Mechanism of Dealkylation of Clionasterol in the Insect *Tenebrio molitor*

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1. 25-³H- and 26-¹⁴C-labelled (24*S*)-24-ethylcholest-5-en-3 β -ol (clionasterol) were synthesized from (24*S*)-24-ethylcholesta-5,25-dien-3 β -ol. 2. These labelled substrates were mixed and administered, together with the hypocholesterolemic agent, triparanol citrate, to *Tenebrio molitor* larvae. 3. The ³H label from the clionasterol substrate was retained in both the desmosterol and the cholesterol isolated from the larvae. 4. Location of this ³H label in the desmosterol showed that dealkylation of the clionasterol involved ³H migration from C-25 to C-24. A possible mechanism for dealkylation is presented.

Most phytophagous and carnivorous insect species can dealkylate C₂₈ and C₂₉ plant sterols to obtain C₂₇ sterols such as cholesterol (VI) (Thompson *et al.*, 1973). There is good evidence that a major route of dealkylation of sitosterol involves the reaction sequence (I) $\rightarrow$ (II) $\rightarrow$ (III) $\rightarrow$ (IV) $\rightarrow$ (VI) (Svoboda *et al.*, 1967, 1971; Morisaki *et al.*, 1972; Svoboda *et al.*, 1975; Chen *et al.*, 1975). During dealkylation the C-25 hydrogen of the C₂₉ sterol is retained in the cholesterol formed (Randall *et al.*, 1972). We now report, in full, evidence that the C-25 hydrogen of clionasterol (VIII) migrates to the C-24 position during dealkylation in *Tenebrio molitor*. Part of this work has been published in preliminary form (Pettler *et al.*, 1974).

Clionasterol (VIII) was chosen as substrate, since a suitable starting material (clerosterol, X) for synthesis of [25-³H]- and [26-¹⁴C]-clionasterol was readily available; the [¹⁴C]sterol was required for reference purposes. Since the side chain of desmosterol (IV) is more amenable to chemical reaction than that of cholesterol (VI), the [26-¹⁴C,25-³H]clionasterol was administered to *Tenebrio* larvae together with the vertebrate hypocholesterolemic agent triparanol, which causes accumulation of compound (IV) (Svoboda & Robbins, 1967). The ³H label in the [³H,¹⁴C]desmosterol produced was then located by chemical reaction.

cholest-5-en-3 β -ol; fucosterol, (24*E*)-stigmasta-5,24(28)-dien-3 β -ol; clerosterol, (24*S*)-24-ethylcholesta-5,25-dien-3 β -ol; triparanol, 2-(4-chlorophenyl)-1-(4-diethylaminoethoxyphenyl)-1-*p*-tolylethanol.

Reagents

n-Butyl-lithium [15% (w/v) solution in hexane] and NaB²H₄ were from Koch–Light Laboratories, Colnbrook, Bucks., U.K. Tris(triphenylphosphin)-rhodium chloride was from Fluka Chemical Co., Glossop, Derbyshire, U.K. Diborane–tetrahydrofuran complex (1M solution in tetrahydrofuran stabilized with 5mol% NaBH₄) was from Ralph N. Emanuel, Wembley, Middx., U.K. Triparanol citrate was a gift from Dr. T. R. Blom, William S. Merrell and Co., Cincinnati, OH, U.S.A. NaB³H₄ (346mCi/mmol) and [¹⁴C]methyl iodide (57mCi/mmol) were from The Radiochemical Centre, Amersham, Bucks., U.K. Desmosterol was synthesized from 3 β -acetoxycholest-5-en-24-one, which was a gift from Dr. I. F. Cook of this Department. Methyltriphenylphosphonium iodide was prepared as described for the corresponding bromo compound (Wittig & Schoellkopf, 1960). Clerosterol [containing 6% of (24*S*)-methylcholesta-5,25-dien-3 β -ol] was extracted from the green alga *Codium fragile* (Rubinstein & Goad, 1974). All other reagents were from BDH, Poole, Dorset, U.K.

Thin-layer chromatography

T.l.c. was carried out on (a) silica gel (Kieselgel G; E. Merck A.-G., Darmstadt, Germany) for non-u.v.-absorbing sterols, (b) Kieselgel GF254 when the steroids were u.v.-absorbing and (c) silica gel (Kieselgel H) impregnated with 20% (w/w) AgNO₃.

Experimental

Nomenclature

Trivial names are used. The systematic names are: cholesterol, cholest-5-en-3 β -ol; desmosterol, cholesta-5,24-dien-3 β -ol; sitosterol, (24*R*)-24-ethylcholest-5-en-3 β -ol; clionasterol, (24*S*)-24-ethyl-

In system (b), compounds were located by examination under u.v. light. In systems (a) and (c), for analytical purposes compounds were located by spraying with conc. H_2SO_4 and heating at 110°C for 10 min, whereas after preparative t.l.c., compounds were detected by examination under u.v. light after spraying with a solution of Rhodamine 6G in acetone. Compounds were removed from the silica by using dry redistilled diethyl ether unless otherwise stated.

Gas-liquid chromatography

A Pye 104 gas chromatograph was used, fitted with a flame ionization detector and 6.5 mm-diameter glass columns (1.5 m or 2.1 m long). Three stationary phases were used, 1.5% OV-1 on Shimalite W (100–120 mesh) and 3% OV-17 or 1% QF-1 on Gas-Chrom Q (100–120 mesh).

For collection of effluent material a metal splitter was inserted between the end of the column and the detector, so that approximately 1 part in 10 passed to the detector, whereas the remainder was collected in glass capillary tubes at ambient temperature (20°C). Radio-g.l.c. was carried out on a Pye-Panax instrument.

Analytical methods

P.m.r. spectra were determined for solutions in $[\text{H}^2]\text{chloroform}$ either at 60 MHz with a Perkin-Elmer R-12 spectrometer or at 100 MHz by the Physico-Chemical Measurements Unit, Harwell, Berks., U.K., with a Varian HA-100D spectrometer. Mass spectra were determined at the latter place with an A.E.I. M.S. 902 mass spectrometer. U.v. spectra were determined for solutions in ethanol. I.r. spectra were determined for compounds either as KBr discs or as solution in carbon tetrachloride.

Radiochemical methods

Radioactivity was measured on an Intertechnique three-channel scintillation spectrometer, model ABACSL40. Samples were dissolved in 10 ml of a scintillation solution consisting of 0.8% (w/v) 5-(4-biphenyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole (butyl-PBD) in toluene. ^3H and ^{14}C radioactivities are quoted after corrections for background, counting efficiency and quenching has been applied.

Growth of insects

Stock cultures of *Tenebrio molitor* (yellow mealworm) were fed on oatmeal containing 5% (w/w) dried yeast and were maintained at a temperature of $25 \pm 2^\circ\text{C}$ and 55% relative humidity.

Synthesis of (24S)-24-ethyl[25- ^3H]cholest-5-en-3 β -ol

The whole synthetic sequence was carried out initially without introduction of radioactivity.

3 β -Tetrahydropyranyloxy-(24S)-24-ethylcholesta-5,25-diene (XI). A solution of POCl_3 (0.22 ml) in dihydropyran (1.75 ml) was added to (24S)-24-ethyl-

cholesta-5,25-dien-3 β -ol (X; 433 mg) and the solution left for 3 h at room temperature (De Ruggieri & De Ferrari, 1958). The solution was diluted with diethyl ether and washed successively with aqueous Na_2CO_3 and water and then evaporated to dryness under reduced pressure. The product was recrystallized from chloroform/methanol to yield 3 β -tetrahydropyranyloxy-(24S)-24-ethylcholesta-5,25-diene (XI); yield 77%; i.r. ν_{max} . (carbon tetrachloride) 1641, 1138, 1116, 1075 and 891 cm^{-1} ; n.m.r. ($[\text{H}^2]\text{chloroform}$, 100 MHz) δ 0.68 (s, 3H, C-18 methyl), 1.02 (s, 3H, C-19 methyl), 0.90 (d, J 6 Hz, C-21 methyl), 0.80 (t, J 8 Hz, C-29 methyl), 1.58 (s, 3H, C-27 methyl), 3.52 and 3.89 (m, 2H and 1H, C-3 proton on sterol and C-6' protons on tetrahydropyranyl ether), 4.64 and 4.71 (2H, $\text{C}=\text{CH}_2$), 4.71 (m, 1H, C-2' proton on tetrahydropyranyl ether), 5.33 (m, 1H, C-6 proton) p.p.m.; m/e 496.428 (M^+ , 1%; $\text{C}_{34}\text{H}_{56}\text{O}_2$ requires m/e 496.428), 412 (6%), 394 (100%), 380 (9%), 379 (4%), 255 (5%) and 213 (4%).

3 β -Tetrahydropyranyloxy-(24S)-24-ethylcholest-5-en-26-ol (XII). Compound (XI) (40 mg), Hg_2Cl_2 (21.2 mg), diethylene glycol dimethyl ether (diglyme) (2.0 ml) and tetrahydrofuran (2.5 ml) were placed in a 5 ml flask fitted with a Suba-Seal cap, through which a needle was placed to allow excess gas to escape. NaBH_4 (3.6 mg) dissolved in the minimum volume of diglyme was then added, resulting in the generation of diborane (Freeguard & Long, 1965). After stirring for $1\frac{1}{2}$ h at room temperature, three to five drops of saturated NaHCO_3 solution and an equal volume of H_2O_2 (30-vol.) were added. The reaction mixture was left for a further 25 min and diluted with diethyl ether. The solution was washed with water and evaporated to dryness under reduced pressure. The crude product was purified by t.l.c. on silica gel developed with chloroform and recrystallized from chloroform/methanol to give 3 β -tetrahydropyranyloxy-(24S)-24-ethylcholest-5-en-26-ol (XII); yield 55%; i.r. ν_{max} . (KBr) 3450, 1116, 1134, 1072 and 1058 cm^{-1} ; n.m.r. ($[\text{H}^2]\text{chloroform}$, 100 MHz), δ 0.66 (s, 3H, C-18 methyl), 1.02 (s, 3H, C-19 methyl), 0.92 (d, J 6 Hz, C-21 methyl), 0.85 (d, J 4.5 Hz, C-27 methyl), 3.47 and 3.87 (m, 4H and 1H, C-3 proton, C-6' protons on tetrahydropyranyl ether and C-26 protons), 4.71 (m, 1H, C-2' proton on tetrahydropyranyl ether), 5.33 (m, 1H, C-6 proton) 0.80 (t, J 6 Hz, C-29 methyl) p.p.m.; m/e 514.437 (M^+ , 2%; $\text{C}_{34}\text{H}_{58}\text{O}_3$ requires m/e 514.439), 430 (5%), 412 (100%), 39.8 (9%), 397 (6%) and 255 (3%).

3 β -Tetrahydropyranyloxy-(24S)-24-ethylcholest-5-en-26-toluene-p-sulphonate (XIV). Compound (XII) (10 mg) was dissolved in pyridine (1.0 ml) and toluene-p-sulphonyl chloride (10 mg) added. After standing in the dark at 5°C overnight, water was added to the reaction mixture, which was then extracted with diethyl ether. The diethyl ether extract was washed with water and evaporated to

Abbreviation: Ts, tosyl.

dryness under reduced pressure. The crude tosylate was purified by t.l.c. on silica gel GF254 developed with chloroform to give 3 β -tetrahydropyranyloxy-(24S)-24-ethylcholest-5-en-26-toluene-*p*-sulphonate (XIV); yield 75%; u.v. $\lambda_{\max}$ (ethanol) 225nm; i.r., $\nu_{\max}$ (KBr), 1362, 1168, 1112, 1131, 1080 and 1072cm⁻¹; n.m.r. ([²H]chloroform, 60 MHz) δ , 0.68 (s, C-18 methyl), 1.01 (s, C-19 methyl), 2.45 (s, aromatic methyl), 3.52 and 3.87 (m, C-3 proton and C-6' protons on tetrahydropyranyl ether), 4.67 (m, C-2' proton on tetrahydropyranyl ether), 5.31 (m, C-6 proton), 7.21 and 7.35, 7.70 and 7.84 (4H, aromatic protons) p.p.m.

3 β -Tetrahydropyranyloxy-(24S)-24-ethylcholest-5-ene (XV). Excess LiAlH₄ was added to compound (XIV) (10mg) dissolved in tetrahydrofuran (1.0ml), the solution was stirred for ½ h and then refluxed for 1 h. After cooling, ethyl acetate was added dropwise until there was no further reaction. An equal volume of saturated sodium potassium tartrate solution was added and the mixture stirred for 15 min. The mixture was extracted with diethyl ether, the extract was washed with water and evaporated to dryness under reduced pressure. The crude product was purified by t.l.c. on silica gel developed with benzene to give 3 β -tetrahydropyranyloxy-(24S)-24-ethylcholest-5-ene (XV); yield 65%; i.r. $\nu_{\max}$ (KBr) 1120, 1142, 1180 and 1061cm⁻¹; n.m.r. ([²H]chloroform, 100 MHz) δ 0.69 (s, 3H, C-18 methyl), 1.00 (s, 3H, C-19 methyl), 3.52 and 3.89 (m, 2H and 1H, C-3 proton and C-6' protons on tetrahydropyranyl ether), 4.72 (m, 1H, C-2' proton on tetrahydropyranyl ether), 5.32 (m, 1H, C-6 proton) p.p.m.; *m/e* 498.445 (*M*⁺, 1%; C₃₄H₅₈O₂ requires *m/e* 498.444), 414 (25%), 399 (20%), 396 (100%), 381 (14%), 303 (16%), 273 (9%), 255 (17%), 231 (11%) and 213 (17%).

(24S)-24-Ethylcholest-5-en-3 β -ol (VIII). HCl (2M; four or five drops) was added to compound (XV) (10mg) dissolved in acetone (1.5ml) and a few drops

of ether. The reaction mixture was left at room temperature for 5h, and shaken periodically (De Ruggieri & De Ferrari, 1958). The solution was diluted with diethyl ether, washed with water and evaporated to dryness under reduced pressure. The crude product was purified by t.l.c. on silica gel developed with chloroform to give (24*S*)-24-ethylcholest-5-en-3 β -ol (VIII; clionasterol), which was recrystallized from chloroform/methanol; yield 78%; i.r. $\nu_{\max}$. (carbon tetrachloride) 3600cm⁻¹; n.m.r. ([²H]chloroform, 100MHz) δ 0.67 (s, 3H, C-18 methyl), 1.01 (s, 3H, C-19 methyl), 0.91 (d, *J* 6Hz, C-21 methyl), 0.81 (d, *J* 7Hz, C-26, C-27 methyls), 0.83 (t, *J* 6Hz, C-29 methyl), 3.53 (m, 1H, C-3 proton), 5.34 (m, 1H, C-6 proton) p.p.m.; *m/e* 414.387 (*M*⁺, 100%; C₂₉H₅₀O requires *m/e* 414.386), 399 (40%), 396 (46%), 381 (35%), 329 (46%), 381 (35%), 329 (46%), 303 (72%), 275 (18%), 273 (36%), 255 (54%), 231 (40%) and 213 (58%).

Analysis of this compound by g.l.c. (OV-1 and QF-1) showed the presence of one major component (95%) corresponding to clionasterol, and a minor component (5%) which co-chromatographed with 24-methylcholest-5-en-3 β -ol.

Preparation of [25-³H]clionasterol. The hydroboration of compound (XI) (33mg) was repeated with B₂³H₆ generated *in situ* from NaB³H₄ (25mCi) as described above. A sample of the crude product was subjected to t.l.c. on silica gel developed with 5% (v/v) ethyl acetate in benzene, and radioactivity scanning indicated the presence of two radioactive compounds, the major one co-chromatographing with compound (XII). The mixture was purified by t.l.c. in the same system yielding 25-³H-labelled 3 β -tetrahydropyranyloxy-(24*S*)-24-ethylcholest-5-en-26-ol (XIII; 5.31mCi). The C-26-tosylate of this compound was reduced with LiAlH₄ and the product treated with acid to give [25-³H]clionasterol (1.10mCi) as described above.

Location of label in [25-³H]clionasterol. Location of ³H label in the [³H]clionasterol was carried out by (a) base equilibration of the aldehyde (XVI) obtained by oxidation of compound (XIII), and (b) mass and n.m.r. spectroscopy of compound (XIII) prepared by the previous procedure, but by using NaB³H₄ instead of the NaB³H₄ at the hydroboration step.

(a) A portion of the 3 β -tetrahydropyranyloxy-(24*S*)-24-ethyl[25-³H]cholest-5-en-26-ol (XIII; 2.6 $\times$ 10⁶d.p.m.) was diluted with non-radioactive carrier material (12.5mg) and dicyclohexylcarbodi-imide (20mg) added (Pfitzner & Moffatt, 1965). The mixture was dissolved in 1.2ml of the following mixture: benzene (40.0ml), dimethyl sulphoxide (20.0ml), pyridine (0.96ml) and trifluoroacetic acid (0.48ml). The reaction was carried out at 50°C for 3h and the mixture then left overnight at room temperature. Ethyl acetate was added and the precipitate of dicyclohexylurea filtered. The filtrate was washed

twice with 0.1M-sodium phosphate buffer (pH 7.2), followed by water and dried over Na₂SO₄ and evaporated to dryness under reduced pressure. The crude product was purified by t.l.c. on silica gel developed with 5% ethyl acetate in benzene to give 3 β -tetrahydropyranyloxy-(24*S*)-24-ethyl[25-³H]cholest-5-en-26-al (XVI); yield 70%; i.r., $\nu_{\max}$. (carbon tetrachloride) 1725, 1116, 1131, 1074 and 1057cm⁻¹; n.m.r. ([²H]chloroform, 60MHz) δ 0.68 (s, C-18 methyl), 1.00 (s, C-19 methyl), 3.52 and 3.89 (m, C-3 proton and C-6' protons on tetrahydropyranyl ether), 4.71 (m, 1H, C-2' proton on tetrahydropyranyl ether), 5.31 (m, 1H, C-6 proton), 9.65 (δ -CH-CHO) p.p.m.; *m/e* 512.422 (*M*⁺, 1%; C₃₄H₅₆O₃ requires *m/e* 512.423), 410 (100%), 396 (6%), 395 (4%), 382 (4%) and 255 (6%).

The 3 β -tetrahydropyranyloxy-(24*S*)-24-ethyl[25-³H]cholest-5-en-26-al (XVI) was recrystallized from methanol to constant specific radioactivity (284034d.p.m. of ³H/mg). This was dissolved in a solution containing KOH (54mg) dissolved in ethanol (3ml) and water (0.06ml), and left at room temperature for 2h, with periodic agitation. The solution was extracted with diethyl ether, washed with water and evaporated to dryness under reduced pressure to yield recovered compound (XVI). This was purified by t.l.c. on silica gel developed with 5% ethyl acetate in benzene, recrystallized twice from methanol and radioassayed (10500d.p.m. of ³H/mg).

(b) The hydroboration of compound (XI) (32mg) was repeated with NaB³H₄ (2.75mg). The crude product obtained was purified by t.l.c. on silica gel developed with chloroform to give 3 β -tetrahydropyranyloxy-(24*S*)-24-ethyl[25-²H]cholest-5-en-26-ol; *m/e* 431 (*M*—84, 6%), 413 (100%), 399 (8%), 398 (7%) and 255 (3%) (a molecular ion was not observed in this spectrum); n.m.r. ([²H]chloroform, 100MHz) δ 0.68 (s, C-18 methyl), 1.01 (s, C-19 methyl), 0.92 (d, *J* 6Hz, C-21 methyl), 3.48 and 3.84 (m, 5H, C-3 proton, C-6' protons on tetrahydropyranyl ether, C-26 protons), 4.69 (m, 1H, C-2' proton on tetrahydropyranyl ether), 5.32 (m, 1H, C-6 proton) p.p.m.

*Synthesis of (24*S*)-24-ethyl[26-¹⁴C]cholest-5-en-3 β -ol (XIX)*

This reaction sequence was initially carried out in non-radioactive form.

3 β -Hydroxy-(24*S*)-24-ethyl-26-norcholest-5-en-25-one (XVIII). (24*S*)-Ethylcholesta-5,25-dien-3 β -ol (VIII; 325mg) was dissolved in pyridine (10ml) and OsO₄ (159mg) added. The mixture was stirred for 5h and then sodium metabisulphite (2.8g) was added, together with water (12ml). The resulting solution was stirred for a further 2h with gentle warming, and ethanol (1.9ml) was added during this period. The solution was diluted with water, extracted with chloroform, the chloroform extract washed with water and evaporated to dryness under reduced

pressure. The crude product was washed several times with light petroleum (b.p. 40–60°C)/diethyl ether (1:1, v/v) to remove any traces of starting sterol. To the product dissolved in pyridine (10ml) and ethanol (36ml), sodium periodate (500mg) and 0.5M-H₂SO₄ (1.8ml) were added, and the mixture was stirred for 16h at room temperature. After addition of water, the mixture was extracted with diethyl ether, the extract washed with water and evaporated to dryness under reduced pressure. The crude product was subjected to t.l.c. on silica gel developed with 5% methanol in chloroform, yielding 3 β -hydroxy-(24S)-24-ethyl-26-norcholest-5-en-25-one (XVIII; Sucrow, 1966); yield 66%; i.r., $\nu_{\max}$ (KBr), 3520 and 1720 cm⁻¹; n.m.r. ([²H]chloroform, 100 MHz) δ 0.67 (s, 3H, C-18 methyl), 1.00 (s, 3H, C-19 methyl), 0.92 (d, *J* 6 Hz, C-21 methyl), 0.86 (t, *J* 7 Hz, C-29 methyl), 2.09 (s, 3H, -C(=O)-CH₃), 3.52 (m, 1H, C-3 proton), 5.33 (m, 1H, C-6 proton) p.p.m.; *m/e* 414.349 (*M*⁺, 6%; C₂₈H₄₆O₂ requires *m/e* 414.350), 257 (28%), 255 (100%), 213 (5%).

(24S)-24-Ethylcholesta-5,25-dien-3 β -ol (X). To a suspension of methyltriphenylphosphonium iodide (50.4mg) in dry benzene, *n*-butyl-lithium in hexane (15%, w/w; 64 μ l) was added and the mixture left at room temperature for 1h. Compound (XVIII) (45mg) dissolved in benzene (1ml) was then added. After a further 16h, the reaction mixture was poured into water, extracted with diethyl ether, the diethyl ether extract washed with water and evaporated to dryness under reduced pressure. The crude product was purified by t.l.c. on silica gel developed with 10% ethyl acetate in benzene yielding 24(S)-24-ethylcholesta-5,25-dien-3 β -ol (X); yield 34%; i.r., $\nu_{\max}$ (carbon tetrachloride) 1640 and 890 cm⁻¹; n.m.r. ([²H]chloroform, 100 MHz) δ 0.68 (s, 3H, C-18 methyl), 1.02 (s, 3H, C-19 methyl), 0.80 (t, *J* 7 Hz, C-29 methyl) 0.91 (d, *J* 6 Hz, C-21 methyl), 1.57 (s, C-27 methyl) 3.52 (m, 1H, C-3 proton), 4.65 and 4.72 (2H, >C=CH₂), 5.34 (m, 1H, C-6 proton) p.p.m.; *m/e* 412.371 (*M*⁺, 100%; C₂₉H₄₈O requires *m/e* 412.371), 397 (16%), 394 (17%), 379 (9%), 327 (6%), 314 (19%), 301 (9%), 273 (10%), 271 (33%), 255 (13%), 231 (11%), 229 (10%), 213 (16%).

(24S)-24-Ethylcholest-5-en-3 β -ol (VIII). Sterol (X) (20mg) in benzene (5ml) was hydrogenated in the presence of Wilkinson's catalyst [Tris(triphenylphosphine)rhodium chloride] for 16h (Birch & Walker, 1966) and the reaction mixture chromatographed on a 5g alumina column (Brockmann grade III), eluted with 50% diethyl ether in light petroleum (b.p. 40–60°C). The crude product was purified by t.l.c. on silica gel developed with 20% ethyl acetate in light petroleum (b.p. 60–80°C) to give (24S)-24-ethylcholest-5-en-3 β -ol (VIII; yield 75%), which was characterized by i.r., n.m.r. and mass spectrometry.

Preparation of [26-¹⁴C]clionasterol (XIX). Slightly modified conditions from the above were used for the Wittig reaction when [¹⁴C]methyl iodide was used. [¹⁴C]Methyl iodide (500 μ Ci) was cooled to solid CO₂/acetone temperature and methyl iodide (2.85mg) in dry benzene (0.1ml) added, followed by triphenylphosphine (6.5mg) in dry benzene (0.2ml). The mixture was allowed to warm to room temperature and kept for 48h, during which time crystals of [¹⁴C]methyltriphenylphosphonium iodide were obtained. The benzene was removed under a stream of dry N₂ and three drops of dry benzene were added, followed by 3 drops of *n*-butyl-lithium in hexane (15%, w/w). The solution turned orange immediately and was left for 20min, after which compound (XVIII) (7mg) dissolved in the minimum volume of benzene was added. The resulting gel was kept for 24h and the crude product was extracted and purified as described above to yield (24S)-24-ethyl-[26-¹⁴C]cholesta-5,25-dien-3 β -ol (50 μ Ci).

Approximately half the (24S)-24-ethyl[26-¹⁴C]-cholesta-5,25-dien-3 β -ol was hydrogenated and purified as described above, yielding (24S)-24-ethyl-[26-¹⁴C]cholest-5-en-3 β -ol (XIX; 16 μ Ci). Radioautography of a thin-layer chromatogram of a sample of this product on AgNO₃-impregnated silica gel H developed with 4% diethyl ether in purified chloroform showed the presence of only one radioactive compound which co-chromatographed with clionasterol (VIII).

Feeding and extraction procedures

Young larvae of *Tenebrio molitor* (about 1500) were removed from the stock culture and fed for 10 days on a synthetic diet (Bieber & Monroe, 1969) containing 0.26% (w/w) triparanol citrate. The larvae were then starved for 24h and then maintained for a further 10 days on the diet coated with [25-³H,26-¹⁴C]clionasterol (84 μ Ci of ³H; 6 μ Ci of ¹⁴C) and 0.26% triparanol citrate. The insects were macerated in ethanol, the homogenate was saponified and the non-saponifiable material (190mg; 3.83 $\times$ 10⁶ d.p.m. of ¹⁴C) extracted. The extracted lipids were then subjected to column chromatography on alumina (Woelm acid washed; Brockmann grade III) eluted successively with (i) light petroleum (b.p. 40–60°C), (ii) 6% (v/v) diethyl ether in light petroleum (b.p. 40–60°C), (iii) 9% (v/v) diethyl ether in light petroleum (b.p. 40–60°C), (iv) 30% (v/v) diethyl ether in light petroleum (b.p. 40–60°C), and (v) diethyl ether. The fraction eluted with 30% (v/v) diethyl ether in light petroleum (b.p. 40–60°C) contained the sterols and was subjected to t.l.c. on silica gel developed with chloroform. The band co-chromatographing with a cholesterol marker was eluted and radioassayed. Radio-g.l.c. (3% OV-17) of this sterol fraction (15.8mg, 2.91 $\times$ 10⁶ d.p.m. of ¹⁴C)

showed the radioactivity to be distributed between cholesterol (17%), desmosterol (23%), 24-methylcholest-5-ene-3 β -ol (3%) and clionasterol (57%). The radioactivity associated with the C-28 sterol can be explained by the presence of a small percentage of this sterol in the administered clionasterol.

Carrier desmosterol (1 mg) was added to the sterol fraction, which was then acetylated and separated into bands corresponding to the acetates of (i) cholesterol plus clionasterol (1.38×10^6 d.p.m. of ^{14}C) and (ii) desmosterol (4.71×10^5 d.p.m. of ^{14}C) by t.l.c. on AgNO₃-impregnated silica gel developed with 40% benzene in hexane. The acetates of cholesterol and clionasterol were separated by preparative g.l.c. (3% OV-17) and a sample of these sterols was radioassayed. Samples of the acetates of the isolated cholesterol and desmosterol and also a portion of the administered [25- ^3H , 26- ^{14}C]clionasterol were diluted with the corresponding carrier material and recrystallized to constant specific radioactivity from chloroform/methanol. The remaining desmosteryl acetate was also diluted with carrier material and used to locate the position of the ^3H label.

Hydroboration of desmosteryl acetate

Diborane/tetrahydrofuran solution (0.26 ml; 1 M-diborane in tetrahydrofuran) was added at 0°C over a 1 min period to a cooled solution of desmosteryl acetate (48 mg) in tetrahydrofuran (0.34 ml). The reaction was left at 0°C for 10 min and then at room temperature for a further 2 h, with periodic shaking. The reaction was cooled in ice and saturated NaHCO₃ (0.21 ml) followed by 30-vol. H₂O₂ (0.21 ml) were added. The mixture was left at room temperature for 10 min, then poured into water and extracted with diethyl ether. The diethyl ether extract was washed with water, dried over anhydrous MgSO₄ and evaporated to dryness under reduced pressure. The product was separated by t.l.c. on silica gel developed with 5% methanol in chloroform into two compounds, 6 α ,24 ξ -dihydroxy-5 α -cholestan-3 β -yl acetate (XX) and 5 α -cholestan-3 β ,6 α -24 ξ -triol (XXI). Samples of both products when fully acetylated gave triacetates with identical R_F on t.l.c., mass and n.m.r. spectra: m/e 386.369 ($M-60$, 15%; C₃₁H₅₀O₄ requires m/e 386.371), 471 (6%) 426 (100%), 411 (14%), 401 (18%), 373 (20%), 360 (70%), 356 (70%), 351 (27%), 313 (29%), 296 (20%), 288 (21%), 283 (19%), 255 (26%), 245 (22%), 228 (72%), 213 (76%); n.m.r. ([^2H]chloroform, 100 MHz) δ 0.64 (s, 3H, C-18 methyl), 2.01 and 2.03 (6H and 3H, C-3, C-6, C-24 acetoxy methyl protons) 4.63 (m, C-3, C-6, C-24 protons).

Compounds (XX) and (XXI) were recrystallized to constant specific radioactivity from methanol and were oxidized after further addition of carrier material.

6,24-Dioxo-5 α -cholestan-3 β -yl acetate (XXII)

Compound (XX) (20 mg) dissolved in dichloromethane (1 ml) was added to an excess of bispyridyl chromium(VI) oxide and the mixture stirred for 1½ h at room temperature (Collins, 1968). The mixture was poured into diethyl ether and washed once with dilute HCl, once with satd. NaHCO₃ solution and three times with water. Removal of the diethyl ether under reduced pressure and purification of the product by t.l.c. on silica gel developed with 15% ethyl acetate in benzene gave 6,24-dioxo-5 α -cholestan-3 β -yl acetate (XXII; yield 30%), which was recrystallized from chloroform/methanol; n.m.r. ([^2H]chloroform, 100 MHz) δ 0.66 (s, 3H, C-18 methyl), 0.76 (s, 3H, C-19 methyl), 1.05 and 1.11 (d, J 6 Hz, C-26 and C-27 methyl group), 0.91 (d, J 6 Hz, C-21 methyl), 2.01 (s, 3H, C-3 acetoxy methyl protons), 4.65 (m, C-3 protons) p.p.m.; m/e 458.340 (M^+ , 85%; C₂₉H₄₆O₄ requires m/e 458.340), 443 (11%), 398 (85%), 383 (24%), 373 (24%), 355 (12%), 313.253 (100%; C₂₂H₃₃O requires m/e 313.253), 295 (15%), 271 (17%), 243 (14%), 229 (17%).

5 α -Cholestane-3,6,24-trione (XXIII)

Jones reagent [2.3 ml from 6.25 g of CrO₃ in 25 ml of 20% (v/v) H₂SO₄] was added to compound (XXI) (28 mg) dissolved in acetone (14 ml) and the reaction was terminated after standing at room temperature for 5 min by addition of methanol. The solution was extracted with diethyl ether, the extract washed with water and evaporated to dryness under reduced pressure. The crude product was purified by t.l.c. on silica gel developed with 15% ethyl acetate in benzene yielding 5 α -cholestane-3,6,24-trione (XXIII; yield 60%), which was recrystallized from chloroform/methanol to constant specific radioactivity; i.r. ν_{max} . (carbon tetrachloride) 1720 and 1230 cm⁻¹; m/e 414.310 (M^+ , 56%; C₂₇H₄₂O₃ requires m/e 414.313), 371 (10%), 329.249 ($M-C_5H_9O$, 100%; C₂₂H₃₃O₂ requires m/e 329.248), 313 (10%), 285 (18%), 245 (12%).

Results and Discussion

Synthesis of [25- ^3H]- and [26- ^{14}C]-clionasterol

Base equilibration of the aldehyde (XVI) derived from compound (XIII) produced greater than 96% decrease in the specific radioactivity of ^3H , thus demonstrating that at least that percentage of the ^3H label in the synthetic clionasterol was present at C-25. In addition, there was no discernible doublet resonance for the C-27 methyl group in the n.m.r. spectrum of 3 β -tetrahydropyranyloxy-(24S)-ethyl-[25- ^2H]cholest-5-en-26-ol produced in an analogous manner to the corresponding ^3H -labelled sample (contrast the spectrum of 3 β -tetrahydropyranyloxy-(24S)-ethylcholest-5-en-26-ol; XII). Furthermore,

the complex pattern of resonances at approx. δ 3.47 p.p.m. in the latter compound is considerably simplified in the ^2H -labelled sample (elimination of large vicinyl coupling between C-26 hydrogens and C-25 proton). These differences between the n.m.r. spectra of the ^2H -labelled and non- ^2H -labelled samples of 3 β -tetrahydropyranyloxy-(24*S*)-ethylcholest-5-en-26-ol provided further evidence for the location of the ^2H at C-25. The mass-spectral evidence also confirms this conclusion.

The starting material for the syntheses of both [25- ^3H]- and [26- ^{14}C]-clionasterol was clerosterol (X), in which the configuration at C-24 is (24*S*) (Rubinstein & Goad, 1974), so that clionasterol of the same C-24 configuration should be produced. It was, however, possible that some equilibration at C-24 in the keto compound (XVIII) could have occurred during the synthesis of the [^{14}C]clionasterol. However, evidence from 220 MHz n.m.r. spectra of the final product of each reaction sequence showed that no discernible epimerization at C-24 had occurred. This was deduced by comparison with a

220 MHz n.m.r. spectrum of an authentic sample of the (24*R*)-epimer, sitosterol, extracted from *Larix decidua*, which showed different positions for the resonances of the C-29 (triplet) and the C-21 (doublet) protons (Thompson *et al.*, 1972; Rubinstein *et al.*, 1976). Further evidence that no apparent epimerization had occurred was obtained from o.r.d. analysis, which showed that each sample of clionasterol had a negative Cotton effect and a small but significant difference in molar rotation compared with sitosterol.

Dealkylation of [25- ^3H ,26- ^{14}C]clionasterol

[25- ^3H ,26- ^{14}C]Clionasterol was administered to *Tenebrio molitor* larvae together with triparanol citrate and the resulting cholesterol and desmosterol were isolated as their acetates. The $^3\text{H}/^{14}\text{C}$ atomic ratios of the latter sterols are in close agreement with that of the administered clionasterol and indicate that the ^3H on C-25 is retained during dealkylation (Table 1). Chemical transformation of the desmosteryl acetate (V) into the diol (XX) and triol (XXI) occurred with almost complete retention of ^3H , which was nearly all eliminated on oxidation to the corresponding dione (XXII) and trione (XXIII) respectively. These results demonstrate clearly that the ^3H atom in the desmosterol is located at C-24, so that during dealkylation of the clionasterol ^3H migration from C-25 to C-24 has occurred. A similar conclusion has been obtained independently by Fujimoto *et al.* (1974) for the migration of the C-25 hydrogen of sitosterol to C-24 during conversion into desmosterol in the silkworm *Bombyx mori*. Migration of the C-25 hydrogen of 24-alkyl sterols to C-24 during dealkylation may be rationalized by invoking a mechanism (Scheme 1) analogous to that proposed previously for the chemical transformation of fucosterol 24,28-epoxide into desmosterol by boron trifluoride etherate (Ohtaka *et al.*, 1973).

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Table 1. $^3\text{H}/^{14}\text{C}$ ratios and final specific radioactivities of the sterols isolated from *Tenebrio molitor* after administration of [25- ^3H ,26- ^{14}C]clionasterol, and of their subsequent chemical transformation products

The $^3\text{H}/^{14}\text{C}$ atomic ratios are based on the $^3\text{H}/^{14}\text{C}$ radioactivity ratio of the initial clionasterol.

Substance	$^3\text{H}/^{14}\text{C}$ radio-activity ratio	$^3\text{H}/^{14}\text{C}$ atomic ratio
Administered clionasterol (VI)	14.11	1:1
Clionasteryl acetate recovered from insects (g.l.c.)	14.82	1.05:1
Cholesteryl acetate isolated from insects	14.93	1.05:1
Desmosteryl acetate isolated from insects	13.62	0.96:1
6 α ,24 ξ -Dihydroxy-5 α -cholestan-3 β -yl acetate (XX)	13.07	0.92:1
5 α -Cholestan-3 β ,6 α ,24 ξ -triol (XXI)	12.25	0.86:1
6,24-Dioxo-5 α -cholestan-3 β -yl acetate (XXII)	0.66	0.04:1
5 α -Cholestane-3,6,24-trione (XXIII)	0.36	0.02:1

Scheme 1. Possible mechanism of dealkylation

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