Synthesis of the Enantiomers of Reduced Haloperidol

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Reduced haloperidol (RHAL) is the best known metabolite of haloperidol (HAL), having been identified in humans, rats, and guinea pigs. Since RHAL contains an asymmetric center, it can exist in two possible enantiomeric forms. However, the enantiomeric composition of the RHAL formed from HAL *in vivo* has never been reported. As a first step toward the enantiomeric analysis of biological samples, we have developed an efficient and stereospecific synthesis of (+)- and (−)-RHAL from readily available commercial materials. We have also identified an enantioselective chromatographic method using a chiral HPLC stationary phase which can detect as little as 1% of either enantiomer in synthetic samples of RHAL enantiomers.

KEY WORDS: haloperidol; reduced haloperidol; enantiomers; chiral; high-performance liquid chromatography (HPLC).

INTRODUCTION

Haloperidol (HAL)[4] is one of the most widely prescribed drugs for the treatment of acute and chronic psychoses. Our current knowledge regarding the metabolic disposition of HAL in animals and humans includes an oxidative N-dealkylation pathway that leads to presumably CNS inactive fragments (1,2) and a reductive pathway (Scheme I) that produces what has come to be known as reduced haloperidol (RHAL) (3–5). Unlike the former metabolites, RHAL exerts marked CNS activity following systemic administration (2,6), which might reflect the activity of the compound itself or be a result of the rapid establishment of an equilibrium between HAL and RHAL (7,8). In any event, given the large interindividual differences in plasma levels of HAL as well as the response to HAL treatment, monitoring of both HAL and RHAL plasma levels in patients undergoing HAL therapy has been suggested as a better clinical indicator than HAL levels alone (3,9–11). Interestingly, there are contradictory reports that suggest that a high RHAL/HAL ratio might correlate with either a good or a bad patient response to HAL treatment (11–13).

The reduction of the carbonyl group of HAL to the alcohol group of RHAL introduces a center of asymmetry in the molecule. However, nothing has been reported about the stereochemical nature of the RHAL produced in animals and/or humans. A priori, the reduction of HAL *in vivo* could produce either a single enantiomer of RHAL or enantiomeric mixtures of varying composition, depending on the stereospecificity of the enzymes involved in the reduction. Since the respective pharmacological properties of the enantiomers of RHAL are not known, and all clinical assays for plasma RHAL up to now have not addressed the stereochemical question, one could hypothesize that the current uncertainty about the therapeutic significance of RHAL levels might be due to interpersonal variations in the specific enantiomer of RHAL that accumulates in the patient during HAL therapy. This would of course require the involvement of at least two ketone reductases of opposite stereoselectivities.

It has recently been shown that rats, guinea pigs, and dogs metabolize the carbonyl group of fenofibrate in a highly stereospecific way, while human metabolism is completely nonstereospecific (14). Thus, studying the stereoselectivity of the metabolic reduction of HAL across species might also yield relevant information that could be useful in extrapolating animal data to humans [e.g., long-term administration of HAL to primates as a model of extrapyramidal side effects in humans (15,16)].

A necessary first step in the pursue of any of these studies is the availability of the pure RHAL enantiomers. We have developed simple and stereospecific syntheses of both enantiomers of RHAL, from readily available commercial reagents. This report also describes a method for the determination of enantiomeric ratio in synthetic samples of RHAL by enantioselective chromatography on an HPLC chiral stationary phase.

MATERIALS AND METHODS

All reagents were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, and were of the highest purity available. They were used as received without purification, except for 4-chloro-4′-fluorobutyrophenone, which was distilled prior to use. All solvents were obtained from EM Science, Gibbstown, New Jersey, and were used as received except for THF, which was distilled from sodium metal under nitrogen prior to use. TLC was conducted on glass plates precoated with silica gel 60 F-254, 0.25-mm thickness, from E. Merck, Darmstadt, Germany. MPLC was performed using E. Merck silica gel 60 (230–400 mesh).

Enantioselective HPLC analyses were carried out using a Varian Model 5000 HPLC system equipped with a Rheodyne injector with a 20-μl loop, a Chiralcel OJ column (250 mm × 4.6-mm ID) obtained from J. T. Baker, Phillipsburgh, New Jersey, and an Applied Biosystems Model 783 UV detector.

(R)-(+)-α-(3-Chloropropyl)-4-fluorobenzenemethanol [(+)-2]. A solution of 20.1 g of 4-chloro-4′-fluorobutyrophenone in 50 ml of THF was added dropwise to a solution

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[4] HAL, haloperidol; HPLC, high-performance liquid chromatography; MPLC, medium-pressure liquid chromatography; RHAL, reduced haloperidol; TLC, thin-layer chromatography; s, singlet; d, doublet; t, triplet; m, multiplet; lf, liquid film; ee, enantiomeric excess.

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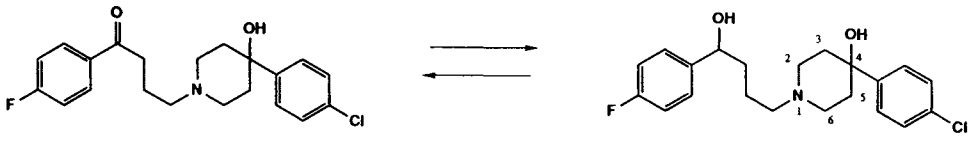


HAL RHAL

Scheme I

of 36.1 g of (+)-B-chlorodiisopinocamphenylborane in 50 ml of THF, at −25°C. The mixture was stirred at this temperature for 16 hr. The solvent was removed *in vacuo* and the residue was taken up into 500 ml of diethyl ether. To this solution was carefully added 21.2 ml of diethanolamine (exothermic). The mixture was stirred under nitrogen overnight. The white salt that formed was separated by filtration through Celite. The filtrate was concentrated to give 30.8 g of a light-yellow oil. Purification by MPLC (hexane:ethyl acetate, 4:1) provided 18.63 g (92% yield) of (+)-2 as a colorless oil; R_f = 0.29 (hexane:ethyl acetate, 3:1); $[\alpha]_D$ + 37.0° (*c* = 1.0, $CHCl_3$). *Elemental analysis*. Calculated for $C_{10}H_{12}ClFO$: C 59.27; H 5.97; Cl 17.49. Found: C 59.50; H 6.07; Cl 17.58. ^1H NMR ($CDCl_3$): 1.7–1.9 (4H, m); 3.5 (2H, m); 3.8 (1H, s); 4.6 (1H, t, J = 5.6 Hz); 7.0 (2H, m); 7.3 (2H, m). ^{13}C NMR ($CDCl_3$): 28.86; 36.26; 44.93; 73.25; 115.39 (J_{CF} = 21.3 Hz); 127.48 (J_{CF} = 7.9 Hz); 140.05; 162.23 (J_{CF} = 245.1 Hz). MS: 202/204 (M, 1.4/0.4); 185/187 (M—OH, 15.4/4.9); 125 (100). IR(lf): 3300 cm^{-1}.

(S)-(−)-α-(3-Chloropropyl)-4-fluorobenzenemethanol [(−)-2]. This compound was prepared in a manner similar to (+)-2, using (−)-B-chlorodiisopinocamphenylborane as the reducing agent. $[\alpha]_D$ −41.0° (c = 1.2, $CHCl_3$). *Elemental analysis*. Calculated for $C_{10}H_{12}ClFO$: C 59.27; H 5.97; Cl 17.49. Found: C 59.45; H 6.07; Cl 17.79. ^1H NMR, MS, and IR spectra were identical to those described above for (+)-2.

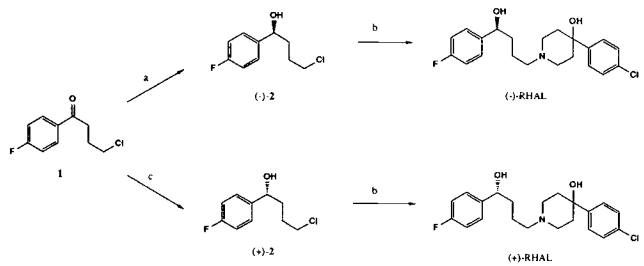
(R)-(+)-4-(Chlorophenyl)-1-[4-(4-fluorophenyl)-4-hydroxybutyl]-4-piperidinol [(+)-RHAL]. A mixture of 6.0 g of (+)-2, 5.0 g of 4-(4-chlorophenyl)-4-piperidinol, 5.0 g of sodium bicarbonate, and 0.6 g of sodium iodide in 250 ml of DMF was heated at 80°C for 16 hr. The mixture was evaporated *in vacuo*, and the residue was partitioned between chloroform (250 ml) and water (250 ml). The organic extract was dried over magnesium sulfate and concentrated *in vacuo*. The residue was purified by MPLC ($CHCl_3$: methanol, 19:1) to give 8.24 g (92.4% yield) of (+)-RHAL as a white solid, mp 124–129°C, R_f = 0.25 ($CHCl_3$:methanol, 9:1), determined by enantioselective HPLC to contain 1.6% of the (−)-enantiomer (96.8% ee). Recrystallization from ethyl acetate/diisopropyl ether afforded material of similar enantiomeric purity (97.0% ee), mp 130–132°C, $[\alpha]_D$ +65.4° (c = 1.2, $CHCl_3$). *Elemental analysis*. Calculated for $C_{21}H_{25}ClFNO_2$: C 66.75; H 6.67; Cl 9.38; F 5.03; N 3.71. Found: C 66.65; H 6.62; Cl 9.74; F 4.96; N 3.82. ^1H NMR ($CDCl_3$): 1.60–1.85 (4H, m); 1.85–2.00 (1H, m); 2.00–2.25 (3H, m); 2.40–2.65 (4H, m); 2.65–2.85 (1H, m); 2.97 (1H, broad d, J = 11.1 Hz); 4.6 (1H, broad s); 6.98 (2H, t, J = 8.6 Hz); 7.20–7.35 (4H, m); 7.42 (2H, d, J = 8.5 Hz). ^{13}C NMR ($CDCl_3$): 24.13; 37.68; 37.97; 40.27; 48.46; 50.08; 58.84; 70.75; 73.19; 114.92 (d, J_{CF} = 21.1 Hz); 126.27; 127.26 (d, J_{CF} = 7.9 Hz); 128.40; 132.81; 141.51; 146.47; 161.66 (d, J_{CF} = 244.2 Hz). ^{13}C NMR (DMSO-d_6): 22.91; 37.74; 48.83; 49.06; 57.96; 69.47; 71.57; 114.52 (d, J_{CF} = 20.9 Hz); 126.80; 127.55 (d, J_{CF} = 8.1 Hz); 127.70; 130.76; 142.55; 149.06; 160.94 (d, J_{CF} = 241.7 Hz). MS: 377/379 (M, 10.4/3.6); 224/226 (100/33). IR(KBr): 3400 cm^{-1}.

(S)-(−)-4-(4-Chlorophenyl)-1-[4-(4-fluorophenyl)-4-hydroxybutyl]-4-piperidinol [(−)-RHAL]. This compound was prepared from (−)-2 in a manner similar to the synthesis of (+)-RHAL described above. Following MPLC purification, the sample was determined to contain 2.2% of the (+)-enantiomer (95.6% ee). Recrystallization from ethyl acetate/diisopropyl ether increased the optical purity to 98% ee, mp 131.5–133.5°C, $[\alpha]_D$ −67.0° (c = 1.0, $CHCl_3$). *Elemental analysis*. Calculated for $C_{21}H_{25}ClFNO_2$: C 66.75; H 6.67; Cl 9.38; F 5.03; N 3.71. Found: C 66.56; H 6.53; Cl 9.60; F 5.30; N 3.81. ^1H NMR, ^{13}C NMR, MS, and IR were identical to those described above for (+)-RHAL.

Enantioselective HPLC. For the determination of enantiomeric purity, the Chiralcel OJ column was equilibrated with hexane:isopropanol (96:4) at a flow rate of 1.5 ml/min. The detection wavelength was set at 220 nm. Samples of RHAL were dissolved in the mobile phase at a concentration of about 0.1 mg/ml and injected into the HPLC system. The retention times of (−)-RHAL and (+)-RHAL were about 26 and 31 min, respectively.

RESULTS

The method developed by H. C. Brown and co-workers for the stereospecific reduction of ketones (17) was employed for the key reduction of commercially available ketone **1** in a highly stereoselective manner (Scheme II). By using either (+)- or (−)-B-chlorodiisopinocamphenylborane, (+)- and (−)-2 were prepared in a 92% yield and with enantiomeric excess (ee) in the 95–97% range, as determined after conversion into RHAL[5]. The (−)-enantio-



Scheme II. (a) (−)-B-Chlorodiisopinocamphenylborane, THF, −24°C. (b) 4-(4-Chlorophenyl)piperidinol, $NaHCO_3$, NaI, DMF, 80°C. (c) (+)-B-Chlorodiisopinocamphenylborane, THF, −24°C.

[5] The synthesis of (+)- and (−)-2 has been included in a recent patent (H. C. Brown, U.S. 4,868,344), but no physical characterization of the compounds is provided.

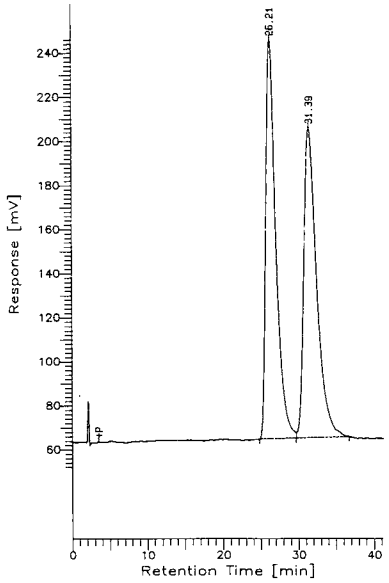


Fig. 1. Separation of the enantiomers of RHAL by enantioselective HPLC using a Chiralcel OJ column. Eluant, hexane:isopropanol (96:4) at 1.5 ml/min; detection, 220 nm; sample concentration, 0.1 mg/ml. Retention times: (−)-RHAL, 26.21 min; (+)-RHAL, 31.39 min.

mer of 2 was assigned the *S*-stereochemistry, based on the enantioselective ketone reduction work of H. C. Brown (17,18) and the known stereochemistry of (*S*)-(−)-4-chloro-1-phenyl-1-butanol (19,20).

Intermediates (+)- and (−)-2 were reacted with 4-(4-chlorophenyl)-4-piperidinol in DMF to produce, after workup and chromatography, (+)- and (−)-RHAL, respectively, as colorless crystalline solids that were fully characterized by ^{1}H NMR, ^{13}C NMR, MS, IR, and elemental analysis. As indicated above, the ee of (+)- and (−)-RHAL prepared by this method was 95–97%, as determined by HPLC on a chiral stationary phase. The optical purity of the isomers could be increased further by recrystallization from ethyl acetate/diisopropyl ether. An example chromatogram of the separation of racemic RHAL is shown in Fig. 1. The separation was adequate for detection of amounts of the undesired enantiomer down to at least 1%, as demonstrated in Fig. 2.

Interestingly, in the ^{13}C NMR spectrum of either enantiomer of RHAL in $CDCl_3$, the piperidine carbon atoms are all nonequivalent. This suggests that there is a strong hydrogen bond between the piperidine nitrogen atom and the alcohol group that restricts the free rotation of the piperidine ring around the N–C_7 bond. When the ^{13}C NMR spectrum was recorded in DMSO-d_6, C_2 and C_3 coalesced with C_6 and C_5, respectively, obviously the result of lack of intramolecular hydrogen bonding in this solvent.

DISCUSSION

The enantiomers of RHAL are now readily available in a high optical purity by a straightforward and short sequence from readily available commercial reagents. The enantiomeric excess of RHAL samples can be determined by enantioselective HPLC. The Chiralcel OJ column used contains silica coated with cellulose *p*-methylbenzoyl derivative and separation is thought to occur as a result of hydrogen bonding and steric interactions (21). This method of enantiomeric purity determination is simple and direct, i.e., does not require derivatization with a chiral reagent, which avoids errors resulting from the purity of the reagent or differing rates of reaction of the reagent with the respective enantiomers.

The resolution (*R*) of the enantiomers, as measured from a chromatogram of a 0.1 mg/ml sample of racemic RHAL, was 1.7 and was calculated as shown below.

$$R = \frac{2(t_2 - t_1)}{w_1 + w_2}$$

where t_2 and t_1 are the retention times and w_1 and w_2 are the widths of the peaks at baseline. The capacity factor, k', of (−)-RHAL was 11.4 and that of (+)-RHAL was 13.9. This results in a selectivity, α, of 1.22. The detection limit of RHAL using the conditions described and a criterion of $S/N = 3$ was found to be 2 μg injected (0.1 μg/ml × 20 μl). Thus it may be difficult to apply this method to the analysis of biological samples due to the relatively low sensitivity of the UV detection (vs electrochemical or fluorescence detection) and low detection wavelength, where many matrix components could interfere.

The availability of the RHAL enantiomers should allow their separate biological evaluation. Racemic RHAL is known to be a high-affinity ligand for the sigma receptor, the relevance of which is still unknown (2). It will be particularly interesting to explore the sigma and dopaminergic activity of (+)- and (−)-RHAL as well as their overall pharmacological activity, vis-à-vis HAL itself. Assuming that a sufficiently sensitive analytical method for the enantiomeric analysis of RHAL in serum samples can be developed, clinicians may in turn be able to correlate serum levels of a given enantiomeric

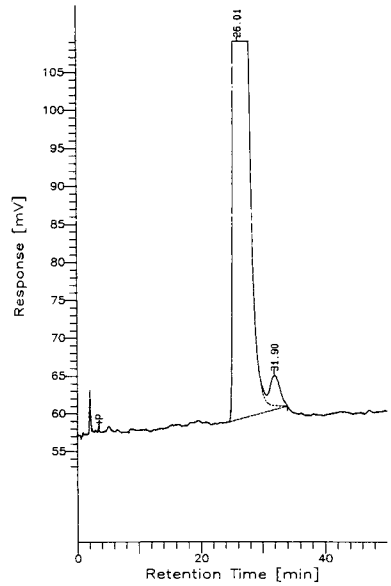


Fig. 2. Detection of about 1% (+)-RHAL contamination in a sample of (−)-RHAL; chromatographic conditions were the same as for Fig. 1.

form of RHAL with clinical efficacy or incidence of side effects during HAL therapy.

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