Short Communication

Preparative liquid chromatographic separation of isomers of 4-amino-3-(4-chlorophenyl)butyric acid

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ABSTRACT

A liquid chromatography method was developed for the chiral resolution of [4-amino-3-(4-chlorophenyl)butyric acid isomers. The compound was derivatized in two steps: protection of the amino group with di-*tert*.-butyl carbonate and reaction with (*S*)-α-methyl benzyl amine to obtain the diastereomeric mixture *RS* and *SS*. Chromatography was carried out on silica gel (5–20 μm) employing *n*-hexane–ethyl acetate as eluent. ^{1}H NMR spectroscopy and analytical high-performance liquid chromatography indicated that the separated fractions were pure.

INTRODUCTION

We are particularly interested in the synthetic preparation of GABA derivatives and especially in GABA-B analogues such as baclofen [4-amino-3-(4-chlorophenyl) butyric acid; β-(*p*-chlorophenyl)-GABA] [1]. Baclofen is used to relieve symptoms of spasticity in patients suffering from sclerosis and spinal lesions [2–5]. The enantiomers were found to have different properties, so the optical resolution of racemates is essential [6]. β-Phenyl-GABA have been resolved by fractional crystallization of cinchonidine [7] or α-methylbenzylamine [8] salts. Resolution based on diastereoisomeric salt has the disadvantage that crystallization is crucial and that the optical purity of the salts relies mainly on chiroptical measurements depending of the concentration of the analyte, solvent and temperature. In high-performance liquid chromatography (HPLC), optical resolution of baclofen enantiomers for preparatives purposes was achieved using a chiral mobile phase [9]. The same resolution by the liquid chromatographic separation of diastereoisomers has been suggested but literature data on the subject are incomplete [5]. Preparative high-performance liquid chromatography (HPLC) seemed the most appropriate method for this purpose in

order to avoid the disadvantage of crystallization and to perform an easy work-up, with a view to extending it to various GABA derivatives. In this paper we describe the results for the HPLC resolution of baclofen after derivatization. A direct scale-up from the analytical to the preparative mode was achieved with only slight changes in the chromatographic conditions. Unmodified silica was used as the stationary phase and n-hexane–ethyl acetate as the mobile phase, with UV detection.

EXPERIMENTAL

Baclofen was kindly supplied by Ciba-Geigy.

Analytical HPLC was carried out with an LKB Model 2249 metering pump at a flow-rate of 1 or 1.5 ml/min with an injection valve (20-μl loop). Detection was performed with an HP 1040 photodiode-array spectrophotometer connected to an HP 9000 S300 computer. UV spectral characteristics were measured by HPLC with diode-array detection to confirm peak homogeneity and purity. A Spherisorb 5 Sil (5 μm) column (250 mm × 4.6 mm I.D.) from Laboratory Data Control (LDC) was used with n-hexane–ethyl acetate in various proportions as the eluent. The samples were dissolved in ethyl acetate. Preparative HPLC separations were made with an LDC Constametric III metering pump at a flow-rate of 6 ml/min on a 40 mm I.D. column of silica gel (5–20 μm; 100 g), with n-hexane–ethyl acetate in various proportions as the eluent. Detection was performed with an LDC multi-wavelength spectromonitor D at 260 nm. Three samples (mass injected = 300, 380 and 438 mg) were dissolved in 10 ml of pure ethyl acetate, owing to their low solubility in n-hexane. All experiments were performed at ambient temperature. The solvents used were of analytical-reagent grade from Merck.

Derivatization was undertaken in two steps (Fig. 1): the first involved treatment of baclofen with di-tert.-butyl carbonate to protect the amino group [10], and the second was reaction with (S)-α-methylbenzlamine according to the mixed anhydride method [11]. This furnished a mixture of RS (compound 1) and SS (compound 2) diastereomers, resolved by chromatography. This pathway has been reported previously but no experimental details were given [5].

The purity of the separated components was checked by thin-layer chromatography (TLC), analytical HPLC and routine ^{1}H NMR spectroscopy at 80 MHz. The different fractions were compared with pure RS and SS compounds prepared unambiguously from the corresponding R and S isomers of baclofen following the same reaction scheme.
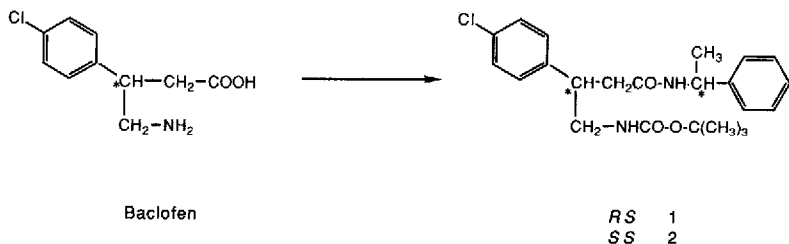

Fig. 1. Scheme of the reaction.

RESULTS

Analytical HPLC resolution of baclofen isomers has been widely published [12–14] but preparative HPLC has not been extensively studied [5,6,9], and this justified our work to set up a procedure and to optimize the resolution.

Derivatization of baclofen in two steps gave a mixture of diastereoisomers as a glassy viscous oil. The enantiomeric ratios of the starting racemate 1 and 2, obtained by NMR and HPLC, are similar (ratio $\approx$ 1). At 80 MHz the diastereomers were readily distinguished by their spectra in that the chemical shifts for the $CH(CH_3)$ methyl doublets differed by 0.10 ppm: $\delta = 1.28$ ppm (d, $J = 6.9$ Hz, compound 1, RS) and 1.38 ppm (d, $J = 6.9$ Hz, compound 2, SS). More complete data at 300 MHz will be published elsewhere. The analytical HPLC data are summarized in Table I. An example of an analytical chromatogram is given in Fig. 2: the RS isomers (compound 1) is eluted before the SS isomer (compound 2). An analytical HPLC study of the effect of the percentage of n-hexane (20–60%) on the capacity factors (identification), selectivity of resolution (α) and resolution (R) was undertaken. An increase in n-hexane concentration (at a flow-rate of 1.5 ml/min) resulted in a corresponding increase in retention. The changes in identification (k'_1, 0.64–4.79; k'_2, 1.20–10.05) α (1.80–2.10) and R (2.52–5.74) are significant. When developing an analytical method for scale-up to the preparative mode, it is desirable to have an analytical resolution (R) greater than 2 and a capacity factor (identification) of less than 5, the latter because of the shorter run times obtained and a corresponding increase in the throughput (mg/h of chemical) for the purification. In fact, for an n-hexane–ethyl acetate (60:40) mobile phase, where $k'_1 = 4.79$ and $k'_2 = 10.05$, the retention times in the preparative HPLC become prohibitive, with $t_1 = 220$ and $t_2 = 501$ min.

The first attempted resolution by recrystallization of the diastereomeric mixture in various solvents such as n-hexane or diisopropyl ether was unsuccessful, leading only to enrichment of some of the diastereomers; thus from the starting material (mass $m = 1118$ mg; ratio $\approx$ 1) we collected three fractions ($m = 300$, 380 and 438 mg) with the corresponding ratios 0.55, 0.59 and 2.00.

In a second attempt, using those fractions, we undertook a preparative separation by chromatography and tried to optimize the conditions by modifying the

TABLE I

ANALYTICAL HPLC: CAPACITY FACTORS (k') SELECTIVITY OF RESOLUTION (α) AND RESOLUTION (R) OF COMPOUNDS 1 AND 2 (RS AND SS)

Capacity factor identification $= (t_x - t_0)/t_0$; separation factor $\alpha = (t_2 - t_0)/(t_1 - t_0)$; peak resolution $R = 2(t_2 - t_1)/(w_1 + w_2)$; $w =$ width at baseline; $t_0 =$ retention time of an unretained compound; $t_x =$ retention time of compound 1 or 2.

Mobile phase (n-hexane–ethyl acetate)	Flow-rate (ml/min)	k'_1	k'_2	α	R
20:80	1	0.65	1.22	1.87	2.66
20:80	1.5	0.64	1.20	1.80	2.52
50:50	1.5	2.68	5.49	2.05	3.93
60:40	1.5	4.79	10.05	2.10	5.74

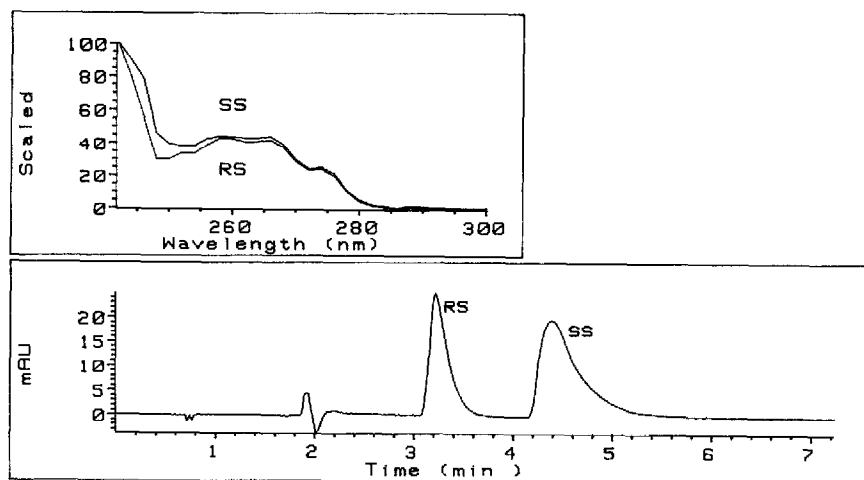Fig. 2. Analytical HPLC of starting material and UV spectrum of each diastereomer. Eluent, n-hexane–ethyl acetate (20:80). Detection at 260 nm.

mobile phase composition. The preparative data are given in Table II. A chromatogram of a preparative separation is shown in Fig. 3. The average recovery of the preparative procedure, calculated on three injections, was 86% with a mean injected mass of 370 mg. The average isomer ratio, calculated on three injections, was 0.97 (RS/SS). No conclusion can be drawn from the loading effects; the injected masses are too close, as also are the yields observed. Nevertheless, the lower yield (74%) is certainly due to the tailing of the peaks caused by a much less effective eluting solvent, n-hexane–ethyl acetate (60:40). This gives rise to a higher loss of compounds during the fractionation. Much larger loadings could be made by using a less effective eluting solvent [e.g., n-hexane–ethyl acetate (60:40)] but with the disadvantage of a higher run time (e.g., $t_1 = 220$, $t_2 = 501$ min).

TABLE II

PREPARATIVE HPLC: RETENTION TIMES (t_1 AND t_2) OF COMPOUNDS 1 AND 2 (RS AND SS)

Flow-rate, 6 ml/min; m_{1+2}, m_1, m_2 = collected masses of compounds $1+2$, 1 and 2.

Mobile phase composition (n-hexane–ethyl acetate)	Retention time (min)		Mass injected (mg)	Ratio[b]	Mass recovered (mg)			Ratio[a]
	t_1	t_2			m_{1+2}	m_1	m_2	
20:80	41	67	438	2.09	420 (96)[c]	284	135	2.10
40:60	70	106	380	0.52	308 (81)[c]	108	200	0.51
60:40	220	501	300	0.55	221 (74)[c]	75	146	0.54

[a] Ratio defined as m_1/m_2 (RS/SS).
[b] Initial ratio calculated from analytical HPLC.
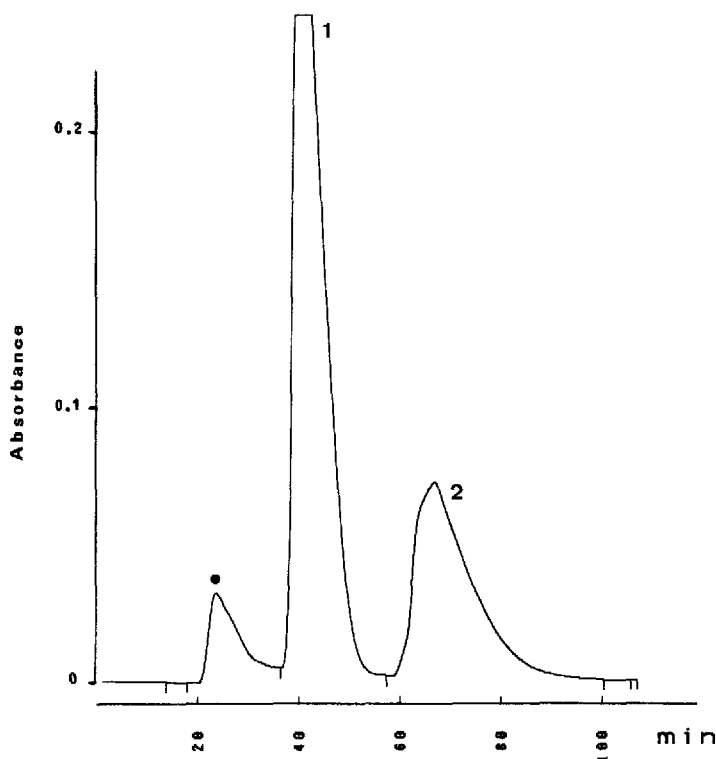[c] Yield (%) in parentheses.

Fig. 3. Preparative chromatogram. Eluent, n-hexane–ethyl acetate (20:80). Detection at 260 nm. Mass injected = 438 mg; volume injected = 10 ml; ratio m_1/m_2 = 2.10. ● = Impurity.

It was possible to demonstrate by ^{1}H NMR spectroscopy and analytical HPLC that the separated fractions were pure (>99%). Preparative HPLC proved to be a rapid and economic method for isolating the isomers of GABA analogues. Hydrolysis of the diastereomers in hydrochloric acid medium leads to the enantiomers [6,15].

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