Acid Catalyzed Hydrolysis of Brotizolam, A Thienotriazolodiazepine: Spectroscopic Study

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The acid catalyzed hydrolysis of the thienotriazolodiazepine, Brotizolam, 2-bromo-4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (**1**), has been studied spectrometrically (ir, pmr, cmr, and ms). The cleavage reaction of the azomethine bond is reversible and the open-ring compound is in equilibrium with the ring closed compound (protonated form of the parent drug).

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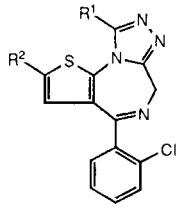
1,4-Benzodiazepines, first synthesized in the early 1960's [1], are well known derivatives extensively used as minor tranquilizers. Since they have very interesting properties as psychotropic drugs [2], a great effort has been made in recent years in order to develop new members of this family. Among the newer benzodiazepine compounds are those containing different heterocyclic rings annellated to the basic benzodiazepine system, such as imidazobenzodiazepines [3] and triazolobenzodiazepines [4], showing many interesting pharmacological properties [5].

More recently, a small family of thienotriazolodiazepines which differ from the triazolobenzodiazepines by the presence of a thiophene ring instead of a benzene ring, has been developed. To our knowledge, only three members are included in the latter group: Brotizolam (We 941) (**1**) [6], Etizolam (**2**) [7], and We 973 (**3**) [8].



1, $R^1 = CH_3$, $R^2 = Br$
2, $R^1 = CH_3$, $R^2 = C_2H_5$
3, $R^1 = C_6H_{11}$, $R^2 = Br$

Comparative pharmacological studies carried out with thienotriazolo- and triazolobenzodiazepines have demonstrated that the former compounds are particularly potent compared with the benzodiazepines [9], the question, therefore arises whether they possess specific behavioural activity. The importance of understanding the drug behaviour after its administration led us to carry out a study on the hydrolytic products of the thienotriazolodiazepine, Brotizolam, at room temperature and at different acid concentrations.

The aim of this paper is the study of the degradation products of the above mentioned drug Brotizolam by spectroscopic techniques, and therefore the establishment of its mechanism of hydrolysis in acidic media.

Taking into account that the pk_a calculated for the drug under study [10] is 2.76, the strategy we developed to attain our objective was as follows:

Brotizolam (**1**) (0.1 g, 0.25 mmole) was treated with 0.1 *M* hydrochloric acid (0.5 ml) and the solution was allowed to stand at room temperature until the equilibrium was reached (3 hours) [11]. After evaporation to dryness under vacuum, the thus obtained solid was immediately chromatographed (tlc). The solid residue was 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. Thus, the chromatogram revealed the presence of a mixture of two products (R_f = 0.65 and R_f = 0.61). Any attempt to separate the components of this mixture (column chromatography either on Alumina or Silica gel with a variety of solvents) failed, presumably due to the fact that both components were in an equilibrium on the chromatographic adsorbents used [13]. Therefore, the above mixture was studied by spectroscopic techniques (ir, pmr, cmr, and ms), in order to determine its compositon.

The ir spectrum (potassium bromide) showed, in addition to the NH bands at 3500-3300 and 3100-2400 cm^{-1},

Table 1

PMR Spectrum of the Mixture of Protonated Brotizolam (**4**) and the Aminoketone Derivative **5** in Deuteriochloroform [a]

Compound No.	CH_3-	-CH_2-	H-3	Aromatic protons	NH_3^+
4	2.81 (s)	5.04 (s)	6.72 (s)	7.26-7.68	
5	2.62 (s)	4.70 (m)	6.99 (s)		9.3 (br m)

[a] s, singlet; m, multiplet; br, broad.

characteristic bands at 1670 (C=O) and 1620 cm^{-1} (C=N). The pmr spectrum (deuteriochloroform) revealed that the crude was, in fact, a mixture of protonated Brotizolam and the corresponding protonated aminoketone derivative **5**, the 5-bromo-2-(3-aminomethyl-5-methyl-4*H*-[1,2,4]triazolo-4-yl)thieno-3-yl 2-chlorophenyl ketone hydrochloride, formed by scission of the azomethinic bond present in the parent drug (see Table 1) [13].

In the pmr spectrum of the mixture, the NH proton of the protonated imine derivative **4** could not be observed because of broadening due to quadrupole interaction of the nitrogen [14]. Moreover, according to the pmr estimate, the equilibrium mixture established under the already reported conditions was composed of 50% of **4** and 50% of **5**.

The cmr spectrum (deuteriochloroform) confirmed the above spectroscopic results. In fact, as it can be seen in Table 2, the most characteristic signal for aminoketone **5** at δ 186.9 (C=O), together with the resonance of the azomethine carbon of protonated Brotizolam (**4**) at δ 164.8 [15] are present in the spectrum of the mixture. Besides, the presence of two methylene carbons and the expected duplicity in the rest of the signals was observed (Table 2).

Table 2

Carbon-13 Chemical Shifts (in ppm from TMS) of the Mixture of Protonated Brotizolam (**4**) and the Aminoketone **5** in Deuteriochloroform

Carbon No.	**4**	**5** [a]
2	110.0	116.5
3	129.1	130.1 [b]
3a	130.9	130.7
4	164.8	186.9
6	46.5	34.5
6a	152.6	154.2
9	150.0	140.9
10a	136.8	135.8
1′	138.2	137.3
2′	133.0	132.6
3′	130.9 [b]	130.2 [b]
4′	131.7	132.6 [b]
5′	127.7	127.4
6′	130.9 [b]	131.0 [b]
CH_3	12.1	10.9

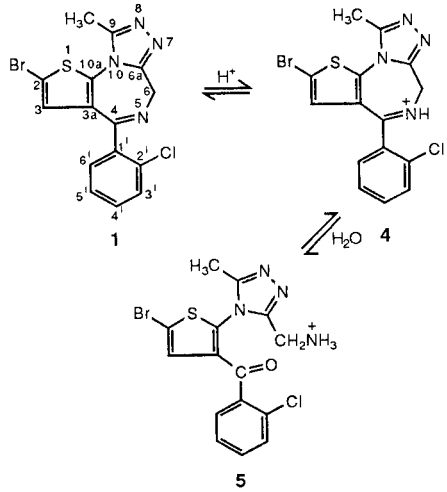
[a] The numbering system is that of thienotriazolodiazepines. [b] These assignments are interchangeable in the same compound.

Since in the literature we have found no report on the mass spectrum of Brotizolam, we considered it would be interesting to record it. Thus, in the mass spectrum of **1** the parent peak appeared at m/z 392 (74%) with the characteristic $M^+ + 2$ and $M^+ + 4$ pattern [16] of compounds containing chlorine, bromine, and sulfur atoms m/z (%) 394 (100) ($M^+ + 2$), 396 (30) ($M^+ + 4$). Moreover, a fragmentation pattern similar to that of triazolobenzodiazepines [17] was observed. In fact, peaks at m/z (%) 363 (8)/365 (10)/367 (3) and 357 (3)/359 (3) were noted, probably produced by loss of a C_2H_5 radical and a chlorine atom from the parent peak, respectively. The peaks at m/z (%) 316 (22)/318 (22), 289 (13)/291 (13) and 210 (15)/212 (5) could be formed by successive losses of CH_3CN, HCN, and a bromine atom from the peak at m/z 357/359. On the other hand, the parent peak could also give rise to the peak at m/z (%) 245 (30)/247 (10) by losses of a CH_2CN group and a bromine atom. Afterwards, the mass spectrum of the mixture was recorded, showing the characteristic peaks of protonated Brotizolam (**4**), together with those of the ketone **5**, whose parent peak appeared as expected at m/z 411/413/415.

On the other hand, Brotizolam (**1**) was also treated with a higher acid concentration (hydrochloric acid, 6*M*) and a mixture in the ratio 1:1 of the protonated drug **4** and the aminoketone **5** was also detected by nmr spectroscopy. A similar behaviour has been observed by other authors [7] in the acidic hydrolysis of Etizolam (**2**), a member of the thienotriazolodiazepine family, though the mentioned study has been carried out spectrophotometrically.

With these results in hand, we can conclude that the hydrolysis of Brotizolam under the reported acidic conditions implies a reversible azomethine bond cleavage reaction, always yielding a 1:1 equilibrium mixture of the protonated drug **4** and the aminoketone **5**. Although these compounds could not be separated by chromatographic methods, spectroscopic techniques (ir, pmr, cmr, and ms) can be used accurately for detection, identification, and determination of both components (Scheme 1).

Scheme 1



1 4 5

EXPERIMENTAL

Infrared spectra were recorded in potassium bromide on a Perkin-Elmer 1430 spectrophotometer and only noteworthy absorptions (cm^{-1})

are reported. The pmr spectra were measured at 250 MHz and cmr spectra at 62.89 MHz on a Bruker WP-250 instrument at ambient temperature, using solutions in deuteriochloroform with tetramethylsilane as internal standard. The ms spectra were determined on a Hewlett-Packard 5930 spectrometer. 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 Aluminum oxide 90 active neutral (0.063-0.200 nm, 70-230 mesh).

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