The Sterols of the Echinoderm *Asterias rubens*

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1. Twenty-two sterols were identified in the starfish *Asterias rubens* (Phylum, Echinodermata; Class, Asteroidea). 2. The major 4-demethyl sterols had a Δ^7 bond and the C_{27} compound 5 α -cholest-7-en-3 β -ol predominated over other mono- and di-unsaturated sterols belonging to the C_{26} , C_{27} , C_{28} and C_{29} series. 3. Small amounts of cholest-5-en-3 β -ol and 5 α -cholestan-3 β -ol were also present. 4. The minor sterols identified all contained either one or two methyl groups at C-4 and are considered to be potential biosynthetic precursors of 5 α -cholest-7-en-3 β -ol. 5. Three sterols possessing a 9 β ,19-cyclopropane ring were also isolated and were probably derived by the starfish from a dietary source.

The early studies on the sterols of echinoderms soon established that the Echinoidea (sea urchins), Ophiuroidea (brittle stars) and Crinoidea (sea lilies) contained predominantly Δ^5 sterols whereas the Asteroidea (starfish) and Holothuroidea (sea cucumbers) contained Δ^7 sterols (Bergmann, 1962). This work also revealed that the sterols of these animals were complex mixtures probably beyond the analytical techniques available at that time for complete resolution and identification. The advent of more sophisticated instrumentation and chromatographic methods has now rendered the identification of the individual components of a sterol mixture a feasible undertaking. In a recent study Gupta & Scheuer (1968) were able to identify a number of C_{27} , C_{28} and C_{29} sterols present in one species from each of the five classes of echinoderm and to confirm the preponderance of Δ^7 sterols in sea cucumbers and starfish. The origin of the Δ^7 sterols present in starfish is of particular interest and it was first proposed by Bergmann (1962) that they arise by modification of dietary Δ^5 sterols. This suggestion gained support from the demonstration that starfish can convert ingested radioactive cholest-5-en-3 β -ol into 5 α -cholest-7-en-3 β -ol (Fagerlund & Idler, 1960; Smith & Goad, 1971a). However, the role of sterol synthesis *de novo* in starfish had not been reported and therefore as a prelude to such studies we have characterized the sterols of *Asterias rubens* paying particular attention to the 4-methyl sterols, which are possible sterol precursors. Part of this work has been reviewed previously (Goad *et al.*, 1970, 1972).

Experimental

General methods

Melting points were determined on a Reichert hot-stage apparatus and are corrected. I.R. spectra were

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measured on a Perkin Elmer 137 spectrometer with samples in KBr discs. N.m.r. spectra were measured at 100 MHz by the PhysicoChemical Measurements Unit, Harwell, Bucks., U.K., or at 60 MHz on a Perkin Elmer R12 instrument with C^2HCl_3 as solvent. G.l.c. was generally as described by Lenton *et al.* (1971) with either 1% SE-30 or 3% OV-17 as stationary phase. For preparative g.l.c. a 1:9 effluent splitter was used; the desired peaks were trapped by condensation in glass capillary tubes at ambient temperature. Mass spectra (m.s.) were measured on an AEI MS12 instrument; for combined g.l.c.–mass spectrometry (g.l.c.–m.s.), a Pye 104 gas chromatograph was coupled via a Biemann separator to the MS12 instrument. Optical-rotatory-dispersion (o.r.d.) curves were measured on 1 mg/ml solutions in methanol on a Bellingham and Stanley Polarmatic 62 by Dr. J. R. Green. Column chromatography of the non-saponifiable lipid was performed on Woelm alumina Brockmann grade III (M. Woelm, Eschwege, West Germany) eluted with increasing proportions of diethyl ether in light petroleum (b.p. 40–60°C) (Goad & Goodwin, 1966). Argentation column chromatography of steryl acetates was on 14% (w/w) $AgNO_3$ -impregnated Woelm acid alumina (M. Woelm) eluted with diethyl ether–light petroleum (b.p. 40–60°C) mixtures (Goad & Goodwin, 1967). For t.l.c. three systems were used; system I: Kieselgel G developed with chloroform containing 2% (v/v) of ethanol; system II: 10% (w/w) $AgNO_3$ -impregnated Kieselgel G developed with benzene–cyclohexane (1:1, v/v); system III: 10% (w/w) $AgNO_3$ -impregnated Kieselgel G developed with chloroform (freshly purified and ethanol free). The Liebermann–Burchard test was performed as described by Kemp *et al.* (1967).

Extraction of sterols from *Asterias rubens*

A. rubens (19 kg, collected off the coast of Anglesey, North Wales) were homogenized and saponified

under reflux with ethanolic KOH followed by extraction into diethyl ether–light petroleum (b.p. 40–60°C) (1:1, v/v) to give the non-saponifiable lipid (53.4g). This was chromatographed on columns of

alumina (Brockmann grade III) to give fractions containing the 4-methyl sterols (5.94g) and 4-demethyl sterols (12.96g). Rechromatography of the 4-methyl sterol-containing fraction on alumina followed by

I

II

III

IV R = H
VII R = CH₃
X R = CH₂CH₃

V R = H
VIII R = CH₃
IX R = CH=CH₂
XI R = CH₂CH₃

VI

XII

XIII

XIV

XV

XVI

XVII

XVIII

XIX

XX

XXI

XXII

preparative t.l.c. on system I gave the 4,4-dimethyl sterols (69 mg) and 4-monomethyl sterols (185 mg).

Separation of the 4-demethyl sterols

A portion of the 4-demethyl sterol fraction (approx. 1.5 g) was acetylated with pyridine-acetic anhydride (1:2, v/v) and the resulting steryl acetates (1.6 g) subjected to argentation column chromatography. The column fractions were monitored by g.l.c. and the individual steryl acetates were then obtained from

the various fractions by careful preparative t.l.c. on system III and crystallized from diethyl ether-methanol before determination of physical properties. All R_F values given below are for system III.

26,27-Dinorengosta-7,22-dien-3 β -yl acetate (I, acetate). R_F 0.40; m.p. 135–138°C; m.s.: m/e 412 (100%, M^+), 397 (15%, $M^+ - CH_3$), 342 (14%, $M^+ - \text{acetate}$), 313 (42%, $M^+ - C-20$ to $C-26-2H$), 255 (45%, $M^+ - C-20$ to $C-26 - \text{acetate}$), 229 (23%), 97 (31%); n.m.r.: (δ , p.p.m.) 0.54 (s, C-18), 0.82 (s, C-19), 0.93 and 0.97 (d, C-25 and C-26), 0.97, 1.03 (d, C-21), 2.01 (s, CH_3CO_2-), 4.65 (m, 3 α -H), 5.18 (m, C-7), 5.23 (m, C-22 and C-23); i.r.: $\nu_{\max}$. 1735, 965 (Δ^{22} trans), 840 and 827 (Δ^7) cm^{-1} .

5 α -Cholest-7-en-3 β -yl acetate (V, acetate). R_F 0.61; m.p. 118–118.5°C (lit. m.p. 118–119°C, Buser, 1947); m.s.: m/e 428 (100%, M^+), 413 (13%, $M^+ - CH_3$), 368 (6%, $M^+ - \text{acetate}$), 315 (13%), 288 (6%), 255 (45%, $M^+ - C-20$ to $C-27 - \text{acetate}$), 229 (20%), 213 (18%, $M^+ - C-20$ to $C-27-42 - \text{acetate}$); n.m.r.: (δ , p.p.m.) 0.53 (s, C-18), 0.83 (s, C-19), 0.81 and 0.89 (d, C-26 and C-27), 0.88 and 0.95 (d, C-21), 2.01 (s, CH_3CO_2-), 4.70 (m, 3 α -H), 5.15 (m, C-7); i.r.: $\nu_{\max}$. 1735, 840 and 827 (Δ^7) cm^{-1} .

5 α -Cholest-7-en-3 β -ol (V). Obtained by preparative g.l.c. of 4-demethyl sterol mixture, m.p. 119–123°C (lit. m.p. 123°C, Buser, 1947); m.s.: m/e 386 (100%, M^+), 371 (31%, $M^+ - CH_3$), 273 (21%, $M^+ - C-20$ to $C-27$), 255 (62%, $M^+ - C-20$ to $C-27 - \text{water}$), 231 (26%), 229 (23%), 213 (25%, $M^+ - C-20$ to $C-27-42 - \text{water}$); n.m.r.: (δ , p.p.m.) 0.54 (s, C-18), 0.80 (s, C-19), 0.82 and 0.89 (d, C-26 and C-27), 0.86 and 0.94 (d, C-21), 3.58 (m, 3 α -H), 5.17 (m, C-7); i.r.: identical to literature (Scallen & Krueger, 1968) and an authentic sample, $\nu_{\max}$. 840 and 827 (Δ^7) cm^{-1} .

5 α -Cholesta-7,22-dien-3 β -yl acetate (IV, acetate). R_F 0.45; m.p. 128–131°C (lit. m.p. 130–131.5°C, Sakai & Tsuda, 1963); m.s.: m/e 426 (100%, M^+), 411 (30%, $M^+ - CH_3$), 342 (23%, $M^+ - C-22$ to $C-27$), 313 (60%, $M^+ - C-20$ to $C-27-2H$), 255 (53%, $M^+ - C-20$ to $C-27 - \text{acetate}$), 229 (24%), 213 (17%, $M^+ - C-20$ to $C-27-42 - \text{acetate}$); n.m.r.: (δ , p.p.m.) 0.54 (s, C-18), 0.82 (s, C-19), 0.81 and 0.89 (d, C-26 and C-27), 0.97 and 1.04 (d, C-21), 2.01 (s, CH_3CO_2-), 4.65 (m, 3 α -H), 5.18 (m, C-7), 5.27 (m, C-22 and C-23); i.r.: $\nu_{\max}$. 1735, 965 (Δ^{22} trans), 827 and 840 (Δ^7) cm^{-1} .

(24S)-24-Methyl-5 α -cholesta-7,22-dien-3 β -yl acetate (VII, acetate). R_F 0.50; m.p. 173–175°C [lit. m.p. for (24R) isomer 184–186°C, Barton & Cox, 1948; no literature value for the (24S) form]; m.s.: m/e 440 (100%, M^+), 380 (5%, $M^+ - \text{acetate}$), 342 (26%, $M^+ - C-22$ to $C-28$), 313 (90%, $M^+ - C-20$ to $C-28-2H$), 255 (65%, $M^+ - C-20$ to $C-28 - \text{acetate}$), 229 (28%), 213 (20%, $M^+ - C-20$ to $C-28-42 - \text{acetate}$); n.m.r.: (δ , p.p.m.) 0.54 (s, C-18), 0.81 (s, C-19), 0.78 and 0.85 (d, C-26 or C-27), 0.81 and 0.87 (d, C-27 or C-26), 0.87 and 0.96 (d, C-28), 0.97 and 1.04 (d, C-21), 2.01 (s, CH_3CO_2-), 4.68 (m, 3 α -H), 5.14 (m,

C-7), 5.20 (m, C-22 and C-23); i.r.: $\nu_{\max}$. 1735, 965 (Δ^{22} trans), 840 and 827 (Δ^7) cm^{-1} .

(24S)-24-Methyl-5 α -cholesta-7,22-dien-3 β -ol (VII). M.p. 159–160°C [lit. m.p. for (24R) epimer 176–177°C, Barton & Cox, 1948; no m.p. available for the (24S) compound]; m.s.: m/e 398 (57%, M^+), 383 (18%, $M^+ - CH_3$), 300 (22%, $M^+ - C-22$ to $C-28$), 271 (100%, $M^+ - C-20$ to $C-28-2H$), 255 (50%, $M^+ - C-20$ to $C-28 - \text{water}$), 229 (28%), 213 (18%, $M^+ - C-20$ to $C-28-42 - \text{water}$); n.m.r.: (δ , p.p.m.) 0.54 (s, C-18), 0.80 (s, C-19); i.r.: $\nu_{\max}$. 970 (Δ^{22} trans) cm^{-1} .

24-Methylene-5 α -cholest-7-en-3 β -yl acetate (IX, acetate). R_F 0.22; m.p. 147–149°C (lit. m.p. 136–138°C, Barton *et al.*, 1972); m.s.: m/e 440 (40%, M^+), 425 (19%, $M^+ - CH_3$), 380 (5%, $M^+ - \text{acetate}$), 356 (62%, $M^+ - C-23$ to $C-28$), 313 (100%, $M^+ - C-20$ to $C-28-2H$), 255 (29%, $M^+ - C-20$ to $C-28 - \text{acetate}$), 229 (12%), 227 (14%), 213 (17%, $M^+ - C-20$ to $C-28-42 - \text{acetate}$); n.m.r.: (δ , p.p.m.) 0.54 (s, C-18), 0.81 (s, C-19), 0.93 and 1.00 (d, C-21), 0.99 and 1.06 (d, C-26 and C-27), 2.02 (s, CH_3CO_2-), 4.66 (m, 3 α -H), 4.70 (s, C-28), 5.16 (m, C-7); i.r.: $\nu_{\max}$. 1735, 1635 and 883 ($\Delta^{24(28)}$), 840 and 825 (Δ^7) cm^{-1} .

5 α -Cholesta-7,24-dien-3 β -yl acetate (VI, acetate). R_F 0.31; m.s.: m/e 426 (100%, M^+), 411 (15%, $M^+ - CH_3$), 342 (19%, $M^+ - C-22$ to $C-27$), 313 (58%, $M^+ - C-20$ to $C-27-2H$), 255 (40%, $M^+ - C-20$ to $C-27 - \text{acetate}$), 229 (19%), 213 (11%, $M^+ - C-20$ to $C-27-42 - \text{acetate}$).

24Z-Ethylidene-5 α -cholest-7-en-3 β -yl acetate (XII, acetate). R_F 0.40; m.s.: m/e 454 (14%, M^+), 356 (95%, $M^+ - C-23$ to $C-29$), 313 (100%, $M^+ - C-20$ to $C-29-2H$), 255 (18%, $M^+ - C-20$ to $C-29 - \text{acetate}$), 253 (6%), 229 (8%), 227 (9%), 213 (13%, $M^+ - C-20$ to $C-29-42 - \text{acetate}$).

24E-Ethylidene-5 α -cholest-7-en-3 β -yl acetate (XIII, acetate). R_F 0.44; m.s.: m/e 454 (23%, M^+), 356 (78%, $M^+ - C-23$ to $C-29$), 313 (100%, $M^+ - C-20$ to $C-29-2H$), 255 (28%, $M^+ - C-20$ to $C-29 - \text{acetate}$), 253 (17%), 229 (10%), 227 (10%), 213 (19%, $M^+ - C-20$ to $C-29-42 - \text{acetate}$).

Separation of the 4,4-dimethyl sterols

The 4,4-dimethyl sterols were acetylated with pyridine-acetic anhydride and separated by preparative t.l.c. on system II into three bands: fraction 1, R_F 0.39, 18.4 mg; fraction 2, R_F 0.35, 20.5 mg; fraction 3, R_F 0.33, 21.1 mg. The fractions were examined by g.l.c. and m.s. The further analysis of these fractions is described in the Results section. The following n.m.r. spectra (δ , p.p.m.) were obtained: fraction 1, 0.58 (d, 9 β ,19-cyclopropane), 0.82, 0.89, 0.95 (21H, $-CH_3$ groups), 2.03 (s, CH_3CO_2-), 4.54 (m, 3 α -H); fraction 2, 0.68 (s, C-18), 0.88 (C-30, C-31, C-32), 1.00 (s, C-19), 1.60 and 1.68 (C-26 and C-27), 2.03 (s, CH_3CO_2-), 4.52 (m, 3 α -H), 5.10 (m, C-24); fraction 3 (mixture) 0.59, 0.87, 0.97, 0.99, 1.58, 1.66,

2.02, 4.50, 5.07; n.m.r. for components of fraction 3 after separation by preparative g.l.c.: 4,4-dimethyl-5 α -cholesta-8,24-dien-3 β -yl acetate (XVIII, acetate), 0.62 (s, C-18), 0.89 (C-30, C-31), 1.02 (s, C-19), 1.61 and 1.69 (C-26 and C-27), 2.01 (s, CH₃CO₂—), 4.45 (m, 3 α -H), 5.09 (m, C-24); 4,4-dimethyl-5 α -cholesta-7,24-dien-3 β -yl acetate (XIX, acetate), 0.55 (s, C-18), 0.90 (C-30, C-31), 1.69 and 1.61 (C-26 and C-27), 2.01 (s, CH₃CO₂—), 4.43 (m, 3 α -H), 5.10 (m, C-7 and C-24).

Separation of the 4-monomethyl sterols

The 4-monomethyl sterols were acetylated and separated into two bands by preparative t.l.c. on system II; fraction 4, R_F 0.37, 118mg; fraction 5, R_F 0.31, 32 mg. These were examined by g.l.c. and m.s. Fraction 4 (60.5mg) was treated with OsO₄ (54.0mg) in pyridine (2.0ml) and the mixture left overnight after which it was stirred for 45min with 5% (w/v) sodium sulphite (25ml). The steryl acetates were extracted twice into chloroform which was then washed successively with water, 10% (v/v) HCl and further portions of water. The chloroform was removed, after drying over anhydrous Na₂SO₄, and the resulting residue dissolved in dry diethyl ether (15ml) and treated with LiAlH₄ to convert the steryl acetates into the 3 β -hydroxy compounds. After extraction of the free sterols (67.0mg) they were subjected to preparative t.l.c. [Kieselgel G developed with chloroform-methanol (9:1, v/v)] and the two major bands eluted exhaustively with diethyl ether to give band A (R_F 0.6), 23.8mg, and band B (R_F 0.2), 9.6mg. Band A was identified as 31-norcycoloartanol (XXI), m.p. 132–135°C (lit. m.p. 128–132°C, Berti *et al.*, 1967; m.p. 132°C, Ayengar & Rangswami, 1967); m.s.: m/e 414 (60%, M^+), 399 (70%, $M^+ - CH_3$), 396 (100%, $M^+ - \text{water}$), 381 (80%, $M^+ - CH_3 - \text{water}$), 341 (24%), 301 (34%, $M^+ - C-20$ to C-27), 288 (63%, $M^+ - \text{ring A}$), 283 (25%, $M^+ - C-20$ to C-27 - water) (lit. m.s., Berti *et al.*, 1967); n.m.r.: (δ , p.p.m.) 0.18 and 0.44 (dd, 9 β , 19-cyclopropane), 0.84 (s, C-18), 0.91 (s, C-30 and C-32), 0.91 and 0.96 (d, C-26 and C-27), 0.91 and 1.00 (d, C-21), 3.22 (m, 3 α -H). Band B was identified as 4 α -methyl-5 α -cholestane-3 β ,7 α ,8 α -triol, m.p. 180–184°C (lit. m.p. 178–180°C, Rahman *et al.*, 1970); m.s.: m/e 434 (28%, M^+), 419 (10%, $M^+ - CH_3$), 416 (87%, $M^+ - \text{water}$), 401 (9%, $M^+ - CH_3 - \text{water}$), 398 (100%, $M^+ - 2 \times \text{water}$), 383 (11%, $M^+ - CH_3 - 2 \times \text{water}$), 303 (17%, $M^+ - C-20$ to C-27 - water), 285 (17%, $M^+ - C-20$ to C-27 - 2 $\times$ water). Treatment of fraction 4 (34.4mg) with *m*-chloroperbenzoic acid in chloroform resulted in epoxidation of the Δ^7 bond of compound XX; preparative t.l.c. [Kieselgel G developed with ethyl acetate-light petroleum (b.p. 40–60°C) (1:9, v/v)] gave unchanged 31-norcycoloartanyl acetate (22mg, R_F 0.60), m.p. 98–101°C (lit. m.p. 98–

99°C, Berti *et al.*, 1967; m.p. 102–103°C, Ayengar & Rangswami, 1967). A portion (8.5mg) of the purified 31-norcycoloartanol (XXI) was oxidized with Jones' reagent and the resulting 31-norcycoloartan-3-one (5.8mg) was purified by t.l.c. on system I; m.p. 102–104°C (lit. m.p. 104–106°C, Berti *et al.*, 1967); m.s.: m/e 412 (68%, M^+), 397 (31%, $M^+ - CH_3$), 328 (30%), 299 (100%, $M^+ - C-20$ to C-27), 288 (18%, $M^+ - \text{ring A}$), 260 (42%); o.r.d.: $[\alpha]_{300} + 550^\circ$, $[\alpha]_{268} - 270^\circ$, unchanged by addition of HCl or after reflux of 31-norcycoloartan-3-one (2.9mg) in ethanol (3.0ml) containing 0.3ml of 20% (v/v) H₂SO₄ (Sanghvi *et al.*, 1967).

Fraction 5 was homogeneous by g.l.c.–m.s. and was identified as 4 α -methyl-5 α -cholesta-7,24-dien-3 β -yl acetate (XXII, acetate); n.m.r.: (δ , p.p.m.) 0.54 (s, C-18), 0.82 (s, C-19), 0.86 (s, C-30), 1.60 and 1.68 (d, C-26 and C-27), 2.04 (s, CH₃CO₂—), 4.40 (m, 3 α -H), 5.16 (m, C-7 and C-24). Reduction with LiAlH₄ in dry diethyl ether gave 4 α -methyl-5 α -cholesta-7,24-dien-3 β -ol (XXII), m.p. 137–139°C (lit. m.p. 139°C, Charles *et al.*, 1968); m.s.: m/e 398 (50%, M^+), 383 (30%, $M^+ - CH_3$), 314 (11%), 285 (100%, $M^+ - C-20$ to C-27 - 2H), 227 (10%, $M^+ - C-20$ to C-27 - 42 - water). Oxidation of compound XXII with Jones' reagent gave 4 α -methyl-5 α -cholesta-7,24-dien-3-one, m.p. 103–106°C; m.s.: m/e 396 (25%, M^+), 312 (19%), 283 (100%, $M^+ - C-20$ to C-27 - 2H), 243 (16%), 69; o.r.d.: curve unchanged by HCl or H₂SO₄ treatment.

Results

The 4-demethyl sterol mixture obtained from *A. rubens* gave an immediate blue colour characteristic of Δ^7 sterols with the Liebermann-Burchard reagent (Moore & Baumann, 1952). The n.m.r. spectrum of the 4-demethyl sterol mixture showed resonances at δ 0.54 p.p.m. and 0.80 p.p.m. for the C-18 and C-19 methyl protons respectively thus confirming that Δ^7 sterols were the major components (Scallen & Krueger, 1968). In addition absorption at 4.6–4.7 p.p.m. indicated the presence of a sterol with a terminal methylene group in the mixture. This was further supported by peaks at 1650 and 887 cm⁻¹ in the i.r. spectrum of the sterols. The absence of cholesta-5,7-dien-3 β -ol or other $\Delta^{5,7}$ dienes was indicated by the u.v. spectrum of the mixture.

G.l.c. analysis (Table 1) revealed that the mixture was complex with eight component peaks and the major peak (C) had a retention time identical with that of authentic 5 α -cholest-7-en-3 β -ol (V). Peaks B, E and G were shown by g.l.c.–m.s. of the trimethylsilyl ethers to contain more than one component and altogether eleven sterols were identified in the mixture by this method (Tables 1 and 2). Compounds in all the g.l.c. peaks gave mass-spectral fragmentations indicating the presence of Δ^7 sterols with prominent ions

Table 1. *G.l.c. analysis of the sterols of A. rubens*

For details see the text.

Peak	Sterol	Relative retention time on:		Percentage composition
		3% OV-17	1% SE-30	
A	26,27-Dinorergosta-7,22-dien-3 β -ol (I)	0.78	0.70	1.8
B	5 α -Cholestan-3 β -ol (II)	1.00	1.00	5.2
	Cholest-5-en-3 β -ol (III)			
	5 α -Cholesta-7,22-dien-3 β -ol (IV)	1.12		
C	5 α -Cholest-7-en-3 β -ol (V)	1.18	1.10	52.6
	5 α -Cholesta-7,24-dien-3 β -ol (VI)*			Trace
D	24-Methyl-5 α -cholesta-7,22-dien-3 β -ol (VII)	1.34	1.24	23.7
E	24-Methyl-5 α -cholest-7-en-3 β -ol (VIII)	1.58†	1.43	9.9
	24-Methylene-5 α -cholest-7-en-3 β -ol (IX)			
F	24-Ethyl-5 α -cholesta-7,22-dien-3 β -ol (X)	1.68	1.63	4.1
G	24-Ethyl-5 α -cholest-7-en-3 β -ol (XI)	1.92†	1.83	2.7
	24-Ethylidene-5 α -cholest-7-en-3 β -ol‡ (XII, XIII)			

* This sterol was present in such small amounts that it was only detected after partial resolution of the steryl acetate mixture by AgNO₃-impregnated alumina chromatography.

† Broad heterogeneous peaks.

‡ Shown after separation to be a mixture of the *E*- and *Z*- isomers.

at *m/e* 255 for loss of the side chain and trimethylsilanol and ions at *m/e* 229 and 213 for cleavages of ring D with concomitant loss of trimethylsilanol (Gustafsson *et al.*, 1966; Knights, 1967; Brooks *et al.*, 1968). The mass-spectral fragmentations for the trimethylsilyl ethers of the sterols present in the various g.l.c. peaks are listed in Table 2. The main component, peak C, was the trimethylsilyl ether of 5 α -cholest-7-en-3 β -ol (V), whereas peaks D and F contained the trimethylsilyl ethers of 24-methyl-5 α -cholesta-7,22-dien-3 β -ol (VII) and 24-ethyl-5 α -cholesta-7,22-dien-3 β -ol (X) respectively. The Δ^{22} bond in the side chains of the latter two compounds was indicated by the presence of strong ions at *m/e* 343 and 345 for loss of the side chain with and without two additional hydrogens (Wyllie & Djerassi, 1968) and in particular by the ion at *m/e* 372 for cleavage of the C-20–C-22 bond (Eneroth *et al.*, 1965; Gustafsson *et al.*, 1966; Lenfant *et al.*, 1967; Brooks *et al.*, 1968). Similar fragmentations were observed for one of the three components in peak B and were assigned to the presence of 5 α -cholesta-7,22-dien-3 β -ol (IV). In addition to this sterol, mass spectrometry also established the presence in peak B of small amounts of 5 α -cholestan-3 β -ol (II) and cholest-5-en-3 β -ol (III). The Δ^5 bond of the latter compound was confirmed by a strong ion at *m/e* 129 together with the complementary ion at *m/e* 329, which can be attributed to the cleavage of the C-1–C-10 and C-3–C-4 bonds and which are typical of Δ^5 sterols (Eneroth *et al.*, 1964; Brooks *et al.*, 1968). On the g.l.c. stationary phase OV-17 the 5 α -cholestan-3 β -ol and cholest-5-en-3 β -ol, although having the

same chromatographic mobilities, separated from the 5 α -cholesta-7,22-dien-3 β -ol and had retention times identical with the authentic compounds (Table 1).

Peak A had a molecular ion at *m/e* 442 demonstrating it to be a di-unsaturated C₂₆ sterol and its identification as 26,27-dinorergosta-7,22-dien-3 β -ol (I) is discussed elsewhere (Goad *et al.*, 1972). Peak E was found to contain two components identified as the trimethylsilyl ethers of 24-methyl-5 α -cholest-7-en-3 β -ol (VIII) and 24-methylene-5 α -cholest-7-en-3 β -ol (IX). The position of the side-chain unsaturation in the latter compound was revealed by fragments at *m/e* 386, 371 and 296, which were derived from an ion arising by cleavage of the C-22–C-23 bond (Knights, 1967; Wyllie & Djerassi, 1968). Similarly, peak G was observed to contain the trimethylsilyl ethers of 24-ethyl-5 α -cholest-7-en-3 β -ol (XI) and 24-ethylidene-5 α -cholest-7-en-3 β -ol (XII), the side-chain double-bond position in the latter sterol again being indicated by the ions at *m/e* 386, 371 and 296 for splitting of the C-22–C-23 bond (Knights, 1967).

To confirm some of the above identifications the 4-demethyl sterol mixture was acetylated and subjected to chromatography on a column of alumina impregnated with AgNO₃. This permitted a preliminary fractionation of the mixture based on the number and position of double bonds in the component sterols (Morris, 1963). The various column fractions were monitored by g.l.c. and their compositions are indicated in Table 3. Further separation into individual steryl acetates was achieved by preparative t.l.c. on AgNO₃-impregnated Kieselgel G.

Table 2. *G.l.c.-m.s. data obtained by analysis of the trimethylsilyl ethers of the sterol mixture of A. rubens*

For details see the text. Relative abundances of ions above m/e 200 are given in parentheses; for mixed g.l.c. peaks relative abundances of ions are only presented for the major component.

Peak	Sterol	M^+ (molecu- lar ion)	$M^+ - CH_3$	$M^+ -$ tri- methyl- silyl	Mass-spectral fragmentation ions					
					$M^+ -$ side chain	$M^+ -$ side chain - 2H	$M^+ -$ side chain - tri- methyl- silyl	$M^+ -$ side chain - tri- methyl- silyl - 26	$M^+ -$ side chain - tri- methyl- silyl - 42	Other diagnostic ions
A	26,27-Dinoregosta-7,22-dien-3 β -ol	442 (78)	427 (44)	352 (12)	345 (24)	343 (46)	255 (100)	229 (56)	213 (26)	372 (14)
B	5 α -Cholestan-3 β -ol	460	445	370	—	—	257	—	215	—
	Cholest-5-en-3 β -ol	458	443	368	—	—	255	—	213	329, 129
C	5 α -Cholesta-7,22-dien-3 β -ol	456 (62)	441 (28)	366 (10)	345 (20)	343 (40)	255 (100)	229 (54)	213 (36)	372 (6)
	5 α -Cholest-7-en-3 β -ol	458 (100)	443 (18)	368 (12)	345 (6)	—	255 (80)	229 (26)	213 (34)	—
D	24-Methyl-5 α -cholesta-7,22-dien-3 β -ol	470 (56)	455 (26)	380 (6)	345 (30)	343 (51)	255 (100)	229 (52)	213 (25)	372 (12)
E	24-Methyl-5 α -cholest-7-en-3 β -ol	472 (100)	457 (18)	382 (9)	345 (12)	—	255 (89)	229 (25)	213 (36)	—
	24-Methylene-5 α -cholest-7-en-3 β -ol	470 (51)	455 (34)	380 (9)	345 (8)	343 (100)	255 (77)	229 (34)	213 (62)	372 (12), 386 (53), 371 (15), 296 (8)
F	24-Ethyl-5 α -cholesta-7,22-dien-3 β -ol	484 (70)	469 (21)	—	345 (30)	343 (85)	255 (100)	229 (56)	213 (47)	372 (23)
G	24-Ethyl-5 α -cholest-7-en-3 β -ol	486 (100)	471 (20)	396 (6)	345 (9)	—	255 (87)	229 (25)	213 (39)	—
	24-Ethylidene-5 α -cholest-7-en-3 β -ol	484	469	394	345	343	255	229	213	372, 386, 371, 296

The major component, obtained in fraction 5-1, was identified as 5 α -cholest-7-en-3 β -yl acetate and all the physical characteristics (m.p., m.s., n.m.r. and i.r.) for both the acetate and the free sterol were identical with those of the authentic compound. The three $\Delta^{7,22}$ sterols present in fractions 10-1 and 10-2 were separated and fully characterized as 26,27-dinoregosta-7,22-dien-3 β -yl acetate, 5 α -cholesta-7,22-dien-3 β -yl acetate and (24*S*)-24-methyl-5 α -cholesta-7,22-dien-3 β -yl acetate. In all three cases the Δ^{22} bond was established to have the *trans* configuration on the basis of t.l.c. mobility on AgNO₃-impregnated Kieselgel G, which permits a clear separation of the Δ^{22} *cis* and *trans* isomers (I. Rubinstein, unpublished work), and by the peak in the i.r. spectrum at 970 cm⁻¹, which is characteristic of a Δ^{22} *trans* bond. The configuration of the C-24 methyl group of the (24*S*)-24-methyl-5 α -cholesta-7,22-dien-3 β -ol (VII) was deduced from the m.p. results and in particular from the n.m.r. spectrum. The m.p. values of both the free sterol (159–160°C) and the acetate (173–175°C) were significantly different from those of authentic (24*R*)-24-methyl-5 α -cholesta-7,22-dien-3 β -ol (174°C) and its acetate (184–186°C), which were prepared by reduction of ergosta-5,7,22-trien-3 β -yl acetate, which has the (24*R*) configuration (Shopee, 1964). When the n.m.r. spectra of these two acetates were compared striking differences were observed for the resonances of the side-chain methyl-group protons and the two spectra were not superimposable (Table 4). Similar differences in the n.m.r. spectra of (24*R*)-24-methylcholest-5-en-3 β -ol and (24*S*)-24-methylcholest-5-en-3 β -ol have been reported previously and have been proposed as criteria for the assignment of C-24 configuration (Thompson *et al.*, 1972). It is therefore concluded that the C₂₈ sterol (VII) of *A. rubens* is principally, if not entirely, the (24*S*) isomer.

T.l.c. of fractions 15-1 to 15-2 on AgNO₃-impregnated Kieselgel G gave three components, which were identified on the basis of their t.l.c. mobilities, g.l.c. retention times and mass-spectral data. The most-polar constituent was shown by m.s. to be a C₂₇ sterol diene. The presence of a strong molecular ion (m/e 426) established a Δ^7 bond since Δ^5 sterols readily lose acetate and give only a very weak molecular ion (Knights, 1967). The mobility of this compound on AgNO₃-impregnated Kieselgel G t.l.c. suggested its tentative identification as the acetate of 5 α -cholesta-7,24-dien-3 β -ol (VI). The two less-polar compounds on AgNO₃-impregnated Kieselgel G were established as the *Z*- and *E*-isomers respectively of 24-ethylidene-5 α -cholest-7-en-3 β -yl acetate (XII and XIII). Both gave mass spectra with molecular ions at m/e 454 and fragment ions at m/e 356 and 313 for the cleavage of the C-22–C-23 bond characteristic of 24-ethylidene sterols (Brooks *et al.*, 1972).

The most-polar compound eluting in fractions

Table 3. *Fractionation of the steryl acetates obtained from A. rubens*

The steryl acetates were chromatographed on a 100g column of alumina impregnated with AgNO₃ (14%, w/w) and eluted with 500ml portions of increasing percentages of diethyl ether in light petroleum (b.p. 40–60°C). Fractions were monitored by g.l.c. on 1% SE-30 and the percentage compositions are given by the values in parentheses.

Fraction	Eluent	Wt. (mg)	Sterols
0-1	Light petroleum	26	—
0-2	Light petroleum	10	—
5-1	Diethyl ether–light petroleum (5:95, v/v)	744	5 α -Cholest-7-en-3 β -yl acetate (100)
5-2	Diethyl ether–light petroleum (5:95, v/v)	167	5 α -Cholest-7-en-3 β -yl acetate (10), 24-methyl-5 α -cholesta-7,22-dien-3 β -yl acetate (90)
10-1	Diethyl ether–light petroleum (10:90, v/v)	313	26,27-Dinorergosta-7,22-dien-3 β -yl acetate (15), 5 α -cholesta-7,22-dien-3 β -yl acetate (15), 24-methyl-5 α -cholesta-7,22-dien-3 β -yl acetate (70)
10-2	Diethyl ether–light petroleum (10:90, v/v)	64	26,27-Dinorergosta-7,22-dien-3 β -yl acetate (10), 5 α -cholesta-7,22-dien-3 β -yl acetate (90)
15-1	Diethyl ether–light petroleum (15:85, v/v)	7	5 α -Cholesta-7,22-dien-3 β -yl acetate (60), 24-ethylidene-5 α -cholest-7-en-3 β -yl acetate (40)
15-2	Diethyl ether–light petroleum (15:85, v/v)	17	5 α -Cholesta-7,24-dien-3 β -yl acetate (30), 24-ethylidene-5 α -cholest-7-en-3 β -yl acetate (70)
100-1	Diethyl ether	103	24-Methylene-5 α -cholest-7-en-3 β -yl acetate (100)
100-2	Diethyl ether		

Table 4. *Comparison of the n.m.r. spectra obtained for the (24S)-24-methyl-5 α -cholesta-7,22-dien-3 β -yl acetate isolated from A. rubens and for synthetic (24R)-24-methyl-5 α -cholesta-7,22-dien-3 β -yl acetate*

The spectra were recorded at 100MHz with a sweep width of 250Hz with C²HCl₃ as solvent and tetramethylsilane as the internal reference. Chemical shifts are given in δ (p.p.m.).

	δ (p.p.m.)				
	C-18	C-19	C-21	C-26 and C-27	C-28
(24S)-24-Methyl-5 α -cholesta-7,22-dien-3 β -yl acetate (<i>A. rubens</i>)	0.541	0.807	1.035, 0.970	0.869, 0.807 0.850, 0.784	0.937, 0.869
(24R)-24-Methyl-5 α -cholesta-7,22-dien-3 β -yl acetate (synthetic)	0.547	0.816	1.052, 0.983	0.871, 0.813 0.855, 0.790	0.948, 0.880

100-1 and 100-2 was identified as 24-methylene-5 α -cholest-7-en-3 β -yl acetate from its i.r., n.m.r. and m.s. characteristics. The acetates of 5 α -cholestan-3 β -ol (II), cholest-5-en-3 β -ol (III), 24-methyl-5 α -cholest-7-en-3 β -ol (IX), 24-ethyl-5 α -cholest-7-en-3 β -ol (XI) and 24-ethyl-5 α -cholesta-7,22-dien-3 β -ol (X), previously identified by g.l.c.–m.s. analysis of the sterol mixture (Table 2), were not detectable in the fractions from the AgNO₃-impregnated alumina column chromatography of the steryl acetates (Table 3) and it is concluded that this was because they were only present in the initial sterol mixture in amounts of less than 1–2% of the total. However, sterol (X) was obtained by fractional crystallization of the sterol mixture, which produced, after fifteen recrystallizations from

methanol, material containing (24S)-24-methyl-5 α -cholesta-7,22-dien-3 β -ol (65%) and 24-ethyl-5 α -cholesta-7,22-dien-3 β -ol (35%). Preparative g.l.c. of this material gave the latter compound (X) which was identified by g.l.c. retention time and mass spectrometry. The m.p. of the sterol (161–164°C) was lower than the values previously reported for both the (24R) or the (24S) isomers (169°C and 172°C respectively, Bergmann & McTigue, 1948), and therefore the C-24 configuration cannot be assigned. However, it is noteworthy that hitodesterol, a compound previously isolated from the starfish *Asterias amurensis* (Matsumoto & Wanai, 1955) and *Asterina pectinifera* (Toyama & Takagi, 1955), was concluded to be (24S)-24-ethyl-5 α -cholesta-7,22-dien-3 β -ol (α -spinasterol).

Sterols containing methyl groups at C-4, obtained in low yield from the non-saponifiable lipid of *A. rubens*, were resolved into the 4,4-dimethyl and 4 α -methyl compounds by preparative t.l.c. The 4,4-dimethyl sterols were acetylated and examined by AgNO₃-impregnated Kieselgel G t.l.c. Three bands were observed, the least polar (fraction 1) had an R_F (0.39) identical with that of marker 24,25-dihydrolanosteryl acetate and cycloartanyl acetate; the intermediate band (fraction 2) had the same chromatographic mobility as lanosteryl acetate and cycloartenyl acetate (R_F 0.35) and the most-polar material (fraction 3) had an R_F (0.33) that did not correspond to any available standard.

Analysis by g.l.c. showed that the major component of fraction 1 had a retention time corresponding to cycloartanyl acetate but the unsymmetrical shape of the peak indicated heterogeneity. A number of other very minor components that could not be identified were revealed. G.l.c.-m.s. confirmed that the major g.l.c. peak consisted predominantly of cycloartanyl acetate. The molecular ion was at m/e 470 as required for the acetate of cycloartanol (XIV), whereas the fragmentation ion at m/e 288 for the loss of ring A established the presence of the 9 β ,19-cyclopropane ring (Audier *et al.*, 1966). Mass spectra taken repeatedly across the g.l.c. peak showed that the leading edge contained a C₂₉ steryl acetate (M^+ at m/e 456). This was identified as the acetate of 4,4-dimethyl-5 α -cholest-7-en-3 β -ol (XV) on the basis of its g.l.c. retention time, which was too long for it to be the Δ^8 isomer, and by the presence of ions in the mass spectrum at m/e 241 (Knights, 1967) and at 274 and 122 for a *retro*-Diels-Alder reaction characteristic of Δ^7 sterols (Galli & Maroni, 1967). The n.m.r. spectrum of fraction 1 was also consistent with the presence of cycloartanyl acetate as the main component with the 9 β ,19-cyclopropane hydrogen atoms resonating at 0.58 p.p.m. although the second anticipated doublet at 0.37 p.p.m. (Berti *et al.*, 1967) was masked by the tetramethylsilane used as internal standard. A portion of the mixture was converted into the free sterol and a small quantity of the cycloartanol obtained free of the other minor constituents in the fraction by preparative g.l.c. This material gave the red-yellow colour with the Liebermann-Burchard reagent typical of 9 β ,19-cyclopropane sterols (Ohta & Shimizu, 1957) and the g.l.c. retention time and mass spectrum were again identical with those of authentic cycloartanol (XIV). No evidence was found for the presence of 24,25-dihydrolanosterol in fraction 1.

G.l.c. examination of fraction 2 showed two constituents with retention times corresponding to the acetates of lanosterol (XVI, 83%) and cycloartenol (XVII, 17%). G.l.c.-m.s. substantiated these identifications with both g.l.c. peaks having molecular ions at m/e 468, and the fragmentation ion at m/e 286 for

loss of ring A in the mass spectrum of the latter compound again confirmed the presence of the 9 β ,19-cyclopropane ring (Audier *et al.*, 1966). The presence of a Δ^{24} bond in both compounds was indicated by the peak in the mass spectra at m/e 69 (Galli & Maroni, 1967) and further substantiated by the peaks at 1.60 and 1.68 p.p.m. in the n.m.r. spectrum for the C-26 and C-27 methyl proton resonances (Scallen & Krueger, 1968) and by the broad peak centred at 5.10 p.p.m. and integrating for the one olefinic proton located at C-24. The singlets for the C-18 and C-19 methyl group protons and the other resonances for the C-21, C-4 and C-14 methyl group protons were all close to those reported previously for lanosteryl acetate (Barton *et al.*, 1970). Conversion of the acetates of fraction 2 into the free alcohols followed by preparative g.l.c. gave small amounts of lanosterol (m.p. 134–136°C; lit. 140°C, Ourisson *et al.*, 1964) and cycloartenol, which were indistinguishable from the authentic compounds by g.l.c., m.s. and response to the Liebermann-Burchard reagent.

Fraction 3 was found by g.l.c. analysis to contain two components that were both shown by g.l.c.-m.s. to be the acetates of C₂₉ sterols (M^+ at m/e 454) containing two double bonds. Mass-spectral fragments at m/e 341 arising from the loss of an unsaturated side chain (Wyllie & Djerassi, 1968) and peaks at m/e 69 indicated that both compounds possessed a Δ^{24} bond (Galli & Maroni, 1967) and therefore only differed in the position of their nuclear unsaturation. The behaviour of the compounds on g.l.c. suggested that the compound of shorter retention time was the acetate of the Δ^8 compound 4,4-dimethyl-5 α -cholesta-8,24-dien-3 β -ol (40%, XVIII) and the compound of longer retention time was the acetate of the Δ^7 isomer 4,4-dimethyl-5 α -cholesta-7,24-dien-3 β -ol (60%, XIX). To substantiate these identifications the steryl acetates were obtained by preparative g.l.c. Both compounds gave an immediate blue colour with the Liebermann-Burchard test confirming the presence of a Δ^8 or Δ^7 bond (Moore & Baumann, 1952) and in accord with the absence of a 14 α -methyl group (Goad *et al.*, 1967). In the mass spectra of both compounds several fragmentation ions were of approximately equal intensity but a number of important differences were observed (Table 5). In the Δ^8 isomer the molecular ion (m/e 454) was the major peak whereas in the Δ^7 compound the major peak was the ion at m/e 341 which arose by loss of the side chain and two hydrogen atoms (Wyllie & Djerassi, 1968). Further evidence for the presence of the Δ^7 bond in the acetate of compound XIX was provided by the intensity of an ion at m/e 122, which has been attributed to the loss of ring A in Δ^7 sterols (Galli & Maroni, 1967). An ion at m/e 266, which may have arisen by cleavage across ring B as reported in the mass spectrum of 4,4-dimethyl-5 α -cholest-8-en-3 β -ol (Scallen *et al.*, 1971), supported the location of the Δ^8

Table 5. Mass-spectral data for the acetates of the 4,4-dimethyl and 4-monomethyl sterols from *A. rubens*

For details see the text. The retention time is relative to 5 α -cholestan-3 β -ol on 1% SE-30. Relative intensities of ions above m/e 220 are given in parentheses.

	Retention time	Mass-spectral fragmentation ions								
		M^+	$M^+ - \text{CH}_3$	$M^+ - \text{acetate}$	$M^+ - \text{acetate} - \text{CH}_3$	$M^+ - \text{side chain}$	$M^+ - \text{side chain} - 2\text{H}$	$M^+ - \text{side chain} - \text{acetate}$	$M^+ - \text{side chain} - \text{acetate} - 4\text{H}$	Other important ions
Fraction 1										
4,4-Dimethyl-5 α -cholest-7-en-3 β -yl acetate*	1.83	456	441	396	381	343	—	203	241	301
Cycloartanyl acetate	2.21	470 (11)	455 (14)	410 (100)	345 (91)	357 (11)	—	297 (80)	—	288 (30)
Fraction 2										
Lanosteryl acetate	2.13	468 (71)	453 (100)	—	393 (61)	—	—	297 (10)	—	271 (8), 69
Cycloartanyl acetate	2.41	468 (76)	453 (71)	408 (100)	393 (86)	357 (8)	—	297 (19)	—	286 (55), 69
Fraction 3										
4,4-Dimethyl-5 α -cholest-8,24-dien-3 β -yl acetate	2.18	454 (100)	439 (25)	394 (8)	379 (11)	—	341 (14)	283 (4)	241 (7)	301 (4), 266, 69
4,4-Dimethyl-5 α -cholest-7,24-dien-3 β -yl acetate	2.83	454 (58)	439 (25)	394 (8)	379 (11)	343 (4)	341 (100)	283 (5)	241 (10)	301 (8), 122, 69
Fraction 4										
4 α -Methyl-5 α -cholest-7-en-3 β -yl acetate	—	442 (75)	427 (10)	382 (100)	367 (30)	329 (4)	—	269 (90)	227 (40)	
31-Norcycloartanyl acetate	1.83	456 (14)	441 (6)	396 (100)	381 (54)	343 (9)	—	283 (20)	—	288 (26)
Fraction 5										
4 α -Methyl-5 α -cholest-7,24-dien-3 β -yl acetate	1.94	440 (47)	425 (27)	380 (46)	365 (29)	329 (3)	327 (100)	269 (21)	227 (29)	267 (72), 108, 69

* The mass spectrum of this compound was obtained by g.l.c.-m.s., the other mass spectra were determined on samples obtained by preparative g.l.c.

bond in the acetate of compound XVIII. In the n.m.r. spectra of the two steryl acetates the signals for the C-18 methyl group protons were consistent with the identification of compound XVIII as the $\Delta^{8(9)}$ compound (0.62 p.p.m.) and compound XIX as the Δ^7 isomer (0.54 p.p.m.), and eliminated the possibility that either compound contained a $\Delta^{8(14)}$ bond (Scallen & Krueger, 1968; Barton *et al.*, 1970; Scallen *et al.*, 1971). The chemical shifts for the C-26 and C-27 methyl protons at 1.61 and 1.69 p.p.m. confirmed the Δ^{24} bond in both compounds XVIII and XIX, and the olefinic proton region (5.10 p.p.m.) integrated for one proton at C-24 in compound XVIII and for two protons at C-7 and C-24 in compound XIX, thus confirming the identifications.

The 4-monomethyl sterols were acetylated and found to separate as two bands (fraction 4, R_F 0.37 and fraction 5, R_F 0.31) on t.l.c. on AgNO_3 -impregnated Kieselgel G. Both these fractions gave single peaks when examined by g.l.c. However g.l.c.-m.s. of fraction 4 revealed two components and the leading edge of the g.l.c. peak gave a mass spectrum (Table 5) identical with that reported for the acetate of 4 α -methyl-5 α -cholest-7-en-3 β -ol (XX) (Galli & Maroni, 1967). The major component of fraction 4 had a mass spectrum identifying it as the acetate of 31-norcycoloartanol (XXI) (Berti *et al.*, 1967; Ayengar & Rangswami, 1967) with, in particular, ions at m/e 343 for loss of a saturated side chain and m/e 288 for the loss of ring A by the typical cleavage of 9 β ,19-cyclopropane sterols. A small peak at 5.16 p.p.m. in the n.m.r. spectrum of fraction 5 was assigned to the C-7 proton of compound XX. To identify these compounds further a portion of fraction 4 was treated with OsO_4 to hydroxylate the Δ^7 bond of compound XX (Rahman *et al.*, 1970). The products were reduced and then separated into two major bands by t.l.c. The least-polar material had the same retention time on g.l.c. (1% SE-30 and 3% OV-17) as authentic 31-norcycoloartanol. In the n.m.r. spectrum a pair of doublets at 0.18 and 0.44 p.p.m. for the 9 β ,19-cyclopropane ring protons and all other resonances were as required for 31-norcycoloartanol. Also the m.p. values, mass spectra and response to the Liebermann-Burchard test of the free sterol and the acetate agreed with those of the authentic compounds. The more-polar material from the OsO_4 reaction was identified as 4 α -methyl-5 α -cholestane-3 β ,7 α ,8 α -triol, the expected product from compound XX, by its m.p. and mass spectrum.

Fraction 5 was shown by g.l.c.-m.s. to contain only one component (Table 5), which was characterized as the acetate of 4 α -methyl-5 α -cholesta-7,24-dien-3 β -ol (XXII). The major fragmentation ion above m/e 220 in the spectrum of both the acetate and the free sterol (M^+ at m/e 398) was derived by loss of the unsaturated side chain accompanied by two hydrogen atoms (m/e 327 and m/e 285 respectively) (Wyllie &

Djerassi, 1968). The presence of a Δ^{24} bond was revealed by the peak at m/e 69 (Galli & Maroni, 1967). The position of the ring double bond was located by the prominent ions at m/e 227 and m/e 108 (Galli & Maroni, 1967) and by the singlet in the n.m.r. spectrum at 0.54 p.p.m. for the C-18 methyl protons (Scallen & Krueger, 1968). The n.m.r. spectrum also confirmed the Δ^{24} bond with signals at 1.60 and 1.68 p.p.m. for the C-26 and C-27 methyl protons (Scallen & Krueger, 1968) and the olefinic multiplet at 5.16 p.p.m. integrated for two protons as expected for a $\Delta^{7,24}$ compound. Also the free sterol (XXII) crystallized as needles, m.p. 137–139°C, in agreement with the literature m.p. of 139°C (Charles *et al.*, 1968).

To confirm the 4 α -configuration of the C-4 methyl group of the 31-norcycoloartanol (XXI) and 4 α -methyl-5 α -cholesta-7,24-dien-3 β -ol (XXII), portions of the sterols were oxidized to the corresponding 3-ketones. The o.r.d. curves of both the 31-norcycoloartan-3-one and the 4 α -methyl-5 α -cholesta-7,24-dien-3-one gave positive Cotton effects with no hemiketalization after addition of HCl, as expected for 4-methyl steroids (Djerassi *et al.*, 1959; Djerassi, 1960; Sanghvi *et al.*, 1967; Pyrek, 1969). Further, little change was observed in the specific amplitudes ($[\alpha]_{303}$, $[\alpha]_{268}$) of either ketone after acid treatment, which would be expected to epimerize a 4 β -methyl group, if present, to the more stable 4 α -methyl confirmation (Mazur & Sondheimer, 1958; Sanghvi *et al.*, 1967; Djerassi *et al.*, 1959; Pyrek, 1969) with a resultant large increase in specific amplitude.

Discussion

In all, 22 sterols have been identified in the sterol mixture obtained from *A. rubens*. These can be broadly regarded as falling into two groups, the 4-demethyl sterols (compounds I–XIII), which constitute the major proportion of the mixture, and the C-4 methyl sterols (compounds XIV–XXII), which are present in only small amounts and are usually regarded as 4-demethyl sterol precursors (Schroepfer *et al.*, 1972; Goad, 1970). The wide range of Δ^7 C₂₆, C₂₇, C₂₈ and C₂₉ 4-demethyl sterols now encountered in *A. rubens* confirms and expands the earlier findings with other starfish (Bergmann, 1962 and references therein; Fagerlund & Idler, 1959; Gupta & Scheuer, 1968) and the recent identifications based upon g.l.c. and mass spectrometry (Matsumo *et al.*, 1972; Kobayashi *et al.*, 1972; Grossert *et al.*, 1973; Voogt, 1973). The structure originally proposed for stellatenol [(24*S*)-24-methyl-5 α -(20*S*)-cholesta-7,22-dien-3 β -ol, Bergmann & Stansbury, 1944; Shoppee, 1964] now seems doubtful and the assignment of the (20*S*) configuration was possibly a consequence of the analysis of a sterol mixture that was inseparable with

the techniques available. However, it is notable that the C-24 methyl group was given the (24*S*) configuration (Bergmann & Stansbury, 1944), which is in accord with our present conclusions based upon the n.m.r. data for the C₂₈ sterol (VII) isolated from *A. rubens*.

The identification of small amounts of the biosynthetically related 4,4-dimethyl- and 4 α -methyl sterols (XV, XVI, XVIII, XIX, XX, XXII) together with a small amount of 5 α -cholesta-7,24-dien-3 β -ol (VI) suggests that biosynthesis of 5 α -cholest-7-en-3 β -ol (V) *de novo* occurs in *A. rubens*. Injected [2-¹⁴C]mevalonic acid is incorporated into a number of these compounds (Smith & Goad, 1971*b*; Goad *et al.*, 1972), substantiating this view. The isolation of three 9 β ,19-cyclopropane sterols (XIV, XVII, XXI) from *A. rubens* is interesting, and some evidence for the occurrence of cycloartenol (XVII) in two other echinoderms (holothuroids) has been reported previously (Nomura *et al.*, 1969). Cycloartenol (XVII) is a 4-demethyl sterol precursor in higher plants and algae (Goad & Goodwin, 1972 and references therein) and its biosynthesis by animals has never been observed. It therefore seems likely that the 9 β ,19-cyclopropane compounds (XIV, XVII, XXI) of *A. rubens* are of dietary origin. However, the possibility exists that *A. rubens* can convert dietary cycloartenol (XVII) via cycloartanol (XIV) into 31-norcycloartanol (XXI), which then accumulates owing to the inability of the starfish to open the 9 β ,19-cyclopropane ring; it is notable that rat liver homogenates also fail to carry out this latter reaction (Gibbons *et al.*, 1971).

The various 4-demethyl sterols of *A. rubens* are probably derived in two ways. A part of the 5 α -cholest-7-en-3 β -ol (V) may be produced by synthesis *de novo* (Smith & Goad, 1971*b*), the remainder being obtained by metabolism of dietary cholest-5-en-3 β -ol (III) (Fagerlund & Idler, 1960; Smith & Goad, 1971*a*; Goad *et al.*, 1972). This latter route could explain the low amount of cholest-5-en-3 β -ol (III) present in *A. rubens*, and the identification of 5 α -cholestan-3 β -ol (II) may be particularly significant, since this compound is produced by reduction of cholest-5-en-3 β -ol in *A. rubens* (Smith *et al.*, 1972) and is then desaturated to give 5 α -cholest-7-en-3 β -ol (Smith & Goad, 1971*a*; Goad *et al.*, 1972). As Δ^5 sterols generally predominate in the marine environment (Bergmann, 1962) the conversion of various dietary Δ^5 C₂₆, C₂₇, C₂₈ and C₂₉ sterols into their Δ^7 counterparts can explain the presence of the latter compounds in *A. rubens* and other starfish. The relative proportions of the 4-demethyl sterols will then reflect the preferred diet of the starfish. Thus C₂₇ sterols may predominate in *A. rubens*, since this species is a carnivore feeding primarily on molluscs (Feder & Christensen, 1966) in which cholesterol is one of the principal sterol components (Bergmann, 1962; Idler & Wiseman, 1971, 1972).

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