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STEROIDS FROM SPONGES

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Abstract—The sponges *Stelleta clarella Tethya aurantia, Lissodendoryx noxiosa, Haliclona permollis* and *Haliclona sp.* were examined for steroids. All sponges contained C_{27}-C_{29}, Δ^5, mono and diunsaturated sterols. In addition, the sponge *Tethya aurantia* contained Z - 24 - propylidene - cholest - 5 - en - 3β - ol (**19**) and the 5α,8α-peroxides of cholesta - 5,7 - dien - 3β - ol, ergosterol, ergosta - 5,7,24(28) - trien - 3β - ol and 24ξ - ethyl - cholesta - 5,7 - dien - 3β - ol (**29**, **30**, **31** and **32**). The sponge *Stelleta clarella* also contained 24 - nor - cholesta - 4,22 - dien - 3 - one (**21**), cholesta - 4,22 - dien - 3 - one (**22**), 24ξ - methyl - cholesta - 4,22 - dien - 3 - one (**24**), ergosta - 4,24(28) - dien - 3 - one (**25**), (E) - stigmasta - 4,24(28) - dien - 3 - one (**28**), 5α - cholestanol (**5**), 5α - ergostanol (**7**) and 5α - poriferastanol (**9**). The possible biosynthetic significance of these hitherto undescribed peroxides and enones from marine sources is discussed. A synthesis of **19** is also described.

The sponges represent an ancient group of animals and contain perhaps the greatest diversity of sterols. The elegant work of Bergmann *et al.* resulted in the isolation of a number of new sterols such as 24-methylenecholesterol,[1] neospongosterol[2] and chondrillasterol.[3] Bergmann was aware that a number of other sponge sterols[4] are in fact mixtures. With the recent advances in chromatographic techniques, it is now possible to resolve most of these complex mixtures and their examination is now clearly warranted. Thus, the recent reexamination of the sponges *Cliona celata*[5] and *Hymeniacidon perleve* Montague has resulted in the isolation of 24 - norcholesta - 22 - en - 3β - ol (**1**) and 5α - cholest - 22 - en - 3β - ol (**2**). Even more unexpected is the isolation[6] of aplysterol (**3**) and 24,28 - dehydroaplysterol (**4**), the first examples of 26-alkylation in steroid biosynthesis. It has been suggested that the distribution of **3** and **4** is restricted to the family *Verongidae* and therefore sterol distribution can be used in sponge taxonomy.[7] Only scanty information is available regarding the origin of sterols in *Porifera.* Incorporation experiments indicated that the sponge *Grantia compressa*[8] could biosynthesize sterols whereas the sponges *Suberites domuncula* and *Verongia aerophoba*[9] failed to incorporate either 1-^{14}C-acetate or 2-^{14}C-mevalonate. Radio labeling experiments using CH_3-^{14}C methionine in the sponge *Verongia aerophoba*[9] resulted only in nonradioactive **3** and **4**. It is conceivable, therefore, that these recently isolated C_{26}, C_{29} (aplysterol skeleton) and C_{30} sterols are of planktonic origin. This would also account for their wide distribution.

In view of these observations and as a direct consequence of our efforts to isolate biologically interesting compounds from marine sources together with a systematic examination of the marine life from the central California coast, we had occasion to examine a number of sponges for their steroid and terpenoid content. We now report on the steroids from five California sponges: *Stelleta clarella* de Laub., is a massive pale yellow sponge with protruding spicules and should be handled with care. *Tethya aurantia* California de Laub., is an orange globular sponge which generally inhabits deep waters and can be collected intertidally only at very low tides. *Lissodendoryx noxiosa* de Laub., is a drab colored sponge and usually has a foul odor. An aqueous extract of this sponge is toxic to mice and elicits all symptoms of histamine poisoning[10] which can be reversed by an injection of phenergan. We are currently examining the odor constituent of this sponge. Our preliminary examination suggests that this sponge contains relatively large amounts of sphingolipids. *Haliclona permollis* (Bowerbank) and *Haliclona sp.* both are drab to pale yellow in color. *Haliclona sp.* was toxic to mice and extremely hemolytic towards mouse red blood cells. Its hemolytic activity is destroyed within a few days on storage at 0°.

Gas chromatographic analysis of the sterol mixtures from all five sponges indicated that they are composed of 6 to 10 components. Further separation of these mixtures was effected by repeated preparative layer chromatography of the corresponding acetates using a minor variant of the procedure described by Idler and Safe.[11] The enriched fractions thus obtained were finally separated into individual components by preparative gas chromatography.

All sponges contained Δ^5-mono and diunsaturated sterols (Table 1). The structures of these sterols were established by a combination of their mass and NMR spectra; whenever sufficient quantities were obtained, determination of m.ps and optical rotations as well as direct comparison with authentic samples were also effected.

In addition to C_{27}-C_{29} sterols (Table 1) the sponge *Tethya aurantia* also contained a C_{30} sterol. The mass spectrum of this sterol depicted a molecular ion peak at *m/e* 426 ($C_{30}H_{50}O$), a base peak at *m/e* 314 corresponding

Table 1. Steroid composition of sponges

Compound	*Stelleta clarella*	*Tethya aurantia*	*Lissodendoryx noxiosa*	*Halicolana permollis*	*Haliclona sp.*
5	7·5%	<1%	<2%	—%	<1%
6	7·5	51·0	53·0	30·0	51·0
9	1·5	—	—	—	—
8	0·25	0·8	0·8	0·5	2·0
10, **11**	1·7	4·6	2·9	—	—
12	4·0	7·0	11·0	21·0	14·0
13	13·5	24·0	25·0	26·0	20·0
14	1·0	<1·0	—	—	—
15	<1·0	<1·0	1·6	1·0	1·0
16, **17**	11·0	9·0	8·0	17·0	2·0
18	0·5	—	<1·0	—	—
19	—	0·2	—	—	—
22	2·0	—	—	—	—
23	6·5	—	—	—	—
24	4·0	—	—	—	—
25	23·0	—	—	—	—
21	0·5	—	—	—	—
28	13·0	—	—	—	—

to a McLafferty rearrangement ion[12] generated by the $\Delta^{24(28)}$ double bond, peaks at *m/e* 271 and 269 corresponding to the loss of the entire side chain with or without transfer of two hydrogens[13] and an ion of mass 231 representing a typical ring D cleavage.[14] The presence of only a very weak molecular ion in the mass spectrum of the corresponding sterol acetate suggested the Δ^5-location of the nuclear double bond. The C_{30} sterol of *Tethya aurantia* is thus 24-propylidene-cholest-5-en-3β-ol (**19**) which has previously been encountered[15] in the scallop *Placopecten magellanicus*. The identity of **19** and the (Z) stereochemistry of the $\Delta^{24(28)}$ double bond was established by comparing the mass spectrum and gas chromatographic retention time of the natural sterol with those of a synthetic sample prepared as follows: Ozonization of fucosterol acetate in the presence of a small amount of pyridine selectively cleaved the $\Delta^{24(28)}$ double bond to furnish 24-ketocholesteryl acetate (**20**) which was subjected to a Wittig reaction with n-propyl triphenylphosphorane using various bases (n-BuLi, NaH and KOH), a number of solvents (ether, THF, mixture of ether and THF and DMSO), various temperatures (33°–90°) and time (6 h–5 days) to furnish **19** in poor yield (10–15%). This should be contrasted with the appreciably higher yields in Wittig syntheses from **18** of 24-methylene cholesterol[16] (**13**) (71%) and isofucosterol[17] (**17**) (35%). The spectral and physical characteristics of our synthetic sample compared well with those reported[15] previously.

The more polar fractions from *T. aurantia* furnished a crystalline mixture of peroxides which after acetylation and preparative TLC furnished two fractions (*A* and *B*). The mass spectrum of fraction *A* depicted a molecular ion peak at *m/e* 470 ($C_{30}H_{46}O_4$) and important ions at *m/e* 410 (M^+–CH_3COOH), 378 (410–O_2), 213 (ring D cleavage + $O_2 + H_2O$). The NMR spectrum of *A* depicted C-18 and C-19 Me signals at 0·80, 0·90 δ, C-26,27 Me at 0·98 δ ($\Delta^{24(28)}$), [Δ^{22} sterols depict C_{26} and C_{27}, Me at 0·85 in contrast to C_{26} and $C_{27}CH_3$ (0·98 δ) of $\Delta^{24(28)}$ sterols],[17a] C-6,7 H, 6·20, 6·55 (d, J = 8 Hz), C-3 H, 5·00 (c) CH_3COO, 2·00 (s) and C-28 H, 4·70 (c). The above data established the presence of the Δ^6 and $\Delta^{24(28)}$-double bonds and the 5α,8α - epidioxy 3β - acetate groupings and thus suggested ergosta - 6,24(28) - dien - 5α,8α - epidioxy - 3β - acetate (**31a**) as the structure for fraction *A*. The mass spectrum of fraction *B* [*m/e* 486 (M^+), 426 (M^+-CH_3COOH), 384 (426-O_2); *m/e* 470 (M^+), 410 (M^+-CH_3COOH), 378 (410-O_2); *m/e* 458 (M^+), 398 (M^+-CH_3COOH), 366 (398-O_2)] suggested that it was composed of 3 compounds. The identity of these compounds as cholest - 6 - en - 5α,8α - epidioxy - 3β - ol (**29**), ergosta - 6,22 - dien - 5α,8α - epidioxy - 3β - ol (**30**) and 24ξ - ethyl - cholest - 6 - en - 5α,8α - epidioxy - 3β - ol (**32**) was established by sodium-ammonia reduction to furnish a mixture of triols (**33**, **34**, **35**), which were compared (GC-MS) with those derived by a similar sequence from authentic samples of 7-dehydrocholesterol (**29**), ergosterol (**30**) and 24 - (R) - ethyl - $\Delta^{5,7}$ - cholestadien - 3β - ol peroxides (**32**) prepared by photosensitized oxygenation.[18]

Ergosterol peroxide (**30**) has been isolated previously from the fungi *Daedalea quercina*[19] L., *Aspergillus fumigatus* fre,[20] *Trichophyton schonleini*,[21] *Rhizoctonia repens*,[22] *Cantharellus cibarius*,[23] *Lampteromyces Japonicus*,[24] and *Penicillum rubrum*.[25] It has been suggested that since fungal extracts contain pigments, ergosterol peroxide could be produced by photosensitized oxygenation[26] of ergosterol. In the case of *Fusarium moniliforme*[27] and *Cantharellus cibarius*, since isolation was effected in the absence of oxygen, ergosterol peroxide (**30**) is a true metabolite and could be an intermediate in cervisterol biosynthesis.[23]

The *in vivo* migration of the steroidal Δ^8 double bond to the Δ^5 position proceeds through the intermediate

$\Delta^{5,7}$-diene[28] and it has been suggested that ergosterol peroxide (**30**) might be a precursor to ergosterol.[29,30]

It is pertinent to note that singlet type oxygen could be generated by activation of molecular oxygen by haem proteins, central constituents of mixed function oxygenases of animals, insect and microorganism,[31,32] and in that case the formation of peroxides, even though carried out by $^1\Delta O_2$, would be a biological process. This point is substantiated by the recent isolation of prostaglandin endoperoxides on aerobic incubation[33] of arachidonic acid with a microsomal fraction of the vesicular gland of sheep. Whether indeed haem proteins are involved in the oxygenation of steroids in the sponge *Tethya aurantia* is not known.

In addition to sterols the sponge *Stelleta clarella* also contained $\alpha:\beta$-unsaturated ketones and stanols (Table 1). One member of this group depicted a molecular ion peak at *m/e* 368 ($C_{26}H_{40}O$) and important fragment ions at *m/e* 298 and 271 corresponding to cleavage of a $C_{20\text{-}22}$ bond with transfer of one hydrogen and loss of the entire side chain (C_7H_{13}) respectively. Its NMR spectrum depicted olefinic protons at 5·60 (C_6, H), 5·20 (C_{22}, C_{23}, H) and C-19 H_3 at 1·20 δ.[34] These data in conjunction with the UV spectrum (λ_{max} 232(hexane)) established the nuclear Δ^4-3-ketone moiety and suggests strongly that it is 24 - nor - cholesta - 4,22 - dien - 3 - one (**21**). Similar reasoning indicated that compounds **22**, **23**, **24** are cholesta - 4,22 - dien - 3 - one, cholest - 4 - en - 3 - one,[35] and ergosta - 4,22 - dien - 3 - one respectively. The mass spectrum of compound **25** ($C_{28}H_{44}O$) depicted in addition to the usual ions corresponding to loss of the side chain with transfer of two hydrogens (*m/e* 269) and ring D cleavage (*m/e* 231) a base peak at *m/e* 312 representing a McLafferty rearrangement due to the $\Delta^{24(28)}$ double bond. These data in conjunction with the NMR signals for C-28, C-4, and C-19 H_3 at 4·66, 5·57 and 1·20 respectively and the UV spectrum (λ_{max} 230(hexane)) suggested that **25** is ergosta - 4,24(28) - dien - 3 - one. Similarly **28** is (E) - stigmasta - 4,24(28) - dien - 3 - one as shown by comparison with an authentic specimen. To our knowledge this is the first report of the occurrence of Δ^4-3 ketones from marine sources.

The co-occurrence (Table 1) of stanols, Δ^4-3-ketones, and the corresponding Δ^5-3β-hydroxy-sterols is of particular interest and significance because it suggests that the sponge *Stelleta clarella* contains 3β-hydroxy dehydrogenase, Δ^5-isomerase and Δ^4-reductase systems. In addition to various lysases,[36] these enzymes are essential for the conversion of sterols to steroid hormones and their presence in various mammalian tissues is now well established.[37] It has recently been shown that gonads of the mussel *Mytilus edulis*[38] contain both 3β-hydroxy steroid dehydrogenases and Δ^5-isomerases and can convert sterols to steroid hormones. The available biochemical information is also suggestive of the presence of these enzymes in the mollusk *Crassostrea gigas*,[39] the starfish *Asterias rubens*,[40,41] *Porania pulvillus*[40] and *Marthasterias glaciallis*.[41] It should be noted that 24 - nor - cholest - 22 - en - 3β - ol (**1**) and cholest - 22 - en - 3β - ol (**2**) from the sponge *Hymeniacidon perleve* could arise by the reduction of the Δ^5 double bond of the corresponding diunsaturated sterols by a sequence outlined earlier.

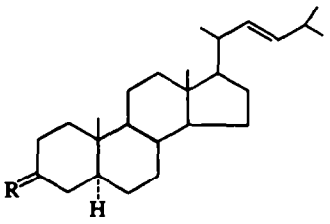
EXPERIMENTAL

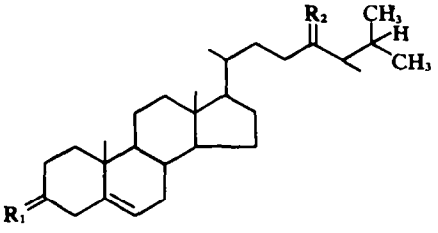
M.ps were determined with a Kofler hot stage apparatus and are uncorrected. IR spectra were obtained for a soln in $CHCl_3$ or as KBr pellets with a Perkin–Elmer 421 spectrometer. ^{1}H NMR spectra were obtained in $CDCl_3$ as a solvent and TMS as internal reference on a Varian XL-100 spectrometer. Optical rotations were recorded with a Perkin–Elmer model 141 spectropolarimeter for soln in $CHCl_3$. Low resolution mass spectra were obtained by Messrs. R. G. Ross and R. Conover with A.E.I. MS-9 and Atlas CH-4 spectrometers. GLC-MS were obtained by Miss Annemarie Wegmann using a Varian MAT 711 spectrometer. GLC was carried out with a Hewlett Packard hp 402 instrument. ORD and CD curves were determined for soln in dioxane by Mrs. R. Records with a JASCO ORD/UV 5 spectrometer with CD attachment. High resolution measurements were obtained with an MS-9 instrument by peak matching using perfluoro kerosene as a standard or with a MAT 711 instrument on line to the ACME Computer facility of the Stanford University Medical Center.

All sponges were collected in the Monterey, Pacific Grove (central California) area and were identified by spicule analysis. *Haliclona permollis*, *Stelleta clarella* were collected intertidally by Mr. Roy Johnson whereas *Lissodendoryx noxiosa*, *Haliclona sp.* were collected intertidally by one of the authors (Y.S.). *Tethya aurantia* was collected by Dr. W. Gladfelter during scuba dives near Pacific Grove. A second collection of *Tethya aurantia* was made by Mr. R. Johnson intertidally near Carmel. The quantities of the sponges available for chemical analysis were between 100 to 600 g wet weight. Sponges were steeped in 95% EtOH right after collection and were stored in dark bottles prior to extraction.

Extraction of sponges. The extraction procedure employed for *Stelleta clarella* typifies the general procedure. The sponge (600 g) was ground in 95% EtOH and finally steeped in a total of 3 l EtOH for 24 h. The mixture was filtered through Celite and the process repeated thrice with the filter cake. The filtrates were combined and evaporated to a gummy residue (with occasional addition of BuOH to prevent foaming) under vacuum and below 80°. Water (300 ml) was added to the gummy residue and the mixture was extracted thrice with ether. The ether extract, after evaporation furnished a dark gum (6·0 g).

The dark gum (2·5 g) was applied to a silica gel column (2·5 × 45 cm) and the column successively washed with hexane (1000 ml), hexane-benzene (1-1) (1000 ml), benzene (2000 ml), benzene-ether (9-1) (2000 ml), benzene-ether (7-3) (2000 ml), benzene-ether (1-1) (1000 ml) and ether (1000 ml), successively. Fraction (500 ml) was collected and examined by analytical TLC over silica gel HF 254 using a variety of solvent systems. Benzena-ether (9:1) and (7-3) eluent which revealed two major spots (one fluorescent and the other nonfluorescent) was combined and subjected to preparative TLC using benzene-ether (8-2) as solvent. The fluorescent, more mobile band was thus separated from the nonfluorescent sterol fraction. Analytical GLC of the sterol fraction over OV 3, 3% on Gas Chrom Q indicated the presence of 3 major and 5 minor components. This fraction was further fractionated by argentation silica gel preparative layer chromatography using benzene-hexane (6-4) as eluent (two developments) of the corresponding acetate into 6 bands which according to decreasing mobility were composed of the following constituents. Band 1: GC depicted one major (relative retention time 1·00; 90%); band 2: 2 minor peaks (r.rt. 1·10 and 1·55; 7 and 3%); band 3: one major (r.rt. 1·60, 80%) and 3 minor peaks (r.rt. 0·66, 0·90, 1·10 and 1·60; 3%, 10%, 6%); band 4: two major (r.rt. 1·55 and 1·61; 24, 60%) and two minor (r.rt. 1·10, 1·28; 2% and 10% peaks; band 5: one peak (r.rt. 1·28).





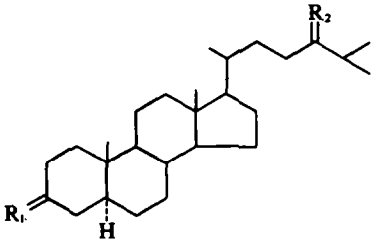
1: R = OH (H)

8: R = OH (H); Δ^5

8a: R = OAc (H); Δ^5

3: R_1 = OH (H); R_2 = CH_3 (H)

4: R_1 = OH (H); $R_2 = CH_2$



2: R_1 = OH (H); R_2 = H (H); Δ^{22}

5: R_1 = OH (H); $R_2 = H_2$

6: R_1 = OH (H); $R_2 = H_2$; Δ^5

7: R_1 = OH (H); R_2 = CH_3 (H)

9: R_1 = OH (H); R_2 = CH_2CH_3 (H)

10: R_1 = OH (H); $R_2 = H_2$; Δ^5, Δ^{22} (trans)

11: R_1 = OH (H); $R_2 = H_2$; Δ^5, Δ^{22} (cis)

12: R_1 = OH (H); R_2 = CH_3 (H); Δ^5, Δ^{22}

13a: R_1 = OH (H); $R_2 = CH_2$; Δ^5, $\Delta^{24(28)}$

13a: R_1 = OAc (H); $R_2 = CH_2$; Δ^5, $\Delta^{24(28)}$

14: R_1 = OH (H); R_2 = CH_3 (H)

15: R_1 = OH (H); $R_2 = CH_2CH_3$; Δ^5, Δ^{22}

16: R_1 = OH (H); $R_2 = CHCH_3$; Δ^5, $\Delta^{24(28)}$(E)

17: R_1 = OH (H); $R_2 = CHCH_3$; Δ^5, $\Delta^{24(28)}$(Z)

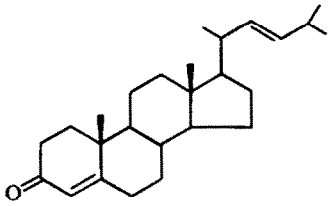
18: R_1 = OH (H); $R_2 = CH_2CH_3$; Δ^5

18e: R_1 = OAc (H); $R_2 = CH_2CH_3$; Δ^5

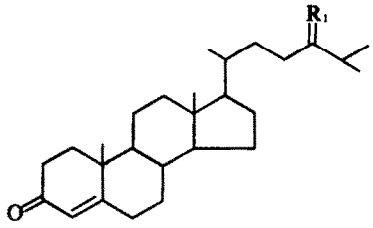
19: R_1 = OH (H); $R_2 = CHCH_2CH_3$; Δ^5, $\Delta^{24(28)}$(Z)

19a: R_1 = OAc (H); $R_2 = CHCH_2CH_3$; Δ^5, $\Delta^{24(28)}$(Z)

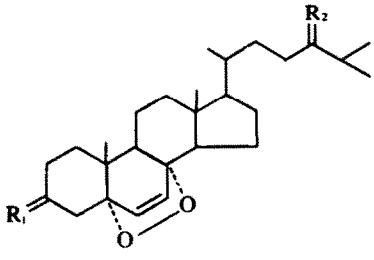
20: R_1 = OAc (H); R_2 = O; Δ^5



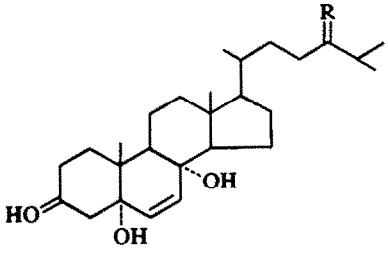
21



22: $R_1 = H_2$; Δ^{22}
23: $R_1 = H_2$
24: $R_1 =$; Δ^{22}
25: $R_1 = CH_2$; $\Delta^{24(28)}$
26: $R_1 =$
27: $R_1 =$; Δ^{22}
28: $R_1 = CHCH_3$; $\Delta^{24(28)}$(E)



29: $R_1 =$  ; $R_2 = H_2$
29a: $R_1 =$  ; $R_2 = H_2$
30: $R_1 =$  ; $R_2 =$  ; Δ^{22}
30a: $R_1 =$  ; $R_2 =$  ; Δ^{22}
31: $R_1 =$  ; $R_2 = CH_2$; $\Delta^{24(28)}$
31a: $R_1 =$  ; $R_2 = CH_2$; $\Delta^{24(28)}$
32: $R_1 =$  ; $R_2 = CH_2CH_3$



33: $R = H_2$
34: $R =$  ; Δ^{22}
35: $R = CH_2CH_3$

Gas chromatography coupled with mass spectrometry of combined bands 1 and 2 showed that the major peak was composed of equal amounts of cholesterol and cholestanol and one of the minor peaks consisted of 24ξ - ethyl - Δ^5 - cholesten - 3β - ol and poriferastanol. Repeated silver nitrate preparative layer chromatography further fractionated this mixture into saturated stanols and Δ^5 sterols (Table 1) which were finally purified by preparative gas chromatography. Bands 3 and 4 could be fractionated into the individual components only on preparative gas chromatography.

The fluorescent fraction was purified by preparative TLC (benzene as solvent and 2 developments) into a compact, strongly fluorescent band; however analytic GC showed it to be composed of 3 major (r.rt. 1·00, 1·28, 1·59) and 3 minor (r.rt. 0·66, 0·91, 1·10) components. Further fractionation of this band could only be effected by preparative TLC over 15% $AgNO_3$ impregnated silica

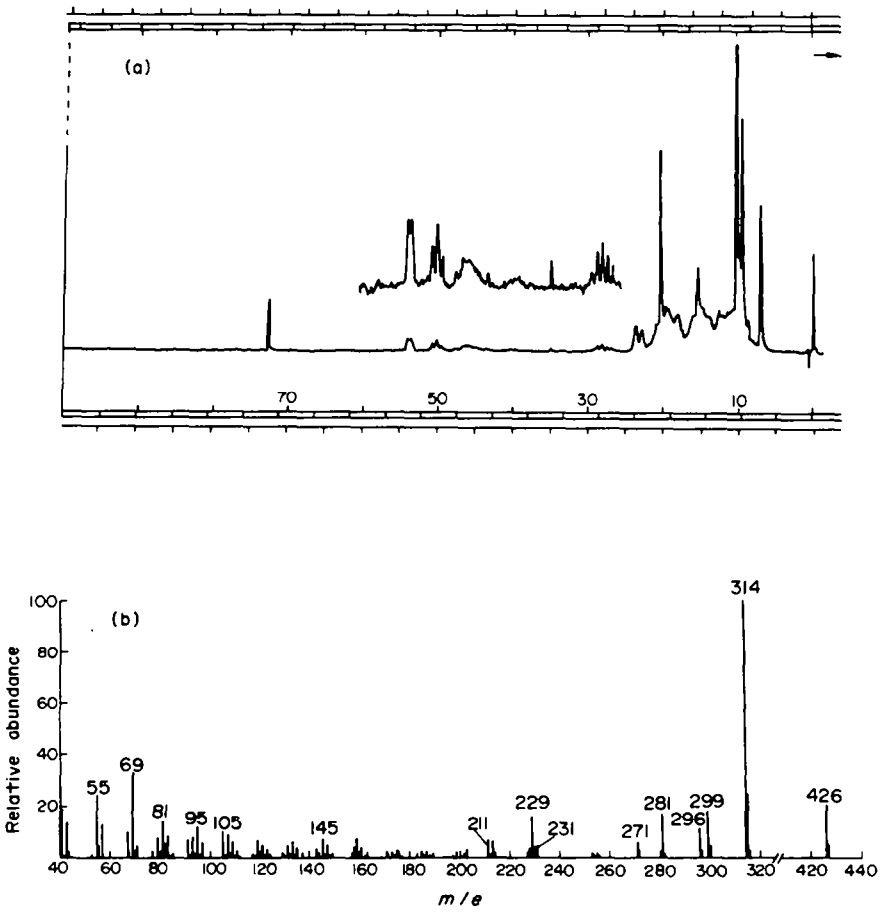


Fig. 1. *a.* 100 MHz NMR spectrum of synthetic (Z)-24-propylidene-cholest-5-en-3βyl-acetate (**19a**). *b.* 70 eV Mass spectrum of synthetic **19**.

gel plates into the following 3 fractions. Fraction 1 (most mobile): three major (r.rt. 1·0, 1·26 and 1·57) and two minor (r.rt. 0·90, 1·10) peaks; fraction 2 (intermediate mobility): two major (r.rt. 1·0, 1·57) and two minor (r.rt. 0·90, 1·10) peaks; fraction 3: one peak (r.rt. 1·26).

Repeated TLC of fraction 1 furnished the following major fractions:

Fraction 1-A: one major (**23**) (1·00, 65%) and two minor (**24, 27**) (r.rt. 1·10 and 1·55; 24% and 10%).

Fraction 1-B: one major (**28**) (r.rt. 1·57; 48%) and three minor (**22, 23, 24**) (r.rt. 0·90, 1·0, 1·10; 15%, 15% and 20%).

Fraction 1-C: one major (**28**) (r.rt. 1·58, 83%) and 3 minor (**21, 22, 24**) (r.rt. 0·65, 0·90, 1·10; 3%, 8%, 4%).

Final separation of these fractions was achieved by preparative gas chromatography.

Physical constants[4] of steroids isolated

Cholestanol (**5**). r.rt. 1·00, m.p. 142°; $[\alpha]_D$ + 24·0°; NMR: C-18, 0·65, C-19, 0·80, C-26,27, 0·83 (d, J = 6·5 Hz), C-3, 3·3–3·7 (b, 1H); MS 388 (79, M^+), 373 (40), 370 (11), 355 (20), 331 (4), 316 (4), 262 (16), 248 (16), 234 (70), 233 (100), 231 (13), 217 (36), 216 (41), 215 (92), 201 (13), 191 (7), 190 (9), 175 (8), 166 (19), 165 (44), 149 (23), 147 (28), 135 (26), 133 (26), 122 (26), 121 (40), 108 (50), 107 (60), 93 (42), 91 (25), 81 (60), 79 (35), 69 (38), 67 (41), 55 (58), 43 (51), 41 (39).

Cholesta - 5,22(cis) - dien - 3β - ol (**11**). r.rt. 0·89, m.p. 134–135·5°; NMR C-18, 0·70, C-19, 1·00, C-26,27, 0·85 (d, J = 6·5 Hz), C-21, 0·99 (d, J = 6·0 Hz), C-3, 3·60 (c), C-22,23, C-6, 5·28 (c); MS (acetate) *m/e* 366 (90%, M^+-CH_3COOH), 351 (100), 253 (8), 211 (10), 202 (19), 201 (8), 143 (59), 123 (40), 105 (64), 95 (31), 81 (35), 71 (35), 69 (41), 57 (60), 55 (57), 43 (64), 41 (55).

Cholesta - 5,22(trans - *dien* - 3β - *ol* (**10**). r.rt. 0·95, m.p. (acetate) 129–30°, $[\alpha]_D^{19} = -19·2°$; NMR (acetate), C-18, 0·70, C-19, 1·02, C-26,27, 0·87 (d, J = 6·5 Hz), C-21, 1·00 (d, J = 6·0 Hz), CH_3COO, 2·02, C-3, 4·60 (c), C-6,22,23, 5·20–5·40 (c); MS (acetate) *m/e* 366 (90, M^+-CH_3COOH), 351 (100), 253 (8), 211 (11), 143 (58), 127 (32), 105 (62), 91 (14), 81 (35), 71 (35), 69 (43), 57 (60), 55 (57), 43 (64), 41 (58).

24 - *Nor* - *cholesta* - 5,22(trans) - *dien* - 3β - *ol* (**8**). r.rt. 0·65, m.p. 137–138·5°; NMR C-18, 2·70, C-19, 1·01, C-26,27, 0·95 (d, J = 6·5 Hz), C-3, 3·50, C-6, 22,23, 5·10–5·40; MS *m/e* 370 (M^+, 100), 355 (17), 352 (27), 337 (18), 300 (55), 285 (24), 272 (23), 271 (43), 267 (10), 256 (17), 255 (75), 231 (10), 229 (11), 213 (22), 199 (12), 197 (11), 187 (10), 175 (11), 133 (14), 161 (22), 159 (38), 145 (35), 119 (20), 109 (19), 107 (28), 105 (28), 97 (54), 95 (25), 93 (25), 91 (27), 81 (37), 79 (21), 69 (23), 67 (21), 55 (55), 43 (33).

Ergosta - 5,22 - *dien* - 3β - *ol* (**12**). r.rt. 1·10, m.p. 157–158°; $[\alpha]_D^{19} = -39·4°$; NMR (acetate), C-18, 0·70, C-19, 1·02, C-26,27 H, 0·83 (d, J = 6·5 Hz), CH_3COO, 2·02, C-3, 4·60 (c), C-6 H, 5·36 (c), C-22,23, 5·17 (c); MS *m/e* 398 (M^+, 14), 380 (76), 365 (7), 300 (6), 255 (51), 228 (6), 213 (11), 173 (7), 161 (16), 159 (27), 147 (23), 145 (32), 133 (28), 107 (30), 105 (35), 95 (30), 93 (31), 91 (29), 81 (65), 79 (24), 69 (100), 67 (33), 55 (67), 43 (31).

24 - ξ - *Methyl* - *cholest* - 5 - *en* - 3β - *ol* (**14**). r.rt. 1·26; MS *m/e*

400 (61, M^+), 385 (12), 382 (25), 367 (15), 315 (23), 300 (5), 289 (30), 273 (22), 271 (20), 255 (24), 231 (17), 213 (22), 163 (20), 161 (26), 159 (30), 149 (13), 147 (22), 145 (30), 107 (50), 95 (49), 81 (57), 69 (100), 55 (76), 43 (93).

Ergosta - 5,24(28) - dien - 3β - ol (**13**). r.rt. 1·27, m.p. (acetate) 130–131°; $[\alpha]_D^{20} = -3·95°$; NMR (acetate), C-18, 0·62, C-19, 0·98, C-26,27,21, 0·96 (d, J = 6·5 Hz), CH$_3$COO, 1·98, C-3, 4·60 (c), C-28, 4·61, 4·65, C-6, 6·34 (c); MS *m/e* 398 (M^+, 7, 383 (7), 380 (28), 314 (53), 299 (13), 296 (13), 281 (14), 271 (26), 255 (15), 31 (8), 229 (13), 213 (22), 199 (8), 173 (11), 161 (21), 159 (28), 147 (26), 145 (43), 133 (30), 121 (30), 109 (30), 107 (52), 105 (51), 95 (51), 93 (44), 91 (42), 83 (37), 81 (72), 69 (65), 55 (100), 43 (46), 41 (70).

24ξ - Ethyl - cholesta - 5,22 - dien - 3β - ol (**15**). r.rt. 1·37; NMR C-18, 0·71, C-19, 1·08, C-26,27, 0·94 (d, J = 6·5 Hz), C-3, 3·40–3·80 (br), C-22,23, 5·13 (c), C-6, 5·37 (c); MS *m/e* 412 (50%, M^+), 397 (4), 394 (7), 369 (14), 351 (15), 314 (5), 300 (23), 272 (16), 271 (27), 255 (40), 231 (5), 229 (5), 213 (13), 159 (27), 151 (13), 147 (20), 145 (24), 133 (30), 123 (23), 121 (16), 119 (17), 109 (20), 107 (28), 105 (22), 97 (50), 95 (33), 93 (25), 91 (20), 85 (16), 83 (82), 81 (57), 79 (22), 71 (24), 69 (62), 67 (26), 57 (35), 55 (100), 43 (48), 41 (35).

24ξ - Ethyl - cholest - 5 - en - 3β - ol (**18**). r.rt. 1·56; NMR C-18, 0·68, C-19, 1·02, C-26,27, 0·83 (d, J = 6·5 Hz), CH$_3$COO, 2·02, C-3, 4·60 (c), C-6, 5·35 (c); MS *m/e* 414 (81%, M^+), 399 (25), 396 (54), 381 (24), 329 (23), 303 (41), 289 (4), 275 (10), 273 (18), 255 (22), 231 (15), 229 (7), 228 (7), 213 (28), 199 (8), 187 (8), 185 (7), 178 (11), 173 (12·5), 171 (8), 163 (21), 161 (27), 160 (17), 159 (29), 158 (12), 148 (30), 146 (44), 144 (13), 135 (26), 133 (29), 121 (30), 120 (28), 107 (50), 105 (38), 95 (52), 93 (36), 81 (62), 79 (28), 69 (45), 57 (57), 55 (74), 43 (100).

24 - Ethyl - cholestan - 3β - ol (**9**). r.rt. 1·56; NMR, C-18, 0·68, C-19, 0·89, C-3, 3·50 (b,c); MS *m/e* 416 (100%, M^+), 401 (17), 398 (4), 383 (8), 290 (6), 275 (5), 248 (14), 234 (53), 233 (61), 219 (31), 217 (53), 163 (45), 151 (7), 149 (16), 133 (17), 123 (24), 121 (27), 109 (20), 108 (42), 107 (47), 95 (45), 93 (30), 83 (13), 81 (45), 79 (20), 71 (18), 69 (35), 67 (26), 57 (36), 55 (50), 43 (63), 41 (27).

(E) - Stigmasta - 5,24(28) - dien - 3β - ol (**16**). r.rt. 1·56, m.p. 124°; $[\alpha]_D^{25} = -41·2°$; NMR (acetate), C-18, 0·71, C-19, 1·00, C-26,27, 0·97 (d, J = 6·5 Hz), C-29, 1·57, (d, J = 7·0 Hz), C-31, 4·60 (c), C-28, 5·17 (q, J = 7 Hz), C-6, 5·35 (c); MS *m/e* 412 (10, M^+), 379 (2), 314 (100), 299 (18), 296 (7), 281 (16), 271 (11), 229 (17), 69 (31), 55 (52), 43 (14).

(Z) - Stigmasta - 5,24(28) - dien - 3β - ol (**17**). r.rt. 1·60; m.p. 127–129°; NMR (acetate), C-18, 0·70, C-19, 1·00, C-26,27, 0·97 (d, J = 6·5 Hz), C-29, 1·57 (d, J = 7 Hz), C-3, 4·60 (c), C-28, 5·17 (q, J = 7 Hz), C-6, 5·35 (c), C-25, 2·80 (septet, 1H, J = 6 Hz); MS *m/e* 412 (53, M^+), 397 (9), 394 (20), 379 (10), 314 (100), 299 (59), 296 (43), 281 (60), 271 (36), 255 (16), 253 (14), 231 (20), 229 (50), 213 (32), 211 (27), 199 (14), 197 (16), 187 (13·5), 185 (12·5), 175 (13), 173 (16), 171 (13), 161 (29), 159 (30), 158 (20), 145 (37), 143 (22), 135 (23), 133 (29), 131 (21), 121 (26), 119 (28), 107 (41), 95 (45), 93 (34), 91 (36), 81 (50), 79 (31), 69 (55), 55 (90), 43 (34).

(Z) - 24 - Propylidene cholest - 5 - en - 3β - ol (**19**). r.rt. 1·86; MS *m/e* 426 (10, M^+), 408 (4), 314 (100), 299 (26), 296 (28), 281 (31), 271 (14), 269 (8), 253 (10), 229 (27), 213 (15), 211 (15), 199 (8), 197 (15), 173 (8), 160 (14), 159 (11), 158 (11), 147 (13), 145 (18), 143 (14), 135 (14), 133 (14), 131 (10), 121 (13), 119 (14), 109 (14), 107 (9), 105 (21), 95 (24), 93 (18), 91 (20), 83 (14), 81 (28), 79 (17), 78 (12), 69 (44), 67 (18), 55 (48), 43 (37).

24 - Nor - cholesta - 4,22 - dien - 3 - one (**21**). r.rt. 0·66; NMR, C-18, 0·72, C-19, 1·20 C-26,27, 0·95 (d, J = 6·5 Hz), C-21, 0·99 (d, J = 6 Hz), C-4, 5·60 (s), C-22,23, 5·20 (c); MS *m/e* 368 (80, M^+), 298 (100), 271 (60), 270 (34), 245 (36), 243 (10), 229 (22), 195 (26), 187 (10), 175 (20), 161 (14), 147 (32), 145 (14), 133 (14), 124 (32), 123 (20), 119 (20), 109 (20), 107 (20), 105 (22), 98 (40), 96 (30), 94 (20), 81 (16), 55 (30), 41 (24).

Cholesta - 4,22 - dien - 3 - one (**22**). r.rt. 0·91; MS *m/e* 382 (60, M^+), 367 (7), 298 (100), 283 (20), 271 (47), 270 (31), 256 (15), 245 (36), 243 (10), 229 (21), 227 (10), 176 (20), 165 (10), 163 (10), 159 (10), 149 (24), 147 (40), 145 (15), 143 (7), 132 (20), 124 (32), 123 (24), 121 (22), 111 (17), 109 (20), 107 (23), 105 (23), 95 (45), 93 (24), 81 (24), 79 (24), 77 (14), 67 (21), 55 (75), 41 (34).

Cholest-4-en-3-one (**23**). r.rt. 1·00; MS *m/e* 384 (40, M^+), 369 (16), 342 (15), 327 (4), 299 (7·5), 271 (10), 269 (5·5), 261 (21), 260 (14), 247 (7), 245 (6), 229 (34), 187 (7), 161 (8), 148 (10), 147 (19), 145 (7), 135 (19), 122 (12), 124 (100), 123 (15), 121 (15), 119 (11), 109 (15), 107 (18), 105 (15), 95 (20), 93 (17), 91 (18), 83 (10), 81 (20), 79 (16), 69 (18), 67 (14), 57 (20), 55 (28), 43 (30).

Ergosta-4,22-dien-3-one (**24**). r.rt. 1·10; MS *m/e* 396 (M^+, 60), 381 (5), 353 (26), 312 (9), 298 (50), 283 (15), 271 (63), 269 (46), 257 (10), 253 (17), 245 (38), 229 (17), 175 (13), 161 (16), 149 (24), 147 (30), 133 (16), 124 (24), 123 (25), 121 (18), 109 (37), 107 (25), 105 (22), 95 (40), 93 (30), 91 (26), 83 (51), 81 (45), 79 (21), 69 (93), 67 (37), 55 (100), 43 (43).

Ergosta - 4,24(28) - dien - 3 - one (**25**). r.rt. 1·28; m.p. 115°, $[\alpha]_D^{20} = +89·1°$; NMR, C-18, 0.72, C-19, 1·20, C-26,27, 1·00 (d, J = 6·5 Hz), C-4, 5·57(s), C-28, H, 4·60, 4·66; MS *m/e* 396 (41, M^+), 381 (12), 313 (60), 312 (100), 298 (27), 297 (36), 281 (10), 269 (48), 256 (12), 245 (12), 243 (14), 231 (20), 229 (16), 227 (15), 189 (8), 187 (7), 175 (9), 173 (8), 161 (10), 149 (16), 148 (11), 147 (18), 145 (8), 143 (10), 135 (26), 133 (15), 131 (10), 124 (29), 123 (15), 121 (16), 119 (15), 107 (22), 105 (17), 95 (30), 93 (17), 91 (22), 81 (29), 79 (20), 69 (32), 55 (50), 43 (16).

(E) - Stigmasta - 4,24(28) - dien - 3 - one (**28**). r.rt. 1·58; m.p. 94–95°; $[\alpha]_D^{20} = +80·0°$; NMR, C-18, 0·71, C-19, 1·18, C-26,27, 0·97 (d, J = 6 Hz), C-4, 5·59 (s), C-28, 5·11 (q), C-29, 1·55 (d, J = 6 Hz); MS *m/e* 410 (26, M^+), 312 (100), 297 (31), 269 (12), 256 (12), 245 (14), 243 (17), 231 (23), 229 (15), 227 (15), 189 (9), 187 (6), 148 (10), 147 (13), 136 (10), 135 (19), 133 (12), 132 (12), 124 (18), 123 (14), 121 (14), 119 (10), 109 (13), 107 (16), 105 (14), 97 (12), 95 (24), 93 (12), 91 (16), 83 (17), 81 (23), 69 (31), 67 (14), 55 (54), 43 (15).

Preparation of ergosta - 4,22 - dien - 3 - one (**24**), *stigmasta - 4,22 - dien - 3 - one* (**27**), *ergosta - 4,24(28) - dien - 3 - one* (**25**) *and (E) - stigmasta - 4,24(28) - dien - 3 - one* (**28**). The Δ^4 - 3 - ketones **24**, **25**, **27** and **28** were prepared from the corresponding sterols by Oppenauer oxidation. In a general procedure a mixture of sterol (20 mg), cyclohexanone (0·2 ml), dry toluene (3–5 ml) and aluminum isopropoxide (25 mg) was refluxed with vigorous stirring for 30 min. Removal of solvent followed by TLC furnished the corresponding Δ^4-3-ones.

Ergosta - 4,22 - dien - 3 - one (**24**). m.p. 128–9°; $[\alpha]_D^{20} = +44·6°$; NMR, C-18, 0·73, C-19, 1·19, C-26,27, 0·83, 0·84 (d, J = 6·5 Hz), C-21 and C-28, 0·90, 1·01 (d, J = 6 Hz), C-22, 23, 5·20 and C-4, 5·73 (b) mass spectrum and retention times identical to that of natural **24**.

Ergosta - 4,24(28) - dien - 3 - one (**25**). m.p. 111–113°; NMR and mass spectrum identical to those of the natural **25**.

(E) - Stigmasta - 4,24(28) - dien - 3 - one (**28**). m.p. 95–96°; NMR and mass spectra identical to those of the natural **28**.

Stigmasta - 4,22 - dien - 3 - one (**27**). m.p. 119–120°; NMR C-18, 0·73, C-19, 1·18; C-26,27,21, 0·80–1·10 (c), C, 22,23, 5·15 (c), C-4, 5·75 (s); MS *m/e* 410 (35, M^+), 367 (34), 349 (3), 312 (7), 298 (25), 271 (53), 269 (22), 255 (10), 247 (23), 231 (7), 229 (10), 175 (8), 173 (5), 161 (10), 149 (17), 147 (18), 145 (8), 137 (11), 135 (11), 133 (12), 124 (14), 123 (124), 121 (13), 119 (10), 109 (15), 107 (27), 105 (16), 97 (40), 95 (31), 93 (22), 91 (18), 83 (51), 81 (43), 79 (21), 69 (50), 56 (23), 55 (100), 43 (35), 41 (35).

Synthesis of (Z) - 24 - propylidene - cholest - 5 - en - 3β - ol (**19**)

(a) *24 - Keto cholesteryl acetate* (**20**). Dichloromethane (100 ml) was saturated with O_3 (45 min, Dry Ice-acetone bath). This soln was poured in one stream in a cooled (Dry Ice-acetone) soln of fucosterol acetate (1·0 g) in dichloromethane (30 ml) containing 3–5 drops of pyridine. The mixture was vigorously stirred for 15 min and Zn powder (2·0 g) and AcOH (10·0 ml) were added.

The mixture was allowed to come to room temp and filtered. The residue was washed with dichloromethane the combined filtrates were evaporated to furnish a colorless gum. Preparative TLC furnished unreacted fucosterol acetate (200–300 mg) and **20** (600 mg) which was crystallized as needles from aqueous MeOH; m.p. 129–130°; $[\alpha]_D^{20} = -42{\cdot}6°$; lit.[15] m.p. 127·5–128°; $[\alpha]_D = -41°$; NMR, C-18, H, 0·68, C-19, H, 1·02, C-26,27, H, 1·09 (d, J = 6·5 Hz), C<u>H</u>$_3$COO, 2·02, C-3, H, 4·60 (b), C-6, H, 5·38 (c); MS *m/e* 382 (100%, M^+-CH_3COOH), 367 (7), 296 (11), 281 (4), 274 (2), 261 (5), 255 (16), 228 (5), 213 (12), 159 (11), 147 (20), 143 (8), 133 (12), 121 (11), 107 (19), 105 (18), 95 (14), 93 (15), 91 (13), 81 (25), 79 (11), 71 (22), 69 (8), 67 (13), 55 (20), 43 (49), 41 (15).

(b) *Wittig reaction.* To a stirred suspension of propyl triphenyl phosphine bromide (4·45 g) in ether (35 ml) was added BuLi (4·0 ml, 1·6N). The mixture was stirred at room temp under N_2 for 5 h. Compound **20** (450 mg) in ether (20 ml) was added and the mixture stirred for 12 h. Dry THF (30 ml) was added and the ether distilled until the temp of the vapor rose to 60°. The mixture was then stirred vigorously while refluxing for 80 h. The mixture was cooled and poured into water and extracted with ether. The ether layer was washed with water, 2N HCl and water, dried over Na_2SO_4 and evaporated to furnish a gum which was acetylated with Ac_2O and pyridine (15 ml each). The excess reagent was removed under vacuum and the residue subjected to preparative TLC (benzene) to furnish impure **19** (30 mg, 10%). This reaction was repeated several times and the pooled products subjected to preparative layer chromatography after acetylation. The isolation of the band, which was slightly more mobile than fucosterol acetate, followed by several crystallizations furnished pure **19a** purity (95%); r.rt. 1·86; m.p. 98–100°; $[\alpha]_D^{20} = -38{\cdot}2°$; NMR, (Fig 1) C-18, 0·68, C-19, 1·00, C-26,27, 0·97 (d, J = 6 Hz), CH_3COO, 2·02, C-25, H, 2·80 (heptet, J = 6·5 Hz), C-3, 4·60 (b), C-28, 5·00 (t, J = 6 Hz), C-6, 5·36 (c); MS *m/e* 408 (22%, M^+-CH_3COOH), 296 (10), 281 (16), 255 (6), 253 (16), 228 (7), 213 (11), 211 (6), 161 (9), 159 (10), 147 (15), 145 (19), 143 (7), 137 (7), 135 (12), 133 (6), 121 (11), 119 (10), 109 (13), 107 (17), 105 (16), 97 (13), 95 (24), 93 (17), 91 (13), 83 (18), 81 (34), 71 (16), 69 (40), 67 (18), 58 (14), 57 (34), 55 (40), 43 (53), 41 (36). Saponification of **19a** furnished **19** (purity > 95% < 98%); m.p. 110–112°; $[\alpha]_D^{19} = -33°$; lit.[15] m.p. 110–111°; $[\alpha]_D^{20} = -27°$ for naturally occurring material; NMR C-18, 0·69, C-19, 1·00, C-26,27, 0·97 (d, J = 6·5 Hz), C-25, 2·80 (hept, J = 6·5 Hz), C-3, 3·52 (c), C-28, 5·02 (t, J = 6·0 Hz), C-6, 5·36 (c); MS (Fig 1) *m/e* 426 (20, M^+), 314 (100), 299 (18), 296 (11), 281 (17), 271 (6), 229 (16), 213 (6), 211 (6), 169 (6), 145 (7), 133 (7), 119 (6), 107 (9), 95 (12), 91 (8), 83 (8), 81 (5), 79 (8), 67 (10), 57 (12), 55 (24), 43 (13), 41 (13). The relative retention times, mass spectra and NMR spectra were identical with those of the natural material.

Tethya aurantia. The material eluted with benzene-ether (7-3) by column chromatography of crude *Tethya aurantia* extract was further purified by preparative layer chromatography (benzene-ether (1:1)). The pale band on elution with ether, and repeated crystallization from hexane furnished shiny needles; m.p. 111–113°; NMR 0·80 (s), 0·89 (s), 4·00 (c,b), 4·75 (c, weak < 1H), 5·2 (c, weak < 1H), 6·24 (d, J = 8·0 Hz), 6·52 (d, J = 8·0 Hz). Weak signals at 4·75 (24-methylene) and 5·20 (C-6, H) indicated that the crystals were composed of a mixture. This was confirmed by high resolution mass measurements which demonstrated the presence of four compounds: *A* *m/e* 444 ($C_{29}H_{48}O_3$), 412 (444-O_2, $C_{29}H_{48}O$); *B* 442 ($C_{29}H_{46}O_3$), 410 (442-O_2, $C_{29}H_{46}O$); *C* 428 ($C_{28}H_{44}O_3$), 396 (428-O_2, $C_{28}H_{44}O$); *D* 416 ($C_{27}H_{42}O_3$), 384 (416-O_2, $C_{27}H_{42}O$): Further fractionation of this mixture was not possible, however the corresponding acetates could be separated into the following components:

(*a*) *Ergosta - 5,7,24(28) - trien - 3β - yl acetate 5α,8α - peroxide* (**13a**). NMR C-18, 0·80, C-19, 0·90, C-26,27, 0·98 (d, J = 6·5 Hz), C-28, 4·70 (c), C-6,7, 6·20, 6·55 (d, J = 8 Hz), C-3, 5·00 (c), C<u>H</u>$_3$COO, 2·00 (s); MS *m/e* 470 (3%, M^+), 455 (1), 410 (7), 392 (14), 378 (100), 243 (6), 241 (4), 213 (6), 211 (6), 199 (6), 197 (6), 95 (5), 158 (30), 145 (18), 143 (20), 135 (14), 133 (11), 123 (13), 121 (15), 119 (14), 109 (24), 107 (24), 105 (22), 97 (17), 95 (37), 93 (30), 91 (22), 83 (31), 81 (53), 79 (30), 77 (12), 71 (16), 69 (56), 67 (36), 60 (25), 57 (35), 55 (78), 45 (23), 43 (66), 41 (48).

(*b*) *Cholesta - 5,7 - dien 3β - yl acetate 5α,8α - peroxide* (**29a**); *24ξ - ethylcholesta - 5,7 - dien - 3β - yl acetate 5α,8α - peroxide* (**32**) and *24β - methylcholesta - 5,7,22 - trien - 3β - yl acetate 5α,8α - peroxide* (**30**). The NMR of this mixture differed from that of **31a** by the absence of a signal at 4·70 δ. The identity of the 3 components was established as follows. The above mixture (10 mg) in ether (10 ml) was added to a soln of Na (200 mg) in ammonia (40 ml) at Dry Ice-acetone temp. After 1 h, the reaction was quenched with ammonium chloride and the residue, after evaporation of ammonia, was extracted with chloroform and the chloroform extract examined by GC-MS.

GLC Peak 1: retention time and MS identical to those derived from **29** by a similar sequence; MS *m/e* 382 (61%, M^+-2 × H_2O), 364 (100), 349 (48), 253 (20), 251 (48), 209 (60), 207 (28), 197 (65), 195 (60), 183 (32), 181 (34), 169 (30), 167 (23), 165 (30), 157 (28), 155 (35), 135 (32), 91 (30), 81 (26), 69 (40).

GLC Peak 2: retention time and MS identical to those derived from **32**; MS *m/e* 410 (40%, M^+-2 × H_2O), 392 (100), 377 (36), 253 (17), 251 (40), 209 (40), 207 (30), 197 (60), 195 (30), 135 (30), 43 (100).

GLC Peak 3: retention time and MS identical to those derived from **30**; MS *m/e* 394 (M^+-2 × H_2O, 50%), 376 (56), 361 (15), 251 (100), 69 (50).

A second batch of *Tethya aurantia* (100 g) from Carmel was checked for peroxide content. Isolation was attempted in the absence of oxygen and light, however complete exclusion of either was not possible. A total of 40 mg of peroxides mixture was isolated.

24 - (*R*) - *Ethyl* - $\Delta^{5,7}$ - *cholestadien* - 3β - *ol.* Stigmasterol i-methyl ether (2·05 g) in MeOH (15 ml)-EtOAc (40 ml) was hydrogenated over PtO_2 (300 mg) for 24 h at 30 psi. The mixture was filtered and the filtrate evaporated to furnish a gum to which glacial AcOH (50 ml) and anhyd zinc acetate (4·5 g) were added and the mixture refluxed with vigorous stirring for 4 h, cooled and poured into water and extracted with ether. The ether layer was washed with $NaHCO_3$aq, dried over Na_2SO_4 and evaporated to a white solid which was crystallized thrice from EtOH to furnish pure stigmasta-5-en-3β-yl-acetate (1·7 g); m.p. 127°; [4]NMR C-18, 0·68, C-19, 1·02, C-26,27, 0·83 (d, J = 6·5 Hz), C<u>H</u>$_3$COO, 2·02, C-3, 4·60 (c), C-6, 535 (c) which on saponification furnished the free sterol, m.p. 136–7°;[4] $[\alpha]_D^{20} = -31{\cdot}9°$; MS *m/e* 412 ($M^+$).

A mixture of stigmasta - 5 - en - 3β - *yl - acetate* (0·85 g) N-bromosuccinimide (0·43 g) and n-hexane (20 ml) was heated under reflux with two photo flood lamps for 8 min. γ-Collidine (0·5 ml) was then added and the mixture was cooled and filtered. The filtrate was evaporated under vacuum and the residue refluxed for 30 min with xylene (10 ml) and γ-collidine (1·0 ml) under N_2. The mixture was poured into hexane, the hexane extract thoroughly washed with water, dried over Na_2SO_4, and evaporated to furnish a gum which was purified by preparative TLC over 15% $AgNO_3$ impregnated silica gel to furnish pure 24(R) - etylcholesta - 5,7 - dien - 3β - yl acetate; m.p. 151°. LAH cleavage of the acetate furnished 24(R) - ethylcholesta - 5,7 - dien - 3β - yl acetate; m.p. 151°. LAH_4 cleavage of the acetate furnished 24(R) - ethylcholesta - 5,7 - dien - 3β - ol; m.p. 137–140°; $[\alpha]_D = -107°$; NMR C-18, 0·63, C-19, 0·93, C-26,27, 0·83 (d, J = 6·5 Hz), C-3, 3·65, C-6,7, 5·42, 5·56 (c); MS *m/e* 412 (51%, M^+), 379 (82), 353 (35), 253 (37), 211 (22), 199 (20), 197 (14), 157 (31), 145 (45), 143 (52), 119 (28), 95 (23), 69 (36), 57 (42), 43 (100).

7 - *Dehydrocholesterol, ergosterol and* 24 - (*R*) - *ethyl* - $\Delta^{5,7}$ - *stigmastadien* - 3β - *ol peroxides.* The steroidal $\Delta^{5,7}$-diene (200 mg) was dissolved in 95% EtOH (200 ml), eosin-Y (10–20 mg) was

added and a stream of air was bubbled through the soln with stirring. The soln was irradiated with a pyrex flood lamp and cooled by a water jacket. After 2 h, the mixture was evaporated to a bright red gum which was chromatographed over silica gel plates using benzene-ether (1-1) as eluent. The nonfluorescent band was eluted with ether. Evaporation of the ether extract followed by crystallization of the residue from hexane furnished the pure peroxides.

7-Dehydrocholesterol peroxide (**29**). m.p. 150°; $[\alpha]_D^{20} = -5{\cdot}0°$; NMR C-18, 0·80, C-19, 0·91, C-26,27,21, 0·80–0·95 (c), C-3, 3·95, C-6,7, 6·24 (d, J = 8 Hz) and 6·55 (d, J = 8 Hz); MS *m/e* 416 (2, M^+), 398 (4), 384 (100), 364 (6), 351 (23), 325 (10), 208 (3), 195 (2), 169 (3), 157 (4), 156 (3), 155 (3), 150 (5), 141 (3), 117 (4), 107 (3), 105 (4), 94 (6), 92 (4), 81 (9), 69 (8), 67 (4), 46 (11), 55 (14), 44 (19), 43 (15), 41 (13); **29** furnished the acetate **29a**; m.p. 153–154°.

Ergosterol peroxide (**30**). m.p. 176–8°; $[\alpha]_D^{19} = -25°$; NMR C-18, 0·82, C-19, 0·91, C-26,27,21, 0·85–1·00 (c), C-3, 3·90, C-22-23, 5·22 (c), C-6,7, 6·26 (d, J = 8 Hz, 1H) and 6·54 (d, J = 8 Hz, 1H); *m/e* 428 (1, M^+), 396 (100), 363 (23), 337 (8), 271 (4), 253 (8), 251 (4), 211 (4), 199 (2), 197 (2), 159 (5), 152 (5), 143 (5), 109 (7), 107 (7), 105 (5), 95 (8), 93 (8), 91 (6), 84 (59), 83 (23), 81 (17), 79 (7), 70 (32), 69 (41), 67 (12), 57 (28), 55 (38), 44 (26), 43 (28), 41 (35); **26** furnished the acetate **30a**; m.p. 197–199°.

24(R) - Ethylcholesta - 5,7 - dien - 3β - ol peroxide (**32**). m.p. 145–6°; $[\alpha]_D^{19} = 0{\cdot}0°$; NMR C-18, 0·80, C-19, 0·88, C-3, 4·0, C-6,7, 6·24, 6·52 (d, J = 8 Hz, 1H each); MS *m/e* 444 (13%, M^+), 426 (19), 412 (48), 401 (10), 398 (9), 393 (10), 379 (14), 161 (14), 152 (21), 145 (10), 135 (11), 133 (9), 123 (11), 121 (12), 119 (10), 109 (14), 107 (22), 105 (13), 98 (17), 95 (28), 93 (25), 91 (14), 85 (18), 83 (16), 81 (40), 79 (15), 71 (24), 69 (37), 67 (20), 57 (45), 55 (61), 43 (100), 41 (45).

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Note added in proof. Subsequent to submission of our manuscript there appeared a paper by E. Fattorusso, S. Magno, C. Santacroce and D. Sica, *Gazz. Chim. Ital.* **104**, 409 (1974) recording the isolation of ergosterol peroxide and 5,8-epidioxycholesta-6,22-dien-3β-ol from the sponge *Axinella cannabina*.