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[A0012]

SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-HYDROXY DERIVATIVES OF DIGITOXIGENIN AND 3-EPIDIGITOXIGENIN

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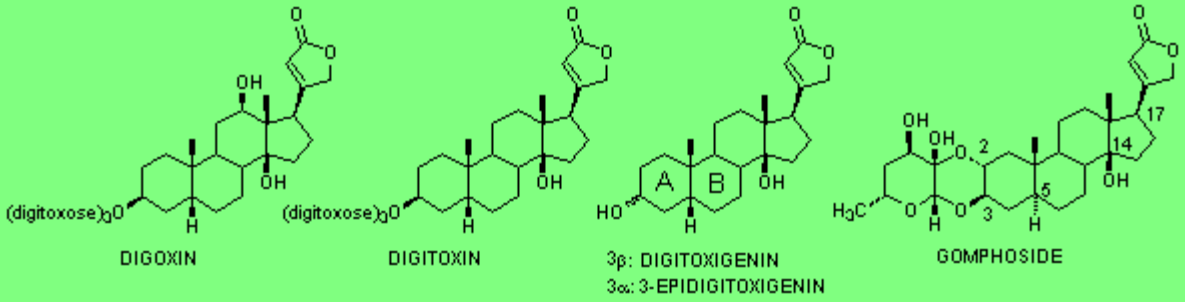
Introduction

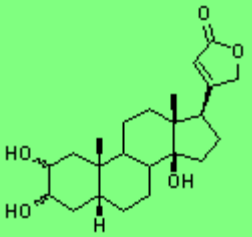
Digitalis cardiac glycosides are well known drugs clinically used for treatment of congestive heart failure.[1] Their action is mainly due to inhibition of Na^+,K^+-ATPase, an enzyme located in the cell membrane and promoting the outward transport of Na^+ and the inward transport of K^+.[2] The most potent inhibitors of Na^+,K^+-ATPase are cardenolides such as digoxin, digitoxin, digitoxigenin and gomphoside (**Figure 1**). The first three compounds have some common features, typical of digitalis: 17b-unsaturated lactone; 14b-hydroxy; A/B and C/D *cis* ring junctions; 3b-hydroxy or 3b-glycosyl linkage with digitoxose. A quite different molecule is gomphoside, an A/B *trans* cardiac glycoside from *Asclepias fruticosa* RBr,[3] in which the aglycone (gomphogenin) is linked to a 4,6-dideoxyhexosulose trhough its 2a- and 3b-hydroxy groups.

Figure 1



Templeton[4] and, more recently, Repke[5] explored the possibility to obtain novel and highly potent digitalis derivatives by functionalization of the 2a-hydroxy group of gomphogenin.
With the aim of having analogues of gomphoside in the A/B *cis* digitalis skeleton, and evaluating the importance of different configuration at positions 2 and 3, we planned the synthesis of the 2-hydroxy derivatives of digitoxigenin and 3-epidigitoxigenin (**Figure 2**).

Figure 2

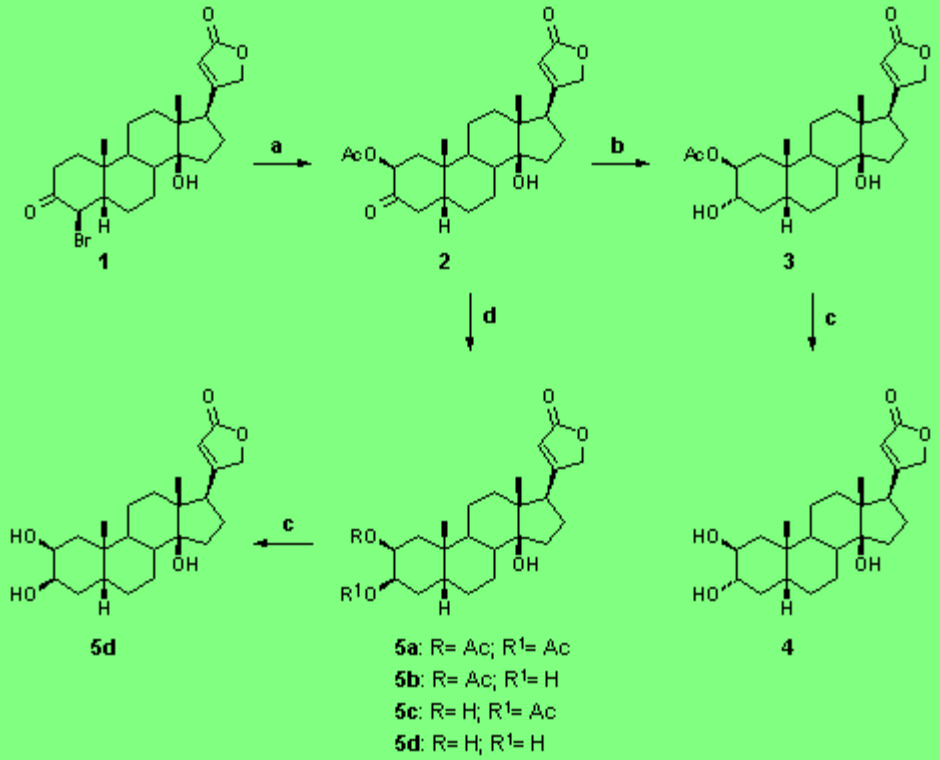


Three of the four isomers of 2,3-dihydroxy derivatives of the A/B *cis* series: 2b,3a **4**; 2b,3b **5d** and 2a,3a **8** were synthesized.

Chemistry

Treatment of the known 4b-bromo-3-oxo-14b-hydroxy-5b-card-20(22)-enolide **1**[4] (**Scheme 1**) with anhydrous potassium acetate in refluxing acetic acid,[6] gave the key compound 2b-acetoxy-3-oxo derivative **2** (60% yield). The reduction of **2** with lithium tri-*tert*butoxyaluminum hydride in THF gave the 2b-acetoxy-3a-hydroxy compound **3** (52% yield). Hydrolysis of the acetoxy group with 10% aq. HCl in methanol gave the desired 2b,3a,14b-trihydroxy-5b-card-20(22)-enolide **4** (70% yield).
The 2b,3b-dihydroxy derivative **5d** was obtained by exploiting the high selectivity of L-Selectride[7] in reducing the 3-keto group of compound **2** to axial 3b-hydroxy group. The reaction gave, together with the 2b,3b,14b-trihydroxy-5b-card-20(22)-enolide **5d** a mixture of acetates: 2b,3b-diacetoxy **5a**, 2b-acetoxy-3b-hydroxy **5b**, and 3b-acetoxy-2b-hydroxy **5c** in roughly equal amount. The mixture was hydrolized with 10% aq. HCl in methanol to give compound **5d** in an overall yield of 70% from **2**.

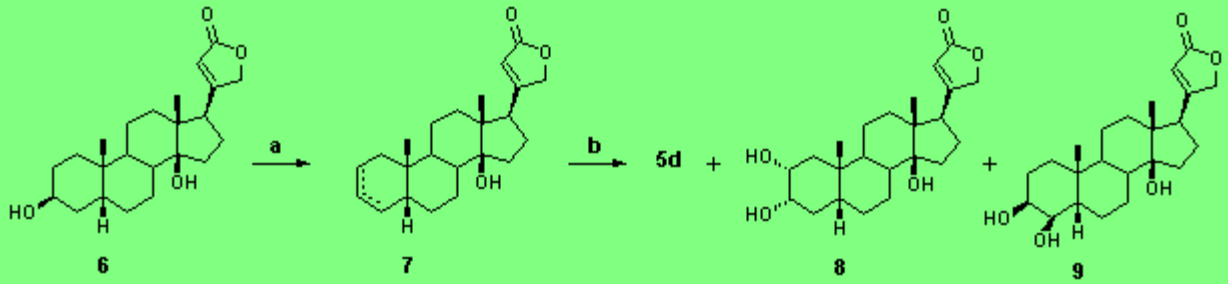
Scheme 1



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; **d**: L-Selectride, THF, 0 °C.

The 2a,3a-dihydroxy compound **8**, was prepared as described in **Scheme 2**. Digitoxigenin **6** was reacted with trifluoromethansulfonic anhydride in pyridine to give a mixture of D^2 and D^3 derivatives **7** in a 6/4 ratio that become 8/2 after crystallization from acetone. This mixture was reacted as such 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. Purification by silica gel chromatography gave the desired 2a,3a-dihydroxy compound **8** (20% yield), together with **5d** (25% yield) and the 3b,4b-dihydroxy derivative **9** (25% yield).

Scheme 2



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%; **8** 20%; **9** 25%).

Biological Data

The preliminary biological data of some of the synthesized compounds, in comparison with digitoxigenin and 3-epidigitoxigenin, in the displacement of the specific [^{3}H]-ouabain binding[8] on Na^+,K^+-ATPase are reported in **Table 1**.

Table 1

Compound	Binding[a]	Compound	Binding[a]
Digitoxigenin	7.2	**5d**	6.2
3-Epidigitoxigenin	6.0	**8**	5.0
4	5.4	**9**	NT[b]

[a]Average of three values (-log IC_{50}). 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[8a] isolated from dog kidney and purified according to J◆rghensen.[8b]
[b]Not tested.

The dihydroxy derivatives tested showed a lower binding affinity when compared with the corresponding parent compounds: 2b,3a-dihydroxy derivative **4** and 2a,3a-dihydroxy derivative **8** *vs* 3-epidigitoxigenin; 2b,3b-dihydroxy derivative **5d** *vs* digitoxigenin. These results are in agreement with previous finding [9] that supplementary hydroxy groups in an aglycone molecule reduce the affinity for the digitalis receptor on Na^+,K^+-ATPase. It is also confirmed that hydrophilic groups in alpha position are more detrimental for the affinity than hydrophilic groups in beta position.

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