UNIVERSITY OF CALIFORNIA, SAN DIEGO

Chemistry of Inhibition of Bee Venom Phospholipase A_2 by the Marine Natural Products Manoalide and Luffariellolide

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Chemistry

by

Barbara Mueller Potts

Committee in charge:

Professor D. John Faulkner, Chair
Professor Edward A. Dennis
Professor William H. Fenical
Professor Daniel F. Harvey
Professor Trevor C. McMorris

1992

ABSTRACT OF THE DISSERTATION

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The marine natural products manoalide and luffariellolide are potent anti-inflammatory agents obtained from sponges of the genus *Luffariella*. Manoalide and its analogs inhibit various secreted forms of phospholipase A_2 (PLA_2) by forming covalent adducts with lysine residues on the enzymes. In order to investigate the chemical mechanism by which this occurs, model reactions employing a primary amine in place of the lysine residue were studied by ^1H NMR spectroscopy. The analogs which were studied include manoalide methyl analog, which contains both the γ-hydroxybutenolide and δ-lactol rings of manoalide; luffariellolide, which contains only the γ-hydroxy-butenolide ring; and synthetic precursors to manoalide methyl analog.

An analog containing only the δ-lactol ring reacted with amines to form an imine. Amines reacted at the γ-hydroxybutenolide ring of luffariellolide to produce γ-

(alkylamino)butenolides, which are cyclized forms of the corresponding imines. Manoalide methyl analog reacted similarly to luffariellolide, indicating that the γ-hydroxybutenolide ring is the key pharmacophore. The γ-(*n*-butylamino)butenolide derivative of luffariellolide reacted with hydroxylamine to form an oxime with concommitant release of *n*-butylamine.

When the luffariellolide-PLA_2 and manoalide-PLA_2 adducts were treated with hydroxylamine, the PLA_2 activity was substantially recovered, but the activity was not recovered if the luffariellolide-PLA_2 adduct was reduced with sodium borohydride prior to hydroxylamine treatment. Similar experiments were carried out on the marine natural product scalaradial. The binding sites of manoalide and luffariellolide on bee venom PLA_2 were sought. [^{3}H]-$NaBH_4$ reduction of the putative imine linkage between the drugs and lysine residues on PLA_2 resulted in up to 10-fold incorporation of tritium versus control enzyme (not treated with drug). The drug-PLA_2 adducts were digested with proteolytic enzymes or cyanogen bromide and the resulting peptides were chromatographed by reversed phase HPLC, but it was not possible to recover peptides which retained the expected levels of radioactivity using this method. Unique hydrophobic peptides obtained from luffariellolide-treated PLA_2 were identified by microsequence analysis, which indicated that lysine-85 might be a binding site for luffariellolide. This residue lies on the proposed interfacial binding site of bee venom PLA_2.

Finally, the structure elucidation of luffalactone is presented along with the revised structure of dehydromanoalide.