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Kinetics and mechanism of hydrolysis of amidals: their relative stability compared to structurally related acetals and acylals

Jianbing Chen, Peter A. Crooks, Anwar Hussain *

University of Kentucky, College of Pharmacy, Lexington, KY 40536–0082, USA

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Abstract

The preparation, kinetics and mechanism of degradation of four amidals (**1–4**), formed from the reaction of benzamide, *N*-methylbenzamide, nicotinamide, and *N*-methylnicotinamide with 3,4-dihydro-2H-pyran, are reported. The hydrolyses of the *N*-methyl amidals **2** and **4** were found to follow first-order kinetics. The degradation of amidal **2** was studied in detail and was catalyzed not only by specific acid catalysis, but also by a general acid catalysis; the second-order rate constant for the involvement of H_3PO_4 was about 4 M^{-1} h^{-1}. Amidal **3** was resistant to acid-catalyzed degradation in 0.05 M phosphate buffer at pH 3.0 and 37° C, whereas the phenyl analogue, **1**, under similar conditions, exhibited a $t_{1/2}$ value of 98.4 days. *N*-methylation of the carboxamide moiety in both the phenyl and pyridyl amidals (i.e., **1** and **3**, respectively) had a marked accelerating effect on the rate of hydrolysis, and this was attributed to the inductive effect of the *N*-methyl group which stabilizes the proposed transition state in the degradation mechanism. In acid media, amidals of 3,4-dihydro-2H-pyran were found to hydrolyze much more slowly than acetals and acylals of 3,4-dihydro-2H-pyran due to the greater stability of the protonated amidal species to unimolecular C-N bond cleavage. Substitution of an *N*-nicotinoyl group in place of the *N*-benzoyl moiety in the *N*-methyl-3,4-tetrahydro-2H-pyran amidal **2** resulted in a much slower rate in the acid-catalyzed hydrolytic cleavage reaction. The results indicated that the amidals formed from carboxamides and 3,4-dihydro-2H-pyran undergo degradation to the parent carboxamide via an acid-catalyzed unimolecular mechanism.

Keywords: Hydrolysis; Amidal; Prodrug

1. Introduction

The chemical properties and kinetics of degradation of acetals and acylals have been extensively studied and several hydrolytic mechanisms have been proposed (Fife and Brod, 1968, 1970; Fife and Jao, 1968; Weeks et al., 1968; Anderson and Fife, 1969; Brown and Buice, 1973; Fife and De, 1974). A number of compounds of this type have been evaluated as potential prodrug forms of pharmacologically active molecules (Hussain et al., 1974, 1978, 1979; Hussain and Rytting, 1974; Hussain and Truelove, 1979). Interestingly, relatively little work has been reported on the properties of the structurally related amidals and their mechanism of degradation. The purpose of this

* Corresponding author.

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report was to study the kinetics of hydrolysis of the model amidals **1–4**, and to compare their stability and degradation mechanism to structurally related acetals and acylals. A further objective of this study was to determine, based upon their kinetic behavior, whether such amidals could be utilized as prodrug forms for drugs containing a primary or secondary carboxamide group.

2. Experimental

2.1. Materials

N-Methylbenzamide was purchased from Janssen Chimica (Beerse, Belgium), 3,4-dihydro-2H-pyran and *p*-toluenesulfonic acid was obtained from the Aldrich Chemical Co. (Milwaukee, WI). Chromatographic silica gel (100–200 mesh) used for column chromatography, monobasic sodium phosphate (analytical grade) for buffers, and HPLC-grade acetonitrile for the mobile phase were all purchased from Fisher Scientific (Fair Lawn, NJ).

2.2. Apparatus

The ^{1}H-NMR and ^{13}C-NMR spectra were taken on a Varian VXR-300 NMR Spectrometer (operating at 75 MHz for ^{13}C and 300 MHz for ^{1}H) (Varian Associates, Palo Alto, CA) using tetramethylsilane (TMS) as internal standard. Melting points were determined on a Capillary Melting Point Apparatus (Arthur H. Thomas Co., Philadelphia, PA) and are uncorrected. The CHN analyses were performed by Atlantic Microlab, Inc. (Norcross, GA).

The high-performance liquid-chromatography (HPLC) system consisted of a Spectroflow 400 Solvent Delivery System (ABI Analytical, Kratos Division, Ramsey, NJ), a Rheodyne sample valve equipped with a 20 μl loop (Rheodyne, Cotati, CA), a Spectroflow 773 Absorbance Detector (Kratos, Ramsey, NJ), and a Hewlett-Packard 3390A Reporting Integrator (Hewlett-Packard, Avondale, PA). The HPLC column was a μ-Bondapak C_{18} (30 cm × 3.9 mm i.d.) (Waters, Bedford, MA). All pH readings were carried out on a digital pH/millivolt meter Model 601 (Orion Research, Cambridge, MA).

2.3. N-(2′-Tetrahydropyran)benzamide (1)

To a solution of benzamide (6.05 g, 50 mmol) and a catalytic amount of *p*-toluenesulfonic acid (50 mg) in chloroform (100 ml) was added 3,4-dihydro-2H-pyran (10 ml, 110 mmol). The reaction mixture was stirred at room temperature overnight and then washed with water (2 × 50 ml), dried over magnesium sulfate, filtered and evaporated. The resulting residue was purified on a silica gel column, by elution with ethyl acetate/hexane (2:3), and recrystallized from a mixture of ethyl acetate and hexane to afford the desired compound as white crystals (8.4 g, 82%), m.p. 127–128° C. ^{1}H-NMR ($CDCl_3$): δ 7.30–7.80 (m, 5H, aromatic H), 6.72–6.86 (d, 1H, NH), 5.22–5.35 (m, 1H, 1′-CH), 3.58–4.05 (m, 2H, 6′-CH_2), 1.42–1.96 (m, 6H, 3′-, 4′- and 5′-CH_2) ppm. ^{13}C-NMR ($CDCl_3$): δ 166.8 (CO), 133.9 (aromatic C), 131.4, 128.2, and 127.0 (3 × aromatic CH), 78.2 (2′-CH), 67.2 (6′-CH_2), 31.2, 24.8, and 22.7 (C3′-, C4′- and C5′-CH_2) ppm.

Anal. – Calcd for $C_{12}H_{15}NO_2$: C, 70.24; H, 7.32; N, 6.83%. Found: C, 70.08; H, 7.42; N, 6.83%.

2.4. N-(2′-Tetrahydropyran)-N-methylbenzamide (2)

To a mixture of 3,4-dihydro-2H-pyran (20 ml, 220 mmol), and *N*-methylbenzamide (2.0 g, 14.8 mmol) was added a catalytic amount of *p*-toluenesulfonic acid (50 mg). The reaction mixture was stirred at room temperature overnight, and then concentrated, and fractionated on a silica gel column by elution with acetone/hexane (1:2). The eluate containing compound **2** was evaporated under reduced pressure to afford the product as a viscous oil (2.82 g, 87%). ^{1}H-NMR ($CDCl_3$): δ 7.30–7.60 (m, 5H, aromatic H), 4.62 (m, 1H, 2′-CH), 3.20–4.10 (m, 2H, 6′-CH_2), 2.70–3.00 (3H, NCH_3), and 1.25–1.96 (m, 6H, 3′-, 4′- and 5′-CH_2) ppm. ^{13}C-NMR ($CDCl_3$): δ 171.6 (CO), 135.9 (aromatic C), 129.8, 128.1, and 126.8 (3 × aromatic CH), 86.1 (2′-CH), 67.7 (6′-CH_2),

29.8, 24.6, and 22.8 (3′-, 4′-, and 5′-CH_2), 27.8 (NCH_3) ppm.

Anal. – Calcd for $C_{13}H_{17}NO_2$: C, 71.20; H, 7.81; N, 6.39%. Found: C, 71.12; H, 7.86; N, 6.31%.

2.5. *N-(2′-Tetrahydropyran)nicotinamide (3)*

To a suspension of nicotinamide (2.0 g, 16.4 mmol) in toluene (100 ml), was added 3,4-dihydro-2H-pyran (10 ml, 110 mmol) and a catalytic amount of *p*-toluenesulfonic acid (50 mg). The mixture was refluxed overnight, the solvent evaporated under reduced pressure, and the product purified on a silica gel column (acetone/hexane, 1:1). The crude product was recrystallized from a mixture of ethyl acetate and hexane, to afford white crystals (2.60 g, 76.9%), m.p. 93–94° C. ^{1}H-NMR ($CDCl_3$): δ 9.03 (s, 1H, aromatic H), 7.25–8.70 (m, 4H, aromatic H and NH), 5.24–5.40 (m, 1H, 2′-CH), 3.56–4.08 (m, 2H, 6′-CH_2), 1.42–2.00 (m, 6H, 3′-, 4′-, and 5′-CH_2) ppm. ^{13}C-NMR ($CDCl_3$): δ 165.0 (CO), 151.9, 148.2, and 135.2 (3 × aromatic CH), 129.6 (aromatic C), 123.1 (aromatic CH), 78.3 (C2′-CH), 67.3 (6′-CH_2), 31.0, 24.8, and 22.6 (C3′-, C4′- and C6′-CH_2) ppm.

Anal. – Calcd for $C_{11}H_{14}N_2O_2$: C, 64.08; H, 6.80; N, 13.59%. Found C, 64.16; H, 6.87; N, 13.65%.

2.6. *N-(2′-Tetrahydropyran)-N-methylnicotinamide (4)*

To a suspension of *N*-methylnicotinamide (2.0 g, 14.7 mmol) in toluene (100 ml) was added 3,4-dihydro-2H-pyran (15 ml, 165 mmol), and a catalytic amount of *p*-toluenesulfonic acid (50 mg). The mixture was refluxed for 7 days, then evaporated to dryness under reduced pressure and purified on a silica gel column, eluting with acetone/hexane (1:1), to afford **4** as a light yellow oil (0.2 g, 6.2%). The product could not be obtained in crystalline form, and elemental analyses obtained were unsatisfactory. However, purity of the dried, solvent-free oil, as estimated by HPLC analysis and UV spectrophotometry in dioxane, was determined to be better than 98% area percent. ^{1}H-NMR ($CDCl_3$): δ 7.20–8.80 (m, 4H, aromatic H), 4.38–4.60 (m, 1H, 2′-CH), 3.10–4.05 (m, 2H, 6′-CH_2), 3.05 (m, 3H, NCH_3), 1.2–2.0 (m, 6H, 3′-, 4′- and 5′-CH_2) ppm. ^{13}C-NMR ($CDCl_3$): δ 169.2 (CO), 150.7, 147.8, and 134.8 (3 × aromatic CH), 131.8 (aromatic C), 123.1 (aromatic CH), 86.3 (2′-CH), 67.7 (C′-CH_2), 29.1, 24.5, and 22.8 (C3′-, C4′- and C5′-CH_2), 27.9 (NCH_3) ppm.

2.7. *Analytical procedure*

The degradation kinetics of compounds **1–4** were followed by HPLC. Detection of compounds **1** and **2** was carried out at 240 nm, whereas detection at 260 nm was utilized for compounds **3** and **4**. The mobile phase utilized for the analyses consisted of a mixture of 0.05 M phosphate buffer at pH 4.0 and acetonitrile (3:1 for the analysis of

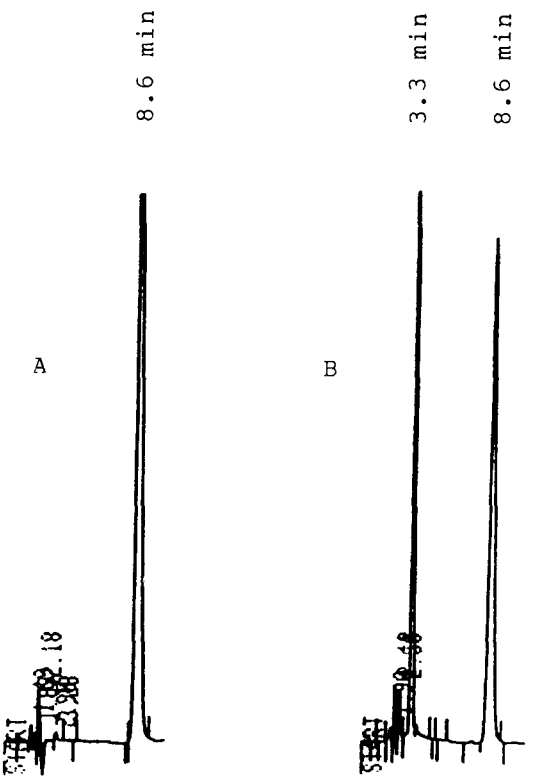


Fig. 1. HPLC chromatogram of **2** in 0.05 M phosphate buffer, pH 3.0 at 37° C at time 0 min (A) and after 2 h (B). The peak with a retention time of 3.3 min represents *N*-methylbenzamide, while that with a retention time of 8.6 min represents compound **2**.

1 and 2, and 4:1 for the analysis of 3 and 4). The flow rate was 1.0 ml/min. Fig. 1 shows a typical HPLC chromatogram for the hydrolysis of 1 at time 0 min and after 2 h, both at pH 3.0.

2.8. Kinetic measurements

All kinetic studies were performed in aqueous solutions at 37° C. The buffers were 0.05, 0.1 and 0.2 M phosphate buffer, and pH values were adjusted to the desired value with 0.1 N HCl or 0.1 N NaOH. A stock solution containing 1 mg/ml in methanol of each of the four compounds was prepared and 0.1 ml of this stock solution was mixed with 9.9 ml of the desired buffer, which was equilibrated at 37° C, to initiate the degradation. 1 ml of each of the solutions was withdrawn at suitable time intervals and the degradation was quenched by freezing in dry ice-methanol. Samples were then analyzed by direct injection onto the HPLC column.

3. Results and discussion

The hydrolyses of amidals 1, 2 and 4 were found to follow first-order kinetics, and pseudo first-order rate constants were determined from the disappearance of the compounds as a function of time. Amidal 3 was found to be resistant to acid-catalyzed degradation at pH 3, in 0.05 M phosphate buffer at 37° C. The effect of buffer concentration on the rate of hydrolysis was studied using compound 2 as a model. As shown in Fig. 2 and Table 1, significant buffer catalysis was only observed in the acidic pH range (e.g., pH 2.4), whereas no buffer effect was observed in the neutral pH range (e.g., pH 6.0). This indicates that the degradation of 2 is catalyzed not only by specific acid catalysis but also by a general acid catalysis, involving the unionized buffer species, H_3PO_4. The second-order rate constant calculated for the involvement of H_3PO_4 was approx. 4 M^{-1} h^{-1}. The data in Table 1 also show that the rate of decomposition of 2 was dependent upon hydrogen ion concentration.

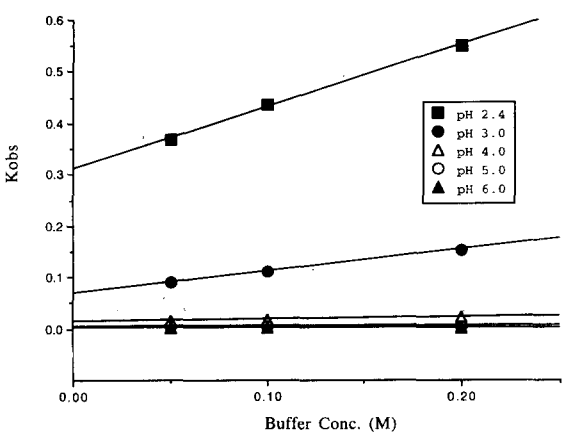
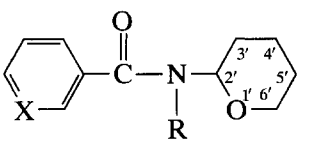


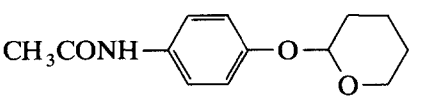
Fig. 2. Effect of buffer concentrations on the observed hydrolysis rate constants of compound 2 at different pH values.

Table 1
Observed hydrolysis rate constants (k_{obs}) of compound 2 at different pH values and various phosphate buffer concentrations

[Buffer] (M)	k_{obs} ($\times 10^{-3}$) (h^{-1})				
	pH 2.4	pH 3.0	pH 4.0	pH 5.0	pH 6.0
0.05	368	89.8	15.7	4.1	0.41
0.10	437	111	17.9	4.9	0.41
0.20	551	153	21.9	5.0	0.41
k_o	311	72.9	13.7	4.0	0.41



1: X = CH, R = H
2: X = CH, R = CH_3
3: X = N, R = H
4: X = N, R = CH_3



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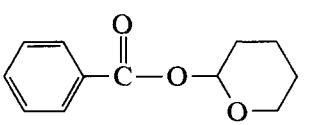

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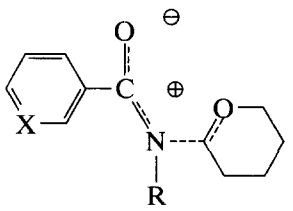

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a) X = CH, R = H

b) X = $\overset{+}{N}$H, R = H

c) X = CH, R = CH_3

d) X = $\overset{+}{N}$H, R = CH_3

In order to compare the rates of hydrolyses of compounds **1–4**, the degradation of all four compounds was studied at pH 3.0 in 0.05 M phosphate buffer at 37° C. The data obtained are shown in Table 2. It can be clearly seen that introduction of an *N*-methyl group into the carboxamide moiety of compounds **1** and **3**, to afford **2** and **4**, respectively, has a marked accelerating effect on the rate of acid-catalyzed hydrolysis. However, substitution of a 3-pyridyl group for the phenyl group in compounds **1** and **2**, resulted in compounds having greater stability towards acid-catalyzed degradation.

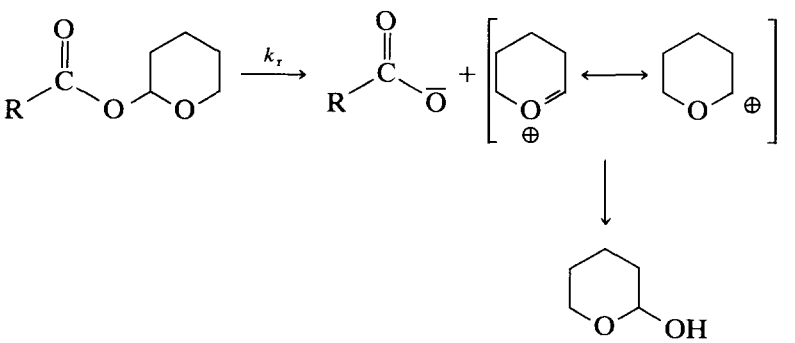
In previous communications (Hussain et al., 1973; Repta and Hack, 1973), it was shown that compounds of structures **5** and **6**, prepared from the corresponding phenol or carboxylic acid and 2,3-dihydro-2H-pyran, undergo rapid hydrolysis, especially in acidic media (see Table 2). For example, the acetamenophen acetal **5** has a half-life of less than 20 min at pH 3, whereas the corresponding benzoic acid derivative **6**, has a half-life

Table 2
Comparison of observed hydrolysis rate constants and half-lives of compounds **1–6** in 0.05 M phosphate buffer at pH 3.0 and 37° C

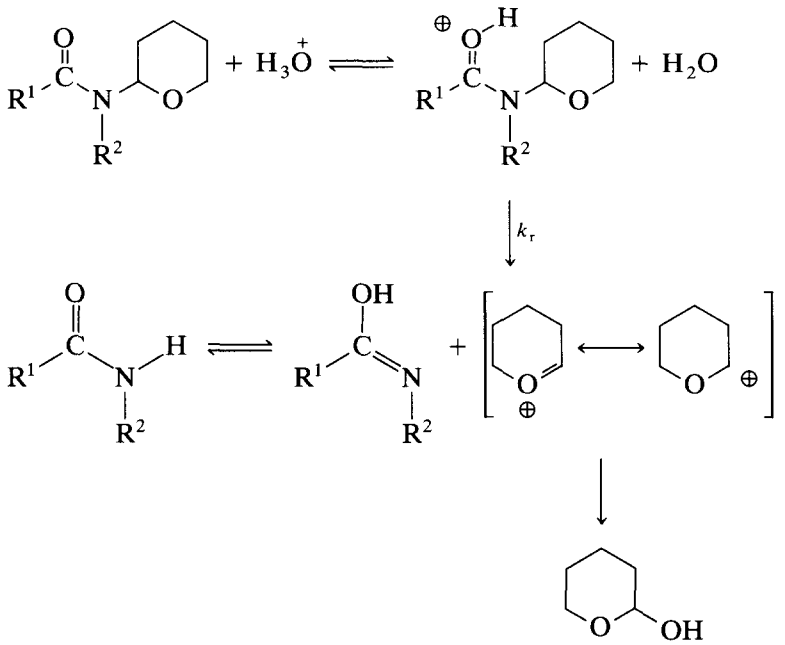
Compounds	k_{obs}	Half-life
1	7.0×10^{-3} day^{-1}	98.4 days
2	2.5×10^{-5} s^{-1}	7.7 h
3	n.d. [a]	no degradation within 2 weeks
4	3.9×10^{-6} s^{-1}	49.5 h
5	6.6×10^{-4} s^{-1}	17.5 min
6	4.5×10^{-2} s^{-1}	15.5 s

[a] Not determinable, decomposition too slow.

of seconds at the same pH. It is well-known that the rate of hydrolysis of acetals such as **5** is pH-dependent and proceeds via protonation of the acetal O atom, followed by unimolecular cleavage of the C-O bond. Factors which govern the rate of degradation are the presence of electron-donating or withdrawing substituents in the phenyl ring, and the relative stability of the generated carbonium ion. On the other hand, the hydrolysis of acylals such as **6** is generally first-order with a non-dependency on acid catalysis over the pH range 3–9 (Hussain et al., 1974, 1979; Hussain and Truelove, 1979). This is attributable to the carboxylate anion being a good leaving group, since it is resonance stabilized (see Scheme 1). Amidals formed from 2,3-dihydro-2H-pyran are much more stable to acid hydrolysis than either acetals or acylals. This can be attributed to the carboxamide moiety being a poor leaving group, making cleavage of the 2′-C-N bond difficult. The mechanism of degradation most likely involves an initial pre-equilibrium



Scheme 1.



Scheme 2.

protonation by hydroxonium ion followed by a rate-determining unimolecular decomposition to afford the enol form of the amide and the resonance-stabilized carbonium (see Scheme 2). The rate-enhancing effect of *N*-methylation results from the inductive effect of the methyl group, which stabilizes the transition state **7**; in addition, the presence of the *N*-methyl group also renders the carboxamide group more basic, and hence more susceptible to protonation. The relative stability of the 3-pyridyl amidals compared to their phenyl counterparts, at pH 3, is most likely due to these amidals existing as protonated pyridinium species under the conditions studied, since the pK_a of the pyridinium moiety is expected to be in the range 3–5. Such protonation would significantly decrease the basicity of these amidals, and prevent protonation of the carboxamide oxygen atom.

In conclusion, the greater stability of amidals to acid-base hydrolytic cleavage, compared to acetals, over a wide range of pH, makes their utility as prodrugs of amido drugs less attractive. However, it may be that these derivatives could be cleaved enzymatically, generating the parent compound. Work is underway to examine the stability of these compounds in biological fluids.

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