Chemicoenzymatic Synthesis of Lysofungin

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Received March 6, 1992 (Revised Manuscript Received May 8, 1992)

Minute quantities of a new phospholipid antifungal agent which we have named lysofungin were recently isolated from a fermentation of *Aspergillis fumigatus* in extremely low titers (38 μg/L). From the limited studies which could be carried out, lysofungin appeared to be a fungicidal, broad-spectrum, water-soluble compound deserving of further investigation. Through extensive NMR and MS studies, the novel lyso phosphatidyl inositol structure 1 shown in Figure 1 was elucidated for lysofungin.[2] Since the proposed structure contains two centers of chirality (D- vs L-*myo*-inositol and 1- vs 3-glycero), there are four possible diastereomers of the indicated structure which are consistent with the spectroscopic data.

Efforts to increase the titer have so far not been successful. Due to the promising biology exhibited by lysofungin, a synthetic approach to the diastereomer with the "natural" configurations (D-*myo*-inositol and 3-glycero) was desired.

Development of synthetic methodology directed at phosphatidyl inositol preparations has recently undergone a surge in popularity.[3] This increase in interest is due in part to the recognition of their diverse biological functions[4] as protein membrane anchors and as the initial source of polyphosphoinositol calcium modulators, arachadonic acid, and diacyl glycerol mediators. However, synthetic preparations of olefin containing phosphatidyl inositols remain scarce due to the difficulty in differentiating the nine potential hydroxyl groups and in keeping the double bonds intact upon removal of the necessary protecting groups.

After considering a lengthy total synthesis, a chemicoenzymatic synthesis starting with a commercially available natural product was appealing. It has been shown that the major component of soybean phosphatidyl inositol is 1-stearoyl(or palmitoyl)-2-linoleoyl-3-glycerophosphatidyl-D-*myo*-inositol (2).[5] This starting material incorporates all of the chiral centers and ligands present in the desired 1. As shown in Scheme I, the required transformations are to remove the saturated ester and migrate the linoleate to the primary position.

Attempts to selectively hydrolyze the primary ester by chemical means were unsuccessful. To our knowledge, no preparations touted as phospholipase A_1 (PLA_1) enzymes are commercially available; however, *Rhizopus arrhizus* lipase has been reported to exhibit PLA_1 activity using various phospholipid substrates.[6,7] The problem of selectively removing the stearate was solved using this lipase which, under mild conditions, stereoselectively cleaves the primary ester of 2 to form the lyso phosphatidyl inositol 3. The secondary ester then undergoes an intramolecular

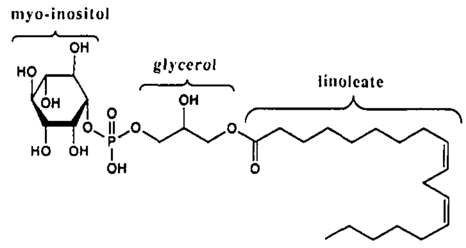


Figure 1. Proposed structure of lysofungin (1).

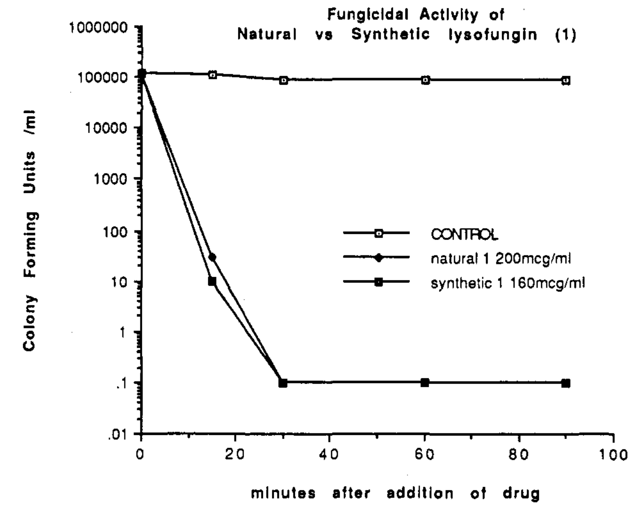
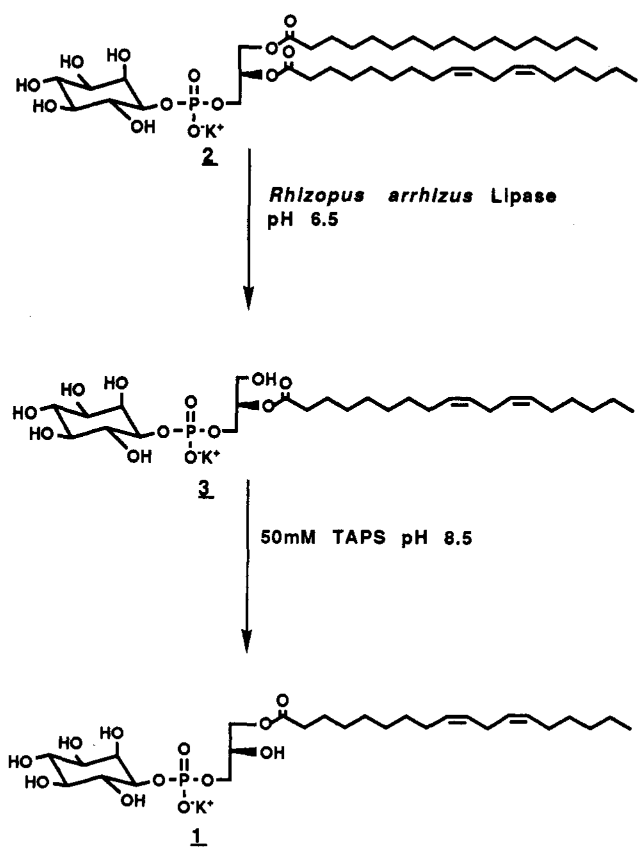


Figure 2. Effects of natural and synthetic lysofungin on the viability of *Saccharomyces cerevisiae*.

Scheme I



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(2) Hensens, O.; Zink, D.; Liesch, J. manuscript in preparation.

(3) (a) Potter, B. *Nat. Prod. Rep.* **1990**, 1–24. (b) Young, R.; Downes, C.; Eggleston, D.; Jones, M.; Macphee, C.; Rana, K.; Ward, J. *J. Med. Chem.* **1990**, *33*, 641–646. (c) Cobb, J. E.; Johnson, M. R. *Tetrahedron* **1991**, *47*(1), 21–30.

(4) (a) Saltiel, A. R.; Cuatrecasas, P. *Am. J. Physiol.* **1988**, *255*, C1–C11.

(5) (a) Myher, J.; Kuksis, A. *Biochim. Biophys. Acta* **1984**, *795*, 85–90.

(6) Rhizopus arrhizus Lipase is available from Sigma Chemical Co.

(7) (a) Slotboom, A.; Haas, G.; Bonsen, P.; Burbach-Westerhuis, G.; van Deenen, L. *Chem. Phys. Lipids* **1970**, 15–29. (b) Tamer, A.; Record, M.; Fauvel, J.; Chap, H.; Douste-Blazy, L. *Biochim. Biophys. Acta* **1984**, *793*, 213–220. (c) Tyagi, S.; Burnham, D.; Lambeth, *J. Biolog. Chem.* **1989**, 12977–12982.

transacylation to the more sterically favorable primary position of 1 by mild treatment at pH 8.5 in the presence of 50 mM TAPS buffer. Semisynthetic 1 exhibits identical physical properties as the natural product lysofungin including TLC, HPLC, ^{1}H-NMR, ^{13}C-NMR, and MS(EI). However, the four possible diastereomers of lysofungin would be expected to exhibit very similar physical properties, so the absolute configuration of lysofungin remains ambiguous.

As shown in Figure 2, semisynthetic 1 exhibits fungicidal activity vs *Saccharomyces cereviseae* with similar potency as is observed for natural lysofungin. At 0 min, stationary-phase cells were treated with the indicated amounts of natural lysofungin, synthetic lysofungin, or untreated. After 30 min exposure to either compound, no colony-forming units were observed at the indicated concentrations.

Now that synthetic lysofungin is available, more extensive studies can be carried out to determine its antifungal potential and the role of similar compounds in the cell.

Experimental Section

Phosphatidyl Inositol 2 to 3. A 4-mL aqueous suspension was prepared consisting of deoxycholate (12 mg, 0.03 mmol), bovine serum albumin (18 mg), $CaSO_4$ (5 mM), and pH 6.5 borate buffer (100 mM). This suspension was added with sonication and stirring to a dried sample of soybean phosphatidyl inositol 2 (40 mg, .049mmol). *Rhizopus arrhizus* lipase (one million units, Sigma) was then added, and the reaction was stirred at room temperature. After 2 h the reaction was quenched by the addition of 4 mL of 50% MeOH. The resulting solution was loaded onto an open RPC-18 column (5 mL of Baker octadecyl, 40 micron) and eluted with a stepwise MeOH/water gradient. The desired crude 3 (25 mg) eluted in the 70–75% MeOH fractions.

3 to Lysofungin 1. Crude 3 was dissolved in 2.5 mL of 50 mM TAPS buffer (pH 8.5) with stirring at room temperature. The reaction was monitored by HPLC (Dupont Zorbax 25-cm column, UV absorbance at 205 nm, 67% 10 mM potassium phosphate (pH 6.5) 33% acetonitrile, 1 mL/min, retention times 11.2 min for 3 and 14.2 min for 1). After 18 h the reaction was applied to an open RPC-18 column (5 mL of Baker octadecyl, 40 μm) and eluted with a MeOH/water gradient. The desired 1 (15 mg) eluted with 80% MeOH and upon lyophilization from water was obtained as a white solid.

^{1}H-NMR (CD_3OD): 0.92 (t, J = 6 Hz, 3 H), 1.28–1.42 (m, 14 H), 1.56–1.68 (m, 2 H), 2.07 (dd, J = 7 and 6 Hz, 4 H), 2.45 (t, J = 8 Hz, 2 H), 2.78 (t, J = 6 Hz, 2 H), 3.20 (t, J = 9 Hz, 2 H), 3.38 (dd, J = 3 and 8 Hz, 1 H), 3.63 (t, J = 10 Hz, 1 H), 3.77 (t, J = 9 Hz, 1 H), 3.92 (ddd, J = 3, 7, and 10 Hz, 1 H), 3.98 (dd, J = 2 and 7 Hz, 2 H), 4.06–4.12 (m, 1 H), 4.15 (dd, J = 4 and 8 Hz, 1 H), 4.21 (t, J = 3 Hz, 1 H), 5.3–5.4 (m, 4 H).

^{13}C-NMR (CD_3OD): 14.4, 23.6, 26.0, 26.5, 28.2 (2×), 30.2 (2×), 30.3, 30.5, 30.7, 32.7, 34.9, 66.3, 67.8 (d, J = 5.8 Hz), 70.0 (d, J = 7.7 Hz), 72.9, 73.1, (d, J = 1.9 Hz), 73.3 (d, J = 5.5 Hz), 74.1, 76.3, 78.4 (d, J = 6.1 Hz), 129.1, 129.1, 130.9, 130.9, 175.4.

FAB-MS (negative ion) indicated a MW of 596 (observed (M - H) at m/z 595).

Fungicidal Biology. *Saccharomyces cerevisiae* MY1117, a presumed wild-type, diploid strain of unknown genotype was obtained from the Merck culture collection and maintained on YEPD (1% yeast extract, 2% peptone, 2% glucose, and 1.5% agar) slants at 40 °C. To determine the effects of lysofungin on cell viability, cultures in early stationary phase were diluted to approximately 1 × 10^5 cells/mL in sterile saline, aliquoted into tubes containing the appropriate drug, and incubated at 30 °C. Samples were removed periodically, diluted in sterile saline, and plated on SDA (Sabouraud's Dextrose Agar, Difco). Colonies were enumerated after 36–48 h of incubation at 30 °C. The limit of detection of the assay was 20 CFU/mL.

Acknowledgment. We would like to thank O. Hensens for valuable discussions and D. Zink and J. Liesch for performing mass spectral analyses.

Palladium-Catalyzed Cross-Carbonylation of Aryl Iodides and 1-Aryl-2-alkyn-1-ones

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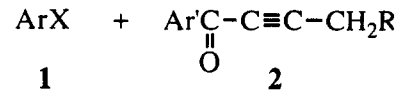
Received March 30, 1992

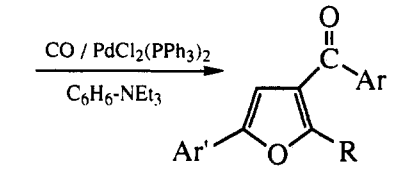
Palladium-catalyzed carbonylation of aryl halides is a useful tool for the preparation of a variety of aromatic carbonyl compounds; the reaction of intermediary aroylpalladium complexes with various nucleophiles and organometallic reagents gives the products.[1] While effective methods for cyclo-carbonylation reactions of haloalkenes including 2-alkenoyl-1-iodobenzenes[2] and allyl acetates[3] to give cyclic ketones have also been developed, intermolecular cross-carbonylation of aryl halides with alkenes and alkynes using palladium catalysts are less common.[4] We report herein our findings that cross-carbonylation of aryl iodides (**1a–c**) or bromothiophene (**1d**) and 1-aryl-2-alkyn-1-ones (**2a–c**) proceeds efficiently in the presence of dichlorobis(triphenylphosphine)palladium ($PdCl_2(PPh_3)_2$) using triethylamine as base to give 2-alkyl-3-aroyl-5-arylfurans (**3–8**) in good yield (eq 1 and Table I).[5]



1 **2**

a; C_6H_5I
b; 4-$CH_3C_6H_4I$
c; 4-ClC_6H_4I
d; 2-Bromothiophene

a; Ar'=C_6H_5, R=n-C_4H_9
b; Ar'=C_6H_5, R=C_2H_5
c; Ar'=4-$CH_3C_6H_4$, R=C_2H_5



(1)

3; Ar=C_6H_5, Ar'=C_6H_5, R=n-C_4H_9
4; Ar=C_6H_5, Ar'=C_6H_5, R=C_2H_5
5; Ar=C_6H_5, Ar'=4-$CH_3C_6H_4$, R=C_2H_5
6; Ar=4-$CH_3C_6H_4$, Ar'=C_6H_5, R=n-C_4H_9
7; Ar=4-ClC_6H_4, Ar'=C_6H_5, R=n-C_4H_9
8; Ar=2-thienyl, Ar'=C_6H_5, R=C_2H_5

(1) Heck, R. F. *Palladium Reagents in Organic Syntheses*; Academic Press: London, 1985.
(2) (a) Tour, J. M.; Negishi, E. *J. Am. Chem. Soc.* **1985**, *107*, 8289. (b) Negishi, E.; Miller, J. A. *J. Am. Chem. Soc.* **1983**, *105*, 6761. (c) Negishi, E.; Wu, G.; Tour, J. M. *Tetrahedron Lett.* **1988**, *51*, 6745. (d) Negishi, E.; Tour, J. M. *Tetrahedron Lett.* **1986**, *40*, 4869. (e) Wu, G.; Shimoyama, I.; Negishi, E. *J. Org. Chem.* **1991**, *56*, 6506.
(3) (a) Matsuzaka, H.; Hiroe, Y.; Iwasaki, Ishii, Y.; M.; Koyasu, Y.; Hidai, M. *J. Org. Chem.* **1988**, *53*, 3832. (b) Iwasaki, M.; Kobayashi, Y.; Li, J. P.; Matsuzaka, H.; Ishii, Y.; Hidai, M. *J. Org. Chem.* **1991**, *56*, 1922.
(4) (a) Sen, A.; Lai, T. W. *J. Am. Chem. Soc.* **1982**, *104*, 3520. (b) Pisano, C.; Consiglio, G.; Sironi, A.; Moret, M. *J. Chem. Soc., Chem. Commun.* **1991**, 421.
(5) The relevant palladium-catalyzed carbonylation reactions of aryl halides with terminal alkynes, 2-methyl-3-butyn-2-ol[6] and 3-aryl-1-propynes,[7] to give 3(2*H*)-furanones and 3-arylidenebutenolides, respectively, have been recently reported. The initial step in these reactions is considered to involve generation of the corresponding 1-aryl-2-propyn-1-ones.[8]

0022-3263/92/1957-4754$03.00/0