Hemicholinium and Related Lipids: Inhibitors of Protein Kinase C

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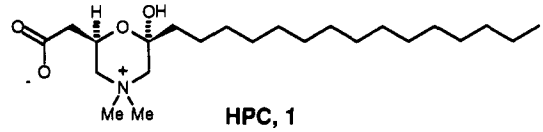
Palmitoylcarnitine, an intermediate of long-chain fatty acid metabolism, competitively inhibits protein kinase C (PKC) when activated by the phospholipid cofactor.[1,2] PKC is a critical component of cellular signal transduction,[3,4] and it is the receptor for phorbol esters,[5] which promote tumor growth. Consequently, interest is intense in developing selective and potent inhibitors.[6] In this study, we test hemipalmitoylcarnitinium[7,8] (HPC, 1), which



HPC, 1

potently inhibits carnitine palmitoyltransferase, as an inhibitor of PKC. Sphingoid bases,[9] *N,N,N*-trimethylsphingosine,[10] and other quaternary ammonium lipids,[11] e.g., palmitoylcholine, hexadecyltrimethylammonium, and phosphatidylcholine, inhibit PKC. For a comparison with HPC and palmitoylcarnitine, we have prepared conformationally constrained choline analogues, viz., hemicholinium lipids (HnC, 2) and related compounds, 3 and 4 (Figure 1) to profile the structural requirements for optimal inhibition.

Chemistry. 1-Bromo-2-alkanones mixed with 2-(*N,N*-dimethylamino)ethanol in nitromethane produced crystalline hemicholiniums [2, n = 10, 12–18] with yields ranging from 20 to 69% (Figure 1). The reaction of 1-alkenes with NBS, $FeCl_3$, CrO_3, and H_2SO_4 in aqueous acetone[12] produced the 1-bromo-2-alkanones in yields ranging from 30 to 55%. Condensation of 3-(*N,N*-dimethylamino)propanol with 1-bromo-2-heptadecanone produced 3. Recently, we corrected[13] an older report[14] of seven-membered hemiketals forming in such reactions. Reaction of H15C with acidic methanol produced 4. All products were characterized by ^1H NMR, ^{13}C NMR, IR, mass spectroscopy, and elemental analyses.

The ^1H NMR spectra of 2 in $CDCl_3$ suggest that approximately 3% is the open (keto) form, eq 1. A triplet at 2.53 ppm ($COCH_2R$) and a singlet at 5.18 ppm (NCH_2CO) indicate the open form. In DMSO-d_6 or D_2O, these peaks are absent. The ^1H NMR spectra reveal separate signals for axial and equatorial methyl groups on nitrogen. The signals for the methylenes next to oxygen and nitrogen contain lines for each hydrogen, which we assign to one conformation of the ring—axial hydroxy. Our recent kinetic studies[15] of three aryl derivatives have quantified the dynamic ring–chain tautomerism that others had demonstrated over 40 years ago.[16] From these studies, we estimate that the equilibrium constant for eq 1 is approximately 100. In the crystalline state, these compounds exist in the closed form.

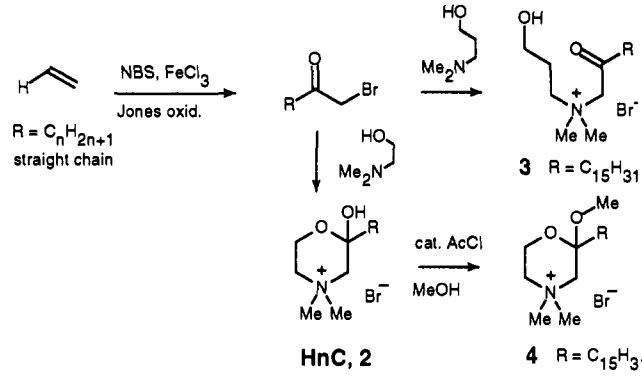
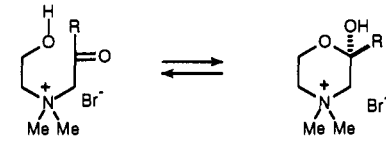


Figure 1. Synthetic scheme for the preparation of 2–4.



Biochemistry. We evaluated compounds as inhibitors of mostly purified rat brain PKC in a histone kinase assay, which was a modification of that described previously.[17] Compounds 2–4, dissolved in DMSO, were included in the assay at concentrations ranging from 0.16 to 160 μg/mL, with a final concentration of 2.4% DMSO. HPC was dissolved in water and thus, had no DMSO in the assay. The assay tubes, which contained tetradecylphorbol acetate (TPA, 3 nM), phosphatidylserine (PS, 8 μg/mL), EGTA (1 mM), glycerol (4%), and rat brain PKC in 20 mM Tris-Cl buffer, pH 7.4, were placed on ice. The reactions were started by adding a mixture of [γ-^{32}P]ATP (20 μM), $MgCl_2$ (10 mM), *p*-nitrophenyl phosphate (5 mM), and lysine-rich histone (240 μg/mL). After incubation at 30 °C for 10 min, an aliquot was removed and radioactivity determined. The IC_{50} for each agent was determined as the concentration that is calculated to inhibit PKC activity by 50%. In each experiment two control agents (staurosporine and trifluoperazine) were included to assure assay quality. This assay condition (1 mM EGTA, no calcium, 3 nM TPA, 8 μg/mL PS) resulted in a 4- to 7-fold activation of PKC above that activity detected without inclusion of TPA. Inclusion of 1.6 mM calcium without TPA results in 7- to 10-fold activation above PS only. These controls were included in each experiment. Finally, no-enzyme blanks were done and their value subtracted from all assay results before any further calculations.

Results and Discussion. The data (Table I) reveal that a chain length of 12 is needed for good inhibition; H10C inhibits approximately 10-fold worse than the longer chain analogues. H12C–H18C, the open chain analogue 3, and the methyl ketal 4 show similar activity. These cationic lipids inhibit better than the zwitterions, DL-palmitoylcarnitine and HPC. A carboxylato group detracts from inhibition. HPC, a cyclic isomer of DL-palmitoylcarnitine, inhibits worse than the ester.

PKC inhibitors can be classified into two groups depending on whether they interact with the protein at the catalytic site, or at the diacylglycerol or phospholipid regulatory sites. Calphostin[18] and sphingosine would interact at a regulatory site while H-7[19] and staurosporine[20] would interact at the catalytic site. Our inhibitors probably

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Table I. Inhibition of Rat Brain PKC by Hemicholinium Lipids and Related Compounds[a]

compound	IC_{50}, μM	compound	IC_{50}, μM
H18C	6.5, 14.9		
H17C	7.1	HPC[b]	49.6, 32.6, 47.4
H16C	7.6	4	3.9, 8.2
H15C	7.3, 5.1, 7.5	3	6.9, 12
H14C	7.6, 7.6	DL-palmitoylcarnitine	15.8, 20.4
H13C	12, 6.6		
H12C	13, 12		
H10C	170, 118		

[a] Values listed represent the mean of duplicate runs. Multiple values for compounds represent assays at different times with different enzyme preparations. [b] Sample was dissolved in water. No DMSO in the assay.

interact at the phospholipid regulatory site, where sphingosines and palmitoylcarnitine interact. Chain-length data suggest that 16 is optimal for acylcarnitines,[2] but 18 is optimal for sphingosines.[9] Our data on hemicholiniums show very little chain-length discrimination. Our data include odd-chain compounds, but we have found no "odd–even" effects.

The potency of our inhibitors attests to the design of hemicholiniums as conformationally constrained analogues of biological quaternary ammonium ions. We envision even more potent inhibitors of PKC by elaboration of the ring. Our immediate goals are to evaluate the selectivity of the compounds with various kinases and to modify the structures to increase potency. Our ultimate goal is to develop a selective, potent inhibitor of PKC that will have therapeutic value.

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Supplementary Material Available: The procedures for the preparation of 1-bromo-2-alkanones, **2** (n = 10, 12–18), **3** (n = 15), and **4** (n = 15) and the procedure for assay of PKC inhibition (6 pages). Ordering information is given on any current masthead page.

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