Two New Brominated Tyrosine Derivatives from the Sponge Druinella (=Psammaplysilla) purpurea

D. Martin James, Holly B. Kunze, and D. John Faulkner

J. Nat. Prod., **1991**, 54 (4), 1137-1140• DOI:
10.1021/np50076a040 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 4, pp. 1137-1140, Jul-Aug 1991

TWO NEW BROMINATED TYROSINE DERIVATIVES FROM THE SPONGE *DRUINELLA* (=*PSAMMAPLYSILLA*) *PURPUREA*

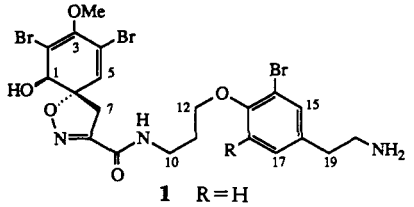
D. MARTIN JAMES, HOLLY B. KUNZE, and D. JOHN FAULKNER*

Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92093-0212

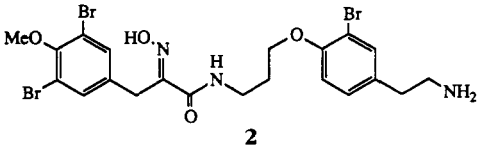
ABSTRACT.—Two new tyrosine-derived metabolites, 14-debromoaraplysillin I [**1**] and 14-debromoprearaplysillin I [**2**], together with the known compound araplysillin I [**3**], have been isolated from the sponge *Druinella* (=*Psammaplysilla*) *purpurea*.

Marine sponges of the order Verongida are characterized by their ability to synthesize brominated tyrosine derivatives, many of which possess antimicrobial activity. Chemical modification occurs both on the side chain and aromatic ring of the brominated tyrosine precursors, giving rise to a broad range of biosynthetically related compounds (1–5). In this paper we report the isolation and structural elucidation of two new antimicrobial metabolites, 14-debromoaraplysillin I [**1**] and its presumed biosynthetic precursor 14-debromoprearaplysillin I [**2**].

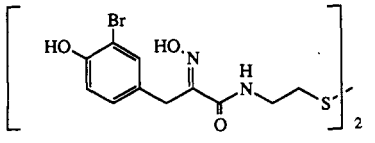
A specimen of *Druinella* (=*Psammaplysilla*) *purpurea* Carter (order Verongida, family Druinellae=Aplysinellidae) was collected by hand using scuba (−15 m) near Praslin, Seychelles. The MeOH extract of the lyophilized sponge showed moderate antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*. The MeOH extract was chromatographed on Sephadex LH-20 using CH_2Cl_2-MeOH (1:1) as eluent, and the active fractions were rechromatographed on Si gel. Araplysillin I [**3**] was isolated as one of the active constituents and identified by comparison of



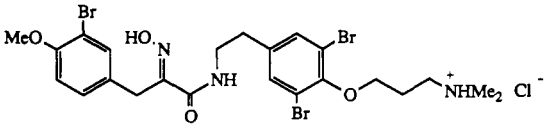
1 R=H
3 R=Br



2



4



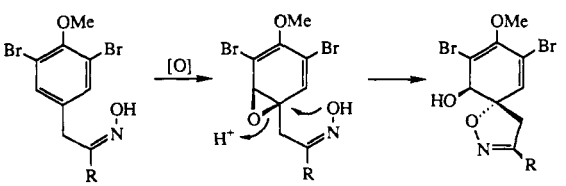
5

spectral data with literature values (6). 14-Debromoaraplysillin I [**1**] and 14-debromoprearaplysillin I [**2**] were also isolated from the active fraction and purified by hplc on an amino column using EtOAc-MeOH (9:1) as eluent. Both 14-debromoaraplysillin I [**1**] and 14-debromoprearaplysillin I [**2**] showed mild antimicrobial activity against *S. aureus* and *B. subtilis* at a loading of 100 μg/disk.

14-Debromoaraplysillin I [**1**] was obtained as a clear glass that colored on standing. The hrfabms of **1** contained a cluster of peaks at *m/z* 635.9360, 638, 640, and 642 for the molecular formula $C_{21}H_{24}Br_3N_3O_5$. The ^{1}H- and ^{13}C-nmr spectral data of **1** were very similar to those of araplysillin I [**3**], except that the ^{1}H-nmr spectrum of **1** contained signals due to a 1,2,4-trisubstituted benzene ring at δ 7.40 (d, 1H, J = 2 Hz), 7.09 (dd, 1H, J = 8.4, 2 Hz), 6.83 (d, 1H, J = 8.4 Hz) in place of the singlet ascribed to H-15 and H-17 in araplysillin [**3**]. These data showed good agreement with the spectral data reported for the monobrominated aromatic rings of psammaplin A [**4**] (7–9) and aplysamine 2 [**5**] (10). All other spectral data are in accord with a structure in which the bromine at C-14 (or C-18) in araplysillin I [**3**] is replaced by hydrogen.

14-Debromoprearaplysillin I [**2**] was isolated as a clear glass. The high resolution mass spectrum of [**2**] contained a molecular ion at *m/z* 619.9377 for the formula $C_{21}H_{24}Br_3N_3O_4$. The ^{1}H-nmr data for the C-10–C-20 portion of **2** were almost idential to those of **1**, but the signals assigned to the spirocyclooxazoline ring system of **1** were not present in **2** and were replaced by signals at δ 7.48 (s, 2H), 3.84 (s, 2H), and 3.82 (s, 3H), which were assigned to a 3,5-dibromo-4-methoxybenzyl moiety. However, comparison of the nmr spectral data of **2** with those of aplysamine 2 [**5**] (10) revealed that the position of the dibrominated and monobrominated aromatic rings could not be assured from these data alone, although biosynthetic considerations and the mass spectral fragmentation pattern strongly favored the proposed structure. The positions of the bromine atoms were confirmed by two NOEDS experiments (11); irradiation of the H-19 signal at δ 2.68 (t, 1H, J = 6.5 Hz) caused enhancements of the H-15 signal at 7.32 (d, 1H, J = 1.8 Hz) and the H-17 signal at 7.02 (dd, 1H, J = 8.4, 1.8 Hz), while irradiation of the H-1 and H-5 signals at 7.48 (s, 1H) caused an enhancement of the signals due to the H-7 methylene protons at 3.84 (s, 2H). The *E* geometry of the oxime was assigned on the basis of the chemical shift of the C-7 signal at δ 28.0 (t); the corresponding signal for the (*Z*)-oxime is found at >35 ppm (7).

The biosynthesis of the spirocyclic isoxazolines is thought to involve the intramolecular cyclization of the hydroxyl group of an oxime with an unstable intermediate arene oxide (12) as shown in Scheme 1. Spiroisoxazolines have been obtained by oxidation of benzyl oximes, although in low yields (13). The isolation of the isoxazole **1** and the oxime **2** from the same sponge is the first reported instance in which a spiroisoxazoline has been isolated together with its



SCHEME 1. Proposed biosynthetic pathway from the prearaplysillins to the araplysillins.

proposed precursor. These results lend further weight to the biosynthetic hypothesis shown in Scheme 1.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer. Uv spectra were recorded on a Perkin-Elmer Lambda 3B spectrophotometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a 10 cm microcell. ^{1}H-nmr spectra were recorded at 360 MHz ($\delta_{TMS}=0$) on a custom built instrument. ^{13}C-nmr spectra were recorded at 50 MHz ($\delta_{TMS}=0$) on a Bruker WP-200 spectrometer. Mass spectra were obtained from the UC Riverside regional facility. All solvents were distilled from glass prior to use.

COLLECTION, EXTRACTION AND ISOLATION PROCEDURES.—A specimen of *D. purpurea* (146 g dry wt) was collected by hand using scuba (−15 m) near Praslin, Seychelles. A voucher specimen (P1122) has been deposited in the SIO Benthic Invertebrate Collection. The sample was frozen upon collection and stored at −20° for just over 1 month. The frozen sample was lyophilized and exhaustively extracted with hexane, CH_2Cl_2, EtOAc, and MeOH successively. The MeOH extract, which exhibited antimicrobial activity, was chromatographed on Sephadex LH-20, eluting with CH_2Cl_2-MeOH (1:1). The antimicrobial factions were combined and chromatographed on Si gel using a solvent system consisting of CH_2Cl_2-MeOH-NH_4OH (87:12:1) to give araplysillin I [**3**] (1.9 g, 1.3% dry wt) and a second antimicrobial fractions that was further purified by hplc on an amino column, eluting with EtOAc-MeOH (9:1) to give 14-debromoaraplysillin I [**1**] (57 mg, 0.039% dry wt) and the oxime **2** (25 mg, 0.017% dry wt.)

14-DEBROMOARAPLYSILLIN I [**1**].—Glass: $[\alpha]_D$ +21° [c = 0.47, $CHCl_3$-MeOH (1:1)]; ir [$CHCl_3$-MeOH (1:1)] 3690, 3630, 3420, 3020, 2945, 1570, 1600, 1535, 1500, 1465, 1255, 1015 cm^{-1}; uv ($CHCl_3$) 240 nm (ε 23000), 287 nm (ε 16700); ^{1}H nmr ($CDCl_3$) δ 7.39 (d, 1H, J = 2 Hz, H-15), 7.09 (dd, 1H, J = 8.4, 2 Hz, H-17), 7.00 (br t, 1H, NH), 6.83 (d, 1H, J = 8.4 Hz, H-18), 6.30 (s, 1H, H-5), 4.32 (s, 1H, H-1), 4.12 (t, 2H, J = 5.7 Hz, H-12), 3.86 (d, 1H, J = 18.5 Hz, H-7), 3.74 (s, 3H, OMe), 3.55 (m, 2H, H-10), 2.95 (d, 1H, J = 18.5 Hz, H-7), 2.94 (t, 2H, J = 7.2 Hz, H-20), 2.67 (t, 2H, J = 6.8 Hz, H-19), 2.10 (m, 2H, H-11); ^{13}C nmr ($CDCl_3$) δ 159.4 (s, C-9), 154.0 (s, C-8), 153.4 (s, C-13), 147.7 (s, C-3), 133.4 (d, C-15), 134.4 (s, C-14), 131.1 (d, C-5), 128.8 (d, C-17), 121.1 (s, C-2), 113.4 (s, C-3), 113.3 (d, C-18), 112.2 (s, C-16), 91.7 (s, C-6), 73.4 (q, OMe), 73.4 (t, C-12), 60.1 (d, C-1), 42.9 (d, C-20), 38.9 (d, C-7), 38.0 (d, C-10), 37.3 (d, C-19), 28.9 (d, C-11); hrfabms *m/z* 635.9360 ($C_{21}H_{24}{}^{79}Br_3N_3O_5$ requires 635.9344).

14-DEBROMOPREARAPLYSILLIN I [**2**].—Glass: ir [$CHCl_3$-MeOH (1:1)] 3410, 3015, 2930, 1670, 1605, 1530, 1495, 1470, 1420 cm^{-1}; uv ($CHCl_3$) 240 nm (ε 6750), 280 nm (ε 3500); ^{1}H nmr ($CDCl_3$) δ 7.48 (s, 2H, H-1, H-5), 7.32 (d, 1H, J = 1.8 Hz, H-15), 7.10 (br t, 1H, J = 6.1 Hz, NH), 7.02 (dd, 1H, J = 8.3, 1.8 Hz, H-17), 6.78 (d, 1H, J = 8.6 Hz, H-18), 4.06 (t, 2H, J = 5.8 Hz, H-12), 3.84 (s, 2H, H-7), 3.82 (s, 3H, OMe), 3.60 (dt, 2H, J = 6.1, 5.8 Hz, H-10), 2.96 (t, 2H, J = 6.5 Hz, H-20), 2.68 (t, 2H, J = 6.5 Hz, H-19), 2.06 (m, 2H, H-11); ^{13}C nmr ($CDCl_3$) δ 163.4 (s, C-9), 153.8 (s, C-3), 152.4 (s, C-13), 150.9 (s, C-8), 135.6 (s, C-6), 133.4 (d, C-5 and C-1), 133.3 (d, C-15), 133.2 (s, C-18), 128.8 (d, C-17), 117.7 (s, C-2), 114.0 (d, C-14), 111.5 (s, C-16), 67.9 (t, C-12), 60.5 (q, OMe), 42.5 (t, C-21), 37.3 (t, C-10), 37.0 (t, C-17), 28.4 (t, C-11), 28.0 (t, C-7); eims *m/z* (intensity) 409 (13), 407 (33), 405 (13), 307 (20), 305 (45), 303 (23), 292 (18), 290 (29), 288 (16), 187 (40), 185 (54), 183 (38), 181 (32); hrfabms *m/z* 619.9377 ($C_{21}H_{24}{}^{79}Br_3N_3O_4$ requires 619.9395).

ACKNOWLEDGMENTS

The specimen of *D. purpurea* was identified by Mary Kay Harper. The research was supported by grants from the National Institutes of Health (CA49084) and the California Sea Grant College Program (R/MP-46) and by a Scripps Undergraduate Research Fellowship (to HBK).

LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **1**, 551 (1984).
2. D.J. Faulkner, *Nat. Prod. Rep.*, **3**, 1 (1986).
3. D.J. Faulkner, *Nat. Prod. Rep.*, **4**, 539 (1987).
4. D.J. Faulkner, *Nat. Prod. Rep.*, **5**, 613 (1988).
5. D.J. Faulkner, *Nat. Prod. Rep.*, **7**, 269 (1990) and references cited therein.
6. A. Longeon, M. Guyot, and J. Vacelet, *Experientia*, **46**, 548 (1990).
7. L. Arabshahi and F.J. Schmitz, *J. Org. Chem.*, **52**, 3584 (1987).
8. E. Quiñoá and P. Crews, *Tetrahedron Lett.*, **28**, 3229 (1987).
9. A.D. Rodriguez, R.K Akee, and P.J. Scheuer, *Tetrahedron Lett.*, **28**, 4989 (1987).
10. R. Xynas and R.J Capon, *Aust. J. Chem.*, **42**, 1427 (1989).
11. A.E. Derome, "Modern NMR Techniques

for Chemistry Research," Pergamon Press, Oxford, 1987, pp. 97–127.
12. R.J. Andersen and D.J. Faulkner, *Tetrahedron Lett.*, 1175 (1973).
13. K.T. Okamoto and J. Clardy, *Tetrahedron Lett.*, **28**, 4969 (1987).

Received 29 November 1990