THE SYNTHESIS OF DEUTERIUM LABELLED MONO-HYDROXY METABOLITES OF PHENYTOIN

John A Hoskins

MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey, UK

Summary

The synthesis of the 3-hydroxyphenyl and 4-hydroxyphenyl metabolites of 5,5-diphenylhydantoin (Phenytoin) labelled with deuterium in either of the two phenyl rings and 5(4-hydroxy-[$^2H_4$]phenyl)-5-[$^2H_5$]phenylhydantoin is described.

Key words: deuterium-labelling, 5(3-hydroxyphenyl)-5-phenyl-hydantoin, 5-(4-hydroxyphenyl)-5-phenylhydantoin, phenytoin metabolites.

INTRODUCTION

Phenytoin (5,5-diphenylhydantoin) is an important drug used primarily for the treatment of grand-mal epilepsy. It is largely metabolised and in man the major metabolite is 5(4-hydroxyphenyl)-5-phenylhydantoin. The 3-hydroxylated isomer is formed to a minor extent in man but is a major metabolite in the dog.

The compounds described in this paper were prepared for a study on the pharmacokinetics of the drug during chronic administration in pregnancy, using therapeutic doses of 5-[$^2H_5$]phenyl-5-phenylhydantoin. They were required both as

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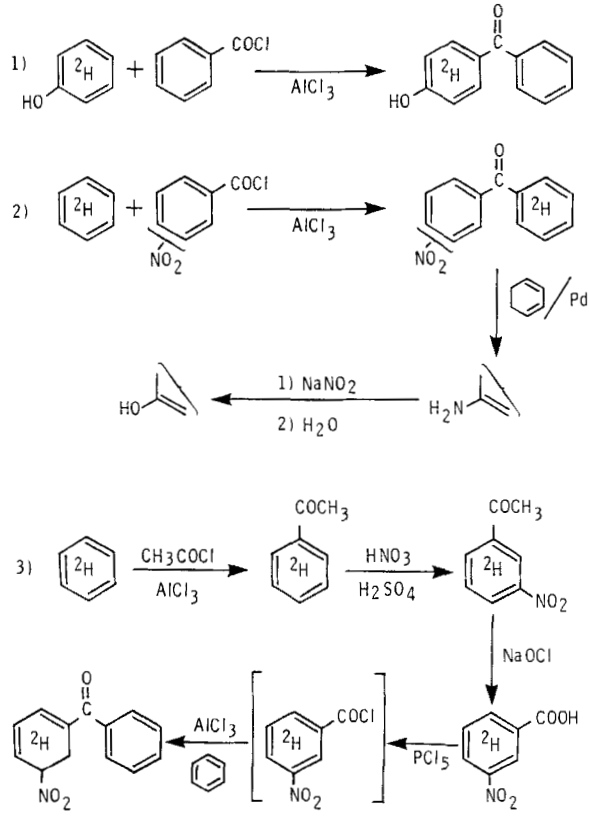
standards and to determine any intermolecular or intramolecular isotope effects in vivo (1). 5(4-Hydroxyphenyl)-5-[$^2H_5$]-phenylhydantoin has been prepared previously (2,3,4) but by a method, acknowledged by one set of authors (2), which allowed appreciable deuterium exchange to occur. The other compounds are believed to be novel.

Scheme

Preparation of the deuterium labelled benzophenones (diphenyl methanones)



DISCUSSION OF MATERIALS AND METHODS

Phenyl ring substituted analogues of phenytoin may be prepared simply in the laboratory by reaction of a substituted benzophenone (diphenylmethanone) with potassium cyanide and an ammonia source in a suitable solvent. This paper is concerned mainly with the production of the benzophenones. Their conversion to a hydantoin is a common reaction for all the precursors. The reaction sequences used to make the hydroxybenzophenones for the hydantoin synthesis are shown in the scheme. The labelled starting materials used were [$^2H_6$]benzene (99.5 atom% D) and [$^2H_6$]phenol (98+ atom% D). Although 100 atom% D benzene is available it is uneconomic to use it for these syntheses because when benzene undergoes a Friedel-Crafts reaction there is inevitably some exchange of hydrogen on the aromatic ring. This exchange was a major problem in the design of the syntheses. It was because of it that the direct preparation of 4-hydroxyphenyl-[$^2H_5$]phenylmethanone from anisoyl chloride and [$^2H_6$]benzene (2,3,4), a concurrent Friedel-Crafts reaction and demethylation which requires strong heating with excess aluminium chloride,was not used. The product of this synthesis shows appreciable loss and scrambling of deuterium between the two aromatic rings. The conditions described here for the various Friedel-Crafts reactions are critical (5). The order and admixture of the chemicals, their relative amounts, and the temperature and time of reaction were chosen to maximise yield and minimise movement of deuterium. Each step in the reaction sequences was monitored by mass spectrometry. Any movement of label was seen by the comparison of ion clusters with those corresponding from unlabelled compounds. The number of

metatheses in the syntheses may seem large but other, shorter routes tried, while chemically sound, resulted in unacceptable loss or exchange of label.

The yields of the various synthetic steps were generally good with the exception of the final hydantoin synthesis. The yield of product from this reaction seems to be very dependent upon the quality of the potassium cyanide used.

EXPERIMENTAL

Several of the intermediate compounds were not prepared in an analytically pure form but the actual purity was always assessed by capillary gas chromatography (g.l.c.) generally using a 25m OV-1 column. Where organic extracts were 'dried by filtration' this means filtration through 'Whatman' phase separating paper. Mass spectrometric results are discussed at the end of this section.

For brevity the symbols HCl, H_2SO_4 and NaOH are used to represent hydrochloric acid, sulphuric acid and sodium hydroxide solution respectively. Literature references to melting points are for the unlabelled compound: unless otherwise stated they are taken from ref. 6.

3-Hydroxyphenyl-[$^2H_5$]phenylmethanone

a) 3-Nitrophenyl-[$^2H_5$]phenylmethanone

To a 100 ml round bottomed 3-necked flask, fitted with a water condenser and drying tube, was added aluminium chloride (8.02 g; 20% mole excess), then nitromethane (10 ml), then m-nitrobenzoylchloride (10.21 g; 10% mole excess) with stirring and the mixture heated to 40° in a oil bath. [$^2H_6$]Benzene (4.10 g) was added in portions and the reaction allowed to proceed at 40° for 24 h. To the cooled mixture were added an excess of crushed ice and 10 M HCl and the organic components

extracted with chloroform. The chloroform solution was extracted with 3 M NaOH (2 x 50 ml; CAUTION - exothermic) to remove nitromethane then with water (50 ml), dried by filtration and the volatile part removed by distillation. The crystalline residue, yield 10.72 g (92%) was purified by recrystallisation (ethanol) to give a material m.p. 92.5°-94° (lit. (7) 94.5°-95.5°).

b) 3-Aminophenyl-[$^2H_5$]phenylmethanone

(By catalytic transfer hydrogenation (8,9)). To 3-nitrophenyl-[$^2H_5$]phenylmethanone (928 mg, 4 mM) in ethanol (16 ml) in a 50 ml conical flask was added palladium black (232 mg) followed by 1,4-cyclohexadiene (4 ml, >40 mM). The flask was flushed with nitrogen, stoppered, and placed in an ultrasonic bath for 2 h. No attempt was made to control bath temperature which rose to approx. 40°. After filtration through a 'filteraid' volatile material was removed by distillation. The residual oil (yield 93%) was dissolved in 2 M HCl (20 ml) and the solution extracted with ether (2 x 10 ml). The aqueous solution was reduced under vacuum to low bulk and cooled to give off-white crystals of the amine hydrochloride, yield 0.65 g (68%), m.p. 185°-187° (lit. 187°).

c) 3-Hydroxyphenyl-[$^2H_5$]phenylmethanone

3-Aminophenyl-[$^2H_5$]phenylmethanone (650 mg) in 5 M H_2SO_4 (25 ml) was cooled to approx. -5° and diazotised by the dropwise addition of ice-cold sodium nitrite solution (0.9 M). This mixture was then added in small portions to boiling water (300 ml). After cooling the organic component was extracted with chloroform (3 x 50 ml) and the chloroform extract itself extracted with 1 M NaOH solution (2 x 50 ml). Acidification and re-extraction with chloroform (3 x 50 ml) gave, after removal of solvent, an oil which crystallised on standing, yield 0.62 g. Sublimation (110°-120°/1 torr) gave a pale yellow product, yield 0.47 g (85%) m.p. 116°-116.5° (lit. 116°).

4-Hydroxyphenyl-[$^2H_5$]phenylmethanone

Prepared as for the 3-hydroxy isomer starting from 4-nitrobenzoylchloride. Differences in method only are noted.

a) 4-Nitrophenyl-[$^2H_5$]phenylmethanone

A more vigorous reaction: reaction time 40° for 5 h, then room temperature for 16 h. Yield of crude material 10.87 g (94%), recryst. (ethanol) to m.p. 135°-136° (lit. 138°).

b) 4-Aminophenyl-[$^2H_5$]phenylmethanone

Crude amine (yield 92%) had m.p. 117°-118° (lit. 122°). The hydrochloride salt dissolves sparingly in water to give a cloudy solution.

c) 4-Hydroxyphenyl-[$^2H_5$]phenylmethanone

Prepared from the crude amino compound. Solution for diazotisation effected only by boiling in a mixture of 5 M H_2SO_4 (25 ml) and glacial acetic acid (10 ml). No separation or crystallisation occurred on subsequent cooling below 0°. Yield of sublimed product was 0.77 g (82%), m.p. 134°-135° (lit. 135°).

(4-Hydroxy-[$^2H_4$]phenyl)phenylmethanone

After the method of Cullinane and Edwards (10): a Friedel-Crafts reaction between [$^2H_6$]phenol (1 g) and benzoyl chloride (freshly distilled, 1.5 g) with aluminium chloride (2.83 g) in nitrobenzene (10.6 ml). All the reagents were mixed in a 50 ml round-bottomed flask, fitted with a water condenser and drying tube, and heated in an oil-bath at 60° for 18 h. After cooling crushed ice and sodium bicarbonate solution were added in excess. The resultant mixture was acidified with a few drops of 10 M HCl and extracted with chloroform (4 x 25 ml). The chloroform solution was dried by filtration and the product extracted with 2 M NaOH (2 x 20 ml). Acidification with 10 M HCl gave the crude ketone as an oil. Extraction (ethyl acetate) and treatment with charcoal gave finally fawn crystals, 1.44 g (71%):

m.p. 133.5°-134.5° (lit. (10) 135°). G.l.c. showed that the material was chemically pure and did not contain the ortho isomer.

(3-Hydroxy-[$^2H_4$]phenyl)phenymethanone

a) Methyl-[$^2H_5$]phenylmethanone

To aluminium chloride (79.3 g, 10% mole excess) in a 500 ml round bottomed, 3-necked flask fitted with a water condenser and drying tube was added 1,2-dichloroethane (50 ml, alumina dried), then [$^2H_6$]benzene (50 g, 0.6 M). After cooling in ice acetyl chloride (51.4 g, 10% mole excess) was added, with stirring, over 0.5 h. The mixture was allowed to warm to room temperature and then heated to 50° for 1 h. Next the cooled mixture was poured into an excess of crushed ice and 10 M HCl (10:1) and organic components extracted into ethyl acetate. After washing with 0.5 M NaOH and drying readily volatile material was removed by distillation under vacuum. On cooling the residue a considerable quantity of crystalline material separated*: this was removed by filtration and the filtrate distilled. A major fraction distilling at 203° (range 199°-205°) was collected (lit. (11) [$^2H_5$]acetophenone 201°-202°).

b) (3-Nitro-[$^2H_4$]phenyl)methylmethanone

After the method of Morgan and Watson (12): acetophenone in concentrated sulphuric acid is nitrated at low temperature by a nitric/sulphuric acid mixture. Low temperatures are very important both when dissolving the acetophenone in acid and for the nitration: a mush of aqueous ethanol/solid CO_2 was used for

* Analysis of the crystalline material by mass spectrometry showed it to be 4,4'-diacetyl-2,2',3,3',5,5',6,6'-[$^2H_8$]bibenzyl formed by alkylation (with dichloroethane) and acetylation (with acetyl chloride) of the [$^2H_6$]benzene. The unlabelled compound has been reported before (5) but this is a novel synthesis. The crude material had m.p. 163°-165°.

cooling. A crude yield of 82% was obtained which after purification gave pale yellow crystals m.p. 79°-80° (lit (12) 79.5°) shown to be a single pure compound by g.l.c.

c) 3-Nitro-[$^2H_4$]benzoic acid

To (3-nitro-[$^2H_4$]phenyl)methylmethanone (6 g) in methanol (60 ml) in a 1000 ml conical flask was added 3 x 100 ml aliquots of sodium hypochlorite solution (10%) containing 2% of added 4 M NaOH with vigorous shaking and ice cooling. The mixture was allowed to stand, with occasional shaking, for 2 h after the addition was complete. Excess hypochlorite was removed by titration with saturated sodium thiosulphate solution (KI/starch indicator). Extraction with chloroform (2 x 20 ml) removed unreacted ketone and then the aqueous solution was acidified to give a crude product in quantitative yield. Purification by solution in alkali, filtering and acidification gave a product with m.p. 141°-142° (lit. (13) 142°).

d) (3-Nitro-[$^2H_4$]phenyl)methanone.

Into a 250 ml 3-necked flask was weighed phosphorus pentachloride (3.13 g) then 3-nitro-[$^2H_4$]benzoic acid (2.5 g). Warming in an oil-bath to 40° gave the crude acid chloride. It was found necessary then to heat the mixture at 85°-90° under water-pump vacuum for 10 min to complete the reaction and possibly remove some phosphorus oxychloride. Aluminium chloride (2.20 g, 10% mole excess) was dissolved in a mixture of benzene (2.33 g, 100% mole excess) and nitromethane (5 ml) and the mixture added to the crude acid chloride. The reaction was carried out at 40° for 24 h and worked up as for the $^2H_5$ compound. A yield of 3.03 g (90%) from the acid was obtained, m.p. 94.5°-95° (lit. (7) 94.5°-95.5°) containing only traces of impurities by g.l.c.

e) (3-Amino-[$^2H_4$]phenyl)phenylmethanone.

Prepared as for the $^2H_5$ compound, yield 84%, m.p. (HCl) 185°-187° (lit. 187°).

f) (3-Hydroxy-[$^2H_4$]phenyl)phenylmethanone.

Prepared as for the $^2H_5$ compound, recovered yield 90%, m.p. 116.5°-117° (lit. 116°).

(4-Hydroxy-[$^2H_4$]phenyl)-[$^2H_5$]phenylmethanone

[$^2H_5$]benzoic acid (1.4 g) was prepared by a haloform reaction from [$^2H_5$]acetophenone, (as for 3-nitro-[$^2H_4$]benzoic acid above) and converted to [$^2H_5$]benzoyl chloride by reaction with an equimolar amount of phosphorus pentachloride (2.3 g). After the initial reaction was complete the bath temperature was raised to 160° and some phosphorus oxychloride was removed by distillation. To the residue, cooled in ice, was added [$^2H_6$]phenol (1 g) in nitrobenzene (10.6 ml). Crushed aluminium chloride (2.83 g) was added and the reaction allowed to proceed at 60° for 18 h. The mixture worked up as before and the crude material purified by sublimation, final yield (based on phenol) 0.46 g (23%) m.p. 136°-136.5° (a sublimed commercial sample of p-hydroxybenzophenone had m.p. 135°-135.5°).

5(4-Hydroxy-[$^2H_4$]phenyl)-5-phenylhydantoin and
5(4-hydroxyphenyl)-5-[$^2H_5$]phenylhydantoin

(After Starchansky and Kostenbauder (14)). In a Parr bomb (25 ml) was placed a solution of potassium cyanide (65 mg) in a mixture of dimethyl sulphoxide (5 ml) and 0.01 M NaOH (5 ml), then a solution of labelled 4-hydroxybenzophenone (32 mg) in dimethyl sulphoxide (5 ml). Finally crushed 'ammonium carbonate' (4.5 g) was added, the bomb sealed and placed in an oven at 140° for 1.5 h. After cooling, the contents of the bomb were added to 1 M NaOH (15 ml) and the mixture extracted with ether (20 ml) to

remove unreacted benzophenone. The aqueous part was acidified with 10 M HCl and on cooling and swirling a white ppt. of the hydantoin formed.

Yield of [$^2H_5$] 20%, [$^2H_4$] 39%.

5(3-Hydroxy-[$^2H_4$]phenyl)-5-phenylhydantoin and
5(3-Hydroxyphenyl)-5-[$^2H_5$]phenylhydantoin

Prepared as the para isomers. The meta compounds are considerably more soluble in water than the para and finally are extracted into chloroform and purified by preparative t.l.c. (Silica Gel G 1000 μm: chloroform, methanol, acetic acid (90:10:1 v/v)) and/or recrystallisation from aqueous acetone.

Yield of [$^2H_5$] 42%, [$^2H_4$] 30%.

5(4-Hydroxy-[$^2H_4$]phenyl)-5-[$^2H_5$]phenylhydantoin

Prepared as the other para isomers. Yield 23%.

Mass Spectrometry

Mass spectra of the compounds were taken by probe introduction into either a 70-70 VG Micromass or a MS30 double focussing mass spectrometer operated in the electron impact mode with an ionisation potential of 70 eV and a trap current of 200 μA, or 100 μA respectively.

The mass spectra showed no consistent evidence for enhancement of the expected deuterium content of the compounds by measurement of the $m^{+\cdot}/(m+1)^{+\cdot}$ ratios (cf. 2). Comparison of $m^{+\cdot}/(m-1)^{+\cdot}$ and $m^{+\cdot}/(m-2)^{+\cdot}$ ratios for both the labelled and unlabelled compounds showed that on average the labelled compounds contained ~15% of species with a one less deuterium atom than expected and probably a trace of a species with two deuterium atoms less. The expected deficit from the known isotopic composition of the [$^2H_6$]benzene and [$^2H_6$]phenol is 3.3%

of a species with one deuterium atom less for the compounds derived from benzene and a maximum of 14% for those derived from phenol. The $^2H_9$ compounds prepared from both labelled substrates are calculated to contain the species with $^2H_8$ 17.5% and $^2H_7$. 0.5%. The $^2H_9$ compounds prepared contained approx. double these amounts.

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