A Ready Synthesis of 5α,14β-Cholest-7-en-3β-ol

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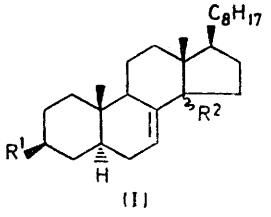
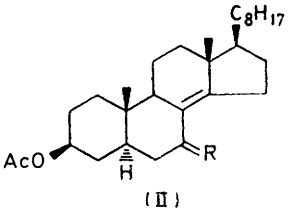
Reduction of 3β-acetoxy-8α,14α-epoxy-5α-cholestan-7-one or 3β-acetoxy-5α-cholest-8(14)-en-7-one with zinc dust in methanolic N-sulphuric acid yields 3β-acetoxy-5α,14β-cholest-7-ene with minor amounts of 3β-acetoxy-5α,14β-cholestan-7-one, and constitutes a novel, simple synthesis of 14β-steroids.

PREVIOUS work on the intermediary role of some sterols in the biosynthesis of cholesterol indicates that the presence of a *cis*-BC-ring junction, rather than the *trans*-ring junction of natural compounds, does not prevent the conversion of a sterol into cholesterol by liver enzymes.[1] On the basis of this observation we considered that sterols with a *cis*-CD-ring junction might also be enzymically transformed. In addition, sterols with stereochemistry different from that of natural compounds could inhibit the biosynthesis of cholesterol, as has been shown for the triterpenoid euphol, which differs from lanosterol only in stereochemistry.[2] We therefore became interested in the synthesis of diastereoisomers of cholesterol precursors; in particular we considered that 5α,14β-cholest-7-en-3β-ol (Ia), having a *cis*-CD-ring junction, would be useful for biological studies since 'natural' 5α-cholest-7-en-3β-ol (Ib) is transformed [3] into



(I)

	R^1	R^2
a;	OH	β-H
b;	OH	α-H
c;	H	β-H
d;	OAc	β-H
e;	OAc	α-H



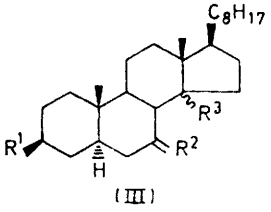
(II)

	R
a;	:O
b;	H_2
c;	H, α-OAc
d;	H, β-OH

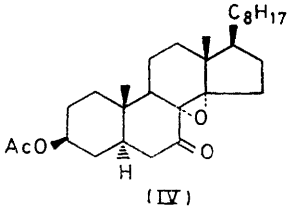
cholesterol by liver enzymes without apparent intervention of position 14.

The synthesis of 5α,14β-cholest-7-ene (Ic) has been reported recently.[4] In the light of this, 3β-acetoxy-5α-cholest-8(14)-en-7-one [5] (IIa) was treated with lithium in liquid ammonia.[4] The reaction gave poor yields of 3β-acetoxy-5α,14β-cholestan-7-one (IIIa), owing to the presence of the 3β-acetoxy-group. Moreover the 14α-diastereoisomer (IIIb) was also isolated. In our hands, reduction of (IIa) with zinc dust in acetic acid under the reported[4] conditions did not proceed. Use of granulated zinc in acetic acid–tetrahydrofuran afforded the ketone (IIIa) in high and reproducible yields but on no more than a 100 mg scale.

The 'non-natural' 7-ketone (IIIa) showed n.m.r. signals for the C-18 and C-19 protons in good agreement



(III)



(IV)

	R^1	R^2	R^3
a;	OAc	:O	β-H
b;	OAc	:O	α-H
c;	OAc	:N·NHTs	β-H
d;	OAc	H_2	β-H
e;	OH	:O	β-H
f;	OH	:O	α-H
g;	OAc	H_2	α-H
h;	OH	H_2	β-H
i;	OPr	H_2	β-H
l;	OPr	H_2	α-H

with values calculated on the basis of Zürcher's rules.[6] The mass spectrum differed from that [7] of the 'natural' stereoisomer (IIIb) only in the relative intensities of the most significant peaks. Reduction [8] of the tosylhydrazone (IIIc) with sodium borohydride afforded 3β-acetoxy-5α,14β-cholestane (IIId), which was also obtained by hydroboration [9] of 3β-acetoxy-5α-cholest-8(14)-ene (IIb), followed by protolysis in boiling propionic acid, fractional crystallization of the mixture of 14α- and 14β-stereoisomers, saponification, and acetylation.

Reduction of Δ^4-3-oxo-steroids with a 4 000-fold excess of zinc dust in acetic acid affords Δ^3-5α-steroids and/or Δ^3-5β-steroids.[10] Moreover, in the reduction of (IIa) with zinc the formation of 3β-acetoxy-5α,14β-cholest-7-ene (Id) in small amounts was shown by g.l.c.–mass spectrometry. Therefore the possibility of obtaining 3β-acetoxy-5α,14β-cholest-7-ene (Id) by reduction of the $\Delta^{8(14)}$-7-ketone (IIa) with zinc was investigated.

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Reduction of (IIa) by the method of McKenna *et al.*[10] did not yield reproducible amounts of (Id), the ketone (IIa) being usually recovered almost quantitatively. However reduction of (IIa) at 20 °C with 450 times the calculated amount of zinc dust in methanolic N-sulphuric acid gave (Id) as the major product, with minor amounts of the ' non-natural ' ketone (IIIa). The reaction was complete in *ca.* 2 min and gave reproducible results.

The mass spectrum of (Id) differed from that [11] of the diastereoisomer (Ie) only in the relative intensities of the most significant peaks; the n.m.r. spectrum showed a multiplet at δ 5.33, and calculated values [6] for the signals of the C-18 and C-19 protons agreed with those observed. The product was identical with the compound obtained by reduction of the tosylhydrazone (IIIc) with lithium hydride.[12] As expected no reaction of (Id) was observed in the selenium dioxide test.[5]

Formation of the ketone (IIIa) from (IIa) was not unexpected; in fact zinc dust–N-sulphuric acid is reported to saturate the double bond conjugated with the aldehyde group of fluorocurarine chloride.[13] The intermediary formation of either allylic alcohols or acetates in reductions with zinc–mineral acid of αβ-unsaturated ketones has been postulated;[14] on the other hand this has been considered improbable in the reduction of αβ-unsaturated ketones with an excess of zinc in acetic acid.[10] Under our conditions (zinc dust in methanolic N-sulphuric acid) 5α-cholest-8(14)-ene-3β,7α-diol diacetate [15] (IIc) and 5α-cholest-8(14)-ene-3β,7βdiol 3-acetate (IId) gave (Id) in 30 and 45% yield, respectively.

Similar reduction of 3β-acetoxy-8α,14α-epoxy-5α-cholestan-7-one [5] (IV) afforded (Id) as the major product. This represents a ready two-step synthesis of (Id) from the natural diastereoisomer (Ie). The intermediary formation of (IIa) can be postulated: (IIa) is in fact obtained in quantitative yield by reduction of (IV) with an excess of zinc in methanolic 0.05N-sulphuric acid in 2 min.

5α,14β-Cholest-7-en-3β-ol (Ia) shows a markedly lower m.p. and g.l.c. retention time than the diastereoisomer (Ib). These properties are typical of all 14β-steroids synthesized by us.

EXPERIMENTAL

I.r. data relate to Nujol mulls, n.m.r. data to 1% solutions in $CDCl_3$, with tetramethylsilane as internal standard, and $[\alpha]_D$ values to 1% solutions in $CHCl_3$. Mass spectra were obtained with an LKB 9000 gas chromatograph–mass spectrometer either by g.l.c. (2 m silanized glass column of 3% SE 30 on GasChrom Q, operating at 240—260°) or by direct insertion (d.i.). Column chromatography was carried out with hexane–benzene or benzene–ether on silica gel G–Celite (50 : 50 v/v). Reactions and column chromatographic fractions were monitored by t.l.c., g.l.c., and (when necessary) g.l.c.–mass spectrometry.

Granulated zinc (mm 0.5-1; Carlo Erba) and zinc dust (puriss.p.a. pulv. Fluka) were employed.

3β-*Acetoxy*-5α,14β-*cholestan*-7-*one* (IIIa).—(*a*) A solution of the enone (IIa) (100 mg) in tetrahydrofuran (2 ml) and acetic acid (8 ml) was stirred at 4 °C for 16 h with granulated zinc (16 g) (added in one portion). After work-up as usual the crude residue was chromatographed. 95% Hexane–benzene eluted a mixture of 3β-acetoxycholestenes (15 mg). 60% Hexane–benzene eluted the *ketone* (IIIa) (60 mg), m.p. 78—79° (from methanol), $[\alpha]_D^{21}$ +52°, $\nu_{max.}$ 1 710 and 1 735 cm^{-1}; δ 0.96 (3 H, s, 13β-Me; calc.[6] 0.958), 1.06 (3 H, s, 10β-Me; calc.[6] 1.075), and 4.90 (1 H, m, 3α-H); M^+ (d.i.) 444 (Found: C, 78.35; H, 11.05. $C_{29}H_{48}O_3$ requires C, 78.3; H, 10.9%). The *tosylhydrazone* (IIIc) had m.p. 140—141° (decomp.) (from hexane–ether), $[\alpha]_D^{22}$ +122°, $\nu_{max.}$ 3 310, 1 730, and 1 605 cm^{-1} (Found: C, 70.55; H, 9.2; N, 4.55. $C_{36}H_{56}N_2O_4S$ requires C, 70.55; H, 9.2; N, 4.55%).

(*b*) The enone (IIa) (450 mg) was reduced with lithium–ammonia according to ref. 4. The crude residue was chromatographed. 85% Benzene–ether eluted a mixture of ketones (IIIe) and (IIIf). The mixture was acetylated and chromatographed. 60% Hexane–benzene eluted the ketone (IIIa) (58 mg), m.p. 78°; 50% hexane–benzene eluted 3β-acetoxy-5α-cholestan-7-one (IIIb) (30 mg), m.p. 149°, $[\alpha]_D^{20}$ −36° (lit.,[16] m.p. 149—149.5°, $[\alpha]_D$ −36°).

3β-*Acetoxy*-5α,14β-*cholestane* (IIId).—(*a*) The tosylhydrazone (IIIc) (300 mg) was reduced with sodium borohydride. The product (IIId), an oil (171 mg), resisted all efforts at crystallization; $\nu_{max.}$ 1 730 cm^{-1}; δ 0.84 (3 H, s, 10β-Me; calc.[6] 0.800) 0.95 (3 H, s, 13β-Me; calc.[6] 0.950) and 4.90 (1 H, m, 3α-H); g.l.c. (240 °C) relative retention time w.r.t. (IIIg) 0.87, M^+ (g.l.c.) 430. The corresponding oily *alcohol* (IIIh) showed M^+ (g.l.c.) 388 (Found: C, 83.35; H, 12.35. $C_{27}H_{48}O$ requires C, 83.45; H, 12.45%). The *3,5-dinitrobenzoate* showed m.p. 155—156° (from acetone), $[\alpha]_D^{21}$ +38°, $\nu_{max.}$ 1 740 and 3 120 cm^{-1} (Found: C, 70.1; H, 8.7; N 4.6. $C_{34}H_{50}N_2O_6$ requires C, 70.05; H, 8.65; N, 4.8%).

(*b*) An excess of diborane was bubbled at 45 °C during 3 h into a stirred solution of the acetate (IIb) (0.95 g) in dry bis-(2-methoxyethyl) ether (25 ml). The solution was then cooled to −80 °C (solid CO_2–acetone), propionic acid (2 ml) was added, and the solution was stirred for 1 h. After it had reached room temperature, more propionic acid (25 ml) was added and the solution was heated at 140 °C for 48 h. Work-up yielded a pale yellow oil (1.0 g), which was chromatographed. Fractions eluted with hexane (0.42 g) contained a mixture of propionates (IIIi) and (IIIl). Crystalline (IIIl) was obtained by chilling the methanolic solution at −15 °C; the mother liquors were evaporated to yield (IIIi) (250 mg) as an oil, $\nu_{max.}$ 1 730 cm^{-1}, M^+ (g.l.c.) 444. The alcohol (IIIh) was transformed into the acetate (IIId) and the 3,5-dinitrobenzoate, both identical with those described above.

3β-*Acetoxy*-5α,14β-*cholest*-7-*ene* (Id).—(a) *By use of zinc–sulphuric acid; general procedure.* To a solution of the steroid (500 mg) in methanol (360 ml) containing zinc dust (64 g), 98% sulphuric acid (9.8 ml) was added dropwise with stirring, and with the temperature kept at 20 °C. After 2 min surplus zinc was filtered off and the solution concentrated *in vacuo* to 50 ml. Ether was added and the ethereal solution was washed with 5% sodium hydrogen carbonate then dried and evaporated.

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(i) *From* 3β-*acetoxy*-5α-*cholest*-7-*ene* (Ie). Compound (Ie) was oxidized to the epoxide (IV) by a modified procedure [5] in high yield. To a solution of (Ie) (4.28 g) in carbon tetrachloride (40 ml) and acetic acid (95 ml) a solution of chromium trioxide (6.42 g) in acetic acid (5 ml) and water (4 ml) was added dropwise, with stirring and with the temperature kept at 30—35 °C. Stirring was continued for 2 h, water was added, and the mixture was worked up to afford a residue which was chromatographed. Benzene eluted 3β-acetoxy-8α,9α-epoxy-5α-cholestan-7-one (1.18 g), m.p. 176°, $[\alpha]_D^{21}$ −32.5° (lit.,[5] m.p. 173—175°; $[\alpha]_D$ −32.3 ± 0.3°), $\nu_{max.}$ 1 690 and 1 725 cm^{-1}; δ 0.67 (3 H, s, 13α-Me), 1.07 (3 H, s, 10β-Me), and 4.62 (1 H, m, 3α-H); M^+ (d.i.) 458. 99% Benzene–ether eluted the 8α,14α-epoxide (IV) (1.84 g), m.p. 142°, $[\alpha]_D^{21}$ −69.9° (lit.,[5] m.p. 142—142.5°; $[\alpha]_D^{21}$ −69.8 ± 0.8°), $\nu_{max.}$ 1 718 cm^{-1}, δ 0.96 (3 H, s, 13β-Me), 1.11 (3 H, s, 10β-Me), and 4.65 (1 H, m, 3α-H); M^+ (d.i.) 458. By reduction with zinc the epoxide (IV) gave an oily mixture which was chromatographed. 95% Hexane–benzene eluted *compound* (Id) (250 mg) as an oil which resisted all efforts at crystallization. 60% Hexane–benzene eluted compound (IIIa) (60 mg). Compound (Id) showed $\nu_{max.}$ 1 730 cm^{-1}, δ 0.77 (3 H, s, 10β-Me; calc.[6] 0.792), 0.82 (3 H, s, 13β-Me; calc.[6] 0.833), 4.63 (1 H, m, 3α-H), and 5.33 (1 H, m, 7-H); M^+ (g.l.c.) 428; g.l.c. retention time relative to (Ie) 0.85 (Found: C, 81.1; H, 11.15. $C_{29}H_{48}O_2$ requires C, 81.25; H, 11.3%), and gave a negative selenium dioxide test.[5]

(ii) The epoxide (IV) (520 mg) in methanolic 0.05N-sulphuric acid (400 ml) was shaken with zinc dust (80 g) for 2 min. Work-up yielded compound (IIa) (500 mg) sufficiently pure for reduction with zinc in methanolic N-sulphuric acid. From the residue of this reduction compound (Id) (250 mg) and (IIIa) (40 mg) were isolated by chromatography.

(iii) The residue from reduction of compound (IIc) [15] was chromatographed. 95% Hexane–benzene eluted a mixture of mono- and di-unsaturated compounds (280 mg) and 20% benzene–hexane eluted starting material (175 mg). The mixture of less polar compounds was then chromatographed on silica gel G—Celite–silver nitrate (50 : 50 : 15) (35 g); hexane eluted pure (Id) (140 mg).

(iv) Reduction of compound (IIa) with sodium borohydride yielded 5α-*cholest*-8(14)-*ene*-3β,7β-*diol* 3-*acetate* (IId), m.p. 126—127°, $[\alpha]_D^{21}$ +27.4°, $\nu_{max.}$ 3 350 and 1 725 cm^{-1}; δ 0.76 (3 H, s, 10β-Me; calc.[6] 0.733), 0.84, (3 H, s, 13β-Me; calc.[6] 0.858), 4.11 (1 H, m, 7α-H),[17] and 4.68 (1 H, m, 3α-H) (Found: C, 78.3; H, 11.05. $C_{29}H_{48}O_3$ requires C, 78.3; H, 10.9%). The mixture from reduction of (IId) with zinc was chromatographed as reported for (IIc), yielding (Id) (223 mg).

(*b*) The tosylhydrazone (IIIc) (250 mg) was reduced according to ref. 12. The crude product was purified by chromatography. 95% Hexane–benzene eluted (Id) (150 mg).

5α,14β-*Cholest*-7-*en*-3β-*ol* (Ia).—Saponification of the acetate (Id) with methanolic potassium hydroxide gave the *alcohol* (Ia), m.p. 90°, $[\alpha]_D^{21}$ −6.21°, $\nu_{max.}$ 3 250 cm^{-1}, g.l.c. (240 °C) retention time relative to (Ib) 0.86; M^+ (d.i.) 386 (Found: C, 83.6; H, 11.75. $C_{27}H_{46}O$ requires C, 83.85; H, 12.0%). The 3,5-*dinitrobenzoate* showed m.p. 164—165° (from acetone), $[\alpha]_D^{21}$ 0°; $\nu_{max.}$ 3 095 and 1 740 cm^{-1} (Found: C, 70.3; H, 8.5; N, 4.8. $C_{34}H_{48}N_2O_6$ requires C, 70.3; H, 8.35; N, 4.8%).

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