EI-1511-3, -5 and EI-1625-2, Novel Interleukin-1β Converting Enzyme Inhibitors Produced by *Streptomyces* sp. E-1511 and E-1625

II. Structure Determination

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The structures of three novel interleukin-1β converting enzyme inhibitors, EI-1511-3, -5 and EI-1625-2 were determined by spectroscopic methods. Their absolute configurations were also determined by analyses of the CD spectra of the inhibitors and their oxidation products with chromium trioxide.

In the preceding paper[1], we have described the taxonomy, fermentation of the producing strain and isolation of novel interleukin-1β converting enzyme inhibitors, EI-1511-3 (**1**), -5 (**2**) and EI-1625-2 (**3**). In this paper, we present the physico-chemical properties and structure determination of these inhibitors.

Results

Identification of Known Compounds

Known manumycin-related compounds were produced together with novel compounds **1**, **2** and **3** by this strain[1], and were identified as manumycins A (**4**)[2], B (**5**)[3], G (**6**)[4], U-56,407 (**7**)[5] and *ent*-alisamycin (**8**, an enantiomer of alisamycin). The physico-chemical data of **8** agreed with those of alisamycin[6] except for the sign of the specific rotation $\{[\alpha]_{578}+124°(CHCl_3)\}$, which is opposite to that of alisamycin $\{[\alpha]_{578}-122°(CHCl_3)^{6)}\}$.

Physico-chemical Properties

The physico-chemical properties of novel compounds **1**, **2** and **3** are summarized in Table 1. Their UV spectra showed the presence of conjugated polyenes. Their IR spectra suggested the presence of secondary amides and ketones conjugated with double bonds. The molecular formulae were determined by high resolution FAB-MS.

Fig. 1. Structures of EI-1511-3 (**1**), -5 (**2**) and EI-1625-2 (**3**).

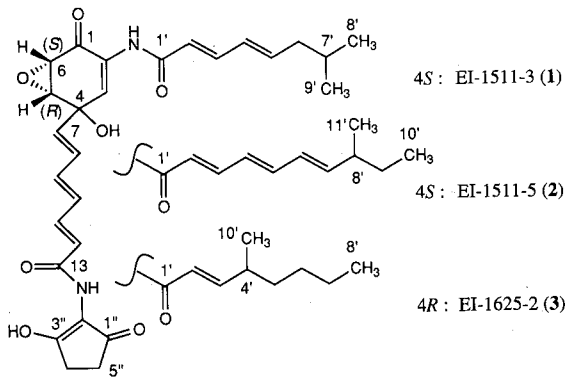


Table 1. Physico-chemical data of EI-1511-3 (**1**), -5 (**2**) and EI-1625-2 (**3**).

	EI-1511-3 (**1**)	EI-1511-5 (**2**)	EI-1625-2 (**3**)
Appearance	Yellow powder	Yellow powder	Yellow powder
MP	165~168°C	194~197°C	105~107°C
$[\alpha]_D$	+231° (*c* 0.23, MeOH)	+325° (*c* 0.15, MeOH)	−17° (*c* 0.10, $CHCl_3$)
Formula	$C_{27}H_{30}O_7N_2$	$C_{29}H_{32}O_7N_2$	$C_{27}H_{32}O_7N_2$
FAB-MS *m/z*	495 $(M+H)^+$	521 $(M+H)^+$	497 $(M+H)^+$
HR-FAB-MS calcd	495.2131	521.2287	497.2288
found	495.2124	521.2289	497.2277
IR ν_{max} (KBr) cm^{-1}	3379, 1684, 1672, 1616, 1523, 1367, 1001	3313, 1687, 1653, 1620, 1608, 1539, 1527, 1371, 1001	3317, 1674, 1624, 1522, 1367, 1005
UV λ_{max}^{MeOH} nm (ε)	314 (42,600), 278 (58,600)	309 (50,300)	321 (37,600), 282 (32,400), 265 (31,000)
CD λ_{ext}^{MeOH} nm ($\Delta\varepsilon$)	314 (+14.2), 280 (−19.9)	342 (+38.7), 300 (−52.9)	322 (−3.7), 277 (+9.9)

Structure Determination of EI-1511-3 (1)

The NMR data of **1** (Tables 2 and 3) were obtained from ^{1}H, ^{13}C, DEPT, COSY, NOESY, HETCOR and HMBC experiments. Upon comparison of these data (chemical shifts of ^{1}H and ^{13}C, and ^{1}H-^{1}H coupling patterns) with those reported for the known manumycins, an epoxycyclohexene moiety (C1~C6), a triene moiety (C7~C12), two secondary amide groups, an enolized cyclopentanedione (C1″~C5″) and a tertiary alcohol were found as common partial structures. In particular, ^{4}J coupling between 3-H and 5-H, and the existence of a hydrogen-bonded OH (enol), were characteristic.

The upper side chain, the novel portion of **1**, was presumed to have a diene and *iso*-butyl groups, based on analyses of 1D NMR, HETCOR and COSY spectra. However, 4′-H and 5′-H were observed at the same chemical shift (6.15 ppm). So, the connectivity and the stereochemistry of C4′~C5′ double bond remained to be confirmed. In $CDCl_3$, higher order couplings and severe overlapping of the olefinic protons were observed, which may have led to a misinterpretation of the ^{1}H NMR signals in the earlier studies[7]. On the other hand, these signals were simplified and well resolved in pyridine-d_5 (see experimental section). By the use of this solvent, the all *E* configuration of the double bonds was confirmed by the ^{3}J values (14.8~15.1 Hz) of their protons. The couplings between 8-H and 9-H were also unclear in $CDCl_3$. But they were clearly detected in pyridine-d_5, which revealed the C8~C9 connectivity. The geometries of the double bonds were also established by NOEs (Fig. 2).

All the partial structures mentioned above were connected based on C-H long range couplings observed in the HMBC spectra (Fig. 2). Correlations of C1′ *versus* 2′-H, and of C3 and C1′ *versus* 2-NH showed the connection of the upper side chain to the cyclohexene ring (C2-N-C1′-C2′), which was also supported by the NOE between 2-NH and 2′-H. The correlations of C4 and C7 by 4-OH and 7-H revealed that the tertiary hydroxyl group and the lower side chain were attached to C4. The latter was also confirmed by NOEs of 3-H and 5-H with 7-H and 8-H. The correlations of C13 and C3″ with 13-NH, and of C13 with 12-H indicated the linkage of cyclopentanedione moiety to the end of the lower side chain (C12-C13-13NH-C3″). Over all, the gross planar structure of **1** was elucidated.

Structure Determination of EI-1511-5 (2)

The epoxycyclohexene ring with the lower side chain is common to manumycin class compounds including **1**, **2** and **3**. The NMR signals of this portion were identical to each other within the experimental error (Tables 2 and 3). Thus, only the differences from **1** (*i.e.* structure determination of the upper side chains) will be described on **2** and **3**.

Table 2. ^{13}C NMR data of EI-1511-3 (**1**), -5 (**2**) and EI-1625-2 (**3**) in $CDCl_3$[a].

Position No.	EI-1511-3 (**1**)[b]	EI-1511-5 (**2**)[c]	EI-1625-2 (**3**)[b]
1	188.62 (s)	188.63 (s)	188.41 (s)
2	128.16 (s)	128.18 (s)	128.04 (s)
3	126.29 (d)	126.31 (d)	126.66 (d)
4	71.25 (s)	71.26 (s)	71.22 (d)
5	57.46 (d)	57.47 (d)	57.44 (d)
6	53.02 (d)	53.02 (d)	52.96 (d)
7	136.24 (d)	136.27 (d)	136.34 (d)
8	131.65 (d)	131.66 (d)	131.60 (d)
9	139.62 (d)	139.62 (d)	139.57 (d)
10	131.76 (d)	131.77 (d)	131.76 (d)
11	143.56 (d)	143.56 (d)	143.48 (d)
12	121.55 (d)	121.56 (d)	121.63 (d)
13	165.43 (s)	165.43 (s)	165.50 (s)
1′	165.17 (s)	165.07 (s)	164.97 (s)
2′	120.94 (d)	121.56 (d)	121.49 (d)
3′	143.75 (d)	143.67 (d)	153.39 (d)
4′	129.11 (d)	127.49 (d)	36.56 (d)
5′	144.28 (d)	142.08 (d)	35.81 (t)
6′	42.41 (t)	128.22 (d)	29.44 (t)
7′	28.33 (d)	146.67 (d)	22.74 (t)
8′	22.37 (q)	38.83 (d)	14.01 (q)
9′	22.37 (q)	29.54 (t)	19.51 (q)
10′		11.74 (q)	
11′		19.73 (q)	
1″	197.29 (s)	197.29 (s)	197.41 (s)
2″	114.95 (s)	114.96 (s)	115.04 (s)
3″	173.84 (s)	173.84 (s)	174.14 (s)
4″	25.66 (t)	25.67 (t)	25.74 (t)
5″	32.17 (t)	32.18 (t)	32.18 (t)

[a] δ ppm from TMS as an internal standard.
[b] 100 MHz.
[c] 125 MHz.

^{1}H-^{1}H spin couplings observed in the COSY spectrum revealed that the upper side chain of **2** was an 8-methyl-2,4,6-decatrienoylamino group. The connection of this group to C-2 was established by long range C-H couplings of C1 and C3 with 2-NH, and of C1′ with 2-NH and 2′-H observed in the HMBC spectrum. The all *E* configuration of the triene was confirmed by the ^{3}J values (14.7~15.2 Hz) and NOEs (Fig. 2).

Structure Determination of EI-1625-2 (3)

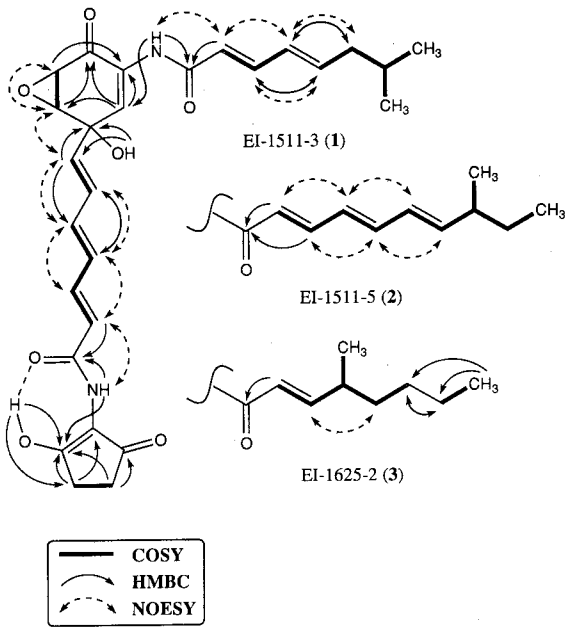
The upper side chain of **3** was assumed to carry a 2-hexenyl substituent. The 2-hexenyl group was also found in manumycins A (**4**)[2] and B (**5**)[3]. ^{1}H and ^{13}C NMR chemical shifts of this group in each compound were similar to each other. However, overlapping of 6′-

Table 3. ^{1}H NMR data of EI-1511-3 (**1**), -5 (**2**) and EI-1625-2 (**3**) in $CDCl_3$[a].

Position No.	EI-1511-3 (**1**)[b]	EI-1511-5 (**2**)[b]	EI-1625-2 (**3**)[c]
3	7.41 d 2.7	7.41 d 2.7	7.40 d 2.5
5	3.71 dd 3.8, 2.7	3.71 dd 3.8, 2.7	3.70 dd 3.7, 2.5
6	3.65 d 3.8	3.65 d 3.8	3.64 d 3.7
7	5.87 m	5.86 m	5.85 m
8	6.59 m	6.59 m	6.59 m
9	6.59 m	6.59 m	6.59 m
10	6.42 m	6.42 m	6.41 m
11	7.33 dd 14.8, 11.2	7.33 dd 14.7, 11.3	7.32 dd 14.6, 11.0
12	6.05 d 14.8	6.05 d 14.7	6.08 d 14.6
2-NH	7.56 br s	7.56 br s	7.56 s
4-OH	2.96 br s	3.01 br s	3.32 br s
13-NH	7.55 br s	7.56 br s	7.70 s
2′	5.84 d 14.8	5.89 d 15.0	5.81 d 15.4
3′	7.24 dd 14.8, 10.1	7.29 dd 15.0, 11.3	6.82 dd 15.4, 8.1
4′	6.15 m	6.23 dd 15.0, 11.3	2.30 m
5′	6.15 m	6.56 dd 15.0, 10.5	1.37 m
6′	2.07 dd 6.4, 6.4	6.11 dd 15.2, 10.5	1.25 m
7′	1.72 m	5.84 dd 15.2, 7.9	1.26 m
8′	0.91 d 6.7	2.14 m	0.88 t 6.8
9′	0.91 d 6.7	1.36 dq 7.3, 7.3	1.05 d 6.8
10′		0.87 t 7.3	
11′		1.02 d 6.7	
4″	2.61 m	2.61 m	2.59 m
5″	2.53 m	2.53 m	2.54 m
3″-OH	13.50 s	13.51 s	13.57 br s

[a] δ ppm from TMS as an internal standard, multiplicity, J in Hz.
[b] 500 MHz.
[c] 400 MHz.

Fig. 2. Results of 2D NMR analyses.



H_2 and 7′-H_2 in the ^{1}H NMR spectra prevented establishment of the linkage of C6′ to C7′. This linkage was confirmed by long range C–H correlations of C6′ *versus* 5′-H, 7′-H_2 and 8′-H_3, and of C7′ *versus* 6′-H_2 and 8′-H_3. The ^3J value (15.4 Hz) between 2′-H and 3′-H showed an *E* configuration of the double bond.

Absolute Configuration

For manumycin class compounds, the absolute configurations of C4~C6 have been determined by the exciton chirality method[8]. The CD spectra of the antibiotics indicate the configuration of C4, and the spectra of their epoxyquinone derivatives show the configurations of C5 and C6[9]. The configurations of C4~C6 of **6~8**, except for C4 of **6**, were unknown. So, absolute stereochemistry of these compounds, in addition to that of the novel compounds **1~3**, was also investigated.

Compound **1** showed an apparent positive CD couplet (314 nm $\Delta\varepsilon = +14.17$, 280 nm $\Delta\varepsilon = -19.93$) in methanol. Compounds **2** and **6~8** also showed the same CD patterns, which indicated the 4*S*-configurations of these compounds. The same result for **6** has been reported[4]. On the other hand, **3** showed the opposite negative CD couplet (322 nm $\Delta\varepsilon = -3.7$, 277 nm $\Delta\varepsilon = +9.9$), which revealed a 4*R*-configuration.

Epoxyquinone derivatives of **1~3** and **6~8** were prepared by the known method, CrO_3 oxidation[9]. All the derivatives showed the same CD patterns as reported for

Table 4. ^{1}H NMR chemical shifts of 3-H (δ ppm).

Compound	$\delta_{Py\text{-}d_5}$	δ_{CDCl_3}	$\Delta\delta$ ($\delta_{Py\text{-}d_5}$ - δ_{CDCl_3})	Stereo[a]
Manumycin A[3]	7.94	7.39	0.55	*trans*
Manumycin B[3]	7.94	7.39	0.55	*trans*
Manumycin C[3]	8.21	7.42	0.79	*cis*
Manumycin D[3]	8.28	7.57	0.71	*cis*
EI-1511-3 (**1**)	8.15	7.41	0.74	*cis*
EI-1511-5 (**2**)	8.15	7.41	0.74	*cis*
EI-1625-2 (**3**)	8.12	7.40	0.72	*trans*
Manumycin G (**6**)	8.15	7.41	0.74	*cis*
U-56,407 (**7**)	8.15	7.41	0.74	*cis*
ent-Alisamycin (**8**)	8.15	7.40	0.75	*cis*

[a] Relative stereochemistry between 4-hydroxyl group and 5,6-epoxy group.

manumycin A (**4**)[9], indicating the 5*R*/6*S*-configuration. Thus, compounds **1**, **2** and **6**~**8** have 4*S*/5*R*/6*S*-configurations and **3** has a 4*R*/5*R*/6*S*-configuration. Compound **6** is the C4-enantiomer of alisamycin. Consequently, the absolute configuration of alisamycin was deduced to be 4*R*/5*S*/6*R*. Together with **1**~**3** and **6**~**8**, the epoxyquinone derivatives were subjected to biological assay[10].

To elucidate the absolute configurations of C5 and C6, the determination of the relative configurations of C5 and C6 *versus* C4 by ASIS (aromatic solvent induced shift, $\Delta\delta = \delta^{\text{pyridine-}d_5} - \delta^{\text{chloroform-}d}$ for 3-H)[3] was also investigated (see experimental section). For **1**, **2** and **6**~**8**, ASIS afforded the same results (5*R*/6*S*) as those of CD experiment. However, an opposite result (5*S*/6*R*) to that of CD experiment was obtained for **3**.

Discussion

Many compounds of the manumycin class are known. All of them including **1**~**3** have the common 5,6-epoxycyclohex-2-enone with the same lower side chain. The differences among them are in the upper side chains and/or in the absolute configurations. Compounds **1**, **2** and **6**~**8** have the same configuration as manumycin C[3] (=UCF-B[11]) and asukamycin[7,12], which are opposite to alisamycin[6]. The configuration of **3** is the same as that of manumycin A[9] (=UCF1-C[11]) and B[7] (=UCF1-A[11]), which is enantiomeric to manumycin F[4]. It is of much interest that all four possible diastereomers of this group are produced by various strains.

Manumycins and the related compounds have antibabterial, antifungal, cytotoxic and/or Ras farnesyltransferase inhibiting activities[11]. However, the structure-activity relationships involving the absolute configurations are still not clear, and no report has been described on inhibition of interleukin-1β converting enzyme by this type of compound. Our studies on biological properties of **1**~**8** and some of their oxidized derivatives will be presented in the following paper[10].

Experimental

General

Reagents and solvents were obtained from commercial suppliers and were used without further purification. Column chromatography was performed on Wakogel C-200 100~200 mesh silica gel (WAKO Pure Chemical Ind., Ltd., Osaka, Japan). Physico-chemical and spectral data were measured on following instruments: MP, Yanaco micro melting point apparatus; $[\alpha]_D$, Jasco DIP-370 digital polarimeter; UV, Shimadzu UV-2200 UV-VIS spectrophotometer; CD, Jasco J-500A spectropolarimeter; IR, Jeol JIR-RFX3001 spectrophotometer; ^{1}H and ^{13}C NMR, Bruker AM500 and Jeol JMN-α400 spectrometers; FAB-MS, Jeol JMS-HX110/110A spectrometer.

^{1}H NMR Data of EI-1511-3 (**1**), -5 (**2**) and EI-1625-2 (**3**) in Pyridine-d_5

1 (400 MHz): δ ppm (integration, multiplicity, *J* in Hz, assignment) 10.50 (1H, br s, 13-NH), 9.53 (1H, br s, 2-NH), 8.15 (1H, d, 2.7, 3-H), 7.65 (1H, dd, 14.8, 11.3, 11-H), 7.58 (1H, dd, 15.0, 10.9, 3′-H), 7.02 (1H, dd, 15.1, 11.5, 8-H), 6.74 (1H, dd, 14.8, 11.5, 9-H), 6.71 (1H, d, 14.8, 12-H), 6.48 (1H, dd, 14.8, 11.3, 10-H), 6.47 (1H, d, 15.0, 2′-H), 6.20 (1H, d, 15.1, 7-H), 6.19 (1H, ddd, 15.0, 10.9, 0.5, 4′-H), 5.98 (1H, dt, 15.0, 7.5, 5′-H), 4.04 (1H, dd, 3.9, 2.7, 5-H), 3.90 (1H, d, 3.9, 6-H), 2.42 (4H, s, 4″-H_2 and 5″-H_2), 1.89 (2H, ddd, 7.5, 7.1, 0.5, 6′-H_2), 1.53 (1H, m, 7′-H), 0.78 (6H, d, 6.6, 8′-H_3 and 9′-H_3).

2 (500 MHz): δ ppm 9.54 (1H, br s, 2-NH or 13-NH), 8.15 (1H, d, 2.7, 3-H), 7.65 (1H, dd, 14.8, 11.2, 3′-H), 7.63 (1H, dd, 14.7, 11.3, 11-H), 7.01 (1H, dd, 15.1, 11.0, 8-H), 6.72 (1H, dd, 15.0, 11.0, 9-H), 6.70 (1H, d, 14.7, 12-H), 6.54 (1H, d, 14.8, 2′-H), 6.53 (1H, dd, 14.9, 10.6, 5′-H), 6.46 (1H, dd, 15.0, 11.3, 10-H), 6.33 (1H, dd, 4.9, 11.2, 4′-H), 6.19 (1H, d, 15.1, 7-H), 6.10 (1H, dd, 15.3, 10.6, 6′-H), 5.74 (1H, dd, 15.3, 7.8, 7′-H), 4.04 (1H, dd, 3.9, 2.7, 5-H), 3.89 (1H, d, 3.9, 6-H), 2.42 (4H, s, 4″-H_2 and 5″-H_2), 2.01 (1H, m, 8′-H), 1.24 (2H, dq, 7.3, 7.3, 9′-H), 0.92 (3H, d, 6.7, 11′-H), 0.78 (3H, t, 7.3, 10′-H).

3 (400 MHz): δ ppm 8.12 (1H, d, 2.7, 3-H), 7.63 (1H, dd, 14.9, 11.2, 11-H), 7.04 (1H, dd, 15.1, 7.8, 3′-H), 7.01 (1H, dd, 15.1, 10.0, 8-H), 6.72 (1H, dd, 14.9, 10.0, 9-H), 6.69 (1H, d, 14.9, 12-H), 6.45 (1H, dd, 14.9, 11.2, 10-H), 6.35 (1H, d, 15.1, 2′-H), 6.19 (1H, d, 15.1, 7-H), 4.03 (1H, dd, 3.7, 2.7, 5-H), 3.89 (1H, d, 3.7, 6-H), 2.41 (4H, s, 4″-H_2 and 5″-H_2), 2.14 (1H, m, 4′-H), 1.25~1.12 (6H, m, 5′-H_2, 6′-H_2 and 7′-H_2), 0.88 (3H, d, 6.8, 9′-H), 0.76 (3H, t, 6.8, 8′-H).

Investigation of ASIS (Aromatic Solvent Induced Shift)

$\Delta\delta$ values of **1**~**3** and **6**~**8** in comparison with the known manumycins are summarized in Table 4. Ac-

cording to the empirical rule stated in the literature[3], the relative stereochemistry between 4-hydroxyl group and 5,6-epoxy group of **3** should have been *cis* by the observed value. However, the stereochemistry determined by exciton chirality method was *trans* (see text). Thus, this may be the case of an exception of the rule.

CrO_3 Oxidation of EI-1511-3 (**1**)

5.3 mg of CrO_3 was dissolved in 0.5 ml of 60% aq AcOH. This solution was added to an AcOH solution of **1** (8.9 mg) in five portions over 6 hours and stirred for an additional 1 hour at room temperature. The reaction mixture was added with 4 ml of 2 N HCl and extracted three times with 5 ml each of ether. The organic layer was washed with satd. NaCl solution, dried over anhydr. Na_2SO_4, and concd. *in vacuo* with a rotary evaporator. The resulting crude product (1.7 mg) was chromatographed on a silica gel column developed with $CHCl_3$ to afford epoxycyclohexanone KT-8110 (1.2 mg): $[\alpha]_D^{30} = +53.5°$ (*c* 0.12, MeOH); FAB-MS *m/z* 276 $(M+H)^+$; HR-FAB-MS *m/z* 276.1237 $(M+H)^+$, $\Delta+0.1$ mmu for $C_{15}H_{18}O_4N$; UV (MeOH) λ_{max} nm (ε) 327 (22,700), 268 (23,600); CD (MeOH) λ_{ext} nm ($\Delta\varepsilon$) 371 (+7.33), 320 (−12.71), 260 (+5.06), 229 (−3.81); ^1H NMR δ ppm (integration, multiplicity, *J* in Hz) 7.82 (1H, br s), 7.62 (1H, d, 2.3), 7.33 (1H, dd, 15.0, 9.9), 6.19 (2H, m), 5.89 (1H, d, 15.0), 3.92 (1H, d, 3.7), 3.83 (1H, dd, 3.7, 2.3), 2.09 (2H, dd, 6.6, 6.6), 1.73 (1H, m), 0.92 (6H, d, 6.7).

CrO_3 Oxidation of EI-1511-5 (**2**)

In the same manner as the preparation of KT-8110, oxidation of **2** (7.0 mg) with CrO_3 gave 1.9 mg of KT-8112: $[\alpha]_D^{28} = +115°$ (*c* 0.047, MeOH); FAB-MS *m/z* 302 $(M+H)^+$; HR-FAB-MS *m/z* 302.1389 $(M+H)^+$, $\Delta-0.3$ mmu for $C_{17}H_{20}O_4N$; UV (MeOH) λ_{max} nm (ε) 343 (8,800), 300 (8,100); CD (MeOH) λ_{ext} nm ($\Delta\varepsilon$) 376 (+3.77), 320 (−6.51), 246 (+1.17), 224 (−2.94); ^1H NMR δ ppm (integration, multiplicity, *J* in Hz) 7.82 (1H, br s), 7.62 (1H, d, 2.2), 7.38 (1H, ddd, 14.4, 11.4, 0.7), 6.62 (1H, dd, 14.6, 10.6), 6.24 (1H, dd, 14.6, 11.4), 6.13 (1H, dd, 15.4, 10.6), 5.94 (1H, d, 14.4), 5.68 (1H, dd, 15.4, 7.6), 3.91 (1H, d, 3.7), 3.83 (1H, dd, 3.7, 2.2), 2.16 (1H, m), 1.37 (2H, dq, 7.4, 7.4), 1.03 (3H, d, 6.8), 0.87 (3H, t, 7.4).

CrO_3 Oxidation of EI-1625-2 (**3**)

In the same manner as the preparation of KT-8110, oxidation of **3** (5.7 mg) with CrO_3 gave 0.6 mg of an epoxycyclohexanone derivative; FAB-MS *m/z* 278 $(M+H)^+$; UV (MeOH) λ_{max} nm (ε) 315 (10,200), 240 (12,800); CD (MeOH) λ_{ext} nm ($\Delta\varepsilon$) 367 (+4.48), 307 (−8.04), 244 (+4.61), 226 (−4.02); ^1H NMR δ ppm (integration, multiplicity, *J* in Hz) 7.83 (1H, br s), 7.61 (1H, d, 2.3), 6.94 (1H, dd, 15.3, 7.9), 5.88 (1H, dd, 15.3, 1.2), 3.92 (1H, d, 3.7), 3.83 (1H, dd, 3.7, 2.3), 2.34 (1H, m), 1.49~1.34 (2H, m), 1.33~1.22 (4H, m), 1.07 (3H, d, 6.7), 0.89 (3H, t, 6.9).

CrO_3 Oxidation of U-56,407 (**6**)

In the same manner as the preparation of KT-8110, oxidation of **6** (9.7 mg) with CrO_3 gave 1.9 mg of KT-8108: $[\alpha]_D^{28} = +117°$ (*c* 0.038, MeOH); FAB-MS *m/z* 302 $(M+H)^+$; HR-FAB-MS *m/z* 302.1395 $(M+H)^+$, $\Delta+0.3$ mmu for $C_{17}H_{20}O_4N$; UV (MeOH) λ_{max} nm (ε) 332 (4,000), 303 (3,900); CD (MeOH) λ_{ext} nm ($\Delta\varepsilon$) 372 (+0.94), 317 (−1.51), 246 (+0.38), 226 (−0.81); ^1H NMR δ ppm (integration, multiplicity, *J* in Hz) 7.83 (1H, br s), 7.62 (1H, d, 2.2), 7.38 (1H, ddd, 14.7, 11.4, 0.5), 6.63 (1H, dd, 14.7, 10.6), 6.24 (1H, dd, 14.7, 11.4), 6.15 (1H, dd, 14.9, 10.6), 6.00 (1H, dd, 14.9, 7.6), 5.94 (1H, d, 14.7), 3.91 (1H, d, 3.7), 3.83 (1H, dd, 3.7, 2.2), 2.05 (2H, ddd, 6.7, 6.7, 0.8), 1.70 (1H, m), 0.91 (6H, d, 6.6).

CrO_3 Oxidation of Manumycin G (**7**)

In the same manner as the preparation of KT-8110, oxidation of **7** (10.1 mg) with CrO_3 gave 0.16 mg of KT-8109: FAB-MS *m/z* 288 $(M+H)^+$; HR-FAB-MS *m/z* 288.1242 $(M+H)^+$, $\Delta+0.6$ mmu for $C_{16}H_{18}O_4N$; UV (MeOH) λ_{max} nm (ε) 328 (39,700), 302 (40,700); CD (MeOH) λ_{ext} nm ($\Delta\varepsilon$) 375 (+8.72), 319 (−15.09), 245 (+3.05), 225 (−10.05); ^1H NMR δ ppm (integration, multiplicity, *J* in Hz) 7.82 (1H, br s), 7.62 (1H, d, 2.3), 7.38 (1H, dd, 14.5, 11.3), 6.62 (1H, dd, 14.9, 10.2), 6.25 (1H, dd, 14.9, 11.3), 6.13 (1H, dd, 15.2, 10.2), 5.98 (1H, dd, 15.2, 6.8), 5.94 (1H, d, 14.5), 3.91 (1H, d, 3.7), 3.83 (1H, dd, 3.7, 2.3), 2.41 (1H, m), 1.04 (6H, d, 6.8).

CrO_3 Oxidation of *ent*-Alisamycin (**8**)

In the same manner as the preparation of KT-8110, oxidation of **8** (10.6 mg) with CrO_3 gave 0.46 mg of KT-8111: FAB-MS *m/z* 302 $(M+H)^+$; HR-FAB-MS *m/z* 302.1388 $(M+H)^+$, $\Delta-0.5$ mmu for $C_{17}H_{20}O_4N$; UV (MeOH) λ_{max} nm (ε) 327 (8,000), 268 (9,100); UV ($CHCl_3$) λ_{max} nm (ε) 335 (4,600), 271 (6,300) [lit[6] 336 (17,700), 270 (22,400)]; CD (MeOH) λ_{ext} nm ($\Delta\varepsilon$) 371 (+2.14), 319 (−4.41), 259 (+1.27), 230 (−1.77); CD ($CHCl_3$) λ_{ext} nm ($\Delta\varepsilon$) 376 (+1.72), 328 (−3.32), 264 (+0.61) [lit[6] 375 (−9.37), 328 (+16.69), 266 (−6.34)]; ^1H NMR δ ppm (integration, multiplicity, *J* in Hz) 7.82 (1H, br s), 7.61 (1H, d, 2.2), 7.31 (1H, dd, 15.0, 10.0), 6.17 (2H, m), 5.90 (1H, d, 15.0), 3.91 (1H, d, 3.7), 3.83 (1H, dd, 3.7, 2.2), 2.12 (1H, m), 1.76 (4H, m), 1.67 (1H, m), 1.29 (2H, m), 1.19 (1H, m), 1.15 (2H, m).

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