Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 2133–2136

TETRAHEDRON: *ASYMMETRY*

Enzymatic resolution of (±)-*threo*-methylphenidate

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Received 23 April 1998; accepted 12 May 1998

Abstract

The resolution of (±)-*threo*-methylphenidate by enzymatic hydrolysis with α-chymotrypsin or subtilisin carlsberg to afford (2S,2′S)-(−)-*threo* and (2R,2′R)-(+)-*threo*-methylphenidate hydrochlorides in high enantiomeric purities is described.

1. Introduction

(±)-*threo*-Methylphenidate hydrochloride (**1**, Ritalin® hydrochloride) is a mild nervous system stimulant. It is marketed for the treatment of children with Attention Deficit Hyperactivity Disorder (ADHD). (2R,2′R)-(+)-*threo*-Methylphenidate hydrochloride (**2**) has been reported to be between five[1] and 38 times[2] more active than the corresponding (2S,2′S)-(−)-*threo*-methylphenidate hydrochloride (**3**). Several classical resolution methods have been reported for the resolution of (±)-*threo*-methylphenidate (**1**).[1,3–5] However, an enzymatic resolution of (±)-*threo*-methylphenidate (**1**) has not been reported. Enzymatic resolution of acids, including α-amino acids, by the hydrolysis of the corresponding esters is a well-established and synthetically useful method. β-Substituted β-amino acids, possessing only one asymmetric center, have also been resolved by enzymatic hydrolysis of esters.[6] Resolution of α,β-disubstituted β-amino acids by enzymatic hydrolysis of esters is not known to the best of our knowledge.[6] In this paper we describe our results on the enzymatic resolution of (±)-*threo*-methylphenidate (**1**), which possesses α,β-disubstituted β-amino acid structural features.

2. Results and discussion

The enantioselective hydrolysis of **1** was carried out using different lipase and protease enzymes. α-Chymotrypsin and subtilisin carlsberg were found to exhibit selectivity towards the hydrolysis of

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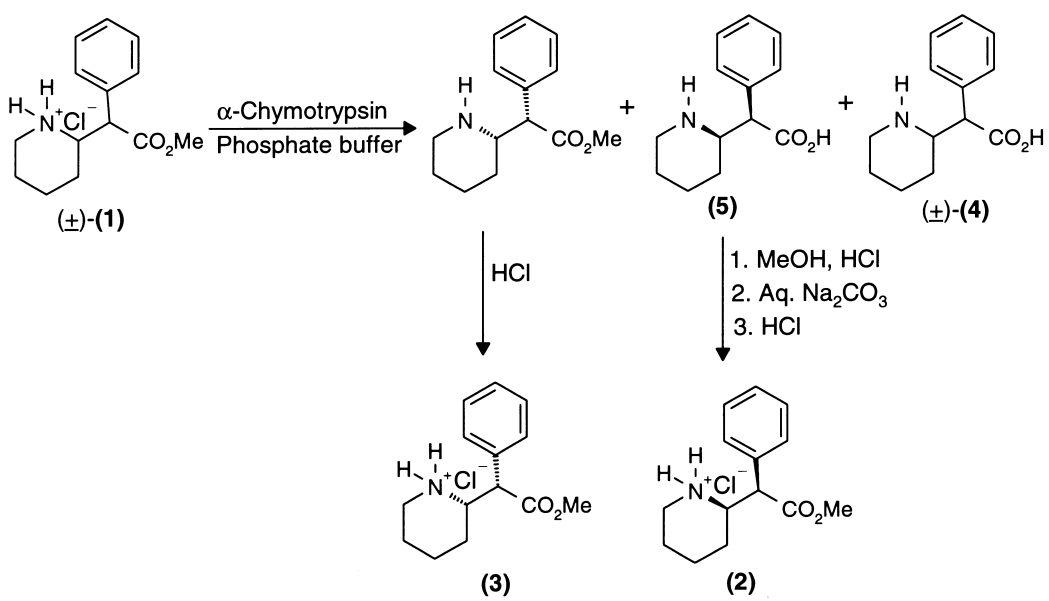
0957-4166/98/$19.00
PII: S0957-4166(98)00220-1

(2R,2′R)-enantiomer (**2**). In both cases the hydrolysis was slow. The progress of the reaction was monitored by HPLC using a Chiralpak AD column and the hydrolysis was continued until <2% of the (2R,2′R)-enantiomer (**2**) remained unreacted. Hydrolysis of **1** with α-chymotrypsin furnished a heterogeneous mixture from which pure (2S,2′S)-(−)-*threo*-methylphenidate hydrochloride (**3**) was isolated in 30% yield (>99% ee) after extractive work-up, and conversion to the hydrochloride salt (Scheme 1). HPLC analysis of the solid, which precipitated during the hydrolysis, indicated it to be (±)-*threo*-ritalinic acid (**4**, yield 30%). Esterification of this acid with methanol in the presence of HCl gas yielded (±)-*threo*-methylphenidate hydrochloride (**1**) as confirmed by HPLC. This result was surprising because we expected the acid to be enriched in (2R,2′R)-*threo*-ritalinic acid (**5**). To ensure that there was no epimerization of the (2R,2′R)-enantiomer at either or both of the stereogenic centers during the hydrolysis, pure (2R,2′R)-enantiomer (**2**) was subjected to enzymatic hydrolysis under identical conditions. The resulting (2R,2′R)-*threo*-ritalinic acid (**5**) was found to be highly soluble in the aqueous medium and did not precipitate. The aqueous layer was then lyophilized and the residue was esterified with methanol in the presence of HCl gas to yield pure **2** as indicated by HPLC. No (2S,2′S)-*threo*-enantiomer (**3**) or corrsponding *erythro* product could be detected by HPLC. These results suggest that there was no racemization of the (2R,2′R)-enantiomer during the enzymatic hydrolysis and that the (±)-ritalinic acid (**4**) emerged as a result of the hydrolysis of some of the (2S,2′S)-enantiomer. The differences in the solubilities of the (±)- and (2R,2′R)-*threo*-ritalinic acids (**4** and **5**) in the aqueous medium led to selective crystallization of the former during enzymatic hydrolysis and made their separation possible. Thus, the soluble (2R,2′R)-ritalinic acid (**5**) was isolated from the aqueous layer by lyophilization and esterified with methanol in the presence of HCl gas. After a basic work-up, the free base of **2** with a (2R,2′R):(2S,2′S) ratio of 90:10 was obtained. Treatment of this free base with HCl gas followed by crystallization of the resulting HCl salt with methanol and *t*-butyl methyl ether yielded pure (2R,2′R)-(+)-*threo*-methylphenidate hydrochloride (**2**) in 16% yield (>98% ee). Both the enantiomers of *threo*-methylphenidate hydrochloride were prepared in this way in high enantiomeric purities even though the isolation of the (2R,2′R)-enantiomer was cumbersome. Similar results were obtained with subtilisin carlsberg yielding pure **3** in 26% yield (>99% ee), pure **2** in 14.5% yield (98% ee), and **4** in 28% yield.



Scheme 1.

In an attempt to invert the selectivity during the hydrolysis, a mixture of an organic solvent and phosphate buffer was used as the solvent and ChiroCLEC-BL as the enzyme. Hydrolysis of **1** with ChiroCLEC-BL in a mixture of phosphate buffer and DMF did not yield any appreciable hydrolysis. A biphasic mixture of phosphate buffer and toluene at pH 7.2 led to hydrolysis of **1** but it did not affect the selectivity. The (2R,2′R)-isomer underwent preferential hydrolysis, and the results were the same as observed with subtilisin carlsberg.

Carbonic anhydrase has been reported to exhibit an opposite stereochemical preference to that observed with proteases during hydrolysis of (±)-N-acylamino acid esters.[7] To test if carbonic anhydrase will hydrolyse the (2S,2′S)-enantiomer preferentially, (±)-*threo*-methylphenidate (**1**) was treated with this enzyme in a phosphate buffer at pH 7.5. This, however, did not lead to any hydrolysis.

3. Summary

In summary, the resolution of (±)-*threo*-methylphenidate by enzymatic hydrolysis with α-chymotrypsin or subtilisin carlsberg afforded (2S,2′S)-(−)-*threo*- and (2R,2′R)-(+)-*threo*-methylphenidate hydrochlorides in high enantiomeric purities.

4. Experimental

α-Chymotrypsin was available from Sigma Chemical Company. Subtilisin carlsberg (Altus 10) and Chiro-CLEC-BL (Altus 16) were purchased from Altus Biologics Inc. Chiral HPLC measurements were performed on a Rainin Dynamax system using a Daicel Chiralpak AD column (4.6×250 mm) and a mixture of hexane:ethanol:methanol:TFA (96:2:2:0.1 ml) as the mobile phase (isocratic at a flow rate of 0.8 ml/min and UV detector at 230 nm). The retention times of the (2S,2′S)- and (2R,2′R)-*threo*-methylphenidates were 13 and 15 min respectively. The retention times of *threo*-ritalinic acids were 19 and 21 min.

4.1. Enzymatic hydrolysis

α-Chymotrypsin (6.0 g) was dissolved in 275 ml of phosphate buffer (pH 7.2) and 5.0 g of (−)-*threo*-methylphenidate hydrochloride (**1**) was added. The pH was adjusted to 7.6 with 1 N NaOH. The mixture was stirred at room temperature for 48 h while maintaining the pH 7.5–7.6 with 1 N NaOH (~7 ml), and the progress of the reaction was monitored by chiral HPLC for disappearance of the (2R,2′R)-enantiomer. The reaction mixture was treated with 250 ml of ethyl acetate and filtered. The solid was washed with 20 ml of ethyl acetate, 20 ml of water and dried to afford (−)-*threo*-ritalinic acid (**4**, 1.3 g, 30%).

The organic layer was separated from the biphasic filtrate, and the aqueous layer was extracted with 2×250 ml of ethyl acetate. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The resulting oil was dissolved in 30 ml of isopropyl acetate and cooled to 5°C. To it was added a 3 ml solution of HCl gas in isopropyl acetate (3.8 M). The solid was collected by filtration and washed with 5 ml of isopropyl acetate. The solid was dried at 50°C under reduced pressure to afford pure (2S,2′S)-(−)-*threo*-methylphenidate hydrochloride (**3**, 1.5 g, 30%); mp=222–224°C; $[\alpha]_D^{20}=-85$ (c=1.0, MeOH) (lit[1] mp=209–210°C; $[\alpha]_D^{20}=-75$ (1% in MeOH)); ee=99%; IR (KBr, cm^{-1}) 1739; ^1H NMR (CD_3OD, δ) 1.33–1.55 (m, 3H), 1.64–1.92 (m, 3H), 3.1 (dt, 1H, J=3.5 and 12.6 Hz), 3.4–3.5 (m, 1H), 3.7 (s, 3H), 3.83 (dt, 1H, J=3.5 and 10.0 Hz), 3.96 (d, 1H, J=10.0 Hz), 7.25–7.44 (m, 5H); ^{13}C NMR

(CD_3OD, δ) 22.79, 23.27, 27.58, 46.64, 53.4, 55.23, 59.2, 129.59, 129.64, 130.38, 135.25, 173.24; MS (m/e) 234 (MH^+). Anal. calcd for $C_{14}H_{20}ClNO_2$: C, 62.33; H, 7.47; N, 5.19; Cl, 13.14. Found: C, 62.21; H, 7.32; N, 5.44; Cl, 13.16.

The aqueous phase was lyophilized, and the residue containing **5** [mp=238–240°C (dec.); IR (KBr, cm^{-1}) 1658; ^1H NMR (CD_3OD, δ) 1.33–1.55 (m, 3H), 1.57–1.68 (m, 1H), 1.74–1.88 (m, 2H), 3.02 (dt, 1H, J=3.5 and 12.5 Hz), 3.38–3.5 (m, 1H), 7.22–7.36 (m, 5H); ^{13}C NMR (CD_3OD, δ) 23.08, 23.7, 28.28, 45.78, 58.23, 60.93, 128.44, 129.75, 129.86, 139.15, 177.76; MS (m/e) 220 (MH^+)] was suspended in 140 ml of methanol. HCl gas was bubbled into this suspension, and the mixture was heated at 45–50°C for 16 h. The mixture was filtered and concentrated under reduced pressure. The residue was treated with aqueous sodium carbonate and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The oil was dissolved in 20 ml of isopropyl acetate and cooled to 5°C. A solution of HCl gas in isopropyl acetate (3 ml, 3.8 M) was added. The solid was collected by filtration and washed with 5 ml of isopropyl acetate. The solid was dried at 50°C under reduced pressure. The solid was suspended in 6 ml of methanol and heated to reflux to obtain a solution. To this solution was added 10 ml of *t*-butyl methyl ether. The mixture was cooled to room temperature and filtered to collect the solid. The solid was washed with 5 ml of *t*-butyl methyl ether and dried at 50°C under reduced pressure to afford pure (2R,2′R)-(+)-*threo*-methylphenidate hydrochloride (**2**, 0.8 g, 16%); mp=222–224°C; $[\alpha]_D^{20}$=+84 (c=1.0, MeOH) (lit[1] mp=210–211°C; $[\alpha]_D^{20}$=+88 (1% in MeOH)); ee=98%; IR (KBr, cm^{-1}) 1739; ^1H NMR (CD_3OD, δ) 1.35–1.58 (m, 3H), 1.65–1.93 (m, 3H), 3.11 (dt, 1H, J=3.5 and 12.6 Hz), 3.4–3.5 (m, 1H), 3.7 (s, 3H), 3.84 (dt, 1H, J=3.5 and 10.0 Hz), 3.99 (d, 1H, J=10.0 Hz), 7.25–7.44 (m, 5H); ^{13}C NMR (CD_3OD, δ) 22.78, 23.23, 27.54, 46.63, 53.4, 55.2, 59.18, 129.59, 129.62, 130.36, 135.25, 173.22; MS (m/e) 234 (MH^+). Anal. calcd for $C_{14}H_{20}ClNO_2$: C, 62.33; H, 7.47; N, 5.19; Cl, 13.14. Found: C, 62.31; H, 7.36; N, 5.15; Cl, 13.11.

Similarly, enzymatic hydrolysis of 1.5 g of **1** with 225 mg of subtilisin carlsberg yielded pure **3** (0.4 g, 26%), **4** (0.35 g, 14%), and pure **2** (0.22 g, 14.5%).

Acknowledgements

We thank Ms. Preeti Minhas for optical rotations.

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