Synthesis of 20-Hydroxy-17,21-cyclopregnane Derivatives: Potential C-20 Oxo Steroid Oxidoreductase Inhibitors

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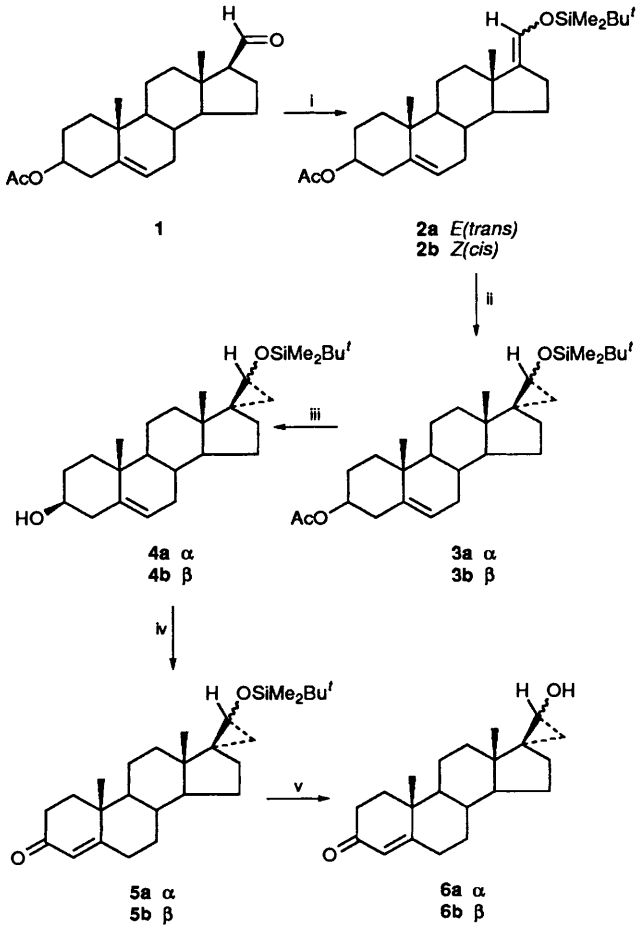
20α- and 20β-Hydroxy-17α,21α-cyclopregnane derivatives, potential mechanism-based-inhibitors of 20α- and 20β-steroid oxidoreductases, have been synthesized by conversion of the steroid 17β-aldehyde into the 20-*tert*-butyldimethylsilyl enol ether followed by addition of methylene prepared from the Simmons–Smith reagent. Enzyme inhibition studies show that neither 20α- nor 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one were inhibitors of the respective enzymes from *Streptomyces hydrogenans*.

Steroid 17β,20α- and 3α,20β-oxidoreductases (cortisone reductase) (EC 1.1.1.62 and EC 1.1.1.53, respectively) are involved in the metabolism of progesterone.[1] To provide a probe for studying the function of these enzymes, the potential mechanism-based-inhibitors 20α- **6a** and 20β-hydroxy-17α,21α-cyclopregn-4-en-3-one **6b** have been synthesized. The cyclopregnane alcohol, acting as the normal enzyme substrate, on oxidation to a highly electrophilic cyclopropanone at the enzyme active-site can be attacked by an active-site nucleophile. Enzyme inhibition by these compounds is based on a proposed covalent bond formation. Support for this process is found in the inhibition of yeast aldehyde dehydrogenase by cyclopropanone hydrate.[2] The high reactivity of the cyclopropanone is demonstrated by its facile acetal formation and reaction with amine nitrogen.[3]

Results and Discussion

Scheme 1 shows the synthesis of the 20α- and 20β-hydroxy-17α,21α-cyclopregnane isomers **6a** and **6b**. The aldehyde **1**[4] was prepared according to a reported method.[5] Treatment of the aldehyde **1** with *tert*-butyldimethylsilyl triflate and triethylamine in ether[6,7] gave a mixture of the silyl enol ethers **2a** and **2b** in the ratio *E*:*Z* (7:3) based on comparison of their vinyl 20-H signal in the NMR spectrum of the reaction product. The major isomer **2a** crystallized from ether–methanol and after chromatographic separation of the mother liquor the minor isomer **2b** was obtained. Separate treatment of each isomer with the Simmons–Smith reagent generated from zinc–silver couple and diiodomethane[8] gave the cyclopregnane derivatives **3a** and **3b**. Regioselective methylene addition occurred at the more electrophilic siloxy 17,20-double bond rather than to the 5,6-double bond. Selective addition also occurred from the α-face as shown by NMR (see below). α-Face addition was expected to result from steric hindrance by the 18-Me group. Of the two isomers, the *Z* isomer, in which reaction was complete in 18 h, reacted more readily than the *E* isomer, which required more reagent for 36 h, as observed by TLC and NMR. Alkaline hydrolysis of the acetate in **3a** and **3b** gave the alcohols **4a** and **4b**, which on Oppenauer oxidation yielded the conjugated ketones **5a** and **5b**. Desilylation with fluoride ion[9] yielded the 20α- and 20β-cyclopropanols **6a** and **6b**. The 20-hydroxy groups in the cyclopregnanes are configurationally analogous to the 20-hydroxy epimers in the normal pregnanes.



Scheme 1 *Reagents:* i, TBDMSiOTf, Et_3N, Et_2O; ii, Zn–Ag, CH_2I_2, Et_2O; iii, KOH, MeOH; iv, cyclohexanone, $Al(Pr^i)_3$, C_6H_5Me; v, Bu_4NF, THF

Nuclear Magnetic Resonance.—^1H and ^{13}C NMR data are given in Tables 1 and 2, in which assignments are based on published data,[10] homonuclear correlation (COSY) spectra,[11] inverse C/H correlation *via* HSQC[13] and internal consistency. Nuclear Overhauser Effect (NOE) measurements were used to establish the structure of the silyl enol ethers **2a** and **2b**. Thus, irradiation of the 18-Me protons in **2a** gave a 1.7% enhancement

Table 1 ^{1}H Chemical shifts (J in Hz)[a,b]

Compd.	10-Me	13-Me	3α-H	4-H	6-H	20-H	20-H	21-H	Other
1	1.02s	0.76s	4.59m		5.37d (J 5.2)	9.76d (J 2.0)			2.02s (3β-OAc)
2a	1.04s	0.81s	4.60m		5.38d (J 5.2)	5.98t (J 2.4)			0.11s (6 H, Me_2) 0.92s (9 H, $SiCMe_3$) 2.03s (3β-OAc)
2b	1.03s	0.89s	4.62m		5.30d (J 4.6)	6.03t (J 1.8)			0.09s (6 H, $SiMe_2$) 0.92s (9 H, $SiCMe_3$) 2.03s (3β-OAc)
3a	1.02s	0.79s	4.61m		5.38d (J 4.8)			3.14dd (J 3.5, 7.0)	0.077s, 0.087s (6 H, $SiMe_2$) 0.89s (9 H, CMe_3) 2.03s (3β-OAc)
3b	1.03s	0.96s	4.61m		5.38d (J 4.4)	0.72dd (H_b) (J 3.1, 5.3)	0.20t (J 5.8)	3.32dd (J 3.0, 6.3)	0.053s, 0.084s (6 H, $SiMe_2$) 0.88s (9 H, CMe_3) 2.03s (3β-OAc)
4a	1.01s	0.86s	3.52m		5.37d (J 5.2)			3.13dd (J 3.5, 7.0)	0.081s, 0.092s (6 H, $SiMe_2$) 0.89s (9 H, CMe_3)
4b	1.02s	0.97s	3.53m		5.36d (J 5.2)	0.72dd (H_b) (J 3.1, 5.3)	0.20t (J 5.8)	3.32dd (J 3.1, 6.3)	0.057s, 0.087s (6 H, $SiMe_2$) 0.88s (9 H, CMe_3)
5a	1.18s	0.83s		5.73s				3.13dd (J 3.5, 7.1)	0.80s, 0.092s (6 H, $SiMe_2$) 0.89s (9 H, CMe_3)
5b	1.19s	0.99s		5.72s		0.70dd (J 3.1, 5.4)	0.21t (J 5.9)	3.32dd (J 3.08, 6.3)	0.053s, 0.087s (6 H, $SiMe_2$) 0.88s (9 H, CMe_3)
6a	1.18s	0.85s		5.73s		0.90d (H_b) (J 3.8, 7.2)	0.22dd (J 3.5, 5.7)	3.28dd (J 3.5; 7.2)	
6b	1.20s	1.02s		5.73s		0.84dd (H_b) (J 3.1, 5.6)	0.33t (J 6.1)	3.51dd (J 3.1, 6.6)	

[a] For solutions in $CDCl_3$ ($SiMe_4$ internal standard) on a Bruker AM300 instrument. [b] Couplings are based on a first-order interpretation of the multiplet pattern. No evidence of strong second order effects was observed.

Table 2 ^{13}C Chemical shifts[a]

	Compound										
Carbon	**1**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**5a**	**5b**	**6a**	**6b**
1	37.00	37.01	37.00	37.03	37.06	37.32	37.32	35.75	35.74	35.74	35.75
2	27.71	27.78	27.81	27.77	27.81	31.69	32.01	34.28	34.06	33.96	33.75
3	73.77	73.92	73.98	73.92	74.00	71.77	71.82	199.48	199.60	199.56	199.60
4	38.06	38.14	38.16	38.12	38.15	42.34	42.35	123.79	123.70	123.82	123.77
5	139.66	139.68	139.78	139.59	139.69	140.72	140.80	171.42	171.67	171.39	171.50
6	122.18	122.48	122.48	122.59	122.58	121.68	121.65	32.96	32.93	32.93	32.96
7	31.75	31.78	31.87	28.68	31.98	32.04	31.72	32.12	32.13	32.07	32.07
8	31.40	31.54	31.78	32.20	31.96	32.27	32.01	36.08	35.74	36.06	35.75
9	50.00	50.47	50.40	50.17	50.22	50.30	50.32	53.88	54.78	53.85	54.84
10	36.65	36.74	36.75	36.70	36.71	36.65	36.65	36.69	38.72	38.70	38.69
11	20.53[b]	20.92	21.15	20.53	20.76	20.61	20.82	20.56	20.78	20.53	20.71
12	38.26	24.68	26.12	34.43	35.83	34.50	35.88	33.98	35.68	34.09	35.96
13	44.62	41.72	43.27	40.49	41.82	40.52	41.82	40.55	41.85	40.69	41.85
14	56.39	55.64	56.32	55.63	55.54[b]	55.74	55.55	54.77	53.94[b]	54.59	53.88[b]
15	24.89	24.36	36.58	24.79	25.86	24.83	29.71	24.64	25.67	24.63	25.60
16	20.01[b]	36.11	24.91	32.00	34.24	28.83	34.26	28.60	32.00	28.19	33.80
17	62.78	133.95	129.67	36.27	34.86	36.30	34.87	36.19	34.72	37.30	36.58
18	13.73	19.30	16.68	16.65	15.18	16.68	15.19	16.75	15.91	16.75	16.22
19	19.31	19.35	19.31	19.32	19.36	19.43	19.45	17.43	17.45	17.42	17.45
20	204.75	129.85	130.82	19.40	19.98	19.43	19.98	19.41	19.91	19.62	20.18
21				52.03	55.70[b]	52.06	55.78	52.00	55.44[b]	51.51	55.41[b]
3-OCOMe	170.42	170.45	170.40	170.46	170.49						
3-OCO*Me*	21.39	21.44	21.45	21.43	21.44						
$SiMe_2$		−5.27	−5.42	−4.79	−5.36	−4.77	−5.36	−4.78	−4.88		
		−5.22	−5.35	−4.71	−4.86	−4.68	−4.86	−4.71	−5.39		
CMe_3		18.35		18.15	18.00	18.17	18.00	18.15	17.98		
CMe_3		25.76		25.89	25.86	25.91	25.86	25.88	25.84		

[a] For solution in $CDCl_3$ ($SiMe_4$ internal standard). [b] Numbers are interchangeable within the column.

of 20-H whereas similar irradiation of **2b** gave 1.0%. Comparison of the NOE observed from irradiation of 20-H in **2a** showed an NOE enhancement of the 12α (1.9%) and 12β (4.4%) protons and no NOE effect on the 16α and 16β protons, whereas the opposite effect was observed on irradiation of 20-H in **2b**, *i.e.* no NOE with 12α and 12β but an NOE with the 16α (2.0%) and the 16β proton (1.6%). Irradiation of 20-H in **2a** showed a small enhancement (0.6%) in the 13-Me protons (18-H_3) while none was observed on similar irradiation in **2b**. These data are in agreement with the assignment of **2a** as the *E* and **2b** as the *Z* isomer.

These assignments are also in agreement with the NOE

enhancement observed for cyclopregnane derivatives **5a** and **5b** where irradiation of the 18-Me protons gave enhancement of the 20-H of 5.9 and 1.2%, respectively. Irradiation of 20-H in **5a** shows an NOE enhancement of the 12β (3.0%) and 18-Me (2.5%) protons whereas 20-H irradiation in **5b** showed enhancement in the 16β (2.5%) proton and only a weak effect on the 18-Me (0.4%). These results are also consistent with the assignment of **5a** and **5b** as the *S* and *R* isomers, respectively, and with their formation from **2a** and **2b**. The downfield (high frequency) shift of the 18-Me signal in the 20α isomers, relative to that in the corresponding 20β isomers, is also in agreement with the proximity of the 18-Me and the 20-silyl ether oxygen.

The corresponding structures for the β-face addition products are not consistent with the NOE enhancement observed for **5a** and **5b**. In the β-face addition products both isomers would be expected to show NOE enhancement between the 13-Me protons and the cyclopropyl methylene protons at C-21 with little effect on the downfield (high frequency) C-20 cyclopropyl proton in **5a** whereas enhancement of 20-H is observed. Furthermore, irradiation of the downfield (high frequency) 21-H (0.8 ppm) in **5a** shows an NOE enhancement in the 12α proton (2.0%) and similar irradiation of the downfield (high frequency) 20-H (0.70 ppm) in **5b** also shows enhancement in 12α (8.6%) in agreement with these protons being located on the steroid α-face. Consistently, irradiation of upfield (low frequency) 21-H (0.22 ppm) in **5b** shows an enhancement of the 16α proton (3.6%). The upfield (low frequency) 21-H in **5a** was not irradiated because of overlap with the $SiMe_2$ peaks. The 12α/β and 16α/β protons were assigned independently by C/H correlation and NOE measurements from the 13-Me. The 21 protons were assigned by their proximity to the 12-H or 16-H, as determined by NOE. NOE measurements for the 8, 11β, 15β and 16β protons were used for ^1H and ^{13}C assignment purposes, principally in ring D.

Enzyme Inhibition Studies.—Neither 20β- **6b** nor 20α-hydroxy-17α,21α-cyclopregn-4-en-3-one **6a** was effective as an inhibitor of steroid 17β,20α- and 3α,20β-oxidoreductases (cortisone reductase), respectively, from *Streptomyces hydrogenans*.

Experimental

^1H and ^{13}C NMR spectra were recorded on a Bruker AM300 instrument and are reported in Tables 1 and 2. Compounds were run on TLC (silica gel, Merck type 60H) in EtOAc–light petroleum (b.p. 35–60 °C) (LP). Flash chromatography was carried out on silica gel Merck type 60 for column chromatography or Terochem flash chromatography type. Ether refers to diethyl ether. Granular zinc (10 mesh, Aldrich Chemical Co. Inc. Madison, Wisconsin) was used. M.p.s were measured on a Gallencamp apparatus and temperatures are uncorrected. Elemental analyses were performed by W. Baldeo, School of Pharmacy, University of London, England, UK.

3β-*Acetoxy*-20α- **2a** *and* 3β-*Acetoxy*-20β-tert-*butyldimethylsiloxy*-21-*norpregna*-5,17-*diene* **2b**.—To a stirred and cooled (ice-bath) solution of the aldehyde **1**[4] (4.2 g), prepared as described in ref. 5, in ether (200 cm^3) and triethylamine (5.1 cm^3) under an argon atmosphere was added, over 5 min by syringe though a rubber septum, *tert*-butyldimethylsilyl triflate (5.65 cm^3). Stirring was continued at room temperature[7] for 6 h, after which TLC showed the absence of starting material. The reaction mixture was washed with aqueous $NaHCO_3$ and brine, dried (Na_2SO_4) and evaporated to give a residue which on recrystallization gave the (20*E*)-silyl enol ether **2a** (2.35 g), m.p. 155–159 °C (from Et_2O–MeOH) (Found: C, 73.1; H, 10.0. $C_{28}H_{46}O_3Si$ requires C, 73.3; H, 10.1%). Flash chromatography of the mother liquor gave fractions on elution with 10–20% CH_2Cl_2–LP which yielded the (20*Z*)-enol ether **2b** (1.3 g), m.p. 96–99 °C (from Et_2O–MeOH) (Found: C, 73.2; H, 10.0. $C_{28}H_{46}O_3Si$ requires C, 73.3; H, 10.1%).

3β-*Acetoxy*-20α-tert-*butyldimethylsiloxy*-17α,21α-*cyclopregn*-5-*ene* **3a**.—To a Zn–Ag couple (10 g, 0.153 mol of Zn), prepared as described by Denis *et al.*,[8] stirred under argon in ether (35 cm^3) was added CH_2I_2 (4 cm^3) at a rate sufficient to maintain gentle reflux (*ca.* 1 h). The (20*E*)-silyl enol ether **2a** (2.1 g) was added in one portion and after being heated under reflux for 18 h, the solution was again treated with the same amount of the above reagents; the mixture was then heated under reflux for a further 18 h. After this, the organic layer was separated and washed with saturated aqueous $NaHCO_3$ to give a residue which on recrystallization gave the cyclopropanosilyl ether **3a** (1.24 g), m.p. 154.5–160 °C (from ether–MeOH) (Found: C, 73.4; H, 10.3. $C_{29}H_{48}O_3Si$ requires C, 73.7; H, 10.2%).

3β-*Acetoxy*-20β-tert-*butyldimethylsiloxy*-17α,21α-*cyclopregn*-5-*ene* **3b**.—Similar treatment of the (20*Z*)-silyl enol ether **2b** (1.12 g) with one portion of Zn–Ag couple (5.5 g) and CH_2I_2 (1.6 cm^3) for 18 h, as described for **2a**, yielded the cyclopropane silyl ether **3b** (1.0 g), m.p. 126–130 °C (from Et_2O–MeOH) (Found: C, 73.5; H, 10.1. $C_{29}H_{48}O_3Si$ requires C, 73.7; H, 10.2%).

20α-tert-*Butyldimethylsiloxy*-17α,21α-*cyclopregn*-5-*en*-3β-*ol* **4a**.—The crude reaction product **3a** (1.2 g) from the Simmons–Smith reaction with **2a** was treated with 0.5 mol dm^{-3} KOH/MeOH (120 cm^3) for 1 h (TLC), after which the mixture was poured into water and extracted with ether. The extract was washed with water and 5% aqueous $Na_2S_2O_3$ to give a crude residue which on flash chromatography with 15% EtOAc–LP as eluent yielded fractions, which gave **4a** (375 mg), m.p. 170–174 °C (from ether–MeOH) (Found: C, 72.4; H, 10.9. $C_{27}H_{46}O_2Si{\cdot}H_2O$ requires C, 72.3; H, 10.8%).

20β-tert-*Butyldimethylsiloxy*-17α,21α-*cyclopregn*-5-*en*-3β-*ol* **4b**.—Treatment of **3b** (200 mg) with 0.5 mol dm^{-3} KOH/MeOH (20 cm^3) as described for **3a** gave **4b** (145 mg), m.p. 210–212 °C (from ether–MeOH) (Found: C, 75.2; H, 10.6. $C_{27}H_{46}O_2Si$ requires C, 75.3; H, 10.8%).

20α-tert-*Butyldimethylsiloxy*-17α,21α-*cyclopregn*-4-*en*-3-*one* **5a**.—To compound **4a** (184 mg) in anhydrous toluene (45 cm^3) under argon was added freshly distilled cyclohexanone (5 cm^3, 48.2 mmol); the solution was then distilled until toluene (6.5 cm^3) had been removed. Aluminium isopropoxide (400 cm^3) in toluene (15 cm^3) was then added dropwise (over 0.5 h) to the residue, and the solution again distilled slowly to remove toluene (15 cm^3). After 45 min, the reaction mixture was cooled and subjected to steam distillation. The residue was extracted with CH_2Cl_2 and the organic layer evaporated to give a product, which on flash chromatography in 10% EtOAc–LP, gave **5a** (150 mg), m.p. 181–185 °C (from ether–MeOH) (Found: C, 75.5; H, 10.4. $C_{27}H_{44}O_2Si$ requires C, 75.6; H, 10.3%).

20β-tert-*Butyldimethylsiloxy*-17α,21α-*cyclopregn*-4-*en*-3-*one* **5b**.—Compound **4b** (216 mg) was treated with cyclohexanone (7 cm^3) and $Al(Pr^iO)_3$ (480 mg) as described for **4a** to give, on flash chromatography in 10% EtOAc–LP, **5b** (180 mg), m.p. 134–137 °C (from ether–LP) (Found: C, 75.4; H, 10.5. $C_{27}H_{44}O_2Si$ requires C, 75.6; H, 10.3%).

20α-*Hydroxy*-17α,21α-*cyclopregn*-4-*en*-3-*one* **6a**.—The silyl ether **5a** (121 mg) in tetrahydrofuran (THF) (5 cm^3) was treated

with 1 mol dm^{-3} Bu_4NF–THF[13] (0.7 cm^3) for 1.5 h (TLC) at room temperature. The mixture was poured into water and extracted with CH_2Cl_2 to give, on flash chromatography in 10% EtOAc–LP, **6a** (108 mg), m.p. 163–167 °C (from CH_2Cl_2–LP) (Found: C, 79.1; H, 9.4. $C_{21}H_{30}O_2 \cdot \frac{1}{4}H_2O$ requires C, 79.1; H, 9.6%).

20β-*Hydroxy*-17α,21α-*cyclopregn*-4-*en*-3-*one* **6b**.—Treatment of **5b** (152 mg) in THF (5 cm^3) with 1 mol dm^{-3} Bu_4NF–THF (0.8 cm^3) as described for **5a** gave, after flash chromatography with 25% EtOAc–LP **6b** (69 mg), m.p. 182–192 °C (decomp.) (from CH_2Cl_2–LP) (Found: C, 79.05; H, 9.6. $C_{21}H_{30}O_2 \cdot \frac{1}{4}H_2O$ requires C, 79.1; H, 9.6%).

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