The Selective Oxidation of Protected Cholestanol Derivatives using the Gif System

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Oxidation of 3β,5α6β-triacetoxycholestane using the Gif system (iron cluster–metallic zinc–pyridine–aqueous acetic acid) gives, as major product, the side-chain fission ketone 3β,5α,6β-triacetoxy-5α-pregnan-20-one. The 15- and 16-keto derivatives of the parent triacetate are also formed. Comparable results are obtained in the oxidation of cholestanol acetate and of acetoxycholestan-6-one ethylene ketal.

The degradation of cholesterol to useful steroidal hormones has fascinated chemists for a long time.[1] Interest waned due to the availability of easily degraded diosgenin, hecogenin *etc.*, and bile acids, but has been revived by recent progress in microbiology.[2]

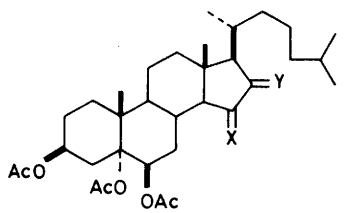
Recently we have described a new system for the oxidation of saturated hydrocarbons.[3–5] In its most developed form, it consists of an iron catalyst in pyridine–aqueous acetic acid, containing metallic zinc, stirred under oxygen or air at room temperature. For convenience we call this the Gif system. It oxidises saturated hydrocarbons selectively in secondary positions; little oxidation at primary positions and almost none at tertiary positions is observed. We now report our first experiments on the oxidation of suitably protected cholestanol derivatives with this system.

Cholesterol was converted into 3β,5α,6β-triacetoxycholestane (**1**) in the usual way. This compound was oxidised to give, as the major product of oxidation, the 20-ketone (**2**) (4.7%), conveniently characterised as its ethylene ketal. An authentic specimen of this latter compound was prepared from pregnenolone acetate. The other major oxidation products were the 15-ketone (**3**) (3.1%) and the 16-ketone (**4**) (2.3%). The evidence for the structures of these compounds is as follows: in the ^{1}H n.m.r. spectra the chemical shifts of the characteristic C-18 and C-19 methyl signals are diagnostic of the substitution pattern. These can be calculated from the corresponding chemical shifts of the parent steroid.[6,7] For the 15-ketone (**3**), the calculated values for the C-18 and C-19 methyl singlets (δ_H 0.76 and 1.20) are in excellent correlation with the observed values (δ_H 0.78 and 1.20); and this was also the case for the 16-ketone (**4**) with the calculated (δ_H 0.85 and 1.23) and observed (δ_H 0.85 and 1.24) values. This evidence is corroborated by molecular rotation difference studies.[8] The 15-ketones are known to possess a large positive value for the molecular rotation difference (+106)[9] whereas the 16-ketones have a large negative value (−507).[10] We found for the 15-ketone (**3**) the value +124 and for the 16-ketone (**4**) −326. The mass spectra of these compounds were also in good agreement with the literature precedents.[11,12]

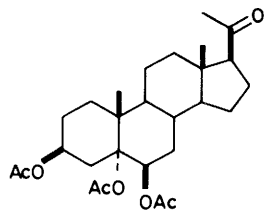
The oxidation of cholestanol acetate (**5**) by the Gif system was also interesting. Again the major product was the 20-ketone, (**6**) (2.0%). The second most important oxidation products were the 6-ketone (**7**) and the 7-ketone (**8**) present in approximately the same amount (1.6% each).

Finally we have examined the oxidation of the ethylene ketal of the 6-ketone (**7**). After separation of starting material, the polar ketonic fraction was converted into the ketal with ethylene glycol. In this way the diketal (**9**) of 3β-acetoxy-5α-pregnane-6,20-dione was obtained (1.2%) and compared with an authentic specimen.

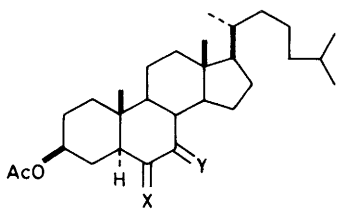
It is clear that the Gif system gives selective oxidation of the cholestane skeleton. We must point out, however, that we have so far only isolated the major products of the oxidation. We are now examining the minor products which are present in limited number. Of course most of the starting material is recovered unoxidised. The yields quoted above are not corrected for recovered starting material.



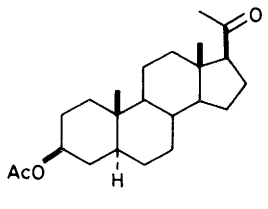
(**1**) X = Y = H_2
(**3**) X = O, Y = H_2
(**4**) X = H_2, Y = O



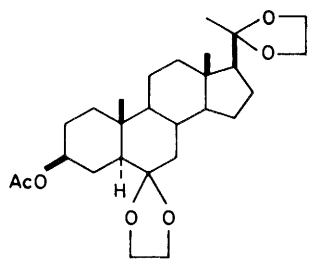
(**2**)



(**5**) X = Y = H_2
(**7**) X = O, Y = H_2
(**8**) X = H_2, Y = O



(**6**)



(**9**)

Experimental

M.p.s were determined with a Kofler hot-stage apparatus and are uncorrected. ^{1}H N.m.r. spectra were obtained for solutions in deuteriochloroform and a 400 MHz Bruker WM 400 instrument was generally used. Optical rotations were measured with a Perkin-Elmer 141 polarimeter in chloroform solutions. I.r. spectra were recorded with a Perkin-Elmer 297 spectrophotometer and mass spectra with various instruments: AEI MS-9, AEI MS-50, Riber 1010. Exact mass measurements were carried

out with a VG 70-35E spectrometer in a dynamic mode. H.p.l.c. was performed with a Waters Associates liquid chromatograph equipped with the Lambda-Max 480 LC spectrophotometer, variable u.v. detector, and the R 401 differential refractometer unit. Preparative normal-phase Ultrasphere-Si and reverse-phase Ultrasphere-ODS, 5μ, 10 mm × 25 cm columns and SDS 'Purex' grade solvents were used in h.p.l.c. work.

T.l.c. was carried out on T.L.C.-Ready-foils F1500/LS 254, and column chromatography with Merck silica gel 60.

5α-*Cholestane*-3β,5α,6β-*triol Triacetate* (**1**).—This was prepared by known literature methods,[13] m.p. 149—150 °C (from methanol); $[\alpha]_D$ −33° (*c* 1.0); $\nu_{max.}(CCl_4)$ 1 720—1 760 cm^{-1} (acetates); δ_H 0.68 (3 H, s, 18-H_3), 0.86 (6 H, d, *J* 7.5 Hz, 26 and 27-H_3), 0.90 (3 H, d, *J* 7.5 Hz, 21-H_3), 1.19 (3 H, s, 19-H_3), 1.99, 2.06, and 2.07 (9 H, 3 s, 3Ac), 2.81 (1 H, m, 4α-H), 4.73 (1 H, m, 3α-H), and 5.85 (1 H, br s, 6α-H); *m/z* 546 (M^+ not observed), 426, and 384 (100%); MNH_4^+, 564 (100%).

3β,5α,6β-*Triacetoxy*-5α-*pregnan*-20-*one Ethylene Ketal*.—A solution of pregnenolene acetate (500 mg) in benzene (100 ml) was heated with ethylene glycol (2.5 ml) and toluene-*p*-sulphonic acid (trace) for 6 h in a Dean–Stark apparatus. After having cooled, the reaction mixture was washed with water, dried (Na_2SO_4), and evaporated. The residue was dissolved in dichloromethane (4 ml) and treated with a solution of 85% *m*-chloroperbenzoic acid (0.5 g) in dichloromethane (6 ml) for 1 h at room temperature.[14] Excess of peracid was decomposed by addition of sodium hydrogen sulphite. The mixture was washed successively with 5% aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and evaporated to yield the crude *epoxide* which without further purification was used in the following steps.

The epoxide was refluxed in acetic acid (35 ml) for 1 h to afford 3β,6β-*diacetoxy*-5α-*hydroxy*-*pregnan*-20-*one* which was subsequently heated in acetic anhydride (10 ml) with toluene-*p*-sulphonic acid (150 mg) at 100 °C for 20 min. After evaporation of the reaction mixture to dryness, the residue was dissolved in benzene (100 ml) and treated with ethylene glycol (2.5 ml) and toluene-*p*-sulphonic acid (trace) in a Dean–Stark apparatus (see above) to reintroduce the partially hydrolysed ketal function. The crude product was purified by column chromatography over silica gel. Elution with toluene–ether (15%) afforded 3β,5α,6β-*triacetoxy*-5α-*pregnan*-20-*one ethylene ketal* (460 mg, 74.7%), m.p. 93—96 °C (from hexane); $[\alpha]_D$ −27° (*c* 0.5); $\nu_{max.}(CHCl_3)$ 907 (C–O) and 1 722, 1 734, and 1 739 cm^{-1} (acetates); δ_H 0.78 (3 H, s, 18-H_3), 1.19 (3 H, s, 19-H_3), 1.28 (3 H, s, 21-H_3), 1.99 (3 H, s, Ac), 2.07 (6 H, s, Ac), 2.79 (1 H, m, 4α-H), 3.85 (4 H, m, OCH_2CH_2O), 4.68 (1 H, m, 3α-H), and 5.80 (1 H, br s, 6α-H); *m/z* 520 (M^+), 505, 400, 358, and 87 (100%); $(MC_4H_7O_2)^+$, 607, 521, 461, 401 (100%), and 341 (Found: C, 67.1; H, 8.6. $C_{29}H_{44}O_8$ requires: C, 66.90; H, 8.52%).

3β-*Acetoxy*-5α-*pregnane*-6,20-*dione*.—Pregnenolone acetate was converted with nitrosyl chloride in dichloromethane into its 5α-chloro-6β-nitro derivative. Without any purification this compound was then treated with anhydrous pyridine at room temperature for 6 h to afford 6-nitropregnenolone acetate.[15] After column chromatography over silica gel with hexane–ether (30%) as eluant, 6-nitropregnenolone acetate was obtained as a crystalline solid, $\nu_{max.}(CCl_4)$ 1 530, 1 520, and 1 360 cm^{-1} (NO_2); δ_H 0.67 (3 H, s, 18-H_3), 1.18 (3 H, s, 19-H_3), and 2.10 and 2.21 (6 H, 2 s, Ac and 21-H_3).

6-Nitropregnenolone acetate as described above was refluxed with excess of zinc powder in glacial acetic acid overnight.[16] Unused zinc was then filtered off and the filtrate was distilled *in vacuo*. The residue was dissolved in ether and washed successively with several portions of 5% aqueous sodium hydrogen carbonate, then with water, dried ($MgSO_4$), and evaporated. The crude product was applied to a column of silica gel and eluted with hexane–ether (50%) to afford 3β-acetoxy-5α-pregnane-6,20-dione, m.p. 154—156 °C (from hexane–ether) (lit.,[17] 157—159 °C); $[\alpha]_D$ +23.5° (*c* 2.0) (lit.,[17] +28° in MeOH); $\nu_{max.}(CHCl_3)$ 1 680—1 730 (acetate and ketones); δ_H 0.61 (3 H, s, 18-H_3), 0.77 (3 H, s, 19-H_3), 2.02 (3 H, s, Ac), 2.12 (3 H, s, 21-H_3), 2.55 (1 H, m, 17α-H), and 4.66 (1 H, m, 3α-H); *m/z* 374 (M^+), 356, 330, 314 (100%), and 299; MNH_4^+, 392 (100%) (Found: C, 73.75; H, 9.1. Calc. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15%).

3β-*Acetoxy*-5α-*pregnane*-6,20-*dione Acetate Bis*(*ethylene Ketal*) (**9**).—3β-Acetoxy-5α-pregnane-6,20-dione (see above) was heated with ethylene glycol and trace amounts of toluene-*p*-sulphonic acid in dry benzene in a Dean-Stark apparatus overnight and the reaction mixture was worked up in the usual manner. Column chromatography of the residue over silica gel with hexane–ether (50%) as eluant afforded pure 3β-*acetoxy*-5α-*pregnane*-6,20-*dione bis*(*ethylene ketal*) (**9**), m.p. 182—184 °C (hexane–ether); $[\alpha]_D$ +1.5° (*c* 1.0); $\nu_{max.}$(KBr) 1 730 (acetate C=O), 1 245 and 1 050 cm^{-1} (C–O–C); δ_H 0.77 (3 H, s, 18-H_3), 0.96 (3 H, s, 19-H_3), 1.29 (3 H, s, 21-H_3), 2.03 (3 H, s, Ac), 3.72—4.04 (8 H, m, 2 × OCH_2CH_2O), and 4.69 (1 H, m, 3α-H); *m/z* 462 (M^+), 444, and 87 (100%); $(MNH_4)^+$, 480, 463 (100%), and 87 (Found: C, 70.4; H, 9.1. $C_{27}H_{42}O_6$ requires C, 70.10; H, 9.15%).

3β-*Acetoxy*-5α-*cholestan*-6-*one* (**7**).—3β-Acetoxy-5α-cholestan-6-one (**7**) was prepared from cholesterol acetate *via* its nitro derivative according to the literature,[16] m.p. 128—130 °C (from methanol–pentane); $[\alpha]_D$ −17.1° (*c* 1.9); $\nu_{max.}(CHCl_3)$ 1 685—1 740 cm^{-1} (acetate and ketone C=O); δ_H 0.63 (3 H, s, 18-H_3), 0.74 (3 H, s, 19-H_3), 0.84 (6 H, dd, *J* 7 Hz, 26- and 27-H_3), 0.88 (3 H, d, *J* 6.5 Hz, 21-H_3), 1.99 (3 H, s, Ac), and 4.65 (1 H, m, 3α-H); MNH_4^+, 462 (100%).

Oxidation of Steroids.—General procedure. In a typical reaction, steroid (2 mmol), zinc (1.31 g, 10 equiv.), iron-cluster[3] (5—8 mg), pyridine (30 ml), water (2 ml), and acetic acid (2.3 ml, 20 equiv.) were placed in a 100-ml conical flask and stirred under the static pressure of an oxygen-filled balloon at room temperature for 5 h.* After the completion of the reaction (no more zinc), the mixture was cooled on an ice-bath and carefully acidified with an ice-cooled solution of 25% sulphuric acid and then extracted with ether (~1 l). The extracts were washed successively with 5% aqueous sodium hydrogen carbonate, saturated aqueous sodium chloride, and water, dried ($MgSO_4$), and evaporated to yield the crude oxidation mixture.

Oxidation of 5α-*Cholestane*-3β,5α,6β-*triol Triacetate* (**1**). The crude oxidation mixture obtained (as described above) was chromatographed on silica gel and eluted with hexane–ether (30%) to give first the unchanged starting material (670 mg, 61.4%). Further elution afforded an impure mixture of 15- and 16-*ketone* compounds and then the 20-*ketone*. Further purifications were affected as follows:

(*a*) By h.p.l.c. [normal phase; hexane–20% ethyl acetate (3 ml min^{-1}) and reverse phase; acetonitrile–5—10% water–0.5% acetic acid (4 ml min^{-1})], the retention time of the 16-ketone being shorter than that of the 15-ketone in both normal- and reverse-phase systems. *The* 15-*ketone* (**3**) (35 mg, 3.1%), m.p. 87—89 °C (from hexane); $[\alpha]_D$ −10° (*c* 2.9); $\nu_{max.}(CCl_4)$ 1 690—1 780 cm^{-1} (acetate and ketone C=O); δ_H 0.78 (3 H, s, 18-H_3), 0.86 (6 H, d, *J* 6.5 Hz, 26- and 27-H_3), 0.99 (3 H, d, *J* 6.5 Hz, 21-H_3), 1.20 (3 H, s, 19-H_3), 1.99, 2.06, and 2.07 (9 H, 3 s, 3 Ac), 2.15, 2.42, and 2.69 (3 H, 3 m, 14α-, 16α-, and 16β-H), 2.83 (1 H, m, 4α-H),

* Some of the earlier oxidation experiments were carried out using iron powder and a carboxylic acid in aqueous pyridine under an atmosphere of oxygen,[3-5] with results similar to those described here.

4.71 (1 H, m, 3α-H), and 5.93 (1 H, br s, 6α-H); *m/z* 560 (M^+ not observed), 398, 356, 314 (100%), 299, 296, 253, 211, 145, and 105; MNH_4^+ 578 (100%) [Found: *m/z*, 314.2197. $C_{21}H_{30}O_2$ (M^+ − 2 CH_3CO_2H − CH_2CO − C_6H_{12}) requires *m/z*, 314.2244] (Found: C, 70.6; H, 9.4. $C_{33}H_{52}O_7$ requires C, 70.68; H, 9.35%). *The 16-ketone* (**4**) (26 mg, 2.3%), m.p. 150—153 °C (from methanol); $[\alpha]_D$ −90.2° (*c* 1.3); $\nu_{max.}(CCl_4)$ 1 695—1 780 cm^{-1} (acetate and ketone C=O); δ_H 0.85 (3 H, s, 18-H_3), 0.87 (6 H, d, *J* 6.5 Hz, 26- and 27-H_3), 0.97 (3 H, d, *J* 6.5 Hz, 21-H_3), 1.24 (3 H, s, 19-H_3), 2.01, 2.09, and 2.11 (9 H, 3 s, 3 Ac), 2.84 (1 H, m, 4α-H), 4.74 (1 H, m, 3α-H), and 5.90 (1 H, br s, 6α-H); MNH_4^+, 578 (100%) (Found: C, 70.7; H, 9.34. $C_{33}H_{52}O_7$ requires C, 70.68; H, 9.35%). *The 20-ketone* (**2**) (45 mg, 4.7%), m.p. 175—177 °C (from benzene–hexane); $[\alpha]_D$ −8.9° (*c* 3.0); $\nu_{max.}(CCl_4)$ 1 705 (ketone C=O), and 1 720—1 770 cm^{-1} (acetate C=O); δ_H 0.63 (3 H, s, 18-H_3), 1.19 (3 H, s, 19-H_3), 1.99, 2.06, 2.08, and 2.10 (12 H, 4 s, 3 Ac and 21-H_3), 2.53 (1 H, t, *J* 8 Hz, 17α-H), 2.80 (1 H, m, 4α-H), 4.71 (1 H, m, 3α-H), and 5.85 (1 H, br s, 6α-H); *m/z* 476 (M^+ not observed), 356, 314 (100%), 299, 281, 159, and 121; MNH_4^+, 494 (100%); [Found: *m/z*, 356.2410. $C_{23}H_{32}O_3$ (M^+ − 2 CH_3CO_2H) requires *m/z* 356.2350] and [Found: *m/z*, 314.2216. $C_{21}H_{30}O_2$ (M^+ − 2 CH_3CO_2H − CH_2CO) requires *m/z* 314.2244] (Found: C, 68.4; H, 8.5. $C_{27}H_{40}O_7$ requires C, 68.04; H, 8.46%).

(*b*) The 20-ketone obtained from column chromatography was dissolved in benzene (50 ml) and the mixture was refluxed with ethylene glycol (1 ml) and toluene-*p*-sulphonic acid (trace) for 4 h in a Dean–Stark apparatus. After having cooled, the reaction mixture was washed with water, dried (Na_2SO_4), and evaporated. Column chromatography over silica gel with toluene–ether (17%) as eluant afforded 3β,5α,6β-triacetoxy-5α-pregnan-20-one ethylene ketal (19 mg), m.p. 93—96 °C, which was identical with the authentic specimen previously described.

Oxidation of 5α-Cholestan-3β-yl Acetate (**5**). The crude oxidation mixture was applied on a column of silica gel and eluted with toluene–ether (0.5%). First, unchanged starting material was recovered (615 mg, 71.5%). Gradual increase of the solvent polarity to toluene–ether (3%) allowed the isolation of the 6- and 7-ketone as a 1:1 mixture (28.1 mg). Separation of the mixture by h.p.l.c. [normal phase; hexane–1.5% isopropyl alcohol (3 ml min^{-1})] gave the 6-ketone (**7**) (14 mg, 1.6%), m.p. 127—130 °C (from methanol) (lit.,[18] 128—130 °C); δ_H 0.63 (3 H, s, 18-H_3) and 0.74 (3 H, s, 19-H_3), which was identical with the 6-ketone previously described; and the 7-ketone (**8**) (14 mg, 1.6%), m.p. 141—144 °C (from ether–methanol) (lit.,[18] 142—145 °C); δ_H 0.64 (3 H, s, 18-H_3) and 1.09 (3 H, s, 19-H_3), which had identical properties when compared with an authentic sample.

Further elution afforded the 20-ketone (**6**) (14.5 mg, 2.0%), m.p. 140—144 °C (from methanol), $[\alpha]_D$ + 73° (*c* 0.3); δ_H 0.60 (3 H, s, 18-H_3), 0.82 (3 H, s, 19-H_3), 2.02 (3 H, s, Ac), 2.10 (3 H, s, 21-H_3), 2.52 (1 H, t, *J* 9 Hz, 17α-H), and 4.69 (1 H, m, 3α-H); *m/z* 360 (M^+) and 300 (100%), whose spectral properties were identical with those of authentic 3β-acetoxy-5α-pregnan-20-one.

Oxidation of 3β-Acetoxy-5α-cholestan-6-one Ethylene Ketal. The crude oxidation mixture was chromatographed on a silica gel column. Elution with benzene–ether (6%) yielded, first, unchanged starting material (850 mg, 87.2%), then a mixture of monoketones and finally an impure fraction of the 20-ketone. This fraction was evaporated and subsequently refluxed in benzene (20 ml) with ethylene glycol (0.8 ml) and toluene-*p*-sulphonic acid (trace) in a Dean–Stark apparatus for 8 h. After the usual work-up the residue was purified by column chromatography over silica gel. Elution with benzene–ether (12%) afforded the diketal (**9**) (12 mg, 1.2%), m.p. 173—180 °C (from hexane) (authentic specimen, 182—184 °C, see above) which was identical in all aspects with the authentic sample.

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