Cyclobutanone Approach to the Synthesis of Cardenolides[‡]

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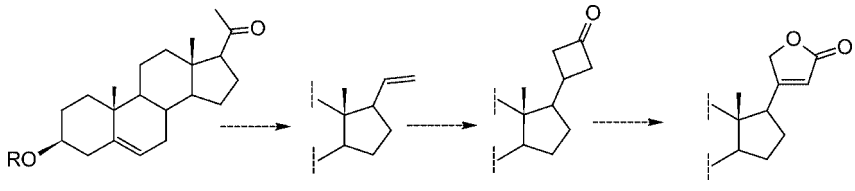
17β-(3′-Oxocyclobutyl)androstane, prepared by the thermal [2 + 2] cycloaddition of dichloroketene to 3β-acetoxypregna-5,20-diene, is the key intermediate in the new, efficient synthesis of steroids bearing the 17β-butenolide fragment that characterizes cardenolides. The six-step synthesis of 3β-*tert*-butyldimethylsilyloxy-14α-carda-5,20(22)-dienolide was achieved with a total yield of 32%.

Introduction

Cardenolides[1] are plant steroids that occur as glycosides and possess powerful cardiotonic activity. They are considered to be "the most ingested drugs in medicine".[1a] Since the pioneering studies by Ruzicka et al.[2] and the first synthesis of digitoxigenin[3] in 1962, enormous efforts have been directed towards the synthesis of cardenolides.[1,4] Despite the long history of research in this area, the interest in cardenolides continues. Recently reported work on cardenolides includes the total synthesis of digitoxigenin[1,5] and ouabain,[6] biosynthesis[7] and the search for new, less toxic digitalis-like compounds with better pharmacological properties for therapeutic use.[8] It has recently been reported that cardenolides have a growth inhibitory effect on cancer cell lines[9] and show antiproliferative[10] and cytotoxic[11] activity. New methods for the introduction of the 17β-butenolide moiety[4] and synthetic approaches to complex cardenolides have also been reported.[12]

The 17β-butenolide moiety is one of the features of cardenolides that is crucial for their biological activity. The synthesis of cardenolides with the 14α configuration from commercially available substrates involves the construction of the β-oriented heterocyclic substituent at the C-17 position and inversion of the configuration accompanied by functionalization of the C-14 atom. In most cases, 21-hydroxy-20-ketones[4,13] with a 17β-oriented side-chain or steroidal 17-ketones[4,14] were selected as substrates. Recently, 17-oxoandrostanes, readily available by chemical or biological degradation of sitosterol, have also become promising starting materials.[4,15,16] Structurally similar, planar steroid lactones with Δ^4 or Δ^5 unsaturation and/or a 5α,14α configuration, which lack the 14-OH group, are also of considerable biological importance, for example, spironolactone, canrenone and others.[1b]

This work is a continuation of our interest in steroidal cyclobutanones.[17] Since the transformation of pregnane derivatives into 17β-butenolide steroids requires a two-carbon side-chain elongation, the [2 + 2] cycloaddition reaction of the appropriate olefinic substrate with a reactive ketene to give cyclobutanone appeared to be an attractive approach to the four-carbon side-chain moiety that is characteristic of cardenolides. Further oxidation of the cyclobutanone to a lactone followed by dehydrogenation should furnish the target compound with the side-chain fragment characteristic of cardenolides. Cycloaddition of dichloroketene to the pregn-20-ene derivative appeared to offer a promising entry to a suitably substituted cyclobutanone precursor. The main steps in the planned synthetic pathway to steroid butenolides are shown in Scheme 1.



Scheme 1

[‡] Steroidal Cyclobutanones, 6. Part 5: Ref.[17a]

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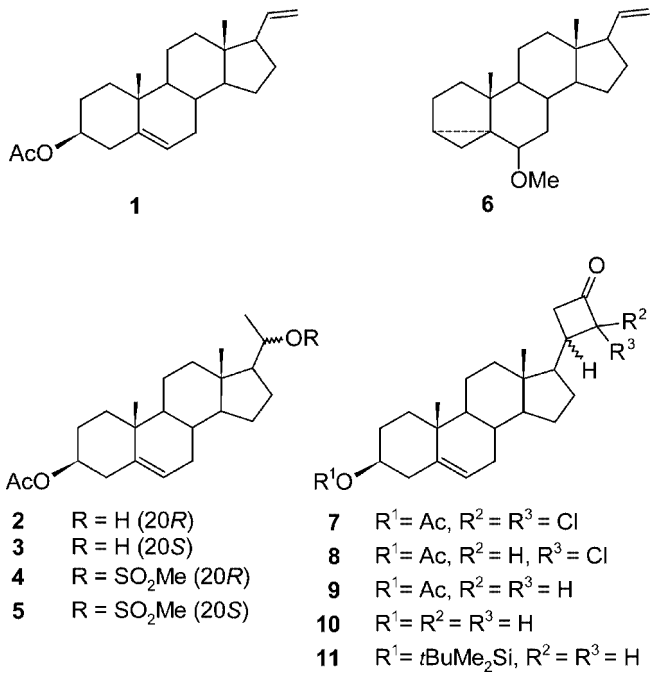
Results and Discussion

Starting olefin **1**, 3β-acetoxypregna-5,20-diene,[18] was obtained from the commercially available 3β-acetoxypregn-5-en-20-one following the procedure reported earlier.[19] We found, however, that reduction of this ketone with sodium borohydride in methanol gave a mixture of the alcohols (20*R*)-**2**[19] and (20*S*)-**3**[20] in a 9:1 ratio, as estimated by the integration of the ^{1}H NMR signals at δ = 0.76 and 0.67 ppm, respectively, arising from the 18-CH_3 group. The mixture of **2** and **3**,which gave two distinct spots on TLC plates, was used without separation in the next step. Mesylation in pyridine/dichloromethane gave the productasa mixture of chromatographically indistinguishable isomers **4** [δ_H = 0.79 (singlet due to the 18-CH_3 group) and 3.00 (singlet due to the CH_3SO_2 group)][19] and **5** (δ_H = 0.72 and 2.98). The mesylates were eliminated by treatment with potassium *tert*-butoxide in refluxing toluene to give the diene **1**.[19] The 6β-methoxy-3α,5-cyclo-5α-pregn-20-ene **6**,[21] with a protected Δ^5 double bond, was then used as the substrate for the preparation of the cyclobutanone by reaction with a ketene. Olefin **6** was prepared from 3β-hydroxy-pregna-5,20-diene following a procedure previously described in the literature.[21] However, the reaction of **6** with dichloroketene[22] generated from trichloroacetyl chloride and zinc failed. Likewise, when olefin **1** was treated with dichloroketene generated by the reaction of dichloroacetyl chloride with triethylamine[23] in hexane or chloroform, the expected cycloaddition product did not form either.

Regioselective [2 + 2] cycloaddition of **1** and dichloroketene generated in situ from trichloroacetyl chloride and activated zinc in diethyl ether gave the best results under sonification conditions. In experiments performed without sonification the yields of the cycloaddition product were appreciably lower.[24] After chromatographic purification the reaction afforded dichlorocyclobutanone **7** in 58% isolated yield. We have previously found that purification of the crude reaction product on a SiO_2 column is usually accompanied by slow decomposition of the dichlorcyclobutanones.[17b] The dichlorocyclobutanone **7** had spectral characteristics (IR, ^{1}H and ^{13}C NMR, MS) in accordance with the proposed structure. Compound **7** was formed as a mixture of C-20 epimers in an approx. 2:1 ratio, as evidenced by the ^{1}H NMR spectrum (see Expt. Sect.). It is supposed that the major diastereoisomer of **7** has a (20*R*) configuration for the following reasons: a) the approach of dichloroketene to the Δ^{20} double bond should occur from the rear side of the steroid skeleton;[8] b) semiempirical calculations (AM1, CAChe, Fujitsu) show that the s-*trans* rotamer of olefin **1** is lower in energy than the s-*cis* rotamer by approx. 0.18 kcal/mol. Consequently, the major dichlorocyclobutanone (20*R*)-**7** should form via a transition state that results from the approach of dichloroketene to the olefin **1** in *s-trans* conformation from the side opposite to the 18-CH_3 group. The endocyclic double bond in ring B of **1** was inert towards dichloroketene under the reaction conditions. Mild reduction[25] of the dichlorocyclobutanone **7** with zinc in acetic acid at room temperature afforded monochlorocyclobutanone **8** as a mixture of four diastereoisomers in 60% yield.

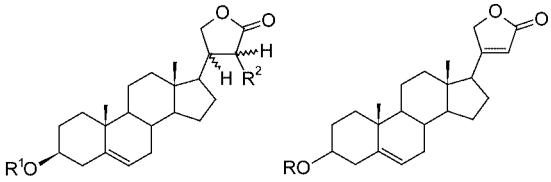


2 R = H (20*R*)
3 R = H (20*S*)
4 R = SO_2Me (20*R*)
5 R = SO_2Me (20*S*)

7 R^1 = Ac, R^2 = R^3 = Cl
8 R^1 = Ac, R^2 = H, R^3 = Cl
9 R^1 = Ac, R^2 = R^3 = H
10 R^1 = R^2 = R^3 = H
11 R^1 = *t*$BuMe_2Si$, R^2 = R^3 = H

The monodehalogenation step was evidenced by the shift in the IR absorption of the four-membered ring carbonyl moiety from $\tilde{\nu}_{max}$ = 1805 cm^{-1} in dichlorocyclobutanone **7** to $\tilde{\nu}_{max}$ = 1793 cm^{-1} in **8**. In the ^{1}H NMR spectrum of **8** a new signal at δ = 4.53–4.48 ppm, ascribed to the proton in the CHCl group, was observed. The full dehalogenation of dichlorocyclobutanone **7** could be effected with activated[22] zinc in boiling acetic acid. The formation of cyclobutanone **9** was evidenced by the IR absorption of the carbonyl group at $\tilde{\nu}_{max}$ = 1775 cm^{-1}. The yield of the two-step synthesis of cyclobutanone **9** was about 50%. However, when the crude cycloaddition product **7** was immediately reduced under the above conditions, the two-step synthesis gave cyclobutanone **9** in a much higher yield of 82%. The Baeyer–Villiger oxidation of cyclobutanone **9** with hydrogen peroxide under basic conditions was accompanied by hydrolysis of the 3-acetate group and afforded 3-hydroxy-17-lactone **12** in 64% yield after chromatography. This unsatisfactory yield was attributed to the oxidation of the A,B ring fragment of the steroid as polar impurities were observed on TLC plates. The hydroxy lactone **12**, an amorphous mixture of C-20 epimers, exhibited an IR absorption at $\tilde{\nu}_{max}$ = 1772 cm^{-1}. The ^{1}H NMR spectrum of **12** showed signals arising from protons of the 21-CH_2 group at δ = 4.47, 4.38, 3.94 and 3.83 ppm and a two-proton multiplet due to the 22-CH_2 group at δ = 2.65–2.44 ppm. The 3-OH group of **12** was acetylated with acetic anhydride/pyridine to give the crystalline acetate **13**. Since the yield of lactone **12** prepared from **9** could not be improved by changing the reaction conditions, at this stage of the synthesis we used a silyl group to protect the 3-OH group before the oxidation step. Thus, acetate **8** was hydrolyzed under mild basic conditions by using potassium carbonate in methanol to afford a quanti-

tative yield of alcohol **10**. This was silylated by reaction with *tert*-butyldimethylsilyl chloride and imidazole in DMF to give silyl derivative **11** in 92% yield. The Baeyer–Villiger oxidation of ketone **11** with 30% H_2O_2in a basic methanol/tetrahydrofuran solution resulted in the formation of lactone **14** in 87% yield after short-column chromatography. Lactone **14** was formed as an approx. 1:1 mixture of C-20 epimers, as estimated from the integration of the low-field signals ascribed to the protons of the 21-CH_2 group at δ = 4.47, 4.37 (isomer A) and 3.93, 3.83 ppm (isomer B) in the ^{1}H NMR spectrum. The attempted dehydrogenation of lactone **13** with benzeneseleninic anhydride[26] in boiling chlorobenzene failed. This result confirmed the previously reported inertness of γ-lactones under direct dehydrogenation conditions.[26] Dehydrogenation of lactone **14** was achieved, however, by using the phenylselenylation–oxidation procedure.[27] The α-phenylselenyl lactone **15** was isolated in 75% yield from the reaction of lactone **14** with lithium diethylamide and phenylselenylchloride in tetrahydrofuran at –70 °C. As expected, the α-phenylselenyl lactone **15** was formed as a mixture of four diastereoisomers. This was evidenced by its ^{1}H NMR spectrum which was rather complex (see Expt. Sect.). However, four signals ascribed to the 18-CH_3 protons at δ = 0.73, 0.71, 0.65, and 0.62 ppm and to the 19-CH_3 group at δ = 1.02, 1.01, 1.00 and 0.98 ppm could be distinguished in the spectrum.



12 $R^1 = R^2 = H$
13 $R^1 = Ac$, $R^2 = H$
14 $R^1 = tBuMe_2Si$, $R^2 = H$
15 $R^1 = tBuMe_2Si$, $R^2 = C_6H_5Se$

16 $R = tBuMe_2Si$
17 $R = H$

The final step in the synthesis was the oxidation of compound **15** with 30% H_2O_2 in tetrahydrofuran/acetic acid, which gave a crystalline product in 67% yield. The IR absorptions[13a,13b] at $\tilde{\nu}_{max}$ = 1785, 1750 and 1630 cm^{-1} and the ^{1}H NMR spectral properties of this product are in agreement with the cardenolide structure **16**. A low-field signal at δ = 5.85 ppm arising from the 22-H atom and two signals of characteristic multiplicity at δ = 4.83 and 4.69 ppm, ascribed to the protons at C-21, were observed. Full assignment of the signals in the ^{13}C NMR spectra of cardenolide **16** and of the three 17β-cyclobutyl-androstane derivatives **9**–**11** is shown in Table 1. Deprotection of the TBDMS ether **16** afforded the known 3β-hydroxycardenolide **17**.[13a,13b] The ^{1}H NMR spectrum of compound **17** was in perfect agreement with the literature data.[13a,13b]

Conclusions

The sequence of reactions that leads to the butenolide fragment of cardenolides from readily available 3β-acetoxypregna-5,20-diene is relatively simple and efficient. An important step in the synthesis is the cycloaddition of dichloroketene to the unsaturated Δ^{20} steroid. This is another example of the synthetic utility of cyclobutanones in the synthesis of biologically significant compounds.[28] The six-step synthesis of **16** from **1** was achieved with a total yield of 32%. Since the transformation of 14α-card-20(22)-enolide to Δ^{14} olefin and 14β-hydroxy derivatives has already been reported,[29] this method may be adapted to the synthesis of variously substituted or modified cardenolides with 14α and 14βconfigurations. It is also expected that this side-chain elongation procedure can be applied to the 5β-steroid series as well.

Table 1. ^{13}C NMR chemical shifts of the cyclobutanones **9**–**11** and cardenolide **16**

Carbon atom	δ [ppm]			
	9	**10**	**11**	**16**
C-1	37.1	37.3	37.5	37.2
C-2	27.8	31.6	32.2	31.6
C-3	73.8	71.6	72.6	72.4
C-4	38.1	42.3	42.9	42.6
C-5	139.6	140.7	141.6	141.7
C-6	122.3	121.4	121.0	120.7
C-7	31.9	31.9	32.0	31.9
C-8	31.9	31.9	32.0	32.0
C-9	50.1	50.1	50.3	50.0
C-10	36.7	36.5	36.7	36.5
C-11	20.8	20.8	20.9	20.2
C-12	38.7	38.8	38.9	37.9
C-13	42.7	42.6	42.7	44.2
C-14	56.1	56.0	56.2	56.5
C-15	24.4	24.4	24.5	24.3
C-16	27.0	27.0	27.1	25.8
C-17	57.1	57.1	57.2	50.7
C-18	13.1	13.1	13.2	12.9
C-19	19.4	19.4	19.5	19.3
C-20	25.3	25.2	25.3	171.4
C-21	51.7	51.7	51.8	73.4
C-22	52.7	52.6	52.8	116.0
C-23	208.0	208.4	208.4	174.2
$Si(CH_3)_2$			–4.4	–4.7
$C(CH_3)_3$			18.4	18.1
$C(CH_3)_3$			26.1	25.8
CH_3CO_2	170.3			
CH_3CO_2	21.5			

Experimental Section

General Remarks: M.p. values were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were determined with a FT-IR Bruker FS 113V spectrometer for solutions inchloroform. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini 300 VT spectrometer (300 and 75.5 MHz, respectively) operating in the Fourier transform mode using solutions in deuteriochloroform. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane as the internal standard. The DEPT technique was used to assign the multiplicity of the carbon signals in the ^{13}C NMR spectra. The additivity rules and comparison with data reported for compounds of similar structure were helpful for signal assignment. Electron-impact mass spectra were recorded with an AMD 402 spectrometer using ionization energy of 70 eV. Optical

rotations were recorded with a Perkin–Elmer 243 B polarimeter. Solvents were dried and distilled according to the standard procedures. Reaction progress and the purity of compounds were monitored by TLC using precoated aluminium-backed silica plates (E. Merck, no. 5554). Silica gel 60 (Merck 70–230 mesh, no. 7734) was used for flash chromatography.

6β-Methoxy-3α,5-cyclo-5α-pregn-20-ene (6): Compound **6** was prepared according to the literature procedure.[21] M.p. 65–70 °C (Et_2O). ^1H NMR: δ = 5.72–5.83 (m, 1 H, 20-H), 4.99 (d, J = 1.1 Hz, 1 H, 21-H), 4.93–4.96 (m, 1 H, 21-H), 3.33 (s, 3 H, OCH_3), 2.78 (t, J = 3.0 Hz, 1 H, 6-H), 1.031 (s, 3 H, 19-CH_3), 0.648 (s, 3 H, 18-CH_3), 0.42–0.46 (m, 1 H, 4-H) ppm.

3β-Acetoxy-21,21-dichloro-21,23-cyclo-24-nor-20ξ-chol-5-en-23-one (7): A solution of trichloroacetyl chloride (213 mg, 1.16 mmol) in anhydrous diethyl ether (7.5 mL) was added dropwise over 2 h to a suspension of 3β-acetoxypregna-5,20-diene (**1**)[19] (200 mg, 0.58 mmol) and zinc dust (114 mg, 1.74 mmol) in anhydrous diethyl ether (7.5 mL) under argon. During the addition of trichloroacetyl chloride, the reaction mixture was irradiated in the water bath of a sonicator at a bath temperature of 25–30 °C. The sonication was continued for an additional 2 h after the addition of trichloroacetyl chloride was completed. Then the reaction mixture was kept under argon at room temperature for 18 h. Thereafter the mixture was filtered, the solution diluted with benzene and then washed with water and 5% aqueous sodium hydrogen carbonate. The organic layer was dried with magnesium sulfate and the solvent was removed in vacuo. The residue was purified by chromatography on silica gel with benzene as eluent to give compound **7** (156 mg, 58%, slowly decomposed[17b] on silica gel) as an oil; two diastereoisomers were formed in a 2:1 ratio, as estimated from the integration of the signals at δ = 0.78 and 0.72 ppm in the ^1H NMR spectrum of the crude product. IR: $\tilde{\nu}$ = 1805, 1725, 1250, 1025, 810, 680 cm^{-1}. ^1H NMR: δ = 5.38 (br. d, J = 4.9 Hz, 1 H, 6-H), 4.60 (m, 1 H, 3α-H), 3.22–3.03 (m, 2 H, CH_2CO), 2.03 (s, 3 H, CH_3CO_2), 1.03 and 1.02 (two s, 3 H, 19-CH_3), 0.78 and 0.72 (two s, 3 H, 18-CH_3) ppm. ^{13}C NMR: δ = 192.3 and 191.8 (C=O), 170.3 (CH_3COO), 139.7 and 139.5 (C-5), 122.2 (C-6), 90.1 and 88.2 (CCl_2), 73.86 and 73.80 (C-3) ppm. MS: m/z (%) = 410 (2) [M^+ – ketene], 394 (98), 392 (100), 352 (70), 350 (96), 213 (54), 145 (53), 44 (93). $C_{25}H_{34}Cl_2O_3$ (453.45): calcd. C 66.22, H 7.56; found C 66.17, H 7.56.

3β-Acetoxy-21-chloro-21,23-cyclo-24-nor-20ξ,21ξ-chol-5-en-23-one (8): Zinc dust (750 mg, 11.5 mmol) was added in portions to the crude product obtained from the reaction of compound **1** (1.000 g, 2.92 mmol) with dichloroketene stirred in acetic acid (50 mL) at room temperature. The mixture was stirred at room temperature for 1 h. Then the reaction mixture was filtered, and the filtrate was poured into water. After workup, the crude product was purified by chromatography on silica gel (30 g) with benzene as the eluent to give compound **8** (730 mg, total yield of 60% for the two steps) as a mixture of four diastereoisomers. M.p. 135–145 °C. IR: $\tilde{\nu}$ = 1793, 1725, 1254 cm^{-1}. ^1H NMR: δ = 5.38 (br. d, J = 4.9 Hz, 1 H, 6-H), 4.66–4.54 (m, 1 H, 3α-H), 4.53–4.48 (m, 1 H, CHCl), 3.17–3.06 (m, 1 H, CH_2CO), 2.82–2.73 (m, 1 H, CH_2CO), 2.04 (s, 3 H, CH_3CO_2), 1.04 and 1.03 (two s, 3 H, 19-CH_3), 0.79 and 0.72 (two s, 3 H, 18-CH_3) ppm. ^{13}C NMR: δ = 199.06 and 198.89 (C=O), 170.27(CH_3CO_2), 139.56 and 139.47 (C-5), 122.20 (C-6), 73.76 (C-3), 66.17 and 65.08 (CHCl) ppm. MS: m/z (%) = 388 (4), 336 (100), 304 (16), 296(43). $C_{25}H_{35}ClO_3$ (419.00): calcd. C 71.66, H 8.42; found C 71.47, H 8.61.

3β-Acetoxy-24-nor-21,23-cyclochol-5-en-23-one (9): The crude product obtained from the reaction of 3β-acetoxypregna-5,20-diene **1** (2.000 g, 5.84 mmol) with dichloroketene was dissolved in acetic acid (100 mL) and activated zinc dust (2000 mg, 30 mmol) was added. The mixture was stirred at 100 °C for 1 h. Then an additional portion of activated zinc (1000 mg, 15 mmol) was added and stirring was continued at 100 °C for 1 h. The mixture was then filtered and the filtrate poured into water. The usual workup gave a crude product which was purified by column chromatography on silica gel with benzene/ethyl acetate (50:1) as eluent to give compound **9** (1.847 g, total yield of 82% for the two steps). M.p. 130–132 °C (methanol). $[\alpha]_D$ = –60.3 (c = 1, $CHCl_3$). IR: $\tilde{\nu}$ = 1775, 1725, 1250, 1030, 680 cm^{-1}. ^1H NMR: δ = 5.38 (br. d, J = 5.1 Hz, 1 H, 6-H), 4.60 (m, 1 H, 3α-H), 3.14–3.00 (m, 2 H, CH_2CO), 2.87–2.70 (m, 2 H, H_2CO), 2.03 (s, 3 H, CH_3CO_2), 1.03 (s, 3 H, 19-CH_3), 0.70 (s, 3 H, 18-CH_3) ppm. MS: m/z (%) = 384 (1) [M^+], 324 (100), 309 (11), 282 (14), 145 (12), 105 (15), 93 (16). $C_{25}H_{36}O_3$ (384.56): calcd. C 78.08, H 9.44; found C 77.87, H 9.55.

3β-Hydroxy-24-nor-21,23-cyclochol-5-en-23-one (10): A saturated methanolic solution of potassium carbonate (40 mL) was added to a solution of compound **9** (1.340 g, 3.48 mmol) in methanol (130 mL). The reaction mixture was stirred at room temperature for 4 h. The usual workup gave chromatographically pure compound **10** (1.174 g, 98%). M.p. 175–176 °C (methanol). $[\alpha]_D$ = –70.8 (c = 0.25, $CHCl_3$). IR: $\tilde{\nu}$ = 3610, 3470, 1775, 1230, 1040 cm^{-1}. ^1H NMR: δ = 5.35 (br. d, J = 5.2 Hz, 1 H, 6-H), 3.52 (m, 1 H, 3α-H), 3.14–3.00 (m, 2 H, CH_2CO), 2.87–2.70 (m, 2 H, CH_2CO), 1.02 (s, 3 H, 19-CH_3), 0.70 (s, 3 H, 18-CH_3) ppm. MS: m/z (%) = 342 (100) [M^+], 300 (47), 267 (57), 231 (42), 213 (42), 159 (39), 145 (60), 133 (42), 105 (80), 91 (54), 41 (58). $C_{23}H_{34}O_2$ (342.52): calcd. C 80.64, H 10.01; found C 80.42, H 10.09.

3-(*tert*-Butyldimethylsilyloxy)-24-nor-21,23-cyclochol-5-en-23-one (11): A solution of compound **10** (1.000 g, 2.92 mmol), *tert*-butyldimethylchlorosilane (1.100 g, 7.30 mmol) and imidazole (1000 mg, 14.6 mmol) in anhydrous dimethylformamide (10 mL) was stirred at room temperature for 1 h. The solution was then poured into water and extracted with diethyl ether. The organic layer was washed with water, dried with magnesium sulfate and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel with benzene as eluent to yield compound **11** (1.222 g, 92%). M.p. 149–150 °C (methanol/acetone). $[\alpha]_D$ = –43 (c = 1, $CHCl_3$). IR: $\tilde{\nu}$ = 1775, 1250, 1075, 885, 870, 835 cm^{-1}. ^1H NMR: δ = 5.32 (br. d, J = 5.2 Hz, 1 H, 6-H), 3.48 (m, 1 H, 3α-H), 3.14–3.00 (m, 2 H, CH_2CO), 2.87–2.70 (m, 2 H, CH_2CO), 1.01 (s, 3 H, 19-CH_3), 0.89 [s, 9 H, $C(CH_3)_3$], 0.70 (s, 3 H, 18-CH_3), 0.06 [s, 6 H, $Si(CH_3)_2$] ppm. MS: m/z (%) = 456 (1) [M^+], 399 (100), 357 (10), 323 (16), 171 (17), 159 (25), 145 (40), 119 (25), 105 (32), 93 (35), 75 (99). $C_{29}H_{48}O_2Si$ (456.78): calcd. C 76.25, H 10.59; found C 76.45, H 10.79.

Crystallization of **11** from MeOH gave the dimethyl acetal of **11**. M.p.161–164 °C. IR: no carbonyl absorption. ^1H NMR: δ = 5.32 (br. d, J= 5.2 Hz, 1 H, 6-H), 3.47 (m, 1 H, 3α-H), 3.16 (s, 3 H, OCH_3), 3.12 (s, 3 H, OCH_3), 0.99 (s, 3 H, 19-CH_3), 0.89 [s, 9 H, $C(CH_3)_3$], 0.61 (s, 3 H, 18-CH_3), 0.06 [s, 6 H, $Si(CH_3)_2$] ppm.

3β-Hydroxy-14α,20ξ-card-5-enolide (12): A solution of NaOH in CH_3OH (0.5 M, 5 mL) and H_2O_2 (30%, 1.5 mL) was added to a solution of cyclobutanone **9** (645 mg, 1.68 mmol) in CH_3OH/THF (1:1, 50 mL) and the mixture was stirred overnight at room temp. The solution was acidified with HCl (0.1 N), benzene and H_2O were added and the organic layer was washed with brine, dried with $MgSO_4$, filtered and the solvent evaporated to give the crude product (726 mg). This was purified by chromatography on a silica-gel column with benzene/hexane mixtures as eluent to give pure lactone **12** (382 mg, 64%). M.p. 245–247 °C (MeOH). IR: $\tilde{\nu}$ = 3606, 3015, 2942, 1772 (C=O), 1380, 1088 cm^{-1}. ^1H NMR: δ = 5.35 (br.

d, J = 5.2 Hz, 1 H, 6-H), 4.47 (t, J = 8.5 Hz, 1 H, 21-H, isomer A), 4.38 (dd, J = 8.5, 7.9 Hz, 1 H, 21-H, isomer B), 3.94 (dd, J= 8.7, 9.6 Hz, 1 H, 21-H, isomer A), 3.83 (dd, J = 9.8, 8.7 Hz, 1 H, 21-H, isomer B), 3.58–3.48 (m, 1 H, 3α-H), 2.65–2.44 (m, 2 H, 22-H), 1.02 (s, 3 H, 19-CH_3), 0.59 (s, 3 H, 18-CH_3) ppm. MS: *m*/*z* (%) = 358 [M^+] (12), 339 (100), 324 (29). HRMS: calcd. for $C_{23}H_{34}O_3$: 358.25079; found 358.25146.

3β-Acetoxy-14α,20ξ-card-5-enolide (13): Acetic anhydride (1 mL) was added to a solution of compound **12** (129 mg, 6 mmol) in pyridine (1 mL) and the mixture was stirred for 2 h at room temp. It was then poured into ice/H_2O and extracted with benzene. The organic layer was washed with HCl (0.1 N), $NaHCO_3$ (0.1 N) and brine, dried with $MgSO_4$ and the solvent evaporated to give **13** (115 mg, 80% yield) as a mixture of C-20 epimers. M.p. 197–202 °C (Me_2CO). IR: ṽ = 3016, 2947, 1773 (lactone C=O), 1725 (acetate), 1375, 1366, 1254, 1087, 1028, 626 cm^{-1}. ^1H NMR: δ = 5.38 (br. d, J = 4.9 Hz, 1 H, 6-H), 4.55–4.66 (m, 1 H, 3α-H), 4.47 (dd, J = 8,2, 8.5 Hz, 1 H, 21-H, isomer A), 4.38 (dd, J = 7.9, 8.2 Hz, 1 H, 21-H, isomer B), 3.93 (dd, J= 9.0, 9.6 Hz, 1 H, 21-H, isomer A), 3.84 (dd, J = 9.8, 8.7 Hz, 1 H, 21-H, isomer B), 2.63–2.47 (m, 2 H, 22-H), 2.01 (s, 3 H, CH_3CO), 1.02 (s, 3 H, 19-CH_3), 0.707 (s, 3 H, 18-CH_3, isomer A), 0.69 (s, 3 H, 18-CH_3, isomer B) ppm. MS: *m*/*z* (%) = 340 [M^+ – AcOH] (100). HRMS: calcd. for $C_{23}H_{32}O_2$ [M^+ – AcOH]: 340.24023; found 340.24114.

3β-(*tert*-Butyldimethylsilyloxy)-14α,20ξ-card-5-enolide (14): A 0.5 M methanolic solution of sodium hydroxide (2.5 mL, 1.25 mmol) and a 30% solution of hydrogen peroxide (0.5 mL, 4 mmol) were added to a solution of compound **11** (500 mg, 1.1 mmol) in methanol (50 mL) and tetrahydrofuran (50 mL). The reaction mixture was stirred at room temperature for 10 min, then poured into water and extracted with diethyl ether. The organic layer was washed with water and dried with magnesium sulfate. After evaporation of the solvent, the residue dissolved in benzene was passed through silica gel to give compound **14** (448 mg, 87%) as a mixture of two diastereoisomers in a 1:1 ratio, as estimated from the integration of the signals at δ = 4.47, 4.37 and 3.93, 3.83 ppm in the ^1H NMR spectrum. M.p. 201–205 °C. IR: ṽ = 1775, 1250, 1175, 1085, 1025, 885, 870, 835 cm^{-1}. ^1H NMR: δ = 5.33 (br. d, J = 5.2 Hz, 1 H, 6-H), 4.47 (dd, J = 8.2, 8.5 Hz, 1 H, 21-H, isomer A), 4.37 (dd, J = 8.2, 7.9 Hz, 1 H, 21-H, isomer A), 3.93 (dd, J = 9.0, 9.3 Hz, 1 H, 21-H, isomer B), 3.83 (dd, J = 9.0, 9.6 Hz, 1 H, 21-H, isomer B), 3.48 (m, 1 H, 3α-H), 2.65–2.48 (m, 2 H, 22-H), 1.00 (s, 3 H, 19-CH_3), 0.89 [9 H, s, $C(CH_3)_3$], 0.70 and 0.69 (s, 3 H, 18-CH_3), 0.06 [s, 6 H, $Si(CH_3)_2$] ppm. ^{13}C NMR: δ = 177.29 and 176.66 (C-23), 141.37 (C-5), 120.73 (C-6), 73.10 and 72.76 (C-21), 72.48 (C-3) ppm. MS: *m*/*z* (%) = 472 (1) [M^+], 457 (12), 416 (100), 339 (22), 159 (39), 75 (99). $C_{29}H_{48}O_3Si$ (472.78): calcd. C 73.67, H 10.23; found C 73.72, H 10.54.

3β-(*tert*-Butyldimethylsilyloxy)-22ξ-(phenylseleno)-14α,20ξ-card-5-enolide (15): A 2 M solution of lithium diisopropylamide in tetrahydrofuran/heptane/ethylbenzene (Aldrich) (0.63 mL, 1.26 mmol) at –30 °C was added to a solution of compound **14** (100 mg, 0.21 mmol) in anhydrous tetrahydrofuran (3 mL) under argon. The reaction mixture was stirred at –30 °C for 30 min and then slowly warmed to 0 °C within 0.5 h. The solution was then cooled to –70 °C and a solution of phenylselenyl chloride (241 mg, 1.26 mmol) in tetrahydrofuran (3 mL) was added dropwise. The mixture was stirred at –70 °C for 2 h and for a further 1 h at –30 °C. Then a saturated aqueous ammonium chloride solution (5 mL) was added and the mixture was left to warm to room temperature. Diethyl ether was then added and the organic layer was washed with water and dried with magnesium sulfate. The solvent was evaporated in vacuo and the reaction products were separated by column chromatography. Elution with benzene gave fraction A (two diastereoisomers of **15**) and fraction B (two further diastereoisomers of **15**) as yellow oils. The total yield of **15** was 99.5 mg (75% yield). IR (KBr): ṽ = 1770, 1752, 1250, 1185, 1177, 1092, 887, 870, 836, 776 cm^{-1}.

^1H NMR: fraction A: δ = 7.71–7.67 (m, 2 H, C_6H_5), 7.40–7.25 (m, 3 H, C_6H_5), 5.30 (br. d, J = 5.0 Hz, 1 H, 6-H), 4.27–4.00 (m, 2 H, 21-H), 3.75 (d, J= 3.8 Hz, 0.5 H, 22-H),3.58 (d, J= 5.4 Hz, 0.5 H, 22-H), 3.47 (m, 1 H, 3α-H), 2.45 (m, 1 H, 20-H), 1.00 and 0.98 (s, 3 H, 19-CH_3), 0.890 and 0.885 [s, 9 H, $C(CH_3)_3$], 0.65 and 0.62 (s, 3 H, 18-CH_3), 0.058 and 0.053 [s, 6 H, $Si(CH_3)_2$] ppm; fraction B: δ = 7.71–7.65 (m, 2 H, C_6H_5), 7.40–7.25 (m, 3 H, C_6H_5), 5.33 (br. d, J = 5.3 Hz, 1 H, 6-H), 4.38–3.84 (m, 2 H, 21-H), 3.84 (d, J = 5.7 Hz, 0.5 H, 22-H), 3.70 (d, J = 6.5 Hz, 0.5 H, 22-H), 3.49 (m, 1 H, 3α-H), 1.017 and 1.006 (s, 3 H, 19-CH_3), 0.89 [s, 9 H, $C(CH_3)_3$], 0.73 and 0.71 (s, 3 H, 18-CH_3), 0.065 and 0.063 [s, 6 H, $Si(CH_3)_2$] ppm. MS: *m*/*z* (%) = 628 [M^+] (74), 569 (36), 493 (32), 337 (27), 199 (33), 158 (100). HRMS: calcd. for $C_{35}H_{53}O_3SiSe$: 629.29291; found 629.29204.

3β-(*tert*-Butyldimethylsilyloxy)-14α-carda-5,20(22)-dienolide (16): Acetic acid (0.1 mL) and a 30% solution of hydrogen peroxide (0.3 mL, 2.5 mmol) were added to a solution of compound **15** (60 mg, 0.1 mmol) in tetrahydrofuran (3 mL). The reaction mixture was stirred at 0 °C for 15 min. After warming to room temp., stirring was continued for 45 min. Then the mixture was poured into water and extracted with diethyl ether. The usual workup gave a crude product, which was purified on a silica-gel column with benzene/ethyl acetate (20:1) as eluent to yield compound **16** (33.5 mg, 67%). M.p. 183–185 °C (heptane). $[\alpha]_D$ = –40 (c = 0.25, $CHCl_3$). IR: ṽ = 1785, 1750, 1630, 1255, 1090, 888, 870, 837 cm^{-1}. ^1H NMR: δ = 5.85 (d, J = 1.6 Hz, 1 H, 22-H), 5.32 (br. d, J= 5.2 Hz, 1 H, 6-H), 4.83 (dd, J = 17.6, 1.6 Hz, 1 H, 21-H), 4.69 (dd, J = 17.6, 1.6 Hz, 1 H, 21-H), 3.48 (m, 1 H, 3α-H), 1.00 (s, 3 H, 19-CH_3), 0.89 [s, 9 H, $C(CH_3)_3$], 0.64 (s, 3 H, 18-CH_3), 0.06 [s, 6 H, $Si(CH_3)_2$] ppm. FAB-MS: *m*/*z* (%) = 471 [M^+ + H] (100), 412 (7), 338 (17). HRMS: calcd. for $C_{29}H_{47}O_3Si$: 471.32944; found 471.33029.

3β-Hydroxy-14α-carda-5,20(22)-dienolide (17): A solution of silyl ether **16** (20 mg, 0.04 mmol) in acetic acid/tetrahydrofuran/water (3:1:1, 4 mL) was stirred at 90–95 °C for 1 h. Then the reaction mixture was poured into water and extracted with diethyl ether. After evaporation of the solvent, the residue dissolved in benzene/ethyl acetate (2:1) was passed through a pad of silica gel to give compound **17** (14.5 mg, 95%). M.p. 244–247 °C (Me_2CO) (ref.[13a] m.p. 240–245 °C, ref.[13b] m.p. 235–240 °C). $[\alpha]_D$ = –44.0 (c = 0.215, dioxane) [ref.[13c] $[\alpha]_D$ = –46.6 (dioxane)]. ^1H NMR: δ = 5.85 (d, J = 1.3 Hz, 1 H, 22-H), 5.37 (d, J= 5.2 Hz, 1 H, 6-H), 4.83 (dd, J = 17.5, 1.9 Hz, 1 H, 21-H_A), 4.69 (d, J = 17.3 Hz, 1 H, 21-H_B), 3.53 (m, 1 H, 3β-H), 1.01 (s, 3 H, 19-CH_3), 0.64 (s, 3 H, 18-CH_3) ppm.

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