137. Minor and Trace Sterols in Marine Invertebrates. XII[1]). Occurrence of 24(*R*+*S*)-Isopropenylcholesterol, 24(*R*+*S*)-Methylcholesta-5,25-dien-3β-ol, and 24(*R*+*S*)-Methylcholesta-7,25-dien-3β-ol in the Caribbean Sponge, *Verongia cauliformis*[2])

by **W.C.M.C. Kokke**[a])[b]), **C.S. Pak**[b]), **William Fenical**[a]), and **Carl Djerassi**[b])

A joint contribution from (a) the Institute of Marine Resources, Scripps Institution of Oceanography, La Jolla, California 92093,

and (b) the Department of Chemistry, Stanford University, Stanford, California 94305

(21.III.79)

Summary

In addition to the two new sterols verongulasterol **11** and 25-dehydroaplysterol **13** of *Verongia cauliformis*[3]), which were reported earlier [2] [3], the minor and trace sterols of this sponge include five new sterols listed in the title (with the exception of the known 24*S*-methyl-cholesta-5,25-dien-3β-ol (codisterol, **1b**)). The isolation of the 24(*R*)-epimer of codisterol is of interest, as this compound is a possibly biosynthetic precursor for aplysterol **12**, 25-dehydroaplysterol **13**, and verongulasterol **11** (all 24*R*) which occur in the same sponge [2]. A partial synthesis from fucosterol (**4**) of 24(*R*+*S*)-isopropenylcholesterol (**9**), and of 24-isopropylcholesterol (**10**) is described.

Introduction. – About 5000 sponges are known to occur in nature [4]. *Goad* [5] lists 78 species from which the sterols have been studied[4]). Generally speaking, the available information [5] confirms what one would expect: sponges, being filter feeders, normally contain a complex mixture of sterols, but the components of the mixture are not necessarily only dietary sterols, as it has been demonstrated [6] that some sponges are able to modify dietary sterols.

Pseudaxinyssa sp. (Class *Desmospongiae,* Order *Halichondrida*) was reported [7] to contain two new sterols, 24-isopropylcholesterol (**10**) and 24-isopropyl-22-dehydrocholesterol, which were, for all practical purposes, the only sterols in that sponge. Because an isopropyl substituent in the side chain was unprecedented[5]), and because the unusual simplicity of the mixture of *Pseudaxinyssa* sterols, these might well be modified dietary sterols.

---

[1]) For paper XI in this series, *cf.* [1].

[2]) Inshore Marine Shallow Water Ecosystem Project Contribution No. 47.

[3]) In two publications [2] [3] this sponge was misnamed *Verongula cauliformis.*

[4]) Part of the work was done before the advent of modern methods for separation and analysis.

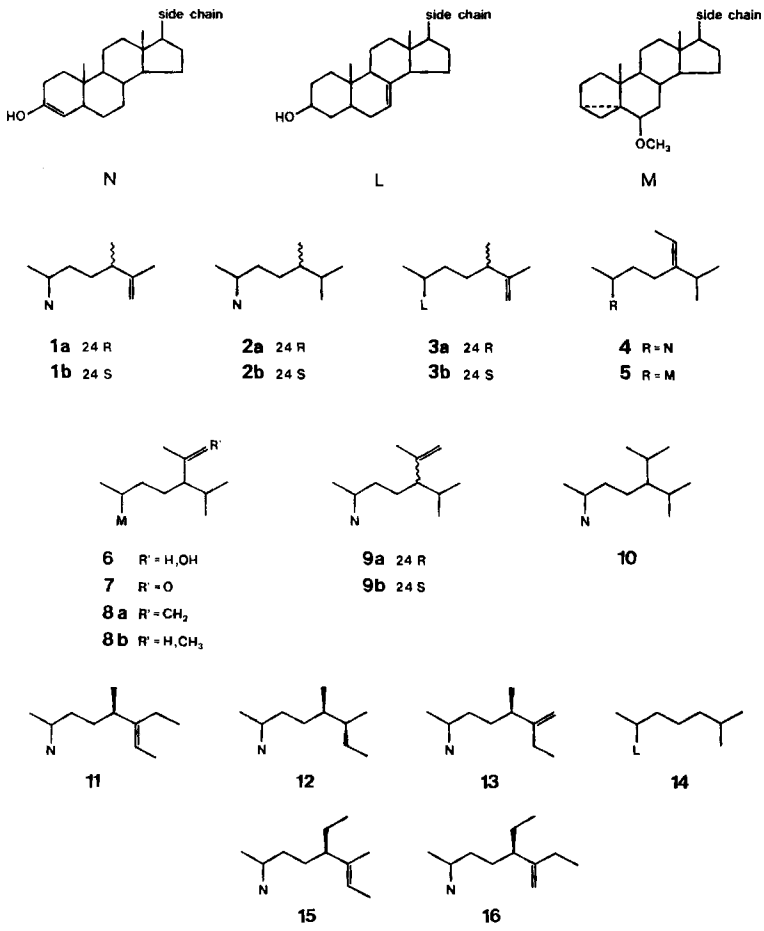
[5]) The occurrence of such side chains had been predicted by a biosynthetic computer program [9].

0018-019X/79/4/1310-09$01.00/0

So far, only the genus *Verongia* has been demonstrated to be homogeneous as far as sterols is concerned [8]. The genus *Pseudaxinyssa* is not homogeneous, as we did not find interesting sterols in *P. pitys*[6]).

We report the isolation of a compound, structurally related to the sterols of *Pseudaxinyssa sp.* [7], viz. 24-isopropenylcholesterol (**9**), and of several other novel marine sterols, which occur as minor or trace sterols in the sponge *Verongia cauliformis*[3]) (Class *Desmospongiae,* Order *Verongida*).

Results and discussion. – *24-Isopropenylcholesterol and 24-Isopropylcholesterol.* Two new sterols of mol.-wt. 426 have been isolated from *V. cauliformis* (*cf.* experimental part); one of these sterols (verongulasterol, **11**) has already been reported [2], the other (obtained in 0.5% yield)[7]) was identified as 24-isopropenylcholesterol (**9**).



[6]) *Wratten & Faulkner* [10] isolated isonitrile dichlorides from this sponge. The main sterols were found to be (GC./MS.) 24-methylcholesta-5,7,22-trien-3β-ol, and its 24-ethyl homolog.

[7]) Yield in % of total free sterols.

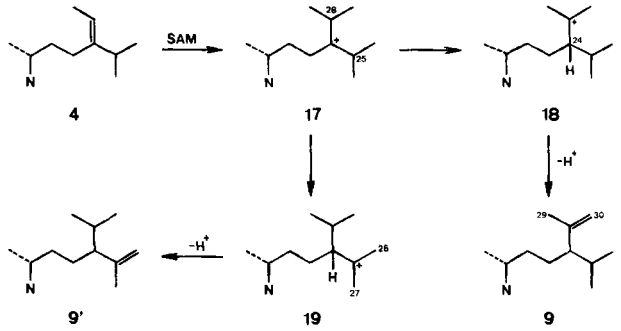
The mass spectrum of **9** is qualitatively identical to, and quantitatively slightly different from the mass spectra of the following sterols with the same mol.-wt.: stelliferasterol (**15**) [12], strongylosterol (**16**) [13], and verongulasterol (**11**) [2]. As explained before [2], the ring fragmentation in the mass spectra of these sterols is consistent with the presence of a C(5),C(6)-double bond, whereas the occurrence of both a 314 and 328 peak is indicative of the presence of a C(25),C(26)-double bond. In the 360-MHz-NMR. spectrum ($CDCl_3$) two olefinic singlets (δ 4.734, 4.603 fine splitting) and a methyl at a double bond (δ 1.563) define an isopropenyl moiety; further NMR. data are included in the *Table*.

To confirm the assigned structure, a partial synthesis was carried out starting with fucosterol (**4**). The double bond in the nucleus of fucosterol was protected, in the usual manner, as the i-methyl ether (**5**) by solvolysis of fucosteryl tosylate in methanol in the presence of potassium acetate. The i-methyl ether **5** was subjected to hydroboration to give alcohol **6**, which was then oxidized to the ketone **7**. A *Wittig* reaction on the ketone **7** followed by hydrolysis of the i-methyl ether afforded the desired 24-isopropenylcholesterol **9**.

The 360-MHz-NMR. spectra of synthetic and natural **9** are almost superimposable, although there are minor differences. Synthetic **9** is obviously a 1:1 mixture of both epimers at C(24), which is reflected in the occurrence of two C(18) methyl peaks, and in a complex isopropyl region (*i.e.* four overlapping nonequivalent methyl doublets) in the NMR. spectrum *(cf. Table)*. One of the epimers could be isolated (reverse phase HPLC. [11]) in almost pure form from the synthetic mixture, but the configuration at C(24) of this compound could not be ascertained. The methyl region in the 360-MHz-NMR. spectrum of natural **9** was equally complex, but the ratio of the intensities of the C(18) singlets indicated it to be a 23:77 mixture of two epimers. The minor component of the natural mixture should be the same as the epimer obtained in pure form from the synthetic mixture.

A slight modification of the above synthetic scheme allowed us to prepare 24-isopropylcholesterol (**10**), one of the *Pseudaxinyssa* sterols [7], which has not been synthesized before. Thus the double bond in the side chain of the i-methyl ether **8a** was hydrogenated; deprotection then gave 24-isopropylcholesterol (**10**). *Hofheinz*'s natural product [7] and our synthetic material were identical by 360-MHz-NMR. *(cf. Table)* and gas chromatographic mobility.

The biosynthetic implications of these isopropyl-containing marine sterols are interesting. First, sterols **9** and **10** are unique, branched side chain sterols which almost certainly arise from biological methylation of fucosterol by means of *S*-adenosylmethionine (SAM.). However, biosynthetically it is not clear whether the double bond of 24-isopropenylcholesterol terminates at position 28 or 25. The initial bioalkylation product of fucosterol would be the carbenium ion **17** which now can undergo an interesting bifurcation process. Migration of the C(28) proton to C(24) affording **18** followed by loss of either the C(29) or C(30) proton would provide 24-isopropenylcholesterol (**9**). However, one could equally well visualize migration of the C(25) proton in **17** to provide the isomeric tertiary carbenium ion **19** and this, upon loss of a C(26) or C(27) proton, would provide 25-dehydro-24-isopropylcholesterol (**9'**).



Suitable distinctions between these two paths could only be accomplished by appropriate labeling experiments. In either event either **9** or **9'** would appear to be plausible biosynthetic intermediates to *Hofheinz*'s [7] 24-isopropylcholesterol (**10**).

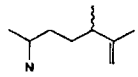
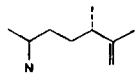
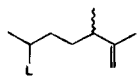
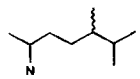
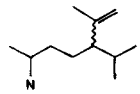
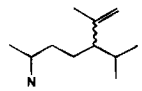
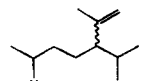
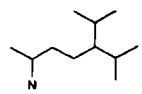
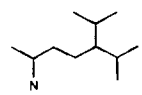
24-Methylcholesta-5,25-dien-3β-ol and its $\Delta^{7,25}$-isomer. The minor sterol of the methylidene sterol fraction (Fr. 6, *cf.* experimental part), obtained in 0.2% yield[7]), was identified by 220-MHz-NMR. as 24-methylcholesta-5,25-dien-3β-ol (**1**), thus as either codisterol (**1b**) or its as yet unknown epimer at C(24) ('epicodisterol', **1a**), or as a mixture of both.

Since epicodisterol **1a** would have a 24*R* configuration [15], it would be a conceivable biosynthetic precursor for aplysterol (**12**) [16], 25-dehydroaplysterol (**13**) [2], and verongulasterol (**11**) [2] in the same sponge, all of which have the 24*R* configuration. Thus it was necessary to establish the relative configuration at C(24).

24-Methylcholesta-5,25-dien-3β-ol (**1**) from *V. cauliformis* was shown to be a 23:77 mixture of C(24)-epimers by 360-MHz-NMR. *(cf. Table);* the minor component had NMR. data in agreement with those of a reference sample of codisterol (**1b**). Hence it was assumed that the main component was epicodisterol (**1a**). To check this assumption the mixture was selectively hydrogenated, using a homogeneous catalyst [17], to give a mixture of what should be campesterol (**2a**) and dihydrobrassicasterol (**2b**) if our assumption was correct. Indeed, 360-MHz-NMR. data *(Table)* for this mixture of hydrogenation products prove that it is a mixture of the expected compounds, whose NMR. data are known [14]. In this manner we established that *V. cauliformis* contains epicodisterol (**1a**) – an observation that may be of considerable biogenetic significance [3].

A trace sterol (yield[7]) 0.02%) of the methylidene sterol fraction (Fr. 6, *cf.* experimental Part) had the same mol.-wt. as codisterol (**1b**). The mass spectrum of this trace sterol supports a Δ^7-sterol nucleus with a double bond in the side chain: a 246 peak (loss of the side chain and part of the D-ring is characteristic for Δ^7-sterols [18], and a 271 peak (loss of side chain and 2 H, characteristic for sterols with a double bond both in the ring and in the side chain [19]). The side chain fragmentation with a low 314 peak (*McLafferty* rearrangement) and a 328 peak not resolved from the background spectrum, indicates either a 24(28), 24(25), or 25(26) double bond.

Table. *Methyl regions of 360-MHz-NMR. spectra of some selected sterols*[8][9])

		C(18)	C(19)	C(21)	C(26)	C(27)	C(28)	C(29)
1	 Natural product	0.674	1.008	0.916 *d*, *J* = 6.5		1.636 (23%) (24*S*) 1.651 (77%) (24*R*)	0.987 *d*, *J* = 6.9 0.991 *d*, *J* = 6.9	
1b	 Ref. compound.[10])	0.672	1.006	0.911 *d*, *J* = 6.5		1.636	0.992 *d*, *J* = 6.9	
3	 Natural product	0.528	0.794	0.920 *d*, *J* = 6.4		1.641 (31%) (24*S*) 1.651 (69%) (24*R*)	0.989 *d*, *J* = 6.9	
2	 Made from **1** above[11])	0.680	1.008	0.911 (24*R*) *d*, *J* = 6.8 0.918 (24*S*) *d*, *J* = 6.5	0.851 *d*, *J* = 6.8	0.802 (65%) *d*, *J* = 7.2 0.783 (35%) *d*, *J* = 6.8	0.772 *d*, *J* = 7.2	
9	 Natural product	0.666 (77%) 0.672 (23%)	1.006	0.803 *d*, *J* = 6.0	0.925 *d*, *J* = 4.3	0.908 *d*, *J* = 4.3		1.564
9	 Synthetic	0.665 (50%) 0.672 (50%)	1.006	0.801 *d*, *J* = 5.8	0.924 *d*, *J* = 5.0	0.907 *d*, *J* = 4.4		1.563
9a or **9b**	 Synthetic[12])	0.672 (24ξ)	1.005	0.798 *d*, *J* = 6.4	0.903 *d*, *J* = 6.5	0.907 *d*, *J* = 6.1		1.559
10	 Natural product[13])	0.678	1.009	0.943 *d*, *J* = 6.5	0.826 *d*, *J* = 6.6	0.844 *d*, *J* = 6.6	0.863 *d*, *J* = 7.5	0.863 *d*, *J* = 7.5
10	 Synthetic[13])	0.677	1.008	0.942 *d*, *J* = 6.5	0.824 *d*, *J* = 5.6	0.842 *d*, *J* = 5.6	0.862 *d*, *J* = 7.8	0.862 *d*, *J* = 7.9

[8]) Measured in $CDCl_3$; shifts are the δ values; internal standard $CHCl_3$ or TMS.

[9]) All percentage ratios in the *Table* were derived from peak heights.

[10]) Reference sample from Dr. *R. M. K. Carlson*.

[11]) Hydrogenated product prepared from codisterol/epicodisterol mixture **1**, isolated from *V. cauliformis; cf.* [14] for NMR. data for campesterol (**2a**) and dihydrobrassicasterol (**2b**).

[12]) Sterically pure compound isolated from the synthetic mixture.

[13]) Assignment for C(26)–C(29) of **10** was arbitrary.

The angular methyl groups display an NMR. shift (compound **3**, *Table*) as expected for Δ^7-sterols on the basis of *Zürcher*'s rules [20]. Indeed, there is an excellent agreement (shift of angular methyl groups and of olefinic proton) between this spectrum and that of lathosterol (**14**)[14]). As the NMR. spectrum also shows numerical similarities with that of codisterol (**1b**) (shift of methyl at double bond, methyl doublets, methylidene **1b**: δ 1.636, 0.992 and 0.911, 4.659; **3**: δ 1.641, 0.989 and 0.920, 4.657) it becomes obvious that we have isolated a new sterol, viz. 24-methyl-cholesta-7,25-dien-3β-ol (**3**).

Although the methyl protons between δ 0.5 and 1.0 ppm do not exhibit any separation which might indicate a C(24) epimeric structure, the C(27) allylic methyl protons showed up as two singlets at δ 1.651 and 1.641, indicating **3** to be a mixture of epimers. The relative configuration at C(24) was tentatively assigned to be 24*R* (69%) for δ 1.651, and as 24*S* (31%) for δ 1.641, on the basis of allylic methyl shifts observed for codisterol (**1b**) (δ 1.636) and epicodisterol (**1a**) (δ 1.651).

We are indebted to Dr. *S.J. Wratten* and to Professor *D.J. Faulkner*, Scripps Institution of Oceanography, for a gift of the *P. pitys* sterols. Financial support to Standford University was provided by *NIH* grants GM-06840 and AM-04257. We acknowledge Dr. *Lois Durham*'s help with the 360-MHz-NMR. spectra, and access to the *Stanford* 360-MHz-NMR. facility (NSF grant GP-23633 and *NIH* grant RR-0711), and to the NMR./MS. facility at UCSD (*NIH* grant RR-708). *V. cauliformis* was identified by Dr. *K. Ruetzler*, Smithsonian Institution, Washington, D.C.

Experimental Part

General. High pressure liquid chromatography (HPLC.) was performed on a *Whatman Partisil* M9 10/50 ODS-2 reverse phase column (50 cm × 8 mm) using a *Haskel* model 28303 pump, an *Ashrof* gauge, and a *Waters Associates* dual cell refractometer detector (pressure 1000 psi., flow rate 7 ml/min, abs. methanol as eluent). Gas chromatographic analysis was performed on a *Hewlett-Packard* 402A chromatograph with a standard 402A flame ionization detector. Melting points were measured on *Thomas Hoover* capillary melting point apparatus. Optical rotation was measured on *Perkin-Elmer* 142 spectro-polarimeter. 60-MHz-NMR. spectra were run on *Varian* T60 and 360-MHz-NMR. were run on *Bruker* HX 360 in $CDCl_3$ as solvent.

All chemical shift values are in ppm with respect to internal TMS, and coupling constants are given in Hz. Low resolution mass spectra were run on an *MS*9 spectrometer, while high resolution mass spectra were run on *MAT*711 double focussing spectrometer equipped with a PDP-11/45 computer for data acquisition and reduction. Unless stated otherwise, relative retention times were determined using a OV25 column at 256°.

For isolation and monitoring of purification the *La Jolla* co-authors used a *Waters* HPLC. set-up (M6000 pump, UK6 injector, R401 differential refractometer, two μC_{18} columns (4 mm I.D. × 30 cm) in series, eluent methanol/water 92:8); a *Hewlett-Packard* 5710A and 402 gas chromatograph with flame ionization detector; a LKB9000 GC./MS.; a *Varian* HR220/*Nicolet* TT100 FT-NMR. instrument. Abbreviations: i.V. = *in vacuo*, RT. = room temperature.

Collection of sponge and isolation of sterols. The thin, rope-like sponge *Verongia cauliformis*[3]) was collected using SCUBA (−8 m), April, 1977, at Carrie Bow Cay, Belize, Central America. The freshly-collected sponge was chopped and immediately stored in a 95% ethanol. Extract was made in the following manner: The drained, preserved sponge (drained weight 2138 g) was homogenized in $CHCl_3$ (800 ml) and MeOH (1600 ml). To the homogenate in a separation funnel 3 l of water was added till a substantial $CHCl_3$ layer was formed. This dark brown layer was removed, and the homogenate, in aqueous MeOH, was extracted with $CHCl_3$ till the bottom layer was only light brown. All

[14]) Isolated from the Pacific starfish *Dermasterias imbricata;* it is the main sterol. 220-MHz-NMR. ($CDCl_3$) include angular methyl groups at 0.534 and 0.796, and an olefinic proton(s) at 5.15 ppm.

$CHCl_3$ layers were combined and filtered through *Celite*. The ethanol extract, obtained by draining the preserved sponge, was taken to dryness, and the residue was partitioned between $CHCl_3$ and water. The bottom layer was combined with the filtered $CHCl_3$-phase of the homogenized sponge, and then taken to dryness. Yield 19.9 g of extract.

The sterol mixture was obtained by silica gel column chromatography of the extract, eluting with hexane, benzene and diethyl ether mixtures. Yield of crude sterols 2.7 g; further purification by recrystallization and work-up of the material from the mother liquor by: 1) chromatography over a silica gel column, and 2) preparation of the acetates, purification of those over a silica gel column, and saponification. Yield of pure sterols 1.82 g.

Separation of the sterols. An initial examination of the total free sterols by GC./MS. revealed the presence of two minor sterols (each about 1% at best) of mol.-wt. 426 (to be referred to as A and B; A has a shorter retention time than B), which were potentially new sterols, because only a few marine sterols of that mol.-wt. were known. The main problem in the attempted isolation of any minor sterol from this mixture would be to get rid of the main sterol (aplysterol **12**, about 71% of the mixture), thus producing a sample strongly enriched in the minor sterol of interest. The sterol **12** has one double bond, sterols of mol.-wt. 426 have two degrees of unsaturation, thus argentic silica gel TLC. (in the manner of *Idler*)[15]) was used in the first isolation step, because it was assumed that A and B, with probably two double bonds, should have a lower Rf value than that of **12**. Two distinct bands showed up[16]), one caused by mainly **12** (acetate), the other by methylidene sterols. In a typical experiment, aimed at the isolation of trace sterols, we did 60 silver nitrate plates.

Information on, and work-up of the prep. TLC. plates. Distance in mm from the origin, description: 179, top of plate/84–96, Fr. 1/86, front of the broad band/78–84, Fr. 2/72–78, Fr. 3/58–72, Fr. 4A/43–58, Fr. 4B/29–43, Fr. 5/17–29, Fr. 6 (= methylidene sterol fraction).

As reported earlier, the main methylidene sterol of *V. cauliformis* is 25-dehydroaplysterol (**13**) [2]; it was isolated from Fr. 6 by reverse phase HPLC.; a minor sterol, 24-methylcholesta-5,25-dien-3β-ol (**1**), and a trace sterol, 24-methylcholesta-7,25-dien-3β-ol (**3**), from Fr. 6 gave one peak in HPLC. and they required separation by prep. GC.[17]) (3% OV25, 265°).

Sterol A (24-isopropenylcholesterol, **9**)[18]) was found both in Fr. 4A (7.8%) and in Fr. 4B (7.4%); sterol B (verongulasterol, **11**) occurred only in Fr. 4B (15.5%). The sterols **9** and **11** were sufficiently enriched in Fr. 4B to allow isolation by prep. GC. Sterol **9** was also isolated from Fr. 4A in the following manner: 1) argentic silica gel TLC. to remove the bulk of **12** (one of the main components (35.9%) of Fr. 4A); 2) reverse phase HPLC.; 3) prep. GC.

Fucosterol i-methyl ether (**5**). To the suspension of fucosteryl tosylate (226 mg, 0.14 mmol) in methanol, fused potassium acetate (450 mg, 4.7 mmol) was added. The reaction mixture was refluxed for 9 h under nitrogen. After cooling, the solvent was removed and the residue was dissolved in 50 ml of water and extracted with ether. The ether layer was washed with saturated $NaHCO_3$-solution, saturated NaCl-solution and dried over $MgSO_4$. The solvent was removed i.V. to give 161 mg (92%) of oil. An analytical sample was prepared using HPLC., $[\alpha]_D^{19} = +47.8°$ ($c = 1.40$, $CHCl_3$). – NMR. (60 MHz); 5.13 (*qa*, $J = 6$, 1 H); 3.30 (*s*, 3 H); 2.73 (*m*, 1 H); 1.53 (*d*, $J = 6$, 3 H); 1.02 (*s*, 6 H); 0.93 (*d*, 6 H); 0.72 (*s*, 3 H). – MS.: 426 (13, M^+), 411 (12), 371 (66), 328 (52), 296 (43), 253 (21), 55 (100).

$C_{30}H_{50}O$ Calc. 426.3861 Found 426.3859

24-Ethyl-3a,5-cyclo-6β-methoxycholestane-28-ol (mixture of epimers at C(24) and C(28) (**6**). To a solution of i-methyl ether (**5**), (158 mg, 0.37 mmol) in 10 ml of dry THF, 2 ml of 1M BH_3/THF (2 mmol) was added slowly *via* syringe. After stirring 9½ h at RT., 1 ml of water was added to destroy

---

15) 20×20 cm Plates poured using a slurry made of silica gel for TLC. (*E. Merck* No. 7741) with two luminescent indicators, 55 g; silver nitrate 7.75 g; water 120 g/thickness of layer 0.75 mm when wet/freshly poured plates dried in an oven at 100° for 1 h and then used immediately/applied 25–27 mg of sterol acetate per plate/developer hexane/benzene 3:2, two developments.

16) Visualization under long wave UV. light, as soon as the benzene, which interferes, has evaporated.

17) Retention times, relative to cholesterol, 3% SP 2250, 260°: **1** 1.30; **3** 1.49.

18) Note that **9** does not behave like a normal methylidene sterol in silver nitrate TLC. This indicates that the double bond is sterically hindered. Off hand one would have expected to find (the acetate of) **9** in Fr. 5 or 6.

excess hydride. Then 2 ml of 6N NaOH was added followed by 1.5 ml of 30% H_2O_2-solution. After stirring for 1 h at RT., the organic layer was separated from the aqueous layer which was saturated with K_2CO_3. The combined organic layer was dried over $MgSO_4$. Removal of the solvent gave 200 mg of crude product which was subsequently column chromatographed (20 g of silica gel, hexane/ether gradient 30–50%) to give 145 mg (88%) of pure alcohol **6** as colorless oil. An analytical sample was prepared using HPLC., $[\alpha]_D^{19} = +49.9$ ($c = 1.16$, $CHCl_3$). – NMR. (60 MHz); 3.70 (*m*, 1 H); 3.27 (*s*, 3 H); 2.72 (*m*, 1 H); 1.15 (*d*, $J = 6$, 3 H); 0.70 (*s*, 3 H). – MS.: 444 (50, M^+), 429 (60), 412 (71), 389 (99), 255 (35), 55 (100).

$C_{30}H_{52}O_2$ Calc. 444.3967 Found 444.3980

*24(*R + S*)-acetyl-3a,5-cyclo-6β-methoxycholestane* (**7**). To a solution of pyridine (0.25 ml) in 10 ml of methylene chloride, 135 mg (1.35 mmol) of chronium trioxide was added. After stirring for 15 min at RT., the alcohol **6** (100 mg, 0.23 mmol) in 2 ml of methylene chloride was added. The black solution was stirred for 13 h at RT. After addition of 10 ml of 5% NaOH-solution, the whole mixture was extracted with ether. The ether layer was washed with cold 5% HCl-solution, saturated NaCl-solution and dried over $MgSO_4$. The solvent was removed i.V. to give 98 mg (99%) of ketone **7**. An analytical sample was prepared using HPLC., $[\alpha]_D^{21} = +38.9°$ ($c = 0.56$, $CHCl_3$). – NMR. (60 MHz); 3.30 (*s*, 3 H); 2.73 (*m*, 1 H); 2.10 (*s*, 3 H); 1.03 (*s*, 3 H); 0.97 (*s*, 3 H); 0.90 (*d*, $J = 6$, 6 H); 0.72 (*s*, 3 H). – MS.: 442 (52, M^+), 427 (54), 410 (98), 387 (100), 289 (40), 255 (53), 213 (38).

$C_{30}H_{50}O_2$ Calc. 442.3811 Found 442.3814

*24(*R + S*)isopropenyl-3a,5-cyclo-6β-methoxycholestane* (**8a**). To oil free KH (74 mg, 1.85 mmol), 4 ml of dry DMSO (refluxed over and distilled from CaH) was introduced unter nitrogen. After stirring for 15 min, methyltriphenylphosphonium bromide (651 mg, 1.82 mmol) in 6 ml of DMSO was added. Stirring was continued for 1 h at RT. To remove DMSO, excess methyltriphenylphosphonium bromide, and phosphine oxide, the whole mixture was passed through a silica gel column (25 g, ether/hexane 1:1). Evaporation of the solvent resulted in a oily residue which was rechromatographed on silica gel (ether/hexane 1:1) to give 30 mg (75%) of the olefin **8a** as an oil. An analytical sample was obtained using HPLC., $[\alpha]_D^{21} = +44.6°$ ($c = 0.735$, $CHCl_3$). – NMR. (60 MHz): 4.68 (*m*, 1 H); 4.57 (*m*, 1 H); 3.28 (*s*, 3 H); 2.72 (*m*, 1 H); 1.58 (*s*, 3 H); 1.00 (*s*, 6 H); 0.87 (*d*, $J = 6$, 6 H); 0.70 (*s*, 3 H). – MS.: 440 (14, M^+), 425 (22), 408 (20), 385 (40), 355 (10), 353 (15), 218 (10), 213 (11), 55 (100).

$C_{31}H_{52}O$ Calc. 440.4018 Found 440.4043

24-Isopropyl-3a,5-cyclo-6β-methoxycholestane (**8b**). The olefin **8a** (10 mg) in 2 ml of EtOAc was hydrogenated over PtO_2 to give 9 mg of saturated i-ether **8b** as an oil. An analytical sample was obtained using HPLC., $[\alpha]_D^{21} = +48.3°$ ($c = 0.30$, $CHCl_3$). – NMR. (60 MHz): 4.38 (*s*, 3 H); 2.72 (*m*, 1 H); 1.02 (*s*, 3 H); 0.98–0.78 (15 H); 0.72 (*s*, 3 H). – MS. (MAT 711): 442.4172 (74), 427.3933 (49), 410.3883 (100), 387.3619 (85), 384.3743 (24), 255.2072 (20), 229.1945 (9), 214.1782 (5), 213.1642 (12).

$C_{31}H_{54}O$ Calc. 442.4172 Found 442.4172

24-Isopropyl-cholesterol (**10**). To a solution of i-ether **8b** (6 mg) in aqueous dioxan, a couple of crystals of *p*-toluenesulfonic acid was added. The mixture was refluxed for 1 h. After cooling, the solvent was removed i.V. The residue was subjected to HPLC. for analysis, m.p. 135–136° (methanol), $[\alpha]_D^{19} = -41°$ ($c = 0.17$, $CHCl_3$). – NMR. (360 MHz): 5.30 (*m*, 1 H); 3.444 (*m*, 1 H); 0.953 (*s*, 3 H); 0.887 (*d*, $J = 6.5$, 3 H); 0.807 (*d*, $J = 8.0$, 6 H); 0.828 (*d*, $J = 5.6$, 3 H); 0.769 (*d*, $J = 5.6$, 3 H); 0.622 (*s*, 3 H). – MS.: 428 (100, M^+), 410 (48), 395 (30), 343 (40), 317 (51), 311 (27), 273 (28), 250 (33), 231 (26), 213 (39).

$C_{30}H_{52}O$ Calc. 428.4018 Found 428.4023

Co-injection with the natural product: one peak (3% OV25, oven temperature 260°), retention time relative to cholesterol 1.95.

(24ξ)-Isopropenyl-cholesterol (**9**). The i-methyl ether **8a** (10 mg) in 6 ml of aqueous dioxan and a few crystals of *p*-toluenesulfonic acid were refluxed for 1 h. After working up as usual, 7 mg of **9** was obtained, m.p. 123–124° (methanol). – NMR. (360 MHz): 0.665 (*s*, 3 H); 0.672 (*s*,

3 H); 0.801 (*d*, $J=6$, 3 H); 0.907 (*d*, $J=4$, 3 H); 1.006 (*s*, 3 H); 1.563 (*s*, 3 H); 4.603 (*m*, 1 H); 4.737 (*m*, 1 H). – MS.: 426 (82, M^+), 411 (15), 393 (15), 328 (17), 314 (28), 299 (45), 271 (57), 229 (17), 213 (30), 55 (100).

$C_{30}H_{50}O$ Calc. 426.3861 Found 426.3853

Co-injection with natural product: one peak (3% OV25, oven temperature 260°); retention time relative to cholesterol 1.86. The epimeric mixture of **9** was subjected to HPLC. (methanol, 850 psi, ret. time 58–61 min) to give 84% pure isomer (**9a** or **9b**).

REFERENCES

[1] *C. Delseth, L. Tolela, P.J. Scheuer, R.J. Wells & C. Djerassi,* Helv. *62*, 101 (1979).
[2] *W.C.M.C. Kokke, W.H. Fenical, C.S. Pak & C. Djerassi,* Tetrahedron Letters *1978*, 4373.
[3] *C. Djerassi, N. Theobald, W.C.M.C. Kokke, C.S. Pak & R.M.K. Carlson,* Pure appl. Chemistry, in press.
[4] *P.R. Bergquist,* 'Sponges', University of California Press, Berkeley and Los Angeles (1978).
[5] *L.J. Goad,* in 'Biochemical and Biophysical Perspectives in Marine Biology', Ed. D.C. Malins, & J.R. Sargent, Academic Press, New York and London, vol. III, pp. 213–318, 1976.
[6] *L. Minale, R. Riccio, O. Scalona, G. Sodano, E. Fattorusso, S. Magno, L. Mayol & G. Santacroce,* Experientia *33*, 1550 (1977).
[7] *W. Hofheinz,* Helv. *62*, 1307 (1979).
[8] *M. de Rosa, L. Minale & G. Sodano,* Comp. Biochem. Physiol. *46B*, 823 (1973).
[9] *C. Djerassi, R.M.K. Carlson, S. Popov & T. Varkony,* in 'Marine Natural Products Chemistry', Ed. D.J. Faulkner & W.H. Fenical, Plenum Press, New York and London, p. 111, 1977.
[10] *S.J. Wratten & D.J. Faulkner,* J. Amer. chem. Soc. *99*, 7367 (1977).
[11] *S. Popov, R.M.K. Carlson, A. Wegmann & C. Djerassi,* Steroids *28*, 699 (1976).
[12] *N. Theobald, R.J. Wells & C. Djerassi,* J. Amer. chem. Soc. *100*, 7677 (1978).
[13] *M. Bartolotto, J.C. Braekman, D. Daloze & B. Tursch,* Bull. Soc. chim. Belges *87*, 539 (1978).
[14] *I. Rubinstein, L.J. Goad, A.D.H. Clague & L.J. Mulheirn,* Phytochemistry *15*, 195 (1976).
[15] *I. Rubinstein & L.J. Goad,* Phytochemistry *13*, 481 (1974).
[16] *P. de Luca, M. de Rosa, L. Minale, R. Puliti, S. Sodano, F. Giordano & L. Mazzarella,* J. chem. Soc. Chem. Comm. *1973*, 825.
[17] *A.J. Birch & K.A.M. Walker,* J. chem. Soc. *C 1966*, 1894.
[18] *L.G. Partridge, I. Midgley & C. Djerassi,* J. Amer. chem. Soc. *99*, 7686 (1977).
[19] *S. Wyllie & C. Djerassi,* J. org. Chemistry *33*, 1305 (1968).
[20] *R.F. Zürcher,* Helv. *46*, 2054 (1963); *N.S. Bhacca & D.H. Williams,* 'Applications of NMR Spectroscopy in Organic Chemistry', Holden-Day, Inc., San Francisco, Ch. 2, 1964.