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STEROLS FROM THE SPONGES *CLIONA CELATA* GRANT AND *HYMENIACIDON PERLEVE* MONTAGU

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Abstract—The sterols of *Cliona celata* have been identified as cholesterol (predominant), 24-norcholesta-5,22-dien-3β-ol, 22,23-dehydrocholesterol, 24-methylenecholesterol, brassicasterol, 22,23-dihydrobrassicasterol, poriferasterol, and clionasterol, while those found in *Hymeniacidon perleve* were cholestanol (predominant), 24-norcholesta-22-en-3β-ol. 22,23-dehydrocholestanol, 24-methylenecholestanol, fucosterol, brassicasterol, β-sitosterol, and neospongosterol.

THE extensive researches of Bergmann on the sterols in sponges were carried out before the introduction of efficient chromatographic methods, and he was aware[1] that much of the data accumulated probably referred to sterol mixtures and not to individual compounds. Thus most of the earlier work now requires revision with the aid of modern techniques. Although about 250 species of sponge can be found in British waters virtually nothing is known of their sterol content, and indeed very little is known of their chemistry. This, no doubt, stems from the fact that the great majority are rather small and either rare, or at best, locally abundant, and the difficulties of collection are considerable. We report here on the sterols in two British species, *Cliona celata* and *Hymeniacidon perleve*.

Cliona celata

This massive dull yellow sponge is common and widely distributed. It was first examined by Dorée[2] using material collected from Plymouth and was subsequently reinvestigated by Bergmann.[3] Dorée's "clionasterol"[2] was found by Bergmann[3] to be a mixture of clionasterol and poriferasterol, and in the present work *C. celata*, collected at Port Erin, Isle of Man, has been shown to contain a mixture of eight sterols (Table 1).

GLC of the sterol fraction revealed the presence of at least eight different compounds (Fig 1) while the mixed acetates could be separated by TLC on silica gel/$AgNO_3$ plates (Fig 2) into six components. In this respect we confirm the observations of Idler and Safe;[22] resolution of the steryl propionates[4, 17] was not noticeably better. Fairly pure samples of all the acetates, except 3, 5b and 7, were obtained by repeated TLC and crystallization; 3 and 7 were further purified by preparative GLC, but 5b remained as a mixture with 3 and 7.

Sterol 1, a minor component, was difficult to separate from higher homologues, and proved to be the C_{26} norcholestadienol (I) recently identified in the scallop *Placopecten magellanicus*[5] and other bivalves,[5] in the tunicate *Halocynthia roretzi*[19] and elsewhere.[23] It had a much shorter GLC retention time than cholesterol or 22,23-dehydrocholesterol, indicating a lower homologue, which was confirmed by a prominent peak at *m/e* 352 (30%) (M-CH_3CO_2H, $C_{26}H_{40}$) in the mass spectrum of the

acetate (Δ^5-steryl acetates, including all those derived from this sponge, do not give molecular ion peaks). A further major peak at *m/e* 255 (M-CH_3CO_2H—s.ch) shows that the side chain is C_7 and unsaturated. The NMR spectrum of the acetate includes a broad doublet at τ 4·63 (H-6), a broad hump at 5·30 (H-3), a doublet at 7·68 (allylic CH_2), and singlets at 7·98 (CH_3CO_2), 8·98 (19-CH_3), and 9·31 (18-CH_3), all typical of Δ^5-steryl acetates. An additional multiplet at τ 4·80, arising from the vinylic protons in the side chain, is exactly like that in the spectrum of brassicasteryl acetate (see IV), while a doublet centred at τ 9·06 can be attributed to the Me protons of an isopropyl group. Additional support for structure (I) can be deduced from the mass spectrum but owing to the presence of impurity peaks, this is not reliable. However, the GLC retention time of the acetate was identical with that of the synthetic acetate of (I) kindly supplied by Dr. Oehlschlager.[9]

Hydrogenation of the acetate over palladized charcoal in isopropanol gave two compounds, the fully reduced stanol (acetate) and the sterol (acetate) with a saturated side chain. The mass spectrum of the stanyl acetate shows peaks at *m/e* 416 (M, 28%), 356 (M-CH_3CO_2H, 30%) and 341 (M-CH_3CO_2H—CH_3, 21%), corresponding to ions at *m/e* 430, 370 and 355 in the spectrum of cholestanyl acetate, while below *m/e* 300 the two spectra are virtually identical apart from intensity variations. Similarly, the NMR spectrum is almost identical with that of cholestanyl acetate. The partially reduced compound, as expected, was very similar to cholesteryl acetate. No molecular ion was observed in the mass spectrum, the M-CH_3CO_2H ion appearing at *m/e* 354 (94%), followed by 339 (18%), and thereafter the spectrum was almost superimposable on that of cholesteryl acetate. The NMR spectrum, consisting principally of a multiplet at τ 4·80, a broad hump at 5·4, singlets at 7·97, 8·98 and 9·32, and two overlapping Me doublets with peaks at 9·06, 9·11 and 9·16, was almost the same as that of cholesteryl acetate.

Sterol 2 is a C_{27} compound, the base peak in the mass spectrum of the acetate falling at *m/e* 366 (M-CH_3CO_2H, $C_{27}H_{42}$). Other significant peaks appear at *m/e* 351 (8%), 282 (5%), and at 255 and 253, the two latter resulting from loss of an unsaturated C_8 side chain, and loss of side chain and 2H, respectively. The ion at *m/e* 282 (M-CH_3CO_2H—C_6H_{12}) can be attributed to vinylic cleavage with hydrogen transfer, characteristic of Δ^{22}-sterols.[6] In agreement the GLC retention time of the acetate was identical with that of a reference sample of 22,23-dehydrocholesteryl acetate. The NMR spectrum [complex multiplet (3H) at τ 4·73, a broad signal at 5·40, a doublet at 7·66, singlets at 7·99, 8·99 and 9·31, and doublets at 9·01 and 9·16] is also consistent with this structure, and the stanyl acetate obtained on hydrogenation was identical (GLC, NMR and mass spectrum) with cholestanyl acetate (see XII). Sterol 2 is thus 22,23-dehydrocholesterol (II). Two peaks immediately preceding cholesterol are observed in the GLC trace of the original sterol mixture (Fig 1). We believe that these correspond to the *cis* and *trans* isomers of 22,23-dehydrocholesterol which have recently been isolated by Idler and Wiseman.[20]

Sterol 3 was cholesterol (III) and it constituted more than half the total mixture. A small amount was purified by preparative GLC, and the sterol, its acetate and the stanyl acetate obtained by hydrogenation had GLC retention times identical with those of authentic samples. The mass spectrum of the steryl acetate showed the base peak at *m/e* 368 (M-CH_3CO_2H, $C_{27}H_{44}$) and the expected cracking pattern.

Sterol 4 was a C_{28} sterol, the propionate showing a base peak in the mass spectrum

at *m/e* 380 (M-$CH_3CH_2CO_2H$, $C_{28}H_{44}$). Other significant peaks at *m/e* 365 (7%), 337 (7%) (loss of $CH(CH_3)_2$), 282 (6%) (loss of C_7H_{15} and hydrogen transfer),[6] and 255 (loss of C_9 side chain) suggested that the compound might be brassicasteryl acetate and the NMR spectrum is entirely consistent with that structure. In fact the GLC retention time of the sterol and its acetate were identical with those of brassicasterol and its acetate, respectively, and on hydrogenation the acetate gave ergostanyl acetate, identified by GLC, MP and MMP. Hence sterol 4 is brassicasterol (IV).

Sterol 5a is isomeric with sterol 4, distinguishable by strong absorption at 885 cm^{-1} in the IR, and a doublet (2H) at τ 5·28 in the NMR spectrum of the acetate, both of which disappeared on hydrogenation. This is indicative of an exocyclic methylene group and peaks at *m/e* 255 and 253 in the mass spectrum show that the side chain is unsaturated. By direct comparison (GLC, NMR, IR) with an authentic sample the acetate was found to be that of 24-methylenecholesterol (chalinasterol; V), and the reduced product was similarly identified as ergostanyl acetate.

Sterol 5b had almost the same GLC retention time as sterol 5a and ran with sterols 3 and 7 on TLC plates. It comprised 3–5% of this mixture and is regarded as 22,23-dihydrobrassicasterol[14] (VI) on the following grounds. (a) The mass spectrum of the mixture, after acetylation, had M-CH_3CO_2H peaks at *m/e* 396 (35%), 382 (9%) ($C_{28}H_{46}$), and 368 (100%), corresponding to loss of acetic acid from the acetates of VII, VI and III, respectively, and hence the molecular formula of 5b is $C_{28}H_{48}O$. (b) The GLC retention time of 5b was intermediate between that of cholesterol and β-sitosterol, and the stanol, obtained by hydrogenation of the mixture, had the same retention time as ergostanol.

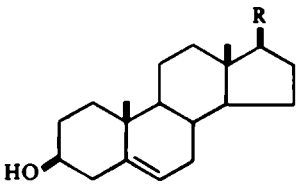
Sterol 6, a very minor component, was a C_{29} sterol, the mass spectrum of the propionate showing an M-$CH_3CH_2CO_2H$ peak at *m/e* 394 (29%) ($C_{29}H_{46}$) and another at 255 (loss of unsaturated C_{10} side chain). This at once suggested poriferasterol (VII), already known[3] in *C. celata*, and comparison of the acetate with authentic poriferasteryl acetate showed that their GLC retention times were identical. The NMR spectrum of the acetate was identical to that of stigmasteryl acetate (the 24α-epimer of poriferasteryl acetate). The derived stanyl acetate ($C_{29}H_{52}O$, mass spectrum) had the same GLC retention time as poriferastanyl acetate, and its NMR spectrum was likewise identical to that of stigmastanyl acetate. The MP of the sterol was somewhat low but clearly distinguishable from that of stigmasterol (Table 1).

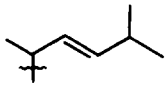
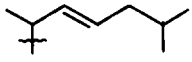
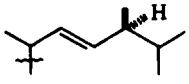
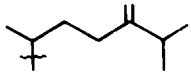
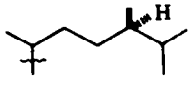
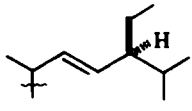
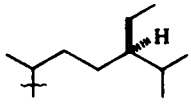
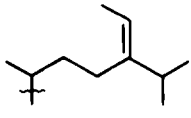
Sterol 7 has the molecular formula $C_{29}H_{50}O$ (mass spectrum) and is either clionasterol (VIII) or its 24α-epimer, β-sitosterol. The NMR spectrum of the acetate was identical with that of β-sitosteryl acetate. The GLC retention time was also identical with that of β-sitosteryl acetate, and similarly the GLC retention time of the reduced acetate was identical with that of stigmastanyl acetate. We assume that sterol 7 is clionasterol (VIII) as it is already known in *C. celata*[3] but unfortunately no sample was available and our GLC system cannot separate 24α- and β-epimers.

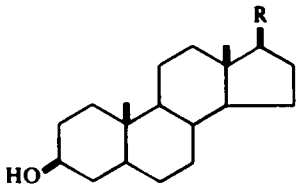
Hymeniacidon perleve

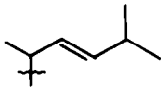
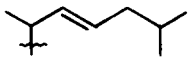
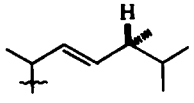
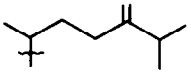
The sterol fraction of this large orange-red sponge amounted to *ca* 1% of the dry weight. A GLC trace (Fig 1) revealed the presence of at least six sterols (Table 2), and a seventh was isolated, although not pure, after acetylation and separation by TLC on silica gel/$AgNO_3$ plates (Fig 2).

Sterol 1 is a new C_{26} stanol containing one double bond [acetate, M^+, *m/e* 414



Sterol	*R*		*Name*
I			24-Norcholesta-5,22-dien-3β-ol
II		(*cis* and *trans*)	22,23-Dehydrocholesterol
III			Cholesterol
IV			Brassicasterol
V			24-Methylenecholesterol
VI			22,23-Dihydrobrassicasterol (24-α = campesterol)
VII			Poriferasterol
VIII			Clionasterol (24-α = β-sitosterol)
IX			Fucosterol



Sterol	*R*	*Name*
X		24-Norcholesta-22-en-3β-ol
XI		22,23-Dehydrocholestanol
XII		Cholestanol
XIII		Neospongosterol
XIV		24-Methylenecholestanol

(44%)]. In the acetate, IR absorption at 960 and 970 cm^{-1}, but not at 800 or 840 cm^{-1}, the absence of signals at τ 4·5 and 7·7 in its NMR spectrum, the large molecular ion and the absence of an M-CH_3CO_2H peak in its mass spectrum, together establish the absence of Δ^5 unsaturation, while a major peak at *m/e* 257 (76%) confirms that the sterol nucleus is saturated. The NMR spectrum of the acetate includes a multiplet at τ 4·77 (2H) exactly like that in the spectrum of the C_{26} sterol (I), together with singlets (each 3H) at τ 7·98 (CH_3CO_2—), 9·18 (19-CH_3) and 9·34 (18-CH_3), a doublet (3H) at 9·02 (20-CH_3), and a doublet (6H) at 9·06 [$(CH_3)_2CH$]. Additional significant peaks in the mass spectrum of the acetate appear at *m/e* 399 (5%), 344 (100) (loss of C_5H_9 and transfer of hydrogen),[6] 329 (18), 315 (62) (loss of C_7H_{13} and transfer of 2H), and 255 (10). These data are consistent with structure (X), 24-norcholesta-22-en-3β-ol.*

Sterol 2 is a new C_{27} stanol. The acetate showed a strong IR doublet at 960–970 cm^{-1}, as does 22,23-dehydrocholesterol, but no absorption at 800 or 840 cm^{-1} indicating the absence of Δ^5 unsaturation which was confirmed by the absence of signals at τ 4·5 and 7·7 from its NMR spectrum. Instead, olefinic absorption was observed at τ 4·75 (2H) together with a broad signal at 5·30 (1H), singlets (each 3H)

* Added in proof: direct comparison shows that the same stanol is present in the tunicate *Halocynthia roretzi*.[25]

at 7·99, 9·18, and 9·34, and doublets at 9·01 (3H) and 9·14 (6H). In the mass spectrum of the acetate the molecular ion appeared at *m/e* 428 ($C_{29}H_{48}O_2$, 35%) with peaks at 368 (25), 344 (65), 327 (15), 315 (35), 284 (35), 269 (18), 257 (100), and 255 (36). This fragmentation pattern is characteristic of Δ^{22} sterols,[6] and since the acetate gave cholestanyl acetate (identified by GLC, NMR, MP and MMP) on hydrogenation, the combined evidence establishes that this new sterol is 22,23-dehydrocholestanol (XI).

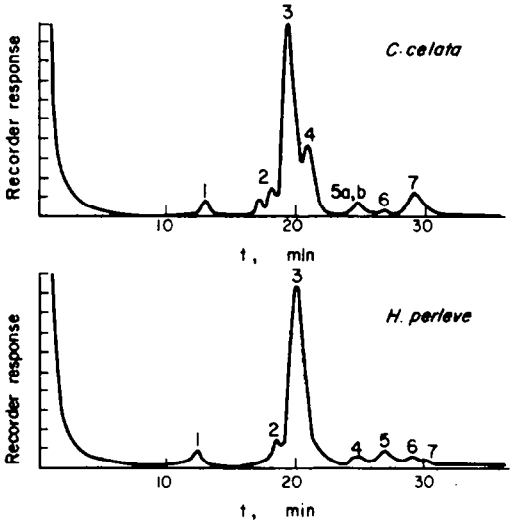


FIG 1. GLC traces of *C. celata* and *H. perleve* sterol mixtures of 3% Hi-Eff-8BP.

Sterol 3, the major component, was identified as cholestanol (XII) by GLC, NMR, IR and mass spectrum, and MP and MMP of the acetate and free stanol.

Sterol 4 could not be obtained free from sterol 6 (β-sitosterol, VIII). This is another Δ^{22}-stanol as shown by IR absorption at 960 and 970 cm^{-1} (but not at 800 or 840 cm^{-1}), olefinic absorption in the NMR spectrum of the acetate at τ 4·79 (2H, m) only, identical with that in the spectrum of brassicasterol, and a strong molecular ion peak in its mass spectrum at *m/e* 442 (68%, $C_{30}H_{50}O_2$) with the base peak at *m/e* 257. The cracking pattern,* *m/e* 442 (68%), 427 (5), 382 (5), 344 (68), 329 (15), 315 (57), 284 (15), 269 (8), 257 (100), and 255 (25) is also typical of Δ^{22} sterols,[6] this compound having a C_9 side-chain. As the acetate on hydrogenation gave campestanyl acetate (see VI) (identified by GLC, MS and MP), sterol 4 appears to be neospongosterol (XIII). Our best sample of the acetate had MP 133–145°; Bergmann[16] reported 142–143°. (The high field NMR spectrum of the acetate showed singlets at τ 7·98, 9·18 and 9·34, and overlapping doublets at τ 9·02, 9·10 and 9·17, but overlapping peaks from the β-sitosteryl acetate impurity made interpretation somewhat difficult.)

Sterol 5 is a new C_{28} stanol. The acetate had the lowest R_f value on silica gel/$AgNO_3$ plates, and showed strong absorption in the IR at 885 cm^{-1}, typical of terminal

* There was also a strong impurity peak at *m/e* 396 (90%) due to β-sitosteryl acetate (M-CH_3CO_2H).

olefins, but no absorption at 800 or 840 cm^{-1} characteristic of Δ^5 sterols. In the mass spectrum significant peaks are seen at *m/e* 442 ($C_{30}H_{50}O_2$, M^+, 7%), 427 (5, M-CH_3), 358 (63, M-C_6H_{12}), 343 (25, M-CH_3-C_6H_{12}), 315 (38, M-s.ch.-2H), 255 (25, M-s.ch.-2H-CH_3CO_2H), and 215 (25, M-s.ch.-CH_3CO_2H-part of ring D). The side chain is thus C_9, and the loss of C_6H_{12} from the molecular ion could result from a McClafferty rearrangement of a Δ^{24}-sterol.[6] As the acetate gave ergostanyl acetate on hydrogenation (identified by GLC, NMR, MP and MMP) the sterol is evidently (XIV). This is confirmed by the NMR spectrum of the acetate which comprises a doublet (2H) at τ 5·32 ($J \sim 5$ Hz, $> C{=}CH_2$) protruding above the broad signal from H-3, singlets (each 3H) at 7·99 (CH_3CO_2—), 9·18 (19-CH_3), and 9·35 (18-CH_3), a doublet (6H) at 8·98 (—$CH(CH_3)_2$), and a doublet (3H) at 9·06 (20-CH_3). This is fully consistent with structure (XIV), the new sterol being 24-methylenecholestanol. The best sample did not melt sharply.

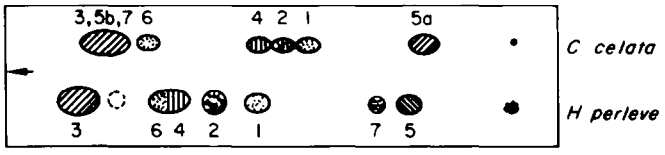


FIG 2. The chromatograms of *C. celata* and *H. perleve* steryl acetates on silica gel/20% $AgNO_3$ (CH_2Cl_2).

Sterol 6 is β-sitosterol [24α-epimer of (VIII)], identified by GLC, NMR and its mass spectrum. The low m.p. of the acetate (125–126°) indicates that it is in fact β-sitosteryl acetate (m.p. 127°[21]) and not its 24β-epimer, clionasteryl acetate (m.p. 144°[3]). Hydrogenation of the acetate gave stigmastanyl acetate (identified by GLC, NMR, MS and m.p.).

Sterol 7, m.p. 123–126° (lit.[7] 124°) is fucosterol (IX). It had the same GLC retention time as authentic IX, and the acetate agreed closely with published IR[7] and NMR[8] values. The mass spectrum of the acetate showed significant peaks at *m/e* 394 ($C_{29}H_{46}$, M-CH_3CO_2H, 67%), 352 (12), 296 (100), 255 (16), 253 (21) and 213 (16). On hydrogenation it gave a stanol identical (GLC, NMR) with stigmastanyl acetate.

Another sample of *H. perleve* also contained brassicasterol (IV) which was identified (as the acetate) by GLC, NMR and mass spectrum.

DISCUSSION

The common sponge sterols, poriferasterol and clionasterol, have been found previously in *Cliona* spp.,[1] and they occur together with cholesterol in *Verongia*, *Ianthella* and *Haliclona* spp.[1] 24-Methylenecholesterol has been reported in *Craniella crania*[16] but the other sterols in Table 1 are new to the Porifera; 22,23-dihydro-

TABLE 1. STEROLS OF CLIONA CELATA

		Sterol	% of mixture*	mp Free sterol	mp Acetate	Ref.
1	(I)	24-Norcholesta-5,22-dien-3β-ol	3·0		131–132·5°	
2	(II)	22,23-Dehydrocholesterol	11·5	133–136° (134·5–135·5°)	130–132° (127–128°)	10
3	(III)	Cholesterol	57·5	150–151°	114–115°	13
4	(IV)	Brassicasterol	13·5	147–148·5° (148°)	152–154° (152°)	11
5a	(V)	24-Methylenecholesterol	4·0	138–142° (142°)	132–136° (135–136°)	12
5b	(VI)	22,23-Dihydrobrassicasterol				
6	(VII)	Poriferasterol	0·5	151–154° (155–156°)	140–143° (140–141°)	3
7	(VIII)	Clionasterol	10·0	141·5–142·5° (137·5–138·5°)	137–141·5° (137°)	3

* As determined by GLC

[a] Stigmasterol[3] has mp 170°, acetate mp 144°

brassicasterol[14] has been tentatively identified in the scallop *Placopecten magellanicus*[20] but was not isolated, and could not be obtained pure in this investigation. The presence of C_{26} sterols in marine invertebrates has been realised for some time[5,18,19,24] and their occurrence in plankton[17] suggests that this may be the common origin of all the C_{26} marine animal sterols.[17]

Only cholestanol has been reported previously in *Hymeniacidon* spp. It constitutes *ca* 75% of the mixture in *H. perleve*. The stanols (X, XI and XIV) are new, and no doubt other stanols await discovery. Fucosterol was reported, tentatively, for the first time in the animal kingdom recently (in *Placopecten magellanicus*[20]); it is common in algae.

TABLE 2. STEROLS OF HYMENIACIDON PERLEVE

	Sterol		% of mixture*	mp Free sterol	mp Acetate	Ref.
1	24-Norcholesta-22-en-3β-ol	(X)	3	85 and 119–121°	117–121°	
2	22,23-Dehydrocholestanol	(XI)	5	117–117·5°	104–105°	
3	Cholestanol	(XII)	75	125 and 142·5–143·5° (125 and 141–142°)	110·5–113° (109–110°)	15
4	Neospongosterol	(XIII)	8		133–145° (141–142°)	16
5	24-Methylenecholestanol	(XIV)	7	116–118°	123–125°	
6	β-Sitosterol	(24α-VIII)	2		125–126° (126–127°)	21
7	Fucosterol	(IX)		123–126° (124°)	116–118° (118–119°)	7

* As determined by GLC

TABLE 3. GLC RETENTION TIMES* OF C. CELATA AND H. PERLEVE STEROLS

Sterols†	Hi-Eff-8BP[a]			OV-17[b]			QF-1[c]		
	Authentic	*C. celata*	*H. perleve*	Authentic	*C. celata*	*H. perleve*	Authentic	*C. celata*	*H. perleve*
24-Norcholesta-5,22-dien-3β-ol	0·67	0·67	0·66	0·66	0·66	0·67	0·64	0·64	0·70
cis-22,23-Dehydrocholesterol	0·88	0·88		0·87	0·87		0·87	0·87	0·98
trans-22,23-Dehydrocholesterol	0·93	0·92	0·92	0·93	0·93	0·93	0·87		
Cholestanol	0·99		0·98	1·00	1·00	1·00	1·13		1·13
Cholesterol	1·00	1·00		1·00	1·00		1·00	1·00	
Brassicasterol	1·07	1·06		1·13	1·13	1·14	1·06	1·06	
Desmosterol	1·25		1·24	1·19			1·09		
Campesterol	1·27	1·28		1·30	1·30		1·30	1·28	
Stigmasterol	1·29			1·40	1·40		1·37	1·38	1·40
24-Methylenecholesterol	1·38	1·37	1·34	1·33	1·33	1·34	1·27	1·28	
β-Sitosterol	1·49	1·51	1·51	1·58	1·59	1·62	1·53	1·53	1·54
Fucosterol	1·62			1·67			1·55		

* Relative to cholesterol

† 24α- and β-Epimers cannot be distinguished on these columns

[a] 3% Hi-Eff-8BP on 100/120 mesh GasChrom Q (Applied Sciences Laboratories) 6′ × ⅛″; col. T = 245°, det. T = 245°, He = 20 ml/min; retention time of cholesterol = 19·1 min

[b] 3% OV-17 on 60/80 mesh GasChrom Q, 7′ × ¼″; col. T = 230°, det. T = 250°, He = 20 ml/min; retention time of cholesterol = 17·0 min

[c] 2% QF-1 on 100/120 GasChrom Q, 6′ × ⅛″; col. T = 200°, det. T = 250°, He = 16 ml/min; retention time of cholesterol = 18·6 min

EXPERIMENTAL

NMR spectra were recorded on a Varian HA-100 spectrometer in $CDCl_3$ and mass spectra on an AEI MS9 instrument at 70 eV. Preparative GLC was performed on an F21 instrument using a 2 m × 8 mm stainless steel column of 2·5% silicone OV-1 on Chromosorb G at 240°; carrier gas N_2; analytical GLC details are given in Table 3.

Acetates and propionates were prepared in pyridine at room temp in the usual way, and hydrogenations were effected over Pd–C in isopropanol. Acetates were separated (TLC or PLC) on silica gel HF/10% $AgNO_3$ plates in CH_2Cl_2–ether (1–3%) or occasionally, hexane–ether (1–3%), recovered by elution with ether, and crystallized from MeOH or MeOH–$CHCl_3$. The procedure was repeated as required. Free sterols were regenerated in the normal manner and crystallized several times from MeOH.

Extraction of Cliona celata.

Wet sponges (2·2 kg dry weight after extraction) were cut into small pieces and covered with MeOH. After several days the yellow extract was drawn off and fresh solvent added. The process was repeated twice. The solvent was then removed *in vacuo* and the residual mixture was centrifuged to remove solid material. The aqueous phase and the dried solid were extracted with cold EtOAc. Evaporation of the combined extracts left a red mush (32 g) and more of this (58 g) was obtained by further acetone extraction (Soxhlet) of the sponge tissue. This material was dissolved in hot MeOH and left overnight. The crystals which separated were collected and recrystallized from MeOH until almost colourless. The combined mother liquors were evaporated and the residual oil was chromatographed on a column of H and W silica gel M.F.C. in CH_2Cl_2 with increasing amounts of ether, and then MeOH. The sterol fractions were combined with the crystalline product and recrystallized from MeOH to yield ~12 g mixed sterols, mp 136–142°, giving a green Liebermann–Burchard reaction. The mixture was acetylated and separated by repeated PLC. Small amounts of the acetates of III and VIII were further purified by preparative GLC.

Extraction of Hymeniacidon perleve.

Wet sponges (*ca* 470 g dry weight) were cut into small pieces, steeped in MeOH overnight and then extracted with boiling acetone overnight. The combined extracts were concentrated on a rotary evaporator leaving an aqueous suspension which was extracted with chloroform. This yielded a red oil (*ca* 18 g) which was saponified (probably unnecessary) and the resulting red oil (*ca* 18 g) was chromatographed on a column of silica gel M.F.C. (500 g) in petrol (b.p. 40–60°) with gradually increasing concentrations of ether. The combined sterol fractions (4·7 g) (Liebermann–Burchard reaction blue, changing to green in 2 hr) were acetylated and the acetates separated by PLC.

The following are new:

24-*Norcholesta*-5,22-*dien*-3β-*ol*. The *acetate* was obtained as crystals (from MeOH), mp 131–132·5°, (Found: M^+-CH_3CO_2H, 352·3117. $C_{26}H_{40}$ requires 352·3130). The mass spectrum showed significant impurity peaks at *m/e* 394 and 408. The *propionate* had mp 113–115°, (Found: M^+-$CH_3CH_2CO_2H$, 352·2741). Hydrogenation of the acetate was incomplete after several hr. Two reduction products were separated by TLC on silica gel in petrol (b.p. 40–60°)/ether (97:3) and examined by NMR and mass spectrometry (discussion).

24-*Norcholesta*-22-*en*-3β-*ol*. The *acetate* was obtained as plates, mp 116–121° (from MeOH), (Found: M, 414·3553 $C_{28}H_{46}O_2$ requires 414·3498). Hydrolysis gave the free *sterol* (X) as leaflets, mp 85° and 119–121° (from MeOH). (Found: M, 372·3406. $C_{26}H_{44}O$ requires 372·3392).

24-*Methylenecholestanyl acetate* formed plates, mp 123–125° (from MeOH), (Found: M, 442·3801. $C_{30}H_{50}O_2$ requires 442·3810). Hydrolysis with methanolic KOH gave the free *sterol* (XIV) as leaflets, mp 110–115° (from MeOH), (Found: C, 84·3; H, 12·2. $C_{28}H_{48}O$ requires C, 83·9; H, 12·1%). On hydrogenation of the acetate it gave ergostanyl acetate, mp 145·5–147°, mmp 144–146·5°, identical (GLC, NMR) with an authentic sample.

22,23-*Dehydrocholestanyl acetate* crystallized from MeOH in plates, mp 104–105°. (Found: M, 428·3655. $C_{29}H_{48}O_2$ requires 428·3654). Hydrolysis gave the free *sterol* (XI) as flakes, mp 117–117·5° (from MeOH), (Found: M, 386·3573. $C_{27}H_{46}O$ requires M, 386·3548). Hydrogenation of the acetate gave cholestanyl acetate, mp and mmp 119–123·5° identical (GLC, NMR) with an authentic sample.

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