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Note

Resolution of antihypertensive aryloxypropanolamine enantiomers by reversed-phase chromatography of (–)-menthyl chloroformate derivatives

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Flavodilol, 7-[2-hydroxy-3-(propylamino)propoxy]flavone (I), is an antihypertensive agent [1,2] related in structure to propranolol, 1-isopropylamino-3-(1-naphthoxy)-2-propanol (II), and other β-adrenergic antagonists which have in common an aryloxy or heteroaryloxy moiety attached to a propanolamine side-chain. It is well documented that the individual enantiomers of agents in this class exhibit marked differences in potency or mode of action [3–5]. As a result it is important to have methods for the analytical resolution of such enantiomers and for the precise measurement of their enantiomeric purity.

Of the methods available for the analysis of enantiomers the following have been applied to aryloxypropanolamines: (1) optical rotation measurements [3,5], (2) nuclear magnetic resonance (NMR) spectroscopic methods [6], (3) circular dichroism (CD) spectroscopic methods [7] and (4) liquid chromatographic (LC) resolutions involving chiral columns [8,9] and separation of precolumn derivatized diastereomers using chiral derivatization agents (CDAs). The CDAs which have been used for the high-performance liquid chromatographic (HPLC) resolution of propranolol and related β-blockers include *R*(+)- and *S*(–)-1-phenylethyl isocyanate (PEI) [10–13], *R*(+)-1-phenylethyl isothiocyanate [14], *R*(+)-1-(1-naphthyl)ethyl isocyanate [15,16], N-trifluoroacetyl-(*S*)-(–)-prolylchloride [17], *R,R*(+)-tartaric anhydrides [18], 2,3,4,6-tetra-O-acetyl-β-glucopyranosyl isothiocyanate [19] and (+)-1-(9-fluorenyl)ethyl chloroformate [20].

In our initial studies (+)-PEI was employed under reversed-phase HPLC conditions similar to those reported for the resolution of propranolol (II) [10]. As

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observed for propranolol [10] long retention times (20–30 min) were required for the complete resolution of flavodilol (I). Furthermore, in the analysis of individually resolved enantiomers it was desirable to employ a CDA approaching 100% in optical purity. The optical purity of (+)- or (−)-PEI from commercial sources in our hands was not reliably 100%.

The optical purities of the CDAs were examined in at least three of the above cases reported for the resolution of β-blockers and were proven to be very high [14,17,18] as required for the accurate measurement of enantiomeric purity. The CDAs employed in those cases and others [15,19] also yielded excellent separation coefficients, an additional requirement for the precise measurement of purity.

We have found that the use of (−)-menthylchloroformate [(−)-MCF], which is readily available in high (100%) optical purity, is likewise advantageous as a CDA in both analytical and preparative resolutions of aryloxypropanolamines and we have developed an assay method for the assessment of optical purity of resolved enantiomers. The resolution method is based on the chromatographic separation of diastereomeric (−)-menthyl carbamate derivatives (Fig. 1) which may be prepared in situ as part of the sample preparation.

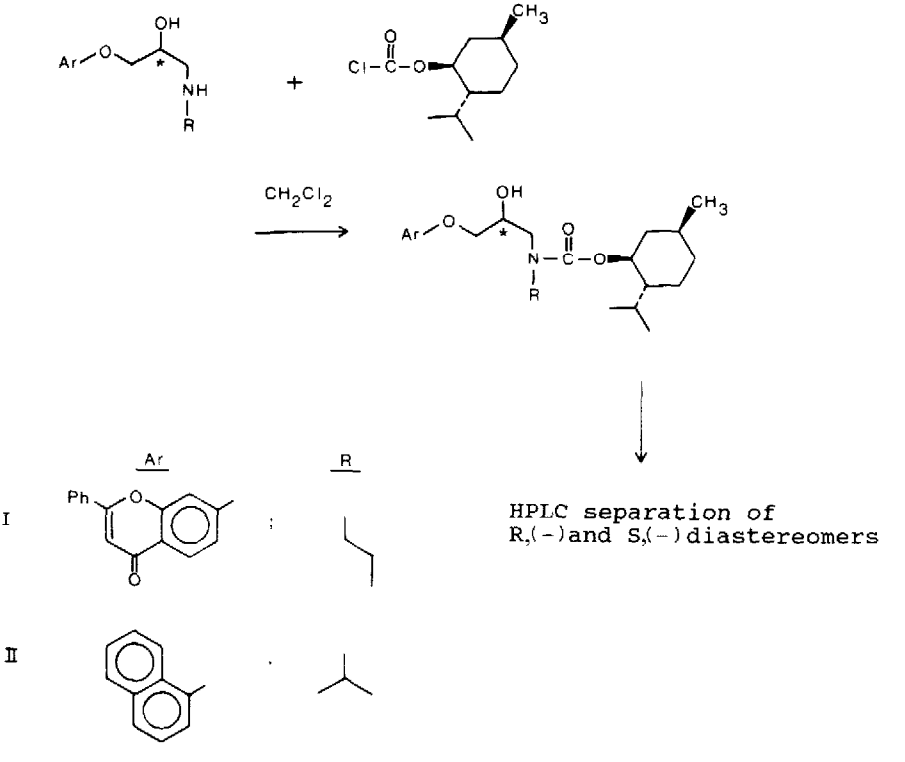

Fig. 1. Derivatization of aryloxypropanolamines with (−)-MCF.

The use of (−)-MCF as a CDA in analytical gas chromatography (GC) and HPLC resolutions has been reported [21–24] including its use in the preparative resolution of a β-blocker by normal-phase LC of an O-carbonate derivative [25]. However, the use of (−)-MCF as a CDA for the resolution of N-carbamate derivatives of β-blockers in either an analytical or a preparative method has not been reported. We describe below such a method using (−)-MCF.

EXPERIMENTAL

Racemic flavodilol maleate was prepared by a modification of the reported method [2]. *R*(+)- and *S*(−)-flavodilol maleates were prepared by fractional recrystallization of the racemates as the di-O-toluoyl tartrate salts in a manner similar to the method described for propranolol [3] followed by standard conversion via the free base to the maleate salt or by a modification of the method reported by Klunder et al. [6] for the chiral synthesis of propranolol. Racemic propranolol and samples of *R*(+)- and *S*(−)-propranolol as the hydrochlorides were obtained from Ayerst Laboratories (New York, NY, U.S.A.). L(−)-MCF and *R*(+)-PEI [as *R*(+)-methylbenzyl isocyanate] were purchased from Aldrich (Milwaukee, WI, U.S.A.).

Instrumentation

Analytical HPLC was performed on a Perkin Elmer Series 4B unit with an LC-95 detector and a Hewlett Packard 3392 integrator. A Dupont Golden Series (Mac Mod) Zorbax C_8, 3-μm, 8 cm×6.2 mm column was employed for the resolution of flavodilol using (−)-MCF (method Ia) or (+)-PEI (method Ib). An identical column packed with Spherisorb C_8, 3 μm, was employed for the resolution of propranolol using (−)-MCF (method IIa).

Enantiomeric assay method

Chiral derivatization method using (−)-MCF (methods Ia and IIa). A 0.10 *M* stock solution was prepared by dissolving 2.19 g (0.01 mol) of (−)-MCF in 100 ml dry methylene chloride. This solution was stable for months if kept dry. A 0.1-mmol sample of the analyte as the maleate salt (I) or hydrochloride salt (II) was liberated to the free base by extraction with 50 ml of 10% sodium carbonate and 50 ml of dichloromethane. The organic layer was separated, washed with 50 ml of saturated sodium chloride, dried with magnesium sulfate and filtered, followed by directly adding 2.0 ml (0.2 mmol) of the above solution and 0.2 ml of triethylamine to form the diastereomeric carbamates in situ. In the case of propranolol (method IIb) no triethylamine was added. The derivatization was complete in 0.5 h (method Ia) and in 4 h (method IIa).

Chiral derivatization method using (+)-PEI (method Ib). A 0.068 *M* stock solution was prepared by dissolving 1.0 g (6.8 mmol) of (+)-PEI in 100 ml of dry methylene chloride. A 0.1-mmol sample of the analyte as the free base in solution was prepared as above followed by direct addition of 1.5 ml (0.1 mmol) of the stock solution. The derivatization is complete within minutes.

High-performance liquid chromatography

Method Ia. The mobile phase was methanol–water (78:22) containing 0.2% acetic acid and 0.2% triethylamine (triethylammonium acetate). The flow-rate was 2.0 or 3.0 ml/min. The detector wavelength was 254 nm at 0.2 a.u.f.s.

Method IIa. The mobile phase was methanol–water (72:28) containing 0.2% triethylamine and acetic acid as above with a flow-rate of 2.0 ml/min. The detector in this case is set at 220 nm at 0.2 a.u.f.s.

Method Ib. The mobile phase was methanol–water (60:40) containing 0.2% triethylamine and acetic acid as above with a flow-rate of 2.0 ml/min. The detector wavelength was 254 nm at 0.2 a.u.f.s.

RESULTS AND DISCUSSION

The resolution of flavodilol (I) was initially performed with (+)-PEI using a method similar to the method described for the resolution of propranolol [10]. A moderate improvement was observed by employing C_8 packing versus C_{18}. Using method Ib the retention times for resolved peaks were 22.8 min for S(−) and 24.3 min for *R*(+) [separation coefficient (α) = 1.07] with near baseline separation achieved. In those cases of assaying for enantiomeric purity of resolved samples the minor enantiomer appeared as a shoulder and longer retention times were required to precisely measure less than 5%.

A substantial improvement was observed using (−)-MCF for the resolution of flavodilol. Using method Ia the retention times observed for baseline resolution were 8.8 min for *S*(−) and 10.2 min for *R*(+) (α = 1.20) at a flow rate of 2.0 ml/min or 5.5 min for *S*(−) and 6.4 min for *R*(+) (α = 1.18) at a flow-rate of 3.0 ml/min (Fig. 2). The percentage composition using method Ia was reproducible to the 0.2% level.

By using method Ia to monitor the classical resolution of flavodilol by recrystallization of di-O,O-ditoluoyl tartrate salts from ethanol it was determined that

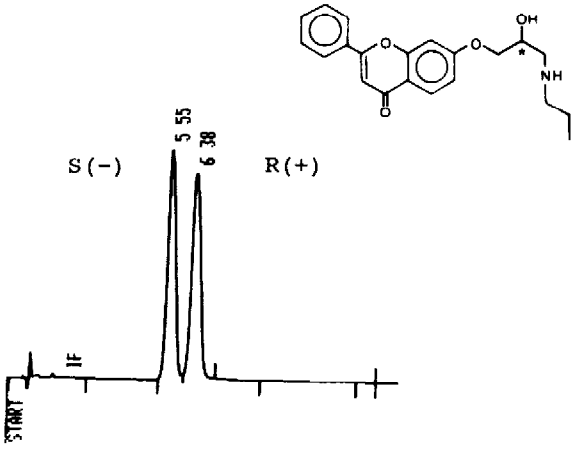

Fig 2. HPLC resolution of racemic flavodilol using method Ia (3.0 ml/min).

seven recrystallizations were necessary to achieve an enantiomeric purity of 98.5% or greater (Fig. 3). The absolute configuration of (−) as *S* and (+) as *R* was determined by analysis of samples prepared by the chiral synthesis of flavodilol

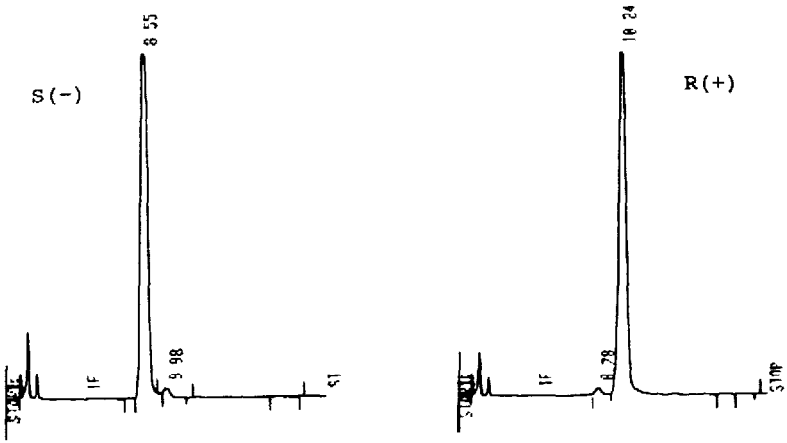

Fig. 3. HPLC resolution of individual flavodilol enantiomers using method Ia (2.0 ml/min).

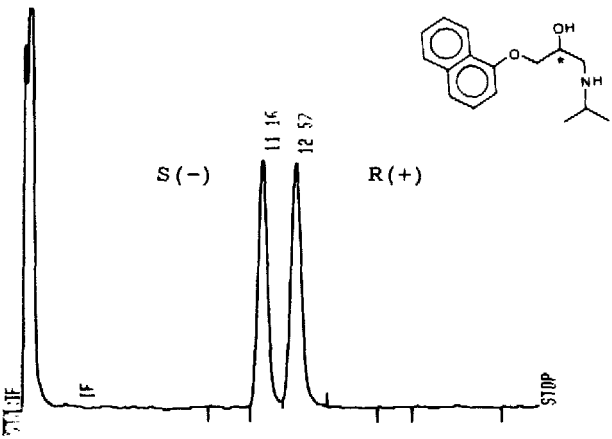

Fig. 4. HPLC resolution of racemic propranolol using method IIa (2.0 ml/min).

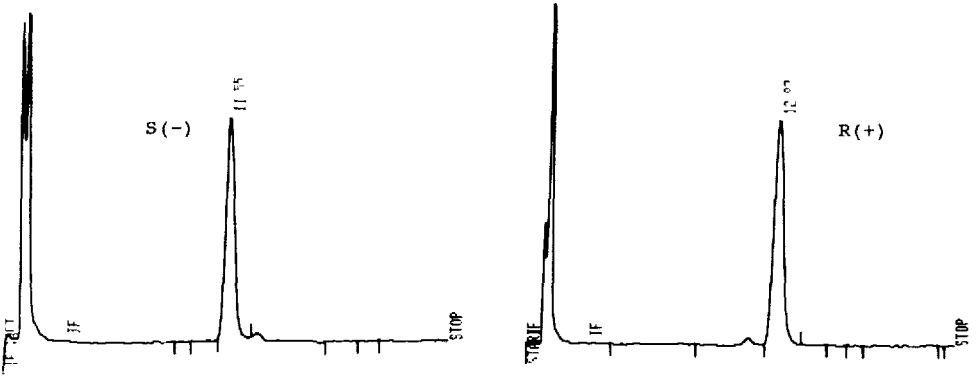

Fig. 5. HPLC resolution of individual propranolol enantiomers using method IIa (2.0 ml/min).

by the method of Klunder et al. [6]. The optical purity of each enantiomer obtained by this method was 96.3 for $S(-)$ and 96.4% for $R(+)$.

Finally, samples of racemic propranolol as well as the individual enantiomers of propranolol were assayed using (−)-MCF as a CDA. Under conditions IIa baseline resolution was achieved with retention times of 11.2 min for $S(-)$ and 12.6 min for $R(+)$ ($\alpha=1.14$) (Figs. 4 and 5). The absolute configurations are based on literature assignments [7]. Optical purities by this assay method exceeded 99.0% but were not further investigated.

CONCLUSIONS

The CDA (−)-MCF was found to offer several advantages in the resolution of aryloxypropanolamines. The derivatization reaction is facile and provides the stable carbamate (urethane) diastereomers which may be analyzed directly. Racemization in pure samples of individual diastereomers has not been observed. The reagent is readily available commercially and is possibly the lowest-priced CDA available. It is derived from (−)-menthol which is isolated from natural sources in 100% optical purity. The (−)-menthol moiety possesses three chiral centers in the cyclohexane ring and as such is a rigid, yet stereochemically rich structure. Furthermore, in reversed-phase chromatography the non-bonding, lipophilic interactions are predominant, and recognition of the highly lipophilic (−)-menthol moiety is taken advantage of and the separation of (−)-menthyl carbamates is thus facilitated.

The above HPLC resolution method using (−)-MCF fulfills most of the requirements which have been well outlined for enantio separations [18] and has been found applicable to the preparative resolution of flavodilol (I). The separation coefficients achieved in analytical resolutions ($\alpha=1.14$–1.20) are comparable to those in several previous reports [14,17,19] ($\alpha=1.14$–1.28) though they are not as high as those obtained with $R(+)$-1-(-1-naphthyl)ethyl isocyanate [15] ($\alpha=1.33$–1.50) or the $R,R(+)$-tartaric anhydrides [18] ($\alpha=2.40$–4.99). The (−)-menthol moiety as a CDA suffers from imparting no increase in chromophoric absorption of the derivative over the parent molecule. Because it is UV-transparent at the wavelengths commonly used, however, there are in turn no complications due to reagent absorptivity.

In the analysis of pharmaceuticals it is of increasing importance to have methods available for the precise measurement of enantiomeric purity. Chiral columns or CDAs approaching 100% in optical purity are required for such analyses to avoid misassignment of an optical antipode in the chiral column packing or CDA as a minor enantiomer. The reagent (−)-MCF is a CDA with the requisite optical purity. The above resolution method should be applicable to the analysis of most aryloxypropanolamines as well as many other primary and secondary amines.

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