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Raspacionin-A: A Novel Rearranged Triterpenoid From The Mediterranean Sponge *Raspaciona aculeata*

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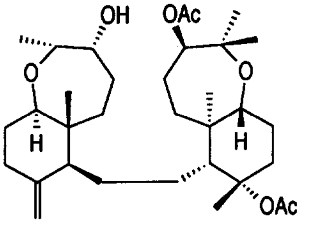
Key words: sponge, raspacionin-A, triterpenoid, 2D-NMR, X-ray structure.

Abstract: *A new rearranged triterpenoid, raspacionin-A (3), has been isolated from the encrusting red sponge Raspaciona aculeata. The structure was unambiguously determined by X-ray diffraction studies on its deacetyl derivative (4). All ^1H- and ^{13}C-NMR resonances of 3 and 4 were assigned by an extensive use of 1D and 2D NMR techniques. The absolute stereochemistry was assigned by applying Mosher's method to 4.*

Raspaciona aculeata is a red encrusting sponge which, being completely devoid of epibiotic organisms, probably is chemically protected against predators. Recently,[2] the main secondary metabolite of *R. aculeata*, raspacionin (**1**), has been isolated from the diethyl ether soluble fraction of the acetone extract of the sponge. A single crystal X-ray diffraction analysis revealed for **1** a new triterpenoid skeleton characterized by two *trans*-perhydrobenzoxepine groups linked by an ethylene bridge. Even though triterpenoids have been rarely found in marine organisms,[3] triterpenoids related to raspacionin (*e. g.* sipholenol-A, **2**) were previously[4-7] isolated from the Red Sea sponge *Siphonocalina siphonella* which belongs to the Haplosclerida order whereas *Raspaciona aculeata* is an Axinellida sponge belonging to the Raspaillidae family. However, until now the taxonomical collocation of *R. aculeata* is a matter of discussion having been alternatively placed in the order Poecilosclerida[7] or Axinellida.[9-10] In this paper we report the structure of a second metabolite from *R. aculeata*, raspacionin-A (**3**), which exhibits a novel rearranged triterpenoid skeleton.

R. aculeata was extracted as previously[2] reported. The TLC analysis (SiO_2, petroleum ether/diethyl ether

1:1) of the diethyl ether soluble fraction revealed, along with the main spot (*Rf* = 0.45) associated with raspacionin (1), a second less polar fraction (*Rf* = 0.70) and some more polar components (*Rf* = 0.15). Raspacionin-A (3) was isolated from the less polar fraction by HPLC (Spherisorb column, CH_3CN/H_2O 8:2) which splitted 3 from a third triterpenoid, raspacionin-B, actually under investigation.



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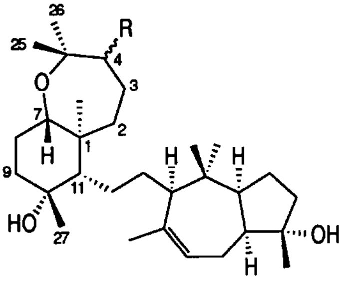
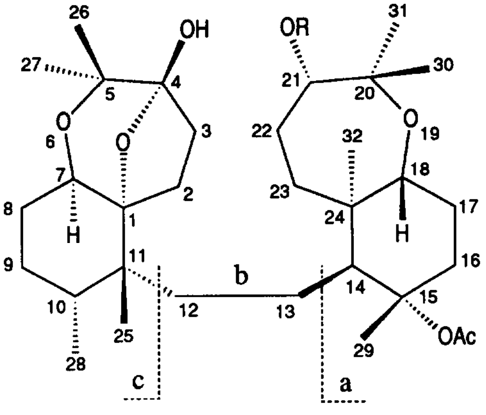


2 R = β-OH 7 R = α-OH



3* R = $COCH_3$ 4* R = H

* Relative stereochemistry

Raspacionin-A (3), $C_{34}H_{56}O_8$ deduced from the ^{13}C-NMR data combined with the HREIMS of the main mass fragments, is an amorphous optically active powder, exhibiting in the IR spectrum two diagnostic bands at 3422 (OH) and at 1728 cm^{-1} (C=O). The ^1H-NMR spectra, displaying the protons of 7 methyl singlets, 2 acetoxy methyls, 3 methines linked to oxygens, showed relevant analogies with the NMR spectra of 1. The differences were mainly due to the absence of the protons (H-4 and H-11) of two methines and of the exomethylene system of 1, substituted in 3 by two non-protonated downfield carbons (δ ^{13}C 105.64 and 90.84) and by a $>CHCH_3$ system [(δ ^1H, 1.23 (1H, m) and 0.80 (3H, d)], respectively. All the ^1H-NMR resonances were connected to the corresponding carbons by direct 2D ^1H-^{13}C heterocorrelation (Table 1). Three downshifted signals at δ 4.70 (H-21, δ ^{13}C 80.80), 4.05 (H-7, δ ^{13}C 71.26) and 3.45 (H-18, δ ^{13}C 70.79) were assigned to the protons of oxygenated methines, two of them attributable to the X part of isolated A_2B_2X systems. In fact, a HOHAHA experiment[11] correlated the signal at δ 4.70 (H-21) with those at δ 2.13 (H-22), 1.38 (H-22), 1.23 (H-23), 2.26 (H-23), whereas the signal at δ 3.45 (H-18) was connected to those at δ 1.42 (H-17), 1.77 (H-17), 1.59 (H-16) and 1.86 (H-16). Three different ^1H-^{13}C long range heterocorrelations were performed using both the normal pulse sequence with delays adjusted for J = 9 Hz and J =10 Hz and the Coloc sequence[12] with delay for J =10 Hz. The ^{13}C-NMR signal at δ 74.97 (C-20) exhibited in addition to some ^2J correlations with the protons at C-21, C-30 and C-31, highly diagnostic long-range ^3J cross-peaks with the protons at C-22 (δ 1.38 and 2.13) and C-18 (δ 3.45) which allowed to connect the two A_2B_2X systems as shown in the partial structure **a** of 3. The same experiments revealed ^1H-^{13}C long-range correlations between the protons of the methyl resonating at δ 1.52 (CH_3-15) and the carbons C-16 (δ 33.43) and C-14 (δ 51.43). The latter was also correlated to the protons of C-32 (δ 1.05), which in turn were connected with C-23 (δ ^{13}C 36.33). An ethylene substituent, partial structure **b** of **3**, was linked at C-14 on the basis of 1D (selective decouplings) and 2D (COSY, HOHAHA) ^{1}H-^{1}H NMR experiments. ^{1}H-^{13}C long range couplings between C-15 (δ 85.02) and H-13 (δ 0.94) and between C-12 (δ 32.65) and H-14 (δ 2.05) confirmed the connection between the partial structures **a** and **b**. The absence of further ^{1}H-^{1}H couplings of the protons at C-12 suggested a quaternary nature for C-11. The comparison of the ^{1}H and ^{13}C-NMR chemical shifts of **1** and **3** revealed that the partial structure **a** of **3** has to possess the same perhydrobenzoxepine system of **1** but with a relative stereochemistry of the substituents which differs both at C-21, on the basis of the resonances of protons and carbons of the couple of β-methyls, and at C-14, on the basis of the more downshifted ^{13}C-NMR value of C-32 (δ 20.68; δ 12.81 in **1**).

Finally, the HOHAHA experiment displayed for the partial structure **c** an isolated -CH_2-CH_2- system with ^{1}H-NMR resonances at δ 2.32 (H-3), 1.57 (H-3) and 1.82 (H-2's) and also an isolated -CH-CH_2-CH_2-CH(CH_3)- moiety with ^{1}H-NMR resonances at δ 4.05 (H-7), 1.31 (H-8), 1.65 (H-8), 1.32 (H-9's), 1.23 (H-10) and 0.80 (CH_3-10). C-10 (δ ^{13}C 39.93) of the second partial structure was linked to a quaternary carbon (C-11, δ ^{13}C 41.04) bearing a methyl (δ ^{1}H 0.98, δ ^{13}C 18.81) on the basis of diagnostic ^3J couplings between C-10 and C-11 and the protons of CH_3-11 and CH_3-10, respectively. Further ^2J couplings between C-11 and H-12 (δ 1.71) and ^3J between C-12 (δ ^{13}C 32.65) and the protons of CH_3-11 (δ 0.98) allowed to link C-11 to the ethylene bridge between C-11 and C-14. Other key ^{1}H-^{13}C correlations were exhibited by C-1 (δ 90.84), with the protons at CH_3-11 (δ 0.98), at C-12 (δ 1.32 and 1.71) and at C-2 (δ 1.82), allowing to link the oxygenated tertiary C-1 to C-2 and C-11. The structure of **3** was completed by an oxygenated tertiary carbon (C-5, δ ^{13}C 77.40) bearing two methyls (δ ^{1}H 1.25 and 1.31) and further linked to a hemiketal carbon (C-4, δ ^{13}C 105.64), which in turn on the basis of a series of ^{1}H-^{13}C heterocorrelations was connected with C-3. All these data well fitted with a partial structure **c** characterized by a perhydrobenzoxepine system with an oxygen bridge between C-4 and C-1 which, bearing in mind the biogenetic origin from a squalene precursor, should induce a 1,2 shift of the methyl from C-1 to C-11 leading to a rearranged triterpenoid skeleton.

With the aim of confirming the suggested structure, raspacionin-A (**3**) was submitted without success to acetylation. On the contrary, the methanolysis of **3** led to the hydrolysis of the acetoxy group at C-21 giving a crystalline compound, 21-deacetyl-raspacionin-A (**4**), obtained from *n*-hexane/diethyl ether 1:1. All the ^{1}H- and ^{13}C-NMR data (Table 1) confirmed the suggested structure. Of course, H-21 was shifted to high fields (δ 3.61) whereas C-21 (78.97), C-22 (29.48) and C-20 (76.45) exhibited the expected deacetylation shifts. However, in spite of the extensive NMR analysis an alternative structure of **3** with an oxygen bridge between C-5 and C-8 (not C-7) cannot be rigorously left out. In fact, the overlap of three resonances (H-9's and H-8) at ≈ δ 1.32 in both **3** and **4**, does not allow to distinctly observe all the couplings of the protons from C-7 to C-10.

In order to confirm unambiguously the structure and to establish the relative stereochemistry of all the ten chiral C atoms, an X-ray analysis on **4** was performed. The structure was solved using SIR88[13] and refined, for the positional and anisotropic thermal parameters of 78 independent non-hydrogen atoms, to a R factor of 0.053 by means of full-matrix least-squares cycles. In the crystal, the asymmetric unit is formed by two molecules which display a high degree of geometrical and conformational similarity. They are related by a non-crystallographic twofold axis, which lies in the *a-c* plane at an angle of about 7° with the [1 0 1] direction. On superposition, the r.m.s deviation of the corresponding atoms of the two molecules is only 0.15Å with the largest differences (< 0.34 Å) associated to acetoxy groups. The pseudo symmetry caused problems in the structure determination, which were ultimately overcome with the SIR88 package by a careful examination of

Table 1. 500 MHz NMR Data of Raspacionin-A (3) and 21-Deacetyl-Raspacionin-A (4)[a].

	3				4			
carbon	δ $^{13}C^{b}$	m^{c}	δ $^{1}H^{b}$	m; *J* in Hz	δ $^{13}C^{b}$	m^{c}	δ$^{1}H^{b}$	m; *J* in Hz
1	90.84	s	-		89.96	s	-	
2	23.41	t	1.82		23.76	t	1.74	
			1.82				1.88	
3	31.33	t	1.57		32.74	t	1.41	
			2.32				2.40	ddd; 12.50, 9.60, 3.30
4	105.64	s	-		105.72	s	-	
5	77.40	s	-		76.64	s	-	
7	71.26	d	4.05	bdd; 10.82; 5.16	71.31	d	4.00	dd; 10.30; 4.78
8	28.52	t	1.31		28.40	t	1.31	
			1.65				1.69	
9	27.66	t	1.32		27.55	t	1.33	
			1.32				1.33	
10	39.93	d	1.23		39.65	d	1.27	
11	41.04	s	-		41.25	s	-	
12	32.65	t	1.32		33.19	t	1.29	
			1.71				1.74	
13	25.14	t	0.94		25.17	t	0.98	
			1.99				1.86	
14	51.43	d	2.05		51.74	d	1.96	
15	85.02	s	-		85.03	s	-	
16	33.43	t	1.59		33.57	t	1.58	
			1.86				1.88	
17	26.76	t	1.42		26.79	t	1.39	
			1.77				1.74	
18	70.79	d	3.45	dd; 11.40; 5.5	70.21	d	3.46	dd; 11.03; 4.41
20	74.97	s	-		76.45	s	-	
21	80.80	d	4.70	d; 10.08	78.97	d	3.61	bd; 9.2
22	26.22	t	1.38		29.48	t	1.41	
			2.13				2.16	
23	36.33	t	1.23		36.61	t	1.24	
			2.26	bdd; 13.53, 13.53			2.14	
24	41.34	s	-		41.41	s	-	
25	18.81	q	0.98		19.20	q	0.96	
26	24.85	q	1.25		24.75	q	1.23	
27	18.41	q	1.31		18.86	q	1.41	
28	15.14	q	0.80	d; 6.64	15.17	q	0.80	d; 6.62
29	25.14	q	1.52		25.29	q	1.51	
30	24.21*	q	1.15		24.34*	q	1.25	
31	23.21*	q	1.15		21.80*	q	1.10	
32	20.68	q	1.05		20.76	q	1.05	
O$\underline{C}$OCH$_3$-15	170.78	s	-		170.83	s	-	
O$\underline{C}$OCH$_3$-21	171.15	s	-		-	-	-	
OCO$\underline{C}$H$_3$-15	23.41	q	1.95		23.08	q	1.95	
OCO$\underline{C}$H$_3$-21	21.45	q	2.04		-	-	-	

[a] CDCl$_3$; BRUKER AMX-500 spectrometer. Chemical shifts referred to C$\underline{H}$Cl$_3$ at 7.26 ppm and to $\underline{C}$DCl$_3$ at 77.00.

[b] Assignments aided by ^{1}H-^{13}C HETCOR, ^{1}H-^{1}H COSY, HOHAHA, ^{1}H-^{1}H spin decoupling experiments.

[c] Deduced by DEPT sequence.

* Starred values in the same column can be interchanged.

difference Fourier synthesis. In Fig. 1 is reported a perspective view of one molecule together with the atomic numbering utilized in the X-ray work. In Table 2 are listed final atomic parameters: unprimed and primed labels refer to the two molecules respectively.

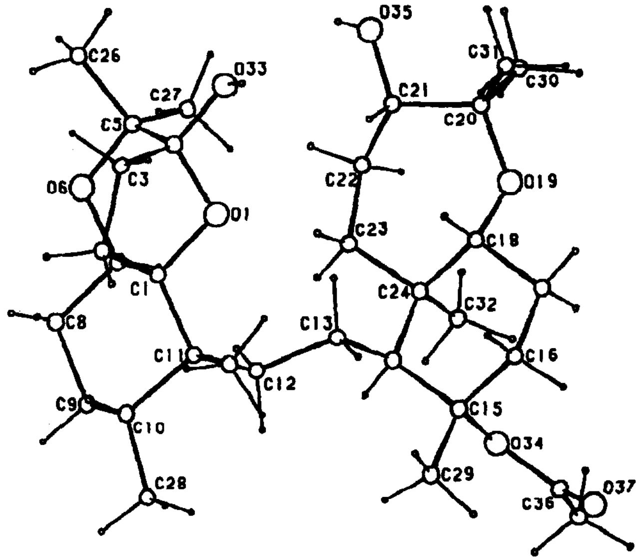


Figure 1. Perspective drawing of one molecule of raspacionin-A deacetylderivative (compound **4**), with the atom labelling scheme used in the X-ray work.

The molecule **4** presents a new rearranged triterpenoid skeleton which strongly reminds the biogenetically correlated raspacionin (**1**). The structure is characterized by a *trans*-perhydrobenzoxepine system linked to an epoxy-perhydrobenzoxepine moiety through an (α/β) axial ethylene bridge.

Geometrical parameters involving non-hydrogen atoms are given in Table 3 (deposited). The general trend of bond lengths and bond angles agree well with the values found for **1** and similar compounds.[14,15]

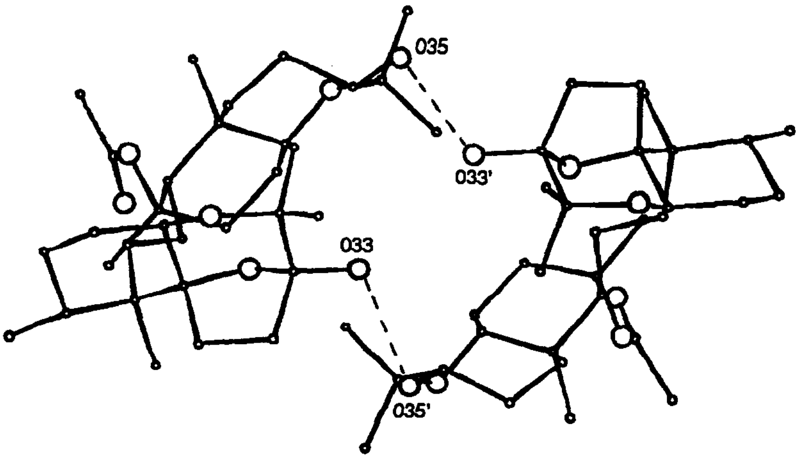


Figure 2. The two molecules of **4** related by a pseudo crystallographic two-fold axis and linked by two hydrogen bonds (dashed lines) involving the hydroxyl groups.

Table 2. Positional Parameters and Equivalent Isotropic Temperature Factors ($Å^2$) with esd in Parentheses for Compound 4 $B_{eq}= {}^4/_3 \Sigma_i \Sigma_j a_i a_j$

Atom	x	y	z	B_{eq}	Atom	x	y	z	B_{eq}
O1	0.1880(2)	0.5000	0.3824(2)	4.04(5)	O1'	0.6721(2)	0.3767(1)	0.7766(2)	4.33(5)
C1	0.0740(3)	0.5279(2)	0.3253(4)	4.25(8)	C1'	0.7540(4)	0.3463(2)	0.8721(3)	4.52(8)
C2	-0.0236(3)	0.4848(2)	0.3349(4)	5.3(1)	C2'	0.7820(5)	0.3864(2)	0.9804(4)	5.7(1)
C3	0.0455(4)	0.4451(2)	0.4319(4)	5.10(9)	C3'	0.6763(5)	0.4273(2)	0.9458(4)	6.2(1)
C4	0.1678(3)	0.4732(2)	0.4853(4)	4.28(8)	C4'	0.5895(4)	0.4032(2)	0.8324(4)	5.07(9)
C5	0.1658(3)	0.5182(2)	0.5786(3)	4.43(8)	C5'	0.5060(4)	0.3574(2)	0.8580(4)	5.5(1)
O6	0.0690(2)	0.5560(1)	0.5234(2)	4.83(6)	O6'	0.5818(3)	0.3174(1)	0.9360(2)	5.52(6)
C7	0.0712(3)	0.5769(2)	0.4090(3)	4.40(8)	C7'	0.6796(4)	0.2971(2)	0.8954(3)	4.84(9)
C8	-0.0413(4)	0.6126(2)	0.3615(4)	5.8(1)	C8'	0.7528(5)	0.2575(2)	0.9896(4)	6.2(1)
C9	-0.0566(3)	0.6285(2)	0.2308(5)	6.3(1)	C9'	0.8697(5)	0.2404(2)	0.9621(4)	6.1(1)
C10	-0.0540(3)	0.5787(2)	0.1508(4)	5.8(1)	C10'	0.9420(4)	0.2904(2)	0.9396(4)	5.8(1)
C11	0.0683(3)	0.5457(2)	0.1976(4)	4.62(9)	C11'	0.8665(3)	0.3273(2)	0.8346(3)	4.46(8)
C12	0.1770(3)	0.5830(2)	0.1939(3)	4.10(8)	C12'	0.8307(3)	0.2925(2)	0.7183(3)	4.09(8)
C13	0.2984(3)	0.5548(2)	0.2005(3)	3.78(7)	C13'	0.7824(3)	0.3214(2)	0.5979(3)	3.95(7)
C14	0.3953(3)	0.5975(1)	0.1905(3)	3.48(7)	C14'	0.7697(3)	0.2785(1)	0.4949(3)	3.60(7)
C15	0.4352(3)	0.5847(2)	0.0777(3)	4.02(7)	C15'	0.8648(3)	0.2919(2)	0.4284(3)	3.99(7)
C16	0.5180(3)	0.5349(2)	0.1002(3)	4.23(8)	C16'	0.8239(3)	0.3411(2)	0.3470(3)	4.21(7)
C17	0.6232(3)	0.5394(2)	0.2144(3)	4.33(8)	C17'	0.6918(3)	0.3388(2)	0.2677(3)	4.25(8)
C18	0.5838(3)	0.5519(2)	0.3247(3)	3.51(6)	C18'	0.6021(3)	0.3255(2)	0.3376(3)	3.48(6)
O19	0.6918(2)	0.5590(1)	0.4224(2)	4.05(5)	O19'	0.4862(2)	0.3204(1)	0.2517(2)	4.03(5)
C20	0.7004(3)	0.5357(2)	0.5371(3)	4.14(8)	C20'	0.3834(3)	0.3455(2)	0.2779(3)	4.15(8)
C21	0.5860(3)	0.5493(2)	0.5763(3)	3.94(7)	C21'	0.3765(3)	0.3273(2)	0.4015(3)	3.98(7)
C22	0.5385(3)	0.6074(2)	0.5419(3)	4.01(7)	C22'	0.4147(3)	0.2684(2)	0.4316(3)	4.46(8)
C23	0.4497(3)	0.6102(2)	0.4146(3)	3.76(7)	C23'	0.5525(3)	0.2624(2)	0.4904(3)	4.37(8)
C24	0.5037(3)	0.6035(1)	0.3087(3)	3.51(6)	C24'	0.6372(3)	0.2729(2)	0.4116(3)	3.62(7)
C25	0.0649(4)	0.4952(2)	0.1179(4)	5.7(1)	C25'	0.9409(4)	0.3774(2)	0.8175(4)	5.8(1)
C26	0.1319(4)	0.4948(2)	0.6856(4)	5.7(1)	C26'	0.4227(4)	0.3799(3)	0.9286(4)	7.0(1)
C27	0.2867(3)	0.5480(2)	0.6217(4)	5.03(9)	C27'	0.4296(4)	0.3312(2)	0.7426(4)	6.1(1)
C28	-0.0846(4)	0.5965(3)	0.0194(5)	8.1(2)	C28'	1.0645(4)	0.2719(3)	0.9262(5)	7.3(1)
C29	0.3279(4)	0.5790(2)	-0.0348(4)	5.5(1)	C29'	0.9932(4)	0.2992(2)	0.5147(4)	4.99(9)
C30	0.8128(3)	0.5624(2)	0.6205(4)	5.7(1)	C30'	0.2760(4)	0.3238(2)	0.1780(4)	5.6(1)
C31	0.7164(4)	0.4734(2)	0.5338(4)	5.5(1)	C31'	0.3899(4)	0.4077(2)	0.2708(4)	5.5(1)
C32	0.5802(4)	0.6555(2)	0.3075(4)	4.57(8)	C32'	0.6218(4)	0.2220(2)	0.3274(4)	4.75(9)
O33	0.2633(2)	0.4375(1)	0.5346(3)	5.16(6)	O33'	0.5175(3)	0.4399(1)	0.7536(3)	6.19(7)
O34	0.5018(2)	0.6350(1)	0.0610(2)	4.70(6)	O34'	0.8694(2)	0.2409(1)	0.3600(2)	4.88(6)
O35	0.6155(2)	0.5397(1)	0.7026(2)	5.09(6)	O35'	0.2564(2)	0.3370(1)	0.4107(3)	5.24(6)
C36	0.5543(4)	0.6415(2)	-0.0262(4)	5.5(1)	C36'	0.9502(4)	0.2312(2)	0.3015(4)	5.8(1)
O37	0.5574(3)	0.6065(2)	-0.0986(3)	7.24(9)	O37'	1.0173(3)	0.2662(2)	0.2808(3)	7.75(9)
C38	0.6068(4)	0.6979(3)	-0.0193(5)	6.8(1)	C38'	0.9417(5)	0.1725(2)	0.2620(4)	6.8(1)

All the deviations from the mean of the corresponding values in the two independent molecules are within the experimental errors. The mean values of the intra-ring C-C and C-O bond lengths are respectively 1.536(5) and 1.438(4)Å, the average valency angle involving tetrahedral carbon atoms is 112.1(6)°. The conformation of the *trans*-perhydrobenzoxepine system is like that in raspacionin molecule (**1**). In the remaining condensed polycyclic moiety, the cyclohexane is also in a near ideal chair conformation whereas the epoxyoxepine system, which presents the 1-4 oxygen bridge in *cis* with respect to the hydrogen at C7, shows the oxepine ring in a boat form. Puckering parameters[16] are reported in Table 4 (deposited).

The two molecules in the independent unit are linked as dimer by two hydrogen bonds (Fig. 2), which involve the four hydroxyl groups: O33-H·······O35' = 2.825(4) and O33'-H·······O35 = 2.809(5)Å. Each

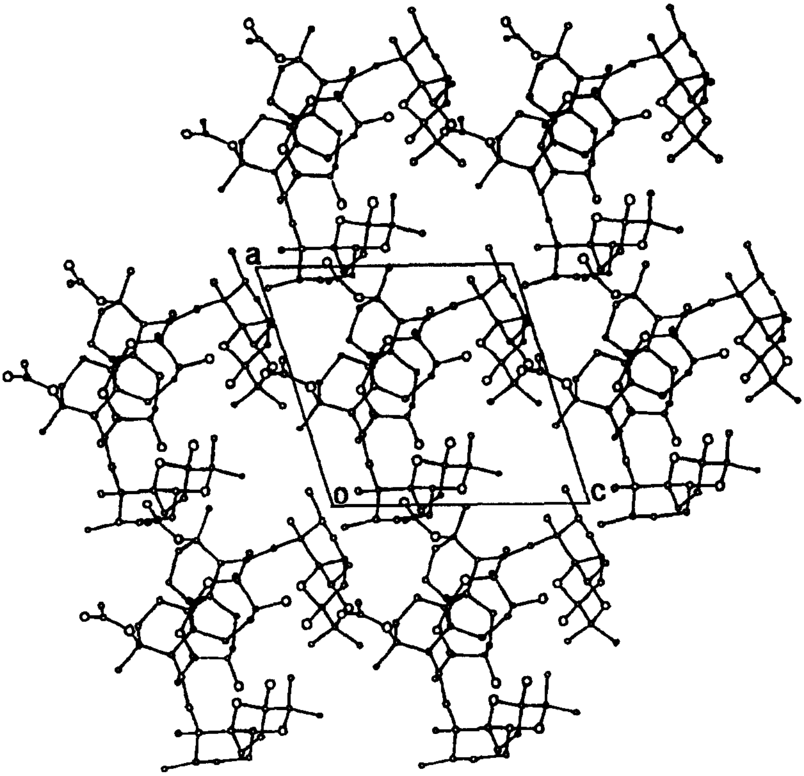


Figure 3. Crystal packing in the *a-c* plane, showing the pseudo-orthorhombic symmetry of the crystal. The H-atoms and the molecules related by the screw axis have been omitted for clarity.

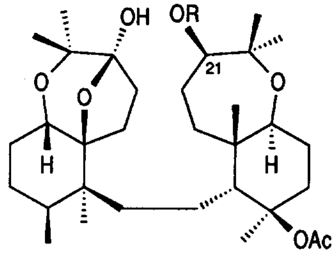
dimer is surrounded by six nearest neighbours in a pseudo-orthorhombic lattice, which leaves channels parallel to the unique axis *b*, partially filled with methyl groups (Fig. 3). The hydroxyl groups O35 and O35' are also engaged in weak intermolecular interactions with the carboxyl oxygens O37 and O37' of traslated molecules: O35-H·······O37$_{(x,y,z-1)}$ = 3.066(5) and O35'-H·······O37'$_{(x+1,y,z)}$ = 3.222(4)Å.

In order to establish the absolute stereochemistry of raspacionin-A we have followed the high field NMR Mosher's method recently applied[19-21] by Kakisawa's group to assign the absolute stereochemistry to sipholenol-A (**2**) and to other marine terpenoids. The deacetyl-raspacionin-A (**4**) was esterified with both *(R)*- and *(S)*-α-methoxy-α-trifluoromethylphenylacetic (MTPA) chloride, giving the *(S)*- and *(R)*-MTPA ester, **5** and **6**, respectively. The ^{1}H-NMR measurements of the Δδ (δ*S*-δ*R*) of **5** and **6**, compared with those of *(S)*- and *(R)*-MTPA ester of 4-*epi*-sipholenol-A (**7**),[20,21] revealed an extraordinary parallelism (Table 5) suggesting a *R* absolute stereochemistry at C-21 of **4**.

Table 5. Selected ^{1}H-NMR Data[a] for the MTPA Esters of **4** and Comparison with the Δδ (δ*S*-δ*R*) Values of the MTPA Esters of **7**.[20,21]

4				7			
proton	*S*-MTPA	*R*-MTPA	Δδ (Hz)	proton	*S*-MTPA	*R*-MTPA	Δδ (Hz)
23a	2.319	2.306	+6.99	2a	1.79	1.77	+10.0
23b	1.277	1.277	0.0	2b	1.27	1.27	0.0
22a	2.286	2.188	+48.17	3a	2.159	2.074	+42.5
22b	1.487	1.430	+26.84	3b	1.52	1.43	+45.0
21	4.950	4.901	+23.90	4	4.964	4.960	+2.0
18	3.439	3.430	+1.840	7	3.207	3.210	-1.5
32	1.050	1.046	+2.57	24	0.972	0.956	+8.0
30	1.070	1.185	-62.50	25	1.09	1.187	-48.5
31	1.050	1.074	-11.40	26	1.033	1.066	-16.5
OCH_3	3.550	3.462	+44.86	OCH_3	-	-	+3.5

[a] All the ^{1}H-NMR values on the same line are assigned, indipendently by the numbering, to corresponding protons of **4** and **7**.



5 R = (*S*)-MTPA
6 R = (*R*)-MTPA

EXPERIMENTAL

General experimental procedures. Fourier Transform IR spectra were recorded with a Perkin-Elmer 1760-X FT-IR. Low resolution mass spectra were determined on a VG TRIO-2000. High resolution mass spectra were obtained on Kratos MS 50 spectrometer. HPLC was performed on a Waters apparatus equipped with a differential refractometer. Enraf-Nonius CAD-4F diffractometer, SDP software and MicroVAX 3100 computer were used in the X-ray work. Commercial Merck silica gel (70-230 mesh ASTM) was used for column chromatography. Analytical TLC was carried out using precoated silica gel Merck F_{254} plates.

NMR experiments. 1D and 2D NMR spectra were recorded at room temperature with a Bruker AMX-500 spectrometer (^{1}H, 500.13 MHz; ^{13}C, 125.75 MHz), equipped with X32 data system. Solvent peak ($CDCl_3$) at δ 7.26 for proton and 77.00 for ^{13}C, low field on respect to TMS, was used as reference. The DEPT experiments were performed using polarization transfer pulses of 135°. Two dimensional experiments were performed using standard Bruker microprograms.

^{1}H-^{1}H COSY. The ^{1}H-^{1}H COSY spectrum was obtained acquiring 96 transients each for 512 values of evolution period. The spectral width used was 6000 Hz with 2K time domain points in T_2 and 512 points in T_1. Before Fourier transformation the data were multiplied with sine-bell and zero filling was applied in the F_1 dimension. The recycle delay time was 1.0 sec.

^{1}H-^{13}C shift correlation proton decoupled. The experiment was performed with a relaxation delay of 1.0 sec. Polarization transfer delays were set to average one bond coupling of $J_{(C\text{-}H)}$=135 Hz. The spectral width was 29411 Hz in F_2 and 3676 Hz in F_1 dimension. 320 scans for each of the 256 increments were acquired. The number of data points was 256 in T_1 and 2K in T_2. The resulting data matrix was multiplied, before Fourier transformation, with sine-bell in both dimensions and zero filled once in F_1.

^{1}H-^{13}C long-range shift correlation. All the parameters are the same of those utilized for the direct hetero-correlation besides the polarization transfer delay that was set to an average $J_{(C\text{-}H)}$= 9 and 10 Hz and the number of scans that was 896. *COLOC.* COLOC experiment was performed using a spectral width of 29411.7 Hz in F_2 and 3676 in F_1. 960 scans were acquired for each of the 256 increments. The number of data points was 2K in F_2 and 256 in F_1. The experiment was optimized for a long range $J_{(C\text{-}H)}$ of 10 Hz. The resulting data matrix was multiplied before Fourier transformation with a sine bell function in both dimensions.

HOHAHA. For the HOHAHA experiment, the spectral width was 5050 Hz. The data set was 2048 points in T_2 and 512 in T_1. 64 scans were accumulated. The mixing time chosen was 50 ms; the recycle delay time was 1.0 sec. Gaussian multiplication (LB=-7, GB=0.1 in F_2 and LB=-8, GB=0.1 in F_1) was applied in both dimensions before Fourier transformation.

Isolation of raspacionin-A (3). The sponge *Raspaciona aculeata* was collected in Blanes (NE, Spain) during January 1990. The diethyl ether soluble material (970 mg) from the acetone extract of the fresh sponge (dry weight 9.5 g) was fractionated on a silica gel flash column using light petroleum with increasing amounts of diethyl ether as eluant to afford a fraction (75 mg) containing **3** as main metabolite. Further purification by HPLC using a Spherisorb column and CH_3CN/H_2O 8:2 as eluant , gave 33 mg of raspacionin-A (**3**) along with a further triterpenoid raspacionin-B (7.5 mg) whose structure is until now undetermined.

Characterization of 3. Raspacionin-A: oil $[\alpha]_D$ -3.95° (*c* 1.17, $CHCl_3$); IR (liquid film, $CHCl_3$) ν_{max} 3422, 1728 cm^{-1}; EIMS, *m/z* (%) 534, M^+- C_3H_6O (15); 516, M^+-C_3H_6O-H_2O (10); 498, M^+-C_3H_6O-$2H_2O$ (5); 474, M^+-C_3H_6O-$C_2H_4O_2$ (12); 456, M^+-C_3H_6O-$C_2H_4O_2$-H_2O (12); 438, M^+-C_3H_6O-$C_2H_4O_2$-$2H_2O$ (8); 414, M^+-C_3H_6O-$2C_2H_4O_2$ (8); 356, M^+-$2C_3H_6O$-$2C_2H_4O_2$ (3); 330 (7); 181 (100); 43 (85). HREIMS *m/z* 534.3550 ($C_{31}H_{50}O_7$ requires 534.3558). ^1H- and ^{13}C-NMR data, see Table 1. ^1H-^{13}C long-range heterocorrelations observed at J =10 Hz: C-1 (1.82, H's-2; 1.71, H-12; 1.32, H-12 or H-8; 0.98, H's-25); C-2 (1.57, H-3); C-3 (1.82, H's-2); C-4 (1.31, H's-27; 1.57, H-3); C-5 (1.57, H-3; 1.31, H's-27); C-7 (1.82, H's-2; 1.31, H-8); C-10 (1.32, H's-9 or H-12; 0.98, H's-25; 0.80, H's-28); C-11 (1.71, H-12; 1.32, H's-9 or H-12; 0.98, H's-25; 0.80, H's-28); C-12 (2.05, H-14); C-14 (1.52, H's-29; 1.05 H's-32); C-15 (2.05, H-14; 1.86, H-16; 1.52, H's-29; 1.42, H-17; 0.94, H-13); C-16 (1.52, H's-29); C-20 (4.70, H-21; 3.45, H-18; 2.13, H-22; 1.38, H-22; 1.15, H's-30 or H's-31); C-21 (1.38, H-22; 1.23, H-23); C-23 (1.05, H's-32); C-24 (1.38, H-22; 1.05, H's-32); C-25 (1.71, H-12); C-26 (1.31, H's-27); C-27 (1.25, H's-26). Additional ^1H-^{13}C long-range heterocorrelation observed at $J_{(C\text{-}H)}$= 9 Hz: C-4 (1.25, H's-26); C-5 (1.25, H's-26; 1.31, H's-27); C-9 (0.80, H's-28); C-13 or C-29 (2.05, H-14); C-14 (1.86, H-16; 0.94, H-13); C-16 (1.77, H-17); C-17 (1.86, H-16; 1.59, H-16); C-18 (1.86, H-16; 1.23, H-23); C-20 (1.15, H's-30 and H's-31); C-23 (1.05, H's-32); C-32 (2.26, H-23). Additional ^1H-^{13}C long-range heterocorrelations observed in the COLOC experiment ($J_{(C\text{-}H)}$=10 Hz): C-4 (1.25, H's-26); C-5 (1.25, H's-26); C-12 (0.98, H's-25); C-32 (2.26, H-23).

Methanolysis of 3 and characterization of 4. 3 (22 mg) was treated with 10 mL of a solution of KOH (3% in CH_3OH) stirring at room temperature. The reaction was monitored by TLC (petroleum ether/diethyl ether 4:6) and stopped after 1 h. After removal of the solvent under vacuum the residue obtained was partitioned between diethyl ether and water. Evaporation of the extract give 26.7 mg of residue that was purified on silica gel Pasteur pipette using petroleum ether/diethyl ether 1:1 as eluant to give pure compound **4** (crystalline, *n*-hexane/diethyl ether 1:1; M.P. 208-211°C); $[\alpha]_D$ 9.56° (*c* 0.63, $CHCl_3$); IR (liquid film, $CHCl_3$) ν_{max} 3395, 1735 cm^{-1}; EIMS, *m/z* 472, M^+-$C_2H_4O_2$-H_2O (10). ^1H- and ^{13}C-NMR data, see Table 1. ^1H-^{13}C long-range heterocorrelations observed at $J_{(C\text{-}H)}$ =10 Hz: C-1 (1.74, H-2 or H-12; 0.96, H's-25); C-4 (1.88, H-2); C-5 (1.41, H's-27); C-10 (0.80, H's-28); C-11 (0.96, H's-25); C-12 (0.96, H's-25; 1.96, H-14); C-13 (1.96, H-14);C-14 (1.58, H-16; 1.05, H's-32); C-15 (0.98, H-13; 1.51, H's-29; 1.95, $OCOC\underline{H}_3$-15); C-16 (1.51, H's-29); C-20 (3.46, H-18; 1.10, H's-31); C-23 (1.05, H's-32; 1.41, H-22); C-24 (1.39, H-17); C-29 (1.96, H-14); C-30 (1.10, H's-31); C-31 (1.25, H's-30).

Crystal Structure Determination. A prismatic single crystal of size 0.32x0.14x0.12 mm, obtained by crystallization from *n*-hexane/diethyl ether mixture (1:1), was selected for the crystallographic study. Accurate cell parameters were obtained by least-squares refinement of the setting angles of 24 reflections at medium θ ($24° < \theta < 30°$), using Ni-filtered Cu Kα radiation and Enraf-Nonius CAD-4F diffractometer on line with a MicroVax Digital computer. Crystal data: $C_{32}H_{54}O_7$, M_w = 550.78: monoclinic, space group $P2_1$ with Z = 4, a = 11.522(3), b = 24.237(4), c = 11.678(3)Å, β = 107.08(1)°, V = 3117(2)$Å^3$, D_c =1.174 g/cm^3. 6594 independent reflections (θ_{max}=75°) were collected at room temperature, using ω-2θ scan mode, as suggested by peak-shape analysis. During the data collection the intensities of three standard reflections were monitored every 5 h (3% variation) in order to check the crystal and equipment stability. The intensities were corrected for Lorentz and polarization factors, but not for the absorption effect (μ =6.13 cm^{-1}). Several attempts to resolve the structure with MULTAN82[22] proved to be completely unsuccessful. The structure was solved using SIR88[13] package and difference Fourier syntheses. The positional and anisotropic temperature parameters for the 78 non-hydrogen atoms (y coordinate of O1 was fixed to define the origin) were refined by full-matrix (on F) least-squares method. At convergence, all hydrogen atoms were located on the basis of difference Fourier synthesis and geometrical considerations and included in the last refinement, as fixed atoms with isotropic thermal parameters set equal to B_{eq} of the parent atoms. Final discrepancy index $R = \Sigma||F_o|-|F_c||/\Sigma|F_o|$ is 0.053 for the 5843 observed reflections with $I \geq 2.5\sigma(I)$; R_w = 0.062 with $w^{-1} = [\sigma^2(F_o)+0.03F_o+1.0]$.[23]

For the crystallographic work, atomic scattering factors were taken from Cromer and Waber,[24] Enraf-Nonius SDP software[25] and MicroVAX 3100 computer were used. Structure factors, hydrogen atom parameters, anisotropic thermal parameters and complete molecular geometry (Table 3) have been deposited.

Preparation of *(S)*- and *(R)*-MTPA ester of 4. *(S)*- and *(R)*-MTPA esters of raspacionin-A (**4**) were prepared by treating both terpenoid available (2 mg) with *(R)*- and *(S)*-MTPA chloride in dry pyridine (100 µL) for 14 h at room temperature. The esters were purified by chromatography in a Pasteur pipette (SiO_2; petroleum ether/diethyl ether 8:2). *(S)*-MTPA ester: EIMS *m/z* (%) 648, M^+-120 (1); *(R)*-MTPA ester: EIMS *m/z* (%) 648, M^+-120 (1). The ^1H-NMR assignments reported in Table 5 were supported by a series of 2D experiments

(^{1}H-^{1}H COSY, HOHAHA, HETCOR).

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