a competitive external nucleophile by an S_N2 mechanism in an aprotic solvent[10] than both **1a** and **1h**, which have been used as mustard simulants for substitution reactions.

Experimental Section

The synthesis of mustard has been described previously;[1] it was prepared inhouse by the U.S. Army. *Caution*: The compound is a toxic vesicant and should only be used by trained professionals in properly equipped facilities. The rest of the sulfide substrates were obtained commercially either from Fairfield Chemical Company or from Aldrich. The oxaziridine compound was prepared at Drexel University.[4] All of the compounds used in this study were greater than 95% pure by ^1H and/or ^{13}C NMR and were used as received.

The competition rates were typically measured by ^1H NMR on a Varian XL200 FTNMR. The spectra of the reaction mixtures were obtained by using 1 pulse and a 90° flip angle. The concentrations of the two competing sulfides were equal at 0.1 M while the concentration of the *N*-sulfonyloxaziridine varied from 0.002 to 0.1 M and was typically 0.1 M. The competition rate was independent of the oxidant concentration within experimental error. However, the measured rates were more reproducible when the reactivity of the two sulfides was similar. For cases in which the sulfoxide yields were very small because the oxidant concentation was small, ^{13}C NMR was used since the ^1H NMR signals of the reaction mixture were too overlapped for accurate determinations of the sulfoxide peak areas.

The observed first-order rate was determined for **1e** in the presence of excess **2** by using ^1H NMR. The spectrum was recorded at 18 °C on a Varian VXR-400S FTNMR. The sweep width was narrowed to 1.6 ppm to observe only the methylene groups in **1e** (see Figure 1). Sixty-four transients were accumulated for each spectrum using a 90° pulse width and a repetition rate of 3.74 s. The progress of the reaction was monitored by measuring the simultaneous disappearance of the CH_2 resonances of the reactant and the appearance of the CH_2 rsonances of the sulfoxide product with time. The resonances were expanded and digitally integrated to obtain the peak areas.

Registry No. 1, 505-60-2; **1a**, 693-07-2; **1b**, 352-93-2; **1c**, 544-40-1; **1d**, 139-66-2; **1e**, 5535-49-9; **1f**, 4837-01-8; **1g**, 699-12-7; **1h**, 542-81-4; **1i**, 5271-38-5; **1j**, 4303-41-7; **1k**, 116037-20-8; **1l**, 4303-40-6; **1m**, 126823-30-1; **2**, 86428-23-1; **3**, 5819-08-9; **4**, 36176-89-3.

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Eudistomidins B, C, and D: Novel Antileukemic Alkaloids from the Okinawan Marine Tunicate *Eudistoma glaucus*†

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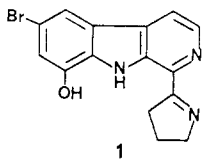
Received January 10, 1990

Marine tunicates have proven to be a rich source of intriguing structures and interesting biological activities.[2] In our continuing survey of bioactive compounds from Okinawan marine organisms,[3] we have reported a novel β-carboline, named eudistomidin A (**1**), with powerful calmodulin antagonistic activity from the Okinawan tunicate *Eudistoma glaucus*.[4] This paper describes the isolation and structure elucidation of eudistomidins B (**2**), C (**3**), and D (**4**), three new pharmacologically active components from this tunicate. The configuration at C-10 of eudistomidin C (**3**) was established by synthesis of 10-(*R*)-*O*-methyleudistomidin C. This tunicate also contained four known compounds, eudistomins D (**5**), E (**6**), H (**7**), and I (**8**), previously isolated from the Caribbean tunicate *Eudistoma olivaceum*.[5]



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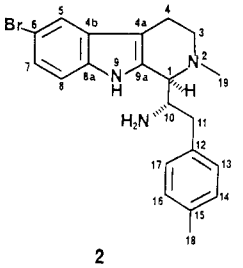

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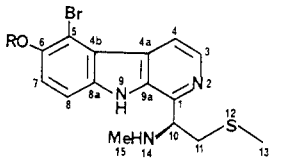

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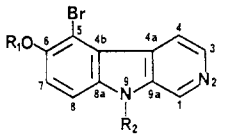
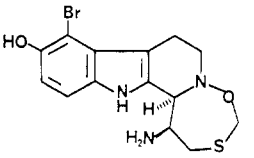
15 R = Me



4 R_1 = H R_2 = Me
16 R_1 = R_2 = Me
5 R_1 = R_2 = H



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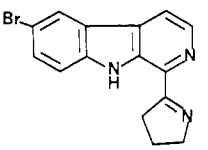

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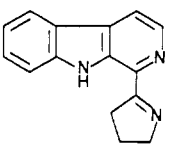

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† This paper is dedicated to Professor Kenneth L. Rinehart, Jr., in honor of his 60th birthday.

(1) (a) Mitsubishi Kasei Institute of Life Sciences. (b) Tohoku University. (c) Kanazawa University. (d) Present address: Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan. (e) Present address: Faculty of Science and Technology, Keio University, Yokohama 223, Japan.

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0022-3263/90/1955-3666$02.50/0

Eudistomidins B (2), C (3), and D (4) showed potent cytotoxicity against murine leukemia L1210 (IC_{50} = 3.4, 0.36, and 2.4 μg/mL) and L5178Y (IC_{50} = 3.1, 0.42, and 1.8 μg/mL) cells, respectively. In addition, eudistomidin B (2) activated rabbit heart muscle actomyosin ATPase[6] by 93% at 3×10^{-5} M, while eudistomidin C (3) exhibited calmodulin antagonistic activity[4] (IC_{50} = 3×10^{-5} M). Eudistomidin D (4) induced Ca^{2+} release from the sarcoplasmic reticulum[7] (SR), about 10 times more potent than caffeine, a well known SR Ca^{2+}-inducer.

The green-colored colonial tunicate *Eudistoma glaucus* collected at Ie Island, Okinawa, was extracted with methanol–toluene (3:1). The extract was partitioned between toluene and water, and the aqueous layer was subsequently extracted with chloroform, ethyl acetate, and 1-butanol. The toluene soluble materials exhibiting cytotoxicity against L1210 were subjected to chromatographies on silica gel columns with chloroform/methanol and chloroform/1-butanol/acetic acid/water, followed by a LH-20 column with chloroform/methanol to afford eudistomidins B (2, 0.002% wet weight) and C (3, 0.001%). In addition, the toluene soluble materials yielded known β-carbolines, eudistomidin A (1) and eudistomins H (7), and I (8). Chromatography of the 1-butanol extract on a LH-20 column with chloroform/methanol and then a silica gel column with chloroform/1-butanol/acetic acid/water furnished eudistomidin D (4, 0.0004%), while the chloroform-soluble materials gave the known compounds, eudistomins D (5) and E (6).

The molecular formula, $C_{21}H_{24}N_3Br$, of eudistomidin B (2) was determined by HRFABMS (*m/z* 398.1269 $(M + H)^+$, Δ +3.7 mmu). The presence of a primary amino group in 2 was indicated by the positive ninhydrin test. The ^1H NMR spectrum disclosed three deuterium-exchangeable protons which should be assigned to a NH_2 and a NH group, suggesting that a remaining nitrogen was tertiary. The ultraviolet spectrum of 2 in methanol, λ_{max} 231 (ε 26 900) and 291 (6300) nm, was characteristic of indole chromophore.[8]

The ^1H NMR spectrum of 2 showed two methyls (δ 2.88 and 2.92) and eight aliphatic (δ 2.38–5.19) and seven aromatic protons (δ 6.59–7.52) along with three NH protons, while the ^{13}C NMR studies of 2 revealed the presence of 21 carbons including two methyls (δ 32.42 and 39.75), three sp^3 methylenes (δ 15.00, 33.27 and 45.80), two sp^3 methines (δ 63.59 and 63.73), seven aromatic methines (δ 113.61–128.87), and seven aromatic quaternary carbons (δ 105.53–137.57). The assignment of the protonated carbons was made by the ^1H–^{13}C COSY data (Table I). The presence of an indole ring (C-4a–N-9 and C-9a) suggested by the UV absorptions and the ^1H and ^{13}C resonances of 2 was confirmed by the HMBC[9] data (Table I). A bromine on the benzenoid ring was placed at C-6 from comparison of the ^1H and ^{13}C resonances with those of 5- or 6-brominated indole derivatives.[10]

The ^1H–^1H COSY spectrum showed cross peaks for H_2-3/H_2-4, H-1/H-10, and H-10/H_2-11, indicating the partial structures CH_2–CH_2 (C-3 and C-4) and CH–CH–CH_2 (C-1, C-10, and C-11) in 2. One methyl group (δ 2.92, C-19) was fixed at N-2 by observation of three-bond couplings for the methyl protons (H_3-19) to C-1 and C-3 in the HMBC spectrum (Table I) to make the partial structure CH_2–CH_2–$N(CH_3)$–CH–CH–CH_2 (C-4–N-2–C-11 and C-19). A remaining NH_2 group was connected to C-10 (δ 63.73), judging from the ^1H and ^{13}C chemical shifts. The HMBC spectrum showed cross peaks for H_2-3 and H_2-4 to C-4a, establishing the connectivity of the tetrahydro-β-carboline (C-1–C-9a). Four aromatic protons of A_2B_2 system at δ 6.59 and 7.05 and six aromatic carbons resonancing at δ 128.52 d, 128.87 d, and 134.10 s (2 C each) implied the presence of a para disubstituted benzene ring (C-12–C-17). Another methyl group (δ 2.88, C-18) was connected to C-15, since in the HMBC spectrum (Table I) C-18 showed a cross peaks to H-14 and H-16 (δ 6.52). Finally, the remaining bond C-11–C-12 was the only possible way to complete the structural assignment of 2. This connectivity was confirmed by long-range couplings for H_2-11 to C-12, C-13, and C-17 in the HMBC spectrum. The EIMS fragmentation [*m/z* 397 $(M)^+$/399 $(M + 2)^+$, 262 $(M - C_9H_{12}N)^+$/264, and 183 (262 – Br)] supported the proposed structure 2.

Table I. ^1H and ^{13}C NMR Chemical Shifts (ppm) of Eudistomidin B (2) and Protons to Which a Long-Range Connectivity Was Observed in the HMBC Experiment[a]

position	^{13}C (δ)	^1H (δ)	*J*(H,H), Hz	HMBC (^1H)
1	63.59 (d)	5.19 (d)	8.9	H_2-3, H_2-11, H_3-19
3	45.80 (t)	2.86 (m)		H_3-19
		3.28 (dd)	5.3, 12.2	
4	15.00 (t)	2.38 (dd)	4.2, 15.6	
		2.82 (m)		
4a	105.53 (s)			H_2-3, H_2-4, H-9
4b	122.40 (s)			H-9
5	121.05 (d)	7.52 (d)	1.6	
6	113.61 (s)			H-5, H-7, H-8
7	126.91 (d)	7.39 (dd)	1.6, 8.7	H-5, H-8
8	113.87 (d)	7.42 (d)	8.7	H-7
8a	135.49 (s)			H-5, H-9
9		11.08 (s)		
9a	137.57 (s)			
10	63.73 (d)	4.18 (m)		H-1, H_2-11
11	33.27 (t)	3.03 (dd)	9.7, 14.7	
		3.23 (dd)	3.1, 14.7	
12	134.10 (s)			H_2-11, H-13 (H-17)
13	128.87 (d)	7.05 (d)	7.5	H_2-11, H-14 (H-16)
14	128.52 (d)	6.59 (d)	7.5	
15	134.10 (s)			H-13 (H-17)
16	128.52 (d)	6.59 (d)	7.5	H-18, H-13 (H-17)
17	128.87 (d)	7.05 (d)	7.5	H_2-11, H-14 (H-16)
18	32.42 (q)	2.88 (s)		H-14 (H-16)
19	39.75 (q)	2.92 (s)		

[a] $CDCl_3/CF_3CO_2D$ (10:1).

The CD spectrum (λ_{ext} 216 (Δε –14.4), 237 (+15.0), and 279 (–4.2) nm) of 2 indicated an α-configuration of H-1.[11] The coupling constant between H-1 and H-10 (8.9 Hz) and observation of cross peaks for H-1/H_2-11 and H-10/H_3-19 in the NOESY spectrum suggested the H-1, H-10 trans relationship and therefore 10*S* configuration.

The UV spectrum of eudistomidin C (3) (λ_{max} 218, 236, 253 (sh), 287 (sh), 298, and 368 nm) was characteristic of β-carboline compounds.[6] The molecular formula, $C_{15}H_{16}ON_3SBr$, for 3 was established by HRFABMS (*m/z* 366.0254 $(M + H)^+$, Δ –2.2 mmu). The ^{13}C NMR spectra showed 15 carbons consisting of two methyls (δ 15.90 and 33.69), one sp^3 methylene (δ 38.75), one sp^3 methine (δ 62.25), four aromatic methines (δ 112.74–138.09), and seven

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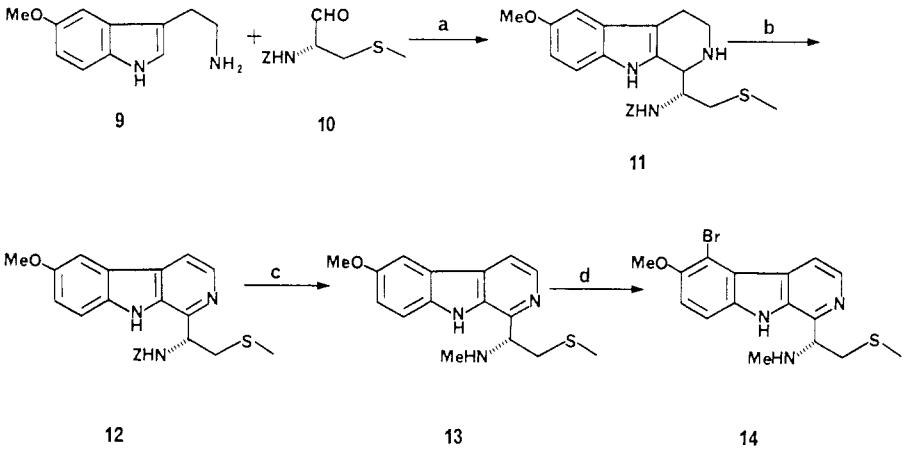
Scheme I. Synthesis of 6-*O*-Methyleudistomidin C (14)[a]



[a] Reagents: (a) TFA, −78 °C; (b) DDQ, benzene, 10–15 °C; (c) LAH, TFA, reflux; (d) Br_2, AcOH, 0–25 °C.

Table II. ^1H and ^{13}C NMR Chemical Shifts (ppm) of Eudistomidin C (3) and Protons to Which a Long-Range Connectivity Was Observed in the HMBC Experiment[a]

position	^{13}C (δ)	^1H (δ)	J(H,H), Hz	HMBC (^1H)
1	140.06 (s)			H-3, H-10, H_2-11
3	138.09 (d)	8.37	5.3	
4	117.45 (d)	8.61	5.3	H-3
4a	130.73 (s)			H-3
4b	121.83 (s)			H-4, H-8
5	104.30 ns)			H-7
6	149.50 (s)			H-8
7	119.81 (d)	7.25 (d)	8.4	
8	112.74 (d)	7.49 (d)	8.4	
8a	137.68 (s)			H-7
9		11.2[b] (s)		
9a	136.51 ns)			H-4
10	62.25 (d)	4.68 (t)	6.9	H_2-11, H_3-15
11	38.75 (t)	3.16 (d)	6.9	H-10, H_3-13
13	15.90 (q)	1.93 ns)		H_2-11
15	33.69 (q)	2.50 ns)		H-10

[a] MeOH-d_4. [b] Acetone-d_6.

sp^2 quaternary carbons (δ 104.30–149.50) as shown in Table II. The ^1H NMR studies revealed two methyls (δ 1.93 and 2.50), one methylene (δ 3.16), one methine (δ 4.68), and four aromatic protons (δ 7.25–8.61). The protonated carbons were all assigned unambiguously by HMQC[12] experiment (Table II).

The two aromatic protons at δ 8.37 and 8.61 showing AB pattern (J = 5.3 Hz) were assignable to H-3 and H-4 of the pyridine ring in β-carboline skeleton, while the remaining aromatic AB protons at δ 7.25 and 7.49 (J = 8.4 Hz) were attributed to H-7 and H-8 of the benzenoid ring, due to lack of cross peak for C-4a to any benzenoid proton and the presence of ^{13}C–^1H long-range couplings for C-4a/H-3, C-4b and C-9a/H-4, C-8a/H-7, and C-4b/H-8 in the HMBC spectrum (Table II). One bromine and one hydroxy group were fixed at C-5 (δ 104.30) and C-6 (δ 149.50), respectively, since cross peaks were observed for C-5/H-7 and C-6/H-8 in the HMBC spectrum. The coupling constant between H-10 and H-11 (6.9 Hz) and the resonances of 10-CH (δ_H 4.68 and δ_C 62.25) and 11-CH_2 (δ_H 3.16 and δ_C 38.75) and the two methyls (δ_H 1.93 and δ_C 15.90, C-13; δ_H 2.50 and δ_C 33.69, C-15) indicated the presence of the partial structure of $CH_3NHCHCH_2SCH_3$ (C-10–S-12–C-13 and N-14–C-15). This was also supported by observation of ^1H–^{13}C long-range couplings for C-10/H_2-11 and H_3-15, and C-11/H_3-13. The remaining bond C-1–C-10 was connected to make the complete structure of eudistomidin C (3), since cross peaks were showed for C-1/H-10 and H_2-11 in the HMBC spectrum (Table II).

The stereochemistry at C-10 of 3 was assigned by the following synthesis. Pictet–Spengler cyclization of 5-methoxytryptamine (9) with *S*-methyl-*N*-(benzyloxycarbonyl)-L-cysteinal[13] (10) in the presence of trifluoroacetic acid afforded 1,2,3,4-tetrahydro-β-carboline (11), which was transformed into the corresponding β-carboline (12) through DDQ oxidation (Scheme I). Reduction of 12 with LAH gave compound 13, which was brominated to afford 10(*R*)-*O*-methyleudistomidin C (14). Compound 14 showed identical ^1H and ^{13}C NMR spectra with those of 6-*O*-methyl derivative (15) of natural eudistomidin C (3). The only difference between 14 and 15 was the sign of $[\alpha]_D$, namely −13.8° for 14 while +12.5° for 15. Therefore, the absolute configuration at C-10 of eudistomidin C (3) was concluded to be *S*.

The molecular formula, $C_{12}H_9ON_2Br$, of eudistomidin D (4) was established by HRFABMS (m/z 276.9978 (M + H)$^+$, Δ +0.2 mmu). The ^1H and ^{13}C NMR and UV spectra[8] were typical of β-carbolines. The bromine and OR (R = H or Me) groups were located at C-5 and C-6, respectively, from comparison of the ^1H and ^{13}C resonances with those of eudistomidin C (3). The remaining methyl group (δ 4.56) can be assigned to either 6-*O*-methyl or 9-*N*-methyl. Methylation of 4 with diazomethane gave the corresponding 6-*O*-methyl derivative 16 (HRFABMS, m/z 291.0146 (M + H)$^+$, Δ +1.4 mmu). Thus the structure of eudistomidin D was assigned as 4 (6-methyleudistomin D). This structure was supported by the NOE experiment in which a significant NOE enhancement (7%) of the 9-methyl signal was observed on irradiation of H-1 (δ 9.21).

Recently, a series of β-carboline alkaloids have been isolated from marine tunicates, eudistomins A-Q[5] and R-T[12] from *Eudistoma olivaceum*, eudistomin K sulfoxide[13] from *Ritterella sigillinoides*, woodinine[14] from *Eudistoma fragum*, and eudistomidin A[4] from *Eudistoma glaucus*. These β-carboline compounds as well as eudis-

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tomidins B–D (2–4) from *Eudistoma glaucus* may be derived from an amino acid tryptophan. In eudistomidins B (2) and C (3), unusual amino acids, *p*-methylphenyl-L-alanine and *S*-methyl-D-cysteine are involved in addition to tryptophan, respectively. Eudistomidin C (3) might be a possible precursor of eudistomin E (6) or vice versa, or both compounds derived from a same intermediate, judging from the remarkable structure similarity between them.

Experimental Section

General Methods. Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi 260-50 infrared spectrophotometer. UV spectra were measured on a JASCO UVIDEC-660 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-500 and a JEOL GX-500 spectrometer in CD_3OD or $CDCl_3/CF_3COOD$ (10:1) with an internal TMS standard (0 ppm). Mass spectra were obtained on a Shimadzu GC-MS QP-1000A spectrometer operating at 70 eV (for EI) or a JEOL HX-100 spectrometer by using glycerol as a matrix (for FABMS). Wako gel C-300 (Wako Chemical) was used for silica gel column chromatography.

Extraction, Isolation, and Separation. The green-colored tunicate *Eudistoma glaucus* was collected at Ie Island, Okinawa, by SCUBA (-5 to -10 m) and kept frozen until used. The methanol–toluene (3:1, 2 × 1000 mL) extract of the specimen (1.5 kg) was evaporated under reduced pressure to give a crude extract (2.9 g), which was partitioned between toluene (2 × 500 mL) and 1 M NaCl (1000 mL). The aqueous layer was subsequently extracted with chloroform (2 × 500 mL), ethyl acetate (2 × 500 mL), and 1-butanol (2 × 500 mL). The toluene-soluble materials were chromatographed over a silica gel column (3.0 × 45 cm) with $MeOH/CHCl_3$ (2:98) to give a mixture of eudistomins H (7) and I (8), eudistomidin A (1, 0.0003%), and two active fractions A (800–850 mL) and B (990–1080 mL). After chromatography over silica gel columns (2.0 × 40 cm) using $CHCl_3$/*n*-BuOH/AcOH/H_2O (3:6:1:1) and a Sephadex LH-20 column (3.0 × 90 cm; $CHCl_3$/MeOH, 1:1), the less polar fraction A yielded eudistomidin B (2, 0.002% wet weight of the tunicate), while the polar fraction B furnished eudistomidin C (3, 0.001%). The mixture of 7 and 8 was separated by C_{18} reversed-phase HPLC (Yamamura Chemical, AM-323, 1.0 × 25 cm, 5 μm) with $MeOH/H_2O$ (2:1) to give eudistomins H (7, 0.009%) and I (8, 0.001%). Chromatography of chloroform solubles (1.0 g) over a silica gel (2.5 × 90 cm; $MeOH/CHCl_3$, 6:94) and a LH-20 (3.0 × 90 cm; $CHCl_3$/MeOH, 1:1) column furnished eudistomins D (5, 0.0001%) and E (6, 0.0002%). 1-Butanol extracts yielded eudistomidin D (4, 0.0004%) after purification over a LH-20 (3.0 × 90 cm; $CHCl_3$/MeOH, 1:1) and a silica gel (1.0 × 40 cm; $CHCl_3$/*n*-BuOH/AcOH/H_2O, 3:6:1:1) columns.

Eudistomidin B (2): yellow foam; mp 81–83 °C; $[\alpha]_D^{22}$ -54° (*c* 0.2, MeOH) and -76.4° (*c* 0.3, $CHCl_3$); IR (film) 3350, 3030, 2950, 2850, 2800, 1600, 1500, 1460, 1380, 1060, and 800 cm^{-1}; UV (MeOH) λ_{max} 231 (ε 26 900) and 291 (6300) nm; EIMS *m/z* 399 $(M + 2)^+$, 397 $(M)^+$, 333, 331, 305, 303, 264, 262, 134 (100), and 91; FABMS *m/z* 400 $(M + 2 + H)^+$ and 398 $(M + H)^+$; HRFABMS *m/z* 398.1269 ($(M + H)^+$, calcd for $C_{21}H_{25}N_3Br$, 398.1232); CD (MeOH) λ_{ext} 216 (Δε -14.4), 237 (+15.0), and 279 (-4.2) nm; ^1H and ^{13}C NMR (see Table I).

Eudistomidin C (3): yellow solid; mp 120–122 °C; $[\alpha]_D^{22}$ +15.6° (*c* 0.2, MeOH); IR (KBr) 3150, 2930, 2860, 1560, 1420, 1280, 1260, and 800 cm^{-1}; UV (MeOH) λ_{max} 218 (ε 21 300), 236 (23 700), 253 (sh), 287 (sh), 298 (13 100), and 368 (3800) nm; EIMS *m/z* 306 $(M - CH_3SCH_2 + 2)^+$ and 304 $(M - CH_3SCH_2)^+$; FABMS *m/z* 368 $(M + 2 + H)^+$ and 366 $(M + H)^+$; HRFABMS *m/z* 366.0254, ($(M + H)^+$, calcd for $C_{15}H_{17}ON_3SBr$, 366.0276); CD (MeOH) λ_{ext} 250 (Δε +2.7) nm; ^1H and ^{13}C NMR (see Table II).

6-*O*-Methyleudistomidin C (15). To a solution of eudistomidin C (3, 2.8 mg) in MeOH (2 mL) was added excess CH_2N_2 ether solution, and the resulting reaction mixture was allowed to stand overnight. Evaporation of solvents gave a residue which was passed through a silica gel column (0.5 × 10 cm) with $CHCl_3/MeOH$ (96:4) to afford 6-*O*-methyleudistomidin C (15, 2.2 mg): $[\alpha]_D^{22}$ +12.5° (*c* 0.2, MeOH); FABMS *m/z* 382 (M + 2 + H)$^+$ and 380 $(M + H)^+$; HRFABMS *m/z* 380.0423 ($(M + H)^+$, calcd for $C_{16}H_{19}ON_3SBr$, 380.0432); ^1H and ^{13}C NMR (see ^1H and ^{13}C NMR data for 14).

Pictet–Spengler Cyclization of 5-Methoxytryptamine (9) with Cysteinal 10. To a stirred solution of 5-methoxytryptamine (9, 330 mg) and *S*-methyl-*N*-(benzyloxycarbonyl)-L-cysteinal[13] (10, 1.0 g) in toluene (45 mL) at -78 °C was added trifluoroacetic acid (0.3 mL). The mixture was stirred for 2 h. After evaporation of the solvent, the residue was passed through a silica gel column (2.5 × 45 cm) with $CHCl_3/MeOH$ (97.5:2.5) to afford compound 11 (618 mg): yellow foam; mp 75–80 °C; $[\alpha]_D^{22}$ +4.6° (*c* 1.4, $CHCl_3$); UV (MeOH) λ_{max} 230 (ε 21 000) and 279 (1800) nm; EIMS *m/z* 425 $(M)^+$; HRFABMS *m/z* 426.1848 ($(M + H)^+$, calcd for $C_{23}H_{28}O_3N_3S$, 426.1852); ^1H NMR ($CDCl_3$) δ 8.09 (br s), 7.36–7.18 (m), 6.94 (d, *J* = 1.9 Hz), 6.82 (m), 5.51 (d, *J* = 7.5 Hz), 5.15 (s, 2 H), 4.51 (m), 2.82 (m, 4 H), and 2.18 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 156.38 (s), 153.93 (s), 136.13 (s), 134.15 (s), 131.39 (s), 128.10 (d, 2C), 127.82 (s), 127.65 (d, 2C), 127.35 (d), 111.49 (d), 111.26 (d), 110.67 (s), 100.49 (d), 66.54 (t), 55.83 (q), 53.96 (d), 52.26 (d), 42.95 (d), 35.93 (t), 21.94 (t), and 15.68 (q).

DDQ Oxidation of Compound 11. A dry benzene solution of 11 (400 mg in 50 mL) was cooled to 10 °C. DDQ (2,3-dichloro-5,6-dicyanobenzoquinone, 850 mg) was added to the solution, and the mixture was stirred at 10–15 °C for 4 h. The reaction mixture was filtrated, and the residue was washed with hot chloroform (3 × 20 mL). The combined filtrate was evaporated to dryness to give a residue which was purified over a silica gel column (1.5 × 45 cm) with $CHCl_3/MeOH$ (97:3) to afford compound 12 (180 mg): yellow solid; mp 130–131 °C; $[\alpha]_D^{22}$ +4.5° (*c* 1.7, $CHCl_3$); UV (MeOH) λ_{max} 212 (ε 34 800), 232 (28 500), 298 (16 000), and 364 (3300) nm; EIMS *m/z* 421 $(M)^+$; HRFABMS *m/z* 422.1527, ($(M + H)^+$, calcd for $C_{23}H_{24}O_3N_3S$, 422.1538); ^1H NMR ($CDCl_3$) δ 9.42 (br s), 8.34 (d, *J* = 5.2 Hz), 7.85 (d, *J* = 5.2 Hz), 7.60 (d, *J* = 2.4 Hz), 7.2–7.3 (m, 7 H), 6.06 (d, *J* = 8.4 Hz), 5.51 (Br s), 5.16 (s, 2 H), 3.94 (s, 3 H), 3.30 (dd, *J* = 6.4 and 15.4 Hz, 2 H), and 2.18 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 156.91 (s), 154.28 (s), 142,98 (s), 137.48 (d), 136.25 (s), 135.61 (s), 134.72 (s), 129.40 (s), 128.35 (d, 2C), 127.94 (d, 2C), 127.70 (d), 122.03 (s), 118.46 (d), 114.07 (d), 112.66 (d), 103.65 (d), 67.13 (t), 56.01 (q), 52.26 (d), 38.27 (t), and 16.09 (q).

Reduction of Compound 12 with LAH. To a solution of excess of $LiAlH_4$ in tetrahydrofuran (15 mL) was added compound 12 (40 mg). The reaction mixture was refluxed for 4 h. The excess of $LiAlH_4$ was decomposed with ice–water. The chloroform extract was dried over sodium sulfate. Evaporation of the solvent gave the residue, which was subjected to a silica gel column (1.5 × 40 cm) with $CHCl_3/MeOH$ (97:3) to give compound 13 (23.3 mg): yellow solid; mp 50–53 °C; $[\alpha]_D^{22}$ -37.7 °C (c, 1.0, MeOH); UV (MeOH) λ_{max} 216 (ε 29 800), 232 (36 000), 248 (sh), 280 (sh), 298 (16 000), 362 (5100) nm; HRFABMS *m/z* 302.1313 ($(M + H)^+$, calcd for $C_{16}H_{20}ON_3S$, 302.1327); ^1H NMR ($CDCl_3$) δ 10.31 (br s), 8.34 (d, *J* = 5.4 Hz), 7.85 (d, *J* = 5.4 Hz), 7.56 (d, *J* = 2.2 Hz), 7.46 (d, *J* = 8.9 Hz), 7.22 (dd, *J* = 8.9 and 2.2 Hz), 4.32 (d, *J* = 10.6 and 3.1 Hz), 3.94 (s), 3.12 (dd, *J* = 13.8 and 3.1 Hz), 2.95 (dd, *J* = 13.8 and 10.6 Hz), 2.48 (br s), and 2.20 (s); ^{13}C NMR ($CDCl_3$) δ 154.10 (s), 145.09 (s), 137.83 (d), 137.80 (s), 135.08 (s), 129.26 (s), 121.87 (s), 118.34 (d), 113.48 (d), 112.43 (d), 103.77 (d), 65.36 (d), 56.12 (q), 39.50 (t), 34.93 (q), and 14.98 (q).

Bromination of Compound 13. To an acetic acid (3.0 mL) solution of compound 13 (20 mg) was added bromine (10 μL). The reaction mixture was allowed to stand at room temperature overnight. The residue after evaporation of the solvent was purified over a silica gel column (1.0 × 20 cm) with $CHCl_3/CH_3OH$ (96:4) to furnish compound 14: $[\alpha]_D^{22}$ -13.8° (*c* 0.2, MeOH); UV (MeOH) λ_{max} 217 (ε 28 900), 237 (26 000), 253 (sh), 297 (15 000), and 366 (4300) nm; HRFABMS *m/z* 380.0429 ($(M + H)^+$, calcd for $C_{16}H_{19}ON_3SBr$, 380.0432); ^1H NMR ($CDCl_3$) δ 10.4 (br s, H-9), 8.54 (d, *J* = 5.5 Hz, H-4), 8.39 (d, *J* = 5.5 Hz, H-3), 7.46 (d, *J* = 8.7 Hz, H-8), 7.27 (d, *J* = 8.7 Hz, H-7), 4.20 (dd, *J* = 11.2 and 3.3 Hz, H-10), 4.00 (s, OCH_3), 3.05 (dd, *J* = 13.9 and 3.3 Hz, H-11), 2.85 (dd, *J* = 13.9 and 11.2 Hz, H-11), 2.47 (s, NCH_3), and 2.21 (s, SCH_3); ^{13}C NMR ($CDCl_3$) δ 151.58 (s, C-6), 141.29 (s, C-1), 136.31 (d, C-3), 135.98 (s, C-9a), 134.76 (s, C-8a), 123.66 (s, C-4b), 118.50 (s, C-4a), 118.40 (d, C-4 and C-7), 112.60 (d, C-8), 103.26 (s, C-5), 58.64 (q, OCH_3), 57.99 (d, C-10), 35.40 (t, C-11), 31.95 (q, C-15), and 16.44 (q, C-13).

Eudistomidin D (4): yellow solid; mp 180 °C dec; IR (KBr) 3500, 3100, 1640, 1540, 1520, 1480, 1320, and 820 cm^{-1}; UV (MeOH) λ_{max} 211 (ϵ 13 600), 270 (9800), 315 (6800), and 416 (1800) nm; (MeOH + KOH) λ_{max} 254 (ϵ 7000), 299 (8600), 342 (sh), and 503 (700) nm; EIMS m/z 278 $(M + 2)^+$, 276 $(M)^+$, 234, 232, 198, 184, 183, and 168; FABMS m/z 279 $(M + 2 + H)^+$ and 277 $(M + H)^+$; HRFABMS m/z 276.9978 $((M + H)^+$, calcd for $C_{12}H_{10}ON_2Br$, 276.9976); ^1H NMR (CD_3OD) δ 9.21 (s, H-1), 9.10 (d, J = 5.4 Hz, H-4), 8.47 (d, J = 5.4 Hz, H-3), 7.66 (d, J = 8.7 Hz, H-7), 7.49 (d, J = 8.7 Hz, H-8), and 4.56 (s, 9-NCH_3); ^{13}C NMR (CD_3OD) δ 148.8 (d, C-1), 148.4 (s, C-6), 138.7 (d, C-3), 135.3 (s, C-9a), 134.7 (s, C-8a), 130.9 (s, C-4a), 121.6 (s, C-4b), 118.2 (D, C-7), 117.7 (d, C-4), 111.4 (d, C-8), 101.5 (s, C-5), and 37.0 (q, 9-NCH_3).

***O*-Methyleudistomidin D (16).** Treatment of eudistomidin D (4, 1.2 mg) with CH_2N_2 in MeOH gave the corresponding methylated compound (16, 1.2 mg): FABMS m/z 293 $(M + 2 + H)^+$ and 291 $(M + H)^+$; HRFABMS m/z 291.0146 $((M + H)^+$, calcd for $C_{13}H_{12}ON_2Br$, 291.0132); ^1H NMR ($CDCl_3$) δ 8.83 (d, J = 6.4 Hz, H-4), 8.62 (s, H-1), 7.88 (d, J = 9.0 Hz, H-8), 7.52 (d, J = 6.4 Hz H-3), 7.43 (d, J = 9.0 Hz, H-7), 4.35 (s, OCH_3), and 4.02 (s, NCH_3).

Acknowledgment. We thank Z. Nagahama for help with collections and M. Hamashima for technical assistance. This study was supported in part by Grant-in-Aid (63010043) for Cancer Research from Ministry of Education, Science, and Culture, Japan.

Supplementary Material Available: ^1H and ^{13}C NMR spectra, correlation spectra, and high-resolution mass spectra (15 pages). Ordering information is given on any current masthead page.

Synthesis of Optically Active 3(*R*)-[(Alkylsulfonyl)oxy]thiolanes from 2(*R*)-Hydroxy-4-(methylthio)butanoic Acid or D-Methionine

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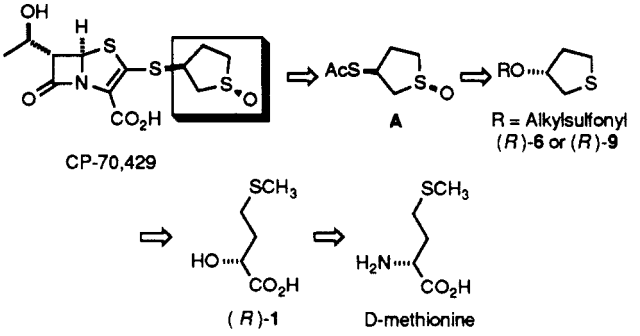
Received December 15, 1989

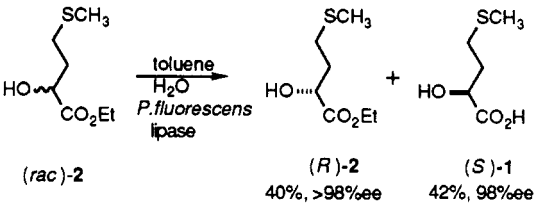
The highly active penem antibiotic CP-70,429 featured a 1(*R*)-oxo-3(*S*)-(thiolanylthio) substituent at carbon 2. The side-chain precursor, 1(*R*)-oxo-3(*S*)-(acetylthio)-thiolane (A), was synthesized initially by Volkmann et al.[1] starting from L-aspartic acid and through the intermediacy of 3(*R*)-[(methylsulfonyl)oxy]- or 3(*R*)-[(*p*-tolylsulfonyl)-oxy]thiolanes, (*R*)-**6** and (*R*)-**9**. An alternate approach to the synthesis of these optically active thiolanes is presented (Scheme I). We started from 2(*R*)-hydroxy-4-(methylthio)butanoic acid ((*R*)-**1**), which was prepared from D-methionine or, more efficiently, by a novel lipase hydrolysis of racemic ethyl 2-hydroxy-4-(methylthio)butyrate (*rac*-**2**).

For our initial work, the diazotization of D-methionine[2] provided a source of optically active 2(*R*)-hydroxy-4-(methylthio)butanoic acid ((*R*)-**1**), although the yield was low. Racemic 2-hydroxy-4-(methylthio)butanoic acid (*rac*-**1**) is readily available as its calcium salt, which is used

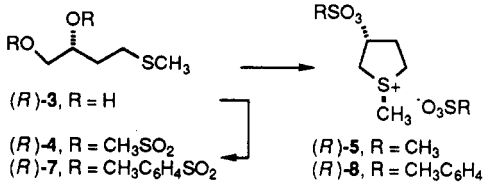
Scheme I



as a methionine substitute in animal feed. However, there have been no reports in the literature for the resolution of this material. The commercially available calcium salt of *rac*-**1** was esterified and the resulting racemic ethyl ester (*rac*-**2**) was resolved by stereospecific hydrolysis with the lipase from *Pseudomonas fluorescens*[3] to give the *R* ester and the *S* acid. While optically active acid (*R*)-**1** was reported also from microbiological reduction of 2-oxo-4-(methylthio)butanoic acid,[4] *the current resolution makes both (R)-2 and (S)-1 readily available chiral starting materials.*



Since the ester group in (*R*)-**2** was activated by the neighboring hydroxy group, the reduction to diol (*R*)-**3** could be accomplished with sodium borohydride. Diol (*R*)-**3** has been prepared previously through resolution of the racemic bis-phthalate half ester of *rac*-**3** with L-amphetamine.[5] The current synthesis afforded (*R*)-**3** with 96% ee, while the literature method yield (*R*)-**3** of 90% ee.



For the thiolane synthesis, diol (*R*)-**3** was converted to its dimesylate (*R*)-**4** with mesyl chloride in pyridine. Dimesylate (*R*)-**4** could be characterized by NMR and TLC but slowly cyclized to the sulfonium salt (*R*)-**5** upon standing. Heating (*R*)-**4** in benzene gave the crude sulfonium salt (*R*)-**5** in 70–80% yield from diol (*R*)-**3** with a trans/cis ratio of 5/2 (estimated by NMR). The pure trans diastereomer was isolated in 40% yield by recrystallization of the crude reaction mixture. Use of tosyl chloride gave similarly a 40% yield of one diastereomer (*R*)-**8** after crystallization, but the determination of the diastereomer ratio was not possible due to the presence of side products in the filtrate including 3-hydroxy-1-methylthiolanium tosylate. The latter was the result of cyclization of 2-

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