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## Note

# Enantiomer separation of metoprolol and its analogues and metabolites by capillary column gas chromatography after derivatization with phosgene

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Phosgene has been used as a derivatization reagent in the analysis of 2-amino alcohols by both gas[1] and liquid[2] chromatography. The advantage of the oxazolidinone structure for the separation of the enantiomers of some β-adrenoreceptor blocking drugs has been reported[3]. Also, enantiomers of chiral diols, α-hydroxy acids and N-methylamino acids can be separated after derivatization with phosgene[4].

Metoprolol {1-isopropylamino-3-[4-(2-methoxyethyl)phenoxy]-2-propanol} is a β-adrenoreceptor blocking drug and the racemate is widely used to treat hypertension, angina pectoris and cardiac arrythmias. Metabolites of metoprolol retaining the 2-amino alcohol side-chain can be gas chromatographed after oxazolidinone formation and trimethylsilylation of remaining hydroxylic and carboxylic groups[5,6].

We report here on the enantiomeric separation of metoprolol and related compounds and two of its major metabolites as oxazolidinones. The related compounds include a homologue based on 3-butanol and compounds with alternative N-alkyl substituents.

## EXPERIMENTAL

### *Gas chromatography*

A Carlo Erba Model 2101 gas chromatograph with a split inlet and a flame ionization detector was used. The procedure for the preparation of the chiral capillary column has been described earlier[7].

### *Reagents and chemicals*

Phosgene in toluene (2 *M*) was obtained from Fluka (Buchs, Switzerland).

The compounds studied are listed in Tables I–III; general structures are given in Table I. The compounds were available from the Department of Organic Chemistry, AB Hässle, except where stated otherwise.

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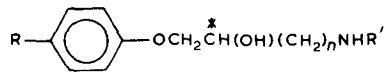
TABLE I

STRUCTURES OF THE COMPOUNDS STUDIED



| *Compound* | *R* | *n* | *R'*** |
|---|---|---|---|
| Metoprolol | $CH_2CH_2OCH_3$ | 1 | iso-$C_3H_7$ |
| α-Hydroxylated metoprolol (H 119/66) | $CH(OH)CH_2OCH_3$ | 1 | iso-$C_3H_7$ |
| Metoprolol acid (H 117/04) | $CH_2COOH$ | 1 | iso-$C_3H_7$ |

* C* is the asymmetric carbon atom.
** For R' substituted compounds, see Table III.

For the formation of phosgene derivatives[1], typically 1 mg of sample was dissolved in 1 ml of buffer (pH 12; ionic strength 1) and mixed with 1 ml of dichloromethane, then 30 μl of 2 *M* phosgene in toluene were added and the mixture was agitated for 10 min. After separation of the phases, an aliquot of the organic phase was evaporated and dissolved in a suitable amount of dichloromethane.

The metabolites of metoprolol were available as oxazolidinones[1]. Before gas chromatography, the alcoholic hydroxy group was converted into its trimethylsilyl ether with N,O-bis(trimethylsilyl)acetamide (BSA) and the acid into its methyl ester with diazomethane[8] in diethyl ether–methanol. The metoprolol acid was also isolated from urine from a healthy volunteer who had taken a therapeutic dose of metoprolol (292 μmole). The method described previously[1] was followed except that diazomethane was used instead of BSA.

## RESULTS AND DISCUSSION

### *Metoprolol homologue*

A homologue of metoprolol with an extra methylene group between the hydroxy and amino groups was derivatized with phosgene and chromatographed. Its identity has been verified previously[1]. The results are shown in Table II. The separation of the five-membered ring is superior to that of the expanded six-membered ring. With the former pair baseline separation was obtained, whereas the latter gave *ca.* 40% valley (= degree of overlapping between two adjacent peaks). It is interesting that of corresponding HFB derivatives only the enantiomers of metoprolol were separated, and not the homologue, although here the derivatives eluted within a reasonable time at 170°C[7].

TABLE II

ENANTIOMERIC SEPARATION OF METOPROLOL AND HOMOLOGUE

Column temperature, 200°C. Carrier gas, hydrogen (100 kPa).

| *Compound* | *n (Table I)* | α | $t_R$ *(min) (peak 2)* |
|---|---|---|---|
| Metoprolol | 1 | 1.025 | 38 |
| H 170/31 | 2 | 1.019 | 100 |

*Metoprolol analogues*

Oxazolidinones of metoprolol and a number of analogues with different N-alkyl substituents were prepared and chromatographed. The separation factors are given in Table III. It can be seen that the N-alkyl substituent has little influence on the enantiomeric resolution of these derivatives. The cyclic oxazolidinone is of primary importance. With all these derivatives, baseline or near baseline separations (< 10% valley) were achieved.

TABLE III

ENANTIOMERIC SEPARATION OF METOPROLOL AND ANALOGUES

Column temperature, 195°C. Carrier gas, hydrogen (100 kPa).

| *Compound* | *R' (Table I)* | $\alpha$ | $t_R$ *(min) (peak 2)* |
|---|---|---|---|
| H 100/11 | $CH_3$ | 1.030 | 37 |
| H 173/09 | $C_2H_5$ | 1.031 | 41 |
| H 117/78 | $n$-$C_3H_7$ | 1.026 | 53 |
| Metoprolol | iso-$C_3H_7$ | 1.032 | 44 |
| H 105/29 | *tert.*-$C_4H_9$ | 1.028 | 38 |

*Metabolites of metoprolol*

Metoprolol is extensively metabolized in man and only *ca.* 5% of the dose can be recovered as unchanged metoprolol in urine[9]. The major metabolic pathway is demethylation of the 2-methoxyethyl side-chain with subsequent oxidation to a carboxylic acid. This acid accounts for *ca.* 50% of the dose. $\alpha$-Hydroxylation represents 10% of the dose[9]. As both have the 2-amino alcohol side-chain intact, they can cyclize with phosgene. Remaining polar groups were masked with BSA[5].

An enantiomeric separation of $\alpha$-hydroxymetoprolol is shown in Fig. 1. We assume that the separation ($\alpha$ = 1.031) is due to the asymmetric carbon atom in the

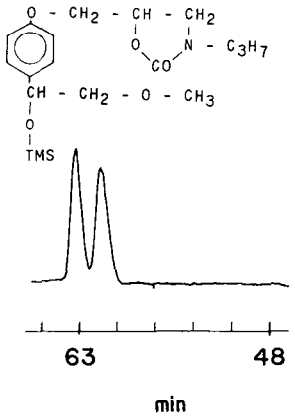



Fig. 1. Enantiomer separation of metoprolol with an $\alpha$-hydroxylated side-chain.

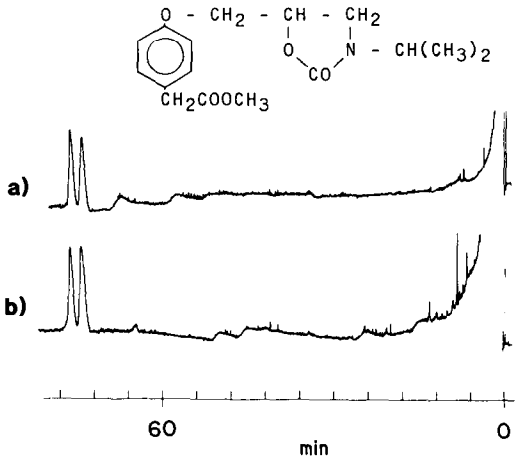



Fig. 2. Enantiomer separation of metoprolol acid; (a) reference compound; (b) isolated from urine.

amino alcohol side-chain, although a new chiral centre has been formed by hydroxylation of the α-carbon in the short side-chain.

The enantiomers of metoprolol acid were separated as their oxazolidinone methyl esters ($\alpha$ = 1.027; Fig. 2a). Fig. 2b shows the separation of the metabolite isolated from the urine of a healthy volunteer who had taken a therapeutic dose of metoprolol. Comparison of these two chromatograms indicates that the enantiomeric ratio was not changed significantly by this metabolic conversion route of metoprolol in this individual. In this chromatographic system, metoprolol acid elutes well separated from other urinary compounds. Using a non-polar capillary chromatographic system (CP-Sil 8), there was little difference in the chromatogram compared with that after trimethylsilylation.

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