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Absolute stereochemistry and antitumor activity of iejimalides

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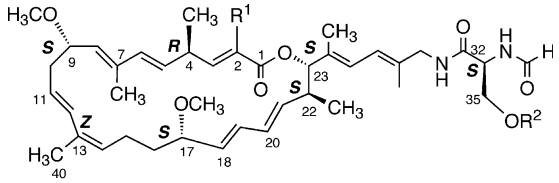
Abstract—The absolute configurations at five chiral centers, except for C-32(*S*) reported previously, in iejimalides A, C, and D, potent cytotoxic 24-membered macrolides isolated from a tunicate *Eudistoma* cf. *rigida*, were assigned as 4*R*, 9*S*, 17*S*, 22*S*, and 23*S* on the basis of detailed analysis of NMR data and chemical means. Furthermore, the structures proposed for iejimalides A, C, and D were revised to their 13*Z*-isomers. Iejimalides A–D (**1**–**4**) exhibited antitumor activity in vivo.

1. Introduction

Iejimalides obtained from a marine tunicate *Eudistoma* cf. *rigida* are unique 24-membered macrolides having two methoxy groups, four diene units, and an *N*-formyl-L-serine terminus, and exhibit potent cytotoxic activity in vitro.[1,2] The gross structures have been elucidated by means of 2D NMR data, and the absolute configuration of the serine residue (C-32) has been determined to be L-form (32*S*) by chiral HPLC analysis of their hydrolysates. Potent cytotoxic activity of iejimalides has attracted great interest as one of the challenging targets for total synthesis[3,4] or as a candidate of new antitumor drugs. Since a limited amount of samples isolated from the tunicate, the stereochemistry of five chiral centers (C-4, C-9, C-17, C-22, and C-23) remains unsolved, and details of its antitumor activity have not been examined. Recently, we have isolated a relatively large amount of iejimalides from a tunicate *Cystodytes* sp. collected off Ie Island, Okinawa,[5,6] and the structure of iejimalide B (**2**) including the relative and absolute stereochemistry was elucidated by detailed analyses of NMR data, distance geometry calculation, and synthesis of a degradation product.[7] In this paper, we describe the revised structures and absolute stereochemistry of iejimalides A (**1**), C (**3**), and D (**4**) on the basis of NMR data and chemical means.



2. Results and discussions

First, the structure of iejimalide A was suggested to be the 13*Z*-isomer of the original structure by the ^{13}C chemical shift of C-40 (δ_C 20.6) and the ROESY correlation for H-11/H_3-40. Since iejimalide A (**1**) was unstable in $CHCl_3$ (or $CDCl_3$) solution, measurements of NMR data of **1** were carried out as a MeOH-d_4 solution (Table 1). Spectral data of iejimalide B (**2**) were re-measured in MeOH, and ^1H–^1H coupling constants (Table 2) and ROESY data of **2** in MeOH were obtained. ^1H–^1H coupling constants in MeOH-d_4 and $CDCl_3$ were revealed to be similar to each other, except

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for H-14/H-15b, H-15a/H-16a, and H-15b/H-16b. Thus, the solution conformations of **2** in MeOH and $CHCl_3$ were deduced to be close to each other. To elucidate the relative stereochemistry of iejimalide A (**1**), a comparison of ^1H–^1H coupling constants of **1** and those of iejimalide B (**2**) was performed. The ^1H–^1H coupling constants were obtained by detailed analyses of resolution-enhanced and/or differential homonuclear decoupling ^1H NMR spectra in MeOH-d_4, and ^1H chemical shifts and ^1H–^1H coupling constants of **1** and **2** are summarized in Tables 1 and 2. Using these coupling constants and ROESY data, conformational analyses of all bond-rotations for **1** were performed.

For the C-1–C-11 portion (Fig. 1), both *anti*-relations for H-3–H-4 and H-4–H-5 were suggested by relatively large *J* values (H-3/H-4: 9.4 Hz, H-4/H-5: 8.9 Hz) and ROESY correlations for H-2/H-4, H-3/H-5, and H-4/H-6. ROESY correlations were observed for H-5/H_3-42 and H-6/H-8, indicating the presence of *S-trans*-diene at C-5–C-8. Orientation of the methyl group (C-43) at C-4 to the outside of the ring was indicated by ROESY correlations for H-3/H_3-43 and H-5/H_3-43. The *J*(H-8/H-9) (9.5 Hz) and *J*(H-9/H-10b) (9.7 Hz) values were typical for both *anti*-relations, while the relatively small *J*(H-9/H-10a) value (2.7 Hz) was attributed to a *gauche*-relation for H-9/H-10a. ROESY correlations for H-8/H-10b, H-8/H_3-41, and H-10a/H_3-41 also supported that the methoxy group at C-9 was oriented to the outside of the macrolactone ring. Therefore, the conformation of the C-1–C-11 portion and the relative configuration at C-4 and C-9 were assigned as shown in Figure 1. Conformational analyses from ^1H–^1H coupling constants and ROESY data revealed for the C-8–C-18 and C-15–C-27 portions in **1** were close to the corresponding portions for iejimalide B (**2**) as shown in Figures 2 and 3, respectively. The ^1H–^1H coupling constants for H-14/H-15b (6.5 Hz), H-15a/H-16a (6.3 Hz), and H-15b/H-16a (4.5 Hz) H-15b/H-16b (6.5 Hz) of iejima-

Table 1. ^1H NMR data of iejimalides A–D (**1**–**4**) in CD_3OD

	1	2	3	4
2	5.79	—	5.78	—
3	6.87	6.65	6.87	6.65
4	3.06	3.26	3.06	3.26
5	5.59	5.58	5.59	5.58
6	6.02	5.99	6.01	5.99
8	5.16	5.13	5.16	5.13
9	4.27	4.26	4.27	4.27
10a	2.66	2.69	2.66	2.70
10b	1.98	1.93	2.34	1.91
11	5.64	5.62	5.63	5.62
12	6.49	6.51	6.48	6.51
14	5.26	5.24	5.26	5.24
15a	2.46	2.57	2.46	2.58
15b	2.35	2.32	1.99	2.32
16a	1.61	1.63	1.62	1.64
16b	1.39	1.36	1.40	1.36
17	3.40	3.32	3.41	3.32
18	5.42	5.40	5.42	5.40
19	6.03	6.00	6.03	5.99
20	6.11	6.11	6.11	6.11
21	5.49	5.43	5.49	5.42
22	2.61	2.63	2.61	2.63
23	5.13	5.16	5.13	5.16
26	6.35	6.36	6.36	6.35
27	6.25	6.26	6.24	6.24
29	3.91	3.89	3.89	3.89
32	4.54	4.54	4.33	4.74
34	8.21	8.21	8.22	8.22
35a	—	3.88	4.33	4.34
35b	3.91	3.81	4.27	4.27
36	1.80	1.81	1.81	1.81
37	1.82	1.83	1.83	1.83
38	1.14	1.10	0.95	1.10
39	3.09	3.01	3.09	3.00
40	1.81	1.85	1.83	1.85
41	3.29	3.28	3.29	3.28
42	1.81	1.80	1.82	1.80
43	0.95	0.96	1.15	0.96
44	—	1.83	—	1.83

Table 2. ^1H–^1H coupling constants of iejimalides A (**1**) and B (**2**) in CD_3OD

H/H	1	2
H-2/H-3	15.4	—
H-3/H-4	9.4	10.3
H-4/H-5	8.9	8.8
H-4/H_3-43	6.8	6.7
H-5/H-6	15.5	15.4
H-8/H-9	9.5	9.5
H-9/H-10a	2.9	2.6
H-9/H-10b	9.7	10.1
H-10a/H-11	5.1	4.5
H-10b/H-11	9.7	10.2
H-11/H-12	15.7	15.4
H-14/H-15a	10.0	10.9
H-14/H-15b	6.5	5.8
H-15a/H-16a	6.3	4.9
H-15a/H-16b	10.1	11.1
H-15b/H-16a	4.5	4.7
H-15b/H-16b	6.5	5.5
H-16a/H-17	10.3	10.2
H-16b/H-17	3.8	3.0
H-17/H-18	7.8	8.2
H-18/H-19	15.1	15.4
H-19/H-20	9.7	10.4
H-20/H-21	15.0	15.1
H-21/H-22	8.9	9.4
H-22/H-23	9.9	9.7
H-22/H_3-38	6.8	6.7
H-26/H-27	11.8	11.3

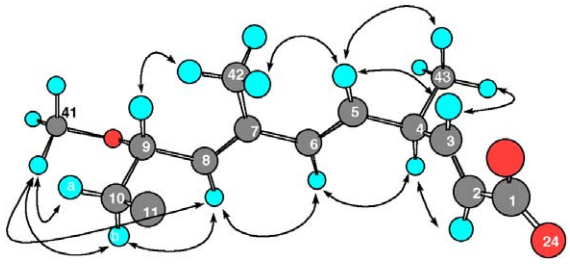

Figure 1. ROESY correlations and relative stereochemistry for the C-1–C-11 part of iejimalide A (**1**). ROESY correlations are illustrated by arrows. *J* in Hz (H/H): H-2/H-3: 15.4, H-3/H-4: 9.4, H-4/H-5: 8.9, H-5/H-6: 15.5, H-8/H-9: 9.5, H-9/H-10a: 2.9, H-9/H-10b: 9.7.

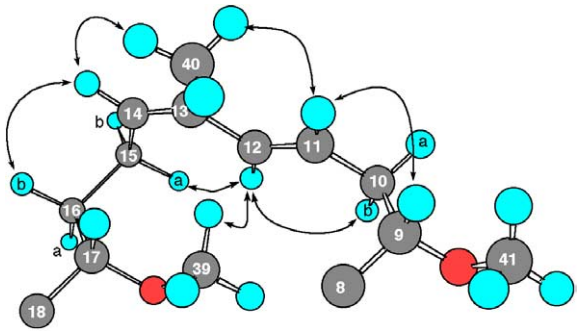

Figure 2. ROESY correlations and relative stereochemistry for the C-8–C-18 part of iejimalide A (**1**). ROESY correlations are illustrated by arrows. *J* in Hz (H/H): H-10a/H-11: 5.1, H-10b/H-11: 9.7, H-11/H-12: 15.7, H-14/H-15a: 10.0, H-14/H-15b: 6.5, H-15a/H-16a: 6.3, H-15a/H-16b: 10.1, H-15b/H-16a: 4.5, H-15b/H-16b: 6.5, H-16a/H-17: 10.3, H-16b/H-17: 3.8.

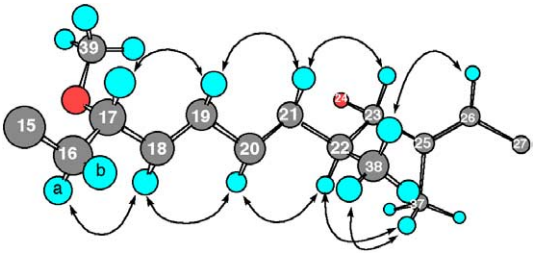

Figure 3. ROESY correlations and relative stereochemistry for the C-15–C-27 part of iejimalide A (**1**). ROESY correlations are illustrated by arrows. *J* in Hz (H/H): H-17/H-18: 7.8, H-18/H-19: 15.1, H-19/H-20: 9.7, H-20/H-21: 15.0, H-21/H-22: 8.9, H-22/H-23: 9.9.

lide B (**2**) in MeOH-d_4 were different from those (H-14/H-15b: 4.6 Hz, H-15a/H-16a: 2.3 Hz, H-15b/H-16a: 2.0 Hz, and H-15b/H-16b: 3.0 Hz)[8] in $CDCl_3$, indicating that the solution conformation for C-14–C-16 portion of **2** in MeOH-d_4 was more flexible than that in $CDCl_3$. The absolute stereochemistries of the remaining four chiral centers in iejimalide A (**1**) were elucidated to be 4*R*, 9*S*, 17*S*, 22*S*, and 23*S*, since the CD spectra of **1** and **2** showed similar Cotton patterns to each other (Fig. 4).

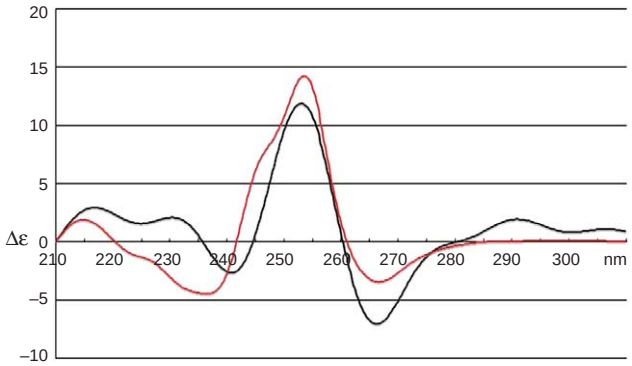

Figure 4. CD spectra of iejimalides A (**1**, black) and B (**2**, red).

Relative and absolute stereochemistries of iejimalides C (**3**) and D (**4**) were elucidated on the basis of spectral data of 35*O*-sulfate derivatives of iejimalides A (**1**) and SO_3-pyridine to afford its 35-*O*-sulfate ester, of which the spectral data including ^{1}H NMR (Fig. 5) and the CD spectra [λ_{ext} 252 nm (+10.4)] (Fig. 6) were the same as those of natural specimen of iejimalide D [**4**; λ_{ext} 252 nm (+9.9)]. Similarly, relative and absolute stereochemistries of iejimalide C (**3**) were elucidated by comparison with spectral data of 35-*O*-sulfate derivative of iejimalide A (**1**). Thus, the absolute configurations at five chiral centers of the macrolactone ring in iejimalides C (**3**) and D (**4**) were assigned as 4*R*, 9*S*, 17*S*, 22*S*, and 23*S*, which were the same as those of iejimalides A (**1**) and B (**2**).

Tumor panel experiments for iejimalides A–D (**1**–**4**) were carried out using 39 human cancer cell lines. The mean log GI_{50}'s of **1**–**4** based on the growth inhibition parameter were −6.31, −6.67, −6.11, and −6.28, respectively. COMPARE analyses of the mean graphs for **1**–**4** were negative, indicating that there are no standard anticancer drugs with a high correlation coefficient ($R < 0.5$).

Effects of iejimalides A–D (**1**–**4**) on the life-span of mice bearing ip-innoculated P388 leukemia were examined. Iejimalides C (**3**) and D (**4**) administered ip daily for 2 days (Days 1 and 5) were active (T/C 150%) at a dose of 200 μg/kg/day, while **1** and **2** were less active (T/C 120%) at the same dose and schedule.

3. Experimental

3.1. NMR and CD spectral measurements

^{1}H NMR spectra of iejimalides A–D (**1**–**4**) were measured on a Bruker AMX-600 spectrometer using 2.5 mm microcells for CD_3OD (Shigemi Co., Ltd.,). Resolution-enhanced ^{1}H NMR spectra were obtained by treatment of FIDs with sine-bell window function. For homonuclear decoupling experiments, 16*K* FIDs (1024 scans) were accumulated for a spectral width of 4800 Hz. CD spectra were recorded on a JASCO J-720 spectropolarimeter in MeOH at 25 °C. Integration times and spectral resolution were set to 64 times and 1 nm, respectively.

3.2. Sulfonation of iejimalides A (1) and B (2)

To a solution of iejimalide B (**2**, 0.1 mg) in pyridine (20 μL) was added a solution of SO_3-pyridine (1 mg) in DMF (10 μL), and stirring was continued at room temperature for 1 h. After evaporation of the solvent, the residue was extracted with EtOAc. The EtOAc-soluble materials were subjected to Sep-Pak C18 cartridge (CH_3CN/H_2O, 80:20) and then C_{18} HPLC (DEVELOSIL ODS-HG-5, Nomura Chemical Co., Ltd., 4.6 × 250 mm; $CH_3CN/H_2O/CF_3CO_2H$, 70:30:0.05, flow rate, 3 mL/min; UV detection at 230 nm) to afford the 35-*O*-sulfate ester of iejimalide B (0.1 mg, t_R 13 min), of which the spectral data

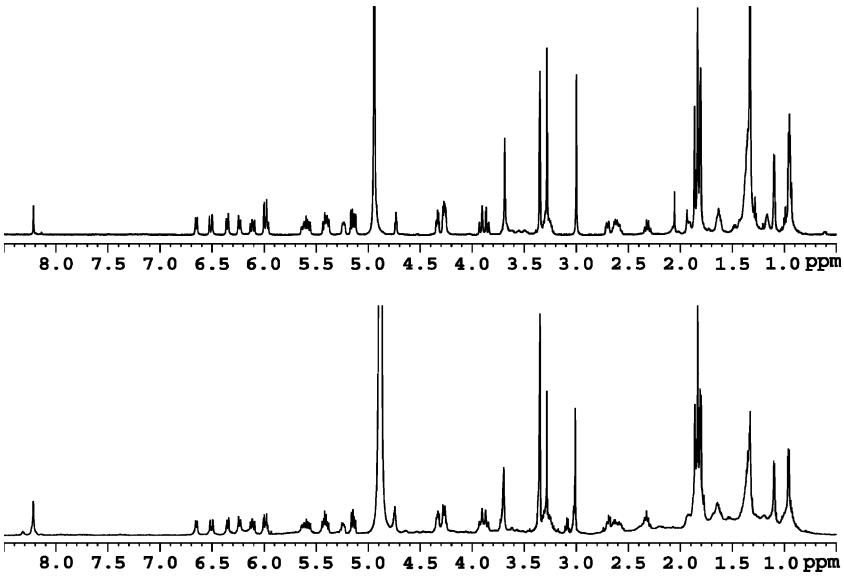

Figure 5. ^{1}H NMR spectra of natural iejimalide D (**4**) (upper) and sulfate ester derived from iejimalide B (**2**) (lower) in CD_3OD.

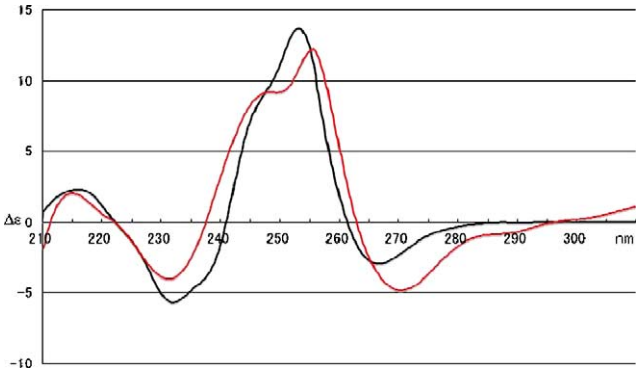

Figure 6. CD spectra of iejimalide D (**4**, black) and sulfate ester derived from iejimalide B (**2**, red).

were identified with those of natural iejimalide D (**4**). Iejimalide A (**1**, 0.1 mg) was treated by the same procedure as described above to afford 35-*O*-sulfate ester of iejimalide A (0.1 mg, t_R 14 min), of which the spectral data were identified with those of natural iejimalide C (**3**).

3.3. Evaluation of cell growth inhibition profile

A human cancer cell line panel combined with a database was used. The system was developed according to the method of the National Cancer Institute,[8,9] modified by the Japanese Foundation for Cancer Research (JFCR).[10,11] The cancer panel experiments for iejimalide A–D (**1**–**4**) were carried out in JFCR, and the inhibition profile was compared with those of more than 200 standard compounds including various anticancer drugs. The following cancer cell lines were used in cancer panel experiments: breast cancers HBC-4, BSY-1, HBC-5, MCF-7, and MDA-MB-231; brain tumor U251, SF-268, SF-295, SF-539, SNB-75, and SNB-78; colon cancers HCC-2998, KM-12, HT-29, HCT-15, and HCT-116; lung cancers NCI-H23, NCI-H226, NCI-H522, NCI-H460, A549, DMS273, and DMS114; melanoma LOX-IMVI; ovarian cancers; OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3; renal cancers RXF-631L and ACHN; stomach cancers St-4, MKN-1, MKN-7, MKN-28, MKN-45, and MKN-74; and prostate cancers DU-145 and PC-3. The cell lines were cultured in RPMI 1640 supplemented with 5% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37 °C in humidified air containing 5% CO_2. The GI_{50} values for these cell lines were determined by colorimetric analysis after staining with sulforhodamine B.

3.4. Evaluation of antitumor activity

P388 cells were transplanted ip into $CD2F_1$ mice on Day 0. Drugs were administered once a day on Days 1 and 5.[12] Antitumor efficacy against the above systems was expressed as a percentage of the median or mean survival time (MST) of the control group.

$$\mathrm{T/C}\ (\%) = \frac{\text{MST of the test group}}{\text{MST of the control group}} \times 100.$$

In these experiments, 6–12 mice were used for the control group and 6 mice for drug-treated group.

Acknowledgments

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