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Tauroacidins A and B, New Bromopyrrole Alkaloids Possessing a Taurine Residue from *Hymeniacidon* Sponge

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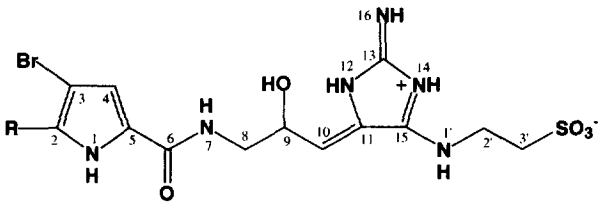
Abstract: Two new bromopyrrole alkaloids, tauroacidins A (**1**) and B (**2**), with tyrosine kinase inhibitory activity have been isolated from an Okinawan marine sponge *Hymeniacidon* sp. and the structures were elucidated on the basis of spectral data and chemical means. Tauroacidins A (**1**) and B (**2**) are rare bromopyrrole alkaloids possessing a taurine residue. © 1997 Elsevier Science Ltd.

During our search for bioactive substances from marine organisms,[1] we have investigated extracts of an Okinawan marine sponge *Hymeniacidon* sp. and isolated two new bromopyrrole alkaloids possessing a taurine residue, tauroacidins A (**1**) and B (**2**), with tyrosine kinase inhibitory activity. Here we describe the isolation and structure elucidation of **1** and **2**.

n-BuOH-soluble materials of MeOH extract of the sponge collected off Ishigaki Island, Okinawa, were subjected to Sephadex LH-20 (MeOH) and C_{18} columns ($CH_3CN/H_2O/CF_3CO_2H$) followed by C_{18} HPLC ($MeOH/H_2O/CF_3CO_2H$) to yield tauroacidins A (**1**, 8 x 10^{-5} %, wet weight) and B (**2**, 5 x 10^{-5} %).

HRESIMS [*m/z* 526.9164, $(M-H)^-$, Δ +0.9 mmu] of tauroacidin A {**1**, $[\alpha]_D^{28}$ -4.3° (*c* 0.15 MeOH)}, indicated the molecular formula to be $C_{13}H_{16}N_6O_5SBr_2$. The IR spectrum of **1** suggested the presence of OH and/or NH (3420 cm^{-1}), amide carbonyl (1690 cm^{-1}), and sulfonate groups (1210 and 1040 cm^{-1}).[2] The UV absorption [λ_{max} 273 nm (ε 9700)] was attributable to a substituted pyrrole chromophore.[3] The ^1H and ^{13}C NMR data showed signals due to a 2,3-dibromopyrrole carbonyl moiety.[4] The ^{13}C chemical shifts at C-2' (δ_C 39.8) and C-3' (δ_C 49.1) corresponded well to those of the taurine residue (ca. δ_C 39 and 48, respectively) of melemeleone A.[5] The presence of the taurine residue in **1** was also supported by amino acid analysis of the acid hydrolysis products of **1**. The whole structure of tauroacidin A (**1**) was elucidated by analyses of 2D NMR data [^1H-^1H COSY, NOESY, and decoupled HMBC (D-HMBC)[6]] (Fig. 1). The ^1H-^1H COSY spectrum of **1** showed proton networks from NH-7 to H-10 and OH-9 and from NH-1' to H_2-3'. The presence of the aminoimidazole ring including three quaternary carbons (C-11, C-13, and C-15) was revealed



1 R = Br (9*S*/9*R* = 6 : 4)
2 R = H (9*S*/9*R* = 1 : 1)

Table 1. ^{1}H and ^{13}C NMR Data of Tauroacidins A (**1**) and B (**2**) in DMSO-d_6.

positn.	**1** δ_H (m, J [a])	**1** δ_C (m)	**2** δ_H (m, J [a])	**2** δ_C (m)
1.	12.70 (s)		11.84 (br.s)	
2.		104.7 (s)	6.99 (br.s)	121.3 (d)
3.		97.8 (s)		94.9 (s)
4.	6.95 (s)	113.0 (d)	6.87 (br.s)	111.8 (d)
5.		127.9 (s)		126.7 (s)
6.		159.1 (s)		159.9 (s)[d]
7.	8.22 (t, 6.0)		8.20 (t, 6.2)	
8.	3.32 (m)[b,c]	44.5 (t)	3.34 (m)[b,c]	44.3 (t)
9.	4.61 (m)	67.3 (d)	4.62 (m)	67.2 (d)
9-OH.	5.94 (d, 4.2)		5.95 (br.s)	
10.	6.17 (d, 4.2)	116.4 (d)	6.18 (m)	116.5 (d)
11.		131.2 (s)		131.1 (s)
12.	9.31 (br.s)		9.32 (br.s)	
13.		165.9 (s)		165.8 (s)[c]
14.	10.51 (s)		10.50 (s)	
15.		167.6 (s)		167.6 (s)[c]
16.	8.05 (br.s)		8.05 (br.s)	
1'	9.64 (br.t, 3.0)		9.65 (m)	
2'	3.67 (dt, 3.0, 7.2)[c]	39.8 (t)	3.67 (m)[c]	39.5 (t)[e]
3'	2.75 (t, 7.2)[c]	49.1 (t)	2.74 (t, 7.5)[c]	49.2 (t)

a) in Hz. b) These signals were overlapped with H_2O signal. c) 2H. d) These signals were assigned on the basis of D-HMBC correlations. e) This signal was overlapped with DMSO signal.

by the D-HMBC cross-peaks for H-10/C-15, NH-14/C-13, and NH-14/C-15. D_2O-Exchangeable protons at δ_H 9.31 (NH-12) and 8.05 (NH-16) were assigned by NOESY correlations for H-9/NH-12 and NH-12/NH-16. Attachment of the pyrrole ring to N-7 via an amide bond was deduced from the NOESY cross-peak for H-4/NH-7 and D-HMBC correlations for H-4/C-6 and NH-7/C-6. The NOE for NH-1'/H-10 indicated *Z*-geometry of the double bond at C-10. The D-HMBC cross-peaks for NH-1'/C-11 and NH-1'/C-15 and the NOE for NH-1'/H-10 implied that the taurine residue was attached to C-15 of the imidazole ring. The negative ion FAB MS/MS spectrum of the pseudomolecular ion (*m/z* 527) of **1** showed several product ions supporting the proposed structure (Fig. 2). Thus the structure of tauroacidin A was concluded to be **1**. In order to dertermine the absolute configuration at C-9, tauroacidin A (**1**) was subjected to ozonolysis and then hydrolysis.[7] Amino acid analysis of the acid hydrolysate showed the presence of one molar equivalent of isoserine. The hydrolysate was treated with HCl/*i*-PrOH and then (*S*)-MTPACl to afford the *N,O*-bis-(*R*)-MTPA/*i*-propyl ester of isoserine, of which HPLC analyses revealed the presence of (*S*)- and (*R*)-isoserines in the ratio of ca. 6:4, showing that tauroacidin A (**1**) was a 6:4 mixture of 9*S*- and 9*R*-isomers.

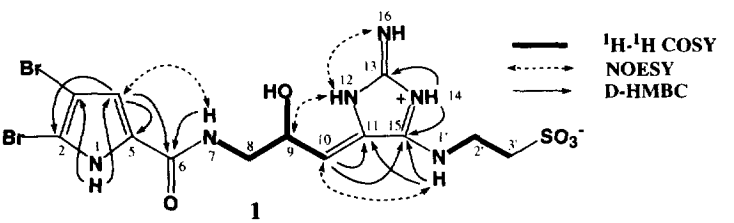


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Fig. 1. 2D NMR Correlations for Tauroacidin A (**1**)

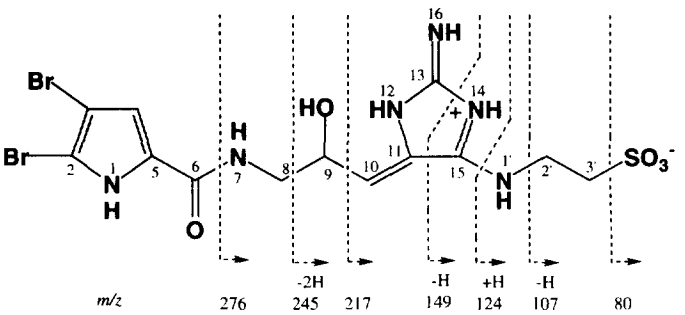

Fig. 2. Negative FABMS/MS Fragmentations of *m/z* 525 from Tauroacidins A (**1**)

The molecular formula, $C_{13}H_{17}N_6O_5SBr$, of tauroacidin B (**2**), optically inactive, was suggested by HRESIMS. The ^1H and ^{13}C NMR data of **2** differed from **1** only in the presence of an sp^2 methine signal [H-2: δ_H 6.99 (m), C-2: δ_C 121.3 (d, $^1J_{CH}$ = 185 Hz)], indicating the presence of 3-bromopyrrole carbonyl moiety[4,8] in **2**. Thus the structure of tauroacidin B (**2**) was elucidated to be 2-debromo form of **1**. Tauroacidin B (**2**) was revealed to be racemic (9*S*/9*R* = 1:1) at C-9 from HPLC analyses of the *N,O*-bis-(*R*)-MTPA/*i*-propyl ester of isoserine contained in the hydrolysate of **2** obtained by the same procedure as **1**.

Tauroacidins A (**1**) and B (**2**) are new bromopyrrole alkaloids possessing a taurine residue attached to the aminoimidazole ring, which may be biogenetically related to mauritamide A, known bromopyrrole alkaloids poessessing a taurine residue at C-11 from sponges.[9] Tauroacidins A (**1**) and B (**2**) exhibited inhibitory activity against EGF receptor kinase and *c-erbB*-2 kinase (IC_{50}, 20 μg/mL each).

EXPERIMENTAL

General Procedure. Optical rotations were recorded on a JASCO DIP-360 polarimeter. The IR and UV spectra were taken on a JASCO FT/IR-5300 and a JASCO Ubest-35 spectrophotometers, respectively. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 and a JEOL EX-400 spectrometers, respectively. ESI mass spectra were obtained on a JEOL SX-102A spectrometer. Standard amino acid analyses were performed with Hitachi amino acid autoanalyzer Model 835.

Sponge Materials. The sponge *Hymeniacidon* sp. (order Halichondrida; family Halichondriidae) was collected off Ishigaki Island, Okinawa, and kept frozen until used. Soft and compressible sponge is somewhat fleshy texture. No ectosomal skeleton is just a thickening in this region of the mesohyl. Spicules are in loose tracts or without orientation. Spicules are styles 439 x 12 μm that are curved along the upperthird of the shaft. The voucher specimen (SS-361) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (5.5 kg, wet weight) was extracted with methanol (3 L x 2). Part (100 g) of the methanolic extract (335.8 g) was partitioned between ethyl acetate (500 mL x 3) and water, and the aqueous layer was extracted with *n*-butanol (1 L x 3). The *n*-butanol soluble material (18.6 g) was subjected to a Sephadex LH-20 column (MeOH), a C_{18} column (Develosil ODS-LOP, Nomura Chemical, 45 x 490 mm; $CH_3CN/H_2O/CF_3CO_2H$, 20:80:0.1), and C_{18} HPLC (Develosil ODS-HG-5, Nomura Chemical, 10 x 250 mm; $MeOH/H_2O/CF_3CO_2H$, 46:54:0.1; flow rate, 2.5 mL/min; UV detection at 260 nm) to yield tauroacidins A (**1**, 8 x 10^{-5} % wet weight, t_R 30 min) and B (**2**, 5 x 10^{-5} %, t_R 18 min).

Tauroacidin A (1). A colorless amorphous solid; $[\alpha]_D^{28}$ -4.3° (*c* 0.15, MeOH); UV (MeOH) λ_{max} 273 (ε 9700) and 312 nm (3800); IR (film) ν_{max} 3420 (br), 1690, 1630, 1210, and 1040 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); ESIMS (Neg., MeOH) *m/z* 525, 527, and 529 [$(M-H)^-$, ca. 1:2:1]; HRESIMS *m/z*

526.9164 (M-H)$^-$, calcd for $C_{13}H_{15}N_6O_5S^{79}Br^{81}Br$, 526.9173.

Tauroacidin B (2). A colorless amorphous solid; $[\alpha]_D^{28}$ 0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 272 (ε 10000) and 310 nm (4000); IR (film) ν_{max} 3420 (br), 1690, 1630, 1210, and 1040 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); ESIMS (Neg., MeOH) *m/z* 447 and 449 [(M-H)$^-$, ca. 1:1]; HRESIMS *m/z* 447.0098 (M-H)$^-$, calcd for $C_{13}H_{16}N_6O_5S^{79}Br$, 447.0088.

Hydrolysis of Tauroacidins A (1). Tauroacidin A (**1**, 0.1 mg) was treated with 6 N HCl (100 μL) at 110 °C for 24 h. After evaporation of the slovent, standard amino acid analysis of the reaction mixture showed the presence of 1 mole of taurine.

Absolute Stereochemistry at C-9 of Tauroacidins A (1) and B (2). A solution of tauroacidin A (**1**, 0.2 mg) in MeOH (100 μL) was treated with ozone at -78 °C for 2 min. After excess ozone was removed by N_2 gas, to the residue were added HCO_2H (100 μL) and 35 % H_2O_2 aq (100 μL). The mixture was stirred for 1 h at 0 °C and then 5 h at room temperature. After evaporation, the residue was hydrolyzed with 6 N HCl (100 μL) at 110 °C for 24 h in a sealed tube. Standard amino acid analysis of the hydrolysate showed the presence of 1 mole each of taurine and isoserine. The hydrolysate was treated with 9 % HCl/*i*-PrOH (100 μL) at 110 °C for 30 min, and then treated with CH_2Cl_2 (100 μL), 4-(*N,N*-dimethylamino)pyridine (0.1 mg), Et_3N (10 μL), and (*S*)-MTPACl (5 μL) at 40 °C for 2 h. After addition of *N,N*-dimethyl-propane-1,3-diamine (5 μL), the reaction mixture was subjected to HPLC analysis [YMC Pack SIL-06, 4.6 x 250 mm; flow rate, 1 mL/min; UV detectoin at 240 nm; eluent, hexane/*i*-PrOH, 95:5]. Retention times of *N,O*-bis-(*R*)-MTPA/*i*-propyl ester derivatives of authentic (*S*)- and (*R*)-isoserine were 13.4 and 16.3 min, respectively. Both (*S*)- and (*R*)-isoserine derivatives were found in the hydrolysate of **1** in the ratio of ca. 6:4. HPLC analysis of *N,O*-bis-(*R*)-MTPA-isoserine *i*-propyl ester derived from the hydrolysate of tauroacidin B (**2**) was performed under the same condition as described above, and both (*S*)- and (*R*)-isoserine derivatives were found in the ratio of ca. 1:1.

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