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# MINOR AND TRACE STEROLS IN MARINE INVERTEBRATES IX.[1] VERONGULASTEROL - A MARINE STEROL WITH A NOVEL SIDE CHAIN ALKYLATION PATTERN

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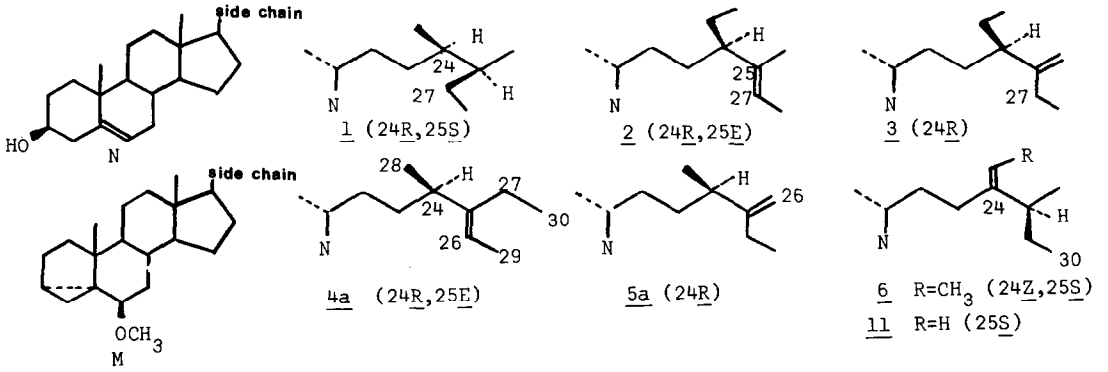
The isolation and structure elucidation of marine sterols such as aplysterol (1),[2] stelliferasterol (2)[3] and strongylosterol (3)[4] represent the first evidence that bioalkylation[5] of the cholesterol side chain can occur at the terminus (C-27) as well as at the central positions 24 (and thence C-28), 23, and 22. By incorporating a plausible set of biosynthetic assumptions into a computer program, we predicted[6] the occurrence of sterols with "extended" side chains resulting from bioalkylation at both termini, i.e., C-26 and C-27. We now wish to report the first isolation of such a sterol - verongulasterol (4a) - from the Belize sponge Verongula cauliformis (class Desmospongia, subclass Keratosa, family Verongidae) together with a new member of the aplysterol (1) class, namely $\Delta^{25(26)}$-dehydroaplysterol (5a). Like the other sponges of the Verongidae family which have been examined so far,[2,7] V. cauliformis contains very little cholesterol (<5% of total sterols) and predominantly sterols with "extended" side chains. We believe, therefore, that these novel sterols may play a significant biological role in membrane function and we are initiating relevant experiments to answer this extraordinarily interesting question.

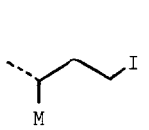
The gas chromatographic trace of the total free sterol fraction from Verongula cauliformis closely resembled that of various Verongia sterols[7] - the main peak (71%) being due to aplysterol (1) as shown by isolation via argentic silica gel TLC.[8] Preparative gas chromatography of the aplysterol-depleted fraction on OV25 or SP2250 columns yielded 0.2% of verongulasterol ($M^+$ 426 = $C_{30}H_{50}O$), whose mass spectrum was qualitatively identical and quantitatively only slightly different from that[3,9] of stelliferasterol (2); particularly noteworthy are the two McLafferty rearrangement peaks at m/e 314 and 328, which are diagnostic[9] for $\Delta^{25}$- double bonds (e.g., 2,3). The 360 MHz ($CDCl_3$) NMR spectrum (see Table 1) showed a marked resemblance to that of isostelliferasterol (6),[3] but the latter's side chain substitution pattern was excluded by decoupling experiments - irradiation at $\delta$ 1.9 ppm (allylic proton region) resulting in collapse of the C-30 methyl triplet at 0.993 ppm. The mass spectrometric and NMR data are consistent with structure 4, the E stereochemistry of the $\Delta^{25}$ double bond being based on the chemical shift of the C-26 vinylic proton signal.[10] Because of the unprecedented nature of this side chain, we chose to verify this structure assignment by synthesis and at the same time establish the stereochemistry at C-24, since this feature is of considerable biosynthetic importance.




The starting material was the recently described[3] 3α,5-cyclo-6β-methoxy-23-iodonorcholane (7), which was condensed with ethyl 2-methylpropioacetate to the β-keto ester 8 and then heated under reflux (1 hr) with 6N ethanolic KOH to provide a mixture of C-24 isomeric ketones 9. Wittig condensation with triphenylethylphosphonium bromide (KH in DMSO, 24 hr, 25° C) and regeneration of the $\Delta^5$-3β-hydroxy grouping (pTsOH in aqu. diox., 2 hr, reflux) gave an inseparable mixture of the 24R (4a) and 24S (4b) epimers (m.p. 101-102°, $[\alpha]_D^{21}$ -14.7° (c, 0.102, $CHCl_3$) of verongulasterol whose GC mobility on 3% OV25 at 255° C (rel. ret. 2.20 relative to cholesterol) and mass spectrum were identical with those of the natural sterol. The NMR spectral comparison summarized in Table 1, notably the downfield C-21 and upfield C-28 methyl signals of natural verongulasterol, are only consistent[10] with the 24R stereochemistry (4a). It should be noted that the particular Wittig reaction conditions employed by us furnished only the 25E isomers, in contrast to the more stringent conditions (n-butyl lithium, THF, 24 hr. reflux) in the stelliferasterol (2) series[3] where both E and Z isomers were produced. Verongulasterol, therefore, is 24R, 26,27-trimethylcholesta-5,25E-dien-3β-ol (4a) - the first example of bioalkylation of the sterol side chain at C-26 and C-27.

Reverse phase HPLC[8] of the "methylene sterol" fraction from the original argentic silica gel TLC separation led in 4% yield to a sterol of $M^+$ = 412 ($C_{29}H_{48}O$), m.p. 130-131.5° C, whose mass spectrum - except for the obvious 14 mass unit shifts of the $M^+$,$M^+$-$H_2O$, $M^+$-$CH_3$ and $M^+$-($H_2O$+$CH_3$) peaks - was qualitatively identical with those of stelliferasterol (2) and verongulasterol (4a). Since the NMR spectrum (Table 1) showed the presence of a terminal methylene grouping and the mass spectrum (m/e 314 and 328 peaks[9]) the presence of a $\Delta^{25}$-olefin, only the known[11] clerosterol (10b) (or its C-24 epimer 10a) or the hitherto unknown 25-dehydroaplysterol (5a) (or its epimer 5b) structures are plausible alternatives for this sterol constituent of V. cauliformis. A decision in favor 5 could be reached by NMR decoupling - irradiation of the C-24 and C-27 allylic proton signals (both at δ 2.0 ppm) causing the collapse of the methyl doublet at 0.996 (C-28 Me) and the methyl triplet at 1.030 (C-29 Me). Confirmation of this structure and proof of the C-24 stereochemistry was accomplished synthetically in exactly the same fashion described above for verongulasterol (4a) with the exception that triphenylmethylphosphonium bromide was employed in the Wittig condensation with the ketone 9. The resulting mixture (m.p. 125-125.5°, $[\alpha]_D^{21}$ -31° (c, 0.148, $CHCl_3$), GC retention time 1.67 relative to cholesterol) of

C-24 epimers 5a and 5b had exactly the same mass spectrum and GC mobility as the natural sterol to which we assign the 24R stereochemistry on the basis[10] of the slight upfield shift (see Table 1) of its C-28 methyl NMR signal. Since the absolute configuration of aplysterol (1) is known (24R,25S)[2], the trivial name 25-dehydroaplysterol (5a) unambiguously defines the correct stereostructure of our new marine sterol. It is noteworthy that with the exception of the two sterols lacking a chiral center at C-24 - 24(28)-dehydroaplysterol (11)[2] and isostelliferasterol (6)[3] - all other naturally occurring marine sterols with "extended" side chains (i.e., 1, 2, 3, 4a, 5a) possess the 24R configuration - a feature which suggests a common biogenetic origin.



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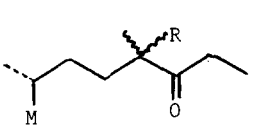
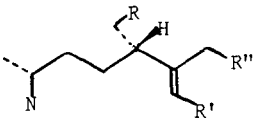
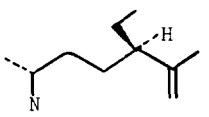


8 R=$CO_2$Et

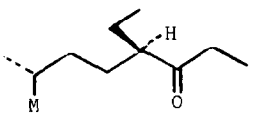
9 R=H



| | R | R' | R" |
|---|---|---|---|
| 4b | H | Me | Me |
| 5b | H | H | Me |
| 10b | Me | H | H |



10a



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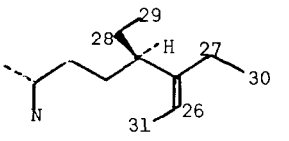

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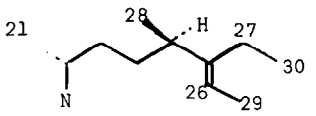
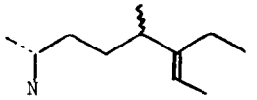
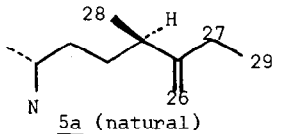
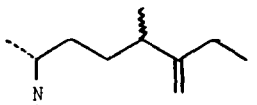
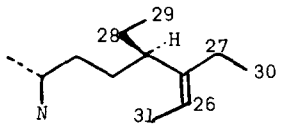
The existence in nature of the $C_{30}$ sterol stelliferasterol (2)[3] and its double bond isomers 3[3] and 6[4] demonstrates that formal triple biomethylation at C-24, C-27 and C-28 is feasible. Since triple biomethylation at C-24, C-26 and C-27 has now also been established through the isolation of verongulasterol (4a), there is no a priori reason why $C_{31}$ sterols - formally the products of quadruple biomethylation - should not also exist in the marine environment. Indeed, a recent GC-MS analytical study[12] of the trimethylsilylated sterol fraction of the tunicate Ascidia mentula indicated the presence of traces of $C_{31}$ and $C_{32}$ monohydroxy sterols of unknown constitution, in which all of the "extra" carbon atoms are in the side chain. Based on the results summarized in our present paper, formal quadruple biomethylation at C-24, C-26, C-27 and C-28 seems eminently plausible. Therefore as a reference standard, we have synthesized[13] such a sterol, viz. 24R-ethyl-26,27-dimethylcholesta-5,25Z-dien-3β-ol (13), by Wittig condensation with triphenylethylphosphonium bromide of the 26-norketone 12 derived[10] from strongylosterol (3).[4] The required extraordinarily drastic conditions (n-butyl lithium in THF, 6 days reflux) led almost exclusively to the 25Z isomer, whose NMR spectral data are collected in Table 1. As expected, the mass spectrum was virtually identical with that[3,9] of stelliferasterol (2) except for the required 14 mass unit shifts above m/e 400. A search for this and related $C_{31}$ sterols among marine organisms is now underway.

Acknowledgment: Financial support from the National Institutes of Health (grants No. GM-06840 and AM-04257) is gratefully acknowledged as is access to the 360 MHz NMR spectrometer facility at Stanford funded by NSF grant GP 23633 and NIH grant RR 0711 and the U.C. San Diego NMR/MS facility supported by NIH grant RR-708. We thank Dr. K. Ruetzler, Smithsonian Institution, Washington, D.C. for taxonomic identification.

Table 1. 360 MHz NMR Chemical Shifts of Verongulasterol (4a), 25-dehydroaplysterol (5a) and Synthetic Reference Compounds

| | C-18 Me | C-21 Me | C-28 Me | C-30 Me | C-19 Me | C-29 Me | C-31 Me | C-26 H |
|---|---|---|---|---|---|---|---|---|
|  4a (natural) | 0.668 | 0.908 | 0.947 | 0.993 | 1.006 | 1.586 | -- | 5.179 |
|  4a + 4b (synth.) | 0.667 | 0.902<br>0.907 | 0.947<br>0.953 | 0.995 | 1.005 | 1.587 | -- | 5.146<br>5.179 |
|  5a (natural) | 0.672 | 0.911 | 0.996 | -- | 1.006 | 1.030 | -- | 4.690<br>4.705 |
|  5a + 5b (synth.) | 0.670 | 0.909 | 0.996<br>1.003 | -- | 1.005 | 1.031 | -- | 4.693<br>4.710 |
|  13 (synth.) | 0.660 | 0.903 | -- | 0.996 | 1.004 | 0.996 | 1.58 | 5.100 (5%)<br>5.300 (95%) |

## References and Notes


1. For paper VIII in the Stanford series see N. Theobald, J. N. Shoolery, C. Djerassi, T. R. Erdman and P. J. Scheuer, J. Am. Chem. Soc., in press.
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13. We are indebted to our colleague Dr. N. Theobald for this experiment.


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