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Enantiomeric separation of amino alcohols on dinitrobenzoyl-diaminocyclohexane chiral stationary phases

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ABSTRACT

A silica-bonded chiral stationary phase, containing the N,N'-3,5-dinitrobenzoyl derivative of 1*R*,2*R*-diaminocyclohexane, was used to separate the enantiomers of some amino alcohols with β-blocking activity after their conversion to oxazolidin-2-ones. The influence of mobile phase composition (mixtures of hexane with dichloromethane, chloroform, dioxane and isopropanol) on the enantioselectivity and efficiency of the column was evaluated. Furthermore, a tandem arrangement of the chiral column and its racemic version was used to resolve all the stereoisomers of one amino alcohol containing two stereogenic centres.

INTRODUCTION

The development of adrenergic blocking agents (β-blockers) has been one of the most important pharmacological and therapeutic innovations in recent years. These drugs are widely used in the treatment of angina, cardiac arrhythmias, hypertension [1] and anxiety [2].

The stereoisomerism of β-blockers is due to the presence of a stereogenic centre in the arylpropanolamine side-chain. Owing to the different pharmacological activity of the enantiomers (*S*-propranolol is about 100 times more active than the *R* enantiomer [2]), there is an increasing need to separate racemic mixtures of such compounds at analytical and preparative levels.

Resolution by HPLC of racemic β-blockers has been achieved, after cyclization to oxazolidin-2-ones, using Pirkle-type chiral stationary phases (CSPs) [3,4]. Microbore and standard analytical columns packed with a CSP derived from *trans*-1,2-diaminocyclohexane have been employed in the resolution of a large series of β-blockers and related compounds after conversion to oxazolidin-2-ones with phosgene or 1,1-carbonyldiimidazole [5,6]. Underivatized enantiomers of β-blockers have been separated on columns packed with tris(3,5-dimethylphenylcarbamate) cellulose supported on silica gel [7], immobilized bovine serum albumin [8], α_1'-acid glycoprotein [9] and ovomucoid [10]; recently, a CSP derived from a 3,5-dinitrobenzoyl-aminophosphonate has been developed for the direct separation of underivatized β-blockers [11].

In the present communication we describe the

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results obtained in the direct HPLC resolution of the enantiomers of two β-blocking agents using a π-acid CSP containing the N,N'-3,5-dinitrobenzoyl derivatives of *trans*-1,2-diaminocyclohexane covalently bonded to a silica matrix (DACH–DNB CSP). This kind of stationary phase is obtained by a three-step modification of silica microparticles consisting of covalent binding of chiral (or racemic) *trans*-1,2-diaminocyclohexane via reactive epoxy groups and subsequent derivatization with 3,5-dinitrobenzoyl chloride. A large number of racemic compounds have been separated on this CSP, including sulphoxides, selenoxides, phosphinates, phosphinoxides, derivatized acids, amines and amino acids containing at least one π-basic function in their structure [12].

EXPERIMENTAL

Apparatus

Chromatography was performed using a Waters M510 HPLC pump, a U6K injector, and an M490 programmable multi-wavelength detector and temperature control module; chromatographic data were collected and processed on a Waters 840 data and chromatography control station.

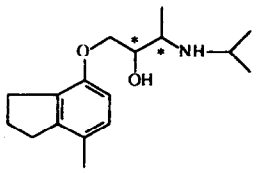
The following columns were used: *R,R*-DACH–DNB CSP (250 × 4 mm I.D.) and its racemic version (150 × 4 mm I.D.) at a flow-rate of 1 ml/min at 25°C; the wavelength used was 275 nm. Synthesis, column packing and kinetic evaluation are described elsewhere [5,6,12].

Chemicals

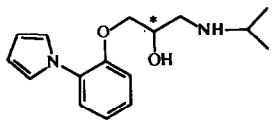
HPLC-grade solvents were obtained from Merck (Darmstadt, Germany); 1,1'-carbonyldiimidazole and 20% phosgene solution in toluene were from Fluka (Buchs, Switzerland); the racemic analytes were supplied by the following manufacturers: (2*RS*, 3*RS*)-1-(2,3-dihydro-7-methyl-1H-inden-4-yl)oxy-3-(1-methylethyl)-amino-butan-2-ol hydrochloride from ICI-Pharma (Milan, Italy) and (2*RS*)-1-(2-(1-pyrrolyl)-phenoxy)-3-isopropylamino propan-2-ol hydrochloride from Ciba-Geigy (Basle, Switzerland). Conversion of racemic amino alcohols into oxazolidin-2-ones was accomplished as previously described [6] with 90% yield.

RESULTS AND DISCUSSION

All the racemic mixtures of amino alcohols (compounds **1–3**) could be efficiently separated after their conversion to oxazolidin-2-ones; chromatographic data pertinent to these resolutions are listed in Table I. No separations were observed when chromatography of the underivatized amino alcohols was attempted because of the strong interaction between the amino group and the stationary phase. Cyclization to the



compound 1 : *erythro*
compound 2 : *threo*



compound 3

TABLE I

CHROMATOGRAPHIC DATA FOR THE RESOLUTION OF COMPOUNDS 1–3 AS OXAZOLIDIN-2-ONES ON THE *R,R*-DACH–DNB CSP

Eluents employed are: (A) *n*-hexane–dioxane (50:50); (B) *n*-hexane–dichloromethane (40:60); (C) *n*-hexane–chloroform (25:75); (D) *n*-hexane–2-propanol (40:60).

Entry	Compound	k_1' [a]	α [b]	R_s [c]	Eluent
1	**1**	7.69	1.30	2.6	A
2	**1**	7.69	1.20	1.6	B
3	**1**	8.12	1.22	1.2	C
4	**1**	10.15	1.20	1.1	D
5	**2**	8.19	1.57	5.2	A
6	**2**	8.12	1.36	3.9	B
7	**2**	8.63	1.40	2.8	C
8	**2**	10.51	1.38	2.2	D
9	**3**	9.93	1.29	2.2	A
10	**3**	8.30	1.26	1.6	B
11	**3**	8.56	1.27	1.5	C
12	**3**	8.95	1.30	1.4	D

[a] The capacity factor of the first eluted enantiomer.
[b] The enantioselectivity factor.
[c] The resolution factor.

oxazolidin-2-ones reduces both the basicity of the analytes and their conformational flexibility, thus leading to good values of enantioselectivity and chromatographic efficiency. The enantioselectivity factors (α) for compounds **1** and **2** show a significant dependence on eluent composition, higher values being observed when 1,4-dioxane is used as polar modifier in the mobile phase (Fig. 1); for compound **3** the degree of enantioselectivity observed is almost constant with the four eluents used. Resolution factors (R_s) show a greater dependence on the nature of the mobile phase as a consequence of variations in both α and efficiency of separation: the lowest R_s values are obtained using 2-propanol as a polar modifier, because of the higher viscosity of the eluent and thus lower diffusion coefficients of the analytes: passing from dichloromethane to chloroform or 2-propanol results in a loss of resolution in spite of similar α and k_1' values (Table I, entries 2, 3, 4; 6, 7, 8; 10, 11, 12).

Of the two diastereoisomers of compounds **1** and **2**, only the erythro form is active: it is therefore desirable to have a stereoselective method able to separate and quantify all four possible isomers, *e.g.* to check the stereochemical stability (*in vitro* and/or *in vivo*) of both stereogenic centres of the drug. From the data listed in Table I it is clearly seen that the chiral column does not afford the required diastereoselectivity: in fact, the separation factors between the first eluted enantiomers of the erythro and threo forms (ratio of the k' values listed in Table I) are always smaller than 1.10, leading to incomplete resolution of the four stereoisomers (overlapping of the first two eluting peaks); a common practice to improve global selectivity in such situations is to connect in series columns with different, complementary selectivities and similar mobile phase requirements [12,13]: by using two columns in series, one packed with the *R,R*-DACH–DNB CSP and the other with its racemic analogue, the complete separation of the four stereoisomers of compounds **1** and **2** is achieved using a ternary mobile phase (chromatogram C, Fig. 2). The composition of the mobile phase was chosen in order to obtain the highest values of enantioselectivity together with a good chromatographic efficiency.

It is worth noting that the tandem arrangement between the chiral column DACH–DNB CSP and a silica column (LiChrosorb Si 60), successfully employed to resolve the four stereoisomers of the racemic ephedrine and ψ-ephedrine [6], it is not feasible in the present

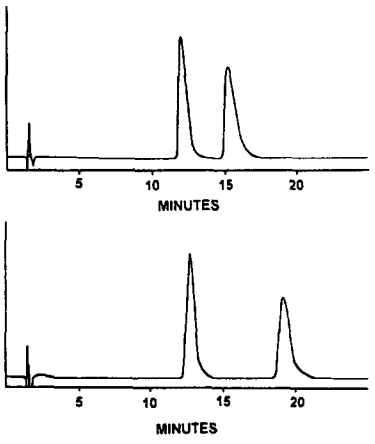


Fig. 1. Chromatographic analysis of compounds **1** (top) and **2** (bottom) using eluent A. See Table 1 for other conditions.

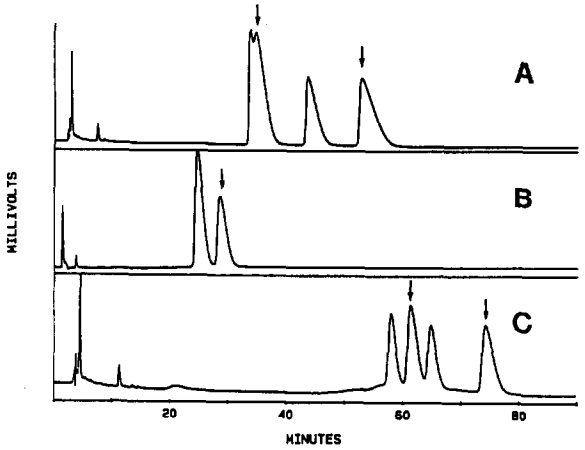


Fig. 2. Chromatographic analysis of the four stereoisomers of compounds **1** and **2**. Eluent: *n*-hexane–dioxane–dichloromethane (60:30:10); (A) *R,R*-DACH–DNB CSP; (B) Racemic version of the CSP; (C) tandem arrangement: *R,R*-DACH–DNB and the racemic version coupled in series. Arrows indicate peaks relative to the *threo* isomer.

case because of the opposite diastereoselectivities afforded by the two phases (the erythro isomer is most retained on the silica column).

CONCLUSIONS

In conclusion, a method for the separation and quantization of the optical isomers of β-blocking agents is reported. The separation of the enantiomers of chiral drugs having more than one stereogenic centre is, in principle, not significantly different from that for compounds with a single stereogenic centre. However a coupled achiral–chiral system is often necessary to compensate for the weak diastereoselectivity of a chiral column.

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