SYNTHESIS OF DEUTERIUM-LABELLED DRUGS OF ABUSE FOR USE AS INTERNAL STANDARDS IN QUANTIFICATION BY SELECTED ION MONITORING. II. DEUTERIUM-LABELLED BENZODIAZEPINES: CHLORDIAZEPOXIDE, DEMETHYLDIAZEPAM, DIAZEPAM, AND OXAZEPAM.

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SUMMARY

Four deuterium-labelled benzodiazepines were prepared for use as internal standards in the quantification of chlordiazepoxide, demethyldiazepam, diazepam, and oxazepam at low levels in body fluids by selected ion monitoring. The method described provides a way to introduce cleanly and in high isotopic purity five deuterium atoms into each drug.

Key Words: Selected ion monitoring, Benzodiazepines, Chlordiazepoxide-d_5, Demethyldiazepam-d_5, Diazepam-d_5, Oxazepam-d_5

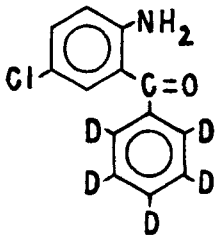
INTRODUCTION AND DISCUSSION

In our previous paper,[1] we described the synthesis of four deuterium-labelled drugs of abuse for use or internal standards in the quantification of the unlabelled drugs by selected ion monitoring. A detailed account of the use of one of these standards has recently been published.[2] In addition, we discussed the need for standards containing more than three deuterium atoms per molecule and having high isotopic purity.

In this paper, we describe the synthesis of four deuterium-labelled benzodiazepines each containing five deuterium atoms and each of high isotopic purity. Chlordiazepoxide and diazepam are two of the

most commonly prescribed tranquilizers, and although oxazepam is a drug in its own right it is also a terminal metabolite of diazepam,[3] while demethyldiazepam is the major diazepam metabolite detected in blood.[4] Labelled variants of these compounds were necessary for our analytical studies of the minor tranquilizers, particularly those compounds which have been identified as special interest substances with abuse potential.[5]

The key intermediate in the preparation of the desired benzodiazepines is 2-amino-5-chlorobenzophenone. The most accessible positions for labelling with deuterium are those of the aromatic ring bearing five hydrogen atoms. The desired intermediate is 2-amino-5-chlorobenzophenone-2',3',4',5',6'-d_5 (1).



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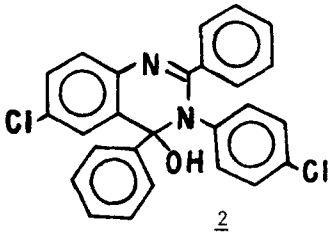
From 1, all of the necessary benzodiazepine-d_5 compounds could be prepared using known literature procedures for the preparation of undeuterated benzodiazepines provided no exchange occurs during these reactions. Much of the chemistry of the benzodiazepines has been reviewed.[6,7]

The usual way of preparing 2-amino-5-chlorobenzophenone is to condense p-chloroaniline with benzoyl chloride in the presence of zinc chloride and then hydrolyze the resulting intermediate, 2, with acid.[8] However, if benzoyl-d_5 chloride is used in place of benzoyl



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chloride and the intermediate is hydrolyzed with acetic acid/sulfuric acid mixtures as described in the literature, the resulting benzophenone derivative contains no deuterium. This difficulty can be circumvented by using DOAc and D_2SO_4 for the hydrolysis, but in this case exchange occurs with the other aromatic hydrogens and a benzophenone bearing from 5 to 8 deuteriums is obtained.[9] Since we would prefer only 5 deuterium atoms in a compound of high isotopic purity, we have devised an alternative route to 1. Our approach is based on a synthesis of 2-amino-5-chlorobenzophenone described in the patent literature[10] and involves the reaction of phenyl-d_5 magnesium bromide with 6-chloro-2-methyl-4H-3,1-benzoxazine-4-one (3). The source of the deuterium is bromobenzene-d_5 which is much less expensive than benzoly-d_5 chloride and the yield (approximately 50 percent) consumes much less of the labelled material than the benzoyl chloride route which requires a large excess of the reagent.

Following is the overall scheme for the preparation of the four benzodiazepine-d_5 compounds: Chlordiazepoxide-d_5 (8), demethyldiazepam-d_5 (5), diazepam-d_5 (11), and oxazepam-d_5 (13). Detailed experimental procedures are given for the preparation of 1, while the other steps are summarized in the Table with literature references to the corresponding preparations of the undeuterated compounds.

The isotopic compositions of the deuterium-labelled compounds were determined by comparison of their chemical ionization mass spectra with the corresponding spectra of the unlabelled compounds. A mixture of methane and ammonia (approximately 8:1) was used as the reagent gas. Under these ionization conditions the mass spectra of all of the compounds show abundant protonated-molecule ions and virtually no fragment ions. The deuterium labelled 2-amino-5-chlorobenzophenone (1) was found to have the following isotopic composition: 96.0 percent of the molecules contained 5 deuteriums, 3.4 percent contained 4 deuteriums, 0.4 percent contained 3 deuteriums, while the d_2, d_1, and d_0 species amounted to less than 0.2 percent. The deuterium contents of the other labelled compounds

(5, 8, 11, and 13) were found to be the same, indicating that hydrogen-deuterium exchange occurring during the subsequent synthetic steps was negligible.

SCHEME. PREPARATION OF DEUTERIUM-LABELLED BENZODIAZEPINES

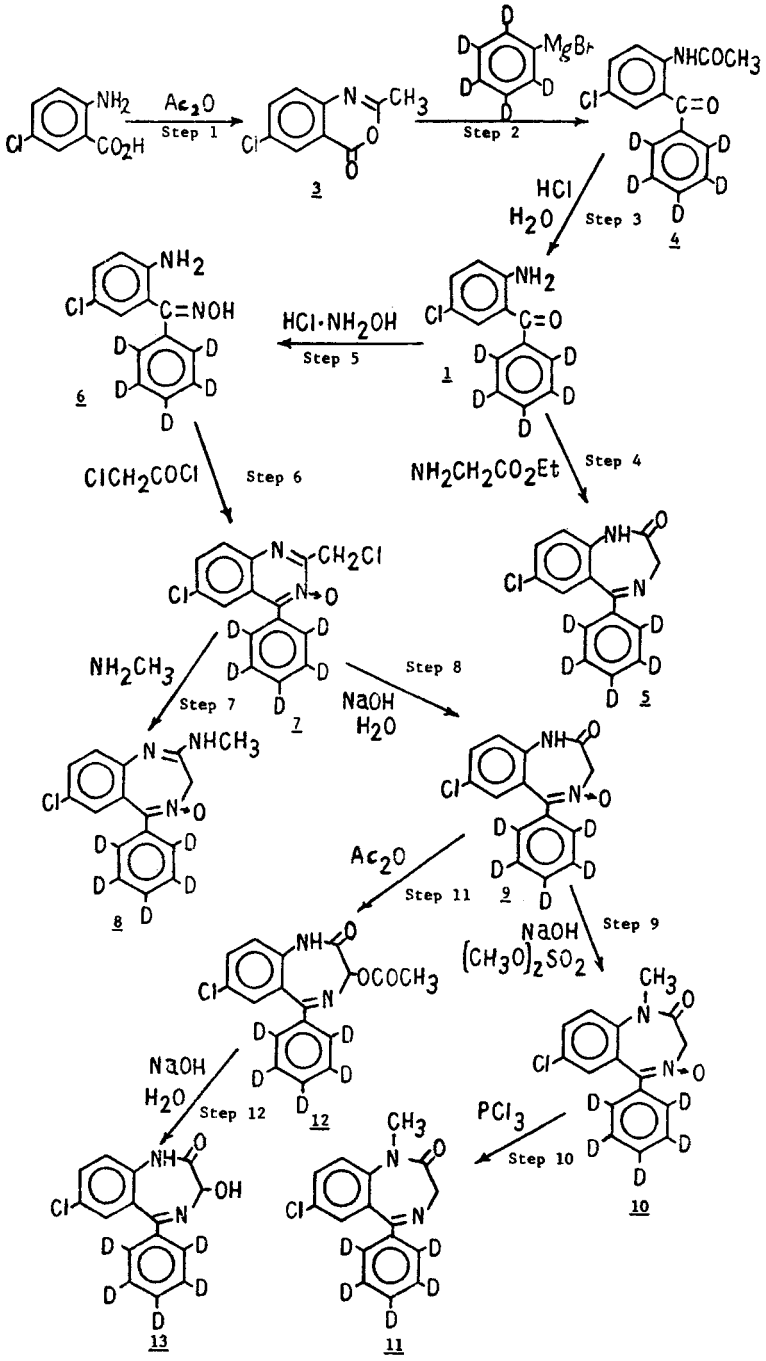

TABLE. PREPARATION OF DEUTERIUM-LABELLED BENZODIAZEPINES

Compound	Step Indicated in Scheme	Yield,(a) percent	(b) Mp,°C	(c) Lit.mp,°C	Ref.
3	1	83	123-125	121-124.5	10
4	2	51	114-115.5	115-116	10
1	3	95	96-98	97-98	10
5	4	41	214.5-215.5	216-217	11,12
6	5	72	164-165	164-167	13
7	6	73	134-136	133-134	13
8	7	61	229-231	236-236.5	14
9	8	78	233-234(dec.)	238-239 235-236(dec.)	15 12
10	9	81	184-185.5	179-180 188-189	15 12
11	10	49	131-132	122-124 125-126	15 12
12	11	83	236-237(dec.)	242-243	16
13	12	78	194-195(dec.)	203-204	16

(a) Yields are indicated for the purified compound. Average yields are indicated when the reaction was run more than once.

(b) Melting points are uncorrected.

(c) Literature melting points are for undeuterated compounds.

EXPERIMENTAL

6-Chloro-2-methyl-4H-3, 1-benzoxazine-4-one (3)

2-Amino-5-chlorobenzoic acid, Aldrich Chemical Company, Inc., 15 g, was added to 50 ml of acetic anhydride and the solution refluxed for 1.5 hr at which time 25 ml of the solvent was distilled off. The product crystallized from the cool solution, was filtered off, washed with acetic anhydride, and dried in a vacuum desiccator. The yield was 14.0 g (83 percent), mp 123-125 °C (lit.[10] mp 121-124.5 °C).

2-Acetamido-5-chlorobenzophenone-2',3',4',5',6'-d_5 (4)

To a solution of 10.0 g (51 mmole) of 3 in 100 ml of benzene

and 50 ml of ether was added the Grignard reagent prepared from 8.7 g (53.5 mmole) of bromobenzene-d_5 (Diaprep, Inc. - Division of Aldrich Chemical Company, Inc.) and 1.4 g (58 mg-atom) of magnesium metal in 20 ml of ether. The Grignard reagent was prepared in a separate flask and transferred to a dropping funnel with a syringe. The Grignard reagent was added over 45 minutes with the reaction temperature maintained at 0 to -2 °C (ice/salt bath). Following addition, the reaction mixture was stirred 0.5 hr at 0 °C and then 1 hr at 25 °C. The reaction mixture was then recooled to 0 °C and 50 ml of 6N hydrochloric acid added. The solution was then warmed to 25 °C and stirred for 0.5 hr. The organic layer was separated, washed twice with water, dried ($MgSO_4$), filtered, and the solvent removed on the rotary evaporator. The yellow oil was triturated under hexane until the crude product formed into a granular solid. The hexane was removed on the rotary evaporator and the crude solid (15.3 g) crystallized from 2-propanol to give 7.8 g, mp 111-113 °C. This material was then recrystallized from 2-propanol to give 7.3 g (51 percent), mp 114-115.5 °C (lit.[10] mp 115-116 °C for undeuterated compound). The IR spectrum shows only a weak C-D adsorption at 2260 cm^{-1}. The mother liquors would not provide any additional product on concentration.

2-Amino-5-chlorobenzophenone-2',3',4',5',6' -d_5 (1)

A mixture of 4, 7.3 g (26.2 mmole), 40 ml of 95 percent ethanol, and 40 ml of 6N hydrochloric acid was refluxed with stirring for 3 hrs. The ethanol was then removed on the rotary evaporator. The remaining aqueous solution was cooled in an ice bath and made basic with conc. ammonium hydroxide. The product was extracted into methylene chloride and the remainder into chloroform. The extracts were combined, washed with water, dried ($MgSO_4$), filtered, and the solvent removed on the rotary evaporator leaving 5.9 g (95 percent) of a bright-yellow, crystalline solid with mp 96-98 °C (lit.[10] mp 97-98 °C for the undeuterated compound).

ACKNOWLEDGMENT

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