MINOR AND TRACE STEROLS IN MARINE INVERTEBRATES 30.[1] ISOLATION, STRUCTURE

ELUCIDATION AND PARTIAL SYNTHESIS OF 26-METHYLSTRONGYLOSTEROL AND 28-METHYLXESTOSTEROL

- TWO MARINE STEROLS ARISING BY A NOVEL QUADRUPLE BIOMETHYLATION SEQUENCE

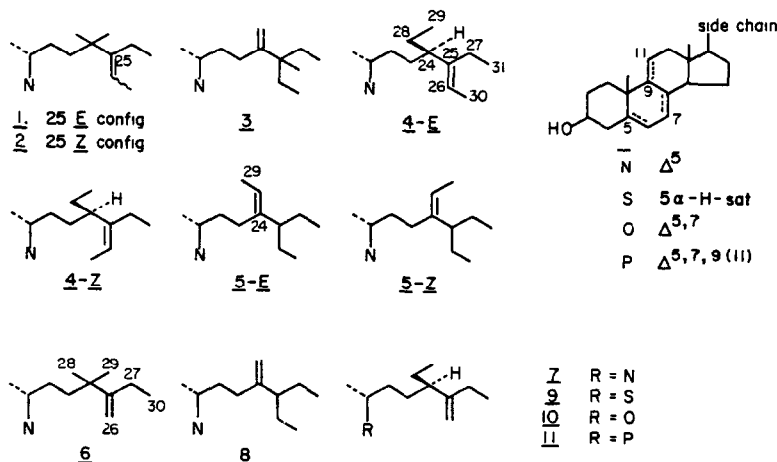Lian Niang Li[2] and Carl Djerassi[*]

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Abstract: Two new sterols, 26-methylstrongylosterol (4) and 28-methylxestosterol (5), arising from a hitherto unknown quadruple biomethylation sequence, together with the biogenetically important "missing link", durissimasterol (6), were isolated from the Indo-Pacific sponge Strongylophora durissima.

Recently we documented the first examples of quadruple biomethylation in the sterol side chain - xestospongesterol (1),[3] isoxestospongesterol (2)[3] and 25-methylxestosterol (3).[4] The eventual isolation of a sterol resulting from an alternative quadruple biomethylation sequence at positions 24,26,27 and 28 has been predicted earlier[5] and one such candidate - 24R-ethyl-26, 27-dimethylcholesta-5,25(26)-dien-3β-ol (26-methylstrongylosterol) (4-Z) - was synthesized.[5] We now wish to report the first occurrence of two sterols, 26-methylstrongylosterol (4) and 28-methyl xestosterol (5), arising from such a quadruple biomethylation pattern together with a biogenetically important "missing link",[6] now named durissimasterol (6), in the Indo-pacific sponge Strongylophora durissima, in which the main sterol is strongylosterol (7).[7]

The mass spectrum of 26-methylstrongylosterol (4) (M^+ 440) was identical with that of the synthetic[5] Z isomer (4-Z), but the doubling of the relevant 360 MHz proton NMR peaks (Table 1) indicated that the natural sterol was a mixture of E and Z isomers of 4. Verification was provided by synthesis via a known procedure,[5] using benzene instead of THF[5] in the Wittig condensation. Reverse-phase HPLC yielded 4-E [mp 90-91° C, $[\alpha]_D^{20}$ -21° (c, 0.002, $CHCl_3$)] and 4-Z [mp 107-108° C, $[\alpha]_D^{20}$ -15° (c, 0.017, $CHCl_3$)], both of which had the same GC mobility (rrt 2.26;cholesterol=1.00) as the natural compound. The C-26 and C-31 proton signals were more shielded in the E isomer,[8] (Table 1) while a septet at 2.475 ppm (C-24 allylic proton) was observed in the Z isomer. Final proof of the stereochemical assignment was provided by the ^{13}C NMR spectra, in which an upfield shift of 7.8 ppm and a downfield shift of 5.2 ppm respectively were observed for C-24 and C-26 in the Z isomer.[9]

The mass spectra of the second new sterol, 28-methylxestosterol (5) (M^+ 440), and of xestosterol (8) (M^+ 426) were practically identical below their m/z 314 base peak and showed virtually no peaks in the region $M^+ \rightarrow$ m/z 314, thus pointing to the presence of a $\Delta^{24(28)}$-double bond.[11] Attachment of a methyl group to the $\Delta^{24(28)}$-double bond of 5 was deduced from the NMR (Table 1) doublet at 1.679 ppm, which was coupled to the olefinic proton at 5.25 ppm, while the six-proton triplet at 0.930 ppm indicated the presence of a 30,31-dimethyl moiety. The stereo-

chemistry of the $\Delta^{24(28)}$-double bond was proven by comparison with the synthetic isomers 5-E
and 5-Z, obtained by Wittig condensation of 26,27-dimethyl-6β-methoxy-3α,5-cyclocholestan-24-
one[10] with triphenylethylphosphonium bromide, followed by removal of the protecting group [5-E:
mp 116-117°C, $[\alpha]_D^{20}$ -37° (c, 0.009, CHCl$_3$); 5-Z: mp 130-131°C, $[\alpha]_D^{20}$ -15° (c, 0.02, CHCl$_3$)]
and comparison of their NMR spectra (notably a pentet at 2.40 ppm (C-25H) in 5-Z vs. 1.8 ppm in
5-E) with those[12] of isofucosterol (Z isomer, C-25 H at 2.8 ppm) and fucosterol (E isomer, C-25
H at 2.2 ppm). This stereochemical assignment was confirmed by the ^{13}C NMR spectra, in which an
upfield shift of 7.76 ppm for C-25 was observed in the Z isomer. The NMR spectra (Table 1) of
synthetic and natural 5-E were identical as were their mass spectra and GC rrt (2.26). While
4 and 5 possess the same GC rrt, they can be distinguished by reverse-phase HPLC (Whatman
Partisil M9 10/50 ODS-2 column) rrt:1.20 (4) vs. 1.12 (5).

The third new sterol, named durissimasterol (6) (M$^+$ 426), showed typical peaks (m/z 213, 231,
253, 271) of a Δ^5-3β-hydroxy sterol,[13] an intense peak at m/z 328 (typical[11,14] of a McLafferty
rearrangement in a Δ^{25}-unsaturated side chain), and a base peak at m/z 98 (C$_7$H$_{14}$ due to "reverse"
McLafferty rearrangement); the absence of a m/z 314 peak suggested the presence of a C-24
quaternary center.[3] The NMR spectrum (Table 1) pointed directly to structure 6, which is an
important "missing link" in the proposed[3,6] biosynthesis of (iso)xestospongesterol (1,2).
For confirmation, 6 [mp 142-143°C, $[\alpha]_D^{20}$ -36° (c, 0.006, CHCl$_3$)] was synthesized by Wittig con-
densation of the appropriate ketone (obtained as a by-product in an earlier[3] synthesis) with tri-
phenylmethylphosphonium bromide (n-BuLi,THF,4 hr, reflux), followed by deprotection of the i-
methyl ether. The NMR (Table 1) and mass spectra, as well as GC rrt (1.91) of the natural and
synthetic sterols, were identical.

Three new minor sterols with M$^+$ 428, 424 and 422 were identified as strongylostanol (9),
7-dehydrostrongylosterol (10) and 7,9(11)-didehydrostrongylosterol (11)) by comparison of their
360 MHz proton NMR (identical side chain proton signals) and mass spectra with those of strongy-
losterol (7). Thus the high resolution mass spectrum of strongylostanol (9) (M$^+$ 428.4037) dis-

played the diagnostic peaks ($\underline{m/z}$ 215,233,255 and 273) for the nucleus of a saturated stanol. The absence of a C-6 vinylic proton in its NMR spectrum and the expected[15] chemical shifts for the C-18 and C-19 methyl groups (0.631 and 0.797 ppm) were only consistent with a 3β-hydroxy-5α-saturated nucleus. The nature of the extra unsaturation in the nuclei of $\underline{10}$ and $\underline{11}$ was demonstrated easily by the characteristic mass and NMR spectra of such $\Delta^{5,7}$ and $\Delta^{5,7,9(11)}$-unsaturated 3β-hydroxy sterols.[16,17]
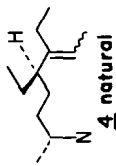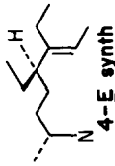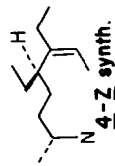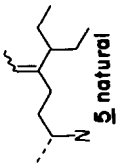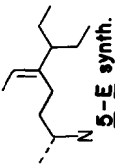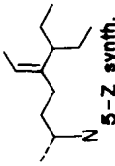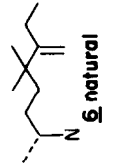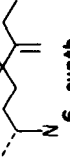
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Table 1. Selected ^{1}H 360 MHz NMR Chemical Shifts (C_6D_6) of 26-Methylstrongylosterol (5), 28-Methylxestosterol (5), 26-Methylstrongylosterol (4), 28-Methylxestosterol (5) and Durissimasterol (6).

Compound	C-18 Me	C-19 Me	C-21 Me	C-29 Me	C-30 Me	C-31 Me	C-26 H	C-28 H
4 natural	0.659(48%) / 0.666(52%)	0.941	1.027 (d,J=6.4) / 1.034 (d,J=6.2)	0.885 (t,J=7.4) / 0.920 (t,J=7.4)	1.665 (d,J=6.7) / 1.625 (d,J=6.7)	1.074 (t,J=7.4) / 1.025 (t,J=7.4)	5.44 / 5.24	
4-E synth	0.666	0.940	1.034 (d,J=6.2)	0.919 (t,J=7.5)	1.626 (d,J=6.7)	1.025 (t,J=7.5)	5.254 (q,J=6.7)	
4-Z synth.	0.658	0.941	1.026 (d,J=6.5)	0.884 (t,J=7.4)	1.663 (d,J=6.7)	1.074 (t,J=7.4)	5.444 (q,J=6.7)	
5 natural	0.664	0.949	1.060 (d,J=6.7)	1.679 (d,J=6.6)	0.930 (t,J=7.5)	0.930 (t,J=7.50)		5.250 (q,J=6.3)
5-E synth.	0.664	0.949	1.060 (d,J=6.5)	1.678 (d,J=6.7)	0.929 (t,J=7.3)	0.929 (t,J=7.3)		5.250 (q,J=6.8)
5-Z synth.	0.660	0.942	1.036 (d,J=6.5)	1.645 (d,J=6.8)	0.905 (t,J=7.4)	0.905 (t,J=7.4)		5.530 (q,J=6.7)
6 natural	0.657	0.944	0.990 (d,J=6.5)	1.088[a]		1.077 (t,J=7.4)	4.98 / 4.94	
6 synth.	0.658	0.944	0.990 (d,J=6.50)	1.088[a]		1.077 (t,J=7.4)	4.978 / 4.94	

[a] Six-proton signal due to C-28 and C-29 methyl groups.