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SYNTHESIS OF DEUTERIUM LABELLED TRIAZOLOBENZODIAZEPINES

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SUMMARY

Alprazolam and Triazolam - anxyolitic and hypnotic agents - were labelled with deuterium. First synthesis: through CD_3COOD acylation of the appropriate Hydrazones, followed by thermal cyclization. Second synthesis: deuterium exchange through acid catalized carbocation intermediates in a CD_3COOD/D_2SO_4 mixture. The products were used as internal standards for GC/MS/SIM quantitative analysis in forensic case work.

Key Words: ALPRAZOLAM, TRIAZOLAM, DEUTERIUM, SYNTHESIS

INTRODUCTION

Alprazolam and Triazolam are anxyolitic and hypnotic agents (1,2). The detection and quantitation of these drugs in post mortem blood samples can be difficult due to the low dose (0.25-3.00 mg) and short half life (2.5-3.0 hrs). The GC/MS/SIM technique in the CI^- ionisation mode using Estazolam as an internal standard has been applied to the analysis of Triazolam in serum (3).

The use of deuterium analogues as internal standards in the MS/SIM quantitation of drugs has long been recognized as the method of choice both in clinical and forensic toxicology. Our laboratory recently introduced such a method for Triazolam (4). This paper will discuss two deuterium labelling methods used for Alprazolam and Triazolam.

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DISCUSSION AND RESULTS

Key compounds for the synthesis of the D_3-Alprazolam (3a) and D_3-Triazolam (3b) are the hydrazones (1a, 1b) which were synthesized by the method of Meguro et al (5). The ring closure with CD_3COOD is a two step reaction; acylation was carried out with equimolar amounts of CD_3COOD and N,N'-carbonyl-diimidazole (CDI) and the second step by heat treatment under vacuum. This synthetic method has been used by Hsi (6,7,8,9) to label Alprazolam and Triazolam with ^{13}C and ^{14}C for clinical experimentation.

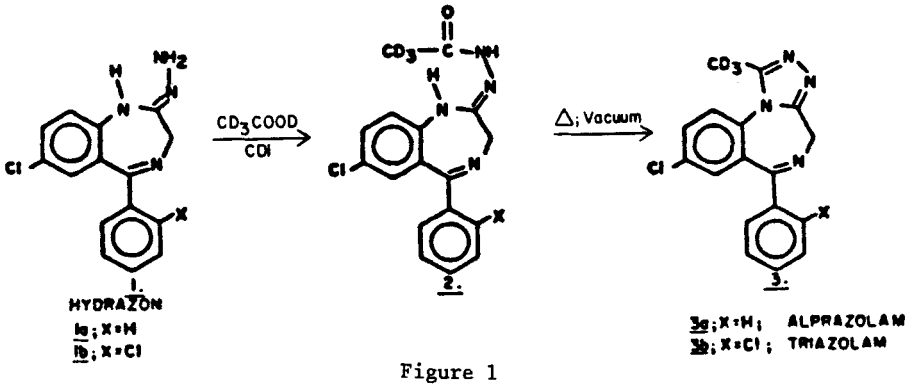

Figure 1

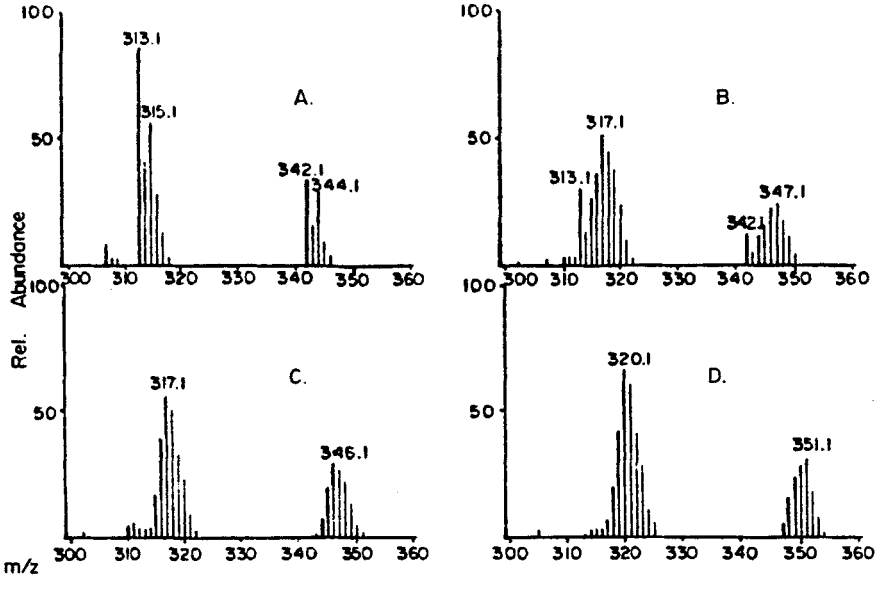

Figure 2

In a CD_3COOD/S_2SO_4 mixture at 200°C Triazolam was found to incorporate up to six deuterium atoms. The deuterium exchange of Triazolam was examined every 24 hours (4 days) by MS in EI^+ mode (Figure 2).

The acid catalysed deuterium exchange process has been used for the deuterium labelling of several drugs such as: Imipramine (10, 11, 12, 13, 14), Verapamil (15), Nomifensin (16, 17), Zoxazolamine (18), and Tryptophan (19).

The 360 MH_2 ^1H NMR spectrum of Triazolam shows two doublets at δ 4.2-5.6. The plausible explanation by Campbell et al (20) is that the nonplanar seven members benzodiazepine ring is thought to exist in a boat conformation.

There are two equivalent boat conformations and interconversions of the ring exchanges the geminal ring protons producing two doublets. These two protons rapidly exchange with deuterium. Further exchanges proceed on the B ring's A, B, C, and D protons leaving the A ring protons (E.F.G.), two doublets (E,F), and one quartet (G) intact. (Figure 3).

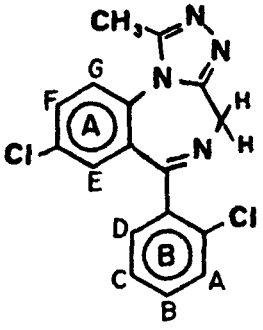

Figure 3.

The complex isotope clusters of the EI^+ spectra (Figure 2) were compared with the labelled and unlabelled Triazolam in CI^- mode (Figure 4). The CI^- spectra are quite simple consisting mainly of the molecular ions at 306 and 312 amu; the mixture shows well resolved ion clusters, optimal for selective ion monitoring.

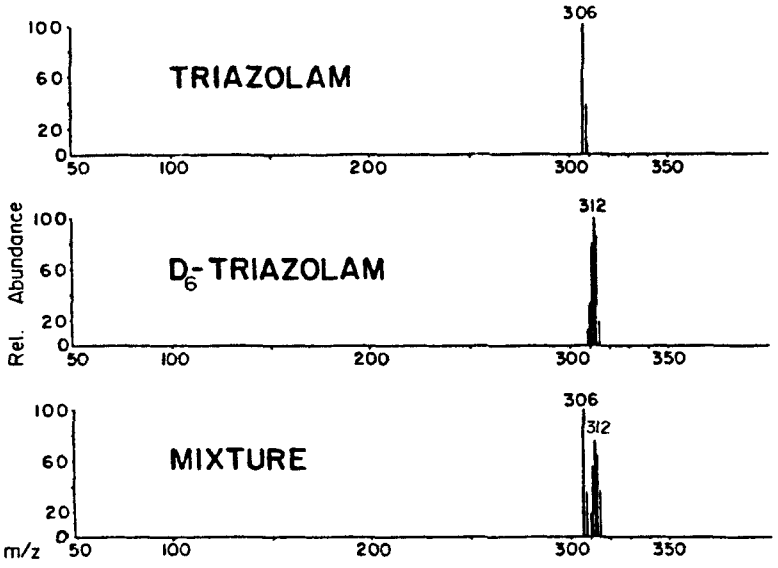

Figure 4

Several other acids and media such as DCl/D_2O; CH_3COCl/D_2O; and $(F_3CCO)_2O/D_2O$) were tested with unsatisfactory results. Alprazolam deuterium exchange was slower in CD_3COOD/D_2SO_4 than Triazolam, only the seven membered ring H-C-H exchanged to give D_2-Alprazolam.

EXPERIMENTAL

Pure Triazolam and Alprazolam were purchased from the Upjohn Company, Kalamazoo, U.S.A. CD_3COOD, D_2SO_4, $(F_3CCO)_2O$, DCl and D_2O were obtained from MSD Isotopes, Dorval, Quebec, Canada.

The GC/MS analyses were carried out with a FINNIGAN 1020 in EI^+ mode and a VG 12-250 in CI^- mode. The ^1H NMR spectra were obtained with a NICOLE 360 instrument.

Synthesis of D_3-ALPRAZOLAM and D_3-TRIAZOLAM. To a stirred solution of CD_3COOD (500 mg, 3.19 mmols) in 20 mL of anhydrous tetrahydrofuran (THF) was added a 10% excess of CDI (1.464 g, 9.03 mmols). After the evolution of gases subsided, the mixture was stirred under N_2 at room temperature for 1 hour, cooled to 0°C and a cooled solution of Hydrazone 1a (2.335 g, 8.20 mmols) or Hydrazone 1b (2.587 g, 8.20 mmols) in 45 mL of anydrous THF was added in one portion. The mixture was stirred at 0°C for 1 hour and allowed to

come to room temperature overnight. The mixture was concentrated under reduced pressure and the residue distributed in Et_2O - hexane, filtered, and the solid washed repeatedly with Et_2O, water and then dried. The dried solid was heated under reduced pressure (18 mm) at 220°C for 2-3 minutes. After the heat treatment the residue was crystallized from a benzene - hexane mixture and analyzed.

D_3-Alprazolam: Yield: 1.50 g. Mp 226-228°C. Mass spectrometric data obtained at 70 eV and 200°C gave m/e in EI^+ mode: 311(70), 282(55), 276(80), 249(22), 239(22), 204(100); in CI^- mode: 312. ^{1}H NMR δ ($CDCl_3$): 7.15-7.65 (8H; m: aromatic) 4.2-5.6 (2H; two doublets; H-C-H).

D_3-Triazolam: Yield: 1.20 g. Mp 222-224°C. Ms m/e in EI^+ 345(50), 316(100), 280(28), 241(70); in CI^- mode: 309. ^{1}H NMR δ ($CDCl_3$): 7.15-7.65 (7H; m; aromatic) 4.2-5.6 (2H; two doublets; H-C-H).

Deuterium exchange in ALPRAZOLAM and TRIAZOLAM. A solution of Alprazolam or Triazolam (500 μg) in CD_3COOD/D_2SO_4 (3 ml; ratio 3:1) was heated under N_2 at 200°C in a Reacti-Vial™ (1.0 mL from Pierce, U.S.A.). The reaction mixture was analysed after every 24 hours. A 10.0 μL sample was withdrawn, 100 μL 5N sodium hydroxide solution was added and vortexed with 100 μL methylene chloride. After centrifugation, the organic layer was separated, evaporated and the residue dissolved in 30 μL methanol. Three μL of the methanol solution was analysed by GC/MS. When the deuterium exchange stopped (4 days), the pH of the mixture was adjusted to pH 10 with conc. NaOH at 0°C and extracted with methylene chloride (3 x 5 mL). The combined extract was washed with water, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The products were purified on silica gel preparative TLC plates with toluene - methylene chloride - ethanol - ammonia (17.5-8-4-0.5 mL) and analysed.

D_2-Alprazolam: Yield: 300 μg, MS data m/e in EI^+ mode: 310(60), 281(50), 275(85), 248(20), 238(20), 204(100); in CI^- mode: 311. ^{1}H NMR δ ($CDCl_3$) 7.15-7.65 (8H; m; aromatic) 2.6(3H: s; $-CH_3$).

D_6-Triazolam: Yield: 250 μg, MS data m/e in EI^+ mode: 351(65), 320(30); in CI^- mode: 312. ^{1}H NMR δ ($CDCl_3$) 7.26-7.65 (3H; m: aromatic) 2.6(3H; s; $-CH_3$).

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