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Short Communication

Direct chromatographic separation of the enantiomers of phaclofen, saclofen and hydroxysaclofen

Influence of the anionic moiety

Claude Vaccher, Pascal Berthelot and Michel Debaert*

Laboratoire de Pharmacie Chimique, Université de Lille II, B.P. 83, 3 Rue du Pr. Laguesse, 59006 Lille Cédex (France)

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ABSTRACT

The direct resolution of three analogues of baclofen (phaclofen, saclofen and hydroxysaclofen), potent γ-aminobutyric acid B antagonists, is achieved by HPLC on an enantioselective crown ether column. The effects of the anionic group are discussed. Perchloric acid and methanol as organic modifier is used as mobile phase. The absolute configuration is proposed by comparison with enantiomers of baclofen (β-*p*-chlorophenyl-γ-aminobutyric acid). The above-mentioned compounds are easily and completely resolved.

INTRODUCTION

γ-Aminobutyric acid (GABA) is a major depressant neurotransmitter involved in the control of neuronal activity in mammals [1]. Receptors for GABA have been subdivided into two distinct classes designated, according to their binding properties, GABA-A and GABA-B receptors [1–4]. Baclofen [4-amino-3-(4-chlorophenyl) butyric acid] (**1**) is the only selective agonist for GABA-B receptors: its *R*-(−) enantiomer is about 100 times more active than the *S*-(+) enantiomer [5]. Selective GABA-B antagonists are of importance to understand the functions of GABA-B receptors. Phaclofen [3-amino-2-(4-chlorophenyl)propylphosphonic acid: phosphonobaclofen] (**2**), saclofen [3-amino-2-(4-chlorophenyl)propylsulphonic acid: sulphonobaclofen] (**3**) and hydroxysaclofen [3-amino-2-(4-chlorophenyl)-2-hydroxypropylsulphonic acid] (**4**) (Fig. 1) have been recently developed in this way by changing the carboxylic acid group to a phosphonic or a sulphonic group. They have proved to be potent antagonists and are currently used as biological tools. As these compounds have a chiral centre at C-3, a detailed investigation of their pharmacodynamic properties could furnish a knowledge of the behaviour of each enantiomer. In this regard there is a real need for the resolution of the enantiomers of these antagonists. As a first step to achieving this, a chromatographic method to separate the

* Corresponding author.

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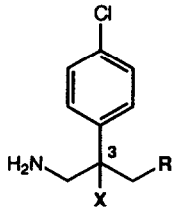
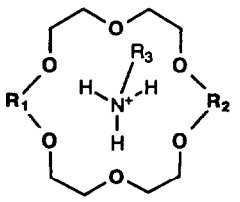


(1) R = CO_2H X = H
(2) R = PO_3H_2 X = H
(3) R = SO_3H X = H
(4) R = SO_3H X = OH



18-crown-6 ether : chiral selector of the CROWNPAK CR

Fig. 1. Chemical structures.

isomers has to be determined, preferably without a derivatization procedure.

We recently described the enantiospecific separation of the agonist baclofen (**1**) [6]. To directly resolve the enantiomeric components, we used a chiral crown ether moiety as chiral selector (Fig. 1). Our interest in the GABA-B receptors [7] prompted us to extend this study to the separation of **2**, **3** and **4**. A comparison was possible to investigate the importance of the anionic group of the molecules in the separation.

EXPERIMENTAL

Chromatography

High-performance liquid chromatography (HPLC) was developed on an LKB Model 2249 metering pump and detection was achieved with a Hewlett-Packard (HP) 1040 photodiode array spectrophotometer connected to an HP 9000 S300 computer. The routine detection wavelengths were 200, 220 and 225 nm. The column was a 150 × 4 mm I.D. Crownpak CR(+) (5 μm) column (Daicel, Baker, France). The sample loop was 10 μl and was made using a Rheodyne 7125 injector. Mobile phase elution was made isocratically using perchloric acid, diluted to obtain the required pH, and methanol as organic modifier. The flow-rate was 0.9 ml/min or 1.5 ml/min. The temperature of the column was controlled by the use of a circulating water through a jacket surrounding the column. Temperature was measured in the water bath. The temperatures used were 30 and 40°C.

TABLE I

ANALYTICAL HPLC: CAPACITY FACTORS (k'), SELECTIVITY OF RESOLUTION (α) AND RESOLUTION (R_s) OF **1–4**

Eluent: $HClO_4$ pH 2–CH_3OH (90:10).

Compound	Flow-rate (ml/min)	Temperature 30°C				Temperature 40°C			
		k'_S	k'_R	α	R_s	k'_S	k'_R	α	R_s
1	0.9	8.4	16.1	1.92	8.01	7.2	11.3	1.57	5.78[a]
	1.5					7.0	11.0	1.57	4.45
2	0.9	3.0	5.5	1.82	4.68	2.3	3.6	1.57	3.75
	1.5					2.3	3.6	1.57	2.89
3	0.9	3.5	7.5	2.15	7.31	2.6	4.9	1.85	6.04
	1.5					2.6	4.9	1.86	4.84
4	0.9	3.1	9.1	2.95	9.98	2.4	5.7	2.40	7.92
	1.5					2.4	5.7	2.40	6.64

[a] Ref. 6.

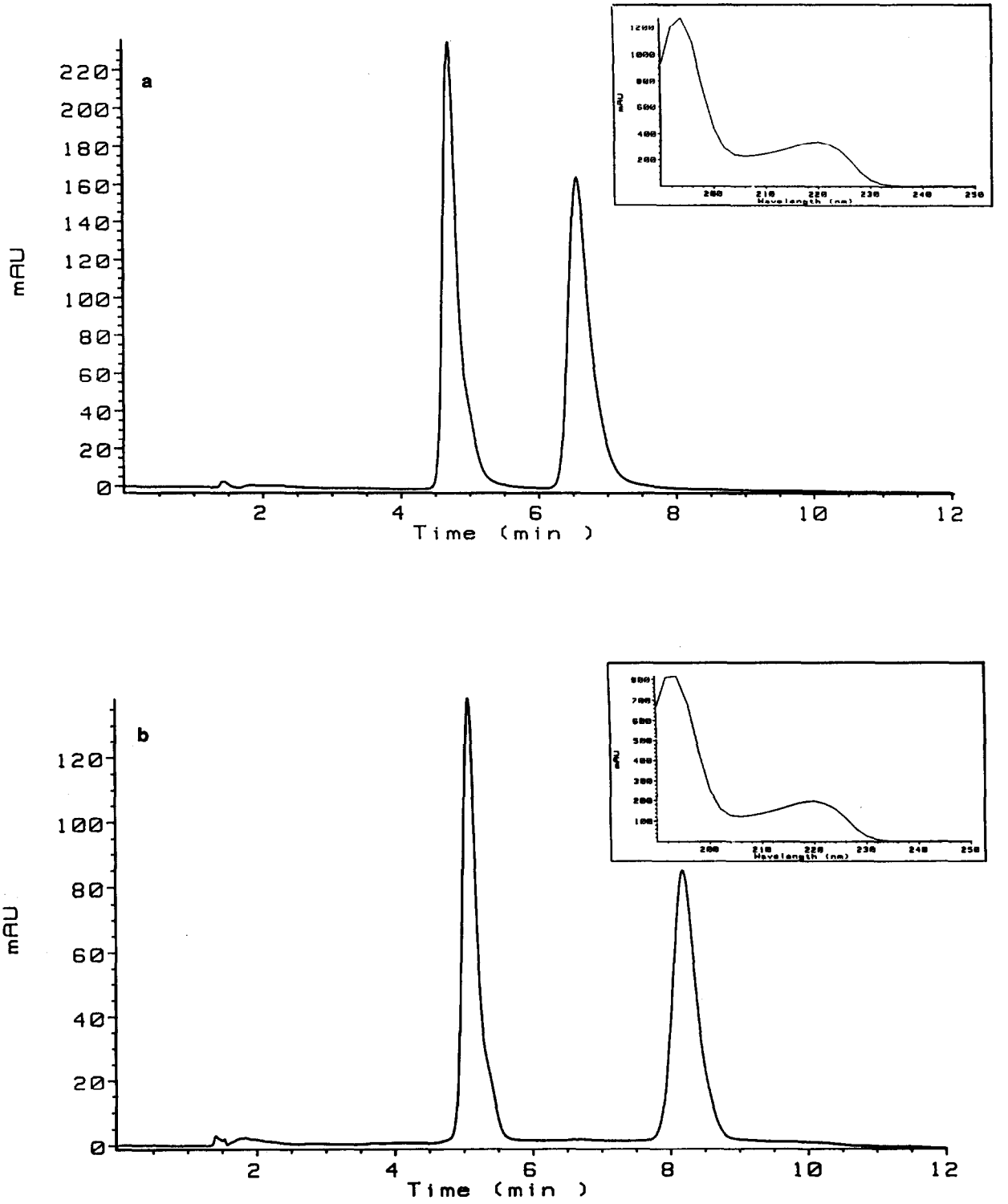


Fig. 2.

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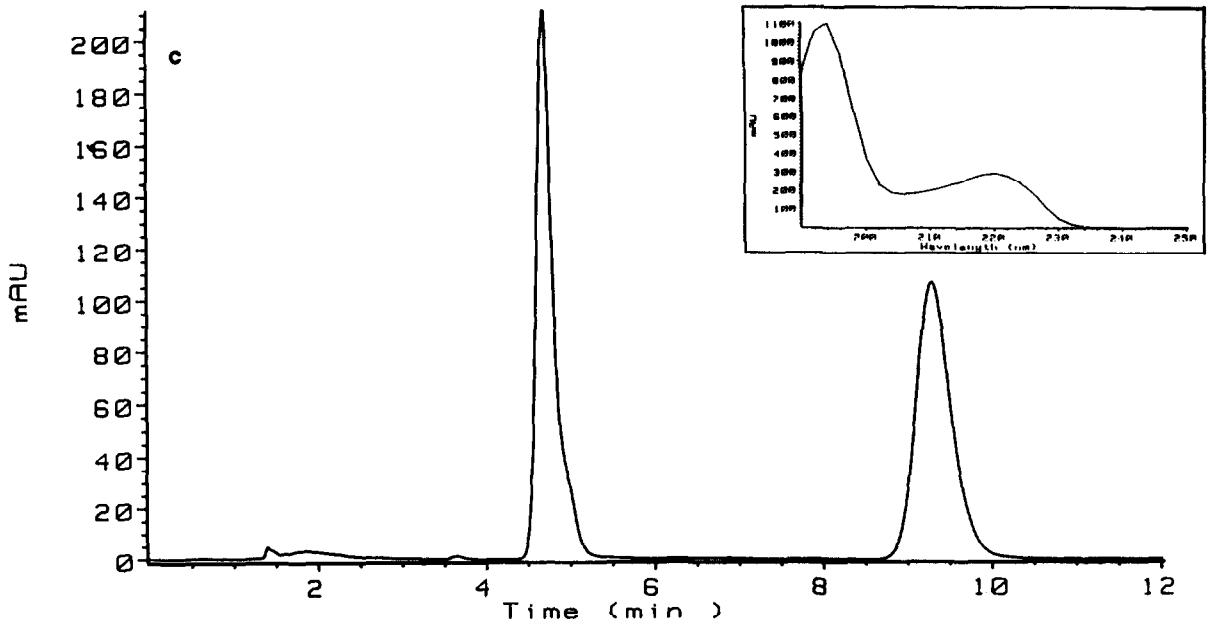

Fig. 2. Chromatographic resolution of (a) **2**, (b) **3** and (c) **4** ($\lambda = 225$ nm). Eluent: $HClO_4$ pH 2–CH_3OH (90:10). Temperature: 40°C. Flow-rate: 0.9 ml/min. The others conditions are described in the Experimental section. Inserts show the UV spectrum of the first eluted peak.

Reagents and materials

Baclofen (**1**) was kindly supplied by Ciba-Geigy. Compounds **2–4** were purchased from Tocris-Neuramin (Langford, Bristol, UK): only their racemates were available. Water used was purified through a Milli-Q unit. The methanol was gradient grade from Merck. Perchloric acid was of analytical grade from Prolabo. The required pH was obtained after dilution as described in the technical note supplied with the column: concentrated acid (70%, 13.6 g) is diluted in 1 l of water to give a pH 1.0 solution, further dilution (100 ml to 1 l) gives a pH 2.0 solution. All the solutions were filtered (0.45 μm), degassed and purged with helium. The mobile phase used was $HClO_4$ pH 2–CH_3OH (90:10). All amino acids were dissolved in the mobile phase to a concentration of about 1.6 m*M* calculated in racemate (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 the apparatus were thoroughly washed with water at the end of each day.

RESULTS AND DISCUSSION

The enantiomeric separation of compounds **1–4** on the crown ether chiral stationary phase is summarized in Table I. For **2**, **3** and **4** adequate resolution can be easily achieved using a $HClO_4$ pH 2–CH_3OH (90:10) solution as mobile phase and representative chromatograms ($\lambda = 225$ nm) are shown at a flow-rate of 0.9 ml/min and 40°C (Fig. 2). The UV spectrum is shown in Fig. 2 for the first eluted peak: as expected, the UV absorbance of the separate enantiomers was identical. Moreover, the UV spectra of compounds **2–4**, are, of course, very similar due to the same conjugation.

The designation of k'_S and k'_R as the first and the second peaks respectively was proposed by analogy with the chromatography, under similar conditions, of authentic samples of the enantiomers of baclofen.

The lower the temperature, the better the resolution becomes (α, R_s increase). The factors α and R_s were found to decrease with increasing temperature from 30 to 40°C: as expected, the resolution increased at the expense of increased

retention times as well as broadened peak shape. The separation remained very satisfactory even at high temperature (40°C), at high-flow-rate (1.5 ml/min) and with an organic modifier in the mobile phase ($R_s \gg 1$).

Under identical eluting conditions the order of elution was (Table I) (Fig. 3): **1** (7.0) > **3** (2.6) > **2** (2.3) (40°C, k'_S in parentheses), and **1** (11.0) > **3** (4.9) > **2** (3.6) (40°C, k'_R in parentheses). The same observations can be made at 30°C. So **2** and **3** are much less retained. These compounds are certainly more hydrophilic than baclofen. The substituted constant Π is a good measure of hydrophobicity [8] and the increment values can be calculated for the -COOH, $-SO_3H$, $-PO_3H_2$ moieties: −0.32 (**1**), −1.76 (**3**) and −2.48 (**2**), respectively. They are in perfect accordance with capacity factor order: **2** and **3** constitute a group quite different from **1**. Similar chromatographic behaviour was observed between glutamic acid and its phosphono analogue where the phosphono function is considerably more hydrophilic than the carboxylic one [9]. The factors α and R_s follow the order: α **1**, **2** (1.57) < **3** (1.86), and R_s **2** (3.75) < **1** (5.78) < **3** (6.04) (Fig. 3).

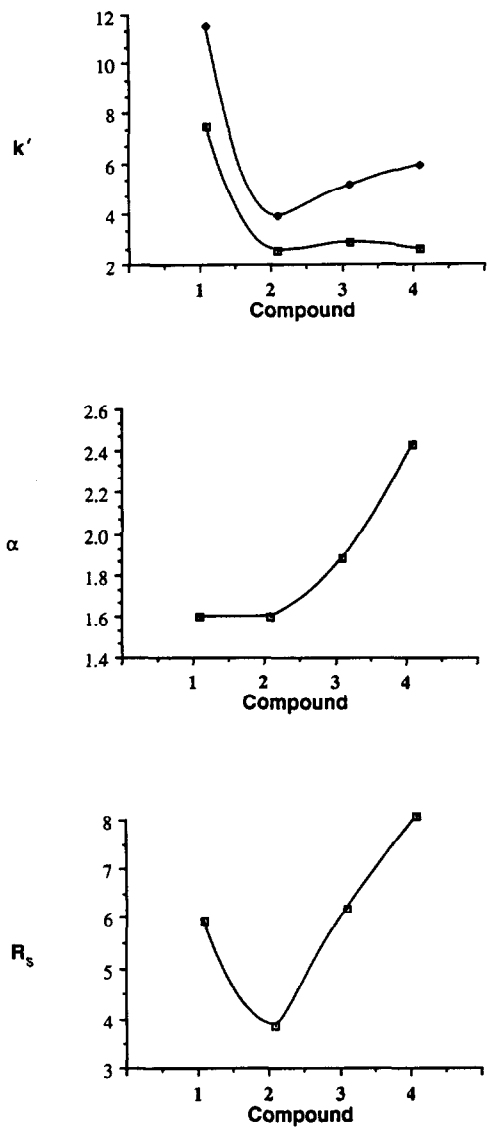

Fig. 3. Variation of the capacity factor k' (□ = k'_1; ◆ = k'_2), enantioselectivity α and resolution R_s of compounds **1–4**. Eluent: $HClO_4$ pH 2–CH_3OH (90:10). Temperature: 40°C. Flow-rate: 0.9 ml/min. (λ = 225 nm). Other conditions are described in the Experimental section.

Between **3** and **4** the presence of a polar OH substituent increases the possibility of chiral discrimination due to the "three-point" interaction, which may involve a supplementary hydrogen bonding and/or dipole interactions between **4** and the chiral stationary phase. In this case steric bulk may also be a factor that affects separation on the column. It can either enhance or decrease enantioselectivity. This depends on whether or not a bulky group prevents the ammonium group from forming a strong inclusion complex. It has been observed that when the primary amine is further removed from the stereogenic centre, steric bulk (due to OH in hydroxysaclofen) seems to enhance the enantioselectivity [10]: **3** (1.86) < **4** (2.40) (40°C, α in parentheses), and **3** (4.84) < **4** (6.64) (40°C, R_s in parentheses). But if a bulky substituent and the amino group are α to the stereocentre, this induces a decrease in enantioselectivity [10].

The good separation of optical isomers of **2–4** make this chromatographic method suitable (i) to quantify optical purity and for studies in pharmacological distribution and (ii) for preparative separation of the enantiomers. In the second case better resolution could, if necessary, easily be obtained at lower pH or without organic modifier or at lower temperature. This method has the advantage of being less time-consuming and less troublesome than the derivatization procedures.

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