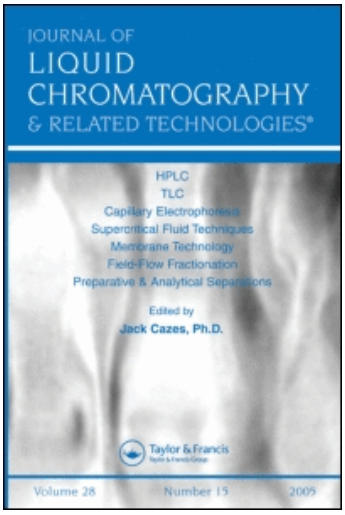

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LIQUID CHROMATOGRAPHIC ANALYSIS OF THE ENANTIOMERIC COMPOSITION OF NOREPHEDRINE AND NORPSEUDOEPHEDRINE BENZYLIC INVERSION PRODUCTS

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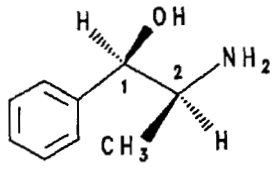
ABSTRACT

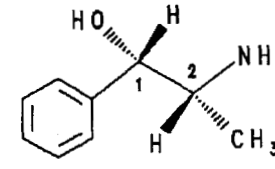
Three of the four stereoisomers of 1-phenyl-2-amino-1-propanol (phenylpropanolamine) are available as the single enantiomers. The S,S-stereoisomer is available only as a component of racemic norpseudoephedrine (S,S- and R,R-1-phenyl-2-amino-1-propanol). The 1S,2S-norpseudoephedrine was prepared from 1R,2S-norephedrine via a benzylic inversion synthetic procedure. This reaction sequence was found to invert the configuration of the benzylic hydroxyl-group in norephedrine and norpseudoephedrine to yield the corresponding diastereomer. The configuration of the product was determined using reversed-phase liquid chromatography following derivatization with 2,3,4,6-tetra-O-acetyl-ß-D-glucopyranosyl isothiocyanate (GITC).

INTRODUCTION

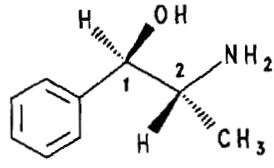
Phenylpropanolamine or 1-phenyl-2-amino-1-propanol contains two chiral centers giving rise to four different stereoisomeric forms or two pairs of enantiomers (Scheme 1). The enantiomeric



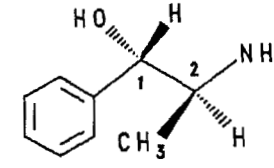
1R,2S-NOREPHEDRINE



1S,2R-NOREPHEDRINE



1R,2R-NORPSEUDOEPHEDRINE

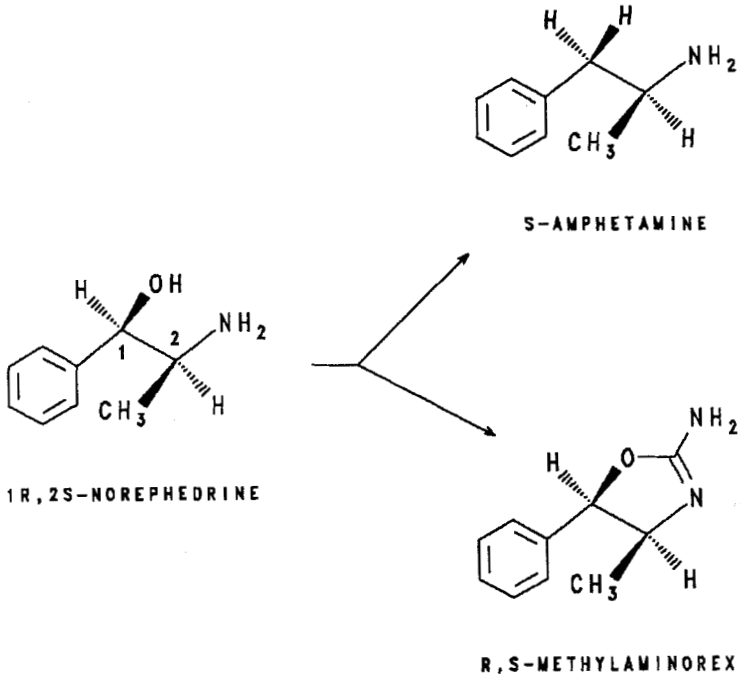


1S,2S-NORPSEUDOEPHEDRINE

Scheme 1. The structures of the stereoisomers of 1-phenyl-2-amino-1-propanol.

pair with the 1S,2R and 1R,2S-configurations are commonly called norephedrines, while the enantiomeric pair with the 1R,2R- and 1S,2S-configurations are called norpseudoephedrines. Also, racemic norephedrine (a mixture of equal parts of 1S,2R- and 1R,2S-norephedrine) is most commonly referred to as phenylpropanolamine. These compounds possess sympathomimetic activity and have been used in therapy as bronchodilators, nasal decongestants and anorexic agents (1). These amines have also been employed by clandestine chemists for synthesis of substances of abuse including amphetamines and methylaminorex (2,3).

Norephedrine and norpseudoephedrine contain the structural elements of amphetamine in chiral form. A number of methods have



Scheme 2. The synthesis of amphetamine and methylaminorex using norephedrine and norpseudoephedrine starting materials.

been reported for the displacement of the 1-hydroxyl group (benzylic hydroxyl) present in these compounds with hydrogen to yield amphetamines (2,4). Also, earlier studies have demonstrated that these displacements occur without altering the configuration of the carbon atom at position 2 (2). Therefore, displacements with 1R, 2S-norephedrine and 1S,2S-norpseudoephedrine yield S-amphetamine, while similar displacements with 1R,2S-norephedrine or 1R,2R-norpseudoephedrine yield R-amphetamine (Scheme 2). Similarly, the configuration is also conserved when

the norephedrines and norpseudoephedrines are used in the synthesis of methylaminorex derivatives (Scheme 2) (3).

The study of the stereochemistry of the reactions to form amphetamine and methylaminorex required each stereoisomer of norephedrine and norpseudoephedrine as a starting material. Currently only the S,R- and R,S-enantiomers of norephedrine, the R,R-enantiomer of norpseudoephedrine and the racemate of norephedrine are commercially available. Therefore it was necessary to synthesize S,S-norpseudoephedrine and develop a suitable method for analysis of the enantiomeric composition of this product. This paper reports the synthesis of S,S-norpseudoephedrine from R,S-norephedrine by benzylic inversion and reversed-phase liquid chromatographic methods for the analysis of enantiomeric purity of the product.

EXPERIMENTAL

Reagents and chemicals

Samples of S,R- and R,S-norephedrine and R,R-norpseudoephedrine were obtained from Aldrich Chemical Company (Milwaukee, WI). Reagent grade phenylisothiocyanate (PIT) was purchased from Polysciences (Warrington, PA). HPLC grade methanol and acetonitrile were obtained from J. T. Baker Chemical and acetonitrile were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). All other chemicals were reagent grade and were used without further purification.

Synthesis of 1S,2S-norpseudoephedrine

A 10% aqueous sodium bicarbonate solution (40 mL) was added to a solution of 1R,2S-norephedrine (2.5 g, 16.5 mmoles) in ethyl acetate (20 mL) and the resulting mixture cooled in an ice bath.

Benzoyl chloride (2.3 mL, 20 mmoles) was then added dropwise over a 30 minute period, and the mixture allowed to warm to room temperature and stirred for 4 hours. The white precipitate which formed was isolated by filtration, washed with water (3 X 25 mL) and dried at 90°C for 14 hours to yield 4.2 g of 1R,2S-N-benzoylnorephedrine (mp 168-170°C). The N-benzoyl intermediate (3.2 g) was added portionwise with stirring to thionyl chloride (25 mL) at room temperature, and the mixture stirred at reflux for 1 hour after the addition was complete. The reaction mixture was then cooled in an ice bath and added dropwise to ice water (60 mL). The resulting mixture containing a white precipitate was stirred at room temperature overnight. Ethanol (30 mL) was added and the reaction mixture stirred at reflux for 4 days. The mixture was cooled to room temperature and concentrated to 30 mL under reduced pressure. Upon cooling (ice bath) benzoic acid precipitated and was removed by filtration. The filtrate was then made basic with 10% aqueous NaOH and the solution saturated with NaCl. The aqueous solution was extracted with ethyl acetate (5 X 25 mL) and the combined organic extracts dried over anhydrous sodium sulfate. Filtration followed by evaporation of the filtrate solvent under reduced pressure yielded a brown oil. The oil was dissolved in ethyl acetate (50 mL) containing decolorizing carbon and stirred at reflux for 30 minutes. This mixture was hot filtered and the filtrate cooled in an ice bath. An equal volume of carbon tetrachloride was added and the mixture cooled (freezer) to yield the product as white needles. The product was isolated by filtration and dried under reduced pressure at room temperature. The diastereomeric product, 1S,2R-norephedrine, was synthesized using the same method described above, starting with 1R,2R-norpseudoephedrine. The structures of

the intermediates and products were confirmed by IR (KBr) and ^{1}-H NMR (deuterated DMSO). The purity of the products was established by GC-MS and the liquid chromatographic analysis.

Instrumentation

The liquid chromatograph consisted of a Waters Associates Model 6000A pump, U6K injector, Model 440 UV detector with a dual wavelength accessory operated at 254 and 280 nm, and a Houston Instruments OmniScribe dual pen recorder. Infrared spectra were recorded on a Perkin-Elmer Model 1710 Fourier transform infrared (FTIR) spectrophotometer. Ultraviolet spectra were recorded on a Shimadzu Instruments Model UV-160 spectrophotometer. Nuclear magnetic resonance spectra (1H) were determined using a Varian EM-360 60 MHz spectrometer.

PIT derivatization procedures

Samples of the amines (approximately 2 mg) were dissolved in 0.45 N NaOH and extracted as the bases into chloroform. To this solution 10 uL of PIT was added and the reaction mixture evaporated to dryness under a stream of air. The resulting residue was dissolved in 1 mL of HPLC grade acetonitrile and 10 uL of this solution injected into the liquid chromatograph.

GITC Derivatization Procedures

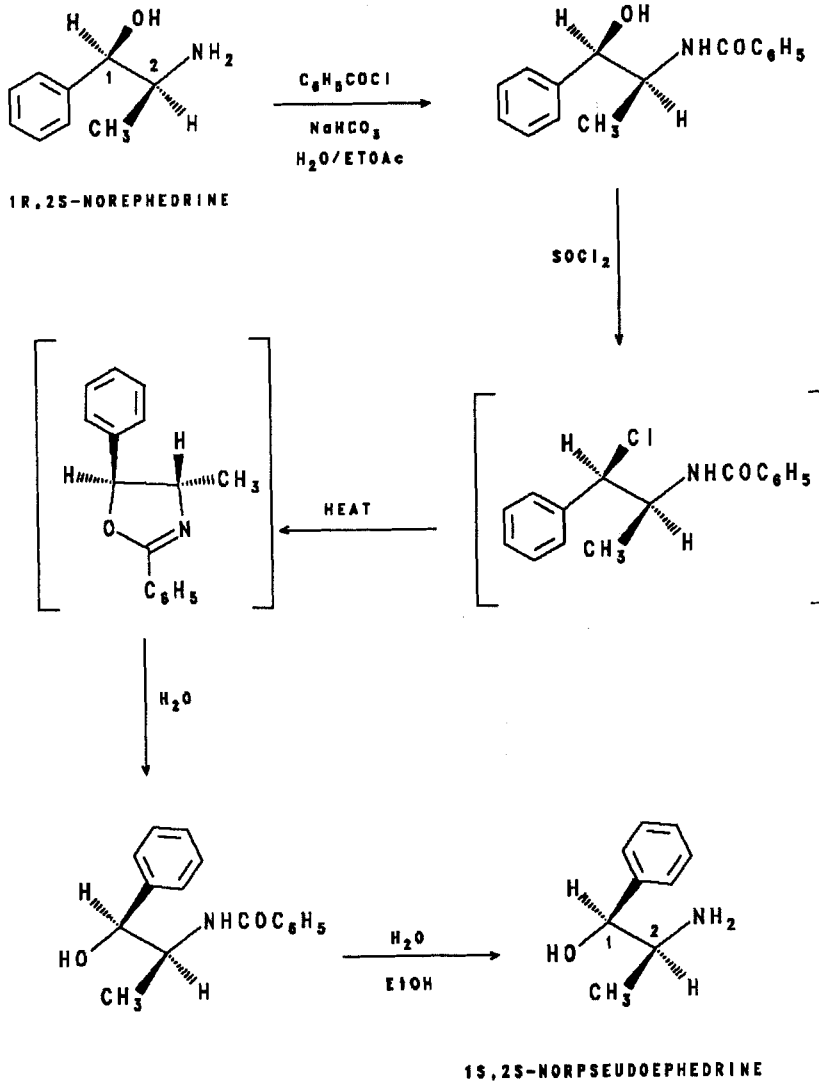
Samples of the amines (approximately 2 mg) were dissolved in 0.45 N NaOH and the bases extracted into chloroform. The chloroform extracts were combined and a 10% molar excess of GITC added as a solution in chloroform. After 10 minutes at room temperature, the solution was evaporated to dryness under a stream of air. The resulting residue was dissolved in 1 mL of HPLC grade methanol and 10 uL injected into the liquid chromatograph.

Liquid Chromatographic Procedures

The analytical column was 30 cm X 3.9 mm i.d. packed with uBondapak C_{18} (Waters Associates). The analytical column was preceded by a 7 cm X 2.1 mm i.d. guard column packed with CO:Pell ODS (Whatman). The derivatives were dissolved in HPLC grade acetonitrile or methanol (1.0 mg/mL) and chromatographed using a mobile phase of methanol, water and acetic acid (45:54:1 for the PIT derivatives and 50:49:1 for the GITC derivatives). The mobile phase flow rate was 1.5 mL/min and the detector was operated at 0.2 AUFS. A 10 uL aliquot of each derivative solution was injected into the liquid chromatograph.

RESULTS AND DISCUSSION

The synthesis of S,S-norpseudoephedrine from R,S-norephedrine was accomplished using previously reported methods as outlined in Scheme 3 (3). Treatment of R,S-norephedrine with benzoyl chloride in a solvent mixture of aqueous base and ethyl acetate afforded the R,S-N-benzoylnorephedrine. Reaction of this intermediate with thionyl chloride resulted in benzylic chlorination with retention of configuration at carbon 1 (2). Intramolecular displacement of chloride by the benzoyl amide oxygen atom afforded an intermediate oxazoline. This intramolecular Sn2 reaction should result in inversion at the benzylic carbon (carbon 1), yielding an oxazoline with the S,S-configuration. Aqueous hydrolysis of this unstable intermediate should then afford the inversion intermediate, 1S,2S-N-benzoylnorpseudoephedrine. Finally, the N-benzoyl moiety was cleaved by hydrolysis in aqueous ethanol, yielding the proposed 1S,2S-norephedrine upon workup. This same synthetic method was applied with 1R,2R-norpseudoephedrine in an attempt to prepare 1S,2R-norephedrine.



Scheme 3. Benzylic inversion synthesis of 1S,2S-norpseudoephedrine from 1R,2S-norephedrine.

Since both the 1R,2R- and 1S,2R-stereoisomers are available in enantiomerically pure form, the inversion reaction with these compounds served as an excellent model system.

The stereochemistry of the inversion reactions described above was initially analyzed by reversed-phase liquid chromatography. It was anticipated that the products formed from these reactions would have a diastereomeric relationship to their corresponding starting materials; the desired product 1S,2S-norpseudoephedrine is a diastereomer of the starting material 1R,2S-norephedrine. Since diastereomers have different physicochemical properties, it should be possible to separate these compounds using an achiral stationary phase. Both the starting norephedrine and norpseudoephedrine are basic, primary amines with weak chromophores. Therefore, to enhance chromatographic properties and increase UV detectability, the compounds were derivatized prior to analysis with phenylisothiocyanate (PIT). Reaction of these amines with PIT yields stable, non-basic thiourea derivatives that are readily detected at 254 nm and provide good retention and peak shape. This is illustrated by the chromatogram in Figure 1 where the PIT derivatives of both the starting 1R,2S-norephedrine and the inversion product were separated using a C_{18} stationary phase and a mobile phase of methanol, water and acetic acid (45:54:1). The peak of higher retention (peak 2) is the starting norephedrine, while the peak eluting earlier is the product. This chromatogram clearly demonstrates that the product formed has different properties and suggests, based on the mechanisms of the synthesis, that it is the desired diastereomeric product, 1S,2S-norpseudoephedrine. The retention of the derivatized product matched that of PIT-derivatized samples of known 1R,2R-norpseudoephedrine.

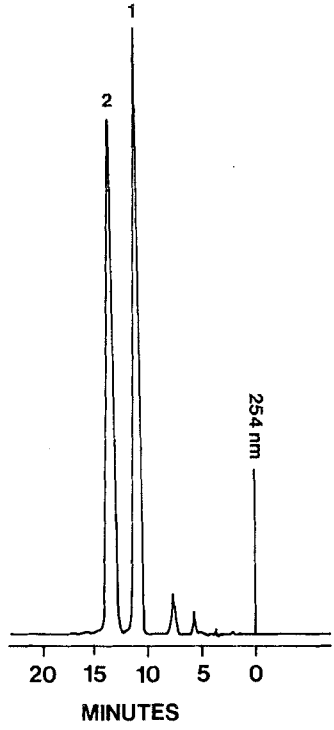


Figure 1. Reversed-phase liquid chromatographic separation of the PIT derivatives of norpseudoephedrine (1) and norephedrine (2) from the inversion reaction of 1R,2S-norepdedrine.

As shown in Figure 2, similar results were obtained upon reversed-phase chromatographic analysis of the PIT derivatives from the 1R,2R-norpseudoephedrine inversion reaction. Again, the significant difference in retention for the product (peak 2) and the starting norephedrine strongly suggests that inversion to form a diastereomer has occurred. This was confirmed by

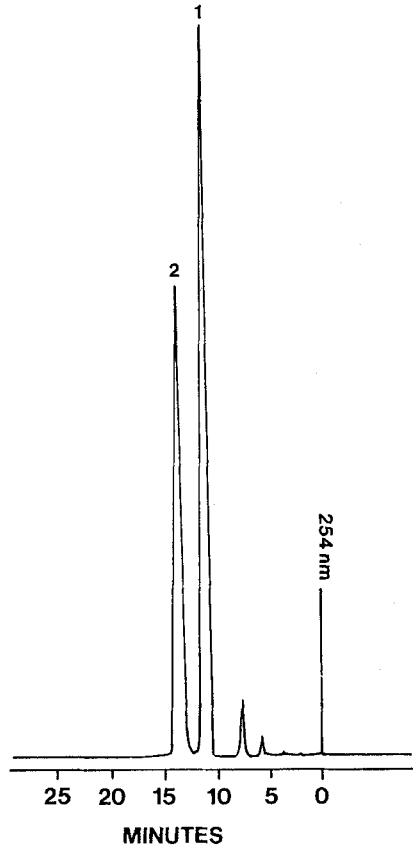


Figure 2. Reversed-phase liquid chromatographic separation of the PIT derivatives of norpseudoephedrine (1) and norephedrine (2) from the inversion reaction of 1R,2R-norpseudoephedrine.

chromatographic analysis of PIT derivatives of commercially available 1R,2R-norpseudoephedrine and 1S,2R-norephedrine.

While the analysis of PIT derivatives of the starting amines and products described above provides evidence in support of the contention that the inversion reactions proceeded as anticipated,

it does not provide evidence for the absolute stereochemistry of the products. To accomplish this, the enantiomeric composition of the inversion reactions described above was also analyzed by reversed-phase liquid chromatography. A variety of liquid chromatographic methods have been reported for the chiral separation of enantiomers. Typically these methods make use of chiral stationary phases, chiral mobile phase additives or precolumn diastereomeric derivatization (5-8). In many cases, the chemical derivatization of enantiomeric amines with chiral reagents to yield diastereomeric products offers the advantages of speed and convenience (2,8,9). Many chiral derivatizing agents are available commercially and the resulting diastereomeric products can be resolved on standard achiral stationary phases (2,8,9). Thus the chiral analysis of a sample requires only the additional chiral derivatizing agent. One such agent, 2,3,4,6-tetra-O-acetyl-ß-D-glucopyranosyl isothiocyanate (GITC) has been used for the analysis of a variety of chiral primary and secondary amines similar to the norephedrines and norpseudoephedrines (2,9). In this study, the derivatization of the norephedrine and norpseudoephedrine products was accomplished in chloroform solution at room temperature using a slight excess of GITC (2). The resulting thiourea diastereomers were then analyzed by reversed-phase chromatography on a C_{18} column with a mobile phase of methanol, water and acetic acid (50:49:1). Application of this analysis to the benzylic inversion reactions yielded the chromatograms in Figures 3 and 4. Figure 3 shows the chromatographic analysis of the GITC derivatives of the starting 1R,2R-norpseudoephedrine and inversion product. The retention of the inversion product (peak 2) is consistant only with the retention of the GITC derivative of 1S,2R-norephedrine. Similar results were obtained upon analy-

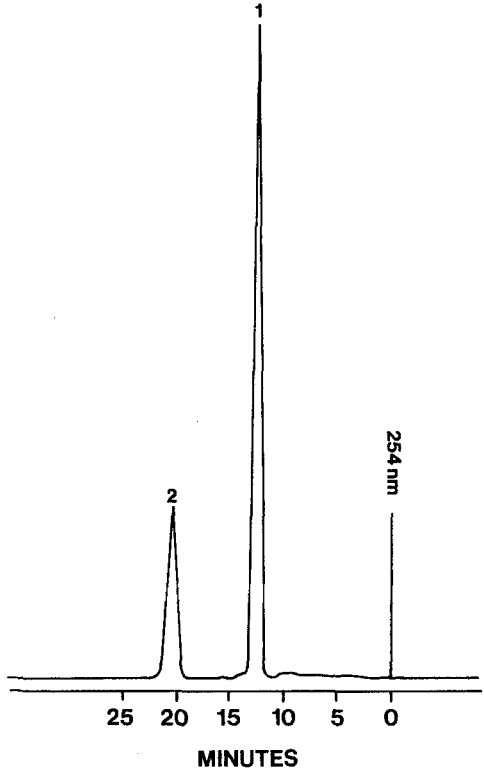


Figure 3. Reversed-phase liquid chromatographic separation of the GITC derivatives of 1R,2R-norpseudoephedrine (1) and 1S,2R-norpehedrine (2) from the inversion reaction of 1R,2R-norpseudoephedrine.

sis of the inversion reaction beginning with 1R,2S-norephedrine (Figure 4). These chromatographic results show that only a single enantiomer was formed by the benzylic inversion reaction and, coupled with the results in Figure 1, show that only a single norpseudoephedrine was formed, 1S,2S-norpseudoephedrine. Therefore, analysis of the GITC derivatives support the conclusion

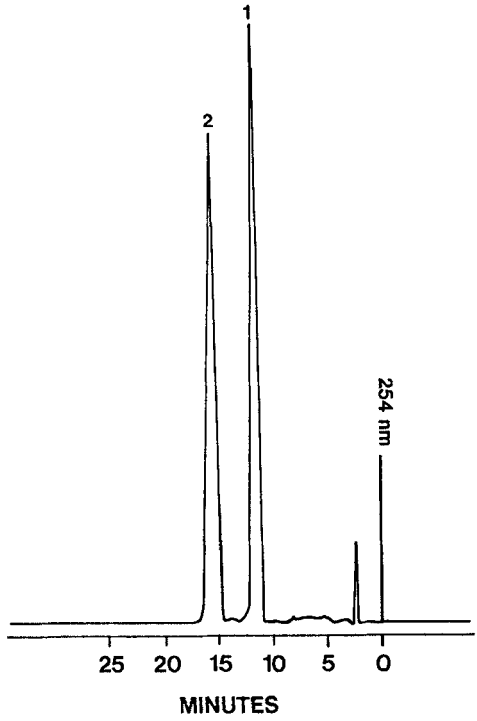


Figure 4. Reversed-phase liquid chromatographic separation of the GITC derivatives of 1S,2S-norpseudoephedrine (1) from the inversion reaction of 1R,2S-norephedrine and 1R,2S-norephedrine (2).

that the synthetic method employed produced the diastereomeric product via inversion at only the benzylic position (carbon 1) without affecting the configuration at the 2-position.

The liquid chromatographic separation of the GITC derivatives from 1R,2S-norephedrine, 1S,2R-norephedrine, 1R,2R-norpseudoephedrine and the synthetic 1S,2S-norpseudoephedrine is shown in Figure 5. This system provides adequate resolution of

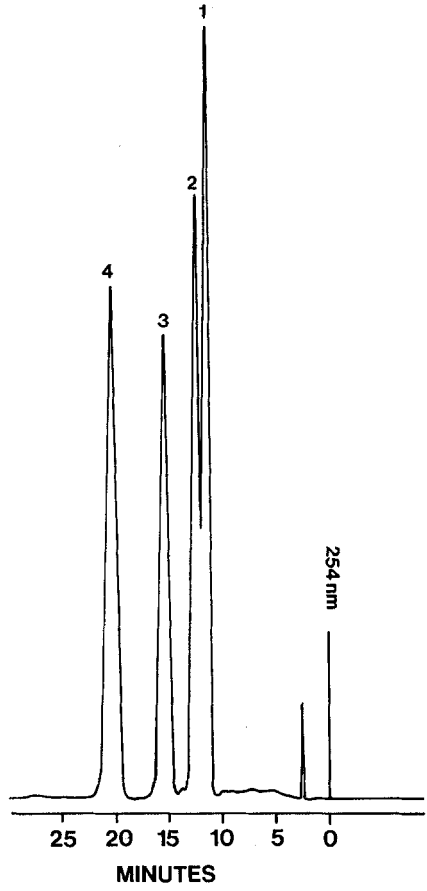


Figure 5. Reversed-phase liquid chromatographic separation of the GITC derivatives of 1S,2S-norpseudoephedrine (1), 1R,2R-norpseudoephedrine (2), 1R,2S-norephedrine (3) and 1S,2R-norpehedrine (4).

all four stereoisomers in a reasonably short analysis time. The GITC derivative of the 1S,2R-enantiomer has the highest capacity factor, followed in order by the GITC derivatives of the 1R,2S-, 1R,2R and 1S,2S-enantiomers. The peaks in this chromato-

gram were identified by analysis of individual GITC derivatives of the 1R,2S- 1S,2R- and 1R,2R-stereoisomers. Therefore, the GITC-derivatization method is a very useful technique for monitoring the stereochemical course of many synthetic procedures. The derivatization reaction is very convenient and the resulting diastereomers can be separated on standard achiral stationary phases.

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