Asmarines G and H and Barekol, Three New Compounds from the Marine Sponge *Raspailia* sp.

Amira Rudi,[†] Hagit Shalom,[†] Michael Schleyer,[‡] Yehuda Benayahu,[§] and Yoel Kashman*[,†]

School of Chemistry, Tel-Aviv University, Ramat Aviv 69978, Israel, Oceanographic Research Institute (ORI), Durban, Republic of South Africa, and Department of Zoology, Tel-Aviv University, Ramat Aviv 69978, Israel

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Four asmarines, the known A and F and two new ones G and H, four diterpenes, the known chelodane, barekoxide, and zaatirin and a new one, barekol, and methyl 3-oxo-cholan-24-oate were isolated from the Kenyan sponge *Raspailia* sp. The structures of all these compounds were established on the basis of MS and NMR data, and the absolute configuration of barekol was determined from the CD measurements of its keto-derivative. The ^{15}N chemical shifts of the N atoms of the heterocycles of the asmarines were measured from ^{15}N HMBC experiments, and the influence of various structural features on the δ_N values studied.

In search of new biologically active substances from marine organisms,[1−3] we investigated the Indian Ocean sponge *Raspailia* sp. (Demospongiae, Poecilosclerida, Microcionina, Raspailiidae). Sponges have been shown to be a rich source of nitrogen-atom-containing compounds. Among the many interesting compounds with novel heterocyclic systems[4] are the asmarines (A−F),[5] previously isolated by us from the Red Sea (Dahlak archipelago, Eritrea) *Raspailia* sp.

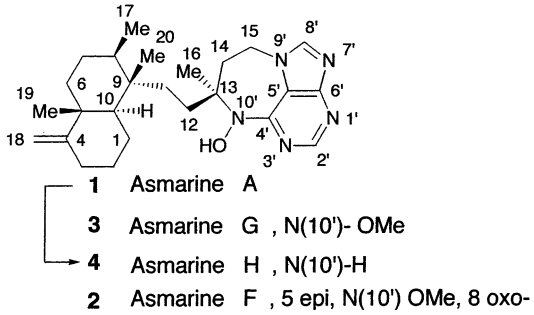
The presently investigated Indian Ocean *Raspailia* sp. is quite different from the Red Sea species and appears zoologically closer to the Kenyan *Aulospangus involutus*. The ethyl acetate extract of the Indian Ocean sponge exhibited cytotoxicity against several tumor cells. Indeed, from this extract, we isolated asmarine A (**1**), previously[5] shown by us to be cytotoxic. Unlike the Red Sea sponge, the major asmarine in the present sponge was asmarine F (**2**), which is less active than A. Together with compounds **1** and **2**, isolated in 0.33% and 0.93% yield, respectively, two other asmarines, designated G (**3**) and H (**4**), were obtained in minute amounts, 0.1% and 0.01% yield, respectively.

Compound **3** was assigned the molecular composition of $C_{26}H_{39}N_5O$ by HREIMS (*m*/*z* 437.3142) and its ^{13}C NMR spectrum. Comparison of the ^{13}C resonances of **3** with those of asmarine A (Table 1) revealed a high degree of similarity, except for an additional methyl peak at δ_C 64.5 q (δ_H 3.90 s, 3H) and small changes in the resonances of C-13, 14, and 16. The presence of two proton signals at δ_H 8.22 and 8.96 s (in d_6-DMSO, or 8.05 and 8.12 in $CDCl_3$) and the appropriate five C atom resonances, characteristic of an adenine heterocycle,[6] as well as the expected CH correlations between the adenine and the diazepine ring atoms,[6] established the tetrahydrodiazepino purine (THDAP) ring system as in **1** (and **2**). Furthermore, the δ_C values and the HMBC results determined, for compound **3**, the same alicyclic ring system as that in **1**. Eventually, the extra OCH_3 group was placed on the N(10′)-OH group on the basis of the following evidence: (a) its relative downfield chemical shift (δ_C 64.5), similar to the same group in the methylation product of **1** (δ_C 66.0) as well as a +2 ppm shift in C-13; (b) the *m*/*z* 218 (100%) and 188 (60%) fragments in the MS (Figure 1); (c) an NOE between this OCH_3 group and CH_3-16; and (d) the ^{15}N chemical shift value of 189.9 ppm for N(10′), vide infra.

Table 1. ^{13}C NMR (100 MHz, $CDCl_3$) of Asmarines G, H, and A[a]

position	3	4	1
1	21.9 CH_2	21.9	21.8
2	28.4 CH_2	28.6	28.6
3	33.4 CH_2	34.1	33.2
4	159.7 C	160.1	160.6
5	39.8 C	40.0	40.1
6	36.9 CH_2	38.2	37.2
7	27.1 CH_2	27.2	27.4
8	36.2 CH	36.5	36.7
9	39.2 C	39.1	39.3
10	48.4 CH	48.7	48.6
11	31.5 CH_2	31.2	31.2
12	32.7 CH_2	32.8	33.0
13	66.3 C	56.1	64.2
14	37.9 CH_2	37.1	36.7
15	42.1 CH_2	43.7	42.3
16	24.3 CH_3	26.5	21.8
17	15.7 CH_3	15.8	15.9
18	102.6 CH_2	102.8	102.5
19	20.7 CH_2	20.8	20.1
20	18.2 CH_3	18.3	18.3
2′	152.9 CH	151.4	151.7
4′	150.5 C	149.7	149.0
5′	109.9 C	111.3	109.3
6′	159.5 C	159.6	158.7
8′	143.0 CH	144.3	143.1
N-OMe	64.5 CH_3		

[a] All assignments were confirmed from 2D NMR spectra.



The fourth asmarine (H, **4**), obtained following RP-18 HPLC, produced an M^+ peak at *m*/*z* 407 for a molecular formula of $C_{25}H_{37}N_5$ (10° unsaturation as in asmarines A

* To whom correspondence should be addressed. Tel: +972-3-6408419. Fax: +972-3-6409293. E-mail: kashman@post.tau.ac.il.
[†] Tel-Aviv University.
[‡] Oceanographic Research Institute (ORI).
[§] Tel-Aviv University Depatrment of Zoology.

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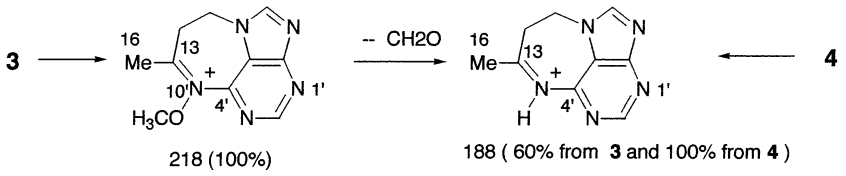


Figure 1. Major MS fragments of compounds **3** and **4**.

Table 2. ^{15}N Chemical Shifts (40 MHz, d_6-DMSO) Deduced from an ^{15}N HMBC Experiment[a]

	N-1′	N-3′	N-7′	N-9′	N(10′)-R	R
1[b]	233.3	247.0	245.8	153.9	166.5	OH
2	224.0	239.2	120.0	113.5	186.9	OCH_3
3	236.7	247.8	244.3	152.9	189.9	OCH_3
4	243.0	231.2	243.8	155.9	116.6	H

[a] Chemical shifts in ppm upfield from liquid NH_3. [b] Previously reported values[5] were wrong due to miscalibration.

Table 3. ^1H and ^{13}C NMR Data of Barakol (**9**) (400 and 100 MHz, $CDCl_3$)

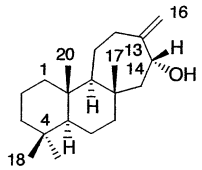
position	^{13}C	^1H		HMBC[a]
1	39.9 CH_2	1.70	0.80	20
2	18.6 CH_2	1.49	1.35	
3	41.9 CH_2	1.38	1.12 dt (4.0, 13.3)	18, 19
4	33.3 C			18, 19
5	56.4 CH	0.80		7a, 7b, 18, 19, 20
6	18.6 CH_2	1.60	1.45	
7	45.1 CH_2	1.55	1.23	8a, 8b, 9, 17
8	35.9 C			17
9	60.3 CH	0.89		12a, 15a, 15b, 17, 20
10	38.6 C			20
11	24.1 CH_2	1.80	1.28	
12	33.2 CH_2	2.45 bd (14.7)	2.20 dt (3.9, 15.2)	14, 16a, 16b
13	155.0 C			12a, 12b, 14, 15a, 15b, 16a, 16b
14	70.8 CH	4.30 dd (10.8, 4.2)		15a, 15b, 16a, 16b
15	55.4 CH_2	1.68 dd (13.5, 4.3)	1.41	7a, 7b, 14, 17
16	110.4 CH_2	5.03 s	4.87 s	12a, 14
17	20.3 CH_3	0.95 s		
18	33.3 CH_3	0.84 s		19
19	21.6 CH_3	0.79 s		18
20	15.7 CH_3	0.77 s		9

[a] Proton showing long-range correlation to indicated proton.

and G). The 1D and 2D NMR data suggest a strong resemblance between **4** and **1** (Table 1) except for the resonance of C-13, which, in **4**, is 8 ppm upfield shifted (to δ_C 56.1), and the resonance of C-16, which in **4** is 4.7 ppm downfield shifted. The position of C-13 became clear from its CH correlations to CH_3-16, which itself had further correlations to C-12 and C-14. The absence of an oxygen atom, the identity of the alicyclic portions of **1** and **4**, and the upfield shift of C-13 suggested for **4** the N-deoxy asmarine A structure, i.e., an amine instead of a hydroxyamine. Corroborative evidence also came from the δ_N values (Table 2). Indeed, mild reduction of asmarine A with sodium thiosulfate afforded asmarine H (**4**) quantitatively, verifying the suggested structure.

The ^{15}N NMR spectra of asmarines A, F, G, and H (**1**−**4**) (Table 2) demonstrate well the feasibility of acquiring structural information from these ^{15}N-resonance measurements.[7,12] Thus, comparison of compound **1** with compound **2** clearly indicates the influence of the hybridization of the N atoms. For example N(7′) and N(9′) shift at 126 and 41 ppm, respectively, upfield in compound **2** due to the change from the sp^2 to the sp^3 hybridization.[7] Additionally, a 20 ppm downfield shift is observed for the change from the NOH to the $NOCH_3$ group. A δ_N value, similar to that of the $NOCH_3$ in **2**, is also seen in the case of **3** (189.9 ppm), where the four purine N atoms remain almost unchanged in comparison with those of **1**. In the case of compound **4**, a 10 ppm downfield and 16 ppm upfield shift for N(1′) and N(3′) were recorded, and, as expected in the absence of a hydroxylamine, an upfield change of about 50 ppm of the N(10′)H to 116.6 ppm was observed. The latter N(1′) and N(3′) shifts may result from a small contribution of the N(3′)H tautomer.

An additional five less polar compounds (**5**−**9**) were isolated from the sponge in addition to the asmarines. Four of the compounds, **5**−**8**, were identified as chelodane (**5**), zaatirin (**6**), barekoxide (**7**), and methyl 3-oxo-cholan-24-oate (**8**); these had previously been isolated from the Red Sea *Raspailia* species.[5] It is noteworthy that chelodane (**5**) is the biogenetically appropriate diterpene to, together with adenine, produce three of the asmarines. The three diterpenes (**5**−**7**) were first isolated from the Red Sea sponge *Chelonaplysilla erecta*, which was collected near Ras Zaatir.[8] The latter sponge is from a different family and did not contain any of the asmarines.



9 Barekol

7 Barekoxide (16-methyl, 13,14-epoxy)

Compound **9**, barekol, showed an HREIMS [M^+] ion at *m/z* 290.2605 for a molecular formula of $C_{20}H_{34}O$ and, therefore, possessed four degrees of unsaturation. In the presence of a single exo-methylene (δ_C 110.4 t and 155.0 s), compound **9** had to be tricyclic. Two-dimensional NMR experiments, including COSY, HMQC, and HMBC, were mainly used for the structure elucidation of **9** (Table 3). Comparison of the NMR data of **5**−**7** and **9** clearly pointed to a barekoxide skeleton and the replacement of compound **7**'s epoxide by a 14-ol-13(16)-ene moiety.

Furthermore, on the basis of NOEs, an all-*trans* stereochemistry, as in barekoxide and 3-bromobarekoxide, for which X-ray diffraction analysis had been performed,[9] could also be suggested for **9**. On the basis of the assumption that barekol is obtained from **7** by acid-catalyzed opening of the epoxide, barekoxide was treated with a trace of acid in $CDCl_3$ and the transformation monitored via NMR. Indeed, after 14 days, barekol was obtained cleanly as a single product in ca. 90% yield (Figure 3).

Circular dichroism was used to determine the absolute configuration of **9**.[10] The 13-oxo derivative (**10**) was prepared by ozonolysis (Figure 3). Whereas the *trans*-decalin portion of **10** is rigid, the third ring is not. A cycloheptane

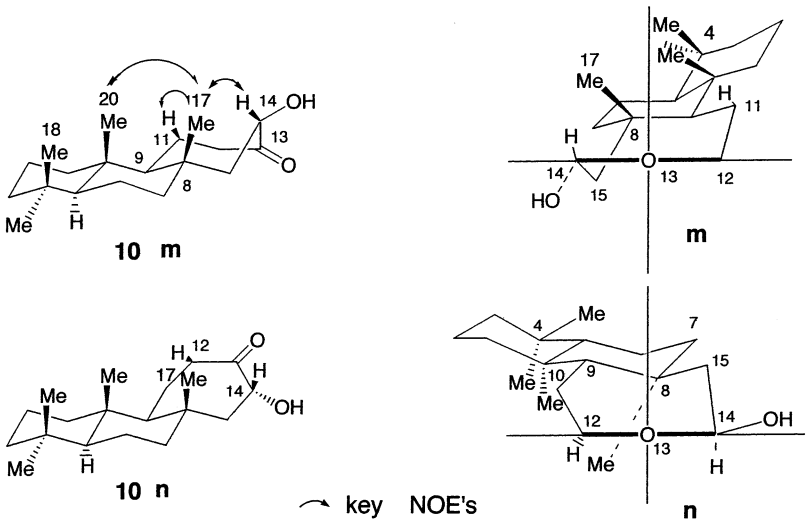


Figure 2. Conformations **m** and **n** of **10** and their projections.

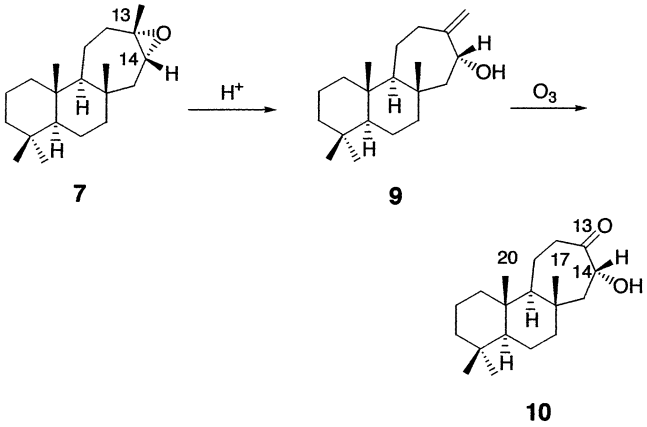


Figure 3. Transformation of barekoxide to barekol and compound **10**.

ring and, moreover, one with an sp^2 carbon atom (C-13) can adopt several conformations. Yet, a strong NOE measured between CH_3-17 and the readily recognizable axial H-14 proton (δ 4.30 dd, $J_{ax,ax} = 10.8$, $J_{ax,eq} = 4.2$ Hz) limited the possible conformations of this ring to two major ones: one, **m** (Figure 2), in which the quasi-axial H-11 is spatially close to CH_3-17 (C-11, 12, 15, and 18 and C-12, 13, 14, and 15 are each approximately in one plane), and the other, **n**, in which the quasi-axial H-12 is spatially close to CH_3-17 (C-11, 12, 14, and 15 are each approximately in one plane) (Figure 2). The distinction between **m** and **n**, in favor of **m**, was achieved from an NOE between CH_3-17 and the pseudoaxial H-11 (and no easily identifiable enhancement of H-12). The CH_3-17 group also shows the expected (from the all-*trans* structure) NOE to the CH_3-20 group.

The projections of **10** in two conformations **m** and **n**, according to the requirements of the octant rule, are given in Figure 2. Knowing the **m** conformation of **10**, see above, the absolute configuration could be determined by CD measurements.[10]

As seen in Figure 2, a strong negative Cotton effect is expected from **10** in conformation **m**. Both the 14-OH group, positioned α to the carbonyl and expected to cause the strongest perturbation of the n$\rightarrow\pi^*$ absorption, and the decalin part are both in negative octants. Conformation **n**, on the other hand, where the 14-OH group is approximately in the nodal plane of the carbonyl and the decalin part is mainly in a positive octant, is expected to give only a weak positive effect. Hence, from the negative Cotton effect ($\Delta\xi = -0.91$), the absolute configurations of **10** and **9**, as depicted in Figure 3, were determined to be in agreement with the absolute configuration suggested for 3-bromobarekoxide by X-ray diffraction analysis.[9]

Asmarine analogues are presently the topic of a synthetic project for structure−activity relationship studies. In this context, it is interesting to note that both asmarines G and H are less active than asmarine A.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR were recorded on Bruker Avance 400 and ARX-500 spectrometers. EIMS and FABMS were recorded on a Fisons Autospec Q instrument. All chemical shifts are reported with respect to TMS (δ_H 0) and $CDCl_3$ (δ_C 77.0). The CD spectrum was recorded on an Aviv 202 spectrophotometer.

Animal Material. The sponge was collected at Wasini Island, Shimoni Channel 4°40′ S; 39°22′ E, southern Kenya (8−14 m, March 7, 2002). Its habitat is a sandy bottom with scattered calcareous substrate overgrown by diverse sponge assemblages, among them *Raspailia* sp. seldom appears. The whole area is exposed to strong tidal currents and thus is appropriate for the growth of filter feeders including sponges, tunicates, and sea pens. The nearby mangrove trees and sea grass beds are an indication of the high productivity of the site. The voucher specimen is deposited at the Zoological Museum of Tel Aviv University under the collection number ZMTAU SF 25366

Extraction and Isolation. The freeze-dried sponge (3 g) was extracted with EtOAc to give, after evaporation, a brown gum (290 mg). The gum was partitioned between aqueous methanol, *n*-hexane, and $CHCl_3$. The hexane fraction (135 mg) was subjected to Si gel vacuum liquid chromatography (VLC, eluting with 9:1 *n*-hexane/EtOAc to pure EtOAc) to produce, according to increasing polarity, zaatirine (**6**) (4.5 mg), barekoxide (**7**) (12.5 mg), chelodane (**5**) (12.5 mg), methyl 3-oxocholane-24-oate (**8**) (4.5 mg), barekol (**9**) (11.5 mg), asmarine F (**2**) (28 mg), and asmarine G (**3**) (2.5 mg).

The $CHCl_3$ fraction (81 mg) was chromatographed on a Sephadex LH-20 column eluted with 1:1 $CHCl_3$/MeOH to yield asmarine A (10 mg) and a mixture of asmarines A and H. The latter mixture was separated on an RP-18 Purospher STAR column (65:35:0.1 CH_3CN/H_2O/TFA) to obtain asmarine H (**4**) (1 mg), in addition to asmarine A (**1**).

Asmarine G (3): oil; $[\alpha]^{25}_D$ +40° (*c* 1.22, CH_2Cl_2); IR (neat) ν_{max} 2932, 1594, 1532, 1457, 1387 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) 8.12 (1H, s, H-2′), 8.05 (1H, s, H-8′), 4.40 (2H, s, H-18), 4.30 (1H, m, H-15a), 4.20 (1H, m, H-15b), 3.88 (3H, s, N−OCH_3), 1.53 (3H, s, Me-16), 0.95 (3H, s, Me-19), 0.66 (3H,

s, Me-20), 0.57 (3H, d, J = 6.5 Hz, Me-17); for ^{13}C NMR, see Table 1; EIMS m/z 437.4 [M^+] (50), 407.4 (10), 218.2 [$C_{10}H_{12}N_5O$] (100), 188 (60); HREIMS m/z 437.3142 (calcd for $C_{26}H_{39}N_5O$, 437.3146).

Asmarine H (4): oil; $[\alpha]^{25}_D$ +10° (c 0.37, CH_2Cl_2); IR (neat) ν_{max} 2931, 1620, 1561, 1388, 1056 cm^{-1}; ^{1}H NMR (d_6-DMSO, 500 MHz) 8.22 (1H, s, H-2′), 7.89 (1H, s, H-8′), 4.44 (1H, s, H-18a), 4.42 (1H, s, H-18b), 4.33 (2H, bs, H-15), 1.28 (3H, s, Me-16), 6.97 (3H, s, Me-19), 0.70 (3H, s, Me-20), 0.67 (3H, d, J = 6.7 Hz, Me-17); for ^{13}C NMR, see Table 1; EIMS m/z 407 [M^+] (55), 392 (15), 216 (10), 188 (100); HREIMS m/z 407.3045 (calcd for $C_{25}H_{37}N_5$, 407.3041).

Reduction of Asmarine A (1) to Asmarine H(4).[11] Asmarine A (10 mg) in 80% aqueous ethanol (5 mL) was treated with $Na_2S_2O_3$ (5 mg). After 3 h at 60 °C, the cooled solution was evaporated. The residue was taken into $CHCl_3$ (20 mL) and H_2O (10 mL), and the organic phase was washed with water, dried, and evaporated to afford asmarine H (9 mg).

Barekol (9): white powder; $[\alpha]^{25}_D$ −29° (c 1, $CHCl_3$); IR (neat) ν_{max} 1461, 1388, 3602, 2972 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; EIMS m/z 290 [M^+] (15), 272 [$M^+ - H_2O$] (35), 257 (40), 191 (100) [$C_{14}H_{23}^+$]; HREIMS m/z 290.2605 (calcd for $C_{20}H_{34}O$, 290.2601).

Ozonolysis of Barkenol (9) to 10. Ozone was passed for 20 s through a solution of **9** (5 mg) in CH_2Cl_2 (10 mL) at −78 °C. Dimethyl sulfide (1 drop) was then added and the solution left overnight at room temperature. The residue, after evaporation, was filtered through Si gel eluted with 1:9 ethyl acetate/n-hexane to afford compound **10** (3 mg), $[\alpha]^{25}_D$ −18° (c 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3488, 2927, 1701, 1466, 1389 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.47 (1H, dd, J = 12.3, 2.5 Hz, H-14), 2.69 (1H, ddd, J = 19.6, 4.7, 2.7 Hz, H-12a), 2.36 (1H, ddd, J = 19.6, 12.9, 3.5 Hz, H-12b), 1.18 (3H, s, CH_3-17), 0.86 (3H, s, CH_3-18), 0.81 (3H, s, CH_3-20), 0.80 (3H, s, CH_3-19); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 216.0 (C, C-13), 72.5 (CH, C-14), 60.8 (CH, C-9), 56.2 (CH, C-5), 53.5 (CH_2, C-15), 43.5 (CH_2,C-7), 41.8 (CH_2, C-3), 41.0 (CH_2, C-12), 39.3 (CH_2, C-1), 38.7 (C, C-10), 36.3 (C, C-8), 33.4 (CH_3, C-18), 33.3 (C, C-4), 21.5 (CH_3, C-19), 20.5 (CH_3, C-20), 18.4 (CH_2, C-6), 18.4 (CH_2, C-2), 17.8 (CH_2, C-11), 15.5 (CH_3, C-20); EIMS m/z 292 [M^+] (40), 263 (40), 191 (55), 123 (100).

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