Raspacionin B, a Further Triterpenoid from the Mediterranean Sponge Raspaciona aculeata

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RASPACIONIN B, A FURTHER TRITERPENOID FROM THE MEDITERRANEAN SPONGE *RASPACIONA ACULEATA*

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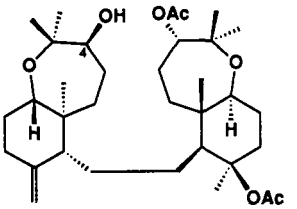
ABSTRACT.—A new triterpenoid, raspacionin B [**4**], has been isolated from the Mediterranean sponge *Raspaciona aculeata*. The structure and relative stereochemistry of **4** were elucidated by means of spectral methods, mainly 1D and 2D nmr. The absolute stereochemistry was proposed by comparison of its cd curve with those of related compounds. The triterpenoid skeleton of raspacionin B, characterized by the presence of a cyclopropane ring, is related to those of terpenoids co-occurring in the same sponge.

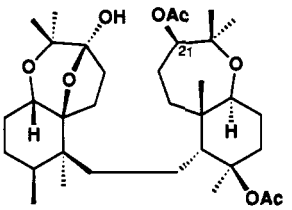
Recently chemical studies on the Mediterranean red sponge *Raspaciona aculeata* Johnston (order Axinellida, family Raspailiidae) led to the characterization of two new triterpenoids, raspacionin [**1**] and raspacionin A [**2**] (1,2), structurally related to some triterpenoids found in the Red Sea sponge *Siphonocalina siphonella* (3–7). In fact, both the series of secondary metabolites display either one or two perhydrobenzoxepine systems.

The structures of **1** and **2** were suggested by analysis of their spectral data, their relative stereochemistries were secured by single crystal X-ray analysis (1,2), and, finally, their absolute stereochemistry was determined by application of high field ^{1}H-nmr to Mosher's method (G. Cimino, R. de A. Epifanio, A. Madaio, R. Puliti, and E. Trivellone, unpublished results), already successfully applied to sipholenol A [**3**] by Kakisawa's group (8,9).

A third triterpenoid, raspacionin B [**4**], co-occurs along with **1** and **2** in *R. aculeata*. In this paper we report the structural characterization of **4**.



1



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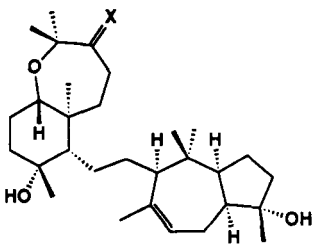
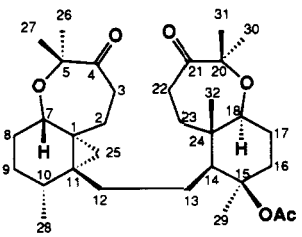


3 X=β-OH, H
5 X=O



4

RESULTS AND DISCUSSION

Raspacionin B [**4**] was isolated as previously reported (2) by hplc [Spherisorb column ODS2, MeCN-H_2O (8:2)] of the chromatographic fraction from *R. aculeata* extract containing as the main metabolite raspacionin A [**2**].

TABLE 1. Nmr Data (500 MHz) of Raspacionin B [**4**].[a]

Position	δ ^{13}C[b]	m[c]	δ ^{1}H[b]	m, *J* in Hz[d]	Long-range ^{1}H-^{13}C correlations (*J* = 10 Hz)
1	31.90	s	—		1.69 (H-12) 2.31 (H-3)
2	33.51	t	1.48	ddd, 13.2, 6.6, 2.8	0.01 (H-25) 2.31 (H-3) 3.23 (H-3)
			1.93	bdd, 13.2, 13.2	
3	38.70	t	2.31	bdd, 10.3, 6.6	1.93 (H-2)
			3.23	ddd, 13.2, 10.3, 2.8	
4	217.93	s	—		1.29 (H's-26) 1.48 (H-2)
5	82.58	s	—		1.35 (H's-27)
7	77.64	d	3.45	dd, 9.6, 6.1	1.25 (H-9)
8	26.46	t	1.12		1.31 (H-9)
			1.48		
9	28.91	t	1.25		
			1.31		
10	27.98	d	1.96	bq, 7.1	0.88 (H's-28)
11	34.65	s	—		0.88 (H's-28) 1.69 (H-12) 1.25 (H-9)
12	40.68	t	1.12		
			1.69	ddd, 13.6, 13.6, 3.0	
13	25.62	t	1.15		
			1.31		
14	57.22	d	0.56	bd, 4.0	
15	83.79	s	—		1.47 (H's-29) 2.82 (H-16)
16	33.00	t	1.22		
			2.82	ddd, 14.9, 3.4, 3.4	
17	22.89	t	1.48		
			1.58	m	
18	80.05	d	2.90	dd, 11.5, 4.4	1.13 (H-23) 2.82 (H-16)
20	82.58	s	—		2.90 (H-18)
21	217.36	s	—		1.28 (H's-30 or H's-31) 1.77 (H-23)
22	35.06	t	2.10	bdd, 11.0, 6.6	1.13 (H-23)
			3.22	ddd, 14.0, 11.0, 2.4	
23	40.11	t	1.13	bdd, 14.0, 13.6	3.22 (H-22)
			1.77	ddd, 13.6, 6.6, 2.4	
24	42.25	s	—		0.56 (H-14) 1.13 (H-23) 1.48 (H-17)
25	17.54	t	0.85	d, 5.1	1.96 (H-10)
			0.01	d, 5.1	
26	26.83	q	1.29	s	1.35 (H's-27)
27	20.94	q	1.35	s	1.29 (H's-26)
28	18.07	q	0.88	d, 7.1	
29	24.75	q	1.47	s	
30	20.46	q	1.28[e]	s	
31	26.52	q	1.26[e]	s	
32	12.41	q	1.10	s	
-OCOCH$_3$	170.05	s	—		2.01 (OCOC*H*$_3$)
-OCOCH$_3$	22.57	q	2.01	s	

[a]$CDCl_3$; BRUKER AMX-500 spectrometer. Chemical shifts referred to $CHCl_3$ at 7.26 ppm and to $CDCl_3$ at 77.00.

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

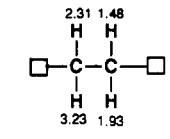
[c]Deduced by DEPT sequence.

[d]Assigned by analysis of the *J*-resolved and ^{1}H-^{1}H spin decoupling experiments.

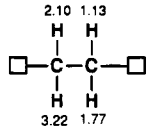
[e]Values may be interchanged.

Raspacionin B [**4**] is an amorphous optically active powder ($[\alpha]^{25}D + 10.8°$) with elementary composition $C_{32}H_{50}O_6$, determined by hreims measurements on the fragment ion at *m/z* 472.3146 $[M - COMe_2]^+$ and from ^1H- and ^{13}C-nmr data. Two ir bands at 1732 and 1714 cm^{-1} suggested the presene of different C=O groups, further supported by the ^{13}C-nmr resonances at δ 217.93, 217.36, and 170.05.

Analysis of the ^1H-nmr spectrum (Table 1) confirmed the correlation of the carbon skeleton of **4** and raspacionin [**1**], suggesting for **4** a related structure characterized by two perhydrobenzoxepine rings linked by an ethylene bridge but displaying two ketone groups at C-4 and C-21. In fact, ^1H-^1H COSY and the HOHAHA experiments showed two isolated -CH_2CH_2- systems (partial structures **a** and **b**) both linked to a carbonyl group on the basis of diagnostic downfield resonances of some protons (H-3, δ 3.23 and 2.31; H-22, δ 3.22 and 2.10). The HOHAHA experiment also revealed a pair of spin sequences indicating two isolated -$CHCH_2CH_2$- partial structures **c** and **d**.



a



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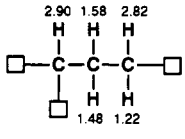
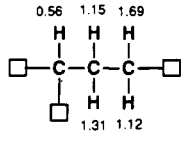


c



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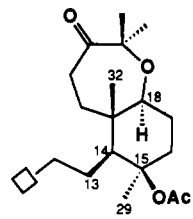
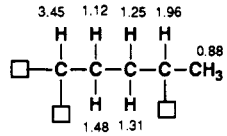


e



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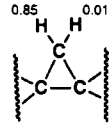

g

Seven singlets were observed in the ^1H-nmr spectra. Five of them were assigned, by 2D experiments (Table 1), to two methyls linked to an oxygenated tertiary carbon (C-20, δ ^{13}C 82.58), one to the methyl of an acetoxy group (δ ^1H, 2.01), one to an angular methyl (C-32, δ ^{13}C 12.41) and, finally, one to a methyl geminal to an acetoxy group at C-15 (δ ^{13}C 83.79). On the basis of spectral data of model compounds and some diagnostic heterocorrelations, **b**, **c**, and **d** were connected as shown by the partial structure **e**. A comparison with sipholenone [**5**] confirmed the suggested partial structure **e**, since some relevant acetylation shifts were observed, in particular for C-15 (δ ^{13}C 83.79; 71.39 in **5**). Direct and long-range ^1H-^{13}C HETCOR experiments (Table 1) further supported partial structure **e**. In particular the ^1H-^{13}C long-range correlations with a 10 Hz coupling displayed a series of diagnostic cross peaks (Table 1) connecting C-21 (δ 217.36) to H_3-30 (δ 1.28) and H-23 (δ 1.77); C-24 (δ 42.25) to H-14 (δ 0.56), to H-23 (δ 1.13), and to H-17 (δ 1.48); C-18 (δ 80.05) to H-23 (δ 1.13) and H-16 (δ 2.82); and, finally, C-15 (δ 83.79) to H_3-29 (δ 1.47) and H-16 (δ 2.82).

Relative stereochemistry at C-14, C-15, C-18, and C-24 was suggested by com-

parison with model compounds (1,2) and confirmed by nOe experiments which proved cis relationships between H-14, H-18, and H_3-29 and by the upfield chemical shift values of C-32 (δ 12.41) and C-13 (δ 25.62) which supported their cis orientation.

The absence of further ^1H-^1H couplings of the proton at C-12 suggested, analogously to raspacionin A [**2**], a quaternary nature for C-11. The analysis of the HOHAHA experiments revealed, in addition to the isolated -CH_2CH_2- and -$CHCH_2CH_2$- systems, the presence of the partial structure **f**, and of an isolated methylene, δ 0.85 and 0.01, which was placed in a cyclopropane ring (**g**) because of its high field shifts. All these data suggested an arrangement as that reported in **4**, which was confirmed by a series of diagnostic ^1H-^{13}C long-range heterocorrelations (Table 1) connecting C-25 (δ 17.54) to H-10 (δ 1.96); C-11 (δ 34.65) to H_3-28 (δ 0.88), to H-9 (δ 1.25), and to H-12 (δ 1.69); C-1 (δ 31.90) to H-12 (δ 1.69) and H-3 (δ 2.31); C-7 (δ 77.64) to H-9 (δ 1.25); C-10 (δ 27.98) to H_3-28 (δ 0.88); C-5 (δ 82.58) to H_3-27 (δ 1.35); C-4 (δ 217.93) to H_3-26 (δ 1.29) and H-2 (δ 1.48).

Relative stereochemistry at C-7, C-1, and C-11 was suggested by a ROESY experiment which revealed a positive effect between H-7 (δ 3.45) and H-2 (δ 1.93) supporting a trans orientation between H-7 and C-25. In addition, H-7 displayed a positive nOe with the protons of C-27 (δ 1.35).

No evidence was obtained for the stereochemistry at C-10, which remains undetermined even though the absence of nOe effects between H-25 and H-10 favors a structure where the methyl at C-10 and the cyclopropane methylene (C-25) bridge are cis oriented. Regrettably, H-25 (δ 0.85) and H-28 (δ 0.88) cannot give detectable nOe effects because their chemical shifts are too close. The downfield ^{13}C-nmr chemical shift of C-12 (δ 40.56) further supports this hypothesis. According to the previously reported structures (1,2), we assume that both H-7 and H-18 must exhibit the same relative stereochemistry. No evidence was obtained about the absolute stereochemistry of **4**, but its co-occurrence with **1** and **2**, and the exhibition of a positive cd absorption at ca. 302 nm, analogous to the 4-oxo-derivative of **1** and the 21-oxo-derivative of **2**, suggested the same absolute stereochemistry for all triterpenoids co-occurring in *R. aculeata*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ft-ir spectra were recorded with a Perkin-Elmer 1760-X FT-IR. Optical rotations were recorded on a JASCO DIP 370 polarimeter, and cd measurements were carried out on a JASCO-710 dicograph. Low resolution ms was determined on a Kratos MS 30. Hrms was obtained on a Kratos MS 50 spectrometer. Hplc was performed on a Waters apparatus equipped with a differential refractometer. Commercial Merck Si gel (70–230 mesh ASTM) was used for cc. Analytical tlc was carried out using precoated Si gel Merck F_{254} plates.

EXTRACTION AND ISOLATION OF **4**.—*R. aculeata* was collected in Blanes (NE, Spain) during January 1990, by hand using SCUBA in an overhang at a depth of 10–15 m. A voucher specimen is deposited at the Centre d' Estudios Avanzados (Blanes). Fresh sponge (12.5 g dry wt after extraction) was cut into small pieces and exhaustively extracted with Me_2CO at room temperature. Solvent was removed under reduced pressure, and the aqueous residue was extracted with Et_2O. After evaporation, the Et_2O-soluble fraction (oil, 1.3 g) was chromatographed on a Si gel flash column. Elution started with petroleum ether and the polarity of the eluent gradually increased with Et_2O to afford the main metabolite raspacionin [**1**] (150 mg), tlc R_f 0.45 [petroleum ether-Et_2O (1:1)], and a fraction containing a mixture of triterpenoids homogeneous by tlc (110 mg), R_f 0.70. This fraction was further purified by means of reversed-phase hplc carried out using a Spherisorb ODS2 column (12 × 250 mm, particle size 5 μm, flow 2.5 ml/min) and MeCN-H_2O (8:2) as eluent to obtain raspacionin A [**2**] (60 mg) and raspacionin B [**4**] (20 mg).

Raspacionin B [**4**].—Amorphous white compound: $[\alpha]^{25}D + 10.8°$ ($c = 0.21$, $CHCl_3$); cd (concn, 2.83×10^{-3} M; EtOH) 20° [θ] 302.90 + 10490; ir ν max (liquid film, $CHCl_3$) 2987, 2951, 2870, 1732, 1714 cm^{-1}; eims *m/z* (rel. int.) $[M-C_3H_6O]^+$ 472 (0.15), $[M-C_3H_6O-C_2H_4O_2]^+$ 412 (100), $[M-C_3H_6O-C_2H_4O_2-CO]^+$ 384 (10), $[M-C_3H_6O-C_2H_4O_2-2CO]^+$ 356 (0.15), $[M-2C_3H_6O-$

$C_2H_4O_2]^+$ 354 (11), $[M-2C_3H_6O-C_2H_4O_2-CO]^+$ 326 (7), $[M-2C_3H_6O-C_2H_4O_2-2CO-CH_2]^+$ 312 (7), 298 (10), 269 (8), 190 (90), 176 (85); hreims *m/z* 472.3146 ($C_{29}H_{44}O_5$ requires 472.3188); ^1H and ^{13}C nmr data see Table 1; relevant nOe data δ 0.56 (1.47, H_3-2%; 2.90, H-18); 2.90 (0.56, H-14; 1.28, H_3-30; 1.47, H_3-29; 1.58, H-17); 3.45 (1.35, H_3-27; 1.93, H-2).

4-Oxo-derivative of raspacionin.—Cd (concn, 2.19×10^{-3} M; EtOH) 20° $[\theta]_{302.00}$ +2479.

21-Oxo-derivative of raspacionin A.—Cd (concn, 7.20×10^{-3} M; EtOH) 20° $[\theta]_{300.40}$ +2292.

NMR EXPERIMENTS.—1D and 2D nmr spectra were recorded at room temperature with a Bruker AMX-500 spectrometer (^1H, 500.13 MHz; ^{13}C, 125.76 MHz), equipped with an X32 data system. 1D nOe experiments were performed with a Bruker WM 250 spectrometer. ^1H- and ^{13}C-nmr chemical shifts were referenced to $CHCl_3$, resonating at δ 7.26 and 77.00 ppm, respectively. The DEPT spectra were obtained using polarization transfer pulses of 135°. Two-dimensional experiments were performed using standard Bruker microprograms.

^1H-^1H COSY.—The ^1H-^1H COSY spectrum was obtained acquiring 96 transients each for 512 values of evolution period. Before Fourier transformation, the data were multiplied with sine-bell in both dimensions and zero filling was applied in the F_1.

^1H-^{13}C shift correlation proton decoupled.—Polarization transfer delays were set to average one-bond coupling of $J_{(C\text{-}H)}$ = 135 Hz, and 320 scans for each of the 256 increments were acquired. The resulting data matrix was multiplied, before Fourier transformation, with π/5 shifted sine-bell in F_2 and π/10 shifted squared sine-bell in F_1 dimensions and zero-filled once in F_1.

^1H-^{13}C long range shift correlation.—All the parameters are the same as those utilized for the direct hetero-correlation, except for the polarization transfer delay that was set to an average $J_{(C\text{-}H)}$ = 10 Hz and the number of scans that was 896 for each of the 128 increments.

HOHAHA.—For the HOHAHA experiment 96 scans were accumulated for each of the 512 increments. The mixing time chosen was 50 msec. A π/12 and π/10 shifted squared sine-bell was applied in F_2 and F_1 dimensions, respectively, before Fourier transformation.

ROESY.—For each of the 256 increments, 64 scans were accumulated. The mixing time chosen was 150 msec. A π/3 shifted squared sine-bell was applied in both dimensions before Fourier transformation.

HMQC.—For each of the 512 increments, 64 scans were accumulated. The resulting data matrix was multiplied, before Fourier transformation, with a π/3 shifted sine-bell in F_2 and a π/8 shifted sine-bell in F_1.

2D J-resolved.—For each of 256 t_1 increments, 64 transients were collected. The resulting data matrix was multiplied, before Fourier transformation, with sine-bell in both dimensions.

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