# Stereochemistry of the Reduction of 3β-Benzoyloxycholest-5-en-4-one with Sodium Borohydride

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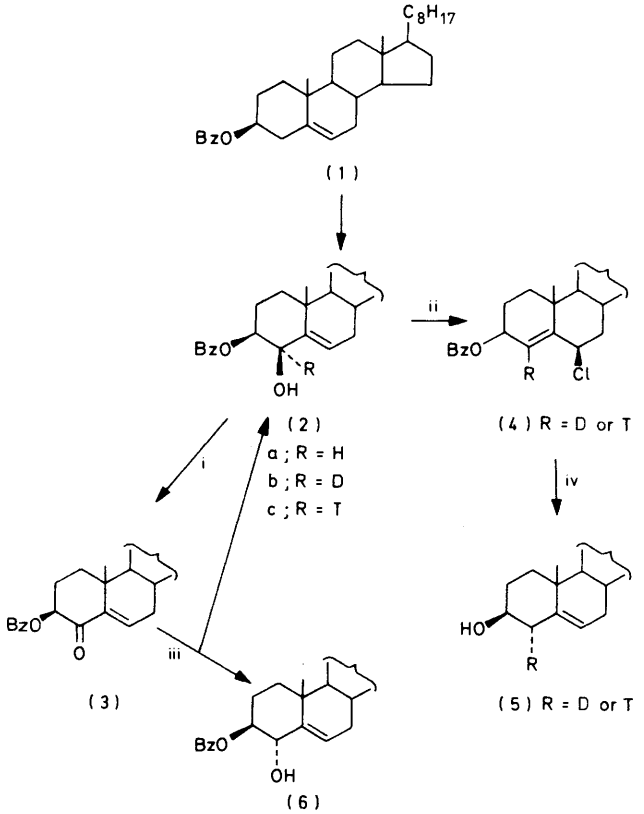
Reduction of 3β-benzoyloxycholest-5-en-4-one (3) with sodium borohydride under various conditions affords only minor amounts of 3β-benzoyloxycholest-5-en-4β-ol (2a). The major product is the 4α-isomer (6). Using sodium borodeuteride it has been shown that the 3β-benzoyloxycholest-5-en-4β-ol obtained is deuteriated at the 3α-position in relatively alkaline conditions, but at 4α- under conditions near neutrality. A plausible mechanism has been proposed for this interesting result.

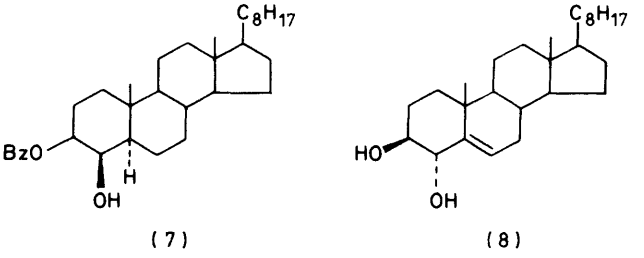
THE use of 4α- and 4β-labelled steroids in the study of $\Delta^5$-3-keto-isomerases and in related biosynthetic work is well established;[1-4] 4α-labelled cholesterol in particular has been used several times,[1a,2a,3,4] usually prepared by the route outlined in Scheme 1.[5,6]




SCHEME 1 *Reagents*: i, $MnO_2$ or $Ph_3BiCO_3$; ii, $SOCl_2$–pyridine; iii, $NaBH_4$; iv, $LiAlH_4$

3β-Benzoyloxy- (or -acetoxy-) cholest-5-ene (1) is treated with selenium dioxide in acetic acid [7] to give the allylic alcohol (2a) in 20—40% yield. The free hydroxy-function is then oxidised with chromium trioxide–pyridine to give the enone (3) in low yield (*ca.* 20%). As we now report, higher yields can be obtained by using freshly prepared manganese dioxide (50%) or, better still, with triphenylbismuth carbonate [8] (70%). The enone is then reduced back to the alcohol (2b or c) with suitably labelled sodium borohydride. Treatment with thionyl chloride–pyridine gives the 4-labelled 3β-benzoyloxy-6β-chlorocholest-4-ene (4), which, on reduction with lithium aluminium hydride, furnishes the 4α-labelled cholesterol (5).[5,6]

The crucial step in this sequence is the reduction of the enone (3), which is used to introduce the label. This step was originally reported as occurring cleanly and in high yield.[5] A good yield has also been reported for a similar reduction in the androstane series.[1a] In our hands, however, the reduction gave the 4α-hydroxy-epimer (6) as the major product. The desired alcohol (2) was produced in minor amounts (3—5%). A similar observation was made earlier in the androstane series.[9] A search of the literature revealed that other workers [2a,4,6] also obtained low yields (10—13%) of the 4β-hydroxy-derivative (2), but the nature of the other products was apparently not investigated. The initial conditions described [5] ($NaBH_4$–dry dioxan) were first tried without success; the reaction was slow and did not occur cleanly. Crude analysis of the complex mixture obtained showed




the presence of only 3—4% of the 4β-hydroxy-derivative (2a). The major product was the 4α-epimer (6), isolated in 20% yield. Its structure was easily deduced *via* n.m.r. spectroscopy and by reoxidation to the enone (3). In contrast to the allylic alcohol (2a), the oxidation of the epimer (6) was rapid and almost quantitative. Other products isolated from the reduction included the saturated alcohol (7) (17%), resulting from an initial 1,4-reduction of the enone (3), and the diol (8) (10%).

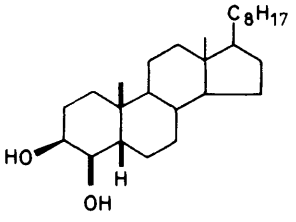
Other solvent systems were also investigated. In

protic media, the reduction was fast and relatively clean but, in every case, the major product was the undesired 4α-hydroxy-derivative (6). Other reducing agents gave similar results (summarized in the Table).

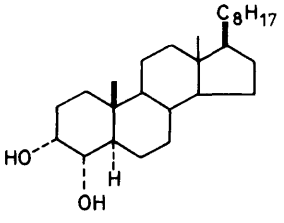
Products from the reduction of the enone (3) with various reagents

| Solvent | Reducing agent | Yield (%) 4β-OH (2) | 4α-OH (6) | Other products |
|---|---|---|---|---|
| Dry dioxan | $NaBH_4$ | 3—4 | 20 | (7) 18, (8) 7—8 |
| Dioxan (aged) | $NaBH_4$ | Traces | 36 | (7) 30 |
| Dioxan + $H_2O$ (5%) | $NaBH_4$ | Traces | 60 | (7) 30 |
| THF–MeOH | $NaBH_4$ | 3—5 | *ca.* 90 | |
| THF–10% HCl | $NaBH_3CN$ | | *ca.* 90 | |
| Diethyl ether | $Zn(BH_4)_2$ | | 100 | |

Commercial sodium borohydride samples contain varying amounts of sodium methoxide, a side-product in its production [$4NaH + B(OMe)_3 \longrightarrow NaBH_4 + 3$ NaOMe]. We therefore investigated the effect of added base on the course of the reduction. Addition of methanolic sodium hydroxide to the reaction medium resulted in a slight increase in the yield of the 4β-hydroxy-compound (2) (to 10%). More significantly, however, the 4α-epimer (6), normally the major product, was almost absent. The diols (9) (10—15%) and (10) (30—35%) were the principal products.




(9)




(10)

This unexpected result could be explained by postulating a different mechanism for the reduction which operates within a particular pH range (Scheme 2). Base-catalysed enolisation, followed by protonation from the α-side, shifts the benzoyloxy-group to the 4-position to give 4β-benzoyloxycholest-5-en-3-one (11). Reduction furnishes the 4β-benzoyloxycholest-5-en-3β-ol (12) which, in basic media, is in equilibrium with its isomer (2). The keto-steroid (11) can also rearrange to the enone (13), which is then reduced to a complex mixture.

In the absence of the reducing agent the enone (13) could be isolated in 45% yield; but perhaps the most compelling evidence for the proposed mechanism comes from the observation that, when sodium borodeuteride is used, the 4β-hydroxy-derivative isolated is deuteriated at the 3α-position. The label is very easily located by 400 MHz n.m.r. spectroscopy. The complex signal at δ 4.95 from the 3α-proton disappears and the doublet at δ 4.36 collapses to a singlet. When the reduction is carried out without deliberate addition of base, using sodium borodeuteride from a freshly opened bottle, the small amount of 4β-allylic alcohol (5%) isolated is deuteriated at the expected 4α-position (2b).

In conclusion, it appears that the course of the reduction is dependent on the age and quality of the borohydride used. Caution must therefore be exercised when using this reaction in the preparation of labelled substrates for mechanistic or biochemical studies. A more certain route to 4α-labelled cholesterol is by the method introduced by Marquet *et al.*[9]

These results are compatible with part of the earlier literature,[2a,4,6] but are not in agreement with earlier results [5] obtained by one of us, nor with the yield of the analogue of the tritiated derivative (2c) recently reported by Akhtar and his colleagues.[1a]

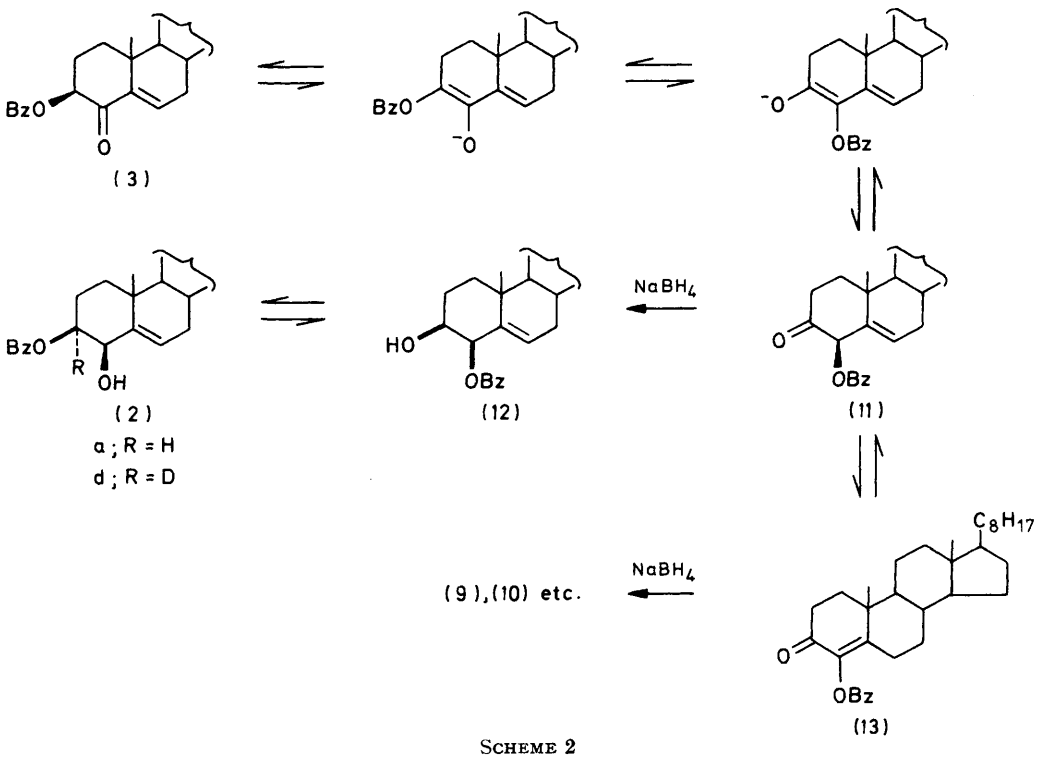
## EXPERIMENTAL

M.p.s were measured on a Köfler hot-plate and are uncorrected. N.m.r. spectra were run in deuteriochloroform with tetramethylsilane as internal standard. Optical rotations are for chloroform solutions (*C* 1.0).

3β-*Benzoyloxycholest*-5-*en*-4β-*ol* [7] (2a).—To a suspension of 3β-benzoyloxycholest-5-ene (1) (10 g) in acetic acid (100 ml) was added selenium dioxide (2 g) in water (1 ml) and acetic acid (100 ml). The mixture was heated under reflux for 5 min. Solid sodium acetate (7.5 g) was added and the hot solution filtered through Celite. The crystalline solid which separated on cooling was filtered off and washed with a little hot acetone and dried, yield 2 g (*ca.* 20%), m.p. 208—210 °C (from AcEOt–MeOH), $[\alpha]_D$ −29.7° (lit.,[7] m.p. 210 °C, $[\alpha]_D$ −30.7); $\nu_{max.}$ ($CHCl_3$) 3 590, 1 720, and 1 270 $cm^{-1}$; δ 5.7 (1 H, m, 6-H), 4.95 (1 H, ddd, seen as a doublet of a triplet, *J* 12 Hz, *J* 4 Hz, 3α-H), and 4.36 (1 H, d, *J* 4 Hz, 4α-H); *m/e* 506 ($M^+$).

3β-*Benzoyloxycholest*-5-*en*-4-*one* (3).—(*a*) *Chromium trioxide.*[5] To a suspension of the alcohol (2a) (1.5 g) in pyridine (1.5 ml) was added 75 ml of $CrO_3$ (10 g) in water (10 ml) and pyridine (100 ml). The resulting solution was kept at 50—55 °C for 4 h, poured into water, and extracted with diethyl ether. The crude product (1.4 g) was purified by chromatography ($SiO_2$; $CH_2Cl_2$ as eluant) to give the pure enone (3) (250 mg, 16%), m.p. 158—160 °C (acetone), $[\alpha]_D$ −38°.

(*b*) *Manganese dioxide* ($MnO_2$).[10] A mixture of the alcohol (2a) (300 mg) and freshly prepared $MnO_2$ (3 g) in dichloromethane (40 ml) was stirred at room temperature overnight. The suspension was filtered through Celite, the solvent evaporated, and the residue purified by chromatography ($SiO_2$; chloroform–hexane, 2 : 1 as eluant) to give pure enone (3) (130 mg, *ca.* 50% based on recovered starting material).




SCHEME 2

(*c*) *Triphenylbismuth carbonate* ($Ph_3BiCO_3$). A mixture of the alcohol (2a) (1.0 g), $Ph_3BiCO_3$ (1.2 g), and anhydrous sodium carbonate (1.0 g) in wet tetrahydrofuran (THF) (20 ml with 1 drop of water) was heated under reflux for 18 h. A further portion of $Ph_3BiCO_3$ (1 g) was added and the refluxing continued for another 6 h. Filtration through Celite, evaporation, and purification of the residue by chromatography gave the pure enone (3) (700 mg, 70%).

*Reduction of the Enone* (3).—(*a*) *Sodium borohydride* ($NaBH_4$)–*dry dioxan*.[5] To the enone (3) (200 mg) in dry dioxan (20 ml, freshly distilled from sodium diphenylketyl) was added solid $NaBH_4$ (24 mg). The reaction mixture was stirred overnight at room temperature and then poured into brine, extracted with dichloromethane, and the organic layer washed with water and dried. Evaporation and chromatography of the residue ($SiO_2$; $CH_2Cl_2$, then chloroform–diethyl ether, 8 : 2 for the more polar fractions) gave 3β-benzoyloxy-5α-cholestan-4β-ol (7) (35 mg, *ca.* 17%), m.p. 242—246 °C; δ 4.92 (1 H, m, 3α-H) and 3.92 (1 H, m, 4α-H); the 4β-alcohol (2a), (7 mg, 3—4%), 3β-benzoyloxycholest-5-en-4α-ol (6) (40 mg, 20%), m.p. 202—204 °C (from acetone), $[\alpha]_D$ +1.9° $\nu_{max.}$ ($CHCl_3$) 3 590, 1 720, and 1 270 $cm^{-1}$; δ 5.93 (1 H, m, 6-H), 4.77 (1 H, m, 3α-H), and 4.39 (1 H, br d, *J* 12 Hz, 4α-H) (Found: C, 80.3; H, 9.9. $C_{34}H_{50}O_3$ requires C, 80.58; H, 9.94%); cholest-5-ene-3β,4α-diol (8) (15 mg, *ca.* 10%), m.p. 218—220 °C (lit.,[11] m.p. 222—223 °C); δ 5.70 (1 H, m, 6-H), 4.05 (1 H, br d, 4α-H), and 3.3 (1 H, m, 3α-H).

(*b*) $NaBH_4$–*wet dioxan*. To the enone (3) (40 mg) in dioxan containing 5% water (4 ml) was added solid $NaBH_4$ (25 mg). After 2 h at room temperature, the reaction mixture was worked up in the usual manner and purified by chromatography to give the 4α-alcohol (6) (24 mg, 60%) and the alcohol (7) (12.6 mg, 30%).

(*c*) $NaBH_4$, *THF–methanol*. Solid $NaBH_4$ (5 mg) was added to the enone (3) (20 mg) in THF–methanol (1 : 1; 2 ml). After *ca.* 10—15 min at room temperature, the reaction mixture was poured into water (5 ml) and extracted with dichloromethane (2 × 5 ml). The combined organic phase was dried and evaporated to give a white crystalline solid (18.8 mg, *ca.* 90%) consisting of the 4α-alcohol (6) with a trace of the 4β-epimer (2a).

Repetition of this reduction on a 200-mg scale using sodium borodeuteride from a freshly opened bottle and careful separation by chromatography ($SiO_2$; dichloromethane–pentane, 8 : 2) afforded the minor 4β-alcohol (2b) (10 mg, *ca.* 5%) with deuterium in the 4α-position; δ 5.7 (1 H, m, 6-H) and 4.95 (1 H, simplifies to dd, *J* 12 Hz, *J* 4 Hz); the signal at δ 4.39 disappears; *m/e* 507 ($M^+$).

(*d*) *Zinc borohydride* [$Zn(BH_4)_2$]–*diethyl ether*. To the enone (3) (26 mg) in diethyl ether (2 ml) was added $Zn(BH_4)_2$ in diethyl ether (*ca.* 0.1M; 1 ml). After 15 min at room temperature, acetone (0.5 ml) was added, and the solution filtered a few minutes later through a short silica-column. Evaporation gave pure 4α-alcohol (6) (25.9 mg, 100%).

(*e*) *Sodium cyanotrihydroborate*(1−)($NaBH_3CN$)–*THF–hydrochloric acid* (10%). The pH of a mixture of the enone (3) (27 mg) and $NaBH_3CN$ (25 mg) in THF (3 ml) was lowered, by addition of hydrochloric acid (10%), to *ca.* 2, and was occasionally adjusted as the reaction progressed. After 2 h at room temperature a further portion of $NaBH_3CN$ (25 mg) was added. The pH was again adjusted and the mixture left overnight. It was poured into water and extracted with dichloromethane. The organic layer was dried and evaporated to give the pure 4α-alcohol (6) (25 mg, 90%).

(*f*) $NaBH_4$, *THF–methanol and added base*. To the enone (3) (220 mg) in THF (20 ml) was added methanolic sodium hydroxide (50 mg NaOH in 20 ml MeOH) followed by solid $NaBH_4$ (70 mg). The mixture was stirred at room tempera-

ture for 30 min, poured into half-saturated brine (20 ml), and extracted with diethyl ether–dichloromethane (9 : 1). The organic layer was washed with water, dried, and evaporated. Chromatography of the residue ($SiO_2$; dichloromethane–hexane, 2 : 1, followed by $CH_2Cl_2$, followed by $CH_2Cl_2$–$Et_2O$ gave the 4β-alcohol (2a) (18.5 mg, *ca.* 9%), m.p. and mixed m.p. 208—211 °C. Other compounds isolated included 5β-cholestane-3β,4β-diol (9) (10—15%), m.p. 189—192 °C; the diacetate had m.p. 145—149 °C (lit.,[12] m.p. 193—194 °C, diacetate m.p. 145—148 °C); and 5α-cholestane-3α,4α-diol (10) (30—35%), m.p. 212—215 °C; the diacetate had m.p. 156—161 °C (lit.,[11] m.p. 212—214 °C, diacetate, m.p. 162—164 °C).

When the above reduction was repeated using sodium borodeuteride, the 4β-alcohol isolated was deuteriated at the 3α-position; δ 5.7 (1 H, m, 6-H) and 4.38 (1 H, s, 4α-H); the signal at 4.95 had disappeared; *m/e* 507 ($M^+$).

*Oxidation of the* 4α-*Alcohol* (6) *to the Enone* (3).—A mixture of the alcohol (6) (39 mg), $Ph_3BiCO_3$ (100 mg), and sodium carbonate (100 mg) in THF (4 ml) was heated under reflux for 2–3 h, cooled, and filtered through Celite. Evaporation of the filtrate and purification of the residue by preparative layer chromatography ($SiO_2$; dichloromethane–hexane, 3 : 1) gave the pure enone (38.3 mg, *ca.* 95%), m.p. 160—161 °C (from acetone), mixed m.p. 157—161 °C.

4-*Benzoyloxycholest*-4-*en*-3-*one* (13).—To the enone (3) (200 mg) in THF (8 ml) was added sodium hydroxide (3 mg) in methanol (8 ml). After 25 min at room temperature, the light-yellow solution was poured into water and extracted with dichloromethane. The organic layer was dried, evaporated, and the residue purified by chromatography ($SiO_2$; dichloromethane–hexane, 1 : 1) to give the pure enone (13) (92 mg, 46%) as an oil. The oil solidified to a white powder after being left on a watch glass for some time, m.p. 56—59 °C, $[\alpha]_D$ +91° (*C* 2); $\lambda_{max.}$ (hexane) 232 nm (ε 48 000); $\nu_{max.}$ 1 725, 1 680, and 1 620 $cm^{-1}$; δ 8.0 and 7.5 (5 H, br, aromatic H), 1.3 (3 H, s, 19-Me), and 0.9 (3 H, s, 18-Me); *m/e* 504 ($M^+$) (Found: C, 78.45; H, 9.4. $C_{34}H_{48}O_3 \cdot H_2O$ requires C, 78.12; H, 9.64%).

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