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Separation and structural elucidation of the hydrolysis compounds of loprazolam☆

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Abstract

The hydrolysis of loprazolam in acidic and basic aqueous media was studied using spectroscopic and chromatographic techniques (UV, HPLC, ^{1}H NMR and GC–MS). 2-Amine-5-nitro-2′-chlorobenzophenone was found as the major hydrolysis derivative in basic media, whereas in acidic media there are two major derivatives, clonazepam and the open compound obtained by scission of the 4,5-azomethine bond of loprazolam.

1. Introduction

Loprazolam, 6-(2-chlorophenyl)-2-[(4-methyl - piperazin - 1 - yl) - methylene - 8 - nitro - 1*H* - imidazo[1,2-*a*][1,4]benzodiazepin-1-one methanesulphonate, is a water-soluble benzodiazepine which has been shown in clinical studies to possess hypnotic properties similar to those of other benzodiazepines [1–4]. Introduction of the imidazole moiety into the benzodiazepine nucleus seems to increase the hypnotic potency [5].

The study of the stability and chemical structure of loprazolam and its derivatives at pH values of physiological importance is of special interest because their absorption by the human body depends on their nature and on the relationship between ionic and non-ionic species and their action is a function of their chemical form in the organism [6,7].

The 1,4-benzodiazepines undergo degradation under hydrolytic reaction conditions which implies the breaking of the diazepine ring at two bonds, the 4,5-azomethine and the 1,2-amide linkages, respectively. This reaction is catalysed by acids and bases, and the final degradation products are a substituted benzophenone and a glycine derivative [8–10]. Hydrolytic breakdown of 1,4-benzodiazepines usually occurs at high temperatures [11,12]. However, some 1,4-benzodiazepines undergo hydrolysis at room temperature, opening the diazepine ring by breaking of the azomethine bond, thus leading to a benzophenone but not to the glycine derivative [11–13]. A similar behaviour has also been established unambiguously for the mechanism of the hydrolytic reaction of triazolam [14] and brotizolam [15], a triazolobenzodiazepine and a thienotriazolodiazepine, respectively, resulting from a reversible scission of the imine bond.

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The aim of this work was the study of the degradation products of loprazolam in acidic and basic aqueous media. Towards this end, spectroscopic and chromatographic techniques (UV, HPLC, ^{1}H NMR and GC–MS) were used.

2. Experimental

2.1. Reagents

Loprazolam was kindly supplied by Hosbon (Barcelona, Spain). A 10^{-3} mol/l stock solution of the drug was prepared in ultrapure water (Millipore, Bedford, MA, USA) and stored in the dark at 4°C. Experimental loprazolam solutions were prepared by dilution of the stock solution with the appropriate Britton–Robinson buffer. All chemicals used were of analytical-reagent grade (Merck, Darmstadt, Germany).

2.2. Apparatus

UV–visible spectra were measured with a Shimadzu (Tokyo, Japan) UV-260 spectrophotometer. ^{1}H NMR spectra (at 250 MHz) were measured on a Bruker (Madrid, Spain) WP-250 instrument at room temperature using solutions in deuterochloroform with tetramethylsilane (SDS, Peypin, France) as internal standard. Mass spectra were obtained with a Varian (Harbor City, CA, USA) Model 3400 gas chromatograph coupled to a Varian Saturn II ion-trap mass detector and equipped with a 300×0.25 mm I.D. DB1701 J&W (0.25 μm) column, at 300°C using a flow-rate of 1 ml/min and an injection volume of 1 μl. Liquid chromatograms were obtained with a Hewlett-Packard (Madrid, Spain) HP-1050 liquid chromatograph, equipped with a UV detector and a 200×4.6 mm I.D. column packed with Hypersil ODS (5 μm). The mobile phase used was methanol–water–phosphate buffer (pH 7.25) (55:25:20, v/v/v) at a flow-rate of 1.5 ml/min [16].

3. Results and discussion

The UV–visible spectra of loprazolam at different pH values were registered. At pH < 3 the absorption spectrum changes with time, indicating a probable acid hydrolysis, such as that for other benzodiazepines and imidazobenzodiazepines. Moreover, at pH > 10 a variation of the spectrum with time was also observed. This behaviour was similar to that of some thienodiazepines, such as clotiazepam [17] and benzodiazepinoxazoles [18]. For pH values between 3 and 10, the spectrum of loprazolam solutions does not change.

In order to establish the number and nature of the hydrolysis products, solutions of loprazolam at different pH values and hydrolysis times were chromatographed using the liquid chromatograph described under Experimental. Acidic loprazolam solutions showed initially only one chromatographic peak at 8.7 min, corresponding to neutral loprazolam. As the hydrolysis advances, two new peaks appear at retention times of 2.3 and 5.1 min. Fig. 1 shows the chromatogram obtained for a loprazolam solution at pH 1.2 after 2 h of hydrolysis. When hydrolysis ends, neutralization of the loprazolam solution leads to

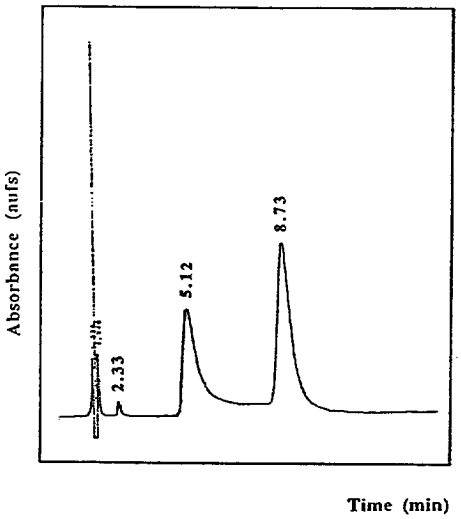

Fig. 1. Chromatogram obtained for a loprazolam solution at pH 1.2 after 2 h of hydrolysis.

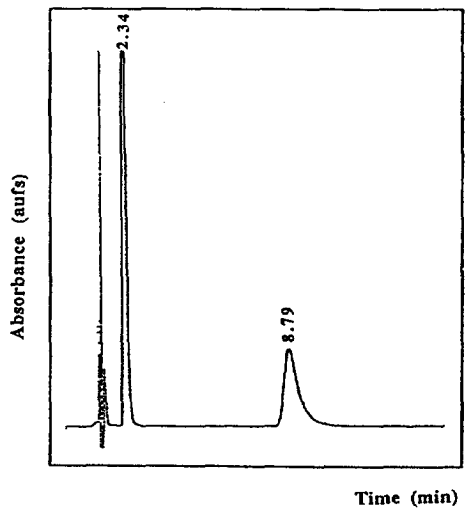

Fig. 2. Chromatogram obtained for a loprazolam solution at pH 12.5 after 2 h of hydrolysis.

transformation of the peak at 5.1 min in the initial compound, showing that the conversion of loprazolam in the compound corresponding to the peak at 5.1 min is reversible. However, the peak at 2.3 min does not disappear when neutralization is accomplished.

On the other hand, basic loprazolam solutions after 2 h of hydrolysis showed a small peak corresponding to loprazolam and a large peak at 2.3 min. Fig. 2 shows the chromatogram obtained for a loprazolam solution at pH 12.5 after 2 h of hydrolysis. This hydrolysis process was irreversible.

The different fractions from liquid chromatography of both types of hydrolysis mixtures were collected and evaporated to dryness and mass and ^{1}H NMR spectra were measured for each fraction.

The solid corresponding to the HPLC peak at

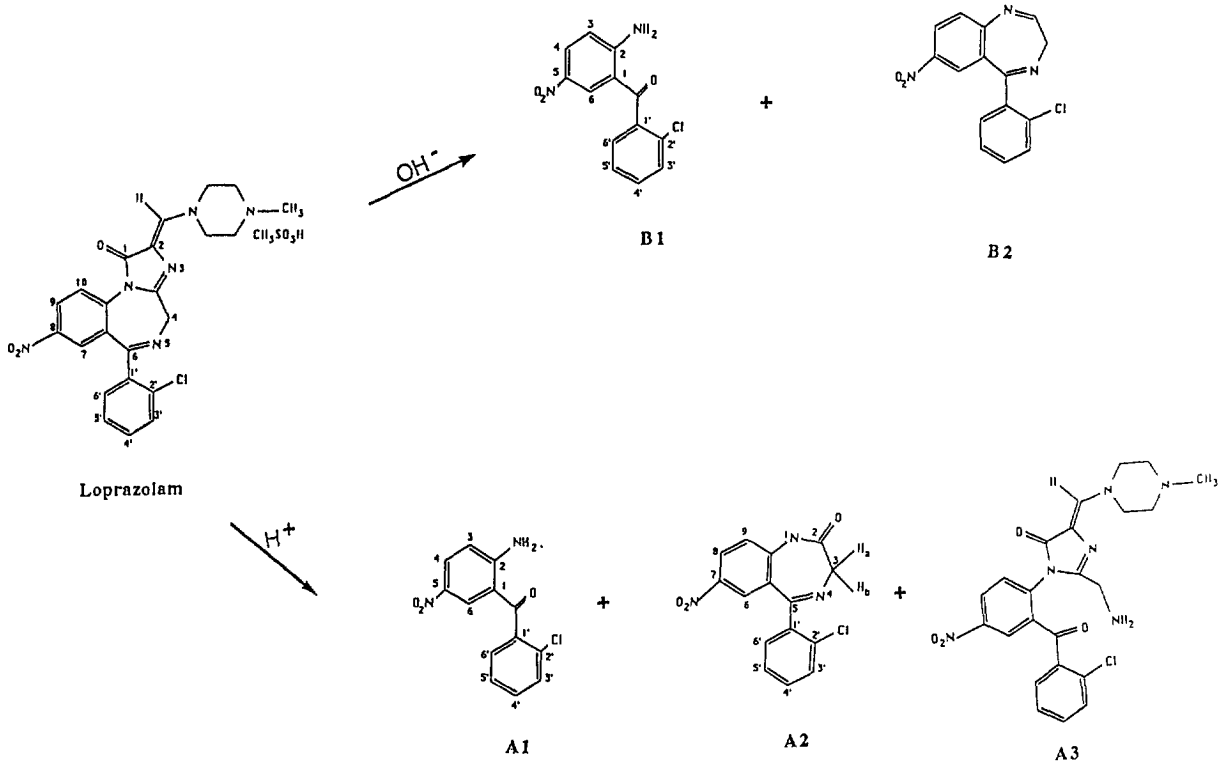

Fig. 3. Structures of the derivatives obtained in the acid and basic hydrolysis of loprazolam.

2.3 min in the basic hydrolysis was dissolved in methanol and injected into the GC–MS system. Two peaks were observed. The first, which was the larger, was assigned as 2-amino-5-nitro-2′-chlorobenzophenone (B1) and the second was identified as compound B2. This compound could originate as a secondary hydrolysis product or could be an impurity in the initial solid loprazolam. This peak also appeared when loprazolam was chromatographed.

The ^{1}H NMR spectrum corresponding to the liquid chromatographic peak at 2.3 min confirmed that this compound was 2-amino-5-nitro-2′-chlorobenzophenone (B1), because the signals due to the N-methylpiperazine and =CH groups in the ^{1}H NMR spectrum for the initial loprazolam had disappeared in the ^{1}H NMR spectrum of this fraction.

In relation to the acid hydrolysis, the liquid chromatographic peak at 2.3 min is the same as in the basic hydrolysis and corresponds to 2-amino-5-nitro-2′-chlorobenzophenone (A1), whereas the peak at 8.7 min is loprazolam. Finally, a solution in methanol of the yellow solid obtained from the fraction corresponding to the peak at 5.1 min was injected into the GC–MS system and two peaks were observed. The first was identified as clonazepam (A2). The second, the major peak, has a mass greater than 420 and its mass spectrum showed only fragments and not the molecular ion. This compound is probably the open derivative of loprazolam (A3) formed by scission of the 4,5-azomethine bond. This was confirmed by the presence in the ^{1}H NMR spectrum of this fraction of a triplet at $\delta = 3.56$ ppm and a singlet at $\delta = 3.48$ ppm, corresponding to the CH_2 group near to NH_2 and to the amino group, respectively.

Fig. 3 shows the structures of the derivatives obtained in the acid and basic hydrolysis of loprazolam. The two major derivatives obtained in the acid hydrolysis, the open derivative and clonazepam, suggest that in the human body the active form of loprazolam is not only the closed loprazolam but also clonazepam, another 1,4-benzodiazepine previously used in pharmacy.

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