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A NEW TRITERPENOID SKELETON FROM THE MEDITERRANEAN SPONGE *RASPACIONA ACULEATA*: STRUCTURE OF RASPACIONIN

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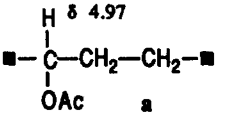
Abstract: A new squalene-derived triterpenoid, raspacionin (1), has been isolated from the sponge *Raspaciona aculeata*. The structure of 1, characterized by two *trans*-perhydrobenzoxepine moieties linked by an ethylene brigde, was suggested by spectral evidence and unambigously established by single-crystal X-ray diffraction analysis.

The sponge *Raspaciona aculeata* offers some intriguing goads to chemical investigations. Firstly, its taxonomic classification is a matter of discussion. In fact, *R. aculeata* belongs to the family Raspailiidae which alternatively has been placed into the order Poecilosclerida[1] or Axinellida[2,3] on the basis of skeletal features. Secondly, this sponge seems to display some allelochemical activities since it never bears epibiotic larvae or propagula nor is covered by other grown-up organisms. On the contrary, an area 2-5 mm thick, free of living organisms, always sourrounds the sponge in habitats where the substratum is saturated.

We now report the first chemical studies on the liposoluble extracts from *R. aculeata*. The structure of the main metabolite, named raspacionin (1), has been unequivocally established; it displays a new triterpenoid skeleton characterized by two *trans*-perhydrobenzoxepine groups linked by an ethylene bridge. *R. aculeata* was collected in Blanes (NE Spain), during January 1990, by hand using SCUBA in an overhang at a depth between 10-15 m. The fresh sponge (dry weight: 4 g) was exhaustively extracted with acetone. The diethyl ether soluble fraction (1.5 g) from the acetone extract was sequentially fractionated on two silica gel columns (1, *n*-hexane-diethyl ether 95:5; 2, methylene chloride-diethyl ether 95:5) obtaining 31 mg of the main metabolite (1) (TLC Rf= 0.45, petroleum ether-diethyl ether 1:1) along with some minor compounds.

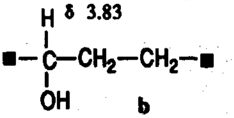
Raspacionin (1): $C_{34}H_{56}O_7$ (deduced by MS and ^{13}C-NMR data); optically active crystalline (colourless elongated prisms from *n*-heptane) compound; mp 188-189°C; $[\alpha]_D$ 31.4° (c 1.5, $CHCl_3$); IR ν_{max} 3470, 1732, 1730 cm^{-1}; MS m/z (%): 456 (M^+-2 AcH, 5), 438 (30), 220 (40), 218 (55), 205 (30), 187 (40), 147 (50), 125 (100), 107 (60). ^{1}H- and ^{13}C-NMR assignments are reported in Table.

The ^{13}C-NMR spectrum of 1 revealed the presence of 4 sp^2 and 30 sp^3 carbons easily assigned, by DEPT sequence and ^{1}H-^{13}C HETCOR, to 2 $-O-CO-CH_3$, 1 $\geq C=CH_2$, 6 CH, 10 CH_2, 7 CH_3 and 5 quaternary carbons. In particular, the ^{13}C-resonances of four methines and three carbons devoid of protons, falling in the region between 70 and 85 ppm, suggested the presence in the molecule of some downshifting substituents; most probably a hydroxy moiety and two oxygen bridges, in addition to the above reported acetoxy groups. The ^{1}H-NMR spectrum of **1** confirmed these structural features, exhibiting resonances at δ 4.97 (-C$\underline{H}$-OAc), 3.83 (-C$\underline{H}$-OH, downshifted after acetylation to δ 4.99), 3.68 and 3.41 (protons of methines linked to ether oxygen). Starting from these four protons, ^{1}H-^{1}H spin-decoupling experiments, ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR allowed to connect all the resonances of the following isolated partial units (**a** - **d**):



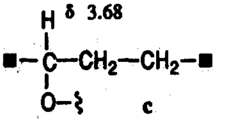
$$\blacksquare-\overset{H\ \delta\ 4.97}{\underset{OAc}{C}}-CH_2-CH_2-\blacksquare$$

a



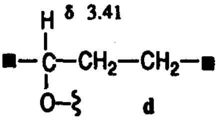
$$\blacksquare-\overset{H\ \delta\ 3.83}{\underset{OH}{C}}-CH_2-CH_2-\blacksquare$$

b



$$\blacksquare-\overset{H\ \delta\ 3.68}{\underset{O-}{C}}-CH_2-CH_2-\blacksquare$$

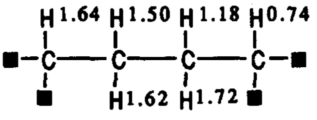
c



$$\blacksquare-\overset{H\ \delta\ 3.41}{\underset{O-}{C}}-CH_2-CH_2-\blacksquare$$

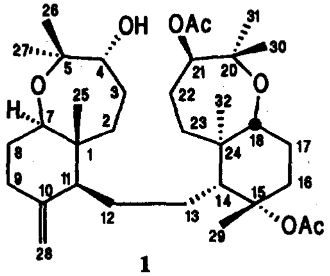
d

The ^{1}H-NMR spectrum was completed by the resonances assignable to the exomethylene protons (δ 4.89 and 4.60), to a tertiary acetoxy group (δ 1.95), to seven tertiary methyls, and to the *isolated* system **e**.



$$\blacksquare-\overset{H1.64}{\underset{\blacksquare}{C}}-\overset{H1.50}{\underset{H1.62}{C}}-\overset{H1.18}{\underset{H1.72}{C}}-\overset{H0.74}{\underset{\blacksquare}{C}}-\blacksquare$$

e



1

Two ^{1}H-^{13}C long-range HETCOR experiments (J = 8 and 10 Hz) displayed some highly diagnostic correlations (Table) which allowed to link the exomethylene group to the partial units **c** and **e**. Furthermore, the latter in turn was connected, through a quaternary carbon bearing a methyl and an acetoxy group, to the fragment **d**. Correlations, involving protons and carbons of the angular methyls (CH_3-1, CH_3-24), led to correctly place the remaining fragments **a** and **b**, both furthermore jointed to downshifted quaternary carbons (^{13}C-NMR δ 77.57 and 77.33) bearing a couple of methyls.

At this point, some alternative triterpenoid structures could be suggested according to the nature of the groups linked by the ether bridges. In order to clarify the whole structure, including the relative stereochemistry, the X-ray diffraction analysis was carried out, using a single crystal (from *n*-heptane) of **1**, mounted on an Enraf-Nonius CAD-4F diffractometer (Ni filtered Cu Kα radiation). CRYSTAL DATA: $C_{34}H_{56}O_7$, Mw = 576.8, orthorhombic, space group $P2_12_12_1$ with a = 7.117(1), b = 19.757(2), c = 27.156(4) Å, V = 3819(1) Å^3, Z = 4, D_c = 1.003 g cm^{-3}.

The structure was solved using direct methods (MULTAN82)[4] and difference Fourier syntheses and refined anisotropically (non hydrogen atoms) by full matrix least-squares procedures. Present discrepancy R factor is 0.057 on 2766 observed reflections (θ_{max} 75°) with $I \geq 3\,\sigma(I)$ and 371 refined parameters[5]. Fig 1 shows a view of the X-ray model of raspacionin and indicates the relative stereochemistry of the chiral centres. In absence of atoms with strong anomalous scattering, the absolute configuration can not be determined.

The refinement, including desordered water molecules, is in progress. Details of the molecular geometry and crystallographic packing will be reported elsewhere.

TABLE - 500 MHz ^{1}H-NMR and 125 MHz ^{13}C-NMR data of raspacionin (1)^a

Carbon	δ ^{13}C^b	m^c	δ ^{1}H^b	m; J in Hz	long-range ^{1}H-^{13}C correlation	
					(J = 8Hz)	(J = 10Hz)
1	42.80	s	-		0.70(CH$_3$-1) 1.64(H-11)	0.70(CH$_3$-1)
2	33.90	t	1.48 1.65		0.70(CH$_3$-1)	0.70(CH$_3$-1)
3	26.10	t	1.82 2.00			
4	77.00	d	3.83	bd; 5	1.12(CH$_3$-5 eq)	
5	77.57	s	-		1.12(CH$_3$-5 eq) 1.27(CH$_3$-5 ax)	1.12(CH$_3$-5eq) 1.27(CH$_3$-5ax)
7	76.01	d	3.68	dd; 11.7, 4.9		
8	33.02	t	1.62 1.38		1.95(H-9)	
9	35.74	t	1.95 2.24	bd; 11.8	4.60(H-28)	4.60(H-28) 4.89(H-28)
10	147.17	s	-			1.64(H-11)
11	53.65	d	1.64		0.70(CH$_3$-1) 4.89(H-28)	0.70(CH$_3$-1)
12	27.59	t	1.50 1.62			0.74(H-14)
13	25.48	t	1.18 1.72			
14	58.18	d	0.74	bs	0.94(CH$_3$-24)	2.80(H-16) 1.50(H-12) 0.94(CH$_3$-24)
15	83.81	s	-		1.53(CH$_3$-15)	2.80(H-16) 1.53(CH$_3$-15
16	33.21	t	2.80 1.31	bd; 14.5	1.51(H-17)	1.51(H-17)
17	26.59	t	1.39 1.51			
18	76.74	d	3.41	dd; 11.6, 4.6		
20	77.33	s	-			
21	79.14	d	4.97	d; 6.8		1.20(CH$_3$-20eq)
22	23.08	t	1.81 2.00			
23	35.32	t	1.45 1.27		2.00(H-22) 0.94(CH$_3$-24)	0.94(CH$_3$-24)
24	43.37	s	-		0.94(CH$_3$-24)	0.94(CH$_3$-24)
25	12.20	q	0.70	s	1.64(H-11)	
26	29.11	q	1.12	s		1.27(CH$_3$-5ax)
27	21.23	q	1.27	s		1.12(CH$_3$-5eq)
28	107.38	t	4.89 4.60	bs bs		2.24(H-9)
29	25.26	q	1.53	s		
30	21.56	q	1.15	s		1.20(CH$_3$-20eq)
31	28.96	q	1.20	s		1.15(CH$_3$-20ax)
32	12.81	q	0.94	s	0.74(H-14) 1.27 (H-23)	1.27(H-23)
$O\underline{C}OCH_3$-15	170.16	s	-			
$O\underline{C}OCH_3$-21	170.16	s	-			
$OCO\underline{C}H_3$-15	22.51	q	1.95	s		
$OCO\underline{C}H_3$-21	21.23	q	2.17	s		

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[a] CDCl$_3$; TMS=0; Bruker WM 500 Spectrometer; numeration according to ref. 7.

[b] Assignments aided by ^{1}H-^{13}C HETCOR, ^{1}H-^{1}H COSY, spin decoupling experiments and by comparison with related compounds (7-11).

[c] Deduced by DEPT sequence.

Raspacionin (1) adds to the very poor group of triterpenoids from sponges[6]. At the best of our knowledge, squalene-derived triterpenoids have been found only in the sponges *Siphonochalina siphonella*[7-11], *Jaspis stellifera*[12, 13] and a species of the genus *Stelletta*[14].

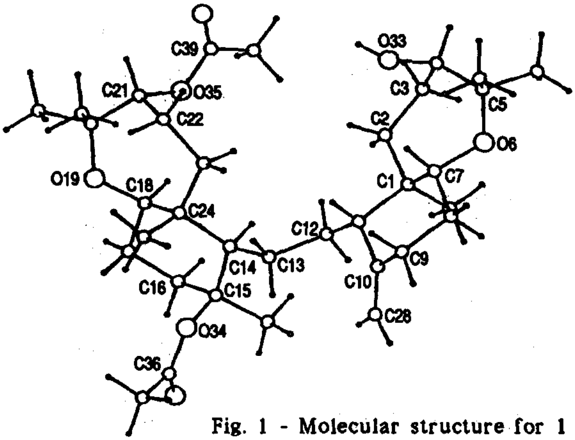
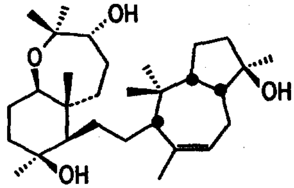


Fig. 1 - Molecular structure for 1



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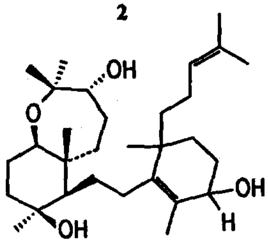

3

The unique in nature carbon skeleton of 1 is structurally related to those of the sipholane and siphonellane metabolites, found in the Red Sea sponge *S. siphonella*, which exhibit frameworks characterized by a *trans*-decahydrobenzoxepine part linked, by an ethylene bridge, either to a *cis*-octahydroazulene moiety [e. g. sipholenol (2)] or to a substituted cyclohexene group [e. g. siphonellinol (3)]. Analogously to the biogenetic origin suggested [8] for the metabolites from *S. siphonella*, a tetraepoxy-squalene precursor should lead to 1 through two independent cyclization initiations.

Studies, aimed at establishing the absolute configuration of 1 and at investigating its role in the life cycle of the sponge, are in progress.

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