Indole Alkaloids from the Sea Anemone *Heteractis aurora* and Homarine from *Octopus cyanea*

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The two new indole alkaloids 2-amino-1,5-dihydro-5-(1*H*-indol-3-ylmethyl)-4*H*-imidazol-4-one (**1**), 2-amino-5-[(6-bromo-1*H*-indol-3-yl)methyl]-3,5-dihydro-3-methyl-4*H*-imidazol-4-one (**2**), and auramine (**3**) have been isolated from the sea anemone *Heteractis aurora*. Both indole alkaloids were synthesized for the confirmation of the structures. Homarine (**4**), along with uracil (**5**), hypoxanthine (**6**), and inosine (**7**) have been obtained from *Octopus cyanea*.

Introduction. – Sea anemones are ocean dwelling, solitary members of the phylum Cnidaria and the class Anthozoa invertebrates. The phylum Cnidaria includes anemones, corals, jellyfish, and hydras. Sea anemones have a basic radial symmetry with tentacles surrounding a central mouth opening. The tentacles are used to catch food and transfer it to their mouth. At the tentacles and other parts of the sea anemone are stinging capsules (nematocysts) containing small threads that are forcefully ejected along with the venom when stimulated mechanically or chemically. Sea anemones use nematocysts for capturing prey, as well as for defence purposes against predators [1] and in interspecies aggression [2]. The venom of sea anemones contains a variety of active compounds like toxins affecting voltage-gated Na^+ and K^+ channels, acid-sensing ion channels, pore-forming toxins (actinoporins), and protease inhibitors. The use of toxins affecting voltage-gated ion channels seems logical since these targets are an important component of the action potential in the signal transduction process of vertebrates and invertebrates. Actinoporins are highly toxic to fish and crustaceans which may be the natural prey of sea anemones [1].

The octopuses are cephalopods of the order Octopoda which are characterized by their eight arms usually bearing suction cups. Octopuses are highly intelligent animals whose behaviour is easily modified by experience. They live in many diverse regions of the ocean, especially in holes in rocky areas from the interdidial area to at least 45 m. *Octopus cyanea* occurs in the Indo-Pacific [3]. Octopuses use different toxins for prey acquisition, as well as in defence. Cephalotoxins from posterior salivary glands of *Octopus vulgaris* have a strong paralysing effect on crabs [4]. Tetrodotoxin appears to be actively applied by the blue-ringed octopus *Hapalochlaena* spp. for acquisition of prey. Tetrodotoxin, the major neurotoxin involved in envenoming, is secreted by the two posterior salivary glands and inoculated during the bite. However, it was detected predominantly in the arms representing about 45% of the body mass by weight,

followed by the abdomen, where the posterior salivary glands are located, and the cephalothorax [5].

In this article, the structure determination of the new natural indole alkaloids **1** and **2**, together with auramine (**3**) from the sea anemone *Heteractis aurora* is described. The structures of both indole alkaloids **1** and **2** were confirmed by synthesis. Homarine (**4**), uracil (**5**), hypoxanthine (**6**), and inosine (**7**) were isolated from *Octopus cyanea*.

Results and Discussion. – *Isolation and Structure Determination of Compounds* **1**–**4**. The fresh yellow sea anemones of *Heteractis aurora* and a frozen octopus of *Octopus cyanea* were cut, homogenized, and exhaustively extracted with EtOH. Both EtOH extracts were concentrated to dryness. The extract of *Heteractis aurora* was submitted to CC with SiO_2 to afford three main fractions. The second fraction was chromatographed over a further SiO_2 column followed by PTLC (prep. thin layer chromatography) to yield the compounds **1**–**3** (*Fig.*).

The EtOH extract of *Octopus cyanea* was successively partitioned between H_2O and hexanes, AcOEt, and BuOH. The BuOH extract was separated over a SiO_2 column followed by PTLC to give homarine (**4**), uracil (**5**), inosine (**6**), and hypoxanthine (**7**) (*Fig.*).

Compound **1** was found to possess the molecular formula $C_{12}H_{12}N_4O$ by HR-ESI-MS at *m*/*z* 229.1083 ($[M+H]^+$). The ^{1}H- and ^{13}C-NMR data for **1** (*Table 1*) showed the signals of five aromatic H-atoms (δ(H) 6.96, 7.05, 7.14, 7.32, 7.53), five quaternary C-atoms (δ(C) 110.1, 127.4, 136.0, 171.9, 188.9), one CH group (δ(C) 60.8), and one CH_2 group (δ(C) 27.4). Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those reported for methyl 2-(1*H*-indol-3-yl)-2-oxoacetate [6] and 2-amino-5-(3-aminopropyl)-1,5-dihydro-4*H*-imidazol-4-one [7] let us propose a 3-substituted indole and a 5-substituted 2-amino-1,5-dihydro-4*H*-imidazol-4-one as subunits for **1**. The HMBCs H–C(5′)/C(2′) and H–C(5′)/C(4′) confirm the ^{13}C assignments of the imidazolinone ring. The HMBC

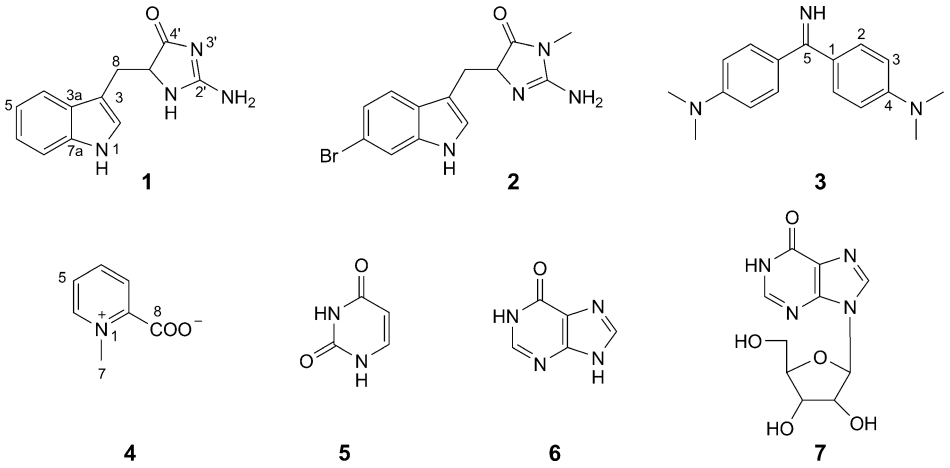


Figure. *Structures of compounds* **1**–**7**

Table 1. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **9**. δ in ppm, J in Hz. Arbitrary numbering, see *Scheme 1*.

Position	**1**[a])		**9**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)
2	7.14 (*d*, *J* = 2.3)	123.3	6.75 (*d*, *J* = 2.4)	123.8
3		110.1		106.9
3a		127.4		127.6
4	7.53 (*d*, *J* = 8.0)	118.5	7.27 (*d*, *J* = 8.0)	118.4
5	6.96 (*ddd*, *J* = 8.0, 8.0, 1.1)	118.3	6.83 (*ddd*, *J* = 8.0, 8.0, 1.1)	118.1
6	7.05 (*ddd*, *J* = 8.0, 8.0, 1.1)	120.8	7.00 (*ddd*, *J* = 8.0, 8.0, 1.1)	120.7
7	7.32 (*d*, *J* = 8.0)	111.2	7.20 (*d*, *J* = 8.0)	111.2
7a		136.0		135.7
8	2.78 (*dd*, *J* = 15.0, 7.2), 3.11 (*dd*, *J* = 15.0, 4.0)	27.4	3.23 (*dd*, *J* = 14.8, 2.3), 3.38 (*dd*, *J* = 14.8, 5.5)	24.8
2′		171.9		167.5
4′		188.9		184.3
5′	3.98–4.03 (*m*)	60.8	4.46–4.49 (*m*)	62.2
1″				135.1
2″,6″			7.53–7.56 (*m*)	128.6
3″,5″			7.42–7.46 (*m*)	128.6
7″			5.32 (*d*, *J* = 12.1), 5.43 (*d*, *J* = 12.1)	68.1
8″				150.8

[a]) Measured in (D_6)DMSO

interactions H–C(5′)/C(8), H–C(5′)/C(3), CH_2(8) (δ(H) 3.11, 2.78)/C(3a), CH_2(8)/C(2), CH_2(8)/C(3), and CH_2(8)/C(4′) revealed the connection between the indole moiety in position 3 and the imidazolinone ring in position 5′ through the CH_2(8) group. Therefore, compound **1** was identified as 2-amino-1,5-dihydro-5-(1*H*-indol-3-ylmethyl)-4*H*-imidazol-4-one.

Compound **2** showed two ions at *m*/*z* 320 and 322 with relative intensities 100 : 98 in the EI-MS suggesting the presence of one Br-atom. The molecular formula was established as $C_{13}H_{13}{}^{79}BrN_4O$ by HR-ESI-MS (*m*/*z* 321.0347 ($[M+H]^+$)). The most remarkable differences in the ^{13}C-NMR data of the indole part of **2** in comparison with **1** were the upfield shift for the signals of C(6) ($\Delta\delta = -7.1$), the downfield shifts for the signals of C(5) ($\Delta\delta = +3.0$), and C(7) ($\Delta\delta = +2.7$) due to the presence of Br-atom at C(6) (*Table 2*). The ^{1}H- and ^{13}C-NMR data of the 6-bromoindole moiety of **2** showed a good agreement with those reported for methyl 2-(6-bromo-1*H*-indol-3-yl)-2-oxoacetate [6]. The ^{13}C-NMR shifts in (D_6)DMSO of the signals C(2′) (δ(C) 157.6) and Me–N(3′) (δ(C) 25.7) of the imidazolinone ring of **2** were in good accordance with those reported for the imidazolidinone ring of 6-bromo-2′-de-*N*-methylaplysinopsin C(3′) (δ(C) 157.7) and Me–N(4′) (δ(C) 25.6) [8]. The HMBCs Me–N(3′)/C(2′), Me–N(3′)/C(4′), and H–C(5′)/C(4′), H–C(5′)/C(2′) confirm the position of the Me group at 3′-position and the ^{13}C assignment of the imidazolinone ring. The connection between the indole and imidazolinone moieties through the CH_2(8) of **2** was evident from the HMBCs of CH_2(8) (δ(H) 3.08, 3.22)/C(4′), CH_2(8)/C(2), CH_2(8)/C(3),

Table 2. *^{1}H- and ^{13}C-NMR Data of Compounds* **2** *and* **11**–**14**. δ in ppm, J in Hz. Arbitrary numbering, see *Scheme 2*.

Position	**2**[a])		**11**[b])		**12**[c])		**13**[a])		**14**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
2	7.16 (*d*, *J* = 2.1)	125.3	6.78 (br. *s*)	126.6	7.08 (br. *s*)	125.3	6.91 (*d*, *J* = 2.3)	124.9	7.15 (*d*, *J* = 2.0)	124.6
3		108.2		107.1		111.5		107.7		110.5
3a		126.3		126.1		127.9		126.7		126.6
4	7.51 (*d*, *J* = 8.5)	120.4	7.01 (*d*, *J* = 8.5)	120.1	7.46 (*d*, *J* = 8.5)	120.8	7.38 (*d*, *J* = 8.5)	120.2	7.49 (*d*, *J* = 8.4)	120.5
5	7.09 (*dd*, *J* = 8.5, 1.8)	121.3	6.75 (*dd*, *J* = 8.5, 1.8)	123.0	7.09 (*dd*, *J* = 8.5, 1.8)	122.8	7.05 (*dd*, *J* = 8.5, 1.8)	121.3	7.07 (*dd*, *J* = 8.4, 1.8)	121.1
6		113.7		113.0		115.7		113.7		113.6
7	7.52 (*d*, *J* = 1.8)	113.9	7.15 (*d*, *J* = 1.8)	115.2	7.47 (*d*, *J* = 1.8)	115.1	7.48 (*d*, *J* = 1.8)	114.0	7.49 (*d*, *J* = 1.8)	113.8
7a		136.8		137.7		138.7		136.7		136.9
8	3.08 (*dd*, *J* = 14.4, 6.7), 3.22 (*dd*, *J* = 14.4, 3.4)	26.3	2.90 (*dd*, *J* = 16.0, 7.4), 2.97 (*dd*, *J* = 16.0, 5.3)	26.2	3.10 (*dd*, *J* = 14.6, 8.0), 3.28 (*dd*, *J* = 14.6, 5.1)	28.6	3.20 (*dd*, *J* = 14.8, 2.5), 3.38 (*dd*, *J* = 14.8, 5.8)	25.0	2.79 (*dd*, *J* = 14.8, 7.5), 3.08 (*dd*, *J* = 14.8, 4.1)	27.2
2′		157.6						167.9		172.1
10 or 4′		174.7		172.2		175.7		184.5		189.1
9 or 5′	4.48–4.52 (*m*)	59.9	3.86–3.90 (*m*)	53.7	4.39–4.43 (*m*)	55.8	4.38–4.39 (*m*)	62.3	3.96–3.99 (*m*)	60.9
Me–N(3′)	2.85 (*s*)	25.7								
1′ or 1″						157.7		150.0		
2′ or 2″						80.6		83.9		
Me$_3$–C(2″)					1.37 (*s*)	28.6	1.54 (*s*)	27.7		

[a]) Measured in (D_6)DMSO. [b]) Measured in D_2O, CF_3COOD, MeOH as internal ^{13}C standard (δ(C) 49.5). [c]) Measured in CD_3OD.

CH_2(8)/C(3a), and H–C(5′)/C(3). Compound **2** was identified as 2-amino-5-[(6-bromo-1*H*-indol-3-yl)methyl]-3,5-dihydro-3-methyl-4*H*-imidazol-4-one, an aplysinopsin-type alkaloid.

Aplysinopsin has been isolated before from the sponge *Aplysinopsis* sp. [9]. Aplysinopsin-type indole alkaloids have previously been obtained from the sponge *Dercitus* sp. [10], the scleractinian coral *Dendrophyllia* sp. [8], and the marine sponge *Hyrtios erecta* [11]. These alkaloids showed a selective inhibitory activity against the neuronal isozyme of nitric oxide synthase (nNOS). Nitric oxide (NO) is an important second messenger and regulates many physiological processes, *e.g.* inflammation, regulation of blood pressure, platelet adhesion, and neurotransmission. An excessive production of NO causes different disease states such as post-ischemic stroke damage, schizophrenia, development of colitis, tissue damage, and pathological inflammation. Therefore, a rational control of NO production is assumed to be an efficient approach to treat these diseases [11].

The molecular formula $C_{17}H_{21}N_3$ of compound **3** was determined by HR-EI-MS at *m*/*z* 267.1736 (M^+). The ^{13}C-NMR data of **3** were in good agreement with those reported for auramine [12], which is a yellow dye used in the manufacture of paints, textiles, and rubber products. Auramine, together with rhodamine, is used as fluorescent dye in the fluorescence microscopy to visualize acid-fast bacteria, like those of *Mycobacteria*. Compound **3** shows bacteriostatic activity against *Streptococcus pyogenes* and *Staphylococcus aureus* [13].

The HR-ESI-MS (*m*/*z* 138.0549 ($[M+H]^+$)) of compound **4** led to a molecular formula of $C_7H_7NO_2$. Comparison of the ^{13}C-NMR data of **4** with those of homarine [14] showed full accord. Homarine is a fairly ubiquitous metabolite in marine invertebrates [15] and has been detected before in the mantle, arms, and liver of *Octopus ochellatus* [16] and urine of *Octopus vulgaris* [17]. It has been isolated also from a lot of other organisms like the echiura *Urechis unicintus* [18], the ascidian *Eusynstyela latericius* [19], and corals [14][15]. Homarine (**4**) has been suggested to serve as an active constituent of the innate immune system in the octocoral *Leptogorgia virgulata*. It may act synergistically with cofactors in this coral to mount a response to microbial invasion and disease [14]. Homarine from *Leptogorgia virgulata* inhibited significantly the growth of the fouling benthic marine diatom *Navicula salinicola* at naturally occurring concentrations [20]. The antarctic soft coral *Gersemia antarctica* produces homarine, which is released into the surrounding water column as predator deterrent compound and antibacterial metabolite [15]. Perhaps homarine of *Octopus cyanea* protects its body against bacterial infection.

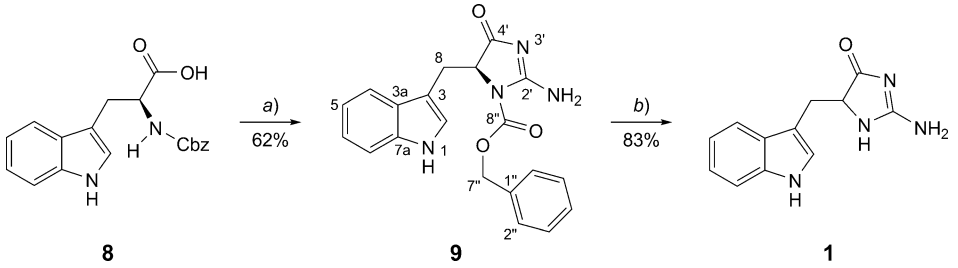
MS, ^{1}H- and ^{13}C-NMR data of compounds **5**–**7** were identical with those of authentic samples of uracil, hypoxanthine, and inosine. Hypoxanthine has also been found before as homarine in the urine of *Octopus vulgaris* [17].

Synthesis of the Alkaloids **1** *and* **2**. The preparation of both compounds **1** and **2** and the comparison of their spectroscopic data with those of the natural products should confirm the structures. A further aim of the synthesis should be the determination of the absolute configuration of the two chiral compounds by comparison of the optical rotations with authentic references.

Compound **1** was prepared through a simple two-step sequence based on a literature-known procedure [21] (*Scheme 1*). Cbz-protected (Cbz = (benzyloxy)car-

bonyl) L-tryptophan (**8**) was treated with NHS (*N*-hydroxysuccinimide) and DCC (*N*,*N*'-dicyclohexylcarbodiimide) to form an activated NHS-ester which was then introduced in an aq. sodium cyanamide solution, furnishing the protected imidazolinone **9** (*Scheme 1*). Deprotection of **9** under hydrogenolytic conditions was carried out two times independently and gave the desired compound **1** which was NMR spectroscopically identical with the natural product **1**. The $[\alpha]_D^{23}$ values of the both synthetic samples were determined as -60.0 (MeOH) and -6.1 (MeOH), respectively, which indicates a partial racemization at the stereogenic center C(5′) with (*S*) configuration of **1**. As the optical rotation of the natural compound **1** is $[\alpha]_D^{23} = +3.2$ (MeOH), its configuration must be (*R*). The synthesis of compound (*R*)-**1** has been described before in an U.S. patent [22].

Scheme 1. *Synthesis of Compound* **1**



8 **9** **1**

a) 1. THF, DCC, NHS, 0°, 3 h. 2. NaHNCN, H_2O, r.t., 16 h; 62%. *b*) MeOH, Pd/C, H_2, r.t., 1 h; 83%.

The synthesis of alkaloid **2** started with a racemic mixture of *N*-acetyl-6-bromotryptophan (**10**) which was treated with the enzyme L-aminoacyclase in a literature-known procedure [23] [24] (*Scheme 2*). *N*-Acetyl-6-bromo-D-tryptophan and 6-bromo-L-tryptophan (**11**) were obtained, and the amino group of **11** was protected with Boc_2CO (Boc = *t*-Butyloxycarbonyl) to give **12**. Compound **12** reacted in the same way as **9** with NHS and DCC to an activated NHS-ester which formed with sodium cyanamide the Boc-protected imidazolinone **13**. Compound **13** was deprotected to **14** with $SnCl_4$ in MeCN. The imidazolinone **14** was methylated with MeI in MeCN/EtOH 1:1, and the desired product **2** was obtained. The NMR spectra of the natural and the synthetic alkaloid **2** were identical. The comparison of the optical rotations of the natural $[\alpha]_D^{23} = +4.4$ (MeOH) and the synthetic alkaloid $[\alpha]_D^{23} = -2$ (MeOH) with (*S*) configuration showed in the same way as for compound **1** a partial racemization. The configuration of the natural alkaloid **2** should be (*R*).

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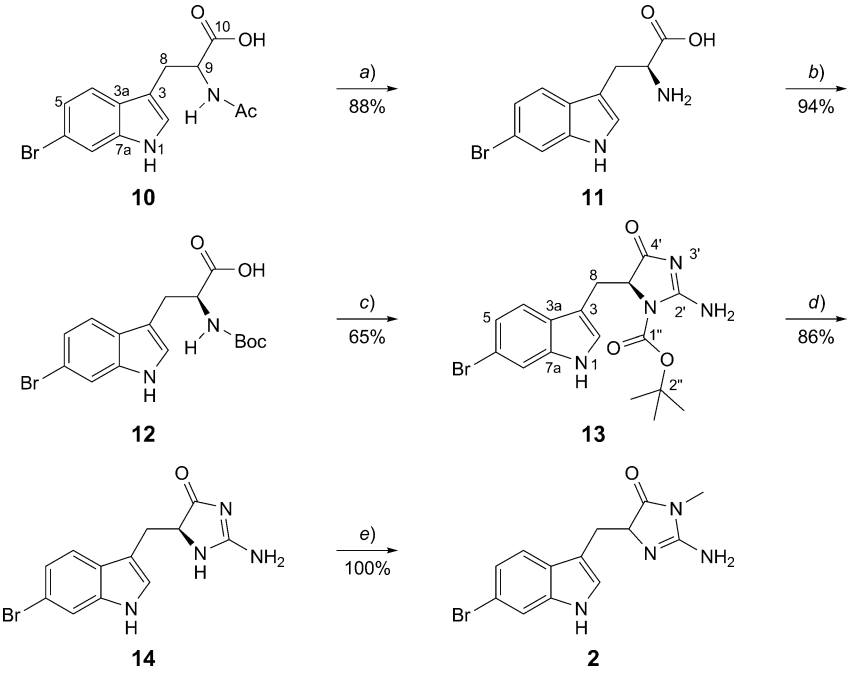
Scheme 2. *Synthesis of Compound* **2**



a) Borate buffer, $CoCl_2 \cdot 6\ H_2O$, pH = 8.5, L-aminoacyclase, 38°, 48 h; 88%. *b*) 1,4-Dioxane, H_2O, KOH, Boc_2O, r.t., 16 h, 94%. *c*) 1. THF, DCC, NHS, 0°, 2 h. 2. NaHNCN, H_2O, r.t., 16 h; 65%. *d*) MeCN, $SnCl_4$, 0°, under Ar, 45 min; 86%. *e*) MeCN, EtOH, MeI, r.t., 24 h; 100%.

Experimental Part

General. All reactions were performed in oven-dried glassware. THF was distilled from sodium diphenyl ketyl. Solvents for chromatography were purchased technical grade and distilled prior to use. All starting compounds were purchased from commercial sources and used as received. TLC: silica gel *60* F_{254} precoated plates (*Merck*); detection with 10% molybdophosphoric acid in EtOH. Column chromatography (CC): silica gel *60 M* (SiO_2, 40–63 μm; *Macherey-Nagel*). PTLC: silica gel *60* PF_{254} precoated plates (*Merck*). Optical rotations: *Jasco P-1020* polarimeter. NMR: *Bruker-Avance 300*; δ in ppm, *J* in Hz; (D_6)DMSO or CD_3OD as solvent and internal standard ((D_6)DMSO: δ(H) 2.50, δ(C) 39.5. CD_3OD: δ(H) 3.31, δ(C) 49.0). HR-EI-MS and HR-ESI-MS: *Bruker APEX IV* and *Thermofisher Scientific Orbitrap Elite*; in *m*/*z*. EI-MS: *Finnigan MAT-8500*; in *m*/*z*.

Animal Material. The yellow sea anemones of *Heteractis aurora* and the octopus of *Octopus cyanea* were collected in December 2006, near Bali, Indonesia, at a depth of 3–15 meter, and were identified by *F. Pfeifer*, Bayreuth.

Extraction and Isolation. The fresh sea anemones of *Heteractis aurora* (300 g) were sliced, homogenized with EtOH, and exhaustively (4×) extracted with EtOH. The solvent was removed under reduced pressure to yield the residue (14 g) which was submitted to CC (SiO_2; hexanes, hexanes/AcOEt gradient, $CHCl_3$, $CHCl_3$/MeOH gradient) to afford three fractions (*Frs. I–III*). *Fr. II* (2.0 g) was separated by CC (SiO_2; $CHCl_3$, $CHCl_3$/MeOH gradient 18:1, 18:3) followed by PTLC ($CHCl_3$/MeOH 18:3) to give compounds **1** (20 mg), **2** (22 mg), and **3** (30 mg).

The octopus of *Octopus cyanea* (320 g) were frozen, sliced, homogenized with EtOH, and directly extracted (4×) with EtOH. The extract was evaporated to dryness and the residue (10 g) was

successively partitioned between hexanes/H_2O, AcOEt/H_2O, and BuOH/H_2O. The BuOH extract (0.8 g) was subjected to CC (SiO_2; $CHCl_3$, $CHCl_3$/MeOH gradient) to yield two fractions (*Frs. A* and *B*). *Fr. A* (60 mg) gave, after purification with PTLC ($CHCl_3$/MeOH/H_2O 21 : 3.5 : 0.5), the compounds **5** (3 mg), **6** (2 mg), and **7** (15 mg), and *Fr. B* (28 mg) gave compound **4** (15 mg).

*2-Amino-1,5-dihydro-5-(1*H*-indol-3-ylmethyl)-4*H*-imidazol-4-one* (**1**). $[\alpha]_D^{23} = +3.2$ ($c = 1.00$, MeOH). IR (ATR): 3200 (broad), 1693, 1629, 1571, 1482, 1456, 1308, 1265, 1227, 1093, 1009, 741, 718. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 228 (3, M^+), 130 (100), 98 (14). HR-ESI-MS: 229.1083 ($[M+H]^+$, $C_{12}H_{13}N_4O^+$; calc. 229.1084).

*2-Amino-5-[(6-bromo-1*H*-indol-3-yl)methyl]-3,5-dihydro-3-methyl-4 H-imidazol-4-one* (**2**). $[\alpha]_D^{23} = +4.4$ ($c = 1.00$, MeOH). ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 322 (12.7, M^+), 320 (13.0, M^+), 208 (100). HR-ESI-MS: 321.0347 ($[M+H]^+$, $C_{13}H_{14}{}^{79}BrN_4O^+$; calc. 321.0346).

Auramine (*=4,4′-Carbonimidoylbis[*N,N*-dimethylbenzenamine]*; **3**) ^{13}C-NMR ((D$_6$)DMSO): 175.6 (C(5)); 154.7 (C(1)); 134.1 (C(3)); 116.7 (C(4)); 111.6 (C(2)); 39.8 (Me_2N–C(1)). HR-EI-MS: 267.1736 (M^+, $C_{17}H_{21}N_3^+$; calc. 267.1736).

Homarine (*=1-Methylpyridinium-2-carboxylate*; **4**). ^{13}C-NMR (CD_3OD): 165.3 (C(8)); 155.6 (C(2)); 147.1 (C(4)); 146.6 (C(6)); 127.7 (C(3)); 127.7 (C(5)); 47.6 (C(7)). HR-ESI-MS: 138.0549 ($[M+H]^+$, $C_7H_8NO_2^+$; calc. 138.0549).

*Synthesis of Benzyl (5*S*)-2-Amino-4,5-dihydro-5-(1*H*-indol-3-ylmethyl)-4-oxo-1*H*-imidazole-1-carboxylate* (**9**). To a soln. of *N*-Cbz protected L-trypthophan **8** (2.000 g, 5.92 mmol) in THF (60 ml) was added DCC (1.236 g, 6.00 mmol) and NHS (691 mg, 6.00 mmol). The soln. was stirred for 3 h at 0° and filtered by suction filtration. The filtrate was added to a soln. of NaHNCN (1.135 g, 17.74 mmol) in H_2O (60 ml) and stirred for 16 h at r.t. THF was removed under reduced pressure and the soln. was adjusted to pH 12 with diluted NaOH and washed with CH_2Cl_2 (2 × 50 ml). The aq. layer was acidified to pH 2.2 with dil. HCl and extracted with AcOEt (2 × 80 ml). The combined org. extracts were dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was purified by CC (SiO_2; $CHCl_3$/MeOH 90 : 10) to afford pure **9** (1.329 g, 3.67 mmol, 62%). Amorphous solid. R_f (SiO_2, $CHCl_3$/MeOH 9 : 1) 0.4. $[\alpha]_D^{23} = +45.6$ ($c = 1.00$, MeOH). IR (ATR): 3394, 1710, 1633, 1456, 1392, 1340, 1294, 1228, 1124, 1024, 745, 699. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 385.1267 ($[M+Na]^+$, $C_{20}H_{18}N_4NaO_3^+$; calc. 385.1271), 361.1299 ($[M-H]^-$, $C_{20}H_{17}N_4O_3^-$; calc. 361.1306).

*2-Amino-1,5-dihydro-5-(1*H*-indol-3-ylmethyl)-4*H*-imidazol-4-one* (**1**). A mixture of **9** (1.000 g, 2.76 mmol) and Pd/C 10% (300 mg, 10 mol-%) in MeOH (55 ml) was hydrogenated at r.t. under atmospheric pressure until TLC showed complete consumption of the starting material (1 h). The catalyst was removed by suction filtration and the filtrate evaporated to dryness. The resultant viscous oil was purified by prep. MPLC (*MN Nucleodur C-18*, *100–12*; MeCN/H_2O (1% ammonia) gradient 10% to 30%, 45 min). Pure alkaloid **1** (0.523 g, 2.29 mmol, 83%) was obtained after *lyophilization* as a white fluffy powder. R_f (*RP-18*, H_2O/MeCN 3 : 1, 1% ammonia) 0.3. $[\alpha]_D^{23} = -6.1$ ($c = 1.00$, MeOH). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 229.1085 ($[M+H]^+$, $C_{12}H_{13}N_4O^+$; calc. 229.1084), 251.0903 ($[M+Na]^+$, $C_{12}H_{12}N_4NaO^+$; calc. 251.0903), 227.0936 ($[M-H]^-$, $C_{12}H_{11}N_4O^-$; calc. 227.0938).

*6-Bromo-*L*-tryptophan* (*=(2*S*)-2-Amino-3-(6-bromo-1*H*-indol-3-yl)propanoic Acid*; **11**). *N*-Acetyl-6-bromo-D,L-tryptophan (**10**, 3.50 g, 10.8 mmol) was dissolved in borate buffer (850 ml) containing $CoCl_2 \cdot 6\ H_2O$ (29.73 mg, 0.125 mmol), and the pH was adjusted with borax soln. to pH = 8.5. L-Aminoacylase (4.15 g, *Amano Enzyme Inc.*, Nagoya, Japan) was added, and the slurry was stirred for 48 h at 38°. The mixture was brought to pH = 5 by addition of 10% HCl and extracted with AcOEt (3 × 500 ml). After drying with $MgSO_4$ and evaporation of the volatiles, crude *N*-acetyl-6-bromo-D-tryptophan was obtained. The residual aq. phase was passed through a column filled with *Dowex* 50 × 2–200 resin (70 × 35 mm) and eluted with MeOH to get rid of surplus enzyme. The desired amino acid **11** was obtained by eluting the column with MeOH/NH_4OH 9 : 1 (750 ml). After removal of the volatiles, the residue was passed through a plug of SiO_2 (70 × 40 mm) by eluting with MeOH/NH_4OH 9 : 1. The eluent containing the desired product was concentrated to dryness. The obtained crude product was purified by MPLC (*MN Nucleodur 100–50-RP 18 ec*, AcOH/H_2O 1 → 100% MeOH) to give **11** (1.34 g, 4.73 mmol, 88%). The yield is based on the maximum of theoretical recovery from the resolution of a racemic mixture (5.40 mmol). $[\alpha]_D^{23} = -18$, $c = 1.0$, MeOH). ^{1}H- and ^{13}C-NMR: *Table 2*.

*(2S)-3-(6-Bromo-1*H*-indol-3-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoic Acid* (**12**). To a suspension of 6-bromo-L-trytophan (**11**, 500 mg, 1.77 mmol) in 1,4-dioxane (5 ml) and H_2O (5 ml), 1M KOH (1.85 ml, 1.85 mmol) was added. After dissolving of the starting material, Boc_2O (443 mg, 2.03 mmol) was added in one portion, and the mixture was stirred for 16 h at r.t. The soln. was adjusted to pH = 4 by slow addition of 1M HCl, diluted with H_2O (50 ml), and extracted with AcOEt (3 × 50 ml). After drying ($MgSO_4$) and removal of all volatiles, the title compound **12** (634 mg, 1.65 mmol, 94%) was obtained which can be used without further purification. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 405.0419 ($[M+Na]^+$, $C_{16}H_{19}{}^{79}BrN_2NaO_4^+$; calc. 405.0420).

tert-Butyl *(5S)-2-Amino-5-[(6-bromo-1*H*-indol-3-yl)methyl]4,5-dihydro-4-oxo-1*H*-imidazole-1-carboxylate* (**13**). A soln. of **12** (290 mg, 757 μmol) in THF (7.5 ml) was cooled to 0° and DCC (161 mg, 780 μmol) and NHS (89.7 mg, 780 μmol) were added. After stirring for 2 h at 0°, the soln. was allowed to warm to r.t. and filtrated into a soln. of NaHNCN (145 mg, 2.27 mmol) in H_2O (7.5 ml). The mixture was stirred at r.t. for 16 h. THF was removed under reduced pressure, the mixture was basified to pH = 12 with 2M KOH, and H_2O (50 ml) was added. After washing with CH_2Cl_2 (2 × 25 ml), the aq. phase was adjusted to pH = 2.2 with 2M HCl and extracted with AcOEt (3 × 50 ml). The combined org. extracts were dried ($MgSO_4$) and all volatiles were removed *in vacuo*. Purification of the crude product by CC (SiO_2; $CHCl_3$/MeOH 9 :1) furnished the title compound **13** (199 mg; 490 μmol, 65%). $[\alpha]_D^{23} = +50.1$ (c = 1.18, MeOH). ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 407.0711 ($[M+H]^+$, $C_{17}H_{20}{}^{79}BrN_4O_3^+$; calc. 407.0713).

*(5S)-2-Amino-5-[(6-bromo-1*H*-indol-3-yl)methyl]-1,5-dihydro-4*H*-imidazol-4-one* (**14**). A soln. of **13** (60.9 mg, 150 μmol) in MeCN (5 ml) was chilled in an ice bath and $SnCl_4$ (189 μl, 1.62 mmol) was added under an inert gas atmosphere. Stirring was continued for 45 min at 0°, and the mixture was partitioned between AcOEt (100 ml) and 1M KOH (100 ml). After separation of both phases, the aq. layer was extracted with AcOEt (3 × 100 ml), and the combined org. phases were dried ($MgSO_4$). All volatiles were removed, and the crude product was purified by MPLC (*MN Nucleodur C-18*, 100–12; MeCN/H_2O (1% ammonia) gradient 10% to 35%, 45 min) to obtain the pure compound **14** (39.6 mg, 129 μmol, 86%). $[\alpha]_D^{23} = -82.0$ (c = 1.13, MeOH). ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 307.0192 ($[M+H]^+$, $C_{12}H_{12}{}^{79}BrN_4O^+$; calc. 307.0189).

*2-Amino-5-[(6-bromo-1*H*-indol-3-yl)methyl]-3,5-dihydro-3-methyl-4*H*-imidazol-4-one* (**2**). To a soln. of **14** (41.2 mg, 134 μmol) in MeCN (30 ml) and EtOH (10 ml), MeI (2.00 ml, 32.1 mmol) was added, and the mixture was stirred at r.t. for 24 h. Evaporation of all volatiles yielded compound **2** as hydroiodide (60.2 mg, 134 μmol, 100%). $[\alpha]_D^{23} = -2$ (c = 0.10, MeOH). ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 321.0348 ($[M+H]^+$, $C_{13}H_{14}{}^{79}BrN_4O^+$; calc. 321.0346).

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