Asmarines I, J, and K and Nosyberkol: Four New Compounds from the Marine Sponge *Raspailia* sp.

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Five asmarines, the known A and F and three new ones, I, J, and K, and four diterpenes, the known chelodane, barekoxide, and zaatirin and a new one, nosyberkol, were isolated from the Nosy Be Islands (Madagascar) sponge *Raspailia* sp. The structures of all these compounds were established on the basis of MS and NMR data. A biogenesis for the various *Raspailia* sp. diterpenes is suggested.

In the search for bioactive substances from marine invertebrates,[1,2] we found the Indian Ocean and Red Sea *Raspailia* sp. sponges to contain combined adenine-diterpene secondary metabolites known as the asmarines.[3,4] Thus far, we have reported the structure of eight asmarines, A−H, with differing levels of cytotoxicity, isolated from several collections of the *Raspailia* sp. sponge.[3,4] Below we report three additional asmarines, I, J, and K (**3**−**5**), isolated together with the known asmarines A and F (**1** and **2**),[3] a new diterpene designated nosyberkol (**9**), and three known diterpenes [zaatirin (**6**), chelodane (**7**), and barekoxide (**8**)],[8] all isolated from a *Raspailia* sp. collected from Nosy Be, Madagascar.

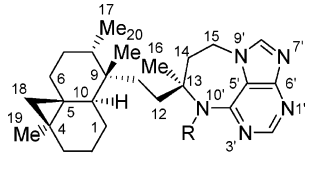
Similar to the extracts of the earlier investigated *Raspailia* spp., the ethyl acetate extract of the Nosy Be collection exhibited mild cytotoxicity against several tumor cell lines.[5] Asmarines A (**1**) and F (**2**) were isolated in 0.5% and 0.4% yield, respectively. Together with them, asmarines I, J, and K (**3**, **4**, and **5**) were isolated, following repeated Sephadex LH-20 and RP-18 HPLC chromatography, in minute amounts only, in 0.12%, 0.02%, and 0.02% yield, respectively.

Asmarine I (**3**) was assigned the molecular composition of $C_{25}H_{37}N_5O$ by HREIMS (*m/z* 423.2996, calcd 423.2990) and its ^{13}C NMR spectrum. Comparison of the NMR data of **3** with those of asmarine A (**1**) revealed a high degree of similarity, except for the appearance of two characteristic cyclopropane protons (δ_H 0.05, d; 0.43, d) and the disappearance of one of the terminal methylene groups. Two singlets at δ_H 8.21 and 8.34, of one proton each, together with the appropriate ^{13}C NMR resonances of the heterocyclic system (Table 1) established the same tetrahydrodiazepino purine (THDAP) ring system for **3** as had been found for **1**. The 10 degrees of unsaturation of **3** required, in addition to the THDAP ring system and in the absence of additional double bonds, a tricyclic ring system. The 1D and 2D NMR data suggested a tricyclo[5.4.0.0^{1,3}]undecane ring system. Two- and three-bond long-range CH correlations established the location of the four methyl groups as well as the angular position of the cyclopropane ring. CH correlations between CH_3-17 and CH_3-20 and their neighboring carbon atoms suggested they were adjacent and in the same positions as the 17- and 20-methyl groups in the asmarines A−H. Furthermore, the HMBC correlations of the third decaline methyl group (CH_3-19) (Figure 1) to C-3, -4, and -5, as well as to the cyclopropane methylene (CH_2-18) led to the conclusion that the CH_3-19 group is positioned on C-4, vicinal to the cyclopropane. The latter suggestion was unambiguously confirmed from the appropriate CH correlations of both H-18a and H-18b, the two cyclopropane protons, to C-3, -4, -5, -6, -10, and -19, thus determining the planar structure of **3**.

Table 1. ^{13}C NMR Data of Asmarines I, J, and K (100 MHz, $CDCl_3$)[a]

position	**3**	**4**	**5**
1	19.8 CH_2	19.7	21.2
2	23.0 CH_2	23.0	24.0
3	31.9 CH_2	31.9	31.7
4	17.2 C	17.2	153.0
5	26.2 C	26.1	39.0
6	27.4[b] CH_2	27.4	38.2
7	27.6[b] CH_2	27.6	27.0
8	35.4 CH	35.3	38.0
9	39.1 C	39.0	40.0
10	40.8 CH	40.0	49.0
11	35.8 CH_2	31.7	31.0
12	23.0 CH_2	23.0	32.0
13	69.4 C	59.3	56.0
14	35.4 CH_2	34.4	36.0
15	42.8 CH_2	43.5	42.0
16	23.6 CH_3	25.6	16.0
17	14.3 CH_3	14.3	15.8
18	24.7 CH_2	24.5	105.0
19	22.2 CH_2	22.3	33.0[c]
20	19.9 CH_3	19.8	20.5
2′	145.9 CH	146.7	151.6
4′	145.0 C	146.1	149.6
5′	109.3 C	110.3	109.3
6′	155.3 C	156.6	158.0
8′	144.5 CH	143.0	143.3

[a] All assignments were confirmed from 2D NMR spectra; the C atoms' multiplicity was determined by a DEPT experiment. [b] Interchangeable. [c] C-19 in compound **5** is a methyl.



3 Asmarine I R = OH
4 Asmarine J R = H

The relative stereochemistry of the tricyclic alicyclic part of **3** was elucidated mainly on the basis of NOE measure-

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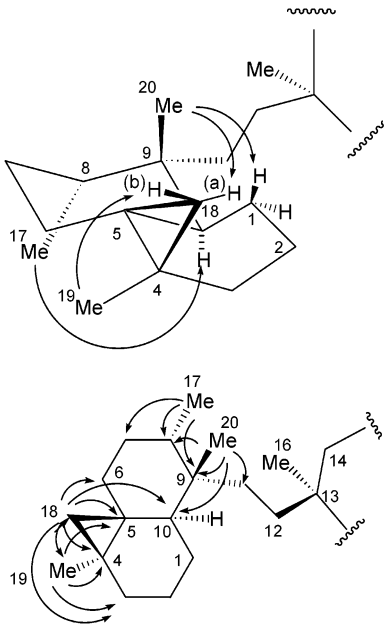


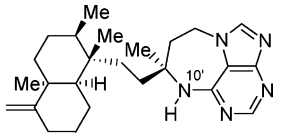
Figure 1. Key NOEs (top) and selective HMBC correlations for the alicyclic part of **3** and **4**.

ments (Figure 1), the starting points being H-10 (recognizable from a 1D-NOE experiment) and 18a and 18b. A 10.2 Hz coupling constant of H-10 (δ_H 1.55 brd) established its axial α-orientation, which was confirmed by an NOE to CH_3-17, which, therefore, is also axial and on the same α-side as H-10. The stereochemistry of CH_3-20 (axial), and hence the 11,12-ethylene bridge to the THDAP system (equitorial), was established from an NOE between CH_3-20 and one cyclopropane proton, H-18a (pointing inward). An NOE between CH_3-19 and the second, outward pointing cyclopropane proton, H-18b, agreed well with the suggested stereochemistry (Figure 1).

Agreement was found between the ^{13}C NMR shifts of the alicyclic part of asmarine I (**3**) and the relevant shifts reported for cacospongionolide, which possesses the same stereochemistry,[6] compared with values reported for dytesinines A and B, where methyls C-17 and C-20 are on the same face of the molecule.[7] The stereochemistry of CH_3-17 and CH_3-20 in **3** is of interest from a biogenetic point of view, as it differs from the stereochemistry of these methyls in asmarines A and F (**1** and **2**)[3] and requires an alternate mechanism (Figure 2, path c).

The fourth asmarine (J, **4**) obtained, following Sephadex LH-20 and RP-18 HPLC chromatography, produced an M^+ peak at *m/z* 407.3038, calcd 407.3041, for a molecular formula of $C_{25}H_{37}N_5$ (10 degrees of unsaturation, as in asmarines A and I). The 1D and 2D NMR data suggested a strong resemblance between **4** and **3** (Table 1), except for the resonances near N(10′); specifically, C-13 and C-16 were −10.1 and +2 ppm shifted, respectively, relative to **3**. These shifts and one oxygen atom less in **4** than in **3** (MS) suggested asmarine J (**4**) to be N(10′) deoxy **3**. Thus, the hydroxylamine functionality of **3** is replaced in **4** with an amine, a relationship similar to that found for asmarines A and H[4] and asmarines B and K (**5**) (vide infra).

The structure of asmarine K (**5**), the third asmarine that was obtained in minute amounts, $C_{25}H_{37}N_5$ (*m/z* 407.3043, calcd 407.3041), was readily determined by comparing its NMR data with that of asmarines H and B. Compound **5** possessed the same THDAP ring system as asmarine H[4] and the alicyclic decalin system as that of asmarine B.[3] Therefore, asmarine K (**5**) is the N(10′) deoxy analogue of asmarine B.



5 Asmarine K

[Asmarine B ; N(10′)OH]

Four additional less polar compounds (**6**–**9**) were isolated

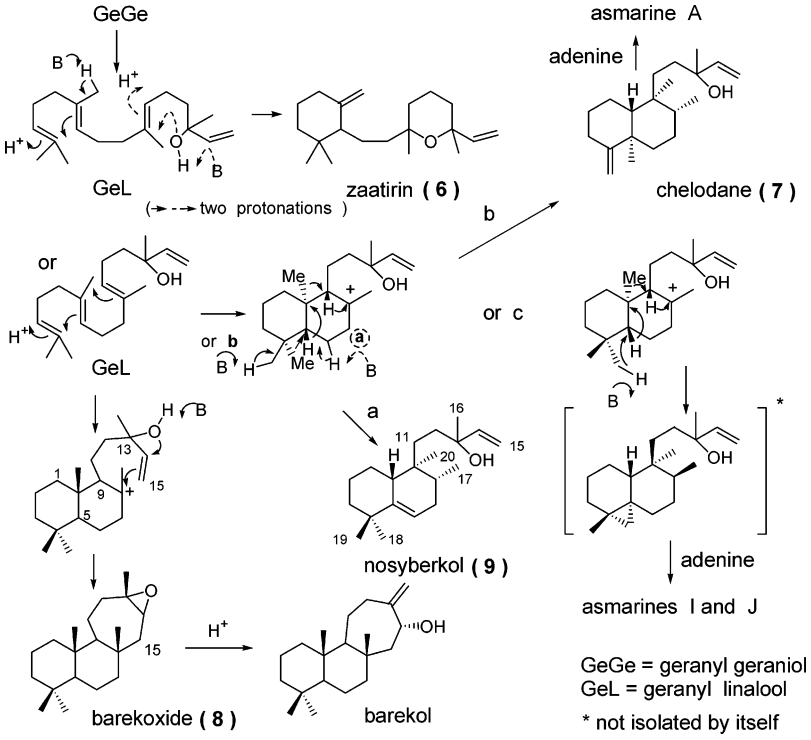


Figure 2. Suggested biogenesis for *Raspailia* sp. diterpenes and asmarines (a–c alternative routes).

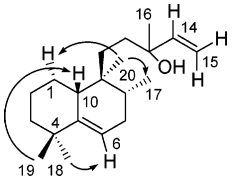


Figure 3. Key NOEs for **9**.

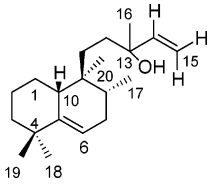
Table 2. ^{1}H and ^{13}C NMR Data for Compound **9** (400 and 100 MHz, $CDCl_3$)[b]

position	δ_C[a]	δ_H	HMBC
1	27.6 CH_2	1.68, 1.03	
2	22.2 CH_2	1.56, 1.50	
3	40.9 CH_2	1.40, 1.20	18, 19
4	36.6 C		18, 19
5	146.0 C		3a, 7a, 10, 18, 19[c]
6	116.2 CH	5.43	7a
7	31.6 CH_2	1.82, 1.70	17
8	33.3 CH	1.47	6, 17, 20
9	36.0 C		17, 20
10	39.7 CH	2.11 bd (13.7)	6, 20
11	30.6 CH_2	1.35, 1.28	20
12	35.1 CH_2	1.45	16
13	73.5 C		11a, 11b, 12, 14, 15a, 15b, 16
14	145.1 CH	5.92 dd (17.3, 10.7)	15a, 15b, 16
15	111.8 CH_2	5.20 d (17.3), 5.07 (10.7)	
16	27.7 CH_3	1.30 s	
17	14.9 CH_3	0.79 d (6.7)	
18	29.7 CH_3	1.06 s	19
19	29.0 CH_3	1.00 s	18
20	16.2 CH_3	0.62 s	

[a] All relevant carbon resonances are in good agreement with literature data for comparable resonances in akaterpin.[9] [b] All assignments were confirmed from 2D NMR spectra; the carbon multiplicity was determined by a DEPT experiment. [c] Proton 'a' refers to the more downfield of each geminal pair.

from the sponge, in addition to the five asmarines (**1**−**5**). Three of the compounds were identified as zaatirine (**6**), chelodane (**7**), and barekoxide (**8**). These had previously been isolated from the Red Sea and Kenyan *Raspailia* sp.[4] as well as from the Red Sea sponge *Chelonaplysilla erecta*.[8] Separation of compounds **7** and **9**, which possessed a very similar polarity, was achieved by chromatography on $AgNO_3$-impregnated silica gel. Nosyberkol (**9**) showed an HREIMS [M^+] ion at *m/z* 290.2609 for a molecular formula of $C_{20}H_{34}O$, calcd 290.2679, and, therefore, possessed four degrees of unsaturation. In the presence of two double bonds (δ_C 146.0 C, 116.2 CH and 145.1 CH, 111.8 CH_2) compound **9** had to be bicyclic. Two-dimensional NMR experiments, including COSY, HMQC, and HMBC, were mainly used for the structure elucidation of **9** (Table 2). Comparison of NMR data of **9** with that of chelodane (**7**) clearly indicated that they possessed the same side chain but were different in the decalin portion of the molecule. While chelodane possesses the clerodane-type skeleton, nosyberkol carries the quite rare halimane-type skeleton.[9] In **9**, the terminal geranyl-linalool (GeL)-derived gem-dimethyl is intact and only CH_3-20 is shifted (a 1,2-shift). In **7**, one of the gem-dimethyls is also shifted (Figure 2).

The relative stereochemistry of the decalin system of **9** was determined on the basis of coupling constants and NOE measurements. An axial orientation was suggested for H-10 (δ 2.11, bd), on the basis of its 13.7 Hz coupling contant with the vicinal axial H-1. NOEs between CH_3-19 and H-10 and between its geminal methyl group CH_3-18 and the olefinic proton H-6 established the positions of the latter two methyl groups (CH_3-18, equatorial and CH_3-19, axial). NOEs between H-1α, axial (δ_H 1.03) and CH_3-20 and between CH_3-20 and CH_3-17 completed the relative stereochemistry of **9**. The ^{13}C chemical shifts of **9** are in excellent agreement with the values reported for the halimane ring system in akaterpin.[9] The configuration of the hydroxyl group at C-13 was not determined.



9 Nosyberkol

The three new asmarines (I−K, **3**−**5**) are less cytotoxic than asmarine A (**1**) or asmarine B[5] (GI_{50} = 5−10 μM, for a variety of tumor cells).[5]

Experimental Section

General Experimental Procedures. Optical rotations were obtained with a Jasco P-1010 polarimeter. IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400 and ARX-500 spectrometers. All chemical shifts are reported with respect to TMS (δ_H 0) and $CDCl_3$ (δ_C 77.0). EIMS and FABMS were recorded on a Fisons Autospec Q instrument.

Animal Material. The brown sponge was collected from Nosy Be, Madagascar, 13°, 34′, 709″ south and 47°, 117′, 729″ east, by scuba at a depth of 10−15 m (May 2003). A voucher specimen (identified by Prof. J. Vacellet) is deposited at the University of La Reunion under the collection number AM 1285.

Extraction and Isolation. The freeze-dried sponge (5 g) was extracted with EtOAc/MeOH (1:1) to give, after evaporation, a brown gum (600 mg). The gum was partitioned between aqueous methanol, *n*-hexane, and $CHCl_3$. The *n*-hexane fraction (250 mg) was subjected to silica gel vacuum liquid chromatography eluting with *n*-hexane to pure EtOAc to produce, according to increasing polarity, zaatirine (**6**) (6 mg), barekoxide (**8**) (10 mg), chelodane (**7**) (8 mg), nosyberkol (**9**) (6 mg), and asmarine F (**2**) (20 mg). Compounds **7** and **9** were separated by chromatography on 2% $AgNO_3$-impregnated silica gel eluted with 3% ethyl acetate in *n*-hexane.

The $CHCl_3$ fraction (260 mg) was chromatographed repeatedly on a Sephadex LH-20 column eluted with *n*-hexane/$CHCl_3$/MeOH (2:1:1) to yield asmarine A (**1**) (25 mg), asmarine I (**3**) (6 mg), asmarine J (**4**) (1 mg), and asmarine K (**5**) (1 mg).

Asmarine I (3): oil; $[\alpha]^{25}_D$ +24° (*c* 0.6, MeOH); IR (neat) ν_{max} 2935, 1595, 1532, 1460, 1387 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz)[10] δ 8.34 (1H, s, H-2′), 8.21 (1H, s, H-8′), 4.45 (1H, m, H-15a), 4.38 (1H, m, H-15b), 2.48 (1H, ddd, 2.5, 9.5, 15.7 Hz, 11a), 2.43 (1H, ddd, 2.5, 9.5, 15.7 Hz, 11b), 1.57 (3H, s, Me-16), 0.95 (3H, s, Me-19), 0.91d (3H, *J* = 6.5 Hz, Me-17), 0.89 (3H, s, Me-20), 0.43 (1H, d, *J* = 4.2 Hz, H-18a), 0.05 (1H, d, *J* = 4.2 Hz, H-18b); for ^{13}C NMR, see Table 1; CIMS *m/z* 424 [MH^+] (65) 408 (100), 183 (50); HREIMS *m/z* 423.2996 (calcd for $C_{25}H_{37}N_5O$, 423.2990).

Asmarine J (4): oil; $[\alpha]^{25}_D$ +20° (*c* 0.6, MeOH); IR (neat) ν_{max} 2930, 1590, 1460 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz)[10] δ 8.31 (1H, s, H-2′), 8.26 (1H, s, H-8′), 4.59 (2H, brs, H-15), 1.55 (3H, s, Me-16), 1.02 (3H, s, Me-19), 0.98 (3H, d, *J* = 6.5 Hz, Me-17), 0.95 (3H, s, Me-20), 0.51 (1H, d, *J* = 4.2, H-18a), 0.01 (1H, d, *J* = 4.2 Hz, H-18b); for ^{13}C NMR, see Table 1; EIMS *m/z* 407 [M^+] (30) 188 (100); HREIMS *m/z* 407.3038 (calcd for $C_{25}H_{37}N_5$, 407.3041).

Asmarine K (5): oil; $[\alpha]^{25}_D$ +28° (*c* 0.5, MeOH); IR (neat) ν_{max} 2930, 1595, 1530, 1460 cm^{-1}; ^{1}H NMR ($CHCl_3$, 500 MHz)[10] δ 8.30 (1H, s, H-2′), 8.05 (1H, s, H-8′), 4.70 (2H, s, H-18), 4.35 (2H, m, H-15), 1.40 (3H, s, Me-16), 1.15 (3H, s, Me-19), 0.87 (3H, s, Me-20), 0.69 (3H, d, *J* = 6.7, Me-17); for ^{13}C NMR, see Table 1; EIMS *m/z* 407 [M^+] (50) 392 (10), 216 (10), 188 (100); HREIMS *m/z* 407.3043 (calcd for $C_{25}H_{37}N_5$, 407.3041).

Nosyberkol (9): $[\alpha]^{25}_{D}$ +16.8° (*c* 0.25, $CHCl_3$); IR (neat) ν_{max} 3600, 3480, 2900, 1620, 1430, 1330 cm^{-1}; for ^1H and ^{13}C NMR, see Table 2; EIMS *m/z* 291 [MH^+] (20), 272(5); HREIMS *m/z* 291.2683 (calcd for $C_{20}H_{35}O$, 291.2679).

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