Igzamide, a Metabolite of the Marine Sponge Plocamissma igzo

Eric Dumdei, and Raymond J. Andersen

J. Nat. Prod., **1993**, 56 (5), 792-794• DOI:
10.1021/np50095a022 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 5, pp. 792-794, May 1993

IGZAMIDE, A METABOLITE OF THE MARINE SPONGE *PLOCAMISSMA IGZO*

ERIC DUMDEI and RAYMOND J. ANDERSEN,*

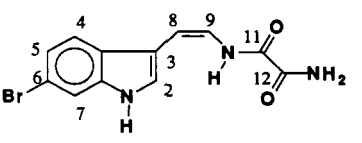
Departments of Chemistry and Oceanography, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

ABSTRACT.—Igzamide [**1**], a new brominated tryptamine derivative, has been isolated from the northeastern Pacific sponge *Plocamissa igzo*.

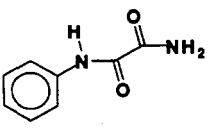
A number of metabolites that are formally derivatives of 6-bromotryptophan or 6-bromotryptamine have been isolated from marine sponges. Examples are the aplysinopsins (1–3), bromotopsentin (4–6), and dragmacidon (7), which are all cytotoxic against murine leukemia L1210 in vitro. As part of our ongoing studies of secondary metabolites from northeastern Pacific marine invertebrates (8), we have undertaken an investigation of the extract of the sponge *Plocamissa igzo* (de Laubenfels) (class Demospongiae, order Poecilosclerida, family Plocamiidae) because it exhibited in vitro cytotoxicity against the L1210 murine leukemia cell line (9). An L1210 cytotoxicity bioassay-guided fractionation of the extract resulted in the isolation of igzamide [**1**], a new brominated tryptamine derivative containing an oxalic acid diamide functionality.

Specimens of *P. igzo* were collected at a depth of 2 m at Anthony Island, B.C. and frozen on site. Freeze-dried sponge (500 g) was extracted sequentially at room temperature with CH_2Cl_2 (700 ml), EtOAc (700 ml), and MeOH (700 ml). The EtOAc fraction was evaporated to dryness to give a viscous brown oil (1.7 g) that showed cytotoxic (L1210): ED_{50} 5 μg/ml) activity. Chromatographic fractionation of the brown oil using a wide variety of normal phase, reversed-phase, and gel permeation supports consistently resulted in fractions that had lower biological potency than the crude EtOAc extract. The most cytotoxic fraction obtained from a Sephadex LH 20 chromatography of the crude EtOAc extract displayed ^{1}H-nmr signals indicating that metabolites containing 6-bromoindole residues were major components of the fraction. Further purification of this fraction by sequential application of Sephadex LH 20 [EtOAc-MeOH-H_2O (40:10:4)] and reversed-phase hplc [H_2O-MeOH (65:35)] chromatographies yielded pure samples of igzamide [**1**] (8 mg) and 6-bromoindole-3-carboxaldehyde (2 mg) (10). Neither of these metabolites was cytotoxic enough to account for the biological activity of the crude EtOAc extract or the Sephadex LH 20 fraction that they came from, although igzamide [**1**] did show some very weak cytotoxicity (L1210: ED_{50} 19 μg/ml). Characterization of the more potent cytotoxic agents in the *P. igzo* extracts has thus far proven to be elusive.

Igzamide [**1**] was obtained as a yellow solid that gave a parent ion in the hreims at *m/z* 306.9961/308.9944, appropriate for a molecular formula of $C_{12}H_{10}N_3O_2Br$ (ΔM +0.4/+0.7 mmu), requiring nine sites of unsaturation. Absorption bands at 3300, 1678, 1613, and 1536 cm^{-1} in



1



2

the ir spectrum of igzamide were attributed to amide functionalities. The ^{1}H-nmr spectrum of igzamide contained a series of deshielded resonances that spanned the chemical shift region from δ 6.15 to 11.52 and integrated for a cumulative total of ten protons. Analysis of COSY, HMQC, and difference nOe data for igzamide identified one subset of resonances that could be assigned to a 3,6-disubstituted indole fragment [DMSO-d_6: δ 11.52, bs (NH); 7.53, s (H-2); 7.55, d, J = 7.4 Hz (h-4), 7.19 (dd, J = 1.8, 7.4 Hz (H-5); 7.61, d, J = 1.8 Hz (H-7)] and a second subset of resonances that could be assigned to an enamide fragment [δ 6.15, d, J = 9.1 Hz (H-8); 6.70, dd, J = 7.6, 9.1 Hz (H-9); 9.48, d, J = 7.6 Hz H-10)]. Irradiation of the olefinic resonance at δ 6.15 (H-8) induced nOe's in the vicinal olefinic resonance at δ 6.70 and the indole resonance at δ 7.55 (H-4). The nOe observed between H-8 and H-4 placed the enamide fragment at C-3 of the indole nucleus and the nOe observed between H-8 and H-9, in conjunction with the observed H-8/H-9 coupling constant of 9.1 Hz, established the enamide olefinic geometry as *Z*.

The remaining portions of igzamide had to account for $C_2H_2NO_2Br$ and two sites of unsaturation. A pair of resonances at δ 157.2 and 161.4 in the ^{13}C-nmr spectrum of **1** were assigned to the amide carbonyls of an oxalic acid diamide by comparison with the chemical shifts of the carbonyl carbons in the synthetic model compound **2** (δ 158.9 and 162.3). The primary amide protons in this fragment of igzamide [**1**] gave resonances at δ 8.07 (bs) and 8.36 (bs) in the ^{1}H-nmr spectrum. Placement of the bromine atom at C-6 of the indole ring was consistent with the observation of an nOe between the indole NH resonance (δ 11.52, bs) and the H-7 resonance (δ 7.61, d, J = 1.8 Hz).

The (*Z*)-6-bromo-8,9-didehydrotryptamine fragment present in igzamide is also found in the halocyamines, a pair of tetrapeptide-like substances isolated from the ascidian *Halocynthia roretzi* (11). A comparison of the ^{1}H- and ^{13}C-nmr chemical shift assignments reported above and in the Experimental section for the 6-bromo-8,9-didehydrotryptamine fragment in igzamide [**1**] with the values reported for the same fragment in the halocyamines showed excellent agreement.

Igzamide is a new member of a rather small family of 6-bromotryptophan and 6-bromotryptamine derivatives that have been reported from marine sponges (3–7, 12). The enamide functionality in **1**, which is quite rare in nature, has been previously encountered in clionamide (13) and the celenamides (12) isolated from the northeastern Pacific sponge *Cliona celata* and in the tunichromes (14). A search of the literature failed to turn up any other sponge metabolites containing an oxalic acid diamide substructure.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H- and ^{13}C-nmr spectra were recorded on Bruker WH400 and AMX-500 spectrometers. ^{1}H chemical shifts are referenced to the residual DMSO-d_6 signal (2.49 ppm), and ^{13}C chemical shifts are referenced to the DMSO-d_6 solvent peak (39.5 ppm). Low resolution and high resolution eims were recorded on Kratos AEI MS-59 and AEI MS-50 mass spectrometers.

Reversed-phase hplc purifications were performed on a Perkin-Elmer Series 2 liquid chromatograph attached to a Perkin-Elmer Spectrophotometer LC-55 using a Whatman Magnum-9 Partisil 10 ODS-3 column.

SPONGE MATERIAL.—A voucher sample of *P. igzo* is deposited in the invertebrate collection housed in the British Columbia Provincial Museum, Victoria, British Columbia (Voucher sample number 992-220-1.

IGZAMIDE [**1**].—Yellow solid: uv (MeOH) λ max 294 (ε 4900), 228 (ε 12,800) nm; Ft-ir (thin film) 3300.1, 1678.2, 1613.7, 1536.5 cm^{-1}; ^{1}H nmr (DMSO-d_6, 400 MHz) δ 11.52 (bs), 9.48 (d, J = 7.6 Hz), 8.36 (bs), 8.07 (bs), 7.61 (d, J = 1.8 Hz), 7.55 (d, J = 7.4 Hz), 7.53 (s), 7.19 (dd, J = 7.4, 1.8 Hz), 6.70 (dd, J = 9.1, 7.6 Hz), 6.15 (d, J = 9.1 Hz); ^{13}C nmr (DMSO-d_6, 125 MHz) δ 161.4 (C-11), 157.2 (C-12), 136.7 (C-7a), 130.8 (C-3a), 124.4 (C-2), 122.3 (C-5), 120.3 (C-4), 117.9 (C-9), 114.7 (C-6 or

C-3), 114.3 (C-7), 109.8 (C-3 or C-6), 105.3 (C-8); eims *m/z* (rel. int.) 309 (4.4), 307 (4.9), 264 (4.6), 262 (3.7), 149 (13), 129 (10), 97 (12), 87 (10), 85 (19), 84 (28), 83 (23), 82 (16), 81 (16), 43 (100); hreims *m/z* 308.9944/306.9961 ($C_{12}H_{10}N_3O_2Br$ calcd 308.9937/306.9957).

ANILINE OXALAMIDE **2**.—Aniline (50 mg) was dissolved in dry CH_2Cl_2, and excess oxalyl chloride was added with rapid stirring. After 1 h, NH_3 gas was bubbled through the reaction mixture, and insoluble oxalyldiamide was removed by filtration. Compound **2** was purified via Si gel flash chromatography (EtOAc): yield 35 mg; Ft-ir (film) 3395.4, 3300.3, 1659.2, 1651.8, 1598.3, 1537.9, 1445.6, 1401.9, 751.0 cm^{-1}; ^{13}C nmr (DMSO-d_6, 50 MHz) δ 162.3, 158.9, 137.8, 128.7, 124.3, 120.3; eims *m/z* (rel. int.) 164 (54), 121 (11), 120 (59), 119 (25), 93 (58), 92 (35), 77 (100), 66 (31), 65 (30), 64 (11), 51 (33), 44 (60); hreims *m/z* 164.0591 ($C_8H_8N_2O_2$ calcd 164.0585).

ACKNOWLEDGMENTS

Financial support was provided by grants to RJA from the Natural Sciences and Engineering Research Council of Canada and the National Cancer Institute of Canada. The authors thank M. Le Blanc and the crew of the J.P. Tully for assisting in the collection of *P. igzo* and Dr. W. Austin for identifying the sponge.

LITERATURE CITED

1. R. Kazlauskas, P.T. Murphy, R.J. Quinn, and R.J. Wells, *Tetrahedron Lett.*, 61 (1977).
2. K.H. Hollenbeak and F.J. Schmitz, *J. Nat. Prod.*, **40**, 479 (1977).
3. A.A. Tymiak, K.L. Rinehart, and G.J. Bakus, *Tetrahedron*, **41**, 1039 (1985).
4. K. Bartik, J.C. Braekman, D. Daloze, C. Stoller, C. Huysecom, G. Vanevyver, and R. Ottinger, *Can. J. Chem.*, **65**, 2118 (1987).
5. S. Tsujii, K.L. Rinehart, S.P. Gunasekera, Y. Kashman, S.S. Cross, M.S. Lui, S.A. Pomponi, and M.C. Diaz, *J. Org. Chem.*, **53**, 5446 (1988).
6. S.A. Morris and R.J. Andersen, *Can. J. Chem.*, **67**, 677 (1989).
7. S. Komoto, Y. Kashman, O.J. McConnell, K.L. Rinehart, A. Wright, and F.J. Koehn, *J. Org. Chem.*, **53**, 3116 (1988).
8. S. Miao and R.J. Andersen, *J. Org. Chem.*, **56**, 6275 (1991).
9. NIH Publication No. 84-2635, National Cancer Institute, Bethesda, Maryland, 1984.
10. S.J. Wratten, M.S. Wolfe, R.J. Andersen, and D.J. Faulkner, *Antimicrob. Agents Chemother.*, **11**, 411 (1977).
11. K. Azumi, H. Yokosawa, and S. Ishi, *Biochemistry*, **29**, 159 (1990).
12. R.J. Stonard and R.J. Andersen, *Can. J. Chem.*, **58**, 2121 (1980).
13. R.J. Andersen, *Tetrahedron Lett.*, 2541 (1978).
14. R.E. Bruening, E.M. Oltz, J. Furukawa, K. Nakanishi, and K. Kurstin, *J. Am. Chem. Soc.*, **107**, 5298 (1985).

Received 8 September 1992