we able to shift the 2-DHPP/4-DHPP equilibrium in solution such that the 4-DHPP isomer became the dominant species. Slowly warming the 2-DHPP/4-DHPP mixture to room temperature resulted in the more facile reversion of the 4-DHPP isomer to its components relative to that of the 2-DHPP isomer. Thus, the formation of the 2-DHPP structure appears to be both the thermodynamic as well as the kinetic product under our reaction conditions.

The 2-DHPP material is noted to darken on prolonged standing at room temperature. The colored material must be present in only trace quantities as it cannot be detected spectroscopically; no loss of chemical reactivity for **2** is observed either. The resulting purple-red color is believed to result from a thermally induced solid-state electrocyclic rearrangement of the dihydropyridine ring. Such a transformation would produce an azaglutaconaldehyde derivative which is expected to be highly colored.[10]

The solution chemistry of **2** mirrors that of traditional phosgene reactions. In aprotic media, **2** can be used to produce carbonates, chloroformates, isocyanates, and ureas in high yield. Furthermore, this material can be used as a highly efficient dehydrating agent; at room temperature, carboxylic acids are converted to their corresponding anhydrides with isolated yields ranging from 80 to 95%. The procedure used for the preparation of the anhydrides is analogous to those published previously.[11]

In conclusion, the reaction of pyridine with phosgene to produce a 2:1 salt generates the 2-DHPP structure, **2**, rather than the previously proposed bis(pyridinium salt), **1**. The 2-DHPP isomer is indicated to be both the kinetically and thermodynamically preferred isomer in solution. Furthermore, this type of diadduct may be common to the chemistry of thiophosgene which is also observed to produce a sensitive yellow crystalline precipitate upon reaction with pyridine.[12] The excellent thermal stability of the solid material (**2**), coupled with its facile reversion to its components in solution, allows this salt to be used as a convenient storage system for phosgene.[13]

Experimental Section

General: ^1H NMR and ^{13}C NMR spectra were obtained on Varian XL 200 and XL 300 spectrometers, respectively. The ^{15}N NMR spectra were obtained on a Varian XL 300 spectrometer. The solid-state ^{13}C CP/MAS spectra were acquired on a Nicolet NT-150 wide-bore spectrometer (^{13}C, 37.7 MHz) at the Colorado State University Regional NMR Center (an USF Regional Instrumentation Facility, Grant No. CHE 8208821).

The solution IR data was acquired on a Nicolet 7198 FTIR spectrometer with a MCT(HgCdTe) detector. The system was outfitted with a class II Imw HeNe laser and a Nicolet IR-80 data processor. The variable-temperature IR cell was a SPECAC P/N 21.000 low-temperature solution cell containing 0.1-mm AgCl windows. All nonvariable-temperature infrared measurements were made by using a Perkin-Elmer Model 598 IR spectrometer.

All manipulations of materials and reaction solutions were carried out under an anhydrous nitrogen atmosphere; whenever possible, a Vacuum Atmospheres Model HE-43-2 DRI-LAB glovebox was used.

The elemental analyses were performed by Galbraith Analytical Laboratories.

General Procedure for Solution Analysis of Pyridinium Salts by IR or NMR. In a typical experiment, the pyridinium salt was weighed out on an analytical balance inside a glovebox under a nitrogen atmosphere. A measured amount of the desired deoxygenated, anhydrous solvent was added to form a homogeneous solution. The resulting solution was partitioned among the various IR cells and precison-bore NMR tubes to be used for study. All tubes and cells were stoppered and sealed before their removal from the glovebox for analysis.

1-(2-(Chloroformyl)-2-azacyclohexa-3,5-dienyl)pyridinium Chloride (2). To 48.9 g (0.618 mol) of pyridine in pentane (500 mL) was added 30.7 g (0.309 mol) of phosgene dissolved in pentane (200 mL) over a 15-min time period. The temperature of the reaction solution was maintained at 4 °C (ice–water bath) during the addition time; the reaction was observed to be slightly exothermic. A voluminous yellow-white precipitate formed spontaneously as the phosgene solution was added. After the addition was completed, the reaction mixture was stirred for an additional 30 min and then filtered. The filtrate was washed twice with 500 mL of anhydrous, deoxygenated pentane and then dried under vacuum (~30 mmHg) at room temperature for 3 h. The resulting light yellow material amounted to 73.6 g or a 92.4% yield: mp 84–87 °C dec; IR (KBr) 1752 cm^{-1} (C=O, br); IR (CH_2Cl_2, −66 °C) 1762 (w), 1734 (vs), 1689 cm^{-1}; ^1H NMR (CD_2Cl_2, 200 MHz, −55 °C) δ 9.54 (d, J = 6.2 Hz, 2 H), 8.43 (t, J = 7.65, 7.65 Hz, 1 H), 8.03 (dd, J = 6.2, 7.65 Hz, 2 H), 7.51 (d, J = 9.5 Hz, 1 H), 7.46 (d, J = 8 Hz, 1 H), 7.33 (dd, J = 4.2, 3.5 Hz, 1 H), 5.61 (dd, J = 8, 4.2 Hz, 1 H), 5.55 (dd, J = 9.5, 3.5 Hz, 1 H); ^{13}C NMR (CD_2Cl_2, 75 MHz, −55 °C) δ 147.5, 146.4, 144.1, 129.3, 128.8, 127.8, 107.4, 106.3, 64.3; ^{13}C NMR (solid state, 37.7 MHz) δ 146, 130, 108, 64. Anal. Calcd for $C_{11}H_{10}Cl_2N_2O$: C, 51.39; H, 3.92; N, 10.90; Cl, 27.58. Found: C, 51.60; H, 4.13; N, 11.09; Cl, 27.24.

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Registry No. 1, 117371-70-7; 2, 117371-69-4; pyridine, 110-86-1; phosgene, 75-44-5.

Supplementary Material Available: Proton and carbon NMR spectra for **2** (4 pages). Ordering information is given on any current masthead page.

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(13) This material can be stored at room temperature for over a year with essentially no loss of chemical reactivity. Rigorous exclusion of moisture is required. This diadduct salt (**2**) has been termed phosgene-in-a-can.

Iejimalides A and B, Novel 24-Membered Macrolides with Potent Antileukemic Activity from the Okinawan Tunicate *Eudistoma* cf. *rigida*

Jun'ichi Kobayashi,*[1a] Jie-fei Cheng,[1a] Tomihisa Ohta,[1b] Hideshi Nakamura,[1a] Shigeo Nozoe,[1b] Yoshimasa Hirata,[1c] Yasushi Ohizumi,[1a] and Takuma Sasaki[1d]

Mitsubishi Kasei Institute of Life Sciences, Minamiooya, Machida, Tokyo 194, Japan, Pharmaceutical Institute, Tohoku University, Sendai 980, Japan, Faculty of Pharmacy, Meijo University, Nagoya 468, Japan, and Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan

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Recently, increasing attention has been paid to tunicates, since their metabolites frequently possess interesting pharmacological activities.[2] In our continuing search for bioactive compounds from Okinawan tunicates,[3] we have

(1) (a) Mitsubishi Kasei Institute of Life Sciences. (b) Tohoku University. (c) Meijo University. (d) Kanazawa University.

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encountered the compound tunicate *Eudistoma* cf. *rigida*,[4] extracts of which exhibited strong antileukemic activity. In this paper we describe the isolation and structure elucidation of two novel 24-membered macrolides, designated iejimalides A (**1**) and B (**2**), with potent cytotoxicity against L1210 (IC_{50} = 62 and 32 ng/mL) and L5178Y (IC_{50} = 22 and 1.0 ng/mL) murine leukemia cells in vitro. This is the first isolation of macrocyclic lactones from a tunicate.

Isolation and Characterization. The tunicate *Eudistoma* cf. *rigida* was collected at Ie Island, Okinawa, by using SCUBA (−5 to −15 m), and kept frozen until used. The tunicate was extracted with methanol/toluene (3:1), and the extract was partitioned with toluene and 1 M aqueous NaCl. The toluene-soluble material was repeatedly chromatographed on silica gel columns with methanol/chloroform (2.5:97.5) followed by reversed-phase HPLC on ODS with acetonitrile/water (8:2) to give iejimalides A (**1**, 0.0003% wet weight) and B (**2**, 0.0003%) as a colorless noncrystalline solid. IR spectra of **1** and **2** showed the same absorptions at 3350, 1715, and 1650 cm^{-1}, which were assignable to bands of NH/OH, ester, and amide carbonyl groups, respectively. The broad UV absorptions at 231 (ϵ 26 000) nm for **1** and at 235 (ϵ 27 000) nm for **2** indicated the presence of a tetrasubstituted diene chromophore.[5] In the positive ion FABMS, **1** and **2** showed each quasi-molecular ion at m/z 784 and 798 (M^+ + diethanolamine + H), while the negative ion FABMS revealed each (M^- − H) ion at m/z 677 and 691, suggesting molecular weights of **1** and **2** to be 678 and 692, respectively. The two amide NH protons (δ 6.7–6.8) appeared in the ^1H NMR spectra of **1** and **2**. In combination with the ^1H and ^{13}C NMR data (Table I), the molecular formulas of **1** and **2** were concluded to be $C_{40}H_{58}O_7N_2$ and $C_{41}H_{60}O_7N_2$, respectively. From comparison of those NMR and MS data, the only structural difference between **1** and **2** was demonstrated to be in that **2** contained an extra methyl group at C-2. Structure elucidation was carried out mostly with iejimalide B (**2**), mp 71–73 °C, $[\alpha]^{23}_D$ −17.6° (*c* 0.17, $CHCl_3$).

Outline for the Structure Determination. Extensive 500-MHz NMR analyses were carried out by using iejimalide B (**2**). Detailed interpretation of COSY[6] spectrum and ^1H–^1H coupling constants gave rise to the proposed partial structures: C-2–C-6 (A), C-7–C-12 (B), C-13–C-23 (C), C-25–N-30 (D), and C-32–C-35 (E). Examination of DEPT[7] and ^1H–^{13}C COSY[8] (supplemental material) spectra established the presence of a total of 41 carbons including two ester and/or amide carbonyls, one formyl carbonyl, two methoxys, five methylenes, five tertiary methyls, two secondary methyls, six sp^3 and thirteen sp^2 methines, and five quaternary sp^2 carbons. The phase-sensitive NOESY[9] (PSNOESY) in addition to conventional NOESY and ^{13}C chemical shifts[10] of olefinic methyls afforded useful information on the geometries of five trisubstituted double bonds. Five segments (A–E) were separated by two carbonyls and three quaternary carbons. A number of long-range (two- and three-bond) couplings observed through HMBC[11] experiments were very useful for confirming assignments of ^1H and ^{13}C NMR signals and establishing the connectivities among five segments (A–E) to construct a whole molecule.

The following detailed discussion is based mainly on these NMR spectral analyses.

Partial Structures A–E. The structure of segment A was disclosed as follows. In the COSY spectrum (supplementary material) a proton at δ 6.62 (labeled H-3) showed couplings to protons at δ 1.79 (H_3-44) and δ 3.15 (H-4). The chemical shift of H-3 along with the absence of other couplings in the COSY spectrum implied that this proton was attached to the β-carbon of the α,β-unsaturated carbonyl group. The HMBC data[12] (supplementary ma-

Table I. ^1H and ^{13}C NMR Chemical Shifts (ppm) of Iejimalide B (2) and NOE Correlations Observed through NOESY Experiments

position	^{13}C δ (m)	^1H δ (m)	J(H,H), Hz	NOE[a] (^1H)
1	167.39 (s)			
2	125.33 (s)			
3	145.41 (d)	6.62 (dd)	10.3, 1.4	H-5
4	37.86 (d)	3.15 (m)	9.0, 10.3, 6.8	H-6, H_3-44
5	131.67 (d)	5.46 (dd)	15.5, 9.0	H-3, H_3-42[b]
6	133.16 (d)	5.86 (d)	15.5	H-4, H-8
7	133.72 (s)			
8	131.58 (d)	5.08 (d)	9.3	H-6
9	76.70 (d)	4.12 (ddd)	9.3, 2.6, 9.3	H_3-42[b]
10	40.46 (t)	2.32, 2.68 (m)		
11	124.61 (d)	5.51 (ddd)	4.7, 10.5, 15.7	H_3-40[b]
12	129.52 (d)	6.48 (d)	15.7	
13	136.74 (s)			
14	128.37 (d)	5.18 (dd)	10.7, 5.6	
15	22.76 (t)	1.90, 2.55 (m)		H_3-40
16	34.71 (t)	1.32, 1.62 (m)		
17	79.52 (d)	3.27 (m)		H-19
18	132.87 (d)	5.38 (dd)	14.6, 5.5	H-20
19	131.99 (d)	6.03 (qq)	14.6, 10.4	H-21
20	130.76 (d)	6.03 (qq)	14.6, 10.4	H-18
21	135.69 (d)	5.39 (dd)	14.6, 4.2	H-19
22	40.62 (d)	2.55 (m)	10.0, 4.2, 6.8	H-20
23	82.72 (d)	5.14 (d)	10.0	H-26[b]
25	132.93 (s)			
26	124.99 (d)	6.28 (d)	11.3	H-23,[b] H_3-36[b]
27	121.20 (d)	6.13 (dd)	11.3, 0.95	H-29, H_3-37[b]
28	134.56 (s)			
29	46.85 (t)	3.90 (d)	5.9	H-32, H-27, H_3-36
30 (NH)		6.80 (t)	5.9	H-32
31	170.27 (s)			
32	52.39 (d)	4.51 (m)		H-29, H-30
33 (NH)		6.87 (d)	7.1	
34	161.56 (d)	8.28 (s)		
35	62.21 (t)	3.65 (m)		
		4.20 (dd)	11.4, 2.6	
35-OH		3.37 (br s)		
36	14.93 (q)	1.75 (s)		H-29, H-26[b]
37	11.77 (q)	1.77 (s)		H-27[b]
38	16.57 (q)	0.93 (d)	6.8	
39	56.09 (q)	2.96 (s)		
40	20.57 (q)	1.80 (s)		H-11,[b] H-15
41	55.74 (q)	3.26 (s)		
42	12.92 (q)	1.74 (s)		H-5,[b] H-9[b]
43	21.26 (q)	1.05 (d)	6.8	
44	11.97 (q)	1.79 (s)		H-4

[a] Cross-peaks resulting from ^1H–^1H couplings are not shown here. [b] NOE also observed in phase-sensitive NOESY experiments.

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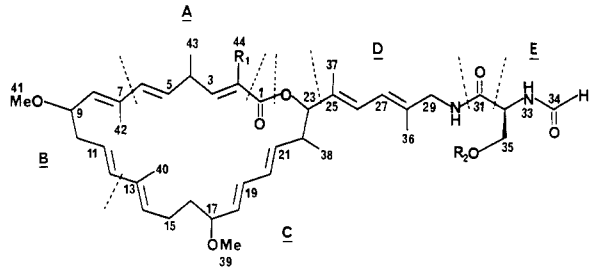

1 $R_1 = H$ $R_2 = H$

2 $R_1 = Me$ $R_2 = H$

3 $R_1 = Me$ $R_2 = Ac$

terial) showed a cross-peak from H-3 to the carbonyl (C-1) at δ 167.39, confirming the connectivity of C-1–C-3. In the COSY spectrum H-4 was coupled to H_3-43 (δ 1.05) and H-5 (δ 5.56), the latter in turn showed a coupling to H-6 (δ 5.86) with a trans coupling constant of 15.5 Hz. In the NOESY spectrum (Table I) an obvious cross-peak was observed between H-4 and H_3-44, which allowed assignment of *E* geometry to Δ^2-double bond. The higher field resonance[10] (δ 11.97) of the olefinic methyl (C-44) also provided a supporting evidence to this assignment.

Connectivity of the backbone chain of segment B was deduced from cross-peaks observed for H-8 (δ 5.08)/H-9 (δ 4.12), H-9/H_2-10 (δ 2.32 and 2.68), H_2-10/H-11 (δ 5.51), and H-11/H-12 (δ 6.48) in the COSY spectrum. One methoxy group (δ 3.26) was fixed at C-9 (δ 76.70) by observation of a three-bond coupling for this methoxy proton (H_3-41) to C-9 in the HMBC spectrum. Since the COSY spectrum revealed a coupling for H-8 to a methyl group at δ 1.74, this methyl (C-42) was connected at C-7. Evidence of *E* configuration assigned to the Δ^7-double bond arose from the higher field chemical shift (δ 12.92) of C-42 and the PSNOESY experiments in which a clear cross-peak was observed for H-9/H_3-42. The geometry of the Δ^{11}-double bond was unambiguously assigned as trans ($J_{11,12}$ = 15.7 Hz).

For segment C the COSY spectrum afforded the following proton connectivities: H-14 (δ 5.18)/H_3-40 (δ 1.80), H-14/H_2-15 (δ 1.90 and 2.55), H_2-15/H_2-16 (δ 1.32 and 1.62), H_2-16/H-17 (δ 3.27), H-17/H-18 (δ 5.38), H-18/H-19 (δ 6.03), H-19/H-20 (δ 6.03), H-20/H-21 (δ 5.39), H-21/H-22 (δ 2.55), H-22/H-23 (δ 5.14), H-22/H_3-38 (δ 0.93). The HMBC spectrum showed a three-bond coupling for methoxy protons at δ 2.96 (H_3-39) to C-17, establishing the substitution of this methoxy group at C-17. The configuration of the Δ^{13}-double bond was assigned as *E* due to the presence of a cross-peak for H_2-15/H-40 in the PSNOESY spectrum. The conjugated trans diene system (C_{18}–C_{21}) in segment C was deduced from a typical ^1H resonance pattern[13] consisting of two quartets centered at δ 6.03 (H-19 and H-20, $J_{18,19} = J_{20,21}$ = 14.6 Hz, and $J_{19,20}$ = 10.4 Hz) and a complex pattern at δ 5.4 (H-18 and H-21). The lower field chemical shifts (δ(H) 5.14 and δ(C) 82.72) of 23-methine strongly suggested that this carbon should bear an oxygen.

The presence of a NH group adjacent to the 29-methylene in segment D was evidenced by the vicinal coupling (*J* = 5.9 Hz) between these protons. In the COSY spectrum H-26 (δ 6.28) showed couplings to H_3-37 (δ 1.77) and H-27 (δ 6.13), which in turn coupled to H_3-36 (δ 1.75) and H_2-29 (δ 3.90). The higher field resonances[10] for the C-36 (δ 14.93) and C-37 (δ 11.77) methyls indicated that the configurations of Δ^{25}- and Δ^{27}-double bonds were both *E*. The NOESY spectra showing cross-peaks for H-26/H_3-36, H-27/H_2-29, and H-27/H_3-37 provided further evidence for assignment of configurations of the two double bonds.

The formamide functionality in segment E was deduced from its typical resonances (δ(H) 8.28 and δ(C) 161.56) and COSY correlation between the formyl proton (H-34) and NH proton (H-33) at 6.67. The proton (δ 3.65 and 4.20) and carbon (δ 62.21) chemical shifts of 35-methylene suggested that this carbon should be attached to an oxygen atom. Treatment of iejimalide B (**2**) with acetic anhydride in the presence of pyridine furnished the monoacetate (**3**) (FABMS, *m/z* 840, M^+ + diethanolamine + H), the ^1H NMR spectrum of which differed from that of **2** only in the chemical shifts of H_2-35 (**3**, δ 4.33 and 4.45) and H-32 (δ 4.51–4.79). In the COSY spectrum H-32 showed cross-peaks to H-33 and H_2-35. Thus the presence of a hydroxy group at C-35 was established. The HMBC spectrum afforded unambiguous connectivity from C-31 to C-32, namely, C-31 (δ 170.27) showed long-range connectivity to H-35. Amino acid analysis of the hydrolysis product (6 N, HCl, 115 °C) of **2** showed the presence of 1 mol of serine in the molecule. The absolute configuration of serine was established to be *S* by using a chiral HPLC column. This L-serine was concluded to have been generated from the segment *E* in **2**.

Whole Structure. Partial structures A–E were connected on the basis of HMBC and NOESY data. The HMBC spectrum showed three-bond couplings for C-42 to H-6 and H-8, while the NOESY spectrum revealed cross-peaks for H-6/H-8 and H-5/H_3-42, confirming connection from segment A to B. Since H-23 was coupled to C-1 in the HMBC spectrum, segment A was connected to segment C through an ester linkage (C-1, O-24, and C-23). The connectivity of partial structure D to E was deduced from a cross-peak for C-31 to H-29 in the HMBC spectrum and cross peaks for H-29/H-32 and H-30/H-32 in the NOESY spectrum. One end (C-25) of the side chain consisting of segments D and E was attached to C-23 on the basis of the fact that a three-bond coupling was detected for C-23 to H-26 in the HMBC spectrum and a cross-peak for H-23/H-26 in the NOESY spectrum. Finally, the remaining bond C-12–C-13 was the only possible way to complete the structural assignment of **2**, constructing the 24-membered macrocyclic lactone ring. This connectivity was confirmed by a long-range coupling for H-12 to C-40 in the HMBC spectrum.

The structure of iejimalide A (**1**) was determined from comparison of the ^1H and ^{13}C NMR data with those of **2**. Compounds **1** and **2** showed essentially identical ^1H and ^{13}C NMR spectra to each other, except that **1** revealed an extra olefinic methine resonance (δ(H) 5.71 and δ(C) 118.96) instead of the methyl (δ(H) 1.79 and δ(C) 11.77) at C-2. Iejimalide A (**1**) was thus established to be 2-demethyl form of iejimalide B (**2**).

The absolute stereochemistry at C-32 was determined as *S* among six chiral centers in **1** and **2**. The relative stereochemistry at C-22 and C-23 was assigned trans by the *J* = 10.0 Hz between H-22 and H-23. The stereochemistry at C-4, C-9, and C-17 remains to be resolved.

Discussion

Iejimalides A (**1**) and B (**2**) are the first macrocyclic lactones from a tunicate. These two 24-membered macrolides bear no biosynthetic resemblance to known tu-

(12) Selective data of two- and three-bond long-range ^{13}C–^1H couplings observed for **2** through HMBC experiments: C-1/H-3, C-1/H-23, C-9/H-41, C-17/H-39, C-23/H-26 C-31/H-29, C-31/H-35, C-40/H-12, C-42/H-6, C-42/H-8.

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nicate metabolites such as β-carbolines,[14] cyclopeptides,[15] and polycyclic aromatic alkaloids.[3d,e,16,17] It is noted that a rare *N*-formyl-L-serine unit (C-31–C-35) is included in the side chains of macrolides **1** and **2**, although some marine macrolides[18] contain a *N*-formyl group in their side chains. Recently, a number of macrolides have been isolated from marine microorganisms,[18a,19] sponges,[18d,20] nudibranch eggs,[18b,18c] and bryozoans.[21] There remain questions as to whether those macrolides as well as **1** and **2** were produced by host animals or symbiotic microorganisms.

Experimental Section

General Methods. All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured on a Hitachi 265-50 infrared spectrometer. UV spectra were taken on a JASCO UVIDEC-660 spectrometer. Optical rotations were obtained on a JASCO DIP-360 polarimeter. ^1H and ^{13}C NMR spectra were recorded on JEOL GX-500 and Bruker AM-500 spectrometers. The 7.27 ppm resonance of residual $CHCl_3$ and 76.9 ppm of $CDCl_3$ were used as internal references for ^1H and ^{13}C NMR, respectively. Mass spectra (FABMS) were obtained on a JEOL HX-100 spectrometer by using diethanolamine as a matrix. Amino acid analysis was performed on an IRICA A-5500 analyzer.

Collection, Extraction, and Separation. *Eudistoma* cf. *rigida*, a purple-colored compound tunicate, was collected at Ie Island, Okinawa, in 1987 by using SCUBA (−5 to −15 m), frozen, and shipped via air to Tokyo. The tunicate (1.6 kg, wet weight) was extracted with methanol/toluene (3:1, 2.5 L × 2). After addition of 1 M NaCl (1.2 L), the mixture was extracted with toluene (500 mL × 4). The toluene-soluble material (2.5 g) was chromatographed on a silica gel column (Wako gel C-300, Wako Chemical, 25 × 200 mm) with methanol/chloroform (2.5:97.5) to give an active fraction (850–950 mL), which was again passed through a silica gel column (Wako gel C-300, 15 × 300 mm) with the same solvent system followed by HPLC separation (Develosil ODS-5, Nomura Chemical, 10 × 250 mm) with acetonitrile/water (8:2) to afford iejimalides A (**1**, t_R 18.3 min, 0.0003% wet weight) and B (**2**, t_R 21.6 min, 0.0003%).

Iejimalide A (1): a colorless noncrystalline solid; mp 71–73 °C; $[\alpha]^{23}_D$ −36.4° (*c* 0.17, $CHCl_3$); IR ($CHCl_3$) 3350, 2925, 2850, 1715, 1600, 1530, 1450, 1380, 1260, 1220, 1150, 1080, 990, and 970 cm^{-1}; UV (MeOH) 231 nm (ε 26 000); ^1H NMR ($CDCl_3$) δ 8.29 (s br, H-34), 6.85 (m, H-33), 6.80 (t, *J* = 5.9 Hz, H-30), 6.62 (dd, *J* = 9.6 and 15.4 Hz, H-3), 6.47 (d, *J* = 15.5 Hz, H-12), 6.27 (d, *J* = 10.9 Hz, H-26), 6.12 (d, *J* = 10.9 Hz, H-27), 6.02 (m, H-19 and H-20), 5.90 (d, *J* = 15.5 Hz, H-6), 5.71 (d, *J* = 15.4 Hz, H-2), 5.53 (ddd, *J* = 5.0, 9.4 and 15.5 Hz, H-11), 5.45 (dd, *J* = 15.5 and 8.9 Hz, H-5), 5.43 (dd, *J* = 14.0 and 5.0 Hz, H-21), 5.42 (dd, *J* = 14.0 and 4.2 Hz, H-18), 5.21 (dd, *J* = 10.7 and 5.6 Hz, H-14), 5.12 (d, *J* = 10.0 Hz, H-23), 5.11 (d, *J* = 9.6 Hz, H-8), 4.50 (m, H-32), 4.19 (dd, *J* = 11.0 and 2.6 Hz, H-35), 4.14 (ddd, *J* = 2.7, 9.6 and 9.6 Hz, H-9), 3.89 (d, *J* = 5.9 Hz, H-29), 3.65 (m, H-35), 3.37 (m, OH), 3.33 (m, H-17), 3.26 (s, 3 H, H-41), 3.04 (s, 3 H, H-39), 2.96 (m, H-4), 2.65 (m, H-10), 2.55 (m, 2 H, H-15 and H-22), 2.36 (m, H-10), 2.05 (m, H-15), 1.80 (s, 3 H, H-40), 1.75 (s, 3 H, H-37), 1.67 (6 H, H-42 and H-36), 1.61 (m, H-16), 1.36 (m, H-16), 1.10 (d, 3 H, *J* = 6.8 Hz, H-43) and 0.93 (d, 3 H, *J* = 6.8, H-38); ^{13}C NMR ($CDCl_3$) δ 170.60 (s, C-31), 166.09 (s, C-1), 161.61 (d, C-34), 152.24 (d, C-3), 136.54 (s, C-13), 135.47 (d, C-21), 134.56 (s, C-28), 133.77 (d, C-6), 133.72 (s, C-7), 132.68 (d, C-18), 131.90 (d, C-19), 131.75 (d, C-5), 131.00 (s, C-25), 130.51 (d, C-8), 129.54 (d, C-20), 128.45 (d, C-12), 128.44 (d, C-14), 125.04 (d, C-26), 124.55 (d, C-11), 121.19 (d, C-27), 118.96 (d, C-2), 82.63 (d, C-23), 79.63 (d, C-17), 76.64 (d, C-9), 62.21 (t, C-35), 56.12 (q, C-39), 55.78 (q, C-41), 52.41 (d, C-32), 46.64 (t, C-29), 41.73 (d, C-4), 40.20 (d, C-22), 39.69 (t, C-10), 34.67 (t, C-16), 22.72 (t, C-15), 21.25 (q, C-43), 20.57 (q, C-40), 16.60 (q, C-38), 14.94 (q, C-42), 12.93 (q, C-36), and 11.97 (q, C-37); FABMS (positive ion), *m/z* 784 (M^+ + diethanolamine + H), (negative ion) *m/z* 677 (M^- − H).

Iejimalide B (2): a colorless noncrystalline solid; mp 69–71 °C; $[\alpha]^{23}_D$ −17.6° (*c* 0.17, $CHCl_3$); IR ($CHCl_3$) 3350, 2925, 2850, 1715, 1650, 1530, 1450, 1380, 1250, 1220, 1100, 1080, 990, and 970 cm^{-1}; UV (MeOH) 235 nm (ε 27 000); ^1H and ^{13}C NMR (see Table I); FABMS (positive ion), *m/z* 798 (M^+ + diethanolamine + H), (negative ion) *m/z* 691 (M^- − H).

Iejimalide B Acetate (3). Iejimalide B (**2**, 1.0 mg) was dissolved with 0.1 mL of pyridine and 0.1 mL of acetic anhydride at room temperature overnight. After evaporation of organic solvents, the residue was passed through a silica gel column (Wako-gel C-300, 5 × 100 mm) with methanol/chloroform (1:99) to afford iejimalide B monoacetate **3** (0.8 mg): FABMS (positive ion), *m/z* 840 (M^+ + diethanolamine + H); ^1H NMR ($CDCl_3$) δ 8.28 (s, H-34), 6.57 (d, *J* = 8.1 Hz, H-33), 6.62 (t, *J* = 5.9 Hz, H-30), 6.61 (d, *J* = 10.4 Hz, H-3), 6.49 (d, *J* = 16.5 Hz, H-12), 6.28 (d, *J* = 11.7 Hz, H-26), 6.13 (d, *J* = 11.7 Hz, H-27), 6.03 (m, H-19 and H-20), 5.86 (d, *J* = 15.4 Hz, H-6), 5.51 (m, H-11), 5.46 (m, H-5), 5.39 (H-18 and H-21), 5.18 (dd, *J* = 10.7 and 5.6 Hz, H-14), 5.14 (d, *J* = 10.2 Hz, H-23), 5.08 (d, *J* = 9.5 Hz, H-8), 4.79 (m, H-32), 4.45 (dd, *J* = 11.5 and 6.6 Hz, H-35), 4.33 (m, dd, *J* = 11.5 and 4.9 Hz, H-35′), 4.14 (dt, *J* = 9.3 and 2.6 Hz, H-9), 3.92 (d, *J* = 5.9 Hz, H-29), 3.26 (m, H-17), 3.25 (s, 3 H, H-41), 3.12 (m, H-4), 2.96 (m, 3 H, H-39), 2.68 (m, H-10), 2.54 (m, H-15 and H-22), 2.34 (m, H-10′), 2.03 (m, H-15′), 1.80 (s, 3 H, H-40), 1.79 (s, 3 H, H-37), 1.77 (s, 3 H, H-44), 1.75 (s, 3 H, H-36), 1.75 (s, 3 H, H-42), 1.62 (m, H-16), 1.32 (m, H-16′), 1.05 (d, 3 H, *J* = 6.7 Hz, H-43), and 0.93 (d, 3 H, *J* = 6.6 Hz, H-38).

Detection of L-Serine. Iejimalide B (**2**, 1.0 mg) was hydrolyzed in 6 N HCl at 115 °C for 20 h in a degassed sealed tube. After evaporation of the solvent in vacuo, the hydrolysate was subjected to amino acid analysis resulting in detection of serine. The hydrolysate was also chromatographed on Chiralpak WH (Daicel Chemical Ind. Ltd., 4.6 × 250 mm) with 1.0 mM $CuSO_4$ aqueous solution (flow rate 1.5 mL/min), monitoring at 254 nm. Both L-serine in the hydrolysate and that as control were detected at t_R 10.5 min, while D-serine as control appeared at t_R 7.5 min.

Determination of Antitumor Activity in Vitro. Antitumor activity was determined by using mouse leukemia cell lines L5178Y and L1210 according to the method previously reported.[3c]

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Supplementary Material Available: ^1H–^1H COSY, ^1H–^{13}C COSY, NOESY, PSNOESY, and HMBC spectra for compound **2** and ^1H–^1H COSY spectrum for compound **1** (7 pages). Ordering information is given on any current masthead page.

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