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RESOLUTION OF RACEMIC AMINOALCOHOLS (β-BLOCKERS), AMINES AND ACIDS AS ENANTIOMERIC DERIVATIVES USING A CHIRAL α_1-ACID GLYCOPROTEIN COLUMN

JÖRGEN HERMANSSON
Apoteksbolaget AB, Central Laboratory, Box 3045, S-171 03 Solna (Sweden)
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SUMMARY

A 3-cm long α_1-acid glycoprotein column (commercially available as Enantio-Pac) has been used for the enantiomeric resolution of β-blockers (propranolol, alprenolol, metoprolol, oxprenolol, pindolol). The method is based on the formation of simple enantiomeric derivatives using a solution of phosgene in toluene. A separation factor of 5.7 was obtained for the enantiomers of propranolol. The separation of the mandelic acid methyl and ethyl esters as well as an acetyl derivative of a primary amine is also demonstrated.

INTRODUCTION

The α_1-acid glycoprotein column (α_1-AGP column) has been used for the resolution of many racemic drugs[1,2]. Furthermore, the column has also been used in bioanalysis for the separation and quantitation of the enantiomers of disopyramide present in human plasma[3].

Although the α_1-AGP column is a powerful tool for the direct resolution of chiral molecules from different compound classes, such as different amines[1,2], non-proteolytic compounds and acids[4], not all compounds are resolved with high separation factors. Preparation of simple enantiomeric derivatives is one way of increasing the resolution of compounds giving low separation factors, or no resolution at all, in underivatized form. The alternative approach to direct resolution of chiral molecules is the use of diastereomeric derivatives. However, this indirect technique must be carefully controlled to avoid errors caused by different reaction rates of the enantiomers with the chiral reagent, or by racemization during derivatization. It has been demonstrated that the enantiomers of ketamine react at different rates with the chiral reagents, N-trifluoroacetyl-(*S*)-prolyl chloride and (*S,S*)-N-trifluoroacetyl proline anhydride[5]. However, these problems can be avoided by preparation of enantiomeric derivatives, using a non-chiral reagent, followed by chromatography on a chiral column. The present study reports the direct separation, on a 3-cm long α_1-AGP column, of the enantiomeric 2-oxazolidone derivatives of β-receptor blocking agents. The separation of esters and an acetyl derivative of a primary amine is also reported.

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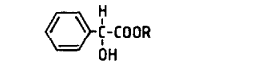
EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a Waters Model 6000 A pump, a Waters U6K injector and a Shimadzu SPD-2A variable-wavelengh UV detector operated at 215 nm. An α_1-AGP silica column (30 × 3.0 mm I.D. or 100 × 3.0 mm I.D., containing 147 mg of α_1-AGP per gram of solid phase) with a mean particle diameter of 10 μm was used[2]. This column is now available as EnantioPac® from LKB Produkter AB (Bromma, Sweden).

Chemicals

Racemic alprenol hydrochloride, metoprolol tartrate, oxprenolol hydrochloride, propranolol hydrochloride, practolol and pindolol were obtained from drug manufacturers. Mandelic acid methyl ester, mandelic acid ethyl ester and mandelic acid were purchased from Sigma (St. Louis, MO, U.S.A.). Trans AJ 126[6] was kindly supplied by Professor J. L. G. Nilsson (Apoteksbolaget AB, Central Laboratory, Solna, Sweden). The chemical structures are shown in Figs. 1 and 2. Analytical grade 2-propranol was obtained from E. Merck (Darmstadt, F.R.G.). Phosgene (20% in toluene) was purchased from Fluka (Buchs, Switzerland).



	R
Mandelic acid	H
Mandelic acid methyl ester	CH_3
Mandelic acid ethyl ester	C_2H_5

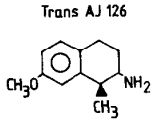


Fig. 1. Chemical structures.

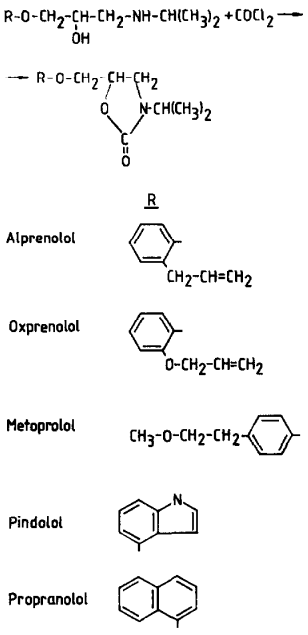


Fig. 2. Reaction of β-receptor blocking agents with phosgene.

Preparation of oxazolidone of β-receptor blocking agents

Racemic oxazolidone derivatives of the β-receptor blocking agents were formed by reaction with phosgene[7,8]. About 1 mg of the "β-blocker" was added to a screw-cap conical test-tube. The substance was dissolved in 0.5 ml of diethyl ether and 0.05 ml of 0.5 *M* sodium hydroxide, and the mixture was shaken for 1 min before the addition of 50 μl of phosgene solution. The mixture was agitated at room temperature in a rotating extraction apparatus. After 1 h of reaction time the tube was centrifuged at 500 *g*, and the organic phase was transferred to a new tube and evaporated to dryness. The residue was dissolved in the mobile phase. A reaction scheme is shown in Fig. 2.

Acetylation of trans AJ 126

Trans AJ 126 hydrochloride (1 mg) was treated with base and extracted into diethyl ether. The diethyl ether layer was transferred to a new tube and evaporated to dryness under nitrogen. The residue was dissolved in 200 μl of methylene chloride and 50 μl of triethylamine. Acetic acid anhydride (250 μl) was added, and after 15 min of reaction time at 40°C the solution was evaporated to dryness under a stream of nitrogen. The residue was dissolved in the mobile phase.

RESULTS AND DISCUSSION

Many racemic drugs have been resolved using an α_1-acid glycoprotein column (α_1-AGP column)[1–3]. However, it is impossible to design a chiral phase that resolves all classes of compounds with high separation factors. Therefore, it is interesting to investigate the possibilities of increasing the separation factors, of compounds giving low enantioselectivity, by the preparation of simple enantiomeric derivatives.

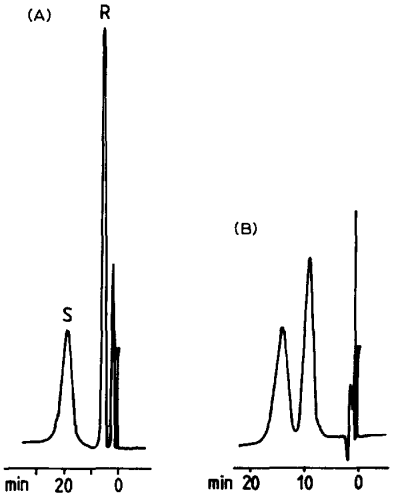


Fig. 3. Separation of the enantiomers of propanolol and pindolol as oxazolidones. Column, 30 × 3.0 mm I.D.), 147 mg of α_1-AGP per gram of solid phase. Mobile phase, (A) 15% (v/v) 2-propanol in phosphate buffer (pH 7.0; μ = 0.02), (B) as in A but 4% 2-propanol; flow-rate, 0.5 ml/min.

TABLE I

CHROMATOGRAPHIC DATA FOR OXAZOLIDONES OF β-BLOCKERS

Conditions: column, 30 × 3.0 mm I.D., 147 mg of α_1-AGP per gram of solid phase; mobile phase, phosphate buffer (pH 7.0; μ = 0.02) with the addition of 10% 2-propanol.

	k'_1*	α
Alprenolol	8.98	1.57
Oxprenolol	28.3	1.64
Metoprolol	0.40	1.00
Pindolol	4.64	1.65
Propranolol	26.8	5.71

* Capacity factor of the first eluted enantiomer.

Separation of non-proteolytic enantiomeric derivatives

Phosgene derivatives. Recently, Wainer *et al.*[7] reported the resolution of the enantiomers of the aminoalcohol norephedrine as its 2-oxazolidone derivative. They also demonstrated that oxazolidone formation proceeds without racemization. The enantiomers of β-receptor blocking agents have also been separated as oxazolidones by gas chromatography[8]. Wainer *et al.* resolved the enantiomers of propranolol as the 2-oxazolidone derivatives on a chiral HPLC column[9]. They obtained a separation factor of 1.09.

The present study reports the separation of the enantiomers of β-receptor blocking agents as the enantiomeric 2-oxazolidone derivatives, using a 30-mm long α_1-AGP column and a mixture of phosphate buffer (pH 7; μ = 0.02) and 2-propanol as the mobile phase. Typical chromatograms are shown in Fig. 3A and B, where the enantiomers of propranolol and pindolol are separated. (*R*)-Propranolol is eluted with a lower capacity factor than the (*S*)-form, therefore it is reasonable to assume that this is valid for the other β-blockers tested. However, a reversal of the elution

Fig. 4. Resolution of metoprolol enantiomers as oxazolidones. Column as in Fig. 3; mobile phase, phosphate buffer (pH 7.0; μ = 0.02); flow-rate, 0.5 ml/min.

Fig. 5. Separation of the enantiomers of acetylated trans AJ 126. Column, 100 × 3.0 mm I.D., 147 mg of α_1-AGP per gram of solid phase; mobile phase, 10% (v/v) 2-propanol in phosphate buffer (pH 7.0; μ = 0.02); flow-rate, 0.5 ml/min.

order of enantiomers has been observed in a series of closely related compounds[10]. Table I summarizes the chromatographic data obtained using 10% (v/v) 2-propanol in phosphate buffer (pH 7.0) as the mobile phase.

The chiral carbon of the β-receptor blocking agents is located in the propanolamine side-chain, which is the same in all the compounds studied. This means that these parts of the molecules are also identical after the formation of oxazolidones, and it is only the aromatic parts of the molecules that differ.

From the data presented in Table I it can be seen that the aromatic part of the structure has a marked influence on the separation factor and the retention. Propranolol, with an unsubstituted naphthalene ring, gives a separation factor of 5.71, whereas metoprolol, with a *p*-methoxyethyl-substituted benzene ring as the aromatic part, gives no resolution of the enantiomers and very low retention. However, a decrease of the 2-propanol concentration in the mobile phase markedly increases both the retention and the separation factor. A separation factor of 1.95 was obtained for the metoprolol enantiomers with a mobile phase of phosphate buffer (pH 7.0) with no addition of alcohol (Fig. 4). Alprenolol and oxprenolol, which contain a benzene ring substituted in the *ortho*-position, give separation factors of the same magnitude (Table I). However oxprenolol, which is more hydrophilic than alprenolol, gives capacity factors that are more than three times higher on the α_1-AGP column. The opposite retention order was obtained using a normal reversed-phase column (LiChrosorb RP-8)[11]. A reasonable explanation of the higher retention of oxprenolol on the α_1-AGP column may be that the oxygen in the allyloxy group interacts specifically with the chiral phase.

Ester and acetyl derivatives. Preparation of ester derivatives of acids and acetyl derivatives of amines can be an effective way of increasing the separation factors of acids and amines, respectively. This can be illustrated by the following examples: the enantiomers of mandelic acid have been separated as ester derivatives, and the enantiomers of trans AJ 126, a primary amine (structure see Fig. 1), have been separated after acetylation. The resolution of the acetylated trans AJ 126 enantiomers is shown in Fig. 5; a separation factor of 4.95 was obtained, and the peaks had capacity factors of 0.88 and 4.33.

The mandelic acid enantiomers were not resolved on an α_1-AGP column. However, the enantiomers can be separated as methyl and ethyl esters. The chromatographic data are summarized in Table II, and a chromatogram of the separation of

TABLE II

CHROMATOGRAPHIC DATA FOR MANDELIC ACID AND ITS ESTERS

Conditions: column, 100 × 3.0 mm I.D., 147 mg of α_1-AGP per gram of solid phase; mobile phase, phosphate buffer (pH 7.0; μ = 0.02) with the addition of 2% 2-propanol.

	k'_1*	α
Mandelic acid	1.13	1.00
Mandelic acid methyl ester	1.04	1.27
Mandelic acid ethyl ester	1.47	1.93

* Capacity factor of the first eluted enantiomer.

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