L-Tartaric acid-mediated Isolation of Optically Pure L-Penicillamine from Racemic Penicillamine

Seon Yi Park, Bang Sook Lee, and Sung Keon Namgoong

Department of Chemistry, Seoul Women's University, Seoul 139-774, Korea

Received March 15, 2002; Accepted September 28, 2002

Abstract: Optical resolution of the unprotected racemic penicillamine was successfully achieved by L-tartaric acid in methanol/acetic acid. This process based on diastereomeric interaction gave the optically pure L-penicillamine in 80% yield.

Keywords: L-penicillamine, L-tartaric acid, racemic penicillamine, resolution, optical purity

Introduction

The non-naturally occurring amino acid L-penicillamine, L-(+)-2-amino-3-mercapto-3-methylbutyric acid, is much more toxic than the D-isomer in therapeutic bases [1,2]. Nonetheless, L-penicillamine (L-PA) has been interesting since it was reported to be one of the peptide building blocks in HIV protease inhibitors [3-5]. At present, industrial production of L-PA is an urgent problem in relation with AIDS chemotherapeutics.

\[
\text{HS} \quad \text{CH}_3
\]
\[
\text{CH}_3 \quad \text{H}_2\text{N} \quad \text{COOH}
\]
\[\text{L-penicillamine}\]

Several methods using chiral auxiliaries to obtain L-PA from protected derivatives of either D-enantiomer or its racemate have been published. In the case of basic resolving agents, L-PA was isolated via diastereomeric salt formation of N-formyl-S-benzyl-D,L-penicillamine with brucine and other alkaloids [6,7]. Another procedure was concerned with the resolutions of N-formyl-isopropylidene-D,L-penicillamine with optically pure amines such as (+)-norephedrine and L-(+)-threo-1-(p-nitrophenyl)-2-amino-1,3-propanediol [2,8]. In the case of an acidic chiral auxiliary, (1S)-(+)10-camphorsulfonic acid (S)-CSA has been applied to achieve asymmetric transformation of the racemic 5,5-dimethylthiazolidine-4-carboxylic acid [9].

These procedures apparently require adequate protection and deprotection of the functionalities in penicillamine. Moreover, chiral resolving agents are mostly expensive. The latter procedure has serious problems impossible to isolate L-PA in final deprotection stage even though asymmetric transformation of the racemic penicillamine (rac-PA) derivative is successful [9]. None of those processes are not suitable for the mass production of L-PA. Here we wish to describe our industrially applicable process to isolate L-PA and this is the unprecedented case in the resolution of rac-PA.

Experimental

General Methods

Melting points are uncorrected. \(^1\)H NMR spectra were recorded with a Bruker AVANCE 500 (500 MHz). The specific rotation was measured on Autopol III automatic polarimeter (Rudolph research Co.) with a quartz cell of 1.0 dm path length. All solvents and reagents were used without further purification.

The Resolution of rac-PA with (S)-CSA

(1S)-(+)10-Camphorsulfonic acid [(S)-CSA, 3.15 g, 13.6 mmol] was dissolved in 13 mL of absolute ethanol under nitrogen. rac-PA (2.00 g, 13.4 mmol) was slowly added to the solution. The solution was stirred for 1 hr at room temperature and a mixture of very hygroscopic insoluble salts were filtered, washed with 7 mL of absolute ethanol and dried. Yield of a mixture of the

To whom all correspondence should be addressed.
(e-mail: sknam@mail.swu.ac.kr)
salts: 2.05 g (80%) as a white powder. The crude salts (2.05 g) were directly dissolved in 11 mL of absolute ethanol under nitrogen. Triethylamine (1.90 mL, 13.6 mmol) was added dropwise to this solution. The resulting reaction mixture was stirred for 1 h at room temperature. The precipitate was filtered, washed with 6 mL of absolute ethanol and dried. Overall yield from the reaction of rac-PA with (S)-CSA: 760 mg (76%) as a white solid; $^1$H NMR (500 MHz, D$_2$O) δ 3.72 (s, 1H, α-H of L-PA), 1.59 (s, 3H, CH$_3$), 1.50 (s, 3H, CH$_3$) ppm; $^{13}$C NMR (125 MHz, D$_2$O) δ 171, 65, 44, 30, 28 ppm; $[^a]_D^{25}$ +25.2° (c 1.00, 1 N NaOH) [lit. $[^a]_D^{25}$ +63° (c 1.00, 1 N NaOH) in case of L-PA with 100% e.e.].

**General Procedure for the Resolution of rac-PA with L-TA**

L-Tartaric acid (L-TA, 15.1 g, 101 mmol) was dissolved in 80 mL of methanol under nitrogen.

40 mL of acetic acid and rac-PA (10.0 g, 67.0 mmol) was slowly added to the solution. The solution was stirred for 25 min at room temperature and the precipitated salt, L-PA · L-TA was filtered through membrane filter (0.45 μm). The salt was successively washed with 40 mL of acetic acid and 80 mL of chloroform and dried. Yield of L-PA · L-TA: 6.31 g as a white powder; $^1$H NMR (500 MHz, D$_2$O) δ 4.70 (s, 2H, α-H of L-TA), 3.78 (s, 1.8H, α-H of L-PA), 1.60 (s, 6H, CH$_3$), 1.52 (s, 6H, CH$_3$) ppm; $^{13}$C NMR (125 MHz, D$_2$O) δ 175, 171, 73, 65, 44, 31, 28 ppm; $[^a]_D^{25}$ +53.2° (c 1.00, 1 N NaOH). The crude salt (6.31 g) was directly dissolved in 32 mL of absolute ethanol under nitrogen. Triethylamine was added dropwise to this solution until it was adjusted to pH 7. The reaction mixture was stirred for 1 h at room temperature. The insoluble L-PA was filtered, washed with 15 mL of absolute ethanol and dried. Yield: 3.98 g (80%) as a white solid; mp 190-194°C (lit. 190-194°C); $^1$H NMR (500 MHz, D$_2$O) δ 3.72 (s, 1H, α-H of L-PA), 1.59 (s, 3H, CH$_3$), 1.50 (s, 3H, CH$_3$) ppm; $^{13}$C NMR (125 MHz, D$_2$O) δ 171, 65, 44, 30, 28 ppm; $[^a]_D^{25}$ +63° (c 1.00, 1 N NaOH) [lit. $[^a]_D^{25}$ +63° (c 1.00, 1 N NaOH).

**Results and Discussion**

To avoid complicated protection/deprotection chemistry in the published resolution processes of rac-PA and to develop industrially applicable procedure, we have systematically studied the direct resolution methods of L-PA by the diastereomeric interactions of unprotected rac-PA with relatively cheaper and widely applicable chiral resolving agents, (1S,3S)-10-camphorsulfonic acid [(S)-CSA] and L-tartaric acid (L-TA) [10-12].

First trial to resolve rac-PA started with (S)-CSA. Rac-PA was prepared from the racemization [13] of D-PA which is a degradation product of penicillin G [14]. Preliminary solubility test under various solvent conditions with each of diastereomeric salts, D-PA · (S)-CSA and L-PA · (S)-CSA indicated that absolute ethanol is the best solvent for selective crystallization of much less soluble salt, L-PA · (S)-CSA. L-PA · (S)-CSA was five fold less soluble than D-PA · (S)-CSA in absolute ethanol. However, the marked solubility difference between those two diastereomers did not satisfy the isolation of optically pure L-PA · (S)-CSA when rac-PA was treated with (S)-CSA in absolute ethanol. Optical purity of L-PA was recorded in the range from 30 to 40% after removal of (S)-CSA. This result is not clearly explained. It may be attributed to molecular aggregation between both diastereomeric salts to prevent from single crystal formation of L-PA · (S)-CSA when these salts coexist in solution.

The other resolution of rac-PA was carried out with L-TA. To investigate solubility of each of rac-PA · L-TA salts, these salts were prepared in acetic acid at room temperature for 30 min respectively [15]. The salts were not formed even under reflux in protic solvents such as water, methanol and ethanol. NMR spectra showed that the chemical shifts of diastereomeric salts were not changed at all after reaction as compared with them of each of the reactants, PA and L-TA. Solubility difference between the two diastereomeric salts was quite remarkable in acetic acid at room temperature. 1 g of D-PA · L-TA salt was completely soluble in 7.5 mL of acetic acid whereas 0.8 g of L-PA · L-TA was precipitated under the identical condition. On the basis of this finding, several approaches to L-tartaric acid-mediated resolution of rac-PA were performed as shown in Scheme 1 and Table 1.

All the reactions presented here were conducted in the condition of atmospheric nitrogen not to form inter
molecular disulfide linkage by air oxidation of penicillamines themselves. The reaction of rac-PA with 1-TA gave three possible kinds of mixture of salts, presumably (1-PA)ₙ·1-TA, (p-PA)ₙ·1-TA (n=1~2) and 1-PA·1-TA·p-PA as a precipitated form at room temperature for 30 min in acetic acid. NMR study revealed that all the proton peaks of the mixed salt split into three sets of peaks respectively. The ratios of these salts on ¹H NMR spectra were not reproducible whenever the same reactions were repeated. After neutralization of the salt with triethylamine in absolute methanol, optical purity of resulting 1-PA was recorded below 30% in general.

The salt formation reactions at even higher temperatures such as 50°C and 65°C did not greatly improve the optical purity and yield of 1-PA (entry 1 and 2 in Table 1) and these reactions seriously reduced them at higher temperatures than 70°C. In fact, the reaction time and temperature in these reactions were not so important to get better optical purity and yield of 1-PA. The reason not to form selective precipitates of 1-PA salt in acetic acid solution is more complicated and difficult to explain due to the presence of diverse 1-TA salts even though the result of these reactions is similar to that of (S)-CSA salt formation reaction with rac-PA. Methanol was used as a co-solvent with acetic acid to completely dissolve 1-TA and to effectively remove the contaminants in the process of selective crystallization of 1-PA. The optical purity of 1-PA depended upon both ratio and amount of co-solvents when the equimolar amount of 1-TA was used in the resolutions of rac-PA salts (entry 3-6). Higher degree of optical purity was observed if the ratio of methanol and amount of co-solvents were increased in those reactions. The result in entry 6 was rather exceptional in this sense. The optical purity was the best in a case of co-solvent ratio of 3:2 if 1-TA was in equimolar use (entry 5). The variation of the reaction conditions such as the reaction time and solvent in the neutralization reaction of the salt in entry 3-6 did almost not change the yields of 1-PA in the range of 60%. The binding ratios between 1-PA and 1-TA varied in the range from 1.6:1.0 to 1.9:1.0 in these reactions. On the other hand, the reaction of rac-PA with 1.5 molar equivalents of 1-TA considerably enhanced the optical purity of 1-PA with either 3:2 or 2:1 ratios of the co-solvents (entry 7-9).

Most of all, the reaction of rac-PA with 1.5 molar equivalent of 1-TA and 2:1 ratio of co-solvent gave the ideal optical purity of 1-PA like 100% and the highest yield was produced in absolute ethanol instead of 99%
methanol and 95% ethanol (entry 8 and 9). The proper combination of reaction conditions such as relative ratios of L-TA and co-solvents and amount of solvents seems to be the crucial factor to obtain optically pure L-PA. 25 min and room temperature were also suitable for the best optical purity and yield among most of reaction times and temperatures respectively. In a scale-up experiment (entry 9), final filtration of L-PA through membrane filter (0.45 μm) yielded up by about 4%.

Concerning recovery of the unresolved mixture of D- and L-PA in the co-solvents, the mixture of D- and L-PA mostly existed in the D-form can be isolated through the identical work-up procedure with that of entry 9 after complete evaporation of the filtrate. The overall yield for the recovery of the unresolved mixture of D- and L-PA was 82%. At this time, the value of specific rotation, [α]D25 and the optical purity of the mixture were -47.9° (c 1.00, 1 N NaOH) and 76% on the basis of the D-PA respectively. In the cases of entry 8 and 9, average binding ratio of L-PA · L-TA was 1.80:1.00 ± 0.03 for L-PA versus L-TA and all the peaks of the salt appeared as the singlet form on NMR spectra. A rationale for the selective precipitation of L-PA · L-TA salt in methanol/ acetic acid as co-solvents may be attributed to intramolecular hydrogen bonds between two α-hydroxyl groups of L-TA and carboxyl group of L-PA so that these salts are not able to be solvated. To examine this possibility, molecular modeling study such as ab initio calculation to find out the energy-minimized structures of L-PA · L-TA salts is in progress. The results related with entry 8 and 9 were reproducible through a couple of scale-up reactions and the spectral data coincided with those of authentic L-PA [6].

Conclusions

We have systematically studied the direct isolation methods of L-PA by the diastereomeric interactions of unprotected rac-PA with both chiral resolving agents, (S)-CSA and L-TA. Of the processes, successful optical resolution of rac-PA was observed by 1.5 molar equivalent L-TA in methanol/acetic acid (2:1). This process gave the optically pure L-penicillamine in 80% yield.

Acknowledgement

Analyses for the optical purity of L-PA and other enthusiastic help and advices from Estech Pharma were greatly appreciated.

References


