Chemical Stability of Esters of Nicotinic Acid Intended for Pulmonary Administration by Liquid Ventilation

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Purpose. It has been suggested that fluorocarbon liquid may be a unique vehicle for the delivery of drugs directly to the acutely injured lung. A prodrug approach was used as a means of enhancing the solubility of a model drug (nicotinic acid) in the fluorocarbon. The solubility, the chemical stability of the putative prodrugs, and the sensitivity to enzymatic hydrolysis was investigated.

Methods. The solubility of each nicotinic acid ester was determined in buffer as a function of pH and in perflubron. The octanol/buffer partition coefficient was determined at pH 7.4. The chemical stability of the putative prodrugs was determined as a function of pH, temperature, buffer content, and ionic strength. In addition, sensitivity of the esters to enzymatic degradation was evaluated.

Results. Compared with nicotinic acid, the solubility in perflubron of the esters was significantly enhanced. In aqueous buffers, the esters exhibited pseudo-first order degradation kinetics, with both acid and base catalyzed loss. Studies of the fluorobutyl ester indicate quantitative loss of the putative prodrug and release of the parent nicotinic acid. Porcine esterase accelerated the loss of fluorobutyl ester by a factor of over 200 compared with chemical hydrolysis at pH 7.4.

Conclusions. The properties of the fluorinated esters suggest that they may be suitable candidates for further testing as possible prodrugs of nicotinic acid based upon higher solubility in perflubron, rapid release of the parent drug after simple hydrolysis, and sensitivity to the presence of a model esterase enzyme.

KEY WORDS: prodrugs; stability; nicotinic acid ester; solubility; PFOB.

INTRODUCTION

Partial liquid ventilation is a technique for delivering oxygen and removing carbon dioxide via the pulmonary tree through the use of perfluorocarbon liquids (PFCs). This technique involves instilling PFCs directly into the lungs in conjunction with a conventional gas ventilator. The technique has potential clinical advantages in the treatment of acute lung injury or adult respiratory distress syndrome (1). Perflubron ($C_8F_{17}Br$; PFOB) is an excellent solvent for oxygen and carbon dioxide, dissolving as much as 20 times the amount of oxygen and more than three times as much carbon dioxide as water (1). It is chemically inert, can be autoclaved, and remains stable indefinitely if stored at room temperature. It is not soluble in water or in lipids, nor are most lipids or water soluble in it (2).

Delivering drugs directly into the lungs may have great clinical advantages for the treatment of acute lung diseases. However, delivering drugs into the diseased lung is made difficult by physiologic responses, such as airway obstruction by edematous fluid or pulmonary vascular shunting (3). We (4), along with several other groups (5–7), have hypothesized that if drugs could be successfully dissolved in PFOB, drug delivery to the acutely diseased regions of the lung during liquid ventilation could potentially be greatly enhanced.

One of the strongest barriers to the clinical application of PFOB as a solvent for the administration of drugs to the diseased lung is the extremely poor solvent properties of PFOB (4). Although an excellent solvent for oxygen and carbon dioxide, perfluorocarbon liquids are notoriously poor solvents for molecules typical of drugs (8,9). Several attempts at delivery to the lung by crude suspensions or emulsions have resulted in concerns of dose-to-dose reproducibility (10–12). A variety of methods are currently under investigation to enhance the deliverability of drugs in PFOB, including a spray-drying process to form hollow porous microparticles of drug for suspension in PFOB (7). Williams *et al.* (13) demonstrated enhanced solubility of phenol and phenol derivatives in PFOB in the presence of hydrophobic complexing agents capable of acting as hydrogen bond donors or acceptors.

The prodrug approach is another technique by which solubility in a hydrophobic phase may be enhanced (14–16). The prodrug molecule has little to no pharmacological activity and must convert back to the parent compound to elicit a biologic response. The conversion to the parent compound *in vivo* can be either catalyzed by an enzyme, or by other means (such as simple acid or base hydrolysis) in an aqueous environment. The goal of the present study was to use a prodrug approach to enhance the solubility of a model drug in neat PFOB. The kinetics of the degradation of the model compounds in an aqueous environment will be examined for effects due to pH, temperature, and buffer components. In this study, nicotinic acid was used as a model polar drug. Although nicotinic acid is poorly soluble in PFOB, attachment of a fluorinated carbon chain to form a putative prodrug is proposed as a means to enhance solubility in PFOB. Nicotinic acid plays an important role as a precursor of cofactors such as nicotinamide adenine dinucleotide (NAD) and may reduce lung injury induced by paraquat (17), bleomycin (18), and endotoxin (19).

MATERIALS AND METHODS

Materials

Nicotinic acid, butyl nicotinate (compound **1**), anhydrous dichloromethane, dicyclohexylcarbodiiimide, dimethylaminopyridine, and perfluorooctyl bromide were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). 1*H*, 1*H*-perfluorobutanol, 1*H*, 1*H,* 2*H*, 2*H*–perfluorohexanol and 1*H*, 1*H,* 2*H*, 2*H*–perfluorooctanol were obtained from Lancaster (Windham, NH). 1-Octanol was obtained from Fisher Scientific, (Fair Lawn, NJ, USA). Acetonitrile, n-hexane and 2-propanol were high-performance liquid chromatography (HPLC) grade and were also purchased from Fisher Scien-

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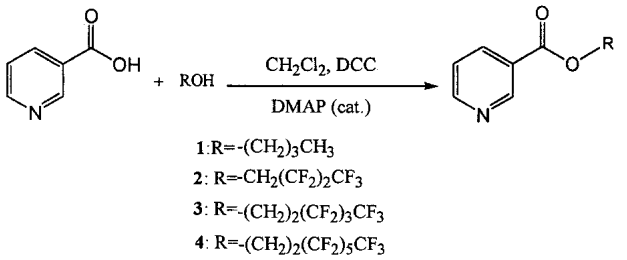

Fig. 1 Synthesis of fluorinated nicotinic acid ester prodrugs.

tific. All chemicals for preparation of buffers were analytical grade. Porcine liver esterase (EC 3.1.1.) was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Synthesis of Fluorinated Nicotinic Acid Esters

Comound **1** was commercially available and purified by Kugelrohr distillation prior to use. Fluorinated nicotinic acid esters **2-4** were synthesized by following the procedure described by Morishita *et al.* (20) (Fig. 1). In short, nicotinic acid (15 mmol) was mixed with the corresponding alcohol (10 mmol) in anhydrous dichoromethane (CH_2Cl_2). Successively, dicyclohexylcarbodiimide (DCC) (11 mmol) and dimethylaminopyridine (DMAP) (1.2 mmol) were added to the solution. The mixture was stirred at room temperature overnight. The product was purified by Kugelrohr distillation (oven temperature approximately 70°C, ambient temperature approximately 25°C, rotation 60 rpm). Typical yields were greater than 80%. The purity of the esters was determined by both GC/MS and HPLC. All synthesized esters were at least 99% pure. The physical properties of **1-4** are shown in Table I.

Nicotinic Acid 1H, 1H-Perfluorobutyl Ester **2**

^{1}H-NMR (300 MHz, [d6]acetone) δ 5.10 (-O-C*H*$_2$CF$_2$-, 2H, t, $^3J_{HF}$ = 13.5 Hz, t, $^5J_{HF}$ = 1.2 Hz), 7.58 (-C*H*-, 1H, d, J = 8.1 Hz, d, J = 4.8 Hz), 8.35 (-C*H*-, 1H, d, J = 8.1 Hz, "t", J = 1.8 Hz), 8.85 (-C*H*-, 1H, d, J = 4.8 Hz, d, J = 1.5 Hz), 9.16 (-C*H*-, 1H, d, J = 2.1 Hz). - ^{19}F-NMR (282 MHz, [d6]acetone) δ -80.71 (-CF$_3$, 3F), -119.70 (-CF$_2$-, 2F), -127.11 (-CF$_2$-, 2F). - ^{13}C-NMR (75 MHz, [d6]acetone) δ 60.59 (T, t, $^2J_{CF}$ = 27.8 Hz), 124.71 (D), 125.44 (S), 137.92 (D), 151.49 (D), 155.33 (D), 164.42 (S). - IR (KBr) [cm^{-1}]: 2971, 1746 (ν(C=O)), 1282, 1230, 1184, 1130. - GC/MS m/z (relative intensity, %): 305 (6, $C_{10}H_6F_7NO_2^{\bullet+}$), 106 (100), 78 (64), 51 (48). Anal. Calcd. for $C_{10}H_6F_7NO_2$: C, 39.34; H, 1.98; N, 4.59. Found: C, 39.25; H, 2.02; N, 4.57.

Nicotinic acid 1H, 1H, 2H, 2H-Perfluorohexyl Ester **3**

^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.60 (-O-CH$_2$C*H*$_2$CF$_2$-, 2H, t, $^3J_{HF}$ = 18.4 Hz, t, $^3J_{HH}$ = 6.4 Hz, t, $^4J_{HF}$ = 1.6 Hz), 4.65 (-O-C*H*$_2$CH$_2$CF$_2$-, 2H, t, $^3J_{HH}$ = 6.4 Hz), 7.54 (-C*H*-, 1H, d, J = 8.0 Hz, d, J = 4.8 Hz), 8.27 (-C*H*-, 1H, d, J = 8.0 Hz, "t", J = 2.0 Hz), 8.78 (-C*H*-, 1H, d, J = 4.8 Hz, d, J = 1.6 Hz), 9.20 (-C*H*-, 1H, d, J = 2.0 Hz). ^{19}F-NMR (376 MHz, $CDCl_3$) δ -80.51 (-CF$_3$, 3F), -114.21 (-CF$_2$-, 2F), -124.93 (-CF$_2$-, 2F), -126.45 (-CF$_2$-, 2F). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 30.45 (T, t, $^2J_{CF}$ = 21.3 Hz), 57.17 (T, t, $^3J_{CF}$ = 4.6 Hz), 123.36 (D), 125.41 (S), 137.10 (D), 150.98 (D), 153.81 (D), 164.82 (S). IR (KBr) [cm^{-1}]: 2978, 1731 (ν(C=O)), 1283, 1220, 1133. - GC/MS m/z (relative intensity, %): 369 (3, $C_{12}H_8F_9NO_2^{\bullet+}$), 123 (15), 106 (100), 78 (52), 51 (43). Anal. Calcd. for $C_{12}H_8F_9NO_2$: C, 39.02; H, 2.18; N, 3.80. Found: C, 39.24; H, 2.16; N, 3.88.

Nicotinic Acid 1H, 1H, 2H, 2H-Perfluorooctyl Ester **4**

^{1}H-NMR (300 MHz, [d6]acetone) δ 2.86 (-O-CH$_2$C*H*$_2$CF$_2$-, 2H, t, $^3J_{HF}$ = 18.9 Hz, t, $^3J_{HH}$ = 6.2 Hz), 4.71 (-O-C*H*$_2$CH$_2$CF$_2$-, 2H, t, $^3J_{HH}$ = 6.2 Hz), 7.54 (-C*H*-, 1H, d, J = 8.1 Hz, d, J = 4.8 Hz), 8.30 (-C*H*-, 1H, d, J = 8.1 Hz, "t", J = 1.5 Hz), 8.80 (-C*H*-, 1H, d, J = 5.1 Hz, d, J = 1.8 Hz), 9.14 (-C*H*-, 1H, d, J = 1.5 Hz). - ^{19}F-NMR (282 MHz, [d6]acetone) δ -80.60 (-CF$_3$, 3F), -112.81 (-CF$_2$-, 2F), -121.32 (-CF$_2$-, 2F), -122.33 (-CF$_2$-, 2F), -125.68 (-CF$_2$-, 2F). - ^{13}C-NMR (75 MHz, [d6]acetone) δ 30.95 (T, t, $^2J_{CF}$ = 21.0 Hz), 58.03 (T, t, $^3J_{CF}$ = 3.8 Hz), 124.50 (D), 126.59 (S), 137.61 (D), 151.36 (D), 154.71 (D), 165.45 (S). - IR (KBr) [cm^{-1}]: 2978, 1734 (ν(C=O)), 1283, 1235, 1196, 1143. - GC/MS m/z (relative intensity, %): 469 (2, $C_{14}H_8F_{13}NO_2^{\bullet+}$), 123 (18), 106 (100), 78 (43), 51 (34). Anal. Calcd. for $C_{14}H_8F_{13}NO_2$: C, 35.82; H, 1.72; N, 2.99. Found: C, 36.02; H, 1.73; N, 3.08.

Differential Scanning Calorimetry (DSC)

The melting point determination was performed on a differential scanning calorimeter (TA 2920 DSC, TA instruments, New Castle, DE, USA). Approximately 2 mg of samples were weighed into the DSC aluminum pans. An empty pan was used as reference. The samples were cooled to –30°C at the scan rate of 3°C/min and equilibrated at –30°C for 30 min. Then the samples were heated at a scan rate of 5°C/min from –30°C to 60°C. All experiments were conducted in triplicate.

HPLC

The HPLC system consisted of a Model P2000 pump with a Model UV1000 UV detector (both from Thermo Sepa-

Table I.

Esters	Aqueous solubility (μg/mL)				
	pH 2	pH 4	pH 5	pH 6	S_o[b]
1	4652.6 ± 17.3	2148.7 ± 271	1950.2 ± 9.0	1934.0 ± 19.5	1904.0 ± 17.0
2	1267.0 ± 0.5	148.3 ± 0.5	134.4 ± 0.7	132.8 ± 0.6	132.0 ± 0.2
3	420.1 ± 5.5	13.8 ± 1.2	10.8 ± 0.2	10.6 ± 0.2	10.3 ± 0.2
4	3.9 ± 0.1	[a]	—[a]	—[a]	—[a]

[a] Not determined.
[b] Fitted values from Eq. (1).

ration Products [TSP], San Jose, CA, USA), a Model AS1000 autosampler (TSP), SCM1000 vacuum membrane degasser (TSP) and Dell Optiplex computer. The UV detector was set at 262 nm (21). The injection volume was 20 μL. For the determination of physicochemical properties of nicotinic acid and **1–4** from aqueous solution, a Supelcosil LC-18-S reverse phase column (250 × 4.6 mm, 5μm) and a C-18 precolumn (20 × 4.0 mm, 5 μm) from Supelcol (Bellefonete, PA, USA) were used. The flow rate of mobile phase was 1.5 mL/min. A Dynamax Microsorb silica gel column from Rainin Instrument Co. Inc., (Woburn, MA, USA) was used for the determination of the solubility of compounds **1–4** and nicotinic acid in PFOB. The mobile phase was a mixture of n-hexane and 2-propanol (50:1, v/v). The flow rate was 2 mL/min. This change in mobile phase was necessary due to the extremely hydrophobic properties of PFOB. For all compounds, and in both assays, the typical limit of detection was 0.1 μg/mL.

Solubility Determination

Solubility of each ester in water was determined as a function of pH. The buffers used were acetate (pH 4–5) and phosphate (pH 6-8) solutions at a total concentration of 0.02 M. For pH 2, hydrochloric acid was used. A constant ionic strength of 0.1 was maintained for each buffer by adding the calculated amount of sodium chloride. Excess amounts of **1–4** were added to 5 mL of the buffers in 10 mL vials. For determination of solubilities in PFOB, samples were prepared in 2 mL HPLC vials by adding 500 mg of PFOB and excess amounts of nicotinic acid or compounds **1–4**. In all cases, the vials containing the mixture were capped, vortexed, and sonicated prior to placing in a temperature-controlled shaking water bath at 25°C. The samples were shaken at a rate of 120 rpm for 12 h. The samples were then centrifuged and the clear supernatants were diluted and analyzed by HPLC.

Determination of Apparent Partition Coefficients

The apparent partition coefficients (log P_{app}) of nicotinic acid and compounds **1–4** were determined in 1-octanol/ phosphate buffer at 25°C. The ionic strength of the 0.02 M phosphate buffer (physiologic pH 7.4) was adjusted to a value of 0.1 by adding a calculated amount of sodium chloride. The 1-octanol was saturated with phosphate buffer for 24 h by stirring vigorously prior to use. A known amount of the ester was added to 1-octanol along with a suitable volume of phosphate buffer (volume ratio 1:50 for **1** and 1:100 for **2–4**). The mixture was shaken at a rate of 120 rpm for 12 h. After shaking, the phases were separated by centrifugation. For **1–3**, the concentrations of compounds in the respective phases before and after partition were determined by HPLC. For **4**, only the concentration in the octanol was determined with the concentration in the aqueous phase calculated by difference. All experiments were performed in triplicate.

Degradation Kinetics

Kinetic Measurements

The degradation of esters **1–4** was investigated in different buffer solutions in the pH range of 2–8 at 60°C. The buffers used were acetate (pH 4–5) and phosphate (pH 6–8) at a total buffer concentration of 0.02M. For pH 2, hydrochloric acid was used. A constant ionic strength of 0.1 for each buffer was maintained by adding a calculated amount of sodium chloride. The pH values of buffer solutions were adjusted with 0.1 M hydrochloric acid or 0.1 M sodium hydroxide. Due to the poor aqueous solubility of some of the esters, acetone was added as a cosolvent. An appropriate amount of compound was dissolved in buffer and acetone to give a final concentration of 200 μg/mL in 5% acetone for **1–2** and 50 μg/mL in 15% acetone for **3–4**. The solutions were placed in a temperature controlled water bath at 60°C. The samples were sealed in crimp-cap vials and weights before and after incubation revealed no loss of solvent. The kinetic studies were carried out over a time period of at least three half-lives. At appropriate time intervals, samples were withdrawn and quantitatively analyzed for remaining ester and parent nicotinic acid by HPLC. All experiments were carried out in triplicate. To verify the formation of nicotinic acid from **2**, an aliquot of a degrading solution was extracted with ethyl acetate. The extract was brought to dryness, derivatized with trimethylsilane and analyzed by GS/MS (performed at the Mass Spectrometry Facility of the University of Kentucky).

Effect of Temperature

The degradation of **1–4** was monitored in a 0.02 M phosphate at pH 7.4 with ionic strength of 0.1 with 5% acetone for **1–2** and 15% acetone for **3–4**. The experiments were carried out at 50, 60, 70, and 80°C.

Effect of Ionic Strength

The influence of ionic strength on the degradation of **1** and **2** was investigated by adding various amounts of sodium chloride. The studies were conducted in acetate (pH 5) and phosphate (pH 7.4) buffer of 0.02M concentrations with 5% acetone and the ionic strength values ranging from 0.05–0.5 at 60°C.

Effect of Buffer Concentration

The effect of buffer on the degradation was examined with phosphate buffer at pH 6 and 7.4 at concentrations 0.02, 0.04, 0.08m and 0.12M with 5% acetone. Degradation of **2** was conducted at 80°C, and ionic strength of 0.5.

Effect of Solvent

The effect of solvent on the degradation rate was determined by comparing degradation rate constants gathered from 5% and 30% acetone for **1**, **2** and 15% and 30% acetone for **3** in 0.02 M phosphate buffer at pH 8 with ionic strength of 0.1. This experiment was conducted at 60°C.

Enzymatic Stability

In vitro enzymatic hydrolysis of esters **1–4** was determined using porcine liver esterase. All experiments were carried out in 0.02 M phosphate buffer at pH 7.4 and ionic strength of 0.1 at 37°C. The esterase was diluted with phosphate buffer with 5% acetone for **1–2** and 15% acetone for **3–4** and preincubated 60 min at 37°C prior to use. Compounds **1** and **2** were present at 200 μg/mL while **3** and **4** were present at 50 micrograms/mL. Samples were sealed in crimp-cap vials and showed no loss of mass during the incubation.

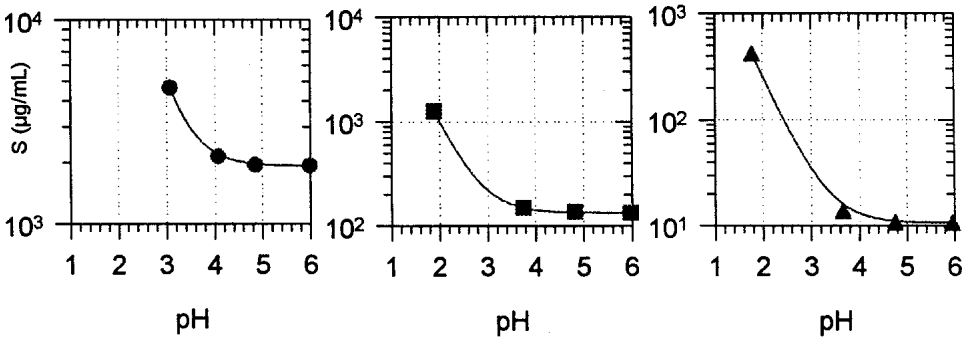

Fig. 2 Aqueous solubilities (S) of prodrugs (**1,** ●; **2,** ■; **3,** ▲) as a function of pH at 25°C. Each point was the average of three determinations. The standard deviations are smaller than the symbols (listed in Table II).

Solutions of **1**–**4** were prepared in phosphate buffer and porcine esterase solution was added to achieve the enzymatic concentration of 100 U/L. Two hundred and fifty microliters of the samples were withdrawn at appropriate time intervals. The reaction was quenched and diluted with addition of 750 μL of cold acetonitrile followed by vortexing for 30 s. After centrifugation at 14,000 rpm for 5 min, the supernatant was analyzed by HPLC. Pseudo-first order rate constants for enzymatic hydrolysis were determined from linear slopes obtained by plotting the logarithm of residual ester concentration vs. time. All experiments were carried out in triplicate. Durrer has reported that 1% acetonitrile did not influence esterase activity (22). The extent to which 15% acetone influences the enzyme activity is not known, but it highly likely to have some effect. Our intention is to compare the observed rate of hydrolysis of the prodrugs in the presence and absence of a model esterase enzyme and not to draw direct conclusions about the rate of reaction expected *in vivo*.

RESULTS AND DISCUSSION

Solubility in Aqueous Solution

The structures of the fluorinated nicotinic acid esters **1**–**4** are shown in Fig. 1. In the present study, the putative prodrugs were designed to be soluble in the extremely hydrophobic solvent PFOB. In addition to solubility in PFOB, the esters also exhibit some solubility in water (Table I). The solubilities of **1**–**3** in different pH buffers are shown in Fig. 2. At pH 2, the compounds, acting as weak bases, exhibit higher aqueous solubilities than at more alkaline pH values (Table I). At constant pH, the aqueous solubilities of the fluorinated compounds decreased as the length of the fluorinated side chain increased. This observation can be attributed to the increase of lipophilicity as the chain length increases. At pH = 2, compound **4** still exhibited extremely low aqueous solubility (Table I). The solubility of **4** at higher pH values could not be determined as the value fell below the sensitivity of the assay. Figure 2 shows the solubility behavior of **1**, **2**, and **3** as a function of pH. The lines in Fig. 2 are obtained through curve fitting the solubility data to Eq. (1):

$$S_t = S_0 (1 + [H^+] / K_a) \quad (1)$$

where S_t is the total measured solubility of the proposed prodrug, $[H^+]$ is the hydrogen ion in molar concentration, K_a is the acid dissociation constant for the protonated amine, and S_0 is the intrinsic solubility of the free base. Estimates of S_0 and pK_a were obtained through curve fitting.

Lowering the pH is a common method used for improving the solubility of weakly basic drugs through ionization (23) and Equation 1 shows how K_a can modulate this effect. The apparent pK_a of **2** is significantly smaller than that of **1** (Table II). The fluorine atoms have a stronger electron withdrawing effect than hydrogen atoms even through a single methylene group. Although there is a distance of several atoms between the side chain and the pyridine nitrogen, the electron withdrawing effect can still be observed (13). Surprisingly, the apparent pK_a values of **1** and **3** are similar despite the fact that **1** has no fluorines and **3** has two methylene groups between the fluorocarbon chain and the ester group. For **3**, perhaps the added distance between the fluorocarbon

Table II.

Ester	Mw	mp (°C)	pK_a[e]	$\log P_{app}$[d]	Solubility in perflubron (M)
1[a]	179.22	<–30	3.222 ± 0.003	3.91 ± 0.01	0.17 ± 0.01
2	305.15	3.59 ± 0.04	2.825 ± 0.002	4.97 ± 0.06	0.62 ± 0.09
3	369.24	–2.80 ± 0.18	3.366 ± 0.007	5.43 ± 0.10	0.84 ± 0.01
4	469.20	21.54 ± 0.21	[b]	[b]	[c]

[a] Refer to Fig. 1 for the chemical structures and names.
[b] Not determined because of poor water solubility.
[c] Miscible in all proportions.
[d] P_{app} is an apparent partition coefficient between 1-octanol and phosphate buffer (pH 7.4) at 25°C. The value for the parent nicotinic acid is –2.47.
[e] K_a is the apparent ionization constant, uncorrected for ionic strength.

chain and the ester decreases the electron withdrawing effect of fluorine atoms on the pyridine base. Another possibility might be enhanced self-association of the fluorocarbon derivative (**3**) compared to hydrocarbon analog (**1**) (9) resulting in an apparent increase in the pK_a compared to the monomer (24). The pK_a values for n-butyl nicotinate **1** have been reported to be 3.23 and 3.15 by spectrophotometry at room temperature and from partition experiment at 37°C, respectively (15). In the present studies, the pK_a values were obtained by fitting the experimental data to Equation 1. For compound **1**, our apparent pK_a value of 3.22 ± 0.003 compares favorably to the results of Larsen *et al.* (15).

Solubility in PFOB

The data in Table II show that the fluorinated compounds **2–4** have solubilities in PFOB higher than that of the non-fluorinated compound **1**. However, we were surprised to find that even **1** has some ability to dissolve in PFOB. On the other hand, nicotinic acid is insoluble in PFOB. Compound **2** exhibits a 3.5 fold greater solubility in PFOB than **1**. Compound **4** is miscible in all proportions with PFOB and appears to be much more soluble than **3**. Why there is such a substantial change in the solubility results of **3** compared to **4** is unknown, but is typical of solubility behavior of other compounds in perfluorocarbon liquids (8,9). These results show that fluorination of the side chain does enhance the solubility of **2–4** in PFOB.

Apparent Partition Coefficients

The apparent partition coefficients of **1–4** were determined in a 1-octanol-pH 7.4 buffer system at 25°C. All the compounds are markedly more hydrophobic than the parent drug, as illustrated by the log P_{app} values (Table II). The nonfluorinated butyl nicotinate **1** is less lipophilic than the fluorinated butyl nicotinate **2**. In an earlier study of **1**, Lee *et al.* (25) reported a log $P_{oct/w}$ of 2.47 in octanol-water at 32°C. The difference in result of the present study from that of Lee *et al.* may be due to the temperature differences in the experimental conditions.

Chemical Stability

Order of Reactions

Figure 3 shows the loss of **1–4** in phosphate buffer solution at 60°C, pH 7.4. The degradation of **1–4** displays pseudo first-order kinetics over several half-lives. The observed rate constants (k_{obs}) for the overall degradation rate was calculated by linear regression analysis of the slope of these lines, based upon Eq. (2):

$$\ln([\text{ester}]_t/[\text{ester}]_0) = -k_{obs}\, t \qquad (2)$$

where $[\text{ester}]_t$ and $[\text{ester}]_0$ are the concentration of the putative prodrug at time t and initial time, respectively. As shown in Fig. 3, the pseudo-first-order rate constants for the esters are, in order of decreasing stability: butyl, perfluorinated octyl, perfluorinated hexyl and perfluorinated butyl nicotinates. The degradation studies of nicotinic esters carried out by other groups also show the pseudo first order degradation (15,21). The n-hexyl and n-octyl alkyl nicotinic esters exhibited no hydrolysis even after 5 weeks in pH 7.4 buffer at 37°C

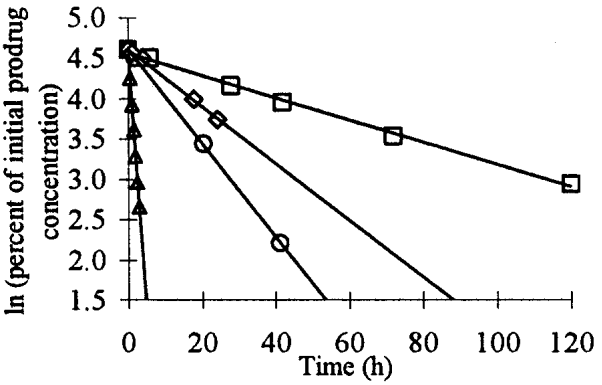

Fig. 3. Pseudo-first order plots for the hydrolysis of prodrugs (**1,** □; **2,** △; **3,** ○; **4,** ◇) in 0.02 M phosphate buffer pH 7.4 (μ = 0.1) at 60°C. For **1** and **2**, the buffer contained 5% acetone, while for **3** and **4**, the buffer contained 15% acetone.

(21). In our hands, the perfluorinated hexyl nicotinic ester **3** and perfluorinated octyl nicotinic ester **4** are susceptible to chemical hydrolysis. Wernly–Chung *et al.* (21) concluded that the factors influencing the rate of hydrolysis of nicotinic acid esters are not steric but electronic in nature, as electron-withdrawing substituents seem to enhance the rate of the reaction. This agrees with our observation that the electron withdrawing effect of fluorine affects the reaction rate of chemical hydrolysis, when comparing the results of **1–3.** If it is assumed that the electron withdrawing capability of the fluorocarbon chain in **3** is roughly similar to that in **4**, it is not completely clear why **4** degrades so much more rapidly than **3**. Additional experiments related to the nature of the transition state are needed to address this issue.

Figure 4 shows a typical degradation curve of **2** and the subsequent appearance of the degradation product (nicotinic acid) as well as the mass balance at pH 7.4. Although we did not identify the products of degradation other than nicotinic acid for all putative prodrugs, the results in Fig. 4 suggest that

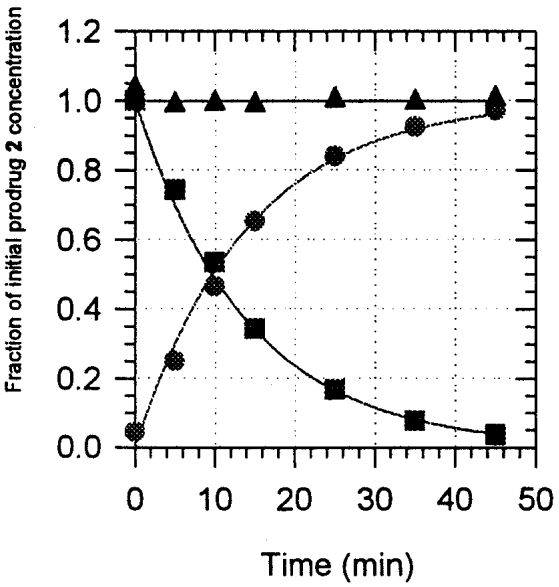

Fig. 4. Degradation of **2** (■), appearance of nicotinic acid (●), and the corresponding mass balance (▲) as a function of time in 0.02M phosphate buffer pH 7.4 (μ = 0.1) at 60°C.

nicotinic acid is released upon the degradation of the fluorinated esters.

pH-Rate Profile

The putative prodrugs **1**, **2**, and **4** were selected for the pH-rate profile study over pH range 2–8 at 60°C. For all experiments, pseudo-first-order degradation kinetics are observed. The influence of pH on overall rate of hydrolysis of the esters is shown in Fig. 5. The pH–rate profile exhibits a U shaped curve with three pH regions. The degradation of **1**, **2**, and **4** were fitted to a model including specific acid- and base-catalysis together with a spontaneously or water catalyzed hydrolysis. The pH-rate profile can be described by equation 3,

$$k_{obs} = k_H[H^+] + k_0 + k_{OH}[OH^-] \quad (3)$$

where k_{obs} is the observed rate constant for the degradation of the esters and k_H, k_0, and k_{OH} are the rate constant for hydronium ion, water-catalyzed hydrolysis, and hydroxide ion catalyzed degradation, respectively. The rate constants obtained by fitting Eq. (3) for **1**, **2**, and **4** at 60°C are shown on Table III. The pH-rate profile showed that the maximum stability is at pH 4-5. Larsen *et al.* (15) also reported similar results, however observing an inflection point at about pH 3. The authors explained this by the existence of spontaneous or water-catalyzed reaction for both of the protonated form of nicotinate and the free base of nicotinate. In Fig. 5, the degradation is accelerated in the basic pH region. The esters exhibit slower degradation rates in the acidic pH region (pH<4), where they are protonated (based on pK_a, Table II). The k_{OH} of the fluorinated ester **2** is approximately 54 fold greater than that of unfluorinated ester **1** in basic solution. In acidic medium, the k_H of **2** is approximately 500 fold greater than **1**. As shown by the pH-rate profiles, **2** degraded faster than the other esters.

Temperature Dependence of Hydrolysis

The effect of the temperature on the stability of **1–4** was determined by measuring the hydrolysis rate at 50–80°C at pH 7.4 buffer and ionic strength of 0.1. The temperature effect followed the Arrhenius equation (Eq. 4),

$$\ln k_{obs} = \ln A - E_a/RT \quad (4)$$

where A is the pre-exponential factor and E_a the activation energy, T the absolute temperature and R the gas constant. The relationship between temperature and observed rate constant has been drawn according to Equation 4 in Fig. 6 and the corresponding activation energies are summarized in Table III. As shown in Fig. 6, the degradation of all the putative prodrugs followed the Arrhenius relationship over the temperature region studied and fluorine-containing compounds **(2**, **3**, and **4)** degraded faster than **1**.

Table III.

Esters	k_H ($M^{-1} \times h^{-1}$)	k_0 (h^{-1})	k_{OH} ($M^{-1} \times h^{-1}$)	E_a (kJ mol^{-1})
1	1.43×10^2	4.77×10^{-4}	2.75×10^4	94.5 ± 1.6
2	7.29×10^4	1.35×10^{-2}	1.50×10^6	91.4 ± 0.3
3	[a]	[a]	[a]	101.8 ± 1.2
4	4.29×10^3	3.71×10^{-3}	1.86×10^4	102.5 ± 1.3

[a] Not determined.

Buffer Effect

Phosphate buffers have been observed to accelerate the rate of drug degradation (26). The effect of the buffer concentration on the hydrolysis of **2** was examined with phosphate buffer (pH 6 and 7.4) at concentrations of 0.02, 0.04, 0.08 and 0.12 M. Figure 7 shows that the degradation rate in 0.12 M phosphate buffer at pH 7.4 is expected to be faster than in unbuffered solution (extrapolation to zero buffer content). In phosphate buffers at pH 6–8, the major buffer species are $H_2PO_4^-$and HPO_4^{2-}, both of which may act as a catalyst for the ester degradation (26). In the pH range of 6-8, k_{obs} would be expected to follow Eq. (5):

$$k_{obs} = k_0 + k_{H_2PO_4^-}[H_2PO_4^-] + k_{HPO_4^{2-}}[HPO_4^{2-}] \quad (5)$$

where $k_{H_2PO_4^-}$ and $k_{HPO_4^{2-}}$ are the rate constants denoting catalysis by $H_2PO_4^-$ and HPO_4^{2-}, respectively. Rearranging and divided by the total phosphate buffer concentration (B_T) gives Eq. (6):

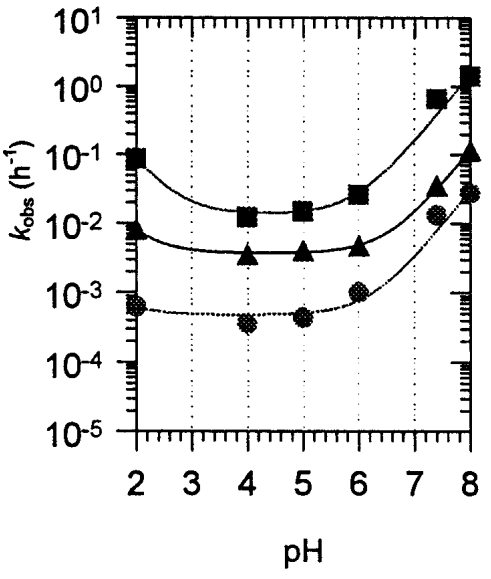

Fig. 5. Degradation (k_{obs}) of prodrugs (**1,** ●; **2,** ■; **4,** ▲) as a function of pH at 60°C. The solid lines are the curve fit to the experimental data.

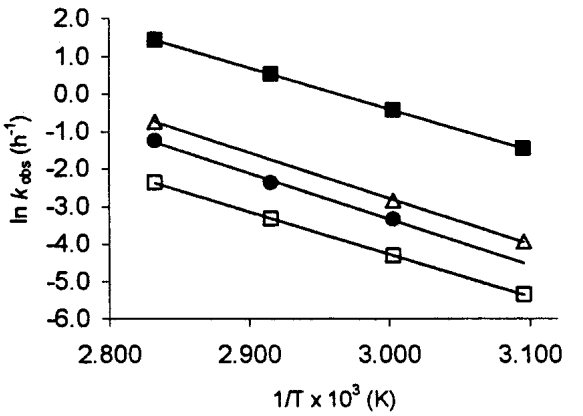

Fig. 6. Arrhenius plot for prodrugs (**1,** □; **2,** ■; **3,** △; **4,** ●) in 0.02 M phosphate buffer pH 7.4 (μ = 0.1).

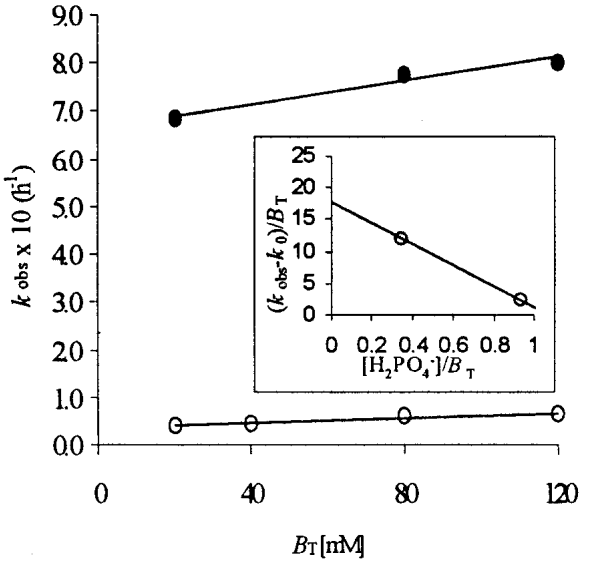

Fig. 7. Degradation (k_{obs}) **2** at 60°C (μ = 0.5) vs. total phosphate buffer concentration (B_T). Closed circles: pH 7.4; open circles: pH 6.0. The linear least-squares slope of these lines is k′; the positive intercept is k_0. The inset figure shows the dependence of the apparent rate constant $k' = (k_{obs} - k_o)/B_T$, on the buffer composition. The 0 and 1 intercepts of the inset figure are $k_{HPO_4^{2-}}$ and $k_{H_2PO_4^-}$, respectively.

$$k' = (k_{obs} - k_o)/B_T = k_{H_2PO_4^-} \times ([H_2PO_4^-]/B_T) + k_{HPO_4^{2-}} \times ([HPO_4^{2-}]/B_T) \quad (6)$$

Thus a plot of the k_{obs} vs. B_T at constant pH results in k_0 as the intercept and k' as the slope (Fig. 7). The inset figure is the apparent rate constant k' vs. the fraction of the acid buffer component present $[H_2PO_4^-]/B_T$, which gives $k_{HPO_4^{2-}}$ and $k_{H_2PO_4^-}$ as the 0 and 1 intercepts, respectively. The values of $k_{HPO_4^{2-}}$ and $k_{H_2PO_4^-}$ are 1.8×10^{-3} M^{-1} h^{-1} and 1.3×10^{-4} M^{-1} h^{-1} indicating that HPO_4^{2-} is a stronger catalyst than $H_2PO_4^-$.

Ionic Strength Effect

The influence of ionic strength on the degradation of **2** was investigated by adding various amounts of sodium chloride. The studies were conducted in acetate (pH 5) and phosphate (pH 7.4) buffer of 0.02M concentrations with the ionic strength values ranging from 0.05–0.5 at 60°C. The results showed that the ionic strength effect on the degradation is negligible at pH 5 and 7.4. No kinetic salt effects were observed indicating that at least one of the reacting molecules is uncharged.

Solvent Effect

Because acetone was used as a cosolvent to enhance the solubility of the putative prodrugs in aqueous solution during stability studies, the solvent effect was determined by comparing the observed degradation rate constants (Table IV) obtained with 5% and 30% acetone for **1**, **2** and with 15% and 30% acetone for **3** in phosphate buffer (pH 8) of 0.02 M and ionic strength of 0.1. In all compounds tested higher acetone concentrations resulted in decreased reaction rates. This may be explained as an effect on the transition state. The transition state of the hydrolysis reaction is polar whereas acetone is a solvent less polar than water. Therefore, increasing the amount of acetone in solution will tend to destabilize the transition state, and the reaction will be expected to be slower (27).

Table IV.

	Observed rate constants (h^{-1})		
Esters	5% acetone	15% acetone	30% acetone
1	0.0273 ± 0.0003	[a]	0.0166 ± 0.0002
2	1.382 ± 0.0001	[a]	1.345 ± 0.001
3	[a]	0.193 ± 0.005	0.134 ± 0.003

[a] Not determined.

Enzymatic Stability

It is anticipated that, to be effective in the intended application, nicotinic acid prodrugs should be rapidly converted back to the parent compound. Further, it would be expected that enzymatic hydrolysis would be responsible for a major fraction of recovery of the parent compound *in vivo*. To confirm that the putative prodrugs can be hydrolyzed enzymatically, the stability of the esters was evaluated using porcine esterase *in vitro*. The data concerning chemical and enzymatic stability of **1–4** are reported in Table V. The half-lives of hydrolysis in pH 7.4 buffer were calculated by the extrapolation of the Arrhenius plot (Fig. 6) at 37°C. The results show that esterase indeed accelerate the degradation of **1-3** compared to chemical hydrolysis in pH 7.4 buffer. Durrer *et al.* (22) reported that esterase catalyzes n-alkyl nicotinic ester hydrolysis with increasing activity from methyl to n-octyl nicotinate. However, branched alkyl chains appear to decrease enzymatic hydrolysis because of the steric hindrance (22). Although the fluorine atom is close in size to the hydrogen atom, a fluorocarbon chain is rather stiff compared to a hydrocarbon chain (28) and such stiffness may cause significant steric effects to decrease the esterase activity. This may help explain why compound **4** did not appear to be a good substrate for the esterase. From the results, it may be expected that **1-3** would be rapidly converted into the parent drug after administration. Identification of all the products of esterase-catalyzed degradation of **1–4** awaits further experimentation.

CONCLUSIONS

Esters **1–4** show a higher solubility in PFOB and are more lipophilic than the parent drug, nicotinic acid. Although

Table V.

	$t_{1/2}$	
Esters	pH 7.4 buffer[a] (h)	Esterase (100 U/L) (min)
1	646	170
2	12	3
3	178	163
4	316	b

[a] $t_{1/2}$ was calculated by the extrapolation of the Arrhenius plot at 37°C.
[b] $t_{1/2}$ > 24 h.

the hydrocarbon derivative is surprisingly soluble, the fluorinated esters show improved solubility in PFOB. The chemical degradation of putative prodrugs in aqueous solution followed the pattern previously shown for similar esters. It was shown that the buffer concentration had only a slight influence on the degradation rate, and ionic strength had no influence on the rate of hydrolysis. Compound **1,** with simple aliphatic side chain, is relatively stable compared to the other esters, especially under basic conditions. Under aqueous conditions, loss of **2** results in a quantitative appearance of the parent nicotinic acid. Compounds **1–3** were rapidly degraded *in vitro* by esterase catalysis. The properties of the fluorinated esters **2** and **3** suggests that they may be suitable candidates for further testing as possible prodrugs of nicotinic acid based on higher solubility in PFOB, more rapid release the parent drug following simple hydrolysis, and sensitivity to the presence of a model esterase enzyme as compared to **1**.

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