128. On Cardioactive Steroids

Communication XV

A Stereoselective Synthesis of (21*R*)-21-Methyldigitoxigenin, a Fully Active Cardenolide with a Wide Margin of Safety: a Contribution to the Topology of the *Digitalis* Receptors[1])

by **Karel Wiesner*, Thomas Y.R. Tsai, Ravindra Kumar,** and **Hariharan Sivaramakrishnan**

Natural Products Research Center, University of New Brunswick,
Fredericton, New Brunswick, Canada E3B 6E2

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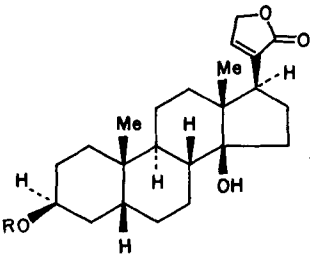
Summary

Two efficient preparations of the title compound, one from common C_{19}-steroids, the other from digitoxigenin, are described. The less active minor epimer (21*S*)-methyldigitoxigenin was also obtained and characterized. The positive inotropic effects and margins of safety of the two C(21)-epimers (tested as glucosides) are discussed in terms of the topological properties of the *Digitalis* receptors.

Introduction. – In the first communication of this series [2], we have described the preparation of the isocardenolide **Ia** which was submitted in the form of its β-glucoside **Ib** (experimental drug *Actodigin*) to Prof. *R. Mendez* (Instituto Nacional di Cardiologia Ignacio Chavez, Mexico City) for evaluation. *Actodigin* was studied extensively by *Mendez et al.* [3] in the failing heart of the dog heart lung preparation and in the intact anesthesized dog. It was found that the minimal therapeutic dose *(MTD)* of *Actodigin* was a smaller percentage of both the irregularity dose *(ID)* and lethal dose *(LD)* than was the case with oubaine and dihydro-oubaine. Thus, *Actodigin* displayed a greater 'margin of safety' than the natural *Digitalis* glycosides used in therapy.



Ia R = H
Ib R = glucose

[1]) For Communication No. XIV, see [1]. Systematic names of all compounds are given in the *Exper. Part.*

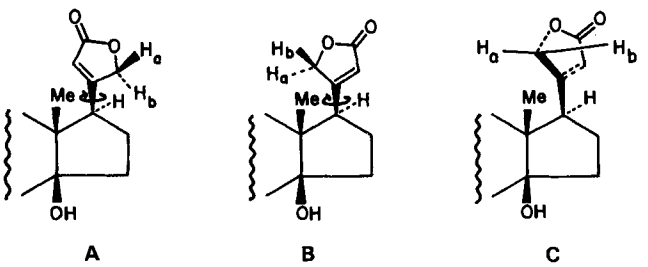
Already on the basis of these first results, Prof. *Mendez* raised the question whether there might not be two separate receptors for the inotropic effect and the toxicity of *Digitalis* glycosides in the heart muscle[2]).

In subsequent communications [4], we described a powerful new methodology for the synthesis of cardenolides, bufadienolides and their analogues which has enabled us to prepare many new derivatives. Some of these compounds submitted to Prof. *Mendez* as glucosides exceeded our original lead *'Actodigin'*, both in potency and margin of safety [5] and, as a consequence of this, the two-receptor hypothesis deserves some consideration.

In the present paper we wish to describe the preparation of cardenolide analogues with an additional chiral center in the lactone ring which were designed to explore the two-receptor hypothesis in greater detail[3]).

Discussion. – Our reasoning about the topology of the *Digitalis* receptor(s) was as follows. If the two-receptor hypothesis is correct, then it would seem, on superficial examination of the problem, that the topology of both receptors must be remarkably similar, since prior to our work a linear relationship was observed between the positive inotropic effect and the toxicity of the various natural cardenolide derivatives examined.

However, this need not be necessarily true. It is, for example, conceivable that both receptors (**A** and **B**) would require contact with the same face (β or α) of the steroid molecule but with a different face of the two non-identical faces of the unsaturated lactone. The geometry of one receptor might thus require rotamer **A** and the geometry of the other receptor, rotamer **B**.



A natural cardenolide could fit both receptors, since the lactone may rotate and present H_a to one and H_b to the other receptor. If we, however, replace one of the two diastereotopic H-atoms H_a and H_b, respectively, by a larger group, a cardenolide would result which is indistinguishable from the natural compound by one receptor and presents the larger group instead of a H-atom to the other one. Thus, if this simple idealized situation, in fact, exists in the heart muscle, epimeric 21-alkyl-cardenolides could have remarkable pharmacological properties. One of the epimers could show a

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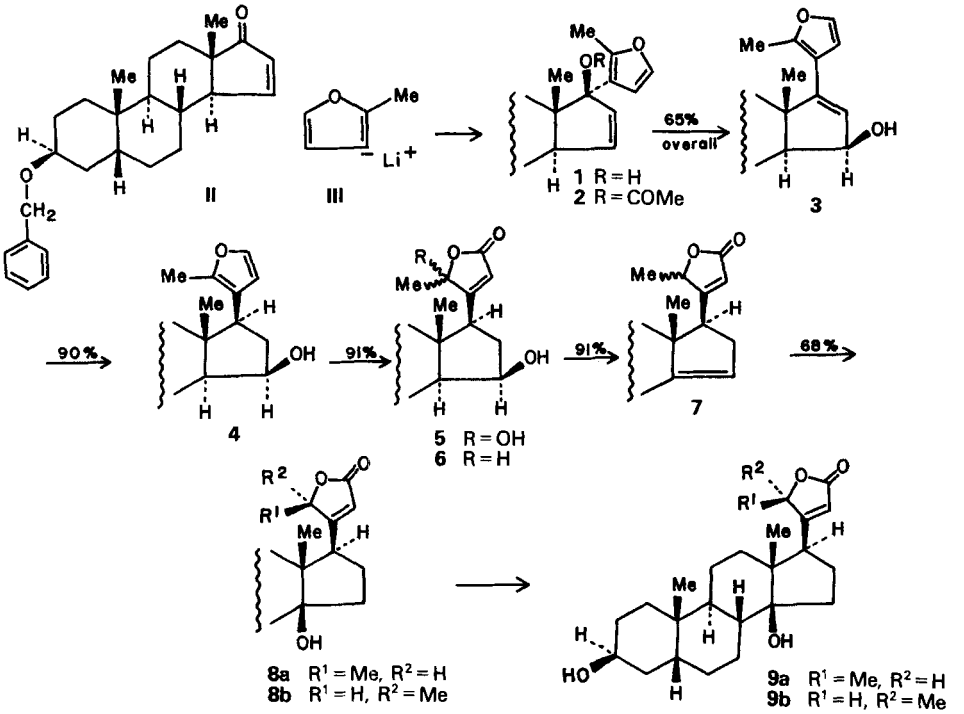
[2]) Pharmacologists are clearly divided on this question, but a complete discussion of the pharmacological literature in the framework of a chemical paper is not possible in the interest of brevity.

[3]) No pharmacology will be discussed, but the parameters (single injection effective dose (*ED*), minimal therapeutic dose (*MTD*), irregularity dose (*ID*), lethal dose (*LD*); *cf.* [3] and [5]) as reported to us by Prof. *Mendez* will be quoted in the appropriate place.

Scheme 1



positive inotropic effect without being toxic, the other epimer could be cardiotoxic without being inotropic[4]).

To test whether at least a partial dissociation of inotropy and toxicity in cardenolides on the basis of the above considerations is possible, we have decided to prepare first the two epimeric 21-methyldigitoxigenins[5]).

Our new synthetic methodology [4b] was eminently suitable for this task.

The standard starting material, the α,β-unsaturated ketone **II** was treated with the furyllithium derivative **III** which was obtained by metallation (BuLi) of 2-methyl-3-bromofuran [7]. The synthesis proceeded as shown in *Scheme 1* uneventfully in the usual high yields to the 15'β-hydroxy derivative 4. Compound **4** was oxidized with *m*-chloroperbenzoic acid, and the crude epimeric hydroxylactones **5** were reduced with $NaBH_4$ to the epimeric 21-methylcardenolides **6**. Since the epimers **6** could not be separated, the crystalline mixture was dehydrated with $SOCl_2$ and pyridine and the equally inseparable mixture **7** was converted to **8a** and **8b** by our modification of the method of *Engel* [4b]. The beautifully crystalline epimers, **8a** (major) and **8b** (minor),

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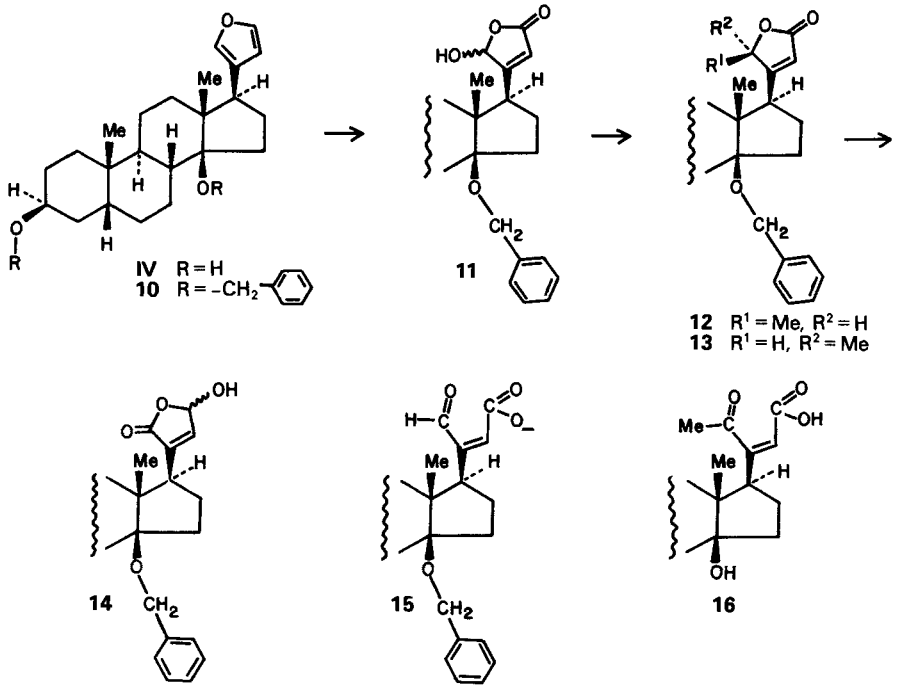
[4]) This simple theory and the synthesis of epimeric methyldigitoxigenins was discussed by *K.W.* in the Opening Lecture at the First International Conference on Chemistry and Biotechnology of Biologically Active Natural Products (Federation of European Chemical Societies), Varna, Bulgaria, September 21–26, 1981.

[5]) These two compounds have been prepared in a very small yield and quantity besides a variety of other products by *Repke et al.* by a direct base-catalyzed methylation of digitoxigenin (*cf.* [6]) without any mention of their potential significance for receptor topology.

Scheme 2



were separated by preparative TLC and debenzylated by hydrogenolysis over Pd/C to the two epimeric 21-methyldigitoxigenins **9a** and **9b**. The ratio in which the two epimers were obtained was 3.5:1. The problem of the configuration of the C(21) chiral center was dealt with very simply by submitting a sample of **9a** to Dr. *E.J. Gabe* (National Research Council of Canada, Ottawa) who solved the crystal and molecular structure in a few weeks and has already published the result [8]. Thus, the (21*R*)-21-methyldigitoxigenin formula **9a** can be rigorously assigned to the major epimer, leaving the (21*S*)-21-methyldigitoxigenin structure **9b** for the minor one.

Since the glucoside of **9a** has turned out to have good potency, and a high margin of safety *(vide infra)*, the development of a short and high yield process for the preparation of **9a** directly from digitoxigenin was desirable. This endeavour has been successful and is illustrated in *Scheme 2*.

The furan derivative **IV** (*cf.* [2]), easily prepared by treatment of digitoxigenin with diisobutylaluminum hydride, was first protected by benzylation, and the product **10** was oxidized with *m*-chloroperbenzoic acid to the epimeric lactols **11**. In this case, a slightly larger amount of the regioisomeric lactols **14** was formed than we observed previously in our synthesis of digitoxigenin [4b]. This was ascribed to the presence of the 14-benzyloxy group. The epimers **14** were separated with no difficulty and saved for use in the chemistry of the methylactodigins.

A solution of the epimers **11** was now treated in THF with an excess of MeLi at −78°. The reaction mixture was then acidified and the two epimeric products **12** and

13 were separated without any difficulty. The yields of the two compounds (**12** and **13**) were 76.5% and 10.1%, respectively.

Debenzylation by hydrogenolysis gave our well known methyldigitoxigenins **9a** and **9b**. It is clear that the reaction **11**→**12** + **13** involves, first, the abstraction of a proton from **11**, followed by an attack of MeLi on the aldehyde group of the resulting carboxylate ion **15**. In this respect, this process is similar to the reaction **5**→**6** *(Scheme 1)* which must proceed *via* a hydride attack on the intermediate keto acid **16** or its carboxylate salt. The remarkable aspect of the two processes is that they are both highly stereoselective and that they both yield the same major epimer. This, of course, is contrary to expectation since diastereoisomers should *(ceteris paribus)* result on delivering a methyl anion to **15** and a hydride ion to **16**. We must, consequently, conclude that the aldehyde in **15** and the methyl ketone in **16** present preferentially their opposite faces to the attack of the methyl anion or hydride ion, respectively. It is relatively easy to justify *post factum* this unexpected result by postulating a stabilization of different rotamers by H-bridges or steric repulsion in the two analogous reactions. We would like to point out the fortunate circumstance that the more potent and less toxic (*R*)-configuration is produced by both methods with considerable stereoselectivity.

It remains, finally, to compare the parameters of the epimers **9a** and **9b** submitted for pharmacological testing as glucosides with each other and with digitoxin. As seen in the *Table,* the glucoside of the (*R*)-epimer **9a** is only very slightly less potent than digitoxin, but its toxicity is dramatically reduced as measured by both ratios *ID/MTD* and *LD/MTD*. Consequently, it seems that the inotropy receptor does have the expected topological properties.

Table. *Potency and Toxicity of Cardenolide Derivatives*[3])

Compound	*ED*	*ID/MTD*	*LD/MTD*
Glucoside **9a**	0.7	45	128
Glucoside **9b**	3.0	33	65
Digitoxin	0.45	2.92	5.07

On the other hand, the toxicity receptor does not show an analogous geometry. Blockade of either of the two faces of the lactone ring reduces toxicity very strongly. This may mean that the topology of the toxicity receptor is such, that both diastereotopic H-atoms H_a and H_b of a natural cardenolide come into contact with the receptor and that the conformation of the cardenolide required by the receptor corresponds neither to **A** nor **B**, but, possibly, to the rotamer **C** (see above).

The lactone ring might be even inserted into a cleft of the toxicity receptor. It is clearly not possible to make finalized conclusions about the two receptor hypothesis on the basis of simple pharmacological parameters obtained on our two compounds. It is imperative to study receptor affinities, Na^+K^+ATPase inhibitions, *etc.* on the most sophisticated level. While this is beyond our experimental capability, it is hoped that the present study and the easy availability of the epimeric methyldigitoxigenins will stimulate some highly competent investigator to take up the problem.

We wish to thank Dr. *E. J. Gabe*, Ottawa, Canada, for solving the crystal and molecular structure of our major product **9a**. We also acknowledge financial support by the *Natural Sciences and Engineering Research Council*, Ottawa, Canada, the *New Brunswick Heart Foundation* and *Advance Biofactures Corporation*, New York.

Experimental Part

General. See [9].

2-Methyl-3-(3'β-benzyloxy-17'β-hydroxy-5'β-androst-15'-en-17'α-yl)furan (**1**). To a stirred solution of 2-methyl-3-bromofuran (3.22 g, 0.02 mol) in abs. Et_2O (35 ml), BuLi (7.50 ml; 2.4M solution in hexane) was added, under N_2 at −70°, and stirring was continued for 1 h. Compound **II** (3.78 g, 0.01 mol) in abs. Et_2O (35 ml) was then added dropwise within 10 min, and the mixture was stirred for 1 additional h. The mixture was kept cool, and H_2O (10 ml) was added slowly to it. More Et_2O (100 ml) was added and the org. layer was washed with H_2O and brine and dried ($MgSO_4$). Evaporation *in vacuo* gave **1** in quant. yield. It was used for the next step without further purification. IR ($CHCl_3$): 3600, 3450 (OH). ^{1}H-NMR ($CDCl_3$): 0.97 (*s*, 3H–C(18')); 1.00 (*s*, 3H–C(19')); 2.33 (*s*, CH_3–C(2)); 3.67 (br. *s*, H–C(3')); 4.47 (*s*, PhCH_2); 6.03–5.60 (*m*, H–C(15') and H–C(16')); 6.12 (*d*, H–C(4)); 7.23 (*d*, H–C(5)); 7.30 (*s*, 5 arom. H). MS: 460 ($C_{31}H_{40}O_3^+$). MS (HR): 460.2981 (M^+, calc. 460.2977).

2-Methyl-3-(3'β-benzyloxy-17'β-acetoxy-5'β-androst-15'-en-17'α-yl)furan (**2**). Compound **1** (4.60 g, 0.01 mol) was acetylated with Ac_2O (5.5 ml) in pyridine (80 ml) in the presence of a catalytic amount of 4-dimethylaminopyridine (100 mg) at r.t. for 24 h. The mixture was evaporated at 40° *in vacuo* to dryness. The residue was redissolved in Et_2O, washed with 5% citric acid, 5% $NaHCO_3$, brine, dried and evaporated *in vacuo*. The product **2** was used for rearrangement without further purification.

2-Methyl-3-(3'β-benzyloxy-15'β-hydroxy-5'β-androst-16'-en-17'-yl)furan (**3**). The crude compound **2** (5.02 g, 0.01 mol) was heated under reflux in aq. acetone (200 ml, 25% H_2O) in the presence of $CaCO_3$ (2.30 g) for 15 h. The mixture was filtered, the residue was washed with acetone, and the combined filtrates were evaporated under reduced pressure. The residue was dissolved in Et_2O, washed with 5% $NaHCO_3$, dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography on silica gel using 7% Et_2O/hexane to yield 3 g (65% overall from **II**) of pure **3** as a foam. IR ($CHCl_3$): 3620, 3475 (OH). ^{1}H-NMR ($CDCl_3$): 1.05 (*s*, 3H–C(18')); 1.23 (*s*, 3H–C(19')); 2.32 (*s*, CH_3–C(2)); 3.70 (br. *s*, H–C(3')); 4.47 (*s*, PhCH_2); 4.63 (br. *s*, H–C(15')); 5.70 (*d*, J = 3, H–C(16')); 6.30 (*d*, J = 2, H–C(4)); 7.22 (*d*, J = 2, H–C(5)); 7.30 (*s*, 5 arom. H). MS: 460 ($C_{31}H_{40}O_3^+$). MS (HR): 460.2985 (M^+, calc. 460.2977).

2-Methyl-3-(3'β-benzyloxy-15'β-hydroxy-5'β-androstan-17'β-yl)furan (**4**). The allylic alcohol **3** (4.60 g, 0.01 mol) was dissolved in EtOH (220 ml) and the solution was hydrogenated with 10% Pd/$CaCO_3$ (460 mg) as catalyst, in the presence of AcONa (5% aq. solution, 6.6 ml) at r.t. The catalyst was filtered off, and the filtrate was evaporated *in vacuo*. The residue was redissolved in Et_2O/CH_2Cl_2 3:1 and washed with H_2O. The org. layer was dried ($MgSO_4$) and evaporated. The product was purified by column chromatography using 5% Et_2O/hexane. The yield of the oily compound **4** was 4.16 g (90%). IR ($CHCl_3$): 3620, 3480 (OH). ^{1}H-NMR ($CDCl_3$): 0.83 (*s*, H–C(18')); 1.0 (*s*, 3H–C(19')); 2.20 (*s*, CH_3–C(2)); 3.67 (br. *s*, H–C(3')); 4.32 (br. *s*, H–C(15')); 4.50 (*s*, PhCH_2); 6.30 (*d*, J = 2, H–C(4)); 7.22 (*d*, J = 2, H–C(5)); 7.33 (*s*, 5 arom. H). MS: 462 ($C_{31}H_{42}O_3^+$). MS (HR): 462.3140 (M^+, calc. 462.3133).

3β-Benzyloxy-15β-hydroxy-21-methyl-5β,14α-card-20(22)-enolide (**6**; mixture of 21-epimers). A mixture of **4** (1.386 g, 3 mmol), AcONa (615 mg) and AcOH (450 mg) in $CHCl_3$ (60 ml) was treated with *m*-chloroperbenzoic acid (1.14 g, 6.6 mmol) and stirred at r.t. for 2 h. The mixture was diluted with $CHCl_3$ (250 ml), extracted with 5% aq. Na_2SO_3, and 5% $NaHCO_3$, dried ($MgSO_4$) and evaporated. The crude lactol **5**, thus obtained, was reduced with $NaBH_4$ (650 mg) in a CH_2Cl_2 (300 ml)/H_2O (60 ml) at r.t. for 8 h. The mixture was then acidified with 5% citric acid, diluted with more CH_2Cl_2, and the org. layer was washed with 5% $NaHCO_3$ and brine, dried ($MgSO_4$) and evaporated. The products **6** could not be separated. Chromatography on silicic acid with 5% CH_2Cl_2 in Et_2O yielded 1.3 g (90.6%) of the pure epimeric mixture as a crystalline solid. IR ($CHCl_3$): 3620, 3500 (OH), 1745 (C=O). ^{1}H-NMR ($CDCl_3$): 1.03 (*s*, 3H–C(18) and 3H–C(19)); 1.44 (*d*, J = 6, CH_3–C(21)); 3.72 (br. *s*, H–C(3)); 4.27 (br. *s*, H–C(15)); 4.48 (*s*, PhCH_2); 4.91 (*m*, H–C(21)); 5.86 (*s*, H–C(22)); 7.33 (*s*, 5 arom. H). MS: 478 ($C_{31}H_{42}O_4^+$). MS (HR): 478.3073 (M^+, calc. 478.3083).

3β-Benzyloxy-21-methyl-5β-carda-14,20(22)-dienolide (**7**). The hydroxylactones **6** (4.78 g, 0.01 mol) were dissolved in CH_2Cl_2 (100 ml) and pyridine (25 ml) and treated with $SOCl_2$ (9 ml) at −78°. The mixture was slowly allowed to attain 0° and stirred at 0° for ½ h. Aq. $NaHCO_3$ (5%, 10 ml) was then added, and the org. layer was washed with 5% citric acid and brine. Drying and evaporation gave crystalline material which was purified by column chromatography using 7% acetone/hexane. The yield of the epimeric mixture **7** was 4.18 g (91%). IR ($CHCl_3$): 1735 (C=O). ^{1}H-NMR ($CDCl_3$): 0.94 (*s*, 3H–C(18)); 0.97 (*s*, 3H–C(19)); 1.45 (*d*, J = 7, CH_3–C(21)); 3.69 (br. *s*, H–C(3)); 4.48 (*s*, PhCH_2); 4.92 (*m*, H–C(21)); 5.24 (br. *s*, H–C(15)); 5.84 (*s*, H–C(22)); 7.33 (*s*, 5 arom. H). MS: 460 ($C_{31}H_{40}O_3^+$). MS (HR): 460.2981 (M^+, calc. 460.2977).

(21R)- and (21S)-3-O-Benzyl-21-methyldigitoxigenin (= (21R)- and (21S)-3β-Benzyloxy-14β-hydroxy-21-methyl-5β,14β-card-20(22)-enolide; **8a** *and* **8b**, *respectively).* The epimeric mixture **7** (460 mg, 1 mmol) was dissolved in acetone (20 ml). AcOH (0.9 ml), H_2O (2 ml), and *N*-bromoacetamide (158.0 mg) were added, and the mixture was stirred for ½ h at 5–10°. It was then diluted with CH_2Cl_2, washed with 5% aq. Na_2SO_3, dried ($MgSO_4$) and evaporated *in vacuo*. The residue was redissolved in CH_2Cl_2/MeOH 1:1 (20 ml) and stirred initially in an ice bath and later at r.t. with *Raney*-Ni (5.0 g) in the presence of AcONa (300 mg) for 1 h. The *Raney*-Ni was filtered off, and the filtrate was washed with H_2O, dried over anh. $MgSO_4$ and evaporated. The crude product, on purification by prep. TLC using hexane/AcOEt 4:1 yielded 252 mg of **8a** (m.p. 222–223°) and 72.2 mg of **8b** (m.p. 210–212°). The pure compounds were crystallized from $CHCl_3$/hexane and $CHCl_3$/Et_2O, respectively. **8a**: IR ($CHCl_3$): 3600, 3450 (OH), 1735 (C=O). ^{1}H-NMR ($CDCl_3$): 0.91 (*s*, 3H–C(18)); 0.97 (*s*, 3H–C(19)); 1.39 (*d*, J = 6, CH_3–C(21)) 3.72 (br. *s*, H–C(3)); 4.48 (*s*, PhCH_2); 4.84 (*m*, H–C(21)); 6.15 (*s*, H–C(22)); 7.33 (*s*, 5 arom. H). MS: 478 ($C_{31}H_{42}O_4^+$). MS (HR): 478.3073 (M^+, calc. 478.3083). Anal. calc. for $C_{31}H_{42}O_4$ (478.65): C 77.78, H 8.84; found: C 77.51, H 8.80.

8b: IR ($CHCl_3$): 3600, 3450 (OH), 1735 (C=O). ^{1}H-NMR ($CDCl_3$): 0.91 (*s*, 3H–C(18)); 0.97 (*s*, 3H–C(19)); 1.39 (*d*, J = 6, CH_3–C(21)); 3.69 (br. *s*, H–C(3)); 4.48 (*s*, PhCH_2); 4.81 (*m*, H–C(21)); 6.09 (*s*, H–C(22)); 7.30 (*s*, 5 arom. H). MS: 478 ($C_{31}H_{42}O_4^+$). MS (HR): 478.3080 (M^+, calc. 478.3083).

(21R)-21-Methyldigitoxigenin (= (21R)-3β,14β-Dihydroxy-21-methyl-5β,14β-card-20(22)enolide; **9a**). A solution of **8a** (478 mg, 1 mmol) in benzene/EtOH 1:4 (100 ml) was hydrogenated at atmospheric pressure and r.t. in the presence of 10% Pd/C (50 mg) for 2 h. The catalyst was filtered off and washed with EtOH. The combined filtrates were evaporated under reduced pressure, and the product was purified by crystallization from $CHCl_3$/hexane. Compound **9a** was obtained in a 90% yield (350 mg), m.p. 246–247°. UV (EtOH): 218 (4.22). IR ($CHCl_3$): 3600, 3450 (OH), 1720 (C=O). ^{1}H-NMR ($CDCl_3$): 0.91 (*s*, 3H–C(18)); 0.98 (*s*, 3H–C(19)); 1.44 (*d*, J = 7, CH_3–C(21)); 4.16 (br. *s*, $w_{½}$ = 9, H–C(3)); 4.88–5.02 (*m*, H–C(21)); 6.18 (*s*, H–C(22)). MS: 388 ($C_{24}H_{36}O_4^+$). MS (HR): 388.2611 (M^+, calc. 388.2613). Anal. calc. for $C_{24}H_{36}O_4$ (388.53): C 74.19, H 9.34; found: C 74.05, H 9.37. For X-ray structure, see [8].

(21S)-21-Methyldigitoxigenin (= (21S)-3β,14β-Dihydroxy-21-methyl-5β,14β-card-20(22)-enolide; **9b**). Compound **8b** was catalytically debenzylated exactly as described for **8a**. The product **9b** (m.p. 241–243°) was obtained in a yield of 88% and recrystallized from $CHCl_3$/hexane. UV (EtOH): 221 (4.08). IR ($CHCl_3$): 3600, 3475 (OH), 1740 (C=O). ^{1}H-NMR ($CDCl_3$): 0.92 (*s*, 3H–C(18)); 0.97 (*s*, 3H–C(19)); 1.44 (*s*, J = 7, CH_3–C(21)); 4.15 (br. *s*, $w_{½}$ = 9, H–C(3)); 4.95–4.80 (*m*, H–C(21)); 6.11 (*s*, H–C(22)). MS: 388 ($C_{24}H_{36}O_4^+$). MS (HR): 388.2608 (M^+, calc. 388.2613). The entire MS of **9a** und **9b** were identical.

3-(3'β,14'β-Dibenzyloxy-5'β,14β-androstan-17'β-yl)furan (**10**). A solution of **IV** (4.53 g) in dioxane (460 ml) was heated under reflux with NaH (2.42 g, 57% dispersion in oil) and a catalytic amount of 18-crown-6 ether for 4 h. Benzyl bromide (6.04 ml) was then added and refluxing was continued for 4 h. After cooling, the mixture was filtered through *Celite* and evaporated. The product **10** was crystallized from $CHCl_3$/hexane (m.p. 140–142°) and was obtained in a yield of 86.4% (5.87 g). IR ($CHCl_3$): no (OH). ^{1}H-NMR ($CDCl_3$): 0.87 (*s*, 3H–C(18')); 1.0 (*s*, 3H–C(19')); 3.75 (br. *s*, $w_{1/2}$ = 7, H–C(3')); 4.5–4.65 (2*s*, 2 PhCH_2); 6.19 (*s*, H–C(2)); 7.12 (*s*, H–C(4)); 7.17 (*s*, H–C(5)); 7.36 (*s*, 10 arom. H). MS: 538 ($C_{37}H_{46}O_3^+$). MS (HR): 538.3449 (M^+, calc. 538.3446). Anal. calc. for $C_{37}H_{46}O_3$ (538.74): C 82,48, H 8.61; found: C 82.71, H 8.50.

3,14-Di-O-benzyl-21-hydroxydigitoxigenin (= 3β,14β-Dibenzyloxy-21-hydroxy-5β,14β-card-20(22)-enolide, **11**; *mixture of 21-epimers).* A solution of **10** (5.87 g) in CH_2Cl_2 (235 ml) was treated with *m*-chloroperbenzoic acid (5.56 g) in the presence of AcONa (2.41 g) and AcOH (1.76 g) for 2 h at 0°. The excess of the peracid was then destroyed with Me_2S and the mixture was diluted with more CH_2Cl_2. The org. layer was washed with sat. aq. $NaHCO_3$ and brine, dried over anh. $MgSO_4$, and evaporated. The regioisomers **11** and **14** were separated by chromatography on silica gel using acetone/hexane mixtures. Both regioisomers were crystalline mixtures. Compound **11** was obtained in a yield of 67% (4.16 g) and compound **14** in a yield of 27% (1.67 g). The material **11** contained besides the two epimeric lactols also a significant amount of the aldehyde tautomer. It was used without any further purification for the next reaction.

*(21R)- and (21S)-3,14-Di-O-benzyl-21-methyldigitoxigenin (= (21R)- and (21S)-3β,14β-Dibenzyloxy-21-methyl-5β,14β-card-20(22)-enolide; **12** and **13**, respectively).* A solution of **11** (4.16 g) in dry THF (425 ml) was treated with MeOH (18.9 ml, 1.5M solution) at −78° under N_2 for 20 min with stirring. The mixture was then acidified at 0° and diluted with $CHCl_3$. The org. layer was washed with 5% citric acid, aq. $NaHCO_3$ and brine, dried over anh. $MgSO_4$ and evaporated. The products were separated by chromatography in hexane/acetone 9:1. Compound **12** was obtained (3.125 g, 75.6%) as foam and **13** (418 mg, 10.1%) as a crystalline solid which was further crystallized from Et_2O/hexane (m.p. 136–138°). **12**: IR ($CHCl_3$): 1725 (C=O). ^{1}H-NMR ($CDCl_3$): 1.01 (*s*, 3H–C(18)); 1.04 (*s*, 3H–C(19)); 1.36 (*d*, J = 7, CH_3–C(21)); 3.75 (br. *s*, $w_{½}$ = 7, H–C(3)); 4.51 (*s*, 2H, PhCH_2O–C(3)); 4.78–4.56 (*m*, 2H, PhCH_2O–C(14)); 4.78–4.92 (*m*, H–C(21)); 5.71 (*s*, H–C(22)); 7.34 (*s*, 10 arom. H). MS: 568 ($C_{38}H_{48}O_4^+$). MS (HR): 568.3554 (M^+, calc. 568.3552). **13**: IR ($CHCl_3$): 1720 (C=O). ^{1}H-NMR ($CDCl_3$): 1.0 (*s*, 3H–C(18)); 1.05 (*s*, 3H–C(19)); 1.40 (*d*, J = 7, CH_3–C(21)); 3.76 (br. *s*, $w_{½}$ = 7, H–C(3)); 4.51, 4.61 (2*s*, 2 PhCH_2); 4.94–4.78 (*m*, H–C(21)); 5.68 (*s*, H–C(22)); 7.36 (*s*, 10 arom. H). MS: 568 ($C_{38}H_{48}O_4^+$). MS (HR): 568.3563 (M^+, calc. 568.3552). Anal. calc. for $C_{38}H_{48}O_4$ (568.76): C 80.24, H 8.51; found: C 79.98, H 8.52.

(21R)-21-Methyldigitoxigenin (**9a**). A solution of **12** (3.125 g) in benzene/EtOH 1:3 (80 ml) was hydrogenated at r.t. and atmospheric pressure over 10% Pd/C (700 mg). The catalyst was filtered off and the solution evaporated to dryness. The product was crystallized from $CHCl_3$/hexane and gave 1.96 g (92%) of pure **9a** (m.p. 246–247°). The product was identical in all respects with the sample of **9a** described above.

(21S)-21-Methyldigitoxigenin (**9b**). A solution of **13** (418 mg) in benzene/EtOH 1:3 (8 ml) was hydrogenated at r.t. and atmospheric pressure over 10% Pd/C (78 mg). The catalyst was filtered off, and the solution was evaporated. Crystallization from $CHCl_3$/hexane yielded (268 mg, 94%) of pure **9b** (m.p. 241–243°). The product was identical in all respects with the sample of **9b** described above.

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