Bioorganic & Medicinal Chemistry Letters, Vol.2, No.10, pp 1263-1266, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

PHOSPHOLIPASE A_2 INHIBITION BY MANOALIDE: DEVELOPMENT OF SIMPLE ANALOGUES AND NECESSARY FUNCTIONAL GROUPS FOR INHIBITION+)

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(*Received* 17 *March* 1992)

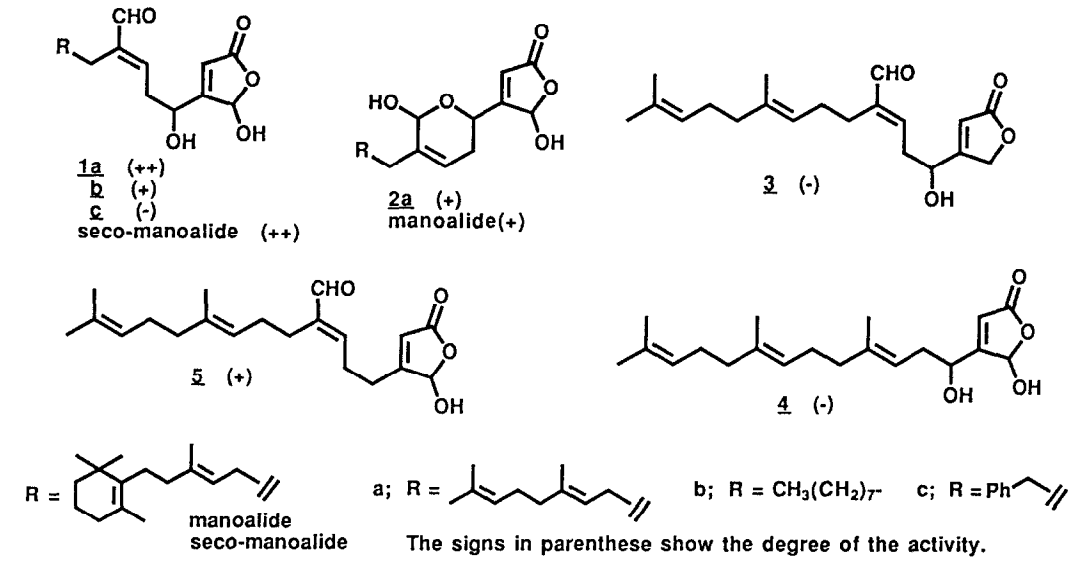
Abstract: Manoalide analogue 1a inhibited bovine pancreatic phospholipase A_2 to the same extent as seco-manoalide. Two aldehyde groups in a manoalide molecule are indispensable to the enzyme inhibition.

Manoalide, which was isolated from a marine sponge *Luffariella variabillis*,[1] showed anti-inflammatory properties *in vivo*.[2] Phospholipase A_2(PLA_2), which hydrolyzes the ester linkage at the C-2 position of glycero-phospholipid, is known as one of the key enzymes in the metabolism of arachidonic acid.[3] Two kinds of PLA_2s, isolated from cobra[4] and bee[5] venoms, were shown to be inhibited by natural manoalide, and their inhibition mechanisms were proposed. Recently, we found independently similar inactivation phenomena of bovine pancreatic PLA_2 by synthetic manoalide and its analogues, and started to elucidate the inhibition mechanism. Bovine pancreatic PLA_2 is thought to be one of the most suitable materials for such purposes, since a great deal of information concerning the three dimensional structure[6] and kinetic properties of the enzyme are available.

Our studies on the enzymatic inhibition of bovine pancreatic PLA_2 by manoalide have been focused upon the following four points. 1. Development of a simple analogue for manoalide which possesses an equal inhibitory ability to that for *d,l*-manoalide. 2. Determination of the functional groups in the manoalide molecule which are responsible for the enzyme inhibition. 3. Identification and characterization of the amino acid residues of this PLA_2 which are modified by a manoalide analogue. 4. Understanding of the reaction mechanism of manoalide with these amino acid residues. In this paper, we report the development of a manoalide analogue which is as effective as *d,l*-manoalide in the inhibition of bovine PLA_2 and discuss the chemical structure of manoalide responsible for the enzyme inhibition.

The inhibition activities of *d,l*-manoalide, seco-manoalide, and analogues 1a, 1b, 1c, and 2a were estimated by measuring the residual PLA_2 activity towards the micelles of dilauroylphosphatydylcholine mixed with an anionic surface-active agent, sodium cholate, since the hydrolytic activity of bovine PLA_2 is significantly activated by the presence of anionic surface-active agents, unlike snake venom PLA_2s. The results are summarized in Figs. 1, 2, and 3.[7] As seen from Figs. 2 and 3, an analogue of 1a exhibited inhibition comparable to that by seco-manoalide. An analogue of 2a was found to be an inhibitor as potent as manoalide. It is of interest

Fig 1



that seco-manoalide is a stronger inhibitor than manoalide, and that an analogue of 1c showed no inhibitory activity (Fig.1). Thus, compounds 1a and 2a were concluded to be simple and effective analogues of seco-manoalide and manoalide.

In order to determine the chemical structure of the manoalide molecule responsible for the enzyme inhibition, the inhibitory activities of homologues 3,[8] 4, and 5[9] were measured by the method described above. Monoaldehyde derivatives 3 and 4 showed no inhibitory activity at all. Dehydroxy derivative 5 showed a much weaker inhibitory activity than analogue 1a. These results indicate that the two aldehyde groups of manoalide are indispensable to inhibit the enzymatic activity of bovine pancreatic PLA_2. The same conclusion was also obtained for cobra[4] and bee[5] venom PLA_2s.

The synthesis of analogues 1a, b, c, 2a, and 4 was performed by our highly effective methods to prepare manoalides as described previously (Fig. 4).[10] Thus, allyl chlorides 7a, b, and c were prepared from the corresponding halides 6a, b, and c by a reaction with the anion of dimethylhydrazone of pyruvaldehyde dimethylacetal followed by acid treatment, Peterson olefination, reduction, and chlorination (40% yield of 7a from 6a). These allylic chlorides were coupled with carbon monoxide and 2-trimethylsilyl-4-tributylstannylfuran catalyzed by palladium(0) in the presence of triphenylphosphine to give the corresponding ketones. The ketones were reduced with lithium aluminum hydride, and the alcohols were protected to afford silylfuran derivatives 8a, b, and c (89% yield of 8a from 7a). Photosensitized oxygenations of 8a, b, and c followed by acid treatment yielded analogues of seco-manoalide 1a, b, and c, respectively (60% of 1a from 8a), through compound 9. Compound 2a was quantitatively obtainded by photoirradiation of 1a. Compound 4 was synthesized from farnesyl chloride by a reaction with the anion derived from 3-(ethoxyethyloxy) tributylstannylfuran (71% yield), followed by photosensitized oxygenation in

Fig 2

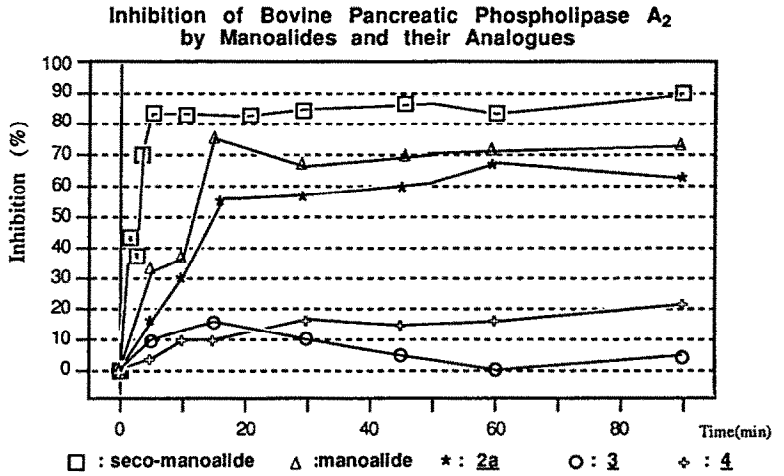


Fig 3

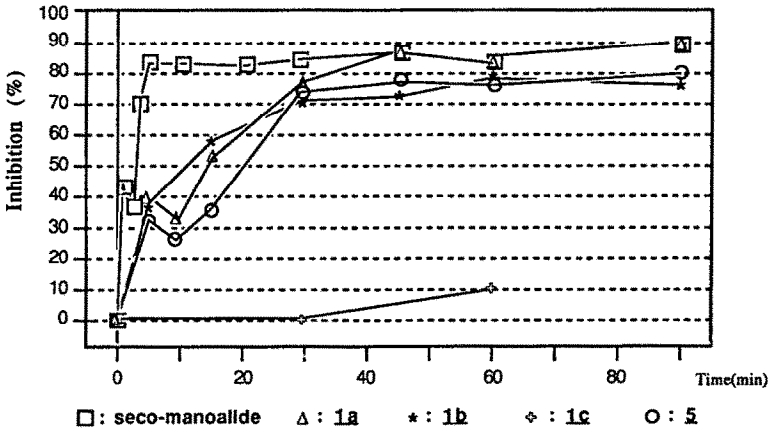
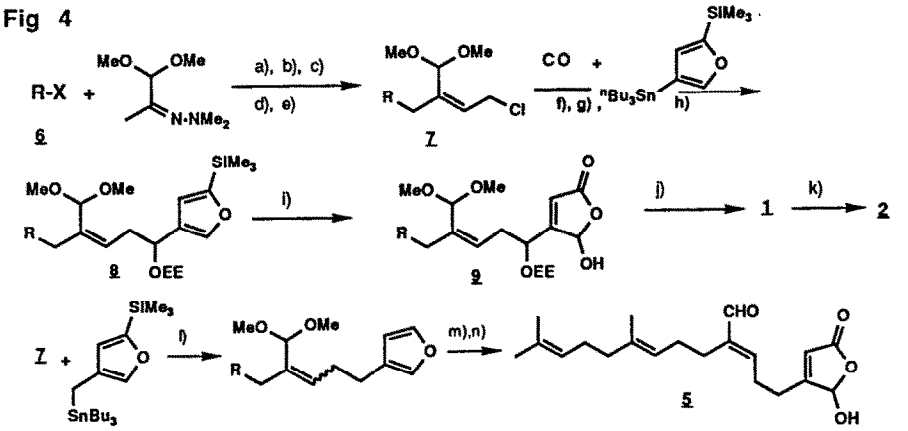


Fig 4



a) Li, Et_2NH / THF, PhH, HMPA b) H_3O^+ c) $Me_3SiCH_2CO_2Bu^t$, LDA /THF, d) DIBAL CH_2Cl_2 e) MsCl, LiCl, collidine / DMF
f) $Pd(dba)_2$, PPh_3 /THF g) LAH /Et_2O h) $CH_3OCH(CH_3)Cl$, $(iso\text{-}Pr)_2NEt$ / CH_2Cl_2 i) $^1O_2$ / MeOH j) 2N-HCl / THF
k) hv / benzene l) BuLi / THF m) $^1O_2$ / MeOH n) 2N-HCl / MeOH

the presence of N,N-diisopropylethylamine and then acid treatment[11] (45% yield). This alkoxystannane route may open the way for easy supply of 1a and 2a because of its simplicity.[9]

Thus, we have obtained a good analogue of manoalide 1a which can be supplied in sufficient quantity to study the inhibition mechanism of bovine pancreatic PLA_2 by manoalide. We have also revealed that the two aldehyde groups of manoalide are indispensable to the enzyme inhibition. It is of great interest to identify the amino acid residues modified by manoalide analogue, 1a. In the accompanying paper, it is described that only two of the eleven native lysine residues were modified by 1a or 2a under the present conditions.

Acknowledgement: We are grateful to the Fujisawa Foundation, the Study for Medicinal Resources, for partial support of this work.

References and Notes

+) A part of this work was reported at the 58th of Annual Spring Meeting of Japan Chemical Society, abstract paper p.1183(1989), and at the 34th Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics, abstract paper p.213(1990).

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7. Assey conditions are as follows. $PLA_2(4.4x10^{-6}M)$ was incubated with manoalide or its analogue($4.4x10^{-4}M$) in 50mM Tris-HCl buffer(pH 8.0) in the presence of 8.3%(v/v) dioxane at 40°C for 20 minutes. Residual PLA_2 activity toward the mixed micelles of 1.1 mM dilauroylphosphatydylcholine with 5.4mM sodium cholate in the presence of 10mM $CaCl_2$ was measured by a pH-stat method at 25°C and pH 7. The titration volumes recorded during the enzymatic hydrolysis of the substrate were cerrected by subtracting the volume due to the spontaneous reaction in he absence of the enzyme.
8. Compound 3 was derived from 9 by reduction and acid treatment ($NaBH_4$ in EtOH, 2N-HCl MeOH). See S.Katsumura, S.Fujiwara, and S.Isoe, *Tetrahedron Lett.*, **28**, 1191(1987).
9. Compound 5 was called manoalog by Dennis.[4] Our simple and efficient synthesis of 5 will be described elsewhere along with an easier route to 1a and 2a.
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