Total Syntheses of (±)-Aerothionin, (±)-Homoaerothionin, and (±)-Aerophobin-1

Shigeru NISHIYAMA and Shosuke YAMAMURA*
Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Kohoku-ku, Yokohama 223
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(±)-Aerothionin, (±)-homoaerothionin, and (±)-aerophobin-1 have been successfully synthesized using phenolic oxidation of the methyl pyruvate oxime derivative with thallium(III) trifluoroacetate as a key step. The stereostructure of aerophobin-2 has been also established by comparison of the ^{1}H NMR spectrum with those of related compounds.

Recently, enlarged synthetic attention has been focused on marine natural products having physiological activities.[1] Among them, several sponges have served attractive bromine-containing metabolites derived from tyrosine, which in biosynthetic pathway seem to involve phenolic oxidation affording dimerization, cyclization and so on.

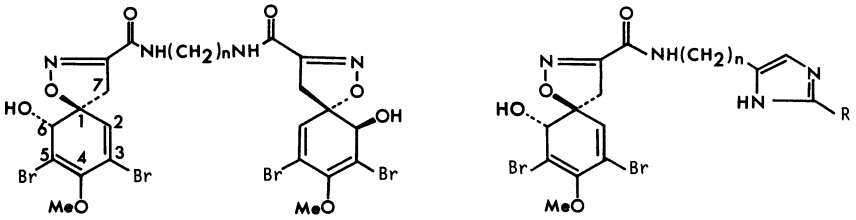
As a part of our approach directed toward total syntheses of these natural products using thallium(III) salt-induced phenolic oxidation as a key step,[2] we have already accomplished total synthesis of bastadins isolated from the sponge *Ianthella basta*.[3] Along this line, our attention turned to aerothionin (**1**),[4] homoaerothionin (**2**),[4] aerophobin-1 (**3**),[5] and -2 (**4**)[5] which are also metabolites of such sponges as *Aplysia fistularis, Verongia thiona* and *V. aerophoba*. Especially, the absolute stereostructure of **1** was established by an X-ray crystallographic analysis coupled with circular dichroism,[6] and its synthetic effort was also made by Thomson *et al.*,[7] wherein sodium borohydride reduction of tetradehydroaerothionin did not yield natural *trans, trans*-aerothionin, but *cis, cis*-aerothionin.

Configurations of the hydroxyl group at C^6-position in aerophobin-1 (**3**) and its congener (**4**) possessing 2-aminohomoistamine moiety instead of histamine in **3** remained unsettled. From a biogenetic point of view, however, they should have the same stereostructures as that of **1**. Accordingly, it was expected that the spiroisoxazoline derivative (**14a**) would be a potent intermediate for syntheses of aerothionin (**1**), homoaerothionin (**2**) and aerophobin-1 (**3**), respectively, and the ketone (**11**) conceivable as a precursor of **14a** might be obtained by the two-electron oxidation process (*i.e.* **9**→**11**) involving attack of a hydroxyimino group to a carbonium ion generated on phenolic oxidation. We describe herein total syntheses of **1, 2,** and **3** as well as determination of aerophobin-2 (**4**).

When a readily accessible azulactone (**5**)[4] was submitted to alkaline hydrolysis followed by oximation and benzylation, the tribenzyl derivative (**6**) was obtained in 31% yield, together with the enamine (**7**) in 14% yield, from which on treatment with the same reaction sequence **6** was further obtained. The structure of **6** could be confirmed by its successful conversion to a known alkaline degradation product of **1**,[4] *N,N'*-bis[3-(3,5-dibromo-2-hydroxy-4-methoxyphenyl)-2-hydroxyiminopropionyl]-1,4-butanediamine (**8**) by treatment with 1,4-butanediamine followed by hydrgenolysis. Furthermore, transesterification of **6** in methanol containing K_2CO_3 afforded the corresponding methyl ester, which on hydrogenolysis led to the desired dihydroxy derivative (**9**) in moderate yield.

The methyl ester (**9**) so far obtained was oxidized with thallium(III) trifluoroacetate (TTFA) in trifluoroacetic acid (room temp, 4 h) to afford **10, 11** and **12** as isolable products in 21, 27, and 3% yields, respectively. The aspect of the TTFA oxidation is compatible, except for the dimer (**12**), with the results reported by Forrester *et al.*,[8] in which they examined several oxidation reactions of phenols [A] using MTA, $Pb(OAc)_4$, Ag_2O, Br_2, and TBCO as oxidant to afford [B] and [C].

As seen in the case of *cis,cis*-aerothionin formation,[7] on treatment of **11** with $NaBH_4$, the undesired **13a** was also obtained in high yield together with trace amount of **14a**. However, evaluation of several reaction conditions provided an improvement to pro-



1 n = 4
2 n = 5
3 n = 2, R = H
4 n = 3, R = NH_2

Fig. 1. Some novel spiroisoxazolines from marine sponges.

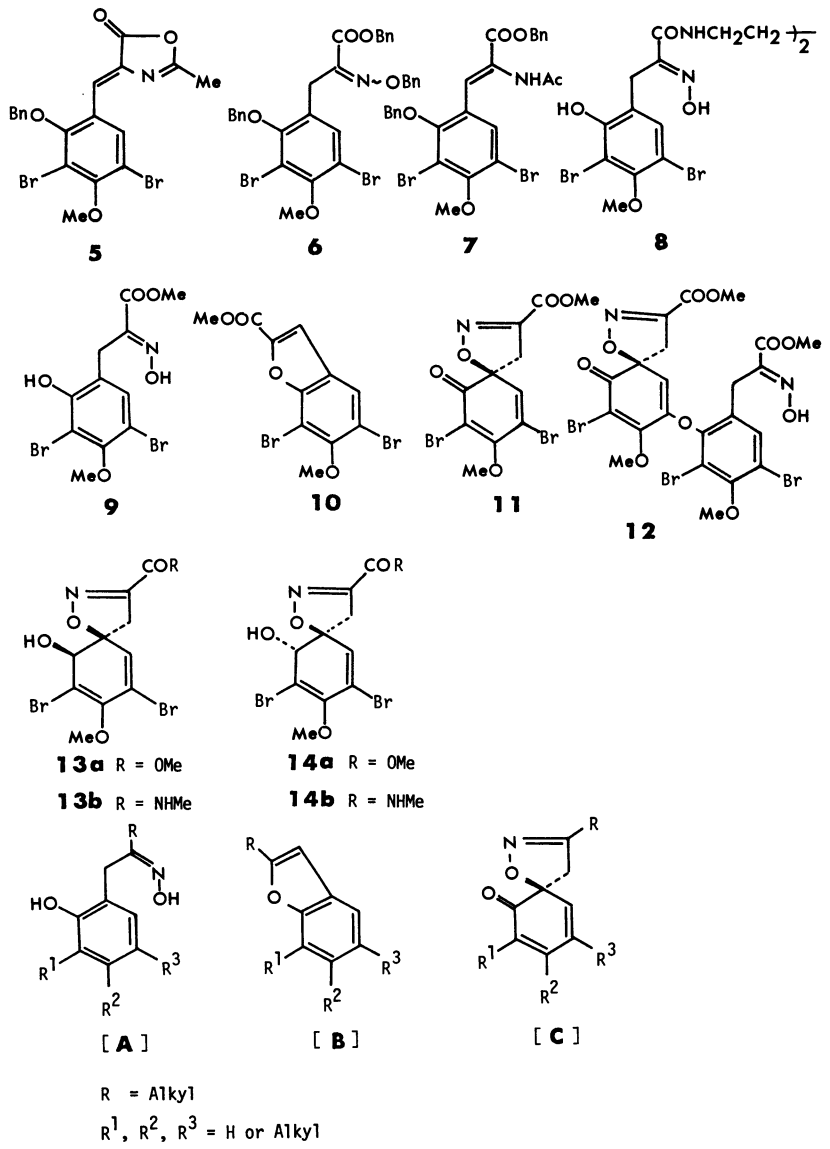


Fig. 2.

duce **14a** in preparative scale. Namely, reduction of **11** was carried out with excess $Zn(BH_4)_2$ in ether (room temp, 7 min) to afford a 1.3:1 mixture of **13a** and **14a**, in 70% yield, which were easily separable on preparative TLC. The stereostructures of **13a** and **14a** could be established on the basis of exhaustive comparison of both ^1H NMR spectra with those of **1** and *cis,cis*-aerothionin which has a cis vicinal relationship between a hydroxyl group and an oxygen atom in the spiroisoxazoline unit, as seen in Table 1.

Finally, amidation of **14a** with 1,4-butanediamine, 1,5-pentanediamine and histamine (room temp, overnight) successfully afforded aerothionin (**1**), homoaerothionin (**2**) and aerophobin-1 (**3**), in 18, 4.4 and 82% yields, respectively. Interestingly, on the other hand, the ester (**13a**), which has the trans relationship between the hydrogen atom attached to the carbon atom bearing the hydroxyl group and the oxygen atom in a spiroisoxazoline ring, was quite unstable to amines. The amides (**1**), (**2**), and (**3**) thus obtained were indistinguishable with authentic samples under the full range of spectral (IR, ^1H NMR, and mass) and chromatographical (TLC and HPLC) means. However, in the cases of **1** and **2**, a possibility that each product consists of a mixture of the natural product and its concomitant diastereomer, could not be ruled out. Unfortunately, up to date we have no reliable solution for it.

The stereostructure of aerophobin-2 could be established as depicted in **4** on the basis of exhaustive comparison of the ^1H NMR spectrum with those of **13b** and **14b**: particularly, the resonances of δ 4.14 and 6.35 ascribed to C^6-H and C^2-H are rather agreeable with those of δ 4.08 and 6.40 in **14b** than those of δ 4.40 and 6.55 in **13b**. Furthermore, while *cis,cis*-aerothionin, **13a** and **13b** have the broad signals attributable to the two geminal protons at C^7-position in their ^1H NMR spectra, the NMR signals due to the corresponding

Table 1. ^{1}H NMR Chemical shifts (δ) of aerothionin (1), *cis,cis*-aerothionin,[7] aerophobin-1 (3), aerophobin-2 (4)[5] and related compounds[a]

Proton	1[b]	*cis,cis*-Aerothionin[b]	3[c]	4[c]	13a[b]	14a[b]	13b[c]	14b[c]
C^2-H	6.53	6.60	6.40	6.35	6.58	6.52	6.55	6.40
C^6-H	4.18 (7.5)	4.52 (7.5)	4.08	4.14	4.53 (7.5)	4.22 (7.5)	4.40	4.08
C^6-OH	5.40 (7.5)	4.97 (7.5)			4.98 (7.5)	5.38 (7.5)		
C^7-H	3.15 (18)	3.2—3.5	3.03 (18)	3.20 (17.5)	3.42	3.17 (18)	3.3—3.4	3.05 (18)
	3.85 (18)		3.77 (18)	3.85 (17.5)		3.85 (18)		3.78 (18)
OMe	3.77	3.73	3.73	3.79	3.68[d]	3.73[d]	3.75	3.73
NMe							2.85	2.83
$-CH_2-$	1.62	1.62	2.83 (7.5)	2.01 (7)				
				2.67 (7)				
Imidazole			6.90	6.47				
			7.67					
$OCNCH_2-$	3.2—3.5	3.2—3.5	3.52 (7.5)	3.43 (7)				
NH	7.60	7.55						
COOMe					3.70[d]	3.83[d]		

a) Coupling constants (in Hz) are given in parentheses. b) Acetone-d_6 as solvent. c) Methanol-d_4 as solvent. d) May be reversed.

protons in **1**, **2**, **3**, **14a**, and **14b** are observed as two doublets because of an interaction of the hydroxyl group with one of the geminal protons. Accordingly, it is reasonable that aerophobin-2 (**4**) exhibiting two doublets at δ 3.20 and 3.85 should possess the same structure unit as those of the latter compounds.

Experimental

All the melting points were obtained on a Mitamura Riken melting point apparatus and uncorrected. IR spectra were recorded on a JASCO Model A-202 spectrophotometer. ^{1}H NMR spectra were obtained on a Varian EM-390 NMR spectrometer (90 MHz) using tetramethylsilane as an internal standard. High resolution mass spectra were obtained on a Hitachi M-80 GC-MS spectrometer operating with an ionization energy (70 eV). Preparative and analytical TLC were carried out on silica-gel plates (Kieselgel 60 F_{254}, E. Merck A.G. Germany) using UV light and/or 5% molybdophosphoric acid in ethanol for detection, unless otherwise stated. HPLC were performed on a JASCO Trirotar-II apparatus using a 4.6×250 mm Develosil 60-3 column with hexane–AcOEt (v/v 1:1) at 1 ml/min flow rate.

Benzyl 2-Benzyloxyimino-3-(2-benzyloxy-3,5-dibromo-4-methoxyphenyl)propionate (6). The azulactone (**5**)[4] (8.22 g) was hydrolyzed at 100 °C for 9 h with KOH (11 g) in dioxane (50 ml)–H_2O (50 ml). After addition of hydroxylamine hydrochloride (3.7 g), the reaction mixture was allowed to stand at room temperature overnight, and then acidified with 2M HCl(1M=1 mol dm^{-3}) and extracted with AcOEt. The AcOEt layer was washed with brine, dried over anhydrous Na_2SO_4, and then evaporated. The residue was treated with benzyl chloride (6 ml) and K_2CO_3 (6 g) in DMF (50 ml) at room temperature overnight. The mixture was partitioned between AcOEt and H_2O, and then the AcOEt layer was washed with brine, dried over anhydrous Na_2SO_4, and then evaporated under reduced pressure to leave a crude syrup, which was submitted to silica-gel column chromatography (250 g) using benzene as eluant to give **6** (3.42 g) and **7** (1.4 g). **6**: mp 83—85 °C from hexane–AcOEt; IR (film) 1720, 1600, and 1580 cm^{-1}: ^{1}H NMR ($CDCl_3$) δ=3.85(3H, s), 3.88(2H, s), 4.88(2H, s), 5.12(2H, s), 5.22(2H, s), and 7.2—7.5(16H, complex); Found: *m/z* 651.0244. Calcd for $C_{31}H_{27}NO_5{}^{79}Br_2$: M, 651.0254. **7**: mp 159—160 °C from hexane–AcOEt; IR (film) 3250, 1730, 1660, 1575, and 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=1.93(3H, s), 3.90(3H, s), 4.90(2H, s), 5.25(2H, s), 7.2—7.5(10H, complex), and 7.55 (1H, s); Found: *m/z* 586.9952. Calcd for $C_{26}H_{23}NO_5{}^{79}Br_2$: M, 586.9943.

Methyl 2-Hydroxyimino-3-(3,5-dibromo-2-hydroxy-4-methoxyphenyl)propionate (9). A mixture of the tribenzyl derivative (2.16 g) and K_2CO_3 (0.91 g, 2 equiv mol) in dioxane (10 ml)–MeOH (10 ml) was stirred at room temperature for 40 min. The mixture was partitioned between AcOEt and H_2O, and the organic layer was washed with 2M HCl and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was hydrogenated at room temperature overnight in the presence of catalytic Pd–C (10%) in dioxane (20 ml)–AcOH (20 ml) to afford the methyl ester (**9**) (0.97 g): mp 148—149 °C from hexane–AcOEt; IR (film) 3300, 1730, 1700 sh., 1650, 1590, and 1550 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=3.85 (3H, s), 3.88(3H, s), 3.92(2H, s), and 7.42(1H, s); Found: *m/z* 396.8984. Calcd for $C_{11}H_{11}NO_5{}^{79}Br^{81}Br$: M, 396.9005.

N,N′-Bis[3-(3,5-dibromo-2-hydroxy-4-methoxyphenyl)-2-hydroxyiminopropionyl]-1,4-butanediamine (8). A mixture of **6** (2.76 g, 4 mmol) and 1,4-butanediamine (0.16 ml, 1.6 mmol) was kept at room temperature for 6 d, and then heated at 60 °C for 6 h. The resulted syrup was passed through a silica-gel column (20 g) using hexane–AcOEt (2:1) as eluant to afford the desired amide (1.63 g): mp 108—110 °C from hexane–AcOEt. A 857 mg portion of the product was submitted to hydrogenolysis in the presence of catalytic Pd–C (10%) in dioxane (40 ml)–AcOH (40 ml) at room temperature for 3 d to yield **8** (564 mg): mp 189—190 °C from ether–acetone [lit,[4] mp 188.5—189.5 °C].

TTFA Oxidation of 9. To a solution of TTFA (1.33 g, 2.4 mmol) in TFA (30 ml) was added the ester (**9**) (0.43 g, 1.1 mmol) in TFA (10 ml). The mixture was stirred at room temperature for 4 h, and then partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give a brownish residue, which was further separated by preparative TLC using hexane–AcOEt (2:1) to afford **10** (81 mg), **11** (114 mg), and **12** (12 mg), respectively. The physical data of these products are as follows: **10**: mp 141.5—143 °C from hexane–AcOEt; IR (film) 1720 sh., 1600, 1575, and 1550 cm^{-1};

^{1}H NMR (acetone-d_6) δ=3.97(6H, s), 7.70(1H, s), and 8.07(1H, s); Found: m/z 363.8763. Calcd for $C_{11}H_8O_4{}^{79}Br^{81}Br$: M, 363.8768. **11** as a colorless oil: IR (film) 1710, 1670, 1585, and 1530 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=3.27(1H, d, J=18Hz), 3.65(1H, d, J=18Hz), 3.93(3H, s), 4.20(3H, s), and 6.78(1H, s); Found: m/z 394.8809. Calcd for $C_{11}H_9NO_5{}^{79}Br^{81}Br$: M, 394.8826. **12** as a colorless oil: IR (film) 3300, 1725, 1680, 1650 sh., 1595, and 1555 cm^{-1}: ^{1}H NMR ($CDCl_3$) δ=3.10(1H, d, J=18Hz), 3.57(1H, d, J=18Hz), 3.80(3H, s), 3.83(3H, s), 3.90(3H, s), 3.8—3.9(2H, overlapped with MeO signals), 4.32(3H, s), 4.92(1H, s), and 7.50(1H, s); Found: m/z 712. Calcd for $C_{22}H_{19}N_2O_{10}{}^{79}Br^{81}Br$: M, 712. The molecular ion peak at m/z 712 has not been detected by high resolution mass spectrum, but observed in quite low intensity on measurement of its normal mass spectrum.

Reduction of 11 with Zinc Borohydride. The dienone (**11**) (103 mg) in CH_2Cl_2 (3 ml) was treated, at room temperature for 7 min, with excess $Zn(BH_4)_2$ in ether (2 ml), and then the mixture was quenched as usual to give a crude product, which was separated by preparative TLC using hexane–AcOEt (5:1) to afford **13a** (41 mg) and **14a** (30 mg). Their physical data are as follows: **13a** as a syrup: IR (film) 3470, 1730, 1620, 1595, and 1575 cm^{-1}; Found: m/z 394.8987. Calcd for $C_{11}H_{11}NO_5{}^{79}Br_2$: M, 394.9002. **14a** as a syrup: IR (film) 3450, 1725, 1630, 1590, and 1580 cm^{-1}; Found: m/z 396.8979. Calcd for $C_{11}H_{11}NO_5{}^{79}Br^{81}Br$: M 396.8983.

Synthesis of Aerothionin (1). A mixture of **14a** (19 mg, 0.05 mmol) and 1,4-butanediamine (2 µl, 0.02 mmol) was allowed to stand at room temperature overnight. Direct separation of the mixture by preparative TLC [benzene–acetone (5:1)] afforded aerothionin (**1**) (3 mg, 18% yield based on 1,4-butanediamine) and unreacted **14a** (7.2 mg). The IR and ^{1}H NMR spectra of the synthetic compound (**1**) were completely identical with those of an authentic sample, and the both aspects in HPLC (retention time, 7 min) were indistinguishable.

Synthesis of Homoaerothionin (2). The ester (**14a**) (8 mg, 0.02 mmol) was treated with 1,5-pentanediamine (1 µl, 0.009 mmol) according to essentially the same procedure as described above to yield homoaerothionin (**2**) (0.3 mg, 4.4%) and unreacted **14a** (7.5 mg). The IR, ^{1}H NMR, and HPLC (retention time, 6.5 min) of the product were identical with those of an authentic sample.

Synthesis of Aerophobin-1 (3). A mixture of **14a** (4.5 mg, 0.01 mmol) and histamine (12.5 mg, 0.1 mmol) in MeOH (0.1 ml)–dioxane (0.1 ml) was kept at room temperature overnight, and then directly purified by preparative TLC using $CHCl_3$–MeOH (3:1) to afford aerophobin-1 (**3**) (4.4 mg, 82%) (IR and ^{1}H NMR spectra).[5] Further acetylation of the synthetic compound (**3**) was carried out using Ac_2O–pyridine (1:1) as usual to afford the corresponding *O*-acetyl derivative, whose spectral data were superimposable with those of an authentic sample.

Syntheses of 13b and 14b. A mixture of **6** (1.06 g) and methylamine (40% aq solution, 1.5 ml) in THF (10 ml) was kept at room temperature for 13 h. The resulted mixture was acidified with 2M HCl, and then partitioned between AcOEt and H_2O. The organic layer was washed with aq $NaHCO_3$ and dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the crystalline residue was recrystallized from hexane–AcOEt to give the corresponding amide (0.6 g, 65%): mp 112—113 °C. In the next step, a 544 mg portion of the amide was hydrogenated at room temperature for 8 h in dioxane (15 ml)–AcOH (15 ml) in the presence of catalytic Pd–C (10%) to afford the hydroxy derivative (272 mg, 73%): mp 143—145 °C from hexane–AcOEt. According to essentially the same procedure as described in **9** the hydroxy amide (450 mg, 1.1 mmol) was oxidized with TTFA (1.26 g, 2.3 mmol) in TFA to give the spiroisoxazoline derivative [41 mg, 9.2% (mp (dec)<170 °C)] as well as the benzofuran (14 mg, 3.4%) and the dimer (98 mg, 24%).

To a solution of the spiroisoxazoline derivative (16 mg) in CH_2Cl_2 was added excess $Zn(BH_4)_2$. After stirring at room temperature for 1.5 h, the reaction mixture was quenched by usual manner. The crude product was separated by preparative TLC using hexane–AcOEt (1:1) to give **13b** (4.6 mg, 29%) and **14b** (4.2 mg, 27%), the physical data of which were shown below: **13b**: mp 182—185 °C (dec.) from hexane–AcOEt; IR (film) 3350, 1640, 1600, 1565, and 1545 cm^{-1}; Found: m/z 393.9172. Calcd for $C_{11}H_{12}N_2O_4{}^{79}Br_2$: M, 393.9164. **14b** as a syrup: IR (film) 3360, 1660, 1630 sh., 1595, 1580, and 1550 cm^{-1}; Found: m/z 395.9141. Calcd for $C_{11}H_{12}N_2O_4{}^{79}Br^{81}Br$: M, 395.9142.

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