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Cyclic Hemiketals from the Sponge *Raspailia* (*Raspaxilla*) sp.

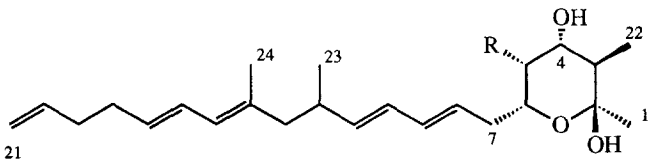
Carlos M. Cerda-García-Rojas and D. John Faulkner*

Scripps Institution of Oceanography, University of California at San Diego,
La Jolla, California 92093-0212

Abstract: The Palauan sponge *Raspailia* (*Raspaxilla*) sp. contains two cyclic hemiacetals, raspailols A (**1**) and B (**2**) that do not fall into any established chemotaxonomic group. The structure and stereochemistry were determined by spectroscopic methods.

The metabolites of marine sponges generally fall into clear chemotaxonomic groups. Compounds that lie outside of these chemotaxonomic groups are of interest because they either represent the start of a new group or may in some cases be of putative symbiotic origin. During our examination of sponges from Palau, we encountered a sponge of the genus *Raspailia* (subgenus *Raspaxilla*)[1] that contained two cyclic hemiketals, raspailols A (**1**) and B (**2**), that are unlike the acetylenic ethers previously isolated from sponges of the genus *Raspailia*[2] or the triterpenes found in the related sponge *Raspaciona aculeata*,[3] and do not belong to any established chemotaxonomic group.

The small orange, sweet-smelling sponge *Raspailia* (*Raspaxilla*) sp. was collected by hand using SCUBA (- 25 m.) from the Western Reef at Palau, Western Caroline Islands, and was stored frozen until extraction. The crude acetone extract of the sponge showed slight activity against *Bacillus subtilis* and the ^{1}H NMR spectrum contained an interesting group of signals in the olefinic region. Chromatography of the crude extract on silica gel followed by HPLC on a cyanopropyl column, using acetonitrile-water (53:47) as eluant, gave raspailols A (**1**, 0.53% dry wt.) and B (**2**, 0.38% dry wt.).

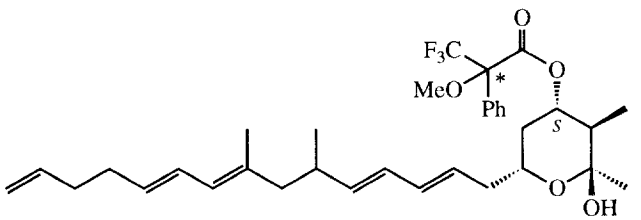


1 R = H
2 R = Me[25]

Table 1. ^{13}C and ^{1}H NMR data for raspailols A (**1**) and B (**2**) in C_6D_6 solution.

	1			**2**		
C#	δ_C	δ_H	(mult, *J*)	δ_C	δ_H	(mult, *J*)
1	28.2	1.17	(s, 3 H)	28.1	1.14	(s, 3 H)
2	98.9			98.6		
3	47.2	1.25	(dq, 10, 7)	40.4	1.50	(dq, 10, 7)
4	69.7	3.57	(ddt, 11, 10, 5)	72.6	3.62	(dt, 11, 5)
5	41.3	1.69	(ddd, 12, 5, 2)	38.4	1.62	(m)
		1.13	(dt, 12, 11)			
6	68.2	3.82	(dtd, 11, 7, 2)	70.8	3.94	(td, 7, 2)
7	39.7	2.34	(br dt, 14, 7)	36.3	2.40	(br dt, 14, 7)
		2.16	(br dt, 14, 7)		2.13	(br dt, 14, 7)
8	128.6	5.68	(br dt, 14, 7)	128.8	5.59	(br dt, 14, 7)
9	133.2	6.12	(m)	133.0	6.14	(m)
10	129.0	6.08	(m)	129.0	6.10	(m)
11	138.5	5.54	(br dd, 14, 7)	138.5	5.55	(br dd, 14, 7)
12	35.1	2.34	(sept, 7)	35.1	2.35	(sept, 7)
13	48.0	2.10	(m)	48.0	2.10	(m)
		1.95	(br dd, 15, 7)		1.96	(br dd, 15, 7)
14	134.5			134.5		
15	127.3	5.91	(br d, 11)	127.3	5.92	(br d, 11)
16	127.6	6.31	(ddt, 15, 11, 2)	127.6	6.31	(ddt, 15, 11, 2)
17	131.8	5.56	(br dt, 15, 7)	131.8	5.56	(m)
18	32.7	2.10	(m, 2 H)	32.7	2.10	(m, 2 H)
19	34.1	2.05	(m, 2 H)	34.1	2.05	(m, 2 H)
20	138.4	5.75	(ddt, 17, 11, 7)	138.4	5.75	(ddt, 17, 11, 7)
21	114.9	5.01	(d, 17)	114.9	5.01	(d, 17)
		4.97	(d, 11)		4.97	(d, 11)
22	12.2	1.12	(d, 3 H, 7)	12.4	1.07	(d, 3 H, 7)
23	20.1	0.96	(d, 3 H, 7)	20.1	0.97	(d, 3 H, 7)
24	16.5	1.62	(br s, 3 H)	16.6	1.62	(br d, 3 H)
25				4.7	0.89	(d, 3 H, 7)
2-OH		1.30	(br s)		1.04	(br s)
4-OH		0.68	(br d, 5)		0.70	(br d, 5)

Raspailol A (**1**), $[\alpha]_D$ = + 62°, was isolated as a clear oil of molecular formula $C_{24}H_{38}O_3$ (*m/z* = 374.2831). The infrared spectrum contained a strong hydroxyl band at 3410 cm^{-1} and the ultraviolet absorptions at 234 and 241 nm suggested the presence of conjugated dienes. The ^1H and ^{13}C NMR spectra were assigned using the COSY, HMQC, and HMBC experiments as shown in Table 1. The COSY spectrum revealed two contiguous spin systems from Me-22 to H-13, which included the H-8 to H-11 diene, and from H-15 to H-21. The two systems were linked through a tri-substituted olefinic bond as indicated by HMBC correlations from the H-15 signal at δ 5.91 to signals at 16.5 (C-24) and 48.0 (C-13) and from the olefinic methyl signal at δ 1.62 (H-24) to signals at 48.0 (C-13), 134.5 (C-14), and 127.6 (C-15). The remaining unassigned methyl signal at δ 1.17 showed HMBC correlations to the ketal carbon at 98.9 (C-2) and to a signal at 47.2 (C-3). The (8*E*,10*E*,14*E*,16*E*) geometry of the olefinic bonds was deduced from the magnitude of the coupling constants of the olefinic proton signals, the carbon chemical shift of the olefinic methyl signal (δ 16.5),[4] and observation of a 13% nuclear Overhauser enhancement of the H-16 signal on irradiation of the olefinic methyl signal. The relative stereochemistry about the six-membered ring was assigned by interpretation of the coupling constants of the H-3 to H-6 signals. The stereochemistry at the hemiketal carbon was assumed to be the same as for compound **2** (see below) because the chemical shifts of C-1 and C-2 are almost identical for the two compounds. The relative stereochemistry at C-12 could not be determined. The absolute configuration at C-4 was determined by Mosher's method (see table 2), in which the ^1H NMR chemical shift differences for selected signals in the spectra of the (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetate esters (**3**) and (**4**) were compared.[5]



3 * = (*R*)
4 * = (*S*)

Table 2. Selected ^1H NMR chemical shift values for (*R*)- and (*S*)-MTPA esters (**3**) and (**4**).

H#	**3** (*R*)	**4** (*S*)	$\Delta_{(R-S)}$
1	1.39	1.41	-0.02
3	1.65	1.67	-0.02
22	0.83	1.00	-0.17
4	5.11	5.09	+0.02
5_{ax}	1.35	1.20	+0.15
5_{eq}	2.16	2.11	+0.04
6	3.99	3.97	+0.02
7	2.29	2.27	+0.02

Raspailol B (**2**), $[\alpha]_D = +111°$, which was obtained as a colorless oil, has the molecular formula $C_{25}H_{40}O_3$ and was therefore a homologue of compound **1**. The infrared and ultraviolet spectra of **2** were almost identical to those of **1**. The ^1H and ^{13}C NMR spectra both contained an additional methyl signal which was placed on the tetrahydropyran ring at C-5 (see Table 1). The coupling constants of the ring proton signals indicate that the methyl group is axial. The relative stereochemistry about the pyran ring (Figure 1) was determined by a series of NOEDS experiments: irradiation of the H-3 signal caused enhancement of the CH_3-1 (2.4%), CH_3-22 (5.7%) and CH_3-25 (5.4%) signals, irradiation of the H-4 signal caused enhancement of the H-5 (8.7%), H-6 (7.7%), OH-4 (3.6%), and OH-2 (7.8%) signals, and irradiation of the H-6 signal caused enhancements of the H-4 (7%), H-5 (7%), and H-8 (4.6%) signals. Since raspailols A (**1**) and B (**2**) are closely related and both have positive optical rotations, it seems reasonable to assume that they have the same absolute configuration.

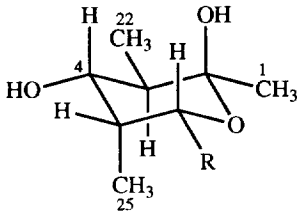


Figure 1. Relative stereochemistry about the pyran ring in raspailol B (**2**).

EXPERIMENTAL

General Methods: The solvents used for extractions and chromatography were either freshly distilled from reagent grade or, for reversed phase HPLC, were purchased as HPLC-grade. ^1H and ^{13}C NMR spectra were obtained in C_6D_6 or $CDCl_3$ on a Varian Unity 500 spectrometer (UCSD NMR Facility). UV spectra were recorded on a Perkin-Elmer Lambda 3B spectrophotometer and IR spectra were obtained on a Perkin-Elmer 1600 series FTIR spectrometer. Optical rotations were measured on an Autopol III polarimeter from Rudolph Research. Low resolution mass spectra were measured on a Hewlett-Packard 5988A spectrometer and high resolution mass spectra were obtained from the Regional Mass Spectrometry Facility at the University of California, Riverside.

Collection, Extraction, and Isolation Procedures: The orange sponge *Raspailia* (subgenus Raspaxilla) sp. (collection # 93-124) was collected by hand (- 25 m) using SCUBA in Palau. A portion (37 g) of the frozen sponge was cut into small pieces and extracted with acetone (300 mL) for 3 h using a sonicator. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. Evaporation of the solvents gave crude EtOAc (253 mg) and aqueous (160 mg) extracts, both of which showed mild activity against *Bacillus subtilis* in the disk diffusion assay. TLC of the EtOAc extract on silica plates, using 7:3 hexane-EtOAc as eluant, showed two major UV-active spots at R_f 0.25 and 0.3. Flash column

chromatography on Merck silica gel 60 (70-230 mesh ASTM), using 7:3 hexane-EtOAc as eluant, yielded 20 fractions. Fraction 10 (30 mg) contained mainly raspailol A (**1**) and fraction 5 (20 mg) contained raspailol B (**2**) mixed with several minor components. The intermediate fractions contained mixtures of **1** and **2**. The compounds were separated by HPLC on a Dynamax 60A cyanopropyl column (25 x 1 cm) using 53:47 CH_3CN-H_2O as eluant (3 mL/min) to obtain compound **1** (49 mg, 0.53% dry wt.), retention time 30 min, and compound **2** (35 mg, 0.38% dry wt.), retention time 34 min.

Raspailol A (1): Colorless oil; $[\alpha]_D$ = + 62° (C_6H_6, *c* = 0.46); UV (hexane) λ_{max} (log ϵ) 234 (4.66), 241 nm (4.67); IR (film) 3408, 2923, 1453, 1381, 1160, 1078, 1016, 990, and 917 cm^{-1}; ^1H NMR see Table 1; ^{13}C NMR see Table 1; CIMS (CH_4, 70 eV) *m/z*: 375 (M+1), 357 (M+1-H_2O), 339 (M+1-2H_2O), 303, 285, 259, 239, 203; EI-HRMS Obsd. *m/z* = 374.2831, $C_{24}H_{38}O_3$ requires 374.2821.

Raspailol B (2): Colorless oil; $[\alpha]_D$ = + 111° (C_6H_6, *c* = 0.14); UV (hexane) λ_{max} (log ϵ) 234 (4.66), 241 nm (4.66); IR (film) 3417, 2924, 1458, 1381, 1177, 1086, 1019, 990, 961 and 912 cm^{-1}; ^1H NMR see Table 1; ^{13}C NMR see Table 1; FAB-HRMS Obsd. *m/z* = 387.2879 (M-1); $C_{25}H_{39}O_3$ requires *m/z* = 387.2899.

Preparation of (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetates (3) and (4): Raspailol A (**1**, 0.6 mg) was treated with CH_2Cl_2 solutions of dicyclohexylcarbodiimide (3 mg in 130 μL), *N,N*-dimethylaminopyridine (0.3 mg in 30 μL) and either (*R*)- or (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (0.4 mg in 40 μL), at room temperature for 24 h. In each case, the reaction mixture was chromatographed on a 3 cc silica Sep-Pak using hexane-EtOAc (7:3) as eluant. Fractions 1 and 2 (3 ml each) were combined and concentrated to dryness. The residue was redissolved in hexane-EtOAc 8:2 (500 μL), filtered and purified by HPLC on a silica column (1 x 50 cm), eluting with hexane-EtOAc (8:2) at 3 mL/min using UV detection (229 nm). The retention times of (*R*)-MTPA ester (**3**, *ca.* 0.5 mg) and (*S*)-MTPA ester (**4**, *ca.* 0.4 mg) were 20 and 22 minutes, respectively.

(*R*)-MTPA ester (3): ^1H NMR ($CDCl_3$) δ 0.83 (d, 3H, *J* = 7 Hz), 0.94 (d, 3H, *J* = 7 Hz), 1.35 (q, 1H, *J* = 12 Hz), 1.39 (s, 3H), 1.65 (m, 1H), 1.69 (br s, 1H), 1.92 (br dd, 1H, *J* = 15, 7 Hz), 2.15 (m, 7H), 2.29 (br dt, 1H, *J* = 14, 7 Hz), 2.36 (sept, 1H, *J* = 7 Hz), 3.55 (s, 3H), 3.99 (m, 1H), 4.95 (br d, 1H, *J* = 11 Hz), 5.01 (br d, 1H, *J* = 17 Hz), 5.11 (td, 1H, *J* = 11, 5 Hz), 5.53 (m, 2H), 5.56 (m, 1H), 5.76 (br d, 1H, *J* = 11 Hz), 5.81 (ddt, 1H, *J* = 17, 11, 7 Hz), 5.99 (m, 2H), 6.24 (br dd, 1H, *J* = 15, 11 Hz), 7.35 (m, 3H), 7.52 (m, 2H).

(*S*)-MTPA ester (4): ^1H NMR ($CDCl_3$) δ 0.94 (d, 3H, *J* = 7 Hz), 1.00 (d, 3H, *J* = 7 Hz), 1.20 (q, 1H, *J* = 12 Hz), 1.41 (s, 3H), 1.67 (m, 1H), 1.69 (br s, 1H), 1.92 (br dd, 1H, *J* = 15, 7 Hz), 2.11 (m, 1H), 2.15 (m, 6H), 2.27 (br dt, 1H, *J* = 14, 7 Hz), 2.36 (sept, 1H, *J* = 7 Hz), 3.52 (s, 3H), 3.97 (m, 1H), 4.95 (br d, 1H, *J* = 11 Hz), 5.01 (br d, 1H, *J* = 17 Hz), 5.09 (td, 1H, *J* = 11, 5 Hz), 5.52 (m, 2H), 5.56 (m, 1H), 5.76 (br d, 1H, *J* = 11 Hz), 5.81 (ddt, 1H, *J* = 17, 11, 7 Hz), 5.98 (m, 2H), 6.23 (br dd, 1H, *J* = 15, 11 Hz), 7.36 (m, 3H), 7.49 (m, 2H).

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REFERENCES AND NOTES

1. A specimen of the sponge has been deposited in the SIO Benthic Invertebrate Collection (Registry number P1151).
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