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Short communication

Chiral resolution of 4-substituted pyrrolidin-2-ones using high-performance liquid chromatography on cellulose and amylose chiral stationary phases

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Abstract

Analytical HPLC methods using derivatized cellulose and amylose chiral stationary phases were developed for the separation of the enantiomers of 4-substituted pyrrolidin-2-ones. Separations were performed using normal-phase methodology with a mobile phase of *n*-hexane–alcohol (ethanol or 2-propanol) in various percentages, and a silica-based cellulose tris-3,5-dimethylphenylcarbamate (Chiralcel OD), or amylose tris-(*S*)-1-phenylethylcarbamate (Chiralpak AS). The mobile phase and the chiral stationary phase were varied to achieve the best resolution. The effects of alcohol concentration, various aliphatic alcohols in the mobile phase were studied. The effects of substitution were analysed. Baseline separation was easily obtained in many cases.

Keywords: Mobile phase composition; Chiral stationary phases, LC; Enantiomer separation; Pyrrolidinones

1. Introduction

GABA (γ-aminobutyric acid) is an important neurotransmitter both in the central and peripheral tissues. We recently described 3-substituted GABA [1,2,19] as potent and specific $GABA_B$ receptor agonists and antagonists. Some of the most efficient ones were commercially available as racemates from Tocris-Cookson (Langford Bristol, UK) till 1991 and are used as neurochemical ligands in pharmacology. We recently described their direct analytical enantiospecific chromatographic separation on a chiral crown ether (CR_+) column with perchloric acid as mobile phase [3].

4-Substituted pyrrolidin-2-ones (structure **1**; Fig. 1) are molecules of interest (i) from a therapeutic point of view [such as Piracetam (Nootropyl®) or Oxiracetam (Neuractiv®) in the treatment of cognitive disorders or Rolipram for treating chronic and acute inflammatory diseases], and (ii) – since they are precursors of the corresponding 3-substituted GABAs – both from a biological aspect (as cyclic GABA and prodrug of GABA) and from a synthetic aspect (as keys intermediates). As **1** has a chiral center, it is interesting to resolve these compounds (i) to investigate the pharmacodynamic properties of each of their enantiomers (stereoselective activity of Rolipram has been demonstrated [12]), and (ii) to

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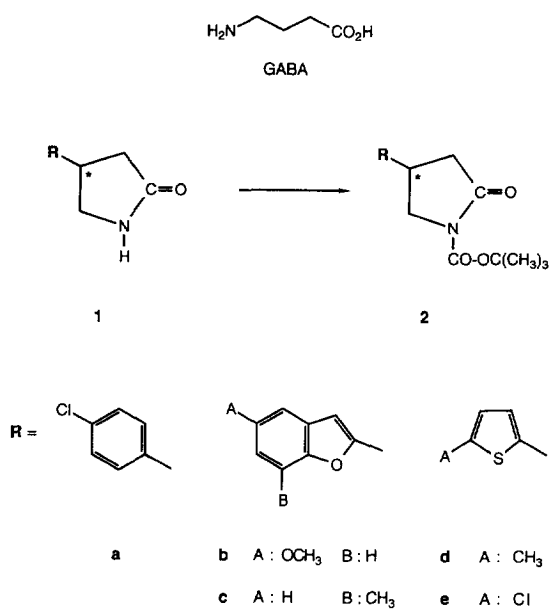

Fig. 1. Chemical structures of compounds **1** and **2**.

obtain pure enantiomers, precursors of the enantiomers of the corresponding 3-substituted GABAs.

As a first step to this, a method has to be developed that would allow the chromatographic separation of these optical isomers. In order to obtain a more rapid method, direct resolution, without any pre-derivatization of the enantiomeric components, was studied. Separation of enantiomers by chiral HPLC is now well established with over 50 different commercially available chiral phases (CSP). To the best of our knowledge only a limited amount of work using liquid chromatography for the analytical or preparative resolution of lactams **1** has been published. It is useful to mention the following described methods: a Pirkle-type CSP without derivatization for 3-substituted lactam [4]; a protein-bonded phase for **1a** but without experimental details [5]; a silica normal achiral phase, the chiral 3- or 4- or 5-substituted lactams being derivatized with chiral isocyanates to afford diastereomeric ureides [6]. Cellulose and amylose esters and carbamates derivatives coated onto a silica gel backbone have been used extensively for chiral resolution under normal-phase conditions [7–11]. The chiral separations are mainly performed with mobile phases containing an alkane, such as *n*-hexane and a lower alcohol. In literature: Rolipram [4-(3-cyclopentyloxy)-4-methoxyphenylpyrrolidin-2-one] was resolved on microcrystalline cellulose triacetate (CTA) and 95% ethanol [12] or on Chiralcel OD with *n*-hexane–2-propanol [13]; Oxiracetam (4-hydroxy-2-oxo-1-pyrrolidineacetamide) was resolved on Chiralcel OC with *n*-hexane–ethanol [14]; a normal-phase displacement method was used for the resolution of 5-vinylpyrrolidin-2-one on Chiralcel OD with *n*-hexane–2-propanol [15].

In this study, we examined the direct separation of **1**, and the derivatives **2** on different CSPs of polysaccharide-derived types and particularly on (tris-3,5-dimethylphenylcarbamate) cellulose (Chiralcel OD-H) which shows a particularly high optical resolving ability among all the phenyl carbamate derivatives of cellulose developed so far [9]. Preliminary works were undertaken on CTA column (250×10 mm I.D.; 10 μm) (Merck, France) according to Ref. [12]. However these experiments were not succesful: no resolution was observed whatever solvent used. For example **1d**: t=22.6 min (k'=0.41) and **2d**: t=26.7 min (k'=0.66) with 1 ml/min ethanol (t_0=16.1 min).

2. Experimental

Chiral chromatography was carried out on a Chiralcel OD-H column (250×4.6 mm I.D.; 5 μm), and on a Chiralpak AS column [Tris-(*S*)-1-phenylethylcarbamate; 250×4.6 mm I.D.; 10 μm] (Daicel, Baker France) using a LKB 2249 metering pump model equipped with a HP 1040 photodiode array spectrophotometer. Chromatographic data were collected and processed on a HP 9000 5300 computer. The column eluate was monitored at 230, 254, and 260 nm (4 nm bandwith) with a reference at 550 nm (100 nm bandwith). The sample loop was 10 μl (Rheodyne 7125 injector). Mobile phase elution was isocratic using *n*-hexane and a modifier (ethanol or 2 propanol) at various percentages. The flow was 0.5 or 0.8 ml/min. The column void time (t_0) was measured by injection of 1,3,5-tri-*tert*.-butylbenzene as a non-retained sample. Retention times were mean values of two replicate determinations. All separations were carried out at ambient temperature (near 23°C).

Compounds **1** used in this study were synthesized

as previously described [1,2,16,19]. Compounds **2** were obtained following literature procedure [17,18]. Derivatization of **1** was performed with di-*tert.*-butyl dicarbonate in methylene chloride in the presence of 4-dimethylaminopyridine and triethylamine. The compounds were purified through crystallisation and/or preparative HPLC (SiO_2/AcEt–*n*-hexane). All new compounds **2** are in accordance with their proposed structures checked by ^1H and ^{13}C NMR spectroscopy at 300 MHz [18]. The ethanol, 2-propanol and *n*-hexane were HPLC grade from Merck or Baker. All the solutions were filtered (0.45 μm), degassed and purged with helium. Compounds were chromatographed by dissolving them in the mobile phase to a concentration of about 0.75 m*M* (which corresponds to 7.5 nmol injected) and passed through a 0.45-μm membrane filter prior to loading the column.

3. Results and discussion

The results of the chiral separation of the **1**, **2** racemates chromatographed are summarised in Table 1. Fig. 2, showing compounds **1d** and **2d**, gives an example typical of the separations achieved for the majority of compounds under test. The UV spectra are shown in Fig. 2 for the two eluted peaks: as expected, the UV absorbance of the separate enantiomers were identical and are, of course, very similar

Table 1
HPLC resolution: retention times (t, min), retention factors (k'), enantioselectivity factor (α) and resolution (R_s) of **1** and **2**

Compound	Eluent (ml/min)	Flow-rate	Chiralcel OD-H				Chiralpak AS			
			t_1	k'_1	α	R_s	t_1	k'_1	α	R_s
1a	A	0.5	28.42	3.60	1.02	0.60				
	C	0.5	16.91	1.74	1	++				
2a	A	0.5	24.30	2.93	1.40	7.90	19.61	2.13	1.04	1.33
	C	0.5	16.00	1.59	1.39	5.92				
	D	0.5	32.92	4.33	1.35	6.97				
1b	A	0.5	36.10	4.84	1	++				
2b	A	0.5	27.54	3.46	1.23	3.93	26.47	3.23	1.09	1.40
	C	0.5	19.04	2.08	1.26	3.67				
	D	0.5	45.55	6.37	1.27	4.48				
1c	A	0.5	23.50	2.80	1.04	0.82				
2c	A	0.5	17.37	1.81	1.42	6.30	14.46	1.31	1.11	1.26
	C	0.5	12.99	1.10	1.44	5.33				
	D	0.5	24.67	2.99	1.53	6.65				
1d	A	0.5	20.06	2.25	1.10	1.91				
	B	0.8	21.64	4.56	1.11	1.90				
2d	A	0.5	16.82	1.72	1.17	2.92	15.04	1.40	1.23	2.54
	A	0.8	10.52	1.73	1.17	2.85				
	B	0.5	23.11	2.74	1.17	3.28				
	B	0.81	4.42	2.71	1.17	2.97				
	D	0.5	22.49	2.62	1.16	2.79				
	D	0.8	14.05	2.60	1.17	2.55				
1e	A	0.5	25.07	3.05	1.11	2.12				
	B	0.8	28.99	6.45	1.11	2.38				
2e	A	0.5	22.48	2.64	1.28	5.08	20.01	2.15	1.35	4.70
	A	0.8	13.86	2.55	1.28	4.40				
	D	0.5	30.89	4.17	1.28	5.71				

t_0: 6.18 (0.5 ml/min); 3.90 (0.8 ml/min) for Chiralcel OD-H and t_0: 6.26 (0.5 ml/min) for Chiralpak AS; Eluents A: hexane–ethanol: (90:10); B: hexane–ethanol (95:5); C: hexane–ethanol (80:20); D: hexane–2-propanol (90:10); ++ unresolved; Concentration ca. 0.75 m*M*.

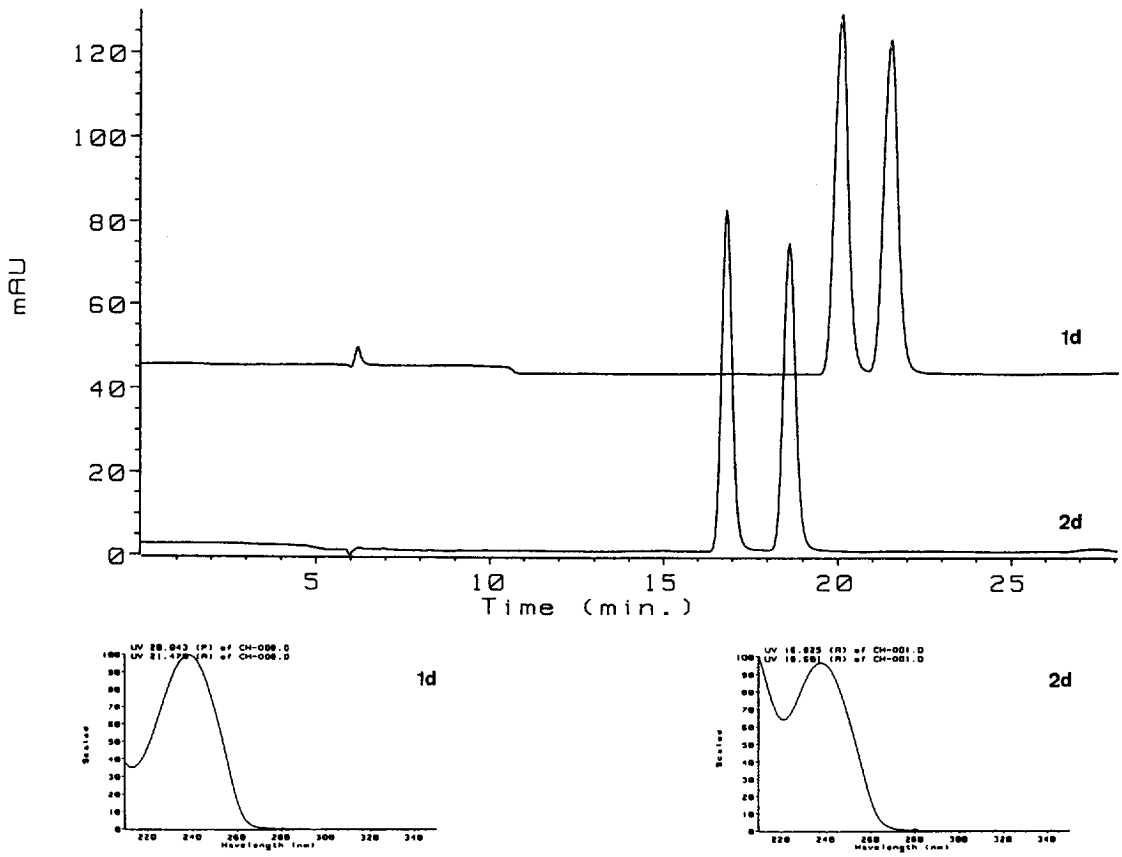

Fig. 2. Chromatograms (λ=230 nm) and UV spectra obtained for **1d** and **2d** (eluent A; 0.5 ml/min).

for compounds **1** and **2**. Fig. 3 illustrates the results obtained for the other derivatized lactams **2**.

The retention factors (k'), enantioselectivity factor (α) and resolution (R_s) of every solute may be regulated over a wide range by the addition of an alcohol. The influence of the percentage of ethanol or 2-propanol and the nature of the alcohol in the mobile phase was thoroughly studied.

Most striking is the influence of the type of alcohol in the mobile phase. The retention times and retention factors k' decrease on changing the mobile phase modifier from 2-propanol (eluent D) to ethanol (eluent A) as expected from the higher polarity of the ethanol [20,21]. On Chiracel OD-H, for example, the values of k' and α for **2d** were 2.98 and 1.19, and 1.82 and 1.17 with eluent D and eluent A, respectively, at a flow-rate of 0.8 ml/min. The use of 2-propanol for **1** is not well-suited since a prohibitive increase in the retention times was observed.

It can be seen that a decrease in the concentration of polar modifier (ethanol) in the mobile phase gives an increase in k' and R_s in a general manner both for **1** and **2** on Chiralcel OD-H columns [10,20]. It must be remarked that (i) those effects are much more important for compounds **2a**, **2b**, and **2c**, and (ii) the enantioselectivity α decreases when the percentage of polar modifier (ethanol) decreases for compounds **2b**, **2c**; this is in contrast to the other compounds **2a**, **2d**, and **2e** where α remains more or less constant.

The Chiralcel OD-H column gives better results compared to Chiralpak AS for unprotected lactams **1**. The retention times of the two resolved peaks, under the same eluting conditions, for the latter column reach 1–2 h. For compounds **2** the Chiralpak AS column remains satisfactory with shorter k' but with a high decrease in α and R_s for compounds **2a**, **2b**, and **2c**. For example, with **2a** the values for k', α, and R_s were 2.83, 1.40, 7.90, and 2.13, 1.04, 1.33 for

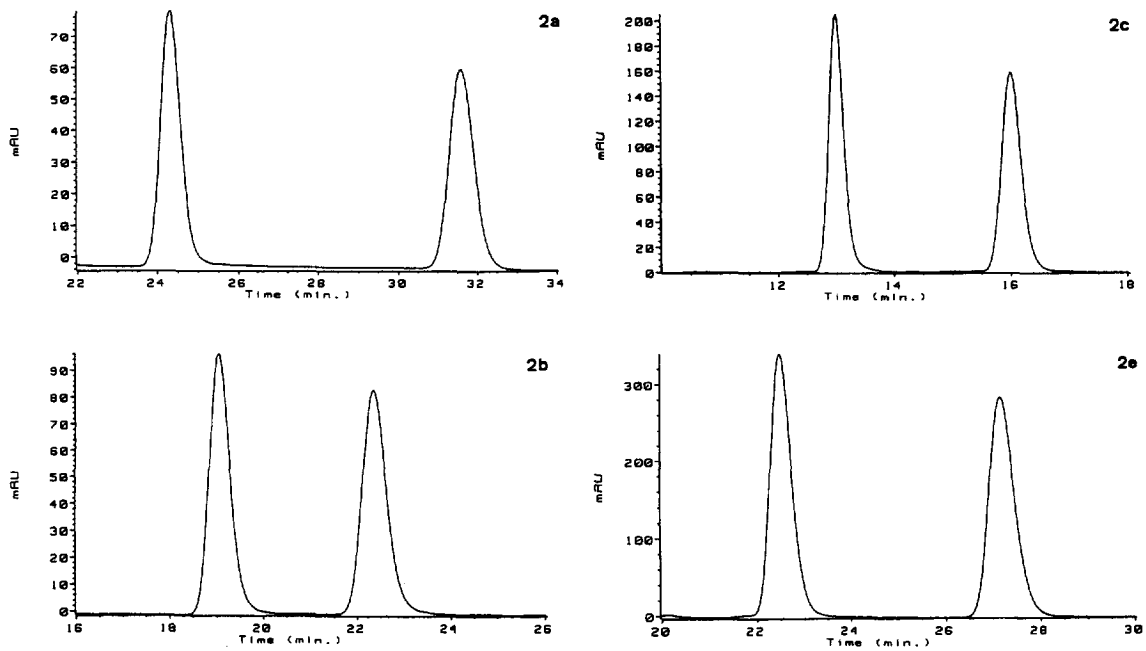

Fig. 3. Chromatograms (λ=230 nm) obtained for **2a** (eluent A; 0.5 ml/min); **2b** (eluent C; 0.5 ml/min); **2c** (eluent C; 0.5 ml/min); **2e** (eluent A; 0.5 ml/min).

the Chiralcel OD-H and Chiralpak AS phases, respectively, at a flow-rate of 0.5 ml/min. For **2d**, and **2e**, α and R_s are less affected. The most important adsorbing site for chiral recognition may be the carbamate residues near chiral glucose units. Cellulose and amylose differ only in the configuration on the 1-4 position of the D-glucose units. This results in a different spatial arrangement between cellulose and amylose. Recently it was proposed that possible structures may be a 3/2 helical chain conformation for modified cellulose and a 4/1 helical chain conformation for modified amylose. It was also postulated that these different higher order structures are responsible for the different chromatographic behaviors exhibited by cellulose- and amylose-modified CSPs [9].

The N-substitution of lactams **1** by a steric bulk such as *tert.*-butoxycarbonyl is necessary to observe a significant enantioselectivity for some compounds (**1a**->**2a**; **1b**->**2b**; **1c**->**2c**) and in all cases induces a greater separation. Moreover the N-substitution induces a lower k'. This is illustrated in the chromatograms **1d** and **2d** (Fig. 2).

In some eluting conditions, we note a marked difference in the enantioseparation between **2a**, **2b**, and **2c** on the one hand and **2d** and **2e** on the other. This may be due to the higher volume of the first ones or to their greater ability to induce π–π interactions with the carbamate substituent.

The good separation of optical isomers of **1** and particularly of **2**, especially on a Chiralcel OD-H column, (i) makes this chromatographic method suitable for quantifying optical purity and for studies in pharmacological distribution, and (ii) opens the way to the rapid preparative HPLC isolation of individual enantiomers. With respect to this second point, if necessary, better resolution could easily be obtained by varying the organic modifier percentage and/or the flow-rate.

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