A Novel Synthesis of 3β-Hydroxy-5α-cholest-8(14)-en-15-one

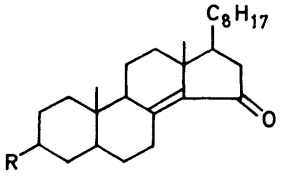
By **Mario Anastasia,* Alberto Fiecchi,** and **Antonio Scala,** Institute of Chemistry, School of Medicine, University of Milan, via Saldini 50, I-20133 Milano, Italy

Jones oxidation of 5α-cholesta-8,14-dien-3β-yl acetate furnishes 9α-hydroxy-15-oxo-5α-cholest-8(14)-en-3β-yl acetate (4a). Treatment of (4a) with zinc dust and sulphuric acid followed by saponification gives 3β-hydroxy-5α-cholest-8(14)-en-15-one. On the basis of chemical and spectroscopic evidence, the product obtained by oxidation of 5α-cholest-8,14-dien-3β-ol is formulated as 9α-hydroxy-5α-cholest-8(14)-ene-3,15-dione (4b) and not as 14α-hydroxy-5α-cholest-8-ene-3,7-dione (3) as reported by others.

A NUMBER of 15-oxygenated sterols act as inhibitors of sterol synthesis in animal cell culture systems.[1,2] In particular, subcutaneous administration to rats of 3β-hydroxy-5α-cholest-8(14)-en-15-one (1a) causes a reduction in serum levels and an inhibition of hepatic sterol synthesis.[3]

A classic chemical approach for the introduction of a 15-oxygen function into a sterol was introduced by Barton and his associates.[4,5] Barton and Laws[5] reported the formation of 15-oxoergosta-8(14),22 dien-3β-yl acetate upon acid treatment of the reaction mixture obtained by the action of perphthalic acid upon ergosta-7,14,22-trien-3β-yl acetate. This path was until now the only practical one.

15-Oxo-5α-cholest-8(14)-en-3β-yl acetate (1b) was first obtained by Wintersteiner and Moore[6] as one component of a mixture of five compounds by chromic acid oxidation of 5α-cholest-8(14)-en-3β-yl acetate.

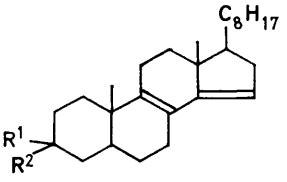


(1)

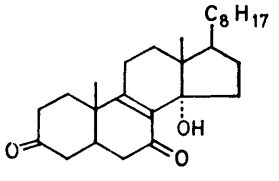
a; R = OH
b; R = $MeCO_2$
c; R = H

More recently Djerassi *et al.*[7] isolated 5α-cholest-8(14)-en-15-one (1c) as a by-product of the oxidation of 5α-cholest-7-ene with peracids followed by treatment with mineral acids.

In this paper we report a simple route for high-yield preparation of 3β-hydroxy-5α-cholest-8(14)-en-15-one (1a) starting from 5α-cholesta-8,14-dien-3β-yl acetate (2b). The idea for this new synthesis originated from a recent communication by Whalley *et al.*[8] According to these authors, Jones or Sarett oxidation of 5α-cholesta-8,14-dien-3β-ol (2a) gives substantial amounts of a ketone provisionally formulated as 14α-hydroxy-5α-cholest-8-ene-3,7-dione (3). In our opinion, the spectral properties reported for the enedione (3) are not in line with the proposed structure. In particular, the u.v. absorption maximum (260 nm) indicates an 8(14) double bond in the molecule[7] and the frequency of the carbonyl absorption in the i.r. spectrum ($\nu_{max.}$ 1 689 cm^{-1}) appears too high for a Δ^8-en-7-one.[9] In addition the



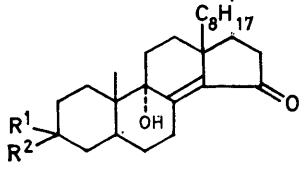
(2)



(3)

a, R^1 = β-OH; R^2 = H
b, R^1 = β-$MeCO_2$; R^2 = H
c, R^1R^2 = O

signal observed in the ^1H n.m.r. spectrum at δ 3.95 suggests the presence of an allylic proton deshielded by a carbonyl group. A $\Delta^{8(14)}$-15-one system would more reasonably agree with the reported properties. According to this interpretation, Jones oxidation of an 8,14-diene system should give a 15-oxo-unsaturated sterol with a hydroxy-group probably in an allylic position. We performed the Jones oxidation at 10 °C on 5α-cholesta-8,14-dien-3β-yl acetate (2b) and obtained an oxo-acetate to which we attributed the structure of 9α-hydroxy-15-oxo-5α-cholest-8(14)-en-3β-yl acetate (4a). The elemental analysis and mass spectrum were in accord with the molecular formula ($C_{29}H_{46}O_4$). The



(4)

a; R^1 = β-$MeCO_2$; R^2 = H
b; R^1R^2 = O

u.v. spectrum showed the presence of an αβ-unsaturated ketone group ($\lambda_{max.}$ 254 nm). The i.r. spectrum [$\nu_{max.}$ 1 703 (αβ-unsaturated ketone group in a five-membered ring) and 1 730 cm^{-1} (acetate)] confirmed this, and

further indicated the presence of a hydroxy-group ($\nu_{max.}$ 3 460 cm^{-1}). The latter was shown to be tertiary by its stability to a further excess of Jones reagent and to esterification. Moreover the hydroxy-group was shown to be contained in a CO·C=C·CR(OH) system by reduction with zinc dust and acetic acid to 15-oxo-5α-cholest-8(14)-en-3β-yl acetate (1b). The location at C-9 and the α-configuration of the hydroxy-group was deduced from the ^1H n.m.r. spectrum of the enone (4a). The positions of the 10- and 13-methyl signals were all within 0.04 p.p.m. of the values calculated by addition to the values of the 10- and 13-methyl groups of 5α-cholest-8(14)-en-15-one [7] (1c) of the shifts due to 3β-acetoxy- and 9α-hydroxy-groups.[10]

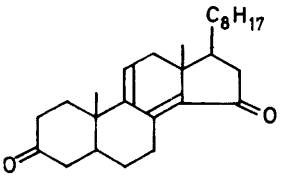
Moreover, the ^1H n.m.r. spectrum displayed a one proton broad doublet (*J ca.* 14 Hz) at δ 3.95, which, although at a higher frequency than expected, undoubtedly corresponds to the 7β-proton; a molecular model reveals that this (allylic) proton lies precisely within the plane of the C-15 carbonyl group, and sufficiently close to be deshielded by it. After exchange with deuterium the ^1H n.m.r. spectrum of the labelled derivative confirmed that one of the exchanged protons is responsible for the downfield signal discussed.

Also in line with the proposed structure was the high optical rotation ($[\alpha]_D$ +153°); in fact many 3β-oxygenated derivatives of 5α-cholest-8(14)-en-15-one (1c) are strongly dextrorotatory.[6,7]

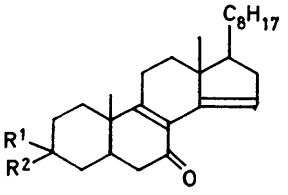
Reduction of (4a) with zinc dust and sulphuric acid in methanol afforded 15-oxo-5α-cholest-8(14)-en-3β-yl acetate (1b) in high yield; saponification completed the synthesis of 3β-hydroxy-5α-cholest-8(14)-en-15-one (1a).

Upon repeating the Jones oxidation * on 5α-cholesta-8,14-dien-3β-ol (2a) we isolated by crystallization a compound, the m.p. and n.m.r. spectrum of which were similar to those given for the supposed compound (3). The u.v. spectrum of our compound showed $\lambda_{max.}$ 254 nm (ε 12 600) in contrast with the reported value (260 nm) for (3); $[\alpha]_D^{20}$ was +182° in contrast to the reported value of +73° for (3). The structure (4b) was attributed to the oxidation product on the basis of its spectral and chemical properties. The positions of the 10- and 13-methyl signals in its ^1H n.m.r. spectrum are in accord with the calculated values. The i.r. frequency of its unsaturated carbonyl group ($\nu_{max.}$ 1 695 cm^{-1}) is low for such a cyclopentenone derivative but agrees with the value observed by Djerassi *et al.*[7] for the carbonyl group of (1c).

Treatment of the compound (4b) with toluene-*p*-sulphonic acid in boiling benzene for 10 min gave a product of molecular formula $C_{27}H_{40}O_2$ (elemental analysis and mass spectrum) and showing i.r. bands at 1 718 (six-membered ring C=O) and 1 690 cm^{-1} (αβ-unsaturated five-membered ring C=O); the u.v. spectrum showed $\lambda_{max.}$ 324 nm (ε 4 300) due to a dienone in which the second double bond is in conjugation with the original αβ-unsaturated ketone. Its ^1H n.m.r. spectrum showed a typical broad peak at δ 4.05 (1 H, d, *J* 18 Hz) for the 7β-allylic proton deshielded by the 15-ketone, and a double doublet at δ 5.85 (1 H, J_{AX} 7, J_{BX} 3 Hz) due to the C-11 vinylic proton. These data strongly suggested that the dehydrated compound has formula (5).



(5)



(6)

a; R^1 = β—OH; R^2 = H
b; R^2 = β—$MeCO_2$; R^2 = H
c; R^1R^2 = O

Whalley *et al.*[8] reported that treatment of the supposed 14α-OH compound (3) gave, with phosphoryl chloride–pyridine, a product devoid of hydroxy-groups, for which the structure of 5α-cholesta-8,14-diene-3,7-dione (6c) was suggested. We have synthesized the compound (6c) from the well known 7-oxo-5α-cholesta-8,14-dien-3β-yl acetate (6b),[6,11] by saponification and Sarett oxidation. The compound (6c) showed $\lambda_{max.}$ 224 (ε 16 000) and 29 nm (ε 5 000), $\nu_{max.}$ 1 720 and 1 675 cm^{-1}, and a signal integrating for one proton at δ 6.58 (for the 15-H) in the ^1H n.m.r. spectrum. The observed chemico-physical properties of (6c) completely agree with those described for (6b),[6,11] while strongly differing from those reported by Whalley *et al.* for (3).[8] In our hands treatment of (4b) with $POCl_3$–pyridine gave unidentified products.

The Scheme shows what we consider to be the most probable mode of formation of the observed product of Jones oxidation of a sterol containing an 8,14-diene system; chromic acid initially forms a 14α,15α-epoxide.[12] Protonation of the bridged oxygen followed by nucleophilic attack at C-9 by water, from the rear face of the molecule, then generates an allylic diol, which, on oxidation of the secondary hydroxy-group, produces the αβ-unsaturated ketone.

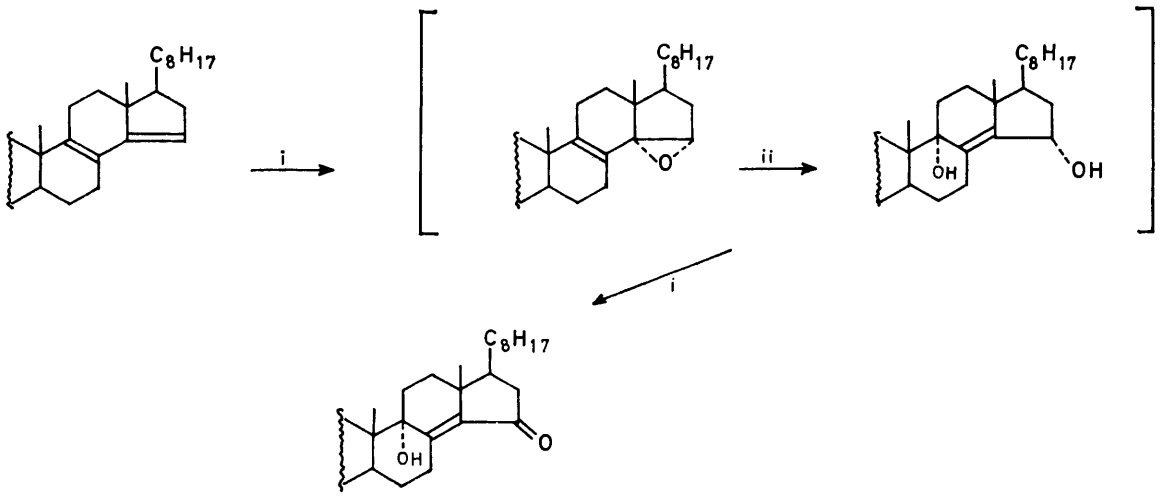
The reported route appears to be general in scope and useful for planning syntheses of oxygenated steroids.

The biological relevance of compounds (4a), (4b), and (5) is under investigation.

EXPERIMENTAL

I.r. spectra were obtained for solutions in chloroform with a Perkin-Elmer 257 spectrometer. ^1H N.m.r. spectra were determined on Varian HA-100 and XL 100 spectrometers for solutions in deuteriochloroform with tetramethylsilane as internal reference. Routine optical rotations were recorded with a Perkin-Elmer model 141 spectropolarimeter for solutions in chloroform. Mass spectra were determined with an LKB 9000 gas chromatograph–mass spectrometer

* In our hands oxidation of compound (2a) with chromium trioxide in pyridine over 48 h gave the 3-ketone (2c) in high yield. No trace of the supposed compound (3) was formed, in contrast to the results of Whalley *et al.*,[8] who reported a 91% yield of the latter compound.



SCHEME Reagents: i, Jones reagent; ii, H^+, H_2O

operating at 20 eV by use of the direct inlet system. The progress of all reactions and column chromatographies (silica gel G–celite 50 : 50 v/v) was monitored by t.l.c. [silica gel G (HF_{254}) microplates], and g.l.c. (2 m silanized glass column; 3% SE 30 on Gas Chrom Q support; 240—260 °C). Column chromatography was carried out with benzene as eluant on silica gel G–Celite (50 : 50 v/v).[5]

9α-Hydroxy-15-oxo-5α-cholest-8(14)-en-3β-yl Acetate (4a).—Jones reagent was added, at 10 °C, to a solution of 5α-cholesta-8,14-diene-3β-yl acetate (2b) (1 g) in acetone (75 ml) until present in excess. The *product* (4a), recovered by extraction with dichloromethane, was crystallized from di-isopropyl ether (yield 0.70 g) and had m.p. 194—195°; $[\alpha]_D^{20}$ +153°, $\nu_{max.}$ 3 460, 1 730, 1 703, and 1 630 cm^{-1}; $\lambda_{max.}$ 254 nm (ε 13 000); δ 3.95br (1 H, d, *J ca.* 14 Hz, H-7β), 0.97 (3 H, s, 18-H_3; calc:[7,10] 0.968), and 0.82 (3 H, s, 19-H_3; calc:[7,10] 0.862); *m/e* 458 (M^+) (Found: C, 76.2; 10.2. $C_{29}H_{46}O_4$ requires C, 75.9; H, 10.1%).

The ketone (4a) was refluxed for 60 h in methan[^{2}H]ol, in the presence of sodium methoxide. After usual work-up and reacetylation, tetradeuteriated (4a) was obtained (M^+ 462), lacking the broad one proton doublet at δ 3.95.

15-Oxo-5α-cholest-8(14)-en-3β-yl Acetate (1b).—Zinc dust (1 g) was added in four portions during 2 h to a stirred solution of the steroid (4a) (350 mg) in methanol (50 ml). 98% Sulphuric acid (0.5 ml) in methanol (10 ml) was added dropwise at room temperature during the same time. Work-up and crystallization from methanol afforded *plates* (0.75 g), m.p. 134—135°;[6] $[\alpha]^{20}$ +118°; $\nu_{max.}$ 1 743, 1 705, and 1 630 cm^{-1}; $\lambda_{max.}$ 260 nm (ε 12 500); δ 4.18br (1 H, d, *J ca.* 14 Hz, H-7β), 0.98 (3 H, s, 18-H_3; calc:[7,10] 0.968), and 0.72 (3 H, s, 19-H_3; calc:[7,10] 0.720); *m/e* 442 (M^+) (Found: C, 78.5; H, 10.6. $C_{29}H_{46}O_3$ requires C, 78.7; H, 10.5%).

The same product was obtained when (4a) (200 mg) in acetic acid (30 ml) was refluxed with zinc dust (500 mg) for 1 h, and the reaction mixture was chromatographed.

3β-Hydroxy-5α-cholest-8(14)-en-15-one (1a).—Saponification of the acetate (1b) with methanol–potassium hydroxide gave the alcohol (1a), m.p. 145—146° (lit.,[6] 145—146°), identical (n.m.r. and mass spectra) with an authentic sample[6] (Found: C, 80.7; H, 11.1. Calc. for $C_{27}H_{44}O_2$: C, 80.9; H, 11.1%).

9α-Hydroxy-5α-cholest-8(14)-ene-3,15-dione (4b).—5α-Cholesta-8,14-dien-3β-ol (2a) (1 g) was oxidized by Jones reagent as described for (1b). The *product* had m.p. 204—205° (dec.) (from benzene–hexane or di-isopropyl ether); $[\alpha]_D^{20}$ +182°; $\nu_{max.}$ 3 430, 1 720, 1 695, and 1 625 cm^{-1}; $\lambda_{max.}$ 254 nm (ε 12 600); δ 3.97br (1 H, d, *J ca.* 13 Hz, H-7β), 1.05 (3 H, s, 19-H_3; calc:[7,10] 1.054), and 0.99 (3 H, s, 18-H_3; calc:[7,10] 1.002); *m/e* 414 (M^+) (Found: C, 78.4; H, 10.5. $C_{27}H_{42}O_3$ requires C, 78.2; H, 10.2%).

Treatment of 9α-Hydroxy-5α-cholest-8(14)-ene-3,15-dione (4b) *with Toluene-p-sulphonic Acid.*—Toluene-*p*-sulphonic acid (0.1 g) in benzene (40 ml) was refluxed and part of the solvent (20 ml) was distilled off. The enone (4b) (0.3 g) was added and the solution refluxed for 10 min. The pale yellow solution was worked up in the usual way to afford *5α-cholesta-8(14),9(11)-diene-3,15-dione* (5) (0.2 g), m.p. 139—140° (from di-isopropyl ether); $[\alpha]_D^{20}$ +82°; $\nu_{max.}$ 1 718, 1 690, and 1 640 cm^{-1}; $\lambda_{max.}$ 324 (ε 4 300) and 233 nm (ε 11 000); δ 5.85 (1 H, d, J_{AX} 7 Hz, J_{BX} 3 Hz, H-11), 4.05br (1 H, dd, *J ca.* 18 Hz, H-7β), 1.13 (3 H, s, 19-H_3; calc:[7,10] 1.054). and 0.95 (3 H, s, 18-H_3; calc.:[7,10] 0.935); *m/e* 396 (M^+) (Found: C, 82.1; H, 10.1. $C_{27}H_{40}O_2$ requires C, 81.8; H, 10.2%).

5α-Cholesta-8,14-diene-3,7-dione (6c).—3β-Hydroxy-5α-cholesta-8,14-dien-7-one (6a) {$[\alpha]_D^{20}$ −22°; $\nu_{max.}$ 3 400, 1 668, and 1 605 cm^{-1}; $\lambda_{max.}$ 298 (ε 5 000) and 224 nm (15 600); δ 6.4 (1 H, m, 15-H), 1.13 (3 H, s, 19-H_3; calc:[9,10] 1.18) and 0.81 (3 H, s, 18-H_3; calc:[9,10] 0.83)} (1 g) dissolved in pyridine (30 ml) was oxidized with chromium trioxide (0.6 g) in pyridine (2 ml) over 12 h. Usual work-up afforded *5α-cholesta-8,14-diene-3,7-dione* (6c) (0.8 g), m.p. 175°; $[\alpha]_D^{20}$ 0°; $\nu_{max.}$ 1 720 and 1 675 cm^{-1}; $\lambda_{max.}$ 298 (ε 5 000) and 224 nm (15 000); δ 6.5 (1 H, m, 15-H), 1.35 (3 H, s, 19-H_3; calc:[9,10] 1.35), and 0.83 (3 H, s, 18-H_3; calc:[9,10] 0.86); *m/e* 396 (M^+) (Found: C, 81.6; H, 10.5. $C_{27}H_{40}O_2$ requires C, 81.8; H, 10.2%).

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