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Hydrolysis Mechanisms for Indomethacin and Acemethacin in Perchloric Acid

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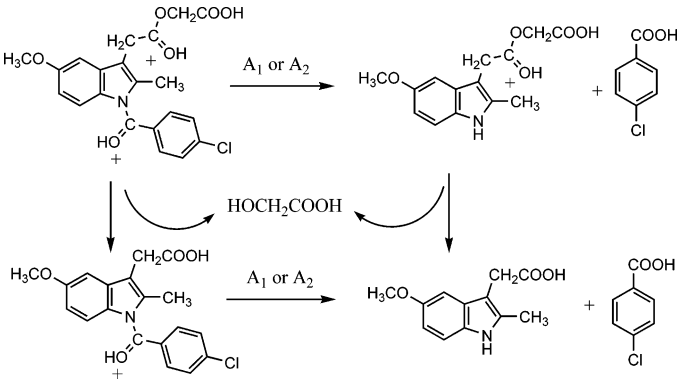
Received December 13, 2005

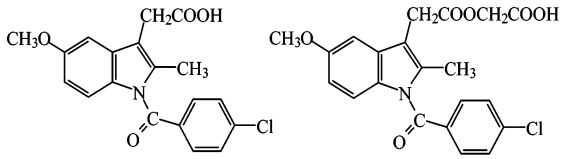


The acid-catalyzed hydrolysis reactions of the antiinflammatory drugs indomethacin and acemethacin were investigated at 25.0 °C in a number of strongly concentrated perchloric acid media. The reaction rates were evaluated by UV measurements, and the intermediate species were detected by UV−vis, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy measurements. A switchover from an A-2 to an A-1 mechanism as a function of the medium acidity is reported for the acid-catalyzed hydrolyses of the amide group of both indomethacin and acemethacin. In the A-2 hydrolysis, two water molecules are involved in the rate-determining step. An analysis of the kinetic data collected for acemethacin by the different techniques used reveals a complex mechanism, indomethacin being a metabolite intermediate species in the hydrolysis of acemethacin. The rate constants for the hydrolysis of the acemethacin ester group were considerably larger compared to those of the amide group.

Introduction

The indole moiety is a fundamental constituent for a number of both natural and synthetic compounds with biological activity. This functional group represents probably one of the most important structural types in drug development;[1] pharmaceutical compounds such as nonsteroidal antiinflammatories (NSAIDs) or analgesic and antipyretic drugs such as indomethacin (**1**) and acemethacin (**2**) (Scheme 1) contain the indole ring. These compounds are widely used in the treatment of pain, arthritis,[2] cardiovascular[3] and Alzheimer[4] diseases, and cancer prevention;[5] however, despite the adverse side effects reported, especially those related to the gastrointestinal tract,[6] so far the use of NSAIDs drugs has become widespread.

SCHEME 1. Indomethacin (1) and Acemethacin (2)



(1) (2)

Acemethacin can be synthesized by hydroxyacetic acid esterification of indomethacin (1-(4-chlorobenzoyl)-5-methoxy-

(1) Smith, A. L.; Stevenson, G. I.; Swain, C. J.; Castro, J. L. *Tetrahedron Lett.* **1998**, *39*, 8317.

(2) Schuna, A. A. *J. Am. Pharm. Assoc.* **1998**, *38*, 728.

(3) (a) Goodnight, S. H. *Curr. Opin. Hematol.* **1996**, *3*, 355. (b) Rodriguez, L. A.; Varas, C.; Patrono, C. *Epidemiology* **2000**, *11*, 382.

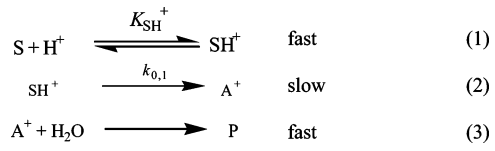
(4) (a) Sloane, P. D. *Am. Fam. Physician* **1998**, *58*, 1577. (b) Flynn, B. L.; Theesen, K. A. *Ann. Pharmacother.* **1999**, *33*, 840.

(5) (a) Shiff, S. J.; Rigas, B. *Gastroenterology* **1997**, *113*, 1992. (b) Jones, M. K.; Wang, H.; Peskar, B. M.; Levin, E.; Itani, R. M.; Sarfels, I. J.; Tarnawski, A. S. *Nat. Med.* **1999**, *5*, 1418.

(6) Matos, C.; Lima, J. L. C.; Reis, S.; Lopes, A.; Bastos, M. *Biophys. J.* **2004**, *2*, 946.

10.1021/jo052561k CCC: $33.50
Published on Web 04/14/2006

SCHEME 2



2-methyl-1*H*-indole-3-acetic acid). Despite the close similarity of their UV−vis absorption curves, which prevent the spectral features from a clear distinction between the two compounds, in a previous paper we have evaluated the protonation constant of the indomethacin amide group in perchloric acid medium ($pK_{InH^+} = -4.0$) and the two constants for the protonation of the acemethacin ester ($pK_{AcH^+} = -2.0$) and amide ($pK_{AcH_2^+} = -4.2$) groups;[7] although N-protonation has been suggested for the amide group,[8] it is generally agreed that protonation occurs at the oxygen site.[7] In addition to basic hydrolyses of indomethacin and acemethacin,[9] acidic hydrolyses are also feasible; to properly elucidate the latter mechanisms, the kinetic study of the acid-catalyzed hydrolyses of the two drugs has been undertaken by monitoring spectrophotometrically the reaction rates in different perchloric acid concentrations, the intermediates and reaction products both being analyzed by UV−vis, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy.

A number of different methods are available to deal with the treatment of kinetic data at medium and high acidity levels; to reliably determine the reaction mechanisms, the following methods have been used: Zucker−Hammett,[10] Bunnett,[11] LFER,[12] and Cox−Yates[13] (or excess acidity) methods; the three first treatments introduce the medium acidity in terms of the Hammett acidity function H_0,[14] whereas the last one does so in terms of the excess acidity function, X.[15,16] To discern the reaction order on the basis of reacting species different from the proton, Ingold referred to the acid-catalyzed hydrolysis reactions as A-1 and A-2 mechanisms.[17] Over the last years, a number of methods have been put forward to differentiate between the A-1 and A-2 mechanisms; these are applicable at high and low acidity levels, respectively, and will be reflected hereafter. Depending on the relative concentrations of the protonated (SH^+) and nonprotonated (S) substrate forms, two different situations can be differentiated in each of the A-1 and A-2 mechanisms.

A-1 Mechanism. If the starting material is the S species, that is, the predominantly unprotonated substrate form, then the mechanism shown in Scheme 2 applies.

The reaction Scheme 2 involves a fast proton-transfer preequilibrium of the substrate S to give SH^+; this species evolves in the rate-determining unimolecular step to some intermediate species A^+, which subsequently reacts speedily to products. The criteria employed to elucidate the A-1 mechanism are described next.

(a) The Zucker−Hammett criterion.[10] The reaction rate defined as the disappearance of the total substrate concentration can be expressed in the form

$$v = -\frac{d(c_s + c_{SH^+})}{dt} = k_{obs}(c_s + c_{SH^+}) = k_{0,1}\, c_{SH^+} \frac{f_{SH^+}}{f_{\ddagger}} \qquad (4)$$

where f_{SH^+} and $f_{\ddagger}$ represent the activity coefficients of the protonated substrate and transition state, respectively. From the mathematical definition of the K_{SH^+} constant, eq 1, and the Hammett acidity function H_0,[14] eq 5 is arrived at when $c_{SH^+} > c_S$, that is, when the predominantly protonated substrate prevails at high acidity levels.

$$\log k_{obs} = \log k_{0,1} + \log\left(\frac{f_{SH^+}}{f_{\ddagger}}\right) \qquad (5)$$

Therefore, if, for an A-1 mechanism, the hydrolysis follows the reaction in Scheme 2 and the ratio $f_{SH^+}/f_{\ddagger} \approx 1$ for all acid concentrations, then the k_{obs} values become independent of the medium acidity and will remain constant.

(b) Linear Free Energy Relationship (LFER). Bunnett and Olsen[11,18] extended the linear free energy relationships to acid-catalyzed reactions,[19] obtaining the equation

$$\log k_{obs} + H_0 = \phi(H_0 + \log c_{H^+}) + \log \frac{k_{0,1}}{K_{SH^+}} \qquad (6)$$

where the solvation parameter ϕ represents the response of the equilibrium to the change in the medium, that is, increase in c_{H^+} and subsequent decrease in a_{H_2O}, and measures the effects brought about by the changes of hydration. If primary anilines are taken as reference bases ($\phi = 0$),[19] then positive ϕ values are expected for bases with higher solvation requirements and negative when the hydration of SH^+ is comparatively low. Hence, the parameter ϕ fluctuates between −1 and +1 for the A-1 mechanism and is evaluated as the sum of (1) the ϕ_e contribution, related to the protonation equilibrium, and (2) the $\phi_{\ddagger}$ contribution, more sensitive to changes in the mechanism, related to the rate-determining step. Lucchini et al.[20] have detailed the equations that correlate the rate constants with the medium acidity through the free-energy relationships, arriving at the following general equation:

$$\log k_{obs} - \log\left(\frac{c_{SH^+}}{c_S + c_{SH^+}}\right) = \log k_{0,1} + (\phi_{\ddagger} - \phi_e)(H_0 + \log c_{H^+}) \qquad (7)$$

At high acidities, when $c_{SH^+} > c_S$, the predominantly protonated substrate prevails, then eq 7 converts to

$$\log k_{obs} = \log k_{0,1} + (\phi_{\ddagger} - \phi_e)(H_0 + \log c_{H^+}) \qquad (8)$$

(7) Hoyuelos, F. J.; García, B.; Ibeas, S.; Muñoz, M. S.; Navarro, A. M.; Peñacoba, I.; Leal, J. M. *Eur. J. Org. Chem.* **2005**, 1161.
(8) Liler, M. *Reaction mechanisms in sulphuric acid*; Academic Press: London, 1971; p 189.
(9) a)Archontaki, H. A. *Analyst* **1995**, *120*, 2627. (b) Hajratwala, B. R, Dawson, J. E. *J. Pharm. Sci.* **1977**, *66*, 27. (c) Cipiciani A.; Ebert, C.; Linda, P.; Rubessa, F.; Savelli, G. *J. Pharm. Sci.* **1983**, 72, 1075−1076. (d) Arcos, J.; Lopez-Palacios, J.; Leal, J. M.; Sanchez Batanero, P.; Mata. F. *Bull. Soc. Chim. Fr.* **1991**, *128*, 314. (e) Alibrandi, G.; Coppolino, S.; D'Aliberti, S.; Ficarra, P.; Micali, N.; Villari, A. *J. Pharm. Sci.* **2003**, *92* (8), 1730.
(10) Zucker, L.; Hammett, L. P. *J. Am. Chem. Soc.* **1939**, *61*, 2791.
(11) Bunnett, J. F. *J. Am. Chem. Soc.* **1961**, *83*, 4956; 4968.
(12) Bunnett, J. F.; Olsen, F. P. *Chem. Commun.* **1965**, 601.
(13) Cox, R. A.; Yates, K. *Can. J. Chem.* **1983**, *61*, 2225.
(14) Hammett, L. P.; Deyrup, A. J. *J. Am. Chem. Soc.* **1932**, *54*, 2721.
(15) Cox, R. A.; Yates, K. *J. Am. Chem. Soc.* **1978**, *100*, 3861.
(16) Cox, R. A.; Yates, K. *Can. J. Chem.* **1981**, *59*, 2116.
(17) Ingold, C. K. *Structure and Mechanism in Organic Chemistry*, 2nd Ed.; Cornell University Press: Ithaca, NY, 1969; Chapter XIV.
(18) Bunnett, J. F.; Olsen, F. P. *Can. J. Chem.* **1966**, *44*, 1917.
(19) Bunnett, J. F.; Olsen, F. P. (a) *Can. J. Chem.* **1966**, 44, 1899; (b) *J. Chem. Soc., Chem. Commun.* **1965**, 601.
(20) Lucchini, V.; Modena, G.; Scorrano, G.; Tonellato, U. *J. Am. Chem. Soc.* **1977**, *99*, 3387.

If eq 8 is fulfilled, then the process obeys the A-1 pattern and the log k_{obs} vs (H_0 + log c_{H^+}) plot gives a straight line, the slope ($\phi_{\ddagger} - \phi_e$) being in the range −1 to +1.

(c) The Excess Acidity Method.[13] Combination of eq 4 and K_{SH^+} leads to

$$\log k_{obs} - \log\left(\frac{c_S}{c_S + c_{SH^+}}\right) - \log c_{H^+} = \log\left(\frac{k_{0,1}}{K_{SH^+}}\right) + \log\left(\frac{f_S f_{H^+}}{f_{\ddagger}}\right) \quad (9)$$

where the last term, the activity coefficients ratio term, is related to the excess acidity function X,[15,16] in the form

$$\log\left(\frac{f_S f_{H^+}}{f_{\ddagger}}\right) = m^{\ddagger} \log\left(\frac{f_S f_{H^+}}{f_{SH^+}}\right) = m^{\ddagger} m^* \log\left(\frac{f_B f_{H^+}}{f_{BH^+}}\right) = m^{\ddagger} m^* X \quad (10)$$

where B is a hypothetical reference base on which the acidity function X is defined; the excess acidity, defined as the log($f_B f_{H^+}/f_{BH^+}$) term, represents the extra medium acidity brought about by the solvent nonideal behavior, that is, the difference between the actual solution acidity and the stoichiometric acidity. The excess acidity displays the very useful feature of being 0 in the standard state of activity coefficients unity. When $c_{SH^+} > c_S$, that is, for substrates predominantly protonated, then eq 10 leads to

$$\log k_{obs} - \log\left(\frac{c_{SH^+}}{c_S + c_{SH^+}}\right) = \log k_{0,1} + (m^{\ddagger} - 1) m^* X \quad (11)$$

To find out the suitable $m^{\ddagger}$ value and thereby establish the reaction mechanism, the proper m^* value corresponding to the protonation equilibrium in high acidity media must be previously determined; the parameter m^* measures the ability of the set of bases to become stabilized by solvation and reflects the equilibrium sensitivity to the large changes in the medium required to complete the protonation; therefore, the value m^* = 0 for H_3O^+ in water denotes the highest solvation requirements, m^* = 1 corresponds to solvation of primary anilines, and higher values denote weaker solvation. A suitable plot of the left-hand side of eq 11 vs X should give a straight line, the intercept providing the log $k_{0,1}$ value. For A-1 the activation parameter should be $m^{\ddagger} > 1$, and in fact, it normally fluctuates between 2 and 3.[21,22] This treatment was applied to deduce the mechanism of a variety of hydrolyses, including esters,[23] substituted benzaldehydes,[24−27] and the chromic acid oxidation of propanal[28] and glycolic acid.[29]

A-2 Mechanism. The A-2 mechanism is similar to A-1, but the protonated substrate reacts with a nucleophile species in the rate-determining step (Scheme 3) where n represents the number of molecules covalently bound to the protonated substrate, forming the transition state.[30] The A-2 hydrolysis involves a rapid preequilibrium to form the SH^+ form which, in turn, is attacked by the nucleophile Nu (water) in the rate-determining step.

SCHEME 3

$$S + H^+ \underset{}{\overset{K_{SH^+}}{\rightleftharpoons}} SH^+ \quad \text{Fast} \quad (12)$$

$$SH^+ + nNu \xrightarrow{k_{0,2}} A^+ \quad \text{Slow} \quad (13)$$

$$A^+ \longrightarrow P \quad \text{Fast} \quad (14)$$

(a) The Zucker−Hammett Criterion.[10] The reaction rate for the A-2 mechanism, Scheme 3, can be expressed as

$$v = -\frac{d(c_S + c_{SH^+})}{dt} = k_{0,2} c_{SH^+} \frac{a_{H_2O}^n f_{SH^+}}{f_{\ddagger}} \quad (15)$$

Reasoning analogous to that applied to A-1 leads to the following expression:

$$k_{obs}\left(\frac{c_S + c_{SH^+}}{c_S}\right) = \frac{k_{0,2}}{K_{SH^+}} c_{H^+} \frac{f_S f_{H^+} a_{H_2O}^n}{f_{\ddagger}} \quad (16)$$

If $c_{SH^+} < c_S$, then eq 16 converts to

$$\log k_{obs} = \log c_{H^+} + \log\left(\frac{k_{0,2}}{K_{SH^+}}\right) + \log\left(\frac{f_S f_{H^+} a_{H_2O}^n}{f_{\ddagger}}\right) \quad (17)$$

If the Zucker−Hammett assumption is correct,[10] according to which the activity coefficients ratio term becomes unity, then the k_{obs} value for A-2 must be directly related to proton concentration in the form

$$\log k_{obs} = \log c_{H^+} + \text{constant} \quad (18)$$

(b) Linear Free Energy Relationship (LFER). Bunnett[11a] observed that the linear (log k_{obs} + H_0) vs log a_{H_2O} plot for a variety of reactions enabled the equation

$$\log k_{obs} + H_0 = w \log a_{H_2O} + \text{constant} \quad (19)$$

A number of empirical mechanistic criteria have been detailed for hydrolysis reactions that follow the A-2 pattern, according to which the w parameter must vary between +1.2 and +3.3 for O- and N-protonated substrates, the nucleophile playing a critical role in the rate-determining step;[12] the parameter w bears relation to the hydration change between the transition state and the reactants. In this treatment, the assumption was made that the activity coefficients ratio term is solvent-independent for species with the same charge. Application of Lucchini et al.'s[20] correlation to eq 16 leads to eq 20, representative of an A-2 mechanism.

$$\log k_{obs} - \log\left(\frac{c_{SH^+}}{c_S + c_{SH^+}}\right) - \log a_{H_2O} = \log k_{0,2} + (\phi_{\ddagger} - \phi_e)(H_0 + \log c_{H^+}) \quad (20)$$

The left-hand side vs (H_0 + log c_{H^+}) plot should lead to a straight line.

(21) Cox, R. A.; Yates, K. *Can. J. Chem.* **1979**, *57*, 2944.
(22) Lajunen, M.; Uotila, R. *Acta Chem. Scand.* **1992**, *46*, 968.
(23) Cox, R. A.; Goldman, M. F.; Yates, K. *Can. J. Chem.* **1979**, *57*, 2960.
(24) Cox, R. A.; Yates, K. *J. Org. Chem.* **1986**, *51*, 3619.
(25) Ali, M.; Satchell, D. P. N. *J. Chem. Soc., Perkin Trans. 2* **1991**, 575; **1993**, 1825.
(26) Satchell, D. P. N.; Wassef, W. N. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1855.
(27) Motie, R. E.; Satchell, D. P. N.; Wassef, W. N. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1087.
(28) Alvarez, M. P.; Montequi, M. I. *An. Quim.* **1992**, *88*, 149.
(29) Montequi, M. I.; Alvarez, M. P. *An. Quim.* **1989**, *85*, 331.
(30) Yates, K.; McClelland, R. A. *J. Am. Chem. Soc.* **1967**, *89*, 2686.

(c) Excess Acidity Method. Starting from eqs 10 and 16, a kinetic analysis similar to that performed for the A-1 mechanism can be established. If $c_{SH^+} < c_S$, that is, if the substrate predominantly unprotonated prevails, then the relevant rate equation derived for this mechanism is as follows[21]

$$\log k_{obs} - \log\left(\frac{c_S}{c_S + c_{SH^+}}\right) - \log c_{H^+} - n \log a_{Nu} = \log\left(\frac{k_{0,2}}{K_{SH^+}}\right) + m^{\ddagger} m^* X \quad (21)$$

where n stands for the number of water molecules in the transition state and the parameter $m^{\ddagger}$ must be close to unity; if the K_{SH^+} constant is already known, then the left-hand side vs X plot gives the $k_{0,2}$ value as the intercept. This equation has been applied successfully to hydrolyses of benzoic acid derivatives[31,32] and azopyridines.[33,34]

In summary, both the LFER and excess acidity methods are reliable for the A-1 and A-2 hydrolyses. The slope parameters m^*, $m^{\ddagger}$, ϕ_e, and $\phi_{\ddagger}$ are related to each other as $\phi_{\ddagger} \approx 1 - m^{\ddagger} m^*$ and $m^{\neq} \approx (1 - \phi_{\ddagger})/(1 - \phi_e)$; that is, $m^* \approx 1 - \phi_e$.[20] Once the mechanism is deduced, the excess acidity method provides reliable solvent-independent $k_{0,i}$ ($i = 1, 2$); the values deduced compare favorably with those determined in buffered media, leaving out the disadvantages inherent to the behavior of the activity coefficients or the different H_0 acidity functions.[25]

Results and Discussion

The very weak bases indomethacin and acemethacin undergo acid−base equilibria in the acidic range 1.7−11.6 M $HClO_4$. Acemethacin displays two protonation equilibria corresponding to the ester (pK_{AcH^+}) and amide ($pK_{AcH_2^{2+}}$) groups, whereas indomethacin undergoes only a single protonation of the amide group (pK_{InH^+}).[7] To rationalize the kinetics of the respective hydrolyses, the overall acidity interval was split into region 1 (1.7−7.5 M $HClO_4$), region 2 (8.0−10.0 M $HClO_4$), and region 3 (10.4−11.6 M $HClO_4$). In region 1, the amide group was found to be predominantly unprotonated, and the reaction was rather slow; in region 3, the substrate was found to be predominantly protonated and hydrolyzed fast. In region 2, the hydrolysis proceeded at an intermediate speed. In regions 1 and 3, the indomethacin and acemethacin UV spectral curves were quite similar, with well-defined isosbestic points, whereas in region 2 only acemethacin displayed UV spectral curves without a clear isosbestic point, which denotes the existence of a complex reaction. As an example, Figures 1 and 2 show sets of spectral curves for both indomethacin and acemethacin in 9.22 M $HClO_4$.

In regions 1 and 3, for acemethacin, and in regions 1−3 for indomethacin, the k_{obs} rate constants were calculated by nonlinear least-squares fitting, using the single-exponential equation[35]

$$A_t = A_\infty - (A_0 - A_\infty)e^{-k_{obs}t} \quad (22)$$

whereas in region 2 the data for acemethacin were fitted to the

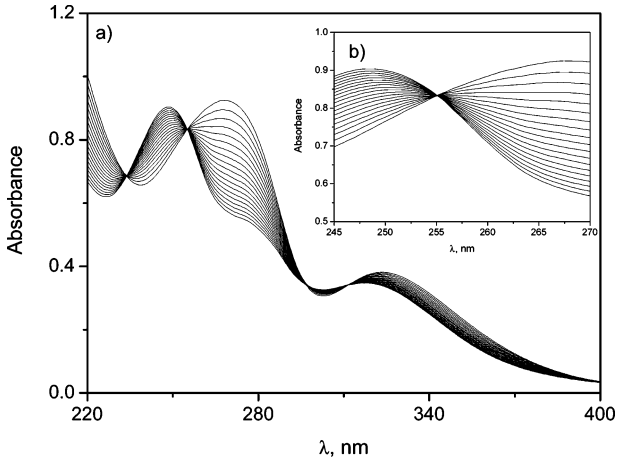


FIGURE 1. UV−vis absorption spectral curves corresponding to hydrolysis of (a) indomethacin in 9.22 M $HClO_4$, time interval 600 s, and (b) magnified view in the 245−270 nm range.

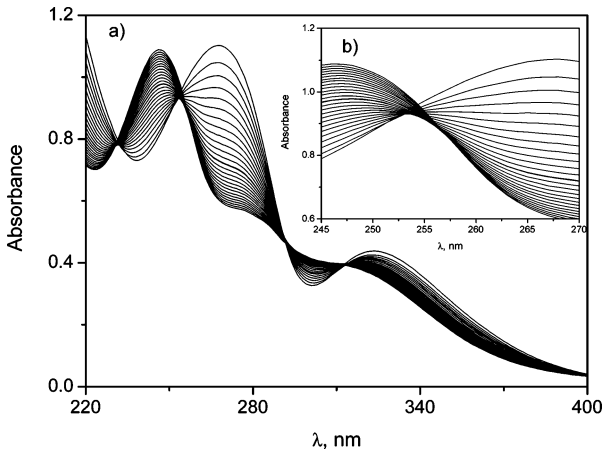


FIGURE 2. UV−vis absorption curves corresponding to hydrolysis of (a) acemethacin in 9.22 M $HClO_4$, time interval 600 s, and (b) magnified view in the 245−270 nm range.

double-exponential equation

$$A_t - A_\infty = \alpha e^{-k_{obs}t} + \beta e^{-k'_{obs}t} \quad (23)$$

where A_0 and A_∞ stand for the initial and final absorbance readings, respectively, and A_t represents that at different reaction times.

The rate constants k_{obs} and k'_{obs} for the disappearance of acemethacin and k_{obs} for indomethacin at different perchloric acid concentrations are listed in Table 1. The acidity effect on k_{obs}, plotted in Figure 3, reveals that the kinetic behavior for the two drugs are nearly identical, consistent with the observation that the A-2 hydrolysis switched over to an A-1 with increasing medium acidity, being 8.0 M $HClO_4$ (roughly) the acid concentration at which the change in mechanism occurs, that is, on the border between regions 1 and 2.

For acemethacin the values for k_{obs} deduced in region 2 on the basis of eq 23 (Figure 3) matched perfectly well with those obtained in the regions 1 and 3 on the basis of eq 22. The rate of hydrolysis, k_{obs}, increased progressively with increasing acid

(31) Cox, R. A.; Smith, C. R.; Yates, K. *Can. J. Chem.* **1979**, *57*, 2952.
(32) Cox, R. A.; Yates, K. *Can. J. Chem.* **1982**, *60*, 3061.
(33) Cox, R. A.; Onyido, I.; Buncel, E. *J. Am. Chem. Soc.* **1992**, *114*, 1358.
(34) Ghosh, K. K.; Rajput, S. K.; Krihnani, K. K. *J. Phys. Org. Chem.* **1992**, *5*, 39.
(35) Moore, P. *J. Chem. Soc., Faraday Trans.* **1972**, *68*, 1890.
(36) von Dell, H. D.; Doersing, M.; Fischer W.; Jacobi, H.; Kamp, R.; Köhler G.; Schöllnhammer G. *Arzneim. Forsch.* **1980**, 30-II-8a, 1391.

TABLE 1. Rate Constants for Indomethacin and Acemethacin as a Function of $HClO_4$ Concentration (c_{HClO_4}), Hammett Acidity Function, H_0, Excess Acidity Function, X, and Water Activity a_{H_2O}

				indomethacin	acemethacin	
c_{HClO_4} (M)	H_0	X	log a_{H_2O}	$k_{obs} \times 10^5$ (s^{-1})	$k_{obs} \times 10^5$ (s^{-1})	$k'_{obs} \times 10^5$ (s^{-1})
0.922	−0.28745208	0.181	1.662	0.283		
1.38	−0.53706339	0.301	1.619	0.417		
1.73	−0.70744830	0.403	1.587		0.683	
1.84	−0.76074035	0.439	1.577	0.600		
2.30	−0.96957324	0.594	1.535	0.800	0.823	
2.77	−1.17755975	0.770	1.492	0.933		
2.88	−1.22914696	0.816	1.482		1.029	
3.23	−1.38616260	0.960	1.448	1.050		
3.46	−1.49464458	1.061	1.425		1.418	
3.69	−1.60222114	1.163	1.401	1.233		
4.03	−1.76999344	1.323	1.364		1.510	
4.15	−1.82933105	1.379	1.351	1.467		
4.61	−2.07048089	1.606	1.295	1.617	1.896	
5.07	−2.32504421	1.841	1.235	1.833		
5.18	−2.38895687	1.899	1.219		2.093	
5.53	−2.59662524	2.086	1.167	2.033		
5.76	−2.73821895	2.212	1.131		2.236	
5.99	−2.88454035	2.340	1.092	2.200		
6.34	−3.11341513	2.538	1.030	2.319	2.638	
6.45	−3.18817181	2.602	1.009	2.417		
6.91	−3.50727983	2.873	0.916	2.567	2.630	
7.37	−3.84528504	3.157	0.812	2.700		
7.49	−3.93323576	3.230	0.784	3.031	2.838	34.01
7.83	−4.19771576	3.451	0.697	3.283		
8.06	−4.37624522	3.599	0.636		3.265	36.41
8.29	−4.56181914	3.753	0.571	3.380	4.083	31.64
8.30	−4.56589694	3.757	0.570	3.400		
8.64	−4.85176223	3.993	0.466	4.055	4.621	33.52
8.76	−4.94988044	4.075	0.429	4.400		
8.87	−5.04729564	4.155	0.392	5.305	5.381	33.88
8.99	−5.15090293	4.241	0.352	6.709	6.876	33.68
9.1	−5.24684207	4.320	0.315	7.071	7.697	35.26
9.22	−5.35256434	4.407	0.273	7.250	10.10	35.99
9.45	−5.55832838	4.576	0.190	13.35	13.94	37.38
9.68	−5.76642607	4.747	0.104	19.35		
9.79	−5.87017832	4.831	0.060		31.73	34.17
10.02	−6.08645722	5.006	−0.033		41.24	43.32
10.14	−6.19912319	5.097	−0.082	40.83		
10.37	−6.42407854	5.275	−0.183		71.63	
10.60	−6.65169672	5.454	−0.287	96.83	123.5	
10.83	−6.88401655	5.632	−0.395		175.5	
11.06	−7.12115943	5.810	−0.508	213.3	262.3	
11.29	−7.36324572	5.988	−0.626		354.0	
11.52	−7.61039087	6.163	−0.749	700.0	533.5	

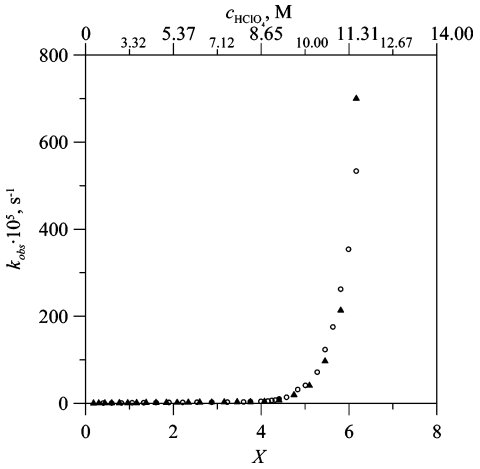


FIGURE 3. k_{obs} vs X and k_{obs} vs c_{HClO_4} plots for indomethacin (▲) and acemethacin (○) at 25 °C.

concentration, whereas k'_{obs} remained (roughly) constant, the average value being 3.5×10^{-4} s^{-1}. At acidity levels below 8.0 M $HClO_4$, the reaction proceeded through a slow nucleophilic attack of the protonated substrate by two water molecules (A-2 mechanism). At the highest acidity, above 8.0 M $HClO_4$, the reaction proceeded by rate-determining decomposition of the protonated substrate (A-1 mechanism).

Acemethacin: Hydrolysis Mechanisms of the Amide Group. A-2 Mechanism. The above kinetic criteria were applied to the data pairs collected for the hydrolysis of acemethacin below 8.0 M $HClO_4$, where $c_{SH^+} < c_S$, and they all pointed to an A-2 mechanism. The kinetic criterion by Zucker−Hammett, eq 18, is fulfilled; the log k_{obs} vs log c_{H^+} plot (Figure 4) led to a straight line ($r = 0.995$) with slope 1.04, close to unity. The requirements by the Bunnett method, eq 19, were also met; the (log $k_{obs} + H_0$) vs a_{H_2O} led to a straight line (Figure 5) with slope parameter $w = 3.3$ ($r = 0.999$). The LFER Lucchini correlation was also fulfilled (eq 20); the plot [log k_{obs} − log($c_{SH^+}/(c_S + c_{SH^+})$) − log a_{H_2O}) vs (H_0 + log c_{H^+}) led to a straight line (Figure 6) with slope ($\phi_{\ddagger} - \phi_e$) = 0.363 (r = 0.995). Finally, the excess acidity method was found to be very useful in the obtaining of mechanistic information. The [log k_{obs} − log($c_S/(c_S + c_{SH^+})$) − log c_{H^+} − n log a_{Nu}) vs X plot, eq

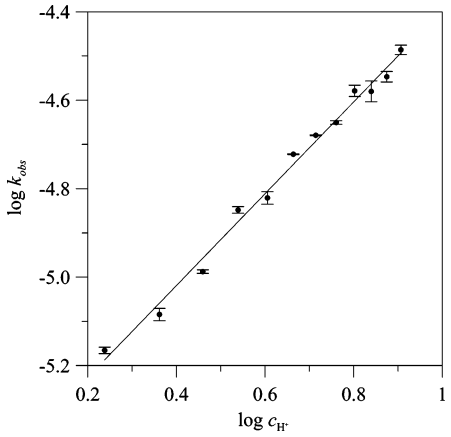


FIGURE 4. log k_{obs} vs log c_{H^+} plot for acemethacin. A-2 Zucker−Hammett criterion (eq 18).

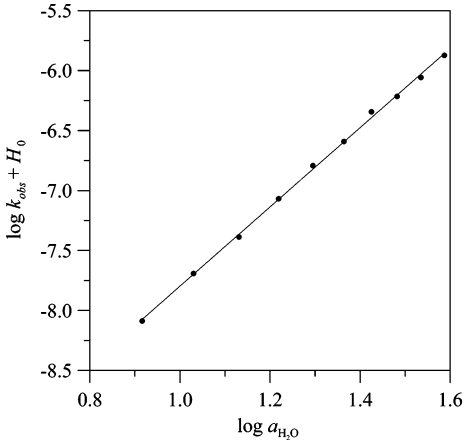


FIGURE 5. (log k_{obs} + H_0) vs log c_{H^+} plot for acemethacin. A-2 Bunnett criterion (eq 19).

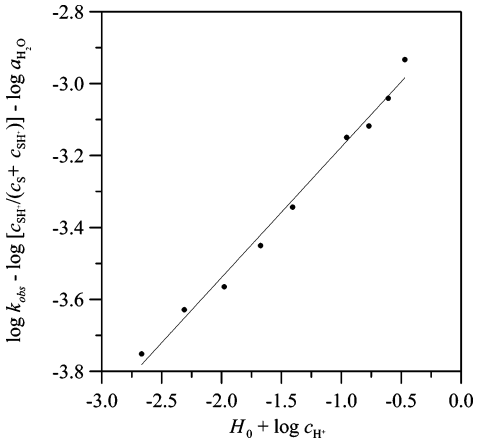


FIGURE 6. log k_{obs} − log(c_{SH^+}/(c_S + c_{SH^+})) − log a_{H_2O} vs (H_0 + log c_{H^+}) plot for acemethacin. A-2 LFER Lucchini correlation (eq 20).

21, attained linearity when $n = 2$ (Figure 7), n being the number of water molecules in the transition state; the ordinate yielded the rate constant $k^A_{0,2} = 2.04 \times 10^{-5}$ M^{-1} s^{-1}. Therefore, the rate-determining step involves the nucleophilic attack by two water molecules to the protonated substrate. If one recalls that $m^* = 0.60$,[7] then the value $m^{\ddagger} = 0.98$ close to unity is deduced, in good agreement with the criterion by Cox−Yates for an A-2 mechanism (Scheme 4, eqs 12−14).

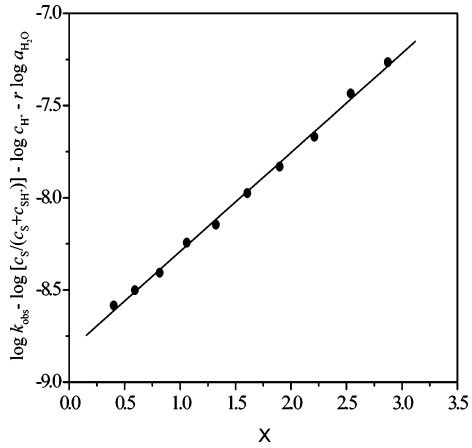


FIGURE 7. (log k_{obs} − log(c_S/(c_S + c_{SH^+})) − log c_{H^+} − 2·log a_{H_2O}) vs X plot (eq 21) for an A-2 mechanism for acemethacin in $HClO_4$, showing the involvement of two water molecules. $T = 25$ °C.

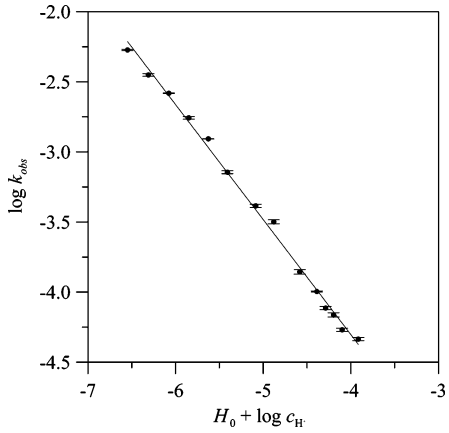


FIGURE 8. log k_{obs} vs (H_0 + log c_{H^+}) plot for acemethacin. A-1 LFER analysis (eq 8).

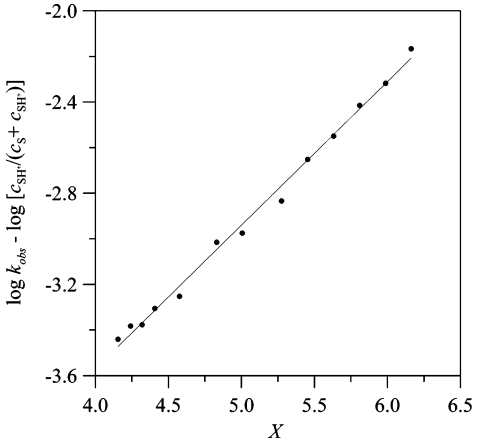
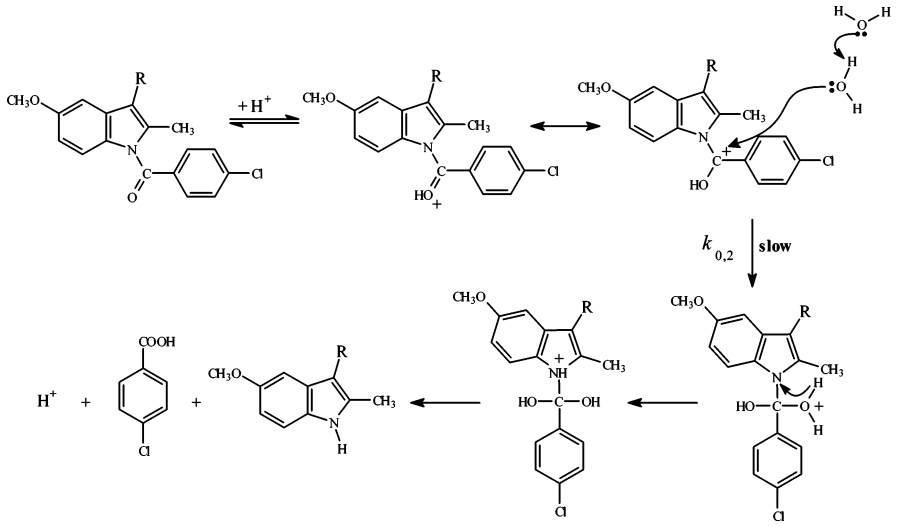


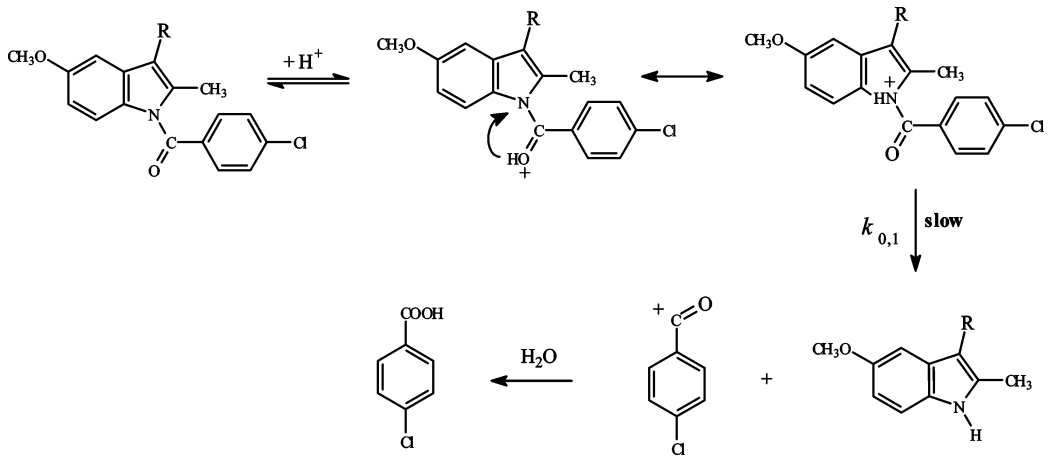
FIGURE 9. log k_{obs} − log(c_{SH^+}/(c_S + c_{SH^+})) vs X plot (eq 11) for acemethacin in $HClO_4$ for an A-1 mechanism. $T = 25$ °C.

A-1 Mechanism. In the region above 8.0 M $HClO_4$, where $c_{SH^+} > c_S$, all the mechanistic criteria analyzed point to an A-1 hydrolysis. According to the LFER method, the linear log k_{obs} vs (H_0 + log c_{H^+}) plot (eq 8, Figure 8, $r = 0.998$), yield the slope ($\phi_{\neq} - \phi_e$) = −0.82, a value in the range −1 and +1 that meets the mechanistic requirements; hence, the constant value of the rate-determining step $k^A_{0,1} = 1.17 \times 10^{-6}$ s^{-1} is deduced

SCHEME 4. A-2 Mechanism for the Hydrolysis of the Amide Group of Acemethacin (R = CH_2COOCH_2COOH) and Indomethacin (R = CH_2COOH)



SCHEME 5. A-1 Mechanism for the Hydrolysis of the Amide Group of Acemethacin (R = CH_2COOCH_2COOH) and Indomethacin (R = CH_2COOH)



from the ordinate. The Excess Acidity criterion for an A-1 mechanism, eq 11, is also fulfilled, as shown in Figure 9 (r = 0.998), with slope ($m^{\ddagger} - 1$)m^* = 0.60 and the ordinate yielding the rate-determining step $k^{A}_{0,1} = 1.17 \times 10^{-6}$ s^{-1}. Introduction of the value $m^* = 0.60$ reported earlier[7] yields the value $m^{\ddagger}$ = 2.0, concurrent with the Cox−Yates requirement for an A-1 mechanism (Scheme 5, eqs 1−3).

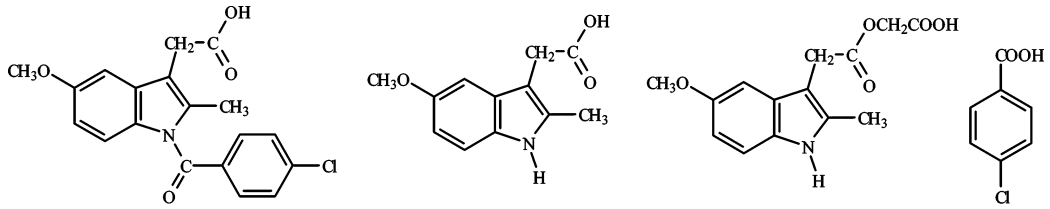
Indomethacin. To deduce the hydrolysis mechanism for indomethacin, the same equations and mechanistic criteria were applied and revealed a switchover from an A-2 to an A-1 mechanism as a function of the medium acidity.

A-2 Mechanism. The Zucker−Hammett criterion, eq 18, was fulfilled within the acidity region below 8.0 M $HClO_4$, where $c_{SH^+} < c_S$: the log k_{obs} vs log c_{H^+} plot was linear (r = 0.999) with slope 1.12, close to unity. The LFER analysis, eq 19, was also fulfilled: the (log $k_{obs} + H_0$) vs log a_{H_2O} plot yielded a straight line (r = 0.999) with slope w = 3.08. Furthermore, the LFER Lucchini correlation, eq 20, was also fulfilled: the [log $k_{obs} - \log(c_{SH^+}/(c_S + c_{SH^+})) - \log a_{H_2O}$) vs ($H_0 + \log c_{H^+}$) plot led to a straight line, and yielded a slope ($\phi_{\ddagger} - \phi_e$) = 0.285 (r = 0.978). The excess acidity method was found to be very useful in the obtaining of mechanistic information; the [log k_{obs} − log-($c_S/(c_S + c_{SH^+})$) − log $c_{H^+} - n\log a_{Nu}$) vs X plot, eq 21, attained linearity when n = 2, n being the number of water molecules in the transition state, and the ordinate yielding the rate constant $k^{I}_{0,2} = 1.05 \times 10^{-5}$ M^{-1} s^{-1}. Therefore, the rate-determining step is the nucleophilic attack of the protonated substrate by two water molecules. If one recalls that $m^* = 0.60$,[7] then the value close to unity $m^{\ddagger}$ = 1.04 is deduced, in accordance with the criterion by Cox−Yates for an A-2 mechanism, (Scheme 4).

A-1 Mechanism. In the acidity region above 8.0 M $HClO_4$, where $c_{SH^+} > c_S$, the criteria tested pointed to an A-1 mechanism. According to the LFER analysis, the log k_{obs} vs (H_0 + log c_{H^+}) plot (eq 8) leads to a straight line, r = 0.998). The slope ($\phi_{\ddagger} - \phi_e$) = −0.87, in the range −1 to +1, concurrent with the mechanistic requirements. The excess acidity criterion for an A-1 mechanism, eq 11, is also fulfilled (r = 0.997), with an slope ($m^{\ddagger} - 1$)m^* = 0.63; introduction of the m^* = 0.60 value reported earlier,[7] yields the value $m^{\ddagger}$ = 2.05, concurrent with the Cox−Yates requirement for an A-1 mechanism (Scheme 5), and provides the rate-determining step $k^{I}_{0,1} = 3.94 \times 10^{-6}$ s^{-1}.

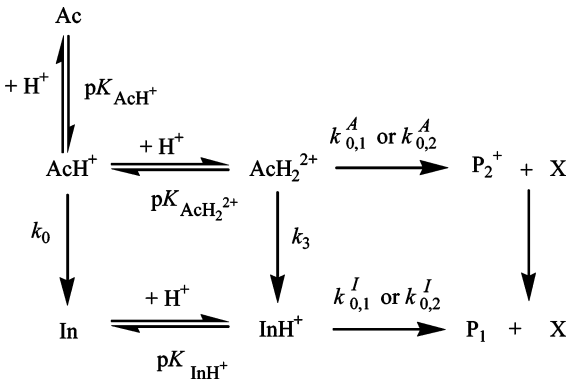
The above results confirm that the two antiinflammatory drugs follow the same hydrolysis pattern for the amide group, with rate constants of the same order of magnitude. In both cases,

SCHEME 6. Indomethacin (3), (2-Methyl-5-methoxy-1*H*-indol-3-yl)acetic Acid (4), (2-Methyl-5-methoxy-1*H*-indol-3-yl)ethoxycarbonylmethylacetic Acid (5), and *p*-Chlorobenzoic Acid (6)



(3) (4) (5) (6)

SCHEME 7. Mechanism for Hydrolysis of Acemethacin



the NMR and GC data have enabled the identification of the reaction intermediates; for indomethacin, the intermediates and reaction products were those that resulted from the hydrolysis of the amide group (4 and 6 forms, Scheme 6), whereas for acemethacin, the intermediates were the 3, 4, 5, and 6 forms; this feature indicates that the ester and amide groups hydrolyze concurrently. On the other hand, in the second acidity range acemethacin undergoes both the ester and amide hydrolyses, and displays a more complex kinetic behavior compared to indomethacin (Figure 2); this feature tells the difference between the two substrates. Finally, Figure 10 shows that the two antiinflammatory drugs yield the same reaction products. In accordance with these results, the hydrolysis mechanism put forward for acemethacin is as follows:

In Scheme 7 AcH^+ represents the acemethacin form protonated at the ester site, AcH_2^{2+} represents acemethacin protonated

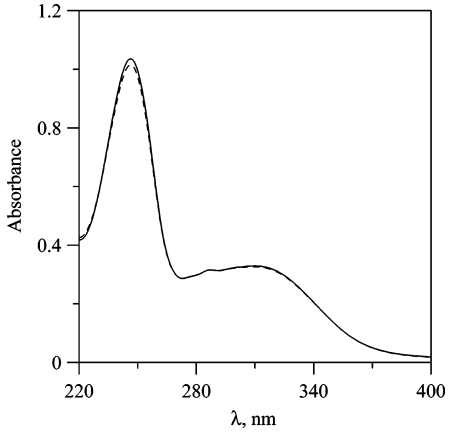


FIGURE 10. UV−vis absorption spectral curves of the hydrolysis products of indomethacin (dashed line), and acemethacin (solid line) in 8.64 M $HClO_4$, at 99.9% completion 25 °C. Initial indomethacin and acemethacin concentrations, 5×10^{-5} M.

both at the ester and amide sites, InH^+ represents indomethacin protonated at the amide site, P_2^+, is the indole protonated at the ester site resulting from hydrolysis of the amide group of acemethacin (acid form 5, Scheme 6), P_1 is the indole in the acid form 4, X is the *p*-chlorobenzyl, acid form 6, $k_{0,i}^{A}$ and $k_{0,i}^{I}$ ($i = 1, 2$) represent the step-determining rate constants for hydrolysis of the amide group for AcH_2^{2+} and InH^+ according to an A-1 (higher acidity) or A-2 (lower acidity) mechanism, respectively. Schemes 4 and 5 show the A-2 and A-1 hydrolysis mechanisms for acemethacin and indomethacin; k_3 refers to hydrolysis of the acemethacin ester group at acidity levels above 7 M $HClO_4$. Assuming that above 8.0 M $HClO_4$ the AcH_2^{2+} and InH^+ species follow A-1 hydrolyses, InH^+ being a reaction intermediate, then application of the kinetic formalism to the hydrolysis of acemethacin (Scheme 7) leads to the equations

$$c_{AcH_2^{2+}} = (c_{AcH_2^{2+}})_0 e^{-(k_{0,1}^{A}+k_3)t} \quad (24)$$

$$c_{InH^+} = \frac{k_{0,1}^{I}(c_{InH^+})_0}{(k_{0,1}^{A} + k_3 - k_{0,1}^{I})}(e^{-(k_{0,1}^{A}+k_3)t} - e^{-k_{0,1}^{I}t}) \quad (25)$$

which are consistent with the fitting of the experimental data (eqs 22 and 23). Therefore, for acemethacin (Table 1), k'_{obs} equals $(k_{0,1}^{A} + k_3) = 3.5 \times 10^{-4}$ s^{-1}, and $k_{obs} = k_{0,1}^{I} = 3.94 \times 10^{-6}$ s^{-1}. In view that $k_{0,1}^{A} = 1.17 \times 10^{-6}$ s^{-1}, it follows that $k_3 \gg k_{0,1}^{A}$, and hence, above 8.0 M $HClO_4$, the hydrolysis of the acemethacin ester group to produce InH^+ prevails, the acidity-independent value $k'_{obs} \approx k_3$ being consistent with an A-1 mechanism (eq 5). The noncatalyzed acemethacin hydrolysis of the ester group (k_3 roughly constant) can be justified because above 8.0 M $HClO_4$ the ester group is fully protonated ($pK_{AcH^+} = -2.0$), the AcH^+ concentration being constant over the whole acidity range. Table 2 summarizes the equilibria and rate constants referred to in Scheme 7.

Conclusions

Indomethacin and acemethacin hydrolyze in perchloric acid above 1.7 M. The reaction products for both indomethacin and acemethacin are the same. The reaction mechanism, which depends on the medium acidity, switched from a bimolecular addition A-2 mechanism involving two water molecules, to a unimolecular addition A-1 mechanism.The hydrolysis of acemethacin is a complex reaction and involves indomethacin as an intermediate reaction; hence, the hydrolyses of indomethacin and acemethacin are concurrent. The reaction rate of hydrolysis of the ester group is two orders higher compared to that of the amide group.

Experimental Section

Acemethacin and indomethacin were purchased. All other materials were of the highest commercially available grade and used

TABLE 2. Dissociation Equilibrium (p*K*) and Rate Constants of the Determining Step (*k*) at 298 K for the Hydrolyses of Acemethacin and Indomethacin in $HClO_4$

pK_{AcH^+} (acemethacin ester protonation)	-2.0[7]
$pK_{AcH_2^{2+}}$ (acemethacinium amide protonation)	-4.2[7]
pK_{InH^+} (indomethacin amide protonation)	-4.0[7]
$k^A_{0,2}$ (amide acemethacin hydrolysis rate constant, A-2 mechanism)	2.04×10^{-5} M^{-1} s^{-1}
$k^A_{0,1}$ (amide acemethacin hydrolysis rate constant, A-1 mechanism)	1.17×10^{-6} s^{-1}
$k^I_{0,2}$ (indomethacin hydrolysis rate constant, A-2 mechanism)	1.05×10^{-5} M^{-1} s^{-1}
$k^I_{0,1}$ (indomethacin hydrolysis rate constant, A-1 mechanism)	3.94×10^{-6} s^{-1}
k_3 (ester acemethacin hydrolysis rate constant)	3.5×10^{-4} s^{-1}

without further purification. All solutions were prepared using deionized water. Perchloric acid was used to attain the required medium acidity. The acid solutions of the proper concentration were prepared by careful addition of appropriate weights of commercial $HClO_4$ to a 100 mL reagent bottle by dilution with doubly distilled deionized water. Since the substrates are not very soluble in water, the substrate stock solutions were prepared in 5 mL volumetric flasks by accurately weighing the mass samples (9−10 mg) and dissolving in freshly distilled acetonitrile, giving concentrations of 5×10^{-3} M. To rule out the occurrence of photochemical decomposition, the flasks were wrapped up in aluminum foil. Aliquots (20 μL) of the relevant stock solution were syringed into a thermostated cell (25.0 ± 0.1 °C) containing 2 mL of the appropriate aqueous $HClO_4$ solution, the final substrate concentration being $\sim 5 \times 10^{-5}$ M in 1% v/v acetonitrile/H_2O.

Kinetics. The spectrophotometer used to monitor the course of the reaction was fit out with a diode array detection system and a temperature cell-holder adapter, electrically regulated and controlled by computer. The kinetics were investigated by repetitive scans in the 200−450 nm range. The rate constants were evaluated by monitoring the disappearance with time of the substrate absorption A_t at the different maxima recorded in the $\lambda = 260-280$ nm range. The course of the reaction was monitored at least up to three half-lives, the absorbance value at infinity (A_∞) being attained after four half-lives. The computer program used in the fitting of kinetic data is based on the Marquardt−Levenberg method; after introducing the initial estimated values (A_0, A_∞, and k_{obs}), the process is iterated until the convergence is achieved for the minimum value of the χ^2 parameter;[35] the average rate constants deviation of the repetitive scans was <5%. The fulfillment of the Lambert−Beer Law was checked at all relevant wavelengths within the working acidity range; the concentration effect on the rate constant was negligible over the acidity range. Full protonation of indomethacin and acemethacin was attained only at about 74 wt % $HClO_4$, therefore the two substrates remained unprotonated in dilute acid media. The excess acidities values, *X*, for perchloric acid were taken from Cox−Yates[15] and the proton concentrations c_{H^+} regarded as the acid molarities.[16]

Intermediate Analyses. The analyses of the intermediates and reaction products were performed using a gas chromatograph (GC) equipped with a mass selective detector. The ^1H and ^{13}C NMR spectra were recorded with a 200 MHz instrument. A solution of indomethacin (0.25 g, 0.0007 mol) or acemethacin (0.25 g, 0.0006 mol) (Scheme 1) in acetonitrile (8 mL) was stirred magnetically for 2 h with 8 mL $HClO_4$ 70 wt % aq. The resulting solution was then slowly added to 8 mL ethanol and stirred for 12 h at 293 K; in view of the high volatility of the acidic form, esterification was needed for the proper GC detection. Samples of the crude product were pulled out for GC−MS analyses; the isolated products of acemethacin hydrolysis, identified by ^1H and ^{13}C NMR and mass spectroscopy (MS) analyses, are shown in Scheme 6.

Acknowledgment. The financial support by Junta de Castilla y León, project BU26-02, and Ministerio de Ciencia y Tecnología, project BQU2002-01061, Spain, is gratefully acknowledged. Thanks are due to Dr. R. Díez and Dr. J. Delgado (Burgos University) for characterization of the compounds.

JO052561K