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Note

Resolution of the enantiomers of ephedrine, norephedrine and pseudoephedrine by high-performance liquid chromatography

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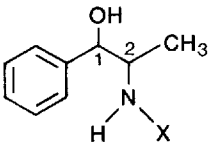
Ephedrine [1-phenyl-2-(methylamino)-1-propanol, EPH, Fig. 1] and the related compounds pseudoephedrine (PSE, Fig. 1) and norephedrine (NOR, Fig. 1) are adrenergic agents widely available in asthma, ophthalmic, cold, and allergy products. The hydroxypropylamino structural element of EPH, PSE and NOR contains two asymmetric centers, and each of these compounds exists as a pair of enantiomers (Fig. 1). Reasons for the interest in the stereochemistry of EPH and related compounds are several, including differences between the enantiomers in their pharmacological actions [1, 2], and reports of stereoselective metabolism of these compounds [3]. Studies on the chromatographic



X	Compound	Absolute Configuration
CH_3	ephedrine	1R,2S-(-)
CH_3	pseudoephedrine	1R,2R-(-)
H	norephedrine	1R,2S-(-)

Fig. 1. The chemical structures and absolute configuration of the compounds studied. The numbering of C_1 and C_2 of the propyl group is also shown.

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resolution of the enantiomers of EPH, PSE and NOR have been few, and, with one exception, limited to gas—liquid chromatography (GLC). Beckett and Testa [4, 5] studied the resolution of the enantiomers of EPH, PSE and NOR as their N-[N'-(trifluoroacetyl)-L-prolyl] derivatives. Poor resolution of the enantiomers of EPH and PSE was attained [4, 5], and another disadvantage of the technique is that the chiral reagent used undergoes partial racemization during its synthesis and/or storage [6, 7]. Frank et al. [8] investigated the resolution of the enantiomers of EPH as their N,O-bis(pentafluoropropionyl) derivatives by GLC on glass capillary columns coated with a chiral stationary phase. Baseline resolution of the EPH enantiomers was not achieved [8]. König and Benecke [9] synthesized new chiral GLC stationary phases and used them in glass capillary columns to separate the enantiomers of a variety of compounds including NOR but not including EPH or PSE.

Wainer et al. [10] recently described the use of a chiral high-performance liquid chromatography (HPLC) column for the resolution of EPH. The procedure involves a 2-h reaction of EPH with 2-naphthaldehyde followed by recrystallization of the oxazolidines produced. The diastereomeric oxazolidines are incompletely resolved by the chromatography system used [10]. PSE and NOR were not studied [10].

In this communication, a new, simple and rapid procedure for the resolution of the enantiomers of EPH, PSE and NOR is described. The method is based on derivatization with the chiral reagent 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) and separation of the resulting diastereomeric thioureas by reversed-phase HPLC.

EXPERIMENTAL

Chemicals and reagents

(±)-Norephedrine hydrochloride was obtained from Eastman Organic Chemicals (Rochester, NY, U.S.A.); (±)-ephedrine hydrochloride, (−)-ephedrine, (−)-pseudoephedrine, and (+)-pseudoephedrine were purchased from Sigma (St. Louis, MO, U.S.A.); (+)-norephedrine was from Aldrich (Milwaukee, WI, U.S.A.); triethylamine and ammonium phosphate (monobasic) were obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.); GITC was purchased from Polysciences (Warrington, PA, U.S.A.), acetonitrile (distilled-in-glass grade) from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.).

Chromatography

A Waters Assoc. (Milford, MA, U.S.A.) HPLC system consisting of a Model M-6000 solvent delivery system, a Model U6K injector, and a Model 440 absorbance detector was used. Separations were carried out on a Beckmann Instruments (Berkeley, CA, U.S.A.) 150 mm × 4.6 mm HPLC column packed with Ultrasphere ODS of 5-μm particle size. The mobile phase was prepared by mixing 400 ml of acetonitrile with 600 ml of water containing 1.4 g of monobasic ammonium phosphate. The two components were vacuum-filtered before mixing. The mobile phase was delivered at 1.0 ml/min, and the column effluent was monitored at 254 nm. Chromatograms were recorded using a Perkin-Elmer (Norwalk, CT, U.S.A.) Model 56 recorder.

Preparation of derivatives

A 1-mg sample of the compound to be derivatized, in the free-base form or as the hydrochloride, was treated with 200 μl of acetonitrile in a test tube. If the compound was in the hydrochloride form, 20 μl of triethylamine was added. A 50-μl aliquot of acetonitrile containing 3 mg of GITC was then added, the mixture was briefly swirl-mixed, and allowed to stand at room temperature for 10 min. Acetonitrile, 500 μl, was added, and 3—5-μl aliquots were injected into the HPLC system.

RESULTS

Fig. 2 shows the resolution of EPH, PSE and NOR. The following values were obtained for the separation factor α [11] and resolution factor R [12]: EPH: α = 1.19, R = 2.73; PSE: α = 1.09, R = 1.32; NOR; α = 1.12, R = 1.71. The diastereomer derived from GITC and the levorotatory enantiomer of EPH, PSE, or NOR elutes before the derivative formed from the corresponding (+)-enantiomer (Fig. 2). The retention times are given in Fig. 2.

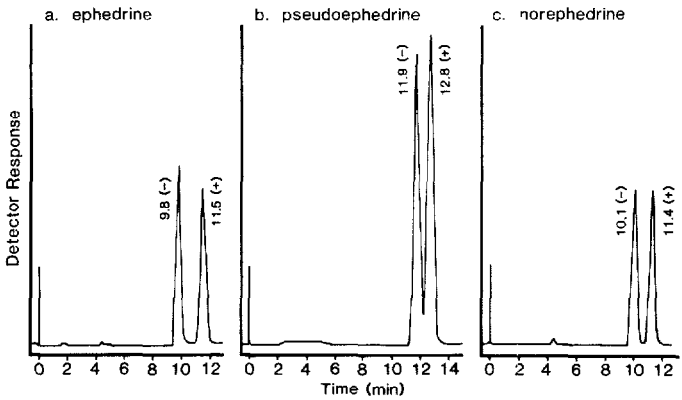

Fig. 2. Resolution of a, (±)-EPH; b, (±)-PSE; and c, (±)-NOR. The retention times in min and the enantiomeric identity of each peak are given.

DISCUSSION

GITC has been shown recently to be a useful chiral reagent for the HPLC resolution of a variety of amines and amino acids [13—15]. Using this reagent, baseline resolution of the enantiomers of EPH and NOR was achieved (Fig. 2). The extent of separation of the enantiomers of PSE was smaller (Fig. 2). If the resolution factor R = 1.0, the resolution of two equal-area peaks is approximately 98% complete [12]. The value of R = 1.32 achieved in the resolution of (±)-PSE, therefore, represents a near-complete separation of the peaks. Fig. 3 shows the structure of the derivative of NOR formed with GITC.

The GITC derivative of the levorotatory enantiomer of each of the three compounds studied eluted before that of its dextrorotatory antipode. Since all three levorotatory compounds possess the R-configuration at C_1 (Fig. 1), it appears that, at least in this series, the order of elution is governed by the configuration around the hydroxyl-bearing C_1 rather than by that around the amino-bearing C_2.

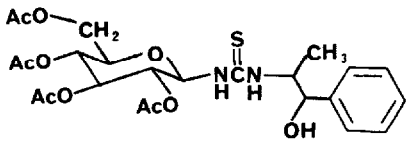

Fig. 3. The chemical structure of the derivative of NOR formed with GITC.

The procedure described has significant advantages: the derivatization method is extremely simple; the chiral reagent is commercially available and is chemically and stereochemically stable; the resolution of EPH, PSE and NOR achieved with GITC is considerably better than that described in previously published GLC [4, 5, 8, 9] or HPLC [10] methods. Thiourea derivatives of GITC have high UV absorption [13, 14], and therefore the present method may be adaptable to the determination of the enantiomers in biological fluids. Such an extension of the procedure would be a useful alternative to the stereospecific radioimmunoassays for (+)-PSE [16] and for the enantiomers of EPH [17].

In conclusion, GITC is a useful reagent for the resolution of the enantiomers of EPH, PSE and NOR by reversed-phase HPLC.

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