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SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF CARDENOLIDES SUBSTITUTED AT THE BUTENOLIDE PART

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Abstract - Deprotonation of the digitoxigenin lactone moiety with NaH in N-methylpyrrolidone yields an enolate that reacts at the 21- or 22-position depending on the electrophile. Lactone substituted derivatives of digitoxigenin have been prepared and their inhibition of the cardiac Na^+ pump and the inotropic effect of some of the compounds have been studied. Structure-activity relationships are discussed in terms of the Höltje-Anzali model.

Introduction

The Na^+ pump of animal cells generates and maintains the Na^+ and K^+ gradients across the cell membrane. These ion gradients are essential for the electrical excitability of the plasma membrane. In addition, the Na^+ gradient serves as an energy source for the transmembrane transport of specific substances, e.g. sugar and amino-acid import and Ca^{2+} transport out of the resting cell.[1,2] Cardioactive glycosides inhibit the Na^+ pump, thereby raising the intracellular Na^+ concentration. The resulting decrease in the Na^+ gradient across the cell membrane of cardiac myocytes reduces the energy available for transport of Ca^{2+} out of the cell by Na^+/Ca^{2+} exchange. This ultimately leads to the (medicinally used) positive inotropic effect of these substances.[3]

The molecular basis of the Na^+ pump is a Mg^{2+}-dependent Na^+- and K^+-activated ATPase in the cell membrane. This ATPase consists of a large catalytic α subunit and a smaller β subunit the function of which is still unknown. The complete amino-acid sequences of α subunits of various species have been determined. The protein chain spans the plasma membrane at least eight times. Two of the extracellular regions are assumed to be involved in binding the steroid moiety of the cardiac glycosides; however, the precise site and the mode(s) of binding have not yet been determined.[1,2] Structure-activity relationships and the results of affinity labeling and site directed mutagenesis studies have been discussed in terms of a number of different binding models.[4,5,6,7] For the work described below we shall restrict ourselves to the model described recently by Höltje and Anzali[7] which seems to take into account most of the experimental results. In essence, it postulates that the H1-H2-extracellular region is involved in binding to the sugar part and most of the steroid nucleus, whereas there exist specific interactions between the 14-OH group and the lactone ring, respectively, and the triade Tyr-Thr-Trp(308-310) from the H3-H4 extracellular loop (see Figure 1 and the discussion below). The merits of the Höltje-Anzali model can be ascertained by experiment. It was the aim of our work to (i) develop approaches to cardenolide analogues substituted at the lactone ring, (ii) study their structure-activity relationships by means of electrophysiological methods, and (iii) test the Höltje-Anzali model with the new results. Ultimately, we are interested in using active derivatives to localize the lactone binding site of the ATPase by affinity labeling.

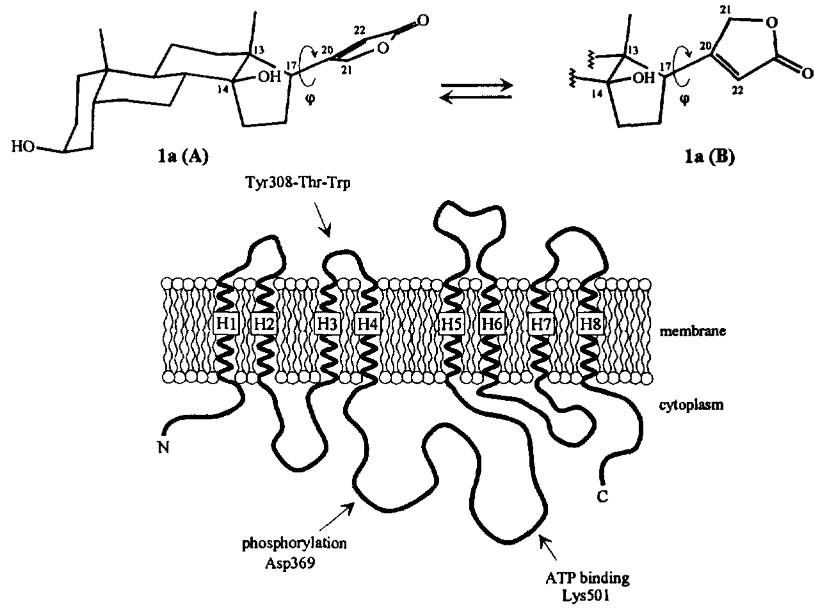
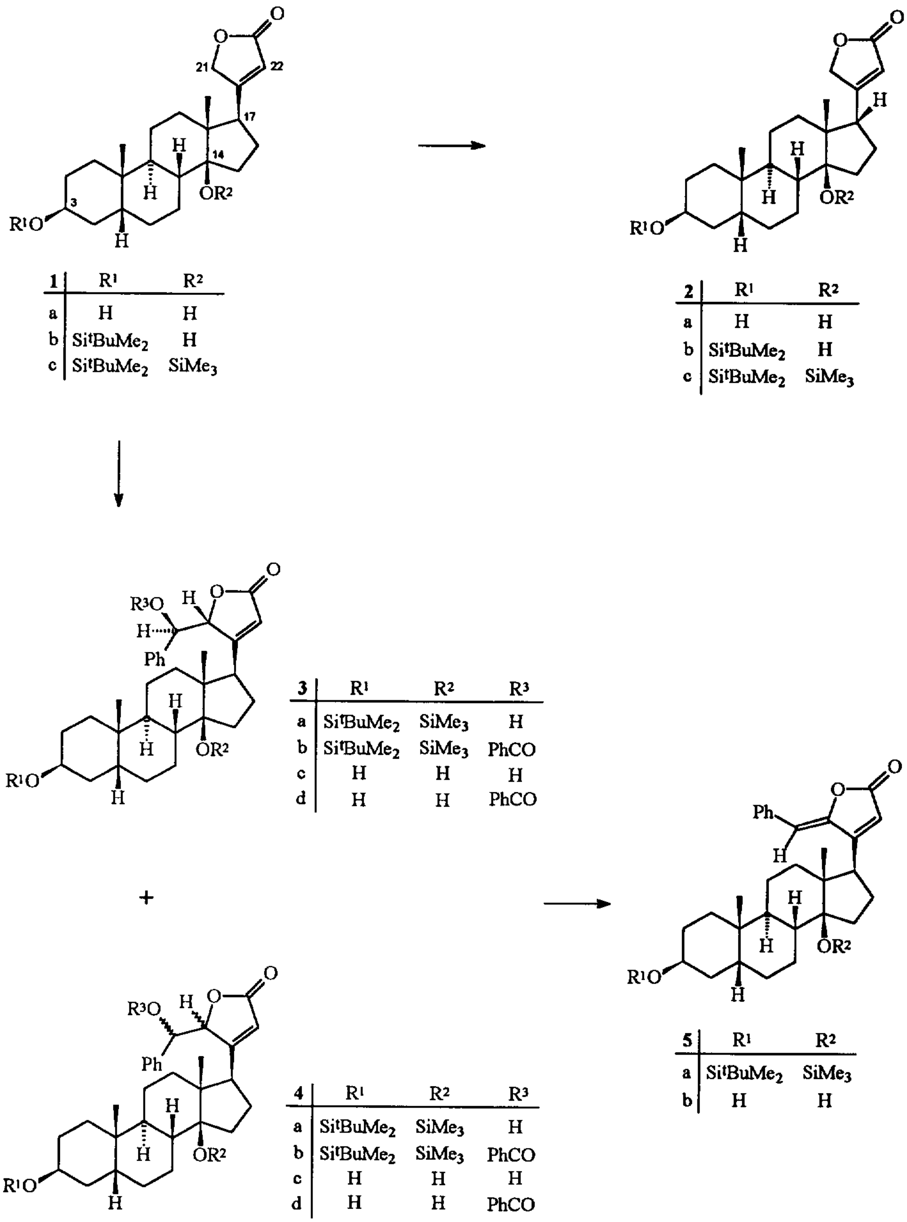


Figure 1. Model representing the α subunit Na,K-ATPase topology[2] and the so-called 14/21- and 14/22-conformations of digitoxigenin (**1a** (A) and **1a** (B), respectively).

Protecting group chemistry for digitoxigenin (1a)

Treatment of **1a** with tert-butyldimethylsilyl triflate yielded selectively the monosilyl ether **1b**. After some experimentation the 14-OH-group could be converted in high yields into its trimethylsilyl ether **1c** with trimethylsilyl triflate. Removal of the silyl protecting groups was cleanly achieved by treatment of **1c** with p-toluenesulfonic acid (0.1 equiv.) in methanol.

On attempted deprotection of **1c** with TBAF only the 14-silylether was cleaved (**1c**→**1b**). On prolonged action of TBAF **1b** rearranged to give the 17-stereoisomer **2b**. Under the same conditions **1a** formed the 17-epimer **2a**. This seems to be the most convenient protocol for the formation of 17βH-digitoxigenin, a compound previously obtained by treatment of **1a** with sodium acetate / sodium tosylate in boiling DMF.[8,9,10,11] Details of the structure of **2a** have now been established by X-ray analysis (see Figure 2).



1	R1	R2
a	H	H
b	Si^{t}BuMe$_2$	H
c	Si^{t}BuMe$_2$	SiMe$_3$

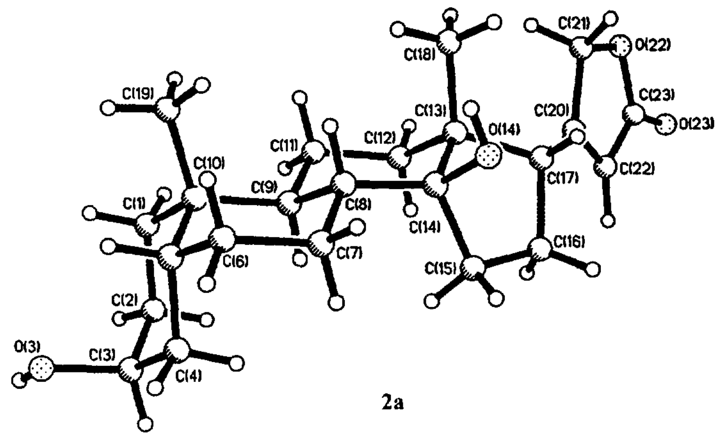
2	R1	R2
a	H	H
b	Si^{t}BuMe$_2$	H
c	Si^{t}BuMe$_2$	SiMe$_3$

3	R1	R2	R3
a	Si^{t}BuMe$_2$	SiMe$_3$	H
b	Si^{t}BuMe$_2$	SiMe$_3$	PhCO
c	H	H	H
d	H	H	PhCO

+

4	R1	R2	R3
a	Si^{t}BuMe$_2$	SiMe$_3$	H
b	Si^{t}BuMe$_2$	SiMe$_3$	PhCO
c	H	H	H
d	H	H	PhCO

5	R1	R2
a	Si^{t}BuMe$_2$	SiMe$_3$
b	H	H



2a

Figure 2. X-ray crystal structure of **2a**.

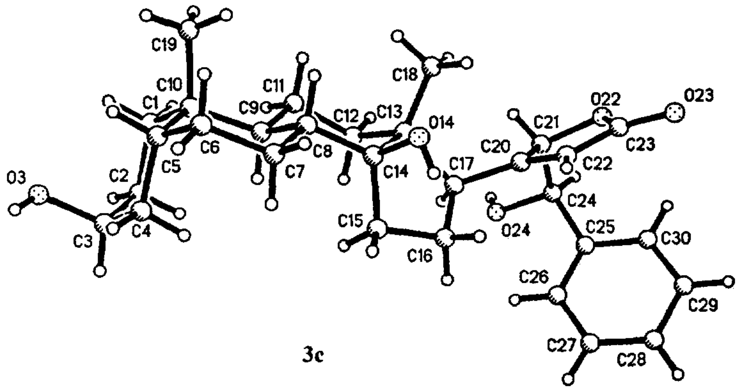
Substitution reactions at the lactone ring

As reported by Lindig and Repke,[12,13] the lactone ring of digitoxigenin can be deprotonated with sodium hydride in DMF. In our studies deprotonation of **1a** and **1c** was performed with sodium hydride in N-methylpyrrolidone (NMP). In view of the results reported above this deprotonation obviously leads to the kinetic lactone enolates. On trapping with H_2O (D_2O) we never observed the formation of **2a** and **2c**, respectively. Thus, the enolate precursors of **2a** and **2c** appear to be the thermodynamic enolates.

Trapping of the kinetic enolates obtained from **1a** and **1c** leads to either 21- or 22-substituted derivatives depending on the electrophile. Thus, deprotonation of **1c** with less than one equiv. of sodium hydride in NMP at ambient temperature followed by treatment with benzaldehyde yielded the benzylidene derivative **5a** (61%). The configuration at the newly formed double bond was determined by NOED. The ^{1}H NMR spectrum of **5a** displays two olefinic singlets at δ = 5.98 and 6.05. Saturation of the signal at δ = 5.98 led to the appearance of the signals of the aromatic ortho protons and that of 17α-H. The NOE at the aromatic proton signals means that the δ = 5.98 signal corresponds to the proton of the benzylidene group (H_b) and an enhancement at the 17α-H signal is only possible, when the configuration at the benzylidene double bond is (Z). In addition, an NOED was observed at the $OSiMe_3$ signal when δ = 6.05 (22-H) was saturated.

Removal of the protecting groups of **5a** with p-toluenesulfonic acid in methanol yielded **5b** (72%).

The elimination reaction could be avoided, when sodium hydride (<1 equiv.) and benzaldehyde were added to a solution of **1c** at lower temperature (-20°C). Under these conditions a mixture of diastereomers **3a** and **4a** was obtained. Separation and removal of the protecting groups furnished **3c** and **4c**, respectively. The configuration at the newly formed stereocenters of **3a** was determined by X-ray analysis of **3c** (Figure 3). The (R) configuration at C-21 in **3a** indicates that the reactive conformation of the enolate is close to the 14/22 conformation of **1c** (see Figures 1 {structure **1a** (B)} and 3). **3a** and **4a** were converted into the corresponding benzoates **3b** and **4b** with benzoic acid, dicyclohexylcarbodiimide and a catalytic amount of DMAP.[14] **3b** and **4b** were readily deprotected with p- toluenesulfonic acid (0.1 equiv.) in methanol (**3b** → **3d**, **4b** → **4d**).



3c

Figure 3. X-ray crystal structure of **3c**.

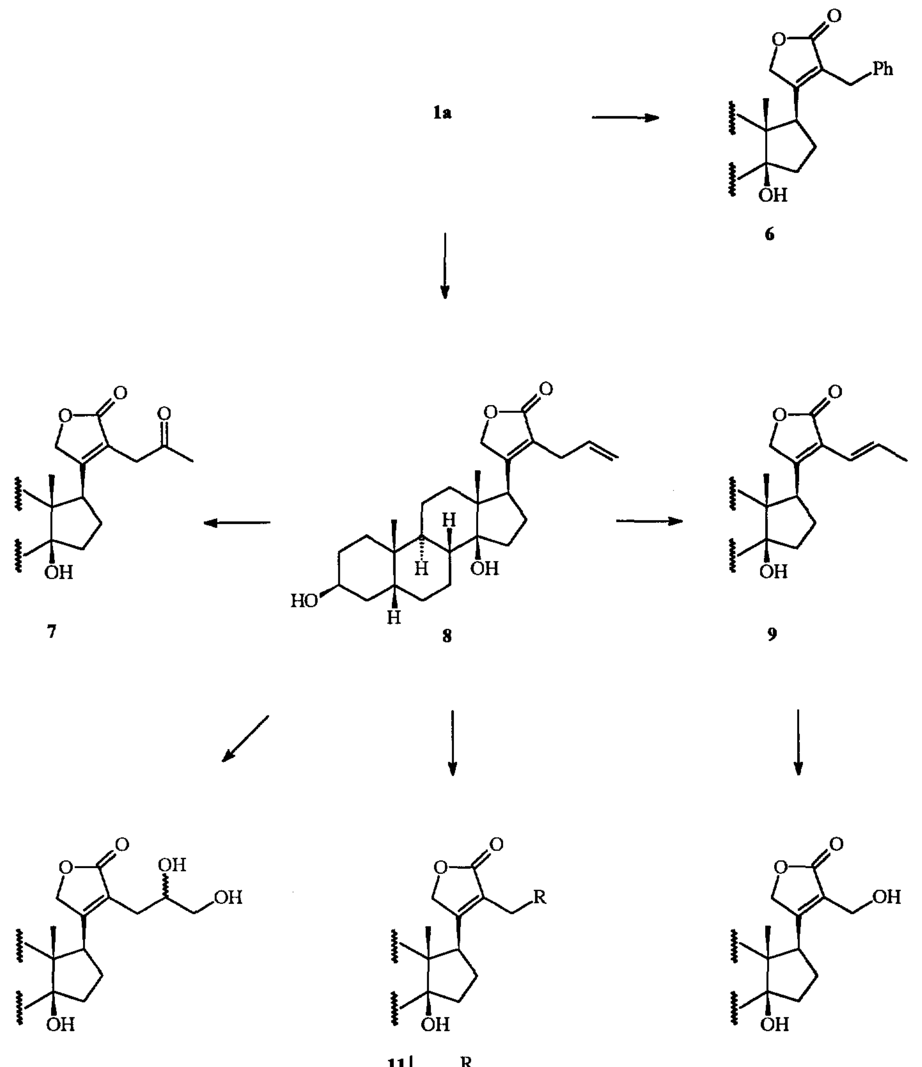
On treatment of free digitoxigenin (**1a**) with sodium hydride and benzaldehyde only the elimination product **5b** could be obtained. 21-substitution was also observed when the lactone enolate was trapped with either phenylselenenyl bromide (whereupon a mixture of stereoisomeric 21-phenylselenenyl derivatives was obtained, see Experimental, formulae not shown) or a brominating reagent.[15] In agreement with results reported by the Repke group,[13] reaction of the lactone enolate with allyl bromide led to substitution in the 22-position. Under our conditions **8** was formed from **1a** in 79% yield. Similarly, 22-benzyl-digitoxigenin (**6**) was prepared in 78% yield.

The reason for the regioselectivity observed in the reactions of the lactone enolate with different electrophiles has not been studied explicitly. The results seem, however, to indicate that soft electrophiles prefer the attack at the 22 position.

The allyl group of **8** was functionalized to get a variety of 22-substituted digitoxigenin derivatives. Hydroboration of **8** with BH_3·THF followed by oxidation with hydrogen peroxide provided **11a** in moderate yield. The sequence (i) ozonolysis at -78°C and (ii) reduction with $NaBH_4$ converted **8** into **11c**, whereas aldehyde **11b**, a rather unstable compound, was obtained from **8** by ozonolysis followed by PPh_3 treatment. In order to introduce two OH-functions stereoselectively into the side chain of **8**, the Sharpless method of asymmetric dihydroxylation[16] was employed. However, a 1:1 mixture of diastereomers **10a** and **10b** was obtained (80%). The product ratio was about the same using either AD-mix-α or AD-mix-β.

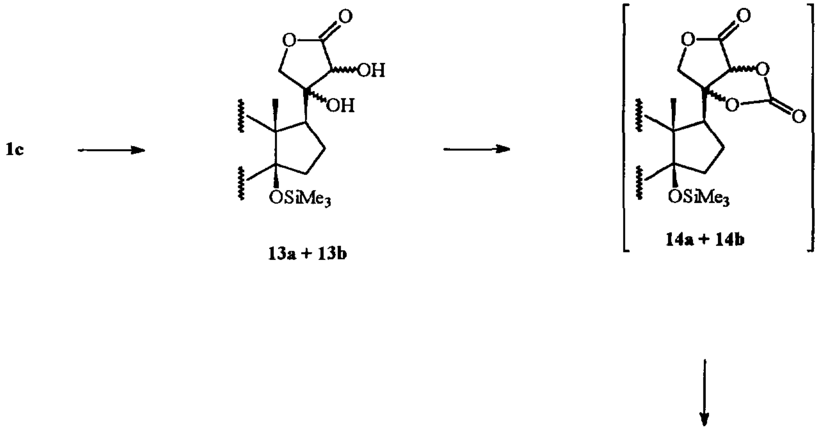
Isomerisation of the allylic double bond with a hydrogenated cationic Ir-catalyst[17] furnished the conjugated diene **9**. On the basis of the 15.8 Hz coupling constant between the vinylic H's, the configuration at the double bond is assigned (E). Ozonolytic cleavage of this double bond at -78°C followed by reduction with $NaBH_4$ provided **12** (41%). After Wacker-oxidation of **8** with a catalytic amount of $PdCl_2$, CuCl and oxygen[18] ketone **7** was isolated in 71% yield.

A route to 22-hydroxy-cardenolides as established by Lindig[19] was optimized. Silyl protected digitoxigenin derivative **1c** was dihydroxylated with osmium tetroxide in pyridine as reported by Schüpbach et al.[20] to afford

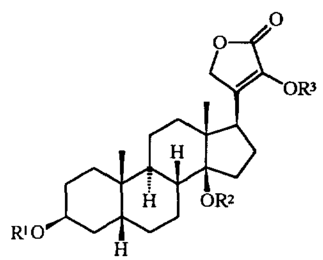


11	R
a	CH_2CH_2OH
b	CHO
c	CH_2OH

a mixture of diastereomeric cis diols **13a** and **13b**. **13a** and **13b** were treated with carbonyldiimidazole (or phosgene) in pyridine to generate carbonates **14a** and **14b**.[21] Formation of **14a** and **14b** could be observed by TLC, although they turned out to be unstable towards silica. Thus, the carbonates were not isolated. Addition of sodium hydride to the reaction mixture led to the elimination of CO_2 and provided **15a** in 86% yield. Esterification of **15a** with benzoic acid and acetic acid, respectively, occurred under the conditions mentioned above to yield **15b** and **15c**, respectively. Removal of the protecting groups of **15a**, **15b** and **15c** with p-toluenesulfonic acid in methanol then provided the desired compounds **15d**, **15e**, and **15f** in good yields.



15	R^1	R^2	R^3
a	Si^tBuMe_2	$SiMe_3$	H
b	Si^tBuMe_2	$SiMe_3$	COPh
c	Si^tBuMe_2	$SiMe_3$	$COCH_3$
d	H	H	H
e	H	H	COPh
f	H	H	$COCH_3$



Pharmacological properties of 21- and 22-substituted digitoxigenin derivatives

The inhibition of the Na^+,K^+ pump of sheep cardiac Purkinje fibres by the new compounds was studied by means of electrophysiological methods as described previously.[22] In addition a patch clamp method[23] was applied to isolated Purkinje cells. The concentrations of the compounds required for half-maximal pump inhibition (apparent K_D values K_D') are listed in Table 1. Compounds **8** and **15d - f** were tested for an impact on the contraction of the fibres. They exerted a positive inotropic effect. Details of this work will be published elsewhere. The K_D' value of **1a** was determined to be $7 \cdot 10^{-7}$ mol/l. With a few exceptions introduction of a substituent in either the 21- or 22-position caused the compounds to be less active by 2-4 orders of magnitude. In agreement with results previously reported from the Repke group[24] the 22-hydroxy compound **15d** is only slightly less active than **1a**, the same holds for the 22-benzoyloxy derivative **15e**. The 22-acetoxy analogue **15f** is as active as **1a**. One may ask the question whether the increase in activity of **15f** when compared with **15d** has the same origin as the stronger activity of the 16-ester derivatives of gitoxigenin (16β-hydroxy-digitoxigenin) in comparison to gitoxigenin itself.[24] Also very remarkable is the difference in the K_D' values of **7** and **15f** which seems to indicate that the oxygen directly attached to the lactone ring is involved in binding to the enzyme (it is also conceivable that a hydrogen bond between the 14-OH group and this oxygen at the 22-position forces the lactone ring into the conformation that binds to the enzyme).

Table 1. 21- and 22-substituted digitoxigenin derivatives: K_D' values and torsional angles and force-field energy differences of the so-called 14/21- and 14/22-conformations (see text).

Compound	K_D' [mol/l]	Torsion angle φ[a)] 14/21	14/22	ΔE[b)] [kJ/mol]
1a	$7 \cdot 10^{-7}$	-115	112	≈ 0
1c	$7 \cdot 10^{-5}$	-112	111	-4
2a	$1 \cdot 10^{-4}$	--	--	--
3c	$1 \cdot 10^{-4}$	≈ 12[c)]	≈ 112	21
3d	inactive	--	--	--
4c	$8 \cdot 10^{-5}$	configuration at C-21 and C-1' unknown		
4d	inactive	--	--	--
5b	$>2 \cdot 10^{-4}$	-79[c)]	69[c)]	≈ 0
6	$8 \cdot 10^{-4}$	-115	112	-17
7	$3 \cdot 10^{-5}$	-110	113	-4
8	$4 \cdot 10^{-5}$	-110	109	≈ 0
9	$2 \cdot 10^{-4}$	-110	112	≈ 0
10a + 10b	$1 \cdot 10^{-4}$	≈ -112	≈ 110	≈ 0 for (2'R), 7 for (2'S)
11a	$1 \cdot 10^{-4}$	-110	113	≈ 0
11c	$1 \cdot 10^{-3}$	-110	112	≈ 0
12	$6 \cdot 10^{-5}$	-110	110	4
15d	$3 \cdot 10^{-6}$	-111	108	12
15e	$9 \cdot 10^{-6}$	-110	109	11
15f	$9 \cdot 10^{-7}$	-114	108	6

a) φ = ∠ C13-C17-C20-C22, in degrees.

b) ΔE < 0 means that the 14/21-conformation is preferred.

c) Conformation different from 14/21 or 14/22.

Discussion of the new structure-activity relationships in terms of the Höltje-Anzali model

Rotation of the lactone moiety in normal cardenolides around the 17-20 bond is known to result in two low energy conformations of comparable energy (14/21 and 14/22, respectively, see Figure 1).[6] The C13-C17-C20-C22 torsional angle difference of these two conformations is roughly 180°. The special features of the Höltje-Anzali model as far as binding of the lactone ring to the Na,K-ATPase is concerned are three-fold: (i) the lactone ring binds to the H3-H4 region of the enzyme, (ii) the binding conformation is **1a** (A), the 14/21-conformation (see Figure 1), (iii) the main binding interactions of the steroid to the enzyme in the H3-H4 region are hydrogen bonds between the steroid and the triade Tyr-Thr-Trp(308-310), i.e. a hydrogen bond between Trp310 and the lactone C=O and another one between the Tyr308 hydroxy group and the 14β-OH (see Figure 4). The geometry of the Tyr-Thr-Trp fragment as used in the Höltje-Anzali model was taken from the crystal structure of a picornavirus capsid protein (2plv-1).[7]

For our substituted cardenolides, we have evaluated the relative potential energies of different conformations which were obtained on rotation of the lactone ring around the C-17 - C-20 bond, using the CHARMm force field. For the lowest energy conformations obtained in these (vacuum) calculations, the torsional angles and the energy differences are summarized in Table 1. In those cases where a hydrogen bond between the 14β-OH group and the 22-OR substituent was possible, the 14/22-conformation was clearly preferred. Docking of **1a** to the Tyr-Thr-Trp fragment was performed using interactive graphics. The structure of the tripeptide was left as obtained from the X-ray data bank in order to leave the conclusions apt to simple experimental testing. The steroid **1a** in its 14/21-conformation was positioned in the way described by Höltje and Anzali as to permit the formation of the hydrogen bonds indicated above (see Figure 4). As can be seen from Figure 4 the front part of the Höltje-Anzali model is occupied by Tyr308. Therefore, introduction of a substituent into either the 21- or 22-position in all cases (including the OR substituents) caused the lactone moiety to prefer a conformation with the substituent at the lactone ring pointing to the back (which is empty in the Höltje-Anzali model) where the repulsive interactions of the substituent with Tyr308 are minimized. The conclusion that can be drawn from this observation is that the Höltje-Anzali model in its present minimal form is unable to explain the structure-activity relationships of cardenolides substituted in the lactone ring.

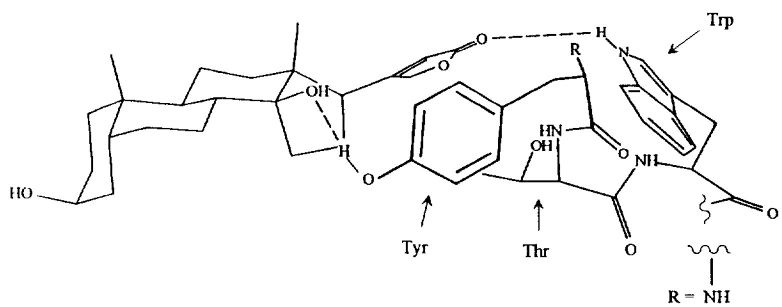


Figure 4. Digitoxigenin (14/21-conformation) and the tripeptide Tyr-Thr-Trp arranged according to the Höltje-Anzali model.

Outlook

Suitable compounds derived from **15e** and **15f** will be used to localize the binding site of the lactone ring by affinity labeling techniques. The results will be reported in due course.

EXPERIMENTAL

General: NMR: GEMINI 200 (Varian), GEMINI 300 (Varian), AM-400 (Bruker); MALDI-TOF mass spectra were recorded on a VOYAGER™-RP spectrometer (PerSeptive Biosystems). Force field calculations were carried out using QUANTA 4.1 / CHARMm program package (Molecular Simulations Inc., Burlington MA, 1994) on a Silicon Graphics workstation. For other methods and instrumentation, see ref.[25]. Micro analyses were performed by the laboratory Ilse Beetz, Kronach. CH_3, CH_2, CH groups and quaternary carbons when identified by APT are indicated by „-" (CH_3, CH) and „+" (CH_2 and C_q), respectively. The assignments of the ^{13}C resonances were aided by the results in ref.[26].

3β-(*tert*-Butyl-dimethyl-silyloxy)-14-hydroxy-5β,14β-card-20(22)-enolide (1b)

To a solution of digitoxigenin (**1a**) (751 mg, 2.00 mmol) in dry CH_2Cl_2 (60 ml) pyridine (405 µl, 5.02 mmol) and tert-butyldimethylsilyl trifluoromethanesulfonate (550 µl, 2.40 mmol) were added. The mixture was stirred at 20°C for 1 h. Quenching with aqu. $NaHCO_3$ (1 per cent, 150 ml), followed by usual work-up (CH_2Cl_2), and FC (CH_2Cl_2-ethyl acetate 30:1) provided **1b** (941 mg, 96 %).- M.p. 212-215 °C (CH_2Cl_2-petrol).- IR ($CHCl_3$): 3672, 3560-3280, 1783, 1745, 1621, 1064 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.01 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.87 (s, 3 H, CH_3-18), 0.88 (s, 9 H, $C(CH_3)_3$), 0.92 (s, 3 H, CH_3-19), 1.10 - 1.97 (om (overlapping multiplets)), 2.02 - 2.25 (m, 2 H), 2.71 - 2.83 (m, 1 H, 17α-H), 4.04 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 4.81 + 4.99 (AB (ABX), 2 H, CH_2-21), 5.86 (X (ABX), 1 H, 22-H), $|J_{21,21'}|$ = 18.0 Hz, $J_{21,22}$ = 1.6 Hz, $J_{21',22}$ = 1.9 Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = -4.39 (-) and -4.35 (-) ($Si^tBu(\underline{C}H_3)_2$), 16.25 (-) (C-18), 18.56 (+) ($\underline{C}(CH_3)_3$), 21.70 (+), 21.99 (+), 24.29 (-) (C-19), 26.31 (-) ($C(\underline{C}H_3)_3$), 27.22 (+), 27.38 (+), 29.16 (+), 30.20 (+), 33.61 (+), 34.72 (+), 35.77 (+), 36.14 (-) and 36.47(-) (C-5, C-9), 40.60 (+) (C-12), 42.39 (-) (C-8), 50.09 (+) (C-13), 51.45 (-) (C-17), 67.61 (-) (C-3), 73.94 (+) (C-21), 86.10 (+) (C-14), 118.09 (-) (C-22), 175.00 (+) and 175.15 (+) (C-20, C-23).- MS: m/z (%) = 488 (0.5), 473 (2), 431 (100), 413 (5), 355 (15), 337 (8), 175 (18), 75 (59).- $C_{29}H_{48}O_4Si$ (488.78), HRMS: calcd for $C_{25}H_{39}O_4Si$ $[M - {}^tBu]^+$ 431.2618, found 431.2616.

3β-(*tert*-Butyl-dimethyl-silyloxy)-14-trimethylsilyloxy-5β,14β-card-20(22)-enolide (1c)

Trimethylsilyl trifluoromethanesulfonate (1.59 ml, 8.76 mmol) was carefully added to a solution of **1b** (1.222 g, 2.500 mmol) in dry pyridine (60 ml). The reaction mixture was stirred at 20°C for 3.5 h and then diluted with CH_2Cl_2 (600 ml). Quenching with aqu. $NaHCO_3$ (1 per cent, 300 ml), followed by usual work-up (CH_2Cl_2), and MPLC (petrol - tert-butyl methyl ether - triethylamine 25:1:0.1 → 5:1:0.1) provided **1c** (1.260 g, 90 %).- M.p. 193 - 196 °C (CH_2Cl_2-petrol).- IR ($CHCl_3$): 1783, 1746, 1633, 1251, 1076, 1053, 836 cm^{-1}.- ^1H NMR (400 MHz, $CDCl_3$): δ = 0.00 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.09 (s, 9 H, $Si(CH_3)_3$), 0.84 (s, 3 H, CH_3-18), 0.86 (s, 9 H, $C(CH_3)_3$), 0.89 (s, 3 H, CH_3-19), 1.00 - 2.08 (om), 2.51 - 2.57 (m, 1 H, 17α-H), 4.02 ($w_{1/2} \approx 7$ Hz, 1H, 3α-H), 4.72 + 4.75 (AB (ABX), 2 H, CH_2-21), 5.81 (X (ABX), 1 H, 22-H), $|J_{21,21'}|$ = 17.5 Hz, $J_{21,22} = J_{21',22}$ = 1.7 Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = -4.39 (-) and -4.35 (-) ($Si^tBu(\underline{C}H_3)_2$), 3.41 (-) ($Si(CH_3)_3$), 18.60 (-) (C-18, and probably $\underline{C}(CH_3)_3$), 21.27 (+), 23.90 (+), 24.35 (-) (C-19), 26.32 (-) ($C(\underline{C}H_3)_3$), 27.22 (+), 27.78 (+), 29.08 (+), 30.30 (+), 34.39 (+), 34.94 (+), 36.23 (+), 36.39 (-) and 37.31 (-) (C-5, C-9), 41.25 (-) (C-8), 42.18 (+) (C-12), 51.18 (-) (C-17), 51.47 (+) (C-13), 67.67 (-) (C-3), 74.39 (+) (C-21), 91.93 (+) (C-14), 117.44 (-) (C-22), 174.56 (+) and 174.81 (+) (C-20, C-23).- MS: m/z (%) = 560 (2, $M^{+\cdot}$), 545 (2), 503 (100), 413 (13), 337 (10), 251 (13), 157 (24), 75 (63), 73 (45).- $C_{32}H_{56}O_4Si_2$ (560.96), calcd C 68.53, H 10.07, found C 68.57, H 10.02.

3β-(*tert*-Butyl-dimethyl-silyloxy)-14-hydroxy-5β,14β,17α-card-20(22)-enolide (2b)

A solution of tetrabutylammonium fluoride (1.1 mol/l in THF, 129 µl, 141 µmol) was added to **1c** (31 mg, 54 µmol), dissolved in dry THF (3 ml). The reaction mixture was stirred at 20°C for 6.5 h, diluted with CH_2Cl_2 (30 ml) and washed with saturated brine (30 ml). The aqueous layer was extracted with CH_2Cl_2 (3 x 30 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. FC (petrol-ethyl acetate 3:1 → 1:1) yielded **1b** (8 mg, 30 %) and **2b** (10 mg, 37 %).- IR ($CHCl_3$): 3600, 3580-3280, 1786, 1747, 1627, 1253, 1063 cm^{-1}.- ^1H NMR (400 MHz, $CDCl_3$): δ = - 0.01 (s, 3 H, $Si^tBu(C\underline{H}_3)_2$), 0.00 (s, 3 H, $Si^tBu(C\underline{H}_3)_2$), 0.86 (s, 9 H, $C(CH_3)_3$), 0.91 (s, 3 H, CH_3-19), 1.01 (s, 3 H, CH_3-18), 1.05 - 1.90 (om), 2.00 - 2.17 (m, 2 H), 3.16 (t, 1 H, 17β-H), 4.02 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 4.67 - 4.73 (m, 1 H, 21-H), 4.77 - 4.83 (m, 1 H, 21-H'), 5.85 - 5.87 (m, 1 H, 22-H), $J_{16,17}$ = 9.4 Hz.- MS: m/z (%) = 473 (2, $[M-CH_3]^+$), 431 (100), 413 (3), 75 (69), 73 (15).- FAB MS: m/z = 511.5 ($[M+Na]^+$), 489.5 ($[M+H]^+$).- $C_{29}H_{48}O_4Si$ (488.78), HRMS: calcd for $C_{25}H_{39}O_4Si$ $[M - {}^tBu]^+$ 431.2618, found 431.2619.

17βH-Digitoxigenin (2a)

A solution of tetrabutylammonium fluoride (1.1 mol/l in THF, 4.3 ml, 4.7 mmol) was added to digitoxigenin (**1a**) (350 mg, 0.935 mmol), dissolved in dry THF (17.5 ml) (colour change to brown). The reaction mixture was stirred at 50°C for 16 h and allowed to cool to 20°C. Then CH_2Cl_2 (150 ml) and water (150 ml) were added. Usual work-up (CH_2Cl_2) and repeated FC (petrol-ethyl acetate 2:3 → 1:2) furnished **2a** (265 mg, 76 %)

and recovered **1a** (30 mg, 9 %).- M.p. 199 - 201 °C (CH_2Cl_2-heptane), ref.[27] 190 - 192 °C.- IR ($CHCl_3$): 3617, 1787, 1749, 1627, 1043, 1033 cm^{-1}.- ^1H NMR (400 MHz, $CDCl_3$): δ = 0.94 (s, 3 H, CH_3-19), 1.02 (s, 3 H, CH_3-18), 1.05 - 1.93 (om), 2.00 - 2.17 (m, 2 H), 3.17 (t, 1 H, 17β-H), 4.11 ($w_{1/2} \approx 8$ Hz, 1 H, 3α-H), 4.66 - 4.72 (m, 1 H, 21-H), 4.77 - 4.83 (m, 1 H, 21-H'), 5.85 - 5.87 (m, 1 H, 22-H), $J_{16,17}$ = 9.6 Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = 18.63 (-) (C-18), 20.50 (+), 21.28 (+), 24.13 (-) (C-19), 24.84 (+), 26.74 (+), 28.30 (+), 29.96 (+), 31.36 (+), 31.88 (+), 33.73 (+), 35.71 (+), 36.11 (-) and 36.38 (-) (C-5, C-9), 42.14 (-) (C-8), 48.77 (-) (C-17), 49.24 (+) (C-13), 67.25 (-) (C-3), 74.24 (+) (C-21), 86.60 (+) (C-14), 117.27 (-) (C-22), 171.82 (+) and 174.54 (+) (C-20, C-23).- $C_{23}H_{34}O_4$ (374.52), MS: m/z (%) = 374 (3, $M^{+\cdot}$), 356 (17), 246 (12), 203 (100), 111 (46).

X-ray Structural Analysis of 2a

2a·CH_2Cl_2, $C_{23}H_{34}O_4 \cdot CH_2Cl_2$, crystallizes triclinic, space group P1, $\underline{a}$ = 7.291(3), $\underline{b}$ = 8.331(3), $\underline{c}$ = 10.725(4) Å, α = 92.64(3), β = 108.70(3), γ = 106.90(3)°, V = 5834(4) $Å^3$, Z = 1, D_c = 1.308 Mg/m^3, T = 293 K. The structure was refined on F^2 to R = 0.048, wR2 = 0.088 for 1635 independent reflections collected on a Siemens P4 diffractometer (2θ ≤ 45°, MoKα, ω-scan). Further details of the structure investigation may be obtained from Fachinformationszentrum Energie, Physik, Mathematik GmbH, D-76012 Eggenstein-Leopoldshafen (Germany), on quoting the deposition number CSD-405466. Any request should be accompanied by the full literature citation of this paper.

3β-(*tert*-Butyl-dimethyl-silyloxy)-14-trimethylsilyloxy-5β,14β,17α-card-20(22)-enolide (2c)

To a solution of **1c** (50 mg, 90 μmol) in DMF (1 ml) a suspension of sodium hydride [100 μl of 17 mg NaH (55-65 per cent dispersion in mineral oil) in DMF (500 μl), 84 μmol] was added (colour change to yellow). The mixture was stirred at 20°C for 2 d and then heated to 70°C for 24 h (colour change to orange). After cooling to 20°C the reaction was quenched with saturated aqu. NH_4Cl (200 μl). The mixture was diluted with CH_2Cl_2 (30 ml) and washed with saturated brine (30 ml). The aqueous layer was extracted with CH_2Cl_2 (2 x 30 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Repeated LC (petrol-ethyl acetate 60:1 then 10:1) yielded **2c** (13 mg, 24 %) and recovered **1c** (8 mg, 16 %) as main products.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.02 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.14 (s, 9 H, $Si(CH_3)_3$), 0.89 (s, 9 H, $C(CH_3)_3$), 0.93 (s, 3 H, CH_3-19), 0.97 (s, 3 H, CH_3-18), 1.02 - 2.19 (om), 3.00 (t, 1 H, 17β-H), 4.05 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 4.63 - 4.76 (m, 1 H, 21-H), 4.78 - 4.90 (m, 1 H, 21-H'), 5.84 - 5.90 (m, 1 H, 22-H), $J_{16,17}$ = 9.4 Hz, $|J_{21,21'}|$ = 17.7 Hz, $J_{21,22}$ = 1.5 Hz, $J_{21',22}$ = 1.7 Hz.- MS: m/z (%) = 560 (0.2, $M^{+\cdot}$), 545 (1), 503 (100), 413 (17), 157 (11), 75 (80), 73 (45).- $C_{32}H_{56}O_4Si_2$ (560.96), HRMS: calcd for $C_{31}H_{53}O_4Si_2$ $[M - CH_3]^+$ 545.3482, found 545.3481.

(Z)-21-Benzylidene-3β-(*tert*-butyl-dimethyl-silyloxy)-14-trimethylsilyloxy-5β,14β-card-20(22)-enolide (5a)

To a stirred solution of **1c** (31 mg, 55 μmol) in dry N-methylpyrrolidone (NMP) (0.8 ml) a suspension of NaH [25 μl of 32 mg NaH (55-65 per cent dispersion in mineral oil) in NMP (700 μl), 28 μmol] was added at 20°C (colour change to orange). After 30 min benzaldehyde (5 μl, 50 μmol) was added (colour change to red). The reaction mixture was stirred for another 30 min and then quenched with saturated aqu. NH_4Cl (150 μl) (formation of a white precipitate). Dilution with CH_2Cl_2 (20 ml) and usual work-up (CH_2Cl_2), followed by LC (petrol-ethyl acetate 60:1) furnished **5a** (22 mg, 61 %).- IR ($CHCl_3$): 1750, 1645, 1591, 1248, 1078, 1053, 832 cm^{-1}.- ^1H NMR (300 MHz, NOED, $CDCl_3$): δ = 0.04 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.14 (s, 9 H, $Si(CH_3)_3$), 0.84 (s, 3 H, CH_3-18), 0.90 (s, 9 H, $C(CH_3)_3$), 0.92 (s, 3 H, CH_3-19), 1.08 - 2.28 (om), 2.77 - 2.87 (m, 1 H, 17α-H), 4.07 ($w_{1/2} \approx 8$ Hz, 1 H, 3α-H), 5.98 (s, 1 H, 1'-H), 6.05 (s, 1 H, 22-H), 7.28 - 7.45 (3 H, arom. H), 7.77 - 7.83 (2 H, arom. H_o).- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = -4.35 (-) and -4.32 (-) ($Si^tBu(\underline{C}H_3)_2$), 3.49 (-) ($Si(CH_3)_3$), 18.50 (-) (C-18), 18.59 (+) ($\underline{C}(CH_3)_3$), 21.58 (+), 23.93 (+), 24.38 (-) (C-19), 26.34 (-) ($C(\underline{C}H_3)_3$), 27.27 (+), 29.13 (+), 29.77 (+), 30.33 (+), 34.41 (+), 34.95 (+), 36.25 (+), 36.43 (-) and 37.39 (-) (C-5, C-9), 41.39 (-) (C-8), 42.49 (+) (C-12), 48.04 (-) (C-17), 51.13 (+) (C-13), 67.71 (-) (C-3), 92.35 (+) (C-14), 110.30 (-) (C-1'), 116.06 (-) (C-22), 129.24 (-) (arom. C), 129.33 (-) (arom. C), 131.07 (-) (arom. C), 133.55 (+) (arom. C_i), 151.14 (+) (C-21), 164.95 (+) and 170.25 (+) (C-20, C-23).- MS: m/z (%) = 648 (32, $M^{+\cdot}$), 633 (4), 591 (80), 501 (4), 426 (5), 425 (5), 340 (7), 296 (7), 271 (13), 157 (49), 75 (100), 73 (71).- $C_{39}H_{60}O_4Si_2$ (649.07), HRMS: calcd 648.4030, found 648.4030.

(Z)-21-Benzylidene-3β,14-dihydroxy-5β,14β-card-20(22)-enolide (5b)
a) A solution of p-toluenesulfonic acid monohydrate (2.6 ml of 14 mg p-TsOH·H_2O in methanol (20 ml), 9.2 µmol) was added to **5a** (61 mg, 94 µmol), dissolved in CH_2Cl_2 (1.4 ml) (a white precipitate formed, which had been disappeared after 16 h). The mixture was stirred at 20°C for 66 h, diluted with CH_2Cl_2 (15 ml) and washed with aqu. $NaHCO_3$ (2.5 per cent, 15 ml). The aqueous layer was extracted with CH_2Cl_2 (3 x 15 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. LC (petrol-ethyl acetate 2:1) yielded **5b** (32 mg, 72 %).- b) To a solution of digitoxigenin (**1a**) (41 mg, 109 µmol) in dry NMP (1.5 ml) a suspension of NaH [100 µl of 29 mg NaH (55-65 per cent dispersion in mineral oil) in NMP (900 µl), 81 µmol] was added at -20°C (colour change to yellow). The mixture was stirred at -20°C for 30 min. Then benzaldehyde (15 µl, 144 µmol) was added. As TLC monitoring showed no disappearance of the starting material, 45 min later the reaction mixture was allowed to warm to 0°C. During the following 45 min, the colour changed from yellow to dark red. 1.5 h after additon of benzaldehyde the reaction was quenched with saturated aqu. NH_4Cl (400 µl) (a white precipitate formed). Dilution with CH_2Cl_2 (15 ml), usual work-up (CH_2Cl_2), followed by FC (petrol-ethyl acetate 2:1 → 1:2) provided **5b** (35 mg, 68 %) as main product.- M.p. 152-155 °C (methanol-water).- IR ($CHCl_3$): 3623, 3580-3390, 1747, 1649, 1591, 1448, 1137, 1032, 953 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.87 (s, 3 H, CH_3-18), 0.96 (s, 3 H, CH_3-19), 1.10 - 2.40 (om), 2.80 - 2.92 (m, 1 H, 17α-H), 4.12 - 4.20 (m, 1 H, 3α-H), 6.03 + 6.26 (2 x s, 2 x 1 H, 22-H, 1'-H), 7.28 - 7.45 (3 H, arom. H), 7.76 - 7.85 (2 H, arom. H_o).- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = 16.23 (-) (C-18), 21.94 (+), 21.99 (+), 24.24 (-) (C-19), 27.02 (+), 28.41 (+), 30.16 (+), 30.41 (+), 33.31 (+), 33.84 (+), 35.92 (+), 36.11 (-) and 36.50 (-) (C-5, C-9), 41.27 (+) (C-12), 42.72 (-) (C-8), 47.45 (-) (C-17), 49.98 (+) (C-13), 67.34 (-) (C-3), 86.63 (+) (C-14), 110.06 (-) (C-1'), 116.37 (-) (C-22), 129.25 (-) (arom. C), 129.40 (-) (arom. C), 131.11 (-) (arom. C), 133.53 (+) (arom. C_i), 151.09 (+) (C-21), 165.43 (+) and 170.59 (+) (C-20, C-23).- MS: m/z (%) = 462 (90, $M^{+\bullet}$), 444 (23), 426 (19), 401 (11), 353 (6), 335 (4), 283 (5), 264 (13), 250 (21), 212 (100), 203 (34), 198 (40).- $C_{30}H_{38}O_4$ (462.62), calcd for $C_{30}H_{38}O_4$·CH_3OH C 75.27, H 8.56, found C 75.08, H 8.40.

Reaction of 1c with benzaldehyde at -20°C
To a stirred solution of **1c** (201 mg, 357 µmol) in dry NMP (5 ml) a suspension of NaH [200 µl of 36 mg NaH (55-65 per cent dispersion in mineral oil) in NMP (1000 µl), 180 µmol] was added at -20°C. After 30 min benzaldehyde (46 µl, 455 µmol) was added at -20°C. Stirring was continued for 40 min at -20°C, then the reaction was quenched with saturated aqu. NH_4Cl (600 µl) (formation of a white precipitate). Dilution with CH_2Cl_2 (100 ml), usual work-up (CH_2Cl_2), followed by repeated MPLC (toluene - 2-propanol 100:0.25 and petrol-ethyl acetate 12:1, rsp.) provided **3a** (larger R_f) (73 mg, 31 %), **4a** (smaller R_f) (78 mg, 33 %) and recovered **1c** (63 mg, 31 %).- According to ^1H NMR, the crude product consisted of recovered starting material **1c** and the products **3a** and **4a** in a ratio of 1:0.9:0.9. No elimination product **5a** could be detected.

3β-(*tert*-Butyl-dimethyl-silyloxy)-21-((R)-hydroxybenzyl)-14-trimethylsilyloxy-5β,14β,21(R)-card-20(22)-enolide (3a)
IR ($CHCl_3$): 3590, 3550-3250, 1748, 1622, 1247, 1076, 1050, 836 cm^{-1}.- ^1H NMR (200 MHz, H,H COSY, $CDCl_3$): δ = 0.02 (s, 6 H, $Si^tBu(\underline{C}H_3)_2$), 0.10 (s, 9 H, $Si(CH_3)_3$), 0.86 (s, 3 H, CH_3-18), 0.89 (s, 9 H, $C(CH_3)_3$), 0.90 (s, 3 H, CH_3-19), 1.00 - 2.05 (om), 2.30 - 2.45 (m, 1 H, 17α-H), partly hidden: 2.42 (d, 1 H, OH), 4.04 ($w_{1/2}$ ≈ 7 Hz, 1 H, 3α-H), 4.88 (dd, 1 H, 1'-H), 5.07 (dd, 1 H, 21-H), 5.92 ($w_{1/2}$ ≈ 3 Hz, 1 H, 22-H), 7.28 - 7.46 (5 H, arom. H), $J_{21,22}$ = 1.4 Hz, $J_{21,1'}$ = 4.2 Hz, $J_{1',OH}$ = 5.1 Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = -4.37 (-) and -4.33 (-) ($Si^tBu(\underline{C}H_3)_2$), 3.37 (-) ($Si(CH_3)_3$), 18.58 (+) ($\underline{C}(CH_3)_3$), 19.24 (-) (C-18), 21.32 (+), 23.78 (+), 24.34 (-) (C-19), 26.33 (-) ($C(\underline{C}H_3)_3$), 27.24 (+), 29.05 (+), 29.10 (+), 30.30 (+), 34.22 (+), 34.92 (+), 36.19 (+), 36.41 (-) and 37.20 (-) (C-5, C-9), 41.28 (-) (C-8), 42.08 (+) (C-12), 49.80 (-) (C-17), 51.24 (+) (C-13), 67.69 (-) (C-3), 74.43 (-) (C-1'), 87.86 (-) (C-21), 92.15 (+) (C-14), 119.27 (-) (C-22), 127.25 (-) (arom. C), 129.05 (-) (arom. C), 129.09 (-) (arom. C), 139.53 (+) (arom. C_i), 173.41 (+) and 175.33 (+) (C-20, C-23).- MS: m/z (%) = 666 (0.2, $M^{+\bullet}$), 609 (0.3), 560 (1), 427 (2), 413 (1), 338 (2), 289 (2), 251 (1), 167 (1), 157 (6), 107 (6), 105 (8), 75 (100).- $C_{39}H_{62}O_5Si_2$ (667.08), HRMS: calcd 666.4136, found 666.4137.

3β-(*tert*-Butyl-dimethyl-silyloxy)-21-((Ξ)-hydroxybenzyl)-14-trimethylsilyloxy-5β,14β,21(Ξ)-card-20(22)-enolide (4a)

IR ($CHCl_3$): 3600, 3550-3250, 1749, 1628, 1249, 1079, 1060, 837 cm^{-1}.- ^1H NMR (200 MHz, H,H COSY, $CDCl_3$): δ = 0.02 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.07 (s, 9 H, $Si(CH_3)_3$), 0.82 (s, 3 H, CH_3-18), 0.89 (s, 12 H, CH_3-19 and $C(CH_3)_3$), 0.95 - 2.10 (om), 2.25 - 2.38 (m, 1 H, 17α-H), 4.03 ($w_{1/2}$ ≈ 7 Hz, 1 H, 3α-H), 5.14 (d, 1 H, 1'-H), 5.19 - 5.24 (dd, 1 H, 21-H), 5.80 ($w_{1/2}$ ≈ 3 Hz, 1 H, 22-H), 7.28 - 7.39 (5 H, arom. H), $J_{21,22}$ = 1.5 Hz, $J_{21,1'}$ = 3.3 Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = -4.37 (-) and -4.33 (-) ($Si^tBu(\underline{C}H_3)_2$), 3.37 (-) ($Si(CH_3)_3$), 18.58 (+) ($\underline{C}(CH_3)_3$), 18.96 (-) (C-18), 21.26 (+), 23.76 (+), 24.33 (-) (C-19), 26.33 (-) ($C(\underline{C}H_3)_3$), 27.22 (+), 28.95 (+), 29.10 (+), 30.27 (+), 34.22 (+), 34.92 (+), 36.17 (+), 36.39 (-) and 37.19 (-) (C-5, C-9), 41.17 (-) (C-8), 41.57 (+) (C-12), 50.07 (-) (C-17), 51.22 (+) (C-13), 67.68 (-) (C-3), 74.27 (-) (C-1'), 88.47 (-) (C-21), 92.22 (+) (C-14), 119.61 (-) (C-22), 126.74 (-) (arom. C), 128.74 (-) (arom. C), 128.98 (-) (arom. C), 138.44 (+) (arom. C_i), 173.58 (+) and 175.08 (+) (C-20, C-23).- MS: m/z (%) = 666 (0.2, $M^{+\bullet}$), 609 (0.2), 560 (7), 545 (3), 503 (100), 413 (5), 338 (21), 251 (12), 157 (40), 106 (88), 105 (85), 77 (80).- $C_{39}H_{62}O_5Si_2$ (667.08), HRMS: calcd 666.4136, found 666.4149.

3β,14-Dihydroxy-21-((R)-hydroxybenzyl)-5β,14β,21(R)-card-20(22)-enolide (3c)

A solution of p-toluenesulfonic acid monohydrate (2.9 ml of 14 mg p-TsOH·H_2O in methanol (20 ml), 10.3 μmol) was added to **3a** (68 mg, 102 μmol), dissolved in CH_2Cl_2 (0.3 ml). The mixture was stirred at 20°C for 59 h, diluted with CH_2Cl_2 (20 ml) and washed with aqu. $NaHCO_3$ (2.5 per cent, 20 ml). The aqueous layer was extracted with CH_2Cl_2 (3 x 20 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. LC (petrol-ethyl acetate 3:2) furnished **3c** (41 mg, 84 %).- M.p. 196 - 199 °C (methanol).- IR (KBr): 3650-3150, 1741, 1707, 1626, 1031, 711 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.88 (s, 3 H, CH_3-18), 0.94 (s, 3 H, CH_3-19), 1.05 - 2.21 (om), 2.22 - 2.33 (m, 1 H, 17α-H), 2.41 - 2.50 (broad d, 1 H, PhCHO$\underline{H}$), 4.08 - 4.18 (m, 1 H, 3α-H), 4.88 (broad t, 1 H, 1'-H), 4.97 - 5.03 (dd, 1 H, 21-H), 6.15 ($w_{1/2}$ ≈ 3 Hz, 1 H, 22-H), 7.33 - 7.48 (5 H, arom. H), $J_{21,22}$ = 1.5 Hz, $J_{21,1'}$ = 4.4 Hz, $J_{1',OH}$ ≈ 4 Hz.- ^{13}C NMR (50 MHz, APT, CD_3OD): δ = 17.39 (-) (C-18), 22.87 (+) (probably 2 x C), 24.64 (-) (C-19), 28.22 (+), 28.88 (+), 30.91 (+), 31.12 (+), 33.66 (+), 34.51 (+), 36.81 (+), 37.05 (-) and 37.76 (-) (C-5, C-9), 41.90 (+) (C-12), 43.11 (-) (C-8), 50.48 (-) (C-17), 51.46 (+) (C-13), 67.98 (-) (C-3), 73.80 (-) (C-1'), 87.46 (+) (C-14), 90.26 (-) (C-21), 119.79 (-) (C-22), 128.09 (-) (arom. C), 129.18 (-) (arom. C), 129.45 (-) (arom. C), 141.76 (+) (arom. C_i), 176.59 (+) and 179.48 (+) (C-20, C-23).- MS: m/z (%) = 462 (3), 444 (4), 429 (1), 374 (12), 356 (30), 338 (27), 323 (6), 203 (29), 107 (100), 105 (98), 79 (53), 77 (80).- FAB MS: m/z = 481.4 ($[M+H]^+$).- $C_{30}H_{40}O_5$ (480.64), calcd for $C_{30}H_{40}O_5$·0.5 CH_3OH C 73.76, H 8.52, found C 73.78, H 8.48.

X-ray Structural Analysis of 3c

3c, $C_{30}H_{40}O_5$, crystallizes orthorhombic, space group P2(1)2(1)2(1), with $\underline{a}$ = 8.215(6), $\underline{b}$ = 11.568(6), $\underline{c}$ = 27.077(8)Å, V = 2573(2) Å^3, Z = 4, D_c = 1.241 Mg/m^3, T = 173 K. The structure was refined on F^2 to R = 0.0914, wR2 = 0.1776 for 1965 independent reflections (2θ ≤ 50°, MoKα, ω-scan).- Further details of the structure investigation may be obtained from Fachinformationszentrum Energie, Physik, Mathematik GmbH, D-76012 Eggenstein-Leopoldshafen (Germany), on quoting the deposition number CSD-405467. Any request should be accompanied by the full literature citation of this paper.

3β,14-Dihydroxy-21-((Ξ)-hydroxybenzyl)-5β,14β,21(Ξ)-card-20(22)-enolide (4c)

The protecting groups of **4a** were removed as described for **3c**. FC (petrol-ethyl acetate 1:2) provided **4c** (84 %).- Melting range (m.r.): 136 - 140 °C (methanol).- IR (KBr): 3650-3150, 1741, 1710, 1622, 1029, 702 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.83 (s, 3 H, CH_3-18), 0.92 (s, 3 H, CH_3-19), 1.00 - 2.19 (om), 2.20 - 2.31 (m, 1 H, 17α-H), 2.57 - 2.68 (m, 1H, PhCHO$\underline{H}$), 4.07 - 4.17 ($w_{1/2}$ ≈ 7 Hz, 1 H, 3α-H), 5.13 - 5.22 (om, 2 H, 21-H, 1'-H), 6.02 ($w_{1/2}$ ≈ 3 Hz, 1 H, 22-H), 7.28 - 7.41 (5 H, arom. H).- ^{13}C NMR (50 MHz, APT, CD_3OD): δ = 17.19 (-) (C-18), 22.76 (+), 22.80 (+), 24.56 (-) (C-19), 28.17 (+), 28.79 (+), 30.72 (+), 31.08 (+), 33.55 (+), 34.48 (+), 36.76 (+), 36.99 (-) and 37.72 (-) (C-5, C-9), 41.63 (+) (C-12), 43.06 (-) (C-8), 50.66 (-) (C-17), 51.44 (+) (C-13), 67.96 (-) (C-3), 74.65 (-) (C-1'), 87.47 (+) (C-14), 90.87 (-) (C-21), 120.11 (-) (C-22), 128.25 (-) (arom. C), 129.26 (-) (arom. C), 129.42 (-) (arom. C), 140.42 (+) (arom. C_i), 176.54 (+) and 178.87 (+) (C-20, C-23).- MS: m/z (%) = 462 (0.4), 444 (1), 426 (1), 374 (4), 356 (14), 338

(9), 203 (31), 106 (75), 105 (82), 77 (100).- FAB MS: m/z = 503.5 ($[M+Na]^+$), 481.5 ($[M+H]^+$).- $C_{30}H_{40}O_5$ (480.64), calcd for $C_{30}H_{40}O_5 \cdot 0.5\ CH_3OH$ C 73.76, H 8.52, found C 73.64, H 8.56.

21-((R)-Benzoyloxybenzyl)-3β-(*tert*-butyl-dimethyl-silyloxy)-14-trimethylsilyloxy-5β,14β,21(R)-card-20(22)-enolide (3b)

A solution of **3a** (63 mg, 94 μmol) and DMAP (2 mg, 12 μmol) in dry CH_2Cl_2 (1 ml) was added to DCC (25 mg, 123 μmol) and benzoic acid (15 mg, 123 μmol) (formation of a white suspension). After stirring at 20°C for 28 h, the mixture was filtered and the precipitate was washed with a little CH_2Cl_2. The filtrate was diluted with CH_2Cl_2 (20 ml) and washed with water (20 ml). The organic layer was washed with aqu. $NaHCO_3$ (5 per cent, 20 ml). The combined aqueous layers were extracted with CH_2Cl_2 (3 x 40 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. LC (petrol-ethyl acetate 50:1) yielded **3b** (72 mg, 99 %).- IR ($CHCl_3$): 1757, 1722, 1636, 1263, 841 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.03 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.09 (s, 9 H, $Si(CH_3)_3$), 0.89 (s, 12 H, CH_3-19# and $C(CH_3)_3$), 0.91 (s, 3 H, CH_3-18#), 1.05 - 2.08 (om), 2.55 - 2.68 (m, 1 H, 17α-H), 4.06 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 5.13 - 5.18 (m, 1 H, 21-H), 5.96 ($w_{1/2} \approx 3$ Hz, 1 H, 22-H), 6.37 (d, 1 H, 1'-H), 7.33 - 7.64 (8 H, arom. H), 7.98 - 8.06 (2 H, arom. H_o (benzoate)), $J_{21,22} \approx 2$ Hz, $J_{21,1'} = 2.2$ Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = -4.37 (-) and -4.33 (-) ($Si^tBu(\underline{C}H_3)_2$), 3.38 (-) ($Si(CH_3)_3$), 18.59 (+) ($\underline{C}(CH_3)_3$), 19.25 (-) (C-18), 21.45 (+), 23.80 (+), 24.34 (-) (C-19), 26.33 (-) ($C(\underline{C}H_3)_3$), 27.23 (+), 29.11 (+), 29.50 (+), 30.31 (+), 34.28 (+), 34.94 (+), 36.21 (+), 36.41 (-) and 37.25 (-) (C-5, C-9), 41.36 (-) (C-8), 42.76 (+) (C-12), 50.03 (-) (C-17), 51.42 (+?) (C-13), 67.68 (-) (C-3), 72.86 (-) (C-1'), 86.86 (-) (C-21), 92.20 (+) (C-14), 119.97 (-) (C-22), 127.59 (-) (arom. C), 129.09 (-) (arom. C), 129.14 (-) (arom. C), 129.27 (-) (arom. C), 129.60 (+) (arom. C_i), 130.25 (-) (arom. C), 134.03 (-) (arom. C), 136.61 (+) (arom. C_i), 165.39 (+) and 173.11 (+) and 173.98 (+) (C-20, C-23, O$\underline{C}$OPh).- $C_{46}H_{66}O_6Si_2$ (771.19), MALDI-MS: m/z = 793.2 $[M+Na]^+$.

These assignments may have to be reversed.

21-((Ξ)-Benzoyloxybenzyl)-3β-(*tert*-butyl-dimethyl-silyloxy)-14-trimethylsilyloxy-5β,14β,21(Ξ)-card-20(22)-enolide (4b)

4a was converted into the benzoate as described for **3b**. Stirring the reaction mixture at 20°C for 8 h, followed by work-up and LC (petrol-ethyl acetate 50:1) provided **4b** (86 %).- IR ($CHCl_3$): 1752, 1719, 1633, 1270, 1073, 840 cm^{-1}.- ^1H NMR (200 MHz, C,H COSY, $CDCl_3$): δ = 0.04 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.08 (s, 9 H, $Si(CH_3)_3$), 0.90 (s, 9 H, $C(CH_3)_3$), (partly hidden: 0.91 and 0.92, 6 H, CH_3-18, CH_3-19), 1.02 - 2.18 (om), 2.58 - 2.70 (m, 1 H, 17α-H), 4.07 (1 H, $w_{1/2} \approx 6$ Hz, 3α-H), 5.38 - 5.43 (dd, 1 H, 21-H), 5.76 ($w_{1/2} \approx 3$ Hz, 1 H, 22-H), 6.38 (d, 1 H, 1'-H), 7.28 - 7.66 (8 H, arom. H), 8.11 - 8.18 (2 H, arom. H_o (benzoate)), $J_{21,22} \approx 1.7$ Hz, $J_{21,1'} = 2.6$ Hz.- ^{13}C NMR (50 MHz, C,H COSY, APT, $CDCl_3$): δ = -4.34 (-) ($Si^tBu(\underline{C}H_3)_2$), 3.37 (-) ($Si(CH_3)_3$), 18.60 (+) ($\underline{C}(CH_3)_3$), 18.94 (-) (C-18), 21.44 (+), 23.82 (+), 24.36 (-) (C-19), 26.34 (-) ($C(\underline{C}H_3)_3$), 27.25 (+), 29.12 (+), 29.20 (+), 30.30 (+), 34.34 (+), 34.95 (+), 36.23 (+), 36.41 (-) and 37.32 (-) (C-5, C-9), 41.31 (-) (C-8), 42.43 (+) (C-12), 50.66 (-) (C-17), 51.62 (+) (C-13), 67.70 (-) (C-3), 76.00 (-) (C-1'), 85.87 (-) (C-21), 92.21 (+) (C-14), 120.23 (-) (C-22), 127.99 (-) (arom. C), 128.82 (-) (arom. C), 129.02 (-) (arom. C), 129.47 (-) (arom. C), 129.96 (+) (arom. C_i), 130.39 (-) (arom. C), 133.42 (+) (arom. C_i), 134.04 (-) (arom. C), 166.14 (+) and 173.09 (+) and 173.36 (+) (C-20, C-23, O$\underline{C}$OPh).- $C_{46}H_{66}O_6Si_2$ (771.19), MALDI-MS: m/z = 793.4 $[M+Na]^+$.

21-((R)-Benzoyloxybenzyl)-3β,14-dihydroxy-5β,14β,21(R)-card-20(22)-enolide (3d)

The protecting groups of **3b** were removed as described for **3c**. LC (petrol-ethyl acetate 3:2) furnished **3d** (94%).- M.r.: 131 - 134 °C (methanol).- IR ($CHCl_3$): 3617, 3560-3250, 1742, 1718, 1630, 1261, 1107, 708 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.91 (s, 3 H, CH_3), 0.95 (s, 3 H, CH_3), 1.05 - 2.25 (om), 2.53 - 2.67 (m, 1 H, 17α-H), 4.14 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 5.09 (t, 1 H, 21-H), 6.21 (d, 1 H, 22-H), 6.38 (d, 1 H, 1'-H), 7.29 - 7.64 (8 H, arom. H), 7.97 - 8.05 (2 H, arom. H_o (benzoate)), $J_{21,22} = 1.3$ Hz, $J_{21,1'} = 2.1$ Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$, here: quart. C, CH_2 (-) and CH, CH_3 (+)): δ = 16.79 (+) (C-18), 21.85 (-), 21.92 (-), 24.20 (+) (C-19), 26.98 (-), 28.40 (-), 30.12 (-), 30.53 (-), 33.46 (-), 33.80 (-), 35.90 (-), 35.99 (+) and 36.45 (+) (C-5, C-9), 41.33 (-) (C-12), 42.50 (+) (C-8), 49.52 (+) (C-17), 50.35 (-) (C-13), 67.29 (+) (C-3), 72.63 (+) (C-1'), 86.52 (-) (C-14), 87.18 (+) (C-21), 120.54 (+) (C-22), 127.60 (+) (arom. C), 129.14 (+) (probably

2 x arom. C), 129.31 (+) (arom. C), 129.61 (-) (arom. C_i), 130.23 (+) (arom. C), 134.06 (+) (arom. C), 136.60 (-) (arom. C_i), 165.42 (-) and 173.61 (-) and 174.67 (-) (C-20, C-23, O<u>C</u>OPh).- MS: m/z (%) = 462 (3), 444 (1), 426 (1), 338 (4), 212 (4), 122 (25), 105 (100), 77 (35).- FAB MS: m/z = 585.3 ($[M+H]^+$).- $C_{37}H_{44}O_6$ (584.75), calcd for $C_{37}H_{44}O_6 \cdot 0.5\ CH_3OH$ C 74.97, H 7.72, found C 75.26, H 7.44.

21-((Ξ)-Benzoyloxybenzyl)-3β,14-dihydroxy-5β,14β,21(Ξ)-card-20(22)-enolide (4d)
The protecting groups of **4b** were removed as described for **3c**. LC (petrol-ethyl acetate 3:2) yielded **4d** (89%).- M.p. 138 - 140 °C (methanol).- IR ($CHCl_3$): 3622, 3580-3360, 1751, 1720, 1634, 1268, 1110, 710 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.92 (s, 3 H, CH_3), 0.95 (s, 3 H, CH_3), 1.00 - 2.38 (om), 2.58 - 2.71 (m, 1 H, 17α-H), 4.15 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 5.34 (t, 1 H, 21-H), 5.98 (d, 1 H, 22-H), 6.39 (d, 1 H, 1'-H), 7.28 - 7.67 (8 H, arom. H), 8.09 - 8.18 (2 H, arom. H_o (benzoate)), $J_{21,22}$ = 1.5 Hz, $J_{21,1'}$ = 2.5 Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$, here: quart. C, CH_2 (-) and CH, CH_3 (+)): δ = 16.59 (+) (C-18), 21.88 (-) (probably 2 x C), 24.21 (+) (C-19), 26.99 (-), 28.42 (-), 30.11 (-) (probably 2 x C), 33.39 (-), 33.82 (-), 35.90 (-), 36.05 (+) and 36.46 (+) (C-5, C-9), 41.13 (-) (C-12), 42.56 (+) (C-8), 49.87 (+) (C-17), 50.60 (-) (C-13), 67.31 (+) (C-3), 75.80 (+) (C-1'), 86.27 (+) (C-21), 86.62 (-) (C-14), 120.79 (+) (C-22), 128.02 (+) (arom. C), 128.81 (+) (arom. C), 129.05 (+) (arom. C), 129.51 (+) (arom. C), 129.92 (-) (arom. C_i), 130.37 (+) (arom. C), 133.51 (-) (arom. C_i), 134.07 (+) (arom. C), 166.15 (-) and 173.53 (-) and 174.17 (-) (C-20, C-23, O<u>C</u>OPh).- MS: m/z (%) = 462 (4), 444 (1), 426 (1), 338 (2), 212 (4), 122 (63), 105 (100), 77 (54).- FAB MS: m/z = 585.3 ($[M+H]^+$).- $C_{37}H_{44}O_6$ (584.75), calcd C 76.00, H 7.58, found C 76.03, H 7.66.

3β,14-Dihydroxy-21-phenylselenenyl-5β,14β,21(Ξ)-card-20(22)-enolide (a, b)
To a solution of digitoxigenin (**1a**) (500 mg, 1.34 mmol) in dry NMP (15 ml) a suspension of NaH [160 mg NaH (55-65 per cent dispersion in mineral oil) in NMP (2 ml), 4.00 mmol] was added at 0°C (colour change to red). After stirring at this temperature for 30 min a solution of phenylselenenyl bromide (350 mg, 1.48 mmol) in NMP (2 ml) was added (colour change to reddish brown) and the mixture was allowed to warm to room temperature. 1.5 h later the reaction was quenched with saturated aqu. NH_4Cl (5 ml) at 0°C (formation of a white precipitate). The suspension was diluted with CH_2Cl_2 (150 ml) and washed with water (150 ml). The aqueous layer was neutralized with diluted aqu. HCl (1.6 mol/l) and extracted with CH_2Cl_2 (3 x 150 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. FC (petrol-ethyl acetate 4:3 → 1:1, then petrol - ethyl acetate - 2-propanol 1:1:0.1) yielded 441 mg (62 %) of a 5:1 mixture (^1H NMR) of diastereomers **a** and **b** (which could not be separated), recovered digitoxigenin (**1a**) (35 mg, 7 %) and 150 mg (16 %) of a mixture of the diastereomers of 21,22-di-selenenylated digitoxigenin. The following analytical data were obtained from a 2:1 mixture of **a** and **b**:
IR ($CHCl_3$): 3620, 1785, 1750, 1626, 1449, 1030, 992 cm^{-1}.
a: ^1H NMR (200 MHz, $CDCl_3$): δ = 0.85 (s, CH_3-18), 0.95 (s, CH_3-19), 1.00 - 2.38 (om), 2.74 - 2.90 (m, 17α-H), 4.14 ($w_{1/2} \approx 7$ Hz, 3α-H), 5.97 ($w_{1/2} \approx 3$ Hz, 21-H[#1]), 6.21 (d, 22-H[#1]), 7.23 - 7.39 (arom. H), 7.50 - 7.60 (arom. H), $J_{21,22}$ = 1.1 Hz.
b: ^1H NMR (200 MHz, $CDCl_3$): δ = 0.89 (s, CH_3-18), 0.97 (s, CH_3-19), 1.00 - 2.38 (om), 2.74 - 2.90 (m, 17α-H), 4.14 ($w_{1/2} \approx 7$ Hz, 3α-H), 6.26 (d, 21-H[#2]), 6.36 (d, 22-H[#2]), 7.23 - 7.39 (arom. H), 7.63 - 7.72 (arom. H), $J_{21,22}$ = 1.5 Hz.
^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = 16.64 (-) and 16.79 (-) (2 x C-18), 21.84 (+), 21.97 (+), 24.22 (-) (C-19), 26.99 (+), 28.37 (+), 30.03 (+), 30.17 (+), 32.19 (+), 33.46 (+), 33.80 (+), 35.88 (+), 36.00 (-) and 36.47 (-) (C-5, C-9), 40.62 (+) and 41.01 (+) (2 x C-12), 42.20 (-) and 42.44 (-) (2 x C-8), 50.00 (-) and 50.71 (-) (2 x C-17), 50.53 (+) (C-13), 67.30 (-) (C-3), 85.61 (-) and 86.73 (-) (2 x C-21), 85.92 (+) and 86.54 (+) (2 x C-14), 118.57 (-) and 119.05 (-) (2 x C-22), 125.29 (+) and 128.08 (+) (2 x arom. C_i), 129.27(-) (arom. C), 129.67 (-) (arom. C), 129.78 (-) (arom. C), 129.89 (-) (arom. C), 135.28 (-) (arom. C) 136.90 (-) (arom. C), 172.71 (+) and 173.23 (+) and 175.63 (+) and 175.80 (+) (2 x C-20, 2 x C-23).- MS: m/z (%) = 530 (0.4, $M^{+\cdot}$), 512 (0.4), 494 (0.3), 373 (8), 353 (23), 337 (8), 314 (28), 234 (12), 203 (9), 157 (52), 77 (100).- $C_{29}H_{38}O_4Se$ (529.57), HRMS: calcd for $C_{29}H_{38}O_4{}^{80}Se$ 530.1935, found 530.1937.
[#1] These assignments may have to be reversed.
[#2] These assignments may have to be reversed.

22-Allyl-3β,14-dihydroxy-5β,14β-card-20(22)-enolide (8)

To a solution of digitoxigenin (**1a**) (501 mg, 1.34 mmol) in dry NMP (20 ml) a suspension of NaH [67 mg NaH (55-65 per cent dispersion in mineral oil) in NMP (3 ml), 1.7 mmol] was added at 0°C (colour change to orange). The mixture was stirred at this temperature for 30 min. Then allyl bromide (130 μl, 1.54 mmol) was added and the reaction mixture was allowed to warm to 20°C and stirred for another 2.5 h. Quenching with saturated aqu.NH_4Cl (4.5 ml) at 0°C (formation of a white precipitate), dilution with CH_2Cl_2 (200 ml), usual work-up (CH_2Cl_2), followed by FC (petrol - ethyl acetate - 2-propanol 1:1:0.01) afforded **8** (439 mg, 79 %), recovered digitoxigenin (**1a**) (44 mg, 9 %) and 85 mg (ca. 12 %) of probably di- and triallyl-substituted digitoxigenin derivatives.- M.p. 195 - 199 °C (acetone-petrol), ref.[13] 160 - 163 °C.- IR ($CHCl_3$): 3627, 1742, 1651, 1449, 1031 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.82 (s, 3 H, CH_3-18), 0.96 (s, 3 H, CH_3-19), 1.05 - 2.20 (om), 3.03 (d, 3 H, CH_2-1', partly hidden: 17α-H), 4.13 ($w_{1/2} \approx 9$ Hz, 1 H, 3α-H), 4.80 (AB, 1 H, 21-H), 4.98 - 5.15 (om, 3 H, 21-H', CH_2-3'), 5.84 (ddt, 1 H, 2'-H), $|J_{21,21'}| = 18.0$ Hz, $J_{1',2'} = 6.6$ Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = 15.97 (-) (C-18), 21.70 (+), 21.89 (+), 24.18 (-) (C-19), 26.26 (+), 26.95 (+), 28.38 (+), 28.62 (+), 30.12 (+), 33.71 (+), 33.78 (+), 35.88 (+), 35.94 (-) and 36.45 (-) (C-5, C-9), 40.92 (+) (C-12), 42.25 (-) (C-8), 49.17 (-) (C-17), 50.17 (+) (C-13), 67.29 (-) (C-3), 72.16 (+) (C-21), 85.84 (+) (C-14), 116.68 (+) (C-3'), 126.33 (+) (C-22), 134.66 (-) (C-2'), 166.05 (+) (C-20), 175.55 (+) (C-23).- $C_{26}H_{38}O_4$ (414.58), MS: m/z (%) = 414 (12, $M^{+\cdot}$), 396 (37), 378 (13), 235 (19), 203 (100).

22-Benzyl-3β,14-dihydroxy-5β,14β-card-20(22)-enolide (6)

To a solution of digitoxigenin (**1a**) (150 mg, 400 μmol) in dry NMP (6 ml) a suspension of NaH [20 mg NaH (55-65 per cent dispersion in mineral oil) in NMP (1.5 ml), 490 μmol] was added at 0°C (colour change to red). After stirring for 30 min at this temperature, benzyl chloride (55 μl, 480 μmol) was added. The mixture was stirred at 0°C for another 15 min and then allowed to warm to room temperature (colour change to greenish black). 2 h after the addition of benzyl chloride the reaction mixture (now red again) was quenched with saturated aqu. NH_4Cl (1.5 ml) at 0°C (formation of a white precipitate). Dilution with CH_2Cl_2 (75 ml), usual work-up (CH_2Cl_2) and repeated FC (petrol - ethyl acetate - 2-propanol 1:1:0.01) afforded **6** (118 mg, 63%) and recovered digitoxigenin (**1a**) (25 mg, 17 %).- M.p. 201 - 203 °C (ethyl acetate-petrol).- IR ($CHCl_3$): 3613, 1741, 1646, 1443, 1028 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.65 (s, 3 H, CH_3-18), 0.92 (s, 3 H, CH_3-19), 1.05 - 2.20 (om), 3.01 - 3.15 (m, 1 H, 17α-H), 3.55 + 3.64 (AB, 2 H, CH_2-1'), 4.11 ($w_{1/2} \approx 8$ Hz, 1 H, 3α-H), 4.81 + 5.06 (AB, 2 H, CH_2-21), 7.12 - 7.32 (5 H, arom. H), $|J_{21,21'}| = 18.1$ Hz, $|J_{1',(1')'}| = 14.8$ Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = 15.70 (-) (C-18), 21.65 (+), 21.86 (+), 24.17 (-) (C-19), 26.40 (+), 26.95 (+), 28.36 (+), 30.01 (+), 30.05 (+), 33.68 (+), 33.77 (+), 35.85 (+), 35.89 (-) and 36.45 (-) (C-5, C-9), 40.67 (+) (C-12), 42.13 (-) (C-8), 49.42 (-) (C-17), 50.13 (+) (C-13), 67.27 (-) (C-3), 72.23 (+) (C-21), 85.79 (+) (C-14), 126.90 (-) (arom. C_p), 127.72 (+) (C-22), 128.99 (-) (arom. $C_{o,m}$), 139.15 (+) (arom. C_i), 166.38 (+) (C-20), 175.92 (+) (C-23).- MS: m/z (%) = 464 (1, $M^{+\cdot}$), 446 (39), 428 (15), 413 (2), 400 (4), 386 (4), 285 (24), 259 (18), 216 (18), 203 (100), 201 (53), 188 (39), 107 (49), 91 (98).- $C_{30}H_{40}O_4$ (464.64), calcd C 77.54, H 8.68, found C 77.47, H 8.76.

3β,14-Dihydroxy-22-(3-hydroxypropyl)-5β,14β-card-20(22)-enolide (11a)

To a solution of **8** (99 mg, 240 μmol) in dry THF (2.5 ml) a solution of BH_3·THF (1 mol/l in THF, 600 μl, 600 μmol) was added at 0°C. After stirring at this temperature for 1.5 h, aqu. NaOH (3 mol/l, 120 μl, 360 μmol) and H_2O_2 (35 per cent, 30 μl, 357 μmol) were added subsequently (formation of a white precipitate). The mixture was stirred for another 1 h, diluted with CH_2Cl_2 (40 ml) and washed with water (40 ml). The aqueous layer was neutralized with aqu. HCl (1.6 mol/l) and extracted with CH_2Cl_2 (3 x 40 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. FC (petrol-ethyl acetate 1:7) yielded **11a** (48 mg, 46 %).- M.p. 198 - 200 °C (methanol-water).- IR (KBr): 3700-3100, 1732, 1650, 1449, 1031 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.83 (s, 3 H, CH_3-18), 0.97 (s, 3 H, CH_3-19), 1.05 - 2.34 (om), 2.39 (t, 2 H, CH_2-1'), 2.97 - 3.12 (m, 1 H, 17α-H), 3.54 - 3.69 (m, 2 H, CH_2-3'), 4.15 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 4.83 + 5.08 (AB, 2 H, CH_2-21), $|J_{21,21'}| = 18.0$ Hz, $J_{1',2'} = 7.1$ Hz.- ^{13}C NMR (50 MHz, APT, CD_3OD): δ = 16.59 (-) (C-18), 21.34 (+), 22.70 (+), 22.87 (+), 24.57 (-) (C-19), 27.20 (+), 28.17 (+), 28.84 (+), 31.10 (+), 32.50 (+), 33.70 (+), 34.47 (+), 36.78 (+), 36.99 (-) and 37.75 (-) (C-5, C-9), 41.75 (+) (C-12), 43.00 (-) (C-8), 50.35 (-) (C-17), 51.41 (+) (C-13), 62.48 (+) (C-3'), 67.96 (-) (C-3), 73.67 (+) (C-21), 86.51 (+) (C-14), 128.56 (+) (C-22), 168.36 (+) (C-20), 178.11 (+) (C-23).- MS: m/z (%) = 432 (5, $M^{+\cdot}$), 414 (47), 396 (9), 370 (5), 246 (61), 203 (100).- $C_{26}H_{40}O_5$ (432.60), calcd C 72.19, H 9.32, found C 72.05, H 9.22.

Ozonolytic cleavage of 8

A stream of oxygen (40 l/h), containing ozone, was bubbled through a solution of **8** (231 mg, 557 µmol) in methanol (50 ml) at -78°C until the blue colour of the solution persisted (5 min). To remove excess ozone, first oxygen and then argon were bubbled through the reaction mixture for 45 min each. The solution was allowed to warm to 0°C and divided into two equal parts. One of these was treated with triphenylphosphine (110 mg, 419 µmol) and was allowed to warm to 20°C. After stirring at this temperature for 2 h, the mixture was concentrated under reduced pressure. FC (petrol - tert-butyl methyl ether - acetone 2.5:20:1) provided unstable aldehyde **11b** (70 mg, 60 %). The other half of the ozonolysis mixture was treated with solid $NaBH_4$ (63 mg, 1665 µmol) at 0°C and was allowed to warm to 20°C. Additional $NaBH_4$ was added after 2 h 15 min and 4 h at 0°C and after 6 h at 20°C (21 mg, 555 µmol each). Stirring was continued for another 2 h. Then the reaction mixture was concentrated under reduced pressure. Repeated FC (petrol - tert-butyl methyl ether - acetone 1:20:1 → 1:20:5) afforded **11c** (65 mg, 56 %).

22-Formylmethyl-3β,14-dihydroxy-5β,14β-card-20(22)-enolide (11b)

^{1}H NMR (200 MHz, $CDCl_3$, containing tert-butyl methyl ether from FC): δ = 0.81 (s, 3 H, CH_3-18), 0.94 (s, 3 H, CH_3-19), 1.05 - 2.23 (om), 2.82 - 2.93 (m, 1 H, 17α-H), 3.38 (d, 2 H, CH_2-1'), 4.09 - 4.18 (m, 1 H, 3α-H), 4.90 + 5.16 (AB, 2 H, CH_2-21), 9.68 (t, 1 H, CHO), $|J_{21,21'}|$ = 18.3 Hz, $J_{1',CHO}$ = 1.6 Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$, containing tert-butyl methyl ether from FC): δ = 15.99 (-) (C-18), 21.65 (+), 21.87 (+), 24.16 (-) (C-19), 26.07 ?, 26.36 (+), 26.93 (+), 28.38 (+), 30.09 (+), 33.72 (+) (perhaps 2 signals), 35.87 (+), 35.92 (-) and 36.42 (-) (C-5, C-9), 39.25 (+) and 40.87 (+) (C-12, C-1'), 42.19 (-) (C-8), 49.83 (-) (C-17), 50.39 (+) (C-13), 67.25 (-) (C-3), 72.97 (+) (C-21), 85.88 (+) (C-14), 120.59 (+) (C-22), 169.51 (+) (C-20), 175.07 (+) (C-23), 197.26 (-) (CHO).- $C_{25}H_{36}O_5$ (416.55).

3β,14-Dihydroxy-22-(2-hydroxyethyl)-5β,14β-card-20(22)-enolide (11c)

M.p. 243 - 246 °C (methanol-water).- IR (KBr): 3700-3100, 1741, 1651, 1450, 1033 cm^{-1}.- ^{1}H NMR (200 MHz, CD_3OD): δ = 0.84 (s, 3 H, CH_3-18), 0.98 (s, 3 H, CH_3-19), 1.00 - 2.25 (om), 2.42 - 2.54 (m, 2 H, CH_2-1'), 3.08 - 3.21 (m, 1 H, 17α-H), 3.58 - 3.70 (m, 2 H, CH_2-2'), 4.06 ($w_{1/2}$ ≈ 7 Hz, 1 H, 3α-H), 4.91 + 5.09 (AB, 2 H, CH_2-21, partly hidden by solvent signals), $|J_{21,21'}|$ = 18.7 Hz.- ^{13}C NMR (50 MHz, APT, CD_3OD): δ = 16.52 (-) (C-18), 22.69 (+), 22.87 (+), 24.57 (-) (C-19), 27.29 (+), 28.16 (+), 28.64 (+), 28.83 (+), 31.10 (+), 33.67 (+), 34.46 (+), 36.77 (+), 36.98 (-) and 37.74 (-) (C-5, C-9), 41.71 (+) (C-12), 42.99 (-) (C-8), 50.45 (-) (C-17), 51.42 (+) (C-13), 61.09 (+) (C-2'), 67.96 (-) (C-3), 73.84 (+) (C-21), 86.54 (+) (C-14), 125.89 (+) (C-22), 170.05 (+) and 178.04 (+) (C-20, C-23).- MS: m/z (%) = 418 (4, $M^{+\cdot}$), 400 (25), 246 (39), 231 (9), 203 (74), 43 (100).- $C_{25}H_{38}O_5$ (418.57), calcd C 71.74, H 9.15, found C 71.61, H 9.08.

3β,14-Dihydroxy-22-(2(Ξ),3-dihydroxypropyl)-5β,14β-card-20(22)-enolide (10a, 10b)

AD-mix-α (170 mg, consisting of: $K_3Fe(CN)_6$ (119 mg, 364 µmol), K_2CO_3 (50 mg, 364 µmol), $(DHQ)_2$-PHAL (0.9 mg, 1.2 µmol) and $K_2(OsO_2(OH)_4)$ (0.1 mg, 0.2 µmol)) and **8** (50 mg, 121 µmol) were treated with tert-butanol (0.6 ml) and water (0.6 ml). The mixture (2 layers: yellow aqueous layer, colourless organic layer) was stirred vigorously at 20°C for 32 h. Then solid Na_2SO_3 (185 mg, 1470 µmol) was added (mixture became colourless). After stirring for another 30 min the reaction mixture was diluted with CH_2Cl_2 (7 ml) and water (7 ml). Usual work-up (CH_2Cl_2) followed by FC (petrol - ethyl acetate - 2-propanol 1:1:0.3) furnished 45 mg (84 %) of a 1.4:1 mixture (^{1}H NMR in CD_3OD) of **10a** and **10b**. The reaction of **8** with AD-mix-β (containing $(DHQD)_2$-PHAL instead of $(DHQ)_2$-PHAL as chiral ligand) yielded 45 mg (84 %) of a 1:1.2 mixture of **10a** and **10b** which could not be separated.- IR (KBr): 3650-3050, 1716, 1632, 1437, 1019 cm^{-1}.- ^{1}H NMR (200 MHz, CD_3OD): δ = 0.84 + 0.87 (2 x s, 3 H, 2 x CH_3-18), 0.98 (s, 3 H, CH_3-19), 1.10 - 2.28 (om), 2.29 - 2.57 (m, 2 H, CH_2-1'), 3.11 - 3.28 (m, 1 H, 17α-H), 3.38 - 3.56 (m, 2 H, CH_2-3'), 3.75 - 3.92 (m, 1 H, 2'-H), 4.06 ($w_{1/2}$ ≈ 7 Hz, 1 H, 3α-H), 4.92 + 5.10 (AB, 2 H, CH_2-21, partly hidden by solvent signal), $|J_{21,21'}|$ = 17.9 Hz.- In $CDCl_3$ only one singlet for CH_3-18 of both diastereomers is visible at δ = 0.82; CH_2-1' appears as a doublet at δ = 2.49 ($J_{1',2'}$ = 5.1 Hz).- ^{13}C NMR (50 MHz, APT, CD_3OD): δ = 16.40 (-) and 16.62 (-) (2 x C-18), 22.69

(+), 22.91 (+), 24.64 (-) (C-19), 27.28 (+), 27.38 (+), 28.20 (+), 28.89 (+), 29.40 (+), 29.61 (+), 31.13 (+), 33.74 (+), 34.51 (+), 36.80 (+), 37.01 (-) and 37.76 (-) (C-5, C-9), 41.56 (+) and 41.75 (+) (2 x C-12), 43.01 (-) (C-8), 50.31 (-) and 50.41 (-) (2 x C-17), 51.51 (+) (C-13), 66.83 (+) and 67.45 (+) (2 x C-3'), 67.97 (-) (C-3), 71.85 (-) and 72.00 (-) (2 x C-2'), 73.97 (+) (C-21), 86.55 (+) and 86.62 (+) (2 x C-14), 125.94 (+) (C-22), 170.23 (+) and 170.45 (+) and 178.38 (+) (C-20 and C-23).- MS: m/z (%) = 448 (6, $M^{+\cdot}$), 430 (76), 418 (13), 417 (17), 412 (14), 404 (15), 399 (13), 381 (12), 370 (11), 250 (45), 246 (48), 203 (82), 107 (75), 105 (59), 55 (100).- $C_{26}H_{40}O_6$ (448.59), calcd for $C_{26}H_{40}O_6 \cdot H_2O$ C 66.93, H 9.07, found C 66.95, H 8.83.

3β,14-Dihydroxy-22-((E)-1-propenyl)-5β,14β-card-20(22)-enolide (9)

8 (250 mg, 603 μmol) and $[Ir(I)(cod)(PMePh_2)_2]PF_6$ (25 mg, 30 μmol) were dissolved in dry THF (1.2 ml). The red solution (the Ir catalyst did not dissolve completely) was degassed three times by freezing with liquid nitrogen and evaporation, then the reagent was hydrogenated at 0.11 MPa until the colour of the solution disappeared and the catalyst dissolved completely (4 min). The mixture was degassed again (colour change to pale yellow), flushed with nitrogen and stirred under argon at 20°C for 16 h. Solvent evaporation under reduced pressure furnished a crude product which consisted of **9** and educt **8** in a ratio of 95:5 (^{1}H NMR). Repeated FC (toluene - 2-propanol 20:1) yielded pure **9** (200 mg, 80 %).- M.p. 193 - 195 °C (ethyl acetate-petrol).- IR ($CHCl_3$): 3697, 3620, 1741, 1601, 1448, 1032 cm^{-1}.- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.81 (s, 3 H, CH_3-18), 0.96 (s, 3 H, CH_3-19), 1.10 - 1.98 (om), partly hidden: 1.86 (dd, CH_3-3'), 1.99 - 2.20 (m, 2 H), 3.10 - 3.19 (m, 1 H, 17α-H), 4.14 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 4.78 + 5.05 (AB, 2 H, CH_2-21), 6.06 (dd, 1 H, 1'-H), 6.92 (dq, 1 H, 2'-H), $|J_{21,21'}|$ = 18.4 Hz, $J_{1',2'}$ = 15.7 Hz, $J_{1',3'}$ = 1.7 Hz, $J_{2',3'}$ = 6.9 Hz.- ^{13}C NMR (75 MHz, APT, $CDCl_3$): δ = 15.55 (-) and 19.41 (-) (C-18, C-3'), 21.32 (+), 21.47 (+), 23.75 (-) (C-19), 25.54 (+), 26.52 (+), 27.95 (+), 29.68 (+), 33.19 (+), 33.37 (+), 35.45 (+), 35.55 (-) and 36.01 (-) (C-5, C-9), 40.54 (+) (C-12), 42.00 (-) (C-8), 48.17 (-) (C-17), 50.24 (+) (C-13), 66.86 (-) (C-3), 70.64 (+) (C-21), 85.56 (+) (C-14), 118.93 (-) (C-2'), 123.85 (+) (C-22), 132.32 (-) (C-1'), 162.24 (+) (C-20), 173.59 (+) (C-23).- MS: m/z (%) = 414 (4, $M^{+\cdot}$), 396 (58), 378 (60), 363 (7), 203 (65), 55 (81), 43 (100).- $C_{26}H_{38}O_4$ (414.58), calcd for $C_{26}H_{38}O_4 \cdot 0.5$ ethyl acetate C 73.33, H 9.23, found C 73.46, H 9.21.

3β,14-Dihydroxy-22-hydroxymethyl-5β,14β-card-20(22)-enolide (12)

A stream of oxygen (40 l/h), containing ozone, was bubbled through a solution of **9** (75 mg, 180 μmol) in methanol (50 ml) at -78°C until the blue colour of the solution persisted (3 min). To remove excess ozone, first oxygen (25 min) and then argon (10 min) was bubbled through the reaction mixture. The solution was allowed to warm to 20°C and concentrated under reduced pressure to 5-10 ml. After cooling to 0°C the stirred mixture was treated with solid $NaBH_4$ and allowed to warm to 20°C. 1 h later the reaction mixture was concentrated under reduced pressure. Repeated FC (petrol - tert-butyl methyl ether - acetone 1:20:5 and 1:20:1, rsp.) afforded **12** (30 mg, 41 %).- M.p. 211-215 °C (CH_2Cl_2-petrol).- IR (KBr): 3660-3050, 1729, 1648, 1447, 1028 cm^{-1}.- ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.80 (s, 3 H, CH_3-18), 0.94 (s, 3 H, CH_3-19), 1.05 - 2.25 (om), 2.85 - 2.99 (broad t, 1 H, $CH_2O\underline{H}$), 3.01 - 3.12 (m, 1 H, 17α-H), 4.08 - 4.17 (m, 1 H, 3α-H), 4.34 (broad d, 2 H, CH_2-1'), 4.85 (AB, 1 H, 21-H), 5.06 - 5.19 (m, 1 H, 21-H'), $|J_{21,21'}|$ = 18.3 Hz, $J_{1',OH}$ = 3.3 Hz, $J_{17 \text{ or } 1',21'}$ = 0.7 Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = 16.14 (+) (C-18), 21.69 (+), 21.91 (+), 24.18 (-) (C-19), 26.13 (+), 26.95 (+), 28.38 (+), 30.11 (+), 33.67 (+), 33.72 (+), 35.88 (+), 35.98 (-) and 36.43 (-) (C-5, C-9), 40.71 (+) (C-12), 42.33 (-) (C-8), 49.15 (-) (C-17), 50.48 (+) (C-13), 55.25 (+) (C-1'), 67.28 (-) (C-3), 72.66 (+) (C-21), 86.03 (+) (C-14), 127.16 (+) (C-22), 167.84 (+) (C-20), 175.76 (+) (C-23).- MS: m/z (%) = 404 (1, $M^{+\cdot}$), 386 (28), 368 (21), 350 (7), 250 (29), 246 (48), 203 (100).- $C_{24}H_{36}O_5$ (404.54), calcd C 71.26, H 8.97, found C 71.18, H 8.91.

3β,14-Dihydroxy-22-(2-oxopropyl)-5β,14β-card-20(22)-enolide (7)

A mixture of NMP and water (9:1, 1.7 ml) was added to cuprous chloride (24 mg, 240 μmol) and palladium(II) chloride (6 mg, 36 μmol). A stream of oxygen was bubbled through the stirred black suspension at 20°C (colour change to greenish grey). Then **8** (50 mg, 121 μmol) was dissolved in the suspension (colour change to black) and again a stream of oxygen was bubbled through the reaction mixture for 16 h. During this time the colour changed to greenish grey. Then the suspension was diluted with CH_2Cl_2 (20 ml) and washed with ice cold aqu. HCl (0.2 mol/l, 20 ml). The aqueous layer was extracted with CH_2Cl_2 (3 x 20 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. FC (petrol - ethyl acetate - 2-propanol 1:1:0.04), followed by recrystallization (petrol-ethyl acetate) provided **7** (37 mg, 71 %).- M.p. 201 - 204 °C (ethyl acetate-petrol).- IR ($CHCl_3$): 3619, 1742, 1653, 1445, 1028 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.83 (s, 3 H, CH_3-18), 0.95 (s, 3 H, CH_3-19), 1.10 - 2.20 (om), 2.24 (s, 3 H, CH_3-3'), 2.85 - 2.97 (m, 1 H, 17α-H), 3.38 (s, 2 H, CH_2-1'), 4.14 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 4.88 + 5.12 (AB, 2 H, CH_2-21), $|J_{21,21'}|$ = 18.0 Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = 15.82 (-) (C-18), 21.64 (+), 21.89 (+), 24.18 (-) (C-19), 26.42 (+), 26.95 (+), 28.37 (+), 30.11 (+), 30.46 (-) (C-3'), 33.72 (+), 33.77 (+), 35.87 (+), 35.93 (-) and 36.44 (-) (C-5, C-9), 39.02 (+) and 40.84 (+) (C-12, C-24), 42.18 (-) (C-8), 49.80 (-) (C-17), 50.33 (+) (C-13), 67.26 (-) (C-3), 72.80 (+) (C-21), 85.83 (+) (C-14), 122.26 (+) (C-22), 168.78 (+) (C-20), 175.28 (+) (C-23), 204.28 (+) (C-2').- MS: m/z (%) = 430 (2, $M^{+\cdot}$), 412 (27), 294 (2), 259 (5), 246 (52), 231 (13), 203 (100), 43 (71).- $C_{26}H_{38}O_5$ (430.58), calcd C 72.53, H 8.90, found C 72.57, H 8.86.

3β-(*tert*-Butyl-dimethyl-silyloxy)-20,22-dihydroxy-14-trimethylsilyloxy-5β,14β,20(Ξ),22(Ξ)-cardanolide (13a, 13b)

To a solution of **1c** (140 mg, 250 μmol) in dry pyridine (3 ml) solid osmium tetroxide (60 mg, 236 μmol) was added (colour change to brown). The mixture was stirred at 20°C for 18 h and then concentrated under reduced pressure. The brown residue was dissolved in tert-butanol (5 ml) and a solution of Na_2SO_3 (160 mg, 1500 μmol) in water (5 ml) was added to the stirred mixture. After a short time a white precipitate formed. 3 h later the suspension was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (30 ml). Usual work-up and FC (petrol-ethyl acetate 3:1) furnished 117 mg (79 %) of a 2.5:1 mixture (^1H NMR) of diastereomers **13a** (smaller R_f) and **13b** (larger R_f) and recovered **1c** (29 mg, 21 %).-Small samples of almost pure **13a** and **13b** could be obtained by FC. ^1H NMR data were obtained from these samples.

13a: ^1H NMR (200 MHz, $CDCl_3$, containing small amounts of **13b**): δ = 0.02 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.21 (s, 9 H, $Si(CH_3)_3$), 0.89 (s, 9 H, $C(CH_3)_3$), 0.94 (s, 3 H, CH_3-19), 1.13 (s, 3 H, CH_3-18), 1.15 - 2.05 (om), 3.08 (d, 1 H, 22-OH, exchanges with D_2O), 4.04 ($w_{1/2} \approx 8$ Hz, 1 H, 3α-H), partly hidden: 4.08 (d, 1 H, 22-H), 4.25 (s, 2 H, CH_2-21; partly hidden: 1 H, 20-OH, exchanges with D_2O), $J_{22,OH}$ = 5.5 Hz.

13b: ^1H NMR (200 MHz, $CDCl_3$, containing small amounts of **13a**): δ = 0.03 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.23 (s, 9 H, $Si(CH_3)_3$), 0.89 (s, 9 H, $C(CH_3)_3$), 0.94 (s, 3 H, CH_3-19), 1.08 (s, 3 H, CH_3-18), 1.10 - 2.30 (om), 3.03 (d, 1 H, 22-OH, exchanges with D_2O), 3.98 (d, 1 H, 22-H), 4.05 ($w_{1/2} \approx 8$ Hz, 1 H, 3α-H), 4.16 + 4.57 (AB, 2 H, CH_2-21), 4.99 (s, 1 H, 20-OH, exchanges with D_2O), $|J_{21,21'}|$ = 10.1 Hz, $J_{22,22\text{-}OH}$ = 9.9 Hz.

The following analytical data were obtained from a 2.5:1 mixture of **13a** and **13b**:

IR ($CHCl_3$): 3540, 3450-3150, 1781, 1467, 1249, 1055, 987, 833 cm^{-1}.- ^{13}C NMR (50 MHz, APT, $CDCl_3$, characteristic signals): δ = -4.40 (-) ($Si^tBu(\underline{C}H_3)_2$), 3.22 (-) and 3.30 (-) ($Si(CH_3)_3$), 18.38 (-) (C-18), 18.58 (+), ($\underline{C}(CH_3)_3$), 24.31 (-) (C-19), 26.31 (-) ($C(\underline{C}H_3)_3$), 36.28 (-) and 37.60 (-) (C-5, C-9), 39.88 (-) (C-8), 43.06 (+) (C-12), 49.99 (+) and 50.64 (+) (2 x C-13), 57.39 (-) and 59.66 (-) (2 x C-17), 67.61 (-) (C-3), 71.64 (-) and 73.59 (-) (2 x C-22), 76.23 (+) and 77.01 (+) and 77.98 (+) and 78.67 (+) (2 x C-20, 2 x C-21), 93.75 (+) and 94.25 (+) (2 x C-14), 176.02 (+) and 176.42 (+) (2 x C-23).- MS: m/z (%) = 594 (2, $M^{+\cdot}$), 579 (2), 537 (88), 519 (9), 447 (15), 431 (9), 429 (7), 372 (10), 371 (8), 286 (11), 285 (13), 255 (9), 169 (57), 75 (100), 73 (68).- $C_{32}H_{58}O_6Si_2$ (594.97), HRMS: calcd for $C_{28}H_{49}O_6Si_2$ $[M - {}^tBu]^+$ 537.3068, found 537.3040.

3β-(*tert*-Butyl-dimethyl-silyloxy)-20,22-carbonyldioxy-14-trimethylsilyloxy-5β,14β,20(Ξ),22(Ξ)-cardanolide (14a)

To a solution of a mixture of **13a** and **13b** (15 mg, 25 μmol) and DMAP (1 mg, 8 μmol) in dry pyridine (150 μl) a solution of phosgene (1.93 mol/l in toluene, 13 μl, 25 μmol) was added carefully at 0°C (a white precipitate formed). After stirring for 4 h at 0°C the reaction mixture was allowed to warm to 20°C. 14 h later CH_2Cl_2 (10 ml) was added. Usual work-up (CH_2Cl_2), followed by FC (petrol-ethyl acetate 2:1) yielded carbonate **14a** (9 mg, 59 %).- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.02 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.20 (s, 9 H, $Si(CH_3)_3$), 0.89 (s, 9 H, $C(CH_3)_3$), 0.92 (s, 3 H, CH_3-19), 1.00 (s, 3 H, CH_3-18), 1.05 - 2.05 (om), 2.22 - 2.35 (m 1 H, 17α-H), 4.05 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 4.57 + 4.95 (AB, 2 H, CH_2-21), 4.82 (s, 1 H, 22-H), $|J_{21,21'}|$ = 11.5 Hz.- MS: m/z (%) = 605 (2), 563 (37), 519 (3), 501 (11), 429 (22), 401 (7), 355 (7), 337 (18), 265 (22), 75 (100), 73 (97).- FAB MS: m/z = 643.6 ($[M+Na]^+$), 621.6 ($[M+H]^+$).- $C_{33}H_{56}O_7Si_2$ (620.97), HRMS: calcd for $C_{29}H_{47}O_7Si_2$ $[M - {}^tBu]^+$ 563.2860, found 563.2851.

3β-(*tert*-Butyl-dimethyl-silyloxy)-22-hydroxy-14-trimethylsilyloxy-5β,14β-card-20(22)-enolide (15a)
To a solution of a mixture of **13a** and **13b** (96 mg, 161 μmol) in dry pyridine (1 ml) a solution of carbonyldiimidazole (CDI, 640 μl of 78 mg CDI in pyridine (1200 μl), 258 μmol) was added. After stirring for 1.5 h at 20°C, a suspension of NaH [600 μl of 32 mg NaH (55-65 per cent dispersion in mineral oil) in pyridine (1000 μl), 483 μmol] was added. Stirring was continued for 1.5 h. Quenching with diluted aqu. HCl (0.15 mol/l, 80 ml), followed by usual work-up (CH_2Cl_2), and FC (petrol-ethyl acetate 15:1) provided **15a** (80 mg, 86 %).- IR ($CHCl_3$): 3540, 1762, 1474, 1257, 1062, 842 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.01 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.15 (s, 9 H, $Si(CH_3)_3$), 0.88 (s, 9 H, $C(CH_3)_3$), 0.90 (s, 3 H, CH_3-18 or CH_3-19), 0.91 (s, 3 H, CH_3-18 or CH_3-19), 1.00 - 2.15 (om), 2.65 - 2.77 (m, 1 H, 17α-H), 4.04 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 4.65 + 4.70 (AB, 2 H, CH_2-21), (6.29 (s, 1 H, 22-OH, exchanges with D_2O)), $|J_{21,21'}|$ = 16.6 Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = -4.35 (-) ($Si^tBu(\underline{C}H_3)_2$), 3.46 (-) ($Si(CH_3)_3$), 17.94 (-) (C-18), 18.58 (+) ($\underline{C}(CH_3)_3$), 21.21 (+), 23.65 (+), 24.41 (-) (C-19), 25.03 (+), 26.32 (-) ($C(\underline{C}H_3)_3$), 27.06 (+), 29.08 (+), 30.29 (+), 34.90 (+), 35.04 (+), 36.17 (+), 36.35 (-) and 37.49 (-) (C-5, C-9), 40.79 (-) (C-8), 41.29 (+) (C-12), 48.18 (-) (C-17), 51.80 (+) (C-13), 67.65 (-) (C-3), 70.75 (+) (C-21), 93.33 (+) (C-14), 136.84 (+) and 137.76 (+) (C-20, C-22), 171.46 (+) (C-23).- $C_{32}H_{56}O_5Si_2$ (576.98), MS: m/z (%) = 575 (0.7, $[M-H]^+$), 519 (0.6), 486 (3), 429 (26), 75 (100), 73 (34).- FAB MS: m/z = 577.3 ($[M+H]^+$), 487.3 ($[M+H-(CH_3)_3SiOH]^+$).

3β,14,22-Trihydroxy-5β,14β-card-20(22)-enolide (15d)
The protecting groups of **15a** were removed as described for **3c**. FC (petrol-ethyl acetate 1:2) afforded **15d** (91%).- M.p. 145 - 148 °C (methanol).- IR (KBr): 3650-3150, 1752, 1679, 1623, 1453, 1140, 1038 cm^{-1}.- ^1H NMR (200 MHz, $CDCl_3$): δ = 0.87 (s, 3 H, CH_3-18), 0.98 (s, 3 H, CH_3-19), 1.10 - 2.33 (om), 3.19 ($w_{1/2} \approx 12$ Hz, 1 H, 17α-H), 4.09 - 4.17 (m, 1 H, 3α-H), 4.49 + 4.56 (AB, 2 H, CH_2-21), 9.60 ($w_{1/2} \approx 12$ Hz, 1 H, 22-OH), $|J_{21,21'}|$ = 16.1 Hz.- ^{13}C NMR (50 MHz, APT, $CDCl_3$): δ = 16.21 (-) (C-18), 21.80 (+), 21.96 (+), 24.29 (-) (C-19), 25.84 (+), 26.87 (+), 28.34 (+), 30.14 (+), 32.64 (+), 33.78 (+), 35.95 (+), 36.12 (-) and 36.43 (-) (C-5, C-9), 41.02 (+) (C-12), 41.23 (-) (C-8), 47.66 (-) (C-17), 50.67 (+) (C-13), 67.36 (-) (C-3), 71.17 (+) (C-21), 87.93 (+) (C-14), 134.48 (+) and 137.99 (+) (C-20, C-22), 171.74 (+) (C-23).- MS: m/z (%) = 390 (0.7, $M^{+\cdot}$), 372 (40), 354 (15), 339 (4), 259 (9), 246 (56), 231 (8), 203 (100).- $C_{23}H_{34}O_5$ (390.51), calcd C 70.74, H 8.78, found C 70.64, H 8.85.

22-Benzoyloxy-3β-(*tert*-butyl-dimethyl-silyloxy)-14-trimethylsilyloxy-5β,14β-card-20(22)-enolide (15b)
15a was converted into the benzoate as described for **3b**. Stirring the reaction mixture at 20°C for 24 h and work-up, followed by FC (petrol-ethyl acetate 15:1) yielded **15b** (78 %).- IR ($CHCl_3$): 1771, 1746, 1250, 1111, 1048, 836 cm^{-1}.- ^1H NMR (300 MHz, $CDCl_3$): δ = 0.02 (s, 6 H, $Si^tBu(C\underline{H}_3)_2$), 0.18 (s, 9 H, $Si(CH_3)_3$), 0.88 (s, 9 H, $C(CH_3)_3$), 0.91 (s, 3 H, CH_3-19), 0.96 (s, 3 H, CH_3-18), 1.05 - 2.05 (om), 2.90 - 2.99 (m, 1 H, 17 α-H), 4.03 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 4.91 + 5.02 (AB, 2 H, CH_2-21), 7.50 (2 H, arom. H_m), 7.65 (1 H, arom. H_p), 8.14 (2 H, arom. H_o), $|J_{21,21'}|$ = 17.1 Hz, $J_{o,m} \approx J_{o,p} \approx J_{m,p} \approx 7.5$ Hz.- ^{13}C NMR (75 MHz, APT, $CDCl_3$): δ = -4.84 (-) ($Si^tBu(\underline{C}H_3)_2$), 3.08 (-) ($Si(CH_3)_3$), 17.55 (-) (C-18), 18.12 (+) ($\underline{C}(CH_3)_3$), 20.87 (+), 23.67 (+), 23.89 (-) (C-19), 25.42 (+), 25.87 (-) ($C(\underline{C}H_3)_3$), 26.76 (+), 28.58 (+), 29.85 (+), 34.02 (+), 34.48 (+), 35.79 (+), 35.89 (-) and 36.98 (-) (C-5, C-9), 40.88 (-) (C-8), 41.92 (+) (C-12), 47.79 (-) (C-17), 51.79 (+) (C-13), 67.20 (-) (C-3), 69.56 (+) (C-21), 91.40 (+) (C-14), 128.09 (+) (arom. C_i), 128.72 (-) and 130.47 (-) (arom. C_o, C_m), 134.12 (-) (arom. C_p), 135.00 (+) and 154.10 (+) (C-20, C-22), 163.07 (+) and 167.67 (+) (C-23, $\underline{C}$OPh).- MS: m/z (%) = 680 (4, $M^{+\cdot}$), 623 (23), 303 (20), 157 (48), 105 (100), 75 (28), 73 (22).- $C_{39}H_{60}O_6Si_2$ (681.07), HRMS: calcd for $C_{35}H_{51}O_6Si_2$ $[M - {}^tBu]^+$ 623.3224, found 623.3235.

22-Benzoyloxy-3β,14-dihydroxy-5β,14β-card-20(22)-enolide (15e)
The protecting groups of **15b** were removed as described for **3c**. Stirring the reaction mixture for 76 h and work-up, followed by FC (petrol-ethyl acetate 1:1) furnished **15e** (72 %).- M.p. 227 - 229 °C (CH_2Cl_2-petrol).- IR ($CHCl_3$): 3625, 1774, 1753, 1675, 1450, 1257, 1118 cm^{-1}.- ^1H NMR (300 MHz, $CDCl_3$): δ = 0.95 (s, 3 H, CH_3), 0.97 (s, 3 H, CH_3), 1.13 - 1.97 (om), 2.03 - 2.19 (om, 2 H), 2.93 - 3.02 (m, 1 H, 17α-H), 4.12 ($w_{1/2} \approx 7$ Hz, 1 H, 3α-H), 4.95 + 5.21 (AB, 2 H, CH_2-21), 7.46 - 7.56 (2 H, arom. H_m), 7.61 - 7.69 (1 H, arom. H_p), 8.11 - 8.18 (2 H, arom. H_o), $|J_{21,21'}|$ = 17.6 Hz.- ^{13}C NMR (75 MHz, APT, $CDCl_3$): δ = 15.37 (-) (C-18), 21.14 (+), 21.39 (+), 23.73 (-) (C-19), 25.20 (+), 26.48 (+), 27.91 (+), 29.65 (+), 33.24 (+), 33.34 (+), 35.42

(+), 35.49 (-) and 35.99 (-) (C-5, C-9), 39.90 (+) (C-12), 41.72 (-) (C-8), 47.15 (-) (C-17), 49.96 (+) (C-13), 66.82 (-) (C-3), 69.76 (+) (C-21), 85.39 (+) (C-14), 128.10 (+) (arom. C_i), 128.70 (-) and 130.49 (-) (arom. C_o, C_m), 134.12 (-) (arom. C_p), 135.14 (+) and 154.55 (+) (C-20, C-22), 163.25 (+) and 167.67 (+) (C-23, COPh).- MS: m/z (%) = 476 (0.7), 372 (9), 354 (3), 339 (1), 246 (5), 203 (5), 122 (6), 105 (100), 77 (21).- FAB MS: m/z = 517.5 ([M+Na]$^+$), 495.5 (M+H]$^+$).- $C_{30}H_{38}O_6$ (494.62), calcd C 72.85, H 7.74, found C 72.97, H 7.73.

22-Acetoxy-3β-(*tert*-butyl-dimethyl-silyloxy)-14-trimethylsilyloxy-5β,14β-card-20(22)-enolide (15c)
DCC (23 mg, 112 μmol) was treated with a solution of acetic acid (100 μl of 50 μl acetic acid in dry CH_2Cl_2 (1000μl), 87 μmol) at 0°C. A solution of **15a** (50 mg, 87 μmol) and DMAP (1 mg, 12 μmol) in CH_2Cl_2 (0.85 ml) was added (formation of a white suspension). The mixture was stirred for 1 h at 0°C. Work-up was performed as described for the esterification of **3a** (→ **3b**). Subsequent FC (petrol-ethyl acetate 15:1) provided **15c** (44 mg, 82 %).- IR ($CHCl_3$): 1769, 1672, 1448, 1250, 1193, 1053, 838 cm^{-1}.- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.02 (s, 6 H, Si^{t}Bu(CH_3)$_2$), 0.16 (s, 9 H, Si(CH_3)$_3$), 0.87 (s, 3 H, CH_3-18$^\#$), 0.89 (s, 9 H, C(CH_3)$_3$), 0.92 (s, 3 H, CH_3-19$^\#$), 1.05 - 2.00 (om), 2.28 (s, 3 H, $COCH_3$), 2.79 - 2.89 (m, 1 H, 17α-H), 4.05 ($w_{1/2}$ ≈ 7 Hz, 1 H, 3α-H), 4.82 + 4.94 (AB, 2 H, CH_2-21), | $J_{21,21'}$ | = 17.1 Hz.- ^{13}C NMR (75 MHz, APT, $CDCl_3$): δ = -4.84 (-) and -4.80 (-) (Si^{t}Bu(CH_3)$_2$), 3.04 (-) (Si(CH_3)$_3$), 17.37 (-) (C-18), 18.12 (+) (C(CH_3)$_3$), 20.34 (-) ($COCH_3$), 20.85 (+), 23.70 (+), 23.88 (-) (C-19), 25.48 (+), 25.86 (-) (C(CH_3)$_3$), 26.75 (+), 28.61 (+), 29.87 (+), 33.99 (+), 34.49 (+), 35.80 (+), 35.89 (-) and 37.01 (-) (C-5, C-9), 40.89 (-) (C-8), 42.04 (+) (C-12), 47.74 (-) (C-17), 51.68 (+) (C-13), 67.21 (-) (C-3), 69.52 (+) (C-21), 91.33 (+) (C-14), 134.80 (+) and 153.71 (+) (C-20, C-22), 167.22 (+) and 167.71 (+) (C-23, $COCH_3$).- MS: m/z (%) = 618 (5, M$^{+\cdot}$), 561 (36), 471 (4), 450 (3), 411 (3), 267 (7), 241 (23), 157 (100), 75 (78), 73 (61), 43 (59).- $C_{34}H_{58}O_6Si_2$ (619.00), HRMS: calcd for $C_{30}H_{49}O_6Si_2$ [M - ^{t}Bu]$^+$ 561.3068, found 561.3056.
$^\#$ These assignments may have to be reversed.

22-Acetoxy-3β,14-dihydroxy-5β,14β-card-20(22)-enolide (15f)
The protecting groups of **15c** were removed as described for **3c**. FC (CH_2Cl_2-ethanol 50:1) yielded **15f** (70 %).- M.p. 183 - 186 °C (CH_2Cl_2-petrol).- IR ($CHCl_3$): 3625, 1768, 1674, 1450, 1192, 1111, 1032 cm^{-1}.- ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.89 (s, 3 H, CH_3-18), 0.96 (s, 3 H, CH_3-19), 1.14 - 1.97 (om), 2.01 - 2.20 (om, 2 H), 2.29 (s, 3 H, $COCH_3$), 2.84 - 2.93 (m, 1 H, 17α-H), 4.13 ($w_{1/2}$ ≈ 7 Hz, 1 H, 3α-H), 4.87 + 5.12 (AB, 2 H, CH_2-21), | $J_{21,21'}$ | = 17.6 Hz.- ^{13}C NMR (75 MHz, APT, $CDCl_3$): δ = 15.20 (-) (C-18), 20.35 (-) ($COCH_3$), 21.13 (+), 21.39 (+), 23.73 (-) (C-19), 25.23 (+), 26.48 (+), 27.93 (+), 29.66 (+), 33.20 (+), 33.35 (+), 35.42 (+), 35.51 (-) and 35.99 (-) (C-5, C-9), 39.91 (+) (C-12), 41.70 (-) (C-8), 47.09 (-) (C-17), 49.86 (+) (C-13), 66.82 (-) (C-3), 69.74 (+) (C-21), 85.34 (+) (C-14), 134.91 (+) and 154.17 (+) (C-20, C-22), 167.25 (+) and 167.74 (+) (C-23, $COCH_3$).- MS: m/z (%) = 432 (0.1, M$^{+\cdot}$), 414 (4), 372 (29), 354 (4), 246 (40), 231 (6), 203 (63), 43 (100).- $C_{25}H_{36}O_6$ (432.55), calcd C 69.42, H 8.39, found C 69.54, H 8.23.

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Dedicated to Professor Koji Nakanishi

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