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Note

Application of enantioselective capillary gas chromatography to the analysis of chiral pharmaceuticals

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Only *ca.* 20% of chiral drugs are used as pure stereoisomers[1], although it is known from numerous examples that optical isomers may have extremely different biological effects. The use of racemic thalidomide as a sedative some years ago with only the (*S*)-(−)-enantiomer having the disastrous teratogenic effect is a drastic case[2]. The progress in asymmetric synthesis of chiral compounds in recent years[1] has also stimulated the development of new analytical techniques for evaluating enantioselectivity of a synthetic method.

It has been shown that enantioselective gas chromatography (GC) is a versatile and sensitive method for configurational analysis[3]. In particular, the availability of thermostable polymer chiral stationary phases[4–7] and the use of novel derivatives[8] have extended the scope of application of this technique.

In a previous communication[6] we demonstrated the high enantioselectivity of the polymer XE-60-L-valine-(*R*)-α-phenylethylamide for amino alcohols. This study deals with the application of this stationary phase to the enantiomer separation of pharmaceuticals with adrenergic and, particularly, β-blocking effects and of some barbiturates.

EXPERIMENTAL

Gas chromatography

A Carlo Erba Model 2101 gas chromatograph with split inlet and a flame ionization detector was used.

Preparation of chiral capillary columns

A suspension of 16 mg of Silanox (Cabot, Boston, MA, U.S.A.) in 3 ml of chloroform was slowly passed through a 18 m × 0.25 mm I.D. Duran glass capillary. After 15 min drying by passing nitrogen through the column the capillary was installed in a gas chromatograph and heated for 1 h at 300°C under nitrogen. For coating by the static procedure[9] a 0.22% solution of XE-60-L-valine-(*R*)-α-phenylethylamide in methylene chloride (w/v) was used. The preparation of the chiral stationary phase was slightly modified from that described previously[6]. Instead of alkaline hydrolysis, a 1:1 (v/v) mixture of concentrated hydrochloric acid and dioxane was used to convert the cyano groups into carboxylic groups. The completion of the reaction was checked by infrared spectroscopy.

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Formation of derivatives

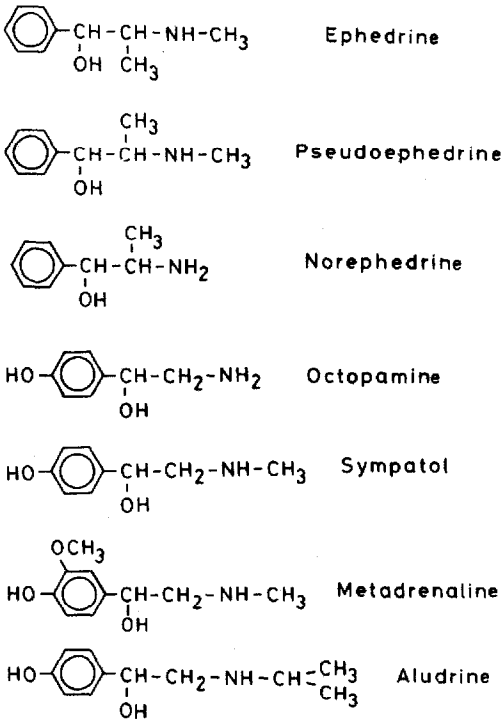
Heptafluorobutyryl derivatives were prepared by heating samples of *ca.* 100–200 μg in a mixture of 150 μl of methylene chloride and 50 μl of heptafluorobutyric acid anhydride for 30 min at 100°C in Wheaton Micro Vials (Wheaton Scientific, Millville, U.S.A.). Excess reagent was removed with a stream of dry nitrogen. For GC the derivatives were dissolved in methylene chloride.

RESULTS AND DISCUSSION

Ephedrine and its analogues (Scheme 1) are potent adrenergic agents with a variety of therapeutic applications. Different vasopressor activity levels have been reported for the enantiomers of ephedrine and pseudoephedrine[10], and a procedure for analyzing the composition of a mixture of enantiomers may be of interest for pharmacokinetic and metabolic studies. Excellent separations of diastereomeric derivatives of several amino alcohols of the ephedrine type have been obtained on a column with a chiral stationary phase after N-acylation with L-α-chloroisovaleryl-chloride and O-trimethylsilylation[11].

In a different approach we have also investigated the diastereomeric N-trifluoroacetyl-L-alanyl-O-trimethylsilyl derivatives of amino alcohols of the ephedrine type[12]. They could be separated on capillary columns with OV-17 or SE-30.

The separation of the N,O-pentafluoropropionyl derivatives of the enantiomers of ephedrine and of some analogues has been carried out by Frank *et al.*[13]



Scheme 1.

TABLE I

SEPARATION FACTORS (α) AND COLUMN TEMPERATURES FOR ENANTIOMER SEPARATION OF AMINO ALCOHOLS OF THE EPHEDRINE TYPE

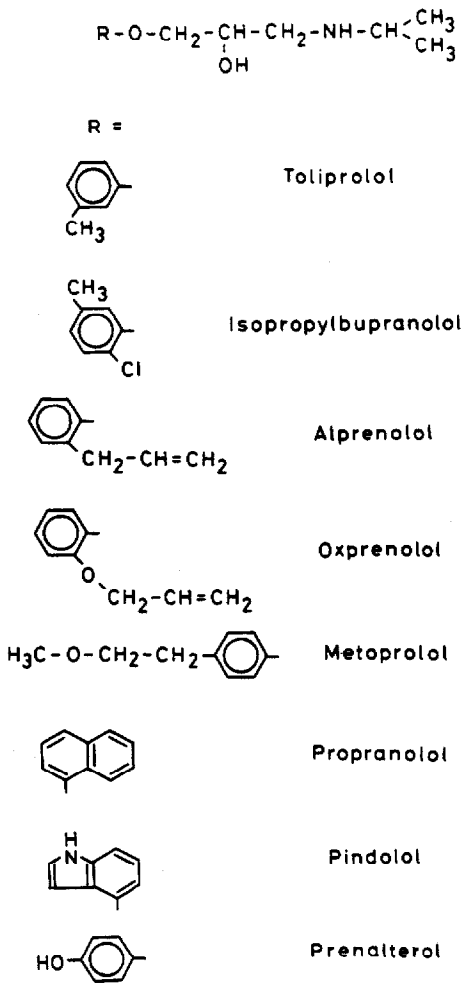
N,O-Bis-heptafluorobutyryl derivatives separated on a 18-m glass capillary column coated with XE-60-L-valine-(*R*)-α-phenylethylamide.

Racemate	*α-Value*	*Column temp. (°C)*
Ephedrine	1.039	120
Pseudoephedrine	1.070	120
Norephedrine	1.089	120
Sympatol	1.037	140
Aludrine	1.043	150
Metadrenaline	1.050	140
Octopamine	1.147	150

using Chirasil-val. Polymer stationary phases obtained by connecting L-valine-(*R*)-α-phenylethylamide to hydrolyzed XE-60[6] or OV-225[7] were found to have a striking enantioselectivity for perfluoroacylated amino alcohol derivatives[6]. The results of the enantiomer separations of ephedrine and of the analogues (Scheme 1) are given in Table I. In the case of ephedrine and pseudoephedrine the (−)-isomers, having (*R*)-configuration at the asymmetric centre next to the hydroxy group, are retarded on XE-60-L-val-(*R*)-α-phenylethylamide.

Procedures for the GC analysis of β-adrenoceptor blocking drugs[14] have been suggested by several authors, mainly for metabolic and pharmacokinetic studies[15–18]. Recently a procedure for the GC and high-performance liquid chromatographic resolution of (*R*)- and (*S*)-propranolol, using a chiral isocyanate for the formation of diastereoisomeric derivatives, was described by Thompson *et al.*[19]. The authors emphasized the different biological activities of the two stereoisomers of the drug. However, even with a very short packed GC column rather high temperatures are necessary to elute the derivatives from the column. More volatile derivatives of β-blockers may be obtained by perfluoroacylation. However, it is known[20,21] that these derivatives are very sensitive to active sites in the column, particularly at the high temperature necessary for elution. Irreversible adsorption and decomposition were experienced, depending very much on the type of stationary phase and on the pretreatment of the glass surface and increasing with retention time. It was also found that the heptafluorobutyryl derivative of metoprolol is less sensitive than the trifluoroacetyl derivative[21].

Our own attempts at enantiomer separation of perfluoroacylated β-blockers were rather discouraging at the beginning, even with capillary columns displaying excellent separation qualities for many other substances. In this investigation we used a batch of XE-60-L-val-(*R*)-α-phenylethylamide thoroughly purified by gel filtration on Sephadex LH-20 and short Duran glass capillaries after Silanox pretreatment without any further deactivation. We succeeded in separating the enantiomers of the drugs listed in Scheme 2. In the case of metoprolol both enantiomers were available in pure form. The (+)-form was eluted prior to the (−)-enantiomer. There was no indication of racemization during derivatization. The separation factors are listed in



Scheme 2.

Table II. An example for the separation is given in Fig. 1. The completeness of acylation was proved by GC–MS investigations in each case.

So far only compounds with isopropyl residues as substituents at the amino group have been separated. Derivatives of β-blockers having *tert.*-butyl substituents were not separated, although bis-acylation was evident from the mass spectra. Presumably the bulky *tert.*-butyl group makes the molecule less accessible to diastereomeric association with the stationary phase.

Of the various types of chiral drugs we have also investigated the barbiturates hexobarbital (Evipan) and prominal. Although these compounds have very low volatility they could both be separated on a 18-m capillary column with XE-60-L-val-(*R*)-α-phenylethylamide at 190°C with α-values of 1.034 and 1.020, respectively, as shown in Fig. 2.

The extension of enantioselective GC to chiral pharmaceuticals opens new

TABLE II

SEPARATION FACTORS (α) AND COLUMN TEMPERATURES FOR ENANTIOMER SEPARATION OF AMINO ALCOHOLS WITH β-BLOCKING ACTIVITY*

O,N-Bisheptafluorobutyryl derivatives separated on a 18-m glass capillary column coated with XE-60-L-valine-(*R*)-α-phenylethylamide.

Racemate	*α-Value*	*Column temp. (°C)*
Toliprolol	1.035	140
Isopropylbupranolol	1.026	150
Alprenolol	1.034	150
Oxprenolol	1.023	150
Metoprolol	1.029	160
Propranolol	1.027	170
Pindolol	1.032	170
Prenalterol*	1.054	140

* Prenalterol has β-adrenergic agonist properties.

possibilities in pharmacokinetic and metabolic studies. Investigations are currently in progress to determine whether the method described in this report is applicable to other types of chiral drug.

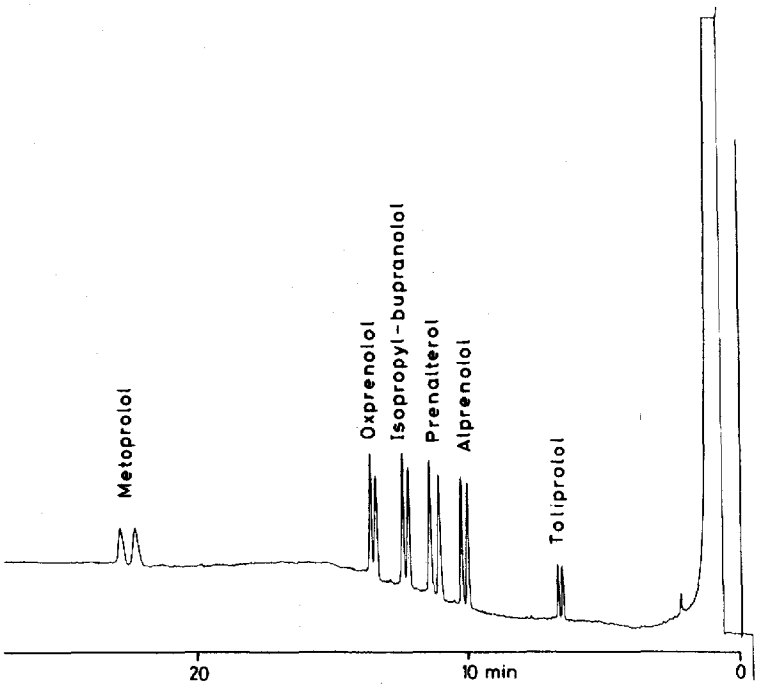


Fig. 1. Enantiomer separation of N,O-heptafluorobutyryl derivatives of some β-blockers on a 18-m Duran glass capillary column coated with XE-60-L-val-(*R*)-α-phenylethylamide. Column temperature, 150°C, 5 min isothermal, temperature programme 1.5°C/min to 160°C. Carrier gas, hydrogen at 0.7 bar.

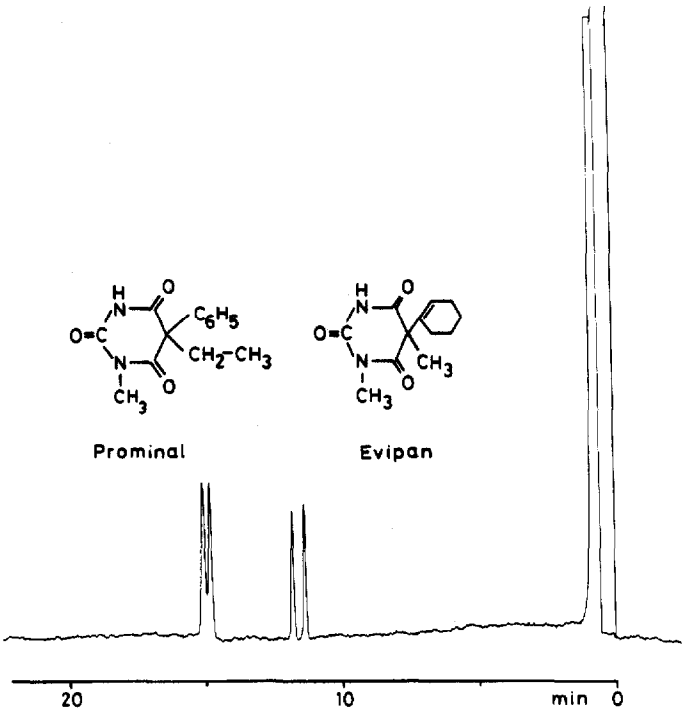


Fig. 2. Enantiomer separation of hexobarbital and prominal. Column as in Fig. 1. Column temperature 190°C. Carrier gas hydrogen at 1.0 bar.

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