Perfragilins A and B, Cytotoxic Isoquinolinequinones from the Bryozoan Membranipora perfragilis

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J. Nat. Prod., **1993**, 56 (8), 1431-1433• DOI:
10.1021/np50098a032 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 8, pp. 1431-1433, August 1993

PERFRAGILINS A AND B, CYTOTOXIC ISOQUINOLINEQUINONES FROM THE BRYOZOAN *MEMBRANIPORA PERFRAGILIS*

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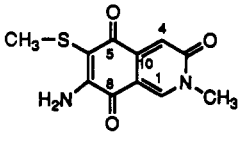
ABSTRACT.—Detailed spectroscopic and cytotoxicity data are reported for two new isoquinolinequinones, perfragilins A [**1**] and B [**2**], which were isolated from the bryozoan *Membranipora perfragilis* collected in South Australia. Both compounds, which are speculated to be of microbial origin, contain a relatively uncommon thiomethyl ether functionality.

The X-ray crystallographic structures of perfragilins A [**1**] and B [**2**], cytotoxic isoquinolinequinones isolated from the cheilostome bryozoan *Membranipora perfragilis* (MacGillivray), were recently reported (1,2). At the time of the original isolation, the available material was inadequate to obtain complete and reliable nmr data for these compounds. We have now isolated sufficient material for spectral analysis and report these data herein along with biological activity data. Both perfragilins A and B were toxic to murine leukemia cells (P388), with perfragilin B being considerably more potent: ED_{50} 0.8 and 0.07 μg/ml, respectively. No antiviral activity was observed for the perfragilins. ^{13}C-nmr assignments were made by HMQC and HMBC experiments.

Although the number of natural products described (3) from bryozoans to date is limited, it includes some very novel and biologically active compounds, for example, the family of macrolide bryostatins (4,5). Nearly all of the other reported bryozoan metabolites are alkaloids, mostly modified physostigmines, substituted indoles, and β-phenethylamines (3).

The isoquinoline and quinone features of perfragilins A and B are reminiscent of mimosamycin [**3**] (6,7). However, the thiomethyl ether substituents render **1** and **2** quite unique. Mimosamycin was reported initially (6,7) from the actinomycete *Streptomyces lavendulae* of terrestrial origin and more recently from several sponges, a *Reneira* sp. (8,9), *Xestospongia caycedoi* (10),



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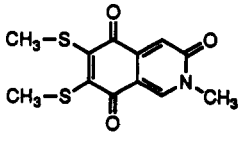

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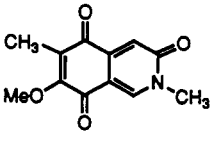

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Xestospongia sp. (11), and a nudibranch (11) that feeds on the last of these. The structural relationship of **1** and **2** to mimosamycin [**3**] prompts us to speculate that the new quinones are of bacterial origin.

EXPERIMENTAL

EXTRACTION AND ISOLATION.—The light orange, fragile, calcareous bryozoan *M. perfragilis* was collected by hand using SCUBA at Rapid Bay, South Australia, and then frozen. The organism was identified by Dr. A. Davis, University of Adelaide, and a voucher specimen is held at the University of Oklahoma, Chemistry Department (F.J.S.) 35-AU-87. The frozen specimens (2.5 kg wet wt) were crushed and soaked in MeOH (3 liters) for 24 h. The MeOH was decanted and the solid residue soaked in 3 liters of $CHCl_3$-MeOH (1:1) at room temperature three times, each for 24 h. The MeOH and combined $CHCl_3$-MeOH extracts were each evaporated under vacuum to give residues of 56.5 and 10.8 g, extracts A and B, respectively. Each of these residues was then triturated with 1 liter of $CHCl_3$, and the $CHCl_3$ extracts were combined and evaporated to give 3.57 g of $CHCl_3$-soluble extract (extract C, ED_{50}, 30 μg/ml vs. P388 cells). The $CHCl_3$-insoluble residues of extracts A and B were then triturated with MeOH, and the MeOH solution was evaporated to give 33 g of residue (extract D, ED_{50} 49 μg/ml vs. P388). A 3.8 g portion of extract D was chromatographed over Sephadex LH-20 (5.9×60 cm column) using MeOH (10-ml fractions). The material eluted from 170–240 ml was the most cytotoxic (ED_{50}, 3.2 μg/ml) and this was chromatographed again over Sephadex LH-20 using CH_2Cl_2-MeOH (4:1) (10-ml fractions). The material eluted from 70–130 ml was purified further by preparative SiO_2 tlc [$CHCl_2$-MeOH-2.8% aqueous NH_4OH (8:7:2)] to give 7 mg pure perfragilin A [**1**] corresponding to a 2×10^{-3}% yield based on wet wt. For processing additional quantities of extract D a simplified separation scheme was employed. The extract was first partitioned between $CHCl_3$ and H_2O, and the $CHCl_3$-soluble material was chromatographed by flash vacuum chromatography over Si gel using hexane-iPrOH (7:3) to give more perfragilin A [**1**] directly.

One gram of extract C was chromatographed over Sephadex LH-20 (150 g) using CH_2Cl_2-MeOH (2:1) as eluent (7-ml fractions). The material eluted from 136–240 ml showed the best level of cytotoxicity, and it was further resolved by SiO_2 preparative layer chromatography [$CHCl_3$-EtOAc-Et_2O (1:1:1)] to give 5 mg of pure perfragilin B [**2**] corresponding to 7×10^{-4}% yield based on wet wt. The last step of the purification could also be effected by normal phase hplc [hexane-iPrOH (8:2)] or RPC_{18} hplc [MeOH-H_2O (80:20)].

Perfragilin A [**1**].—Red needles: mp 219–220°; ir ν max (thin film) 3423, 3322, 1675 (m), 1645 (m), 1572 (vs) cm^{-1}; uv λ max (95% EtOH) 222 nm (12,616), 248 (7000), 330 (15,140), 362 (8785), 440 (2224); ^{1}H-nmr (CD_3OD) δ 2.26 (3H, s, S-Me), 3.64 (3H, s, N-Me), 6.98 (1H, s, H-4), 8.56 (1H, s, H-1); ^{13}C nmr (CD_3OD/$CDCl_3$) δ 16.86 (S-Me), 38.52 (N-Me), 112.61 (C-6, C-9), 116.64 (C-4), 142.76 (C-10), 144.89 (C-1), 155.54 (C-7), 165.37 (C-3), 176.83 (C-8), 178.84 (C-5); HMQC 2.26/16.86, 3.64/38.52, 6.99/116.6, 8.55/144.8; HMBC (4 Hz experiment) 2.27 (S-Me)/112.61 (C-6), 3.64 (N-Me)/165.37 (C-3), 144.88 (C-1), 6.99 (H-4)/178.84 (C-5), 165.83 (C-3), 142.76 (C-10), 122.61 (C-9), 8.55 (H-1)/176.84 (C-8), 165.83 (C-3), 142.78 (C-10), 112.61 (C-9), 38.52 (N-Me); lr fabms *m/z* (%) $[M+1]^+$ 251.1 (81), 221.2 (43), 147 (100).

Perfragilin B [**2**].—Red needles: mp 163°; ir ν max (thin film) 1670 (m), 1623 (s) cm^{-1}; uv λ max (EtOH) 214 nm (14,941), 235 (10,504), 332 (8227), 382 (5946), 465 (1848); ^{1}H nmr ($CDCl_3$) δ 2.67, 2.73 (3H×2, s, S-Me); 3.64 (3H, s, N-Me); 7.07 (1H, s, H-4), 8.23 (1H, s, H-1); ^{13}C nmr ($CDCl_3$) δ 18.14 (S-Me), 18.68 (S-Me), 38.42 (N-Me), 111.92 (C-9), 117.43 (C-4), 139.68 (C-10), 142.44 (C-1), 147.36 (C-6 or C-7), 150.68 (C-7 or C-6), 162.49 (C-3), 175.66 (C-8), 176.66 (C-5); HMQC 2.67/18.14, 2.73/18.68, 3.64/38.42, 7.07/117.43, 8.23/142.44; HMQC (results of 4 and 10 Hz experiments) 2.67/147.36 (C-6 or C-7), 2.73/150.68 (C-7 or C-6), 3.64/162.49 (C-3), 7.07/111.92 (C-9), 162.49 (C-3), 176.6 (C-5), 8.23/139.68 (C-10), 162.49 (C-3); lr eims *m/z* (%) $[M]^+$ 280.9 (28.6), $[M-15]^+$ 265.8 (100), 237.9 (15).

ACKNOWLEDGMENTS

This work was supported by Department of Commerce, NOAA, Office of Sea Grant Project NA86AA-D-SG074. We thank Dr. Andy Davis, University of Adelaide, for assistance in specimen collection and for specimen identification. We gratefully acknowledge NSF Grant CHE 8113507 and the University of Oklahoma Research Associates Fund for funds to purchase a high field nmr.

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Received 28 December 1992