ENANTIOSPECIFIC SYNTHESIS OF [1-^{3}H]-(+)-PSEUDOEPHEDRINE HYDROCHLORIDE

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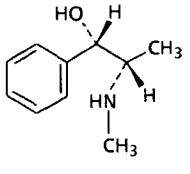
SUMMARY

The naturally occurring dextrorotary enantiomer (+)-pseudoephedrine, 1, was synthesized (1) in the [^{3}H]-labelled form with specific activity 17.5 Ci/mmol suitable for development of a radioimmunoassay procedure. The chirally specific route from *L*-alanine to [1-^{3}H]-*d*-pseudoephedrine hydrochloride was based on the use of α-amino acids as chiral educts for asymmetric products as developed by Rapoport.

Key Words: [^{3}H]-(+)-pseudoephedrine, *d*-ψ-ephedrine, (+)-(*S*,*S*)-2-methylamino-1-phenyl-1-propanol hydrochloride, SUDAFED®, ACTIFED®, nasal decongestant, radioimmunoassay.

INTRODUCTION

d-Pseudoephedrine 1, which was first isolated from the Chinese plant *Ma Huang* by Chou and Read (2) in 1926, is in widespread use as a proven, clinically effective nasal decongestant (3-5).



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In order to determine drug disposition following oral administration of both immediate- and sustained-release pseudoephedrine preparations, a radioimmunoassay procedure for direct plasma analysis was required. Development of the radioimmunoassay required a high specific activity tritium labelled form of pseudoephedrine. The synthesis of [^{3}H]-*dl*-pseudoephedrine and development of a stereospecific radioimmunoassay for 1 in human plasma employing [^{3}H]-*dl*-pseudoephedrine as radioligand have been reported previously (6). This assay involved the use of an antiserum which was stereospecific, having been elicited by immunization of rabbits with a conjugate of bovine serum albumin and an analog of *d*-pseudoephedrine. This stereospecificity was confirmed by the low cross-reactivities observed for other ephedrine isomers (6). Although only about 35% of the [^{3}H]-*dl*-radioligand added could be bound by excess antibody, studies of bioavailability and disposition of pseudoephedrine using it in the radioimmunoassay have been completed successfully (7-9).

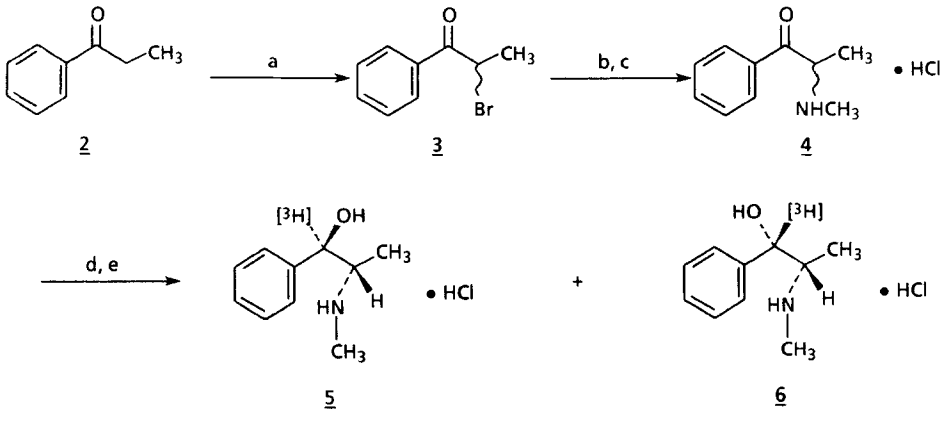
It was expected that the use of enantiomerically pure [^{3}H]-*d*-pseudoephedrine would improve the apparent binding capacity and titer of the antiserum, thus leading to better utilization of antiserum and a more sensitive radioimmunoassay.

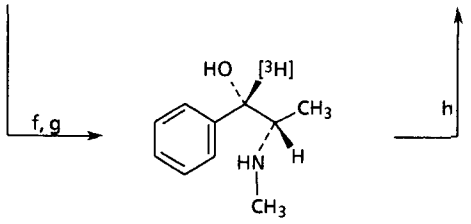
This paper describes the preparation of tritium labelled *d*-pseudoephedrine hydrochloride with a specific activity of 17.5 Ci/mmol by a chirally specific route from *L*-alanine.

RESULTS AND DISCUSSION

The synthesis of [α-^{3}H]-*dl*-pseudoephedrine hydrochloride 6 from propiophenone was reported earlier (6). The compound was prepared by a modification of methods described previously (10-12) for the unlabelled compound. The reaction sequence is summarized in Scheme 1.

Scheme 1





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a) Br_2, CH_2Cl_2, reflux, 60 min
b) CH_3NH_2, EtOH, 0°C, 1.5 h
c) HCl, 0°C, Et_2O, $CHCl_3$; EtOH/acetone
d) [^{3}H]-$NaBH_4$, EtOH, 25°C, 60 min
e) HCl, H_2O, Et_2O, NaOH, Et_2O; MeOH/HCl
f) Ac_2O, reflux, 30 min
g) HCl, H_2O, 100°C, 2 h; 0°C, H_2O, NaOH
h) MeOH, 12*N* HCl; isoamyl alcohol/THF

Bromination of propiophenone gave *dl*-α-bromopropiophenone 3, which was condensed with methylamine gas in ethanol at 0° to give *dl*-ephedrone hydrochloride 4. Treatment of this compound with sodium boro[^{3}H]hydride in ethanol gave a quantitative yield of predominantly the *erythro* compound, [^{3}H]-*dl*-ephedrine hydrochloride 5.

In the Welsh procedure (11), the *erythro* diastereomer undergoes an N→O acyl migration with inversion of configuration upon reaction with refluxing acetic anhydride while the *threo* isomer undergoes the same migration with retention of configuration. Thus, acetylation of the crude ephedrine, followed by hydrolysis in aqueous hydrochloric acid, gave the free base [^{3}H]pseudoephedrine 7, which was converted to 6 (64% yield from ephedrine).

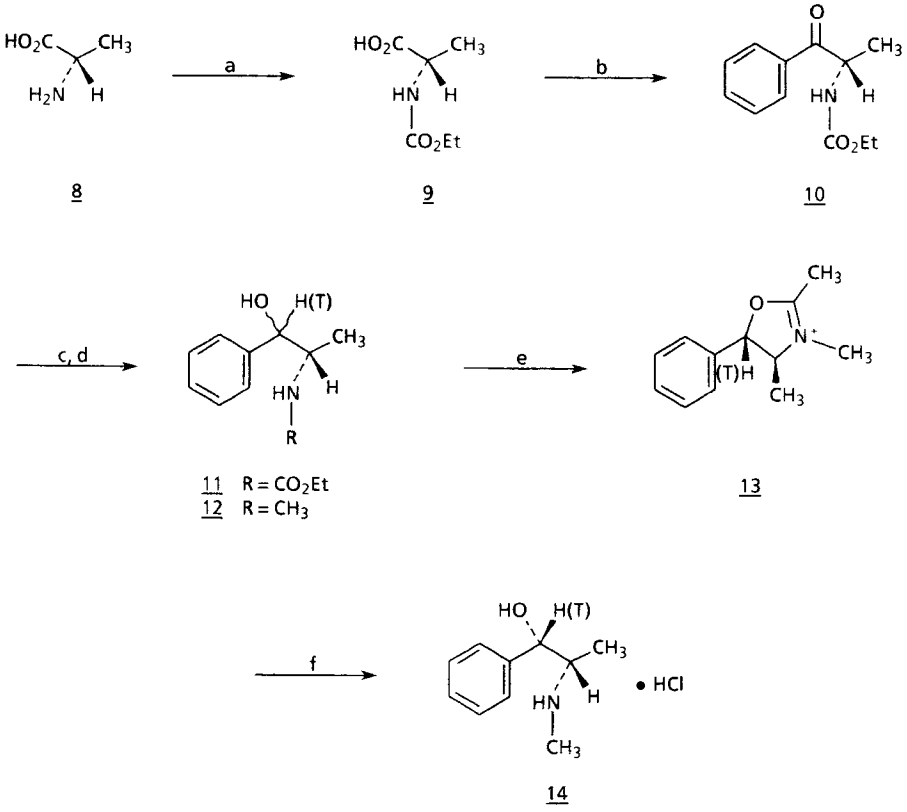
Two recrystallizations from isoamyl alcohol-tetrahydrofuran gave pure [^{3}H]-*dl*-pseudoephedrine hydrochloride 6 with specific activity 5.0 Ci/mmol.

Two useful methods for determination of the diastereomeric purity of the final product were developed during the synthetic work. TLC on silica gel layers in 2-butanone-2-propanol-ammonium hydroxide (6:3:1) clearly separated ephedrine ($R_f = 0.49$) and pseudoephedrine ($R_f = 0.82$). Also, the chemical shift of the α-proton doublet in the ^{1}H NMR spectrum could be used to detect ephedrine (δ 5.24, d, $J = 3$ Hz) in the presence of pseudoephedrine (δ 4.62, d, $J = 10$ Hz) and vice versa.

Following the depletion of the supply of available racemic 6, the enantiospecific synthesis of *d*-pseudoephedrine 1 with a high specific activity tritium label was required for subsequent plasma sample radioimmunoassays and disposition studies to evaluate new delivery forms for SUDAFED® and ACTIFED®. These studies demanded that the [^{3}H]-1 should be chemically stable, should have a high chemical and radiochemical purity, and a minimum specific activity of 15 Ci/mmol.

A chirally specific synthesis of [^{3}H]-labelled 1 from *L*-alanine 8 has now been developed as shown in Scheme 2. The synthesis was based on the work of Buckley and Rapoport (13) on the use of α-amino acids as chiral educts for asymmetric products.

Scheme 2



a) NaOH, Cl·CO_2Et, 15°C, 1 h
b) $(COCl)_2$, C_6H_6, $AlCl_3$, -15°C, 12 h
c) CH_3OH, $NaBH_4$(T), 20°C, 1 h
d) THF, $LiAlH_4$, 60°C, 2 h
e) C_6H_5·CH_3, Ac_2O, 115°C, 7 h; H_2SO_4, 90°C, 1 h
f) H_2O, HCl, 90°C, 4 h; 50% NaOH, C_6H_6; MeOH, HCl; isoamyl alcohol/THF

Treatment of *L*-alanine (*S*-(+)-alanine) 8 with ethyl chloroformate at 15°C at pH 9.0-9.5, according to the procedure of Buckley and Rapoport (13), gave a colorless oil in 84.8% yield and shown by ^{1}H NMR to be the carbamate 9.

Compound 9 was converted to the acid chloride and used in the Friedel-Crafts acylation of benzene as previously described (13). The reaction was performed with anhydrous $AlCl_3$ in CH_2Cl_2 at -15°C

overnight. Crystallization of the crude product from hexane gave a white solid in 45.2% yield shown by ^{1}H NMR and melting point to be the propiophenone 10 in good purity.

Reduction of 10 with $NaBH_4$ in EtOH gave a 96.1% yield of the mixture of diastereomers 11 as an oil.

Reaction of 11 with excess $LiAlH_4$ in THF, followed by aqueous NH_4Cl quench and work-up, gave an 88.6% yield of an oil shown by TLC to be a mixture of the diastereomers 12 (ephedrine and pseudoephedrine).

The *erythro* isomer (ephedrine) in this mixture of diastereomers was converted selectively to the corresponding *threo* isomer (pseudoephedrine) by the use of an adaptation of one of the processes described in a Dow Chemical Company patent (14). Treatment of 12 in toluene with excess Ac_2O at 115°C to form the O,*N*-diacetyl derivatives was followed by treatment with conc. H_2SO_4 at 90-95°C to form the oxazolinium salt 13 which was not isolated. Hydrolysis with aqueous HCl, to open the ring with concomitant retention of the *threo* stereochemistry, was followed by basification and extraction to give 74.6% of a colorless crystalline residue shown to be pseudoephedrine by TLC. After treatment with conc. HCl in methanol, the crude hydrochloride salt was recrystallized from isoamyl alcohol/THF.

The product 14 was obtained as a white solid in 28.9% yield (24.6% overall from the propiophenone 10), and was shown to be of excellent purity by TLC. FT ^{1}H NMR (DMSO-d_6) confirmed the absence of *l*-ephedrine at the 1% level. While the doublet at δ 4.62 (J = 10 Hz) for the α-proton of pseudoephedrine was easily observed, the doublet for the α-proton of ephedrine at δ 5.24 (J = 3 Hz) could not be detected. Determination of specific rotations showed the product to be the desired optical isomer, and confirmed the absence of racemization during the synthesis. For authentic *d*-pseudoephedrine HCl, $[\alpha]_D^{20}$ = +63.9° (c = 0.8, 95% EtOH); for the product, $[\alpha]_D^{20}$ = +63.8°

Thus, *d*-pseudoephedrine HCl of excellent purity has been produced without racemization in 24.6% overall yield from the propiophenone 10.

The radiolabelled synthesis of high specific activity [^{3}H]-*d*-pseudoephedrine hydrochloride 14 from (*S*)-2-[(ethoxycarbonyl)amino]propiophenone 10 was carried out using essentially the same sequence and reaction conditions. Reaction of 10 with [^{3}H]-$NaBH_4$ in EtOH gave a 98.9% yield of the mixture of diastereomers 11 as an oil. Reaction of 11 with excess $LiAlH_4$ in THF gave a 93.9% yield of an oil shown by TLC to contain a mixture of the diastereomers 12 (ephedrine and pseudoephedrine) as expected.

Treatment of the oil with excess Ac_2O to form the O,*N*-diacetyl derivatives was followed by treatment with conc. H_2SO_4 to form the oxazolinium salt 13 (not isolated). Hydrolysis with aqueous HCl was followed by basification and extraction to give an 87.5% yield of a yellowish residue. This was shown

to be essentially pseudoephedrine by TLC. After treatment with conc. HCl, the crude hydrochloride salt was recrystallized from isoamyl alcohol/THF to give the tritiated product 14 in 18.7% yield (17.4% overall yield from the propiophenone 10). The radiochemical purity of 14 was >99% by TLC and plate scanning. The specific activity of 14 was found to be 17.5 Ci/mmol.

The binding of the enantiomerically pure [^{3}H]-14 to antibodies elicited in rabbits in response to immunization with a conjugate of *d*-pseudoephedrine coupled to bovine serum albumin was studied as previously described (6). Whereas these antibodies were capable of binding only approximately 35% of a limiting amount of [^{3}H]-*dl*-pseudoephedrine, they could bind more than twice this proportion of the enantiomerically pure [^{3}H]-*d*-pseudoephedrine, 14, indicating that the stereospecific antibodies preferentially recognized the pure [^{3}H]-*d*-radioligand. The radioimmunoassay employing [^{3}H]-14 has now been successfully applied to the analysis of pseudoephedrine in plasma samples from several pharmacokinetic and bioequivalence studies.

EXPERIMENTAL

Sodium boro[^{3}H]hydride was obtained from Amersham International plc at a batch specific activity of ~63.5 Ci/mmol. *L*-Alanine, ethyl chloroformate, oxalyl chloride, anhydrous aluminium chloride, and lithium aluminium hydride were purchased from Aldrich Chemical Company. Acetic anhydride and benzene were purchased from Mallinckrodt Inc. Sodium borohydride was purchased from EM Science. Isoamyl alcohol was purchased from J.T. Baker Inc. All other solvents and reagents were of reagent purity and were obtained from readily available commercial sources. Methylene chloride, *N,N*-dimethylformamide, tetrahydrofuran, benzene and toluene were dried over Type 4A Molecular Sieves. Thin layer chromatography (TLC) was performed on 5 x 20 cm glass plates pre-coated with 0.25 mm silica gel 60 (E. Merck). Proton NMR spectra were obtained using a Perkin-Elmer R-24B spectrometer (60 MHz) or a Varian FT-80A spectrometer (80 MHz) with tetramethylsilane as internal standard. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Specific rotations were determined on a Perkin-Elmer 141 Polarimeter. Radiochemical purity was determined on the TLC plate with a Bioscan System 200 Imaging Scanner. The specific activity was determined on a weighed sample using a Packard 2000CA liquid scintillation counter and Aquasol-II (DuPont NEN) liquid scintillation cocktail.

L-N-Ethoxycarbonylalanine 9

9 was prepared according to the method of Buckley and Rapoport (13). From 13.35 g of *L*-alanine

(*S*-(+)-alanine) was obtained 20.84 g (84.8%) of a colorless oil; ^{1}H NMR ($CDCl_3$): δ 1.2 (3H,t), 1.4 (3H,d), 4.15 (2H,q), 4.25 (1H,m), 5.5 (1H,bs).

(*S*)-2-[(Ethoxycarbonyl)amino]propiophenone 10

10 was also prepared by the Rapoport procedure (13). From 1.61 g of 9 was obtained, after crystallization from hexane, 1.00 g (45.2%) of white solid 10; m.p. 61.5-62.5°C [Lit. (13) m.p. 62-63°C]; ^{1}H NMR ($CDCl_3$): δ 1.15 (3H,t), 1.32 (3H,d), 4.05 (2H,q), 5.25 (1H,qn), 5.7 (1H,bd), 7.4-7.9 (5H,m).

{(*S*)-2-[(Ethoxycarbonyl)amino]-[1-^{3}H]-1-hydroxy}propylbenzene 11

A solution of 10 (139.5 mg) in EtOH (7.1 mL) was added dropwise during 3 min to a stirred mixture of sodium boro[^{3}H]hydride (13.3 mg, 20 Ci at ~63.5 Ci/mmol) in EtOH (7.1 mL) at 5-10°C under argon. After 60 minutes at 25°C, acetone (1.4 mL) was added, and the mixture was stirred for a further 10 minutes. After evaporation to dryness under reduced pressure, the white solid residue was partitioned between CH_2Cl_2 (25 mL) and saturated aqueous $NaHCO_3$ (21 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 x 14 mL). The combined organic extracts were dried and the solvent evaporated *in vacuo* to give a crude mixture of the diastereomers 11 (140.0 mg, 98.9%) as a colorless oil.

[α-^{3}H]-*l*-Ephedrine and [α-^{3}H]-*d*-Pseudoephedrine 12

To a stirred solution of the crude 11 (140.0 mg) in dry THF (7.4 mL) at 25°C under argon was added $LiAlH_4$ (90.8 mg) and then dry THF (4.4 mL and 3.0 mL). The mixture was stirred at 60°C for 2 h, allowed to cool for 2 h, and stirred at 0°C for 30 min.

After dropwise addition of saturated aqueous NH_4Cl (7.4 mL) and then H_2O (7.4 mL), the reaction mixture was filtered and evaporated to dryness under reduced pressure. The residue was partitioned between CH_2Cl_2 (20 mL) and 1*N* aqueous NaOH solution (20 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic extracts were dried and the solvent evaporated *in vacuo* to give a crude mixture of the diastereomers 12 (97.5 mg, 93.9%) as a colorless oily residue. TLC using 2-butanone:2-propanol:NH_4OH (6:3:1) showed the expected mixture of ephedrine (R_f = 0.50) and pseudoephedrine (R_f = 0.76).

[α-^{3}H]-*d*-Pseudoephedrine Hydrochloride 14

A stirred mixture of 12 (97.5 mg), dry toluene (1.6 mL) and acetic anhydride (0.8 mL) was heated at 115°C for 7 hours under argon. After cooling overnight, 36*N* H_2SO_4 (5 drops) was added and the mixture heated at 90-95°C for 60 min. After cooling for 30 min, H_2O (0.3 mL) was added dropwise followed by 12*N* HCl (2 drops), and the mixture was heated at 90°C for 4 hours. After cooling and addition of 50% NaOH solution (3.0 mL), the mixture was partitioned between H_2O (15 mL) and benzene (15 mL).

The organic layer was dried and the solvent evaporated *in vacuo* to give a yellowish residue (85.3 mg, 87.5%). TLC using 2-butanone:2-propanol:NH_4OH (6:3:1) showed the product to be pseudoephedrine ($R_f = 0.75$). No ephedrine was detected under UV light but a radioactive chromatogram scan was not performed at this stage. Treatment of the crude product in methanol (7 mL) with 12*N* HCl (4 mL) was followed by evaporation of the solvent to dryness under reduced pressure and crystallization of the residue (104.6 mg) from isoamyl alcohol/THF (0.9 mL/13.7 mL).

The mixture was cooled at -10°C overnight and the crystals were filtered, washed with ice-cold THF, and dried *in vacuo* at 56°C overnight.

The yield of off-white solid 14 was 22.2 mg (1.92 Ci; 18.7% yield; 17.4% overall from 10) with specific activity 17.5 Ci/mmol.

TLC using 2-butanone:2-propanol:NH_4OH (6:3:1) showed single-spot material under UV 254 nm light at $R_f = 0.78$ corresponding to authentic 14. Radioactive chromatogram scanning showed the presence of 0.5% impurity at $R_f = 0.52$ corresponding to ephedrine; thus the radiochemical purity was >99%.

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