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# Structure elucidation of EI-1941-1 and -2, novel interleukin-1β converting enzyme inhibitors produced by *Farrowia* sp. E-1941

Fumito Koizumi,[a,*] Yuichi Takahashi,[b] Hiroki Ishiguro,[a] Rieko Tanaka,[a] Shizuo Ohtaki,[b] Mayumi Yoshida,[a] Satoshi Nakanishi[b] and Shun-ichi Ikeda[a]

[a]*Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd, 3-6-6 Asahi-machi, Machida-shi, Tokyo 194-8533, Japan*
[b]*Pharmaceutical Research Center, Kyowa Hakko Kogyo Co., Ltd, 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan*

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**Abstract**—EI-1941-1 (**1a**) and EI-1941-2 (**2a**) accompanied by EI-1941-3 (**3**) have been isolated from culture broth of *Farrowia* sp. E-1941 as the inhibitors of interleukin-1β converting enzyme. The structures of **1a**, **2a**, and **3** were elucidated by the analysis of NMR and MS data, and finally the absolute stereochemistries of **1a** and **2a** were confirmed by optical rotation data, or X-ray crystallographic analysis of *p*-bromobenzoate, **2b**, respectively.


The interleukin-1β converting enzyme (ICE) is a cysteine protease, which cleaves the biologically-inactive 31 kDa precursor to the biologically-active IL-1β,[1] a key mediator of inflammation. A number of peptide-based ICE inhibitors have been reported; however, these compounds have a common defect, presumably due to their low oral bioavailability and poor pharmacokinetic properties such as rapid disappearance from the blood. Therefore, non-peptidyl ICE inhibitors will be needed for suppression of the inflammation disease. Our studies and others on ICE inhibitors led to the discovery of a variety of natural compounds.[2a–2f] During the course of this project, we isolated novel ICE inhibitory compounds, EI-1941-1 and -2, together with a minor component, EI-1941-3.[3] Recently, the isolation and ICE inhibitory activity of these compounds accompanied by the planar structure of them was reported. It is of importance to determine the absolute chemical structure of naturally occurring compounds. Therefore, in this paper, we describe the detailed structure elucidation of EI-1941-1, -2, and -3.

Compound **2a** was obtained as a colorless powder, $[\alpha]_D^{23}$ −307.5 ($c = 0.57$, methanol). The $^{13}C$ NMR spectrum (Table 1) showed 12 carbon signals in good accordance with the high resolution FAB-MS [calcd for $C_{12}H_{15}O_5$ $[M + H]^+$ 239.0919, found 239.0928] to elucidate the molecular formula as $C_{12}H_{14}O_5$, indicating six degrees of unsaturation. The IR spectrum of **2a** suggested the presence of hydroxyl (3367 $cm^{-1}$) and carbonyl (1696 and 1717 $cm^{-1}$) groups. Four singlet signals in $^{13}C$ NMR at 195.4, 165.2, 141.5, and 138.7 ppm indicated the presence of ketone, ester, and fully substituted double bond functionalities, respectively, and the rest of unsaturation index, three, implied that **2a** was composed by a three-ring system. The analysis of $^1H$ NMR and $^1H$–$^1H$ COSY spectrum (Fig. 1) showed the following two connections, –CH(–O–X1)–CH(–O–X2)–CH(–O–X3)– and –$CH_3$–$CH_2$–$CH_2$–CH(–O–X4)–$CH_2$–, attached to quaternary carbons, and the assurance of each unit was supported by NOESY experiments. The coupling constant ($J = 3.7$ Hz) between $\delta_{1a\text{-}H}$ 3.84 and $\delta_{7a\text{-}H}$ 3.55 and signals of 1a-*C*H (δ57.3, $J_{CH} = 186.9$ Hz) and 7a-*C*H (δ 53.3, $J_{CH} = 189.4$ Hz) indicated these carbons to be attached to an oxygen atom forming the *cis*-epoxide.[2f,4] These estimation demonstrated that the former unit, –CH(–O–X1)–CH(–O–X2)–CH(–O–X3)– contains a hydroxyl group and an oxirane ring. Furthermore, signals of C5 methine carbon (δ 78.5) and 5-H methine proton (δ 4.50) suggested that X4 in the later unit would be carbonyl. Additionally, the long range coupling between methylene and hydroxymethine

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* Corresponding author. Tel.: +81 42 725 2555; fax: +81 42 726 8330; e-mail: fumito.koizumi@kyowa.co.jp

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**Table 1.** Summary of $^{13}C$ and $^{1}H$ NMR data for **1a**, **2a**, and **3** in $CD_3CN$

| 1a | | | 2a | | 3 | | |
|---|---|---|---|---|---|---|---|
| No. | $\delta$ $^{13}C$ | $\delta$ $^{1}H$ ( )[a] | $\delta$ $^{13}C$ | $\delta$ $^{1}H$ ( )[a] | No. | $\delta$ $^{13}C$ | $\delta$ $^{1}H$ ( )[a] |
| 1a | 57.8 | 3.73 (1H, dd, 3.7, 1.3) | 57.3 | 3.84 (1H, dd, 3.7, 1.7) | 7 | 70.4 | 4.22 (1H, q, 3.4) |
| 2 | 63.0 | 4.60 (1H, m) | 62.0 | 4.96 (1H, m) | 8 | 66.6 | 4.46 (1H, m) |
| 2a | 148.3 | | 136.7 | | 8a | 136.8 | |
| 3 | 88.3 | 5.51 (1H, s) | 165.2 | | 1 | 167.0 | |
| 5 | 66.4 | 3.85 (1H, m) | 78.5 | 4.50 (1H, m) | 3 | 79.0 | 4.46 (1H, m) |
| 6 | 28.4 | 2.09 (1H, ddd, 17.7, 3.2, 1.8) | 26.8 | 2.54 (1H, ddd, 18.4, 4.7, 1.3) | 4 | 26.3 | 2.76 (1H, ddd, 18.9, 3.9, 1.4) |
| | | 1.96 (1H, br dd, 17.7, 11.1) | | 2.46 (1H, ddd, 18.4,10.0, 1.0) | | | 2.25 (1H, br dd, 18.9, 12.0) |
| 6a | 130.0 | | 141.5 | | 4a | 143.5 | |
| 7 | 195.0 | | 195.4 | | 5 | 197.7 | |
| 7a | 53.5 | 3.42 (1H, dd, 3.7, 1.0) | 53.3 | 3.55 (1H, dd, 3.7, 1.0) | 6 | 41.6 | 2.92 (1H, dd, 16.7, 3.1) |
| | | | | | | | 2.51 (1H, dd, 16.7, 3.8) |
| 8 | 38.0 | 1.50 (2H, m) | 37.1 | 1.70 (IH, m) | 9 | 37.3 | 1.75 (1H, m) |
| | | | | 1.61 (1H, m) | | | 1.65 (1H, m) |
| 9 | 19.2 | 1.45 (1H, m) | 18.8 | 1.42 (2H, m) | 10 | 18.8 | 1.45 (2H, m) |
| | | 1.38 (1H, m) | | | | | |
| 10 | 14.2 | 0.91 (3H, t, 7.2) | 14.0 | 0.92 (3H, t, 7.3) | 11 | 14.0 | 0.94 (3H, t, 7.3) |

$^{13}C$ NMR (125 MHz), $^{1}H$ NMR (500 MHz).
[a] (Integrity, multiplicity, coupling constant).

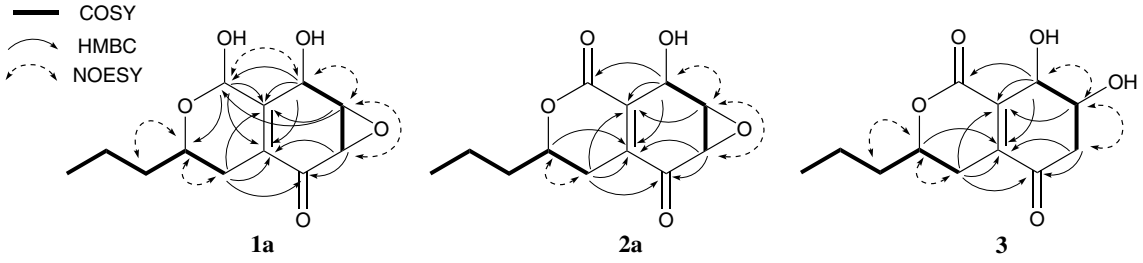
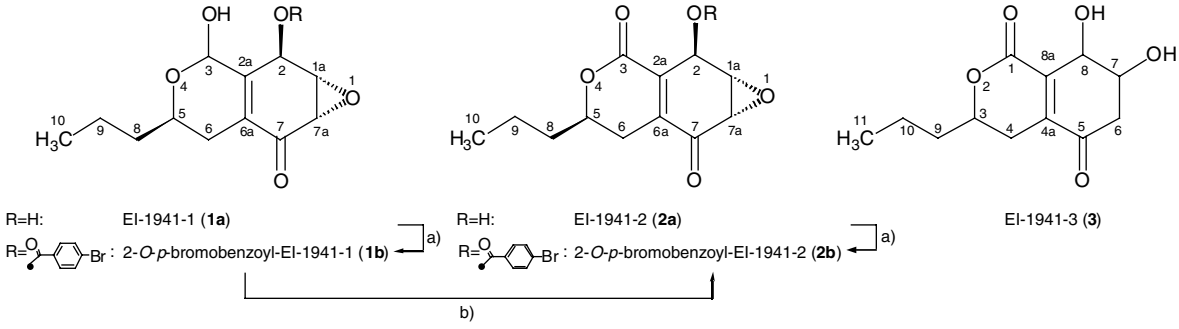



**Figure 1.** Summary of $^{1}H$–$^{1}H$ COSY, HMBC, and NOESY data for **1a**, **2a**, and **3**.

protons in the above two subunits indicated the connection of them through the $sp^2$ carbons. Finally, detailed analysis of HMBC spectrum (Fig. 1) with taking account of the above two subunits into the structure elucidation revealed the planar structure of **2a** as described in Scheme 1, constructing with three fused ring system.

The absolute stereochemical outcome of **2a** was confirmed by the X-ray analysis of *p*-bromobenzoyl derivative (**2b**) as shown in Figure 2 by the absolute-structure determination using anomalous scattering effects of X-ray. Treatment of **2a** with dry pyridine and *p*-bromobenzoylchloride in dichloroethane at ambient temperature for 16 h gave **2b** in 10% yield,[5a] and the product was crystallized to afford colorless single crystals for the X-ray studies. Some structural features of **2b** were revealed by the X-ray analysis as follows. The epoxy ring was oriented almost vertical to the cyclohexenone ring of **2b**, and C2 hydroxyl group was *trans* to the epoxide. The *n*-propyl group attached to the lactone ring was oriented equatorial. Consequently, the absolute configuration of **2a** was deduced to be 2*R*/1a*S*/7a*S*/5*R*, respectively.

Compound **1a** was obtained as a brownish oil, $[\alpha]_D^{27}$ –193.7 ($c = 0.31$, methanol). The molecular formula was determined by the high resolution FAB-MS




**Scheme 1.** Reagents and conditions: (a) *p*-bromobenzoylchloride/pyridine/dichloroethane; (b) PCC/dichloromethane.

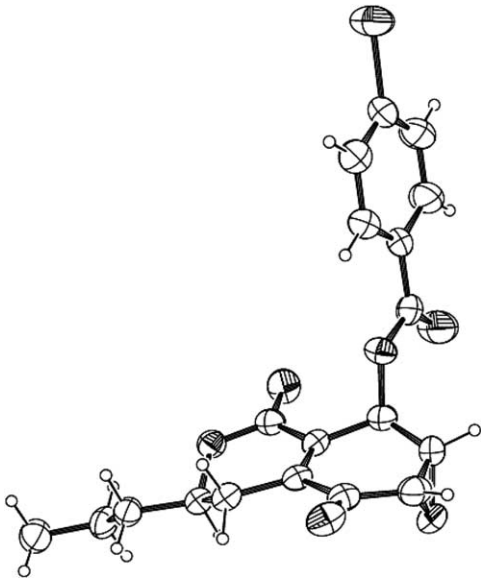


**Figure 2.** The molecular structure of **2b** determined by X-ray crystallographic analysis.

[calcd for $C_{12}H_{17}O_5$ $(M + H)^+$ 241.1076, found 241.1058] and the $^{13}C$ NMR to be $C_{12}H_{16}O_5$. The structure of **1a** was determined by the comparison of its NMR data with those of **2a**. $^1H$ and $^{13}C$ NMR spectra of **1a** and **2a** were almost similar to each other. However, a significant difference between them was found around the lactone moiety of **2a**, such as an appearance of a singlet proton signal at 5.51 ppm and a high field shift of the C3 carbon signal from 165.2 to 88.3 ppm. These were clearly indicating the carbonyl group of **2a** should be replaced by the hydroxymethine group in **1a**. This structure was further supported by the $^1H$–$^1H$ COSY, HMBC, and NOESY data summarized in Figure 1. Interestingly, the structure of **1a** is isomeric with cycloepoxydon, another fungal metabolite, whose oxidation pattern is quite different from that of **1a**.[6]

The stereochemistries of **1a** were estimated by the CD spectra analysis, and by the $^1H$–$^1H$ coupling constants comparing with those of **2a** and other naturally occurring epoxycyclohexenone analogues.[7] The $^1H$–$^1H$ coupling constants between 1a-H and 7a-H (3.7 Hz) and between 1a-H and 2-H (1.3 Hz) were very close to those observed for **2a**, (−)-terremutin, (−)-panepoxydon, and (+)-isoepoxydon while not to those of (+)-epoxydon, which had the *cis* oriented hydroxyl group and the epoxide moiety (Fig. 3). These data demonstrated that 1a-H and 7a-H should be *cis* relation and hydroxyl group should be oriented *trans* to the epoxide moiety. The apparent negative Cotton effect ($\Delta\varepsilon$ −3.0 at 335 nm and $\Delta\varepsilon$ −9.2 at 246 nm) was similar to those observed for (−)-terremutin and (−)-panepoxydon, and different from those observed for (+)-isoepoxydon, suggesting the absolute stereochemistry on C7a of **1a** to be the same to those of (−)-terremutin and (−)-panepoxydon. These results demonstrated that the absolute stereochemistries of **1a** were estimated to be 2*R*/1a*S*/7a*S*. To confirm these estimations, X-ray analysis was first examined, but all attempts to obtain a suitable crystal of **1a**, or 2-*O*-*p*-bromobenzoyl-EI-1941-1 (**1b**), or its regioisomer (see below) for the X-ray crystallography resulted in decomposition of the compounds, and in the case of latter two, a crystal of *p*-bromobenzoic acid was afforded. The stereo- chemistries of **1a** were finally determined by the chemical conversion of **1b** to **2b**. Treatment of **1a** with dry pyridine and *p*-bromobenzoylchloride in dichloroethane at ambient temperature for 16 h gave **1b**[8] and its regioisomer in 4.0% and 1.7% yield, respectively. Further treatment of **1b** with PCC in dichloromethane at ambient temperature for 40 h afforded **2b** as a sole product indicated by the $^1H$ NMR data of the crude sample, but the isolated yield of **2b** after purification with a preparative thin layer chromatography was only 17%

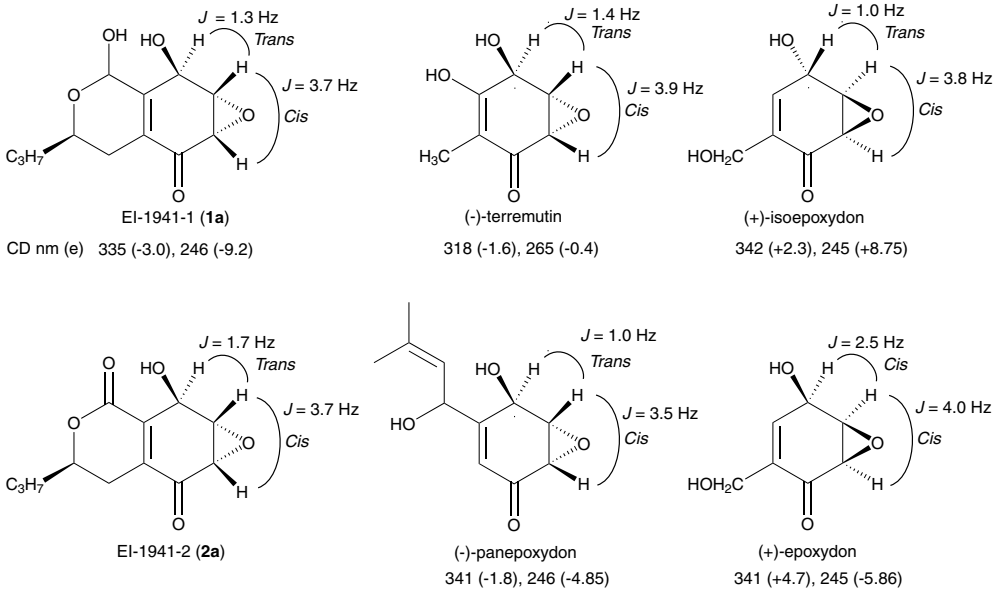



**Figure 3.** The coupling constant around the epoxy-alcohol moiety of **1a**, **2a**, and their congeners.

because of its unstability. The $^1$H NMR and optical rotation of **2b** derived from both **1a** and **2a** were identical to each other, concluding that the stereochemistries of oxirane ring, hydroxyl group and *n*-propyl group were the same to those of **2a**.[5b] These results were in good accordance with the stereochemical estimations described above. Consequently, the absolute stereochemistries of **1a** were deduced to be 2*R*/1a*S*/7a*S*/5*R*, respectively. However, the stereochemical study at C3 of **1a** remained to be examined.

Compound **3** was obtained as a reddish oil, $[\alpha]_D^{23}$ –87.5 ($c = 0.31$, methanol). The molecular formula of **3** was also determined by the high resolution FAB-MS [calcd for $C_{12}H_{17}O_5$ $(M + H)^+$ 241.1076, found 241.1061] and the $^{13}$C NMR to be $C_{12}H_{16}O_5$, corresponding to $H_2$ more than **2a**. $^1$H and $^{13}$C NMR spectra of **3** were closely similar to those of **2a** with the exception of signals around the oxirane ring in **2a**. $^1$H–$^1$H COSY spectrum in addition to $^1$H and $^{13}$C NMR data of **3** (Fig. 1 and Table 1) revealed the connection of –CHOH–CHOH–$CH_2$–, indicating that the epoxide moiety of **2a** was reduced to a hydroxyethylene group. Consequently, the planar structure of **3** was elucidated as described in Scheme 1, that was also supported by the HMBC and NOESY data in all respects, but the stereochemistries of **3** were not determined because of its low availability from fermentation broth.

EI-1941-1 (**1a**) and -2 (**2a**) inhibited the human recombinant ICE activities with the $IC_{50}$ values of 86 and 6.0 nM, respectively. On the other hand, EI-1941-3 (**3**) was inactive against the human recombinant ICE at concentrations up to 4.0 μM.[3] These results indicated that the epoxide moiety of EI-1941-1 and -2 plays an important role to inhibit the ICE activity.

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5. (a) 2-*O*-*p*-Bromobenzoyl-EI-1941-2 (**2b**) as a colorless amorphous crystal; $[\alpha]_D^{23}$ –288.0 ($c = 0.27$, methanol); $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 7.85 (2H, d, $J = 8.6$ Hz), 7.58 (2H, d, $J = 8.6$ Hz), 6.48 (1H, m), 4.53 (1H, m), 4.00 (1H, dd, $J = 3.6$, 1.8 Hz), 3.66 (1H, dd, $J = 3.7$, 1.0 Hz), 2.63 (2H, m), 1.81 (1H, m), 1.67 (1H, m), 1.52 (2H, m), 0.96 (3H, t, $J = 7.4$ Hz); FAB-MS *m/z* 421/423 $[M + H]^+$; HR FAB-MS calcd for $C_{19}H_{18}{}^{79}BrO_6$, $[M + H]^+$ 421.0287, found. 421.0292. Full details of the X-ray analysis of **2b** will be published elsewhere; (b) Optical rotation data of **2b** derived from **1a**; $[\alpha]_D^{26}$ –274.5 ($c = 0.17$, methanol).
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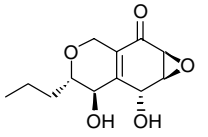

cycloepoxydon

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8. 2-*O*-*p*-Bromobenzoyl-EI-1941-1 (**1b**) as a colorless oil; $[\alpha]_D^{25}$ –141.6 ($c = 0.50$, methanol); $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 7.90 (2H, d, $J = 8.8$ Hz), 7.61 (2H, d, $J = 8.8$ Hz), 6.28 (1H, s), 5.46 (1H, s), 4.00 (1H, m), 3.87 (1H, dd, $J = 3.6$, 1.5 Hz), 3.57 (1H, dd, $J = 3.6$, 1.1 Hz), 2.94 (1H, d, $J = 5.1$), 2.23 (2H, m), 1.59 (1H, m), 1.55 (1H, m), 1.48 (2H, m), 0.94 (3H, t, $J = 7.2$ Hz); FAB-MS *m/z* 445/447 $[M + Na]^+$; HR FAB-MS calcd for $C_{19}H_{19}{}^{79}BrO_6Na$, $[M + Na]^+$ 445.0263, found. 445.0242.