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Chemical Diversity in the Mediterranean Sponge *Raspaciona aculeata*: Structure and Absolute Stereochemistry of Blanesin

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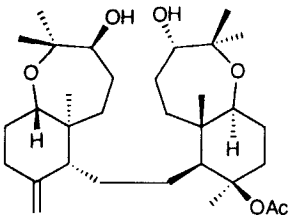
Abstract: Several samples of *Raspaciona aculeata* collected from the same area (Blanes) displayed completely different metabolite patterns characterized either by already known triterpenes, raspacionins, or by blanesin (**2**). Blanesin is a new *ent*-labdane furanoditerpenoid with an α-hydroxyketone in the ring A. Structure and absolute stereochemistry were established by spectroscopic and chemical methods.

Raspaciona aculeata Johnston is a red encrusting sponge that contains a series of unusual triterpenoids[1-4] all characterized by two perhydrobenzoexepine systems linked by an ethylene bridge (e.g. raspacionin, **1**). Continuing our research to investigate the chemistry and the biology of *R. aculeata*, many little samples of the sponge were collected off Blanes (NE Spain) from January to March 1992. The samples were separately extracted. All extracts were characterized by the presence of raspacionins with the surprising exception of a single sample which, analyzed by TLC (petroleum/diethyl ether, 8:2), displayed a completely different metabolite pattern. The sponge was reanalyzed from a taxonomic point of view, but external morphology, shape, size of spicules, amount of spongin were identical to those already described for *R. aculeata* . It has to be noted that other two species of this genus have been also described: *R. robusta* Sarà[5] and *R. calva* Sarà.[5] While *R. calva* is well characterized by the particular shape if its acanthostyles and the enormous size of its tylostyles, *R. robusta* is indistinguishable from *R. aculeata*, given the spicule variability shown by this last species.[6]

The diethyl ether soluble fraction (130 mg) from the acetone extract of *R. aculeata* (dry weight 1g) was characterized by a main metabolite (15 mg, petroleum/diethyl ether, 8:2, *Rf* 0.5), named blanesin (**2**), that was isolated by column chromatography (SiO_2, petroleum and increasing amounts of diethyl ether). No trace of raspacionins was observed. Blanesin (**2**), 3α -hydroxy-*ent* -labda-7-en-13-furan-2-one, is an optically active ($[\alpha]^D_{20}$ = - 14.3°, c 0.1 $CHCl_3$) amorphous powder with elemental composition $C_{20}H_{28}O_3$ deduced by HREIMS on the molecular peak at *m/z* 316.2051 (M^+, 3%;$C_{20}H_{28}O_3$ required 316.2038); other MS fragments: 235 (10%), 161 (3%), 119 (9%), 107 (12%) 91 (18%), 82 (100%); IR: ν_{max} (KBr) 3485, 1703, 853 cm^{-1}; UV λ max (MeOH) 212 (ε 9830), 280 (ε 883) nm .

The ^{1}H-NMR spectrum displayed resonances easily attributable to the protons of a labdane furanoditerpenoid. In fact, three broad singlets (δ 7.36, H-15; δ 7.23, H-16; δ 6.26, H-14) were assigned to the protons of a β-substituted furan ring linked to a methylene (δ 2.38-2.66; H_2-12) that through a second methylene (δ 1.53; H_2-11) was connected to a proton (δ 2.02; H-9) devoid of further vicinal couplings but allylically

coupled to an olefinic proton (δ 5.46; H-7). The proton at δ 5.46 displayed allyl coupling with H_3-17 (δ 1.77) and vicinal coupling with a methylene (δ 1.99-2.02; H_2-6) which in turn was linked to a methine (δ 1.82; dd, J =5, 11 Hz; H-5). The ^{1}H-NMR spectrum was completed by an isolated AB system (δ 2.00-2.57; d, J =13 Hz; H_2-1), by three methyl singlets (δ 0.71, H_3-19; δ 0.77, H_3-20; δ 1.13, H_3-18) and, finally, by a second AB system with resonances at δ 3.90 (H-3, d, J =5.2 Hz) and 3.38 (OH, d, J =5.2 Hz). Acetylation (room temperature, 24h) gave a slightly less polar acetyl derivative which exhibited in the ^{1}H-NMR spectrum the substitution of the second AB system with a singlet at δ 4.95 (H-3). The difficult acetylation of **2**, as well as the comparable polarity with its acetate , might be explained by the presence of a strong hydrogen bond between the alcoholic group and the near carbonyl .



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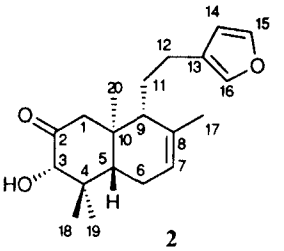

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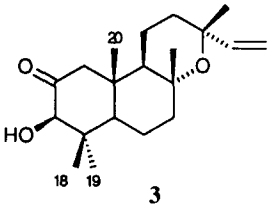

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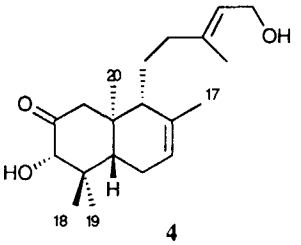

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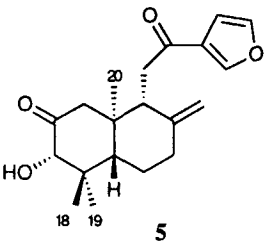

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All these data led to a furanolabdane skeleton with a substitution pattern of the ring A characterized by an α-hydroxy ketone. All ^{1}H- and ^{13}C-NMR resonances (Table 1) were connected by ^{1}H-^{1}H-COSY, ^{1}H-^{13}C -HETCOR. The multiplicity of the isolated alcoholic methine suggested two alternative structures with the OH group either at C-1 or at C-3. The latter was preferred by a series of diagnostic 2D heterocorrelations observed in an HMBC experiment. The ^{1}H-^{13}C long range heterocorrelations between C-3 (δ 82.36) and H_3-18 (δ 1.13) and H_3-19 (δ 0.71) placed the OH group at C-3, whereas other cross peaks (Table 1) completely confirmed the suggested structure. The OH group was equatorially oriented by comparison with model compounds: 8,13-epoxy-3β-hydroxylabd-14-en-2-one (**3**),[7] 3α,15-dihydroxy-*ent*- labda-7,13*E*-dien-2-one (**4**)[8] and 3α-hydroxyaustrofolin (**5**)[9] (Table 1). In fact, the hydroxylated methine of **2** displayed NMR resonances at δ 82.36 (C-3) and δ 3.90 (H-3) according to the chemical shifts (δ^{13}C 82.8, δ^1H 3.89) of the corresponding methine of **3**. The comparison of the ^{1}H-NMR spectra of **2** and **4** further supported the suggested stereochemistry whereas the ^{1}H-NMR chemical shift of H-3 of **5** at δ 4.27 suggests a reanalysis of the proposed[9] orientation of the substituents at C-3. Strangely, a long range coupling was observed in the ^{1}H -^{1}H COSY spectrum of **1** between the axial H-1 (δ 2.00) and H-3 (δ 3.90). This diaxial coupling is unusual but already described for **3**.[7]

Table 1- NMR Data for Blanesin 2 [a,b], 3[7], 4[8] and 5[9]

	1					3	4	5
Position	δ^1H	δ^{13}C	m	H's related to C	n.O.e.	δ^1H	δ^1H	δ^1H
1β	2.00	51.23	t	H_3-20		2.47	2.20	2.41
1α	2.57					2.10	2.64	2.52
2	--	210.89	s	H-3; H_2-1; OH		-	-	-
3	3.90	82.36	d	H_3-1; H_3-18; H_3-19; OH	H-1β, H-5,H_3-18	3.89	3.95	4.27
4	--	44.88	s	H_3-18; H_3-19		-	-	-
5	1.82	48.61	d	H_3-18; H_3-19; H_2-1	H_3-18, H-3, H-1β		1.85	1.63
6	1.99	23.68	t	H-5			1.99	1.53
	2.02						2.11	1.78
7	5.46	122.15	d	H-9; H_2-6; H_3-17			5.55	
8	--	134.62	s	H-11; H_3-17;			-	
9	2.02	53.69	d	H-11; H_3-17; H_3-20; H-7; H_2-12; H_2-6			1.95	2.77
10	--	43.20	s	H_2-1; H_3-20; H-4				
11	1.53	27.71	t	H_2-12				
12	2.38	26.28	t	H_2-11				
	2.66							
13	--	124.46	s	H_2-12; H-14; H-15; H-16; H_2-11				
14	6.26	110.89	d	H_2-12; H-16; H-15				
15	7.36	143.02	d	H-14; H-16				
16	7.23	139.05	d	H_2-12; H-14; H-15				
17	1.77	21.85	q				1.73	
18	1.13	28.11	q			1.18	1.13	1.12
19	0.71	15.77	q		OH	0.76	0.72	1.11
20	0.77	14.55	q		H-1α, H-11	0.68	0.77	0.91

a) Brucker 500 AMX-spectrometer. Chemical shifts referred to $\underline{C}HCl_3$ at 7.26ppm and to $\underline{C}DCl_3$ at 77.00
b) Assignments aided by ^{1}H-^{13}C HETCOR, DEPT, HMQC, HMBC, ^{1}H-^{1}H COSY, ^{1}H-^{1}H spin decoupling experiments

Conversely, we did not observe any coupling between H-3 and H_3-19 as reported for **4**. The comparison of the ^{1}H-NMR resonances between **2** and **3** (Table 1) shows some little differences (H_3-18, H_3-19 and H_3-20). However, our assignment of the ^{1}H and ^{13}C-NMR resonances of the methyls at C-4 and at C-10 was supported by a series of diagnostic cross peaks observed in an HMBC experiment (10 Hz) that linked C-1, C-9, C-10 to H_3-20 and C-3, C-4, C-5 to H_3-18, H_3-19. Finally the ^{13}C-NMR chemical shift of C-11 (δ 27.71) led to assign an equatorial orientation to the methylene substituent at C-9. A series of n.O.e. experiments confirmed this structure. When H-3 was irradiated a strong enhancement was observed for H_3-18, H-5 and H-1β, while when H_3-20 was irradiated a n.O.e. was observed with H-1α and H-11.

The absolute stereochemistry was established by applying the high resolution ^{1}H-NMR to the Mosher method. Blanesin (**2**) was esterified with both *R*- and *S*-α-methoxy-α-trifluoromethylphenylacetic acid chloride (MTPA) giving, respectively, the *S*- and *R*-MTPA esters. The observed ^{1}H-NMR values of $\Delta\delta$ [δ(*S*-MTPA ester) - δ(*R*-MTPA ester)] (Table 2) suggested that the absolute configuration at C-3 is *S*. Particularly diagnostic was the negative $\Delta\delta$ effect observed for the protons of both methyls at C-4 (H_3-18, -0.18; H_3-19, -0.05). Previously, the absolute stereochemistry of **4** was suggested by a negative CD (CH_3CN) at 292 nm of its acetate.[8] Analogously, the CD spectrum (CH_3CN) of the acetate of **2** shows the same sign, but some conflicting data were observed by recording the CD spectra of **2** in different solvents.[10]

Table 2-Selected δ ^{1}H Values [a] for Mosher's Esters of Compound **2**

Position	δ H (*S*)-MTPA ester	δ H (*R*)-MTPA ester	$\Delta\delta$ (δS-δR)
1β	2.16	2.16	0.00
1α	2.56	2.56	0.00
3	5.06	5.07	-0.01
5	1.90	1.93	-0.03
6	1.99	2.02	-0.03
	2.08	2.12	-0.04
7	5.46	5.47	-0.01
18	0.95	1.13	-0.18
19	0.83	0.88	-0.05
20	0.81	0.80	+0.01

[a] 500MHz, $CDCl_3$, chemical shifts are referred to $CHCl_3$ (δ 7.26)

Compounds related to blanesin (**2**) are relatively widespread plant metabolites[7,8,9,11] whereas the oxidative pattern of ring A is rare among the diterpenoids from marine organisms. It has been displayed only by some tricyclic diterpenoids from horny sponges (*Hyatella intestinalis*,[12] *Spongia* sp.[13]) from the Indo-Pacific with no evident taxonomical relationship with *R. aculeata* . However, bicyclic labdanes were isolated from the alga *Caulerpa trifaria*[14] and *Laurencia pinnata*,[15] whereas a clerodane compound was recently found in the Aplysillidae sponge *Chelonaplysilla erecta*.[16]

The finding of different, but related, metabolites in sponges classified under the same name is quite common[17] (e.g. furanosesquiterpenoids in *Dysidea fragilis*), but the presence of metabolite patterns completely different in samples of the same species, collected from the same habitat, by the same marine biologist, identified by the same expert (M.U.) is very unusual. This study raises intriguing reflections upon the potential role of secondary metabolites in taxonomical studies.

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10. The CD spectra of **2** are influenced by the polarity of the solvents: $[\theta]_{296max}$ = -461, (cyclohexane); $[\theta]_{276max}$ =+404, (CH_3CN).The CD profiles of the acetate of **2**, after acetylation of the same sample used for recording the CD of 2, were negative in both solvents: $[\theta]_{301max}$ =-1700, (cyclohexane); $[\theta]_{301max}$=- 1300, (CH_3CN).
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