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Note

Enantiomer separation of phenolic α- and β-receptor active drugs by chiral capillary gas chromatography after derivatization with diazomethane and phosgene

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α- and β-adrenergic stimulants of the adrenaline type are important and widely used drugs with antiasthmatic or cardial activity[1]. Although it is known that the *S*-enantiomer of adrenaline (epinephrine) has 10–100 fold and of isoprenaline (aludrine) up to 1500 fold higher activity than the corresponding *R*-isomer[2], most of these drugs are still used in the racemic form. Nevertheless, there is a strong tendency to use enantiomerically pure pharmaceuticals, and techniques for determining their optical purity have received much attention.

In recent communications[3–6] we have shown that the enantiomers of pharmaceuticals of the amino alcohol type can be separated by capillary gas chromatography (GC) with the chiral stationary phase XE-60-L-valine-(*R*)-α-phenylethylamide after formation of the oxazolidin-2-one derivatives using phosgene as a reagent. In this work we have extended this approach to amino alcohols with phenolic substituents.

EXPERIMENTAL

Chemicals and derivatization

Samples of adrenaline and its analogues were either commercially available or supplied by the Department of Organic Chemistry, AB Hässle. A saturated solution of diazomethane in diethyl ether was prepared in a micro-reactor[7] from N-methyl-N-nitroso-*p*-toluenesulphonamide in 40% aqueous potassium hydroxide solution.

An excess of the diazomethane solution was added to a solution of 0.5–1 mg of sample in 200 μl of methanol and kept for 3 h at room temperature. The excess of reagent and of the solvent were then removed in a stream of nitrogen. To the residue, 400 μl of diethyl ether, 50 μl of 0.5 *M* aqueous sodium hydroxide solution and 50 μl of a 20% solution of phosgene in toluene were added. The mixture was shaken occasionally and kept for 1 h at room temperature. The organic phase was transferred to a dry vial and the solvent and excess of reagent were removed in a stream of nitrogen. A 400-μl volume of dichloromethane was added to the residue, and the solution again taken to dryness in a stream of nitrogen. Finally, the sample was dissolved in 200 μl of dichloromethane for GC analysis.

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Gas chromatography

A Carlo Erba Model 2101 gas chromatograph with a split inlet and a flame ionization detector was used. The preparation of the chiral stationary phases[8] and coating of the glass capillary columns has been described elsewhere[9].

RESULTS AND DISCUSSION

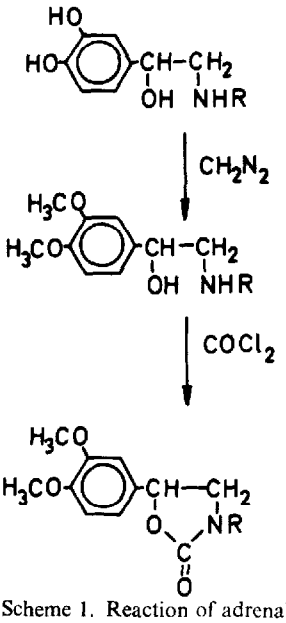
Phosgene is a versatile and convenient reagent for enantiomer separation of mainly bifunctional compounds, namely diols, amino alcohols, aminothiols, N-methylamino acids and hydroxy acids[3–6]. According to our experience, this derivatization method produces chemically and configurationally stable derivatives. This is particularly important for pharmaceuticals like ephedrine and its analogues, which tend to racemize when perfluoroacylation is used to form volatile derivatives for enantiomer separation[10,11]. For ephedrine, racemization of the chiral centre next to the hydroxy group was observed after formation of the N,O-bis(heptafluorobutyryl) derivative[12]. It is also known that these derivatives are highly sensitive to humidity and to active sites in the GC system[13].

Phenolic amino alcohols react with phosgene and methanol at pH 12 to yield a methyl carbonate oxazolidin-2-one derivative[14]. This type of derivative, however, has an unduly long retention time in the chromatographic system, *e.g.*, 90 min for prenalterol at 190°C (1.3 bar H_2).

Catechols such as adrenaline react with phosgene in an aqueous buffer to give a cyclic carbonate oxazolidin-2-one derivative[15]. Due to the polar nature of the cyclic carbonate, this derivative partially decomposed in the chromatographic system. Under the strongly alkaline conditions (sodium hydroxide), used to form oxazolidin-2-one derivatives, the phenolic residue forms a salt and again sufficient volatility cannot be achieved. To avoid these problems, diazomethane is used to form methyl ether derivatives prior to the reaction with phosgene (Scheme 1). This approach has been used before in the stereochemical analysis of a new amino acid isolated from a hydrolyzate of nikkomycin B, an antibiotic with antifungal and insecticidal properties[16].

Using this procedure it was possible to obtain sufficiently volatile derivatives of many sympathomimetic drugs (Table I), which could all be baseline separated on a 15-m glass capillary column containing the chiral phase XE-60-L-valine-(*R*)-α-phenylethylamide. An example is shown in Fig. 1. Some N-methylation was observed in the treatment with diazomethane of compounds having primary amino groups. This may result in ambiguities when synephrine and octopamine or phenylephrine and norfenefrine are present in the same sample. Also, adrenaline and metadrenaline with a 3-OCH_3 group at the aromatic ring give identical derivatives after treatment with diazomethane. For these cases diazoethane may be used in order to distinguish between the original compounds. In all cases gas chromatography–mass spectrometry was applied to confirm the structure of the derivatives.

The separation factors (Table II) are almost identical in each case, independent of the kind of substituent present at the amino group. Except for adrenaline analogues, the enantiomers of the phenolic metabolite of the β-receptor blocking agent alprenolol and the β-receptor agonist prenalterol and its antipode could be separated under the same conditions.



Scheme 1. Reaction of adrenaline and analogues with diazomethane and phosgene.

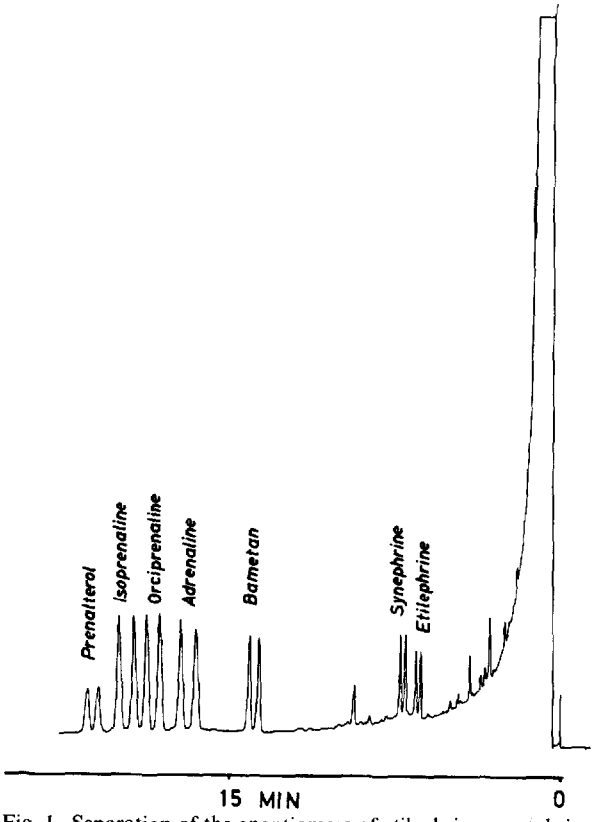
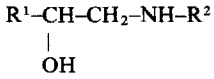


Fig. 1. Separation of the enantiomers of etilephrine, synephrine, bametan, adrenaline, orciprenaline, isoprenaline and prenalterol after reaction with diazomethane and phosgene. Column: 15-m glass capillary containing XE-60-L-valine-(*R*)-α-phenylethylamide; temperature, 190°C. Carrier gas: 1 bar H_2.

TABLE I

STRUCTURES OF RACEMIC COMPOUNDS SEPARATED BY CAPILLARY GAS CHROMATOGRAPHY ON XE-60-L-VALINE-(*R*)-α-PHENYLETHYLAMIDE



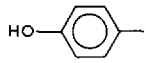
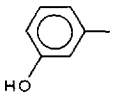
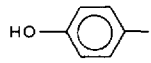
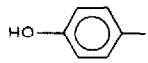
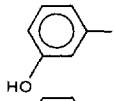
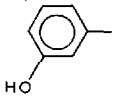
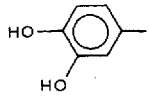
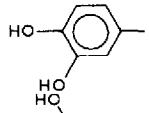
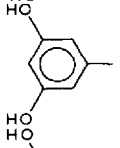
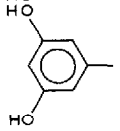
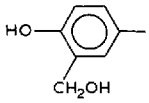
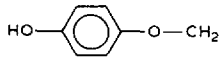
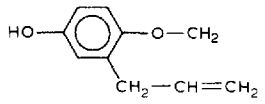
Compound	R^1	R^2
Synephrine		CH_3
Etilephrine		CH_3CH_2
Bametan		$CH_3(CH_2)_3$
Octopamine		H
Norfenefrine		H
Phenylephrine		CH_3
Adrenaline		CH_3
Isoprenaline		$(CH_3)_2CH$
Orciprenaline		$(CH_3)_2CH$
Terbutaline		$(CH_3)_3C$
Salbutamol		$(CH_3)_3C$
Prenalterol		$(CH_3)_2CH$
4-Hydroxyalprenolol		$(CH_3)_2CH$

TABLE II

SEPARATION FACTORS, α, AND COLUMN TEMPERATURES FOR ENANTIOMER SEPARATION OF RACEMIC PHARMACEUTICALS AFTER DERIVATIZATION WITH DIAZOMETHANE AND PHOSGENE (ACCORDING TO SCHEME 1)

Column: 15-m glass capillary containing XE-60-L-valine-(*R*)-α-phenylethylamide.

Compound	α	*Column temp. (°C)*
Synephrine	1.033	190
Etilephrine	1.036	190
Bametan	1.033	190
Octopamine	1.033	200
Norfenefrine	1.042	190
Phenylephrine	1.044	190
Adrenaline	1.041	190
Isoprenaline	1.036	190
Orciprenaline	1.033	190
Terbutaline	1.033	200
Salbutamol	1.029	190
Prenalterol/*R*-antipode	1.026	190
4-Hydroxyalprenolol	1.024	190

In some examples, pure enantiomers were available and the order of elution could be investigated. As already shown for β-receptor blocking drugs[3], the stereoisomers with negative optical rotation are eluted after the (+)-isomers.

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