Anhydride Prodrugs for Nonsteroidal Anti-Inflammatory Drugs

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Received August 13, 2002; accepted November 1, 2002

Purpose. The objective of this study was to synthesize anhydride prodrugs for prolong action to shield the carboxylic acid group from irritative effects and to temporary hydrophobize the drug so that it becomes accessible to aqueous media when the anhydride residue is hydrolyzed.

Methods. Ibuprofen, a nonsteroidal anti-inflammatory agent, was used as a representative drug for anhydride derivatization. Mixed anhydrides of ibuprofen with fatty acids of different chain length were prepared by reacting acid chloride derivatives with the corresponding acid in the presence of acid acceptor and two-phase reaction. Mixed anhydrides were also prepared by dehydration reaction using acetic anhydride and anhydride interchange of symmetric anhydrides. The analgesic effects of mixed anhydride prodrugs were tested using nonsteroidal anti-inflammatory drug rat paw edema model. *In vitro* degradation of mixed anhydrides and drug release were monitored by high-performance liquid chromatography.

Results. Ibuprofen was bound to aliphatic and aromatic acids via an anhydride bond in high reaction yields (>85%) with high mixed anhydride content (>80%). The mix anhydride was purified by chromatography and stored at 4°C to minimize conversion into the symmetric anhydride. These anhydride derivatives hydrolyzed at different time intervals depending on the hydrophobicity of conjugated acid. *In vivo* testing of the ibuprofen anhydride derivatives for analgesic effect indicated an extended action of the drug for over 24 h as a function of the fatty acid chain length.

Conclusion. This study demonstrates the promise of anhydride prodrugs for extending drug action and shielding the carboxylic acid group.

KEY WORDS: mixed-anhydride; prodrugs; ibuprofen; NSAID; prolong-action.

INTRODUCTION

A wide variety of compounds having carboxylic acid groups are biologically active, for example, the nonsteroidal anti-inflammatory drugs (NSAIDs), such as naproxen, ibuprofen, indomethacin, and diclofenac; antibiotics, such as ampicillin and cefmetazole; and herbicides, such as Tordon, Endothal, and Amiben. The presence of free acid groups in these compounds produces local irritation on interaction with mucosal tissues, and at the same time they ionize at physiological pH, which makes the drug poorly absorbed through biologic membrane.

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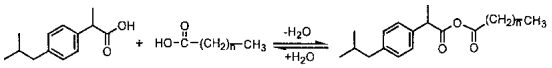
The general approach to overcome these limitations is esterification of the carboxylic acid groups to produce lipophilic and nonirritating prodrug forms, provided that the parent bioactive agent can be released from the prodrug at its sites of activity (1). However, several aliphatic and aromatic esters of carboxylic acid drugs are not sufficiently labile *in vivo* to ensure a sufficiently high rate and extent of prodrug conversion. For example, ethyl esters of naproxen (2) and fenbufen (3) have lower anti-inflammatory activity relative to the free acids because of the resistance of esters to hydrolyze *in vivo*. In the field of angiotensin-converting enzyme inhibitors, ethyl esters of enalaprilic acid and its derivatives improved their oral bioavailability. Plasma enzymes do not hydrolyze these esters fast enough and the necessary conversion of ester to free acid predominantly takes place in the liver (4). As a result of this reason, liver function may be a very important determinant for the bioactivation of enalapril and its therapeutic effect. In another consideration, ester derivative prodrugs have been found cleavable enzymatically to release its bioactive forms and, thus, are dependent on the enzymatic activity, which may vary among individuals, or even in the same individuals at various times during the day or in various sites where the drug is administered. This fact may result in a large variation of drug bioavailability (5–7).

To overcome the above-mentioned limitations, anhydride derivatives of carboxylic acid-bearing drugs are suggested. Unlike the ester bond used in prodrugs, the anhydride bond is more susceptible to hydrolysis and decomposition of its carboxylic acid counterparts in a predictable rate and pattern and is less sensitive to enzymolysis than the esters or amides (8). Studies on polyanhydride as drug carriers show that the anhydrides degrade in a controlled fashion and are biocompatible with the human body tissues, including the brain (9,10). Interestingly, not much attention has been given to the formation of prodrugs based on the hydrolytically degradable anhydride bonds, although biodegradable polymers based on these bonds have been developed and used as erodible carriers for drugs both in animals and in humans (8–10).

This article presents a new class of prodrugs based on mixed anhydrides of carboxylic acid bearing drugs characterized by possessing a high susceptibility to undergo hydrolytic degradation and at the same time providing ample possibilities for varying the water and lipid solubilities of the derivatives. The structure of the anhydride prodrug is illustrated using ibuprofen as drug and linear aliphatic acids as carriers.



Prodrugs will degrade slowly by hydrolysis in a predictable and controlled fashion, eliciting the pharmacological response characteristic of the acids from which they are derived. These prodrugs are characterized to be less irritating to topical and gastric or intestinal mucosal membranes. Prodrugs of carboxylic acid agents may provide increased biomembrane transport so that the parent drugs are more bioavailable from the site of administration, such as the gastrointestinal tract, the rectum, the blood–brain barrier, the skin, or the eye. In addition, anhydride prodrugs may temporarily modify the physi-

cal properties of a drug to allow new formulations. For example, some ibuprofen derivatives as shown in this report are liquid at room temperature, which allows their formulation in soft gelatin capsules. We report here on the synthesis of mixed anhydrides of ibuprofen with various fatty acids, their analysis, *in vitro* degradation, and preliminary *in vivo* antiinflammatory properties.

MATERIALS AND METHODS

Ibuprofen was a gift from Ethyl Corp. (NJ, USA). Acetic anhydride, propionic anhydride, hexanoic anhydride, octanoic anhydride, lauric anhydride, benzoic anhydride, and their respective acids and acid chlorides were purchased from Aldrich (Milwaukee, WI, USA). Thionyl chloride and acetic anhydride were distilled freshly under nitrogen before use, and all solvents were of analytical grade. High-pressure liquid chromatography (HPLC)-grade acetonitrile and dichloromethane were purchased from LabScane (Dublin, Ireland).

HPLC apparatus used for acids and anhydrides was composed of an HP 1050 (Hewlett-Packard, Palo-Alto, CA, USA) modular system, including a diode array UV-detector and an IBM compatible data-system HPCHEM attached with a ThinkJet printer. Injections were made using a Rheodyne (Cotati, CA, USA) injection valve with a 20-μL loop. Samples (10 μL in acetonitrile) were eluted through a C8 column (Supelcosil 8, Supelco, PA, USA) using mixtures of acetonitrile and water at a flow rate of 1 mL/min. Preparative HPLC for the separation of mixed anhydrides was conducted on a Giloson system composed of 305, 306, and 806 Manometric Model pump system, a Rheodyne (Cotati, CA) injection valve with a 5-mL loop, and a 117 UV detector. MODcoI columns, 25 × 2.54 cm with packing media CER-1481 or 1489 (MODcoI, St. Louis, MO, USA) was used with acetonitrile as eluent.

Infrared (IR) spectroscopy (Anelect Instruments FT-IR model fx-6160) was performed for the drugs and anhydride samples cast on NaCl plates using CH_2Cl_2 as solvent. Kontron Instruments (Uvikon model 930) was used to record the UV spectra. ^{1}H NMR spectra ($CDCl_3$/TMS/d/ppm) were obtained by using 300-MHz spectrometer (Varian, CA, USA).

Ibuprofen Analysis

Quantitative analysis of ibuprofen was performed in a C18 Lichospher 100 column (Merck, Dorstadt, Germany, 250 × 4 mm, 5 μm) using a 70:30 v/v mixture of acetonitrile:water as mobile phase at a flow rate of 1 mL/min. Ibuprofen was detected by UV at 270 nm, and its typical retention time was 4.4 min.

Preparation of Ibuprofen Anhydride

Ibuprofen (10 g) was refluxed in acetic anhydride (100 mL) for 30 min followed by the evaporation of acetic anhydride to dryness and the residue was distilled under vacuum (0.3 mm Hg at 170°C) to yield (70%) a viscous liquid of symmetric ibuprofen anhydride. IR (cm^{-1}) 1812, 1748; ^{1}H-NMR 7.05 (d, 8H), 3.65 (m, 2H), 2.45 (dd, 4H), 1.85 (m, 2H), 1.42 (dd, 6H), 0.92 (d, 12H).

Preparation of Ibuprofen Acid Chloride

Ibuprofen was dissolved in thionyl chloride (2 g in 5 mL) and refluxed for 2 h. After distilling off the thionyl chloride using an oil pump (170°C, 0.5 mm Hg) the crude yellow liquid was extracted with hexane (90% yield). IR (film cast) 1800 cm^{-1} (Cl-C=O) ^{1}H NMR: 7.22 (q, 4H); 4.12 (q, 1H); 2.53 (D, 2H); 1.88 (Octa, 1H); 1.62 (D, 3H); and 0.95 ppm (D, 6H).

Preparation of Ibuprofen Sodium

Ibuprofen (10 g) was dissolved in double-distilled water with the addition of sodium hydroxide solution (1 N). The solution was lyophilized under reduced pressure (10 mm Hg) at –50°C for 34 h to obtain a white powder (85% yield). IR (film cast) 1710 cm^{-1} (C=O) ^{1}H NMR: 7.22 (q, 4H); 4.03 (q, 1H); 2.53 (D, 2H); 1.88 (Octa, 1H); 1.62 (D, 3H); and 0.95 ppm (D, 6H).

Synthesis of Mixed Anhydrides

Mixed anhydrides of ibuprofen and fatty acids (C_3–C_{18}) were synthesized by the following methods:

Schotten Baumann Reaction

In this method, an acid and the corresponding acid chloride react in the presence of a tertiary amine as acid acceptor. Pyridine, triethylamine, and crosslinked poly(4-vinylpyridine) (PVP; Reillex® 402, Reilly Industries, NJ, USA) were used as acid acceptors. The acid, acid chloride, and the amine (1:1.1:3 molar ratio) were mixed in dichloromethane (10% by weight, 10 mL) at 0°C with constant stirring of one hour, and evaporated to dryness. The oily residue was weighed and analyzed by HPLC, IR, and ^{1}H NMR.

In a typical reaction, ibuprofen (4.1 mmol) was added with constant stirring to a solution of hexanoyl chloride (3.7 mmol) and PVP (10 mmol pyridine equivalents) in dry dichloromethane (10 mL) immersed in an ice bath. After one hour of stirring at 0°C, the reaction mixture was quickly filtered and washed with 5-mL portions of 0.1 N HCl followed by distilled water and dried over magnesium sulfate. The clear solution thus obtained was evaporated to dryness using a nitrogen stream and analyzed. IR (cm^{-1}) 1800, 1735; ^{1}H-NMR 7.21 (d, 2H), 7.05 (d, 2H), 3.70 (m, 1H), 2.45 (dd, 3H), 2.35 (t, 2H), 1.85 (m, 2H), 1.63 (m, 2H), 1.50 (m, 1H), 1.33 (dd, 4H), 0.88 (d, 9H). Ibuprofen mixed anhydrides with longer fatty acids show similar IR and H NMR spectra with the peak integration at 1.33 ppm increases to reflect the increase in the number of methylenes in the fatty chain.

Two-Phase Reaction

A two-phase reaction was performed between benzoyl chloride (organic phase) and ibuprofen sodium (aqueous medium) with and without the presence of pyridine *N*-oxide (PNO) as phase transfer agent. Ibuprofen sodium (0.1 M) in deionized water (10 mL) was mixed with a dichloromethane solution (10 mL) containing hexanoyl chloride (0.1 M), PNO (6×10^{-6} M), and naphthalene as internal standard for HPLC analysis. Both phases were stirred vigorously at room temperature and samples were withdrawn at the time interval of 5 min, dried over magnesium sulfate, evaporated to dryness, and analyzed by HPLC.

Anhydride Interchange

Symmetric ibuprofen-anhydride obtained above was dissolve in dichloromethane along-with a symmetric fatty acid anhydride like hexanoic anhydride and stirred for 30 min at room temperature followed by evaporation to dryness. The oily residue thus obtained was dissolved in minimal amount of acetonitrile and separated by preparative HPLC to isolate the ibuprofen mixed anhydride.

Dehydration Method

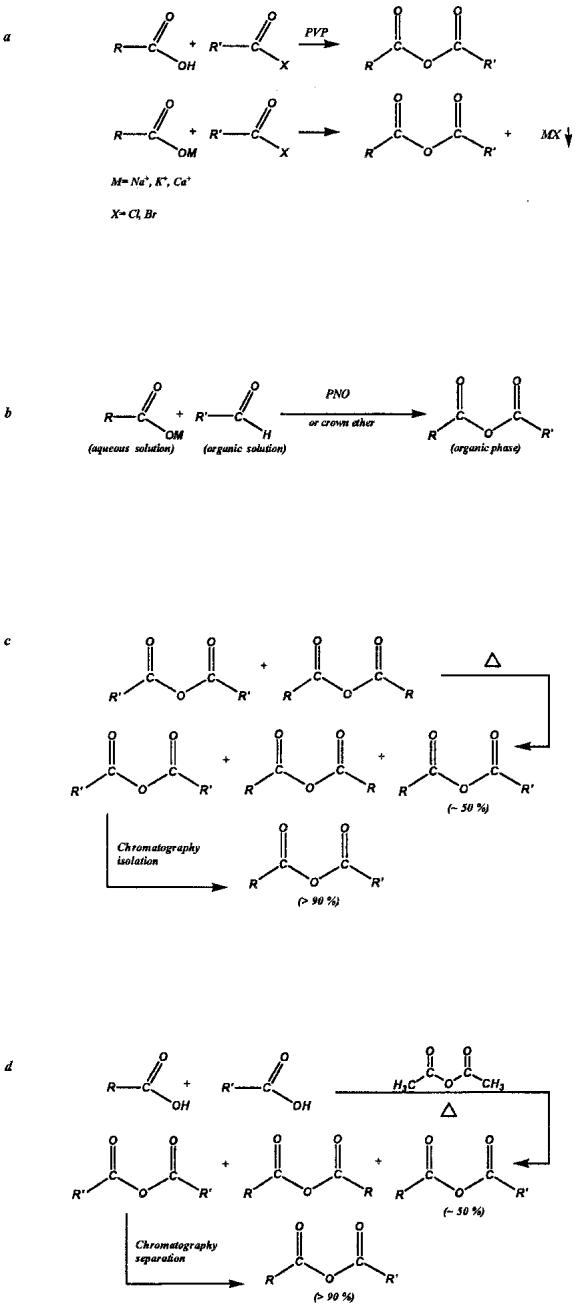
Equivalent amounts of ibuprofen and hexanoic acid were added to a boiling solution of acetic anhydride and refluxed for 1 h followed by evaporation to dryness. The resulting residue was dissolved in acetonitrile (0.5 mL) and immediately separated by preparative HPLC to isolate the mixed anhydride.

In Vitro Degradation of Ibuprofen–Fatty Acid-Mixed Anhydrides

Hydrolytic degradation of ibuprofen-fatty acid mixed anhydrides was studied by suspending a sample of the viscous solution in 10 mL of 0.1 M phosphate buffer pH 7.4 at 37°C with constant shaking (100 rpm). After a fixed time interval, an aliquot of the suspension (5 mL) was withdrawn and ibuprofen concentration was determined by HPLC using citrate buffer (0.01M, pH 3): acetonitrile (60:40 v/v) mixture as mobile phase with UV detection at 264 nm.

In Vivo Analgesic Activity: Randall-Selitto Assay in Rats

Analgesic activity of ibuprofen mixed anhydrides was evaluated using the Randall Selitto NSAID rat paw edema model. For this assay, ibuprofen and mixed anhydrides of ibuprofen with hexanoic and oleic acids (50 mg ibuprofen equivalents) were dispersed in 1.0 mL of sterile water. According to the protocol, female Sprague–Dawley rats (250–300 g, Harlan, Israel), six in each group were injected with 0.05 mL of 10% (w/v) aqueous suspension of yeast in the left hindpaw followed by the injection of 100 μL of drug solution and drug activity was determined by measuring the amount of weight (in g) accumulated on the inflamed paw until the rat withdraws its hindpaw under the weight probe (11). The threshold of the rat to weight pressure was determined every few hours for 24 h.



Scheme 1. Methods for the synthesis of mixed anhydrides.

RESULTS

Ibuprofen mixed anhydrides based on binding ibuprofen to aliphatic and aromatic acids were prepared by four different methods as described in Scheme 1.

The preferred method for the formation of mixed anhydrides with high purity and without further isolation or purification is the Schotten Baumann reaction of an activated carboxylic acid derivative with a carboxylic acid or the salt thereof under mild conditions.

In this method, ibuprofen acid chloride was reacted with hexanoic acid or benzoic acid, and ibuprofen with hexanoyl chloride or benzoyl chloride (Scheme 1a).

In each of these reactions, dichloromethane, petroleum ether, acetonitrile, chloroform, or toluene were used as solvents. No significant differences in the mixed anhydride reaction yields obtained when using ibuprofen acid chloride or ibuprofen acid when reacted with the corresponding acid or acid chloride. The effect of the solvent on the reaction yield and the time to achieve maximal concentration of the mixed anhydride was examined (Fig. 1). Ibuprofen acid chloride-:benzoic acid at a molar ratio 1:1.1 were reacted in the presence of PVP at 0°C in different solvents and progress of the reaction was monitored by HPLC at 30, 90, and 270 min. As shown in Fig. 1, the highest yield was obtained from the reaction in chloroform for 30 min.

Mixed anhydrides of ibuprofen and fatty acids with the chain length C_3–C_{18} were prepared in chloroform for 30 min. All mixed anhydrides were liquids or semisolids at room temperature. The total anhydride yield was >85% and the mixed anhydride content is summarized in Table I.

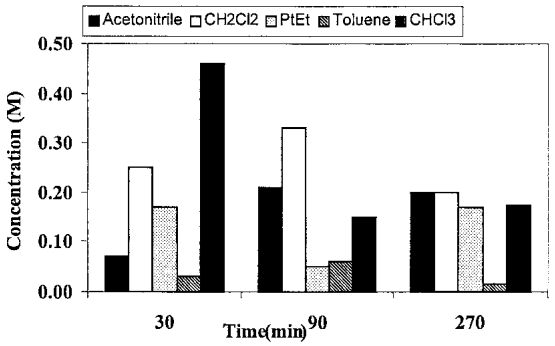
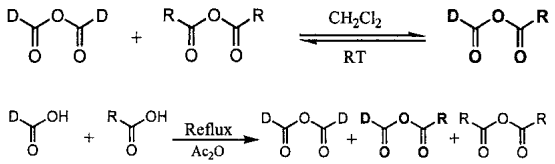


Fig. 1. Effect of solvent on the formation of ibuprofen-benzoic acid-mixed anhydrides. Ibuprofen acid chloride-benzoic acid at a molar ratio 1:1.1 were reacted in the presence of poly(4-vinylpyridine) at 0°C in different solvents and progress of the reaction was monitored by high-performance liquid chromatography at 30, 90, and 270 min.

The results obtained from the two-phase reaction (Scheme 1b) are shown in Fig. 2a and b, which suggested that the formation of mixed anhydride in the presence of PNO was shorter (~20 min) compared with the reaction time without PNO. The mixed anhydride concentration was reduced dramatically over time in the presence of PNO followed by increased concentration of the symmetric anhydride. Possible reasons for these results are that the presence of PNO led to the replacement of chloride with the acid, and anhydride replacement. Accordingly, the symmetric product was formed with a higher concentration compared to the concentration without PNO. Similar results were obtained when 18-crown-6 ether was used as phase transfer agent.

Mixed anhydrides were also prepared by anhydride interchange (Scheme 1c). In this method, two symmetric anhydrides were reacted in solution or in bulk to form the corresponding mixed anhydride.



About 50% yield of mixed anhydrides were obtained as determined by HPLC (Table I). Removal of a water molecule from two carboxylic acids was used for mix anhydride formation (Scheme 1d). Ibuprofen was reacted with fatty acids using acetic anhydride as dehydrating agent to yield over 90% of anhydrides with up to 50% mixed anhydride (Table I). The mixed anhydrides were isolated by chromatography to yield >90% pure compound.

Mixed Anhydrides Analysis

The formation of mixed anhydrides was determined by HPLC as previously described (12). A simple reverse phase HPLC was used for the quantitative analysis of symmetric and mixed aliphatic and aromatic anhydrides. Anhydrides and the corresponding acids were isolated using a C8 (octane substituted silica) reverse phase column with a mixture of acetonitrile:water (70:30 v/v) as mobile phase. No hydrolysis of the anhydrides occurred during analysis.

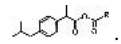
Ibuprofen anhydrides were separated at retention times of between 3 and 9 min using an isocratic flow of a 30:70 v/v water:acetonitrile and UV detection at 254 nm. The retention time was increased with the increase in chain lengths of fatty acids. The detection limit for these anhydride derivatives was set on 10 μg/mL. Ibuprofen acid and the fatty acids were eluted at short retention times (2–5 min) and did not interfere with the analysis of their corresponding anhydrides. For example, the retention times for hexanoic anhydride, ibuprofen-hexanoic anhydride, and ibuprofen anhydride were 3.9, 7.1, and 7.7 min., respectively. This separation method was used for the quantitative separation of mixtures of symmetric and mixed anhydrides either by preparative HPLC or by flash chromatography. The quantitative separation was conducted at low temperature, preferably at refrigeration (4–8°C) to avoid interchange of the mix anhydride to the corresponding symmetric anhydrides.

IR analysis of aromatic and aliphatic anhydrides show peaks at 1780 and 1730 cm^{-1} and at 1805 and 1740 cm^{-1}, respectively. Mixed anhydrides of aromatic and aliphatic acids show peaks at 1800 and 1735 cm^{-1}, which correspond to the mixtures of aliphatic and aromatic anhydrides. An absorption peak at 1700 cm^{-1} indicated the existence of free acid

Table I. Ibuprofen-Fatty Acid Anhydrides Obtained from Different Methods

Ibuprofen-fatty acid anhydride (fatty residue)	Schotten baumann	Two-phase	Anhydride-interchange	Dehydration	High-performance liquid chromatography reverse transcription (min)
CH_3CH_2	96	85	40	52	4.2
$CH_3(CH_2)_4$	97	90	44	55	7.1
$CH_3(CH_2)_6$	95	88	43	38	7.3
$CH_3(CH_2)_{10}$	96	85	48	42	7.5
$CH_3(CH_2)_{16}$	96	90	50	36	9.2

Note: Anhydrides were synthesized from the reaction between ibuprofen acid chloride and the fatty acids in chloroform for 30 min at 0°C using poly(4-vinylpyridine) as acid acceptor (Schotten Baumann); ibuprofen acid chloride and the fatty acids reacted in water:chloroform 1:1 mixture using pyridine *N*-oxide PNO for 30 min at 0°C using poly(4-vinylpyridine (two phase); symmetric anhydrides reacting in bulk at 60°C; ibuprofen and fatty acids reacted with acetic anhydride as dehydrating agent. The total reaction yield was >90% and the mixed anhydride content were determined by high-performance liquid chromatography.

[a] Structure of asymmetric ibuprofen anhydrides  .

[b] Mixed anhydride content was determined by HPLC, the retention times are given in minutes. Rest anhydride content was symmetric anhydrides ibuprofen anhydride and fatty acid anhydride.

[c] Mixed anhydride yields of >90% were obtained after chromatography separation.

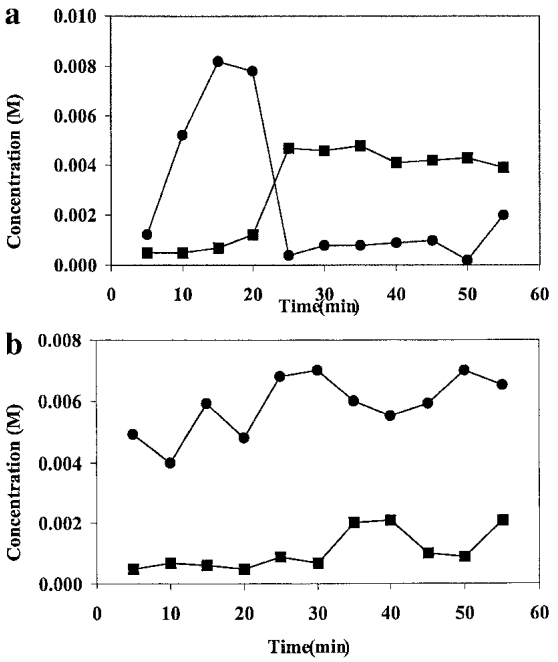


Fig. 2. Formation of ibuprofen-benzoate anhydride by the two phase method with pyridine *N*-oxide (PNO; a) and without PNO (b). Concentrations of ibuprofen-benzoyic acid-mixed anhydride (●) and benzoic acid symmetric anhydrides (■) obtained from the reaction of ibuprofen sodium with benzoyl chloride in the presence of PNO (two-phase reaction method). Ibuprofen sodium and benzoyl chloride (1.1:1 molar ratio) reacted in water:dichloromethane 1:1 mixture at 0°C. The concentration (in molar) of acids and anhydrides was determined by HPLC.

products in the sample, which may be a result of hydrolysis or unreacted acid. NMR spectra of mixed anhydride products of ibuprofen and fatty acids conform with the structure of the molecule. The H NMR spectrum of ibuprofen-hexanoate anhydride is given in Fig. 3, which show a good fit of the NMR data to the molecule. All anhydride derivatives were either liquids or semi solids.

Stability of Mixed Anhydrides

Samples of ibuprofen-hexanoate mixed anhydride (90% pure) were saved in sealed glass containers under dry argon at –20, 4, 25, and at 37°C and the mix anhydride content was followed for 7 days. Samples stored at refrigeration and –20°C did not change the mixed anhydride content whereas the samples stored at 25 or 37°C gradually decreased in mixed anhydride content to a level of about 50% within 24 h, by an anhydride interchange process to form the corresponding symmetric anhydrides. These levels remain constant for the rest of the experiment.

Release Behavior of Ibuprofen from Mixed Anhydrides

The hydrolytic degradation of ibuprofen-fatty acid mixed anhydrides was studied by placing samples of mixed anhydrides in 0.1 M phosphate buffer pH7.4 at 37°C and monitoring the concentration of ibuprofen in the buffer by HPLC. The cumulative release (%) of ibuprofen from mixed anhydrides of ibuprofen with different fatty acids is shown in Fig. 4.

The release rate of ibuprofen was a function of the fatty acid chain length. Typically, 70% of the drug was constantly released for 7 days from the ibuprofen-C_3 derivative, whereas only 30% was released from ibuprofen-C_{18} mixed anhydride during the same time period. A comparison study of these prodrugs at the pH 2 in physiological buffer was also performed; however, no *in vitro* release could be monitored, probably because the insolubility of the drug at acidic pH. These *in vitro* drug release results are in correlation with the results obtained for polyanhydride (14), in which, sebacic acid and dodecanoic acid based polyanhydride had a slower hydrolysis rate in water than adipic acid based polyanhydride. In both cases, the degradation rates were dependent on the length of the alkanoic acid residue, which indicated that longer alkanoic residue have slower degradation rates and thus, anhydride prodrugs having longer alkanoic chains are useful for extended release. In acidic pH, very little ibuprofen was detected in the solution because of the insolubility of ibuprofen in acidic solutions.

The mix anhydrides of ibuprofen were tested for their analgesic activity using the Randall-Selito NSAID rat paw edema model (11,12). The threshold of the inflamed rat paw model to weight pressure was determined every few hours for 24 h.

All ibuprofen mixed anhydrides produced significant analgesic effect in the yeast-inflamed paw test (Fig. 5). The long chain derivative (oleic-ibuprofen mixed anhydride) was very effective in increasing the pain threshold for 24 h, whereas the shorter-chain derivative (hexanoic-ibuprofen mixed anhydride) was effective for 24 h but at a lower threshold, and intact ibuprofen was effective for less than 3 h at a comparable dose. These data demonstrate the effectiveness of the prodrugs for controlled delivery of drugs.

DISCUSSION

Mixed anhydride derivatives of ibuprofen as model drug with benzoic acid and aliphatic acids have been synthesized for use as ibuprofen prodrugs with temporary hydrophobicity. Mixed anhydrides were prepared by four methods with the preferred method is the reaction between ibuprofen chloride and the inert alkanoic acid in an organic solvent in the presence of insoluble poly(vinyl pyridine) as acid acceptor. Reaction yields were in the range of 65–97%, with the mixed anhydride content greater than 85%.

The synthesis of symmetric anhydrides are well known (15–17) and can be formed from two carboxylic acid molecules using a dehydrating agent. Common dehydrating agents are acetic and propionic anhydrides, phosgene, diphosgene, dicyclohexylcarbodiimide, and methoxyacetylene. These methods generally produce the symmetric anhydrides, in addition to the desired mixed anhydrides. The preferred method is the reaction between the carboxylic acid molecule and an activated acid either is an organic solvent and an acid acceptor, or in a mixture of organic and aqueous medium. Examples of active acid derivative include acyl halides, or the reaction product of a dehydrating agent with an acid or its metal salt (15).

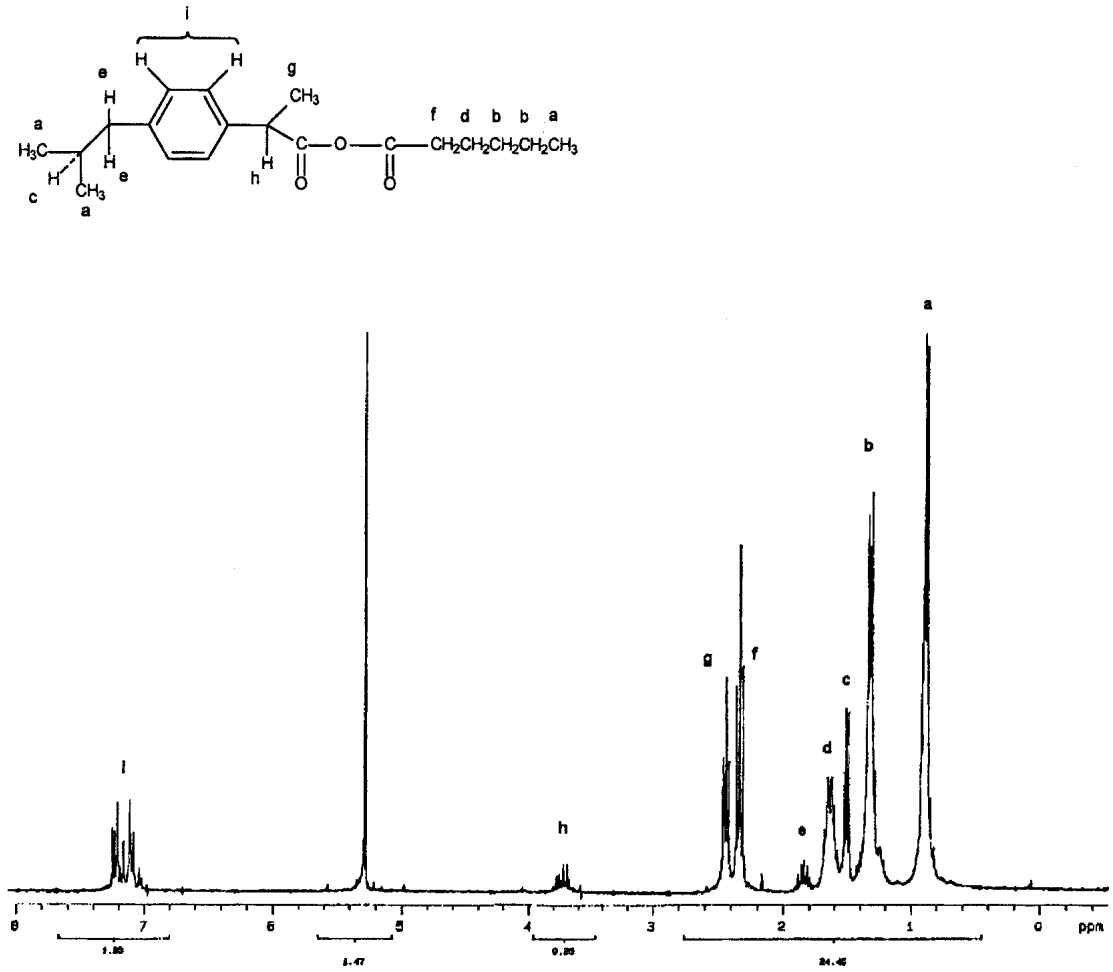


Fig. 3. ^{1}H NMR spectrum of ibuprofen-hexanoate anhydride.

where M is H, or a metal ion such as Na, Ag, Tl, or Ca; X is an halogen ion, or an active acid derivative; and R and R′ are different organic residues of the carboxylic acid molecules.

When M = H, hydrohalogenic acid is released during the reaction that must be eliminated to avoid decomposition of the anhydride product. The majority of acid acceptors used in the anhydride synthesis were pyridine and tertiary alkyl amines (16). These acid acceptors form a salt with the hydrogen halide byproducts, which is mostly removed by filtration. However, the soluble amine salts remaining in the reaction mixture are difficult to remove. Thus, an alternative acid acceptor used is a solid crosslinked PVP (16). This polymer binds to acid byproduct and can be isolated by filtration at the end of the reaction. Hence, we have used a solid amine as an

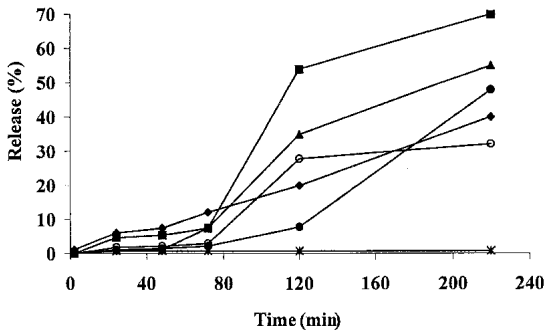


Fig. 4. *In vitro* release of ibuprofen from mixed anhydrides of ibuprofen and fatty acids with different chain length as measured by high-performance liquid chromatography. The measured mixed anhydrides were: ibuprofen-C_3 (■), ibuprofen-C_6 (▲), ibuprofen-C_8 (●), ibuprofen-C_{12} (♦), ibuprofen-C_{18} (○), ibuprofen-C_3 at pH 2 (∗). Experiments were performed in phosphate buffer (pH 7.4), at 37°C.

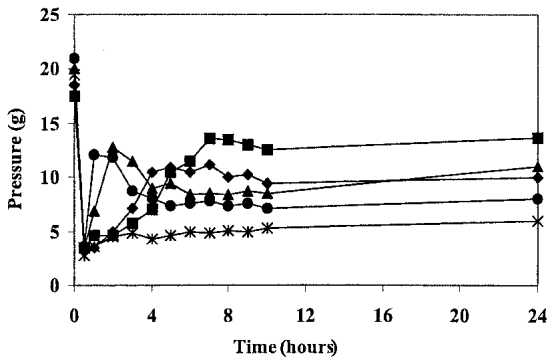


Fig. 5. *In vivo* activity of ibuprofen mixed anhydrides as measured by Randall-Selito test. The measured mixed anhydrides were as follows: oleic-ibuprofen anhydride (■), hexanoic-ibuprofen anhydride (♦), ibuprofen-ibuprofen anhydride (▲), ibuprofen anhydride (●), and yeast (∗).

acid acceptor. An additional problem associated with the mixed anhydride synthesis in solution is the isolation of the product from the symmetric and unreacted starting materials. The most useful method is probably the reaction between the acid or its metal salt and the acyl chloride of another acid.

The free carboxylic acid drug is conjugated to an inert carrier or another drug via a hydrolytically labile anhydride bond to alter the properties of the parent drug because the prodrug properties also depend on the selected carrier. The duration of drug release can be designed by altering the hydrophobicity of the mixed anhydride moiety from 1 to 24 methylenes. This modification will increase the hydrophobicity of the prodrug, slow its hydrolysis, and increase the release time of active parent drug. Hence, it is concluded that *in vivo*/*vitro* hydrolysis of the anhydride conjugate is dependent on the hydrophobicity of the anhydride groups. The main limitation of this approach is the stability of the mixed anhydride, which tend to form the corresponding symmetric anhydride during storage at ambient temperatures. It should be noted that drugs containing reactive functional groups, such as amino, hydroxyl or sulfhydryl, are less prominent to this approach.

In this approach, the release rate of a free drug can be manipulated by using different types of fatty acids. Thus, it can be set at minimal (acidic pH) in the stomach while it can be fast in the intestine, depending on the fatty acid derivative. For systemic or localized delivery via intramuscular or subcutaneous administration, a release of drug for periods of weeks can be obtained with the only degradation byproduct being a fatty acid. The results obtained suggest that anhydride prodrugs can be a good alternate to overcome the problems of carboxylic acid bearing drugs addressed in the introduction part of this report. On one hand, anyone can protect the carboxylic function can be temporarily masked by anhydride bond to overcome stomach irritation. On the other hand, anhydride conjugated drugs with the acids having higher alkane moiety provides higher absorption through lipid-water membrane. In addition, this anhydride prodrug approach can be applied for extended action/release of acidic drugs after administration to the eye, skin, or oral intake.

CONCLUSION

This work provides synthetic and characterization methods for mixed anhydrides of carboxylic acid-bearing drugs and demonstrate the possible use of mixed anhydrides for altering the physical properties of these drugs for improved bioavailability.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the US-Israel Binational grant (BSF) and a grant from the Alex Grass Center for Drug Design and Synthesis.

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