Isolation and Structure Determination of Diazonamides A and B, Unusual Cytotoxic Metabolites from the Marine Ascidian *Diazona chinensis*

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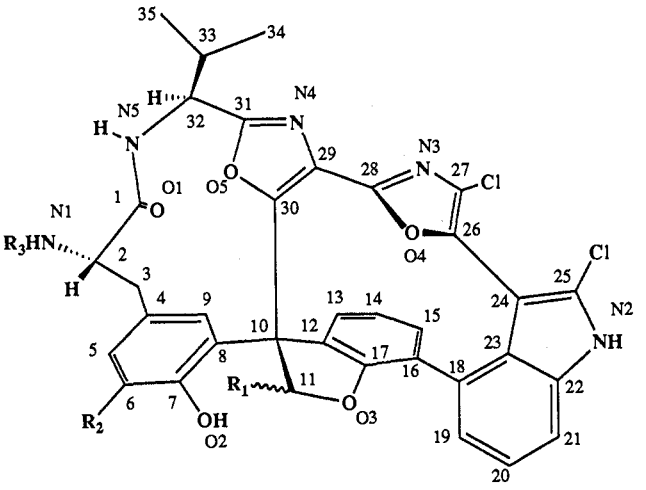
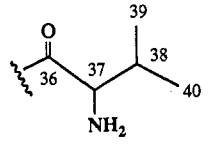
Ascidians are a rich source of unusual secondary metabolites with potent antimicrobial, cytotoxic, and antiviral activities.[2] Recent ecological studies with such diverse ascidian secondary metabolites as the tambjamine class alkaloids,[3] didemnin cyclic peptides,[4] and polyandrocarpidines A–D[4] have demonstrated that these pharmacologically active substances represent defensive adaptations by these soft-bodied organisms. We have investigated the secondary metabolites of the colonial ascidian *Diazona chinensis* (order Phlebobranchia)[5] collected from the ceilings of small caves along the northwest coast of Siquijor Island, Philippines. In this paper, we report the structures of diazonamides A (**1**) and B (**2**), two unusual halogenated cyclic peptides with potent in vitro cytotoxic activity.

The combined organic extract of the lyophilized ascidian (256.2 g dry weight) was partitioned between hexane and methanol, and the methanol-soluble material was further partitioned between butanol and water. Gel filtration of the butanol-soluble material on Sephadex LH-20 with methanol followed by reverse-phase HPLC (ODS-silica) using 9:1 methanol/water gave pure diazonamides A (**1**, 54 mg, 0.021% dry weight) and B (**2**, 132 mg, 0.052%).

A combination of ^{1}H NMR, ^{13}C NMR, and HRFABMS experiments for diazonamides A[6] and B[7] led to assignments for

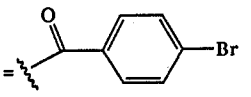


1 R_1 = OH, R_2 = H, R_3 =



2 R_1 = OH, R_2 = Br, R_3 = H

3 R_1 = O2 (acetal), R_2 = Br, R_3 =



several structural subunits, but the large number of unprotonated carbons and heteroatoms prevented connecting the fragments into a complete structure with ^{1}H–^{13}C correlation methods. We established the missing connectivities with a single-crystal X-ray diffraction analysis of the *p*-bromobenzamide derivative of diazonamide B (**3**) made by reacting **2** with *p*-bromobenzoyl chloride in pyridine.[8] Figure 1 shows the results of the crystal structure determination[9] of **3**, including the absolute stereochemistry.

A direct assignment of the diazonamide B (**2**) structure from the X-ray structure of derivative **3** was straightforward in all but one respect. Diazonamide B (**2**) was a hemiacetal at C11, on the basis of a 3.5-Hz coupling of a D_2O-exchangeable proton at δ 7.36 to a δ 6.46 proton that was one-bond coupled to the C11 resonance at δ 106.6 in the XHCORR spectrum. In the conversion of **2** to **3**, the C11 hemiacetal had been converted to an acetal. To assign

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(5) This ascidian was identified as *Diazona chinensis* (Tokioka, 1955) by Dr. Françoise Monniot, Museum National D'Histoire Naturelle Paris, France.

(6) Diazonamide A (**1**): clear glass; $[\alpha]_D$ (*c* 8.8, CH_3OH) −217.3°; UV (MeOH) 298 (sh), 290 (ϵ 5300), 280 (sh), 216 nm (19200); UV (MeOH + NaOH) 350 (sh), 296 (ϵ 4500), 204 nm (100900); IR (KBr) 3600–2800, 1675, 1610, 1570, 1455, 1410, 1305, 1215, 1170, 1150, 1120, 1065, 750 cm^{-1}; HRFABMS obsd ($M^+ + H - H_2O$) *m/z* 765.1998, $C_{40}H_{35}N_6O_6Cl_2$ requires 765.1999; ^{1}H NMR (MeOH-d_4, 360 MHz) δ 4.60 (1 H, dd, *J* = 11.7 and 3.3 Hz, 2-H), 3.38 (1 H, dd, *J* = 12.7 and 11.7, 3-H), 2.78 (1 H, dd, *J* = 12.7 and 3.3, 3-H), 7.19 (1 H, dd, *J* = 8.2 and 1.3, 5-H), 6.78 (1 H, d, *J* = 8.2, 6-H), 7.33 (1 H, d, *J* = 1.3, 9-H), 6.34 (1 H, s, 11-H), 7.00 (1 H, d, *J* = 7.4, 13-H), 6.66 (1 H, dd, *J* = 7.4 and 7.3, 14-H), 6.86 (1 H, d, *J* = 7.3, 15-H), 7.19 (1 H, d, *J* = 7.3, 19-H), 7.37 (1 H, dd, *J* = 8.2 and 7.3, 20-H), 7.45 (1 H, d, *J* = 8.2, 21-H), 4.85 (1 H, d, *J* = 6.5, 32-H), 2.19 (1 H, dqq, *J* = 6.5, 6.8, and 6.8), 1.05 (3 H, d, *J* = 6.8, 34-H), 0.92 (3 H, d, *J* = 6.8, 35-H), 3.88 (1 H, d, *J* = 3.8, 37-H), 2.11 (1 H, dqq, *J* = 3.8, 6.8, and 6.8, 38-H), 1.02 (3 H, d, *J* = 6.8, 39-H), 0.96 (3 H, d, *J* = 6.8, 40-H); ^{13}C NMR (MeOH-d_4, 50 MHz) δ 174.8, 57.2, 38.9, 129.9, 131.1, 111.3, 159.7, 129.6, 131.1, 62.3, 106.1, 127.8, 123.9, 120.9, 131.2, 123.2, 151.0, 131.6, 122.7, 124.3, 112.2, 136.9, 127.6, 98.2, 141.7, 130.1, 129.2, 155.1, 128.4, 155.4, 163.1, 56.6, 33.2, 19.4, 16.4, 175.5, 76.9, 31.7, 19.6, 18.9 for C1–C40, respectively.

(7) Diazonamide B (**2**): amorphous solid; UV (MeOH) 297 (sh), 290 (ϵ 4900), 281 (sh), 215 nm (21000); UV (MeOH + NaOH) 350 (sh), 22 (ϵ 4700), 205 nm (110000); IR (KBr) 3600–2800, 1680, 1610, 1500, 1450, 1410, 1300, 1215, 1175, 1150, 1060, 750 cm^{-1}; HRFABMS obsd ($M^+ + H - H_2O$) *m/z* 743.0590, $C_{35}H_{24}N_5O_5Cl_2Br$ requires 743.0340; ^{1}H NMR (MeOH-d_4, 360 MHz) δ 3.86 (1 H, dd, *J* = 11.6 and 3.3, 2-H), 3.34 (1 H, dd, *J* = 12.7 and 11.6, 3-H), 2.93 (1 H, dd, *J* = 12.7 and 3.3, 3-H), 7.26 (1 H, d, *J* = 1.6, 5-H), 7.34 (1 H, d, *J* = 1.6, 9-H), 6.41 (1 H, s, 11-H), 7.01 (1 H, dd, *J* = 7.5 and 1.0, 13-H), 6.67 (1 H, dd, *J* = 7.5 and 7.5, 14-H), 6.88 (1 H, dd, *J* = 7.5 and 1.0, 15-H), 7.20 (1 H, dd, *J* = 7.2 and 0.8, 19-H), 7.37 (1 H, dd, *J* = 8.1 and 7.2, 20-H), 7.46 (1 H, dd, *J* = 8.1 and 0.8, 21-H), 4.91 (1 H, d, *J* = 6.1, 32-H), 2.18 (1 H, dqq, *J* = 6.1, 6.8, and 6.8, 33-H), 1.02 (3 H, d, *J* = 6.8, 34-H), 0.96 (3 H, d, *J* = 6.8, 35-H); ^{13}C NMR (MeOH-d_4, 50 MHz) δ 171.6, 57.1, 37.3, 133.7, 130.0, 104.0, 157.4, 129.4, 131.6, 62.9, 106.6, 126.8, 123.8, 121.1, 131.3, 123.3, 150.8, 131.2, 122.7, 124.3, 112.3, 136.8, 127.4, 98.1, 141.9, 130.5, 130.0, 154.8, 128.4, 154.4, 162.8, 56.7, 31.9, 19.3, 18.9 for C1–C35, respectively.

(8) The *p*-bromobenzoyl derivative of diazonamide B (**3**): clear hexagonal prisms, mp 287–289 °C dec; HRFABMS obsd ($M^+ + H$) *m/z* 925.9837, $C_{42}H_{28}N_5O_6Cl_2Br_2$ requires 925.9784.

(9) Crystals of **3** are hexagonal, $a = b = 20.084$ (3) Å, $c = 20.233$ (3) Å, space group $P6_3$, $Z = 6$, ρ(calcd) = 1.331 g/cm^3 for $C_{42}H_{28}N_5O_6Cl_2Br_2$. A total of 5640 independent reflections, including Friedel pairs, were measured with graphite-monochromated Cu Kα radiation at 25 °C on a Nicolet R3m diffractometer to a maximum 2θ of 116°. The structure was solved without difficulty by using direct and heavy-atom methods. Full-matrix least-squares refinements with 4094 observed reflections ($|F_o| \geq 3\sigma(|F_o|)$ converged to crystallographic residuals of R = 5.62%, wR = 7.69% with η = 1.00 (6) for the enantiomer shown. The primary programs used were DIRDIF, by P. Beurskens, University of Nijmegen, Netherlands, and SHELXTL, by G. Sheldrick, University of Göttingen, FRG.

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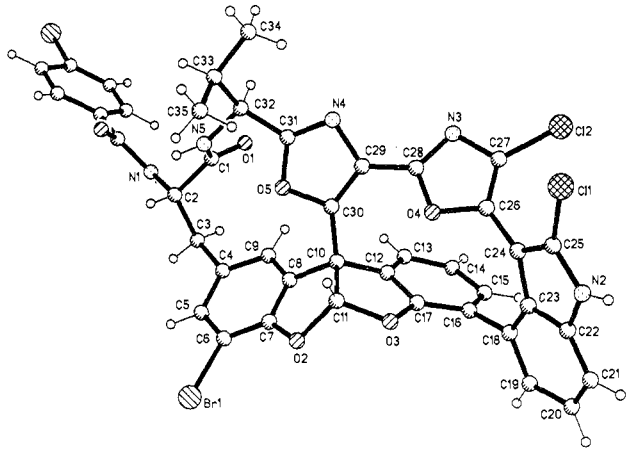

Figure 1. A computer-generated perspective drawing of 3. The absolute stereochemistry shown was determined by using anomalous dispersion measurements in the X-ray diffraction study.

the structure of diazonamide B (2) from 3, two questions had to be answered: was O2 or O3 bonded to C11, and what was the stereochemistry at C11? The hemiacetal ring involved O3 rather than O2 in diazonamide B (2), on the basis of a three-bond correlation observed between the C11 proton and C17. The stereochemistry at C11 proved intractable. Molecular mechanics studies[10] of the hemiacetal 2 and its corresponding aldehyde indicate a small preference (~1 kcal/mol) for the *R* configuration at C11 (OH down in the drawing) in the hemiacetal and a small preference (~1 kcal/mol) for aldehyde conformations in which the *si* face of the aldehyde faces O3, also leading to an *R* configuration at C11. But these calculated differences are too small to assign the C11 stereochemistry securely.

The similarity of ^{1}H and ^{13}C NMR data and the UV and IR spectra for diazonamide A (1) and B (2) indicated an identical polycyclic nucleus for A, except for the replacement of the C6 bromine by hydrogen. Diazonamide A (1) differed from B (2) in containing an extra valine residue. On the basis of a downfield shift of C2-H (δ 4.56 in 1 compared to δ 3.30 in 2) and an 8.5-Hz coupling in 1 of C2-H to the amide proton at δ 7.67 in DMSO-d_6, the valine carboxyl was attached to N1. The absolute stereochemistry of this terminal valine was not determined.

Diazonamides A (1) and B (2) represent an entirely new class of halogenated, highly unsaturated cyclic peptides containing derivatives of at least three common amino acids: a 3,4,5-trisubstituted L-tyrosine (C1-C9), a tryptophan substituted at the 2- and 4-positions of the indole (C18-C27), and an L-valine (C31-C35). Carbons C28-C30 could be the partial carbon skeleton of an undetermined β-hydroxy amino acid with amine nitrogen N4 and carboxyl oxygen O4. The likely biosynthetic origin of the C10-C17 unit is not clear. These structural units have cyclized in an unprecedented manner to form an extremely rigid framework with essentially no conformational freedom for the polycyclic core.

The UV spectra of diazonamides A[6] and B[7] show little evidence of their high degree of unsaturation. The strict steric requirements of the bicyclic framework prevent any appreciable overlap of the conjugated heterocycles. In the crystal structure of diazonamide B *p*-bromobenzamide (see Figure 1), the two oxazole rings are twisted with respect to one another with a dihedral angle of 29°, the chlorooxazole and chloroindole rings have a dihedral angle of 60°, and the chloroindole ring has a dihedral angle of 74° with the C12-C17 phenol.

Diazonamide A has potent in vitro activity against HCT-116 human colon carcinoma and B-16 murine melanoma cancer cell lines, with IC_{50} values less than 15 ng/mL. Diazonamide B is less active.

(10) W. C. Still, N. G. J. Richards, W. C. Guida, M. Lipton, R. Liskamp, G. Chang, and T. Hendrickson, MacroModel V2.0, Department of Chemistry, Columbia University, New York, NY 10027.

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Supplementary Material Available: Crystallographic data for diazonamide B *p*-bromobenzamide (3) (10 pages). Ordering information is given on any current masthead page.

Design of Self-Destructive Electron Acceptors. Highly Efficient Cleavage of C-C Bonds in Photogenerated Radical Anions[†,1]

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Unimolecular fragmentation reactions of radical ions to radicals and ions (mesolytic cleavages[3]) have recently attracted considerable attention,[4,5] especially in the context of photochemically initiated electron-transfer (ET) processes.[6] In systems where the fragmentation is rapid, it may successfully compete with energy-wasting back-electron transfer (BET) processes, yielding high quantum yields of radicals and ions.[6] The fragmentation reaction may, therefore, provide means to rapidly generate these reactive intermediates[7] or serve as a convenient probe of photoinduced electron-transfer (PET) processes.[8]

[†] Dedicated with admiration and appreciation to Professor Ronald Breslow.

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