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SYNTHESIS OF DEUTERIUM LABELLED LORAZEPAM

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SUMMARY

Synthesis of $^{2}H_3$-lorazepam was achieved by modification of literature procedures for the unlabelled drug. The key step in the seven step procedure was the initial one where upon selective exchange of 2-amino-5,2'-dichloro-benzophenone was obtained in deuterated acids. Purifications were carried out by preparative HPLC. The $^{2}H_3$-lorazepam is suitable for use as an internal standard in GC-MS-NICI-SIM quantitative analysis in forensic case work.

Key Words: $^{2}H_3$-lorazepam, deuterium exchange, negative chemical ionization

INTRODUCTION

Lorazepam is a 3-hydroxy-1,4-benzodiazepines, that is used as a sedative/hypnotic and antianxiety agent. Recent work indicates that the kinetics of this drug are complex (1).

One of the aims of forensic toxicology is to establish accurate and specific methods for the quantitative analysis of drugs in complex matrices (2). The method of choice is often GC-MS using deuterium labelled internal standards. The synthesis of deuterium labelled lorazepam, however, has not been reported.

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The synthesis of lorazepam and related drugs was first published by Sternbach et al and has been modified by several authors and patented (3). These references, however, do not give sufficient information to optimise the yield for lorazepam.

In this paper, optimization of the synthesis for labelled lorazepam is described, where in the first step of the seven step procedure, selective deuteration of 2-amino-5,2'-dichlorobenzophenone was achieved by use of deuterated acids.

DISCUSSION AND RESULTS

The classical synthesis of a benzodiazepine starts from the appropriate benzophenone. Previous study had shown that deuterium labelled 2-amino-5-chlorobenzophenone (synthesized by Friedel-Crafts reaction) gave a scrambled product. For this reason, a more complicated synthetic route was explored in this previous study (4).

A simple deuterium exchange on benzodiazepines using Broensted type acids, as catalyst, has recently been introduced in this laboratory (5). The one step reaction has been successfully applied to the direct deuteration of the triazolobenzodiazepines, triazolam and alprazolam. Unfortunately, other benzodiazepines such as lorazepam are irreversibly hydrolysed to their respective benzophenones in acidic media.

The first acid-catalyzed deuterium exchange reaction was reported by Ingold (6). The theories and applications have been reviewed and recent publications indicate that this method can be used to obtain a diverse range of deuterium labelled compounds. Drug related application have been previously reviewed (5).

The five day treatment of 2-amino-5,2'-dichlorobenzophenone (ADBP) **(1)** with $^2H_2SO_4/C^2H_3COO^2H$ at 160°C gave two major and several minor products in addition to the labelled benzophenone **(2)**. Using preparative HPLC, **(2)** was successfully isolated from the by-products (7). ^1H NMR analysis (Table 1) of **(2)** indicated that the reaction had resulted in one deuterium exchange on the triple substituted ("A") ring and two deuterium exchange on the double substituted aromatic ring ("B").

Compound **(2)** was successfully converted to the benzodiazepine by use of carbobenzyloxyglycylchloride. Thus glycine intermediate **(3)** was hydrolysed with HBr/AcOH to obtain **(4)**. Spontaneous ring closure gave only a 10% yield of **(5)** , howewer, treatment of **(4)** with pyridine under reflux gave **(5)** in high

Table 1 - ^{1}H NMR analysis of ADBP and $^{2}H_3$-ADBP.

H	ADBP δ	ADBP J	$^{2}H_3$-ADBP δ	$^{2}H_3$-ADBP J
Aromatic a	6.68	8.85 d.	Exchange = 99.0%	
" b	7.23	8.82(2.48,2.49)q.	7.23	2.28 d.
" c	7.11	2.37 d.	7.11	2.29 d.
" d	7.46	8.00(1.24,1.18)q.	Exchange = 98.0%	
" e	7.42	7.6(1.72,1.69,1.74)h.	7.42	- s.
" f	7.36	7.41(1.43,1.27,1.29)h.	Exchange = 92.0%	
" g	7.30	7.49(1.70,1.71)q.	7.30	- s.
NH_2	6.46	br.	6.50	- br.

yield. The purified, labelled 7,2'-dichlorobenzodiazepine **(5)** was oxidized to **(6)** with an excess amount of freshly washed m-chloroperoxybenzoic acid (MCPBA) (8). The final product was prepared in two steps (9); firstly using the Polonovski reaction, the N-oxide product **(6)** was rearranged to $^{2}H_3$-lorazepamacetate **(7)**, and secondly basic hydrolysis of **(7)** gave $^{2}H_3$-lorazepam **(8)**. The pathway is shown in Scheme A.

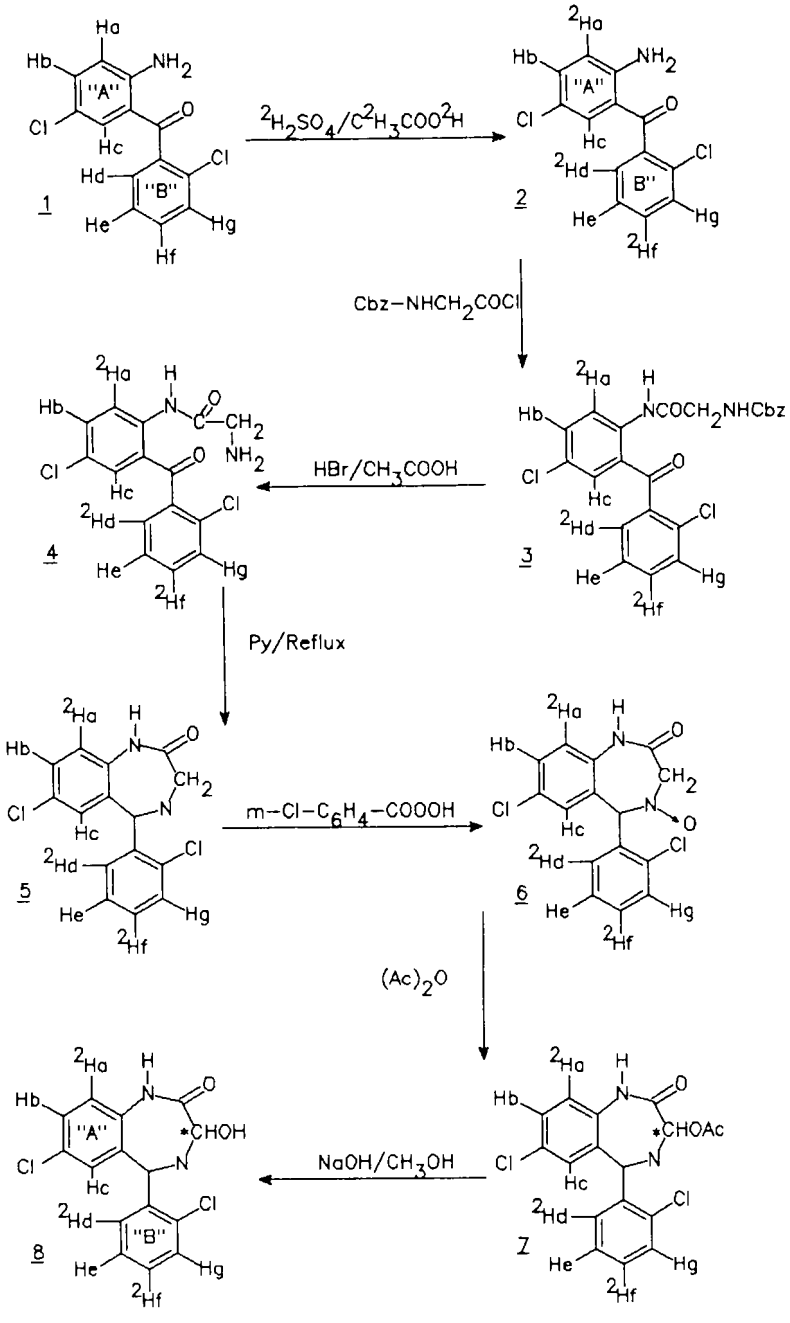
The isotope envelope of the unlabelled and labelled lorazepam in MS/NICI mode is shown in Table 2.

Table 2 - Isotope envelope of unlabelled and labelled lorazepam.

m/z	lorazepam (%)	$^{2}H_3$-lorazepam (%)
300	1.48	-
301	-	-
302	100.00	-
303	15.48	3.00
304	65.96	28.82
305	10.69	100.00
306	9.62	48.13
307	-	68.75
308	-	21.10
309	-	13.46

The m/z 302 and 307 are the ions selected to provide background free SIM quantitation of lorazepam and $^{2}H_3$-lorazepam, respectively.

SCHEME A


Ha
Hb
NH2
"A"
Cl
O
Hc
Hd
"B"
Cl
He
Hg
Hf
1
2H2SO4/C2H3COO2H
2Ha
2Hd
2Hf
2
Cbz–NHCH2COCl
NCOCH2NHCbz
3
HBr/CH3COOH
CH2
NH2
4
Py/Reflux
N
5
m–Cl–C6H4–COOH
6
(Ac)2O
*CHOH
8
NaOH/CH3OH
*CHOAc
7

EXPERIMENTAL

ADBP and carbobenzyloxyglycine (Cbz-Gly) were purchased from Aldrich Company, Milwaukee, Wisconson, USA and MCPBA (85%) from K&K Laboratories, Cleveland, Ohio, USA. $C^2H_3COO^2H$ and $^2H_2SO_4$ were supplied by MSD Isotopes, Dorval, Quebec, Canada.

Preparative HPLC was carried out on a Spectra Physics 8810 pump equipped with a preparative pump head and a Rheodyne 7000L injector valve (5.0 mL loop size). The UV detector was an Applied Biosystem, Model 757, set with a preparative cell and connected to an Eldex Universal fraction collector. Analytical HPLC was carried out on the same instrument using an analytical UV cell, a Rheodyne 7125 injector valve and a loop size of 0.1 mL.

A Delta Pak C_{18}-100 Å analytical column (3.9 x 300 mm) and matching preparative column (19 x 300 mm) were purchased from Nihon Waters Ltd., Tokyo. The two columns were separated with a Rheodyne 7000L switching valve.

GC-MS analyses were performed on a HP 5970 MSD (EI mode) equipped with a HP 1000A series RTE data system and on a VG12-250 (NICI mode) using a VG MASSLAB data system. Chemical ionization was achieved using methane gas. ^1H NMR spectra were obtained on a Bruker AM, 500 MHz high-resolution spectrometer using tetramethylsilane as reference in C^2HCl_3 (δ=ppm, J=Hz).

Deuterium exchange on 2-amino-5,2'-dichlorobenzophenone

One gram of **(1)** was dissolved in 10 mL of $C^2H_3COO^2H$ in a Teflon digesting vessel. After the addition of an aliquot of 10 mL of $^2H_2SO_4$, the vessel was closed, placed in a preheated sand bath and kept for five days at 158-160 °C. Two μL of the reaction mixture was periodically tested by GC-MS (EI mode). When the isotope envelope indicated a deuterium exchange of three hydrogens, the reaction mixture was poured dropwise into 115 mL of previously cooled (10-15°C) 5N NaOH. During this time, (approximately 1 hr) the mixture was kept under constant stirring in an ice bath. After a pH adjustment to pH 12, the mixture was diluted with 100 mL of water and extracted four times with 25 mL of methylenechloride. The combined organic extracts were washed with 25 mL of water and dried with Na_2SO_4. The filtered organic solution was evaporated and the brown residue was dissolved in 10 mL of methanol. Two 5 mL samples were injected onto the preparative column. The flow rate of the methanol mobile phase

was 15 mL/min. The automatic fraction collector was initiated after 3 min and the fractions were collected up to 8 min at 10 sec intervals. The fractions were analysed on the analytical column where the mobile phase was MeOH:H_2O (80:20, v/v) and the flow rate was 1.0 mL/min. The selected purified fractions were combined, evaporated and crystallized in ethanol. **Yield:** 0.40 g **(2)**. Mp 87-89°C; ^{1}**H NMR** δ: See Table 1. **MS** in EI$^+$ mode m/z(%): 268 $M^{+\cdot}$(80), 270(55) 233(100), 198(20), 155(30), 141(25), 113(25), 100(20), 64(20). NICI mode: 232 $[M-Cl]^-$(100), 234(25), 196(20).

2-(Carbobenzyloxyglycyl)amido-5,2'-dichlorobenzophenone-$^2H_3$ (3)-- 1.04 g of phosphorous pentachloride was added in three portions to a cooled and stirred suspension of 1.25 g of Cbz-Gly in 8 mL dry ether. After being stirred for 30 min, Cbz-glycyl-chloride was obtained. To this solution, 1.50 g of **(2)** in 30 mL ether was added dropwise over 40 min. The thick crystal slurry was then continuously stirred for another 2 hr. The pH was adjusted to 11 by the dropwise addition of 25 mL 5N NaOH and stirred for 2 hr. The ether layer was separated and thoroughly washed with water. The collected aqueous phase was extracted with chloroform and the combined organic extracts dried over Na_2SO_4. The filtered solution was evaporated in vacuo and the residue was crystallized in 100 mL ethanol. **Yield:** 2.25 g **(3)**. **MS** in EI$^+$ mode m/z(%): 459 $M^{+\cdot}$(5), 353(10), 295(10), 268(20), 233(20), 181(40), 141(4), 91(100).

7,2'-Dichloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one-$^2H_3$ (5) -- A solution of 1.50 g of **(3)** and 8 mL of 30% HBr in CH_3COOH was stirred for 1 hr at room temperature. After 1 hr, 100 mL of dry ether was added and the resultant mixture stirred for 5 min. The ether solution was decanted and the precipitated solid was washed with 50 mL of dry ether. The solid product was suspended in 25 mL of ether, chilled on an ice bath and then 8 mL of 10% NaOH was added. The resultant mixture was stirred and the ether layer was separated, washed with water and evaporated under pressure to yield **(4)**.

The residue was dissolved in 25 mL of freshly distilled pyridine and refluxed for 16 hr under nitrogen atmosphere. The pyridine was evaporated under pressure and the residue was purified by preparative HPLC (mobile phase: MeOH:H_2O, 80:20, flow rate: 10 mL/min). The automatic fraction collector was initiated

after 5 min and the fractions were collected for 10 min at 10 sec intervals. All fractions were analysed on the analytical column using the same mobile phase and a flow rate of 1.0 mL/min. The selected purified fractions were combined, evaporated and crystallized in ethanol. **Yield:** 1.09 g **(5)**. **^{1}H NMR** δ: 4.39 (2H s. $-CH_2$), 7.06 (1H d. **AH**, J=2.41), 7.25 (1H s. **AH**), 7.41 (1H s. **AH**), 7.43 (1H d. **AH**, J=2.25), 8.14 (1H br. -NH). **MS** in EI^+ mode m/z(%): 307 $M^{+\cdot}$(80), 309(60), 280(80), 278(100), 274(25), 272(100), 244(50), 180(50), 139(30), 121(30), 104(30), 76(40). NICI mode: 271$[M-Cl]^-$(100), 273(25), 272(20).

7,2'-Dichloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one-4-oxyde-$^2H_3$ (6) -- A solution of 984.60 mg of **(5)** in 50 mL of methylenechloride was added dropwise to a stirred solution of 830.10 mg of m-chloroperoxybenzoic acid (previously washed with a buffer solution pH 7.4 and dried in vacuo) in 10mL of methylenechloride at 20-25°C during 90 min. The reaction mixture was stirred for 10 hr at room temperature and then 830.10 mg of m-chloroperoxybenzoic acid was added. After 10 hr of constant stirring, the reaction mixture was brought to pH 8.0 with 25% of NH_4OH and washed thoroughly with water. The solid white precipitate was filtered and dried in vacuo. The combined solids were crystallized in 7 mL of ethanol. **Yield:** 800.00 mg **(6)**. **^{1}H NMR** δ: 4.73 (1H d. -CH, J=12.56), 4.77 (1H d. -CH, J=12.51), 6.95 (1H d. **AH**, J=3.31), 7.36 (1H d. **AH**, J=1.67), 7.37 (1H d. AH, J=2.34), 7.42 (1H d. **AH**, J=1.44), 8.30 (1H br. -NH). **MS** in EI^+ mode m/z(%): 323 M+·(5), 288(75), 245(100), 224(30). NICI mode: 271$[M-Cl-O]^-$(100), 273(25)

3-Acetoxy-7,2'-dichloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one-$^2H_3$ (7) -- A suspension of 1.00 g of **(6)** in 10 mL of $(CH_3CO)_2O$ was stirred and heated on a water bath for 3 hr at 60-70°C. The resultant clear solution was evaporated in vacuo and the residue was dissolved in 5 mL of ethanol and crystallized. **Yield:** 1.00g **(7)**. **^{1}H NMR** δ: 2.32(3H s. $-CH_3$), 6.01 (1H s. -CH), 7.07 (1H d. **AH**, J=2.40), 7.42 (1H d. **AH**, J=1.56), 7.47 (1H d. **AH**, J=2.29), 7.57 (1H d. **AH**, J=0.8), 9.33 (1H s. -NH). **MS** in EI^+ mode m/z(%): 365 $M^{+\cdot}$(20), 323(42), 296(70), 294(100), 288(30), 278(40), 242(50), 180(45), 152(30).

7,2'-Dichloro-3-hydroxy-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one-$^2H_3$ (8) -- One gram of **(7)** was dissolved in 10 mL of methanol and then 6 mL of 4N NaOH was added. After 30 min, a solid precipitate was produced which dissolved upon the

addition of 50 mL water. The solution was acidified with acetic acid and extracted with methylenechloride. After separation, the methylenechloride was washed three times with 20 mL of water, dried on Na_2SO_4 and evaporated in vacuo. The opaque, white solid wax was purified on preparative HPLC (mobile phase: MeOH:H_2O, 30:70, flow rate: 10 mL/min). The automatic fraction collector was initiated after 8 min and the fractions were collected for to 12 min at 10 sec interval. All fractions were analyzed on the analytical column using the same mobile phase and a flow rate of 1 mL/min. The selected fractions were combined, evaporated and crystallized in 2 ml of ethanol. **Yield:** 0.47g **(8)**. **^{1}H NMR δ:** 4.59(1H d. -OH, J=9.95), 5.06(1H d. -CH, J=8.82), 7.10(1H d. AH, J=2.36), 7.42(1H d. AH, J=1.44), 7.48(1H d. AH, J=2.37), 7.63(1H s. AH), 8.62(1H br. -NH). **MS** in EI^+ mode m/z(%): 305[$M-H_2O$]$^{+\cdot}$ (25), 277(60), 242(100), 140(50), 113(55), 76(70). NICI mode:See Table 2.

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