Absolute Stereochemistry of Raspacionin, the Main Triterpenoid from the Marine Sponge Raspaciona aculeata

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ABSOLUTE STEREOCHEMISTRY OF RASPACIONIN, THE MAIN TRITERPENOID FROM THE MARINE SPONGE *RASPACIONA ACULEATA*

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ABSTRACT.—Raspacionin [**1**] is an unusual triterpenoid recently isolated from the red encrusting sponge *Raspaciona aculeata* and characterized by X-ray diffraction studies. The absolute stereochemistry of raspacionin [**1**] has been established by applying high field ^{1}H-nmr to the Mosher method, as recently suggested by Kakisawa's group. The stereochemistry at C-4 of raspacionin was inverted to obtain a less hindered alcohol **4**. After esterification with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride, the MTPA esters were submitted to careful ^{1}H- and ^{13}C-nmr measurements which assigned the *S* absolute stereochemistry at C-4 of raspacionin.

Raspaciona aculeata Johnston (family Raspailiidae) is a red encrusting Mediterranean sponge which possesses a series of unusual triterpenoids named raspacionins (1–4). The structure and relative stereochemistry of two of these, raspacionin [**1**] and raspacionin A [**2**], have recently been established by extensive nmr analysis and by single crystal X-ray diffraction studies (1–3). However the absolute stereochemistry of **1** remains undetermined.

In this paper we report the determination of the absolute configuration of **1** by applying high-field nmr to Mosher's method (5,6) with α-methoxy-α-trifluoromethylphenylacetic (MTPA) esters, as recently proposed and applied successfully by Kakisawa's group to determine the absolute stereochemistry of a series of marine terpenoids (7–10), all having a secondary alcohol function.

To establish the absolute stereochemistry of raspacionin [**1**], the secondary -OH at C-4 first suggested use of the traditional Horeau method (11,12) based on the kinetic resolution of racemic 2-phenylbutyric anhydride during esterification. However, no ester was obtained, probably because of the steric crowding around the secondary alcohol of **1**. Application of the modified Horeau method (13) with racemic 2-phenylbutyryl chloride led to comparable amounts of diastereomeric esters without useful stereoselectivity.

Recently, on the basis of Mosher's assumption (5,6) that the nmr spectra of both (*S*)- and (*R*)-MTPA esters are dominated by the conformation in which the carbinyl proton, the C=O carbonyl bond, and the trifluoromethyl group are located in the same plane, Kakisawa's group suggested a model predicting positive and negative ^{1}H-nmr $\Delta\delta$ ($\delta S - \delta R$) values, determined by the anisotropic effects of the phenyl groups of the (*S*)- and (*R*)-MTPA esters (7–10).

(*R*)- and (*S*)-MTPA esters of raspacionin [**1**] were prepared by treating the terpenoid with (*S*)- and (*R*)-MTPA chloride in dry pyridine at room temperature. The esters were purified by chromatography in a Pasteur pipette. The ^{1}H-nmr positive and negative $\Delta\delta$ values of the esters were irregularly distributed on both sides of the MTPA plane. Analogous anomalies observed for sipholenol A [**3**] by Kakisawa's group (9,10) were rationalized by the steric crowding around the ester moiety. By analogy to their solution to this problem, we inverted the

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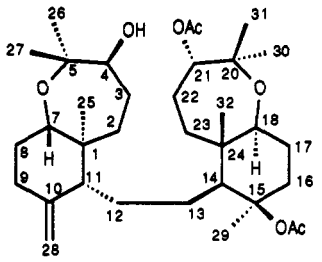

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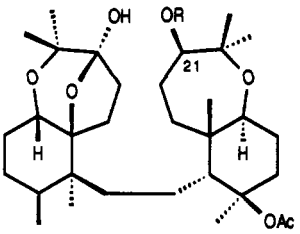
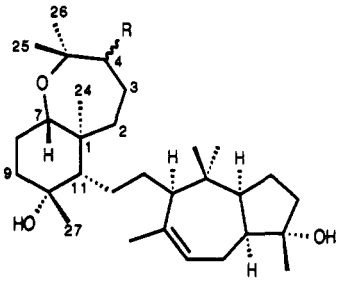
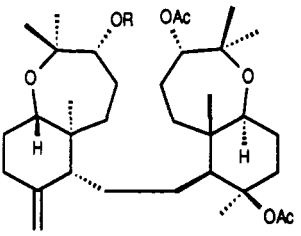


2 R=Ac
8 R=H



3 R=β-OH
7 R=α-OH



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

stereochemistry at C-4 by oxidation with Jones reagent and reduction with $NaBH_4$, which led to 4-*epi*-raspacionin [**4**].

Treatment of 4-*epi*-raspacionin [**4**] with (*R*)- and (*S*)-MTPA chloride yielded the (*S*)- and (*R*)-MTPA esters, **5** and **6**, respectively. All ^{1}H- and ^{13}C-nmr resonances of **4**, **5**, and **6** (Table 1) were assigned by extensive application of 1D and 2D (^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR, HMQC, HOHAHA) experiments. Some selected ^{1}H-nmr $\Delta\delta$ ($\delta S - \delta R$) values are listed in Table 2 and compared with those recorded for 4-*epi*-sipholenol A [**7**] (9,10). The extraordinary correspondence of the $\Delta\delta$ values of (*S*)- and (*R*)-MTPA esters led to assignment of the *S* absolute configuration at C-4 of raspacionin [**1**]. Recently (3), the same method was directly applied to the 21-deacetyl derivative of raspacionin A [**8**], leading to assignment of the *R* absolute stereochemistry at C-21.

These results further support the parallelism between the metabolites of the sponges *R. aculeata* and *Siphonocalina siphonella*, which suggests a reinvestigation of their taxonomic status.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectrum was recorded with a Perkin-Elmer 1760-X FT-IR. Mass spectra were determined on a VG TRIO-2000 and on a Kratos MS 50 spectrometer. Optical rotations were measured with a JASCO DIP 370 polarimeter. Nmr spectra were recorded with a Bruker AMX-500 spectrometer (^{1}H, 500.13 MHz; ^{13}C, 125.75 MHz). Solvent peaks ($CDCl_3$) at δ 7.26 for ^{1}H and at δ 77.00 for ^{13}C were used as reference. The purity of all new compounds was established by nmr analysis. Chromatographic procedures were carried out as previously described (4).

ISOLATION OF RASPACIONIN [**1**].—The isolation of **1** from the Me_2CO extract of the sponge *R. aculeata* was performed as previously reported (1).

OXIDATION OF RASPACIONIN [**1**].—A solution of **1** (14 mg) in 7 ml of Me_2CO was treated with Jones reagent. After 10 minutes a few drops of MeOH were added to destroy the excess of reagent until green in color. After removal of the solvent the residue was partitioned between Et_2O and H_2O. Evaporation of the Et_2O extract gave

TABLE 1. 500 MHz nmr Data of 4-*epi*-Raspacionin [**4**] and its (*S*)- and (*R*)-MTPA Esters **5** and **6**.

Position	Compound								
	4			**5**			**6**		
	$\delta^{13}C$[a]	m[b]	$\delta^{1}H$[a]	$\delta^{13}C$[a]	m[b]	$\delta^{1}H$[a]	$\delta^{13}C$[a]	m[b]	$\delta^{1}H$[a]
1	42.85[c]	s		42.78[c]	s		42.77[c]	s	
2	41.20	t	1.22, 1.80	40.49	t	1.39, 1.82	40.43	t	1.37, 1.81
3	30.63	t	1.51, 2.09	26.86	t	1.60, 2.15	26.74	t	1.52, 2.07
4	79.60	d	3.68	82.91	d	4.98	83.04	d	4.97
5	76.44	s	—	n.d.[d]	s	—	n.d.[d]	s	—
7	75.22	d	3.31	75.81	d	3.35	75.98	d	3.36
8	32.83	t	1.43, 1.66	32.75	t	1.43, 1.66	32.71	t	1.42, 1.69
9	35.66	t	1.94, 2.28	35.59	t	1.94, 2.28	35.58	t	1.94, 2.29
10	146.61	s	—	146.26	s	—	146.20	s	—
11	54.14	d	1.57	53.54	d	1.61	53.57	d	1.62
12	27.79	t	1.41, 1.56	27.49	t	1.42, 1.50	27.48	t	1.43, 1.56
13	25.73	t	1.11, 1.72	25.45	t	1.16, 1.69	25.45	t	1.13, 1.69
14	58.30	d	0.74	58.01	d	0.74	58.03	d	0.75
15	83.66	s	—	83.76	s	—	n.a.[e]	s	—
16	33.15	t	1.29, 2.80	33.13	t	1.33, 2.80	33.12	t	1.32, 2.80
17	26.55	t	1.39, 1.47	26.53	t	1.40, 1.47	26.40	t	1.42, 1.49
18	76.62	d	3.41	76.57	d	3.41	76.81	d	3.42
20	77.38	s	—	n.d.[d]	s	—	n.d.[d]	s	—
21	79.08	d	5.00	78.93	d	5.01	79.04	d	5.03
22	23.09	t	1.78, 2.02	23.11	t	1.82, 1.99	23.13	t	1.83, 2.00
23	35.35	t	1.25, 1.43	35.49	t	1.30, 1.45	35.45	t	1.31, 1.45
24	42.64[c]	s	—	42.71[c]	s	—	42.67[c]	s	—
25	12.48	q	0.68	12.43	q	0.69	12.41	q	0.68
26	24.44	q	1.28	24.07	q	1.11	24.34	q	1.21
27	21.92	q	1.10	22.84	q	1.02	22.94	q	1.06
28	107.70	d	4.60, 4.90	108.08	d	4.63, 4.92	108.12	d	4.63, 4.92
29	25.22	q	1.52	25.33	q	1.52	25.30	q	1.53
30	21.52	q	1.15	21.35	q	1.15	21.48	q	1.16
31	28.94	q	1.20	28.91	q	1.20	28.80	q	1.21
32	12.81	q	0.94	12.78	q	0.94	12.78	q	0.95
$OCOCH_3$-15	170.11	s	—	170.23	s	—	n.a.[e]	s	—
$OCOCH_3$-21	170.20	s	—	170.23	s	—[c]	n.a.[e]	s	—
$OCOCH_3$-15	22.49	q	1.94	22.50	q	1.95	22.47	q	1.96
$OCOCH_3$-21	21.25	q	2.16	21.51	q	2.18	21.34	q	2.19

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

[b]Deduced by DEPT sequence.

[c]Values in the same column can be interchanged.

[d]Not detected, being obscured by solvent.

[e]Not assigned due to poor resolution.

12.5 mg of pure ketone [tlc, SiO_2, petroleum ether-Et_2O (4:6), R_f 0.86); crystalline (*n*-heptane); mp 117–120 °; $[\alpha]^{25}D$ −5.86° (*c*=1.25, $CHCl_3$); eims *m/z* (rel. int.) $[M-2HOAc]^+$ 454 (2); hreims *m/z* 454.3439 ($C_{30}H_{46}O_3$ requires 454.3447); ir (liquid film, $CHCl_3$) ν max 2941, 1733, 1718, 1649 cm^{-1}; selected ^{1}H-nmr data ($CDCl_3$) δ 5.00 (1H, d, *J*=6.9 Hz, H-21), 4.95 (1H, s, H-28), 4.66 (1H, s, H-28), 3.38 (1H, dd, *J*=11.6, 4.7 Hz, H-18), 3.20 (1H, ddd, *J*=13.3, 11.2, 2.1 Hz, H-3), 3.06 (1H, dd, *J*=11.2, 4.7 Hz, H-7), 2.78 (1H, db, *J*=14.5 Hz, H-16), 2.31 (1H, m, H-3), 2.20 (1H, m, H-9), 2.15 (3H, s, $OCOCH_3$-21), 1.94 (3H, s, $OCOCH_3$-15), 1.52 (3H, s, H_3-29), 1.32 (3H, s, H_3-27), 1.25 (3H, s, H_3-26), 1.20 (3H, s, H_3-31), 1.15 (3H, s, H_3-30), 0.96 (3H, s, H_3-32), 0.84 (3H, s, H_3-25), 0.74 (1H, bs, H-14); ^{13}C nmr ($CDCl_3$) δ 217.81 (C-4), 170.25 ($OCOCH_3$-21), 170.10 ($OCOCH_3$-15), 145.90 (C-10), 108.36 (C-28), 83.70 (C-15), 82.21 (C-5), 80.60 (C-7), 78.91 (C-21), 77.45 (C-20), 76.57 (C-18), 57.99 (C-14), 53.23 (C-11), 42.77 (C-1 or C-24), 42.54

TABLE 2. Selected ^{1}H-nmr (500.13 MHz, $CDCl_3$) Data for the MTPA Esters of **4** and Comparison with the $\Delta\delta$ ($\delta S-\delta R$) Values of the MTPA Esters of **7** (8,9).

Proton	Compound					
	4			**7**		
	(*S*)-MTPA	(*R*)-MTPA	Δδ (Hz)	(*S*)-MTPA	(*R*)-MTPA	Δδ (Hz)
H_a-2	1.824	1.811	+6.50	1.79	1.77	+10
H_b-2	1.391	1.368	+11.50	1.27	1.27	0
H_a-3	2.150	2.075	+37.51	2.159	2.074	+42.5
H_b-3	1.604	1.518	+43.01	1.52	1.43	+45
H-4	4.976	4.971	+2.50	4.964	4.960	+2
H-7	3.352	3.356	−2.00	3.207	3.210	−1.5
H-24	—	—	—	0.972	0.956	+8
H-25	0.690	0.676	+7.00	1.09	1.187	+48.5
H-26	1.106	1.206	−50.01	1.033	1.066	−16.5
H-27	1.024	1.061	−18.50	—	—	—
OMe	3.573	3.496	+38.51	—	—	+3.5

(C-24 or C-1), 39.54 (C-2), 35.75 (C-9), 35.42 (C-23), 35.42 (C-3), 33.09 (C-16), 32.57 (C-8), 28.89 (C-31), 27.54 (C-12), 26.51 (C-26) 26.51 (C-17), 25.42 (C-13), 25.22 (C-29), 23.10 (C-22), 22.47 (OCO*C*H_3-15), 21.48 (C-30), 21.23 (OCO*C*H_3-21), 20.27 (C-27), 12.74 (C-32), 11.60 (C-25).

PREPARATION OF 4-*epi*-RASPACIONIN [**4**].—4-*epi*-Raspacionin was prepared by treating a solution of the ketone (12.5 mg) in 1.5 ml of EtOH with 30 mg of $NaBH_4$ (room temperature, 1.5 h). The reaction was monitored by tlc [petroleum ether-Et_2O (4:6)]. The excess of reagent was destroyed with a few drops of glacial HOAc. After evaporation of the solvent, the residue was treated with $CHCl_3$ and filtered. A mixture of **1** and its epimer **4** was obtained and 10 mg was purified on Si gel Pasteur pipette using petroleum ether-Et_2O (7:3) as eluent to give raspacionin [**1**] (8 mg) [tlc, SiO_2, petroleum ether-Et_2O (4:6) R_f 0.60) and 4-*epi*-raspacionin [**4**] (2 mg) [tlc, SiO_2, petroleum ether-Et_2O (4:6), R_f 0.45]; $[\alpha]^{25}D$ −27.48° (c=0.37, $CHCl_3$); eims *m/z* (rel. int.) $[M-HOAc]^+$ 516 (9); hreims *m/z* 516.3798 ($C_{32}H_{52}O_5$ requires 516.3814); ^{1}H and ^{13}C nmr see Table 1.

PREPARATION OF (*S*)- AND (*R*)-MTPA ESTERS OF **4**.—(*S*)- and (*R*)-MTPA esters of **4** were prepared by treating the terpenoid (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/Et_2O), obtaining 1.0 mg of (*S*)- and 1.3 mg of (*R*)-MTPA ester, **5** and **6**, respectively [tlc, SiO_2, petroleum ether-Et_2O (1:1), R_f 0.63 for both esters]. (*S*)-MTPA ester [**5**] eims *m/z* (rel. int.) $[M-HOAc]^+$ 732 (1); hreims *m/z* 732.4201 ($C_{42}H_{59}O_7F_3$ requires 732.4212); ^{1}H and ^{13}C nmr see Table 1. (*R*)-MTPA ester [**6**]: eims *m/z* (rel. int.) $[M-HOAc]^+$ (1); hreims *m/z* 732.4205 ($C_{42}H_{59}O_7F_3$ requires 732.4212); ^{1}H and ^{13}C nmr see Table 1.

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