Journal of Chromatography, 631 (1993) 173–175
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2580

Analytical and preparative high-performance liquid chromatographic separation of thienopyran enantiomers

Charles J. Shaw, Pauline J. Sanfilippo, James J. McNally, Sung Ae Park and Jeffery B. Press

The R. W. Johnson Pharmaceutical Research Institute, 1000 Route 202, Raritan, NJ 08869-0602 (USA)

ABSTRACT

The analytical and preparative enantiomeric resolution of a racemic substituted thienopyran was obtained with a β-cyclodextrin bonded-phase column using isocratic conditions. Baseline separations were obtained with short run times. The analytical method is accurate, reliable and reproducible for measuring enantiomeric excess. A semi-preparative high-performance liquid chromatographic method was used to obtain the enantiomers for pharmacological testing prior to developing an asymmetric synthesis of RWJ 26629.

INTRODUCTION

RWJ 26629 [1,2] is a racemic substituted thienopyran currently under investigation as a potassium channel opener for the treatment of hypertension and other vascular disorders. In the early development phase of this medicinal compound, it was essential to know the primary pharmacological profile of each enantiomer. Enantiomers not only have quantitative differences in comparable activity with the opposite isomer but they can also have pharmacological, therapeutic and pharmacokinetic differences [3–5]. Thus, it is desirable to have each enantiomer available for testing whether they are obtained by (1) asymmetric synthesis, (2) classical resolution or (3) separation by preparative chromatography using an enantiomeric stationary phase. In all cases, a quantitative method is needed to determine enantiomeric excess. The application of cyclodextrin stationary phases for the high-performance liquid chromatographic (HPLC) separation of drug stereoisomers has been demonstrated [6–8]. The enantiomeric excess of RWJ 26629 was determined by HPLC using a β-cyclodextrin column. The analytical method was modified to provide a convenient semi-preparative method for the isolation of the enantiomers prior to the development of an asymmetric synthesis.

Correspondence to: Dr. C. J. Shaw, The R. W. Johnson Pharmaceutical Research Institute, 1000 Route 202, Raritan, NJ 08869-0602, USA.

EXPERIMENTAL

Chemicals

(±)-5,6-Dihydro-6-hydroxy-5,5-dimethyl-2-nitro-7-(2-oxopiperidin-1-yl)-5H-thieno[3,2-*b*]pyran, (−)-(6*S*,7*S*)-*trans*-5,6-dihydro-6-hydroxy-5,5-dimethyl-2-nitro-7-(2-oxopiperidin-1-yl)-7H-thieno-[3,2-*b*]pyran and (+)-(5*R*,6*S*)-5,6-dihyro-6-hydroxy-5,5-dimethyl-2-nitro-7-(2-oxopiperidinyl-1-yl)-7H-thieno[3,2-*b*]pyran were synthesized at The R.W. Johnson Pharmaceutical Research Institute. Specific rotations were obtained in chloroform at 25°C at 589 nm. The specific rotations observed for the (−) and (+) enantiomers are 80.1 and 81.6°, respectively. HPLC-grade ammonium acetate, chloroform, methanol and acetonitrile were obtained from Fisher Scientific (Springfield, NJ, USA).

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Chromatography

A Perkin-Elmer (Norwalk, CT, USA) Model 410 solvent delivery system and Hewlett-Packard (Avondale, PA, USA) Series 1050 diode-array detection system were used for the analytical separations. The solvent delivery system for the preparative separations consisted of two Rainin (Woburn, MA, USA) HPX pumps with 25 ml/min heads and an Apple Macintosh Plus (Cupertino, CA, USA) controller. A Rainin HPX dispensing pump was used to inject a 2-ml sample. A Gilson Model 116 UV detector and Kipp & Zonen BD 41 strip-chart recorder was used to monitor the preparative separations. Resolution of the enantiomers were obtained on an Advanced Separation Technologies (Whippany, NJ, USA) Cyclobond I prepacked HPLC column. Quantitative measurements were made using the Hewlett-Packard 3350 laboratory automation system.

A 10-μl volume of a 1 mg/ml solution of the racemate was injected onto a 250 × 4.6 mm I.D. column, and a mobile phase of methanol–acetonitrile–ammonium acetate (5:23:72) at a flow-rate of 0.7 ml/min was used for the analytical resolution of the enantiomers, which is shown in Fig. 1. Preparative resolution of the enantiomers was obtained with a 250 × 10 mm I.D. column using a 2-ml injection of 15 mg/ml. A mobile phase of acetonitrile–ammonium acetate (13:87) at a flow-rate of 7 ml/min was used with a total elution time of 25 min.

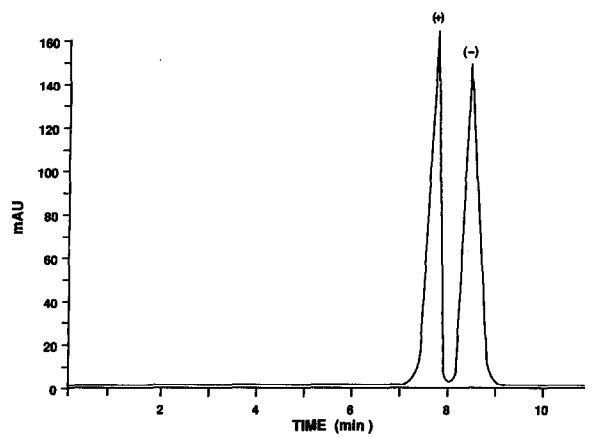

Fig. 1. HPLC of the analytical resolution of racemic RWJ 26629.

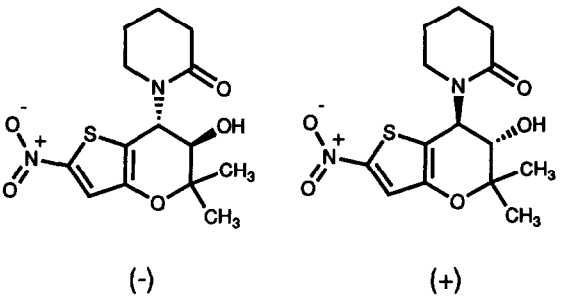

Fig. 2. Structures of the thienopyran enantiomers.

RESULTS AND DISCUSSION

The thienopyran enantiomers were resolved on a single enantiomeric column using isocratic conditions. Samples of the individual enantiomers, which are shown in Fig. 2, were used to determine the elution order. A separation factor (α) of 1.09 and peak resolution factor (R) of 1.35 were obtained [9]. The (+) enantiomer eluted prior to the (−) enantiomer. A correlation coefficent of 0.998 was obtained for the calibration curve which had a concentration range of 0.2–25.0% (w/w). The detection limit was 0.1%, where the limit detection is equal to 1.5 + R_n (maximum amount of peak to peak noise level). Enantiomeric composition of the fractions examined are presented in Table I. The excess of one enantiomer decreasing between experiments I,

TABLE I

ENANTIOMERIC COMPOSITION OF FRACTIONS OBTAINED FROM PREPARATIVE HPLC

Experiment	Fraction	Percent (−) enantiomer
I	1	100
	2	81.8
	3	16.5
	4	4.1
II	1	99.6
	2	97.4
	3	12.0
	4	3.6
III	1	93.2
	2	85.2
	3	12.3
	4	1.7

II and III may be attributed to human error in the collection of the appropriate fractions. Preparative HPLC isolation of the enantiomers using a 10 mm I.D. column offers a simple and direct approach to provide sufficient material for pharmacological testing. It provides the best compromise between resolution, separation time and solvent consumption. A loading study showed that the maximum amount of racemate the 10 mm I.D. column could tolerate without compromise to resolution was 30 mg. A single pass was made for each injection and two fractions were collected for each peak. This method provided 100 mg of the (−) enantiomer in 99.8% enantiomeric excess and 100 mg of the (+) enantiomer in 99.4% enantiomeric excess.

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