SYNTHESIS OF DEUTERIUM LABELLED IBUPROFEN

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SUMMARY

The preparations of [ar-$^2H_4$]-ibuprofen and [ar, 3,3,3-$^2H_7$]-ibuprofen are described. The deuterium was incorporated into the aromatic ring of [ar-$^2H_4$]-ibuprofen which is a metabolically stable position. [ar,3,3,3-$^2H_7$]-ibuprofen was synthesized by the same route using [$^2H_3$]-CH_3I instead of CH_3I for use as a GC/MS internal standard in stable isotope labelled bioavailability studies.

Key Words: [$^2H_4$]-ibuprofen, [$^2H_7$]-ibuprofen, deuterium labelling, non-steroidal anti-inflammatory

INTRODUCTION

Ibuprofen (1) is a widely prescribed non-steroidal anti-inflammatory agent with analgesic and antipyretic properties. It is rapidly metabolized in man and animals primarily on the isobutyl side chain yielding two major metabolites, 2-[4-(2-carboxylpropyl)phenyl] propionic acid and 2-[4-(2-hydroxy-2-methylpropyl phenyl] propionic acid in the urine [1]. More recently, it has been shown that ibuprofen (1) undergoes metabolic chiral inversion about the 2-position of the propionic acid group from the R(-) enantiomer to the S(+) antipode [2]. The reverse process was not observed. This chiral inversion pathway is common to many 2-arylpropionic acids and is the subject of an extensive review [3]. Deuterium labelled ibuprofen was needed for comparative bioavailability studies which involve the coadministration of equal doses of the labelled drug in solution and an unlabelled dosage form. It is necessary in studies of this type that the labelled drug not exhibit any *in vivo* kinetic

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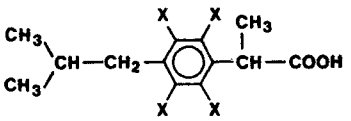
isotope effects [4,5]. Previously, loss of deuterium from [2,3,3,3-$^2H_4$]-ibuprofen during in vivo chiral inversion had been observed [2]. On the basis of this work and the metabolic pathways discussed above, the synthesis of [ar-$^2H_4$]-ibuprofen (2) was undertaken. For use in the analysis of the samples generated by the stable isotope bioavailability studies, [ar,3,3,3-$^2H_7$]-ibuprofen (3) was also prepared as an internal standard for the gas chromatographic/mass spectrometry methodology.



1 X = H
2 X = D

EXPERIMENTAL

Perdeuterated benzene, 99.5 atom %, and [$^2H_3$]-iodomethane, 99+ atom %, were purchased from the Aldrich Chemical Co., Milwaukee, Wisconsin. The labelled benzene was vacuum transferred from calcium hydride before use while the [$^2H_3$]-iodomethane was used without further purification. The remainder of the chemicals and solvents were obtained from standard commercial sources and were used without further purification. Essentially all procedures were carried out under a nitrogen atmosphere with magnetic stirring unless noted otherwise.

The identities of the compounds were established by ^1H NMR, MS and elemental analysis. The spectra were compared in each case with those of authentic compounds. The purity of [$^2H_4$]-ibuprofen was obtained using the GLC procedure in the United States Pharmacopeia, 21st revision. Proton NMR spectra were recorded on a Varian XL200 fourier-transform, quadrature detection spectrometer. Mass spectra were determined on a Finnigan 4600 quadrupole mass spectrometer using 70 eV electron ionization. Microanalyses were performed by Micro-Analysis, Inc., Wilmington, Delaware and deuterium values were calculated from H+D assuming complete deuteration.

[ar-$^2H_5$]-Isobutyrophenone (5)

Anhydrous aluminum trichloride (84.0 g, 0.63 moles) was added to perdeuterated benzene (4) (125 g, 1.50 moles) in a 500 ml reaction flask inside a nitrogen flushed glovebag. The flask was removed from the glovebag and equipped with a mechanical stirrer, an addition funnel containing isobutyryl chloride (63.9 g, 0.60 moles) and a nitrogen inlet system. With vigorous stirring, approximately 2 ml of isobutyryl chloride was admitted and the flask was warmed gently until DCl was evolved. Heating was removed and the remainder of the isobutyryl chloride was added over a period of 2 hours. The reaction mixture was refluxed for 30 minutes and then allowed to cool before addition to approximately 300 g of vigorously stirred ice water. The organic fraction was allowed to separate, followed by washing with 10% sodium carbonate solution, water and then dried over anhydrous magnesium sulfate. The organic layer was distilled effecting removal of excess benzene and isolation of 77.2g (84% yield) of 5.

[ar-$^2H_5$]-Isobutylbenzene (6)

A solution of 5 (91.9 g, 0.60 moles) in 750 ml of diethylene glycol was combined with 90 ml of hydrazine hydrate (85%) and 81 g of potassium hydroxide in a 1000 ml flask equipped with a reflux condenser. The reaction mixture was warmed with stirring until all of the potassium hydroxide had dissolved and then the solution was refluxed for 1 hour. At this point, the apparatus was rearranged for distillation and distillate was collected until the temperature of the reaction mixture rose to 175°C. Distillation was discontinued at this point and the upper organic layer of the distillate was returned to the reaction flask. The reflux condenser was replaced and the contents were refluxed for an additional 3.5 hours. After cooling to room temperature, the aqueous portion of the distillate was recombined with the reaction mixture and extracted with 4 x 40 ml of ether. The combined ethereal extracts were dried over

anhydrous magnesium sulfate and reduced in volume at room temperature on a rotoevaporator. The residue was distilled to yield 53.8 g (0.39 moles, 65%) of 6; ^1H NMR ($CDCl_3$) δ 0.90 (6H, d, J=7, CH_3), 1.86 (1H, m, CH), 2.60 (2H, d, J=6, CH_2), ~7.3 (residual H, aromatic); mass spectrum, m/z (relative intensity) 140 (2.8), 139 (28), 138 (1.4), 137 (0.6), 98 (4.6), 97 (66), 96 (100), 95 (13), 94 (1.8), 93 (1.4), 83 (2.4).

[ar-$^2H_4$]-4-Isobutylacetophenone (7)

Acetyl chloride (10.6 ml, 0.149 moles) was added over a period of 15 minutes with mechanical stirring to a slurry of anhydrous aluminum trichloride (21.7 g, 0.163 moles) in 20 ml of methylene chloride at 0°C under nitrogen. Maintaining the temperature below 5°C, 6 (19.7 g, 0.142 moles) was added dropwise over a period of 30 minutes. The reaction mixture was stirred an additional 30 minutes before warming to 10°C and addition to 25 ml of concentrated hydrochloric acid in 40 ml of ice water. The mixture was warmed to room temperature and the layers were separated. The aqueous layer was washed with 2 x 30 ml of methylene chloride. The combined organic layers were washed with aqueous potassium bicarbonate (7 g in 50 ml of water) and were dried over anhydrous magnesium sulfate. The product solution was filtered and evaporated to yield 25.5 g of crude 7. The product was used without further purification.

[ar-$^2H_4$]-ibuprofen (2)

A mixture of sulfur (7.01 g, 0.219 moles), morpholine (19.1 ml, 0.220 moles) and crude 7 (24.9 g, 0.138 moles) were refluxed in a fume hood under nitrogen for 18 hours. The reaction mixture was allowed to cool to room temperature. Following the addition of 110 ml of glacial acetic acid and 180 ml of concentrated hydrochloric acid, the mixture was refluxed for an additional 7 hours. The volume of the reaction mixture was reduced in vacuo using a rotoevaporator by approximately 250 ml and the concentrate was diluted with 100 ml of water followed by the addition of 150 ml of ether. The ether layer was separated and then extracted with aqueous sodium carbonate (10 g in 150

ml). The aqueous extract was acidified to pH 1-2 with hydrochloric acid and the organic material was extracted with 200 ml of ether. The ether solution was filtered and evaporated to dryness yielding 24.7 g of crude 8.

The residue from above (8) was refluxed with 65 ml of ethanol and 1.9 ml of concentrated sulfuric acid for 5 hours. Approximately 55 ml of ethanol was then distilled off and 100 ml of water was added to the residue. The oil which separated was extracted with 150 ml of ether. The ether extract was washed with aqueous sodium carbonate (5 g in 75 ml), water (50 ml) and then dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo to yield 21.7 g of crude 9.

A solution of sodium ethoxide was prepared under nitrogen by adding 50 ml of absolute ethanol in one portion to 2.83 g (0.123 moles) of metallic sodium cooled by an ice bath. After the initial reaction subsided, the ice bath was removed and the sodium was allowed to completely dissolve. The resultant solution was added dropwise with stirring over a period of 20 minutes to a solution of crude 9 (21.7 g, 0.0967 moles) in diethyl carbonate (80 ml, 0.660 moles) at 100°C under nitrogen. After the addition was complete, the apparatus was rearranged for distillation and the ethanol and the majority of the diethyl carbonate were distilled from the reaction flask until the head temperature reached 90°C. The distillation flask was cooled to 0°C and 40 ml of water with 10 ml of glacial acetic acid were added with stirring. The reaction mixture was then extracted with 100 ml of ether. The ether phase was washed with aqueous sodium carbonate (5 g in 50 ml), water (50 ml) and was then dried over anhydrous magnesium sulfate. Filtration and removal of the solvent in vacuo yielded 27.2 g of crude 10.

A solution of crude 10 (21.6 g, 0.073 moles) in 50 ml of absolute ethanol

was added dropwise under nitrogen to a solution of sodium ethoxide (prepared as described above from 1.71 g of metallic sodium and 60 ml of absolute ethanol). After the addition was complete, methyl iodide (9.1 ml, 0.146 moles) was added slowly and the reaction mixture was refluxed for 3 hours. The apparatus was rearranged for distillation and the excess methyl iodide and ethanol were removed. The residue was diluted with 50 ml of water and extracted with 150 ml of ether. The organic fraction was washed with aqueous sodium bisulfite (2 g/50 ml) followed by 50 ml of water and the solvent was removed in vacuo. The residue was hydrolyzed at reflux in 90 ml of 3.3 N sodium hydroxide. After 1 hour, the reaction mixture was cooled to room temperature and 50 ml of water was added. The solid was converted to the free acid by acidification to pH 1-2 with concentrated hydrochloric acid. The reaction mixture was extracted with 200 ml of ether and the organic layer was washed with 50 ml of water. After drying over anhydrous magnesium sulfate, the solvent was removed in vacuo and the residue was dried under high vacuum (10^{-2} torr).

The resultant oil was added to a 250 ml r.b. flask and was heated in a silicone oil bath for 30 minutes at 210°C until carbon dioxide evolution had ceased. Heating was discontinued and 30 ml of heptane was added after the crude 2 had reached room temperature. The heptane was heated to 60°C and the solution was allowed to cool slowly to -20°C overnight. The crystalline material was isolated on a medium frit and was washed with 10 ml of -20°C heptane followed by air drying. A second recrystallization (2 ml/g) from heptane followed by 3 x 10 ml washings with -20°C heptane afforded 7.83g of pure [ar-$^2H_4$]-ibuprofen (2) after drying in vacuo to constant weight. Overall yield (based on 6) was 33%. ^1H NMR [$(CD_3)_2SO$] δ 0.85 (6H, d, J=7, CH_3), 1.33 (3H, d, J=7, CH_3), 1.80 (1H, m, CH), 2.41 (2H, d, J=7, CH_2), 3.60 (1H, q, J=7, CH), 7.09 (residual H, s, aromatic), 7.18 (residual H, s, aromatic), 12.22 (1H, s, COOH); mass spectrum, m/z (relative intensity) 211 (5.8), 210 (40), 209 (4.6), 168 (22), 167 (90), 166 (21), 165 (100), 123 (50), 122 (24), 121

(25), 111 (42), 95 (31), 94 (31). Anal. Calcd for $C_{13}H_{14}D_4O_2$: C, 74.26; H, 6.71; D, 3.81. Found: C, 74.69; H, 6.62; D, 3.75. Purity (GLC): 99%.

[ar,3,3,3-$^2H_7$]-ibuprofen (3)

A solution of crude 10 (5.11 g, 17.3 mmoles), sodium ethoxide (17.5 mmole) and [$^2H_3$]-methyl iodide (5.00 g, 34.5 mmoles) in 25 ml of absolute ethanol were reacted as described above for [ar-$^2H_4$]-ibuprofen. The hydrolysis, decarbonylation and isolation steps were also carried out in a manner analogous to that described for 2. Recrystallization from heptane yielded 1.98 g (34% yield based on 6) of [ar, 3,3,3-$^2H_7$]-ibuprofen. ^1H NMR ($CDCl_3$) δ 0.89 (6H, d, J=7, CH_3), 1.20 (residual H, CH_3), 1.84 (1H,m,CH), 2.45 (2H, d, J=8, CH_2), 3.70 (1H, s, CH), 7.16 (residual H, s, aromatic), 7.22 (residual H,s, aromatic); mass spectrum, m/z (relative intensity) 214 (9.5), 213 (72), 212 (12.8), 171 (31), 170 (91), 169 (38), 168 (100), 167 (21), 126 (63), 125 (42), 124 (30), 123 (38), 111 (55), 96 (23), 95 (45), 94 (35). Anal. Calcd for $C_{13}H_{11}D_7O_2$: C, 73.22; H, 5.20; D, 6.57. Found: C, 73.69; H, 5.23; D, 6.60.

RESULTS AND DISCUSSION

To accomplish the incorporation of deuterium in the aromatic ring of ibuprofen (1), perdeuterated benzene was chosen as the starting point. The main impetus for this decision was based on economic considerations since the bioavailability studies planned could require the administration of gram quantities of labelled drug to each subject. The synthetic approach taken consisted of two stages. [ar-$^2H_5$]-isobutylbenzene (6) was the first target molecule. Isobutylbenzene has previously been prepared by the reduction of isobutyrophenone or by the isopropylation of benzyl sodium [6]. Many preparative routes for ibuprofen starting from isobutylbenzene have been published or patented and the literature up to 1982 has been reviewed [6]. The route utilized for the synthesis of ibuprofen (2) is given in Figure 1.

The malonic ester synthesis route (steps D through H) originally used by

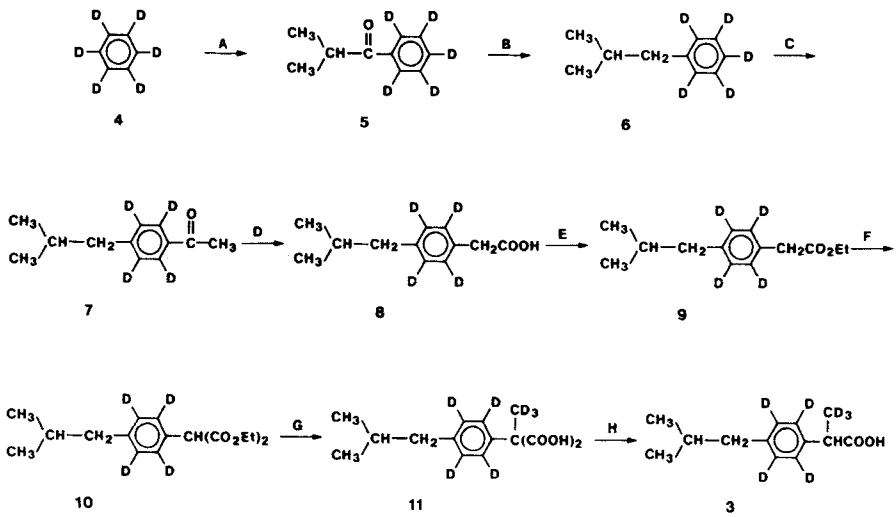


Figure 1.

Nicholson and Adams [7] was chosen because it allowed the incorporation of additional label making the preparation of the GC/MS internal standard, [ar,3,3,3-$^2H_7$]-ibuprofen (3) a convenient and simple proposition.

[ar-$^2H_5$]-isobutyrophenone (5) was prepared by Friedel-Crafts acylation of perdeuterated benzene (4). Reduction of 5 via the Huang-Minlon modification of the Wolff-Kishner reaction yielded [ar-$^2H_5$]-isobutylbenzene (6). The

A: $AlCl_3$, isobutyryl chloride, reflux

B: Diethylene glycol, KOH, $H_2NNH_2 \cdot H_2O$, 175°C

C: $AlCl_3$, acetyl chloride, CH_2Cl_2, 5°C

D: Sulfur, morpholine, Δ; HOAC, HCl, Δ

E: EtOH, conc. H_2SO_4, Δ

F: NaOEt, EtOH, diethyl carbonate, 100°C; HOAC

G: NaOEt, EtOH, CD_3I, Δ; dil. NaOH, Δ, conc. HCl

H: 210°C

reaction was carried out under basic conditions since the acidic conditions of the Clemmensen reduction could be expected to labilize the aromatic deuteriums [8]. GC/MS analysis of 6 indicated retention of essentially all of the label (98.6 atom %). Friedel-Crafts acylation of 6 gave the acetophenone (7) which was converted to the phenylacetic acid (8) via the Kindler modification of the Willgerodt reaction and hydrolysis of the resulting thiomorpholide.

Attempts to directly methylate the protio analogue of 8 in the α-position using lithium diisopropylamide and methyl iodide [9] proceeded in low yield necessitating a lengthy purification. Ultimately, the phenylacetic acid (8) was instead esterified to 9 which on heating with diethyl carbonate and sodium ethoxide followed by acidification produced the malonic ester (10). Deprotonation of 10 with sodium ethoxide, alkylation with either $[^2H_3]$-CH_3I or CH_3I followed by hydrolysis afforded 11 or its $[^2H_4]$-analogue. Decarboxylation of the respective malonic acids at 210°C produced either $[^2H_4]$-ibuprofen (2) or the MS internal standard, $[^2H_7]$-ibuprofen (3) in about 20% overall yield after two recrystallizations.

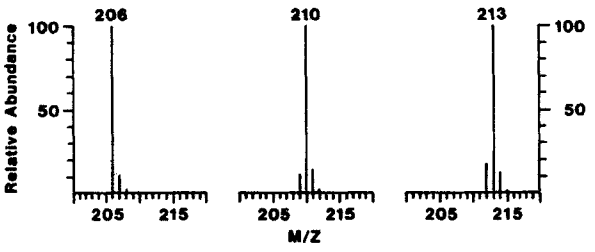


Figure 2.

Mass spectral analysis of purified 2 and 3 indicated 97 and 98 atom % deuterium incorporation, respectively. The chemical purity of 2 obtained by GLC was 99%. The proton NMR spectrum of 2 confirmed the location of label in the aromatic ring and exhibited a doublet centered at 1.33 ppm that was nearly absent in the spectrum of 3 confirming deuteration of the 3-methyl group in the internal standard (3). The parent ion regions of the mass spectra of unlabelled, [$^2H_4$] and [$^2H_7$]-ibuprofen are given in Figure 2.

There is no overlap of the spectra observed at m/z 206, 210 and 213 used to monitor the concentrations of 1, 2 and 3 in serum. The lack of overlap has allowed the construction of linear calibration curves over the range of 0.1-100 μg/ml with zero intercepts for the determination of 2 and 3 in canine and human serum [10]. Studies to demonstrate the *in vivo* bioequivalence of ibuprofen and [$^2H_4$]-ibuprofen in dogs and humans are in progress.

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REFERENCES

1. Mills R.F.N., Adams S.S., Cliffe E.E., Dickinson W. and Nicholson J.S. - Xenobiotica 3: 589 (1973)

2. Wechter W.J., Loughead D.G., Reischer R.J., VanGiessen G.J. and Kaiser D.G. - Biochem. Biophys. Res. Commun. 61: 833 (1974)

3. Hutt A.J. and Caldwell J. - J. Pharm. Pharmacol. 35: 693 (1983)

4. Baillie T.A. - Pharmacol. Rev. 33: 81 (1981)

5. Wolen R.L. and Gruber C.M. Jr. - Drug Absorption and Disposition: Statistical Considerations, Albert K.S. ed., American Pharmaceutical Association, Washington, D.C., 1980 p. 69

6. Nicholson J.S. - Chronicles of Drug Discovery 1: 149 (1982)

7. Nicholson J.S. and Adams S.S. - U.S. Patent No. 3,228,831 (11 Jan 1966)

8. Thomas A.F. - Deuterium Labeling In Organic Chemistry, Appleton-Century-Crofts, New York (1971)

9. Adam W. and Fick H. - J. Org. Chem. 43: 772 (1978)

10. Halstead G.W., Halm K.A. and Theis D.L. - unpublished results