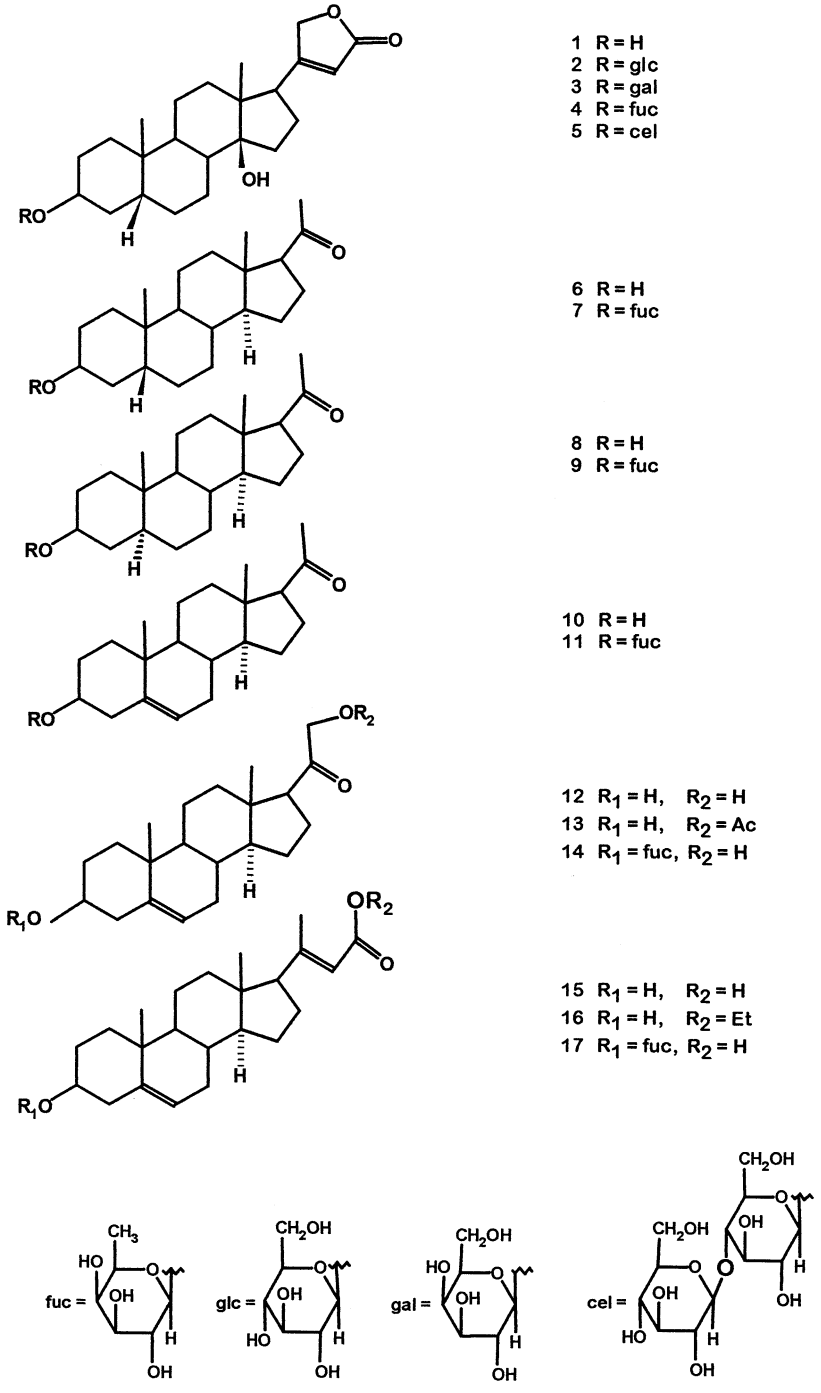

Figure 2 Cardenolides, pregnanes, and their glycosylated products.

lated for $C_{29}H_{44}O_7$ (464.64): C, 69.02; H, 8.79. Found: C, 69.13; H, 8.81.

Results and discussion

For the preparation of steroid glycosides the activated α-bromo-derivatives of D-glucose, D-galactose, D-fucose and D-cellobiose were reacted with the respective steroids. Silver maleinate as "catalyst" showed a superior purity profile for the newly formed glycosides than silver perchlorate, silver salicylate, Fetison's reagent or mercury cyanide which resulted in products with higher impurity profiles, insufficient yield or poor stereoselectivity. Silver maleinate on the other hand forced the carbohydrate moiety exclusively into the desired β-configuration. This was proven by NMR spectroscopy as exemplified by the respective NMR data of unglycosylated **6** and fucosylated **7** 5β-pregnan-3β-ol-20-on (Table 1). As a result of glycosylation chemical shifts of C-3 resp. at H-3 of the steroid changed significantly compared to the unglycosylated steroids. All other signals correspond well to those of the unglycosylated steroid and are in good accordance with the published data.[12,17] In the ^{1}H-NMR the 3α-H shifts from 3.5 to 4.0 ppm. In the ^{13}C-NMR shifts for C-3 towards higher frequencies take place in good accordance with shifts calculated by the respective increments (8.3 ppm). Formation of the β-glycosidic bond can also be proven by the signals originating from the carbohydrate moiety. In the ^{13}C-NMR the signals for C-2′ to C-6′ remain nearly unchanged whereas for C-1′ a typical downfield shift of about 6 ppm to 103 ppm occurs. Such a shift is characteristic for β-configuration whereas α-bonds are characterized by shifts to about 100 ppm only.[14] In the ^{1}H-NMR a significant upfield shift of the anomeric proton from about 5 ppm to 4 ppm is seen. All other carbohydrate-related proton signals remain nearly unchanged. The coupling constants of the doublet for H-1′ of about 8 Hz in D-glucose, D-galactose, D-fucose and D-cellobiose are typical for a trans-configuration of H-1′ and H-2′ and are correlated to β-glycosidic linkage for these glycosides. Applying the Karplus relation[13] β-configuration of the glycosidic bond was inferred.

No evidence was found for isomerisation or degradation of the steroid part or the sugar moiety. The spectral data for the relevant positions C-3 and C-1′ of all synthesized fucosides or other glycosides were consistent with the exemplary data evaluated for fucosylated 5β-pregnan-3β-ol-20-one as shown in Table 1.

The reactivity of activated halogenoses decreased as expected from the hexoses over the deoxysugar fucose to the disaccharide cellobiose. In any case a 72 h reaction time was sufficient to yield about 60 to 70% of peracetylated steroid glycosides. In case of cardenolides as steroidal component no degradation to the $\Delta^{8,14}$ and $\Delta^{14,15}$-anhydrocardenolides, respectively by elimination of the 14β-hydroxyl group was observed. Using the other catalysts indicated above, anhydro compounds were formed from cardenolides up to 30%, with decreased yields of the final products and a time-consuming chromatographic clean-up.

The resulting peracetylated steroid-glycosides were not isolated or purified but subjected directly to alkaline hydrolysis. Removal of the acetyl protecting groups was performed by strong alkaline hydrolysis with 50% sodium hydroxide or sodium methoxide in methanol. These conditions did not hydrolyze the γ-lactone system of the butenolide ring in cardenolides nor degraded the sensitive 20-keto-21-hydroxyfunction of **14**. Prolonged reaction times were necessary when weak bases, such as sodium hydrogen carbonate,[15] trialkylamine[3,16] or *N*-methylpyrrolidine[16] were used for deprotection; only sodium methoxide and sodium hydroxide were suitable for almost complete hydrolysis within 8 h in overall yields of 30 to 50%.

The optimized general reaction sequence followed to obtain 3β-O-glycosyl-steroids is displayed in Figure 1. According to this procedure digitoxigenin **1** was glycosylated easily to the respective β-D-glucoside **2**, β-D-galactoside **3**, β-D-fucoside **4** and β-D-cellobioside **5** in yields of about 40 to 50% referring to the starting material **1** (Figure 2). Similar glycosydation procedures with D-fucose as carbohydrate component were applied to 5β-pregnan-3β-ol-20-one **6**, 5α-pregnan-3β-ol-20-one **8** and pregnenolone **10** to give the respective 3β-O-D-fucopyranosides **7**, **9** and **11** in an overall yield of about 30% (Figure 2). Prior to the glycosylation of pregn-5-en-20-one-3β,21-diol **12** it was necessary to protect the 21-hydroxyl group by formation of the ethyl ester **13**. The subsequent reaction sequence with peracetylated fucosyl bromide yielded 21-hydroxypregn-5-ene-3β-D-fucoside **14**. Norcholenic acids as putative biosynthetic precursors of cardenolides are characterized by a C-23 carboxylic group which has to be protected by ethyl ester formation prior to glycosylation procedures. Preparation of the 23-norchola-5,20(22)E-diene acid-3β-D-fucoside **17** from 3β-hydroxy-23-norchola-5,20(22)E-diene acid ethyl ester **16** showed the robustness and suitability of the described glycosylation procedure for the rapid and efficient preparation of various steroid glycosides.

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