Isolation and Structural Elucidation of Degradation Products of Alprazolam: Photostability Studies of Alprazolam Tablets

NORMA S. NUDELMAN, C. GALLARDO CABRERA

Depto. Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pab. II, P.3 Ciudad Universitaria, 1428 Buenos Aires, Argentina

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ABSTRACT: Accelerated thermal, hydrolytic, and photochemical degradations of alprazolam were performed under several reaction conditions. The stress studies revealed the photolability of the drug as the most adverse stability factor; the main photodegradation products were isolated and properly characterized as: triazolaminoquinoleine; 5-chloro-[5̃-methyl-4H-1,2,4-triazol-4-yl]benzophenone, and 1-methyl-6-phenyl-*4H*-s-triazo-[4,3-α][1,4]benzodiazepinone. Accelerated pH-dependent studies show that the photoinstability increases as the pH decreases; at pH 9.0, photodegradation does not occur, therefore, the photochemical degradation of alprazolam was performed in buffered solutions at pH 2.0 and 3.6. The higher rate of reaction was observed at pH = 2.0; consequently, acidic conditions should be avoided and appropriate light protection is recommended during the drug-development process, storage, and handling. The main degradation route for alprazolam tablets is also photochemical. J Pharm Sci 91:1274–1286, 2002

Keywords: alprazolam; photochemical degradation; alprazolam stability; triazolobenzodiazepines; alprazolam photodegradation products

INTRODUCTION

Alprazolam (AL) is a popular triazolobenzodiazepine; compared with the older benzodiazepines, AL has a shorter half-life, a smaller dose is required for therapeutic efficacy (clinical doses range from 0.25 mg three times daily to a total dose of 4 mg per day),[1] and it is extensively used for the control of panic attacks and in the management of anxiety disorders.[2] Many of the routine human studies of AL have utilized conventional gas chromatography with electron capture detection,[3] or high-pressure liquid chromatography (HPLC) with ultraviolet (UV) detection.[4] Nevertheless, because of the low concentrations found in biological specimens, highly sensitive analytical methods have been recently developed for forensic and clinical applications.[2,5]

Stability studies of other triazolobenzodiazepines, such as triazolam[6] and midazolam,[7] have been reported, but despite the extensive use of AL, to the best of our knowledge, a thorough study of its stability has not been published. The kinetics and equilibrium of the reversible AL ring-opening reaction at 25°C were carefully studied over a pH range of 0.5–8.0.[8] Hydrolysis of the benzodiazepinone ring is one the most frequently observed degradation routes for benzodiazepinones;[9,10] nevertheless, in the case of AL, we have determined that hydrolysis under several different conditions, is not a major degradation source.[11] Similar observations have been reported for this[12,13] and other related triazole benzodiazepinones.[14] The reversible 1,4-benzodiazepinone ring-opening under aqueous acidic conditions was previously described for AL[8] and for triazolam.[6] In preliminary forced stress testing, we have observed that the drug is photolabile, and a

Correspondence to: Norma S. Nudelman (Telephone: 5411-45763355; Fax: 5411-45763346; E-mail: nudelman@qo.fcen.uba.ar)

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photosensitivity reaction has been recently reported in some patients treated with AL.[15–17]

There is a strong demand for harmonized guidelines for photochemical stability studies of drugs and drug products from regulatory bodies dealing with drug registration worldwide.[18,19] The ICH Harmonized Tripartite Guideline[20] as well as the recent FDA draft guidance[21] state that light testing should be an integral part of the stress testing.[22] The present work describes stability testing of AL under several stress conditions, isolation and characterization of the main photodegradation products of AL buffered solutions, and kinetic studies of the photochemical degradation of pharmaceuticals containing AL in solid forms.

EXPERIMENTAL SECTION

Materials

AL, and the synthetic intermediates, were obtained from Gador Labs (Buenos Aires, Argentina) and used as received. HPLC-grade acetonitrile, citric acid, disodium acid phosphate, and triethylamine from Aldrich Chemical Company (Milwaukee, WI) were used as purchased. Liquid chromatography grade methanol was distilled immediately before use. Phosphate-citrate buffer solutions (pH 2.0, 3.6, and 5.0) were prepared according to standard methods.

Mass spectra were recorded on a VG Trio-2 mass spectrometer (Manchester, UK). High-resolution mass spectra were measured on a ZAB-SEQ4F mass spectrometer (Manchester, UK). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-500 spectrometer (Karlsruhe, Germany). NMR samples were dissolved in chloroform-*d* (Aldrich), and NMR spectra were referenced using tetramethylsilane as internal reference. HPLC experiments were performed on a Hewlett-Packard (now Agilent Technologies) 1100 HPLC system consisting of an HP-G1311A Quat pump, HP 61315A UV detector, and reversed-phase HPLC column Lichrosorb RP-8, 5 μm (200 × 4.6 mm). Data acquisition and treatment were performed by using a Hewlett-Packard HP-G2170AA Chem Station (Avondale, PA).

Photostability Studies

For the light-stress exposure testing, a photoreactor provided with a medium-pressure metal halide lamp HPA-400 W (Philips, Belgium) and mirrors was used. This metal halide lamp has a close resemblance to sunlight; the output spectrum is rather uniform across the 350–650 nm region.[23] Distance between the lamp and the samples was 5 cm; likely heating of the samples was monitored and the temperature was always below 60°C. The UV dose from the lamp was measured by a reported method[24] using quinine monohydrochloride dihydrate (2% solution in water) as a chemical actinometer, for calibrating the UV radiation in sunlight-simulating conditions. The ICH establishes that the minimum desired exposure is 200 W per m^2 which corresponds to a quinine actinometer change of 0.5 AU at 400 nm; this change was observed after a 3-h exposure time with a new halide lamp. The calibration of the photolysis system was performed following the recent recommendations for the photostability testing.[25] Linear regression analysis of the absorbance change for the quinine actinometry versus the photolysis exposure time for independent quinine solutions showed almost the same slope within experimental error (± 2–3%). The quinine solutions and the drug samples were exposed after the UV lamp was operating for 2 h to ensure proper equilibrium of the system. Calibration of the lamp was performed at the beginning of each study; a new lamp replaced it when a change in the slope was observed. Thermal controls were performed with the quinine solutions as well as with the drug samples by exposing tightly wrapped cells of each of them during the entire photolysis exposure time: no changes in the absorbances were observed in any of the wrapped cells. In separate runs, the drug samples were exposed for longer times (6–7 h) to enlarge the photolysis extent. The integrated intensity of radiation ΔAbs, at 400 nm after 6-h exposure was of $\Delta Abs = 0.9$ AU of the quinine actinometer. No change in the nature or the relative yields of the photodegraded products was observed when compared with the 3-h exposure time.

Preparation of Photodegradation Products

AL 1.0 g, in a 1000-mL volumetric flask, was dissolved in 130 mL of methanol and made up to volume with citrate buffer (pH 3.6). The solution was irradiated with an HPA lamp for 8 days. The temperature inside the reactor was nearly 60°C, and it was widely demonstrated that AL does not undergo thermal degradation under these conditions (see Results and Discussion). After irradiation, the solution was treated with NaOH up

to pH 8.0; upon cooling, a yellowish precipitate appeared which was removed by filtration. The filtrate was extracted four times with 100 mL of dichloromethane each time. The extracts were washed with water several times and the organic layer (dried with $MgSO_4$) was evaporated to dryness under reduced pressure. This mixture of photodegradation products is called the "extract" below.

Tablets (0.5 mg of AL) were irradiated with the lamp, described previously, for 6 months. After irradiation, the tablets were dissolved in methanol by sonication for 30 min, filtered, and the filtrate was evaporated to dryness under reduced pressure: three main degradation products were isolated and identified. In separate runs, four brands of commercial tablets (containing 0.5 to 2.0 mg of AL as the only clinical drug) were exposed to sunlight for 400–700 days. Two main products were isolated and identified.

Isolation and Purification of Photodecomposition Products

The "Extract"

To isolate the several photodegration products, different chromatographic systems were tested. Repeated attempts of separation by thin-layer chromatography (TLC), using several eluent systems, showed that the "extract" was a complex mixture very difficult to resolve; similar unsuccessful results were obtained using different kinds of column chromatography. This suggests that the products might have very similar structures.

By several successive TLCs, three main products could be isolated. The first sample resolution was performed using preparative silica gel plates (Merck KGaA, TLC silica 60 F_{254} Darmstadt, Germany) with 70:30:0.4 EtOAc/MeOH/triethylamine as eluent. UV light (254 and 366 nm) showed four very close spots that were removed, eluted with MeOH, and worked up with standard procedures. The residue was redissolved in chloroform, filtered, and the filtrate evaporated to dryness. The observed fractions were purified by successive TLCs and HPLC as described below for each photodegradation product.

For HPLC method 1, a mobile phase of water/acetonitrile (60:40) was pumped at 1 mL/min through an HPLC column with UV detection at 220 nm.

HPLC method 2 was a binary gradient reversed-phase HPLC procedure. Mobile phase A was 0.025 M acetate buffer (pH 4.5)/acetonitrile (65:35 v/v) and mobile phase B was 0.025 M acetate buffer (pH 4.5)/acetonitrile (20:80). The following gradient program was used: 0–10 min (linear gradient mobile phase A: 100% to 50%), 10–14 min (isocratic, mobile phase A: 50%) and 14–20 min (linear gradient mobile phase A: 50% to 0%). The flow rate was 1.0 mL/min, with UV detection at 254 nm.

[5-Chloro-2-[5̀-methyl-4H-1′,2′,4′-triazol-4′-yl]-benzophenone],1 (triazolbenzophenone). This photoproduct was firstly purified by preparative TLC silica gel 60 F_{254} (Merck), using isopropyl alcohol (100%) as eluent ($R_{f\ alprazolam}$ 0.20; $R_{f\ triazolbenzophenone}$ 0.27). Spots were visualized by 254-nm UV light, and extracted from the silica with methanol. Solvent was removed by distillation at reduced pressure. The extract was dissolved in chloroform, filtered by suction, and the solvent distilled at reduced pressure. The extract was then purified by preparative TLC cellulose (Aldrich). Plates were developed in aqueous 0.2 N NaOH ($R_{f\ alprazolam}$ 0.35; $R_{f\ triazolbenzophenone}$ 0.49). Spots were removed as described above. The residue was further purified by HPLC using method 1 before the spectroscopic determinations. Sample solutions were prepared by diluting the residue with water/acetonitrile (40:60 v/v); 20-μL aliquots of the sample solution were injected in the HPLC. The fractions corresponding to the main part of each peak were manually collected ($t_{r\ alprazolam}$ 14.3 min; $t_{r\ triazolbenzophenone}$ 12.0 min). Then, each fraction was evaporated to dryness under reduced pressure. The residue was dissolved in chloroform and sonicated for 15 min, after which the solution was filtered and evaporated to dryness under reduced pressure. It is worthwhile to note that this compound, together with compound **3**, were the main degradation products isolated from the irradiated tablets, following the described procedures.

1-[methyl]-6-phenyl-4*H*-s-triazol-[4,3α][1-4]benzodiazepine, 2 (8H-alprazolam). This product was purified by preparative TLC silica gel (Merck TLC silica 60 F_{254}). Plates were developed in chloroform/methanol (100:5 v/v) ($R_{f\ alprazolam}$ 0.56; $R_{f\ 8H\ alprazolam}$ 0.52). Then the extract was purified by preparative TLC cellulose (Aldrich) using aqueous 0.2 N NaOH as solvent ($R_{f\ 8H\ alprazolam}$ 0.65). The residue was redissolved in 0.025 M acetate buffer (pH 4.5)/acetonitrile (40:60 v/v) and then submitted to further purification by HPLC using method 2. ($t_{r\ alprazolam}$ 10.7 min; $t_{r\ 8H\ alprazolam}$ 8.1 min)

Triazolaminoquinoleine, 3. This photoproduct was firstly purified by preparative TLC on silica gel plates (Merck TLC silica 60 F_{254}) using a mixture of ethyl acetate/dichloromethane/triethylamine (70:30:0.4 v/v) as eluent. Spots were visualized by 366-nm UV light. The extract corresponding to **3** was further purified by preparative HPLC using method 1. Because the MW of this compound is the same as that of AL, its identity was confirmed by independent synthesis.

Synthesis of triazolaminoquinoleine, 3. AL 186 mg was dissolved in 2 mL of methanol and 4 mL of 0.33 N HCl was added to generate "*in situ*" the 5-cloro-2-[5′-methyl-4H-1′,2′,4′-triazol-4′-yl]-benzophenone. The pH of this solution was immediately adjusted to 9.0 with 2.0 N KOH, and 2.0 mL of acetic anhydride in 4 mL of THF (tetrahydrofurane) was added with stirring. After 3 h at room temperature, the reaction mixture was evaporated to dryness under vacuum. The residue was dissolved with 0.5 mL of methanol, 10 mL of 2.0 N KOH was added, and the solution was heated for 6 h at 91°C. After cooling, the solution was extracted with 5 mL of benzene. The extract was purified by TLC silica gel using chloroform/isopropyl alcohol/methanol (80:15:5 v/v). ($R_{f\ triazolquinoleine}$ 0.77; $R_{f\ alprazolam}$ 0.62; $R_{f\ subst.\ benzophenone}$ 0.49). **3** was properly characterized[26]; the overall yield was 83%.

The Precipitate

The yellow crystals isolated from the irradiated buffered solution (pH 3.8, 8 days) were sparingly soluble only in methanol; the addition of acid or basic cosolvents did not improve dissolution. A new run was performed by irradiating AL in citrate buffer (pH 2.0) during 10 days. By neutralizing the solution, a yellowish precipitate was obtained. This solid was soluble in ethyl acetate, thus allowing its separation from the buffer salts after several washings. The organic layer was dried ($MgSO_4$) and the solvent distilled at reduced pressure. The TLC analysis revealed the presence of several products. The solid was firstly treated by column chromatography separating fraction 1 (EtAcO/MeOH, 7:3) and fraction 2 (MeOH/triethylamine, 50:0.1). The TLC analysis showed that fraction 1 is a mixture of the three products previously described.

Attempts to resolve fraction 2 by several TLC systems using silica gel were unsuccessful; then by using reverse phase with methanol and formic acid as eluent, several close spots were obtained that were further purified by column chromatography. MeOH pH 2.8 formic buffer (50:50 followed by 70:30) and then MeOH/formic acid 50%, 10:0.8, gave one compound which we call compound **4**. However, TLC analysis of fraction 2 using alumina plates gave three close spots that could be separated by column chromatography using successive solvent mixtures. Thus, ethyl acetate/methanol/triethylamine 50:5:0.5 followed by 50:10:0.5 gave compound **5** and 30:70:0.5 gave compound **6** and compound **4**. The amounts of isolated compounds **4**–**6** were not enough to perform a reliable characterization, and efforts were concentrated on the main products **1**–**3**.

RESULTS AND DISCUSSION

Preliminary studies of AL stability were performed under thermal, hydrolytic, and oxidative stress conditions. Solid samples of AL were stored at 80°C: periodic quantitative HPLC determination did not show significant degradation even after 5 months at 80°C. Buffered AL solutions at pH = 3.5 were heated in sealed ampoules at 80°C for 88 days: HPLC determinations did not show any significant change in AL concentration or appearance of any degradation product. Previous studies reported the observation of changes in the UV-visible spectra of pH < 0.5 AL solutions at 25°C, but the degradation products were not identified.[4,5] Attempts to isolate the degradation products by TLC also failed.[27] AL solutions at pHs ranging from 2.0 to 9.0 were studied in our laboratory at temperatures of 50–80°C to detect hydrolysis but AL seems to be stable under those conditions. Attempts to isolate any degradation product were unsuccessful likely because of the small amounts and/or to the equilibrium between the structures. Only by ^{1}H-NMR determination of the reaction mixture at pH = 0.5 was it possible to characterize the presence of the [5-chloro-2-[3̀-methylamino-5′methyl-1′,2′,4′-triazol-4′-yl]-benzophenone] in small amounts. The ^{1}H-NMR of AL is shown in Figure 1 for further comparisons; the spectrum exhibits the AX system of both geminal protons in C4 as two duplets at 5.5 and 4.3 ppm, J = 12 Hz, and the sharp singlet due to the methyl group at 2.65 ppm. In the ^{1}H-NMR of the mixture obtained by hydrolysis (not shown), the distinct signals due to the open substituted aminobenzophenone are clearly a broad signal at 8.8 ppm attributed to the protonated amino group and the relatively broad singlet for the two equivalents protons at C4 which appears at 4.5 ppm (2H).

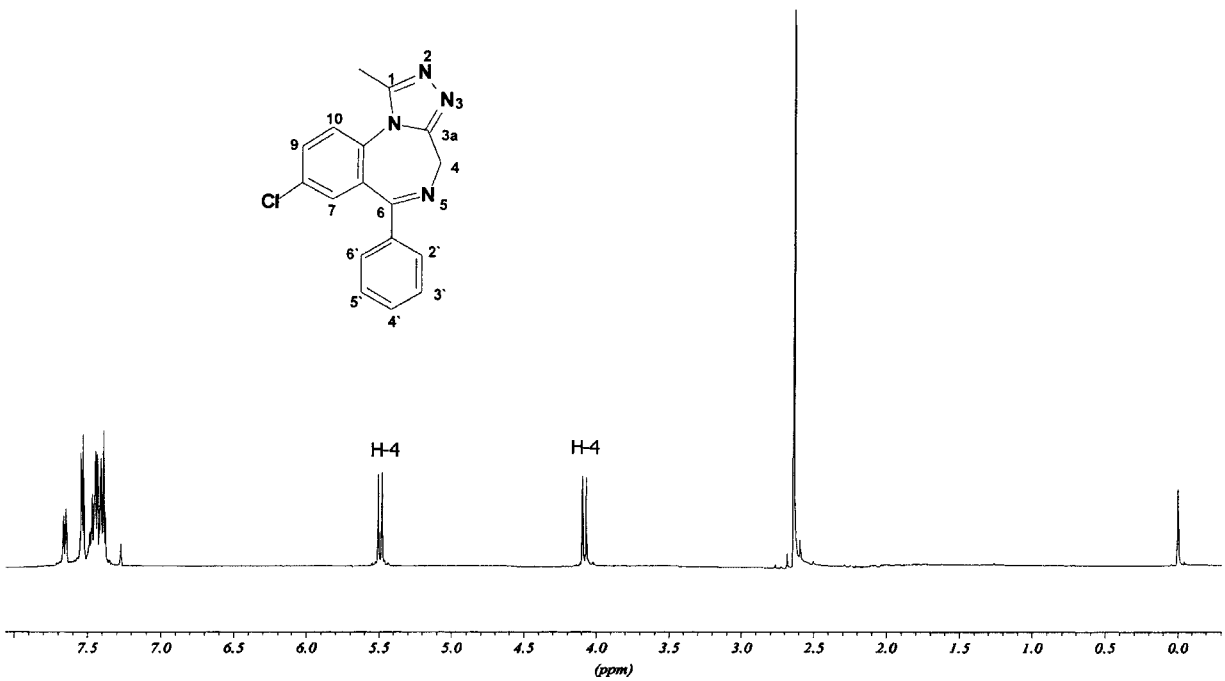

Figure 1. ^{1}H-NMR spectrum of alprazolam.

Several oxidants were tested on AL solutions in methylene chloride and in chloroform, but even after 5 days at the solvent boiling temperature, no significant degradation was observed. The described results showed the relatively high stability of AL under the usual accelerated aging conditions.

The main degradation route for AL was found to be photochemical and most of the following studies were conducted to determine the effect of light irradiation under different conditions. Preliminary studies show that AL solutions at pH > 9.0 are insensitive to photochemical reactions, and the rate of degradation increases as the pH decreases. Figure 2 shows the changes in the UV visible spectra of pH 3.5 buffered AL solutions upon irradiation: an increase in the absorbance at 240 nm and a decrease at the AL $\lambda_{max} = 260$ nm is observed. No new peaks appeared; identical AL solutions protected from light showed no changes in the UV visible spectra after 48 h, which indicates that the changes observed in Figure 2 are not due to conventional hydrolysis as previously reported. These changes are not significant to follow the photostability by spectrophotometric determinations and a fluorometric assay was developed. Solid AL samples as well as tablets containing AL as the only clinical drug were exposed to natural sunlight; after 88 days of irradiation, they showed by TLC the same photodegradation products. This gives confidence to the artificial irradiation stress to reproduce natural photochemical degradation. Longer exposure to sunlight (400–700 days) increases the concentration of the photodegradation products but it did not change the structures and no new compounds appeared.

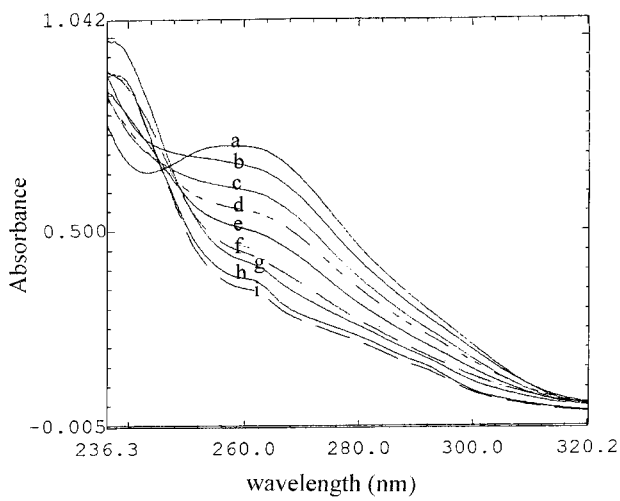

Figure 2. UV spectrum of alprazolam in pH 3.6 buffered solution after irradiation with a metal halide lamp. Irradiation times (h) for curves (a) to (i) equal to: 0, 1, 2, 3, 4, 5, 6, 7, and 9 h, respectively.

Structure Elucidation of the Photodegradation Products

As stated in the Experimental section, the separation and isolation of the degradation products of AL were very difficult likely because of their similar chemical properties. The most important photochemical degradation was observed at pH = 2.0 (citrate buffer). After 8–10 days of irradiation of buffered AL solutions, three main products, **1–3**, were isolated and fully identified.

5-Chloro-[5̂-methyl-4H-1,2,4-triazol-4-yl]-benzophenone, 1 (HRMS MW 294.0667). The electron ionization mass spectra (EI-MS) of this compound showed the molecular ion and some key ion fragments with a pattern typical of compounds containing one chlorine atom. Furthermore, this is the only one that shows a key ion fragment of mass 105 which revealed an aminobenzophenone with one unsubstituted phenyl ring, as previously observed in the EI-MS of degradation products of other benzodiazepinones.[9,10] Another important ion fragment corresponds to the loss of 29 amu (HNN) which is typical of 1,2,4-triazoles. The ion fragment (m/z 192) complementary of that of mass 105, and the other ion fragments of minor relative abundance confirm the structure. Analysis of the ^{1}H-NMR spectrum showed in Figure 3 indicates a methyl group (singlet at high field), the typical pattern for the protons in both phenyl groups with multiplicities consistent with the given structure and the singlet at low field which indicates the unique proton (H3̂) in the triazole ring. A signal at 192.59 ppm in the ^{13}C-NMR of **1** (not shown) confirms the carbonyl C, as well as the other C assignments for the remainder of the signals.

1-[Methyl]-6-phenyl-4*H*-s-triazol-[4,3α][1-4]benzodiazepine (8H-alprazolam), 2 (HRMS MW 274.1216). The EI-MS shows an intense molecular ion of 274 amu, in which the [M + 2] ion is clearly absent. Both results suggest that this compound could be the product of photodechlorination of AL. Consistent with this assumption, the rest of the EI-MS shows fragment ions that are very similar to those observed in the EI-MS of AL, after the separation of one Cl atom (main fragment ions: 245, 204, 103, 89, and 77).

Halogenated aryl compounds easily undergo intra or inter-electron transfer photo-dehalogenation when there is a good electron donor present, given the reduction and/or the hydroxylated (or methoxylated if the reaction is performed in methanol) products.[28] The accumulation of nitrogen atoms in the AL molecule contributes to the reaction occurrence. Photo-dehalogenation for other drugs has been previously reported: e.g., dichlofenac[29] and chlorpromazine[30] give the reduction product and also hydroxylated products[30,31] by photochemical degradation. The typical dd signal for the geminal coupling (J = 12.9 Hz) of the two H4s is clearly observed in the ^{1}H-NMR spectrum (Figure 4), and the singlet for the methyl group which also appears at the same chemical shift as that of AL. The aromatic protons zone is quite different, showing one additional proton; the corresponding shifts and multiplicities for the other aromatic protons are consistent with the assigned structure. The ^{13}C-NMR (not shown) is also very similar to that of AL.

Triazolaminoquinoleine, 3 (HRMS MW 308.0827). This compound shows a strong fluorescence at λ_{exc} 260 nm; the emission spectrum shows a λ_{max} = 410 nm. The first approach to the structure of compound **3** was performed through the analysis of the EI-MS. Unexpectedly for a degradation product, the molecular ion shows the same mass as AL (308 amu), but the rest of the spectrum is completely different. The [M – 1] ion base peak, typical for amines, and the presence of [M – 1 + 2] [M] [M + 2] ions, indicate an amine group and one chloro atom in the molecule. Other main ion fragments are 103, 89, and 77 which are also observed in the EI-MS of AL. The main clues for the elucidation of this unexpected structure arose from a careful analysis of the ^{1}H- and ^{13}C-NMR spectra. The singlet for the methyl group appeared downfield, suggesting a major deprotective effect that could be due to an increased resonance in the rings. The broad signal at 4.69 ppm observed in the ^{1}H-NMR spectrum shown in Figure 5, integrates for 2 H which are exchangeable upon treatment with MeOD. That these 2 H are bonded to the same N (like in a primary amine), was indicated by the ^{13}C spectrum whose peculiar downfield zone is expanded in Figure 6. Only one signal for aliphatic carbon appears which is due to the methyl group. (The typical signal for the methylene C, which is part of the diazepinone group in AL, is not present.) The resonance of two carbons typical for alkenes is observed at 112.44 and 116.59 ppm; there are only two (instead of three) imine C, which appear at 147.35 and 145.46 ppm, respectively. Also, noticeable in the ^{1}H-NMR is the absence of the dd signal typical of the geminal coupling of the protons bonded to C4 in the diazepine ring. In the

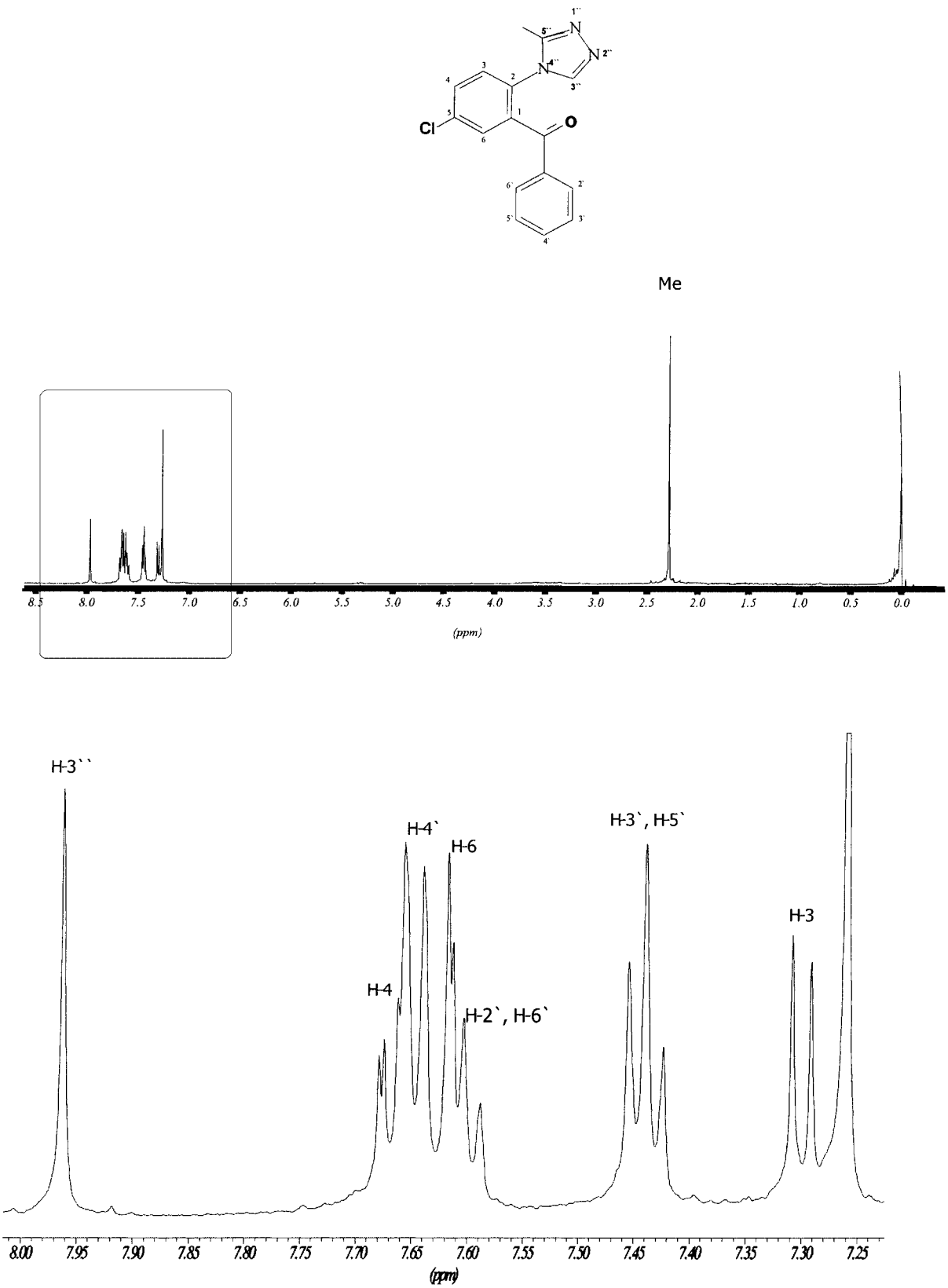

Figure 3. [1]H-NMR spectrum of 5-chloro-[5̃-methyl-4H-1,2,4-triazol-4-yl]benzophenone.

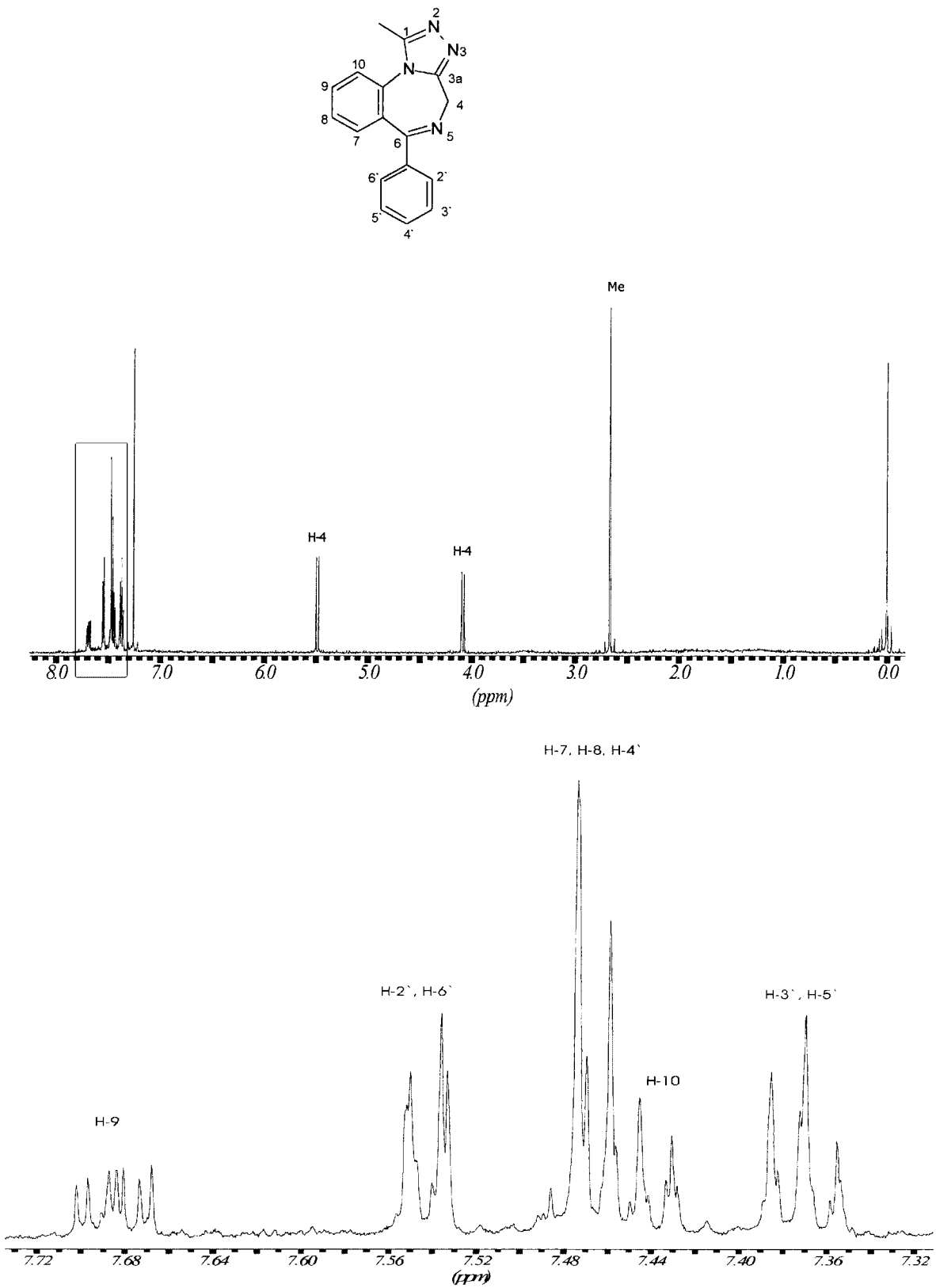

Figure 4. ^{1}H-NMR spectrum of 1-methyl-6-phenyl-*4H*-s-triazo-[4,3-α][1,4]benzodiazepinone.

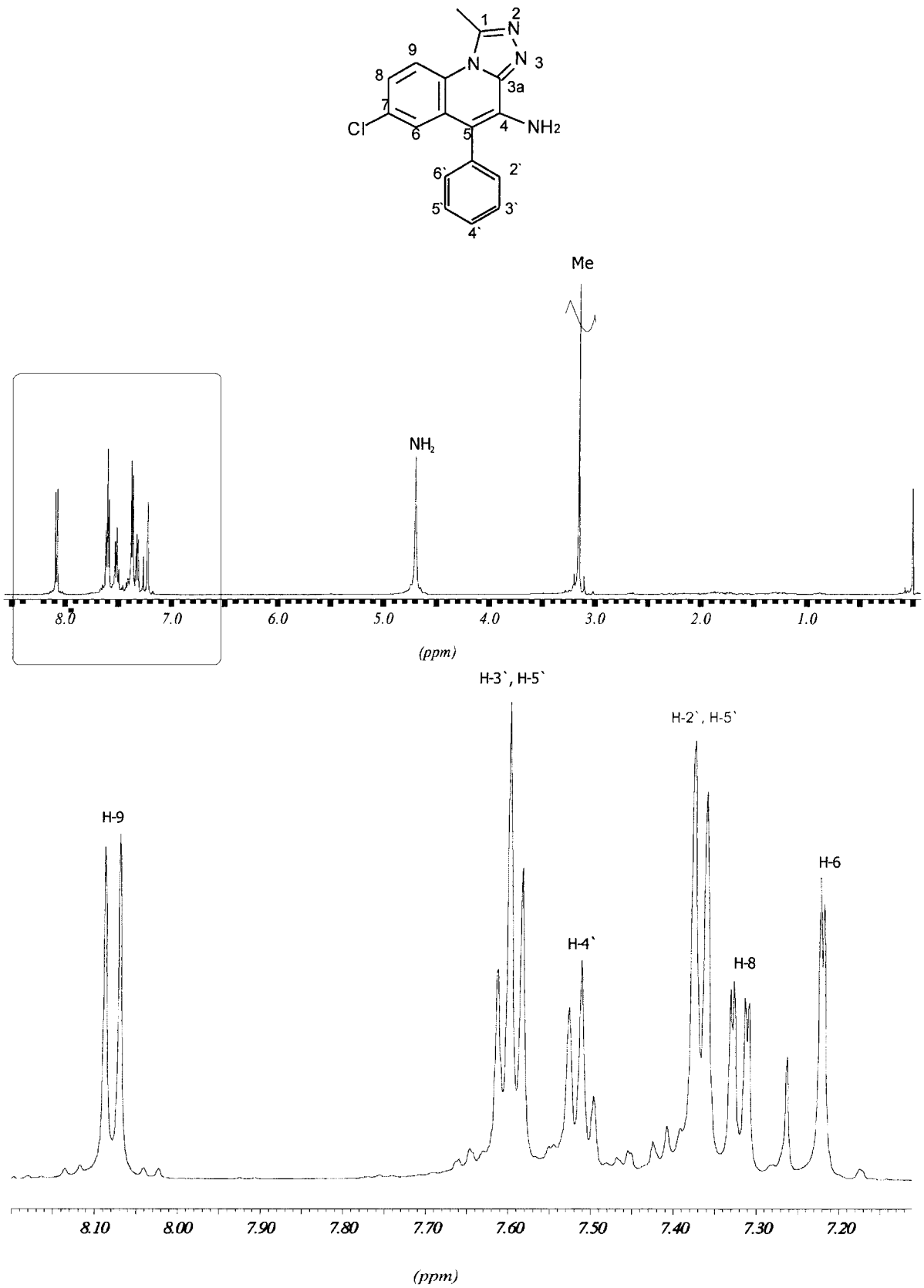

Figure 5. ^{1}H-NMR spectrum of triazolaminoquinoleine.

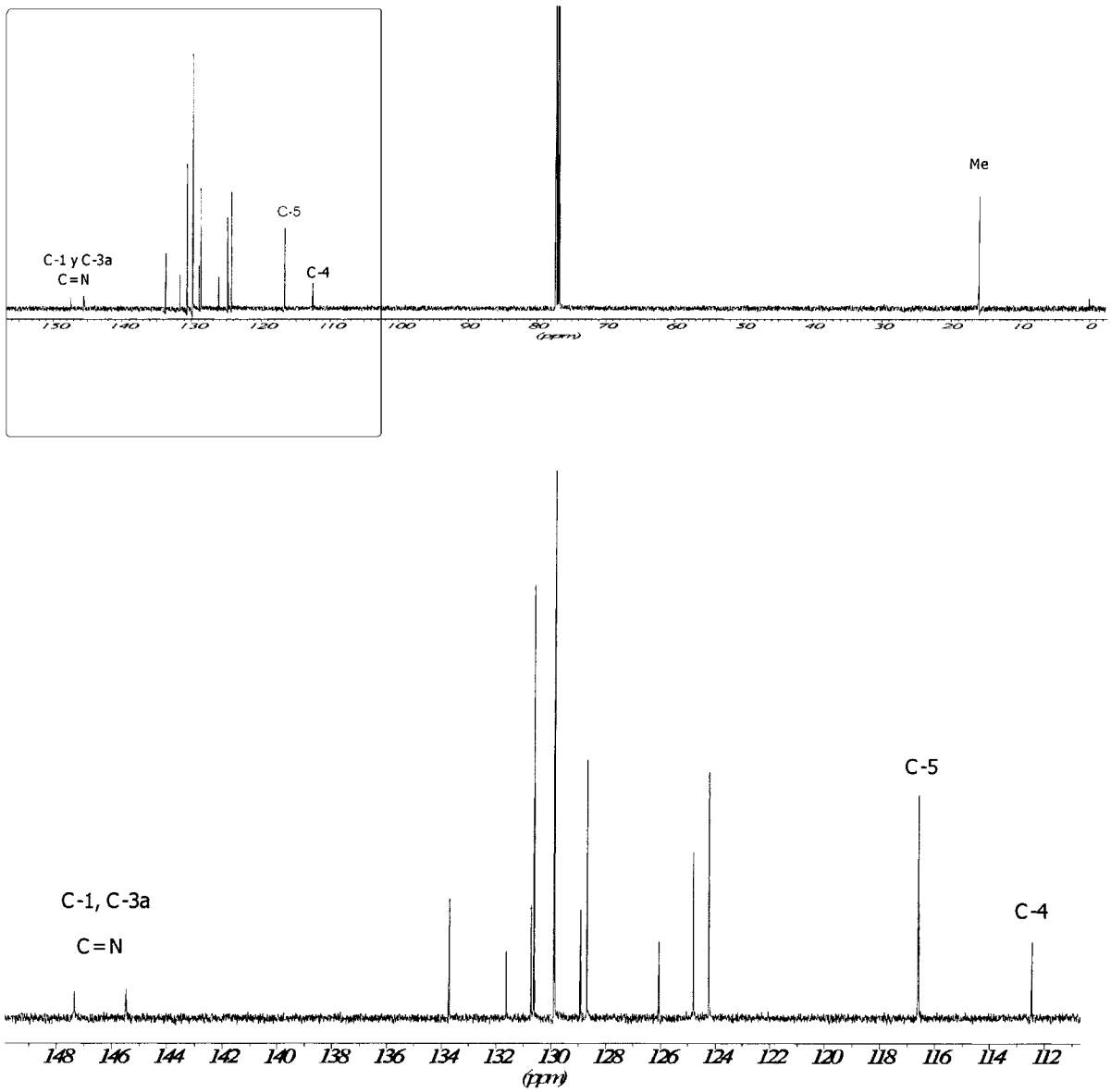

Figure 6. ^{13}C-NMR spectrum of triazolaminoquinoleine.

aromatic zone of the ^{1}H-NMR spectrum, the signals due to the unsubstituted phenyl ring were assigned, according to the integration and the expected multiplicities for the *ortho*- and *para*-coupling, as shown in Figure 5. The other three signals, which integrate for one H each one, exhibit the classical AMX multiplicity of a trisubstituted ring: the doublet at 8.07 ppm (H-9), the dd at 7.32 ppm (H-8), and the d at 7.22 (H-6). The signal that appears at the most downfield position is assigned at H-9 because of the anisotropy that affects the ipso H. Because various rearrangement structures could be consistent with the above results, the structure was fully confirmed by independent synthesis, as described in the Experimental section.

Photostability of Pharmaceuticals Containing AL

To assure that the photodegradation products identified by irradiation of buffered solutions of AL could be related to the degradation undergone by exposure of pharmaceuticals to natural irradiation, commercial tablets containing 0.5, 1.0, and 2.0 mg of AL were irradiated by sunlight for long periods (400–700 days). The contents of AL

and of the degradation products were periodically determined. The knowledge of the different structures of the photodegradation products allowed the development of a reliable HPLC assay for AL and its degradation product. Four commercial pharmaceuticals were studied. A (2.0 mg) and B (1.0 mg) are tablets expended as large doses; C and D (0.5 mg) are packed in 10 tablet blisters. Tablets A and B were separately put in sealed transparent flasks, whereas samples C and D were irradiated in the original package. The tablets become yellowish upon irradiation but no changes in the blister packs were observed even after 2 years of sunlight irradiation. The contents of AL and of the degradation products were periodically determined. Figure 7 shows a typical HPLC run corresponding to tablets D after 408 days of sunlight exposure. The peaks appear in the chromatogram in the order: 8H-alprazolam, **2**, as traces; triazolbenzophenone **1** (5.3%); AL (93.2%), and triazolquinoleine, **3** (1.3%).

The four commercial samples were exposed to sunlight between 495 (tablets D) and 781 (tablets C) days; the concentrations of AL and of the main degradation products were periodically determined. The two main degradation products, namely: triazolbenzophenone, **1**, and triazolaminoquinoleine, **3**, showed identical TLC and HPLC chromatographic behavior with the standards of the previously characterized compounds from the photochemical degradation of buffered solutions

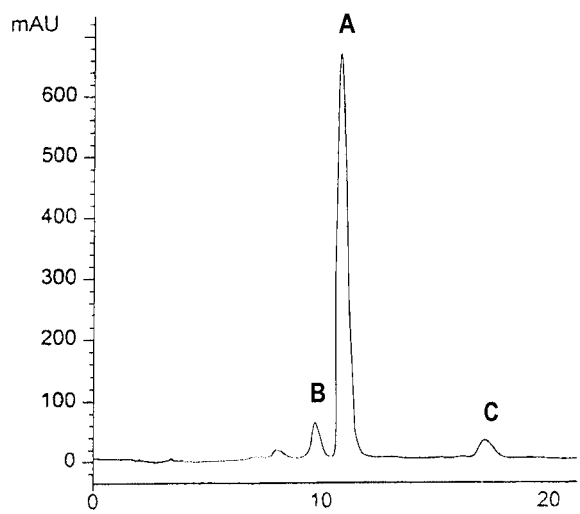

Figure 7. Typical HPLC determination of alprazolam tablets D after 408 days of exposure to sunlight in blister packs. Peaks appear in the order: 8H-alprazolam (traces); 5-chloro-[5̃-methyl-4H-1,2,4-triazol-4-yl]benzophenone (5.3%); alprazolam (93.2%); triazolquinoleine (1.3%).

of AL. Furthermore, compounds **1** and **3** could be easily isolated from the irradiated tablets, purified, and fully characterized by NMR and MS which were identical with the spectra determined with the photodegraded products of AL. Under the present experimental conditions, the degradation of AL and the formation of compounds **1** and **3**, follow a zero order kinetic law; the regression coefficients for the four samples were within acceptable ranges for solid forms. The 8-hydroalprazolam, **2**, appeared only in small amounts. It was observed that the relative rates of degradation of the tablets packed in blister packs are higher than those in large doses; although it was shown that AL is not very sensitive to hydrolysis, it could be possible that the blister better retains the humidity and other likely deleterious factors that could accelerate the degradation. Because there are also small differences in the excipients, their specific effects as well as the possible surface catalysis, cannot be fully neglected.

CONCLUSIONS

AL shows an unusually high stability against the most common degrading factors: humidity, oxygen, and heat. Hydrolytic degradation products resulting from the opening of the diazepinone ring were not amenable of isolation due to the rapid reverse cyclization at the various pHs assayed. However, we have found that the drug is sensitive to both artificial and sunlight, and the photolability increases at low pH. Irradiation of buffered solutions of AL allowed isolation and characterization of three main products: triazolaminoquinoleine (fluorescent); 5-chloro-[5̃-methyl-4H-1,2,4-triazol-4-yl]benzophenone, and 1-methyl-6-phenyl-*4H*-s-triazo-[4,3-α][1,4benzodiazepinone (also called 8H-alprazolam). Irradiation of AL tablets by artificial and sunlight showed the same main degradation products, being 8H-alprazolam formed only as traces. The higher rate of the photochemical reaction was observed at pH = 2.0, consequently, acidic conditions should be avoided, and appropriate light protection is recommended during the drug-development process, storage, and handling.

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