



## Synthesis of a New Phospholipase A<sub>2</sub> Inhibitor of an Aldehyde Terpenoid and its Possible Inhibitory Mechanism

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**Abstract:** 3-(E)-Methoxycarbonyl-2,4,6-trienal compound **A** was stereoselectively synthesized and found to show strong inhibitory activity toward phospholipase A<sub>2</sub> (PLA<sub>2</sub>) from bovine pancreas. As the inhibitory mechanism of PLA<sub>2</sub> by **A**, the irreversible formation of dihydropyridine resulting from the reaction of **A** with lysine residues in PLA<sub>2</sub> is proposed based on the model reactions.

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Recently, it has been reported that some unsaturated aldehyde terpenoids inhibit phospholipase A<sub>2</sub> (PLA<sub>2</sub>),<sup>1</sup> which catalyzes hydrolysis of the ester linkage at the *sn*-2 position of glycerophospholipids. Since the release of arachidonic acid from glycerophospholipids is the rate-limiting step in the production of eicosanoid mediators of inflammation,<sup>2</sup> the inhibitory mechanism of PLA<sub>2</sub> by these aldehyde terpenoids has been of biochemical and medicinal interest. Among these aldehyde terpenoid inhibitors, manoalide and scalaradiol, which are sesterterpenoids isolated from marine sponges,<sup>3,4</sup> are well characterized and their inhibitory mechanisms toward various PLA<sub>2</sub>s have been reported.<sup>5,6</sup> We independently studied to elucidate the inhibitory mechanism of bovine pancreatic PLA<sub>2</sub> by manoalide, and found that two aldehyde groups and the hydrophobic moiety of manoalide were essential for the PLA<sub>2</sub> inhibition. Furthermore, it was concluded that the irreversible modification of Lys-56 by manoalide, which was thought to be included in the interfacial recognition site of the enzyme, was responsible for inhibition of this enzyme.<sup>7</sup> Unfortunately, we could not obtain a clear answer on what irreversible reaction proceeded between manoalide and lysine residues of PLA<sub>2</sub>, since the reaction of manoalide with a primary amine as a model for lysine residue gave unidentified insoluble polymers. In our program on the synthesis of new PLA<sub>2</sub> inhibitors and elucidation of their inhibitory mechanisms, we discovered that 3-(E)-methoxycarbonyl-2,4,6-trienal **A** showed more powerful inhibitory activity than manoalide toward bovine pancreatic PLA<sub>2</sub>, while its *Z* isomer **B** showed no activity. Here, we report the highly stereoselective syntheses of both **A** and **B**, and show that the 3-(E)-methoxycarbonyl-2,4,6-trienal function in **A** is essential for inhibition of bovine PLA<sub>2</sub>. In addition, as the inhibitory mechanism of bovine PLA<sub>2</sub> by **A**, the irreversible formation of a dihydropyridine derivative is proposed based on the reaction of **A** with a primary amine as a model for lysine residues in PLA<sub>2</sub>.

The stereoselective syntheses of both **A** and **B** are shown in Fig. 1. Conjugated ethynyl alcohol **1** was prepared from  $\beta$ -ionone via enol phosphonate,<sup>8</sup> acetylene formation, and then one-carbon elongation (81% for three steps). The key step is highly stereoselective hydromagnesiation on the ethynyl alcohol with iso-

butylmagnesium chloride catalyzed by titanocene dichloride.<sup>9,10</sup> The vinylmagnesium compound thus prepared was successively reacted with carbon dioxide to give the desired (E)-hydroxymethyl carboxylic acid **2** in 73% yield as the sole stereoisomer. Then, synthesis of the compound **A** was achieved by esterification of **2** with 1,1,3,3-tetramethylguanidine and methyl iodide<sup>10</sup> followed by oxidation (71% yield for 2 steps).<sup>11</sup> Stereo-selective synthesis of the Z stereoisomer **B**<sup>12</sup> was successfully realized from  $\gamma$ -hydroxybutenolide **3**<sup>13</sup> retaining its Z stereochemistry by treatment of **3** with methyl iodide (12 equivalent) in the presence of diazabicyclo-undecene (3 equivalent) in DMF (6 hr at 0 °C and then 1 hr at room temperature) in 82% yield. The ratio of Z to E stereoisomer was 98 to 2 by NMR analysis.<sup>14</sup> Thus, stereoselective syntheses of both (E)- and (Z)-methoxycarbonyltrienal compounds were achieved. The various derivatives **C-E** were prepared by the above hydromagnesiation method or by photoisomerization of the corresponding Z stereoisomers.

The inhibitory activities of **A-I**<sup>15</sup> toward bovine pancreatic PLA<sub>2</sub> were tested and their results are shown in Fig. 2 and 3.<sup>16</sup> All 3-(E)-methoxycarbonyl-2,4,6-trienal compounds **A**, **C**, **D**, and **E** showed powerful inhibitory activities toward PLA<sub>2</sub>. On the contrary, the Z isomer **B**, the derivatives **F**, **G**, **H**, and **I** showed no sufficient inhibitory activities toward bovine PLA<sub>2</sub>. Obviously, the 3-(E)-methoxycarbonyl-2,4,6-trienal system is essential for the inhibition of bovine pancreatic PLA<sub>2</sub>. Furthermore, by amino acid analysis of the PLA<sub>2</sub> reacted with **A**, it was found that **A** irreversibly modified six out of eleven lysine residues of bovine PLA<sub>2</sub> and inactivated its hydrolytic ability in 90-100%. Then, our interest was directed to investigating what irreversible products would be formed by the reaction. We used a primary amine as a model for lysine residues of PLA<sub>2</sub> and examined the reaction mechanism between **A** and lysine residues.

Reaction of **A** with n-propylamine in dioxane quantitatively yielded 1,2-dihydropyridine derivative **K** within five minutes at room temperature.<sup>17,18</sup> The reaction must proceed via 6 $\pi$ -electrocyclization of the intermediary azatriene **J** as shown in Fig. 1.<sup>19,20</sup> The corresponding dihydropyridine derivatives were also obtained from the reaction of compounds **C**, **D**, and **E** with n-propylamines. On the other hand, the derivatives **B**, and **F-I**, which showed no significant inhibitory activities toward bovine PLA<sub>2</sub>, only gave the corresponding Schiff bases within 60 minutes at room temperature. These results are the first observation that both the C3 methoxycarbonyl group and the C6 double bond in the 3-(E)-methoxycarbonyl-1-aza-2,4,6-triene significantly contribute to acceleration of the 6 $\pi$ -electrocyclization reaction,<sup>19</sup> and these required functional groups for rapid cyclization to give the dihydropyridine derivatives are in accordance with those for significant inhibition of PLA<sub>2</sub> by **A**, **C**, **D**, and **E**. These assumed model studies were also supported by MALDI-TOF mass spectrum of the irreversibly modified PLA<sub>2</sub> by the analog **D**. The mass spectrum of the modified PLA<sub>2</sub>, which was prepared by the reaction with **D** for 60 minutes at 40 °C, showed a molecular weight ion at MH<sup>+</sup> = 13,960 (theoretical MH<sup>+</sup> = 13,959) which corresponded to the modified PLA<sub>2</sub> by one molecule of **D** to form a dihydropyridine derivative, along with a molecular weight ion at MH<sup>+</sup> = 13,782 (theoretical MH<sup>+</sup> = 13,783) for the unreacted PLA<sub>2</sub>. Thus, the results of the model reactions between the derivatives **A-I** and n-propylamines were well compatible with those of their inhibitory activities toward the enzyme.

In conclusion, 3-(E)-methoxycarbonyl-2,4,6-trienal **A**, **C**, **D**, and **E** would inhibit the hydrolytic activity of bovine pancreatic PLA<sub>2</sub> by the formation of the dihydropyridine derivatives resulting from the reaction with lysine residues of PLA<sub>2</sub> via 6 $\pi$ -electrocyclization of the intermediary Schiff bases.

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Fig. 1

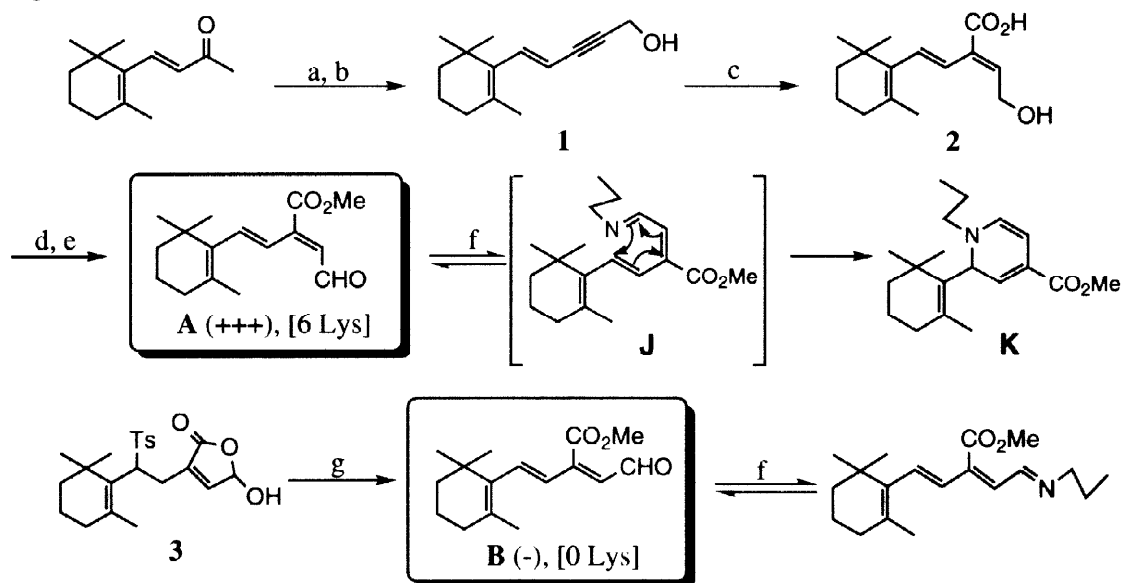
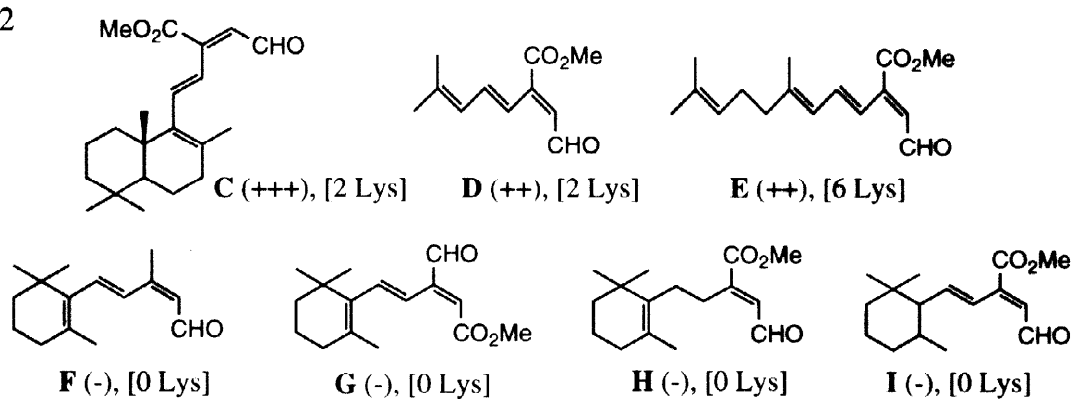
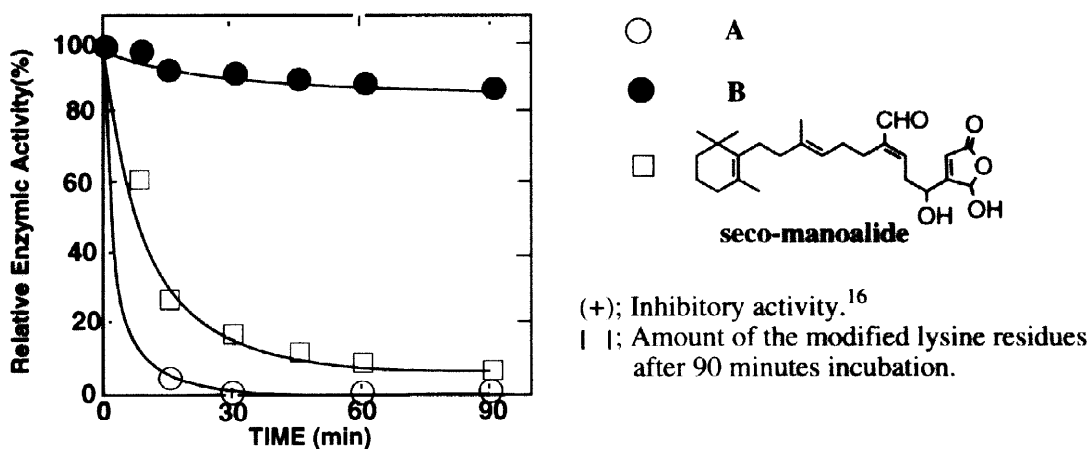


Fig. 2

Fig. 3 Inhibitory Activity of Bovine Pancreatic PLA<sub>2</sub>

## References and Notes

- Potts, B. C. M.; Faulkner, D. J.; Jacobs, R. S. *J. Nat. Prods.*, **1992**, *55*, 1701.
- Dennis, E. A., *The Enzymes*, **16**, p.307, Boyer, P.D. ed., Academic Press, New York (1983).
- Silva, de D. E.; Scheuer, P. J. *Tetrahedron Lett.*, **1980**, *21*, 1611.
- (a) Walker, R. P.; Thompson, J. E.; Faulkner, D. J. *J. Org. Chem.*, **1980**, *45*, 4976. (b) Cimino, G.; Sodano, G.; Spinella, A. *Tetrahedron*, **1987**, *43*, 5401. (c) de Carvalho, M. S.; Jacobs, R. S. *Biochem. Pharmacol.*, **1991**, *42*, 1621.
- Potts, B. C. M.; Faulkner, D. J.; de Carvalho, M. S.; Jacobs, R. S. *J. Am. Chem. Soc.*, **1992**, *114*, 5093, and references cited therein.
- Reynolds, L. J.; Michelich, E. D.; Dennis, E. A. *J. Biol. Chem.*, **1992**, *266*, 16512, and references cited therein.
- (a) Katsumura, S.; Han, Q.; Kadono, H.; Fujiwara, S.; Isoe, S.; Fujii, S.; Nishimura, H.; Ikeda, K. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 1263. (b) Katsumura, S.; Han, Q.; Fujiwara, S.; Isoe, S.; Nishimura, H.; Inoue, S.; Ikeda, K. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 1267. (c) Fujii, S.; Tahara, Y.; Toyomoto, M.; Hada, S.; Nishimura, H.; Inoue, S.; Ikeda, K.; Inagaki, Y.; Katsumura, S.; Samejima, Y.; Omori-Satoh, T.; Takasaki, C.; Hayashi, K. *Biochem. J.*, **1995**, *308*, 297.
- Negishi, E.; King, A.O.; Klima, W.L. *J. Org. Chem.*, **1980**, *45*, 2526.
- Sato, F.; Ishikawa, H.; Watanabe, H.; Miyake, T.; Sato, M. *J. Chem. Soc. Chem. Commun.*, **1981**, 718.
- Kocienski, P.J.; Love, C. J.; Whitby, R.J.; Costello, G.; Roberts, D.A. *Tetrahedron*, **1989**, *45*, 3839.
- Data for **A**;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.07 (s, 6H), 1.47-1.50 (m, 2H), 1.60-1.67 (m, 2H), 1.79 (s, 3H), 2.07 (t, 2H,  $J=6.3\text{Hz}$ ), 3.87 (s, 3H), 6.63 (d, 1H,  $J=7.3\text{Hz}$ ), 6.64 (s, 2H), 10.05 (d, 1H,  $J=7.3\text{Hz}$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  18.93, 21.82, 28.86, 33.30, 34.10, 39.48, 52.71, 123.30, 131.18, 134.39, 137.26, 141.77, 146.73, 167.15, 191.44; IR (NaCl, neat)  $\nu$  1738, 1730, 1678, 1582, 1442, 1246, 1142  $\text{cm}^{-1}$ .
- Data for **B**;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.05 (s, 6H), 1.46-1.49 (m, 2H), 1.59-1.65 (m, 2H), 1.76 (s, 3H), 2.06 (t, 2H,  $J=6.2\text{Hz}$ ), 3.94 (d, 3H,  $J=0.7\text{Hz}$ ), 6.07 (d, 1H,  $J=7.6\text{Hz}$ ), 6.22 (d, 1H,  $J=16.3\text{Hz}$ ), 6.69 (d, 1H,  $J=16.3\text{Hz}$ ), 9.76 (dd, 1H,  $J=0.7, 7.6\text{Hz}$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  18.88, 21.76, 28.84, 33.44, 34.22, 39.54, 52.59, 127.64, 128.15, 135.13, 136.84, 139.76, 150.53, 166.77, 190.72; IR (NaCl, neat)  $\nu$  1772, 1736, 1680, 1582, 1442, 1246, 1142  $\text{cm}^{-1}$ .
- Katsumura, S.; Hori, K.; Fujiwara, S.; Isoe, S. *Tetrahedron Lett.*, **1985**, *26*, 4625.
- Stepwise formation from **3**; methylesterification (MeI,  $i\text{-Pr}_2\text{NEt}$  / DMSO; 88% yield) and then elimination of sulfone (DBU,  $\text{Pd}(\text{Ph}_3\text{P})_4$  / DMSO; 94% yield) gave **2** : **5** mixtures of **A** and **B**.
- The derivatives **F-I** were synthesized by the similar strategy as shown in Fig. 1.
- Bovine pancreatic  $\text{PLA}_2$  was incubated with the analogs **A-I** in 50 mM Tris/HCl buffer at 40 °C and pH 8.0. At appropriate time intervals,  $\text{PLA}_2$  activity toward anionic mixed micelles of 1,2-dilauroyl-*sn*-glycero-3-phosphocholine with cholic acid was measured by the pH-statt method at 25 °C, pH 7.0, and ionic strength 0.2 in the presence of 10 mM  $\text{CaCl}_2$ .<sup>7(c)</sup>
- The dihydropyridine compounds obtained from the reaction of **A**, **C**, **D**, and **E** with *n*-propylamine were unstable for acid. Then, we monitored these reactions by NMR in dioxane- $d_6$  or  $\text{C}_6\text{D}_6$ .
- Data for **K**;  $^1\text{H}$  NMR (400 MHz,  $\text{C}_4\text{D}_8\text{O}_2$ )  $\delta$  0.84 (t, 3H,  $J=7.3\text{Hz}$ ), 1.00 (s, 3H), 1.04 (s, 3H), 1.42-1.62 (m, 6H), 1.76 (s, 3H), 2.01-2.07 (m, 2H), 2.52-2.60 (m, 1H), 2.75-2.81 (m, 1H), 3.62 (s, 3H), 4.68 (dd, 1H,  $J=1.6\text{Hz}, 7.6\text{Hz}$ ), 5.27 (d, 1H,  $J=4.0\text{Hz}$ ), 5.63 (ddd, 1H,  $J=0.4\text{Hz}, 1.7\text{Hz}, 4.0\text{Hz}$ ), 5.89 (dd, 1H,  $J=0.4\text{Hz}, 7.6\text{Hz}$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{C}_4\text{D}_8\text{O}_2$ )  $\delta$  11.35, 19.98, 20.74, 21.14, 27.33, 28.92, 35.25, 35.53, 41.03, 51.49, 53.75, 57.20, 86.83, 123.79, 126.78, 137.60, 137.98, 142.97, 166.14; IR (NaCl, neat)  $\nu$  1724, 1640, 1580, 1466, 1442, 1262  $\text{cm}^{-1}$ ; APCIMS calcd. for  $\text{C}_{19}\text{H}_{30}\text{NO}_2$  ( $\text{M} + \text{H}$ )<sup>+</sup> 304, found 304.
- Maynard, D. F.; Okamura, W. H. *J. Org. Chem.*, **1995**, *60*, 1763, and references cited therein.
- Ren, R. X.-F.; Sakai, N.; Nakanishi, K. *J. Am. Chem. Soc.*, **1997**, *119*, 3619.