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NOTES

EI-2346, a Novel Interleukin-1β Converting Enzyme Inhibitor Produced by *Streptomyces* sp. E-2346

II. Structure Elucidation

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The IL-1β converting enzyme (ICE) is a cysteine protease which cleaves biologically-inactive 31 kDa precursor to biologically-active IL-1β[1,2], a key mediator of inflammation[3,4]. Thus, ICE inhibitors would be useful as anti-inflammatory agents. As described in previous paper[5], we isolated novel ICE inhibitory compound, EI-2346, from culture broth of *Streptomyces* sp. E-2346. In this paper, we describe the structure elucidation of EI-2346.

The physico-chemical properties of EI-2346 (**1**) were summarized in previous paper[5]. The molecular formula of **1** was determined by high resolution FAB-MS to be $C_{22}H_{26}O_9$. The ^1H and ^{13}C NMR data of **1** were summarized in Table 1, and COSY, HMBC, and NOESY correlations were shown in Fig. 2. The ^{13}C NMR spectrum (Table 1) showed 22 carbon signals which supported the molecular formula of **1**. UV and IR spectra of **1** were consistent with

Fig. 1. Structure of EI-2346.

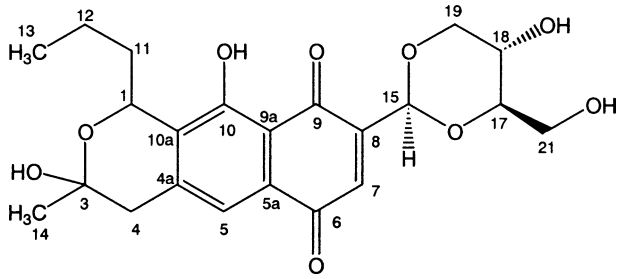

Table 1. ^{13}C and ^1H NMR data for EI-2346.

No.	δC^a (ppm)	δH^b (ppm, multi.-, *J* in Hz)
1	70.6	5.11, m
3	95.1	
4	41.5	2.95, d, *J*=16.5
		2.86, d, *J*=16.5
4a	144.3	
5	120.6	7.33, s
5a	131.1	
6	185.9	
7	136.8	7.10, s
8	146.1	
9	189.9	
9a	114.4	
10	159.0	
10-OH		
10a	134.8	
11	37.1	1.98, m (2H)
12	19.1	1.43, m
		1.27, m
13	14.4	0.89, t, *J*=7.4
14	29.2	1.54, s
15	95.2	5.72, s
17	84.4	3.64, m
18	62.3	3.64, m
19	72.4	4.19, dd, *J*=4.5, 10.3
		3.57, dd, *J*=10.3, 10.3
21	62.4	3.89, dd, *J*=2.0, 12.2
		3.74, dd, *J*=5.1, 12.2

[a] ^{13}C NMR (100 MHz in CD_3OD)
[b] ^1H NMR (400 MHz in CD_3OD)

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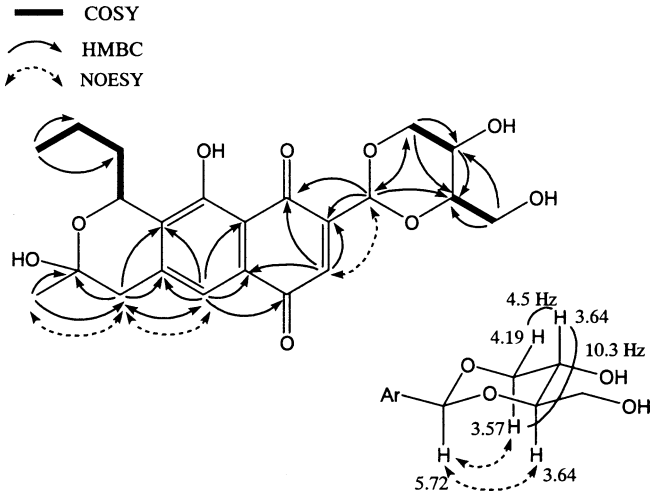
naphthoquinone chromophore, and the UV spectrum of **1** was similar to that of exfoliamycin[6,7] which also has a naphthoquinone chromophore. The ^{1}H and ^{13}C NMR spectra of **1** were similar to those reported for K1115B$_1$[8], another secondary metabolite isolated from *Streptomyces* sp., which has a naphthoquinone structure and a 1,3-dioxan ring. Upon comparison of NMR data (chemical shifts of ^{1}H and ^{13}C, and ^{1}H-^{1}H coupling constants) of **1** with those of K1115B$_1$[8], naphthoquinone and 1,3-dioxan moieties were found as common partial structure. The molecular formula of **1** ($C_{22}H_{26}O_9$) which was determined by high resolution FAB-MS corresponds to H_2O more than that of K1115B$_1$ ($C_{22}H_{24}O_8$). The NMR analysis of **1** indicated that this additional unit was located in the 3,4-double bond of pyran ring of K1115B$_1$. In the ^{1}H and ^{13}C NMR spectra of **1**, signals which were attributed to 3,4-double bond of pyran ring (δ C-3 158.7, δ C-4 100.0, and δ H-4 5.6) in K1115B$_1$ were replaced by those of hemiacetal carbon (δ C-3 95.1) and methylene (δ C-4 41.5, δ H-4 2.95 and 2.86). Therefore, the structure of **1** should be the corresponding 3,4-hydrate derivative of K1115B$_1$. This was also confirmed by the results that ^{1}H and ^{13}C NMR spectra of tricyclic naphthoquinone and tetrahydropyran moieties of **1** were similar to those reported for the known exfoliamycin which also has the same tricyclic structure. Moreover, this structure was supported by the 2D NMR study for **1** (Fig. 2).

Relative configuration of the 1,3-dioxan ring in **1** was determined by coupling constants and NOE experiment as shown in Fig. 2. NOEs were observed between H-15 (δ_H 5.72) and H-17 (δ_H 3.64), H-15 and H-19$_{ax}$ (δ_H 3.57). These results revealed that 1,3-dioxane moiety was chair conformation, and that these protons were of the 1,3-diaxial orientation. A large coupling constant between H-18 and H-19$_{ax}$ (J=10.3 Hz) indicated that these protons were of the 1,2-diaxial orientation and 18-OH was of the equatorial orientation. These results revealed the relative configuration of 1,3-dioxan ring shown in Fig. 2. Stereochemistries at C-1 and C-3 of EI-2346 were not determined yet.

Fig. 2. Summary of COSY, HMBC, and NOESY correlations for EI-2346.



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