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Synthesis of a New Phospholipase A_2 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_2 (PLA_2) from bovine pancreas. As the inhibitory mechanism of PLA_2 by **A**, the irreversible formation of dihydropyridine resulting from the reaction of **A** with lysine residues in PLA_2 is proposed based on the model reactions.

Recently, it has been reported that some unsaturated aldehyde terpenoids inhibit phospholipase A_2 (PLA_2),[1] 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,[2] the inhibitory mechanism of PLA_2 by these aldehyde terpenoids has been of biochemical and medicinal interest. Among these aldehyde terpenoid inhibitors, manoalide and scalaradial, which are sesterterpenoids isolated from marine sponges,[3,4] are well characterized and their inhibitory mechanisms toward various PLA_2s have been reported.[5,6] We independently studied to elucidate the inhibitory mechanism of bovine pancreatic PLA_2 by manoalide, and found that two aldehyde groups and the hydrophobic moiety of manoalide were essential for the PLA_2 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.[7] Unfortunately, we could not obtain a clear answer on what irreversible reaction proceeded between manoalide and lysine residues of PLA_2, 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_2 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_2, 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_2. In addition, as the inhibitory mechanism of bovine PLA_2 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_2.

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

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butylmagnesium chloride catalyzed by titanocene dichloride.[9,10] 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[10] followed by oxidation (71% yield for 2 steps).[11] Stereoselective synthesis of the Z stereoisomer **B**[12] was successfully realized from γ-hydroxybutenolide **3**[13] retaining its Z stereochemistry by treatment of **3** with methyl iodide (12 equivalent) in the presence of diazabicycloundecene (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.[14] 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**[15] toward bovine pancreatic PLA_2 were tested and their results are shown in Fig.2 and 3.[16] All 3-(E)-methoxycarbonyl-2,4,6-trienal compounds **A**, **C**, **D**, and **E** showed powerful inhibitory activities toward PLA_2. On the contrary, the Z isomer **B**, the derivatives **F**, **G**, **H**, and **I** showed no sufficient inhibitory activities toward bovine PLA_2. Obviously, the 3-(E)-methoxycarbonyl-2,4,6-trienal system is essential for the inhibition of bovine pancreatic PLA_2. Furthermore, by amino acid analysis of the PLA_2 reacted with **A**, it was found that **A** irreversibly modified six out of eleven lysine residues of bovine PLA_2 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_2 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.[17,18] The reaction must proceed via 6π-electrocyclization of the intermediary azatriene **J** as shown in Fig.1.[19,20] 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_2, 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 6π-electrocyclization reaction,[19] and these required functional groups for rapid cyclization to give the dihydropyridine derivatives are in accordance with those for significant inhibition of PLA_2 by **A**, **C**, **D**, and **E**. These assumed model studies were also supported by MALDI-TOF mass spectrum of the irreversibly modified PLA_2 by the analog **D**. The mass spectrum of the modified PLA_2, which was prepared by the reaction with **D** for 60 minutes at 40 °C, showed a molecular weight ion at MH^+ = 13,960 (theoretical MH^+ = 13,959) which corresponded to the modified PLA_2 by one molecule of **D** to form a dihydropyridine derivative, along with a molecular weight ion at MH^+ = 13,782 (theoretical MH^+ = 13,783) for the unreacted PLA_2. 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_2 by the formation of the dihydropyridine derivatives resulting from the reaction with lysine residues of PLA_2 via 6π-electrocyclization of the intermediary Schiff bases.

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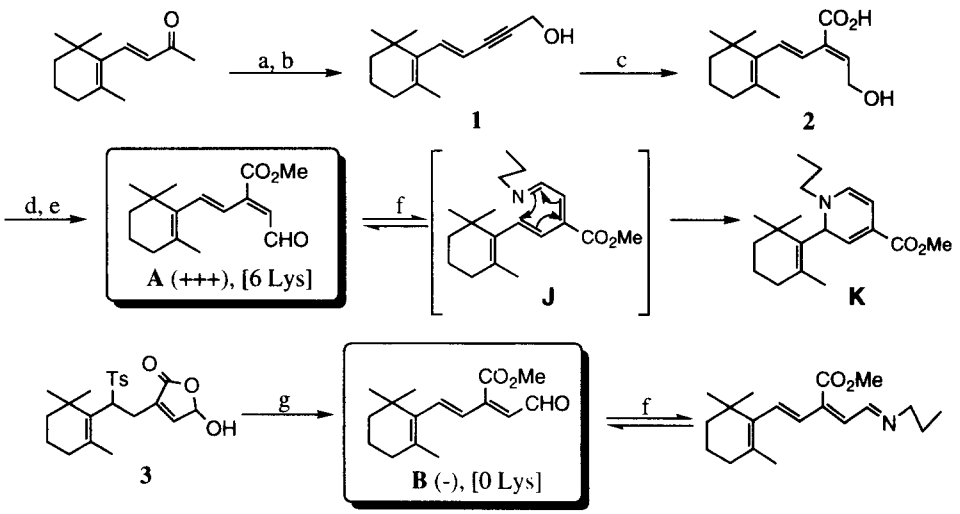
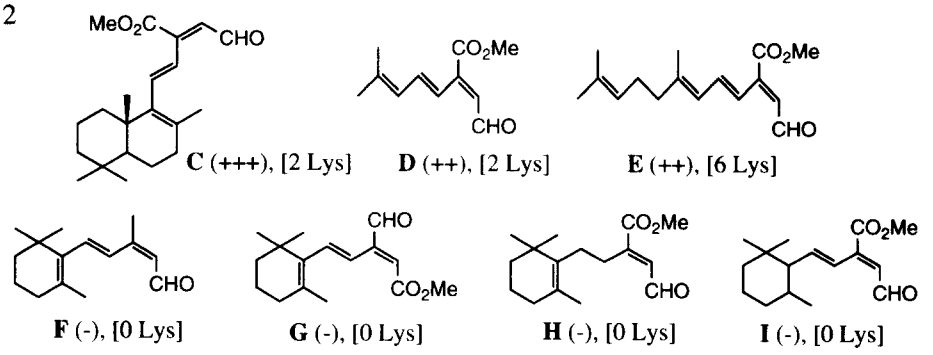
Fig. 1



a) LDA, $ClP(O)(OEt)_2$/THF, then 2eq.LDA; 81% b) n-BuLi, $(CH_2O)_n$/THF; quant.
c) iBuMgCl, Cp_2TiCl_2/ether, then CO_2/THF; 73% d) N,N,N',N'-tetramethylguanidine, MeI/benzene; 71%
e) MnO_2/CH_2Cl_2; quant. f) n-$PrNH_2/C_4D_8O_2$; 5 min. quant.[17] g) DBU, MeI/DMF; 82%.

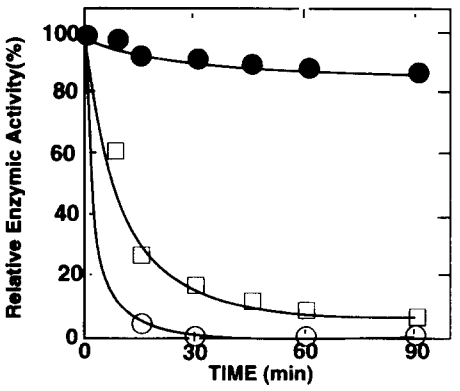
Fig. 2

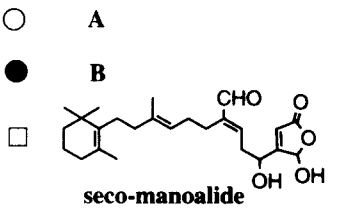


C (+++), [2 Lys] **D** (++), [2 Lys] **E** (++), [6 Lys]

F (-), [0 Lys] **G** (-), [0 Lys] **H** (-), [0 Lys] **I** (-), [0 Lys]

Fig. 3 **Inhibitory Activity of Bovine Pancreatic PLA_2**





(+); Inhibitory activity.[16]
| |; Amount of the modified lysine residues after 90 minutes incubation.

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11. Data for **A**; ^{1}H NMR (400 MHz, $CDCl_3$) δ 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=6.3Hz), 3.87 (s, 3H), 6.63 (d, 1H, J=7.3Hz), 6.64 (s, 2H), 10.05 (d, 1H, J=7.3Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 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) ν 1738, 1730, 1678, 1582, 1442, 1246, 1142 cm^{-1}.
12. Data for **B**; ^{1}H NMR (400 MHz, $CDCl_3$) δ 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=6.2Hz), 3.94 (d, 3H, J=0.7Hz), 6.07 (d, 1H, J=7.6Hz), 6.22 (d, 1H, J=16.3Hz), 6.69 (d, 1H, J=16.3Hz), 9.76 (dd, 1H, J=0.7, 7.6Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ18.88, 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) ν 1772, 1736, 1680, 1582, 1442, 1246, 1142 cm^{-1}.
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14. Stepwise formation from **3**; methylesterification (MeI, i-Pr_2NEt / DMSO; 88% yield) and then elimination of sulfone (DBU, $Pd(Ph_3P)_4$/ DMSO; 94% yield) gave 2 : 5 mixtures of **A** and **B**.
15. The derivatives **F-I** were synthesized by the similar strategy as shown in Fig.1.
16. Bovine pancreatic 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, 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 $CaCl_2$.[7(c)]
17. 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_8 or C_6D_6.
18. Data for **K**; ^{1}H NMR (400 MHz, $C_4D_8O_2$) δ 0.84 (t, 3H, J=7.3Hz), 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=1.6Hz, 7.6Hz), 5.27 (d, 1H, J=4.0Hz), 5.63 (ddd, 1H, J=0.4Hz, 1.7Hz, 4.0Hz), 5.89 (dd, 1H, J=0.4Hz, 7.6Hz); ^{13}C NMR (100 MHz, $C_4D_8O_2$) δ 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) ν 1724, 1640, 1580, 1466, 1442, 1262 cm^{-1}; APCIMS calcd. for $C_{19}H_{30}NO_2$ $(M + H)^+$ 304, found 304.
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