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Note

Preparative resolution of the enantiomers of the β-blocking drug atenolol by chiral derivatization and high-performance liquid chromatography

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Of critical importance for further understanding of the antihypertensive mechanism(s) of the action of β-blocking drugs are studies including the effects of the pure enantiomers. This is especially true for the polar β-blocking drug enantiomers for which very little or no pharmacological information has been published. The enantiomers of these drugs, including one of the most widely used drugs of this class, atenolol (Fig. 1), are currently not available in sufficient quantities for pharmacological studies.

A promising approach for preparative resolution of racemic β-blocking drugs into their pure enantiomers was recently published [1]. The authors described the formation of monoesters of the β-blocking drugs with optically pure symmetrically O,O-disubstituted (*R,R*)- or (*S,S*)-tartaric acid anhydrides for subsequent separation of the diastereomers by high-performance liquid chromatography (HPLC). The advantages compared to previous approaches, derivatizing the secondary amine moiety only, were the greater separation of the diastereomers by

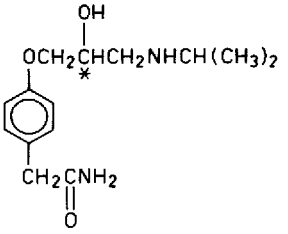

Fig. 1. Chemical structure of atenolol. The asterisk denotes the chiral carbon atom.

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HPLC for preparative resolution and a facile hydrolysis of the diastereomers after their resolution, to yield the pure enantiomers of the β-blocking drugs. This method was demonstrated to be useful for the preparative resolution of the enantiomers of propranolol, the most lipophilic of the β-blocking drugs.

This approach was used in the present investigation to accomplish the resolution of gram quantities of the most hydrophilic of the β-blocking drugs, atenolol, into its pure enantiomers. Because of its polar nature and susceptibility to acid-catalyzed dehydration, the atenolol resolution required method modifications.

EXPERIMENTAL

Reagents

(*R,R*)-Tartaric acid and *p*-toluoyl chloride were purchased from Aldrich (Milwaukee, WI, U.S.A.). (*R,R*)-O,O-Di-*p*-toluoyltartaric acid anhydride [2] and 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) [3,4] were synthesized as previously described. Racemic atenolol was purchased from Sigma (St. Louis, MO, U.S.A.). A small amount of the pure atenolol enantiomers was a kind gift from Imperial Chemical Industries (I.C.I., Macclesfield, U.K.). All solvents were glass-distilled from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Nanograde water was used for all aqueous reagents. All other chemicals were reagent grade or better.

Chiral derivatization, separation and hydrolysis of the atenolol diastereomers

Racemic atenolol (6 g) dissolved in 100 ml of nanograde water was titrated to pH 4.0 with 0.5 *M* trichloroacetic acid and freeze-dried. The solid ion-pair was dissolved in 200 ml of chloroform and a 2 *M* excess of (*R,R*)-O,O-di-*p*-toluoyltartaric acid anhydride (16.0 g) was added and refluxed for 1 h. After cooling, the reaction mixture was extracted twice with 200 ml of 0.2 *M* ammonium acetate (pH 6.9) to remove unreacted atenolol and most of the excess tartaric acid anhydride. The chloroform layer was taken to dryness under reduced pressure and the atenolol diastereomers formed were dissolved in mobile phase and separated using a normal-phase silica column system. The mobile phase eluent fractions containing the diastereomers were collected separately and taken to dryness under reduced pressure. The resulting solids were hydrolyzed at 60°C for 1 h with 100 ml of 1 *M* aqueous ammonia (pH 11.8). Each aqueous solution was adjusted to pH 4.0 with 2 *M* hydrochloric acid and extracted twice with 200 ml of chloroform to remove the tartaric acid formed. After alkalinization with ammonia the aqueous phase was freeeze-dried. The solid was triturated with 50 ml of chloroform and filtered to remove ammonium chloride. The chloroform was evaporated under a stream of nitrogen to yield the free bases of the pure atenolol enantiomers.

Instrumentation

HPLC. The HPLC system consisted of a Model 590 high-pressure pump, a Model U6K injector and a Model 440 UV detector with a 280-nm filter from Waters-Millipore (Milford, MA, U.S.A.). The HPLC column was an 8-μm silica Dynamax axial compression preparative type (Rainin, Woburn, MA, U.S.A.).

Two 25 cm×21.4 mm column modules were connected in series with a guard column module (5 cm×21.4 mm) for atenolol monoester diastereomer resolution. The mobile phase was chloroform–methyl *tert.*-butyl ether–methanol–hexane–acetic acid (100:100:80:30:1, v/v). The flow-rate was 10 ml/min.

GITC derivatives of the resolved atenolol enantiomers were separated on an analytical Spherisorb ODS-1, 5-μm column, 25 cm×4.6 mm (Alltech, Deerfield, IL, U.S.A.), using a mobile phase of acetonitrile–0.05 *M* ammonium acetate buffer, pH 4 (40:60).

Mass spectrometry. A Finnigan/MAT 212 mass spectrometer with an SS-200 data system was modified for fast atom bombardment mass spectrometry (FAB-MS) utilizing an Ion-Tek fast atom gun. The samples were analyzed in a glycerol matrix on a 316 stainless-steel probe tip at ambient temperature using xenon bombardment at approximately 8 keV. Quantitative gas chromatography–mass spectrometry (GC–MS) utilized an LKB 2091 instrument operated under electron-impact conditions.

RESULTS AND DISCUSSION

Several modifications of the method described for the preparative resolution of the propranolol enantiomers by Lindner et al. [1] were necessary in order to successfully resolve the enantiomers of atenolol. Chiral derivatization of racemic atenolol with (*R,R*)-di-*p*-toluoyltartaric acid anhydride proceeded quantitatively as evidenced by HPLC. To be able to achieve the critical step in this approach, i.e. derivatization of the alcoholic group only, the secondary amine function was blocked by ion-pair formation, using an excess of trichloroacetic acid. However, after HPLC separation of the diastereomers and hydrolysis of the esters it was observed that the acetamide moiety (Fig. 1) of the atenolol enantiomers had dehydrated to form a nitrile. This was noticed by MS as a loss of 18 a.m.u. from the molecular ion and the ring fragment of atenolol. The dehydration was determined to be caused by the acidic conditions in the chiral derivatization step, i.e. excess trichloroacetic acid. Dehydration could be avoided by titration of atenolol to pH 4.0 with trichloroacetic acid prior to chiral derivatization. Furthermore, in contrast to the propranolol diastereomers, the atenolol diastereomers could not be purified by recrystallization prior to HPLC separation of the diastereomers, presumably due to their highly polar character. The atenolol diastereomers were instead partially purified by extracting the crude reaction mixture in chloroform with a neutral (pH 6.9) buffer. The use of dilute aqueous ammonia in this step [1] caused substantial hydrolysis of the monoesters of atenolol as indicated by the reappearance of underivatized atenolol. This hydrolysis, which apparently did not occur with the corresponding propranolol monoesters, was probably due to the hydrophilic nature of the atenolol monoesters.

The purified reaction mixture could be separated either by normal- or reversed-phase HPLC. The separation factor and peak symmetry were both better on reversed-phase HPLC. The capacity of the normal-phase silica column was, however, at least ten-fold greater. As preparative resolution was the goal, normal-phase HPLC was used. A chromatogram of a small amount of the reaction mix-

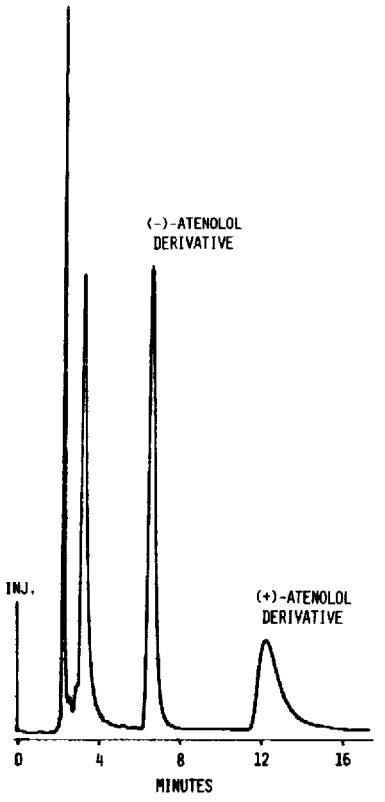

Fig. 2. Normal-phase HPLC of partially purified reaction mixture of racemic atenolol and (*R,R*)-O,O-di-*p*-toluoyltartaric acid anhydride. Detection was made by UV (280 nm).

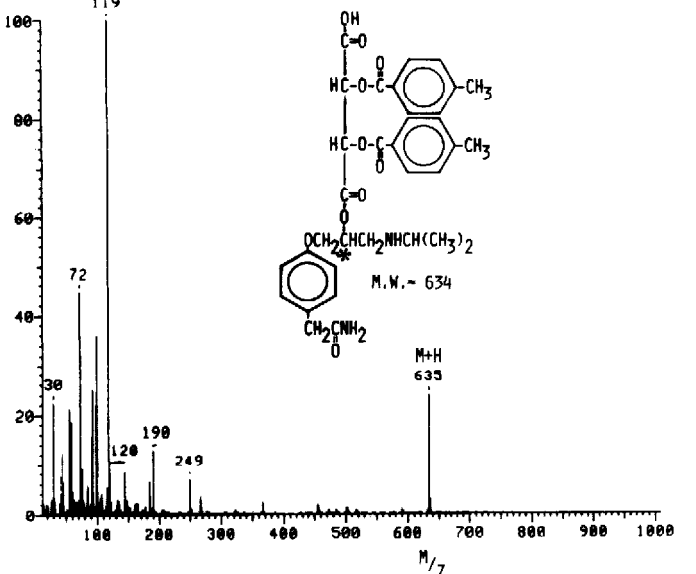

Fig. 3. Positive-ion FAB-MS of the (+)- and (−)-atenolol (*R,R*)-O,O-di-*p*-toluoyltartaric acid monoesters. The two diasteromers, collected from the HPLC effluent in Fig. 2, produced identical spectra.

ture is shown in Fig. 2, using a 21.4 mm I.D. silica column. The two diastereomers were well separated from each other and from other UV-absorbing peaks. Attempts were made to confirm the proposed structure of these highly charged diastereomer derivatives by MS. Direct probe electron impact or chemical ionization MS gave no interpretable information. FAB-MS, however, gave clear spectra, which were identical for the two diastereomers (Fig. 3). The spectra showed intense M+H ions. The base peaks at *m/z* 119 were from the *p*-toluoyl ion. Other minor fragmentation was derived from cleavages at other positions of the tartaric acid moiety. The order of elution of the atenolol diastereomers was determined by separate derivatization of small samples of the optically pure atenolol enantiomers obtained from I.C.I. The nature of the two early peaks was not determined. Up to 300 mg of crude reaction mixture could be chromatographed in a single injection without loss of separation. The resolution of the total reaction mixture originating from 6 g of racemic atenolol required 50 injections, using the 21.4 mm I.D. column.

After resolution of the diastereomers by HPLC, hydrolysis of the monoesters with ammonia, extraction at pH 4 to remove tartaric acid and lyophilization, 1.3

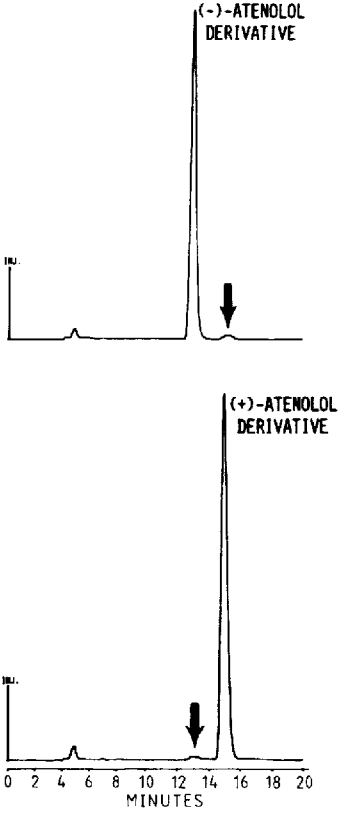

Fig. 4. Determination of the enantiomeric purity of the resolved atenolol enantiomers by reversed-phase HPLC after chiral derivatization with GITC. The arrows denote enantiomeric impurities. Detection was made by UV (280 nm). The peak at 4.8 min is from the chiral reagent.

g of each enantiomer was obtained, i.e. 43% overall recovery. The chemical purity as determined by quantitative HPLC and GC–MS (with racemic atenolol as the pure reference compound) was greater than 97%. The enantiomeric purity, determined by HPLC after chiral derivatization with GITC [4,5] was also greater than 97% for each enantiomer (Fig. 4). The enantiomeric purity could be greatly increased (>99.5%) if a larger fraction between the two diastereomeric HPLC peaks was discarded, however, leading to decreased yields.

CONCLUSIONS

It is concluded that the approach described by Lindner et al. [1] for the chiral separation of β-blocking drugs with some modifications was applicable to preparative-scale resolution of the enantiomers of the most polar and chemically labile of these drugs, atenolol. The use of larger columns and automated injection and fraction collection devices should further improve the efficiency of this approach. Preliminary pharmacological studies of the atenolol enantiomers resolved in this manner indicate that (−)-atenolol possesses a stereoselective central hypotensive action in laboratory animals [6,7], a novel observation for this highly polar drug. The resolution of the atenolol enantiomers has also been of importance for the finding of stereoselective uptake and release of (−)-atenolol by adrenergic neuronal storage granules [8–10].

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