Marine Sterols: 19-Nor-stanols from the Sponge *Axinella polypoides*

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The total sterol content of the marine sponge *Axinella polypoides* is a mixture of 19-nor-stanols, in which the major resolved component has been fully characterized (as the acetate) as 19-nor-5α.10β-ergost-*trans*-22-en-3β-ol (1). The remaining components (2)—(8) have been identified largely on the basis of spectral measurements.

DURING a recent investigation of sterols of sponges [1] we observed that the total sterols of *Axinella polypoides* comprise a complex mixture of C_{25} (trace), C_{26}, C_{27}, and C_{28} fully reduced and side-chain monounsaturated stanols, resolvable into seven components by g.l.c. of the derived acetates. Preliminary structural examination, mainly by mass spectrometry on the unresolved mixture, suggested that these sterols have structures of the nor-stanol type with a C_{18} nucleus. Here we report the fractionation of the mixed acetates and the evidence supporting their structures (1)—(8), which combine the unusual 19-norcholestanol nucleus with conventional saturated and monounsaturated C_7 (24-nor), C_8, C_9, and C_{10} side-chains. A very minor component, which seems to possess, besides a 19-norcholestanol nucleus, a C_{10} side-chain with an unusual alkylation pattern, is being further investigated.

A free sterol fraction, having the same t.l.c. R_F value on silica gel as cholesterol, was obtained by column chromatography of the crude extract on silica gel, and was further resolved into seven fractions by acetylation and chromatography on alumina impregnated with silver nitrate. The results are given in the Table. The most abundant of the resolved individual constituents,

Column chromatography (Al_2O_3–$AgNO_3$) of the 19-nor-stanyl acetates * from *Axinella polypoides*

Fraction no.	Solvent † Ratio (v/v)	Solvent † Volume (l)	Weight of material (mg)	Stanyl acetates
I	100 : 0	0·45	515	(2)—(4)
	100 : 1	0·9		
II	100 : 2	0·3	16	19-Nor-C_{29}-stanol
	100 : 3	0·2		
III	100 : 3	0·25	25	(6)
IV	100 : 4	0·5	123	(1)
V	100 : 5	0·4	24	(5)
VI	100 : 7	0·25	90	19-Nor-C_{27}-Δ^{22}-stanol
	100 : 10	0·25		and (8)
VII	100 : 20	0·3	20	(7)
	0 : 100	0·15		

* Obtained from 1·1 g of crude stanols derived from 120 g (dry weight) of sponge. † Light petroleum (b.p. 40—70°)–benzene.

recovered in fraction IV, was identified as 19-nor-5α,10β-ergost-22-en-3β-yl acetate (1). The presence of a saturated nor-sterol nucleus was indicated by the mass spectrum, which, besides an abundant molecular ion [*m/e* 428 (95%)] and a small M^+ — AcOH peak (7%), showed a major peak at *m/e* 243 accompanied by a minor one at *m/e* 241. It is well known that mass spectra of saturated sterol acetate nuclei carrying an unsaturated side-chain are characterized by a doublet at *m/e* 257 and 255, resulting from loss of the side-chain and AcOH, and loss of side-chain, AcOH, and 2H, respectively.[2] Significant peaks at *m/e* 301 (50%) and 201 (10%) in the spectrum of (1) could be similarly interpreted, the former as a result of loss of the C_9 side-chain and 2H transfer from the C_{18} nucleus, and the latter arising by loss of AcOH, the side-chain, and 42 mass units (part of ring D).[2] The spectrum also indicated the position (C-22) of unsaturation in the C_9 side-chain,[3] showing peaks at *m/e* 385 (3%, loss of isopropyl, allylic cleavage), 330 (71%, vinylic cleavage of the 20,22-bond and H transfer), 325 (26%, M — AcOH — C_3H_7), and 270 (9%, M — AcOH — C_7H_{14}).

Supporting evidence for the location of unsaturation in the side-chain came from the n.m.r. spectrum, which showed olefinic absorptions at δ 5·16 (2H, complex) and a broad signal at δ 4·70 (1H, $W_{\frac{1}{2}}$ 22 Hz, C*H*OAc). The n.m.r. spectrum also showed a three-proton singlet at δ 0·66 (13-Me) and this, together with the lack of further methyl singlet absorption in the range δ 0·75—0·90, typical of the 10-Me protons, indicated a 19-nor-type structure.

This was confirmed as follows. Hydrogenation of compound (1) followed by saponification and oxidation with dichromate gave the ketone (9), *m/e* 386 (M^+), $\nu_{max.}$ 1705 cm^{-1}. Bromination in acetic acid afforded the 2,4-dibromo-derivative which, without further purification, was dehydrobrominated with lithium carbonate–lithium bromide in dimethylformamide to give the phenol (10), *m/e* 382 (M^+), $\lambda_{max.}$ 280 and 285 nm (ε 2215 and 2000), bathochromically shifted to 298 nm by alkali. The estrones have $\lambda_{max.}$ 280 and 285 nm (ε 2300 and 2000).[4] The n.m.r. spectrum showed an aromatic pattern (see Experimental section) consistent with a 3,4-disubstituted phenol system.

The structure of the phenol (10) was confirmed and its stereochemistry defined by comparison (m.p. and mixed m.p.; mass, n.m.r., and u.v. spectra) with authentic 19-norergosta-1,3,5(10)-trien-3-ol, prepared as follows from ergostan-3-one (11). Bromination in acetic acid gave 2,4-dibromoergostan-3-one, and subsequent dehydrobromination produced ergosta-1,4-dien-3-one (12), purified by preparative silica gel t.l.c., *m/e* 396 (M^+),

[1] M. De Rosa, L. Minale, and G. Sodano, *Comp. Biochem. Physiol.*, 1973, **46B**, 823.
[2] B. A. Knight, *J. Gas Chromatog.*, 1967, 273.
[3] S. G. Wyllie and C. Djerassi, *J. Org. Chem.*, 1968, **33**, 305.
[4] A. I. Scott, 'Ultraviolet Spectra of Natural Products,' Pergamon, Oxford, 1964, p. 94.

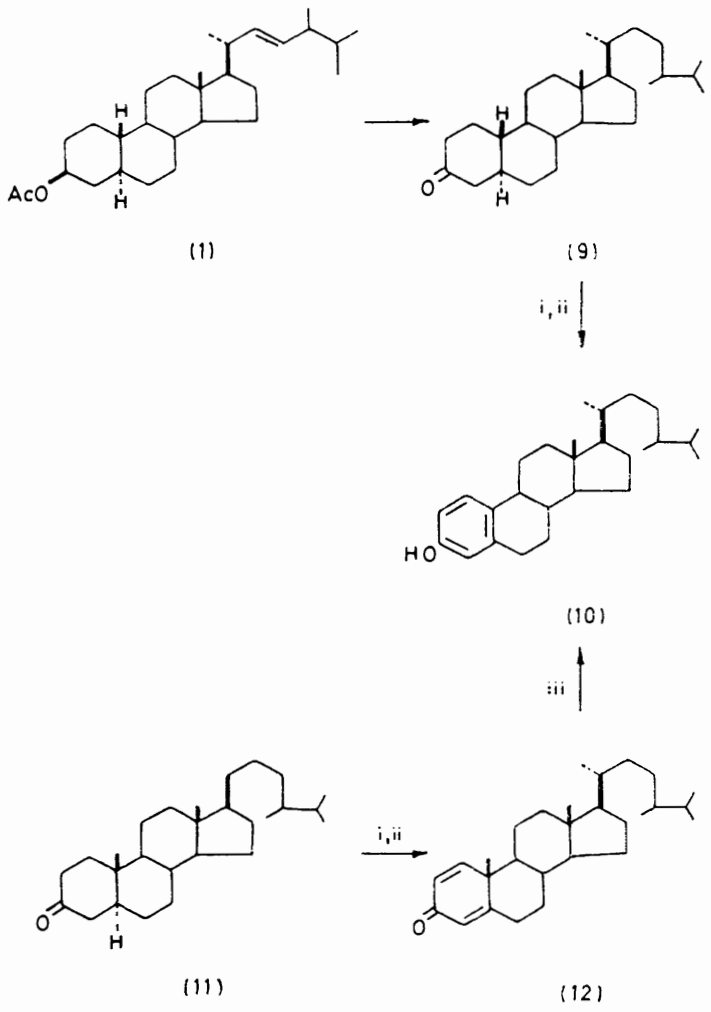
$\nu_{max.}$ 1670 cm^{-1}, $\lambda_{max.}$ 246 (log ε 4·12). Reductive aromatization involving expulsion of the C-10 methyl group by treatment with the radical anion derived from lithium and biphenyl in boiling tetrahydrofuran [5] afforded the phenol (10) in 50% yield.

The stereochemistry of the AB-ring junction (*trans*; 5α) in compound (1) was established by the c.d. curve

A strong i.r. band at 970 cm^{-1} suggested a *trans*-configuration for the 22,23-bond.[8]

The structures (2)—(8) for the remaining components, all showing a broad C*H*OAc n.m.r. signal at δ *ca.* 4·70 ($W_{\frac{1}{2}}$ 22—24 Hz), were largely derived from spectral data.

Fraction I.—The major fraction, although apparently homogeneous on t.l.c. (SiO_2–$AgNO_3$) was resolvable by



SCHEME Reagents: i, Br_2–AcOH; ii, Li_2CO_3-LiBr–Me_2N·CHO; iii, Li–Ph_2–$[CH_2]_4O$

(MeOH) of the ketone (9) (θ_{292} +5210), which agrees completely with those observed for analogous compounds.[6]

The broad nature of the C-3 proton signal in the n.m.r. spectrum of the acetate (1) ($W_{\frac{1}{2}}$ 22 Hz) indicated a degree of coupling consistent with axial orientation; hence the acetoxy-group is equatorial and occupies the 3β-position.[7] The half-band width corresponds to that observed for 3β-acetoxy-5α-cholestane ($W_{\frac{1}{2}}$ 22 Hz) rather than that for 3α-acetoxy-5α-cholestane ($W_{\frac{1}{2}}$ 7 Hz).

g.l.c. into three components (ratio *ca.* 3·1 : 4·3 : 2·4) with retention times of 0·86, 1·00, and 1·26 relative to cholesteryl acetate. The mass spectrum showed a series of peaks at *m/e* 444 (45%), 430 (70), and 416 (45) corresponding to the acetates of fully saturated C_{28}, C_{27}, and C_{26} sterols.

The most significant feature of the mass spectra was the presence of major peaks at *m/e* 261 (22%) and 201 (100), which, together with the absence of peaks at *m/e* 275 and 215, characteristic of fully saturated stanyl

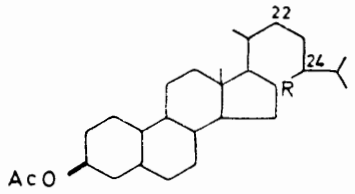
[5] H. L. Dryden, G. M. Webber, and J. J. Wieczorek, *J. Amer. Chem. Soc.*, 1964, **86**, 742.

[6] P. Crabbé, 'Applications de la dispersion rotatoire optique et du dichroisme circulaire optique en chimie organique,' Gouthier–Villars, Paris, 1968, p. 260.

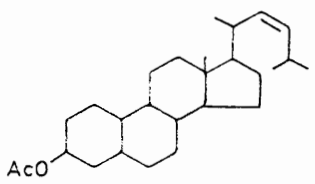
[7] J. T. Edward and J. M. Ferland, *Canad. J. Chem.*, 1966, **44**, 1311.

[8] L. L. Smith, A. K. Dhor, J. R. Gilchrist, and Y. Y. Lin, *Phytochem.*, 1973, **12**, 2727.

acetates [2] [the first arising by loss of the side-chain and part of ring D (42 mass units) and the latter by the same sequence from M^+ — AcOH] was consistent with the structures (2)—(4). The 19-nor-feature was confirmed as



(2) R = H
(3) R = Me
(4) R = Et
(5) R = H, Δ^{22}-*trans*
(6) R = Et, Δ^{22}-*trans*
(7) R = Me, $\Delta^{24(28)}$



(8)

before, by saponification followed by oxidation to a mixture of three ketones, *m/e* 400, 386, and 370 (M^+), which was converted by bromination and subsequent dehydrobromination into a mixture of three phenols showing molecular ions at *m/e* 396, 382, and 368 and having u.v. and n.m.r. spectra virtually identical with those of 19-norergosta-1,3,5(10)-trien-3-ol (10).

Fraction II.—This fraction contained a single component, $[\alpha]_D$ ±0°. The mass spectrum showed a molecular ion at *m/e* 442·3805 (Calc. for $C_{30}H_{50}O_2$: 442·3811). Major peaks at *m/e* 301 (100%; M^+ — side-chain — 2H) and 243 (95%; M^+ — AcOH — side-chain) indicate that this is a further member of the 19-nor-stanol series, carrying a monounsaturated C_{10} side-chain. Other significant peaks at *m/e* 399 (15%), 339 (19), 330 (58), and 270 (9) indicate unsaturation at C-22.[3] Surprisingly, the n.m.r. spectrum showed a vinyl methyl singlet (δ 1·48) together with a 1H olefinic doublet (δ 4·88; *J* 9 Hz).

These spectral data, taken together, cannot be interpreted in terms of a conventional C_{10} side-chain, and therefore we suppose that this component possesses a 19-norcholestanol nucleus with a monounsaturated C_{10} side-chain involving an unusual alkylation pattern. We intend to reinvestigate this component.

Fraction III.—This contained 24-ethyl-19-norcholest-*trans*-22-en-3β-yl acetate (6), as shown by mass and n.m.r. measurements. The mass spectrum showed the molecular ion at *m/e* 442·3815 and the fragmentation pattern was very similar to that of (1). The allylic fragments were seen at *m/e* 399 (loss of isopropyl), and 339 (loss of isopropyl and AcOH), corresponding to the ions at *m/e* 385 and 325 in the spectrum of (1); below *m/e* 339 the two spectra were virtually identical.

The n.m.r. spectrum, showing absorptions for two olefinic protons (δ 5·08, t, *J* 6 Hz), together with the allylic (loss of C_3H_7) and vinylic (loss of C_8H_{16}) fragmentation in the mass spectrum, enabled the structure of the C_{10} side-chain to be determined. The i.r. spectrum (970 cm^{-1}) was in agreement with a *trans*-configuration of the double bond.

Fraction V.—This was characterized as 19-norcholest-*trans*-22-en-3β-yl acetate (5). The mass spectrum showed the molecular ion at *m/e* 414·3485 and the fragmentation pattern [*m/e* 330 (60%, vinyl cleavage of the 20,22-bond and H transfer), 301 (44%, loss of the C_8 side-chain and 2H), 270 (9%, loss of AcOH, side-chain, and part of ring D)] was fully consistent with a norcholestanol nucleus carrying a conventional Δ^{22}-C_8 side-chain.[3] The absence of branching at C-24 suppresses almost completely the allylic cleavage of the 24,25-bond.

The n.m.r. spectrum showed only one methyl singlet, at δ 0·68 (13-Me) [apart from the acetyl signal (δ 2·00)] and a signal for two olefinic protons at δ 5·14.

A strong i.r. absorption at 970 cm^{-1} suggested a *trans*-configuration for the 22,23-double bond.

Fraction VI.—G.l.c.–mass spectrometry showed that this contained a norcholestenyl acetate isomeric with (5), as the major component (85%), together with a minor one with a much shorter g.l.c. retention time and giving a molecular ion at *m/e* 400, corresponding to a bisnorcholestenyl acetate.

The principal component, showing a slightly shorter g.l.c. retention time than (5), was a 19-norcholest-22-en-3β-yl acetate, as confirmed by the mass spectrum, which was virtually identical with that of (5), and the n.m.r. spectrum of the mixture, which showed a two-proton olefinic signal at δ 5·25. We at first supposed that this component was the *cis*-isomer, but the i.r. spectrum of the mixture lacked the characteristic *cis*-1,2-disubstituted ethylene band at 730—760 cm^{-1} and exhibited a strong *trans*-band at 970 cm^{-1}. The difference between the two 19-nor-C_{27}-Δ^{22}-stanols probably arises from other stereochemical features.

G.l.c.-mass spectral measurements on the minor component [*m/e* 400 (8%, M^+), 385 (5), 330 (14), 301 (18), 243 (100), and 201 (20)] suggested that it was 19,24-bisnorcholest-22-en-3-yl acetate (8).

C_{26} Sterols (24-nor) are ubiquitous in sponges.[1] After the discovery of these sterols in marine plankton [9] a common origin from phytoplankton for all the C_{26} marine sterols was suggested. Now the occurrence of a 19-nor-C_{26}-sterol in *Axinella polypoides*, probably originating from a C_{26} sterol in the diet, suggests that one of the processes involved in the biosynthesis of these 19-nor-

[9] J. L. Boutry, A. Alcaide, and M. Barbier, *Compt. rend.*, 1971, **272**, 1022.

stanols could be the removal of the 10-methyl group from sterol substrates.

Fraction VII.—Rechromatography of this fraction on silica afforded an amorphous solid, which could not be crystallized. The small quantity of material precluded further purification and so the compound was not rigorously characterized. Nevertheless, spectral measurements showed that it was probably 24-methylene-19-norcholestan-3β-yl acetate (7).

The mass spectrum showed a molecular ion at 428·3645 (Calc. for $C_{29}H_{48}O_2$: 428·3654). The most intense peak occurred at *m/e* 344, and major peaks were observed at *m/e* 301 (54%), 243 (10), 241 (21), and 201 (20), all associated with a saturated C_{18} nucleus and an unsaturated side-chain. The genesis of the ion at *m/e* 344 has been attributed to the McLafferty cleavage of the side-chain at C(22)–C(23), involving loss of a C_6 fragment, strongly indicative of a 24,28-double bond.[3] A peak at *m/e* 284 (25%), originating by the same sequence from M^+ — AcOH is also apparent. Alternative positions for the side-chain double bond were readily eliminated by the n.m.r. spectrum, which contained a typical $=CH_2$ signal as a doublet (δ 4·67), clearly emerging from the broad C*H*OAc signal. The i.r. spectrum also pointed to the presence of a methylene group ($\nu_{max.}$ 890s cm^{-1}).

EXPERIMENTAL

Sponges (*Axinella polypoides*) 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 60 F_{254}) sprayed with a saturated solution of silver nitrate in acetone and subsequently dried at 100°. Spots were located by spraying with cerium sulphate in sulphuric acid (100 mg in 10 ml). G.l.c. was conducted on a 2 m × 3 mm (i.d.) columns packed with 1% OV-1 on Gaschrom Q (100—200 mesh) at 245° with a nitrogen flow rate of 32 ml min^{-1} on a Carlo Erba Fractovap model GV instrument. I.r. spectra were measured for solutions in carbon sulphide on a Perkin-Elmer 257 Infracord spectrophotometer. U.v. spectra were obtained for solutions in methanol on a Bausch and Lomb Spectronic spectrophotometer. N.m.r. spectra were determined for solutions in [^{2}H]chloroform on a Varian HA-100 spectrometer (tetramethylsilane as internal reference). Mass spectra were measured on an A.E.I. MS 902 instrument at 70 eV. G.l.c.–mass spectrometry measurements were carried out on an A.E.I. MS 30 instrument connected with a Pye gas-chromatograph equipped with a 1·5 m × 1·5 mm (i.d.) glass column packed with 3% SE-30 on Gaschrom Q (100—200 mesh) (temp. 280°). Rotations were measured for solutions in chloroform.

Extraction of Sponge.—Fresh sponge (120 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 (2·6 g) was chromatographed on a column of silica gel (250 g; Merck) to give, on elution with chloroform, a crude sterol fraction (1·1 g).

Fractionation of the Mixed 19-Nor-stanyl Acetates.—The sterol fraction was acetylated with acetic anhydride–pyridine at 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 (1·02 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 t.l.c. and g.l.c., and combined accordingly. The results are summarized in the Table.

Fraction I. This was shown to be a mixture of compounds (2)—(4) by g.l.c.; *m/e* 444 (45%), 430 (70), 416 (45), 384 (48), 370 (93), 356 (70), 261 (22), and 201 (100), δ 4·67br (m, $W_{\frac{1}{2}}$ 23 Hz, 3β-H) and 2·01 (s, $MeCO_2$).

Fraction II. This was crystallized from methanol to give crystals (8 mg) of unknown structure, m.p. 146—148, $[\alpha]_D$ ±0° (*c* 0·4), single peak on g.l.c., single spot on t.l.c., *m/e* 428 (M^+, 45%), 399 (15), 339 (19), 330 (58), 301 (100), 270 (9), and 243 (95), δ 0·68 (3H, s, 13-Me), 1·48 (3H, s), 2·01 (3H, s, $MeCO_2$), 4·67br (1H, m, $W_{\frac{1}{2}}$ 22 Hz, C*H*OAc), and 4·88 (1H, d, *J* 9 Hz), $\nu_{max.}$ 1735, 1250, 940, and 840 cm^{-1}.

Fraction III. This contained *24-ethyl-19-norcholest*-trans-*22-en-3β-yl-acetate* (6) (Found: M^+, 442·3815. $C_{30}H_{50}O_2$ requires *M*, 442·3811), m.p. 116—117° (from methanol), $[\alpha]_D$ +7·5° (*c* 0·4), *m/e* 442 (100%), 399 (7), 339 (50), 330 (53), 301 (50), 270 (8), 243 (90), and 201 (10), δ 0·69 (3H, s, 13-Me), 2·01 (3H, s, $MeCO_2$), 4·68br (1H, m, $W_{\frac{1}{2}}$ 24 Hz), and 5·08 (2H, t, *J* 6 Hz), $\nu_{max.}$ 1740, 1250, 1035, and 970 cm^{-1}.

Fraction IV. Recrystallization from methanol yielded *19-nor-5α,10β-ergost*-trans-*22-en-3β-yl acetate* (1) (88 mg) (Found: C, 80·4; H, 11·5. $C_{29}H_{48}O_2$ requires C, 80·7; H, 11·6%), m.p. 112—113°, $[\alpha]_D$ +16·3° (*c* 1·2), *m/e* 428 (95%), 413 (5), 385 (3), 330 (71), 325 (26), 315 (14), 301 (50), 269 (3), 270 (9), 271 (16), 243 (100), 241 (7), and 201 (10), δ 0·66 (3H, s, 13-Me), 2·01 (3H, s, $MeCO_2$), 4·73br (1H, m, $W_{\frac{1}{2}}$ 22 Hz, C*H*OAc), and 5·17 (2H, m, olefinic), $\nu_{max.}$ 1740, 1250, 1035, and 970 cm^{-1}.

Fraction V. Crystallization from methanol yielded *19-norcholest*-trans-*22-en-3β-yl acetate* (5) (15 mg), m.p. 103—105°, $[\alpha]_D$ +15·8° (*c* 0·4) (Found: M^+, 414·3485. $C_{28}H_{46}O_2$ requires *M*, 414·3497), *m/e* 414 (64%), 399 (5%), 371 (<1), 330 (60), 315 (14), 301 (44), 270 (9), 243 (100), and 201 (9), δ 0·68 (3H, s, 13-Me), 2·00 (3H, s, $MeCO_2$), 4·64br (1H, m, $W_{\frac{1}{2}}$ 22 Hz), and 5·14 (2H, m, olefinic), $\nu_{max.}$ 1740, 1250, 1030, and 975 cm^{-1}.

Fraction VI. This was shown to be a mixture of two components by g.l.c. The principal component (85%) was characterized as a 19-norcholest-22-en-3β-yl acetate (Found: M^+, 414·3485. Calc. for $C_{28}H_{46}O_2$: *M*, 414·3497), δ 0·66 (3H, s, 13-Me), 2·00 (3H, s, $MeCO_2$), 4·17 (1H, m, $W_{\frac{1}{2}}$ 24 Hz), and 5·25 (2H, m, olefinic), $\nu_{max.}$ 1740, 1250, 1030, and 970 cm^{-1}. N.m.r. and i.r. measurements were carried out on the mixture. The first g.l.c. peak showed *m/e* 400 (8%, M^+), 385 (5), 340 (8), 330 (14), 315 (8), 301 (18), 270 (14), 271 (6), 243 (100), 241 (14), and 201 (20); the second peak showed *m/e* 414 (7%), 399 (2), 354 (3), 330 (13), 315 (7), 301 (13), 270 (14), 243 (100), 241 (13), and 201 (18).

Fraction VII. This was rechromatographed on silica gel (2 g) [elution with light petroleum (b.p. 40—70°)–benzene (1 : 1)] to give an amorphous solid (12 mg) *m/e* 428 (25%), 413 (11), 344 (100), 301 (54), 284 (25), 243 (10), 241 (21), and 201 (20), δ 0·66 (3H, s), 2·00 (3H, s) and 4·68 (3H), $\nu_{max.}$ 890 cm^{-1}.

19-Norergostan-3β-yl Acetate.—19-Nor-5α,10β-ergost-22-en-3β-yl acetate (1) (80 mg), dissolved in ethyl acetate (5 ml), was hydrogenated at room temperature and atmospheric pressure over platinized carbon (5%; 100 mg) for 16 h.

The *dihydro-derivative* crystallized from methanol; m.p. 89—90° (Found: M^+, 430·3820. $C_{29}H_{50}O_2$ requires M, 430·3811).

19-*Norergostan*-3-*one* (9).—19-Norergostan-3β-yl acetate was treated with methanolic 10% potassium hydroxide at reflux for 2 h to give the corresponding alcohol. A solution of the crude stanol (70 mg) in benzene (5 ml) was added with stirring and cooling to a solution of potassium dichromate (120 mg) in glacial acetic acid (0·1 ml), concentrated sulphuric acid (0·2 ml), and water (0·6 ml). The mixture was stirred at room temperature for an additional 6 h. A small amount of water was added and the benzene layer was separated, washed with water, aqueous sodium carbonate and water, and finally dried ($MgSO_4$) and evaporated. The crude *ketone* (9) (65 mg) was crystallized from methanol; m.p. 79—80° (Found: M^+, 386·3540. $C_{27}H_{46}O$ requires M, 386·3548), $\nu_{max.}$ ($CHCl_3$) 1705 cm^{-1}, *m/e* 386 (90%) and 217 (100), c.d. (*c* 0·2 in methanol; 20 °C) $[\theta]_{325}$ 0, $[\theta]_{292}$ +5210, $[\theta]_{240}$ 0.

19-*Norergosta*-1,3,5(10)-*trien*-3-*ol* (10).—(i) *From the ketone* (9). A solution of bromine (60 mg) in acetic acid (0·5 ml) was added to the ketone (9) (60 mg) dissolved in acetic acid (3 ml). The mixture was kept at room temperature for 24 h; water (5 ml) was added, and the precipitate was filtered off, washed with water, and dried (yield 53 mg). Its mass spectrum corresponded to a dibromo-derivative (*m/e* 542/544/546). The crude dibromo-ketone was refluxed in dimethylformamide (4 ml) containing lithium bromide (30 mg) and lithium carbonate (30 mg) for 5 h. After cooling, the mixture was poured into 0·5N-hydrochloric acid and extracted with methylene chloride. The residue left after evaporation of the extract was purified by silica gel preparative t.l.c. [benzene–ether (9 : 1)]. Extraction (with chloroform) of the band of R_F 0·8, gave 19-*norergosta*-1,3,5(10)-*trien*-3-*ol* (10), (23 mg), m.p. 145—147° [from light petroleum (b.p. 40—70°)] (Found: M^+, 382·3228. $C_{27}H_{42}O$ requires M, 382·3235), *m/e* 382 (100%), 367 (3), 228 (18), and 213 (70), δ 0·68 (3H, s, 13-Me), 2·80 (3H, m, benzylic), 6·39—6·55 (2H, m, H-2, H-4), and 7·14 (1H, d, *J* 8 Hz, H-1), $\lambda_{max.}$ 280 and 285 nm (ε 2215 and 2000).

(ii) *From ergostan*-3-*one*. Ergostan-3-one (260 mg) was brominated as before, giving a dibromo-derivative, which, without further purification, was refluxed in dimethylformamide (8 ml) in the presence of lithium bromide (30 mg) and lithium carbonate (30 mg) for 6 h. The mixture was worked up as before, giving an oil (220 mg), which was applied to three preparative silica gel plates (20 × 20 cm; Merck). Development with benzene–ether (9 : 1) showed three components, visible under u.v. light. *Ergosta*-1,4-*dien*-3-*one* (12) was recovered as oil (50 mg) from the slowest moving band by extraction with chloroform (Found: M^+, 396·3399. $C_{28}H_{44}O$ requires M, 396·3392), $\lambda_{max.}$ 246 nm (log ε 4·12), $\nu_{max.}$ ($CHCl_3$) 1670 cm^{-1}, δ 0·73 (3H, s, 13-Me), 1·22 (3H, s, 10-Me), 6·08br (1H, H-4), 6·21 (1H, dd, *J* 10 and 1·5 Hz, H-2), and 7·07 (1H, d, *J* 10 Hz, H-1) [the broad signal at 6·08 was simplified to a sharp doublet (*J* 1·5 Hz) by irradiation at δ 2·35 (6-H_2)]. Material from the middle band (54 mg) showed u.v. absorption at 296 nm corresponding to a 4,6-dien-3-one chromophore.

The ergosta-1,4-dien-3-one was subjected to reductive aromatization as follows. Anhydrous tetrahydrofuran was distilled into the reaction apparatus (*ca.* 15 ml) and, under a stream of nitrogen, biphenyl (1·54 g, 0·01 mol) and small pieces of freshly-cut lithium (154 mg, 0·22 g atom) were added. The mixture was stirred vigorously under nitrogen for 1 h, during which time the lithium dissolved to form a deep blue-green solution. Ergosta-1,4-dien-3-one (50 mg), dissolved in anhydrous tetrahydrofuran, was introduced into the adduct solution, and the mixture was refluxed under nitrogen for 2 h. After 15 min of refluxing diphenylmethane (50 mg) was added. After cooling, methanol was added to decompose the excess of the 2 : 1 lithium–biphenyl adduct, and the solvents were removed *in vacuo*. Acidification (2N-HCl) and extraction with ether yielded a syrup which was chromatographed on a silica gel column. After removal of the excess of biphenyl by washing with light petroleum (b.p. 40—70°)–benzene (1 : 1), the *phenol* was eluted with benzene. Further purification by preparative silica gel t.l.c. as before yielded 19-norergosta-1,3,5(10)-trien-3-ol (10) (18 mg), m.p. 144—147°, identical (i.r., n.m.r., u.v., and mass spectra) with the phenol derived from the ketone (9).

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