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Research Article

Resolution, Characterization and Biological Evaluation of Doxylamine Isomers

MR Arunkumar[1], MD Karvekar[1,*]

[1] *Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, Karnataka, India*

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* *Corresponding author.*
MD Karvekar

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ABSTRACT

Chirality plays a vital role in the pharmacological action of drugs, as enantiomers can exhibit vastly different efficacies, toxicities, and metabolisms. Doxylamine succinate, a racemic first-generation antihistamine, can act primarily as one enantiomer. Thus, enantiomeric resolution is essential for the formulation of safer and more potent drugs. A racemic mixture of doxylamine was resolved through diastereomeric salt formation with L(+)-tartaric acid in methanol and then crystallised from an acetone/water mixture. The free enantiomers were released and prepared as succinate salts. Purity and identity were established using melting point, optical rotation, IR, NMR, and mass spectrometry. The antihistaminic activity was measured using isolated guinea pig ileum and tracheal chain models. The analytical methods ensured structural integrity and enantiomeric purity. Optical rotation and spectral information clearly differentiated the isomers. R(+)-Doxylamine succinate possessed the highest antihistaminic activity (95.83%), exceeding that of both the racemic mixture (91.66%) and S(−)-isomer (87.5%). The present study offers an easy and scalable method for resolving doxylamine enantiomers and demonstrates that R(+)-doxylamine possesses superior pharmacological activity, supporting its potential as a monotherapy agent.

Keywords: Resolution; Doxylamine Succinate; Enantiomeric Purity; Antihistaminic Activity

INTRODUCTION

Chirality is of special significance in the pharmacodynamics and pharmacokinetics of drugs because enantiomers frequently exhibit dramatically different biological properties, metabolic fates, and toxicological features. Doxylamine succinate is a first-generation antihistamine and an anticataleptic sedative that is extensively used as an allergy drug and nonprescription sleeping tablet. It is a racemic mixture containing equal amounts of two optical isomers, R(+)- and S(−)-doxylamine. However, one enantiomer might predominantly be the cause of the desired therapeutic effect, while the other can be the source of side effects or have lesser efficacy[1–3]. Therefore, the resolution of racemic mixtures to enantiomerically pure compounds has been on the rise in drug development, both to improve therapeutic efficacy and to adhere to regulatory requirements with an enantiomer-specific assessment. The physicochemical characterizations are critical for determining enantiomeric identity, particularly considering how subtle structural differences between isomers can affect the interaction with biological targets[4–8]. The isolated resolved isomers with pharmaceutical activity hold tremendous value to the pharmaceutical industry, allowing safer, more effective, and more targeted drugs to be developed[9]. The main aim of this study was to resolve the racemic mixture of doxylamine through diastereomeric salt formation with L(+)-tartaric acid and subsequent crystallisation to obtain pure optical isomers.

MATERIALS AND METHODS

The resolution and synthetic work were conducted at the R&D Laboratory of R. L. Fine Chemicals, Yelahanka, Bangalore. Pharmacological studies were conducted at the Laboratory of the Department of Pharmacology, Krupanidhi College of Pharmacy, Bangalore.

All chemicals used in the current project were of Laboratory Reagent and Analytical Reagent quality and were obtained from Lancaster, Sigma Aldrich, NR Chem., Rolex, S.D. Fine Chem. Ltd., and Merck. The chemicals used were doxylamine succinate, methanol, L-(+)-tartaric acid, ethyl

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acetate, succinic acid, ammonia, dichloromethane, toluene, magnesium turnings, and acetone.

Analytical Techniques

The melting points of the synthesised and recrystallised compounds were measured using open capillary tubes and not corrected. Thin-layer chromatography (TLC) using the stationary phase silica gel G and the mobile phase acetone: chloroform (90:10) was used to check the purity. The spots for recrystallisation were detected in an iodine chamber. The instrumental characterisation methods used were infrared (IR) spectroscopy, proton nuclear magnetic resonance (^{1}HNMR), and mass spectroscopy. IR spectra were obtained in 4000–400 cm^{-1} range by KBr pellet technique on Fourier Transform IR Spectrophotometer (Model-1 Shimadzu 8700) at R.L. Fine Chemicals. ^{1}H NMR (200 MHz) spectra were obtained in deuterated chloroform on an AMX–200 liquid-state NMR spectrometer at IISc, Bangalore, and chemical shifts (δ) were given in ppm downfield from the internal standard TMS. Mass spectra were obtained on a Shimadzu spectrophotometer (LCMS-2010) at L.G.C. Promo Chem. Laboratory, Bangalore. Optical rotation was determined using a Jasco Polarimeter (P-2000) at R.L.Fine Chemicals.

Scheme of resolution and characterization of Doxylamine Isomers

Step 1: Resolution of (±) Doxylamine Succinate

a. Basification of Doxylamine Succinate: 250 g of (±) doxylamine succinate salt was dissolved in 700 ml of water in a 1000 ml flask. Sodium hydroxide was added to alkalize the solution, which was checked with red litmus paper which turned blue. Then, 750 ml of toluene was added, and the mixture was shaken well. The organic layer was separated using a separating funnel and the aqueous layer was discarded. The toluene layer was distilled to evaporate the solvent, yielding 150 g of pure (±) doxylamine base in 88% yield.

b. Preparation of (±) Doxylamine Tartrate: Into a 2000 ml round-bottom flask fitted with a stirrer, reflux condenser, oil seal, and thermometer, 100 g (0.370 mol) of (±) doxylamine base was dissolved in 500 ml methanol. The solution was then stirred and heated to 40°C. 55.5 g (0.370 mol) of L-(+)-tartaric acid was added, and the mixture was continued for an hour. Approximately 80% of the methanol was removed by distillation, and the solution was left to cool to room temperature. When 500 ml ethyl acetate was added, a white solid precipitate developed, which was dried by vacuum filtration at 60°C to produce 148 g of (±) doxylamine tartrate (95% yield).

c. Isomerization by Crystallization of R(+)- and S(–)-Doxylamine Tartrate Isomers: The (±) doxylamine tartrate was dissolved in a minimum amount of acetone: water (90:10), heated, and allowed to stand at room temperature for five days. Crystals of R(+)-doxylamine tartrate were formed and filtered. The filtrate was used to purify the S(–) doxylamine tartrate. Ethyl acetate was added to the filtrate, and 50% was distilled off to yield the S(–) isomer. Optical rotation was used to confirm the identity of isomers.

Step 2: Preparation of R(+) Doxylamine Succinate Isomer

a. Basification of R(+) Doxylamine Tartrate: Fifty grams of R(+) doxylamine tartrate were dissolved in 300 ml water, and 40 ml sodium hydroxide was added. The mixture was stirred for 5 min, after which 200 ml toluene was added. The organic layer was separated using a separating funnel and the aqueous layer was discarded. The organic layer was washed twice with 150 ml of water and dried using sodium sulphite to remove moisture. The toluene layer was vacuum-distilled to drive 50% of the solvent, giving 30 g of pure R(+) doxylamine base (93% yield).

b. Formation of R(+) Doxylamine Succinate: Eighteen grams of R(+) doxylamine base were reacted with 11.36 g of succinic acid dissolved in 156 ml of acetone. The solution was warmed at 50°C in a water bath under nitrogen gas until succinic acid was dissolved. The reaction mixture was cooled to 5–6°C and stirred for one hour, resulting in a white precipitate of the R(+) doxylamine succinate isomer (24 g), with a yield of 92.7%.

Step 3: S(–) Doxylamine Succinate Isomer Preparation

a. Basification of S(–) Doxylamine Tartrate: S(–) doxylamine tartrate was dissolved in 300 ml of water and 40 ml of sodium hydroxide and stirred for 5 min. Next, 200 ml of toluene was added. The layers were separated using a separating funnel and the aqueous layer was discarded. The organic layer was washed twice with 150 ml water and dried with sodium sulphite. The organic layer was vacuum-distilled to dry 50% toluene to obtain 30 g of pure S(–) doxylamine base (93% yield).

b. Formation of S(–) Doxylamine Succinate: Eighteen grams of S(–) doxylamine base were reacted with 11.36 g of succinic acid dissolved in 156 ml of acetone. The mixture was warmed to 50°C in a water bath under nitrogen gas until the acid was dissolved. The reaction mixture was cooled to 5–6°C and stirred for one hour to precipitate the S(–) doxylamine succinate isomer (23 g) in 91% yield.

Evaluation of antihistaminic activity

In pharmacological screening, antihistaminic activity was measured using isolated guinea pig ileum and tracheal chain tests. Both sexes of guinea pigs weighing 400–500 g were used. Animals were euthanised by exsanguination and stunning. The ileum was dissected from the junction of the ileocecum, tyrode-washed, and cut to obtain 2–3 cm in length. The segments were suspended in an organ bath of Tyrode's solution at 37°C, which was aerated with oxygen. 0.5 g of tension was applied, and the tissues were equilibrated for 30 min. Histamine dose-response curves

at 0.1, 0.4, and 0.8 μg/ml concentrations were obtained, and 0.4 μg/ml was chosen as the sub-maximal dose. Test samples were prepared by dissolving 10 mg of each sample in dimethyl sulfoxide to obtain the desired concentrations. Standard histamine solutions were prepared in DNS buffer. The percentage inhibition was calculated using the formula: $\%\ inhibition = \frac{(a-b)}{a} \times 100$, where a is the height of the histamine response and b is the height of the test response, both in centimetres.

In the tracheal chain technique, overnight-fasted guinea pigs were sacrificed, and the trachea was removed, cut into separate rings, and tied together to form a chain. The tracheal chain was suspended in an organ bath with Kreb's solution (NaCl 5.9, KCl 0.35, $CaCl_2$ 0.28, $MgSO_4$ 0.11, $NaHCO_3$ 2.1, KH_2PO_4 0.16, and glucose 2.0 g/L), continuously aerated and at 37 $\pm$ 0.5°C. One end of the tracheal chain was attached to an S-shaped aerator tube and the other to an isotonic frontal writing lever. A 400 mg load was used, and the tissue was left to equilibrate for 45 min. Dose-response plots were constructed for histamine at different molar concentrations with a 15-minute interval between the doses. Once the response curve was obtained, test samples were obtained. The percentage of maximum contractile response was plotted against the negative logarithm of the molar histamine concentration to analyse the inhibitory effect of the test compounds.

RESULTS

The racemic doxylamine succinate was effectively resolved by diastereomeric salt formation in the presence of L(+)-tartaric acid as the resolving reagent. Methanol was used for the process of resolution with the formation of racemic doxylamine tartarate. Crystallisation involving a mixture of acetone and water (90:10) resulted in single-crystal separation of the (−)-isomer within a period of five days, with the (+)-isomer extracted from the mother liquor using ethyl acetate. Basification of both salts with sodium hydroxide released the corresponding free bases. Verification of the resolution success was confirmed by measuring the optical rotation and melting point, complemented by spectral analysis.

Melting point analysis

The melting points of the compounds were assessed to verify their identities and purities. Succinic acid had a melting point of 183–185 °C, while the racemic mixture (R, S-doxylamine succinate) melted between 100 and 104 °C. The melting points of the enantiomeric forms differed slightly, such that R-doxylamine succinate melted at 90–93 °C and S-doxylamine succinate at 84–87 °C. R,S-Doxylamine tartarate melted at 141–144 °C, the resolved R-isomer melted at 152–155 °C, and the S-isomer melted at 120–123 °C. (Table 1)

Table 1: Melting points of racemic and resolved doxylamine compounds

Compounds	Melting point
Succinic acid	183 - 185 °C
(R)(S)-Doxylamine succinate	100 - 104 °C
(R)-Doxylamine succinate	90 - 93 °C
(S)-Doxylamine succinate	84 – 87 °C
(L)+ Tartaric Acid	180 – 182 °C
(R)(S)-Doxylamine tartarate	141 – 144 °C
(R)-Doxylamine tartarate	152 – 155 °C
(S)-Doxylamine tartarate	120 – 123 °C

Optical rotation studies

Optical rotation measurements confirmed the chiral purities of the resolved enantiomers. The racemic base was devoid of optical rotation ($[\alpha]$ = 0.07). However, the S(–)-doxylamine base was characterised by a specific rotation of —41, whereas the R(+)-enantiomer exhibited a rotation of +13. Similarly, for the tartarate salts, R, S-doxylamine tartarate possessed a specific rotation of 0.7, R(+)-doxylamine tartarate was +13, and S(–)-doxylamine tartarate was —41. (Table 2)

Table 2: Optical and specific rotation of racemic and resolved doxylamine tartarate isomers

Compounds	Optical rotation	Specific rotation
S (-)-doxylaminetartarate	-4.1025	41
R(+)- doxylaminetartarate	1.3787	13
R,S doxylaminetartarate	0.0070	07

Infrared Spectroscopic analysis

Infrared spectroscopy was used to verify the functional groups of the resolved compounds. The IR spectrum of Doxylamine tartarate (Figure 1) showed prominent peaks at 3321 cm^{-1} (OH stretch), 3057 cm^{-1} (C–H stretch), 2370 cm^{-1} (quaternary ammonium salt), 1728 cm^{-1} (C=O), 1467.83 cm^{-1} (C=N), and 848.68 cm^{-1} (aromatic C–H stretch). S(–)-Doxylamine succinate had similar peaks, with a strong OH stretch at 3423 cm^{-1} and C=O stretching at 1710 cm^{-1}. R(+)-Doxylamine succinate also showed comparable IR characteristics, with minor shifts, such as a C=O stretch at 1714 cm^{-1} and C=N at 1589 cm^{-1}.

Mass Spectrometric analysis

Mass spectrometry was used to validate the molecular weights of the compounds. The mass spectrum of one isolated compound showed a molecular ion peak at m/z 271 (Figure 2), while the other had a peak at m/z = 270 (Figure 3), showing the molecular integrity and validating the structure.

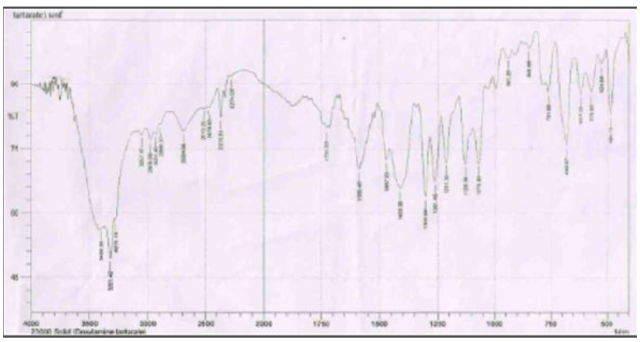

Fig. 1: IR Spectrum of Doxylamine tartarate

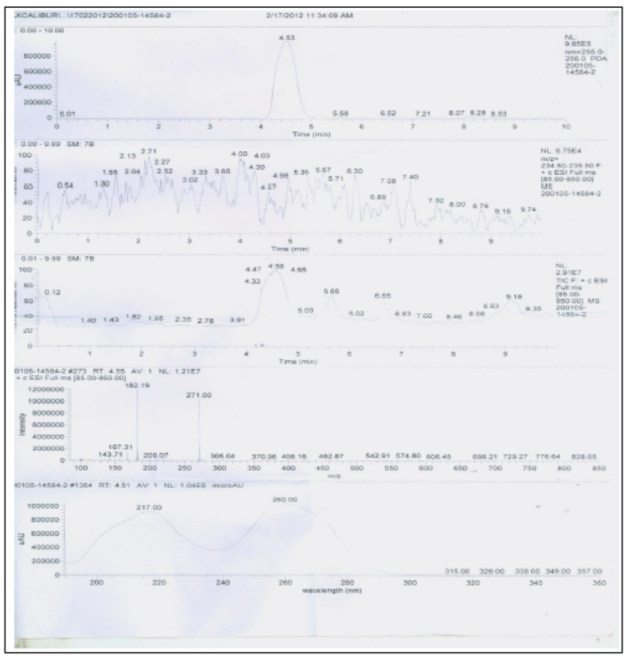

Fig. 2: Mass Spectrum: M+ (271)

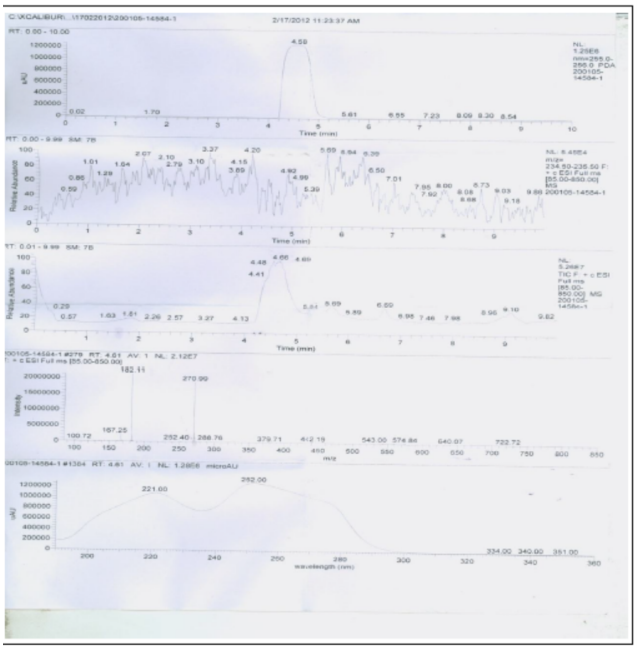

Fig. 3: Mass Spectrum: M+ (270)

NMR Spectroscopic analysis

The proton NMR (^{1}H-NMR) spectra provided elaborate structural information. The racemic mixture showed characteristic peaks: δ 8.56 (d, 1H, pyridine), δ 7.92 (s, 1H, pyridine), δ 7.22–7.8 (m, 8H, aromatic), δ 3.5–3.6 (d, 2H, OCH_2), δ 3.21 (d, 2H, CH_2), and δ 1.89–1.90 (2s, 6H, $N(CH_3)_2$). The resolved isomers contained similar aromatic and alkyl signals and were slightly shifted according to the isomeric environment. Overall, singlets for the dimethylamino group ($N(CH_3)_2$) were always present at δ 1.89–1.92, reflecting the presence of the functional tertiary amine.

Antihistaminic activity

The antihistaminic activity of the racemic and resolved compounds was assessed using guinea pig ileum tissue. R(+)-Doxylamine succinate was the most active, with 95.83% inhibition of histamine, followed by 91.66% for the racemic mixture, and 87.5% for the S(–)-isomer. The same trend was observed for tartarate salts, in which R(+)-doxylamine tartarate showed an inhibition of 95.32%, while racemic and S(–)-isomers showed inhibition of 90.6% and 91.0%, respectively. The same values are presented in Table 3, which indicates that the R(+)-isomer has more antihistaminic activity, which is in close agreement with the action of reference antihistamine drugs.

Table 3: Antihistaminic activity of racemic doxylamine and its resolved isomers (succinate and tratrate salts)

Racemic Doxylamine succinate and its isomers	**% Inhibition**
R,S doxylamine succinate	91.66%
R (+)- Doxylamine succinate	95.83 %
S(-)- Doxylamine succinate	87.5%
Racemic Doxylamine tartarate and its isomers	**% Inhibition**
R,S doxylaminetartarate	90.6%
R (+)- Doxylamine tartarate	95.32 %
S(-)- Doxylamine tartarate	91.0%

DISCUSSION

In the present study, racemic (R,S)-doxylamine succinate was resolved into its enantiomers, R-(+)-doxylamine and S-(–)-doxylamine, and then characterised and evaluated for their pharmacological activity. The characterisation involved melting point determination, infrared (IR) spectroscopy, optical rotation determination, and nuclear magnetic resonance (NMR) spectroscopy. Melting point analysis indicated that the racemic doxylamine succinate had a melting range of 100–104 °C, consistent with reported values for Form I polymorphs.[10] The optically resolved R-(+)-doxylamine succinate and S-(–)-doxylamine succinate had slightly lower melting points of 90–93 °C and 84–87 °C, respectively, indicating successful enantiomer separation. In the same way, the racemic doxylamine tartrate melted at 141–144 °C, while the R-(+)- and S-(–)-enantiomers melted at 152–155 °C and 120–123 °C, respectively, indicating disparities in crystalline structures for the isomers[10].

IR spectroscopy provided information on the functional groups of the compounds. The racemic doxylamine tartrate had a characteristic quaternary nitrogen asymmetric stretching peak at 2370 cm^{-1}, while the R-(+)-enantiomer had this peak at 2360 cm^{-1}. For the succinate salts, the racemic mixture had the quaternary nitrogen peak at 2453 cm^{-1}, while the R-(+)- and S-(–)-enantiomers had peaks at 2337 cm^{-1} and 2672 cm^{-1}, respectively. These differences in the IR absorption bands support the structural differences between the enantiomers.

Optical rotation measurements confirmed the chirality of the resolved compounds. The R-(+)-doxylamine tartrate had a specific rotation of +13°, whereas the S-(–)-enantiomer had a rotation of–41°, indicating the presence of enantiomeric forms. The racemic mixture gave near-zero optical rotation, which was expected for an equimolar mixture of enantiomers. ^{1}H-NMR spectroscopy was used to investigate the proton surroundings of the compounds. Racemic doxylamine tartrate exhibited two singlets at δ 1.895 and 1.908 ppm, reflecting the N-$(CH_3)_2$ protons of the two enantiomers. The S-(–)-enantiomer revealed a single singlet at δ 1.897 ppm, while the R-(+)-enantiomer showed a singlet at δ 1.91–1.92 ppm, indicating effective separation of the enantiomers.

Pharmacological evaluation of antihistaminic activity was conducted using isolated preparations of the guinea pig ileum. R-(+)-Doxylamine succinate produced a greater degree of inhibition of histamine (95.83%) than its S-(–)-enantiomer (87.5%), reflecting enhanced antihistaminic activity. These observations are consistent with previously reported study that the dextrorotatory enantiomer of doxylamine exhibited higher antihistaminic and sedative-hypnotic activity compared to the laevorotatory enantiomer[11].

CONCLUSION

The resolution of racemic doxylamine succinate into its enantiomeric forms with L(+)-tartaric acid and subsequent characterisation proved successful separation and identification of R(+)- and S(–)-doxylamine isomers. The resolved compounds exhibited different physical and spectral characteristics, such as variations in the melting point, optical rotation, and infrared and NMR spectra. Biological testing showed that the R(+)-isomer had a higher antihistaminic activity than the S(–)-isomer. These results demonstrate the possible pharmacological benefit of enantiopure R(+)-doxylamine over the racemic form.

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