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Note

High-performance liquid chromatographic resolution of enantiomers of γ-vinyl-γ-aminobutyric acid

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γ-Aminobutyric acid (GABA) has been established as a major inhibitory neurotransmitter in the central nervous system. The deficiency of GABA in the brain may cause neurological and mental disorders[1–5]. Recently, γ-vinyl-γ-aminobutyric acid (γ-vinyl-GABA; MDL 71,754) has been reported as a new irreversible catalytic inhibitor for GABA-α-oxoglutarate aminotransferase (GABA-T)[6]. Compared with the previous inhibitors of GABA-T, this agent is more selective and less toxic and therefore has more potential for therapeutic use[7]. Through animal experiments it has been reported that only the *S*(+)-γ-vinyl-GABA is active and the *R*(−)-enantiomer has no effect on GABA-T[8]. The resolution of *R*(−)- and *S*(+)-enantiomers of γ-vinyl-GABA has been achieved by Haegele *et al.*[9] using a capillary gas chromatographic (GC) column coated with a chiral stationary phase. The sample was converted to volatile derivatives by acylation and esterification prior to analysis. In this communication, we describe an alternative approach for the resolution of enantiomers of γ-vinyl-GABA. The sample is reacted with *tert.*-butyloxy-L-leucine N-hydroxysuccinimide ester to form diastereomeric derivatives which can then be separated by reversed-phase high-performance liquid chromatography (HPLC). This approach gives excellent resolution of the enantiomers.

EXPERIMENTAL

Materials

R(−)-, *S*(+)-γ-Vinyl-GABA (MDL 71,754), *R*(−)-γ-vinyl-GABA (MDL 71,894) and *S*(+)-γ-vinyl-GABA (MDL 71,890) were supplied by the Merrell Dow Research Institute. The derivatizing reagent, *tert.*-butyloxy-L-leucine N-hydroxysuccinimide ester (Boc-L-Leu-OSu), was obtained from Fluka (Hauppage, NY, U.S.A.). Acetonitrile and water were HPLC grade obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). Potassium phosphate, sodium bicarbonate, tetrahydrofuran (THF) and trifluoroacetic acid (TFA) were standard reagent grade received from MCB (Gibbstown, NJ, U.S.A.).

Derivatization procedure

The procedure for the reaction of Boc-L-Leu-OSu reagent with an amino acid has been described elsewhere[10]. About 2.6 mg (20 μmol) of γ-vinyl-GABA sample

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was placed in a glass tube containing 0.25 μl of 0.2 *M* sodium bicarbonate solution. The solution was stirred for 2 min and 0.25 ml of Boc-L-Leu-OSu in THF (65 mg/ml; 40 μmol) was added. The reaction mixture was stirred continuously at room temperature for 30 min and then evaporated to dryness with a stream of nitrogen. The residue was dissolved in 0.2 ml of TFA and allowed to stand at room temperature for 5 min to remove the *tert.*-butyloxy group. The excess TFA was then removed from the final derivatization products with a stream of nitrogen. The residue of the final derivatization products was dissolved in 10 ml of mobile phase for HPLC analysis.

HPLC analysis

HPLC analyses of the diasteromeric derivatives of γ-vinyl-GABA were performed by a liquid chromatographic system which consisted of a Varian 5000 liquid chromatograph and a Vari-Chrom UV detector (Palo Alto, CA, U.S.A.), a Valco AH60 sample valve equipped with a 100-μl sample loop (Houston, TX, U.S.A.), and a LiChrosorb RP-8 column (10-μm particle size, 25 cm × 4.0 mm I.D.) (MCB, Gibbstown, NJ, U.S.A.). The mobile phase was 0.05 *M* phosphate buffer (pH 7)–acetonitrile (96:4) at a flow-rate of 2 ml/min. HPLC effluent was measured with UV detection at 210 nm. The chromatogram was recorded and integrated by an Automated Laboratory Data System (Computer Inquiry Systems, Englewood Cliffs, NJ, U.S.A.).

RESULTS AND DISCUSSION

The diastereomeric derivatives of *R*(−)- and *S*(+)-γ-vinyl-GABA were easily prepared by reacting the sample with Boc-L-Leu-OSu reagent in basic media. The best yield was obtained with a 1:2 molar ratio of γ-vinyl-GABA:Boc-L-Leu-OSu in 0.2 *M* sodium bicarbonate–THF solution although other ratios and sodium carbonate solutions were also investigated. The derivatization reaction was essentially completed within 15 min, however, for quantitative analyses the reaction time was ex-

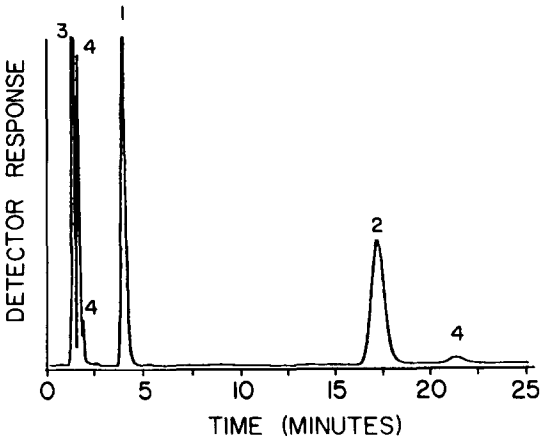

Fig. 1. Typical chromatogram of the diastereomeric derivatives of *R*(−)- and *S*(+)-γ-vinyl-GABA. Peaks: 1 = *R*(−)-enantiomer derivative; 2 = *S*(+)-enantiomer derivative; 3 = L-leucine; 4 = reagent byproduct.

TABLE I

CAPACITY FACTOR FOR DIASTEREOMERIC DERIVATIVES OF $R(-)$- AND $S(+)$-γ-VINYL-GABA AS A FUNCTION OF pH

Capacity factor = (retention volume − void volume)/void volume.

Enantiomeric derivative	*Capacity factor*		
	pH 3.0	*pH 5.0*	*pH 7.0*
$R(-)$	11.4	3.4	2.0
$S(+)$	>50	15.1	11.5

tended to 30 min to assure reproducibility. In order to minimize the formation of byproducts, all the reactions were carried out below 35°C.

The separation of the diastereomeric derivatives of $R(-)$- and $S(+)$-γ-vinyl-GABA and the byproducts produced from the derivatization was accomplished using a LiChrosorb RP-8 column with 0.05 *M* phosphate buffer (pH 7)–acetonitrile (96:4) mixture as the mobile phase. A typical chromatogram is shown in Fig. 1. The $R(-)$ form of the derivative is eluted before the $S(+)$ form. A LiChrosorb RP-18 column provided similar separation results as that of the LiChrosorb RP-8 column but with a relatively longer retention time. However, a LiChrosorb RP-2 was not able to completely resolve the diastereomers of $R(-)$- and $S(+)$-γ-vinyl-GABA from the derivatization byproducts. The capacity factors for the diastereomeric derivatives of $R(-)$- and $S(+)$-γ-vinyl-GABA were markedly increased as the acidity of the mobile phase was increased (Table I). This is due to the conversion of the diastereomeric derivatives of $R(-)$- and $S(+)$-γ-vinyl-GABA from the zwitterion species to the protonated amine species[11].

Since the $S(+)$-enantiomer is the form of biological interest, it is of importance to evaluate this derivatization HPLC method when applied for the assay of low levels of the $R(-)$-enantiomer in essentially pure $S(+)$-γ-vinyl-GABA samples synthesized via a stereospecific method.

In order to evaluate the method, $S(+)$-γ-vinyl-GABA was spiked with the $R(-)$-enantiomer at the 0.5, 1.0 and 2.0% levels, respectively. The samples were then derivatized and analyzed by this method. The results of recovery were excellent as shown in Table II. The chromatograms are reproduced in Fig. 2. The data of Table II reflect good correlation between weight/weight spiking and peak area/area calcu-

TABLE II

RESULTS OF $R(-)$-ENANTIOMER SPIKE RECOVERY STUDY

Level spiked (%)	*Found* (%)*	*Recovery (%)*
0.50	0.49	98
1.00	1.01	101
2.00	2.03	102

* Data was calculated based on net spike area after correction for low level indigenous $R(-)$-enantiomer in sample.

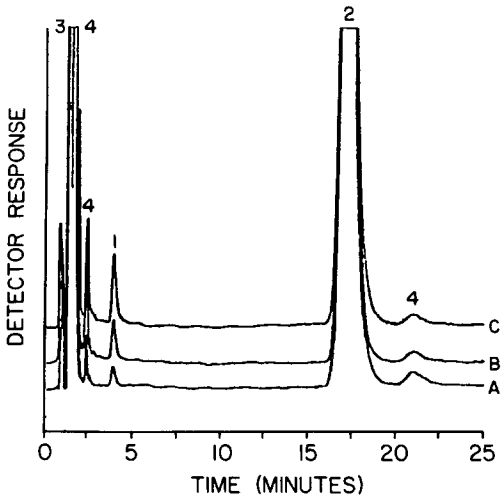

Fig. 2. Determination of low levels of $R(-)$-enantiomer in $S(+)$-γ-vinyl-GABA. Chromatograms: A = 0.5% $R(-)$-enantiomer added; B = 1.0% $R(-)$-enantiomer added; C = 2.0% $R(-)$-enantiomer added. Peaks: 1 = $R(-)$-enantiomer derivative; 2 = $S(+)$-enantiomer derivative; 3 = L-leucine; 4 = reagent byproduct.

lation of the relative percent levels of $R(-)$-enantiomer. The detection limit of the $R(-)$-enantiomer in $S(+)$-γ-vinyl-GABA was 0.1%. The relative standard deviation of the method for a racemic mixture was 0.5% (n = 5).

In order to compare the described HPLC method and the GC method of Haegele *et al.*[9] for the analysis of enantiomeric impurity of $S(+)$-γ-vinyl-GABA, an authentic sample, MDL 71,890-03, was analyzed using these two methods. The percentages $R(-)$-enantiomer found (n = 4) were 0.23 ± 0.03% and 0.24 ± 0.04% for the HPLC method and the GC method[12], respectively. They reflect the very good correlation of the two methods.

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