SYNTHESIS OF DEUTERIUM-LABELLED METHYLPHENIDATE, p-HYDROXYMETHYLPHENIDATE, RITALINIC ACID AND p-HYDROXYRITALINIC ACID

Kennerly Patrick, Clinton Kilts and George Breese
Biological Sciences Research Center (220-H)
School of Medicine, University of North Carolina
Chapel Hill, NC 27514

SUMMARY

The synthesis of threo-dl-methylphenidate (Ritalin®, 1), threo-dl-p-hydroxymethylphenidate (3), threo-dl-ritalinic acid (2), and threo-dl-p-hydroxyritalinic acid (4) with deuterium incorporated in the piperidine ring is described. These compounds were synthesized for use as internal standards for mass fragmentographic assays of methylphenidate and its metabolites. The synthetic scheme described resulted in less than 0.05% $^2H_0$ in the piperidine ring in any of the preparations.

Key words: methylphenidate; metabolites; deuterium; gas chromatography-mass spectrometry.

INTRODUCTION

The central stimulant threo-dl-methylphenidate (methyl threo-dl-2-phenyl-2-(2'-piperidyl)acetate·HCl, Ritalin®, 1) is the drug of choice in the treatment of the hyperkinetic syndrome in children. After oral administration, the drug is rapidly deesterified to ritalinic acid (2), yielding low serum concentrations (1-20 ng/ml) of the parent drug following therapeutic doses (1). Methylphenidate is extensively para-hydroxylated in rats to form threo-p-hydroxymethylphenidate (3) which was reported to be the primary metabolite in rat brain (2). Furthermore, 3 is approximately twice as active as methylphenidate in an animal model of central stimulant activity (3). Deesterified 3 (p-hydroxyritalinic acid, 4) is a urinary metabolite of 1 in man (2).

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Gas chromatographic separation with flame ionization (4), nitrogen/phosphorous specific (5), electron capture (6) or mass fragmentographic (7,8) detection have been used in quantitative assays of methylphenidate and ritalinic acid in biological fluids. High performance liquid chromatographic separation and ultraviolet absorbance detection have also been reported for assays of methylphenidate (9) and ritalinic acid (10). To quantify the relatively low serum concentrations of 1 and to determine to what extent the active metabolite 4 is found in clinical biological fluids, only quantitative mass fragmentographic analysis offers the degree of sensitivity necessary.

The published assays for methylphenidate based on gas chromatography-mass spectrometry (GCMS) have utilized ethylphenidate (ritalinic acid ethyl ester) as the internal standard. Single ion monitoring (SIM) of the perfluoroacylated piperidyl fragment (base peak) of both 1 and ethylphenidate permitted quantification of 1 by signal ratio. These assays suffer from the necessity of completely resolving chromatographically the two ester homologues, methyl- and ethylphenidate. In our hands, significant peak overlap occurred as a function of the condition of the column and relative concentrations.

In the present manuscript the synthesis of compounds 1, 2, 3 and 4, deuterium-labeled in the piperidine ring is described. These compounds are currently being used as internal standards for a highly sensitive and specific GCMS analysis of methylphenidate and its major metabolites in clinical samples.

DISCUSSION

Methylphenidate undergoes extensive biotransformation following its administration to humans (1) and rats (2), yielding at least three metabolites of differential pharmacological activity (3). Electron impact mass spectra of these compounds are characterized by a base peak corresponding to the piperidine ring-containing fragment with no other fragments found in appreciable relative abundance. Therefore, the use of stable isotopically-labelled variants of methylphenidate and its metabolites as internal standards in mass fragmentographic assays requires that the isotope enrichment involve the piperidine ring. Furthermore, a minimum of two deuterium atoms must be incorporated to avoid interference from the natural abundance of ^{13}C as well as problems in attempting to resolve compounds

differing in only one amu inherent to low resolution mass spectrometers. The synthetic scheme described resulted in the formation of methylphenidate and its major metabolites containing a polydeuterated piperidine ring.

The synthesis (Figure 1) was based on a modification of the procedure of Panizzon (11):

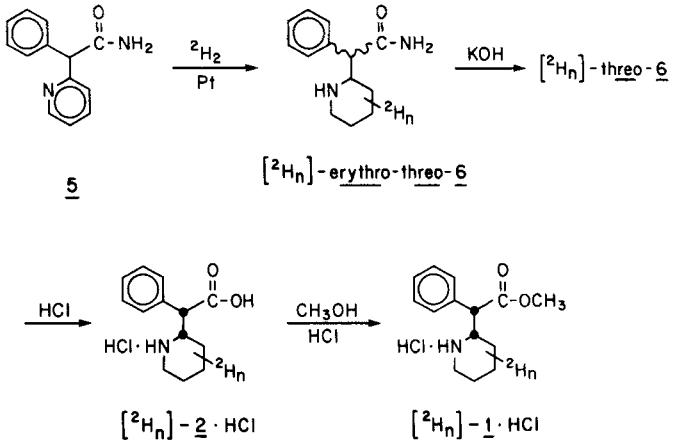

Figure 1. Scheme of the synthesis.

The pyridyl amide 5 was obtained by the partial hydrolysis of the corresponding nitrile. Complete hydrolysis to the phenylpyridylacetic acid was avoided since such systems can spontaneously decarboxylate (12). Deuterogenation of 5 yielded a 70:30 erythro-threo mixture (GLC) of the piperidine compound $[^2H_n]$-6. All possible combinations of deuterium on the piperidine ring were found to occur in the product mixture due to the concomitant exchange of hydrogen atoms during the catalytic reduction step (13). The pentadeuterated product was of greatest abundance while the $[^2H_0]$ species was almost undetectable by mass spectrometry.

Column chromatography on silica succeeded in providing fractions of pure racemic $[^2H_n]$-erythro-6. However, the later eluting threo fractions all contained appreciable amounts of the erythro racemate. Therefore, a KOH epimerization (14) of the 70:30 diastereomeric mixture of 6 was conducted. After α-proton abstraction, the isomerization appears to be driven by an unfavorable α-anionic-nitrogen lone pair interaction, avoided by pyramidal inversion at the α-position. Reprotonation yields the threo stereochemistry. This process provided a product enriched (>90%) in the threo racemate, circumventing the necessity of chromatographic separation. The residual erythro stereoisomers were removed in the subsequent re-

crystallization involved in obtaining deuterated compounds 1·HCl and 2·HCl from the epimerized [^2H_n]-6.

EXPERIMENTAL

Melting points were determined with a Thomas Hoover melting point apparatus using open capillaries and are uncorrected. Electron impact (70eV) mass spectra were obtained using a Finnigan Model 3300 mass spectrometer with sample introduction via a solid probe. A Varian series 2400 gas chromatograph (6 ft x 2 mm id, 1.5% OV-101 on 100-200 Chrom GHP, 180°C, carrier gas: He 30 mL/min; air 150 mL/min; H_2 4.5 mL/min) with thermionic (nitrogen/phosphorus) specific detection was used for GLC determinations of relative percentages of the erythro and the later eluting threo racemates.

No appreciable deuterium-protium exchange occurred during the reactions subsequent to the reduction step. Therefore, the degree of deuterium incorporation is indicated only for deuterated 1·HCl.

erythro-dl-threo-dl-2-Phenyl-2-(2'-piperidyl-2',3',4',5',6'-^2H_n)acetamide Acetate (erythro-threo-6·Acetate). Amide 5 (1.0 g, 4.7 mMol) was dissolved in 5 mL of perdeuteroacetic acid (Aldrich), then 0.02 g of PtO_2 were added. A deuterium inflated 2 mL dropper bulb (Fisher Scientific) was attached to the flask via a thermometer adapter. The reaction was magnetically stirred at ambient temperature until the deuterium uptake ceased (ca. 48 h). The reaction mixture was treated with activated carbon and evaporated to dryness under reduced pressure to obtain [^2H_n]-erythro-threo-6·Acetate (75:25) from EtOAc-hexane (1.19 g, 87%): mp 158-160°C (lit. [15] protium form 158°C).

Threo Enrichment of erythro-dl-threo-dl-2-Phenyl-2-(2'-piperidyl-2',3',4',5',6'-^2H_n)acetamide ([^2H_n]-threo-6). The isomeric mixture [^2H_n]-erythro-threo-6·acetate (0.25 g, 0.88 mMol) was stirred in 50% KOH at 110° for 15 h or until an aliquot contained 10% or less of the erythro isomer (GLC). The mixture was cooled, filtered, and the filtrate washed with portions of cold water. Crystallization from EtOAc provided white granules of [^2H_n]-threo-6 (>90% threo, 0.1 g, 51%): mp 174-175°C (lit. [11] protium form of undesignated erythro:threo composition 173°C); MS m/e (rel. abundance) 147(1.5), 118(12), 89(100), 59(25).

threo-dl-2-Phenyl-2-(2'-piperidyl-2',3',4',5',6'-^2H_n)acetic Acid Hydrochloride ([^2H_n]-threo-dl-ritalinic Acid·HCl, [^2H_n]-2·HCl). Amide threo-6 (0.2 g, 0.88 mMol)was refluxed in 5 mL 4N HCl for 5 h, then evaporated to dryness under reduced pressure. The residue was recrystallized from MeOH/Et_2O to yield deuterated 2·HCl (97% threo, 0.17 g, 74%): mp 228-231°C (lit. [16] protium form, pure threo 234-236°C); MS m/e (rel. abundance) 147(5), 119(7), 89(100), 58(34).

Methyl threo-dl-2-Phenyl-2-(2'-piperidyl-2',3',4',5',6'-^2H_n)acetate Hydrochloride threo-dl-methylphenidate, [^2H_n]-1·HCl). Compound [^2H_n]-2·HCl (0.1 g, 0.38 mmol) was refluxed in 5 mL of methanolic HCl for 4 h, then evaporated to dryness under reduced pressure to yield fine needles of [^2H_n]-1·HCl from MeOH-Et_2O (pure threo, 0.09 g, 89%): mp. 207-209°C (lit. [11] protium form of undesignated erythro:threo stereochemistry 204-208°C); MS m/e (rel. abundance) 119(5), 89(100), 59(24). Deuterium enrichment: ($^2H_0$) = 0.05%, ($^2H_1$) = 0.4%, ($^2H_2$) = 2.3%; ($^2H_3$) = 7.0%, ($^2H_4$) = 15.1%, ($^2H_5$) = 22.8%, ($^2H_6$) = 21.4%, ($^2H_7$0 = 17.9%, ($^2H_8$) = 9.0%, ($^2H_9$) = 3.2%.

[^2H_n]-threo-p-Hydroxymethylphenidate Hydrochloride (3·HCl) and [^2H_n]-threo-p-Hydroxyritalinic Acid Hydrobromide ([^2H_n]-4·HBr). Compounds [^2H_n]-3·HCl and [^2H_n]-4·HBr were prepared by a modification of the method of Patrick et al. (3), differing only in the substitution of deuterium and perdeuteroacetic acid for hydrogen and glacial acetic acid during the reduction step. The yields of 3 and 4 were comparable to those obtained for the protium forms (3). Deutérium enrichment for 3 = 4 = ($^2H_0$) = 0.2%, ($^2H_1$) = 0.4%, ($^2H_2$) = 3.7%, ($^2H_3$) = 13.0%, ($^2H_4$) = 23.0%, ($^2H_5$) = 28.0%, ($^2H_6$) = 20.0%, ($^2H_7$) = 8.5%, ($^2H_8$) = 1.8%, ($^2H_9$) = 0.2%.

Derivatization. Aliquots of solutions containing deuterated compounds 1·HCl, 2·HCl, 3·HCl or 4·HBr for GLC analysis were placed in screw cap vials fitted with teflon liners, then evaporated to dryness under stream of N_2. For compounds [^2H_n]-1·HCl and [^2H_n]-3·HCl, 50 µL of trifluoroacetic anhydride (Aldrich) was added, then the samples were heated at 100° for 15 min. For [^2H_n]-2·HCl and [^2H_n]-4·HBr, 20 µL of pentafluoropropanol was also added before heating. After evaporation of the excess derivatizing reagents, the samples were reconstituted with benzene.

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