Acid Hydrolysis of Diazepam. Kinetic Study of the Reactions of 2-(*N*-Methylamino)-5-chlorobenzophenone, with HCl in MeOH–H_2O

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Abstract □ In the acid hydrolysis of diazepam (**1**), several unusual products, apart from 2-(*N*-methylamino)-5-chlorobenzophenone (**2**) and glycine, were isolated. On the assumption that some of those products could arise from further degradation of **2**, the reaction of this compound with 0.5–2 M HCl was studied, in 1:1 MeOH–H_2O, at 60 and 80 °C. Several unexpected products were isolated from the reaction of **2** with HCl, namely, 2-amino-5-chlorobenzophenone (**3**), 2-(*N,N*-dimethylamino)-5-chlorobenzophenone (**4**), 2-(*N*-methylamino)-3,5-dichlorobenzophenone (**5**), 2-amino-3,5-dichlorobenzophenone (**6**), 2,4-dichloro-10-methyl-9,10-acridinone (**7**), and 2,4-dichloro-9,10-acridinone (**8**). The methyl transfers, the chlorination, and the cyclization reactions that give rise to products **3–8** are unexpected under the present reaction conditions. The rate of reaction of **2**, as well as the rate of formation of compounds **3–6**, was measured at several HCl concentrations.

In most of the kinetic studies of the hydrolysis of diazepam (**1**), 2-(*N*-methylamino)-5-chlorobenzophenone (**2**) is considered to be the final degradation product.[1,2] Diazepam is one of the most frequently prescribed drugs for the treatment of anxiety, sleep disorders, seizure disorders, and alcohol withdrawal,[3] and many structure–activity relationships for 1,4-benzodiazepines have been recently described.[4–6]

Diazepam hydrolysis has been previously studied in acidic[7–10] as well as basic media;[10] micellar catalysis studies in both pH ranges have been recently reported as well.[11] In all these works, kinetics have been followed by the spectrophotometric technique,[12] frequently by monitoring the rate of disappearance of **1** and/or the formation of **2**. It is recommended, when using the spectrophotometric technique, that the final absorbance should be read after at least 10 half-lifes.[13]

We have previously reported that in diazepam hydrolysis in 0.5 M HCl, MeOH–H_2O, at 50 °C the [**2**] reached a maximum after nearly 45 h and then decreased.[14] On the other hand, in early stability studies of pharmaceuticals containing **1**, other products apart from **2** were found;[15a] a specific method to assess diazepam stability, avoiding the interference of those degradation products, was described.[15b] Nevertheless, even in very recent reports,[11,16] the spectrophotometric determination is still used to study diazepam stability.

Taking into account the abundant research existing on this frequently prescribed drug and the unusual finding of further reactions of its main degradation product, it was then of interest to study the overall reactions that **2** undergoes under acidic conditions in which diazepam hydrolysis is usually studied. The present work describes the kinetics of the reaction of **2** at several [HCl], in MeOH–H_2O at 60 and 80 °C. The rate of disappearance of **2**, as well as the rates of formation of its reaction products, has been determined. In addition to its relevance to the hydrolysis of diazepam, this study is basically interesting because of the unexpected reactions that are taking place.

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Experimental Section

Materials. Diazepam (Hoffmann-La Roche Inc., Nutley, N.J.) was verified to be chromatographically pure and was used as received. All other chemicals were reagent grade quality. Methanol,[17a] benzene,[17b] hexane,[17c] cyclohexane,[17c] and THF[17b] were purified and made anhydrous by methods previously described. Methanol–aqueous solutions were prepared using bidistilled deionized water. Hydrochloric acid (Aldrich), 37%, ACS reagent, was used throughout the work, and the results were confirmed by testing p.a. grade HCl from other origins. TLC methods used for the analysis of pharmaceuticals containing diazepam, degraded by accelerated aging, were reported previously.[15a] Melting points are uncorrected and they were determined using an Arthur Thomas melting point apparatus; in some cases the melting was also observed using the Kofler apparatus. The GC system was a Hewlett-Packard model 5830 gas chromatography equipped with a FID detector; dried nitrogen was used as the carrier gas; different column phases (OV-101 1.5%, OV-17 3%, NPGS-8%, and SE-30) on Chromosorb WAW were used to determine each product. The GC quantitative determinations were carried out with a NPGS-8% column. The temperature settings were as follows: oven, 210 °C; injection port, 260 °C; FID temperature, 230 °C. The flow rate used was adjusted to 28 mL/min. This system does not separate the chlorinated products **5** and **6**; those products were detected using a SE-30 column. The UV spectra were recorded on a photodiode array Hewlett-Packard HP 8541 A spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on a Brucker AC-200 spectrometer and referenced to internal TMS. The EI-MS spectra were recorded at 70 eV on a Varian MAT CH-7a model equipped with a Mat 166 data processor and a Varian 1400 GC.

Isolation and Characterization of the Reaction Products—Compound **2** was allowed to react in 0.5 M HCl, 1:1 MeOH–H_2O at 70 °C for 15 days prior to isolation. At this point, **2** had completely reacted as determined by analytical TLC. The reaction products were isolated by column chromatography and each fraction was then purified by successive preparative TLC. The following products were isolated and characterized: 2-amino-5-chlorobenzophenone (**3**), 2-(*N,N*-dimethylamino)-5-chlorobenzophenone (**4**), 2-(*N*-methylamino)-3,5-dichlorobenzophenone (**5**), 2-amino-3,5-dichlorobenzophenone (**6**), 2,4-dichloro-10-methyl-9,10-acridinone (**7**), and 2,4-dichloro-9,10-acridinone (**8**). Characterization of products **3** and **5–8** as well as their independent synthesis have been previously described.[14] Compound **4** was characterized spectroscopically and the structure confirmed by independent synthesis: by thorough methylation of **3** with formic acid and 40% formaldehyde following a conventional procedure,[18] after refluxing for 6 h and regular workup, an oil was obtained that was dissolved in ethanol and left in the freezer overnight. Crystals were obtained, mp 91–92 °C (lit.[19] mp 91–92 °C). ^1H NMR ($CDCl_3$): δ 2.74 (s, 6 H, CH_3); 6.92 [d, J = 5, 0.5, 1H, (H-3)]; 7.25–7.6 [m, 5H, (H-4,6,10,11)]; 7.75 (m, J = 1, 5, 2H, (H-9)]. ^{13}C NMR (CD_3COCD_3): δ 37.3, C-14; 113.1, C-3; 118.1, C-5; 122.8, 123.0, C-1,6; 123.6, C-9,10,12,13; 125.2–125.5, C-11,4; 131.5, C-2. MS: *m/z* (ion, % relative intensity) 261 ($M^{+\bullet}$ + 2) 39, 260 ($M^{+\bullet}$ + 1) 18, 259 ($M^{+\bullet}$) 100, 244 ($M^{+\bullet}$ − 15) 36, 242 ($M^{+\bullet}$ − 17) 94, 207 ($M^{+\bullet}$ − 42) 84, 182 ($M^{+\bullet}$ − 77) 20, 154 ($M^{+\bullet}$ − 105) 2, 147 ($M^{+\bullet}$ − 112) 16, 105 ($PhCO^+$) 12.

Kinetic Measurements—Stock solutions (10^{-2} M) of the substrates **1** or **2** were prepared in methanol. Stock solutions of HCl were prepared in water. Appropriate volumes of the stock solutions were mixed and diluted as required to obtain the desired reactant concentrations in 1:1 MeOH–H_2O solvent. Preliminary studies of the acid hydrolysis of **1** were previously reported.[14] In the present study, aliquots of the solutions of **2** in sealed ampules were placed at once into the thermostat. The solutions were allowed to reach constant

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Table 1—Reaction of 2-(*N*-Methylamino)-5-chlorobenzophenone (2) with 0.5 M HCl in Methanol–H_2O at 60 °C

t, days	[2], [a] 10^3 M	[5 + 6], 10^3 M	[3], 10^3 M	*t*, days	[2], [a] 10^3 M	[5 + 6], 10^3 M	[3], 10^3 M
0	3.32			8.13	1.01	1.42	0.89
0.87	3.25		0.08	18.0	0.35	1.50	0.87
2.3	2.59	0.34	0.39	23.8	0.12	2.04	1.11
3.12	2.44	0.41	0.31	24.2	0.05	2.17	0.90
4.05	2.26	0.56	0.51	26.2	0.06	2.05	0.91
5.14[b]	1.65	0.83	0.76				

[a] $[2]_0 = 3.32 \times 10^{-3}$ M. $t_{1/2} = 6.70$ days. Compound **4** was detected only in trace amounts. At higher reaction times some other compounds were detected in trace amounts (yield ≤3%). [b] From this time on, acridinones **7** and **8** were also detected by TLC.

temperature, an aliquot was taken and worked up, and the concentration was measured by GC; this was considered the $[2]_0$. The rate of disappearance of the reactant as well as the rate of formation of products was monitored by GC at appropriate time intervals, a typical run is shown in Table 1. The reactions were followed for at least 3 half-lifes and the final values were determined after at least 10 half-lifes. Calculations of the rate constants were carried out by a computer program designed to give the best straight line in each case. The first portions of the curves behave as parallel reactions in most of the cases. Nevertheless, the best fit was obtained with a whole set of differential equations corresponding, in each case, to the several parallel and/or consecutive reactions formally represented in the Scheme 1.

The differential equations were derived following classical procedures;[20] a typical set is shown below, where $k_{\psi 1}$, $k_{\psi 2}$, $k_{\psi 3}$, and $k_{\psi 4}$ are the pseudo-first-order rate constant for the reactions **2** → **3**, **3** → **5** + **6**, **2** → **4**, and **2** → **5** + **6**, respectively.

$$dC_3/dt = k_{\psi 1}C_2 - k_{\psi 2}C_3$$

$$dC_2/dt = -k_{\psi 1}C_2 - k_{\psi 2}C_2 - k_{\psi 3}C_2$$

$$dC_{5+6}/dt = k_{\psi 4}C_2 + k_{\psi 2}C_3$$

$$dC_4/dt = k_{\psi 3}C_2$$

The system was solved by numerical integration, and simulation of the system was firstly carried out by an iterative procedure using the TUTSIM program. A good fit between simulated and experimental plots was obtained for the significant portions of each curve. In order to reproduce the entire curves, fitting was also carried out with the SIGMA PLOT program, using the FITTING subroutine; the parameters were then adjusted through the MATH subroutine. In order to simplify the system, consecutive first-order reactions were firstly assumed, then the systems were adjusted to involve, in some cases, parallel reactions too. The experimental points as well as the calculated plots are shown in Figures 1–5. The general equations to obtain the actual concentrations are shown below for the case where mainly consecutive first-order reaction kinetics are observed.

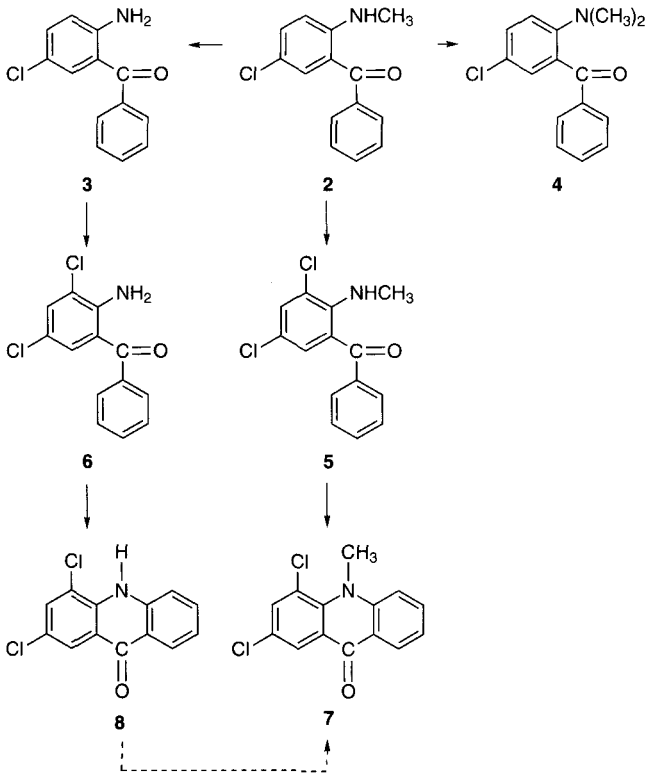
$$[2] = [2]_0 e^{-k_{\psi 1}t}$$

$$[3] = [2]_0 \frac{k_{\psi 1}}{k_{\psi 2} - k_{\psi 1}}(e^{-k_{\psi 1}t} - e^{-k_{\psi 2}t})$$

$$[4] = \frac{k_{\psi 1}}{k_{\psi 4} - k_{\psi 1}}[2]_0(e^{-k_{\psi 1}t} - e^{-k_{\psi 4}t})$$

$$[5 + 6] = [5 + 6]_\infty \left\{1 - e^{-k_{\psi 1}t} - \frac{k_{\psi 1}}{k_{\psi 2} - k_{\psi 1}}(e^{-k_{\psi 1}t} - e^{-k_{\psi 2}t})\right\}$$

The significant portions of the curves were well-reproduced. To fit also the final portions of the curves, another term was added that accounts for the parallel, reversible, and consecutive reactions that are occurring at that time. To obtain smooth lines, plotting of the curves was carried out with the EXCELL program, using the parameters curves was carried out with the EXCELL program, using the parameters given by the SIGMAPLOT. Table 5 shows the rates given by the SIGMAPLOT (in $days^{-1}$) which are compared with the values derived by experiment and those given by the TUTSIM program.

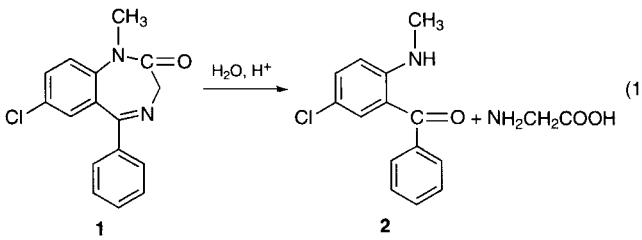


Scheme 1

Rate constants were all obtained at least in duplicate and average results are presented in Tables 2–4. The reproducibility of the rate constants is within 3% for the reaction of **2** and around 5% for the formation of the degradation products. The simulated rates are shown in Tables 2, 4, and 5.

Results

Reaction Products—Reaction **1** shows the total hydrolytic cleavage of diazepam (**1**), as reported in the literature.[1,7–12]It



(1)

is described that the TLC analysis of the acid degradation of diazepam ([HCl] = 0.1 M), at T = 70–85 °C, indicated formation of only two products, which were identified as 2-(*N*-methylamino)-5-chlorobenzophenone (**2**) and glycine, and that the final UV spectrum of the reaction mixture was found to be identical to that of **2**.[8–10] Nevertheless, when the reaction was reinvestigated in our lab, other products, apart from **2** and glycine, were detected by careful TLC. The compounds were fully characterized and their structures confirmed by independent synthesis.[14] Scheme 1 shows the six degradation products isolated. The reaction of **2** with HCl was then studied in the [HCl] range 0.5–2 M. As shown in Scheme 1 several unexpected reactions of **2** occurred to give rise to

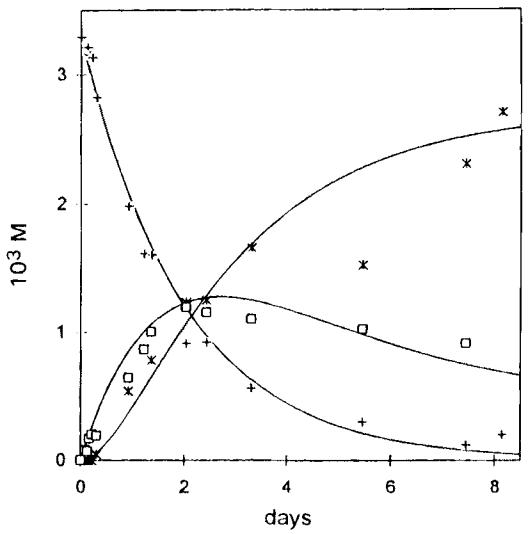


Figure 1—Reaction of 2-(*N*-methylamino)-5-chlorobenzophenone (**2**) with 0.5 M HCl in 1:1 methanol–water, at 80 °C: (+) [**2**], (*) [**5** + **6**], (□) [**3**].

products **3**–**8**. Formally, a demethylation would form compound **3** while a methylation would form **4**. Compounds **5** and **6** could be derived from an electrophilic substitution by chlorine, on **2** and **3**, respectively; alternatively, **6** could be formed by a demethylation of **5**. Although **5** could be also produced from a methylation of **6**, on the grounds of the following results, this pathway seems to be less favored. The condensed ring compounds **7** and **8** would form through a cyclization of the 2-amino group with the ortho-position of the other aromatic ring; cyclization could occur after or before the chlorination. Although every combination of processes should seem possible, the kinetic studies suggest some routes of formation, albeit some steps remain as yet unclear.

Reaction of 2-(*N*-Methylamino)-5-chlorobenzophenone with HCl in MeOH–H_2O at 80 °C—As it was mentioned in the introduction, the spectrophotometric technique was inappropriate to follow the kinetics. Taking into account the amounts of products observed in preliminary studies,[14] a set of several GC conditions was examined, and the systems described in the Experimental Section allowed the determination of the actual concentrations of products **2**–**4** and **5** + **6**. Compounds **7** and **8** cannot be analyzed by GC and their concentrations were estimated by semiquantitative TLC. A range of 0.5–2.0 M HCl and two temperatures, 60 and 80 °C, were selected. Because of the complexity of the reaction, most of the results will be presented in the form of figures for the sake of clarity.

Figure 1 shows the plot of the molar concentrations of **2**, **3**, and **5** + **6**, in the reaction of **2** with 0.5 M HCl at 80 °C, against time. It can be observed that [**2**] decreases steadily, [**3**] increases up to 2 days and then slowly decreases, while [**5** + **6**] increases steadily after a short induction period. At the end of the reaction, only compounds **5** + **6** were detected ([**5** + **6**] = 2.7×10^{-3} M), plus traces of other compounds with higher retention times; analytical TLC showed also the presence of products **7** and **8**. The plot of $\ln([\mathbf{2}]_o/[\mathbf{2}])$ vs t was a straight line, which indicates that the degradation of **2** follows first-order kinetics. The pseudo-first order specific rate coefficient was calculated from the regression plot and found to be 7.97×10^{-6} s^{-1}. Treatment of the data up to 2 days of reaction was consistent with two parallel reactions. The specific rate coefficients for the formation of **3** and **5** + **6** were calculated from the plots of the molar concentrations of each product as a function of $(1 - e^{-kt})$. The results (average of at least duplicate runs) are shown in Table 2 for this and all the other reactions at different [HCl]. Simulation of the complex

Table 2—Reaction of 2-(*N*-Methylamino)-5-chlorobenzophenone (2) with HCl in Methanol–H_2O at 80 °C

	$10^7 k_\psi$,[a] s^{-1}			
[HCl], M	**2**	**3**	**4**	**5** + **6**
0.5	78.3	41.4		41.9
0.5[b]	78.3	41.7		33.6
1.0	44.3	20.4	1.60	15.0
1.0[b]	44.3	19.1	2.34	11.6
1.57	20.6	8.13	7.06	3.10
1.5	20.6	5.79	4.62	2.54
2.0	18.0	6.10	6.23	
2.0[b]	18.0	8.56	6.37	0.35

[a] Pseudo-first-order rate constants. [b] Simulated rate constants.

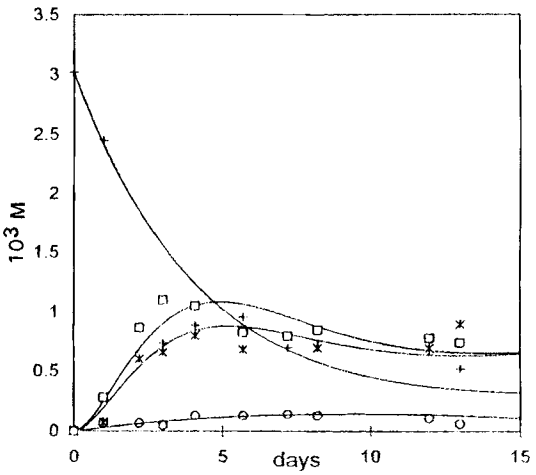


Figure 2—Reaction of 2-(*N*-methylamino)-5-chlorobenzophenone (**2**) with 1.0 M HCl in 1:1 methanol–water, at 80 °C: (+) [**2**], (○) [**4**], (*) [**5** + **6**], (□) [**3**].

reaction kinetics at some [HCl], gave the pseudo-first-order specific rate coefficients which are also shown in Table 2. A fairly good agreement with the experimental rates is observed.

The results of the reaction of **2** with 1.0 M HCl at 80 °C are shown in Figure 2. It can be observed that (a) the overall degradation of **2** is slower than with 0.5 M HCl; (b) the amounts of chlorination products **5** + **6** are always smaller than those of **3**; and (c) a new product, **4**, appeared after a short induction period. Treating the data similarly to that of the reaction with 0.5 M HCl gave the pseudo-first-order rate coefficients shown in Table 2.

The reaction of **2** with 1.52 M HCl at 80 °C is shown in Figure 3. It can be observed that the overall degradation of **2** is even slower than with 1.0 M HCl; the amounts of chlorination products, **5** + **6** are significantly smaller than those of the other degradation products; the concentration of **3** increases up to 5.5 days and then decreases, and the amount of **4** has significantly increased, being the main reaction product.

Finally, the results of the reaction with 2.0 M HCl are shown in Figure 4 (the half-life for the reaction of **2** is 4.82 days). The chlorination products **5** + **6** were detected only as traces; [**3**] increased up to 6 days and then decreased, while [**4**] increased steadily after a short induction period. Compound **4** was the main reaction product, and at the end of the reaction, traces of some other products with higher retention times were detected.

The Reaction of 2-(*N*-Methylamino)-5-chlorobenzophenone with HCl at 60 °C—The reaction with 0.5 M HCl showed a behavior rather similar to that observed at 80 °C, although some peculiarities deserve comment. The degradation of **2** was complete in 25 days; the main reaction products

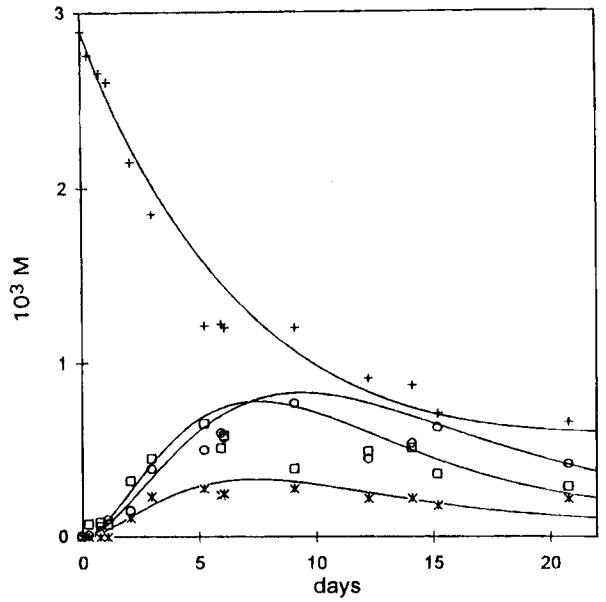


Figure 3—Reaction of 2-(*N*-methylamino)-5-chlorobenzophenone (**2**) with 1.52 M HCl in 1:1 methanol–water, at 80 °C: (+) [**2**], (○) [**4**], (*) [**5** + **6**], (□) [**3**].

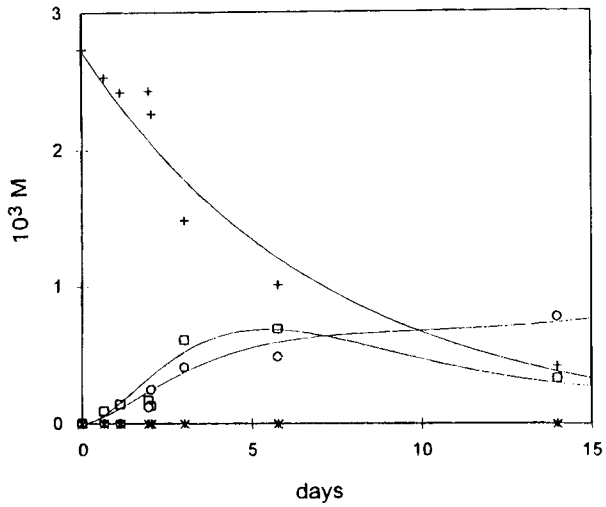


Figure 4—Reaction of 2-(*N*-methylamino)-5-chlorobenzophenone (**2**) with 2.0 M HCl in 1:1 methanol–water, at 80 °C: (+) [**2**], (○) [**4**], (*) [**5** + **6**], (□) [**3**].

Table 3—Reaction of 2-(*N*-Methylamino)-5-chlorobenzophenone (2) with HCl in Methanol–H_2O at 60 °C

	$10^7 k_{\psi 1}$,[a] s^{-1}			
[HCl], M	**2**	**3**	**4**	**5** + **6**
0.5	12.7	5.27		6.35
1.0	9.59	6.10	0.01	0.35
1.53	2.28	0.89	0.74	0.25
0.5[b]	4.25[b]			

[a] Pseudo-first-order rate constants. [b] Reaction carried out in 1:1 THF–H_2O.

were **5** + **6**, which reached an overall concentration of 2.27×10^{-3} M in 24 days. ($[\mathbf{2}]_o = 3.32\ 10^{-3}$ M). Up to 11 days the reaction followed a behavior consistent with parallel reactions. The calculated pseudo-first-order specific rate coefficients for all the [HCl] studied are shown in Table 3. It was observed that with more concentrated HCl solutions, the overall rates of reaction decreased; **4** appeared when working at [HCl] = 1.0–2.0 M. At 1.0 M HCl, **3** was the main degradation product, which reached its maximum concentration in 10 days ($[\mathbf{3}] = 1.6 \times 10^{-3}$ M; $[\mathbf{2}]_o = 3.02 \times 10^{-3}$ M) and then remained

Table 4—Reaction of 2-(*N*-Methylamino)-5-chlorobenzophenone (2) with HCl in Methanol–H_2O

	$10^7 k_2$,[a] $M^{-1}\ s^{-1}$			
[HCl], M	**2**	**3**	**4**	**5** + **6**
	$T = 80$ °C			
0.5	156.6	82.8		83.8
0.5[b]	156.6	83.4		67.2
1.0	44.3	20.4	1.60	15.0
1.0[b]	44.3	19.1	2.34	11.6
1.57	11.5	3.89	3.97	1.97
1.57[b]	11.5	3.69	2.94	1.62
2.0	10.3	4.06	3.53	
2.0	10.3	4.23	3.18	0.18
	$T = 60$ °C			
0.5	25.4	10.54		12.7
1.0	9.59	6.1	0.01	0.35
1.53	1.45	0.58	0.47	0.16
0.5[c]	8.45[c]			

[a] Bimolecular rate constant. [b] Simulated rate constants. [c] Reaction carried out in 1:1 THF–H_2O.

constant up to 35 days, while the [**4**] and [**5** + **6**] increased steadily. At 1.5 M HCl, contrarily to the reaction at 80 °C, formation of chlorination products was not observed; the [**3**] and [**4**] were similar throughout the time ([**3**] was slightly higher); both increased steadily up to 40 days and then slightly decreased. The whole reaction was very slow: after 50 days of reaction the [**2**] was 1.10×10^{-3} M. ($[\mathbf{2}]_o = 2.89 \times 10^{-3}$ M).

To find out the effect of the solvent on the rates and the product distribution and to elucidate if some methyl transfers from and/or to **2** occurred in the absence of methanol, the reaction was also examined in aqueous–THF. It was observed that the overall degradation of **2** was slower in THF–H_2O than in MeOH–H_2O ($t_{1/2} = 12.1$ days, against $t_{1/2} = 6.9$ days, both in 0.5 M HCl at 60 °C). The chlorination product **5** was the main reaction product: after 30 days, [**2**] decreased to 1.15×10^{-3} M and [**5**] increased steadily up to 1.96×10^{-3} M. This was indeed the maximum [**5**] observed under these conditions; it remained constant up to 60 days and then slightly decreased. None of the products produced from methyl transfers (**3**, **4**, **6**) were found. On the contrary, when the reaction mixture was examined by GC–MS after 130 days of reaction, only unidentified products with ion fragments showing incorporation of the butyl moiety of cleaved THF were observed, apart from **5** ($[\mathbf{5}] = 1.30 \times 10^{-3}$ M). Isolation and characterization of these products was considered irrelevant for the present study.

Table 4 shows the calculated bimolecular rate coefficients for all the reactions studied.

Discussion

In spite of the complexity of this reaction, some conclusions can be deduced from the kinetic studies. Furthermore, there are also some comments in previous works that in the light of the present findings become more meaningful. Thus, Hahn et al.[8] in their kinetic studies of the hydrolysis of **1** in aqueous media reported that the TLC analysis of the reaction mixture at low pH values indicated formation of one product that was not observed at pH values above the pK_a (pK_a of diazepam in water is 3.3).[22] "Attempts to isolate sufficient quantities for identification of the compound were unsuccessful due to the small amount present",[8] and the authors tentatively presumed it was an intermediate resulting from the partial hydrolysis of **1** previous to the formation of **2**. Nevertheless, contrarily to what was observed for oxazepam (for which both intermediates were isolated although not fully characterized) this

product did not recycle to **1**. Taking into account that their studies were carried out using 0.1 M HCl and temperatures up to 85 °C, it is probable that some of the products shown in the present paper might have been also formed in the studies of Hahn et al. On the other hand, Bronxton et al.[11] in their acid hydrolysis of diazepam ([HCl] = 0.01–0.26 M, at 68.5 °C) reported that "a very slow subsequent reaction was observed, but it was too slow to obtain reliable results": Points were collected for the first *two* half-lifes and the infinity value was calculated by using a computer program designed to give the best straight-line fit to the data. For similar reasons, Nakano et al.[23] considered the absorbance measured after only 3 half-lifes, as the final value. In the three cases, the spectrophotometric technique was used to follow the kinetics.

As can be observed in Tables 2 and 3 the rate of reaction of **2** diminishes when [HCl] increases. This behavior is opposite to that observed in the acid hydrolysis of diazepam, for which the rate increased with the [HCl] in the range pH = 0.9–2.6 in aqueous media.[8] The diminution in both: the observed pseudo-first-order rate constants (Tables 2 and 3) as well as the calculated bimolecular rate constants (Table 4) for the reactions of **2** with increasing [HCl] would suggest that the unprotonated form of **2** is more prone to undergo the subsequent reactions than the protonated one. Compound **2** is expected to be a very weak base, although its pK_a in 1:1 H_2O–MeOH has not been determined, an approximate value can be easily estimated. We have demonstrated that the effect of a 2-COPh substituent is very similar to that of 2-nitro;[23a] taking into account that the pK_a of 2-nitro-4-chloroaniline in water is −1.03[24] and the electron-donating effect of a CH_3 group on the N, the pK_a of **2** could be conservatively estimated to be not more than −0.5. This means that the ratio [**2**]/[$2H^+$] (where $2H^+$ symbolizes the acid conjugate of **2**) should have nearly the values 6 and 1.6, for [HCl] = 0.5 and 2 M, respectively. Unprotonated **2** should be the reactant species in both cases.

Although only two temperatures were examined, comparison of the data in Tables 2 and 3, as well as in Table 4, indicates that the overall reaction exhibits an important energy of activation, typical of polar reactions. The rates of formation of each one of the reaction products were also very sensitive to the temperature. Some preliminary reactions carried out at 35 and 45 °C allowed a crude estimation of the energy of activation of around 83.6 kJ mol^{-1}.[23b] The energy of activation for the acid hydrolysis of **1** in 0.5 M HCl, 1:1 MeOH–H_2O, was found to be 75.7 kJ mol^{-1} [10^6k_2 (s^{-1} M^{-1}): 1.93 (35 °C), 4.78 (45 °C), 17.4 (60 °C)] which is very similar to the reported value in water: 18.4 kcal mol^{-1} [8] (76.9 kJ mol^{-1}).

Taking into account the reactions shown in Scheme 1, simulation of the rate constants was carried out, considering consecutive first-order reactions and/or parallel reactions in other cases. Table 5 shows experimental and simulated rates in $days^{-1}$, calculated with the SIGMA PLOT and the TUTSIM program for 50–60% and 100% reaction, respectively. In spite of the complexity of the reactions, and the assumptions made to formulate and solve the kinetic equations, a good agreement is found between the partial rate constants calculated from the experimental data and the simulated ones given by adjustment of the derived equations. This gives confidence to the whole scheme of reactions.

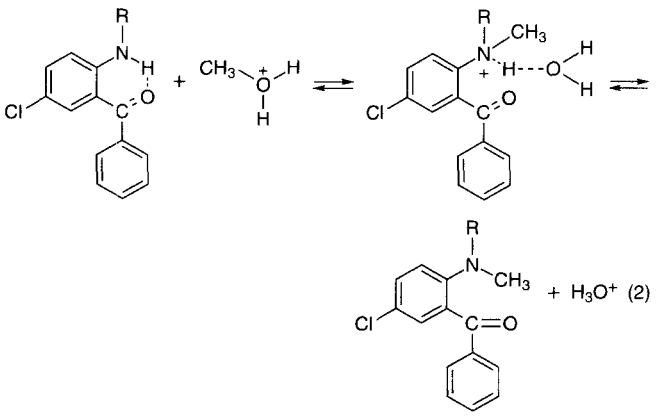
The fact that no products formally arising from a methyl transfer from (or to) **2** were observed in THF strongly suggest that methanol is involved in the methylation and demethylation processes. Similarly, the observation that only **5** was found under those conditions would indicate that the chlorination occurred directly on **2**. 2-(*N*,*N*-Dimethylamino)-3,5-dichlorobenzophenone (**9**) was not detected. Taking into account that the methylation increased at high [HCl], it is

Table 5—Reaction of 2-(*N*-Methylamino)-5-chlorobenzophenone (2) with HCl in Methanol–H_2O at 80 °C

[HCl], M		$10k_{\psi 1}$,[a] $days^{-1}$	$10k_{\psi 2}$,[a] $days^{-1}$	$10k_{\psi 3}$,[a] $days^{-1}$	$10k_{\psi 4}$,[a] $days^{-1}$
0.5	exptl	6.76	3.58	3.62	
	S[b]	5.00	3.90	4.1	
	S[c]		3.60	2.9	
	S[d]		3.90	4.2	
1.0	Exptl	3.85	1.76	1.29	0.14
	S[b]	2.30	2.00	2.60	0.60
	S[c]	3.85	1.65	1.00	0.20
1.57	Exptl	1.8	0.70	2.60	0.61
	S[b]	1.3	0.77	2.20	0.65
1.5	Exptl	1.80	0.50	2.20	0.40
2.0	Exptl	1.55	0.53		0.54
	S[b]	1.40	1.00		0.80
	S[c]	1.55	0.74	0.03	0.55

[a] Pseudo-first-order rate constants. [b] Rate constant simulated with the SIGMA PLOT procedure. [c] Rate constant simulated with the TUTSIM procedure (up to 50–60% reaction). [d] Rate constant simulated with the TUTSIM procedure (100% reaction).

proposed that protonated methanol is the methylating agent, although its equivalent, the methyl cation produced from loss of water, cannot be discarded (eq 2). A similar reaction has



been recently proposed to explain the alkylation by alcohols and protic acids in the Friedel–Crafts reaction.[25] Regarding the demethylation to form **3**, the reversible mechanism would be operating, and protonated water would be the reactant attacking the amino nitrogen.

The appearance of the observed chlorination products is rather unusual under the present reaction conditions. Products chlorinated in the unsubstituted benzene ring were not detected and chlorination occurred at the position expected for an electrophilic aromatic substitution. Nevertheless, no electrophilic reactant was present in the reaction media. The possibility that very reactive impurities could exist in the reagent was thoroughly checked by testing several lots of reagent grade HCl from different origins. No differences were found either in the rates or in the product distributions. Some reactions were also carried out in the presence of *m*-dinitrobenzene, a well-known radical scavenger, but no effects were observed; the same was true for the reactions carried out in the presence of small amounts of $FeCl_3$. The possibility of traces of molecular chlorine and/or of metal salts that could act as catalysts being dissolved in the reagent was also checked and discarded. The fact that the reaction was faster at low [HCl] made it also highly improbable that the reaction could be due to trace impurities present in the reagent.

Some preliminary reactions were also carried out with **3** under the same reaction conditions to find out if the methyl

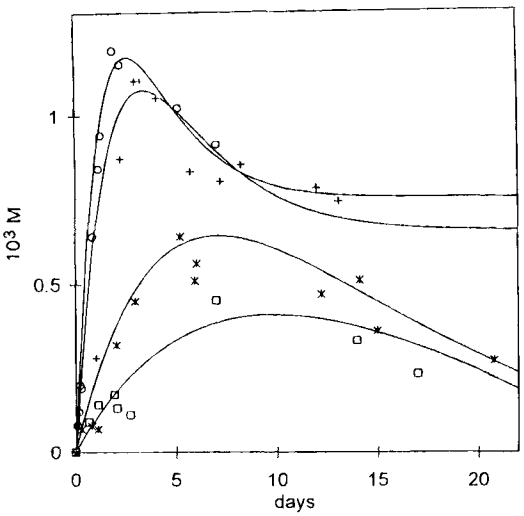


Figure 5—Reaction of 2-(*N*-methylamino)-5-chlorobenzophenone (**2**) with different concentrations of HCl in 1:1 methanol/water, at 80 °C: (○) 0.5 M HCl, (+) 1.0 M HCl, (*) 1.5 M HCl, (□) 2.0 M HCl.

group was required for the chlorination to occur.[23b] It was previously reported that treatment of the *N*-methylaniline with $Ca(ClO_4)_2$ produced *N*-chloro-*N*-methylaniline, which rearranged to give ring-chlorinated products, mainly 2-chloro-*N*-methylaniline.[23] Nevertheless, when **3** was treated with HCl in THF, chlorination also occurred, and **6** was produced, although the reactions were slower than with **2**. The observed results thus indicate that the methyl group is not involved in the chlorination; the substituent effect observed is consistent with that expected for an electrophilic aromatic substitution.

On the other hand, the appearance of the condensed ring products **7** and **8**, under the present reaction conditions, is also amazing. We synthetized those products by HCl *elimination* from 2-(N-methylamino)-2′,5-dichlorobenzophenone (**10**), with sodium hydride in DMSO and further treatment with sulfuryl chloride,[14] conditions that are very different from the present ones. Furthermore, **10** was carefully sought in the reaction mixtures, but it could not be detected in any case. Similarly, no monochlorinated acridinones, namely, 2-chloro-9,10-acridinone (**11**) and/or the 2-chloro-10-methyl-9,10-acridinone (**12**), could be found in the reaction mixtures. The absence of monochlorinated compounds **11** or **12** suggests that the cyclization would occur with the double chlorinated compounds **4** and **5**, i.e. the annelation would occur after the second chlorine atom has attacked the 3-position. A cyclization mechanism involving an intermediate resulting from the chlorination could be envisaged.

Regarding the relevance that the present finding could have on the studies of the acidic hydrolysis of diazepam, the present results strictly apply to the reactions carried out in 1:1 $MeOH-H_2O$ solutions. Nevertheless, it can be observed in Figure 5 that a great extent of demethylation occurred in a few hours at low [HCl]. Most of the studies reported in the literature were carried out with [HCl] ≤ 0.5 M; although they have been run in water, it is probable that any formation of **3** could not have been detected by the spectrophotometric technique (λ_{max} = 380 and 410 nm for **3** and **2**, respectively). Similarly, it can be observed in Figure 1 that **2** has completely reacted with 0.5 M HCl in 8 days at 80 °C and that the amount of **5** + **6** is important (2.8×10^{-3} M). (λ_{max} = 380 nm for **5** + **6**). When monitoring the actual absorbances, as well as the final data, the presumable interferences by products **3**–**8** should be taken into account.

Although, obviously, chlorinated products would be observed only when studying the acidic hydrolysis of diazepam with HCl, it is worth mentioning that in stability studies of solid dosage forms containing diazepam in aqueous solutions other products, apart from **2**, were detected early in the reaction.[15] Nevertheless, even a very recent HPLC method reported to assess diazepam stability considers **2** as the only degradation product.[25]

Gas chromatography is widely used in studies of the *metabolism* of diazepam.[28] The major metabolic pathway for diazepam was shown early on to be the demethylation at the nitrogen in position 1.[29] The method generally used for the study of the fate of diazepam in blood samples was first developed by de Silva et al.[30] and then modified by Steyn and Hundt.[31] The de Silva method[30c] is a quantitative GC procedure capable of differentiating between the intact drug and its major metabolite, the N-demethylated analog. The point related to the present study is the fact that the reported methods are based on a previous treatment of the sample which is heated (often at 100 °C) with 6 N HCl to effect the hydrolysis. It can be easily see in Figures 3 and 4 that the demethylated product, **3**, is the main degradation product in the first days of reaction in 1.52 and 2.0 M HCl, at 80 °C. Taking into account that N-demethylation increases with [HCl], it is reasonable to assume that it would occur to an important extent on exposure of diazepam to 6 M HCl. Then, it is possible that works dealing with the metabolism of diazepam may have overestimated the extent of metabolism of this compound. Even in a recent HPLC method[32] for the determination of benzodiazepines, the urine samples are hydrolyzed by heating with "concentrated" HCl for 15 min at 100 °C. The yield reported for the hydrolysis product, **2**, is 74% in water and 72% in urine. The method is described to separate **2** from the N-desmethyl metabolite, and it is highly probable that under the treatment conditions, demethylation occurs to some extent.

Other GC methods reported for the study of the metabolism of medazepam,[33] nitrazepam,[34,35] clonazepam,[36] and flunitrazepam[36] are also based on an acid hydrolytic treatment of the sample. In all these cases, methods could produce an overestimation of the extent of the metabolism of those compounds.

Regarding the observation of the other "unexpected" products, although these have not been described for diazepam, there are some comments in the literature, describing the observation of halogenated products in studies of clonazepam,[36] flunitrazepam,[36] and bromazepam[37] following treatment with 4 or 6 M HCl. In a study of clonazepam, chlorination was attributed to the presence of free chlorine and traces of metals that could act as catalysts, a fact that was carefully examined and discarded in the present study. In other cases, the authors report that "migration" of halogen atoms occurs in the case of clonazepam and bromazepam.[38] This is not possible in the present study: it can be observed in Table 1, and also in the figures, that this work gives an almost quantitative account of the fate of **2**; e.g., 3.32×10^{-3} M **2** was converted into 2.05×10^{-3} M **5** + **6** after 26.2 days in 0.5 M HCl at 60 °C, and no dechlorinated product was found. The second chlorine atom attached to products **5**, **6**, **7**, and **8** in this study clearly should be provided by the HCl present in the reaction media. Finally, substituted 9-acridinones were detected by heating of the benzophenone derivatives of flunitrazepam and its N-desmethyl metabolite at elevated temperatures in *strongly basic* media.[39,40] This is due to the high reactivity of the *fluorine* group present in the *2′-position* of the phenyl ring, which easily eliminates HF; conditions quite different from the present study with the 2-(*N*-methylamino)-5-chlorobenzophenone in *acidic media*.

Conclusions

The present work demonstrates that 2-(*N*-methylamino)-5-chlorobenzophenone (**2**) reacts with HCl in aqueous methanol to give unexpected products that are formally derived from methyl transfer, chlorination, and cyclization reactions. The same products were isolated from the acid hydrolysis of diazepam in methanol–water. The decrease in the rates of reaction with increasing HCl indicates that unprotonated **2** reacts to form the demethylated, chlorinated, and annelated products. On the contrary, the methylation increases with [HCl], suggesting that protonated methanol is the methylating reactant. Confirming this assumption, methyl transfers were not observed in aqueous THF. The measured rates indicate that these reactions could interfere in the HCl hydrolytic studies of diazepam, especially when they are followed by spectrophotometric techniques. They could also lead to an overestimation of the extent of the metabolism of diazepam, when the samples are exposured to an acid hydrolitic treatment previous to the GC determinations.

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