Conversion of Nor-ketones into Prochiral Terminal Methylene Groups: Synthesis of (24*E*)- and (24*Z*)-[28-^{2}H]Ergosta-5,24(28)-dien-3β-ols

Mario Anastasia,* Pietro Allevi, Pierangela Ciuffreda, Alberto Fiecchi, Pierluigi Gariboldi, and Antonio Scala.
Department of Medical Chemistry and Biochemistry, and Laboratory of Organic Chemistry, Faculty of Sciences, University of Milan, I-20133, Milano, Italy.

The synthesis of 24-prochiral methylene steroids from nor-ketones, illustrated by the preparation of (24*E*)- and (24*Z*)-[28-^{2}H]ergosta-5,24(28)-dien-3β-ols starting from 6β-methoxy-3α,5-cyclo-5α-cholestan-24-one (**3**), is described.

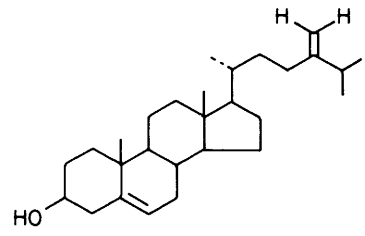
The ketone (**3**) was transformed into (24*E*)- and (24*Z*)-6β-methoxy-3α,5-cyclo-5α-stigmast-24(28)-en-29-als labelled with deuterium at C-28 or at C-29 by using labelled reagents in the different steps of the reaction sequence. Stereospecific decarbonylation of the aldehyde groups then affords the prochiral methylenes.

24-Methylene sterols occupy a key position in a number of biosynthetic processes.[1] In particular ergosta-5,24(28)-dien-3β-ol (24-methylenecholesterol) (**1**) is important as a possible biological precursor[2,3] of brassinolide (**2**), [(2*R*,3*S*,22*R*,23*R*,24*S*)-2,3,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one], a plant growth hormonal steroid isolated from the pollen of rape (*Brassica napus*)[2] in which the 24-methylenecholesterol forms 56% of the sterols.[2,3]

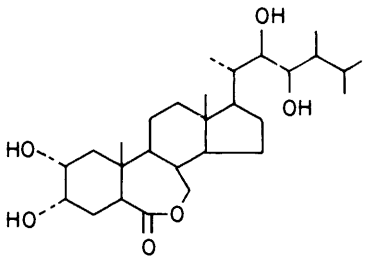
In connection with our chemical[4,5] and biological[6] interest in the brassinolide field we needed to differentiate between the two hydrogens of the terminal methylene group of compound (**1**) in order to study the stereochemistry of the biosynthetic reduction of the 24-methylene group to the (24S)-methyl group of compound (**2**). In fact it has been shown that the enzymatic hydrogenation of non-activated double bonds could occur in both *cis* and *trans* modes of addition.[7,8] In the present paper we report the synthesis of (24*Z*)- and (24*E*)-[28-^{2}H]ergosta-5,24(28)-dien-3β-ols (**15b**) and (**16b**) (Scheme 1) starting with 6β-methoxy-3α,5-cyclo-5α-cholestan-24-one (**3**), using a simple method that gives prochiral terminal methylenes from nor-ketones.[9]

Treatment of the ketone (**3**) with ethoxyethynyl-lithium[10] (Scheme 1) afforded a mixture of lithium salts of epimeric (24*R*)- and (24*S*)-29-ethoxy-6β-methoxy-3α,5-cyclo-5α-stigmast-28-yn-24-ols (**4**) which rearranged in dilute deuteriosulphuric acid to give an inseparable (t.l.c.) mixture of (24*E*)- and (24*Z*)-6β-methoxy-3α,5-cyclo-5α-[28-^{2}H]stigmast-24(28)-en-29-oic acid ethyl esters (**5**). The mass spectrum of the diastereoisomers (**5**) showed, by comparison with that of the non-deuteriated mixture (**6**) [obtained by the rearrangement of compound (**4**) by treatment with dilute sulphuric acid], the presence of a single deuterium atom in the molecule. Its position was assigned as C-28 on the basis of the ^{1}H n.m.r. spectra of compounds (**5**) and (**6**). The spectra were in agreement with the assigned structure and differed only in the olefinic proton region, the spectrum of the deuterate of compound (**5**) lacking the two singlets at δ 5.57 and 5.62 present in the spectrum of the non-deuteriated analogue (**6**) (in a 1:5 ratio as shown by the n.m.r. integration); these signals are attributable to the olefinic protons of the *Z* and *E* components of the mixture, as supported by successive isolation of the alcohols derived from (**6**).

The attempted reduction of the mixture of stereoisomers (**5**) to give the corresponding aldehydes with di-isobutylaluminium hydride at −70 °C gave an over-reduced mixture of separable alcohols that were assigned the structures (**7**) and (**8**) (in a 5:1 ratio). The structure (**7**) was assigned to the more abundant, less polar (in the eluant used) component of the mixture on the basis of its transformation into [28-^{2}H]fucosterol (**9b**) *via* the



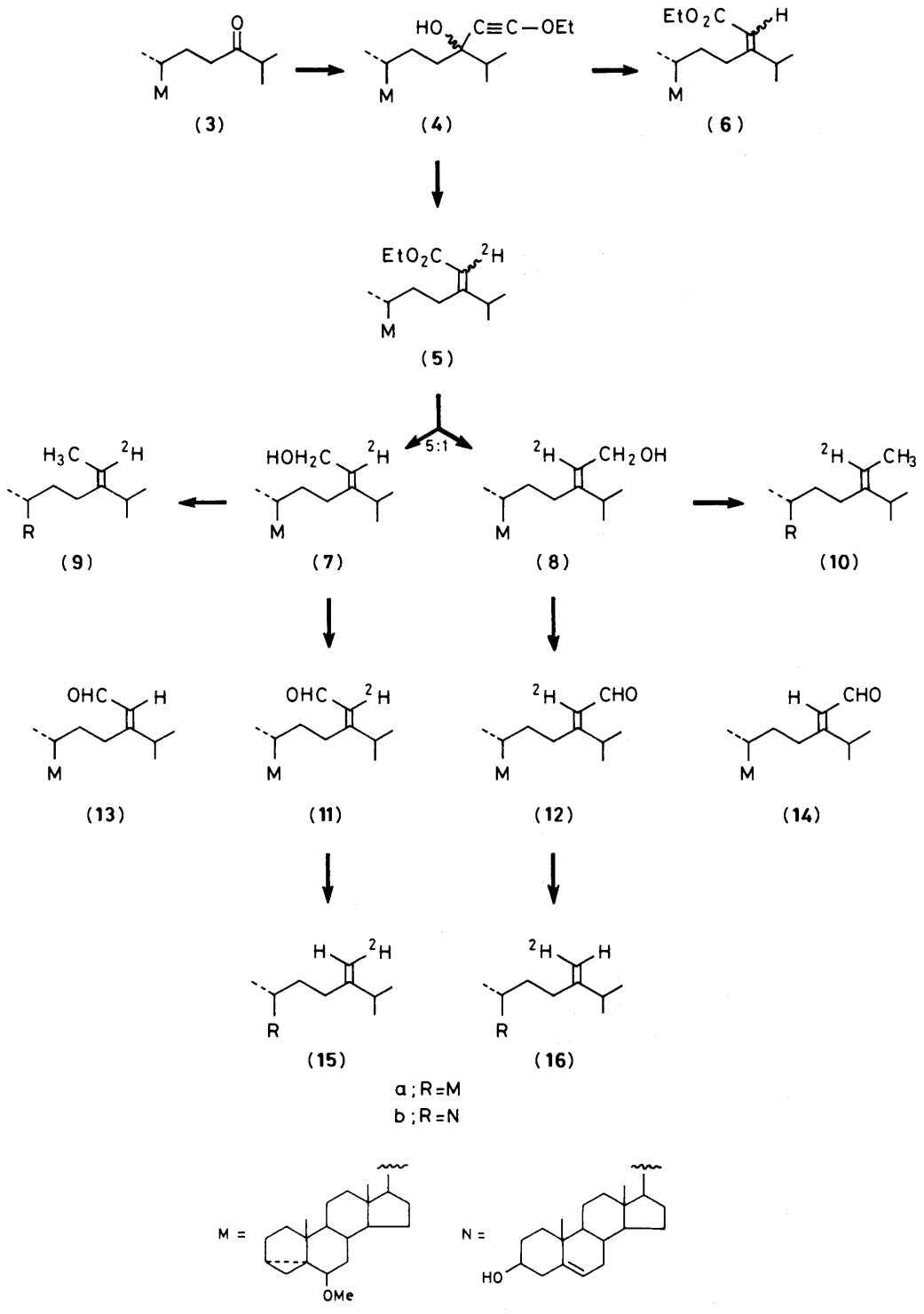
(**1**)



(**2**)

reduction of its methanesulphonic ester and the regeneration of the Δ^5-3β-OH system. A similar sequence of reactions starting with the more polar alcohol (**8**), the minor component of the mixture, afforded [28-^{2}H]isofucosterol (**10b**). Each of the allylic alcohols (**7**) and (**8**) was separately oxidized with chromic trioxide in pyridine to provide the respective aldehydes (**11**) and (**12**) in good yields. The ^{1}H n.m.r. and mass spectra of compounds (**11**) and (**12**) were in agreement with the assigned structures and were consistent with those of the unlabelled aldehydes (**13**) and (**14**) when the substitution of the vinylic hydrogen at C-28 with a deuterium atom is taken into account. The aldehydes (**13**) and (**14**) were independently obtained by 'crossed-aldol' coupling of the ketone (**3**) with trimethylsilyl-acetaldehyde t-butylimine.[11]

The (*E*)- and (*Z*)-aldehydes can be distinguished by the position of the proton at C-25 which resonates at δ 2.40 in the case of the (*Z*)-isomer and at δ 3.59 in the case of the (*E*)-isomer. Decarbonylation of the aldehyde (**11**) with tris(triphenylphosphine)chlororhodium afforded the deuteriated methylene compound (**15a**) which, after removal of the *i*-methyl ether protecting group, gave the (*Z*)-compound (**15b**). A similar sequence of reactions starting with compound (**12**) afforded the product (**16a**) and then the (*E*)-isomer (**16b**). The two

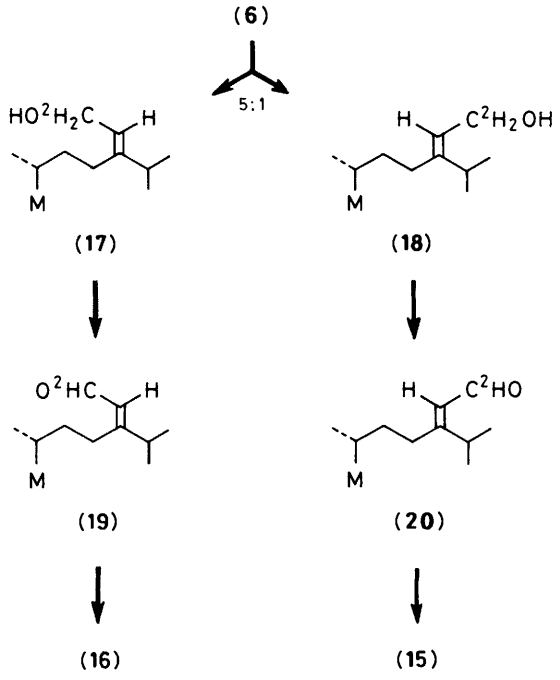


Scheme 1.

deuteriated isomers (**15b**) and (**16b**) can be distinguished both by the position of the C-28 olefinic proton resonance in the ^1H n.m.r. spectrum and by the splitting of the signal. In fact the spectrum of the (*Z*)-isomer (**15b**) shows a split signal due to one proton at δ 4.66, while that of the (*E*)-isomer (**16b**) shows a sharper signal due to one proton at δ 4.72. Separate ozonolysis of compounds (**15a**) and (**16a**) afforded the ketone (**3**).

Using this route the (24*Z*) deuteriated diastereoisomer (**15b**) is obtained in good yield while its (24E)-isomer (**16b**) is obtained in lower yield; the latter isomer is derived from the (*Z*)-ester which is the minor component of the mixture (**5**). However, utilizing the same reaction sequence, it was possible to introduce the deuterium atom at a different step of the synthesis, thus obtaining the isomer (**16b**) from the (*E*)-ester.

Lithium aluminium deuteride reduction of the ester mixture (**6**) (Scheme 2) afforded a mixture of the deuteriated alcohols (**17**) and (**18**) (in a 5:1 ratio). The isomer (**17**) was oxidized to the corresponding aldehyde (**19**) which, by decarbonylation,



Scheme 2.

afforded the methylene sterol (**16a**) and, after regeneration of the Δ^5-3β-OH system, the title compound (**16b**). A similar reaction sequence starting with the (*Z*)-alcohol (**18**) afforded the aldehyde (**20**), the methylene compound (**15a**), and then the (*Z*)-isomer (**15b**).

A comparison of ^{1}H n.m.r. spectra (200 MHz) of the C-29 labelled compounds (**17**)—(**20**) shows that the (*E*)- and (*Z*)-isomers can be distinguished on the basis of the position of the signal due to the olefinic proton at C-28 or that of the allylic proton at C-25. In fact, in the *Z* series the olefinic protons resonate at higher field while the allylic protons at C-25 resonate, as expected,[12] at lower field.

The method described for the synthesis of compounds (**15b**) and (**16b**) appears to be general in scope and is useful for the synthesis of doubly labelled terminal methylene groups with a known orientation of two hydrogen isotopes, starting from a nor-ketone. The use of sulphuric acid and lithium aluminium hydride labelled with the desired hydrogen isotopes (*i.e.* ^{1}H, ^{2}H, ^{3}H) appears to make all six differently labelled terminal methylene groups available.

Experimental

M.p.s are uncorrected. I.r. spectra were recorded for solutions in chloroform or for Nujol mulls. ^{1}H N.m.r. spectra were recorded on a Varian HA-100 or a Varian XL-200 instrument for solutions in [^{2}H]chloroform and are reported as δ values relative to internal Me_4Si. Optical rotations were recorded for chloroform solutions. The mass spectra were determined on a Varian MAT 112 S spectometer by direct inlet methods. The progress of all reactions and column chromatography determinations (silica 230—400 mesh) was monitored by t.l.c. on silica gel (HF_{254}) plates. Hexane–ethyl acetate mixtures were used as developing solvents and spots were detected by spraying with 70% sulphuric acid followed by heating. The $[\alpha]_D$ values are given in °.

(24E)- *and* (24Z)-6β-*Methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*sigmast*-24(28)-*en*-29-*oic Acid Ethyl Esters* (**5**).—Ethoxyethynyl-lithium was prepared by dropwise addition of a solution of n-butyllithium in hexane (1.05 ml of a 1.6M-solution) to a stirred solution of ethoxyethyne (0.420 ml of a 40% solution in hexane) in dry tetrahydrofuran (1 ml) at −50 °C for 1 h and then was added to a solution of 6β-methoxy-3α,5-cyclo-5α-cholestan-24-one (**3**) (500 mg) in dry tetrahydrofuran (3 ml). The mixture was stirred for 1 h at −50 °C, allowed to warm to room temperature, and treated with deuteriosulphuric acid (1 ml of a 10% solution in deuterium oxide). The solution was stirred for 12 h at room temperature, and then sodium hydrogen carbonate (8 ml of a 0.5N-solution) was added, and the reaction mixture was extracted with diethyl ether to afford, after evaporation of the solvent, a residue; this was purified by column chromatography (t.l.c. grade silica gel, 3% ethyl acetate–hexane) to give a mixture of non-crystalline esters (**5**) (370 mg). The mixture of diastereoisomers (**5**) was not separable on t.l.c. and showed the following properties: δ 0.73 (3 H, s, 18-CH_3), 1.00 (3 H, s, 19-CH_3), 2.75 (1 H, br t, 6α-H, *J* 3 Hz), 3.30 (3 H, s, CH_3OCH) and 4.15 (2 H, q, CH_3CH_2OCO, *J* 7 Hz) [Found: C, 79.1; H, 10.8%; *M* (mass spectrum), 485. $C_{32}H_{51}{}^2HO_3$ requires C, 79.12; H, 11.00%; *M*, 485]. The ^{2}H isomeric compounds were 96% isotopically pure.

The mixture (**6**) was obtained in a similar way using sulphuric acid as the isomerizing acid. The ^{1}H n.m.r. spectrum was almost identical with that of compound (**5**), but in addition showed signals at δ 5.57 and 5.62 (1 H, 2 × s, 28-H of *Z*- and *E*-components) [Found: C, 79.1; H, 10.9%; *M* (mass spectrum), 484. $C_{32}H_{52}O_3$ requires C, 79.29; H, 10.81%; *M*, 484].

(24E)- *and* (24Z)-6β-*Methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*stigmast*-24(28)-*en*-29-*ols* (**7**) *and* (**8**).—The ester mixture (**5**) (400 mg) in toluene (15 ml) was cooled to −70 °C and di-isobutylaluminium hydride (300 mg in 2.5 ml of toluene) was added under dry nitrogen. The solution was kept at −70 °C for 4 h before ethyl acetate (0.5 ml) was added. The solution was allowed to warm to room temperature and then poured into a saturated ammonium chloride solution. Work-up followed by rapid chromatography (20% ethyl acetate–hexane) afforded first (24E)-6β-*methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*stigmast*-24(28)-*en*-29-*ol* (**7**) (260 mg) as an oil; δ 0.70 (3 H, s, 18-CH_3), 1.01 (3 H, s, 19-CH_3), 2.24 (1 H, hept, 25-H, *J* 6 Hz), 2.75 (1 H, br t, 6α-H, *J* 3 Hz), 3.30 (3 H, s, CH_3OCH), and 4.11 (2 H, br s, CH_2OH) [Found: C, 81.35; H, 11.47%; *M* (mass spectrum), 443. $C_{30}H_{49}{}^2HO_2$ requires C, 81.20; H, 11.58%; *M*, 443]. The ^{2}H isomeric compound was 96% isotopically pure.

Further elution yielded (24Z)-6β-*methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*stigmast*-24(28)-*en*-29-*ol* (**8**) (52 mg) as an oil; δ 0.70 (3 H, s, 18-CH_3), 1.01 (3 H, s, 19-CH_3), 2.81 (1 H, hept., 25-H, *J* 6 Hz), 2.75 (1 H, br t, 6-H, *J* 3 Hz), 3.30 (3 H, s, CH_3OCH), and 4.11 (2 H, br s, CH_2OH) [Found: C, 81.1; H, 11.65%; *M* (mass spectrum), 443. $C_{30}H_{49}{}^2HO_2$ requires C, 81.20; H, 11.58%; *M*, 443]. The ^{2}H isomeric compound was 96% isotopically pure.

[28-^{2}H]*Fucosterol* (**9b**) *and* [28-^{2}H]*Isofucosterol* (**10b**).—To a stirred solution of the alcohol (**7**) (80 mg) and freshly distilled triethylamine (110 μl) in dry dichloromethane (2.6 ml) at 0 °C under nitrogen was added freshly distilled methanesulphonyl chloride (44 μl). After 30 min at 0 °C the liquid component of the mixture was removed under reduced pressure. Dry diethyl ether (15 ml) and lithium aluminium hydride (450 mg) were added and the mixture stirred at room temperature for 3 h. Water (1 ml), sodium hydroxide (1 ml of a 15% aqueous solution), and water (3 ml) were then added sequentially. Filtration of the mixture and removal of the diethyl ether from the filtrate gave a residue which was chromatographed to afford (24E)-6β-*methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*stigmast*-24(28)-*ene* (**9a**) (48 mg): m.p. 70—71 °C; $[\alpha]_D{}^{20}$ −46 (lit.,[13] for non-deuteriated compound, m.p. 70—71 °C; $[\alpha]_D{}^{18}$ −47.2); δ 0.65 (3 H, s, 18-CH_3), 0.95 (3 H, s, 19-CH_3), 1.53 (3 H, s, 29-CH_3),

2.72 (1 H, br t, 6-H, *J* 3 Hz), 2.30 (1 H, hept., 25-H, *J* 6 Hz), and 3.28 (3 H, s, CH_3OCH) [Found: C, 84.35; H, 12.2%; *M* (mass spectrum), 427. $C_{30}H_{49}{}^2HO$ requires C, 84.24; H, 12.02%; *M*, 427].

The *i*-methyl ether (**9a**) (40 mg) in 10% aqueous dioxane (16 ml) containing a catalytic amount of toluene-*p*-sulphonic acid was heated under reflux for 1 h. After cooling, the usual work-up afforded (24E)-[28-^{2}H]*stigmasta*-5,24(28)-*dien*-3β-*ol* ([28-^{2}H]*fucosterol*) (**9b**) (30 mg): m.p. 123—124 °C (from methanol); $[\alpha]_D{}^{20}$ −40 (lit.,[13] for non-deuteriated compound: m.p. 124 °C; $[\alpha]_D$ −41.80); δ 0.68 (3 H, s, 18-CH_3), 1.03 (3 H, s, 19-CH_3), 1.57 (3 H, s, 29-CH_3), 2.20 (1 H, hept., 25-H, *J* 6 Hz), 3.50 (1 H, m, 3-H), and 5.36 (1 H, m, 6-H) [Found: C, 84.3; H, 11.8%; *M* (mass spectrum), 413. $C_{29}H_{47}{}^2HO$ requires C, 84.19; H, 11.94%; *M*, 413].

Similar treatment of the alcohol (**8**) (70 mg) gave (24Z)-6β-*methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*stigmast*-24(28)-*ene* (**10a**) (50 mg) as an oil; δ 0.65 (3 H, s, 18-CH_3), 0.95 (3 H, s, 19-CH_3), 1.53 (3 H, s, 29-CH_3), 2.72 (1 H, br t, 6-H, *J* 3 Hz), 2.78 (1 H, hept., 25-H, *J* 6 Hz), 3.28 (3 H, s, CH_3OCH) [Found: C, 84.4; H, 12.1%; *M* (mass spectrum), 427. $C_{30}H_{49}{}^2HO$ requires C, 84.24; H, 12.02%; *M*, 427].

The *i*-methyl ether protecting group in (**10a**) was removed to afford (24Z)-[28-^{2}H]*stigmasta*-5,24(28)-*dien*-3β-*ol* (**10b**) (30 mg) ([28-^{2}H]*isofucosterol*): m.p. 130—131 °C; δ 0.68 (3 H, s, 18-CH_3), 1.03 (3 H, s, 19-CH_3), 1.57 (3 H, s, 29-CH_3), 2.78 (1 H, hept., 25-H, *J* 6 Hz), 3.50 (1 H, m, 3-H), and 5.36 (1 H, m, 6α-H) [Found: C, 84.3; H, 11.85%; *M* (mass spectrum), 413. $C_{29}H_{47}{}^2HO$ requires C, 84.19; H, 11.94%; *M*, 413].

All the physicochemical properties except for the mass spectrum were identical with those reported for the non-deuteriated compound.[14]

(24E)- *and* (24Z)-6β-*Methoxy*-3α,5-*cyclo*-5α-[29,29-$^2H_2$]*stigmast*-24(28)-*en*-29-*ols* (**17**) *and* (**18**).—The ester mixture (**6**) (500 mg) in anhydrous diethyl ether (10 ml) was added dropwise to an ice-cold solution of lithium aluminium deuteride (200 mg) in anhydrous diethyl ether (10 ml). After an hour the excess of deuteride was destroyed by addition of saturated aqueous sodium sulphate. The clear dry ethereal solution was filtered from the insoluble aluminium salts and concentrated under reduced pressure to give a crude residue which, after chromatography, gave first (24E)-6β-*methoxy*-3α,5-*cyclo*-5α-[29,29-$^2H_2$]*stigmast*-24(28)-*en*-29-*ol* (**17**) (320 mg) as an oil; δ 0.70 (3 H, s, 18-CH_3), 1.01 (3 H, s, 19-CH_3), 2.24 (1 H, hept., 25-H, *J* 6 Hz), 2.75 (1 H, br t, 6α-H, *J* 3 Hz), 3.30 (3 H, s, CH_3OCH), and 5.34 (1 H, s, 28-H) [Found: C, 81.15; H, 11.65%; *M* (mass spectrum), 444. $C_{30}H_{48}{}^2H_2O_2$ requires C, 81.02; H, 11.78%; *M*, 444]. The ^{2}H compound was 96% isotopically pure.

Further elution yielded (24Z)-6β-*methoxy*-3α,5-*cyclo*-5α-[29,29-$^2H_2$]*stigmast*-24(28)-*en*-29-*ol* (**18**) (68 mg) as an oil; δ 2.81 (1 H, hept., 25-H, *J* 6 Hz), 5.28 (1 H, s, 28-H); all other proton signals are identical with those reported for (**17**) [Found: C, 81.2; H, 11.5%; *M* (mass spectrum), 444. $C_{30}H_{48}{}^2H_2O_2$ requires C, 81.02; H, 11.78%; *M*, 444]. The ^{2}H compound was 96% isotopically pure.

(24Z)- *and* (24E)-6β-*Methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*stigmast*-24(28)-*en*-29-*als* (**12**) *and* (**11**).—The alcohol (**8**) (300 mg) in pyridine (2 ml) was oxidized with chromium trioxide (150 mg) in pyridine at 4 °C for 12 h. The usual work-up and crystallization afforded (24Z)-6β-*methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*stigmast*-24(28)-*en*-29-*al* (**12**) (260 mg), m.p. 81—83 °C (from methanol); $\nu_{max.}$ 1 680 cm^{-1}; δ 0.68 (3 H, s, 18-CH_3), 0.96 (3 H, s, 19-CH_3), 2.74 (1 H, br t, 6-H, *J* 3 Hz), 3.30 (3 H, s, CH_3OCH), 3.59 (1 H, hept., 25-H, *J* 6 Hz), and 10.00 (1 H, s, CHO) [Found: C, 81.7; H, 11.2%; *M* (mass spectrum), 441. $C_{30}H_{47}{}^2H_2O_2$ requires C, 81.58; H, 11.18%; *M*, 441]. The ^{2}H compound was 96% isotopically pure.

Oxidation of the alcohol (**7**) (500 mg) afforded (24E)-6β-*methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*stigmast*-24(28)-*en*-29-*al* (**11**) (450 mg): m.p. 90—92 °C (from methanol); $\nu_{max.}$ 1 680 cm^{-1}; δ 2.40 (1 H, hept., 25-H, *J* 6 Hz); all the other signals are identical with those of (**12**) [Found: C, 81.7; H, 11.1%; *M* (mass spectrum), 441. $C_{30}H_{47}{}^2HO_2$ requires C, 81.58; H, 11.18%; *M*, 441]. The ^{2}H compound was 96% isotopically pure.

(24E)-*and*(24Z)-6β-*Methoxy*-3α,5-*cyclo*-5α-[29-^{2}H]*stigmast*-24(28)-*en*-29-*als* (**19**) *and* (**20**).—The (24*E*)-alcohol (**17**) (400 mg) was oxidized as described above to provide (24E)-6β-*methoxy*-3α,5-*cyclo*-5α-[29-^{2}H]*stigmast*-24(28)-*en*-29-*al* (**19**) (350 mg): m.p. 90—92 °C (from methanol); δ 5.82 (1 H, br s, 28-H); all other signals, apart from that at δ 10.00 which is missing, are similar to those described for (**13**) [Found: C, 81.75; H, 11.05%; *M* (mass spectrum), 441. $C_{30}H_{47}{}^2HO_2$ requires C, 81.58; H, 11.18%; *M*, 441]. The ^{2}H compound was 95.8% isotopically pure.

Oxidation of the alcohol (**18**) (200 mg) provides (24Z)-6β-*methoxy*-3α,5-*cyclo*-5α-[29-^{2}H]*stigmast*-24(28)-*en*-29-*al* (**20**) (180 mg): m.p. 81—83 °C; δ 5.78 (1 H, br s, 28-H); apart from the signal at δ 10.0 which is missing, all the signals are similar to those described for (**14**) [Found: C, 81.6; H, 11.8%; *M* (mass spectrum), 441. $C_{30}H_{47}{}^2HO_2$ requires C, 81.58; H, 11.18%; *M*, 441]. The ^{2}H compound was 95.8% isotopically pure.

(24E)- *and* (24Z)-6β-*Methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*ergost*-24(28)-*enes* (**16a**) *and* (**15a**).—(*a*) The aldehyde (**12**) (200 mg) was dissolved in degassed toluene (5 ml) and was refluxed under nitrogen in the presence of tris(triphenylphosphine)chlororhodium (200 mg) for 1 h. The mixture was filtered through a pad of Celite and the solvent was removed under reduced pressure. The residue was chromatographed (t.l.c. grade silica gel, 15% ethyl acetate–hexane) to afford the deuteriated compound (24E)-6β-*methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*ergost*-24(28)-*ene* (**16a**) (160 mg) as an oil; δ (200 MHz) 0.72 (3 H, s, 18-CH_3), 1.00 (3 H, s, 19-CH_3), 2.20 (1 H, hept., 25-H, *J* 6 Hz), 2.76 (1 H, br t, 6α-H, *J* 3 Hz), 3.30 (3 H, s, CH_3OCH), and 4.72 (1 H, br s, 28-H) [Found: C, 84.25; H, 11.8%; *M* (mass spectrum), 413. $C_{29}H_{47}{}^2HO$ requires C, 84.19; H, 11.94%; *M*, 413]. The ^{2}H compound was 95.7% isotopically pure.

Similar treatment of the aldehyde (**11**) (400 mg) afforded (24Z)-6β-*methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*ergost*-24(28)-*ene* (**15a**) (280 mg) as an oil: δ (200 MHz) 4.64 (1 H, br s, 28-H); all the other physicochemical properties were identical with those of (**16a**) (Found: C, 84.3; H, 11.85. $C_{29}H_{47}{}^2HO$ requires C, 84.19; H, 11.94%). The ^{2}H compound was 95.8% isotopically pure.

(*b*) The aldehyde (**19**) (400 mg) afforded compound (**16a**) (300 mg) (Found: C, 84.3; H, 11.9. $C_{29}H_{47}{}^2HO$ requires C, 84.19; H, 11.94%). The ^{2}H compound was 95.6% isotopically pure.

The aldehyde (**20**) (200 mg) afforded compound (**15a**) (140 mg) (Found: C, 84.1; H, 11.7. $C_{29}H_{47}{}^2HO$ requires C, 84.19; H, 11.94%). The ^{2}H compound was 95.6% isotopically pure.

(24E)- *and* (24Z)-[28-^{2}H]*Ergosta*-5,24(28)-*dien*-3β-*ols* (**16b**) *and* (**15b**).—The (24*E*)-[28-^{2}H]-*i*-methyl ether (**16a**) (100 mg) in 10% aqueous dioxane (40 ml) containing toluene-*p*-sulphonic acid (10 mg) was heated under reflux for 1 h. After cooling, the usual work-up afforded the crude (24*E*)-isomer (**16b**) (90 mg): m.p. 143—144 °C (from aqueous methanol); $[\alpha]_D{}^{20}$ −36; δ 0.69 (3 H, s, 18-CH_3), 1.01 (3 H, s, 19-CH_3), 3.50 (1 H, m, 3-H), 4.70 (1 H, br s, 28-H), and 5.35 (1 H, m, 6-H) [Found: C, 84.25; H, 11.8%; *M* (mass spectrum), 399. $C_{28}H_{45}{}^2HO$ requires C, 84.14; H, 11.85%; *M*, 399]. The ^{2}H compound was 95.6% isotopically pure.

Similar treatment of compound (**15a**) (100 mg) afforded the (24*Z*)-isomer (**15b**) (80 mg): m.p. 143—144 °C (from aqueous methanol); $[\alpha]_D^{21}$ −36; δ 4.64 (1 H, br s, 28-H); all the other proton signals are identical with those reported for (**16b**) [Found: C, 84.25; H, 11.8%; *M* (mass spectrum), 399. $C_{28}H_{45}{}^2HO$ requires C, 84.14; H, 11.85%; *M*, 399]. The ^2H compound was 95.7% isotopically pure (lit.,[15] for non-deuteriated compound, m.p. 143—144 °C; $[\alpha]_D^{20}$ −36.4°).

Ozonolysis of (24E)- *and* (24Z)-6β-*Methoxy*-3α,5-*cyclo*-5α-[28-^{2}H]*ergosta*-24(28)-*dienes* (**16a**) *and* (**15a**).—A solution of labelled *i*-methylene sterol (**16a**) (50 mg) in dichloromethane (5 ml) was ozonized at −70 °C until an excess of ozone was present. Treatment with Zn (20 mg) and acetic acid (0.5 ml) and the usual work-up afforded 6β-methoxy-3α,5-cyclo-5α-cholestan-24-one (**3**) (42 mg): m.p. 127—128 °C; $[\alpha]_D^{20}$ −44 (lit.,[13] 129—130 °C; $[\alpha]_D$ −45.4) [Found: C, 81.15; H, 11.25%; *M* (mass spectrum), 414. Calc. for $C_{28}H_{46}O_2$: C, 81.10; H, 11.18%; *M*, 414].

Similar results were obtained starting with compound (**15a**).

(24E)- *and* (24Z)-6β-*Methoxy*-3α,5-*cyclo*-5α-*stigmast*-24(28)-*en*-29-*als* (**13**) *and* (**14**).—To a stirred solution of lithium diisopropylamide (2.9 mmol) in tetrahydrofuran (10 ml) at 0 °C, trimethylsilylacetaldehyde t-butylimine[11] (479 mg, 2.8 mmol) was added during 5 min under argon. The reaction mixture was stirred for a further 15 min, cooled to −78 °C, and added to the *i*-ketone (**3**) (600 mg, 1.45 mmol). The resulting mixture was warmed to −20 °C during 2.5 h and then quenched with water (3 ml). The reaction pH was adjusted to 4.5 with solid oxalic acid and stirring was continued for a further 30 min. The reaction mixture was poured into brine and extracted with ethyl acetate. After the usual work-up the solid residue was chromatographed (10% ethyl acetate–hexane) to afford first (24E)-6β-*methoxy*-3α,5-*cyclo*-5α-*stigmast*-24(28)-*en*-29-*al* (**13**) (190 mg): m.p. 90—92 °C (from methanol); $\nu_{max.}$ 1 680 cm^{-1}; δ 0.68 (3 H, s, 18-CH_3), 0.96 (3 H, s, 19-CH_3), 2.40 (1 H, hept., 25-H, *J* 6 Hz), 2.74 (1 H, br t, 6-H, *J* 3 Hz), 3.30 (3 H, s, CH_3OCH), 5.82 (1 H, br d, 28-H, *J* 8 Hz), 10.00 (1 H, d, CHO, *J* 8 Hz) [Found: C, 81.8; H, 11.05%; *M* (mass spectrum), 440. $C_{30}H_{48}O_2$ requires C, 81.76; H, 10.98%; *M*, 440].

Further elution yielded (24Z)-6β-*methoxy*-3α,5-*cyclo*-5α-*stigmast*-24(28)-*en*-29-*al* (**14**) (170 mg): m.p. 81—83 °C (from methanol); $\nu_{max.}$ 1 680 cm^{-1}; δ 3.59 (1 H, hept., 25-H, *J* 6 Hz), 5.78 (1 H, br d, 28-H, *J* 8 Hz); all the other proton signals are identical with those reported for (**13**) [Found: C, 81.6; H, 11.05%; *M* (mass spectrum), 440. $C_{30}H_{48}O_2$ requires C, 81.76; H, 10.98%; *M*, 440].

Acknowledgements

This research was supported by the Ministero della Pubblica Istruzione.

References

1 W. R. Nes, *Adv. Lipid Res.*, 1977, **15**, 233.
2 M. D. Grove, G. F. Spencer, W. K. Rohwedder, N. Mandava, J. F. Worley, J. D. Warthen, G. L. Steffens, J. L. Flippen-Anderson, and J. C. Cook, *Nature*, 1979, **281**, 216.
3 H. Abe, T. Morishita, M. Uchiyama, S. Takatsuto, N. Ikekawa, M. Ikeda, T. Sassa, T. Kitsuwa, and S. Marumo, *Experientia*, 1983, **39**, 351.
4 M. Anastasia, P. Ciuffreda, and A. Fiecchi, *J. Chem. Soc., Perkin Trans. 1*, 1983, 379.
5 M. Anastasia, P. Ciuffreda, M. Del Puppo, and A. Fiecchi, *J. Chem. Soc., Perkin Trans. 1*, 1983, 383.
6 R. Cerana, P. Lado, M. Anastasia, P. Ciuffreda, and P. Allevi, *Z. Pflanzenphysiol. Bd.*, 1984, **114**, S., 221.
7 I. A. Watkinson, D. C. Wilton, A. D. Rahimtula, and M. M. Akhtar, *Eur. J. Biochem.*, 1971, **23**, 1.
8 I. S. Rosenfeld, and S. B. Tove, *J. Biol. Chem.*, 1971, **16**, 5025.
9 For a different route to a 24-methylene sterol labelled in the terminal methylene group starting with an acetylenic compound see: D. Arigoni, in 'Molecular Interaction and Activity in Proteins,' Ciba Foundation Symposium 60, November 1978, Excerpta Medica, pp. 243—261.
10 G. Stork, and M. Tomasz, *J. Am. Chem. Soc.*, 1964, **86**, 471.
11 E. J. Corey, D. Enders, and M. G. Bock, *Tetrahedron Lett.*, 1976, 7.
12 R. B. Bates, A. D. Brewer, B. R. Knights, and J. W. Rowe, *Tetrahedron Lett.*, 1968, 6163.
13 D. H. Hey, J. Honeyman, and W. J. Peal, *J. Chem. Soc.*, 1950, 2881.
14 F. Nicotra, and L. Toma, *Gazz. Chim. Ital.*, 1980, **110**, 579.
15 I. J. Massey and C. Djerassi, *J. Org. Chem.*, 1979, **44**, 2448.

Received 6th July 1984; *Paper* 4/1170