ORGANIC LETTERS

2003
Vol. 5, No. 14
2433−2435

Barrenazine A and B; Two New Cytotoxic Alkaloids from an Unidentified Tunicate

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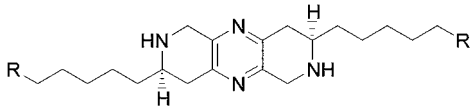
Received April 10, 2003

ABSTRACT



1 Barrenazine A R= CH_2CH_3
2 Barrenazine B R= $CH{=}CH_2$

Two novel compounds, barrenazine A (1) and B (2), were isolated from an unidentified tunicate collected at Barren Islands, Madagascar. The two new compounds are of an unprecedented heterocyclic skeleton, namely 1,3,4,6,8,9-hexahydrodipyridino[3,4-*b*:3′,4′-*e*]pyrazine. The structures of the two alkaloids were elucidated by interpretation of MS, COSY, HMQC, HMBC, NOESY, and ^{15}N-HMBC data. Barrenazine A exhibits mild cytotoxicity against LOVO-DOX colon carcinoma (with a GI_{50} value of 0.9 g/mL)

As part of our continuing program to discover bioactive compounds from marine invertebrates,[1,2] we isolated two new cytotoxic alkaloids, having an unprecedented 1,3,4,6,8,9-hexahydrodipyridino[3,4-*b*:3′,4′-*e*]pyrazine skeleton, designated as barrenazine A (**1**)[3] and B (**2**).[4] Barrenazine A is mildly cytotoxic to a few human cancer cells. In this paper we present the isolation and structure determination of these novel marine metabolites.

Pyrazine ring systems are not widely distributed in marine metabolites and are mostly restricted to a few distinct types of structures. The most prevalent of these are the bis steroidal pyrazines comprised of the anticancer cephalostatins[5] isolated from the African marine worm *Cephalodiscus gilchristi* and the cytotoxic ritterazines[6] isolated from the tunicate *Ritterella tokioka*. Other occurrences of the pyrazine ring include the antibiotic compounds, pelagiomicins,[7] isolated from the marine bactria *Pelagiobacte variabilis*, in which the pyrazine is part of a phenazine system; palythazin and isopalythazine which both consist of a hexahydrodipyranopyrazine,[8] isolated from the zoanthid *Palythoa tuberculosa*; as well as clavulazine,[9] a trihydropyranopyrazine metabolite isolated from the soft coral *Clavulardia viridis*. Other examples of compounds containing a pyrazine nucleus include botrylla-

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(1) Rudi, A.; Chill, L.; Kashman, Y. J. *Nat. Prod.* In press.
(2) Rudi, A.; Levi, S.; Benayahu, Y.; Kashman, Y. *J. Nat. Prod.* **2002**, *65*, 1672−1674.
(3) Barrenazine A (1): an oil; $[\alpha]^{21}_D$ −55.4 (*c* 0.48, MeOH); UV (MeOH) λ max 288 (ϵ 6950), 313 (sh) nm; for ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 387 [M + H] $^+$ (100), 287 (90); HRFABMS *m*/*z* 387.3476 (calcd for $C_{24}H_{43}N_4$, 387.3487).
(4) Barrenazine B (2): an oil; $[\alpha]^{21}_D$ −51.0 (*c* 0.26, MeOH); UV (MeOH) λ max 288 (ϵ 6950), 313 (sh) nm; for ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 383 [M + H] $^+$ (100), 285 (30); HRFABMS *m*/*z* 383.3103 (calcd for $C_{24}H_{39}N_4$, 383.3174).
(5) Pettit, G. R.; Tan, R.; Xu, J.; Ichihara, Y.; Williams, M. D.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 955−958.
(6) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1997**, *62*, 4484−4491.
(7) Imamura, N.; Nishijima, M.; Takadera, T.; Adachi, K.; Sakai, M.; Sano, H. *J. Antibiot.* **1997**, *50*, 8−12.
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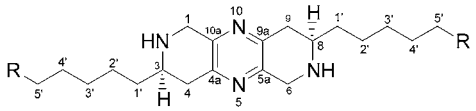
10.1021/ol034615n CCC: $25.00
Published on Web 06/11/2003

zine A and B that were isolated from the red ascidian *Botryllus leachi*.[10]

The unidentified tunicate was collected at Barren Islands (Ban de l'Albatros), North-West of Madagascar.[11] The tunicate (40 g) was homogenized and extracted with chloroform−methanol (2:1) to give a brown gum (1.3 g). The extract was subjected to partition by the method of Kupchan et al.[12] The chloroform fraction was repeatedly chromatographed on Sephadex LH-20 columns, eluting with heptane−$CHCl_3$−MeOH (2:1:1) to afford **1** (15 mg, 0.037% dry weight) and **2** (3 mg, 0.0075% dry weight), as well as additional compounds with other side chains. Whether the real source of these new compounds is the sponge or symbiotic microorganisms is unknown.



1 Barrenazine A R= CH_2CH_3
2 Barrenazine B R= $CH{=}CH_2$

The positive FAB mass spectrum of **1** exhibited a pseudomolecular ion $[MH]^+$ at *m*/*z* 387. The molecular formula $C_{24}H_{42}N_4$ was determined by HRMS. The ^{13}C NMR experiment revealed the presence of only 12 carbon atoms and thus it was deduced that **1** is of a symmetrical structure. A partial structure of barrenazine A could be constructed from HMQC, COSY, HMBC (Figure 1), and HSQC-TOCSY

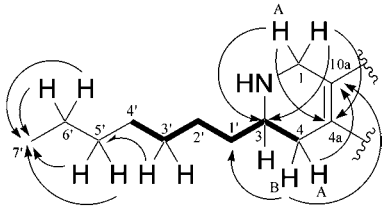


Figure 1. Key: HMBC (arrow with full arrowhead), COSY(—), and HSQC-TCSY (arrow with half arrowhead) correlations in barrenazine A.

data. A set of sequential COSY correlations was observed for protons H-4 through H-4′. HMBC correlations between C-7′ and both H-6′, H-5′, as well as a correlation in the HSQC-TOCSY experiment between C-5′ and H-3′, allowed the assignment of the C-4 to C-7′ segment. $^3J_{CH}$ couplings were observed between C-4a and both H-1A,1B, between C-10a and H-4A,4B, as well as between C-3 and H-1A,1B. Both C-3 and C-1 were positioned downfield (δ_C 44.8, 52.5 ppm) suggesting the presence of a tetrahydropyridino moiety.

This partial structure accounts for all the carbon and hydrogen resonances observed but only for one nitrogen atom. The UV spectrum of **1** showed an absorption at 288 nm (ϵ 6950) suggesting the presence of a pyrazine ring,[13] which was in agreement with the two ^{13}C NMR singnals of C-4a and C-10a (144.9, 147.5 ppm).[14] On the basis of this result and the symmetric nature of compound **1**, two possible symmetric structures (**I** and **II**) that satisfy the molecular formula could be suggested (Figure 2). The actual orientation

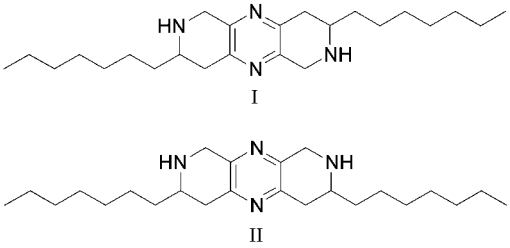


Figure 2. Two possible symmetric combinations that could apply for the structure of barrenazine A.

of the two 3-piperideines units with respect to the pyrazine ring could be determined on the basis of ^{15}NH-HMBC experiment data. In the case of structure **I**, the ^{15}NH-HMBC experiment was expected to give rise to NH correlations of two different nitrogen atoms, namely the nitrogen atoms of the pyrazine ring and the secondary amine moiety. In the case of structure **II**, however, the ^{15}NH-HMBC experiment should give rise to NH correlations due to three different nitrogen atoms: two from the pyrazine ring and one from the amine moiety. ^{15}NH-HMBC experiments were carried out with various delay times (60, 90, 120, and 150 ms). The best results were obtained when the delay was set to 120 ms and $^3J_{NH}$ correlations were observed between H-1B(6B), H-4A,4B(9A,9B) and a single nitrogen atom resonating at 326 ppm (Figure 3) which is consistent with structure **I**. An

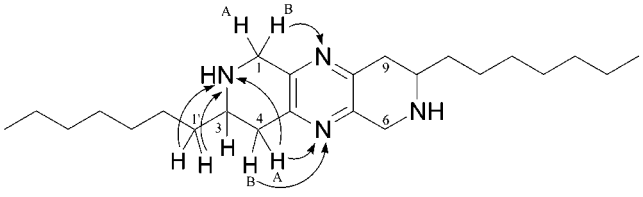


Figure 3. ^{15}N-HMBC correlations in **1**.

additional set of important correlations observed in the ^{15}NH-HMBC spectrum between H-4A,4B(9A,9B), H-1′A,1′B and

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(11) The tunicate was collected at Barren Islands (Ban de l'Albatros) North-West of Madagascar (18°, 17′, 260″ south; 43°, 41′, 200″ east) on 5/2001 by scuba at a depth of 18 m. The tunicate is a massive colonial tunicate in orange balls. The colony of the tunicate is at rest with the state of the stolons. It is impossible to determine whether it is Polyclinidae or Polycitoridae. A voucher specimen is deposited at Museum d'Histoire Naturelle de Paris (voucher no. AM-737).

(12) Kupchan, S. M.; Komoda, Y.; Branfman, A. R.; Sneden, A. T.; Court, W. A.; Thomas, G. J.; Hintz, H. P. J.; Smith, R. M.; Karim, A.; Howie, G. A.; Verma, A. K.; Nagao, Y.; Dailey, R. G., Jr.; Zimmerly, V. A.; Summer, W. C., Jr. *J. Org. Chem.* **1977**, *42*, 2349−2357.

a nitrogen atom resonating at 49 ppm further support the structure suggested for barrenazine A.

Two diastereomers of **1**, both chemically and magnetically symmetric, depicted in Figure 4, agree with the above data

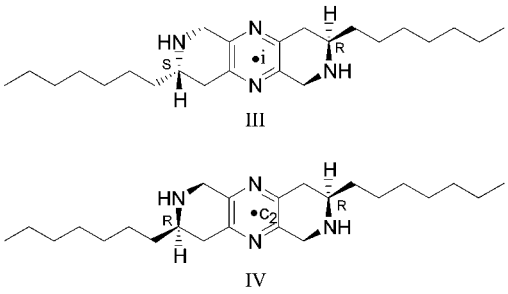

Figure 4. Two possible diastereomers of **1**.

and can be distinguished only by their optical properties. **III**, the *S**,*R** diastereomer, possesses a point of symmetry and thus should not display any optical activity while **IV**, the *R**,*R** diastereomer, exhibits C_2 symmetry and should display optical activity. Since **1** displayed optical activity $[\alpha]^{21}_{D}$ −55 (and CD activity), it was established that the relative stereochemistry is *R**,*R**.

Generally, a half-chair is the predominant conformation for the piperideine ring.[15] The signal of proton H-4B(9B) is a double-doublet presenting 18 Hz, 12 Hz couplings, due to coupling to H-4A and H-3 (and H-9B to H-9A and H-8). Therefore, both H-4B(9B) and H-3(8) must be quasi-axial and the two C-1′−C-7′ heptyl chains must be quasi-equatorial. NOE correlations between H-3 and both H-4A and H-1A (and between H-3 and H-9A, H-6B) further corroborate this result. Moreover, Barrenazine A was converted to its tetramethyl derivative (**3**).[16] From the ^{1}H NMR, ^{13}C NMR, and 2D experiments it became evident that two equivalent methyl groups were quasi-equatorial (δ_H 3.3 ppm, δ_C 52 ppm) and two other equivalent methyl groups were quasi-axial (δ_H 3.0 ppm, δ_C 44 ppm). The large upfield shift of the qausi-axial methyl groups resulted from γ effects by carbons C-10a(5a) and C-4(9). In a selective NOE experiment, a positive NOE enhancement was observed for the quasi-equatorial methyl group upon irradiation of the H-3/8 protons, which were earlier determined to be in a quasi-axial position.

The positive mass spectrum of **2** exhibited a molecular ion $[MH]^+$ at *m*/*z* 383. The molecular formula, $C_{24}H_{38}N_4$, was determined by HRMS. The NMR data of **2** indicated it to be identical to **1** except for the presence of a terminal double bond (δ_H 5.32, 5.28, 5.76 ppm, δ_C 114.8, 138.7 ppm) in both chains of **2** while in **1** both chains are saturated.

Other similar compounds of the same heterocyclic skeleton but with different side chains are undoubtedly present in the tunicate. So far we were not able to separate mixtures of those compounds (some of which are isomers of barrenazine B with the double bond located at different sites of the heptyl chains).

Barrenazine A has been found to exhibit mild cytotoxicity against LOVO-DOX colon carcinoma with a GI_{50} value of 0.900 g/mL. A mixture of Barrenazine A and the above-mentioned similar compounds also exhibited mild cytotoxicity against LN-caP prostate carcinoma and K-562 Leukemia carcinoma with GI_{50} values of 0.594 and 0.180 g/mL, respectively. It is of note that the structurally related tetrahydropyridinopyrazine derivatives exhibit a variety of biological activities, i.e., inhibitory activity against various metalloproteinases[17] and against keratinocyte growth[18] and an inhibitory effect on TNF-a converting enzyme production[19] and on matrix metalloproteinases (MMP).[20] These tetrahydropyridinopyrazines were also shown to be agonists of human melanocortin receptors[21] and of interleukin 1 and therefore useful for the treatment of immunoinflamatory conditions.[22] Therefore, synthesis of analogues with different side chains is in progress.

Acknowledgment. We thank PharmaMar, Madrid, for financial support and for the biological evaluation of the barrenazines.

Supporting Information Available: General experimental procedures and NMR data (^{1}H NMR, ^{13}C NMR, COSY, and HMBC) for barrenazines A and B. This material is available free of charge via the Internet at http://pubs.acs.org.

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