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Thymosiosterol and Δ^{24} Thymosiosterol, new Sterols from the Sponge *Thymosiopsis* sp.

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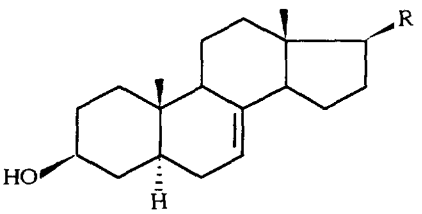
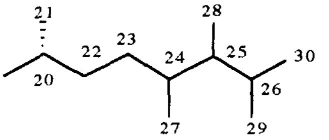
Abstract. Two new sterols were isolated from the sponge *Thymosiopsis* sp. and their structure elucidated by 2D NMR spectral data.

The sponge *Thymosiopsis* sp. is an undescribed species collected near La Ciotat (France). [1] Chromatography of the dichloromethane extract over a silicagel column (dichloromethane-acetone 95:5) gave, as the major constituent, a mixture of sterols (30% of the extract, 0.1 % dry weight). GC-MS [2] analysis of the TMS derivatives indicated two major sterols : retention time 19.14 mn (*m/z* 498) and 20.37 mn (*m/z* 500) corresponding to sterols having M^+ 426 and M^+ 428 respectively. These sterols were unseparable on a C-18 reverse phase, however separation on argentic silicagel plates of the acetylated mixture [3] allowed to obtain pure sterol acetates, which, after CO_3K_2-MeOH hydrolysis and silicagel chromatography afforded pure **1** and **2**. Compound **1**, m.p. 136-137° C, $[\alpha]_D$ +6 (c 0.2, $CHCl_3$), HRCIMS $[M+H]^+$ *m/z* 429.4096 in agreement with $C_{30}H_{53}O$. The ^1H NMR data of **1** displayed a broad singlet at δ 5.15 ppm characteristic for a Δ^7 vinyl proton. [4] The resonances assigned to the 18 and 19 methyl protons (δ 0.51 and 0.77 ppm, respectively) confirmed the Δ^7 unsaturation. [4, 5] The ^{13}C NMR signals at δ 139.6 and 117.4 ppm are also consistant with those of C-7 and C-8 of a Δ^7 nucleus, as well as the signals at δ 11.8 and 13.0 for C-18 and C-19.



1 R =



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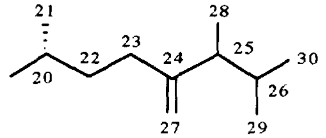

The ^1H NMR spectrum showed signals for seven methyl groups : two singlets for C-18 and C-19 carbons and five doublets. A normal sterol side-chain would require an additional doublet or a triplet, and apparently we had a sterol with an extended side-chain. Combination of COSY and heteronuclear correlations (HMBC, HMQC) experiments allowed to assign all proton and carbon chemical shifts. However, some signals in the ^1H spectrum overlap and it is difficult to describe their multiplicity. HMBC experiments furnished correlations between Me-29 and 30 protons and carbons 25 and 26, correlations of Me-28 protons with carbons 24, 25 and 26, and correlations of Me-27 protons with carbons 23, 24, 25. Other correlations are listed in the table. These observations allowed to assign the 24,26,26-trimethyl cholest-7en-3βol structure. In the NOESY spectrum of **1**, NOE was observed from H3 to H5, confirming the β position of H5, but no significant NOE allowed to assign the configuration at C25 which remains undetermined.

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Compound **2**, m.p. 125-127° C, $[\alpha]_D$ +10 (c 0.2, $CHCl_3$), HRCIMS $[M+H]^+$*m/z* 427.3938, in agreement with $C_{30}H_{51}O$. The ^1H and ^{13}C NMR spectra of **2** also showed the characteristic signals for a Δ^7 sterol. In addition, the ^1H spectrum, showed two doublets at δ 4.66 and 4.69 due to a methylene group. This was confirmed by ^1H-^{13}C NMR correlations which clearly showed correlations between protons 23 at δ 2.03 with carbons resonating at δ 107.6 and 155.1. Correlations between 29 and 30 methyl protons and carbons 25 and 26, of methyl at δ 0.94 with carbons 24, 25, 26, and of exo-methylene protons with carbons 23, 24 and 25 completed the structure. MS fragmentation at *m/z* 314 confirmed that the side-chain bears an unsaturation at the 24 position. Thus **2** was assigned the 24,26,26-trimethyl cholesta-7,24(27)dien-3βol structure.

Table : ^{13}C (75 MHz) and ^1H (300 MHz) NMR data of **1** and **2** (δ ppm, $CDCl_3$)

	Compound 1			Compound 2		
position	^1H (mult., *J* (Hz))	^{13}C	HMBC	^1H (mult., *J* (Hz))	^{13}C	HMBC
1	1.25	34.2	3,5	1.25	34.2	3,5
2	1.70	31.5	3,4	1.70	31.5	3,4
3	3.51 (m, 1H)	71.1	2,4	3.51 (m, 1H)	71.1	2,4
4	1.31	37.1	3,5	1.31	37.1	3,5
5	1.35	40.2	-	1.35	40.2	-
6	1.77	29.7	5,4	1.77	29.7	5,4
7	5.15 (br s, 1H)	117.4	-	5.15 (br s, 1H)	117.4	-
8	-	139.6	-	-	139.6	-
9	1.60	49.4	-	1.60	49.4	-
10	-	38.0	-	-	38.0	-
11	1.45-1.52	21.5	13	1.45-1.52	21.5	13
12	2.01 (dt,1H)-1.23 (m,1H)	39.6	13, 18	2.01 (dt,1H)-1.23 (m,1H)	39.6	13, 18
13	-	43.4	-	-	43.4	-
14	1.77	55.0	8	1.77	55.0	-
15	1.50	22.9	8	1.50	22.9	-
16	1.37	27.9	-	1.37	27.9	-
17	1.18	56.1	13, 16, 18	1.18	56.1	13, 16, 18
18	0.51 (s, 3H)	11.8	12, 13, 14, 17	0.51 (s, 3H)	11.8	12, 13, 14, 17
19	0.77 (s, 3H)	13.0	1, 5, 9, 10	0.77 (s, 3H)	13.0	1, 5, 9, 10
20	1.32	36.4	-	1.37	36.4	-
21	0.88 (d, *J*=6.5, 3H)	18.8	17, 20, 22	0.92 (d, *J*=6.5, 3H)	18.8	17, 20, 22
22	1.08	33.6	-	1.24	38.1	-
23	1.23-1.83	28.3	-	2.03	39.5	-
24	1.40	35.0	-	-	155.1	-
25	0.98	44.6	-	1.80	47.3	-
26	1.62	29.5	29, 30	1.77	30.9	-
27	0.82 (d, *J*=6.7, 3H)	18.7	23, 24, 25	4.66 (d, *J* =1.4, 1H) 4,69 (d, *J* =1.1, 1H)	107.6	23, 24, 25
28	0.73 (d, *J*=6.9, 3H)	11.4	24, 25, 26	0.94 (d, *J*=6.5, 3H)	16.8	24, 25, 26
29*	0.80 (d, *J*=6.8, 3H)	21.9	25, 26	0.81 (d, *J*=6.6, 3H)	21.8	25, 26, 30
30*	0.85 (d, *J*=6.8, 3H)	18.3	25, 26	0.82 (d, *J*=6.5, 3H)	18.9	25, 26, 29

* May be reversed

Sterols bearing such an extended side-chain are seldom found in Nature : a Δ^5 sterol having a side-chain with the same substitution pattern as in **2** was previously described as a trace constituent of the sterol content of a Chrysophyte alga. [6] A Δ^7 sterol, 24,26,26-trimethyl cholesta-7,25(27)-dien-3βol, with a similar side-chain as in **2**, was isolated as the major sterol of the sponge *Aciculites pulchra* [4] (taxonomically unrelated to *Thymosiopsis*); the side-chain of **1** is unprecedented. These sterols are likely biosynthesized by a double methylation at the 26 position. [4] Moreover **1** and **2** possess a Δ^7 nucleus, a rare type of nuclear unsaturation in sponges. [7]

References

[1] The sponge *Thymosiopsis* sp. will be described later (voucher MNHN DJV 63), it is a near relative of *T. cuticulitus* Vacelet & Perez.

[2] CPSil8 column, Chrompack, 100°C to 290°C, 10°C/mn, helium flow rate 1ml/mn.

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[7] Kerr, R.G.; Baker, B.J. *Nat. Prod. Rep.* **1991**, *8*, 465-497.