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Asmarines A-C; Three novel cytotoxic metabolites from the marine sponge *Raspailia* sp.

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Abstract: Three novel nitrogen-containing metabolites, asmarines A-C (**1** - **3**) together with two known diterpenes, zaatirin and chelodane were isolated from the Red Sea sponge *Raspailia* sp. collected in the Dahlak archipelago, Eritrea. The structure of the three new compounds and the known zaatirin and chelodane were established by spectroscopic analysis including X-ray diffraction analysis for asmarine A. Asmarines A-C are cytotoxic to a few human cancer cells. © 1998 Elsevier Science Ltd. All rights reserved.

In connection with our long-standing interest in the chemistry of marine sponges, we investigated the Red Sea sponge *Raspailia* sp. (Demospongiae, Order Poecilosclerida, Family Raspailiidae)[1] collected in the Dahlak archipelago, Eritrea, the Red Sea. In the process of normal chemical examination, we isolated from the sponge five compounds, two previously isolated by ourselves diterpenoids (zaatirin and chelodane[2]) and three new metabolites designated asmarines A-C. The new compounds possess significant cytotoxicity against a variety of cancer cells.

Freshly collected *Raspailia* sp. was frozen on site and kept frozen until needed. Freeze-dried sponge tissue (20g. dry wt.) was extracted sequentially with hexane and ethyl acetate to give a brown gum (1.2g) after evaporation. The latter extract was subsequently partitioned between aqueous methanol and hexane, CCl_4 and $CHCl_3$, and the two latter extracts were fractionated by chromatography on Sephadex LH-20 (eluting with $CHCl_3$; MeOH, 1:1) to give zaatirin, chelodane[2] and asmarines A-C (**1** - **3**) (10, 10, 90, 90 and 4 mg, respectively).

Asmarine A (**1**)[3] was isolated as needle shaped crystals from MeOH and was analyzed for $C_{25}H_{37}N_5O$ by HREIMS[3] and NMR spectral methods (Table 1), indicating 10 units of unsaturation. The ^{13}C NMR ($CDCl_3$) spectrum of **1** are consistent with the following functional groups: (a) a purine heterocycle (δ_C 151.7d, 149.0s, 109.3s, 158.7s and 143.1d for C-2', 4', 5', 6' and 8', respectively), (b) a decalin (δ_C 21.8t, 28.6t, 33.2t, 160.6s, 40.1s, 37.2t, 27.4t, 36.7d, 39.3s and 48.6d for C-1÷10, respectively) substituted by three methyl groups (δ_C 15.9q, 20.1q

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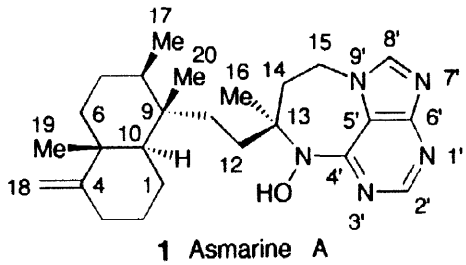
and 18.3q, C-17, 19, 20) and an external methylene (δ_C 102.5t, C-18), (c) an ethylene bridge between two quaternary carbon atoms (δ_C 31.2t and 33.0t) and (d) a $>NCH_2CH_2C(CH_3)N<$ unit (δ_C 42.3t, 36.7t, 64.0s and 21.7q, respectively). As the purine and the substituted decalin count for nine degrees of unsaturation, asmarine A has to possess another ring. 2D NMR spectra, i.e. COSY, TOCSY, HMQC and HMBC experiments (Table 1) revealed, unequivocally, the connection between all the above functionalities leaving one OH group and one C-N bond to be accounted for. Methylation of **1** with MeI under basic conditions[4] resulted in a dimethyl derivative (**4**) in which one incoming methyl group was on N-9' (δ_C 37.1q) and the second was on an oxygen (δ_C 66.0q). The outstanding lowfield of the latter OMe group pointed clearly to a $>NOCH_3$ group, suggesting for **1** a secondary hydroxylamine functionality which closes the missing ring, between C-13 and C-4' (a sp^2 singlet which required an additional substituent). Asmarine A comprises a diterpene, a purine heterocycle and a new N(OH)azacycloheptane ring which is condensed to the purine. To the best of our knowledge, asmarine A possesses an unprecedented pentaazatricyclic ring system. The relative stereochemistry of the diterpene portion of asmarine A was readily established by a NOESY NMR experiment. Thus e.g. correlations between CH_3's 19 and 20 established the *trans* ring junction of the decalin as well as the relative stereochemistry of C-9.

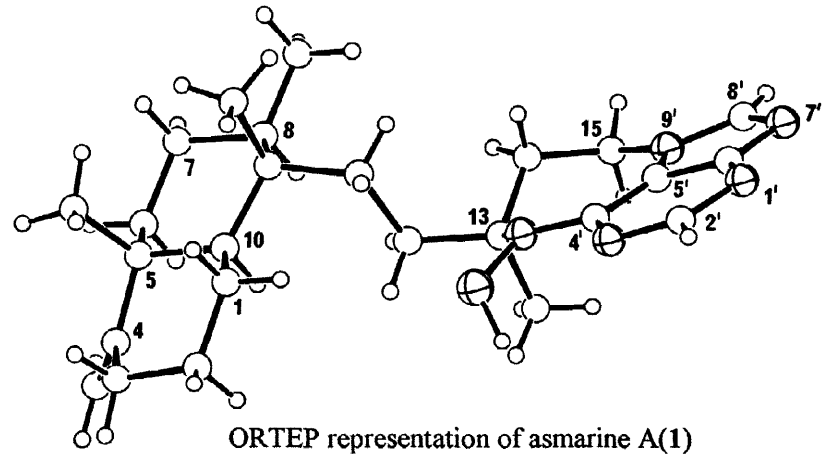
Comparison of the carbon chemical shifts of the diterpene part of **1** with those of chelodane,[2] *vide supra,* (Table 1) proved unequivocally, the identity of the two structures, thus, establishing the relative configurations of four out of the five chiral centers of **1**. Due to conformational mobility around the C-11,12 ethylene bridge, it was impossible to determine the relative stereochemistry of the fifth chiral center, C-13. The latter unresolved feature was elucidated by an X-ray diffraction analysis[5] which also confirmed the entire suggested structure of **1**. The structure was solved by direct methods (SHELXS-86)[6] and refined by full-matrix least-squares (SHELXL-97).[7] Non-hydrogen atoms were treated anisotropically. All the hydrogen atoms could be located in difference-Fourier maps. A total of 3481 unique reflections were obtained after data reduction with Friedel opposites not merged. The final refinement, based on F^2, converged at R=0.057 for 2166 observations having $F_0 > 4\sigma(F_0)$ and R=0.166 for 3481 unique data. At convergence, S=0.93 and $|\Delta\rho| \leq 0.20$ e.Å^{-3}.

Table 1. ^{1}H (500MHz) and ^{13}C (125 MHz) NMR Data of Asmarine A (**1**, in $CDCl_3$)

No.	^{13}C	^{13}C[a]	HMQC	HMBC (H to C)	COSY	No.	^{13}C	HMQC	HMBC (H to C)	COSY (H to H)
1	21.8 t	21.8 t	1.70 d	2,3,10	1b,10	13	64.2 s			
			1.45	2,3,5,10	1a,10	14	36.7 t	2.50 dt	13,15,16	14b,15a
2	28.6 t	28.9 t	1.85 brd	1,3	3a,2b			2.15 dd	12,13,15	14a,15b
			1.21	1,3,10	3a,b,2a	15	42.3 t	4.25 dt	13,14,5'	14a,15b
3	33.2 t	33.2 t	2.25 dt	2,4,5,18	2a,b,3b			4.20 dd		14b,15a
			2.05 dd	1,2,4,5,18	2b,3a	16	21.8 q	1.44 s	11,12,13,14	
4	160.6 s	160.6 s				17	15.9 q	0.70 d	7,8,9	8
5	40.1 s	40.0 s				18	102.5 t	4.60 s	3,4,5,	3a
6	37.2 t	37.2 t	1.50 (2H)	5,7,10,19	7a,b	19	20.1 q	1.00 s	4,5,6,10	
7	27.4 t	27.9 t	1.45 (2H)	5,6,8	6a,b	20	18.3 q	0.65 s	8,9,10,11	
8	36.7 d	36.6 d	1.37	7,9,10	7a,b,18	2'	151.7 d	8.50 s	4',5',6'	
9	39.3 s	39.2 s				4'	149.0 s			
10	48.6 d	48.6 d	1.05 d	1,5,9,20	1b	5'	109.3 s			
11	31.2 t	32.2 t	1.55 dt	9,10,12,20	11b,12a	6'	158.7 s			
			1.25	9,10,12,20	11a,12a	8'	143.1 d	7.95 s	4',5',6'	
12	33.0 t	35.6 t	1.95 dt	11,13,14,16	11a,b,12b	[a] ^{13}C values of the corresponding carbons in chelodane[2].				
			1.43		12a					

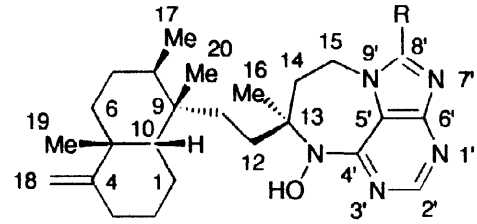


1 Asmarine A



ORTEP representation of asmarine A(1)

The second new compound, asmarine B (**2**), analyzed also for $C_{25}H_{37}N_5O$, from the EIMS *m/z* 423 [M^+] and NMR data.[8] The very similar spectral data of **2** and **1** pointed clearly to a stereoisomer of **1**. 2D NMR experiments suggested that asmarine B possesses the same tricyclic heterocyclic ring system as **1** but differs in the decalin portion. From the latter experiments, it was evident that the planar structure of the decalin of **2** is identical to that of asmarine A and that there is a change in the stereochemistry. The relative stereochemistry was established by d-NOE and NOESY NMR experiments. A correlation between CH_3-19 (1.10 s) and H-10 (1.30 m) pointed clearly to a *cis*-ring junction of the decalin in **2** rather than *trans* in **1** and an enhancement between H-18 (4.70 s) and CH_3-20 (0.80 s) established the stereochemistry of C-9. Moreover, the latter enhancement established the conformation of the *cis* decalin system in **2** i.e. H-10β being axial towards ring B, the trimethylated ring, and equatorial towards ring A (and CH_3-19 *vice versa*). The suggested *cis* decalin system is in full agreement with the ^{13}C chemical shifts of the corresponding portion in popolohuanone F,[9] most characteristic being the 6ppm downfield shift of C-4 and 13ppm upfield shift of CH_3-19, in asmarine B and popolohuanone as compared to the corresponding resonances in asmarine A. That the stereochemistry of C-13 in **2** is the same as in **1** was evident from the same δ_C 64ppm value in both, in contrast to δ_C 58.2 found for the different stereochemistry, see below.



2 Asmarine B R=H
3 Asmarine C R=Me 13-epi

The third new compound isolated, in minute amounts, from the sponge was asmarine C (**3**).[10] Asmarine C (**3**) analysed for $C_{26}H_{39}N_5O$ (*m/z* 437, M^+) - a higher homolog of **1** and **2**. From the NMR data it was evident that compound **3** possesses an 8'-methylpurine system. A suggestion that was also in full agreement with the *m/z* 218 $[C_{10}H_{12}N_5O]^+$ ion in the mass spectrum (cleavage of the C12,13 bond, α to a nitrogen). The ^{13}C resonances also determined the *cis*-chelodane system for **3** as in **2**.. Another difference between **3** and **2** was the chemical shift of C-13, 58.2 ppm in **3** against 64.9 ppm in **2**, suggesting a 13S* configuration instead of the 13R* configuration of asmarines A and B.

Asmarines A and B (**1** and **2**) have been found to have cytotoxic activity.[10] The activity against cell cultures of P-388 murine leukemia, A-549 human lung carcinoma. HT-29

human colon carcinoma and MEL-28 human melanoma are shown in Table 2.

Table 2 Antitumor activity of asmarines A & B (IC50 μM)[11]

Compound	P-388	A-549	HT-29	MEL-28
Asmarin A (**1**)	1.18	1.18	1.18	1.18
Asmarin B (**2**)	0.24	0.12	0.12	0.24

From this table it can be seen that **2** is more active than **1** and shows higher activity against the human lung and human colon carcinoma.

The asmarines are closest in structure to the *Agelas* 9-methyladeninium-7-diterpenoids.[12] However, they possess a new heterocycle which includes a secondary hydroxyamine and are not quaternary salts.

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References and Notes

1. A globular, soft, prickly looking, mustard coloured sponge; which seems to be an unidentified sp.
2. Rudi, A.; Kashman, Y. *J. Nat. Prod.*, **1992**, *55*, 1408-1414.
3. **1**: mp 232°; $[\alpha_D]$ +55° (c = 0.5 $CHCl_3$); ν_{max}^{KBr} 3400,2928,1600,1553,1451,1400,1388,900 cm^{-1}; HREIMS *m/z* 423.2999, clacd. 423.2998.
4. Asmarine A (**1**) (6mg) with K_2CO_3 (5mg) in acetone (2ml) was treated with CH_3I (2 drops) to give after 18h the $NOCH_3$, $(N\text{-}9')CH_3$ derivative(**4**). δ 66.0 q; 37.1 q and δ_H 4.22 s and 4.08 s, respectively.
5. X-ray diffraction measurements were carried out at ca. 293 K on a KappaCCD diffractometer system, using MoKα (λ = 0.7107 Å) radiation. A sphere of 47,576 significant data out to $2\theta = 50^0$ was collected during *ca.* 13h via 0.6° ϕ scans. No corrections for absorption and secondary extinction effects were applied.
   Crystal data: $C_{25}H_{37}N_5O$, formula weight 423.6, orthorhombic, space group $P2_12_12_1$, *a* = 6.913(1), *b* = 7.595(1), *c* = 44.337(1) Å, V = 2327.88Å^3, Z=2, D_{calc} = 1.209 g.cm^{-3}, *F*(000) = 920, μ(MoKα) = 0.76 cm^{-1}, crystal size 0.03x0.20x0.20 mm.
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8. Asmarine B (**2**), an oil; $[\alpha_D]$ +60° (c = 0.5 $CHCl_3$); ν_{max}^{neat} 3400,2927,1606,1553,1451,1404,1388,900 cm^{-1}; δ_C 21.2 t, 24.1 t, 31.6 t, 153.6 s, 39.3 s, 38.1 t, 27.2 t, 38.1 d, 40.5 d, 46.6 d, 31.1 t, 31.6 t, 64.9 s, 36.4 t, 42.3 t, 23.1 q, 15.8 q, 105.7 t, 32.9 q, 19.9 q (C-1÷20), 151.6 d, 149.6 s, 109.3 s, 158.4 s, 143.3 d (the purine carbons).
9. Carney, J.R.; Scheuer P.J.; *Tetrahedron Lett.*, **1993**, *34*, 3727-3730.
10. Asmarine C (**3**): an oil; δ_H 1.51 s, 0.75d, 1.15 s, 0.90 s, 3.53 s (3H each); δ_C 58.2 s, 144.3 d, 141.1 s, 109.5 s, 147.0 s (x2) and 26.8 q, - the other resonances as in **2**.
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