Marine Sterols: Unique 3β-Hydroxymethyl-A-nor-5α-steranes from the Sponge *Axinella Verrucosa*

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The total sterol content of the marine sponge *Axinella verrucosa* is a mixture of unique stanols containing a 3β-hydroxymethyl-A-nor-5α-cholestane nucleus with conventional C_8, C_9, and C_{10} side-chains.

MARINE invertebrates have proved to be sources of unusual sterols containing the cholesterol nucleus with side-chain structures involving new alkylation patterns. Thus gorgosterol,[1] acansterol,[2] and 23-demethylgorgosterol [3] exemplify the attachment of 'extra' carbon atoms at C-22 and C-23; in 29-methylisofucosterol,[4] isolated from *Placopecten magellanicus*, a propylidene group has been elaborated at C-24; and in the more recently discovered sponge sterols aplysterol and 24(28)-didehydroaplysterol [5] an 'extra' carbon atom is attached at C-26.

Modifications of the sterol nucleus are also found. The marine sponge *Axinella polypoides* has recently been shown to contain a 19-nor-5α,10β-cholestan-3β-ols carrying conventional saturated and monounsaturated C_7 (24-nor), C_8, C_9, and C_{10} side-chains.[6] We report here some natural sterols containing a 3β-hydroxymethyl-A-nor-5α-cholestane nucleus and conventional C_8, C_9, and C_{10} side-chains (1)—(6).

These unique stanols were isolated from the marine sponge *Axinella verrucosa*, in which the usual sterols are absent.[7] A free sterol fraction, less polar then the 3β-hydroxy-sterols as indicated by t.l.c. on silica in chloroform (R_F 0·45 as against R_F 0·4), was obtained by chromatography of the crude extract on silica gel, and was further resolved into four fractions by acetylation and chromatography on alumina impregnated with silver nitrate. The results are reported in the Table.

The major fraction, I, apparently homogeneous on t.l.c. (SiO_2–$AgNO_3$), was shown by g.l.c. to be a mixture of three homologues (ratio *ca.* 7·5 : 1·2 : 1·3), with retention times (t_R) 1·00, 1·40, and 1·64 relative to that of cholesteryl acetate. The mass spectrum showed peaks at *m/e* 458, 444, and 430 corresponding to the acetates of fully saturated C_{29}, C_{28}, and C_{27} sterols, and accurate mass measurements confirmed the molecular formulae $C_{31}H_{54}O_2$, $C_{30}H_{52}O_2$, and $C_{29}H_{50}O_2$, respectively.

Furthermore, the mass spectrum showed, besides signals for M^+ — AcOH (*m/e* 398, 384, and 370), two significant peaks at *m/e* 275 and 215, characteristic of fully saturated stanyl acetates,[8] the first arising by loss of the side-chain and part of ring D (42 m.u.) and the second by the same sequences from M^+ — AcOH. G.l.c.–mass spectrometry confirmed that all three components give the same fragmentation pattern (see Experimental section).

Having shown the homologous relationship between these three compounds, we continued the structural work on the mixture, of which the C_{27} component comprised *ca.* 75%.

The n.m.r. spectrum of the free stanol mixture [(1)—(3)] included an eight-line signal (2H) centred at δ 3·60 with the characteristic features of the AB portion of an ABX pattern (*J* 10, 8, and 6 Hz); irradiation at δ 2·2 changed the multiplet into an AB quartet at δ 3·75 and 3·47 (*J* 10 Hz).

This established the presence of a $HC{\cdot}CH_2{\cdot}OH$ grouping, as confirmed by the mass spectrum of the mixture, which included strong peaks corresponding to the elimination of 31 m.u. (CH_2OH) from the molecular ions, and by the conversion of the compounds by oxidation with chromic acid into the corresponding carboxylic acids (7), M^+ 430, 416, and 402, δ (m, $CH{\cdot}CO_2H$) 2·87. Both the mixture of stanols and the mixture of carboxylic acids show the 10- and 13-methyl n.m.r. signals at δ 0·74 and 0·65, respectively.

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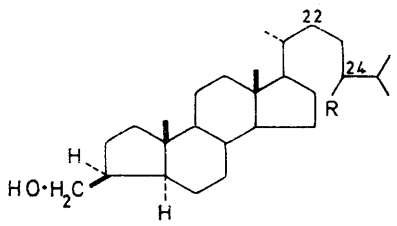
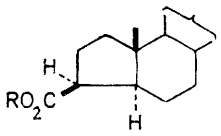
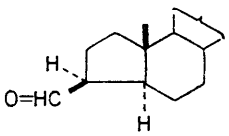
All the foregoing evidence pointed to a hydroxymethyl-A-nor-sterane structure for the stanols.



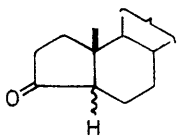
(1) R=H
(2) R=Me
(3) R=Et
(4) R=H, Δ^{22}
(5) R=Me, Δ^{22}
(6) R=Et, Δ^{22}



(7) R=H
(8) R=Me



(9)

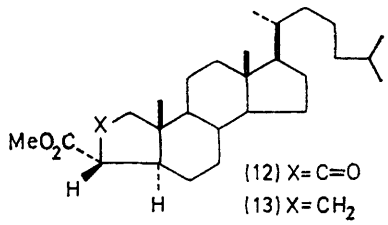


(10) 5α–H
(11) 5β–H

We then attempted to transform the stanols into the corresponding nor-ketones. Treatment with phosphoric trichloride gave very low yields of the exocyclic olefin; and Wieland–Barbier degradation (PhMgBr) of the methyl esters (8) and attempts to obtain the nor-amines by a Schmidt reaction on the acids (7) were unsuccessful. However, oxidation of the free stanols with dicyclohexylcarbodi-imide–dimethyl sulphoxide gave a mixture of the corresponding aldehydes (9), M^+ 414, 400, and 386, δ 9·84 (d, J 1·2 Hz, CHO) and 0·60 (10-Me). The aldehydes were converted into the enol acetates, and ozonolysis of the latter led to the nor-ketones (10), M^+ 400, 386, and 372 as the sole products. An i.r. band at 1735 cm^{-1} (five-membered ring ketone) and base-catalysed deuteriation leading to exchange of three hydrogen atoms suggested an A-nor-cholestan-3-one structure for the major component of the mixture. The *trans*-5α-stereochemistry of the AB ring junction was evident from the results of base-catalysed equilibration, which gave quantitatively, as expected, the more stable AB-*cis*-epimer (11).[9,10] Confirmation was obtained by c.d. measurements on the ketones (10), which exhibited an intense negative Cotton effect (θ_{290} −18,850); a positive Cotton effect (θ_{292} +7890) was exhibited by the AB-*cis*-epimers.[10]

In order to determine the stereochemistry at position 3, we then decided to synthesize the methyl A-nor-cholestan-3-carboxylates for comparison with the esters derived from the natural stanols. Literature compilations offered us two alternative routes, both apparently leading to the 3α-isomer. The first consisted of applying the Favorskii reaction to the 2α-bromocholestan-3-one, which was reported by Evans *et al.*[11] to give a separable mixture of methyl A-nor-5α-cholestane-2α- and -3α-carboxylates. The choice of configurations for the two isomers was made on mechanistic grounds. The second route consisted of Dieckmann cyclization of dimethyl 2,3-seco-5α-cholestane-2,3-dioate to the β-keto-ester (12), followed by conversion into the ethylene thioacetal and subsequent desulphurization to give the deoxo-ester (13), as first reported by Fuchs and Loewenthal.[12] These authors originally assigned the 3β-configuration to the Dieckmann cyclization product, and accordingly to the derived deoxo-ester. The possibility that the β-keto-ester was in fact the 3α-epimer (12) was suggested by Smith,[13] in view of the value of $J_{3,5\alpha}$ (13 Hz), and subsequently supported by studies[14] of the effects of solvent changes on the chemical shift of the 10-methyl protons, and o.r.d. studies of (12) and other relevant compounds. The 3α-ester (13) of Fuchs and Loewenthal[12] melted at 78·5—79°, in contrast to the ester (m.p. 45°) obtained by the Favorskii route.[11] It therefore seems possible that the latter was the 3β-isomer.

The hydroxymethyl group of the natural stanols was expected to have the β-configuration because of the



(12) X=C=O
(13) X=CH_2

apparently low reactivities of the corresponding carboxylic acids and esters: considerable steric repulsion might be expected between the 10-methyl and a 3β-ester group. With this in mind we repeated the two syntheses and found that both gave the 3α-ester (13). The low m.p. of the material reported by Evans *et al.* is now ascribed to contamination by the 2α-isomer, as shown by the n.m.r. spectrum.

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As expected, the synthetic 3α-ester (13) was different from the ' natural ' esters in its t.l.c. and n.m.r. properties. The principal differences in the n.m.r. spectra were the chemical shifts of the 10-methyl protons [δ 0·76 for the ' natural ' esters and 0·72 for (13)], and of the 3-proton [δ 2·87 for the ' natural ' esters and 2·46 for (13), strongly indicating a 3β-configuration for the former]. Since the 3β-isomer suffers a 1,3-diaxial Me–CO_2Me interaction, it should be thermodynamically less stable than the 3α-isomer, into which we expected it would be easily converted. Indeed, treatment of the ' natural ' esters with sodium methoxide gave the 3α-epimers in quantitative yield, which ran concurrently with (13) on t.l.c. The n.m.r. spectra were also identical apart from some minor differences attributable to the presence of the higher homologues in the ' natural ' ester mixture. The mass spectra were superimposable below *m/e* 416 (the molecular ion of the C_{27} component). The major component of the epimerized mixture gave a g.l.c. peak with retention time identical with that of (13).

Thus the C_{27} component of fraction I was identified as 3β-hydroxymethyl-A-nor-5α-cholestane (1). The two minor components are assumed to be the 24-methyl (2) and 24-ethyl (3) derivatives, respectively, on biogenetic grounds and on the basis of the evidence given below.

Fractions II—IV all contained single compounds, which were identified (as the acetates) as the 22,23-dehydro-derivatives (4)—(6) mainly from spectroscopic data (see Experimental section). The mass spectra fragmentation clearly established that the ' extra ' carbon atoms of the C_{28}-Δ^{22}- and C_{29}-Δ^{22}-compounds were in the conventional 24-position. In the spectrum of the acetate of (5) (M^+ 442) a doublet at *m/e* 257 (M^+ — side-chain — AcOH) and 255 (M^+ — side-chain — AcOH — 2H) showed that the side chain is C_9 and unsaturated.[8] Two further principal peaks at *m/e* 344 (loss of C_7H_{13} and H transfer) and 339 (loss of Pr^i and AcOH) located the unsaturation at C-22,[15] and established that the ' extra ' methyl group was at position 24. The mass spectrum of the acetate of (6) showed the molecular ion at *m/e* 456 and the subsequent fragmentation pattern was similar to that of the acetate of (5). The allylic fragment was seen at *m/e* 353 (loss of Pr^i and AcOH), corresponding to the aforementioned ion at *m/e* 339, and below *m/e* 344 the two spectra were virtually identical.

Hydrogenation of compounds (4)—(6) (acetates) yielded the dihydro-derivatives, which were indistinguishable on g.l.c. from the acetates of (1)—(3). Finally, the dihydro-derivative of (4), when subjected to chromic acid oxidation, methylation with diazomethane, and treatment with sodium methoxide, gave methyl A-nor-5α-cholestane-3α-carboxylate, identical with the synthetic sample (g.l.c., m.p. and mixed m.p., n.m.r. and mass spectra).

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EXPERIMENTAL

Sponges (*Axinella verrucosa*), collected in the Bay of Naples, were obtained from the supply department of the Zoological Station, Naples. T.l.c. was carried out on silica gel (Merck F_{254}). G.l.c. was conducted on 2 m × 3 mm (i.d.) columns packed with 1% OV-1 on GasChrom (100—200 mesh) at 245° with nitrogen flow rate 32 ml min^{-1} (Carlo Erba Fractovap GV instrument); retention times (t_R) are given relative to that of cholesteryl acetate. I.r. spectra were measured for solutions in chloroform on a Perkin-Elmer 257 Infracord spectrophotometer, n.m.r. spectra for solutions in [^{2}H]chloroform on a Varian HA-100 spectrometer (tetramethylsilane as internal reference), and mass spectra on an A.E.I. MS 30 instrument at 70 eV. G.l.c.–mass spectrometry was carried out on an A.E.I. MS 30 instrument connected to a Pye gas chromatograph equipped with a 1·5 m × 1·5 mm (i.d.) glass column packed with 3% SE-30 on GasChrom (100—200 mesh) (temp. 270°). Rotations were measured for solutions in chloroform.

Extraction of Sponge.—Fresh sponge (28 g dry weight after extraction) was extracted three times with acetone at room temperature for 3 days; after concentration the aqueous residue was extracted with ether (three times). The combined ethereal extracts were taken to dryness and the residue (5·4 g) was chromatographed on a column of silica gel (250 g; Merck) to give, on elution with chloroform, a crude sterol fraction (0·98 g).

Fractionation of the Mixed Acetates.—The sterol fraction was acetylated with acetic anhydride–pyridine under reflux for 1 h and the mixed acetates were purified by silica gel column chromatography [eluant light petroleum (b.p. 40—70°)–benzene, 1 : 1 v/v]. The combined acetate fractions (0·9 g) were dissolved in light petroleum (b.p. 40—70°), applied to a column of alumina (90 g; neutral, Merck) impregnated with silver nitrate (30 g) and eluted (for solvents see Table). Fractions (40 ml) were collected, analysed by g.l.c., and combined accordingly. The results are summarized in the Table.

Column chromatography (Al_2O_3–$AgNO_3$) of the 3β-acetoxymethyl-A-nor-steranes * from *Axinella verrucosa*

Fractions	Solvent LP–B † Proportions (v/v)	Volume (l)	Amount of material (mg)	Acetates of compounds
I	100 : 0	0·7	520	(1)—(3)
	100 : 1	0·12		
II	100 : 1	0·12	65	(6)
III	100 : 1	0·4	100	(5)
	100 : 1	0·9	75	Mixture
	100 : 2	0·12		
IV	100 : 2	0·32	27	(4)

* Obtained from crude stanols (0·98 g) derived from 28 g dry weight of sponge. † LP = light petroleum (b.p. 40—70°); B = benzene.

Fraction I was shown to be a mixture of three components [acetates (1)—(3)] by g.l.c.; t_R 1·00, 1·40, and 1·64 (ratio *ca.* 7·5 : 1·2 : 1·3), *m/e* 458·4118 (Calc. for $C_{31}H_{54}O_2$: *M*, 458·4124), 444·3972 (Calc. for $C_{30}H_{52}O_2$: *M*, 444·3967), 430·3808 (Calc. for $C_{29}H_{50}O_2$: *M*, 430·3811), δ 4·02 (7-line m, *J* 11, 9, and 7 Hz, CH_2·OAc), 2·01 (s, $MeCO_2$), 0·66 (s, 13-Me), and 0·75 (s, 10-Me). G.l.c.–mass spectrometry gave three peaks: (i) [acetate of (1)] *m/e* 430 (5%, M^+), 370 (25), 275 (10), and 215 (100); (ii) [acetate of (2)] *m/e* 444 (4%, M^+), 384 (18), 275 (8), and 215 (100); (iii)

[acetate of (3)] *m/e* 458 (3%, M^+) 398 (20), 275 (10), and 215 (100).

The free stanol mixture (prepared by treatment of the mixed acetates with methanolic 10% potassium hydroxide under reflux for 2 h) crystallized from methanol and showed the following spectral properties: *m/e* 416 (8%, M^+), 402 (7%, M^+), 401 (7, 416 − Me), 398 (3, 416 − H_2O), 388 (45%, M^+), 387 (6, 402 − Me), 385 (5, 416 − CH_2OH), 384 (2, 402 − H_2O), 373 (28, 388 − Me), 371 (3, 402 − CH_2OH), 370 (10, 388 − H_2O), 357 (13, 388 − CH_2OH), 248 (14), 233 (100), 217 (95), 215 (28), and 203 (56), δ 3·58 (8-line m, *J* 10, 8, and 6 Hz, CH_2·OH), 0·74 (s, 10-Me), and 0·65 (s, 13-Me).

Frction II was crystallized from methanol to give 3β-*acetoxymethyl*-24-*ethyl*-A-*nor*-5α-*cholest*-22-*ene* [acetate of (6)] (35 mg) (Found: M^+, 456·3961. $C_{31}H_{52}O_2$ requires *M*, 456·3967), m.p. 104—106°, $[\alpha]_D$ +12° (*c* 1·65), t_R 1·48, *m/e* 456 (100%), 441 (4), 396 (6), 358 (10), 353 (70), 344 (60), 329 (10), 315 (50), 302 (10), 285 (18), 284 (7), 283 (8), 257 (100), 255 (30), and 215 (15), δ 5·07 (2H, t, *J* 7 Hz, olefinic), 4·03 (2H, 7-line m, *J* 11, 9, and 7 Hz, CH_2·OAc), 2·02 (3H, s, $MeCO_2$), 0·99 (3H, d, *J* 7 Hz, 20-Me), 0·75 (3H, s, 10-Me), and 0·66 (3H, s, 13-Me).

Fraction III was crystallized from methanol to give 3β-*acetoxymethyl*-24-*methyl*-A-*nor*-5α-*cholest*-22-*ene* [acetate of (5)] (65 mg) (Found: M^+, 442·3817. $C_{30}H_{50}O_2$ requires *M*, 442·3811), m.p. 104—105°, $[\alpha]_D$ +32° (*c* 2·1), t_R 1·12, *m/e* 442 (100%), 427 (4), 382 (7), 358 (9), 344 (80), 339 (33), 329 (12), 315 (43), 302 (10), 285 (15), 284 (6), 283 (5), 257 (100), 255 (23), and 215 (13), δ 5·13 (2H, m, olefinic), 4·03 (2H, 7-line m, *J* 11, 9, and 7 Hz, CH_2·OAc), 2·01 (3H, s, $MeCO_2$), 0·98 (3H, d, *J* 7 Hz, 20-Me), 0·91, 0·81, and 0·79 (9H, each d, 24-Me and 25-Me_2), 0·75 (3H, s, 10-Me), and 0·66 (3H, s, 13-Me).

Fraction IV contained 3β-*acetoxymethyl*-A-*nor*-5α-*cholest*-22-*ene* [acetate of (4)] (Found: M^+, 428·3650. $C_{29}H_{48}O_2$ requires *M*, 428·3654), m.p. 64—65° (from methanol), $[\alpha]_D$ +23° (*c* 0·5), t_R 0·88, *m/e* 428 (60%), 413 (5), 368 (6), 353 (5), 344 (90), 329 (13), 315 (31), 302 (8), 285 (5), 284 (8), 257 (100), 255 (15), and 215 (10), δ 5·22 (2H, m, olefinic), 4·03 (2H, 7-line m, *J* 11, 9, and 7 Hz, CH_2·OAc), 2·01 (3H, s, $MeCO_2$), 1·01 (3H, d, *J* 7 Hz, 20-Me), 0·86 (6H, d, *J* 6 Hz, 25-Me_2), 0·76 (3H, s, 10-Me), and 0·66 (3H, s, 13-Me).

Conversion of the Stanol Mixture [(1)—(3)] *into the Corresponding Mixture of Carboxylic Acids.*—A solution of the stanols (1)—(3) (250 mg) in benzene (20 ml) was added with stirring and cooling to a solution of potassium dichromate (400 mg) in glacial acetic acid (0·4 ml), concentrated sulphuric acid (0·8 ml), and water (5 ml). The mixture was stirred at room temperature for 6 h and at reflux for an additional 1 h. After cooling a small amount of water was added and the benzene layer was separated, washed with water, and dried ($MgSO_4$). Removal of solvent left material (160 mg) which was purified on a silica gel column to give, on elution with benzene–ether (95 : 5) the mixture of A-nor-5α-sterane-3β-carboxylic acids (7) (single spot on t.l.c.), *m/e* 430 (M^+), 416 (M^+), and 402 (M^+), and 247 (base peak, M^+ − side-chain − 42), δ 9·93br (exchangeable with D_2O, CO_2H), 2·90 (m, CH·-CO_2H), 0·76 (s, 10-Me), and 0·66 (s, 13-Me), $\nu_{max.}$ 3500, 3300—2500, and 1700 cm^{-1}. A sample (90 mg) of these acids in ether was treated with an excess of ethereal diazomethane. The solvent was removed and the residue was chromatographed on silica gel to give, by elution with light petroleum (b.p. 40—70°)–benzene (3 : 7) the methyl A-nor-5α-sterane-3β-carboxylates (8); single spot on t.l.c. (SiO_2), *m/e* 444 (M^+), 430 (M^+), and 416 (M^+), and 261 (base peak, M^+ − side-chain − 42), δ 3·61 (s, OMe), 2·84 (m, CH·CO_2Me), 0·75 (s, 10-Me), and 0·66 (s, 13-Me).

Base-catalysed Epimerization of the Methyl A-*Nor*-5α-*sterane*-3β-*carboxylate Mixture* (8).—The mixture (30 mg) was refluxed for 24 h in a solution obtained by dissolving sodium (20 mg) in dry methanol (3 ml). The mixture was diluted with water and extracted with ether. Evaporation of the extract left crystals (22 mg), which were recrystallized from methanol to give the 3α-epimers; single spot on t.l.c. (SiO_2), separable from the 3β-epimers in light petroleum (b.p. 40—70°)–benzene (7 : 3; two elutions), *m/e* 444 (M^+), 430 (M^+), and 416 (M^+), and 261 (base peak), δ 3·64 (s, OMe), 2·46 (m, CH·CO_2Me), 0·90 and 0·85 (each d, Me), 0·72 (s, 10-Me), and 0·66 (s, 13-Me).

Conversion of the Stanols (1)—(3) *into the* A-*Nor*-5α-*steran*-3-*ones* (10).—A solution consisting of the stanol mixture (200 mg; *ca.* 0·5 mmol), dry pyridine (0·04 ml, 0·5 mmol), trifluoroacetic acid (0·02 ml, 0·25 mmol) and dicyclohexylcarbodi-imide (0·31 g, 1·5 mmol) in dry benzene (1·65 ml) and dry dimethyl sulphoxide (1·65 ml) was left at room temperature overnight. Benzene (20 ml), ether (5 ml), and oxalic acid dihydrate (0·187 g; 1·5 mmol) were added. After gas evolution had ceased water was added, and after filtration the organic layer was separated and washed with water. Evaporation left a mixture (205 mg) which was purified on a silica column to give, on elution with light petroleum (b.p. 40—70°)–benzene (1 : 1), the A-nor-5α-sterane-3-carbaldehyde mixture (9) (120 mg), *m/e* 414 (M^+), 400 (M^+), and 380 (M^+), and 231 (base peak, M^+ − side-chain − 42), δ 9·84 (d, *J* 1·2 Hz, *CH*O), 2·70 (m, *CH*·CHO), 0·66 (s, 13-Me), and 0·61 (s, 10-Me).

The aldehyde mixture (100 mg) was refluxed for 4 h in isopropenyl acetate (8 ml) containing concentrated sulphuric acid (2 drops). The mixture was left overnight at room temperature and the excess of isopropenyl acetate was then removed *in vacuo*. Water was added and the mixture was extracted with ether. The ether layer was washed with water, aqueous sodium carbonate, and water again, dried ($MgSO_4$), and evaporated. The residue was chromatographed in light petroleum (b.p. 40—70°)–benzene (1 : 1) on silica to give in the first fractions the enol acetate mixture (95 mg), *m/e* 456 (M^+), 442 (M^+), 428 (M^+), 414 (456 − CH_2=C=O), 400 (442 − CH_2=C=O), and 386 (base peak, 428 − CH_2=C=O), δ 6·8 (q, *J* 2 Hz, =*CH*−OAc), 2·12 (s, $MeCO_2$), and 0·69 and 0·63 (each s, 10- and 13-Me or *vice versa*), which was ozonized in ethyl acetate for 5 min at −20°. Ethyl acetate was removed *in vacuo* and to the residue acetic acid (5 ml) and zinc dust (1 g) were added. After 10 min at room temperature ether was added, and after filtration solvents were evaporated off. The residue was chromatographed in light petroleum (b.p. 40—70°)–benzene (1 : 1) on silica to give the A-nor-5α-steran-3-one mixture (10) (50 mg); single spot on t.l.c. (SiO_2) (R_F 0·40 in benzene); $\nu_{max.}$ ($CHCl_3$) 1735 cm^{-1}, θ_{290} −18,850, *m/e* 400 (M^+), 386 (M^+), and 372 (M^+), and 217 (M^+ − side-chain − 42), δ 0·76 and 0·68 (each s, 10- and 13-Me).

Deuteriation of the A-*Nor*-5α-*steran*-3-*ones* (10).—The nor-ketone mixture (10 mg) was added to a solution prepared by dissolving sodium (10 mg) in methan[^{2}H]ol (1 ml),

and the mixture was refluxed for 30 min, then evaporated *in vacuo*. Methan[^{2}H]ol (1 ml) was added to the residue and the mixture was refluxed for 30 min. Deuterium oxide was added and the product was extracted with dry ether (3 times). The combined extracts were washed with deuterium oxide, dried ($MgSO_4$), and evaporated to leave the deuteriated ketones (8 mg), M^+ 403, 389, and 375, base peak *m/e* 220.

Base-catalysed Epimerization of the A-*Nor*-5α-*steran*-3-*ones* (10).—The mixture (10) (20 mg) was treated as above but with methanol to give the 5β-epimers (11) (18 mg); single spot on t.l.c. (SiO_2) (R_F 0·50 in benzene), θ_{292} +7890, *m/e* 400 (M^+), 386 (M^+), and 372 (M^+), and 217 (base peak), δ 0·68 (6H, s, 10- and 13-Me).

Methyl A-*Nor*-5α-*cholestane*-3α-*carboxylate*. Prepared according to the method of Fuchs and Loewenthal,[12] the ester had m.p. 75—77° (lit., 78·5—79°), t_R 0·66, *m/e* 416 (25%, M^+), 401 (32), 341 (8), 276 (16), 271 (4·5), 263 (13), 262 (60), 261 (100), 247 (32), 203 (40), and 201 (35), δ 3·64 (3H, s, OMe), 2·46 (1H, m, C*H*·CO_2Me), 0·90 (d) and 0·85 (d) (9H, 20-Me and 25-Me_2), 0·72 (3H, s, 10-Me), and 0·66 (3H, s, 13-Me).

Methyl A-*Nor*-5α-*cholestane*-2α-*carboxylate*.—Prepared as reported by Evans *et al.*,[11] the 2α-ester had m.p. 93—96° (lit., 97·2—98°), mass spectrum identical with that of the 3α-isomer, δ 3·64 (3H, s, OMe), 2·88 (1H, m, C*H*·CO_2Me), 0·91 (d) and 0·88 (d) (9H, 20-Me and 25-Me_2), 0·71 (3H, s, 10-Me), and 0·67 (3H, s, 13-Me).

By following the same isolation procedure as Evans *et al.* we also obtained a fraction with m.p. 53—55° (lit., 45°), which was assumed by these authors to be the 3α-ester. However, the n.m.r. spectrum of our sample showed that it was a mixture of the 2α- and 3α-isomers (ratio *ca.* 1 : 2 from the areas of the C*H*·CO_2Me signals).

Hydrogenation of the Acetates of (5) *and* (6).—Each sample, dissolved in ethyl acetate was hydrogenated at room temperature and atmospheric pressure over platinized carbon (10%) for 15 h. The dihydro-derivatives were crystallized from methanol. 3β-*Acetoxymethyl*-24-*methyl*-A-*nor*-5α-*cholestane* [acetate of (2)] had m.p. 84—86°, $[\alpha]_D$ +40° (*c* 1·7), t_R 1·40 (Found: M^+, 444·3975. $C_{30}H_{52}O_2$ requires *M*, 444·3967), mass spectrum identical with that of peak 2 of fraction I. 3β-*Acetoxymethyl*-24-*ethyl*-A-*nor*-5α-*cholestane* [acetate of (3)] had m.p. 86—88°, $[\alpha]_D$ +24 (*c* 0·7), t_R 1·64 (Found: M^+, 458·4129. $C_{31}H_{54}O_2$ requires *M*, 458·4124), mass spectrum identical with that of peak 3 of fraction I.

Conversion of 3β-*Acetoxymethyl*-A-*nor*-5α-*cholest*-22-*ene* [*Acetate of* (4)] *into Methyl* A-*Nor*-5α-*cholestane*-3α-*carboxylate* (13).—The dihydro-derivative of the acetate of (4) (15 mg), obtained similarly, was treated with methanolic 10% potassium hydroxide at reflux for 2 h. Work-up afforded the free alcohol, *m/e* 388 (M^+), $[\alpha]_D$ +23° (*c* 1·3), which, without further purification, was submitted to oxidation with chromic acid as described for the stanols (1)—(3). The resulting carboxylic acid was methylated with diazomethane to give, after chromatography on silica, the corresponding methyl ester (6 mg), *m/e* 416 (M^+), δ 2·84 (m, C*H*·CO_2Me), $[\alpha]_D$ +18° (*c* 0·5), which was refluxed for 24 h in methanolic sodium methoxide. Work-up as before afforded the 3α-ester (4 mg), m.p. 74—77° (from methanol) t_R 0·66, identical (n.m.r. and mass spectra, mixed m.p.) with the synthesized sample (m.p. 75—77°).

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