for the specific substrate 5-androstene-3,17-dione (7.5×10^9 M^{-1} min^{-1}) shows that **1** is an excellent substrate.[17]

When the trienol **3** was incubated with the isomerase at pH 4.52 (0.0025 M acetate, 1.7% methanol) and 10.0 °C, a first-order decay was observed at $\lambda = 320$ nm with substrate concentrations of 7–15 μM and an enzyme concentration of 4.4×10^{-10} M. The observed rate was approximately 6-fold greater than the buffer-catalyzed rate. Calculation of k_{cat}/K_m gave $(7.2 \pm 0.4) \times 10^9$ M^{-1} min^{-1}. A spectral scan of the product showed that it consisted almost exclusively of 4,7-estradiene-3,17-dione (**2**). Addition of 60 μM of the competitive inhibitor 19-nortestosterone in the presence of 7.2% methanol caused a marked decrease (ca. 90%) in the enzyme-catalyzed rate, as expected for a reaction occurring at the active site.

The ability of the isomerase to catalyze the conversion of trienol **3** to the α,β-unsaturated ketone **2** is strong evidence for the existence of an enol intermediate in the normal catalytic reaction. Furthermore **3** is trapped by the isomerase ca. 3 times faster than **1** is converted to **2**. The observation of a first-order decay with **3**, coupled with the magnitude of the rate constant, suggests that the reaction is diffusion-controlled with the rate-limiting step being association of **3** and the enzyme.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM 33059). We thank Professors Donald J. Creighton and Dale L. Whalen for helpful discussions.

(17) The rapid enzyme-catalyzed isomerization of **1** to **2** is somewhat surprising in view of the report that the isomerase is incapable of utilizing 5(10),9(11)-dien-3ones as substrates.[18]

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Fluoro Ketone Phospholipid Analogues: New Inhibitors of Phospholipase A_2

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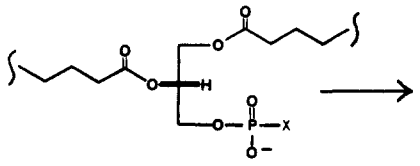
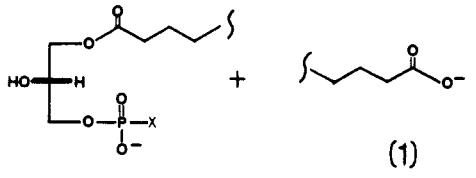
The design of inhibitors of phospholipase A_2 is of recent medicinal interest since this enzyme catalyzes the liberation of arachidonic acid from the phospholipid membrane pool.[1] This represents the rate-determining step in the biosynthesis of prostaglandins, leukotrienes, thromboxanes, and prostacyclin.[2] This enzyme has been isolated from a number of sources and cleaves *sn*-glycero phospholipids specifically at the 2-position as shown in eq 1.[3] Very few rationally designed phospholipase A_2 inhibitors



X = OH, choline, ethanolamine, etc...

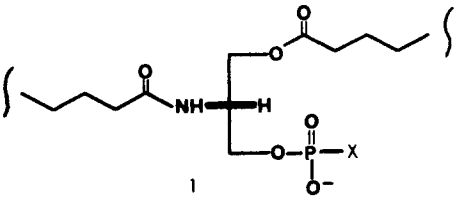


(1)

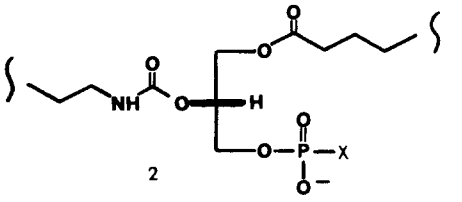
(1) Lapetina, E. G. *Annu. Rep. Med. Chem.* **1984**, *19*, 213–221. Blackwell, G. J.; Flower, R. J. *Br. Med. Bull.* **1983**, *39*, 260–264. Wallach, D. P.; Brown, V. J. R. *Biochem. Pharmacol.* **1981**, *30*, 1315–1324.

(2) Lapetina, E. G. *Trends Pharmaceut. Sci.* **1982**, *3*, 115–118. Cross, P. E. *Annu. Rep. Med. Chem.* **1982**, *17*, 79–88.

have been reported. The most extensively studied have been the phospholipid analogues **1** and **2** in which the enzyme-susceptible



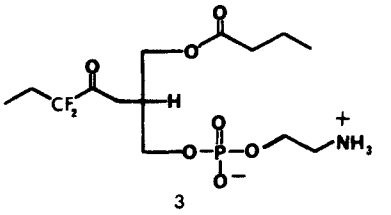
1



2

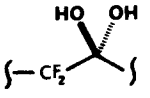
ester linkage has been replaced with an amide[4] or carbamate.[5] The K_i values for these inhibitors are similar in magnitude to the K_m values for the analogous substrates.

We now report a difluoromethylene ketone phospholipid analogue **3** and show that this compound is a tight-binding inhibitor

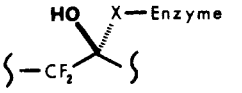


3

of phospholipase A_2. Substrate analogues containing polarized ketones including fluoro ketones and 1,2-diketones have been shown to inhibit hydrolytic enzymes.[6] Since difluoromethylene ketones are predominantly hydrated in aqueous solution, **3** might mimic the tetrahedral intermediate that forms during phospholipase-catalyzed lipolysis and might therefore be considered a transition-state analogue inhibitor.[7] The use of **3** is particularly appealing in the present case since it can bind to the enzyme as either the hydrate **4** or as a hemiketal **5** involving a nucleophile



4



5

present at the active site of the enzyme. This point is important since the structure of the tetrahedral intermediate that forms during phospholipase-catalyzed substrate hydrolysis has not been established, although current evidence favors a mechanism involving the enzyme-assisted attack of a water molecule onto the ester carbonyl group (mimicked by **4**) as opposed to the formation of a covalent acyl-enzyme intermediate[3] (mimicked by **5**).

Compound **3** is an analogue of a short-chain phosphatidylethanolamine and was chosen for this initial study since, unlike long-chain phospholipids, it will have monomeric solution prop-

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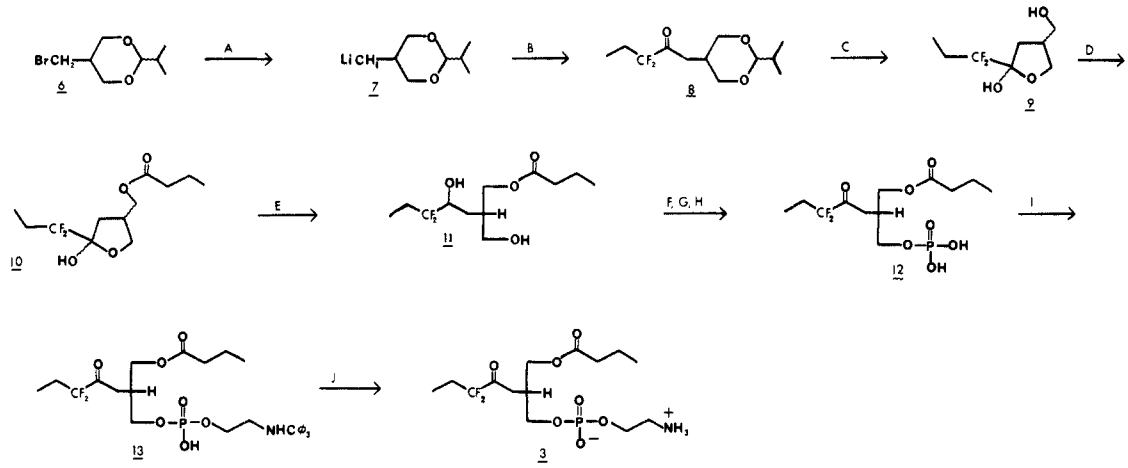
Scheme I[a]



[a] (A) 2.0 equiv of *t*-BuLi, Et_2O, -78 °C; (B) $CH_3CH_2CF_2CO_2CH_3$, -78 to 25 °C; (C) 1 N HCl, dioxane, 25 °C; (D) 1 equiv of butyryl chloride, 1.0 equiv of pyridine, $CHCl_3$, 0 °C; (E) $NaBH_4$, CH_3CH_2OH, 25 °C; (F) 3.0 equiv of $ClPO(OPh)_2$, pyridine, 25 °C; (G) 12.0 equiv of CrO_3·(pyridine)$_2$, CH_2Cl_2, 25 °C; (H) PtO_2, 1 atm of H_2, CH_3OH, 25 °C; (I) trityl-$NHCH_2CH_2OH$, triisopropylbenzenesulfonyl chloride, $CHCl_3$, 25 °C; (J) 25% CF_3CO_2H in CH_2Cl_2, 0 °C.

erties. Thus the interaction of **3** with the enzyme will be interpretable in terms of the standard treatment of enzyme inhibition kinetics in homogenous systems.

The preparation of **3** in racemic form is outlined in Scheme I. Bromide **6**[8] was converted to organolithium **7** and treated with methyl 2,2-difluorobutyrate to give difluoromethylene ketone **8** (35%). Cleavage of **8** under acidic conditions gave cyclic hemiketal **9** (100%). Compound **9** was selectively acylated with butyrl chloride to give **10** (90%) followed by hydride reduction to diol **11** (66%). Selective phosphorylation of the more nucleophilic, nonfluorinated alcohol with diphenyl chlorophosphate followed by oxidation of the fluorinated alcohol with CrO_3·(pyridine)$_2$ and finally hydrogenolysis of the phosphate triester gave **12** in 75% overall yield. Phosphatidic acid analogue **12** was condensed with *N*-tritylethanolamine in the presence of triisopropylbenzenesulfonyl chloride to **13** (45%). Detritylation of **13** with trifluoroacetic acid gave product **3** (90%).

Phosphatidylethanolamine analogue **3** was tested as an inhibitor of snake venom phospholipase A_2. Enzymatic activity toward the monomerically dispersed substrate 1,2-dibutyryl-*sn*-glycero-3-phosphatidylcholine[5] was measured by titration of the liberated butyric acid in a pH state.[9] Compound **3** was found to be a simple competitive inhibitor of phospholipase A_2 with a K_i = 50 μM. It is significant that **3** binds approximately 300-fold tighter than the analogous substrate (1,2-dibutyryl-*sn*-glycero-3-phosphatidylethanolamine, K_m = 14 mM). Furthermore, **3** is bound considerably tighter than amides **1** or carbamates **2**. The ability of **3** to act as a tight-binding inhibitor may be due to its unique ability to form tetrahedral species. The mixture of difluoromethylene alcohols **14**, prepared by borohydride reduction of **13** followed by detritylation, was also inhibitory (K_i = 200 μM). Although somewhat less inhibitory than **3**, **14** is significantly more potent than **1** and **2**. Taken together, these results suggest that **3** and **14** are mimics of a tetrahedral species formed by the attack of water onto the carbonyl group of a phospholipid substrate.

The use of the difluoromethylene ketone unit as an isosteric replacement of ester linkages in phospholipids appears to be an effective strategy for the inhibition of lipolytic enzymes. The determination of the mode of binding of **3** to the enzyme (i.e., hydrate **4** or hemiketal **5**) will have important implications for the catalytic mechanism of enzymatic lipolysis. We hope to extend these results to the preparation of long-chain difluoromethylene ketone phospholipid analogues in order to study the inhibition in heterogenous, substrate/inhibitor aggregates.

Acknowledgment. The generous support of this research by the National Institutes of Health (HL-36235) and Merck and helpful discussions with Professor Paul Hopkins are gratefully acknowledged.

Supplementary Material Available: Experimental procedures and physical data for the preparation of all new compounds and enzyme kinetic data (7 pages). Ordering information is given on any current masthead page.

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(9) Enzyme assays were carried out under Argon at 37 °C in 1 mL of 10 mM $CaCl_2$ containing substrate (1-10 mM) and inhibitors (0.05-0.5 mM). Reactions were started by the addition of phospholipase A_2 (*Naja naja* venom, typically 25 units/mL). Reactions were maintained at pH 8.0 in a pH stat by the addition of 0.01 N NaOH.

Ring Opening of Cyclopropylidenes to Allenes: Reactions with Bifurcating Transition Regions, Free Internal Motions, Steric Hindrances, and Long-Range Dipolar Interactions

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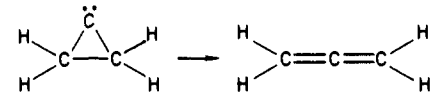
The mechanism of the ring opening of cyclopropylidene to allene,



the prototype bond fission of a cyclic carbene due to ring strain, has implications for many organic reactions. Substituted species only have been examined so far experimentally and these reactions are stereospecific.[1-6] Theoretical work has been limited to the

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