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NOVEL STEROLS FROM THE SPONGE *ESPERIOPSIS EDWARDII*

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Abstract - The sponge *Esperiopsis edwardii* was examined for its steroids. It contains forty-three C27-C29 derivatives with four different types of nucleus: 3β-hydroxy-Δ^5-; Δ^4-; 3β-hydroxy-5αH-; 3α-hydroxy-5αH- and 3-keto-Δ^4-derivatives. The four groups present similar side-chain patterns and identical GC profiles. Those sterols having the 3α-hydroxy-5αH-structural configuration constitute a new group of natural products.

Sponges are a rich source for discovery of marine sterols with new and unique structures[1]. As part of a continuing search for interesting sterols from marine sources together with a systematic investigation of marine life from south America and Antarctic coasts[2], we investigated the sponge of the Pacific coast *Esperiopsis edwardii* for its steroid content. It inhabits deep waters (-8m) and was collected by scuba diving at Chiloé (Chile).

Silica gel column chromatography allowed separation of three sterol groups and GC analysis indicated that each one was a complex mixture constituted by at least nine components. The most polar and abundant fraction (fraction 3) contained twelve Δ^5-derivatives (1A-12A) (Figure 1) and ten Δ^0-derivatives (2B,4B-12B). In this mixture, 24-methyl-cholest-5,24(28)-dien-3β-ol (9A) was the most abundant sterol among the Δ^5-derivatives while the corresponding stanol (9B) was the major among the Δ^0-derivatives.

The next fraction was separated into individual components by HPLC on reversed phase column (10 μm) and separated into individual components by re-chromatography on reversed phase columns (5 μm). Although mass spectra of all of them were very similar to those expected for a 3β-hydroxy-5αH-nucleus, ^{1}H-NMR spectra showed H-3 signal as a multiplet at δ 4.04 instead of the value δ 3.58 expected for a 3α-H in a 5αH-nucleus. The former value fulfilled the requirements for a 3β-H of a 3α-hydroxy-5αH-nucleus, taking into account that equatorial protons attached to hydroxyl substituted carbon atoms appear at lower fields than the axial protons in the epimeric alcohol. Also, values for Me-18 and Me-19 in each case were in good accordance with the proposed 3α-hydroxy-5αH-steroid structure. The ^{13}C-NMR spectrum performed on the total mixture showed a good accordance for nucleus carbons having a 3-substituted-5αH-skeleton being the displacement C-3 (66.60 ppm) of diagnostic value. This value was in accordance with a 3α,5α-array, considering that the other possibility (3β,5β) that also presents a similar C-3 displacement can be fully discarded, for the remaining ^{13}C-NMR spectra values and the Me-19 and H-3 displacement values in the ^{1}H-NMR spectra point to an A/B *trans* arrangement

The above data established the presence of a 3α-hydroxy-substituent in a sterol with A/B *trans* configuration and the combination of data from the individual mass and NMR spectra suggested that the compounds isolated from this fraction were: *(E)-24-Nor-cholest-22-en-3α-ol* (2C), *(E)-27-Nor-24(S)-methyl-cholest-22-en-3α-ol* (3C), *(E)-Cholest-22-en-3α-ol* (4C), *Cholestan-3α-ol* (5C), *(E)-24-Methyl-cholest-22-en-3α-ol* (7C), *24ξ-Methyl-cholestan-3α-ol* (8C), *24-Methyl-cholest-24(28)-en-3α-ol* (9C), *(E)-24ξ-Ethyl-cholest-22-en-3α-ol* (10C), *24ξ-Ethyl-cholestan-3α-ol* (11C) and *24-Ethyl-cholest-24(28)-en-3α-ol* (12C). To the best of our knowledge these compounds are described here for the first time as natural products (Table 1). The most abundant was *24-methyl-cholest-24(28)-en-3α-ol* (9C) coincidently with the structure of the most abundant Δ^5- and Δ^0-derivatives described below.

The third and less polar fraction (fraction 1) furnished a crystalline mixture of α,β-unsaturated-3-ketones as the principal components and two dienones as traces. Characteristic UV absorption (λ_{max} 230(hexane)) indicated the presence of the 3-keto-Δ^4-moiety and all the compounds showed the characteristic *m/z* 124 fragment in their mass spectra[3]. All spectroscopic data obtained for compounds 2D, 4D, 5D, 7D, 8D, 9D and 12D were in good agreement with those described for similar compounds isolated by Djerassi *et al.*[4] from the sponge *Stelleta clarella*. In addition to the mentioned ketones already described from the marine environment the sponge also contained compounds 10D and 11D which are already known only from terrestrial sources[5,6]. Although the amounts of each steroid containing fractions were different, GC profiles of the fractions were almost identical. This fact can be seen from Table 2. The presence as traces of the two dienones did not allow their separation, so ^{1}H-NMR spectrum was performed on the mixture. It depicted olefinic protons at δ 5.80 (H-4) and δ 6.20 (H-6). Mass spectrometric analysis of 7E afforded *m/z* 394 as the molecular ion, and important peaks at M-15 and M-43. Other important fragment ions at *m/z* 296, 295, 269 and 267, corresponding to the cleavage of C20-C22 bond with transfer of one and two hydrogens respectively, were present and also those corresponding to ring D cleavage with and without transfer of two hydrogens (*m/z* 225 and 227). The base peak of the spectrum, *m/z* 136 corresponds to the cleavage of C9-C10 and C7-C8 bonds characteristic of a 3-keto-$\Delta^{4,6}$-diene moiety[7]. All the above data suggested structure 7E for this compound, previously found in a fungus[8]. The other dienone, showed *m/z* 408 as the molecular ion and the same type of nucleus fragmentation as the previous one, plus an

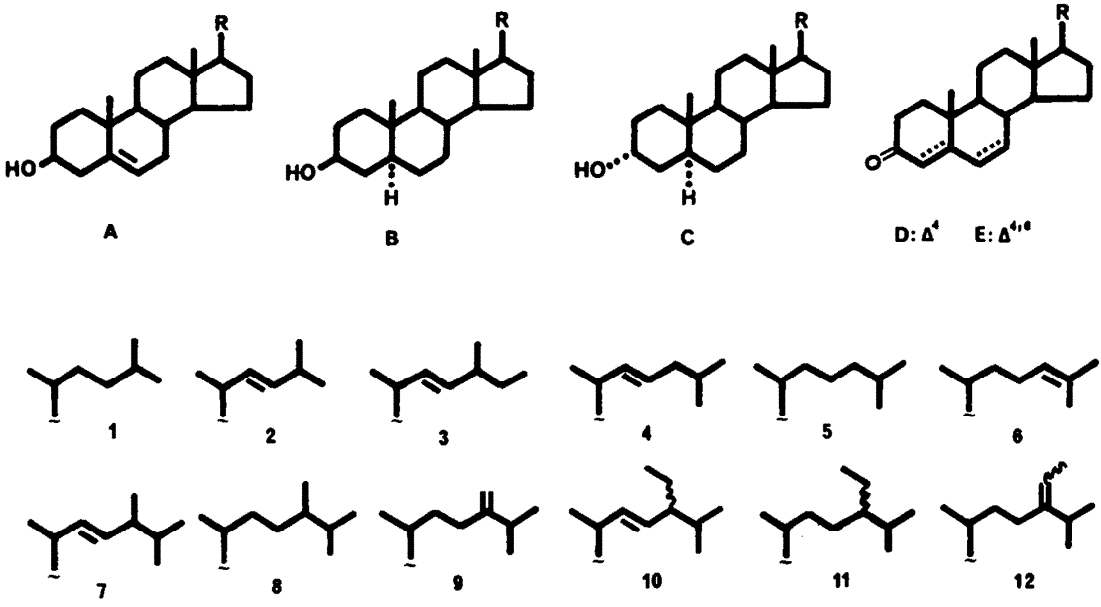

TABLE 1. ^{1}H-NMR, R.RT., and EIMS data for compounds 2C-5C, 7C-12C

100.1 MHz ^{1}H-NMR data ($CDCl_3$), J values in Hz

	H-18	H-19	H-26 H-27	H-28	H-29	H-21	H-3	H-22 H-23	R.RT.*	EIMS(m/z,%)
2C	0.65s	0.77s	0.95d J=6.5	----	----	0.97d J=6.0	4.06m	5.16m	0.85	372(5,M^+),357(8),354(16),339 (12),303(10),283(10),275(25) 273(29),257(41),255(38),233 (50),231(18),215(16),55(100)
3C	0.65s	0.75s	0.82t J=7.0	0.92d J=6.7	----	0.99d J=6.2	4.05m	5.20m	0.96	386(62,M^+), 371(22),353(28), 302(71),287(52),275(43),273 (81),257(100),255(27),232(19) 231(11),215(23),213(18),69
4C	0.66s	0.76s	0.86d J=6.5	----	----	0.99d J=6.8	4.06m	5.20m	0.97	386(50,M^+),371(20),353(30), 302(92),287(54),275(38),273 (63),257(100),255(33),232(14) 231(15),215(22),213(30),69
5C	0.66s	0.77s	0.83d J=6.6	----	----	0.90d J=6.2	4.05m	----	1.00	388(82,M^+),373(10),355(26), 316(8),302(19),275(14),257 (21),234(63),233(100),231(29) 215(99),201(10),55(60)
7C	0.66s	0.76s	0.82d J=6.6	0.91d J=6.9	----	0.99d J=6.6	4.04m	5.15m	1.04	400(65,M^+),385(7),367(3),357 (6),302(61),275(7),273(55), 257(100),255(9),233(11),215 (14),69(78).
8C	0.66s	0.77s	0.83d J=5.0	0.78d J=6.5	----	0.90d J=6.2	4.05m	----	1.08	402(100,M^+),387(6),369(4), 275(10),257(31),233(43),217 (18),215(36),43(17)
9C	0.66s	0.76s	1.02d J=6.9	4.65d J=1.2 4.71s	----	0.93d J=6.5	4.04m	----	1.11	400(16,M^+),385(18),367(7), 316(100),302(20),301(24),298 (5),287(5),285(12),273(65), 257(21),255(12),234(26),233 (33).
10C	0.65s	0.79s	0.82d J=6.5	----	0.81t J=5.8	1.02d J=6.0	4.08m	5.16m	1.13	414(72,M^+),399(7),381(4),371 (12),353(47),316(68),303(16), 302(57),301(22),287(20),285 (20),273(93),257(98),233(25), 217(14),215(32),55(100)
11C	0.65s	0.79s	0.82d J=6.5	----	0.84t J=5.8	0.92d J=6.0	4.04m	----	1.19	416(100,M^+),401(34),398(8), 383(26),355(10),275(16),257 (19),233(81),217(29),55(51)
12C	0.65s	0.79s	0.98d J=6.2	5.15m	1.57d J=5.8	0.99d J=6.0	4.04m	----	1.21	414(76,M^+),399(7),381(3),316 (53),302(53),301(17),298(3), 273(87),257(100),233(21),215 (28)

* R.RT. are relative to cholestan-3α-ol

TABLE 2. Sterol composition of the sponge *Esperiopsis edwardii*

Nucleus / Side chain	A	B	C	D	E
1	0.18(0.2)	----	----	----	----
2	1.68(1.9)	0.09(1.5)	0.45(3.0)	0.08(2.6)	----
3	0.18(0.2)	----	0.04(0.8)	----	----
4	5.12(6.0)	0.27(4.5)	0.27(5.1)	0.24(7.2)	----
5	3.20(3.7)	0.18(3.0)	0.11(2.2)	0.08(3.4)	----
6	0.55(0.6)	0.09(1.5)	----	----	----
7	7.32(8.6)	0.36(6.0)	0.45(8.6)	0.24(7.6)	traces
8	0.46(0.5)	0.09(1.5)	0.07(1.5)	0.01(0.5)	----
9	39.33(46.0)	3.20(52.2)	2.74(52.0)	1.75(54.2)	----
10	6.10(7.0)	0.27(4.5)	0.30(5.8)	0.16(4.7)	----
11	10.21(12.0)	0.64(10.4)	0.45(9.2)	0.32(8.7)	----
12	10.97(13.0)	0.91(15.0)	0.61(11.7)	0.32(10.0)	traces

Open values are % in total mixture; values in parentheses are % in the particular mixture.

important fragment ion at m/z 310 corresponding to a McLafferty rearrangement ion generated by a 24(28)-double bond[9] and the corresponding to the loss of methyl from this fragment (m/z 295). The ^{1}H-NMR spectrum depicted the olefinic quartet for H-28 at δ 5.20. The proposed structure for this compound was 12E which, to the best of our knowledge, has not been described previously.

EXPERIMENTAL

High performance liquid chromatography (HPLC) was carried out on a Micromeritics liquid chromatograph equipped with a 750 solvent delivery system, a 730 manual injector and a refractive index detector. Separations were performed using reverse phase columns Altech R-Sil C-18HL (10 μm) (500 x 10 mm, i.d.) and/or an Altex Ultrasphere ODS (5 μm) (250 x 10 mm, i.d.) using methanol and methanol-water mixtures as eluants. Proton magnetic resonance spectra (^{1}H-NMR) at 100 MHz and carbon-13 magnetic resonance spectra (^{13}C-NMR) at 25.1 MHz were performed on a Varian XL-100-15 spectrometer in the FT mode in deuterochloroform using TMS as internal standard. Other analytical methods are described elsewhere[2].

Sponges were collected in Quetamalhué, Chiloé (Chile) by scuba diving in December 1983 and steeped in acetone right after collection. They were identified by Ruth Desqueyroux-Faundez at the Museum d'Histoire Naturelle (Geneve, Switzerland) as *Esperiopsis edwardii*.

Extraction of sponges. The sponges (2080 g, wet weight) were ground in acetone and extracted with the same solvent. The solvent was filtered and concentrated at reduced pressure until most of the acetone was evaporated. The remaining aqueous residue was reextracted with ethyl acetate. The combined organic extracts were evaporated to a gummy residue (31.8 g) which was chromatographed on a silica gel column eluted with mixtures of petroleum ether-ethyl acetate of increasing polarity. Fractions collected were examined by TLC. The system allowed the separation of three main fractions, *i.e.*, fraction 1 (0.42 g), fraction 2 (0.70 g) and fraction 3 (12.0 g), according to decreasing mobility. Fraction 1, a single spot on TLC (Rf 0.4) showed by analytical GC to be a mixture of at least nine components. Further fractionation of the mixture by HPLC in conditions described and using 90% methanol as eluant allowed the separation of the main components which were analysed by GC-MS and ^{1}H-NMR. The next fraction, namely N° 2, a single spot by TLC (Rf 0.3) also showed by analytical GC to be formed by at least nine components, with a chromatographic profile similar to that of fraction 1. Fractionation of the mixture was achieved by HPLC on the reversed phase columns previously described and using methanol as solvent, to afford pure 3α-hydroxy-5α-stanols (2C-5C and 7C-12C) which were characterized as described above. Further elution of the column afforded fraction 3, a single spot by TLC (Rf 0.2). Analytical GC showed an elution profile similar to those registered for fractions 1 and 2. Fraction 3 was further purified by argentic silica column chromatography of the sterol acetates which allowed the separation of fully saturated stanols and 3β-hydroxy-Δ^5-derivatives with saturated and unsaturated side chains. Separation of the main components by HPLC allowed fully characterization of them by GC-MS, ^{1}H-NMR and comparison with authentic standards.

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