Species-Specific Hydrolysis Kinetics of *N*-Methylated Heroin Derivatives

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The hydroxide-catalyzed hydrolysis of 3,6-diacetylmorphine (heroin) was shown to take place predominantly *via* its positively charged form. *N*-Methylated quaternary derivatives of heroin bearing a permanent positive charge were synthesized, and thus, hydrolysis kinetics of these cationic species could be studied over a wide pH range. Specific rate equations were introduced to characterize either the simultaneous or the consecutive decompositions. The kinetic constants determined for the diester are distinctive for the site of hydrolysis. The rate of 6-acetyl-*N*-methylmorphine was quantified in terms of microscopic kinetic constants of hydrolysis, in which the protonation state of the phenolic OH group had also been taken into account. The site-specific data indicate that the 3-acetoxy moiety is hydrolyzed 6–12 times faster than the 6-acetoxy function. The latter, previously ignored minor pathway was shown to represent a non-negligible 10% of the overall decomposition process. Protonation of the 3-O^- site accelerates the rate of hydrolysis of the 6-acetoxy moiety by a factor of 4, and replacement of the adjacent OH group by MeO or AcO substituents slows the rate of hydrolysis slightly, presumably due to the increased local hydrophobicity caused by the alkyl or acyl moiety.

Introduction. – 3,6-Diacetylmorphine (diamorphine, heroin) is one of the most widely studied chemical entities, mainly due to its narcotic activity with high abuse potential and the related therapeutic, analgesic use in final stages of malignant forms of cancer [1–10].

The chemical studies on heroin have largely been motivated by its pharmacological significance, in the hope that a thorough understanding of its biological behavior at the molecular level might result in the development of compounds with the desired therapeutic activity but without the infamous abuse capacity.

Pharmacokinetic investigations revealed that the concentration of heroin in the blood drops below the detection level after 20 min [5], but its various biological activities last much longer. The biological effects of heroin set in faster than those of morphine [3], and the major metabolite morphine-6-glucuronide is also active. These observations indicate that *a*) heroin undergoes fast hydrolysis in the body; *b*) the receptor-binding entity is presumably not heroin, but one (or some) of its decomposition products [4]; *c*) the diacetylated derivative, however, exhibits higher bioavailability and better transport properties than morphine.

These observations initiated several studies on the hydrolysis of heroin. The effect of temperature and pH on its deacetylation was investigated in aqueous solution and in human plasma, and half-life values were determined [6]. Various chromatographic techniques were developed to optimize stability in drug mixtures [7] and to characterize the pH-rate profile of hydrolysis [8]. The metabolic products of heroin

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were identified from urine [9], and the long-term effects of storage time and temperature were analyzed in pharmaceutical products [10].

A uniform feature of all these studies is that the major hydrolysis pathway (diacetylmorphine → 6-acetylmorphine → morphine) has been taken into consideration as the only decomposition route, despite the fact that also other intermediates are involved in the deacetylation of heroin to morphine [11]. Moreover, no literature information is available on the charge and molecular state of the reactive species in the hydroxide-catalyzed hydrolysis. This shortcoming is especially troublesome in view of two considerations: *1*) The tertiary amino group of diacetylmorphine has a pK_a value of 8.16. Thus, 85% of the heroin molecules occur in positively charged form in blood (pH 7.4). *2*) A positive charge on an adjacent moiety can accelerate the ester hydrolysis by a factor of up to 130 [12–20]. No reliable report can be found on any opposite effects.

The conclusion drawn from the above-mentioned arguments is that heroin hydrolyzes predominantly *via* its cationic form, at least up to a pH value of 8.16. A significant participation of the cationic form in the hydrolytic process can be postulated at $pH > 8.16$ as well.

Although the rate-acceleration upon cation formation is undisputed in the literature, its extent is controversial, as shown by conflicting data from different sources (see data in [13][16] and [19]).

However, these inconsistencies might be caused by the fact that kinetic studies on OH^--catalyzed ester hydrolysis of *N*-protonated compounds typically suffer from two types of problems. On the one hand, at low pH, where the protonated form becomes predominant, the OH^- concentration and, consequently, the rate of hydrolysis becomes low. Thus, a tangible decomposition takes days or weeks, leading to inevitable experimental and evaluation difficulties. At high pH, on the other hand, the mole fraction of the deprotonated form is high, and its contribution to the overall rate is, therefore, significant, even though its inherent propensity to hydrolyze is lower than that of its protonated counterpart. Hence, the observed effect is a composite one. Nevertheless, it can be transformed into component rate constants on the basis of sound theoretical background and carefully chosen experimental conditions. Literature data show, however, that meeting these criteria is rather the exception than the case [12–17][20].

Rate constants purely specific to the cationic form can be directly obtained when the N-atom is quaternized. Although a quaternary N-atom is somewhat different from a protonated tertiary N-atom, their inductive effects on the rest of the molecule are very similar [21], especially in rigid molecules such as morphine and its derivatives.

The hydrolytic behavior of acyloxy-substituted quaternary ammonium compounds can be studied over a wide pH-range, providing a sound experimental basis for the evaluation of the specific rate constants in various cases.

Here, we report the rate constants for ester hydrolysis of 3,6-diacetyl-*N*-methylmorphine, 3-acetyl-*N*-methylmorphine, 6-acetyl-*N*-methylmorphine, and 6-acetyl-*N*-methylcodeine. All kinetic parameters were expressed either in terms of pH-dependent, conditional rate constants or in terms of pH-independent ones. The rate constants for 3,6-diacetyl-*N*-methylmorphine were specifically determined for the decomposition pathways *via* the 3-acetyl- and 6-acetyl-derivatives, respectively. On the other hand, the rate constants of 6-acetyl-*N*-methylmorphine were determined separately for the two protonation forms (*viz*. PhO^- and PhOH) of the parent compound.

These investigations required the introduction and elaboration of specific sets of equations for the evaluation, the preparation of previously undescribed compounds, such as 6-acetyl-*N*-methylmorphine and 3-acetyl-*N*-methylmorphine, the development of an unbiased capillary-electrophoretic technique to simultaneously measure the reactant and product concentrations in reaction mixtures, and the determination of acid-base equilibrium constants, which either characterized the basicity of some of the compounds studied, or served as independent component parameters in the rate equations.

Experimental Part

Materials and Methods. Na_2HPO_4, NaH_2PO_4, NaOH and H_3PO_4 were of analytical grade from *Reanal Chemical Co.* $Na_2B_4O_7$ was from *Sigma*, potassium tetraoxalate from *Merck*, potassium hydrogen phthalate and sodium dodecyl sulfate (SDS) were from *Fluka*. Bidistilled water was used to prepare all buffers.

TLC: *Merck DC 5562* silica gel *60* F_{256}; mobile phases: *a*) $CHCl_3$/MeOH 9:1, *b)* C_6H_6/MeOH 8:2, *c*) $CHCl_3$/acetone/Et_2NH 5:4:1. M.p.: *Büchi 510*; uncorrected; for values, see *Table 1*. NMR: *Varian-Gemini* instrument at 200 MHz; for values, see *Table 1*.

Table 1. *Melting Points and ^1H-NMR Spectroscopic Data of* N-*Methylated Morphine Esters*

	Compound	3-Acetyl-*N*-methylmorphine iodide	6-Acetyl-*N*-methylmorphine iodide	Diacetyl-*N*-methylmorphine iodide	6-Acetyl-*N*-methylcodeine iodide
	Formula	$C_{20}H_{24}INO_3$	$C_{20}H_{24}O_3IN$	$C_{22}H_{26}INO_5$	$C_{21}H_{26}INO_4$
	Melting point	236–238°	258–260°	249–251°	252–253°
	NMR solvent	D_2O	(D_6)DMSO	(D_6)DMSO	(D_6)DMSO
^1H-NMR chemical shifts	H–C(1) H–C(2)	6.9–6.78 (*dd*)	6.55–6.45 (*dd*)	6.9–6.7 (*dd*)	6.8–6.63 (*dd*)
	H–C(5)	5.10 (*d*)	5.10 (*d*)	5.16 (*d*)	5.10 (*d*)
	H–C(6)	5.25 (*m*)	5.25 (*m*)	5.25 (*m*)	5.25 (*m*)
	H–C(7)	5.72 (*m*)	5.65 (*m*)	5.66 (*m*)	5.69 (*m*)
	H–C(8)	5.40 (*m*)	5.35 (*m*)	5.50 (*m*)	5.50 (*m*)
	Me–N (ax.)	3.40 (*s*)	3.40 (*s*)	3.41 (*s*)	3.42 (*s*)
	Me–N (eq.)	3.30 (*s*)	3.28 (*s*)	3.30 (*s*)	3.30 (*s*)
	MeCOOC(3)	2.30 (*s*)	–	2.23 (*s*)	–
	MeOC(3)	–	–	–	3.78 (*s*)
	MeCOOC(6)	–	2.10 (*s*)	2.08 (*s*)	2.10 (*s*)

Morphine derivatives of drug-standard quality (3-acetyl-*N*-methylmorphine iodide (=3ANmM), 6-acetyl-*N*-methylmorphine iodide (=6ANmM), diacetyl-*N*-methylmorphine iodide (=DANmM), 6-acetyl-*N*-methylcodeine (=6ANmC)) were synthesized as described below. 3-Acetylmorphine, 6-acetylmorphine, diacetylmorphine, and 6-acetylcodeine were synthesized according to the methods of *Welsh* [22] and *Wright* [23]. *N*-Methyl-derivatives were prepared from the respective morphine esters: the ester (1 g) was dissolved in MeOH and MeI (10 ml) was added to the soln. which was stirred at 40° for 3 h. The precipitated product was filtered and washed with MeOH. Each crystallized product was found to be homogeneous in TLC tests with mobile phases *a*, *b*, and *c*, respectively. M.p.: see *Table 1*.

pH-Potentiometry: protonation-equilibrium constants were determined with a *Radiometer ABU91* titrator equipped with a *Radiometer PHC2406* combination pH-electrode; concentrations of solns. were 0.03–0.05M; all experiments were performed at 298 ± 0.1 K at 0.2M ionic strength with NaCl as auxiliary electrolyte; all pH-meter readings relative to *NBS* primary standards: 0.05M potassium hydrogen phthalate (pH 4.005), 0.025M KH_2PH_4 + 0.025M Na_2HPO_4 (pH 6.865), 0.01M $Na_2B_4O_7$ (pH 9.180), 0.05M potassium tetraoxalate buffer (pH 1.780).

Cap. electrophoresis: *Unicam Crystal 310 CE* system with automated sampling and thermostat, equipped with a *BioRad* 375-µm o.d., 50-µ-m i.d. fused-silica capillary (65 cm, 50 cm effective length); samples were injected hydrodynamically at 50 mbar for 0.02 min for all runs; a voltage of 25 kV was applied during analysis, and the current did not exceed 45 µA; on-column UV-detection at 210 nm; data collection and handling with a *Unicam 4880* system; the auto-sampling unit was air-thermostated to $15 \pm 0.5°$; the capillary was flushed with 0.1M NaOH soln. for 5 min every day, then running buffer was used to rinse the capillary for 10 min, and between runs, the capillary was flushed with fresh buffer for 4 min; mobile phase: 10 mM NaH_2PO_4, 10 mM $Na_2B_4O_7$ and 25 mM SDS; running-buffer pH was set to 7.75 (lowest pH to obtain baseline separation for diacetyl-*N*-methylmorphine and its degradation products), and under these conditions, no hydrolysis during analysis was observed; non-linear parameter fitting was performed with *STATISTICA 5.1* for *Windows*.

Hydroxyde-catalyzed hydrolysis experiments were carried out in buffer solns. containing 0.05M NaH_2PO_4 and 0.025M $Na_2B_4O_7$ at different pH values (see *Tables 2–5*). 10–12 mg substance of each morphine-derivative ester was separately dissolved in 23.0 ml buffer soln. The pH was adjusted with 2M NaOH or 2M H_3PO_4 solns.

Table 2. *Conditional* (k'_{3ANmM}) and pH-Independent (k_{3ANmM}) *Ester-Hydrolysis Rate Constants* for 3-*Acetyl*-N-*methylmorphine*

pH	k'_{3ANmM} [s^{-1}]
8.87	$6.35 \cdot 10^{-5} \pm 6.24 \cdot 10^{-6}$
9.08	$1.11 \cdot 10^{-4} \pm 1.165 \cdot 10^{-5}$
9.19	$9.99 \cdot 10^{-5} \pm 6.70 \cdot 10^{-6}$
9.40	$1.92 \cdot 10^{-4} \pm 1.61 \cdot 10^{-5}$
$k_{3ANmM} = 7.97 \pm 1.21$ s^{-1}	

Table 3. *Conditional* (k'_{6ANmC}) *and pH-Independent* (k_{6ANmC}) *Ester-Hydrolysis Rate Constants for 6-Acetyl*-N-*methylcodeine*

pH	k'_{3ANmC} [s^{-1}]
10.35	$6.81 \cdot 10^{-5} \pm 4.64 \cdot 10^{-6}$
10.60	$1.08 \cdot 10^{-4} \pm 6.02 \cdot 10^{-6}$
10.63	$1.26 \cdot 10^{-4} \pm 1.13 \cdot 10^{-5}$
10.85	$6.81 \cdot 10^{-5} \pm 8.79 \cdot 10^{-6}$
$k_{6ANmC} = 0.291 \pm 0.013$ s^{-1}	

Table 4. *Conditional* (k'_{6ANmM}) *and pH-Independent* (k_{06ANmM} *and* k_{+6ANmM}) Species-Specific *Ester-Hydrolysis Rate Constants for 6-Acetyl*-N-*methylmorphine*

pH	k'_{6ANmM} [s^{-1}]
7.84	$1.03 \cdot 10^{-6} \pm 1.88 \cdot 10^{-7}$
8.13	$1.63 \cdot 10^{-6} \pm 2.27 \cdot 10^{-7}$
8.38	$1.17 \cdot 10^{-6} \pm 3.26 \cdot 10^{-7}$
8.69	$4.41 \cdot 10^{-6} \pm 3.93 \cdot 10^{-7}$
9.02	$6.17 \cdot 10^{-6} \pm 6.73 \cdot 10^{-7}$
9.33	$1.26 \cdot 10^{-5} \pm 3.53 \cdot 10^{-7}$
9.41	$1.80 \cdot 10^{-5} \pm 6.20 \cdot 10^{-7}$
9.86	$3.35 \cdot 10^{-5} \pm 8.87 \cdot 10^{-7}$
10.25	$6.31 \cdot 10^{-5} \pm 3.34 \cdot 10^{-6}$
10.60	$1.51 \cdot 10^{-4} \pm 7.34 \cdot 10^{-6}$
10.73	$2.09 \cdot 10^{-4} \pm 1.27 \cdot 10^{-5}$
$k_{06ANmM} = 0.375 \pm 0.007$ s^{-1}	
$k_{+6ANmM} = 1.45 \pm 0.49$ s^{-1}	

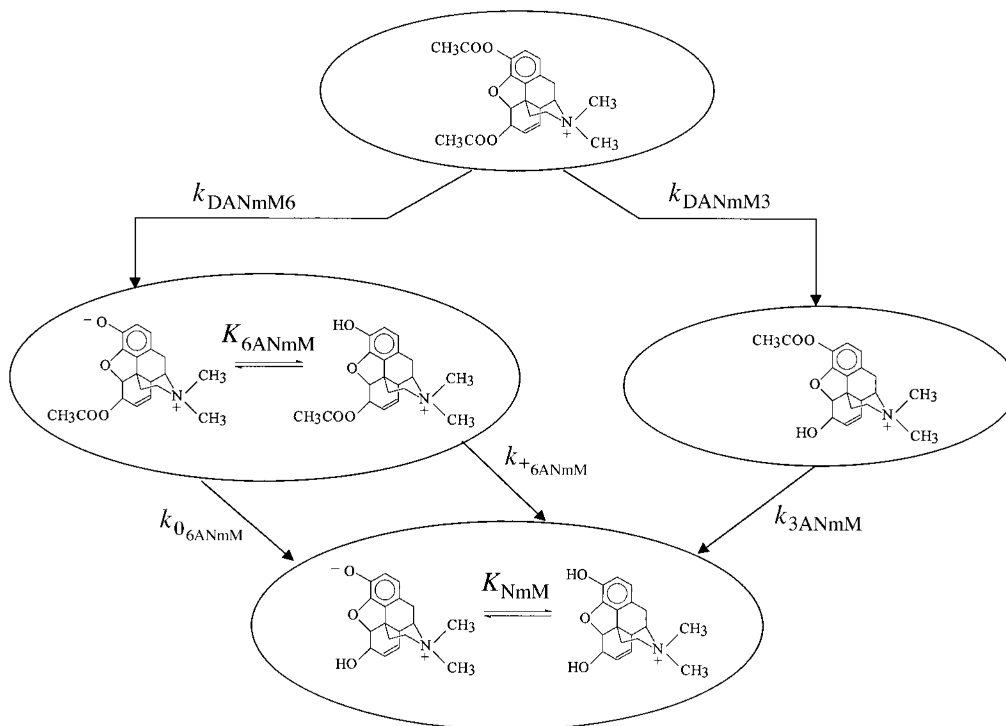
Table 5. *Conditional* (k'_{DANmM6} and k'_{DANmM3}) *and pH-Independent* (k_{DANmM6} and k_{DANmM3}) *Ester-Hydrolysis Rate Constants for Diacetyl-*N*-methylmorphine*

pH	k'_{DaNmM6} [s^{-1}]	k'_{DANmM3} [s^{-1}]
9.38	$1.13 \cdot 10^{-4} \pm 7.93 \cdot 10^{-6}$	$7.82 \cdot 10^{-6} \pm 1.74 \cdot 10^{-6}$
9.54	$1.02 \cdot 10^{-4} \pm 9.68 \cdot 10^{-6}$	$7.45 \cdot 10^{-6} \pm 1.65 \cdot 10^{-6}$
9.60	$1.43 \cdot 10^{-4} \pm 1.17 \cdot 10^{-5}$	$1.39 \cdot 10^{-5} \pm 2.37 \cdot 10^{-6}$
9.63	$1.41 \cdot 10^{-4} \pm 2.24 \cdot 10^{-5}$	$1.53 \cdot 10^{-5} \pm 2.39 \cdot 10^{-6}$
$k_{DANmM6} = 3.62 \pm 0.76\ s^{-1}$		
$k_{DANmM3} = 0.312 \pm 0.066\ s^{-1}$		

keeping the ionic strength at 0.2M with NaCl as auxiliary electrolyte. The progress of hydrolysis was followed by validated capillary electrophoretic methods [27] until at least 50% decomposition at $25 \pm 0.1°$, taking 1.00 ml time-incremented samples out of the reaction mixture and mixing them with 1.00 ml of the mobile phase.

Results and Discussion. – The complete hydrolysis scheme of 3,6-diacetyl-*N*-methylmorphine is depicted in the *Scheme*. For 6-acetyl-*N*-methylmorphine and *N*-methylmorphine, where the free phenolic OH group occurs in two acidity forms, protonation-equilibrium constants also had to be considered. Besides the structures in the *Scheme*, 6-acetyl-*N*-methylcodeine was also studied.

Scheme. *Hydrolysis and Protonation Scheme for 3,6-Diacetyl-*N*-methylmorphine and Its Derivatives.* Constants outside the ellipses, on the one-way arrows, are specific rate constants, whereas those inside the ellipses, on the double arrows, are acid-base equilibrium constants.



Taking into account the types of the hydrolytic processes and the molecules studied, the rate equations were sorted into three classes. Thus, the evaluation procedure was based on the following rate equations and considerations:

1) One ester group, no protonation site on the molecule (*i.e.*, 3-acetyl-*N*-methylmorphine and 6-acetyl-*N*-methylcodeine).

This simplest case uses the routine, second-order rate equation

$$-\frac{\mathrm{d}[\mathrm{e}]}{\mathrm{d}t} = k[\mathrm{e}][\mathrm{OH}^-] \quad (1)$$

where [e] is the ester concentration at any instant, t is time in s. Integration and rearrangement yields:

$$k = \frac{\ln\frac{[\mathrm{e}]_{\mathrm{init}}}{[\mathrm{e}]_t}}{t[\mathrm{OH}^-]} \quad (2)$$

where $[\mathrm{e}]_{\mathrm{init}}$ and $[\mathrm{e}]_t$ are ester concentrations after zero and t seconds in the course of hydrolysis, respectively.

2) One ester group, one protonation site (*i.e.*, 6-acetyl-*N*-methylmorphine).

The phenolic site of 6-acetylmorphine exists in anionic and neutral forms during the hydrolysis, providing the molecule with the corresponding ± 0 and $+1$ gross charges. The differently charged molecules are obviously hydrolyzed at different rates, which can be characterized in terms of the respective $k_{06\mathrm{ANmM}}$ and $k_{+6\mathrm{ANmM}}$ kinetic constants in rate *Eqn. 3:*

$$-\frac{\mathrm{d}[6\mathrm{ANmM}]}{\mathrm{d}t} = k_{0_{6\mathrm{ANmM}}}[6\mathrm{ANmM}_0]\cdot[\mathrm{OH}^-] + k_{+_{6\mathrm{ANmM}}}[6\mathrm{ANmM}_+]\cdot[\mathrm{OH}^-] \quad (3)$$

The concentration of the neutral and cationic forms of the 6-acetyl-*N*-methylmorphine molecule can be given as the product α, the charge-specific mole fraction, and c_{ANmM}, the total concentration:

$$[6\mathrm{ANmM}_0] = \alpha_{0_{6\mathrm{ANmM}}}\cdot c_{6\mathrm{ANmM}} \quad (4)$$

$$[6\mathrm{ANmM}_+] = \alpha_{+_{6\mathrm{ANmM}}}\cdot c_{6\mathrm{ANmM}} \quad (5)$$

where protonation mole fractions can be calculated from species and proton concentrations and protonation constants (K) as follows:

$$\alpha_{0_{6\mathrm{ANmM}}} = \frac{[6\mathrm{ANmM}_0]}{[6\mathrm{ANmM}_0]+[6\mathrm{ANmM}_+]} = \frac{1}{1+K_{6\mathrm{ANmM}}\cdot[\mathrm{H}^+]} \quad (6)$$

$$\alpha_{+_{6\mathrm{ANmM}}} = \frac{[6\mathrm{ANmM}_+]}{[6\mathrm{ANmM}_0]+[6\mathrm{ANmM}_+]} = \frac{K_{6\mathrm{ANmM}}\cdot[\mathrm{H}^+]}{1+K_{6\mathrm{ANmM}}\cdot[\mathrm{H}^+]} \quad (7)$$

Introducing *Eqns. 4–7* into *Eqn. 3*, integration, and rearrangement yield:

$$\ln\frac{[6\mathrm{ANmM}]_{\mathrm{init}}}{[6\mathrm{ANmM}]_t}\cdot\frac{1+K_{6\mathrm{ANmM}}\cdot[\mathrm{H}^+]}{t[\mathrm{OH}^-]} = k_{0_{6\mathrm{ANmM}}} + k_{+_{6\mathrm{ANmM}}}\cdot K_{6\mathrm{ANmM}}\cdot[\mathrm{H}^+] \quad (8)$$

where the protonation constant is determined in an independent, pH-potentiometric experiment (log $K_{6ANmM} = 8.56$). Every other term at the left-hand side of *Eqn. 8* is known from the kinetic experiments. The k_{06ANmM} and k_{+6ANmM} constants can be obtained by non-linear parameter fitting (see *Fig. 2*), if the progress of hydrolysis is monitored at distinct pH-values. These kinetic studies have been carried out in the log $K \pm 2$ pH-range, where both the protonated and unprotonated forms of 6-acetyl-*N*-methylmorphine occur in measurable amounts.

3) Two ester groups, no protonation site (3,6-diacetyl-*N*-methylmorphine).

The hydrolysis of *N*-methylheroin contains both simultaneous and consecutive kinetic elements. The first step is an alternative simultaneous one, producing one of the two isomeric monoester intermediates (3-acetyl-*N*-methylmorphine and 6-acetyl-*N*-methylmorphine). Either of them is subsequently hydrolyzed in a consecutive manner, resulting uniformly in *N*-methylmorphine, the only major end product.

Concerning the first-step hydrolyses, the rate of DANmM hydrolysis can be formulated as follows:

$$-\frac{\mathrm{d[DANmM]}}{\mathrm{d}t} = k_{\mathrm{DANmM}_6} \cdot [\mathrm{DANmM}] \cdot [\mathrm{OH}^-] + k_{\mathrm{DANmM}_3} \cdot [\mathrm{DANmM}] \cdot [\mathrm{OH}^-] \quad (9)$$

The concentration of the isomeric, intermediate monoesters depends on their formation upon hydrolysis of the diester and also on the concluding decomposition, yielding *N*-methylmorphine. The pertinent, second-step rate equations are shown in *Eqns. 10* and *11:*

$$-\frac{\mathrm{d[6ANmM]}}{\mathrm{d}t} = k_{\mathrm{DANmM}_6} \cdot [\mathrm{DANmM}] \cdot [\mathrm{OH}^-] - k_{\mathrm{6ANmM}} \cdot [\mathrm{6ANmM}] \cdot [\mathrm{OH}^-] \quad (10)$$

$$-\frac{\mathrm{d[3ANmM]}}{\mathrm{d}t} = k_{\mathrm{DANmM}_3} \cdot [\mathrm{DANmM}] \cdot [\mathrm{OH}^-] - k_{\mathrm{3ANmM}} \cdot [\mathrm{3ANmM}] \cdot [\mathrm{OH}^-] \quad (11)$$

Rate *Eqns. 10* and *11* can be integrated and rearranged as in [24], yielding *Eqns. 12* and *13:*

$$\frac{[\mathrm{6ANmM}]_t}{[\mathrm{DANmM}]_{\mathrm{init}}} = \frac{k'_{\mathrm{DANmM6}}}{k'_{\mathrm{DANmM6}} + k'_{\mathrm{6ANmM}}} \cdot (\mathrm{e}^{-k'_{\mathrm{DANmM6}} \cdot t} - \mathrm{e}^{-k'_{\mathrm{6ANmM}} \cdot t}) \quad (12)$$

$$\frac{[\mathrm{3ANmM}]_t}{[\mathrm{DANmM}]_{\mathrm{init}}} = \frac{k'_{\mathrm{DANmM3}}}{k'_{\mathrm{DANmM3}} + k'_{\mathrm{3ANmM}}} \cdot (\mathrm{e}^{-k'_{\mathrm{DANmM3}} \cdot t} - \mathrm{e}^{-k'_{\mathrm{3ANmM}} \cdot t}) \quad (13)$$

where primed rate constants are pH-dependent (conditional) ones. The concentrations $[\mathrm{6ANmM}]_t$ and $[\mathrm{3ANmM}]_t$ are analytical data obtained by the capillary-electrophoresis micellary electrokinetic experiments at various t values. The parameters k'_{DANmM6} and k'_{DANmM3} can be obtained by non-linear fitting, where k'_{3ANmM}, k'_{06ANmM}, and k'_{+6ANmM} have previously been determined as described (*vide supra*). The rate constant k'_{6ANmM} is still pH-dependent, composed of known elements of basicity (α_{06ANmM} and α_{+6ANmM}, *Eqns. 6* and *7*), rate (k_{06ANmM} and k_{+6ANmM}, *Eqn. 8*), and hydroxide concentration, as shown in *Eqn. 14:*

$$k'_{\mathrm{6ANmM}} = (\alpha_{0_{\mathrm{6ANmM}}} \cdot k_{0_{\mathrm{6ANmM}}} + \alpha_{+\mathrm{6ANmM}} \cdot k_{+_{\mathrm{6ANmM}}}) \cdot [\mathrm{OH}^-] \quad (14)$$

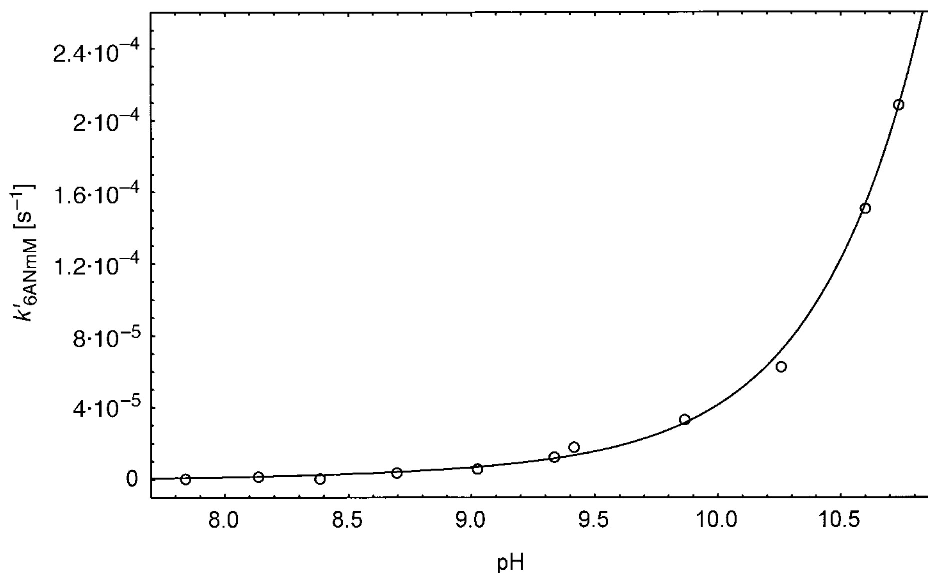


Figure. *The pH-dependence of* k'_{6ANmM}, *the conditional hydrolysis rate constant of 6-acetyl-*N*-methylmorphine.* Circles represent experimental data, the solid line has been obtained by non-linear curve-fitting using *Eqns. 12–14.*

Upon division by the actual OH^- concentration, all conditional rate constants provide the pH-independent kinetic parameters (*Fig.*).

Tables 2–5 contain the pH-dependent and pH-independent hydrolysis-rate constants of 3-acetyl-*N*-methylmorphine, 6-acetyl-*N*-methylcodeine, 6-acetyl-*N*-methylmorphine and 3,6-diacetyl-*N*-methylmorphine, respectively.

The uncertainty of the data ranges between 2% (k_{06ANmM}) and 34% (k_{+6ANmM}), with a typical value of 20% in the pH-independent rate constants. The largest error belongs to the data which needed a three-week-long hydrolysis experiment to achieve sufficient decomposition at relatively low pH, inevitably jeopardizing the stability of the experimental conditions. Taking into account that the data in *Tables 2–5* are delicate kinetic parameters, the small errors rather than the large ones are unusual. Conclusions are drawn from differences within the 95%-certainty limits only.

Comparison of the 3- *vs.* 6-Ac hydrolysis-rate constants can be made in two different data matches. The evident one compares the alternative first-step decomposition-rate constants of 3,6-diacetyl-*N*-methylmorphine. Here, k_{DANmM6} is 12 times larger than k_{DANmM3}, which indicates that 92% of the total first-step decompositions take place along the major hydrolysis pathway. The other assessment on the hydrolyzing propensity of the two sites can be made by comparing k_{3ANmM} and k_{+6ANmM}, in which the 3- and 6-OH groups of the parent compound are esterified, respectively, while the other site is in the OH form. These reciprocal data show a somewhat smaller, 85:15 ratio in favor of the 3-acetyl hydrolysis.

The k_{+6ANmM}/k_{06ANmM} ratio quantifes that protonation of the phenolate site accelerates the hydrolysis at the 6-Ac ester by a factor of 4. The k_{DANmM3} and k_{6ANmC}

values are very similar, providing evidence that the effects of 3-AcO and 3-MeO groups on the hydrolysis rate of the 6-Ac ester site are not significantly different.

The relations $k_{3ANmM} > k_{DANmM6}$ and $k_{+6ANmM} > k_{DANmM3} \approx k_{6ANmC}$ indicate that ester or ether groups slow ester hydrolysis, as compared to the corresponding hydroxy compounds. This phenomenon is apparently due to their local relative hydrophobic effects on the medium, since microscopic basicity determinations show [25][26] that the intramolecular electron-withdrawing and -donating effects of acids and their *O*-alkylated derivatives are almost identical.

The pharmacological implications of these studies will be discussed in a forthcoming paper together with the complete microscopic treatment of heroin hydrolysis [28].

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