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SYNTHESIS OF OXAZOLIDINONE PHOSPHOLIPID ANALOGUE AS A NEW INHIBITOR OF PHOSPHOLIPASE A_2†)

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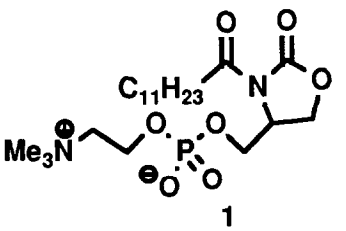
Abstract: (S)- and (R)-3-dodecanoyl-4-phosphatidylcholinohydroxymethyl-2-oxazolidinone (**1**), which are cyclic analogues of the amide phospholipid **7**, were synthesized. The inhibitory activities of these analogues toward phospholipase A_2 were compared with that of the amide analogue **7**.

Design and synthesis of phospholipase A_2 (PLA_2) inhibitors is one of the topics of recent biochemical and medicinal interests, since this enzyme catalyzes the hydrolysis of the ester linkage at the *sn*-2 position of glycerophospholipids and also the release of arachidonic acid from *sn*-2 position of phospholipids is the rate-limiting step in the production of eicosanoid mediators of inflammation.[1] Among various inhibitors of PLA_2, phospholipid analogues have been most extensively examined.[2] In these studies, the enzyme-susceptible ester linkage at the *sn*-2 position of phospholipids has been replaced by an amide,[3] carbamate,[4] or hydrated fluoroketone,[5a] and phosphate group[5b] for mimicking a tetrahedral intermediate in the process of phospholipase A_2 catalyzed hydrolysis. An alternative approach was to design phosphoglyceride analogues including a cyclic γ-lactone.[6] The powerful inhibitory activity of the amide analogue **7** developed by de Haas *et al.*[3c] and that of the γ-lactone analogue **9** by Campbell *et al.*[6a] prompted us to examine the synthesis and inhibitory activity of a new PLA_2 inhibitor, oxazolidinone phospholipid **1**, which is a cyclized form of the amide analogue[3c] and is also an amidated form of the γ-lactone analogue.[6a]



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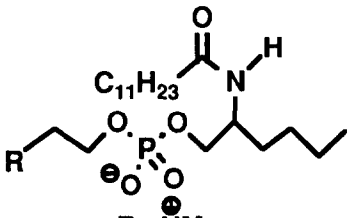

7 : R= $\overset{\oplus}{N}Me_3$
8 : R= OH

Synthesis of oxazolidinone phospholipid **1** from optically active glycidol is as follows. Treatment of R-(+)-glycidol, $[\alpha]_D$ +22.2° (98%ee), with benzoylisocyanate in carbon tetrachloride at room temperature gave epoxycarbamate quantitatively, which was treated with potassium

carbonate in the presence of benzyltriethylammonium chloride to afford 4-benzoyloxymethyl-2-oxazolidinone (**2**), mp 112-113 °C, $[\alpha]_D$ +29.6°, in 92% yield.[7] The benzoyl group of **2** was replaced with alkylsilyl group by a migration from the nitrogen to oxygen. Thus, O-benzoyl-N-alkylsilyl compound **3**,[8] which was prepared by silylation of **2** (TBDMSCl / DMAP/ Et_3N / DMF, 95% yield), was treated with potassium hydroxide in methanol to yield the silyl ether **4** in 93% yield. Acylation of **4** with dodecanoyl chloride in the presence of DMAP and triethylamine in THF gave **5**, which was treated with 2N HCl in THF without purification to afford the alcohol **6** [9] in 74% yield for two steps. The synthesis of (S)-**1**[10] was achieved by the reaction of **6** with 2-chloro-2-oxo-1,3,2-dioxa-phospholane (DMAP / Et_3N / benzene) followed by treatment with trimethylamine in benzene at 60 °C in a pressure bottle for 15h in 39.4% yield.

Inhibition activities of the synthesized oxazolidinone phosphatidylcholine analogues (R)- and (S)-**1**, were tested against the PLA_2 from the snake venom of *A. halys blomhoffii*.[11] They were compared with those for the amide analogues, (R)- and (S)-2-dodecanoylamino-1-hexanol-phosphocholine (**7**). The enzymatic activity was measured toward a monodispersed substrate (R)-1,2-dihexanoyl-glycerol-3-phosphocholine in the presence of 3.3mM calcium chloride by using the pH-stat method at 25 °C, pH 8.2, and ionic strength 0.1. All the analogues were found to be simple competitive inhibitors to the PLA_2. The results are summarized in Fig.1. The (R)-**1** analogue showed 50% inhibition at a concentration of 31μM (IC_{50} value). The binding constant of the analogue to the enzyme ($1/K_i$ value) was calculated to be $4.5 \times 10^4\ M^{-1}$. On the other hand IC_{50} and $1/K_i$ values of (R)-**7** were 5.3 μM and $2.7 \times 10^5\ M^{-1}$ respectively. The diastereomer, (S)-**1**, showed no significant inhibitory activity.

The crystal structure of a complex between an extracellular PLA_2 and the amide analogue (R)-**8** was recently reported.[12] This inhibitor (R)-**8** was also reported to bind to the PLA_2 molecule by three orders of magnitude than natural substrate. De Haas *et al.* proposed that a strong hydrogen bond between the NH group of the inhibitor amide bond and the Nδ1 of the catalytic group His-48 of the enzyme contributes to the higher affinity of the inhibitor compared to those of natural substrates. Concerning this proposal, it is very interesting that the oxazolidinone phospholipid (R)-**1**,which has no hydrogen atom at amide nitrogen, showed significant inhibitory activity. Previously, Campbell *et al.*[6a] suggested by the energy minimization method that a γ-lactone analogue **9** was snugly bound into the active site of PLA_2 molecule. Since our present analogue (R)-**1** possesses a cyclic five membered framework as well as the γ-lactone analogue **9**, the manner of binding to the PLA_2 of (R)-**1** might be similar as that of **9**. Compared with (R)-**7** and **9**, the analysis of the interactiones of (R)-**1** with PLA_2 by computer modeling will be discussed in a detailed paper.

Further studies on the binding effect to calcium ion and on the inhibitory activities toward other PLA_2s of the analogue **1** are also in progress.

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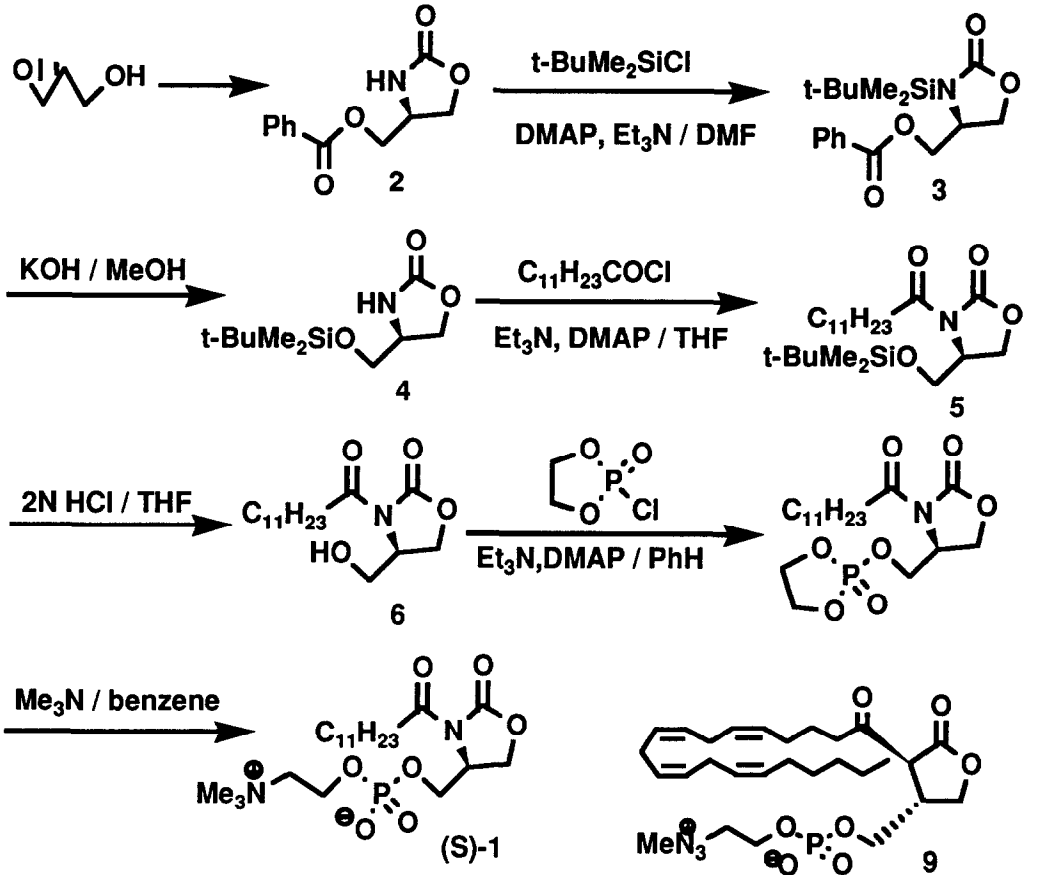

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†)A part of this work was reported at the 63th of Annual Spring Meeting of Japan Chemical Society, the abstract p.1904(1992).

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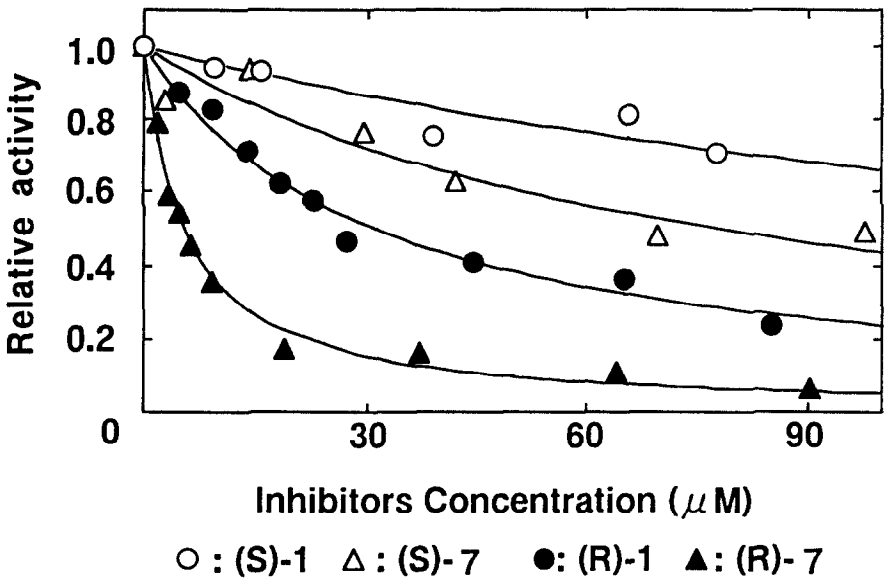
Fig.1 Inhibition of a Phospholipase A_2 from the Snake (*A. halys blomhoffii*) venom by Substrate analogues



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7.S.Katsumura, A.Kondo, and Q.Han, *Chem. Lett.*, **1991**, 1245. R-(+)-glycidol gives R-(+)-4-benzoyloxymethyl-2-oxazolidinone (**2**). The absolute structure drawn in the previous paper must be revised.

8.All new compounds was fully characterized by spectroscopic data.

9.**6**: mp 67.5 - 68.5 °C. [α]$_D$ -51.8 °(c=1.05, $CHCl_3$). ^{1}HNMR ($CDCl_3$, δ): 0.85(t, 3H), 1.30(s,16H), 1.65(m, 2H), 2.65(OH, 1H), 2.95(m, 2H), 3.83(m, 2H), 4.34(m,1H), 4.55(m,1H); ^{13}C NMR($CDCl_3$, δ): 14.1, 22.7, 24.3, 29.1, 29.4, 29.5, 29.6, 31.9, 35.5, 55.8, 62.3, 65.0, 153.7, 174.6.

10.**1**: $[\alpha]_D$ -55.2° (c=1.06, $CHCl_3$). ^{1}H NMR(CD_3OD, δ): 0.90(t, 3H), 1.30(s,16H), 1.63(m, 2H), 2.89(m, 2H), 3.22(s, 9H), 3.63(t, 2H), 4.00(m, 1H), 4.23(m, 3H), 4.46(d, 2H), 4.65(m, 1H); ^{13}C NMR ($CDCl_3$, δ): 14.1, 22.7, 24.3, 29.2, 29.4, 29.5, 29.6, 29.7, 31.9, 35.4, 54.3, 59.4, 63.8, 65.6, 66.2, 154.1, 173.4. Anal. Calcd. for $C_{21}H_{41}N_2O_7PNa{\cdot}H_2O$: C, 46.57; H, 8.74; N, 5.17. Found: C, 46.67; H, 8.98; N, 5.17.

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