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Eudistomidin G, a new β-carboline alkaloid from the Okinawan marine tunicate *Eudistoma glaucus* and structure revision of eudistomidin B

Yohei Takahashi, Haruaki Ishiyama, Takaaki Kubota, Jun'ichi Kobayashi *

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

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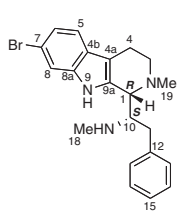
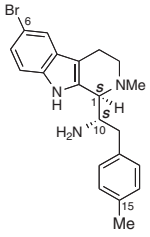
ABSTRACT

A new β-carboline alkaloid, eudistomidin G (**1**), has been isolated from the Okinawan marine tunicate *Eudistoma glaucus*, and the structure was elucidated from spectroscopic data. Furthermore, the structure of eudistomidin B (**2**), which has been isolated from the same tunicate, was revised from **2a** to **2b** by detailed analyses of spectroscopic data. Asymmetric synthesis of the revised structure (**2b**) of eudistomidin B (**2**) and its (1*S*,10*S*)-diastereomer (**2c**) has been accomplished with the Noyori catalytic asymmetric hydrogen-transfer reaction. The absolute configuration of eudistomidin B (**2**) was confirmed to be **2b** possessing (1*R*,10*S*)-configuration, from comparison of the ^{1}H NMR data, CD spectra, $[\alpha]_D$ values, and HPLC analysis of **2b**, **2c**, and natural eudistomidin B.

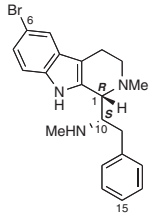
Marine tunicates are well known to be a rich source of secondary metabolites with interesting structures and various biological activities.[1] Among them, many β-carboline alkaloids have been isolated from tunicates of the genus *Eudistoma* so far.[2] In our search for new metabolites from marine tunicates, a new β-carboline alkaloid, eudistomidin G (**1**), has been isolated from the Okinawan tunicate *Eudistoma glaucus* together with a known related β-carboline alkaloid, eudistomidin B (**2**). In this Letter, we describe the isolation and structure elucidation of eudistomidin G (**1**) and structure revision of eudistomidin B (**2**) from **2a** to **2b** as well as total synthesis of **2b**.



eudistomidin G (**1**)



proposed structure (**2a**) of eudistomidin B (**2**)



revised structure (**2b**) of eudistomidin B (**2**)

According to the previous purification procedure,[3] fractions containing eudistomidins were purified by repeated reversed-phase HPLC to afford eudistomidins G (**1**) and B (**2**).

Eudistomidin G (**1**)[4] was obtained as an optically active amorphous solid. The ESIMS spectrum of eudistomidin G (**1**) showed the pseudomolecular ion peaks at *m*/*z* 398 and 400 (1:1), indicating the presence of a bromine atom, and the molecular formula, $C_{21}H_{24}N_3{}^{79}Br$, was established by HRESIMS data (*m*/*z* 398.1235 $[M+H]^+$, Δ +0.9 mmu). UV absorptions (λ_{max} 296, 286, and 231 nm) of **1** implied the presence of tetrahydro-β-carboline ring, while the existence of NH functionality was suggested by the IR absorptions (3211 and 3049 cm^{-1}). The ^{1}H NMR (Table 1) spectrum of **1** included signals due to one NH (δ_H 11.12), eight aromatic protons (δ_H 7.73–6.59), two sp^3 methines (δ_H 5.14 and 4.13), six sp^3 methylenes (δ_H 3.28–2.42), and two *N*-methyls (δ_H 2.92 and 2.87), while the ^{13}C NMR (Table 1) spectrum of **1** showed signals due to six sp^2 quaternary carbons, eight sp^2 methines, two sp^3 methines, three sp^3 methylenes, and two sp^3 methyls.

Connectivities of C-1 to C-10, C-3 to C-4, C-5 to C-6, C-10 to C-11, and C-13 to C-17 were deduced from the ^{1}H–^{1}H COSY, TOCSY, and HMQC spectra of **1** (Fig. 1). HMBC correlations for H-5 to C-7 and C-8a, H-6 to C-4b, H-8 to C-7, NH-9 to C-4b, and H-1 to C-9a, and NOESY correlations for H-1/NH-9, H-4/H-5, and H-8/NH-9 revealed the presence of a 1,2,7-trisubstituted tetrahydro-β-carboline ring. ^{13}C chemical shifts of C-6–C-8 (δ_C 123.88, 117.67, and 115.40, respectively) suggested that a bromine atom was attached to C-7, while the connection of N-2 and C-19 was disclosed by ^{1}H and ^{13}C chemical shifts for CH_3-19 (δ_H 2.92; δ_C 39.79) and HMBC correlations for H-19 to C-1 and C-3. The HMBC cross-peak of H-18 to C-10 and ^{1}H and ^{13}C chemical shifts for CH-10 (δ_H 4.13; δ_C 63.80) and CH_3-18 (δ_H 2.87; δ_C 32.45) indicated that CH_3-18 was attached to C-10 via 10-NH. H-11 showed HMBC correlations for C-12 and C-13 (C-17), suggesting the connectivity of

* Corresponding author. Tel.: +81 11 706 3239; fax: +81 11 706 4989.
E-mail address: jkobay@pharm.hokudai.ac.jp (J. Kobayashi).

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Table 1
^{1}H and ^{13}C NMR data for eudistomidin G (**1**) in $CDCl_3$

Position	δ_C		δ_H	
1	63.83[c]	CH	5.14	(d, 3.2)
3	45.65	CH_2	3.28	(dd, 13.0, 6.0)
			2.95[e]	(m)
4	15.01	CH_2	2.87[e]	(m)
			2.42	(dd, 16.3, 5.2)
4a	105.98	C		
4b	123.82[d]	C		
5	119.53	CH	7.23	(d, 8.5)
6	123.88[d]	CH	7.27	(dd, 8.5, 1.6)
7	117.67	C		
8	115.40	CH	7.73	(d, 1.6)
8a	137.53	C		
9			11.12	(s)
9a	121.62	C		
10	63.80[c]	CH	4.13	(m)
11	33.06	CH_2	3.24	(dd, 15.0, 4.8)
			3.09	(dd, 15.0, 9.7)
12	134.12	C		
13, 17	128.46	CH	6.59[a]	(d, 7.5)
14, 16	128.86	CH	7.04[a]	(dd, 7.5, 7.5)
15	127.77	CH	7.12	(dd, 7.5, 7.5)
18	32.45	CH_3	2.87[b]	(s)
19	39.79	CH_3	2.92[b]	(s)

[a] 2H.
[b] 3H.
[c,d] Interchangeable.
[e] *J*-values were not determined since overlapping with other signals.

Table 2
^{1}H and ^{13}C NMR data for the revised structure (**2b**) of eudistomidin B (**2**) in $CDCl_3$

Position	δ_C		δ_H	
1	63.63	CH	5.07	(d, 2.9)
3	45.85	CH_2	3.30	(m)
			2.94[c]	(m)
4	14.92	CH_2	2.86[c]	(m)
			2.43	(br d, 10.5)
4a	105.59	C		
4b	126.61	C		
5	121.06	CH	7.54	(s)
6	113.71	C		
7	127.06	CH	7.41	(d, 8.5)
8	113.82	CH	7.43	(d, 8.5)
8a	135.53	C		
9			11.19	(s)
9a	122.09	C		
10	63.86	CH	4.01	(m)
11	33.37	CH_2	3.22	(m)
			3.07	(dd, 15.0, 9.4)
12	133.82	C		
13, 17	128.46	CH	6.61[a]	(d, 7.5)
14, 16	128.93	CH	7.07[a]	(dd, 7.5, 7.5)
15	127.93	CH	7.15	(dd, 7.5, 7.5)
18	32.54	CH_3	2.85[b]	(s)
19	39.81	CH_3	2.90[b]	(s)

[a] 2H.
[b] 3H.
[c] Interchangeable.

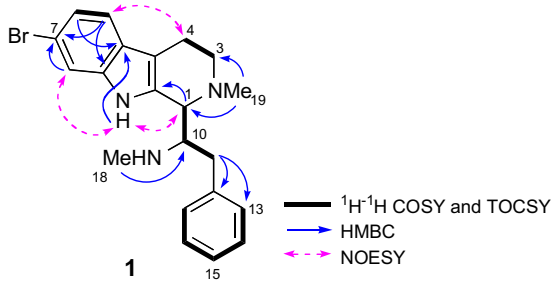


Figure 1. Selected 2D NMR correlations for eudistomidin G (**1**).

C-11 and C-12. Thus, the gross structure of eudistomidin G was elucidated to be **1**.

The relative stereochemistry of **1** was not able to be elucidated from the $^3J_{H\text{-}1/H\text{-}10}$ value (3.2 Hz) and NOESY correlations of H-1/H-10, H-10/H-19, and NH-9/H-11b. The absolute configuration at C-1 in **1** was deduced as *R* from a positive Cotton effect at 228 nm ($\Delta\varepsilon$ +5.6) in the CD spectrum of **1**.[5]

The structure of eudistomidin B (**2**) was reinvestigated, since the total synthesis of the proposed structure (**2a**) of eudistomidin B (**2**) revealed that the NMR data of the synthetic compound were inconsistent with those of natural eudistomidin B (**2**).[7] The molecular fomula of eudistomidin B (**2**)[8] was shown to be $C_{21}H_{24}N_3{}^{79}Br$ (*m*/*z* 398.1235 $[M+H]^+$, Δ +0.9 mmu), and UV and IR absorptions of **2** were the same as those reported previously.[3] The ^{1}H NMR (Table 2) spectrum of **2** measured in $CDCl_3$ showed signals due to one NH (δ_H 11.19), eight aromatic protons (δ_H 7.54–6.61), two sp^3 methines (δ_H 5.07 and 4.01), six sp^3 methylenes (δ_H 3.30–2.43), and two methyls (δ_H 2.90 and 2.85). The ^{13}C NMR (Table 2) spectrum of **2** included 19 signals derived from 21 carbons due to six sp^2 quaternary carbons, eight sp^2 methines, two sp^3 methines, three sp^3 methylenes, and two sp^3 methyls. Among them, two methyls (δ_C 39.81, δ_H 2.90, and δ_C 32.54, δ_H 2.85) were ascribed to those bearing a nitrogen.

The ^{1}H–^{1}H COSY, TOCSY, and HMQC spectra of **2** revealed connectivities of C-1 to C-10, C-3 to C-4, C-7 to C-8, C-10 to C-11, and C-13 to C-17. HMBC and NOESY correlations indicated the presence of a 6-brominated 1,2,6-trisubstituted tetrahydro-β-carboline ring. The connectivity of C-11 and C-12 was suggested by HMBC correlations for H-11 to C-12 and C-13 (C-17). HMBC correlations for H_3-19 to C-1 and C-3 disclosed that one methyl group (C-19) was connected to N-2, while the HMBC correlation for H_3-18 to C-10 revealed that another methyl group (C-18) was not attached to C-15 but attached to N-10. (Fig. 2). These data suggested that the gross structure of eudistomidin B (**2**) was **2b** but not **2a**.[9]

The relative stereochemistry of revised structure (**2b**) of eudistomidin B (**2**) was not able to be assigned from the $^3J_{H\text{-}1/H\text{-}10}$ value (2.9 Hz), and NOESY correlations for H-10/H-19, H-1/H-10, and NH-9/H-11b. The absolute configuration at C-1 in eudistomidin B (**2**) was deduced to be *R* from a positive Cotton effect at 231 nm ($\Delta\varepsilon$ +2.4) in the CD spectrum of **2**.[5,6]

To elucidate the relative and absolute configurations of eudistomidin B, the revised structure (**2b**) of eudistomidin B (**2**) and its (1*S*,10*S*)-diastereomer (**2c**) were prepared in optical active form employing the Noyori catalytic asymmetric hydrogen-transfer reaction[10] (Scheme 1). Treatment of 5-bromotryptamine **3**[7] with carboxylic acid **4**[11] in the presence of EDC and HOBt provided amide **5**. The Bischler–Napieralski reaction[12] in benzene afforded dihydro-β-carboline (**6**). Noyori asymmetric hydrogen-transfer reaction of **6** in DMF afforded tetrahydro-β-carboline, which was

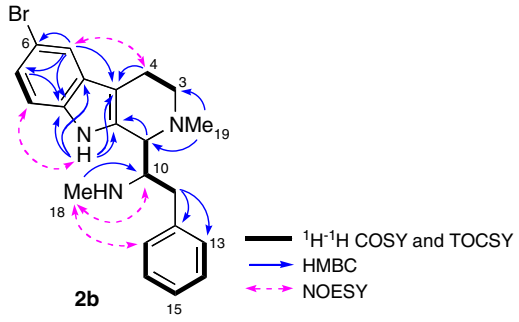
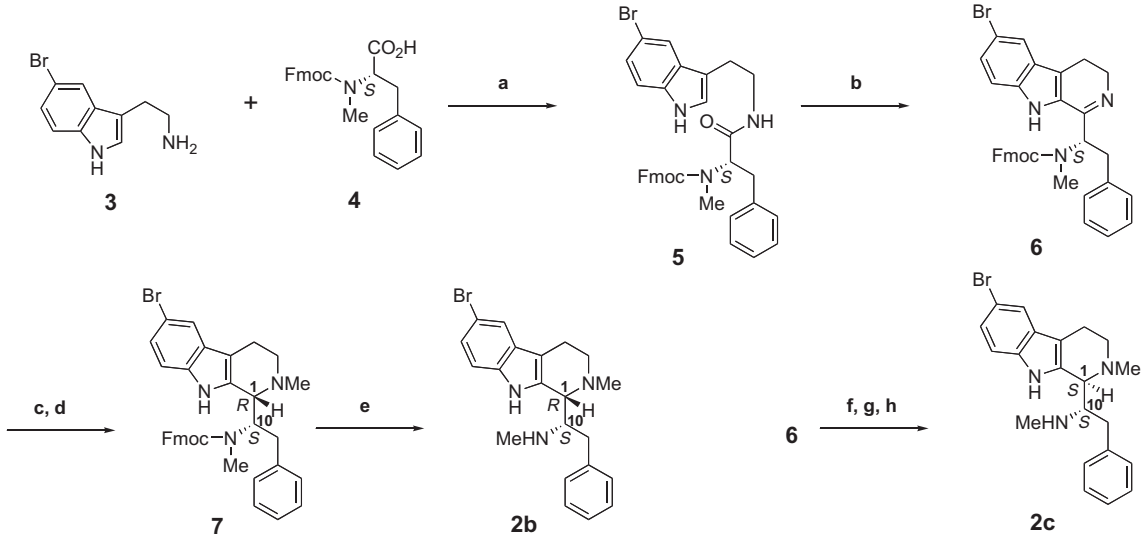


Figure 2. Selected 2D NMR correlations for the revised structure (**2b**) of eudistomidin B (**2**).



Scheme 1. Synthesis of the revised structure (**2b**) of eudistomidin B (**2**) and its (1*S*,10*S*)-diastereomer (**2c**). Reagents and conditions: (a) HOBt, EDC, CH_2Cl_2, 94%; (b) $POCl_3$, benzene, 12%; (c) (*S*,*S*)-TsDPEN–Ru(II) complex, HCO_2H/Et_3N (5:2), DMF; (d) HCHO aq, $NaBH_3CN$, MeCN, 89% for two steps; (e) DBU, CH_2Cl_2, 85%; (f) (*R*,*R*)-TsDPEN–Ru(II) complex, HCO_2H/Et_3N (5:2), DMF; (g) HCHO aq, $NaBH_3CN$, MeCN, 92% for two steps; (h) DBU, CH_2Cl_2, 78%.

methylated with HCHO and $NaBH_3CN$ to yield **7** in 89% (dr >10:1 from ^1H NMR analysis). Removal of Fmoc group in **7** with DBU furnished the revised structure (**2b**) of eudistomidin B (**2**), having (1*R*,10*S*)-configuration. Similarly, by starting from dihydro-β-carboline (**6**), (1*S*,10*S*)-diastereomer (**2c**) was synthesized.

The spectral data (^1H and ^{13}C NMR, CD, and optical rotation)[13] of the synthetic revised structure (**2b**) were coincident with those of natural eudistomidin B (**2**), whereas the spectral data[14] for its synthetic (1*S*,10*S*)-diastereomer (**2c**) were inconsistent with those of natural eudistomidin B (**2**) (Table 3). Thus, the structure of eudistomidin B (**2**) was revised from **2a** to **2b**. Furthermore, synthetic compounds **2b** and **2c**, and natural eudistomidin B (**2**) were subjected to C_{18} HPLC [Luna Phenyl-Hexyl, 4.6 × 250 mm; flow rate 0.5 mL/min: eluent; MeOH/H_2O/TFA (80:20:0.1); UV detection at 280 nm], and it was found that the retention time of natural eudistomidin B (**2**) (t_R 13.0 min) was identical with that of **2b** (t_R 13.0 min) but not that of **2c** (t_R 10.8 min). These results also support that natural eudistomidin B (**2**) has (1*R*,10*S*)-configuration.

Table 3
^1H NMR data for synthetic revised structure (**2b**) of eudistomidin B (**2**) and its synthetic (1*S*,10*S*)-diastereomer (**2c**) in $CDCl_3$

Position	**2b**		**2c**	
1	5.10	(d, 2.0)	5.27	(d, 2.3)
3	3.32	(m)	3.52	(m)
	2.94	(m)	2.98	(m)
4	2.86	(m)	2.88	(m)
	2.42	(br d, 10.6)	2.30	(dd, 16.9, 4.9)
4a				
4b				
5	7.53	(s)	7.49	(d, 2.0)
6				
7	7.43	(d, 8.6)	7.48	(d, 9.0)
8	7.45	(d, 8.6)	7.42	(dd, 9.0, 2.0)
8a				
9	11.04	(s)	11.27	(s)
9a				
10	3.99	(m)	4.10	(m)
11	3.22	(m)	3.27	(m)
	3.07	(dd, 14.9, 9.3)	3.26	(m)
12				
13, 17	6.56[a]	(d, 7.3)	6.50[a]	(d, 7.5)
14, 16	7.07[a]	(dd, 7.3, 7.3)	7.02[a]	(d, 7.5)
15	7.15	(dd, 7.3, 7.3)	7.12	(d, 7.5)
18	2.87[b]	(s)	2.92[b]	(s)
19	2.91[b]	(s)	2.94[b]	(br s)

[a] 2H.
[b] 3H.

The relative stereochemistry of eudistomidin G (**1**) was elucidated from comparison of ^1H NMR data of **1** with those of synthetic diastereomers (**2b** and **2c**) of eudistomidin B (**2**). ^1H NMR data of **1** were quite similar to those of **2b** but not those of **2c**, indicating that the relative stereochemistry of eudistomidin G (**1**) was the same as that of the revised structure (**2b**) of eudistomidin B (**2**). Since the absolute configuration at C-1 in eudistomidin G (**1**) was deduced to be *R* from the CD spectrum, the absolute configuration at C-10 in eudistomidin G (**1**) was assigned as *S*.

Eudistomidin G (**1**) corresponds to the 7-bromo analog of eudistomidin B (**2**). Biosynthetically both **1** and **2** might be derived from tryptophan and L-phenylalanine. Eudistomidins G (**1**) and B (**2**) showed cytotoxicity against L1210 murine leukemia cells (IC_{50}, 4.8 and 4.7 μg/mL, respectively) in vitro. Since eudistomidin alkaloids show various biological activity,[3,15] further biological studies of eudistomidins B and G are in progress.

Acknowledgments

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4. *Eudistomidin G* (**1**): pale yellow amorphous solid; $[\alpha]_D^{21}$ +6.4 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 3211, 3049, 2922, 2851, and 1458 cm^{-1}; UV (MeOH) λ_{max} 366 (ε 200), 296 (sh 1870), 286 (2190), 231 (10,200), and 203 nm (10,400); CD (MeOH) λ_{ext} 293 (Δε +0.11), 228 nm (+5.6), 213 nm (−2.5); ^1H and ^{13}C NMR

($CDCl_3$) see Table 1; ESIMS (pos.) m/z 398 and 400 ($[M+H]^+$, 1:1); HRESIMS (pos.) m/z 398.1235 ($[M+H]^+$, calcd for $C_{21}H_{25}N_3{}^{79}Br$, 398.1226).
5. Stöckigt, J.; Zenk, M. H. *J. Chem. Soc., Chem. Commun.* **1977**, 646.
6. Though we have previously reported[3] that the absolute configuration at C-1 in eudistomidin B (**2**) was assigned to be *S* from a negative Cotton effect at 216 nm ($\Delta\varepsilon$ −14.4) in the CD spectrum[5] of **2**, the 1*R* configuration of eudistomidin B (**2**) was deduced from a positive Cotton effect at 231 nm ($\Delta\varepsilon$ +2.4), considering red shift due to bromine substitution in the indole ring.
7. Ito, T.; Kitajima, M.; Takayama, H. *Tetrahedron Lett.* **2009**, *50*, 4506.
8. *Eudistomidin B* (**2**): pale yellow amorphous solid; $[\alpha]_D^{21}$ +7.5 (*c* 0.5, $CHCl_3$); IR (film) ν_{max} 3211, 3029, 2916, 2855, and 1454 cm^{-1}; UV (MeOH) λ_{max} 364 (ε 170), 301 (sh 1950), 291 (2490), 284 (sh 2410), 230 (12,100), and 204 nm (11,500); CD (MeOH) λ_{ext} 281 ($\Delta\varepsilon$ +0.39), 231 nm (+2.4), and 213 nm (−1.7); ^1H and ^{13}C NMR ($CDCl_3$) see Table 2; ESIMS (pos.) m/z 398 and 400 ($[M+H]^+$, 1:1); HRESIMS (pos.) m/z 398.1235 ($[M+H]^+$, calcd for $C_{21}H_{25}N_3{}^{79}Br$, 398.1226).
9. In this study, eudistomidin B reported previously[3] has proved to be a (2:1) mixture of eudistomidins B (**2**) and G (**1**).
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13. *Synthetic* **2b**: pale yellow amorphous solid; $[\alpha]_D^{20}$ +8.2 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} 361 (ε 280), 301 (sh 1770), 293 (2320), 284 (sh 2700), 227 (12,700), and 205 nm (12,800); CD (MeOH) λ_{ext} 276 ($\Delta\varepsilon$ −0.6), 231 (+4.7), and 213 nm (−4.0); ^1H NMR ($CDCl_3$) see Table 3; ESIMS (pos.) m/z 398 and 400 ($[M+H]^+$, 1:1); HRESIMS (pos.) m/z 398.1230 ($[M+H]^+$, calcd for $C_{21}H_{25}N_3{}^{79}Br$, 398.1226); ^{13}C NMR ($CDCl_3$) δ:135.43 (C-8a), 133.93 (C-12), 128.95 (C-14,16), 128.41 (C-13,17), 127.94 (C-15), 127.15 (C-7), 126.52 (C-4b), 121.90 (C-9a), 121.00 (C-5), 114.02 (C-8), 113.76 (C-6), 105.37 (C-4a), 64.22 (C-10), 64.03 (C-1), 45.61 (C-3), 39.93 (C-19), 33.76 (C-11), 32.54 (C-18), 15.03 (C-4).
14. *Synthetic* **2c**: pale yellow amorphous solid; $[\alpha]_D^{20}$ −11.5 (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} 300 (ε 1710), 290 (2180), 279 (2190), 227 (11,700), and 205 nm (11,100); CD (MeOH) λ_{ext} 278 ($\Delta\varepsilon$ +0.7), 231 (−3.3), and 215 nm (+1.3); ^1H NMR ($CDCl_3$) see Table 3; ESIMS (pos.) m/z 398 and 400 ($[M+H]^+$, 1:1); HRESIMS (pos.) m/z 398.1227 ($[M+H]^+$, calcd for $C_{21}H_{25}N_3{}^{79}Br$, 398.1226).
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