Journal of Chromatography, 238 (1982) 470–475
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,676

Note

Procedure for the chiral derivatization and chromatographic resolution of *R*-(+)- and *S*-(−)-propranolol

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(First received November 26th, 1981; revised manuscript received December 28th, 1981)

Propranolol [1-isopropylamino-3-(1-naphthoxy)-2-propanol] (Fig. 1) is an important beta adrenergic blocking agent which has gained widespread usage in the treatment of angina pectoris, cardiac dysrhythmias and hypertension. Currently the commercially available preparation is a racemic mixture of which only the *S*-(−)-enantiomer has beta adrenergic blocking activity[1] while the *R*-(+)-enantiomer has only a membrane stabilizing effect[2]. These isomers were resolved on a preparative scale by repetitive recrystallizations of the dibenzoyl tartaric acid salts[3]. Analysis of each enantiomer in biological fluids has been accomplished by administering a 1:1 mixture of a deuterium-labeled enantiomer and the unlabeled opposite enantiomer and measuring the concentrations of these isomers by gas chromatographic–mass spectrometric (GC–MS) techniques[4]. This method, however, requires the prior availability of the pure enantiomers and the preparation of deuterium-labeled propranolol. A simpler method involves derivatization with an optically pure chiral reagent and the chromatographic separation of the resulting diastereomers. This technique, employing N-trifluoroacetyl-*S*-(−)-prolyl chloride[5], has been used to quantitate each enantiomer of propranolol by GC[6] and high-performance liquid chromatography (HPLC)[7,8] following administration of the racemic drug. Several investigators have noted, however, that this chiral reagent can racemize during storage[5,8].

We have recently examined the applicability of an alternative chiral reagent, *R*-(+)- or *S*-(−)-1-phenylethyl isocyanate[9,*] for converting racemic propranolol to diastereomeric derivatives which can be resolved chromatographically. Each pure

* The Aldrich Chemical Co. catalog (1981–82, p. 629) lists these compounds as *R*-(−)- and *S*-(+)-, with optical rotations measured on the neat liquid. It is clear from previous work[10] that these absolute configurations should be reversed, *i.e.*, the negative rotating enantiomer has the *S* configuration and the positive rotating enantiomer has the *R* configuration.

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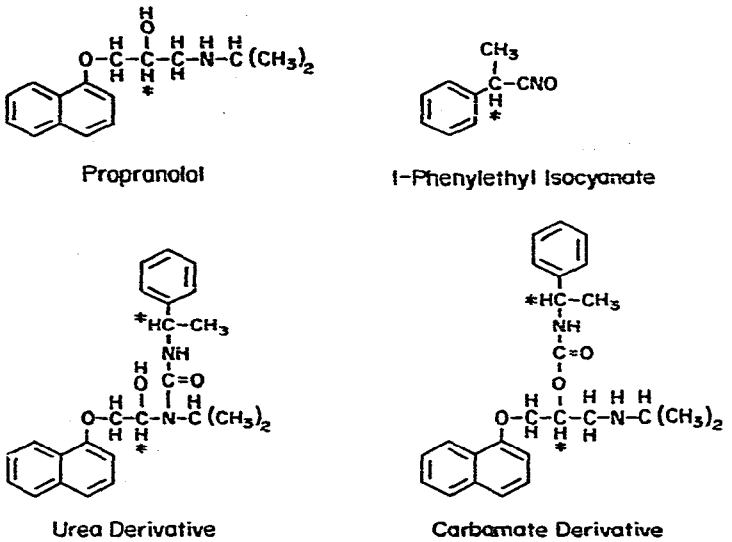

Fig. 1. Structure of propranolol, 1-phenylethyl isocyanate and the possible urea and carbamate derivatives.

enantiomer of 1-phenylethyl isocyanate is commercially available and has been employed for the GC separations of the enantiomers of chiral secondary alcohols after formation of carbamate derivatives[10,11]. Racemization, as described above, does not occur with this reagent and it is chemically quite stable.

In the current study we have found that the reaction with propranolol occurs rapidly at 25°C to form a urea derivative with the secondary amino group of the drug (Fig. 1). The diastereomers have been separated in gram quantities by silica gel chromatography and fully characterized. Subsequent hydrolysis of these isomers produces the optically pure enantiomers of propranolol, thereby providing a method for resolving racemic propranolol without repetitive recrystallizations. This method of resolution is applicable to quantities from the microgram to the gram range. Conditions for GC and HPLC separation have also been developed which demonstrate the potential for using this chiral reagent for micro-scale analyses of the enantiomers of propranolol and of other beta adrenergic blocking drugs in biological fluids.

MATERIALS AND METHODS

R- and *S*-1-phenylethyl isocyanate and *R,S*(+)-propranolol were purchased from Aldrich (Milwaukee, WI, U.S.A.). *R*-(+)- and *S*-(−)-propranolol standards were prepared by the method of Yost and Holtzman[3]. Silica gel (Merck, mesh) was purchased from J. T. Baker (Phillipsburgh, NJ, U.S.A.). Thin-layer chromatography (TLC) plates LK5DF were obtained from Whatman and plastic plates from Kodak (Rochester, NY, U.S.A.). Solvents were ACS reagent grade. Hexamethyldisilazane and trimethylchlorosilane were purchased from Pierce (Rockford, IL, U.S.A.).

Equipment

Infrared (IR) spectra were obtained on a Perkin-Elmer 237B spectrometer. Proton nuclear magnetic resonance (NMR) spectra were obtained at 360 MHz on a

Brucker WH-360 spectrometer at the Middle Atlantic Regional NMR Facility at the University of Pennsylvania*. High-performance liquid chromatography was performed with an Altex Model 110 pump (Beckman, Berkeley, CA, U.S.A.) using a Spherisorb C_{18} 5-μm column (Regis, Chicago, IL, U.S.A.) (25 × 0.46 cm). Peaks were detected with Kratos SF970 fluorometer with a 220-nm excitation and a >340-nm filter.

GC and GC–MS

GC analysis was performed on a Hewlett-Packard (HP) 5710 gas chromatograph equipped with 0.8 m × 2 mm I.D. glass column packed with 5% OV-22 on Supelcoport (80–100 mesh; Supelco, Bellefonte, PA, U.S.A.). Conditions were: helium flow-rate, 30 ml/min; injector temperature, 250°C, and column temperature programmed from 250 to 280°C at 4°C/min and held at the upper temperature. The GC was interfaced via a glass jet separator to an HP 5980 A mass spectrometer and Model 5934A data system. The transfer line was held at 280°C and the ion source at 180°C. The mass spectrometer was scanned repetitively from 50 to 500 a.m.u. using an ionizing energy of 70 eV. Capillary analysis was performed on a HP 5710 gas chromatograph equipped with a Model 18740B capillary inlet system, a nitrogen–phosphorus detector and a 12.5 × 0.2 mm I.D. fused-silica column coated with SP 2100 and deactivated with Carbowax 20M (Hewlett-Packard). The operating conditions were: injector temperature, 250°C; detector temperature, 250°C; and the column temperature was programmed from 230°C to 250°C at 4°C/min and held at the upper temperature.

The diastereomeric propranolol derivatives were trimethylsilylated by treatment with 100 μl of hexamethyldisilazane, 20 μl of trimethylchlorosilane and 100 μl of pyridine at 25°C for 8 h. The resulting derivative was analyzed by GC–MS using the packed column conditions described above. Although no molecular ion was present, M − 15 (*m/z* 463) and M − 90 (*m/z* 388) ions were produced as expected for trimethylsilyl (TMS) derivatives. Other characteristic ions include: *m/z* 373 corresponding to the loss of $\cdot CH(C_6H_5)CH_3$ from M+; *m/z* 359 corresponding to the loss of $NH = C(C_6H_5)CH_3$; and *m/z* 335 corresponding to the loss of the naphthyloxyl group.

RESULTS

Preparation of derivatives of propranolol

Propranolol free base (0.1 g, 0.39 mmol) dissolved in chloroform (30 ml) reacted quickly and quantitatively at room temperature with *R*-(+)-1-phenylethyl isocyanate (40 mg, 0.3 mmol). The chloroform mixture was extracted with 0.1 *M* HCl (50 ml). The pH of the aqueous phase was adjusted to neutrality with 10 *M* NaOH and extracted with chloroform (40 ml). The extract was chromatographed on plastic thin-layer plates developed with acetone. There was a small propranolol spot (R_F 0.24) but no derivative was found (R_F 0.77). Thin-layer chromatography of the reaction mixture on LK5DF plates with development in acetone–acetic acid–water (81:1:1) showed no propranolol (R_F 0.15) or 1-phenylethylamine (R_F 0.26) formed

* The Middle Atlantic NMR Facility is supported by the United States Public Health Service Grant RR 542.

TABLE I

ELUTION OF *R*-(+)- AND *S*-(−)-PROPRANOLOL DERIVATIVE FORMED WITH *R*-(+)- AND *S*-(−)-1-PHENYLETHYL ISOCYANATE FROM A SILICA GEL COLUMN

Volume (ml)	*Propranolol derivative observed*
0– 65	None
65– 87	*R*-(+)-
87– 95	*R*-(+)- + *S*-(−)-
95–127	*S*-(−)-

Column: silica gel (Merck, mesh), 260 × 0.9 cm, eluted with chloroform. The propranolol derivatives were determined by TLC on LK5DF plates developed in chloroform.

from the hydrolysis of the *R*-(+)-1-phenylethyl isocyanate. When LK5DF plates were developed three times in chloroform, the reaction mixture showed a derivative of *R*-(+)-propranolol (R_F 0.067, after three developments R_F = 0.20) and S-(−)-propranolol (R_F 0.053, after three developments R_F = 0.16).

Since the product was not acid extractable, it would indicate that the isocyanate forms a N-substituted urea with the 1-amino group rather than a carbamate with the 2-hydroxyl (Fig. 1).

Three hundred milligrams of the two derivatives were separated on a silica gel column (0.9 × 60 cm) using chloroform as the solvent and following the elution with TLC (Table I). The small region of overlap was discarded.

The IR spectra of the mixture of derivatives and the individual derivatives showed a broad absorption from 1740 cm^{-1} to 1690 cm^{-1}. This region encompasses both expected carbonyl stretching bands for either a carbamate or urea.

The NMR spectrum of the mixture in chloroform shows six doublets of equal intensity in the methyl region, each with J = 7 Hz. The spectrum of each of the separated derivatives had three methyl doublets [*R*-(+)-propranolol (1) (δ 1.49, 1.25,

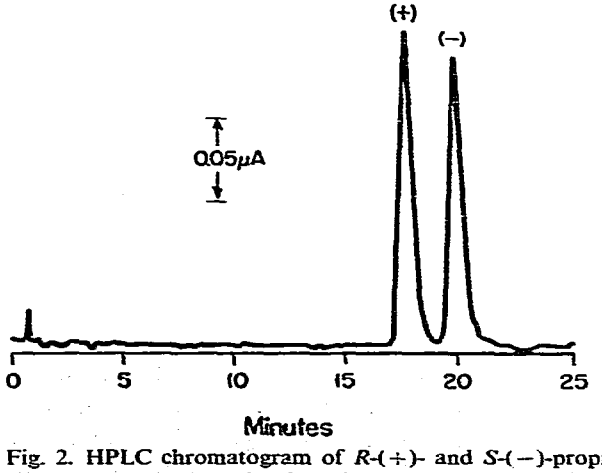

Fig. 2. HPLC chromatogram of *R*-(+)- and *S*-(−)-propranolol derivatized with *R*-(+)-1-phenylethyl isocyanate. The column is a Spherisorb 5-μm C_{18} with an Altex Model 110 pump. The solvent is methanol–water (65:35) runs at 3.2 ml/min (4300 p.s.i.). The detector is a Kratos SF 970 (excitation 220 nm; emission greater than 340 nm).

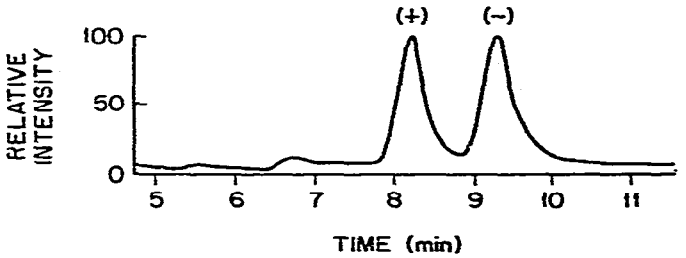

Fig. 3. Total ion current chromatogram from the GC–MS analysis of *R*-(+)- and *S*-(−)-propranolol derivatized with *R*-(+)-1-phenylethyl isocyanate. The column was a 0.8 m × 2 mm I.D. glass column packed with 5% OV-22 on Supelcoport in a HP 5984A GC–MS system. The column was programmed from 250 to 280°C.

1.30), *S*-(−)-propranolol (2) (δ 1.46, 1.27, 1.14)]. The presence of three methyl resonances for each isomer would suggest that the individual methyl groups of the N-isopropyl group were non-equivalent. This is consistent with a urea derivative in which the bulky substituent hinders free rotation.

Chromatographic separation of propranolol derivatives

HPLC of unpurified reaction mixture on C_{18} reversed phase with methanol–water (65:35) at 3.2 ml/min showed two peaks with retention times of 17 min for D-propranolol and 20 min for L-propranolol with a clear baseline separation (Fig. 2). Unreacted propranolol in this system has a retention time of 25 min. The addition of 10–1000 fold excess isocyanate did not affect this pattern, indicating that the carbamate is not formed even when the derivatizing agent is in great excess.

The diastereomeric derivatives of propranolol were separated by GC. The peaks were resolved (resolution, R_s = 1.49) in about 10 min using a short packed column as shown in Fig. 3. Further work demonstrated that the peak shape could be greatly improved and the analysis time considerably shortened by using a fused silica capillary column (R_s = 4.15). Trimethylsilylation of the free hydroxyl group of the propranolol derivatives yielded diastereomers which eluted from the packed column as one peak with a retention time slightly longer than that of the non-silylated diastereomers.

Hydrolysis of the derivative

The derivatives were hydrolyzed by reflux in 1 *M* H_2SO_4 with 50% recovery of D- and L-propranolol as determined by HPLC.

DISCUSSION

The only previously reported applications of resolution of racemic mixture using the optically pure enantiomers of 1-phenylethyl isocyanate involved analyses of chiral secondary alcohols which form carbamates[10,11]. In the present work, we have shown that a chiral amine can also be derivatized using this reagent. In the case of propranolol, with both secondary hydroxyl and amino groups, the reagents react exclusively with the amino group under the conditions specified even with a 100-fold excess of reagent. The resulting diastereomeric urea derivatives are easily separated

by low-resolution liquid and gas chromatographic methods. The magnitude of the difference in chromatographic properties of the diastereomers is surprising, since there are four atoms separating the chiral centers and these are not included in a ring system. Conversion of the free hydroxy group to a TNS ether drastically reduces the physical differences between these diastereomers as evidenced by the fact that a single GC peak was obtained. On the basis of this information, one can speculate that intramolecular hydrogen bonding between the hydroxyl group and the oxygen of the carbonyl group plays a role in enhancing the physical differences between the diastereomers.

In summary, we have shown that diastereomeric urea derivatives are formed between racemic propranolol and *S*-(−)-1-phenylethyl isocyanate. This chiral reagent can be used to resolve the enantiomers of propranolol by preparative liquid chromatography, followed by acidic hydrolysis of the derivatives. Furthermore, the reagent can be used for the analyses of the propranolol enantiomers on a micro scale by HPLC or GC techniques.

This method is potentially adaptable to the quantitation of the enantiomers in biological fluids and represents a possible convenient alternative to the use of N-trifluoroacetyl-*S*-(−)-prolyl chloride for this purpose. Studies along these lines are currently in progress.

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