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Note

Application of high-performance liquid chromatographic chiral stationary phases to pharmaceutical analysis

Resolution of ephedrine

IRVING W. WAINER* and THOMAS D. DOYLE
Division of Drug Chemistry, Food and Drug Administration, Washington, DC 20204 (U.S.A.)
and
ZOHREH HAMIDZADEH and MARY ALDRIDGE
Department of Chemistry, The American University, Washington, DC 20016 (U.S.A.)
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Ephedrine (I, 1-phenyl-2-(methylamino)-1-propanol, Fig. 1) is an adrenergic agent widely used as a vasopressor, analeptic, and antiasthmatic. More than 100 commercial preparations containing this substance are available in the U.S.A. Ephedrine has two adjacent asymmetric carbon atoms; the levorotatory 1*R*,2*S*-stereoisomer, *l*-ephedrine, has been reported to be three times as active as its 1*S*,2*R*-enantiomer, *d*-ephedrine[1].

Although a number of chromatographic procedures[2–4] separate ephedrine from diastereoisomeric pseudoephedrine (1*S*,2*S*- and 1*R*,2*R*-configurations), only two are concerned with the resolution of enantiomeric *l*- and *d*-ephedrine. Beckett and Tes-

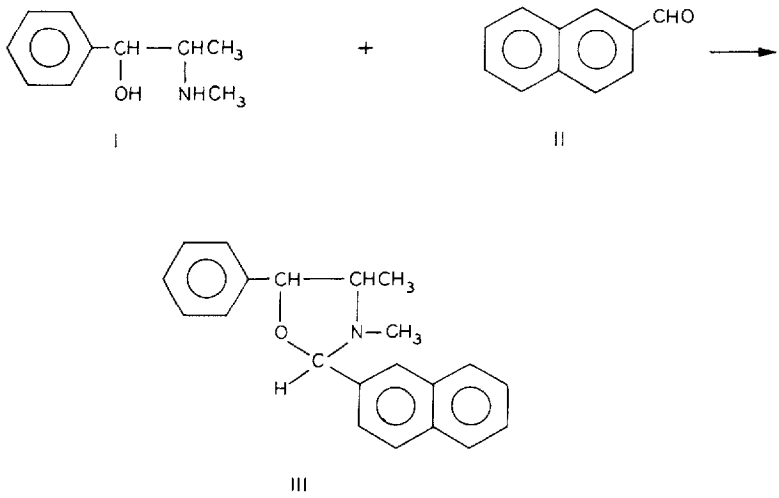

Fig. 1. The reaction of ephedrine (I) with 2-naphthaldehyde (II) to form 3,4-dimethyl-2-(β-naphthyl)-5-phenyloxazolidine (III).

ta[5,6] separated the optical isomers of ephedrine-like compounds via the formation of the diastereoisomeric N-trifluoroacetyl-L-prolyl derivatives, followed by gas–liquid chromatography (GLC); however, only semiquantitative estimates of the *d*- and *l*-isomers of ephedrine and pseudoephedrine were possible.

The direct resolution of the enantiomers of ephedrine and pseudoephedrine was reported by Frank *et al.*[7]. They resolved the enantiomers as N,O-pentafluoropropionyl derivatives on Chirasil-Val, a GLC chiral stationary phase. However, the enantiomers were not adequately separated for pharmacokinetic and regulatory purposes. König and Benecke[8] reported the resolution of a number of amino alcohols, including the N-demethylated analogue of ephedrine, norephedrine[8], on a GLC chiral stationary phase. However, they did not report the resolution of ephedrine. To our knowledge, the resolution of ephedrine and ephedrine-like molecules on high-performance liquid chromatographic (HPLC) chiral stationary phases has not been reported.

We report here the direct enantiomeric resolution of ephedrine by HPLC on a commercially available, ionically bonded chiral stationary phase. The (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine stationary phase was developed by Pirkle *et al.*[9] and is reported to have broad applicability to the resolution of molecules of pharmacological interest[9,10].

The enantiomers of ephedrine were resolved as their cyclic oxazolidine derivatives (III, Fig. 1), which were produced by the condensation of the amino alcohol with 2-naphthaldehyde (II, Fig. 1). The synthesis and absolute configuration of the oxazolidines derived from ephedrine and aromatic aldehydes were described by Neelakantan[11]. After recrystallization the reaction yields a single stereochemically pure product which contains a number of stereospecific sites that can interact with the stationary phase. These sites form the basis for the resolution of the ephedrine enantiomers.

EXPERIMENTAL

Apparatus

The chromatography was performed with a Spectra-Physics (Santa Clara, CA, U.S.A.) Model 8000 liquid chromatograph equipped with an SP 8000 data system, a Spectra-Physics Model 8310 UV–visible detector set at 254 nm, and a temperature-controlled column compartment. The column was a stainless-steel Regis-packed Pirkle Type 1-A (25 cm × 4.6 mm I.D.) with an α-aminopropyl packing of 5-μm spherical particles modified with (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (Regis, Morton Grove, IL, U.S.A.). ^{1}H Nuclear magnetic resonance (NMR) spectra were obtained on a 200 MHz Fourier transform NMR spectrometer (Varian XL-200, Varian Assoc., Instrument Group, Palo Alto, CA, U.S.A.). Optical rotations were measured with a Model 241MC Polarimeter (Perkin-Elmer, Norwalk, CT, U.S.A.). Mass spectra were obtained with a double-focusing, electron-impact mass spectrometer (Varian MAT 311A; Finnigan MAT, San Jose, CA, U.S.A.).

Materials

The *d*- and *l*-isomers of ephedrine free base and the 2-naphthaldehyde were purchased from Aldrich (Milwaukee, WI, U.S.A.). The HPLC solvents were pur-

chased from Burdick & Jackson (Muskegon, MI, U.S.A.). The remaining chemicals and solvents were reagent grade and were used as purchased.

Synthesis procedure

The oxazolidines were synthesized from the free base according to the procedure described by Neelakantan[11]. Ephedrine free base (0.1 mole) and 2-naphthaldehyde (0.1 mole) were dissolved in 100 ml of benzene and refluxed for 2 h. The calculated amount of water was removed by using a Dean-Stark trap. The excess benzene was removed by distillation under reduced pressure and the residue was recrystallized from absolute ethanol.

Chromatographic conditions

The mobile phase was hexane–isopropanol (99.5:0.5). A flow-rate of 1 ml/min and a column temperature of 20°C were maintained throughout the analysis.

RESULTS

The reaction of 2-naphthaldehyde with a 50:50 mixture of *d*- and *l*-ephedrine (Fig. 1) proceeded smoothly and quantitatively. Chromatography of the recrystallized product on the chiral stationary phase produced a chromatogram (Fig. 2a) with two prominent peaks in a 1:1 ratio. The peaks had capacity factors (k') of 6.2 and 7.7, a separation factor (α) of 1.06, and a resolution factor (R_s) of 1.03.

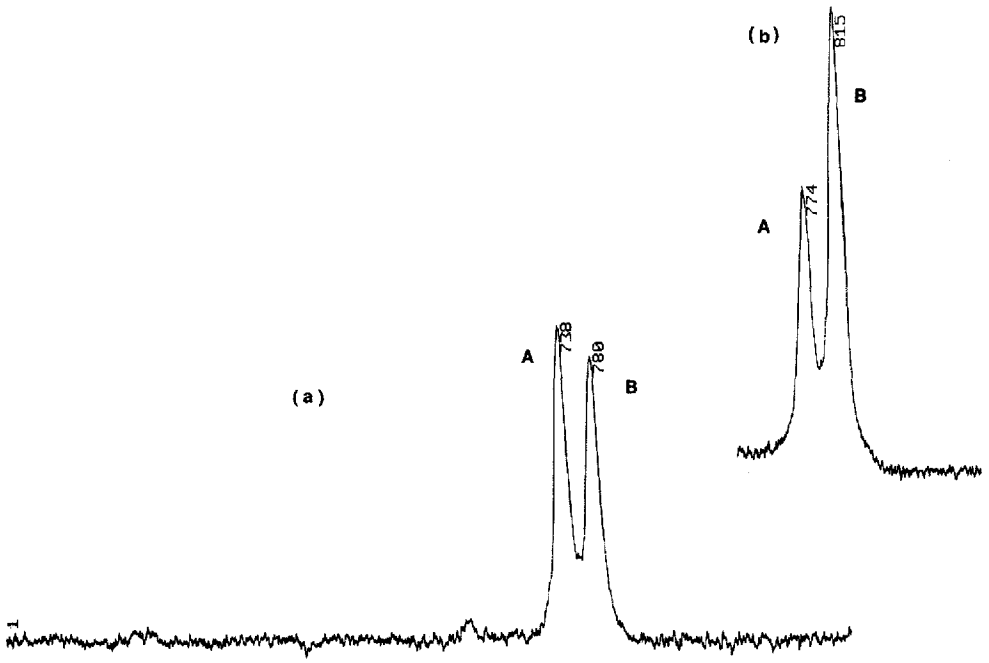

Fig. 2. The chromatograms of mixtures of enantiomeric oxazolidines. Peaks: A = the oxazolidine derived from the cyclization of *l*-ephedrine; B = the oxazolidine derived from the cyclization of *d*-ephedrine. Enantiomeric ratios (A:B) before derivatization: (a) 50:50; (b) 25:75.

The reaction of *l*-ephedrine with 2-naphthaldehyde produced a product whose chromatogram contained a single peak with a capacity factor corresponding to that of compound A in Fig. 2. Similarly, the cyclization of *d*-ephedrine yielded a product corresponding to compound B in Fig. 2. The optical rotations ($[\alpha]_D^{25}$) of the products arising from the derivatization of the *l*- and *d*-enantiomers were $-54.6°$ and $+51.7°$, respectively.

The NMR, infrared, and mass spectra of the enantiomeric products were identical in all respects and were consistent with the formation of a single reaction product, 3,4-dimethyl-2-(β-naphthyl)-5-phenyl oxazolidine. In particular, the mass spectra, which showed molecular ion peaks at *m/z* 301 and base peaks at *m/z* 245, were consistent with condensation and the loss of one molecule of water.

DISCUSSION

The reaction of ephedrine and 2-naphthaldehyde produces a rigid oxazolidine ring system. The cyclization proceeds without racemization and the resulting enantiomers can be directly resolved by HPLC on a chiral stationary phase. The analytical approach is a direct and relatively rapid probe of the enantiomeric purity of ephedrine.

The application of this method to biological systems and to the other members of the ephedrine series is currently under way in our laboratory.

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* *Editor's note:* See also N. Ôi, M. Nagase and T. Doi, *J. Chromatogr.*, 257 (1983) 111.