198. Synthesis of 3β-Hydroxy[21-^{14}C]-5β-pregn-8(14)-en-20-one from Chenodeoxycholic Acid

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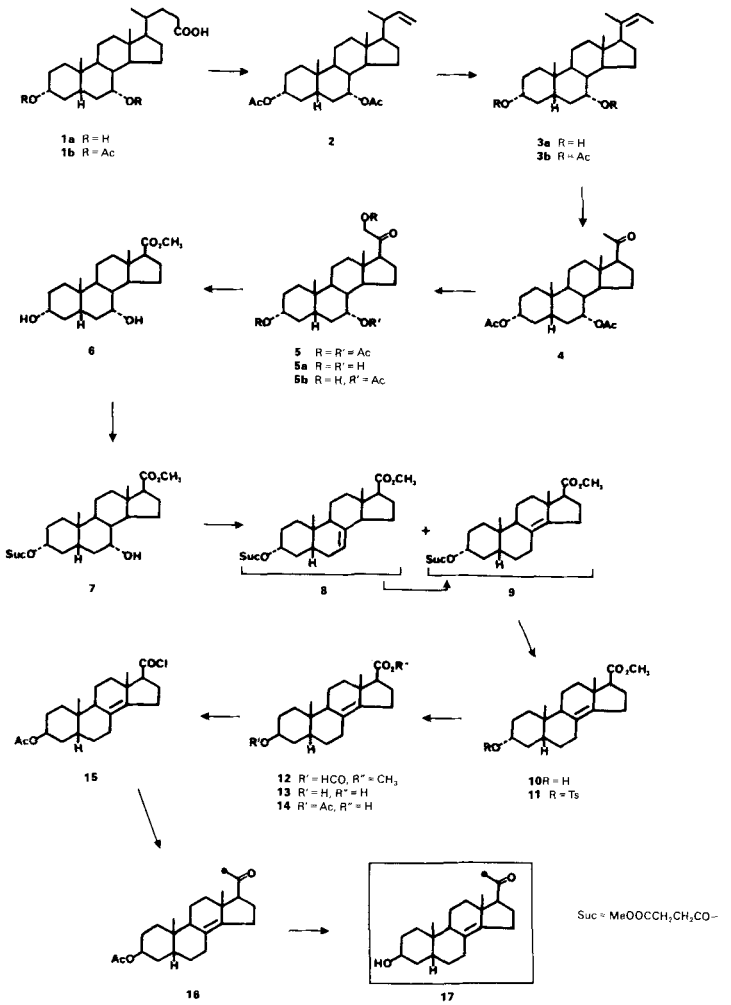
3β-Hydroxy[21-^{14}C]-5β-pregn-8(14)-en-20-one (**17**) was prepared from chenodeoxycholic acid (**1a**). The synthetic sequence involved: *i*) degradation of the bile-acid side chain to an etianic acid; *ii*) formation of the 8(14)-double bond; *iii*) inversion of the configuration at C(3); *iv*) construction of the acetyl side chain at C(17) with the required isotopic label at C(21). Structures of all described products were confirmed by chemical and spectroscopic (IR, ^{1}H-NMR, ^{13}C-NMR, MS) methods.

Introduction. – Although the biosynthetic pathway from cholesterol to cardenolides has been subjected to several studies using different labelled compounds, none of these has satisfactorily demonstrated solutions to the hydroxylation at C(14) with the correct stereochemistry [1]. In connection with our attempts to clarify this intriguing step, we needed 3β-hydroxy-5β-pregn-8(14)-en-20-one (**17**) labelled at either one of the side chain C-atoms. A synthetic transformation of progesterone into compound **17** had been reported [2], but the described procedure did not allow the introduction of a label at the acetyl side chain. For the sake of a simpler synthetic approach and an easier degradation of the natural product resulting from the feeding experiment, we chose to have the label at C(21). Taking into account the *cis* A/B ring junction of the final product, chenodeoxycholic acid (**1a**), a commercial bile acid both inexpensive and readily available, was our choice of starting material. The synthetic approach *(Scheme)* involved four main transformations: *i*) degradation of the bile-acid side chain to afford the corresponding etianic acid; *ii*) dehydration of the OH group at C(7) and isomerization of the double bond to position 8(14); *iii*) inversion of the configuration at C(3); *iv*) construction of the labelled 20-oxopregnane side chain.

Results and Discussion. – Acetylation of 3α,7α-dihydroxy-5β-cholan-24-oic acid (= chenodeoxycholic acid; **1a**) to afford the diacetyl derivative **1b** was performed by refluxing **1a** in Ac_2O and HCl as a catalyst; this drastic condition was needed to accomplish the acetylation of the sterically hindered 7α-OH group in a 5β-steroid compound. Degradation and isomerization of the side chain of **1b** to give the 20(22)-unsaturated derivative **3a** *via* **2** was conducted according to a known procedure [3] with minor modifications (see *Exper. Part*); the choice of Li wire instead of BuLi led to a 85% yield of the isomerized olefin **3a**. Acetylation of **3a** as described above afforded diacetate **3b** in 90% yield. Ozonolysis of this product led to the 20-oxopregnane **4** which, upon treatment with $Pb(OAc)_4/BF_3 \cdot Et_2O$ [4], afforded **5** in 71% yield. Under mild acid hydrolysis conditions, **5** gave a mixture of two products, the free triol **5a** and the 7α-acetoxy-diol **5b**,

Scheme



without isomerization at C(17). Although the mixture could be separated by column chromatography, this was considered unnecessary because both products had a free OH group at C(21) being, therefore, amenable to periodate oxidation [5] to give the mixture of respective etianic acids. Base-catalyzed solvolysis of the mixture obtained after treatment of **5** with $NaIO_4$ and reaction with diazomethane afforded pure **6** in 62% yield from **5**.

For the following synthetic steps, we took advantage of the fact that, due to the different sterical environment of the OH groups at C(3) and C(7) in a 5β-steroid derivative, they show different chemical reactivities. The axial 7α-OH group experiences the steric hindrance of rings A and B in a *cis* configuration, while the equatorial 3α-OH group, lying on the outside of the carbon-skeleton influence, shows no steric impediment. Hence, the latter could be selectively protected by bulky substituents like succinyl ester

without affecting the 7-OH group which afterwards could be eliminated to afford the desired olefin. Selective succinylation of the 3-OH group of **6** yielded compound **7** in 75% yield. Treatment of **7** with anhydrous $ZnCl_2$ in dry acetone gave, after methylation, a mixture of the olefins **8** and **9**. The mixture could be fully separated by preparative HPLC. To improve the yield of the required compound **9**, the conversion of **8** into **9** was accomplished in 68% yield by treatment of **8** with liquid SO_2 in a sealed tube heated at 100° for 35 h; therefore, the overall yield of **9** from **7** was 62%. Basic hydrolysis of **9** afforded **10** in very good yield.

For the inversion of the configuration at C(3), several methods were described.

The simpler though not the most successful procedure requires oxidation of the 3-OH group to a 3-oxo group and further reduction using an appropriate catalyst to obtain the axial alcohol [6] [7]. In the present case, oxidation was performed with *Jones* reagent followed by catalytic reduction to afford an equimolar mixture of both acetylated epimers. In view of this unsatisfactory result, we turned our attention to alternative methods to achieve our purpose. A one-step reaction has been described for the required conversion [8]; this involved the treatment of the steroidal alcohol with Ph_3P and diethyl azodicarboxylate in tetrahydrofuran in the presence of an acid such as benzoic or formic acid. Although good yields of a sterically pure ester of inverted configuration were described, in our case the results obtained with compound **10** were not quite successful. We were able to obtain the 3β-formiate **12** in about 20% yield only after a tedious chromatographic separation. Similar low yields were described for this reaction when applied to methyl cholate [9].

Finally, we decided to employ the well established procedure which produces inversion of configuration by displacement of good leaving groups such as tosylates. The tosyl derivative **11** was obtained in good yield, and treatment of it with dimethylformamide as solvent and reagent [9–11] under proper temperature control allowed the isolation, by column chromatography, of 3β-formiate **12** in 63% yield. Basic hydrolysis of **12** yielded the etianic acid **13** which was acetylated in refluxing AcOH (→**14**). This procedure avoided the formation of the mixed anhydride on the free COOH group.

For the construction of the labelled 20-oxopregnane side chain, a technique using low amounts of the radioactive material in a reaction with the highest possible isotopic yield was needed. Although the use of MgMeI fulfilled the requirement of high isotopic yield in an equimolar reaction [12], in our case the low amount of acyl chloride **15** that should be used made this reaction disadvantageous due to its low yield when processing small quantities of the steroid. Accordingly, we proceeded to the construction of the side chain using an organo-cadmium derivative [13]. Reaction of equimolar quantities of acyl chloride **15** and [$^{14}C_2$]dimethylcadmium obtained *in situ* in a specially designed apparatus (see *Exper. Part*) afforded pure ketone **16** in 36% yield with a high specific activity. Mild acid hydrolysis gave the 3β-hydroxy ketone **17**.

In conclusion, the synthesis described allowed the transformation of a commercial bile acid into a 3β-hydroxy-5β-pregnane-20-one derivative. Similar procedures with suitable modifications could be applied to other bile acids in order to obtain pregnanes with different functionalities [14]. The advantages of the procedure are the accessibility of the starting material, the relatively few steps in the reaction sequence, the simplicity, and the availability of the reagents.

Experimental Part

General. All solvents and reagents were purified and/or dried before using according to generally accepted procedures, unless otherwise stated. $^{14}CH_3I$ was purchased from *New England Nuclear Corp.* Prep. HPLC: *Micromeritics* liquid chromatograph, refractive-index detector. Anal. TLC: F_{254} TLC plates *(Merck)*. Prep.

Table. *^{13}C-NMR Chemical Shifts of Compounds* **2**–**7** *and* **9**–**12**[a])

C-Atom	**2**	**3a**	**3b**	**4**	**5**	**6**	C-Atom	**7**	**9**	**10**	**11**	**12**
C(1)	(34.9)	(35.4)	(34.9)	(34.8)	(34.8)	(35.4)	C(1)	(35.1)	(35.9)	(36.3)	(35.7)	(30.3)
C(2)	26.8	30.7	26.8	26.8	26.7	30.7	C(2)	26.7	26.9	30.9	26.9	25.7
C(3)	74.1	71.9	74.1	73.9	73.9	71.8	C(3)	74.6	74.4	71.4	82.8	70.7
C(4)	(34.7)	39.8	(34.7)	(34.6)	(34.6)	39.6	C(4)	(35.0)	31.8	(36.0)	32.7	(30.0)
C(5)	41.1	41.6	41.1	40.1	40.8	41.5	C(5)	41.3	42.0	42.2	42.1	37.4
C(6)	31.3	34.6	31.3	31.2	31.2	34.9	C(6)	34.7	27.1	27.4	27.9	26.6
C(7)	71.2	68.5	71.2	71.0	71.0	68.3	C(7)	68.2	24.8	25.0	24.6	24.6
C(8)	37.9	39.9	38.0	37.9	37.9	39.8	C(8)	39.6	128.2	128.6	127.7	128.2
C(9)	34.1	33.1	34.4	34.1	34.0	33.0	C(9)	33.0	36.2	36.0	36.0	36.0
C(10)	(34.8)	(35.1)	(34.7)	(34.9)	(34.8)	(35.1)	C(10)	(35.3)	(36.3)	(36.3)	(36.1)	36.6
C(11)	20.7	20.6	20.7	20.7	21.4	20.5	C(11)	20.5	19.3	19.4	19.2	19.7
C(12)	39.4	38.6	38.4	38.5	38.2	38.2	C(12)	38.0	34.2	34.6	34.2	35.7
C(13)	42.6	43.7	43.7	44.1	44.7	44.2	C(13)	44.1	43.2	43.3	43.2	43.3
C(14)	50.4	49.9	50.7	50.6	50.8	50.0	C(14)	50.0	138.9	138.6	139.2	139.0
C(15)	23.6	23.7	23.6	23.8	23.9	(24.0)	C(15)	(24.0)	25.6	25.6	25.6	25.7
C(16)	28.3	24.8	24.7	22.6	22.6	(23.8)	C(16)	(23.8)	23.8	23.8	23.7	24.0
C(17)	55.5	58.9	58.9	63.5	59.1	55.3	C(17)	55.2	56.3	56.3	56.2	56.3
C(18)	11.9	12.8	12.7	13.0	12.9	12.2	C(18)	13.2	19.8	19.8	19.7	19.8
C(19)	22.7	22.8	22.7	22.9	22.8	22.7	C(19)	22.6	23.5	23.6	23.3	23.8
C(20)	41.0	135.1	134.8	208.8	203.3	174.2	C(20)	174.2	173.8	174.1	173.8	174.0
C(21)	20.1	17.6	17.5	31.4	170.0		$O_2C(CH_2)_2CO_2CH_3$	171.5	171.5			
C(22)	144.8	119.0	119.1					172.5	172.5			
C(23)	111.6	13.5	13.5				$O_2C(CH_2)_2CO_2CH_3$	29.1	29.0			
CH_3CO_2	170.2		170.0	169.9	170.0			29.6	29.5			
	170.4		170.2	170.3	170.3		$O_2C(CH_2)_2CO_2CH_3$	51.6	51.7			
CH_3CO_2	21.5		21.4	21.5	20.4		CO_2CH_3	51.0	51.1		51.1	51.2
			21.5		20.6		$CH_3C_6H_4SO_2$				21.6	
CO_2CH_3						51.1	$CH_3C_6H_4SO_2$				127.3	
											129.6	
											134.5	
											144.2	
							HCOO					160.6

[a]) The assignments of values in parentheses are ambiguous; they should not affect other assignments.

column chromatography: silica gel *(Merck)*. M.p.: *Fisher-Johns* apparatus; uncorrected. IR spectra (cm^{-1}): nujol dispersions; *Perkin-Elmer 421* spectrophotometer. ^1H- and ^{13}C-FT-NMR spectra: *Varian XL-100-15* at 100.1 and 25.4 MHz, resp., using TMS as internal standard; δ_C were assigned in the totally ^1H-decoupled spectra and confirmed by analyses of the signal multiplicities determined by the attached proton test (APT) spectra, by comparison to data of model compounds, and in several cases by comparison with calculated chemical shifts, which were obtained from semiempirical rules [15] [16] and known substituent-induced shifts. MS (*m/z*, %): at 70 eV (direct inlet), *Varian-MAT-CH7-A* spectrometer interfaced to a *Varian-MAT-Data-System-166* computer.

3α,7α-Diacetoxy-5β-cholan-24-oic Acid (**1b**). A soln. of *3α,7α-dihydroxy-5β-cholan-24-oic acid* (= *chenodeoxycholic acid;* **1a**; 1.5 g, 4.17 mmol) in Ac_2O (20 ml) and HCl (0.15 ml) was refluxed for 2 h. After cooling, H_2O was added and the solid filtered off. It was crystallized from dil. EtOH giving **1b** (1.6 g, 88%) of m.p. 200–203° ([17]: m.p. 206–208°). IR: 3300–2600 (OH), 1740 (C=O, ester), 1710 (C=O, acid). ^1H-NMR ($CDCl_3$): 0.65 (*s*, CH_3(18)); 0.94 (*s*, CH_3(19)); 2.00 (*s*, CH_3CO_2); 2.02 (*s*, CH_3CO_2); 3.62 (*s*, CO_2CH_3); 4.50 (br., H–C(3)); 4.80 (br., H–C(7)). MS: 356 (11, $M^{+\cdot}$ – 2 AcOH), 255 (10, 356 – side chain), 78 (100).

24-Nor-5β-chol-22-ene-3α,7α-diol Diacetate (**2**). A mixture of **1b** (800 mg, 1.68 mmol), cupric acetate (80 mg, 0.44 mmol), and dry pyridine (0.01 ml, 0.12 mmol) in dry benzene (30 ml) was refluxed under N_2 for 4 h, while $Pb(OAc)_4$ (2.2 g, 4.45 mmol) was added in 4 portions. The solid was collected and the filtrate washed with 5% HCl, 5% Na_2CO_3 soln., and H_2O, dried ($MgSO_4$), and evaporated. The crude residue was chromatographed on silica gel *G* with toluene affording **2** (435 mg, 60%) which was crystallized from MeOH, m.p. 143–144° ([3]: m.p. 134°). IR: 1740 (C=O). ^1H-NMR ($CDCl_3$): 0.72 (*s*, CH_3(18)); 0.94 (*s*, CH_3(19)); 1.03 (*d*, J = 6, CH_3(21)); 2.03 (*s*, 2 CH_3CO_2); 4.65 (br., H–C(3)); 4.85 (br., H–C(7)); 4.95 (*m*, CH_2(23)); 5.65 (*m*, CH(22)). MS: 370 (12, $M^{+\cdot}$ – AcOH), 310 (89, $M^{+\cdot}$ – 2 AcOH), 255 (100, 310 – side chain), 145 (24).

24-Nor-5β-chol-20(22)-ene-3α,7α-diol (**3a**) *and 24-Nor-5β-chol-20(22)-ene-3α,7α-diol Diacetate* (**3b**). To dry ethylenediamine (7.9 ml) heated at 90–110° (bath temp.) under N_2, Li (234 mg) was added portionwise and the stirred soln. heated for 2 h to complete the reaction. To this soln. containing 7.8 equiv. of *N*-lithioethylenediamine, **2** (1.2 g) was added in one portion and the mixture refluxed under N_2 for 15 min. After cooling, H_2O was added and the whole was extracted twice with CH_2Cl_2. The org. extract was washed with H_2O until neutral reaction, dried ($MgSO_4$), and evaporated to afford crude **3a**. Crystallization from hexane gave 825 mg (85%) of m.p. 177–178° ([3]: m.p. 178°). IR: 3300 (OH). ^1H-NMR ($CDCl_3$): 0.53 (*s*, CH_3(18)); 0.90 (*s*, CH_3(19)); 1.58 (*d*, J = 6, CH_3(23)); 1.61 (*s*, CH_3(21)); 3.60 (br., H–C(3)); 3.80 (br., H–C(7)); 5.20 (*m*, CH(22)). MS: 346 (45, $M^{+\cdot}$), 328 (31, $M^{+\cdot}$ – 18), 310 (20, $M^{+\cdot}$ – 2 × 18), 87 (100).

Olefin **3a** was acetylated as described for **1a** and the product crystallized from MeOH to afford **3b** (560 mg, 90%) of m.p. 130–131°. IR: 1745 (C=O). ^1H-NMR ($CDCl_3$): 0.52 (*s*, CH_3(18)); 0.93 (*s*, CH_3(19)); 1.58 (*d*, J = 6, CH_3(23)); 1.62 (*s*, CH_3(21)); 2.02 (*s*, CH_3CO_2); 2.06 (*s*, CH_3CO_2); 4.55 (br., H–C(3)); 4.85 (br., H–C(7)); 5.20 (*m*, CH(22)). MS: 430 (2, $M^{+\cdot}$), 370 (12, $M^{+\cdot}$ – AcOH), 310 (10, $M^{+\cdot}$ – 2 AcOH), 43 (100).

3α,7α-Dihydroxy-5β-pregnan-20-one Diacetate (**4**). Ozone was bubbled through a soln. of **3b** (500 mg, 1.16 mmol) in dry CH_2Cl_2 (10 ml) at −10° until the soln. became blue. After evaporation, the residue was dissolved in acetone (10 ml) and treated with *Jones'* reagent at 0° until the mixture was redish brown. It was poured into ice/H_2O and extracted twice with $CHCl_3$. The org. extract was washed with H_2O, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel (toluene/AcOEt 9:1) affording **4** (385 mg, 79%) which was crystallized from hexane, m.p. 140–141° ([17]: m.p. 133–134°). IR: 1745 (C=O, acetoxy), 1705 (C=O). ^1H-NMR ($CDCl_3$): 0.61 (*s*, CH_3(18)); 0.93 (*s*, CH_3(19)); 2.02 (*s*, CH_3CO_2); 2.04 (*s*, CH_3CO_2); 2.10 (*s*, CH_3(21)); 4.55 (br., H–C(3)); 4.85 (br., H–C(7)). MS: 358 (3, $M^{+\cdot}$ – AcOH), 298 (5, $M^{+\cdot}$ – 2 AcOH), 283 (15, $M^{+\cdot}$ – AcOH – CH_3), 255 (16, 298 – side chain), 43 (100).

3α,7α-21-Trihydroxy-5β-pregnan-20-one Triacetate (**5**). To a soln. of **4** (200 mg, 0.48 mmol) in dry benzene (8 ml), $Pb(OAc)_4$ (400 mg, 0.90 mmol), MeOH (0.36 ml), and $BF_3 \cdot Et_2O$ (1.12 ml) were added. The mixture was maintained at r.t. for 24 h, poured into H_2O and extracted with CH_2Cl_2 (3 × 30 ml). The org. extract was washed with H_2O until neutral reaction, dried ($MgSO_4$), and evaporated to afford crude **5**. It was crystallized from EtOH giving 162 mg (71%) of m.p. 156–157°. IR: 1730 (C=O, acetoxy), 1710 (C=O). ^1H-NMR ($CDCl_3$): 0.66 (*s*, CH_3(18)); 0.94 (*s*, CH_3(19)); 2.04 (*s*, CH_3CO_2); 2.06 (*s*, CH_3CO_2); 2.18 (*s*, CH_3CO_2); 4.90 (br., H–C(7)). MS: 416 (1, $M^{+\cdot}$ – AcOH), 403 (37, $M^{+\cdot}$ – AcOH – CH_3), 356 (4, $M^{+\cdot}$ – 2 AcOH), 315 (6, $M^{+\cdot}$ – AcOH – side chain), 296 (2, $M^{+\cdot}$ – 3 AcOH), 255 (100, $M^{+\cdot}$ – 2 AcOH – side chain).

Methyl 3α,7α-Dihydroxy-5β-androstan-17β-carboxylate (**6**). Triester **5** (150 mg, 0.32 mmol) was refluxed with H_2SO_4 (0.2 ml) in EtOH (10 ml) for 24 h. The mixture was poured into ice/H_2O, extracted twice with AcOEt and the extract washed with H_2O until neutral reaction, dried ($MgSO_4$), and evaporated. The gummy residue was dissolved in MeOH (5 ml) and a soln. of $NaIO_4$ (200 mg) in H_2O (2 ml) was added. The stirred mixture was maintained at r.t.

for 3 h during which a white solid appeared. The mixture was poured into H_2O and extracted with AcOEt. The org. extract was washed with H_2O, dried ($MgSO_4$), and evaporated. The residue was dissolved in 3% NaOH/EtOH soln. (20 ml) and refluxed for 4 h. It was poured into ice/H_2O, acidified with dil. HCl soln. and extracted twice with AcOEt. The extract was washed with H_2O until neutral reaction, dried ($MgSO_4$), and evaporated. The solid residue was dissolved in MeOH and treated with diazomethane. Evaporation afforded **6** which crystallized from benzene/hexane (80 mg, 62%), m.p. 149–151° ([18]: m.p. 152–154°). IR: 3400 (OH), 1735 (C=O). ^{1}H-NMR ($CDCl_3$): 0.66 (*s*, CH_3(18)); 0.91 (*s*, CH_3(19)); 3.50 (br., H–C(3)); 3.68 (*s*, CO_2CH_3); 3.90 (br., H–C(7)). MS: 350 (1, $M^{+\cdot}$), 332 (12, $M^{+\cdot} - H_2O$), 314 (100, $M^{+\cdot} - 2\,H_2O$), 299 (85, $M^{+\cdot} - 2\,H_2O - CH_3$), 273 (24, $M^{+\cdot} - H_2O$ – side chain), 254 (13, $M^{+\cdot} - 2\,H_2O - HCO_2CH_3$).

Methyl 7α-Hydroxy-3α-[(methoxysuccinyl)oxy]-5β-androstan-17β-carboxylate (**7**). The diol **6** (100 mg, 0.29 mmol) was added to a soln. of succinic anhydride (220 mg, 2.2 mmol) in dry pyridine (5 ml). The mixture was refluxed for 1 h, poured into ice/dil. HCl soln., stirred at r.t. for 10 min, and extracted with AcOEt (3 × 50 ml). The extract was washed with H_2O until neutral reaction, dried ($MgSO_4$), and evaporated. The residue was suspended in MeOH and treated with diazomethane. Evaporation gave an oil which was chromatographed on silica gel *G* ($CHCl_3$/EtOH 95:5) yielding **7** (99 mg, 75%) which was crystallized from EtOH, m.p. 118–119°. IR: 3400 (OH), 1735 (C=O). ^{1}H-NMR ($CDCl_3$): 0.66 (*s*, CH_3(18)); 0.93 (*s*, CH_3(19)); 2.62 (*s*, $O_2CCH_2CH_2CO_2$); 3.70 (*s*, CO_2CH_3); 3.72 (*s*, CO_2CH_3); 3.88 (br., H–C(7)); 4.60 (br., H–C(3)). MS: 332 (15, $M^{+\cdot} - C_5H_8O_4$), 314 (100, 332 – H_2O), 300 (7, 332 – MeOH), 299 (82, 332 – H_2O – CH_3), 255 (33, 332 – H_2O – side chain), 254 (6, 332 – H_2O – HCO_2CH_3).

Methyl 3α-[(Methoxysuccinyl)oxy]-5β-androst-7-en-17β-carboxylate (**8**) *and Methyl 3α-[(Methoxysuccinyl)-oxy]-5β-androst-8(14)-en-17β-carboxylate* (**9**). Anh. $ZnCl_2$ (400 mg, 2.96 mmol) was dissolved in dry acetone (10 ml), and **7** (100 mg, 0.22 mmol) was added. The soln. was distilled until it changed to a light yellow syrup. Dry acetone (10 ml) was added and the soln. distilled again; this procedure was repeated 3 times. The addition of $ZnCl_2$/acetone followed by distillation was done 5 times more. The remaining yellow gum was treated with 15% HCl soln. (10 ml) and extracted twice with AcOEt. The org. extract was washed with H_2O until neutral reaction, dried ($MgSO_4$), and evaporated. The residue was suspended in MeOH and treated with diazomethane. After evaporation, the crude product was chromatographed on silica gel (toluene/AcOEt 9:1) giving 77 mg (80%) of **8**/**9**. IR: 1735 (C=O). ^{1}H-NMR ($CDCl_3$): 0.54 (*s*, CH_3(18) of **9**); 0.82 (*s*, CH_3(19) of **8**); 0.84 (*s*, CH_3(18) of **8**); 0.93 (*s*, CH_3(19) of **9**); 2.60 (*s*, $O_2CCH_2CH_2CO_2$); 3.69 (*s*, CO_2CH_3); 3.71 (*s*, CO_2CH_3); 4.60 (br., H–C(3)); 5.10 (br., H–C(7)).

The mixture **8**/**9** was separated by HPLC using a reverse-phase column *Altex Ultrasphere ODS* 5 µm (250 × 10 mm i.d.) with MeOH/H_2O 9:1 at a flow-rate of 3 ml/min yielding 26 mg of **9** and 50 mg of **8**.

Conversion of **8** *into* **9**. Compound **8** (50 mg) in a *Pyrex* tube was cooled to – 60°, and liquid SO_2 (2 ml) was added; no precautions were taken to prevent access of moisture. The tube was sealed and heated at 100° for 36 h. It was cooled again, opened, and the SO_2 was eliminated with a stream of N_2. The orange-brown residue was chromatographed on silica gel (toluene/AcOEt 9:1) giving **9** (34 mg, 68%). IR: 1735 (C=O). ^{1}H-NMR ($CDCl_3$): 0.82 (*s*, CH_3(19)); 0.84 (*s*, CH_3(18)); 2.62 (*s*, $O_2CCH_2CH_2CO_2$); 3.69 (*s*, CO_2CH_3); 3.71 (*s*, CO_2CH_3); 4.75 (br., H–C(3)). MS: 446 (3, $M^{+\cdot}$), 415 (3, $M^{+\cdot}$ – MeOH), 314 (96, $M^{+\cdot} - C_5H_8O_4$), 299 (81, 314 – CH_3), 255 (16, 314 – side chain), 57 (100).

Methyl 3α-Hydroxy-5β-androst-8(14)-en-17β-carboxylate (**10**). A soln. of **9** (100 mg, 0.22 mmol) in 2% KOH/EtOH (50 ml) was refluxed for 6 h. The mixture was poured into 20% aq. HCl soln. and extracted with EtOAc (3 × 50 ml). The extract was washed with H_2O until neutral reaction, dried ($MgSO_4$), and evaporated. The residue was suspended in MeOH and treated with CH_2N_2. Evaporation afforded **10** which was purified by TLC (CH_2Cl_2/MeOH 98:2). Pure **10** (from MeOH; 65 mg, 87%) had m.p. 126–127°. IR: 3400 (OH), 1735 (C=O). ^{1}H-NMR ($CDCl_3$): 0.81 (*s*, CH_3(19)); 0.83 (*s*, CH_3(18)); 3.60 (br., H–C(3)); 3.69 (*s*, CO_2CH_3). MS: 332 (20, $M^{+\cdot}$), 314 (100, $M^{+\cdot} - H_2O$), 299 (91, 314 – CH_3), 255 (10, 314 – side chain), 254 (7, 314 – HCO_2CH_3).

Methyl 3α-Tosyloxy-5β-androst-8(14)-en-17β-carboxylate (**11**). To a soln. of **10** (50 mg, 0.15 mmol) in dry pyridine (5 ml), TsCl (110 mg, 0.58 mmol) was added. The reaction was maintained at r.t. for 24 h and the mixture poured into ice/HCl. It was extracted twice with $CHCl_3$, and the extract was washed with H_2O until neutral reaction, dried ($MgSO_4$), and evaporated. The crude product was purified by TLC (CH_2Cl_2) to give **11** (54 mg, 74%) of m.p. 56–58°. IR: 1730 (C=O), 1600 (C=C). ^{1}H-NMR ($CDCl_3$): 0.78 (*s*, CH_3(19)); 0.83 (*s*, CH_3(18)); 2.46 (*s*, $CH_3C_6H_4$); 3.69 (*s*, CO_2CH_3); 4.50 (br., H–C(3)); 7.25–7.85 (*m*, 4 arom. H). MS: 314 (2, $M^{+\cdot}$ – TsOH), 43 (100).

Methyl 3β-Formyloxy-5β-androst-8(14)-en-17β-carboxylate (**12**). A soln. of **11** (50 mg, 0.10 mmol) in dimethylformamide (2.5 ml) was maintained in a sealed tube at 75° for 50 h. It was poured into H_2O and extracted twice with CH_2Cl_2. The extract was washed with H_2O, dried ($MgSO_4$), and evaporated. The residue was crystallized from

MeOH to afford **12** (23 mg, 63%) of m.p. 150–152°. IR: 1730 (C=O). ^{1}H-NMR ($CDCl_3$): 0.86 (*s*, CH_3(18), CH_3(19)); 3.69 (*s*, CO_2CH_3); 5.25 (br., H–C(3)); 8.07 (*s*, HCO_2). MS: 360 (82, $M^{+\cdot}$), 345 (27, $M^{+\cdot} - CH_3$), 314 (73, $M^{+\cdot} - HCO_2H$), 299 (46, $M^{+\cdot} - HCO_2H - CH_3$), 255 (11, 314 – side chain), 147 (100).

3β-Hydroxy-5β-androst-8(14)-en-17β-carboxylic Acid (**13**). A soln. of **12** (20 mg, 0.06 mmol) in 2% KOH/EtOH (20 ml) was refluxed for 2 h. It was poured into ice/H_2O, acidified with dil. HCl soln. and extracted with AcOEt. The org. extract was washed with H_2O until neutral reaction, dried ($MgSO_4$), and evaporated: **13** as a white crystalline product (17 mg, 96%) which was used without further purification. IR: 3500–2700 (OH, acid), 3300 (OH, alcohol), 1715 (C=O).

3β-Acetoxy-5β-androst-8(14)-en-17β-carboxylic Acid (**14**). Acetylation of **13** (16.5 mg) with glacial AcOH at reflux for 2 h afforded **14** (15.5 mg, 83%) as a solid which was used without further purification. IR: 3400–2600 (OH, acid), 1730 (C=O, ester). 1715 (C=O, acid). ^{1}H-NMR ($CDCl_3$): 0.83 (*s*, CH_3(18)); 0.85 (*s*, CH_3(19)); 2.06 (*s*, CH_3CO_2); 5.10 (br., H–C(3)).

3β-Acetoxy-5β-androst-8(14)-en-17β-carbonyl Chloride (**15**). To a soln. of **14** (15 mg) in dry benzene (0.5 ml), oxalyl chloride (0.5 ml) was added. The mixture was stirred at r.t. for 2 h. Evaporation afforded crude **15** which was used without further purification. IR: 1790 (C=O, acyl chloride), 1730 (C=O, ester).

20-Oxo[21-^{14}C]-5β-pregnan-8(14)-en-3β-yl Acetate (**16**). A small flask containing metallic Mg (2 mg, 0.08 mmol) was connected to the sealed tube containing $^{14}CH_3I$ (1 mCi, 58 mCi/mmol), and the system was evacuated. A soln. of unlabelled CH_3I (1.7 µl, 0.027 mmol) in anh. Et_2O (0.3 ml) was injected into the flask through a silicone-rubber septum, and the mixture was stirred for 45 min. The flask was cooled in liq. N_2 and the breakseal of the tube containing the radioactive reagent was broken. Under these conditions, the labelled-CH_3I glass container was gently warmed for 15 min. The cooling bath was removed and, once at r.t., the mixture was stirred for 1 h. The flask was filled with dry N_2, and $CdCl_2$ (18 mg, 0.1 mmol) was added. The mixture was stirred at r.t. for 2 h. A soln. of **15** (15 mg, 0.04 mmol) in dry benzene (2 ml) was added through the septum to the [^{14}C]dimethylcadmium soln., and the suspension was stirred at r.t. for 18 h and then heated at 50° for 1 h. The mixture was cooled to 0° and conc. HCl soln. (0.5 ml) was added dropwise followed by H_2O (2 ml). The mixture was extracted twice with $CHCl_3$, and the org. extract was washed with H_2O until neutral reaction and dried ($MgSO_4$). The solvent was evaporated and the residue purified by TLC ($CHCl_3$/hexane 98:2) giving pure **16** (5.1 mg, 36%). IR: 1735 (C=O, ester), 1710 (C=O, ketone). ^{1}H-NMR ($CDCl_3$): 0.80 (*s*, CH_3(18)); 0.86 (*s*, CH_3(19)); 2.04 (*s*, CH_3CO_2); 2.12 (*s*, CH_3(21)); 5.10 (br., H–C(3)). Spec. activity 3.66 mCi/mmol.

3β-Hydroxy[21-^{14}C]-5β-pregn-8(14)-en-20-one (**17**). Compound **16** (4.9 mg, 0.014 mmol) in EtOH (10 ml) was treated with conc. H_2SO_4 soln. (3 drops) and refluxed for 24 h. It was poured into H_2O and extracted with AcOEt (3 × 20 ml). The solid obtained by evaporation of the solvent was purified by TLC ($CHCl_3$/EtOH 99:1) affording pure **17** (2.5 mg, 58%) of m.p. 118–120° ([2]: m.p. 123°). IR: 3350 (OH), 1710 (C=O). ^{1}H-NMR ($CDCl_3$): 0.80 (*s*, CH_3(18)); 0.83 (*s*, CH_3(19)); 2.16 (*s*, CH_3(21)); 4.20 (br., H–C(3)); Spec. activity 3.65 mCi/mmol.

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