A SYNTHESIS OF 5-PHENYL-5-PHENYL-$\underline{d}_5$-HYDANTOIN

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SUMMARY

The drug 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin was prepared employing a five step synthesis starting from readily available benzene-$\underline{d}_6$. Two key intermediates, benzoin-$\underline{d}_5$, and benzil-$\underline{d}_5$, were prepared, containing only one fully labelled phenyl-$\underline{d}_5$ group. The precursor benzil-$\underline{d}_5$ was condensed with urea to generate the labelled drug, diphenylhydantoin-$\underline{d}_5$. This new probe possesses only one fully labelled phenyl group making it suitable for metabolic studies and as an internal standard for combined GC-MS-computer analyses of body fluid extracts. Synthetic procedures as well as complete spectral data of precursors and the labelled drug are presented.

Key Words: Bromobenzene-$\underline{d}_5$, benzoin-$\underline{d}_5$, benzil-$\underline{d}_5$, diphenylhydantoin-$\underline{d}_5$, IR and MS data.

INTRODUCTION

Diphenylhydantoin (phenytoin) was introduced in 1938 as an effective agent for the symptomatic control of epilepsy. The drug is also an antiarrhythmic and a highly specific antagonist for digitalis intoxication. This compound is used today in a great many patients under a variety of clinical conditions. Recently, diphenyl-$\underline{d}_{10}$-hydantoin has been prepared and proposed (1) as a useful probe for the determination of deficiency in diphenylhydantoin absorption, distribution, metabolism, and excretion. In a separate study (2) 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin and 5-phenyl-$\underline{d}_5$-5-(para-hydroxyphenyl)-hydantoin have been prepared and used successfully to quantitate the parent drug and its major metabolite in plasma. In connection with our research (3) aimed at the identification of metabolites of the parent drug, considerations of a deuterium isotope effect during metabolism, and the development of a suitable internal standard for combined GC-MS-computer analyses, 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin has also been prepared (Scheme 1).

The synthesis of the labelled compound utilizes a benzene-$\underline{d}_6$, an inexpensive and

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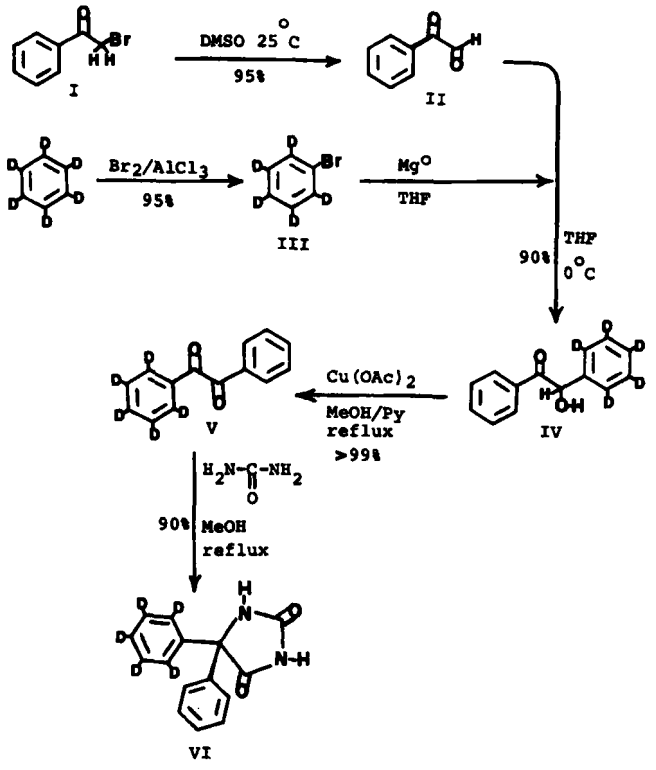
readily available NMR solvent, as the precursor for the preparation of a key intermediate, benzil-$\underline{d}_5$, V. Condensation of V with urea resulted in the preparation of 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin, VI, in a high overall yield (76%).



Scheme 1: Preparation of 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin from benzene-$\underline{d}_6$.

EXPERIMENTAL

<u>α-Bromoacetophenone (I)</u> - Sufficient quantities of α-bromoacetophenone were obtained from acetophenone, bromine, and $AlCl_3$ in ethyl ether following standard procedures (4).

Phenylglyoxal (II) - Alpha-bromoacetophenone (60 g) and 400 ml of dimethylsulfoxide (DMSO) were stirred at room temperature following published procedures (5). After 12 hours, the reaction mixture was evaporated in vacuo to initially removed dimethylsulfide (stench). Vacuum distillation (132°/100 mm Hg) of the products yielded 20 g of a viscous yellow oil, II. NMR, $CDCl_3$, (δ): 9.45 (aldehyde), 8.00 and 7.54 (aromatic H, multiplet). M.S. (rel. int.): M^+ 134 (0%), m/e 105 (100%) and m/e 77 (60%).

Bromobenzene-d_5(III) - A 300 ml three neck flask, fitted with dropping funnel and thermometer, was charged with 200 ml trichlorotrifluoroethane, 0.2 g (1.6 mmol) $AlCl_3$, and 12.04 g (148 mmol) benzene-d_6 (Stohler Isotope Chemicals, Inc.). The reaction mixture was stirred and cooled to 0°C in an ice bath. Slowly, over 1.5 hr., 12.5 g (156 mmol) of bromine were added. The reaction mixture was then poured into a saturated $NaHCO_3$ solution (100 ml) and extracted with excess CH_2Cl_2. Evaporation of the solvent followed by distillation (88-89°/100 mm Hg) yielded 22.7 g (95%) of bromobenzene-d_5 (95%) of bromobenzene-d_5 (III) (> 98% phenyl-d_5 by mass spectral analysis). M.S. (rel. int.): M^+ = 161 and 163 (100% and 97%, respectively), m/e 82 (94%) m/e 54 (42%), m/e 52 (36%) and m/e 40 (13%).

Benzoin-d_5 (IV) - Into a 250 ml round bottom flask, fitted with a rubber septum, was placed 150 ml of dry tetrahydrofuran (THF) and 5.4 g (40 mmol) phenylglyoxal. The mixture was stirred at 0°C in an ice bath. To this cooled solution phenylmagnesium bromide-d_5 (prepared from 6.50 g (40 mmol) bromobenzene-d_5, and 3 g magnesium metal in 40 ml dry THF) was slowly added by cannula over a 30 min. period. The reaction turned from yellow to slate gray after all of the Grignard reagent had been added. The reaction mixture was then poured into 500 ml of 0.1N H_2SO_4 and extracted with excess CH_2Cl_2. Evaporation of the solvent yielded a syrup. Recrystallization from ethanol produced 7.8 g (90%) benzoin-d_5. M.p. 135-137°C. M.S. (rel. int.): M^+ = 217 (2%), m/e 112 (15%), m/e 110 (93%), m/e 107 (14%), m/e 105 (100%), m/e 84 (45%), m/e 82 (58%), m/e 79 (12%), m/e 77 (45%), m/e 54 (20%), and m/e 51 (23%).

Benzil-$\underline{d_5}$ (V) - To a solution of 7.8 g (36 mmol) of benzoin-$\underline{d_5}$ in 150 ml of methanol and 10 ml of pyridine was added 11 g (60 mmol) of $Cu(OAC)_2 \cdot H_2O$. The resulting dark blue solution was refluxed for two hours. At the end of that time the now dark green solution was poured into 1 liter of water. Crystals of benzil-$\underline{d_5}$ that formed were collected and the aqueous solution extracted with excess CH_2Cl_2. Both crystals and extract were combined and the solvent evaporated yielding a yellow-green mixture containing benzil-$\underline{d_5}$ and some copper salts. The benzil-$\underline{d_5}$ was conveniently isolated using a silica gel column eluted with ethylacetate/hexane (9:1). An intense yellow band developed on the column which was collected and the solvent evaporated yielding 7.65 g (99% yield) of benzil-$\underline{d_5}$. M.p. 93-94°, yellow needles; R_f = 0.70 (TLC, alumina, 40% ethylacetate/pentane); M.S. (rel. int.): M^+ = 215 (3%), m/e 110 (71%), m/e 105 (100%), m/e 82 (55%), m/e 77 (50%), m/e 54 (24%), and m/e 51 (20%).

5-Phenyl-$\underline{d_5}$-5-phenylhydantoin (VI) - To a stirred solution of 25 ml methanol, 5 ml H_2O and 7.3 g of KOH at 0° was added 2.4 g (40 mmol) of urea and 4.24 g (20 mmol) of benzil-$\underline{d_5}$. The orange solution was stirred vigorously until it solidified. The reaction mixture was then heated at reflux for one hour. The clear-orange-to-colorless solution was poured into 60 ml of H_2O and an impurity, diphenyl-$\underline{d_5}$-acetylendiurein (5 mg), removed by filtration. The filtrate was acidified with 50% HCl to yield 4.65 g (90% yield) 5-phenyl-5-phenyl-$\underline{d_5}$-hydantoin. M.p. 285-286°C. M.S. (rel. int.): M^+ = 257 (85%), m/e 228 (45%), m/e 227 (39%), m/e 214 (94%), m/e 186 (39%), m/e 185 (100%), m/e 184 (78%), m/e 109 (50%), and m/e/ 104 (44%).

DISCUSSION

A high yield synthesis of diphenylhydantoin has been reported previously (6,7). This unique synthesis required the benzylic rearrangement of benzil and condensation of a reactive intermediate with urea in methanolic KOH. Therefore a synthesis aimed at the preparation of a mononophenyl-$\underline{d_5}$ labelled drug would require the preparation of benzil-$\underline{d_5}$, V. The Friedel-Crafts reaction of hexadeuterobenzene

with phenylacetylchloride in carbon disulfide was initially attempted in order to generate an intermediate, alpha-phenyl-$\underline{d}_5$-acetophenone. However, mass spectral analysis of the resulting product revealed extensive scrambling of the deuterium atoms between the two phenyl groups under the Lewis acid conditions of the reaction. These results sharply contrasted analogous experiments (2) in which excess hexadeuterobenzene and benzoylchloride were successfully coupled, under Friedel-Crafts conditions, to generate benzophenone-$\underline{d}_5$ with no scrambling of deuterium atoms. These experimental variations can be attributed to the type of acid chlorides used and the choice of solvents. The method first attempted here utilized phenylacetylchloride, containing highly exchangeable benzyl protons. The reaction was also carried out in carbon disulfide which solubilized the catalyst, $AlCl_3$, to a much greater extent than the previous reactions (2) utilizing excess hexadeuterobenzene as the reagent and solvent. Therefore, to avoid reaction conditions which would favor deuterium exchange or waste isotopically labelled starting materials, an improved procedure was developed to generate an isotopically pure, benzoin-$\underline{d}_5$. This compound was easily generated by the Grignard condensation of phenyl-$\underline{d}_5$-magnesium bromide with phenylglyoxal at 0°C. The cold temperature appeared to favor the addition of the Grignard reagent specifically on the more reactive aldehyde portion of the molecule. A quantitative oxidation of the intermediate, benzoin-$\underline{d}_5$, with cupric acetate, easily and mildly generated benzil-$\underline{d}_5$. Condensation of benzil-$\underline{d}_5$ with urea under basic reaction conditions has resulted in a high yield (76% overall) for the synthesis of diphenylhydantoin-$\underline{d}_5$ from hexadeuterobenzene, phenylglyoxal, and urea.

The mass spectral comparisons of nonlabelled and labelled diphenylhydantoin are shown in Figure 1-A and 1-B, respectively. Inspection of the molecular ion regions (M^+ 252 and M^+ 257) reveals that five deuterium atoms have been incorporated into the parent drug. Calculations of isotopic enrichment reveal that the labelled drug, 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin, is 97% enriched with five deuterium atoms. Comparisons of the shifts of major mass spectral fragment ions in the mass spectrum

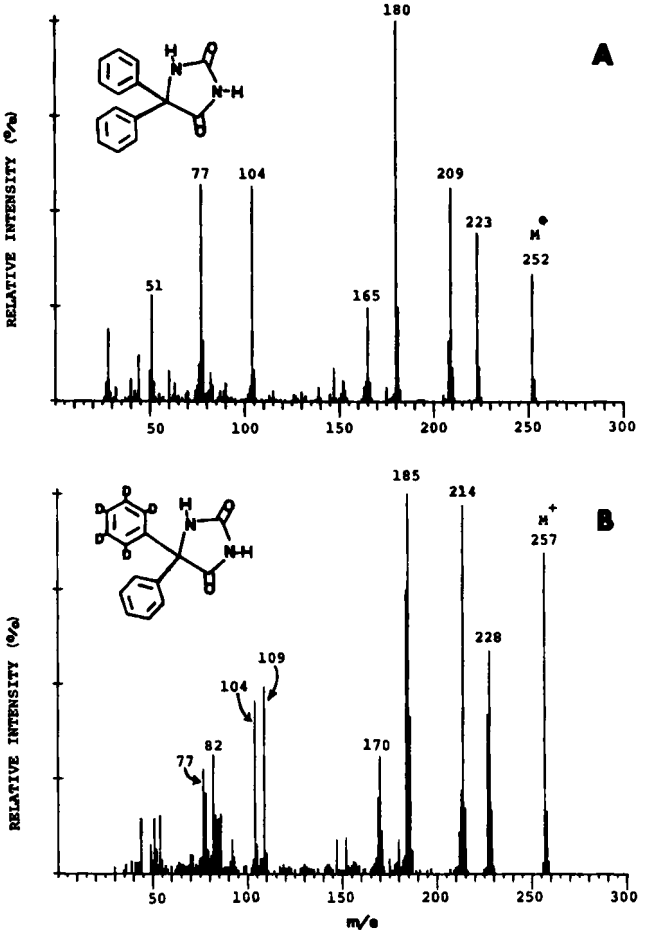
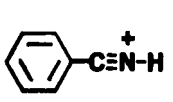


Figure 1-A: Mass spectrum of 5,5-diphenylhydantoin

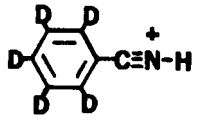
Figure 1-B: Mass spectrum of 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin.

(Mass spectra were recorded on a DuPont 490-F single focusing, magnetic sector instrument by probe distillation directly into the ion source of the mass spectrometer, 70eV).

of the nonlabelled drug (Figure 1-A) at m/e 51, m/e 77, m/e 104, m/e 180, m/e 209 and m/e 223 to the mass spectrum of the labelled drug (Figure 1-B) indicate that only one phenyl group has been labelled with five deuterium atoms. Mass spectral ion doublets (Figure 1-B) seen at m/e 51, and m/e 54, m/e 77 and m/e 82, as well as the pronounced doublet at m/e 104 and m/e 105 clearly reveal that the labelled drug possesses two different phenyl groups contributing to two different sets of fragment ions.



m/e 104



m/e 109

Figure 2-A and 2-B show the infrared spectra of nonlabelled and labelled diphenylhydantoin, respectively. Whereas a mass spectral comparison of nonlabelled and labelled diphenylhydantoin appeared very dissimilar, the infrared spectra of the two compounds appear very similar. However, all infrared absorption bands associated with aromatic C-H stretching and bending appear altered in the spectrum of the labelled drug. In particular, absorption bands at 3070 cm^{-1} and 3040 cm^{-1} (Figure 2-A) are diminished significantly in the spectrum of the labelled drug (Figure 2-B). A new absorption band at 2270 cm^{-1}, although of low intensity, appears only in the spectrum of the labelled diphenylhydantoin-d_5 (Figure 2-B) and is characteristic of C-D stretching vibrations. This new IR band is observed very near the infrared region calculated for such deuterium labelled aromatic groups(8). Also, the absorption bands associated with in-plane and out-of-plane bending of the aromatic protons are significantly diminished in the regions of 1235-1015 cm^{-1} and 785-695 cm^{-1}, respectively, in the spectrum of the labelled drug (Figure 2-B). The amide bands, located near 3250 cm^{-1} and in the 1700 cm^{-1} region, are pronounced in both spectra.

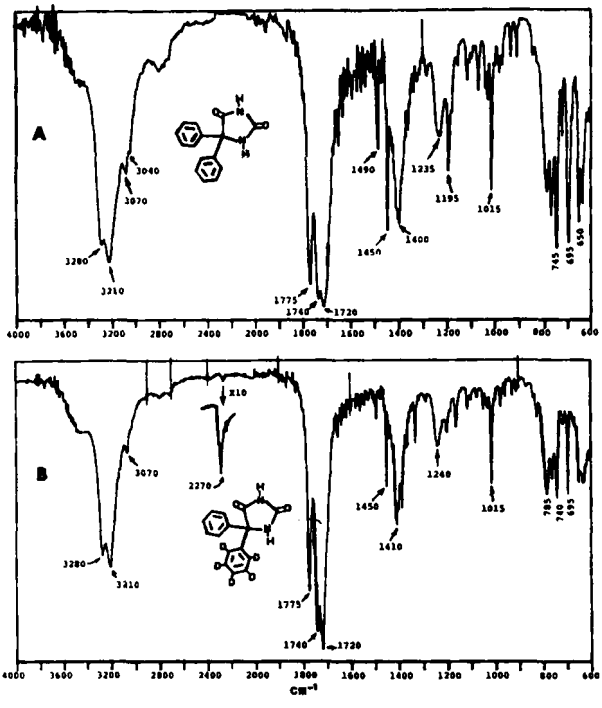


Figure 2-A: Infrared spectra of synthetic, nonlabelled 5,5-diphenylhydantoin.

Figure 2-B: Infrared spectra of 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin. The region from 2400-2300 cm^{-1} has been amplified by a factor of 10.

(Spectra were obtained from KBr pellets using a Beckman 4210 infrared spectrometer with ordinate expansion. Sharp vertical line indicates internal calibration).

CONCLUSION

The synthetic scheme presented here reveals a convenient and high yield method (76%, overall) for the preparation of 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin from an inexpensive and readily available NMR solvent, benzene-$\underline{d}_6$. Previous work (1) has presented a synthesis of diphenyl-$\underline{d}_{10}$-hydantoin in which the isotopic enrichment of the final product was reported to be at least 99.5%. However, inspection of the mass spectral data presented and calculations of isotopic enrichment (M^+ 262/261) revealed that the $\underline{d}_{10}$-labelled drug was only 92% enriched. In contrast, inspection of the mass spectral data presented here, and similar calculations of isotopic enrichment, reveal that the new 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin probe is 97% enriched with phenyl-$\underline{d}_5$.

Because five deuterium atoms have been incorporated into the parent compound, five additional mass units are observed in the mass spectrum of 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin, completely separated from the M^+ and $M^+ + 1$ of the nonlabelled drug. This difference would avoid interferences during quantitation of the parent drug using this isotopically labelled compound as an internal standard, isolated from body tissues and fluids subjected to analytical techniques requiring combined GC-MS-computer methods (9).

If a deuterium labelled diphenylhydantoin is to be administered to patients as previously reported (1) it would seem reasonable to test first if the deuterium labelled phenyl groups of a diphenylhydantoin molecule would reveal a significant isotope effect (10). Metabolic studies have shown that 95% of the parent drug is excreted after a single, *para*-hydroxylation of one aromatic ring (11). If a significant isotope effect were observed, erroneous conclusions could be reached following the oral administration of a deuterium labelled diphenylhydantoin molecule and analysis of isolated parent drug and metabolites. Before deuterium labelled drugs are used for quantitation, a suitable experiment to exclude contribution from an isotope effect should be performed. The molecule presented here, 5-phenyl-5-phenyl-$\underline{d}_5$-hydantoin, is ideally suited for such

a test. The hydroxylation of the parent drug will depend on an enzymatic choice between a non-labelled and deuterium labelled ring system. Analysis of the distribution of fully labelled and partially labelled metabolites of 5-phenyl-5-phenyl-$\underline{d_5}$-hydantoin should reveal the existence of a significant isotope effect during hydroxylation. The results of feeding experiments in rats (3), using 5-phenyl-5-phenyl-$\underline{d_5}$-hydantoin, have revealed no significant biological isotope effect in the para-hydroxylation of the deuterium labelled aromatic ring of the parent drug. Therefore, these results point to the suitability of a deuterium labelled diphenylhydantoin probe for quantitation and metabolic studies of the parent drug in man.

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