SYNTHESIS OF 5-ETHYL-$\underline{d}_5$-5-PHENYL-2-THIOXOBARBITURIC ACID, PRIMIDONE-ETHYL-$\underline{d}_5$ AND PHENOBARBITAL-ETHYL-$\underline{d}_5$

Brian D. Andresen
Department of Pharmaceutical Chemistry
College of Pharmacy, University of Florida
Gainesville, Florida 32610

K. Biemann
Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 92139

SUMMARY

Three deuterium labelled drugs, 5-ethyl-$\underline{d}_5$-5-phenylthiobarbituric acid, primidone-ethyl-$\underline{d}_5$, and phenobarbital-ethyl-$\underline{d}_5$, have been prepared employing a three step synthesis utilizing diethyl phenylmalonate and bromoethane-$\underline{d}_5$. Overall isotopic purity of the labelled drugs was in excess of 95%. Complete synthetic techniques as well as spectral data of labelled intermediate and drugs are presented.

KEY WORDS: Deuterium Labelling, Malonic Ester Synthesis, Thiobarbituric Acid, Primidone and Phenobarbital, Mass Spectral Data.

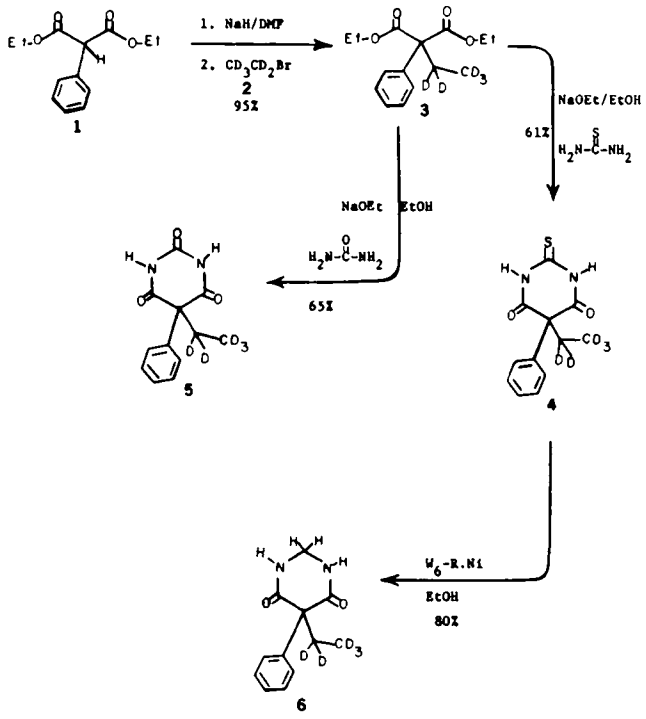
INTRODUCTION

Previous reports concerning the concurrent administration of phenobarbital and primidone in seizure patients have led to conflicting statements (1) pertaining to dose requirements, relative toxicity, and metabolic disposition of these drugs. Therefore, in order to obtain a better understanding (2) of the metabolic fate of these compounds when used separately or in combination, as well as to seek out new metabolites of the parent drug, deuterium labelled primidone and phenobarbital were prepared. Both probes were designed containing a fully labelled ethyl-$\underline{d}_5$ moiety. In the process of preparing these compounds, a new deuterium labelled thiobarbiturate was also generated.

The synthetic pathways leading to these compounds are seen in Scheme 1. A malonic ester condensation of diethyl phenylmalonate, 1, with bromoethane-$\underline{d}_5$, 2, formed diethyl ethyl-$\underline{d}_5$-phenylmalonate, 3. This deuterium labelled intermediate was next condensed with either thiourea or urea to yield 5-ethyl-$\underline{d}_5$-5-phenylthiobarbituric acid, 4, or phenobarbital-ethyl-$\underline{d}_5$, 5, respectively. Catalytic hydrogenation of 4 produced primidone-ethyl-$\underline{d}_5$ in high yield.

0362-4803/79/0516-0681$01.00

Received October 6, 1978
Revised November 20, 1978



Scheme 1. Preparation of 5-ethyl-d_5-5-phenylthiobarbituric acid (4), phenobarbital-ethyl-d_5 (5), and primidone-ethyl-d_5 (6).

EXPERIMENTAL

Diethyl ethyl-d_5-phenylmalonate, 3 - To a solution of 100 ml of dry dimethylformamide and 3 g (0.124 moles) of NaH in a 300 ml three necked flask equipped with a reflux condenser and three rubber septums diethyl phenylmalonate 16.0 g (0.068 moles) was added by cannula under nitrogen. The mixture was initially stirred, heated to 85°, and the evolved hydrogen allowed to escape through a 25 gauge needle. After 90 min the evolution of hydrogen had ceased and the temperature had dropped to 70°. By cannula 6.0 g (0.053 moles) of bromoethane-d_5 was slowly added over a period of 10 min. A small temperature increase to 80° was noted. After 1 hr the temperature had stabilized at 70° and the mixture was added by cannula to a stirring 0.1 N H_2SO_4 ice solution, extracted with ethyl acetate, dried with anhydrous $MgSO_4$, and evaporated to a light yellow oil. To the oil, dissolved in 300 ml of pentane, was added 200 g of preactivated (heated to 320° for 24 hours) aluminum oxide (Woelm aluminum oxide; activity I; 100 mesh) and the

whole evaporated down until a free flowing powder was obtained. The aluminum oxide was then extracted extensively with dry methylene chloride to yield 13.5 g (95%, isolated) of an oil, diethyl ethyl-$\underline{d}_5$-phenylmalonate. M.S. (relative inten.): M^+ 269 (6%), m/e 197 (49%), m/e 196 (47%), m/e 179 (15%), m/e 151 (51%), m/e 136 (100%), m/e 122 (43%), m/e 121 (48%), m/e 93 (53%), and m/e 77 (62%). Rf = 0.60 (E.M. Silica gel plates/ether; I_2 vapor).

5-Phenyl-5-ethyl-$\underline{d}_5$-2-thiobarbituric acid, 4 - To a 50 ml round bottom flask equipped with a stirring bar, reflux condenser, and rubber septum were added thiourea, 1.2 g (0.058 moles) and NaH, 1.0 g (0.0416 moles). While stirring slowly, by cannula, diethyl-ethyl-$\underline{d}_5$-phenylmalonate, 3.5 g (0.0130 moles) in 40 ml absolute ethanol was added. After the addition, a drying tube was added and the whole mixture refluxed for 24 hr. After refluxing, the mixture was added by cannula to 0.1 N H_2SO_4 and ice. The white crystals were filtered and washed with water to yield 2.0 g (61%) of the thiobarbiturate, 4. R_f = 0.57 (Si gel/ether; H_2SO_4/heat and char) m.p. 216-218°. M.S. (relative inten.): M^+ 253 (100%) and m/e 221 (83%).

Phenobarbital-ethyl-$\underline{d}_5$, 5 - To a 50 ml round bottom flask equipped with a stirring bar, reflux condenser, and rubber septum were added 1.0 g (0.0166 moles) of urea and 1.0 g (0.0416 moles) of NaH under nitrogen. While stirring, diethyl ethyl-$\underline{d}_5$-phenylmalonate, 3.4 g (0.0126 moles), in 40 ml dry ethanol was slowly added by cannula. The mixture was refluxed for 24 hours and then added to 50 ml 0.1 N H_2SO_4 and 25 ml crushed ice. After extraction with ether, drying, and evaporation of the solvent to a small volume, the phenobarbital was obtained by column chromatography (Silica gel; E.M. Reagents, 0.5-0.2 mm mesh, 2.5 x 28 cm) in the first 200 ml of dry ethyl ether. The ether was evaporated to yield 2.0 g (65%) of pure phenobarbital. R_f = 0.72 (Si gel/ether; diphenycarbazone and mercuric sulfate positive) m.p. 175-178°.

Primidone-ethyl-$\underline{d}_5$, 6 - To a 50 ml round bottom flask was added 1.5 g (0.00593 moles) of the thiobarbiturate, 4, 25 ml absolute alcohol, and 9 g W_6-Raney nickel (3). Under 1 atm of hydrogen the whole was refluxed for 5 hr. The mixture was filtered hot and the pyrophoric nickel catalyst washed with boiling hot ethanol. Evaporation of the solvent yielded 1.06 g (80%) of primidone-ethyl-$\underline{d}_5$. R_f = 0.66 (Si gel, ether) m.p. 280-282°.

DISCUSSION

Various synthetic methods were initially employed in order to optimize the yield of 3 in the first step of Scheme 1. Although many variations of the malonic ester synthesis have been reported, it was found that the addition of diethyl phenylmalonate and sodium hydride to dry dimethylformamide (4) at a slightly elevated temperature, followed by the addition of bromoethane-d_5, produced the highest yields of 3 with few side reactions. Traces of water in the reaction mixtures were found to catalyze decarboxylation leading to the formation of ethyl 2-ethyl-d_5-phenylacetic acid and ethyl 2,2-diethyl-d_5-phenylacetic acid. These impurities were eliminated employing anhydrous conditions.

In order to utilize all of the valuable bromoethane-d_5 in the first step of Scheme 1, an excess amount of starting material was used. Initial purification of the desired intermediate 3 by vacuum distillations or extractions into various solvent systems failed. However, it was discovered that excess 1 possessed more affinity for and could be trapped on activated aluminum oxide. Simple extraction of a free flowing powder of alumina, coated with the reaction products, following the malonic ester synthesis, produced 3 in a highly purified form.

The condensation of the labelled intermediate 3 with either thiourea or urea as described previously for the preparation of nonlabelled 5-ethyl-5-phenyl-thiobarbituric acid (5,6) or phenobarbital (7), respectively, generated two ethyl-d_5 labelled drugs. Catalytic hydrogenation of 4 using Raney's catalyst (3) yielded 5-ethyl-d_5-5-phenyl-hexahydropyrimidine-4,6-dione, 6, (primidone-ethyl-d_5).

Because phenobarbital-ethyl-d_5 and primidone-ethyl-d_5 are ideally suited for mass spectrometry applications, mass spectral comparisons between the nonlabelled and labelled drugs are presented in Figures 1 and 2, respectively. The molecular ion at m/e 232 for nonlabelled phenobarbital (Figure 1-A) is observed to increase 5 mass units to m/e 237 in the spectrum of the labelled molecule (Figure 1-B). However, the base peak ion at m/e 204 in the mass spectrum of nonlabelled phenobarbital shifts only one mass unit to m/e 205 in the mass spectrum of the labelled molecule. These observations confirm earlier data suggesting the loss of ethylene

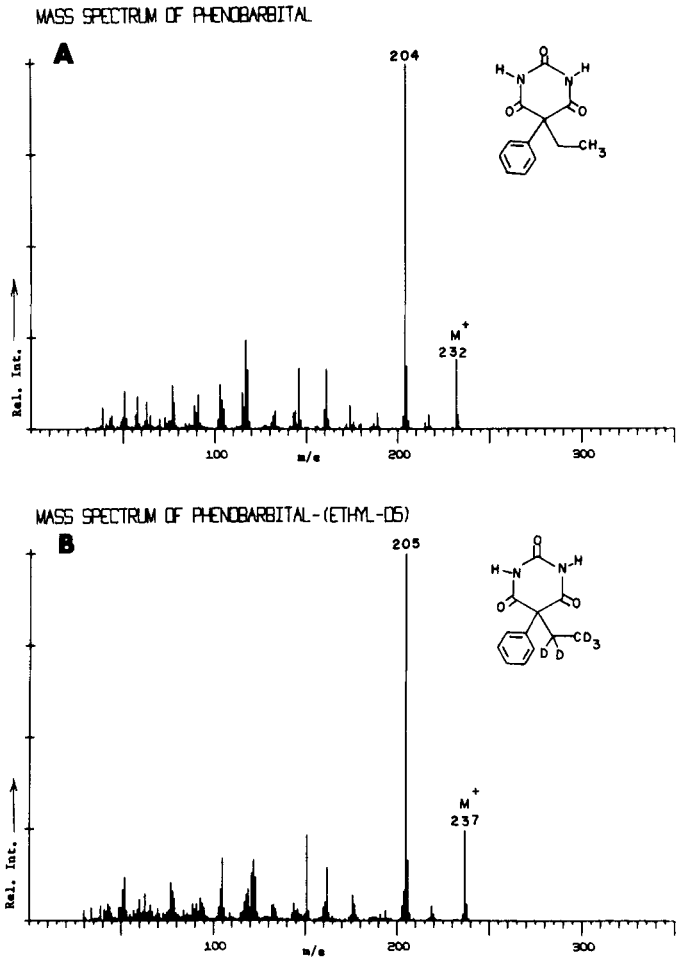
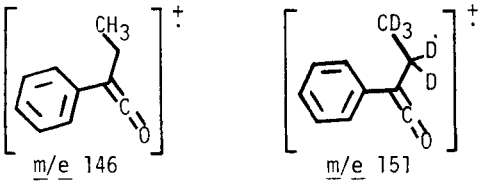


Figure 1: A- Mass spectrum of phenobarbital.
B- Mass spectrum of phenobarbital-ethyl-$\underline{d}_5$.

and migration of a hydrogen or deuterium atom through a McLafferty rearrangement (8) observed with many 5-substituted barbiturate compounds.

In a similar fashion, primidone reveals a molecular ion at $\underline{m/e}$ 218 (Figure 2-A) which shifts to $\underline{m/e}$ 223 in the mass spectrum of primidone-ethyl-$\underline{d}_5$ (Figure 2-B). Also the migration of a hydrogen or deuterium atom and subsequent loss of ethylene is apparent by the shift of the ion at $\underline{m/e}$ 190 to $\underline{m/e}$ 191 for nonlabelled and labelled primidone, respectively. The base peak ion at $\underline{m/e}$ 146 (Figure 2-A) shifts to $\underline{m/e}$ 151 (Figure 2-B). This requires retention of the labelled ethyl side chain and suggests this ion to be an ethyl-phenylketene fragment. High resolution mass

spectral analyses (CEC 110) indeed confirmed that m/e 146 and m/e 151 in Figure 2 were generated from $C_{10}H_{10}O^+$ and $C_{10}H_5D_5O^+$, respectively.



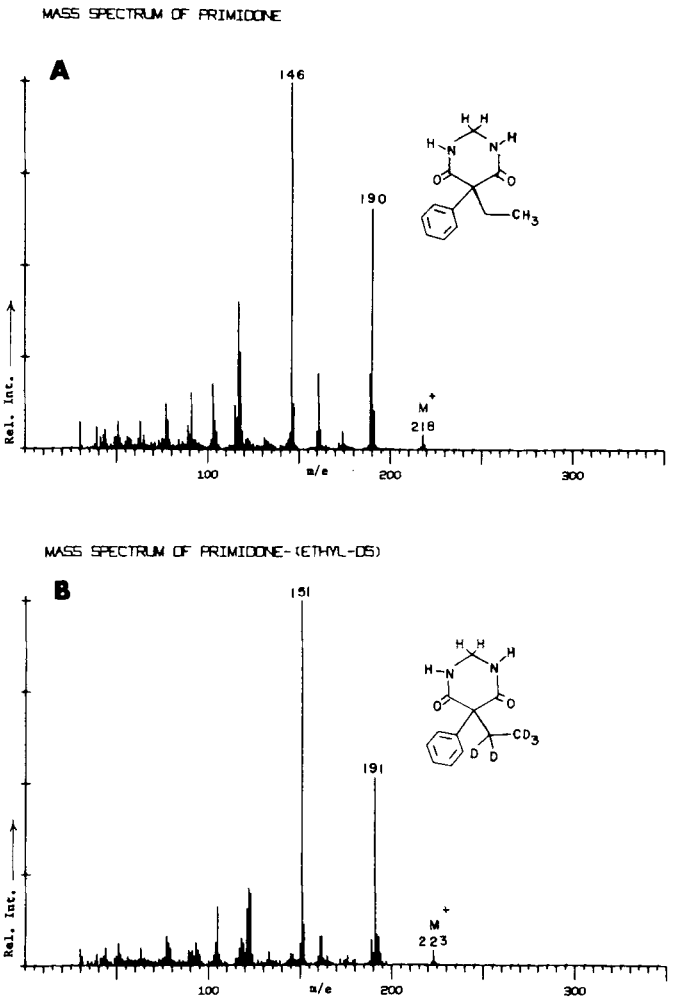


Figure 2: A- Mass spectrum of primidone.
B- Mass spectrum of primidone-ethyl-d_5.
(Spectra were obtained using an Hitachi RMU-6L low resolution mass spectrometer interfaced to an IBM 1800 computer. Samples were volatilized directly into the ion source of the mass spectrometer, 70 eV, EI)

CONCLUSIONS

The reactions presented here reveal a convenient and inexpensive synthesis of three ethyl-$\underline{d}_5$ labelled drugs. In contrast one analogue, phenobarbital-2,4(6), 5-$^{13}C_4$ has been prepared by the reaction of diethyl malonate-1,2-$^{13}C_2$ with bromobenzene in liquid ammonia, followed by condensation of the labelled intermediate with ethyl iodide and urea-^{13}C. Also a variety of deuterium labelled 1-methoxymethylphenobarbital derivatives (10) and 2-thiophenobarbital-ethyl-1-^{14}C and phenobarbital-ethyl-1-^{14}C analogues (11) have recently been prepared. Therefore this new phenobarbital-ethyl-$\underline{d}_5$ probe may provide an alternative to either an expensive or radioactively labelled probe necessary for or used in drug assays and drug metabolism studies.

This new primidone-ethyl-$\underline{d}_5$ analogue has not been reported previously and it also should satisfy the need for an inexpensively labelled probe for metabolism studies. In fact, initial studies and feeding experiments in rats (2) using ethyl-$\underline{d}_5$ labelled primidone and phenobarbital demonstrated and confirmed earlier proposals (12) that the major metabolite of primidone is 2-ethyl-2-phenylmalondiamide. Also, it could be easily shown by using these stable isotopically labelled probes that phenobarbital is never incorporated into the enzymatic pathways leading to the malondiamide. One could also conclude from these labelling experiments that primidone is irreversibly oxidized to phenobarbital which is not metabolically reduced to primidone.

ACKNOWLEDGEMENTS

We are indebted to the National Institutes of Health Grant (RR00317) from the Biotechnology Resource Branch, Division of Research Resources and one author (B.D.A.) greatly acknowledges a National Institutes of Health Training Grant GM01523, a Cottrell Starter Grant administered by Research Corporation, and a University of Florida Division of Sponsored Research Award.

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