Marine Steroids. Part I. Structures of the Principal Aglycones from the Saponins of the Starfish, *Marthasterias glacialis* [1]

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The principal aglycones obtained by hydrolysis of the saponins of *M. glacialis* are identified as 3β,6α-dihydroxy-5α-cholesta-9(11),24-dien-23-one and 3β,6α-dihydroxy-5α-cholest-9(11)-en-23-one. These are the first 23-oxocholestanes to be isolated from natural sources. On catalytic hydrogenation they yield 3β,6α-dihydroxy-5α-cholestan-23-one, which has been synthesised from stigmasterol.

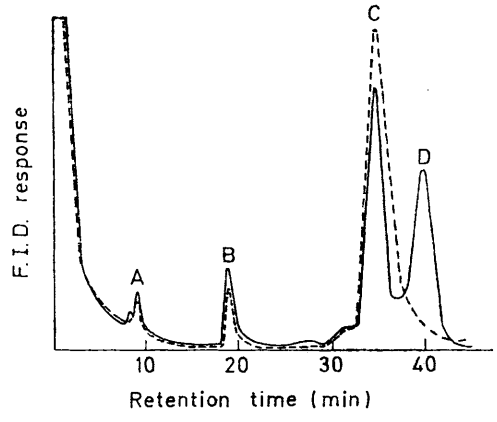
ESCAPE responses of marine invertebrates such as molluscs and sea anemones towards predatory starfish were first documented late in the last century,[2] but the chemical nature of this phenomenon has only recently become clear with the identification of surface-active steroid glycosides as the active principles.[3] Previous work [4] on the saponins of *Marthasterias glacialis* revealed that the conjugating systems consisted of glucose, quinovose, fucose, and sulphate residues, and established that one of the major aglycones was a cholestane derivative ($C_{27}H_{42}O_3$) containing a Δ^{24}-23-ketone system in the side-chain, together with two secondary hydroxy-groups and a trisubstituted olefinic bond in the steroid nucleus. Signals due to the five methyl groups of this aglycone were clearly resolved in the n.m.r. spectrum of its diacetate (marthasterone diacetate). In this paper we describe the elucidation of the nuclear structure of this aglycone and the characterisation of other components of the aglycone mixture.

The Principal Aglycones.—Crude *M. glacialis* saponins were hydrolysed with hydrochloric acid and the water-insoluble aglycone mixture was purified by chromatography on silica gel. Although apparently homogeneous (t.l.c. in several solvent systems), the aglycone mixture was resolved by g.l.c. into four main components (ratio *ca.* 10 : 6 : 2 : 1, showing slight variation amongst different batches of animals). The two major components [Figure (a); C and D] were then separated by t.l.c. of their diacetates (continuous development). The more polar of these, marthasterone diacetate (1), contained the Δ^{24}-23-ketone system previously characterised ($\lambda_{max.}$ 240 nm, $\nu_{max.}$ 1690 cm^{-1}); the other exhibited no selective u.v. absorption and its carbonyl stretching band appeared at 1715 cm^{-1}. This, together with its

[1] A. B. Turner, D. S. H. Smith, and A. M. Mackie, *Nature*, 1971, **233**, 209.
[2] (*a*) H. M. Feder, *Scientific American*, 1972, **227**, 93; (*b*) J. S. Gressert, *Chem. Soc. Rev.*, 1972, **1**, 1.
[3] A. M. Mackie, R. Lasker, and P. T. Grant, *Comp. Biochem. Physiol.*, 1968, **26**, 415.
[4] A. M. Mackie and A. B. Turner, *Biochem. J.*, 1970, **117**, 543.

n.m.r. spectrum, which lacked the olefinic proton signal at τ 3·94 and the olefinic methyl signals at τ 7·85 and 8·11, suggested that it was the 24,25-dihydro-derivative (3) of marthasterone diacetate. The relationship was confirmed by selective hydrogenation (Pd–C; 1 atm; 1 h) of the side-chain double bond of marthasterone (2) to give dihydromarthasterone (4). When this was carried out with the crude aglycone mixture, the product contained *ca.* 80% dihydromarthasterone [g.l.c. analysis; Figure (b)]. Dihydromarthasterone (4) was crystallised from this product (m.p. 167—169°), and further structural work was carried out on this material, which was the most abundant constituent of the original aglycone mixture.

A significant feature of the mass spectrum of dihydromarthasterone ($C_{22}H_{44}O_3$) was the McLafferty-style cleavage of the side-chain at C(20)–C(22), involving loss



G.l.c. separation of *M. glacialis* aglycones: (a) —— crude aglycone mixture; (b) · · · · mixture after hydrogenation (Pd–C; 1 atm; 1 h)

of a C_6 fragment containing the ketone function. This leads to the prominent ion at *m/e* 316 ($M - 100$), and charge localisation in the side-chain does not occur to the same extent as in marthasterone diacetate.[4]

The nuclear double bond of dihydromarthasterone was not attacked under the foregoing hydrogenation conditions, but was reduced after prolonged catalytic hydrogenation (150 h), as shown by the disappearance of the olefinic proton signal at τ 4·68. In the resulting tetrahydromarthasterone (5), the C-19 angular methyl signal showed a large upfield shift (15 Hz), while the angular 13-methyl signal had moved downfield (4 Hz). Zurcher's rules [5] indicated that this was in accordance only with the presence of a $\Delta^{9(11)}$-bond in dihydromarthasterone. Hydrogenation of other nuclear trisubstituted double bonds produced markedly different effects upon the resonance frequencies of the angular methyl groups, and in fact the $\Delta^{9(11)}$-bond was the only one which on reduction caused opposing changes in the positions of these signals (Table 1). Hydrogenation of the side-chain double bond had no appreciable effect upon these resonance frequencies.

Epoxidation of the nuclear double bond of dihydromarthasterone, by means of monoperphthalic acid in ether, was accompanied by loss of the olefinic proton signal at τ 4·68 and the appearance of a one-proton doublet at τ 6·83 (J 6 Hz). Downfield movements of the 10- and 13-methyl signals were also noted (14 and 5 Hz, respectively). Since these were not in

TABLE 1

Effect of hydrogenation of trisubstituted double bonds upon chemical shifts * of angular methyl groups

	10-Me	13-Me
Δ^4	−25	−4
Δ^5	−23	−4
Δ^7	1	12
$\Delta^{9(11)}$	−14	7
Δ^{14}	−1	−25
Dihydromarthasterone ⟶ Tetrahydromarthasterone	−15	4
Marthasterone diacetate ⟶ Tetrahydromarthasterone diacetate	−15	4

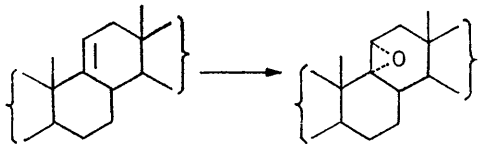
* In Hz (at 100 MHz); a positive value denotes a downfield shift.

agreement with the values tabulated by Zurcher,[5] the effect of the transformation upon the methyl resonance positions of a number of authentic $\Delta^{9(11)}$-steroids was studied. Similar changes were found to occur in the positions of the angular methyl signals following the epoxidation of these model compounds (Table 2).

TABLE 2

Effect of epoxidation of $\Delta^{9(11)}$-bonds upon chemical shifts * of angular methyl groups

	10-Me	13-Me
$\Delta^{9(11)}$-Tigogenin acetate (6)	14	6
$\Delta^{9(11)}$-Pregnanolone acetate (7)	14	7
$\Delta^{9(11)}$-Diacetate (8)	14	9
Dihydromarthasterone	14	5



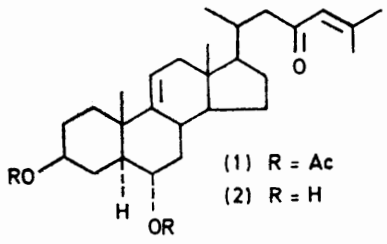
* See Table 1.

There is thus strong evidence that the nuclear double bond of marthasterone and dihydromarthasterone occupies the 9(11)-position.

The location of the two nuclear hydroxy-groups was next investigated. Oxidation of marthasterone with chromic acid in acetone gave a triketone (9) which showed u.v. absorption similar to that of marthasterone itself. Similar oxidation of dihydro- and tetrahydromarthasterone gave the triketones (10) and (11), neither of which showed intense u.v. absorption. The i.r. spectra of these triketones confirmed that only saturated keto-groups were present, thereby eliminating C-12 as a possible site for one of the original hydroxy-groups. Their n.m.r. spectra showed that the angular methyl

[5] R. F. Zurcher, *Helv. Chim. Acta*, 1961, **44**, 1380; 1963, **46**, 2054.

signals had moved to lower field (10-Me, 15 Hz; 13-Me, 5 Hz) and the C-11 olefinic proton was also deshielded to the extent of 16 Hz in the triketones (9) and (10). were consistent only with situation of the oxygen functions at the 3- and 6-positions. Further evidence for this assignment was obtained by oxidation of 3β,6α-



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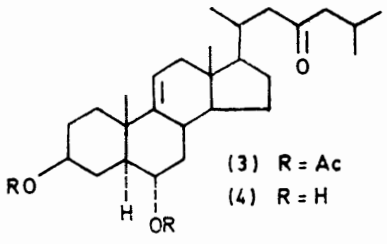
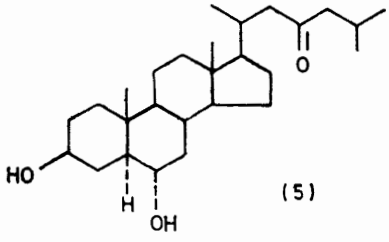
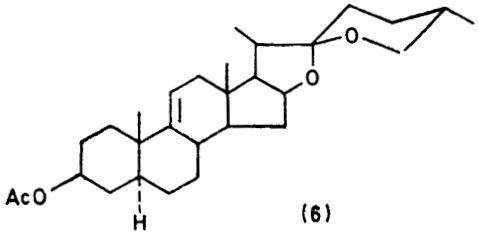
(2) R = H



(3) R = Ac
(4) R = H



(5)



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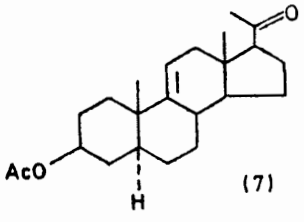

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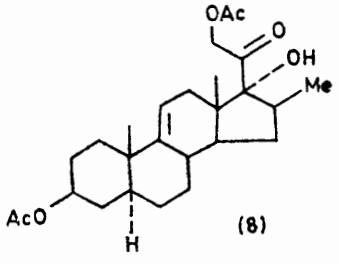

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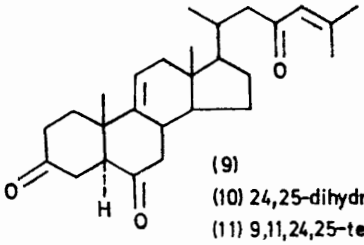
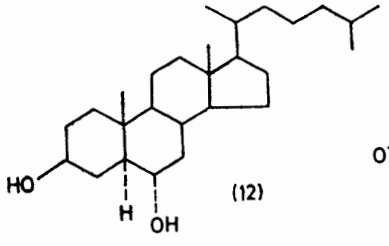


(9)
(10) 24,25-dihydro
(11) 9,11,24,25-tetrahydro



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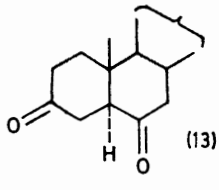

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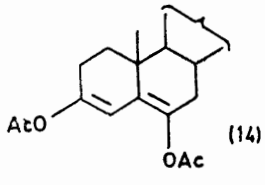

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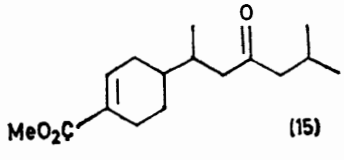


(15)

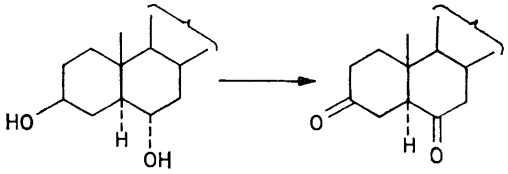
These results indicated that the nuclear oxygen functions were relatively close to the 10-methyl group, but more remote from the 13-methyl group. Reference to literature compilations [5] showed that these changes dihydroxy-5α-cholestane (12) to 5α-cholstane-3,6-dione. This was accompanied by changes in the resonance frequencies of the angular methyl groups which closely paralleled those already mentioned (Table 3).

Attempts to confirm the 3,6-relationship of the new keto-groups by dehydrogenation to the conjugated Δ^4-3,6-dione system with selenium dioxide were unsuccessful. However, treatment of the triketone (10) with isopropenyl acetate produced a dienol diacetate

TABLE 3

Effect of oxidation of 3β,6α-diols to 3,6-diones upon chemical shifts * of angular methyl groups

	10-Me	13-Me
Marthasterone	15	5
Dihydromarthasterone	14	4
Tetrahydromarthasterone	15	6
3β,6α-Dihydroxy-5α-cholestane	13	4



* See Table 1.

mixture having $\lambda_{max.}$ 244·5 nm (ε 3400). The corresponding product mixture from 5α-cholestane-3,6-dione (13) had $\lambda_{max.}$ 245 nm (ε 3400). This absorption corresponds to that of an heteroannular diene chromophore of type (14). The acetylated mixtures from the two compounds were shown by g.l.c. to consist of similar mixtures of components, resulting from the various enolisation pathways open to the ketones. The major products appear to be the $\Delta^{3,5}$- and $\Delta^{2,5}$-3,6-diol diacetates.

G.l.c. retention times for the various cholestane derivatives prepared during the structural work are collected in Table 4, together with the constant ratios

TABLE 4

G.l.c. data for oxygenated cholestanes

5α-Cholestane	t_R	$\frac{t_R[\Delta^{9(11)}\text{-23-one}]}{t_R(9,11\text{-dihydro-23-H}_2)}$	$\frac{t_R(23\text{-one})}{t_R(23\text{-H}_2)}$
3β,6α-Dihydroxy	1·79		
3β,6α-Dihydroxy-23-one	2·65	1·37	1·48
3β,6α-Dihydroxy-$\Delta^{9(11)}$-23-one	2·46		
3β,6α-Diacetoxy	2·56		
3β,6α-Diacetoxy-23-one	3·79	1·36	1·48
3β,6α-Diacetoxy-$\Delta^{9(11)}$-23-one	3·49		
-3,6-dione	1·75		
-3,6,23-trione	2·58	1·33	1·48
-$\Delta^{9(11)}$-3,6,23-trione	2·33		

obtained by comparison of 23-oxo-derivatives with the corresponding 23-unsubstituted cholestanes.

The broad nature of the C-3 and C-6 proton signals in the n.m.r. spectra of the aglycones and their diacetates ($W_{\frac{1}{2}}$ 20—25 Hz) indicates a degree of coupling consistent with axial orientation; hence the hydroxy-groups are equatorial, and occupy the 3β- and 6α-positions. The half-band widths correspond to those observed for 3β,6α-dihydroxy-5α-cholestane and its diacetate. The resonance frequencies of the angular methyl groups in this series of compounds accord with an AB-*trans* ring junction rather than AB-*cis*, and hence the structures proposed for the major aglycones present in *Marthasterias* saponins are those of 3β,6α-dihydroxy-5α-cholesta-9(11),24-dien-23-one [marthasterone (2)] and 3β,6α-dihydroxy-5α-cholest-9(11)-en-23-one [dihydromarthasterone (4)]. These conclusions have been substantiated by a synthesis of tetrahydromarthasterone (5), particularly in regard to the positions and stereochemistry of the oxygen functions.

Synthesis of 3β,6α-*Dihydroxy*-5α-*cholestan*-23-*one* [*Tetrahydromarthasterone* (5)].—Stigmasterol was chosen as the starting material for the chemical synthesis of tetrahydromarthasterone, as its Δ^{22}-bond allows convenient breakdown and reconstitution of the side-chain. Furthermore, its 5,6,22,23-tetrabromide can be partially debrominated to the 22,23-dibromide with sodium iodide [6] (probably owing to relief of steric compression between the 6β-bromine atom and the angular 10-methyl group upon regeneration of the Δ^5-bond), thereby allowing hydration of the nuclear double bond prior to cleavage of the side-chain. The 1,5-dimethyl-3-oxohexyl side-chain of tetrahydromarthasterone (and dihydromarthasterone) is the same as that of the insect juvenile hormone juvabione (15), and it was possible to utilise the method recently employed by Pawson *et al.*[7] for the synthesis of (+)-juvabione in the reconstruction of this side-chain from the bisnorcholane intermediates available from the stigmasterol route.

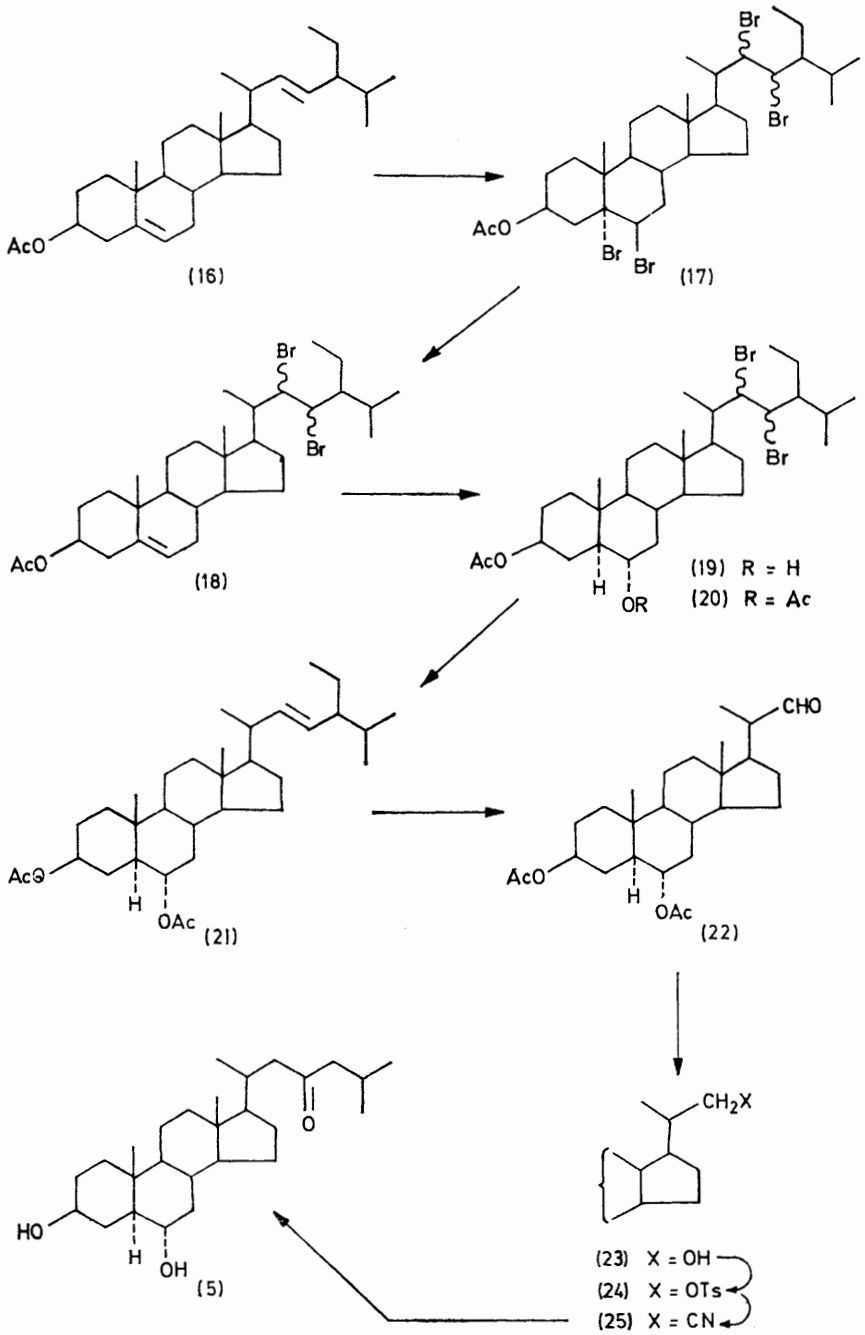
Stigmasterol acetate (16) was converted into the tetrabromide (17) in high yield by bromination in ethereal acetic acid. Partial debromination of this compound by the method of Fernholz and Stavely [6] produced stigmasteryl acetate 22,23-dibromide (18) in 74% yield. Hydroboration of the olefin (18) with diborane in bis-(2-methoxyethyl) ether, followed by oxidation with alkaline hydrogen peroxide, gave 3β-acetoxy-22,23-dibromo-24-ethyl-6α-hydroxy-5α-cholestane (19) in 41% yield after chromatography on silica gel. Acetylation to the diacetate (20), followed by debromination with zinc dust in ethanolic acetic acid, gave the Δ^{22}-compound (21), which was readily degraded by low-temperature ozonolysis to the 22-aldehyde (22). This was reduced to the 22-alcohol (23) with sodium borohydride, and the synthesis was completed using the sequence developed by Pawson *et al.*[7] for the construction of juvabione (see Scheme 1). This involved conversion into the tosylate (24), displacement of the tosyloxy-group with cyanide ion to give the nitrile (25), condensation with isobutyl-lithium, and final hydrolysis with sulphuric acid in aqueous dioxan. The resulting tetrahydromarthasterone (5) was identical with that obtained by prolonged catalytic hydrogenation of marthasterone and of dihydromarthasterone.

This synthesis provides a flexible route to 23-oxo-

[6] E. Fernholz and H. E. Stavely, *J. Amer. Chem. Soc.*, 1939, **61**, 2956.

[7] B. A. Pawson, H. C. Cheung, S. Gurbakani, and G. Saucy, *J. Amer. Chem. Soc.*, 1970, **92**, 336.

cholestanes, since the C_{22} intermediates are readily accessible. 23-Oxocholesterol has previously been prepared from a norcholane derivative,[8,9] and, more recently, 23-ketones of the 24-methyl series have been compounds has been hampered by difficulties in separation owing to their low abundance, and information as to their structures is largely derived from g.l.c.–mass spectral measurements.



SCHEME 1 Synthesis of tetrahydromarthasterone

obtained *via* selective attack of the iodine–silver acetate complex upon the Δ^{22}-bond of ergosterol derivatives.[10]

The Minor Aglycones.—Structural work on these

The most abundant of the minor components (Figure; B), comprising *ca.* 10% of the original aglycone mixture, had a retention time (t_R) of 1·39, relative to that of cholesterol. Hydrogenation of the aglycone mixture (Pd–C; 1 h; 1 atm) produced no change in the t_R of

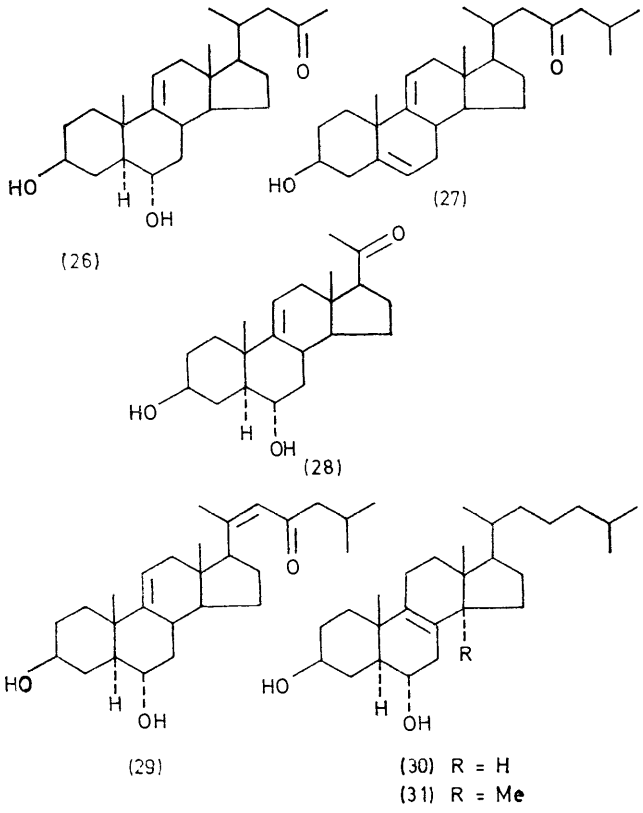
[8] P. Kurath, F. M. Ganis, and M. Radakowich, *Helv. Chim. Acta*, 1957, **40**, 933.

[9] J. E. van Lier and L. L. Smith, *J. Org. Chem.*, 1971, **36**, 1007; *J. Pharm. Sci.*, 1970, **59**, 719.

[10] D. H. R. Barton, J. P. Poyser, P. G. Sammes, M. B. Hursthouse, and S. Neidle, *Chem. Comm.*, 1971, 715.

this component, indicating that it did not contain a Δ^{24}-bond. Prolonged hydrogenation, however, caused disappearance of the compound and appearance of a product having t_R 1·50. The conversion was complete after 7 days, and the course of the hydrogenation was remarkably similar to the conversion of dihydromarthasterone into tetrahydromarthasterone, *i.e.* the reduction of a $\Delta^{9(11)}$-bond.

The mass spectrum of this component, obtained by combined g.l.c.–mass spectrometry showed prominent ions at *m/e* 374 (13%), 356 (18), 341 (36), and 316 (80), and the subsequent fragmentation pattern was similar to that of dihydromarthasterone. The retention time is intermediate between those of the pregnane (C_{21}) and cholestane (C_{27}) derivatives, and is thus in keeping with the postulated cholane (C_{24}) structure (26). However, the mass spectrum also contains weak ions at *m/e* 398 (8%), 383 (7), and 380 (7), and it appears that another compound is present. The molecular weight corresponds to a dehydrated form of dihydromarthasterone, possibly 3β-hydroxycholesta-5,9(11)-dien-23-one (27).



The remaining minor component (Figure; A) has been identified as 3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one (28) by comparison with an authentic sample synthesised from 11α-hydroxyprogesterone.[11] This pregnene, which possesses the same nuclear structure as marthasterone, is present in several other species of starfish (see later).

Discussion.—The sterols of asteroids (starfish) are characterised by the presence of a Δ^7-bond,[12] and in this respect resemble the sterols of the holothurians (sea-cucumbers). On the other hand, the other classes of echinoderms (ophiuroids, crinoids, and echinoids) contain Δ^5-derivatives.

The presence of toxic saponins in both asteroids and holothurians has been long recognised, but only holothuroid saponin aglycones have so far been characterised (as lanostane derivatives).[13] Marthasterone and dihydromarthasterone are the first asteroid saponin aglycones to be characterised.[1] Several papers have now appeared identifying 3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one (28) as a principal aglycone of the saponins of the starfish *Acanthaster planci*[14,15] and *Asterias amurensis*.[16] Dihydromarthasterone and a related 23-alcohol are also present in *A. amurensis*,[17] and a double-bond isomer of marthasterone occurs in *A. planci*.[14] This is 3β,6α-dihydroxy-5α-cholesta-9(11),20(22)-dien-23-one (29). Minor aglycones possessing the same nuclear structure, but lacking the 23-oxo-function, have also been reported in *A. planci*.[18]

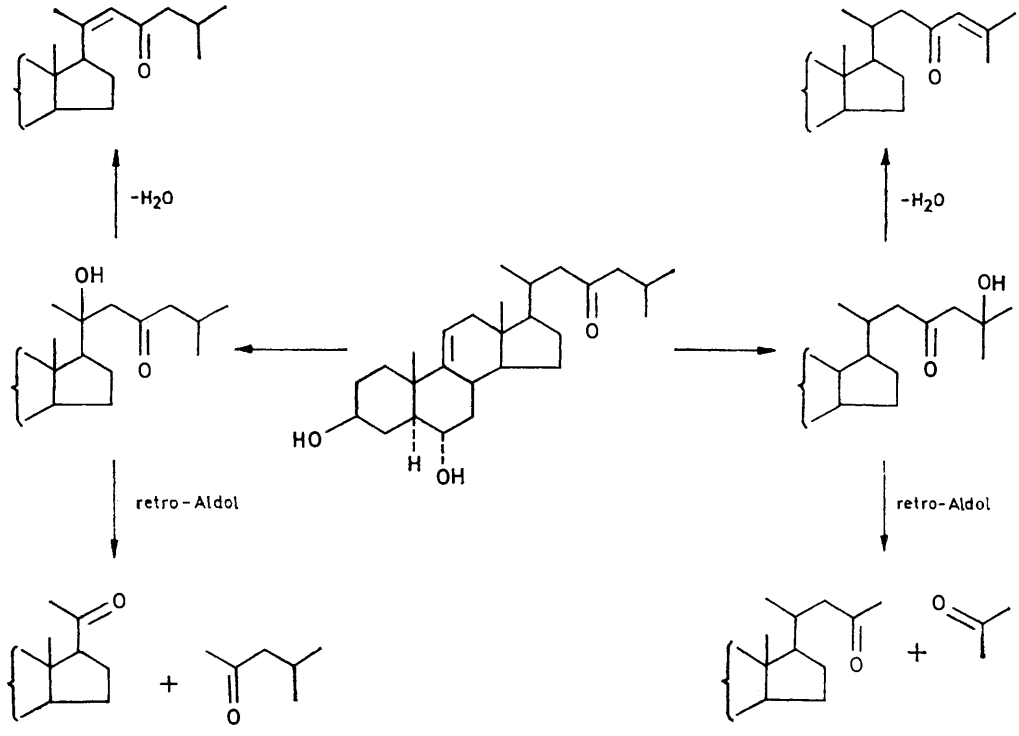
There is clearly a close correlation between the asteroids *Acanthaster planci*, *Asterias amurensis* and *A. rubens*, and *Marthasterias glacialis*, with respect to their saponin content. 3β,6α-Dihydroxy-5α-pregn-9(11)-en-20-one (28) is common to all four, although only a minor aglycone of *M. glacialis*. Marthasterone or dihydromarthasterone constitutes the main aglycone from *M. glacialis* and *A. rubens*, and is a major component of *A. amurensis*, and a side-chain isomer of marthasterone occurs in *A. planci*. Thus the $\Delta^{9(11)}$-bond occurs in every starfish saponin so far characterised, as is also true of the sea-cucumbers,[19] and the $\Delta^{9(11)}$-bond in ring C may be characteristic of asteroid and holothuroid saponins in the same way as the Δ^7-bond in ring B characterises the free sterol content of these two classes of echinoderms.[20]

The 23-oxo-groups in these cholestanes are unique; oxygenation at C-23 has only previously been encountered in nature in the case of 23-hydroxylanosterol. This was isolated from the common fungus, *Scleroderma aurantium*.[21] The nuclear hydroxylation pattern occurs

[11] D. S. H. Smith and A. B. Turner, *Tetrahedron Letters*, 1972, 5263.
[12] W. Bergmann in 'Comparative Biochemistry,' eds. M. Florkin and H. S. Mason, Academic Press, New York, 1962, vol. 3, p. 103.
[13] P. Roller, B. Tursch, and C. Djerassi, *J. Org. Chem.*, 1970, **35**, 2585.
[14] Y. M. Sheikh, B. M. Tursch, and C. Djerassi, *J. Amer. Chem. Soc.*, 1972, **94**, 3278.
[15] Y. Shimizu, *J. Amer. Chem. Soc.*, 1972, **94**, 4051.
[16] S. Ikegami, Y. Kamiya, and S. Tamura, *Tetrahedron Letters*, 1972, 1601; *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 1777.
[17] S. Ikegami, Y. Kamiya, and S. Tamura, *Tetrahedron Letters*, 1972, 3725.
[18] Y. M. Sheikh, B. Tursch, and C. Djerassi, *Tetrahedron Letters*, 1972, 3721.
[19] J. O. Chanley, T. Mezzetti, and H. Sobotka, *Tetrahedron* 1966, **22**, 1857; *cf.* ref. 2*b*.
[20] W. Bergmann, ref. 12, p. 144.
[21] N. Entwhistle and A. D. Pratt, *Tetrahedron*, 1968, **24**, 3949; *cf.* ref. 17.

in the cactus sterols, peniocerol (30),[22] and macdougallin (31).[23]

Recent work [24] has shown that echinoderm sterols are partly dietary in origin and partly biosynthesised by the animals. It has been suggested [14] that the 23-oxo-function could arise biogenetically from a 22,23-olefinic linkage, so prevalent in marine sterols,[25] by rearrangement of an epoxide intermediate. Subsequent hydroxylation at C-20 or C-25, followed by dehydration, would then afford the $\Delta^{20(22)}$- and Δ^{24}-23-ketones (Scheme 2). It may be significant that the postulated 20- and 25-hydroxy-23-oxocholestanes are also possible precursors of the C_{21} and C_{24} compounds, as they can both undergo retro-aldol cleavage with loss of a C_6 or a C_3 ketonic fragment, respectively. The balance between the degraded aglycones and the 23-oxocholestane derivatives would then be determined by the interplay in the starfish tissues of enzymes capable of catalysing the dehydrations and the retro-aldol reactions.



SCHEME 2 Possible biogenetic interrelationships

EXPERIMENTAL

T.l.c. was carried out on silica gel (Merck GF 254) with location by u.v. illumination or spraying with methanolic sulphuric acid (1 : 1 v/v). G.l.c. was conducted on 2 m × 1·5 mm (i.d.) columns packed with 2·5% silicone gum rubber E-301 on AW-DMCS Chromosorb G(80—100 mesh) at 250° (unless otherwise stated) with a nitrogen flow rate of 17 ml min^{-1} on a Perkin-Elmer F-11 instrument. Quoted retention times are relative to cholesterol (t_R 1·0). I.r. spectra were obtained on a Perkin-Elmer 237 grating spectrophotometer. U.v. spectra were measured for solutions in ethanol on a Perkin-Elmer 137 UV 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.

Hydrolysis of the Saponins.—A solution of crude saponin [4] (3·75 g) from *M. glacialis* in 2N-hydrochloric acid (350 ml) was heated at 100° for 6 h. The brown precipitate was collected and purified by t.l.c. [chloroform–ethyl acetate (1 : 1) as irrigant] to give the aglycone mixture (230 mg).

Diacetates of Marthasterone and Dihydromarthasterone.—The aglycone mixture (75 mg) was acetylated in pyridine (1 ml) and acetic anhydride (1 ml) at 100° for 1 h. After hydrolysis of the excess of anhydride and isolation with ether the crude diacetates were separated by t.l.c. [benzene–ether (24 : 1 v/v) as irrigant (3 developments)] and isolated as oils: 3β,6α-*diacetoxy*-5α-*cholesta*-9(11),24-*dien*-23-*one* (1) (21 mg) (Found: M^+, 498·3344. $C_{31}H_{46}O_5$ requires M, 498·3345), $\lambda_{max.}$ 237 nm (ε 11,200), $\nu_{max.}$ (film) 1740, 1695, 1610, and 1250 cm^{-1}, τ 9·36 (s, 13-Me), 9·08 (d, J 6 Hz, 20-Me), 8·97 (s, 10-Me), 8·11 [s, C(26)H_3], 7·96 (s, 3β- and 6α-OAc), 7·85 [s, C(27)H_3], 5·25 (m, 3α- and 6β-H), 4·67 (d, J 4 Hz, 11-H), and 3·94 (s, 24-H), t_R 3·92, *m/e* 498 (4%), 340 (32), 280 (35), 125 (40), 98 (100), and 83 (97); 3β,6α-*diacetoxy*-5α-*cholest*-9(11)-*en*-23-*one* (3) (23 mg) (Found: C, 74·1; H, 9·4. $C_{31}H_{48}O_5$ requires C, 74·4; H, 9·6%), $\nu_{max.}$ (film) 1740, 1715, and 1250 cm^{-1}, τ 9·35 (s, 13-Me),

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9·08 (d, J 6 Hz, 20-Me and 25-Me_2), 8·96 (s, 10-Me), 7·96 (s, 3β- and 6α-OAc), 5·25 (m, 3α- and 6β-H), and 4·66 (d, J 4 Hz, 11-H), t_R 3·39.

Dihydromarthasterone (4).—The aglycone mixture (50 mg) was hydrogenated for 1 h in redistilled propan-2-ol (5 ml) over 10% Pd–C at 1 atm. Filtration and evaporation gave an oil (48 mg) which was crystallised twice from acetone–hexane to give 3β,6α-*dihydroxy*-5α-*cholest*-9(11)-*en*-23-*one*, m.p. 167—169° (Found: C, 78·0; H, 10·7. $C_{27}H_{44}O_3$ requires C, 77·9; H, 10·6), $\nu_{max.}$ 3320 and 1715 cm^{-1}, τ 9·35 (s, 13-Me), 9·07 (d, J 7 Hz, 20-Me and 25-Me_2), 9·04 (s, 10-Me), 6·38 (m, 3α- and 6β-H), and 4·68 (d, J 4 Hz, 11-H), t_R 2·46, *m/e* 416 (5%), 316 (75), 301 (25), 298 (28), 265 (20), 108 (90), 95 (100), 85 (75), and 57 (90).

Tetrahydromarthasterone (5).—A solution of the aglycone mixture (144 mg) in propan-2-ol (20 ml) was hydrogenated over 10% Pd–C at 1 atm for 150 h. Filtration and evaporation left a solid (142 mg) which, after two recrystallisations from acetone–hexane, gave 3β,6α-*dihydroxy*-5α-*cholestan*-23-*one* (85 mg), m.p. 185—187° (Found: C, 77·7; H, 10·7%; M^+, 418·3450. $C_{27}H_{46}O_3$ requires C, 77·5; H, 11·0%; M, 418·3447), $\nu_{max.}$ 3300 and 1712 cm^{-1}, τ 9·31 (s, 13-Me), 9·19 (s, 10-Me), 9·10 (d, J 6 Hz, 20-Me and 25-Me_2), and 6·48 (m, 3α- and 6β-H), t_R 2·65, *m/e* 418 (4%), 400 (10), 361 (10), 318 (100), 303 (33), 300 (25), 285 (30), 127 (33), 122 (40), 95 (51), 85 (51), and 57 (50).

9α,11α-*Epoxy*-3β,6α-*dihydroxy*-5α-*cholestan*-23-*one*.— A solution of dihydromarthasterone (25 mg) in chloroform (2·5 ml) and diethyl ether (5 ml) containing monoperphthalic acid (50 mg) was left for 24 h at room temperature. The solution was extracted with aqueous sodium hydrogen carbonate, washed with water, dried ($MgSO_4$), and evaporated *in vacuo*. The residual oil (26 mg) was purified by t.l.c. (chloroform–ethyl acetate, 1 : 1) to give the 9α,11α-*epoxide* (Found: M^+, 432·3250. $C_{27}H_{44}O_4$ requires M, 432·3240), $\nu_{max.}$ (KBr) 3300 and 1715 cm^{-1}, τ 9·30 (s, 13-Me), 8·92 (s, 10-Me), 9·08 (d, 20-Me and 25-Me_2), 6·85 (d, J 5 Hz, 11β-H), and 6·50 (m, 3α- and 6β-H), t_R 3·38, *m/e* 432 (75%), 284 (25), and 18 (100).

3β,6α-*Diacetoxy*-9α,11α-*epoxy*-5α-*cholestan*-23-*one*.—To a mixture of marthasterone and dihydromarthasterone diacetate (50 mg), isolated by p.l.c. (benzene–ether, 4 : 1) from the acetylated aglycone mixture (see before), was added a solution of monoperphthalic acid (60 mg) in diethyl ether (5 ml). After 18 h at room temperature the ether was evaporated off and the residue (46 mg) was separated by p.l.c. (four developments in benzene–ether, 98 : 2) into 3β,6α-diacetoxy-9α,11α-epoxy-5α-cholest-24-en-23-one (18 mg), $\nu_{max.}$ (film) 1740, 1695, 1625, and 1250 cm^{-1}, τ 9·30 (s, 13-Me), 9·09 (d, J 6 Hz, 20-Me), 8·82 (s, 10-Me), 8·10 [s, C(26)H_3], 7·97 and 7·94 (each s, 3β- and 6α-OAc), 7·85 [s, C(27)H_3], 6·83 (d, J 6 Hz, 11β-H), 5·25 (m, 3α- and 6β-H), and 3·96 (s, 24-H), t_R (260°) 4·77; and 3β,6α-*diacetoxy*-9α,11α-*epoxy*-5α-*cholestan*-23-*one* (20 mg), m.p. 173—175° [Found: C, 72·3; H, 9·3. $C_{31}H_{48}O_6$ requires C, 72·1; H, 9·3%. (M^+ — HOAc), 456·3231. $C_{29}H_{44}O_4$ requires 456·3239. ($M^+ - 2 \times$ HOAc — CH_3), 381·2780. $C_{26}H_{37}O_2$ requires 381·2793], $\nu_{max.}$ (Nujol) 1740, 1715, and 1250 cm^{-1}, τ 9·30 (s, 13-Me), 9·08 (d, J 6 Hz, 20-Me and 25-Me_2), 8·83 (s, 10-Me), 7·97 and 7·94 (each s, 3β- and 6α-OAc), 6·83 (d, J 6 Hz, 11β-H), and 5·25 (m, 3α- and 6β-H), t_R (260°) 4·17, *m/e* 516 (1%), 456 (33), 396 (14), 381 (22), 363 (9), 356 (9), 297 (14), 296 (11), 145 (33), 85 (75), 57 (100), and 43 (80).

3β-*Acetoxy*-9α,11α-*epoxy*-5α-*spirostan*.— $\Delta^{9(11)}$-Tigogenin acetate (100 mg) was epoxidised by the foregoing method. The crude product was crystallised from chloroform–methanol to give the 9α,11α-epoxide (82 mg) as needles, m.p. 259—260° (lit.,[26] 264—265°), τ 9·23 (s, 13-Me), 8·89 (s, 10-Me), and 6·89 (d, J 5 Hz, 11β-H).

3β,21-*Diacetoxy*-9α,11α-*epoxy*-17α-*hydroxy*-16β-*methylpregnan*-20-*one*.— 3β,21-Diacetoxy-17α-hydroxy-16β-methylpregn-9(11)-en-20-one (40 mg) was epoxidised as before. The 9α,11α-*epoxide* (33 mg) crystallised from methylene dichloride–hexane as needles, m.p. 211—214° (Found: C, 67·4; H, 8·1. $C_{26}H_{38}O_7$ requires C, 67·5; H, 8·2%), $\nu_{max.}$ (KBr) 3470, 1760, 1735, 1715, 1280, and 1230 cm^{-1}, τ 9·18 (s, 13-Me), 8·90 (s, 10-Me), and 6·80 (d, J 5 Hz, 11β-H).

3β-*Acetoxy*-9α,11α-*epoxypregnan*-20-*one*.— 3β-Acetoxypregn-9(11)-en-20-one (30 mg) was epoxidised as before. After crystallisation from dichloromethane–hexane, the epoxide (22 mg) had m.p. 159—160° (lit.,[27] 149—152°), τ 9·38 (s, 13-Me), 8·90 (s, 10-Me), 7·98 (s, 3β-OAc), 7·89 (s, 20-Me), 6·82 (d, J 4 Hz, 11β-H), and 5·36 (m, 3α-H).

5α-*Cholest*-9(11)-*ene*-3,6,23-*trione* (10).—A stirred solution of the aglycone mixture (30 mg) in acetone (25 ml; distilled from $KMnO_4$) was treated dropwise with chromic acid.[28] After 15 min at room temperature, the solution was diluted with water (50 ml), the acetone was removed *in vacuo*, and the resulting suspension was extracted with ethyl acetate. The organic layer was washed with water, dried ($MgSO_4$), and evaporated. The residual oil (30 mg) was resolved by multiple-development p.l.c. (benzene–ether, 94 : 6) into 5α-cholesta-9(11),24-diene-3,6,23-trione (11 mg), $\lambda_{max.}$ 239 nm (ε 10,100), $\nu_{max.}$ (KBr) 1717, 1680, and 1610 cm^{-1}, τ 9·31 (s, 13-Me), 9·05 (d, J 6 Hz, 20-Me), 8·90 (s, 10-Me), 8·12 [s, C(26)H_3], 7·86 [s, C(27)H_3], 4·45 (m, 11-H), and 3·96 (s, 24-H), t_R 2·51; and 5α-*cholest*-9(11)-*ene*-3,6,23-*trione* (10 mg), m.p. 140—141° (from EtOH) (Found: M^+, 412·2959. $C_{27}H_{90}O_3$ requires M, 412·2982), $\nu_{max.}$ (KBr) 1720 cm^{-1}, τ 9·31 (s, 13-Me), 9·08 (m, 20-Me and 25-Me_2), 8·90 (s, 10-Me), and 4·46 (m, 11-H), t_R 2·23, *m/e* 412 (M^+, 11%), 397 (5), 394 (5), 356 (20), 355 (75), 314 (26), 313 (100), 312 (97), 297 (31), 127 (33), 108 (33), 105 (23), 85 (40), and 57 (61).

5α-*Cholestane*-3,6,23-*trione* (11).—Tetrahydromarthasterone (10 mg) was oxidised as before, giving the *trione* (9 mg), m.p. 196—197° (from EtOAc) [Found: M^+, 414·3128. $C_{27}H_{42}O_3$ requires M, 414·3138. (M^+ — $C_6H_{12}O$), 314·2247. $C_{21}H_{30}O_2$ requires 314·2246], τ 9·25 (s, 13-Me), 9·07 (d, J 6 Hz, 20-Me and 25-Me_2), and 9·04 (s, 10-Me), t_R 2·58, *m/e* 414 (M^+, 17%), 316 (16), 315 (82), 314 (100), 300 (10), 299 (46), 285 (14), 245 (10), 176 (10), 127 (36), 85 (64), and 57 (64).

Enol Acetylation of 5α-*Cholest*-9(11)-*ene*-3,6,23-*trione*.—A mixture of the trione (10) (14·5 mg), isopropenyl acetate (1·5 ml), and toluene-*p*-sulphonic acid monohydrate (5 mg) was heated under reflux for 24 h. The solvent was removed *in vacuo*; a solution of the residue in ethyl acetate was washed with aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated. The residual brown oil (24 mg) was dissolved in benzene and decolourised by percolation

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[27] C. Djerassi, H. Martinez, and G. Rosenkranz, *J. Org. Chem.*, 1951, **16**, 1278.

[28] P. Bladon, J. M. Fabian, H. B. Henbest, H. P. Koch, and G. W. Wood, *J. Chem. Soc.*, 1951, 2402.

through a short alumina column. The resulting diacetate mixture had $\lambda_{max.}$ 244·5 nm (ε 3400).

5α-Cholestane-3,6-dione.—A solution of 3β,6α-dihydroxy-5α-cholestane [29] was oxidised with chromic acid as already described. Crystallisation of the crude product from dichloromethane–hexane gave the 3,6-dione as needles, m.p. 172—173° (lit.,[30] 172°), τ 9·30 (s, 13-Me), 9·13 (d, *J* 6 Hz, 20-Me and 25-Me_2), and 9·03 (s, 10-Me), t_R 1·75.

Enol Acetylation of 5α-Cholestane-3,6-dione.—A mixture of the 3,6-dione (13) (300 mg), isopropenyl acetate (20 ml), and toluene-*p*-sulphonic acid monohydrate (75 mg) was heated under reflux for 24 h. The mixture was worked up as before, giving a clear oil (270 mg), $\lambda_{max.}$ 245 nm (ε 3400). The incompletely resolved product mixture had a g.l.c. profile similar to that from 5α-cholest-9(11)-ene-3,6,23-trione. Multiple-development t.l.c. (hexane–benzene, 1 : 1) showed three components, only one of which absorbed selectively at 245 nm.

3β-Acetoxy-22,23-dibromo-24-ethylcholest-5-ene (18).—Prepared from stigmasterol acetate tetrabromide [31] in 74% yield by the method of Fernholz and Stavely,[6] the dibromide (18) had m.p. 210—212° (lit.,[6] 212—213°), τ 9·24 (s, 13-Me), 9·06 (s, 10-Me), 5·50 (m, 22- and 23-H), 5·39br (m, 3-H), and 4·61 (m, 6-H).

3β-Acetoxy-22,23-dibromo-24-ethyl-5α-cholestan-6α-ol (19).—Diborane was carried on a slow stream of dry nitrogen into a stirred solution of the foregoing dibromide (10·4 g) in bis-(2-methoxyethyl) ether (200 ml) and chloroform (200 ml) for 2 h, after which time no starting material could be detected by t.l.c. Aqueous 1·0M-sodium hydroxide (16 ml) was slowly added, followed, when effervescence had ceased, by hydrogen peroxide (30% w/v; 50 ml). The mixture was poured into water and extracted with chloroform. The organic extracts were washed with water, iron(II) sulphate solution, and water again, and dried ($MgSO_4$). The residue left after evaporation of the solvent was chromatographed on silica gel. After traces of starting material had been eluted with benzene, elution with benzene–ether (50 : 1) gave the *6α-alcohol* (4·87 g, 45%), m.p. 214—215° (from chloroform–hexane), raised upon recrystallisation to 215—216° (Found: C, 59·0; H, 8·0; Br, 25·4. $C_{31}H_{52}Br_2O_3$ requires C, 58·9; H, 8·2; Br, 25·3%), $\nu_{max.}$ (KBr) 3500, 3430, 1732, 1716, and 1250 cm^{-1}, τ 9·26 (s, 13-Me), 9·16 (s, 10-Me), 7·98 (s, 3β-OAc), 6·60 (m, 6β-H), 5·52 (m, 22- and 23-H), 5·35 (m, 3α-H).

3β,6α-Diacetoxy-22,23-dibromo-24-ethyl-5α-cholestane (20).—Acetylation of the alcohol (19) with acetic anhydride in pyridine at room temperature gave the *diacetate* as needles, m.p. 187—188°, after three recrystallisations from chloroform–methanol (Found: C, 58·5; H, 8·1; Br, 23·7. $C_{33}H_{54}Br_2O_4$ requires C, 58·8; H, 8·0; Br, 23·7%), $\nu_{max.}$ (KBr) 1732 and 1250 cm^{-1}, τ 9·27 (s, 13-Me), 9·10 (s, 10-Me), 7·98 (s, 3β- and 6α-OAc), 5·53 (m, 22- and 23-H), and 5·34 (m, 3α- and 6β-H).

3β,6α-Diacetoxy-24-ethyl-5α-cholest-22-ene (21).—A solution of 3β,6α-diacetoxy-22,23-dibromo-24-ethyl-5α-cholestane (3·5 g) in ethanol (25 ml) and glacial acetic acid (25 ml) was heated under reflux with zinc dust (2·5 g) for 1·5 h. The mixture was filtered and the filtrate was concentrated *in vacuo* to small volume, and taken up in ethyl acetate. The solution was washed with aqueous sodium hydrogen carbonate, then with water, and dried ($MgSO_4$). The residue left after evaporation was crystallised from ethanol to give the *22-ene* (1·6 g, 60%), m.p. 88—89° (Found: C, 77·0; H, 10·4. $C_{33}H_{54}O_4$ requires C, 77·0; H, 10·5%), $\nu_{max.}$ (KBr) 1745—1735, 1253—1235, and 1030 cm^{-1}, τ 9·33 (s, 13-Me), 7·98 (s, 3β- and 6α-OAc), 5·31 (m, 3α- and 6β-H), and 4·93 (m, 22- and 23-H).

3β,6α-Diacetoxy-23,24-bisnor-5α-cholan-22-ol (23).—A stream of ozonised oxygen was bubbled through a solution of the 22-ene (21) (1·5 g) in dry dichloromethane (50 ml) containing dry pyridine (0·5 ml) at −70° until t.l.c. [benzene–ether (9 : 1) as irrigant] showed the absence of starting material. The solution was washed successively with dilute hydrochloric acid, aqueous sodium hydrogen carbonate, and water, and dried ($MgSO_4$). Evaporation to dryness left an oil, which, after column chromatography on silica gel, yielded 3β,6α-diacetoxy-23,24-bisnor-5α-cholan-22-al (22) (500 mg, 40%) as an oil, τ 9·31 (s, 13-Me), 9·12 (s, 10-Me), 8·90 (d, *J* 6 Hz, 20-Me), 8·01 (s, 3β- and 6α-OAc), 5·35 (m, 3α- and 6β-H), and 0·45 (s, 22-H).

A solution of the 22-aldehyde (1·85 g) in chloroform (10 ml) and ethanol (50 ml) containing sodium borohydride (180 mg) was stirred for 3 h at room temperature, concentrated *in vacuo*, diluted with ethyl acetate, washed with water, dried ($MgSO_4$), and evaporated. The residue was crystallised from acetone–hexane to give the *22-alcohol* (1·05 g, 56%) as plates, m.p. 171—172°, raised by recrystallisation to 173—174° (Found: C, 72·0; H, 9·4. $C_{26}H_{42}O_5$ requires C, 71·9; H, 9·7%), $\nu_{max.}$ (KBr) 3530, 1743—1735, 1245, and 1033 cm^{-1}, τ 9·31 (s, 13-Me), 9·10 (s, 10-Me), 8·95 (d, *J* 6 Hz, 20-Me), 7·98 (s, 3β- and 6α-OAc), 6·48 (m, 22-H_2), and 5·33 (m, 3α- and 6β-H).

3β,6α-Diacetoxy-23,24-bisnor-5α-cholane-22-carbonitrile (25).—Toluene-*p*-sulphonyl chloride (1·0 g) was added to a stirred solution of the 22-alcohol (23) (500 mg) in anhydrous pyridine (3 ml) at 0°; when dissolution was complete the mixture was left at 0° for 18 h. The amber solution, containing needles of pyridine hydrochloride, was diluted with ether, washed successively with cold dilute hydrochloric acid, aqueous sodium hydrogen carbonate, and water, and dried ($MgSO_4$). The brown oil left after evaporation was chromatographed on a column of silica gel (30 g). After removal of the excess of tosyl chloride by elution with benzene, the crude 3β,6α-diacetoxy-22-tosyloxy-23,24-bisnor-5α-cholane (24) (610 mg, 90%) was eluted with benzene–ether (9 : 1); τ 9·40 (s, 13-Me), 9·14 (10-Me), 8·93 (d, *J* 6 Hz, 20-Me), 8·00 (s, 3β- and 6α-OAc), 7·56 (s, aromatic Me), 6·15 (AB part of ABX system, J_{AB} 9, J_{AX} 6, J_{BX} 3 Hz, 22-H_2), 5·35 (m, 3α- and 6β-H), and 2·46 (ABq, *J* 8 Hz, aromatic H).

The crude tosylate (24) (610 mg) was dissolved in anhydrous dimethyl sulphoxide (10 ml) and sodium cyanide (50 mg, 1 equiv.) was added. The mixture was stirred at 60° under dry nitrogen for 5 h before being poured on aqueous ammonium chloride. The mixture was extracted with benzene, and the extracts were washed with water, dried ($MgSO_4$), and evaporated. Crystallisation of the residual oil from ethanol gave the *22-carbonitrile* (25) (221 mg, 48%) as needles, m.p. 109—111° (Found: C, 73·1; H, 9·0. $C_{27}H_{41}NO_4$ requires C, 73·1; H, 9·3%), $\nu_{max.}$ (KBr) 2260, 1745, 1732, and 1250 cm^{-1}, τ 9·33 (s, 13-Me), 9·12 (s, 10-Me), 8·85 (d, *J* 6 Hz, 20-Me), 7·99 (s, 3β- and 6α-OAc), and 5·34 (m, 3α- and 6β-H).

3β,6α-Dihydroxy-5α-cholestan-23-one (5).—A solution of

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[31] H. R. Bentley, J. A. Henry, D. S. Irvine, and F. S. Spring, *J. Chem. Soc.*, 1953, 3673.

the nitrile (25) (150 mg) in dry ether (10 ml) was treated dropwise with a solution of isobutyl-lithium in hexane until t.l.c. showed the absence of starting material. A white precipitate formed immediately. Sulphuric acid (6N; 10 ml) in dioxan (20 ml) was added and the mixture was heated under reflux for 3 h, cooled, and extracted with ether. The extracts were washed with aqueous sodium hydrogen carbonate, followed by water, and dried ($MgSO_4$). The brown solid (171 mg) left after evaporation *in vacuo* was purified by t.l.c. [chloroform–ethyl acetate (1 : 1) as irrigant] to give 3β,6α-*dihydroxy*-5α-*cholestan*-23-*one* (55 mg, 39%) as needles, m.p. 185—188° (from acetone–hexane) (Found: C, 77·6; H, 10·9%; M^+, 418·3446. $C_{27}H_{46}O_3$ requires C, 77·5; H, 11·0%; *M*, 418·3447), identical (mixed m.p., t_R, spectral data) with natural tetrahydromarthasterone (see before).

G.l.c.–Mass Spectral Data for Component B.—Component B showed *m/e* 398 (9%), 383 (7), 380 (7), 374 (14), 365 (9), 359 (11), 357 (7), 356 (20), 342 (17), 341 (40), 338 (13), 323 (28), 316 (82), 301 (25), 299 (21), 298 (42), 283 (38), 281 (22), 280 (15), 269 (16), 265 (28), 247 (20), 229 (22), 211 (26), 197 (20), 191 (22), 159 (38), 145 (39), 123 (39), 121 (44), 95 (100), 57 (90).

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