Pergamon

Tetrahedron Letters, Vol. 38, No. 48, pp. 8349-8350, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)10260-X

Poecillanosine, a New Free Radical Scavenger from the Marine Sponge *Poecillastra* spec. aff. *tenuilaminaris*

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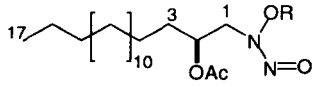
Abstract: A new free radical scavenger, poecillanosine (**1**) was isolated from the marine sponge *Poecillastra* spec. aff. *tenuilaminaris* and its structure elucidated as a nitrosohydroxyalkylamine.

Peroxy radicals are believed to cause a variety of age-related diseases including atherosclerosis, arthritis, ischemia, brain disorders, and cancer.[1] Radical scavengers are therefore potential drug leads for these diseases. While screening for inhibitors of lipid peroxidation using rat brain homogenate,[2] we found significant activity in the lipophilic extract of the marine sponge *Poecillastra* spec. aff. *tenuilaminaris* collected off the Sada Peninsula, 1,000 km west of Tokyo.[3] Bioassay-guided isolation afforded an active substance named poecillanosine (**1**). Its structure was elucidated as a nitrosohydroxalkylamine by spectral analysis of its methyl ether **2**.

The lyophilized, powdered sample (50 g) was extracted with $CHCl_3$/MeOH (1:1) and MeOH, and the combined extracts were partitioned between EtOAc and H_2O. The organic phase was further partitioned between hexane and 90% MeOH. The active hexane layer was separatedted on silica gel with $CHCl_3$/MeOH and on Sephadex LH-20 with MeOH, followed by HPLC on ODS with MeOH to afford poecillanosine (**1**) as a colorless solid (6.5 mg).[4]

Poecillanosine (**1**) was optically active, $[\alpha]^{25}_D$ -20.2° (*c* 0.1, MeOH), and showed a UV maximum at 232 nm (ε 5700) which shifted to 250 nm (ε 7300) upon addition of 0.5 M NaOH. The ^1H and ^{13}C NMR spectra exhibited signals for a terminal methyl, a long methylene chain, a deshielded methine, a methylene



1: R=H
2: R=Me

adjacent to a hetero atom, and an acetoxy group.[4] These spectral features are reminiscent of a nitrosohydroxyalkylamine.[5-7] Since mass spectral data provided no useful information of the molecular weight and since many NMR peaks were doubled, we converted **1** into its more stable methyl ether **2** with CH_2N_2.

Methyl ether **2**[8] had a molecular formula of $C_{20}H_{40}N_2O_4$ as established by HRFABMS (MH^+, *m/z* 373.3063, Δ mmu -0.3). The presence of a methoxynitrosoamino moiety was inferred from a base peak at *m/z* 297.2759 corresponding to a composition of $C_{19}H_{37}O_2$ (Δ mmu -3.5). The ^1H and ^{13}C NMR and HMQC data revealed the presence of $CH_3(CH_2)_n$-, -CH(OAc)-, $-CH_2N=$, and -OMe. Decoupling experiments were consistent with the connectivity from C1 to C3 which was also substantiated by HMBC crosspeaks between 4.12, 4.17/70.0 ppm. Thus **1** is 1-(*N*-nitrosohydroxylamino)-2-acetoxyheptadecane, which was also supported by a negative FABMS (*m/z* 357, 327, 253). Doubled NMR signals are probably due to the presence of tautomers.[5] Finally, stereochemistry of C2 was determined to be *R* by the Horeau method.[9]

Poecillanosine (**1**) not only inhibited lipid peroxidation of rat brain homogenate with an IC_{50} value of 0.04 μM, but also showed cytotoxicity against P388 murine leukemia cells (IC_{50} 1.8 μg/mL). Nitroso-hydroxylamino compounds are a rare class of natural products, previously unknown from marine organisms; only two antibiotics, alanosine [6] and nitrosofungin/propanosine [7] are known from *Streptomyces* spp.

Acknowledgment: We thank Professor Paul J. Scheuer, University of Hawaii for reading the manuscript. Thanks are also due to Dr. Rob van Soest, Institute of Systematics and Population Biology, University of Amsterdam, for identification of the sponge, to the crew of R/V Toyoshio-maru of Hiroshima University for assistance in collecting sponge samples, and to Ms. M. Totsuka for assistance in preparation of freeze-dried samples.

References and Notes

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3. Sponge samples were collected at a depth of 25 m off the Sada Peninsula, immediately frozen, and transported frozen to our laboratory. The sponge was identified as *Poecillastra* spec. aff. *tenuilaminaris* Sollas, 1888 by Dr. Rob W. M. van Soest, and a voucher specimen (ZMA POR. 10121) was deposited at Zoological Museum of the University of Amsterdam.
4. **1**: $[\alpha]^{25}_D$ -20.2° (*c* 1.0, MeOH); UV λ_{max} (MeOH) 232 nm (ε 5700), (H^+/MeOH) 230 (6200), (OH^-/MeOH) 250 (7300); IR (neat) ν_{max} 2920, 2850, 1740, 1460, 1375, 1220 cm^{-1}; ^1H NMR (major in $CDCl_3$) δ 0.88, 1.26, 1.61, 2.03, 2.27, 4.13, 5.43; ^{13}C NMR (major in $CDCl_3$) δ 14.3, 20.8, 22.8, 25.1, 29.7, 32.0, 61.7, 70.6, 171.5.
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8. **2**: $[\alpha]^{25}_D$ -3.0° (*c* 0.2, MeOH); UV (MeOH) λ_{max} 236 nm (ε 8200); IR (neat) ν_{max} 2920, 2850, 1740, 1500, 1460, 1375, 1220, 1055, 1010 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (3H, dt, *J*=6.1, 6.7, H-17), 1.25 (26H, b), 1.66 (2H, m, H-3), 2.06 (3H, s, OAc), 4.05 (3H, s, OMe), 4.12 (1H, dd, 12.8, 4.2, H-1a), 4.17 (1H, dd, 12.8, 7.3, H-1b), 5.37 (1H, m, H-2); ^{13}C NMR ($CDCl_3$) δ 14.1 q (C17), 20.85 q (OAc), 22.7 t (C16), 24.9 t (C4), 29.2 t, 29.3 t, 29.4 t, 29.5 t, 29.58 t, 29.62 t (2C), 29.65 t (3C), 31.7 t (C3), 31.9 t (C15), 61.3 q (OMe), 65.4 t (C1), 70.0 d (C2), 170.0 s (OAc); negative FABMS (diethanolamine/glycerol) *m/z* 371, 357 (base peak), 342, 327, 253.
9. Horeau, A. in *Stereochemistry, Fundamentals and Methods*; Kagan, H. B., Ed., G. Thieme, Stuttgart, 1977, Vol. 3, pp 51- 94. **2** (6.2 mg) was deacetylated with 1.0 M NaOMe/MeOH to afford 3.3 mg of deacetylated methyl ether after purification. This was treated with 2 eq of racemic 2-phenylbutanoic anhydride in pyridine; unreacted acid exhibited an optical rotation of $[\alpha]_D^{23}$ -1.36° (*c* 0.27, benzene).

(*Received in Japan* 14 *August* 1997; *revised* 24 *September* 1997; *accepted* 26 *September* 1997)