Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1889–1894

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Biological Evaluation of 2-Hydroxy Derivatives of Digitoxigenin and 3-Epidigitoxigenin†

M. Gobbini,* G. Marazzi, G. Padoani, L. Quadri, L. Valentino, M. P. Zappavigna and P. Melloni

Prassis Istituto di Ricerche Sigma-Tau, Via Forlanini, 3, 20019 Settimo Milanese (MI), Italy

Received 25 March 1998; accepted 2 June 1998

Abstract—The four stereoisomers of the 2-hydroxy derivatives of digitoxigenin and 3-epidigitoxigenin have been synthesized, their structures established by NMR, and their binding affinity for the digitalis receptor on Na^+, K^+-ATPase evaluated. These derivatives showed lower affinities than the parent compounds. The hydrophilic hydroxy groups in the alpha position are more detrimental to the affinity than hydroxy groups in the beta position.

Introduction

Digitalis cardiac glycosides are well known drugs clinically used to improve myocardial contractility in the treatment of congestive heart failure.[1] Their action is mainly due to inhibition of Na^+, K^+-ATPase, an enzyme located in the cell membrane and promoting the outward transport of Na^+ and the inward transport of K^+.[2] Life-threatening cardiac arrhythmias are the major problem with these compounds. The search for novel inotropic agents with a more favorable therapeutic index has prompted a lot of work on digitalis-like compounds[3] and a recent NIH sponsored trial, demonstrating that digoxin treatment does not increase mortality, has renewed interest in this field.[4]

The most potent inhibitors of Na^+, K^+-ATPase are cardenolides such as digoxin, digitoxin, digitoxigenin, and gomphoside (Fig. 1). The first three compounds have some common features, typical of digitalis: a 17β-unsaturated lactone, a 14β-hydroxy group, A/B and C/D *cis* ring junctions, and 3β-hydroxy or 3β-glycosyl linkage with digitoxose. A quite different molecule is gomphoside, an A/B *trans* cardiac glycoside from *Asclepias fruticosa* RBr,[5] in which the aglycone (gomphogenin) is linked to a 4,6-dideoxyhexosulose through its 2α- and 3β-hydroxy groups.

Templeton[6] and more recently Repke[7] explored the possibility of obtaining novel and highly potent digitalis derivatives by functionalization of the 2α-hydroxy group of gomphogenin.

With the aim of having analogues of gomphogenin in the A/B *cis* digitalis skeleton, and evaluating the importance of different configurations at positions 2 and 3, we planned the synthesis of the four stereoisomers of the 2-hydroxy derivatives of digitoxigenin and 3-epidigitoxigenin (Fig. 2).

Results and Discussion

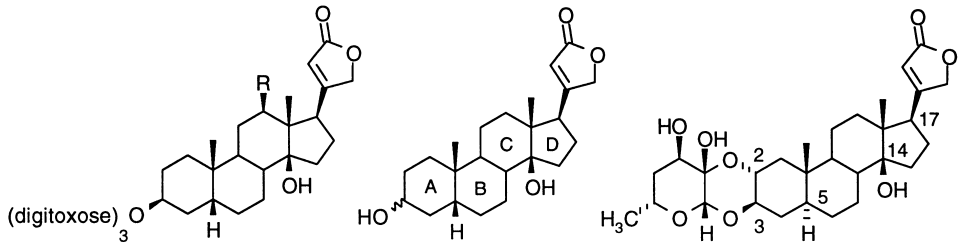
Treatment of the known 4β-bromo-3-oxo-14β-hydroxy-5β-card-20(22)-enolide **1**[6] (Scheme 1) with anhydrous potassium acetate in refluxing acetic acid[8] gave the key compound 2β-acetoxy-3-oxo derivative **2** (60% yield). The configuration at C(2) was straightforwardly assigned because of the low-field axial signal in the ^1H NMR spectrum (d 5.25, dd, *J* 13.7 and 5.9 Hz, H-2α).

Key words: 2-Hydroxy digitalis derivatives; digitoxigenin; 3-epidigitoxigenin; Na^+, K^+-ATPase; binding affinities.

*Corresponding author.

†A preliminary account of this work has been presented at the First International Electronic Conference on Synthetic Organic Chemistry (ECSOC-1), A0012, 1–30 September 1997; http://www.mdpi.org/escoc-1.htm

0968-0896/98/$—see front matter
PII: S0968-0896(98)00119-9



R = OH: Digoxin 3β: Digitoxigenin Gomphoside
R = H: Digitoxin 3α: 3-Epidigitoxigenin

Figure 1.

The reduction of **2** with lithium tri-*tert*-butoxyaluminum hydride in THF gave the 2β-acetoxy-3α-hydroxy compound **3** (52% yield). Hydrolysis of the acetoxy group with 10% aq HCl in methanol gave the expected 2β,3α,14β-trihydroxy-5β-card-20(22)-enolide **4** (70% yield). The equatorial configuration of both 2β- and 3α-hydroxy groups in **4** was confirmed by the width at half height ($W_{h/2} = 25$ Hz) of the C***H***-OH signals in the ^{1}H NMR spectrum at 3.47 and 3.34 ppm.

The 2β,3β-dihydroxy derivative **5d** was obtained by exploiting the high selectivity of L-Selectride®[9] in reducing the 3-keto group of compound **2** to axial 3β-hydroxy group. The reaction gave, together with the 2β,3β,14β-trihydroxy-5β-card-20(22)-enolide **5d**, a mixture of acetates: 2β,3β-diacetoxy **5a**, 2β-acetoxy-3β-hydroxy **5b**, and 2β-hydroxy-3β-acetoxy **5c** in roughly equal amount.

The mixture was hydrolysed with 10% aq HCl in methanol to give compound **5d** in an overall yield of 51% from **2**. The axial configuration of the 3β-hydroxy group in **5d** was confirmed by the signal at 3.96 ppm for the equatorial 3α-hydrogen ($W_{h/2} = 7$ Hz).

The oxidative/reductive procedure to obtain the 2α,3β-dihydroxy derivative **7** is described in Scheme 2. Compound **5d** was oxidized using three equiv of 2-iodylbenzoic acid (IBX)[10] to give a mixture of starting material and 2-oxo-3β-hydroxy derivative **6** in 57% yield. Reduction of **6** with L-Selectride® gave the *trans* diaxal 2α,3β-dihydroxy compound **7** in 18% yield and the *cis* cyclic boronate **8** (18% yield). The axial configuration of 2α- and 3β-hydroxy groups in **7** was shown by the C(2)-H and C(3)-H proton signals at 3.72 and 3.81 ppm ($W_{h/2} = 7$ Hz for both multiplets), respectively. The assignments were established through decoupling experiments.

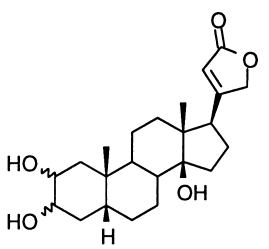
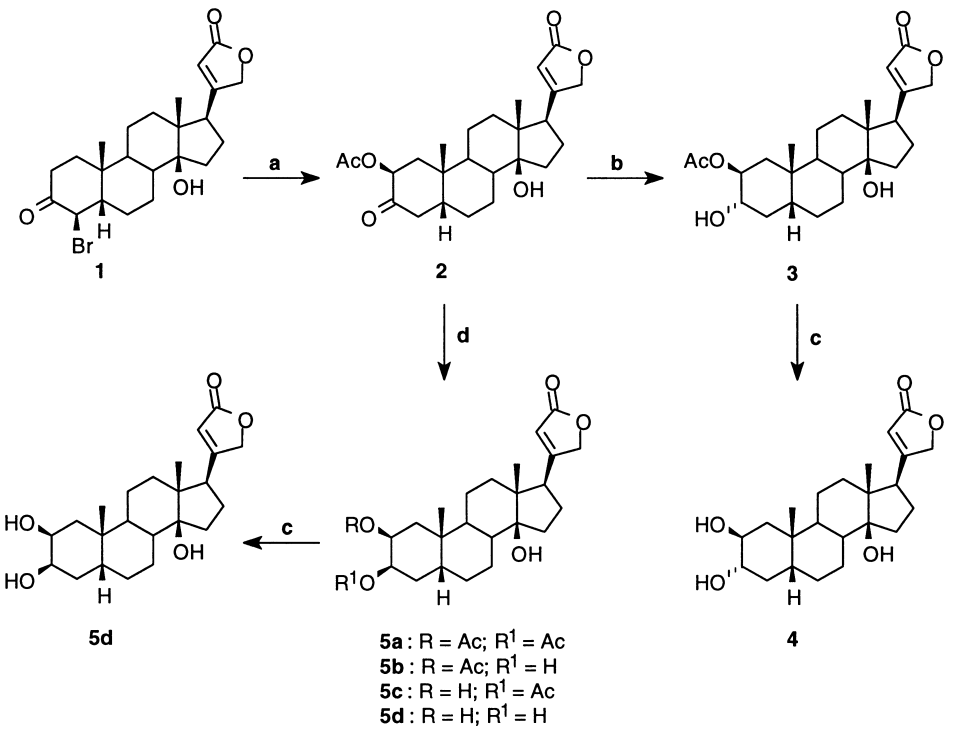


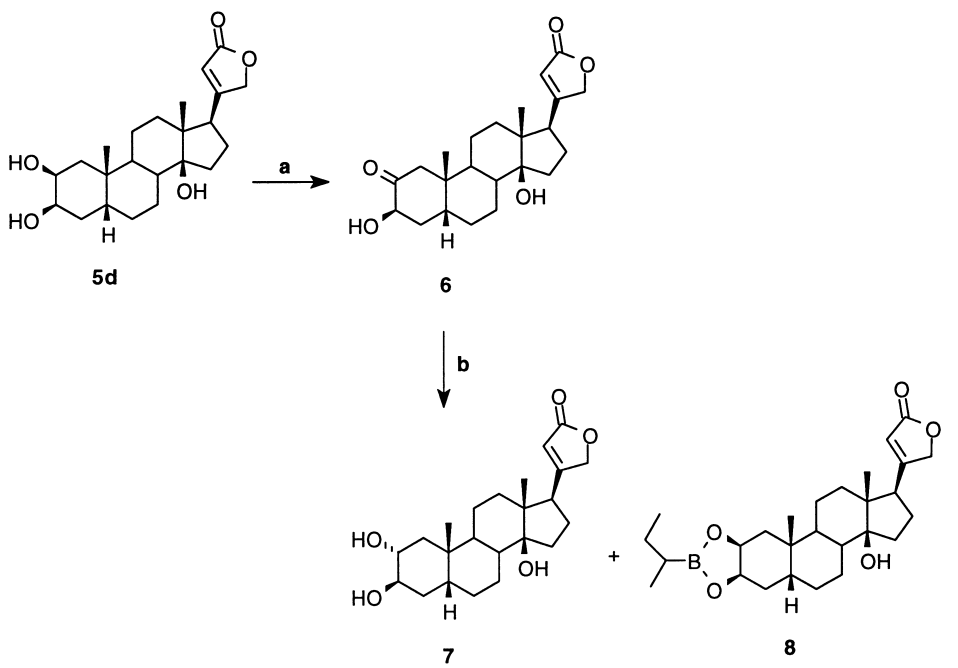
Figure 2.

Finally, the 2α,3α-dihydroxy compound **11** was prepared as described in Scheme 3. Digitoxigenin **9** was reacted with trifluromethanesulfonic anhydride in pyridine to give a mixture of Δ^2 and Δ^3 derivatives **10** in a 6:4 ratio that became 8:2 after crystallization from acetone (61% yield). This mixture was reacted with a catalytic amount of OsO_4 in the presence of 4-methylmorpholine-4-oxide, to give a mixture of three 2,3- and 3,4-dihydroxy compounds. Chromatographic separation on silica gel gave the desired 2α,3α-dihydroxy compound **11** (20% yield), together with the 2β,3β-dihydroxy derivative **5d** (25%) and the 3β,4β-dihydroxy derivative **12** (20% yield). The axial configuration of the 2α-hydroxy group and the equatorial configuration of 3α-hydroxy group in **11** were confirmed also in this case by the observed multiplicity of the signals in the ^{1}H NMR spectrum: 3.88 ppm ($W_{h/2} = 7$ Hz, equatorial 2β-hydrogen) and 3.58 ppm (dt, *J* 11.8 and 3.5 Hz, axial 3β-hydrogen). In **12**, the axial configuration of the 3β-hydroxy group and the equatorial configuration of 4β-hydroxy group were confirmed by the proton signals at 3.93 ppm (m, $W_{h/2} = 7$ Hz, equatorial 3α-hydrogen) and 3.81 ppm (dd, *J* 11.0 and 2.0 Hz, axial 4α-hydrogen).

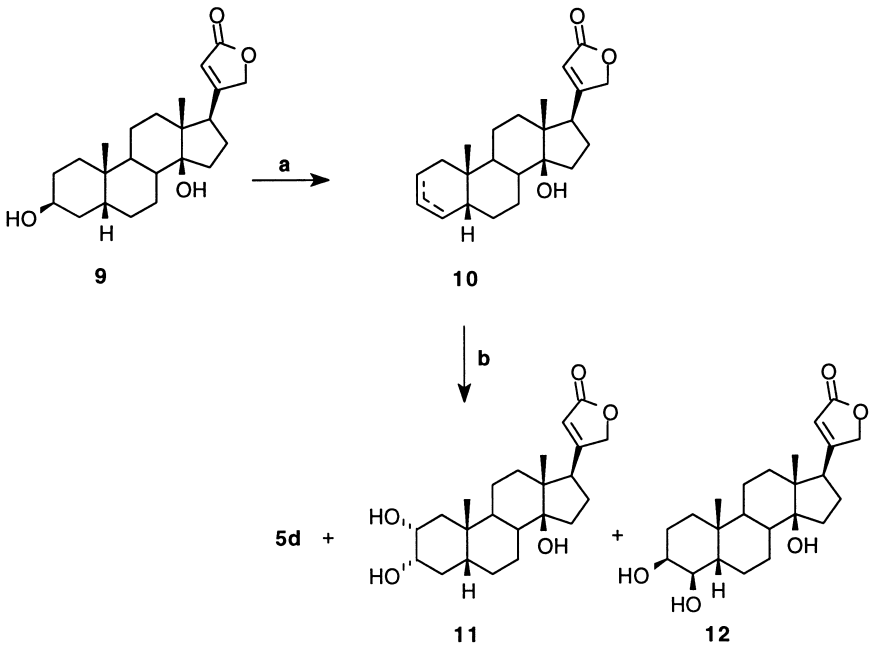
The four 2,3-dihydroxy isomers and the 3β,4β-dihydroxy derivative were evaluated in the displacement of the specific [^{3}H]ouabain binding from Na^+, K^+-ATPase in comparison with digitoxigenin and 3-epidigitoxigenin.[11] The biological data are reported in Table 1.



Scheme 1. Reagents and conditions: (a) AcOK, AcOH, reflux (60%); (b) $LiAlH[OC(CH_3)_3]_3$, THF, 0°C (52%); (c) 10% aq HCl, MeOH, rt (**4**, 70%; **5d**, 51% from **2**); (d) L-Selectride®, THF, 0 °C.



Scheme 2. Reagents and conditions: (a) IBX, DMSO, rt (57%); (b) L-Selectride®, THF, 0°C (**7**, 18%; **8**, 18%).



Scheme 3. Reagents and conditions: (a) $(CF_3SO_2)_2O$, pyridine, 0 °C (70%); (b) OsO_4 (0.005 M in Et_2O), NMO, acetone/water, rt (**5d**, 25%, **11**, 20%; **12**, 20%).

The dihydroxy derivatives showed a lower binding affinity when compared with the corresponding parent compounds and the hydrophylic 2-hydroxy substituent was more detrimental in the 2α than the 2β position. These results are in agreement with previous findings that supplementary hydroxy groups in a digitalis aglycone molecule reduce the affinity for the receptor on Na^+, K^+-ATPase.[3]

Experimental

General

Melting points were measured on a capillary melting point apparatus and were uncorrected. Elemental analyses were performed by Redox, Cologno Monzese, Italy. NMR spectra were recorded on a Bruker AC-300 spectrometer at 300.13 (^{1}H) or 75.48 (^{13}C) MHz. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as internal standard and coupling constants (*J*) values are in Hz. ^{1}H NMR assignments were drawn from classical arguments on chemical shift and coupling constants behavior. ^{13}C NMR signals, reported in Table 2, were attributed on the basis of the multiplicities obtained by Distortionless Enhancement by Polarization Transfer (DEPT) experiments and by comparison with published data for digitoxigenin and affinosides.[12] In two instances (**4** and **12**), the C-2/C-3 resonances were assigned through a 2-D-heterocorrelation experiment (HETCOR, Bruker software). Mass spectral data were obtained with electron impact ionization at 70 eV from a Finnigan INCOS-50 mass spectrometer using the direct exposure probe (DEP). Chromatography was carried out on silica gel (Baker 7024-02) in all instances. Solvents and reagents were used as purchased from Aldrich. Usual workup means washing the extracts with water, brine, drying over Na_2SO_4, filtering, and evaporation in vacuo.

2β-Acetoxy-14β-hydroxy-3-oxo-5β-card-20(22)-enolide (2). A mixture of 4β-bromo-14β-hydroxy-3-oxo-5β-card-20(22)-enolide[6] **1** (6.9 g, 15.3 mmol) and anhydrous potassium acetate (13.5 g, 137 mmol) in acetic acid (80 mL) was refluxed for 1 h. The solution was cooled at

Table 1. Binding affinity on Na^+, K^+-ATPase

Compd	Binding[a]
Digitoxigenin **9**	7.2
3-Epidigitoxigenin	6.0
2β,3α-Dihydroxy **4**	5.4
2β,3β-Dihydroxy **5d**	6.2
2α,3β-Dihydroxy **7**	5.8
2α,3α-Dihydroxy **11**	5.0
3β,4β-Dihydroxy **12**	6.0

[a]Average of three values as $-\log IC_{50}$ (M). The affinity for the receptor site of Na^+, K^+-ATPase was evaluated by the displacement of the specific [^{3}H]-ouabain binding from Na^+, K^+-ATPase receptor[11a] isolated from dog kidney and purified according to Jørghensen.[11b]

Table 2. ^{13}C NMR spectra data

Compd	2	4	5d	7	11	12
1	42.4[a]	44.5	38.6	37.8	41.1[a]	30.2
2	74.5	72.0	68.5[a]	72.3[a]	71.6	27.2[a]
3	207.4	77.3	71.0[a]	71.7[a]	73.4	69.2[b]
4	43.6[a]	35.5	33.7	30.4	31.3	70.9[b]
5	46.4	43.1[a]	36.6	37.2	43.8[b]	44.4
6	27.4[b]	27.6[b]	27.1[b]	28.3[b]	28.1[c]	21.7[c]
7	22.1[c]	22.3[c]	22.5	22.7	22.9[d]	22.2[c]
8	42.5	42.8[a]	42.7	43.1	43.1[b]	42.7
9	38.6	38.9	38.1	39.7	39.5	38.3
10	38.6	38.3	38.0	36.7	36.4	38.0
11	22.5[c]	22.7[c]	22.5	22.7	23.1[d]	22.5[c]
12	40.6	40.9	40.9	41.2	41.1[a]	40.9
13	51.0	51.0	51.0	51.1	51.1	51.0
14	85.8	86.2	86.3	86.6	86.5	86.4
15	33.6	33.4	33.4	33.5	33.4	33.4
16	28.2[b]	28.0[b]	28.1[b]	28.1[b]	28.7[c]	28.1[a]
17	52.1	52.1	52.1	52.3	52.3	52.1
18	16.7	16.4	16.4	16.5	16.5	16.4
19	23.2	23.7	24.1	24.3	24.0	24.2
20	177.1[d]	177.2[d]	177.2[c]	177.2[c]	177.2[e]	177.2[d]
21	75.2	75.3	75.3	75.4	75.3	75.3
22	118.2	117.8	117.8	117.7	117.7	117.8
23	177.7[d]	178.3[d]	178.4[c]	178.5[c]	178.5[e]	178.4[d]
COO	172.1					
Me	21.4					

Values in ppm downfield from TMS, in CD_3OD, (CD_3OD/$CDCl_3$, 9/1 for **2**).
[a,b,c,d,e]Values may be exchanged within a column.

room temperature, poured into water/ice and extracted with dichloromethane. After the usual work up, the crude mixture was crystallized from ethyl acetate to give **2** (4.0 g, 60%): mp 248–251 °C; IR (KBr): 3440, 1735, 1720, 1640 cm^{-1}; MS (*m/z*): 430, 412, 388, 370, 217; ^1H NMR ($CDCl_3$) δ: 5.88 (1H, br s, H-22), 5.25 (1H, dd, *J* 13.7 and 6.2 Hz, H-2α), 5.04 (1H, dd, *J* 18.4, 1.7, H-21), 4.88 (1H, dd, *J* 18.4 and 1.5, H-21), 2.89 (1H, t, *J* 13.7, H-4α), 2.85 (1H, m, H-17), 2.32 (1H, dd, *J* 13.7 and 5.9, H-1α), 1.50 (1H, t, *J* 13.7, H-1β), 1.07 (3H, s, Me-10), 0.90 (3H, s, Me-13). Anal. calcd for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.68; H, 8.20.

2β,3α,14β-Trihydroxy-5β-card-20(22)-enolide (4). Lithium tri-*tert*-butoxyaluminum hydride (1.3 g, 5.12 mmol) was added to a stirred solution of **2** (1.0 g, 2.32 mmol) in anhydrous THF (50 mL) at 0 °C under nitrogen. After 2 h, the mixture was poured into iced aq 10% acetic acid solution and extracted with ethyl acetate. The usual work up followed. The crude product (1.0 g, a 9:1 mixture of 3α/3β-hydroxy derivatives) was crystallized from ethyl acetate to give 2β-acetoxy-3α,14β-dihydroxy-5β-card-20(22)-enolide **3** (0.52 g, 52%).

A solution of the 3β-acetoxy derivative **3** (0.41 g, 0.95 mmol) in methanol (20 mL) and aq 10% hydrochloric acid solution (8.2 mL) was stirred at room temperature for 48 h. The solution was poured into water, neutralized with sodium hydrogen carbonate, and extracted with chloroform/methanol (9/1). The usual work up gave a foam (0.35 g) and the crude product was crystallized from ethyl acetate to give **4** (0.26 g, 70%): mp 179–182 °C; IR (KBr): 3530, 3470, 1713, 1622 cm^{-1}; MS (*m/z*): 390, 372, 354, 201; ^1H NMR (CD_3OD) δ: 5.90 (1H, br s, H-22), 5.03 (1 H, dd, *J* 18.4, 1.7, H-21), 4.92 (1H, dd, *J* 18.4 and 1.5, H-21), 3.47 (1H, ddd, *J* 12.0, 8.7 and 4.2, H-2α), 3.35 (1H, ddd, *J* 12.0, 8.7 and 4.0, H-3β), 2.85 (1H, m, H-17), 1.98 (1H, dd, *J* 13.7 and 4.2, H-1α), 1.00 (1H, dd, *J* 13.7 and 12.0, H-1β), 1.00 (3H, s, Me-10), 0. 89 (3H, s, Me-13). Anal. calcd for $C_{23}H_{34}O_5 \cdot 0.25\ H_2O$: C, 69.93; H, 8.80. Found: C, 70.02; H, 8.82.

2β,3β,14β-Trihydroxy-5β-card-20(22)-enolide (5d). L-Selectride® (2.8 mL of a 1 M solution in THF, 2.8 mmol) was added to a stirred solution of **2** (1.0 g, 2.32 mmol) in anhydrous THF (50 mL) at 0 °C under nitrogen. The solution was allowed to warm to room temperature and after 18 h the mixture was diluted with ethyl acetate and poured into iced aq 10% acetic acid solution. The usual work up followed.

A solution of the crude mixture (0.90 g) in methanol (40 mL) and aq 10% hydrochloric acid solution (18.0 mL) was stirred at room temperature for 72 h. The solution was poured into water, neutralized with sodium hydrogen carbonate and extracted with chloroform/methanol (9/1). The usual work up gave a foam (0.80 g) and the crude product was crystallized from diethyl ether to give **5d** (0.46 g, 51%): mp 212–214 °C; IR (KBr): 3542, 3495, 3450, 1720, 1608 cm^{-1}; MS (m/z): 390, 372, 354, 219, 201, 195; ^1H NMR (CD_3OD) δ: 5.90 (1H, br s, H-22), 5.03 (1H, dd, *J* 18.4, 1.7, H-21), 4.92 (1H, dd, *J* 18.4 and 1.5, H-21), 3.95 (1H, m, $W_{h/2}$ 7 Hz, H-3α), 3.66 (1H, dt, *J* 12.5 and 4.0, H-2α), 2.85 (1H, m, H-17), 0.98 (3H, s, Me-10), 0.88 (3H, s, Me-13). Anal. calcd for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.48; H, 8.84.

2α,3β,14β-Trihydroxy-5β-card-20(22)-enolide (7). 2-Iodylbenzoic acid (IBX) (1.5 g, 5.37 mmol) in dimethylsulfoxide (6 mL) was added to a stirred solution of **5d** (0.7 g, 1.8 mmol) at room temperature. After 20 h, the solution was diluted with ethyl acetate and water. The usual work up followed. The crude product was purified by flash chromatography using dichloromethane/ethyl acetate (7/3) as eluant to give 0.2 g of starting material and **6** (0.4 g, 57%): ^1H NMR ($CDCl_3$) δ: 5.89 (1H, br s, H-22), 4.98 (1H, dd, *J* 18.4, 1.7, H-21), 4.80 (1H, dd, *J* 18.4, 1.5, H-21), 4.11 (1H, bs, H-3α), 2.79 (1H, m, H-17), 1.08 (3H, s, Me-10), 0.88 (3H, s, Me-13).

L-Selectride® (0.44 mL, 1 M solution in THF) was added to a solution of **6** (0.17 g, 0.44 mmol) in anhydrous THF (6 mL) at 0 °C under nitrogen. The solution was allowed to warm to room temperature and after 24 h was diluted with ethyl acetate and poured into iced 5% acetic acid solution. The organic phase was washed with a saturated solution of sodium hydrogen carbonate and then with a saturated solution of sodium chloride, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by flash chromatography using ethyl acetate as eluent to give **8** (0.035 g, 18%) and **7** (0.030 g, 18%): mp 233–236 °C; IR (KBr): 3510, 3400, 1725, 1625, 1610 cm^{-1}; MS (*m*/*z*): 390, 372, 354, 219, 201; ^1H NMR (CD_3OD) δ: 5.89 (1H, br s, H-22), 5.03 (1H, dd, *J* 18.4, 1.7, H-21), 4.92 (1H, dd, *J* 18.4 and 1.5, H-21), 3.81 (1H, m, $W_{h/2}$ 7 Hz, H-3α), 3.71 (1H, m, $W_{h/2}$ 7 Hz, H-2β), 2.82 (1H, m, H-17), 0.93 (3H, s, Me-10), 0.88 (3H, s, Me-13). Anal. calcd for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.52; H, 8.73.

2α,3α,14β-Trihydroxy-5β-card-20(22)-enolide (11) and 3β,4β,14β-trihydroxy-5β-card-20(22)-enolide (12). Trifluorometanesulfonic anhydride (27 mL, 16.0 mmol) was slowly added at 0 °C under nitrogen to a solution of digitoxigenin **9** (6.0 g, 16.0 mmol) in pyridine (24 mL). After 0.5 h, the solution was poured into ice. The filtered precipitate was dissolved in ethyl acetate and dried over Na_2SO_4. The usual workup followed. The crude product was crystallized from acetone to give a mixture of 14β-hydroxy-5β-carda-2,20(22)-dienolide and 14β-hydroxy-5β-carda-3,20(22)-dienolide **10** (2.5 g, 61%) (Δ^2:Δ^3, 8:2).

14β-Hydroxy-5β-carda-2,20(22)-dienolide. ^1H NMR ($CDC1_3$) δ: 5.89 (1H, brs, H-22), 5.65 (1H, m, H-3), 5.54 (1H, m, H-2), 4.98 (1H, dd, *J* 18.4, 1.7, H-21), 4.80 (1H, dd, *J* 18.4, 1.5, H-21), 2.79 (1H, m, H-17), 0.97 (3H, s, Me-10), 0.88 (3H, s, Me-13).

14β-Hydroxy-5β-carda-3,20(22)-dienolide. ^1H NMR ($CDCl_3$) δ: 5.89 (1H, br s, H-22), 5.65 (1H, m, H-3), 5.37 (1H, m, H-4), 4.98 (1H, dd, *J* 18.4, 1.7, H-21), 4.80 (1H, dd, *J* 18.4, 1.5, H-21), 2.79 (1H, m, H- 1 7), 0.95 (3H, s, Me-10), 0.88 (3H, s, Me-13).

The Δ^2:Δ^3 mixture **10** (1.0 g, 3.9 mmol) and OsO_4 (0.65 mL, 0.08 M solution in Et_2O, 0.052 mmol) were added at room temperature to a solution of 4-methylmorpholine-4-oxide (0.55 g, 4.7 mmol) in water (6.5 mL) and acetone (30 mL). After 2 h, a saturated solution of sodium hydrogen sulfite was added. The resulting solution was diluted with water (20 mL) and 20 g of celite was added. The mixture was stirred for 1 h and then filtered. The resulting solution was extracted with dichloromethane. The usual work up followed. The crude product was purified by flash chromatography using dichloromethane/ethyl acetate (95/5) as eluant to give **5d** (0.28 g, 25%), **11** (0.23 g, 20%), and **12** (0.23 g, 20%).

11: Mp 259–262 °C; IR (KBr): 3565, 3460, 1728, 1628 cm^{-1}; MS (*m*/*z*): 390, 372, 354, 219, 201; ^1H NMR (CD_3OD/$CDCl_3$) δ: 5.89 (1H, br s, H-22), 5.03 (1H, dd, *J* 18.4, 1.7, H-21), 4.92 (1H, dd, *J* 18.4 and 1.5, H-21), 3.88 (1H, m, $W_{h/2}$ 7 Hz, H-2β), 3.58 (1H, dt, *J* 11.8 and 3.6, H-3β), 2.82 (1H, m, H-17), 0.90 (3H, s, Me-10), 0.88 (3H, s, Me-13). Anal. calcd for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.52; H, 8.69.

12: Mp 146–150 °C; IR (KBr): 3555, 3475, 3420, 1745, 1630, 1617 cm^{-1}; MS (*m*/*z*): 390, 372, 354, 219, 201, 195; ^1H NMR (CD_3OD) δ: 5.90 (1H, br s, H-22), 5.04 (1H, dd, *J* 18.4, 1.7, H-21), 4.92 (1H, dd, *J* 18.4 and 1.5, H-21), 3.92 (1H, m, $W_{h/2}$ 7 Hz, H-3α), 3.80 (1H, dd, *J* 11.0 and 3.3, H-4α), 2.84 (1H, m, H-17), 0.99 (3H, s, Me-10), 0.88 (3H, s, Me-13). Anal. calcd for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.48; H, 8.72.

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