On the Mechanism of Hydrolysis of the Triazolobenzodiazepine, Triazolam. Spectroscopic Study

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Received August 8, 1986

The hydrolysis of 8-chloro-6-(2'-chlorophenyl)-1-methyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (Triazolam) at room temperature, involves a reversible mechanism. The intermediate is a protonated species and the final product is the ring-opened compound resulting from the reversible scission of the imine bond. The two compounds were determined simultaneously as a function of *p*H with pmr and cmr spectrometry. Spectral data of the benzophenone derivative **II** (ir, cmr, pmr) are reported.

J. Heterocyclic Chem., **24**, 421 (1987).

Since their introduction in the early 1960's, 1,4-benzodiazepines have made a substantial therapeutic contribution matched by few other drugs. As a consequence of their rapid development, the number of published analytical methods for their detection, identification and determination is large and, indeed, still increasing specially for bioanalytical studies. Most of these analytical methods have been based on the physical and chemical properties of the original compounds, as discussed in several pertinent reviews [1-3]. Nevertheless, it has been found that colour reactions of the original 1,4-benzodiazepines are not sufficiently sensitive and that the thermal stability of some compounds is questionable. Therefore, many authors have proposed the conversion of 1,4-benzodiazepines to yield products which can be more easily evaluated by other methods [4].

On the other hand, it is important to examine the chemical structure of these psychotropic drugs and their derivatives in the *p*H range close to physiological, since the drug absorption in the gastrointestinal tract is greatly affected by the nature of protonated and unprotonated species.

Among the newer benzodiazepine derivatives are those containing heterocyclic groups such as imidazolobenzodiazepines [5], thienodiazepines [6] and triazolobenzodiazepines [7], showing many interesting pharmacological properties [8].

During the last few years, in connection with our studies on psychotropic drugs such as benzodiazepines, we have reported spectrometric data of triazolobenzodiazepines [9], together with the kinetic studies on the hydrolysis reaction of 8-chloro-6-(2'-chlorophenyl)-1-methyl-4*H*-[1,2,4]-triazolo[4,3-*a*][1,4]benzodiazepine, Triazolam **I** [10]. In the present paper, we wish to report the spectroscopic study (pmr, cmr and ir) that we have done in order to establish unambiguously the mechanism of the hydrolytic reaction of Triazolam in acidic media at room temperature.

Taking into account that the pK_a calculated for Triazolam **I** [11] is 2.26, the strategy we developed to attain our aim was as follows:

From a stock solution of Triazolam (2.36×10^{-3} *M*) in methanol, solution **T**, an aliquot was diluted in 0.1 *M* hydrochloric acid, solution **A**, while another aliquot was treated with 2.5 *M* hydrochloric acid, solution **B**, and then the latter two solutions were allowed to stand overnight.

After lyophilization of the above solutions (**T**, **A** and **B**), the thus obtained solids were immediately chromatographed (tlc). Towards this end, the three samples were dissolved in chloroform, spotted and developed in a closed tank with chloroform/methanol (9:1). After drying, the plates were visualized using a short wavelength uv lamp (254 nm) and then sprayed with Dragendorff's reagent [12] to produce visible spots.

Solution **T** showed a unique spot (R_f = 0.46) and so did solution **B** (R_f = 0.25). Solution **A** chromatogram showed both spots (R_f = 0.46 and R_f = 0.25), revealing the presence of a mixture of the former two compounds.

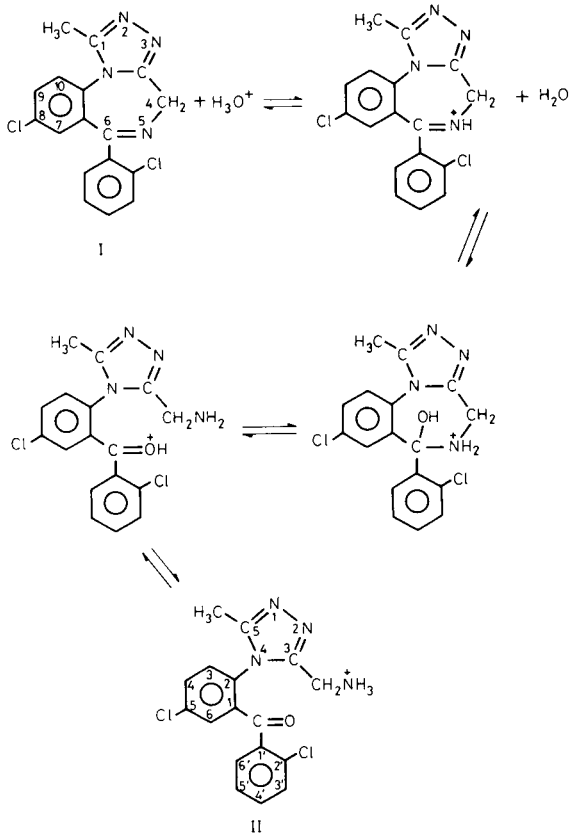
Any attempt to separate the components of solution **A** (column chromatography either on Alumina or Silica gel with a variety of solvents) failed, presumably due to the fact that both components of solution **A** were in an equilibrium on the chromatographic adsorbents employed. Consequently we tried to develop a spectroscopic study of both, solution **A** and solution **B**.

The ir spectrum (potassium bromide) of the lyophylized solid from solution **B** showed bands at 1685 (C=O), 3400-3600 (NH_2) and 2300-3200 cm^{-1} ($^+NH_3$), but no absorption at 1620 cm^{-1}, which is characteristic of the imine bond. On the other hand, in the ir spectrum (potassium bromide) of solution **A** appeared the typical bands of

Triazolam [2] together with those already described for solution **B**.

Based on the observed spectral behaviour of solutions **A** and **B**, a sequential hydrolytic pathway can be proposed:

A protonation takes place when solution **T** is treated with hydrochloric acid followed by a reversible hydrolytic ring opening of the benzodiazepine nucleus, leading, when solution **T** is treated with concentrated hydrochloric acid, to the formation (as the sole product), of a protonated substituted benzophenone derivative: 2',5-dichloro-2-(3-aminomethyl-5-methyl-4*H*-1,2,4-triazol-4-yl)benzophenone **II**, Scheme 1.



Scheme 1

To support the above conclusions, the pmr spectrum was examined and found to be in agreement with the proposed mechanism for the hydrolysis of Triazolam **I** under the reported conditions. In fact, the presence of only one singlet at δ 3.89 for the methylene protons in solution **B**, evidences the fission of the benzodiazepine skeleton, the singlet at δ 2.21 being the signal due to the methyl group. Moreover, while the $^{+}NH_3$ group showed a typical signal at δ 8.91, in the aromatic region the other expected signals, in the correct integration ration, could be observed.

On the other hand, the presence in solution **A**, of a couple of doublets (AB system, J = 13 Hz) for the diastereotopic methylenic protons of the benzodiazepine ring, together with a broadened singlet at 4.01 ppm for the corresponding enantiotopic methylenic protons of the benzophenone derivative **II**, solution **B**, and also a couple of singlets at δ 2.36 and δ 2.78 respectively for the protons of the methyl group in two different derivatives, proved that the so-called solution **A** was in fact, a mixture in the ratio 2:1 of the protonated Triazolam and the ring-opened compound. The rest of the resonance signals were in accord with the above proposition, Figure 1.

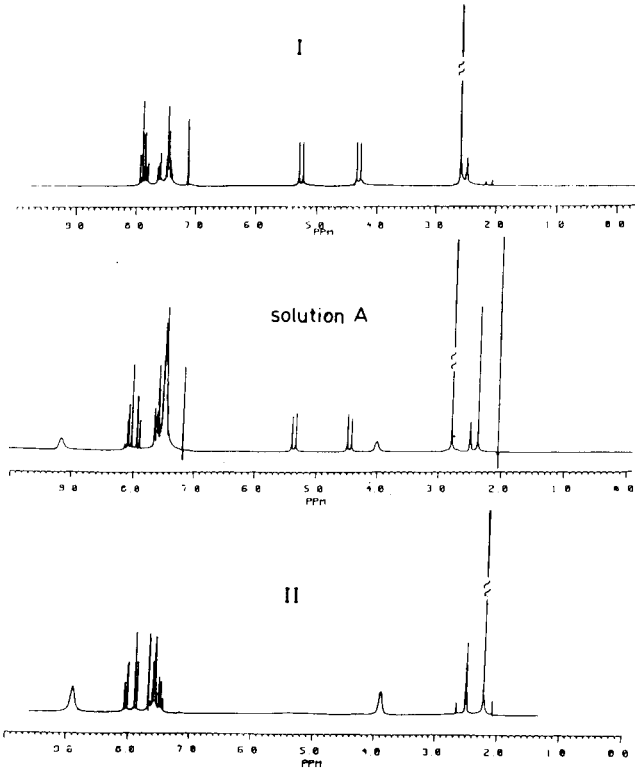


Figure 1. Pmr spectra of **I**, solution **A** and **II** at 200 MHz in DMSO at 23°. All concentrations used were 2% w/v.

Analysis of the cmr data for these compounds, confirmed the above spectroscopic results. In effect, as it can be seen, the most characteristic signal for the benzophenone derivative **II** at δ 191.90 (C=O), together with the resonance of the iminic carbon of Triazolam at δ 167.20, are present in solution **A** [13]. Moreover, the signals for C-3 and C-5 (carbons of the 1,2,4-triazole ring) and the methylene and methyl carbons of both compounds (Triazolam and benzophenone **II**), are also distinguishable, Table 1.

With these results available, the following conclusions can be drawn: The hydrolytic mechanism of Triazolam in the already reported conditions implies, not the fission of the amidine group, but the reversible imine bond cleavage

Table 1

CMR [a] Data for Triazolam **I** and Benzophenone **II** [b]

Compound	CH_3	CH_2	C-3 and C-5	C=N	C=O
I	11.42	45.59	150.82 154.11	166.96	
Solution **A**	11.19	45.04	151.65 153.83	167.20	
	10.08	33.02	149.50 152.84		191.90
II	10.17	33.11	149.59 152.95		191.99

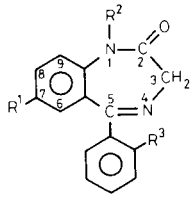
[a] DMSO was used as solvent, in all cases. [b] The triazole carbons designated as C-3 and C-5 were indistinguishable.

reaction leading to an open-ring compound the 2',5-dichloro-2-(3-aminomethyl-5-methyl-4*H*-1,2,4-triazolo-4-yl)-benzophenone, which was characterized as its hydrochloride **II** (mp 176-178°) [14]. The formation ratio of this new compound is a function of the *p*H of the medium. Moreover, from the results already reported we are allowed to propose that an adequate technique for the determination of the pathway for the hydrolysis of Triazolam, a 1,4-benzodiazepine of currently significant clinical importance, is the pmr and cmr spectrometry.

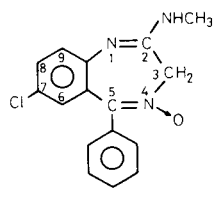
Finally, analysis of bibliographic data on the hydrolytic reaction of 1,4-benzodiazepine derivatives can be summarized as follows:

The reaction is complex and indeed influenced by several factors, for example, the functional groups present in the basic seven-membered structure, their pK_a values, the type of acid used and its concentration, the presence of organic solvents used to achieve complete solution of the reactants, the heterocyclic nucleus joined to the benzodiazepinic ring, *etc* [3].

Studies reported on Diazepam **IIIa** [15], Nitrazepam **IIIb**, Clonazepam **IIIc** and Flunitrazepam **IIId** [16] show that the hydrolytic reaction pathway for these types of compounds involves two parallel reactions: a reversible ring opening at the 1,2-amide linkage or the 4,5-imine bond, followed by the cleavage of the remaining 4,5-imine or 1,2-amide bonds respectively, giving rise to the corresponding substituted benzophenone and glycine derivatives. Chlordiazepoxide **IV**, on the other hand, was reported to degrade sequentially in aqueous solution, yielding 2-amino-5-chlorobenzophenone as the final product. An isolated intermediate in the reaction is the corresponding lactam Demoxepam **IIIe**, which in turn, was formed by hydrolytic cleavage of the methylamino substituent at the 2 position of Chlordiazepoxide **IV** [17-18].



III a-e



IV

a: R^1=Cl, R^2=CH_3, R^3=H

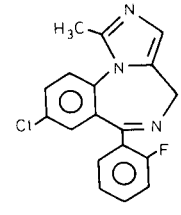
b: R^1=NO_2, R^2=H, R^3=H

c: R^1=NO_2, R^2=H, R^3=Cl

d: R^1=NO_2, R^2=CH_3, R^3=F

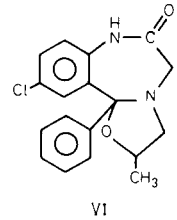
e: R^1=Cl, R^2=H, R^3=H, N_4→O

Nevertheless, the triazolobenzodiazepine studied, Triazolam **I**, presents a different hydrolysis reaction pathway probably due to the existence of the triazole nucleus which stabilizes the product of the first reversible step, the benzophenone derivative **II**, and does not allow the total degradation of the compound. Similar behavior has been reported by Bhattachatyya *et al.* [4] and Walser *et al.* [5] for the hydrolysis reaction of Midazolam *V*, which has an imidazole nucleus joined to the diazepine ring.

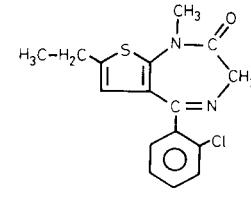


V

It is worthwhile to comment on the different behavior of other psychotropic drugs such as the Oxazolam **VI** [19], which shows an irreversible hydrolytic pathway, and the Clotiazepam **VII** which does not present a hydrolysis reaction in acidic media, [20-21].



VI



VII

EXPERIMENTAL

The melting point was determined on an Electrothermal 1A 6304 apparatus and is uncorrected. The ir spectra were recorded in potassium bromide on a Perkin-Elmer 1430 spectrophotometer and only noteworthy absorptions (cm^{-1}) are listed. The nmr spectra were run on a Bruker WM-200-SY apparatus operating in the pulse-Fourier Transform mode and provided with a computer ASPECT 2000. The pmr spectra were recorded at 200.13 MHz in dimethyl sulfoxide-d_6 solution with tetramethylsilane as the internal standard. The following experimental parameters were selected: the pulse width was 3.8 μs (flip angle ≈60°); acquisition times were 2.02 s for determination of chemical shifts and ^{1}H-^{1}H coupling constants where scans of 2024 Hz sweep width were acquired in 8K data points.

Concerning the ^{13}C data: cmr spectra were run at 50.32 MHz in dimethyl sulfoxide-d_6 solution. The signal of the solvent (δ 39.5 ppm) was used throughout as the internal reference. The following experimental parameters were selected: pulse width 2.3 μs (flip angle ≈35°); acquisition times 0.36 s; interpulse delay 1.5 s; number of scans variable;

sweep width 11363 Hz acquired in 8K data points.

For thin layer chromatography Merck Kieselgel GF 254 plates (0.2 mm thick) were used. The column chromatography was carried out on Merck Kieselgel 60 (0.040-0.063 nm, 230-400 mesh) and Aluminium oxide 90 active neutral (0.063-0.200 nm, 70-230 mesh). To accomplish lyophilization a frozen-drier Biolifar B-10/4E-R-70 was used.

Acknowledgements.

The authors thank Upjohn Laboratories for kindly providing the drug used in this work and to Professor Jesús Hilario Rodriguez Ramos of the Department of Organic Chemistry of the Universidad Autónoma de Madrid, Spain, for the facilities provided for the realization of the pmr and cmr spectra.

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[14] Satisfactory spectroscopic and analytical data was obtained for this new compound **II**; ir (potassium bromide): 1685 cm^{-1} (C=O) 2300-3200 cm^{-1} ($^{+}NH_3$) and 3400-3600 cm^{-1} (NH_2); pmr (dimethyl sulfoxide-d_6): δ 2.21 (s, CH_3, 3H), 3.89 (s, CH_2, 2H), 7.47-7.58 (m, H-3', H-4', H-5' and H-6', 4H), 7.68 (d, H-6, 1H, $J_{4,6}$ = 2.4 Hz), 7.89 (d, H-3, 1H, $J_{3,4}$ = 8.5 Hz), 8.05 (dd, H-4, 1H, $J_{3,4}$ = 8.5 Hz and $J_{4,6}$ = 2.4 Hz), 8.91 (broad s, NH, 3H); cmr (dimethyl sulfoxide-d_6): δ 10.17 (CH_3), 33.11 (CH_2), 149.59 and 152.95 (C-3 and/or C-5), 191.99 (C=O) ppm.

Anal. Calcd. for $C_{17}H_{15}Cl_3N_4O$: C, 51.32; H, 3.77; N, 14.08; Cl, 26.79. Found: C, 51.27; H, 3.77; N, 14.09; Cl, 26.81.

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