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Short communication

GABA-B agonists: enantiomeric resolution of 4-amino-3-(5-chlorothien-2-yl)butyric acid and analogues on chiral crown ether stationary phase

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Abstract

The enantiomeric resolution of 4-amino-3-(5-chlorothien-2-yl)butyric acid, an analogue of baclofen [4-amino-3-(4-chlorophenyl)butyric acid], was examined by HPLC using a chiral stationary phase consisting of a crown ether and perchloric acid–methanol as the mobile phase. Optimization of the separation was achieved by variation of temperature, pH and eluent composition. The absolute configuration may be assigned by comparison with authentic enantiomers of baclofen of known absolute configuration. The best results obtained were $\alpha = 1.15$ and $R_s = 2.72$ at 20°C with $HClO_4$ (pH 1.3)–CH_3OH (90:10). The study was extended to analogues, viz., 3-(substituted thienyl) and 3-(substituted furanyl)-4-amino acids.

1. Introduction

Following our interest in the preparation of 4-amino acids (4-AAs), we recently described [1] potent and specific $GABA_B$ receptors agonists **1–5** [2]; the most efficient one, **3** (Fig. 1), was commercially available as the racemate from Tocris-Cookson (Langford, Bristol, UK) until 1991 and is used as a neurochemical ligand in pharmacology. As this molecule has a chiral centre, a detailed investigation of its pharmacodynamic properties could require a knowledge of the individual biological behaviour of each of the enantiomers. This kind of research in the $GABA_B$ field has recently received much attention [3,4]. As a first step, a method has to be developed that would allow the chromatographic separation of these optical isomers. In order to obtain a more rapid method, the direct resolution, without any prederivatization, of the enantiomeric components was studied with a newly introduced phase using a chiral crown ether moiety as chiral selector.

In this paper, we describe the direct separation and an optimization study of the potent 4-AA **3** and its comparison with the less active 4-AAs **1** and **2** and the inactive 4-AAs **4** and **5**, in order to collect information about the influence on the chromatographic parameters of the heteroaromatic ring and substituents.

2. Experimental

Chromatographic resolution was accomplished with an LKB 2249 metering pump and detection

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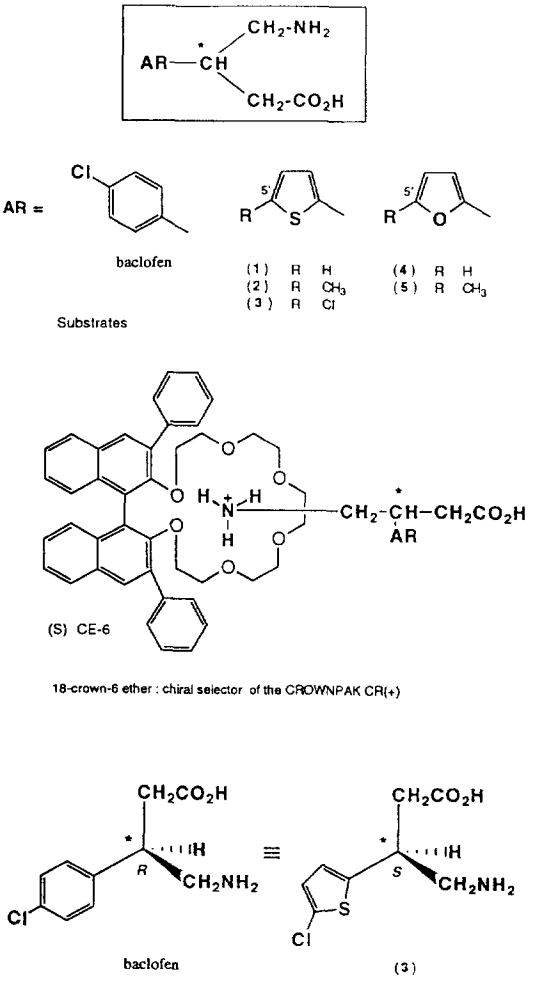

Fig. 1. Structures of substrate 4-AAs and of the selector and illustration of nomenclature.

with an HP 1040 photodiode-array spectrophotometer connected to an HP 9000 S300 computer. The column eluate was monitored at 225, 220 and 200 nm (4 nm bandwidth) with a reference at 550 nm (100 nm bandwidth). The column was Crownpak CR(+) (5 μm) (150 × 4 mm I.D.) packed with a chiral stationary phase (CSP) composed of a chiral crown ether (Fig. 1) (Daicel Chemical Industries, Baker, France). The sample loop was 10 μl (Rheodyne Model 7125 injector). Elution was carried out isocratically using perchloric acid at the required pH and methanol at a flow-rate of 0.9 ml/min. The temperature of the column was controlled with water circulating through a jacket surrounding the column.

Baclofen and its enantiomers were kindly supplied by Ciba-Geigy. The 4-AAs **1–5** were obtained as described previously [1]. Water was purified through a Milli-Q unit (Millipore). Methanol was of gradient grade from Merck and perchloric acid was of analytical-reagent grade from Prolabo. All the solutions were filtered (0.45 μm), degassed and purged with helium. All amino acids were dissolved in the mobile phase at a concentration of about 1.6 m*M* (which corresponds to $1.6 \cdot 10^{-8}$ mol injected) and passed through a 0.45-μm membrane filter prior to injection. To prevent corrosion and decomposition of the stationary phase, the column and all

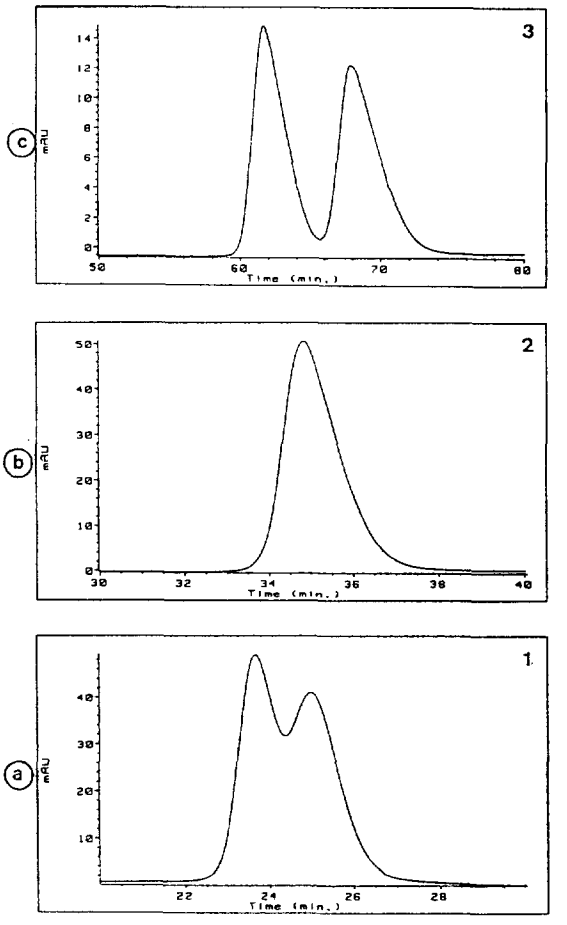

Fig. 2. Chromatograms obtained for (a) **1** (eluent D; 10°C); (b) **2** (eluent D; 20°C; (c) **3** (eluent A; 20°C).

Table 1
Analytical HPLC: retention factors (k'), enantioselectivity (α) and resolution (R_s) of compounds **1–5**

Compound	Eluent[a]	10°C			20°C			30°C			40°C		
		k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s
Baclofen	A				37.0	1.84	10.02	25.4	1.65	8.07	17.4	1.48	6.11
1	A				8.40	1	–[b]	5.57	1	–[b]			
	B				5.33	1	–[b]						
	C				7.66	1.05	0.81						
	D	15.44	1.06	0.91	8.55	1.06	0.91						
2	A				25.20	1	–[b]	15.70	1	–[b]			
	B				14.50	1	–[b]						
	C				20.50	1	–[b]						
	D	41.76	1	–[b]	23.34	1	–[b]						
3	A				36.50	1.11	1.76	23.56	1.10	1.34	15.25	1.09	1.21
	B				21.40	1.15	1.90						
	C				34.80	1.15	2.72						
4	A				3.93	1.09	0.86						
	B				2.66	1.09	0.66	1.70	1.07	0.48	1.12	1	–[b]
5	A				10.46	1.15	1.64						
	B				6.47	1.18	1.52	4.08	1.15	1.21	2.64	1.12	0.98

$k' = (t_s - t_0)/t_0$; $\alpha = (t_2 - t_0)/(t_1 - t_0)$; $R_s = 2(t_2 - t_1)/(w_1 + w_2)$, where w = width at baseline, t_0 = retention time of an unretained compound and t_s = retention times of compounds, S or R.
[a] Eluent A = $HClO_4$ (pH 2); eluent B = $HClO_4$ (pH 2)–CH_3OH (90:10); eluent C = $HClO_4$ (pH 1.3)–CH_3OH (90:10); eluent D = $HClO_4$ (pH 1.1)–CH_3OH (90:10).
[b] No resolution.

the apparatus were thoroughly washed with water at the end of every day.

3. Results and discussion

The enantiomeric separation of 4-AAs **1–5** on the crown ether CSP is summarized in Table 1 and shown in Figs. 2 and 3. According to the structural spatial analogy, one could think that the same elution order may be observed for each pair of enantiomers of the 4-AAs **1–5** as for the enantiomers of baclofen. The designation of k'_S and k'_R as the first and second peaks for baclofen can be deduced by chromatographing samples of pure enantiomers of known absolute configuration. Following the Cahn–Ingold–Prelog rules, (i) for baclofen the substituent order is $CH_2N >$ phenyl $> CH_2CO > H$, but (ii) for 4-AAs **1–5** this order becomes thienyl (or furyl) $> CH_2N > CH_2CO > H$. Hence for an identical spatial structure the order is reversed. For **1–5**, the first and the second peaks should be k'_R and k'_S, respectively.

For each compound we tried to optimize the separation either by addition of an organic modifier to the mobile phase or by varying the temperature or the pH. The enantioselectivity factor (α) and resolution (R_s) slowly increase while significant retention factors (k') reduction are observed with increasing concentration of methanol for **3**. Methanol proportions above 15% are not recommended for this column. For **4** and **5** the selectivity α increases or remains unchanged whereas R_s and k' decrease [5,6]. Variation of the pH of the mobile phase [5,6] between 1.1 and 2.0 influences the retention (k') and enantioselectivity (α) factors of **1–3** as demonstrated in Table 1. The enantioselectivity factor decreases slightly or remains unchanged

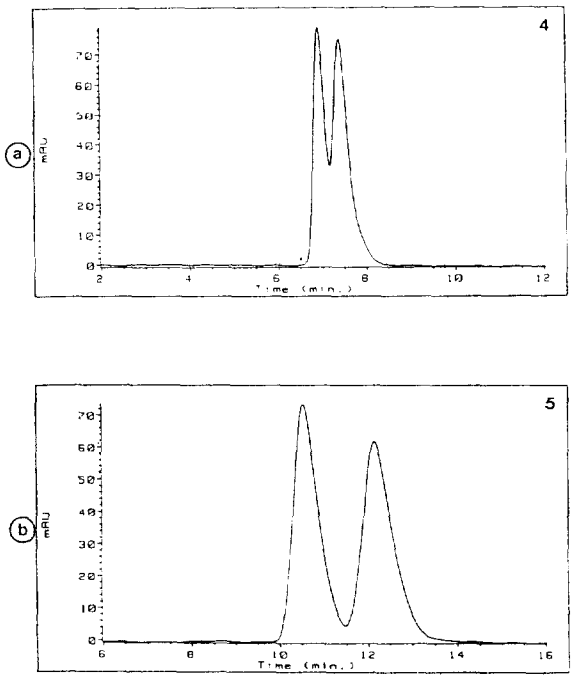

Fig. 3. Chromatograms obtained for (a) **4** (eluent A; 20°C); (b) **5** (eluent B; 20°C).

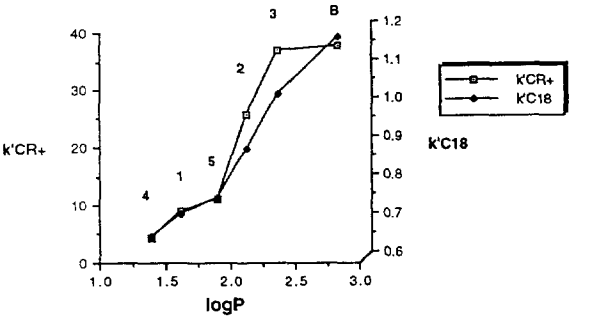

Fig. 4. Retention factors vs. log P: $k'_{CR(+)}$ (eluent A; 20°C); $k'_{C_{18}}$ [eluent CH_3OH–H_2O (60:40); 20°C].

They are in perfect accordance with the order of capacity factors both on Crownpak CR(+) and reversed (C_{18}) stationary phases (Fig. 4). For the latter, the $k'_{C_{18}}$ values are 0.66, 0.72, 0.76, 0.89, 1.03 and 1.18 for **4**, **1**, **5**, **2**, **3** and baclofen, respectively.

The limit of detection (at a signal-to-noise ratio of 3) for each enantiomer of **3** was $6.2 \cdot 10^{-6}$ mol/l, corresponding to $6.2 \cdot 10^{-11}$ mol injected. Fig. 5 illustrates the enantioseparation

with increasing pH, whereas the retention factors are much more affected. The resolution (R_s) also shows a progressive decrease with increasing pH. The large separations exhibited at lower pH are presumably because of the ability of the preferred enantiomers to fill the chiral cavity [5].

The factors k', α and R_s were found to be increased with decreasing temperature from 40 to 10°C. As might be expected, the resolution increased at the expense of lengthened retention times and broadened peak shapes [5–8].

Under the same elution conditions, **1–5** are much less retained than baclofen. The order of elution in each system is (Table 1) **4** < **1** < **5** < **2** < **3** < baclofen. Compounds **1–5** are certainly less hydrophobic than baclofen. The literature data for log P values of the aromatic moieties are 1.36 (1.34), 1.59 (1.81), 1.87 (1.90), 2.10 (2.37), 2.34 (2.52) and 2.81 for **4**, **1**, **5**, **2**, **3** and baclofen, respectively (P = partition coefficients measured in *n*-octanol–water following Rekker [9] or Hansch and Leo [10] (in parentheses)).

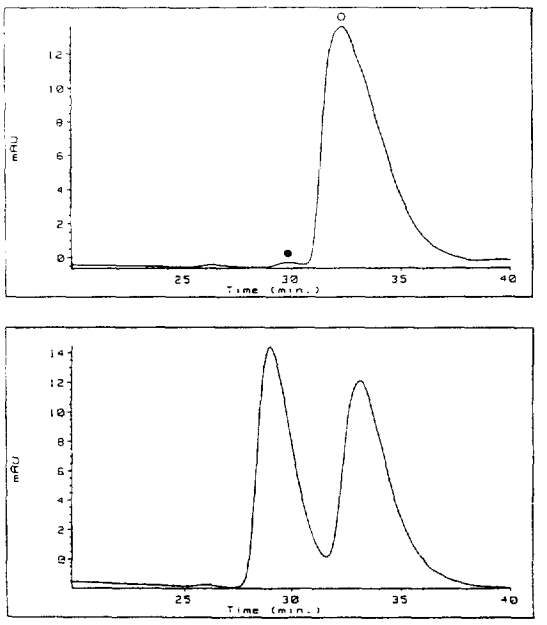

Fig. 5. Chromatograms of **3** (eluent B; 20°C): (a) racemate; (b) a major component (○) with a minor enantiomer (●).

of racemate **3** and its comparison with one of its enantiomers, the minor enantiomer (*R*) eluting first in front of the main component (*S*). These enantiomers were prepared according to a previously described method [11]. The *S* antipode is much more potent than the *R* antipode (bioassays will be published elsewhere). This is in accordance with the fact that the biological activity of baclofen, phaclofen [3] and saclofen [4] resides in the single enantiomer (*R*). Using the crown ether phase we were able to determine the minor enantiomer in a relative proportion of less than 1% [12,13] (Fig. 5: 0.4% *R* versus *S*).

The good separation of the optical isomers of the commercially available 4-AA **3** as efficiently as for the reference baclofen makes this chromatographic method suitable for studies designed to probe the enantiomeric distribution of the antipodes of **3** in biological media.

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